# Help (PDF version)

# EthoVision® XT

## Version 18

Noldus
Information Technology

Version 18

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# Contents

# Configure Detection Settings 351

# Welcome to EthoVision XT!

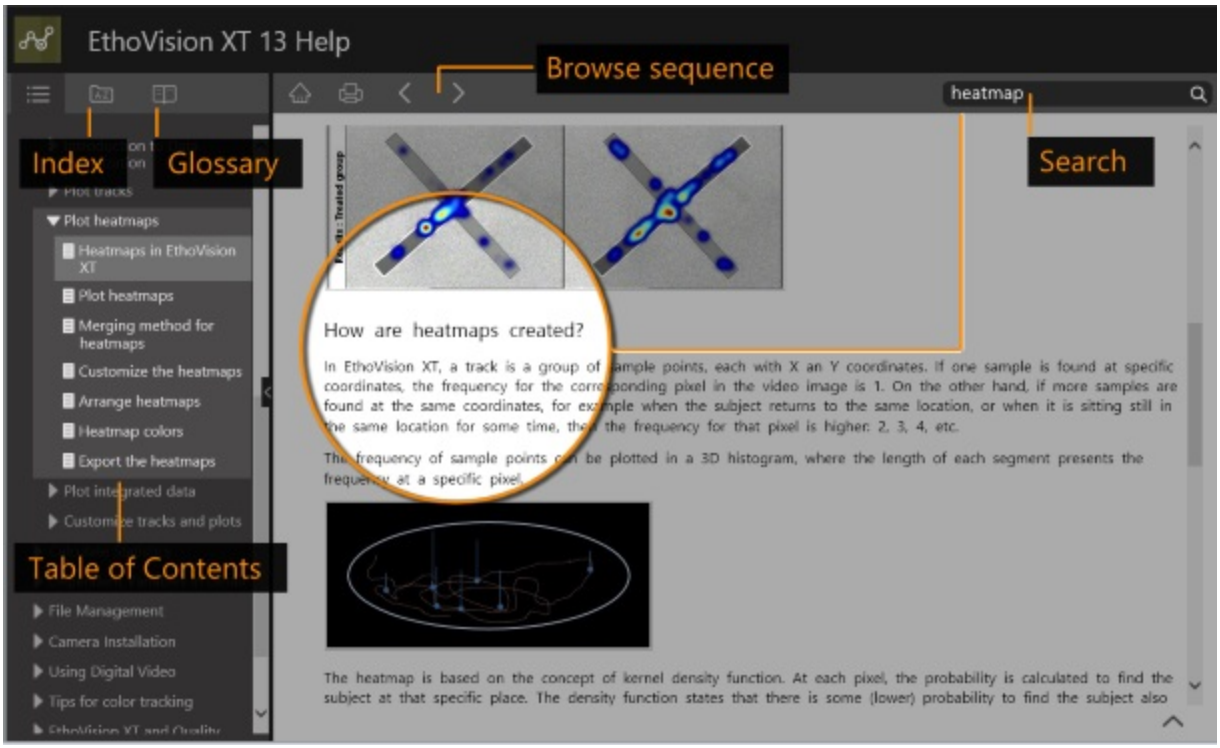
## Main topics and tasks



*See also*

# How to use this help

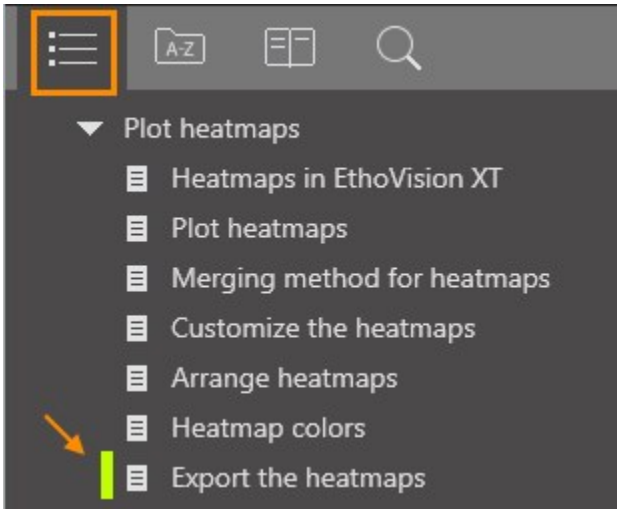
## Main elements




## Tips for using the help

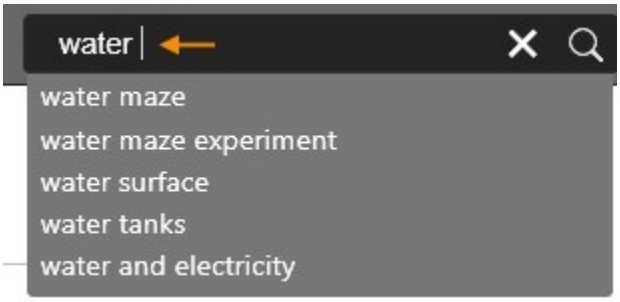
*Table of Contents*

- If you do not see the Table of Contents on the left, enlarge the Help window or zoom out the characters (**Ctrl**+mouse wheel).
- If you want to know the current topic's position within the browse sequence, click the Table of Contents icon and locate the green indicator.




*Search*

- To search for two or more adjacent words, use quotes. For example, “plus maze”.
- While you type, the Help gives you suggestions about recurring terms and sentences. Click one of them to refine your search.

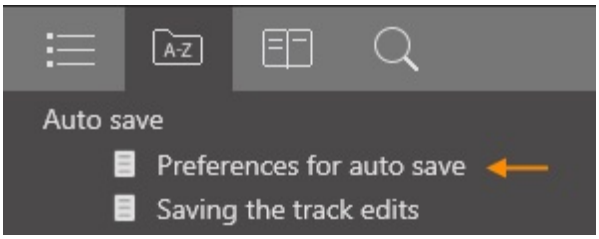



- The search results are shown on the left side pane.
- To go back to the main page, click the EthoVision XT icon at the top-left corner of the screen.

*Index*

- To use the index, type a word in the **Filter keyword** field.

- If an index term (or sub-term) occurs in different topics, these are listed under the term.




- To reset the index, delete the word you typed or click the X button and press **Enter**.

## Notes

- The EthoVision XT Help is in responsive HTML5 format. Depending on the size and shape of the Help window, the buttons are automatically re-arranged, for example in tablet mode.


*See also*

For more manuals, click the following link:

EthoVision XT 18 Other Documentation

Or from the Windows **Start** menu, choose **All Apps** > **Noldus** > **EthoVision XT 18 Other Documentation**.

# Introduction to EthoVision XT

## Learn about

- Video tracking with EthoVision XT
- Video in EthoVision XT
- Modules of EthoVision XT
- What's new in EthoVision XT 18
- Restrictions in EthoVision XT
- For more information

# Video tracking with EthoVision XT

EthoVision XT is an automated video tracking and motion analysis system. Video tracking means that EthoVision XT detects and follows one or more animals, or objects, in a video image (live footage or pre-recorded video file), and tracks their location and movement.

EthoVision XT is a software platform. The *Base* version help you carry out tests like the Open field and the Morris water maze test. With its *Add-on modules*, it offers a wide range of video tracking options, and extensive analysis of locomotion and behavior. For more information, see Modules of EthoVision XT on page 31.

## Overview

The entire process carried out by EthoVision may be summarized as follows: a video camera observes the movement of one or more subjects, and passes images of the subjects to your computer. There they are transformed into a digital signal and optionally encoded as a digital video file. From this digital signal or file, EthoVision first detects the subjects and then extracts the size of the subjects and position of one or more body points of the subjects in each image. That data is then transformed into a series of dependent variables quantifying the behavior of the subjects.

*See also*

- The **EthoVision XT 18 - Quick Start Guide**, which came with the software.
- The **EthoVision XT Video Tutorial** to learn how to set up an experiment in EthoVision XT. In EthoVision XT, choose **Help** > **Video Tutorial**. Note that the Video Tutorial contains audio.

## Image sensing

The starting point of an imaging system is a video camera. A video camera transforms a scene (the area in front of the lens), into an image (a picture taken by the camera). If you have an analog camera the image must be converted into a digital image consisting of pixels. With USB, Gigabit Ethernet (GigE) and Internet Protocol (IP) cameras, digitization occurs within the camera. You can plug them directly into your computer. Instead of using live video from a camera, you can also make a digital video file and use that for tracking.

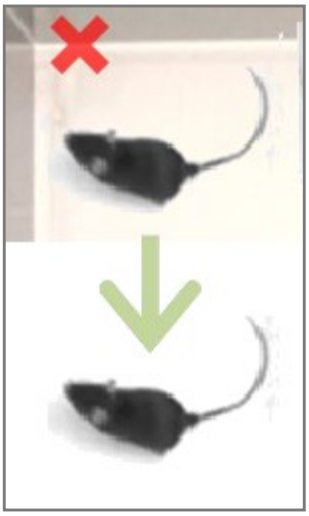
One video image from a camera or digital video file is called a frame. The frame is made by a point which scans the scene in a series of horizontal lines (called fields), starting at the top and working its way down to the bottom. The fields are interlaced, that is, the camera first scans the odd lines, then the even lines. As the

scene is scanned, the brightness (or color) of the scene is transformed into an analog signal describing the intensity of the image at each point of the scan.

The number of frames scanned in each second is called the frame rate, and this determines the maximum possible sample rate for EthoVision. The frame rate differs between cameras. See Cameras supported by EthoVision XT

## Subject detection

When EthoVision XT receives the video frame, it does not "see" a mouse in an open field (for instance), but a bitmap composed of pixels, each of which has a particular gray value. The first thing that EthoVision XT does is to distinguish between the subjects to be tracked, and the background. In order to establish which pixel is part of the animal and which pixel is part the background, EthoVision makes use of different *detection methods*. You can choose which detection method to use, but you can also let EthoVision XT select the best method for you. To do so, choose the *Automated Setup* of the Detection Settings.



## Excluding noise

Whichever detection method you choose, it is possible that some pixels are identified as the subject, that are in fact just system noise or reflections. You can exclude these as follows:

- *Before tracking*:

  In the Arena Settings, define an arena. This means that pixels outside the arena are ignored.

  In the Detection Settings, make various settings to exclude noise.

- *After tracking*:

  Smooth the track to filter out system noise, outliers and small movements.

## Identifying multiple subjects

EthoVision XT can track more than one animal per arena. A few techniques are available, from the basic tracking of unmarked subjects to the state-or-the-art identification technique based on Deep learning (see below). To track multiple subjects, you need the Social interaction add-on module. Modules of EthoVision XT

### *Tracking of unmarked subjects*

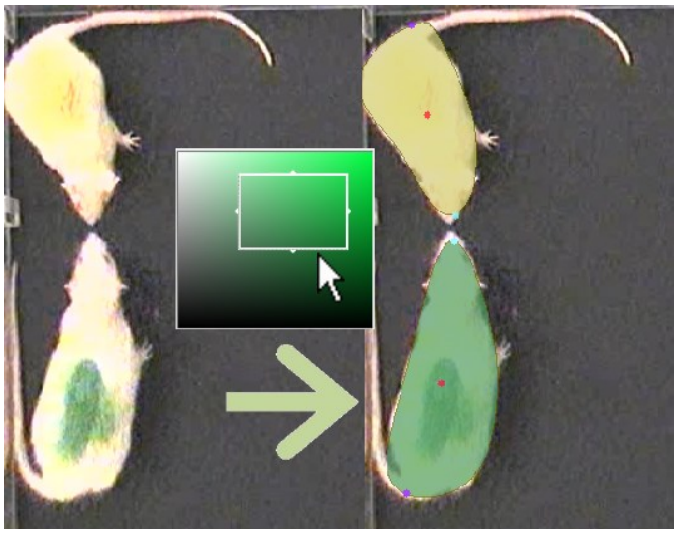
You can track multiple animals without using color markers. For instance, track a shoal of fish to measure the average between-individual distance. However, keep in mind that there may be subject identity swaps, that is, what is labeled subject 1 may be labeled subject 2 later in the track. Therefore individual identification is not guaranteed. The methods listed below keep track of the subjects' identities.

### *Color-marker tracking*

Here below, you see an example of color marker tracking. EthoVision XT follows the color spots on the back of the insects, ignoring the shape of the animals.

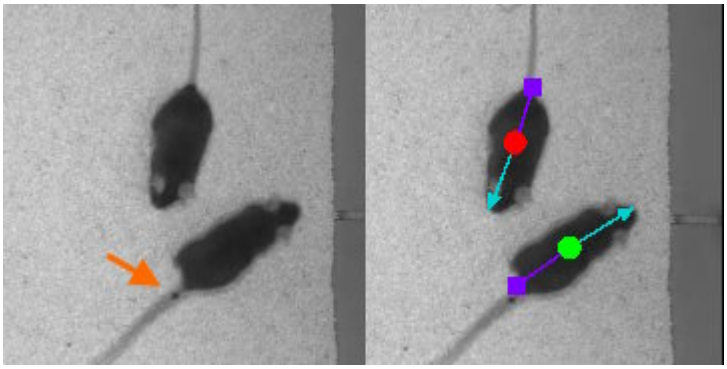
### *Marker-assisted identification*

With Marker-assisted identification, EthoVision XT uses the colors to identify the subjects, but tracks their entire body. This method allows to collect detailed data in social interaction tests, particularly in rodents. Just like for the previous method, you need to tell EthoVision XT which colors to follow.



*Tracking based on Deep learning*

If two subjects look different, or are marked in some way (also in grayscale video), EthoVision XT can discriminate between the two using its neural network. In most cased you need to mark one of the two subjects on its back or at the base of its tail.



Individual discrimination is accomplished after tracking, in a process named *Data Preparation* which involves a thorough review of the tracks and the corresponding video images. The neural network re-assigns the identity labels to the subjects based on their visual appearance. Note that to use Deep learning you need a compatible secondary graphics card (GPU). See Deep learning: Basics and Deep learning: Requirements

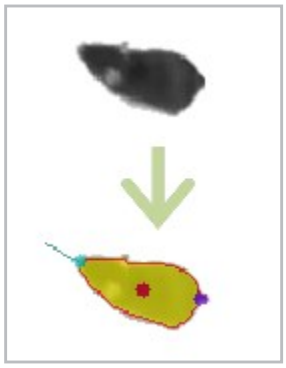
## Video tracking and video recording

You can set EthoVision XT to track the subjects and, at the same time, save the video image to a digital video file. Alternatively, you can choose to save video first, and track the subjects later from that video. See Important things to know about data acquisition.

## Subject position, size and orientation

When EthoVision XT has identified a group of adjacent pixels as a subject, it extracts a few *features* (depending on which add-on you have):

- *Subject position*. This is the x,y coordinates of the body points being tracked: either the center-point only, that is the point mathematically in the center of the shape considered to be the subject, or the center-point, the nose-point and the tail-base point. See Overview of nose-tail base detection



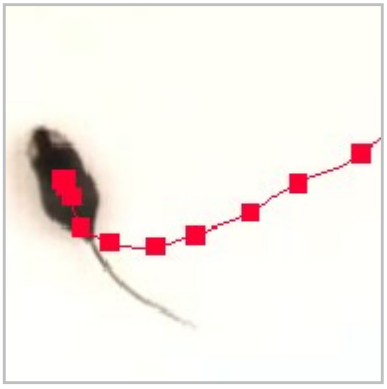
- *Subject area*. This is the area of the surface in the video image (camera image or video file) which composes the detected subject. The area is expressed and exported as squared distance units.
- *Subject area change*. This is the proportion of the subject's area that changes between frames. It is used to estimate the subject's mobility.
- *Subject's head orientation*. When you track the subject's nose point (only for contour-based methods), the software estimates the direction of the gaze. This is indicated with the blue line that originates from the nose point.
- *Subject elongation*. This quantifies how stretched the subject's body is.

For an overview of the output variables related to the subject's body, see Dependent Variables in Detail > Body

## Quantifying behavior

The output of video tracking is a series of numbers representing the x,y coordinates and size of the subject. From the raw data EthoVision XT calculates a number of Dependent variables describing the behavior of the subject. These include speed and distance moved but also complex variables such as the direction of movement relative to an object.

Furthermore, with the Rat and Mouse Behavior Recognition modules, behaviors like grooming and rearing can be detected automatically.



## Data analysis and results export

For all the dependent variables, EthoVision XT can calculate a wealth of descriptive statistics (such as mean, standard deviation, etc.). These are shown for each trial, and for the groups of trials that you may want to define (for example, treated vs. vehicle subjects).

Results and raw data (x,y coordinates and per-sample values of dependent variables) can be exported to text and Excel format.

**NOTE** With custom code written in JavaScript language within EthoVision XT, you can greatly expand analysis capabilities. See a few examples in the following topics:

- JavaScript continuous
- JavaScript event
- JavaScript state

# Video in EthoVision XT

EthoVision XT can work with different types of cameras:

## Analog cameras

To use analog CCD cameras, you need an encoder board, which converts the analog signal to digital. With this board you can also save the video image to a video file directly with EthoVision XT. See Install analog cameras

## Digital cameras

You can plug a digital camera (GigE Vision or USB 3.0) directly into your computer.

The video signal is used directly in EthoVision XT to track live and save video. However, for acquisition the quality of the input signal is crucial, therefore not all digital cameras can be used. See Cameras supported by EthoVision XT

## Mixing camera images

You can feed multiple cameras to the EthoVision XT computer. EthoVision XT mixes the images of up to sixteen cameras. Video is saved with all camera images merged into one. For example, when you use two cameras, the resulting video file contains the two camera views next to each other.

For information on the supported cameras, frame rates, and resolutions, and the maximum number of cameras supported, see Camera Installation.

## Recording video with MediaRecorder

With the Noldus MediaRecorder software you can record video to be used in EthoVision XT for *offline tracking*. An advantage of using MediaRecorder is that you can create separate, full resolution video files from multiple cameras. With EthoVision XT, the images from multiple cameras are merged in one video file.

*See also*

- Camera Installation

# Modules of EthoVision XT

To know which add-on module you have, choose **Help** > **About EthoVision XT** then click the **License Info** button. The modules enabled in your license are marked with a check symbol.


What module is enabled depends on the EthoVision XT license is currently active on that computer. You cannot select and deselect those modules from the software. See also Types of EthoVision XT license

## Base module

The Base module of EthoVision XT allows tracking of the center-point, the nose-point and tail-base point and other relevant features of one subject in one arena.

- Tracking the nose-point is, for instance, relevant in the Elevated plus maze test to determine the number of times the subject puts its head in one of the open arms of the maze without actually entering the arm. Tracking the center-point is then not so useful.
- You can also record your subjects' behavior manually using the Manual Scoring function.
- You can also measure Body elongation on the basis of thresholds that you set yourself.

**NOTE** The Multiple Body Points module is included in the Base module.

You can extend the base functionality with the add-on modules listed below.

## Multiple Arenas module

Allows tracking in more than one arena at the same time. You can track one subject per arena.

## Rat Behavior Recognition module

With this module you can have EthoVision XT detect various behaviors automatically: drinking, eating, jumping, grooming, rearing (supported and unsupported), resting, sniffing, twitching and walking.

## Mouse Behavior Recognition module

With this module you can have EthoVision XT detect various behaviors of mice automatically: digging, drinking, eating, grooming, hopping, rearing (supported and unsupported), resting, sniffing and walking.

**IMPORTANT** The Mouse Behavior Recognition and the Rat Behavior Recognition modules are not interchangeable. For example, you cannot use the Mouse Behavior Recognition to detect digging in rats.

## External Data module

With this module, you can import any external data in ASCII format. External data may include physiological data (e.g. EEG, blood pressure, heart rate, body temperature, etc.) or environmental data (e.g. room temperature, humidity, etc.).

You can synchronize track data and associated external data and subsequently visualize, select and export these data. See External Data

## Quality Assurance module

This module supports you in making experiments which fulfill quality requirements. It helps you protect your data and creates log files so you can easily keep track of everything that happens during a project. See EthoVision XT and Quality Assurance

## Social Interaction module

This module allows you to track more than one animal per arena and study social interactions. For the statistical analysis a number of variables are available like the distance between the animals, and the relative movement. See also Advanced detection settings for tracking color-marked subjects

## Trial & Hardware Control module

With this module, you can make advanced rules to start and stop the trial. You can also control hardware, like, e.g., a pellet feeder and make settings in such a way that the pellet feeder drops a pellet when the animal is in the trigger zone for 30 seconds. See Trial Control Settings in this Help and the EthoVision XT 18 - Trial and

Hardware Control - Reference Manual. To find this manual, in the Window Apps screen choose **Noldus** > **EthoVision XT 18 Other Documentation**.

EthoVision XT 18 Other Documentation

## Track3D module

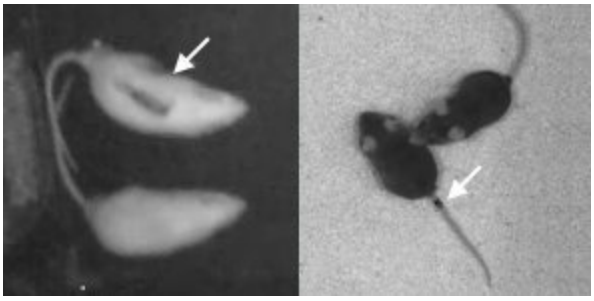
Track3D is a solution for tracking animals in a 3D space. This add-on includes a MATLAB application that converts 2D tracks obtained from two cameras to a three-dimensional track, and calculates a wealth of movement parameters in 3D.

Note that Track3D only works with one tracked individual at a time. For more information, contact Noldus.

# What's new in EthoVision XT 18

## In the spotlight

- *Deep learning technique for two-subject tracking*. EthoVision XT extends the use of neural network models to the case of social interaction test with two subjects. This method requires that the two subjects have some noticeable difference in the appearance. In most cases you need to mark one of the two animals, on the back (see the figure below, left) or on the base of the tail (right).



  For details see the following:

  - Deep learning: Requirements
  - More information on graphics cards (GPUs)

## Apparatuses and video technology

- *Full support for PhenoTyper 2*. EthoVision XT can operate the top unit of PhenoTyper 2 recently released. For details, see the EthoVision XT - PhenoTyper - Reference Manual.
- *Support for 16 digital cameras*. You can record video from up to 16 Basler monochrome digital cameras. See 16-camera configuration
- *Ugo Basile Fear Conditioning System*. The devices of the Ugo Basile FC system are now fully controlled from the EthoVision XT Trial Control Settings. These include the infrared light necessary for tracking. You no longer need an external device to adjust the IR light intensity inside the cubicle.

## Detection and data acquisition

- *Use the first video frame to update the reference image*. When you carry out a series of trials and use either Dynamic Subtraction or Differencing as the detection method, you want to update the reference image based on the

most recent lighting conditions and background. For this, you can let EthoVision XT take the first video frame of the trial and use it as a reference image. See The reference image

- *Manual Scoring*. you can visualize the arenas and the zones while you observe and score behaviors manually. Click the **Show/Hide** button at the top-left corner of the Manual Scoring screen, and choose **Arena features**.

## Data analysis

- *New JavaScript analysis functions*. JavaScript custom analysis variables rely on the tracking data. New functions make it easier to extract tracking data.
  - The function IsSubject[bodypoint]InZone helps you check whether one of the subjects of a multi-subject experiment is in a specific zone.
  - The function IsPointInZone checks that a point (i.e., a body point or a point of interest) lies within a zone. It replaces the old variable IsInZone.
  - The functions GetSubjectArea, GetSubjectChangedArea, GetSubjectElongation, GetSubjectViewDirection extract relevant information of subjects in a multi-subject experiment.

  See the complete list in Commands and functions for JavaScript variables
- *New JavaScript variables for analysis of social behaviour*. New JavaScript code is available for analysis of social events: social contact, approaching, following and leaving. You can find the scripts on the EthoVision XT installation package under Drivers and tools\JavaScript custom variables, or on GitHub. See JavaScript custom variables
- *New option for Relative movement*. The social behavior variable *Relative movement* quantifies the tendency of one subject to move toward or away from another subject. You can now tell the software what *movement* is based on a minimum velocity. See Relative movement
- *Data visualization of multi-day recordings*. In the Integrated Visualization you can now plot data for up to seven days (previous limit was 24 hours).

## Miscellaneous

- *New experiment templates*. Experiment templates are now available for the Ugo Basile Fear Conditioning System, the Ugo Basile Active Avoidance Shuttle Box and for PhenoTyper 2, the latter also in a 16-cage configuration. See Create an experiment from a predefined template
- *Import profile for Doric data*. A profile for importing external data from Doric fiber photometry/optogenetics systems has been added to the list of import profiles. See Doric

- *Video Tutorial*. The EthoVision XT Video tutorial includes a section about two-subject tracking using Deep learning.
- *Onboarding training*. If you are new to EthoVision XT, click the link on the start screen under **Learn more** to access the Noldus Academy's EthoVision XT onboarding training.

See also

EthoVision XT 18 Other Documentation

# Restrictions in EthoVision XT

In the following table you find a summary of the main restrictions in EthoVision XT.

## General

| Feature | Maximum number | Notes |
|---|---|---|
| Subjects per arena | 1 (Base module), up to 16 (Social Interaction module) | The limit of 16 subjects is set by the software. It may be difficult to discriminate between 16 different color marks depending on the light conditions. See Color markers<br><br>We recommend to only use Tracking from video files when you track multiple subjects and multiple body points.<br><br>The technical limit of 16 subjects per arena can be overridden in the software; contact Noldus for the possible solutions. Contact the Noldus Support |
| Arenas | 100 | With the Multiple Arena Module; One arena with the Base Module.<br><br>Arenas must not overlap. The number of arenas does not depend on the number of cameras. You can have for example 96 wells in one camera image, or 16 arenas with 16 camera views (one arena per camera).<br><br>When you combine tracking of multiple subjects (with colors) and multiple arenas, performance may limit the total number of subjects followed simultaneously. |
| Trials | 10000 | |
| Behavior types scored manually | 256 | |
| Hardware commands and signals | 1024 | With the Trial and Hardware Control Module |
| Arena Settings cache | 50 MB | |
| Heatmap dimensions (pixels) | 65536 | |
| Rows or columns in analysis results table | One million | |

| | | |
|---|---|---|
| Free intervals | 2000 | See Free interval |
| Time bins | No restriction | If you create more than 1000 time bins, the group charts are not displayed. |

## Computer

| Feature | Limitations | Notes |
|---|---|---|
| Computer type | Workstation | We recommend the use of Dell workstations especially when using multiple add-on modules (e.g. tracking nose-, center- and tail-base points in multiple subjects simultaneously)<br><br>When tracking for more than 24 hours, restart the PC before a new trial.<br><br>See Hardware |
| Video graphics card | NVIDIA, 4 GB or larger | If you opt to buy your own PC, it must have a stand-alone graphics card that supports Direct3D acceleration, We recommend NVIDIA video cards with a memory of 4 GB or larger.<br><br>For tracking subjects with Deep learning, there are further restrictions. See Deep learning: Requirements |
| Processor and RAM memory | Intel | We recommend an Intel processor; for desktops PCs a 32 GB RAM, and for laptops a 16 GB RAM. |

### *See also*

- System requirements: Hardware
- Ordering a PC from another supplier
- More information on graphics cards (GPUs)

## Video resolution, sample rate and trial duration

| Feature | Maximum | Notes |
|---|---|---|
| Video resolution (camera image) | Depends on camera type | See Cameras supported by EthoVision XT |
| Video resolution (video file) | 1900 x 1200 | Higher resolutions, for example 4K video may work. Test a few sample videos and check for missing samples. Keep **DDS** selected in the Acquisition screen.<br><br>See DDS (Detection determines speed) |
| Sample rate (live tracking) | 60 frames per second; it depends on the camera | See Cameras supported by EthoVision XT |
| Sample rate (tracking from video file) | Not specified | EthoVision XT can handle short video files with a frame rate of 1000, but that also depends on the video format. Test a few sample videos and check for missing samples before running the experiment. |
| Trial duration | 72 hours, but this depends on the tested setup | See Resolution, frame rate, and maximum trial duration |

**IMPORTANT** When tracking from video files at high resolution and/or frame rate, always select **DDS** in the Playback Control window. This way EthoVision XT adjust the analysis speed to the time needed to analyze each frame without skipping frames. See DDS (Detection determines speed)

**IMPORTANT** When tracking for more than 24 hours, always reboot the PC after every trial to refresh the PC resources, the RAM specifically. Failure to do so could result in crashing, which can corrupt the experiment, or the recorded video.

# Applications and add-ons

| Feature | Maximum number | Notes |
|---|---|---|
| Multiple Body Point module (live tracking) | Four arenas per computer | In principle it is possible to track in more than 4 arenas, but it depends on the apparent size of the subjects in the video image (in pixels).<br><br>Sample rate must be 25 samples/s or higher. |
| Multiple Arena module | 100 arenas | The number of arenas may be limited by other factors, for example the number of subjects in each arena and the tracking of multiple body points.<br><br>Contact Noldus if you wish to work with more than 100 arenas. |
| Social Interaction module | 16 subjects per arena | If subject are unmarked, there may be subject identity swaps. |
| CognitionWall (Sylics) | Four arenas, only center-point tracking; up to 72 h recording | In case of recordings > 24 h, we advise to restart the PC after each trial. A workstation is required. |
| Behavior Recognition | Subjects: one per arena<br><br>Arenas: one (live tracking), up to four (offline tracking) | Requires the Multiple Body Point module. Camera must be overhead. Camera frame rate must be at least 25 fps with no missing frames. Cannot work together with Deep learning-based tracking. The PC must be a workstation.<br><br>See Behavior Recognition: Requirements |
| Deep learning for nose-tail base tracking | One or two subjects per arena (up to 4 arenas) | See Deep learning: Requirements |

## *See also*

- Choose your camera
- System requirements
- The test environment

# For more information

## Manuals

For all the other manuals, click the following link:

EthoVision XT 18 Other Documentation

Or from the Windows **Start** menu, choose **All Apps** > **Noldus** > **EthoVision XT 18 Other Documentation**.

For EthoVision XT:

- **EthoVision XT 18 - Quick Start Guide.pdf**

  Recommended when you use EthoVision XT for the first time. It also comes with the software, printed on paper. The Quick Start Guide is also available in four languages: German, French, Italian, and Spanish.

- **EthoVision XT 18- Application Manual.pdf**

  This manual shows you how to use EthoVision XT with standard tests like the Morris water maze test, the Novel Object test and the Sociability/Social novelty test, but also complex setups like Optogenetics and Calcium Imaging.

- **EthoVision XT 18 - Trial and Hardware Control - Reference Manual.pdf**

  An extensive manual about controlling external devices and creating complex routines within EthoVision XT.

For PhenoTyper:

- **PhenoTyper - EthoVision XT 18 - Reference Manual.pdf**
- **PhenoTyper - EthoVision XT 18 - Service Manual.pdf**

For DanioVision:

- **DanioVision DVOC-0041 - EthoVision XT 18 - Reference Manual.pdf**

You can also find all manuals on the MyNoldus portal.

my.noldus.com

## Sample experiments

On the download page of EthoVision XT you can find all the documentation listed above and sample experiments. Open a sample experiment to see how a specific

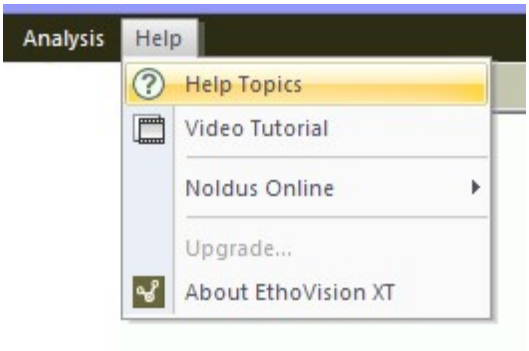
test, for example the Morris water maze test, or the Novel Object Recognition test, is conducted in EthoVision XT.

Browse to

my.noldus.com

Click **Downloads**, then **EthoVision XT** and then **Sample Experiments**. Note that to access the MyNoldus pages you must register and log in.

## Help menu




Choose **Help** > ...

- **Help Topics**. Opens this Help.
- **Video Tutorial**. The Video Tutorial explains how to set up an experiment in EthoVision XT and shows useful examples of how to adjust various settings.
- **Noldus Online**. If your computer is connected to the Internet, you can choose this option to go to the EthoVision XT home page, check for updates, contact Noldus Support or consult the EthoVision XT knowledge base. If you encounter a problem with EthoVision XT, choose **Report an Issue**. The EthoVision XT home page contains all kinds of information about the program as well as examples of how EthoVision XT is used.
- **Upgrade**. See Upgrade EthoVision XT.
- **About EthoVision XT**. Choose this option to see details of exactly which version of EthoVision you are using, the end-user license agreement and the copyright notices for third-party software.

## Information messages

In some parts of the program you see an “i” icon next to specific features. Click the icon to know more about this feature.


## Noldus Support

If you have any problems, questions, remarks or comments, please let us know. You can contact us at our support page:

my.noldus.com

We offer 24 hour support in various time zones.

Before you contact Technical Support, please have the following information available. To find this information, go to the **Help** menu and select **About EthoVision XT**:

- The version number of your copy of EthoVision XT.
- The name of the registered user of EthoVision XT (click **License Info**).
- The license number of your copy of EthoVision XT (click **License Info**).

Our Technical Support department may request a log file and/or a dump file when answering your support question. Please see File locations.

## Other contact information

Browse to

www.noldus.com/about-noldus/meet-noldus

## Make a PC report for Support

This section is needed if Noldus Support has asked you to send a PC report for the EthoVision XT computer.

1. Download the SIW utility as indicated by Noldus Support and save it to the EthoVision XT computer.
2. Double-click on the file called **siw64.exe**.
3. Choose **File** > **Create Report File** > **HTML Report**.
4. Save the HTML file to your Desktop.
5. Send the HTML file as an e-mail attachment to Noldus Support.

## Send video material to Noldus

In order to provide adequate support, it is possible that we request you to supply us with video recordings made by your organization. Since May 25th 2018 the new GDPR rules apply to all people in the EEA (EU + Norway, Iceland and Liechtenstein). For this reason we need your signed consent that you agree with the fact that you have given us permission to use these video recording(s). Please be aware that the person(s) who are recognizable in the video also has/have to give consent that the video is sent to Noldus Information Technology BV and our technology partners, and that it is your responsibility to arrange this consent. More information regarding our Privacy policy can be found at:

https://www.noldus.com/legal/privacy-policy.

## NoldusCare

Your license of EthoVision XT comes with a standard service package of one year. This includes a one-year period of free technical support. With NoldusCare you make sure that you work with the latest version of your software, based on input from our worldwide customer base. Updates, upgrades and new releases are available for free. As well as update meetings, where you can discuss new features with a Noldus consultant. For more information, see our web page:

Browse to

my.noldus.com

and select **NoldusCare**.

# The EthoVision XT screen

## Learn about

- Screen components

## What do you want to do?

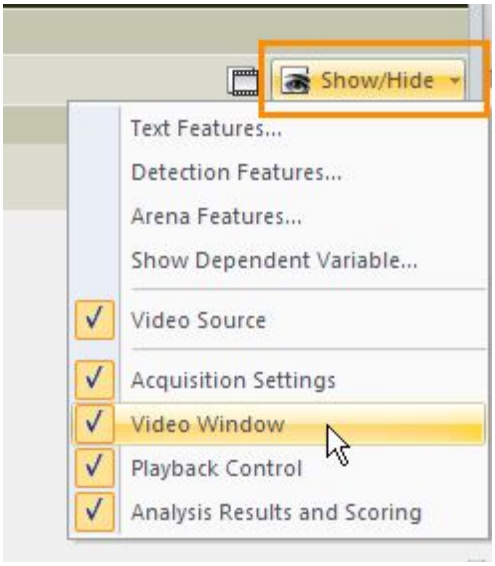
- Customize your EthoVision XT screen

# Screen components

The EthoVision XT interface is made of three main elements: the menu bar (A), the Experiment Explorer (B), and the Working area (C).

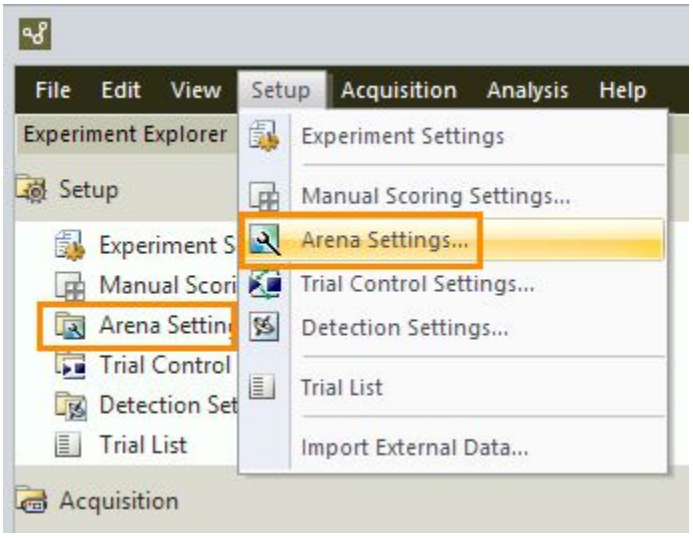

When you click an item in the Experiment Explorer, the Working area shows the elements related to that item.

Use the **Show/Hide** button at the top-right corner to specify which windows and other elements in the Working area you want to show.




## Menu bar

Throughout this Help, instructions like "Choose **File** > **Open**..." refer to menu items. The three menus **Setup**, **Acquisition** and **Analysis** contain the same functions as the Experiment Explorer.

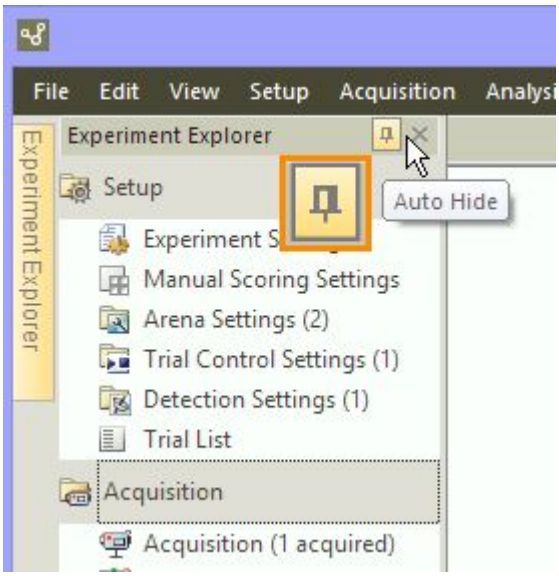



With the **View** menu you can show or hide the Standard toolbar, the Experiment Explorer and the Status bar.

## The Experiment Explorer

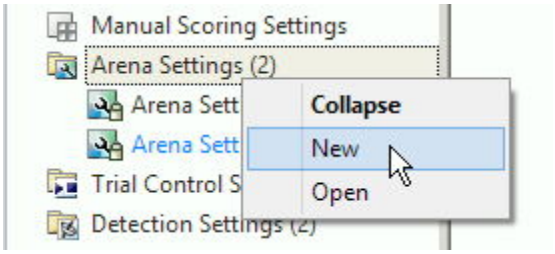
The Experiment Explorer helps you keep an overview of your experiments, including your data and the settings used for tracking. It consists of three main items: **Setup**, **Acquisition** and **Analysis**.

- Expand and collapse. Click an item to view its components.
- View or hide. By default, the Experiment Explorer is visible. To hide it, choose **View** and deselect **Experiment Explorer**.
- Auto hide. You can minimize the Experiment Explorer by clicking the Auto hide button in its upper-right corner.




To expand the Experiment Explorer s again, hover with your mouse over the minimized Experiment Explorer on the upper-left corner of your screen. To expand it permanently, click the **Auto hide** button once more.

- Right-click menus. Most items in the Experiment Explorer have right-click menus to access key functionality. For example, to create a new settings profile.




## Other tools

The Working area shows specific toolbars, which depend on the function that is currently open. For example, when you open the Arena Settings, this toolbar includes the tools for drawing arenas and zones.


The Standard toolbar includes icons for basic functions like creating and opening experiments, saving and copying-pasting. To view the Standard toolbar, choose **View** > **Standard Toolbar**.

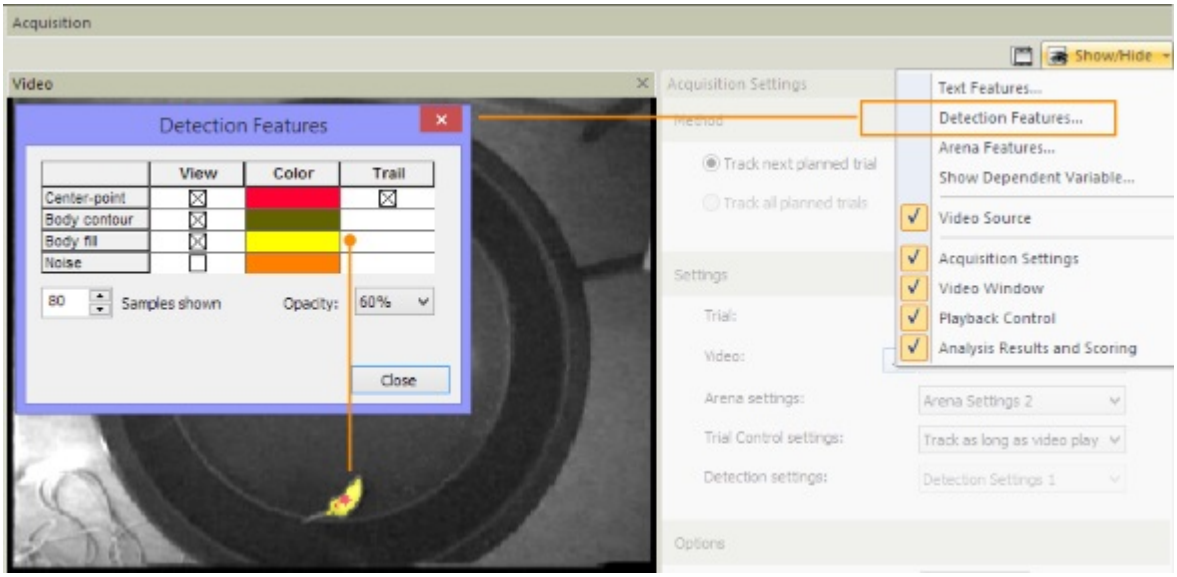
The Status bar is located at the bottom of the screen. It gives information about the status of some keyboard keys: **Caps Lock**, **Num Lock** and **Scroll Lock**. To view the Status bar, choose **View** > **Status Bar**.

# Customize your EthoVision XT screen

## To show and hide windows and other elements

Click the **Show/Hide** button at the top-right corner of the screen.

The menu options that appear are related to the current phase of the experiment. For example, during acquisition, choose **Show/Hide** > **Detection Features** and choose in which colors to show the subject being tracked.

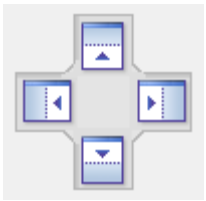



## To position and resize the windows

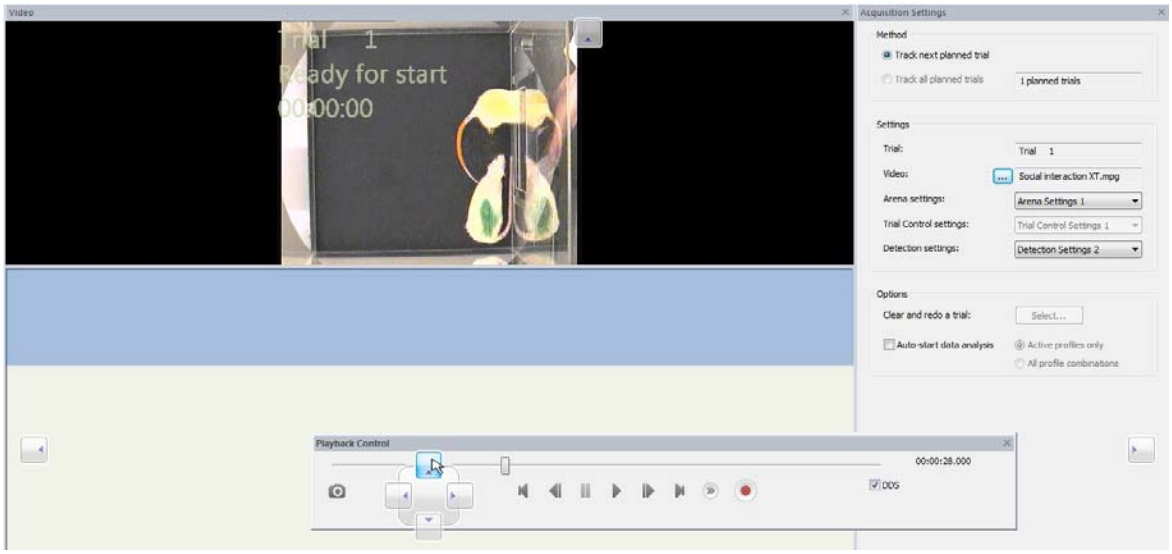
By default, all the windows on your screen are docked. You can change their position and resize them according to your needs.

### *To position a window*

To re-position a window, click its title bar and drag it to the new position. To undock a window, click its title bar and drag it anywhere until you see its margins no longer constrained by other windows. Docking icons appear, to show you where you can position the undocked window.



If you hover with your window over one of the docking icons, the new position of the window is shown in blue.




If you release the window, the window is positioned at the chosen location.

*To resize a window*

Hover the mouse on one of its margins until the cursor changes to this symbol:

Next, drag the margin to the desired position.

# Software Installation

## Main topics and tasks



*See also*



**NOTE** Complete your experiments and make a backup of all of them before installing the new version.

# System requirements

## Before you install EthoVision XT

If you ordered a computer from Noldus Information Technology when you purchased EthoVision XT, the software and any internal hardware has already been installed and tested.

If you did not order a computer, you must install EthoVision XT from the MyNoldus portal. See the Welcome Letter for more information.

Prior to installation, please check the packing list to make sure all the components are present. If any of the components listed is missing or damaged, please let us know.

The contents of your package differ for new and existing users:

- If you are a new EthoVision XT user, you received a printed Quick Start Guide and a Software Activation Key.
- If you are upgrading from older versions of EthoVision XT, you received a printed Quick Start Guide and an Software Upgrade Key to upgrade your current license. See Upgrade EthoVision XT

## Learn about

- Operating system
- Hardware
- Network and user rights

*See also*

- Restrictions in EthoVision XT
- Camera Installation
- EthoVision XT and Quality Assurance

# Operating system

## Windows version

EthoVision XT 18.0 supports Microsoft Windows 11 Pro and was tested with Windows 11 Pro, 64 bits, version 23H2.

**NOTE** EthoVision XT 18 was also installed on Windows 10 Pro, version 22H2 and Windows 11 Pro, version 24H2 for short tests unrelated to the assessment of tracking performance with specific camera configurations. Although we did not report any significant issue, Noldus cannot guarantee that the same performance as on Windows 11 can be obtained on these versions when using a specific camera configuration. See Resolution, frame rate, and maximum trial duration

EthoVision XT is not designed for use with the Touch features of Windows.

## User rights

To install EthoVision XT, you must have administrator rights. In other words: either you are the system administrator or you are a member of the Windows group Administrators and have been assigned administrator rights.

**IMPORTANT** We recommend to turn off automatic updates for device drivers. See Turn off automatic updates for device drivers

**IMPORTANT** We also recommend not to use a comma as decimal separator in the Windows regional settings. To check the regional settings, in Windows choose **Apps** > **Windows System** > **Control Panel**. Under **Clock, Language and Region** click **Change date, time or number formats**, then click **Additional settings**.

## Language support

EthoVision XT has also been tested with Cyrillic, Japanese and Chinese language packs.

Like any software package, it remains possible that minor differences in the operating systems of certain local language versions may affect how well the program runs. If you encounter a problem of this sort, please contact Noldus Technical Support.

## Windows Update

There have been cases of EthoVision XT trials being interrupted by forced Windows Update. It is not straightforward to prevent unwanted updates during data acquisition, especially in Windows 11. If you plan to do long data acquisition

sessions (e.g. 24+ hours), an easy solution is to disconnect the EthoVision XT computer from the network and restart it before starting the new acquisition session.

# Hardware

## Computer

EthoVision XT has been tested with the workstations listed below. If you order a complete solution from Noldus Information Technology, you will obtain one of these computers or its successor, with EthoVision XT installed and ready to use. See also Ordering a PC from another supplier

### *Dell Precision 3680 desktop*

- Processor: Intel Core i7-14700, 20 CPU cores, 3.4 GHz.
- Internal memory: 32 GB DDR5.
- Hard disks: M.2 1TB PCIe NVMe SSD.
- Graphics card: NVIDIA T1000, 4 GB GDDR6.
- PCIe slots (full-height): 1x Gen5 PCIe x16, 1x Gen3 PCIe x4, 1x Gen4 PCIe x4 (open-ended)
- Operating system: Windows 11 Pro.

Dell Precision PCs are recommended when tracking subjects with the Deep learning method. See Deep learning: Requirements

### *Dell 3591 mobile*

- Processor: Intel Core Ultra 7 155H vPro Essentials, 16 cores, up to 4.8 GHz
- Internal memory: 16 GB DDR5.
- Hard-disks: M.2 2280 1 TB PCIe NVMe SSD.
- Graphics card: NVIDIA RTX 1000, 6 GB GDDR6.
- Operating system: Windows 11 Pro.

## Ordering a PC from another supplier

If you choose to order a PC from a supplier other than Noldus or its distributors, you can use the above specifications as a guideline.

### *Use a professional workstation*

We recommend that you use a professional workstation. It is possible to buy consumer-range computers with a high processor speed and plenty of memory, but in order to remain competitive regarding price, the manufacturers often economize

on the underlying system architecture. That means those computers are suitable for home use, but not for running professional scientific software. You should select a computer which is intended for professional use or labeled by the manufacturer as a workstation.

### *Desktop or laptop?*

- If you plan to purchase a desktop computer, always choose a tower *mid-size* or *full tower* size. Do not use mini or small form factor desktops. When in doubt, ask your Noldus representative.
- If you plan to use a laptop or a desktop with an unpowered network interface board (also known as network interface card, NIC), you need a Power over Ethernet Injector to power a GigE camera.
- If you plan to use analog cameras (e.g. for PhenoTyper 1), you need a desktop computer with at least one PCI slot.
- The Ethernet interface board should support data transfer of 1 Gb/s (one port).
- If you plan to use a laptop, please consider the following:
  - Only one camera is supported. The laptop must have a Ethernet port; a USB-Ethernet adaptor won't work.
  - There may be restrictions in terms of maximum resolution and/or maximum recording time. Check carefully what has been tested. See Cameras supported by EthoVision XT
  - Always acquire data with the laptop being plugged in. In Windows, click **Settings**, then type *power*. Click **Power, sleep and battery settings**. Next, click **Screen and sleep**. Next to **When plugged in, put my device to sleep after** select **Never**.

### *Intel processor (CPU)*

We strongly recommend to use a computer with Intel x64-compatible processors, Intel Xeon or i7 CPU. In Intel processors the acceleration required by the IPP (Integrated Performance Primitives) software is guaranteed. That may not be the case for other manufacturers which offer high-quality and fast processors.

IPP is an extensive library of ready-to-use, domain-specific functions. It is used by EthoVision XT during image processing and data acquisition. IPP therefore affects performance of video-tracking. Processors from other manufacturers are compatible with EthoVision XT, but tracking could be significantly slower. Consider this when you plan to purchase a computer to acquire large amount of data from high-resolution video, or analyze video with Behavior Recognition or Deep learning.

- Other processors such as AMD may well work with the IPP although Noldus does not support them. Always test your computer thoroughly before running the actual experiments.
- EthoVision XT won't work on Qualcomm / ARM chips.

### *Important factors to keep in mind*

- The more animals and arenas you track, the more processor load you require.
- Nose-tail tracking requires more processor load than tracking the center-point only.
- The optimal sample rate depends on the species you track. Mice, e.g., require a higher sample rate than rats. See Sample rate for more information about the optimal sample rate.
- A larger image requires more computer resources for tracking, so a larger image does not necessarily lead to better tracking.

### *RAM Memory*

- For desktop computers, we recommend a 32 GB RAM Memory.
- For laptops, a 16 GB RAM Memory should suffice.

### *USB 3.0 port*

Current PCs have USB 3.x ports standard installed. USB ports are necessary if you use a USB 3.0 camera. See Install USB 3.0 cameras

If you use hardware like the USB-IO box, you need an additional USB port.

### *Power supply unit (PSU)*

Powerful graphics cards need additional power. Ensure that the computer has a Power Supply Unit (PSU) that can provide enough power to the graphics card. Take note of the Suggested PSU of a specific card and make sure the PC can handle that. See also More information on graphics cards (GPUs) and Install a graphics card for Deep learning

### *Virtual machines*

Virtualization allows you to run different operating systems on the same computer. However, EthoVision XT was not designed to work on a virtual machine, therefore its use on virtual machines is not supported.

## Ethernet interface board

In order to use high-quality GigE cameras, your computer must have a 1 Gb Ethernet Interface Adapter (board), also known as Network Interface Card (abbreviated NIC). Depending on the number of Ethernet ports you can connect one to four cameras per board. The following boards have been tested successfully:

- CT1000 Pro (1 camera)
- Adlink PCIe-GIE74 (1-4 cameras)
- Basler GigE Interface Card, 4 port PoE (1-4 cameras)
- FS X550AT2-2TP (1-16 cameras)

Make sure that the board has the necessary number of ports when you use multiple cameras. See Install an Ethernet board for GigE cameras and Using multiple GigE cameras

If you have a laptop, you can connect only one GigE camera. The Ethernet port of your laptop will be the dedicated camera port. If you want to connect the laptop to the internet, you need to use a Wi-Fi connection. Note that if your laptop has a USB port with a Ethernet-USB adapter, instead of an Ethernet port, that may not work with GigE cameras.

## Power over Ethernet (PoE) injector

If you bought your GigE camera elsewhere and you are not using an Ethernet board powered from the PC, you need a Power over Ethernet (PoE) injector to power the camera. In all cases it must be a Gigabit PoE injector.

## USB 3.0 interface boards

See Install the USB 3.0 interface board.

## Graphics card for deep learning-based tracking

If you intend to use the Deep learning technique for nose- and tail-base detection, you need a powerful NVIDIA Graphics Processing Unit (GPU). GPUs other than NVIDIA would not work due to lack of compatibility with the NVIDIA TensorRT software component. For suggestions, see Deep learning: Requirements.

**IMPORTANT** Make sure to install latest driver for your graphics card.

## Monitor

Your monitor should be set to at least 24 bit color depth. If you use a dual monitor set-up, both monitors should be at least 24 bit. To check the color depth, search for Display in Windows or open the graphics control panel of your display.

## Camera

For a list of the camera models currently supported, see Camera Installation.

If you would like to use another type of camera, please check with your nearest Noldus representative first.

**IMPORTANT** For correct functioning of cameras, we recommend to turn off automatic updates for device drivers. See Turn off automatic updates for device drivers

## Disk space to store video files

If you are tracking from video files, you need sufficient free disk space to store them. You need to be especially aware of the disk space when you let EthoVision XT save videos while you carry out batch acquisition and for long recordings. See Video file size

If your video files are on a DVD or network drive, first copy them to your hard disk drive before loading them in EthoVision XT.

## External hard disk drives

- *For tracking*. We do not recommend that you do tracking from video files that are stored on an external hard disk. Always store the video files on the hard disk of the EthoVision XT computer.
- *For video playback*. Once you have done tracking, you can store the video files on an external hard disk. When you want to review the video in the Integrated Visualization (see Plot integrated data) EthoVision XT asks you to locate the video files if it does not find them in the default experiment location on the hard disk. Select the folder on the external hard disk where the video files are stored. You can also instruct EthoVision XT to look for video files in an alternate folder. See also Preferences for default folders

  **NOTE** Video might sometimes not play smoothly depending on the size of the video file and the speed of data transfer from the external hard disk.

*See also*

- System requirements
- Restrictions in EthoVision XT
- How to prevent Missed samples

# Network and user rights

## Using EthoVision XT across a network

Please note that it is not possible to install EthoVision XT on one computer and access it from another across a local area network. The program must be installed on the computer where it will be used. However, you can work with EthoVision XT remotely using one of the remote control applications like TightVNC or TeamViewer. See Work with EthoVision XT remotely

You can track from video files stored at other computers in your network (depending on your network's bandwidth), although we recommend to copy the file to you hard disk before tracking. To let EthoVision XT know where to find the video files, choose **File** > **Preferences** and select the location on the network in the **Alternative media location** field.

## Working with EthoVision XT as a restricted user

The user rights for folders and files are different for System administrators, Power users, and restricted (normal) users. Users who have Administrator rights and Power users have enough access rights to use EthoVision XT and its files without any limitations.

For restricted users the following limitations apply:

- As a restricted user you can create new experiments and view, edit and delete your own experiments.
- If you want to make it possible for other users to edit or delete your experiments, your system administrator must change the Windows security rights for the folder that contains the experiments. By default this folder is C:\Users\Public\Public Documents\Noldus\EthoVision XT\Experiments.

  For instance, to set the Modify rights to Everyone: in the Windows Explorer, select the folder mentioned above, right-click it, select **Properties** and then the **Security** tab and set the rights.

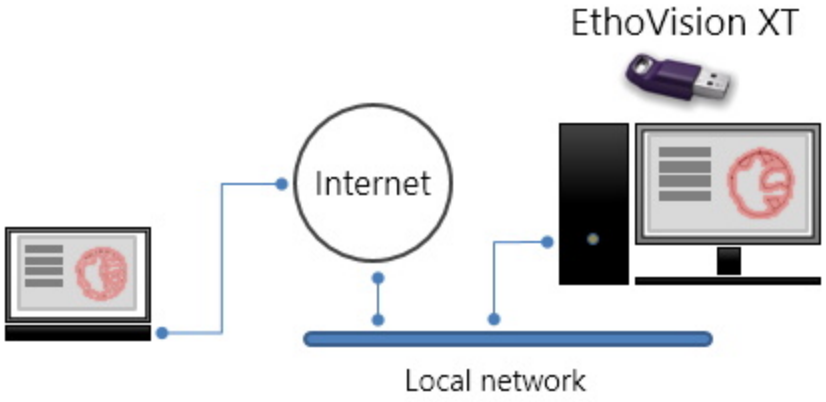
# Work with EthoVision XT remotely

## Aim

You can use utilities like TightVNC or TeamViewer to control EthoVision XT fully remotely.

## Prerequisites

- When using a hardware key, remember to leave the hardware key plugged in the EthoVision XT computer before you leave work if you need to acquire data (i.e., do tracking). However, if you only need to do post-acquisition data analysis and export, you do not need to leave the hardware key inserted. This may actually free up the hardware key to be inserted in another computer at work running EthoVision XT so another user can use it for data acquisition. See EthoVision XT in analysis mode
- When using a software license, make sure that the license on the EthoVision XT computer is active. See Activate your EthoVision XT license




## Procedure

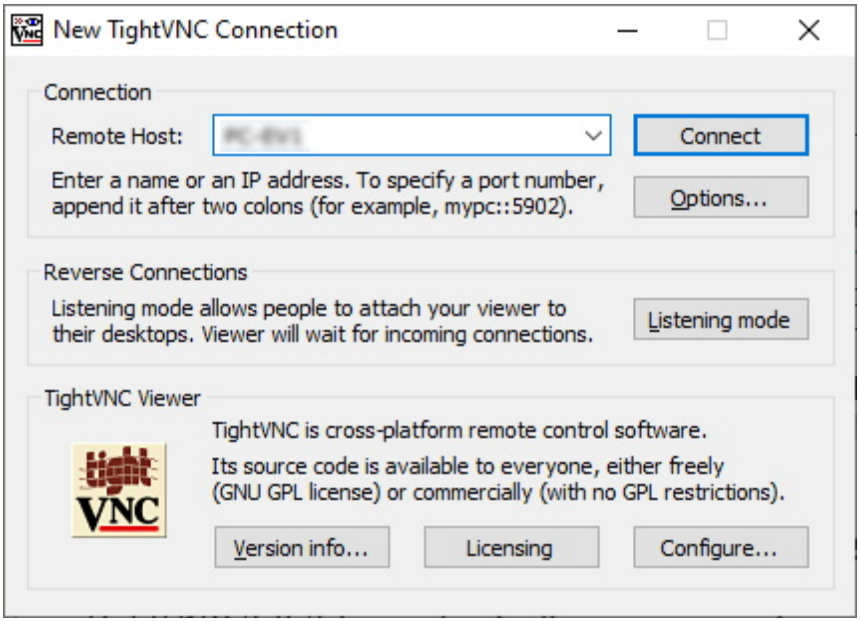
1. Leave the EthoVision XT computer running.
2. On the remote computer use a utility to access EthoVision XT on that PC.
3. Only one user can access a remote EthoVision XT computer at any one time. Of course you can install EthoVision XT on multiple PC so multiple users can work with EthoVision XT.

## TightVNC

www.tightvnc.com/

**IMPORTANT** You must have a VPN connection to the network where the PC you are connecting to is located.

1. Download the installer of **TightVNC for Windows** from the TightVNC website and run it on the PC with EthoVision XT. Use the Full install to install both the Viewer and the Server. Use the default install, but set the passwords for security.
2. Restart the PC, and leave it running in order to be able to connect to it.
3. Install the Viewer on the PC you want to work with.
4. Make the connection to the Local Network where the remote computer is running.
5. Run the Viewer and type the name or the IP address of the PC you want to connect to.




6. Enter the password. You can view the desktop of the remote PC in a window.

## TeamViewer

www.teamviewer.com

1. Download TeamViewer for Windows.
2. When installing TeamViewer on both the work PC and your remote PC, make sure to select **Unattended Access**.
3. Open TeamViewer on the workplace PC. On the **General** tab under **Unattended Access**, select **Start TeamViewer**.

4. Write down the Partner ID (or Your ID) and Password (or take a photo of the screen).
5. Before leaving your work place, launch TeamViewer and leave it running.

# Install EthoVision XT

## Please read this first

- Installing EthoVision XT 18 but does not replace previous versions, for example EthoVision XT 17.
- You can keep working with both versions. However, if you have completed your experiments in the previous version, you can uninstall that previous version.
- We recommend that you complete your experiments and make a backup of all of them before you open them in a new version of the software. See Back up an experiment
- Removing or installing EthoVision XT does not remove your experiments and data already stored on that computer.

## What do you want to do?

- **IMPORTANT** Turn off automatic updates for device drivers
- **IMPORTANT** Disable fast startup
- **IMPORTANT** Disable the Power save options
- Install EthoVision XT

*See also*

- Uninstall EthoVision XT
- Upgrade EthoVision XT
- Read the End-user license agreement
- Acknowledgments and copyright notices
- EthoVision XT trial version
- Uninstall EthoVision XT

# Turn off automatic updates for device drivers

## Aim

To make sure EthoVision XT works with the drivers that were tested by Noldus.

Although the general recommendation from Microsoft to use automatic updates is good, especially for security updates, automatic updates of hardware device drives can sometimes give problems. Follow this procedure to specifically turn off the automatic updates only for device drivers.

## Procedure

1. Click the Windows Start menu and type *change device*.
2. Click **Change device installation settings** in the search results under **Best match**.
3. In the Device installation settings window, select **No (your device might not work as expected)**.
4. Click **Save Changes**.

## Note

There have been cases of EthoVision XT trials being interrupted by forced Windows Update. It is not straightforward to prevent unwanted updates during data acquisition, especially in Windows 11. If you plan to do long data acquisition sessions (e.g. 24+ hours), an easy solution is to disconnect the EthoVision XT computer from the network and restart it before starting the new acquisition session. Do not forget to recover internet connection after you complete your trials.

*See also*

- Disable fast startup
- Disable the Power save options
- Install EthoVision XT

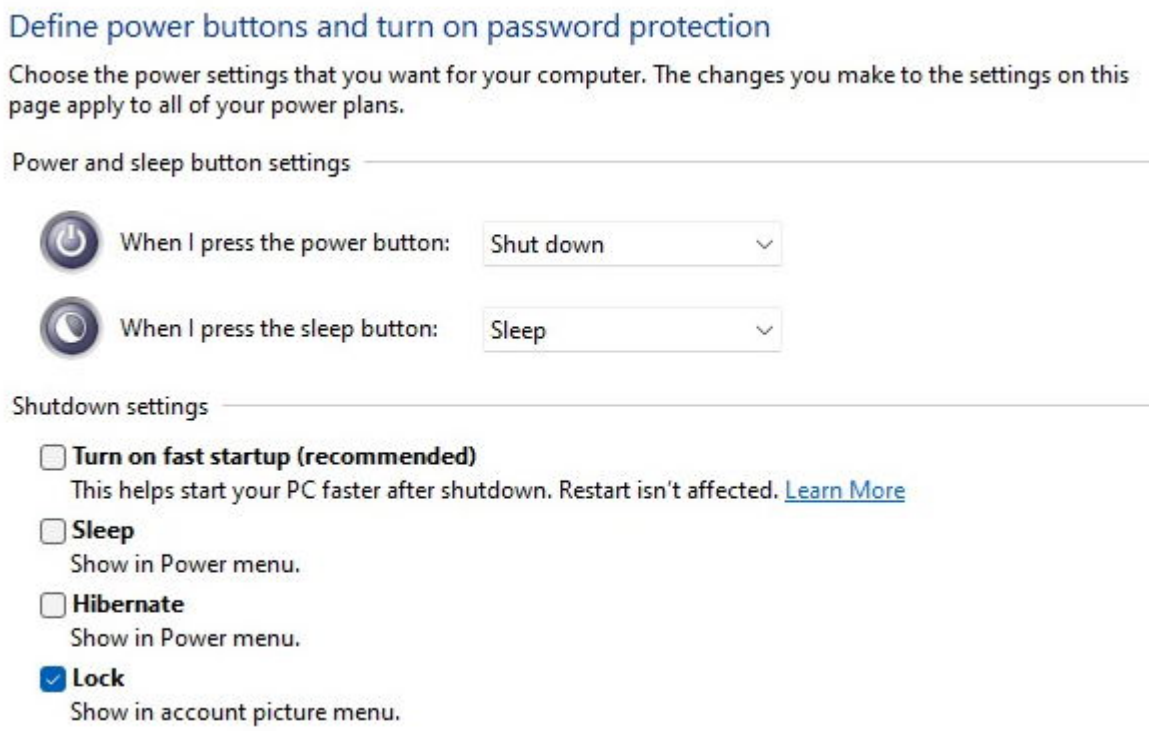
# Disable fast startup

## Aim

To disable the Fast startup option in Windows 10/11, which can have adverse effect on EthoVision XT performance.

## Procedure

1. Click the Windows **Search** icon on the taskbar and enter *control panel*.
2. Click **Control Panel**, choose **System and Security** > **Power options**.
3. Click **Choose what the power buttons do** in the upper left corner.
4. Click **Change settings that are currently unavailable** (this may require an administrator password).
5. De-select the options **Turn on fast start-up**, **Sleep**, and **Hibernate**.




*See also*

- Turn off automatic updates for device drivers
- Disable the Power save options

- Install EthoVision XT

# Disable the Power save options

## Aim

To prevent that the computer is put to sleep or the hard disk is turned off during tracking.

## Procedure

1. Click the Windows **Search** icon on the taskbar and type *edit power plan*.
2. Click **Edit power plan** when it appears in from the search results.
3. Click **Change advanced power settings**.
4. Under **Hard disk** > **Turn off hard disk after**, select 0 (**Never**). For laptops, select **Never** for both **On battery** and **Plugged in**.
5. Click **OK**.

*See also*

- Turn off automatic updates for device drivers
- Install EthoVision XT

# Install EthoVision XT

## Please read this first

- Installing EthoVision XT 18 but does not replace previous versions (that is, EthoVision XT 17 and earlier). If you want to remove previous versions, do so in the Windows Control Panel > **Programs and Features**.
- Close all other programs before installing EthoVision XT and turn off the screen saver and power save options. Check that programs do not run in the background (look in the system tray at the bottom right of your screen).

  We recommend to follow these procedures: Disable the Power save options, Disable fast startup, Turn off automatic updates for device drivers
- **IMPORTANT** For users of Quality Assurance: If you have added EthoVision XT users in User Management and re-install the software, those users and their rights are deleted.

## Procedure

1. Browse to the MyNoldus portal:

   https://my.noldus.com/download/latest/ethovision-xt

   Log in or register (if you are a new user you can use the registration code which you can find on the Welcome letter).
2. Click **Versions** and download the **EthoVision XT - Installation Package [version number].zip**. Save th zip file on your PC.

   **NOTE** The Installation Package is a very large file; download may take some time.

   If you store the zip file on a network drive, copy it to your PC before the next step.
3. Unzip and extract the files on your computer.

   The zip file contains six installation files:

   - EthoVision XT - Setup - [version number].exe
   - EthoVision XT - Package - [version number].msi
   - Tracker Interface NN - x64 Package - [version number].msi
   - Tracker Interface NN - x64 Cabinet - [version number]- 01.cab
   - Tracker Interface MST - x64 Package - [version number].msi

- Tracker Interface MST - x64 Cabinet - [version number]- 01.cab

4. Double-click **EthoVision XT - Setup - [version number].exe**.
5. Select the interface language. You have the choice between **English (United States)** and **Chinese (Simplified)**.
6. Under **Installation type**, select **Standard**.Then click **Next**.

   Select **Custom** if you want to specify the location for the program and the experiments other than those selected by default.

   Application location: C:\Program Files\Noldus\EthoVision XT 18.

   Experiments location: C:\Users\Public\Public Documents\Noldus\EthoVision XT\Experiments

   **IMPORTANT** Do not select a removable disc, like a USB memory drive, as the experiments location.

7. Under **Drivers and Tools**, select the drivers you want to install.

   If you have USB 3.0 cameras, choose **Basler USB camera driver**.

   If you have GigE Vision cameras, choose **Basler GigE camera driver**.

   If you have analog cameras, choose **Euresys Multicam**. This works with the Picolo Alert encoder board.

   **NOTE** If you need to re-install the drivers for your camera, you can find them on the MyNoldus support portal. See also Install the driver software for the digital cameras

8. Click **Next**. Read the end-user license agreement. Select **I agree with the End-User License Agreement** and click **Install**.
9. When the message **Installation is complete!** appears, click **Close**.

## Notes

- To view the manuals other than the EthoVision XT Help, you need a PDF reader like Acrobat Reader or Foxit Reader.
- To uninstall EthoVision XT, do this from the Control Panel. See Uninstall EthoVision XT

*See also*

- Software components installed with EthoVision XT
- Camera Installation

- End-user license agreement

# Software components installed with EthoVision XT

## Components installed automatically

The following components are installed automatically when you install EthoVision XT:

| Name | EthoVision XT 18 | EthoVision XT 17.5 |
|---|---|---|
| Noldus - Basler Pylon Interface - x64 Package - 1 | 1.1.12 | 1.1.10 |
| Noldus - Chart Wrapper Control - x64 Package - 3 | 3.0.6 | 3.0.4 |
| Noldus - Data Storage Converter - x86 Package - 1 | 1.0.10 | 1.0.8 |
| Noldus - Frame Counter - x64 Package - 2 | 2.0.2 | - |
| Noldus - HardwareInterface IoBox - x64 Package - 5 | 5.1.16 | 5.1.10 |
| Noldus - HardwareInterface UgoBasile - x64 Package -1 | 1.0.14 | 1.0.10 |
| Noldus - ImporterInterface LMT - x64 Package - 1 | 1.0.8 | 1.0.6 |
| Noldus - IPP - x64 Package - 8 | 8.2.4. | 8.2.2 |
| Noldus - LeadTools - x64 Package - 21 | 21.0.14 | 21.0.8.0 |
| Noldus - MBRM Interface - x64 Package - 2 | 2.0.6 | 1.3.2 |
| Noldus - MediaLooks ScreenCapture - x64 Package - 2 | 2.0.4 | 2.0.4 |
| Noldus - ParameterInterface LMT - x64 Package - 1 | 1.0.10 | 1.0.8 |
| Noldus - RBRM Interface - x64 Package - 2 | 2.0.6 | 1.3.2 |
| Noldus - Resizer Filter - x64 - Package - 13 | 13.1.10 | 13.1.6 |
| Noldus - SampleGrabber - x64 Package - 15 | 15.0.4 | 14.0.2 |
| Noldus - Tracker Interface NN - x64 Package - 2 | 3.0.8 | 2.0.10 |
| Noldus - Tracker Interface MST - x64 Package - 1 | 1.0.12 | - |

| | | |
|---|---|---|
| Sentinel Runtime | 8.11 | 8.11 |
| Microsoft Visual C++ 2015-2019 Redistributable (x64) / (x86) | 14.29 (*) | 14.29 |

To view the components currently installed on your computer, from the Windows Start menu choose **Settings** > **Apps** > **Apps & features**. Sort the list by install date and locate the items installed with EthoVision XT.

(*) Not installed if a newer version is already present on the PC.

## Additional components

The list of components under **Apps and features** also includes camera drivers and drivers for the PCIe interface boards. These are usually installed when you install EthoVision XT. See also Cameras supported by EthoVision XT

| Name | Version number |
|---|---|
| pylon 7 Camera Software Suite | 7.5.0.15658 |
| Daheng Galaxy SDK | 1.12.2102.9251 |
| Euresys MultiCam | 6.15.1.3573 |
| Noldus - HardwareInterface SerialPort - x64 Package - 1 (*) | 1.1.6 |

(*) You need this component to be able to control hardware devices through a serial (COM) port. You find this component on the EthoVision XT full installation disk, under **Drivers and tools**. For more information, see **Set the port connections for COM ports** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

## Notes

- To view the components currently installed on your computer, from the Windows Start menu choose **Settings** > **Apps** > **Apps & features**. Sort the list by install date and locate the items installed with EthoVision XT.
- There may be components installed with previous EthoVision XT versions:
  - Noldus MainConcept Encoder Package 7.7 (for recording video)
  - Noldus MainConcept Codec Package 8.5.34 (for playback of video files)
  - Noldus MediaLooks AV Filter 5.0.1.0.
  - Components similar to those listed in the first table above, but with lower version number (e.g. Sample Grabber 13).

Please keep them only if you still use previous versions of EthoVision XT. The MainConcept software has been replaced by the LeadTools software in EthoVision XT 16 and 17.

- To add a component, like a camera driver, or the video tutorial, that is not installed yet on your computer, double-click the file **EthoVision XT - Setup - [version number].exe** (see Install EthoVision XT) and select **Custom**, then **Modify**. Select the component you require and click **Update**.
- The Euresys Picolo U4/U8 H.264 encoder boards are no longer supported with EthoVision XT. Those boards do not work on 64-bit software.

*See also*

- Camera Installation
- End-user license agreement

# EthoVision XT trial version

## Aim

To evaluate the full functionality of EthoVision XT for a limited period of time (14 days).

## Prerequisites

- You have received a Software Activation Key from your Noldus Sales Representative.
- You have downloaded and installed the EthoVision XT trial version. You can find the installation files on:

  https://www.noldus.com/download/trial-download-ethovision-xt
- By downloading the trial version you agree:
  - That you shall only use the trial version to review EthoVision XT.
  - That you shall not acquire data that you use to produce scientific research and/or publication.
  - That you shall not hand over or sell the trial version to a third party.

## To start a trial version

1. Start EthoVision XT. A message appears that no valid license key is detected.
2. Click **Activate software license key**.
3. Choose whether to activate a Computer-locked license or a Floating license. See Types of EthoVision XT license
4. **Activate Online** is automatically selected as that is the only option available. Type in the **Activation key** that you received and click OK.

## Notes

- The EthoVision XT trial version is fully functional and includes all add-on modules as the full license, like Multiple Arenas, Social Interaction and Behavior Recognition, except the Quality Assurance Module and Track3D. See Modules of EthoVision XT
- To check the number of days left for evaluation, choose **Help** > **About EthoVision XT**. When the trial period is over, a message is shown.

- Experiments from the EthoVision XT trial version cannot be opened in previous versions of EthoVision XT.

*See also*

- Install EthoVision XT
- Activate your EthoVision XT license

# Uninstall EthoVision XT

## Aim

To remove EthoVision XT, drivers and other components from the computer.

**NOTE** If you have a computer-locked license or a floating license and want to use that license somewhere else, first deactivate the license, then uninstall EthoVision XT. Deactivate an EthoVision XT license

## Procedure

1. In the Windows Search screen, search for *programs*, or *apps* and select **Programs and Features** or **Apps and Features** (depending on the Windows version).
2. In the **Programs/Apps and Features** window, select **EthoVision XT 18** and click **Uninstall**.
3. To make a complete uninstall, also select and uninstall each of the components installed with EthoVision XT. Sort the programs list by **Installed On** and look for the components installed on the same day as EthoVision XT. See Software components installed with EthoVision XT in the topic Install EthoVision XT.

## Notes

- When you uninstall EthoVision XT, the your software license stays on that computer. So if you re-install the software on that computer you do not have to re-activate it.

*See also*

- Install EthoVision XT
- See the list of Software components installed with EthoVision XT

# License activation

## What do you want to do?

- Activate your EthoVision XT license
- Deactivate an EthoVision XT license
- Software license - offline
- Turn a floating license to a computer-locked license
- Turn a computer-locked license to a floating license

## Learn about

- Types of EthoVision XT license
- Duration of the EthoVision XT license
- EthoVision XT in analysis mode

# Activate your EthoVision XT license

## Aim

To activate an EthoVision XT license after purchase. Note that you do not need to follow this procedure if your license is on a USB hardware key.

If you have already an EthoVision XT license and you purchased a new add-on module, see Add new modules to your license

## Background information

When you start EthoVision XT, the software checks that your license is valid. The license may be stored in your hardware key (dongle), in your EthoVision XT computer (Computer-locked license) or in the Noldus server (Floating license). See Types of EthoVision XT license

If no valid license is found, you can activate your license by following the procedure below.

## Important terms

### *Activation key*

An activation key is provided by Noldus to activate your license. You find an activation key on a letter or e-mail sent to you.

### *Computer key*

A Computer key comes into play when you activate your license offline (see below), This is a key that identifies the computer that you are using during activation.

### *License key*

A license key is the unique piece of information that you obtain after you activate the license. You can find this key in EthoVision XT, by choosing **Help** > **About EthoVision XT** > **License Info**. See Types of EthoVision XT license

### *Computer-locked license*

This is a license that is bounded to a specific computer. It is a fixed-seat license intended to be used for long time in the same location. However, when necessary, you can easily transfer this license to another computer. See Types of EthoVision XT license

*Floating license*

This is a license that is not associated with a specific computer. It is intended to be used in multiple computers, although not simultaneously, for example, in one PC at the lab and one or more PCs in the office. See Types of EthoVision XT license

## Prerequisites

- You have installed EthoVision XT. See Install EthoVision XT
- You have received a license **Activation Key** from Noldus. Contact Noldus Support
- If your license is stored in a hardware key (dongle), you do not have to follow this procedure. Simply plug in the key in a USB port of your PC.

## What do you want to do?

- Activate a floating license

  Use a license on different computers at different times. However, if you have two or more floating licenses instead of one, you can use EthoVision XT on multiple computers simultaneously. For this type of license you need an internet connection.

- Activate a computer-locked license - online

  Your license is coupled with a specific computer. Activate your license online. You need an internet connection during activation.

- Activate a computer-locked license - offline

  Your license is coupled with a specific computer. The computer does not have to be connected to the internet, but you need a smartphone or tablet with an internet connection during activation.

  **NOTE** This option is not available if you have purchased a time-limited license or received a license for a free trial. See Duration of the EthoVision XT license

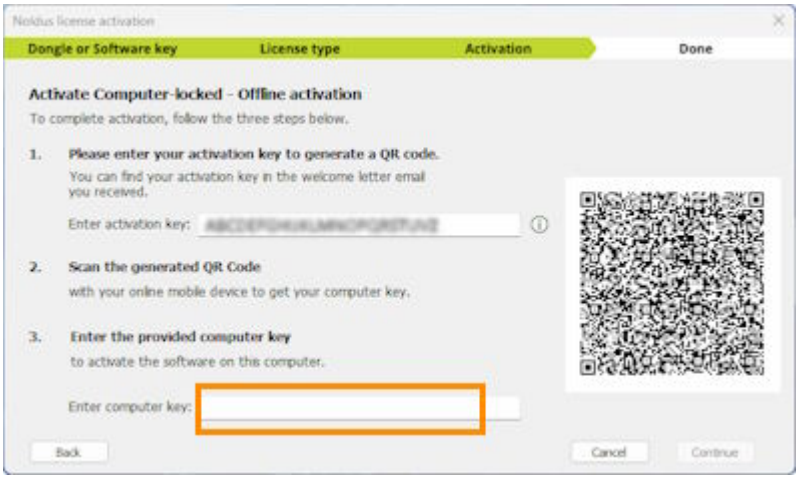
## Activate a floating license

**IMPORTANT** You can only activate and use a floating license when your EthoVision XT computers are connected to the internet. So make sure that your EthoVision XT computer in the lab is always connected.

Follow these steps for each computer.

1. Make sure that your EthoVision XT computer is connected to the internet.

2. Start EthoVision XT.
3. In the window that appears, choose **Use the software activation key** and click **Continue**.
4. Choose **Floating license - Online connection required** and click **Continue**.
5. Enter or copy the **activation key** that you received from Noldus.




6. Click **Continue**.
7. When the license has been activated, a message appears.

Repeat the procedure on the other computers.

## Activate a computer-locked license - online

1. Make sure that your EthoVision XT computer is connected to the internet.
2. Start EthoVision XT.
3. In the window that appears, choose **Use the software activation key** and click **Continue**.
4. Choose **Computer-locked license - Online activation** and click **Continue**.
5. Enter or copy the **activation key** that you received from Noldus.
6. Click **Continue**.
7. When the license has been activated, a message appears.

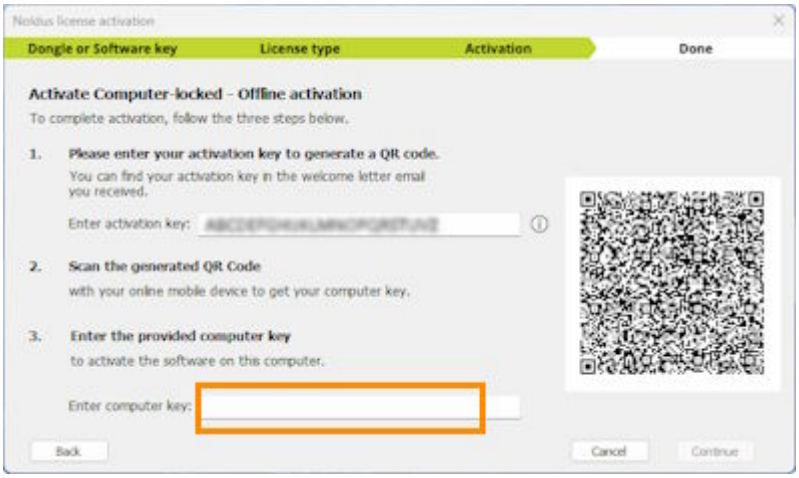
## Activate a computer-locked license - offline

1. Make sure that you have a smartphone connected to the internet that can scan a QR-code.


2. Start EthoVision XT.
3. In the window that appears, choose **Use the software activation key** and click **Continue**.
4. Choose **Computer-locked license - Offline activation** and click **Continue**.
5. Enter or copy the **activation key** that you received from Noldus.
6. A QR code appears on the window. Scan the QR code with your smartphone.

   If the QR code is recognized, the Noldus License Manager web page opens on your smartphone, showing the required Computer Key.


7. Enter this key in the **Enter computer key** field in the Noldus License Activation window.



8. Click **Continue**.
9. When the license has been activated, a message appears.

## Notes

- You cannot activate a term license after the expiration date.
- If activation fails:
  - Try once again and make sure that you enter the correct Activation/ Computer Key, including capital letters and dashes.
  - Contact Noldus to obtain a valid Activation Key.
- If your license is not activated, you can still start EthoVision XT and work in *Analysis mode*. See EthoVision XT in analysis mode
- If the license activation window does not appear after starting EthoVision XT, it may be that the software is in Analysis mode. To activate/deactivate the license, first choose **Help** > **Deactivate License**. The window says **Deactivate Analysis mode**. Choose **Continue**, then restart EthoVision XT.
- See also Upgrade EthoVision XT

# Deactivate an EthoVision XT license

## Aim

To deactivate a license, either to end the license agreement or to transfer the license to another computer.

**EXAMPLE** You have purchased a new computer and you would like to transfer your EthoVision XT license from the old to the new computer.

## Prerequisites

- EthoVision XT is running, and no experiment is open.
- The EthoVision XT computer is connected to the internet, or you have a smartphone connected to the internet.

## What do you want to do?

- Deactivate a computer-locked license
- Deactivate a floating license

See Types of EthoVision XT license

## Deactivate a computer-locked license

1. If an experiment is open in EthoVision XT, close it (**File** > **Close**).
2. Choose **Help** > **Deactivate License**.
3. Choose **Computer-locked** and click **Continue**.
4. Select one of the options depending if your EthoVision XT computer is connected to the internet.
    - **Deactivate online**. Do this if your EthoVision XT computer is connected to the internet. Select this option and click OK.
    - **Deactivate offline**. If your computer is not connected to the internet, you need a smartphone connected to the internet, which can scan a QR-code. (1) Select this option, and click **OK**. (2) A QR code appears on the screen. (3) Scan this code using a smartphone and follow the link within 24 hours. (4) Click **Deactivate** on the screen that appears in your browser.
5. EthoVision XT shows a message that the license has been deactivated and closes.

Note:

- If your smartphone is not connected to the internet, leave the link open, go to a place where your smartphone is connected to the internet and open the linked page.
- When you click **Deactivate offline**, your license is deactivated on your computer, but not yet on the Noldus Server. For this second step you must scan the QR code and follow the associated link and click **Deactivate** on a web browser of your smartphone. Do this within 24 hours.

## Deactivate a floating license

Deactivate a floating license when you need to transfer that license to another computer. Note that, when you have floating licenses, in order to be able to work on one computer you just need to close EthoVision XT on the other computer(s). You do not need to deactivate the license on the other computer(s).

1. Make sure that the EthoVision XT computer is connected to the internet.
2. In EthoVision XT, choose **Help** > **Deactivate License**.
3. A message appears: *The license was successfully deactivated*.
4. Click **Close**. You can now activate that floating license on another computer. See Activate your EthoVision XT license

*See also*

- Activate your EthoVision XT license
- Types of EthoVision XT license

# Turn a floating license to a computer-locked license

## Aim

You may want to turn a floating license to a computer-locked (fixed-seat) license so you can use EthoVision XT on a computer without internet connection.

## Prerequisites

- You have an Activation Key for your license.

## Procedure

1. Open EthoVision XT and deactivate the floating license. Here you need an internet connection. See Deactivate a floating license
2. After deactivation of the license, EthoVision XT closes.
3. Start EthoVision XT on the computer where you want to have the license activated.
4. Choose **Activate software license key**, then **Computer-locked**. Follow the instructions in Activate a computer-locked license - online.

*See also*

- Types of EthoVision XT license

# Turn a computer-locked license to a floating license

## Aim

You may want to turn a computer-locked (fixed-seat) license to a floating license because you want to use EthoVision XT on multiple computers (although not simultaneously).

Note that if you purchased multiple floating licenses, you can work on multiple computers simultaneously, because a license will be active in each computer.

## Prerequisites

- You have the Activation Key for your license.

Procedure

1. Open EthoVision XT and deactivate the computer-locked license. See Deactivate a computer-locked license
2. After deactivation of the license, EthoVision XT closes.
3. Start EthoVision XT on the computer where you want to activate the floating license. Here you need an internet connection.
4. Choose **Activate software license key**, then **Floating - On multiple computers**. Follow the instructions in Activate a floating license.

## Notes

- If you choose to deactivate offline, first click OK, then scan the QR code that appears. Follow the link and click/tap **Deactivate**.

*See also*

- Types of EthoVision XT license

# Types of EthoVision XT license

There are two main types of EthoVision XT license:

- License on a hardware key
- Software license key

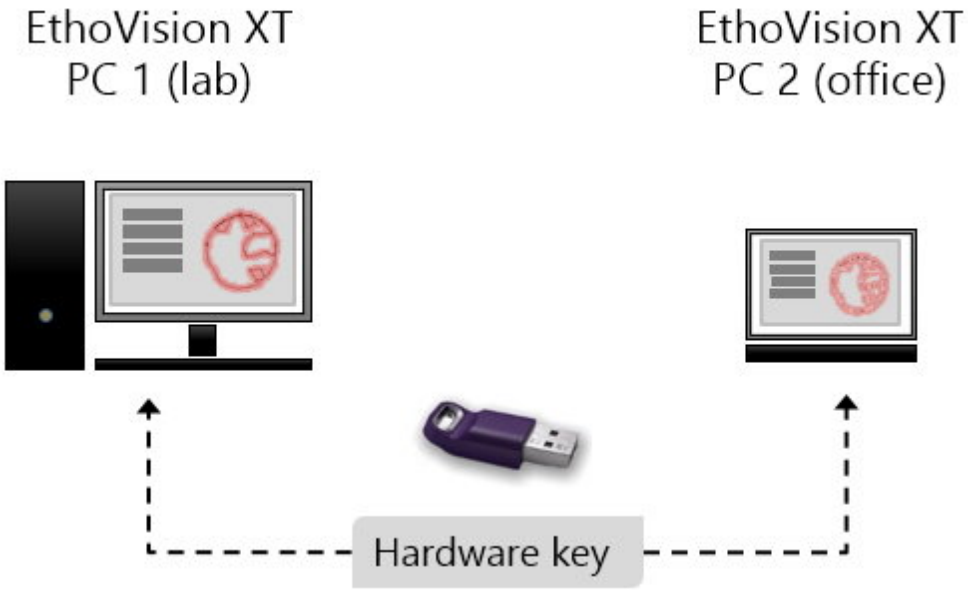
In all cases, to know which modules your license has, choose **Help** > **About EthoVision XT** > **License Info**.

## License on a hardware key

Your EthoVision XT license is stored on a USB key. A USB key looks similar to a USB Flash drive, but does not include any disk storage space.

**IMPORTANT** Make sure that you do not lose the hardware key! You will need to pay for a new license if you do so. Please be careful with the hardware key. It is sensitive and can be easily damaged.

In order to use the full functionality of EthoVision XT on either one or the other computer, you must plug the hardware key in that computer.



Alternatively you can start EthoVision XT without a hardware key plugged in and work in analysis mode to analyze the trials already acquired but not acquire new trials. See EthoVision XT in analysis mode

## Software license key

A software license key allows you to use EthoVision XT without a hardware key plugged in your computer.

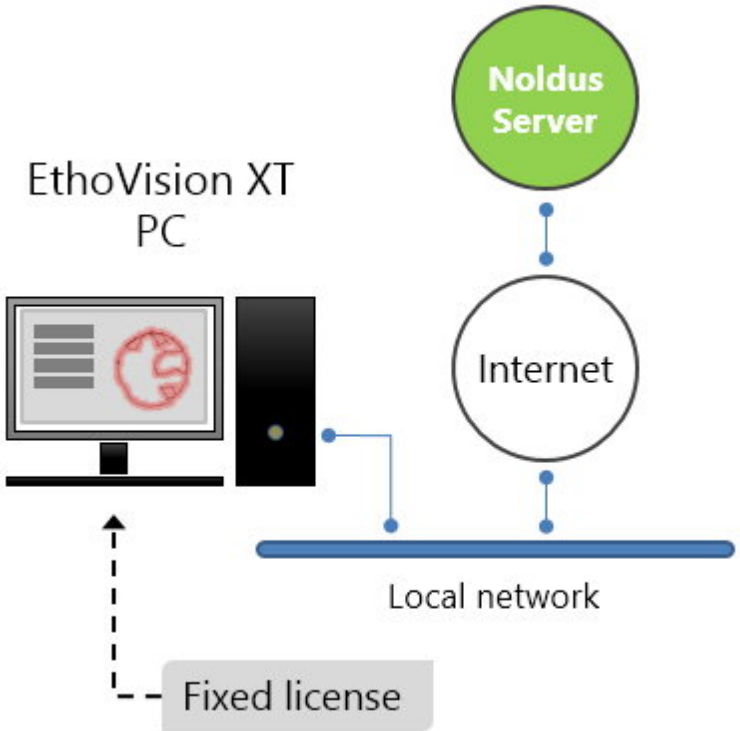
**IMPORTANT** Your software license key is coupled to the hardware of your computer; not to a specific user or user group name.

A software license can be of two types: *Computer-locked* and *Floating*.

### *Computer-locked license*

This license is meant to be used on a specific computer for long time. It is a fixed-seat license associated with that computer. This association is registered on the Noldus Server.

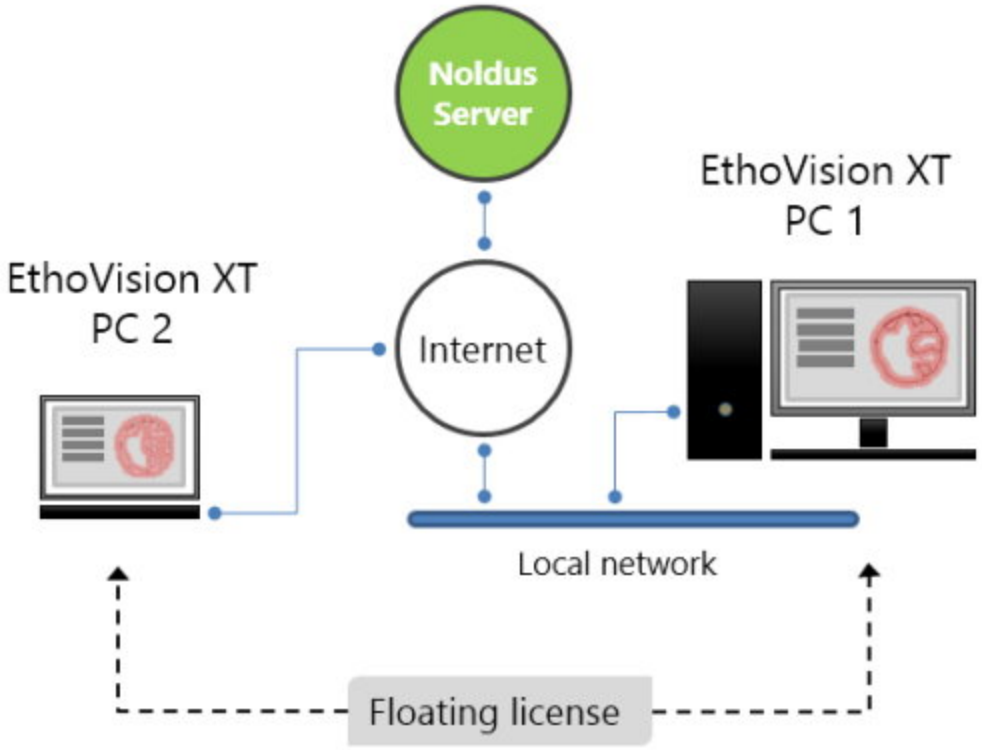
In order to use a computer-locked license on another computer, you must first deactivate that license on the computer where it is currently active. See Deactivate an EthoVision XT license



*Floating license*

This license is handy when you want to use EthoVision XT to acquire data and video on two or more computers (though not simultaneously).

- **EXAMPLE** Activate a floating license on two computers: one in the lab for recording video, and one in the office to acquire the tracks from the resulting video files.
- If you always record data/video on the same computer in the lab and analyze/visualize data on your laptop in the office, a floating license may not be needed. You can activate a computer-locked license on your lab's computer to acquire data and video and install EthoVision XT and use it in *Analysis mode* in your office (see below). See EthoVision XT in analysis mode
- With a floating license you can work on one computer at a time. Make sure that EthoVision XT is closed on the computers other than the one you are using at that time.
- **IMPORTANT** In order to work with a floating license, your EthoVision XT computer must be connected to the internet. When you close EthoVision XT on one computer with a floating license, the license is automatically deactivated, at the condition that the computer is connected to the Noldus Server. At that point you can start using EthoVision XT on another computer.



- With a floating license you can work offline with EthoVision XT for a maximum of 90 days. After that, you must re-connect the EthoVision XT PC to the internet in order to continue to work with the software or to deactivate the license and make it possible to use the license on another PC.

## Notes

- If the hardware key is not plugged in your PC or the software license is not activated, you can still start EthoVision XT and work in *Analysis mode*. This means that you can analyze and visualize previously-recorded data, but cannot acquire more data. See EthoVision XT in analysis mode

*See also*

- Activate your EthoVision XT license
- Modules of EthoVision XT

# Duration of the EthoVision XT license

An EthoVision XT license can be:

## Perpetual license

This is the most common license. You purchase a copy of EthoVision XT on a one-time basis and can use the software forever.

## Time-limited license

This license is generally valid for a period of six or twelve months.

- A time-limited license can only be activated online. See Activate your EthoVision XT license

## Trial license

This is a temporary, free-of-charge license that you can use to evaluate the newest version of the software. See EthoVision XT trial version

- A trial license can only be activated online. See Activate your EthoVision XT license

*See also*

- Types of EthoVision XT license
- Activate your EthoVision XT license
- Upgrade EthoVision XT
- License agreement

# EthoVision XT in analysis mode

## Aim

To carry out analysis and visualization of already-existing track data in EthoVision XT without a license.

## Background information

An EthoVision XT license is needed whenever you acquire new data and record video. So it is important to activate a license on the computer that you use to collect data. However, you can also install EthoVision XT on other computers without a license. If an experiment already contains data, you can open it in EthoVision XT, filter data, calculate statistics and make charts and heatmaps.

**EXAMPLE** Collect the data in your lab. Copy the experiment to your laptop where you have installed EthoVision XT without a license. There you can visualize and analyze your data.

## Procedure

1. If you have not done so yet, install EthoVision XT on a computer.
2. Start EthoVision XT.
3. If EthoVision XT does not find a valid license, a window opens with the message *Your license*.
4. Choose **Start in analysis mode**.

## What you can do in analysis mode

EthoVision XT in analysis mode offers all the functions necessary to refine analysis and produce results.

- Add new zones and modify existing zones in the Arena Settings.
- Edit tracks in the Track Editor screen. For example, remove outliers before running analysis.
- Create behavioral categories in the Manual Scoring Settings, and score those categories manually while reviewing existing video.
- Adjust the Track Smoothing settings.
- Create new Data profiles and select data.
- Create new Analysis profiles and select dependent variables.

- Use all the analysis functions under **Analysis** > **Results**.
- Export data and results.
- Make a backup of your experiment.

Furthermore you can create new Detection Settings profiles, create new Trial Control Settings profiles, and add trials and variables in the Trial List.

## Notes

- To return to or start a full license, you must first deactivate the analysis mode. Close the experiment, then choose **Help** > **Deactivate License**, then click **Continue**. Next, restart EthoVision XT. See Activate your EthoVision XT license

*See also*

- License activation

# Upgrade EthoVision XT

## What do you want to do?

- Upgrade EthoVision XT
- Add new modules to your license

## Read about

- Types of EthoVision XT license
- Modules of EthoVision XT

# Upgrade EthoVision XT

## Aim

To activate the license for a newer version of EthoVision XT.

If you purchased add-on module, see Add new modules to your license

## Prerequisites

- You have an older version of EthoVision XT (17.5 or earlier) and you have just purchased a new EthoVision XT 18 license.
- You received a code (named **Upgrade Key**) from Noldus, either on the Welcome letter or by e-mail.
- If you have a USB hardware key, insert that in a USB port of your EthoVision XT computer.

## Procedure

Choose the procedure that applies.

### *For a software-based license*

1. Download and install EthoVision XT from

   my.noldus.com.

   **NOTE** You do not need to uninstall previous EthoVision XT versions.

2. Start EthoVision XT.
3. Choose **Activate software license key**.
4. Choose whether to activate online or offline and follow the instructions on the screen. See also Activate your EthoVision XT license

### *For a license stored on the hardware key*

1. Download and install EthoVision XT from

   my.noldus.com.

   **NOTE** You do not need to uninstall previous EthoVision XT versions.

2. Start EthoVision XT.

3. Enter the **Upgrade Key** that you received from Noldus and complete the upgrade procedure.

*See also*

- License on a hardware key
- Activate your EthoVision XT license
- Deactivate an EthoVision XT license
- Types of EthoVision XT license
- Modules of EthoVision XT

# Add new modules to your license

## Aim

To make update your EthoVision XT license after you have purchased one or more add-on modules. For example:

- The Multiple Arena module.
- The Rat Behavior Recognition module.

See Modules of EthoVision XT

## Prerequisite

- If you have a software license, the EthoVision XT computer must be connected to the internet, or you have a smartphone connected to the internet.
- If you have a license stored on a hardware key, insert the hardware key in a USB port of your EthoVision XT computer.

## Procedure

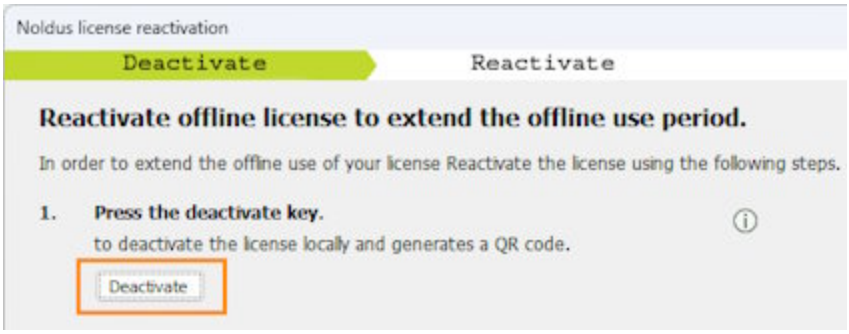
Choose the procedure that applies to upgrade your license with new modules.

### *Software license - online*

If your EthoVision XT computer is connected to the internet, the license is updated automatically the next time that you start the software. You do not have to take any further action.

### *Software license - offline*

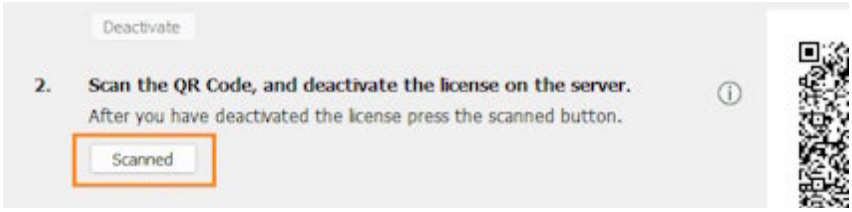
1. Start EthoVision XT.
2. Choose **Help** > **Reactivate Offline**.
3. In the window that appears, click the **Deactivate** button.




4. A QR code appears on the right. Scan that QR code with your smartphone.
5. Tap the link that appears on the smartphone, then on the smartphone tap **Deactivate**.

   **NOTE** The web page that opens on the smartphone shows your current license key and a code that identifies your computer.

   You'll notice nothing changes after you have clicked **Deactivate** on the smartphone screen. That's ok.
6. On the computer screen, click **Scanned**.
7. A new QR code appears. Scan this code and tap the link that appears on the smartphone.



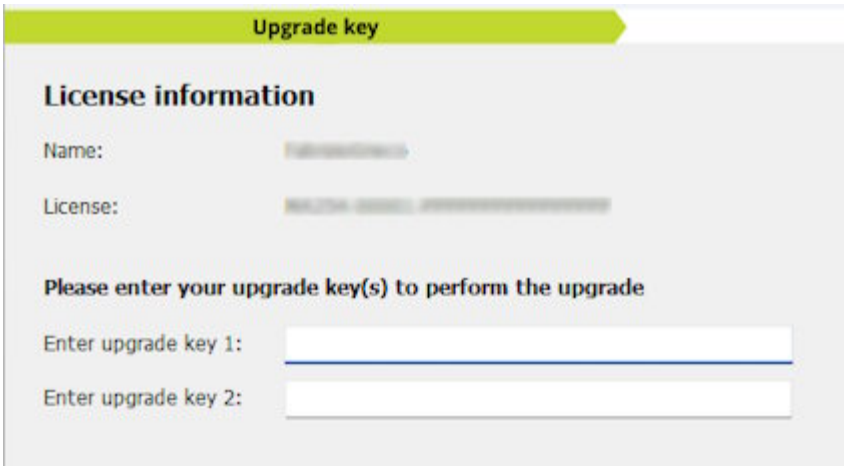

8. On the computer screen, next to **Computer key**, enter the code that appears on the smartphone.


9. A message informs you that the license has been activated, and the software closes. You can now start EthoVision XT and work as usual.

*License stored on a hardware key*

1. Start EthoVision XT. Make sure that no experiment is open.

2. Choose **Help** > **Upgrade**.
3. Enter the **Upgrade Key 1** and, if that apples, the **Upgrade Key 2** that you received from Noldus.




4. Click OK to complete the upgrade procedure.

## Notes

- To know which modules are included in your current license, choose **Help** > **About EthoVision XT** > **License Info**. See also Modules of EthoVision XT

*See also*

- Activate your EthoVision XT license
- Types of EthoVision XT license
- Troubleshooting: Installation and license

# Preferences

## Aim

After installing EthoVision XT, it is a good idea to set general preferences. For example, where to store the experiments, the default measurement units, etc.

The options under **Preferences** are at the application level. This means that they are applied to all new experiments. However, default units can also be changed per experiment.

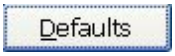
## Procedure

Choose **File** > **Preferences**.

- Preferences for default folders
- Preferences for warnings
- Preferences for auto save
- Preferences for default units
- Preferences for language
- Preferences for video settings

## Notes

- To return to the default settings, click the **Defaults** button.

  Returning to default means that:

  - The default experiment folder will be that specified during installation.

  - All the warnings are selected.

  - Auto save is enabled, with auto save interval is reset to 5 min.

- When you click the **Defaults** button with the **Default Folders** tab open, the preferences for warnings, auto save and default units are also reset (and vice versa).

# Preferences for default folders

## Procedure

Choose **File** > **Preferences** > **Default Folders** tab. See the options below.

## Default folder location

Click **Browse** to specify a default location for new experiments. The initially suggested location is the location specified during installation.

See also File locations on page 1238

## Alternative media location

When visualizing and animating tracks, EthoVision XT needs the corresponding video files. If EthoVision XT does not find the video files in the Media Files folder of the experiment, it looks in the folder specified under **Alternative media location**.

This can happen, for example, when you copy an experiment to another computer and the video files are not stored in the Media Files folder of the experiment, but on a different folder external to the experiment, or even a different drive. When you open the experiment on the second computer, EthoVision does not find the video files.

To have EthoVision XT look for videos in an additional location, click **Browse** and select the correct location.

*See also*

- Preferences

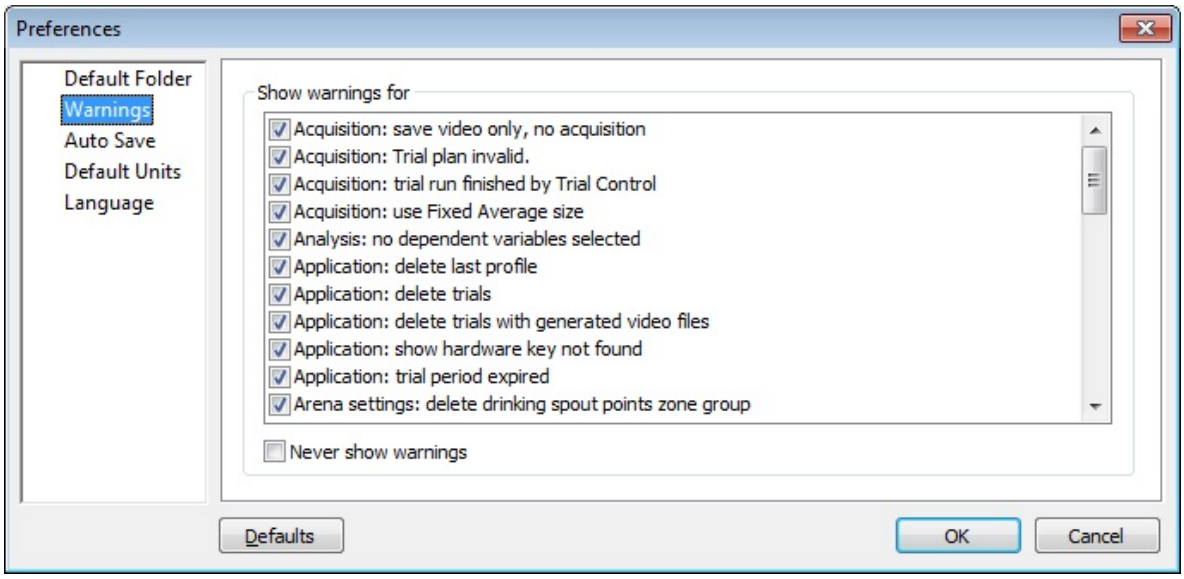
# Preferences for warnings

## Aim

To enable or disable a warning that appears after you perform a certain action.

## To choose which warnings to view

Choose **File** > **Preferences** > **Warnings**.




*See also*

- Preferences

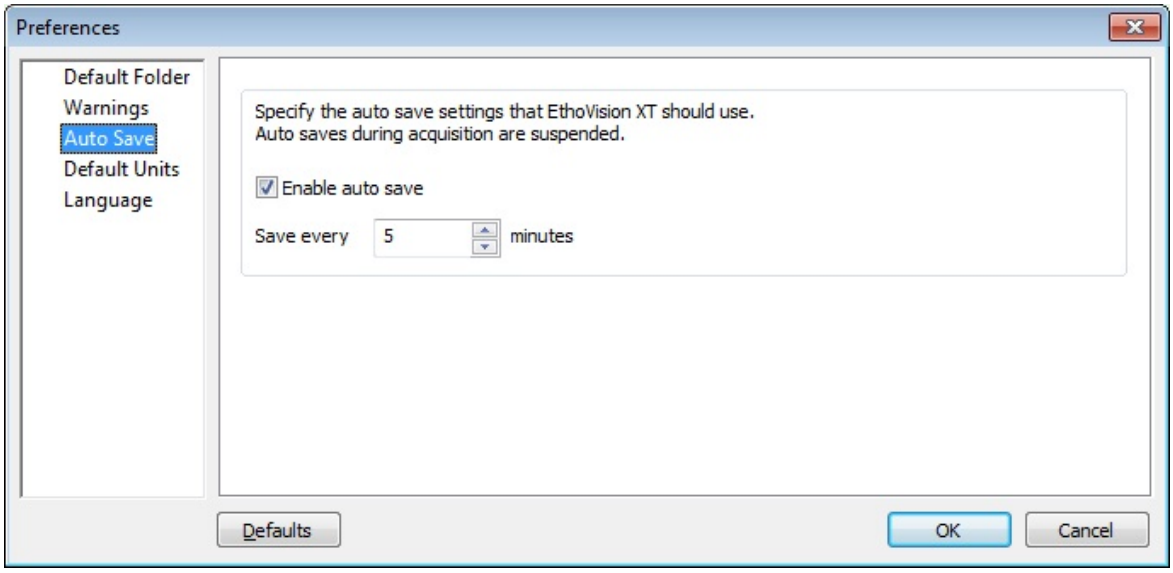
# Preferences for auto save

## Aim

To enable or disable auto save of data and settings.

## To enable auto save

Choose **File** > **Preferences** > **Auto Save**. Select **Enable auto save**. Enter the time interval at which you like to save your data.




## Notes

- Auto save saves all the data (tracks, independent variables, detection settings, data and analysis profiles, etc.), just like when you save the experiment manually (choose **File** > **Save Experiment** or press **Ctrl+S**).
- Auto saves during acquisition are suspended to prevent auto save from interfering with data acquisition.

*See also*

- Preferences

# Preferences for default units

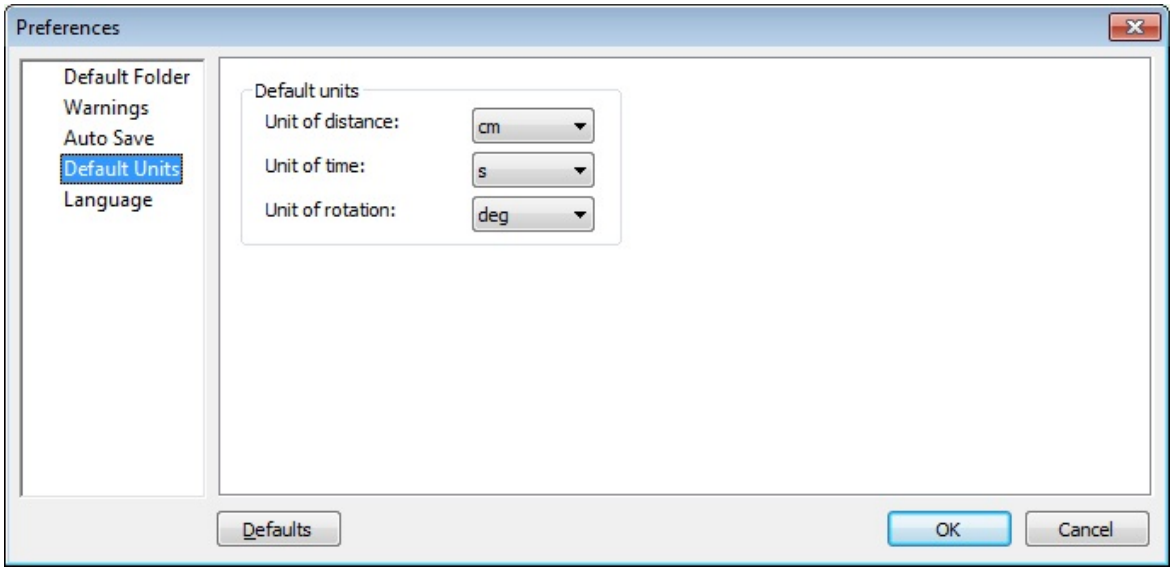
## Aim

To specify the default units for distance, time and rotation for future experiments.

## Procedure

Choose **File** > **Preferences** > **Default Units**.

- **Unit of distance**. Choose between mm (millimeters), cm (centimeters), m (meters) or inch (inches).
- **Unit of time**. Choose between ms (milliseconds), s (seconds), min (minutes) or hr (hours).
- **Unit of rotation**. Choose between deg (degrees; °), rad (radians), grad (gradians; 1 grad = 9/10° or π/200 rad) or rot (rotations).




## Notes

- EthoVision XT measures the distance between two points in pixels. With the Calibration procedure in Arena Settings, these are converted to distance values.
- Changing the default units in Preferences does not affect the experiment currently open. To change the units in your present experiment, do so in the Experiment Settings > Units.
- Changing the units does not affect the raw data, only their presentation.

*See also*

- Preferences

# Preferences for language

## Aim

To specify the language in EthoVision XT. You can choose between US English and Simplified Chinese.

## Procedure

1. Choose **File** > **Preferences** > **Language**.
2. Select either **English (US)** or **Chinese (Simplified)**.
3. Click **OK** and restart EthoVision XT to apply the change.

*See also*

- Language support
- Preferences

# Preferences for video settings

## Aim

To specify how video is processed during tracking. These settings are applied to all new experiments.

## Background information

In EthoVision XT 17.5 and later versions you can set playback of video to be done through the Graphics Processing Unit (GPU or dedicated graphics card) instead of the CPU. This allows greater performance, especially when tracking in demanding conditions like with Behavior Recognition. Up to EthoVision XT 17.0 it was only possible to playback video with the CPU.

## Procedure

1. Choose **File** > **Preferences** > **Video settings**.
2. Next to **Play back and record video with**, Select either **GPU** (default) or **CPU**.
   - Choose **GPU** if you want to have the GPU to process video.
   - Choose **CPU** if you want to have the CPU to process video.
3. Click **OK** to apply the change.

## Notes

- If you are in a middle of an experiment started with EthoVision XT 17.0 or an earlier version and some tracks were acquired before upgrading to EthoVision XT 18, you have two options:
  - *Complete the experiment with the old playback method*. Keep the option **CPU** selected (recommended). The reason is that if you choose **GPU** a video file could look slightly brighter than it would do if you chose **CPU**. In that case you would need to make new detection settings for the next trials. To keep consistent detection settings across trials, choose **CPU**.
  - *Acquire all trials with the new playback method*. Clear the existing trials (i.e., delete the tracks without removing the videos), and re-acquire all trials, with the option set to **GPU** for consistency. See Redo a trial
- For all your new experiments, we recommend to keep the option **GPU** selected.

- What option you choose next to **Play back and record video with** does not affect tracking based on Deep Learning, even though this relies on the GPU. So whatever option you choose, you can use the Deep Learning body-point detection technique, provided that your PC meets specific requirements. See Deep learning: Requirements

*See also*

- Preferences

# License agreement

## Topics and tasks

- End-user license agreement
- Acknowledgments and copyright notices

# End-user license agreement

## Procedure

To open a PDF copy of the End-User License Agreement, do one of the following:

- Browse to the following file:

  C:\Program Files\Noldus\EthoVision XT 18\Documentation\Legal\End-User License Agreement.pdf

- In EthoVision XT, choose **Help** > **About EthoVision XT** > **License Info** > **End-User License Agreement**.

## Notes

- If you installed EthoVision XT on a folder other than the default one, then browse to that folder, and open

  ...\EthoVision XT 18\Documentation\Legal.

*See also*

- File locations
- Acknowledgments and copyright notices

# Acknowledgments and copyright notices

EthoVision® is a registered trademark of Noldus Information Technology bv. EthoVision XT would not be what it is without the use of third-party software. This page lists all software libraries and other software products used in EthoVision XT and where you can find license and compliance information and/or acknowledgments for that product.

For the complete terms and conditions in PDF format, click the link below:

Acknowledgments

This folder is located, by default, on C:\Program Files\Noldus\EthoVision XT 18\Documentation\Legal**Acknowledgments**.

If you installed EthoVision XT on another location, refer to that location.

| Name | Description | Link/ License |
|---|---|---|
| Boost | Open source (Boost community) software library, C++ templates | Boost software license<br>http://www.boost.org/users/license.html |
| Euresys Multicam | Driver to address Picolo boards | Euresys<br>http://www.euresys.com/<br>Terms & conditions<br>http://www.euresys.com/terms-conditions/ |
| FFmpeg | Cross-platform solution to record, convert and stream audio and video | FFmpeg<br>https://ffmpeg.org<br>GNU Lesser General Public License<br>https://ffmpeg.org/legal.html |

| | | |
|---|---|---|
| HASP | Library for software protection | EULA<br><br>https://supportportal.gemalto.com/csm?id=kb_article_view&sys_kb_id=f8ad2d43db19ff04d298728dae96195d&sysparm_article=KB0018933<br><br>Thales Group<br><br>https://cpl.thalesgroup.com/ |
| IPP, MKL, TBB | Software library for signal, image, speech and audio processing (Intel Corporation) | Intel Composer XE<br><br>https://software.intel.com/en-us/license/intel-simplified-software-license |
| LeadTools | Video encoder and decoder software | LeadTools license agreement<br><br>Portions of EthoVision XT were created using LEADTOOLS ©1991-2021, LEAD Technologies, Inc. ALL RIGHTS RESERVED. Portions of this product are licensed under US patents 9,552,527, 10,318,563 and 10,576,653 and foreign counterparts.<br><br>https://www.leadtools.com/help/sdk/v21/licensing/leadtools-software-license-agreement.html |
| LibTorch | C++ frontend for PyTorch machine learning framework | Pytorch<br><br>https://pytorch.org/<br><br>3-clause BSD License<br><br>https://github.com/pytorch/pytorch/blob/main/LICENSE |
| NVIDIA CUDA® | Parallel computing platform for general computing on GPUs | NVIDIA CUDA Toolkit<br><br>https://docs.nvidia.com/cuda/eula/index.html |

| | | |
|---|---|---|
| NVIDIA cuDNN | Library of primitives for deep neural networks | NVIDIA<br>https://docs.nvidia.com/deeplearning/cudnn/reference/eula.html |
| NVIDIA TensorRT | SDK for deep-learning inference | NVIDIA<br>https://developer.nvidia.com/tensorrt<br>Apache License 2.0<br>https://github.com/NVIDIA/TensorRT/blob/master/LICENSE |
| OpenCV | Software library for various computer vision problems | OpenCV<br>http://www.intel.com/content/www/us/en/research/intel-research.html<br>Apache License 2.0<br>https://github.com/opencv/opencv/blob/master/LICENSE |
| Pylon Camera Software Suite | Driver for Basler cameras | Baslerweb<br>https://www.baslerweb.com<br>Pylon End-User License Agreement<br>https://www.baslerweb.com/en/service/pylon-eula/ |
| QR Code generator library | Library for generating QR codes | MIT License<br>https://github.com/nayuki/QR-Code-generator<br>Project Nayuki<br>https://www.nayuki.io/page/qr-code-generator-library |

| | | |
|---|---|---|
| Wix Toolset | Tools to author software installers | Wix Toolset<br><br>http://wixtoolset.org/<br><br>.NET Foundation<br><br>https://dotnetfoundation.org/<br><br>Microsoft Reciprocal License (MS-RL)<br><br>All copyright, patent, trademark attribution notices that are present in the installation software are retained by Microsoft<br><br>https://wixtoolset.org/docs/about/#license |

# Set Up an Experiment

## Main topics and tasks

# Your first EthoVision XT experiment

## Learn about

- Important terms
- Protocols on the web featuring EthoVision XT

## What do you want to do?

- Create an experiment from a predefined template
- Procedure

## Notes

- You can also Create a new experiment based on an existing experiment. To do so, open the existing experiment and choose **File** > **Save as**. In the new experiment, delete the existing trials and settings if needed.
- **IMPORTANT** When you create experiments, do not move, rename or delete experiment files and folders with Windows Explorer. Instead, always manage your experiments from within EthoVision XT. See File Management
- To create an experiment for Live Mouse Tracker, choose **File** > **New** and select the option **Live Mouse Tracker experiment**. For details, see Live Mouse Tracker: Create an experiment and Live Mouse Tracker: Workflow
- For information on how to conduct EthoVision XT experiments with specific apparatuses, like the Open Field, the Morris Water Maze, the Novel Object Recognition and others, see the EthoVision XT 18 - Application Manual in this folder:

  Other Documentation

# Important terms

## Experiment

In EthoVision XT, the experiment is the container of all information related to an experimental setup. For example, you can create an experiment to collect data of a Morris water maze test, and another experiment for a Porsolt forced swim test.

### *Create an experiment from a template (recommended)*

You can create an experiment with already predefined settings, for example a circular arena for a Morris water maze. You can create a template experiment in two ways: a) from one of the predefined templates or b) from one of your preexisting experiments.

- Create an experiment from a predefined template
- Create a new experiment based on an existing experiment

If none of the predefined templates applies to your experiment, create a experiment from scratch (see below).

### *Create an experiment from scratch*

You can create a new experiment with no predefined settings. To do so, choose **File** > **New**. See Create a new experiment from scratch

## DanioVision experiment

A special version of an experiment for tracking zebrafish larvae with DanioVision. For the procedure, see the DanioVision DVOC-0041 - Reference Manual. See Manuals

## GLP experiment

This is a special version of an EthoVision XT experiment made for Quality Assurance. First, certain actions such as editing or deleting data can only be done by specific users. EthoVision XT logs all user actions which can result in changes to the acquired data, for instance altering settings. It is also possible to leave a comment in the log when a user leaves a part of the program.

- EthoVision XT and Quality Assurance

## Live Mouse Tracker experiment

This is a special version of an EthoVision XT experiment made for importing data from Live Mouse Tracker systems. In a Live Mouse Tracker experiment, some elements are not available, like the Track Editor and the Acquisition module. You cannot acquire data directly. Instead, import the Live Mouse Tracker databases into EthoVision XT. You do not need to draw the arena since that is already defined in the Live Mouse Tracker database. You analyze Live Mouse Tracker data just like any other EthoVision XT experiment. Note: EthoVision XT is based on Live Mouse Tracker version 1.0.3.

## Trial

A trial is a single, uninterrupted data acquisition session. The trial starts when you either click the **Start Trial** button or press **Ctrl+F5**.

## Track

A track is the actual data collected by EthoVision XT - a succession of 2D positions of each subject. At track include additional information such as the subject's apparent size in pixels, and other information. The start of a track may or may not coincide with the start of the trial. This depends on your Trial Control Settings. In most cases, with the default Trial Control Settings, EthoVision XT waits one second after the subject has been detected in the arena. Therefore, depending on when the subjects is in view, the track begins some time after the moment that you click the Start trial button.

If you have multiple subjects or multiple arenas in the same video image, each subject produces one track within the trial.

# Create an experiment from a predefined template

## Aim

To create a new experiment based on standard experimental setups like Morris water maze, Elevated plus maze, DanioVision, PhenoTyper, etc.

## Procedure

1. Choose **File** > **New From Template** (**Ctrl+T**), or in the EthoVision XT Startup window, under **New experiment**, click **New from template**.
2. In the Select a template option window, select **Apply a predefined template** and, next, follow the instructions in the guided setup.
3. Step 1- Which video source will you use?

   **From video file**. If you select this option, you can click **Browse** to select the video file. Optionally, you can also open the video file later.

   **Live tracking** (and saving video files). Select this option and click **Sources**. In the window that opens, select the video sources for tracking. You can also set up your video source here. If you plan to co-acquire physiological data, select DAQ Co-acquisition.

   You can only select Live tracking if EthoVision XT detects an encoder board for analog cameras or a digital camera. See Cameras supported by EthoVision XT

4. Step 2 - Which subjects will you track?

   If you select the option **Rodents**, **Fish** or **Arthropods**, select a species from the list. If your species is not in one of these lists, select **Other**.

   Based on the subject you select, different arena configurations are available in the next step of the guided setup, a suitable sample rate is selected and the nose-tail detection is made available.

5. Step 3 - How is the arena configured?

   **Arena template**. Select a type of arena from the list (a picture is shown upon selection).

   **Zone template**. Here you have the choice to select predefined zones for the type of arena you select in the Arena template list.

   **Number of Arenas**. Select the number of arenas in the list.

You can only select multiple arenas, if you have the Multiple Arenas module.

**Rows and columns**. If you have multiple arenas, select the layout of the arenas.

6. Step 4 - How many subjects per arena will you track?

   You can only select multiple animals if you have the Social Interaction module.

   This step is skipped for some single-subject configurations like the Morris water maze.

7. Step 5 - Which features will you track?

   Select the tracked features. Also select whether the animal is Darker, Brighter or Brighter and darker than the background. The features option is skipped for configurations like the Novel object test, where tracking the nose-point is mandatory, or DanioVision where only the center point is tracked.

   You can change your selection later in the Experiment Settings. See Tracked features

8. Step 6 - Initialize template experiment.

   This window gives an overview of the selected settings. Click **Previous** if you want to change settings, otherwise click **Finish**.

9. In the New Experiment window, in the **Name** field, enter a name for your experiment.

   Select **GLP Experiment** if you want EthoVision to help you making a Quality Assurance-compliant experiment.

10. Click **Browse** and select the location in which you want to store your experiment. Do not select a removable disc, like a USB memory drive.
11. Click **OK**. The EthoVision XT Overview window appears.
12. Next: check the Experiment settings.

## Notes

- Each experiment is stored in a folder with the same name as the experiment.
- The default experiment location is the location specified during installation. See File locations
- The pre-defined templates (*.tpl) are stored in C:\ProgramData\Noldus\EthoVision\XT 18\Templates.

Do not move or delete those files!

- For experiments with one or two subjects per arena, you can select to use Deep learning to detect the nose and tail base of your subjects. Note that Deep learning works if a recent graphics card (GPU) is installed on your PC and its driver is up-to-date. See Deep learning: Requirements and More information on graphics cards (GPUs)
- If you select **DanioVision** as arena template, the EthoVision XT interface is adjusted for DanioVision. Unnecessary functions like the Manual Scoring Settings are not available. Detection settings are optimized for detection of zebrafish larvae and the arenas are detected automatically. For more information, see the DanioVision DVOC-0041 - Reference Manual. See Manuals

# Create a new experiment based on an existing experiment

## Aim

To create a new experiment with settings (arena shape, detection settings etc.) imported from an existing experiment. Note that the new experiment will not contain data from the existing experiment.

## Procedure

1. Do one of the following:

   Choose **File** > **New From Template** (**Ctrl+T**), or in the EthoVision Startup window, under **New experiment**, click **New from template**.

2. In the Select a template option window, click **Use a custom template**.
3. Browse to the experiment folder of the existing experiment, click the experiment file (*.evxt) and click **Open**.
4. In the Name field, enter a name for your experiment.

   Select **GLP Experiment** if you want EthoVision to help you making a Quality Assurance-compliant experiment. See EthoVision XT and Quality Assurance

5. Browse to the location in which you want to store your experiment. Then click **OK**. The default location is the location that you specified during installation.
6. The EthoVision XT Overview window appears. Click the buttons to check and adjust settings if necessary.

## Notes

- When you create an experiment based on an existing experiment set to Live tracking, the new experiment imports the camera settings from that existing experiment. Check that the camera settings, for example the frame rate and the exposure time, still apply to the new situation. See Adjust camera settings in EthoVision XT
- If you create an experiment based on an existing experiment, which used Live tracking and was created on a different computer, you must remake the Video source settings for the new experiment.

# Protocols on the web featuring EthoVision XT

Researchers are encouraged to publish test protocols on the internet to improve reproducibility of animal experiments.

Browse to

https://www.bio-protocol.org/en/searchlist?content=ethovision

There you find protocols created with or mentioning EthoVision XT.

*See also*

- Your first EthoVision XT experiment

# Experiment settings

To access the Experiment Settings, in the EthoVision XT Overview window, click **Experiment Settings**, or choose **Setup** > **Experiment Settings**.

In the Experiment Settings specify those aspects of your experiment that remain constant during the entire course of the experiment.

- Experiment information
- Video source
- Enable DAQ co-acquisition of external data
- Number of arenas
- Subjects per arena
- Tracked features
- Body point detection technique
- Analysis options: Activity analysis and Behavior Recognition
- Trial control hardware
- Units

*See also*

- Select multiple cameras
- Adjust camera settings in EthoVision XT
- More about Experiment Settings

# Experiment information

## Experiment mode

The mode depends on your EthoVision XT license and the options you choose while creating a new experiment. The mode can be:

- **Standard** if you created an experiment, without selecting the **DanioVision experiment**, or **GLP experiment** option.
- **DanioVision** if you selected **DanioVision experiment** while you created a new experiment, or created an experiment using a DanioVision template. Manual Scoring Settings are not available and the Arena Settings and Detection Settings are optimized for zebrafish larvae in well plates.
- **Standard GLP** if you selected **GLP experiment** while you created a new experiment. This option is only available if you have the Quality Assurance Module. See EthoVision XT and Quality Assurance
- **DanioVision GLP** if you create a new experiment and select both options **DanioVision experiment** and **GLP experiment**. The experiment is set up for Quality Assurance and optimized for DanioVision.

**IMPORTANT** Once you created an experiment, the mode cannot be changed. Create a new default experiment if you want to change the mode.

For details about setting up a DanioVision experiment, see the DanioVision DVOC-0041 - Reference Manual. See Manuals

## Location

The location where your experiment is stored on your computer. The default location is (unless it changed during installation or in the Preferences):

C:\Users\Public\Public Documents\Noldus\EthoVision XT\Experiments.

To change the default location for experiments, choose **File** > **Preferences**.

## Description

Enter information about your experiment such as the kind of test that you perform, the scientific research question that you aim to answer with this study, etc. To edit the description, use Unicode characters in the Basic Multilingual Plane range. You can enter up to 255 characters.

See http://en.wikipedia.org/wiki/Plane_(Unicode) #Basic_Multilingual_ Plane.

# Video source

## From video file

Select this option if you track from video files acquired with software other than EthoVision XT, for example MediaRecorder. For the list of compatible video formats, see Video file formats.

## Live tracking

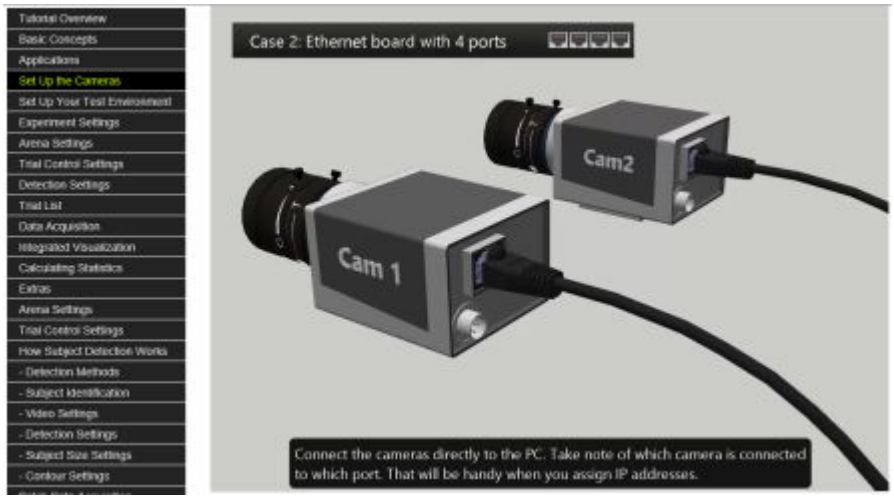
Select this option to acquire data live from the camera image.

**IMPORTANT** If you work with Basler digital cameras, close the camera software Pylon Viewer before operating the camera in EthoVision XT.

### *One digital camera*

Connect the camera to the Ethernet port of the PC. Click the **Refresh** button. Select **1** from the list next to **Number of sources** and select the camera from the list under **Source**.


**TIP** For how to install and connect digital GigE Vision cameras, watch the video tutorial **Set Up the Cameras**. To open the tutorial, choose **Help** > **Video Tutorial**.

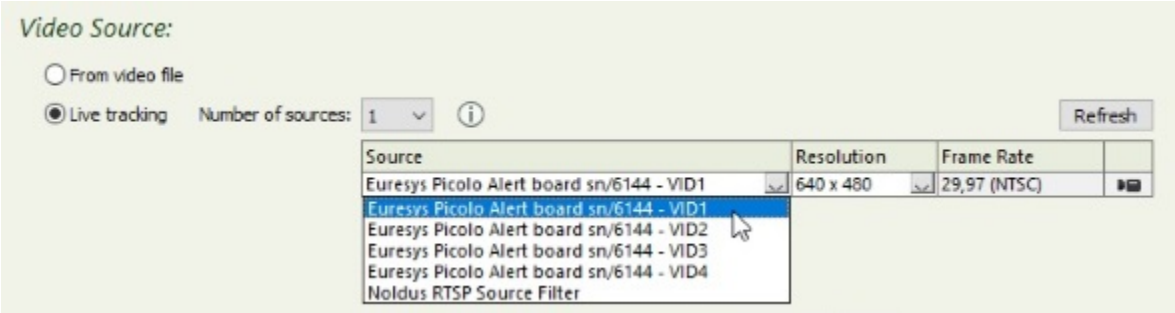


*Multiple digital cameras*

- See Select multiple cameras

*One analog camera*

Connect your camera to the encoder board. Select 1 from the list next to **Number of sources** and select the correct input from the list under **Source**.




*Multiple analog cameras*

- See Select multiple cameras

## Notes

- **IMPORTANT** You cannot use a combination of analog and digital cameras. Always use the same type of camera in one experiment.
- You can use 1, 2, 3, 4, 8, 12 or 16 cameras simultaneously. Using for example 5 cameras is not supported.
- When you use analog cameras, you need an encoder board. See Install analog cameras and Connect analog cameras to the computer
- If you mix the image from multiple cameras with a hardware video mixer, follow the procedure for one analog camera.
- The quality of the camera image can only be adjusted prior to acquisition. For details, see Adjust camera settings in EthoVision XT.

*See also*

- Cameras supported by EthoVision XT
- Check that the camera is set up properly
- Install GigE cameras and Install USB 3.0 cameras
- Install analog cameras

# Enable DAQ co-acquisition

## Aim

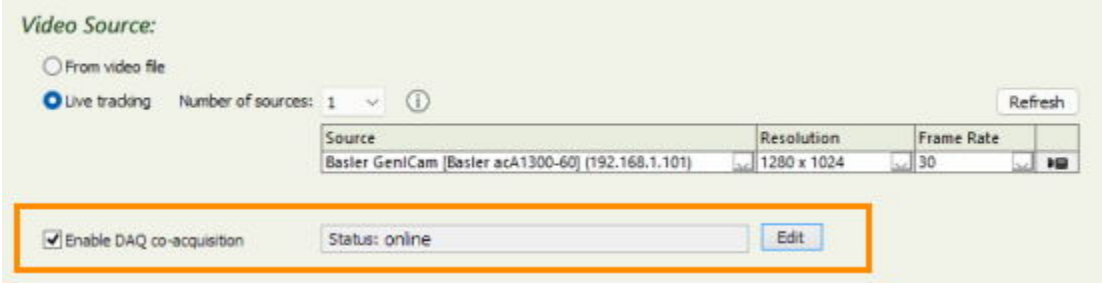
To co-acquire external data with a separate Data AcQuisition (DAQ) system. You can co-acquire, for example, physiological data while you track the subjects.

## Prerequisites

- Under Video Source in the Experiment Settings, you have selected **Live tracking**.
- You have the External Data module.

## Procedure

1. Choose **Setup** > **Experiment Settings**.
2. Under **Video Source** select **Live tracking**.
3. Select **Enable DAQ co-acquisition**.
4. For the complete procedure, see Enable DAQ co-acquisition in EthoVision XT.




*See also*

- External Data

# Select multiple cameras

## Aim

To mix the live view of multiple camera in a picture-by-picture fashion.

**IMPORTANT** When you mix video images of many cameras, the resulting resolution per camera image is reduced. Make sure that this resolution is high enough for proper detection and tracking.

## Prerequisites

- For GigE cameras (including PhenoTyper 2), see Using multiple GigE cameras, Connect the GigE camera to the PC and Assign IP addresses
- For analog cameras (PhenoTyper 1): see Connect analog cameras to the computer

## Multiple digital cameras

EthoVision XT mixes the camera images in a picture-by-picture fashion.

1. Connect your cameras.

   Plug each camera to the port of its Ethernet board. Each camera has its unique link with one of the ports of the Ethernet interface board. If you swap cameras, EthoVision XT won't get the camera image. See Using multiple GigE cameras and Assign IP addresses

2. In the Experiment Settings, select the number of cameras from the list next to **Number of sources**.
3. Select the cameras under **Source**. The camera names will show a number in brackets at the end, which makes it possible to distinguish between the cameras.
4. Select the **Resolution**, the **Frame rate** and the **Color space** for the first camera. The settings are then applied automatically to the other cameras. See Adjust camera settings in EthoVision XT
5. Next to **Merged camera view**, select the resolution of the merged camera view. This is also the resolution of the video file that EthoVision XT creates if you opt for video recording.
6. Click the preview icon to get a preview of the merged camera images.

**EXAMPLE** Selecting four GigE cameras: note the different IP addresses.


**NOTES**

- For how to install and connect digital GigE cameras, watch the video tutorial **Set Up the Cameras**. To open the tutorial, choose **Help** > **Video Tutorial**.
- The video resolution and frame rate that you set in one experiment is selected automatically in your next experiment. When you create a new experiment, always check that the camera settings are those required for that experiment. See also Configure the digital camera

*See also*

- Connect the GigE camera to the PC
- Using multiple GigE cameras
- Tested configurations with GigE cameras
- Check that the camera is set up properly

## Multiple analog cameras

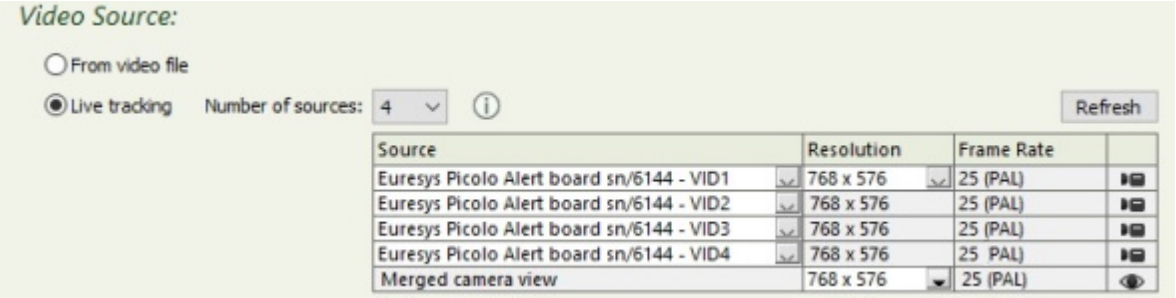
**NOTE** Mixing images from analog cameras using encoder boards other than the Picolo Alert is not supported.

*Two or four analog cameras*

1. Connect your cameras to the inputs of your encoder board. See Connect analog cameras to the computer
2. Next to **Number of sources**, select the correct number of cameras from the list and select the inputs from the list under **Source**. The resolution and frame rate are selected automatically, dependent on whether you have NTSC or a PAL cameras.
3. Next to **Merged camera view**, select the resolution of the mixed image. This is also the resolution of the video file that EthoVision XT creates if you opt for video recording. See Acquire one trial

4. Click the preview icon to get a preview of the merged image.

**EXAMPLE** Selecting four analog cameras:




### *More than four analog cameras*

When using more than four analog cameras, we recommend to use a hardware video mixer also known as Quad Processing Unit. See Connect analog cameras to the computer. If you use PhenoTyper 1, see also the PhenoTyper - EthoVision XT - Reference Manual. See Manuals

### *See also*

- Tested configurations with Analog cameras
- Install analog cameras

# Adjust camera settings in EthoVision XT

## Aim

To adjust the settings of your camera within EthoVision XT. For example, the video resolution, the frame rate, or the exposure.

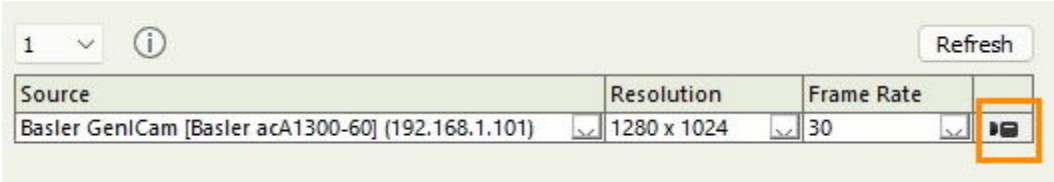
**IMPORTANT** To improve the quality of the image, always try adjusting the lighting and camera aperture before changing the settings in EthoVision XT.

## Prerequisites

- If you work with Basler digital cameras, before using the camera in EthoVision XT close the camera software Pylon Viewer.

## To access the camera options

1. Choose **Setup** > **Experiment Settings**, and click the camera button for the camera you require.




2. Click one of the tabs and adjust the settings (see below).
   - Format tab
   - Image tab
   - Camera/General tab
   - Color tab
   - Address tab
   - More settings

## Format tab

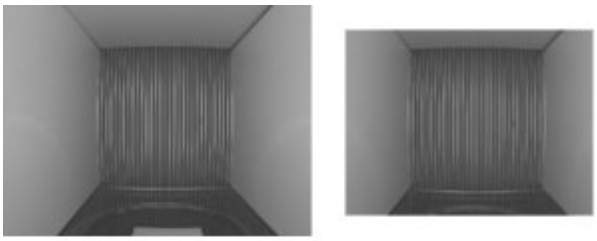
The **Format** tab is only available for the first camera. The settings you specify here are also applied to the other cameras.


**IMPORTANT** If you change the resolution within an experiment, open the Arena Settings profile and Grab the background image. This will update the background image with the new resolution. If the Arena Settings profile is locked, right-click it and select **Duplicate**. In the new profile you can now grab the background image. Next, use that Arena Settings profile to acquire new trials.

*Resolution*

For single arenas and subjects more than a couple of centimeters long, a resolution of 384x288 (analog cameras, PAL) or 320x240 (analog cameras, NTSC) or 640 x 480 (GigE cameras) is sufficient.

For digital cameras: When you reduce the resolution, the field of view also changes. In the following example, reducing the resolution from 800 x 600 to 640 x 480 results in a cropped image.



800 x 600 640 x 480

You can compensate for this if your camera is provided with a zoom lens. Another option is to leave the resolution unchanged and use the Binning function which reduces the size of the image in pixels but does not affect the field of view.

If you opt for a higher resolution in GigE cameras, for example 1280 x 1024, always check that it does not conflict with the frame rate (see below).

- The higher the video resolution, the more accurate your tracking.
- The higher the video resolution, the larger the resulting video files. If disk space is limiting, use the above-mentioned resolution.
- Use a high resolution especially:

- When you track the three body points in general.
- You work with Behavior Recognition. See Behavior Recognition: Requirements
- You track the subjects with the Deep learning technique. See Deep learning: Requirements

### *Frame rate*

This is the number of video images per second that are generated by the camera. Note that the frame rate is not the same as the Sample rate, which is the number of images the EthoVision XT analyzes per second.

- For digital cameras, the frame rate can vary. Common values are 25 and 30. A frame rate higher than 30 is recommended when working with fast-moving animals, or when you want to detect movements like startle responses in fish or rapid turns in flying insects. High frame rates require more processing power. If you select a high frame rate, in some cases you need to lower the video resolution. This window shows a message when that is the case. See the tested configurations in GigE cameras
- For analog cameras, the frame rate is determined by the TV standard of your camera.

### *Color space*

This option is available for digital cameras only.

Color space determines how the information about brightness and color is encoded in the video frames that are sent to EthoVision XT. Note that this may impact the maximal frame rate. The optimal color space differs between cameras. See also Pixel format and color space

- For monochrome cameras: Choose **Y800**. Check the preview image to assess whether the image quality is good.
- For color cameras: If the image is black-and-white instead of color and a Moire pattern appears in the image, try selecting another color space. Normally, the default color space gives a good image quality. If you use a high frame rate or resolution, this reduces the number of animals you can track simultaneously. This also depends on how fast your computer is.

  **TIP** In the Detection Settings, in the Detection Performance pane check the value under **Missed samples** to get an indication of the quality of tracking.

  See also Settings for the color camera and Pixel format and color space

### *Standard*

This is only available for analog cameras. Select the TV standard of your camera. You can find this in the specifications section of your equipment's manual. It will probably be the standard used in your country.

- **PAL**. In Western Europe, China, Indonesia and Australia.
- **NTSC**. In United States, Canada and Japan.

## Image tab

Brightness, Contrast and Saturation are only available for analog cameras. Move the slider, click the arrows or change the number in one of the fields to adjust the settings and check the resulting image.

Click the **Default** button to revert the Image settings to default. The settings on the other tabs will not change. Click the **More Settings** button to open a window with additional settings.

### *Brightness*

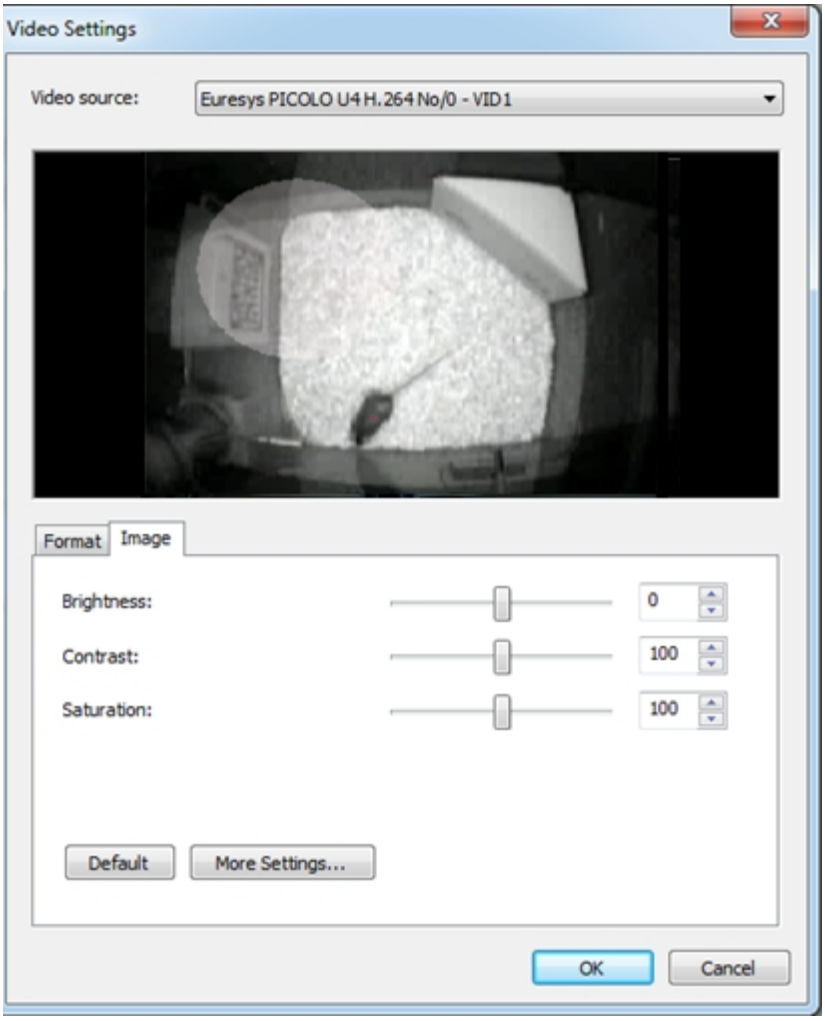
Be aware that a too dark/too bright image may reduce the quality of video tracking. Brightness is not the same as Exposure; the latter is the period of time when the camera sensor is exposed to light.

### *Contrast*

This is the difference in brightness or color that makes objects in an image distinguishable. Use this slider to enhance or reduce the contrast in the image.

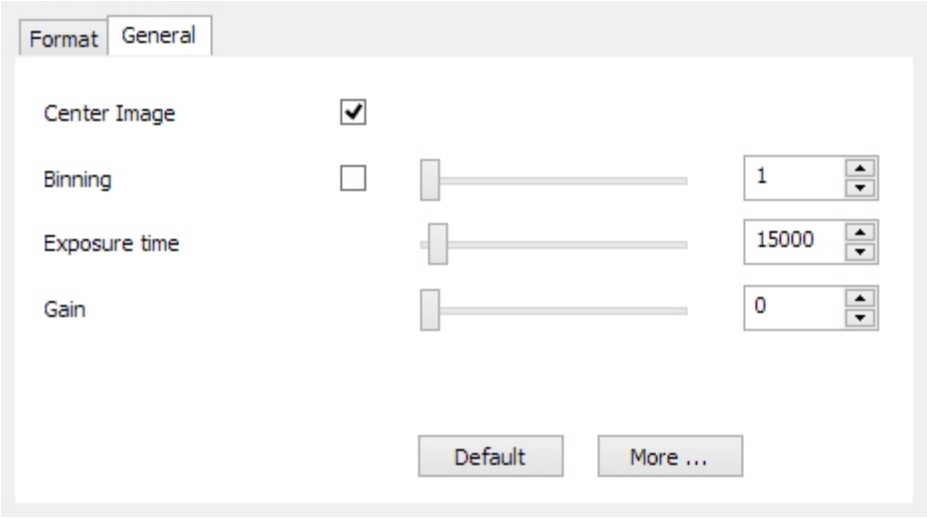
### *Saturation*

This is the strength or purity of the color and represents the amount of gray in proportion to the hue. A saturated color is pure, while an unsaturated color has a large percentage of gray.




## Camera/General tab

Some options are only available for Basler cameras.



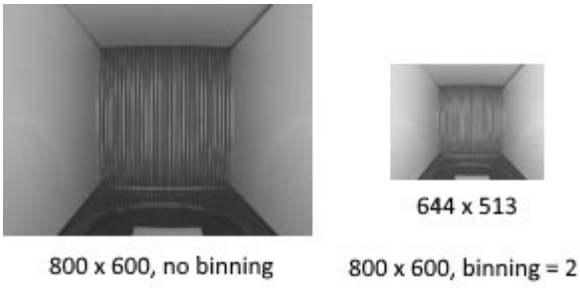

*Center Image*

When selected, **Center Image** ensures that the resulting camera image is centered over the sensor when not capturing the largest possible image. We recommend to keep this option selected. See also Center the camera view

*Binning*

Use Binning when you want to reduce the video size, increase brightness without cropping the video image. When selected, sensor pixels are grouped together to make one image pixel. For example, selecting 2 means that two horizontal and two vertical pixels, thus 4 sensor pixels, make up one image pixel. This increases the sensitivity of the camera but reduces the size of the video image.

In the following figure, setting binning to 2 reduces the video size from 800 x 600 to 644 x 513. Note that the image is just smaller, not cropped.




Note:

- Binning results in a brighter image. Check that detection is still optimal. To reduce brightness, close the aperture ring of the camera lens, or reduce the Exposure time or the Gain.
- When you select or deselect **Binning**, do that for all the cameras in your experiment. Binning should be the same across the cameras.
- If you want to reduce the video size and crop the video image, clear the **Binning** option, keep **Center Image** selected and reduce the resolution.
- You can also crop the video image when your camera lens is multi-focal (that is, the lens has a zoom ring).

### *Exposure time*

Exposure determines the time when the sensor is exposed to light. The higher the value, the brighter the image. Note that, if Exposure is too high, the camera may not be able to send the images at the supposed frame rate. In that case, the window shows that the frame rate is too low. Reduce the Exposure, or reduce the frame rate to have longer time intervals between subsequent video frames.

- For Zebrafish larvae, enter 4000 or 2000, depending on the video resolution chosen; the smaller the resolution, the shorter the exposure time needed.
- For rodents in an open field, a value between 5000 and 20000 is fine.
- For a mosquito filmed under infrared, a value around 10000 is fine.
- All else being equal, faster animals require shorter exposure times.

**TIP** If the image becomes too dark due to the short exposure time, increase the Gain (see below).

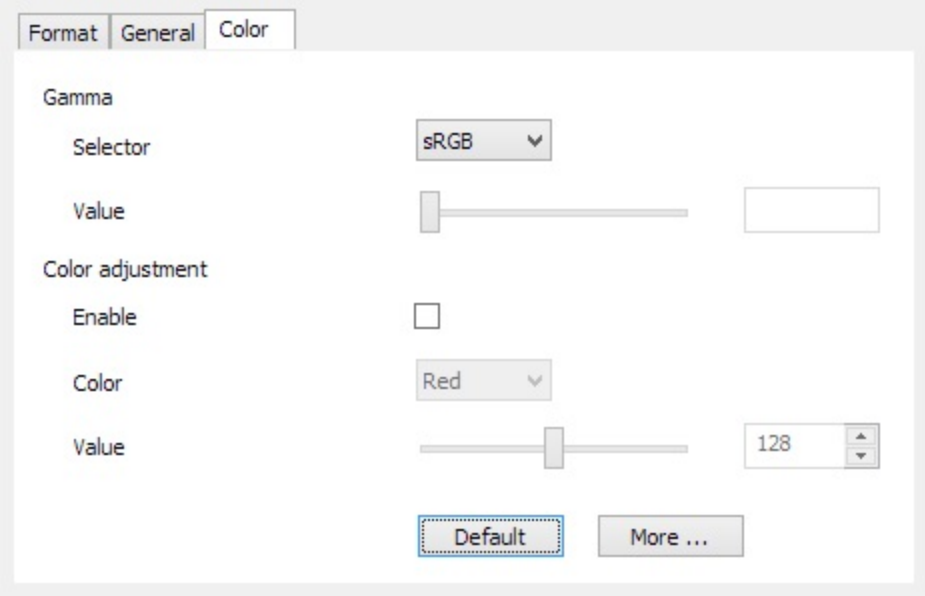
### *Gain*

Adjust the Gain to change the amplification level of the signal coming from the camera sensor. A higher value means more sensitivity. Increase the gain to make the picture brighter, for example in dark environments. Be aware that higher Gain may increase noise in the image.

### *Default/More*

- Click the **Default** button to change the camera settings back to default. The settings on the other tabs will not change.
- Click the **More** button to open the camera software with additional settings. To operate this software, click first the **Cancel** button in the EthoVision XT Video Settings window.

## Color tab




### *Gamma*

Human visual perception is typically non-linear. Adding 1 to a dark or bright color would not result in the same increase in the perceived brightness. Gamma adjusts the brightness of pixel values to account for this non-linearity. Choose **sRGB** to take a colors *as is*. Choose user to fine-tune the color representation and get a color perception that is optimal for you.

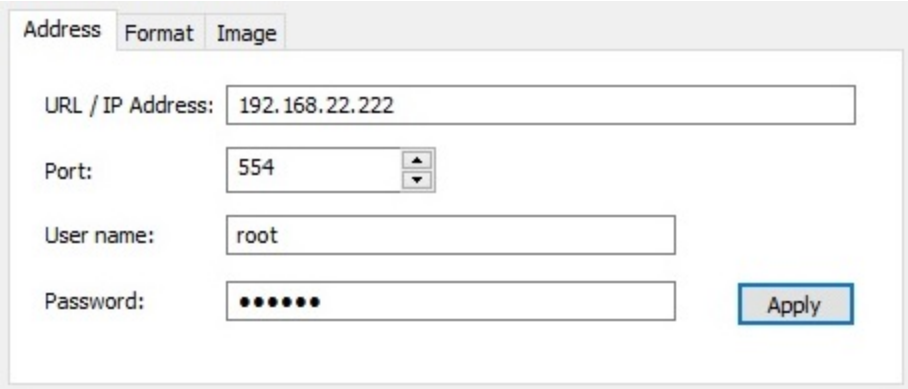
When the gamma correction value is set to 1, the output pixel brightness is not corrected. A value between 0 and 1 results in increased overall brightness; a value greater than 1 results in decreased overall brightness.

### *Color adjustment*

With Color adjustment you can fine tune the color perception by adjusting the settings of colors separately. Select **Enable**, then choose a color from the list and adjust the saturation **Value**. Check the result in the image preview.

## Address tab

This contains the settings for IP cameras.




*IP Address*

Enter the IP address of the IP camera. For details about how to define a IP address for the camera, see the Help of EthoVision XT 17 or earlier, which you can download from my.noldus.com.

*Port*

Enter the port number specified for the IP camera (default for Noldus cameras: 554).

*User name*

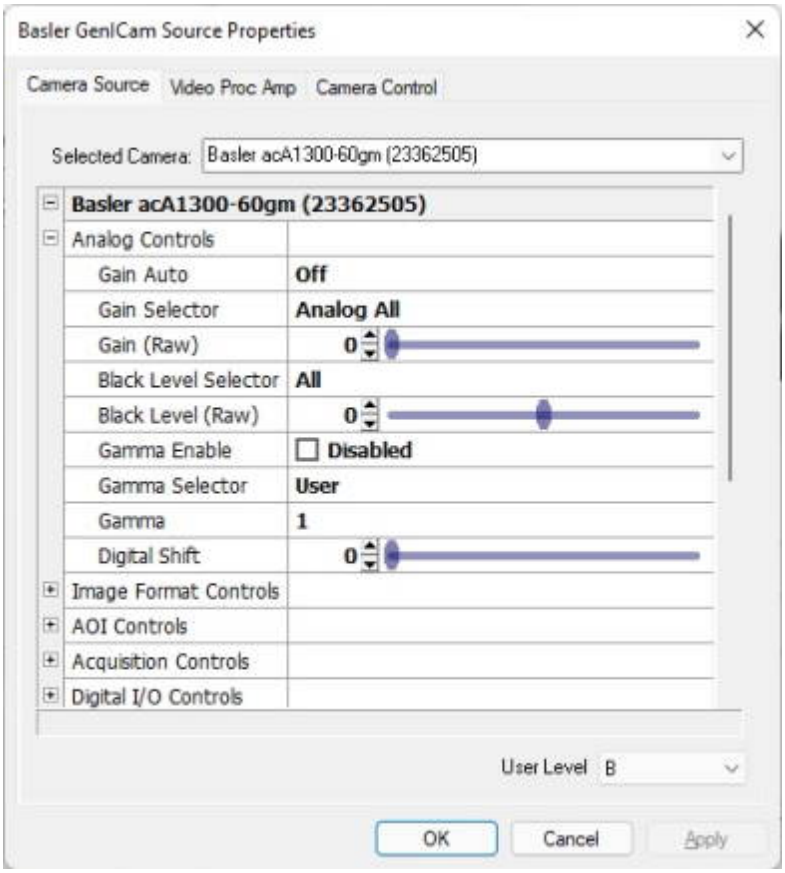
Enter the user name specified for the IP camera. For details on how to define a user, see the Help of EthoVision XT 17 or earlier, or consult the camera's manual.

*Password*

Enter the password specified for the IP camera. For details on how to set a password, see the camera's manual.

## More settings

For some types of cameras the **More** button takes you to additional settings. For example, for GigE cameras:




These settings corresponds to those you can access with the camera software (see Configure the digital camera). The main settings are:

- **Analog Controls** > **Gain**. Increase the value of Gain Raw to increase light intensity. Note that this also increases background noise.
- **AOI Controls** > **Resolution**. See Format tab above for this setting.
- **Acquisition Controls** >
  - **Exposure time**. Increase the value of Exposure time to have a brighter image. However, a long Exposure time may conflict with other settings, in particular frame rate. See Configure the digital camera
  - **Acquisition frame rate**. See Format tab above for this setting.

## Notes

- For digital GigE or USB 3.0 cameras, all settings are available in the camera software. See Configure the digital camera
- When you adjust the camera settings in EthoVision XT, the settings are sent back to the camera once you re-open the experiment.

- The camera settings are saved at the experiment level. So you can create two EthoVision XT experiments with different camera frame rates, exposure levels, etc.

*See also*

- Configure the digital camera
- Check that the camera is set up properly

# Check that the camera is set up properly

## Aim

If a digital camera is not setup properly, you will get dropped video frames which results in either Missed samples or Interpolated samples in EthoVision XT.

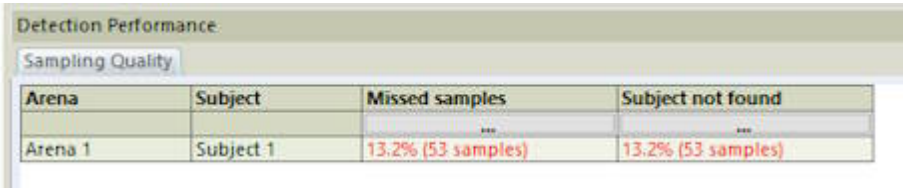
## Prerequisites

- You have followed the steps in Camera Installation to connect and configure the camera
- You have created an experiment in EthoVision XT
- You have made the Arena Settings. This step is necessary to open the Detection Settings.

## Procedure

1. In the EthoVision XT experiment, select **Setup** > **Detection Settings** > **New**.
2. Make sure that the video window shows the live camera image.
3. In the Detection Settings pane, under **Smoothing**, set **Dropped frame correction** to **Off**.
4. Locate the **Detection Performance** pane.
5. Check the value of **Missed samples**. If the percentage of missed samples is low, say less than 1%, then the camera is set up properly.

   If the percentages is higher, there may be an issue with the camera.




6. You have a few options:
   - For GigE cameras: check that the exposure time is not too high for the set frame rate. See Adjust the camera exposure time
   - Reduce the video resolution, the frame, or both.
   - The Ethernet board may not be set up properly. See Settings of the Ethernet board

- See the Troubleshooting: Camera

## Notes

- The value of **Subject not found** is not related to the camera setup. It shows how often the software is not able to find a subject in the arena. If the arena is empty, the value grows to 100%.
- The **Dropped frame correction** set to **Off** enables you to see if EthoVision XT can cope with the actual camera frame rate without missing samples.
- If the camera is set up properly and still the number of Missed samples is high (e.g. > 5%), you can set **Dropped frame correction** to **On**. However, the software interpolates samples to compensate for the missing video frames. See Dropped frames correction

# Number of arenas

## Aim

To specify the number of enclosures (arenas) that you use during a trial.

## Prerequisites

- Your EthoVision XT license includes the Multiple Arenas Module. See Upgrade EthoVision XT
- You want to track animals in two or more arenas simultaneously.

## Procedure

1. Choose **Setup** > **Experiment Settings**.
2. Specify the **Number of Arenas** in the video image.

## Notes

- Examples of multiple arenas: a well plate with 96 wells; two apparatuses for a novel object recognition test placed one next to the other; four open fields; 4 or 16 PhenoTypers.
- It does not matter how many cameras you use. If you use for example four cameras, each pointing to an open field, select 4 as the number of arenas.
- With the Base version of EthoVision XT you can track one subject in one arena. With the Multiple Arenas Module you can track in up to 100 arenas simultaneously.
- **IMPORTANT** If you want to use more than one arena, first check that no samples are missed during acquisition before deciding to work with live tracking. You can display statistics on missing samples during acquisition, in the Analysis Results and Scoring pane, in the Trial Status tab.

# Subjects per arena

## Aim

To specify the number of subjects that are present in each arena during a trial. This setting is valid throughout the experiment, and cannot be changed after you have acquired the first trial.

## Procedure

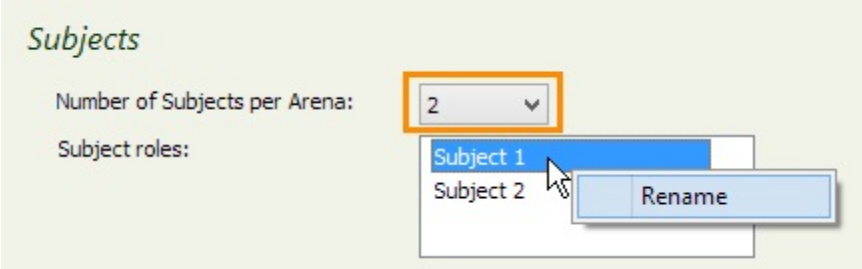
1. Choose **Setup** > **Experiment Settings**.
2. Next to **Number of Subjects per Arena**, select the number of subjects that are present in each arena simultaneously.

   If you intend to use the Deep learning technique for body point detection, you can apply it to one or two subjects per arena. See Body point detection technique
3. Under **Subject roles**, enter the role of the subjects or accept the default ones (Subject 1, Subject 2, etc.).

## Subject roles

To modify the Subject's role name, right-click a name, select **Rename** and type in the new name. Enter generic role names, for instance in a Resident-intruder test with two animals, enter *Resident* and *Intruder*, or *Mutant* and *Wild type*.

Do not enter the ID of the subjects you are going to test. You can specify the ID of your subjects as an independent variable. See Define an independent variable




If you use the Deep learning technique to track two subjects in an arena, the software does not know which role belongs to a specific individual. So it may happen that the resident subject is labeled *Resident* in one trial, and *Intruder* in another trial. To correct this, open the Track Editor and swap the subjects in the trial where the resident subject is labeled as *Intruder*. See Swap subjects

## Notes

- With the Base version of EthoVision XT you can track one subject per arena.
- With the Social Interaction Module, you can track up to 16 subjects per arena. Mind that this is a technical limit; the actual number of subjects that you can track depends on other factors. When using color markers to discriminate between individuals, it may be difficult to discriminate between 16 different color marks depending on the light conditions.
- To change the number of subjects per arena after acquisition, either delete the trials or make a copy of the experiment with a different name (**File** > **Save As**) and delete all trials.
- Contact Noldus if you need to track more than 16 subjects per arena.

# Tracked features

## Aim

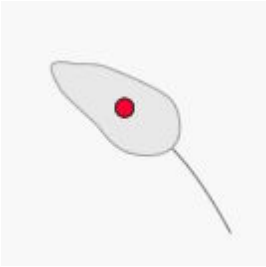
To specify which feature of the subject body is tracked. This choice affects the X,Y coordinates resulting in a Track.

## Procedure

1. Choose **Setup** > **Experiment Settings**.
2. Under **Tracked Features** choose the option that applies to your experiment.

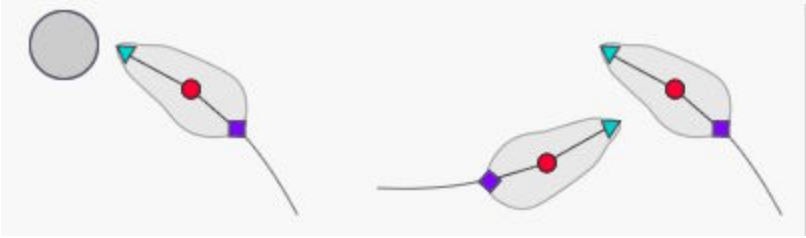
## Center-point detection

To track only the geometric center of the subject detected (or the part of its body being detected). For example, in an open field or Morris water maze test.



## Center-point, nose-point and tail-base detection

Tracking the nose-point is essential in, for example, a Novel Object Recognition test or in a Social interaction test to determine how often and how long your subject touches the object of interest or follows and interacts with the other subject.



Also select this option

- When you track two or more rodents per arena, marked with colors.

- When you want to use the Behavior Recognition function.

## Color marker tracking (treat marker as center point)

If you choose this option, EthoVision XT will only detect the color marker, and track its geometric center. EthoVision XT won't extract the position of body points and any information about the subject's body posture.

Choose this option for social interaction tests where you do not need to track nose and tail base (e.g. color-marked insects or fish).

# Body point detection technique

## Aim

To choose which technique EthoVision XT uses to detect the nose-point and the tail-base points of your subjects.

## Prerequisites

- In the Experiment Settings, under **Tracked Features**, you selected **Center-point, nose-point and tail-base detection**.
- The camera must be placed overhead.

## Procedure

1. Choose **Setup** > **Experiment Settings**.
2. Under **Body Point Detection Technique**, choose the option that applies to your experiment.

## Contour-based

This technique uses foreground/background segmentation based on conventional image processing. The center-point, the nose-point and the tail-base point are derived from the detected body contour. This technique is suitable for any animal with an elongated body and pointed snout, for example rodents, fish, and certain insects.

**NOTE** Contour-based is used by default in older EthoVision XT versions.

## Deep learning

If you choose this option, the nose-point and the tail-base point are detected using a trained neural network. The center-point is detected based on the contour of the detected subject, as described above (except for when there are two subjects in the arena; in that case the center point is also found using the neural network).

**IMPORTANT** Deep learning is currently limited to rodents, and for up to two subjects. If you want to use the Deep learning technique, consider that there are specific restrictions and hardware requirements. See also:

- Deep learning: Basics
- Deep learning: Requirements

## Notes

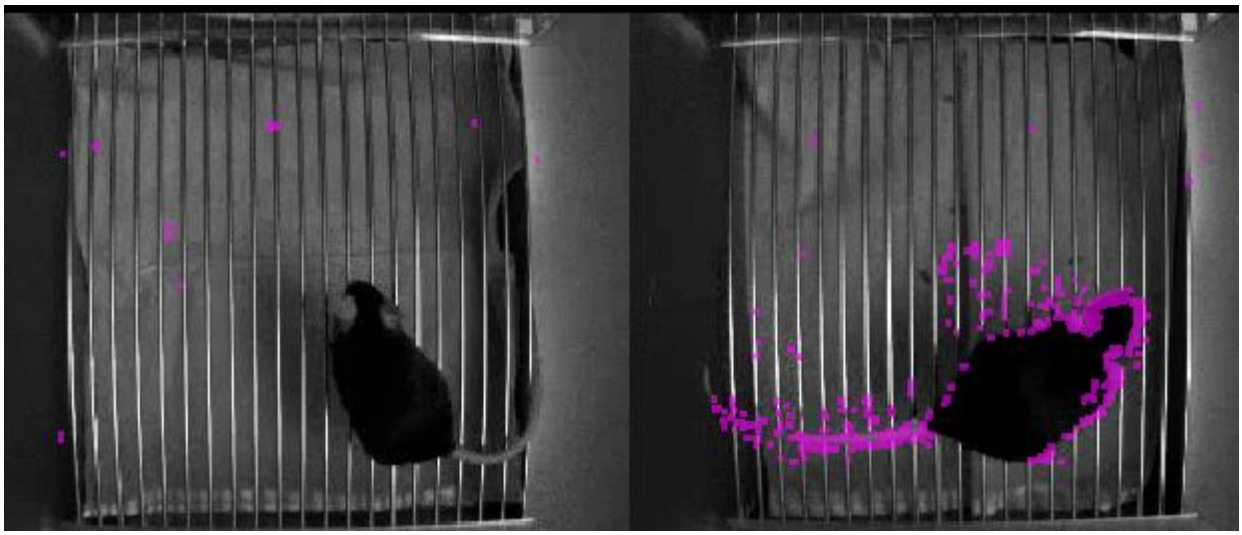
- If you chose **Center-point** as the tracking feature, the center-point is always detected with the Contour-based technique.
- You cannot use the Deep learning technique combined with Behavior Recognition.
- When you select **Deep learning**, you may see this message: *The graphics card driver is outdated or not installed or the graphics card does not support CUDA*. It is possible that the graphics card is too old, or the graphics card driver is not up-to-date. See Deep learning: Requirements

# Activity analysis

## Aim

Activity measures temporal changes in the video image that may have biological meaning. For example, a sudden drop in the levels of Activity in an open field may indicate freezing in a fear conditioning experiment. This can be detected automatically by EthoVision XT, based on the amount of temporal change in the gray scale value of each pixel in the arena.

**EXAMPLE** In the example below: pixels marked in purple indicate temporal change in their gray scale value. Left: No pixel change around the mouse, which is completely immobile. Right: Pixel change when the mouse is active.



Additional examples:

- Detect immobility in a Porsolt swim test. When the subject stays still in the cylinder, Activity is reduced to a minimal level.
- Detect activity of insects flying around a target object. The more insects are flying around the target, the higher the level of Activity.

## To use Activity Analysis

1. Choose **Setup** > **Experiment Settings**, and under **Analysis options** select **Activity analysis**.
2. In the Arena Settings, define the arena.
3. In the Detection Settings, define the level of significant activity. See Activity settings

4. Run your trials. During acquisition, you can monitor the level of activity and, if necessary, use this information to adjust the activity settings to better detect the behavior you are interested in.

   See an example in Fear conditioning: view Activity state

5. To analyze activity, in the Analysis profile choose the variables Activity and Activity state.

## Notes

- Activity analysis is only available when in the Experiment Settings, under **Subjects**, you select **1** Subject per arena.

*See also*

- For how Activity is calculated, see Activity and Activity state as Dependent variables
- Fear conditioning: view Activity state

# Behavior Recognition

## Aim

To active the functions Rat Behavior Recognition and Mouse Behavior Recognition.

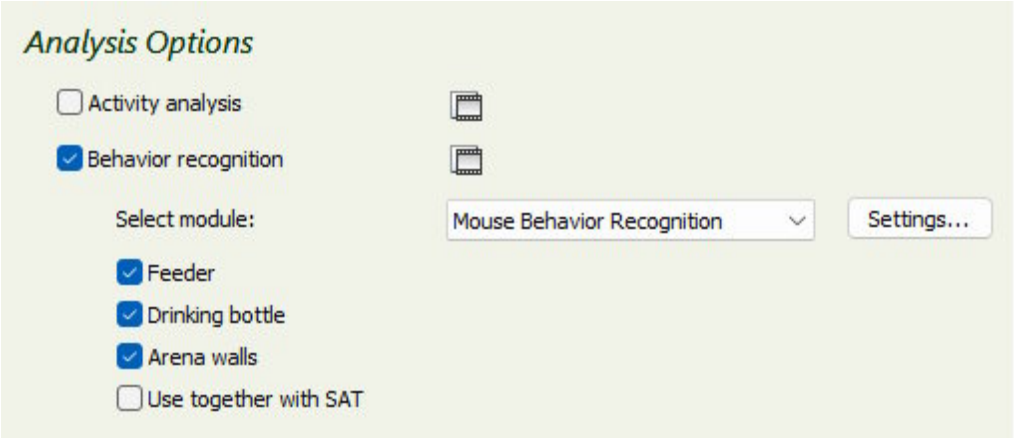
For the list of behaviors recognized with these functions, see Behavior Recognition: Data, performance and accuracy

## Prerequisites

- You have the Rat/Mouse Behavior Recognition module.

## Procedure

1. Choose **Setup** > **Experiment Settings**.
2. Under **Subjects**, choose **1**.
3. Under **Tracked Features**, select **Center-point, nose-point and tail-base detection**.
4. Under **Body Point Detection Technique**, select **Contour-based**.
5. Under **Analysis options**, select **Behavior recognition** and choose the function **Rat Behavior Recognition** or **Mouse Behavior Recognition**.
6. Select the options that apply (see below).




## Options

- **Feeder**. Select this if you want to analyze eating behavior at the feeder. In the Arena Settings, you must draw a zone over the feeder.

- **Drinking bottle**. Select this if you want to detect drinking. In the Arena Settings, you must draw a point over the tip of each drinking bottle spout.
- **Arena walls**. To have EthoVision XT discriminate between rearing to the walls and rearing unsupported. In the Arena Settings, you must draw a zone over the walls.

  See Zones and points for Behavior recognition
- **Settings** (for Mouse Behavior Recognition). When you select **Mouse Behavior Recognition**, click the **Settings** button and select the options that apply to your setup:
  - Select **Bedding present** if the cage includes bedding material ad you want to detect digging behavior.
  - Select **Food available** if there is food for the mice and you want to detect eating behavior.

## Next steps

1. in the Arena Settings, draw the zones important for behavior recognition. See Zones and points for Behavior recognition
2. In the Detection Settings, set a sample rate between 25 and 31 samples per second, and specify the Detection settings for Behavior recognition.
3. Run your trials.

*See also*

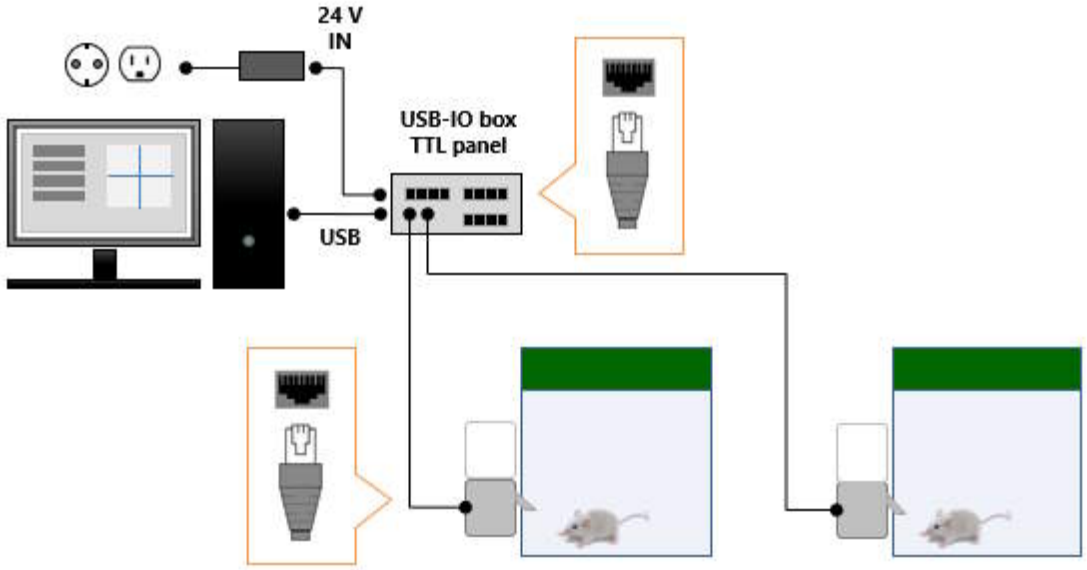
- Behavior Recognition: Requirements
- Choose **Help** > **Video Tutorial** and watch the tutorial about Behavior Recognition.

# Trial control hardware

## Aim

To set the EthoVision XT experiment to control hardware devices.

**EXAMPLE** Enable control of two Pellet dispensers.




## Prerequisites

You must have one of the following interface devices:

- The USB-IO box.
- The Mini USB-IO box.
- The DanioVision DVOC-00xx observation chamber.

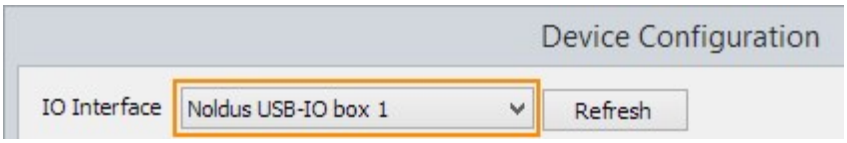
## To enable trial control hardware

1. Connect the interface device (Noldus USB-IO box, Noldus Mini USB-IO box or DanioVision Observation Chamber) to the EthoVision XT computer.

   For details and connection schemes, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual. See Manuals

2. Select **Trial Control Hardware** and click **Settings**.

3. Depending on which device is connected to the EthoVision XT computer, select either **Noldus USB-IO box** or **Noldus Mini USB-IO box**, or the DanioVision version you have. Next, click **OK**.
4. If you have connected multiple USB-IO boxes, in the window that appears, select the interface device from the **IO interface** list. Otherwise, skip this step.




5. For each device connected, under **Device Type** select the port that device is connected to, and under **Device ID** specify the device name or accept the default name. See some examples below.
6. If you use multiple USB-IO boxes, repeat steps 4-5 for each USB-IO box. Specify the hardware connected to that box.

## Examples

### *One Pellet dispenser*

Locate the TTL port connected to the Pellet dispenser, and in that row under **Device type** select **Pellet Dispenser**.

| Ports | Device type | Device ID |
|---|---|---|
| TTL Port 1 | Pellet Dispenser (PTPD-0010) | Pellet Dispenser (PTPD-0010) 1 |
| TTL Port 2 | <No device connected> | <No device type selected> |
| TTL Port 3 | <No device connected> | <No device type selected> |
| TTL Port 4 | <No device connected> | <No device type selected> |

### *One Pellet dispenser and one Lickometer*

Under **Device type**, select **Pellet Dispenser** next to the TTL port connected to the Pellet dispenser. For Lickometer, select **Lickometer** next to the SDI port that is connected to the Lickometer.

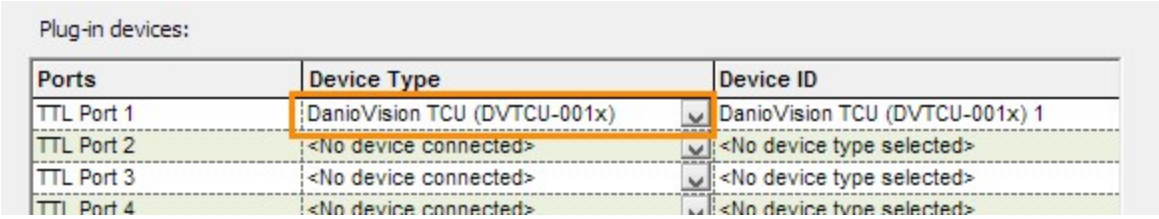
| Ports | Device type | Device ID |
|---|---|---|
| TTL Port 1 | Pellet Dispenser (PTPD-0010) | Pellet Dispenser (PTPD-0010) 1 |
| TTL Port 2 | <No device connected> | <No device type selected> |
| TTL Port 3 | <No device connected> | <No device type selected> |
| TTL Port 4 | <No device connected> | <No device type selected> |
| TTL Port 5 | <No device connected> | <No device type selected> |
| TTL Port 6 | <No device connected> | <No device type selected> |
| TTL Port 7 | <No device connected> | <No device type selected> |
| TTL Port 8 | <No device connected> | <No device type selected> |
| TTL Port 9 | <No device connected> | <No device type selected> |
| TTL Port 10 | <No device connected> | <No device type selected> |
| TTL Port 11 | <No device connected> | <No device type selected> |
| TTL Port 12 | <No device connected> | <No device type selected> |
| SDI Port 1 | Lickometer (PTLM-0010) | Lickometer (PTLM-0010) 1 |
| SDI Port 2 | <No device connected> | <No device type selected> |
| SDI Port 3 | <No device connected> | <No device type selected> |
| SDI Port 4 | <No device connected> | <No device type selected> |

*PhenoTyper*

The connections depend on whether you use PhenoTyper 1 or PhenoTyper 2. For information, see the PhenoTyper 2 - EthoVision XT 18 - Reference Manual. See Manuals

*DanioVision*

The White Light stimulus and the Tapping stimulus (when present) are already assigned. For the Temperature Control Unit, or custom hardware, under **Device type** select the device next to the TTL port you use.




## Notes

- **IMPORTANT** If you use multiple Noldus USB-IO boxes, their identity number must be different. You can set this number using the jumpers inside the USB-IO box. For details, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual. We recommend to test your own configuration of USB-IO boxes and devices before carrying out the actual trials.
- You cannot combine the regular Noldus USB-IO Box with the Noldus Mini USB-IO Box.

- To see what type of USB-IO box you have, check the label at the bottom side of the box: **PTIO-002x** for the Noldus USB-IO Box or **PTIO-003x** for the Noldus Mini USB-IO Box.

*See also*

- For details on how to connect hardware devices, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.
- For DanioVision, the DanioVision DVOC-0041 - EthoVision XT 18 - Reference Manual.
- For PhenoTyper, the PhenoTyper - Reference Manual for general information, and the PhenoTyper - EthoVision XT 18 - Service Manual for details about the Noldus devices that work with PhenoTyper.
- See Manuals

You can also find the manuals on the Noldus website.

my.noldus.com

Choose **Downloads**, then **EthoVision XT** and **Documentation**.

# Units

Specify the units for distance, time and rotation:. Changing the units does not affect the raw data, only their presentation. You can change the displayed units at any time.

## Unit of distance

EthoVision measures the distance between two points in pixels. To convert these to real values you must calibrate your arena. Here, you can choose what unit of distance you prefer: millimeters (mm), centimeters (cm), meters (m) or inches (inch).

## Unit of time:

Milliseconds (ms), seconds (s), minutes (min) or hours (hr).

## Unit of rotation:

Degrees (°), radians (rad), gradians (grad) or rotations (rot).

## Notes

- You can change the units after you have acquired tracks. This only affects the presentation; the data are always correct.
- Changing the units only affects the present experiment. To change the default units for future experiments, choose **File** > **Preferences**. Click **Default units** and select the units you want.
- Settings of units are saved on your computer. If you open another EthoVision XT experiment from a colleague, numbers will be displayed according to your settings, regardless of the settings your colleague used. For example, your colleague tracked an animal moving one inch; on your computer it is shown as 2.54 cm.

# More about Experiment Settings

## Deep-learning for body point detection

- Deep learning: Basics
- Deep learning: Requirements
- More information on graphics cards (GPUs)
- Install a graphics card for Deep learning

## Behavior Recognition

- Behavior Recognition: Requirements
- Behavior Recognition: Data, performance and accuracy

## Live Mouse Tracker

- Live Mouse Tracker: Workflow
- Live Mouse Tracker: Create an experiment

# Deep learning: Basics

## Background information

**NOTE** The following information only applies to tracking of rodents when using the Deep learning function. See also Adjust the settings for nose-tail base detection (Deep learning)

*Deep learning* is a type of machine learning. While machine learning is a general category that encompasses all sorts of mathematical tools that help a computer learn by experience, Deep learning refers to the use of deep neural networks, where "deep" indicates that the networks are made of multiple, hidden layers of *neurons* or *decision nodes*.

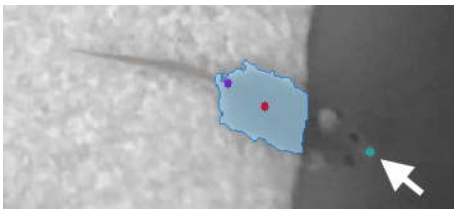
In a deep neural network, intermediate layers are placed between the input layer, which receives the data, for example the RGB values of the pixels that make up a bitmap picture, and the output layer, which represents the categories of classification; for example, when classifying a picture, "the picture is of a cat" or "the picture is not of a cat".

## Deep learning in EthoVision XT

A deep neural network can find structures in unstructured data, like for example pictures and video images. They can recognize recurring patterns, such as the eyes of humans in a number of portraits, and relationships between those patterns. Because of the layered structure of decision nodes, deep neural networks can learn to represent data at various levels, from low levels such as edges, colors, curves, etc. to higher levels such as semicircles (a combination of a curve and a straight edge), squares (a combination of straight edges), up to even higher, more abstract levels (concepts), such as "handwriting", "dark object", "a tail" etc.

EthoVision XT uses a trained network, that is, the network has learned to extract features from a number of video images of rodents of various colors and in various backgrounds, where the nose and the tail-base were previously annotated. During tracking, the network analyzes a portion of the image that includes the detected subject. It creates a map of probability of occurrence for both the nose- and the tail-base points, and finally makes an estimate of the position of the nose point and the tail-base point, based on the highest probability.

With deep learning, the detection of the body points is less dependent on the detected contour of the subject. You can see this effect in cases with low contrast between the subject and the background, like in the following picture. Here, a dark mouse is only partly detected when it rears with the forepaws placed on the cage wall. However, the neural network can find the nose point.



## Convolutional networks

EthoVision XT uses a deep Convolutional Neural Network (CNN) to find the nose- and the tail-base points in each sampled video image. CNNs are particularly suitable to classify images based on spatial relationships. CNNs resemble the structures of the cells in the visual cortex of our brain. The visual cortex has small regions of cells that are sensitive to specific regions of the visual field. Hubel and Wiesel (*Journal of Physiology* **165**: 559-568,1963) showed that some neurons fired only in the presence of edges of a certain orientation. Some neurons fired when exposed to vertical edges and some when shown horizontal or diagonal edges. The basis of convolutional networks is the idea that specialized components in the network have specific tasks, that is, to look for specific characteristics in the image.

## Features detected with Deep learning

When you track the subjects with Deep learning, the main features of the subjects, for example the body center point, are calculated in different ways, either predicted by the neural network or the calculated based on the contour of the detected subject. When tracking multiple subjects with Deep learning, some of those features are not calculated at all. See the tables below for reference.

### *One subject per arena*

| Feature calculated | Technique and other features used |
|---|---|
| Center-point | Contour |
| Nose-point | Deep learning |
| Tail-base point | Deep learning |
| Body elongation | Contour |
| Body area | Contour |
| Body changed area | Contour |
| Head direction line | Contour, nose-point |

Where:

- Body elongation is used to calculate the dependent variable Body elongation and Body elongation state.
- Body area and Body changed area are used to calculate Mobility and Mobility state.
- The Head direction line is used to calculate Head direction and Head directed to zone.

*Two subjects per arena*

| Feature calculated | Technique and other features used |
|---|---|
| Center-point | Deep learning |
| Nose-point | Deep learning |
| Tail-base point | Deep learning |
| Body elongation | Not calculated |
| Body area | Not calculated |
| Body changed area | Not calculated |
| Head direction line | Not calculated |

*See also*

- Deep learning: Requirements

# Deep learning: Requirements

## Main topics

- Graphics card (GPU)
- Video source
- Sample rate
- Subject species, color and size
- Number of subjects and arenas
- Video image
- Video length
- Individual marking (for two-subject tracking)
- Test apparatus and background
- Recording protocol
- Behavior Recognition
- Test results

## Graphics card (GPU)

Neural networks make calculations over huge data matrices, and therefore require substantial computation power. In order for the Deep learning tracking technique to work in EthoVision XT, you need a Graphics Processing Unit (GPU, or graphics card) that is able to sustain those computations.

Furthermore, Deep learning makes use of TensorRT software development kit (version 8.6.1.6) which is built on the cuDNN deep neural network library, which in its turn relies on the CUDA computing platform. For this reason, the GPU driver must support CUDA runtime version 12.2.

If you intend to purchase a GPU for Deep learning, click here below for more information.

- More information on graphics cards (GPUs)
- Install a graphics card for Deep learning

## Video source

Choose:

- When tracking one subject per arena: **Live tracking** limited to up to four arenas, or **From video file**.
- When tracking two subjects per arena: **From video file**.

  Tracking two subjects live using Deep learning may lead to a high number of missing samples, depending on the power of GPU. Always test your setup first. If you note a significant number of missing samples (e.g. > 5%), do not track live. Instead, record video first, then acquire the trial later using Deep learning.

See the recommendations in Resolution, frame rate, and maximum trial duration

## Sample rate

Accuracy of individual discrimination may go down when reducing the sample rate, for example from 25 to 12.5 samples/s. We strongly recommend that you track at the maximum sample rate available, that is, the frame rate of your camera. Choose the sample rate in the Detection Settings. See Sample rate

## Subject species, color and size

- The neural network has been trained with images of rats and mice of uniform color.
- For hooded rats, like Lister and Long-Evans rats:
  - When tracking one subject per arena: Select **Hooded rats** in the Detection settings. This loads a neural network model specific for those animals.
  - When tracking two subjects per arena: the neural network for two interacting subjects has not been trained for the fur patterns of hooded rats. If you want to use hooded rats, test pairs of animals before the actual experiments, to check that the software produces acceptable results.
- The apparent length of the subjects should be at least 10% of the size of arena. We recommend that the apparent length of the subject's body is at least 120 pixels for rats and 50 pixels for mice (nose to tail-base).

See also Adjust the settings for nose-tail base detection (Deep learning)

## Number of subjects and arenas

You can select Deep learning as body point detection technique in experiments with:

- One subject per arena, in a maximum of four arenas.

- Two subjects per arena, in a maximum of four arenas.

**IMPORTANT** Always draw an arena in the Arena Settings for optimal results.

## Video image

- When working with one arena at a time, choose a resolution of 640 x 480 or higher.
- When working with two to four arenas, choose a resolution of 1280x960 or 1280x1024, or similar.
- In any case, use video of resolution equal to or higher than PAL/NTSC (PAL: 704 x 576; NTSC/EIA: 640 x 480). A higher resolution is not necessarily better, also considering that it makes tracking slower when tracking offline, or can cause missing samples when tracking live. First try a low resolution, and switch to a higher resolution if the results are not good. See also subject size below.
- For live tracking, not all video resolutions and frame rates are compatible with Deep learning. See Test results and then click on the camera type for configurations tested with Live tracking combined with Deep learning: GigE cameras, USB 3.0 cameras, and Analog cameras.
- EthoVision XT converts video to grayscale before feeding it to the neural network. Therefore, both monochrome and color video work fine.

## Video length

- One subject per arena: no restrictions. Maximal trial duration tested 72 hours. See Resolution, frame rate, and maximum trial duration
- Two subjects per arena: we recommend to perform trials of at least five minutes. Maximal trial duration tested 1 hour.

  During the trials, the two subjects should be separated for at least three minutes. If the subjects are in contact for most of the trial duration, individual recognition may fail. Also consider that, with two-subject tracking:

  - EthoVision XT saves additional files during acquisition, which increase the storage space needed on your PC. A 1-hour video produces 10 MB of additional files.
  - With long trials, the marker could change or droppings would likely cumulate in the arena. Both factors could potentially interfere with marker recognition.

## Individual marking (for two-subject tracking)

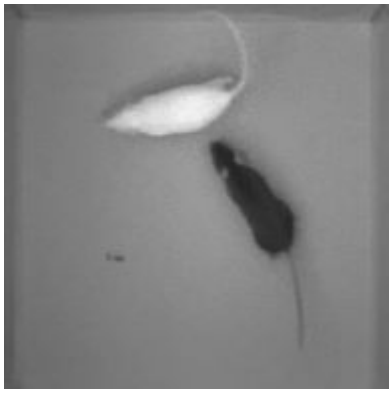
This section refers to tracking two subjects per arena with Deep learning (up to four arenas). If you track one subject per arena, you do not need to mark the animals. For successful application of Deep learning-based discrimination between two individuals, the interacting animals must look different, or be marked.

**IMPORTANT** EthoVision XT does not detect differences in the subject's body size. Also, toe clipping, ear punching or tagging won't work. Differences in fur color and markings (also on the tail base) are essential for successful individual discrimination with EthoVision XT.

For information about non-invasive marking techniques, see Klabukov *et al*. 2023. *Animals* **13**(22): 3452. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10668729/

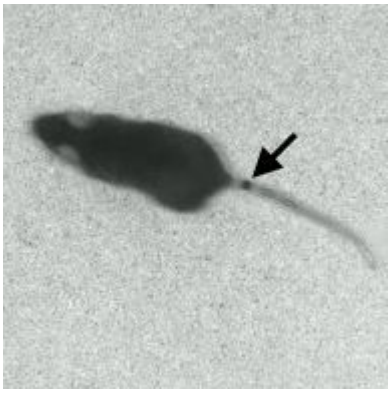
### *Different fur color*

If the two subjects are of different fur color, for example one white and the other brown/black, you do not have to mark them.



### *Tail marks*

Tail marks only work if they are close enough to the abdomen of the subject. Only mark one subject, not the other. Make one ring leaving some space between that and the abdomen:



In the following figure the tail mark is too far from the abdomen. Individual discrimination won't work.

- Advantages: The procedure is painless and easy.
- Disadvantages: It may require weekly marking, which could cause stress. Furthermore, droppings left in the arena could be detected as tail marks and affect individual discrimination. This could especially occur in long trials.

### *Shaving marks*

You can use a hair trimmer to shave a patch of fur of one of the subjects. The patch must be located along the spine of the subject. If you decide to shave both subjects, they must exhibit different patterns. Such marks generally last 1-4 weeks, depending on the stage of the hair cycle.

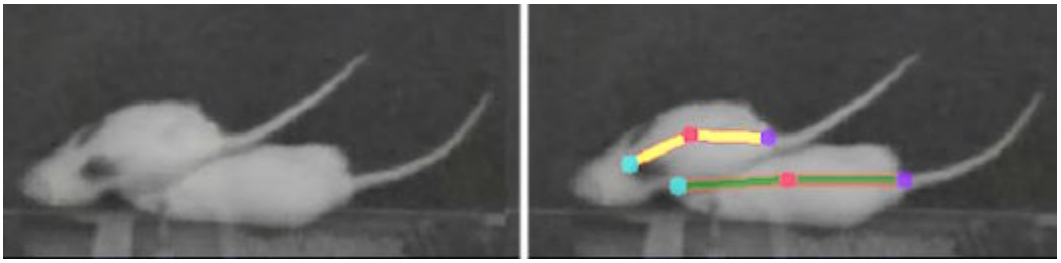
- Advantages: The procedure is painless; grooming and manipulation do not remove shaving marks; it may be performed on rodents of all colors.
- Disadvantages: The marking is temporary due to hair regrowth; you need to monitor the animals regularly to assess the condition of the mark. In long-term experiments, you need to renew the mark as hair regrows. Also the marking may look too irregular, reducing identification rate.

*Fur staining*

White rodents can be marked with non-toxic dye or felt tip pens. When using infrared illumination, make sure that the marks are visible under infrared light. Mark only one of the subjects.


- Advantages: The procedure is easy and painless, and may be used with rodents of all ages.
- Disadvantages: Dyes may fade over time or due to grooming; daily monitoring is needed to assess the condition of the mark; it may be used predominantly on white-furred animals; there is potential adverse response to solvents and odor.

Do not make V-shape markings or separate markings that converge on the back of the subjects, since they could interfere with the detection of the nose point, like in the following example:



*Bleaching*

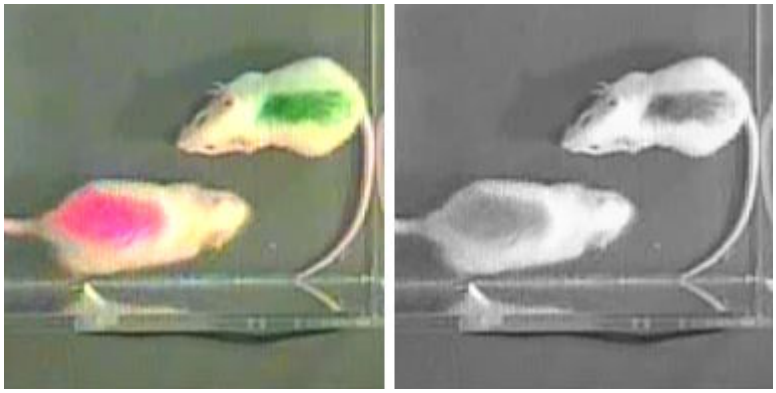
Here the same remarks for shavings apply.

- Advantages: The procedure is painless and bleach marks are not removed during grooming.
- Disadvantages: It may be used on dark-furred rodents only. Furthermore, the software won't work optimally if the part bleached is irregular and so large that it breaks the subject's contour, like in the following example:

**IMPORTANT** Remove bleach solutions to avoid skin damage.

*Color marking*

Color marks could only work when they exhibit different light intensity in grayscale. For example, light pink and blue. In the example below, different colors may look too similar in grayscale.

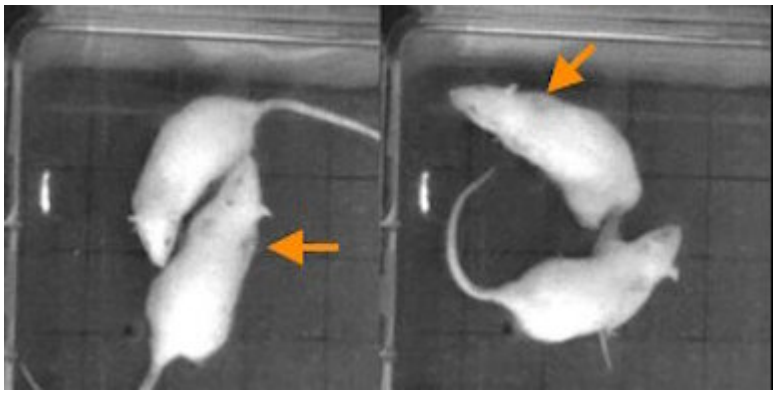


A safer option is to mark one subject, not the other (see above). For more details about color marking, see Tips for color tracking.

*Marker location*

Make sure that the marks are visible in most cases, also when the animal is sitting curled up. In the example below (right), the mark is not visible.

In the following example, the two marks on the rat's shoulders are just too small (left). Combined with the effect of direct lighting (to be avoided in all cases), the marks disappear a few frames later (right). Individual recognition won't work here.

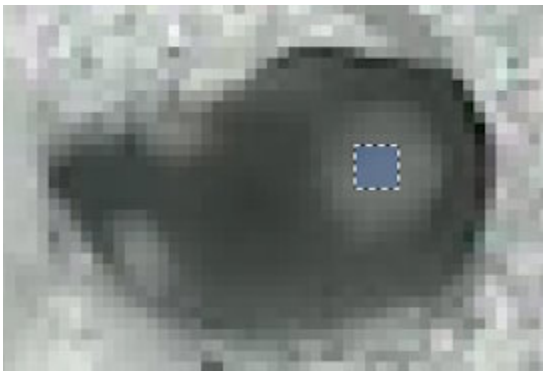


*Size of marks*

The mark must be at least 4 x 4 pixels, after the arena has been rescaled to 256 x 256. This applies to tail marks and marks on the back of the animal.

If in doubt, do the following:

1. Open the video file at zoom level 1:1 (Original size).
2. Make a screenshot of the video window.
3. In a paint program, cut the image to include approximately only the arena.
4. Rescale the arena to 256 x 256 pixels. For rectangular arenas, rescale in such a way that the longest side is 256 pixels. Maintain the original aspect ratio.
5. After rescaling, the marker should be at least 4 x 4 pixels, as shown in the following image.



*Hooded rats*

The neural network for two-subject tracking and identification has not been trained with the color patterns of hooded rats, so we cannot guarantee that it works when tracking two hooded rats. Do some tests before the actual experiments, to verify that it provides acceptable results.

## Test apparatus and background

*Test apparatus*

EthoVision XT's neural networks have been trained with videos of:

- One subject per arena, subjects of uniform color: Open field (regular or with round objects in it), PhenoTyper (with or without bedding material), Elevated plus maze, Three-chamber social approach cage, Barnes maze, Fear conditioning cage with floor grid, Y-maze (with no objects), and a

maze with multiple chambers and openings. See below the note about objects.

- One subject per arena, hooded rats: Open field (regular or with round, rectangular or triangular objects in it), PhenoTyper (with or without bedding material), and Elevated plus maze.
- Two subjects per arena: Open field (with no objects), PhenoTyper (with or without bedding material), and home cage.

### *Size of the arena*

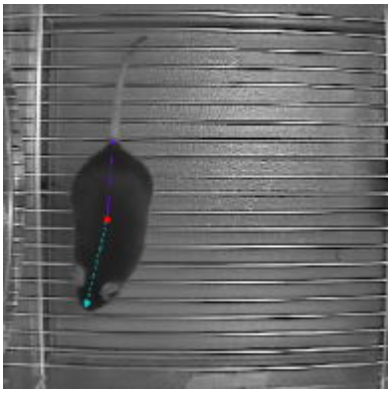
- One subject per arena: No particular requirements, but see Video image above.
- Two subjects per arena: Small containers reduce the probability that the two animals are separated for sufficient time. This condition is necessary for success with Deep learning-based individual recognition.

### *Background*

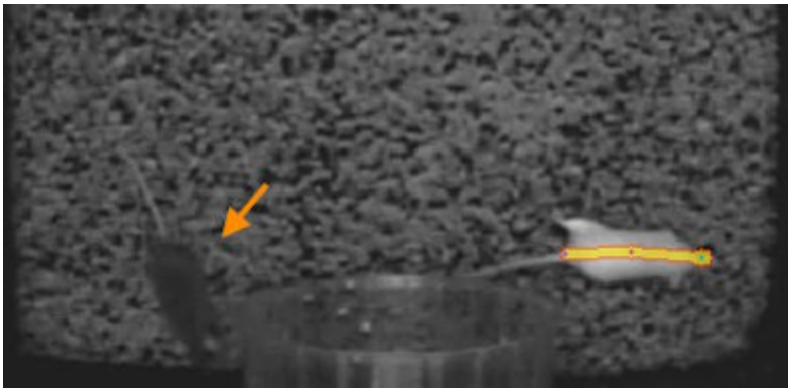
The figure below shows an example of a good background where the rat's apparent size is about 150 pixels.

- If the animal is small relative to the arena, and there is no way to zoom in the camera image, try to improve the contrast with the background, for example by providing more infrared light, in order to compensate for the lower level of spatial detail.
- *Low contrast*. In the following example, the mouse is large enough (about 80 pixels; see above) but the contrast with the background is too low. To solve this, increase lighting, or open up the lens' aperture, or increase the camera gain. See Adjust camera settings in EthoVision XT

- *Floor grids* are compatible with the Deep learning detection technique. If necessary, reduce the amount of light to minimize the reflections and shadows caused by the metal bars.



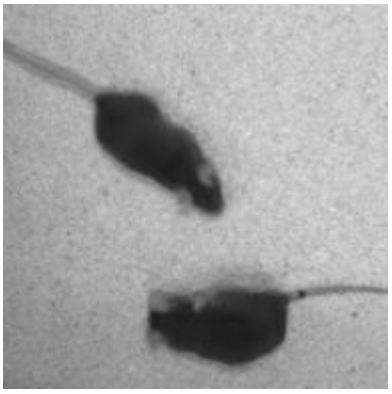
- *Bedding material* can also be used with Deep learning-based tracking. However, there should be enough contrast with the animals. In this example with two mice in a PhenoTyper, the dark individual often went undetected.

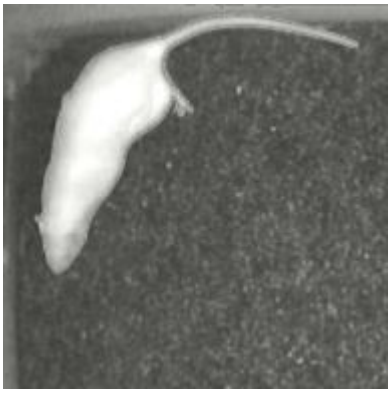


Try increase lighting or, if possible, switch to another type of bedding.

Too much bedding/nest material can cause occlusions when the animal digs in it. In that case the subject detection and discrimination may not work properly.

Here below are examples of setups that worked.



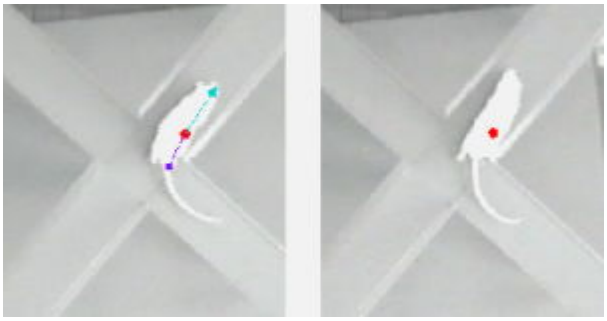


- *Objects*. For one-subject tracking, the apparatus can contain objects, like in the Novel Object Recognition test, or in the Sociability test. Whenever possible, use objects of color different from the subject's color.

- *Blind corners*. When working with multiple arenas simultaneously, check that there are no blind corners, which may reduce detection rate.

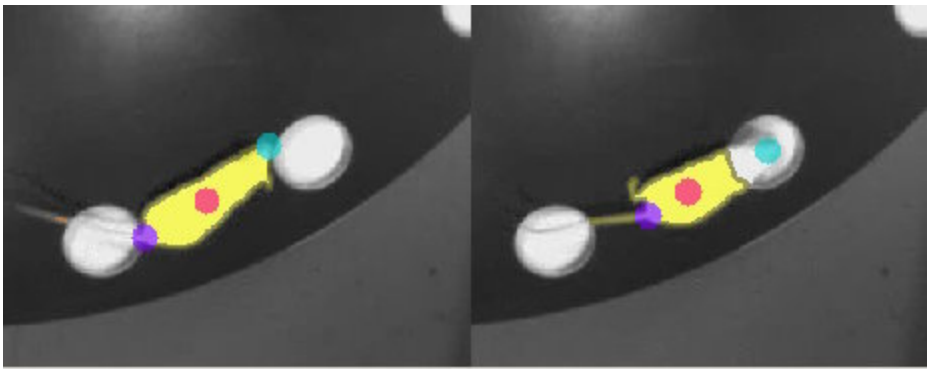
- *Corridors and walls*. For elevated plus mazes, radial mazes and other apparatuses with corridors, make sure that the walls are not of the same color as the subject.

  In the following example, an excess of light from one side of the test room makes the top of the walls of this plus maze look white. A white mouse is still detected but when it touches the walls the nose-point and/or the tail-base point are no longer detected. Dim the lights and adjust their orientation to reduce the reflections.



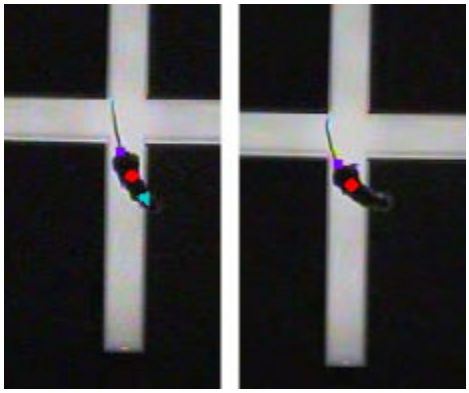
  When you define the Cutout size in the Detection Settings, try not to include walls and objects in the cutout box, especially if those objects are the same color as the animal. See Adjust the settings for nose-tail base detection (Deep learning)

- *Hole boards*. Deep learning-based tracking also performs well in situations of low contrast between the subject and the holes, like in the following example.



**TIP** Define the target hole as a hidden zone. See Shelters and other hidden zones

- *Apparatuses with backlight*
  - One subject per arena: backlight is compatible but may not be ideal. In the following example, the mouse explores a plus maze with an infrared-lit background (left). When the mouse dips the head below the level of the open arms (right), the nose is not found. To solve this, place dim lights on the floor.



  - Two subjects per arena: backlight is not compatible.
- *Tethered animals*
  - One subject per arena: When running experiments with tethered animals, the contour of the subjects seen by EthoVision XT is often disrupted by the fiber. The Deep learning technique may not be able to resolve the position of the nose. We did not test Deep learning with tethered animals. Therefore, run a few tests with sample videos to make sure that detection of the nose point is accurate enough. To improve detection of tethered subjects, use the Dilation and Erosion filter options. See an example in Advanced detection settings: Subject contour

- Two subjects per arena: this setup has not been tested thoroughly, therefore applying Deep learning is not recommended.

## Recording protocol

- One subject per arena: No particular limitations.
- Two subjects per arena: preferably, tracking should start when both animals are in the arena. The software also works when you release one animal first, then the other. However, for best results, ensure that the second animal is released within up to 30 seconds after the first.
- With the Trial Control Settings, you can ensure that the data acquisition starts when both subjects are in the arena. See Start the trial in the Social interaction test

## Behavior Recognition

Unfortunately, it is not yet possible to combine Deep learning for body point detection and Behavior Recognition for behavior classification in the same experiment. When you select **Deep learning** under **Body Point Detection Technique** in the Experiment Settings, the Behavior Recognition option is grayed out.

## Test results

We tested Deep learning with various combinations of PCs, graphics cards and cameras. We recommend to use a high-end workstation when working with Deep learning - based tracking. Click the link that applies based on which camera type you have:

- GigE cameras
- USB 3.0 cameras
- Analog cameras

In all the tests we used live tracking + save video, with one arena per camera. All tests were based on an open-field experiment with a black mouse and a clearly contrasting gray background.

*See also*

- Cameras supported by EthoVision XT
- System requirements > Hardware

# More information on graphics cards (GPUs)

## Aim

- To provide you with information about graphics cards and their software that is needed to perform video tracking with the Deep learning technique.
- This topic does not apply if you track your subject with Contour-based method.

**NOTE** Technically, the GPU indicates the main processor in the graphics card. The graphics card includes other components such as the video memory (VRAM), the printed circuit board (PCB), a power management unit, the PCIe connectors, the display connectors etc. In this topic we assume that GPU coincides with the physical board that you install on your PC.

## GPU microarchitecture

Graphics cards are categorized based on their microarchitecture. Such categories are also known as *generations*.

To know which microarchitecture category your GPU belongs to, browse to

https://en.wikipedia.org/wiki/CUDA

- Under the table **Compute Capability, GPU semiconductors and Nvidia GPU board products** locate the name of the GPU in the column **GeForce**. The name under **Micro-architecture** tells which generation the GPU belongs to.
- In the table **Compute Capability (CUDA SDK support vs. Microarchitecture)** you can see whether that GPU generation works with the CUDA SDK version used with EthoVision XT - currently 12.2.

## GPU speed

When comparing two GPUs for deep learning applications, one of the best indicators is the memory bandwidth, rather than single properties like the GPU memory speed. A GPU's memory bandwidth determines how fast it can move data from/to the memory (VRAM) to the computation cores.

Theoretically, the memory bandwidth depends on:

- The memory clock speed (in Hz).

- The width of the data bus between the card memory and the graphics processor, in bits (also known as memory interface). This is the physical count of bits that may fit along the bus every clock cycle.
- The memory type (the so-called memory clock type multiplier).

Memory bandwidth is measured in GB/s. For example, a graphics card with bandwidth 1000 GB/s is expected to perform two times faster than one with 500 memory bandwidth. Again, this is just a rough indication of the difference in performance, as the real performance may depend on several other factors, including the neural network architecture. If there is no reading from memory at any particular clock cycle, that cycle's worth of bandwidth goes unused and cannot be stored and used later when there's more memory pressure. Because GPS tend to acquire data in spurts, the usable bandwidth is generally lower than the numerically available bandwidth.

Another useful measure is the number of Floating Point Operations per Second (FLOPS). For example:

- For the NVIDIA Quadro P2200: 3.8 TFLOPS (teraFLOPS equal to $10^{12}$ FLOPS).
- For the NVIDIA T1000: 2.5 TFLOPS
- For the NVIDIA GeForce RTX 4060: 15.1 TFLOPS.

For the specifications of graphics cards, see the following web site:

https://www.techpowerup.com/gpu-specs/

## GPUs tested and supported

The following NVIDIA graphics cards have been successfully tested with Deep learning-based tracking.

**TIP** Install the most recent driver version that is available on the NVIDIA web site for that GPU.

### *Desktop*

| GPU | Architecture | Memory | Bandwidth | Driver v. |
|---|---|---|---|---|
| T1000 | Turing | 4 GB GDDR6 | 160 GB/s | 553.09 |
| GeForce RTX 4060 | Lovelace | 8 GB GDDR6 | 272 GB/s | 552.22 |

**NOTE** The GeForce RTX 4060 is a dual-slot GPU, that is, it occupies two PCIe slots. Moreover, there are several versions of this board, with two or three fans. Make sure it can fit in your PC. Both the T1000 and the MSI GeForce 4060 Ventus 2X (dual fan) fit in the Dell Precision 3680 desktop computer.

*Laptop*

| GPU | Architecture | Memory | Bandwidth | Driver v. |
|---|---|---|---|---|
| RTX 1000 Mobile | Lovelace | 6GB GDDR6 | 192 GB/s | 553.09 |

**NOTE** If you install new GPU on a computer that you have used for some time to acquire the data with Deep learning, you must update the neural network model that is stored on your computer. See Update the neural network model after installing a new GPU

## Performance

A few NVIDIA GPUs were tested in an EthoVision XT experiment with four arenas, one subject per arena, Deep learning as a body point detection technique. A 5-minute video of resolution 1280 x 1024 and frame rate 30 fps was used. The average time taken to acquire one trial was as follows:

- Quadro P2200: 4 min 29 s.
- T1000: 4 min 24 s.
- GeForce RTX 4060 Ventus 2X: 1 min 58 s.

## Importance of CUDA

The driver of the graphics card must support the CUDA component. CUDA is a parallel computing platform and programming model created by manufacturer NVIDIA that helps speed up applications by using the power of graphics cards.

However, each graphics card seems to have its own latest driver version in Windows Update. For the cards specified above, Windows Update installs the needed minimal version. However, other cards may need more recent versions than that available with Windows Update. When a driver version is not sufficient for supporting CUDA, EthoVision XT gives the message in the Experiment Settings:

*The graphics card driver is outdated or not installed, or the graphics card does not support CUDA*.

We recommend to download and install the latest driver from the card's manufacturer. This should make the card work properly with CUDA, provided that the hardware supports that version.

To know whether your NVIDIA graphics card is CUDA-enabled, refer to this web page:

https://developer.nvidia.com/cuda-gpus

## CUDA version

If your GPU is already installed on the PC, follow these steps to know which version of CUDA is installed.

1. In Windows Explorer, locate the folder C:\ProgramData\Noldus\Components\Ethovision\TrackerInterfaceNN\[version number]
2. In that folder you find a file named CudaReport.txt.
3. Open this file. The **runtime version** should give an indication of the version of CUDA currently present on your PC.

If you do not see the folder specified above, create an experiment and in the Experiment Settings under **Body Point Detection Technique** select **Deep Learning**. Next, check again the folder in step 1.

**NOTE** The version of CUDA is not the same thing as the version of the driver of the GPU!

## Which GPU should I choose?

- Choose in any case a recent NVIDIA GPU of the series 1000, 2000, 3000 or 4000. However, a very recent GPU or one of a new generation may not be compatible. Contact Noldus if in doubt.
- In principle, a graphics card of the Pascal microarchitecture category, with 4 GB memory, 1024 CUDA cores and memory bandwidth up to 140 GB/s represents the minimum specifications. Click one of the links below depending on the camera you use. In the table that appears, locate the row with a specific graphics card and DL (Deep learning) specified in the rightmost column:
  - GigE cameras
  - USB 3.0 cameras
  - Analog cameras
- The number of CUDA cores could be a good indicator of performance if you compare GPUs within the same microarchitecture (generation). However, when you compare cards between generations (e.g. Pascal vs. Turing) then the difference in the number of CUDA cores does not predict the actual difference in performance. An older card with more CUDA cores may not perform as good as a more recent card with fewer CUDA cores.
- Overall, newer generations perform better; for example, an Ampere GPU should perform better than a Turing GPU.

- GPUs of the Maxwell generation / 900 series are not supported with EthoVision XT 18.
- Note that a more powerful GPU requires more power from the PC. Make sure that your PC has enough power to feed the GPU. See the prerequisites in Install a graphics card for Deep learning.
- Performance of a graphics card not only depends on its own characteristics; it also depends heavily on the sample rate and the resolution chosen in EthoVision XT. See Video source

*See also*

- Install a graphics card for Deep learning
- Deep learning: Requirements

# Install a graphics card for Deep learning

## Aim

To give general information about the installation of an advanced graphics card (Graphics Processing Unit, or GPU).

**NOTE** This topic is not exhaustive and is not focused on a particular graphics card. If you need more details, see the documentation provided by the card's manufacturer.

## Prerequisites

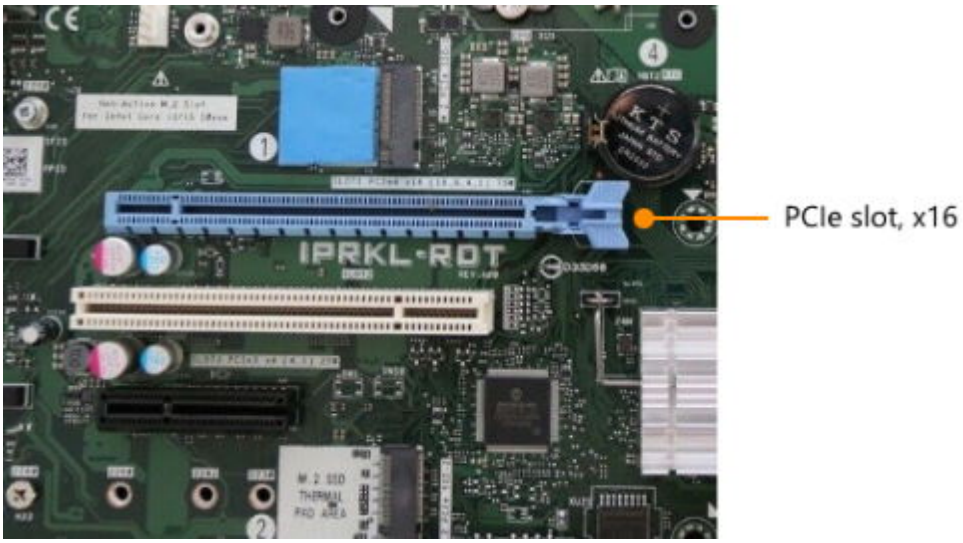
- *Space requirements*. Ensure that there is enough space around the card in its final position. Other cards could reduce ventilation and cause overheating. If necessary, remove those cards and place them on another slot. Plan additional space for the cables.
  - The NVIDIA T1000 needs 15.5 cm x 6.9 cm (6.1" x 2.7"). This card occupies one PCIe slot.
  - The NVIDIA RTX 4060 dual fan needs 24.7 cm x 13 cm x 4.1 cm (9.8" x 5.1" x 1.6"). This card occupies two PCIe slots.
- *Power requirements*. Ensure that the computer has a Power Supply Unit (PSU) that can provide enough power to the graphics card. Some graphics cards need additional power. For example:
  - The NVIDIA T1000 has a maximum power consumption of 50 W and a suggested PSU of 250 W.
  - The NVIDIA RTX 4060 dual fan has a maximum power consumption of 115 W and a suggested PSU of 300 W.
- *Power cables*. Make sure your Power Supply Unit has the correct connectors to support the graphics card. Cheap PSUs may offer fewer options and shorter lengths. Check with your motherboard and graphics card documentation to determine which connector types are needed.
  - The NVIDIA T1000 does not need an additional power supply.
  - The NVIDIA RTX 4060 needs a power cable with a PCIe 8-pin connector.
- A graphics card must support the CUDA software. Check on this web page if you have a NVIDIA graphics card:

  https://developer.nvidia.com/cuda-gpus

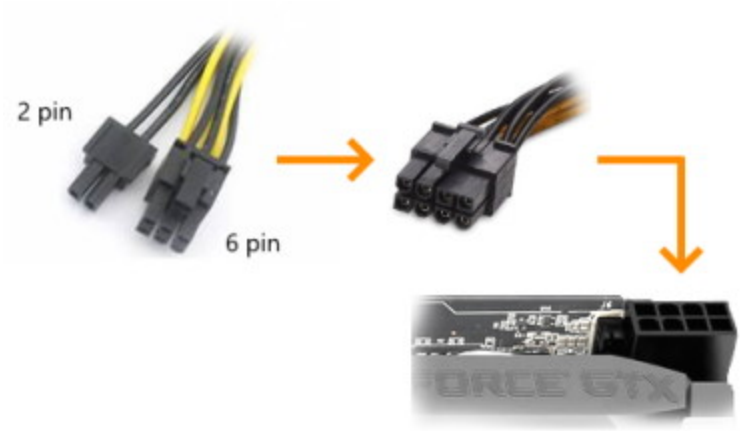
## Procedure

**IMPORTANT** Before installation, disconnect the AC main source and place the computer on a stable surface. Discharge your body's static electricity by touching a grounded surface, for example, the computer chassis. Do not install near water or when your hands or body are wet.

1. Remove the cover/side panel of the computer.
2. Remove any existing graphics card (if there is one).
3. Remove one or two adjacent slot brackets. Some graphics cards are double-wide and require removing two slot brackets.
4. Plug the graphics card into the Primary PCI Express (PCIe) x16 slot on your motherboard. This is often the slot closest to the CPU. Sometimes this slot has a color different from the others (for example, blue instead of black), or has a retention lever on one end.




5. Most graphics cards need additional power. For this reason they have their own power connector. Connect the 6-pin (or 6+2 pin, or 8 pin) power connector from the PC's power supply to the power connector on the card.




**NOTE** The connector and socket on the graphics card have a unique shape and connect one way only.

6. Put the cover back on your computer and reconnect any cables that you removed earlier.
7. Install the drivers that come with the card. When possible, choose to activate the automatic update of the drivers.
8. Restart the computer.

## Update the neural network model after installing a new GPU

Follow the procedure below if you installed a new GPU and you have used the deep learning-based tracking technique on that computer.

Background information: The deep learning trackers use a built and serialized model specific for the GPU. This needs to be rebuilt when a different GPU is used. Do one or both of the two depending on what applies to your situation:

- *When tracking one 1 subject per arena*: Open the folder C:\ProgramData\Noldus\Components\Ethovision\TrackerInterfaceNN\3 (or latest version number).

  Remove the file modelnn.trt and model_hoodednn.trt

  If that does not solve the issue, in the **Control panel** > **Programs and Features** remove the component **Noldus – Tracker Interface NN – x64 Package – 3**. Next, repair EthoVision XT from the installation package (choose **Repair** in the start screen).

- *When tracking two subjects per arena*: Open the folder C:\ProgramData\Noldus\Components\Ethovision\TrackerInterfaceMST\1.

  Remove the file model_mstnn.trt.

If that does not solve the issue, in the **Control panel** > **Programs and Features** remove the component **Noldus – Tracker Interface MST – x64 Package – 1**. Next, repair EthoVision XT from the installation package (choose **Repair** in the start screen).

## Notes

- Install the graphics card into the primary PCI Express x16 slot. If you install the graphics card into the secondary slot, your system may not recognize the graphics card and there will be nothing displayed on the monitor. When your PC has more than one PCI Express x16 slots, you can configure the physical location of the primary slot in the motherboard's BIOS.

# Behavior Recognition: Requirements

## Species

- The Behavior Recognition function has been developed and tested for tracking rats and mice, not other animal species.

## Number of arenas

- When tracking live: one arena.
- When tracking from pre-recorded video files: one to four arenas.

## Subjects

- If you use multiple arenas, the subjects must have similar size. The apparent size of the subjects is specified in the Detection Settings. That size is used for all arenas.
- The subject's length (nose to tail base) in the video image must be at least 60 pixels for rats, and 55 pixels for mice. In all cases its length must not exceed half the arena size.
- The subject's fur must be of uniform color. Hooded animals like Lister and Long-Evans rats have not been tested with Behavior Recognition.
- For rats: the subject must be older than approximately three weeks. In all cases the subject must be able to walk.
- In the Detection Settings, make sure that the subject's tail is not detected. To do so, use the erosion filter. See Advanced detection settings: Subject contour
- Behavior detection is much depending on the subject age and size. For Wistar rats of age 3-5 weeks, the behavior size setting can be used about one week; from age 5 weeks on, it may be used two weeks. We advise to create separate Detection Settings for different age classes.

## Camera and video

- The camera is placed above the subject, and provides a top view of the test setup.
- Because video compression introduces artifacts, live video is preferred over pre-recorded video.
- Video frame size (video resolution) must be greater than 352 x 288 per arena.

- The frame rate of the recorded videos can only be a multiple of the sample rate that you intend to use. For example, for a sample rate of 25 samples per second, the camera/video file frame rate should be 25 or 50 etc. For a sample rate of 30, set the frame rate to 30 or 60.
- The video image must not be overexposed. Details of the subject's fur must be visible.
- If you use pre-recorded video, compression should be as lossless as possible. Reducing compression by decreasing the GOP size (that is, the number of video frames between two full frames) produces better results than increasing video resolution. Values of GOP size of 10-15 worked well, however the lower the better. Reducing GOP size, however, increases file size.
- To check that your video file has artifacts, create a separate experiment with Activity analysis enabled. Set the Activity threshold to a minimum. See Activity settings. If video contains artifacts, you should see purple pixels appearing with a regular rhythm. See also Troubleshooting: Behavior recognition
- Try to avoid evident *fish eye-effect* (barrel distortion) in the video image, which hampers behavior recognition. If necessary, use a lens with lower distortion.

## Test environment

- The test setup must be simple, for example a home cage (without bars) or an open field with no objects around.
- The animal should not climb on top of objects like a shelter. When the animal climbs on top of an object, it gets closer to the camera, and its apparent size changes significantly. This may bias results. See also Behavior Recognition: Data, performance and accuracy
- Lighting must be from the top, not backlighting. Slow changes in lighting in the course of the test are not a problem, but moving spotlights reduce reliability of detection.
- Avoid reflections on the walls.

## Tracking settings

- In the Experiment Settings, you must select **Contour-based** as **Body Point Detection Technique**.
- In the Detection Settings, the sample rate must be set between 25 and 31 samples per second.

## Further recommendations

- During acquisition, keep **DDS** (Detection Determines Speed) selected.
- After acquiring data, visualize the detected behaviors in the Integrated Visualization and check whether no gaps between scored behaviors occur. If gaps occur, which are not due to the fact that the subject is not found, they could be the result of video frames not being analyzed due to high processor load. To decrease processor load, lower the video resolution. Also, try not to use **Differencing** as detection method, and any of the methods named **For occlusions** as the tracking method. See Advanced detection settings: Method

# Behavior Recognition: Data, performance and accuracy

## Data

With the Rat/Mouse Behavior Recognition functions, the following behaviors can be automatically detected: digging (only for mice), drinking, eating, grooming, hopping (for mice), jumping (for rats), rearing (unsupported and supported), resting, sniffing, twitching (for rats), and walking. For the definition of those behaviors, see Behavior Recognition.

For each video frame, behavior is detected analyzing a number of frames before and after the current one.

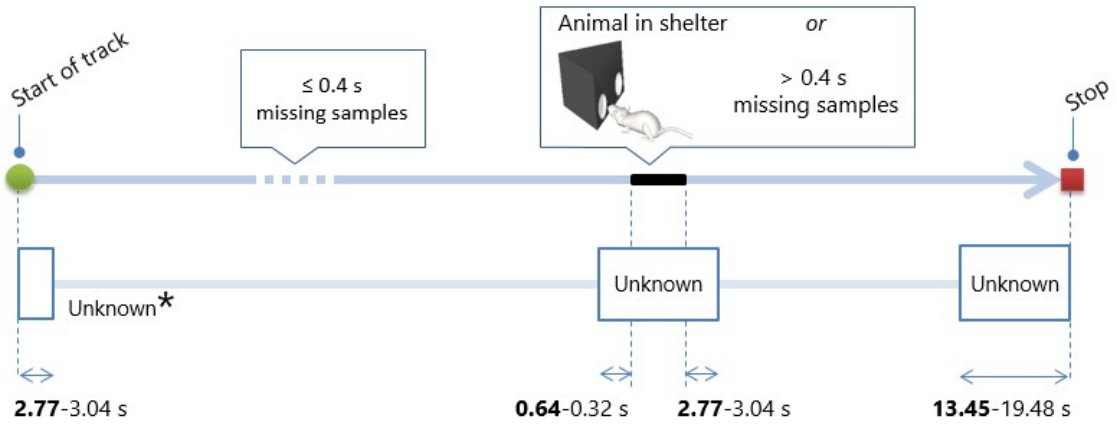
- Behavior data are available from the start of the track, to about 13 s before the end of the track when using a default Trial Control Settings, which has an additional condition for starting the track (see the figure below).
- When the Trial Control Settings does not have the special condition mentioned above, behavior is not scored in the first 3 seconds of the track (see (*) in the figure below).
- When samples are missed or the subject is not found for a maximum of 0.4 seconds, the subject's position and size is interpolated using the adjacent frames.
- When samples are missed or the subject is not found in for more than 0.4 seconds, or when the subject enters a hidden zone, the behavior detector is reset. This means that behavior is not scored some time before the first missing sample (or before entering the hidden zone) and after last missing sample (or after exiting the hidden zone). The behavior is set to Unknown (see the figure below for the exact times).

  We advise you to adjust the duration of the trial in the Trial Control Settings. For example, set 6 minutes of track duration to get at least 5 minutes of automatically-detected behaviors.

Below: Schematic representation of which portions of the track do not result in detection of behavior with the Behavior Recognition function. Times next to Unknown are shown for Mouse Behavior Recognition (in bold) and Rat Behavior Recognition.

*) The first part of the track is scored as Unknown only if you start tracking as soon as the animal is detected in the arena. If your Trial Control rule includes a condition that waits 3 seconds after the animal is detected, then you make sure that behavior is scored from the start of the track. We recommend to use the default Trial Control

Settings for experiments set to Rat or Mouse Behavior Recognition, which include this additional condition.




**NOTE** To visualize or analyze the portions of the tracks scored as Unknown, select Unknown in the behavior property window in the Analysis profile.

## Performance

When Behavior recognition is selected, and you track live, use maximally one arena. To improve performance, and avoid samples being discarded because of the use of too much processor capacity, follow these rules:

- As a detection method, use one of the **Subtraction** methods, not Differencing.
- For the nose-tail tracking, try first **Rodents / Default**, not **Rodents / For occlusions**. Use the latter only if the first does not give good results.

## Accuracy

- Rat Behavior Recognition was trained on Sprague Dawley rats and tested on various strains by DeltaPhenomics and Radboud University Nijmegen, The Netherlands. The details of this validation are in the publication listed below.
- Mouse Behavior Recognition was trained on C57Bl6 mice, and tested on various models and strains including BalbC, CD1 (white), and TgAD models. The module has been beta tested by four experienced animal behavior specialists at different labs (two in USA, two in Europe).

The average recall rate, that is, the fraction of ground truth manually scored behaviors that EthoVision XT recognizes correctly, is 0.70.

See the following paper:

van Dam, E., J.E. van der Harst, C.J.F. ter Braak, R.A.J. Tegelenbosch, B.M. Spruijt, L.P.J.J. Noldus (2013). An automated system for the recognition of various specific rat behaviours. *Journal of Neuroscience Methods* **218** (2), 214–224.

http://dx.doi.org/10.1016/j.jneumeth.2013.05.012

*See also*

- Trial control With Behavior recognition
- Behavior Recognition in the Analysis profile

# Live Mouse Tracker: Workflow

## Background information

- Live Mouse Tracker (https://livemousetracker.org) is a system for prolonged monitoring of mice housed in small groups and in enriched environment.
- Tracking and individual recognition is based on a Kinect depth camera and RFID antennas and readers.
- You can track up to four subjects (tested).
- For the assembling instructions: see https://livemousetracker.org.
- EthoVision XT supports data of Live Mouse Tracker version 1.0.3 (October 4, 2022).

## Prerequisites

- You must have the Social Interaction add-on module in your EthoVision XT license. See Upgrade EthoVision XT

## Procedure

1. Assemble the Live Mouse Tracker setup.
2. Connect all the hardware to the PC.
3. Collect the data with Live Mouse Tracker.
4. In EthoVision XT, create an experiment in Live Mouse Tracker mode.

   Choose **File** > **New Experiment**. Select the check box **Live Mouse Tracker experiment**.
5. Import Live Mouse Tracker data. For this you need the Live Mouse Tracker databases and the video files (optional).
6. In the Analysis profile, choose the dependent variables. See Live Mouse Tracker
7. Visualize and analyze the data.

## Notes

- Live Mouse Tracker works with 3D coordinates (x, y, z). While analysis is mostly based on 2D (x,y) coordinates, some dependent variables are also based on the z-coordinates, for example Rear. See Dependent variables for Live Mouse Tracker

- In the Live Mouse Tracker database, the x and y coordinates are in pixels, while z is in mm. However, EthoVision XT converts pixel coordinates to distance Units specified in the Experiment Settings.
- In Live Mouse Tracker experiments you can also analyze data using the well-known analysis variables like *Velocity* and *In zone*. However, the following variables won't be available in your Analysis profile, because they cannot be calculated with Live Mouse Tracker data:
  - Head direction and Head directed to zone.
  - Turn angle when it is based on head direction.
  - Activity and Activity state.
  - Body elongation and Body elongation state.
  - Mobility and Mobility state.
  - Body contact.
  - Behavior Recognition (all behaviors).

# Live Mouse Tracker: Create an experiment

## Aim

To create an EthoVision XT experiment that is compatible with the Live Mouse Tracker behavioral data acquisition system.

## Background information

An EthoVision XT experiment set to Live Mouse Tracker is a modified version of a standard, multi-subject, multi-body point experiment, with predefined, permanent settings. The main characteristics are:

- In the Experiment Settings, the **Video Source** is **Video file**, the **Number of Arenas** is **1**, the **Number of Subjects** is **4** and under **Tracked Features** the option **Tracker add-in** is selected. We recommend not to change those settings. You can, however, change the settings under **Units**.
- In the Arena Settings, the arena is already calibrated using the scaling factor of Live Mouse Tracker. If you like, you can add zones for analysis beyond the standard readouts of Live Mouse Tracker.
- Trial Control Settings, Detection Settings, Acquisition, and Track Editor have been removed because they are not relevant in Live Mouse Tracker.
- All analysis functionalities of EthoVision XT are kept. So you can, for example, analyze the tracks obtained with Live Mouse Tracker combined with zones using a Data profile, or combine Live Mouse Tracker readouts with the EthoVision XT analysis variables.

For more information about the Live Mouse Tracker acquisition system, see the following link:

https://livemousetracker.org

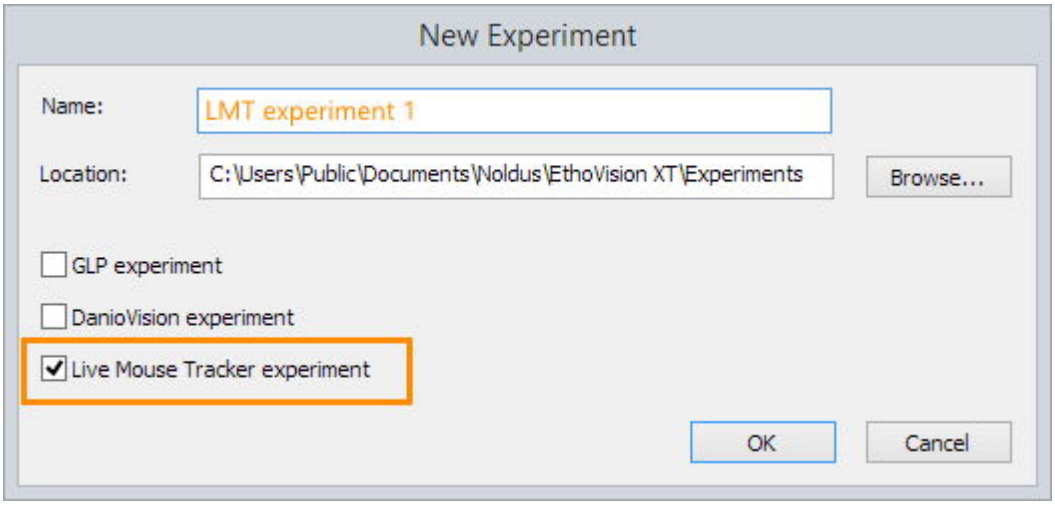
## Prerequisites

- You have collected data using the Live Mouse Tracker acquisition system.
- You must have the Social Interaction add-on module in your EthoVision XT license. See Upgrade EthoVision XT

## Procedure

1. Choose **File** > **New**.
2. Enter a name for the experiment.

3. Select the **Live Mouse Tracker experiment**.
4. Click **OK**.




5. Next: Import Live Mouse Tracker data.

## Notes

- You may notice that in all Live Mouse Tracker experiments, when opening the Experiment Settings, under **Tracked Features**, the option **Tracker add-in** is selected. This is the correct selection in the experiments made for Live Mouse Tracker. Please leave it as it is.

*See also*

- Live Mouse Tracker: Workflow

# Manual scoring settings

## Learn about

- Scoring behaviors manually

## What do you want to do?

- Define the behaviors that you want to record manually
- Edit the manual scoring settings
- Validate the manual scoring settings

# Scoring behaviors manually

## Aim

- To record rodent behaviors that cannot be detected automatically with the Rat and Mouse Behavior Recognition; for example, pouncing or fighting.
- To record behaviors in animals other than rodents; for example, thrashing in fish.

**NOTE** You can also score behaviors after acquisition, for example to correct scoring errors and add data. See Score behaviors manually after acquisition

## Procedure

1. Define the behaviors that you want to record manually.
2. **OPTIONAL** Score behaviors manually during acquisition.

   You score behaviors and events by pressing a predefined key or clicking an appropriate button on the screen as soon as the behavior or event occurs. Score behaviors manually after acquisition.

   **NOTE** This is only possible when you have tracked from a video file, or you tracked live and saved video.

## Notes

- Manual scoring settings are not available in a DanioVision experiment.
- Manually-scored behaviors are stored in a special Manual scoring log associated with the track data.
- If you record video and track later, you can only record events when you do tracking, not during video recording.
- If you are familiar with The Observer XT software, the manual scoring settings in EthoVision XT is a simplified The Observer XT coding scheme. You can export the scored behaviors to The Observer XT for further analysis. See Export manually-scored behaviors
- With the Rat (or Mouse) Behavior Recognition module, EthoVision XT can detect a set of rodent behaviors automatically. See More about Experiment Settings

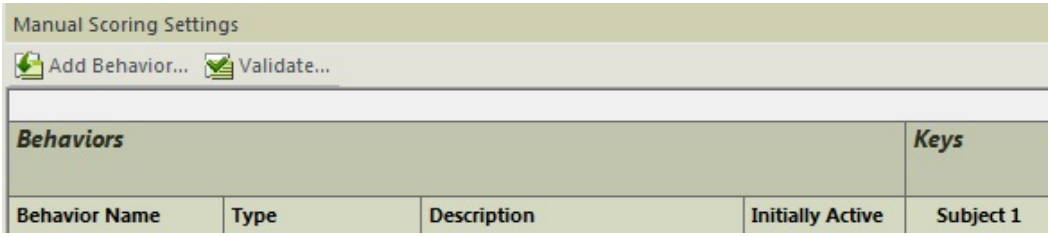
# Define the behaviors that you want to record manually

## Prerequisite

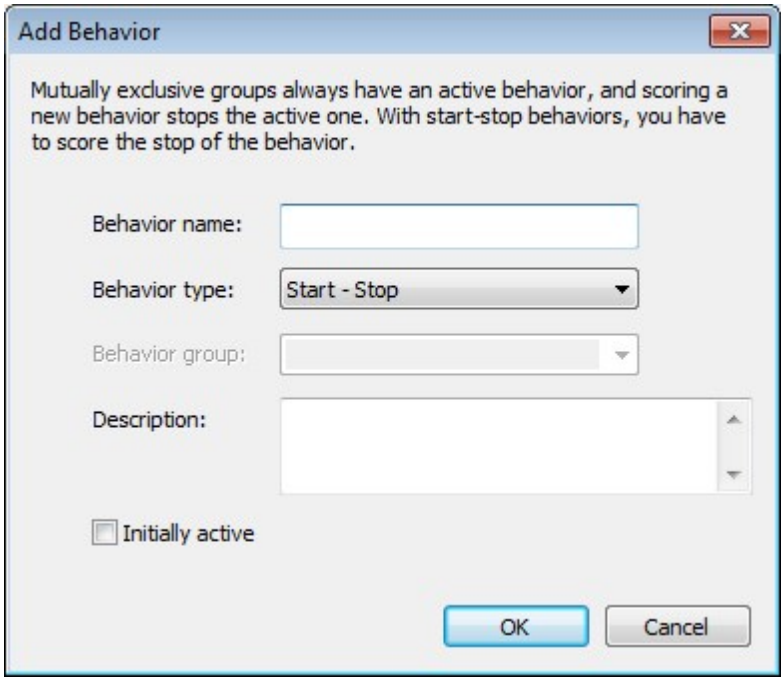
Think in advance how the different categories of behavior relate to each other. You may for example want to score different types of grooming (Unilateral stroke, Bilateral stroke etc.). Each type will be a behavior, however these are best organized in a group. If you plan to score behaviors that do not relate to each other (for example Rearing and Drinking), they do not need to be defined in a group.

## Procedure

1. Choose **Setup** > **Manual Scoring Settings**, or in the Experiment Explorer, click **Manual Scoring Settings**.




2. Click the **Add Behavior** button or press **Ctrl+B**. The Add Behavior window appears.




3. Next to **Behavior name**, enter the name to the behavioral category you want to score. For example, *Follow*, or *Bite*.
4. Next to **Behavior type**, choose the type of behavior. This choice affects how you score the behavior relative to others. See Behavior types
5. If in the previous step you have chosen **Mutually-exclusive**, then the **Behavior group** list becomes available. Type in the name of the group the behavior belongs to, or, if you have already defined it, select it from the list.

   If you have chosen **Start-Stop** or **Point event**, the behavior cannot be part of any group, and therefore the Behavior group list is not available. Go to the next step.
6. Enter a **Description** (optional) for the behavior.
7. If you think that the behavior is active at the start of the trial, select the Initially active option.
8. Click **OK**. The behavior is listed in the table, under the group it belongs to.

| *Behaviors* | | | | *Keys* |
|---|---|---|---|---|
| **Behavior Name** | **Type** | **Description** | **Initially Active** | **Subject 1** |
| Grooming | | | | |
| Unilateral stroke | Mutually exclusive | | ☐ | u |
| Bilateral stroke | Mutually exclusive | | ☐ | b |
| No grooming | Mutually exclusive | | ☑ | 0 |

9. In the cell under **Keys**, select the keyboard key that you want to use for scoring that behavior. See Choose the keys for coding

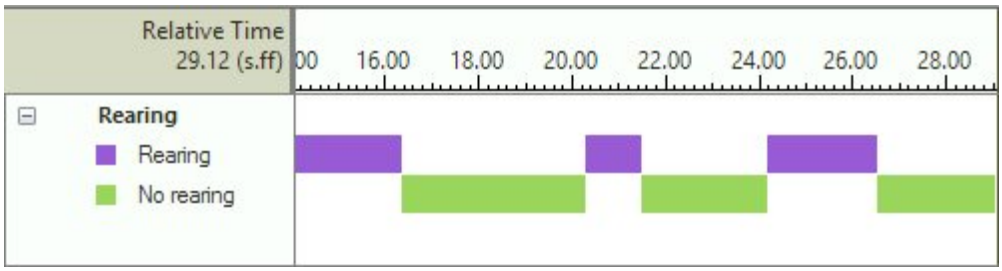
## Behavior types

### *Mutually exclusive*

The behavior will be part of a group, where one item, when scored, automatically switches off the item currently active. For example, you define a group of two behaviors, *Rearing* and *No Rearing*. When the subject shows rearing, score this behavior. *Rearing* is now active. As the subject stops rearing, score *No rearing*. This state is now active, while *Rearing* is stopped automatically.

Within a mutually-exclusive group, only one behavior can be defined as initially active. If one of the behaviors of a Mutually-exclusive group is already selected as Initially active and you check the same option in the Add Behavior window for a second behavior, the program asks you whether you want to remove that option for the first behavior. Click **Yes** to do so. If you click **No**, you return to the dialog, so you can clear the Initially active option.

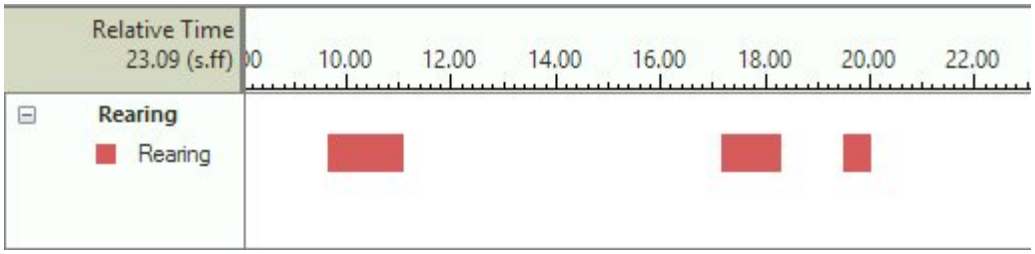
In the Dependent variable plots, a mutually-exclusive group of behaviors would look like this:




### *Start-Stop*

Scoring that behavior is fully independent of other behaviors. To stop that behavior you must press the Stop key.

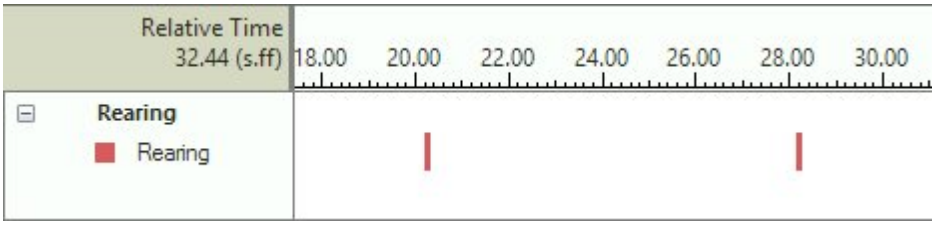
In the Dependent variable plots, a Start-stop behavior would look like this:



*Point event*

With this option you only score a point of time when the behavior occurs. Choose this option when you only want to measure the frequency, not the duration of the behavior. Typical examples of such behaviors are biting and twitching.

In the Dependent variable plots, a Point event would look like this:




## Choose the keys for coding

- It is not required to use key codes. You can also score events using the mouse.
- Choose keys that you can press without taking your eyes off the keyboard (for example A, S, D) rather than keys which are easy to remember. If your scoring settings contain so many behaviors that you cannot remember the keys, it is probably too complex.
- For each cell you can enter one key, among those available a...z, and 0...9.
- If your setup includes multiple subjects and arenas, choose a key for each arena-subject-behavior combination.
- For Start-Stop behaviors, choose a key to Start the behavior and a key to stop it. You can also define the same key for starting and stopping it.
- Only the keys available are listed, not those already in use. For Start-Stop behaviors, you can select the same key for both starting and stopping a behavior.

## Notes

- For mutually-exclusive behaviors, It is always a good idea to define a default behavior that can be scored when the subject does not show any significant behaviors. Choose this behavior as Initially active.
- Names of groups and start-stop behaviors are displayed in a gray row. Names of mutually-exclusive behaviors are displayed in a white row under the corresponding group name.

- Behavior names and group names must be no more than 64 characters long. The characters you use, must be Unicode characters in the Basic Multilingual Plane range. See the following web page:

  http://en.wikipedia.org/wiki/Plane_(Unicode)#Basic_Multilingual_Plane

- *How many behaviors?* You can define as many behaviors as you need. However, since the number of available keys is 36, we advise you to limit the number of arena-subject-behavior combinations to 36, so you can assign a keyboard key to a behavior of a specific subject in a specific arena. If you need to define more combinations, do so without assigning a key code. You can score those behaviors by clicking the corresponding button on the acquisition screen.

  When you need to score behaviors live, keep the scoring settings as simple as possible, with few behaviors. If you try to score many complex behaviors at once then you may make mistakes.

*See also*

- Score behaviors manually
- Analyze a Manually scored behavior
- Export manually-scored behaviors to The Observer XT

# Edit the manual scoring settings

## Rename a behavior or behavior group

Click the name of the behavior or behavior group, make the necessary changes and press **Enter**.

## Modify the key code

Click the cell under **Keys** for a behavior, and type in the new key code, or select another key. If the new key you want to use is assigned to another behavior, it is not available in the **Keys** list. Pressing that key on your keyboard makes no change in the selected cell.

To delete a key code, click the corresponding cell and press Backspace.

## Edit the behavior properties

Double-click the behavior row or right-click and select **Edit Behavior**.

## Create a new behavior group

Create first a behavior that belongs to that group, then from the **Behavior type** list select **Mutually-exclusive**, and in the Behavior group list enter the name of the new group.

## Add a behavior to a specific group

Right-click the behavior group row and select **Add Behavior**. Type in a new name.

## Delete behaviors and behavior groups

You can delete a behavior or behavior group only after you have deleted all your trials. Right-click anywhere on the behavior (or behavior group) row, and select **Delete Behavior** (or **Delete Behavior Group**).

**IMPORTANT** If you delete a behavior group, all behaviors of that group are deleted!

## Move a behavior from one group to another

Double-click a behavior to edit it. In the Edit Behavior window, select the Behavior group you want to move the behavior to.

## Edit manual scoring settings after acquiring data

Choose **Setup** > **Manual Scoring Settings**.

You can:

- Add a new behavior.
- Rename an existing behavior or behavior group.
- Edit the behavior's description.
- Set a behavior as Initially active. This won't modify your existing data, it will only set that behavior as initially active for the next trial.

You cannot:

- Delete behaviors, unless they have been added after data acquisition. If you acquire more trials, those new behaviors cannot be deleted.
- Rename or change the properties of behaviors in a Quality Assurance experiment.

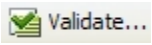
# Validate the manual scoring settings

## Aim

To have EthoVision XT check that the coding scheme is consistent.

## Procedure

In the Manual Scoring Settings screen, click the **Validate** button or press **Ctrl+V**.




The program checks that the manual scoring settings are valid every time you leave the Manual Scoring Setting screen.

## Notes

Manual scoring settings are valid if:

- For Start-Stop behaviors: A key has been selected for both start and stop, or for neither of the two. If you define a start key but not a stop key or vice versa, when leaving the manual scoring settings an error message is displayed.
- For Mutually-exclusive behaviors: All behavior groups contain at least two behaviors, and one is selected as Initially active.
- All behaviors and behavior groups have unique names.

# Arena Settings

## Main topics and tasks

# Introduction to Arena Settings

## Learn about

- Important terms in Arena Settings
- Useful things to know about Arena settings

# Important terms in Arena Settings

## Arena

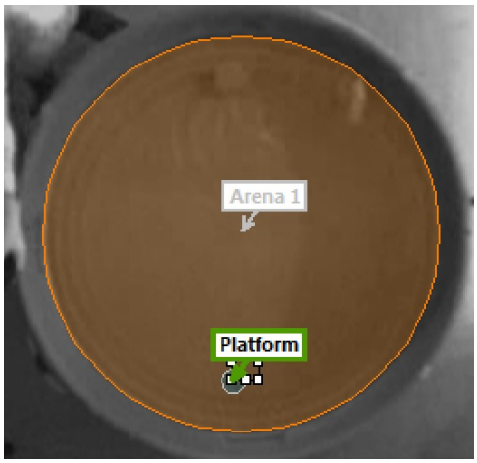
An arena is the region in the video image on the screen in which a moving Subject is tracked by EthoVision XT. You can have one or more (up to 100) separate arenas in one screen. If you have multiple arenas, they must not overlap.

*See also*

- Physical setup of an arena

## Zone

A zone is a specific area of interest within an arena. For example, in a Morris water maze, define the hidden platform as a zone named Platform.




You can use a zone to calculate, for example, the time the animal spent in that zone, or the number of zone visits; you can also use a zone for starting and stopping data acquisition.

## Point

A point is a dimensionless object that you can use as a reference. For example, an odor source that is the target of an insect. You can define the source as a point, to determine whether the animal is heading towards that spot.

## Cumulative zone

A zone that combines two or more existing zones, so you analyze them as one. For example, the two open arms “Open arm 1” and “Open arm 2” of an Elevated plus maze can be analyzed as a Cumulative zone “Open arms”.

## Hidden zone

A zone that you can use to calculate the time that the subject stays out of view, for example in a shelter.

## Zone group

You can organize zones in zone groups. Within one zone group, zones must be non-overlapping.

*See also*

- Organize the zones in your arena
- Cumulative zones
- Shelters and other hidden zones

# Useful things to know about Arena settings

## Define different Arena Settings in the same experiment

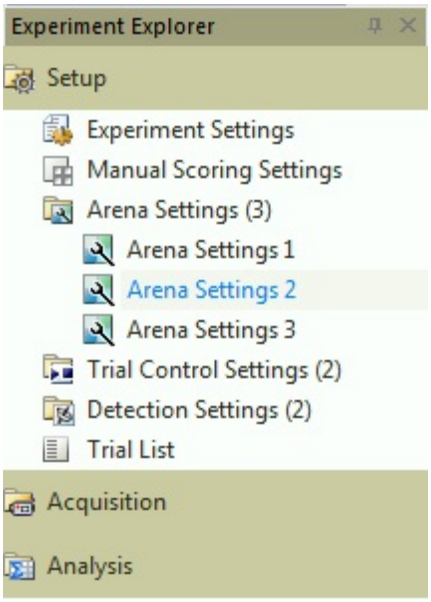
Arena Settings define the configuration of arenas and zones in an experiment. You can create as many Arena Settings as you want. This can be useful if your trials are carried out with different zones configuration. For example, when the position of the hidden platform changes in different trials in a Morris water maze experiment.

## Arena Settings in the Experiment Explorer

All Arena Settings you create are listed under Arena Settings in the Experiment Explorer.

To open Arena Settings, choose **Setup** > **Arena Settings** > **Open**, or in the Experiment Explorer, under **Arena Settings**, click one of the Arena Settings.

The Arena Settings currently active are shown in blue. Acquisition is done using those settings, unless you specify otherwise in the Trial list or in the Acquisition Settings window.



## Read-only Arena Settings

Arena Settings that have been used in acquisition are 'read-only' and can not be renamed or deleted. Read-only Arena Settings are indicated by a small lock symbol in the Arena Settings icon.


You can make an editable duplicate of read-only Arena Settings, edit it and use the new settings for acquisition. Alternatively you can delete all trials, which unlocks the read-only arena settings. In addition:

- Zones that are used in Trial Control cannot be deleted or edited.
- You can add new zones to read-only arena settings.

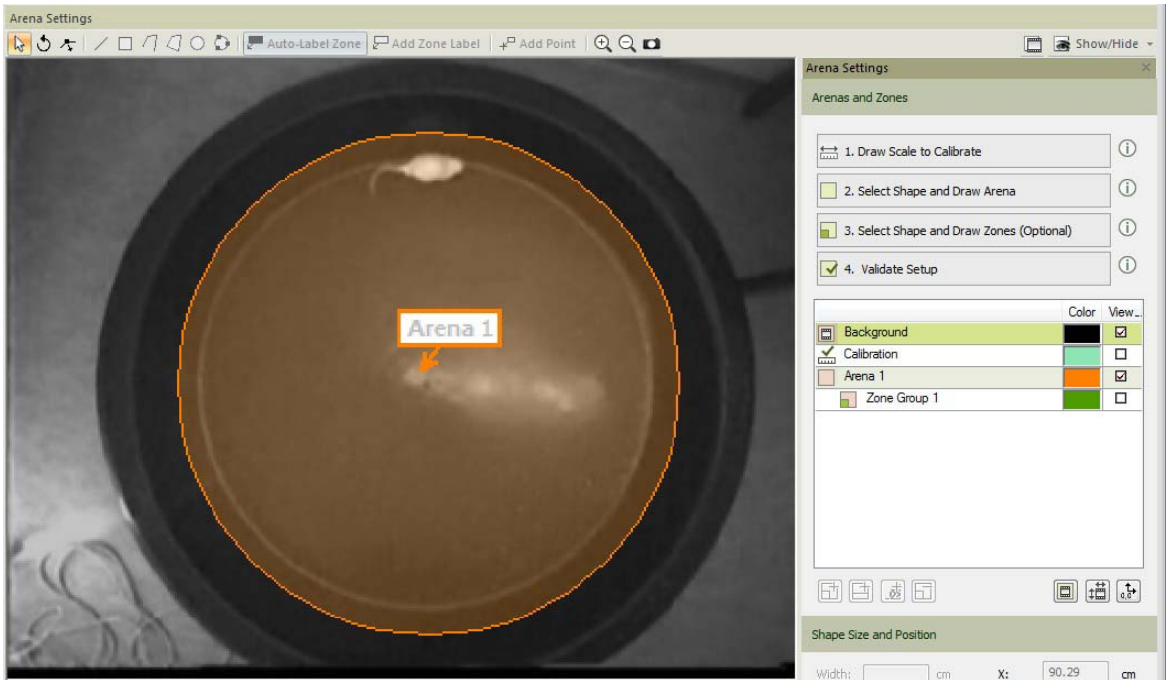
*See also*

- Settings and profiles

## Working with layers

The elements of the Arena Settings are organized in layers.

- To display arenas, zones, calibrations and background image on your screen, select an arena, zone group, zone, calibration or background image by clicking its row in the Arenas and Zones section. This way you can highlight the correct layer.
- To hide a layer, deselect the corresponding check box in the View column.
- To view more than one layer at the same time in the video image, select the appropriate check boxes in the **View** column. In the figure below, Arena 1 and Background are selected in the **View** column and therefore visible.




*See also*

- Organize the zones in your arena

# Make the arena

## What do you want to do?

1. Grab the background image
2. Calibrate the arena
3. Draw the arena
4. Define zones (optional)

   Organize the zones in your arena
5. Validate the Arena Settings

*See also*

- Draw a point
- More about zones
- Work with multiple arenas
- Make a subdivided arena
- Draw a point
- Validate the Arena Settings

# Grab the background image

## Aim

To create a background that you can use to draw arenas and zones.

## Procedure

1. Choose **Setup** > **Arena Settings** > **New** or right-click **Arena Settings** in the Experiment Explorer and select **New**.

   Result: The Grab Background Image window opens.

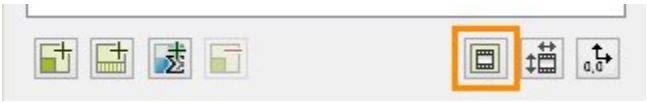
2. If you track from video, click **Browse** and open a video file. In the Video window, if possible, browse to a video frame without animals. Then click **Grab**.

   If you track live, if possible, remove the animal from the arena and click **Grab**.

3. Check that the aspect ratio of the background image is correct, and if necessary, adjust it (see Check the video aspect ratio).

## Notes

- If the Grab Background Image window shows a white background, that may be due to the graphics card settings. See Troubleshooting > The camera image is not shown in the Arena Settings or Integrated Visualization
- You can define and update a background of existing Arena Settings at any time, as long as they are not read-only. To do so, click the **Grab Background Image** button in the lower part of the Arena Settings window.



- For all experiments from video: The default video file shown in the Video window is the last video used to grab a background image.
- For DanioVision experiments: After grabbing the background image, EthoVision XT attempts to detect the well plates and draw the arenas automatically. Check that the outline of the arenas overlap with the well borders. If they do, click **Yes** in the window that appears. For more information, see the DanioVision DVOC-0041 - Reference Manual, which you can open from the **Apps** screen under **Noldus** > **EthoVision XT 18 Other Documentation**.

- When presence of the animal makes it difficult to draw the arena, and it is not possible to grab a background image without the animal, do the following:

  Open the Detection Settings. When the animal moves around in the arena, under **Method** click **Background** and use the **Learn** function. This saves a bitmap of the empty arena.

  Next, In the Arena Settings, click the **Grab Background Image** button, then **Browse** and open the **Bitmap Files** folder within your experiment folder. From the file type list, select **All files (*.*)** to also view image files.

  Choose the bitmap image just saved - its name starts with DetProf_. Next, click **Grab**.

# Calibrate the arena

## Aim

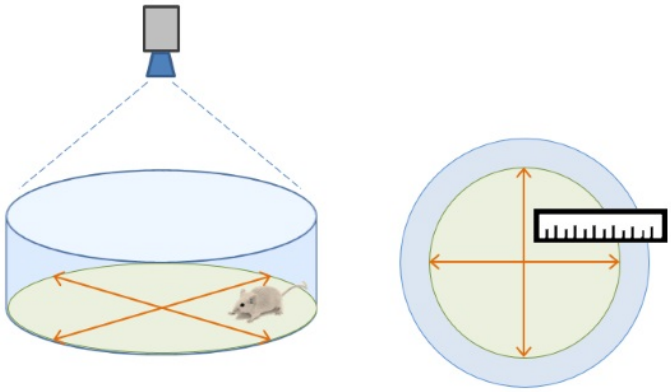
To convert the subject's coordinates expressed in pixels to real-world coordinates.

**IMPORTANT** Avoid barrel and spherical distortion by using correct lenses. Place the camera above the middle of the arena, and perpendicular to the arena surface.

**IMPORTANT** If you use Track3D, do not do calibration within EthoVision XT. Instead, make sure that you set Pixel calibration to ON. For more information, see the Track3D Reference Manual.

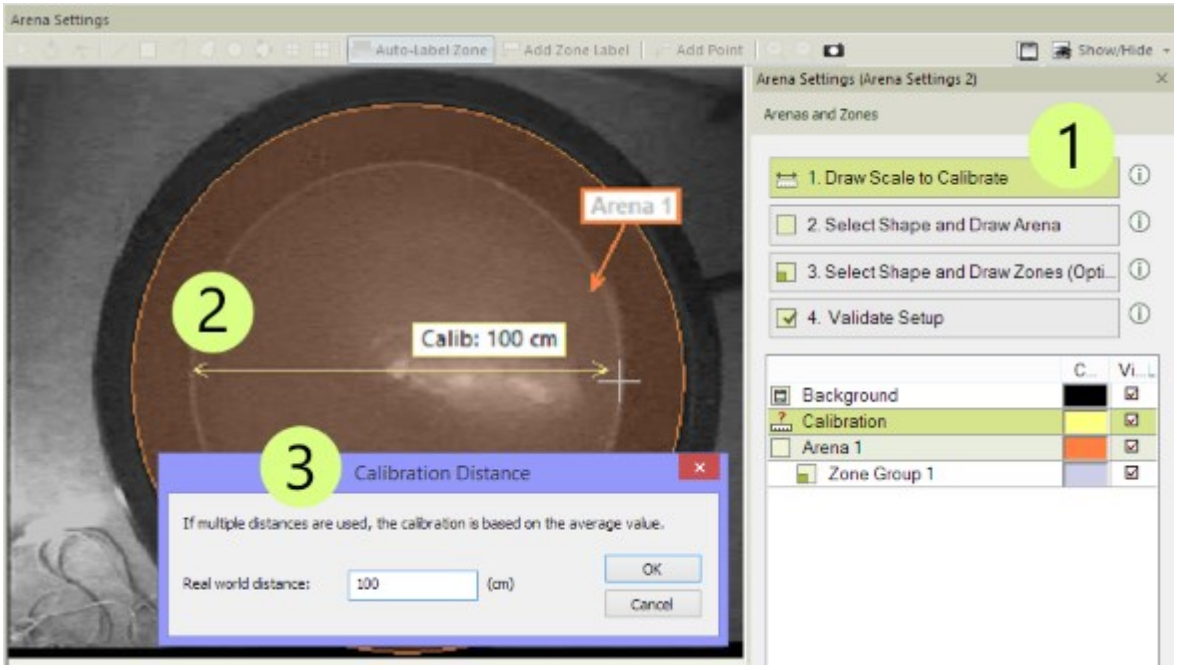
## Prerequisites

- You grabbed a background image.
- You know the real-world size of the arena. Measure this distance at the height of the animal, not, for example, at the top of the arena.



## Procedure

1. In the Arenas and Zones section, click **1. Draw Scale to Calibrate**.
2. Click the mouse button on the first a point in the video image, hold the button down, and drag to the second point in the image. Next, release the mouse button.
3. In the Calibration Distance window, type in the real-world distance between the two points.




## Notes

- Repeat the steps above for all calibration lines. EthoVision XT uses the average of the scale factors obtained from each calibration line. This factor is used for analyze movement of the subject in all directions. Draw calibration lines on both directions, horizontal and vertical.
- See also Modify the coordinate system of the arena.
- For each calibration line, EthoVision XT calculates a scale factor (real world distance entered divided by the length of the line in pixels). If this factor differs by more than 20% from the average of the other lines, a message is shown: “Calibration scale substantially larger or smaller then other existing calibration scales”.

  Click **No** to edit the real world distance. Or click **No** and then **Cancel** to re-draw the last calibration line.

  Click **Yes** to accept the value. Beware that very different scale factors may produce false results.
- If you enter a real world distance smaller than 10 mm (0.39 in) or greater than 10 m (32 ft 9 45/64 in), a message is shown. Click **Yes** to confirm the value or **No** to edit it.
- If you have clicked **Cancel** and you want to repeat the calibration procedure, click again **1. Draw Scale to Calibrate**.

# Draw the arena

## Aim

To define an area of interest so that EthoVision XT ignores all activity outside this area, so only movement inside the arena is tracked.

With multiple arenas within one video image, you can track more animals simultaneously and separately. Each arena is an independent replicate.

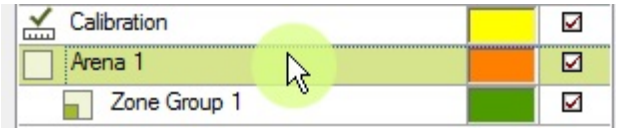
## Prerequisites

- You have calibrated the video image.

## Procedure

1. Click **2. Select Shape and Draw Arena** in the Arenas and Zones section.

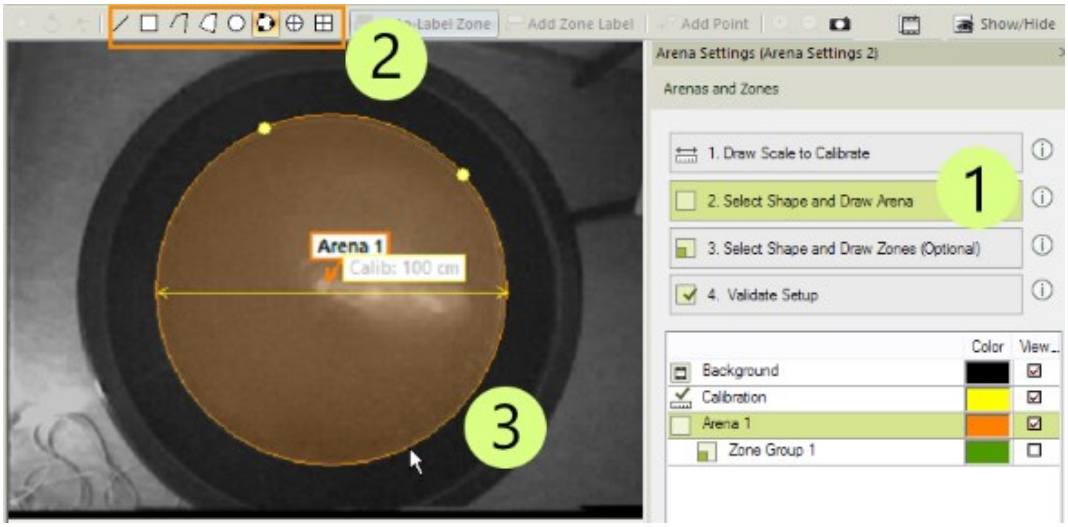
   When working with multiple arenas, click the layer for the arena you want to draw.

2. Select a shape on the Arena Settings toolbar at the top of the window by clicking it or by pressing the corresponding shortcut key.

   To split an arena in quadrants automatically (for example, in a Morris water maze, or an open field), choose **Create subdivided 3-point circle** or **Create subdivided rectangle** . See Draw subdivided shapes

3. Create a shape that covers the region on the screen in which you want EthoVision XT to track the subject. Make sure the arrow point of the arena label is inside the shape you just created.




4. If you have chosen one of the subdivided options, the Add Subdivided Circle (or Rectangle) window appears.

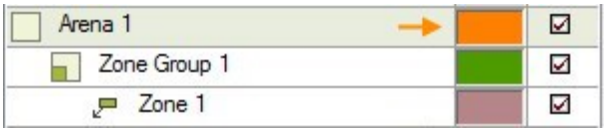
   For circular arenas, enter the number of sectors (for example, 4 for quadrants in a Morris water maze). Default =2, maximum = 50.

   For rectangular arenas, enter the number of rows and columns. A total of rows x columns equal zones will be defined.

   Result: In the Arenas and Zones section, a new zone group named Subdivision Zones is added, listing the new zones. See The Subdivision Zones group

## Notes

- If you do not draw an outline, the whole background image is considered as the arena.
- If you have multiple arenas with the same size and shape, you do not have to draw each arena separately. You just draw one arena and duplicate this one. See Duplicate an arena on page 266.
- The shape is filled with the color visible next to the arena name in the Arenas and Zones section. To change this color, click the color next to the name.




- To create a circle or a square, select the Ellipse ◯ or Rectangle □ tool, respectively, and hold **Shift** down while you draw the shape.

- To export the image of the arena, click the camera button on the toolbar. Give the export file a name and select a picture format (*.png, *.jpg, *.bmp, *.gif).

  The image contains all layers that are currently visible. See Working with layers for how to display layers.

- For an experiment in DanioVision mode, EthoVision XT can detect the arenas automatically. See the DanioVision DVOC-0041 - Reference Manual.
- You cannot delete an arena that has been used for acquisition; it has become ‘read-only’. To edit it for future tracks, duplicate the arena, edit the copy and use these settings for acquisition.

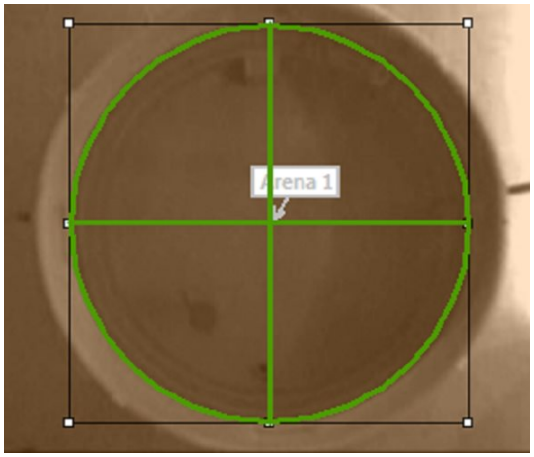
# Make a subdivided arena

When you draw an arena using the subdivided tools, note the following:

If the Auto-Label Zone is active, all sectors are automatically defined as zones, and labeled with S1, S2, etc. A layer named **Subdivision** is added in the Arenas and Zones section together with its zones.


**TIP** When the Auto Label Zone is active, the **Auto-Label Zone** button is highlighted in orange.


If the Auto-Label Zone button is not highlighted, the arena is still divided in sectors, but the resulting zones are not labeled. Click the **Add Zone Label** button to label the zones.



**TIP** To activate/deactivate Auto-Label Zone, click **3. Select Shape and draw Zones (Optional)** in the Arenas and Zones section and click the **Auto-Label Zone** button.


*See also*

- Draw subdivided shapes

# Define zones

## Aim

To define specific regions of the arena to be used in analysis or to start and stop tracking.

**NOTE** Any area falling outside the arena is not considered as a zone.

## Prerequisite

- You have defined the arena.

## Procedure for one zone

1. Click **3. Select Shape and draw Zones (Optional)** in the Arenas and Zones section.

   If multiple zone groups are defined, in the Arenas and Zones section, click the zone group where you want to insert the zone. This group is highlighted in the background image.


2. Select a shape from the toolbar. See also Draw simple shapes
3. Create a shape that covers the area of interest within the arena. Do so at the height of the animal, not, for example, at the top of the cage.

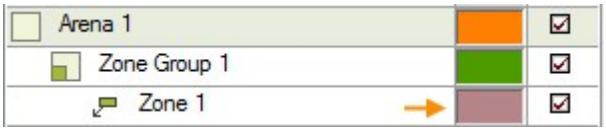
   If you choose one of the subdivided options, select the number of sectors or rows x columns and click **OK**. See also Draw subdivided shapes

4. In the Add Zone Label window that appears, enter the name of the zone or accept the suggested name, then click **OK**.

   **TIP** When you have multiple arenas and want to give the same name to a zone defined in each arena (for example, the Feeder in multiple PhenoTypers), select **Use existing zone** and choose the zone name.


*Notes*

- The zone shape is filled with the color visible next to the zone name in the Arenas and Zones section. Click the color next to the name to change it.

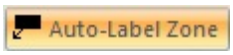



- To create quadrants in an existing arena, draw two perpendicular lines over the arena. Click the **Add zone Label** button and drag the label to one quadrant. Repeat this step for each quadrant.

## Auto-label zone

By default the option Auto-label zones is turned on (the **Auto-Label Zone** button on the toolbar is highlighted in orange). This means that every time you draw a closed shape, the zone label is added automatically.

To switch the auto-label option off, click the **Auto-Label Zone** button on the toolbar or press **A**.




If the auto-label option is off (the button is not highlighted in orange) and the zone has no name, click the **Add Zone Label** button on the toolbar, drag the label to the zone, then click the mouse button. Name the zone and click **OK**.

## To edit the zone

You can resize and re-shape the zone. See Work with shapes on page 257.

# Organize the zones in your arena

## Zone groups

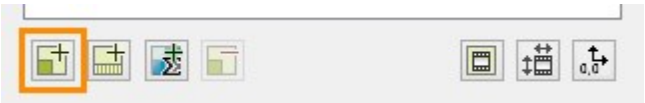
Zones are organized in zone groups. You can add an unlimited number of zone groups to each arena. Within one zone group, zones must be non-overlapping.

**IMPORTANT** If zones within a zone group are overlapping, the overlapping area is excluded from both zones.

## Zones that do not overlap

If two or more zones do not overlap, like the quadrants of a Morris water maze, then define them in the same zone group.

To create a new zone group, select the arena and then click the **Add Zone Group** button in the lower part of the Arena Settings window.



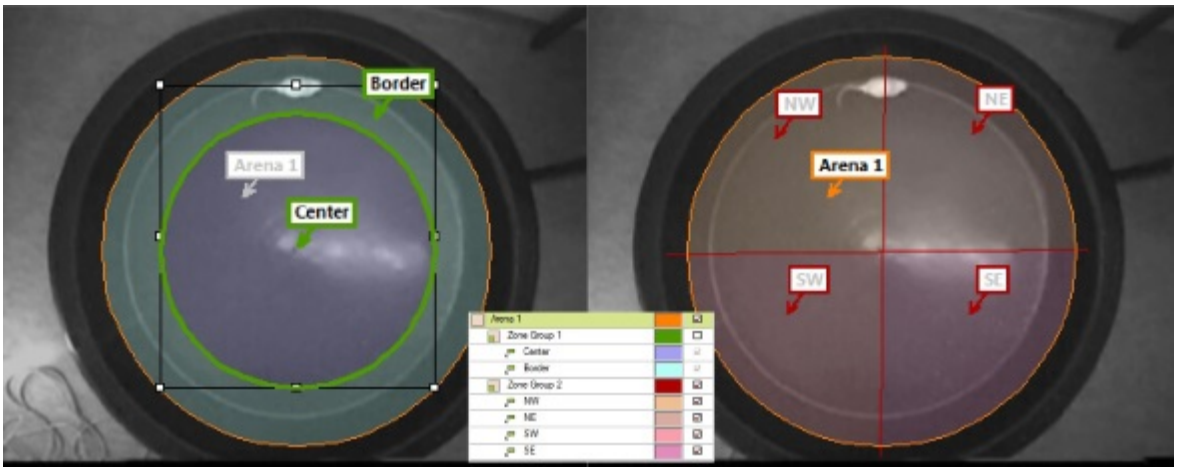
You cannot edit zone groups that contain zones that have been used in Trial Control (for example, to start tracking when the animal is detected in that zone) or have been used as hidden or entry zone. However, you can add new zone groups and there add zones. These zones then can be used, for example, for analysis.

To analyze zones in the same group as one, see Cumulative zones on page 239.

## Zones that do overlap

If you want to define zones that overlap, define them in separate zone groups or use cumulative zones.

**EXAMPLE** In a circular open-field, you want two circular zones (Center and Border) and four quadrant zones (NW, NE, SW, SE). Create two zone groups: one with the two circular zones, and one with the four quadrant zones (see below).


Border
Arena 1
Center
Arena 1
Zone Group 1
Center
Border
Zone Group 2
NW
NE
SW
SE
NW
NE
Arena 1
SW
SE

# Draw a point

## Aim

Define a reference point, for example to calculate the average distance of the subject to the point, or the heading angle.

## Procedure

1. In the Arenas and Zones section, select the zone group in which you want to add one or more Points.
2. Click the **Add point** button on the toolbar.


3. Click the background image to position the point. Note that you can also position a point outside the arena.
4. In the Add Point window, enter the point name and click **OK**.

# Validate the Arena Settings

## Aim

To check that the Arena Settings are consistent, after defining all arenas and zones. For example, that the arena is calibrated, that multiple arenas do not overlap, and that all zones are inside the arena.

## Procedure

Click the **4. Validate Arena Setup** button in the Arenas and Zones section.


If arenas and zones are created correctly, a message will be shown that Arena Settings are valid. If not, an error message appears.

# More about zones

## What do you want to do?

- Copy a zone
- Move an entire arena with its zones
- Define Cumulative zones
- Define Shelters and other hidden zones
- Define an entry zone
- Change the hidden zone subject coordinates
- Zones and points for Behavior recognition

# Copy a zone

## To copy a zone within the same arena

1. Right-click the shape and select **Duplicate**.
2. In the Duplicate window, enter an Enlargement factor and click **OK**.

## To copy a zone to another arena

1. In the Arenas and Zones section, select the zone group into which you want to add a zone.
2. Make a zone that covers the area of interest within the arena.
3. Click the **Label Zone** button on the toolbar and click in the shape you have just created.
4. In the Add Zone window, select **Use existing zone** and select one of the zones from the list.
5. Click **OK**.

## Notes

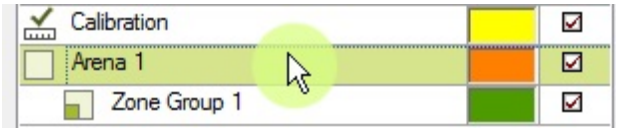
- You cannot copy individual zones from one arena to another. You can redraw the zone in the destination arena and use a label with the same zone name.
- **TIP** If you have multiple arenas with the same size and shape you can duplicate a complete arena including all zones. Then arrange the arenas, using the Multiple Arena Setup.

*See also*

- Duplicate an arena
- The Multiple Arena Setup

# Move an entire arena with its zones

1. In the Arenas and Zones section, click the Arena row to display the corresponding arena in the video image.




2. Drag the mouse cursor over the entire arena in the window on the left, to select all components. Then move the entire selection.

*See also*

- Move, rotate and resize a shape

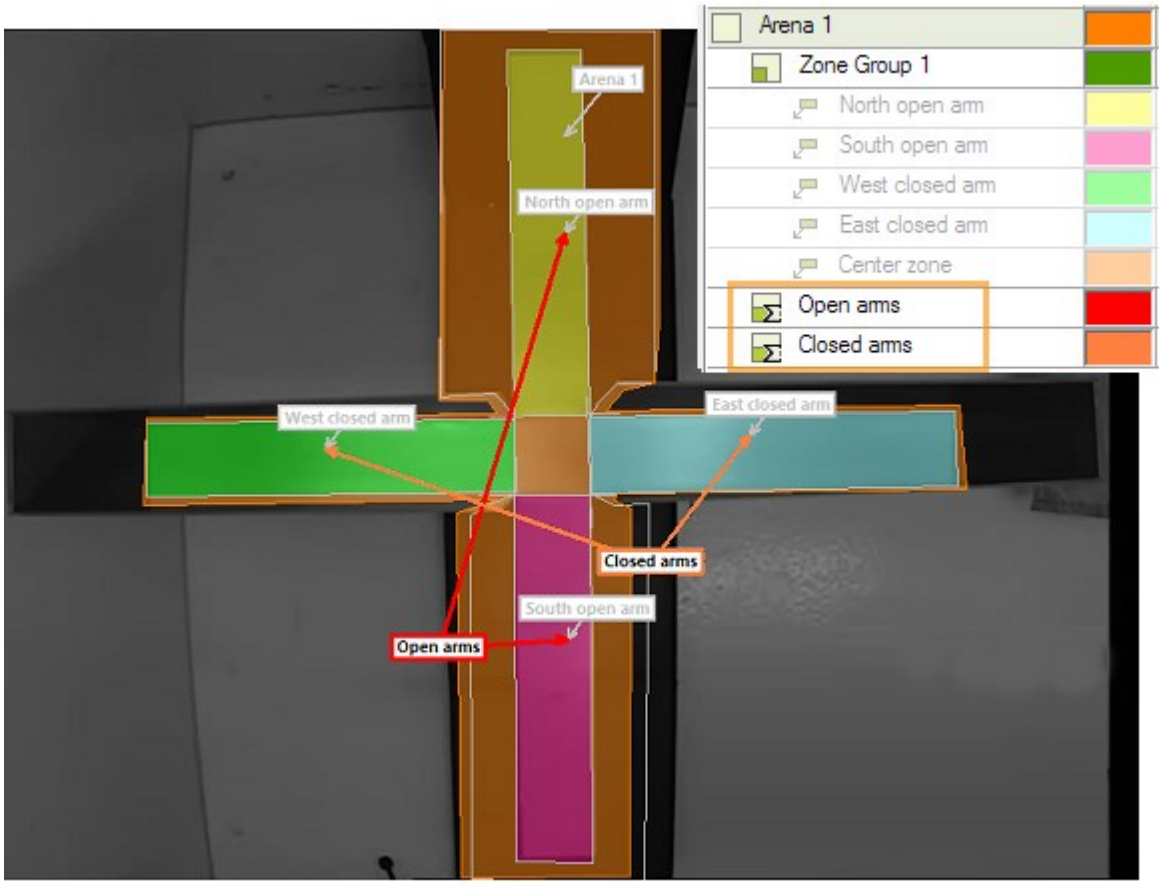
# Cumulative zones

## Aim

To analyze two or more zones, as one. With the procedure below you create a *cumulative zone*, using two or more existing zones.

**NOTE** The original zones are not deleted. The Cumulative zone is added as a new zone, outside the original zone group.

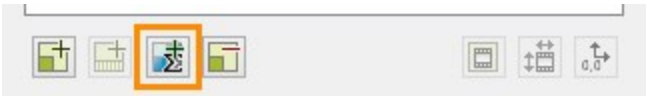
**EXAMPLE** In an Elevated plus maze test, you defined two open arms and two closed arms. To analyze the total time spent in either arm type, define the two open arms as cumulative zone *Open arms*, and the two closed arms as cumulative zone *Closed arms*.




You can add zones from different zone groups to a cumulative zone.

## Procedure

1. In the Arenas and Zones section, click a zone group in which you want to create a cumulative zone.
2. Click the **Add Cumulative Zone** button at the bottom of the Arenas and Zones section.



3. In the Add Cumulative Zone window you have two options:

   **Create new cumulative zone**. Type in the name of the new cumulative zone, click **OK** and select the zones in the window that appears.

   **Use existing cumulative zone**. From the drop-down list, choose the name of an existing cumulative zone in another Arena Settings.

## Duplicate a cumulative zone

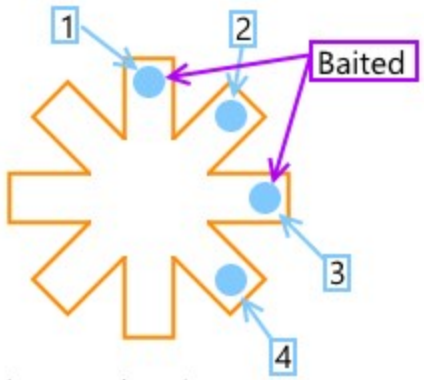
Use the option **Use existing cumulative zone** to replicate a cumulative zone that was defined in another Arena Settings profile, and you want to include it in a new Arena Settings.

Make sure that, when you do this, all the zones that make up the existing cumulative zone are also defined in the new Arena Settings.

**EXAMPLE** In a radial-arm maze test, four zones can be either baited or not baited. In some trials zone 1 and 3 are baited, in some other trials zones 2 and 4 are baited.

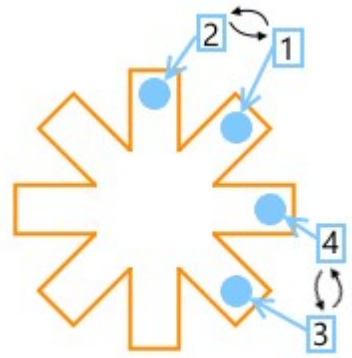
1. In Arena Settings 1, define all zones. Next, define a cumulative zone **Baited** with zones 1 and 3.

   **TIP** you can double-click the label **Cumulative zone 1** and rename it to **Baited**.



Arena settings 1

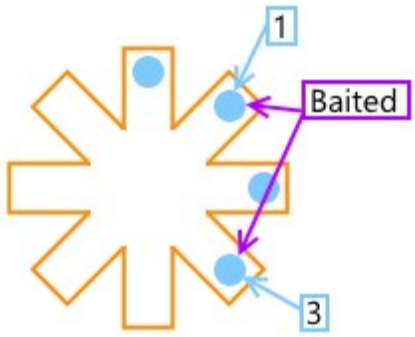
2. Create a new Arena Settings (or duplicate Arena Settings 2). Make sure the all relevant zones are defined.
3. Swap the labels for the zones.



Arena Settings 2

4. If you created Arena Settings 2 anew, create a cumulative zone and choose **Use existing cumulative zone**. Choose the existing cumulative zone from Arena Settings 1.

   If you have duplicated Arena Settings 1 to obtain Arena Settings 2, the cumulative zone is already pointing to the correct zones after you swap the labels.




Arena Settings 2

Note that the same physical zone is labeled with different names in the two Arena Settings profiles; take this into account when you analyze single zones.

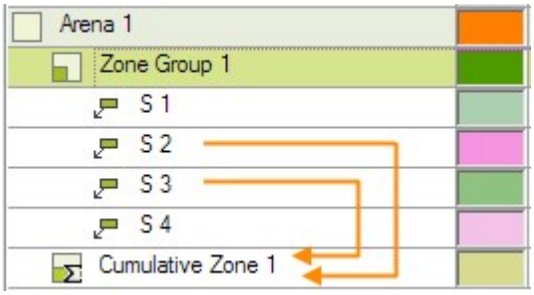
**IMPORTANT** We advise you not to rename the cumulative zone in Arena Settings 2 when pointing to physical zones other than those in Arena Settings1. If you do so the name also changes in Arena Settings 1, and that would create confusion.

## Notes

- A cumulative zone is not the same as a zone group.

  A zone group is one of the layers in the Arena Settings, where zones are always analyzed separately.

  A cumulative zone is just an extra zone, outside the zone group it origins from. For example, zones **S2** and **S3** form **Cumulative zone 1**.



# Shelters and other hidden zones

## Hidden zones and Entry zones

You can define a shelter or any zone where the animal is out of view as a Hidden zone. After you have defined a hidden zone, you must define at least one Entry zone for that hidden zone. The entry zone is used to determine whether the animal has entered or left the hidden zone.

## Entering a hidden zone

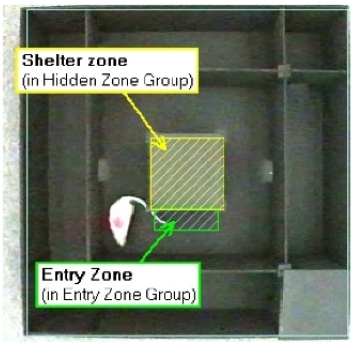
Suppose you have a square shelter defined as hidden zone and an entry zone on the side of the shelter where the animal can enter it (see the figure below, left). When the animal enters the entry zone, one or more body points are tracked in that zone (1). As soon as a body point is not detected anymore (2), that body point is assumed to have entered the hidden zone and this body point is positioned in the center of the hidden zone during the time the animal is inside the shelter.

## Leaving a hidden zone

By default, the animal is considered to have left the shelter when it is next detected, that is, when its body points are “not missing”. However, every time an animal pokes its nose out, this may be recorded as an exit (middle, 2). Subsequently, retracting back into the shelter - without ever really leaving the shelter - would count as entering again. This results in an overestimation of how often the animal enters/ exits the hidden zone. Therefore, EthoVision XT versions 5.1 and up have the possibility to also use the entry zone as leaving zone. The animal is then considered to have left the hidden zone when it was first considered to be in a hidden zone, and subsequently detected outside of the entry zone (see Right, 2).

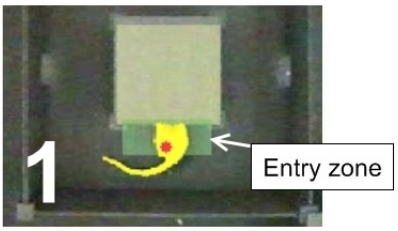
In other words: the exit is counted only when the body point crosses the border between the entry zone and the rest of the arena. We recommend you use this function if you want to prevent an overestimation of entrances into, and exits from the hidden zone.

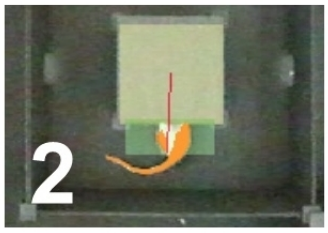
Definitions of the zones:



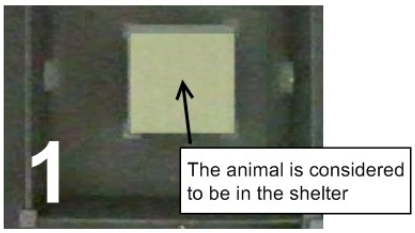

Detection:

When entering

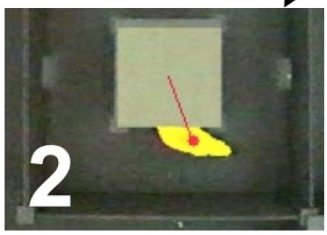


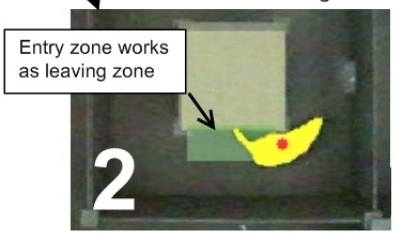

When exiting




A: Without Entry Zone
selected as Leaving Zone




B: With Entry Zone also
selected as Leaving Zone




Left: If the animal's center point is first detected in the entry zone, and then disappears (because the apparent size of the animal becomes smaller than the minimum size set in Detection Settings, then the animal is considered to be in the shelter.

Right: The detection for exiting the hidden zone differs for not using or using the entry zone as a leaving zone also, respectively. (A) The subject is detected as having left the hidden zone as soon as the minimum subject size is detected anywhere within the arena. Depending on the minimum size set, only poking out the nose could count as "exiting". (B) The subject is detected outside the hidden zone, but will only be recognized as "out of the hidden zone" as soon as it crosses the border of the entry zone into the rest of the arena.

## Define a hidden zone

1. At the bottom of the Arenas and Zones section, click the **Add Hidden Zone Group** button.

2. Create a zone covering the area you want to define as a hidden zone.

   If the Auto-label zones option is switched on, the zone is automatically named **Hidden Zone 1**. Right-click the name to rename it. If the auto-label option is switched off, click the **Add Zone Label** button on the toolbar, click the zone and rename the zone.

## Note

- If a hidden zone is included in a larger, overlapping zone, the time spent in the hidden zone is not included in the results for the larger zone. See a note under the topic In zone.

*See also*

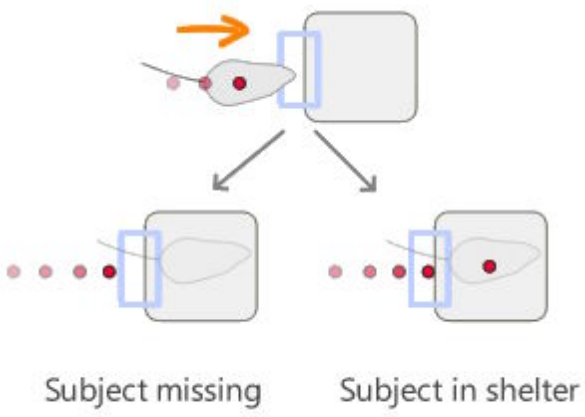
- Define an entry zone

# Define an entry zone

## Aim

To define a region of the arena where the subject must be immediately before moving into the hidden zone and disappearing from view.

It is important that at least one sampled location of the subjects' body point (in the example below, the center point) is found in the entry zone, for the subject to be considered in the hidden zone. Otherwise, the subject is considered missing. So, make the entry zone large enough!




See also the Troubleshooting topic:

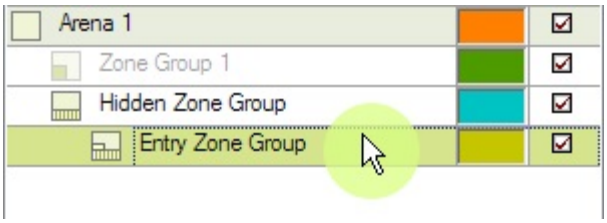
The animal is in the hidden zone but the data show "missing sample"

## Prerequisites

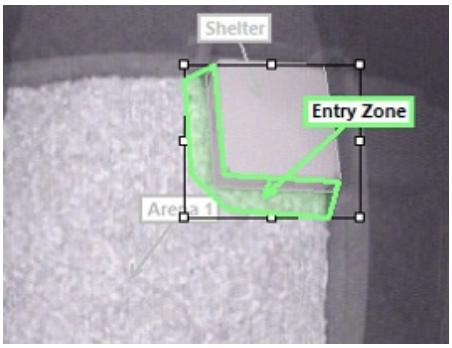
You have defined a Hidden zone. See Shelters and other hidden zones.

## To define an entry zone

1. In the Arenas and Zones section, select the **Entry Zone Group** row.



2. Draw a zone that covers the area, overlapping the hidden zone, to define as an entry zone.



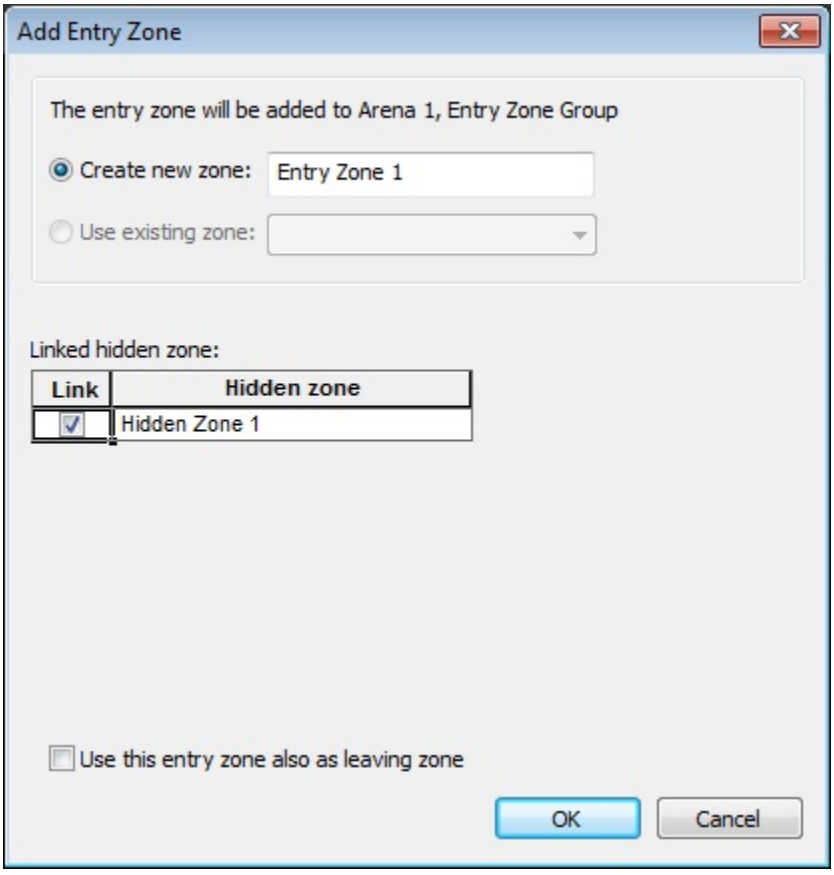

The size of the entry zone is critical, it must be wide enough, but not too wide so that:

*When entering*. The subject never goes straight away from the area outside the entry zone to the hidden zone; There should be at least one instance of the subject's body point within the entry zone, before disappearing from view. If necessary, increase your sample rate to make sure that this happens.

*When exiting*. The subject's body point is far, but not too far from the hidden zone when it crosses the entry zone border to the rest of the arena; just enough for it to be considered as having left the hidden zone. This only applies if you select **Use this entry zone also as leaving zone** (see step 7).

You can use the Integrated Visualization to help you set the right size.

3. If the Auto-label zone is activated, The Add Entry Zone window opens. If the auto-label option is switched off, click the **Add Zone Label** button on the toolbar and click the zone to open the Add Entry Zone window.




4. In the Add Entry Zone window, link this entry zone to the appropriate hidden zone by selecting the **Link** box under **Linked hidden zone**. You can link more than one entry zone to a hidden zone, but you cannot link more than one hidden zone to an entry zone.
5. If you want to define the entry zone also as a leaving zone, select the check box for **Use this entry zone also as leaving zone**. This will be applied on all hidden zones in your Arena Settings. Each entry zone will be also used as leaving zone for the hidden zone associated to it.
6. Click **OK** to assign the label to the entry zone.

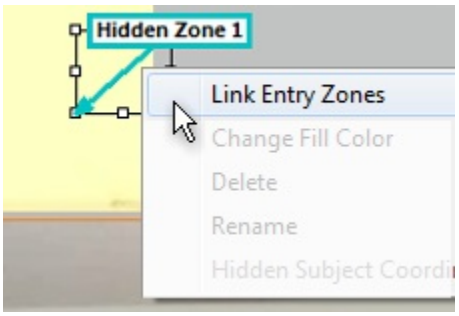
## Notes

- To display the hidden zone while drawing the entry zone, in the Arenas and Zones section, select the check box in the View column of the **Hidden Zone Group** row, next to the **Color** column.
- If you have multiple arenas, create first hidden zones and entry zones in the first arena, then duplicate that arena and its contents. See Duplicate an arena.

## To link a hidden zone to an entry zone

If you did not link an entry zone to its hidden zone when you defined the hidden zone (see above), you can do one the following:

- In the video image, right-click the hidden zone label or right-click the hidden zone row in the Arena Settings window. Select **Link Entry Zones** and select the **Link** check box of the appropriate entry zone in the Links to Entry Zones window.




- In the video image, right-click the entry zone label, or right-click the entry zone row in the Arena Settings window. Select **Link Hidden Zones** and select the **Link** check box of the appropriate hidden zone in the Link to Hidden Zone window.

# Change the hidden zone subject coordinates

## Aim

To specify the coordinates of the subject when it is in the hidden zone.

When a subject enters a hidden zone, its body points are assumed to be in the center of the hidden zone. However, if you have a very large hidden zone, the distance the subject moves from the entry zone to the center of the hidden zone every time it enters the hidden zone, contributes rather significantly to Distance moved. In these cases, you can "move" the coordinates of the subject near the entry zone.

## Prerequisites

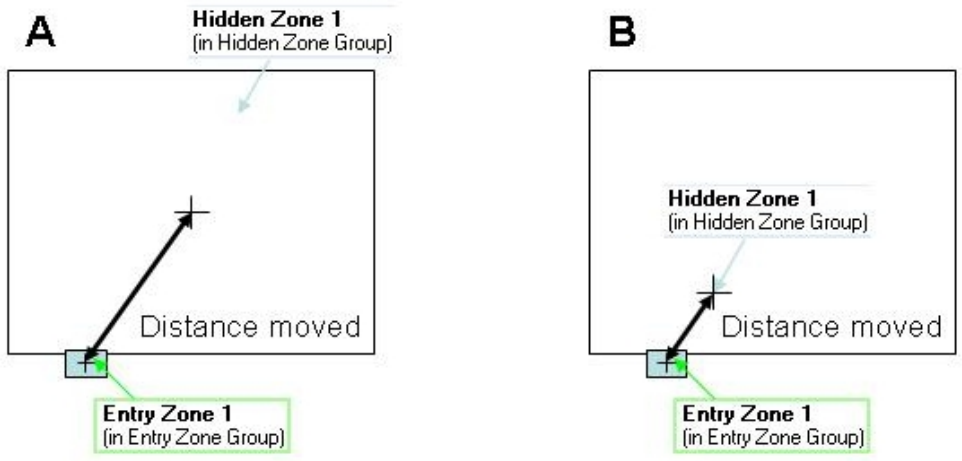
You have defined a hidden zone. See Shelters and other hidden zones.

## To set the hidden zone subject coordinates

1. Right-click the hidden zone label and select **Hidden Subject Coordinates**.
2. Now, select one of two options to set the hidden subject coordinates:

   **Center of the zone, unless the center is outside the zone (then it is the arrow tip location)**. Select this option when you have a small hidden zone (for an example, see **A**).

   **Arrow tip location**. Select this option when you have a large hidden zone (for an example, see **B**).



**A**. Default situation; the hidden subject coordinates are at the center of the hidden zone, thereby making a large contribution to the Distance moved.

**B**. The hidden subject coordinates are set to the arrow tip location, thereby minimizing the contribution to the Distance moved.

# Zones and points for Behavior recognition

## Aim

When you use the Rat/Mouse Behavior Recognition function, defining a few special zones and points significantly increases behavior recognition accuracy.

- Drinking spout points to detect drinking. See To define the drinking spout points
- Feeder zones to detect eating behavior at the feeder. See To define the feeder zones
- Wall zones to detect rearing to wall. See To define the wall zones

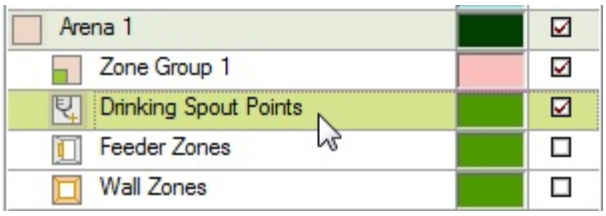
## Prerequisites

- You need the Rat or Mouse Behavior Recognition Module of EthoVision XT.
- You have read important notes about behavior recognition. See Behavior Recognition: Requirements
- In the Experiment Settings under Tracked features you selected Center-point, nose-point, and tail-base detection. Under Behavior Recognition, you selected one or more of the options **Feeder**, **Drinking bottle**, **Arena walls**. If one of the options is not selected, the corresponding layer is not added in the Arena Settings.
- Make sure that the outline of the arena covers all possible regions where all detected body points of the animal can be, including when rearing. See Draw the arena on page 224 for the procedure. Make sure that the aspect ratio of the video image is correct.

## To define the drinking spout points

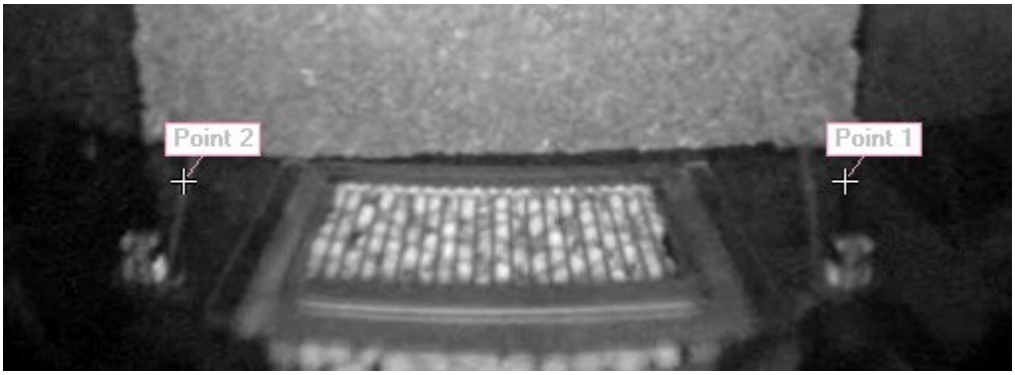
1. In the Arenas and Zones section, click **3. Select Shape and draw Zones (Optional)**.


2. Click the **Drinking Spout Points** zone group.



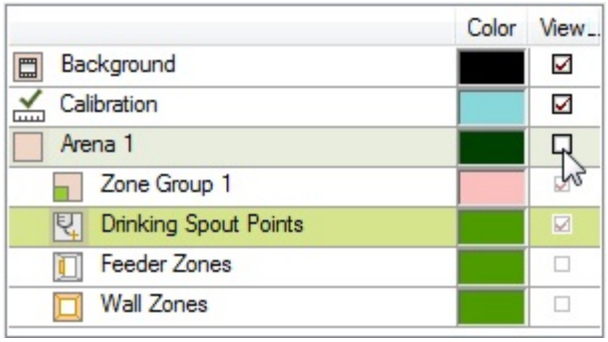

3. Click the **Add Point** button on the toolbar. The mouse pointer changes to a cross.


4. Click the background image to position the point.
5. In the Add Point window, accept the default name (**Spout 1** for the first point), or enter another name. Then click **OK**.




Repeat the steps above to define more points.

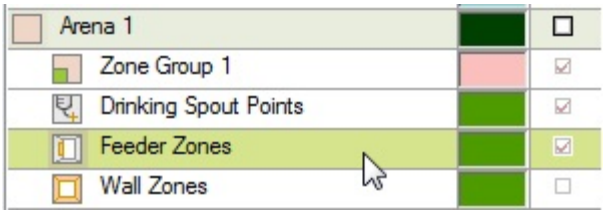
**TIP** To have a better view of the background image and locate the tip of the drinking spout more easily, in the Arenas and Zones section deselect the **View** option for the Arena and other zone groups.



## To define the feeder zones

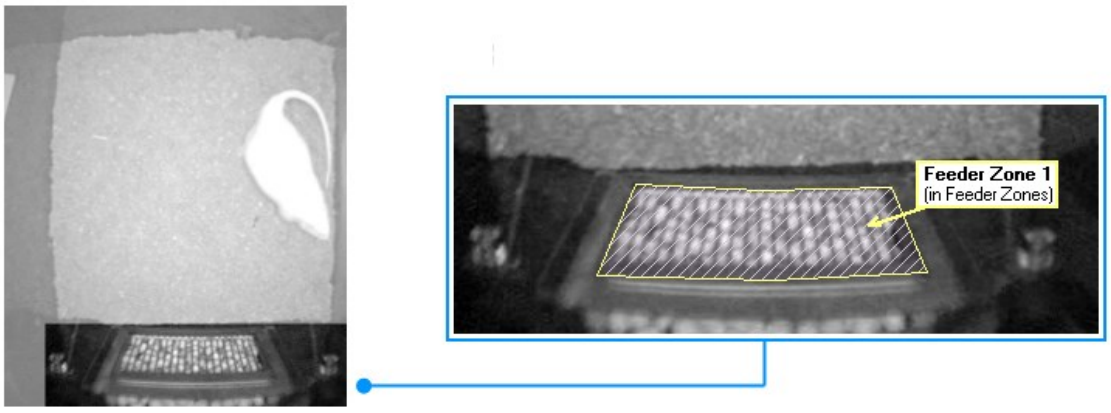
1. In the Arenas and Zones section, click **3. Select Shape and draw Zones (Optional)**.


2. Click the **Feeder Zones** zone group.




3. Using one or more drawing tools available on the toolbar, draw the outline of the feeder.
4. If the Auto-label zone option is activated, the zone is automatically named **Feeder Zone 1**. Right-click the label to rename it.

Repeat the steps 3-4 above to define more feeder zones.

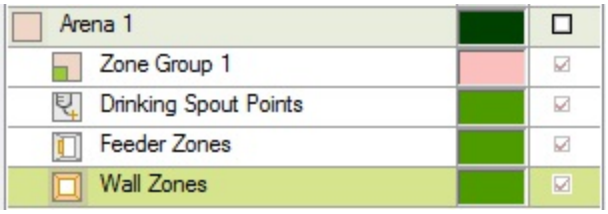



## To define the wall zones

All the high objects that the rat can use for supported rearing should be defined as wall zones.

1. In the Arenas and Zones section, click **3. Select Shape and draw Zones (Optional)**.

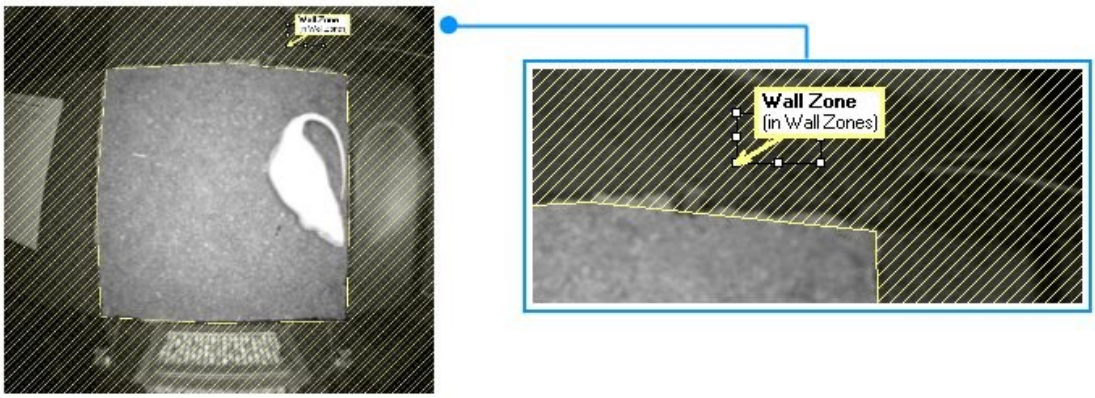

2. Click the **Wall Zones** zone group.



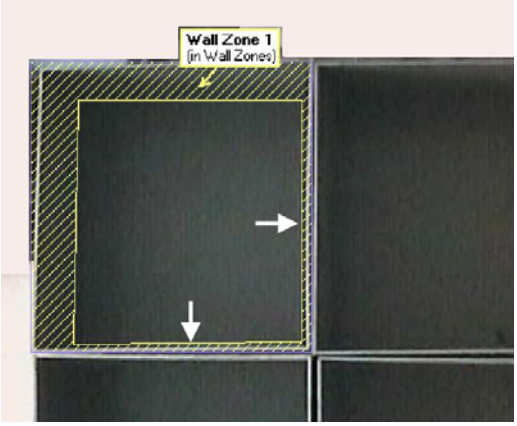

3. Using the polygon drawing tool, draw the outline of the floor of the arena.

   It is important that all sides of the arena are included in the wall zone, also when the side walls are barely visible.

4. If the Auto-label zone option is activated, the zone is automatically named **Wall Zone 1**. Right-click the label to rename it.
5. Make sure that the zone label points to the arena walls in the video image. If it points to the inside of the arena, move the label.




When the camera is not centered on the arena, like in a multiple arena setup, side walls may be partly or totally hidden. Draw the polygon in such a way that the wall zone also covers those regions.




## Notes

- In the Drinking Spout Points group, you can only define points, not zones.
- You cannot delete one of the zone groups Drinking Spout Points, Feeder Zones and Wall Zones. You can delete the zone/point label, but the zone group itself remains. To delete a group, deselect the corresponding option under Behavior Recognition in the Experiment Settings.

# Work with shapes

## What do you want to do?

- Draw simple shapes
- Draw subdivided shapes
- Move, rotate and resize a shape

# Draw simple shapes

## Line, rectangle, ellipse

1. Click the appropriate shape button on the toolbar  or press **L** for a line, **S** for a rectangle, and **E** for an Ellipse.
2. Click the background image to define the first point of the shape, keep the mouse button pressed, and drag to the end point. When ready, release the mouse button.

To create a circle with the Ellipse tool, hold **Shift** down.

## Polyline

1. Click the appropriate shape on the toolbar  or press **O** for an Open Polyline and **Y** for a Closed Polyline.
2. Click in the background image to position the first point, move the mouse pointer to the position of the second point and click in the background image. Next, move the mouse pointer to a new position for the third point, etc.
3. When ready, double-click.

## 3-point circle

1. Click the **3-point circle button** on the toolbar  or press **T**.
2. Click in the background image to position the first point, move the mouse and click to position the second point.
3. Move the mouse to position the 3-Point Circle and click to finish.

When you resize a 3-point circle, it becomes an ellipse. If you don't want that, press **Shift** while you resize the circle.

To show or hide the center of the bounding rectangle of a shape, right-click it and select **Show/Hide center**.

## To copy and enlarge a shape

1. Right-click the shape and select **Duplicate**.
2. In the Duplicate window, enter an Enlargement factor and click **OK**.

# Draw subdivided shapes

## Aim

To automatically divide a circle and a rectangular shape into a number of zones. This applies when:

- You draw an arena and you want to split it in equal zones like the four quadrants in a Morris water maze.


- Within an existing arena, draw a shape and divide it in a number of zones.

Result: Zones are automatically defined with labels S1, S2, etc. provided that the Auto-Label Zone option is active.

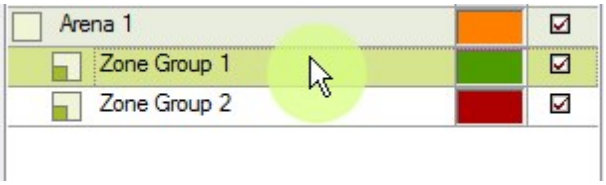
## To subdivide an arena in zones

This applies when you still have to draw the outline of the arena. See Make a subdivided arena.

## To divide a shape within an existing arena

1. In the Arenas and Zones section, click **2. Select Shape and Draw Zones (Optional)**.


2. In the Arenas and Zones section, click the zone group where you want to insert the zones that result from the subdivision.



3. Click one of the buttons on the toolbar:

   For circular shapes, **subdivided 3-point circle**  or press **I**.

   For rectangular shapes, **subdivided rectangle**  or press **U**.

4. Draw the shape.
5. In the Add Subdivided [Circle/Rectangle] window:

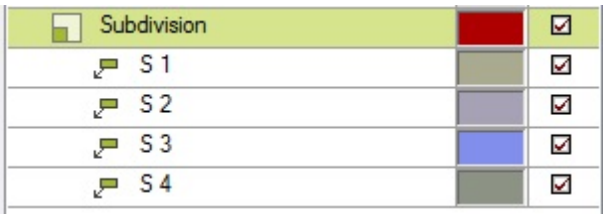
   For circular shapes, enter the number of sectors (for example, 4 for quadrants in a Morris water maze).

   For rectangular arenas, enter the number of rows and columns. A total of rows x columns equal zones will be defined.

Result: in the Arenas and Zones section, the zones are defined in the zone group that you clicked in step 2.

## The Subdivision Zones group

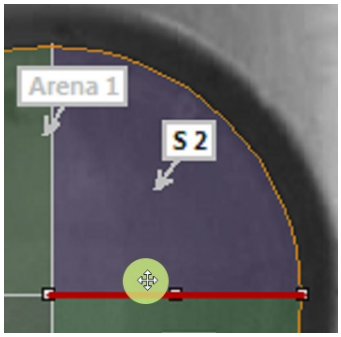
Subdivision Zones is a zone group that includes the zones created in a subdivided arena.




**NOTE** The Subdivision Zones zone group is not created when you subdivide a shape within an existing arena.

## Subdivision segments

A subdivided shape is defined by a number of segments. Move and resize a segment just like with other shapes.


Arena 1
S 2

# Move, rotate and resize a shape

**IMPORTANT** Whenever you edit an arena or zone, make sure the arrow point of the corresponding label stays within the shape.

## To move a shape

1. On the Arena Settings toolbar, click the **Normal mode** button or press **V**.

2. Move the mouse pointer over the shape, so the pointer becomes a move pointer. Then click the object and move it.

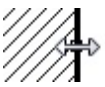
   

## To rotate a shape

1. Click the **Rotation mode** button on the Arena Settings toolbar or press **R**.
2. Move the mouse pointer over the shape, so the pointer becomes a rotation pointer. 
3. Click the object with the left mouse button, keep the mouse button pressed and move the pointer to rotate the shape.
4. Click **Normal mode** again when you are done rotating.

## To resize a shape (or multiple shapes)

1. Click the **Normal mode** button on the toolbar (or press **V**), and select the shape. For multiple shapes, drag the mouse around all shapes.
2. Use the handles to resize the shape.

   To resize a rectangle also at the opposite side, press **Ctrl** and move the resize handle.

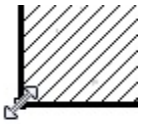
## To rescale a shape

If you rescale an shape, the size changes but the aspect ratio is maintained.

1. Click the **Normal mode** button on the toolbar (or press **V**), then select the shape. For multiple shapes, drag the mouse around all shapes.
2. Use the resize handles at the corners of the shape.

   To rescale the shape with the center point of the shape as the reference point, press **Ctrl** and move the resize handle.

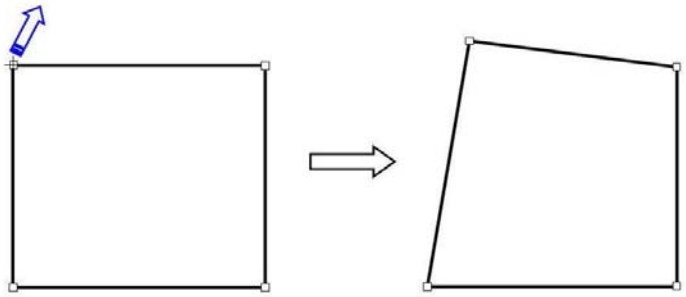
   To rescale the shape with the opposite corner handle as the reference point, press **Shift** and move the resize handle.



## To reshape a shape

1. Click the **Point Edit mode** button on the toolbar (or press **P**).

2. Click and drag the handles at the corners to a new position.



3. Select **Normal mode** when you are done reshaping.

## To add or remove corners in a shape

1. Click the **Point Edit mode** button on the toolbar (or press **P**).

2. To add a corner, click on the contour of the shape. To remove a corner, right-click the corner and select **Remove Point**.

# Work with multiple arenas

## What do you want to do?

- Calibrate multiple arenas
- Duplicate an arena
- Add and delete an arena
- Arrange arenas with the The Multiple Arena Setup

# Calibrate multiple arenas

When the video image includes two or more arenas, by default the calibration is shared. This means that the calibration is used for all arenas.

When you need more accurate calibration, for example when the distance between individual arenas and the camera varies, you can calibrate each arena individually.

## To make calibration unshared

To make the default shared calibration unshared and calibrate individual arenas, in the Arenas and Zones section, right-click **Calibration** and select **Unshare This Calibration**.

Next, calibrate each arena. See Calibrate the arena.

## To make calibration shared

In the Arenas and Zones section, right-click the calibration you want to share and select **Share This Calibration**.

# Duplicate an arena

## Aim

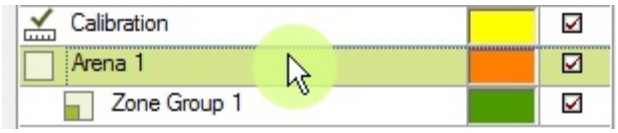
To make copies of an existing arena in the same video image. For example, in an experiment with four PhenoTypers, draw an arena for cage 1, and then copy that arena for the other three cages.

**IMPORTANT** Before duplicating an arena, make sure you have created all the zones for that arena.

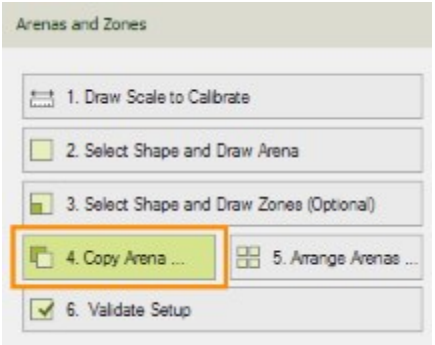
To define arenas in DanioVision experiments, see the DanioVision DVOC-0041 - Reference Manual, which you can open from the **Apps** screen under **Noldus** > **EthoVision XT 18 Other Documentation**.

## Procedure

1. In the Arena Settings window, click the row for the arena you want to duplicate.




2. Click the **Copy Arena** button, or right-click the row and select **Duplicate Complete Arena**.




3. In the Duplicate Arena window, select an option from the drop-down list. If you select the option All other arenas, also select how the arenas are arranged in rows and columns. The multiple arena setup now opens.
4. Select **Duplicate content to existing arenas** if you want to duplicate zones and points to the other arenas.
5. Move the duplicated arena to the appropriate location.

6. Repeat steps above for all consecutive arenas.
7. Adjust the position, shape and size of individual arenas. See Move, rotate and resize a shape.
8. If the arenas are evenly distributed in the video image, use the The Multiple Arena Setup to move, scale, rotate and space all arenas.

## Notes

- To duplicate an arena, you can also right-click the outline of the arena and select **Duplicate**.
- When you copy an arena, calibration is also duplicated if it is unshared. See Calibrate multiple arenas.
- An arena to which another arena is duplicated, loses its previous definitions and contents.
- If you select the option **Duplicate content to existing arenas**, the position of existing arenas is maintained. If you do not select this option, the position of each arena is adjusted based on the number of arenas and their layout. See The Multiple Arena Setup.

*See also*

- The Multiple Arena Setup to adjust all arenas simultaneously.

# Add and delete an arena

You cannot add or delete an arena in the Arena Settings. You can only delete the shape that defines the contour of the arena.

## To add or delete an arena

In the Experiment Settings, increase or reduce the number of arenas in the drop-down list next to **Number of Arenas**.

## Note

You cannot add/delete an arena in the Experiment Settings once you have carried out Acquisition in the Experiment.

# The Multiple Arena Setup

Use the Multiple Arena Setup to configure multiple arenas which are regularly distributed (for example, four open fields placed on a 2x2 grid). Taking into account the number of arenas and their layout (that is, the number of arenas on rows and on columns), EthoVision XT creates a virtual grid in which the arenas are initially evenly distributed. You can then configure the multiple arenas with the Multiple Arena Setup.

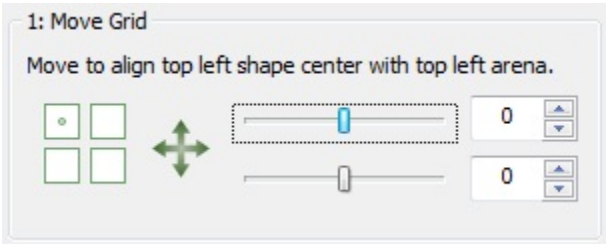
There are two ways to open the Multiple Arena Setup:

- The Multiple Arena Setup window opens automatically, when you copy an arena to all other arenas.
- In the Arenas and Zones section, click the Arrange Arenas button.

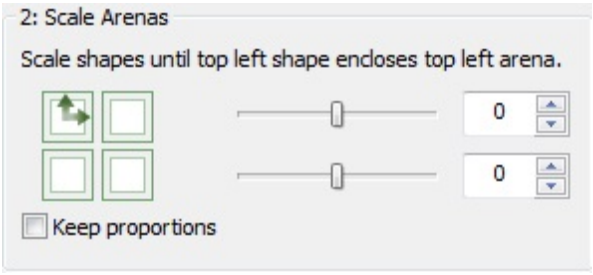
## Arranging arenas

The following options are available in the Arrange Arenas window:

1. **Move Grid**. You can move all shapes (including zone shapes) both horizontally and vertically, by using the slider, by pressing the up/down arrows of the corresponding box or by entering a number in a box.

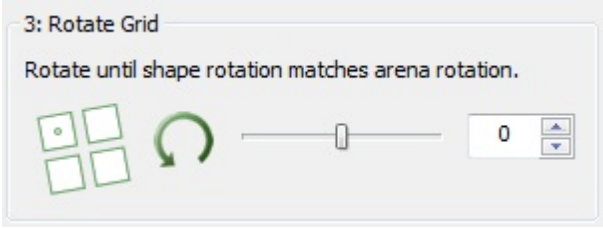



2. **Scale Arenas**. To resize all shapes by using the sliders, by pressing the up/down arrows of the corresponding box or by entering a number in a box.

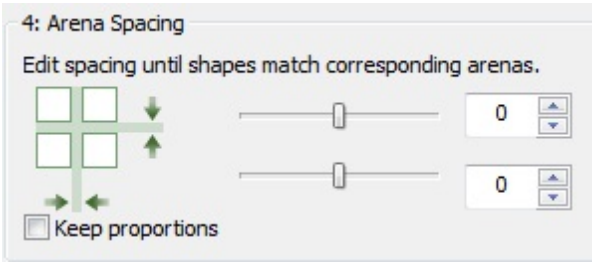



   By default the option **Keep proportions** is not selected. If you select this option, both the height and width of all shapes are changed while the original shape and position is kept. If you do not select the option Keep proportions, you can resize the height and width of all shapes separately.

3. **Rotate Grid**. To rotate all shapes. The shapes rotate around the geometric center of Arena 1.




4. **Arena Spacing**. To change the space between the shapes.




BY default, the option **Keep proportions** is not selected. If you select this option, spacing is done both in horizontal and vertical direction relative to Arena 1. If you do not select Keep proportions, you can change the space between the shapes separately in the vertical and horizontal direction (relative to the first row and first column, respectively).

## To reset the Multiple Arena Setup

Set the values back to zero. Make sure to deselect the option **Keep proportions**. Alternatively, press the **Cancel** button; this resets all configurations.

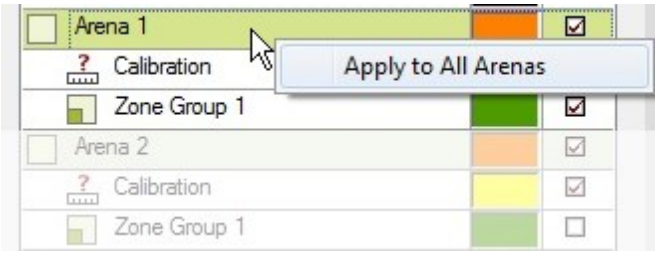
## To view details in multiple arenas

If you have multiple arenas, you can view or hide layers and labels of all arenas for better viewing. In the Arenas and Zones section, right-click an arena and select one of the options:

- To show/hide the border of all arenas, select **Show All Arenas** or **Hide All Arenas**, respectively.
- To show/hide the fill color and the label of all arenas, select **Show All Fills and Labels** or **Hide All Fills and Labels**, respectively.
- To show/hide a zone group or a zone, in all arenas, right-click that group or zone and select **Show in All Arenas** or **Hide in All Arenas**, respectively.

## To apply a color to all arenas

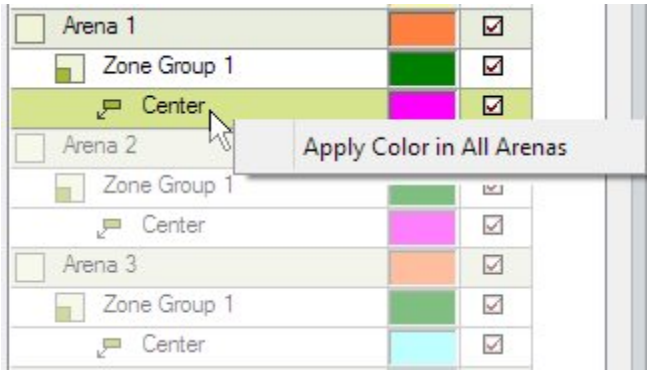
1. Change the color of one arena.
2. In the Arena Settings window, right-click the row of that arena and choose **Apply Color to all arenas**, or right-click the color field and select **Apply to All Arenas**.




## To apply the color of a zone in all arenas

This changes the fill color for a zone in all arenas where a zone is defined with the same name.

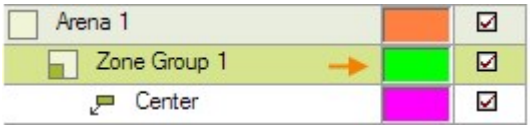
1. Make sure the zone is present in all Arenas, and has the same name for all of them.
2. In the Arena Settings window, change the fill color of the zone in one of the arenas.
3. Right-click the row of that zone and choose **Apply Color to all arenas**, or right-click the color field and select **Apply to All Arenas**.



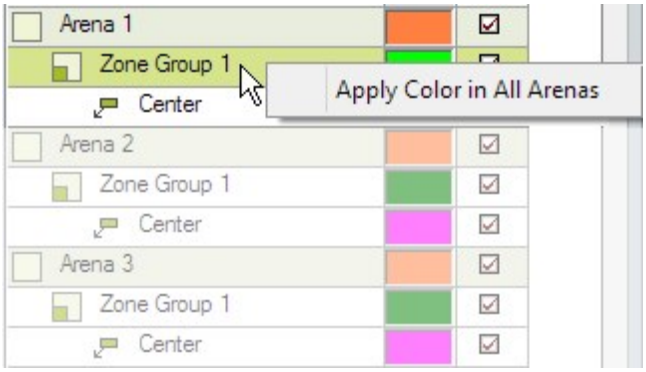

## To change the contour color of a zone in all arenas

**NOTE** The color of the contour of a zone depends on the color selected for the zone group (layer). By changing the contour color of a zone, you also change the contour color of all zones in that group.

1. In the Arena Settings window, change the color for the zone group the zone belongs to. To do so, click the color cell for the row with the zone group name.




2. Right-click the row of that zone group and choose **Apply Color to all arenas**, or right-click the color field and select **Apply to All Arenas**.



# Further information on Arena Settings

## Learn about

- The x-y coordinate system in the arena

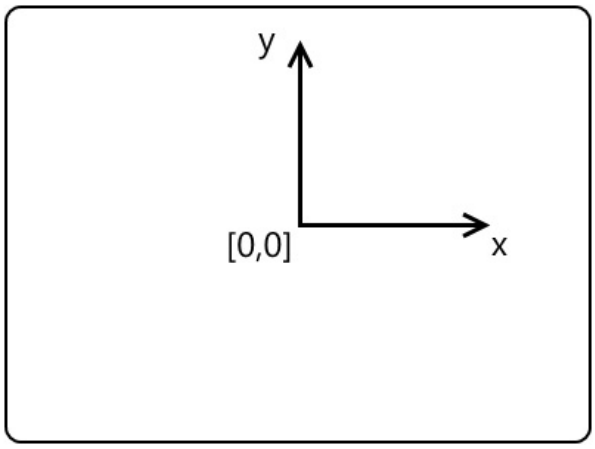
## What do you want to do?

- Modify the coordinate system of the arena
- Check the video aspect ratio
- Adjust the video aspect ratio
- Use the Arena - Hardware mapping

# The x-y coordinate system in the arena

## The default coordinate system

By default, the x-and y- coordinates of your track are expressed relative to the center point of the background image. The positive x-axis points to the right, and the positive y-axis points upward.



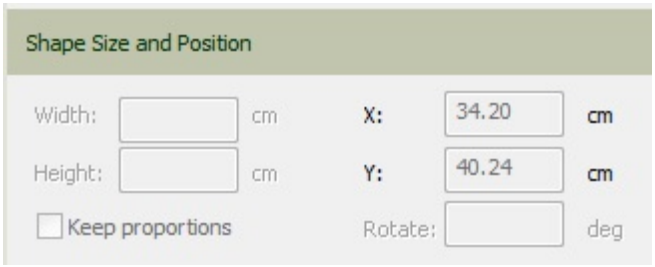

## Position of shapes in the coordinate system

After calibrating the arena, the **Shape size and Position** section at the bottom of the Arena Settings pane shows the real-world coordinates **X** and **Y** of the object currently selected.

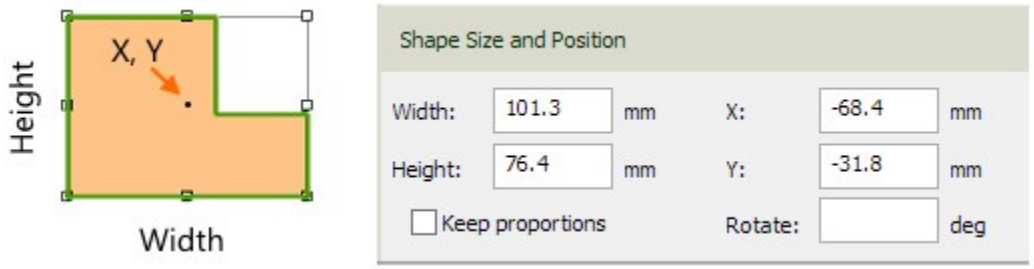
If the arena is not calibrated yet, the coordinates X and Y are given in pixels.

Note:

- If you click the calibration arrow line, the X and Y values are the coordinates of the center of the arrow.




- If you click an object like a zone, X and Y are the coordinates of the geometric center of the object. Width and Height are the width and height of the rectangle around the object, respectively.

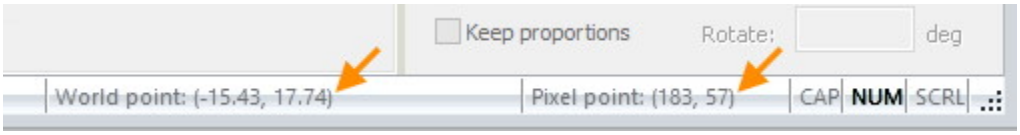



- Remember that the value of X and Y depend on where the origin (0,0) is placed. In the default coordinates system, a negative value means that the center of the object is to the left (for X) or below (for Y) the center of the background image. See Modify the coordinate system of the arena

## World point / Pixel point

In the Status bar, **World point** and the **Pixel point** indicate the current position of the mouse pointer. You can use this function to know the exact position of an object.

**TIP** If you do not see the Status bar, choose **View** > **Status bar**.




- **World point** gives you the coordinates of a point based on the calibration scale and origin, and the distance unit chosen in the Experiment Settings (see Units). The World coordinates can be positive or negative depending on where your axis origin is.
- **Pixel point** gives you the coordinates of a point in pixels. The 0, 0 coordinates correspond to the top-left corner of the video image. The Pixel coordinates are always positive.

# Modify the coordinate system of the arena

## Aim

To have the coordinates system different from the default one. For example:

- You want to export the real-world coordinates and you want these coordinates expressed relative to a point of reference in the real world that is not in the middle of your image.
- You want the dependent variables that use angle (that is, Head direction, heading, Meander, Turn angle), to be expressed relative to a reference line that is different from the default one (horizontal and pointing to the right). For example, you have an external cue to the north-east of your arena and you want to express the animal's orientation relative to this cue. In this case, you rotate the Calibration Axes so the x-axis points towards the cue.

Use the Calibration Axes object to specify the orientation of the real-world coordinate system and to specify the real-world coordinates for one pixel point.

## To modify the coordinates system

1. Click the **Create Calibration Axes** button at the bottom of the Arenas and Zones section.

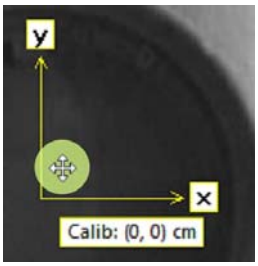
   

2. **OPTIONAL** In the Calibration Coordinate System window, type in the real-world origin of the x-axis **Origin X** and of the y-axis **Origin Y** relative to the current position of the origin on your screen. Do this if you know the origin of the coordinate system is outside the background image. Alternatively, skip this step and go to the next.

   **EXAMPLE** If you type 10 (cm) for Origin X, it means the origin [0,0] of your calibration system is shifted 10 cm to the left relative to the center of the video image.

3. Click and drag the Calibration Axes object to re-position the axes origin.

   To flip the y-axis of the Calibration Axes object, double-click the Calibration Axes object and select **Flipped Y-axis**. Move the Rotation mode arrow to rotate the calibration axes.




## To return to the default coordinates system

Select the Calibration Axes object and press **Delete**.

# Check the video aspect ratio

## The video aspect ratio

The video aspect ratio is the relation of the horizontal to vertical size of a video image. When you pre-record video from analog cameras, the aspect ratio of the resulting video files is not the same as that of the source camera. This results in a distorted background image. In such cases, a perfectly circular Morris water maze may look ellipse-like in EthoVision XT after you grabbed a background image from the video file.

## When is the video aspect ratio not correct?

The problem of the aspect ratio does occur:

- When you record video with a program other than EthoVision XT, like MediaRecorder, and from analog cameras.
- When you recorded video with EthoVision XT and you use that video in a new experiment.

If you record video and do tracking within the same experiment, the video aspect ratio is always correct.

## How do I know that the video aspect ratio is distorted?

- If the shape of the test enclosure as displayed in the background deviates from the shape that you expect. For example, a circular water maze looks like an ellipse, or a square open field looks like a rectangle.
- Place a sheet with a circle under the camera and grab this image as background image. In the Arena Settings, draw a three point circle. If the aspect ratio of the image is correct, this three point circle fits over the circle from the camera image.

See Adjust the video aspect ratio

## Calculate the correct aspect ratio in a video image

1. After grabbing the background image, locate the bitmap file of your background Image in the folder **Bitmap Files** of your experiment folder:

   C:\Users\Public\Public Documents\Noldus\EthoVision XT\Experiments\[experiment name].

2. Open the file in an image editing program (such as Paint.NET).

3. Draw a shape exactly around the arena. Note the dimensions of the shape you have just drawn.

If the ratio (for instance, width/height) of the dimensions of the drawn shape deviates from the ratio of the real-world dimensions of the arena, this means that the image is distorted.

**EXAMPLE 1** You have a perfectly circular water maze with a diameter of 100 cm. When you open the Background Image of this water maze in an image editing program and draw an ellipse around the maze, the ratio of width/height is not exactly 1 but is 1.019. This means that you need to adjust the aspect ratio of the Background Image using this ratio: in the Adjust Aspect Ratio window, in the **Custom** field, divide the width (box on the left) of the image by 1.019. Do not change the height.

**EXAMPLE 2** You have a square open field measuring 40 x 40 cm. When you open the Background Image of the open field in an image editing program and draw a rectangle around the open field, the ratio of width/height is not exactly 1, but is 0.8. This means that you need to adjust the aspect ratio of the Background Image using this ratio. In the Adjust Aspect Ratio window, in the **Custom** field, divide the width of the image (box on the right) by 0.8. Do not change the height.

*See also*

- Adjust the video aspect ratio

# Adjust the video aspect ratio

## Aim

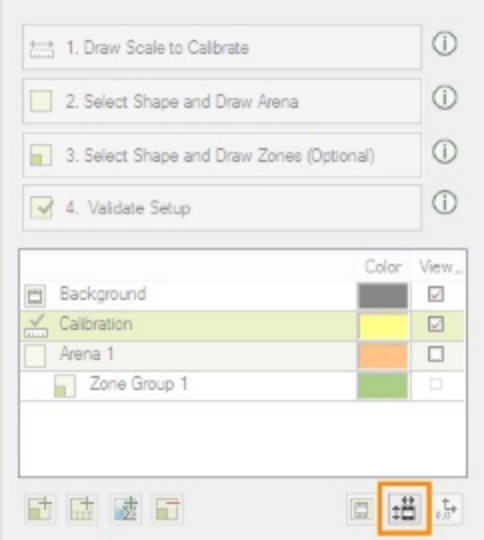
To correct for image distortion that sometimes occurs when recording video from analog cameras.

## Prerequisites

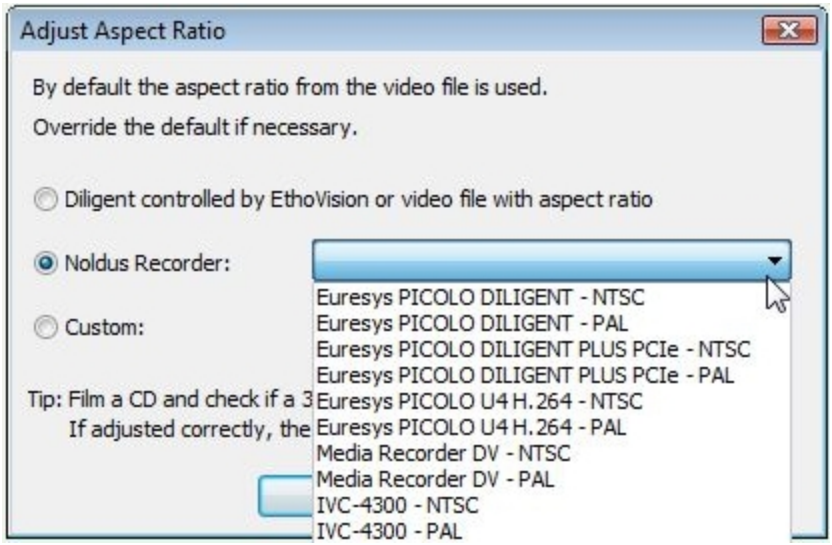
- You track from pre-recorded video files.
- You know that the aspect ratio in the video file differs from that of the original camera image.See Check the video aspect ratio

## Procedure

1. Open the Area Settings.
2. Click the **Adjust Aspect Ratio** button.




3. Choose the option based on the software and hardware you used (see below), then click OK.




## Options

*If you used Noldus MPEG Recorder 1.1/2.0, or MediaRecorder, and analog cameras*

- When you used MediaRecorder with a Picolo H.264 encoder board, choose **Noldus Recorder** and select **Euresys PICOLO U4 H.264**. Make sure you select the TV standard of the camera (**NTSC** or **PAL**).
- When you used an older encoder board (Picolo Diligent or IVC-4300), choose **Noldus Recorder** and select one of the other options.

*If you used MediaRecorder, and digital cameras*

- In most cases you do not need to correct the aspect ratio. However, if you are sure the video image is distorted, select **Custom** and enter the correct aspect ratio.

*If you used video files recorded with EthoVision XT, but in a different experiment*

- Select **Noldus Recorder** and select **Euresys Picolo U4 H.264**.

*If you used video files recorded with programs other than MediaRecorder:*

- Select **Custom** and enter the adjusted aspect ratio. See Calculate the correct aspect ratio in a video image

# Arena - Hardware mapping

If your experiment is set to use of Trial control hardware, the Hardware mapping section at the bottom of the Arena Settings pane is available. Click the **Arena-hardware mapping** button to assign hardware devices to each of your arenas.


If your experiment consists of only one arena, the hardware is automatically assigned to this arena, so you do not have to take further actions.

If you have more than one arena, assign the hardware to the arenas manually.

*See also*

- The section **Assign devices to arenas** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual. To open this manual, on the **Apps** screen choose **Noldus** > **EthoVision XT 18 Other Documentation**.

# Trial Control Settings

## Main topics and tasks



**IMPORTANT** This chapter describes the Trial Control functions of the EthoVision XT Base version only. For a detailed overview of conditions, creating sub-rules and controlling hardware devices via the USB-IO box, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual. To access this manual, on the Apps screen choose **Noldus** > **EthoVision XT 18 Other Documentation**.

# Introduction to Trial Control

## Learn about

- Why use trial control?
- Important terms in Trial Control

# Why use trial control?

## Start and stop data acquisition

Trial Control allows you to automate your experiment. Let EthoVision XT control the start and stop of data acquisition (tracking) depending on time, the behavior of the subjects and other conditions. For example:

- Start tracking when the subject is released and detected in the open field.
- Start tracking at exactly 12:30:00, and stop tracking after one hour.
- Stop tracking when the rat has reached the platform in the Morris water maze, or one minute after the start in any case.
- Stop tracking after the animal has been in the closed arms of the Elevated plus maze for five minutes.
- Stop tracking when the subject has explored an object for five minutes.

*See also*

- The Start-Stop trial rule

## Advanced Trial Control

With the EthoVision XT Trial and Hardware Control Module, you can also:

- Define conditioning routines for the subject, based on sequences of conditions and actions that are repeated a number of times (sub-rules).
- Control hardware devices, like a pellet feeder, or the arms of a radial maze.

Your EthoVision XT license must include the Trial and Hardware Control module.

For more information about advanced trial control options, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

# Important terms in Trial Control

## Trial vs. track

A *Trial* is a single, uninterrupted acquisition session. The trial can be viewed as a container for the data collected in one recording session. It starts when you give the Start command in acquisition and stops when the tracks for all arenas and subjects have stopped.

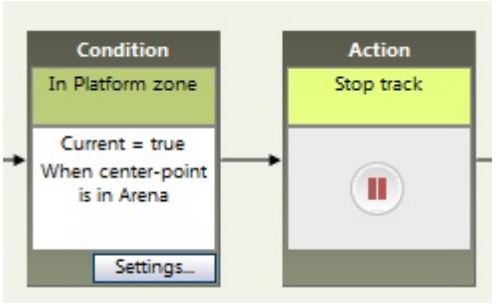
A *Track* is the actual recording of a subject's position and behavior. The start of a track may or may not coincide with the start of the trial. This depends on your Trial Control Settings. If you use the default Trial Control Settings, the track starts 1 second after the animal has been detected in the arena and stops when you stop the trial.

## Conditions and actions

A Condition is a statement that EthoVision evaluates. A condition can be based on time, presence of the subject in a zone, any variable that quantifies behavior, or the state of a device connected to EthoVision XT.

An Action is a command executed on a variable or a hardware device. You can therefore control your experiment by linking conditions with actions.

**EXAMPLE** In a Morris water maze test, stop tracking when the rat is detected on the platform (provided that the platform has been defined as a zone). The action is Stop tracking and the condition is Rat detected on the platform. This is represented by the following:




A condition is followed by an action. The condition checks that the animal is in the zone named “Platform”. The action “Stop track” is taken when the condition is met.

# The Start-Stop trial rule

## Learn about

- The default Start-Stop Trial rule

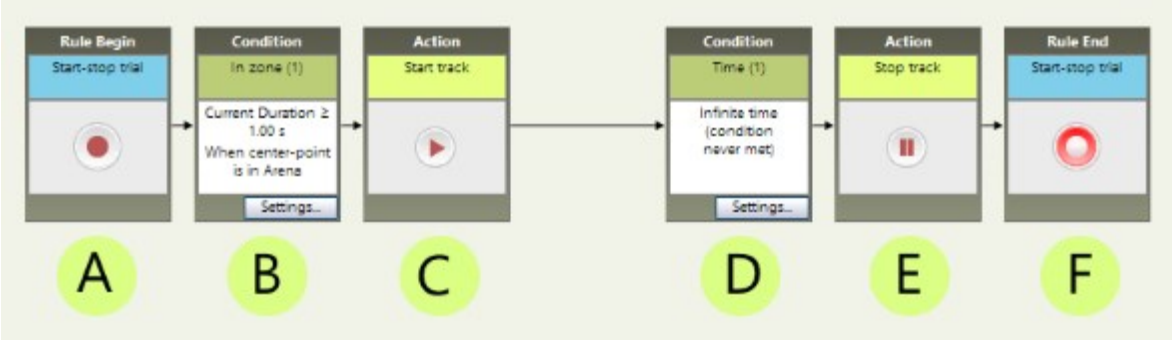
## What do you want to do?

- Control the start of tracking
- Control the end of tracking
- Split a multi-day test in multiple trials

# The default Start-Stop Trial rule

## How to access the Start-Stop Trial rule

- Choose **Setup** > **Trial Control Settings** > **Open** and select an existing Trial Control Settings.
- In the Experiment Explorer, click one of the Trial Control Settings.




With this rule, you control the start and stop of data acquisition (tracking).

## What the Start-Stop Trial rule means

The sequence of boxes A to F can be read as follows:

1. When you start the trial, for example by clicking the Start Trial button, the box **A** (**Rule Begin** - **Start-stop trial**) is activated. Control passes immediately to the box B.
2. EthoVision XT checks that the condition in the box B (**Condition** - **In Zone**) is *true*. The condition states that the center point of the subject (or of any subject in the same arena) must be detected in the arena for an uninterrupted period of one second.

   When you start the trial and the subject is not yet in the arena, the condition is *false*. EthoVision XT waits until the subject has been detected for one second. At that time, the condition becomes *true*. Control passes immediately to the box C.

   **NOTE** In special cases this box is absent or has different settings. See The start-stop trial rule in special cases
3. When the condition B becomes true, the box **C** (**Action** - **Start track**) is activated, and tracking starts.
4. Control passes to the box **D** (**Condition** - **Time**). EthoVision XT checks that the condition is met. This condition is set to wait indefinitely, so that

tracking stops when you give the stop command, or when the end of the video file has been reached, or when the maximum trial duration has been reached. See Control the end of tracking

5. When one of the events specified above occurs, the box **E Action** - **Stop track** marks the end of the tracks in the arena.
6. The box **F Rule End** - **Start-stop trial** marks the stop of the trial; it does not take any action.

## Notes

- If the condition in box **B** is not met immediately, the time that tracking starts (**C**) is different that the time that the trial starts.
- The Start-Stop trial rule only works when you acquire data (live or offline). If you only record video, the Start-Stop trial rule is inactive. You must start and stop video recording manually.

*See also*

- Trial vs. track
- How the trial control instructions are executed
- The start-stop trial rule in special cases

# The start-stop trial rule in special cases

## Multiple arenas

When multiple enclosures are defined as separate arenas, the default Start-Stop trial rule is applied for each arena separately. This means that condition in box B is applied separately for each arena. As soon as an the subject is detected in an arena, tracking starts for that arena, not the others. As a result:

- This way you do not have to release all the animals at the same time.
- Tracking may start at different times in different arenas in the same trial, depending on when a subject is released in its arena.

*See also*

- Trial control in multiple arenas

## Multiple subjects in the same arena

When multiple subjects are present in the arena, tracking starts when the first subject is found. Therefore, tracking starts for all subjects at the same time, even if the others are not yet in the arena. For those subjects, the track starts with a number of missing samples, until the subject is found.

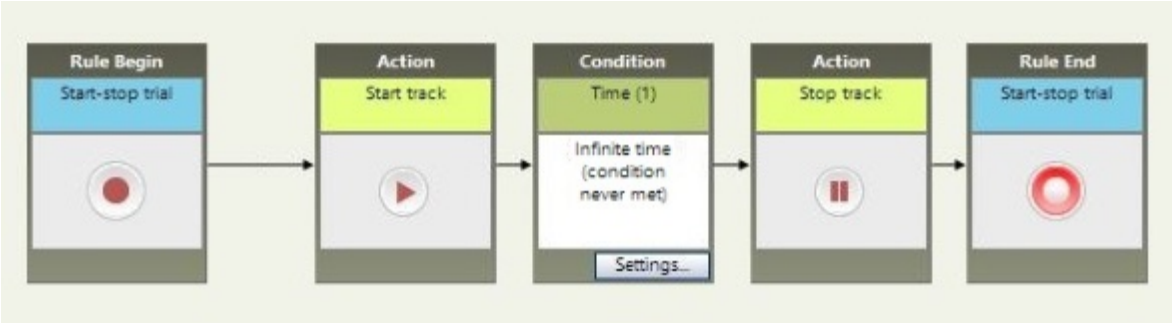
To start tracking only when all subjects are found in the arena, and thereby avoid missing samples at the beginning of the track, see Start the trial in the Social interaction test.

## With DanioVision

If you create a DanioVision experiment, the Condition - In zone box is not present. Tracking starts as soon as you start the trial.

## With Activity analysis

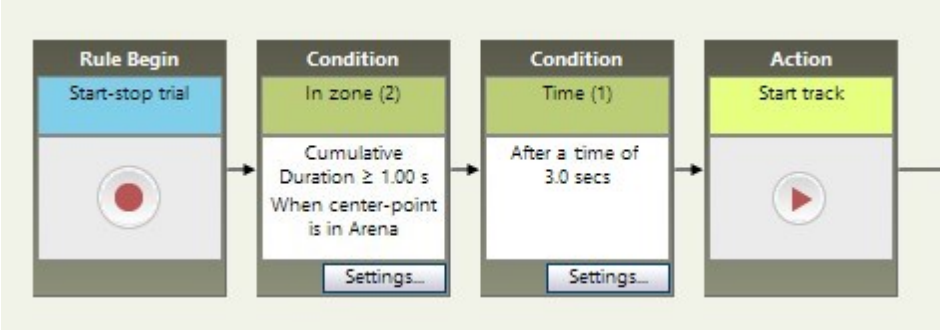
If you select **Activity analysis** under **Analysis Options** in the Experiment Settings, the Condition - In zone box is removed from the default Start-Stop rule. To carry out tracking and activity analysis simultaneously, and start tracking when your subject is detected in the arena for a specific time, insert a new In Zone condition box in the Start-Stop rule.




**NOTE** If you also select **Behavior recognition** in the Experiment Settings, the Start-Stop rule is as described below.

## With Behavior recognition

If you select **Behavior recognition** under **Analysis Options** in the Experiment Settings, a Time condition is added between the Condition - In zone box and the Action Start Track box. This means that EthoVision XT waits 3 seconds after detecting the animal for the first time, before starting actual tracking. This is done because the behavior recognition algorithms need a number of video frames equivalent to about three seconds before the current frame in order to recognize behavior.



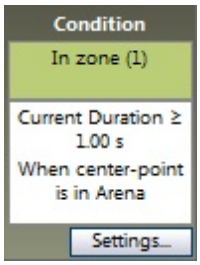

Part of the Start-Stop trial rule of the Trial Control Settings when selecting Behavior recognition in the Experiment Settings.

The time condition “After a time of 3.0 s” is removed automatically from a Trial Control rule if you deselect **Behavior recognition** in the Experiment Settings.

# Control the start of tracking

## To modify the Start track condition

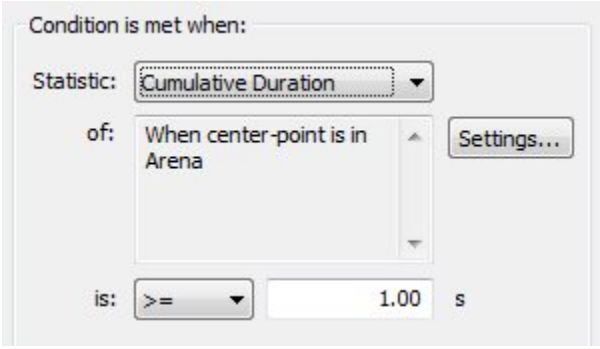
The default Start track condition is a condition based on the variable *In zone*. The condition becomes true when the subject is detected in the arena for one second.




1. Click the **Settings** button.
2. In the window that appears, specify in which zone the subject should be to start tracking. Click **Settings** and choose the zone. See details about the variable In zone
3. Specify the criterion (time, frequency in the zone etc.). From the **Statistic** list, choose one of the statistics. See the details below.

*Statistics available*

- **Current Duration** or **Cumulative Duration** to start tracking when the animal has been detected for some time in the zone. Current Duration is an uninterrupted period of time (for instance, the animal must be detected for 1 s without interruption). Cumulative Duration sums up all periods when the animal is in the zone since the start of the trial.




- **Current** to start tracking as soon as the animal is detected (one sample is sufficient). Note that this condition may become true also when noise is occasionally detected as subject for a very short time, before the animal is released in the arena. Make sure that this does not happen.

- **Frequency** to start tracking when the animal has visited the zone a number of times.
- **Latency to First** to start tracking when the latency to its first visit to the zone is less or greater than a specific time.

## To use a different Start track condition

Click the Condition - In zone box and press **Delete**, then choose another condition (see the examples below).

**EXAMPLE 1** Start tracking exactly 1 minute after clicking the Start Trial button.

1. Click the Condition - In zone box and press **Delete**.
2. Under **Conditions**, click the button next to the variable you want to use (Time in this example), and select the options you require.
3. Insert the new box in the sequence and re-connect the boxes. See Connect two boxes.

**EXAMPLE 2** Start tracking at a time you are not in the lab, for example at 23:00 h.

1. In step 2 above, click the button next to **Time**, choose **After clock time** and enter 23:00:00.
2. Before leaving the lab, choose **Acquisition** > **Open Acquisition** and click the **Start trial** button. The program waits till 23:00 to start tracking.

See also Conditions on page 314.

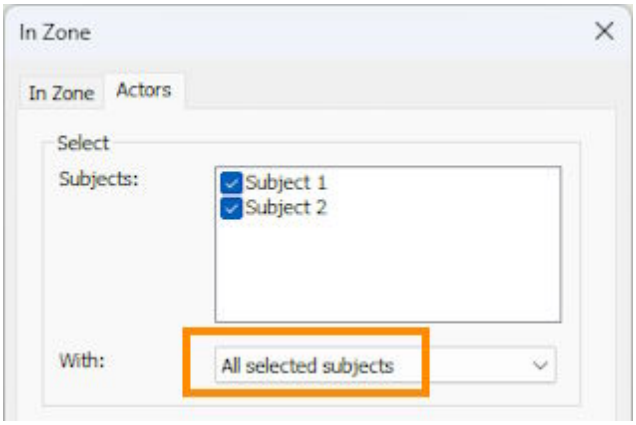
## Examples

**EXAMPLE 1** Start tracking immediately when you start the trial

1. Click the condition box and press **Delete**.
2. Reconnect the first box with the second one. See Connect two boxes

**EXAMPLE 2** Start tracking when both (all) subjects are in the arena

1. Here we assume that the experiment is set to multi-subject tracking.
2. Click the **Settings** button in the condition box.
3. In the window that appears, click the **Actors** tab and select the subjects that should be in the arena at the start of tracking.
4. Select **All selected subjects**.




*See also*

- Details about the variable In zone
- Control the end of tracking

# Control the end of tracking

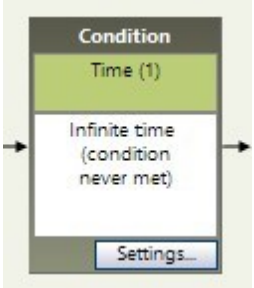
## What do you want to do?

- Stop tracking after some time
- Stop tracking based on the behavior of the subject
- To set a maximum duration for your trials
- Stop video recording after some time

## Stop tracking after some time

This option allows you to create tracks of fixed length.

The default Stop track condition is a Time condition.




Click the **Settings** button, and choose the option you require.

- To stop tracking after some time, choose **After a time of** and set the time. For example, set 5 min if you want to track subjects for five minutes.
- To stop tracking at a specific clock time of the same day, choose **After clock time** and set the time required. To stop tracking after the current day, you must have the Trial and Hardware Control module.

## Stop tracking based on the behavior of the subject

1. Click the Time condition box and press **Delete**.
2. Under **Conditions**, choose the variable that describes the behavior and click the corresponding button.

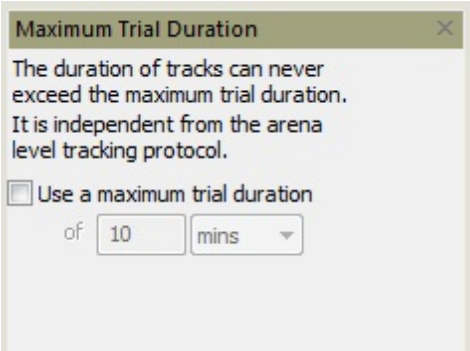
   **EXAMPLE** To stop tracking when the subject is in a zone, click **In zone**.
3. Configure the variable and insert the new box in the Start-Stop trial rule. See Insert a box in a Trial Control sequence

## To set a maximum duration for your trials

If the conditions to stop the trial are never met, EthoVision XT waits indefinitely and the trial never ends. To prevent this from happening, define a maximum trial duration.

**EXAMPLE** In a Novel object test, if the Stop track box is preceded by a condition "when the subject enters the zone with the familiar object", it may happen that the subject completely ignores the familiar object and only pays attention to the novel object. In that case the condition to stop tracking is never met.

In the Maximum Trial Duration pane, select **Use a maximum trial duration** and enter the maximum duration of the trial (in hours, minutes or seconds).




For information about the duration of trials tested with specific cameras and computers, see Resolution, frame rate, and maximum trial duration

Longer trials and videos are possible in principle but they have not been thoroughly tested.

## Stop video recording after some time

All the options above refer to stopping the trial when you do tracking. If you record video and then acquire the tracks (see Record video, then acquire a trial), the Maximum Trial Duration option won't work. This is because the Trial Control Settings, including the Maximum Trial Duration option, are not active when EthoVision XT just records video without acquiring data. You must stop video recording manually.

An alternative strategy is the following:

1. Set the Maximum trial duration (see above).
2. Acquire a trial live + save video.
3. The saved video has a fixed length. Now you can use that video to re-do tracking in the same experiment, or use that video in another experiment (set to offline tracking).

## Notes

- Keep at least one condition between **Start track** and **Stop track**. If you do not do this, tracking stops immediately after tracking starts, resulting in no data.
- If you do not see the Maximum Trial Duration pane, click the **Show/Hide** button on the toolbar and select **Maximum Trial Duration**. If the text in this pane is grayed out, it means that Trial Control Settings are read-only. Open another Trial Control Settings profile or create a new one.
- Instead of using a maximum trial duration, you can also define a condition based on time and place it immediately before the Stop track box. This way you set the exact duration of the tracks. See Stop tracking after some time
- For more information on conditions, see **Overview of conditions** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

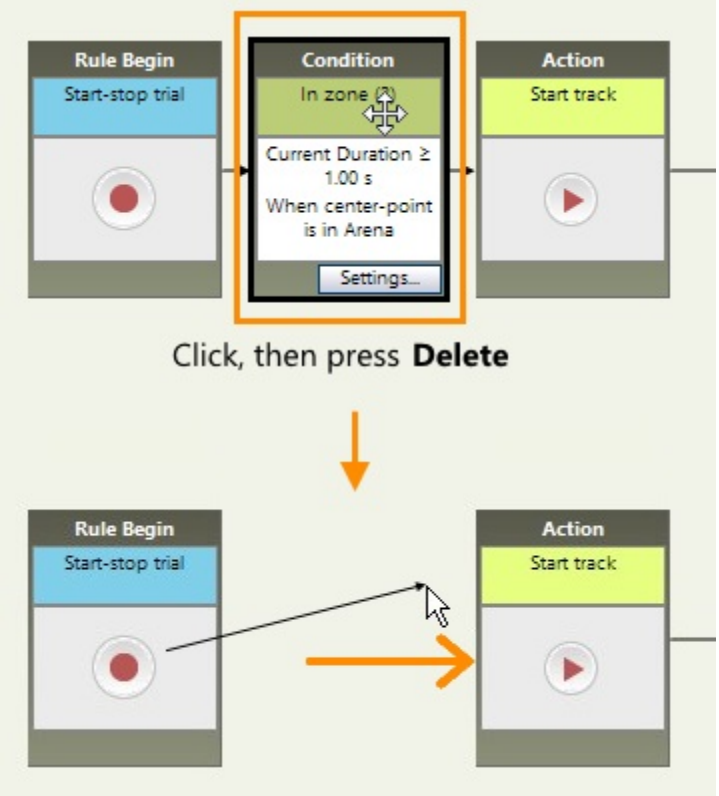
# Split a multi-day test in multiple trials

## Aim

To acquire trials longer than the maximum duration that is tested at Noldus. See Cameras supported by EthoVision XT > Resolution, frame rate, and maximum trial duration.

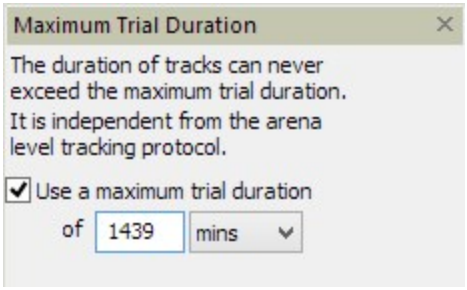
## Procedure

1. In the Trial Control Settings, remove the condition immediately before the **Start track** action box.




2. In the Trial Control Settings, set the maximum trial duration. For example 24 hours. See Control the end of tracking

   **TIP** Set this time to a little less than 24 hours, for example 1439 min (= 24 h minus 1 min). The remaining minute is used as inter-trial interval (see below). You can also choose a shorter inter-trial interval if you like.

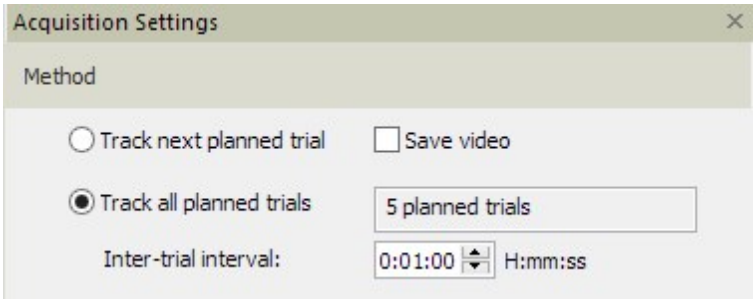



3. Choose **Setup** > **Trial List**. Add a number of trials that cover the total duration of the test for the subject.
   - **EXAMPLE 1** One arena. Each subject is tested over five days. Therefore, add five trials for that subject.
   - **EXAMPLE 2** Subjects in four PhenoTypers (arenas) are tested over five days. Add five trials. Each trial contains data of one day and the four arenas.

   **TIP** Only add the trials for the current subject(s), not for the subjects that come next in the protocol. Because you will use the series of trials (see below), in most cases with a short inter-trial interval you won't have time to clean the cages and release the next subject.

   **TIP** Add a variable "Subject ID" to the Trial List, and enter the ID of the subjects to be tested. So you know which trials refer to which subject.

4. Choose **Acquisition** > **Open Acquisition**. Under **Method**, select **Track all planned trials** with an **Inter-trial interval** of 1 minute. This is done to make sure that EthoVision XT has the time to save the data. If you want to use a shorter time, test it beforehand.




5. Acquire the data.
6. In step 3, add the trials for the next subject(s), and repeat the procedure.
7. In the Data profile, group the trials based on the subject ID. This way you can analyze a multi-trial test as one trial. See Define groups of tracks

# Examples of Start-Stop trial rules

## What do you want to do?

- Start/stop the trial in the Morris water maze test
- Start the trial in the Social interaction test
- Stop the trial in the Eight-arm radial maze test
- Start and stop the trial at specific clock times

# Start/stop the trial in the Morris water maze test

## Aim 1

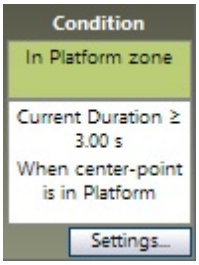
To stop tracking when the animal has found the platform.

## Procedure

1. In the Arena Settings, make sure that the platform has been defined as zone.
2. In the Trial Control Settings, delete the Time condition before the Stop track box.
3. Define an In Zone condition. See also Stop tracking based on the behavior of the subject

   If you want the program to stop recording as soon as the animal is over the platform, select Frequency as Statistic and choose >= 1. Click **Settings** and select the platform zone. See details about the variable In zone

   Sometimes the animal swims over the platform, but it does not stop there. In such cases the program would stop recording while the animal has not 'found' the platform. Instead of selecting **Frequency**, choose **Current Duration** and the minimum time the animal must stay on the platform (for example, 3 s) without interruption. Click **Settings** and select the platform zone.




## Aim 2

To stop tracking either when the rat has found the platform, or when it has been swimming in the water maze for 60 seconds.

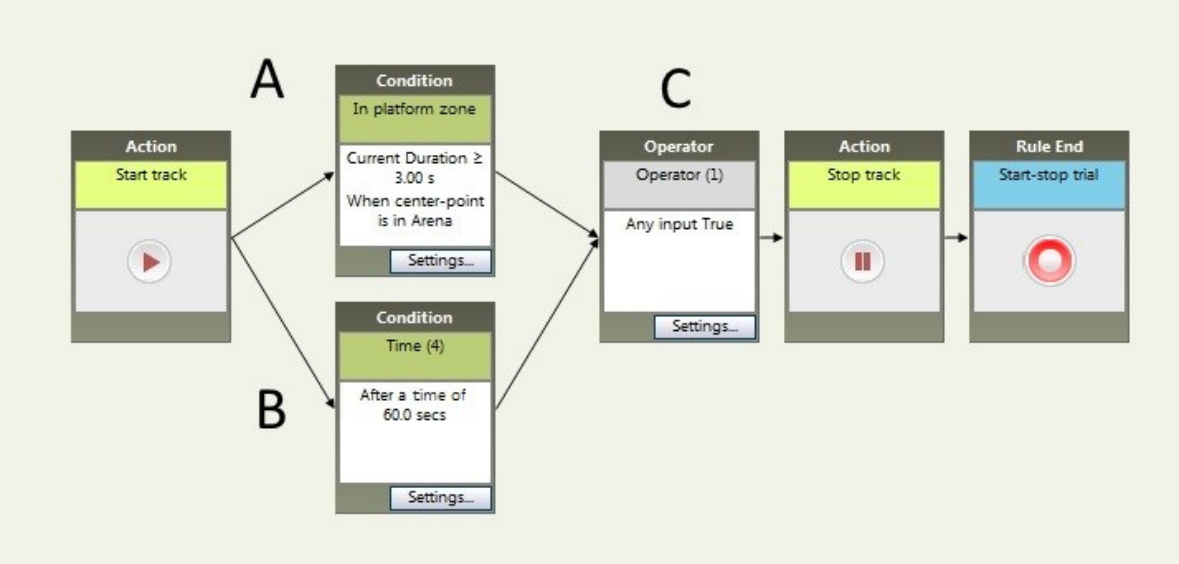
## Procedure

The Arena Settings and the condition “the rat has found the platform” are similar to those in the example above. The condition “rat swimming for 60 s” can be translated to “time from start tracking >= 60 s”.

The track stops when either condition is met. The two conditions are combined with OR logic. See also Operators.

This solution results in tracks of different duration: less than 60 s for the animals that found the platform, and 60 s for the others.

With the Start-Stop trial rule shown below, the trial stops when the subject has been in the platform zone for at least 3 s without break, or the time since the start of tracking is 60 s.




A. In zone condition that specifies that the subject must be for at least 3 seconds over the Platform zone. Select Current duration >= 3s.

B. Time condition that specifies a time of 60 s since the track started.

C. ‘Any’ operator box.

# Start the trial in the Social interaction test

## Aim

To start tracking when all the subjects have been released in the arena

## Procedure

1. Click the **Settings** button in the second box (Condition - In zone).
2. Click **Settings** and then the **Actors** tab.
3. Select all the subjects that need to be in the arena at the time of start.
4. Choose **All selected subjects** below the subject list.

# Stop the trial in the Eight-arm radial maze test

## Aim

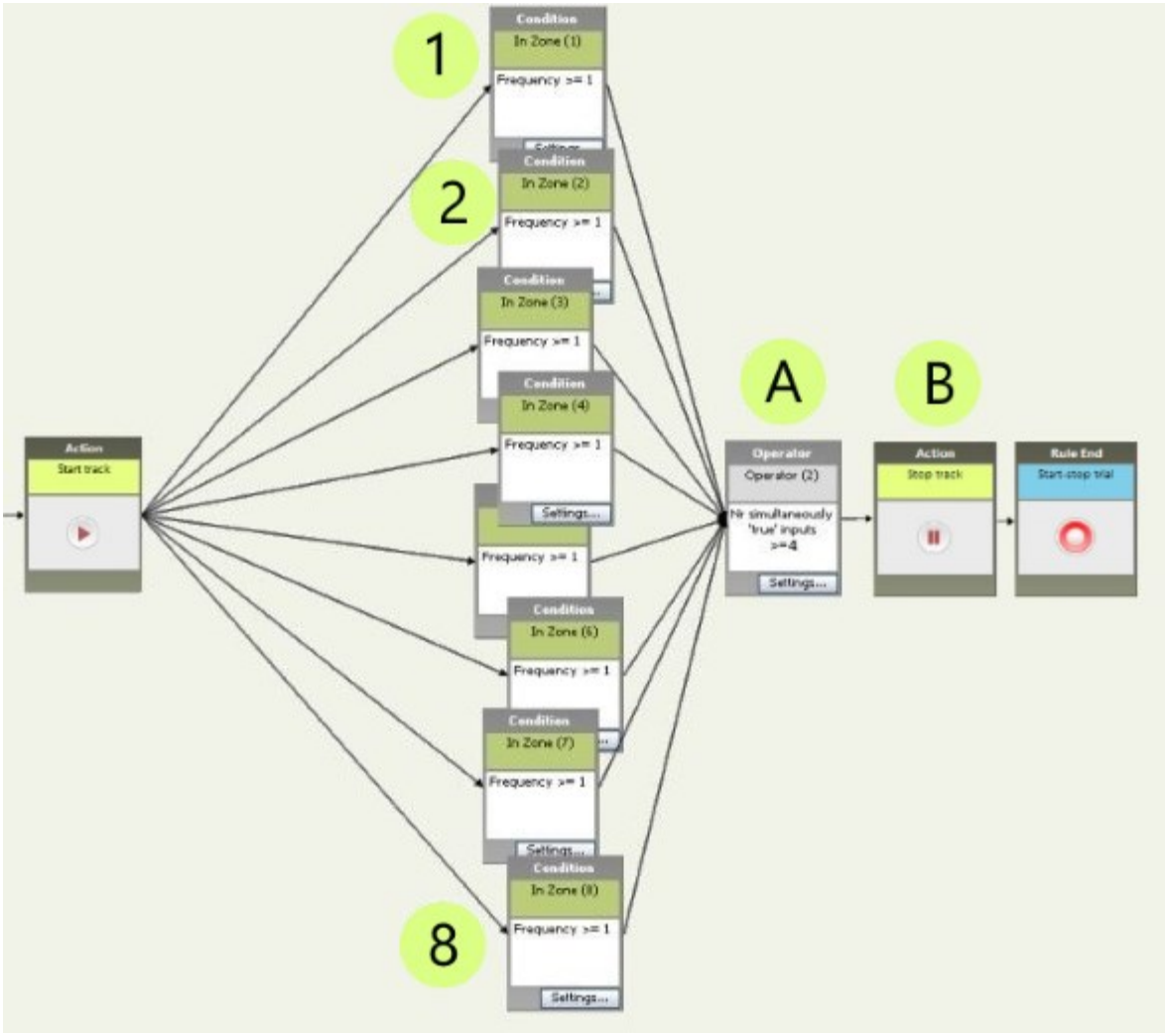
To stop tracking when the subject has been in four arms within 10 minutes

## Procedure

This can be done by combining eight conditions, that is, that the animal must be in the arm specifying that at least four must be met, no matter which arm the animal visits.

1. Create an In zone condition and specify that the Frequency for Arm 1 must be >=1. That is, the animal must have visited Arm 1 at least once. Do the same for each of the other arms.
2. Connect the resulting eight condition boxes in parallel using the N of All operator. See Operators
3. Set the maximum trial duration to 10 minutes to stop tracking in the case the animal fails to visit four arms in the meantime. See To set a maximum duration for your trials

Below: Trial Control sequence for an eight-arm radial maze. The trial must stop when the animal has visited four of the arms at least once. **1, 2,... 8**: **In zone** condition boxes for Arm 1,2,... 8 respectively. A condition is met when the **Frequency** of In zone for that arm is greater than or equal to 1. **A**: **Operator** that checks that at least four of the eight conditions are met. **B**: **Stop track** box. When four conditions are met, the trial is stopped.




*See also*

- Operators on page 332.

# Start and stop the trial at specific clock times

## Aim

To control the start and stop of the trials, without the need for the researchers/ technicians to be in the stable to control EthoVision XT.

## Procedure 1 - One trial

**EXAMPLE** You want to start tracking at 9 AM (9:00) and stop at 7 PM (19:00). You are in the lab the day before.

1. Open the Trial Control Settings and create time conditions as in the figure below.


2. Before leaving the lab, in the Acquisition screen click **Start Trial**. The screens says **Checking start condition**.
3. The software waits until 9 AM on the following day and then starts tracking.
4. Tracking stops on 19:00 (7 PM).

   **NOTE** If you click Start trial before 9:00, it will start tracking on the same day!

## Procedure 2 - Repeated trials using a Series of trials

**EXAMPLE** You plan to record your subjects for 20 days. Each trial should be carried out during the day, from 8 AM (8:00) to 7 PM (19:00).

1. Create a Trial List with the number of planned trials. See Plan your trials
2. In the Trial Control Settings, create a Start-Stop rule similar to the one above, to start tracking from 8 AM to 7 PM.
3. In the Acquisition screen, select **Track all planned trials**. In the Inter-trial interval field, enter the time that covers the interval from the end of one trial to some time before the beginning of the next. In this case the inter-

trial interval runs from 7 PM to 7: 59 AM = 12 h 59 min. Leave some time between the end of the inter-trial interval (7:59 AM) and the Time condition of the rule (8 AM). This way the two instructions will not interfere with each other. At 7:59 AM of the next day, the trial starts but the software waits till 8:00 AM to start tracking.

4. To start the first trial in the series, make sure to click the **Start Trial** button before the time specified in the Time Condition box placed at the left of the **Start track** box (in this case 8 AM).
5. In the remaining days you do not have to be in the lab at that time because the series of trials will do the work for you.

   See also Acquire a series of trials (Batch acquisition)

## Procedure 3 - Repeated trials using Task Scheduler

**EXAMPLE** You plan to record your subjects for 20 days. Each trial should be carried out during the day, from 8 AM (8:00) to 7 PM (19:00).

This is an alternative method that makes use of the Windows Task Scheduler.

- Create batch files to trigger the start of the trials (this replaces the clicking of the Start Trial buttons in the procedures above).
- Use the Windows Task Scheduler to trigger the batch files at a specific time.
- Do not forget to add the planned trials in the Trial List; in this example, 20 trials.

Do the following:

1. Using the Windows Notepad, create a file with extension VBS that performs a key press **Ctrl+F5**. This triggers the start of the trial. Enter the following code:

   ```
   Set WshShell = WScript.CreateObject("WScript.Shell")

   WshShell.AppActivate "EthoVision"

   WScript.Sleep 1000

   WshShell.SendKeys "^{F5}"

   for (i = 0; i < g_aSubjects.length; ++i)
   ```

   And name this file **vbsprogram.vbs**.

   **NOTE** The WScript.Sleep 1000 (1 s delay) ensures that the key press is given when EthoVision XT is in focus.

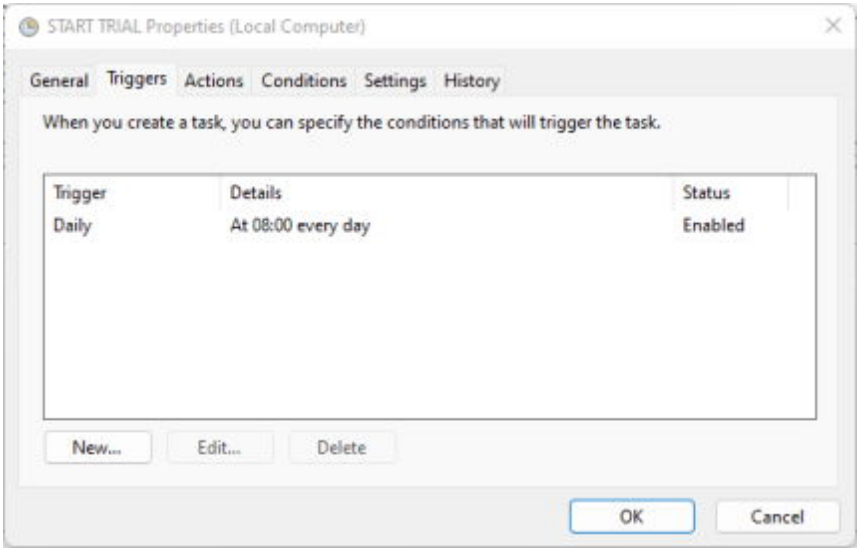
2. Using the Windows Notepad, create a second file, this time with extension BAT, which triggers the vbs script. Enter the following code:

```
wscript "C:\vbsprogram.vbs"
```

And name this file **start_vbsprogram.bat**.

3. Save both files on C:\.
4. Start the Windows Scheduler and define a new basic task, which runs daily. Under **Action**, select **Start a program** and select the batch file **C:\start_vbsprogram.bat**.

   Make sure that the task is repeated daily at the required time:




5. To program the end of the trial, do this in the Trial Control Settings. See Control the end of tracking
6. Leave EthoVision XT open on your screen and close all other unnecessary windows. When Windows runs the task at 8 AM, the trial starts automatically.

## Notes

- If you select the option **Save video** in the Acquisition screen, video recording starts the moment that you click Start Trial. Depending on the condition placed immediately before the Start Track box, video recording starts some time before the actual start of tracking. In the case of Procedure 1, several hours earlier. This means that you record many hours of video when tracking is not yet active.

# Programming Trial Control

Follow this section if you need more information on how to define conditions and actions in the Start-Stop trial rule.

**TIP** Before defining Trial Control in EthoVision XT, draw your experimental procedure as a flowchart, where each block represents either an action or a condition which, when met, triggers other actions or conditions.

## Learn about

- Conditions
- Actions
- External commands
- Operators

### *See also*

- Useful things to know
- Re-use trial control elements
- Working with trial control boxes

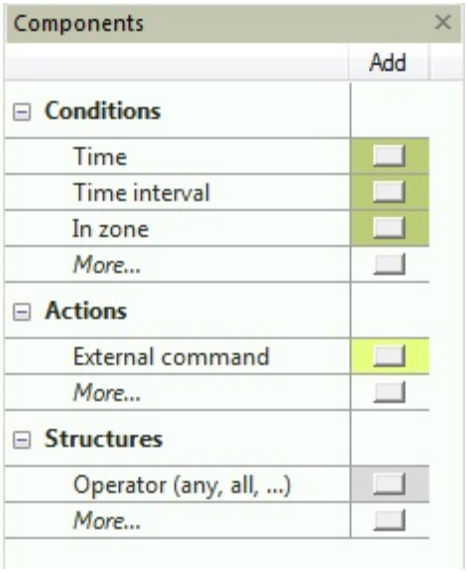
### *Other manuals*

For further information, including information on controlling hardware devices such as the PhenoTyper Top Unit, the Pellet dispenser, PhenoWheel and the Lickometer, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual. To open this manual, choose **Apps** > **Noldus** > **EthoVision XT 18 Other Documentation**.

# Useful things to know

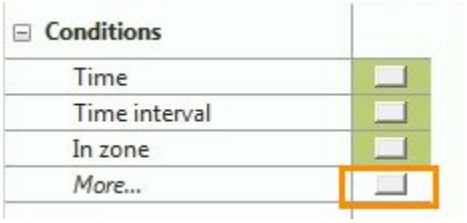
## Components pane

Use the Components pane to choose conditions, actions and other elements for your Start-Stop trial rule.




If you do not see the Components pane, click the **Show/Hide** button on the toolbar and select Components.

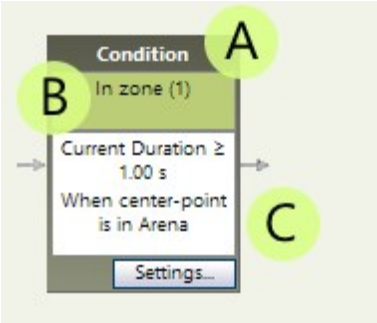
To display the complete list of options, click the buttons next to **More**.




What you see in the Components pane depends on what EthoVision XT license you have on your computer.

## Trial Control boxes

A Trial Control box has the following information:




- A. Type of control (Rule Begin/End, Action, Condition, Operator, Reference). You cannot change this text.
- B. Text describing the control. To change this text, click the **Settings** button and enter the text under Name, for example Drop one food item. You can also add a longer description under Comment (this is not shown).

  Names of Trial Control boxes must be unique, unless you make a copy of an existing box. See Re-use trial control elements
- C. Depending on the type of control, this text shows the option chosen, for example the command to be given, or the sub-rule that the box refers to.

Trial control boxes have different colors:

- Light blue for the Start-Stop Trial rule, sub-rules and sub-rule References.
- Olive green for conditions.
- Light green for actions.
- Grey for operators.

## To zoom the Trial Control rule in and out

- To enlarge the size of all trial control boxes up to their original size, click the **Zoom in** button (**Ctrl+.**).

  
- To reduce the size of all trial control boxes, click the **Zoom out** button (**Ctrl+,**).

  
- To make all trial control boxes visible on your screen, click the **Fit all** button (**Ctrl+/**).

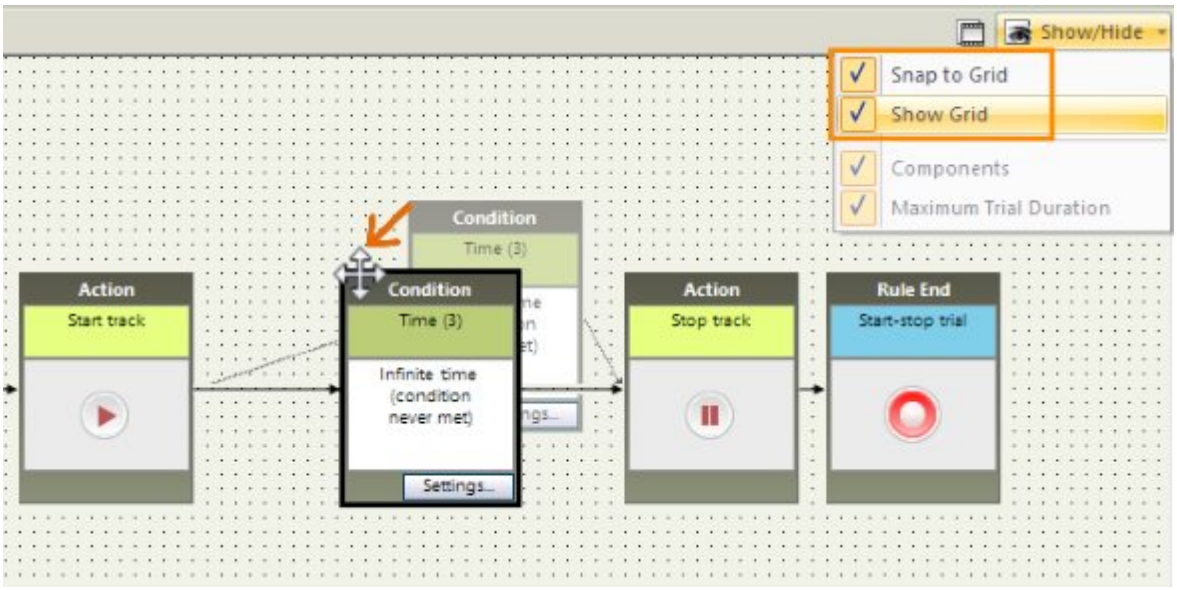
**TIP** Navigate 'from left to right' in the Trial Control window by using the scrollbar at the bottom. Use the **Fit all** button to make all trial control boxes visible.

## To keep Trial Control boxes aligned

The Trial Control boxes automatically snap to a grid. This helps you keep the boxed aligned.

To show the grid, click the **Show/Hide** button on the toolbar and select **Show Grid**.

To disable the grid alignment, click the **Show/Hide** button on the toolbar and deselect **Snap to Grid**.

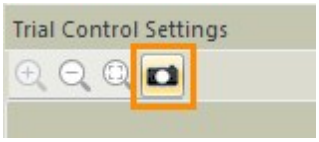



## Trial Control Settings become locked after acquisition

After you have acquired at least one trials, the Trial Control Settings used for that become locked. In the Experiment Explorer, locked settings are indicated by a lock symbol, and cannot be edited. To edit a locked Trial Control Settings profile, duplicate it and edit this copy. See Re-use trial control elements

## To export a picture of the Trial Control Settings

1. Click the **Export image** button on the toolbar.



2. Select a location to save the image to, type in the File name or accept the default one and select an image type from the **Save as type** list, then click **Save**.

The complete Trial Control window is exported, irrespective of the zoom factor.

# Conditions

## Aim

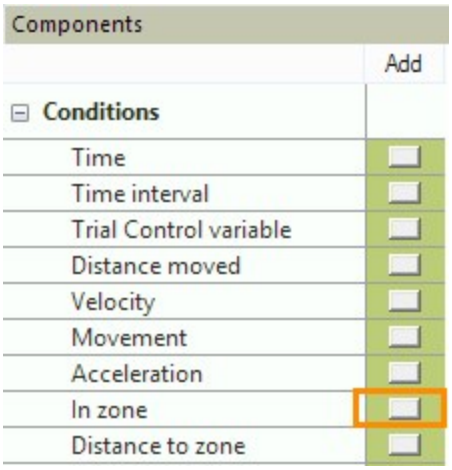
A Condition is a statement that EthoVision checks during the trial. When the Condition is met (True), the program evaluates the next element in the Trial Control sequence.

**EXAMPLES** (Conditions are highlighted in *italics*)

- *When the rat reaches the platform*, stop tracking.
- *When the mouse is detected in the open field*, start tracking.
- *When the animal has visited zone A ten times*, stop tracking.

## To define a condition

1. Open or create new Trial Control Settings.
2. In the Components pane under **Conditions**, locate the type of condition you want to define. and click the button next to it.




**TIP** Combine two or more conditions using the Multi condition. For details, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

3. Next to **Condition name**, type in the name you want to give to the condition, or accept the default name.
4. Specify the condition properties. See Types of condition
5. Enter a **Comment** (optional), then click **OK**.
6. Insert the condition box in the Trial Control rule.

# Types of condition

## *Time*

It defines a time interval that must elapse before an action be taken.

**EXAMPLE** Start tracking two seconds after clicking the Start trial button, or start tracking at 12 PM.

## *Time interval*

This condition makes sense when it is combined with another condition.

**EXAMPLE** Stop tracking when the animal is found in Zone A (In zone condition) between 5 and 10 minutes (Time interval condition).

## *Trial Control variable*

To create a condition that is met when a Trial Control variable has a certain value, or a value within a range, or equal to another variable at the time the condition becomes active. See also To define a Trial Control variable

For the meaning of *becomes active*, see How the trial control instructions are executed on page 343.

**EXAMPLE** Stop tracking when the variable Counter is greater than or equal to 10.

## *Dependent variables*

To define a condition based on the behavior of the subject. This also includes behaviors scored manually. Choose one of the dependent variables to create the condition.

**EXAMPLE 1** In zone condition. Stop tracking when the subject visits the target zone 10 times.

**EXAMPLE 2** Movement condition. Stop tracking when the subject has been in the Not moving state for more than five minutes.

**EXAMPLE 3** Multi condition. Stop tracking when the subject has explored an object for more than 5 minutes. Here, “explored” is defined with a Multi condition: (a) the subject’s nose point is the object zone AND (b) its head is directed to the object. When the two conditions are met simultaneously for a total of 5 minutes, the trial stops.

*Hardware*

To define a condition based on the signal given by a hardware device. To use hardware-based conditions, you must have the Trial and Hardware Control Module. See the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

## Notes

- If the Add a condition window appears, it means that there is at least one condition with the same name in your experiment. Create a new condition, or re-use the existing condition. See Re-use trial control elements.
- For a complex condition like “stop the trial when the rat reaches the platform or it has been swimming for 60 seconds”, see an example for the Start/stop the trial in the Morris water maze test.
- Trial Control conditions cannot be defined based on behaviors scored automatically with Rat or Mouse Behavior Recognition. This because the detection of behavior also depends on video frames that come after the time that the condition is evaluated.
- If you insert a Condition box based on Activity, and then deselect **Activity analysis** in the Experiment settings, your rule becomes invalid. The Condition boxes based on Activity are removed from your sequence and the connecting arrows are removed. Re-connect the boxes.
- See also **Overview of conditions** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

# Actions

## Aim

An Action is a command that EthoVision XT carries out during acquisition.

**EXAMPLES** (Actions are highlighted in *italics*)

- *Start tracking* and *Stop tracking* (see The Start-Stop trial rule).
- *Do C= C+1*. This is an example of an action on a Trial Control variable. See Using Trial Control variables.
- *Start video recording with MediaRecorder*. This is an example of External Command. See External commands
- *Open the door of LeftArm* or *Drop a pellet*. These are actions on hardware devices (only with the Trial and Hardware Control Module).

## Procedure

1. Open or create new Trial Control Settings.
2. In the Components pane under **Actions**, locate the type of action you want to define. and click the button next to it.
3. Next to **Action name**, type in the name you want to give to the action, or accept the default name.
4. Specify the action.
5. Enter a **Comment** (optional), then click **OK**.
6. Insert the action box in the Trial Control rule.

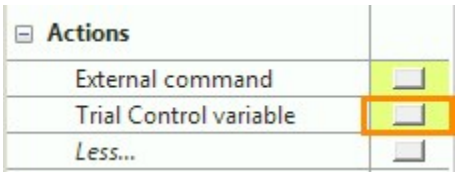
# Using Trial Control variables

## Aim

Trial Control variables help you keeping track of events that occur during the trial. An example of a Trial Control variable is a counter.

## To define a Trial Control variable

1. Open or create new Trial Control Settings.
2. In the Components pane, under **Conditions** or **Actions** click the button next to **Trial Control variable**.



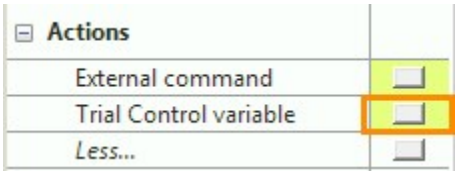

3. Next, click the **Variables** button. The Trial Control Variables window lists the variables currently in the experiment (also those defined in other Trial Control Settings). To add a new variable, click **Add variable**.
4. A new row is appended to the table. Under **Name**, type in the name you want to give to the variable. Under **Initial Value**, enter the value of this variable at the start of the trial (default: 0).
5. Click **OK**. In the TC-variable action/condition window, define the action or condition you require. Click **Cancel** if you do not want to create a condition or action based on that variable at that point.

## Notes

- To delete a variable, click the variable name in the Trial Control Variables window and click the **Delete variable** button.
- To rename a variable, click the variable name in the Trial Control Variables window and edit this name.
- The default name of a new trial control variable is VarN, where N is a progressive number.
- The variable name cannot contain blank spaces.

## To define an Action on a Trial Control variable

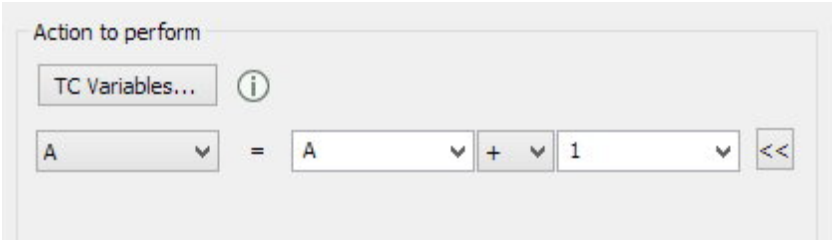
1. In the Components pane, under **Actions** click the button next to **Trial Control variable**. If you do not see this action, under **Actions** click the **More** button.



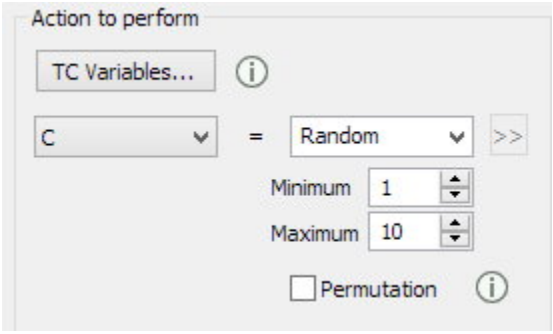

2. If the Add an action window appears, it means that there is at least one action of the same type in your experiment. You are asked to choose between creating a new action, or re-use an existing one. See Re-use trial control elements on page 335.
3. In the TC-Variable Action window, next to **Action Name**, enter the name of the action (for example, *Increment Counter*) or accept the default name.
4. Under **Action to perform**, select the variable from the list. You can also create the variable by clicking **Variables** if you have not yet done so.
5. Next to the **=** symbol, do one of the following:
   - To assign the same value of another variable (for example A = B), select the other variable (B) from the second list.
   - To enter a formula, click the double-arrow button.


     Select the operator from the list and specify the formula in the second and third lists. For example, **A= A + 1**.




   - To assign a random value, select **Random** from the second list, and select the **Minimum** and **Maximum** limits (only integer values, 0 up to 999) for the random value.




For the **Permutation** option, see below.

6. Click **OK**. Insert the resulting Action box in the Trial Control rule.

## Notes

- If your setup includes multiple arenas, each arena receives an instance of the variable. Thus, a variable can have different values in different arenas.
- You cannot combine Random with a formula (for example, to compute A= Random+1). The equivalent solution is the following: define first an action B= Random, and then one more action A= B+1. Place the two resulting Action boxes in sequence.
- For a random variable, select the **Permutation** option to generate a random value that is unique in the sequence of all values in the specified range. For example, if the Minimum is 1 and the Maximum is 5, any value will be generated only after all the other possible values have been generated in previous repeats of the action. That is, a new value **1** can only occur after that all the other possible numbers are generated during previous repeats of the action: 3, 2, 4, 1, 5, **1**. Use the Permutation option to create sequences of unique numbers, for example to activate routines in a "pseudo-random" order but without the repetitions that would occur if you generate fully random numbers (e.g. 2, 4, 1, **1**, 5).

*See also*

- The EthoVision XT - Trial and Hardware Control - Reference Manual. See Manuals

# External commands

## Aim

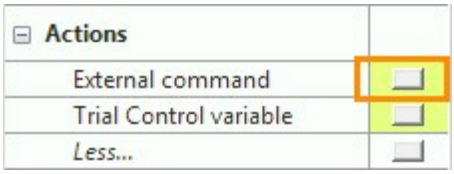
To trigger external software with EthoVision XT.

## Prerequisites

- You need at least the Base version of EthoVision XT.

## Procedure

1. Open or create new Trial Control Settings.
2. In the Components pane, under **Actions** click the button next to **External command**.




3. Next to **Action Name**, enter the name of the action (for example, start recording) or accept the default name.
4. Under **Actions to perform**, select which file you want to run by clicking the ellipsis button.

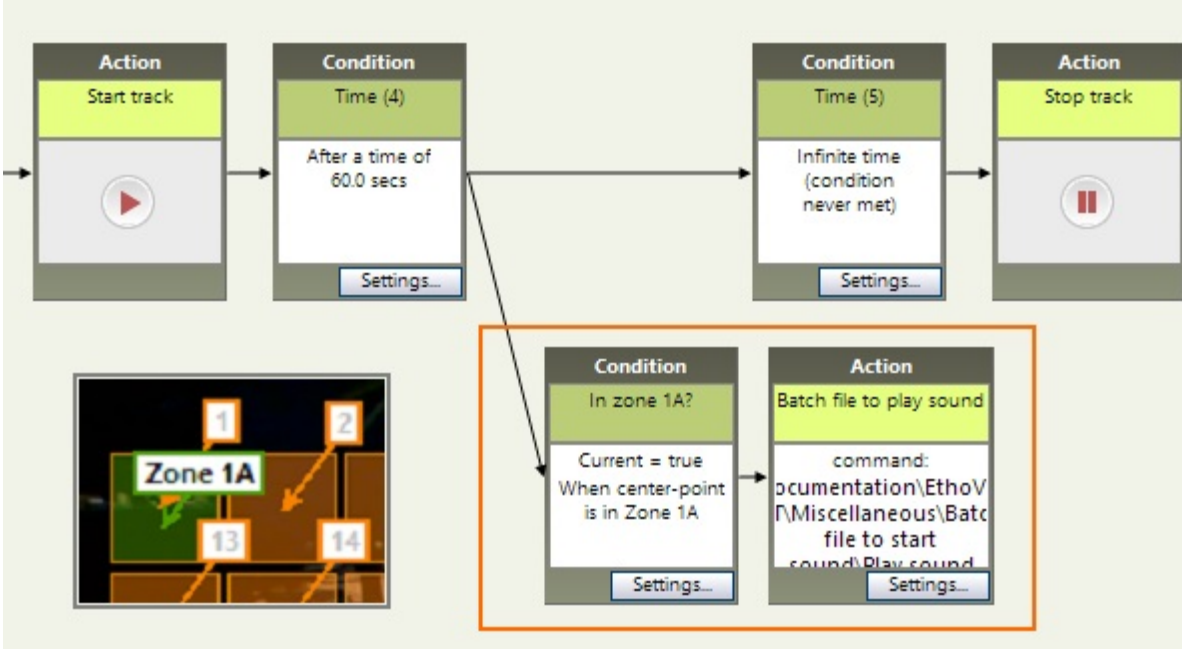

5. Select one of the file types from the list: Executables (*.exe), or Batch Files (*.bat, *.cmd).
6. Browse to the file and click **Open**.
7. Optionally, enter a **Command line option**. Click the Information button to get additional information about defining an External command.

For more details, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

8. To test the command, click the **Test** button.

## Note

If you work with two or more arenas, be aware that an external command is sent out for each arena. For example, the command to play a sound is repeated 96 times for a 96-well plate DanioVision experiment. To prevent this from happening, put a condition before the External command box, which will only become true for one of the arenas, for example Arena 1. In the Arena Settings, create a zone in Arena 1 and call it “Zone 1A”. Make the zone as large as the arena. In the Trial Control Settings, make a condition like “Is subject in Zone A?”, followed by the external command. Make sure that the sequence does not interfere with the rest of the Trial Control rule, like in this example.




## Application examples

- Play a sound
- Trigger video recording with MediaRecorder
- Trigger sound recording with UltraVox XT

# Play a sound

## Aim

To play a sound file while carrying out a trial.

## Procedure

1. Open the Windows Notepad and enter the following text:

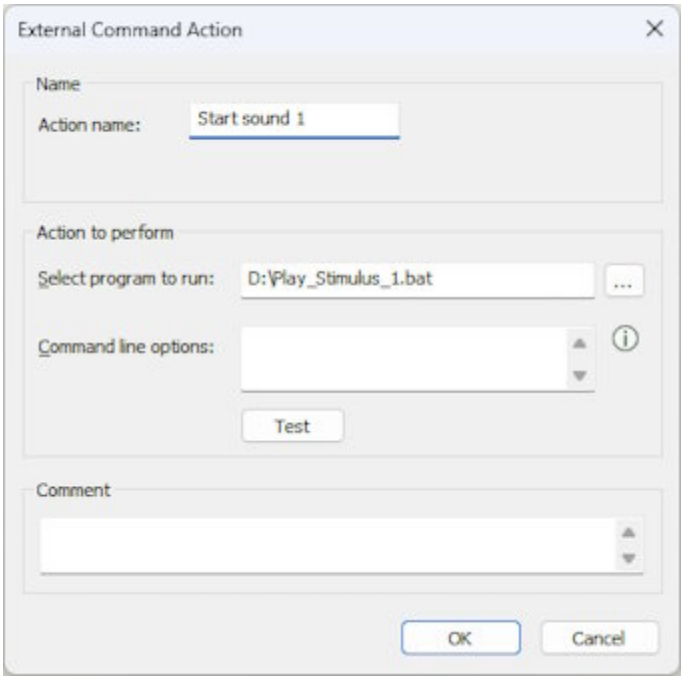
   ```
   @echo off

   set soundFile="D:\Sound Files\Stimulus1.wav"

   start "" "C:\Program Files\Windows Media Player\wmplayer.exe"
   %soundFile%
   ```

   Replace the text within quotes with the actual path to your sound file.

2. Save the file with the *.bat extension.
3. Follow the procedure in External commands to create an External Command action that selects the batch file.




4. Place the External command box in the Trial Control rule.

## Notes

- Create one batch file for each sound file you want to play during the trial. Create multiple External command actions, one for each batch file, and place them in the Trial Control rule.
- wmplayer represents Windows Media Player. You can also use other software to play video, for example VLC.

```
@echo off

set soundFile="D:\Sound files\Stimulus1.wav"

start "" "C:\Program Files\VideoLAN\VLC\vlc.exe" --play-and-exit
%soundFile%
```

- For examples of the **Command line options**, see **External Commands** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

*See also*

- External commands

# Trigger video recording with MediaRecorder

## Aim

To record high resolution video with MediaRecorder while tracking the subjects with EthoVision XT.

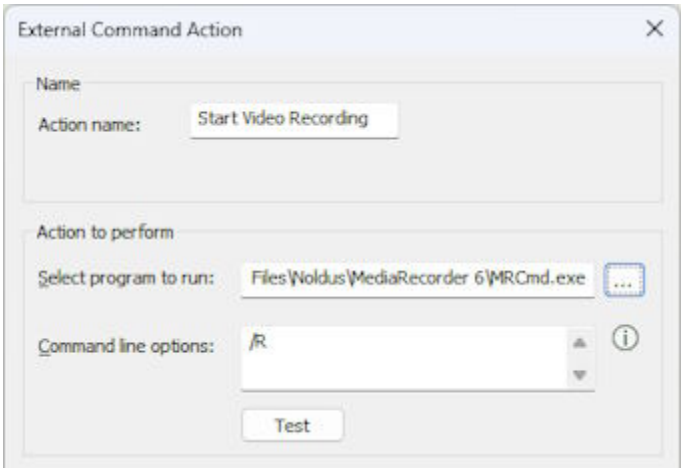
## Prerequisites

- You work with analog video cameras. If you work with digital cameras, EthoVision XT *and* MediaRecorder cannot access the signal from a digital camera simultaneously.

## Procedure

1. In the Trial Control Settings, create a condition that must be true in order to trigger video recording.
2. Follow the procedure in External commands to create an External Command action that starts video recording in MediaRecorder.

   **TIP** To visualize batch (*.bat) files, choose **Batch files (*.bat; *.cmd)** next to **File name**.

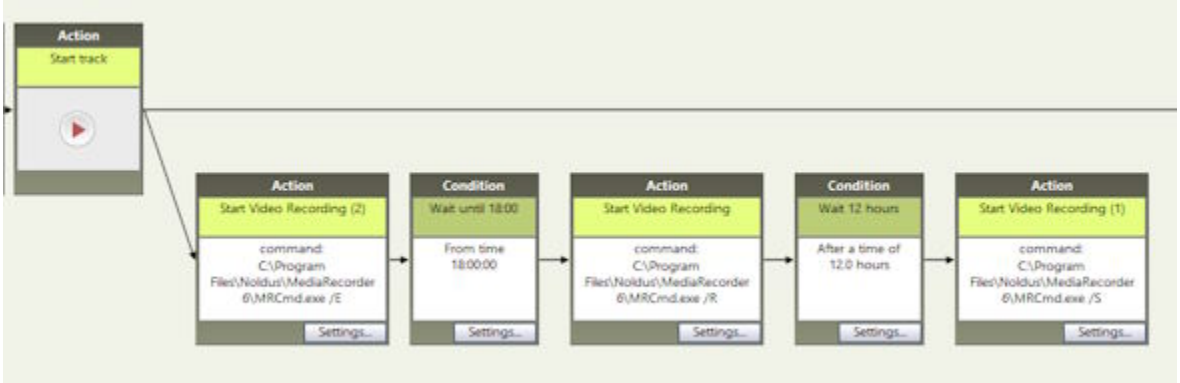



3. Place the second box after the first in the Trial Control rule. See below for examples.

## Example 1

In this example, the trial starts in the early afternoon but the researcher wants to start video recording only after 6 PM (18:00) and end it after 12 hours. The box sequence is as follows (the rest of the Trial Control rule is not shown):

1. Start tracking.
2. Open Media Recorder. Add the Command line option **/E**.
3. Wait until 18:00.
4. Start video recording. Add the Command line option **/R**.
5. Wait 12 more hours.
6. Stop video recording. Add the Command line option **/S**.




## Example 2

In this example, the researcher carries out live tracking during a 24-hour period. The trial starts during the light cycle when the animal is inside the shelter but the researcher wants to start recording video only when the animal leaves the shelter. This saves significant disk space as the resulting video will be shorter.

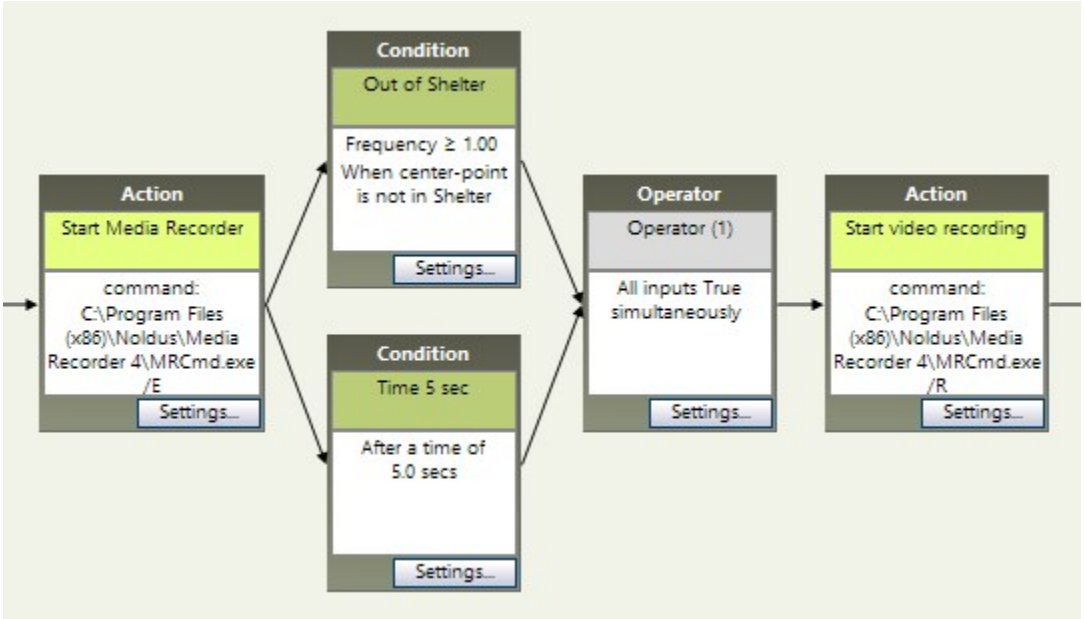
1. Create an External Command action to open MediaRecorder.

   Select **MRCmd.exe** as the program to run and enter **/E** as a Command line option (see the figure below, box at the right).

2. Create an **In Zone** condition "Out of shelter".
3. Create a **Time** condition to make sure that video recording starts when MediaRecorder is already open on the screen. Combine the two condition boxes with an operator of type **All inputs true** (see below for an example).
4. Create an External command similar to the first one, this time with **/R** as a Command line option to start recording.

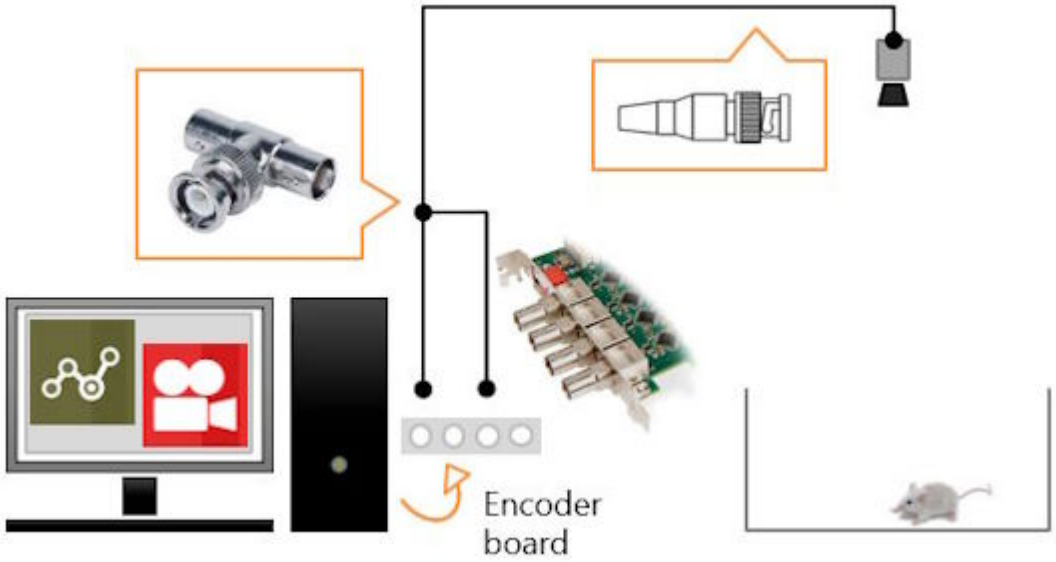
5. You can have EthoVision stop video recording (Command line option: **/S**) after a specific time elapsed after the start.

In the example below, the first action box (left) starts up MediaRecorder. The action box on the right starts video recording when both conditions **Out of Shelter** and **Time 5 sec** become true, that is, the center-point of the animal has left the shelter at least 5 seconds after MediaRecorder was started.




## Notes

- To record video in MediaRecorder and carry out data acquisition in EthoVision XT simultaneously, you need to feed the video signal to two separate channels. Split the video cable that comes from the camera in two, and connect each end to a separate video input of the encoder board. Select one input per program, for example video input 1 for EthoVision XT and 3 for MediaRecorder.




- There may be a delay between the command Start Recording and the moment MediaRecorder actually starts recording. Run a test recording to test how long this delay is.

*See also*

- External commands

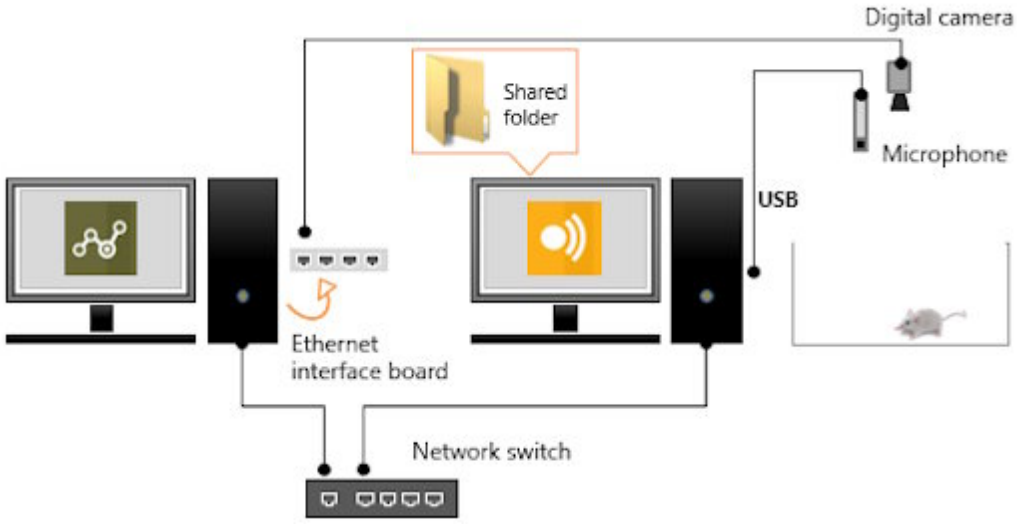
# Trigger sound recording with UltraVox XT

## Aim

To record ultrasonic vocalizations with UltraVox XT 3 while tracking the subjects with EthoVision XT. UltraVox XT is triggered by an EthoVision XT's external command.

## Prerequisites

- We recommend to run UltraVox XT and EthoVision XT on separate computers, preferably connected through a network switch. Alternatively, you can use a local network but that could add delays in communication between the two computers. On both computers, disable the firewall.




- Set the IP addresses of the Ethernet ports on both PCs that match the IP address of the network switch. Here you find an example of IP addresses that were tested:
  - Network switch (Netgear GS310TP): 192.168.0.239.
  - EthoVision XT 18 PC: 192.168.0.201.
  - UltraVox XT 3 PC: 192.168.0.202.
- **IMPORTANT** If you decide to run EthoVision XT and UltraVox XT on the same machine, make sure that you start EthoVision XT and open the experiment **before** starting UltraVox XT. Also check that the PC can handle the recording of video, data and sound simultaneously. Check that there are few or no missing samples in your data before carrying out the actual experiment. For more information, see the UltraVox XT Help.

## Procedure

1. When working with two computer, make the folder **C:\ProgramData\Noldus\UltraVox\XT 3\Synch** shared, so it can be accessed by the SUV batch file.
2. In the EthoVision XT Trial Control Settings, define an external command that runs one of the batch files **SUV.bat** stored on the EthoVision XT computer.

   You can find the SUV batch files on the EthoVision XT full installation package, under **Drivers and tools** > **Utilities** > **UltraVox XT 3 Control**. For more information on this file, see the UltraVox XT Help.

   If you work with two PC and multiple arenas, use the batch file **SUV - 2PC multiple arenas.bat** (see below). We recommend not to run EthoVision XT and UltraVox XT on the same PC when you work with multiple arenas.
3. Place the external command immediately after the Start track box (see the figure below).
4. Define a second instance of the external command and place it immediately before the Stop track box (see the figure below).
5. Open your experiment in UltraVox XT and in the Trial Control Settings under **Start acquisition** select **External program trigger**.
6. Start acquisition by starting the trial in EthoVision XT.
7. After acquisition, import the UltraVox XT data in EthoVision XT using the import profile for UltraVox XT 3. See also Import a complete external data file at once

   **NOTE** For this step you need the External Data add-on module. See Modules of EthoVision XT


## Triggering UltraVox XT with two or more arenas

If you work with multiple arenas, we advise to use the batch file **SUV - 2 PCs multiple arenas.bat**, in such a way that the start command is given only once, for the first arena.

This is the content of the batch file **SUV - 2 PCs.bat** for working with *one arena*:

```
@if exist "\\IP address\Synch\start.txt" del "\\IP address\Synch\start.txt"

@copy "C:\ProgramData\Noldus\Common\Tools\UltraVox XT 3 Control\start.txt" "\\IP address\Synch" > nul
```

This is the content of the batch file **SUV - 2 PCs multiple arenas.bat** for when working with *two or more arenas*:

```
@if exist "\\IP address\Synch\start.txt" del "\\IP address\Synch\start.txt"

@echo off

echo %1

IF %1=="Arena 1" (

@copy "C:\ProgramData\Noldus\Common\Tools\UltraVox XT 3 Control\start.txt" "\\IP address\Synch" > nul

)
```

Note:

- In the Trial Control Settings, and for both Action boxes, add a command line option: **"%an%"** (including quotes).
- In the batch files, replace *IP address* with the IP address of the UltraVox XT computer (suggested: 192.168.0.202, or 10.0.0.9 as suggested in the UltraVox XT 3 Help). You can also use the name of the computer instead, e.g. DESKTOP-ABC123.
- If the arena has a name different from *Arena 1*, replace *Arena 1* with that name in the batch file.

*See also*

- Import profile for UltraVox XT 3
- Import a complete external data file at once
- External commands

# Operators

## Aim

To combine actions, conditions and sub-rules in various ways.

**EXAMPLES** (Operators are highlighted in *italics*)

- When *at least one* of the two conditions A and B is met, then do …

  This is an example of conditions combined by an operator of the "Any" type (OR logic).

- When two conditions are met *at the same time*, then do …

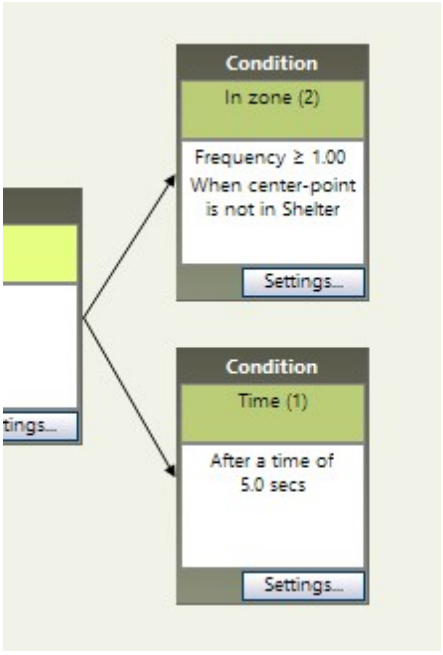
  This is an example of conditions combined by an operator of the "All" type (AND logic).

- When *at least/at most/exactly* 4 of 8 conditions are met, then do …

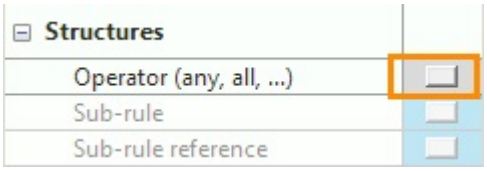
  This is an example of conditions combined by an operator of the "N of All" type.

## To combine conditions/actions/rules:

1. Define the conditions/actions/rules that you want to combine. Place them in your Trial Control sequence as parallel branches. The connecting arrows must originate from the condition/action that precedes the combination of elements you want to define.

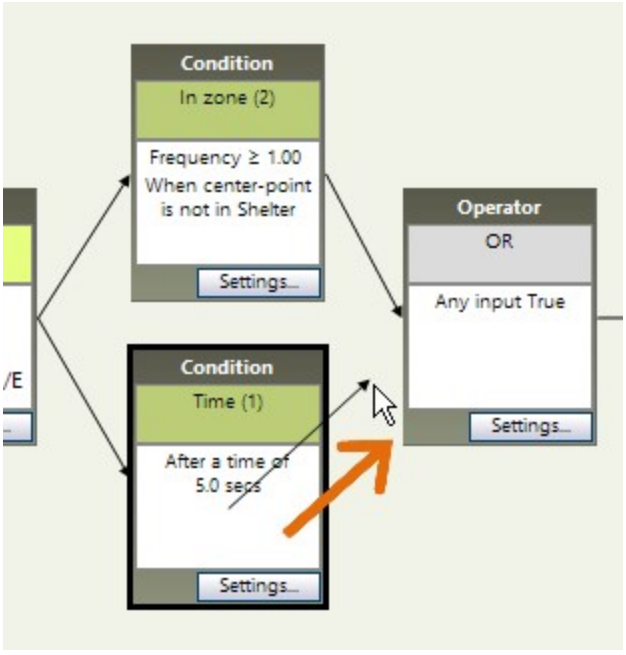


2. In the Components pane under **Structures**, double-click **Operator** or click the button next to it.




3. Under **Name**, enter the Operator name or accept the default name Operator (n), where n is a progressive number.
4. Under Operator triggers when, select the option that applies:
   - **Any** (at least one) of the inputs is 'true'.
   - **All** inputs are simultaneously 'true'.
   - **N of All** inputs are simultaneously 'true'. Specify how many inputs N must be 'true': = (exactly equal to), ≠ (not equal to), >= (at least), <= (a maximum of), etc.

   Where an input being 'true' means a condition being met, an action carried out, or a sub-rule finished.
5. Enter a **Comment** (optional) to describe this operator, and click **OK**.
6. A new Operator box appears in the Trial Control. Place the box right of the elements defined in step 1, and connect each element (or ending element, in the case of a sequence) to the operator.




7. Connect the operator to the next element that should be activated.

## Notes

- Instead of using multiple condition boxes, you can also combine conditions with **Multi condition**. For details, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.
- If the Add an operator window appears, it means that there is at least one operator of the same type in your experiment. For more information, see Re-use trial control elements.
- Names of operators must be unique in your experiment. You cannot define two operators with the same Operator name.
- An Operator can also have just one input box. In that case the operator is of no use, because control passes immediately to the next box as soon as the input condition becomes true or the input action is carried out. EthoVision informs you about this.

# Re-use trial control elements

## Aim

To create a Trial Control element without starting from scratch.

All elements of Trial Control (conditions, actions, operators, sub-rules and sub-rule references) defined in other Trial Control Settings can be duplicated and re-used in your current Trial Control Settings.

## Procedures

### *Re-use a Trial Control element (general)*

Use this procedure to create a Trial Control element from an existing one, even when this is stored in another Trial Control Settings profile.

1. Click the button next to the element you want to create.

2. The Add window appears. Select **Reuse an existing condition**/ **action**. and select the name of the existing element from the list next to the option.

   From the second list, choose the Trial Control Settings profile that contains the element you want to re-use.

3. Click **OK**.
4. A window appears for the type of element chosen. The Name and settings specified here are the same as in the element chosen in step 2.
   - To create an identical copy of the element, click **OK** and go to step 5.
   - In all other cases, edit the settings and click **OK**, and go to the next step.
5. Insert the resulting box in the Trial Control sequence.

### *Copy and paste a Trial Control element*

Use this procedure to quickly copy one or more Trial Control elements that you see on the screen. This applies to actions, conditions, operators and references to sub-rules. See Copy and paste a box

## Notes

- To duplicate an entire Trial Control procedure, In the Experiment Explorer, under **Trial Control Settings**, right-click the profile and select **Duplicate**.
- When you duplicate a Trial Control element, and change any settings of the new element, including name and comment, a window appears showing two options:

  **Apply the new settings only in the current trial control profile**. Choose this option to apply the settings only to the new copy,

  **Apply the new settings in all writable Trial Control profiles**. Choose this option to extend those changes to the original elements in all Trial Control Settings that are writable, that means, not locked after acquisition.

# Working with trial control boxes

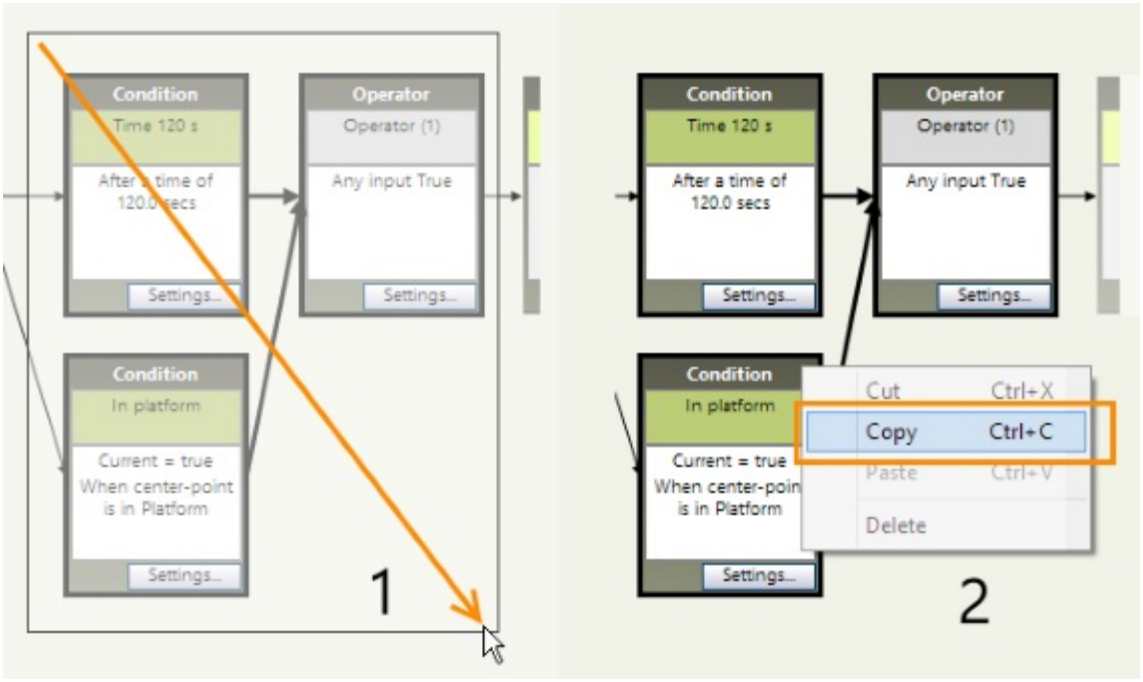
## Create a new box

Click the button that applies in the **Components** pane.

## Copy and paste a box

You can create duplicates of one or more Trial Control boxes. This makes it easier to create procedures with similar actions and conditions.

1. Click the box you want to copy. To select multiple boxes, click and drag the mouse around them.
2. Choose **Edit** > **Copy**, or right-click anywhere and choose **Copy** or press **Ctrl+C**.




3. When necessary, open the Trial Control Settings where you want to insert the new boxes.
4. Choose **Edit** > **Paste**, or right-click anywhere and select **Paste**, or press **Ctrl+V**.

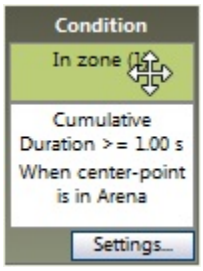
**NOTES**

- The name of the new Trial Control box is the same as that of the original one, followed by (2), (3), etc.

- You cannot copy and paste a sub-rule, only the actions and conditions within it.

## Move a box

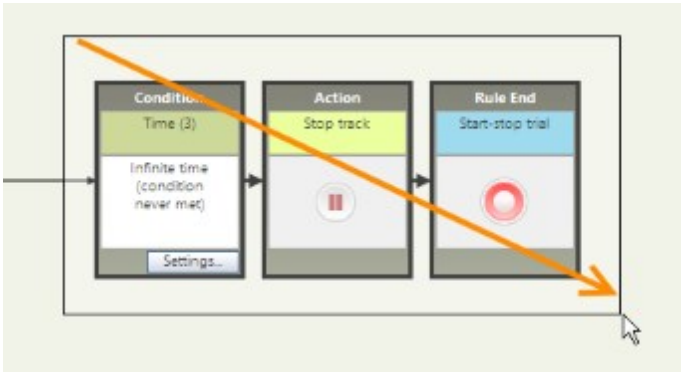
1. Hover the mouse on the margin or the colored area of the box. The mouse cursor changes to a four-headed arrow.




2. Drag the box to the position you require.

To move a group of boxes:

1. Draw a rectangle around the boxes you want to move or click on the boxes you want to select while holding the **Ctrl** key.

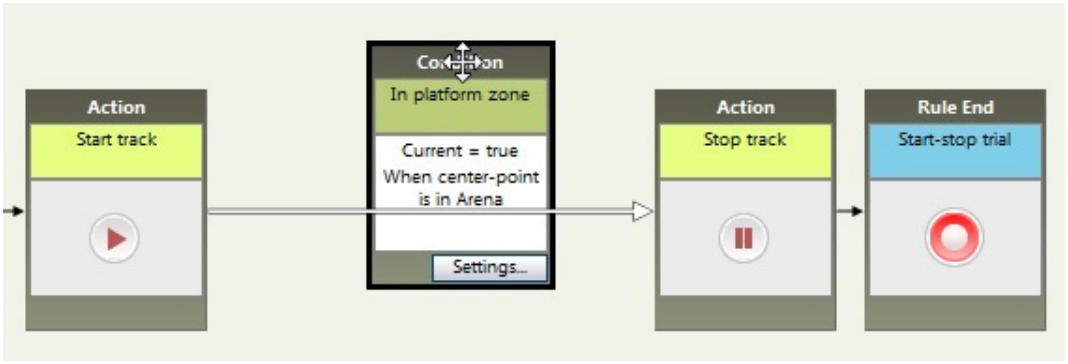



   As a result, the selected boxes get a gray, dark border.

2. Hover the mouse on the margin or the colored area of one of the selected boxes. The mouse cursor changes to a four-headed arrow.
3. Drag the group of boxes to the position you require.

## Insert a box in a Trial Control sequence

1. Drag the Trial Control box between two boxes until the connecting arrow turns white.




2. Release the mouse button. The new box is inserted.

## Connect two boxes

1. Point the mouse to the center of the first box, press and hold the left mouse button and drag toward the center of the other box.
2. Release the mouse button when the pointer has reached the center of the other box. The two boxes are connected.

Operator boxes can have one, two or more input arrows; all other boxes have no more than one input arrow.

All boxes can have 1 or more output arrows, pointing to different boxes.

## Modify the settings in a box

Click the **Settings** button in the lower part of the box. Make the appropriate changes in the window that appears.

## Delete a box

1. Click the title of the box. The box border is highlighted.
2. Press **Delete**.

## Delete a group of boxes

1. Draw a box around the boxes you want to delete or click on the boxes you want to select while holding the **Ctrl** key.
2. Press **Delete**.

You cannot delete the Rule Begin, the Rule End box, the Start track box and the Stop track box.

## Delete a connecting arrow

1. Click the arrow you want to delete. The arrow turns bold to show it is selected.
2. Press **Delete**.

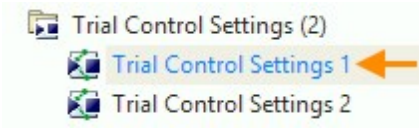
You cannot delete the arrow connecting the Stop track box and the Rule End box.

# Apply trial control to your trials

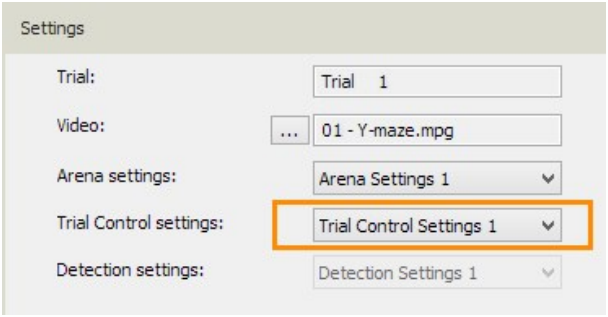
## Apply Trial Control Settings to a single trial

Before starting the trial, do one of the following:

- In the Experiment Explorer, make sure that the Trial Control Settings profile you require is highlighted in blue.




- In the Acquisition screen, under **Settings**, select the Trial Control Settings you require.




Test your setup thoroughly before carrying out the actual trials (see above).

## Apply Trial Control Settings to multiple planned trials

Choose **Setup** > **Trial List**. For each trial planned in the list, select the Trial Control Settings you require in the **Trial Control Settings** column.

For more information, see Prepare the trial list for batch acquisition.

# Further information on Trial Control

## Learn about

- How the trial control instructions are executed
- Trial control when tracking from video files
- Trial control in multiple arenas

## What do you want to do?

- View trial control instructions during acquisition
- Analyze trial control data

# How the trial control instructions are executed

## When EthoVision XT evaluates conditions and actions

During a Trial, EthoVision XT evaluates the Trial Control Settings selected for that trial, and checks the state of conditions and actions at each sample time. Note that the sample time depends on the chosen sample rate, not on the video frame rate.

## How EthoVision XT evaluates conditions and actions

The program remembers which Trial Control box was evaluated (active) in the previous sample. Depending on the type of this box:

- For a Condition box. EthoVision XT checks whether the condition at the current sample time is met. If it is not, the condition becomes false. The program waits until the condition is met. When this happens (condition becomes true; see 3 in the figure below), the program passes control to the next box in the sequence. The condition becomes then inactive (see 4).
- For an Action box. EthoVision XT carries out the action, and passes control to the next box, which becomes active. Then, the Action box becomes inactive (see 4-5).
- For sub-rules and sub-rule references, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

When a box becomes active, the previous becomes inactive.

## Notes

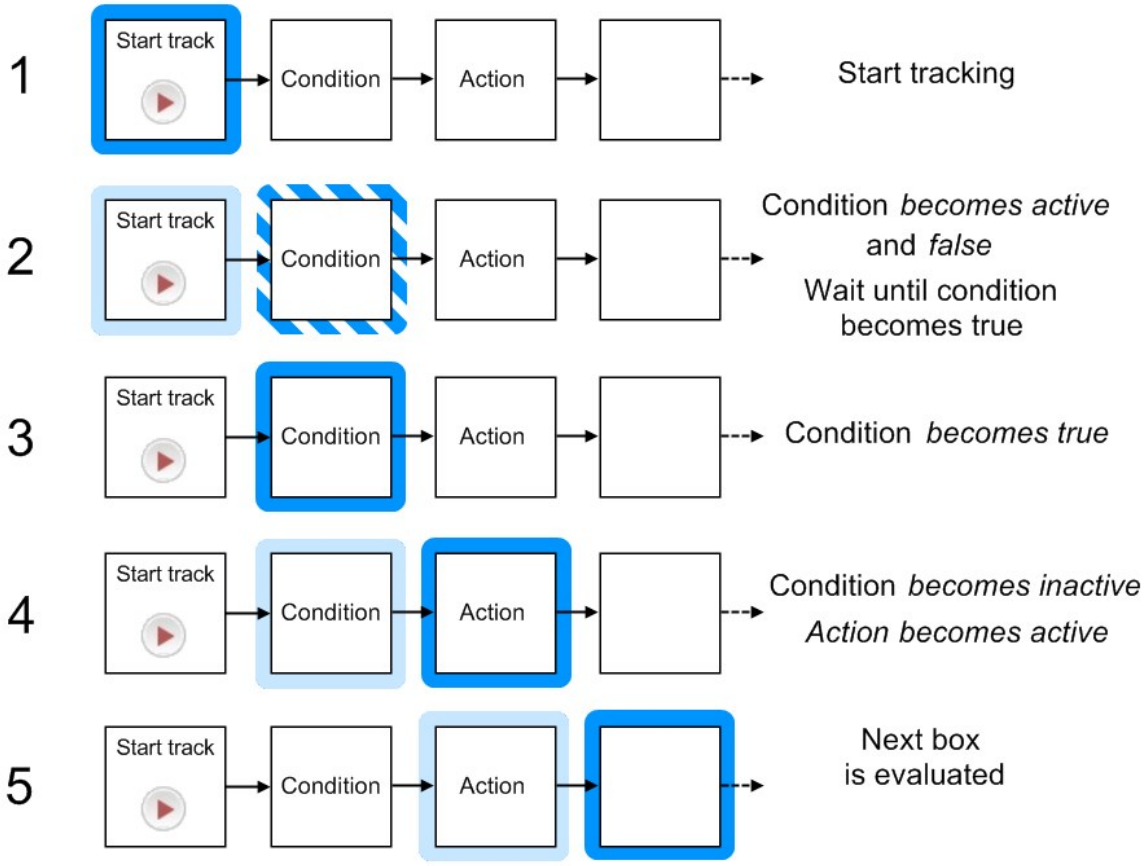
- Boxes combined in parallel using operators are evaluated at the same sample time, in unspecified order. This means that one cannot establish which condition is evaluated/which action is taken first.
- Actions on Trial Control variables are executed immediately. Actions on hardware devices are executed when all boxes that must be evaluated at that sample time have been evaluated.
- If a box being evaluated contains a condition that is immediately true, the program passes control to the next box. Therefore, within one sample time the program can pass control to two or more boxes to the right.
- When you stop the trial or the Maximum trial duration has been reached, all Trial Control boxes are deactivated and hardware devices are reset. See Control the end of tracking

- When the Rule End box of the Start/Stop trial rule is evaluated, data recording stops. From that moment, Trial Control is deactivated, even in those sub-rules that were ongoing in the meantime.

Schematic representation of how Trail Control Instructions are executed. The scheme shows an example of a Start-Stop trial rule.

1. Tracking starts, either manually or because a previous condition has been met.
2. Control passes to a Condition box (for example, “Is mouse on top of Shelter?” which becomes active (hatched outline). The condition is evaluated. Since the condition is not met immediately, it becomes false.
3. The Condition becomes true (solid outline).
4. Control passes to the next box. In this case, it is an Action. Actions are taken immediately.
5. The Action box becomes inactive (light outline). The next box becomes active.

For clarity, step 3 and 4 have been placed separately. In reality, when a condition is met it becomes inactive at the same time, and control passes to the next box.



# Trial control when tracking from video files

## When tracking from pre-recorded video files

EthoVision XT checks conditions using the time in the video file, instead of the real time.

- For conditions based on time. If you select the **DDS** option, Trial Control is carried out at the speed set by EthoVision in order not to skip video images. This results in the video playing faster or slower than normal (1x), depending on the processor load necessary to detect subjects. For example, if detection requires little processor work, the program tracks the subject faster than normal. A Time condition (for example, Time 60 s) is therefore met earlier than at real time.
- For conditions that use clock time. If you define a condition based on clock time, or schedule a sub-rule with clock time, this is translated into the video start time, that is, the date and time the video file used for tracking was created.

  **EXAMPLE 1** You set a Time condition to start tracking After clock time 11:30. The video file was created on March 6, 2008 at 11:00. once you start the trial, the condition is met half an hour later in the video.
  If you had set to start tracking After clock time 10:30, tracking would start immediately after starting the trial.

  **EXAMPLE 2** You set a sub-rule to start at 10:00 (1st day). The video file was created on March 6, 2008 at 11:00. Once you start the trial, the sub-rule never starts, because the planned start occurs before the initial time of the video. To make a sub-rule start when tracking from that video, set the start time between 11:00 and the video end time.

## When recording video, then tracking

You can choose to record video first with EthoVision XT and then acquire the tracks from the resulting video file. In that case:

- When recording video, Trial Control is turned off.
- When you track from that video, Trial Control is activated, but you cannot control hardware devices.

See Record video, then acquire a trial

## When re-doing a trial

For video files recorded with EthoVision XT, you can re-do the corresponding trial (see Redo a trial). Note that the Trial Control log files recorded with the previous instance of the trial are deleted.

# Trial control in multiple arenas

## Each arena gets its copy of Trial Control Settings

If your experimental setup includes two or more arenas, Trial Control is applied to each arena separately. This means that, if a condition is met in one arena, EthoVision XT takes the corresponding action in that arena, not the others.

EthoVision XT evaluates the instructions for each arena within the same sample time. The order in which arenas are evaluated is not stored in your data.

*See also an example*

- Working with trial control in multiple arenas.

## Using zones in multiple arenas

A condition or action is not specific to one arena. If the condition is based on a zone, that zone must be present in all arenas, and have the same name.

If the zone is not present in an arena, Trial Control cannot progress for that arena. Therefore, tracking does not stop unless you set a maximum trial duration or tracking reaches the end of the video. See Control the end of tracking

## Maximum trial duration with multiple arenas

Note the difference:

- If the Trial Control Settings include a maximum trial duration, tracking stops in all the arenas simultaneously.
- If the Trial Control Settings does not have a maximum trial duration, and a condition is set before the Stop track box, tracking does not necessarily stop in all the arenas simultaneously. Consider the following example:

  Start tracking is set when the subject is found for the first time, just like in the default Start-Stop trial rule. A Time condition of 5 minutes is placed before the Stop track box. If the subjects in two arenas are found for the first time at different times, tracking starts at different times in the two arenas. Tracking also stops at different times, after the five minutes applied to each subject independently.

# View trial control instructions during acquisition

## Aim

During acquisition you can view the status of the trial control instructions. This way you can check that the Trial Control Rule works as expected. For example, check that an action is taken when the subject enters a zone. You can also view Trial Control States, which are time intervals defined by two Trial Control Events.

## To view trial control states or events during acquisition

In the Acquisition screen, click **Show/Hide** and select **Show Dependent variables**. Select **Trial Control Event**, or **Trial Control State**.

*See also*

- An example in Conditioning test: view a Trial Control State variable

# Analyze trial control data

## Aim

To analyze the events of the Trial Control rule which occurred during a trial. For example, visualize when a condition became true, or when EthoVision sent a command to an external device. You may want to do this for two reasons:

- To test if the Trial Control rule works as expected.
- To analyze the subject's response to presentation of stimuli in conditioning tests.

## To visualize or calculate statistics of Trial Control data

1. Choose **Analysis** > **Analysis Profile** > **New**. In the Components pane, under **Trial Control**, click the button next to

   **Trial Control event** for example, to visualize when exactly a condition become true.

   **Trial Control state** for example, to visualize the time from the moment a condition became active to when the condition became true.

2. Calculate statistics or visualize the data (choose **Analyze** > **Results** > ...).

For details, see Trial Control in the Analysis profile (page 1116), and **Analysis of Trial Control data** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

## To analyze intervals based on Trial Control data

If you want to calculate statistics and visualize data of dependent variables like velocity in portions of a track based on Trial Control data, do the following:

1. In the Data profile, define an interval based on Trial Control data. You can use:

   Nesting over a Trial Control state or

   Nesting over a Free interval based on Trial Control events.

2. Specify the dependent variables in the Analysis profile.
3. Calculate statistics or visualize the data (choose **Analyze** > **Results** > ...).

## To export Trial Control data

See Export Trial Control data.

# Configure Detection Settings

## Main topics and tasks

# Introduction to Detection Settings

## Learn about

- Useful things to know about Detection Settings
- Adjust detection settings - General procedure
- Detection settings: Automated setup
- Detection settings: Advanced setup

# Useful things to know about Detection Settings

Detection Settings are a set of criteria to track moving subjects. Detection Settings specify, for example, how different the subject is from the background in terms of gray scale or color values, and how many images per second will be analyzed among those available.

You can create multiple Detection settings in the same experiment. For example, you can have one set for detecting white animals, and another to detect dark ones.

**TIP** For a DanioVision experiment, see the DanioVision DVOC-0041 - Reference Manual. To open this manual, on the **Apps** screen choose **Noldus** > **EthoVision XT 18 Other Documentation**.

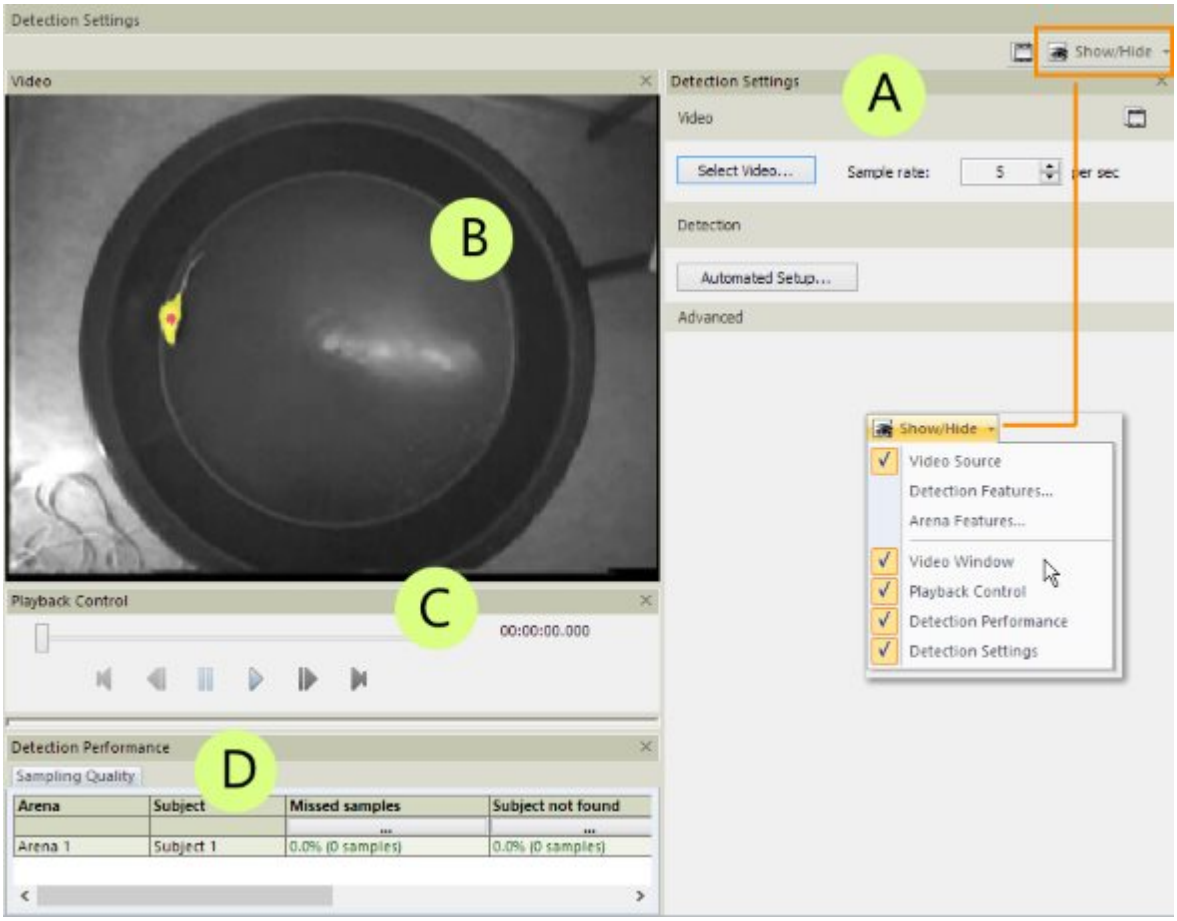
## To open the Detection Settings

Choose **Setup** > **Detection Settings** > choose one of the options.

By default, the following windows are displayed:

- A. Detection Settings
- B. Video, showing the current camera image or video file.
- C. Playback Control
- D. Detection Performance.

Click the **Show/Hide** button on the toolbar to view/hide windows, or change features shown, like the yellow blob representing the subject being detected.




## Automated Setup

EthoVision XT has an Automated Setup function to apply the detection settings that work well in the most common scenarios. In most cases you will obtain good detection using the Automated Setup. In difficult situations, for example if video quality is bad, or if you have multiple animals with color markers that are not distinct enough, the Automated Setup may not give good enough detection. If this is the case, use the advanced settings to create the detection settings manually.

## Detection Settings available in your EthoVision XT license

Which settings are available depends on your EthoVision XT license:

- EthoVision XT Base version: In this version, you can track the center-point, the nose-point and the tail-base point of a single animal.

  The base version also allows tracking of a color marker on a single animal; in this case the color marker is treated as the center-point of the animal.

- Social Interaction Module: This add-on allows you to track two or more animals in one arena, either color-marked or unmarked. Use this module in combination to study social interactions in detail.

  We recommend to only use Tracking from video files if you track the three body points in combination with the Social Interaction module.

- Behavior Recognition Module: For detecting a number of behaviors automatically, including rearing, grooming and sniffing. In the Detection Settings, the Behavior Settings are enabled.

# Adjust detection settings - General procedure

Subject detection works well if there is good contrast between the subject and the background in the video image, and for the whole duration of the trials. Increasing the contrast (for example, by changing the background so it differs as much as possible in color from the subject) is far more effective than any detection setting.

## To adjust the detection settings

1. Select the sample rate and video file. See Video file, image quality and sample rate on page 357.
2. Use the Automated Setup functionality to detect the subject. See Detection settings: Automated setup on page 366.
3. Optionally use advanced detection settings if Automated Setup does not give satisfying detection. See Detection settings: Advanced setup on page 370.

## Notes

- **IMPORTANT** Make sure you carefully follow the order of steps as described in the next topics. If a particular step does not apply to your setup, proceed to the next step.
- Every time you apply changes in the Detection Settings window, you can see the consequences in the Video window.
- To save the detection settings, click the **Save Changes** button at the bottom of the pane. If you have made more changes and you want to return to the last saved settings, click the **Undo Changes** button.
- EthoVision XT offers a number real-time statistics on the quality of detection that you can check while you adjust detection settings.

# Video file, image quality and sample rate

## Learn about

- Video file and image quality
- Sample rate

# Video file and image quality

What you see under **Video** depends on whether you track from video files, or live.

## Select Video

Available when tracking from video files. By default, the Detection Settings shows the same video file used in the Arena Settings, to obtain a background image.


To use another video file, click **Select Video**.

## Image quality

Available when tracking live, and for some cameras, not others. To adjust, for example, the image contrast or brightness, click the **Image Quality** button under **Video**.

**IMPORTANT** Always try adjusting the lighting and camera aperture settings before changing the Image Quality Settings.


In the window that appears, adjust the properties you require.


- Contrast enhances the lighter and darker parts of the image.
- Brightness makes the image lighter
- Saturation increases the color intensity.

Dependent on the camera, some settings may be grayed out.

## Notes

- For some cameras, the Image Settings are not available. In the Experiment Settings, click the camera button and click the Image tab. See Video source
- The Image Quality Settings also affect the image that is saved to a video file.
- To return to the defaults of the camera driver, click the **Default** button.

# Sample rate

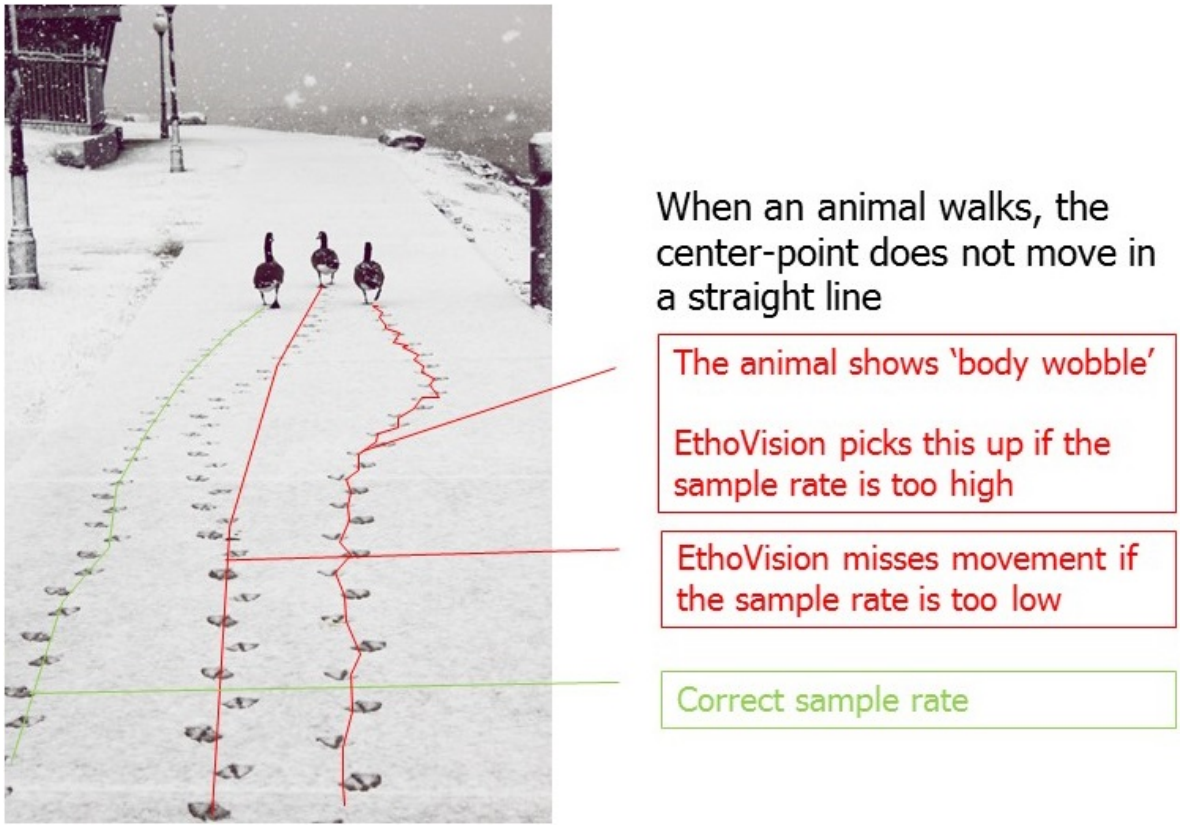
The sample rate is the number of images EthoVision XT analyzes per second. Selecting a certain sample rate does not mean that the program can always analyze data at that rate. If the computer processor load is too high, EthoVision XT may skip a sample and analyze the next one. Skipped samples result in missed samples (see below).

The maximum sample rate is the camera frame rate. The sample rate you set in EthoVision XT can only be the frame rate divided by an integer. For example, for PAL video it is 25, 12.5, 8.33, etc.

**IMPORTANT** Some digital cameras support very high frame rates. However, subject detection at such rates requires a lot of processor capacity. To prevent that EthoVision XT discards samples while tracking live, do not set the frame rate and sample rate too high. Check the percentage of missed samples (see View the detection statistics) to make sure the EthoVision XT can handle the selected frame rate.

## What is the optimal sample rate?

Setting the correct sample rate is very important. If the rate is too high, the noise caused by small movements of your animal will be picked up and give an overestimate of dependent variables such as the distance moved. If the sample rate is too low, you will loose data and get an underestimate of the distance moved. See also the picture below.




## General guidelines for the sample rate

We strongly recommend that you determine the optimum sample rate for your specific setup and animals (see To determine the optimal sample rate). If, for instance, your treatment causes hyperactivity, you will need a higher sample rate for hyperactive animals than somnolent animals.

**NOTE** In the list below, the sample rate for NTSC video (29.97/s) is approximated to 30/s.

### *Rodents*

- Rat, single subject, center-point only: 5/s
- Mouse, single subject, center-point only: 12/s
- Rat/Mouse Behavior recognition: 25-31/s (depending on TV standard)
- Rat/Mouse, three body points: 25/s (PAL) to 30/s (NTSC or digital camera)
- Rat/Mouse, three body points combined with marker-assisted identification: 12.5/s (PAL) to 15/s (NTSC or digital camera)
- Rat/Mouse, three body points combined with Deep learning-based identification: 25/s or higher. See Deep learning: Requirements

*Other mammals*

- Tree shrew (*Tupaia*): 6-12/s

*Fish*

- Zebrafish, adult: 5-6/s
- Zebrafish larva: For analog camera: 25/s (PAL) or 30/s (NTSC). For digital camera: 30/s or 60/s*

  (*) For rapid movements you may want to track with a higher sample rate. It depends on the number of tracked subjects, the video resolution, the camera settings and the processor speed of your computer whether that is possible.
- Damselfish: 5/s
- Goldfish: 0.5/s

*Arthropods*

- Mite: 1/s
- Parasitic wasp: 2/s
- Tick: 3/s

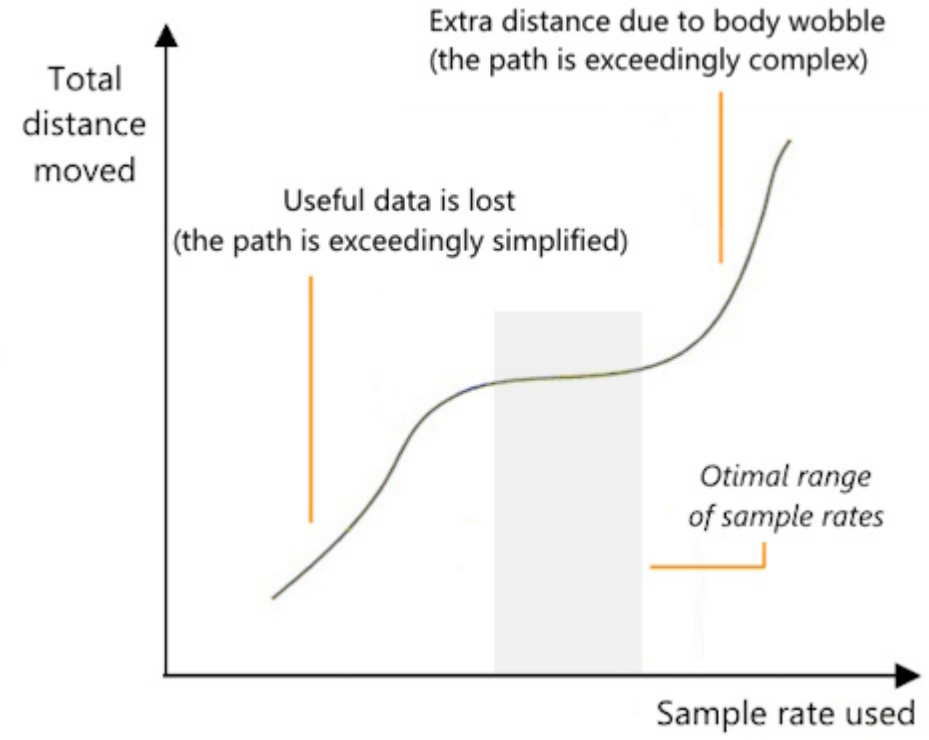
## To determine the optimal sample rate

The optimal sample rate is the minimum sample rate that provides an accurate estimation of the dependent variables (distance, velocity, etc.) without including the redundant information due to phenomena other than the 'real' locomotion. For example, for an animal walking in a straight line the data points will never be in a straight line because the center-point of the subject shifts laterally with each step. In order to distinguish between 'real' movement and effects like the one described above, you can calculate dependent variables like distance moved using the maximum or a lower sample rate.

1. Create new Detection Settings and specify the maximum sample rate. Be aware that the maximum sample rate also depends on the performance of your computer, the number of animals you track, and the video resolution.
2. Acquire data with those Detection Settings.
3. Calculate the dependent variable you are interested in.
4. Export the data to Excel and plot the dependent variable values against the sample rate. In the example below, distance moved is used.

5. Repeat the steps above by selecting smaller sample rates.

Once the data are plotted as in the figure below, there should be a range of sample rates for which the dependent variable value does not change much (plateau). This means that slight changes in the sample rate do no result in loss of information, or addition of redundant information (noise and movements like body wobble).




Low sample rates result in loss of useful information, because the sinuosity of the original path is removed. Therefore, the total distance moved is usually decreased.

High sample rates result in acquisition of redundant information. In the case of body wobbling, and assuming that the animal is moving along a straight line, the lateral shift of the body center causes the total distance moved to be longer than the 'real' one. With Track smoothing you can filter out 'noise' as a result of body wobble. See Smooth the Tracks

## Missed samples

The actual sample rate may be lower than the maximum you set, because an image cannot be captured until the previous one is processed. If the sample rate you define is too high, EthoVision will miss samples (up to 1% is acceptable) and the processor load will be high. The percentage of missed samples is shown in the Detection performance pane and in the Trial List as a System Variable. You can calculate the number of missed samples in acquired tracks with the Number statistic of continuous variables (e.g., velocity). If your processor load is larger than

100, and there are large amounts of missed values, you will have to lower the sample rate.

**TIP** After acquisition you can see the proportion of missed samples in the Trial list as one of the System Variables.

**TIP** When acquiring from video files, in the Playback Control window select the option DDS (Detection Determines Speed). This way, no samples will be missed. See DDS (Detection determines speed)


## Factors affecting the occurrence of missing samples

The following factors may cause the processor load to be too high:

- Computer memory, processor speed and video graphics card capacity: See the system requirements. In general, using a computer with a dual-core CPU helps you to work with higher sample rates than normal computers do.
- Other programs installed: Do not install other video software (for example, video editing programs, DVD burning software), because this can interfere with EthoVision XT's video processing and cause a reduction in performance.
- Other programs are running: Make sure you shut down all other programs, including those running in the background such as e-mail programs and virus scanners. These are usually shown in the System Tray in the bottom-right corner of your screen.
- Image resolution: For live video tracking, In the Experiment Settings you can choose the resolution for your video image.
- Size of arenas: Make arenas as small as possible (but including the entire area the animal can be in).
- Number of arenas: If you track live and use more than four arenas in a trial, check first that no samples are missed. If the number of missed samples is too high, first make a video file, then track from that. More generally, if you track from video files the number of arenas is never a problem as long as you select DDS (Detection determines speed).

  When making detection settings, you could start with making an arena definition with only one area which speeds up the detection process. After

you have finished configuring detection settings for one arena, you can add the others to the arena definition.

- Display options: You can decrease processor load by minimizing the number of Track Features to be displayed (see View the detection features on the video window).
- Detection method: If possible, use the Gray scaling method which requires less processor load than Static subtraction. Static subtraction requires less processor load than Dynamic subtraction and Differencing. See Advanced detection settings: Method

# Detection settings: Automated setup

## Aim

To quickly instruct EthoVision XT how to recognize the subject that moves in the arena.

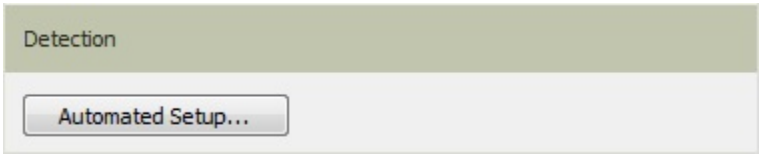
**TIP** For common tests like the Open field and the Morris water maze, choose Automated Setup. If Automated Setup does not work well enough, see Detection settings: Advanced setup.

## Prerequisites

- The subject must be released in the arena. If you work with pre-recorded video, play the video to a point when the subject is well visible.
- The contrast of the subject with the background is crucial for success with video tracking software. Choose **Help** > **Video Tutorial** and watch the video chapter **Set up your Test Environment** for tips on how to improve the contrast between the subject and the background.

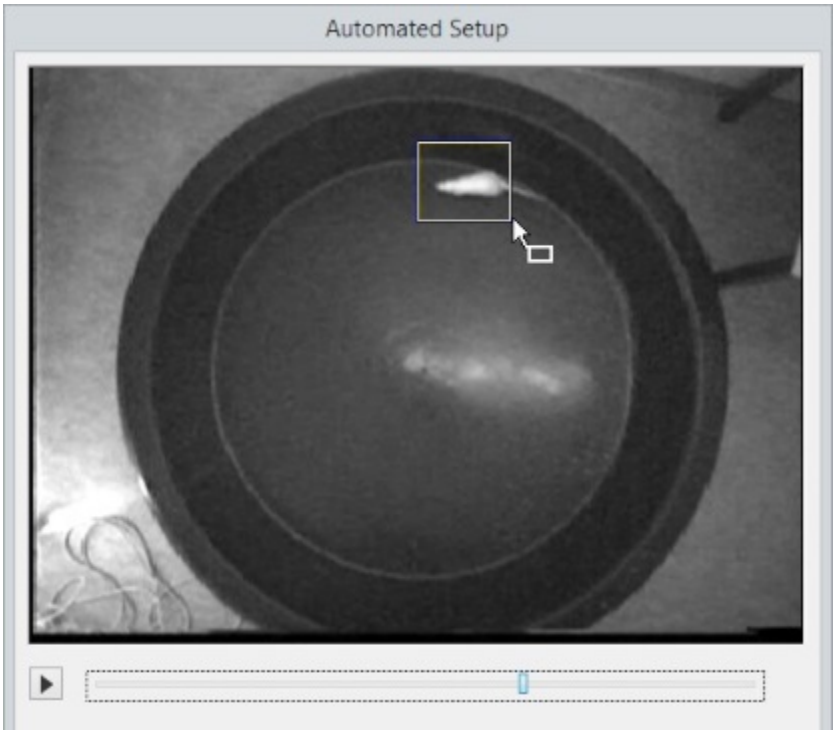
## Procedure

1. In the Detection Settings pane, click the **Automated Setup** button.




2. Choose the type of animal you work with, then click **Next**.


3. Drag the mouse to draw a rectangle around the subject.

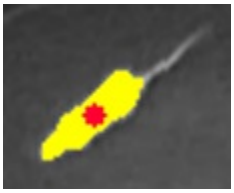



- If you have multiple subjects, draw a rectangle around each of them. Make sure that the subjects are not in contact with one another.
- If you have multiple arenas, draw a rectangle around each subject in every arena.
- For rodents, draw the rectangle around the animal's body. Try to leave the tail out of the rectangle.
- For fish, always include the caudal fin in the rectangle.

4. Move the **Finetune** slider until detection quality is satisfactory.


Moving the slider to the left detects more from the subject, for example it results in including the legs and tail of a rodent, but also more noise. Moving the slider to the right results in detecting less noise, but also less of the subject.

Below you see a detailed image of the same rat as in the picture above, when detected correctly (the red dot marks the detected center point).



5. Check in the Detection Performance pane under **Subject not found** if detection is good. As a guideline, the percentage Subject not found should be lower than 10% if the animal is visible at all times. Realize that the initial frames in which the subject was not detected yet is included in this percentage. If the number of samples (in brackets) under **Subject not found** does not increase or hardly increases anymore, and the percentage Subject not found decreases, detection is good. After acquisition, in the Trial list click the **Show/Hide** button and select Variables. Select **Subject not found** and check the percentage in the **Subject not found** column.
6. If detection quality is satisfactory, click **Yes**. If not, click **No** and continue with the Detection settings: Advanced setup.

## Notes

- If you work with the Deep learning detection technique, the Automated Setup (above) also includes the automatic adjustment of the Cutout window. This makes it less likely that you need to adjust this setting once again. See Adjust the settings for nose-tail base detection (Deep learning)

## The next step

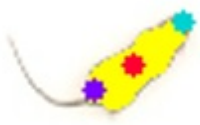
What you do next depends on how many subjects and what EthoVision XT functions you want to use.

- One subject per arena; Center-point detection

  At this point you do not need to do anything anymore. If detection is not good enough, see Detection settings: Advanced setup

- One subject per arena; Nose-tail detection

  See Detect the nose and the tail base. In the picture below you see an example of a mouse with its three body points detected correctly.



- Social interaction (marker-assisted identification)

  See Advanced detection settings: Marker-assisted identification, step 4.

  If not all subjects are detected well after the Automated Setup, click **No**. Click **Automated Setup** again, and repeat the procedure when the subjects are in other locations. If this does not help, see Advanced detection settings for tracking color-marked subjects.

- Social interaction (unmarked subjects)

  See Advanced detection settings for tracking multiple unmarked subjects

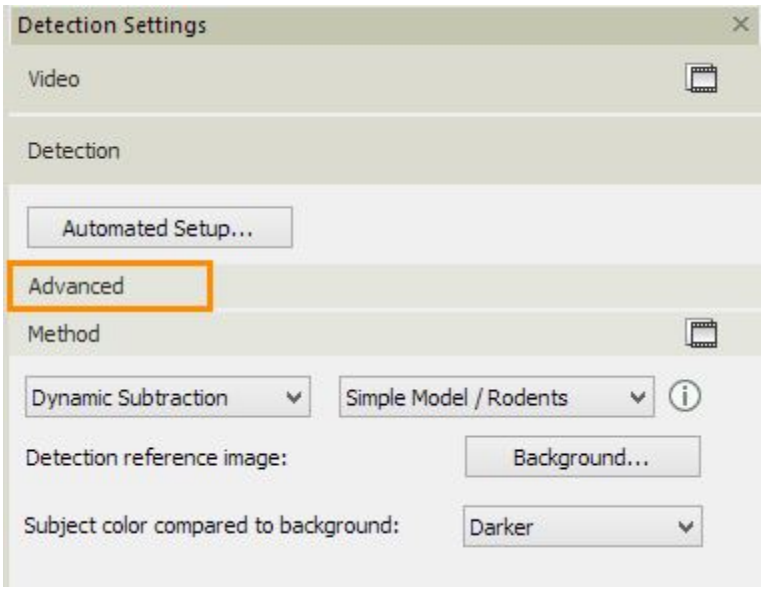
- Rat or Mouse behavior recognition

  See Detection settings for Behavior recognition.

  If detection is not satisfactory after the Automated Setup, continue with Detect the nose and the tail base. Then proceed with Detection settings for Behavior recognition.

# Detection settings: Advanced setup

If you do not get satisfactory detection with the Automated Setup, in the Detection Settings pane click **Advanced**.




## To track one animal per arena, center-point only

Follow the procedures in this order:

1. Advanced detection settings: Method
2. Advanced detection settings: Smoothing
3. Advanced detection settings: Subject contour
4. Advanced detection settings: Subject size (one subject per arena)

## Other cases

- For tracking multiple animals per arena, color-marked

  See Advanced detection settings for tracking color-marked subjects
- For tracking multiple animals per arena, unmarked

  See Advanced detection settings for tracking multiple unmarked subjects
- For tracking center-point, nose-point and tail-base

  See Detect the nose and the tail base
- For Behavior Recognition

See Detection settings for Behavior recognition

- For tracking larvae in DanioVision

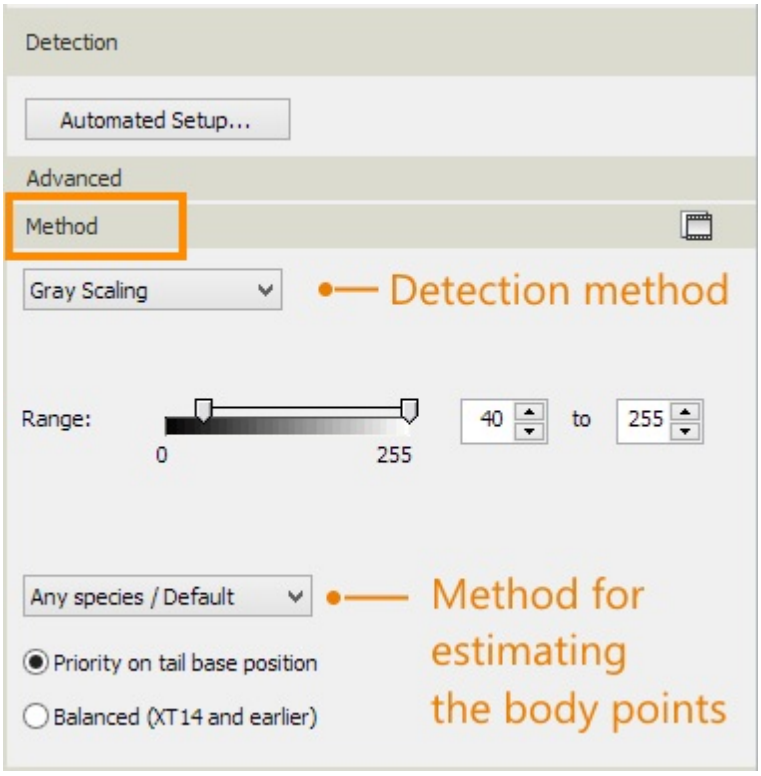
  See the DanioVision DVOC-0041 - Reference Manual. To open this manual, on the **Apps** screen, choose **Noldus** > **EthoVision XT 18 Other Documentation**.

# Advanced detection settings: Method

## Aim

To choose:

- The *detection method*, that is, how EthoVision XT finds the subject over the background. Once you have selected the method, specify the background image (for the detection methods Static Subtraction, Dynamic Subtraction and Differencing only) and the level of contrast between subject and background. See below.
- The *method for estimating the body points*. See Methods for estimating the body points




## Gray Scaling

Choose Gray scaling when:

- The animal's grayness differs from the background in all parts of the arena.
- The background does not change during a trial.
- Lighting is even (minimal shadows and reflections) during the trial.

**EXAMPLE** Tracking a white rat in a uniform black open field with no bright objects.

## Static Subtraction

Choose Static Subtraction when:

- The Gray scaling method does not work, for example because other objects in the arena have a similar color as the animal.
- The background does not change in time.
- The light is constant during the trial.

**EXAMPLE** Tracking a white rat in an open field with unavoidable reflections or bright objects.

## Dynamic subtraction

Choose this method when during trials light conditions gradually change or the background changes, for example because bedding material is kicked around, food pellets are dropped, droppings appear etc.

**EXAMPLE** Tracking a mouse in a home cage provided with bedding material. The activity of the mouse causes the bedding to change appearance in the video image.

## Differencing

Choose this method when there is a lot of variation in contrast between a subject and the background within an arena.

**EXAMPLE** There is a gradient in light intensity over the apparatus that cannot be removed, or a strong difference in the color of the fur of the animal, like in hooded rats.

# Gray Scaling

## Aim

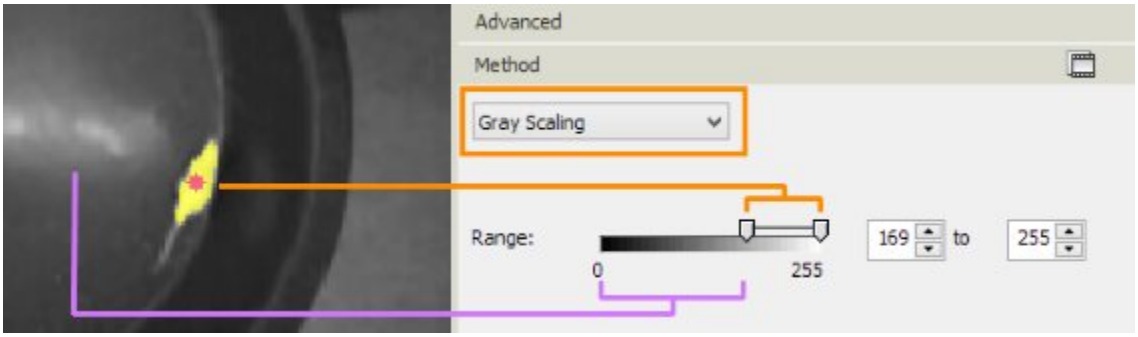
To detect the subject, under the assumption that its color is always different from that of the background (no shadows, gradients etc.).

## How Gray scaling works

The video image is converted to monochrome. Each pixel in the image has a gray scale value, ranging from 0 (black) to 255 (white). With Gray scaling, you define which range of gray scale values should be considered as the subject. The remaining gray scale values are considered as background. The Gray scaling method does not make use of a background image, the subject is determined by its gray scale values only.

## Procedure

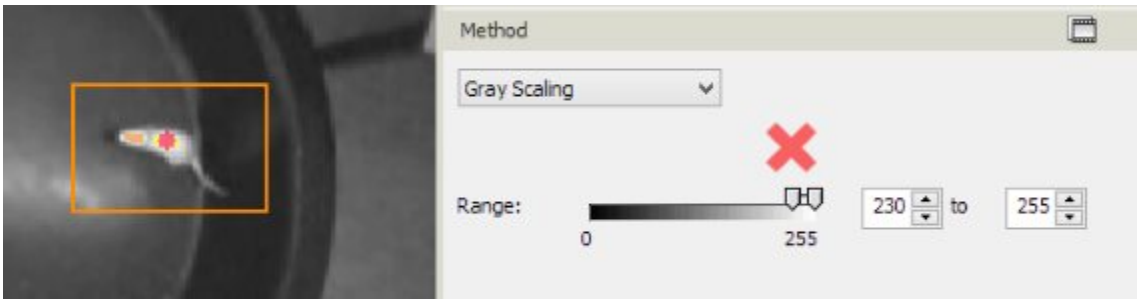
1. In the Detection Settings pane, click **Advanced**, then **Method**. Select **Gray scaling**.
2. Release the subject in the arena, or position the media file at a point where the subject is moving.
3. Move the two sliders next to **Range** or type the values in the corresponding fields to define the lower and higher limits of gray scale values for the subject. Make sure there are no areas in the background with gray scale values that fall in this range, otherwise they will be detected as the subject.



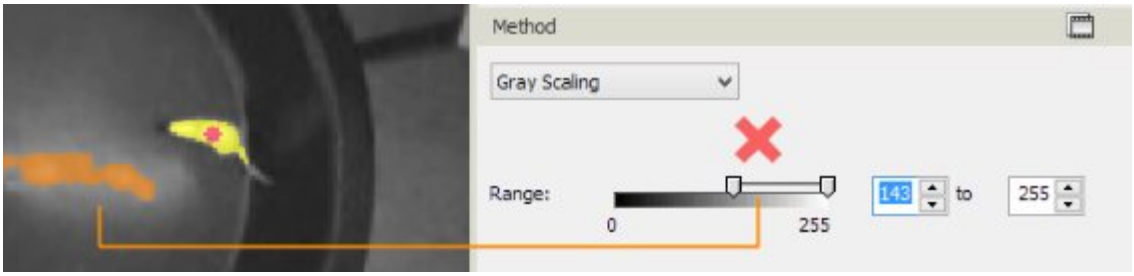

4. In the Video window, check the quality of detection.

   If the detected area (by default, in yellow) is too large, or other objects are detected as the subject, reduce the range in either direction (brighter or darker).

If the detected area is too small relative to the real subject, increase the range at least in one direction (brighter or darker).




Areas marked as noise (by default, in orange), indicate that the gray scale range is too wide: you need to narrow it in at least one direction.




5. Move the sliders until the subject (or the part which is of interest) is detected fully, and the noise is minimized. Check that the subject is properly detected in all parts of the arena by moving the video slider, or by waiting for the live animal to move.

**IMPORTANT** The whole animal's body must be detected for optimal tracking. Proceed with the Contour adjustments to optimize body detection.

# Static Subtraction

## Aim

To detect the subject, assuming that the background does not change over time.

## How Static Subtraction works

The video image is converted to monochrome. Each pixel in the image has a gray scale value, ranging from 0 (black) to 255 (white). With the Static subtraction method, you choose an image of the arena without the subject, named *Reference Image*. When analyzing the images, EthoVision XT subtracts the gray scale value of each pixel in the reference image from the gray scale value of the corresponding pixel in the current image (live or from video files). The pixels with non-zero difference are considered the subject.

You can remove small non-zero differences by defining the contrast between current image and background that must be considered as the subject (see the procedure below). The remaining pixels are considered as the background.

Below: An example of how the Static subtraction detection method works. The gray scale value of each pixel of the reference image is subtracted from the gray scale value of each pixel of the live image. The result is '0' for every pixel; if the difference > '0' and within the gray scale range you have set, these pixels are considered to be the subject. So, with this method your task is to specify the contrast that optimizes the detection of the subject.


## Procedure

1. In the Detection Settings pane, click **Advanced**, then **Method**. Select **Static subtraction**.
2. Click the **Background** button. The **Reference Image** window opens with the image that is currently used as background. The aim is to obtain a reference image that does not contain images of the animals you want to

track. To do so, follow the instructions on the screen in consecutive order. If A fails, move on to B, if that fails move on to C. See also Optimize the reference image.

3. From the **Subject color ...** list, select one of the following, depending on the color of the subject you want to track:

   **Brighter**: For example, to track a Wistar rat in a black open field.

   **Darker**: For example, to track a C57BL6 mouse in an open field with white bedding.

   **Brighter and darker**: For example, to track a DBA2 mouse in a home cage with white background and a black shelter, or a hooded (black and white) rat in a uniform gray open field.

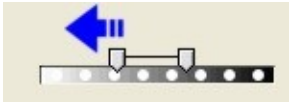
   Depending on the selection above, different contrast sliders become available. For each slider, the contrast varies from 0 (no contrast) to 255 (full contrast). Unlike with Gray scaling, the values selected with the sliders represent the difference between the current and the reference image, not absolute gray scale values.

4. Release the subject in the arena, or position the media file at a point where the subject is moving.

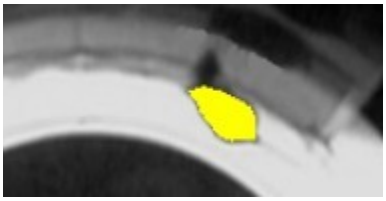
5. Move the appropriate slider or type the values in the corresponding fields to define the lower and higher limits of the contrast that corresponds to the subject. In the Video window, check the quality of detection.

   **EXAMPLE 1** The subject is brighter than the background. Only the whiter area of the subject is detected.

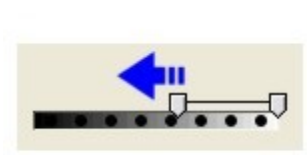
   Move the **Bright** slider to the left to increase the range towards values of lower contrast between subject and background.

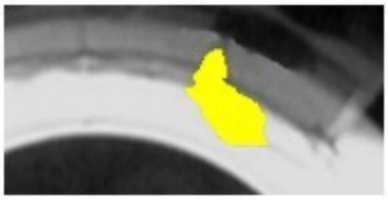


   **EXAMPLE 2** The subject is darker than the background. Its body is detected only partially in the area of lower contrast.

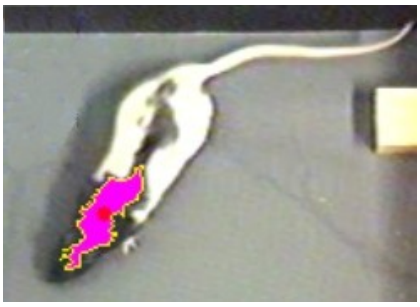


Move the **Dark** slider to the left to increase the range towards lower values of contrast between subject and background.

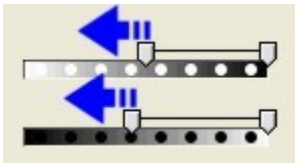




**EXAMPLE 3** The subject is brighter and darker than the background. Only the darker areas of the black fur are detected.



Move the **Bright** slider to the left to increase the range towards less contrast between the subject's white areas and the gray background. Then, move the **Dark** slider to the left to increase the range towards less contrast between the subject's black areas and the background.



6. Move the sliders until the subject (or the part which is of interest) is detected fully, and the noise is minimized. Check that the subject is properly detected in all parts of the arena by playing back different parts of the video file, or by waiting for the live animal to move.

## Notes

When the subject is brighter and darker than the background, detection only works well when there is enough contrast between the areas of different brightness and the background. For example, tracking a hooded rat works well when the background is intermediate between black and white. Rather, use Differencing.

# Dynamic subtraction

## Aim

To detect the subject, while compensating for temporal changes in the background.

**EXAMPLES**

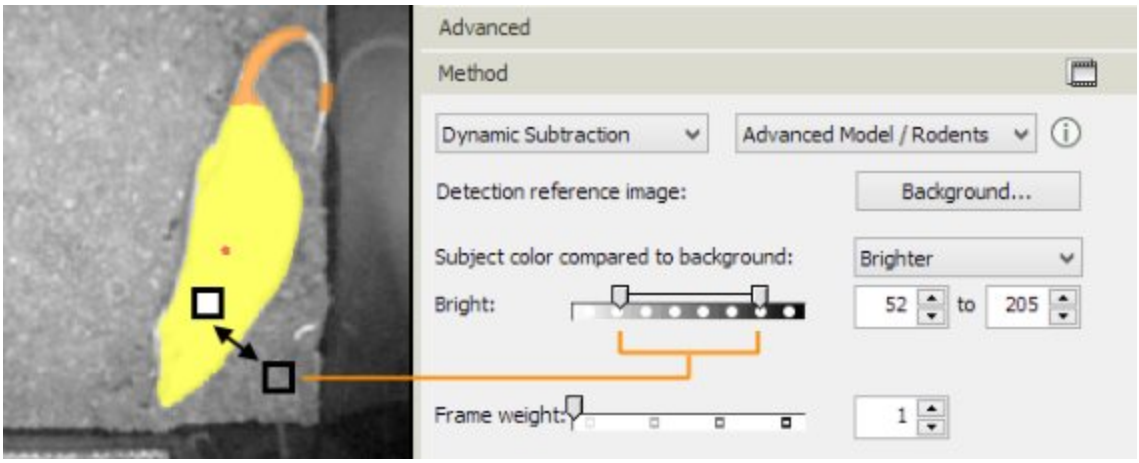
- Detect a rat in a water maze, where other detection methods do not work, usually because of the effect of waves in the pool.
- Detect a mouse in a PhenoTyper or home cage with bedding material, where the animal's activity (e.g. digging) changes the appearance of the background.
- All cases where lighting changes slowly.

## How Dynamic Subtraction works

Like with Static subtraction, EthoVision XT compares each sampled image with a reference image, with the important difference that the reference image is updated regularly. This compensates for temporal changes in the background. See How the reference image is updated in Dynamic subtraction.

## Procedure

1. In Detection Settings pane, click **Advanced**, then **Method**. Select **Dynamic subtraction**.
2. Click the **Background** button. The Reference Image window opens with the image that is currently used as background. The aim is to obtain a reference image that does not contain images of the animals you want to track. To do so, follow the instructions on the screen in consecutive order. If A fails, move on to B, if that fails move on to C. See Optimize the reference image.
3. From the **Subject color ...** list, select one of the options from the list, depending on the color of the subject you want to track.
4. Move the slider next to **Bright**/**Dark** to select the range of the contrast between subject and background.




5. Move the slider next to **Frame weight** or enter the value in the appropriate field to specify how the reference image is updated (range 0-100%):

In typical situations, a value between 1-5 gives a good result.

**IMPORTANT** As much of the animal's body must be detected for good tracking. See Advanced detection settings: Subject contour to optimize body detection.

## Frame weight

- Select a low value if you want to have a large number of past images to contribute to each reference image. As a result, changes in the background are diluted over many images. Choose a low value when the background changes slowly.
- Select a high value if you want to have a small number of past images to contribute to each reference image. As a result, changes in the background are captured over short time. Choose a high value when the background changes rapidly, for example, when the subject is very active and moves the bedding material around.
- If you select 0 as Frame weight, the reference image is not updated. This is the same as using Static Subtraction.
- If you select 100, each sample gets its own reference image with no contribution by the past images. In most cases a Frame weight of 100 does not give good detection, because the subject itself is often removed from the detected image when it moves slowly or sits still.

**TIP** To find the optimal Frame weight, set a value and carry out one or more trials. Evaluate if the tracking was satisfactory. If not, increase or decrease the setting by 20% and try again.

# Differencing

## Aim

To detect the subject, while compensating for spatial and temporal changes in the background.

## How Differencing works

Like with Dynamic subtraction, the Differencing method updates the reference image over time. Differencing makes a statistical (probabilistic) comparison between each pixel in the reference image and the pixels of the current image. The statistical comparison uses the variance in the contrast between the current and reference image to calculate the probability that each pixel is the subject.

The Differencing method takes more processor load than the subtraction methods. Therefore, when using Differencing, make sure you computer meets the system requirements.

## Procedure

1. In the Method section of the Detection Settings window, select **Differencing**.
2. Click the **Background** button. The Reference Image window opens with the image that is currently used as background. The aim is to obtain a reference image that does not contain images of the animals you want to track. To do so, follow the instructions on the screen in consecutive order. If A fails, move on to B, if that fails move on to C. See Optimize the reference image.
3. From the **Subject color ...** list, select one of the options from the list, depending on the color of the subject you want to track.
4. Next, if necessary, adjust the position of the **Sensitivity** slider and change the option selected in the **Background Changes** list.


The **Sensitivity** slider determines what difference in contrast from the background is seen as the animal. For an image with good contrast, there is no need to change the slider. For images with less contrast, adjust the position of the slider to the right or the left until the subject is properly detected.

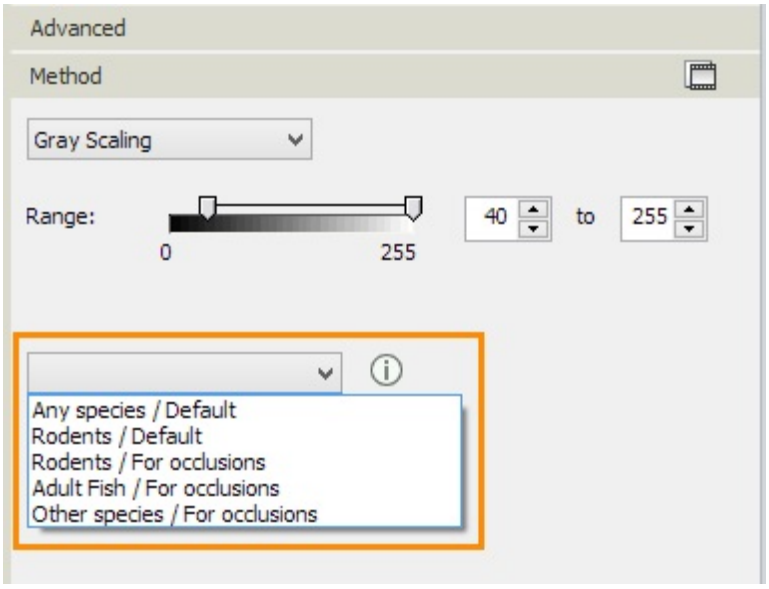
In the **Background Changes** list you can select options that reflect how fast the background changes. For example, a cage with bedding might change a

lot because of animals kicking around the bedding material. If this case, to prevent changes in the background to interfere with detection, select 'Medium fast' or faster. Usually, 'Medium slow' works just fine.

**IMPORTANT** As much as possible of the animal's body must be detected for good tracking.

# Methods for estimating the body points

Depending on the experiment mode (standard vs. DanioVision) and how many subjects and body points you specify in the Experiment Settings, the Method section of the Advanced Detection Settings window lists one or more options.




**NOTE** In some cases, like when tracking the center point of one subject, this group of options is not available, because EthoVision XT applies the only method possible for that experiment.

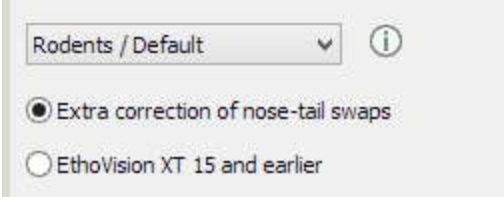
### *Any Species / Default*

- This was called *Shape-based / Any* in EthoVision XT 14 and earlier.
- This method analyzes the contour of the blob detected as subject at each sample to assign the nose-point and the tail-base. If you track rodents, make sure that the tail is fully detected.
- The subject's center-point is calculated as the geometric center of the *area detected as subject*. See Calculation of the center-point below for details.
- This method is automatically activated when you track the center-point in one animal per arena, and you specified **Other** species in the Automated setup.
- If your experiment is from an older EthoVision XT version, you have additional options available. Here you can choose to use the last improvements or keep the settings as they were in the older experiments.
    - Choose **Priority on tail base position** whenever the tail is apparent in the video and well detected. This method finds the tail base and then

assigns the nose-point to the opposite point in the subject contour. If the tail base is not found, then other rules based on the contour are used to find the nose-point. Choose **Balanced (XT 14 and earlier)** if you want to use the same method as in previous EthoVision XT versions, or if for any reason the first option does not give good results.

### *Rodents / Default*

- This was called *Simple Model / Rodents* in EthoVision XT 14 and earlier.
- This method analyzes the shape of the blob detected as subject and builds up a "rodent model". It is more robust than the *Any species / Default* method because it does not require the nose and tail to be visible: it can 'predict' the position of the nose and the tail based on previous samples.
- The subject's center-point is calculated as the geometric center of the *area detected as subject*. See Calculation of the center-point below.
- Choose this method when you want to track a single rodent without occlusions or without difficult tracking conditions, like in an open field or a novel object recognition test. For optimal results, make sure in the detection settings that the tail is removed from the body contour, using Erosion and then Dilation. See Advanced detection settings: Subject contour
- If you upgrade your experiment from an older version of EthoVision XT, select **Extra correction of nose-tail swaps** to use the latest algorithm that aims at correcting nose-tail swaps during tracking. Compared to previous versions, this algorithm corrects swaps faster, reducing the total number of samples with swaps (if they occur). Choose **EthoVision XT 15 and earlier** if you want to keep using the old algorithm.




The extra correction is based on the detection of false backward movements, that is, when the animal looks like it is moving backward when the nose and the tail-base points are swapped.

### *Rodents / For occlusions*

- This was called *Advanced Model / Rodents* in EthoVision XT 14 and earlier.
- This method learns the animal shape and how it moves in the first 15 frames and continually updates its statistics. Therefore, it can handle severe

shape distortions, such as, for example, when the subject's body is occluded or when two subjects come into contact. However, it requires a lot of computer performance.

- The subject's center-point is calculated as the geometric center of the *area detected as subject*. For experiments created with previous EthoVision XT versions, choose how EthoVision XT should find the center point. See Calculation of the center-point below for details.
- Choose this method for rodents in a social interaction context, or when you track rodents that can be occluded, for example, by bars or other objects in the cage. For optimal results, make sure in the detection settings that the tail is removed from the body contour, using Erosion and then Dilation. See Advanced detection settings: Subject contour and Subject contour for nose-tail base detection

### *Adult Fish / For occlusions*

- This was called *Advanced Model / Adult Fish* in EthoVision XT 14 and earlier.
- Choose this method to track adult zebrafish and other fish of similar shape, when viewed from above. Make sure that the whole body of the fish is detected, including the tail. This method is recommended no matter how many fish you track in the same tank.
- The subject's center-point is calculated as the geometric center of the *area detected as subject*. For experiments created with previous EthoVision XT versions, choose how EthoVision XT should find the center point. See Calculation of the center-point below for details.

### *Other species / For occlusions*

- This was called *Advanced Model / Other* in EthoVision XT 14 and earlier.
- This method does not make any assumption about the shape of the subject. Note: with this method you only track the center point.
- The subject's center-point is calculated as the geometric center of the *area detected as subject*. For experiments created with previous EthoVision XT versions, choose how EthoVision XT should find the center point. See Calculation of the center-point below for details.
- Choose this method to track for example insects, crustaceans or large animals, and when multiple subjects move in the same arena, or when occlusions make detection difficult.
- Also choose use this method to track fish from the front view of the water tank, and the Adult Fish method does not give good results.

*DanioVision*

- This method is optimized to track zebrafish larvae on a white background. It is only available in DanioVision experiments.
- Moving the **Sensitivity** slider to the left results in detecting more of the body of the larvae, but also more noise. Moving the slider to the right results in detecting less noise, but also gives less good detection of the larvae.
- For more information, see the DanioVision DVOC-0041 - Reference Manual.

## Method names

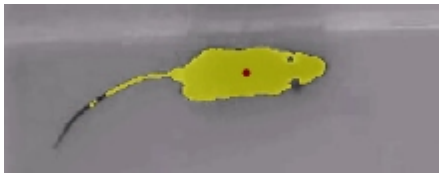
Please note that the names of the methods above differ from those in EthoVision XT 14 and earlier versions:

| EthoVision XT 15-17 | EthoVision XT 12-14 | EthoVision XT 11 and earlier |
|---|---|---|
| Any species / Default | Shape-based / Any | Shape-based (XT 4) |
| Rodents / Default | Simple Model / Rodents | Model-based (XT 5) |
| Rodents / For occlusions | Advanced Model / Rodents | Advanced Model-based (XT 6) |
| Adult Fish / For occlusions | Advanced Model / Adult Fish | - |
| Other species / For occlusions | Advanced Model / Other | - |

## Calculation of the center-point

*For new experiments*

In all new experiments, the subject's center-point is determined using the area of the blob (in yellow, per default) that indicates detected subject. The x, y coordinates of the center-point are the average of the x, y coordinates of each pixel in the blob. For example:

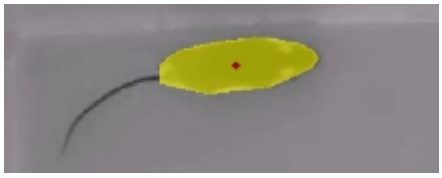


If the tail is detected, that contributes to the position of the center-point.

*Experiments created in EthoVision XT 14 and earlier*

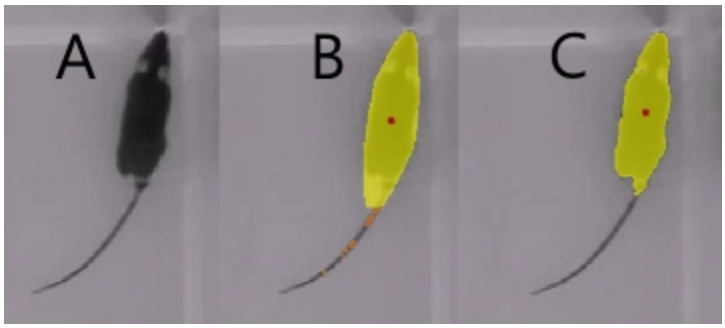
If your experiment was created in a previous EthoVision XT version, and you use one of the For occlusions methods, you can choose whether to use the detected area (as described above) or the *shape model*. Choose **Use center of model (XT14 and earlier)**.

The x, y coordinates of the center-point is the average of the pixels coordinates of the model fit over the detected subject. In many situations the model has a more regular shape than the detected area. For example:



Choose this option **Use center of model (XT14 and earlier)** to keep compatibility with older experiments. Consider that, if detection or the model parameters are not optimal, the model may differ greatly from the subject's image. In this case the center point may shift compared to the expected position (see B vs. C in the next picture). This may add up distance moved on the long run.

Below: A. Subject not detected. B. Center point determined as the model's center. C. Center point determined as the center of the detected area.




If that happens often, choose **Use center of detected area**. Or, for multi-subject tracking, adjust the modeled subject size. See Advanced detection settings: Subject size (multiple animals per arena)

*See also*

- Adjust the settings for nose-tail base detection (Contour-based)
- Subject contour for nose-tail base detection

# Advanced detection settings: Smoothing

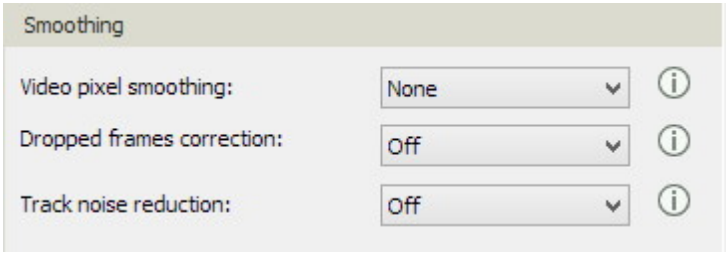
## Aim

To make tracking less depending on noise.

- Use **Video pixel smoothing** to remove fine-grained noise in the video image.
- Use **Dropped frames correction** to compensate for irregular frame rate in low-end cameras.
- Use **Track noise reduction** to reduce jitter of the body points and smooth out the track during acquisition.

## How to access these options

In the Detection Settings window, under **Advanced**, click **Smoothing**.




## Procedure

- Video pixel smoothing
- Dropped frames correction
- Track noise reduction

*See also*

- Smooth the Tracks after acquisition

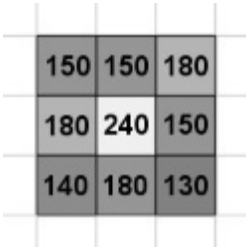
# Video pixel smoothing

## Aim

The **Video pixel smoothing** option reduces the difference between adjacent pixels prior to detection, by smudging the image, that is, replacing the gray scale value of each pixel with the median of the surrounding pixels.

## Background information

How does video pixel smoothing work? In the following example, a bright pixel with gray value 240 is surrounded by darker pixels:




- If you select Video pixel smoothing= Low, that pixel gets the median value calculated among the 8 nearest pixels plus that pixel itself. In that case the median is 150, so that pixel will look darker.
- If you specify Video pixel smoothing= Medium, the median is calculated over the 24 nearest pixels plus the pixel itself.
- If you specify Video pixel smoothing= High, an even bigger group of surrounding pixels is considered.

## Procedure

1. In the Detection Settings, under **Advanced**, open **Smoothing**.
2. Next to **Video pixel smoothing**, choose one of the values:
   - **None** (default): No pixel smoothing. The video image is analyzed for subject detection as it is.
   - **Low**: Each pixel is blended with the 8 nearest pixels (pixel distance =1).
   - **Medium**: Each pixel is blended with the 24 nearest pixels (pixel distance 1 or 2).
   - **High**: Each pixel is blended with the 48 nearest pixels (pixel distance 1, 2 or 3).

## Notes

- Select a moderate Video pixel smoothing value or leave None selected If adjacent pixels in the background are relatively constant. Using more surrounding pixels for the smoothing effect does not bring up better results.
- Select a high Video pixel smoothing value if adjacent pixels in the background are on average very different. For example, when the cage's bedding material looks grainy. In such cases you need to smooth each pixel using more surrounding pixels to compensate for this variation.
- A high Video pixel smoothing level requires a significant amount of processor capacity.
- Using Video pixel smoothing may result in losing information in the video image important for detection. For example, sharp borders of subjects, etc.
- Pixel smoothing does not affect Color marker tracking. It does affect detecting the body contour in Marker assisted tracking.

*See also*

- Advanced detection settings: Smoothing

# Dropped frames correction

## Aim

To interpolate data points when a video frame is not available at the required sample time.

**NOTE** This information does not apply if you use analog cameras, GigE cameras and USB cameras supported with EthoVision XT. Normally those cameras do not skip a significant number of video frames, provided that you set the camera properly, especially the resolution, the frame rate and the exposure time. For digital cameras, see Configure the digital camera

**IMPORTANT** This correction only deals with missing video frames, not when EthoVision XT does not detect the subject in a valid video frame, for example because of wrong detection settings. If you want to interpolate the points when the subject was not found, do this in the Track Editor. See Interpolate points

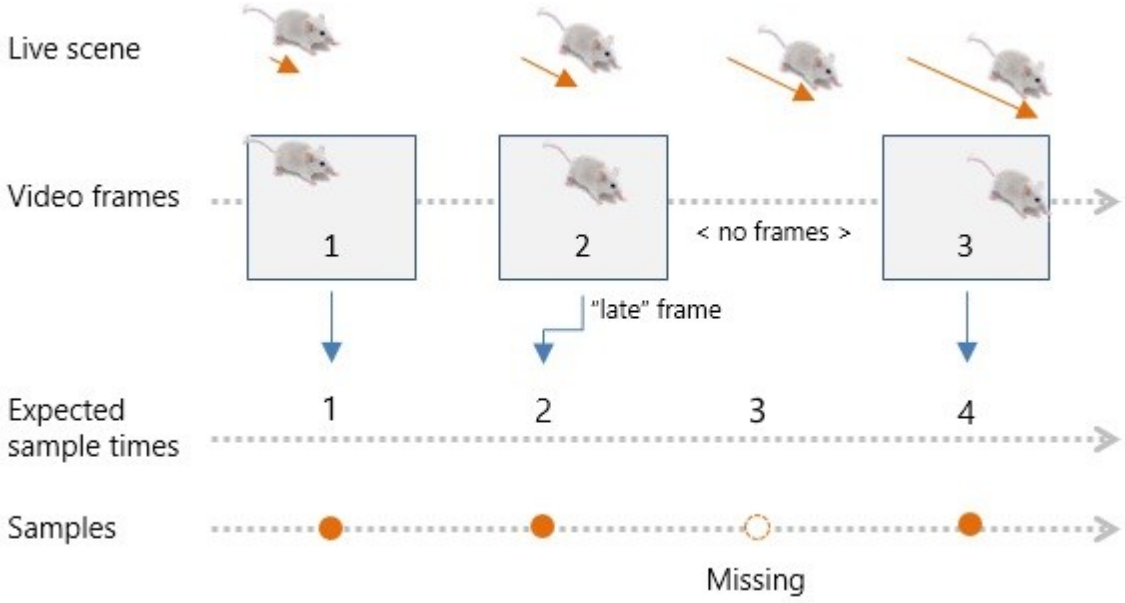
## Background information

Some low-end cameras like webcams and IP cameras are not capable of achieving a fixed video frame rate. With those cameras, the time between two consecutive video frames is not constant enough, so that EthoVision XT cannot assign a video frame to a specific sampled time.

EthoVision XT accepts video frames originated from a camera or a frame grabber, and assigns them to the expected sample time t, t+1, t+2, etc. according to the specified sample rate.

In the figure below, frames 1 and 3 arrive at the expected times. Frame 2 arrives a bit late but it is assigned anyway to its expected time. In all those cases EthoVision XT produces a valid sample.

However, if video frames do not arrive for significant time, no frame is assigned for one or more sample times. The result is a missing sample at time 3. Note that, because a frame was missing, frame 3 is assigned to time 4.




Whenever missing samples occur, EthoVision XT can fill the gaps with interpolation, using the adjacent valid samples.

## Procedure

1. In the Detection Settings, under **Advanced**, open **Smoothing**.
2. Next to **Dropped frames correction**, do one of the following:
   - Select **On** if you want to apply the dropped frames correction. The missing samples will be replaced with valid samples obtained with linear interpolation.
   - Select **Off** (default) to leave the missing samples. If you select **Off**, you can always interpolate the missing samples after acquisition, in the Track Editor. See Interpolate points

## Notes

- Dropped frames correction occurs during tracking. Interpolation is performed only for missing samples due to dropped frames. EthoVision XT interpolates between the last valid sample before a missing sample and the first valid sample after the last missing sample, for a maximum of 10 consecutive dropped frames. If more than 10 dropped frames are found, correction is not done, and the subject remains missing for those samples.
- Dropped frames correction does not change the video time of the valid video frames. For example if the frame rate is not exactly 30 fps, the time

between subsequent frames will not be exactly 1/30 s = 0.03333 s. Small differences in the time between subsequent frames remain.

- Interpolation is only applied to the body points, and to the subject area. The center point is interpolated linearly using the center points in the adjacent samples. The nose- and tail-base point are obtained by doing interpolation on the angles formed by the segment joining the nose-point, the center point and the tail-base point. See How the nose-point and tail-base point are interpolated
- Interpolation is not applied to: body elongation, head direction, area change from the previous sample (used to calculate mobility), and activity. Those values remain missing for the interpolated sample.
- To know the percentage of video frames actually corrected, see View the detection statistics.

*See also*

- Advanced detection settings: Smoothing

# Track noise reduction

## Aim

To reduce the effect of noise in the data during tracking.

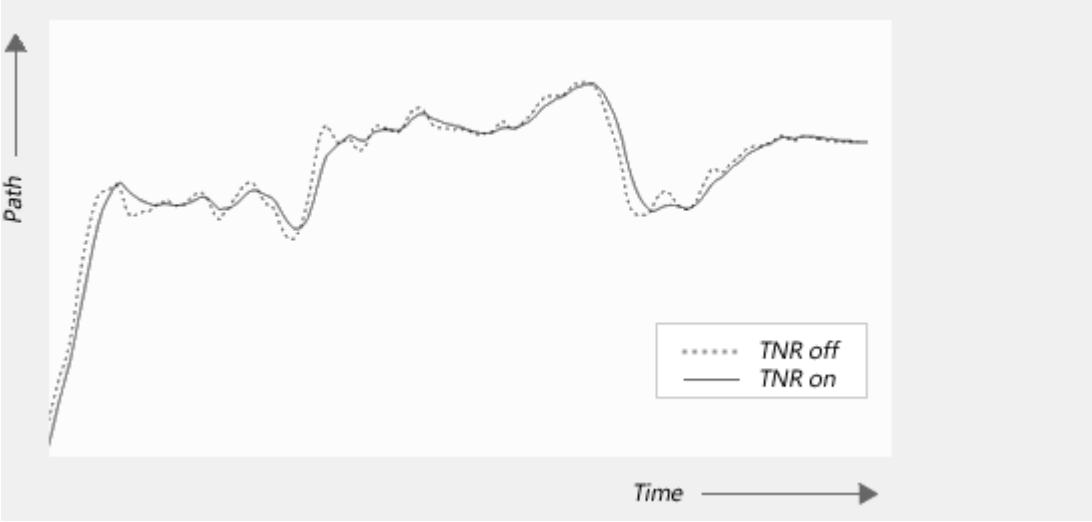
**EXAMPLE** When the center point of an animal is detected in a zone, you want the pellet dispenser to drop a pellet. If the detected center point is moving rapidly because of noise, this may result in a number of consecutive pellets to be dropped, every time the center point crosses the border of the zone. Track noise reduction may solve this problem.

**IMPORTANT** Do not use Track noise reduction if you are particularly interested in rapid movements of the subject, for example, if you study the startle response of zebrafish larvae.

## Background information

If the detected center point of the subject is continuously moving, while in fact the subject is sitting still, the total distance moved will be overestimated. You can use track smoothing to correct for this after you have acquired your data (see Smooth the Tracks), however in some cases you may want to smooth the track *during* acquisition. This may especially be the case if you use Trial and Hardware Control.

The example below shows the effect of track noise reduction (TNR) on the x coordinates of the tracked subject. The chart plots the x coordinate against time. With TNR on, the path is smoothed out.



## Procedure

1. In the Detection Settings, under **Advanced**, open **Smoothing**.
2. Next to **Track noise reduction**, do one of the following:
   - Select **Off** (default) if you do not want to apply track noise reduction.
   - Select **On** if you want to apply track noise reduction.

## Notes

- Track noise reduction makes use of the Gaussian Process Regression method. Track noise reduction is applied during acquisition. Hence, it alters the acquired tracks, which cannot be undone afterwards.

  With Gaussian Process Regression, the sample points are smoothed, using the x,y coordinates of the previous 12 sample points. This differs from the Lowess post-acquisition smoothing method that uses samples before and after the sample point to be smoothed. This is not possible during acquisition, because the x,y coordinates of future samples are not yet known.

- Using Track noise reduction in the Detection Settings influences the acquired track, and therefore it is not possible to change it back after acquisition. This is in contrast to post-acquisition smoothing (see Smooth the Tracks) where you can use profiles to calculate analysis results with and without those filters applied.
- If you use noise-tail tracking, the paths of the nose point and tail base are smoothed independent of the path of the center point.
- If you use Track noise reduction, the sample points may lag behind when compared with the video images, especially when the subject moves fast. This is because the sample position is smoothed using point locations acquired in the previous samples.
- When you use Track nise reduction in combination with hidden zones, the subject may go missing when it is in fact in a hidden zone. This occurs when the last (smoothed) position of the subject before it disappears from view is outside the entry zone. See the Troubleshooting topic: The animal is in the hidden zone but the data show “missing sample”.

*See also*

- Advanced detection settings: Smoothing

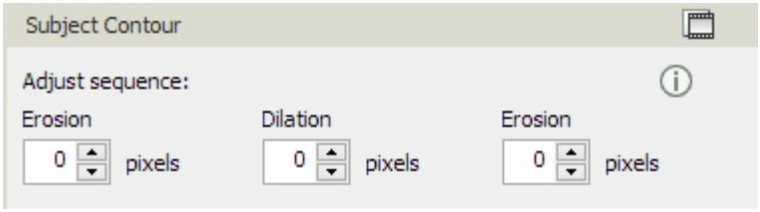
# Advanced detection settings: Subject contour

## Aim

- To eliminate the detection of thin objects such as the rodent's tail.
- To remove the effect of fibers and other thin objects of similar color as the subject, for example in optogenetic stimulation systems.
- To remove the effect of cage bars.
- To 'join up' the parts of the body, when they are detected separately. For example a zebrafish when swimming over a dark object.

## How to access this option

In the Detection Settings window, locate **Subject Contour**.

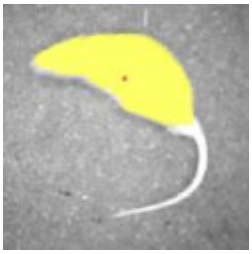



## Procedures

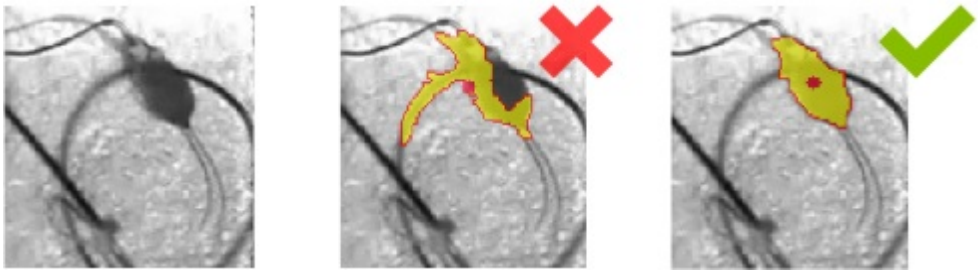
*To remove the rodent's tail, or thin objects*

1. Select **Erosion >0, Dilation >0**. In most cases the second Erosion filter is not necessary.
2. Increase the values **Erosion** and **Dilation** until the rodent's tail or the interfering objects are no longer detected.

   **TIP** Try with equal values for Erosion and Dilation; alternatively, Erosion slightly higher than Dilation. For example, 3, 2, 0.



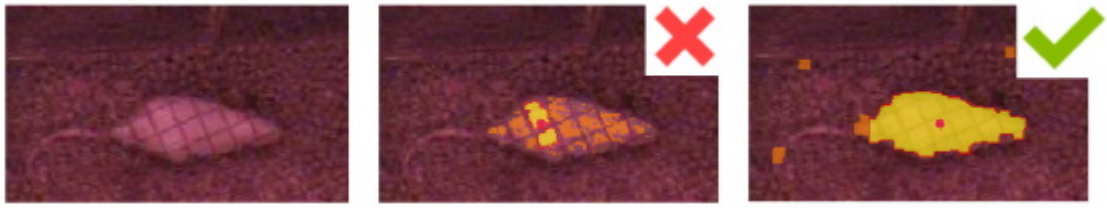
For tethered animals, increase Erosion until only the subject is detected:



*To remove the effect of cage bars*

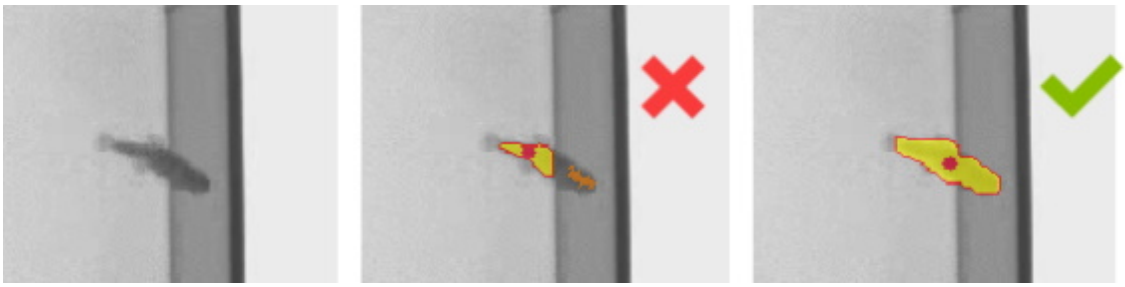
- If the cage bars are the same color as the subject, first erode, then dilate. In the example below, **Erosion 1**, **Dilate 3**, **Erosion 0**.

- If the cage bars are darker (or lighter) than the subject, then first dilate, then erode. In the example below, **Erosion 0**, **Dilation 4**, **Erosion 1**.



*To 'join up' parts of animals like fish and insects*

1. Select **Erosion 0**, **Dilate > 0**, **Erosion > 0**.
2. Increase **Dilation** until the subject's body is fully detected. Then increase Erosion to return to the normal subject size. For example **Erosion 0**, **Dilation 4**, **Erosion 2**.



## How Erosion and Dilation work

*Contour erosion*

Erosion removes an external layer of pixels from the subject's contour. The detected subject appears smaller. **1** means that a layer of 1 pixel is removed. Range: 1 to 10.

*Contour dilation*

Dilation adds a layer of pixels around the subject's contour. The detected subject appears larger. **1** means that a layer of one pixel is added. Range: 1 to 20.

## Combine erosion and dilation

Select **Erosion** and **Dilation** when you want to keep the apparent size of the subject as the original.

When you select at least two filters, erosion and dilation are applied based on the sequence that results from which filters you choose.

- **Erosion > 0**, **Dilation > 0**, Erosion =0. A layer of pixels is removed, then another layer is added to the contour. This is the same as **Erode first, then dilate** in EthoVision XT 15 and earlier.
- Erosion = 0, **Dilation > 0**, **Erosion > 0**. A layer of pixels is added, then another layer is removed from the contour. This is the same as **Dilate first, then erode** in EthoVision XT 15 and earlier.
- **Erosion > 0**, **Dilation > 0**, **Erosion > 0**. A layer of pixels is removed, then another layer is added, and finally a third layer is removed.

  **TIP** Use this combination to improve nose-tail detection in some difficult situations, for example when the subject enters a darker area. Adding the second Erosion filter may help.

## Note

- It is important that the complete body of the animal is detected. If even after setting the Contour adjustments you do not achieve this, go back to the appropriate Detection method and adjust the contrast to improve body detection.
- A reason for why you may want to eliminate the animal's tail is that when the animal sits still and its tail moves, it adds to distance moved. Furthermore, when the tail is not part of the detected blob, the center point is better estimated.
- If you apply Erosion 2, Dilation 0, Erosion 2, the effect is like that of Erosion 4, Dilation 0, Erosion 0.

*See also*

- The video tutorial **Contour Settings**.

# Advanced detection settings: Subject size (one subject per arena)

## Aim

To set a minimum and a maximum apparent size of the subject to prevent objects like droppings or large reflections from being detected during tracking.

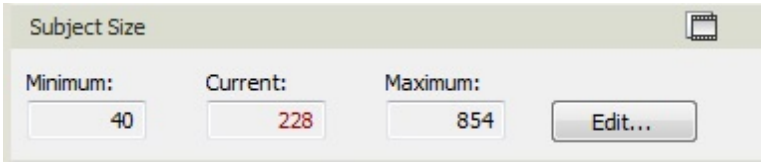
## This topic applies to

All experiments set to:

- Number of Subjects per Arena: 1
- Body point detection technique: Contour-based

## Procedure

1. In the Detection Settings window, locate the **Subject Size**.




2. When EthoVision XT finds the subject, check the value under **Current**. The Current value gives an idea of the detected body size in pixels.
3. Click the **Edit** button and set the **Minimum** and **Maximum** subject sizes. Objects that have the same color as the animal, but are smaller than the Minimum or larger than Maximum size, are not tracked.

You are now ready to acquire data.

## Notes

- The term size here means surface area in video pixels, not length or screen pixels. Enlarging the Video window does not change the subject's size in video pixels.
- Objects whose size is outside the size range, are shown in the color under Noise (default: orange; see View the detection features on the video window).
- Click the video icon next to **Subject Size** and watch the video tutorial.

# Advanced detection settings for tracking multiple unmarked subjects

## Aim

To set EthoVision to track multiple subjects per arena, when the subjects are not color-marked.

- To minimize subject identity swaps, make sure that the subjects are well detected in the whole arena. Make lighting as uniform as possible, and use indirect light, to minimize shadows.
- When subjects crowd closely together EthoVision may temporarily assign the same position to those subjects, or lose track of one of them. This can be minimized or totally eliminated by setting good detection settings. Make sure that the body of the subjects is fully detected, and that the reference image does not contain the image of the subjects.

## This topic applies to:

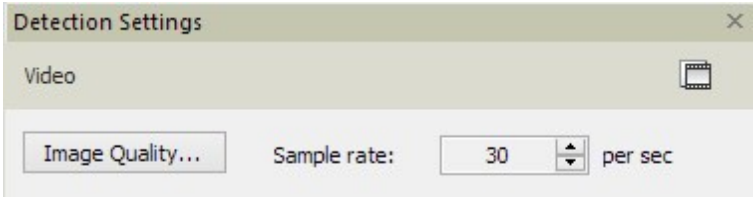
Experiments set to:

- Number of Subjects per Arena: 2 or more.
- Tracked Features: Center-point, nose-point and tail-base detection.
- Body point detection technique: Contour-based

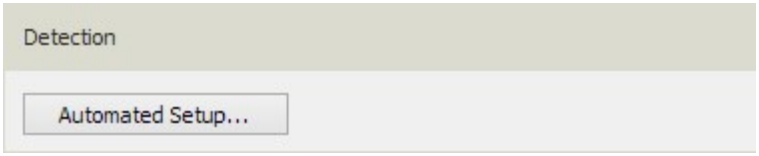
  If you use Deep learning to track two subjects, you do not need to adjust detection settings. Simply choose the sample rate (step 1 below) and skip the remaining steps. See Deep learning: Requirements

## Procedure

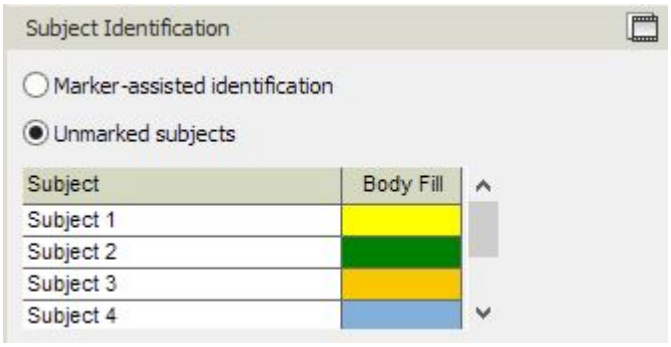
1. In the Detection Settings window, under **Video**, select the image source and sample rate. See Video file, image quality and sample rate.




2. Use the Automated Setup. When the Automated Setup gives good detection, proceed with the next step. If not, create advanced detection settings, follow the procedure in Advanced detection settings: Method.

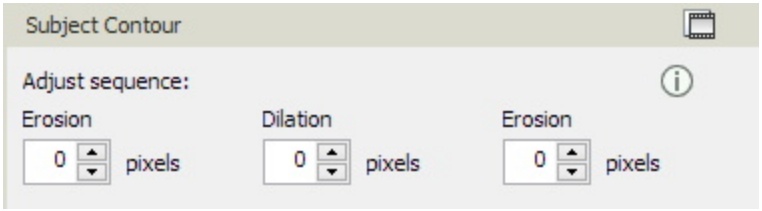



3. In the Detection Settings pane, under **Subject Identification**, select **Unmarked subjects**.




4. **OPTIONAL** Click a cell under **Body fill** and specify the color for a subject. This helps checking identity swaps during tracking.
5. Adjust Advanced detection settings: Smoothing settings.


6. When tracking the three body points: adjust Subject contour for nose-tail base detection. In all other cases see Advanced detection settings: Subject contour.




7. Adjust Advanced detection settings: Subject size (multiple animals per arena).

## Notes

- When tracking unmarked animals, it is not possible to specify which animal is labeled “Subject 1”, which “Subject 2” and so on. EthoVision XT chooses which individual is assigned a label. This also means that the same animal in video 1 is not necessarily labeled with the same name in video 2. To link the identity (or role) of Subject 1, Subject 2, etc. enter that information as an independent variable in the Trial List. See Define an independent variable
- When tracking unmarked subjects, identity swaps may occur, that is, what is labeled Subject 1 may at some point in the track be labeled as Subject 2, and what was Subject 2 is now Subject 1. Always check carefully the tracks, and, if necessary, correct the identity swaps. See Swap subjects

# Advanced detection settings for tracking color-marked subjects

## Aim

Use color marks in one of the following cases:

- When detection of the subject is difficult, for example because its color is similar to that of the arena.
- When you want to track multiple animals per arena, and you want to keep the correct identity of each individual.

## Background information

There are two ways of tracking subjects that are color-marked: Marker-assisted identification and Color marker tracking. The difference between the two is as follows:

- *Marker assisted identification*. EthoVision XT tracks the animal's body but uses the marker to determine the animal's identity. In the Detection settings pane, under **Subject Identification**, select **Marker-assisted identification**.

  See the procedure in Advanced detection settings: Marker-assisted identification on page 408.

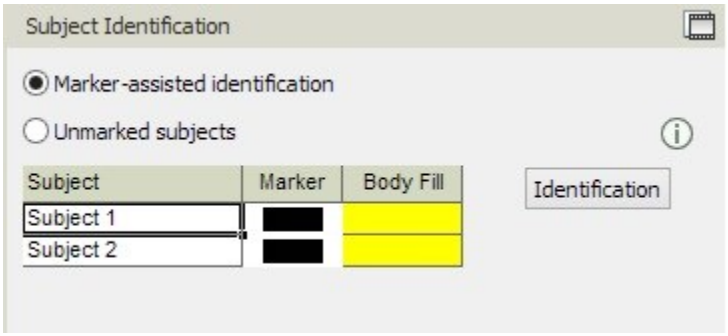
- *Color marker tracking*. EthoVision XT only tracks the marker’s geometric center, and ignores the subject’s body. The actual shape and size of the animal is ignored. To apply this method, in the Experiment settings under **Tracked Features** select **Color marker tracking (treat marker as center-point)**.

  Next, see the procedure Advanced detection settings for color marker tracking on page 421.

In all cases, you can track up to 16 animals simultaneously in the same arena. This is a technical limit; in practice, it may be difficult to discriminate between 16 different color marks depending on the light conditions.

## Procedure

1. Advanced detection settings: Marker-assisted identification




2. Advanced detection settings: Smoothing
3. Advanced detection settings: Subject contour
4. Advanced detection settings: Subject size (multiple animals per arena)

*See also*

- Advanced detection settings for tracking multiple unmarked subjects

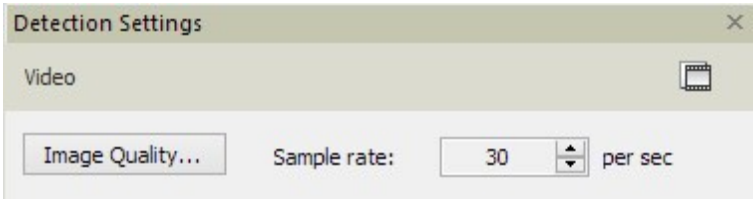
# Advanced detection settings: Marker-assisted identification

## Prerequisites

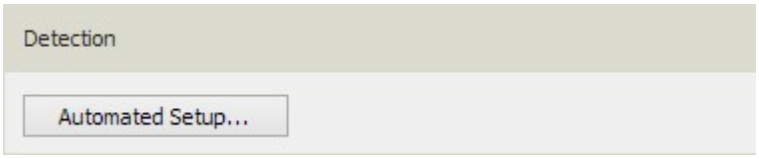
- You have marked the animals with colors.
- You have optimized lighting as suggested in Tips for color tracking.

## To adjust detection settings for marker-assisted identification

1. Put the marked animals in the arena or play the video.
2. In the Detection Settings window, under **Video**, select the image source and sample rate. See Video file, image quality and sample rate.




3. Use the Automated Setup.



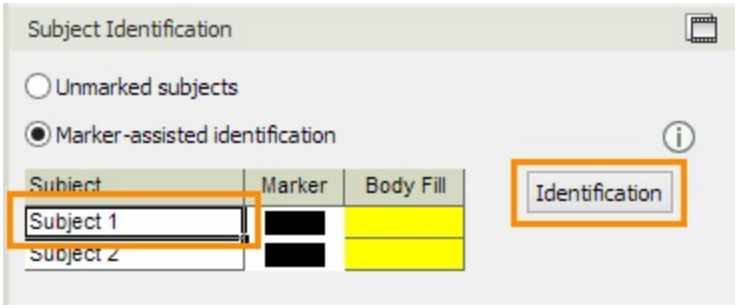

   At this stage EthoVision XT detects the subjects, but does not distinguish between the animals yet. When the Automated Setup gives good detection, proceed with the next step. If not, create advanced detection settings, follow the procedure in Advanced detection settings: Method.

   When done with the Automated Setup, make sure **Marker-assisted identification** is selected in the **Subject Identification** section in the Detection Settings pane. Note:

   - **IMPORTANT** Make sure you select a point in the video where the animals do not touch!
   - If you use three-body point detection, it is normal that the nose is not correctly identified at this point.

- If detection is processor intensive and you track from video, the video slows down, so all frames can be analyzed to enable creating good detection settings.

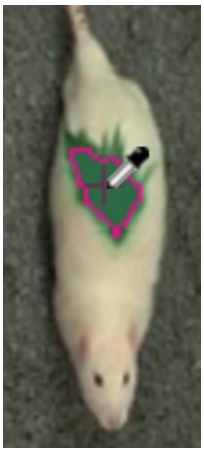
4. In the **Subject Identification** section, click the name of one of the subjects and click the Identification button.




*Result*: The Identification [Subject name] window opens. Optionally enlarge the Video window by dragging its bottom-right corner.

5. Move the mouse pointer to the Video window so the pointer becomes an eyedropper.

6. Move the eyedropper on top of the color marker of the subject you want to identify (see the figure below) and click the left mouse button.



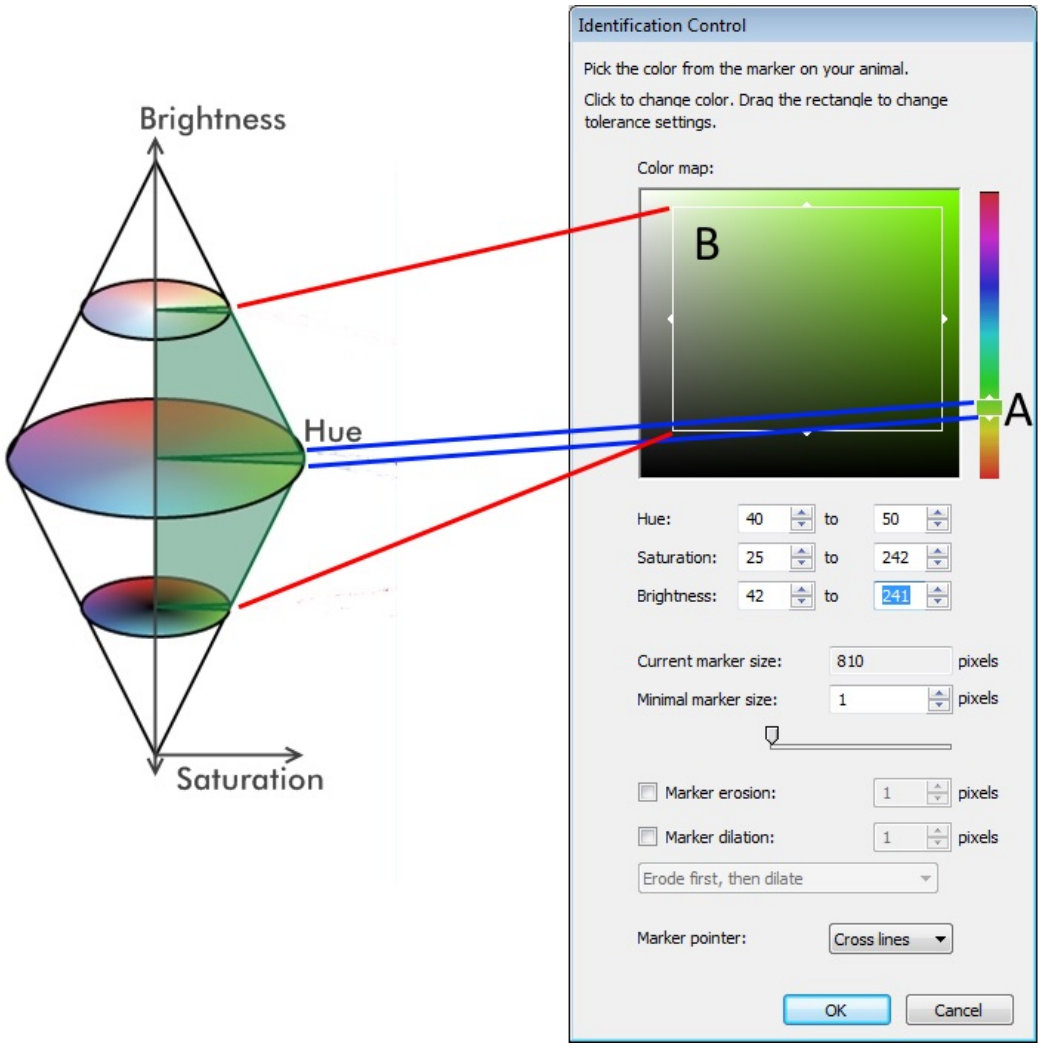
The Identification [Subject name] window now displays the color you just picked and the pixels with the initial color are highlighted in the Video window. In the Identification [Subject name] window, you can change the following (see also the figure below):

**Hue**: Hue is the predominant wavelength of the marker color and represents what is usually referred to as color in everyday life (red, green, blue, etc.). The range of values for Hue of the picked color are shown and this range is represented by the box on the vertical color bar on the right.

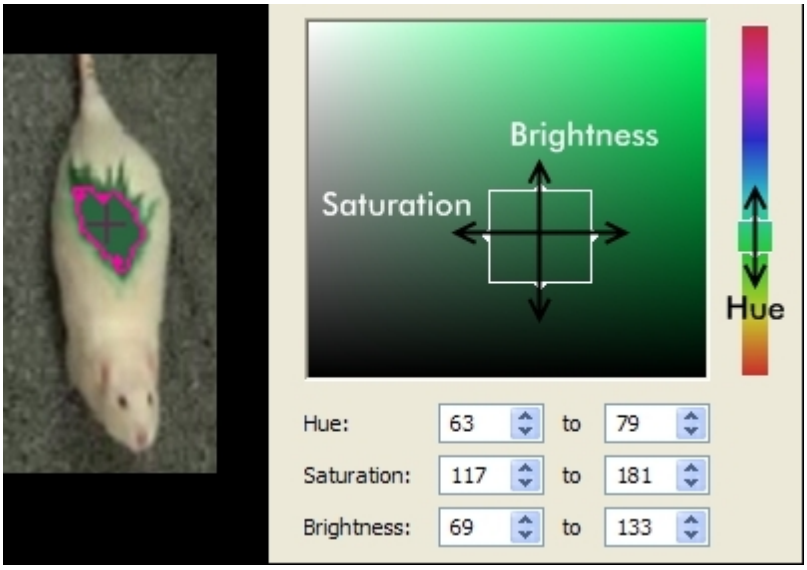
**Saturation**: Saturation represents the purity of a color. Saturation decreases when a pure color is mixed with white; “red” is saturated, “pink” is less saturated. The range of values for Saturation are shown and this range is represented by the width of the box on the Color map.

**Brightness**: Brightness (or Intensity) represents the amount of light reflected by the colored surface. The range of values for Brightness are shown and this range is represented by the height of the box on the Color map. If you set this range too broad, you will not be able to separate the colors well.

Below: A: Color bar. The box represents Hue which corresponds to an angle on the circle in the HSI color model (for example, 0 degrees means red, 240 degrees means blue). B: Color map. The height of the box represents the Brightness (or Intensity) range which corresponds to the vertical position of the color circle. The width of the box represents the Saturation range which corresponds to the horizontal position on the circle between the center and the edge.

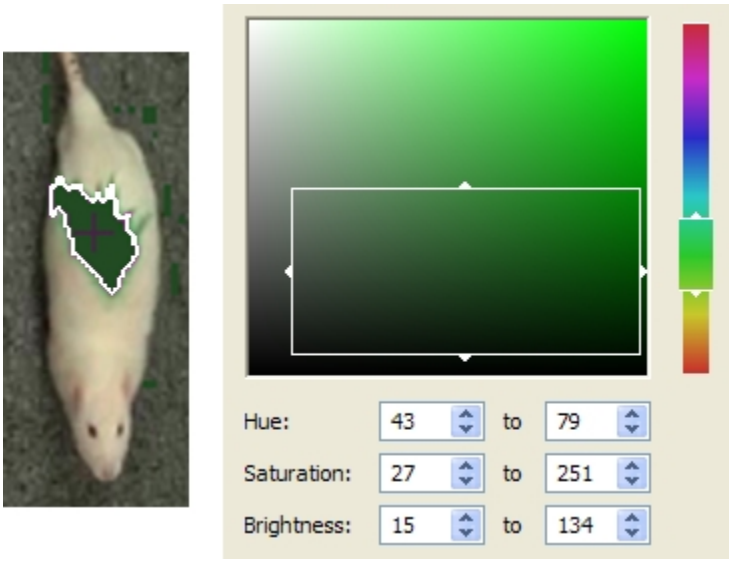



If the marker is not detected completely or not detected in all areas of the arena, expand the range of Hue, Saturation and Brightness slightly.




Change the color range by changing the numbers or by resizing the Hue box on the vertical color bar, or resizing/moving the box in the color map (horizontally to adjust Saturation, vertically to adjust Brightness). As a result, the outline covers (almost) the complete marker.

Below: The color of the marker after fine-tuning the color settings. Most of the marker is now selected as indicated by the white outline.




7. Next, play the video to see in the Video window whether the marker is detected correctly in different parts of the arena.

   If the marker 'dances' then your color settings are too sensitive. Go back to step 5 and make the box larger.

8. Continue with setting the following:

    - **Marker erosion**: Set the number of pixels to erode. By selecting **Erode first, then dilate**, you can make the marker more round to prevent the center-point of the marker to start jittering.
    - **Marker dilation**: Set the number of pixels to dilate. By selecting **Dilate first, then erode**, you can prevent the marker from being masked or divided in two separate markers by, for instance, a grid on top of the arena.
    - **Minimal marker size**: Set the Minimal marker size to prevent noise to be detected as the marker. First, increase the Minimal marker size until noise is not detected anymore. Next, enter a value for the Minimal marker size that is somewhere in between this lower threshold and the value of the Current marker size.

    Marker pointer: Select a **Marker pointer** from the list. With relatively small markers it is useful to select **Cross lines**.

9. Click **OK** when you are done.

Click another subject under **Subject Identification**, and repeat steps 2-8 for all subjects you want to identify. If you used the Automated Setup, estimates for the settings below have been created. Optionally fine-tune these settings if detection of all subjects does not go well in the entire track.

*What next?*

Follow the procedure in this order:

1. Advanced detection settings: Smoothing
2. Advanced detection settings: Subject contour
3. Advanced detection settings: Subject size (multiple animals per arena)

# Advanced detection settings: Subject size (multiple animals per arena)

## Aim

To specify the average (and variation) of size of subjects, when tracking multiple subjects per arena.

**IMPORTANT** When setting the Subject size, make sure the animals do not touch each other.

## To set subject size

1. In the **Subject size** section, click the **Advanced** button.

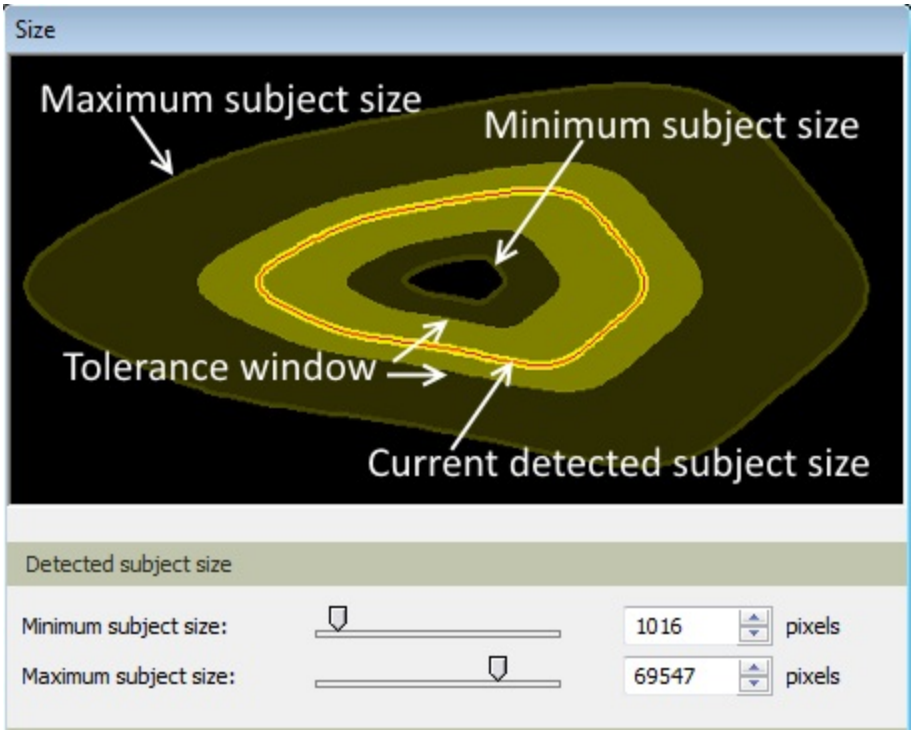
   In the Size window, in the figure at the top, the thin red contour represents the current size of what EthoVision XT assumes is the animal shape.

   - To set the **Detected subject size**, proceed with step 2. Set the Detected subject size to ensure that small or very large objects are not detected as a subject.
   - To set the **Modeled subject size**, proceed with step 3. Set the Modeled subject size to obtain an estimate of the subject's body size, and body position of the different subjects when they overlap or touch.

2. Set the Minimum and Maximum subject size (represented by a green contour):

   - **Maximum subject size**: The largest surface area (in pixels) that is detected as the subject. Objects bigger than the Maximum subject size, for example, the experimenter's arm, are detected as noise and not tracked. Decrease the Maximum subject size until its thick green contour surrounds the thin red contour by a fair margin.
   - **Minimum subject size**: The smallest surface area (in pixels) that is detected as the subject. Objects smaller than the Minimum subject size, such as droppings or disturbed sawdust, are detected as noise and not tracked. Increase the Minimum subject size until its thick green contour is smaller than the thin red contour by a fair margin.

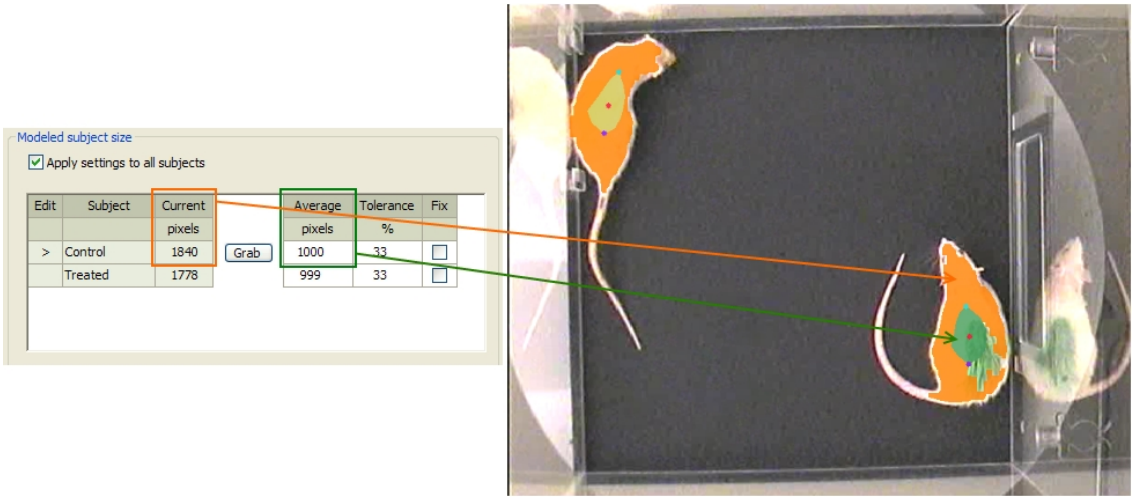
   The two sliders are interdependent. So, after you have set the Minimum subject size, when you next change the Maximum subject size, the slider for the Minimum subject size also moves (although the size in pixels stays the same).




3. In the **Modeled subject size** group, select **Apply settings to all subjects** if your multiple animals have similar sizes.
4. Select one of the subjects to model the subject size for, by clicking the name of the subject.
5. Next, adjust the modeled subject size (under **Average - pixels**) to the detected subject size (under **Current - pixels**):

   You do this by clicking the **Grab** button. Keep clicking the **Grab** button until the modeled (Average) subject size equals the detected (Current) subject size.

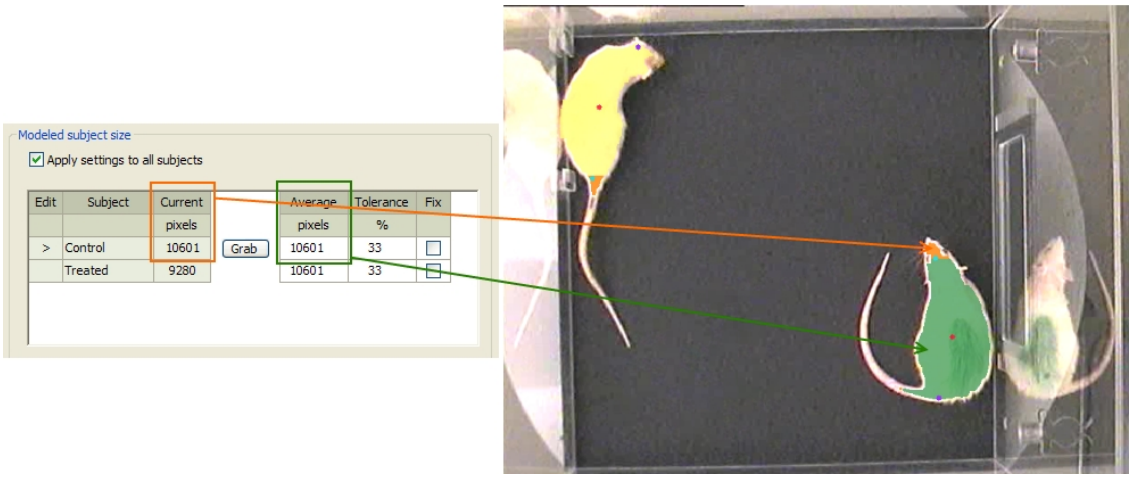
   Below: Part of the **Modeled subject size** group in the Size window (left) and the Video window. In the table, Current shows the current detected subject size in pixels, Average shows the modeled subject size in pixels. The arrows point to the visual feedback you get about the current and average subject size in the Video window.




When the modeled (Average) subject size equals the detected (Current) subject size, the following becomes visible:

- In the Modeled subject size group: the **Average** subject size now equals or is larger than the **Current** subject size (see the table in the figure below).
- In the Video window: the modeled subject size now completely overlaps with the current subject size (see the figure below).
- In the Size window, the bold yellow contour represents the modeled subject size. This now coincides with the detected subject size indicated by the thin red contour.

Below: Part of the **Modeled subject size** group in the Size window (left) and the Video window. The modeled (Average) subject size is now adjusted to the detected (Current) subject size. Compare the table and video window in this figure with those in the figure above.

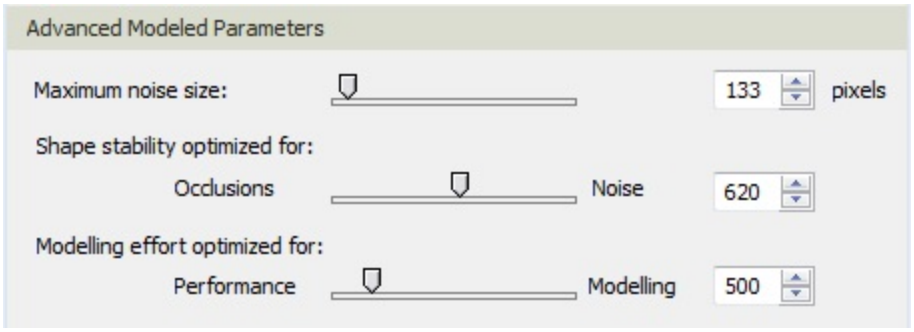


6. You can now set the **Tolerance**. Click the corresponding cell and enter a value.

   The Tolerance determines the deviation of the average subject size. When the Current detected size deviates more from the Average subject size than the Tolerance, then the object is not considered to be the subject anymore and EthoVision starts making an educated statistical guess about the body contour of the animal.

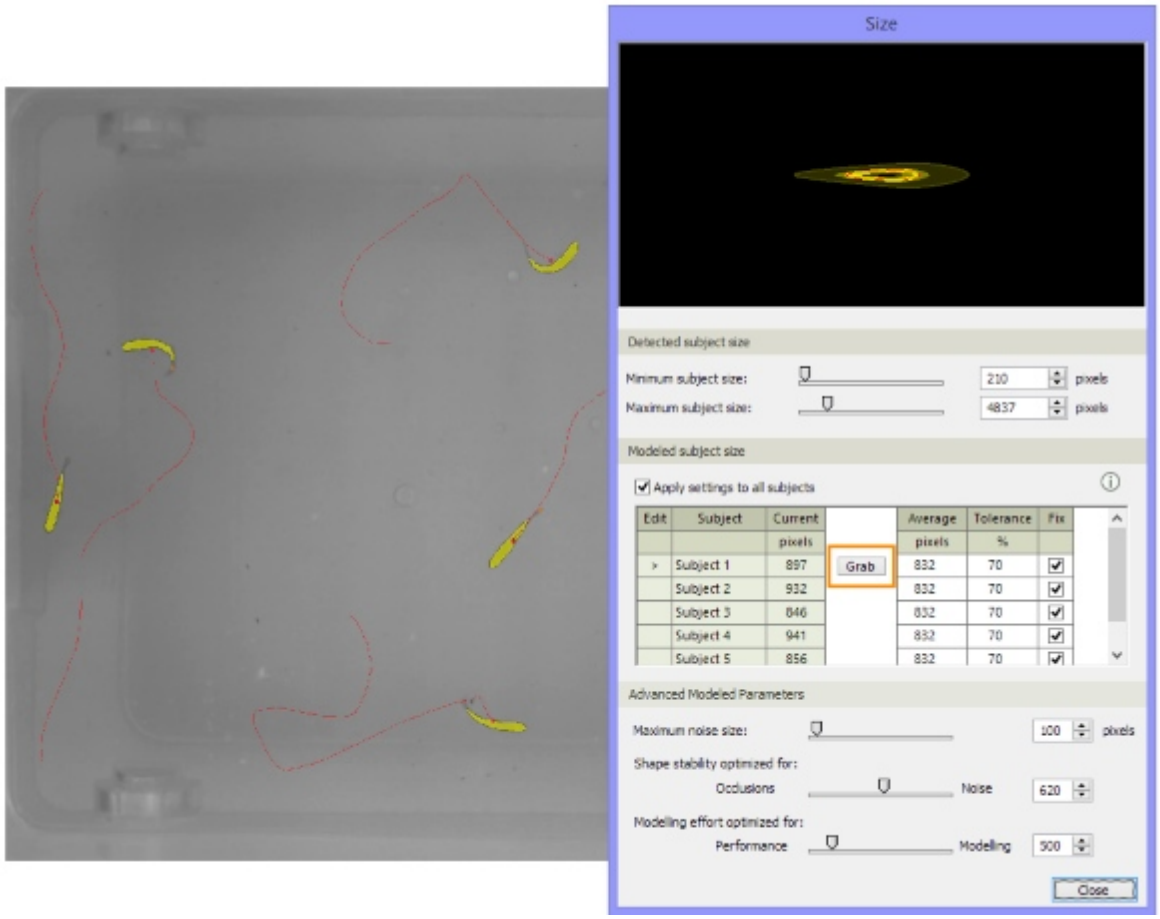
   This is visible in the Video window by a wobbling marker-color area. When this happens when animals do not touch, you should increase the Tolerance.

7. Select the **Fix** check box for each subject.
8. Optionally click **Advanced Modeled Parameters** and change the settings for the **Maximum noise size**, **Shape stability**, and **Modelling effort** (see below).

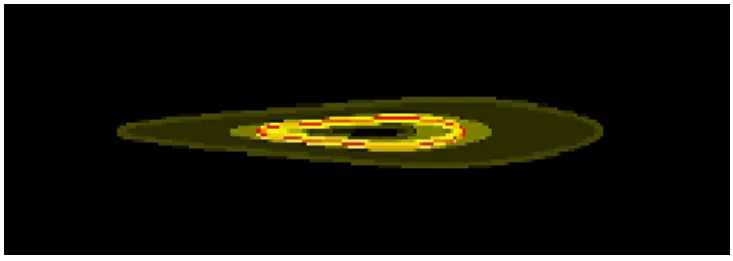



## Subject size when tracking multiple fish

Click **Grab** until the body fill color covers the whole body. Or, enter the body size under **Average** and select **Fix**. Keep **Tolerance** high (70-100%).




Make sure that in the Subject size window, the red contour oscillates within the limits of the Average subject size (bold yellow contour).



If that is not the case, click **Grab** when you see in the Video window that the subjects are detected correctly, or enter the appropriate value of size in the Average cell, then select **Fix**.

# Advanced Modeled Parameters

### *Maximum noise size*

Set the Maximum noise size. The value should be lower than the minimum subject size and but high enough to make sure that small objects with the same contrast as the subject are not detected.

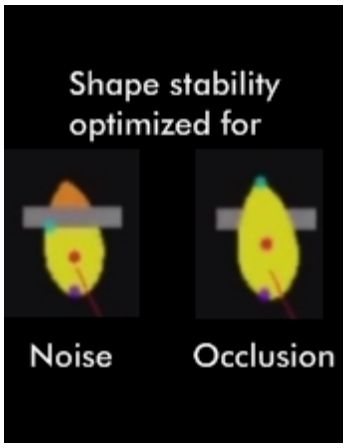
### *Shape stability*

The Shape stability setting is used when you track animals whose body can be occluded by, for example, cage bars or part of the body of another animal. When this happens, the animal's body consists of two separate objects that are close together.

The **Shape stability optimized for** slider has two extreme settings:

- **Occlusions**: When you set the slider close to Occlusions, EthoVision considers separate objects that are close together part of one animal.
- **Noise**: When you set the slider close to Noise, EthoVision considers separate smaller parts not part of the animal.

The figure below shows the animal model as a result of applying the two extreme Shape stability settings. 'Noise' shows that the front of the animal, on the other side of the bar, is not considered to be part of the animal. 'Occlusion' displays the animal body as a whole.




If you are not sure which setting to select, leave Shape stability at the default value of 620.

*Modeling effort*

The Modeling effort setting is used when two animals touch and EthoVision loses the separate shapes. At this point, EthoVision tries to determine which part of the big 'merged' body fill belongs to either animal. This costs a lot of processing load.

The **Modeling effort optimized for** slider has two extreme settings:

- **Performance**: When you set the slider close to Performance, EthoVision is only allowed a short time to determine which part of the 'merged' body fill belongs to which animal. Therefore, Modeling quality is low.
- **Modelling**: When you set the slider to Modelling, EthoVision XT is allowed a longer time per frame to determine which part of the 'merged' body fill belongs to which animal. Therefore, Modelling quality is good, but this costs a lot of processor load.

  We recommend to select Modelling only when you have a computer that exceeds the minimum system requirements.

When you are not sure which setting to select, leave Modeling effort at the default value of 500.

## Tips for setting the Subject Size

- Make sure you do not set the Tolerance too small; it is better get a wrong body size/shape than a wrong location of the animal.
- It is better to set the Average subject size slightly bigger than the actual subject size, especially when you carry out nose-tail tracking.
- If you want to carry out live tracking with multiple similarly-sized animals, it is recommended to first introduce one animal into the arena and make the Subject Size settings for this animal.
- If the subject size changes a lot between trials, it is recommended to create new Detection Settings for this new size.

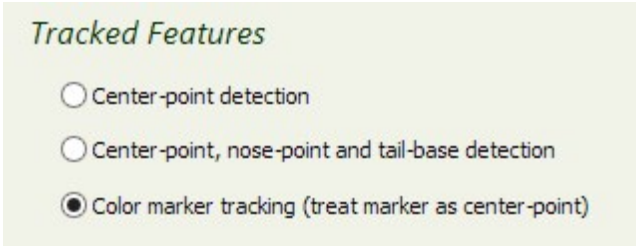
# Advanced detection settings for color marker tracking

## Aim

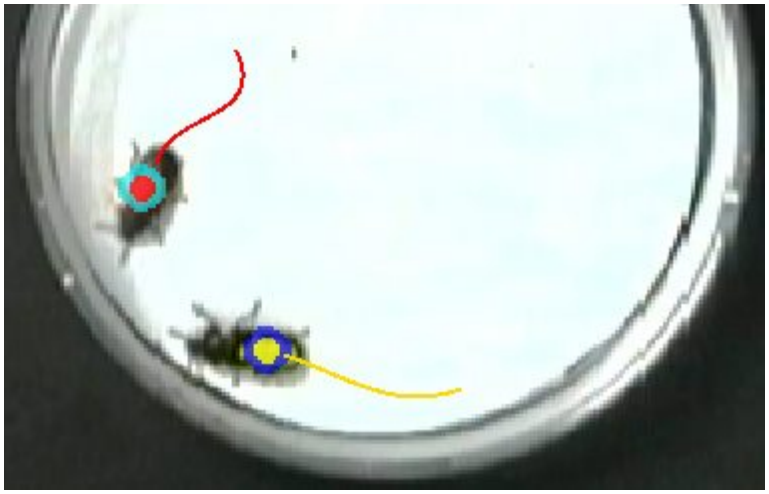
To specify which colors EthoVision XT should follow when tracking color markers.

## Prerequisites

- You have marked the animals.
- In the Experiment Settings under **Tracked Features** you selected **Color marker tracking (treat marker as center-point)**.



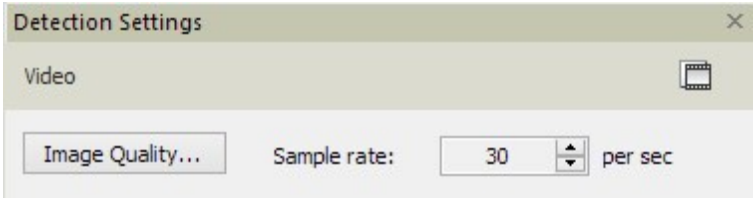

Note that with this option you track only the marker, while the animal's shape is ignored.



## Procedure

1. Put the marked animals in the arena or play the video. Optimize the camera setup, lighting conditions and marker characteristics. See Tips for color tracking

2. In the Detection Settings window, under **Video**, select the image source and sample rate. See Video file, image quality and sample rate.




3. In the Detection Settings window, click **Subject Identification**.


4. Carry out steps 4 to 9 in Advanced detection settings: Marker-assisted identification for all subjects.

No additional settings are necessary, EthoVision XT tracks the markers and makes no estimate of the body contours.

# Detect the nose and the tail base

## Learn about

- Overview of nose-tail base detection
- Optimize nose-tail base detection

## When do I need this information?

In the Experiment Settings, under **Tracked features**, you chose **Center-point, nose-point and tail-base detection**. Choose the procedure based on what you selected under **Body point detection technique**. See Body point detection technique

### *Contour-based technique*

Follow the procedure in this order:

1. Adjust the settings for nose-tail base detection (Contour-based)
2. Adjust Advanced detection settings: Smoothing
3. Adjust Subject contour for nose-tail base detection
4. Adjust Subject size for nose-tail base detection

### *Deep learning*

Follow the procedure: Adjust the settings for nose-tail base detection (Deep learning)

**NOTE** Subject size and contour do not affect the performance of the Deep learning algorithm. Therefore, EthoVision XT should be able to find the nose and tail base points also when the detection of the subject's body is not optimal. However, we advise you to adjust the settings for size and contour, especially if you want to analyze the body's mobility.

# Overview of nose-tail base detection

When you set an experiment for Nose-tail base detection, EthoVision XT analyzes the contour of the area detected as subject at each sample, and assigns Nose-point and Tail-base to two specific pixels of the contour. Furthermore, it determines the direction the animal is supposed to point to (Head direction).

Nose-tail base detection is optimized for rodents and for adult fish, depending on the method used.

## Nose- and tail-base points

The two points are detected independently through one of two complex algorithms. The nose-point is found in all cases, except when the center-point is not found either. The tail-base may not be found in a few cases if detection is good.

Reliable tracking of nose and tail-base is limited by the size of the video image. You can mix four camera images like in the case of a group of PhenoTypers, with good results. Mixing 16 camera images makes the subjects too small for reliable nose and tail-base tracking.

## Head direction

Once the nose-point has been found, two points are determined along the contour lying at a specific distance from the nose-point. The Head direction is the line dividing equally the angle formed by the center and those additional points.

**IMPORTANT** If you use Nose-tail tracking with two or more subjects per Arena, track **from video file** instead of live.

# Adjust the settings for nose-tail base detection (Contour-based)

## Aim

To achieve detection of the nose- and tail-base points of your subjects with high accuracy.

## Prerequisites

- In the Experiment Settings, under **Tracked Features**, you chose **Center-point, nose-point and tail-base detection**.
- You have optimized the lighting and the background. See Optimize nose-tail base detection

## Procedure

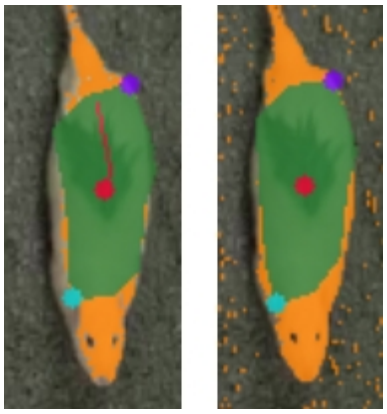
1. Select the image source and sample rate. See Video file, image quality and sample rate.
2. Use the Automated Setup. When the Automated Setup gives good detection, proceed with acquisition. If not, follow the steps below. See Detection settings: Automated setup
3. Under **Video**, *choose the sample rate*.

   Use a sample rate as high as possible. However, for nose-tail tracking in combination with marker-assisted tracking, use a sample rate of 12.5, 15 or 14.98 samples/s depending on the camera.

4. Open the Advanced detection settings: Method. Choose the detection method and the method for estimating the body points. See Methods for estimating the body points

   If you followed the Automated Setup, a **Default** method is selected automatically for one-subject tracking, and **For occlusions** for multiple-subject tracking. See Methods for estimating the body points

In the picture below: Left, a sub-optimal result of body detection (part of the right side of the body is not detected). Right, the result when the contrast settings are optimized; now the complete body is detected. The color of the body contour at this stage is orange (=noise) because the model parameters have not been configured yet.



## Notes

- The maximum sample rate depends on the camera.

# Subject contour for nose-tail base detection

## Aim

To optimize the shape of the detected subject, so that the body points are correctly found.

## Procedure

- When you use one of the following methods for detecting the body points, **Any species / Default**, or **Adult Fish / For occlusions**, make sure the tail is fully detected as part of the subject.
- When you use a **Rodents** method (Default or For occlusions), it is best to remove detection of the rodent's tail. See Advanced detection settings: Subject contour

## Notes

- When you track fish from above, the tail-base point is not positioned on the tail fin, rather more towards the mid-point. That is normal and is not a sign of bad detection.

*See also*

- Advanced detection settings: Subject contour
- Subject size for nose-tail base detection

# Subject size for nose-tail base detection

## Aim

To optimize the size of the detected subject, so that the body points are correctly found.

## Procedure

Choose the option depending on which method for estimating the body points you are using.

- For **Any species / Default** or **Rodents / Default**:

  See Advanced detection settings: Subject size (one subject per arena).

- For **Rodents / For occlusions** and **Other species / For occlusions**:

  See Advanced detection settings: Subject size (multiple animals per arena).

# Optimize nose-tail base detection

Because of the way the nose- and tail base points are found, nose-tail base detection is much depending on the quality of the video image and the experimental setup. Before using this feature, please check the following guidelines:

## Conditions related to the Arenas

- *Light*: Light conditions must be equal across the arena. Try to remove shades, light spots and reflections. For proper detection, the subject's body contour must be kept as constant as possible across the whole arena.
- *Subject/background contrast*: The color of the subject and of the background must be contrasting enough to get a full and clear body contour.
- *Video quality*: Noise and interference reduce the proportion of samples which are correctly detected.
- *Noise reduction*: The Video Pixel Advanced detection settings: Smoothing function can sometimes help getting a more appropriate body contour. However this is of little use if the video has too much noise or too little contrast.
- *Areas hidden to the camera view*: When the animal enters or exits areas hidden to the camera (for instance, a shelter), nose-point and tail-base are wrongly assigned.
- *Number of arenas*: Reliable tracking of nose and tail-base is limited by the size of the video image. You can mix maximally four camera images like in the case of a group of PhenoTypers, with good results.

## Conditions related to the Subjects

- *Subject's apparent size*: The subject must be large enough to get a constant body contour. Small animals and large arenas pose detection problems with nose- and tail-base points. When you mix the image of multiple cameras with a quad unit, like in the case of a group of PhenoTypers, a group of 4 cameras gives good results. When mixing 16 PhenoTypers, the apparent size of the subject is generally too small.
- *Subject's color variation*: For hooded rats, both the flanks (white) and the head (dark) must contrast with the background, otherwise detection of body contour is sub-optimal. In the Detection Settings, next to **Subject color compared to background** select **Brighter and darker than background**. You can also try the Differencing detection method instead of the Subtraction methods.

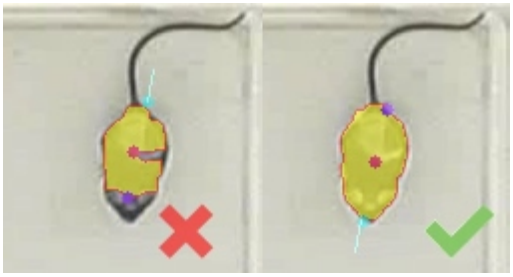
- *Subjects in a water maze*: Tracking nose- and tail-base points in a water maze is impossible because the tail-base is under the water, and it is not possible to obtain a proper body contour.
- *Subject's behavior*: Immobile animals are hard to track because their body contour differs from that of a mobile animal. Nose-points are therefore hard to detect.

## Important choices

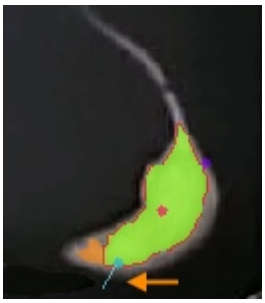
- *Tracking live vs. from video files*. We recommend to track from video files if you use one of the methods named **For occlusions**. Tracking live requires high processor load, which may result in many missing points, especially when you use multi-subject tracking and multiple arenas simultaneously.
- *When tracking from video files*. Keep the DDS (Detection determines speed) option selected, unless you score behaviors manually.
- *Missing tail-base points*: The high percentage of missing tail-base points is an indication of poor detection. The higher this percentage, the greater the probability that the nose-point is not placed in the correct location. To estimate the proportion of missing tail-base points, run some test trials and visualize the Sample list in the Track Editor screen. You can also calculate the Number of samples as a statistic for a dependent variable such as Velocity for the nose point.

## In practice…

The contour of the blob detected as subject is crucial for proper detection of nose- and tail-base points. If only part of the subject is detected, EthoVision XT may swap the pixels assigned as nose-point and tail-base.



Or the nose-point is not placed on the subject's nose tip (for clarity, the nose point is shown together with the Head direction):



If you use a Subtraction method, select a wider range of gray scale values or, for Differencing, adjust the sensitivity to increase the number of pixels detected as subject.

Detection of the tail depends on which method you use for detecting the body points. See Methods for estimating the body points

- When you use the **Any species / Default** method for estimating the body points, make sure that the tail is fully detected.
- When you use one of the **Rodents** method, remove the tail from the detected subject using the Erosion then the Dilation filter. See Subject contour for nose-tail base detection

# Adjust the settings for nose-tail base detection (Deep learning)

## Aim

To achieve detection of the nose- and tail-base points of your subjects using the Deep learning method.

This topic applies to tracking of one subject per arena. If you track two or more subjects per arena with Deep learning, you do need to adjust settings besides the sample rate.

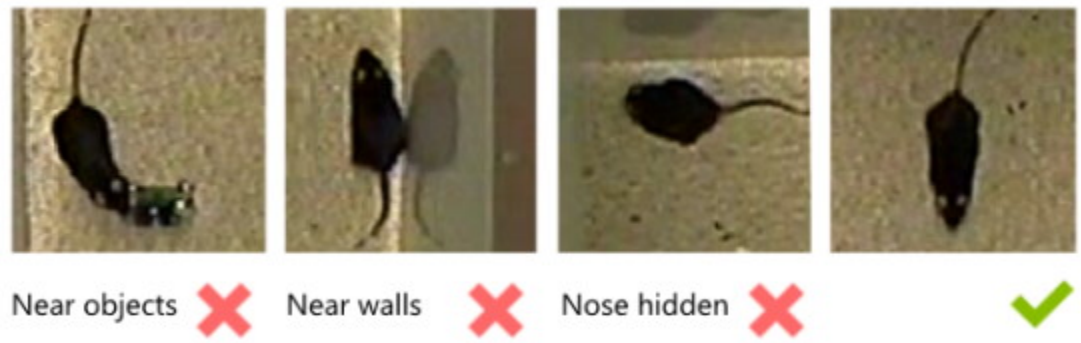
## Prerequisites

- In the Experiment Settings:
  - Under **Subjects**, **1** is selected.
  - Under **Tracked Features**, **Center-point, nose-point and tail-base detection** is selected.
  - Under **Body point detection technique**, **Deep learning** is selected.
- You optimized the lighting and the background based on Deep learning: Requirements.

## Procedure

1. Open the Detection Settings.
2. In the **Video** section (top-right), select the video file (if you track offline) and choose the sample rate. See Video file, image quality and sample rate
3. Under **Advanced** > **Method**, choose the detection method and the contrast range. See Advanced detection settings: Method
4. In the Detection Settings window, under Detection, click the **Automated Setup** button and follow the instructions. When the Automated Setup gives good detection, proceed with the next step. See Detection settings: Automated setup
5. Play the video or wait until the subject in the live image is located (a) far from walls and other objects (especially when the walls and objects provide little or no contrast with the subject), (b) its body is not curled or contracted, and (c) its nose is visible.

   **TIP** For best results, position the video on a frame where the animal is slightly stretched.

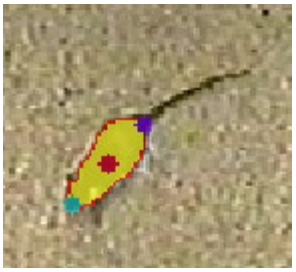



6. If you work with hooded animals, like the Lister rats, or the Long-Evans rats, under **Method**, next to **Deep learning settings**, select **Hooded rats**.
7. Under **Method**, next to **Deep learning settings**, click the **Define** button.

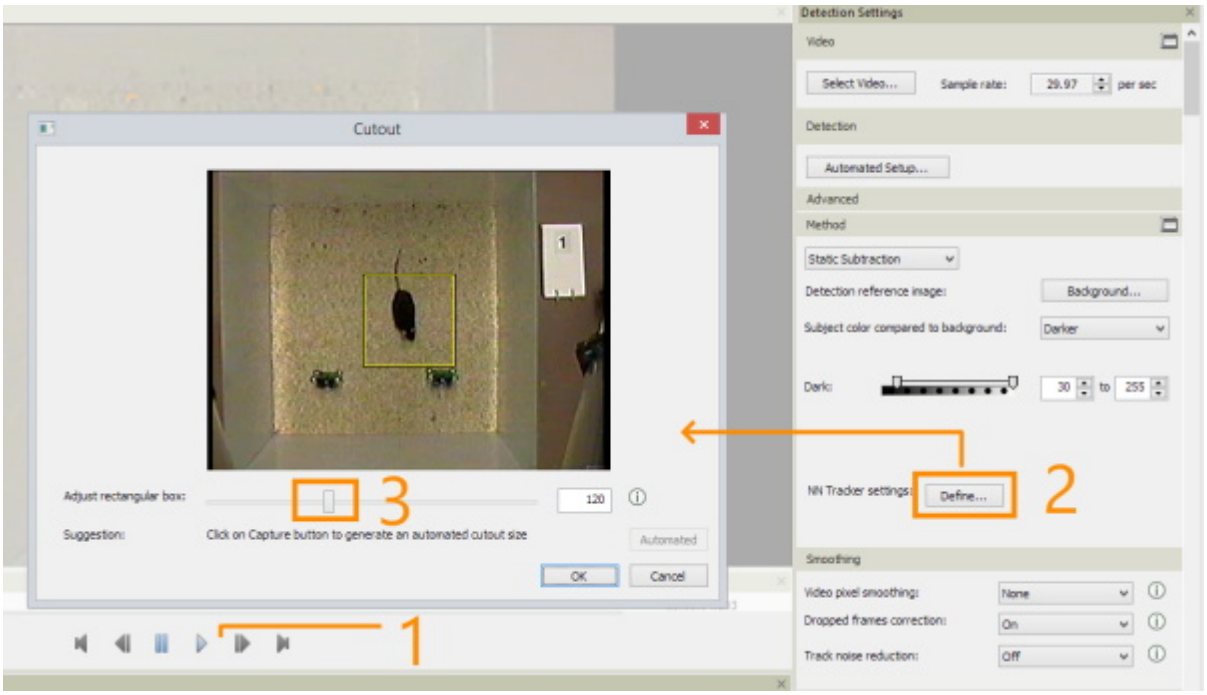
   The **Cutout** window opens. EthoVision XT shows a square box around the subject.

   The size of this box should already be optimized based on the detected subject. The Cutout box should include the whole subject's body leaving some space around it.
8. If that is not the case, click Automated.

   
9. Click **OK**.
10. The Video window now shows the subjects with its nose and tail-base highlighted (nose = light blue).
11. If the nose and tail base are not detected sufficiently well, click **Define** again and move the slider until the box includes the entire body of the subject. See the suggestions below.

- The tail of the rodent does not have to be included.
- **IMPORTANT** Keep some space between the subject and the outline of the Cutout box.

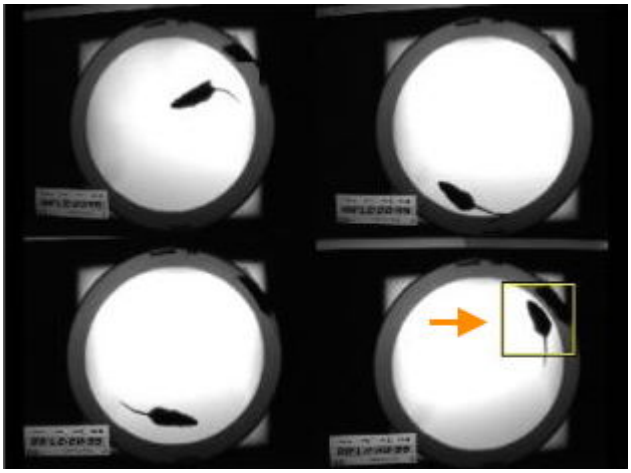



## Notes

- To improve detection, see also Deep learning: Requirements
- The Cutout box size is saved in the Detection Settings. The next time you click **Define**, the Cutout dialog shows last saved value.
- *Which value for the Cutout box size?* The Cutout value is shown for reference. Do not focus on a specific value, because other, similar values may work fine (for example, 135 and 137). However, take note of that value if you know that it works, so you can use it in the next experiment assuming that you use the same camera distance, arena size, etc.


As a rule of thumb, the Cutout box should include the animal also when it is stretched. Make sure that the box includes some space around the animal, at least half the body length.

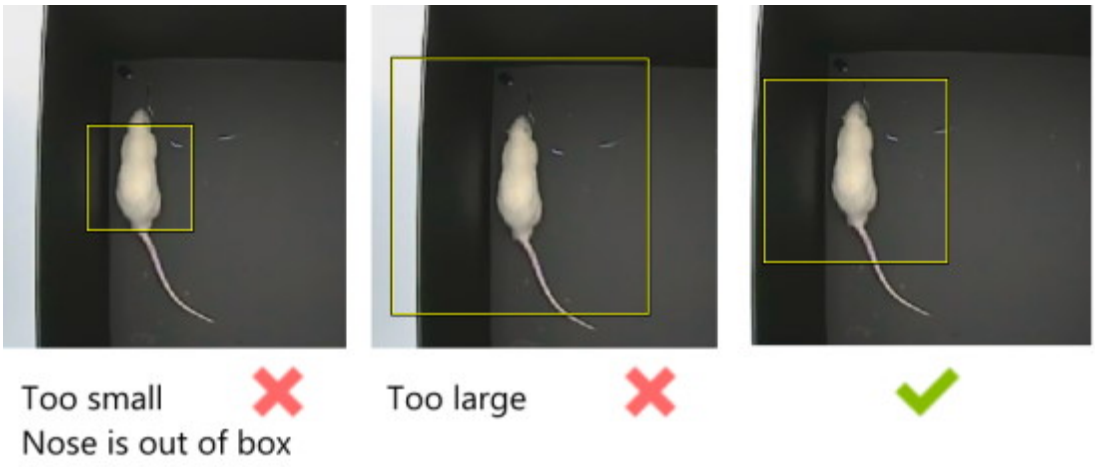
- *Small animals*. With small animals, the Cutout box can quickly become too small or too big. Adjust the Cutout value by small steps, until the nose and tail-base points are detected correctly.
- *Occlusions*. When the subject image is obstructed by e.g. a door, increase the Cutout box size, so that the nose or tail can be found on the other side of the obstruction. This usually improves the detection.
- *Reflections*. If there are reflections at the wall of the apparatus, and these give problems with detection of the nose point try to reduce the size of the Cutout box. See an example in Troubleshooting: The detected nose point is far from the animal's contour
- *Multiple arenas*. When you work with two or more arenas, the Cutout box is displayed only in the last arena. Adjust its size as described above.



## Examples of a good Cutout box size

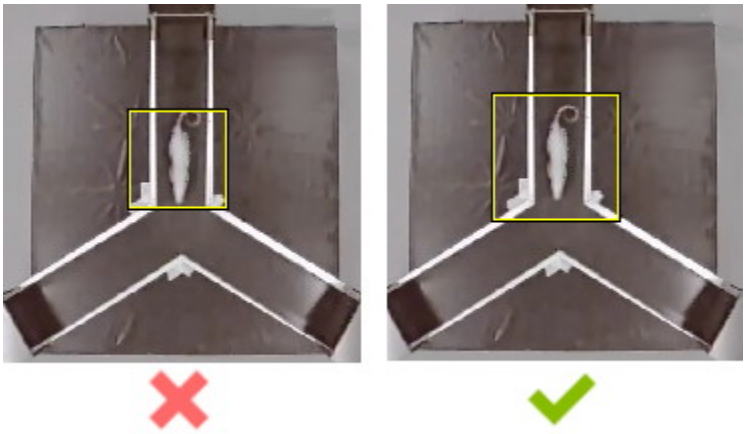
- Subject in an open field

  In the first picture below, detection won't work because the rat's nose is outside the box. In the second picture, detection may work but a large Cutout size increases the risk that other objects are included that are of similar color as the rat.




**NOTE** In this example, the subject is near the wall of the open field. Although that should be avoided, in this case there are no reflections and the walls are as dark as the floor; therefore, the contrast is good. This makes it possible to use an image when the subject is near the walls.

- Subject in a corridor or a T- / Y-maze



*See also*

- Deep learning: Requirements
- The chapter **Methods Settings** in the EthoVision XT Video Tutorial

# Detection settings for Behavior recognition

## Prerequisites

- You have the Rat/Mouse Behavior Recognition Module.
- In the Experiment Settings, under **Tracked Features**, you have selected **Center-point, Nose-point and tail-base tracking**. Under Analysis Options, you have selected Behavior recognition.
- See also Behavior Recognition: Requirements

## Procedure

1. Put the animal in the arena or play the video. Optimize the camera setup, and lighting conditions. Try to reduce reflections on the walls of the arena as much as possible.
2. Select the image source and sample rate. In the Detection Settings pane, under **Video**, select a value of sample rate between 25 and 30 samples/second.

   See Video file, image quality and sample rate
3. Use the Automated Setup as described in Detection settings: Automated setup.
4. When detection is good, open the **Behavior Recognition** section in the Detection Settings pane and follow Advanced detection settings: Behavior recognition.

## Notes

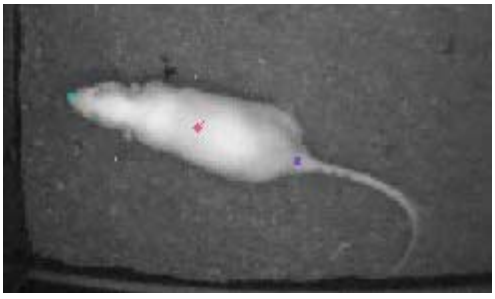
- If detection with the Automated Setup is not good, in the Detection Settings pane, click **Detection** and then **Advanced**. Under **Method**, select one of the following:
  - **Rodents / Default**. This is selected automatically when you use Behavior recognition, and should work in most cases.
  - **Rodents / For occlusions**. Choose this method only when Default does not give good results. This option is optimal when there are objects in the arena (for example a novel object) which create occlusions and make detection of the whole subject's body difficult.

- The quality and amount of light is very important when using Behavior recognition. See Subject exposure

# Advanced detection settings: Behavior recognition

## To adjust Behavior recognition settings

1. In the Detection Settings pane, click **Behavior Recognition**.
2. If you work with video, play the video up to a frame where the subject is walking normally, and its hind limbs can be partially seen; see the figure below. It is important that the animal's body is not contracted or stretched. Also, nose- and tail-base points are correctly detected.

   If you track live, wait until the animal shows a posture like in the figure above.
3. In the Behavior Recognition Settings window, click the **Grab** button.
4. A still image appears showing the detected subject's contour and the detected body points.

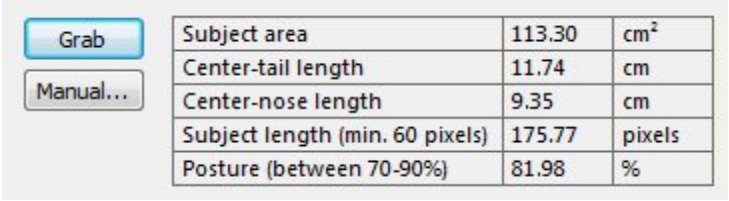
   
   

You can update the grabbed image at any time:

- If you track from video files, position the video to another frame, and click **Grab**.
- If you track live, wait that the posture of the animal is like that described above, and click **Grab**.

EthoVision XT only stores the image grabbed last.

5. In the Behavior Recognition Settings window, make sure that the calculated Subject length is greater than 60 pixels (55 for mice), and that the Posture index is between 70 and 90.



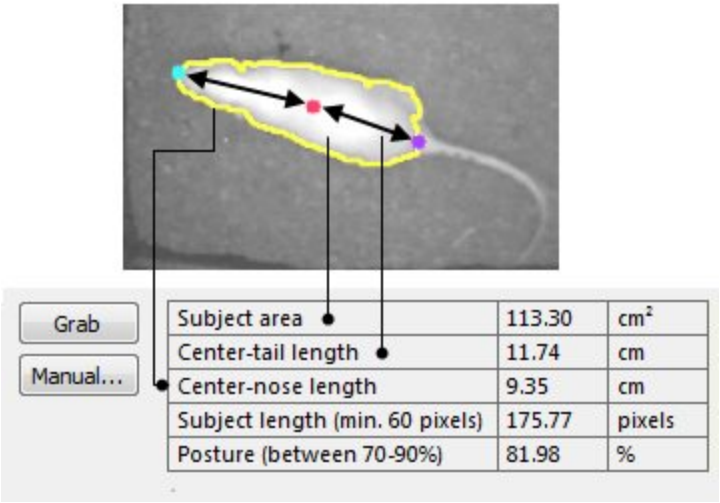

If the Subject length is smaller than 60 pixels (55 for mice), move the camera closer to the animal, or use a higher video resolution. The subject length must not exceed half the arena size.

6. Click **OK** to close the Behavior Recognition Settings window.

## To enter specific size values (optional)

If you know specific size values (for example, from a previous experiment using the same animal size, camera, lighting, camera-arena distance and the same calibration), click **Manual** in the Behavior Recognition Settings window and in the Manual Settings window enter the following values (see the picture below for explanation):

- **Subject area** (in distance unit square)
- **Center-nose length** (in distance unit)
- **Center-tail length** (in distance unit)
- **Posture** (between 70-90).




Then click **OK**. The Behavior Recognition Settings window says *No image saved: Size settings were manually set*.

- Subject size is expressed in the unit selected in the Experiment Settings.
- The value of Subject length in the Behavior Recognition Settings window is the sum of Center-nose length and Center-tail length, expressed in pixels. If this value is lower than 60 (55 for mice), when opening the Acquisition screen an error message appears. To increase subject length, move the camera closer to the animal, or use a higher video resolution.

## Making size-dependent detection settings

Accurate recognition of behavior is based on subject size settings. Since apparent size increases with the subject age, all being equal, we advise you to create detection settings specific for a certain age class. Each Detection Settings profile can only be used for a limited time. For example, for Wistar rats, a Detection Settings profile for rats that are 3-5 weeks old, which can be used about one week, and a Detection Settings profile for rats older than 5 weeks, which can be used for two weeks.

Subjects should not vary in size for more than 10%. If that happens, create more Detection Settings (for example, one for smaller animals and one for larger animals).

**IMPORTANT** EthoVision XT shows a warning message in the following cases:

- When the sample rate set is lower then 25 or higher than 31 samples/s.
- When the Subject length is smaller than 60 pixels for rats, or 55 pixels for mice.
- When the animal is larger than the arena.

# Other settings for behavior recognition

## *Smoothing*

See Advanced detection settings: Smoothing.

## *Subject contour*

For optimal results, we recommend to select Erosion and then Dilation to remove the tail from the detected body. See Advanced detection settings: Subject contour

## *Subject size*

Choose the option depending on the method for estimating the body points.

- If you select **Rodents / Default**:

  See Advanced detection settings: Subject size (one subject per arena).

- If you select **Rodents / For occlusions**:

  See Advanced detection settings: Subject size (multiple animals per arena).

## *Subject exposure*

When using Behavior recognition, the amount of light that the camera receives from the subject is even more important than usual. See Subject exposure

# Subject exposure

## Aim

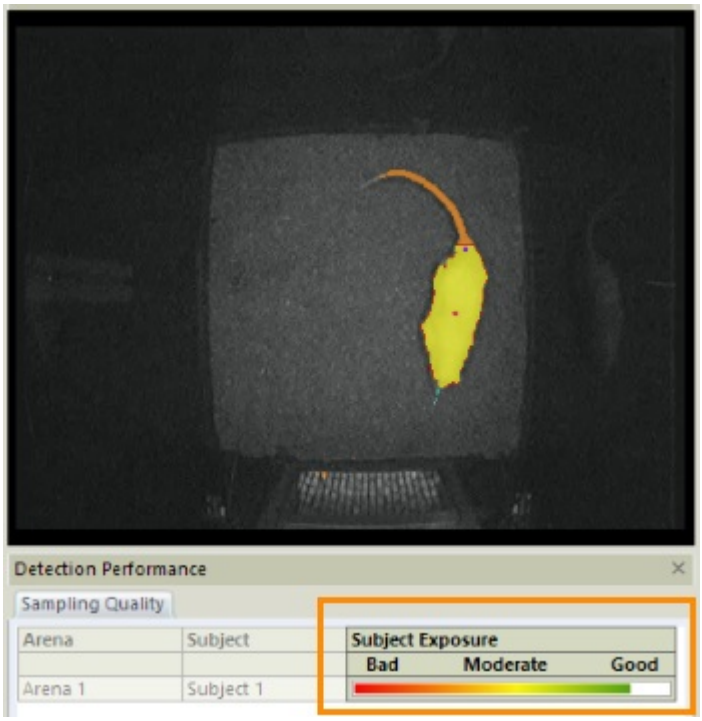
To obtain a balanced image of the subject. This is of critical importance when using Behavior recognition.

## Prerequisites

- Your experiment is set for Behavior recognition.
- You open the Detection Settings.

## Procedure

1. Play the video or let the subject walk in the arena.
2. In the Detection Performance window, locate the **Subject Exposure** indicator.




3. Make sure that the Exposure is Good when the subjects has been in all parts of the arena.
4. When the image is overexposed or underexposed, the subject looks too bright or too dark, respectively. The details of the fur are hardly visible. Try the following:

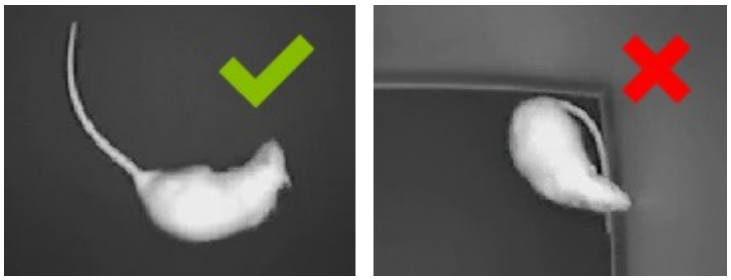
To reduce the subject's exposure, close the aperture of the camera lens. Alternatively, for GigE and USB cameras, reduce the Exposure time.

To increase the subject's exposure, open the aperture of the camera lens or increase the Exposure time. If that is not possible, increase the Gain.

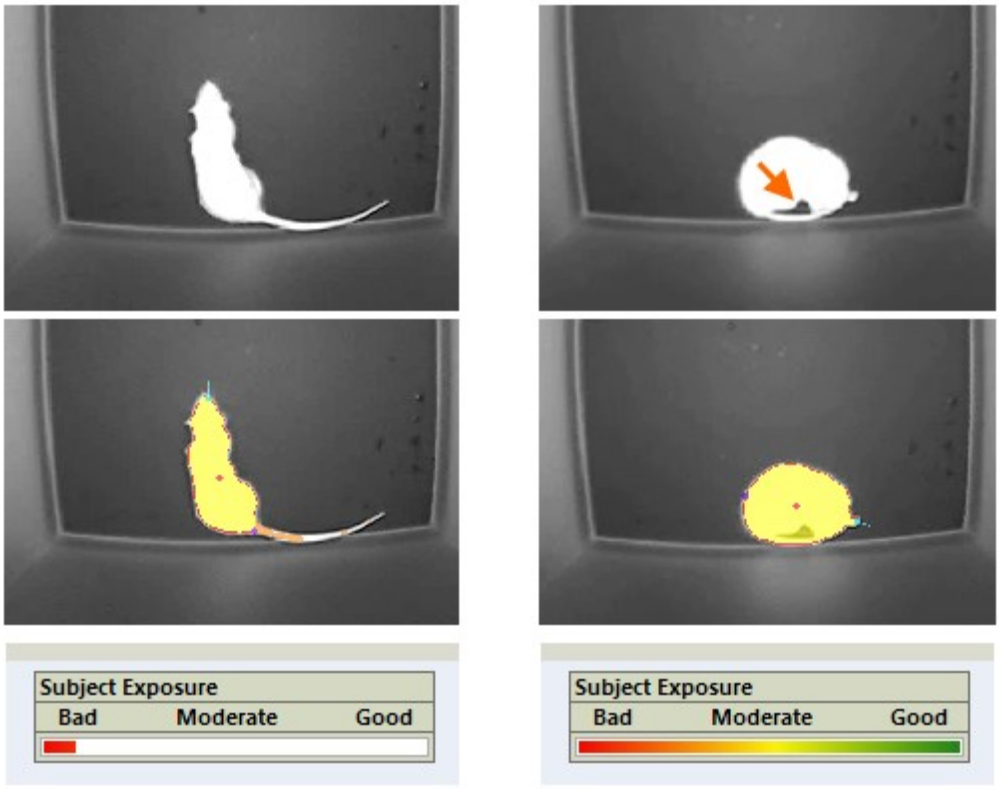
For how to adjust the exposure and gain, see Adjust camera settings in EthoVision XT.

## Notes

Check the exposure level when the subject is walking, or sitting still. The subject's tail should not touch the sides of its body. It that occurs, the estimate of subject exposure may not be reliable.



In the following example, the image of the rat is clearly overexposed (left). However when the tip of the rat's tail touches its body (right), the Subject Exposure level may give the impression that exposure is good.



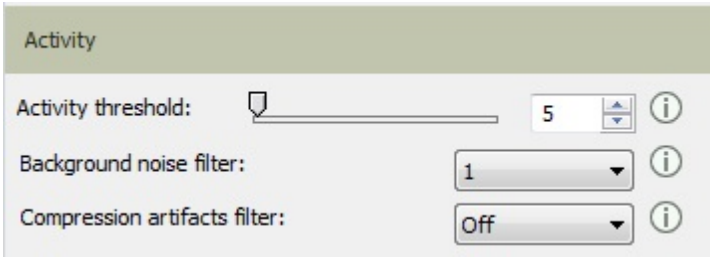

Wait that the subject's tail does not touch its body, and check the Exposure Level for a more reliable indication.

# Activity settings

If you selected Activity analysis in the Experiment Settings, you must create settings for this analysis.

To make it easier to judge whether the settings are correct, make sure the detected Body fill of your subject is not shown in the Video window. Click the **Show/Hide** button in the top-right corner of your window, select **Detection Features** and deselect **Body fill** and **Noise**. Then select **Activity**. Close the Detection Features window and play the video. The detected pixel change between samples is shown in purple.

Open the **Activity** section in the Detection Settings pane.



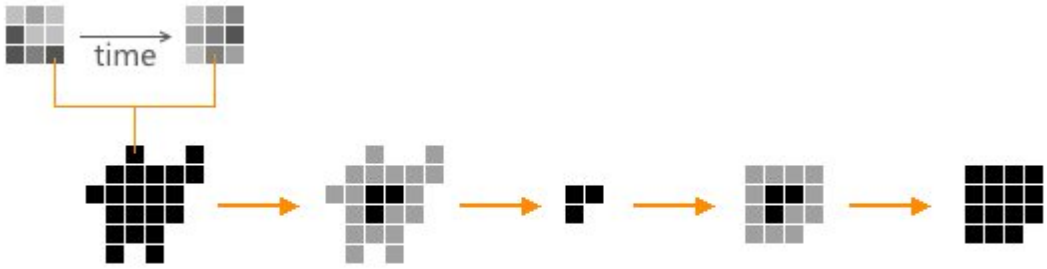

## Activity threshold

This value gives the threshold for the difference in gray scale values, between a sample and the previous sample.

## Background noise filter

Use this filter to remove noise in the video, or camera image. With the background noise filter, a pixel change is only counted as a change, if the surrounding pixels also have changed. The pixels that are not fully surrounded with changed pixels are removed and around the remaining pixels a layer of changed pixels is added. The higher the setting for the background noise filter, the more surrounding pixels are used.

Below: Activity is based on the change in the pixels' gray scale value with time. Left: The black squares represent pixels that have changed between two consecutive samples. First, all pixels that are not completely surrounded by one layer of changed pixels are removed (gray squares). Then, one layer of changed pixels is added around the remaining pixels. The resulting blob of changed pixels looks smoothed (right).




## Compression artifacts filter

Use the compression artifacts filter to compensate for video artifacts that are regularly recurring. With the compression artifacts filter, only the changes that are occurring in a number of consecutive frames are taken into account.

- If you track live, we recommend to leave this setting on the default value **Off**.
- If you track from video, or if you select Redo tracking from an existing video file, select **On**.
- However, if you are interested in very brief or fast occurring changes, leave the setting for the Compression artifacts filter on **Off**.

Create settings in such a way that all activity of your animal is detected and some noise is left. Also try whether lowering the sample rate and using Video Pixel Smoothing improves Activity detection. Then, click the **Show/Hide** button once more and select Detection Features. De-select **Activity** and select **Body fill**. Then create detection settings for your subject. Or, if you need different sample rates for activity analysis and tracking, create separate detection settings for tracking.

It is also possibly to only carry out activity analysis and not create detection settings for tracking. However, if you do so, it may happen that EthoVision XT has so much difficulties to detect the animal, that this decreases the performance of acquisition. This may result in many missed samples. Therefore, while creating activity settings, check that the proportion missed samples does not become too high. See Missed samples on page 363

# Optimize the reference image

## Learn about

- The reference image
- How the reference image is updated in Dynamic subtraction
- How the reference image is updated in Differencing

# The reference image

## Aim

To provide EthoVision XT an image of the background (that is, the arena without the subject) in such a way it can discriminate between the subject to track and everything else.

## Background information

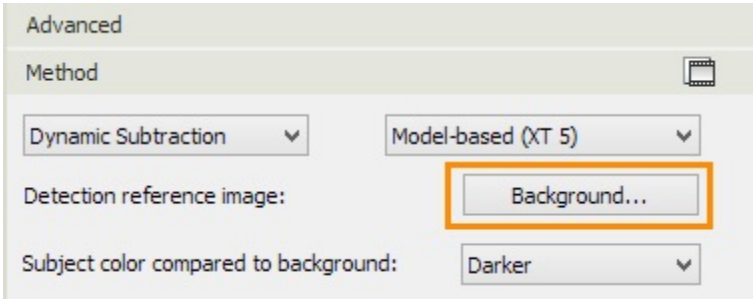
A reference image should not contain the image of the subject. The options below help you create a reference image even when it is not possible to have the subject removed from the video image.

A reference image is used in detection methods Static Subtraction, Dynamic subtraction and Differencing.

Grabbing a reference image is optional. However, if you do not do so, EthoVision XT takes the first video frame available from the camera or video file and considers that as the reference image.

## Procedure

1. Click **Background** in the **Method** section.




2. In the Reference image window, choose **Grab Current (A)**. Scroll through the video until you find an image without animals. If you track live, make sure that there are no animals in the arena, then click **Grab Current (A)**. This image will be the initial reference image. Skip **B** and **C** and click **Close**.

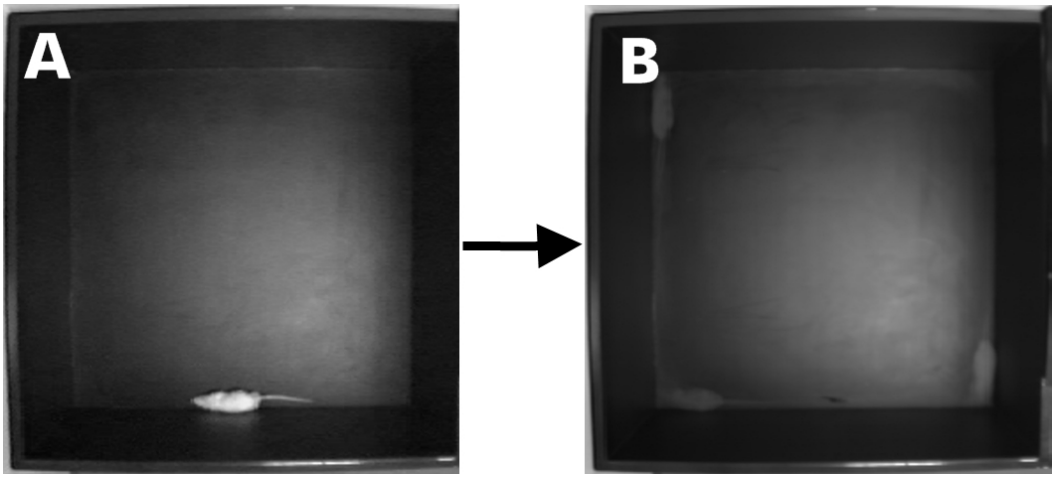
   If your video does not contain images without animals, continue with option **B**. Also continue with option **B** if you track live and you cannot start with an empty arena.


3. **Grab from other (B)**. You may have a video with an identical background as the one you use for tracking, but without animals. Or you may have an image of a background without animals. If this is the case, click **Grab from Other** and select this video file or image file. If you select a video file, the first frame of this file will be used as an initial reference image. If you select an image file, this has to have the same resolution as the video file you use

for tracking. Browse to this file and click **Open**. Skip option **C** and click **Close**. If you do not have such video or image, proceed with option **C**.

4. **Start learning (C)**. With this option EthoVision XT makes an average image of the entire video. If the animals are moving, this process will average out the pixels of the animals, resulting in a reference image without animals.

   In the figure below you see an effect of using this option. In A, the rat is in the view at all times. In B, after applying **Start learning**, the animal is removed from the background.




   If you track live, you have to click **Start Learning**, and subsequently click **Stop Learning** as soon as you have obtained an initial reference image without animals. Click **Close** if you have a good reference image without animals. If you use Dynamic subtraction or Differencing and the reference image is still not good, continue with step 5.

5. **Acquisition settings**: If you run a number of consecutive trials, you may want to choose which image to use as initial reference image. Pay particular attention here, since the video image may not be exactly the same between the end of a trial and the start of the next one.

   - **Use saved reference image**. Choose this option if the background remains constant between the different trials. For example, when there are no moving objects, bedding material, shadows etc.
   - **Use dynamic reference image**. Choose this option if the background changes between the different trials, and you assume that the video image at the end of a trial is the same as that of the start of the next trial. For example, when a trial starts a few seconds or minutes after the end of the previous trial.
   - **Use first frame of each trial**. Choose this option whenever the video image at the end of a trial is likely to differ from that at the start of the next trial. This may be the case for example when you do batch

acquisition from a number of video files, where shadows or reflections appear in the arena in the new video. EthoVision XT takes the first video frame available from the camera (or video file) and considers it as the background. Note that if you choose this option and the subject is already in the arena the moment that you start a new trial, EthoVision XT misses the subject in the first frames and finds it only when it moves away from the starting location. To avoid this, release the subject after you have started the trial.

6. Click **Close**.

## Notes

- By default, the reference images are stored in the folder **Bitmap Files** of your experiment. If the background has not changed, you can use these images as reference images in other experiments.
- If you are tracking from video files, you must play the video forward whilst making dynamic subtraction settings. This is because the program needs to update the reference image. Do not skip through the file, since the reference image will then not be correctly made.
- The option **Acquisition settings** is available only in Dynamic subtraction and Differencing, since in these methods the background is continually updated during the trial.

*See also*

- How the reference image is updated in Dynamic subtraction
- How the reference image is updated in Differencing

# How the reference image is updated in Dynamic subtraction

A video stream is composed of a number of video images (frames). During data acquisition, EthoVision XT analyzes one every x images according to the sample rate specified (see Video file and image quality). When analyzing the sample (image) n, the reference image is obtained by summing up the gray scale values of each pixel from two images:

- The reference image made of pixels which have an average value of previous images.
- The current image, where a square area around the subject detected in the previous sample has been removed. This provides a rough estimate of the current background.

The gray scale values are summed up according to the formula:

$Reference_{i,n} = (1-\alpha) * Reference_{i,n-1} + \alpha * Current_{i,n}$

for each pixel *I*, where:

- $Reference_{i,n}$ = Gray scale value of pixel *I* in the reference image of sample *n*.
- $Reference_{i,n-1}$ = Gray scale value of pixel *I* in the reference image of sample *n–1*.
- $Current_{i,n}$ = Gray scale value of pixel *I* in sample *n* where a square area around the subject previously detected has been removed.
- $\alpha$ = Current Frame weight.

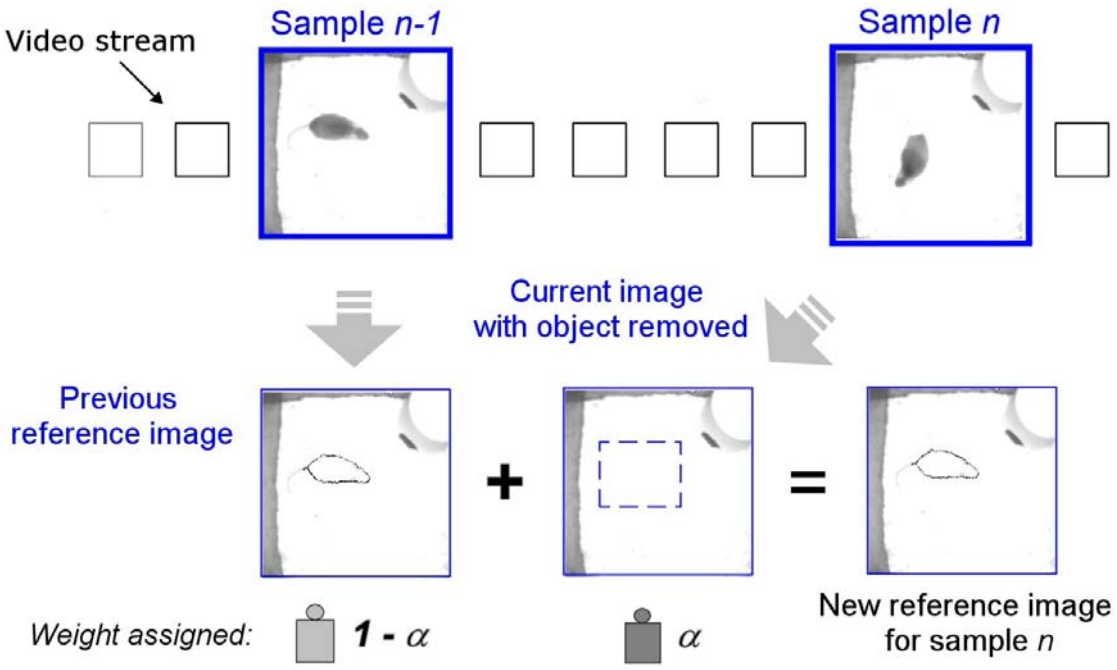
The Current Frame weight determines the relative weight of the two components of the new reference image.

Because the above formula is recursive, that is, each value of $Reference_{i,n}$ is also a function of the previous sample, the value of $\alpha$ determines the number of past images that contribute to the reference image for the sample *n*. The lower $\alpha$, the more past images contribute at least partially to the current reference image.

The extent to which each past image contributes to the current reference image is a power function of 1-$\alpha$. The older an image relative to the current one, the smaller its contribution to the reference image.

**EXAMPLE** If $\alpha$=20%, then 1-$\alpha$ =80%. The first video image contributes by 80% to the second sample, by $80\%^2$ =64% to the third sample, then by $80\%^3$ =51% to the fourth sample, etc. At the 21th sample, the contribution by the first image gets below 1%.

Below: In the Dynamic subtraction detection method, the reference image is updated at each sample. The starting reference image is the one you specify by clicking one of the buttons in the Reference Image window, otherwise it is the first frame analyzed (not shown in the picture). For the general sample n, the reference image is obtained by summing the reference image of the previous sample *n–1* and the current image *n* where the area around the subject estimated from the previous sample has been removed. The current image with subject removed is given the weight $\alpha$ that you specify (see the procedure), while the previous reference image is given the weight (1-$\alpha$). Because of the way it is determined, each reference contains information on a number of past images, depending on the value of $\alpha$.



# How the reference image is updated in Differencing

The Differencing method uses a Gaussian distribution of all pixels in a frame. EthoVision XT keeps a running average of the mean $\mu$ and the variance $\sigma^2$ of the gray value of each pixel to detect unlikely pixels. These pixels are considered to be the subject.

The mean of the gray values is summed up according to the same formula as for Dynamic subtraction.

The variance of the gray values is summed up according to the following formula:

$$Variance_{i,n} = (1-\alpha) * Variance_{i,n-1} + \alpha * (Current_{i,n}. Reference_{i,n})^2$$

for each pixel *i*, where:

- $Variance_{i,n}$ = Variance of gray scale value of pixel *i* in the reference image of sample *n*.
- $Current_{i,n}$ = Mean gray scale value of pixel *i* in sample *n* where a square area around the subject previously detected has been removed.
- $Reference_{i,n-1}$ = Mean gray scale value of pixel *i* in the reference image of sample *n–1*.
- $\alpha$ = Frame weight, which depends on the **Background changes** option. The higher the value set (from Very slow to Very fast), the higher $\alpha$.

The Frame weight determines the relative weight of the two components of the new reference image (see the example in How the reference image is updated in Dynamic subtraction).

# Customize the Detection Settings screen

To achieve optimal subject detection, you need proper feedback about the effect of your settings on the quality of detection. EthoVision offers you a number of statistics for this purpose.

## What do you want to do?

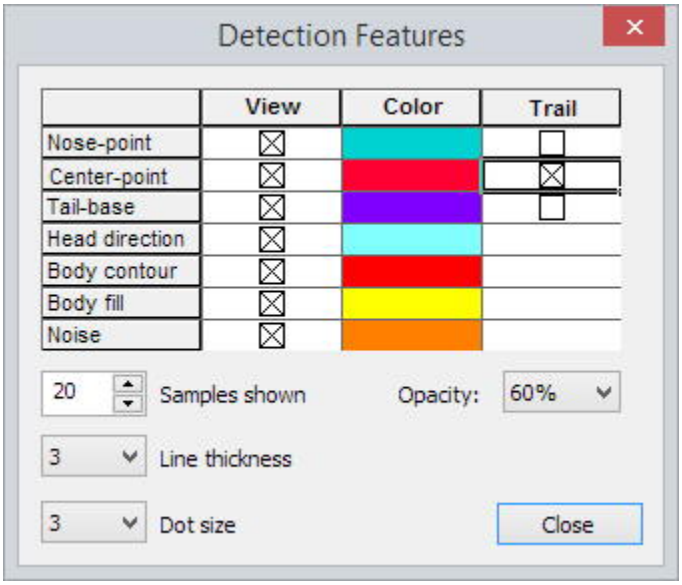
- View the detection features on the video window
- View the detection statistics

# View the detection features on the video window

**IMPORTANT** Displaying detection features can use a lot of processor power and reduce the maximum possible sample rate if you are tracking live.

## Procedure

1. In the Detection Settings screen, click the **Show/Hide** button on the tool-bar and select **Track Features**.
2. Select **View** for the feature you want to view. Choose the color and, for the body points, whether to show the trails.




3. If you have selected to view the body points' trail, choose the number of **Samples** you want to be shown at a time.
4. Adjust the **Line thickness** and the **Dot size**.
5. Click **Close** and check the result in the Video window.

## Detection features

### *Nose-point*

To check that the nose tip is detected correctly.

*Center-point*

To check that the center-point of the subject is detected correctly.

The center-point is the point whose X,Y coordinates are the arithmetic mean of the X,Y coordinates of all pixels detected as subject. For more information on how the nose- and tail-base points are calculated, see Overview of nose-tail base detection.

*Tail-base*

To check that the base of the tail is detected correctly.

*Head direction*

To estimate at what the subject is sniffing. Select this option especially with Novel object and orientation tests.

*Body contour*

To check that the subject's contour (or the part which should be found) is detected.

*Body fill*

To check that the subject's body (or part of it) is detected. For example, in a test where it is important to measure the change in the animal's shape to estimate its mobility.

If you do not select a color for **Body fill**, the body contour will be shown as noise.

*Noise*

To view the pixels that match the criteria for subject detection (depending on the detection method), but other than those detected as subject.

We recommend to keep Noise selected. This way you can see which parts of the video image have gray scale values similar to those of the subject(s) to be detected.

*Activity*

To view the pixels that match the criteria for activity detection (see Activity settings). This setting is only available if you selected **Activity analysis** in the Experiment Settings.

Some of the options above are not available if your experiment is set to Only center-point detection in the Experiment Settings.

### *Line thickness*

This refers to the thickness of the trails, the body contour and the head direction line.

### *Dot size*

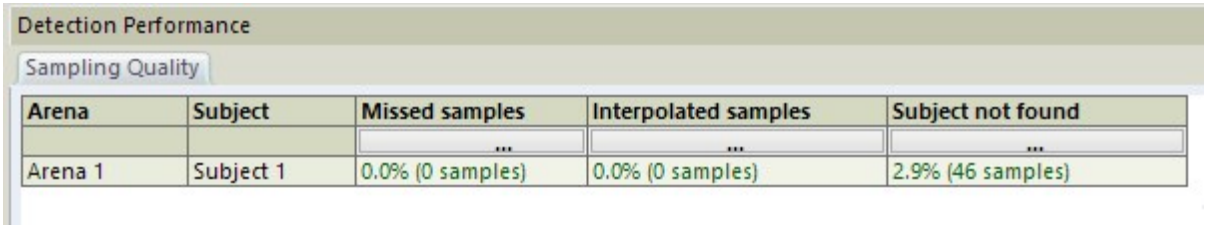
This refers to the size of the body points visualized.

# View the detection statistics

The detection statistics are displayed in the Detection performance pane, which is, by default, displayed at the bottom of the screen.

If the Detection Performance pane is not displayed, click the **Show/Hide** button on the toolbar and select **Detection Performance**.

The Detection Performance pane shows immediate feedback when you change detection settings.




## Missed samples

The percentage and number of samples that were skipped due to lack of processor time. This value tells you whether your PC can handle the sample rate specified (see Sample rate). See also Missed samples for tips on how to increase the maximum sample rate handled by the PC.

The value is reset to zero when you load another video file or click **Save changes** in the Detection Settings pane.

## Interpolated samples

The percentage and number of missing samples *due to missing video frames* that were interpolated. This statistic is only available when in the Detection Settings under **Smoothing** the option **Dropped frames correction** is **On**. See Dropped frames correction

- What is the maximum acceptable percentage of interpolated samples? Only accept a high value of interpolated samples when you are sure that the tracks represent the movement of the subject reliably. If the percentage is as high as 30% and the subject moves fast or makes sharp turns, the resulting track includes lots of artifacts and the statistics are unreliable. In Integrated Visualization check whether the tracks with high percentages of interpolated samples match the actual movement of the subject.
- The value is reset to zero when you load another video file or click **Save changes** in the Detection Settings pane.

**NOTE** This correction is not applied when the subject is not found for other reasons other than the dropped frames, for example when the subject is out of view.

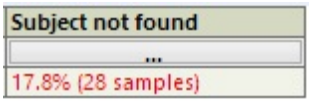
## Subject not found

The percentage and number of samples in which the subject was not found. This information is useful to check the quality of detection in general. When a subject is not found, it means that EthoVision XT processed the image, but did not find anything matching the current Detection Settings. Use **Subject not found** to assess the quality of tracking.

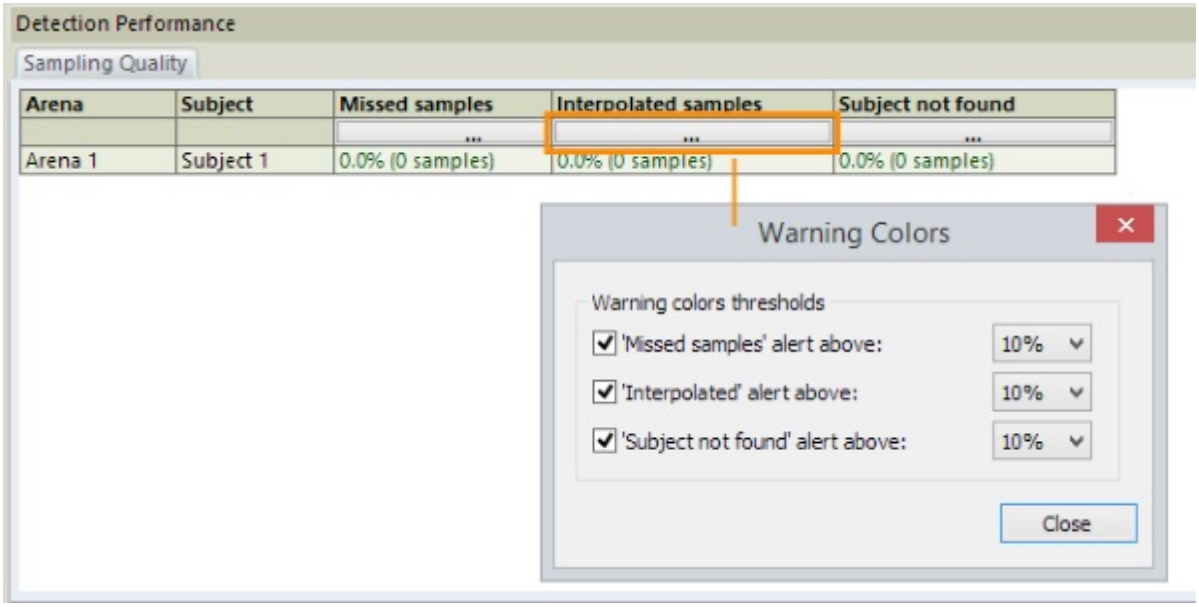
When you select another video file, or click **Save changes**, the value for **Subject not found** is reset to zero.

## Warning colors thresholds

The percentages of missed samples, interpolated samples and samples where the subject is not found are usually displayed in green for each arena and subject. When the values are above the set threshold, they are highlighted in red. This helps you monitoring the quality of tracking, and spotting detection issues.




Click the button under **Missed samples**, **Interpolated samples** or **Subject not found** to change the thresholds.



*See also*

- Detection statistics after acquiring data

# Trial List

## Main topics and tasks

# Introduction to the Trial List

## Learn about

- Important terms in the Trial List
- Useful things to know about the Trial List

# Important terms in the Trial List

## Independent variable

An *independent variable* is a variable that you define to record specific experiment conditions, the identity of your subjects, or the amount of drug injected.

An independent variable potentially determines the value of a dependent variable, such as speed of movement or distance moved. EthoVision XT assumes that the value of the variable stays constant throughout a trial.

Independent variables are especially useful to create analysis groups.

**EXAMPLE** The independent variable *Treatment* with possible values *Control*, *Sham*, and *Treated*. Each subject is assigned one of the values. In the Data profile you can group tracks based on the value of Treatment.

## System variable

A system variables is a variable that EthoVision records during acquisition. For example, the duration of a trial, or the name of the video file used in that trial.

## Trial

A *trial* is a single data acquisition session, no matter how many subjects are tracked simultaneously. For each trial, you can enter the value of independent variables.

In the Trial List you can plan your trials. You can add and delete planned trials. You can also delete acquired trials.

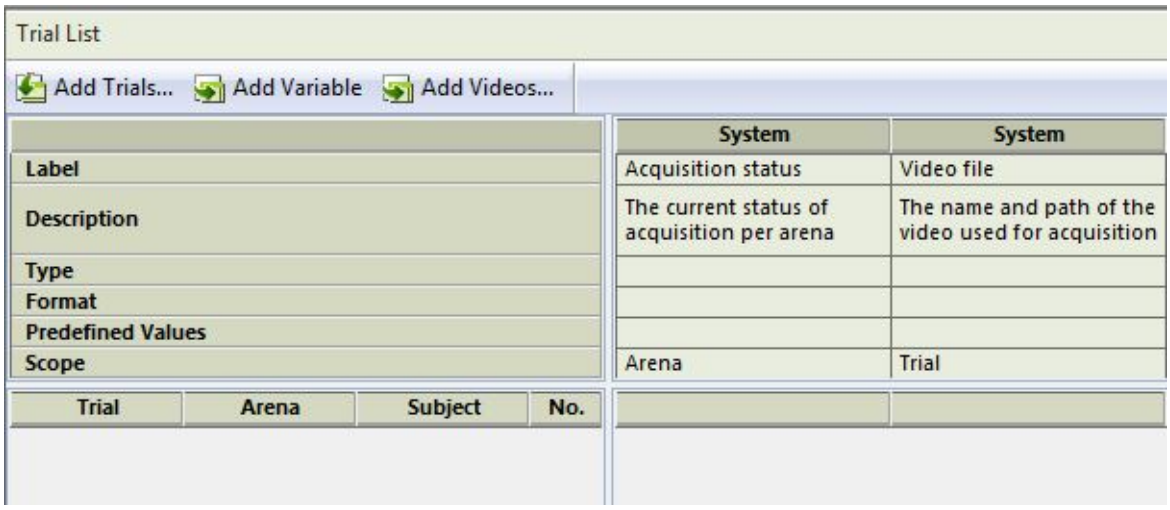
*See also*

- Analyze groups of tracks

# Useful things to know about the Trial List

## To open the Trial List

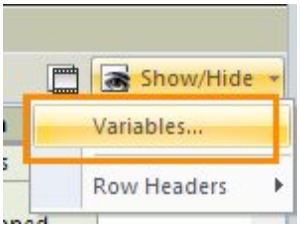
Choose **Setup** > **Trial List**, or in the Experiment Explorer click **Trial List**.




## To show and hide variables in the Trial List

1. Do one of the following:

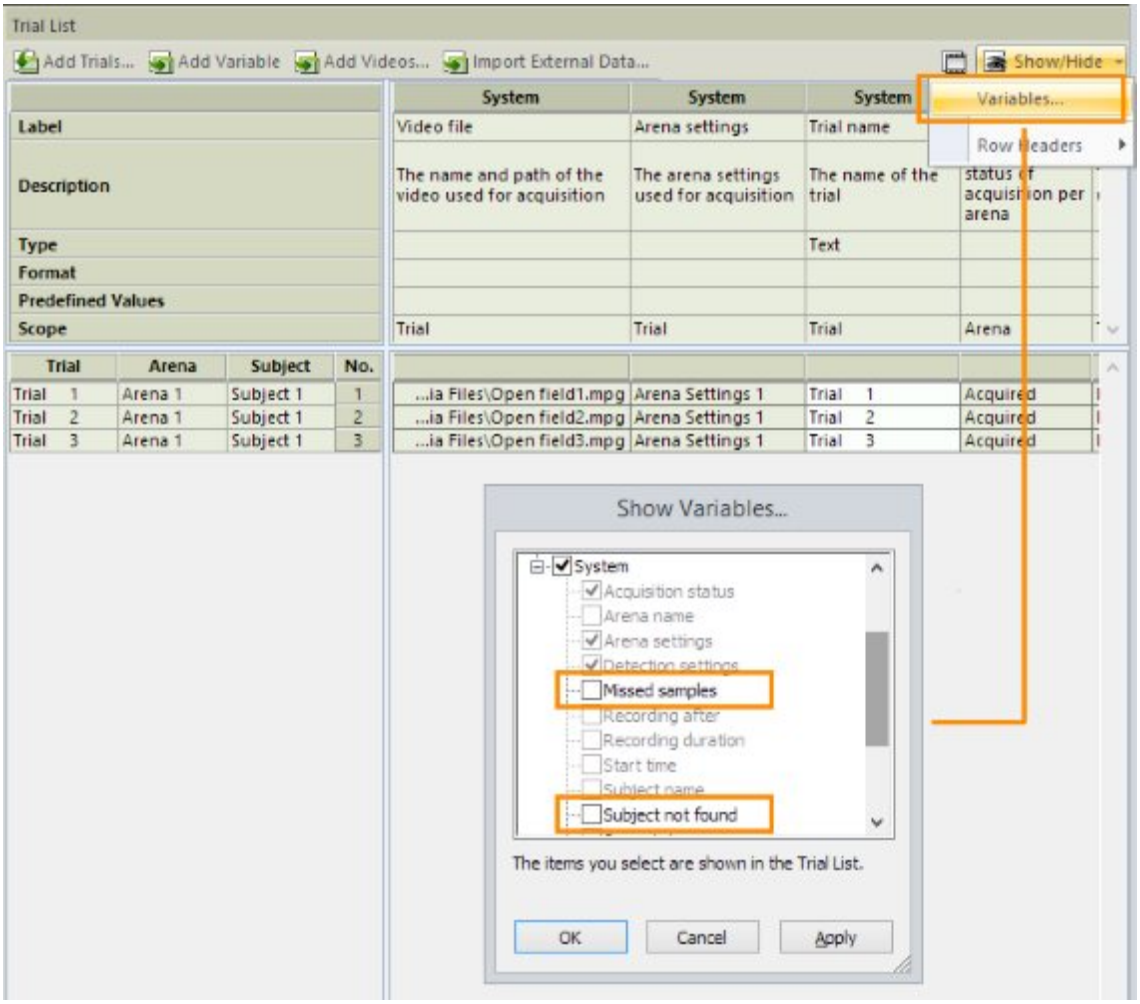
   Click the **Show/Hide** button on the toolbar and select **Variables**.

   Right-click a column header and choose **Show Variable**.

2. In the Show Variables window, select the variables of your choice and click **Apply**.

**TIP** Under **System**, also choose **Subject not found** and **Missed samples**. The two variables give useful information about the reliability of your data.




The statistics are shown in two columns:

| | System | System |
|---|---|---|
| Label | Subject not found | Missed samples |
| Description | Proportion samples not found | Proportion skipped samples |
| Type | Numerical | Numerical |
| Format | x.x % | x.x % |
| Predefined Values | | |
| Scope | Subject | Subject |

| Trial | Arena | Subject | No. | | |
|---|---|---|---|---|---|
| Trial 1 | Arena 1 | Subject 1 | 1 | 0.1 % | 0.0 % |
| Trial 2 | Arena 1 | Subject 1 | 2 | 0.0 % | 0.0 % |
| Trial 3 | Arena 1 | Subject 1 | 3 | 0.0 % | 0.0 % |

*See also*

- System variables

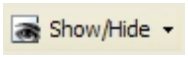
## To sort columns in the Trial List

You can change the order of the columns. Click the column header to select the column of your choice and then drag it to the new position. While you drag it, a red line shows the insertion point of the column.

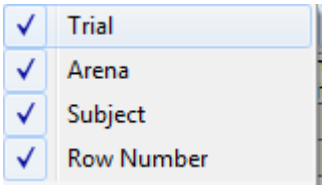
## To show and hide row headers in the Trial List

Do one of the following:

- Click the **Show/Hide** button on the toolbar.

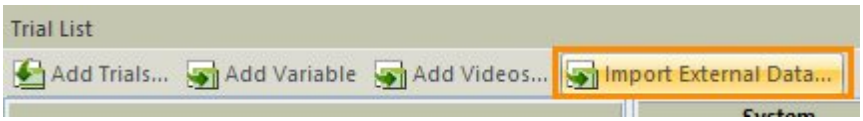
  Select **Row Headers** and then the headers you want to display.

- Right-click on any of the headers (Trial, Arena, Subject, No.) and select which headers you want to display.

## Import External Data button

If you have the External Data Module, the **Import External Data** button is available on the toolbar of the Trial List.




Click this button to import external data like EEG, or Heart Rate into EthoVision XT and synchronize it with the tracks.

# Independent variables

## What do you want to do?

- Define an independent variable
- Delete an independent variable
- Specify Variable values, formats and other properties
- Select System variables

# Define an independent variable

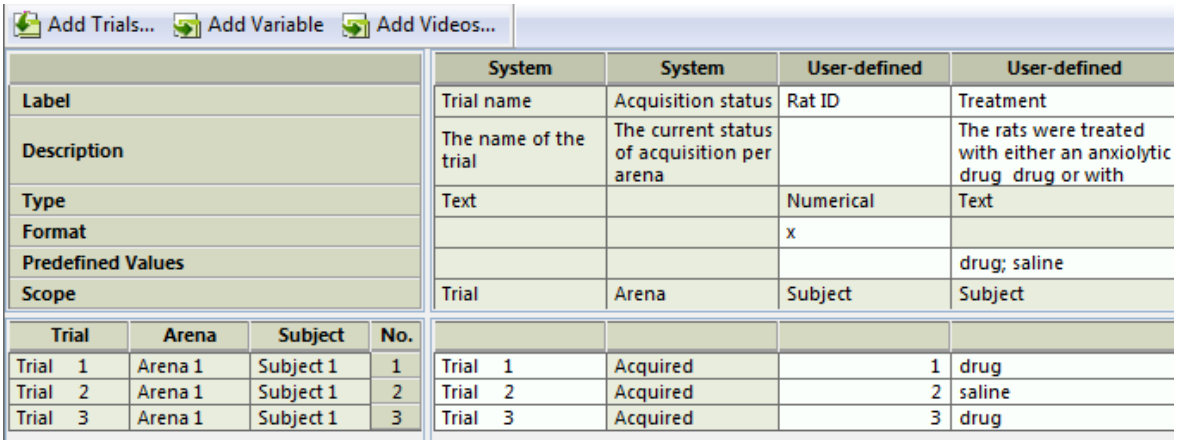
## Aim

To label trials, arenas and subjects using an independent variable, for example the level of the treatment, the ID of the subjects and the day of testing.

## Procedure

1. Open the Trial List.
2. Click the **Add Variable** button.

   A new column with label **User-defined** is inserted at the end of the table.
3. Specify the label (name), description, type, format, predefined values and scope of the variable. See below an example of a Trial List with two independent variables, Rat ID and Treatment.




| | System | System | User-defined | User-defined |
|---|---|---|---|---|
| **Label** | Trial name | Acquisition status | Rat ID | Treatment |
| **Description** | The name of the trial | The current status of acquisition per arena | | The rats were treated with either an anxiolytic drug drug or with |
| **Type** | Text | | Numerical | Text |
| **Format** | | | x | |
| **Predefined Values** | | | | drug; saline |
| **Scope** | Trial | Arena | Subject | Subject |

| Trial | Arena | Subject | No. | | | | |
|---|---|---|---|---|---|---|---|
| Trial 1 | Arena 1 | Subject 1 | 1 | Trial 1 | Acquired | 1 | drug |
| Trial 2 | Arena 1 | Subject 1 | 2 | Trial 2 | Acquired | 2 | saline |
| Trial 3 | Arena 1 | Subject 1 | 3 | Trial 3 | Acquired | 3 | drug |

# Delete an independent variable

## Aim

To remove an independent variable that you no longer need in your experiment.

This action removes the column from the Trial List and also removes the values of the variable assigned to each trial, arena and subject.

## Procedure

Right-click the column header and choose **Delete Variable**.

## Notes

- You can only delete variables that you defined yourself.
- To hide system variables, see System variables.

# Variable values, formats and other properties

## Variable label

In the **Label** field enter the name of the variable. The name must be unique.

Names cannot be longer than 64 characters. The characters you use, must be Unicode characters in the Basic Multilingual Plane range. Please see http://en.wikipedia.org/wiki/Plane_(Unicode)#Basic_Multilingual_Plane.

## Variable description

In the Description field you can enter a short text or any other background information about the variable.

The description is limited to 255 characters. The characters you use, must be Unicode characters in the Basic Multilingual Plane range. Variable type

Click the **Type** field and from the drop-down list choose one of the following options:

- **Text**. A text variable is denoted by alphanumeric characters, composed of letters, numbers or both. For example, the name of the treatment.
- **Numerical**. A variable represented by numbers only, for example, the dose of the drug that you apply.
- **Time stamp**. A variable represented by a time stamp, for example, the start date and time of the trial.
- **Duration**. A variable represented by a duration, for example the duration of the treatment.
- **Boolean**. A variable that is either 'False' or 'True'. For example, the presence of a novel object in the arena.

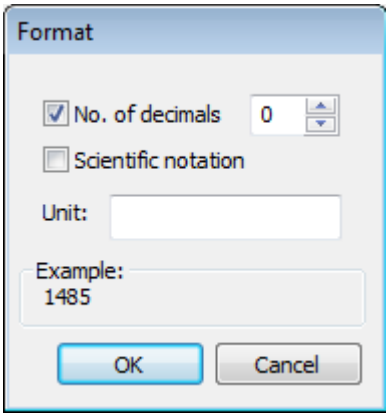
## Variable format

- For Text variables you do not need to specify a format.
- For Numerical variables, double-click in the **Format** field. In the Format window:

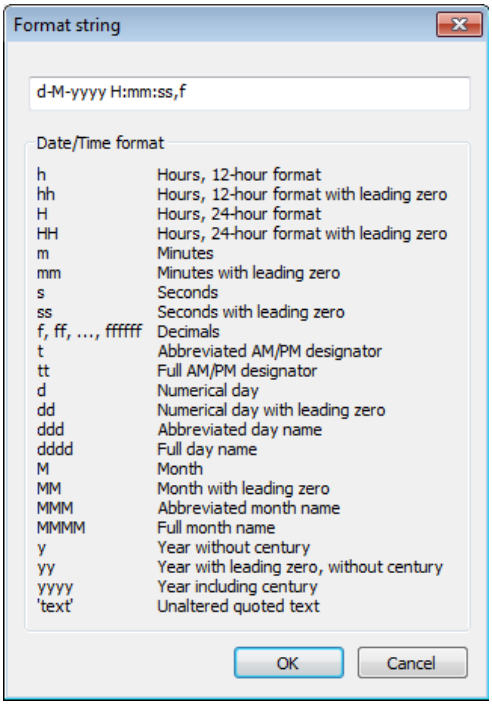
  Select **No. of decimals** and enter the number of decimals (1 to 9). If you work with integer values, leave the check box unselected.

Select Scientific notation if you want to write, for example, 1485 as 1.485e+0.03

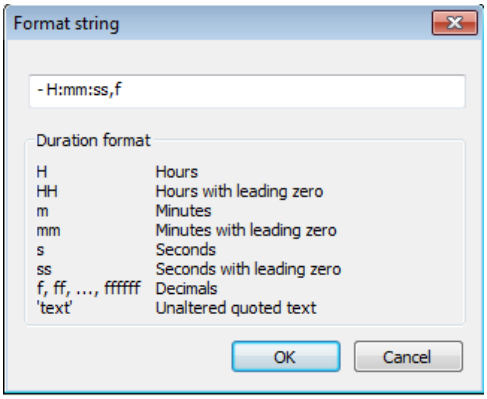
In the Unit field, enter the unit of your variable, for instance, µg/g for the dose of the drug. Then click **OK**. Tip: To enter Greek symbols, use the Windows Characters map.




- For Time stamp variables, click in the **Format** field. In the Format string window, and enter your preferred format in the field at the top.




- For Duration variables, click in the Format field. In the Format string window, enter your preferred format in the field at the top.



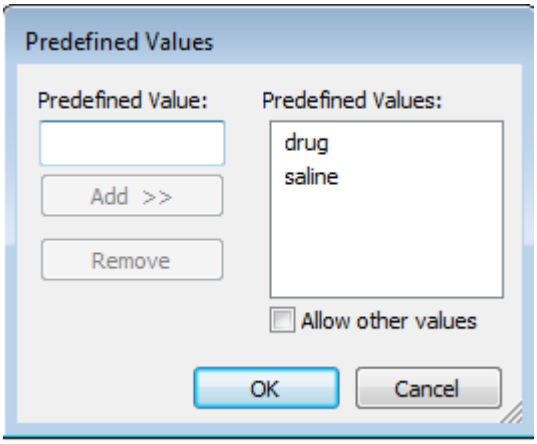

- For Boolean variables you do not need to specify a format.

## Predefined Values

- For Text variables, double-click in the **Predefined Values** field. In the Predefined Values window:

  Under Predefined Value enter one of the possible values of the independent variable, then click **Add**. Repeat this step for all values.

  **EXAMPLE** Your independent variable is the treatment you apply, with possible values *drug* and *saline*, enter *drug*, then click **Add**. Repeat this step for *saline*.




  Keep the **Allow other values** check box selected if you are not sure whether the values that you defined are exhaustive. This allows you to add new values if needed. Clear this option if the values under **Predefined values** are exhaustive.

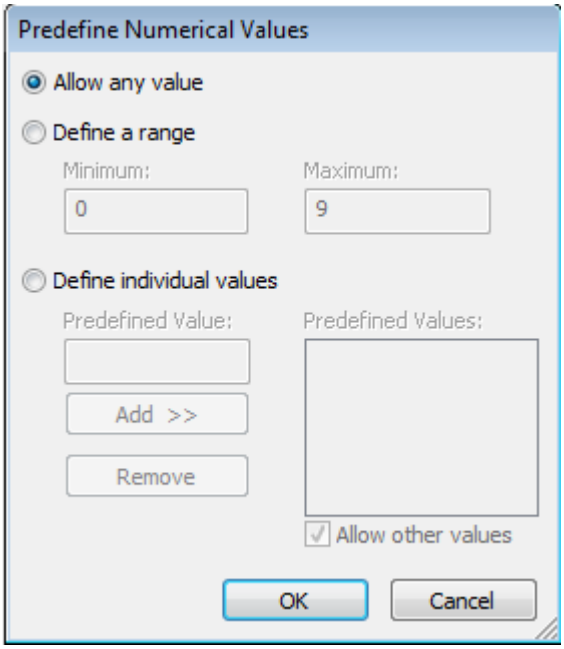
  The characters you use, must be Unicode characters in the Basic Multilingual Plane range.

To delete a predefined value, select it in the **Predefined Values** field and click **Remove**.

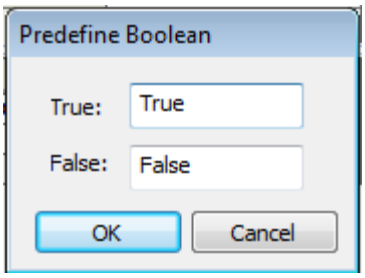
See also:

http://en.wikipedia.org/wiki/Plane_(Unicode)#Basic_Multilingual_Plane.

- For Numerical variables, double-click in the **Predefined Values** field. In the Predefine Numerical Values window, select one of the following options:
  - **Allow any value**. Select this option if you do not want to predefine a range of values or individual values.
  - **Define a range**. Define a minimum and maximum value. For instance, when the age of the rats in your experiment ranges from 6-18 months, enter '6' as the minimum value and '18' as the maximum.
  - **Define individual values**. Select this option when you are, for instance, testing a drug in three dosages: 0.05; 0.1 and 0.5 mg/g. Enter '0.05' in the Predefined Value field and click Add. The value moves to the Predefined Values field. Repeat this procedure for the other two values. Keep the Allow other values check box selected if you are not sure whether the values that you defined are exhaustive. This allows you to add new values if needed. Clear the check box if you are sure that you do not want to add new independent variable values. To delete a predefined value, select it in the Predefined Values field and click Remove.



- For Time stamp and Duration variables you cannot specify any predefined values.
- For Boolean variables, double-click in the **Predefined Values** field. In the Predefine Boolean window, enter values for **True** and **False**. For instance, if your independent variable is *Presence of novel object*, define the values Yes (True) and No (False).




## Variable scope

For the scope of an independent variable you can choose between the following three options: Trial, Arena and Subject.

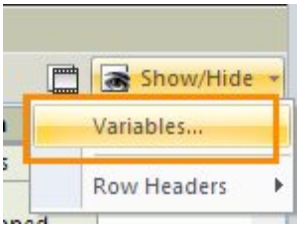
**EXAMPLE** The scope of the variable *Bedding type* is **Trial** if all the arenas in a trial have the same kind of bedding material. Select **Arena** if the bedding material differs for different arenas during the same trial.

For variables whose values may differ between subjects in the same arena and trial, select **Subject**. For instance, in two-subject interaction tests, the variable *sex* (with subject 1 being male, and subject 2 female) or *Status* (subject 1 being resident, and subject 2 intruder).

# System variables

## To view/hide a system variable

1. From the **Show/Hide** menu on the toolbar, choose **Variables**.




2. Select the variables you want to view.

To hide a system variable, clear its check box.

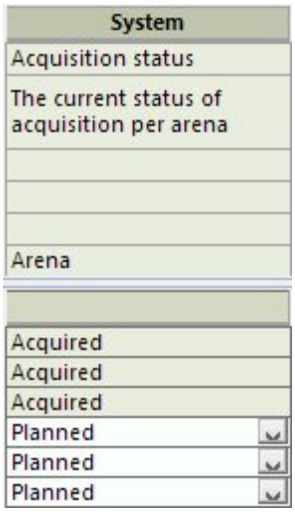
## System variables

### *Acquisition status*

The current status per arena:

- **Planned**. When a trial is added to the Trial List, all the arenas within the trial get the status Planned.
- **To Skip**. You can give an arena the status **To skip** when for any reason you do not want or cannot track a subject in that arena. For example, when the subject that was planned in that arena is no longer available.
- **Skipped**. The status **Skipped** is given to an arena that was previously set to **To skip**. The status **Skipped** is therefore a label given *after* the trial has been acquired (or at least video was recorded for that trial). See also Skip arenas within a trial.
- **Postponed**. This label appears when you choose to record video and track later (see Record video, then acquire a trial). After recording video, the arenas within a trial get the status **Postponed**. After you do tracking from that video, the status changes to **Acquired**.
- **Postponed To Skip**. If you have recorded video without tracking the status of the trials/arenas in the Trial List is **Postponed** (see above). Now you can choose to skip one of the trials/arenas by selecting **Postponed to Skip**. Do this for example if one of the arenas in the video is empty.

- **Waiting**. An arena gets the status **Waiting** when you have started the trial but acquisition did not yet start because you defined a Trial Control profile with a Start recording rule and the starting condition is not yet met.
- **Acquiring**. An arena gets the status **Acquiring** when acquisition is ongoing.
- **Acquired**. An arena gets the status **Acquired** when data has been acquired for that arena.




*Arena name*

The name of the Arena in which the animal was tracked.

*Arena Settings*

The name of the Arena Settings used to acquire your tracks.

*Detection settings*

The name of the Detection Settings used to acquire your tracks.

*Interpolated samples*

The proportion of samples that were interpolated during the trial, expressed in percentage. Interpolation occurs when the camera fails to send the video frames at the expected time. For details, see in Detection settings: Advanced setup > Dropped frames correction.

### *Missed Samples*

The proportion of missed samples, expressed in percentage. EthoVision misses samples if the processor load is too high or the camera delivers fewer video frames than expected. See also Missed samples

### *Recording after*

The length of the interval between the start of the trial and the start of acquisition. This variable has the value 0 unless you defined a condition between the Start trial box and the Start track box in the Start-stop trial rule in the Trial Control settings.

### *Recording duration*

The length of the interval from start of acquisition till stop of acquisition. Recording duration is equal to Trial duration unless you defined a condition between the Start trial box and the Start track box in the Start-stop trial rule in the Trial Control settings.

### *Start time*

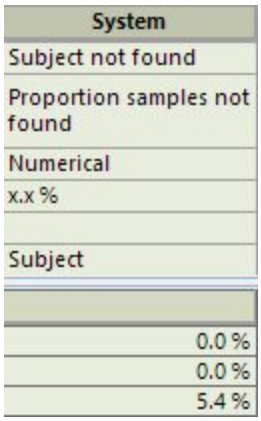
The date/time of the start of the trial.

### *Subject name*

The name of the subject that track refers to. Subject names are specified in the Experiment Settings.

### *Subject not found*

Proportion of time the subject was not found. If the subject is not found, it means EthoVision processed the sample but did not find anything matching the current Detection Settings. Therefore, this system variable is a measure of the quality of detection.




### *Sync status*

The status of the sync-out file. When no external data co-acquisition has been carried out for a specific trial, the status is Planned. As soon as co-acquisition is started on the DAQ system, the status becomes Acquired.

### *Track*

The name of the track file.

### *Tracking source*

The video input on the encoder board connected to the camera. If you use a digital camera, this is the name of the camera. The cell is empty when you track from a video file.

### *Trial Control settings*

The name of the Trial Control profile used during tracking.

### *Trial Duration*

The length of the interval from start of the trial until the stop of the trial.

### *Trial name*

By default, Trial 1, Trial 2, etc. To change a trial name, double-click it and enter a new name.

### *Video file*

The name and location of the video file that was used or will be used for acquisition.

*Video start time*

The date and time the video file that you used for tracking, was created.

## System variable format

For the Time stamp variables (Start time and Video start time) and Duration variables (Recording after, Recording duration and Trial Duration) you can specify the format.

Double-click in the **Format** field. In the Format string window, enter your preferred format in the field at the top and click **OK**.

For the other system variables Label, Description, Type, Predefined Values and Scope you cannot change their format.

*See also*

- The default Start-Stop Trial rule

# Prepare the list of trials

## What do you want to do?

- Plan your trials
- Edit the Trial List
- Skip trials
- Clear trials in the Trial List
- Prepare the trial list for batch acquisition
- Import data from other experiments

  **NOTE** This option is for when you want to merge data from multiple identical experiments.

# Plan your trials

## Aim

- To prepare the list of experimental trials in detail and in advance, so that fewer actions have to be taken during their execution.
- Planning trials in the Trial List also allows you to run a batch acquisition. Select video files for all the trials in your trial list. For each trial you can optionally select separate Arena Settings, Trial Control Settings, and Detection settings. Then you can automatically acquire all trials.

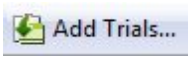
**NOTE** If you do not predefine a trial list, it is built up automatically as you acquire new trials.

**EXAMPLE** Define the independent variables *ID* and *Dose* and fill in the list of trials with the ID of the subjects to be tested, and the drug amount planned for each trial. When you start data acquisition, EthoVision XT informs you that for Trial 1 you need to prepare subject A21034 which has to be injected with 1.0 mg/kg apomorphine.

## To define the list of trials

1. Choose **Setup** > **Trial List**, or in the Experiment Explorer click **Trial List**.
2. To add trials, do one of the following:

   *If you track live or from videos*, click the **Add Trials** button on the toolbar, then enter the number of new trials you want to add.

   *If you track from videos*, you can add trials automatically based on the video files you select. Each video will be associated with one trial. Click the **Add Videos** button on the toolbar, and select your videos. See also Automatically add video files to the Trial List.

3. A number of rows appear in the lower half of the Trial List. Each row represents a trial, that is, an acquisition session to be carried out in one or more arenas. If your setup is made of multiple arenas, each trial is divided in multiple rows, each one representing a track, that is, data of one individual subject tracked in a single trial.
4. For each trial, enter the value of the independent variables in the corresponding cells, when required.

5. For each trial, select the Arena Settings, the Trial Control Settings and the Detection Settings you want to use.

| | | | | System | System | System | System |
|---|---|---|---|---|---|---|---|
| Label | | | | Acquisition sta | Arena settings | Trial Control settings | Detection settings |
| Description | | | | The current status of acquisition per arena | The arena settings used for acquisition | The trial control settings used for acquisition | The detection settings used for acquisition |
| Type | | | | | | | |
| Format | | | | | | | |
| Predefined Values | | | | | | | |
| Scope | | | | Arena | Trial | Trial | Trial |
| Trial | | Arena | Subject | No. | | | |
| Trial | 1 | Arena 1 | Subject 1 | 1 | Planned | Training | Track for one minute | Detection Settings 1 |
| Trial | 2 | Arena 1 | Subject 1 | 2 | Planned | Training | Track for one minute | Detection Settings 1 |
| Trial | 3 | Arena 1 | Subject 1 | 3 | Planned | Training | Track for one minute | Detection Settings 1 |
| Trial | 4 | Arena 1 | Subject 1 | 4 | Planned | Training | Track for one minute | Detection Settings 1 |
| Trial | 5 | Arena 1 | Subject 1 | 5 | Planned | Probe | Track as long as video plays | Detection Settings 1 |

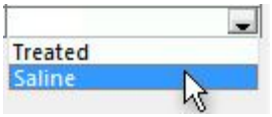
## Notes

- **TIP** If you do not see the columns Arena Settings, Trial Control Settings and Detection Settings, click the **Show/Hide** button on the toolbar, select **Variables** and under **System** select the items you want to view.
- You can also edit the Trial List before, during or after acquisition. See Edit the Trial List
- If trials are already present in the table, the trials you add are appended.

# Edit the Trial List

## Enter values in the Trial List

Click a cell in the Trial List and enter the value you require, or select it from the list of values available.




Filling the Trial List table is not mandatory. You can also enter those values in the data acquisition screen.

## Delete values from the trial list

Select the appropriate cell and click **Delete**. Or, right-click the appropriate cell and select **Delete**.

To delete trials, see Delete trials or Clear trials in the Trial List

## Copy and paste values

You can copy and paste (part of) the Trial List:

- To/from Excel. You can copy a list of trials from Excel, for example, when you have made your list of independent variables and its values. You can copy/ paste multiple columns but make sure the columns are in the exact same order as in the Trial List.
- Within the same Trial list. You can copy and paste part of Trial List to newly added cells, for example, when you have added new trials.

Do the following:

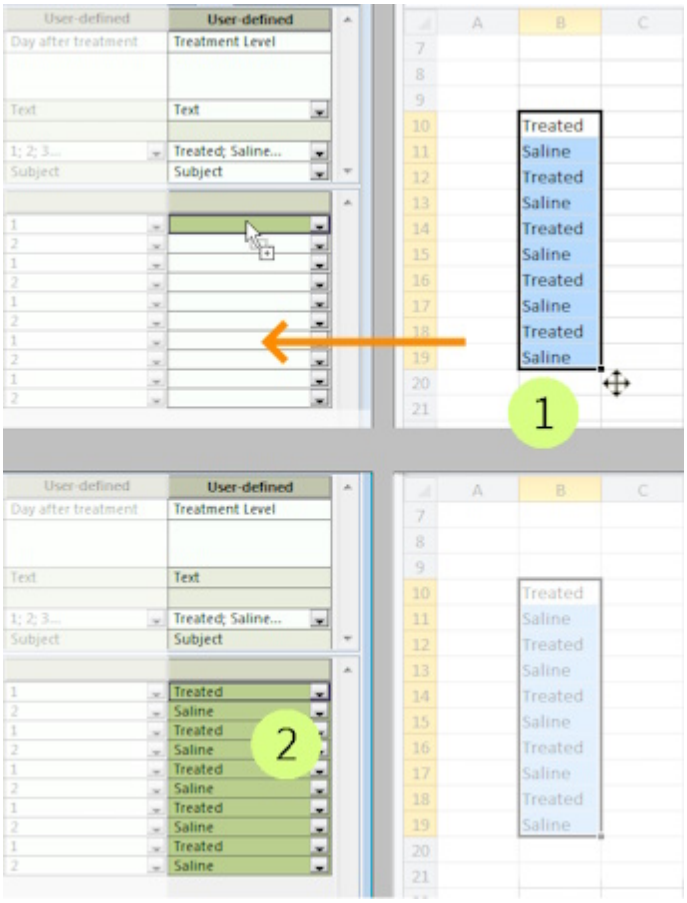
1. Select one or more cells in the Trial List or in the Excel worksheet.
2. Right-click one of the selected cells and select **Copy** or press **Ctrl+C**.
3. Click a cell in the Trial List or in the Excel worksheet and either right-click and select **Paste** or press **Ctrl+V**.

You can also select multiple adjacent cells to copy to. Click the first cell of the range, hold down **Shift** and click the last cell, then paste.

You can also select nonadjacent cells using the **Shift** and **Ctrl** key. It is only possible to paste in such a selection if there is just a single value on the clipboard.

## Drag and drop cell values from Excel

You can also drag and drop cells from an Excel worksheet. Drag the cells to the first cell in the Trial List where you want the values to be copied, then release the mouse button.




## Notes

- A value can only be pasted if the format of the value corresponds to the format required by the cell. For instance, it is not possible to paste a cell with a non-numerical independent variable to a cell with a numerical variable. If you do that, you will get a warning. The existing cell value remains unchanged.
- Check that the copied values of numerical independent variables are either within the predefined range or correspond to individual predefined values of numerical independent variables in the Trial list.
- You can change the order of variables by selecting a column and dragging it to the new position.

# Skip trials

## Aim

To skip one or more of the planned trials because of unforeseen circumstances. For instance, when a subject is no longer available.

You can skip:

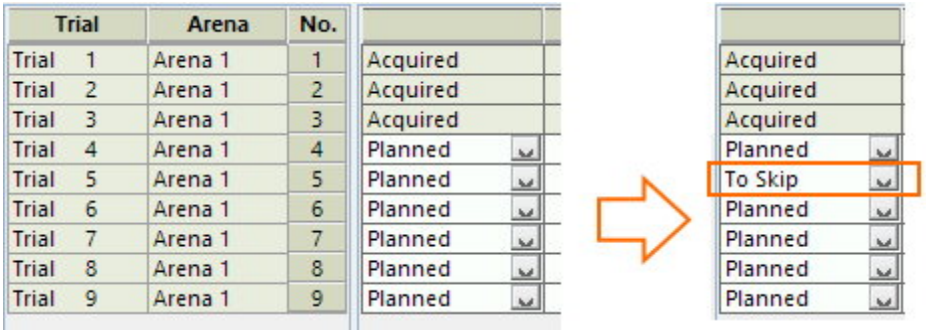
- An entire trial. See the procedure below.

  **EXAMPLE** You track mice in an open field. For trial 5, the subject is no longer available. That means that after acquiring trial 4 you want to proceed with trial 6.

- One or more arenas within a trial. See Skip arenas within a trial

## Procedure

1. Open the Trial List, and click **Show/Hide** > **Variable** > select **Acquisition status**.
2. In the **Acquisition status** column select **To Skip** for the corresponding trial.




3. Proceed with acquiring your trials. If for any reason you want to acquire the trial set to **To Skip**, set it back to **Planned** in the Trial List and open the Acquisition screen.

## Notes

- You can also skip a trial in the Acquisition screen. When the text on the video window shows the name of the trial that you want to skip, click the **Skip trial** button. EthoVision is ready to acquire the next planned trial.

- If your trials include multiple arenas, and you want to skip an entire trial, select **To Skip** for all the arenas in that trial. Otherwise you can skip specific arenas within a trial. See Skip arenas within a trial
- If you have recorded video and you want to skip trials/arenas before acquiring data, in the Trial List change the status from **Postponed** to **Postponed To Skip**.

# Skip arenas within a trial

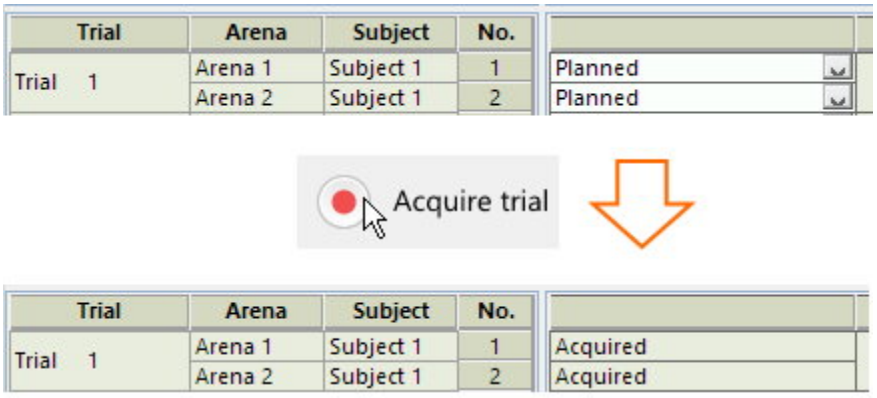
## Aim

To skip one or more of the arenas within a trial because those arenas are empty or the subjects there should not be tracked.

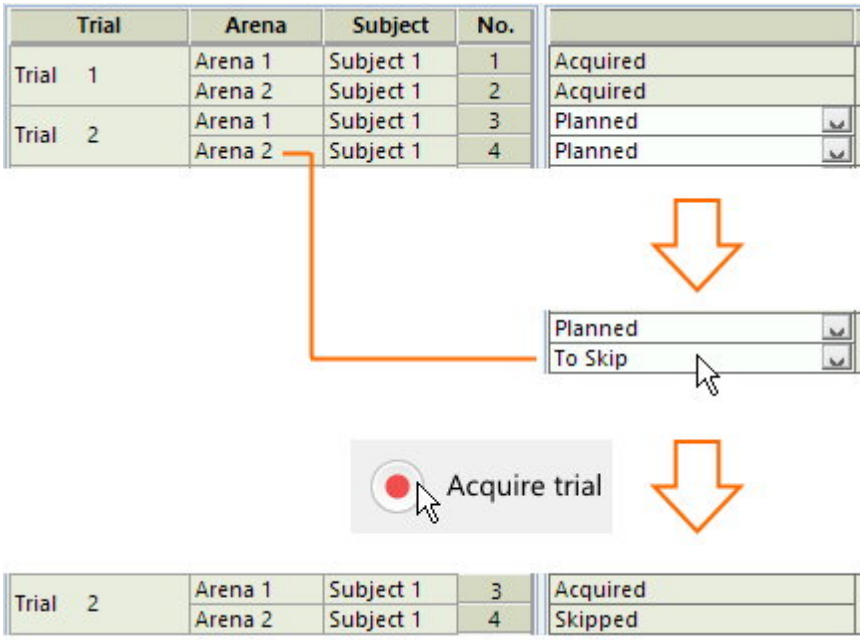
**EXAMPLE** You track fish larvae in a well plate with 24 wells. Therefore, each trial contains 24 arenas. When some wells are empty, it does not make sense to do tracking in empty arenas. Therefore, skip those arenas before acquiring the trial.

## Background information

- Before you acquire a trial which includes two or more arenas, all arenas are set to Planned. After acquiring the trial, they are all set to **Acquired**.




- The **To Skip** option in the Trial List allows you to skip some arenas in a specific trial, so you can proceed with tracking in the remaining arenas. For example, in Trial 2, Arena 2 is empty, so you want to skip Arena 2. After you acquire Trial 2, Arena 1 is set to **Acquired** and Arena 2 is set automatically to **Skipped**.




## Procedure

1. Open the Trial List, and click **Show/Hide** > **Variable** > select **Acquisition status**.
2. In the **Acquisition status** column select **To Skip** for the corresponding arena.
3. Proceed with acquiring your trials.

## Notes

- When you skip arenas within a trial, only the subjects in the non-skipped arenas are tracked.
- You can clear a trial with one or more skipped arenas. After clearing a trial, all arenas are set to **Planned** (or **Postponed** if you chose to record video and track later).

# Delete trials

## Aim

To remove the trial from the Trial List and the experiment. This includes:

- The track data
- The manually-scored data
- The Trial Control events
- The Hardware log
- The video file (when requested)

**Delete Trials** also removes the corresponding row from the trial list.

## Procedure

1. Do one of the following:
   - Click the trial name in the list, press **Delete**.
   - Right-click the trial name in the list, select **Delete Trials**.
2. If the trial is associated to a video file recorded with EthoVision XT, you are asked whether you want to rename it for backup purposes, or delete it. Choose the option that applies and click **Yes**.

## Notes

- A video file is renamed with a number 0001 appended to its original name. The renamed video file is kept in the Media Files folder of the experiment.
- With Clear Trials you can remove the tracks, keep the row empty and when necessary acquire that trial again. See Clear trials in the Trial List and Redo a trial

*See also*

- Clear trials in the Trial List

# Clear trials in the Trial List

## Aim

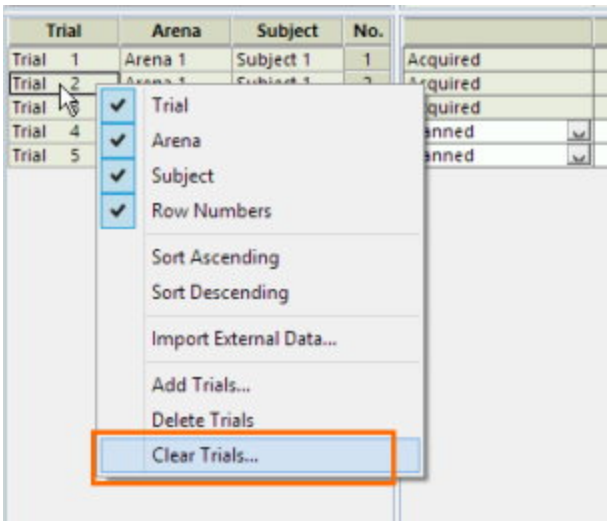
To delete the tracks, the associated data and the recorded videos of one or more trials, but leave those trials as empty rows in the Trial List, so you can redo those trials at later time.

**IMPORTANT** When you clear a trial, the data scored manually (Manual Scoring log), the events of Trial Control and the hardware events associated with the trial are deleted.

**NOTE** *Clear Trials* is not the same as Delete trials. When you delete a trial, the tracks are deleted and the corresponding row is removed from the Trial List.

## Procedure

1. Right-click the name of the trial that you want to clear and select **Clear Trials**.




2. Choose one of the options that appear. These depend on whether you tracked from external video files or live (+save video).

   When tracking from external video files:

   - **Clear tracks**. The tracks are deleted. In the Trial list, the link to the video file used for tracking located in the **System - Video file** column is removed. Choose this option if you want to re-do the trial using a video file different from the current one.

- **Clear tracks but re-use the video reference**. As above, however the link to the video file is retained. You can open Acquisition to track from that video file.

When tracking live (and optionally saving video):

- **Clear tracks**. The tracks are deleted. This option is available if you acquired tracks without saving video.
- **Clear tracks but re-use the video**. To delete the tracks, not the video file used to acquire those tracks. You can then track again from the video file.
- **Clear tracks and video**. To delete the tracks and the associated video and redo the trial anew.
- **Clear video**. To clear a trial that has video only (its current status is Postponed). You can then record a new video and acquire tracks.

## Notes

- When you clear a trial, the Arena Settings, Trial Control Settings and Detection Settings that were selected in the Trial List are retained.

*See also*

- Redo a trial
- Skip trials
- Skip arenas within a trial

# Prepare the trial list for batch acquisition

## Aim

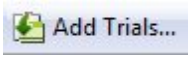
To make a list of the trials to be acquired in batch, either live or from video files.

## Prerequisites

- Click the **Show/Hide** button on the toolbar and select **Variables**. When you track from pre-recorded video files, make sure that Video file is selected.
- Optionally select to display Arena settings, Trial Control settings and Detection settings.

## Prepare the Trial List for batch acquisition

1. Add the trials you need.

2. **OPTIONAL** If you defined multiple Arena Settings, Trial Control settings or Detection settings: for each trial select which settings you want to use. Either select settings for all the trials or for none at all.

   **TIP** If you want to use the same Arena Settings, Trial Control settings or Detection Settings for all the trials, select them in the Acquisition Settings window.

3. Add video files to the planned trials, either automatically or manually. See the options below.

   You can either select different video files for different trials or the same video for multiple trials. Choose the latter way of working if you made a long video with multiple trial recordings. If you choose the same video, EthoVision will start tracking at that point in the video where the previous trial stopped. To make sure that tracking starts and stops at the correct time point, you must define appropriate Trial Control settings.

## Automatically add video files to the Trial List

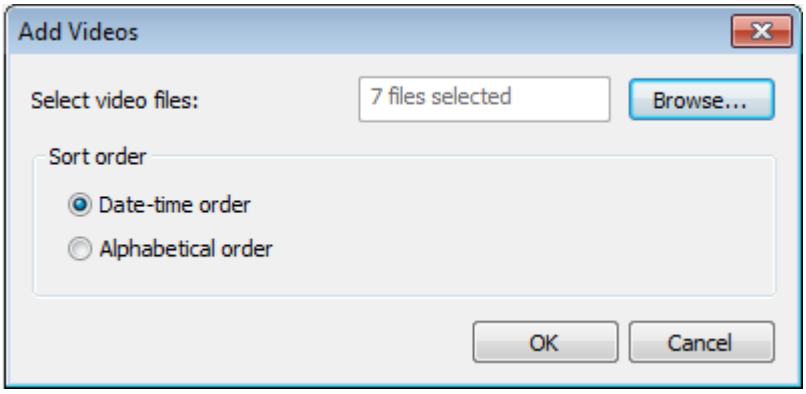
1. Click the **Add Videos** button on the toolbar.

2. In the Add Videos window, select the video files you want to use and click **Open**.
3. Select one of the options under **Sort order** and click **OK**:

   **Date-time order**. Selecting this option links the video file with the earliest date/timestamp to Trial 1, the next video file to Trial 2, etc.

   **Alphabetical order**. Selecting this option links the video files to the trials in alphabetical order.




## Manually add video files to the trial list

Click one of the cells in the **Video file** column. In the Select Video window, select the video file and click **Open**. Do this for the other planned trials.

## Notes

- If you select more video files than the number of planned trials in the Trial List, EthoVision will add additional trials to the Trial List.
- If you manually linked videos to some of the planned trials, and for the remaining trials you use the automatic option, EthoVision only fills the gaps, that is, it adds videos to those trials which do not have video references yet.

## To carry out batch acquisition

Choose **Acquisition** > **Open Acquisition**. In the Acquisition Settings window, select **Track all planned trials**.

*See also*

- Acquire a series of trials (Batch acquisition)

# Acquire Data

## Main topics and tasks



**NOTE** If you plan to use hardware devices like a pellet dispenser or the PhenoTyper, see also the EthoVision XT 18 - Trial and Hardware Control - Reference Manual, which you can find on the **Apps** screen, under **Noldus** > **EthoVision XT 18 Other Documentation**.

# Introduction to Data Acquisition

## Learn about

- Important terms in acquisition
- Important things to know about data acquisition

# Important terms in acquisition

## Trial vs. track

A trial is a single acquisition session. A track is the data collected during a trial. A trial can contain one or more tracks. See Trial vs. track on page 286.

## Batch acquisition

Acquisition of a series of trials, either live, or from pre-recorded video files.

## Inter-trial interval

When you choose a live series of trials, an inter-trial interval between two consecutive trials allows you to take the subject from the maze or cage, clean the maze/cage and release the next subject before the next trial starts automatically.

## Planned trial

When you add trials in your Trial List, their default acquisition status is Planned. EthoVision XT acquires the planned trials in the order specified in the Trial List: Trial 1, Trial 2, etc.

You can skip a planned trial whenever you cannot acquire data, for example when the subject assigned to that trial is no longer available (see below).

## Skipping a trial

It may happen that a trial planned in the Trial List cannot be acquired, for example when the animal to be tested is not available. You can skip one or more trials and acquire a trial further down in the Trial List.

See Skip trials

When you skip a trial (and all arenas within it), you can always set it back to **Planned** to acquire data at a later time. See Carry out trials in an order different from that specified in the trial list. Furthermore, you can also skip single arenas within a trial, for example not to acquire data in empty wells in a well plate. See Skip arenas within a trial

## Start-Stop Trial rule

This is the rule in the Trial Control Settings that determines when actual tracking (data acquisition) starts and stops. For example, start tracking when the animal is detected in the arena. See The default Start-Stop Trial rule.

# Important things to know about data acquisition

## Four methods to acquire data

Choose the method that best suits your setup and experimental protocol:

- *Acquire data live*. The animals are tracked as they move in your experimental setup.

  The disadvantage of this method is that you do not have a video backup, so if you need to repeat data acquisition you have to repeat the test itself.

  See Acquire a trial live or Acquire a series of trials - live.

- *Acquire data live and save video simultaneously*. Like above, in addition EthoVision XT records video to a digital video file. You can use the video to re-do tracking if needed.

  See Acquire a trial live + save video or Acquire a series of trials live + save video

- *Record video with EthoVision XT, then acquire data*. This is a two-steps method. First, record video to a digital video file as the animals move in your experimental setup. Afterwards, open the video file and acquire the data. This way you are free to track data later. See also Advantages of using video files below.

  See Record video, then acquire a trial, or Record a series of videos, then acquire a series of trials.

- *Acquire data from video files recorded with other programs*. Use an external program, like MediaRecorder, to record video to a file while the animals move in the experimental setup. Later on, open the video file and acquire the data.

  See Acquire a trial from a video file recorded with other programs, or Acquire a series of trials from video recorded with other programs.

  See also Video file formats.

## Advantages of using video files

- You have a video backup. A video file is very handy since it allows you to acquire data for a trial once again, if needed. For example, if you realize that the detection settings were not optimal when you acquired the data. See Redo a trial on page 530.

- You make sure that tracking is reliable. When you track from a video file, let EthoVision XT take as much time as it needs to analyze each sample, independent of the actual frame rate. For example, if the detection method used requires a lot of time per sample, acquisition may be slower than real time, but you ensure that no sample is missed. See DDS (Detection determines speed) on page 586.

## Settings used for data acquisition

You can view which settings were used for a particular trial.

In the Experiment Explorer, click **Trial List**. Click the **Show/Hide** button on the toolbar and in the Show Variables window, select **Arena Settings**, **Detection Settings** and **Trial Control Settings**.

In the Experiment Explorer, settings profiles are marked with a lock icon, meaning that they cannot be edited. To edit a settings profile, make a copy.

## To view your trials

Choose **Setup** > **Trial List**. Click the **Show/Hide** button on the toolbar. In the Show Variables window, select **Acquisition status**. For an explanation of Acquisition status, see System variables on page 477.

## Correct tracking errors

Good detection settings do not generally result in tracking errors. Before editing tracks, make sure that the lighting conditions and the detection settings are optimized.

See Edit the Tracks

# Acquire one trial

## Learn about

- Before you start acquisition

## What do you want to do?

- Acquire a trial live
- Acquire a trial live + save video
- Record video, then acquire a trial
- Acquire a trial from a video file recorded with other programs

*See also*

- Acquire a series of trials (Batch acquisition)

# Before you start acquisition

## Screen saver and power options

Turn off the screen saver and make sure that the power save options of your computer are turned off.

## Video cameras and video files

Make sure that the video cameras are connected to your PC's encoder board (for analog cameras) or USB/Ethernet ports (for digital cameras). See Camera Installation

## Use the correct settings

Make sure that the Arena Settings, Trial Control Settings and Detection Settings you want to apply are selected in the Acquisition Settings window.

## Enter the independent variables for the next trial

In the Analysis Results and Scoring pane, click the **Independent Variables** tab and enter or select the values of the independent variables in the row highlighted in green. You can enter independent variables values at any time in the Trial List. See Independent Variables tab

## When co-acquiring external (physiological) data

If you want to acquire external data during the trial (only live, not from video files), make sure that the external data recording device is switched on and connected to the EthoVision XT computer. In EthoVision XT, in the Experiment Settings under **Video Source** select **Enable DAQ co-acquisition**. See also External Data.

**NOTE** You must have the External Data Module in order to use this functionality.

## Auto-start data analysis

If you want to start analysis immediately after finishing the trial (or series of trials, in the Acquisition Settings window select **Auto-start data analysis**.

## To speed up analysis

- Hide the Analysis Results and Scoring pane. To do this, click the **Show/Hide** button and then deselect **Analysis Results and Scoring**.

- For tracking offline: Select **DDS** in the Playback Control window. This way EthoVision XT plays the video faster than 1x if subject detection requires little processor time.

# Acquire a trial live

## Aim

To carry out video tracking in a live situation. Video is not recorded.

## Prerequisites

1. in the Experiment Settings, under **Video Source** select **Live tracking**.
2. Choose **Acquisition** > **Open Acquisition**. In the Acquisition Settings window, select **Track next planned trial**; do not select **Save video**.
3. Make sure at least one trial is planned. To add a new trial, in the Playback control window click the **New trial** button or press **Ctrl+F3**.

4. **IMPORTANT** Make sure that video resolution, frame rate and duration do not exceeds the values reported in Resolution, frame rate, and maximum trial duration.

## Procedure

1. Choose **Acquisition** > **Start Trial**, or press **Ctrl+F5**, or click the **Start trial** button in the Playback control window.

2. Release the animal in the arena. As soon as data recording starts, the Video window says **Acquiring**.
3. The trial stops when the condition before the Stop track box is met, or when you choose **Acquisition** > **Stop Trial**, or press **Ctrl+F6**, or click the **Stop trial** button.

## What next?

- If you need to enter values of independent variables, click the Independent Variables tab in the Analysis Results and Scoring pane. Edit the values where needed.

- Remove the animal from the arena and repeat the procedure with the next trial.

# Acquire a trial live + save video

## Aim

To carry out video tracking in a live situation. Video is recorded for backup purposes. This is also useful when one needs to re-do tracking.

## Prerequisites

1. in the Experiment Settings, under **Video source** select **Live tracking**. Choose **Acquisition** > **Open Acquisition**. In the Acquisition Settings window, select both **Track next planned trial** and **Save video**.
2. Make sure at least one trial is planned. To add a new trial, in the Playback control window click the **New trial** button or press **Ctrl+F3**.

3. **IMPORTANT** Make sure that video resolution, frame rate and duration do not exceeds the values reported in Resolution, frame rate, and maximum trial duration.

## Procedure

1. Choose **Acquisition** > **Start Trial**, or press **Ctrl+F5**, or click the **Start trial** button in the Playback control window.

EthoVision XT starts recording video, and waits that the condition to start tracking is met.

2. Release the animal in the arena.
3. The trial stops when the condition before the Stop track box is met, or when you choose **Acquisition** > **Stop Trial**, or press **Ctrl+F6**, or click the **Stop trial** button.

Video recording stops at the same time.

## What next?

- If you need to enter values of independent variables, click the **Independent Variables** tab in the Analysis Results and Scoring pane. Edit the values where needed.
- Remove the animal from the arena and proceed with the next trial.

# Record video, then acquire a trial

## Aim

To record video while the test is carried out. Video tracking is done at a later stage.

**IMPORTANT** With this method, the Trial Control rule is applied in the second stage (acquire trial), not during the first stage (save video). To stop video recording, after a fixed time, do so manually (see below) or see the suggestions in Stop video recording after some time.

## Prerequisites

1. in the Experiment Settings, under **Video source** select **Live tracking**.
2. Choose **Acquisition** > **Open Acquisition**. In the Acquisition Settings window, select **Save video only, track later**.
3. Make sure at least one trial is planned. To add a new trial, in the Playback control window click the **New trial** button or press **Ctrl**+**F3**.

4. **IMPORTANT** Make sure that video resolution, frame rate and duration do not exceeds the values reported in Resolution, frame rate, and maximum trial duration.

## Procedure

*Record video*

1. Choose **Acquisition** > **Start Trial**, or press **Ctrl**+**F5**, or click the **Start trial** button in the Playback Control window.

The Video window shows a message **Recording video**.

2. To stop video recording, choose **Acquisition** > **Stop Trial**, or press **Ctrl**+**F6**, or click the **Stop trial** button in the Playback Control window.

Remove the animal from the arena. You can now proceed with the next subject or track the data from the video just saved (see below).

*Acquire the trial from the recorded video*

1. In the Acquisition Settings window, select **Track next saved video file**. The trial name shown next to Trial refers to the first saved video in your Trial List that you still have to track from.
2. Position the video at the point you require.

3. Select **DDS** in the Playback Control window to make sure that samples are not missed. See DDS (Detection determines speed) on page 586
4. Choose **Acquisition** > **Start Trial**, or press **Ctrl+F5**, or click the **Start trial** button in the Playback Control window.

The video is played forward and tracking takes place.

5. Acquisition stops automatically based on the Trial Control Settings, or when the video file has reached the end, or when you choose **Acquisition** > **Stop Trial**, or press **Ctrl+F6**, or click the **Stop trial** button.

## What next?

- If you need to enter values of independent variables, click the I**ndependent Variables** tab in the Analysis Results and Scoring pane. Edit the values where needed.
- If you wish to discard the track and re-do tracking from that video, see Redo a trial on page 530.

# Acquire a trial from a video file recorded with other programs

## Aim

To carry out video tracking from a video file recorded with a program other than EthoVision XT.

## Prerequisites

1. The video file is compatible with EthoVision XT. See Video file formats.
2. In the Experiment Settings, under **Video Source**, select **From video file**.
3. In the Arena Settings, Check the video aspect ratio.
4. **OPTIONAL** Make a Trial List and add a video to each trial.
5. Choose **Acquisition** > **Open Acquisition**. If no trial is planned, to add a new trial, in the Playback control window click the **New trial** button or press **Ctrl+F3**.

## To acquire a trial from a video file

1. In the Acquisition Settings window select **Track next planned trial**.
2. in the Acquisition Settings window, under **Settings** click the **Video** button.

Select the video file you require. To have files of all formats listed, select **All files** from the Files of type list.

3. Position the video at the point you require.

4. Select **DDS** in the Playback Control window to make sure that samples are not missed. See DDS (Detection determines speed) for more information.
5. To start the trial, choose **Acquisition** > **Start Trial**, or press **Ctrl+F5**, or click the **Start trial** button in the Playback Control window.

The video is played forward and tracking takes place.

6. Acquisition stops automatically when the video file has reached the end, or when the condition before the Start track box in the Trial Control rule is met, or when you choose **Acquisition** > **Stop Trial**, or press **Ctrl+F6**, or click the **Stop trial** button.

## What next?

- If you need to enter values of independent variables, click the Independent Variables tab in the Analysis Results and Scoring pane. Edit the values where needed.
- You cannot Redo a trial obtained with this acquisition method.

# Acquire a series of trials (Batch acquisition)

## What do you want to do?

- Acquire a series of trials - live
- Acquire a series of trials live + save video
- Record a series of videos, then acquire a series of trials
- Acquire a series of trials from video recorded with other programs

## Learn about

- Choosing the optimal reference image in a series of trials

*See also*

- Acquire one trial
- Split a multi-day test in multiple trials

# Acquire a series of trials - live

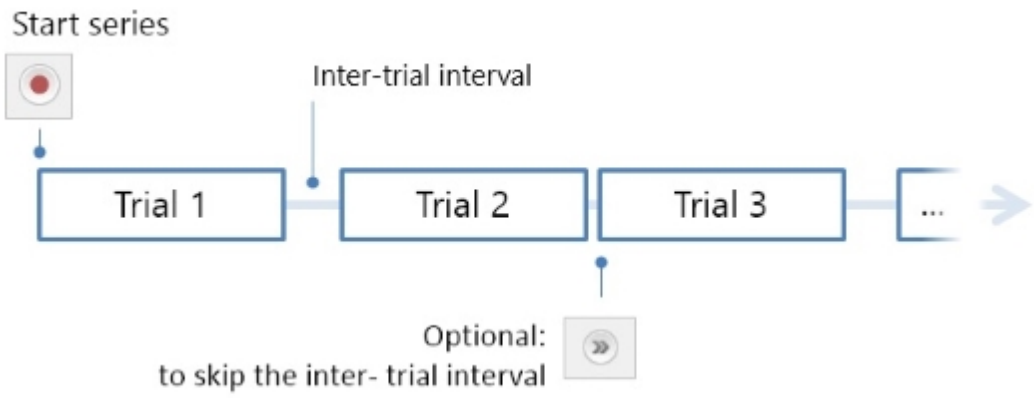
## Aim

To carry out video tracking in a live situation, for a number of times (trials), in a predefined list. Video is not recorded.

## Trials and inter-trial intervals

In the following example, the duration of the trials is assumed to be constant (for example, 5-minutes trials in an open field). Click the **Start series** button to start acquisition. The inter-trial interval is not applied before the start of the first trial. You specify the inter-trial interval in the Acquisition Settings window.

If you want to start the next trial before that end of the inter-trial interval, click the **Skip countdown** button.

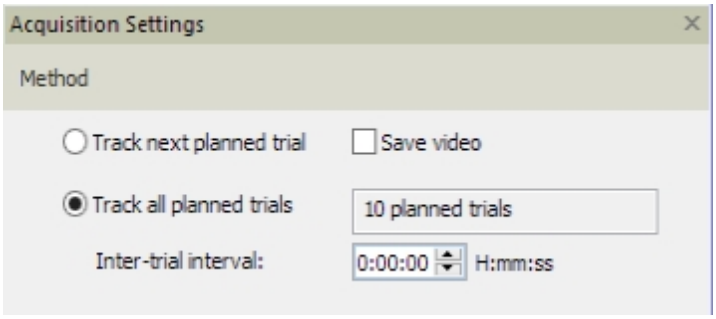



**IMPORTANT** The serial acquisition is interrupted automatically when the available disk space gets lower than 100 MB. EthoVision XT checks the available disk space before starting a new trial. Close the experiment, clean up some disk space, and then resume acquisition.

## Prerequisites

1. See also Before you start acquisition.
2. In the Experiment Settings, under **Video Source** select **Live tracking**.
3. Make sure that the Trial list includes all trials that you plan to acquire. If necessary, open the Trial List and add trials. Select a combination of Arena Settings, Trial Control Settings and Detection Settings for each planned trial.

4. Choose **Acquisition** > **Open Acquisition**. In the Acquisition Settings window, select **Track all planned trials** and specify the **Inter-trial interval** (H:mm:ss).



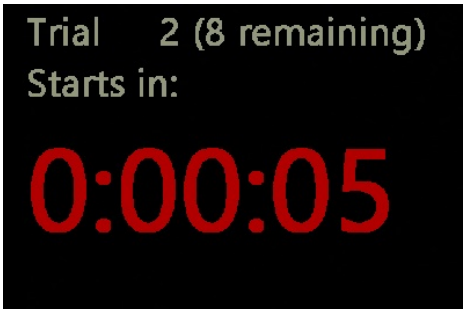

   The name of the first planned trial in the Trial List appears in the field next to Trial.

## Procedure

1. Choose **Acquisition** > **Start Trial**, or press **Ctrl+F5**, or click the **Start series** button in the Playback Control window.

2. Release the animal for the first trial in the arena.
3. The trial is stopped automatically according to the rules set in the Trial Control Settings. Remove the animal from the arena, clean the experiment setup and prepare the next animal.

   The video window shows the countdown (inter-trial interval).




4. At the end of the countdown, the next trial starts. Release the animal in the arena.
5. To stop the series of trials:

   The series stops automatically when the last planned trial has been tracked.

You can stop the series manually at any time. Choose **Acquisition** > **Stop Trial**, or press **Ctrl**+**F6**, or click the **Stop series** button in the Playback Control window.

If you stop batch acquisition manually, all trials acquired up to that time are saved, including the current one that was interrupted. Click the **Start series** button again to complete the series. If necessary, redo the trial that was interrupted. See Redo a trial.

## Notes

- If cleaning the experiment setup takes less time than the Inter-trial interval, and you do not want to wait until the end of the countdown, skip the remaining time and start the next trial. Click the **Skip interval and Start trial** button (or press **Ctrl+F5**).

- During the inter-trial interval, you can view information on when the next trial starts. In the Acquisition Settings window, next to **Track all planned trials** you see **[name of next trial] starts in [H:mm:ss]**.

  In the Video window you see **[name of next trial] (Number of trials remaining) Starts in [H:mm:ss]** (the last, in red). This text is always shown, even when you specify to hide the overlay text.

- Automatic analysis does not start if you stop the series manually.
- If you save video while tracking, saving the video at the end of each trial takes time. The Inter-trial interval countdown only starts after the video has been saved. For this reason, the total time between the end of the i-th trial and the start of the i+1-th trial may be longer than the inter-trial interval.

# Acquire a series of trials live + save video

## Aim

To carry out video tracking in a live situation, for a number of times (trials), in a predefined list. Video is recorded.

## Prerequisites

1. For general recommendations, see Acquire a series of trials - live.
2. In the Acquisition Settings window, select both **Track all planned trials** and **Save video**.

## To acquire a series of trials live + video

See the procedure Acquire a series of trials - live on page 514.

The difference is that:

- Video for the current trial is being recorded to a file on the hard disk. Video recording starts at the start of the trial, not at the start of tracking.
- For each trial, video recording is stopped before the start of the inter-trial interval.
- Saving the video at the end of each trial takes time. The **Inter-trial interval** countdown only starts after the video has been saved. For this reason, the total time between the end of the i-th trial and the start of the i+1-th trial may be longer than the inter-trial interval.

# Record a series of videos, then acquire a series of trials

## Aim

To record a number of video files while the tests are carried out. Video tracking is done in a later phase.

**IMPORTANT** With this method, Trial Control is not applied during video recording. This means that for example you cannot stop video recording automatically after 10 minutes. To stop video recording, do so manually (see below). Trial Control is applied in the second phase (acquire trial).

## Prerequisites

1. For general recommendations, see Acquire a series of trials - live.
2. Choose **Acquisition** > **Open Acquisition**. In the Acquisition Settings window, select **Save video only, track later**.
3. If no trials are planned, click the **New trial** button in the Playback Control window.

## Procedure

*Record a series of video files*

1. Choose **Acquisition** > **Start Trial**, or press **Ctrl+F5**, or click the **Start trial** button in the Playback Control window.

   The Video window says **Recording video**.

2. Release the animal in the arena.
3. To stop video recording, choose **Acquisition** > **Stop Trial**, or press **Ctrl+F6**, or click the **Stop trial** button in the Playback Control window.

Repeat the procedure for more video files.

*Acquire a series of trials from the video files*

1. In the Acquisition Settings window, select **Track all saved video files**.
2. Make sure that the video file is positioned at its beginning or, in any case, at the point where you want to start data acquisition.

   **OPTIONAL** Select **Detection determines speed** in the Playback Control window to make sure that samples are not missed. See DDS (Detection determines speed) for the advantages of applying this option.
3. Choose **Acquisition** > **Start Trial**, or press **Ctrl+F5**, or click the **Start trial** button in the Playback Control window.

   The video is played forward and tracking takes place.
4. Acquisition stops automatically when the video file has reached the end and EthoVision XT automatically proceeds with the next saved video in your Trial List.
5. When all the videos have been analyzed, a message informs you that acquisition is done.

## Notes

- If you wish to discard the tracks and re-do tracking from that video, see Redo a trial.
- To make sure that tracking starts and stops at the correct moment in the video, you must define appropriate Trial Control settings.

# Acquire a series of trials from video recorded with other programs

## Aim

To carry out video tracking from a number of video files, recorded with a program other than EthoVision XT.

## Prerequisites

For general recommendations, see Acquire a trial from a video file recorded with other programs.

## Procedure

1. Release the animals in the arena and record video to a digital media file using the appropriate hardware and software. We recommend using MediaRecorder.

   At the end of this step you should have a number of video files.

2. Make a Trial List for batch acquisition and add one video for each trial in the list. See Prepare the trial list for batch acquisition.

3. Acquire a series of trials from the video files.

   (a) Choose **Acquisition** > **Open Acquisition**. In the Acquisition Settings window select **Track all planned trials**. The name of the first planned trial in the Trial List appears in the field next to Trial and the associated video file opens in the Video window.

   (b) Make sure that the video file is positioned at its beginning or, in any case, at the point where you want to start data acquisition. Select **DDS** in the Playback Control window to make sure that samples are not missed.

   (c) Choose **Acquisition** > **Start Trial**, or press **Ctrl+F5**, or click the **Start series** button in the Playback Control window.

## Notes

- To make sure that tracking starts and stops at the correct time point, you must define appropriate Trial Control settings.

- If you stop batch acquisition manually, all trials acquired up to that time are saved, including the current one that was interrupted. Click the **Start series** button again to complete the series. If necessary, redo the trial that was interrupted. Redo a trial
- If you wish to track data once again from that video, add a trial and follow the procedure for single trial acquisition See Acquire one trial.
- The lighting conditions could be different between the start of subsequent trials. When using subtraction of differencing for subject detection, you can update the reference image based on the first video frame. So you keep the reference image matched with the current conditions. See **Use first frame of each trial** in Optimize the reference image > The reference image

# Choosing the optimal reference image in a series of trials

## Background information

The background image is the image that the software compares with the incoming images from a camera (or video file) in order to find the subject. A good background image is critical for obtaining high quality tracking data. See The reference image

When you acquire multiple trials in a series, a reference image is used at the start of each new trial. Choosing the reference image is important especially when there is a long temporal gap between the end of one trial and the start of the next one. Follow the guidelines below.

This topic applies when:

- You use one of the detection methods Dynamic subtraction, Differencing, or DanioVision.
- You Acquire a series of trials (Batch acquisition), or carry out trials manually one after the other.

## Procedure

1. Create your series of trials in the Trial List. See Prepare the list of trials
2. In the Detection Settings, choose **Automated Setup** or choose the detection method.
3. Under **Method**, click the Background button. Obtain the reference image for the current video (or live image). See The reference image
4. In the Background Image window, under **Acquisition Settings**, choose one of the following:
    - **Use saved reference image**. The saved reference image is the image that you obtained in the previous step. The software will use this image at the start of each trial. Choose this option if you are 100% sure that the background does not change with time (or between video files). For example, when there is no bedding material, the background is lit uniformly, and the camera is always exactly in the same position relative to the arena.
    - **Use dynamic reference image**. As explained in How the reference image is updated in Dynamic subtraction, a dynamic reference image keeps changing during a trial. With this option, for each new trial, the software will use the most recent dynamic reference image available,

that is, the one that was created at the end of the previous trial. Choose this option if the live (or video file) image at the end of a trial is comparable to that at the start of the next trial. For example, when a trial starts a few seconds/minutes after the end of the previous trial.

- **Use first frame of each trial**. With this option, the software takes the first video frame available from the camera (or video file) and uses it as the reference image. Choose this option whenever you suspect that the video image at the end of a trial is likely to differ from that at the start of the next trial. For example when lighting conditions change, or the bedding material looks different between two trials. When you specify long inter-trial intervals, that is often the best option. See Trials and inter-trial intervals

## Notes

- Consider that the video image could also change between trials if the camera moves slightly relative to the camera. For example if you place a Petri dish under the camera not exactly on the same spot each time. This effect is more important when you track small animals. Any shift in the reference image, even a few pixels large, could affect detection in the next trial.
- You should not use the option **Use first frame of each trial** when the subject is already in the arena at the start of each trial. If you do so, EthoVision XT misses the subject in the first frames and finds it only when it moves away from the starting point.

# Additional information on data acquisition

## Learn about

- Working with trial control

## What do you want to do?

- Carry out trials in an order different from that specified in the trial list
- Acquire data for some arenas, not others
- Redo a trial
- Rename or delete a trial
- Rename or delete a generated video file
- Score behaviors manually during acquisition
- Use a remote control to start and stop acquisition
- Export track data live
- Auto-start data analysis
- Search the Troubleshooting: Data acquisition

# Working with trial control

## Starting data acquisition

- Data acquisition starts depending on when the condition in the Action box Start track is met. In a default DanioVision experiment, this condition is not present, so tracking starts as soon as you start the trial.
- If conditions to start data acquisition are never met within an arena, data are not recorded for that arena. A track file is created, but contains no track data.
- See The Start-Stop trial rule

## Stopping data acquisition

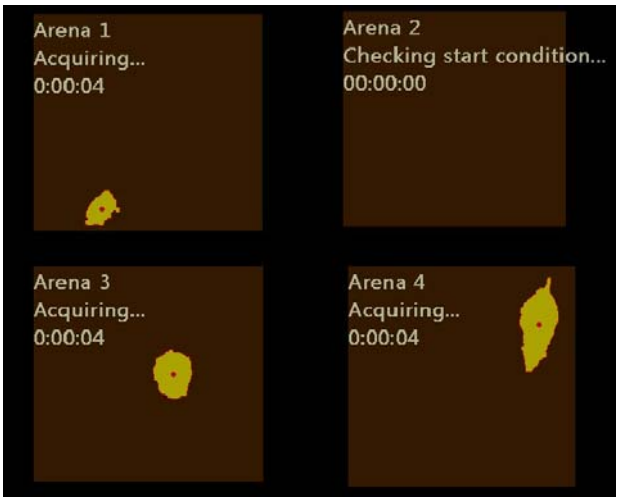
- If you use the default Trial Control profile, data acquisition stops when you give the Stop command (by clicking the **Stop** button or pressing **Ctrl+F6**).
- If conditions to stop data acquisition are never met within an arena, data are recorded until the video ends or (in the case of live tracking) indefinitely unless you have specified a maximum trial duration in the Trial Control.
- See The Start-Stop trial rule

## Starting and stopping data acquisition with multiple arenas

When your trial includes multiple arenas, a copy of the Trial Control rule is applied to each arena separately. This means that actual data acquisition may start and stop at different times in different arenas, depending on when the start/stop conditions are met in those arenas. See Trial control in multiple arenas

- When starting the trial  , actual data acquisition starts independently for each arena depending on when the animal is detected.

  **EXAMPLE** The time indicated in each arena is the time since the start of the actual acquisition in the arena. Acquisition started first in Arena 1, 3 and 4 because the animals were already in those arenas when the trial started. In Arena 2 acquisition has not started yet, because the animal was not yet released in the cage.




- Data acquisition stops independently for each arena unless you stop acquisition manually .

  **EXAMPLE** A condition has been defined so that data recording stop when the animal is detected in its arena for 10 cumulative minutes (Cumulative duration). Data recording may stop at different elapsed times in different arena, if for example the animals have been released in the arenas at different times, and therefore the 10-minute threshold is reached at different times in the four arenas.

# Carry out trials in an order different from that specified in the trial list

## Example

Carry out Trial 5 before Trial 4.

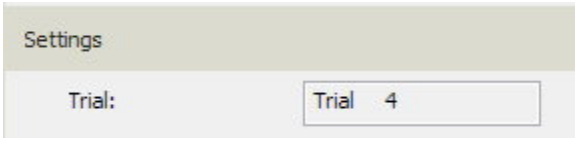
1. Skip Trial 4 and let EthoVision XT acquire data for Trial 5.
2. If you want to carry out Trial 4 at a later time, in the Trial List set this trial back to **Planned**.

**NOTE** This procedure also applies when you track subjects in multiple arenas simultaneously. Set all arenas to To Skip.

## Procedure

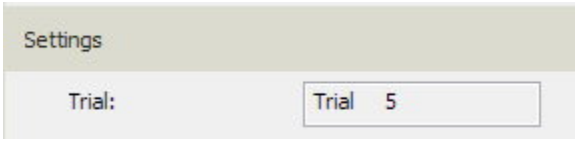
To skip the current trial:

1. Make sure that the Acquisition Settings window shows the name of the trial you want to skip.




2. Click the **Skip Trial** button in the Playback Control window.

*Result*: The Acquisition Settings window shows the next trial number.




3. Carry out the trials as usual.

To carry out the skipped trial

1. In the Experiment Explorer, click Trial List. Click the **Show/Hide** button on the toolbar, select **Variables** and make sure **Acquisition status** is selected in the Show Variables window.

2. Locate the trial and change its Acquisition status from **To Skip** to **Planned**. Make sure that you do this for all the arenas you want to use in that trial.
3. Go to the Acquisition module and carry out the trials as usual. After that, its Acquisition status changes from **Planned** to **Acquired**.

## Notes

- To skip a trial other than the current one in the Acquisition screen, open the Trial List. Click the **Show/Hide** button on the toolbar, select **Variables** and make sure **Acquisition status** is selected in the Show Variables window. Locate the trial you want to skip, and change the Acquisition status for that trial from **Planned** to **To skip**. See Skip trials
- You can also Skip arenas within a trial.

*See also*

- Acquire data for some arenas, not others

# Acquire data for some arenas, not others

## Aim

To acquire data only it the arenas that contain subjects.

**EXAMPLE** For Trial 32, a few arenas in a well plate are empty. Nevertheless, you want to track the subjects in the remaining arenas.

## Procedure

1. In the Experiment Explorer, click **Trial List**.
2. Locate the **Acquisition status** column. If it is not present, click the **Show/ Hide** button on the toolbar, select **Variables** and select **Acquisition status**.
3. Locate the trial for which you want to skip arenas, and change the Acquisition status for those arenas from **Planned** to **To Skip**.
4. Carry out the trials as usual.

## Notes

- After acquisition, the Acquisition status of the skipped arenas changes from **To Skip** to **Skipped**. See Skip arenas within a trial
- You can no longer acquire data for the **Skipped** arenas because the trial itself has been acquired. To acquire data for those arenas, add a new trial and acquire data anew.

*See also*

- Skip arenas within a trial

# Redo a trial

## Aim

To record video or track data once again for a trial previously acquired.

**IMPORTANT** When you redo a trial, you remove the tracks, the events scored manually and the trial control event data (including hardware device data) recorded during that trial.

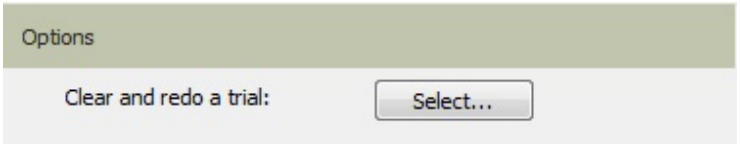
**EXAMPLE 1** You have acquired a trial and realize that you have acquired the data with the wrong detection settings, or the tracks obtained from that trial contain too many missing samples.

**EXAMPLE 2** You have acquired video using EthoVision for Trial 12 and realize that something went wrong during the test. You want to record new video and assign it to Trial 12.

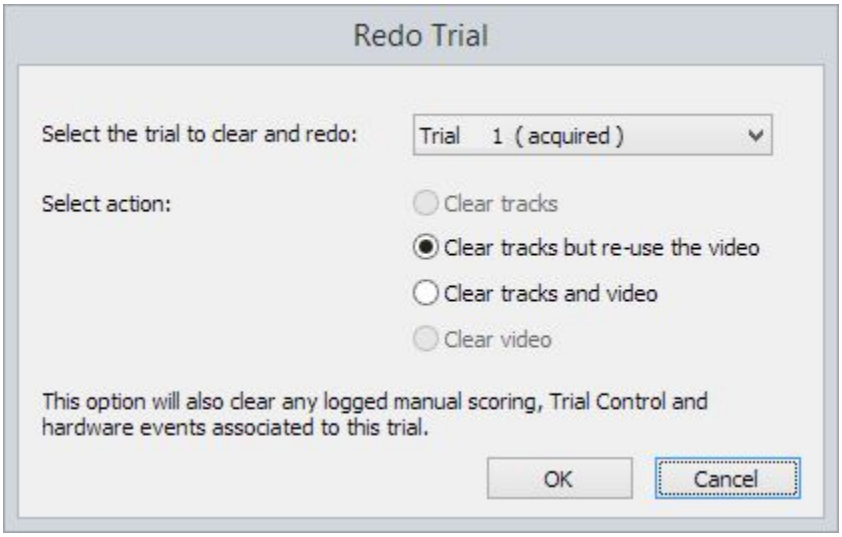
## Procedure

We assume at this point that you have carried out a trial and a video file corresponding to that trial has been saved.

1. In the Acquisition Settings window, under **Options** next to **Clear and redo a trial**, click the **Select** button.




2. In the Redo Trial window, select the trial that you want to redo.




3. Select one of the options, then click **OK**.

   When tracking from video files:

   - **Clear tracks**. The tracks are deleted. Choose this option if you want to re-do the trial using a video file different from the current one.
   - **Clear tracks but re-use the video reference**. As above, however the link to the video file is retained. You can open Acquisition to track from that video file.

   When tracking live:

   - **Clear tracks**. The tracks are deleted. This option is available if you acquired tracks without saving video.
   - **Clear tracks but re-use the video**. To delete the tracks, not the video file used to acquire those tracks. You can then track again from the video file.
   - **Clear tracks and video**. To delete the tracks and the associated video and redo the trial anew.
   - **Clear video**. To clear a trial that has video only. You can then record a new video and acquire tracks. This is the only option available if you acquire data by saving the video first.

   See also Clear trials in the Trial List

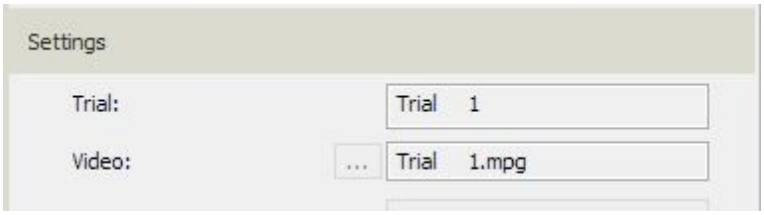
4. In the Acquisition Settings pane, under **Method**,

   - If you cleared a trial that used a video file not made with Ethovision XT, choose **Track next planned trial**. This is the option for redoing the trial from that existing video file.

- If you cleared a trial with a video file made with EthoVision XT, and deleted that video file, choose **Track next planned trial**. Alternatively, choose **Save video only, track later** to record a new video file for that trial without tracking.
- If you cleared a trial with a video file made with EthoVision XT, but kept the video file, choose **Track next saved video file**. With this option you re-do the trial for that video file.
- Choose **Track all planned trials** if you have a list of planned trials and you want to acquire data for all of them in one go.

5. Under **Settings**, next to **Trial**, you see the number of the trial that you are about to acquire.

   The **Video** box shows either **Live video** or the video file you will track from, depending on the options chosen above.




6. If necessary, click the button next to **Video** and select the video file to track from.
7. You can now start the trial. Click the **Start trial** button in the Playback Control window.

## Notes

- The options available next to **Select action** vary depending on how you acquire the trials. For example, if you track from pre-recorded video files, then only the first two options **Clear tracks** and **Clear tracks but re-use the video reference** are available.
- Before re-doing a trial, always check in the Acquisition Settings pane under **Method** which option is selected, and under **Settings** the video file you are going to acquire the trial from.
- If you track from video recorded with programs other than EthoVision XT, and you want to delete the video file corresponding to the trial you want to redo, you must delete the video manually.

*See also*

- Plan your trials
- Skip trials
- Clear trials in the Trial List
- Acquire one trial

# Rename or delete a trial

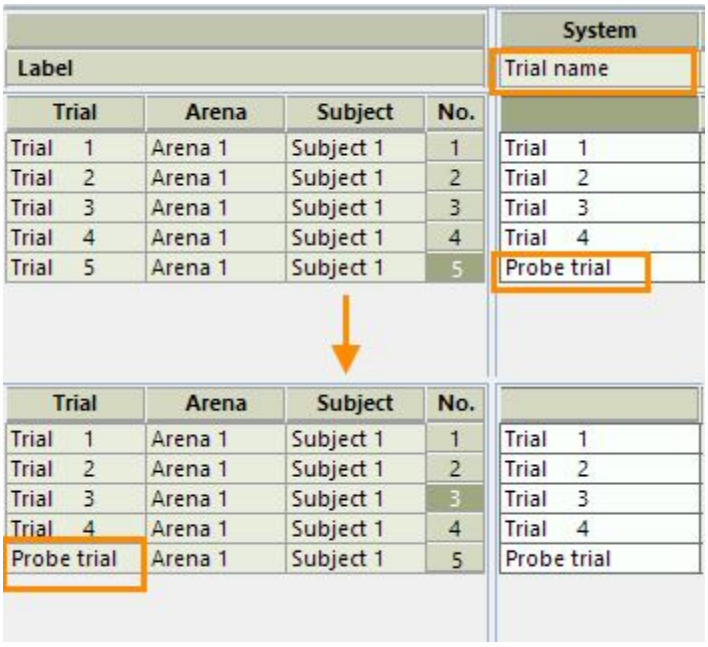
## To rename a trial

Trials are given default names depending on the order they are added in your Trial List: Trial 1, Trial 2, etc. (first column of the Trial List).

To rename your trials, edit the Trial name variable in the Trial List.

1. Choose **Setup** > **Trial List**.
2. Click the **Show/Hide** button on the toolbar and select **Variables**. In the Show Variables window, select **Trial name**.
3. In the Trial name column, double-click the cell corresponding to the trial you want to rename. Next, enter the new name.
4. The name under **Trial** is changed automatically, matching what is entered under Trial name.

**EXAMPLE** You want to rename Trial 5 to Probe Trial.




## To delete a trial

Deleting a trial means that all the tracks and external data files (if these have been imported in EthoVision XT) are removed from your PC. To delete a trial, in the Trial List, click a trial and press **Delete**, or right-click a trial and select **Delete Trials**.

*Note*

- If you have tracked from video files generated with EthoVision XT, you are asked whether you want to keep the video file associated with the trial or delete this file too. See Rename or delete a generated video file.
- If you have tracked from video files generated with programs other than EthoVision, the video file associated with the trial is kept on your PC.
- To delete multiple trials, select the trials in the first column of the Trial List, then press Delete or right-click and select **Delete Trials**.
- In a multiple-arena experiment, you cannot delete tracks acquired in some arenas within a trial. You can only delete all tracks, that is, the entire trial.
- If you delete a trial and then add a new one, this is not given the name of the deleted trial. For example, if you have 10 trials in your Trial List and you delete Trial 4, when adding a new trial this is named Trial 11. To avoid this, do not delete the trial but clear it: right-click the trial in the Trial List and select **Clear Trials**.

*See also*

- Clear trials in the Trial List
- Redo a trial

# Rename or delete a generated video file

Video files recorded within EthoVision XT are given the same name as the corresponding trial: Trial 1.mpg, Trial 2.mpg, etc. If you want to rename or delete a video file, you must first delete the corresponding trial.

If you want to rename a video file without deleting its trial, make a copy of the video file with the File Explorer and give this copy the new name.

1. Choose **Setup** >**Trial List**.
2. In the Trial List, right-click the row corresponding to the video you want to delete and select **Delete**.
3. In the window that appears:
   - Choose **Rename generated video files for backup** if you want to rename the file and delete the trial.
   - Choose **Delete generated video files** if you want to delete the video file and the trial.

   **IMPORTANT** In either case the tracks and the external data files associated with that trial will be deleted!
4. Click **Yes** to confirm.

# Use a remote control to start and stop acquisition

## Aim

To start and stop data acquisition when standing far from the EthoVision XT computer.

## Prerequisites

- You need a remote control with **Page-up** (**<**) and **Page-down** (**>**) buttons, such as those typically used for PowerPoint presentations.

## Procedure

1. Close EthoVision XT.
2. Browse to my.noldus.com, log in or register. Click **Downloads**, then **EthoVision XT**, then **Drivers and Tools**, and download the file for remote control. Save **remote_control.zip** somewhere to your EthoVision XT computer.
3. Extract and double-click **remote_control.reg** file and when a message appears click **Yes**. You can now use the remote control.
4. Switch on and connect the remote control. This is usually done by plugging a USB receiver into the EthoVision XT computer.
5. Start EthoVision XT and choose **Acquisition** > **Open Acquisition**.
6. To start a trial, press **Page-up** (**<**) on the remote control.
7. To stop a trail, press **Page-down** (**>**) on the remote control.

## Notes

- Signals from the remote control will only be recognized:
  - While acquiring data, and if the trial is not finished yet.
  - When you are not entering independent values.
  - When you are not browsing through the video.
- For earlier versions, download the full installation package and look for the Remote Control folder under **Utilities**.

- If your PC is connected to a network through Wi-Fi, you could control EthoVision XT from a smartphone using one of the apps available, provided that the network's security settings allow communication.
- See also How do I start data acquisition on multiple PCs simultaneously?
- If you have the USB-IO box and the TTL Port Tester PTTB-001x, you could have EthoVision XT start tracking when you press the button on the TTL Port Tester. To do so, connect the TTL Port Tester to a TTL port of the USB-IO box. In the Trial Control Settings, create a condition based on the TTL Port Tester that becomes true when the button 1 is pressed. Place the condition box between the **Start Trial** box and the **Start track** box. You use the TTL port tester as a remote control. Note that with this solution you must click the **Start trial** button in EthoVision XT, which then waits for the button press.

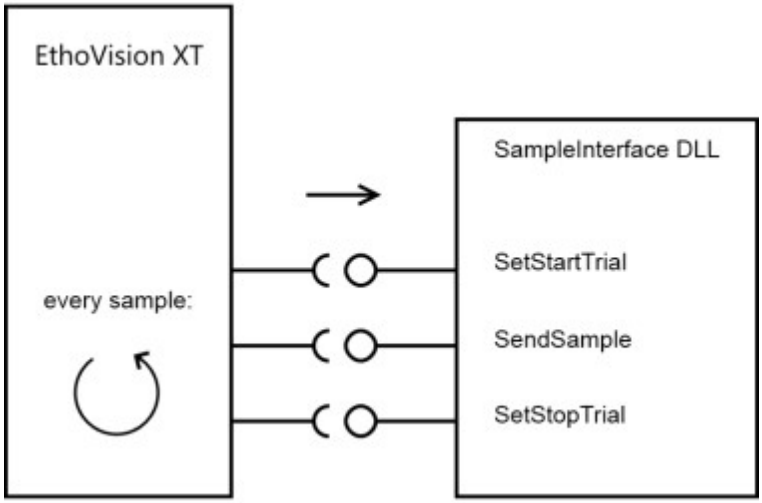
# Export track data live

## Aim

To crease a sample interface that exports the track samples real-time, that is, during data acquisition.

## Procedure

To enable external data export, you can write a DLL (COM DLL, also known as COM component) in C++. The DLL should implement the sample interface as follows:




Furthermore the DLL should include the instructions for processing the samples, for example:

- Write the sample data to a local file;
- Send the sample data over the network to an external application

If you need help with writing the code for your DLL, please contact Noldus.

## Notes

- The sample interface is specific to the EthoVision XT version you are using.

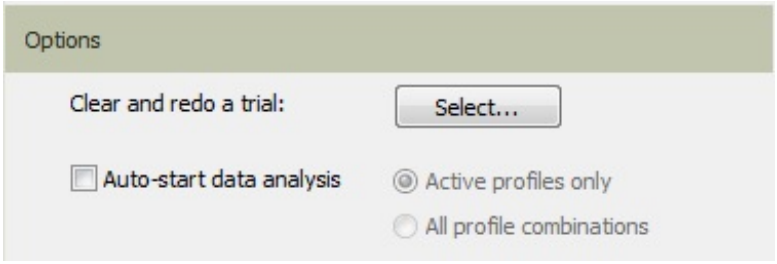
# Auto-start data analysis

The Auto-start data analysis option in the Acquisition Settings window allows you to automatically start batch calculations immediately after data acquisition has ended, without the need to give any additional command.

**EXAMPLE** You have a large set of video files, and you plan to start the batch data acquisition before leaving the lab in the evening. By selecting Auto-start data analysis, you make sure that analysis starts immediately after the end of the last trial, so that the results are ready the next morning.

## To enable auto-start data analysis

1. Define your series of trials in the Trial List.
2. In the Acquisition module, before starting acquisition, in the Acquisition Settings window, under **Options**, select **Auto-start data analysis**.




3. Select one of the two options:
   - **Active profiles only**. To calculate the statistics using only the combination of the Track smoothing profile, Data profile and Analysis profile currently active (highlighted in blue in the Experiment Explorer).
   - **All profile combinations**. To calculate the statistics using all combinations of the Track smoothing profiles, Data profiles and Analysis profiles in your experiment.
4. Carry out data acquisition as usual.

## Notes

- If you choose the second option, to minimize processing time and disk space occupied by results, make sure that the Track smoothing, Data and Analysis profiles in your experiment are those you want to use for analysis. Remove any duplicate or non-interesting profiles.
- If you want to analyze data using certain profile combinations, not others, do not select **Auto-start data analysis**. Instead, do batch analysis after acquisition; there you can choose those profiles combinations.

- The analysis does not start automatically when you stop tracking manually.
- If you move to another window and then return to the Data acquisition screen, you have to set the Auto-start data analysis option again.
- The Auto-start data analysis option is not available when you select **Save video only, track later** as acquisition method.
- Once analysis is started, a window with a progress bar opens. You can see that there are a few stages:
    - Step 1 - Data Preparation (for when tracking two subjects with Deep learning). For details see Prepare the data in multi-subject trials
    - Step 2 - Track Smoothing, for adjusting the tracks based on a Track smoothing profile.
    - Step 3 - Data Selection, for choosing tracks, and track segments based on a Data profile.
    - Step 4 - Analysis, for calculating the values of the dependent variables specified in an Analysis profile.

# Score behaviors manually

## What do you want to do?

- Score behaviors manually during acquisition
- Score behaviors manually after acquisition
- Edit the duration of manually-scored behaviors
- Replace a behavior with another one
- Score behaviors in different arenas
- Score behaviors of two or more subjects
- Delete a manually-scored behavior

# Score behaviors manually during acquisition

## Aim

To record behaviors manually, while EthoVision XT tracks the subjects.

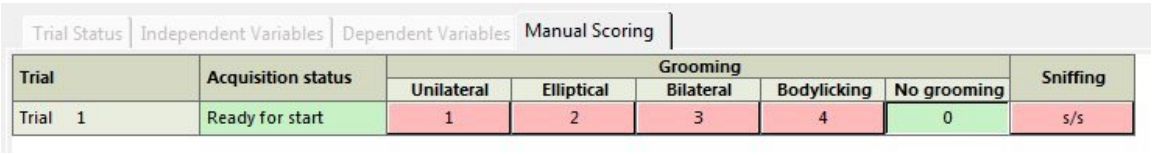
**NOTE** You can also Score behaviors manually after acquisition.

## Prerequisites

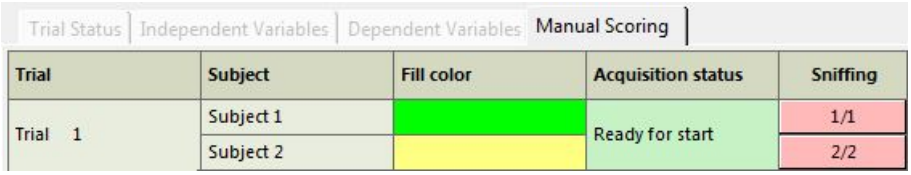
- You have defined behaviors in the Manual Scoring Settings.
- In the Acquisition screen locate the Analysis Results and Scoring pane, and click the **Manual scoring** tab. If you do not see the Analysis Results and Scoring pane, click the **Show/Hide** button and select Analysis Results and Scoring.
- If you track from video files, in the Playback Control window, deselect **DDS**. So you make sure that the video file is played at the normal speed.

## Behavior buttons and key codes

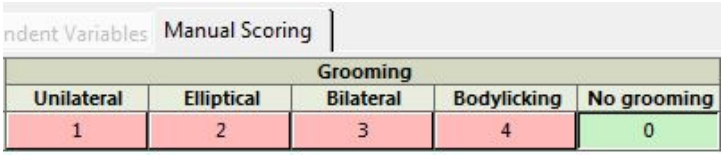
Each behavior has its corresponding buttons (with key code, if defined) for each arena and subject. Mutually-exclusive behaviors are shown under their group name.




For multiple animals per arena, you can use the fill color as a reference to identify animals.




If at least one trial is set to Planned in your Trial List, the buttons for behaviors defined as Initially active in the Manual Scoring Settings are highlighted in green (see **No grooming** in the picture below), indicating that when you start the trial this is recorded automatically as initial behavior.

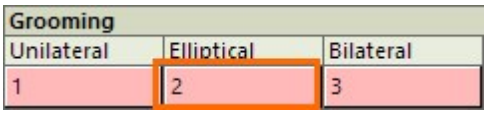



## Procedure

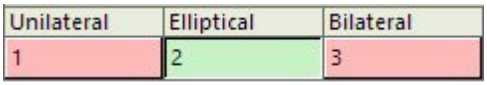
1. Start the trial.

2. To score a behavior for a specific subject, arena and behavior, press the key for that combination, or click the button for that combination.

   Result: The button of the scored behavior is highlighted in green. The behavior's start has been scored.

   For mutually-exclusive behaviors, the button of the previous behavior is reset.

3. When the subject no longer shows that behavior, do the following:
   - For Mutually-exclusive states, score the behavior that is active next.
   - For Start-Stop states, press the stop key or click the button for that behavior.

   Result: The button of the behavior is reset. For mutually-exclusive behaviors, the button of the new behavior is highlighted in green.

## Notes

- Data can only be recorded after tracking has started. If you start the trial and tracking starts some time later as a result of Trial control conditions setting some delay, any scoring action between the start of the trial and the tracking is not recorded.

- You cannot pause recording behaviors even when you track from a video file. However, after acquisition you can Score behaviors manually after acquisition.
- If you want to change the initial state of a subject for the next trials, do so in the Manual Scoring Settings. Initial behavior for trials already acquired remains as it is.
- To modify the coding scheme, stop the trial and click **Manual Scoring Settings** in the Experiment Explorer.
- For more efficient data scoring you can use the X-keys programmable keyboards. You can for example press and hold a key as long as the behavior lasts. Contact Noldus Information Technology for more information.
- To improve clarity in the video image during the observation, choose which body points to show, and adjust their size. See The video window > Show/hide body points and other track features
- If you want to show the arena and zones during the scoring sessions, click the **Show/Hide** button at the top-left corner, and choose **Arena features**.

# Score behaviors manually after acquisition

## Aim

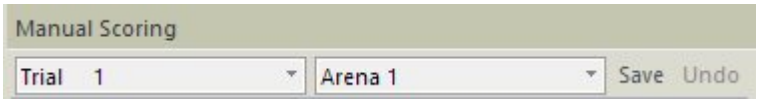
To add new or edit existing behavior scores in your trials.

## Prerequisites

- You have defined behaviors in the Manual Scoring Settings.
- You have acquired one trial and its corresponding video.

## Procedure

1. Choose **Acquisition** > **Manual Scoring**.
2. Choose the trial, and when it applies, the arena in which you want to score behaviors.




3. To enable the scoring mode, click the **Start scoring mode** button.

4. Follow one or more of the procedures below.

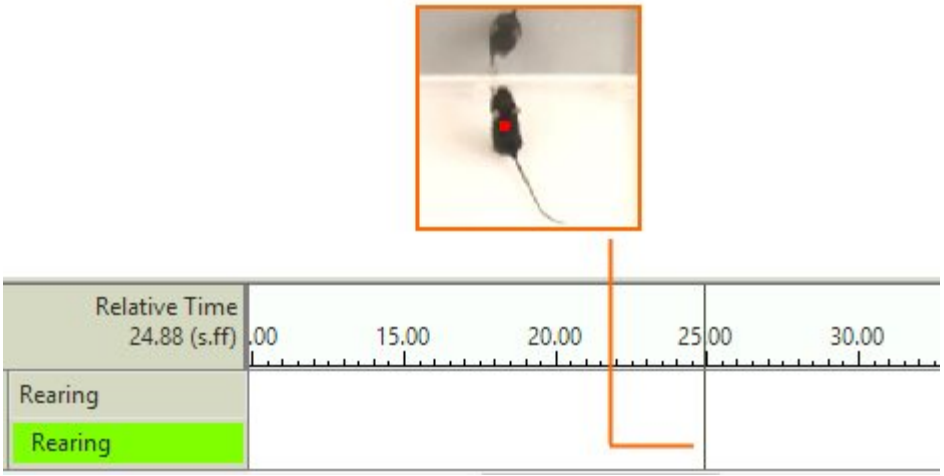
   **TIP** Before editing existing data, click the **Save** button on the toolbar. This will save a copy of the current behavior scores. In the case you make mistakes when editing, you can always revert to that copy by clicking **Undo**. For more information, see Saving the track edits.

5. When finished, click the **Stop scoring mode** button.

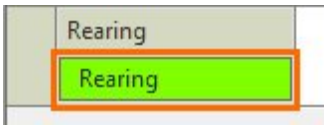
## To score a behavior with a duration

This applies to behaviors defined as Mutually exclusive and Start-stop. See Define the behaviors that you want to record manually

1. Play the video to the point where the behavior starts.



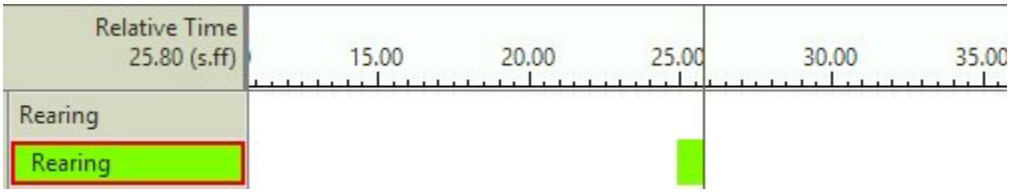

2. To score the behavior at that point in the video, click the color button with the behavior name at the left side of the plot.



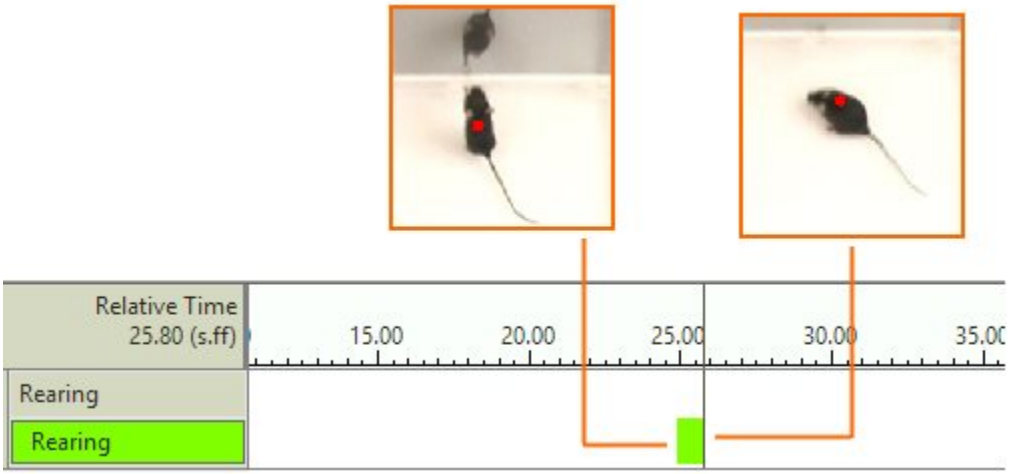

   Result: The button is highlighted in red.

3. Play the video forward. As you play the video, a colored bar appears at the left of the hairline.

   **NOTE** If you play the video backward, the color bar is reduced in length.




4. Pause the video where the behavior stops. To score the end of the behavior, click again the button at the left, so it is not highlighted anymore.



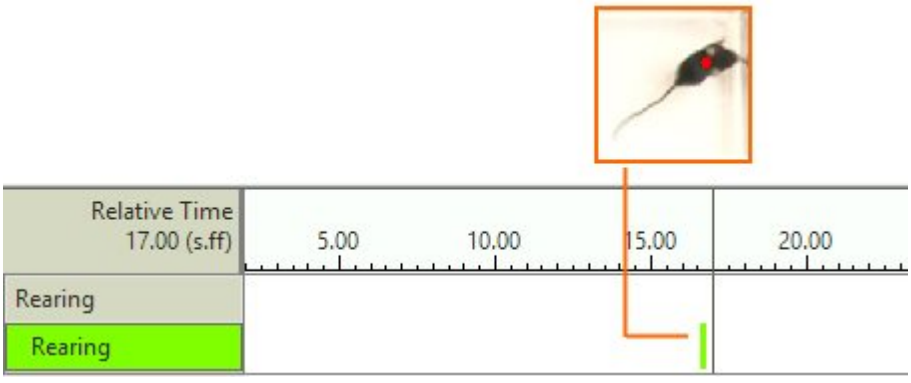

5. Repeat steps 1-4 to add more instances of the behavior.

## To score a point event

Point events are events with no duration. See Define the behaviors that you want to record manually

1. Play the video to when the behavior occurs.
2. To score the behavior at that point in the video, click the color button with the behavior name at the left side of the plot.


3. Play the video further. The point event is displayed as a vertical segment.



## Notes

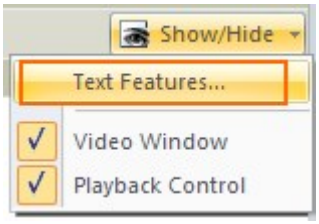
- After clicking **Start scoring mode** button, the video image shows **[On]** and message next to the button changes to **Scoring on**.

  When you play the video, behavior scores do not change until you click the corresponding button at the left side of the plot.

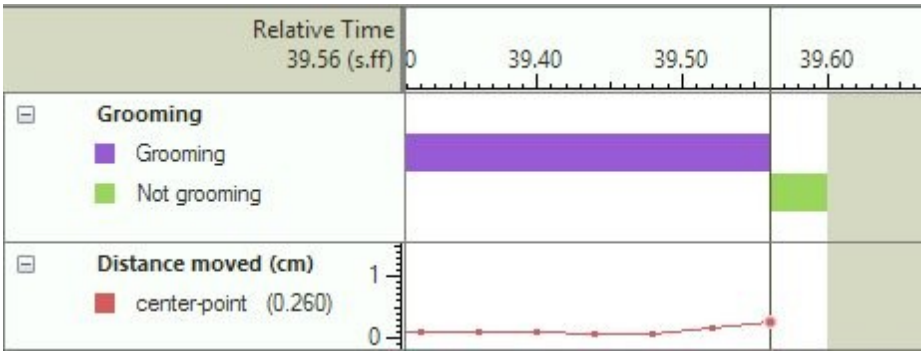
- If you want to hide the center point to make the subject more visible, choose **Track Visualization** or **Integrated Visualization** or **Track Editor**, and de-select the body points on the right-hand pane. Next, close and re-open the experiment.
- When you click the button for a behavior, the name of the behavior is displayed on the video image.

  To show/hide and customize this text, choose **Show/Hide** > **Text Features**.

- When you score the start of a behavior (either Mutually exclusive or Start-Stop) and then you reach the end of the trial without scoring the end of the same behavior, that behavior is considered to last until the last sample.

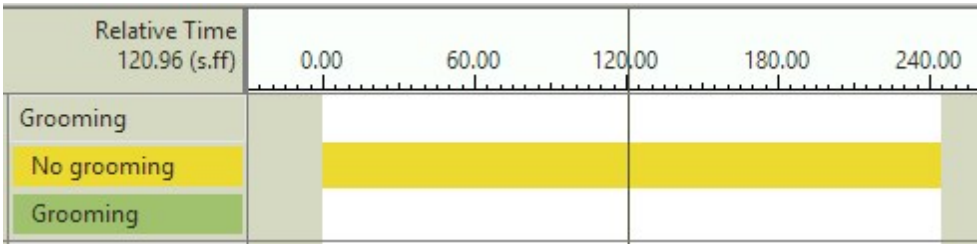
  If the behavior is defined as Start-stop, a complementary behavior ("not-behavior") is added at the end of the behavior you scored. You can see this in the Integrated visualization. The example below shows *Grooming* and the complementary *Not grooming*. The plot of distance has been added to visualize the last sample of the track.

This means that the statistics of *Not grooming* is increased by 1.

- Behaviors that are defined as **Initially Active** in the Manual Scoring Settings, are shown as active throughout the trial, if you do not edit them.

  For example, *No grooming* was defined as Initially Active and no behaviors were scored during acquisition. When opening the Manual Scoring screen, the data look like this:




- If you want to show the arena and zones during the scoring sessions, click the **Show/Hide** button at the top-left corner, and choose **Arena features**.

*See also*

- Edit the duration of manually-scored behaviors
- Replace a behavior with another one
- Delete a manually-scored behavior
- Score behaviors in different arenas
- Score behaviors of two or more subjects

# Edit the duration of manually-scored behaviors

## Aim

To increase or reduce the duration of instances of behaviors already scored in your trial. This way you can improve the match between what you see in the video and the behavior scores.

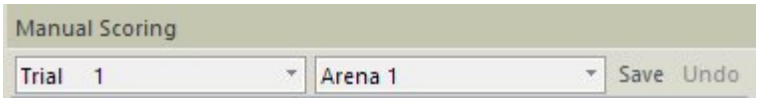
**NOTE** This applies to all behavior types but Point events.

## Prerequisites

- You have scored behaviors manually. See Score behaviors manually

## Procedure

1. Open the Manual Scoring screen (**Acquisition** > **Manual Scoring**).
2. Choose the trial, and when it applies, the arena in which you want to score the behaviors.

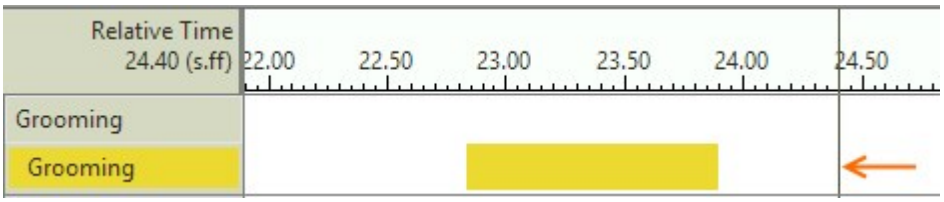



3. Follow one or more of the procedures below.

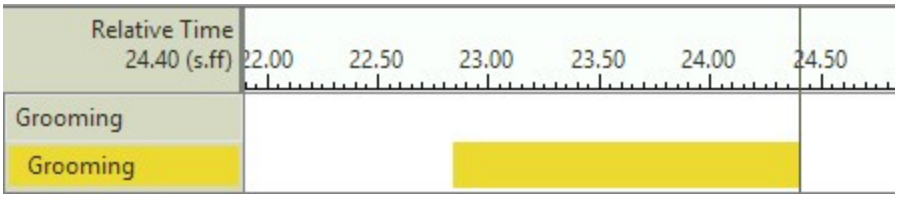
**TIP** Before editing existing data, click the **Save** button on the toolbar. This will save a copy of the current behavior scores. In the case you make mistakes when editing, you can always revert to that copy by clicking **Undo**. For more information, see Saving the track edits.

## To extend the duration to a specific time point

1. Play the video up to when the behavior ends (here indicated with the arrow).



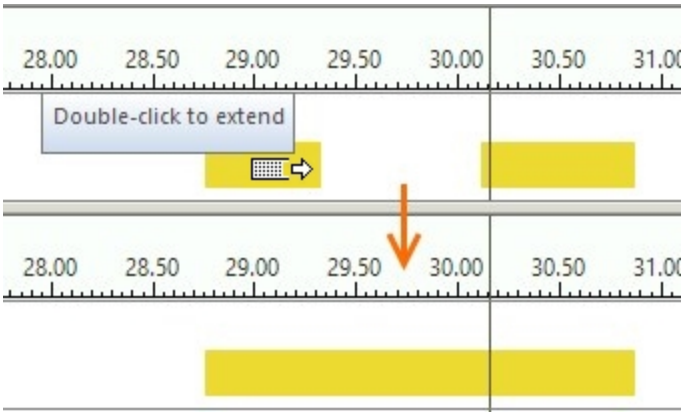
2. Hover with mouse over the existing color bar, right-click and choose **Extend to current**, or double-click the existing score.
3. Result: The color bar now ends at the specified time.




## To fill the gap between two behavior scores

Follow this procedure if you scored two instances of the same behavior, and you want to fill the gap between the two instances since they actually refer to one bout of behavior. The frequency of the behavior will be reduced by 1.

1. Position the video at the start of the second color bar.
2. Point to the first color bar, and double-click or right-click and select **Extend to current**.



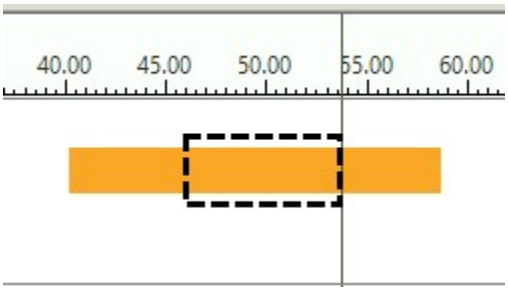

## To create a gap between two behavior scores

This applies to Start-stop behaviors only.

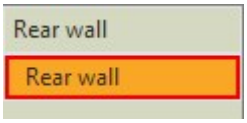
Follow this procedure if you scored an instance of a Start-stop behavior, and you want to insert a gap between the original start and end points. The frequency of the focal behavior will increase by 1. The gap will appear as *Not [behavior name]* in the analysis.

1. Click the **Start scoring mode** button.

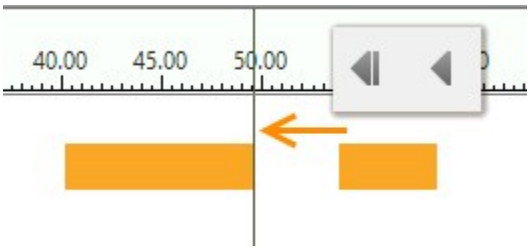
2. Position the video to where you want the end of the gap (= the second start point of the behavior).




3. Click the button for the behavior.




4. Play the video *backward*. **TIP** Use the frame-by-frame controls for accurate scoring. Pause the video at the start of the gap (= the first end point of the behavior).

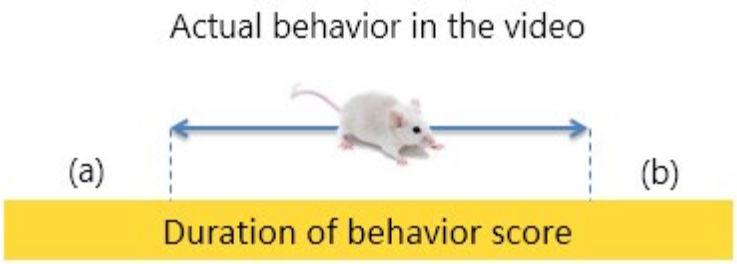



5. When finished, click the **Stop scoring mode** button.

## To reduce the duration to a specific time point

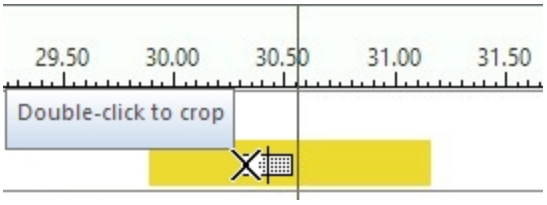
Follow this procedure if the behavior score is too long relative to what shown in the video.

Do one or both the following, depending on whether the color bar occurs before (a) or after (b) the actual behavior in the video, or both (a) and (b).

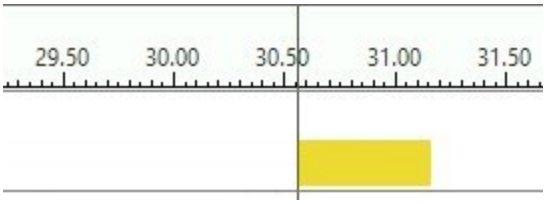



*(a)*

1. Position the video where the behavior starts.
2. Point to the color bar at the left of the hairline. The mouse pointer turns to a delete symbol on the left side.

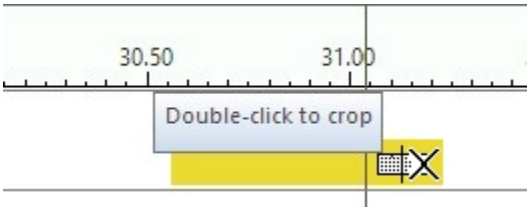



3. Right-click and choose **Crop to current**, or double-click the color bar.
4. Result: The color bar is shortened.

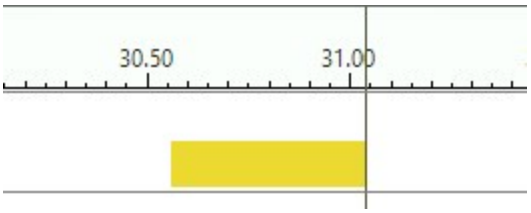



*(b)*

1. Position the video where the behavior ends.
2. Point to the color bar at the right of the hairline. The mouse pointer turns to a delete symbol on the right side.



3. Right-click and choose **Crop to current**, or double-click the color bar.
4. Result: The color bar is shortened.




**NOTE** Which side of the color bar is deleted by **Crop to current** depends on where the mouse pointer is located relative to the hairline.

# Replace a behavior with another one

## Aim

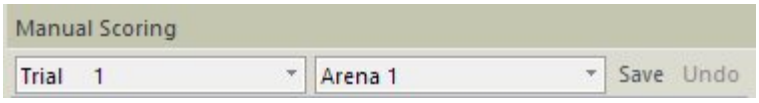
To replace the score of a behavior with that of another behavior from the same Mutually-exclusive group.

## Prerequisites

- You scored behaviors of a group defined as Mutually-exclusive. See Define the behaviors that you want to record manually
- You have opened the Manual Scoring Screen (**Acquisition** > **Manual Scoring**).

## Procedure

1. Choose the trial, and when it applies, the arena in which you want to score behaviors.

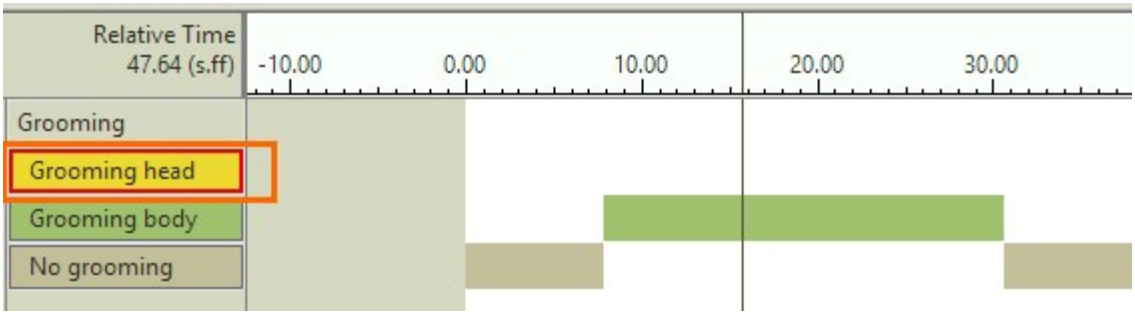



2. To enable the scoring mode, click the **Start scoring mode** button.

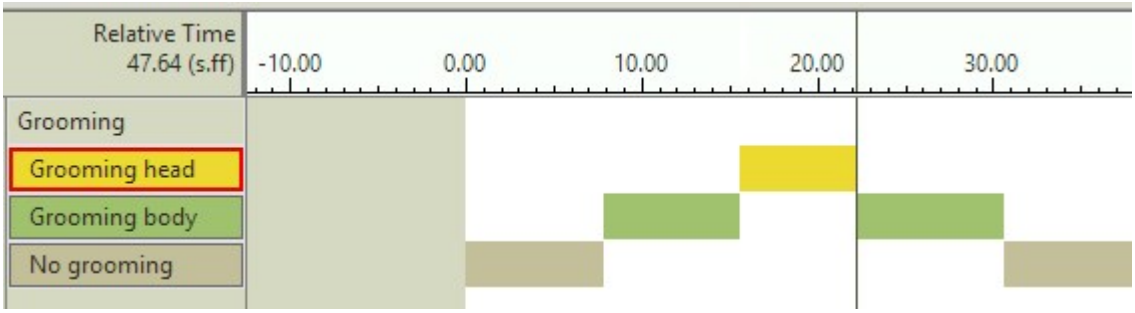
**TIP** Before editing existing data, click the **Save** button on the toolbar. This will save a copy of the current behavior scores. In the case you make mistakes when editing, you can always revert to that copy by clicking **Undo**. For more information, see Saving the track edits.

3. Position the video to the point where the new behavior starts.


4. Click the button for the new behavior.




5. Play the video forward. **TIP** Use the frame-by-frame controls for accurate scoring.
6. Pause the video when the behavior ends.




7. To confirm, click the behavior button again. To quit editing, click the **Stop scoring mode** button.

*See also*

- Delete a manually-scored behavior

# Delete a manually-scored behavior

## Aim

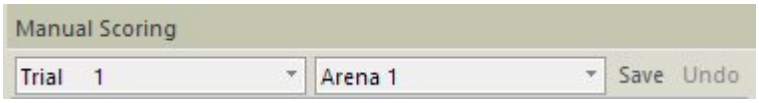
To remove an instance of behavior scored manually.

## Prerequisites

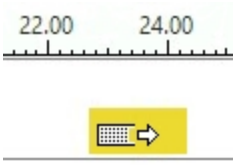
- You have opened the Manual Scoring screen (**Acquisition** > **Manual Scoring**).

## Procedure

1. Choose the trial, and when it applies, the arena in which you want to score behaviors.



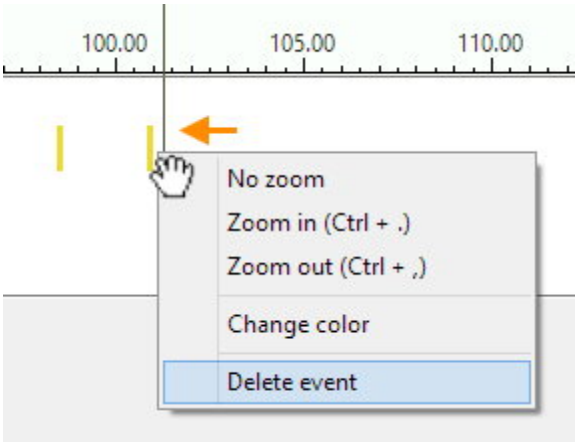

2. Hover with the mouse on the color bar (or vertical segment) which represents the instance you want to delete. The cursor changes to a symbol depending on where this instance is relative to the hairline.




   **TIP** If you are not sure of the results of editing, click first the **Save** button on the toolbar. You can always return to the last saved data by clicking **Undo**. See Saving the track edits

3. Right-click and choose **Delete event**.
4. The same is valid for point events:

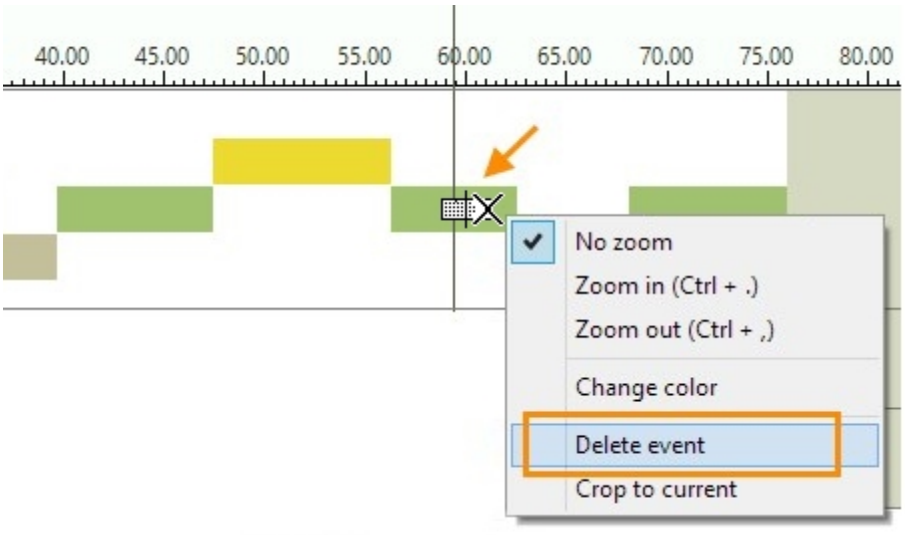
   **TIP** When deleting point events, make sure that the hand cursor is slightly at the right of the event you want to delete.



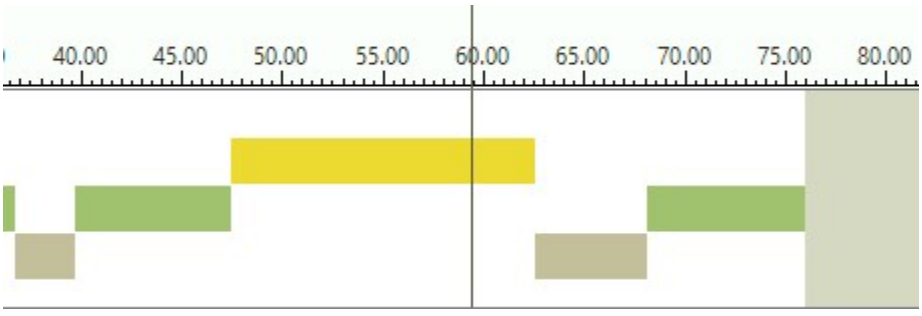

## Notes

- If you delete a behavior from a mutually-exclusive group, the previous behavior is extended up to the end of the color bar that you delete.

  **EXAMPLE**




  Result:



- You can only delete a behavioral category defined in the Manual Scoring Settings, but only after deleting all the trials. Note that if you remove all trials, you remove all your data! See Edit the manual scoring settings

# Score behaviors in different arenas

## Aim

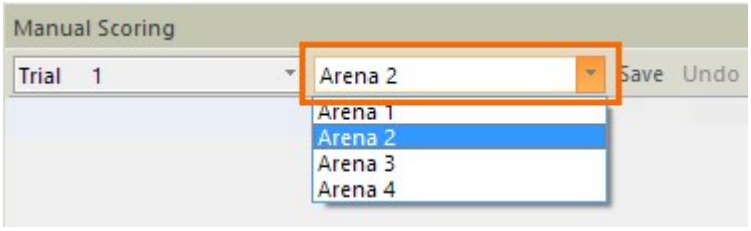
To edit behaviors manually for subjects that were tracked in different arenas.

## Prerequisites

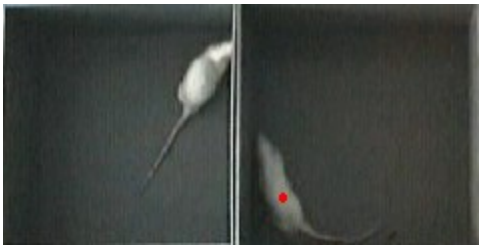
- You have defined two or more arenas in the Arena Settings.
- You have defined behaviors in the Manual Scoring Settings.
- You have acquired one trial and its corresponding video.
- You have opened the Manual Scoring screen (**Acquisition** > **Manual Scoring**).

## Procedure

1. In the Manual Scoring screen, select the arena in which you see the subject you want to score behaviors for.




   Result: The body points of the subjects in the selected arena are displayed.



2. To enable the scoring mode, click the **Start scoring mode** button.

3. Score/edit the behavior for the subjects in that arena. See Score behaviors manually after acquisition.

4. Repeat the procedure for the remaining arenas.

# Score behaviors of two or more subjects

## Aim

To edit behaviors manually in a social interaction context.

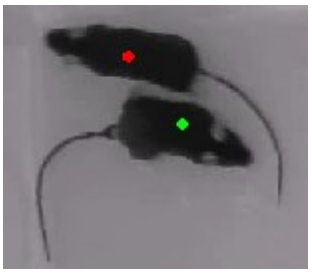
**IMPORTANT** If you tracked unmarked subjects, always check that there is no subject swap in your tracks. If that is the case, correct the swaps in the Track Editor screen. Otherwise you take the risk to score a behavior for the wrong subject. See Swap subjects

## Prerequisites

- You have defined behaviors in the Manual Scoring Settings.
- You have acquired one trial and its corresponding video.
- You have opened the Manual Scoring screen (**Acquisition** > **Manual Scoring**).

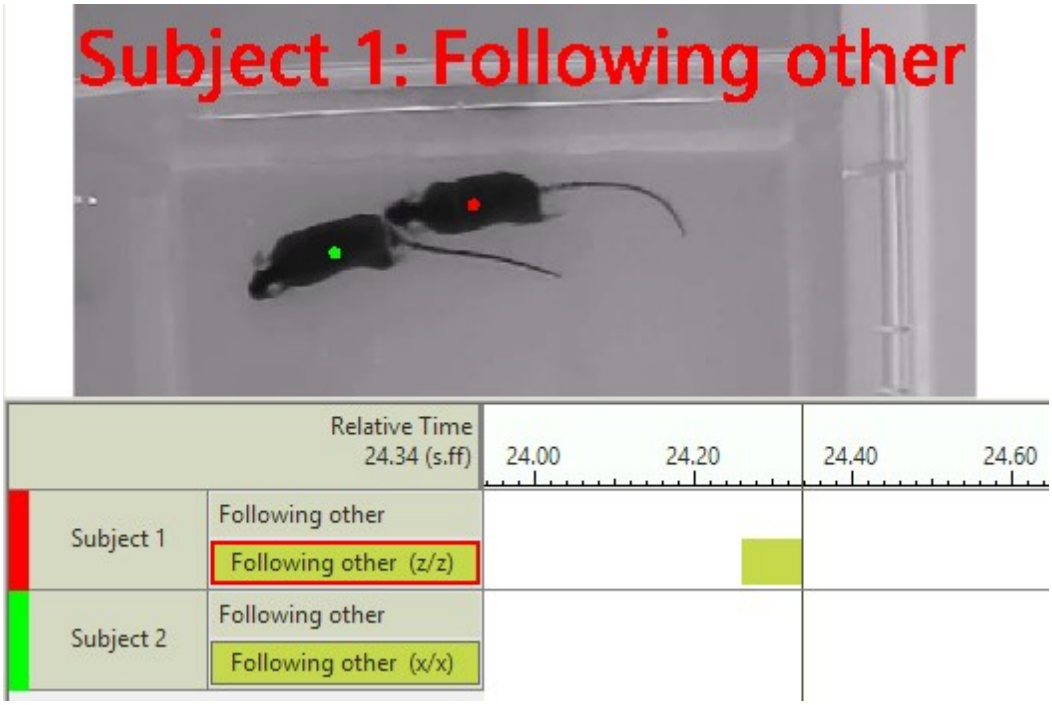
## Procedure

1. In the Manual Scoring screen, subjects are indicated with different colors.



2. To score the behaviors of a subject, locate the plot marked with the same color.

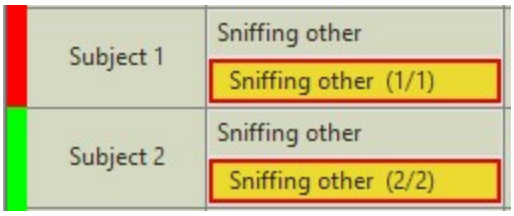

3. Score the behavior as described in Score behaviors manually after acquisition.
4. When you score a behavior with duration, the subject name is also indicated on the video image.



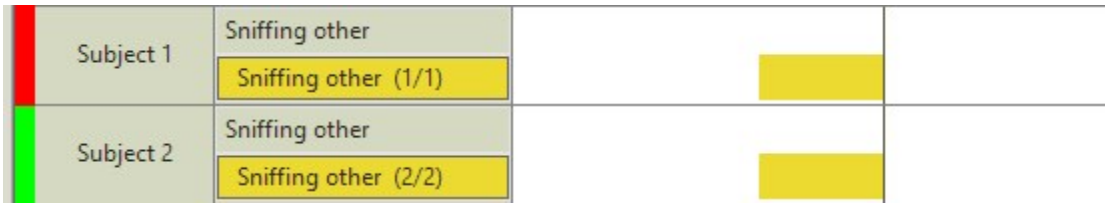

## Simultaneous scoring of behavior

Behaviors are always associated with a subject. If you want to score a behavior valid for all subjects simultaneously, score the same behavior for each subject. To do so,

1. Position the video at the start of the behavior.
2. Click the button for each subject involved.

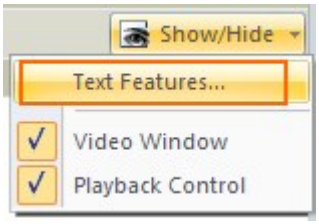



3. Next, play the video until the end of the behavior.
4. Finally, click the button for that behavior again, and for each subject.



## Notes

- **TIP** Behaviors like proximity can be automatically measured. See Social dependent variables
- **TIP** Before editing existing data, click the **Save** button on the toolbar. This saves a copy of the current behavior scores. In the case you make mistakes when editing, you can always revert to that copy by clicking **Undo**. For more information, see Saving the track edits.
- To show/hide and customize the overlay text, choose **Show/Hide** > **Text Features**.

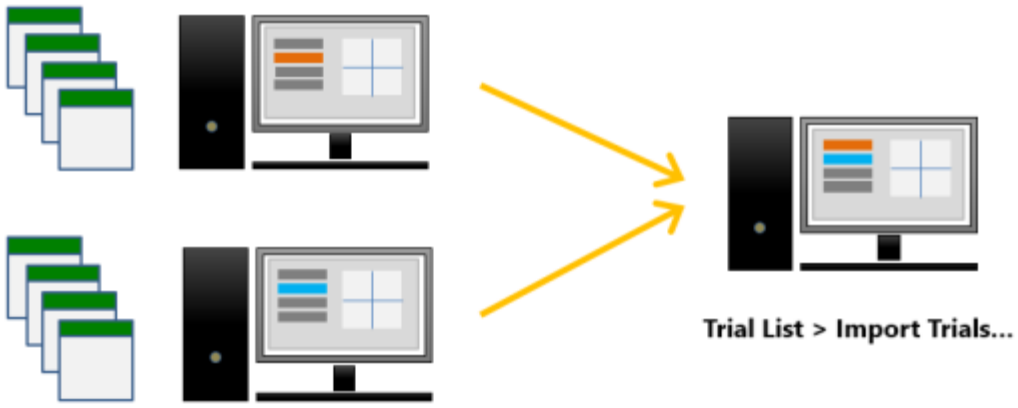


# Import data from other experiments

## Aim

To analyze data collected in two or more (identical) experiments.

**EXAMPLE** You run experiments with a number of PhenoTyper cages. Each group of four PhenoTypers is controlled by a computer with EthoVision XT. Therefore, you have as many experiments as computers. You would like to analyze all the data from the original (source) experiments in one *master experiment*.




## Prerequisites

- You have two or more EthoVision XT experiments containing data.
- The setup must be identical between the original experiments. Import of different experiments (e.g. am open field and a PhenoTyper) won't work.
- The original experiments and the master experiment must have identical settings. For example:
  - The same detected features (e.g. center point);
  - The same number of arenas and the same number of subjects per arena;
  - The same Arena Settings, with for example the same zone names, Detection Settings and Trial Control Settings.
  - The same Behavior Recognition settings.
  - The same behavioral events defined in the Manual Scoring Settings.
  - The same hardware device names, mapped on the same arenas. For example, Lickometer 1 must be mapped on Arena 1 in all experiments.

**TIP** When you create the first of the original experiments, adjust the settings and then, before acquiring the first trial, save it with a different name to create an identical copy (**File** > **Save As**). This is going to be your second original experiment.

## Step 1 - Create the empty master experiment

1. Start EthoVision XT on the PC that contains one of the original experiments.
2. Choose **File** > **New from Template**. Choose **Use a custom template** and select one of the original experiments. Select the file with the name of that experiment and the extension **evxt**.
3. Complete the procedure, and give the new experiment a name, for example *Master experiment*.
4. If you intend to work with the master experiment on a different PC, copy the original experiments and the master experiment to another PC.

## Step 2 - Import the trials into the master experiment

1. If needed, copy the original experiments to the PC where the master experiment is stored.
2. Open the master experiment (see above).
3. Choose **Setup** > **Trial List**.
4. Delete the empty trials in the master experiments. These rows were generated and left empty when you created the experiment from the custom template. You do not need them, so select all rows, right-click and choose **Delete Trials**. See Delete trials
5. Click the **Import Trials** button.


6. In the window that opens, select **EthoVision XT data (trials)** and click **OK**.
7. Locate the experiment that you want to import. Select the file with the extension **evxt** and click **Open**. To locate this file, open the folder that has the same name as the experiment.
8. Select which trials you want to import. If you want to copy the video files into the master experiment, select **Also import the associated video files**.


**NOTE** This option only applies when the video files were generated by EthoVision XT in experiments set to Live tracking. If your original experiments are set to Offline tracking, the video files that were used to do tracking are not copied; however, the master experiment preserves the link to those video files.

9. After import, which may take some time depending on the number and length of the tracks, the imported trials are shown in the trial list.

## Result of the trial import

What is imported in the master experiment:

- The trials (track data, independent variables, manually-scored events, hardware events)
- The settings associated with the trials (Arena Settings, Trial Control Settings, Detection Settings, Manual Scoring Settings).
- The physiological data associated with the trials.

## Notes

- If two settings profiles have the same name but slightly differ, for example one detection settings profile **Detection Settings 1** in the first experiment set to Gray Scaling 40 to 255 while the **Detection Settings 1** in the second

experiment is set to Gray Scaling 45 to 255, the second imported experiment is renamed; in this example **Detection Settings 1 (1)**.

- If two settings profiles are exactly the same in the original experiments, they are imported with the same name.
- There may be subtle differences between the master experiment and one of the original experiments which cause import to fail. For example, in the master experiment a Trial Control Settings profile contains a condition named **Time (1)**, while the same settings profile in experiment 1 or 2 contains the same condition named **Time (2)**. Although the settings profiles have the same name, EthoVision XT detects that their content is different. Please follow the procedures above to make sure that the master experiment is an exact copy of the original experiments.
- When you select the option **Also include the associated video files** and only for video files recorded with EthoVision XT, those video files are copied to the Media Files folder of the master experiment. If you do not select that option, you can still view the videos in the master experiment, provided that they are still stored in the original experiment.
- If you record video with MediaRecorder or software other than EthoVision XT, the video files are not copied to the master experiment anyway, also when they are stored in the **Media Files** folder of the original experiments. However, the Trial List shows the link to the original video files.

# Import Live Mouse Tracker data

## Aim

To import Live Mouse Tracker (LMT) data into an EthoVision XT experiment.

## Background information

You are going to import the information contained in the LMT database. The LMT database consists of a number of tables, each containing specific data, for example the subjects, the coordinates of the detected subjects, the list of events like Stop or Contact, the configurations the output variables, etc.

A Live Mouse Tracker database does not contain video. To import video into EthoVision XT, the original video files must be stored in the folder where the LMT database is stored.

## Prerequisites

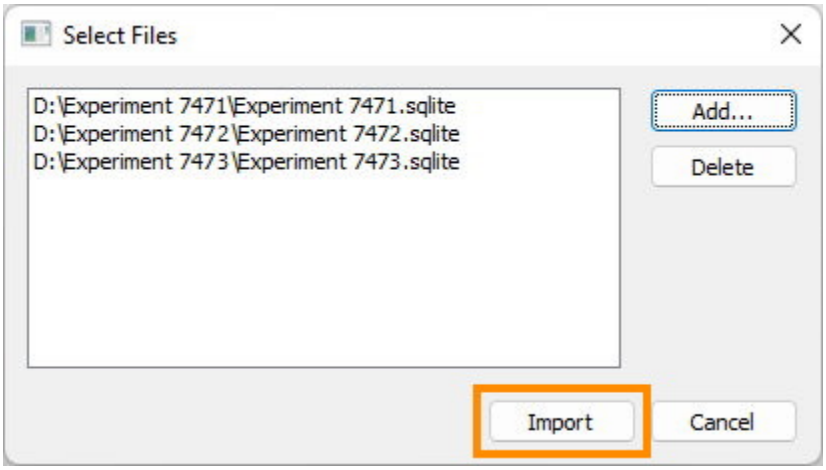
- You use EthoVision XT 17.5 or a later version.
- Your EthoVision XT license includes the Social Interaction add-on module. See Upgrade EthoVision XT
- You have created an EthoVision XT experiment of type Live Mouse Tracker. See Live Mouse Tracker: Create an experiment
- You have one or more Live Mouse Tracker database files (*.sqlite). EthoVision XT supports import of data of LMT version 1.0.3.
- **IMPORTANT** We strongly recommend that you keep databases and video of different Live Mouse Tracker recordings in different folders. This to prevent that EthoVision XT merges video of different experiments in one. when importing the databases.

## Procedure

1. Open the Live Mouse Tracker experiment in EthoVision XT.
2. Choose **Setup** > **Trial List**.
3. Click the **Import Trials** button on the toolbar.
4. in the window that opens, select **Live Mouse Tracker data (databases)** and click **OK**.
5. The Select files window opens. To add a database, click the **Add** button, select the database you want to import and click **Open**.

- Repeat this step to select a new database. You can only select one database file at a time.
- To remove one file from the list of added files, select that file and click **Delete**.
- You cannot add a database twice in the same experiment.

6. Once you have completed the list of the databases to be imported, click the **Import** button.




**NOTE** Import of long recordings may take several minutes.

7. Each database is imported as a new trial.

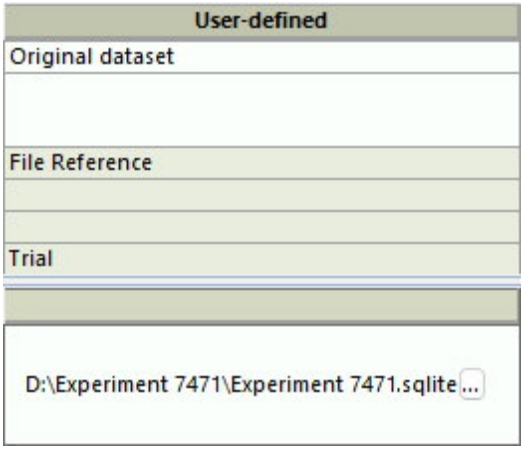
| Trial | Arena | Subject | No. | |
|---|---|---|---|---|
| Trial 1 | Arena 1 | A | 1 | Acquired |
| | | B | 2 | |
| | | C | 3 | |
| | | D | 4 | |
| Trial 2 | Arena 1 | A | 1 | Acquired |
| | | B | 2 | |
| | | C | 3 | |
| | | D | 4 | |
| Trial 3 | Arena 1 | A | 1 | Acquired |
| | | B | 2 | |
| | | C | 3 | |
| | | D | 4 | |

8. You are ready to analyze the data. Open an Analysis profile and add the Live Mouse Tracker variables. See Dependent Variables in Detail > Live Mouse Tracker

## Notes

- Each Live Mouse Tracker database results in one trial when imported into EthoVision XT. Check that in the Trial List.

- Live Mouse Tracker makes 10-minute video fragments. At import, EthoVision XT merges those fragments in one video file, which is imported and linked to the trial.
- During the transition from one video fragment to the next one, Live Mouse Tracker does not record video frames. This means that there is a gap between adjacent video fragments. An offset between video and data may occur when visualizing the trial. EthoVision XT cannot compensate for this, however it places the next 10-minute fragment at the correct time point and restores synchronicity between video and data.
- Calculation of some behaviors is based on fixed thresholds that were used during data acquisition. For example the speed thresholds that are used to define the behavior category *Move*. You cannot change those thresholds cannot be modified in EthoVision XT.
- When you import Live Mouse Tracker data, the Trial List contains two additional independent variables:
  - **RFID**. This shows the RFID code of each subject.
  - **Original dataset**. This shows the location and the name of the original database file that was imported (*.sqlite).




*See also*

- Live Mouse Tracker: Workflow
- Live Mouse Tracker: Create an experiment
- Dependent Variables in Detail > Live Mouse Tracker

# View real-time information

## What do you want to do?

- Choose a variable to view real-time

## Application examples

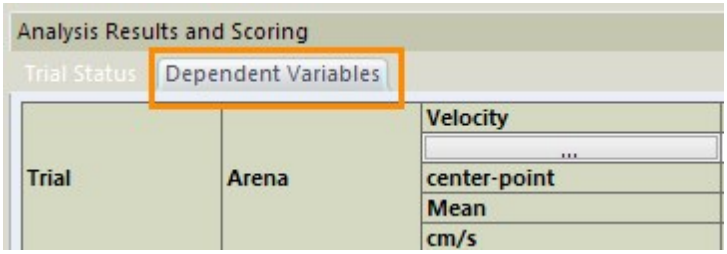
- Open field: view the Movement variable
- Porsolt swim test: view the Mobility state variable
- Fear conditioning: view Activity state
- Conditioning test: view a Trial Control State variable

# Choose a variable to view real-time

By default, the dependent variables *Velocity* (with the statistic Mean) and *Movement* (with the statistic Cumulative duration, and only for the Moving state) are already displayed. You can specify any dependent variable, except behaviors recognized automatically.

## To choose a variable

1. In the Analysis Results and Scoring pane, click the **Dependent Variables** tab.




2. Click the **Show/Hide** button on the toolbar, and select **Show Dependent Variable**.
3. Select the variable you want to monitor during the trial. Make sure that you specify the correct body point and statistic.

   **NOTE** You cannot select a Free interval variable to view during acquisition.

Below you find four examples.

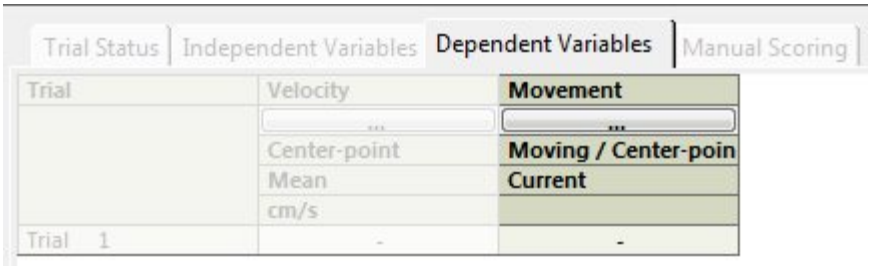
## To remove a variable

On the **Dependent Variables** tab of the Analysis Results and Scoring pane, right-click anywhere in the variable column and select **Delete**.

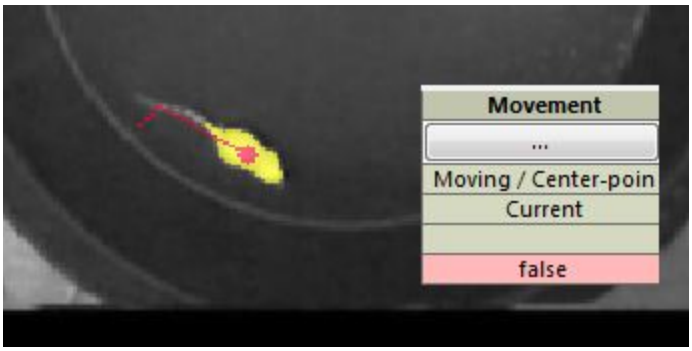
## Open field: view the Movement variable

In this example, the aim is to calibrate the *Movement* variable in order to detect bouts of locomotor activity. *Movement* is based on the subject's speed.

1. Follow the procedure above to choose Movement. Click its properties button. On the **Trial Statistics** tab, choose **Current**.




2. During the trial, watch the subject and monitor the current value of Movement in the Dependent Variables tab. Note when the behavior of the subject and Movement do not match.
   - If the subject is walking and the cell under Moving-Current says false, you must reduce the Start velocity and Stop velocity thresholds.




   - Conversely, if the subject is still and the cell Moving-Current says true, you must increase those thresholds. Note that only displacement in space of the animal should be scored as Moving, not other movements like grooming or body axis curling.
   - If there are many rapid transitions false-true-false but the animal seems not to change its behavior, then you can increase the difference between Start velocity and Stop velocity, or increase the Averaging interval, for example from 1 to 3.
3. Click the properties button for **Movement** and adjust the settings accordingly.

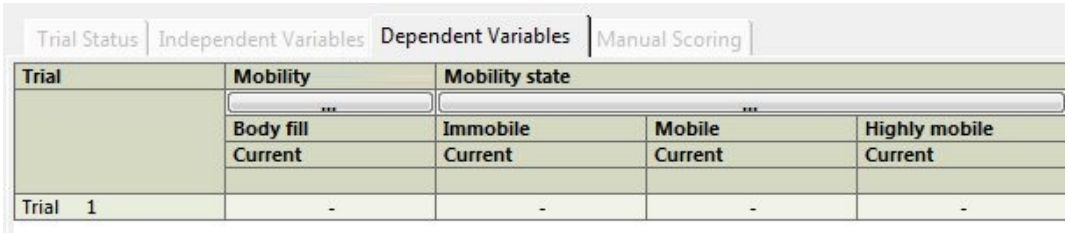

4. Check again the match between behavior and the values in the **Movement** cell. If necessary repeat steps 3-4 until you have a good agreement between the two.

See also Movement in the Analysis profile.

## Porsolt swim test: view the Mobility state variable

In this example, the aim is to calibrate the *Mobility state* variable in order to quantify swimming behavior. *Mobility state* has three possible states and is based on the temporal change in the subject's detected area.

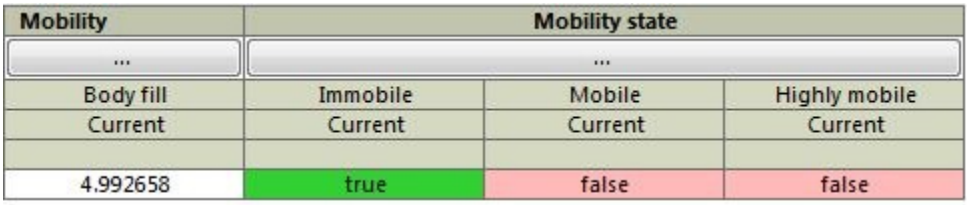
1. Add Mobility and Mobility state. For both variables select **Current** as a Trial Statistic, and leave the other settings as they are.




2. During the trial, watch the subject and monitor the current values of Mobility and Mobility state in the Dependent Variables tab. Note when the behavior of the subject and Mobility state do not match.

   First, focus on the mobility thresholds, then on the averaging interval.

   For example, to calibrate the Immobile state, watch the subject and check the value of Immobile-Current. If the subject swims (or struggles) and Immobile-Current is true, take note of the running values of Mobility. This tells you how much the animal's area changes with time. You must set the Immobile below threshold smaller than those values.




   The Immobile-Current value is true (second column), and the rat in the video is swimming. Mobility (first column) shows that the change in the area is around 5%. For correct scoring of the Immobile state, the Immobile below threshold of Mobility state should be set below 5%.

3. Click the properties button for Mobility state and change the threshold accordingly.


4. Watch the subject again and monitor the value of Immobile-Current. If the subjects moves and Immobile-Current says true, the threshold must be lowered further.

- At some point the animal still floats, something you would like to score as Immobile. Check that Immobile-Current is true. If not, the corresponding threshold must be increased slightly.
- If there are rapid transitions between Immobile, Mobile, Strongly Mobile, that cannot be accounted for in the video, you can increase the difference between the two thresholds, and/or increase the Averaging interval.

5. Repeat the steps above to adjust the Highly mobile above threshold. The aim is to obtain Highly mobile-Current equal to true only when the animal struggles.
6. Visualize the trial (Plot Integrated Data) to view the events of Mobility state together with the video. You can always refine your thresholds in the Analysis profile to re-calculate the three states.

See also Mobility state in the Analysis profile.

## Fear conditioning: view Activity state

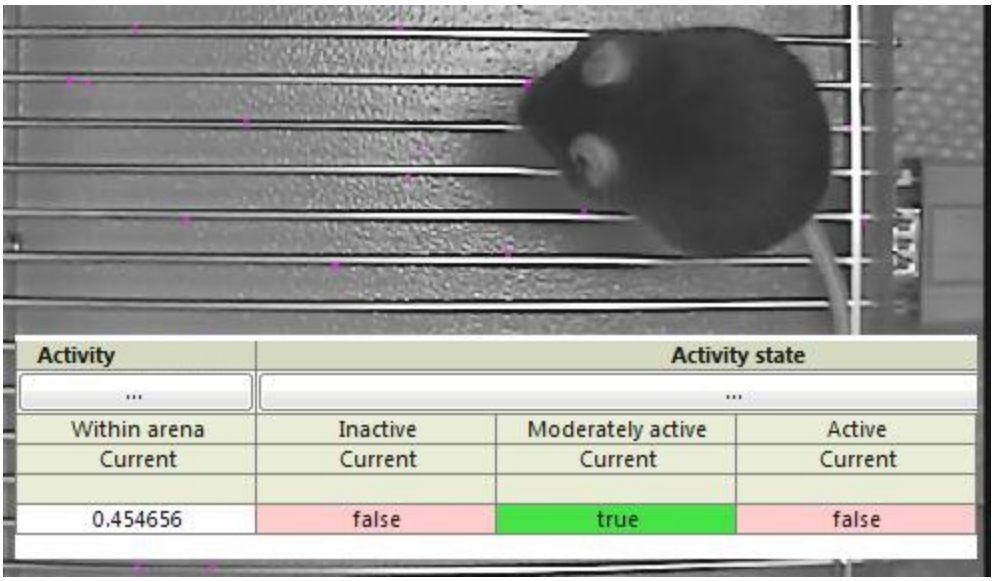
In this example, the aim is to calibrate the *Activity state* variable in order to quantify activity levels and freezing. *Activity state* has four possible states and is based on the change in the pixels in the whole arena.

1. Select **Activity analysis** in the Experiment Settings and define the Activity settings in the Detection Settings.
2. Add Activity and Activity state to the **Dependent Variables** tab of the **Analysis Results and Scoring** pane.
3. Choose the number of states (minimum two and maximum four).
4. Leave the threshold values as they are, and set the minimum duration of a state. For example, if you think that inactivity should last at least 0.2 s, then enter 0.2.

   Select **Current** as a Trial Statistic for both variables.

5. During the trial, watch the subject and monitor the current values of Activity state in the Dependent Variables tab. Note when the behavior of the subject and Activity state do not match.

   When the animal shows the behavior that should be scored as Inactive, monitor the running values of Activity. This tells you how much the video image area changes with time. If the value of Inactive-Current is false, you must reduce the Inactive below threshold smaller than those values.




In the example above, the mouse in the video is still, but the Inactive-Current value is false (second column). Activity (first column) shows that the change in the pixels is around 0.5%. For correct scoring of the Inactive state, the Inactive below threshold should be set below 0.5%

6. Click the properties button for Activity state and change the threshold accordingly.


With the new threshold, if episodes of inactivity are not detected, then adjust the threshold in the other direction.

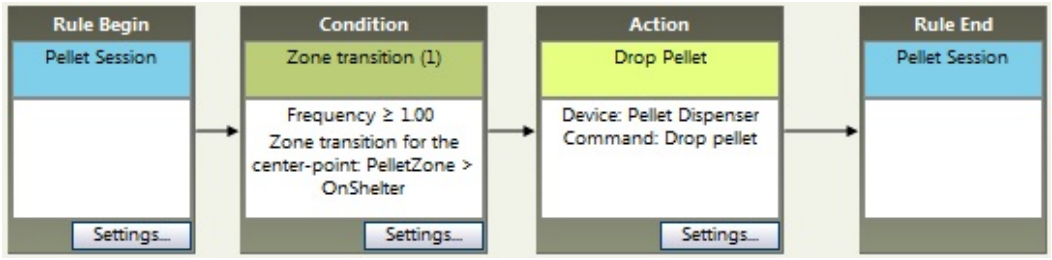
7. If you have more than two states, repeat the steps above to adjust the other thresholds. The aim is, for example, to obtain the value of Highly mobile-Current equal to true only when the animal struggles.

See also Activity state in the Analysis profile.

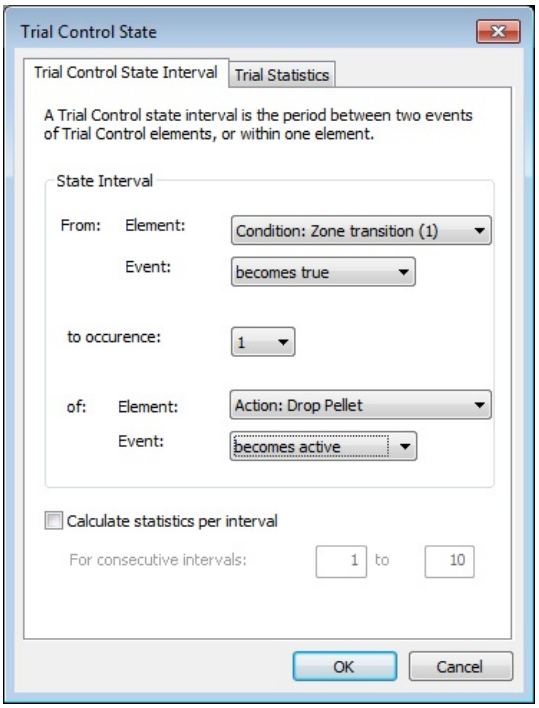
## Conditioning test: view a Trial Control State variable

In this example the aim is to test whether a pellet is dropped when the animal enters the trigger zone. This is also useful to check that the zone is defined correctly.

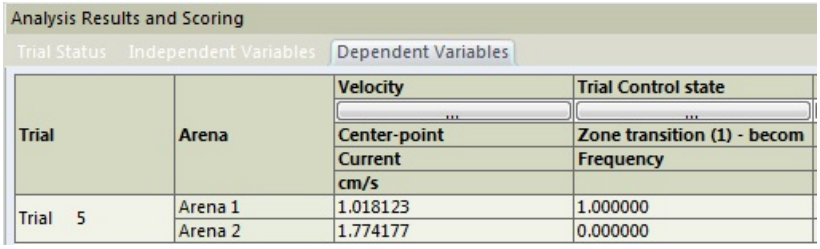
1. Define the Trial Control rule with a Zone transition Condition to define the transition to the trigger zone and an Action to let the pellet dispenser drop a pellet. See the figure below for an example.




2. Open the Acquisition screen.
3. Choose **Show/Hide** > **Show Dependent Variable** > **Trial Control State**.
4. Define the Trial Control State as shown in the figure below. Select **Frequency** in the **Trial Statistics** tab.




5. Start the trial and open the Dependent variables tab of the Analysis Results and Scoring pane to view the Trial Control State. If the animal moves to the trigger zone and a pellet is dropped the Frequency increases with 1.




| Trial | Arena | Velocity | Trial Control state |
|---|---|---|---|
| | | ... | ... |
| | | Center-point | Zone transition (1) - becom |
| | | Current | Frequency |
| | | cm/s | |
| Trial 5 | Arena 1 | 1.018123 | 1.000000 |
| | Arena 2 | 1.774177 | 0.000000 |

For simple Trial Control events, like the animal entering a zone, define a Trial Control Event instead of a Trial Control State.

# The data acquisition screen

## Learn about

- The video window
- The Playback Control window
- Analysis results and scoring pane

# The video window

## Show and hide the Video Source

Click the **Show/Hide** button on the toolbar and select **Video Source**.

If you choose not to display the video source, you can still display the center-point and other features of the subject to monitor the tracking.

Displaying the video footage requires significant processor power. If viewing the video footage during acquisition is not necessary in your experiment, deselect **Video Source** to reduce processor load and consequently increase your maximum attainable sample rate.

## Show/hide the overlay text

1. Click the **Show/Hide** button on the toolbar and select **Text Features**.
2. Select **Hide Information** or **Show Information**.
3. If you select **Show information**, choose one of the following:

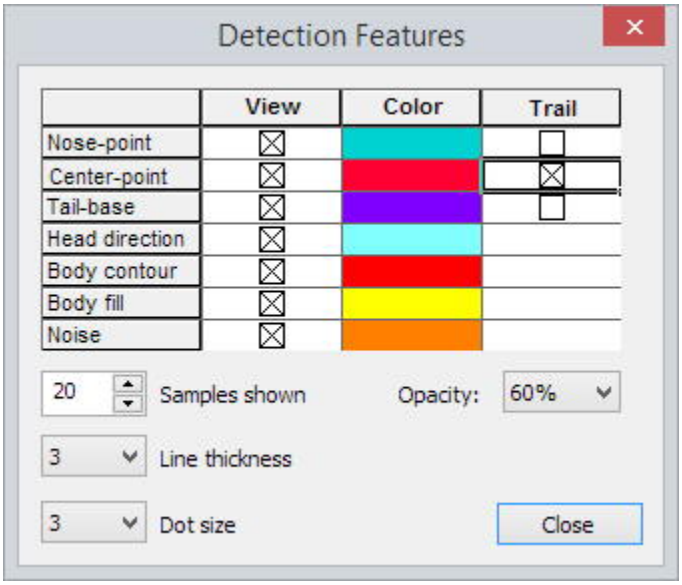
   **Per Arena** or **Per Trial**.

4. Select whether to show the trial name, acquisition status and/or acquisition elapsed time. Furthermore you can select the text color and opacity.

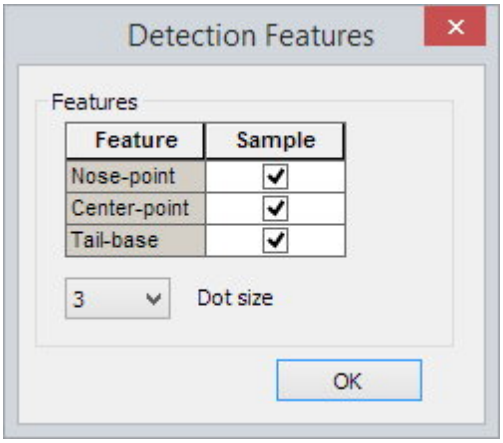
If no trial has been planned, the Video window shows **No Trial Planned**.

## Show/hide body points and other track features

1. Click the **Show/Hide** button on the toolbar and select **Detection Features**.
2. Select the features you want to display and their colors and opacity.
3. Adjust the **Line thickness** and the **Dot size**. Check the result in the video window, then click **Close**.

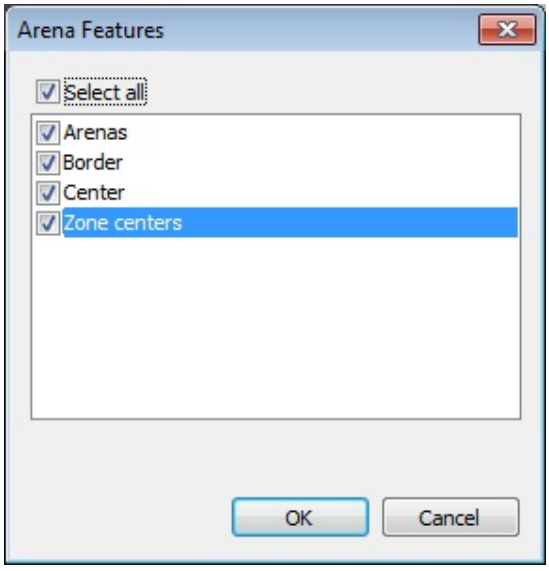



When you are in the Manual Scoring screen, you can only choose which body point to show and adjust the dot size.

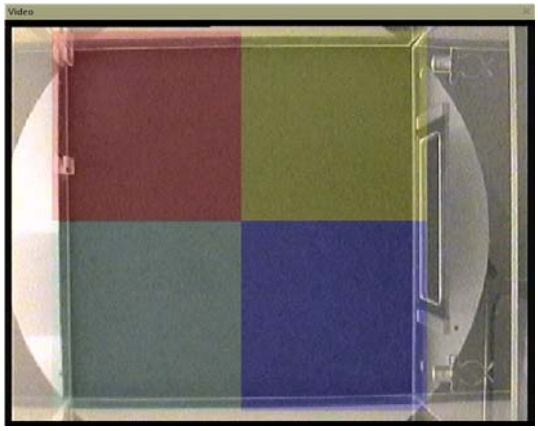



## Show/hide arenas and zones

1. Click the **Show/Hide** button on the toolbar and select Arena Features.
2. In the Arena Features window, select the Arena Features you want to display.




In this example, the four quadrants of an open field have been selected for display.




The arenas and zones are highlighted in the colors selected in the currently active Arena Settings. If you want to change the color of a zone or arena, open those Arena Settings, and change the fill color.

## Acquisition status

You can check the status of acquisition in the Video window:


- **Trial number**. Displayed as long as the recording starts. If you carry out batch acquisition, the remaining number of trials is shown in brackets.
- **Ready for start**. Displayed until data recording starts.
- **In progress**. Displayed as long as recording lasts. This text is displayed if you selected to show the text features **Per trial**.
- **Acquiring**. Displayed as long as recording lasts. This text is displayed if you selected to show the text features **Per arena**.
- **Timer**. Displayed as long as recording lasts.
- **No Trial Planned**. Displayed when no trial is planned to the trial list. To acquire data, add a trial, or in the Trial List change the trial status.
- **Starts in**. Displayed only when you track live in a series of trials. It shows the time until acquisition of the next trial starts.
- **Recording video**. Displayed when you record video with the option **Save video only, track later** selected.

# The Playback Control window

## Show/hide the Playback Control window

Click the **Show/Hide** button on the toolbar and select Playback Control to show/hide the Playback Control window.

Use the Playback Control to:

- Start and stop trials, and add/skip trials in your list.
- Position video files on the frame you require (when you track from video files).

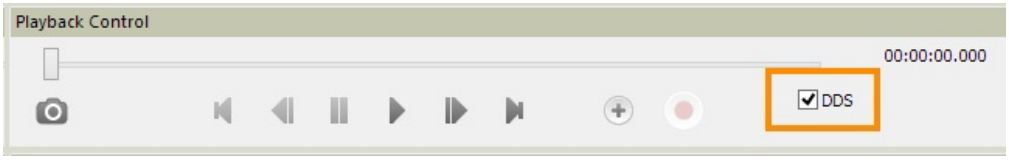
## Playback functions

For the description of the playback control buttons, see Playback Control, Acquisition and Visualization.

**NOTE** Clicking the playback control buttons does not result in acquiring data!

## DDS (Detection determines speed)

Detection determines speed is only available if you track from video. When you select this option, during acquisition images from the video file are analyzed at the maximum speed that the processor can handle. EthoVision varies the speed depending on how much data it has to process at each frame.




We recommend to keep this option selected, so that video frames are not missed with high processor load.

**IMPORTANT** Do not select **DDS** when you score behaviors manually or determine thresholds.

## Why use Detection determines speed?

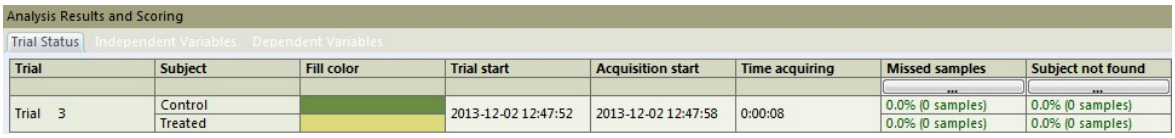
- To track faster when EthoVision XT can process images at a speed higher than the regular playback speed.

If you select this option, the program tracks at a speed higher than normal (1x). For example, when you use a fast detection method such as Gray scaling.

- To ensure that no sample is missed when tracking is very demanding.

  If you select this option, when EthoVision cannot keep up with the regular playback speed it tracks at a speed lower than normal (1x) in order not to miss any sample. For example, when you track from high resolution video, in many arenas and with a high sample rate combined with a detection method that requires significant processor time.

# Analysis results and scoring pane




## Show/hide the Analysis Results and Scoring pane

Click the **Show/Hide** button on the toolbar and select **Analysis Results and Scoring**.

**IMPORTANT** You can reduce processor load, for example when tracking from many arenas, by closing the Analysis Results and Scoring pane. To do so, from the **Show/Hide** menu deselect **Analysis Results and Scoring**.

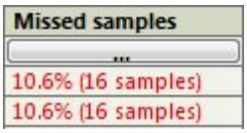
## Trial Status tab

Shows information about the current Trial (to be acquired or being acquired). If no trials are planned, the Trial column says No Trial planned.

- **Fill color** (only visible when tracking multiple subjects per arena). Shows the color associated with each subject. You can assign a new color in the Subject identification settings.
- **Trial start**. Shows the time the current trial has started. If the current trial has not started yet, Trial start shows '00:00:00'.
- **Acquisition start.** Shows the time that tracking has started. If the current trial has not started yet, Acquisition start shows '00:00:00'.

  If you track from video files, Trial start is the time that the video was recorded. Acquisition start is the Trial Start + the video time elapsed at the start of data recording.
- **Time acquiring**. The time elapsed since the start of data recording for a subject in a specific arena. Time acquiring can differ per arena because Trial Control is applied in each arena independently.

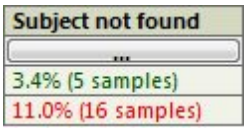
  The real time information is only available after you start the trial and until you stop it.
- **Missed samples**. The percentage and the number (in parentheses) of samples missed up to the current sample.




- **Interpolated samples**. The percentage and number (in parentheses) of missing samples that were interpolated.

  This statistic is only available when in the Detection Settings under **Smoothing** the option **Missing sample interpolation** is **On**. See Dropped frames correction in Advanced detection settings: Smoothing

- **Subject not found**. The percentage and the number (in parentheses) of samples in which the subject was not found up to the current sample.




### *To adjust the warning color thresholds*

When the values under Missed samples, Interpolated samples and Subject not found exceed a specific threshold, they are shown in red. This helps you monitoring the quality of tracking during acquisition.

To change a threshold, click the button under one of the headers.

### *Detection statistics after acquiring data*

After acquisition you can view the proportion of missed samples and samples in which the subject was not found in different parts of the software.

- In the Trial list, click **Show/Hide** on the toolbar and select **Variables** and select **Missed samples** and/or **Subject not found**.
- In the Statistics and Charts screen, click **Show/Hide** on the toolbar and select **Independent Variables** and select **Missed samples** and/or **Subject not found**.
- In the Track Visualization or in the Heatmaps screen, click **Show/Hide** on the toolbar and select **Layout**. Under **Available**, drag **Missed samples** and/or **Subject not found** to the On Columns or On Rows box.

## Independent Variables tab

Analysis Results and Scoring

Trial Status | Independent Variables | Dependent Variables

| Trial | Subject | Acquisition status | Dose | Day | Marker color |
|---|---|---|---|---|---|
| | | | | | |
| Trial 1 | Control | Acquired | 0.0 mg/Kg | 1 | Green |
| | Treated | | 0.5 mg/Kg | | Yellow |
| Trial 3 | Control | Acquired | 1.0 mg/Kg | 1 | Green |
| | Treated | | 1.0 mg/Kg | | Yellow |
| Trial 4 | Control | Acquired | 0.0 mg/Kg | 1 | Green |
| | Treated | | 0.0 mg/Kg | | Yellow |
| Trial 5 | Control | Ready for start | | | |
| | Treated | | | | |

The **Independent Variables** tab is a simplified version of the Trial List. It shows the user-defined independent variables and the Acquisition status for the acquired trials and the planned trials.

Row colors

- Before acquisition, the current trial (about to be acquired) is highlighted in green. Acquisition status says Ready to start.
- During acquisition, the current trial is highlighted in red. Acquisition status says Acquiring.

## Dependent Variables tab

Analysis Results and Scoring

Trial Status | Independent Variables | Dependent Variables | Manual Scoring

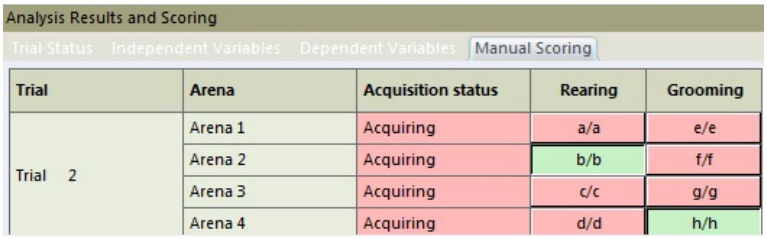
| Trial | Arena | Velocity | Movement | |
|---|---|---|---|---|
| | | ... | ... | |
| | | Center-point | Moving / Center-poin | Not Moving / Center- |
| | | Current | Cumulative Duration | Cumulative Duration |
| | | mm/s | s | s |
| Trial 14 | Arena 1 | 15.116163 | 0.033333 | 6.866598 |
| | Arena 2 | 0.293859 | 0.000000 | 6.899931 |
| | Arena 3 | 1.493565 | 0.099999 | 6.799932 |
| | Arena 4 | 0.727787 | 0.000000 | 6.899931 |

In the **Dependent Variables** tab you can view any dependent variable in real time during acquisition, for each subject and arena. By default, it shows the following variables: Velocity and Movement.

To view more variables during a trial, see Choose a variable to view real-time.

**IMPORTANT** Adding variables may reduce performance and increase missing samples. Select only the variables and its properties you are interested in. For example, if you are interested in monitoring the Immobile state during a Porsolt swim test, add the **Mobility state** variable, then select Immobile and deselect the other two states under **Calculate for state** in the Mobility State properties window.

# Manual Scoring tab




| Trial | Arena | Acquisition status | Rearing | Grooming |
|---|---|---|---|---|
| Trial 2 | Arena 1 | Acquiring | a/a | e/e |
| | Arena 2 | Acquiring | b/b | f/f |
| | Arena 3 | Acquiring | c/c | g/g |
| | Arena 4 | Acquiring | d/d | h/h |

See Score behaviors manually during acquisition

# External Data

## Main topics and tasks

# Introduction to External data

## Learn about

- Work with external data
- Enable DAQ co-acquisition in EthoVision XT
- On-Off vs. Time Code (TCAP) synchronization signal

# Work with external data

## External data types

You can import external data, like for example heart rate, EEG, GSR or accelerometer data into EthoVision XT and synchronize these data with the tracks. EthoVision XT can import data in ASCII format with a constant sample rate or a time stamp column in the file.

**NOTE** It is also possible to import European Data Format (EDF) Files, or BioSemi Data Format (BDF) files, which is the 24 bit version of EDF, into EthoVision XT. Import of EDF+ or BDF+ files, which may contain annotations, is not supported.

## Procedure

Working with external data in EthoVision XT involves some basic steps:

1. When possible, connect the EthoVision XT computer to one of the input channels of the external DAQ system. This allows you to synchronize EthoVision XT and the external data automatically. Contact Noldus for a cable that serves this function. See Connect EthoVision XT to the DAQ system
2. Enable DAQ co-acquisition in EthoVision XT.
3. Acquire the trials, while the DAQ system acquires its own data.

   See Acquire a trial live for the general procedure.
4. After data acquisition, import the external data file(s) and assign them to a Trial, Arena, or Subject.

   See Import a complete external data file at once

   Tracking data and external data are automatically synchronized in case you used DAQ co-acquisition. If not, you can use manual synchronization.
5. Visualize external data together with video and acquired tracks.

## Notes

- The external data are saved on the computer dedicated to the DAQ device, not on the EthoVision XT computer.
- If you want to visualize tracks according to the values of a dependent variable, make sure that you specify that dependent variable in the active Analysis profile. To edit a profile, click it in the Experiment Explorer and make the necessary changes.

# Connect EthoVision XT to the DAQ system

## Aim

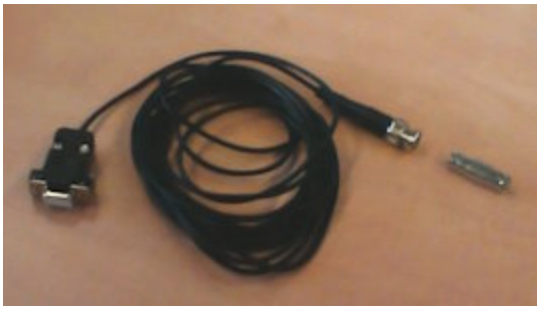
To ensure that EthoVision XT sends a synchronization signal to the DAQ system during data acquisition.

## Prerequisites

You must have:

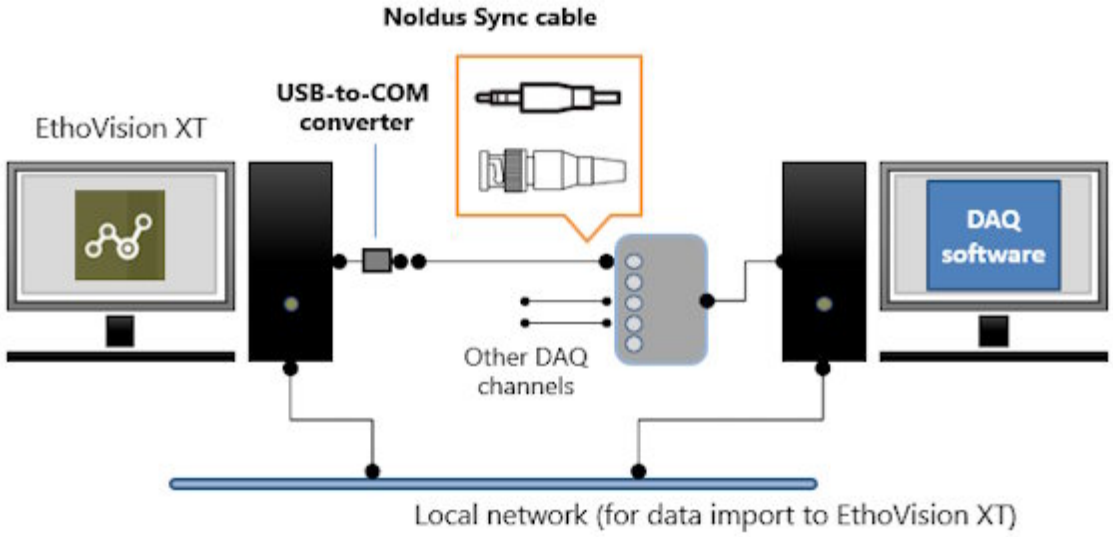
- A free USB port on your EthoVision XT computer.
- A USB-to-Serial (COM) converter.

- A sync cable from Noldus. This cable has connectors that depend on the DAQ system you have. Contact Noldus to purchase one.



**OPTIONAL** Connect the two computers through a local network for easy data transfer from the DAQ system to the EthoVision XT computer. See Import external data: General information

## Connection scheme




## Notes

- It is also possible to ensure synchronicity between EthoVision XT and the DSI Ponemah system without a sync signal. See Sync the computer clocks for EthoVision XT and DSI Ponemah

*See also*

- Enable DAQ co-acquisition in EthoVision XT

# Enable DAQ co-acquisition in EthoVision XT

## Aim

To program EthoVision XT to send a synchronization signal to the external DAQ system.

## Procedure

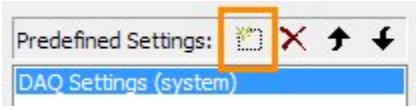
1. In EthoVision XT, open your experiment.
2. Choose **Setup** > **Experiment Settings**.
3. Under **Video Source**, choose **Live tracking** and select the **Enable DAQ co-acquisition** box. Click the **Edit** button.
4. Under **Predefined Settings**, select the item corresponding to your DAQ system, or create a new one (see below).

## Notes

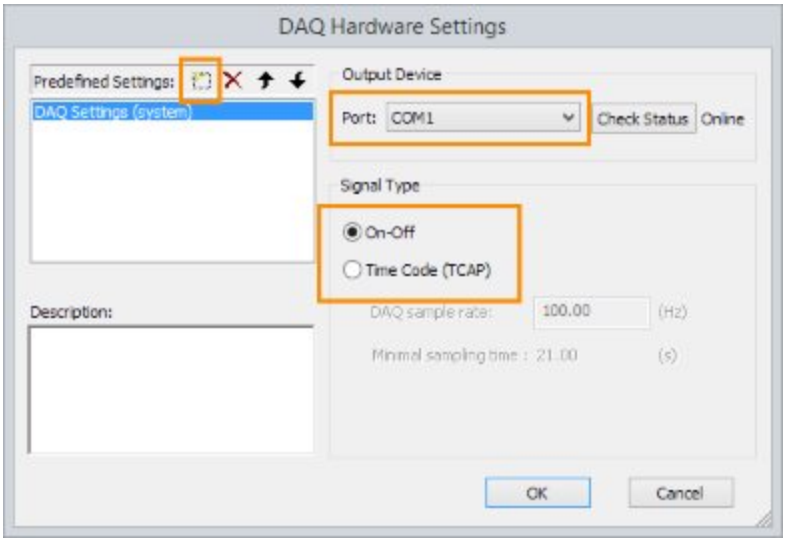
- When you have acquired data, the Experiment Settings are read-only (settings are grayed out).
- You can only co-acquire external data when you carry out Live Tracking.

## To create a new DAQ Hardware Settings profile

1. In the DAQ Hardware Settings window, click the **New** button.




2. A new field appears at the bottom of the list of predefined profiles. Type a name for the new profile.




3. Under **Output device**, select one of the COM ports from the list. This COM port is used to connect the EthoVision XT computer to the DAQ system.

   Click the **Check Status** button to check the availability of the port.

   It may be that either all COM ports on your PC are occupied, or your PC does not have a COM port. In those cases, contact Noldus for a USB-to-COM converter.

4. Under **Signal Type**, select:

   - **On-Off** if the external signals are sampled at low rate (less than 10 Hz) and without interruption (thus no scheduled sampling).
   - **Time Code** (TCAP) in all other cases. Enter the Sample rate (in Hz) with which you sample data on your DAQ system.

   If you want to sample data at a rate higher than 2 KHz, set 2000 Hz.

   Depending on the Sample rate, the Minimal sampling time changes; this indicates the minimum time the data should be acquired on the DAQ system for synchronization to work properly.

5. Click **OK**.

## To delete a DAQ Hardware Settings Profile

In the DAQ Hardware Settings window, select a Profile and click **Delete** or press **Delete**.

## To change the order of DAQ Hardware Settings Profiles

In the DAQ Hardware Settings window, select a Profile and click the **Move Up** / **Move Down** button or press ALT-arrow down / ALT-arrow up on your keyboard.

## To add a description to a DAQ Hardware Settings Profile

In the DAQ Hardware Settings window, select a Profile, click in the **Description** box and type in text. Click on another Profile and click **Yes** to save the Description.

*See also*

- On-Off vs. Time Code (TCAP) synchronization signal

# On-Off vs. Time Code (TCAP) synchronization signal

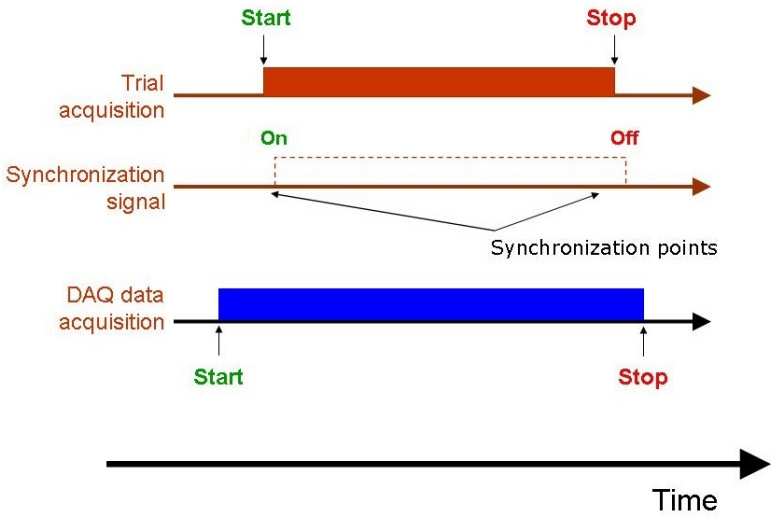
In order to synchronize external data and EthoVision XT tracking data, you can send out a synchronization signal with time information (SyncOut) from the EthoVision XT computer to the DAQ device. This signal is sampled by the DAQ computer and after acquisition imported into the EthoVision computer as a home coming signal where it is compared with the original synchronization signal.

On-Off and Time Code (TCAP: Time Code Auxiliary Parity) are two types of signals that reflect two ways of synchronizing external data (for example, heart rate) with tracking data.

## On-Off

This signal is sent from EthoVision XT to the DAQ device shortly after a trial is started and stopped. Note that the On and Off signal are not sent at the exact moments of trial start and stop. The difference in offset between the synchronization signal and reference signal is calculated at the start and at the end of the recording. In order for synchronization to be successful, the DAQ recording must start before starting acquisition in EthoVision XT, and end after stopping acquisition in EthoVision XT.

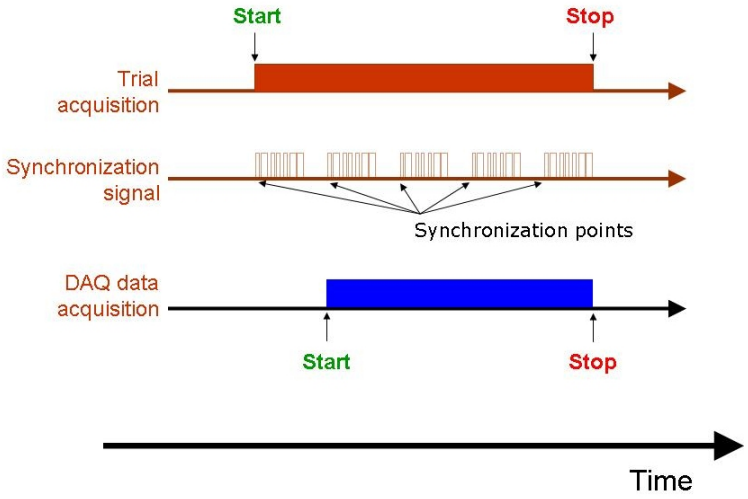
A dialog in EthoVision XT informs you to start and stop data acquisition on your DAQ device. Furthermore, the DAQ recording should not be stopped during acquisition in EthoVision XT, which means that you cannot use the On-Off signal when performing scheduled sampling (that is, starting and stopping DAQ acquisition at regular intervals).



The relationship between Trial acquisition, the On-Off synchronization signal from EthoVision XT and acquisition on the external DAQ system.

## Time Code

A series of bits called a *frame* is sent from the EthoVision XT computer to the external DAQ device. Each frame contains the current date and time and additional information. The difference in offset between the synchronization signal and the reference copy is calculated for several time points during acquisition (by default, for 10 time points), while the gain is calculated through regression analysis of offset values obtained at different points in time (that is, different frames). Therefore, synchronization of tracking and external data is more accurate using the Time Code signal because it is done for more than two offset points and not just at the start and stop of acquisition as with the On-Off signal.




The relationship between Trial acquisition, the Time Code synchronization signal from EthoVision XT and acquisition on the external DAQ system.

# Import external data in EthoVision XT

## Learn about

- Import external data: General information

## What do you want to do?

- Import a complete external data file at once
- Import one or more specific data sets
- Create a new custom import profile
- Edit a custom import profile

*See also*

- Examples of import profiles

# Import external data: General information

## External data files

To import external data you need to link it to an acquired Trial, an Arena or a Subject.

You can import external data with the following characteristics:

- It is stored in ASCII format.
- The sample rate is constant, or when it is not constant, the data file contains time stamps.
- The file contains at least 15 data lines. It preferably contains a header with information describing the data sets. This information about the data set, such as data set name, date and time information, sample rate, is typically stored in the header section of a DAQ export file
- You can import European Data Format (EDF) or BioSemi Data Format (BDF) data files. Import of EDF+ or BDF+ files is not supported.

*Notes*

- It is not necessary that external data are recorded when you do the tracking; for example, when you track from video files. However, you must ensure synchronization between the external data and the video file.
- You can only import external data into an acquired Trial. This is indicated in the Trial List by 'Acquired' in the System Variable Status column.

## Data sets within a file

An external data file can contain one or more data sets. For example, you test three rats, each in its own arena, and for each rat you have acquired ECG data. The resulting external data file contains three ECG data sets. In EthoVision XT you now have the option:

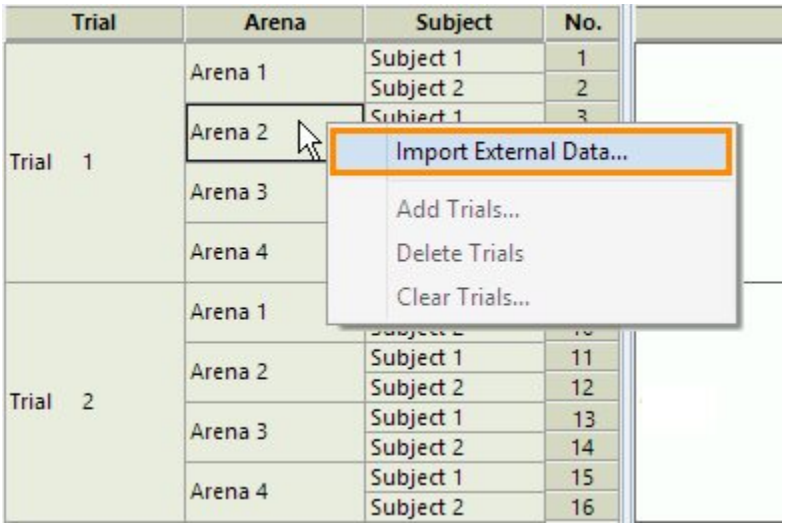
- to link the complete external data file (i.e. all three data sets at once) to a Trial, Arena, or Subject.
- to link one or more data sets from an external data file to a Trial, Arena, or Subject.

# Import a complete external data file at once

## To import an external data file

1. Choose **Setup** > **Trial List**.
2. Click the row for the trial you want to import the data to.
3. Do one of the following:

   To import data to a specific arena or subject within that trial, right-click that arena or subject and select **Import External Data**.




4. Next, you can either:
   - Select a predefined Import Profile. Select a predefined Import Profile from the list under **Files of type**. Next, locate the external data file, select the filename and click **Open**.
   - Create a new custom import profile.

## Notes

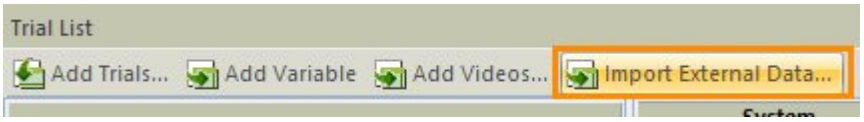
- To import specific data sets in an external data file in the Trial List click the **Import External Data** button. See Import one or more specific data sets

*See also*

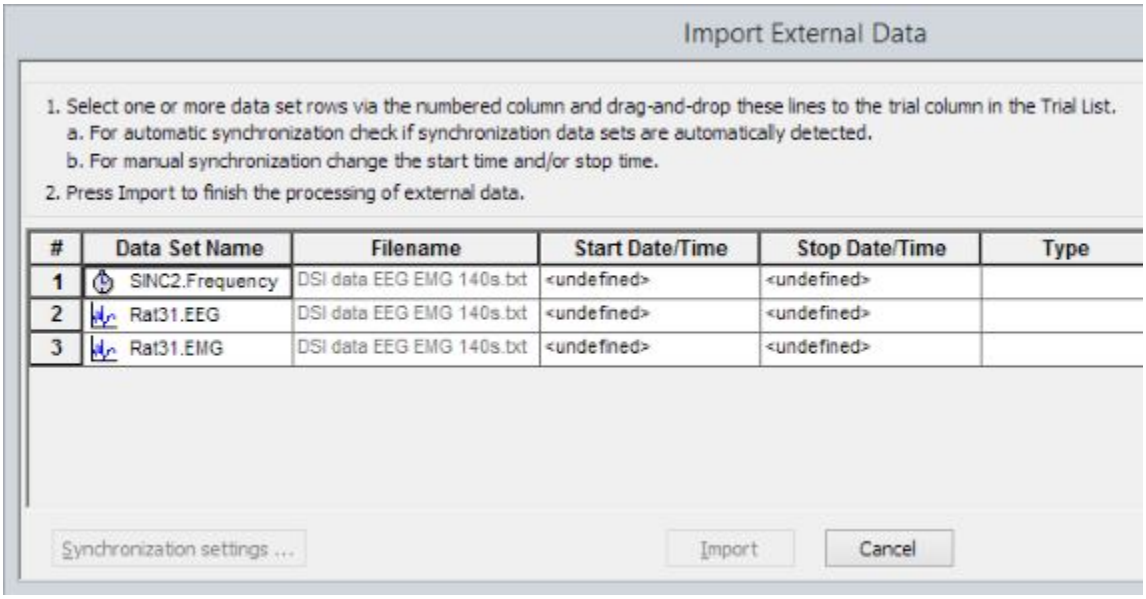
- Create a new custom import profile

# Import one or more specific data sets

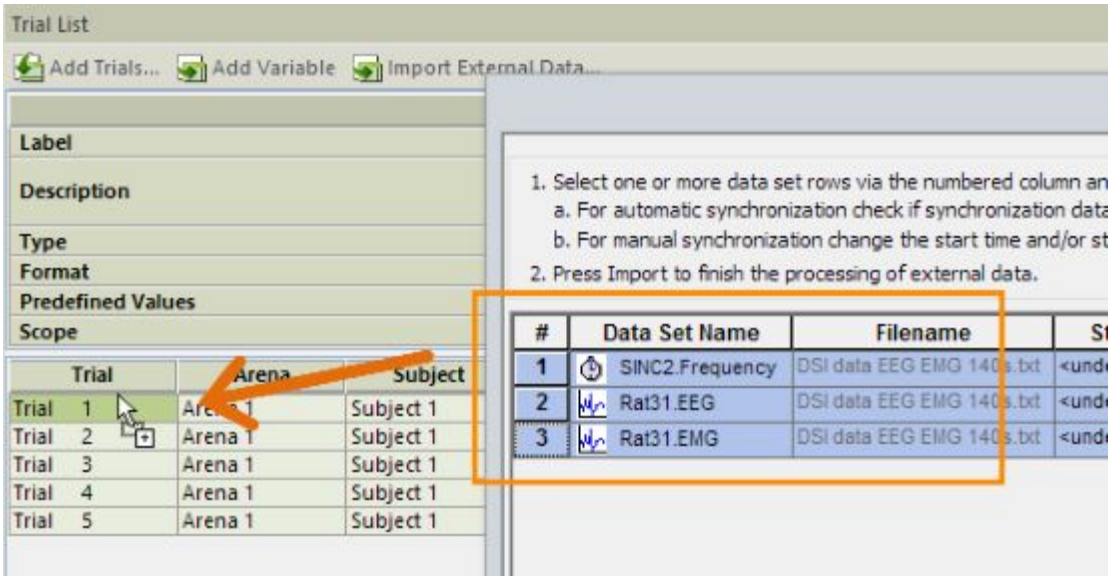
1. Choose **Setup** > **Import External Data**, or in the Trial List, click the **Import External Data** button.




2. Select a predefined Import profile from the drop-down list under **Files of type**. Next, locate the external data file, select the filename and click **Open**. A second Import External Data window shows one or more Data Set Names. The Trial list also appears.




3. To add external data to a Trial, select on or more Data Set rows and drag-and-drop it to the Trial list; you can choose to drag-and-drop external data to a Trial, Arena or a Subject (see the figure below).




4. Click the **Import** button in the Import External Data window to finish import.

EthoVision XT offers predefined Import profiles for a number of export formats from DAQ systems. If your file type is not in the list, create a Custom Import Profile.

# Create a new custom import profile

## Aim

To import external data as ASCII files when EthoVision XT does not have a predefined import profile (see step 4 in **Import a complete external data file at once**). With an import profile you create a template based on the structure of the external data file.

## Prerequisites

- Your license for EthoVision XT includes the External Data Module
- You exported your external data as ASCII files.

## Procedure

1. Choose **Setup** > **Import External Data**.
2. Click the **Custom Import Profiles** button and then the **New** button.
3. Browse to the external data file.
4. Build the import profile by specifying the structure of the data file. This is done by dragging and dropping cells. To make a selection undone, select the cell to which content was dropped and press **Delete**.

   Follow the procedure in these sections:

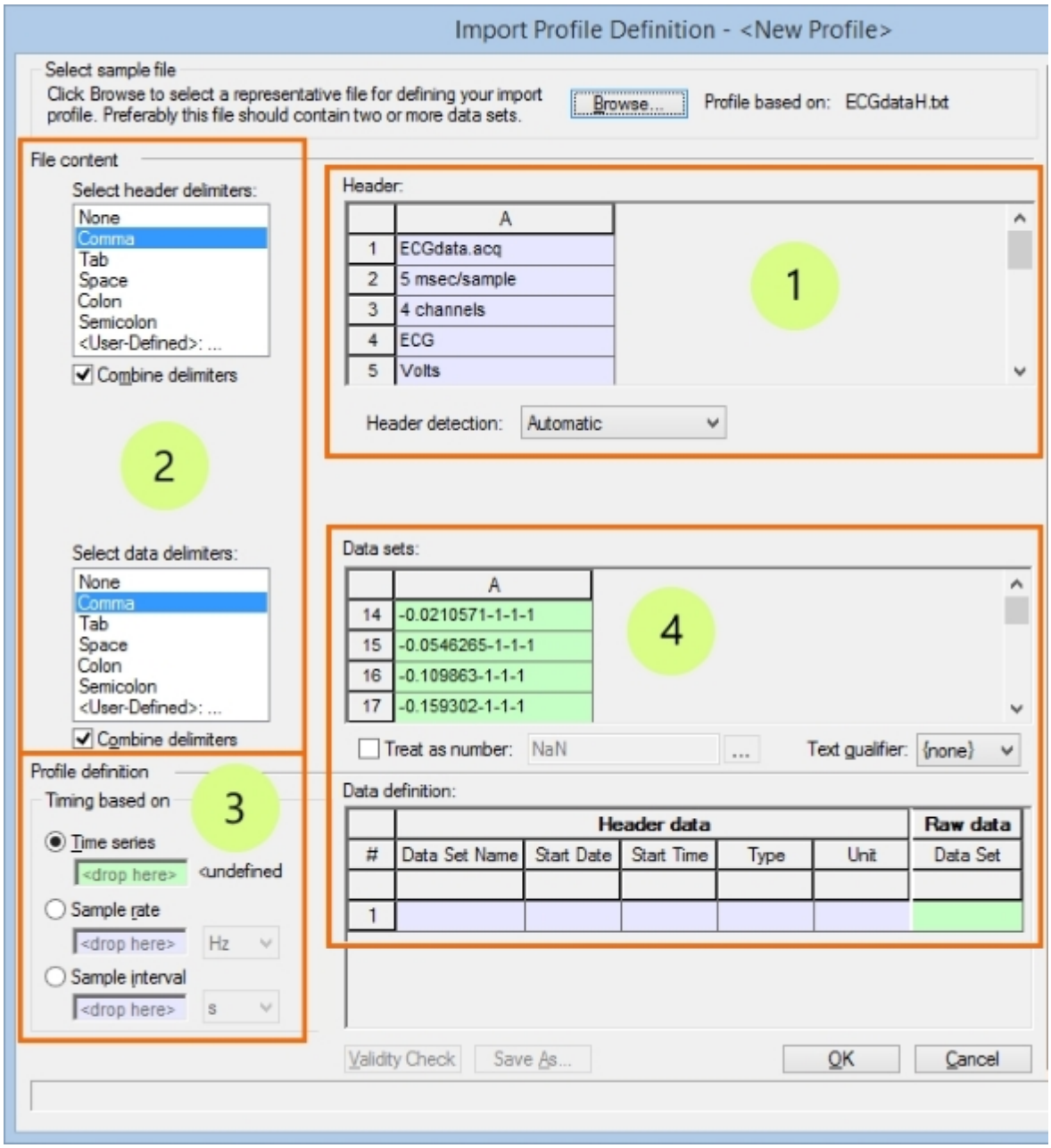
   1 - Header

   2 - Delimiters

   3 - Time information

   4 - Data
5. Import a text file with the new import profile

The numbers in this figure correspond with the numbers of the headers in the text below.




# 1 - Header

EthoVision XT usually automatically detects header and data information in the file. If not, specify which part of the data file contains the header. Choose an option from the **Header detection** list:

- **Automatic** (default). EthoVision XT is set to automatically detect the header and data sections in the data file. Choose one of the other options if automatic header detection does not work.

- **Specify tag**. Select this option if the data file has a variable number of header lines and the header always ends with the same word. Specify the phrase (with either nominal or numerical information) that indicates the end of the header part of the file. If necessary, also specify the number of rows between the header line that contains this phrase and the data.

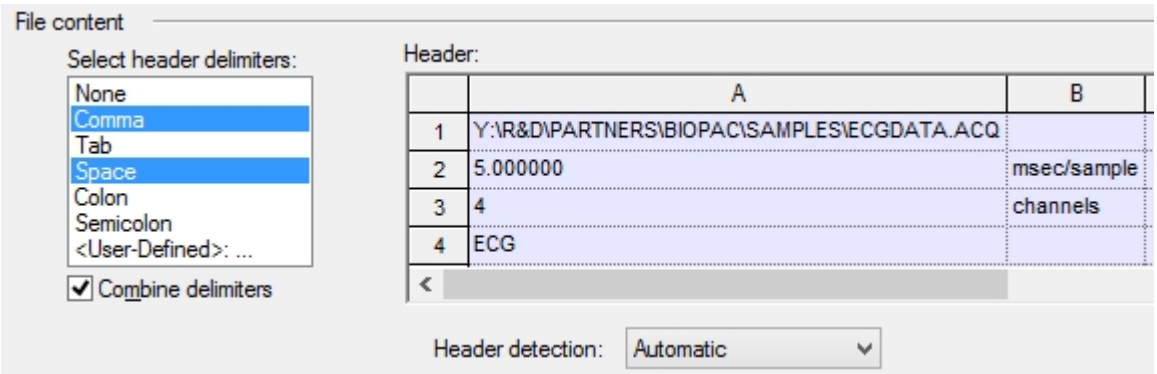
  **EXAMPLE** The header always ends with a line containing the text [Data]. After the line with this text there is always an empty line before the data starts. Enter [Data] in the End tag field and 1 in the Extra rows field.

- **Specify row number**. If the data file always has the same number of rows in the header, select this option and specify the number of header rows.

## 2 - Delimiters

EthoVision XT uses the comma as the default delimiter to separate text in the header and data sets. However, the data file may have other delimiters. If this is the case, select the correct ones from the **Select header delimiters** and **Select data delimiters** lists. You can also select multiple delimiters.

Some DAQ software enables you to select the type of delimiter when saving the DAQ data to an ASCII export-file. In that case you select the same delimiter in the **File content section** of the Profile Definition window. A comma or semicolon are advised as delimiters.

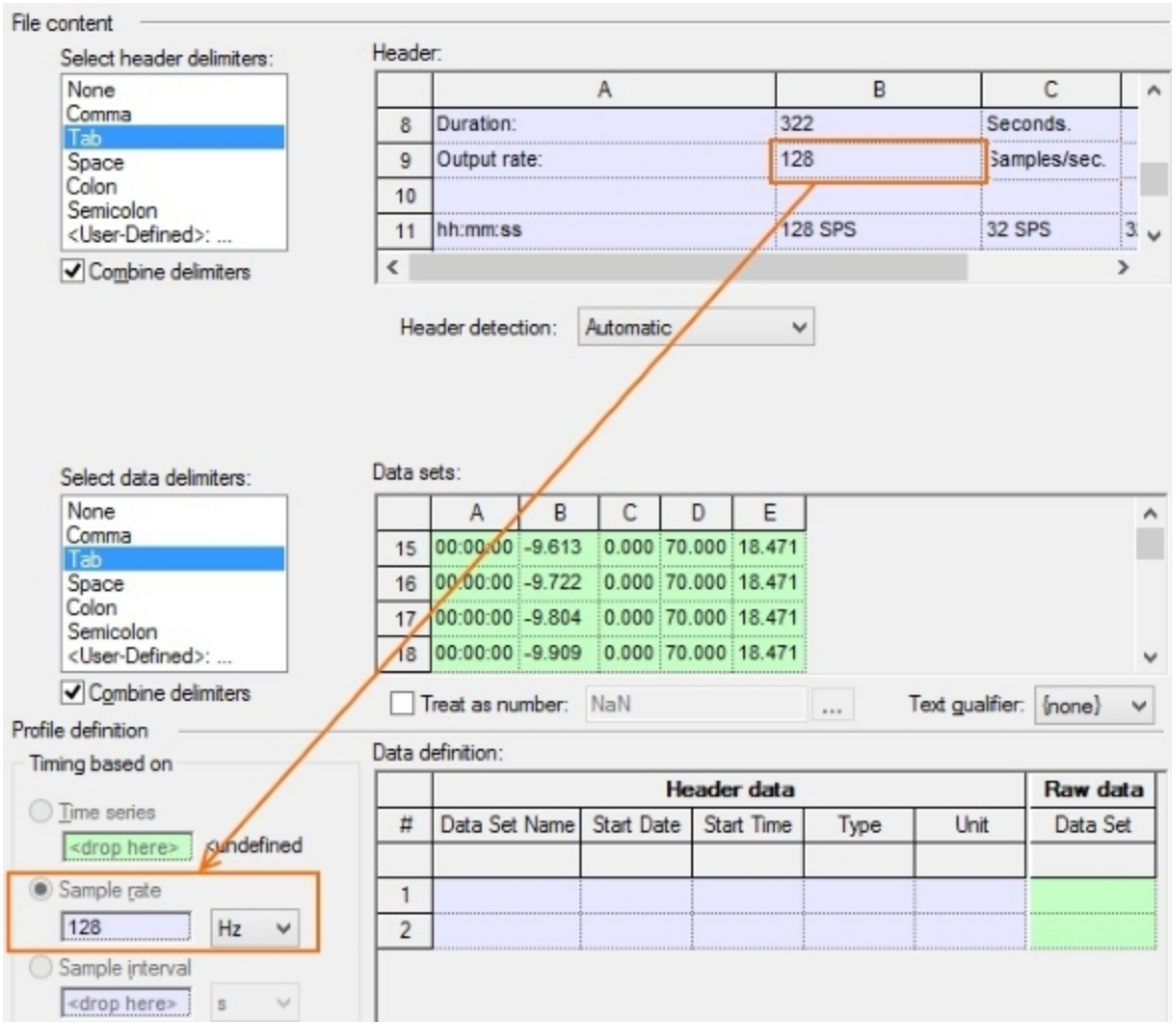



## 3 - Time information

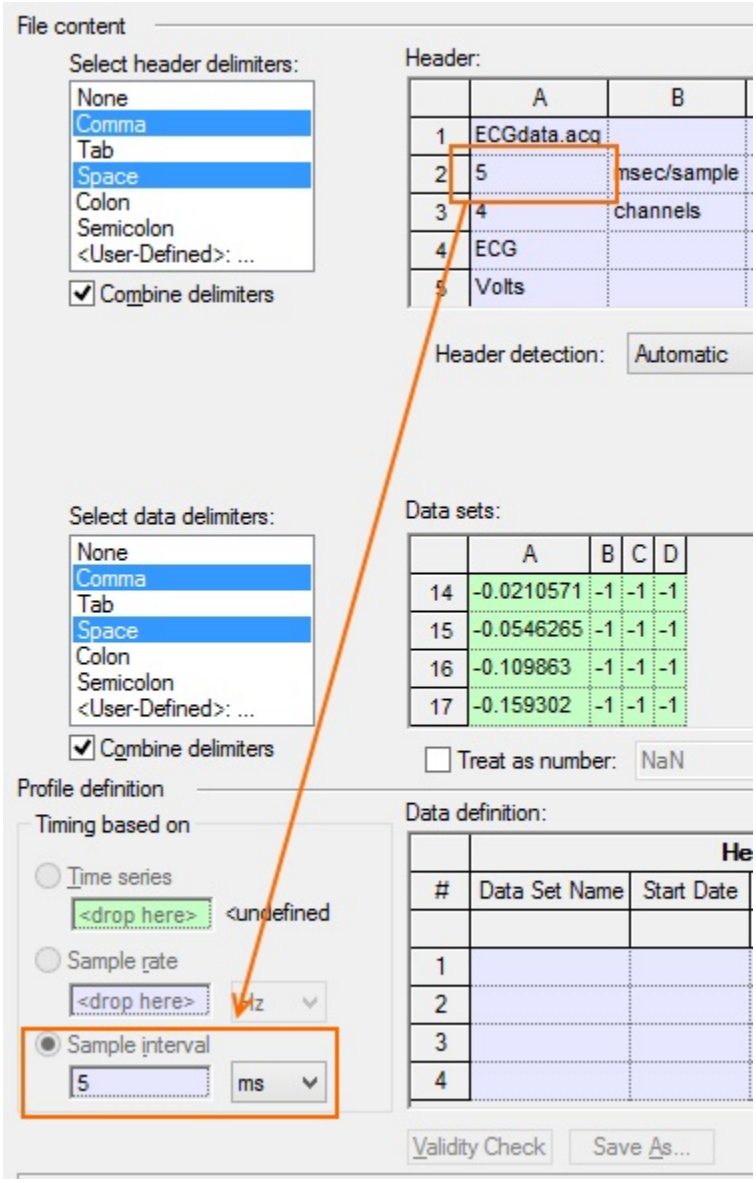
Specify which cell or column contains the time information. This can be a column with time stamps, a cell with the sample rate, or a cell with the sample interval, which is the time between samples. Drag and drop the cell or column with time information to the appropriate field in the **Profile definition** section.

1. **Sample rate**. If the header contains a cell with the sample rate, select the **Sample rate** button under **Timing based on** in the **Profile definition**

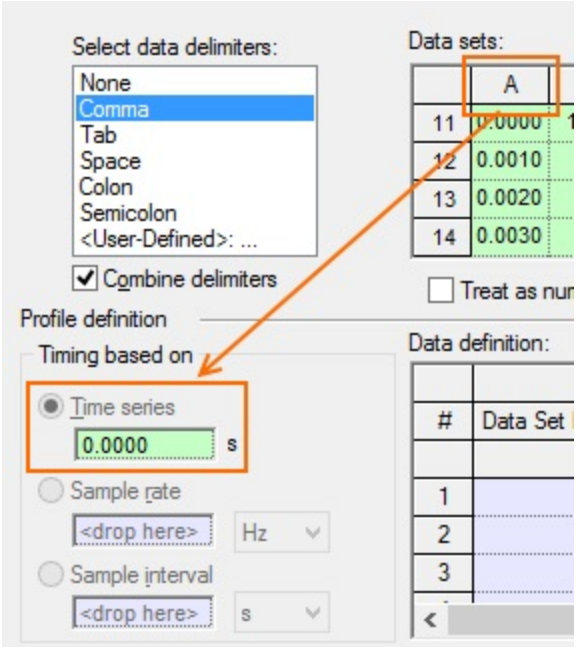
section. Drag the cell with the sample rate to the **Sample rate** box and select the appropriate unit (Hz or kHz) from the list.




2. **Sample interval**. If the header contains a cell with the sample interval, select the **Sample interval** button in the **Profile definition** section. Drag the cell with the sample interval to the **Sample interval** box. Select the unit of time from the list.

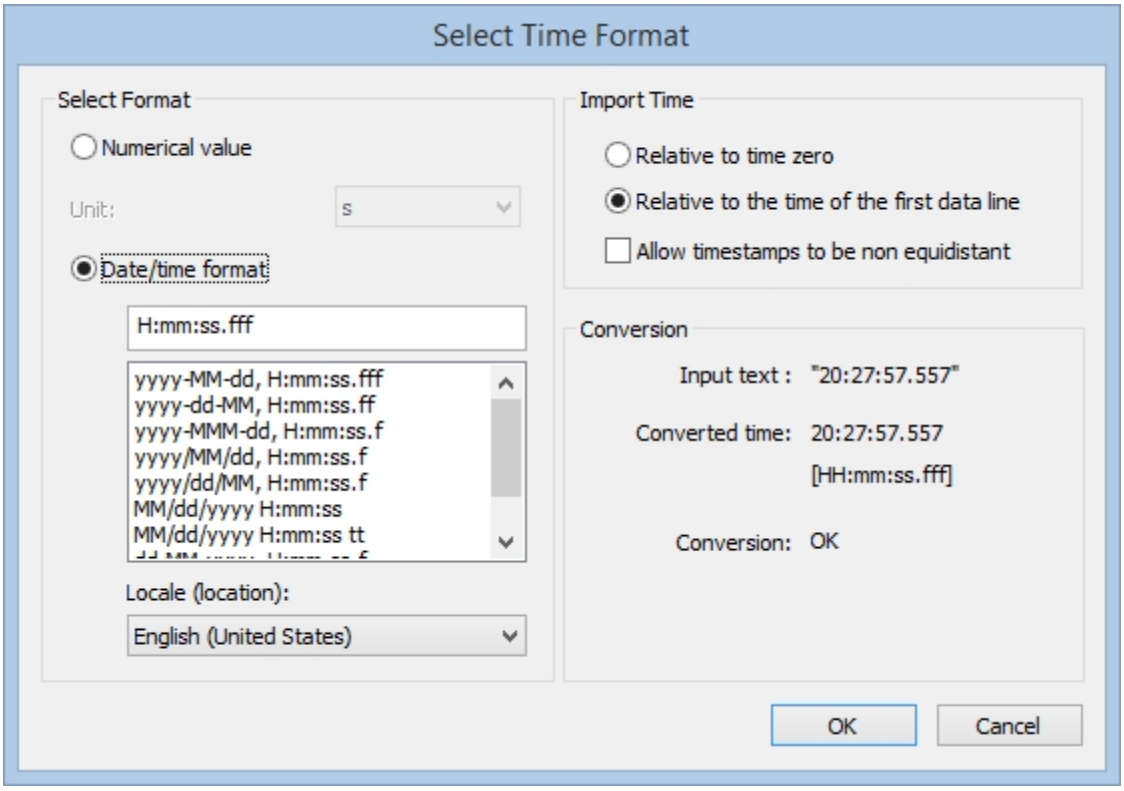



3. **Time series**. If the header does not contain information on sample rate, select the **Time series** button under **Timing based on**. Under **Data sets** in the **File content** section, select the column with time stamps and drag this to the **Time series** box. The column with time stamps now appears grayed.




The **Select Time Format** window opens. If the time matches one of the predefined formats, EthoVision XT automatically selects one. **Converted time** shows the conversion of the text to time and **Conversion** is **OK**.

You can also define your own format by typing an **H** for each number representing 'hour', an m for 'minute', an **s** for second and a **f** for each decimal of a second (see the next picture).



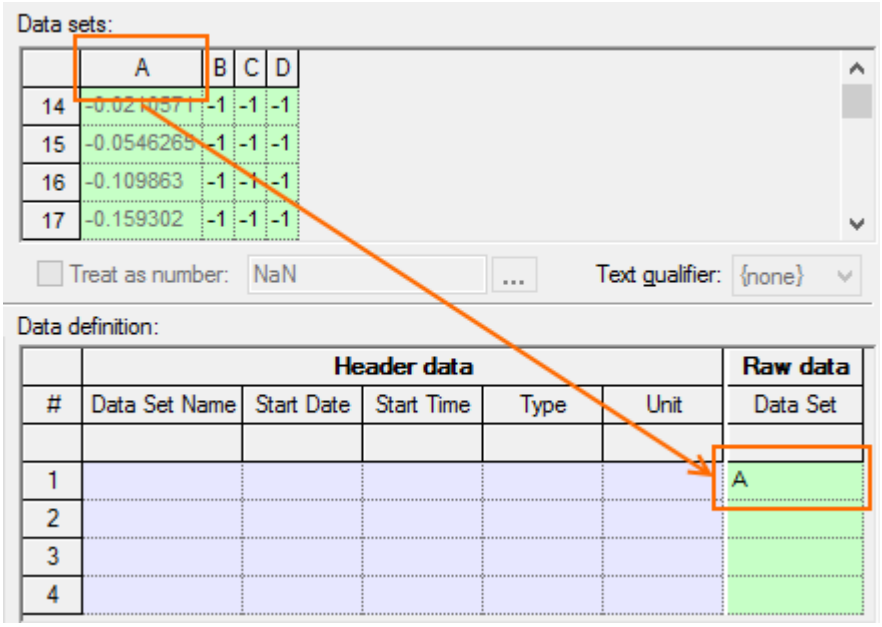

Under **Import Time**, select one of the options.

- **Relative to time zero**. Suppose the first row in the imported external data set has time 00:00:05. When the option Relative to time zero is selected, the time stamp of the first row of the imported data set will remain 00:00:05.
- **Relative to the time of the first data line**. Suppose the data set you want to import starts at 14:28:00 and has samples every 5 seconds. With the option Relative to the time of the first data line, the first row of the imported data will get the time stamp 00:00:00. The second row will have time stamp 00:00:05. The options under Import Time are grayed out when your time stamps contain the date the file was created. In this case the option Relative to time zero is used.
- Select the option **Allow timestamps to be non equidistant** when the time stamps do not represent regular intervals. For example, 0, 490, 572, etc...

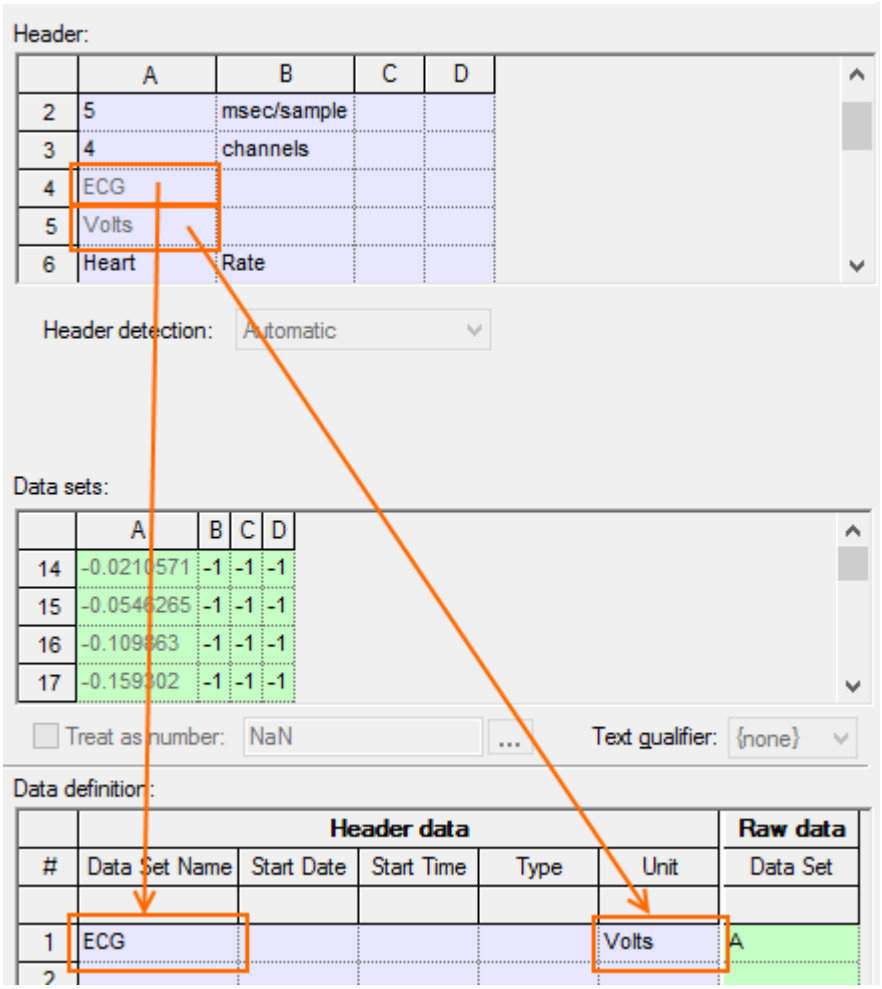
## 4 - Data

Under Data definition in the Profile definition section there are two parts. The lilac part is labeled **Header Data** where you drag cells to from the lilac **Header** section. The green part is labeled **Raw data** where you drag Data sets to from the green cells in the **Data sets** section.

1. Drag the first column under **Data Sets** to the first empty cell in the **Raw data** - **Data Set** column. As a result, the letter of the original column appears in the cell and the column in the **Data Sets** section is grayed.



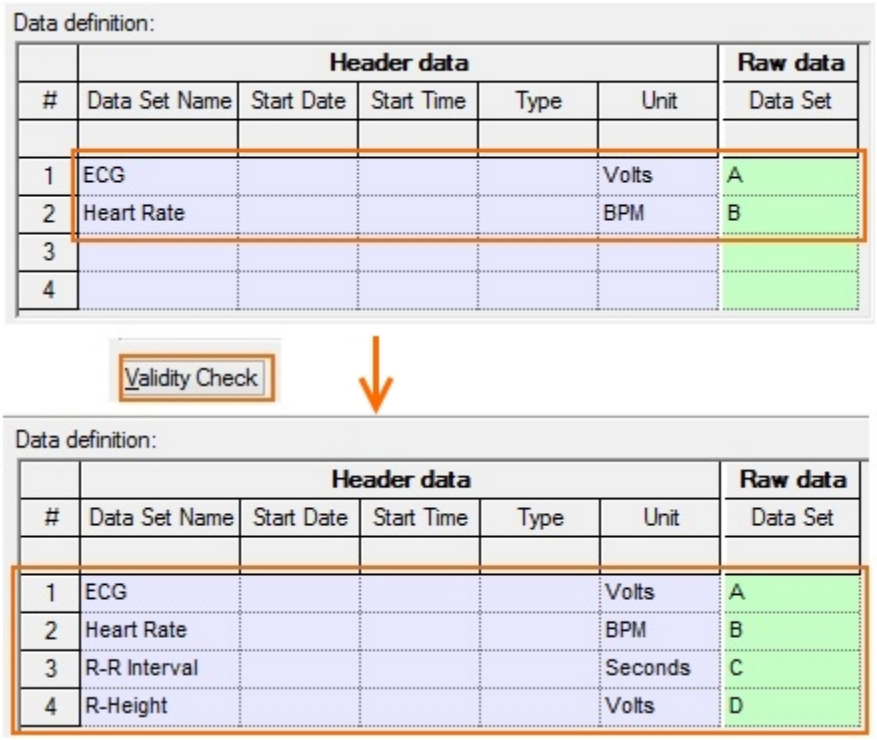

2. Now locate the data set name, the unit and, optionally, other information in the header and drag those cells to the **Header data** cells in the same row.




If you drag and drop the **Start Date** and/or **Start Time** a window opens in which you can define the format. Accept the default format, or see step 3 of 3 - Time information for the procedure to change it.

*Multiple data sets*

1. Follow the procedure above to define the next dataset. Once two datasets are defined, the **Validity Check** button becomes active.
2. Click this button. EthoVision XT now automatically fills the other rows in the **Data definition** field.



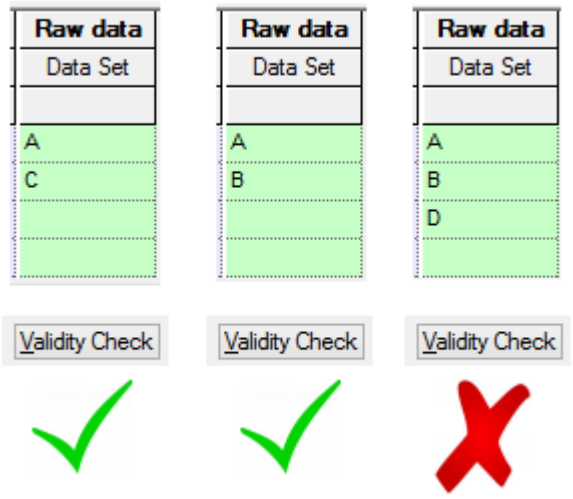

3. Check that the correct columns are selected.

*How the Validity check is applied*

EthoVision XT assumes that your header and data set info are ordered in a regular way in your external data file (e.g., left-right, with/without empty cells in between).

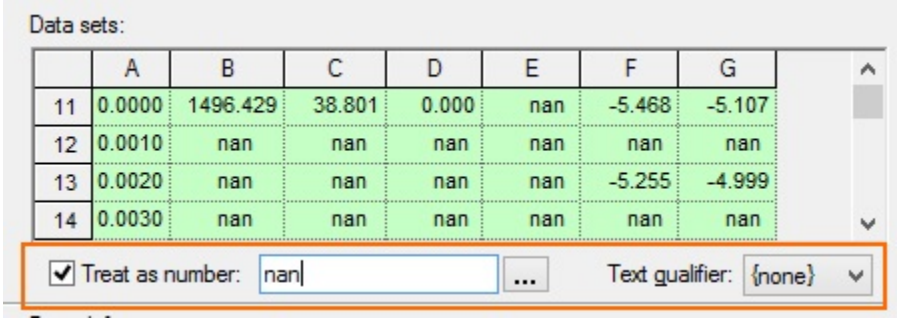
**EXAMPLE** Your external data file contains four Data Sets in columns A, C, E and G. Columns B, D, F are empty. When you drag columns A and C to the first two rows in the **Raw Data column** and next click the **Validity Check** button, EthoVision XT automatically assigns columns E and G to rows 3 and 4, thereby taking into account the empty columns between Data Sets.

The distance between the columns should be the same. For example, dropping columns A and C and clicking **Validity Check** works. The columns E, G, I, K etc are automatically added to the other rows. Dropping columns A and B, and clicking **Validity Check** also works. All other columns are then automatically added to the other rows. However, dropping A, B, and D and clicking Validity Check does not work, because there is an empty column between B en D, but not between A and B.




*Data sets with missing samples*

If your data set contains missing samples indicated by non-numeric symbols, specify this symbol and select the **Treat as number** checkbox.




Alternatively, click the button next to the **Treat at number** field to select one or more predefined symbols. To select a specific text, click **<User Defined>**, click **OK** and enter this text after a comma (,).

If text is identified by a character, select this from the **Text qualifier list**.

## Save the import profile

When all the information is in the **Import Profile Definition** sheet, click the **Save As** button and give the profile a name.

## Import a text file with the new import profile

The newly created import profiles is now in the **Files of Type** list.

1. Locate the external data file and select the filename.
2. Click **Open**.

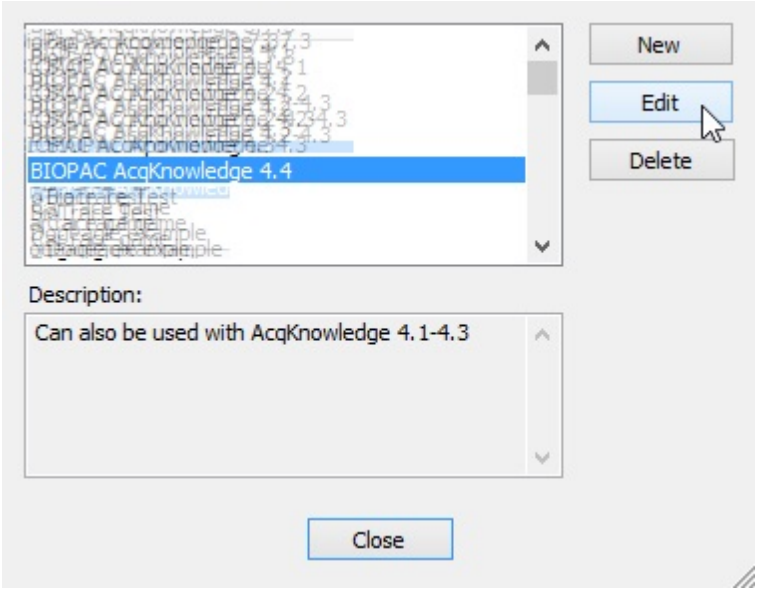
## Notes

- A missing sample is converted to a ‘zero’. As a result, it is plotted as a ‘zero’ in the Visualization.
- The import profile is stored with the extension *.eip.

# Edit a custom import profile

If you want to import an external data file that is very similar to, but not exactly the same as, another data file for which you already have an Import profile, you can edit the existing Import profile.

1. In the Import External Data window, click **Custom Import Profiles**.
2. Select the Import Profile from the list in the Import Profiles window and click **Edit**.




   In the **Select Sample File** group you see the original sample file behind Profile based on.

3. Click **Browse** in the **Select Sample File** group to select the new external data file and click **Open**.
4. Follow the instructions 4-8 under Create a new custom import profile.
5. Click **Save Profile As** when you are finished filling in the Data definition sheet.
6. Type in the name for the Import Profile and click **OK**. Close the Profile Definition window.
7. Close the Import Profiles window. Make sure you select the right Import Profile.
8. Select the external data file and click **Open** to finish import.

## Note

- You cannot edit the import profile for European Data Format files.

# Examples of import profiles

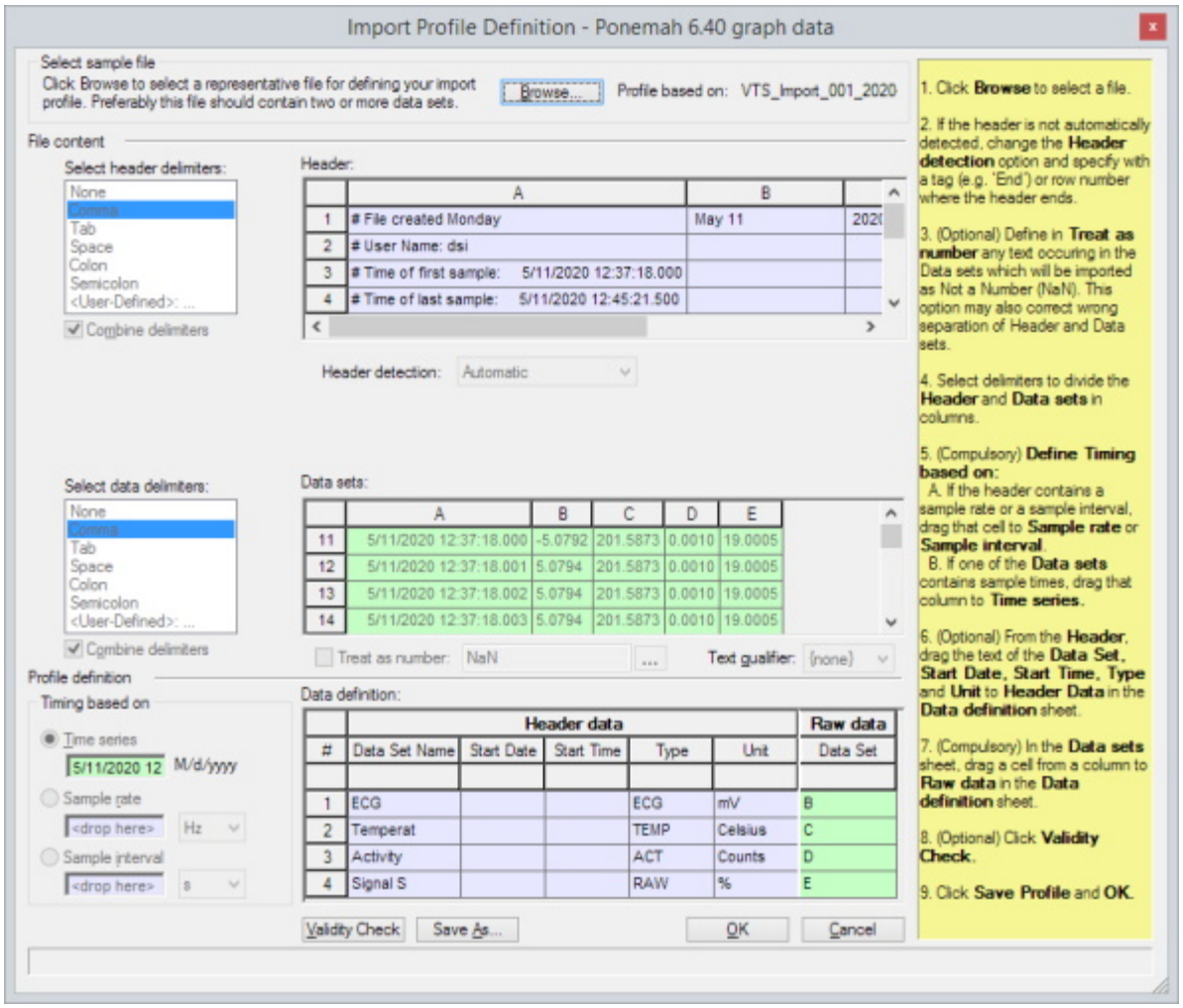
## DAQ systems/software

- DSI Ponemah 6.40 and DSI DataQuest A.R.T. 4.3
- BIOPAC AcqKnowledge 4.4
- Doric
- Polar Precision Performance 4.0
- European Data Format files
- Noldus UltraVox XT 3

# DSI

## DSI Ponemah 6.40

The following picture shows the import profile for Ponemah 6.40. With this profile you can import one of more data columns.




- Note that the second column of the data file (B) contain time stamps and must be dragged to the Time series field.
- **IMPORTANT** Import of data from Ponemah 6.40 to EthoVision XT works when the time format in Windows is set without AM/PM. When exported, the time should be in a format like 10:57:55.000.
- To export the data in Ponemah, choose **Action** > **Start Review**. Select all signals you wan to export, and the time range. In the graph window, choose **File** > **ASCII Output**. Select the channels and the data range. Select the

option to **Include Time in Output**. Next, click **Convert to ASCII**. The file is ready for import into EthoVision XT.

## DSI DataQuest A.R.T. 4.3

For DataQuest, the profile specifies that the first column of the data file (A in Data Sets) contains the time stamps. Column B contains the sync data, and the remaining columns C-G contain activity and physiological data.

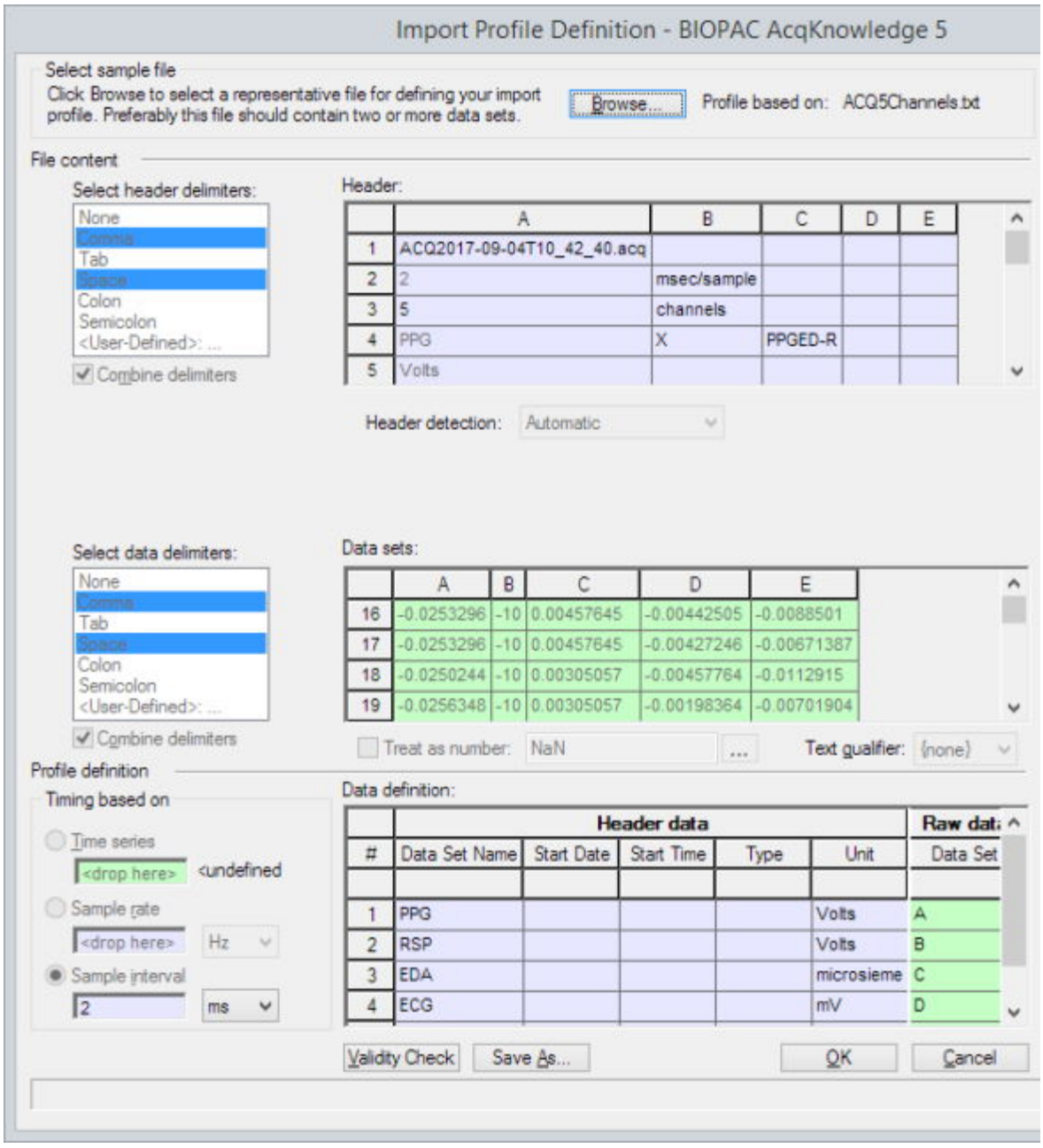
# BIOPAC

For the BIOPAC system, AcqKnowledge is the software used to record, analyze and export the data. This is an example of an export file of AcqKnowledge 5. The file contains information about the sample interval. In this case the sample interval is 2 ms.

ACQ2017-09-04T10_42_40.acq
2 msec/sample
5 channels
PPG, X, PPGED-R
Volts
RSP, Y, RSPEC-R
Volts
EDA, X, PPGED-R
microsiemens
ECG, Y, RSPEC-R
mV
TimeCode signal
Volts

| CH1 | CH5 | CH9 | CH13 | CH16 | | |
|---|---|---|---|---|---|---|
| 55222 | 55222 | 55222 | 55222 | 55222 | | |
| -0.0253296 | | -10 | 0.00457645 | -0.00442505 | -0.0088501 | |
| -0.0253296 | | -10 | 0.00457645 | -0.00427246 | -0.00671387 | |
| -0.0250244 | | -10 | 0.00305057 | -0.00457764 | -0.0112915 | |
| -0.0256348 | | -10 | 0.00305057 | -0.00198364 | -0.00701904 | |
| -0.0256348 | | -10 | 0.00305057 | -0.00183105 | -0.00762939 | |
| -0.0250244 | | -10 | 0.00305057 | 0.000152588 | -0.00610352 | |
| -0.0253296 | | -10 | 0.00457645 | 0.000610352 | -0.00701904 | |
| -0.0256348 | | -10 | 0.00457645 | 0.000762939 | -0.0106812 | |
| -0.0253296 | | -10 | 0.00305057 | 0.000457764 | -0.0088501 | |
| -0.0256348 | | -10 | 0.00457645 | -0.00167847 | -0.00701904 | |
| -0.0256348 | | -10 | 0.00305057 | -0.00213623 | -0.00823975 | |

The following picture shows the Import Profile Definition window in EthoVision XT for a BIOPAC AcqKnowledge 5 file.



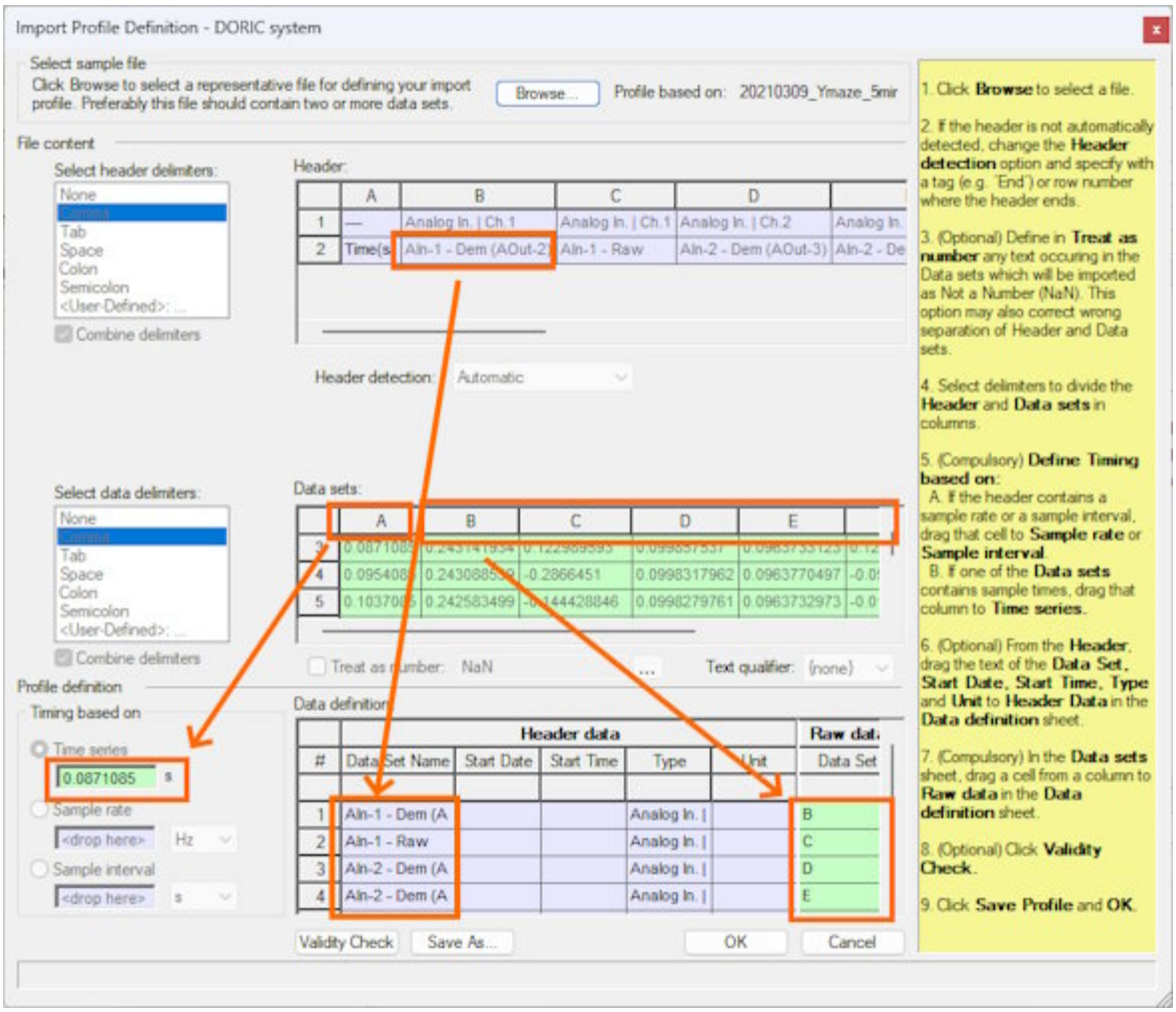

The header contains information about the sample interval (milliseconds), and information about the channels. In this example, the ECG, heart rate, the R-R interval and R-peak height were measured.

The sample rate is calculated based on the sample interval. In this case the samples are taken at an interval of 2 milliseconds. This corresponds to a sample rate of 500 Hz.

# Doric

Doric (***.doric**) data files can be exported as CSV files to EthoVision XT.

The following figure shows an import profile for CSV files with multiple channels. The first column of the CSV includes the time stamps. The other columns contain data of the channels recorded. The arrows indicate where you need to drag the cell contents in the **Header** section and in the **Data sets** section in order to create a similar import profile.




*See also*

For more information, see https://neuro.doriclenses.com/

# Polar

The Polar system is used for monitoring heart rate. After data acquisition the data can be transferred to a computer (not available with all Polar systems). The data is stored in a HRM file. These HRM files can be directly imported into EthoVision XT.

EthoVision XT cannot be connected to the Polar system. Therefore, Polar heart rate data and EthoVision tracking data always need to be synchronized manually.

To facilitate manual synchronization of Polar heart rate data and tracking data you can do the following:

- If the Polar software is running on the EthoVision XT computer: Synchronize the Polar watch with the clock of the EthoVision XT computer.

  Please see the Polar Precision Performance software Reference Manual for a description of how to do this. Please note that this is not possible for all Polar systems.

- If the Polar software is not running on the EthoVision XT computer: Synchronize the EthoVision XT computer and the Polar computer. Synchronize the Polar watch with the clock of the Polar computer.

The settings on the Polar receiver determine what header information is written to the HRM file. Please refer to you Polar manual for a detailed description of the settings.

Below: An example of part of a Polar HRM file.

```
[Params];
Version;105
Monitor;1
Mode;0
Date;20011209
StartTime;22:21:13.0
Length;0:12:54.8
Interval;5
Upper1;250
Lower1;10
Upper2;250
Lower2;10
Upper3;250
Lower3;10
ActiveLimit;0
MaxHR;193
RestHR;70

[HRData];
185;
188;
188;
183;
178;
```

The file header contains information about date and time, the length of the recording period (00:12:54:08) and the sample interval (5 sec). It also contains information about settings of the upper and lower limit of the heart rate.

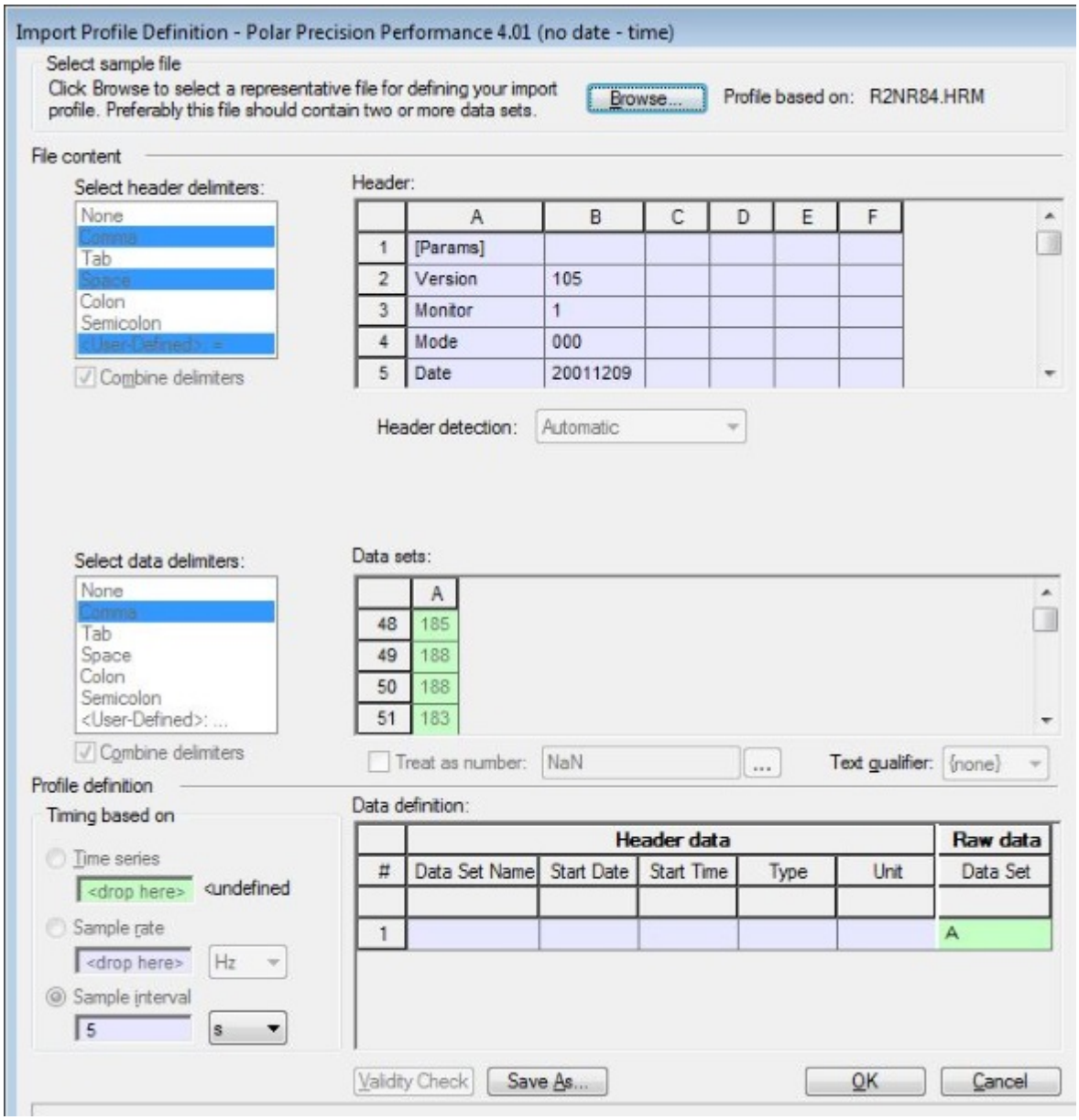
Depending on the settings of the receiver, the HRM file can contain additional information. This does affect subsequent import of HRM files acquired with different type of receivers.

The HRData column show the mean heart rate (in beats per minute) over a 5-s period.

## Polar Precision Performance 4.01 (no date - time)

This is an example of the Import Profile Definition window in EthoVision XT for the profile Polar Precision Performance 4.01 (no date - time) obtained with the Polar file described above.

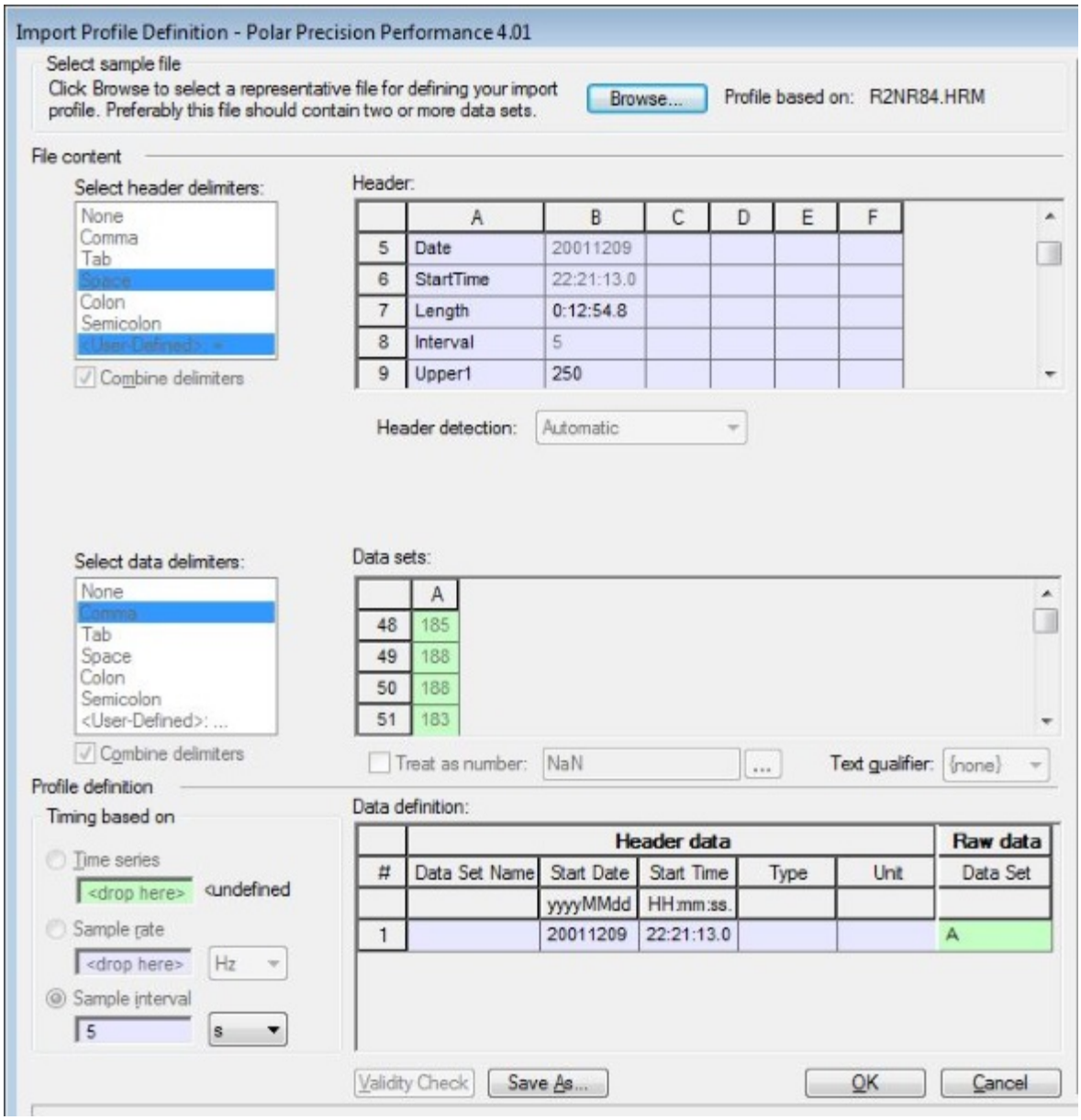
Note that in the Data definition section the cells under Start Date and Start Time are empty, meaning that the import profile does not read date and time information in the Polar file.




## Polar Precision Performance 4.01

This is an example of the Import Profile Definition window in EthoVision XT for the profile Polar Precision Performance 4.01 obtained with the Polar file described above. This profile reads the date and time information contained in the export file header.

Note that in the Data definition section the cells under Start Date and Start Time contain the information read from the Header section (the corresponding cells in the Header section are grayed out).




## Interbeat interval data

Interbeat interval data obtained with a Polar system have the same format as HRM data. However, the file does not contain the time stamps that EthoVision XT needs for time information.

# UltraVox XT 3

## Background information

Ultravox XT is software for analysis of ultrasound emitted by rodents and other animals. EthoVision XT can import Ultravox XT files (ETX) using a specific import profile.

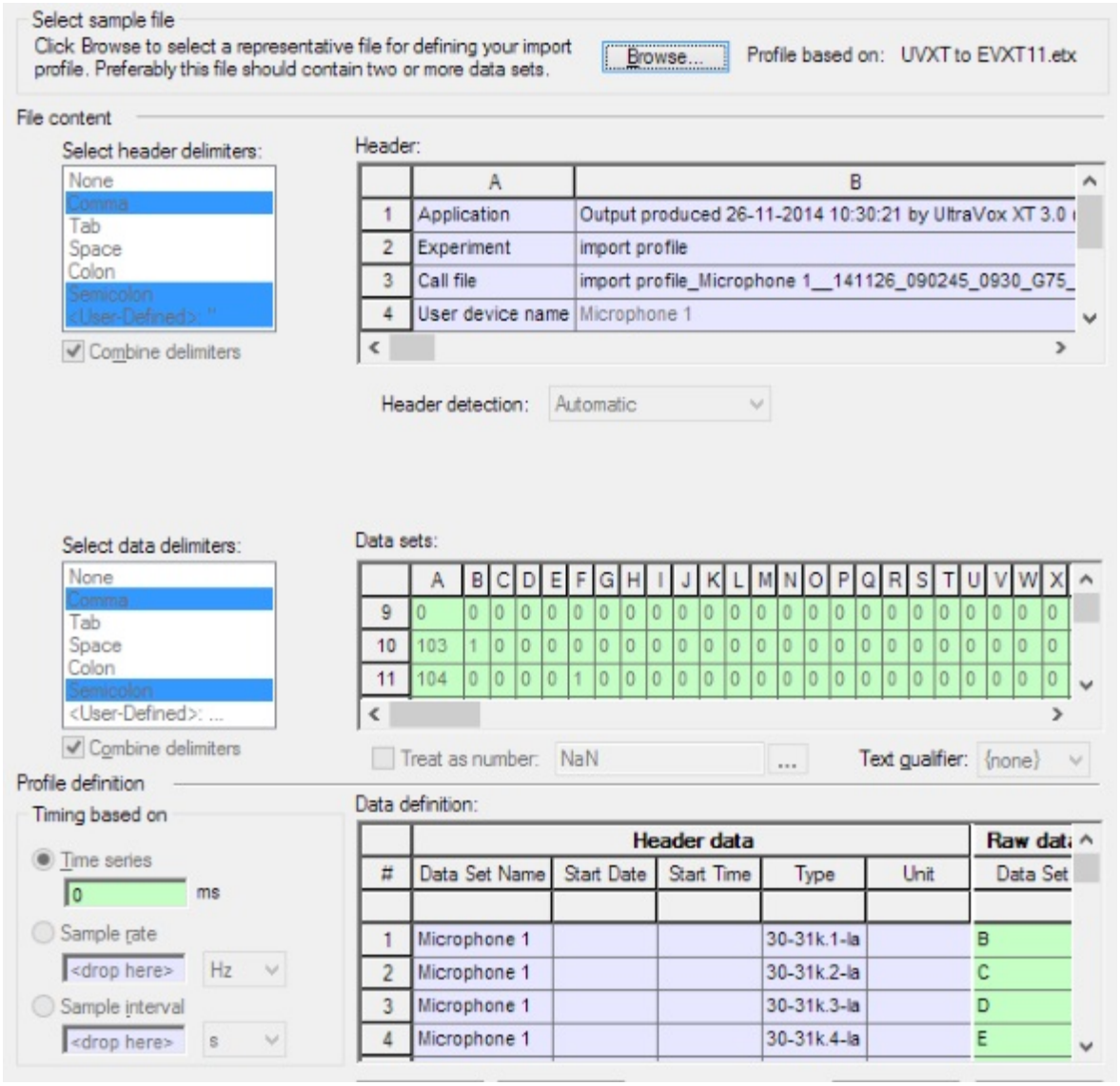
The following picture shows an example of an ETX file for EthoVision XT 11 and later. The first column contains the time stamps in milliseconds. The other columns show 0 (not a call start) or 1 (call start time). The 0 in the row following a 1 marks the end of that call. Each column represents a call category (call name, or call name*pattern label). Note that the time stamps are not equidistant.

| Application | Output produced 17-Nov-14 15:24:01 by UltraVox XT 3.0 (B77) | | | | | | |
|---|---|---|---|---|---|---|---|
| Experiment | Sample | | | | | | |
| Call file | Sample_Imported_3-B6-P112013-06-04 13-07-15 1474_140929_102507_0343_G0_1.UVC | | | | | | |
| User device name | Imported | | | | | | |
| Date | 29-09-2014 | | | | | | |
| Start time | 10:25:07.343 | | | | | | |
| Time (msec) | 52-65k.Chevron | 52-65k.Composite | 84-99k.Descend | 84-99k.Flat | 52-65k.Squiggle | 84-99k.U-shape | |
| End | | | | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 1162 | 1 | 0 | 0 | 0 | 0 | 0 | |
| 1188 | 0 | 0 | 1 | 0 | 0 | 0 | |
| 1189 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 1210 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 1337 | 0 | 1 | 0 | 0 | 0 | 0 | |
| 1360 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 1716 | 1 | 0 | 0 | 0 | 0 | 0 | |
| 1742 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 1914 | 1 | 0 | 0 | 0 | 0 | 0 | |
| 1945 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 2145 | 0 | 0 | 0 | 1 | 0 | 0 | |
| 2162 | 0 | 0 | 0 | 0 | 0 | 0 | |

## The UltraVox XT 3 import profile

The following figure shows an example of the Import Profile Definition window in EthoVision XT for the import profile of Ultravox XT 3.

In the Data definition section, the cells under Data Set Name contain the information read from the Header section (the corresponding cells in the Header section are grayed out). The cells in the Type column contain the call types and the green cells in the Data Set section contain the data sets.




## Install the UltraVox XT 3 import profile

If you do not see UltraVox XT 3 in the list of import profiles when you attempt to import the call data (that is, during Import a complete external data file at once), it may not be installed.

Install the import profile from the UltraVox XT installation USB Flash drive.

1. Close EthoVision XT.
2. On the UltraVox XT 3 installation USB Flash drive, open the folder **Extras\Import Profiles**.
3. Double-click the file **EthoVision XT 11 and Newer.bat**.
4. You should now see the UltraVox XT 3 import profile in the list.

## Notes

- The UltraVox XT 3 import profile is installed in the following folder:

  C:\ProgramData\Noldus\Common\Profiles.

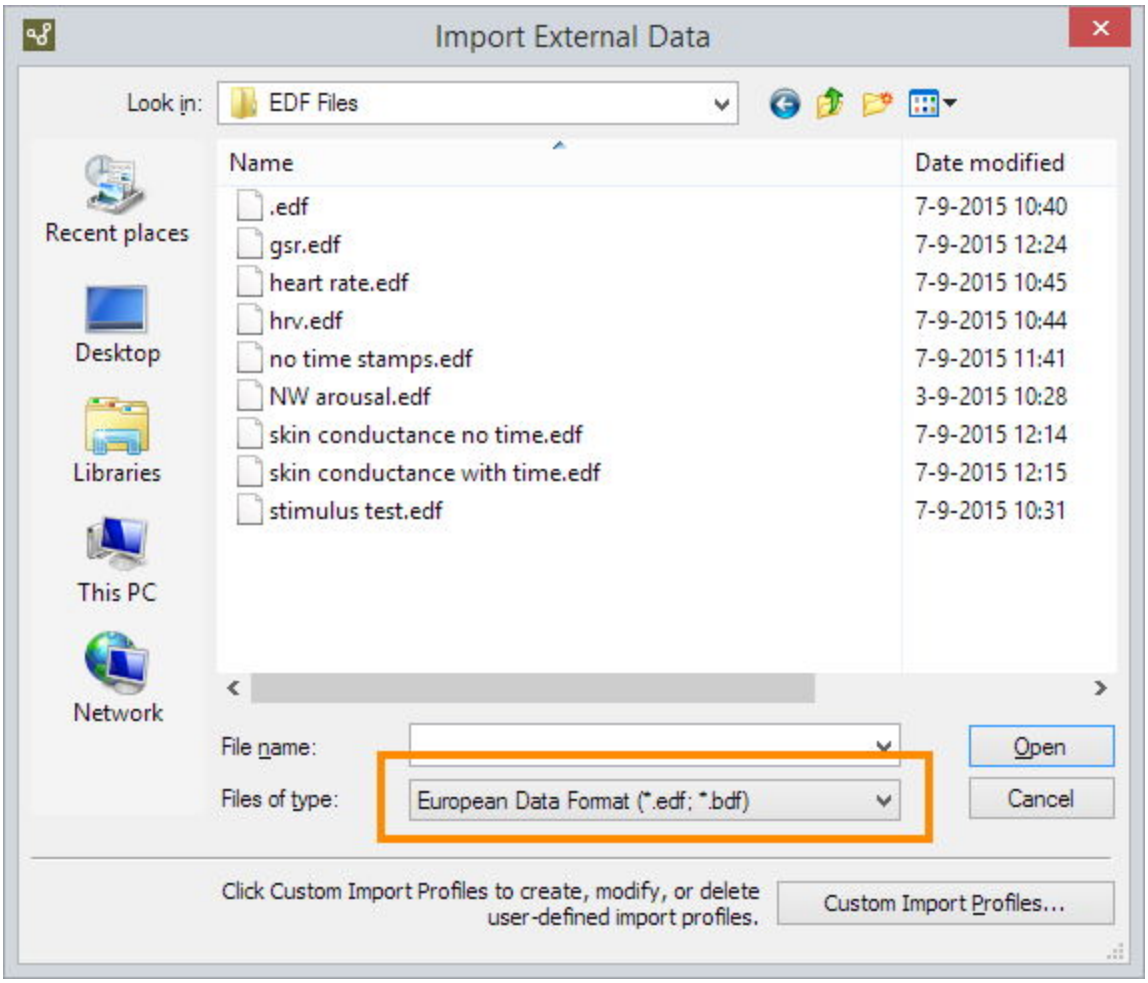
  It includes the folder UltraVox XT 3 and a file, UltraVox XT 3.eip.


- You can find the UltraVox XT installation files on my.noldus.com. Log in, and locate **Downloads** > **UltraVox XT** > **Current Version**.

# European Data Format files

European Data Format (EDF) files and BioSemi Data Format (BDF) files are binary files. EDF files are 16 bit and BDF files are 24 bit. The import profile for EDF and BDF files cannot be edited, as for BIOPAC and other import profiles.

Simply import the files by selecting the **European Data Format** in the import window.




## Notes

- Import of EDF+ and BDF+ files, which may contain annotations, is not supported.

# Synchronization of tracking data and external data

## What do you want to do?

- Synchronize data automatically
- Synchronize data manually at import
- Synchronize data manually after import

## Learn about

- Other ways to ensure synchronicity between video and data streams

# Synchronize data automatically

## Aim

To synchronize EthoVision XT data and external data using the synchronization signal sent to the external DAQ system during live tracking.

## Procedure

1. Import the external data that was simultaneously acquired during live tracking into EthoVision XT.
2. In the Import External Data window, you see:

   one or more 'Normal Data Sets' (indicated by a diagram icon).

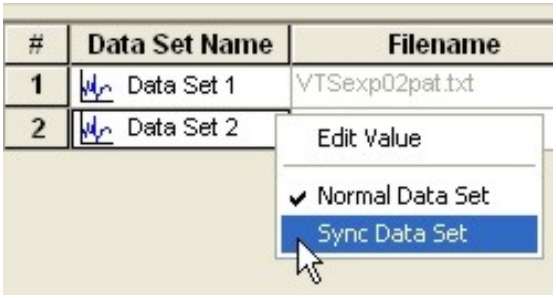
   one or more 'Sync Data Sets' (indicated by a clock icon).

   The **Link Variable** cell for a Sync Data Set shows which Trial was acquired simultaneously with the external data.

   A Sync Data Set is automatically detected when you use the Time Code (TCAP) synchronization signal. When you use the On-Off signal, it may be necessary to select **Sync Data Set** from the context menu to tell EthoVision XT that the data set contains the synchronization signal:

   In the Import External Data window, right-click the Data Set that you want to change to Sync Data Set and select **Sync Data Set**.

3. If necessary, adjust the Synchronization Settings (this step) or proceed to step 4.

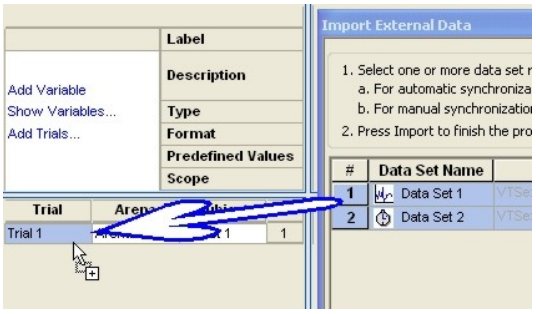
   Click the **Synchronization Settings** button to open the Synchronization Settings window. Here you can set values for the following parameters that

determine how the information from the synchronization signal is extracted from the imported external data file (default values in brackets):

- **Number of offset values** (10) is the number time points in the synchronization signal used to calculate offset points. This only applies to the Time Code signal. The offset values are used for the calculation of average offset and gain. A higher value increases the accuracy of synchronization, but also increases calculation time.
- **Smoothing factor** (1) is the number of sample points over which an average signal level is calculated. A higher value reduces spikes on the signal, but also reduces the time accuracy of the synchronization. The Smoothing Factor is used for both On-Off and Time Code signal.
- **Signal-to-noise-ratio** (50) determines the tolerance for detecting high-low signal transitions. A higher value increases this tolerance, but also increases the chance of detecting false transitions.
- **Number of samples prescan TCAP** (180000) is the maximum number of sample points used for auto-detection of a Time Code signal in the DAQ samples. For example, 180 seconds at 1 kHz or 30 minutes at 100 Hz. This setting only applies when you started your DAQ system before you started tracking in EthoVision XT.

Click in the boxes to set a value for each of the parameters. Click **Defaults** if you want to return to the default values or click **OK** to finish.

4. In the Import External Data window, select one or more Data Sets you want to link, click on one of the Data Sets with the left mouse-button, keep the left mouse-button pressed and link the Data Sets with a drag-and-drop action to the corresponding Trial, Arena or Subject.




5. Click **Import** to finish linking the Data Sets.

## Notes

- The **Synchronization Settings** button in the Import External Data window is only available if **Enable DAQ co-acquisition** is selected in the Experiment Settings.

- The information in the synchronization signal is sampled by the DAQ system. During import into EthoVision XT some digital signal processing takes place to deal with noise, signal distortion, spikes etc. This processing is determined by the Synchronization Settings above.

*See also*

- Enable DAQ co-acquisition in EthoVision XT
- Connect EthoVision XT to the DAQ system

# Synchronize data manually at import

## Aim

To synchronize the tracking data and the external data if it was not possible to connect the EthoVision XT computer and the external DAQ system.

You can manually synchronize external and tracking data during import of external data into EthoVision XT:

- *Shift the offset*. This means that the complete external Data Set is shifted in time. The duration of the external Data Set remains unchanged. Shifting is done by changing the Start Date/Time of a Data Set.
- *Stretch or shrink the external data set*. This means that the duration of the external data set is changed to match the duration of an acquired Trial. Stretching the external data set is done by changing the Start Date/Time and/or the Stop Date/Time. This way you can either stretch or shrink the duration of the data set.

Manual synchronization of external data and tracking data is accurate to within one millisecond. However, note that the sample rate of EthoVision XT, and therefore the resolution of the derived data, can never be higher than the video frame rate.

## When do I need to Shift the offset?

For example, you started acquisition on your DAQ system 10 seconds after you started acquisition in EthoVision XT. During import, shift your external data set 10 seconds to match the offset of the Trial.

## When do I need to Stretch/Shrink?

You need stretching/shrinking in the scenario that you carry out acquisition during a prolonged period of time during which the clocks of the EthoVision XT computer and the DAQ computer start running 'out of sync' (you started with synchronized clocks (see below how to synchronize computer clocks).

**EXAMPLE** You carry out tracking for 24 hours and after this period you notice that the DAQ computer is 10 seconds ahead of the EthoVision XT computer. This means you need to shrink the external data set by changing the Stop Time of the data set to 10 seconds earlier.

## To facilitate manual synchronization

You can facilitate manual synchronization by synchronizing the clock of the EthoVision computer and the computer on which the external DAQ system is running, before acquisition. You can use one of the following methods:

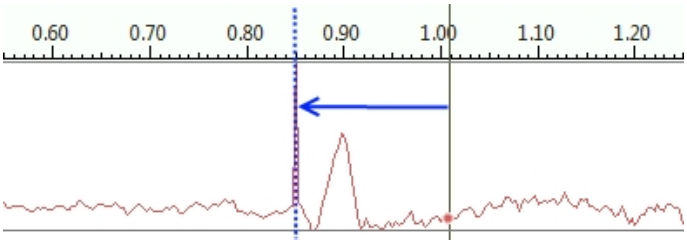
- If the computers are situated next to each other, double-click the clock in the taskbar at the bottom of the screen on both computers. In the Date & Time tab, set both clocks to the same time.
- In all cases: use an online clock, when connected to the Internet, to set both clocks to the same time (see description above); or use NetTime, a simple Network Time Protocol (SNTP).

If, for some reason, it is not possible to synchronize the computer clocks there is a more advanced method to facilitate manual synchronization. This method only works when the external data file contains information about date and time of acquisition.

1. Acquire tracking data in EthoVision XT and external data on the DAQ system and make sure on both systems a synchronization event is recorded.

   For example, you can use a relay to create a peak on your DAQ system and simultaneously switch on a light which is visible in the video.

2. Import the external data into EthoVision XT.
3. Visualize the data by choosing **Analysis** > **Results** > **Plot Integrated Data**. Go to the position in the video where the swiping action occurs. Note down the time stamp.
4. In the external data window, move the hairline to the position where the peak, caused by the swiping action, occurs. Note down the corresponding time.

   **EXAMPLE** The swiping action in the video occurred at 1.01. The corresponding peak in the external data occurred at 0.85 in the external data plot, so 16 seconds earlier.

5. In the Trial list, remove the external data. To do so, select the columns that contain the external data sets, right-click and select **Delete Variable**.

6. Change the start time of the external data file by doing one of the following:
   - Open the external data file (for example, with Notepad) and change the start time.
   - Import the external data file. In the Import External Data window, you can change the start time of the external data set (see below).

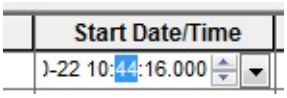
   **EXAMPLE** The difference in offset was 16 seconds (with the external data starting earlier than the tracking data). This means that the start time of the external data set should be set to 16 seconds later.
7. If you have edited the external data file, re-import it into EthoVision XT.

Manual synchronization of tracking data and external data is accurate to within one millisecond.

## To shift an external data set

1. Import the external data that was simultaneously acquired during live tracking into EthoVision XT.
2. In the Import External Data window, double-click the Start Date/Time cell of the external data set you want to shift.
3. In this cell, click the year, month, day, hours, minutes, seconds or milliseconds and change them by using the arrow-up/arrow-down on your keyboard, the up/down arrow at the right side of the cell or just type in the desired value.

   When you change the Start Date/Time of a Data Set, the Stop Date/Time also changes. As a result the duration remains the same.




4. Finish import.

## To stretch/shrink an external data set

Stretching or shrinking can be used in the unlikely case that during acquisition the clock of the external DAQ system starts running out of sync with the clock of the EthoVision computer. This probably only occurs when live tracking is performed for a prolonged period of time. For example, in EthoVision XT, the clock time is determined by the camera frame rate. If the camera frame rate is not accurate, for example when a low-end consumer model is used, the time stamps in EthoVision gradually become out of sync with the DAQ computer clock. Then you need to manually increase or decrease the duration of the external data set.

1. Import the external data that was simultaneously acquired during live tracking into EthoVision XT.

   Shift the external data set if necessary (see above).

2. To stretch the Import External Data window, double-click the Stop Date/Time cell of the external data set you want to stretch.
3. In this cell, click the year, month, day, hours, minutes, seconds or milliseconds and change them by using the arrow-up on your keyboard or the up-arrow at the right of the cell.

   You can shorten the external data set, by changing the Stop Date/Time to earlier.

4. Finish import.

# Synchronize data manually after import

## Aim

To adjust the offset between external data and video/track data.

**NOTE** You change the offset of the original signal. The offset is automatically applied to all the EthoVision XT variables derived from that signal. Choose **Analysis** > **Results** > **Integrated Visualization**.

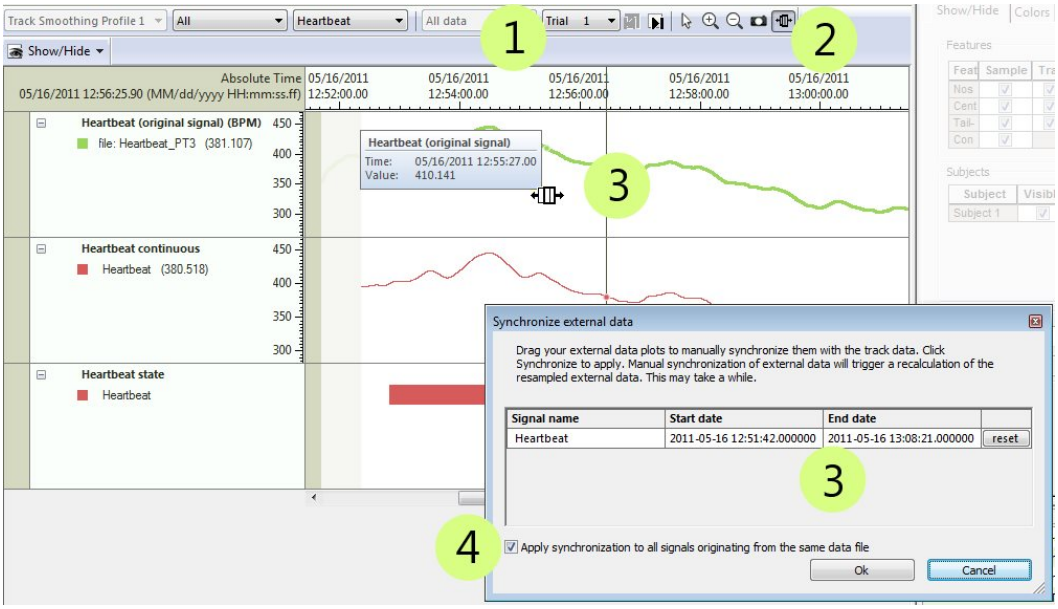
## Procedure

1. From the lists available on the toolbar, choose the Track smoothing profile, the Data profile, the Analysis profile and the trial to visualize.
   - Make sure that the signals are well visible; zoom in/out if necessary.
   - If you want to re-synchronize using an event visible in a specific video frame, position the video to that frame.
2. Click the External data synchronization button on the toolbar, or choose **Show/Hide** > **External data synchronization**.

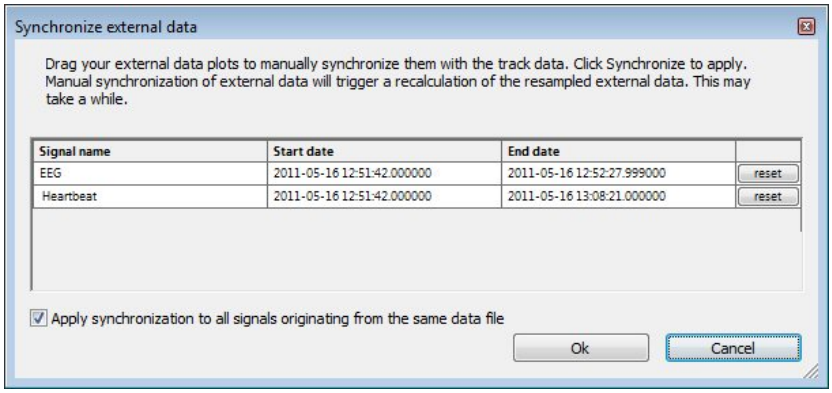
   If no original signal is displayed in a chart, EthoVision asks you to select the original signal for synchronization.

   Take note of the original start and top time in the Synchronize external data window. If you change the offset (next step) and click **OK**, the start/stop times are overwritten and cannot be recovered.

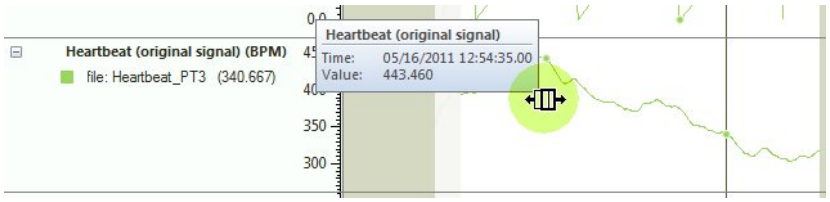



3. You can synchronize data in two ways:

   - In the Synchronize external data window, enter the new Start date and time and the Stop date and time for the chosen signal.




   - In the plot area, hover the mouse over the original signal and drag to the position you require.




   While you drag the mouse, the time plot of the original signal is shifted to the left or to the right. The Synchronize external data window shows the new Start and Stop time of the original signal. Release the mouse button when ready.

4. Select the option **Apply synchronization to all signals originating from the same data file** if you also want to apply that offset to the EthoVision XT variables derived from the other signals imported from the same data file.

   Make sure to keep this option selected when you import data sets from the same data file (for example, call data imported from UltraVox XT 3), and you want to maintain the original offset for all those data sets.

5. When ready, click **OK**.

## Notes

- The shift in the offset is also applied to signals derived from the original signal, which are not currently visualized.
- After clicking **OK**, the shift in offset cannot be undone! To restore the original offset you must know the original Start and top time and enter them in the Synchronize external data window.
- To stretch the data in time, enter new values for Start and Stop time in such a way that the duration of the data set is longer than the original. First enter Start time, then Stop time.
- To shrink the data in time, enter new values for Start and Stop time in such a way that the duration of the data set is shorter than the original. First enter Start time, then Stop time.
- If your data plots include EthoVision XT variables derived from signals other than the one you synchronize, these are not synchronized automatically, unless the original signals come from the same file and you select **Apply synchronization to all signals originating from the same data file**.

  In all other cases, to synchronize those variables, open the signal they are derived from, and repeat the procedure above for that signal.

- Re-synchronization may affect your data selection when this contains Nesting functions based on those external data. For example, your Data profile contains a Nesting function based on Temperature state, which selects the intervals when Temperature > 30 ºC. If you re-synchronize Temperature and Temperature state, then also the intervals based on Temperature state will shift accordingly. Plot the data to check the changes.
- To undo the changes and return to the current offset between data sets, click **Reset** in the Synchronize external data window.

If you synchronize external data at least once, re-opening the Synchronize external data window and clicking **Reset** does not restore the offset when you imported the data. It simply restores the last saved offset.

# Other ways to ensure synchronicity between video and data streams

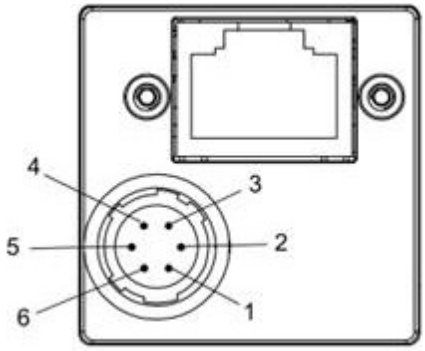
## Aim

Whenever you want to ensure high synchronicity between your video and the external data streams, for example EMG, this topic describes the possible solutions.

This topic applies to Basler digital cameras.

## Method 1 - Send a signal at every video frame

Many Basler digital cameras including the acA1300-60gm GigE camera have a 6-pin I/O connector on the back.




The pin number 2 is for input (named **Line1** in Pylon Viewer), while pin number 4 is for output (**Out1** in Pylon Viewer). You can connect pin 4 to a channel of the DAQ system that reads the time that the video frame is sent out.

**NOTE** Which pin number serves a specific function may vary depending on the camera model. Please refer to the camera documentation.

1. Set the camera for example to free-running at 60 fps and get an electrical signal on the camera output **Out1** pin when the camera makes a new frame. Settings can be made using the Pylon Viewer application. For details, see the Pylon Viewer manual and the camera manual.
2. Connect the camera output to the data recording channel. This way you know down to microseconds accuracy when the camera creates a video frame.

## Method 2 - Send a TTL pulse to the DAQ system

For this you need a USB-IO box and the Trial and Hardware Control add-on module.

1. Connect a TTL port of the USB/IO box to one of the channels of the DAQ system. For this you need a cable with a RJ45 connector at the end of the USB-IO box and a connector at the other end that matches the DAQ system.
2. In the Trial Control Settings of EthoVision XT, make a sub-rule with an action on the chosen TTL port that sends a pulse of 1 second at regular intervals, for example every 5 seconds.
3. During the trial, the pulses are recorded by the DAQ systems.

For more information:

- The chapter **Experiments with Calcium Imaging** in the EthoVision XT 18 - Application Manual, which includes an example with the Inscopix nVoke system.
- The EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

See Manuals

## Note

- To connect the camera to a DAQ system, you can use the standard cable available from Basler, or make your own using a Hirose connector HR10-7P-6S(73).

# Sync the computer clocks for EthoVision XT and DSI Ponemah

## Aim

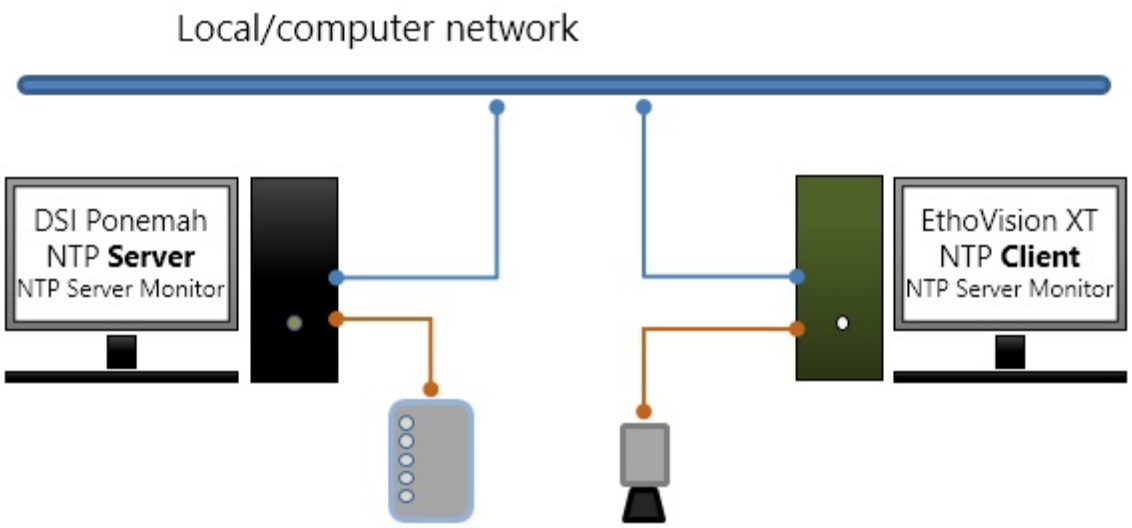
To ensure high synchronicity between EthoVision XT data and physiological data collected with DSI Ponemah.

## Background information

Synchronization between the EthoVision XT system and the DSI Ponemah system is based on a Network Time Protocol (NTP), a networking protocol for computer clock synchronization.

https://en.wikipedia.org/wiki/Network_Time_Protocol

The NTP Time Server runs on the DSI Ponemah computer and acts as time reference. The NTP Client runs on the EthoVision XT and adjusts that PC's clock time based on the NTP Time Server.




## Prerequisites

- The NTP Server is already installed on the DSI Ponemah computer.
- You need two installation files:
  - ntp-time-server-monitor-104.exe (NTP Server Monitor)

- ntp-4.2.8p15-v2-win32-setup.exe (NTP Client).

Download the Time Server Monitor software from

https://www.meinbergglobal.com/english/sw/ntp-server-monitor.htm

Download the NTP Client for Windows from

https://www.meinbergglobal.com/english/sw/ntp.htm#ntp_stable

## Procedure 1 - DSI Ponemah

1. On the DSI computer, create the following folder structure:

   C:

   C:\NTP\

   C:\NTP\ETC\

   C:\NTP\Installation\

2. Copy the file **ntp-time-server-monitor-104.exe** to C:\NTP\Installation.
3. Double-click the file and follow the instructions.

   As the **Destination Folder**, choose C:\NTP.

   As the **Select Program Manager Group**, keep **Meinberg** selected.

   Click **Next>** and continue installation.

4. When installation is done, a new icon **NTP Time Server Monitor** appears on the desktop.
5. Right-click on the icon and choose **Run as administrator**.
6. In the NTP Time Server Monitor window, click the **NTP Status** tab. You should see that a line highlighted in green. That indicates that the service is running correctly.

   In the next steps you take note of the IP address of the DSI computer. Note that the DSI computer has two network cards; one is connected to the matrix (2.0) and the other one should be connected to the network which connects that computer to the EthoVision XT computer.

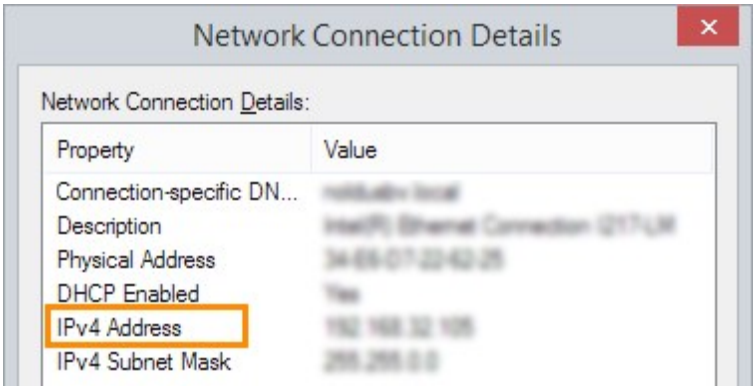
7. On the DSI computer, open the Network Connections.

8. Locate the item that corresponds to the network port to be connected to the EthoVision XT computer.

   **TIP** To find the correct port, temporarily disconnect the network cable from one of the network ports and check which item shows the message **Network cable unplugged**.


   That helps you find which item corresponds to which network port.

9. Right-click the network adapter item and choose **Status**, then click **Details**.
10. Take note of the IP address next to **IPv4 Address**.




## Procedure 2 - EthoVision XT

This procedure is meant to install the NTP Client on the computer which runs EthoVision XT. The NTP client adjusts the computer clock in such a way that it will be in sync with the clock of the NTP Server. This ensures that the system time of the EthoVision XT computer is in sync with the system time of the DSI computer.

1. On the EthoVision XT computer, create the following folder structure:

   C:\NTP

   C:\NTP\ETC

   C:\NTP\ETC\ntp.conf

   C:\NTP\Installation

   C:\NTP\Installation\ntp-4.2.8p15-v2-win32-setup.exe

C:\NTP\Installation\ntp-time-server-monitor-104.exe

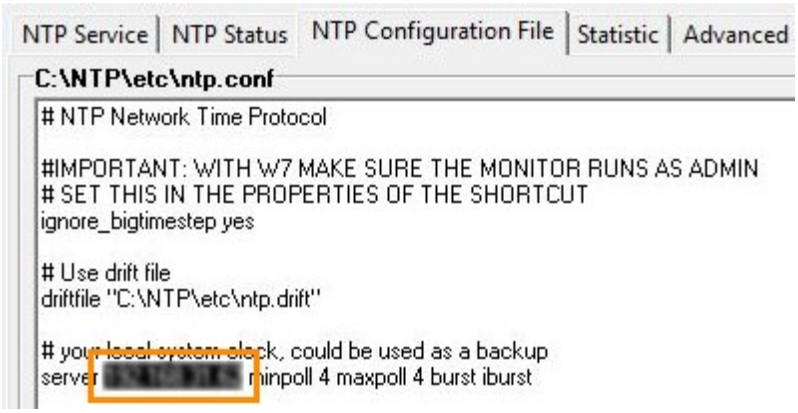
2. Double-click **ntp-4.2.8p15-v2-win32-setup.exe**.
3. Choose C:\NTP as the installation folder. Click **Next** and under **Choose Components** keep the default settings.
4. Under **Files have been installed**, keep C:\NTP\etc\ntp.conf selected file. This is the file that you copied in the first step, and contains all the necessary settings, except for the IP address of the DSI computer.

   De-select **Create an initial configuration file with the following settings**.

5. Click **Next** and under **Setting Up NTP Service**, select **Create and use a special NTP account (recommended)**. Leave all selections as they are.
6. Click **Next** and enter a name and password. Next, complete installation.

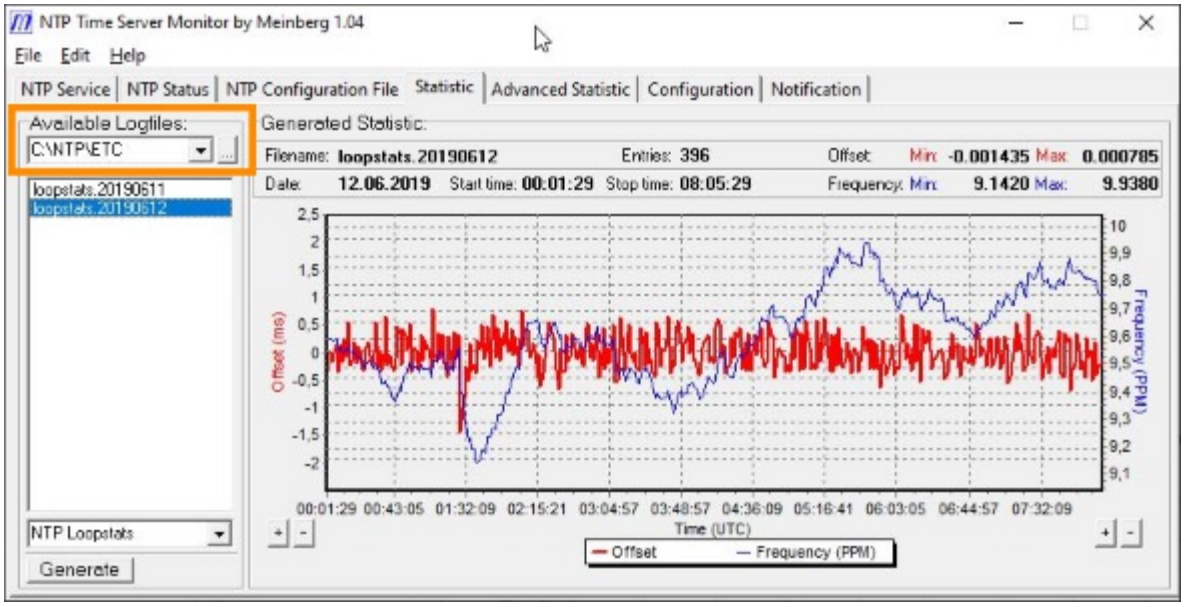
   After this, the Service is installed and started automatically. If the service does not start after the installation, because you used an existing network account, do the following: (1) Start **Services** in Windows (see Administrative Tools in the Windows Control Panel). (2) Select the **Network Time Protocol Daemon** service. (3) Change the Logon properties to the desired account (when you do that, the account will be given services rights). (4) Start the Service.

7. On the EthoVision XT computer, double-click the file **ntp-time-server-monitor-104.exe** (for details see in Procedure 1 above).
8. When installation is done, run the **NTP Time Server Monitor** ad administrator and click the **NTP Configuration file** tab. There change the IP address by entering the one you found at the end of Procedure 1 (see also below).




Once ready, click the **Save configuration** button. At this point you will be asked to restart the service. **TIP** You can always restart the service in the Windows Services.

9. Click the **NTP Status** tab you should see the green line indicating that the service is running correctly.
10. Click the **Statistic** tab in the NTP Time Server Monitor window. A few minutes later you can view the statistics of the NTP synchronization.
11. To monitor the offset between the server and the client, under **Available Logfiles** click the browse button and in the folder C:\NTP\ETC\ select the loop statistics file. Typically, one file is generated per day. Click that file in the list to view the time-offset plot (in red). In most cases the offset, that is the difference in the clock time between the computers, is within one millisecond.




## The NTP.CONF file

The ntp.conf file is a text file which contains the lines as shown below. You can open the file with the Windows Notepad.

Make sure that the IP address shown in bold is the IP address of the NTP Time Server, that is, your DSI-computer.

# NTP Network Time Protocol

#IMPORTANT: WITH W7 MAKE SURE THE MONITOR RUNS AS ADMIN

# SET THIS IN THE PROPERTIES OF THE SHORTCUT

ignore_bigtimestep yes

# Use drift file

driftfile "C:\NTP\etc\ntp.drift"

# your local system clock, could be used as a backup

server **123.456.78.901** minpoll 4 maxpoll 4 burst iburst

# generate statistics

enable stats

statsdir "c:\NTP\etc"

statistics loopstats

# End of generated ntp.conf --- Please edit this to suite your needs

## Notes

- When the two computers are in the same network domain, the domain administrator can use his/her existing account. However it is always possible to create a separate account as described above. The account must have the rights to start the NTP Service.

*See also*

- Import profiles for DSI physiological data

# Edit the Tracks

## Main topics and tasks

# Introduction to editing tracks

## Learn about

- Why edit data?
- Important definitions in Track editing

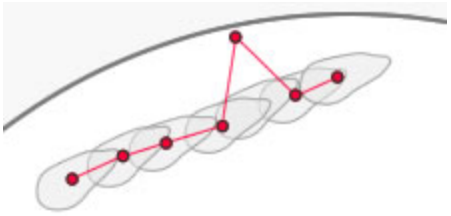
# Why edit data?

## Correcting tracking errors

Sometimes EthoVision XT tracks a reflection instead of the subject, or confuses the nose-point and tail-base. If that occurs, you can correct such errors in the Track Editor screen.

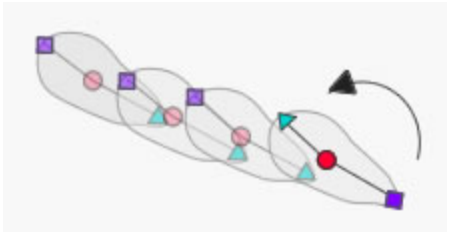
## Examples

- *Reflections in a water maze*. EthoVision XT tracks accidentally a reflection next to the maze's border. There are “spikes” in the resulting track.

  **TIP** Make sure that light is not directed to the water surface. Watch the video tutorial **Set up your test environment** (**Help** > **Video Tutorial**).

- *Nose-tail base swaps*. In experiments set to nose-tail base tracking, the nose-point could be detected as the tail-base, and vice versa. In the last sample of the following track, the two body points are inverted. The Track Editor offers some ways to correct such swaps.

  **TIP** Try out the Deep learning tracking technique, which is superior at finding the subject’s nose-point.

## Notes

- **IMPORTANT** The Edit Tracks function in EthoVision XT is only intended for minor changes of a few points. If you have many errors, there is a problem with your experimental set-up, trial control or detection settings, and you should correct those problems rather than try to manipulate the raw data.

For example, if EthoVision XT tracks the arm of the operator who released the animal in the water maze, do one or both the following:

- In the Trial Control Settings, select to start tracking a few seconds after the subject is detected in the arena.
- In the Detection Settings, set a maximum subject size.

# Important definitions in Track editing

## Sample vs. body point

A sample is the set of coordinates found at any sampling time. This means that:

- If your experiment is set to Only center-point detection, a sample is the subject's center point (one pair of X, Y coordinates).
- If your experiment is set to Center-point, nose-point and tail-base detection, a sample includes the center-point, nose-point and tail-base point (three pairs of X,Y coordinates).

Within a sample, one or more *points* can be missing. Missing points are shown in a different color.

*See also*

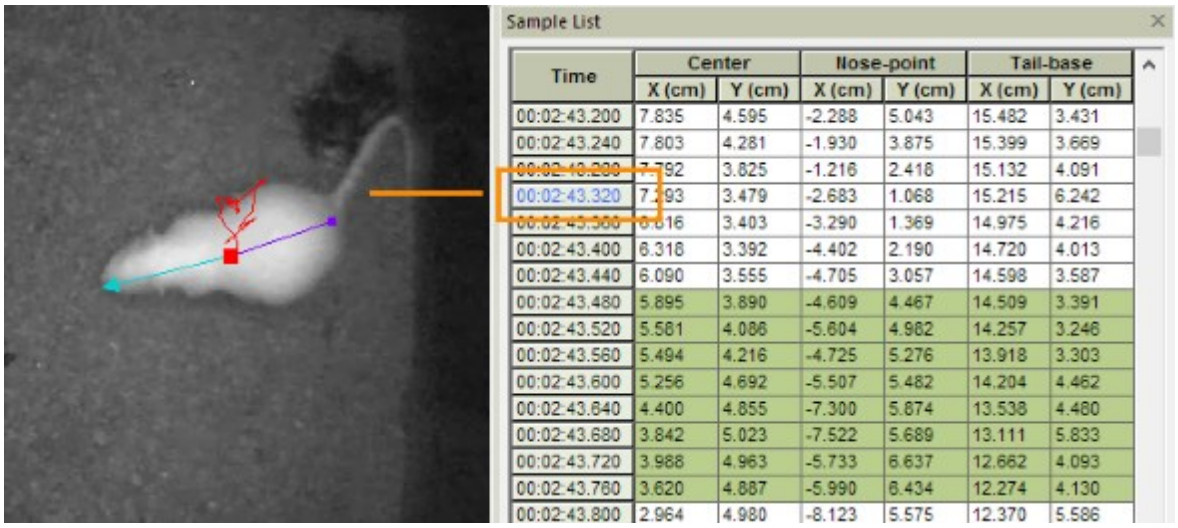
- Customize the missing sample points

## Sample List

The Sample List is the list of coordinates of the subject’s body points and surface area, for each sample time. It is the actual raw data used in all the subsequent analysis functions (from Track Smoothing to Results).

To access the Sample List window, choose **Acquisition** > **Edit Tracks**. If the Sample List is not shown, choose **Show/Hide** > **Sample List**.

**IMPORTANT** When you select samples in the Sample List, selected samples are highlighted in green, while the sample time that corresponds to the current video image is the one shown in blue in the Time column. These two selections are independent of each other, meaning that the video image may show a sample time that is not within the range selected. For example:




## Save edits


You can save a copy of the track that includes the last edits. If you make changes after clicking **Save**, you can always go back to that copy if you are not satisfied with those changes.

*See also*

- Saving the track edits on page 710.

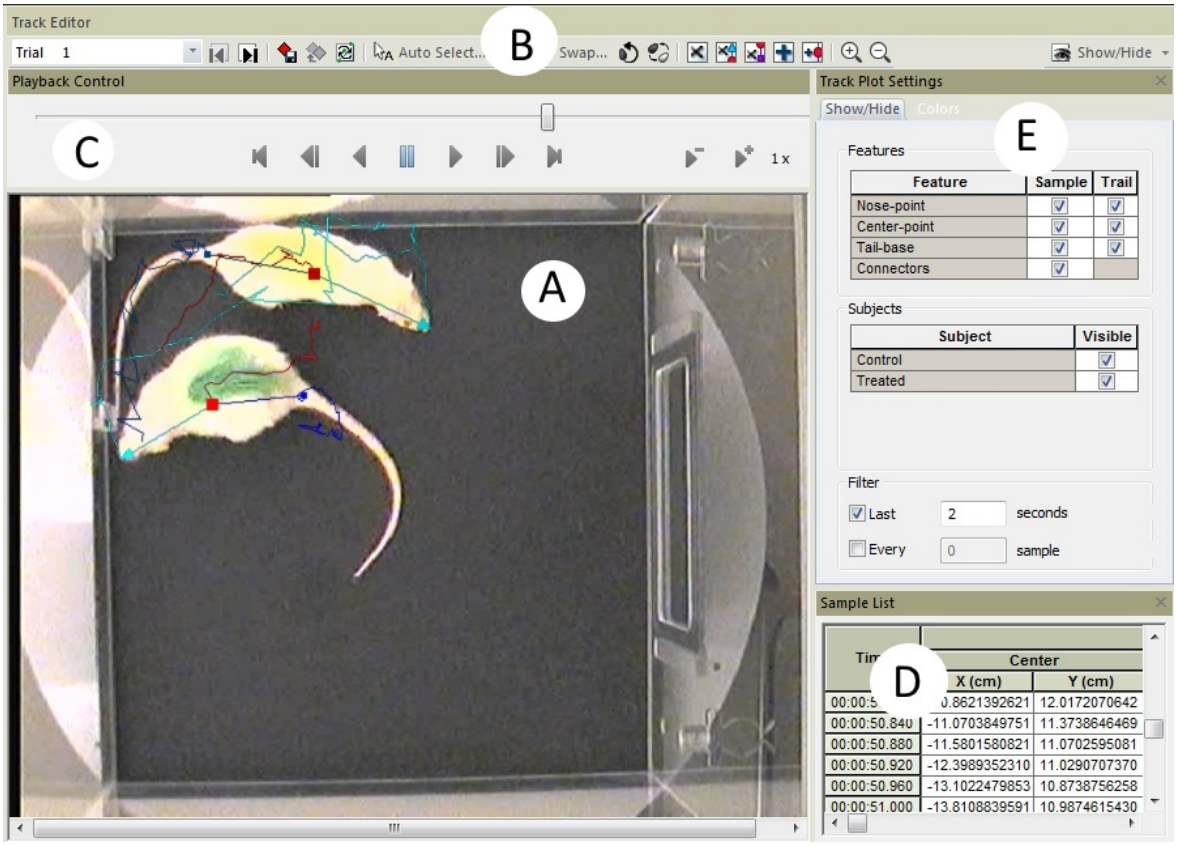
# Customize your screen before editing

## What do you want to do?

- Visualize the tracks in the Track Editor screen
- Improve the readability of the tracks before editing

# Visualize the tracks in the Track Editor screen

To access the Track Editor screen, choose **Acquisition** > **Edit Tracks**, or in the Experiment Explorer, under **Acquisition** click **Track Editor**.



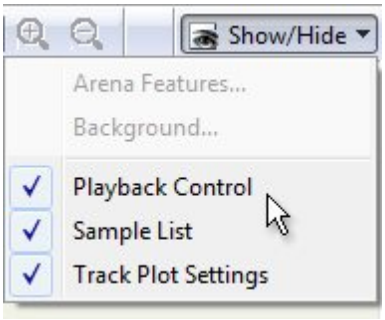

In the track editing screen you can view the following objects.

- A. The track area, where you can view:
  - The background. This can be of uniform color, the captured image of the arenas from the Arena Settings, or the content of the video file.
  - Arenas and zones as overlay objects.
  - Tracks (samples and paths).
- B. The toolbar, which contains tools for:
  - selecting the trials to be edited.
  - managing track edits and reverting to the original data if needed.
  - selecting and editing data points.

The toolbar also contains:

  - The **Show/Hide** button for choosing the elements to display.
- C. The Playback Control window.
- D. The Samples List window, listing the coordinates of the data points and the surface area of the subjects tracked.
  - The **Time** column shows the time elapsed since the start of the trial. If data recording started after the start of the trial or ended before the end of the trial, the Samples List shows asterisks (*****) for the samples not collected before the start and after the end of data recording.
  - The current time (also shown in the Playback Control window) is marked in blue.
  - Missing points are listed with coordinates and surface area '-'.
- E. The Track Plot Settings pane, for customizing the tracks and body points.

To display the Playback Control window, the Samples List and the Track Plot Settings pane, click the **Show/Hide** button on the toolbar and make sure they are selected.




## To visualize the track you want to edit

Select the trial name from the list on the toolbar.


You can visualize one trial at a time. To switch from one trial to the previous or next, click the **Previous Trial** button or the **Next Trial** button on the toolbar, respectively.

To play back tracks, use the buttons in the Playback Control window. Set the play back speed you require.

To visualize the whole tracks, clear the options under Filter in the Track Plot Settings pane and click the **Jump to end** button in the Playback Control window.

## Colors

In the Colors tab of the Track Plot Settings pane, by default, **Subject** is selected under **Level**, that is, the track color does not change between tracks of the same subject. If you select Track, the color of tracks can change between tracks, according to the value of the variable selected under Variable for those tracks.

*See also*

- Improve the readability of the tracks before editing
- Customize the track colors

# Improve the readability of the tracks before editing

## Aim

To locate and edit the tracks more easily.

## What do you want to do?

- Zoom in and out the track plots
- Visualize part of the track
- Visualize one sample every n-th
- Hide the tracks you do not need
- Hide unnecessary track features
- Adjust the line thickness and dot size
- Customize the missing sample points
- Customize the background

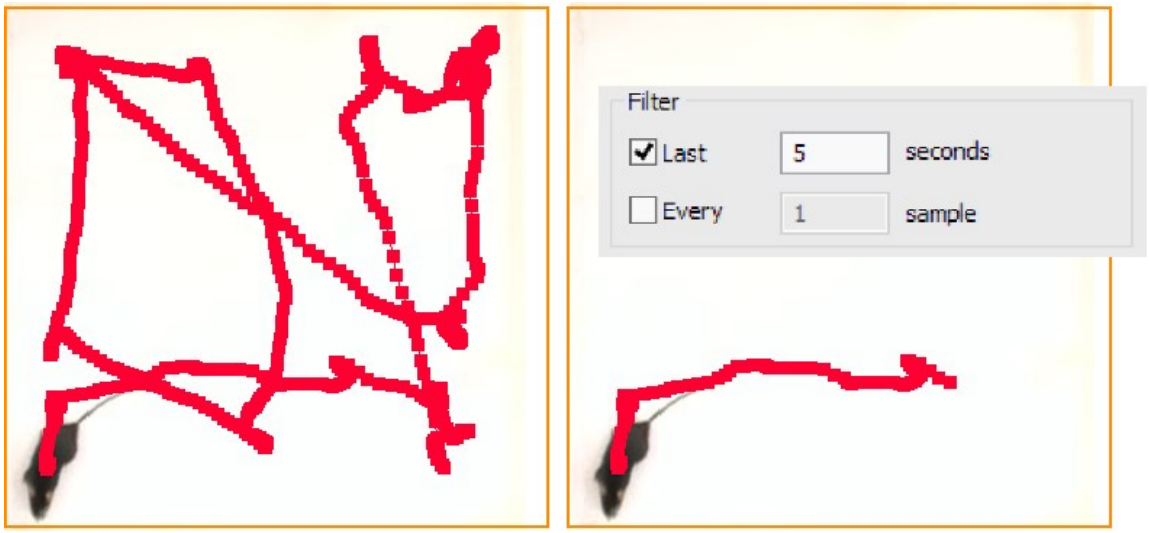
## Zoom in and out the track plots

Click the **Zoom in** button (**Ctrl**+.) or the **Zoom out** button (**Ctrl**+.) on the toolbar, respectively.

If the data points are out of view after zooming in, use the scrollbars to find them.

## Visualize part of the track

In the Track Plot Settings pane, click the **Show/Hide** tab, and under **Filter** select **Last** and enter the time interval. This way, only the last few seconds are visualized before the current sample.



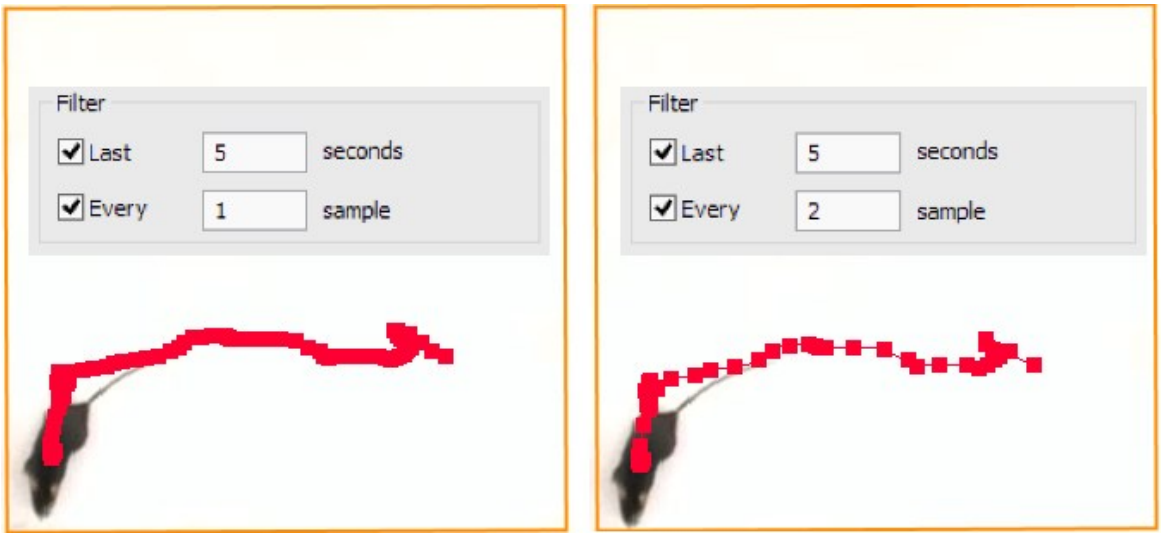

The current sample is highlighted in blue in the Samples List.

## Visualize one sample every n-th

This option is handy when you edit tracks with high sample rates or of slow-moving animals.

To show one sample every n-th, click the **Show/Hide** tab, and under **Filter** select **Every** and enter n in the corresponding field.

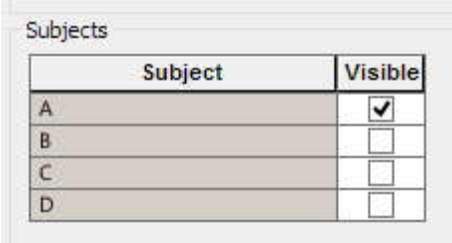
- If you select 0, only the current sample point is shown.
- If you select 1, this is the same as not selecting the Last option (all samples are displayed).



## Hide the tracks you do not need

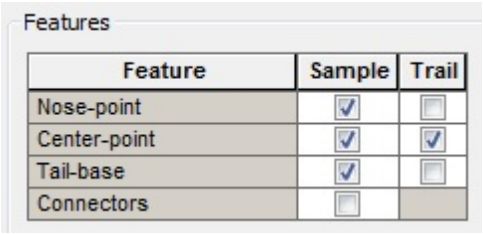
When two or more subjects move in the same arena, the tracks become a dense population of points. If you edit the track of subject A, you can hide the tracks of the other subject B which you do not edit at that time.

To hide tracks of subjects, in the Track Plot Settings pane click the **Show/Hide** tab. Under **Subjects**, clear the box corresponding to those subjects.



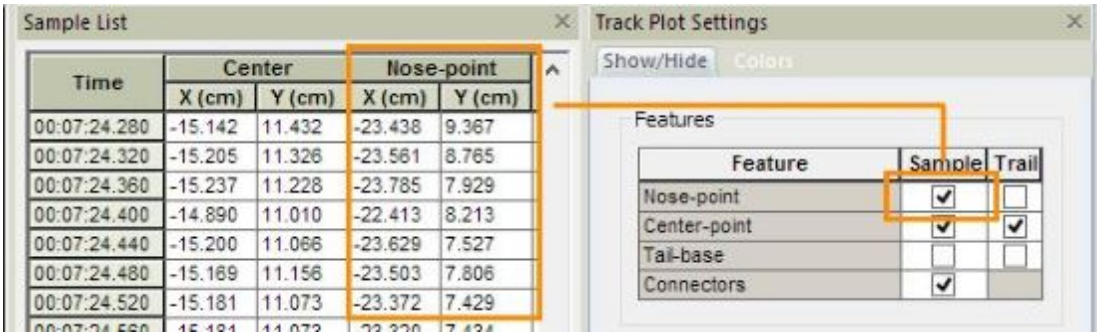

## Hide unnecessary track features

To hide unnecessary features like body points and connectors, in the Track Plot Settings pane click the **Show/Hide** tab. Under **Features**, clear the corresponding box for **Sample** or **Trail**.




*Notes*

- The nose-point and the tail-base point are not selected by default.
- What you choose under **Features** also determines which body points are shown in the Sample List. For example:

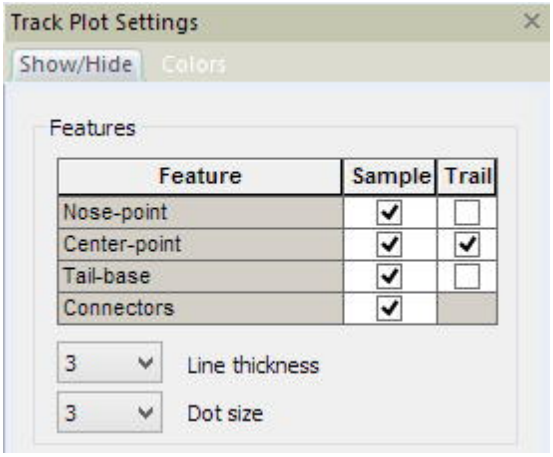


## Adjust the line thickness and dot size

Sometimes dots are too small and therefore scarcely visible, or too large make the tracks difficult to edit. The tracks may be hardly visible especially when using high resolution video.

In the Track Plot Settings pane click the **Show/Hide** tab. Under **Features**, adjust the following:

- **Line thickness**. Adjust the thickness of the trails and the segments joining the body points (Connectors).
- **Dot size**. Adjust the size of the body points visualized.




## Customize the missing sample points

Missing sample points are always visualized in the track plot. You can change the color of missing points to distinguish them more easily from the actual data. The current color for missing points is indicated next to Missing Samples Color at the bottom of the Colors tab in the Track Plot Settings pane. To change the color for missing points, click this button and select the new color in the Color window.


*Notes*

- Remember that *within a sample*, nose-point and tail-base can be missing independently. For example, the nose-point may be missing, while the corresponding tail-base may not. However, if the center-point is missing, then the other two points are too.
- If the experiment is set to Only center-point detection or Color marker tracking, the term *sample* coincides with the only point (center).

# Customize the background

## *Background color or image*

Click the **Show/Hide** button on the toolbar and select **Background**. In the Background window, you can choose between the following:

- **Plain**. The background is uniform. Click the button next to it and choose the color you require. Click **Other** to select among more colors.
- **Captured image**. The background image captured in the Arena Settings that was used to acquire the data. This image does not change as you play the tracks back.
- **Video file**. The video footage from the video file used to acquire the data. The video file is synchronized with the track as you play it back.

  **TIP** Select Video file every time you want to compare the track data with the orientation and behavior of the animal. For example, when you want to verify that the nose point and the tail base point have been detected properly.

## *Arenas*

Click the **Show/Hide** button on the toolbar and select **Arena Features**. Select the arena features you want to have displayed superimposed on the background.

# Select the samples to edit

## What do you want to do?

- Select samples manually

  Choose this option to select a single sample or one or more ranges of samples.

- Select samples automatically

  Choose this option to let EthoVision find the samples that match a specific criterion. For example, find all missing samples to interpolate them.

**IMPORTANT** If you select a body point in the video image, all body points of that sample are automatically selected, no matter whether they are actual data points or missing points.

# Select samples manually

## To select a single sample

Do one of the following:

- Hover the mouse on a body point. The mouse pointer changes to a four-headed arrow.

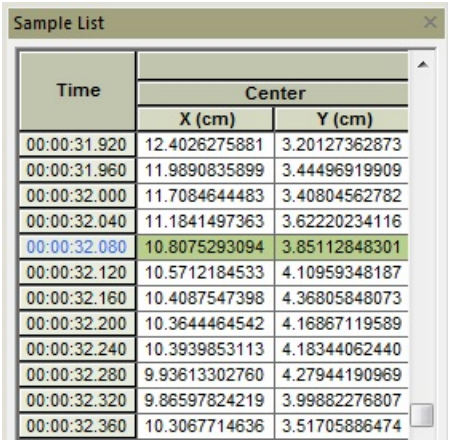
  

  Next, click the point. The sample is highlighted in the track and in the Sample List.

  To cancel the selection, press **Esc** or click on the background image.

- Click the **Show/Hide** button on the toolbar and select **Sample List**. In the Samples List window, click any cell corresponding to the sample you want to select. Scroll down the list if necessary to locate the sample. The line is highlighted in green.

  | Time | Center | |
  |---|---|---|
| | X (cm) | Y (cm) |
| 00:00:31.920 | 12.4026275881 | 3.20127362873 |
| 00:00:31.960 | 11.9890835899 | 3.44496919909 |
| 00:00:32.000 | 11.7084644483 | 3.40804562782 |
| 00:00:32.040 | 11.1841497363 | 3.62220234116 |
| 00:00:32.080 | 10.8075293094 | 3.85112848301 |
| 00:00:32.120 | 10.5712184533 | 4.10959348187 |
| 00:00:32.160 | 10.4087547398 | 4.36805848073 |
| 00:00:32.200 | 10.3644464542 | 4.16867119589 |
| 00:00:32.240 | 10.3939853113 | 4.18344062440 |
| 00:00:32.280 | 9.93613302760 | 4.27944190969 |
| 00:00:32.320 | 9.86597824219 | 3.99882276807 |
| 00:00:32.360 | 10.3067714636 | 3.51705886474 |

  Next, right-click the row and select **Jump to Time** to display the selected samples on the plot.

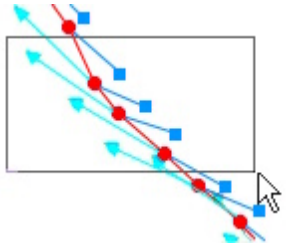
**NOTE** If you click the **Time** column, you select all subjects for that sample.

## To select a range of samples

You can select a range of samples in different ways.

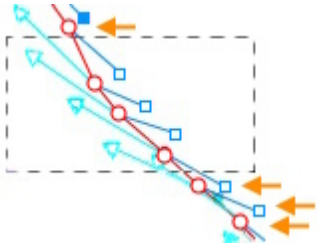
### *Select a range of samples with the mouse*

1. Press and hold your left mouse button, and drag the mouse like when drawing a rectangle.
2. All samples within this rectangle are selected.
3. Release the mouse button.



**NOTES**

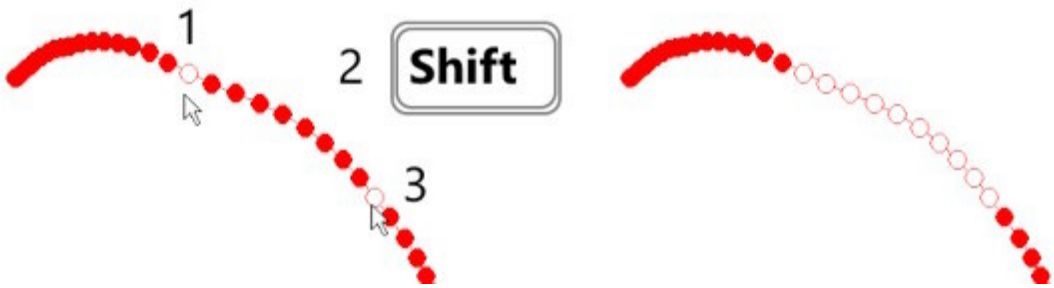
- Use this method to quickly select samples in a specific region.
- This method is not handy if you want to select one subject in a trial with multiple subjects. Rather use the Sample List (see below).
- To cancel the selection, press **Esc**.
- **NOTE** If body points lie outside the selection rectangle are selected, but they belong to the samples at least partially included in the rectangle, they are selected anyway. In the example above, some tail-base and center-points outside the rectangle are selected (see the points indicated by the arrows) because their corresponding nose-points and/or center points lie within the rectangle.



### *Select the first and last samples*

1. Click the first sample of the range you want to select.
2. Press and hold down the **Shift** key.

3. Click the last sample of the range you want to select. The sample range is highlighted.




**NOTES**

- To cancel a range selection, press **Esc** or click on the background image. To select another range, repeat steps 1 to 2.
- You cannot select multiple sample ranges with this method.

### *Choose the sample range from the Samples List*

Choose this method when the ones described above do not allow you to easily select a range because of the high density of sample points.

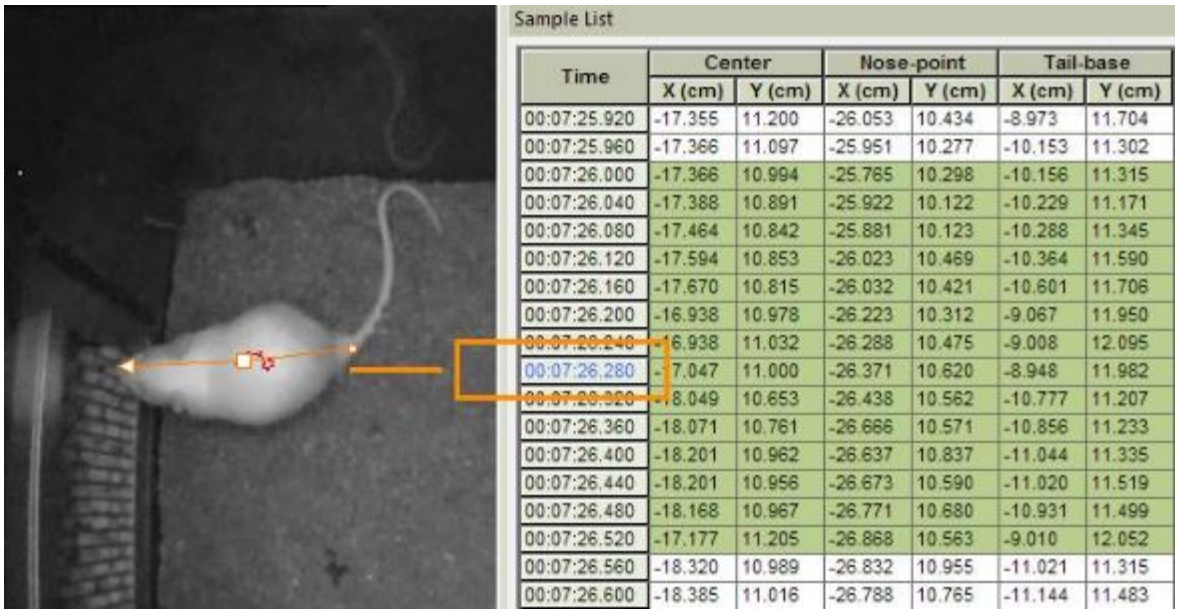
1. Choose **Show/Hide** > **Samples List**.
2. In the Samples List window, click a cell corresponding to the first sample of the range. The line is highlighted in green.

   **TIP** Clicking a row in the Sample List does not move the video to that position. Double-click that row to move the video, so you can see where the subject is at the time you have just selected.

3. Press and hold the left mouse button, and drag down to the last sample you want to select. Release the button when ready.

   Right-click one of the rows highlighted in green and select **Jump to Time** to display the selected samples in the plot.

**TIP** The time highlighted in blue always corresponds to the current video image. The current sample is highlighted (white fill, and orange outline).

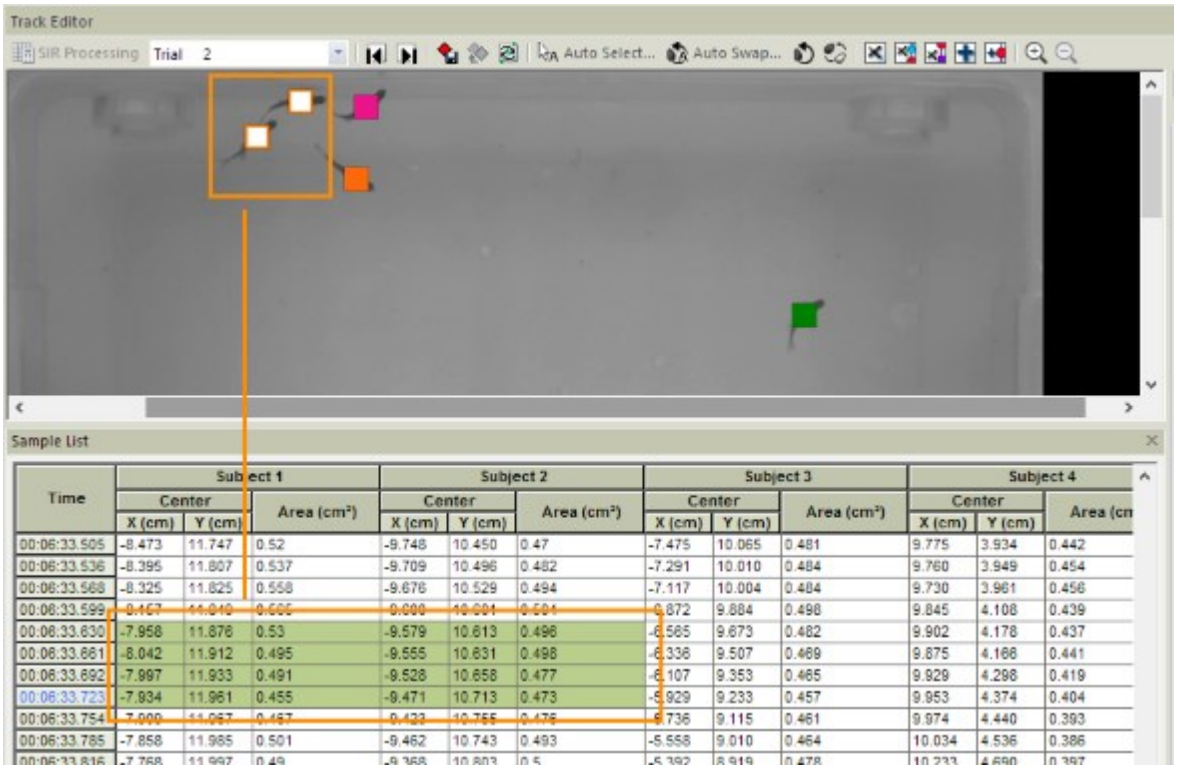


Sample List

| Time | Center | | Nose-point | | Tail-base | |
|---|---|---|---|---|---|---|
| | X (cm) | Y (cm) | X (cm) | Y (cm) | X (cm) | Y (cm) |
| 00:07:25.920 | -17.355 | 11.200 | -26.053 | 10.434 | -8.973 | 11.704 |
| 00:07:25.960 | -17.366 | 11.097 | -25.951 | 10.277 | -10.153 | 11.302 |
| 00:07:26.000 | -17.366 | 10.994 | -25.765 | 10.298 | -10.156 | 11.315 |
| 00:07:26.040 | -17.388 | 10.891 | -25.922 | 10.122 | -10.229 | 11.171 |
| 00:07:26.080 | -17.464 | 10.842 | -25.881 | 10.123 | -10.288 | 11.345 |
| 00:07:26.120 | -17.594 | 10.853 | -26.023 | 10.469 | -10.364 | 11.590 |
| 00:07:26.160 | -17.670 | 10.815 | -26.032 | 10.421 | -10.601 | 11.706 |
| 00:07:26.200 | -16.938 | 10.978 | -26.223 | 10.312 | -9.067 | 11.950 |
| 00:07:26.240 | -16.938 | 11.032 | -26.288 | 10.475 | -9.008 | 12.095 |
| 00:07:26.280 | -17.047 | 11.000 | -26.371 | 10.620 | -8.948 | 11.982 |
| 00:07:26.320 | -18.049 | 10.653 | -26.438 | 10.562 | -10.777 | 11.207 |
| 00:07:26.360 | -18.071 | 10.761 | -26.666 | 10.571 | -10.856 | 11.233 |
| 00:07:26.400 | -18.201 | 10.962 | -26.637 | 10.837 | -11.044 | 11.335 |
| 00:07:26.440 | -18.201 | 10.956 | -26.673 | 10.590 | -11.020 | 11.519 |
| 00:07:26.480 | -18.168 | 10.967 | -26.771 | 10.680 | -10.931 | 11.499 |
| 00:07:26.520 | -17.177 | 11.205 | -26.868 | 10.563 | -9.010 | 12.052 |
| 00:07:26.560 | -18.320 | 10.989 | -26.832 | 10.955 | -11.021 | 11.315 |
| 00:07:26.600 | -18.385 | 11.016 | -26.788 | 10.765 | -11.144 | 11.483 |

## Notes

- To select samples for *all* subjects in the arena, click the cell in the **Time** column. for the first sample of the range, then drag down to the last sample you want to select.
- You can also select samples for two or more subjects that are adjacent in the Sample List (e.g. Subject 1 and 2). Click the first sample for Subject 1, and drag down to the last sample you want to select for Subject 2.




| Time | Subject 1 | | | Subject 2 | | | Subject 3 | | | Subject 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Center | | Area (cm²) | Center | | Area (cm²) | Center | | Area (cm²) | Center | | Area (cm |
| | X (cm) | Y (cm) | | X (cm) | Y (cm) | | X (cm) | Y (cm) | | X (cm) | Y (cm) | |
| 00:06:33.505 | -8.473 | 11.747 | 0.52 | -9.748 | 10.450 | 0.47 | -7.475 | 10.065 | 0.481 | 9.775 | 3.934 | 0.442 |
| 00:06:33.536 | -8.395 | 11.807 | 0.537 | -9.709 | 10.496 | 0.482 | -7.291 | 10.010 | 0.484 | 9.760 | 3.949 | 0.454 |
| 00:06:33.568 | -8.325 | 11.825 | 0.558 | -9.676 | 10.529 | 0.494 | -7.117 | 10.004 | 0.484 | 9.730 | 3.961 | 0.456 |
| 00:06:33.599 | -8.167 | 11.849 | 0.605 | -9.600 | 10.601 | 0.501 | -6.872 | 9.884 | 0.498 | 9.845 | 4.108 | 0.439 |
| 00:06:33.630 | -7.958 | 11.876 | 0.53 | -9.579 | 10.613 | 0.496 | -6.585 | 9.673 | 0.482 | 9.902 | 4.178 | 0.437 |
| 00:06:33.661 | -8.042 | 11.912 | 0.495 | -9.555 | 10.631 | 0.498 | -6.336 | 9.507 | 0.469 | 9.875 | 4.166 | 0.441 |
| 00:06:33.692 | -7.997 | 11.933 | 0.491 | -9.528 | 10.658 | 0.477 | -6.107 | 9.353 | 0.465 | 9.929 | 4.298 | 0.419 |
| 00:06:33.723 | -7.934 | 11.961 | 0.455 | -9.471 | 10.713 | 0.473 | -5.929 | 9.233 | 0.457 | 9.953 | 4.374 | 0.404 |
| 00:06:33.754 | -7.900 | 11.957 | 0.467 | -9.433 | 10.755 | 0.475 | -5.736 | 9.115 | 0.461 | 9.974 | 4.440 | 0.393 |
| 00:06:33.785 | -7.858 | 11.985 | 0.501 | -9.462 | 10.743 | 0.493 | -5.558 | 9.010 | 0.464 | 10.034 | 4.536 | 0.386 |
| 00:06:33.816 | -7.768 | 11.997 | 0.49 | -9.368 | 10.803 | 0.5 | -5.392 | 8.919 | 0.478 | 10.233 | 4.690 | 0.397 |

# Select samples automatically

## Aim

To easily find:

- Missing points.
- Samples separated by a distance greater or smaller than a specific value. For example, to find points lying far from the normal path.
- Samples with a surface area greater or smaller than a specific value. For example, to find all points where the detected blob is too large to be the actual subject.
- Samples with the distance between nose-point and tail-base greater or smaller than a specific value.

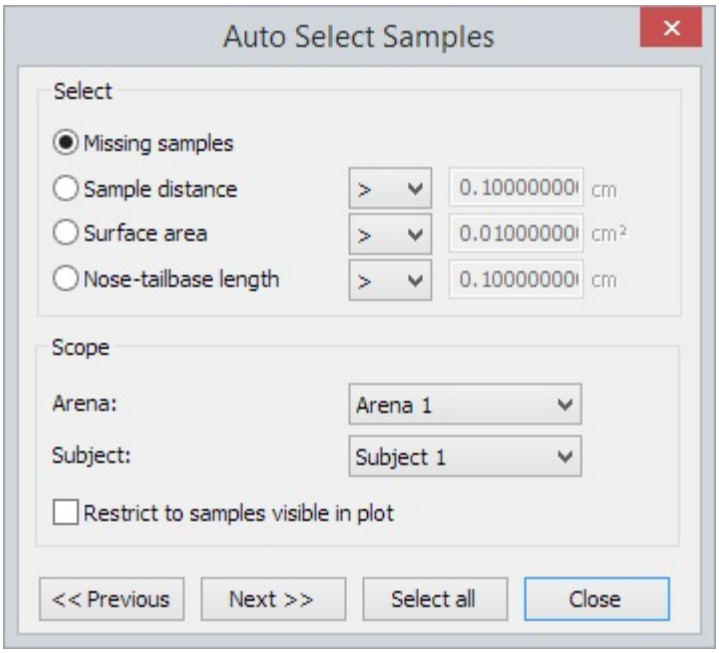
## To access this function

Choose **Acquisition** > **Edit Tracks**, and select the trial you want to view.

## Procedure

1. On the toolbar, click the **Auto Select** button.


2. In the Auto Select Samples window, under **Select** choose the option you require. For more information, see the notes below.




3. Under **Scope**:
   - Select the arena you want to search. If you want to find samples in all arenas, select **All arenas**.
   - Select the subject you want to search. If you want to find samples in all subjects, select **All subjects**.
4. Click **Select All**.

   *Result*: The samples matching the criterion are highlighted in the track plot. In the Samples List, the corresponding rows are highlighted in green.
5. If you chose **Missing samples** in step **2**, click the **Next>>** and **<<Previous** buttons to move to the next or previous missing sample, respectively. There, you can decide whether to Interpolate points or leave the sample as missing.
6. If necessary, change the settings under **Select** and click the **Select All** button. When you are satisfied with the selection, click **Close**.

## Options

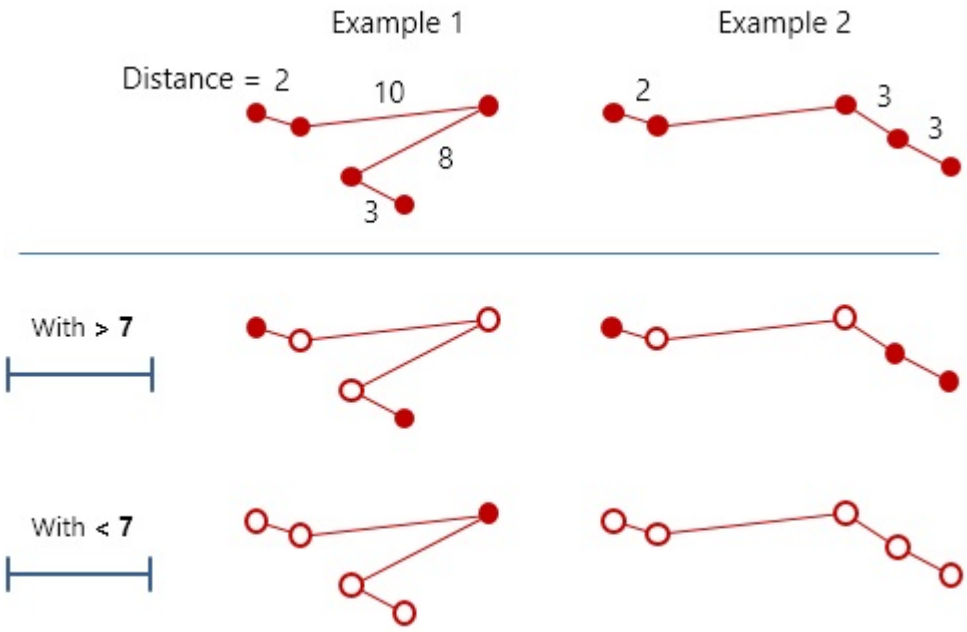
### *Missing samples*

Option **Missing samples**. Choose this option to select all missing samples in the track, for example to interpolate them.

### *Sample distance*

EthoVision XT selects all the non-missing points separated from the previous or the next by a distance greater *or* smaller than the threshold you specify. To select samples, EthoVision XT always uses the distance between center points.

In the following example with two short tracks, the open circles represent the samples selected after using a distance threshold, indicated with the segment.




**NOTE** Using > and < does not result in complementary selections. Some samples in the figure above are selected in both cases.

**TIP** To remove outliers, for example data points resulting from detection of reflections in a water maze, select **>** and enter a value of distance. This value must be greater than a realistic estimate of the maximal distance that the subject could move between two consecutive samples. Note that this value also depends on the sample rate. The higher the sample rate, the shorter the distance moved by the subject between two consecutive samples. Next, do Delete points and then Interpolate points to replace the outliers.

### *Surface area*

For this option, specify the value in the measurement unit selected in the Experiment Settings.

*Nose-tailbase length*

This is only available if your experiment is set to Center-point, nose-point and tail-base detection. Nose-tailbase length is the total length of the segments between nose-point and center-point, and that between center-point and tail-base point.

*Restrict to samples visible in plot*

Select this option if you want to select only the samples that match the **Select** criterion *and* that are currently displayed in the track plot. The other samples that match the criterion but are not displayed, are not selected.

**NOTE** If you select this option and then play back the track, the samples that are not currently visualized, but that match the selection criterion under **Select**, are *not* selected. To have them selected, pause the video and click **Select all** once again.

## To deselect samples

Do one of the following:

- Press **Esc**.
- Click anywhere on the background image that is not a point.
- Right-click anywhere on the background image.

# Swap subjects and body points

## What do you want to do?

*Swap subjects*

- Swap subjects
- Prepare the data in multi-subject trials

*Swap body points*

- Swap nose-point and tail-base
- Swap nose-point and tail-base point manually
- Swap nose-point and tail-base point automatically

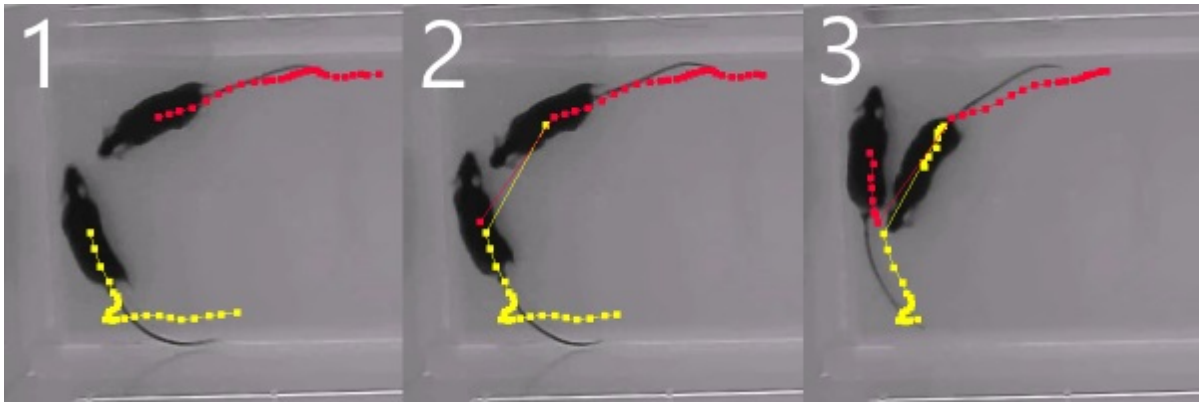
# Swap subjects

## Aim

To exchange the identities of two subjects within one sample (or range of samples, or the entire track), after checking that these were assigned incorrectly by EthoVision XT.

Below: An example of subject identity swap. 1. before the swap (Subject 1 = yellow track. Subject 2 = red track). 2. Moment of subject swap; the mouse with yellow track becomes Subject 2 and the mouse with red track becomes Subject 1 (see the last samples exchanged between the yellow and the red tracks). 3. After the swap.

To correct the subject identity swap, swap the subjects for all samples beginning from time 2, up to a new swap (if present) where subjects are labeled correctly again.

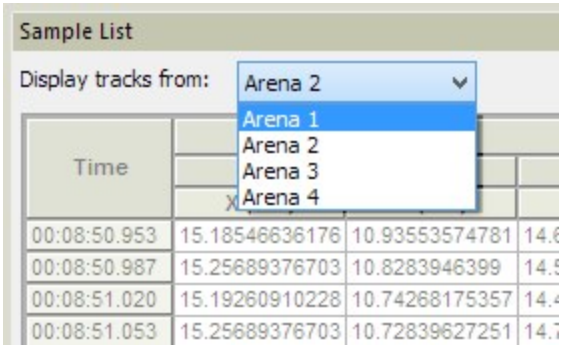



**NOTES**

- If a large number of samples contain body points assigned to the wrong subject, it may mean that the detection settings defined to discriminate between subjects are not optimal, or that you need to improve the lighting or the color marking of your setup.
- You can click **Save** on the toolbar so that if you are editing many swaps and make a mistake, you do not loose your previous edits. See Saving the track edits.

## Procedure

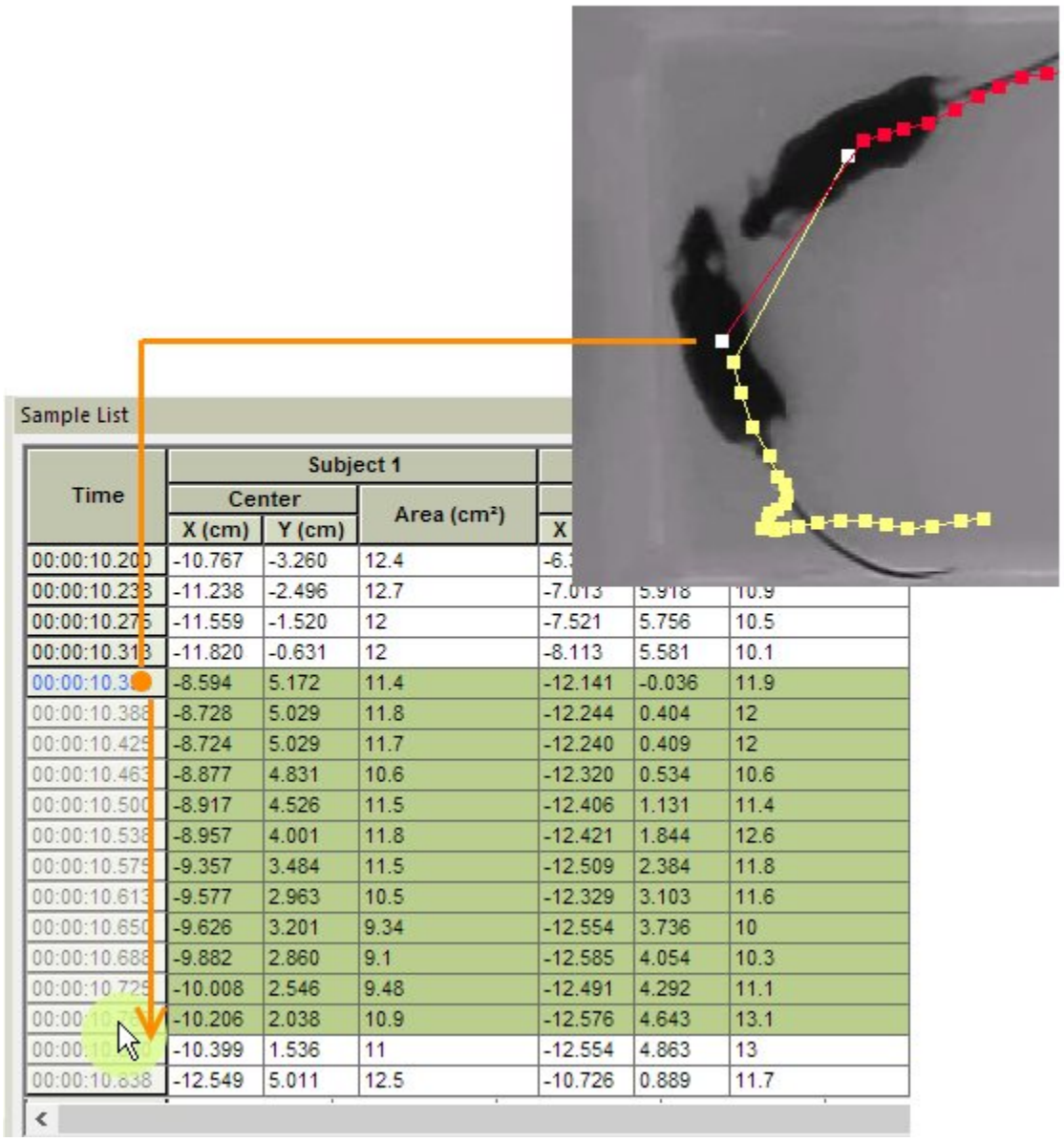
1. If you work with multiple arenas, choose first the arena in the Samples List.




2. Play the track until you find samples with wrongly-assigned subjects.

   **TIP** Play frame-by-frame to position the video when the first samples are swapped (see the figure below).

3. Select the sample (or range of samples) in which you want to swap the subjects. To do so, in the Samples List, double-click the time value highlighted in blue, then drag to select the rows corresponding to all the samples to swap. The last row selected should be immediately before the next swap (where body points are assigned correctly again).




**IMPORTANT** If you drag on the **Time** column, all subjects in the trial are selected. If you have more than two subjects (for example, three subjects and you want to swap Subject 1 and 3), do not drag in the **Time** column. Instead, select the cells of one of the two subjects to swap. In the next step you will be asked to select the other subject.

Sample List

| Time | Subject 1 | | | Subject 2 | | |
|---|---|---|---|---|---|---|
| | Center | | Area (cm²) | Center | | Area (cm²) |
| | X (cm) | Y (cm) | | X (cm) | Y (cm) | |
| 00:00:10.313 | -11.820 | -0.631 | 12 | -8.113 | 5.581 | 10.1 |
| 00:00:10.350 | -8.594 | 5.172 | 11.4 | -12.141 | -0.036 | 11.9 |
| 00:00:10.388 | -8.728 | 5.029 | 11.8 | -12.244 | 0.404 | 12 |
| 00:00:10.425 | -8.724 | 5.029 | 11.7 | -12.240 | 0.409 | 12 |
| 00:00:10.463 | -8.877 | 4.831 | 10.6 | -12.320 | 0.534 | 10.6 |
| 00:00:10.500 | -8.917 | 4.526 | 11.5 | -12.406 | 1.131 | 11.4 |
| 00:00:10.538 | -8.957 | 4.001 | 11.8 | -12.421 | 1.844 | 12.6 |
| 00:00:10.575 | -9.357 | 3.484 | 11.5 | -12.509 | 2.384 | 11.8 |
| 00:00:10.613 | -9.577 | 2.963 | 10.5 | -12.329 | 3.103 | 11.6 |
| 00:00:10.650 | -9.626 | 3.201 | 9.34 | -12.554 | 3.736 | 10 |
| 00:00:10.688 | -9.882 | 2.860 | 9.1 | -12.585 | 4.054 | 10.3 |
| 00:00:10.725 | -10.008 | 2.546 | 9.48 | -12.491 | 4.292 | 11.1 |
| 00:00:10.763 | -10.206 | 2.038 | 10.9 | -12.576 | 4.643 | 13.1 |
| 00:00:10.800 | -10.399 | 1.536 | 11 | -12.554 | 4.863 | 13 |
| 00:00:10.838 | -12.549 | 5.011 | 12.5 | -10.726 | 0.889 | 11.7 |

Result: The selected samples are highlighted.

**TIP** To swap subject identity throughout an entire track, click the header for one of the two subjects (e.g. click the header **Subject 1**).

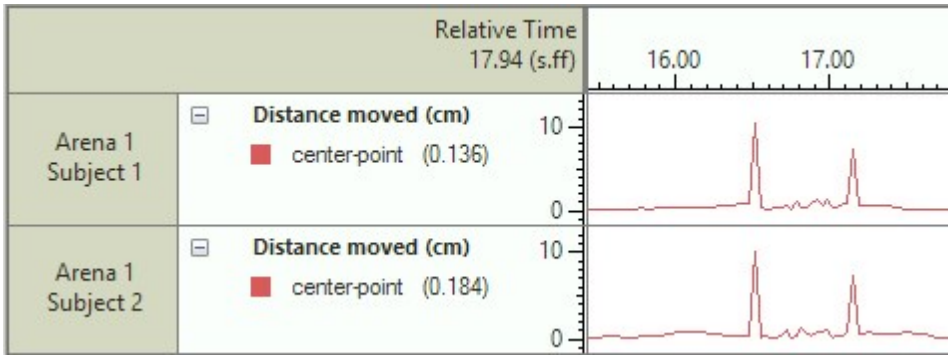
4. Click the **Swap subjects** button on the toolbar, or press **D**.


5. If you have more than two subjects, the Swap Subject window appears. Select **Swap Selected Samples**. Select the second subject of the pair you want to swap, then click **Apply**.

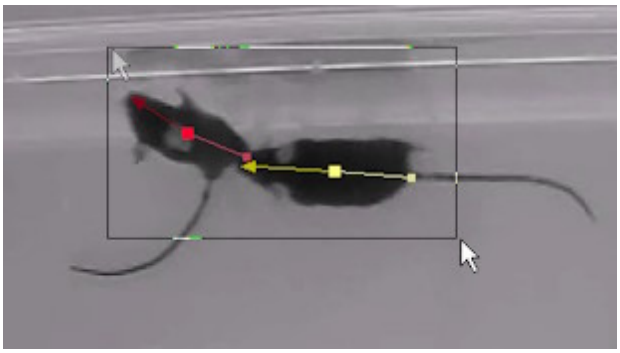
Result: The subjects in the selection are swapped. You can check this in the Samples List, where the coordinates and the area are swapped between subjects and for the rows selected.

## Notes

- To search for subject swaps, plot the dependent variable *Distance moved* in the Integrated Visualization. The peaks in the plot appearing for two subjects indicate a quick displacement of the body points, that is a suspect subject swap.




- To quickly swap two subjects for just one sample, drag the mouse around the subject's body points in the video window. Make sure that the Track Editor only display one sample.



Then click the **Swap subjects** button .

- In the **Time** column of the Samples List, the cell highlighted in blue corresponds to the sample highlighted in white in the video window.
- If you select several samples and choose to have one sample every n-th visualized, you also swap the body points in the selected range that are not visualized.
- In the Swap Subjects window, select **Swap all samples** to swap the subjects for the entire track length.

# Prepare the data in multi-subject trials

## Aim

To fix potential identity swaps after tracking multiple subjects with the Deep learning body point detection technique.

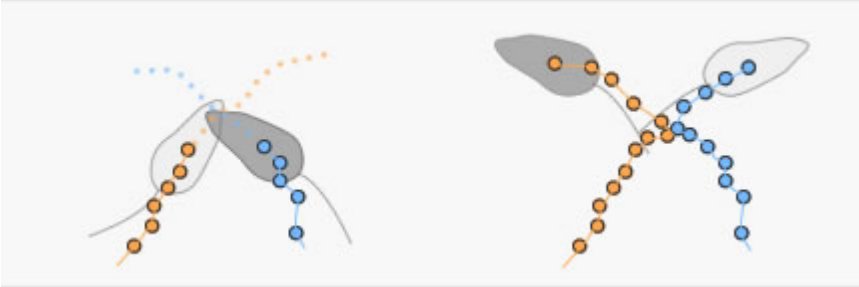
## This topic applies to

Experiments set as follows: (see Experiment settings)

- Number of Subjects per Arena: 2.
- Tracked Features: Center-point, nose-point and tail-base detection.
- Body Point Detection Technique: Deep learning.

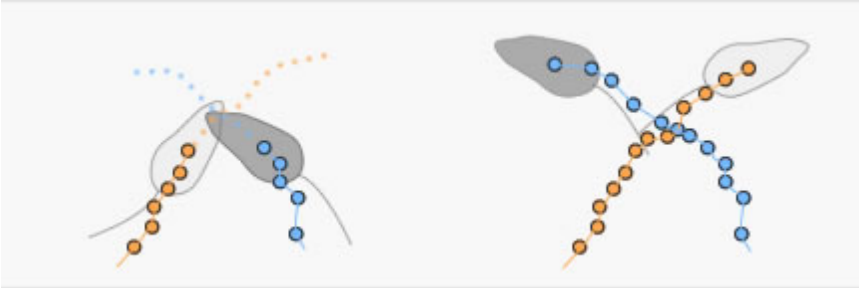
## Background information

When EthoVision XT tracks unmarked subjects, there is chance that subject identity is swapped. This could occur for example when two subjects come into contact with each other. In the example below, the dotted line is the true trajectory of the two subjects. After the crossing, the subject *orange* is labeled as *blue*, and *blue* is labeled as *orange*.



To reduce or entirely remove identity swaps, when using Deep learning, the software works in two steps:

- Step 1 (Tracking) - The software finds and tracks the subjects' body points.
- Step 2 (**Data Set Preparation**) - The software reviews the tracks and sorts them based on the visual differences between the subjects. This step is carried out after tracking, and automatically when you calculate/visualize the data. As a result, the occurrence of identity swaps is reduced.



Recognition of individuals is based on the differences in the appearance of the subjects' fur. For how to mark your subjects, see the recommendations in Deep learning: Requirements

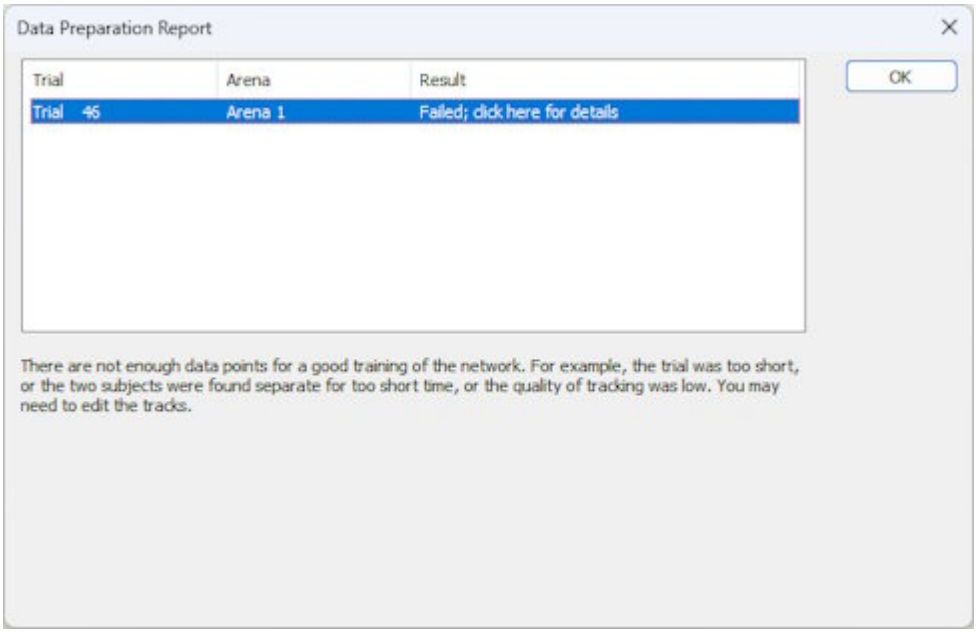
## Procedure

The Data Set Preparation is done automatically the first time that you calculate the statistics or visualize the tracks. You can also run this procedure manually in the Track Editor.

1. Choose **Acquisition** > **Edit Tracks**.
2. Select one trial from the list on the toolbar that is marked with **(not yet prepared)**.
3. Click **Prepare Data**.
4. Wait until the trial name shows "(ready)". Depending on a few factors, including the length of the video and its resolution, this process may take some time.
5. You can now edit the tracks or run analysis.

## Error messages

If for any reason, the Data Set Preparation procedure fails for at least one trial/ arena, the Data Preparation Report shows in which trial and arena that happened, and what the possible causes could be.




- **Not enough data points available to reliably train the network**. This occurs if the trial was short, or the two animals were separated for too short time. Another possibility is that the animals were separated, but at least one of them was curled up for long time. In all those cases the software cannot create a high number of reliable images of the two subjects.

  In general, we recommend to acquire a trial of at least five minutes, at the maximum sample rate (25 samples/s or higher).

  Although the message may sound pessimistic, there are cases when the individual discrimination worked well. For example when the visual difference between the two subject is very clear. In such a situation a relatively small number of video frames may still be ok to create a reliable network for individual discrimination.

  Check the identity labels of the two animals and edit the tracks when necessary.

- **No data points available to train the network**. This could occur when tracking was so bad because of poor detection (e.g. not enough contrast, or presence of reflections) or because the two subjects were in contact for the entire duration of the trial. In such a situation there are no data points at all available for training the network; individual discrimination fails, no matter how long the trial lasted.

  We advise to check the lighting conditions, the contrast between the subjects and the background, and repeat the trial.

- **The subjects look too similar**. This occurs when the two subjects are not marked and there is no significant difference in their visual appearance.

Check the suggestions about marking animals: Deep learning: Requirements > Individual marking (for two-subject tracking)

## Notes

- The Data Set Preparation may take some time, depending on the length of the video and the power of the GPU.
- The Data Set Preparation is not applied to trials where the subjects are tracked with the Contour-based technique for body point detection. For the difference between Contour-based and Deep learning, see Body point detection technique.
- The trial where data preparation failed is still added to your results table. However, there may be significant identity swaps. There are two options:
  - Open the trial in the Track Editor and fix the identity swaps. See Swap subjects
  - Exclude the problematic trials in your Data profile. See Filter tracks
- Check that you have the latest version of the driver for the secondary graphics card which does most of the work in deep learning - based tracking.

*See also*

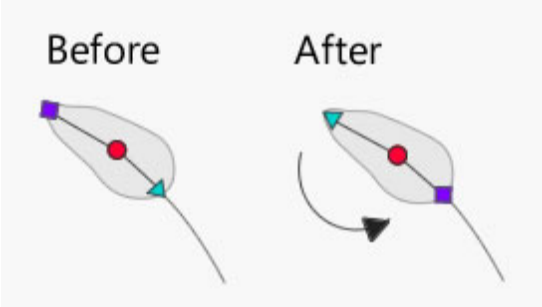
- Advanced detection settings for tracking multiple unmarked subjects
- Introduction to editing tracks
- Swap subjects
- Deep learning: Basics
- Deep learning: Requirements

# Swap nose-point and tail-base

## Aim

To exchange the nose-point and the tail-base within a sample (or range of), after checking that these were assigned incorrectly by EthoVision XT.

In the example below, the nose-point and the tail-base point are assigned incorrectly (left). The Swap action puts the nose-point and the tail-base point back in the correct place.




To check that nose-point and tail-base were mistakenly exchanged, choose **Analysis** > **Results** > **Integrated Visualization**. Play back the tracks with the video on the background. Check that the nose-point and tail-base do correspond to the actual orientation of the subject.

## Procedure

Choose **Acquisition** > **Edit Tracks**.

Choose one of the two options:

- Swap nose-point and tail-base point manually. Look for the samples with exchanged nose-point and tail-base, and give the Swap command for those samples.
- Swap nose-point and tail-base point automatically. Define a criterion for automatic detection of changes in orientation of the nose-point and tail-base. EthoVision XT searches your tracks for the samples where a sudden change in orientation has occurred. It is up to you to swap nose-point and tail-base for those samples.

## Notes

- By selecting the tracking methods **Rodents / For occlusions** or **Adult Fish / For occlusions**, you can minimize the occurrence of nose-tail swaps.

However, this method requires much processor load, so make sure you do not miss samples.

- If a large number of samples contain wrongly-assigned nose-point and tail-base, it may mean that the detection settings for nose-point and tail-base detection are not optimal, or that you need to improve the lighting or other settings of your setup.
- You can click **Save** on the toolbar so that if you are editing many swaps and make a mistake, you do not loose your edits.
- When you swap nose- ant tail-base points, the Head direction line is recalculated too. This influences the calculation of *Head direction*, *Head directed to zone* and *Turn angle* and *Angular velocity* when they are based on Head direction.

# Swap nose-point and tail-base point manually

## Aim

To correct body point swaps that may have occurred when tracking the nose-point and the tail-base point.

## Procedure

1. Play the track until you find samples with wrongly-assigned nose-point and tail-base. See To check that nose-point and tail-base were mistakenly exchanged, choose Analysis > Results > Integrated Visualization. Play back the tracks with the video on the background. Check that the nose-point and tail-base do correspond to the actual orientation of the subject.
2. Select the sample (or range of) in which you want to swap the nose-point and tail-base.

   Result: The selected samples are highlighted.
3. Click the **Swap nose and tail** button on the toolbar, or press the **W** key.


*Result*: The nose-point and tail-base within each sample in the selection are swapped. In the Samples List, the coordinates are swapped between Nose-point and Tail-base for those samples.

## Notes

- When you swap nose- and tail-base points, the Head direction line is recalculated as the prolongation of the segment joining the center-point and the tail-base point. This influences the calculation of *Head direction*, *Head directed to zone* and *Turn angle* and *Angular velocity* when they are based on Head direction.

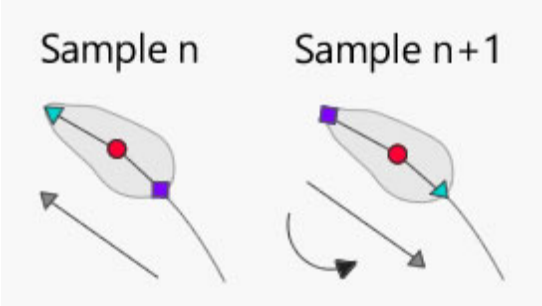
# Swap nose-point and tail-base point automatically

With automatic swapping, EthoVision looks for the samples where the subject made a turn exceeding a user-specified threshold relative to the previous sample. These turns are interpreted as nose-point and tail-base being incorrectly assigned. You can then select those samples and let EthoVision exchange the corresponding nose-point and tail-base.

Use this method when a large number of consecutive samples have body points wrongly assigned.

## What does 'turn' mean here?

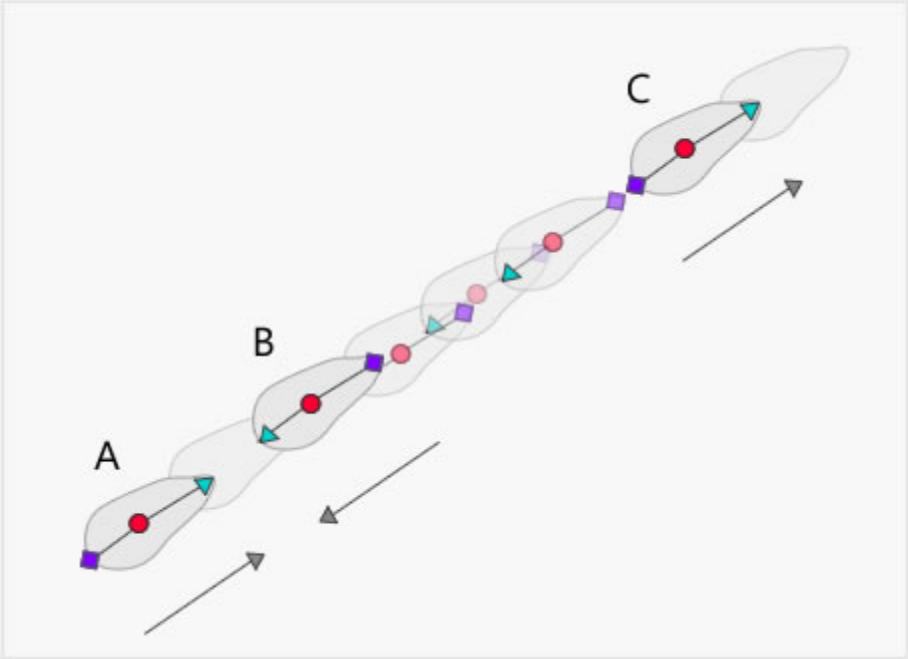
Turn is referred to the change in orientation of the nose-tail base vector. The nose-tail base vector is the vector connecting the nose-point and the tail-base point within a sample (see the dotted arrow in the picture below). With automatic swapping, if the change in the vector's direction from sample *n* to *n+1*, is larger than the angle you specify, the sample *n+1* is considered for swapping. Then, it is up to you to decide whether to swap the points.




If the nose-point or the tail-base is missing, the nose-tail base vector is the vector connecting the center with the other point. If both nose- and tail-base points are missing, the sample is not considered for swapping anyway.

If samples following *n+1* have a vector's turn lower than the specified threshold, they too are assumed to have nose-point and tail-base detected wrongly. Therefore, they are included in the swapping interval (see samples under B in the figure below).

**EXAMPLE** A: the body points are assigned correctly. B: the nose-tail base vector (indicated with the black arrow) makes a turn greater than the specified threshold (for example, 150°). This is the start of the swapping interval. C: a new significant turn of the nose-tail base vector is found. Samples between B and C (not included) are swapped. The samples after C are considered correct, until a new significant turn is found.

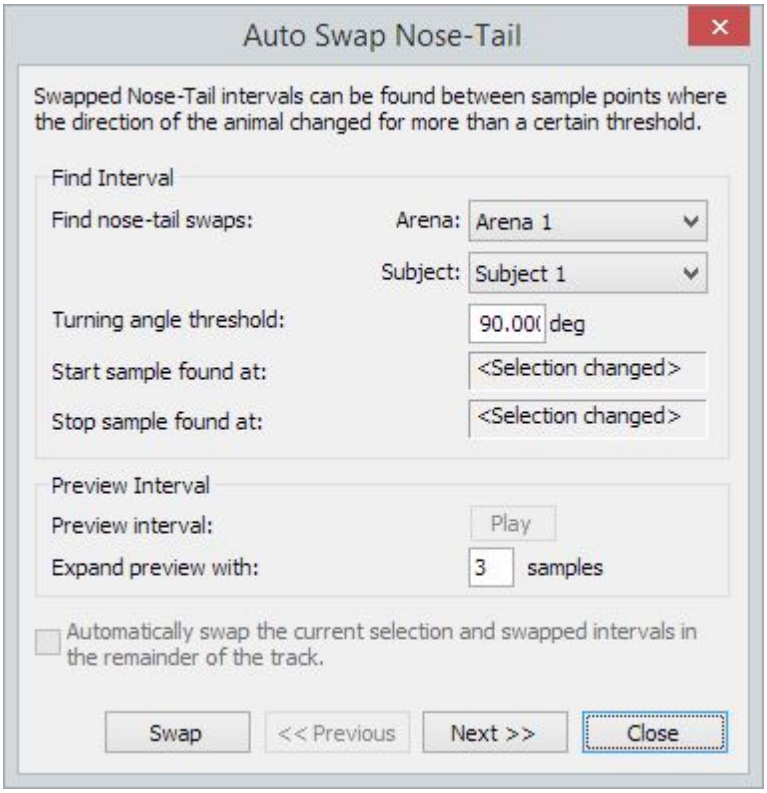



## To swap body points automatically

1. Position the track at the beginning and check whether the first sample in your track is orientated correctly.

   To check this: If you have tracked data from a video file, make sure that the video file is on the background. If you have tracked live, make sure that the nose-tail base orientation matched the direction of movement.

2. Click the **Auto Swap** button on the toolbar, or press **Ctrl+F**.

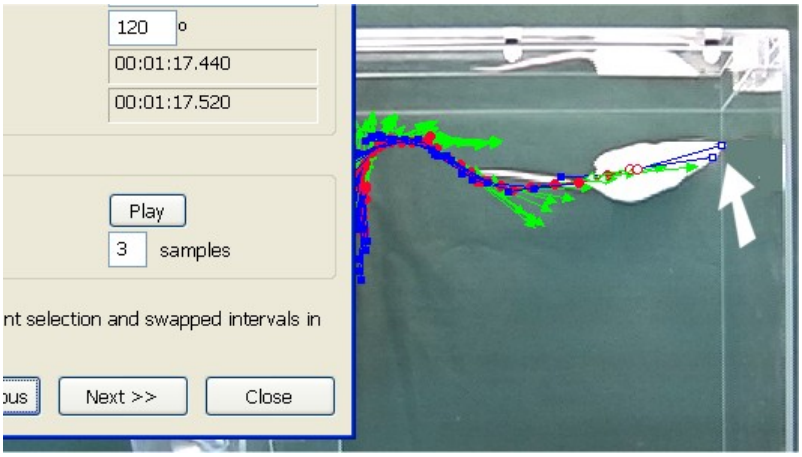



3. In the Auto Swap Nose-Tail window, under **Find Interval**, do the following:
   - From the **Find nose-tail swaps in the track** list, select the arena and the subject you want to search. For single-arena setups and single-subject setups, only one arena/subject is available, so go to the next step.
   - In the **Turning angle threshold** field, enter the threshold angle. The first sample with a change in orientation of the nose-tail base vector larger than this threshold is considered for swapping.
4. Click the **Next >>** button.

   Result: A number of samples are highlighted (selected). Under **Find Interval**, the following times are displayed:
   - **Start sample found at:** The time of the sample in the track with turning angle greater than the threshold relative to the previous sample. It is the start of the interval of samples considered for swapping.
   - **Stop sample found at:** The time of the sample immediately preceding the sample with turning angle greater than the threshold. It is the end of the interval of samples considered for swapping.
5. Do one of the following:

If the first sample of the selected interval in your track is oriented correctly, click the **Next >>** button until the first sample of the new interval is not oriented correctly.

If the first sample of the selected interval is not oriented correctly, this is the start of the interval of samples to be swapped. Proceed to step 6.




6. Once you have selected an interval of samples that does not match the orientation of the animal, do one of the following:
   - If you want to let EthoVision XT swap the intervals of samples throughout the track based on the above criteria, select the option **Automatically swap the current selection and swapped intervals in the remainder of the track**, and click the **Swap** button in the Auto Swap Nose-Tail window.
   - If you only want to swap the selected interval, make sure that the option above is cleared and click the **Swap** button in the Auto Swap Nose-Tail window.

   Result: The nose-point and tail-base in the selected intervals are swapped.
7. Do one of the following:
   - If you want to search for the next interval to be swapped, go back to step 5.
   - If the track contains only samples oriented correctly, click the **Close** button.

To swap nose-point and tail-base in another arena, select that arena from the appropriate list.

## Notes

- When you click **Next>>** for the first time, the interval from the start of the track (0:00:00.000) to the first change in the nose-tail vector orientation greater than the threshold is displayed. If you click the Next>> button more times, other intervals are displayed depending on where in the track other significant turns are found.
- Select the Automatic swap option above only if you are confident that the angle threshold specified in step B results in identifying the correct intervals to be swapped. This usually happens when you have checked the intervals yourself a few times.
- If you select points outside the Auto Swap Nose-Tail window, the **Start sample found at** and **Stop sample found at** fields show **<Selection changed>**. In this case the Automatic swap option is not available. Click the **<<Previous** button to return to one of the sample intervals found by the program.
- If you want to view the movement of the subject around the selected sample, click the **Play** button in the **Preview Interval** group. If you want to view additional samples immediately before the start and after the end of the selected interval, enter the number of such samples in the Expand preview with box.
- To go back in the track and select the previous intervals that start and stop at samples where the turn angle exceeds the threshold, click the **<<Previous** button.
- If you want to undo the swap action, click the **Swap** button again.

# Delete, move and interpolate points

## What do you want to do?

- Delete points
- Move points
- Interpolate points

## Learn about

- More information about interpolation

# Delete points

## Aim

To remove a sample (or some of the points of your sample) from your data set.

Deleting points means that they are set to missing.

**IMPORTANT** If you delete a point, you cannot recover the original coordinates, you can only interpolate it. If you delete an entire sample, the surface area is also lost. If you are not sure about the consequences of setting points to missing, save the edits first. You can always return to your original track data.

## Procedure

1. Select the sample (or range) that contains the points you want to set to missing.
2. Do one or more of the following:

   If your track contain only center-points, click the **Set to missing** button on the toolbar or press **Ctrl+Del**.

   If your data also contains nose-point and tail-base, to set all points to missing, click the **Set to missing** button or press **Ctrl+Del**.

   To set only nose-points to missing, click the **Set nosepoint to missing** button or press **Ctrl+Shift+M**.

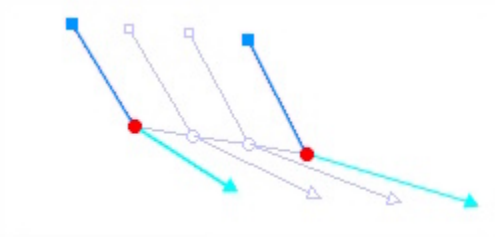
   

   To set only tail-base points to missing, click the **Set tailbase to missing** button or press **Ctrl+M**.

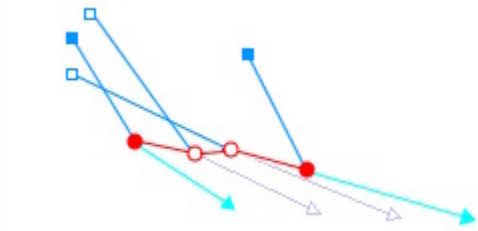
   

## What “Set to missing” means

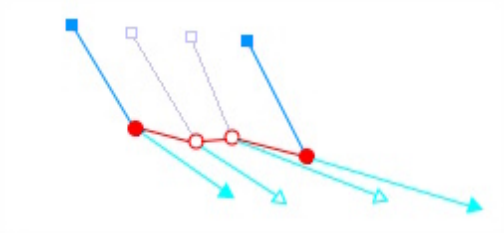
- If you have set the whole selection to missing:



- If you have set only the nose-points to missing:



- If you have set only the tail-bases to missing:



Missing points are shown as linear interpolations between the non-missing points of their own type. However, their coordinates are not in the track data, and are not subject to analysis. For example, if sample n is missing, the distance moved between the two non-missing points n–1 and n+1 is given by the straight line connecting the two points of the same type (nose-point to nose-point, etc.).

- In the Samples List, the points' X,Y coordinates are removed from the Samples List. If you set the sample to missing, the surface area is also removed. Missing values are indicated by "-".
- If you set a nose-point to missing, the Head direction value for that sample is removed.

# Move points

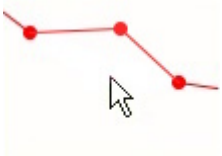
## Aim

To change the X,Y coordinates of a point on the track plot.

## Procedure

1. Hover the mouse on the point you want to move. The mouse cursor changes to a four-headed arrow.

2. Press and hold the left mouse button and drag to the position you require.
3. Release the mouse button. The point is moved to the new position and its X,Y coordinates are updated in the Samples List.



4. To de-select the point, press **Esc** or click on the background image.

## Notes

- If you move a missing sample, this becomes non-missing and therefore included in your data.
- You cannot move a sample outside the arena.
- If your experiment is set to Center-point, nose-point and tail-base detection, a sample is made of three points (no matter whether those are actual data points or missing). You cannot move a sample as a whole, you must move each point separately (center-, nose-, and tail-base points).
- If you move the nose-point, the corresponding value of Head direction for that sample is unchanged.

# Interpolate points

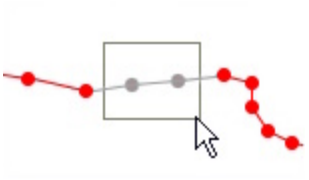
## Aim

To replace one or more missing points (or samples) or points clearly misplaced with points calculated on the basis of the position of the first previous and next non-missing points.

If your experiment is set to Center-point, nose-point and tail-base detection, you can choose between two types of interpolation: interpolate all selected body points or the center-points only (see below).

## Procedure

1. If you want to interpolate non-missing points clearly misplaced, set them first to missing (see Delete points). In other cases go to step 2.
2. Select the missing sample (or the range of) you want to interpolate.



**TIP** To select missing samples automatically, click the **Auto Select** button on the toolbar, then choose **Missing samples**. Click the **Next>>** button to move the video to the next missing sample.

3. Click one of the following buttons on the toolbar:

   **Interpolate the selection** (or press **Ctrl+I**). Click this button if you want to interpolate all the body points of the selected samples. See the notes below for how points are interpolated.

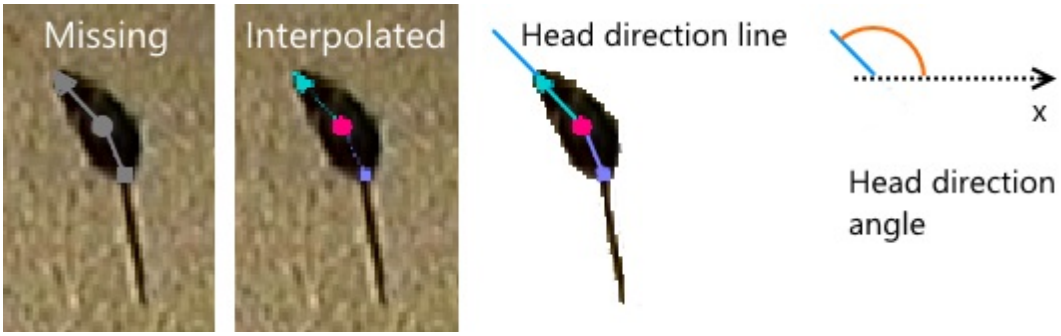
   **Interpolate center points** (or press **Ctrl+Shift+I**). Click this button if you want to interpolate the Center points for the selected samples, and leave the nose-point and tail-base as they are currently (no matter whether missing or not).

   If your experiment is set to Only center-point detection, only the first button is available. Click that button to interpolate the center-points.

4. A message appears after you have clicked the interpolate buttons, informing you that EthoVision XT has extended the range of samples to be interpolated, if needed (see the note below). Click **Yes** if you want to continue. If you are not sure about the result of the interpolation, click **Cancel**.

## Notes

- The interpolated points are highlighted in the track plot and get the color of non-missing points. In the Samples List, the points obtain X,Y coordinates.
- The Subject area is interpolated linearly.
- When you visualize the data, missing points are shown as linear interpolation between the first previous and next valid points. However, their coordinates are not calculated (you can check this in the Samples List). This means that missing points are actually not in the data set. If you want to include them in your data set, you must first interpolate them.
- In some cases interpolation may result in points being located outside the arena. In those cases, they are automatically set to missing. This could be the reason why you still see missing points after you have carried out interpolation.
- If you are not sure whether interpolation gives good results, save the edits first. See Saving the track edits
- If you interpolate the nose- and tail-base points, the Head direction line is recalculated as the prolongation of the segment that joins the center-point and the nose-point after interpolation.




This has consequences on all variables based on the Head direction line: *Head direction*, *Head directed to zone* and *Turn angle* and *Angular velocity* when they are based on head direction.

# More information about interpolation

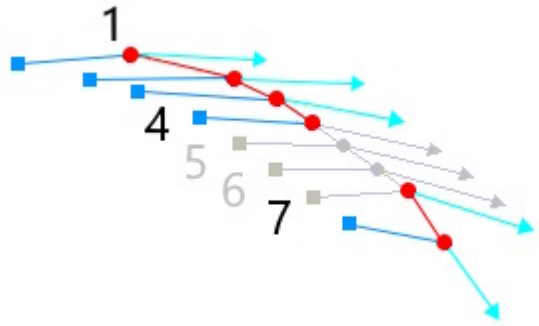
## Interpolation range

To interpolate points, EthoVision XT needs complete samples immediately before and after the sample to be interpolated. 'Complete samples' means that:

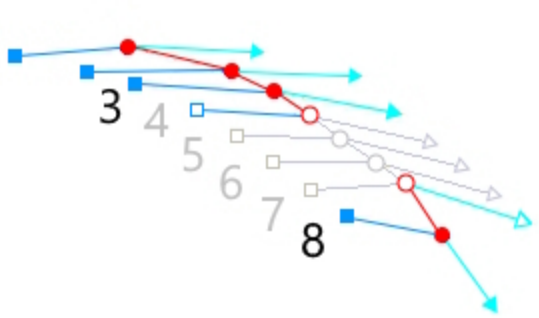
- For Only center-point detection, the Center point of the adjacent samples must not be missing.
- For Center-point, nose-point and tail base detection:
  - If you chose **Interpolate the selection** on the toolbar, all the three points of the adjacent samples must not be missing.
  - If you chose **Interpolate center points** on the toolbar, the Center-point of the adjacent samples must not be missing.

If the samples adjacent to the selected range are not complete, EthoVision XT searches for the first complete sample before and after that sample (or range). Therefore, all (incomplete) samples in the middle are included in the range to interpolate.

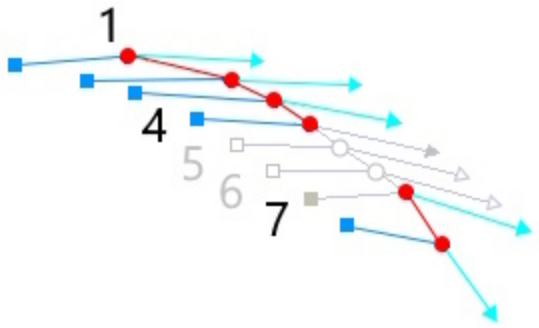
**EXAMPLE** The missing samples 5 and 6 in the picture below have been selected for interpolation. Samples 4 and 7 are adjacent to the range, but not complete: sample 4 lacks the nose-point, while sample 7 lacks the tail-base point (missing points and connectors look faded here).




- If you choose to interpolate the entire selection, EthoVision XT needs the coordinates of the three points for both adjacent samples in order to calculate the coordinates of the three points of samples 5 and 6. Therefore, samples 4 and 7 are not used. Instead, the complete samples 3 and 8 are used as adjacent samples, so the interpolating range is extended to samples 4 and 7 (see the open dots below). The coordinates of the missing points of samples 4 to 7 are calculated.



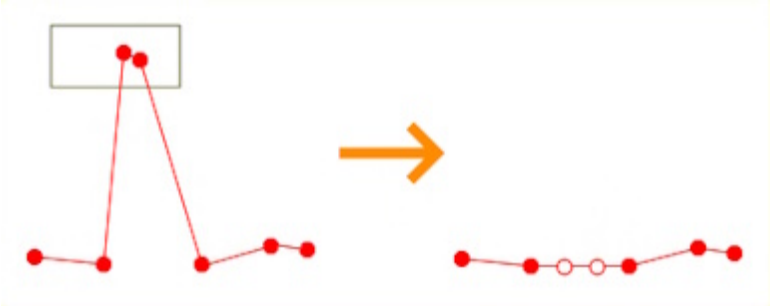

- If you interpolate only the center points, EthoVision XT needs the coordinates of the center points for the adjacent samples in order to calculate the coordinates of the center-points 5 and 6. Sample 4 and 7 are used because they are the first previous and next samples with valid center-points, respectively. The interpolating range is not extended (see the open dots below). The coordinates of the missing center-points, not nose-point and tail-base points, of samples 5 and 6 are calculated.




## How the center point is interpolated

The center-points of the selected samples are a linear interpolation between the first previous non-missing center-point and the first next non-missing center-point found around the interpolating (expanded) range.

Below: Linear interpolation of two center points. Left: Select the two points. Right: After interpolation. The arrows indicate the interpolated points.



If your track contains nose-point and tail-base, interpolation of center-points is independent of whether you choose to also interpolate nose-point and tail-base. In any case, center-points are interpolated first.

## How the nose-point and tail-base point are interpolated

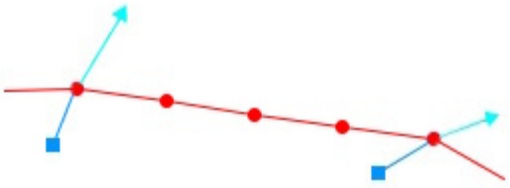
Nose-points and tail-base points are always interpolated after the center-points have been found (see above), and their interpolation is dependent on the orientation of the non-missing nose-points and non-missing tail-base points immediately preceding and following those missing samples, respectively.

Before interpolation, the missing points are indicated as a linear interpolation between the first previous and the first next non-missing points of their type.

The direction of movement does not play any role in interpolation.

Consider the following example with three missing samples (missing nose- and tail-base points are not shown for clarity):

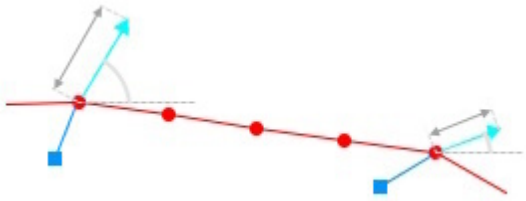
First, center points are linearly interpolated:



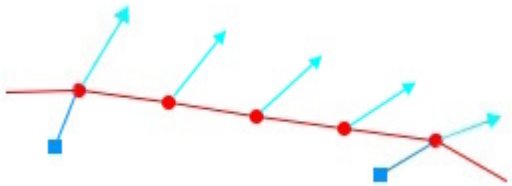
Second, for the two adjacent non-missing samples, the program determines:

- The length of the segment joining the center and the nose- (or tail-base) points.
- The angle formed by this segment and the horizontal x-axis generating from the center point (this angle can be either positive or negative depending on whether the segment lies above or below the axis).

The picture below shows this step for the nose point only:



Third, the program calculates the interpolated values of segment length and angle for the missing points, using linear interpolation of the values obtained in the previous step.



So for example:

- If the length of the center-nose point segment for the two adjacent samples is 50 and 10 mm, respectively, then the length of the three segments joining the interpolated nose-points and their center points will be 40, 30 and 20 mm respectively.
- If the orientation of the center-nose point segment for the two adjacent samples is 90 and -10 degrees, respectively, then the orientation of the three segments joining the interpolated nose-points and their center points will be 65, 40 and 15 degrees respectively.

The second and third step is repeated to interpolate the tail-base points.

## Interpolating points at the beginning or end of the track

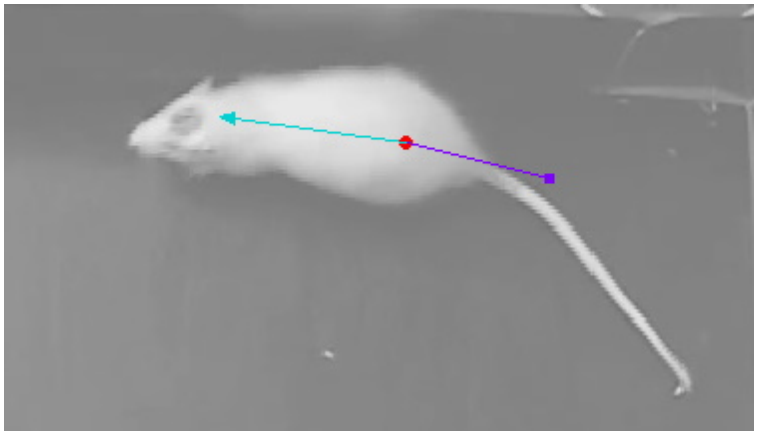
If the range of missing points to interpolate is at the beginning or the end of the track, the missing points are not interpolated. This is also the case when you want to interpolate nose- or tail-base points at the beginning/end of the track and the corresponding center points are non-missing.

# Synchronize video and tracks

## Aim

To correct the delay between video image and tracks in the various visualizations: Track Editor, Track Visualization, Integrated Visualization.

**NOTE** Normally you do not need to edit the Offset between video and tracks, because this is calculated by the software. Follow the procedure below if you notice that the tracks are lagging behind the video, like in the example below.



## Prerequisites

You acquired data using the method *Live tracking and Save video*.

## Procedure

See Troubleshooting: Data visualization > There seems to be a delay between video and track

# Manage your edits

## Learn about

- Saving the track edits

## What do you want to do?

- Save the track edits
- Undo track edits

# Saving the track edits

## Aim

To save edits done up to that time. If you make more changes after saving, and you are not satisfied with those changes, you can always recover the edits made at the point of Save.

**NOTE** This and the following topics apply to editing tracks and manually-scored behaviors.

**EXAMPLE** You want to interpolate points, but are not sure whether the result is satisfactory. Click **Save** to create a copy of the track with the current edits, then do the interpolation:

- If the result of interpolation is good, click **Save** again. This replaces the previous track edits.
- If the result is not good, you can revert to the data of the last Save by clicking **Undo**.

## What is the difference between saving the experiment and saving in the Track Editor?

### *Save experiment*

To save your edited tracks at any time, choose **File** > **Save Experiment** or press **Ctrl+S**. However, once you have saved the data, you cannot revert to the first edit. In other words, you cannot undo any save action. However, you can always return to your original track data.

### *Save in the Track Editor*

With **Save** in the Track Editor you can revert to the previous set of changes. You do so with the **Undo** action. This is handy when you want to make sure you can recover the data after you carry out some editing.

**IMPORTANT** The Save action persists until you close the experiment. If you save, then make further changes to the data and close the experiment, all changes are saved but when you re-open the experiment you cannot revert to the Save point.

## Notes

- Every time you click **Save**, the new copy replaces the previous one. This means that you can always keep only one copy of edited data.

- The original (unedited) data are always stored in your experiment. To revert to the original tracks, and discard all changes, click the **Undo all** button on the toolbar.
- Auto save in EthoVision XT works like a normal Save command. Therefore, if an auto save action occurs, all changes made so far to the currently edited trial are applied. However, if you make further changes and an auto save action occurs, you can always revert to the saved copy that was made before the auto save action. To alter your auto save settings, choose **File** > **Preferences** > **Auto Save**.

*See also*

- Save the track edits

# Save the track edits

## Aim

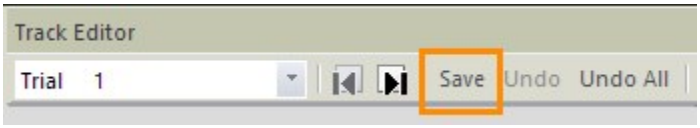
To create a copy of your tracks and manually-scored behaviors, that serves as a restore point. In the case you make further changes and you are not satisfied with them, you can revert to the restore point.

## Prerequisites

- You have acquired at least one trial.
- Open the Track Editor (**Acquisition** > **Edit Tracks**).

## Procedure

1. Edit the Tracks.
2. When you want to save the changes, click the **Save** button on the toolbar.




3. Continue with editing.
4. If you are not satisfied with the changes, click **Undo** to return to the saved copy.

## Notes

- The tracks that are visualized and analyzed are always include the changes you made. If you want to visualize or analyze the original data, click the **Undo all** button on the toolbar. However, keep in mind that you lose the edits!

# Undo track edits

## Aim

To revert to previously saved data after you have changed the data further and you do not want to keep the last edits.

**NOTE** The procedure below is also valid when you edit data in the Manual Scoring screen. There you also find the **Save** and **Undo** buttons.

## Prerequisites

- In the Track Editor, or in the Manual Scoring screen, you have done edits after saving the data at least one time.

## Procedure

1. Click the **Undo** button on the toolbar.


   *Result*: A message appears, asking you whether you want to lose the changes since the last save.

2. If you click **Yes**, the original track plot is restored.

   If you click **No**, you return to the main screen without losing the changes.

## Notes

- To return to the original data, click **Undo all**. However, keep in mind that you lose all changes.
- If you edit data in one trial and then select another trial in the trial selection list on the toolbar, the changes you have made to that trial are saved automatically, including those you made after saving.
- If you open another EthoVision XT screen, for example, to calculate statistics, or close the experiment after making changes to the track, those changes are saved, and retained when you switch back to the Track Editor screen. However, the saved copy (restore point) is always deleted when closing the experiment. Therefore, you cannot revert to the saved copy.

  Consider the following sequence of actions:

1. Edit data, then click **Save**.

2. Make a second run of changes, then close the experiment.

3. Re-open the experiment and choose **Acquisition** > **Edit Tracks**.

The screen shows also shows the second set of changes made, but you cannot revert to the saved copy (restore point).

# Smooth the Tracks

## Main topics and tasks

# Why smooth the tracks?

In any tracking system, there are three sources of noise that potentially affect the values of dependent variables such as Distance moved or Velocity:

- *System noise*. The noisy output of tracking systems means that when you track an object that does not move, the position of the object irregularly oscillates between two or more locations. This way the track may show continuous movement of the object that corresponds to a real-world distance of, for example, 1 cm, while in fact the animal is sitting still. As a result, the data show that the immobile object traveled quite a distance.
- *Outliers resulting from tracking noise*. Occasionally the tracking system generates a single outlier which obviously affects a dependent variable such as Velocity.
- *Small movements of the animal ('body wobble')*. When you track a moving animal using a high sample rate, sideways movements from the wobbling of the animal's body are also tracked. This results in an overestimation of, for instance, the total distance moved.

## Smoothing methods

### *Smoothing (Lowess)*

Use this method to eliminate small movements, such as body wobbling during locomotion, that might affect dependent variables such as total distance moved. See The Lowess smoothing method

### *Minimal Distance Moved*

Use Minimal Distance Moved when the animal sits still, yet the body points move slightly due to system noise or breathing. See The Minimal Distance Moved smoothing method

### *Maximal Distance Moved*

Use Maximal distance moved to remove outliers due to erratic detection, for example in a water maze test when some water reflection is detected as the subject, or in DanioVision experiments when the subject does not move and EthoVision XT detects the margins of the well as the subject. See The Maximum Distance Moved smoothing method

## Track smoothing during acquisition

The track smoothing methods listed above affect the tracks *after* acquisition. In some cases you may already want to smooth the track *during* acquisition. This may especially be the case if you use Trial and Hardware Control. As an example, if the center-point of an animal is detected in a zone, you want the pellet dispenser to drop a pellet. If the detected center-point is moving rapidly because of noise, this may result in a number of consecutive pellets to be dropped, every time the center-point crosses the border of the zone. Track smoothing does not solve this problem. Instead, use Track noise reduction in the detection settings to smooth the tracks during data acquisition.

# The Lowess smoothing method

Lowess stays for **Lo**cally **we**ighted **s**catter plot **s**moothing. This method fits a curve to the dataset, using a least square regression modified as follows:

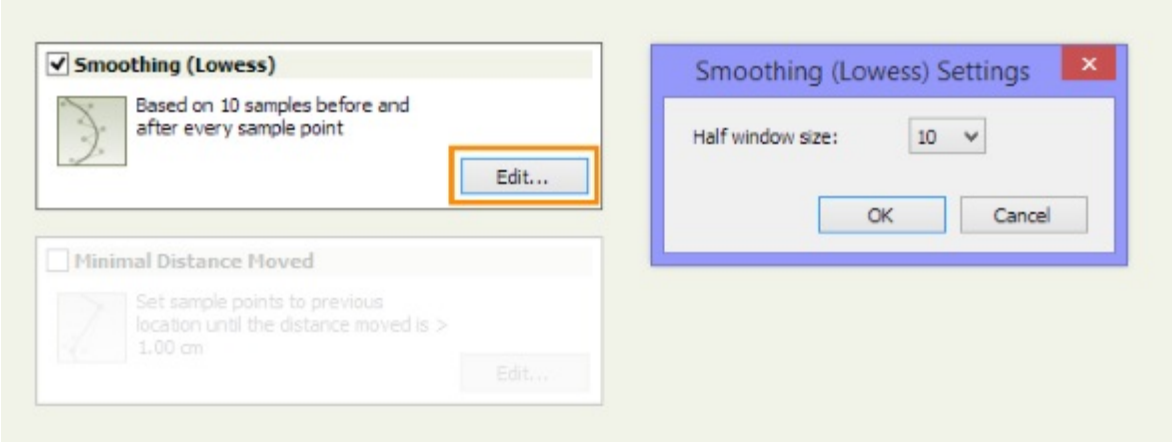
- The Lowess method uses a moving time window (or half size window) that contains a subset of sample points.
- The Lowess method uses a 2-degree (non-linear) polynomial fit. This fit is applied to the center-point. If you use center-point, nose-point and tail-base detection, the fit is also applied to the angle between center-point and nose-point and between the center-point and tail-base. (see Notes below).
- The Lowess method is weighted; sample points nearest to the point being fitted have a larger influence on the fit than sample points further away.

The Lowess method has been successfully applied on track data from EthoVision.

## To apply Lowess smoothing

1. Choose **Acquisition** > **Track Smoothing Profile** > **Open**.
2. Select the **Smoothing (Lowess)** check box and click the **Edit** button.
3. Change the number of sample points in the **Half window size** list.

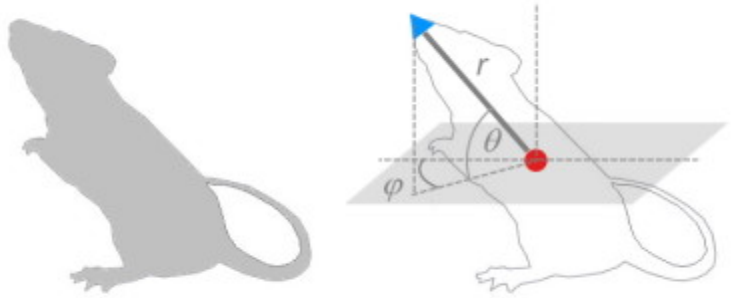
   The default value is 10 which is the recommended value. Selecting a larger half window size results in a smoother path. Selecting a smaller half window size results in a path that more resembles the original path. With a lower sample rate, you should use a larger half window size to get the same smoothing as with a higher sample rate.



## Notes

- Smoothing is applied after data acquisition and after data editing, but before the Minimal Distance Moved method. The smoothed data, not the original data, are used for analysis and for export. Applying Smoothing does not change the original data.
- *Smoothing and body points*. Smoothing is applied to the center-point, and subsequently, if you also tracked the nose-point and the tail-base, to the angle formed by the three body points.
- *Smoothing and missing samples*. When up to 200 consecutive samples are missing, they are interpolated (see Interpolate points). Next, their x,y coordinates are re-calculated based on the Lowess method. The Subject area for missing samples is interpolated linearly, but is not smoothed.

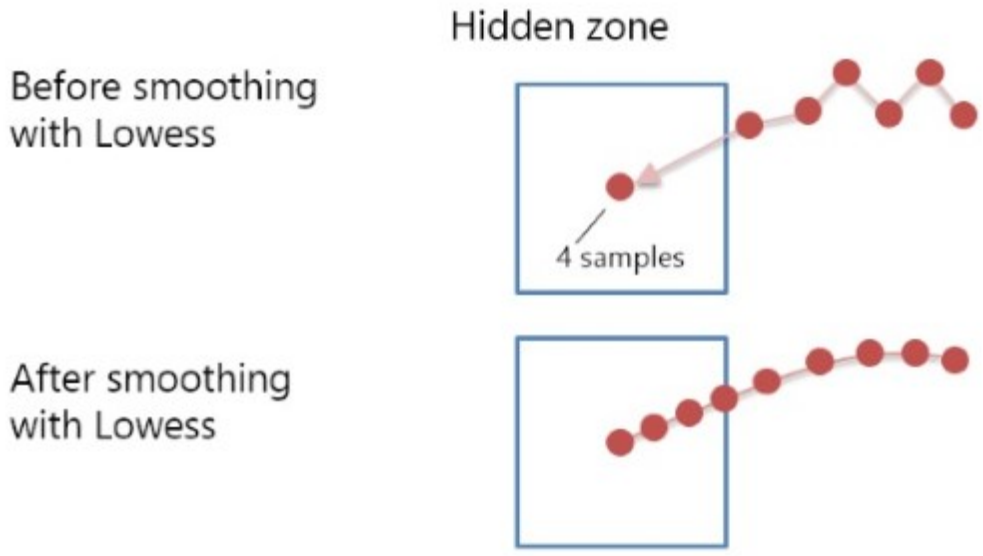
  If the subject is missing for more than 200 samples (this corresponds to 8 seconds for a sample rate of 25), that track segment stays missing, and is not smoothed.
- *Smoothing of Live Mouse Tracker data*. When you apply Lowess smoothing to Live Mouse Tracker data, EthoVision XT takes the 3D coordinates of the body points (x-, y- and z-coordinates). The 3D position of the nose-point and the tail-base point are represented with spherical coordinates bound to the center-point.
  - For example, the coordinates of the nose point are based on the length *r* and angles θ and φ of the segment joining the center-point and the nose-point. The z-coordinate of the nose- (or tail-base) point includes the angle θ formed between the x-y plane and the segment joining that body point and the center-point.




  - Smoothing of Live Mouse Tracker data is done as described in How the nose-point and tail-base point are interpolated, with the difference that the vectors and the angles are drawn in 3D instead of 2D like in typical EthoVision XT nose-tail data.

- *Smoothing and hidden zones*. Samples assigned to a hidden zone are smoothed too. For this reason, after smoothing with the Lowess method, part of the samples in the hidden zone are "moved" outward, producing the effect shown below. Those samples add up to the total distance moved in your results. How many samples are moved depends on the original trajectory.

  Below: An example of the effect of Lowess smoothing on the location of samples originally assigned to a hidden zone. After smoothing, part of the samples assigned to the hidden zone center are moved toward the adjacent samples outside the zone. A similar effect can be seen when the animal exits the hidden zone.




*See also*

- For more information on the Lowess method, see http://en.wikipedia.org/wiki/Lowess.
- For more information on the application of the Lowess method on EthoVision tracks, see:
  - Drai and Golani, 2001. SEE: a tool for the visualization and analysis of rodent exploratory behavior, *Neurosci. & Biobehav. Rev.* **25**(5): 409-426.
  - Hen, I. et al. 2004. The Dynamics of Spatial Behavior: How can robust smoothing techniques help? *J. Neurosci. Methods* **133**(1-2): 161-172.
  - Kafkafi, N. et al., 2005. Genotype-environment interactions in mouse behavior: a way out of the problem, *Proc. Natl. Acad. Sci. USA*, **102**(12): 4619-4624.

*Acknowledgments*

We gratefully acknowledge the ongoing collaboration with Prof. Ilan Golani, Prof. Yoav Benjamini and their colleagues at Tel Aviv University. Their pioneering work on the detailed analysis of animal movement has been a source of inspiration for the developers of EthoVision and for many of its users around the world.

# The Minimal Distance Moved smoothing method

## Aim

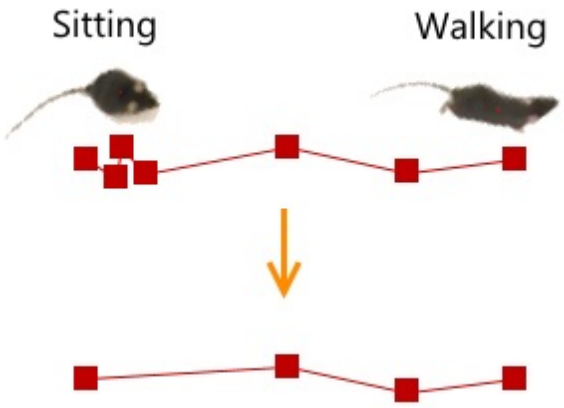
To filter out small movements of the subject's center point that are caused by random noise, not to the subject's spatial displacement.

## Working principle

With the Minimal Distance Moved method, you can pick out the data when the subject moved a minimal distance from one sample to the next.

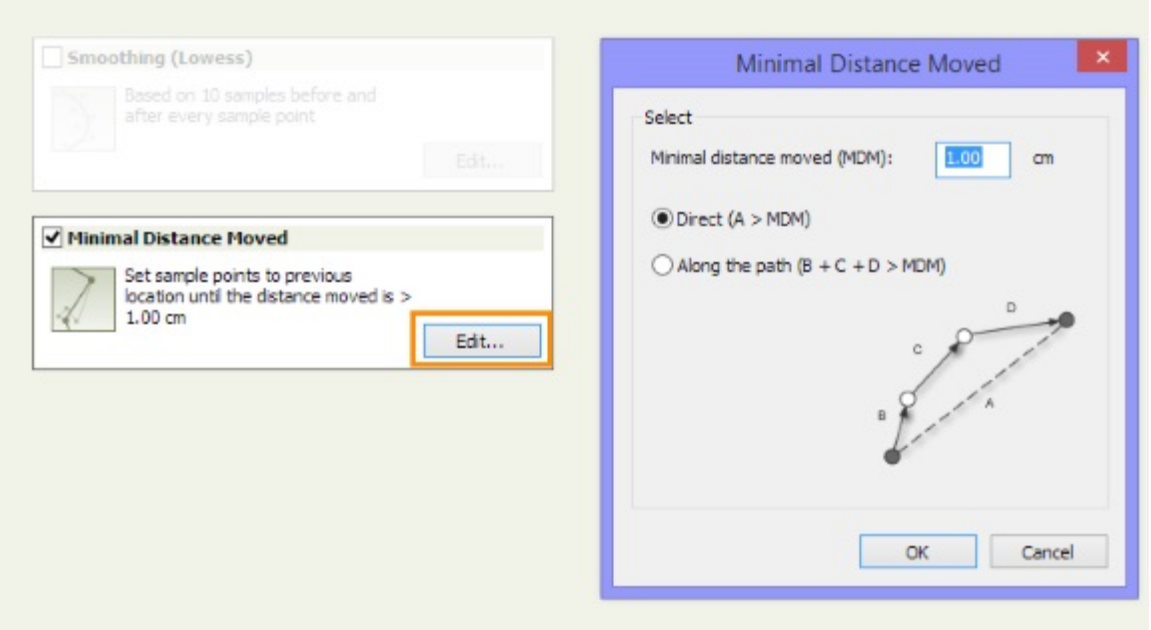
- When the distance moved between the current and the previous sample is above (or equal to) the threshold for Minimal Distance Moved, the current sample is used for analysis.
- If the distance moved between the current and the previous sample does not exceed the threshold, the current sample is "moved back" to the previous sample.

The result of this filter is that small movements of the body points, supposedly due to noise, are removed, and the trajectory of the subject becomes simpler.




## Procedure

1. Choose **Acquisition** > **Track Smoothing Profile** > **Open**.
2. Select the **Minimal Distance Moved** check box and click the **Edit** button.




3. Under **Select**, enter the Minimal Distance Moved threshold.
4. Choose one of the two options (see below for an example):

   **Direct (A > MDM)**. To select samples on the basis of the shortest distance (beeline distance) between samples. Select Direct to exclude movements such as breathing, when the animal is sitting still.

   **Along the path (B + C + D > MDM)**. To select samples on the basis of the actual path between samples. Select Along the path whenever you want to filter samples according to the distance moved along the path.

5. Click **OK**.

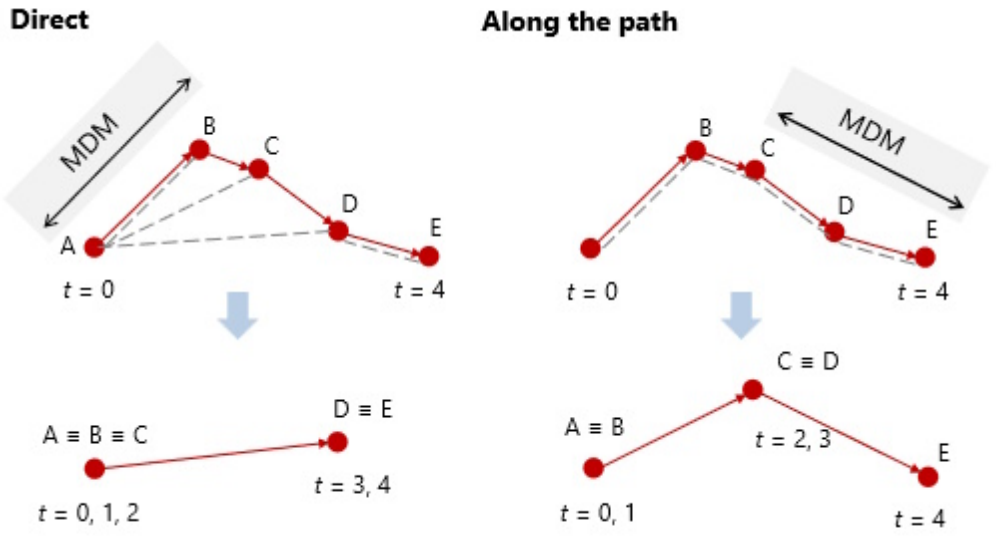
## Notes

- Minimal Distance Moved is only applied to the center-point.
- Under some circumstances, using the Minimal Distance Moved filter might affect dependent variables other than Distance Moved and Velocity. To apply Minimal Distance Moved to some dependent variables, not others, create two Track Smoothing profiles: one with Minimal Distance Moved selected, the other without Minimal Distance Moved selected. Run batch calculations using both smoothing profiles.

## Direct vs. Along the path Minimal Distance Moved

As shown by the case of Distance moved in the figure, calculation of the dependent variables is affected by what Minimal Distance Moved option you choose.

- **Direct** (left). The program calculates the shortest distance between a sample and the next (AB, dashed line). If this distance is shorter than the threshold distance MDM, sample B is set to sample A. Then the program calculates the distance to the second next sample (AC), etc. until it finds a segment (AD) longer than MDM. Samples B and C are both set to sample A. This procedure is then repeated up to the end of the track.
- **Along the path** (right). EthoVision XT calculates the distance between a sample and the next (AB, dashed line). If this distance is shorter than the distance threshold, sample B is set to sample A. Then the program calculates the cumulative distance along the path to the second next sample (AB + BC, etc.) In this example, sample B is set to sample A. However, the distance AB + BC is greater than MDM, so C stays in its place. Next, the sample D is set to sample C because CD < MDM. However, CD + DE is longer than MDM, so sample E stays in its place. The procedure is repeated up to the end of the track.




In both cases, all five samples are selected for analysis. However, some samples have been "moved" to a previous sample in such a way that their own value of distance moved is zero.

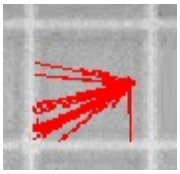
- With the Direct method, samples B and C overlap with A, and E with D.
- With Along the path, sample B overlaps with A, and D with C.

The resulting total distance moved can therefore differ depending on which option is chosen.

# The Maximum Distance Moved smoothing method

## Aim

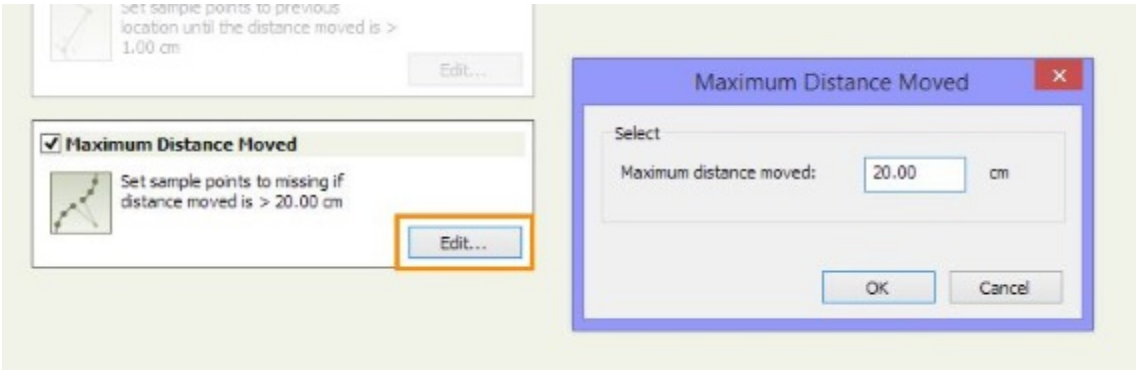
To remove outliers in the track, due to erratic detection of objects within the arena. For example, in a DanioVision experiment, if detection is not optimal, the margin of the well is sometimes detected as the fish.



**IMPORTANT** If you see many errors, adjust the Arena Settings and/or the Detection Settings.

## Procedure

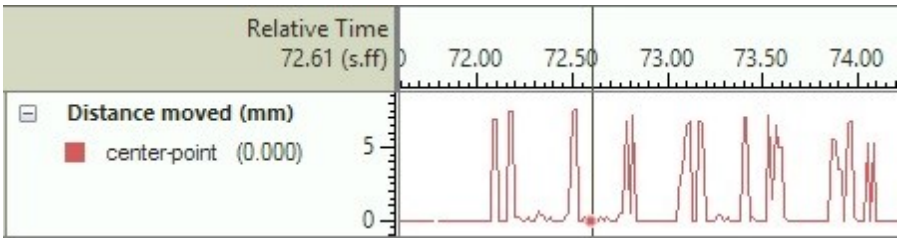
1. Choose **Acquisition** > **Track Smoothing Profile** > **Open**.
2. Select the **Maximum Distance Moved** check box and click the **Edit** button.



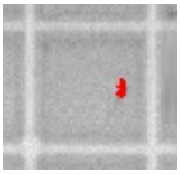

3. Enter the threshold value of distance moved, above which the sample will be set to missing. If the distance moved from sample 1 to 2 is longer than the threshold, sample 2 is set to missing.
4. Click **OK**.
5. Plot the tracks to see the result.

## Notes

- **TIP** To know which value of distance to use as a threshold, plot the values of distance moved in the Integrated Visualization, and look at the spikes corresponding to the outliers. Take a value well below the value of the spikes. Be aware that a threshold being too low can remove good data.




Result (compare this with the picture at the top):



- The Maximum Distance Moved method is applied after data editing, and after the Smoothing (Lowess) and the Minimal Distance Moved methods (when selected). The smoothed data, not the original data, are used for analysis and for export. Applying the Maximum Distance Moved method does not change the original data.

# Introduction to Data Analysis

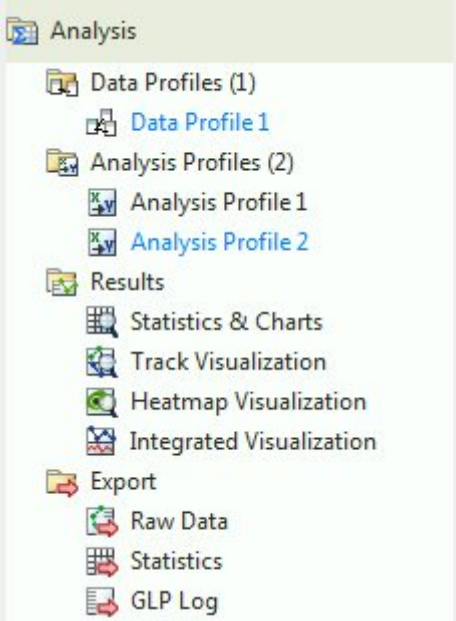
## Main topics and tasks

# Overview of data analysis

## Learn about

- Analysis functions
- Important terms in data analysis

# Analysis functions




## For data preparation:

- **Data Profiles**. For choosing the data points subject to analysis. You can filter tracks, create groups, select specific time periods or track segments, etc.
- **Analysis Profiles**. For choosing the behavioral endpoints, like speed, distance moved, time spent in a zone, etc.

## For the analysis results:

- **Statistics and Charts**. For obtaining a table with summary statistics of the dependent variables per trial and summary statistics and charts per group of trials.
- **Track Visualization**. For visualizing data as tracks plotted over a static background video image.
- **Heatmap Visualization**. For visualizing the location where your animals spent most time.
- **Integrated Visualization**. For visualizing the track together with video, and plotting dependent variables against time.
- **Export** (**Raw Data**, **Statistics** and **GLP log**). To export raw data, dependent variables and their summary statistics, and the GLP log (available with the Quality Assurance module).

## Note

In general, the analysis results depend on a few factors:

- Whether you applied Noise reduction during acquisition.
- Whether the tracks are smoothed, and how.
- Which tracks and data points are selected in the current Data profile.
- Which dependent variables and statistics are selected in the current Analysis profile.

# Important terms in data analysis

## Dependent variables

In EthoVision XT, a Dependent variable is a variable that quantifies the behavior of a subject or marks an event occurred in a trial.

- **EXAMPLE 1** To quantify locomotor activity, choose *Distance moved*, or *Velocity*, or *Movement* as Dependent variables.
- **EXAMPLE 2** To calculate how many food pellets were dropped by a pellet dispenser, choose *Hardware continuous* as a Dependent variable.

Dependent variables are calculated for each sample of the track, when data are available. The table below shows the Distance moved calculated for a few samples. X and Y are the coordinates of the center of the subject's body. Note that distance moved is not calculated at time = 0, because there is no previous sample to use as a reference.

| Time | X | Y | Distance moved |
|---|---|---|---|
| 0 | -8.7393 | -26.1678 | – |
| 0.08 | -6.8267 | -26.9699 | 2.0740 |
| 0.16 | -4.7220 | -27.0748 | 2.1074 |
| 0.24 | -3.2380 | -26.6227 | 1.5513 |

### *State dependent variables*

Some dependent variables are state variables, that is, they have discrete values. For example, *In zone* with values 1 (in zone) and 0 (not in zone). The following table shows the *In zone* variable calculated for the zone A and for a few samples. When the animal’s body point is found within zone A, then *In zone*=1, otherwise *In zone*=0. In the example below, the subject's center point enters the zone at 0.16 s.

| Time | X | Y | In zone |
|---|---|---|---|
| 0 | -8.7393 | -26.1678 | 0 |
| 0.08 | -6.8267 | -26.9699 | 0 |
| 0.16 | -4.7220 | -27.0748 | 1 |
| 0.24 | -3.2380 | -26.6227 | 1 |

## Statistics

In EthoVision XT, Statistics are descriptive statistics of the per-sample values of a Dependent variable.

- **EXAMPLE 1** To quantify locomotor activity, choose the *Total* statistic for the dependent variable *Distance moved*. Choose the *Mean* statistic for the dependent variable *Velocity*.

  The Total distance moved is the sum of the per-sample values of distance. In the example of the table above, the total is 5.73 cm.

- **EXAMPLE 2** To calculate the time spent in zone, choose the Total statistic for the dependent variable In zone.

## Trial statistics vs. Group statistics

For each dependent variable you can specify two types of statistics:

- **Trial Statistics**

  Statistics calculated per trial.

  **EXAMPLE** Your experiment contains 100 trials of one animal in one arena. Choose Total as a Trial Statistic for the dependent variable *Distance moved*. You obtain 100 values of Total Distance moved.

- **Group Statistics**

  Summary statistics of the Trial Statistics, calculated over all the trials specified in the data profile, or in a group.

  **EXAMPLE** You have defined two groups of tracks, Treated and Saline, and you want to calculate the *Mean* and *Standard deviation* of Distance moved and other variables per group.

  In the example above with the 100 trials, with Group Statistics you would obtain the average and standard deviation of the 100 values of distance moved.

# Analysis advisor

## What do you want to do?

- Simple calculations (e.g. total distance moved, average velocity)
- Zone visits (entries and time spent)
- Other analyses with zones
- Analysis in relation to the path
- Analysis of regular time intervals
- Analysis of event transitions and sequences

## Notes

- To analyze groups of tracks, define first your groups in the Data profile. See Define groups of tracks.
- **TIP** If none of the dependent variables represents an adequate behavioral endpoint for your research question, please contact Noldus, so we can discuss your analysis requirements. EthoVision XT contains functions which can process the raw data based on JavaScript code. See Custom Variables
- For information on analysis related to specific apparatuses, like the Open Field, the Morris Water Maze, the Novel Object Recognition and others, see also the EthoVision XT 18 - Application Manual, which you can find here:

  Other Documentation

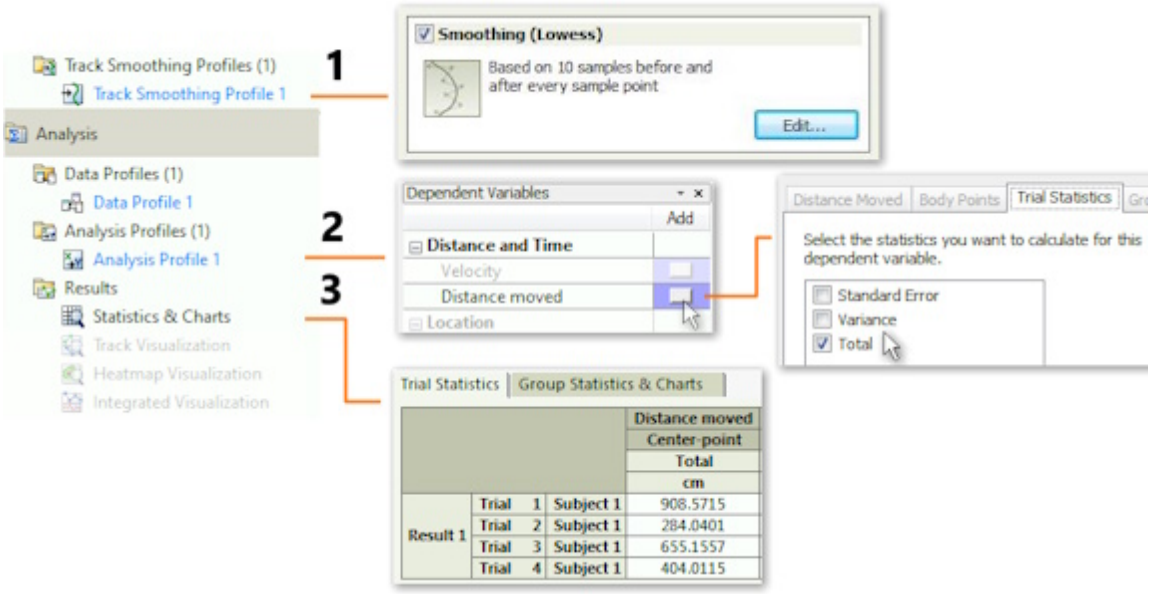
# Simple calculations (e.g. total distance moved, average velocity)

## What do you want to do?

- Calculate the results for the whole arena
- Calculate the results per zone

## Calculate the results for the whole arena

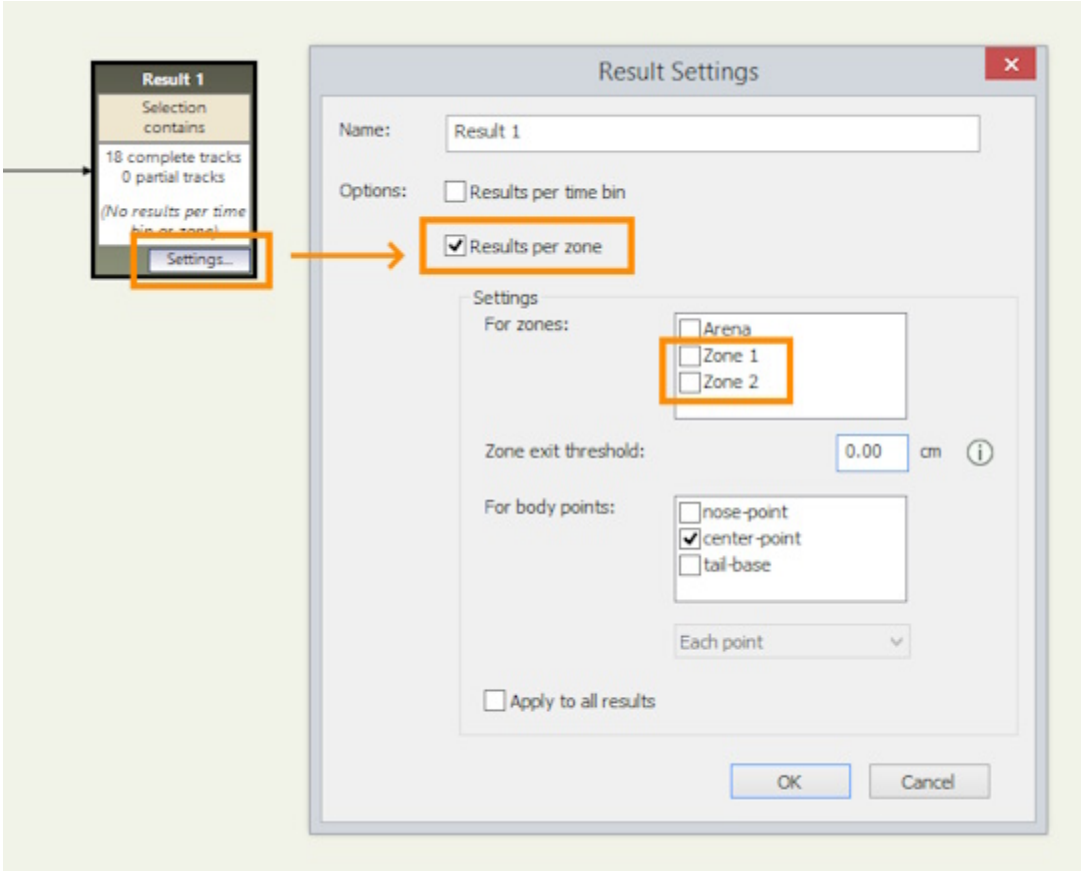
1. **TIP** Smooth the tracks to get more realistic measures of distance. See Smooth the Tracks.
2. In the Experiment Explorer, click an Analysis profile, and then click the button next to the dependent variable if it is not already selected. For example, *Distance moved* or *Velocity*. In the Trial Statistics tab, select the statistics you want to calculate (for example, *Total* for Distance moved, and *Mean* for Velocity).
3. In the Experiment Explorer, click **Statistics and Charts**, then click the **Calculate** button.




## Calculate the results per zone

1. Create a new Data profile or open an existing one.

2. Click the **Settings** button on the **Result** box. In the Result Settings window, select **Results per zone**.




3. Under **Settings** - **For zones**, select the zones you want to analyze your data for.
4. Under **Settings** - **For body points**, select which body points should be in the selected zones. This option is only available if you track the three body points.
5. Click **OK**.
6. In the Analysis profile, define the variables you want to calculate.
7. In the Experiment Explorer, under **Results**, click **Statistics and Charts**, then click the **Calculate** button.

## Notes

- The Zone exit threshold works this way: once the animal's body point is detected in the zone, the animal is considered to be in the zone until its distance from the zone border exceeds that threshold. Use this option to remove false re-entries resulting from random movements of the body point around the zone border.

If you enter a value greater than 0, you make sure that when the animal exits the zone border for a short distance (less than the threshold), that data will be included in the results, for that zone. Click the button ⓘ next to this option for an example. For more information, see In zone.

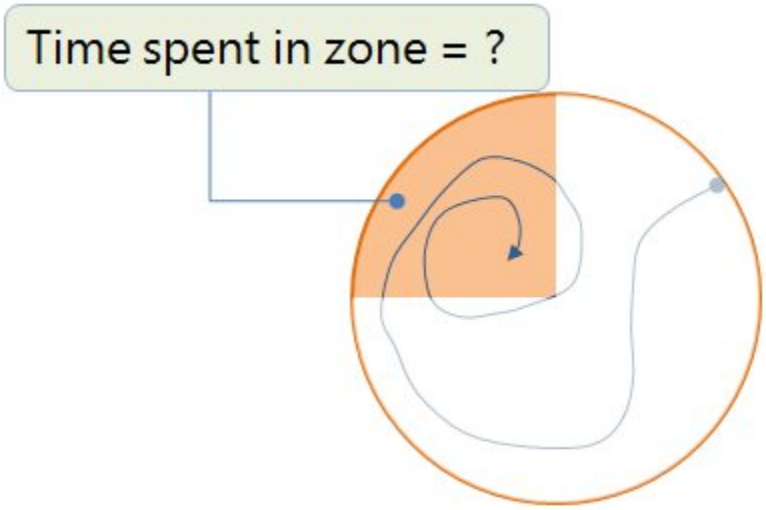
- Select the **Apply to all results** if you want to copy the settings specified in the Results Settings window to other Result boxes. This applies if you have defined more than one Result box in that Data profile.

# Zone visits (entries and time spent)

## What do you want to do?

- Calculate the number of entries and time spent in specific zones
- Calculate the number of entries and total time spent in two or more zones
- Calculate the number of entries and time spent in an intersection of zones
- Analyze the visits to target zones and errors
- Find out which zone the subject visited first
- Count the number of times that the subject was in a zone for more than N seconds
- Calculate the time that the three body points were in a zone

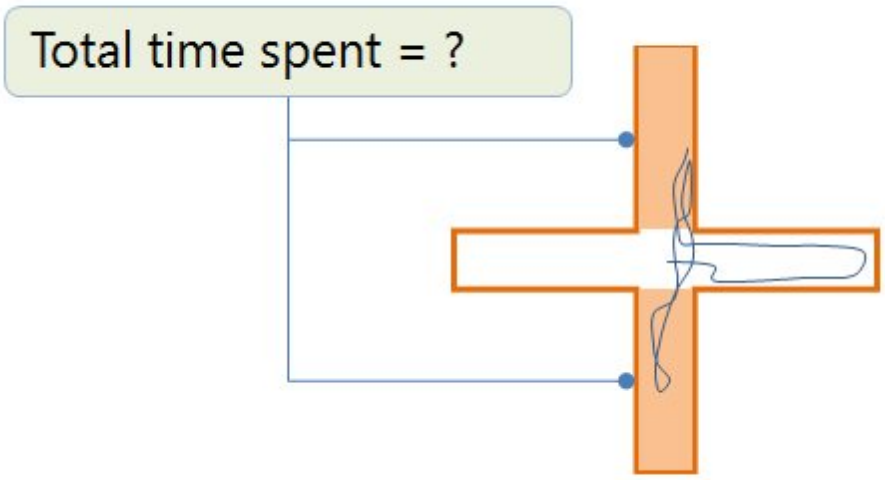
## Calculate the number of entries and time spent in specific zones




1. In the Analysis profile, under **Location**, click the button next to **In zone**. Specify the zones you are interested in. In the **Trial Statistics** tab, select **Frequency** (for zone entries) and **Cumulative Duration** (for time spent).
2. Click **Statistics and Charts**, then **Calculate**.

## Calculate the number of entries and total time spent in two or more zones

If you want to group results for two or more zones, like the open arms of an Elevated plus maze:

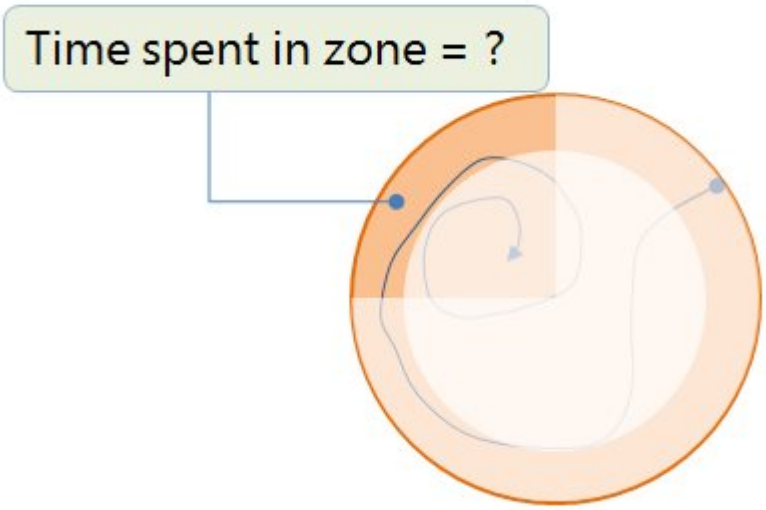



1. In step 1 above, specify the zones you are interested in, and make sure that When in any of the selected zones is selected. In the **Trial Statistics** tab, select **Frequency** and **Cumulative Duration**.
2. Click **Statistics and Charts**, then **Calculate**.

Alternatively, in the Arena Settings make a Cumulative zone from the selected zones and calculate the frequency and cumulative duration in this zone.

## Calculate the number of entries and time spent in an intersection of zones

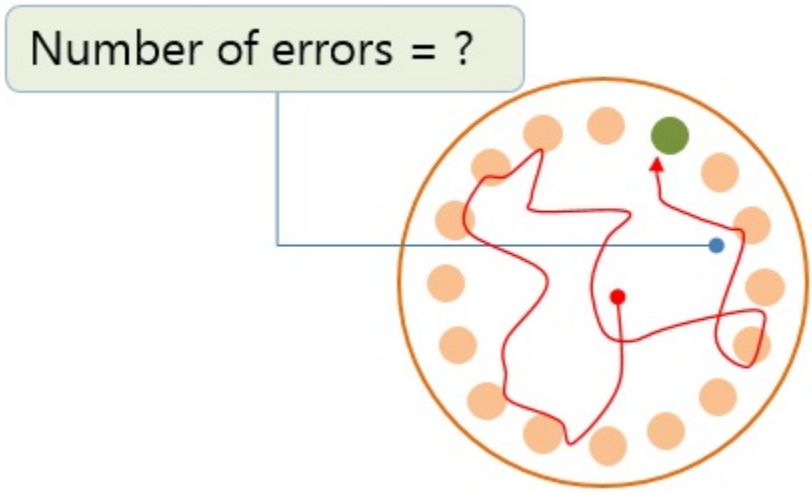
When two zones are defined in different layers, it is possible to analyze their intersection. In this example, the intersection of the quadrant “North-West” and the annulus-shape “Maze border”.




1. In the Analysis profile, under **Location**, click the button next to **In zone**. Specify the zones you are interested in, and make sure that **When in all selected zones** is selected. In the **Trial Statistics** tab, select **Frequency** and **Cumulative Duration**.
2. Click **Statistics and Charts**, then **Calculate**.

## Analyze the visits to target zones and errors

For example, in a Barnes maze test, calculate how many non-target holes were visited and the time to the first visit to the escape hole (target visit).



1. In the Analysis profile, specify the dependent variable **Target visits and errors**. Select the target zones and the non-target zones. In the **Trial Statistics** tab, select **Total number** and **Latency to First**.

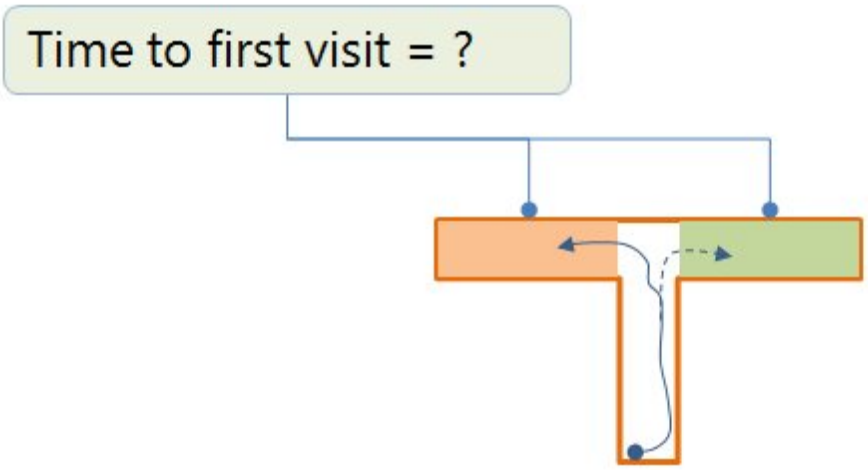
   **NOTE** Latency is always calculated from the start of the track, even when you define time bins and nesting intervals.

2. Click **Statistics and Charts**, then **Calculate**.

Use the variable Target visits and errors also for a Radial-arm maze test.

## Find out which zone the subject visited first

For example, in a dual choice test, in a T maze, leaf disc, or a Y-maze, etc.




1. In the Analysis profile, specify the dependent variable **In zone**. In the **Trial Statistics** tab, select **Latency to first**.

   **NOTE** Latency is always calculated from the start of the track, even when you define time bins and nesting intervals.

2. Click **Statistics and Charts**, then **Calculate**.
3. Under **Export**, click **Statistics**. In the exported file check which zone has a lower Latency value.

## Count the number of times that the subject was in a zone for more than N seconds

1. In the Analysis profile, specify the dependent variable **Free interval**. Define an interval:

   **From**. Dependent variable: **In zone** (specify the zone in the settings); Statistic: **Current Duration** > N seconds.

   **To**. Dependent variable: **In zone** (specify the same zone); Statistic: Current; Value: When not in zone = true.

2. In the **Trial Statistics** tab, select **Frequency**.
3. Click **Statistics and Charts**, then **Calculate**.

If you want to calculate the actual duration of each of those visits, export the results table, then add N seconds to each result under Cumulative duration.

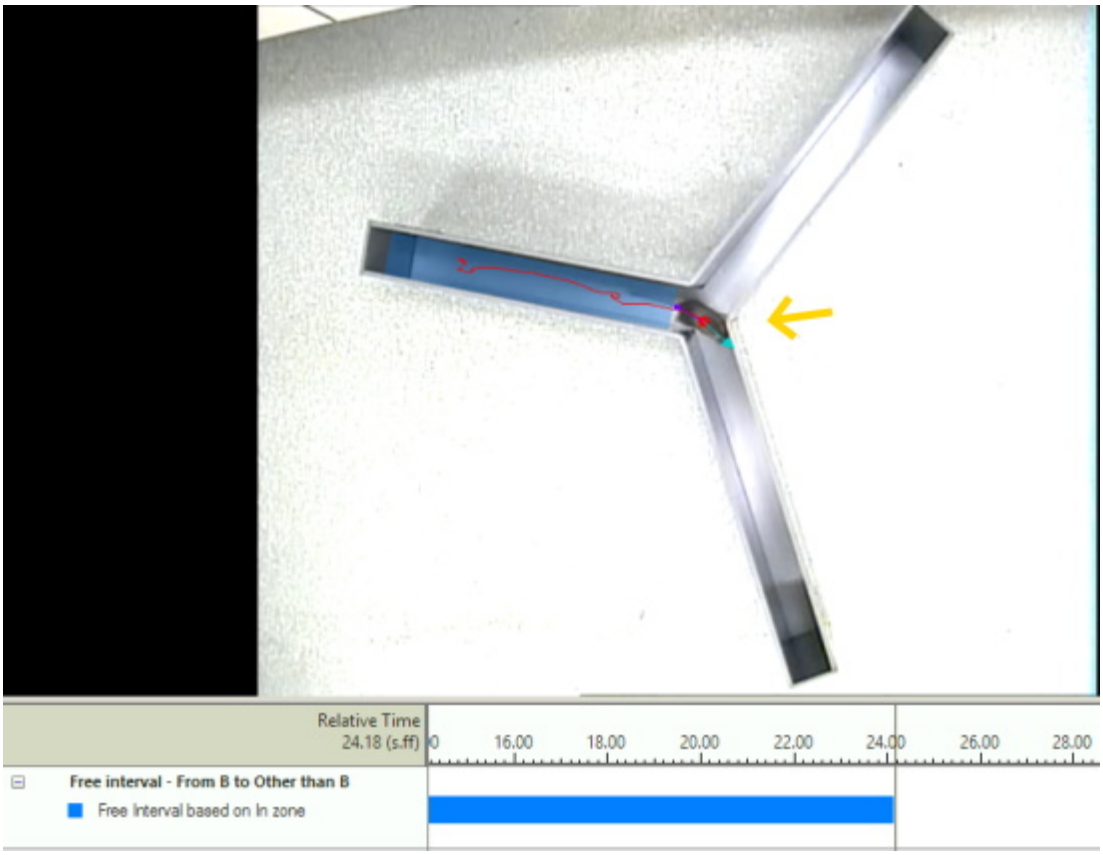
## Calculate the time that the three body points were in a zone

With this solution, the animal is considered

- in a zone only when all its body points are within the zone
- out of the zone only when all its body points are outside the zone

**EXAMPLE** A Y-maze test, with arm zones A, B, C.

1. In the Arena Settings, define the main zones of interest.
2. In the Arena Settings, create additional zone groups, one for each main zone. In the example of a Y-maze, 3 zone groups. In each group, define a cumulative zone, including all zones but the focal zone. For example, “Other than A”, “Other than B”, etc. Each of these cumulative zones must be in separate zone groups. See Cumulative zones
3. In the Analysis profile, from each main zone A, B, C, define a Free Interval, which goes from **In zone** (Frequency > 0, for zone 1, with all three points) to **In zone** (Current = true, for “Other than zone 1”). Repeat this step to create more Free intervals.
4. Calculate the statistics or visualize the Free interval.

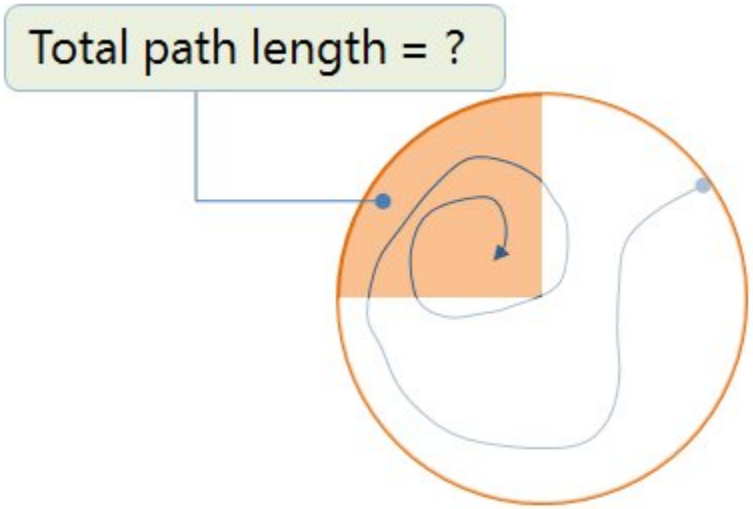

Relative Time
24.18 (s.ff)
16.00
18.00
20.00
22.00
24.00
26.00
28.00
Free interval - From B to Other than B
Free Interval based on In zone

# Other analyses with zones

## What do you want to do?

- Calculate path length and speed for specific zones
- Calculate path length and speed for a combination of zones
- Calculate the distance traveled before reaching the platform in a Morris water maze
- Calculate the distance traveled or time before entering a zone
- Calculate the distance traveled or time before a specific zone visit
- Find where a behavior occurred
- Find out when the subject disappears from the arena

## Calculate path length and speed for specific zones




In this case you calculate dependent variables for track segments. First, you specify the track segments When in zone with a Data profile and then you choose the dependent variables in an Analysis profile.

1. In the Data profile, in the **Result** box, click the **Settings** button and then select **Results per zone**. Specify the zones you are interested in.

2. In the Analysis profile, click the button next to the dependent variable you require, e.g Distance moved or Velocity. In the **Trial Statistics** tab, select the statistic (e.g., Total for distance moved and Mean for velocity).
3. Click **Statistics and Charts**, then **Calculate**.

## Calculate path length and speed for a combination of zones

1. In the Data profile, in the Components pane, under **Nesting** click the button next to **In zone**. Specify the zones you are interested in. Select the option that you require:
   - **When in any of selected zones** to analyze when the animal was in one or another zone.
   - **When in all selected zones** to analyze when the animal was in all the zones simultaneously. This assumes that the zones are at least partially overlapping: for instance, a quadrant and a border zone in a water maze. If zones are not overlapping, data are not selected since the subject's body points cannot be in two non-overlapping zones at the same time.

   For more information, see Nesting over In zone.
2. In the Analysis profile, click the button next to the dependent variable (e.g. *Velocity*).
3. Click **Statistics and Charts**, then **Calculate**.

## Calculate the distance traveled before reaching the platform in a Morris water maze

Use the function Free Interval in the Data profile to select the interval from the track start to when the animal stays on the platform for say five seconds.

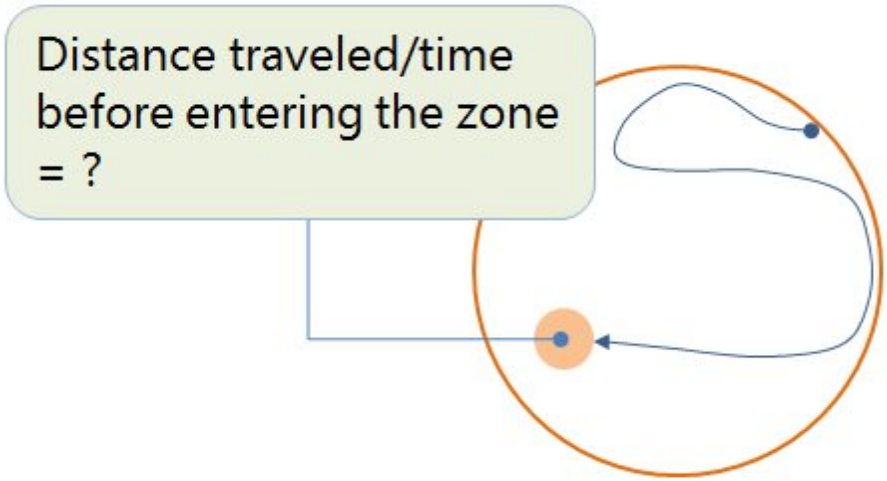
1. In the Data profile, in the Components pane, under **Nesting** click the button next to **Free interval**. Specify the interval:
   - Start criterion: Time (Track start).
   - Stop criterion: Dependent Variable In zone. Statistic: Current duration >=5 s".
   - Select **Ignore last interval if incomplete**. This will consider only cases when the animal reaches the platform.
2. In the Analysis profile, click the button next to the **Distance moved**. In the **Trial Statistics** tab, select **Total**.
3. Click **Statistics and Charts**, then **Calculate**.

EthoVision XT only gives results for those trials where the animal stayed on the platform for five seconds (here below, Trial 1). In all other cases, including when the animal swims over the platform, but does not stop there, EthoVision XT shows "-"; see Trial 2.

| | Distance moved |
|---|---|
| | center-point |
| | Total |
| | cm |
| Trial 1 | 734.7192 |
| Trial 2 | - |

## Calculate the distance traveled or time before entering a zone

For example, in a Barnes maze, you want to calculate the Total distance moved before the animal enters a zone.




*For distance traveled*

1. In the Data profile, in the Components pane, under **Nesting** click the button next to **Latency to zone**. Specify **1st visit**, the zone and keep Interval before zone visit selected.
2. In the Analysis profile, click the button next to the **Distance moved**. In the **Trial Statistics** tab, select **Total**.
3. Click **Statistics and Charts**, then **Calculate**.

*For time*

If you want to know the time before the animal enters a zone, you do not need a Data profile.

1. In the Analysis profile, click the button next to the **In zone**. Choose the zone and in the **Trial Statistics** tab, select **Latency to first**.

   **NOTE** Latency is always calculated from the start of the track, even when you define time bins and nesting intervals.

2. Click **Statistics and Charts**, then **Calculate**.

## Calculate the distance traveled or time before a specific zone visit

In this example, we want to know the distance traveled or the time taken to stay in a zone for more than a specific time.

*For distance traveled:*

1. In the Data profile, in the Components pane, under **Nesting** click the button next to **Free interval**. Specify the interval "From Time (track start) to [dependent variable]. For example,

   "From track start to Current duration >=20 s". This will pick out the interval until the animal stays in a zone for at least 20 s.

2. In the Analysis profile, click the button next to the **Distance moved**. In the **Trial Statistics** tab, select **Total**.
3. Click **Statistics and Charts**, then **Calculate**.

*For time:*

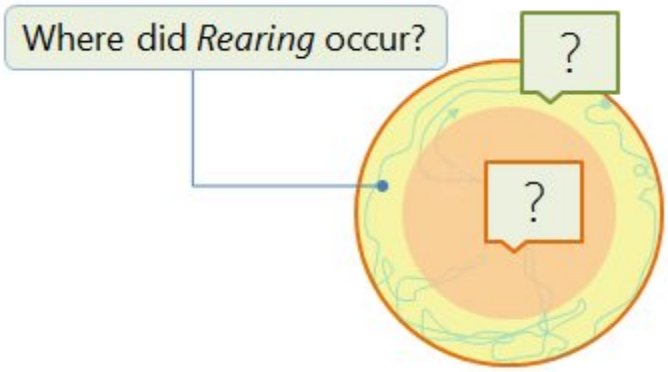
1. In the Analysis profile, under **Custom Variables** click the button next to the **Free Interval**. Define the interval. In the **Trial Statistics** tab, select **Cumulative duration**.
2. Click **Statistics and Charts**, then **Calculate**.

## Find where a behavior occurred

The term "Behavior" here indicates a state like *Movement*, *Mobility*, *Activity*, *Body elongation*, behaviors scored manually, or behaviors detected automatically.

*Quantitative analysis*

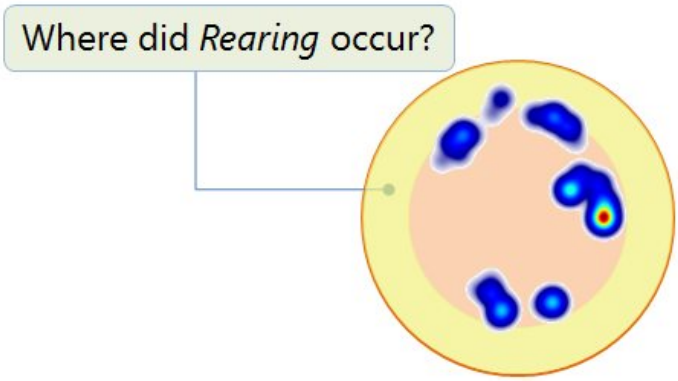
If you are interested in quantitative results (for example, the number of occurrences per zone). Specify the zones in a Data profile and select the behavior in an Analysis profile.




1. In the Data profile, in the **Result** box, click the **Settings** button and then select **Results per zone**. Select the zones you are interested in.
2. In the Analysis profile, select the behavior you are interested in. In the **Trial Statistics** tab, select **Frequency**.
3. Click **Statistics and Charts**, then **Calculate**.

*Qualitative analysis*

If you are interested in a visualization. Select the behavior and optionally the zones of interest in a Data profile and then create a heatmap of the frequency of visits to the zones.




1. In the Data profile, in the Components pane, under **Nesting**, click the button next to the behavior you want to visualize.

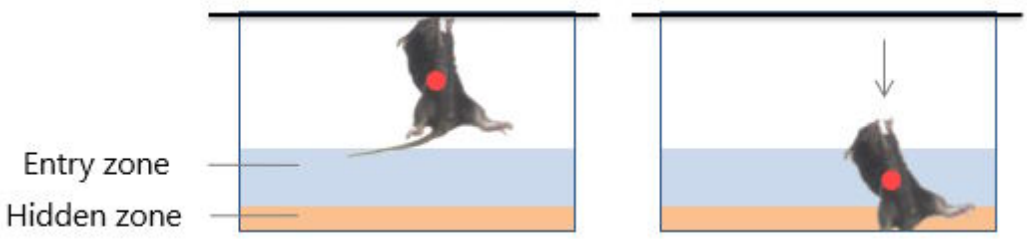
**OPTIONAL** If you are interested in particular zones, in the **Result** box, click the **Settings** button and then select **Results per zone**. Specify the zones you are interested in.

2. Click **Heatmap Visualization**, then **Plot Heatmaps**.

## Find out when the subject disappears from view

There are situations when you want to know when the subject is no longer found in the arena. In the wire hanging test, used to evaluate abnormalities in muscle strength, the time that the subject remains hanging on the wire needs to be recorded. A way to do this is to measure the time until the subject falls from the wire and disappears from view.

1. Make sure that the camera view does not include the bottom of the experimental cage. So when the animal falls, the software does not see it anymore.
2. In the Arena Settings, define a hidden zone at the bottom of the arena. Define an entry zone that should occupy the lower half of the arena. Make sure that the entry zone partly overlaps with the hidden zone. See Shelters and other hidden zones




3. Acquire the tracks. When the subject falls, its center point is first found in the entry zone, then it disappears, so it is automatically assigned to the hidden zone.
4. In the Analysis profile, click the button next to **In Zone**. Select the hidden zone and on the **Trial statistics** tab choose **Latency to first**.
5. Click **Statistics and Charts**, then **Calculate**.

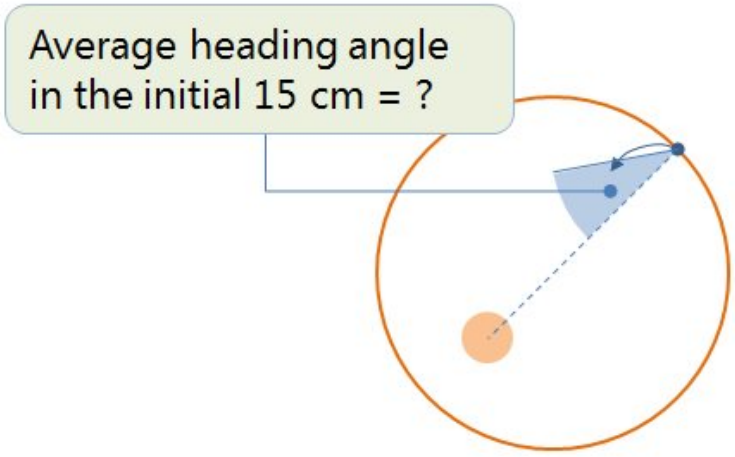
# Analysis in relation to the path

## What do you want to do?

- In a water maze experiment, calculate the initial heading angle
- Split the track in a number of segments based on the distance traveled

## In a water maze experiment, calculate the initial heading angle

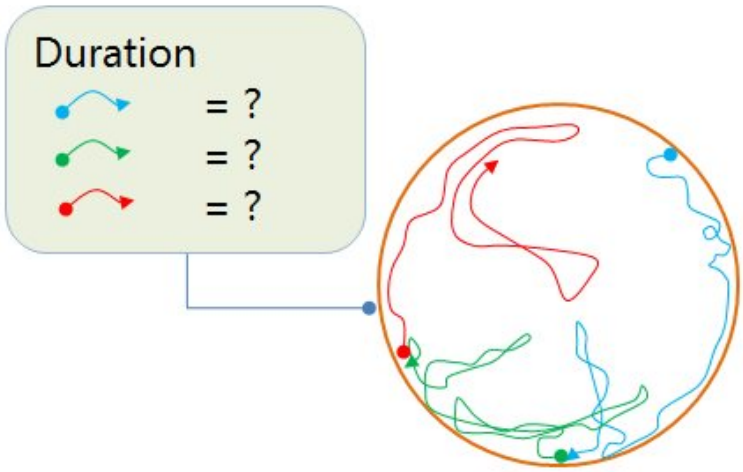
That is, calculate the deviation from the optimal route, for example in the first 15 cm of the swim path.




Specify the track segment From track start until the animal swam 15 cm with a Data profile. Then select the dependent variable Heading to point in an Analysis profile.

1. Smooth the tracks to obtain a swim path less influenced by noise.
2. In the Data profile, in the Components pane, under **Nesting**, click the button next to **Free Interval**. Specify the interval “from Track start to Total distance moved >= 15 cm”. See Nest over a Free interval
3. In the Analysis profile, specify the dependent variable **Heading to point**. Select the center point of the platform. In the **Trial Statistics** tab, select **Mean**.
4. Click **Statistics and Charts**, then **Calculate**.

# Split the track in a number of segments based on the distance traveled




Specify the track segments in the analysis profile.

1. In the Analysis Profile, under **Custom Variables**, click **Free interval**. Specify the interval "From **Distance moved - Total >=0**" "To **Distance moved - Total >=100 cm**". Select the option **Calculate statistics per interval**. In the **Trial Statistics** tab, select **Cumulative Duratio**n.
2. Click **Statistics and Charts**, then **Calculate**.

*Results*

The duration is shown in the columns, for each 1-m segment (interval occurrence 1, interval occurrence 2, etc.).

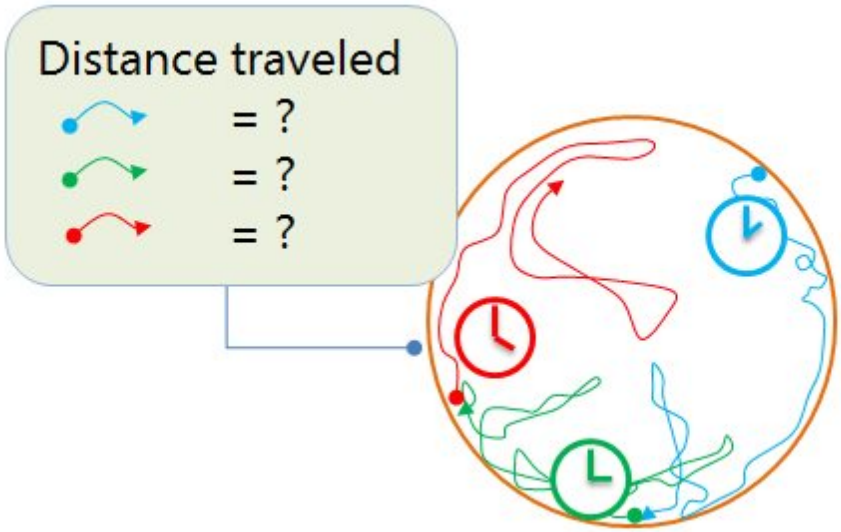
| | Free Interval based on Distance moved / interval occurrence 1 | Free Interval based on Distance moved / interval occurrence 2 |
|---|---|---|
| | Cumulative Duration | Cumulative Duration |
| | s | s |
| Trial 1 | 12.1333 | 22.2666 |

*See also*

- Free interval in the Analysis profile

# Analysis of regular time intervals

## Split the track in a number of regular intervals, and then calculate statistics for each interval




Specify the track segments in a Data profile and select the dependent variable Distance moved in an Analysis profile.

1. In the Data profile, in the **Result** box, click the **Settings** button and then select **Results per time bin**. Specify the length of the single time interval.
2. In the Analysis profile, specify the dependent variable. In the **Trial Statistics** tab, select the statistic (for example, Total for Distance moved).
3. Click **Statistics and Charts**, then **Calculate**.

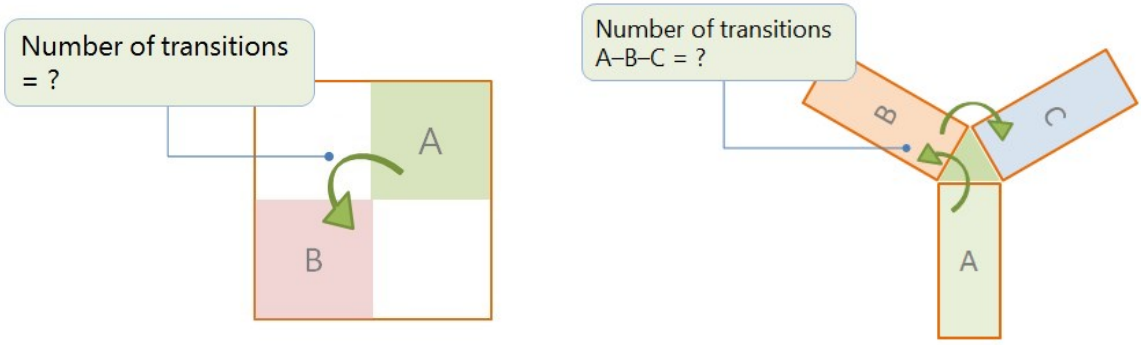
# Analysis of event transitions and sequences

## What do you want to do?

- Calculate transitions between zone visits
- Analyze bouts of behavior

## Calculate transitions between zone visits

For example, how many times did the subject go from zone A to zone B? Or, in a Y-maze experiment, how many times did the subject go from zone A, to B, then C? Or, in a radial maze experiment, how many 8-arm visits were made without any revisits?




1. In the Analysis profile:

   For zone alternations (like in a Y-maze test), choose the dependent variable **Zone alternation**. See Zone alternation

   For transitions in general, choose **Zone transition** and define the sequence (for example, in a radial maze test, Arm 1 > Arm 3 > Arm 5).

   Select the sequence of zone visits. If necessary, select more sequences. See Zone transition.

   In the **Trial Statistics** tab, select the statistic **Total number**.

2. Click **Statistics and Charts**, then **Calculate**.

## Analyze bouts of behavior

If you define a bout of behavior as a group of events where the interval *t* between them is lower than a specified limit ($t < t_{max}$), you can detect those bouts with the *Free intervals* function. For example, look for bouts of movement, where single instances of the state *Moving* are separated by less than five seconds.

In the Analysis profile, choose the dependent variable **Free interval**.

For an example, see **Detect bouts of behavior** in Examples of Free intervals in the Analysis profile.

# Basics of data selection

## Aim

To carry out analysis on a selection of data, not all data.

## Learn about

- The Data Profiles screen
- Create your data selection
- Work with data selection boxes

## Notes

- You can create as many Data profiles as you want, containing different criteria for data selection.
- Data selection is optional. If you do not select data, the default data selection profile is used, which contains all data in your experiment.

# The Data Profiles screen

## To access the Data Profiles screen

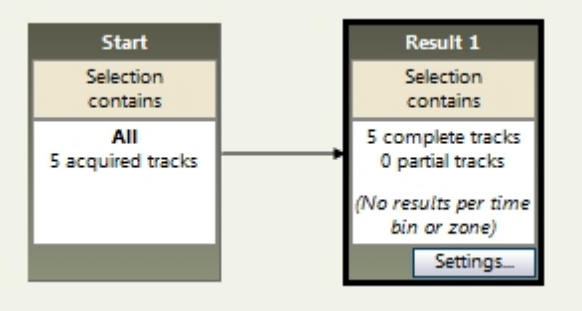
- Choose **Analysis** > **Data Profile** > **New**. Enter a name for the new Data profile and click **OK**.
- In the Experiment Explorer, in the **Analysis** folder, right-click **Data Profiles**, select **New**, enter a name and press **Enter**.

You can also open an existing data profile. Choose **Analysis** > **Data Profile** > **Open**.

## Default Data profile

The Data Profile contains two selection boxes:

- The **Start** box (left) contains all tracks currently stored in the experiment.
- The **Result 1** box (right), contains the data used for analysis. The two boxes are connected through an arrow. This means that in the default Data profile all data are used for analysis.




See Create your data selection

# Create your data selection

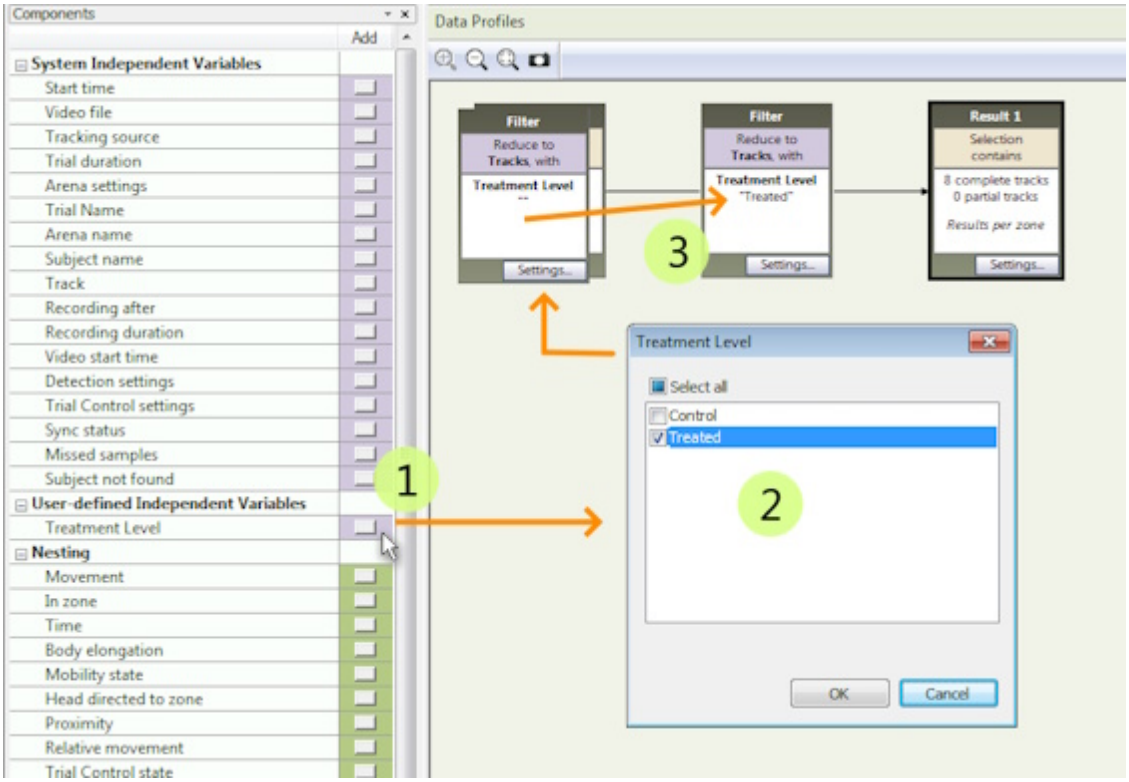
By default, all tracks in your experiments are selected. To refine your selection:

1. In the Components pane, click the button next to the variable you want to use.

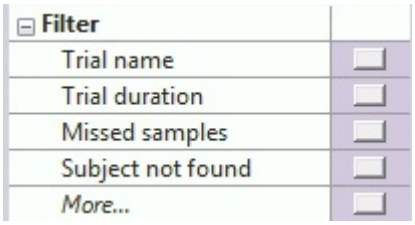
   If you do not see the Components pane, click the **Show/Hide** button on the toolbar and select **Components**.

2. A new box appears in the top-left corner of the Data Profiles window, and a new window appears on top, listing all possible values or characteristics of the chosen variable. For example, which treatment level you want to select for analysis. Choose the values that specify your selection and click **OK**.

3. Drag the box over the arrow that connects the two preexisting boxes. When the arrow turns white, release the mouse button.




If you do not see all buttons as shown above, click the **More** button.




## To create complex selections

You can define complex data selections by inserting two or more selection boxes in the sequence.

- See Combining selection boxes

## To create different selections in the same Data profile

By clicking the button next to **Result** (last button in the Components pane), you get a new **Result** box.

Additional Result boxes allow you to view the results in the same table for different data selections. For example, create groups based on an experimental treatment and compare the results.

- See Define groups of tracks.

# Work with data selection boxes

This topic contains instructions for the basic operations with selection boxes. See also Create your data selection

## Move a selection box

1. Click the margin of the box. The mouse cursor changes to a four-headed arrow.


2. Drag the box to the position you require.
3. Release the mouse button. The new box is inserted.

To move a group of selection boxes:

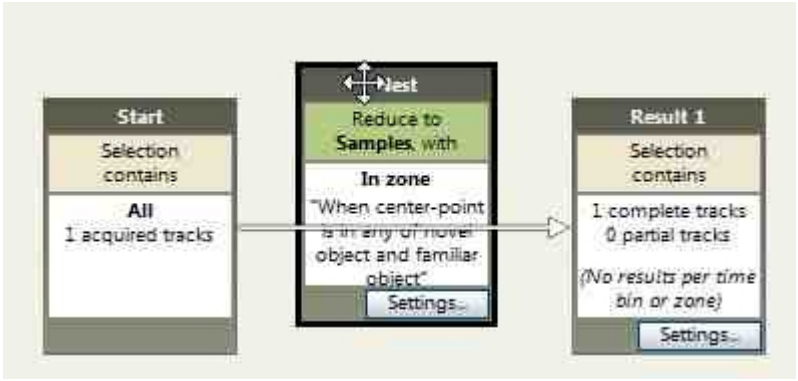
1. Draw a box around the boxes you want to move or click on the boxes you want to select while holding the **Ctrl** key.

   As a result, the border of the selected boxes becomes darker.

2. Hover the mouse on the margin or the colored area of one of the selected boxes. The mouse cursor changes to a four-headed arrow.
3. Drag the group of boxes to the position you require.

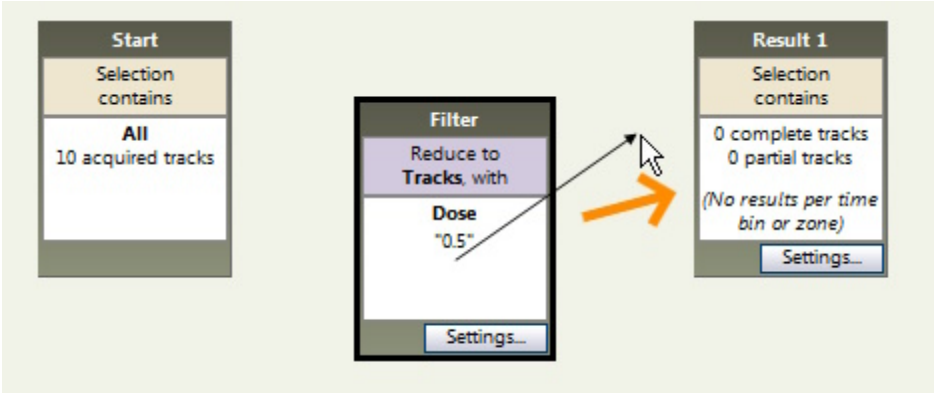
## Insert a box in a data selection sequence

1. Drag the selection box between two boxes, until the connecting arrow turns white.



## Connect two selection boxes

1. Point the mouse to the center of the first box, press and hold the left mouse button and drag toward the center of the other box.




2. Release the mouse button when the pointer has reached the center of the other box. The two boxes are connected.

You cannot create connections from the Result box to any other box, and from any box to the **Start** box.

## Change the selection criteria in a selection box

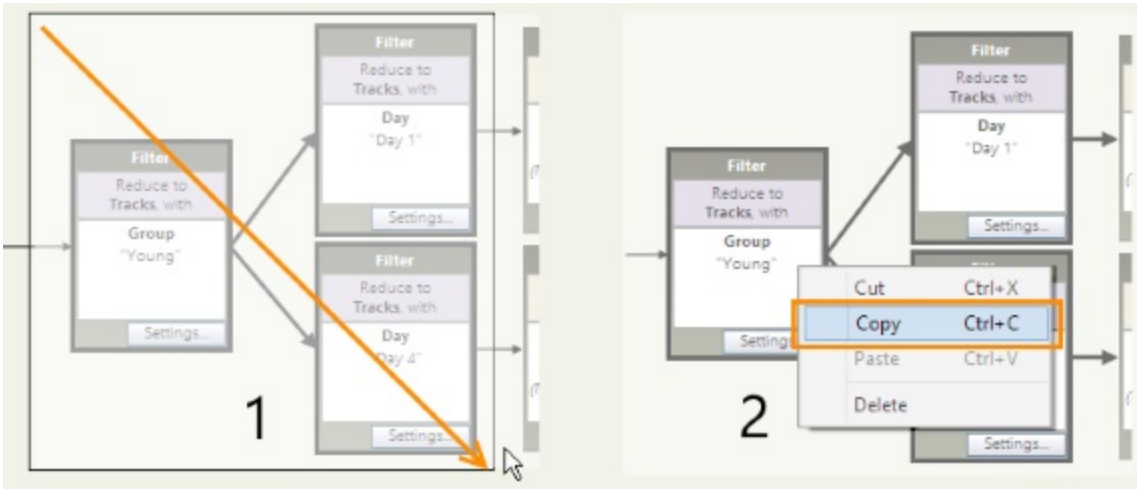
Follow the instructions below when you have inserted a selection box, and you want to adjust the settings to restrict/widen your selection. For example, when you have selected drug dose = 0.01 and 0.05 in the first instance, and you want to remove 0.05.

1. Locate the selection box that specifies the criterion you want to change.
2. Click the **Settings** button.
3. Select the appropriate values in the window that appears.

## Copy and paste a selection box

Follow this procedure to quickly duplicate Filter, Nesting and Results boxes. If needed, you can always change the settings in the new selection box that is created.

1. Select one or more selection boxes in the Data profile.
2. Choose **Edit** > **Copy**, or right-click and choose **Copy**, or press **Ctrl+C**.




3. If necessary, open the Data profile where you want to copy the boxes to.
4. Choose **Edit** > **Paste**, or right-click and select **Paste**, or press **Ctrl+V**.

Note that the new selection box is given the same name as the original one, followed by a number (2), (3), etc.

## Delete a selection box

1. Click the title of the box so the mouse pointer changes to a four-headed arrow.

   To select a group of selection boxes, draw a box around the selection boxes that you want to delete, or click on each of those boxes while holding the **Ctrl** key.

2. Press **Delete**, or choose **Edit** > **Delete**, or right-click and choose **Delete**.

You cannot delete the **Start** box. You can delete a **Result** box only if another one has been inserted in the Data Profiles window.

If you delete a box within a sequence, the arrows connecting the adjacent boxes are lost. Therefore, you must re-connect the adjacent boxes (see above).

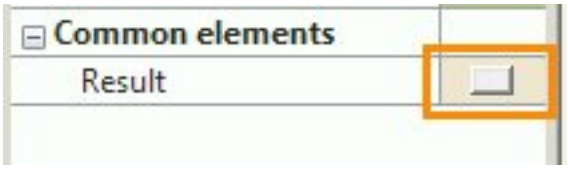
## Delete a connecting arrow

1. Click the connecting arrow you want to delete. The arrows turns bold to show it is selected.
2. Press **Delete**. The arrow is deleted.

## Create a new Result box

Create a new Result box when you want to display analysis results from different data selection criteria at the same time. For example, to calculate statistics for two groups of tracks.

1. In the Components pane, under **Common elements**, click the button next to **Result**.

   The new **Result** box appears in the top-left corner of the Data Profiles window. Connect the selection box to other boxes.

2. Drag the new **Result** box to the right-hand side of the Data Profiles window, below the first Result box.

   **TIP** Click **Settings** in the **Result** box and edit its name (see below).

3. Complete the selection sequence by making a branching so that each data selection criterion ends up in its own Result box.

See also

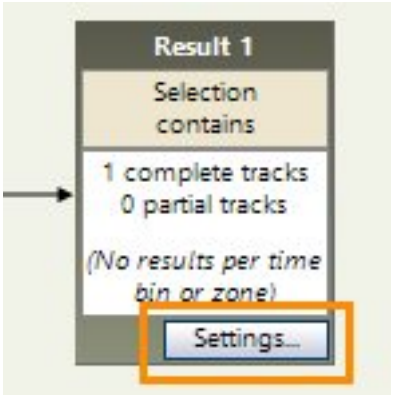
- Multiple selections in a data profile

## Edit the Result box name

The Result boxes are named automatically as you create them: Result 1, Result 2, etc.

The **Result** box name is shown in the Analysis results (statistics and track plots), so it is good to give a name that reminds you from which data selection that result comes from.

1. Click the **Settings** button in the bottom-right corner of the box.




2. Enter the new name in the **Name** field.

## Snap to grid

The data selection boxes automatically snap to a grid. You can change this by clicking the **Show/Hide** button on the toolbar and selecting/deselecting the two Grid options (**Snap to Grid** and **Show Grid**).

## Zoom in and out

The toolbar of the Data Profiles window shows three zoom icons:

- **Zoom in** (**Ctrl+.**). Click this button until all data selection boxes fit in the window.

- **Zoom out** (**Ctrl+,**).

- **Fit all**. Click this button to fit all data selection boxes into the window.

The Data Profiles window is ‘dynamic’: this means that it expands when you move data selection boxes to the right. In this case, you can navigate ‘from left to right’ in the Data Profiles window by using the scrollbar at the bottom. use the **Zoom to fit** button on the toolbar to make all data selection boxes visible.

# Analyze Entire Tracks

## Main topics and tasks

# Filter tracks

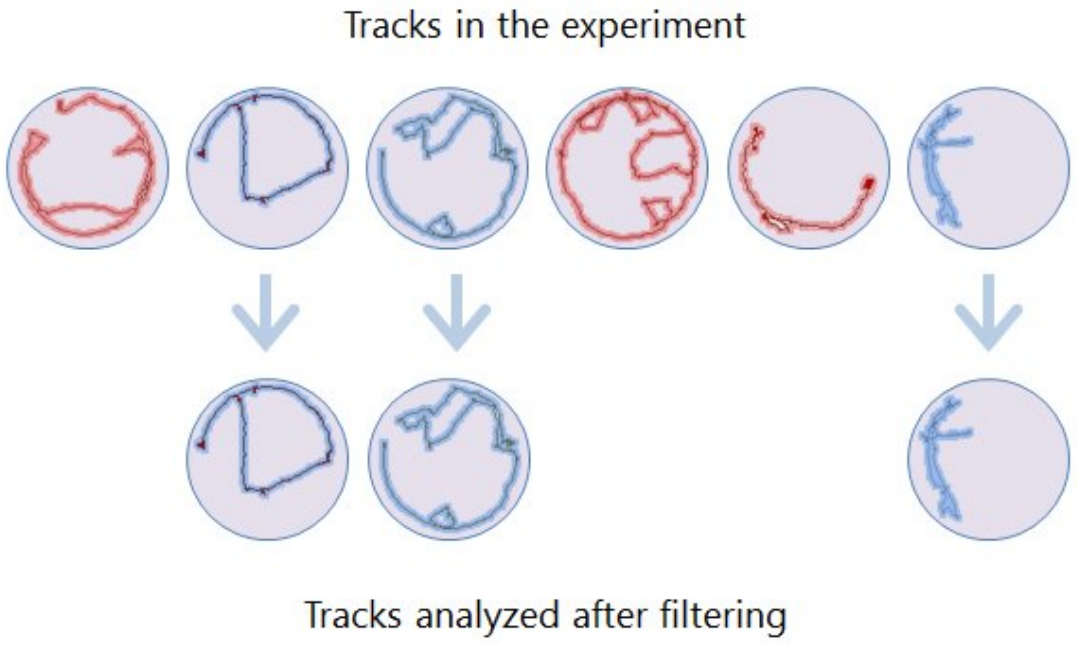
## What do you want to do?

- Filter tracks
- Variables available for filtering

# Filter tracks

## What is Filtering?

Filtering is the process of picking out entire tracks, either manually or based on the value of independent variables (System or User-defined). Filtered tracks and the corresponding video and external (physiological) data are subject to analysis.

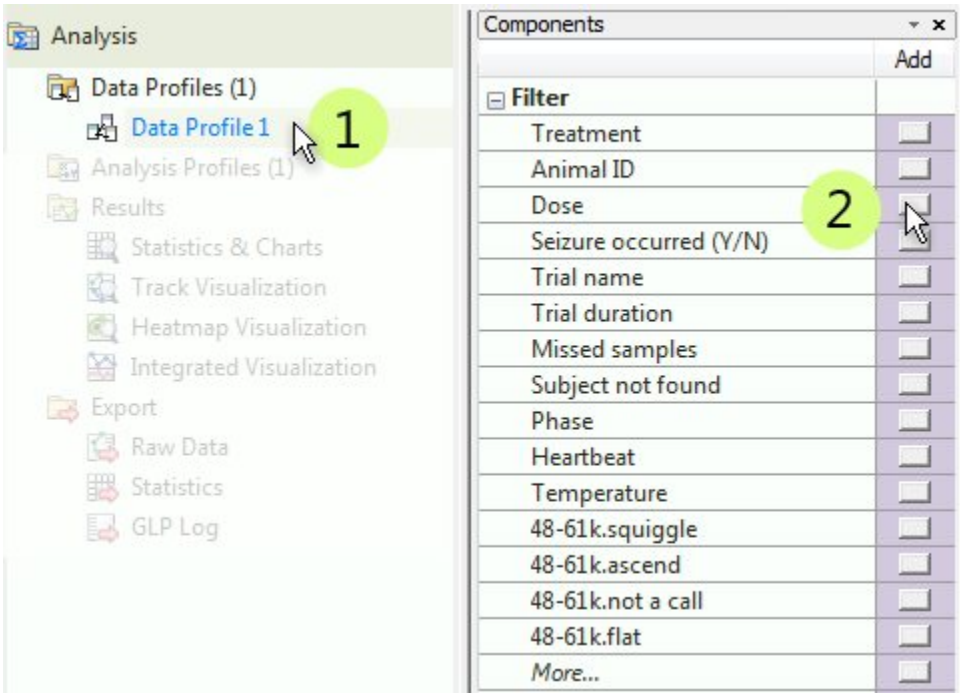



Unlike filtering, Nesting picks out segments of tracks. You can combine Filter and Nesting criteria.

- See Basic rules for combining selection boxes.

## To filter tracks

1. Create a new data profile or open an existing one.
2. Locate the variable in the Components pane that you want to use for filtering data, and click the corresponding button.




If you do not see item you are looking for, click the button next to **More**.

See the next section for details on the independent variables available for filtering.

3. A **Filter** box appears in the top-left corner of the Data Profiles screen. The Independent Variable values window opens on top.
4. Select the values of the variable for the tracks you want to analyze, and click **OK**.
5. Drag the **Filter** box to the desired position in the selection sequence between the Start and the Result box.
6. Repeat steps 2 to 5 to add more **Filter** boxes and refine your selection.

## Notes

- Scope of the variable. Within a single trial, you can select different data sets depending on the Scope of the variable used (Trial, Arena or Subject). Depending on your setup, a trial may consist of one or more arenas. On its turn, each arena can include one or more subjects.
  - By filtering by a variable with **Trial** scope, you analyze all data for the trials that you specify, no matters how many subjects and arenas those trials contain.

        - By filtering by a variable with **Arena** scope, you analyze all subjects recorded in the arenas that you specify, and you ignore the data recorded in other arenas in the same trial.
        - By filtering by a variable with **Subject** scope, you analyze the tracks referring to the subjects that you specify, and ignore the tracks of other subjects recorded in the same arena and trial.
    - If the Independent variable values window does not list any value available for selection, it means that no values have been assigned to any trial in the Trial List.
    - You can combine different filtering criteria to create complex data selections. To do so, insert multiple Filter boxes in the same sequence.
    - You cannot filter tracks according specific values of external (physiological) data. For example, filter all tracks for which average heart rate was higher than 600 bpm.
    - If you want to edit an existing **Filter** box, click the **Settings** button of that box.

See also

- Create your data selection

# Variables available for filtering

## System variables

- **Start time**. Filters the tracks belonging to the trials started at the times you select from the list.

  Tracks acquired from video files have the time of creation of the video files as their start date/time.

  Tracks that belong to the same trial have equal Start Time (that is, the time you started the Trial). If you have applied Trial Control with Start conditions, actual data recording may have started at different times in each track. Therefore, if you want to select individual tracks according to when actual data recording started, filter tracks by the variable Recording after (see below).

- **Video file**. Filters the tracks recorded from the video files you select from the list.
- **Tracking source**. Shows the camera source if you track live. Since EthoVision allows one camera source per experiment, this variable will show only one camera.
- **Trial Duration.** Filters the tracks belonging to the trials of the duration you select from the list.

  Tracks that belong to the same trial have equal Trial Duration (that is, the difference between the Stop time and the Start time of the trial). If you have applied Trial Control with Start or Stop conditions, the duration of actual data recording may be different for each track. Therefore, if you want to select individual tracks according to the duration of actual data recording, filter tracks by the variable Recording Duration (see below).

- **Arena settings**. Filters the tracks recorded using the Arena Settings profile you select from the list.
- **Trial name**. Filters the tracks belonging to the trials you select from the list.

  Tracks recorded in the same trial have the same Trial name. If you want to select individual tracks, filter by the variable Arena name or Track (see below).

- **Arena name**. Filters the tracks recorded in the arenas you select from the list.

  This option is handy if you have a multiple arena setup, and you want to analyze data from some arenas, not others.

- **Subject name**. Filters the tracks of the subjects you select from the list (available when tracking multiple subjects per arena).
- **Track.** Filters the tracks you select from the list.

  Each track name corresponds to data of one individual subject recorded in one arena and in one session (trial). If you want to select data according to the recording sessions (which, in the case of multiple arena setups, may contain multiple tracks), filter by Trial name (see above).

- **Recording after.** Filters the tracks that started a specific time after the start of the Trial. Select this time from the list.

  This option is handy if you apply Trial Control with Start conditions. If this is the case, actual data recording for each track may have started later than the trial's start time. You can filter tracks started a certain time after the start of the Trial they belong to. For example, in a multiple arena setup, Trial 1 started at 10:23:00, but actual data recording started after 5 seconds in Arena 1 and after 30 seconds in Arena 2. You can select those time lags from the list to filter specific tracks.

  If you do not use Trial Control with Start conditions, data recording starts at the start of the Trial. In this case, the Filter Variables window for Recording after shows only the '0' value.

- **Recording duration**. Filters the tracks recorded for the time you select from the list.

  This option is handy if you apply Trial Control with Start and Stop conditions. If this is the case, actual data recording may be shorter than the trial duration. You can filter tracks that have a certain duration, independent of the trial's duration. For example, in a two-arena setup, a trial lasted 15 minutes. In Arena 1, actual data recording lasted 15 minutes while in Arena 2 12 minutes because a Stop condition was met for that arena before the trial stopped. You can select those duration values from the list to filter specific tracks.

  If you do not use Trial Control, actual data recording lasts as long as the Trial, so Recording duration is the same as trial Duration (see above). In this case, the Filter Variables window for Recording Duration shows the same values as that for Duration.

- **Video start time**. Filters the tracks recorded from the video file created (or last saved) on the date and time you select from the list.
- **Detection settings**. Filters the tracks recorded using the Detection Settings profiles you select from the list.

The Detection profile variable has Trial Scope; this means that within a trial, all tracks have the same Detection Profile. If you want to know which Detection Settings were used for a specific trial, open the Trial List.

- **Trial control settings**. Filters the tracks recorded using the Trial Control profile you select from the list.

  If you want to know which Trial Control profile was used in a certain trial, make sure the System Variable Trial control settings is selected for display in the Trial List.

- **Sync status**. Filters the tracks corresponding to the status of the synchronization between track and co-acquired external data.

  **Planned**. Filters the tracks for which no external data co-acquisition has been carried out.

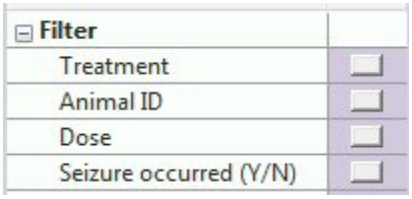
  **Acquired**. Filters the tracks for which co-acquisition was carried out.

- **Missed Samples** - Filters the tracks corresponding to the proportion of missed samples caused by, for example, a too high processor load.
- **Subject not found** - Filters the tracks corresponding to the proportion of samples which were processed by EthoVision but in which no subject was detected.

## User-defined variables

This list depends on the variables that have been defined in the Trial List.

Click the button next to the variable you want to use as a criterion for filtering data.




See also the note about the Scope of the variable in To filter tracks.

## External data

If you import external data into one or more trials, in the Components pane under Filter then type of physiological variables are listed. Click the button next to the variable you want to use to filter data.


In the window that appears on top, select the imported data files you want analyze. Tracks linked with those data files are selected for analysis.

If you filter by External data, the data being subject to analysis also includes the tracks and the video files associated.

What track data are selected with external data files depends on how you linked the external data files to the track data during import (whether at the Trial, Arena or Subject level; see Import external data: General information). For example, if ECG data files are linked at the Trial level, entire trials are filtered. If ECG data files are linked to Subjects, only the tracks for the subjects linked to those ECG files are filtered.

See also

- Import external data in EthoVision XT

# Analyze groups of tracks

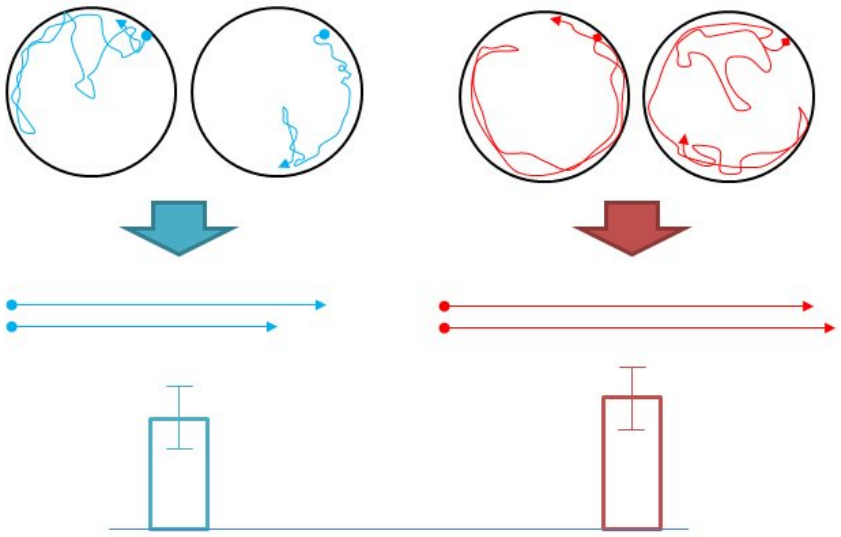
## What do you want to do?

- Define groups of tracks
- View the results for groups of tracks

# Define groups of tracks

## Aim

To compare groups of tracks. For example, to compare the tracks of animals treated with a drug with the tracks of control animals.



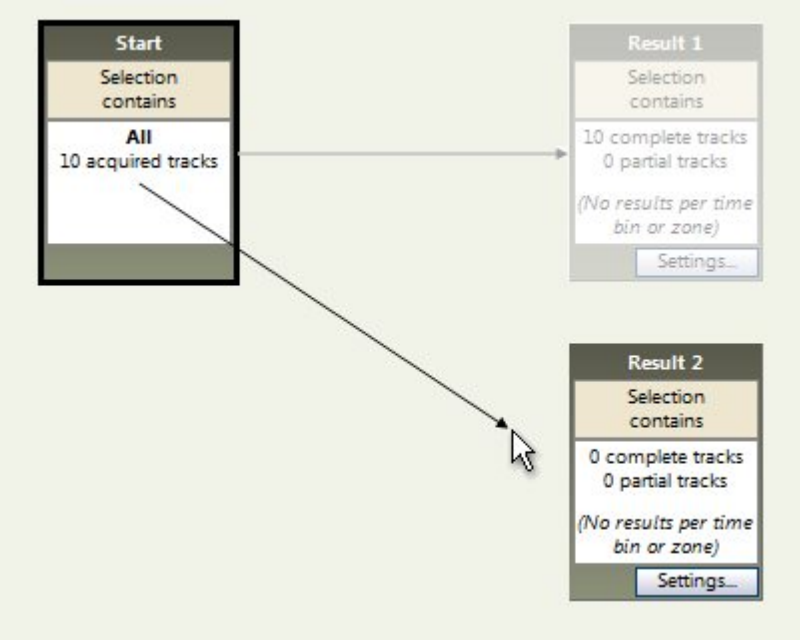
You can easily create groups by filtering tracks according to one or more independent variables, and linking that filter criterion to a specific Result box.

## Procedure

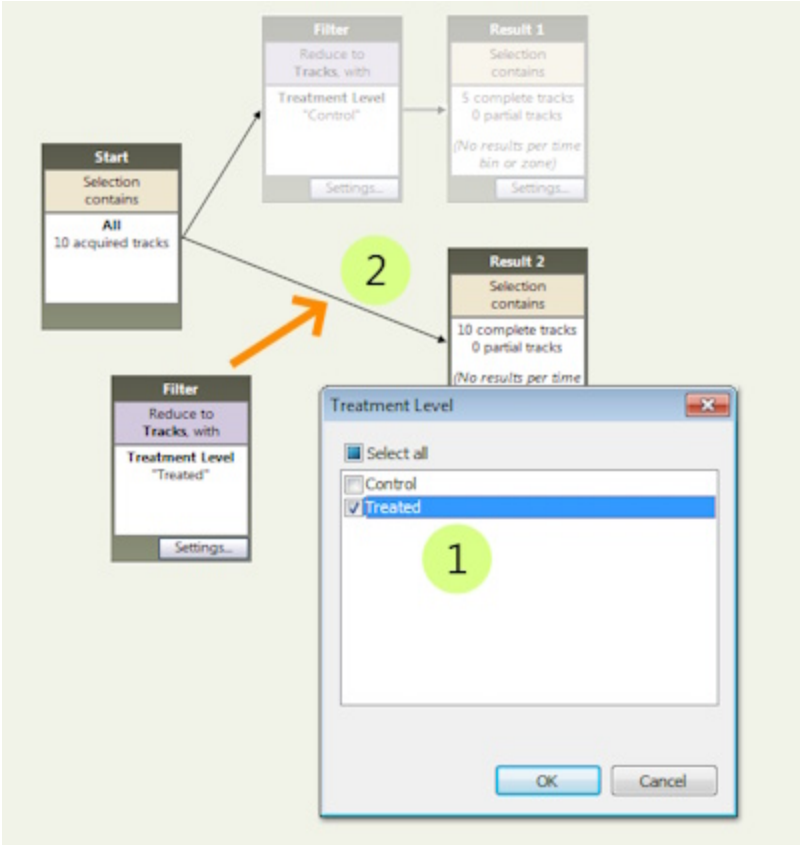
1. Create a new Data profile.
2. To add a Result box, in the Components pane under **Common elements** click the button next to **Result**.


   The new **Result** box is by default named **Result 2**. Place this box somewhere under the **Result 1** box.

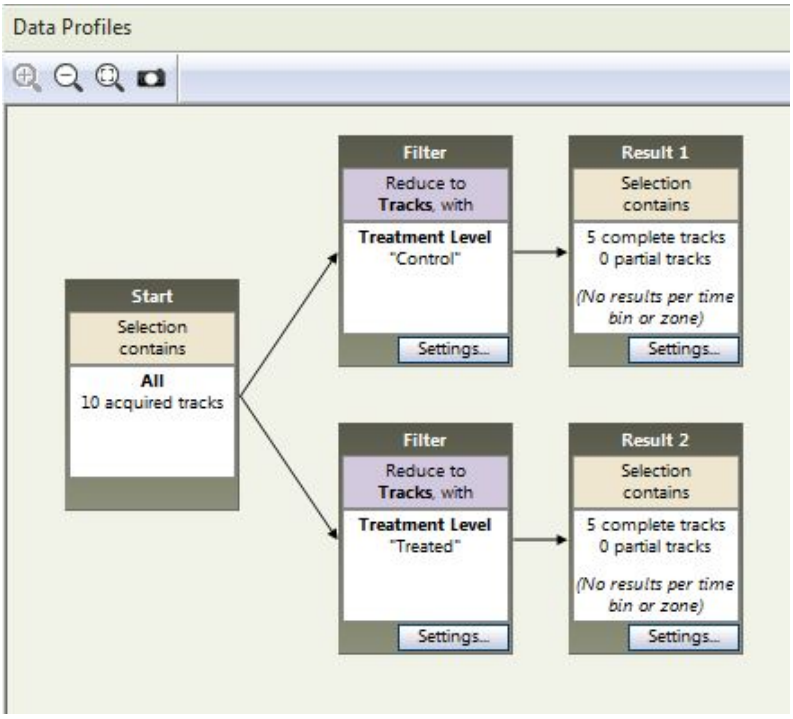
3. Connect the **Start** box to the **Result 2** box.




4. Filter your tracks by the independent variable Treatment Level:
   - Click the button next to Treatment Level. Choose **Control** and place the resulting Filter box between **Start** and **Result 1**.
   - Click again the button next to Treatment Level. Choose **Treated** and place the resulting Filter box between **Start** and **Result 2**.




5. The Data Profiles window should look like this:



The two Result boxes receive the data filtered according to the independent variable Treatment Level. **Result 1** receives five tracks filtered with Treatment Level = Control. **Result 2** receives other five tracks filtered with Treatment Level = Treated.

6. **TIP** The name of the **Result** box is shown in the results (statistics and track plots), so it is good to give a name that reminds you from which data selection that result comes from. Click **Settings** in a Result box and rename it. For example, rename *Result 1* to *Control group*.

*See also*

- Edit the Result box name

# View the results for groups of tracks

## Prerequisite

You have defined one or more groups of tracks.

## To view the results for groups of tracks

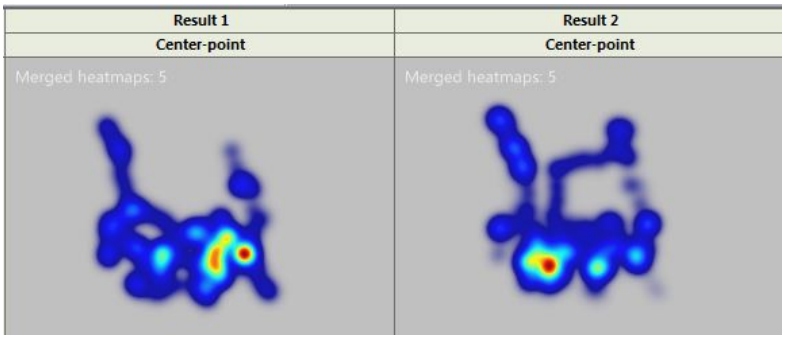
1. Create a new Analysis profile and choose the dependent variables (for example, Distance moved).
2. In the Experiment Explorer, under **Results**, choose the option you require (Calculate Statistics, Heatmaps etc.).

In the analysis results, you can see which output is produced by which Result box. Look up the **Result** header. For example, in the Statistics & Charts screen, click **Group Statistics and Charts**:

Trial Statistics | Group Statistics & Charts

| | Treatment Level | Distance moved | | |
|---|---|---|---|---|
| | | Center-point | | |
| | | Total | | |
| | | N | Mean | Standard Error |
| | | | m | m |
| Result 1 | Control | 5 | 66.2128 | 19.2582 |
| Result 2 | Treated | 5 | 171.0094 | 103.5069 |

In the Heatmaps screen, click **Group Mean** and then click the **Plot Heatmaps** button.



# Analyze Track Segments

## Main topics and tasks

# Analyze track segments: main concepts

## Learn about

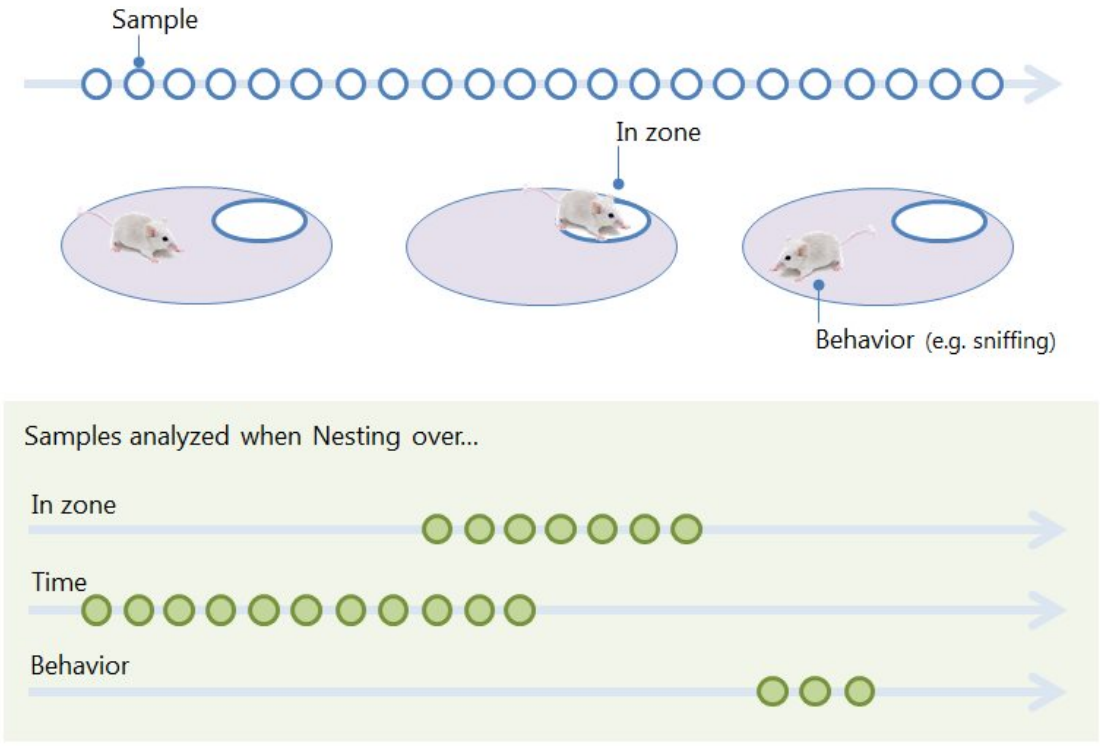
- Analyzing track segments
- How to select track segments

# Analyzing track segments

In EthoVision XT you can pick out segments of tracks specified by time intervals or the behavior of the subject. To do so, use the **Nesting** function in the Data profile.

When Nesting is activated, analysis is carried out on the samples within those track segments, and the corresponding video and external (physiological) data. The track segments outside the selection are not included in the analysis.

Below: A track represented by samples on a time line. The sketch shows a mouse entering a zone someway in the middle of the track, then sniffing air towards the end. The time lines at the bottom represent the samples selected for analysis when nesting over a zone, over time (for example, the first half of the track), or over a behavior (in this example, sniffing), respectively.




## An important difference

Selecting a variable in the Data profile is not the same as selecting the same variable in the Analysis profile. For example:

- When you choose In zone under Nesting in the Data profile, you select the samples when the animal was in a zone. Then, in the Analysis profile you choose a dependent variable, like Velocity. As a result, EthoVision XT calculates the velocity of the subject when it was in the zone.

- When you choose In zone under Location in the Analysis profile, EthoVision XT calculates statistics for the variable In zone, for example, the time spent in a zone, or the number of zone entries, but not the velocity of the subject when it was in that zone.

## Nesting options

- Nesting over time, to analyze a time interval defined by a Start time and a Stop time.

  **EXAMPLE** Analyze the first 15 minutes or each track.

- Nesting over zones, to analyze the track segments when one or more subjects are within a zone or a combination of zones.

  **EXAMPLE** Analyze the behavior of the mouse when its nose point was in a zone defined around the novel object.

- Nesting over behavioral states, to analyze the track segments corresponding to the state of one or more subjects (for example, Movement, or Grooming).This option includes manually-scored behaviors and behaviors recognized automatically.

  **EXAMPLE 1** Analyze all the samples collected when the animal's body was stretched (this can be done with the Body elongation dependent variable).

  **EXAMPLE 2** Analyze the locations where the animal was sniffing.

- Nesting over trial control states, to analyze the track segments corresponding to the time between two events of Trial Control.

  **EXAMPLE** Analyze the time from when the cue light switched on to when the mouse consumed the food item delivered.

- Nesting over subjects, to analyze the track segments based on the behavior of another subject. This is available when your arena contains multiple subjects, like in a social interaction test.

  **EXAMPLE** The researcher wants to analyze the track segments of Subject 1 when Subject 2 was In proximity of Subject 1.

## Notes

- Track segments defined with Nesting are left-closed [A, B). That is, the start sample A, not the stop sample B, is included in the analysis.
- To analyze entire tracks, use the Filter function. See Filter tracks.
- You can also combine Filter and Nest boxes to create complex selections. See Complex Data Selections

# How to select track segments

1. Create a new data profile or open an existing one.
2. Locate the variables under **Nesting** or **Nesting over Subjects** in the Components pane that you want to use to select track segments, and click the corresponding button.

   A **Nest** box appears in the top-left corner of the Data Profiles screen. A new window opens on top.
3. Select the values that define the track segments you want to analyze, and click **OK**. See the next topics for details.
4. Drag the **Nest** box to the desired position between the **Start** and the **Result** box. See Insert a box in a data selection sequence.
5. Repeat steps 2 to 4 to add more Nest boxes and refine your selection.

   If you want to edit an existing Nest box, click the **Settings** button in that box.
6. Choose **Analysis** > **Results** > then one of the options available to obtain numerical and graphical results for the track segments selected.

## Notes

- If you have tracked two or more subjects per arena, you have two main nesting options:
  - **Nesting**: Choose this category if you want to analyze the track segments that correspond to the behavior of the subject of that track.
  - **Nesting over Subjects**: Choose this category if you want to analyze the track segments that correspond to the behavior of subjects other than the subject of that track, or a combination of subjects. See Nesting over Subjects.

# Nesting over time

## Aim

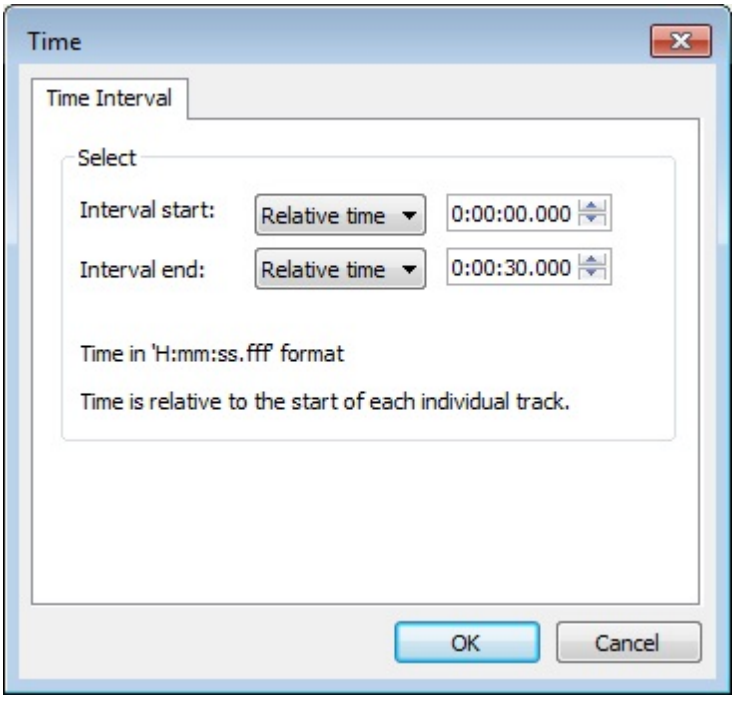
To pick out the samples in one time interval, from time $t_{start}$ to time $t_{end}$.

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Time**.


**NOTE** Choosing **Time** under **Nesting over subjects** leads to the same data selection.

2. In the Time window, enter the Interval start and Interval end.

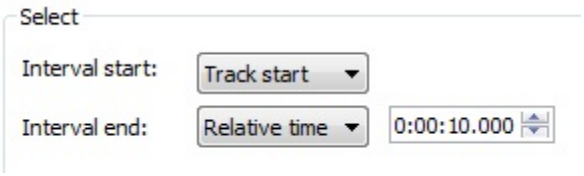


- **Relative time** is the time since the start of the track. Enter the relative time. In EthoVision XT 17.5 and later, you can specify any time within the trial, even after 24 hours.
- **Track start**/**Track end** mark the start and end of each track, respectively. Therefore, they may mean different relative times between tracks.

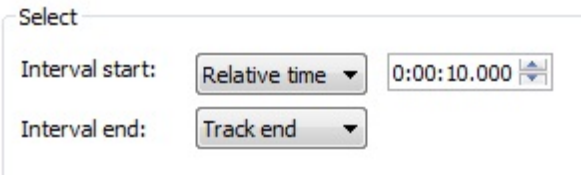
3. Complete the procedure from step **4** in How to select track segments.

**EXAMPLES**

- To analyze the first 10 minutes of each track:




- To analyze the from 10 minutes to the end of each track:

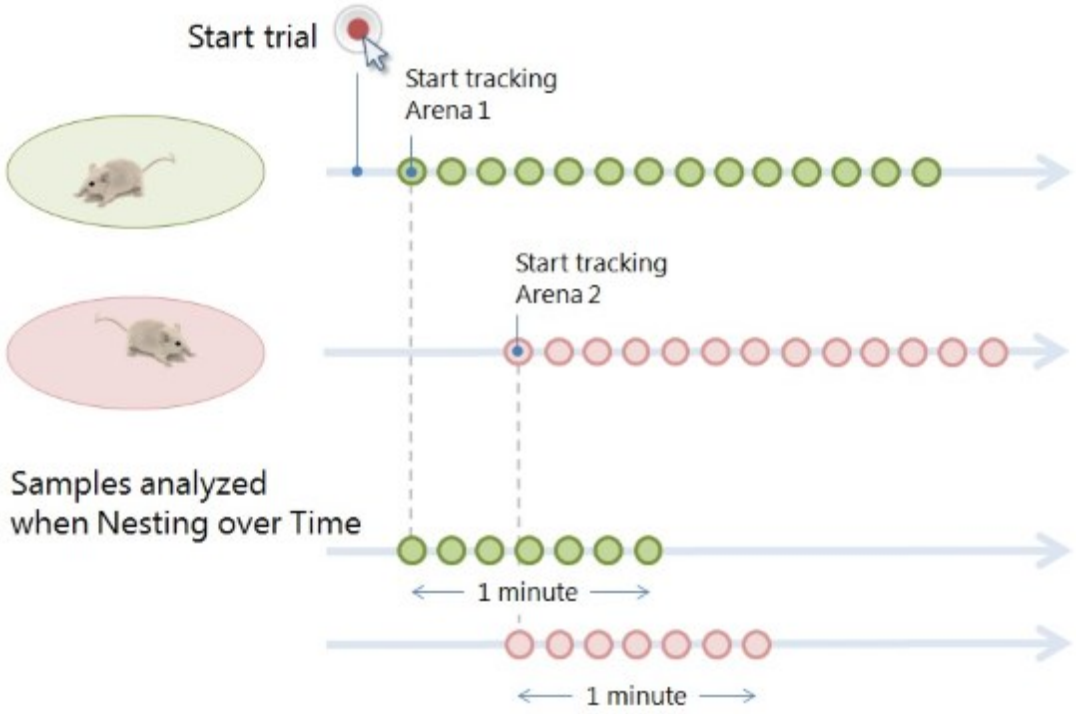



## Notes

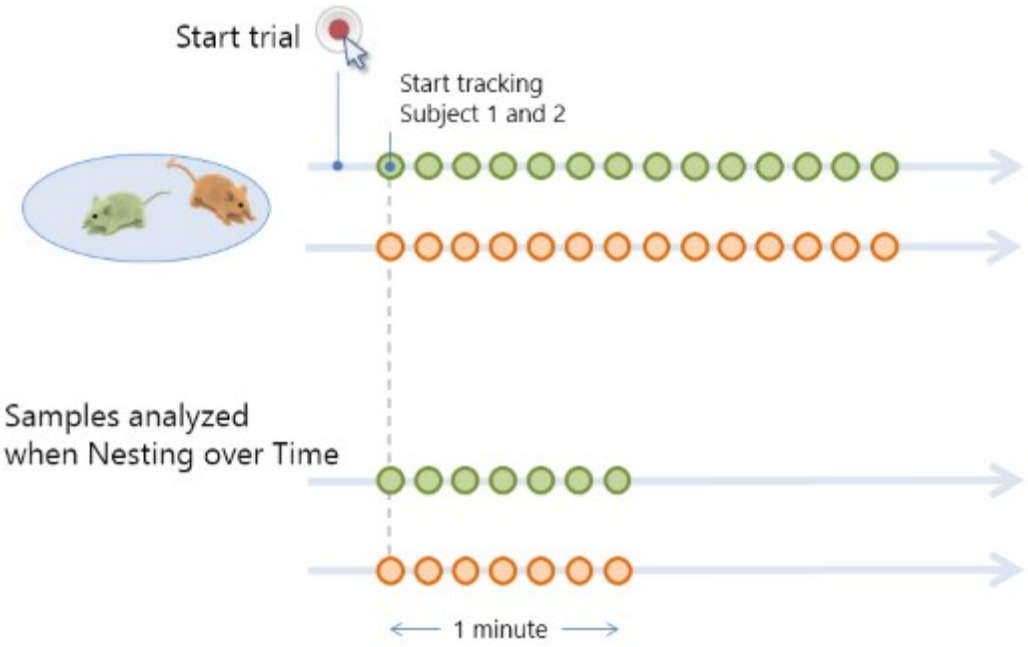
- Time intervals are applied in each track independently (see also the notes below).
- Default: Interval start 0:00:00.000 (h:mm:ss.fff), Interval end 0:00:30.000.
- 0:00:00.000 represents the time that tracking started for that arena. With multiple arenas, tracks may actually start at different times within the same trial (see below).
- If you want to split the data in regular intervals (for example, a one-hour recording in six 10-minutes intervals), do not nest over time. Instead, in the Data profile choose Results per time bin on page 840.
- **TIP** If you want to select a track segment that lasts some time relative to an event (for example, select the 10 seconds after the presentation of a stimulus, or select up to when an object is explored for 1 minute), then choose Free interval instead. See Nesting over a Free interval.

- *Nesting over time when working with multiple arenas*. if you apply Trial Control with Start and Stop conditions for each arena separately, actual data recording may start and stop at different times in each arena.

  The example below shows the effect of Nesting over the first minute of the tracks, in an experiment with two arenas. Dots represents the samples on the timeline. Tracking started automatically as soon as the animal was detected in the arena. Because the mouse in Arena 1 was released (and therefore detected) earlier than in Arena 2, tracking started earlier in that arena. The tracks have equal duration set in the Trial Control Settings, for example 5 minutes. When nesting over time, we select the first minute of each track (from Track start to 0:01:00.000). As a result, the two track segments do not overlap completely on the time line.

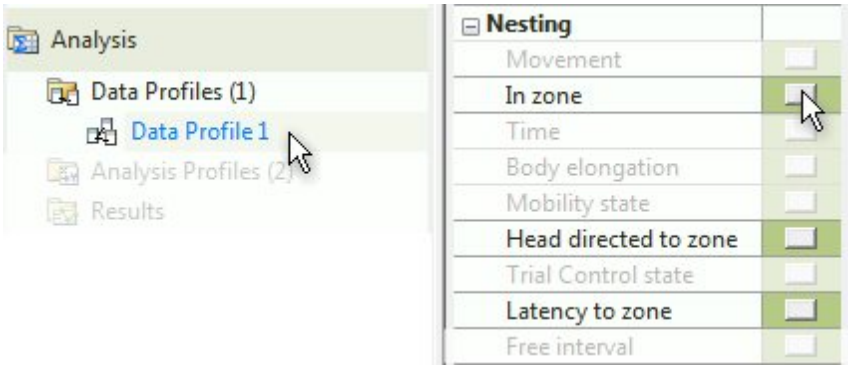



- *Nesting over time when working with multiple subjects per arena*. Within an arena, tracking starts at the same time for all subjects. Therefore, nesting over time results in the same interval being selected for all the subjects.




Note that tracking starts for all subjects even when one is not released yet in the arena. In that case the track for that subject starts with missing samples.

# Nesting using zones




- Nesting over In zone
- Nesting over Latency to zone
- Nesting over Head directed to zone

# Nesting over In zone

## Aim

To pick out the data collected when one or more subjects were in a specific zone, or in a combination of zones.

Nesting over In zone is only necessary when you want to analyze track segments by combining zones or subjects. If you simply want to calculate statistics of distance, velocity etc. for each zone, without combining subjects or zones, see Calculate the results per zone.

## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on that subject being in specific zones, choose **In zone** under **Nesting**. See the procedure below. See also B in Nesting vs. Nesting over subjects.
- To select data of a subject based on other subjects being in specific zones, choose **In zone** under **Nesting over Subjects**. There you find the **Actors** tab where you can specify which subjects (Actors) were in the selected zone(s). For an example, see C, D in Nesting vs. Nesting over subjects. See the procedure in Nesting over subjects.

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **In Zone**.
2. In the In Zone window, under In the following zones, deselect **Arena** and select the zones in which the animal must be.
3. Choose the remaining options to specify which combination of zone (any, all, not in any) and body points. For explanation, see Dependent Variables in Detail > In zone.
4. Click **OK**. Proceed with step **4** in How to select track segments.

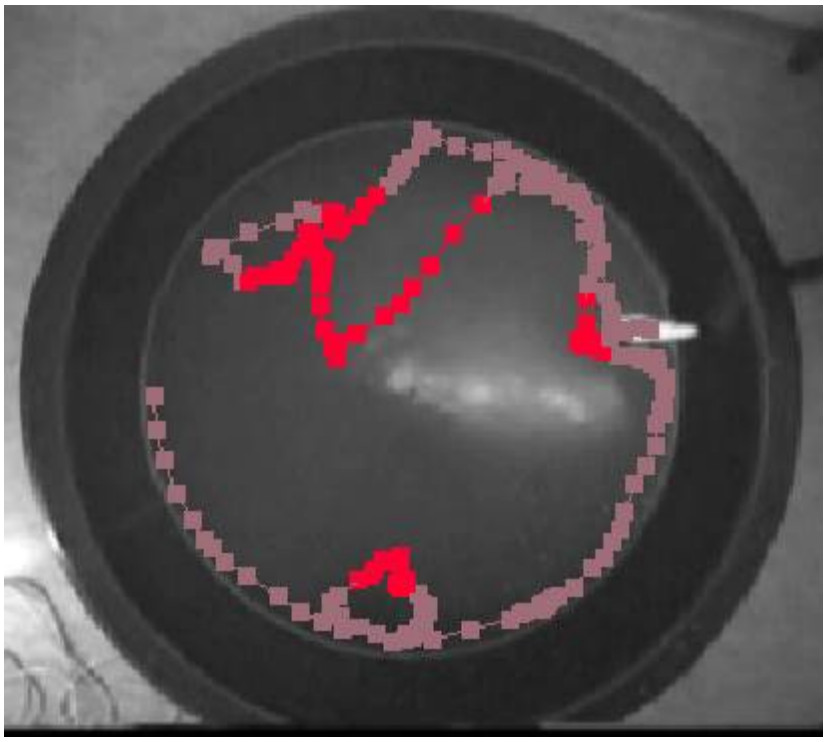
## Notes

- If your experiment is set to **Only center-point detection**, the **From following body points** options are not available. Calculations are based on the center-point of the body.
- Select **When in all selected zones** only when the zones are overlapping at least partially (for example, North Quadrant and Center of an open field). If

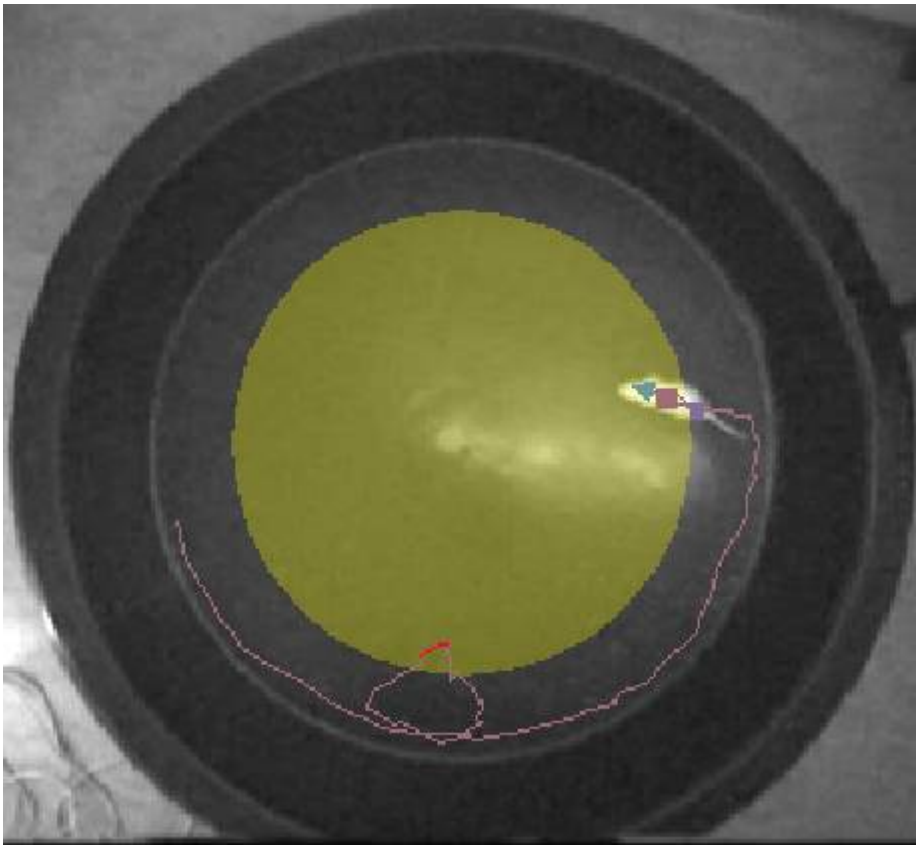
two zones are not overlapping, selecting **When in all selected zones** results in no data being selected, because a body point can never be in two different locations at the same time.

## Application examples

**EXAMPLE 1** A Center zone has been defined in an open field. When you do Nesting and choose Center under **In the following zones**, the samples in the center of the open field are selected for analysis. When you visualize the track, selected samples are displayed in bright colors; non-selected samples are dimmed out (see below).



**EXAMPLE 2** For the same open field as above, but when also tracking the nose and tail-base points, if you select the three body points and select **All selected points** from the list, a sample is only selected when the three body points are within the Center zone. In the example below, the tail-base is outside the Center zone (highlighted in yellow). Therefore, that sample is not selected for analysis.

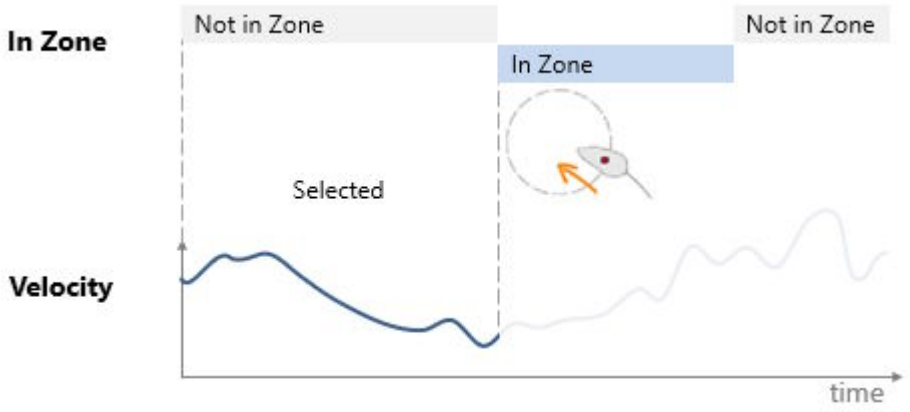


# Nesting over Latency to zone

## Aim

To pick out the data collected before or after the subject has entered a specific zone.

**EXAMPLE** Calculate the velocity of the subject up to when it entered a zone for the first time.



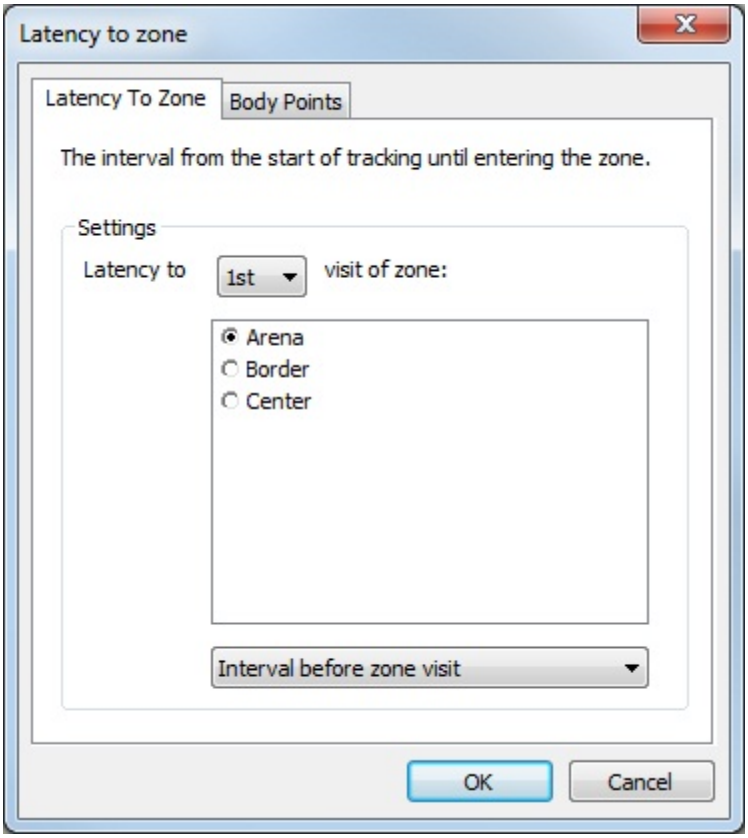

## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on the latency to enter a zone by that subject, choose **Latency to zone** under **Nesting**. See the procedure below.
- To select data of a subject based on the latency to enter a zone by other subjects, choose **Latency to zone** under **Nesting over subjects**. There you find the **Actors** tab where you can specify which subjects (Actors) entered the selected zone. See Nesting over subjects

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Latency to zone**.
2. In the Latency to Zone window, under **Settings**, next to **Latency to**, choose which zone visit you want to use for your data selection.




3. Select the zone of interest and choose from the list at the bottom of the window whether you want to analyze the **Interval before zone visit** (as in the example above, under Aim) or the **Interval after zone visit**.
4. Click the **Body Points** tab (when available), and specify the body points that Latency is based on. Next, select one of the following:
   - **All selected points**. The data is analyzed before/after all the selected body points were simultaneously in the zone.
   - **Any selected point**. The data is analyzed before/after at least one of the selected body points entered the zone.
5. Click **OK**. Proceed with step **4** in How to select track segments.

## Note

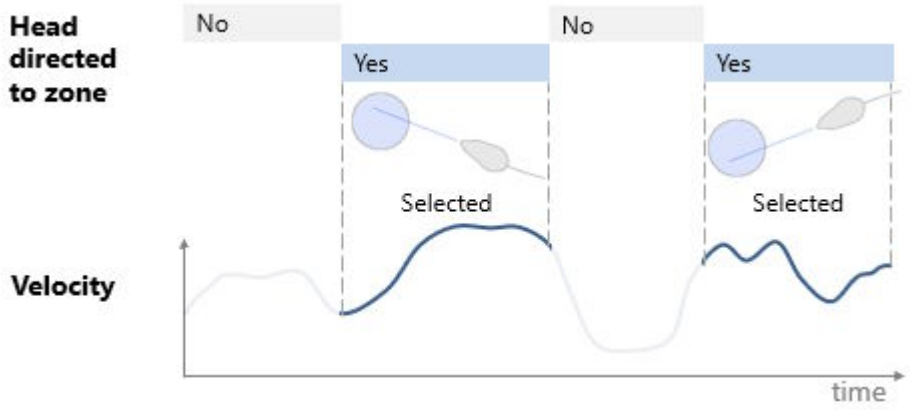
- If you select **Latency to zone** under **Nesting**, different subjects in the same arena are analyzed in different track segments because the nesting criterion is defined based on each subject separately.
- If you select **Latency to zone** under **Nesting over Subjects**, different subjects are analyzed in the same track segments, because the nesting criterion is determined by the Actors specified and is valid for all the subjects. See Nesting over Subjects

# Nesting over Head directed to zone

## Aim

To pick out the data collected when the head of the subject (or more subjects, named Actors) is pointing to a zone or a circular area around a point. The zone or point must be defined in the Arena Settings.

**EXAMPLE** Calculate the velocity of the subject every time it was pointing to the target zone.




## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

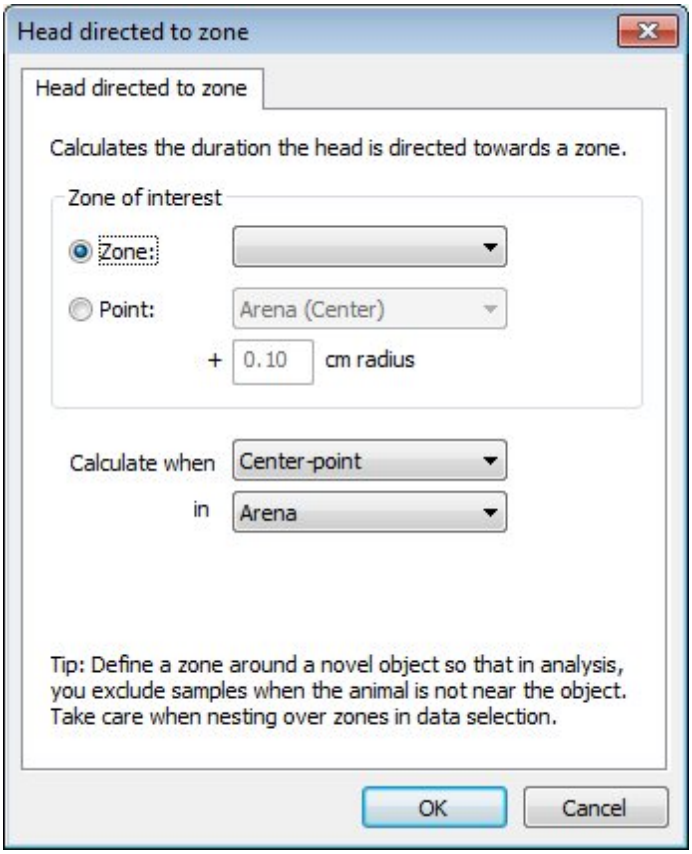
- To select data of a subject based on when that subject points to a zone, choose **Head directed to zone** under **Nesting**. See the procedure below.
- To select data of a subject based on when other subjects point to a zone, choose **Head directed to zone** under **Nesting over subjects**. There you find the **Actors** tab where you can specify which subjects (Actors) were pointing to the selected zone. See Nesting over subjects

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Head directed to zone**.
2. In the Head Directed to Zone window, select:
   - The **Zone of interest** (a zone or a point of interest + a specific radius).

- When to calculate the Head directed to zone state. Use this option to restrict the selection criterion (e.g. calculate Head directed to zone only when the subject was in an area around the target zone, not the entire arena).

For details about how Head directed to zone is calculated, see Dependent Variables in Detail > Head directed to zone.




3. Click **OK**. Proceed with step **4** in How to select track segments.

*See also*

- Nesting over Subjects

# Nesting over behavioral states


## Aim

To pick out the data collected when the subject was in a specific behavioral state. Click the options here below for information.

- Nesting over Activity state
- Nesting over Acceleration state
- Nesting over Body elongation state
- Nesting over Body angle state
- Nesting over Movement
- Nesting over Mobility state
- Nesting over Proximity
- Nesting over Body contact
- Nesting over Relative movement
- Nesting over Side by side
- Nesting over behaviors scored manually
- Nesting over behaviors of Rat/Mouse Behavior Recognition
- Nesting over behaviors of Live Mouse Tracker

**IMPORTANT** You may have defined thresholds during acquisition for behavioral states, like Movement, Activity state or Mobility State in the Analysis Results and Scoring Pane. Those thresholds are not copied to Data Selection. So you have to adjust the thresholds here as well.

# Nesting over Activity state

## Aim

To pick out data the data collected when the subject was in one of the states defined by the dependent variable *Activity state*.

**EXAMPLE** Select all samples when the rat was in inactive (freezing), and based on this selection, make a heatmap to highlight where freezing occurred.

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Activity state**.
2. In the Activity State window:
   - Under **Outlier filter**, enter an **Averaging interval** to smooth the values of acceleration.
   - Under **Number of states**, enter how many different categories you want to split the data in.
   - Under **Thresholds**, enter the thresholds that defines the various activity states.
   - Under **Calculate nesting for**, select the state that the intervals are based on.
   - Under **State duration threshold**, enter the minimal duration of the state chosen in the previous step.

   For details about how Activity state is calculated, see Dependent Variables in Detail > Activity state.
3. Click **OK**. Proceed with step **4** in How to select track segments.

## Notes

- You can only nest over Activity state if you selected **Activity analysis** in the Experiment Settings, and for experiments with one subject per arena.
- If you want to select track segments based on more states (e.g. Active and Highly active), create one Nest box for each state, and combine them with OR logic. This selects the time when the subject was in one of those states (e.g. either Active or Highly active). See Basic rules for combining selection boxes
- For Activity state, it is possible to select different thresholds for nesting (see above) and for analysis (see Activity state in the Analysis profile). You

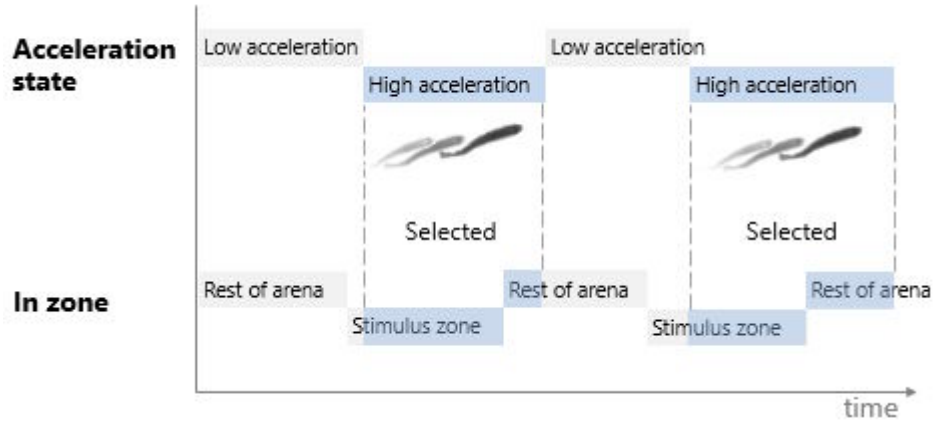
should either use the same thresholds or make the thresholds in your analysis profile more restrictive, so that the variable specified in the Analysis profile is in effect a fine-tuning of your nesting criteria.

# Nesting over Acceleration state

## Aim

Pick out the data collected when one or more subjects were in one of the states High acceleration or Low acceleration.

**EXAMPLE** Select the time intervals when the fish performed swim bursts (for this, use *Acceleration state*). Next, calculate how much time the fish was in the stimulus zone compared with the rest of the arena during those time fragments (for this, use the variable *In zone*).




## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on the Acceleration state of that subject, choose **Acceleration state** under **Nesting**. See the procedure below.
- To select data of a subject based on the Acceleration state of other subjects, choose **Acceleration state** under **Nesting over Subjects**. There you find the **Actors** tab where you can specify which subjects (Actors) were in a certain Acceleration state. See Nesting over subjects

## Procedure

1. In the Data Profile, under **Nesting** click the button next to Acceleration state.
2. In the Acceleration State window:
   - Under **Outlier filter**, enter an **Averaging interval** to smooth the values of acceleration.

- Under **Threshold**, enter the threshold that defines the state *High acceleration* and *Low acceleration*.
- Under **Calculate nesting for**, select the state that the intervals are based on.

For details about how Acceleration state is calculated, see Dependent Variables in Detail > Acceleration state.

3. Click the **Body Points** tab (when available), and specify the body points that Acceleration is based on. Choose whether Acceleration state is based on all the chosen points being in the same state simultaneously (**All selected points**) or at least one being in that state (**Any selected point**).
4. Click **OK**. Proceed with step **4** in How to select track segments.

*See also*

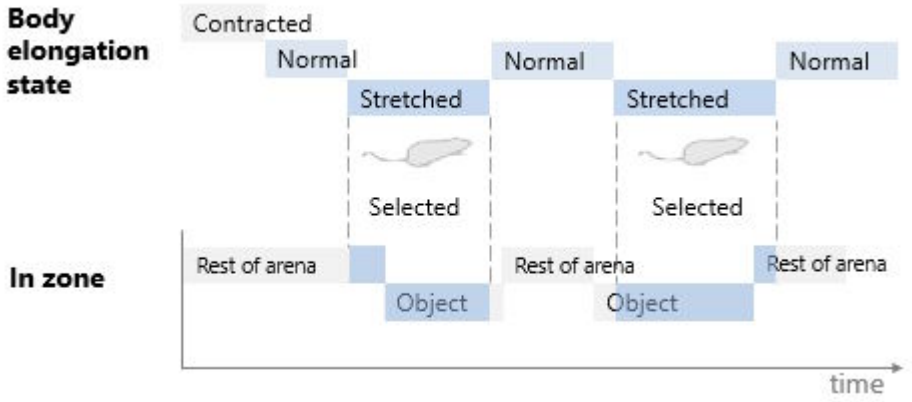
- Nesting over Subjects

# Nesting over Body elongation state

## Aim

To pick out data collected when the subject was in one of the Body elongation states: *Stretched*, *Normal* or *Contracted*.

**EXAMPLE** Analyze the time that the subject spent in the zones Object and Rest of arena when it was in the *Stretched* state. The aim is to test whether the subject spent more time stretched near the object than when in the rest of the arena.




## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on the Body elongation state of that subject, choose **Body elongation state** under **Nesting**. See the procedure below.
- To select data of a subject based on the Body elongation state of other subjects, choose **Body elongation state** under **Nesting over Subjects**. There you find the **Actors** tab where you can specify which subjects (Actors) were in a certain Body elongation state. See Nesting over subjects

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Body elongation state**.
2. In the Body Elongation State window:
   - Under **Outlier filter**, enter an **Averaging interval** to smooth the body elongation values.

- Under **Threshold**, enter the thresholds that defines the states *Stretched*, *Normal* and *Contracted*.
- Under **Calculate nesting for**, select the state that the intervals are based on.

For details about how Body elongation state is calculated, see Dependent Variables in Detail > Body elongation state.

3. Click **OK**. Proceed with step **4** in How to select track segments.

*See also*

- Nesting over Subjects

# Nesting over Body angle state

## Aim

To pick out data collected when the subject was in one of the body angle states: *Straight* or *Bent* (with the *Bent* state which can be further split into *Bent counterclockwise* and *Bent clockwise*).

## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on the Body angle state of that subject, choose **Body angle state** under **Nesting**. See the procedure below.
- To select data of a subject based on the Body angle state of other subjects, choose **Body angle state** under **Nesting over subjects**. There you find the **Actors** tab where you can specify which subjects (Actors) were in a certain Body angle state. See Nesting over subjects

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Body angle state**.
2. In the Body Angle State window:
   - Under **Outlier filter**, enter an **Averaging interval** to smooth the body angle values.
   - Under **Threshold**, enter the angle value that defines the state *bent*.
   - Under **Calculate nesting for**, select the Body angle state that defines the intervals.

   For details about how Body angle state is calculated, see Dependent Variables in Detail > Body angle state.
3. Click **OK**. Proceed with step **4** in How to select track segments.

*See also*

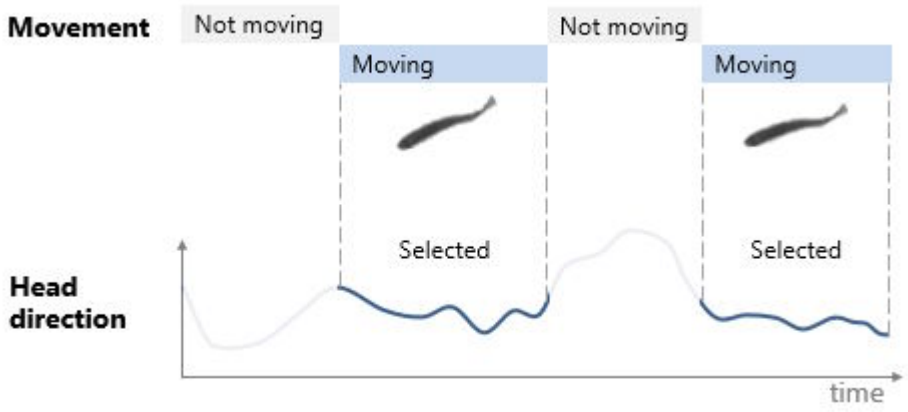
- Nesting over Subjects

# Nesting over Movement

## Aim

To pick out the data collected when the subject was in one of the movement states: *Moving* or *Not moving*.

**EXAMPLE** Calculate the average Head direction (an estimate of body orientation in fish) when the subject was *Moving*.




## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on the Movement state of that subject, choose **Movement** under **Nesting**. See the procedure below.
- To select data of a subject based on the Movement state of other subjects, choose **Movement** under **Nesting over subjects**. There you find the **Actors** tab where you can specify which subjects (Actors) were in a certain Movement state. See Nesting over subjects

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Movement**.
2. In the Movement window:
   - Under **Outlier filter**, enter an **Averaging interval** to smooth the velocity values.
   - Under **Threshold**, enter the **Start velocity** and the **Stop velocity** that the states *Not moving and Moving* are based on.

- Under **Calculate nesting for**, select the Movement state that defines the intervals.

**NOTE** The default values are an example and may not apply to your experiment. The threshold values also vary between species. If the subject is very slow, like a walking tick, you must reduce the two thresholds to detect true movement. See also Dependent Variables in Detail > Movement.

3. Click the **Body Points** tab (when available), and specify the body points that Movement is based on. Choose whether Movement is based on all the chosen points moving simultaneously (**All selected points**) or not (**Any selected point**).
4. Click **OK**. Proceed with step **4** in How to select track segments.

## Notes

- In some cases the number of samples available for smoothing can be less than the averaging interval entered. For example, in tracks with missing samples or at the beginning of the track. In such cases EthoVision XT uses the samples available in the specified interval. See Averaging interval
- When the velocity is between the two thresholds, the current state of the subject does not change relative to the previous sample.
- For *Movement*, it is possible to select different thresholds for nesting (see above) and for analysis (see Movement in the Analysis profile). You should either use the same thresholds or make the thresholds in your analysis profile more restrictive, so that the variable specified in the Analysis profile is in effect a fine-tuning of your nesting criteria.

*See also*

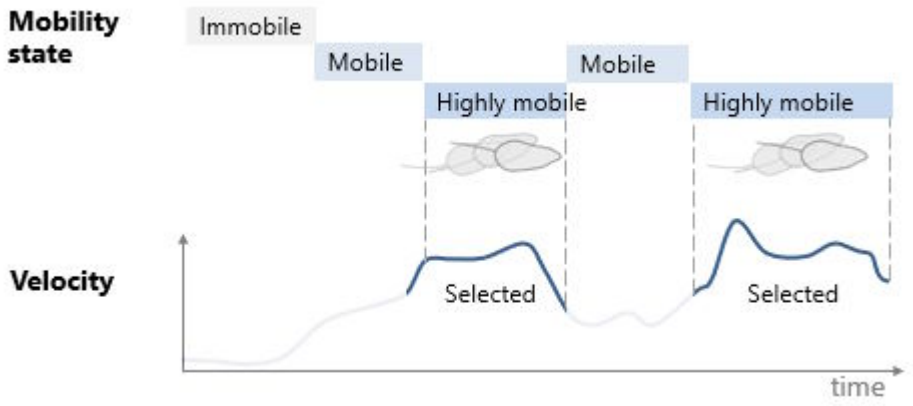
- Nesting over Subjects

# Nesting over Mobility state

## Aim

To pick out data collected when the subject was in one of the Mobility states: *Immobile*, *Mobile* or *Highly Mobile*.

**EXAMPLE** Calculate the average velocity for when the subject was in the state *Highly mobile*.




## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on the Mobility state of that subject, choose **Mobility state** under **Nesting**. See the procedure below.
- To select data of a subject based on the Mobility state of other subjects, choose **Mobility state** under **Nesting over subjects**. There you find the **Actors** tab where you can specify which subjects (Actors) were in a certain Mobility state. See Nesting over subjects

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Mobility state**.
2. In the Mobility State window:
   - Under **Outlier filter**, enter an **Averaging interval** to smooth the Mobility values.
   - Under **Threshold**, enter the thresholds of the mobility variable that define the states *Immobile*, *Mobile* and *Highly mobile*.

- Under **Calculate nesting for**, select the Mobility state that defines the intervals.

For details about how Mobility state is calculated, see Dependent Variables in Detail > Mobility state.

3. Click **OK**. Proceed with step **4** in How to select track segments.

## Notes

- For Mobility state, it is possible to select different thresholds when you define Mobility state in Nesting (see above) and in the Analysis profile (see Calculate statistics: procedure). You should either use the same thresholds or set the thresholds in your analysis profile more restrictive, so that the variable specified in the Analysis profile is in effect a fine-tuning of your nesting criteria.
- In some cases the number of samples available for smoothing can be less than the averaging interval entered. For example, in tracks with missing samples or at the beginning of the track. In such cases EthoVision XT uses the samples available in the specified interval. See Averaging interval

## Notes

- If you select Mobility state under **Nesting over subjects**, and you select two or more subjects in the **Actors** tab of the settings window, you have the following options:
  - To analyze the track segment when at least one of those subjects was in the selected Mobility state, choose **Any selected subject**.
  - To analyze the track segments when all the selected subjects were in the selected Mobility state simultaneously, choose **All selected subjects**.

*See also*

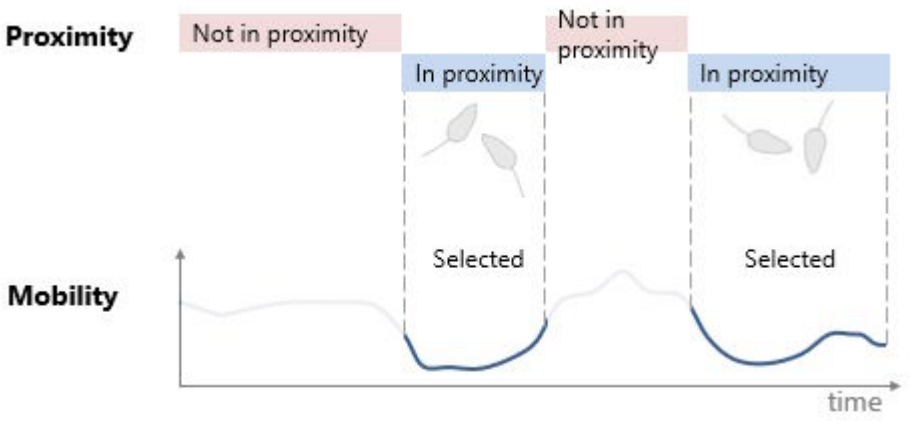
- Nesting over Subjects

# Nesting over Proximity

## Aim

To pick out data collected when one or more subjects (Actors) were within a user-defined distance from one or more other subjects (Receivers).

**EXAMPLE** Calculate the average mobility when the subject was in proximity of other subjects.




## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on the Proximity behavior of that subject, choose **Proximity** under **Nesting**. In the **Receivers** tab you specify which subject was in proximity of the focal subject. See the procedure below.
- To select data of a subject based on the Proximity behavior of other subjects, choose **Proximity** under **Nesting over Subjects**. There you find the **Actors** tab and the **Receivers** tab where you can specify which subjects (Actors) were in proximity of which other subjects (Receivers). See Nesting over subjects

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Proximity**.
2. In the Proximity window, select the Proximity state that defines the intervals to analyze.
   - Enter the **In proximity threshold** and the **Not in proximity threshold**.

- Under **Calculate nesting for**, choose which state defines the intervals.

For details about how proximity is calculated, see Dependent Variables in Detail > Proximity.

3. Click the **Body Points** tab (when available), and specify the body points that define proximity. Choose whether proximity is based on all the chosen points being in proximity simultaneously (**All selected points**) or not (**Any selected point**).
4. Click **OK**. Proceed with step **4** in How to select track segments.

*See also*

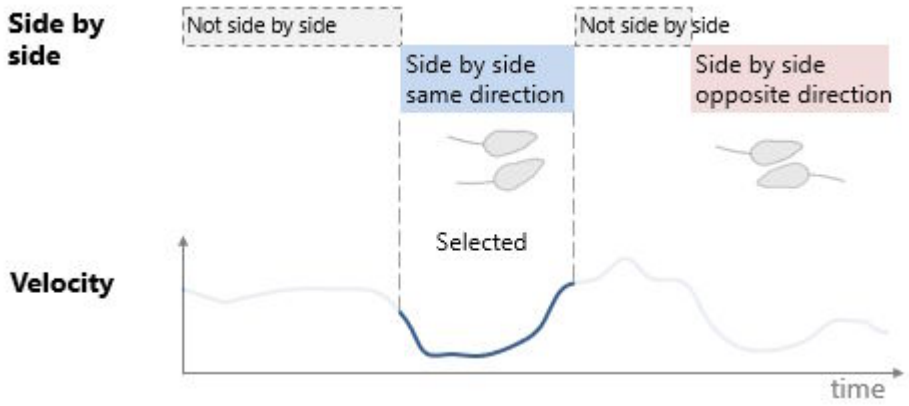
- Nesting over Subjects

# Nesting over Side by side

## Aim

To pick out data occurring when two subjects were side by side within a proximity threshold distance.

**EXAMPLE** Calculate the average velocity of the subject when it was side-by-side with another subject, pointing to the same direction.




## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on the Side-by-side behavior of that subject, choose **Side by side** under **Nesting**. In the **Receivers** tab you specify which subject was side-by side with the focal subject. See the procedure below.
- To select data of a subject based on the Side-by-side behavior of other subjects, choose **Side by side** under **Nesting over Subjects**. There you find the **Actors** tab and the **Receivers** tab where you can specify which subject (Actor) was side by side with which other subjects (Receivers). See Nesting over subjects

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Side by side**.
2. In the Side by side window, either enter the proximity threshold distance or accept the default value (see below for details).

3. Under **Calculate nesting for**, choose which state of the Side by side variable defines your selection: either **Side by side, same direction** or **Side by side, opposite direction**.

   For details about the *Side by side* variable, see Dependent Variables in Detail > Social > Side by side.

4. Click the **Receivers** tab and select which subjects should be in proximity with the focal subject
5. Click **OK**. Proceed with step **4** in How to select track segments.

## Note

- The default proximity threshold distance is 5.26 cm. This corresponds to the threshold used in Live Mouse Tracker, that is, 2 x 15 = 30 pixels, where 15 is the original value of MAX_DISTANCE_HEAD_HEAD_GENITAL_THRESHOLD in Live Mouse Tracker, and the scale factor for converting pixels to cm is 10/57.
- If you select multiple subjects A, B, etc. in the **Receivers** tab, and select the option **All selected subjects**, then the track segments are selected when the focal subject was side by side with A, B, etc. simultaneously. That may not occur often. Instead, select **Any selected subject** if you want to analyze the track segments when the focal subject was side by side either with A, or B, etc.

*See also*

- Nesting over Proximity
- Nesting over subjects

# Nesting over Body contact

## Aim

To pick out the track segments corresponding to when one the subject was in contact or not in contact with another subject.

## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on that subject being in contact with any other subject, choose **Body contact** under **Nesting**. In the **Receivers** tab specify which subject was in contact with the focal subject. See the procedure below.
- To select data of a subject based on when other subjects were in contact with any subject, choose **Body contact** under **Nesting over Subjects**. There you find the **Actors** tab where you can specify which subjects were in contact with any other subject. See the notes below and also Nesting over subjects.

## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Body contact**.
2. In the Body contact window, select the state that defines the intervals to analyze.

   For details on how Body contact is calculated, see Dependent Variables in Detail > Body contact.
3. Click **OK**. Proceed with step **4** in How to select track segments.

## Notes

- **TIP** You can nest over "No contact" to filter out all the instances of body contact. For example, if contact situations result in subject identity swaps or nose-tail swaps.
- If you select Body contact under **Nesting over subjects**, the **Actors** tab is also available. The track segments are selected based on the Body contact of the Actors. For example, if the arena contains three subjects and in the **Actors** tab you select Subject 1 and Subject 3, the data are selected when both Subject 1 and 3 are in contact with any subject, or when at least one of them is in contact with any other subject. For details and examples, see Nesting over subjects. With **Nesting over subjects**, you cannot specify

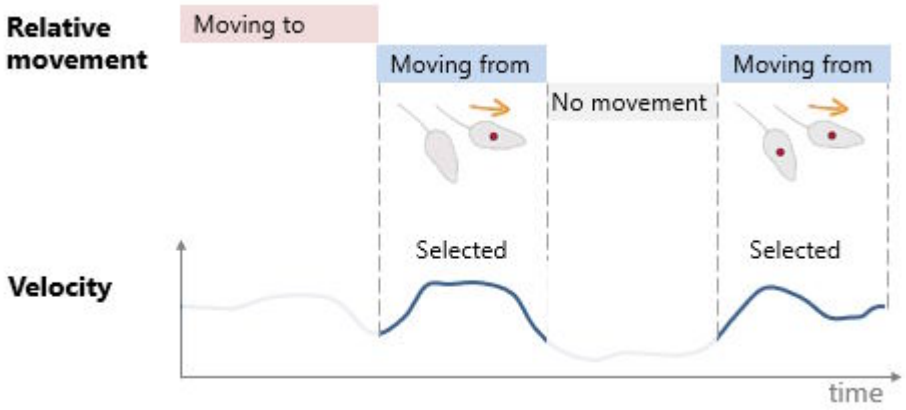
body contact between two specific subjects. If you select Subject 1 and Subject 3 in the **Actors** tab, you will not get the track segments when those two subjects are in contact. Instead, you get the track segments when, depending on the options you choose, both subjects (or at least one) are in contact with *any subject*.

# Nesting over Relative movement

## Aim

To pick out data collected when one or more subjects (Actors) were moving to or away from one or more other subjects (Receivers).

**EXAMPLE** Analyze the velocity when the subject was moving away from another subject.




## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on the Relative movement of that subject, choose **Relative movement** under **Nesting**. In the **Receivers** tab you specify which subject the focal subject was moving to/from. See the procedure below.
- To select data of a subject based on the Side-by-side behavior of other subjects, choose **Side by side** under **Nesting over Subjects**. There you find the **Actors** tab and the **Receivers** tab where you can specify which subject (Actor) was moving to/from which other subjects (Receivers). See Nesting over subjects

## Procedure

1. In the Data Profile, under **Nesting** click the button next to Relative movement.
2. In the Relative Movement window:
   - Enter the **Maximum interaction distance**.

- Enter the **Minimum velocity** that defines movement.
- Under **Calculate nesting for**, choose the state that defines the intervals.

For details about how Relative movement is calculated, see Dependent Variables in Detail > Relative movement.

3. Click the **Body Points** tab (when available), and specify the body points that define movement. Choose whether movement is based on all body points moving simultaneously (**All selected points**) or not (**Any selected point**).
4. Click **OK**. Proceed with step **4** in How to select track segments.

## Notes

On the **Receivers** tab:

- To select the samples when one subject moves to the other, select both subject 1 and subject 2, and choose **Any selected subject** from the list.
- To select the samples when one specific (focal) subject moves toward other subjects simultaneously, do not select the focal subject under Receivers. Select all the other subjects and choose **All selected subjects** from the list.

*See also*

- Nesting over Subjects
- Dependent Variables in Detail > Relative movement

# Nesting over behaviors scored manually

## Aim

To can pick out the track segments in which a behavior was scored manually. To learn more about manually scoring behaviors, see Manual scoring settings and Score behaviors manually during acquisition.

**EXAMPLE** You manually scored freezing behavior, and you want to visualize where in the arena it occurred during the trial. First, nest over the manually-scored behavior *Freezing* (see below for the procedure), then create the heatmaps.

## Nesting vs. Nesting over Subjects

Notice the difference when there are multiple subjects in the same arena:

- To select data of a subject based on the behavior of that subject, choose that behavior under **Nesting**. See the procedure below.
- To select data of a subject based on the behavior of other subjects, choose that behavior under **Nesting over Subjects**. There you find the **Actors** tab where you can specify which subject (Actor) was performing that behavior. See Nesting over subjects

## Procedure

1. In the Data Profile, under **Nesting** click the button next to the behavior's name, or the group of behaviors it belongs to.
2. In the window that appears, select the behavior and click **OK**.

   If you want to analyze the track segments in which the behavior was not active, select **Not [behavior name]**.
3. Click **OK**. Proceed with step **4** in How to select track segments.

## Notes

- If the behavior is part of a mutually-exclusive group, under Nesting you see the name of the group, not the individual behavior.
- If you select a behavior from a mutually-exclusive group, the other behaviors of the same group are automatically left out of analysis and visualization.

# Nesting over behaviors of Rat/Mouse Behavior Recognition

## Aim

To pick out the samples collected when the subject was in one or more of the behavioral states detected using the Rat or Mouse Behavior Recognition function:

| | |
|---|---|
| Digging (only for mice) | Rearing unsupported |
| Drinking | Rearing supported |
| Eating | Resting |
| Grooming | Sniffing |
| Jumping (only for rats) | Walking |
| Merged behavior | |

**EXAMPLE** You want to visualize where in the arena *Grooming* occurred. First, nest over *Grooming* (see the procedure below), then create heatmaps.

## Procedure

1. In the Data Profiles screen, under **Nesting**, click the button corresponding to the behavior you want to use to select the track segments.
2. In the window that appears, select the properties and which state you want to consider for nesting. Then, click **OK**. See also Behavior Recognition in the Analysis profile.

   If you want to analyze the track segments in which the behavior was not active, select **Not [behavior name]**.
3. Click **OK**. Proceed with step **4** on How to select track segments.

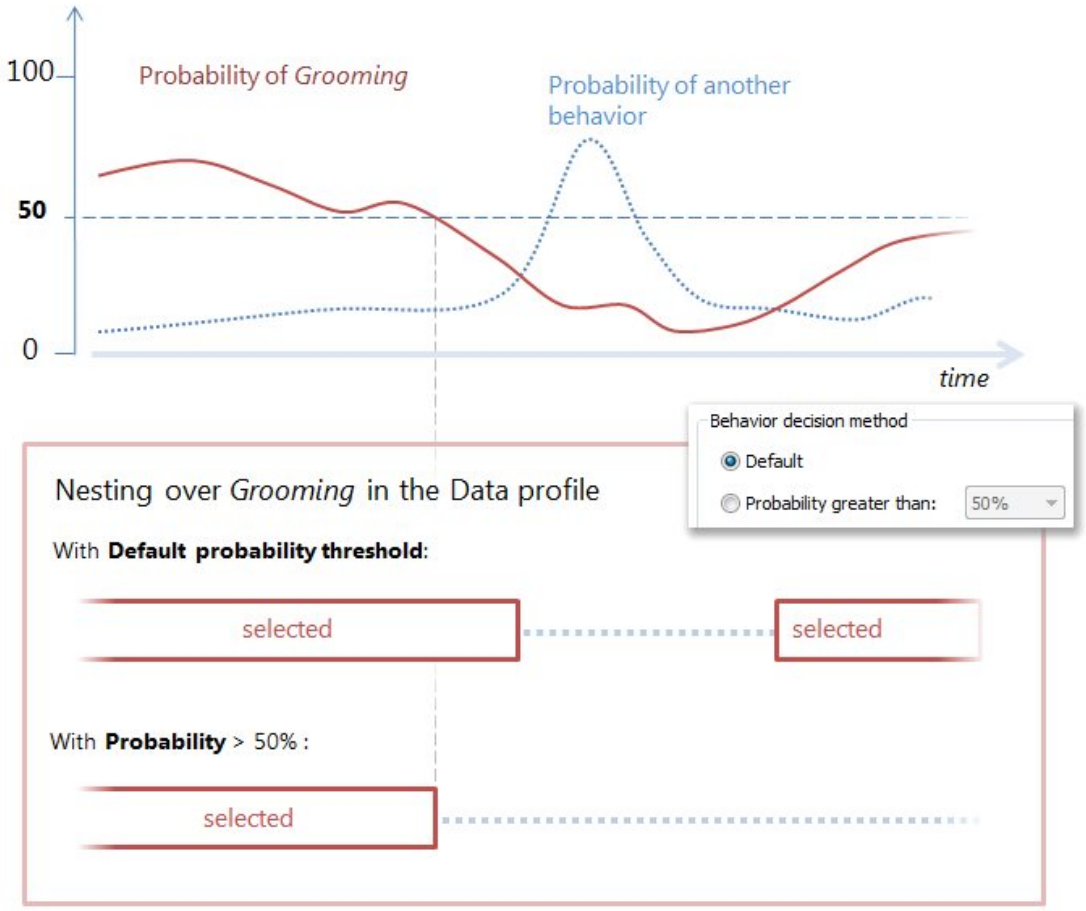
## Options

### *Behavior decision method*

The Behavior decision method acts as a filter that you can use to ignore samples where the behavior's probability is below a certain value. Default means that EthoVision uses the original scores (thus no filtering based on probabilities).

The figure below shows the effect of Behavior decision method on the track segment selected for analysis. Top: The probability of Grooming, and another

behavior plotted against time (for simplicity, other behaviors are ignored here). Bottom: When Nesting over Grooming. With **Default** selected, analysis is done on the samples for which the probability of Grooming exceeds the probability of any other behavior (default behavior recognition). With a set **Probability** > 50%, analysis is done on the samples for which the probability is higher than 50%. The higher the value, the more conservative the selection.




- If you want to select the samples when a behavior probability was lower than a certain value, choose that value, then under Calculate nesting for, select Not **[behavior name]**.
- To choose a specific probability threshold, it is best to view the per-sample values of probability for that behavior. In the Analysis profile, under Rat/ Mouse Behavior Recognition select Behavior probability, and select the behavior(s) you are interested in. Choose **Plot Integrated Data** to see the probability values plotted against time.

## Notes (all behaviors)

- This nesting option is only available if you have the Rat or Mouse Behavior Recognition add-on module.
- Since *Hopping* (mouse behavior) and *Twitching* are events with no duration, it is not possible to nest over those behaviors.
- If you want to pick out samples based on two or more behaviors and analyze them together as if they were one (for example, *Rearing unsupported* and *Rearing supported*), click next to **Merged Behavior** instead. See Notes about Merged behavior
- If you want to calculate duration of a behavior as a percentage of a track, but excluding the time scored as *Unknown*, make two nesting criteria, one with **[behavior name]** and the other with **Not [behavior name]** selected. Combine the resulting boxes with OR logic and then in the Analysis profile define the behavior you are interested in, and under **Trial statistics** choose the statistic **Cumulative Duration within Nesting (%)**.
- The Behavior duration threshold acts as a filter to ignore short transitions between states. See also Behaviors detected with Behavior recognition in the Analysis profile.

## Notes about Merged behavior

- *Nesting over Merged behavior*. When you select multiple behaviors under Merged behavior, the track segments are selected in which the subject was in one of the selected states, and then combined with OR logic.
- *Nesting over "Not" Merged behavior*. The Merged Behavior window does not allow you to select **Not [behavior name]**. If you want to select the samples when two or more behaviors were not active, then make a Nesting criterion for each behavior, selecting **Not [behavior name]**, then place the resulting Nest boxes in a linear sequence (see Basic rules for combining selection boxes).

  Note that the track segments are not selected when the behavior is *Unknown*, or when no behavior is scored in that segment (this is represented as a gap between behaviors).

# Nesting over behaviors of Live Mouse Tracker

Currently it is possible to select track segments based on the behavior *Look* of Live Mouse Tracker data. Data are selected when they occur at the same time as the chosen state.

## Procedure

1. In the Data profile, under **Nesting**, or **Nesting over Subjects**, choose **Look**.
2. Choose the state of this variable, either **Look_up** or **Look_down**.
   - To select data of a subject based on the *Look* behavior of that subject, choose **Look** under **Nesting**.
   - To select data of a subject based on the *Look* behavior of other subjects, choose **Look** under **Nesting over Subjects**. There you find the **Actors** tab where you can specify which subject (Actor) performed that behavior. See Nesting over subjects
3. Click **OK**. Proceed with step **4** in How to select track segments.

### *See also*

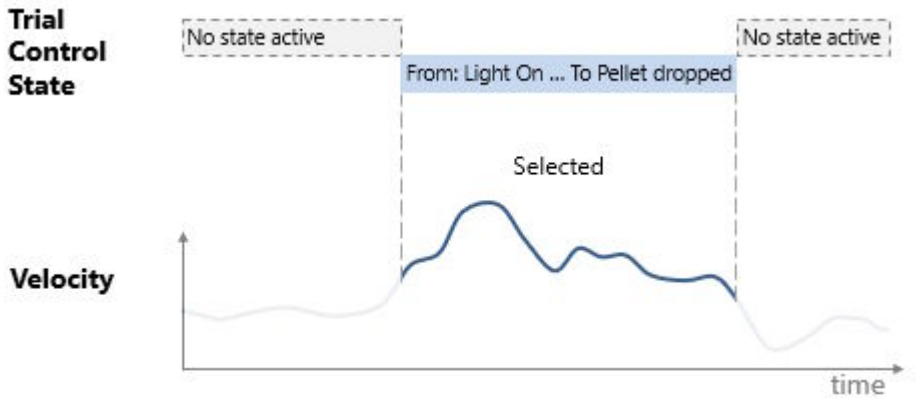
For an overview of Live Mouse Tracker analysis variables, see Live Mouse Tracker.

# Nesting over a Trial Control state

## Aim

To pick out the track segments corresponding to a Trial Control state. A Trial Control state is an interval defined by two events occurred during the trial (a condition met, a sub-rule started, an action taken etc.). To learn more about Trial Control states, see Trial Control state in the Analysis profile.

**EXAMPLE** Calculate the average velocity of the subject between the time that the light went on to the time that the pellet was given.




## To nest over a Trial Control state

1. In the Data Profile, under **Nesting** click the button next to Trial Control state.
2. In the Trial Control State window, select the Trial Control elements that define the start and the stop of the interval to analyze.

   For details about how you can define a Trial Control state, see Dependent Variables in Detail > Trial Control state.
3. Click **OK**. Proceed with step **4** in How to select track segments.

## Notes

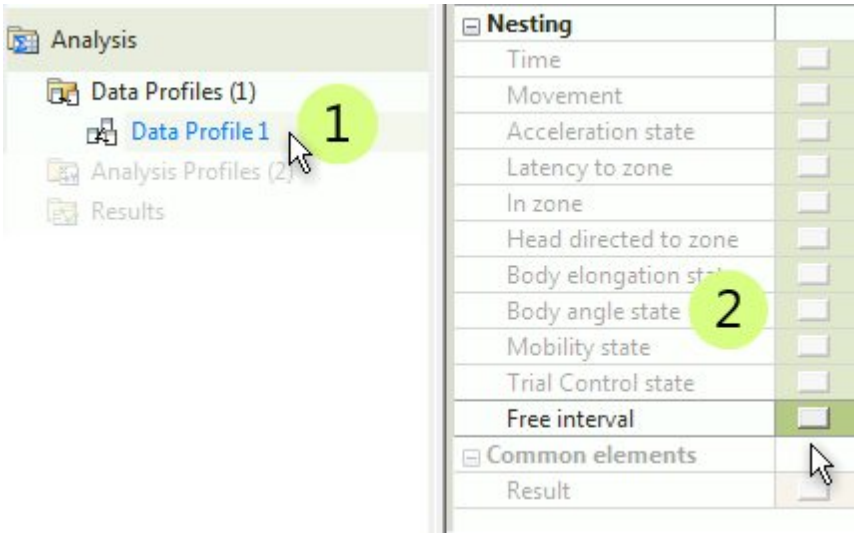
- If the Trial Control state occurs more times in your trial, like those defined with events in a subrule, there will be multiple selection intervals in one trial. You have the following options:
  - Select **Calculate result for interval** if you want to restrict your selection to just one interval, for example the first one, or the third one.

Next to **For consecutive intervals**, enter the number of the interval (**1** for the first).

- Leave **Calculate result for interval** not selected if you want to select all the resulting intervals. Analysis is based on all those intervals merged in one.

- The **Actors** tab in the Trial Control state window is of no use, because Trial Control states occur at the trial and arena level, independent of the subjects. You cannot assign a Trial Control state to a specific subject.

# Nesting over a Free interval




With Nesting over a Free interval you can define the beginning and the end of a track segment, and analyze the dependent variables within that segment.

For example, define an interval that goes from the start of the track to when the subject has explored an object for a total of 30 seconds. Then, analyze the zones that the subject visited, or calculate the average speed in that interval.

- Nest over a Free interval
- Examples of Nesting over a Free interval

*See also:*

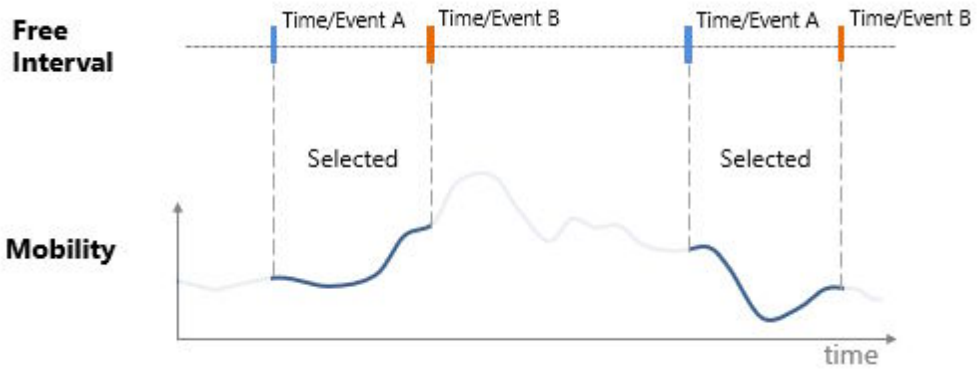
- Free interval in the Analysis profile

# Nest over a Free interval

## Aim

To pick out the track segments corresponding to a Free interval, i.e., the interval included between event (or time) A and event (or time) B. See Examples of Nesting over a Free interval

**EXAMPLE** Analyze the mobility of the subject in the five seconds immediately following administration of a stimulus. In this example, event A is the onset of the stimulus. Event B occurs 5 seconds after A.




## Nesting vs. Nesting over Subjects

If your trials contain multiple subjects in the same arena and you want to analyze data of one subject based on the behavior of another subject, click **Free Interval** under **Nesting over Subjects**. See Free interval based on multiple subjects.

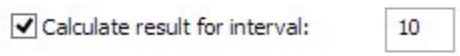
## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Free Interval**.
2. In the Free Interval window, specify the criteria that define the start and stop of the interval.

   For details about how you can define a free interval, see Dependent Variables in Detail > Free interval.
3. Click **OK**. Insert the resulting **Nest** box in the appropriate position between the **Start** and the **Result** box. See How to select track segments

## Notes

- **IMPORTANT** If a start criterion is not met in a track, EthoVision XT does not select any interval for that track.
- The following dependent variables are not available for the definition of start/stop criteria: *Activity state*, *Acceleration state*, and the behaviors of Rat and Mouse Behavior Recognition.
- Note the difference:
  - You define a Free Interval in the **Data Profile** when you want to analyze any dependent variable *within the resulting track segment*. For example, to calculate the average velocity of the subject in a certain track segment.
  - You define a Free Interval in the **Analysis Profile** when you want to analyze the interval itself. For example you can calculate the duration of an interval. However, you won't get, for example, the average velocity in the resulting track segment. See Free interval in the Analysis profile
- *Multiple occurrences of the interval*. If the interval defined in the Data profile results in multiple occurrences within a track, you can nest over a specific interval occurrence. Select **Calculate results for interval** and enter the number of the occurrence. For example, 10 to nest over the 10th occurrence of the interval. Other occurrences are not used in the analysis.

  If you do not select **Calculate results for interval**, analysis is done in all occurrences merged into one result.
- *Nest over a Free interval based on external data*. If you imported external (physiological) data, you can analyze the track segments that correspond to a free interval based on external data. For example, Nest over a Free interval that goes from the track start to when Heart rate reaches 400 bpm.

  **TIP** If you want to select the track segments between two values of external data, for example from Heart rate = 300 to Heart rate = 400, then choose Nest over external data (state) instead.

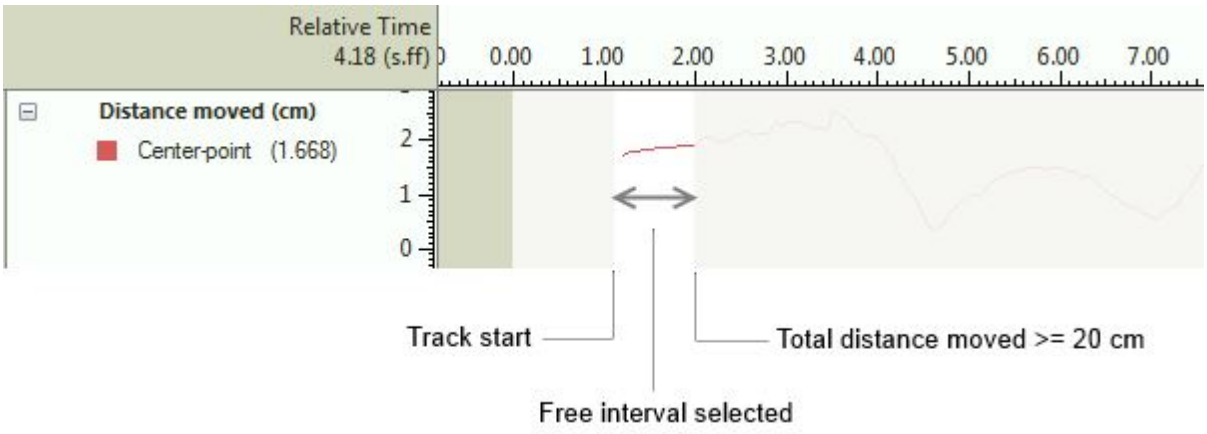
# Examples of Nesting over a Free interval

## Example 1: Analyze the first 20 cm of swim path in a water maze

*Aim*

Suppose you want to analyze the movement of the subject in the initial swim path, say in the first 20 cm. A Data profile with Free interval "From track start to Total distance moved 20 cm" is defined.

The double arrow indicates the duration of the resulting interval. In the Analysis profile you can then for example define the heading angle of the subject, which will be calculated for that interval.




*Solution*

In the Data profile, under **Nesting** choose **Free interval**, with:

- Start criterion: Track start.
- Stop criterion: Dependent variable *Distance moved*, Statistic *Total*, is >= 20 cm.

Note that the Total Distance moved is unlikely to be exactly 20 cm at a given sample time; for this reason, the end criterion says ">= 20 cm".

Next, in the Analysis profile choose the variable you want to calculate, for example Heading to point, or Turn angle. Then choose **Analysis** > **Results** > **Statistics and Charts**.

*See also*

- Analysis advisor: Analysis in relation to the path

## Example 2: Analyze the time up to when the subject has explored an object for 30 seconds

*Aim*

In a novel object experiment, the trial lasts for example 5 minutes. Subjects often differ in the amount of time spent exploring the objects. The researcher wants to analyze the same exploration time across subjects, for the object labeled as zone "Familiar object".

*Solution*

In the Data profile, under **Nesting** choose **Free interval**, with:

- Start criterion: Track start.
- Stop criterion: Dependent variable *In zone*, Statistic *Cumulative duration*, is >=30 s. *In zone* is a dependent variable defined for the zone "Familiar object" and the body point "nose-point".

Next, in the Analysis profile choose the variable you want to calculate, for example *In zone*, to calculate the number of exploration events in the 30 s of exploration time. Then choose **Analysis** > **Results** > **Statistics and Charts**.

*See also*

- Zone visits (entries and time spent)
- Other analyses with zones

## Example 3: Compare two intervals, 5-s before vs 5-s after a stimulus

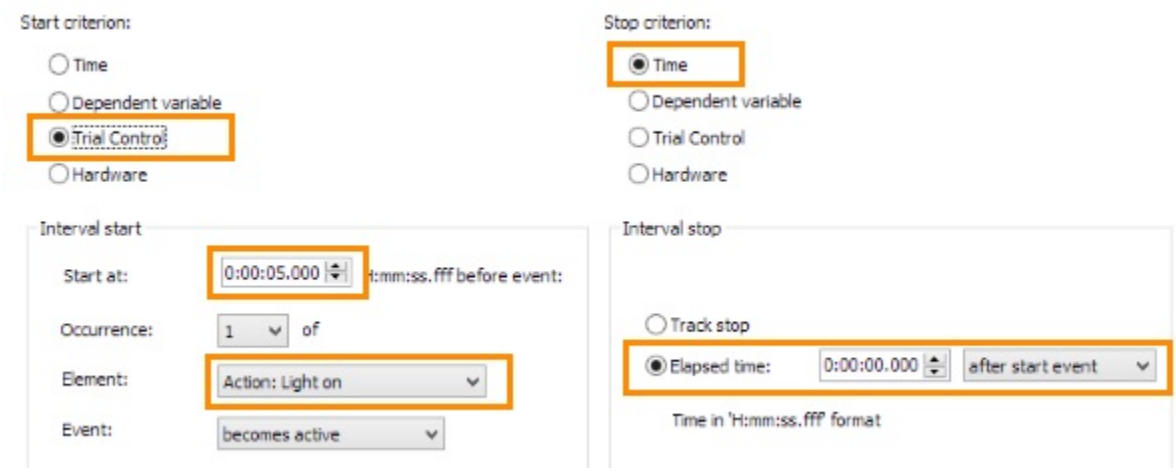
*Aim*

In a DanioVision experiment, a white light stimulus is presented to zebrafish larvae. The researcher wants to compare the movement parameters of the animals in the 5 s before the onset of the stimulus, with those in the 5 s thereafter.

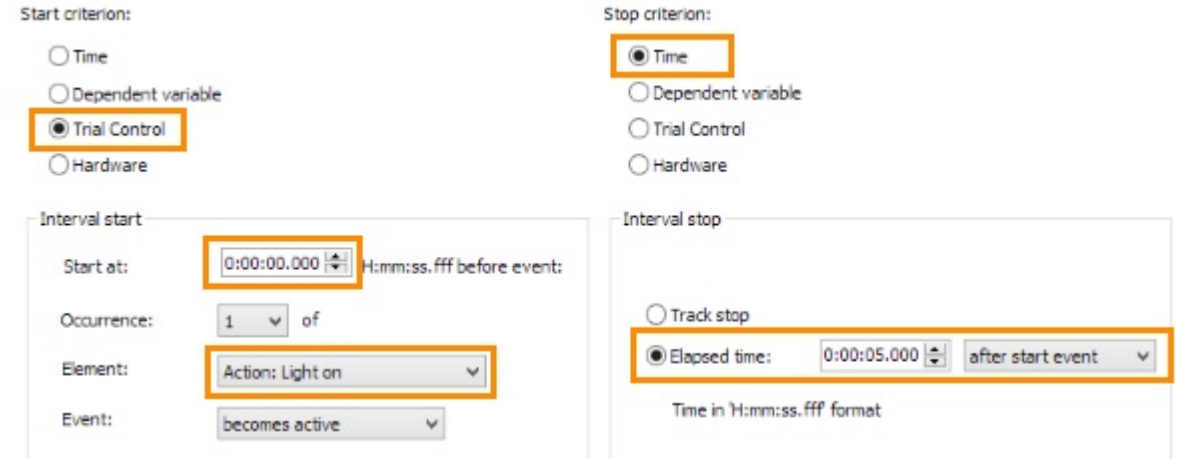
*Solution*

In the Data profile, create two Free intervals based on Trial Control.

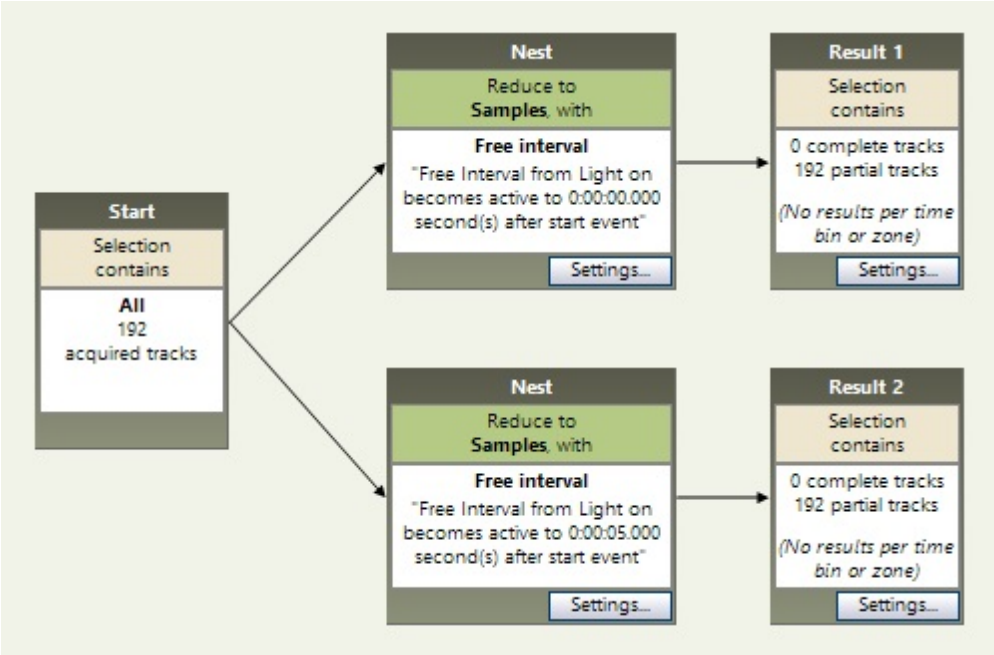
The first free interval goes from 5 seconds before “Action: Light on” to 0 s after start event (which is “Action: Light on”).




The second free interval goes from 0 s before “Action: Light on” to 5 s after start event (which is “Action: Light on”).




Connect the two **Nest** boxes to different **Result** boxes. To create an additional **Result** box, under **Common elements** click the button next to **Result**. See Multiple selections in a data profile




Next, in the Analysis profile choose the variable you want to calculate, for example *Movement*, to quantify movement in the selected periods of time. Then choose **Analysis** > **Results** > **Statistics and Charts**.

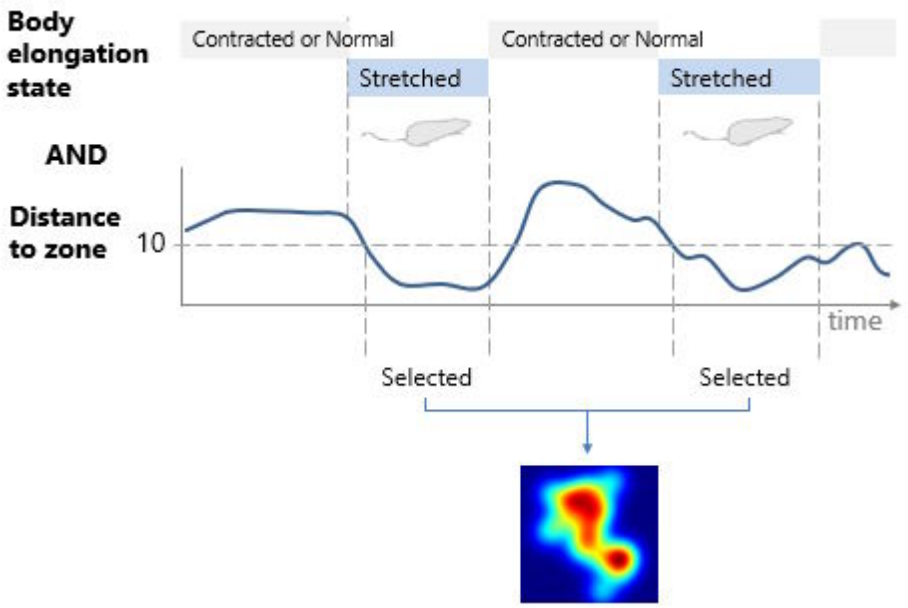
# Nesting over a Multi condition

## Aim

To pick out the track segments corresponding to a Multi condition. To get an idea of what a Multi condition is, see Multi condition in the Analysis profile.

You can also define complex selections by combining two or more Nesting boxes. See Multiple nesting criteria. However, there are limitations. For example, you cannot select the points when “Distance to zone was less than 10 cm” using a Nest box. However, you can do that by creating a Multi condition.

**EXAMPLE** Select all the points in the track when (a) the subject’s Body elongation state was *Stretched* and (b) its *Distance from the zone* Object was less than 10 cm. Then, create a heatmap.




## Procedure

1. In the Data Profile, under **Nesting** click the button next to **Multi condition**.
2. In the Multi Condition window, specify the single conditions that define the Multi condition.
3. Combine the conditions with either **All conditions are true** (AND logic) or **Any condition is true** (OR logic).

   For details, see Dependent Variables in Detail > Multi condition.

4. Click **OK**. Insert the resulting **Nest** box in the appropriate position between the **Start** and the **Result** box. See How to select track segments

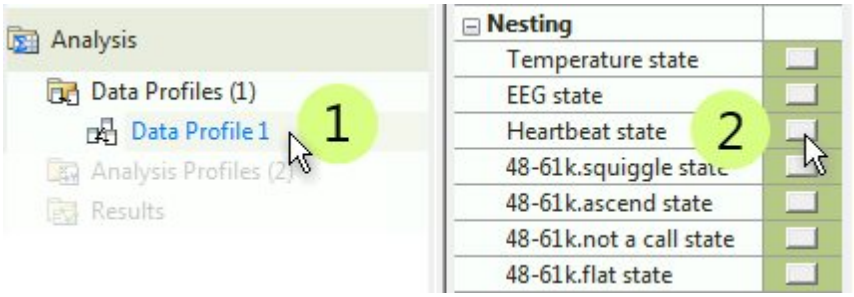
## Nesting vs. Nesting over Subjects

If your trials contain multiple subjects and you want to analyze one subject in Multi condition based on the behavior of another subject, click **Multi condition** under **Nesting over Subjects**. See Nesting vs. Nesting over subjects

## Notes

- Note the difference:
  - You define a Multi condition in the **Data Profile** when you want to analyze any dependent variable *within the resulting track segment*. For example, to calculate the average velocity of the subject when a Multi condition is true.
  - You define a Multi condition in the **Analysis Profile** when you want to analyze the interval itself. For example when you want to know the total duration of the Multi condition being true. However, you won't get, for example, the average velocity in the resulting track segment. See Free interval in the Analysis profile

# Nesting over External data




## Aim

To pick out the track segments and the associated external data corresponding to a specific range of values in the external data signal.

**EXAMPLE** Select all intervals when *heart rate* was above a certain value. Then analyze distance moved, velocity, and movement.

## Procedure

1. In the Data profile, under **Nesting**, click the **Add** button next to **[data set name] state**.
2. Define the state variable. For details, see External data - state in the Analysis profile.
3. Click **OK**. Proceed with step **4** on How to select track segments.

## Notes

- The track segments are based on the values of external data calculated on the re-sampled signal, not the original (imported) signal.
- If you have chosen the variable under **Nesting over Subjects**, the **Actors** tab is also available. Select the subjects whose state define the nesting intervals. If you select two or more subjects, select:
  - **Any selected subject**. To analyze the time that at least one subject was in that state.
  - **All selected subjects**. To analyze the time that all subjects were in that state simultaneously.

# Nesting over Subjects

## Learn about

- Nesting vs. Nesting over subjects
- Nesting over subjects

*See also*

- How to select track segments

# Nesting vs. Nesting over subjects

## Aim

To select track segments based on the behavior of one or more subjects. In experiments with multiple animals per arena, the Data profile offers two types of nesting options,

## Prerequisite

- You have the Social Interaction Module.

## Nesting

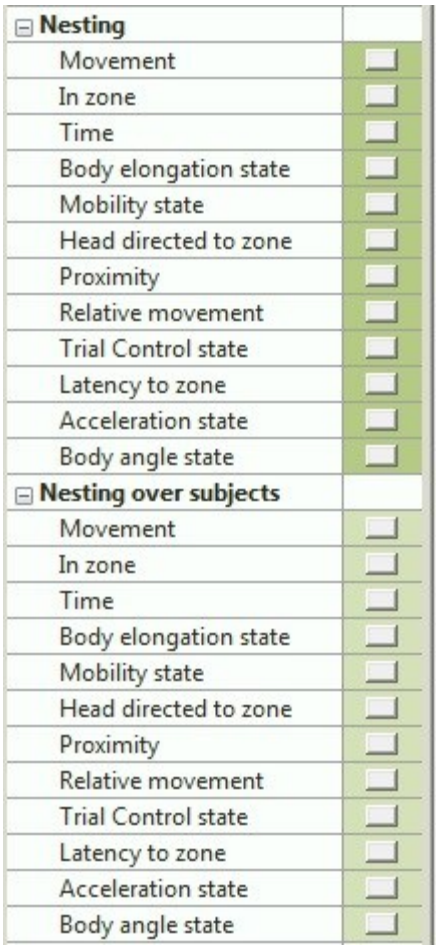
To analyze the track segments of a subject based on that subject's behavior, choose an option under **Nesting**, and follow the procedure on How to select track segments on page 782.

Result: Each subject will be analyzed in different track segments (intervals), according to its own behavior (see B in the figure below).

## Nesting over Subjects

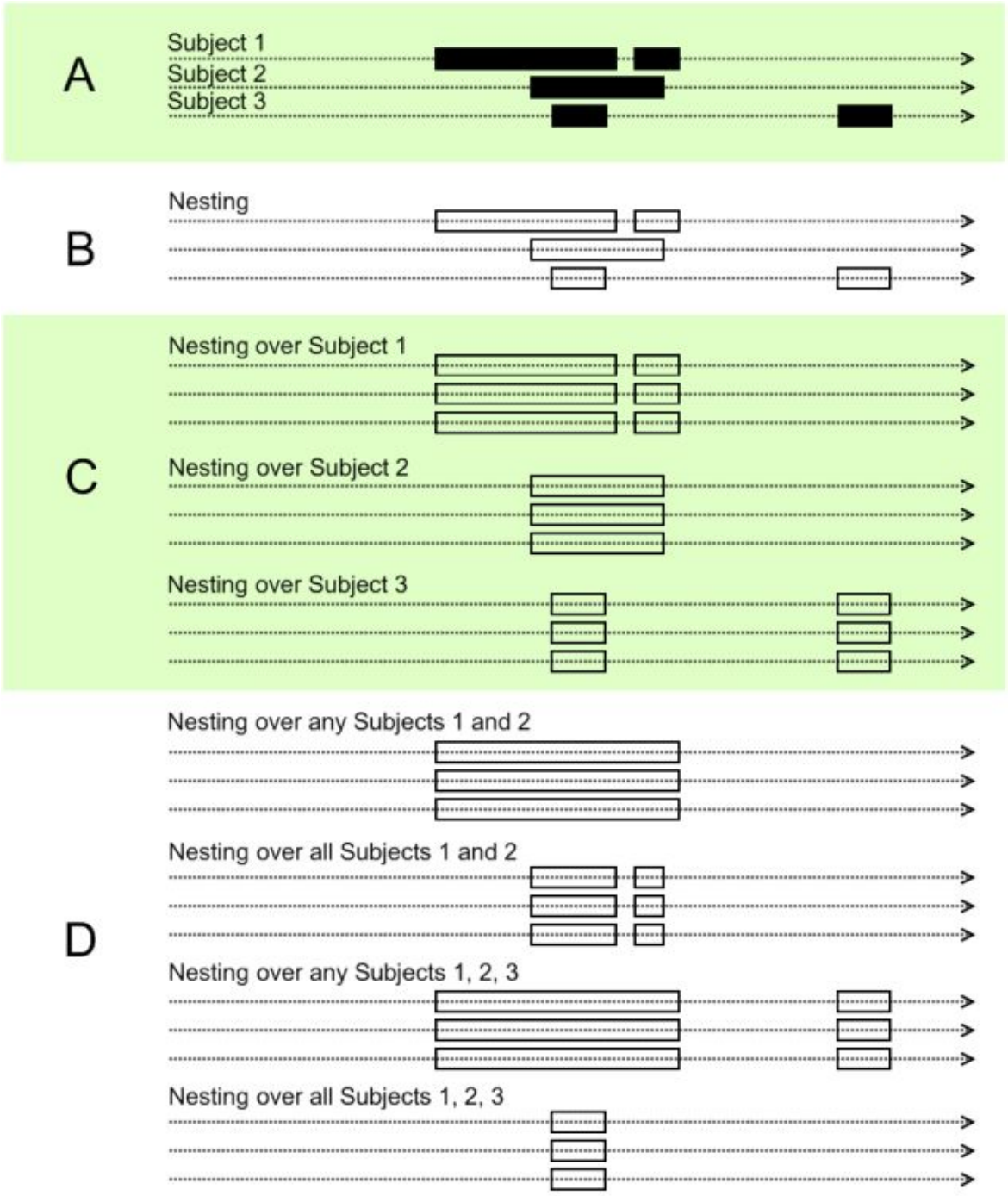
To analyze the track segments of a subject based on the behavior of a specific subject (or combination of subjects, called **Actors**), choose an option under **Nesting over Subjects**, and select the **Actors**. See Nesting over subjects on page 837.

Result: Within the same arena, subjects will be analyzed in equal track segments, according to the behavior of the Actors (see C and D in the figure below).




## Notes

- Arenas are independent replicates. Therefore, nesting intervals are applied to each arena separately depending on the state of their subjects.
- Selecting **Time** under **Nesting** or **Nesting over Subjects** makes no difference, because within one arena the tracks of multiple subjects start at the same time.




Effect of Nesting in one arena with three animals. Dotted lines: tracks. Black bars: time intervals when a behavioral state or Trial Control state is active (for example: In zone for the variable In zone, or Highly mobile for Mobility). White bars: data selected for analysis. **A**: Original data. **B**: Results of Nesting. Track segments are selected according to the state of the corresponding subject. **C**: Results of Nesting over Subjects, with one subject selected in the **Actors** tab. **D**: Results of Nesting over Subjects, with two or more subjects selected in the **Actors** tab. For reasons of

space, the combinations “Any/All of Subjects 1 and 3” and “Any/All of Subjects 2 and 3" are not displayed.

# Nesting over subjects

## Aim

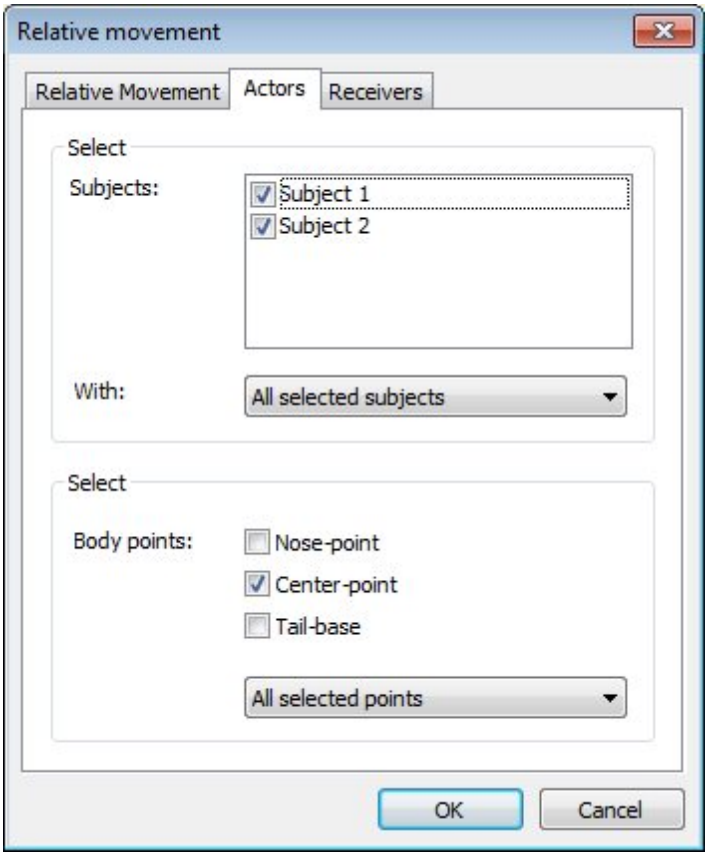
To analyze the track segments of a subject based on the behavior of a specific subject or combination of subjects, called Actors.

This option is only available if you track two or more subjects simultaneously in the same arena.

## Prerequisites

## Procedure

1. In the Data profile, choose the variable under **Nesting over Subjects**.
2. Click the **Actors** tab of the nesting window. See also How to select track segments




3. Select the actors of the behavior defining the interval. Select the subject that should show the behavior of interest. If you select two or more subjects

in the **Actors** tab, select one of the two options from the list immediately below the **Subjects** box:

- **All selected subjects**: To analyze the samples when all the actors show that behavior simultaneously.
- **Any selected subject**: To analyze the samples when at least one actor shows that behavior.

4. If the **Actors** tab also contains the **Body points** options, select one or more body points of the subjects selected above.
5. For the dependent variables of social interaction, a **Receivers** tab is also available. Receivers are the subjects towards which the behavior of the Actor is directed. For example:
   - To analyze when Subject 2 is moving to Subject 1, Subject 2 is the **Actor** and Subject 2 is the **Receiver**.
   - To analyze when Subject 1 is in proximity of Subject 2, Subject 1 is the **Actor** and Subject 2 is the **Receiver**.

   Click the **Receivers** tab and select the subjects that you want to define Receivers (see an example below).
6. Click **OK**. Proceed with step **4** in How to select track segments.

**IMPORTANT** Be careful when selecting the same subject under Actors and Receivers. In such case you may also get unwanted selections, for example Subject 1's nose point (Actor) In proximity of Subject 1's tail base (Receiver). The resulting selection has little meaning.

#### EXAMPLES

- *Social interactions*. You want to analyze the time that the nose-point of Subject 1 was in proximity of the tail-base point of Subject 2 (ano-genital sniffing).

  Solution: Subject 1 is Actor, Subject 2 is Receiver.

  - Under **Nesting over Subjects**, click the button next to **Proximity**.
  - In the **Actors** tab select Subject 1 and deselect Subject 2. Under **Body points**, select **Nose-point** only.
  - In the **Receivers** tab select Subject 2 and deselect Subject 1. Under **Body points**, select **Tail-base** point only.

  If you want to select the time that the nose point of Subject 1 was in proximity of the tail-base point of Subject 2, or vice versa, create two nesting boxes, one that specifies Subject 1's nose in proximity of Subject 2's

tail base, and the other that specifies Subject 2's nose in proximity of Subject 1's tail base. Next, combine the two boxes with OR logic (see Basic rules for combining selection boxes).

- *One Actor, no Receivers*. You want to select the track segments when Subject 2 was moving, and quantify the distance moved by other subjects in that period of time.

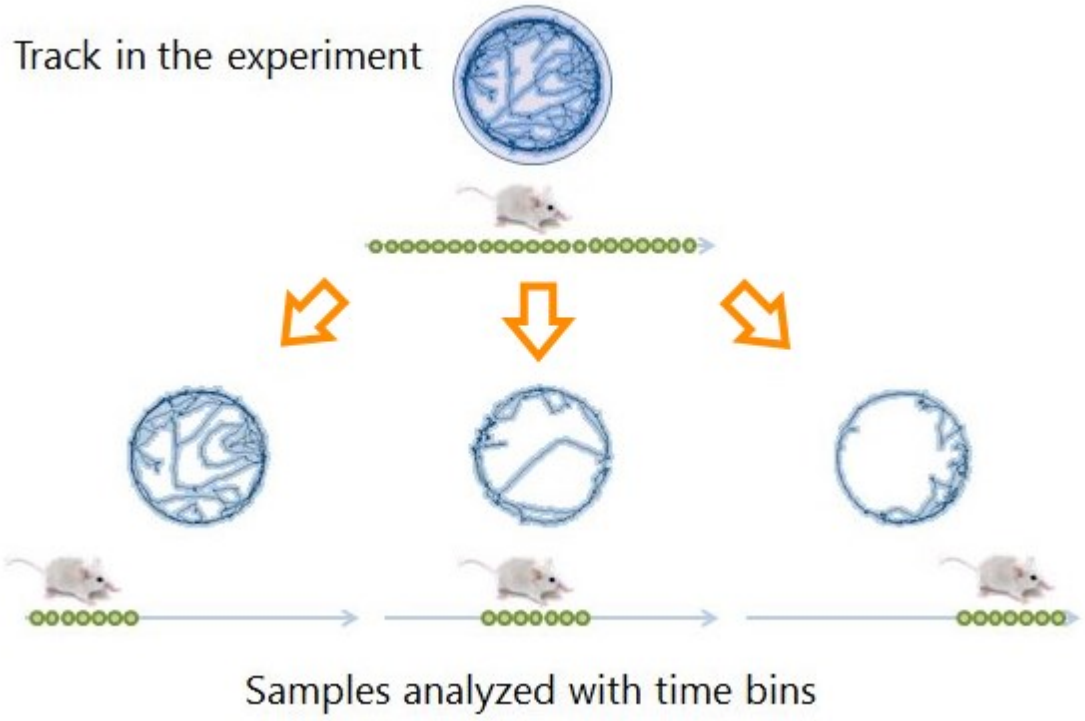
  Solution: Subject 2 is the Actor. Under **Nesting over Subjects**, click the button next to **Movement**.

  - In the **Actors** tab select Subject 2 and deselect the other subjects.
  - In the Analysis profile, select **Distance moved**. The statistics results refer to each subject in the specified interval.

# Results per time bin

## Aim

To split tracks into segments of equal duration. You can then show results for each time bin. Time bins are different from Nesting over time.

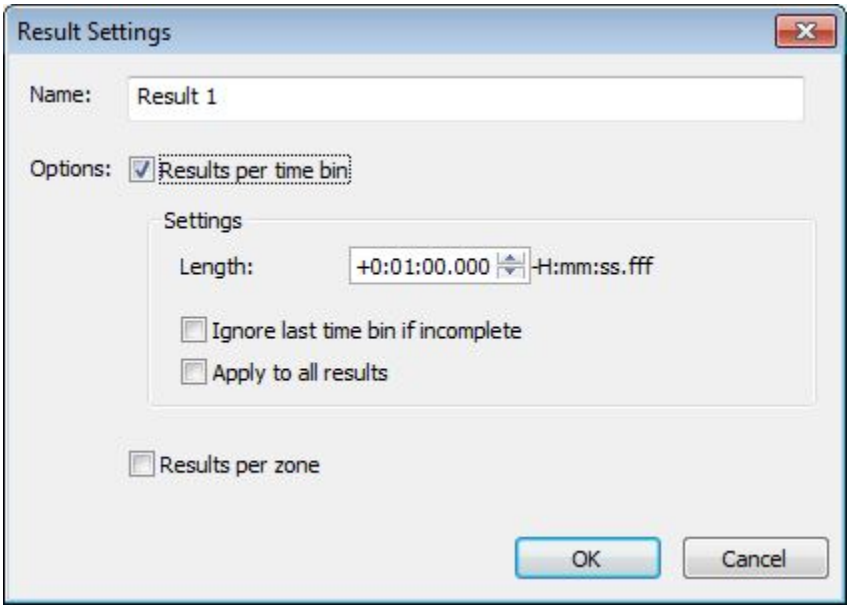



## Procedure

1. In the Data profile, click the **Settings** button on the **Result** box.

   If your data profile contains more Result boxes, open the one corresponding to the data you want to be split in time bins.

2. In the Result Settings window, select **Results per time bin**.




3. Under **Settings**, select:
   - The **Length** of the single interval (range: 0.001 second to 24 hours).
   - **Ignore last time bin if incomplete**. To exclude the last interval in the case this is of a shorter duration than the Length you have set.
   - **Apply to all Results**. Select this option if your data profile contains more than one Result box and you want to apply time bins to all of them.
4. Click **OK**.

## Notes

- **Results per time bin** is applied for each track separately.
  - In a setup with multiple arenas, tracking can start at different times for each arena, depending on when the Trial Control's Start conditions are met in the arenas. Therefore, the same time interval may refer to different 'real' times for different arenas.
  - In a setup with multiple subjects per arena, tracking starts simultaneously for all the subjects in each arena. Therefore, the same time interval refers to the same 'real' time for all the subject in that arena.
- If you select a time bin length that is short compared to the sample rate, bear in mind that (1) Increasing the number of time bins increases the calculation time. (2) If the length of the time bins is shorter than the sample time, only some of the time bins will contain a sample.

- If the length of the time bins is not a multiple of the sample length, the time bins will differ in the number of samples they contain. For example with a sample rate of 1 per second and a time bin length of 1.5 second, half the time bins will contain 1 sample and the others will contain 2.
- If you use time bins in combination with Nesting or Filtering, the time bins outside the data selection are shown in the table headers but with no results. In the example below, time bins of 10 seconds were used and a time interval was created with Nest over Time from 20 to 30 seconds.

Trial Statistics | Group Statistics & Charts

| | | | | Distance moved | Velocity |
|---|---|---|---|---|---|
| | | | | Center-point | Center-point |
| | | | | Total | Mean |
| | | | | cm | cm/s |
| Result 1 | Trial 2 | Control | Start-0:00:10 | - | - |
| | | | 0:00:10-0:00:20 | - | - |
| | | | 0:00:20-0:00:30 | 61.2553 | 6.2251 |
| | | | 0:00:30-0:00:40 | - | - |
| | | | 0:00:40-0:00:50 | - | - |
| | | | 0:00:50-0:01:00 | - | - |
| | | | 0:01:00-0:01:10 | - | - |
| | | Treated | Start-0:00:10 | - | - |
| | | | 0:00:10-0:00:20 | - | - |
| | | | 0:00:20-0:00:30 | 113.2858 | 11.5128 |
| | | | 0:00:30-0:00:40 | - | - |
| | | | 0:00:40-0:00:50 | - | - |
| | | | 0:00:50-0:01:00 | - | - |
| | | | 0:01:00-0:01:10 | - | - |

- If you specify more than 1000 time bins in your Data profile, the group charts are not displayed. See Group charts

# Complex Data Selections

## Main topics and tasks

# Combining selection boxes

Selection boxes can be combined in a variety of ways to create complex data selections.

## Learn about

- Basic rules for combining selection boxes
- Order of selection boxes in a sequence

## What do you want to do?

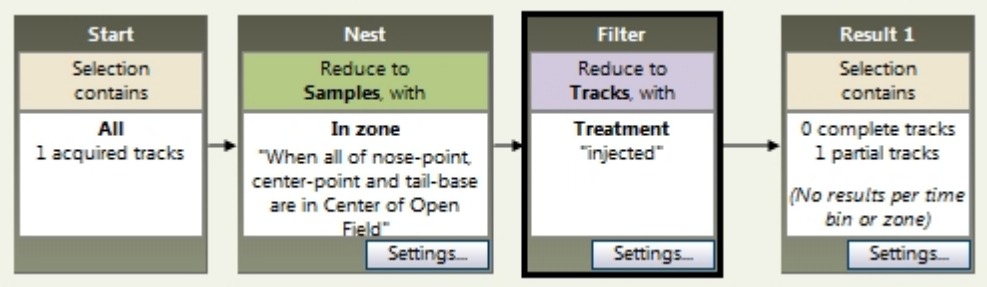
- Use Multiple filters
- Use Multiple nesting criteria

# Basic rules for combining selection boxes

## Boxes in a linear sequence = AND logic

When you line up boxes in a single sequence, the data in the **Result** box (and thus selected for analysis) satisfy all conditions set by the boxes (AND logic).

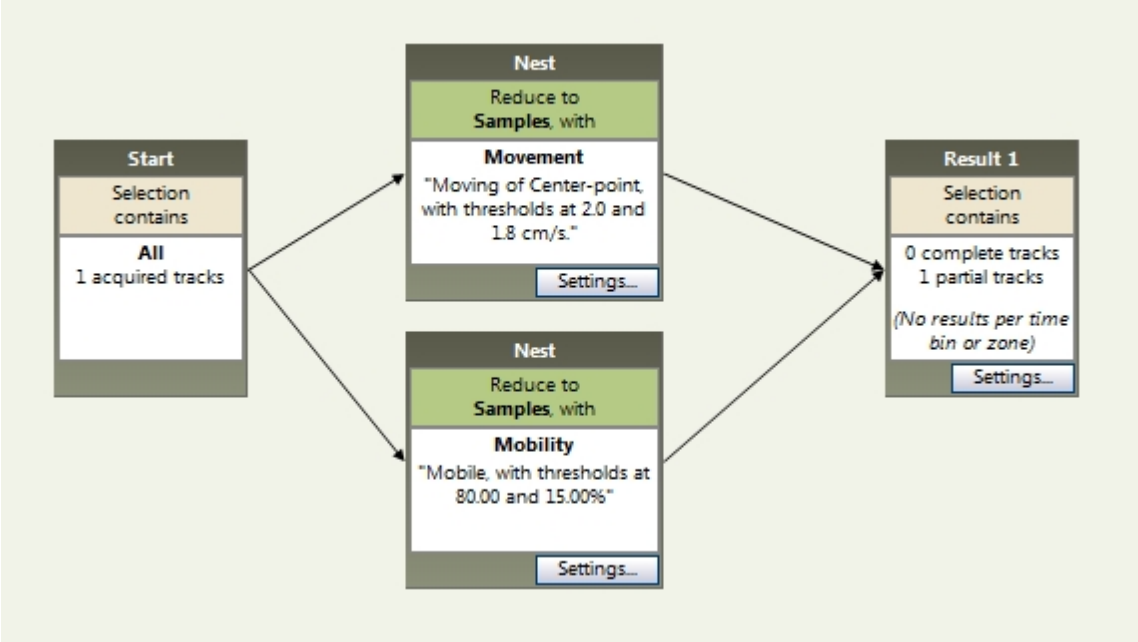
**EXAMPLE** Select the samples in the center of the open field (**Nest**; second box), AND only for subjects with the value of the independent variable Treatment = Injected (**Filter**; third box).




## Boxes in parallel = OR Logic

When you split a sequence in two or more branches which end in the same **Result** box, the data in the **Result** box (and thus selected for analysis) satisfy either one condition or another set in those branches. (OR logic).

**EXAMPLE** Select all samples when the animal was either Moving (**Nest**, top box) OR Mobile (**Nest**; bottom).


Start
Selection contains
All
1 acquired tracks
Nest
Reduce to Samples, with
Movement
"Moving of Center-point, with thresholds at 2.0 and 1.8 cm/s."
Settings...
Nest
Reduce to Samples, with
Mobility
"Mobile, with thresholds at 80.00 and 15.00%"
Settings...
Result 1
Selection contains
0 complete tracks
1 partial tracks
(No results per time bin or zone)
Settings...

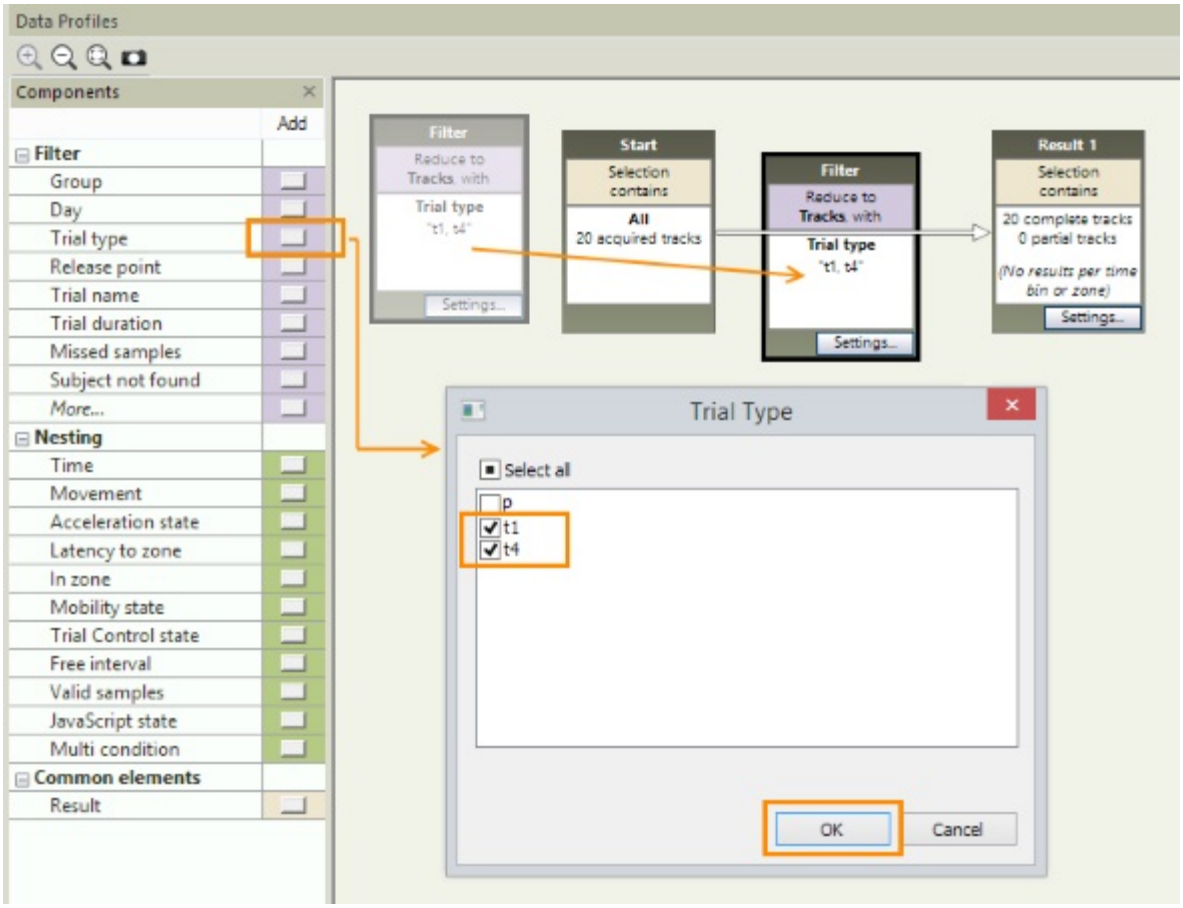
# Multiple filters

## To filter by two or more values of a variable

Specify those values in one **Filter** box.

**EXAMPLE** A Morris water maze experiment contains a number of trials of different type, either training or probe. Training trials are marked with t1, t2, etc. according to their position in a sequence, using the user-defined variable *Trial type*. You want to analyze the tracks for type t1 and t2, not probes (p).

1. Create a **Filter** box corresponding to the filtering variable.
2. Specify the values of the variable.
3. Insert the **Filter** box in the sequence.



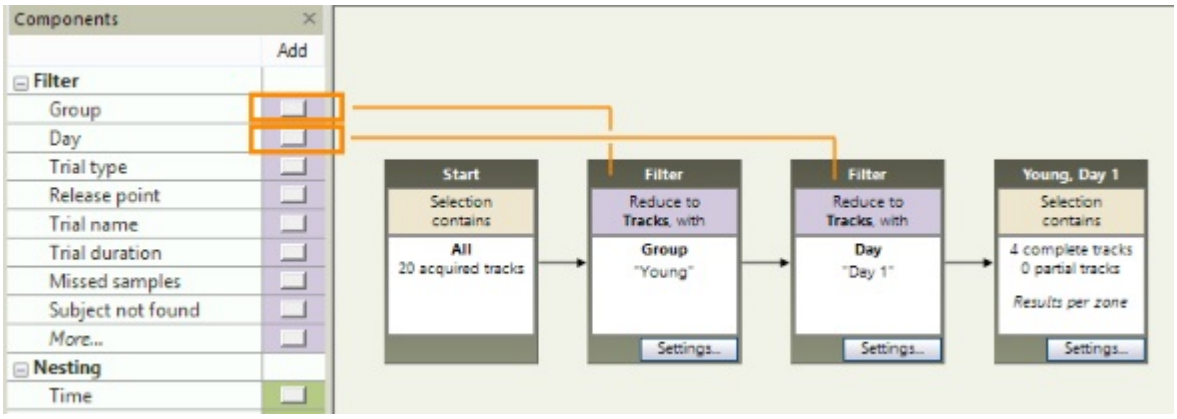
## To filter by two or more variables

Create one box per variable.

Connect them between the **Start** box and the **Result** box using the logic you require. See Basic rules for combining selection boxes.

**EXAMPLE** Your experiment contains a number of tracks recorded for two groups of subjects, *Young* and *Aged*, and on a variable number of days after the treatment. You want to calculate the statistics for the *Young* subjects, and for the *Day 1* after the treatment.

We assume here that *Group* and *Day* have been entered as user-defined variables. Since the selection criteria depend on different variables, place the **Filter** boxes one after the other; which comes first does not matter.



# Multiple nesting criteria

## To nest over multiple values of the same variable

- For variables like *In zone*, specify those values in one **Nest** box.
- For variables with more than 2 states, like *Body elongation state*, *Activity state* and *Mobility state*, create one **Nest** box per value, and combine the resulting boxes with OR logic.

**EXAMPLE 1** With the variable *In zone*

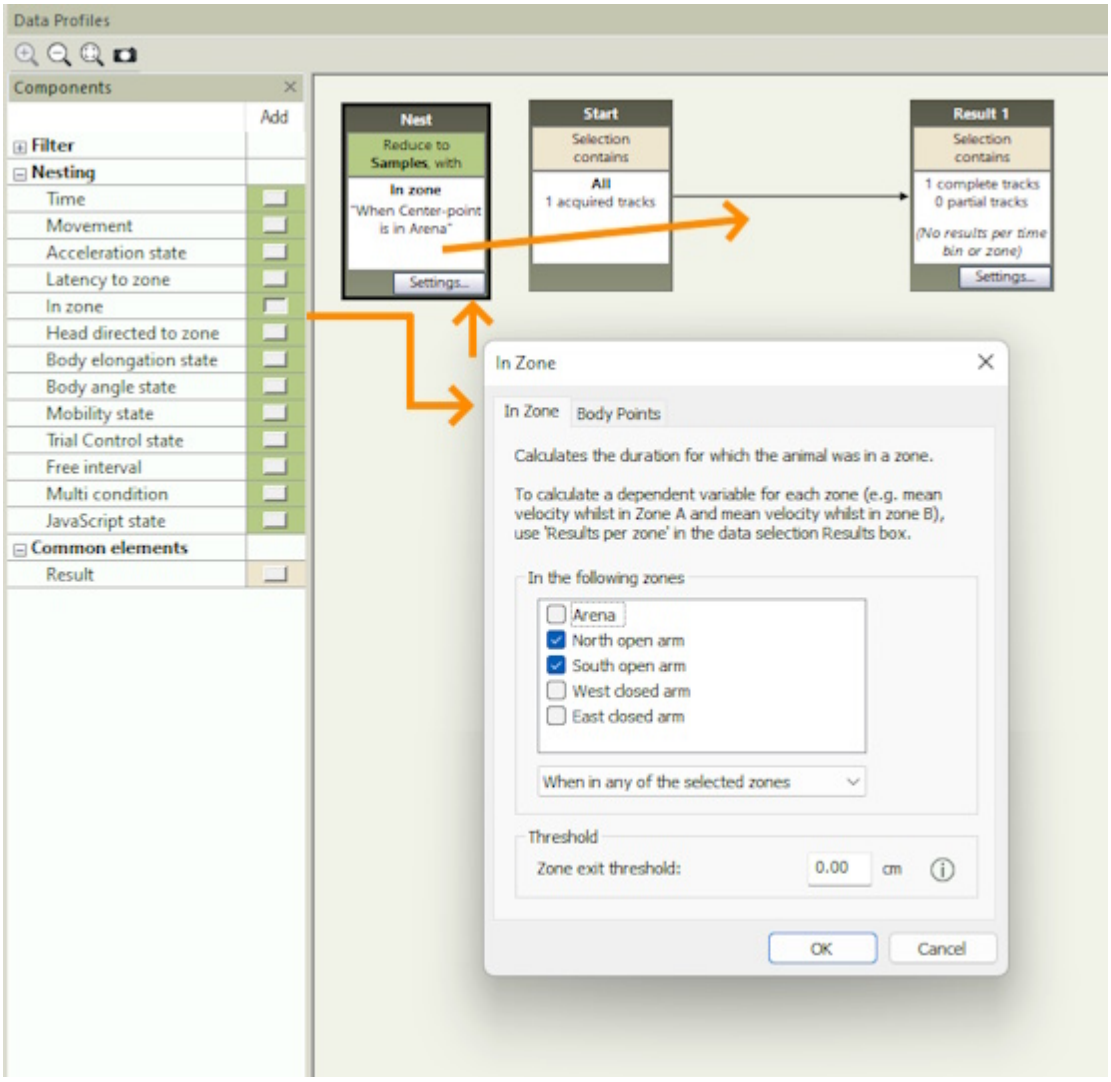
You want to visualize the track segments when the subject was entirely in one of the open arms of the Elevated plus maze. Each open arm has been defined as a zone.

1. Create a **Nest** box corresponding to the variable; in this example, *In zone*.
2. Specify your selection.
3. Insert the **Nest** box in the sequence.

Analysis is done on the samples collected when the center point, nose-point and tail-base of the subject were all in either in open arm 1 or in open arm 2 of the plus maze.

**NOTES**

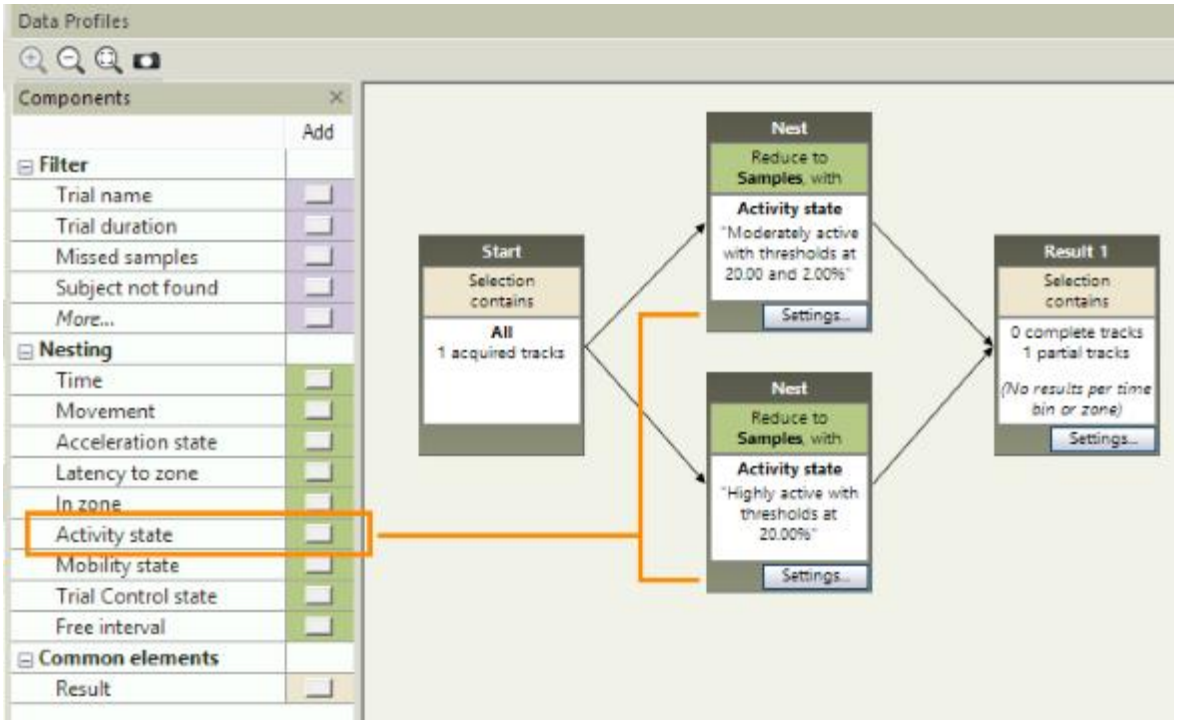
- If the zones do not overlap, from the **In the following zones** list select **When in any of the selected zones** (OR logic).
- If the zones do overlap, and you want to select the time when the subject was in the intersection of those zones, from the **In the following zones** list select **When in all selected zones** (AND logic).




**EXAMPLE 2** With the variable *Activity state*

You want to select the track segments when the subject was either Active or Highly active. It is assumed here that Activity state is defined with 3 possible states: *Inactive*, *Moderately active*, *Highly active*.

1. Create a **Nest** box for the variable *Activity state*. Under **Calculate nesting for**, choose **Moderately active**.
2. Create a second **Nest** box for *Activity state*. Under **Calculate nesting for**, choose **Highly active**.
3. Combine the Nest boxes with OR logic. See Boxes in parallel = OR Logic.

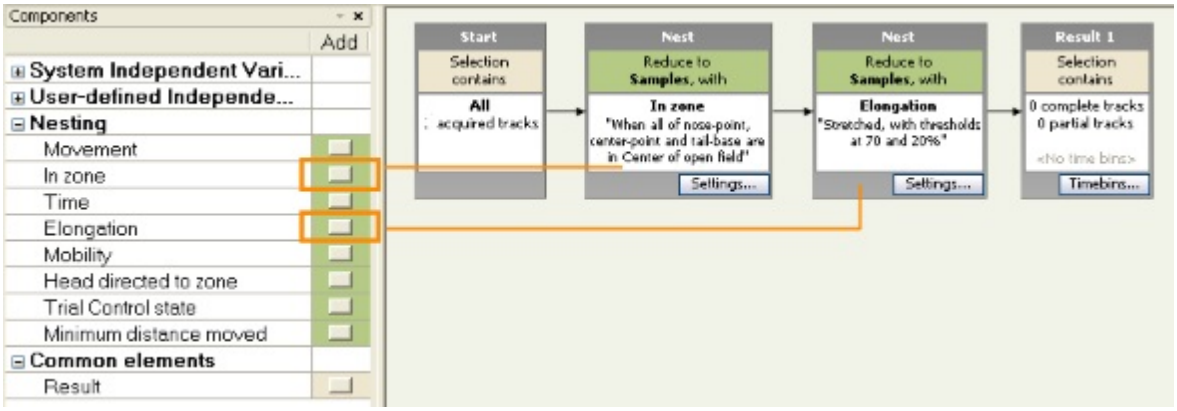



## To nest over two or more variables

Create one **Nest** box per criterion.

To refine nesting according to two or more nesting criteria, create the corresponding **Nest** boxes and connect them between the **Start** box and the **Result** box using the logic you require.

**EXAMPLE** You want to visualize the track segments when the animal was completely in the *center* of the open field AND its body was *stretched*. The center of the open field was defined as a zone.



If you want to nest over multiple values of the same variable (for example, nest over *Highly mobile* AND *Mobile* of the variable *Mobility*), create one **Nest** box per value and connect them using the logic you require (AND, OR or Multiple Results).

# Order of selection boxes in a sequence

In some instances the order in which you place selection boxes in the Data selection sequence may be important

## Filter boxes

- If two or more **Filter** boxes refer to different variables (for example, one to filter trials, another one to filter drug doses), then the order you place the Filter boxes in does not matter.
- If two or more **Filter** boxes refer to the same variable, then selection will only work if the elements selected in the second box are also selected in the first box.

**EXAMPLE** If you filter male subjects in one **Filter** box and female subjects in the second **Filter** box, the selection contains no data.

## Nest boxes

The order you place the **Nest** boxes relative to **Filter** boxes does not matter.

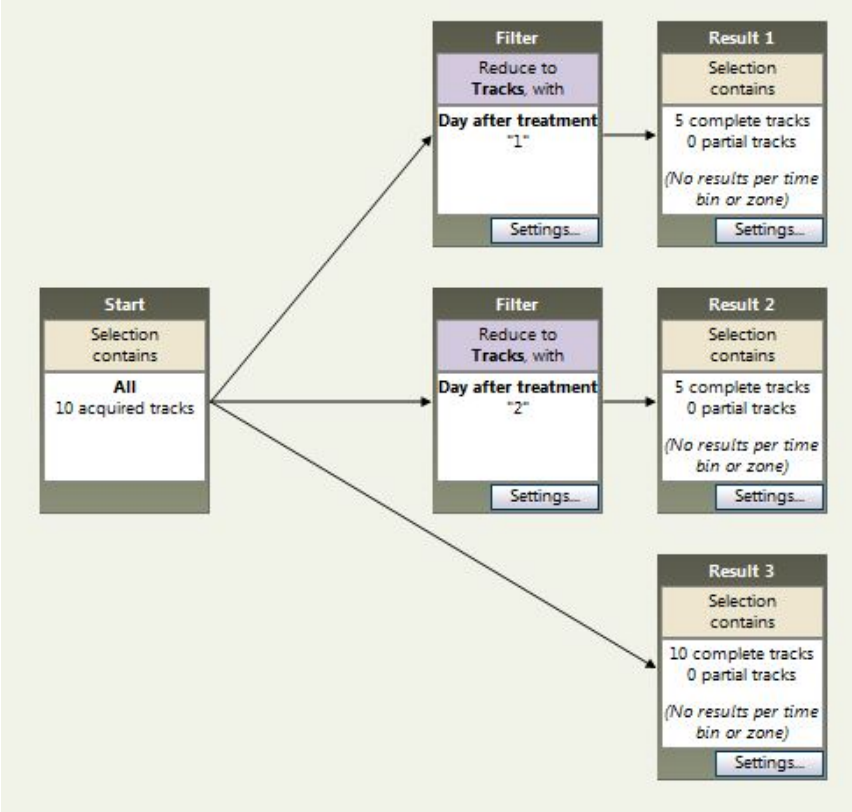
# Multiple selections in a data profile

You can create different data selections in the same Data profile. Each sequence of boxes must end in a separate **Result** box. When you analyze the data using that Data profile, the results (statistics, track plots, or heatmaps) are displayed for each selection separately.

**TIP** With large data sets, you may have a large number of possible data selections. Create multiple Data profiles, each specifying one of those selection criteria, and then run batch calculations for each combination between Data profiles and Analysis profiles.

**EXAMPLE** Tracks have been recorded for a number of animals at Day after treatment 1 and 2. *Day after treatment* is an independent variable. You want to calculate statistics for the two groups separately, but also with all tracks pooled together.

Connect each Filter sequence to a separate **Result** box. To add a **Result** box, click the button next to **Result** in the **Components** pane.




The first **Result** box receives the data after filtering with the independent variable Day after treatment = 1. The second **Result** box receives the data after filtering with Day after treatment =2. The third **Result** box receives all data. The statistics are calculated separately for each criterion.

**TIP** click the **Settings** button in a **Result** box to change its name, Enter a name associated with the selection (for example, “Day 1”). You will see this name in the results table.

In the analysis results, you can see which output is produced by which **Result** box. Look up the **Result** header.

*See also*

- Analyze groups of tracks

# Visualize Data

## Main topics and tasks

# Introduction to Data visualization

## Learn about

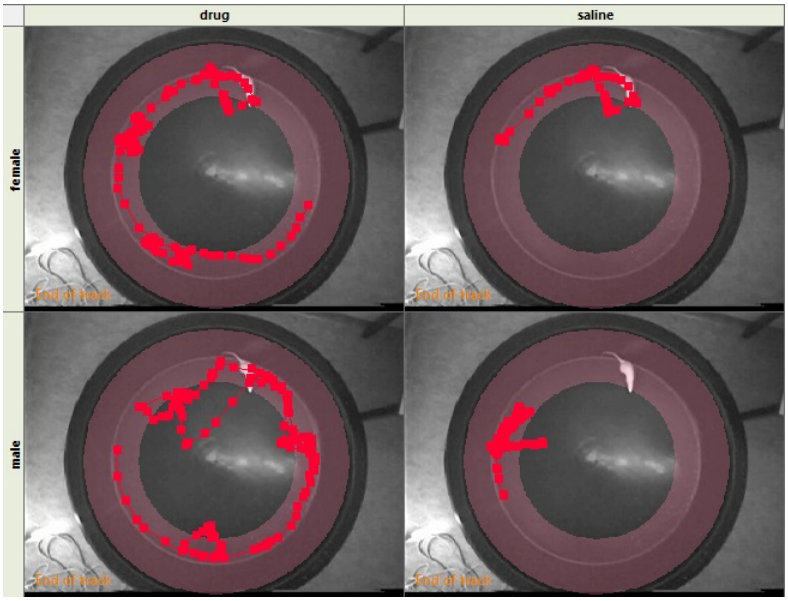
- Important terms in data visualization
- Useful things to know in data visualization

# Important terms in data visualization

## Track plot

Visualization of one or multiple trials, where the subject's position is displayed over a static (background) image. Use track plots to visually inspect trials and compare the effect of experimental treatments.

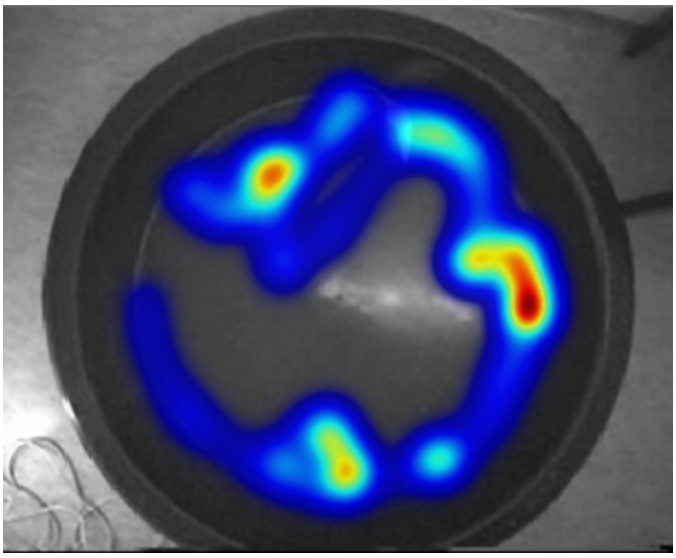
- See Plot tracks




## Heatmap

Visualization of one or multiple trials, where the density of sample points is represented as a color. It is useful to find hotspots.

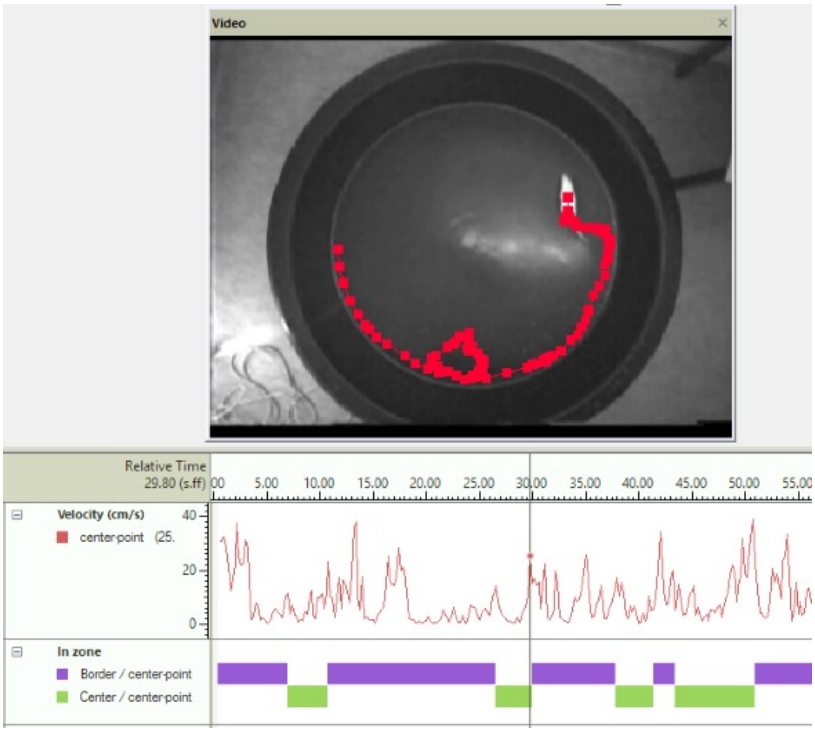
- See Plot heatmaps



## Integrated data

Combines visualization of tracks (see above) with video (when present), and dependent variables and external data (when present) plotted against time.

- See Plot integrated data

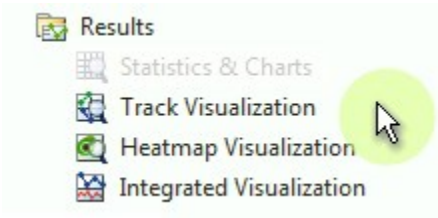


# Useful things to know in data visualization

## To visualize the data

Do one of the following:

- Choose **Analysis** > **Results** > one of the options **Plot Tracks**, **Plot Heatmaps**, or **Plot Integrated Data**.
- In the Experiment Explorer, under **Results**, click **Track Visualization**, **Heatmap Visualization**, or **Integrated Visualization**.

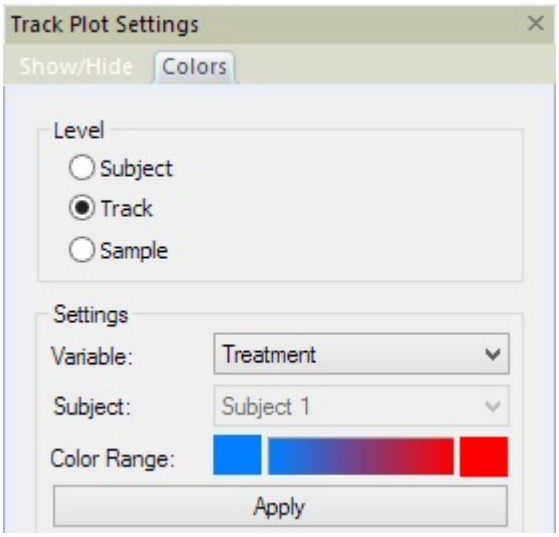



## To visualize a specific data set

On the toolbar, choose the Track Smoothing profile, Data Profile and Analysis Profile, and a trial (for Integrated visualization).


## To customize track colors, data points etc.

Choose the options on the right-hand pane.

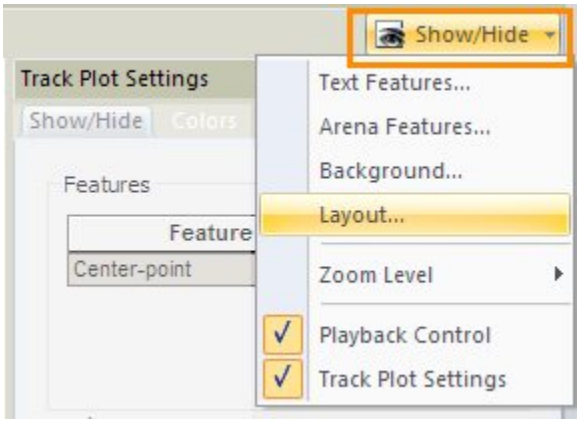



*See also*

- Customize tracks and plots

## To customize the elements on your screen

Click the **Show/Hide** button at the top-right corner and choose the options you require.




*See also*

- Customize tracks and plots

## To move and resize windows

- To move a window, click its title bar and drag it to the desired position. You can either dock a window or let it float on the screen.

- To resize a window, point with your mouse to the window's left or top margin so the mouse pointer turns to a double arrow. Drag the margin to the desired position.

# Plot tracks

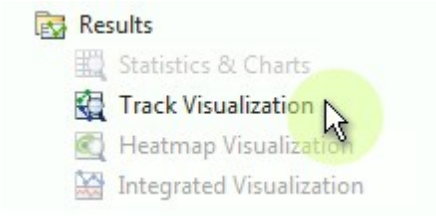
## What do you want to do?

- Plot tracks
- Sort track plots
- View and play back tracks
- Export track plots

# Plot tracks

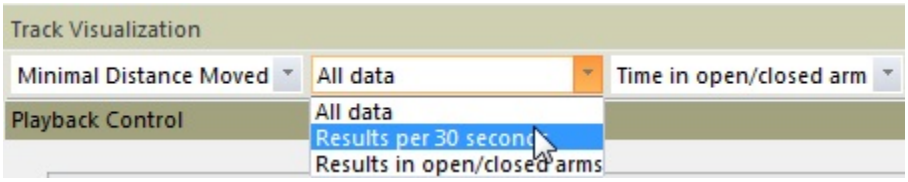
## Aim

Choose **Track Visualization** to visualize a set of tracks. This function is particularly useful when you want to have an overview of your data acquired so far. If you want to visualize data from one specific trial together with video or external data, see Plot integrated data.




## Procedure

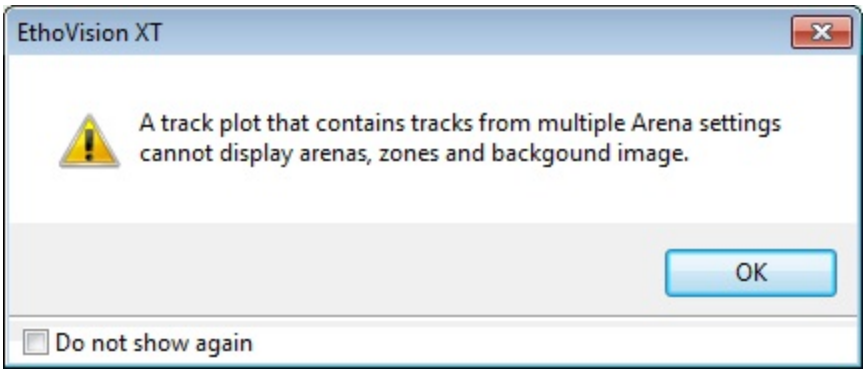
1. Choose **Analysis** > **Results** > **Plot Tracks**, or in the Experiment Explorer, under **Results**, click **Track Visualization**.
2. On the toolbar, choose the Track Smoothing profile and the Data profile that you want to use. The two profiles define the samples to plot.




3. See Customize tracks and plots on page 916.

## Notes

- If you want to plot tracks in colors depending on the value of a dependent variable, like velocity, make sure that that variable is defined in one of the Analysis profiles. Then, repeat the steps above and choose the correct Analysis profile.
- If your data selection contains nesting criteria, all samples are plotted, however the samples excluded by nesting are displayed in a different color.
- You may sometimes get the following message:




You have recorded data with more than one Arena Settings, and the layout of the plot matrix is such that tracks obtained with different Arena Settings are expected to be plotted in the same plot. Add the independent variable **Arena Settings** to the layout (see Sort the track plots by a new variable). Alternatively, in your Data profile filter data from one of the Arena Settings.

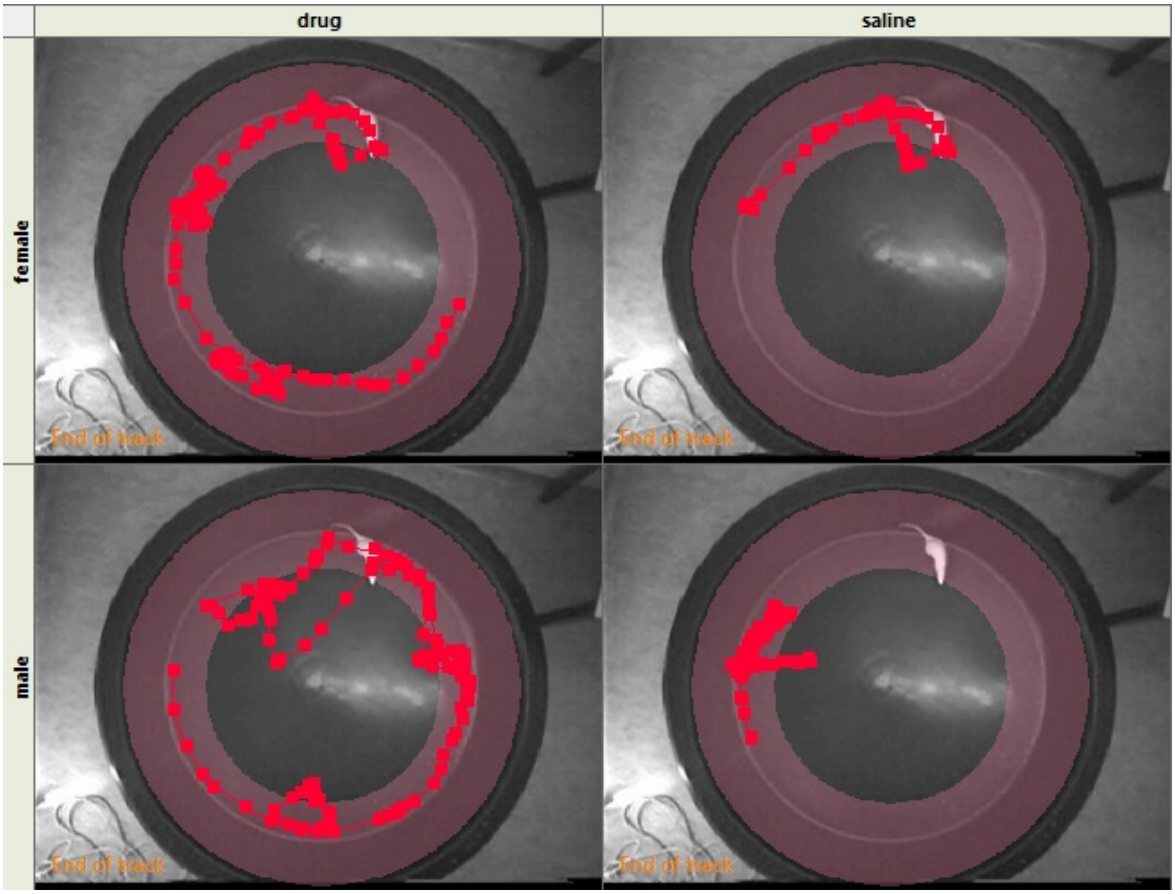
- Sometimes a variable number of cells in the plot matrix are empty. This is caused by the fact that there are no tracks corresponding to the values of the independent variable specified in the plot headers. To create a more compact matrix, choose a different set of independent variables to sort track plots (see Sort track plots).

# Sort track plots

## Aim

Track plots are displayed in the form of a matrix. You can sort rows and columns according to one or more independent variables.

**EXAMPLE** Subjects of both sexes were assigned to two different treatment levels (drug or saline). You want to visualize tracks according to Subject sex (on the rows) and Treatment (on the columns).




By default, plot headers do not include the name of an independent variable and the variable's value for that row/column. To show the variables' name, right-click a header and select **Show Name**. The values of the variables and the resulting sorting order are not removed. To hide the variables' name, right-click the header and deselect **Show Name**.

## Sort the track plots by a new variable

Do one of the following:

- Click the **Show/Hide** button on the toolbar, and select **Layout**. In the Layout window, select **Arrange by headers**. Under **Available**, click the variable you want to use. Next, click one of the buttons next to **On Columns** or **On Rows**, depending on whether you want to sort columns or rows by that variable.
- Right-click the header of a column or a row, depending on where you want to place the new variable. Next, select **Insert**. In the Select Independent Variables to Insert window, from the **Independent Variable Name** list, select the variable you want to use, then click **OK**.

**NOTE** The variable list does not include the variables already present in the plot headers.

## Remove a sorting variable

Do one of the following:

- Click the **Show/Hide** button on the toolbar and select **Layout**. Click the variable name under **On Columns** or **On Rows**, and click the deselect button next to it.

- Right-click a header for that variable and select **Delete**.

If you remove all variables from one side of the matrix, the headers will be empty. All tracks are plotted on one row or column.

## Sort the plots in ascending or descending order

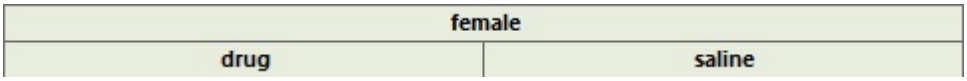
By default, track plots are sorted in ascending order of the corresponding variables (numeric or alphabetic).

- To sort variable values in descending order, right-click a header for that variable and select **Sort Descending**.
- To sort variable values in ascending order, right-click a header for that variable and select **Sort Ascending**.

You cannot change the plot order from the Layout window.

## Re-arrange multiple sorting levels

When two or more variables are inserted or moved to the same side of the matrix, they form a multi-level structure. In the example below, plots are first sorted by *Subject sex* (top level), and then by *Treatment* (bottom level).



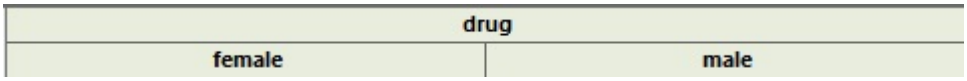

To re-arrange sorting levels, do one of the following:

- Click the **Show/Hide** button on the toolbar and select **Layout**. Click the variable you want to move. To move the variable one level up, click the **Up** button. To move the variable one level down, click the **Down** button.

- Right-click a track plot header for a variable. To move a variable one level up, select **Move up**. To move a variable one level down, select **Move down**. Or click a header for that variable, and drag it to a lower or upper level.

In the example below, the variable *Treatment* has been moved above *Subject sex*. As a result, plots are first sorted by Treatment, then by Subject sex (compare this with the previous figure).




## Swap variables from columns to rows and vice versa

You can re-arrange your plots by moving the variables shown in the rows to columns and vice versa. Do one of the following:

- Click the variable name under **On Columns** or **On Rows**, and click the **Place Horizontal** or **Place Vertical** button.

- Right-click a row/column header for that variable and select **Place Horizontal** or **Place Vertical**, or click a row/column header and drag it to a new position.

# View and play back tracks

## Zoom the track plots in and out

The Zoom buttons are located on the toolbar.


- To zoom in, click the **Zoom in** button or press **Ctrl+.** .
- To zoom out, click the **Zoom out** button or press **Ctrl+,** .
- To fit all track plots on the screen, click the **Fit all** button .

To zoom in/out to predefined percentages, from the **Show/Hide** menu, select **Zoom level**, and choose one of the values available (6% to 800%).

## Customize the length of tracks

See Customize the elements and length of the tracks on page 917.

## Play back the tracks

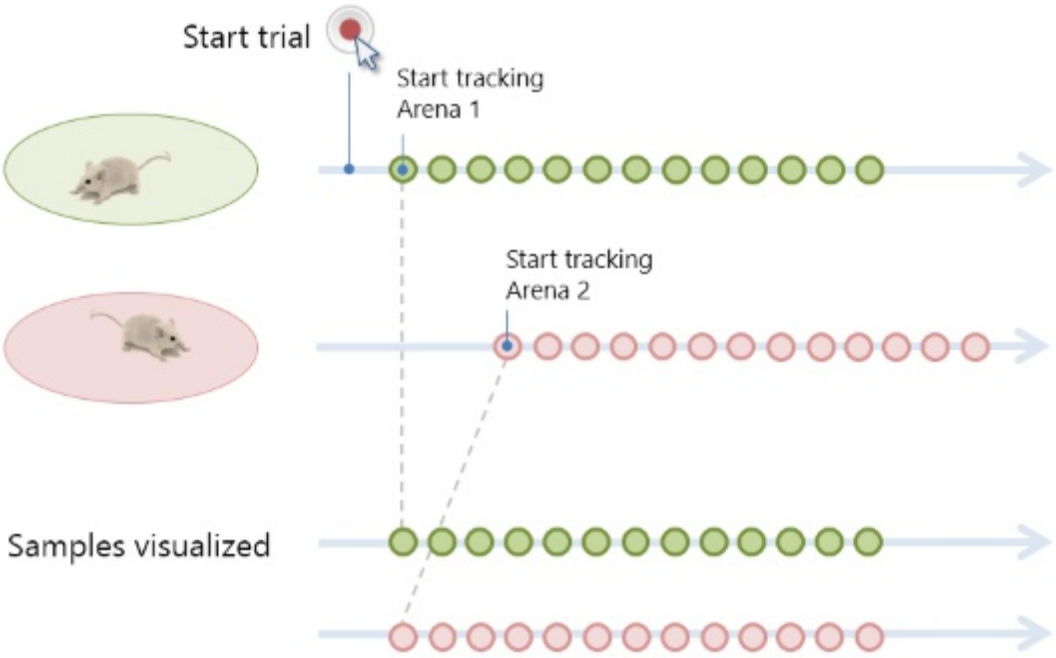
Click the buttons in the Playback Control window. See Playback Control, Acquisition and Visualization

## View the tracks in multi-arena setups

In trials with multiple arenas, all tracks are played back starting from the first sample. If the tracks started at different times in different arenas, the samples visualized at a given time refer to different 'real times' in those arenas.

To preserve the time difference between arenas, choose Integrated Visualization.

Below: In a multi-arena setup, data recording may start at different times in different arenas. When tracks are plotted together, their starting times are aligned. This means that samples visualized simultaneously for the two arenas may refer to two different moments in the real time line.


Start trial
Start tracking
Arena 1
Start tracking
Arena 2
Samples visualized

# Export track plots

## Export a single track plot

1. Click the plot you want to export, so that its border becomes highlighted in red.
2. On the toolbar, click the **Export image** icon.

3. Under **Export**, choose **Selected plot**.
4. Select the image resolution in pixels.
5. Click **Export**. In the Export Image window, enter the name and location of your image file, choose the image format and click **Save**.

## Export multiple plots as separate files

Each plot is exported as a single image file.

1. Hold the **Ctrl** key down and click the plots you want to export. If you do not make a selection, all plots will be exported.
2. On the toolbar, click the **Export image** icon.

3. Under **Export**, choose **To separate image files**.
4. Select the image resolution in pixels, and click **Export**.

## Export multiple plots as one file

The matrix grid is exported as a single image file.

1. Select the plots you want to export. If you do not make a selection, all plots will be exported.
2. On the toolbar, click the **Export image** icon.

3. Under **Export**, choose **To one image file**.
4. Select the image resolution in pixels, and click **Export**.

## Notes

- The available image resolutions depend on the aspect ratio of the original background pictures. The zoom level chosen for the plots affects the default resolution.

## Available image formats

- **Portable Network Graphic (*.png)** (default). A picture format using lossless compression.
- **JPEG file (*.jpg)**. A compression format for color images, particularly photographs. It can reduce file sizes to about 5% of their original size, but some detail might be lost in the compression.
- **Windows Bitmap (*.bmp)**. A non-compressed format generally resulting in large files.
- **Graphics Interchange Format (*.gif)**. A lossless format limited to 256 colors. This makes the GIF format suitable for storing graphics with relatively few colors such as simple diagrams.

# Plot heatmaps

## Learn about

- Heatmaps in EthoVision XT
- The heatmap color scale

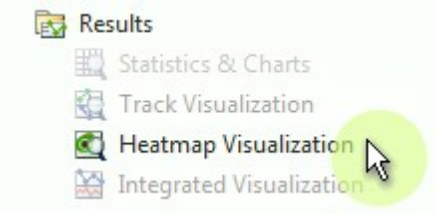
## What do you want to do?

- Plot heatmaps
- Choose the Merging method for heatmaps
- Customize the heatmaps
- Arrange heatmaps
- Choose the Heatmap colors
- Export the heatmaps

# Heatmaps in EthoVision XT

## What are heatmaps?

A heatmap is a graphical representation of the subject's position in the 2D space where the density of utilization of a specific place is represented as a color. Heatmaps facilitate identification of "hotspots" and clustering of data points.



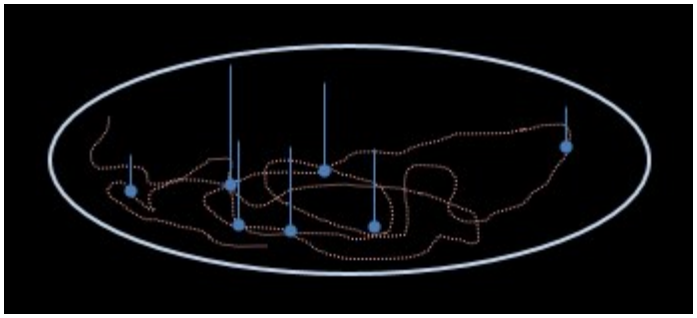

Choose **Heatmap Visualization** to generate heatmaps.

You can generate heatmap for single tracks or groups of tracks, and sort heatmaps according to various criteria. You can combine heatmaps with data selection to visualize for example the subject's position while it shows a specific behavior. Finally, you can export heatmaps to a high-resolution graphics file.

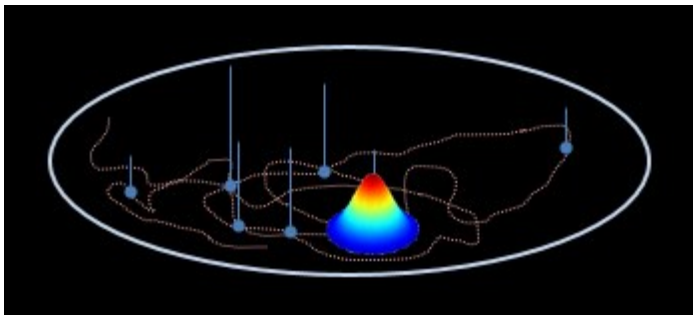
## How are heatmaps created?

In EthoVision XT, a track is a group of sample points, each with x an y coordinates. If one sample is found at specific coordinates, the frequency (= number of samples) for the corresponding pixel in the video image is 1. If more samples are found exactly on that pixel, for example when the subject returns to the same location, or when it sits still for some time in that location, then the frequency for that pixel is higher: 2, 3, 4, etc.

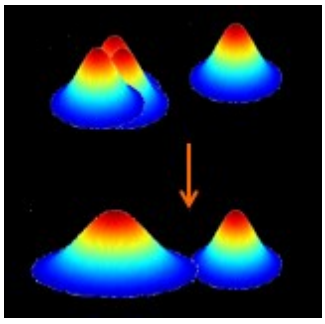
**IMPORTANT** Here by *frequency* we mean the number of samples that are found on a specific pixel, not the number of entries in a zone or the like. The number of samples can be plotted in a 2D histogram, where the length of each column indicate how much time the body point of the subject was found exactly at that location.



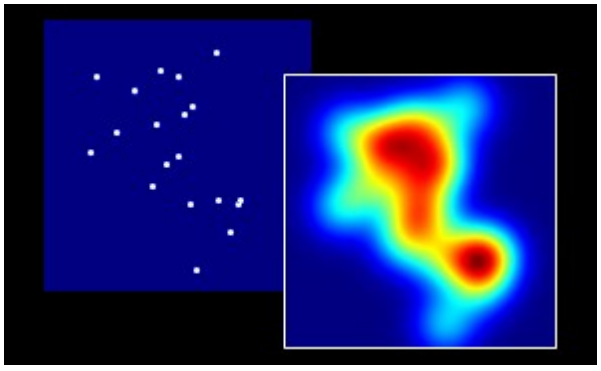
The heatmap is created from that histogram, based on the concept of kernel density estimation. At each pixel, the probability is calculated to find the subject at that specific location. The density function states that there is some (lower) probability to find the subject also in the surroundings of the focal pixel. This can be represented with a bell-shaped three-dimensional curve.



The Kernel density function is calculated at each location. The single 3D bell curves for neighboring locations are merged together, resulting in higher and wider curves.



However, heatmaps in EthoVision XT are a 2D representation of the hilly landscape that results from the kernel density estimation. It's like viewing the hills and valleys from the top. The colors represent the altitude of the landscape.

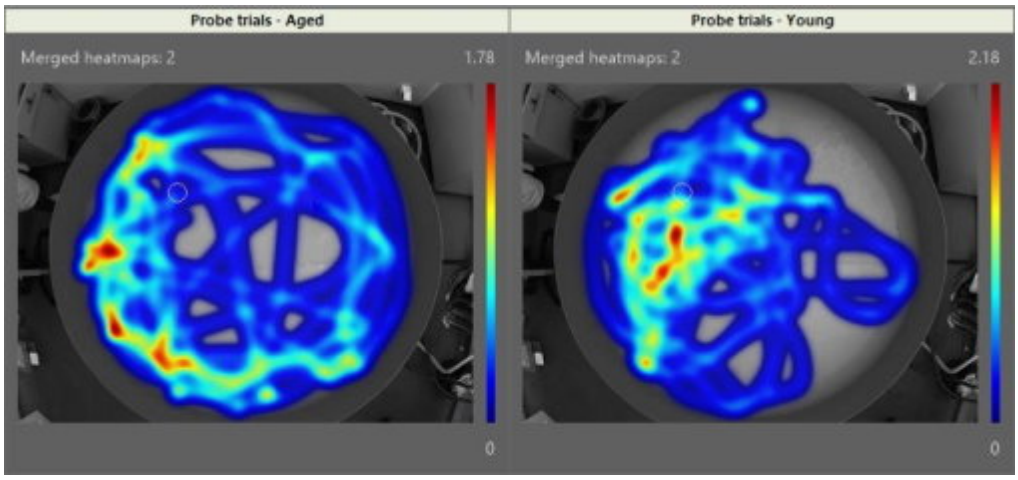


In EthoVision XT, the colors may represent the cumulative time in a particular pixel, or the fraction of the track that is found of that pixel. This depend on what you choose under Color level.

# Examples of heatmaps

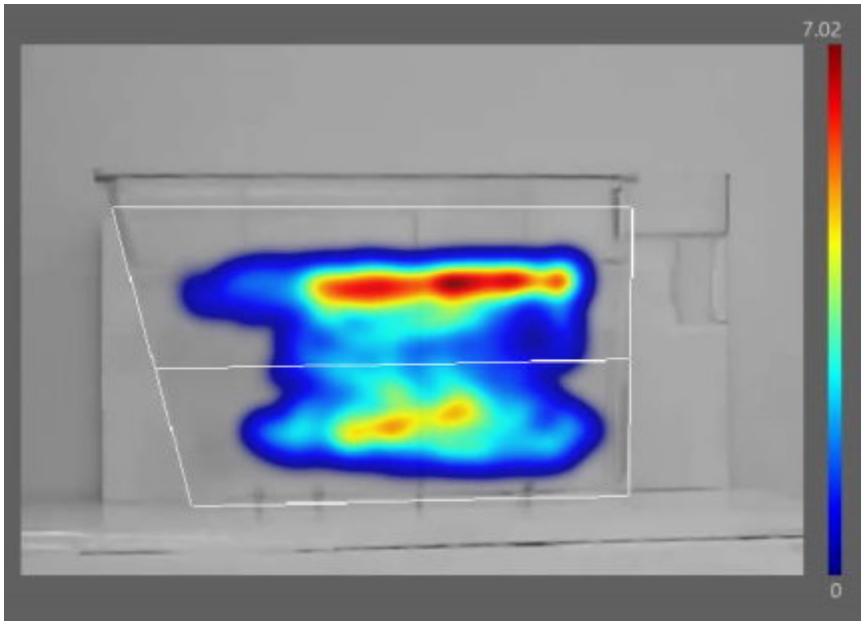
## *Morris water maze test*

In this example, we compare the swim pattern between two groups of rats in probe trials. The young rats (right) display more focused search then the old ones (left).



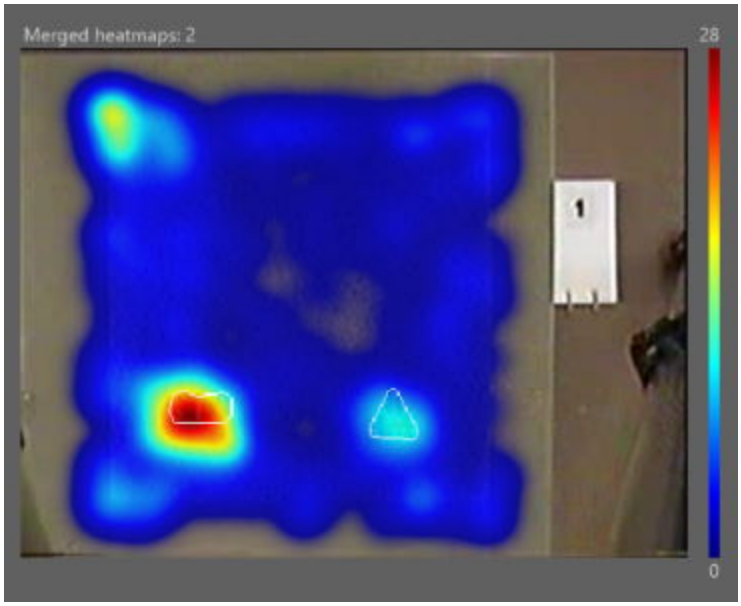

## *Zebrafish in a novel tank diving test*

In this example we want to show the bias toward top-swimming after the fish was treated with an anxiolytic drug. The two zones, Top and Bottom, are outlined in white.



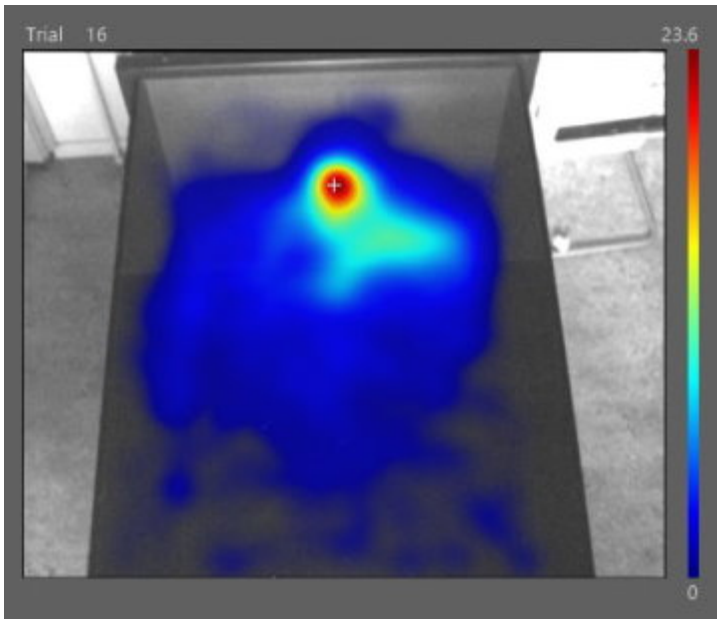
*Novel Object Recognition test*

In this test the subject explores two objects, one familiar and one novel. The heatmap clearly show the difference in the "density" of location points around the two objects. The two object zones are outlined in white.




*Mosquito in a wind tunnel*

A mosquito tends to reach the odour source located behind the upwind screen of the flight chamber. The middle of the screen is indicated with a cross.



## Notes

- Be careful when interpreting color heatmaps. First, the colors lack the natural perceptual ordering found, for example, in gray scale color maps. The changes between colors lead to perception of gradients that aren't actually present, making actual gradients less prominent.

*See also*

- The heatmap color scale

# The heatmap color scale

## Aim

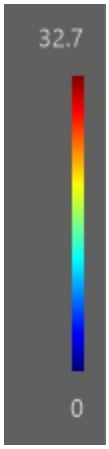
To help estimate how much time the subject spent in a region of the arena.

Because heatmaps create gradients by definition, they are not meant to accurately measure the time that the subject spent in a certain zone. For that, calculate the statistics for the variable *In zone*. See Analysis advisor

## To access this option

Choose **Analysis** > **Results** > **Plot Heatmaps**.

Locate the scale at the right-hand side of the heatmap.




## Scale maximum

You find the maximum value at the top of the scale. It is expressed in:

- Seconds, if you choose **Cumulated** as a Merging method for heatmaps.
- A fraction of the track duration, if you choose **Mean** as a Merging method for heatmaps (see also a note below).

To calculate the maximum, EthoVision XT finds the pixel of the heatmap with the highest number of recorded positions and calculates the total time spent in a circular area around that position. The circular area depends on the Color smoothing that is currently set. The larger the smoothing value, the larger the circular are around the maximum, the higher the value in the scale because more point locations around the “peak” pixel are included in the calculation.

This correction is made because the highest absolute density of sample points (shown in dark red by default) is usually found in one or few pixels per track; taking

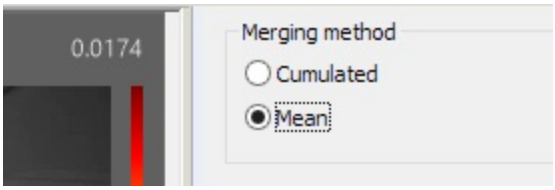
only the cumulated time for those pixels would do not provide a realistic estimate of the time spent by a subject in a certain location.

## Scale minimum

You find the minimum value at the bottom of the scale. This is always zero seconds (or fraction of the track duration; see above for Maximum). It represents the locations where the subject was never found.

## Notes

- The scale is plotted independently for each heatmap. The Maximum value only depends on the original data points that form that heatmap.
- In order to make a good judgment when comparing heatmaps, make sure that the total track duration is the same or very similar between groups or subjects.
- If you choose **Over heatmap** as **Color level**, the scale maximum also changes. For example, the maximum for that heatmap is represented with blue or green, not red. That happens because the overall maximum is found in other heatmaps. See Heatmap colors
- If you choose **Mean** instead of **Cumulated** as a Merging method for heatmaps, the maximum is expressed as a fraction of the total time that the subject was in that region. If the subject keeps moving across the arena, this value can be rather low, for example 0.01. It means that the region of peak occurrence contained 1% of the positions.




*See also*

- Customize the heatmaps

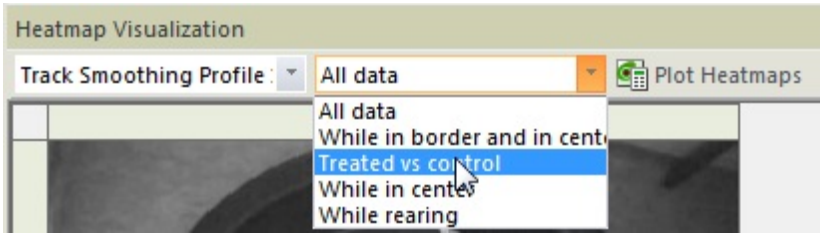
# Plot heatmaps

## Aim

To create heatmaps from the tracks currently selected in the Data profile.

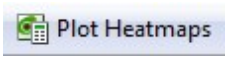
## Procedure

1. Choose **Analysis** > **Results** > **Plot Heatmaps**, or in the Experiment Explorer, under **Results**, click **Heatmap Visualization**.
2. On the toolbar, choose the Track Smoothing profile and the Data profile that you want to use. The two profiles specify the set of samples to be used in the heatmaps.




3. In the Heatmap Settings pane, click the **Show/Hide** tab. Choose the **Features** (body points) and the **Subjects** to visualize.
4. Under **Presets**, click one of the buttons to arrange the layout. See Arrange heatmaps on page 887.

   Under **Merging**, specify how EthoVision should normalize data of different tracks when putting them in a single heatmap. See Merging method for heatmaps on page 883.
5. Click the **Plot Heatmaps** button.

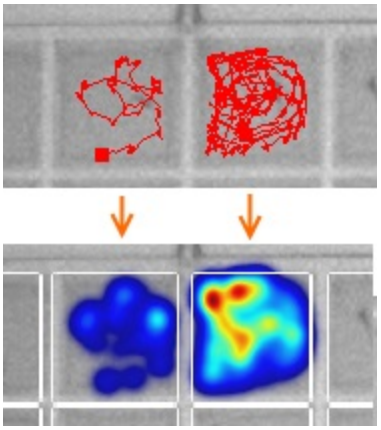



6. **OPTIONAL** Customize the heatmaps on page 885.
7. **OPTIONAL** Export the heatmaps on page 894.

## Notes

- By default, heatmaps are displayed with overlay text and without arena and zone outlines.
- If your video image includes two or more arenas, the heatmap is calculated across arenas, not per individual arena. This means that, if the track in one

arena has fewer samples than the track in another arena, the heatmap in the first arena is less likely to show high-density colors. For example:



This is generally not a problem in DanioVision or PhenoTyper experiments because the track duration is comparable between arenas. For other setups, compare heatmaps from different arenas only when the duration of the tracks is more or less the same, or export the tracks and create the heatmaps in an external application.

- If you want to know where a behavior occurs, in your Data profile nest over that behavior (see Nesting over behaviors scored manually or Nesting over behaviors of Rat/Mouse Behavior Recognition), and then plot the heatmaps.
- When you combine tracks from different arena settings, the corresponding heatmaps may have different size and aspect ratios. The tracks are first aligned at the upper-left corner before they are combined. Therefore, a specific point in the combined heatmap may represent different locations for different tracks.

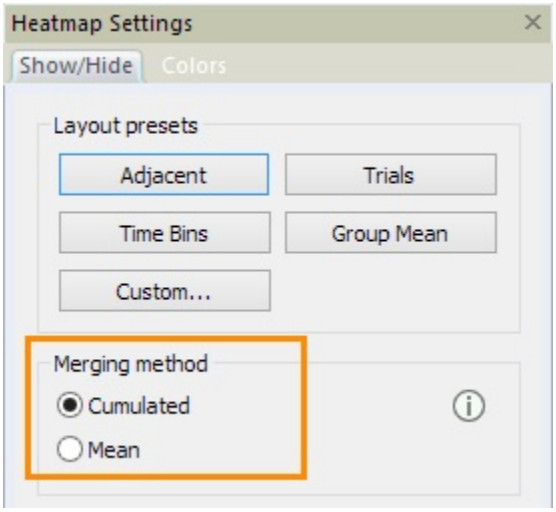
# Merging method for heatmaps

## Aim

To specify how data of different tracks are merged in one heatmap.

## Procedure

1. Choose **Analysis** > **Results** > **Plot Heatmaps**, or in the Experiment Explorer, under **Results**, click **Heatmap Visualization**.
2. In the Heatmap Settings pane, click the **Show/Hide** tab. Choose one of the options under **Merging method**.




## Cumulated

Each sample in a track gets a weight equal to 1/sample rate. Therefore, the color of a pixel in the heatmap represents the total time that the subject(s) body point was at that location. Choose this option when you want to make a heatmap based on all the possible locations of the animals for a group of tracks. With this method, a subject's position is over-represented in tracks longer than others.

## Mean

Each sample in a track gets a weight equal to 1/track length. The color of a pixel represents the average proportion of a track that is found at that location. Choose this option when you want to make a heatmap that represents the average distribution for that group, and you want to compare averages between two or more groups of tracks.

**EXAMPLE** Consider two tracks, recorded at 25 samples/s. One track is 10 samples long, the other 1000 samples long. The total track duration is 1010/25 = 40.4 seconds. A subject's center point is found at location P two times in track 1 (=20% of track 1), and 100 times in track 2 (=10% of track 2). When the two tracks are merged:

- With **Cumulated**, the color of pixel P represents a total time of (2 x 1/25) + (100 x 1/25) = 4.08 s. This means that, in total, 4.08s / 40.4 s = 10.1% of the data points are at that location. The longer track has a greater weight in this calculation.
- With **Mean**, the color of pixel P represents [(2/10) + (100/1000)]/2 = 0.15 = 15% of the track duration. This means that on average, 15% of the data points are at that location. Short and long tracks have the same weight in this calculation.

## Notes

- Merging makes sense when two or more tracks share the same value of an independent variable chosen as a header. For example, two tracks with the same of Treatment level = Control. If Control is one of the headers, the two tracks contribute to the same heatmap.

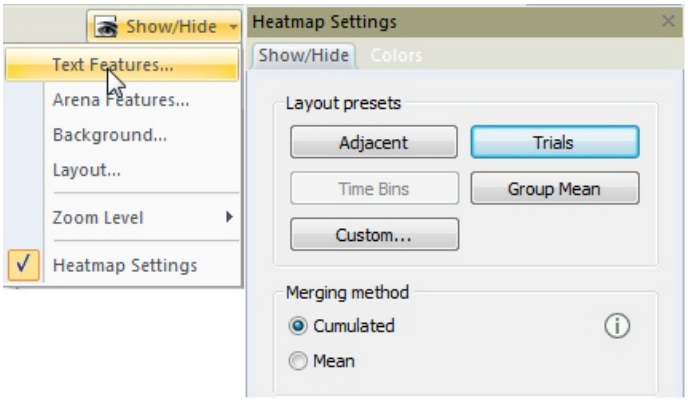
# Customize the heatmaps

## Aim

To choose which text, arena feature or background image to be displayed with the heatmaps.

## How to access these options

Click the **Show/Hide** button on the toolbar.




## Show and hide the overlay text

To facilitate orientation in a heatmap matrix, you can display the value of independent variables used to arrange the heatmaps, and the number *n* of tracks used to create the current heatmap: *Merged heatmaps: [n]*.

1. From the **Show/Hide** menu, select **Text Features**.
2. Under **View**, select **Show information** if you want to see overlay text over the heatmap.
3. Under **Text properties**, choose the text **Fill color** and **Opacity**.

**NOTE** Even when selecting **Show information**, the overlay text is not shown if that information is redundant. For example, independent variables are not shown when the heatmaps have headers; For the same reason, the *Merged heatmaps: [n]* text is not shown when heatmaps are based on single tracks.

## Show and hide arenas, zones and points

1. From the **Show/Hide** menu, select **Arena Features**.

2. In the Arena Features window, choose the arenas, zones, zone centers and points of interest you want to view over the heatmap.
3. Select the feature outline color. Next, click **OK**.

**NOTE** Arenas and zones are displayed as outlines, not semi-transparent regions like in the Track Visualization. Zone centers and points of interest are displayed as crosses.

## Customize the background of the heatmap

1. From the **Show/Hide** menu, select **Background**.
2. Choose:
   - **Plain - by range minimum** to have a uniform background that is the same as the color for the minimum value in the heatmap. To change the color, click the **Colors** tab in the Heatmap Settings window and change option under **Display**.
   - **Plain - custom** to have a uniform background. Click the button next to this option to select a color. To select colors other than the basic ones, click the button **Other**.
   - **Captured image** to have the image captured in the Arena Settings as a background.

If a heatmap is based on tracks with different Arena Settings, it is displayed with the background in the color currently selected next to **Plain - custom**.

## Zoom the heatmap in/out

You can find the **Zoom in** (**Ctrl+ .**) and **Zoom out** (**Ctrl+ ,**) buttons on the toolbar.

To fit all heatmaps on the screen, click the **Fit all** button. 

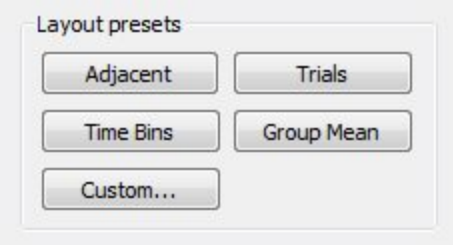
To zoom in/out to predefined percentages, from the **Show/Hide** menu, select **Zoom level**, and choose one of the values available (6% to 800%).

# Arrange heatmaps

You can arrange heatmaps in two ways: with Layout Presets (below), or manually arranging headers in the matrix (Custom layout).

## With Layout presets

In the Heatmap Settings pane, click the **Show/Hide** tab. Under **Layout presets**, click one of the following:




- **Adjacent**. The heatmaps are arranged from left to right, and from top to bottom to fill the screen as much as possible. The following order is used: first Result container, then trial name, then subject name, then body point. With Adjacent, each heatmap represents a single track per arena. Tracks from multiple arenas are combined in the same heatmap. With this preset, the Merging method **Cumulated** is selected automatically.

  Clicking **Adjacent** is the same as selecting **Show/Hide** > **Layout** > **Arrange adjacently**.

- **Trials**. The matrix is arranged in this way:
  - On rows: Trial names.
  - On columns: Result containers, subject names and body-points (if more than one), in this order.

  With Trials, each heatmap represents a single track, and the Merging method Cumulated is selected automatically.

- **Time bins**. This button is available if you define time bins for at least one Result container in your Data profile. Choose this option for example to inspect behavioral changes over time. The matrix is arranged this way:
  - On rows: Result containers, trial names, subject names, and body points in this order.
  - On columns: Time bins ordered by starting time.

  With Time bins, each heatmap represents a segment of a single track.

With the Time bins preset, the Merging method **Cumulated** is selected automatically.

- **Group Mean**. Choose this option when you create two or more Result containers in your Data profile and you want to compare the corresponding heatmaps, just like you would do with Group statistics, or when you just want to inspect the behavior of a whole group of tracks.

  The matrix is arranged this way:

  - On rows: No independent variable. Heatmaps are always arranged in one row.
  - On columns: Result containers, subjects and body-points in this order.

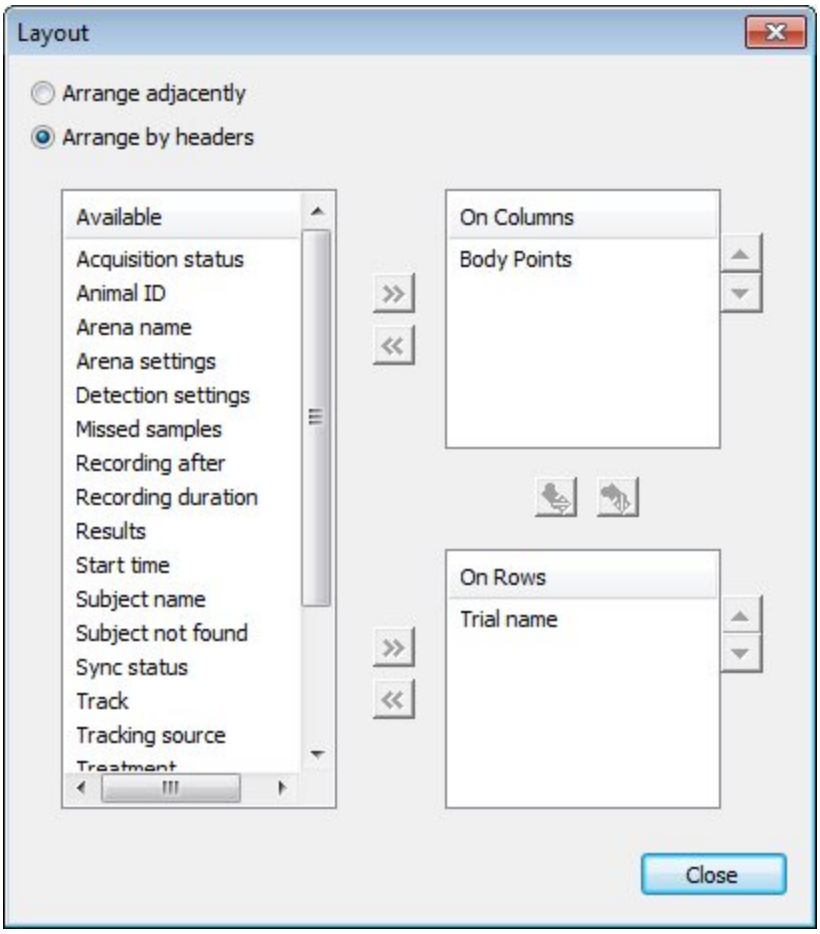
  With Group mean, each heatmap always represents a group of tracks, unless a Result container only contains one track. The Merging method **Mean** is selected automatically.

  **NOTE** This button is not available in experiments defined as **DanioVision experiment**.

## Custom layout

Besides the Layout presets, you can re-arrange the heatmaps by moving and adding/removing the matrix headers.

1. In the Heatmap Settings pane, under **Presets** click the **Custom** button, or click the **Show/Hide** button on the toolbar and select **Layout**.
2. In the Layout window, select **Arrange by headers**.




3. To place headers on the other side of the matrix, select the corresponding element under **On Columns** / **On Rows** and click the **Place Horizontal** or **Place Vertical** button.

To alter the sorting order, select an element and click the arrow buttons to place it in a upper/lower position.

To add a header, select the corresponding element under **Available** and click the arrow button next to **On Columns** or **On Rows**, depending on whether you want to place the new header.

To remove a header, select its name under **On Columns**, or **On Rows** and click the arrow button.

## Notes

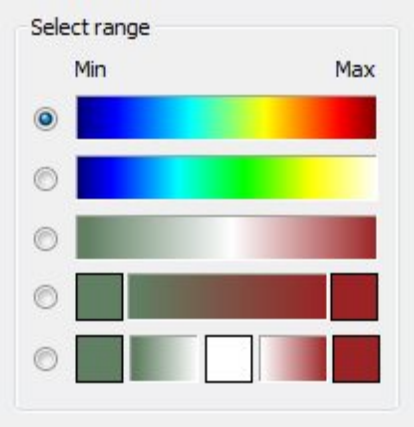
- **TIP** You can also re-arrange the heatmaps from the matrix itself.
  - Right-click a header and select **Place Horizontal**/**Place Vertical** to place the headers on the other side of the matrix; or **Move Up**/**Move Down** to modify the sorting order.
  - To add an independent variable as a new header, right-click the header row/column and select Insert, then choose the independent variable.
  - To remove an independent variable as a header, right-click it and select **Delete**.
- You can only remove a header if that does not generate ambiguities in the heatmap matrix. For example, if your Data profile contains two Result containers, you cannot remove the Results header. For the same reason, you cannot delete the Body Points header because mixing body points in a heatmap does not make sense.
- The **Arrange adjacently** option is equal to clicking the **Adjacent** button under **Presets**.

*See also*

- Sort track plots
- Merging method for heatmaps

# Heatmap colors

## Color range




In the Heatmap Settings pane, click the **Colors** tab. Under **Select range**, select one of the five color ranges:

- From dark blue to dark red (default).
- From dark blue to white.
- From green to red.
- A custom range where the end colors can be changed.
- A custom range where the end and middle colors can be changed.

For the last two options, to change a color, click the corresponding button and choose the color you require.

The end colors represent the minimum and the maximum density of data points per pixel. Whether the colors represent the same value in different heatmaps depends on what you choose as **Color level** (see below).

## Color level

In the Heatmap Settings pane, click the **Colors** tab. Under **Color level**, specify how the color range is mapped to the range of location frequencies between different heatmaps:

- **Per heatmap**. When you choose this option, the colors are mapped to the range of density of points in each heatmap separately. This means that a specific color may represent different densities of points in different heatmaps. Choose this option to compare the utilization of space within heatmaps.
- **Over heatmaps**. When you choose this option, The colors are mapped to the global range of density of points, calculated over all the heatmaps plotted on your screen. This means that a specific color represents the same density of points in different heatmaps. Choose this option to compare the utilization of space between heatmaps.

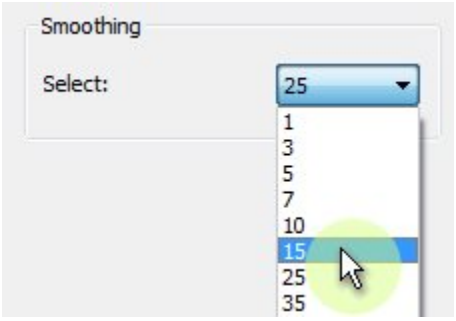
  When you choose this option:

  - The maximum color (e.g. dark red) is only displayed in the heatmap(s) with the highest absolute value of point density.
  - The way heatmaps will look like also depends on what Merging method for heatmaps you choose.

## Color smoothing

With Smoothing, you can specify the distance around a point at which the influence of the point will be felt when determining the color. Larger values result in greater smoothing, but smaller values may show finer details and variation.

1. In the Heatmap Settings pane, click the **Colors** tab.
2. Under **Smoothing**, choose the smoothing intensity (range **1 - 125**).

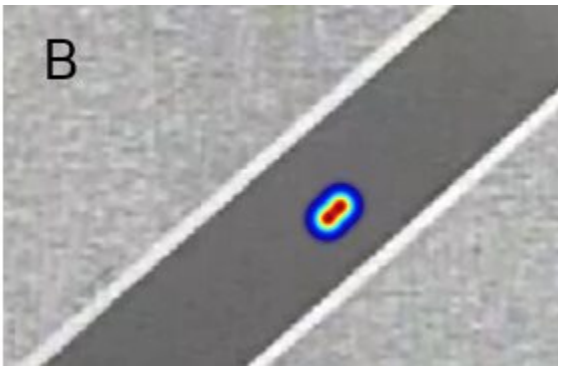



Choose a small value when you work with small arenas, like in well plates, and when you want to view small-scale differences in location.

## Notes

- If your trials include two or more arenas, the options **Per heatmap**/**over heatmaps** do not have an effect on the difference in the color mapping between arenas. However, the duration of the tracks in two different arenas may influence the heatmaps. See an example in Plot heatmaps

- The value of Smoothing is the standard deviation of the Gaussian kernel density function centered on a sample. The heatmap is created by summing up the density functions for the selected data points. Smoothing also influences how many points are represented by a multicolor blob. The following example, shows heatmaps created from two data points. **A**: with Smoothing = 1 pixel. **B**: with Smoothing = 3 pixel.

# Export the heatmaps

You can export a single heatmap, some heatmaps or the whole matrix of heatmaps to image files. A great advantage of this function compared to copy and paste is that you can rescale the heatmap to a resolution higher than that of the original video, resulting in quality pictures ready for publications.

## Export a single heatmap

1. Click the heatmap you want to export, so that its border becomes highlighted in red.
2. Right-click the heatmap and select **Export image**, or on the toolbar click the **Export image** icon.

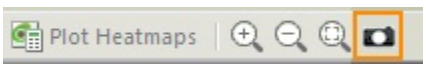

3. Select the image **Resolution** (width x height) in pixels.
4. Click **Export**. In the Export Image window, enter the name and location of your image file, choose the image format and click **Save**.

**TIP** You can also copy and paste single heatmaps: click a heatmap, right-click and select **Copy** or press **Ctrl+C**, then paste the picture to an external program. You cannot copy/paste multiple heatmaps.

## Export multiple heatmaps

1. Select the heatmaps you want to export.
   - To export all heatmaps in the matrix, make sure that no heatmap is highlighted. Click outside the heatmaps area to remove any selection.
   - To export multiple, non-adjacent heatmaps, hold **Ctrl** down and click the individual heatmaps.
   - To export multiple, adjacent heatmaps, hold **Shift** down, then click the first, then the last of the heatmaps you want to export.

   *Result*: The border of the selected heatmaps becomes highlighted in red.
2. On the toolbar click the **Export image** icon.




   If you have selected some heatmaps, you can also right-click anywhere over the selected heatmaps and choose **Export image**.

3. Under **Export**, choose one of the options: **To separate image files** or **To one image file**.
4. Select the image **Resolution** (width x height) in pixels.

   **TIP** To ensure that each heatmap has a sufficient resolution, under **Export** select first **To separate image files**, select the resolution you require and then choose **To one image file**. The image resolution (width, height, or both) is increased by a number of times depending on how many heatmaps are in the rows and columns.
5. Choose **Independent variable as overlay** to have the trial information (headers) displayed on the exported images.
6. Click **Export**. In the Export Image window, enter the name and location of your image file, choose the image format and click **Save**.

## Notes

- The available image resolutions depend on the aspect ratio of the original background pictures. The zoom level chosen for the plots affects the default resolution.
- To choose the appropriate information to display on the heatmaps, see Custom layout in Arrange heatmaps.
- The suggested export file name is Heatmap Image + a progressive number. For heatmaps exported to separate files, the name of the exported file is the name you enter in the Export Image window followed by the row headers and the column headers. For example Heatmap Image 0001 - Trial 1 - Center point.

# Plot integrated data

## What do you want to do?

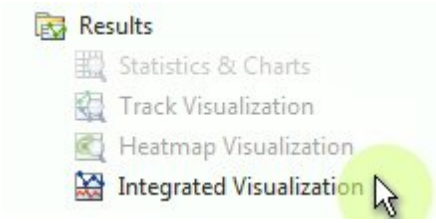
- Plot integrated data
- View and interpret variable plots
- Customize the dependent variable plots
- Export the integrated visualization
- Create a video from your data

## Learn about

- Dependent variable plots
- External data plots
- Trial control events pane

# Plot integrated data

Choose **Integrated Visualization** to visualize a set of tracks obtained from one tracking session (trial), together with video, external data (if applicable), and plots of your dependent variables (including manually scored behaviors and external data).




This function is particularly useful when you want to compare tracks with the corresponding video.

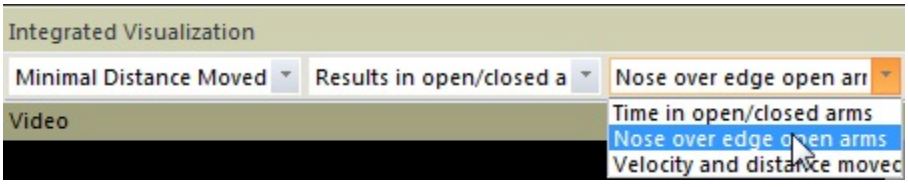
**IMPORTANT** You can visualize up to 200 plots at the same time. If the number of subjects/arenas times the number of dependent variables currently selected exceeds this limit, filter the subjects or arenas in your Data Profile, or remove some dependent variables from your Analysis Profile.

## Prerequisite

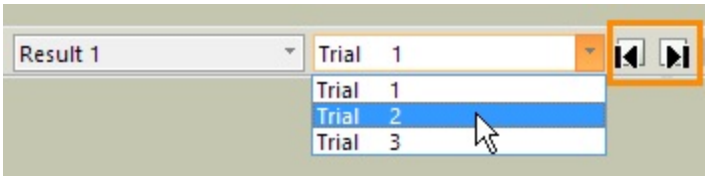
Make sure that the dependent variables you want to plot are specified in the active Analysis profile.

## To plot integrated data

1. Choose **Analysis** > **Results** > **Plot Integrated Data**, or in the Experiment Explorer, under **Results**, click **Integrated Visualization**.
2. On the toolbar, choose the Track Smoothing profile, the Data profile and the Analysis Profile that you want to use.




3. On the toolbar, choose the selection result and the trial you want to visualize. By default, the first trial is selected. To select the next/previous trial, click the buttons next to the drop-down list.




The selection result list is available if your current Data profile includes two or more selection results.

4. **OPTIONAL** Customize tracks and plots on page 916.
5. View and interpret variable plots on page 901.

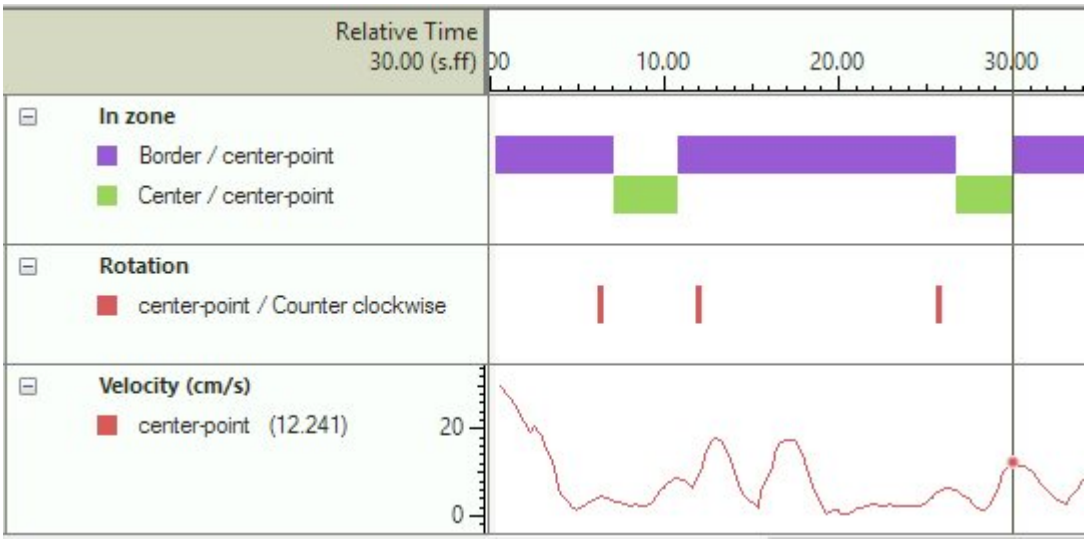
## Notes

- In the Integrated Visualization screen you can also find one or more External Data plots, depending on how many external data files have been imported in that trial. See External data plots
- Not all playback speeds might be available. Depending on the video format, you may not be able to play the video at a specific speed.
- If your data selection contains nesting criteria, all samples are plotted but the samples excluded by nesting are shown shaded.
- You can visualize up to 200 plots per trial. If the number of dependent variables in your analysis profile times the number of subjects/arenas exceeds this limit, a message informs you that not all charts are visualized. Filter the subjects/arenas in your Data Profile, or remove some variables in your Analysis Profile.
- The begin time in the video window is the time of the first sample acquired in all arenas.
- The end time in the video window is the time of the last sample from all arenas. Within each arena, the last sample is marked by the message End of track over the top-left corner of the corresponding arena.
- For multi-arena setups, the tracks are synchronized, that is, samples of different arenas visualized at a given moment were acquired at the same absolute time.

# Dependent variable plots

Dependent variable plots show the values of the dependent variables selected in your active Analysis profile, on a time line. Dependent variables are displayed in different ways depending on their type (see the picture below):

- States like *In zone*: colored bars indicating the state's duration.
- Point events like *Rotation*: vertical segments indicating the event's occurrence, with no duration.
- Continuous variables like *Velocity*: values are plotted as a time series, with one value per sample time,



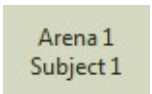

## Procedure

To plot a dependent variable:

1. Define the variable. Calculate statistics: procedure.
2. See Plot integrated data.

## Scope of the Dependent variable plot

When the trial includes more than one arena or subject, the plot's header indicates which arena and the subject it refers to.



## Why use Dependent variable plots?

Use Dependent variable plots are especially useful:

- To check whether you set correct thresholds for state variables like Body elongation, Movement, Mobility and Activity.

  a) Open your analysis profile, double-click the variable's name and enter your best estimate for the thresholds.

  b) Plot integrated data. Play back your data, watch the video and check whether the states shown in the plot correspond with the states of the animals in the video.

  If necessary, repeat steps a and b.

- To check that you have scored behaviors manually at a good level of accuracy.

  a) In your Analysis profile, select the manually scored behaviors you want to check.

  b) Plot integrated data. Play back your data, watch the video and check whether the states shown in the plot correspond with the behavior of the animals in the video.

  If necessary, export the data to The Observer XT, where you can correct the errors.

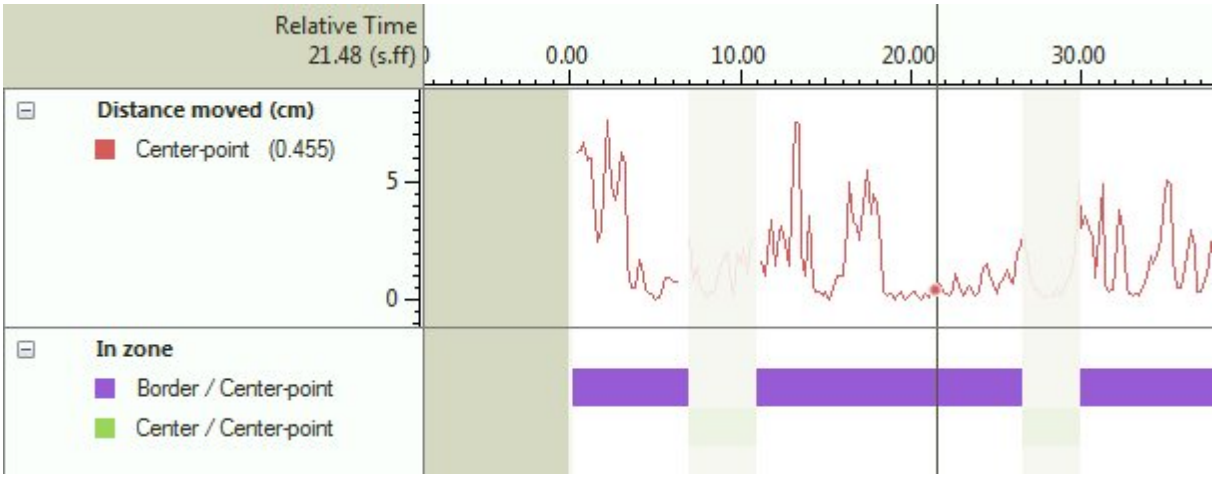
- To view the behaviors of Behavior Recognition together with their probability values. This helps when you want to refine the definition of those behaviors based on their probability value.
- To check whether your Trial Control Settings do what you want them to do. You can visualize Trial Control events and Trial Control states. For more information about Trial Control, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

# View and interpret variable plots

## Effect of data selection on plots

If the data profile contains nesting criteria, the selected intervals are shown with a white background. The non-selected intervals are shown semi-transparent enabling you to see the excluded data in these intervals.

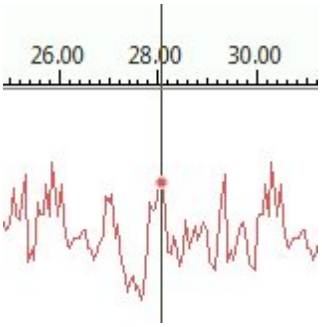
This example shows a plot of the dependent variables 'Distance moved' and 'In zone' after nesting over the border zone of the open field.



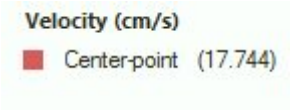

If the data profile contains time bins, these are not visualized.

## Navigate through the data

Use the scrollbar at the bottom to run through the visualization. The hairline represents the current time in the trial, and is always fixed in the middle of the plot area. For numerical variables, the current sample is highlighted with a circle.




The current value of the variable is also shown near the variable name.

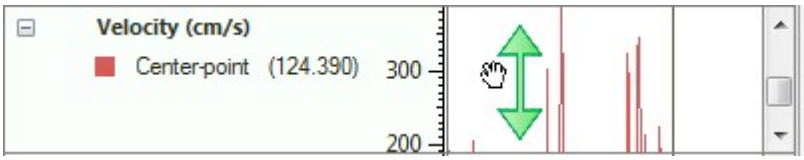



To move from the start to the end of the trial, you can also:

- Click anywhere in the plot, hold the left mouse button and drag the background.
- Hold the **Shift** key down and scroll with the mouse wheel. This way you zoom in/out while the cursor moves forward/backward by one time span.

  **NOTE** Each wheel step corresponds to the time span chosen from the **Show/Hide** menu.

If part of the data plot is not visible at the current zoom level, you can either zoom out or move the plot line. Use the scrollbar at the right end of the plot area, or drag the background of the plot. See also View and interpret variable plots

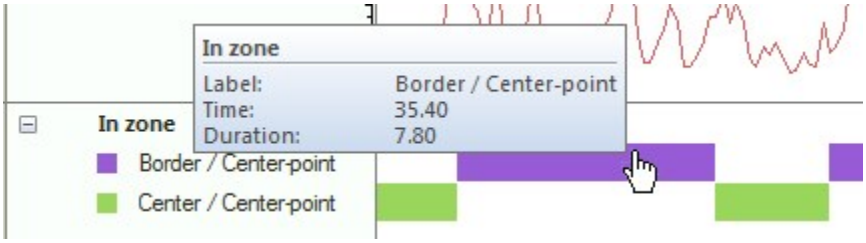



**TIP** To visualize the whole vertical data range, zoom out the plot until you no longer see the vertical scrollbar.

## Visualize a single variable value

- *For discrete variables* (states or events). Each state or event is shown as a colored bar. When a variable includes multiple states like In zone (with different zones being defined) or Mobility (with its states Immobile, Mobile, Highly mobile), these are shown as parallel channels.

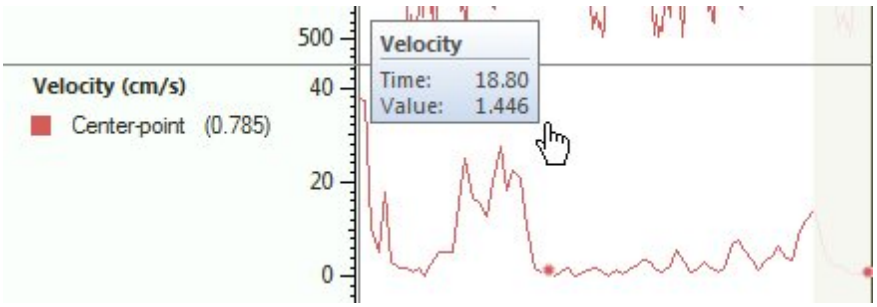
  Keep the mouse pointer above a colored bar. A tool tip is shown with the following information:




  - The name of the dependent variable.
  - Label: the name of the state or event.

- Time: The sample time the mouse pointer points to.
- Duration: the length of the state, in seconds. For point events like Rotation or Trial Control events, the duration is not shown.

- *For numerical variables* (including external data). Values are connected with lines. Keep the mouse pointer over the data plot. A tooltip is shown with the following information:

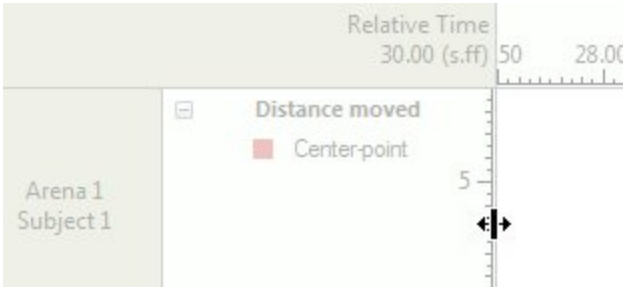



- The name of the dependent variable.
- The sample Time selected.
- The value of the dependent variable at that time. This is indicated by a circle on the plot line.

**IMPORTANT** Although the plot lines may look continuous, the numerical variable values are not interpolated and do not become continuous. For data selection and analysis, the discrete samples are used.

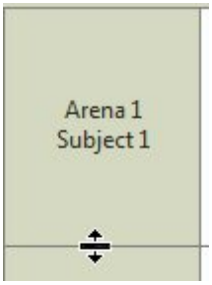
## Resize the plots

- *Resize the time window displayed*. Point to the y-axis in the upper part of the plot, so the mouse pointer turns to a double arrow. Drag the y-axis to the desired position to resize the time window.

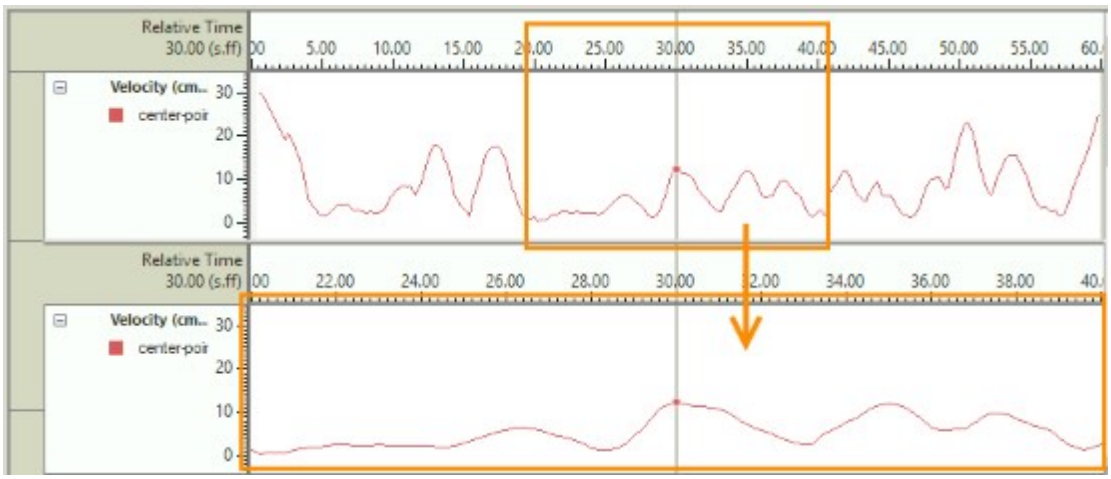



- *Resize the width of the plot*. Point to the lower margin of the gray cell on the far left of the plot so the mouse pointer turns to a double arrow. Drag the margin to the desired position to have a larger or narrower plot.

**NOTE** The data of continuous variables are stretched/condensed when you resize the plot, but the range of data does not change.




## Adjust the time scale (x-axis)




Do one of the following:

- Click the **Zoom in** or **Zoom out** button on the toolbar, then click the time scale one or more times.
- Right-click the plot and select **Zoom in** or **Zoom out**. Next, click the time scale one or more times.
- Press **(Ctrl+ .)** and **(Ctrl+ ,)** to zoom in/out, respectively.
- Click the time scale, hold the **Ctrl** key down and turn the mouse wheel forward (to zoom in) and backward (to zoom out).

For the first two methods: To stop zooming in/out and return to the normal mouse pointer, click the **No Zoom** button on the toolbar, or right-click the plot and select **No zoom**.

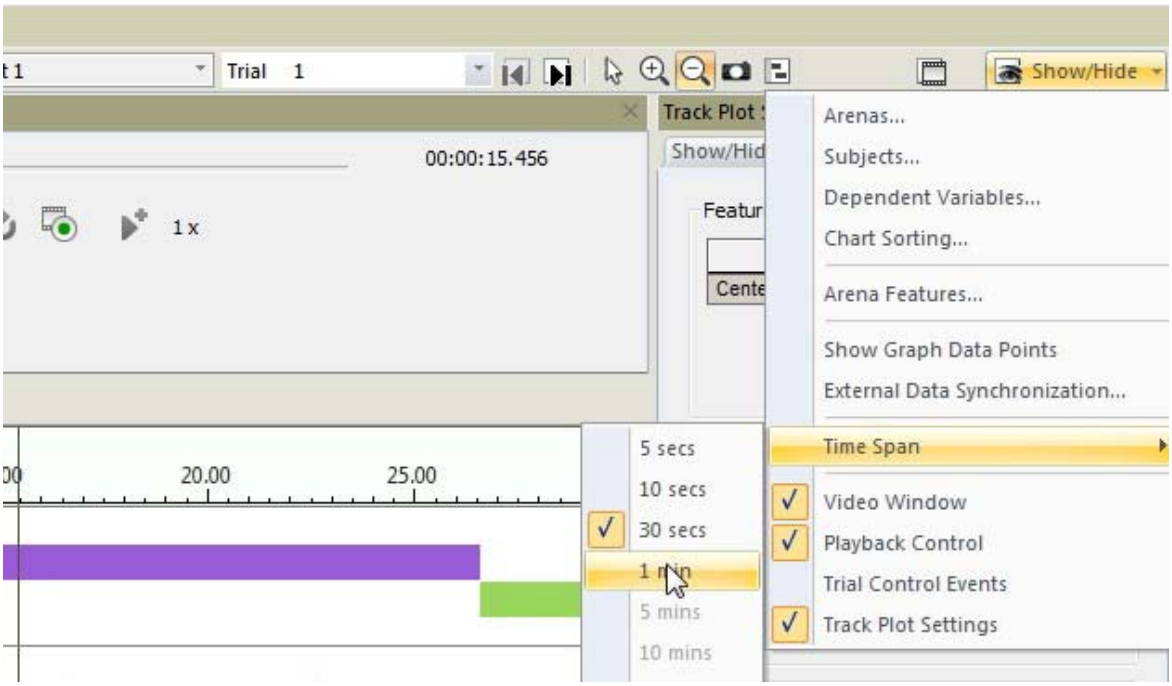
*Notes*

- Every time you zoom in/out, the plot scale is enlarged or reduced by a factor of 2.

- The shortest time that you can display is 0.1 seconds, the maximum time seven days.
- When you zoom in/out by clicking the plot, the clicked time becomes the new current time.

## Set a specific time span

The time span is the duration of the time visible in the time axis at any moment. To set a specific time span, click the **Show/Hide** button on the toolbar, then click **Time span** and select one of the values available (minimum 5 sec; maximum 1 hour).




**NOTE** The available values depend on the length of your trial. For example, if your trial is 10 minutes long, the 30 minutes and the 1 hour options are not available.

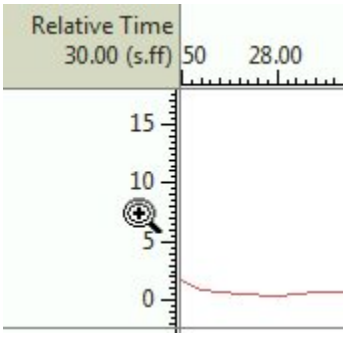
## Zoom the y-axis in/out

You can adjust the range of values for continuous variables (dependent variables and external data). If you have two or more continuous data plots, the variable scale changes only for the plot you have clicked.

1. Do one of the following:
   - Click the **Zoom in** button or the **Zoom out** button on the toolbar.
   - Right-click the plot and select **Zoom in** or **Zoom out**.

   *Result*: The mouse pointer turns to a magnifier symbol.

2. Click the y-axis scale one or few times.




3. To stop zooming in/out and return to the normal mouse pointer, click the **No Zoom** button on the toolbar, or right-click the plot and select **No zoom**.

Every time you zoom in/out, the plot scale is enlarged or reduced by a factor of 2.

**TIP** To zoom in/out the y-axis in all plots, hold the **Ctrl** and **Alt** keys down and scroll with the mouse wheel.

# Customize the dependent variable plots

## Show and hide the plots

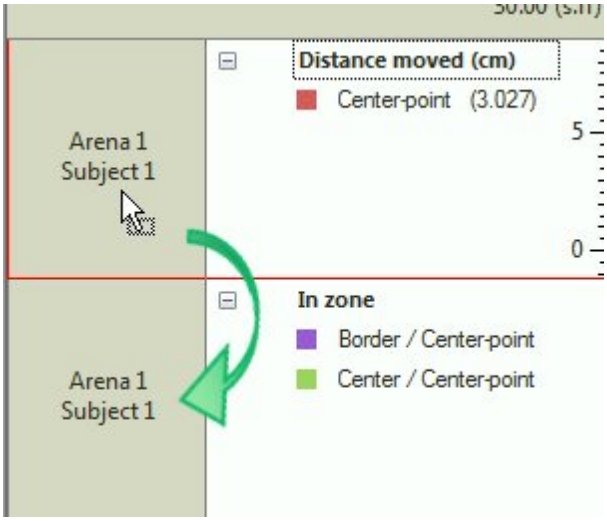
Click the **Show/Hide** button, select **Dependent Variables** and then choose the items you want to display. By default, all external data files are selected for that trial unless the associated tracks have been filtered out in the Data selection.

## Sort the plots

You can change the order in which data plots are displayed by dragging them up and down on the screen.

1. Click the leftmost column next to the plot you want to move.

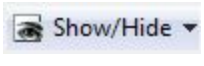
   Result: The cursor changes and the plot border is highlighted in red.



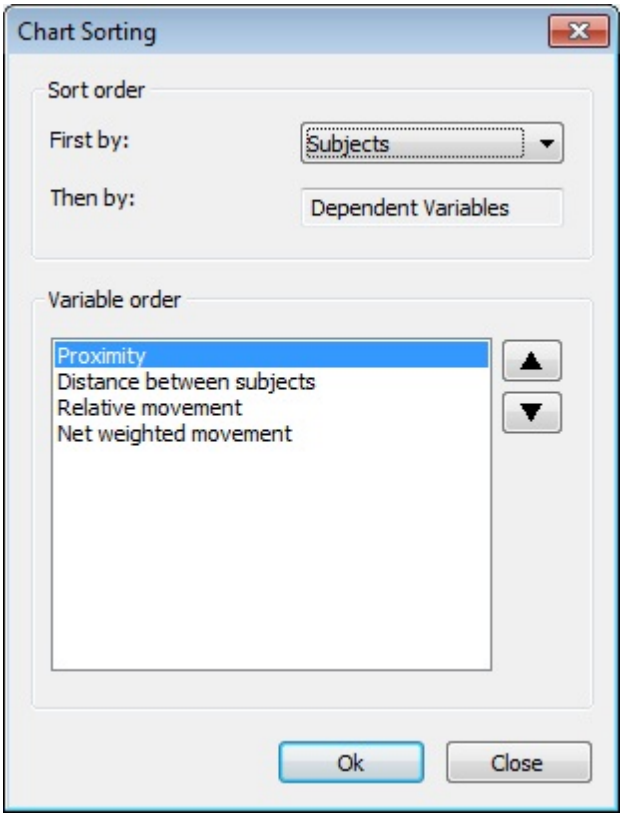

2. Drag the plot up or down to the position you require. Release the mouse button to insert the plot.

You can also sort plots by dependent variable, subject name, or arena name. To do so:

1. Click the **Show/Hide** button, then select **Chart Sorting**.

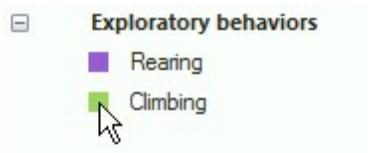



2. In the Chart Sorting window, if there are multiple sorting factors, like dependent variables and arena names, choose them under **Sort order**.
3. Under **Variable order**, click the arrow buttons to sort the dependent variables.




## Change the color of a plot

1. Click the square symbol next to the name of the body point, subject, or behavioral state.



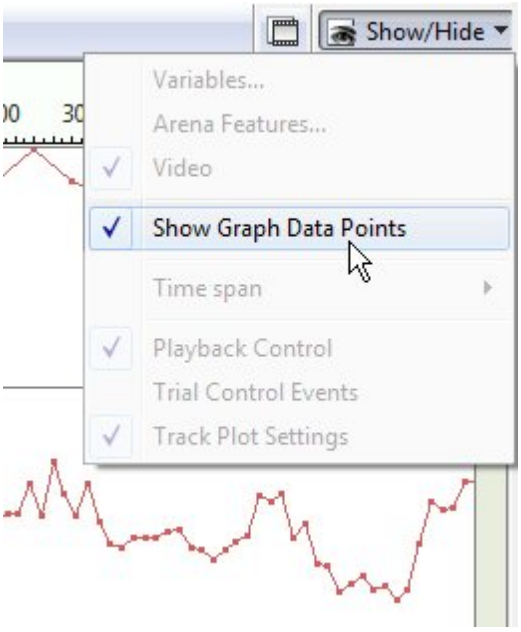

   Alternatively, click next to the data line (for numerical variable plots) or the color bar (for the discrete variables).

2. In the Color window, choose a new color and click **OK**.

**NOTE** For color bars, you can also choose a hatch pattern.

## Show and hide the data points

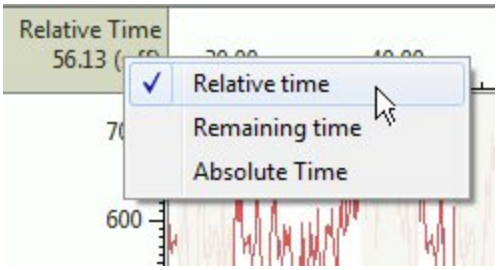
Click the **Show/Hide** button on the toolbar, and select/deselect **Show Graph Data Points**.




## Specify the time mode

You can choose to display time values on the x-axis as elapsed time, remaining time (that is, time to the end of the trial) or the actual time.

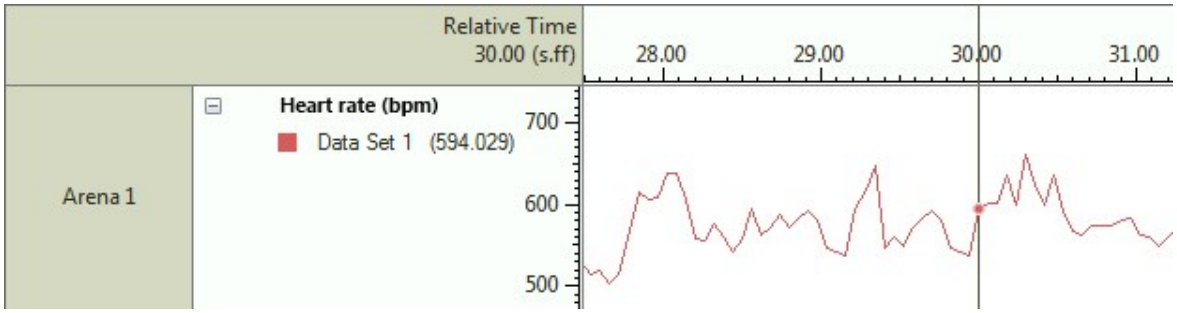
1. Right-click the top-left corner of the chart with the time label.




2. Select one of the following:
   - **Relative time**. The time elapsed since the start of the trial. The 0:00:00.00 time on the far left side corresponds to the start of the trial.
   - **Remaining time**. The time to the end of the trial. The 0:00:00.00 time on the far right side corresponds to the end of the trial.
   - **Absolute time**. The actual date and time the trial was acquired (for example 11/13/2017 16:29:30.00).

# External data plots

External data are shown in the form of X-Y plots. The start and end time of the tracks does not always coincide with the start and end time of the co-acquired external data. When you visualize the integrated data, you can only view the external data acquired in the same time interval as the tracks associated with the external data file. External data samples acquired before the start and after the end of the tracks are not plotted.

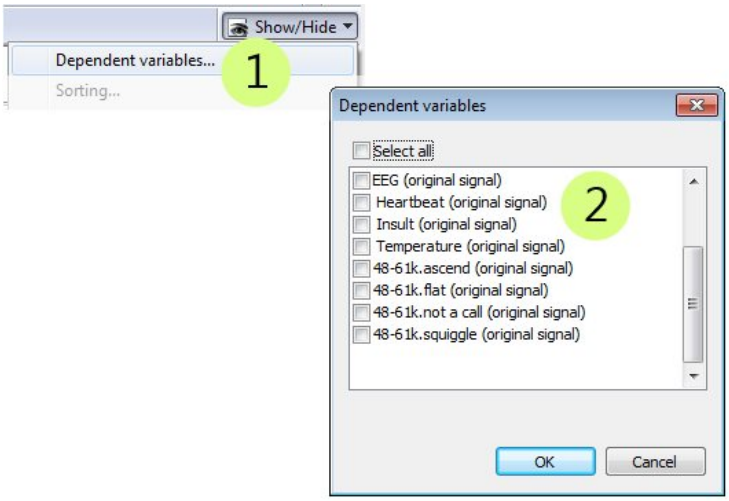



The hairline shows the current video/track position. The current value of heart rate is shown next to the data set name.

By default the external data are not plotted in the Integrated Visualization.

## Plot the original signals

1. Choose **Show/Hide** > **Dependent Variables**.
2. Select the [data set name] (original signal) that you require.




By default the original signal is plotted in green.

## Plot the resampled signals

1. Define the re-sampled signal in the Analysis profile. See External data (resampled).
2. Choose **Analysis** > **Results** > **Integrated Visualization**.

By default, resampled signals are plotted in red.

- Select **Show/Hide** > **Show Graph Data Points** to better view the original and resampled signals.
- If you upsample a signal with a sample rate much lower than the EthoVision sample rate, and you choose **Missing value** as the Upsampling method, it may be difficult to see the data points. Choose **Show/Hide** > **Show Graph Data Points** and zoom in the plot.

## Synchronize the external data with the tracks

Click the **Synchronize External Data** button.

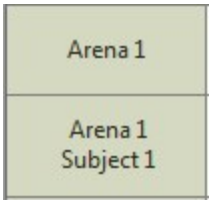
Either change the **Start date and time** and **Stop date and time**, or move the external data plot to the correct position. For details, see Synchronize data manually after import.

## The plot's titles

The titles are shown at the left of the plots and vary according to what level the external data have been linked at.

- If linked to the subject level, the title shows the Arena and Subject names.
- If linked to the arena level, the title shows the Arena name.
- If linked to the whole trial, the title is not shown.

In the example below, the first plot was linked at the Arena level, the second at the subject level.



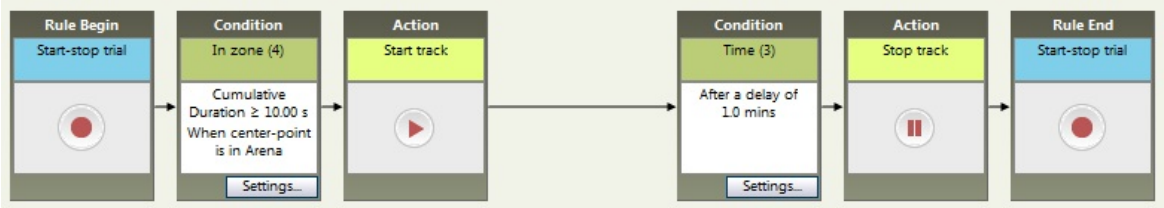

See also Customize tracks and plots.

# Trial control events pane

The Trial Control Events pane gives you an overview of the events that occurred during the trial as a result of the Trial Control Settings you specified.


The corresponding Trial Control profile is shown below.

The first condition (the center-point of the mouse is in the arena for more than 2 second) is met after 2 seconds. At that moment the track is started. One minute later, the track is stopped. During playback, the hairline indicates the current position.




## Customize the visualization of the Trial Control Events pane

By default, the Trial Control Events pane is shown in the lower right corner of the screen. Choose one of the following options to change the visualization of the pane:

- To show the pane, click the **Show/Hide** button on the toolbar and select **Trial Control Events**.
- To move the pane, click its title bar and drag it to the desired position. You can either dock the pane or let it float on the screen.
- To resize the pane, point with your mouse to either the pane's upper margin or its left margin so the mouse pointer turns to a double arrow. Drag the margin to the desired position to have a larger pane. Alternatively, click the small black triangle in the upper right corner of the pane to have a full view of the pane and hide the Track Plot Settings pane.

# Export the integrated visualization

## Aim

To export Dependent variable plots in form of image files.

## Procedure

1. Make sure that no other window is on top of the plots. Zoom the plots in/ out to reach the level of detail you require for the export picture.
2. On the toolbar, click the **Export** image icon.

3. In the Export Image window, enter the name and location of your image file.
4. From the **Save as type** list, select one of the available formats, then click **Save**.

*See also*

- Adjust the time scale (x-axis)
- Zoom the y-axis in/out

# Create a video from your data

## Aim

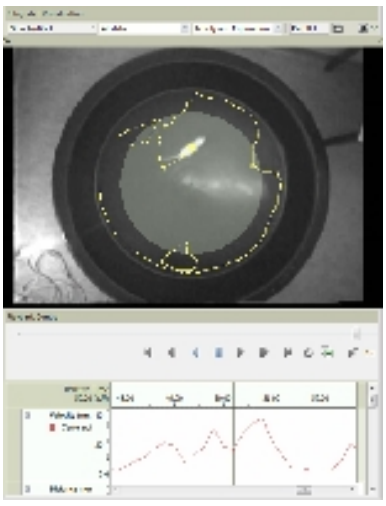
To export the animated track and the plots of the dependent variables to a video file, for instance for presentation purposes.

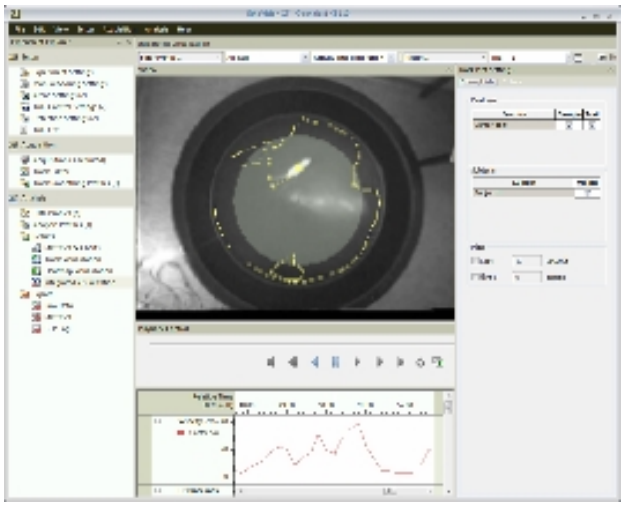
## Procedure

1. Choose **Analysis** > **Results** > **Integrated Visualization**.
2. From the toolbar, select the Analysis profile that contains the variables to plot.
3. If you do not want the Track Plot Settings pane be part of the screen capture, click the **Show/Hide** button on the toolbar and deselect **Track Plot Settings**.
4. Position the video where you want your recording to start, or click the **Jump to begin** button to start from the beginning of the trial.

5. Click the **Start screen recording** button.

6. In the Screen Recording window, under **Region**, choose whether you want to capture the **Video, tracks and plots** (see the example below, left) or the **Full screen** (right):





Note that with **Full screen** you also record the Experiment Explorer and the desktop (if visible).

7. Choose the **Frame rate** (default: 25 fps) and the **Resolution** (default width: 1280; see the note below).
8. Choose the video file **Saving location** and **File name**.
9. Click **Start**. EthoVision XT shows the message:


10. Play the video at the speed you require.
11. To stop screen recording, click the **Stop screen recording** button.

## Notes

- If you choose **Video, tracks and plots**, the Track Plots Settings pane is also recorded. To remove this from your video, see step 3.
- Video resolution is available in a number of steps. The values of width are 320, 480, 640, 800, 1024, 1280 (default), and 1600 pixels. The values of height are adjusted in each step, based on the Region option you choose, and the current size of the EthoVision screen.
- The video file is saved in DivX format; the file extension is *.mpg.
- The suggested video file name is **Screen Recording n**, where n is a progressive number (0001, 0002, etc.).

# Customize tracks and plots

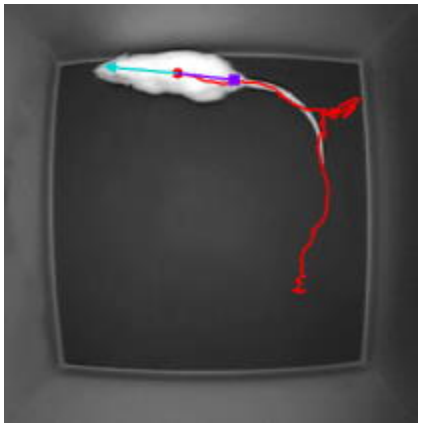
## What do you want to do?

- Customize the elements and length of the tracks
- Customize the track colors
- Display background, arenas and zones

# Customize the elements and length of the tracks

## Aim

To optimize visibility of data points.



## To access these options

Choose **Analysis** > **Results** > **Plot Tracks** or **Plot Integrated Data**. In the Track Plot Settings pane, and click the **Show/Hide** tab.

## What do you want to do?

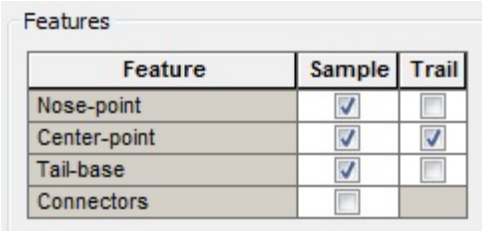
- Show and hide body points
- Adjust line thickness and dot size
- Show and hide subjects
- Show part of the track

## Show and hide body points

Under **Features**, select the following:

- **Nose-point** (only for nose-tail tracking).
- **Center-point**.
- **Tail-base** (only for nose-tail tracking).

- **Connectors** (only for nose-tail tracking) to display the line connecting the nose-point and the tail-base to the center-point.




To view the body points, select the option under **Sample** next to that body point.

To view the line connecting samples for a body point, select the **Trail** option next to that body point.

The nose-point is always represented by a filled triangle, the center-point by a filled circle and the tail-base by a filled square. You cannot change the shape of body points in your tracks.

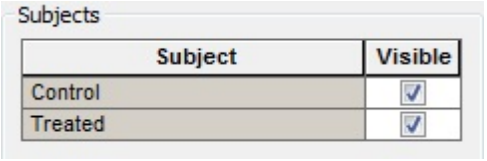
## Adjust line thickness and dot size

In the Show/Hide pane on the right, under **Features**, choose a value for:

- **Line thickness**. This changes the thickness of the tracks and the connectors, that is, the segments joining the body point.
- **Dot size**. This changes the size of the body points along the tracks.

## Show and hide subjects

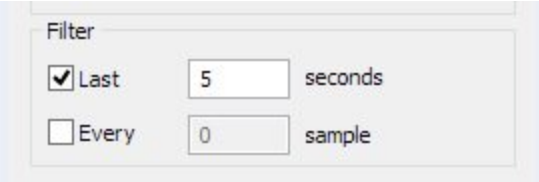
When you track multiple subjects in the same arena, under **Subjects** select the **Visible** option for the subjects you want to have displayed and clear the selection for the others.



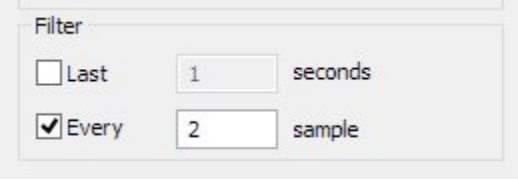

## Show part of the track

Under **Filter**, choose how many samples you want to display at the same time.

- **Last ... seconds**. Choose this option to visualize only part of it, for instance only the last 5 seconds.



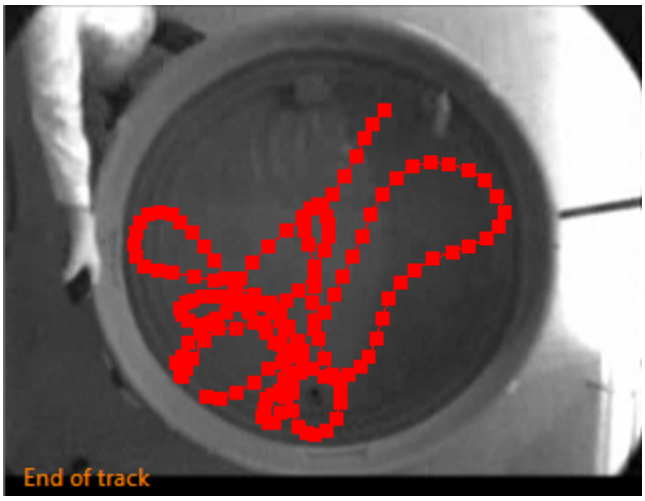

- **Every ... sample**. If the subject moves slowly, the samples are very dense. Choose this option to visualize, for example, every 5th sample.



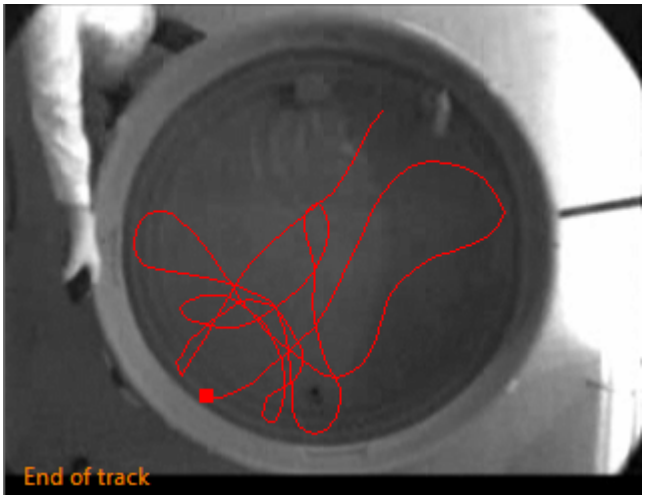

## Tips

- To show the entire track, deselect **Last ... seconds** and click the **Jump to End** button in the Playback Control window.

- To show all samples in the track, select **Every ... sample** and enter **1**.




- To only show the last sample, select **Every ... sample**, and enter **0** next to it. Make sure that in the **Features** box the option under **Sample** is selected for the body point you want to plot (see above in this topic).


End of track

# Customize the track colors

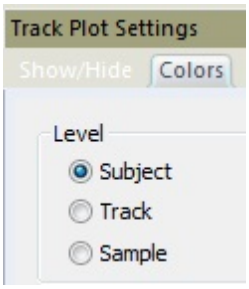
## Aim

To plot data in colors depending on the subject, the treatment level, or other variables of interest.

## To access these options

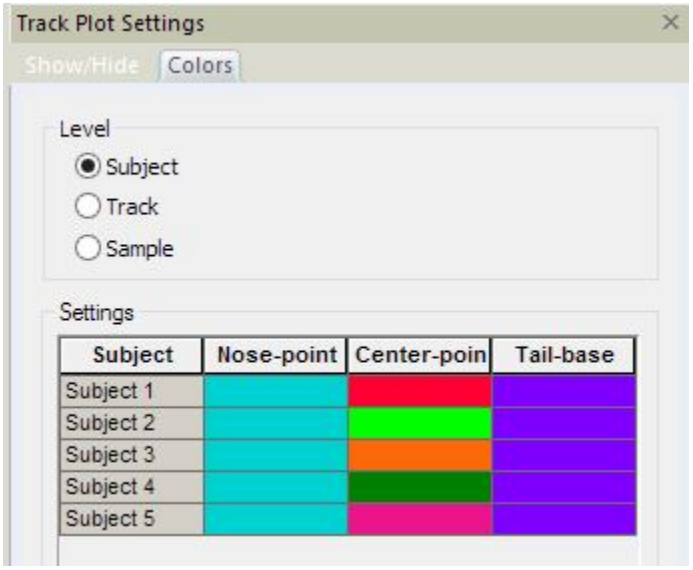
Choose **Analysis** > **Results** > **Plot Tracks** or **Plot Integrated Data**. In the Track Plot Settings pane, and click the **Colors** tab.

Specify how colors should vary, for example between arenas, or within tracks.




## Subject

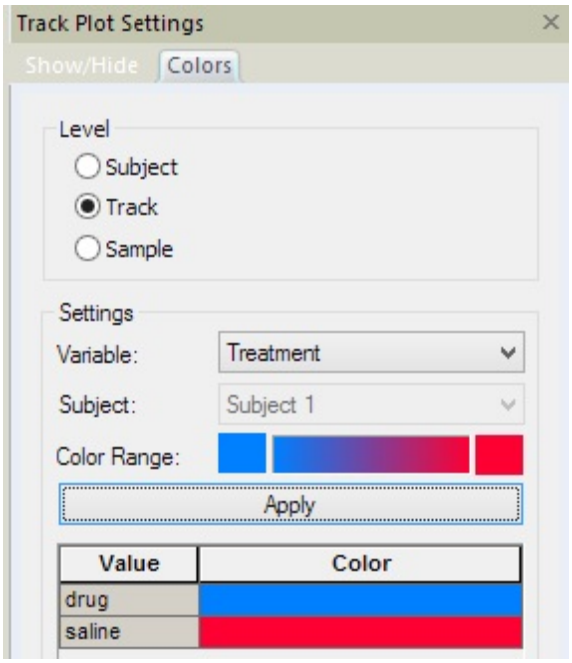
Under **Level**, select **Subject**. To change the color for a subject and a body point, click the corresponding cell under **Settings**.



- If you tracked more than one subject per arena, choose this option to have the tracks of different subjects displayed in different colors. By default, different subjects have different colors for the center-point, and the same color for the nose- and tail-base points.
- If you tracked subjects in multiple arenas, the track of Subject 1 in Arena 1 is displayed in the same color as that of Subject 1 in Arena 2, Arena 3, etc.

## Track

Under **Level**, select **Track** to have tracks in colors based on the value of an independent variable, for example to show subjects in colors based on the treatment level.

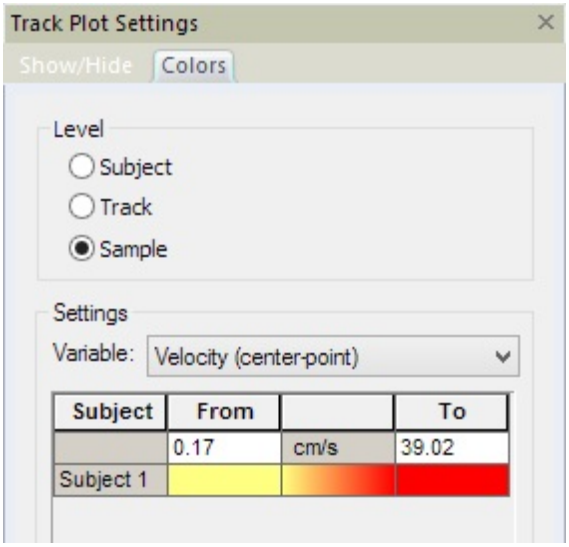



1. Select an independent variable from the **Variable** list.
2. Click the buttons next to **Color Range** and select the colors that you want at the ends of the range. Next, click **Apply** to assign colors automatically.

   Alternatively, for each value of the variable selected, click the color cell and choose the color you want to assign to the tracks with that value.
3. Do the same for each subject in the arena.

## Sample

Under **Level**, select **Sample** to have the samples within tracks displayed in a color depending on the value of a dependent variable, for example velocity. This way you can see where changes in behavior took place.



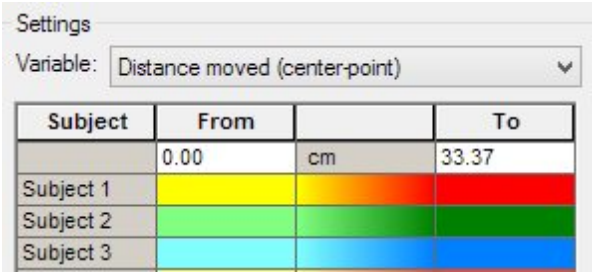

1. Select a dependent variable from the **Variable** list.

   The list shows the dependent variables selected in the currently active Analysis profile. If you do not see the variable you want, open the Analysis profile and include that variable, or from the list on the toolbar select the analysis profile that contains the variable.

2. Click the cell under **From/To** and choose a color for the lowest/highest value of the dependent variable, respectively. EthoVision calculates the spectrum of colors in between and shows it in the middle cell.

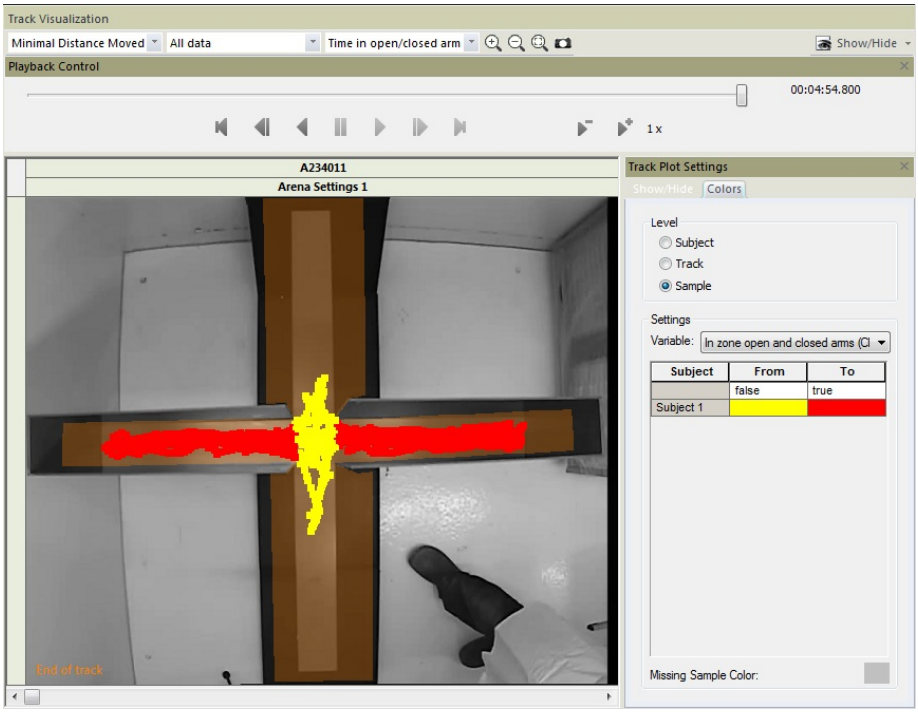
   The lowest and highest values are shown under **From** and **To**. For State variables, those are **false** and **true**. For continuous variables, those are the minimum and maximum value measured for that variable in the trial. For example, 0 to 33.37 for *Distance moved*.

3. If you tracked more than one subject per arena, repeat step 2 for each subject.




Below: Example of a track plot where **Sample** is selected under **Level**. Samples are displayed in red when the subject was in any of the closed arms of the Elevated plus maze. In the Analysis profile, a dependent variable *In zone* is defined for the Center-point in any of the closed arm (the **When in any of the selected zones** option is selected in the **In zone** settings dialog). When plotting the tracks, samples in any of

the open arms are displayed in the color selected for the **true** value of the In zone variable. Other samples are displayed in the color under **false**.




## Customize the color of missing samples

Missing sample points are always visualized in the track plot. You can change the color of missing samples to distinguish them more easily from the actual data. The current color for missing samples is indicated next to **Missing Sample Color**. To change the color for missing samples, click the button and select a new color in the Color window.

# Display background, arenas and zones

## Aim

To customize how the background image, the arena and the zones are displayed in Track Visualization or Integrated Visualization.

## Customize the background (for Track Visualization)

1. Choose **Analysis** > **Results** > **Plot Tracks**.
2. On the toolbar, click the **Show/Hide** button and select **Background**.
3. Select one of the following, then click **OK**.
    - **Plain**. To have a uniform background. Click the button next to this option to select a color. To select colors other than the basic ones, click the button **Other**.
    - **Captured image**. If you want to have the captured image from the Arena Settings as background.

## Show/hide the video image (for Integrated Visualization)

Choose **Analysis** > **Results** > **Plot Integrated Data**.

The Video window is shown by default. To hide the Video window, click the **Show/Hide** button on the toolbar and deselect **Video Window**.

## Show/hide arenas and zones in the track plots

Arenas, zones and points can be displayed on top of the background as overlay objects. The color of each arena and zone depends on the color chosen in the Arena Settings.

1. Choose **Analysis** > **Results** > **Plot Tracks** or **Plot Integrated Data**.
2. Click the **Show/Hide** button on the toolbar and select **Arena Features**.
3. Select the arena feature you want to have displayed.

Arenas and zones are shown in a semi-transparent color, so their appearance also depends on what you select as background.

If you want to change the color of an arena or zone group, open the appropriate Arena Settings, click the correct Color row and select the color of your choice.

For a multi-arena setup, you cannot select to view some arenas and not others.

# Calculate Statistics

## Main topics and tasks

# Calculate statistics

## What do you want to do?

- Calculate statistics: procedure
- Edit the Analysis profile
- Export the statistics result

## Learn about

- Statistics available

# Calculate statistics: procedure

## Aim

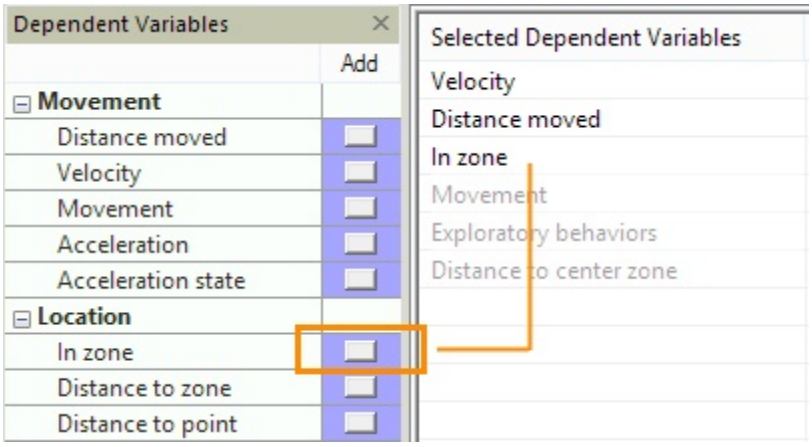
To calculate descriptive statistics of one or more dependent variables.

## Prerequisite

- You have smoothed data (optional).
- You have selected data in a Data profile (optional).

## To calculate statistics

1. Choose **Analysis** > **Analysis Profile** > **Open** (to open an existing profile) or **New** (enter a name for the new profile), then click **OK**.
2. Choose the dependent variable. Click the **Add** button next to the dependent variable you want to use for analysis. If you do not see that variable name, click the **More** button.

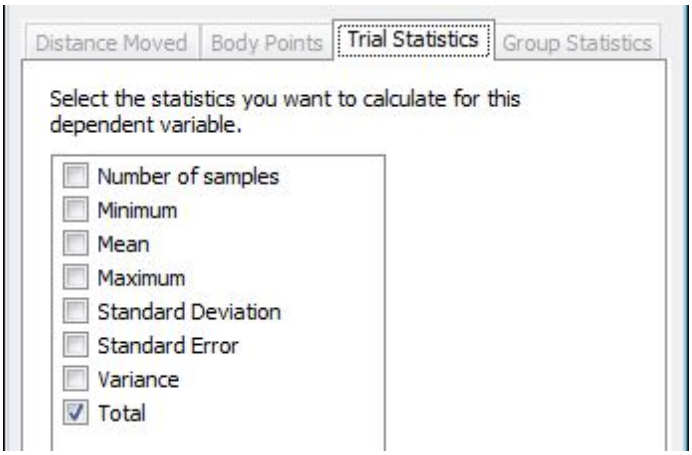



**TIP** In most cases Distance moved and Velocity are already selected.

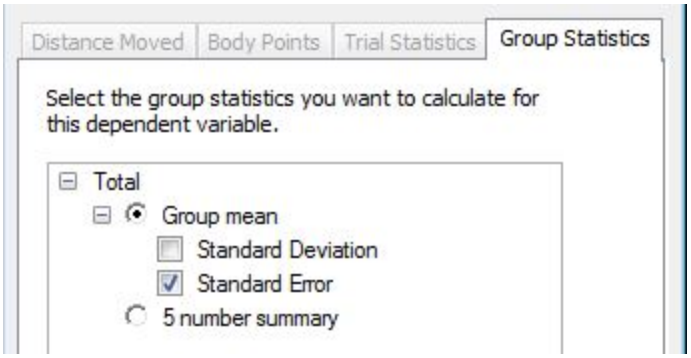
3. In the dependent variable-specific tab, select the properties of the dependent variable.

   For details on dependent variables, see Dependent Variables in Detail.

4. Choose the Trial statistics. Click the **Trial Statistics** tab, and choose the statistics you want to calculate for each trial.



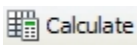

5. Choose the Group statistics. Click the **Group Statistics** tab, choose the statistics you want to calculate as a summary for a group.




**EXAMPLE** You want to calculate the average and standard error of the Total distance moved, for Control and Treated subjects (we assume here that you create such groups in the Data profile; see Analyze groups of tracks.

Choose Distance moved as a dependent variable, **Total** as a Trial statistic, and **Mean** and **Standard Error** as Group statistics.

6. Repeat steps **2** to **5** to add more dependent variables.
7. Choose **Analysis** > **Results** > **Statistics and Charts**, then click the **Calculate** button on the toolbar.




8. The **Statistics and Charts** table appears on the screen.

## Notes

- Some dependent variables are available if you have one or more of the following add-on modules: Social Interaction, Rat/Mouse Behavior Recognition, Trial and Hardware Control.
- You only can select specific body points if your experiment is set to Center-point, nose-point and tail-base detection.
- You can change the properties or delete a dependent variable already selected. You can also add multiple instances of a dependent variable, with different settings. See Edit the Analysis profile
- You can also calculate statistics in batch mode. See Batch calculation

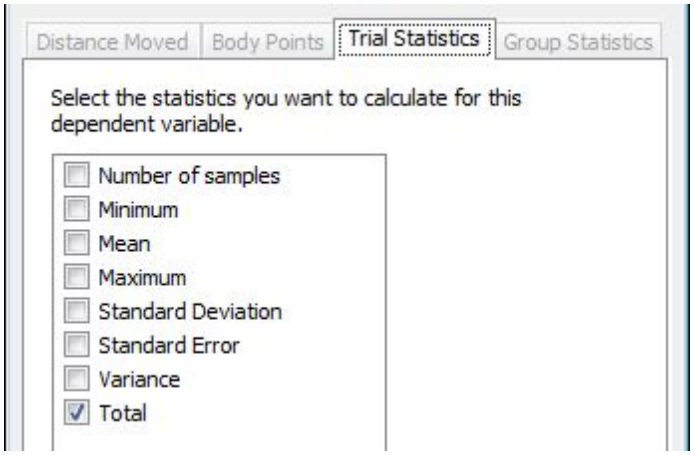
*See also*

- The statistics result

# Statistics available

Statistics are a summary of the values of the dependent variable calculated for all the samples in your tracks (or the segments you have selected in the active Data profile).

For each dependent variable you select in the Analysis profile, you can choose **Trial Statistics** and **Group Statistics**. Click on the corresponding tab to select the statistics you want to have in the results table.




*See also*

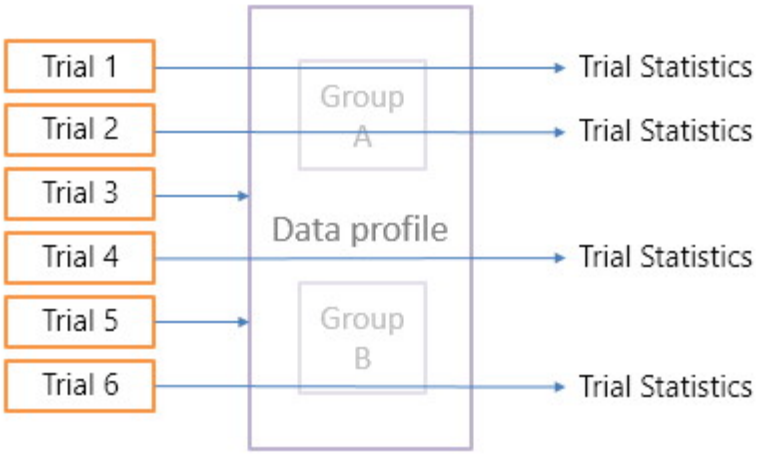
- List of Trial statistics
- List of Group statistics

# List of Trial statistics

## Aim

Trial Statistics give you summary statistics of individual trials. If a trial includes two or more tracks (subjects), the statistics are by default shown for each subject separately.

In the figure below, you find an example of how Trial statistics results are created, The Data profile filters tracks and/or intervals within tracks. If the Data profile contains groups of tracks, these groups are ignored in the results. To view results per group, choose **Results** > **Group Statistics and Charts**.




Below you find information about the Trial statistics.

## Cumulative Duration

The cumulative duration of the state variable in the whole track, or in the track segment selected. For example, the time that the animal spent in a zone (for the variable *In zone*), or the time that the subject was in the state *Mobile*.

When a state does not occur at all in a track or track segment, the value of Cumulative duration is zero.

## Cumulative Duration (%)

The Cumulative Duration divided by:

- If you do not define time bins: The duration of the track (this corresponds to the Recording duration independent variable, not Trial duration). This is also the case when you define nesting intervals or results per zone.

- If you define time bins: The duration of a time bin defined.

This statistic is expressed as percentage.

## Cumulative Duration within Nesting (%)

The Cumulative Duration divided by:

- If you calculate results per zone: The total time the animal is in the zone.
- If you defined track segments with Nesting: The total duration of the track segments defined.
- If you defined time bins: The duration of a time bin defined.
- If you combine any of the three above: The duration of the time period shared by all selection criteria (the time periods of each criterion are combined with AND logic).

This statistic is expressed as percentage.

## Frequency

The total number of occurrences of a state (only for state variables).

## Latency to First

The time from the start of the track to the first occurrence of the behavior (for example, the first time in the track that the animal is in a zone). Latency is always calculated from the start of the track, even when you define time bins and nesting intervals. See the note below.

## Latency to Last

The time from the start of the track to the last occurrence of the behavior. Latency is always calculated from the start of the track, even when you define time bins and nesting intervals.

### *Notes about Latency to First/Last*

- Latency to First and Latency to Last are calculated from the start of the track. This is the time when EthoVision XT collects the first sample (missing or not). If the animal is not yet in the arena, and tracking has started, EthoVision XT collects a number of missing samples. The time that the animal is missing is included in Latency to First/Last. Obviously this overestimates the “true” latency, which should be calculated from the time that the animal is released. To prevent this from occurring, make sure that

the Trial Control rule includes a Start track condition in such a way that tracking only starts when the animal is first found in the arena.

**TIP** To calculate the time from an event to another event in the trial, select a Free interval in the Analysis profile. See Free interval

- When two or more animals are tested simultaneously in the same arena, The meaning of Latency to First/Last much depends on how you set this condition. Suppose you start your trial by releasing one animal, then a little later the second animal. You want to calculate the Latency of "In Zone A" for both subjects. Consider the two scenarios:
  - If you set the Trial Control to start tracking *when any subject is found in the arena*, tracking starts when the first animal is released. Latency is calculated from that point also for the second animal, which enters the arena later. This means that Latency for this animal will be overestimated.
  - If you set the Trial Control to start tracking *when all subjects are found in the arena*, tracking starts when both animals are found. That is, when the second animal is released and the first is detected (also when in a hidden zone). Latency is calculated from this point, so Latency for the second animal is correct. However, for the first-released animal, the time that it was in the arena before the second was released is not counted in Latency. This means that Latency may be underestimated, for example when the first animal enters Zone A before the second animal is released. For this reason, try to release the animals at the same time, or one immediately after the other.

## Mean

- For linear variables like *Distance moved*, *Velocity*, *Distance to zone*, or Distance between subjects, and the variables *Turn angle*, *Angular Velocity* and *Meander*, the arithmetic mean is used, that is, the sum of the per-sample values divided by the number of samples.
- For the angular variables *Heading*, *Head direction*, and *Heading to Point*, the mean is calculated according to the circular mean formula, where $\alpha$ is the per-sample angle, and *N* is the number of samples:

$$\bar{\alpha} = \operatorname{atan} \frac{(\sum \sin\alpha)/N}{(\sum \cos\alpha)/N}$$

The circular mean value can also be represented by the angle of the vector resultant *R* obtained by summing up all the vectors that represent the per-sample angle values.

- For state variables like *In zone*, *Movement* and the behaviors scored manually or automatically recognized, the sum of the duration of occurrences of the state, divided by the number of occurrences.

## Minimum

The lowest value of the variable.

**NOTE** For the state variables, this is the minimum duration of the instances of a state, for example *Moving*. When the state does not occur at all in a track or track segment, the value of Minimum is zero.

## Maximum

The highest value of the variable.

**NOTE** For the state variables, this is the maximum duration of the instances of a state, for example *Moving*. When the state does not occur at all in a track or track segment, the value of Maximum is zero.

## Number of samples

The total number of valid values of that variable (only for numerical variables like *Distance moved*).

## Standard Error

The standard deviation divided by the square root of the number of samples. This is also the case for angular variables *Heading*, *Head direction*, and *Heading to Point*.

## Standard Deviation

For most variables, also including *Turn angle*, *Angular Velocity* and *Meander*, the sample standard deviation of the mean:

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{N - 1}}$$

where:

$x$ is the individual variable value, and $\bar{x}$ the mean of the variable;

$N$ is

- For numerical variables like *Distance moved* or *Velocity*, the number of samples used to calculate the mean;
- For state variables like *In zone*, *Mobility* and the behaviors scored manually or automatically recognized, the number of occurrences of the state used to calculate the mean (for example, when a subject visited a zone 4 times, then *N*=4).

For the angular variables *Heading*, *Head direction*, and *Heading to Point*, the circular standard deviation is given by

$$s = \sqrt{-2 \times \ln(R/N)}$$

where *R* is the resultant vector length (see the text above for the circular mean) and *N* the number of samples.

## Total

The sum of all the values.

## Variance

- For most variables, including *Turn angle*, *Angular Velocity* and *Meander*, the square of the standard deviation.
- For the angular variables *Heading*, *Head direction*, and *Heading to Point*, the variance is given by 1 - *R*/*N*, where *R* is the resultant vector length (see the text above for the circular mean) and *N* the number of samples.

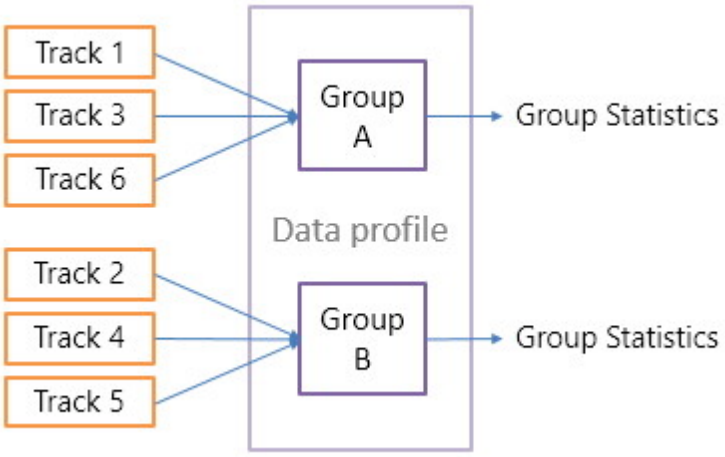
## Notes (for all statistics)

- Not all statistics listed above are available for every dependent variable. To view the list of statistics for a specific variable, double-click its name under **Selected Dependent Variables** and click the Trial Statistics tab.
- Values of latency and duration can only be a multiple of the sample interval (= 1 second divided by the sample rate).
- To add or remove statistics for a dependent variable, double-click that variable under Selected Dependent Variables and change your selection.

# List of Group statistics

## Aim

Group statistics are a summary of trials in a specific experimental group. Here a "group" refers to the tracks selected in a **Result** box in the Data profile. See Define groups of tracks




Choose between **Group mean** and **5 number summary**.

**NOTE** Group statistics are not available when you only select Standard deviation, Standard error or Variance as Trial statistics.

## Group mean

- For most variables, including *Turn angle*, *Angular Velocity* and *Meander*, the Group mean is the arithmetic mean of the trial statistics, calculated for each group.
- For the variables *Heading*, *Head direction*, and *Heading to Point*, the group mean is calculated as the circular mean, with *N* being the number of trials in the group.

If you select **Group mean**, the following options are available:

- **Standard Deviation**. For most variables, the sample standard deviation of the trial statistics, calculated for each group. For the variables *Heading*, *Head direction*, and *Heading to Point*, the group standard deviation is calculated as the circular standard deviation (see above), with *N* being the number of trials in the group.

- **Standard Error**. The Standard deviation divided by the square root of the number of tracks used to calculate the Standard deviation. This is also the case for angular variables *Heading*, *Head direction*, and *Heading to Point*.

## 5 number summary

This is the Minimum, the Lower quartile, the Median, the Upper quartile and the Maximum value of trial statistics calculated for each group.

The method used to calculate quartiles is the *N + 1 Basis interpolation*, which is also used in SPSS. If the sample size is lower than 3, the lower quartile is the same as the minimum and the upper quartile is the same as the maximum value, which is also the same as in SPSS.

**NOTE** With 5 number summary you can also plot data in form of Box and whiskers charts. You can also show the original data points for better interpretation. See Group charts > Customize the group charts

### *See also*

- List of Trial statistics
- Define groups of tracks
- View the results for groups of tracks

# Edit the Analysis profile

## Aim

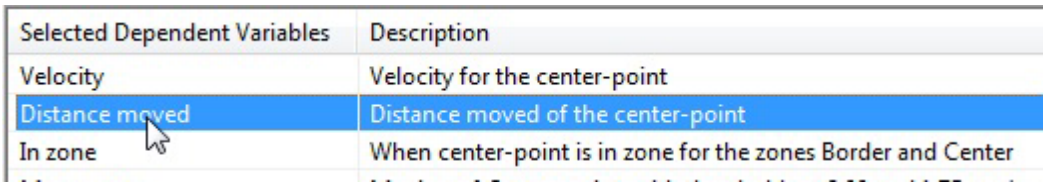
- To change the properties and settings of a dependent variable
- To make multiple instances of a dependent variable
- To rename a dependent variable
- To remove a dependent variable from the Analysis profile

After editing the Analysis profile, re-run analysis (**Analysis** > **Results** > ...).

## To modify the properties of a dependent variable

You can change the properties of a dependent variable at any time, for example to add one more statistic.

1. Open the Analysis profile that contains the dependent variable you want to modify.
2. Under **Selected Dependent Variables**, double-click the dependent variable.




3. Edit the variable's properties.

   For how to modify the variable's properties, choose the variable in Dependent Variables in Detail and see the **How to specify** section.

4. Choose **Analysis** > **Results** > **Statistics and Charts**, then click the **Calculate** button.

You can also modify a dependent variable directly in the Statistics result. Right-click the dependent variable header and select **Properties**. Change the properties of the variable, then click the **Calculate** button.

## To calculate multiple instances of a dependent variable

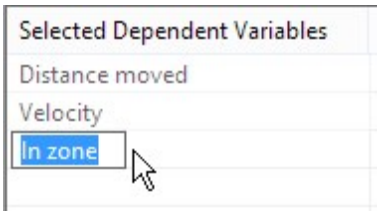
You can add multiple instances of a dependent variable to an Analysis profile. This is handy when you want to compare the effect of different settings on the same dependent variable. For example, two instances of *Mobility* with different Mobility thresholds.

1. Open the Analysis profile that contains the dependent variable.
2. Right-click the dependent variable under **Selected Dependent Variables** and select **Duplicate**. A new row is appended under **Selected Dependent Variables**.
3. Double-click the new row and set the properties you require for the new instance of the dependent variable.

Additional instances of a dependent variable are given names with a progressive number (for example, Distance moved 2, Distance moved 3, etc.). You can rename those variables (see below).

## To rename a dependent variable

1. Open the Analysis profile that contains the dependent variable.
2. Under **Selected Dependent Variables**, right-click the variable's name and select **Rename**.
3. Type in the name you want to give and press **Enter**.




## To remove a dependent variable from the Analysis profile

Under **Selected Dependent Variables** click the variable’s name and press **Delete**, or right-click the variable's name and select **Delete**.

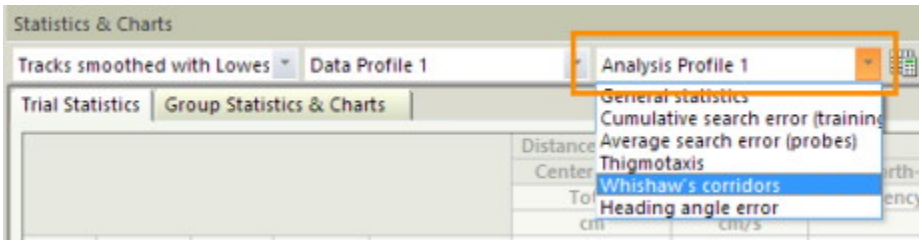
# Export the statistics result

## Aim

To export the statistics of the dependent variables in the results table to an external program. For example, export the values of average velocity to SPSS.

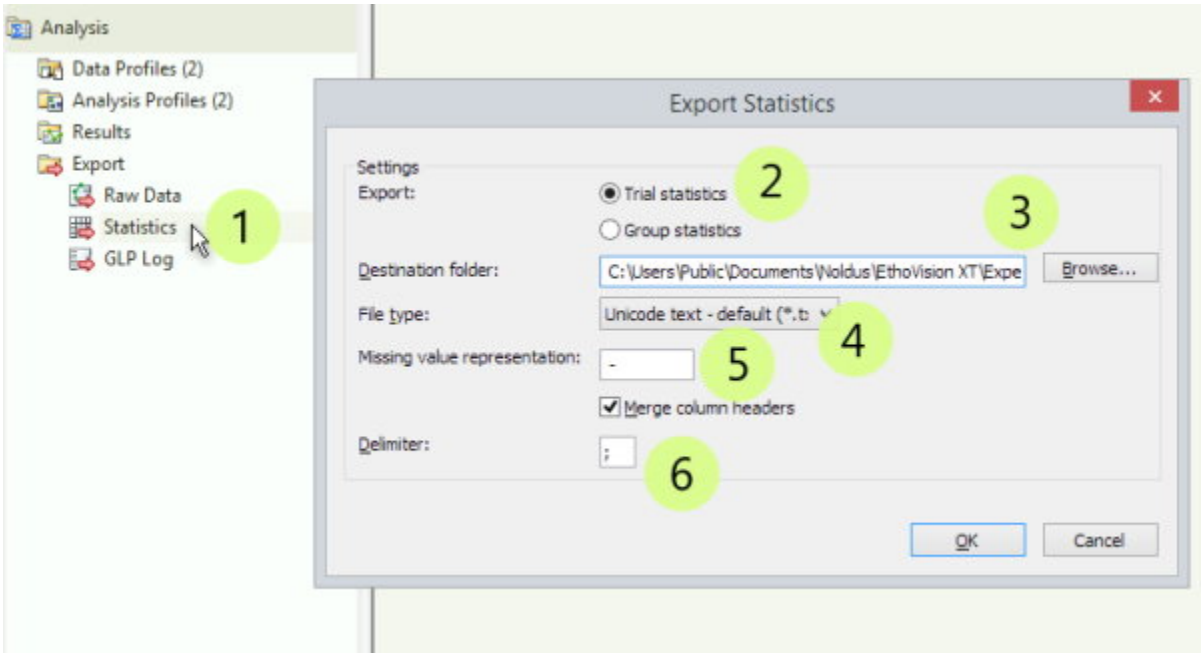
## Prerequisites

- The statistics result is open on your screen.
- Select the Data profile and Analysis profile from the lists on the toolbar.




- If the table layout does not match the requirements of the program you want to export the result to, see The statistics result.

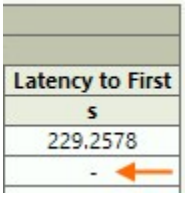
## Procedure



1. Choose **Analysis** > **Export** > **Statistics**, or in the Experiment Explorer under **Analysis** > **Export**, click **Statistics**.
2. Under **Export**, choose to export **Trial Statistics** or **Group Statistics**.
3. Click the **Browse** button to select the location for your export file. Its suggested location is the **Export Files** folder located in your experiment folder.
4. Under **File Type**, select either **ANSI text** (*.txt), **Excel** (*.xlsx), or **Unicode text - default** (*.txt).
5. Next to **Missing value representation**, enter the character that you want to export when the value for a particular cell is missing.
6. For text export, enter the **Delimiter** character you want to use to separate columns in the exported file.
7. Click **OK**.

## Options

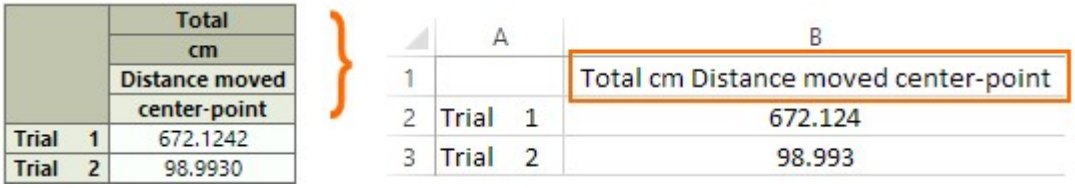
- **Missing value representation**. Missing values in a statistic result are statistics that could not be calculated for that particular cell. For example, if the subject never visited a zone during trial 2, the result for the statistic *Latency to first* and for the dependent variable *In zone* "-".



| Latency to First |
|---|
| s |
| 229.2578 |
| - |

By default, missing values are exported as "-". Make sure the destination program recognizes the character you specify as missing value.

- **Merge column headers** (selected by default in new experiments). Keep this option selected if you want to export to applications like Excel or SPSS. In this example, the column headers are merged after export to Excel.



| | Total cm Distance moved center-point |
|---|---|
| Trial 1 | 672.1242 |
| Trial 2 | 98.9930 |

| | A | B |
|---|---|---|
| 1 | | Total cm Distance moved center-point |
| 2 | Trial 1 | 672.124 |
| 3 | Trial 2 | 98.993 |

If you do not select this option, each of the four column headers in the results table above will appear in separate rows in the export file.

Note that the order of the text elements in the header depends on the order of the headers in the results table. You can change the order using the Layout function. Modify the layout of the results table

Here an example where the units **cm** were at the bottom level of the results table. They are now at the end of the header.

| | A | B |
|---|---|---|
| 1 | | Distance moved Center-point Total cm |
| 2 | Trial 1 | 549.155 |

## Notes

- The name of the export file is Statistics-[Experiment name]. If you export results multiple times to the same location, EthoVision attaches a number at the end of the file name: (1), (2), etc.
- Choose **Unicode text** to export according to the Unicode character set. This supports most world languages, including Chinese. Choose **ANSI text** to export the data as text files according to the ANSI character set. Choose this option only if the application in which you will open the exported file cannot handle the Unicode character set.
- The values exported have a variable number of decimals, depending on the number of integers. The total number of significant digits exported is always six (for example, 1.23456 or 1234.56), or seven if the unsigned number is smaller than one (0.123456).

  Because of the restriction mentioned above, there may be a very small difference between the values in the results table and in the export file. This difference is in the order of 1/10000th of the measurement unit; see below for an example (left: results table; right: export file in text format).

| | | Distance moved | Velocity |
|---|---|---|---|
| | | center-point | center-point |
| | | Total | Mean |
| | | cm | cm/s |
| Result 1 | Trial 1 | 158.8827 | 3.2558 |
| | Trial 2 | 151.8787 | 3.1641 |
| | Trial 3 | 395.5340 | 8.7122 |

```
"";"";"Distance moved";"Velocity";
"";"";"center-point";"center-point";
"";"";"Total";"Mean";
"";"";"cm";"cm/s";
"Result 1";"Trial    1";158.883;3.25579;
"Result 1";"Trial    2";151.879;3.16414;
"Result 1";"Trial    3";395.534;8.7122;
```

- To change the layout of the exported table, follow Modify the layout of the results table and then export the results.

## Export settings for specific applications

- For R: Export to text. Make sure that R can recognize "-" as a missing value. Use the na.strings to include all possible "no data" characters or strings, for example:

  ```
  loadfile <- read.table("exportfile.txt" , header = TRUE , sep=";" ,
  na.strings = c("-" , "NODATA", ....) )
  ```

- For SPSS: Select the option **Merge column headers**.
- For Systat: Each case must be written in one line. Separator (for text files): space or comma.
- For SAS: For text files, use the DELIMITER option to specify the text delimiter if this is other than space. For Excel files, use the PROC IMPORT procedure with the SHEET option to specify the worksheet to import. Use the GETNAMES option to read variable names from the first row of the worksheet.

*See also*

- Export the raw data (track and dependent variables)
- File management: Export data

# The statistics result

## Learn about

- Trial Statistics result
- Group Statistics result

## What do you want to do?

- Modify the layout of the results table
- Sort the results table

*See also*

- Export the statistics result

# Trial Statistics result

## Aim

The Trial Statistics tab gives the statistics for each trial separately.

## Procedure

Choose **Analysis** > **Results** > **Statistics and Charts**. The **Trial Statistics** tab opens.

## Default layout

In the Trial Statistics table, each row corresponds to a subject tracked in one arena during a specific trial (see also the figure below).

The row headers (**A**) tell you which Trial, Arena and Subject a specific cell corresponds to. Remember that, depending on your experiment design, one Trial may contain one or more Arenas, and one arena may contain one or more Subjects.

The column headers (**B**) show the type of dependent variable (e.g. velocity), the body point (e.g. center point), the type of statistic (e.g. mean) and the measurement unit.

Additional headers appear depending on your data selection:

- Selection results. If you define two or more **Result** boxes in the Data profile, the name of the boxes are shown in the first cell (**C**).
- Time bins. If you selected **Results per time bin** in your Data Profile, extra rows are added, one per time bin, and the headers show the corresponding time periods. For example: 0:00:00 - 0:00:20, 0:00:20 - 0:00:40, etc. (**D**).
- Results per zone. If you selected **Results per zone** in your Data Profile, the zone names are displayed on rows, together with the body points in those zones (**E**).

What you see in the result table depends on which Data profile and the Analysis profile are selected on the toolbar.

Trial statistics with Selection results and Time bins:

Trial Statistics | Group Statistics & Charts

| Selection Result | Trial | Arena | Subject | Time bin | Distance moved | Velocity |
|---|---|---|---|---|---|---|
| | | | | | Center-point | Center-point |
| | | | | | Total | Mean |
| | | | | | cm | cm/s |
| Probe trials - Aged | Trial 17 | Arena 1 | Subject 1 | 0:00:00-0:00:20 | 519.6055 | 25.9836 |
| | | | | 0:00:20-0:00:40 | 375.8164 | 18.7932 |
| | | | | 0:00:40-0:01:00 | 338.3362 | 16.9190 |
| | | | | 0:01:00-0:01:20 | 339.2490 | 16.9646 |
| | | | | 0:01:20-0:01:40 | 351.3205 | 17.5683 |
| | | | | 0:01:40-0:02:00 | 309.7266 | 15.4883 |
| | Trial 19 | Arena 1 | Subject 1 | 0:00:00-0:00:20 | 472.4253 | 23.6307 |
| | | | | 0:00:20-0:00:40 | 411.3268 | 20.5746 |
| | | | | 0:00:40-0:01:00 | 370.4868 | 18.5318 |
| | | | | 0:01:20-0:01:40 | 380.1698 | 19.0161 |
| | | | | 0:01:40-0:02:00 | 371.2257 | 18.5378 |
| | | | | 0:01:40-0:02:00 | 304.4441 | 15.2283 |
| Probe trials - Young | Trial 18 | Arena 1 | Subject 1 | 0:00:00-0:00:20 | 279.7904 | 13.9951 |
| | | | | 0:00:20-0:00:40 | 377.7851 | 18.8968 |
| | | | | 0:00:40-0:01:00 | 427.2948 | 21.3732 |
| | | | | 0:01:00-0:01:20 | 456.4434 | 22.8312 |
| | | | | 0:01:20-0:01:40 | 473.1444 | 23.6272 |
| | | | | 0:01:40-0:02:00 | 366.1238 | 18.3135 |
| | Trial 20 | Arena 1 | Subject 1 | 0:00:00-0:00:20 | 436.4320 | 21.8304 |
| | | | | 0:00:20-0:00:40 | 201.9910 | 10.1036 |
| | | | | 0:00:40-0:01:00 | 300.2108 | 15.0166 |
| | | | | 0:01:00-0:01:20 | 331.9414 | 16.6038 |
| | | | | 0:01:20-0:01:40 | 337.9815 | 16.8777 |
| | | | | 0:01:40-0:02:00 | 437.3528 | 21.8765 |

Trial Statistics with Results per zone:

Trial Statistics | Group Statistics & Charts

| Selection Result | Trial | Arena | Subject | Zone | Distance moved | Velocity |
|---|---|---|---|---|---|---|
| | | | | | Center-point | Center-point |
| | | | | | Total | Mean |
| | | | | | cm | cm/s |
| Aged | Trial 1 | Arena 1 | Subject 1 | In North-West | 341.2893 | 16.0232 |
| | | | | In North-East | 536.0222 | 14.7165 |
| | | | | In South-East | 536.3492 | 14.6449 |
| | | | | In South-West | 392.1395 | 16.0903 |
| | Trial 5 | Arena 1 | Subject 1 | In North-West | 250.9833 | 9.5795 |
| | | | | In North-East | 296.3371 | 17.9008 |
| | | | | In South-East | 342.5168 | 13.6106 |
| | | | | In South-West | 451.6392 | 8.7529 |
| | Trial 17 | Arena 1 | Subject 1 | In North-West | 576.4086 | 17.0947 |
| | | | | In North-East | 374.5388 | 22.8957 |
| | | | | In South-East | 584.8364 | 19.3143 |
| | | | | In South-West | 698.8702 | 17.6211 |
| | Trial 19 | Arena 1 | Subject 1 | In North-West | 590.9939 | 20.0310 |
| | | | | In North-East | 422.5905 | 21.2801 |
| | | | | In South-East | 443.2660 | 18.7587 |
| | | | | In South-West | 854.0082 | 18.1603 |

# Group Statistics result

## Aim

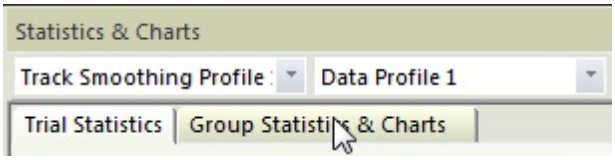
The **Group Statistics & Charts** tab shows the statistics for the groups of trials defined in your Data Profile.

- In the Group Statistics result table, each group is labeled as the corresponding Result container in the Data profile. The statistics are calculated for the trials/arenas/subjects included in that Result container. For how to group data, see Analyze groups of tracks.
- The dependent variables (e.g. velocity) are those specified in the currently-selected Analysis profile.

**NOTE** If you use the default Data profile, all trials are grouped. The Group Statistics & Charts table shows the statistics for all trials together.

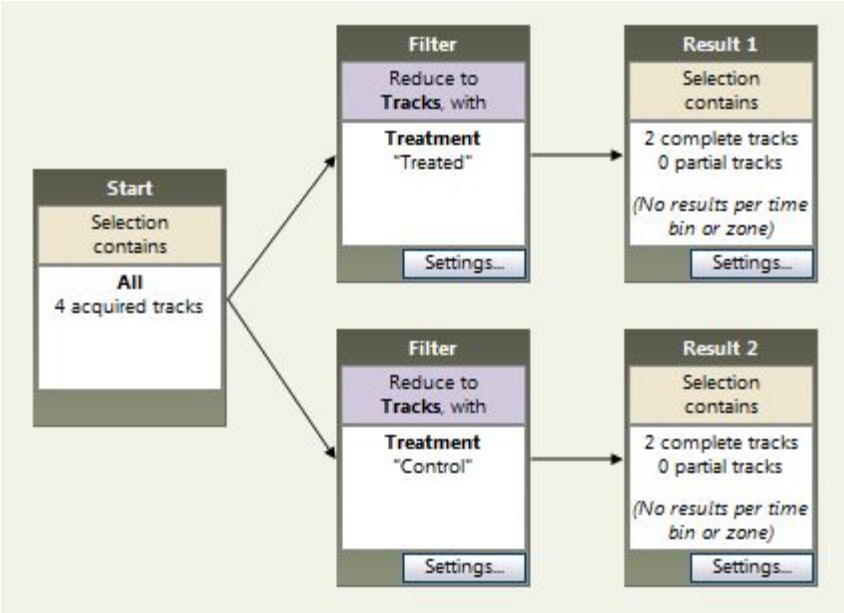
## Procedure

Choose **Analysis** > **Results** > **Statistics and Charts** and click the **Group Statistics & Charts** tab.




## Example

In the following example, the Data profile includes two Result containers: Result 1 for treated subjects, Result 2 for control subjects. The groups have been created with **Filter** boxes, each selecting a value of the independent variable Treatment, Control or Treated. Each Result Container contains two tracks.




The Group Statistics table looks like this:

Trial Statistics | Group Statistics & Charts

| | Treatment | Distance moved | | | Velocity | | |
|---|---|---|---|---|---|---|---|
| | | Center-point | | | Center-point | | |
| | | Total | | | Mean | | |
| | | N | Mean | Standard Error | N | Mean | Standard Error |
| | | | cm | cm | | cm/s | cm/s |
| Result 1 | Treated | 2 | 379.7723 | 90.3332 | 2 | 12.8730 | 1.2020 |
| Result 2 | Control | 2 | 264.8913 | 34.2463 | 2 | 9.8362 | 3.2838 |

The statistics are displayed for each Result container. Result 1 contains the tracks marked as Treated; Result 2 contains the tracks marked as Control.

In addition, a chart is created automatically for each dependent variable in the table. See Group charts

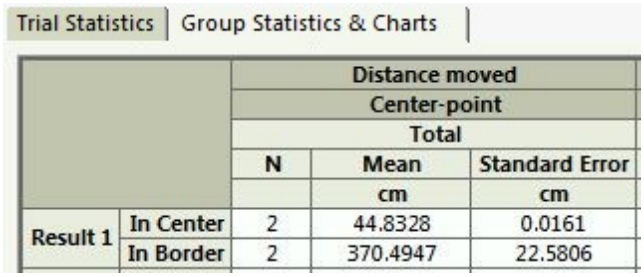
## How to read the Group statistics

- Statistics should be read as follows: [Lower level header] of [Higher level header] is [value in cell]. For example:

| | Treatment level | Distance moved | | |
|---|---|---|---|---|
| | | Center-point | | |
| | | Total | | |
| | | N | Mean | Standard Error |
| | | | cm | cm |
| Result 1 | Control | 2 | 415.3275 | 22.5645 |
| Result 2 | Treated | 2 | 464.8215 | 244.9276 |

415.3275 cm is the **Mean** of the per-trial **Total Distance moved** for the tracks contained in **Result 1**.

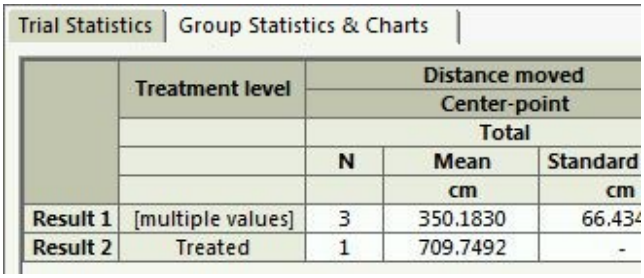
- **N** is the number of tracks used to calculate the group statistic for that dependent variable.
- Group statistics are split in multiple rows when you define results per time bin and/or zone. In the example below, results are split per zone defined in the Data profile, under **Results per zone**. See Calculate the results per zone



Trial Statistics | Group Statistics & Charts

| | | Distance moved | | |
|---|---|---|---|---|
| | | Center-point | | |
| | | Total | | |
| | | N | Mean | Standard Error |
| | | | cm | cm |
| Result 1 | In Center | 2 | 44.8328 | 0.0161 |
| | In Border | 2 | 370.4947 | 22.5806 |

- By default, user-defined independent variables are displayed in the table. To show more independent variables, click the **Show/Hide** button and choose **Independent Variables**.
- If tracks in a group have different values of an independent variable, the text **[multiple values]** is displayed for that group:



Trial Statistics | Group Statistics & Charts

| | Treatment level | Distance moved | | |
|---|---|---|---|---|
| | | Center-point | | |
| | | Total | | |
| | | N | Mean | Standard |
| | | | cm | cm |
| Result 1 | [multiple values] | 3 | 350.1830 | 66.43 |
| Result 2 | Treated | 1 | 709.7492 | - |

## How group statistics are calculated

For most dependent variables, The group Mean is the arithmetic mean of the values of the dependent variable reported in Trial Statistics. For angle variables like *Heading*, *Heading to point*, and *Head direction*, (but not for *Turn angle*) the circular statistics are used.

Group statistics are not weighted. For example The total distance moved is 100 cm for trial 1, and 200 cm for trial 2. The resulting group mean is 150, independent of how many samples each track is made of.

Similarly, the group Standard error, Standard deviation, and the 5-number summary (Minimum, First Quartile, Median, Third Quartile and Maximum) are calculated from the single values of the dependent variables reported in Trial Statistics.

## Notes

- The number of decimals in the Trial Statistics and Group Statistics is fixed to four. When exporting the results table, the number of decimals varies depending on the number of integers in the value. See Export the statistics result
- The dependent variables displayed in Group Statistics & Charts are those defined in the Analysis profile chosen from the list in the toolbar.
- The statistics displayed in Group Statistics & Charts are those selected under Group Statistics for that variable in the currently active Analysis profile. Group statistics are always measures of central tendency, either a Mean (with Standard deviation and/or Standard error) or the 5-number summary (Minimum, First Quartile, Median, Third Quartile and Maximum). You cannot calculate, for example, the group total of distance moved.
- The group statistics can be calculated for all the trial statistics, except for the standard error, the standard deviation and the variance. For example, you cannot calculate the group mean of the trials' standard deviations of a variable.
- To add and remove statistics, right-click any column header for the dependent variable for which you want to add or remove the statistics, and select **Properties**. On the **Trial Statistics** and **Group Statistics** tabs, select the statistics that you require.
- See also Analyze groups of tracks.

# Modify the layout of the results table

## Aim

To make sure your results are grouped and displayed in the most useful way possible. This is particularly the case when exporting the results to another application.

**NOTE** This applies to Trial Statistics and Group Statistics.

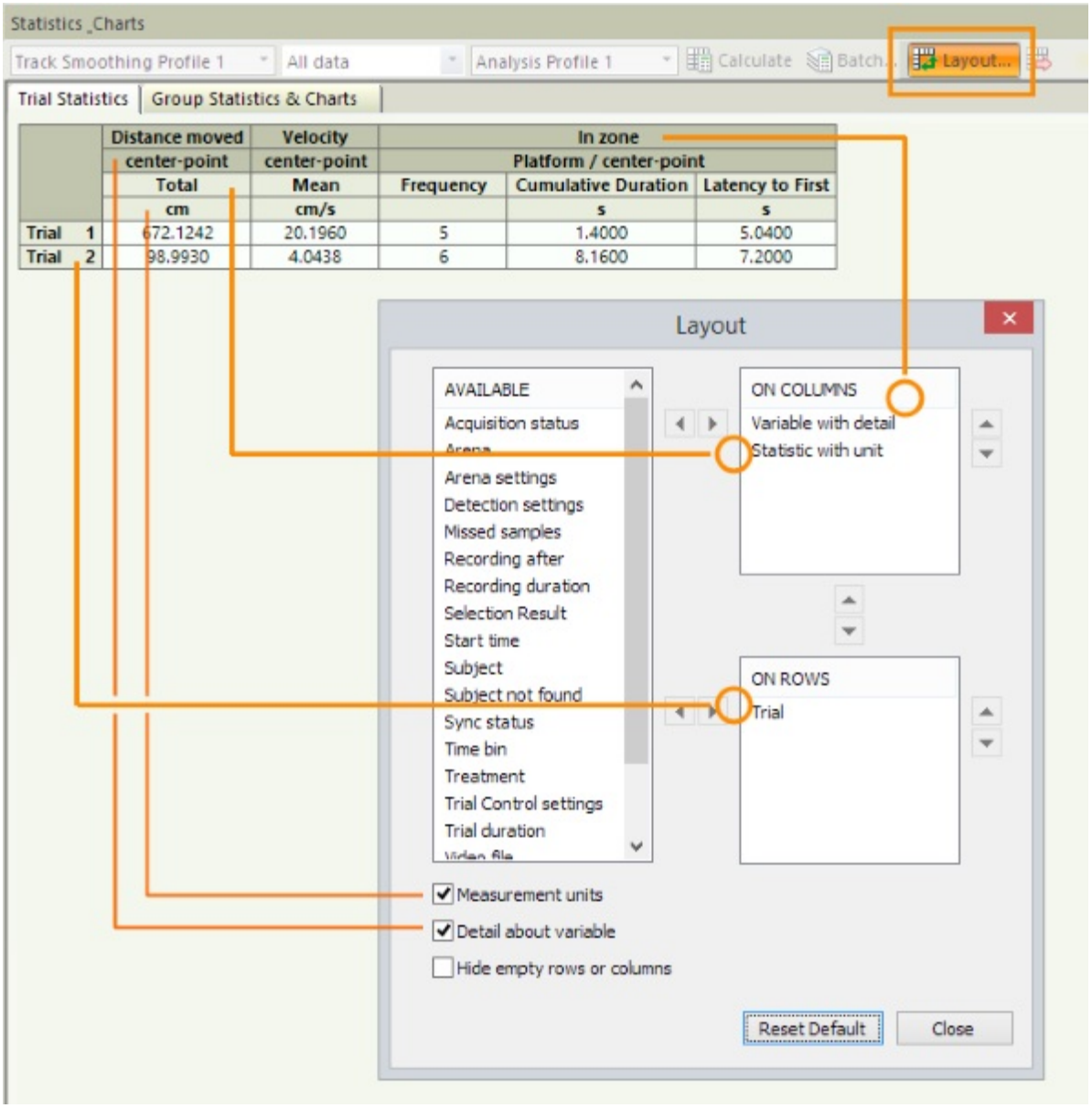
## Prerequisites

- You have run analysis (**Analysis** > **Results** > **Statistics and Charts** > **Calculate**).

## General procedure

Click the **Layout** button on the toolbar, or choose **Show/Hide** > **Layout**.

By default, the results table shows the dependent variables and their statistics on the columns, and the trials on the rows. In the following example you can see how you can customize the layout of the table.




## What do you want to do?

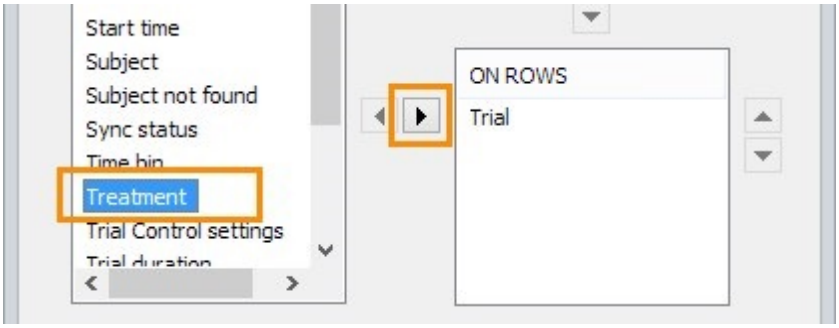
- Add a header to the table
- Remove a header from the table
- Special headers in the Layout window
- Move a header between rows and columns
- Swap rows and columns
- Modify the table hierarchy
- Show and hide part of the table

- Remove empty rows or columns
- Reset the table layout
- Move the headers in the Group statistics results table

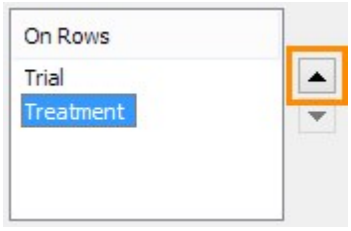
## Add a header to the table

**EXAMPLE** In the Trial Statistics page, add the independent variable *Treatment*, so that each row (trial) is labeled with the value of Treatment, either *Compound* or *Vehicle*.

1. Click the **Layout** button on the toolbar.
2. In the **Available** box, click the header you want to add.
3. Click the arrow button which points to the **On Rows** box.




4. **OPTIONAL** Move the header up in the list to have trials separated based on the values of *Treatment*.




**NOTES**

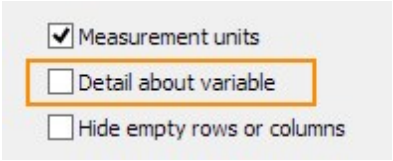
- If you click the arrow button which points to **On Columns**, the variable is placed in columns.
- **TIP** To add a new dependent variable, do this in the Analysis profile, then return to **Analysis** > **Results** > **Statistics and Charts**.

## Remove a header from the table

**EXAMPLE** Remove the Arena name from your table headers.

1. Click the **Layout** button on the toolbar.
2. In the **On Columns**/**On Rows** box, click **Arena**.
3. Click the arrow button which points to the **Available** box.


- To remove measurement units, in the **Layout** window de-select **Measurement units**.
- To remove the detail of a dependent variable, like the body point name, in the **Layout** window de-select **Detail about variable**.

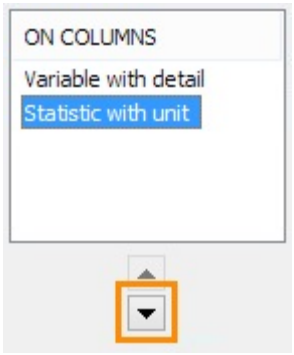



**NOTE** Removing an element from the headers does not aggregate the results under that header. For example, if you remove **Arena** all results are still displayed for each arena separately. If you want to aggregate results, see Define groups of tracks.

## Move a header between rows and columns

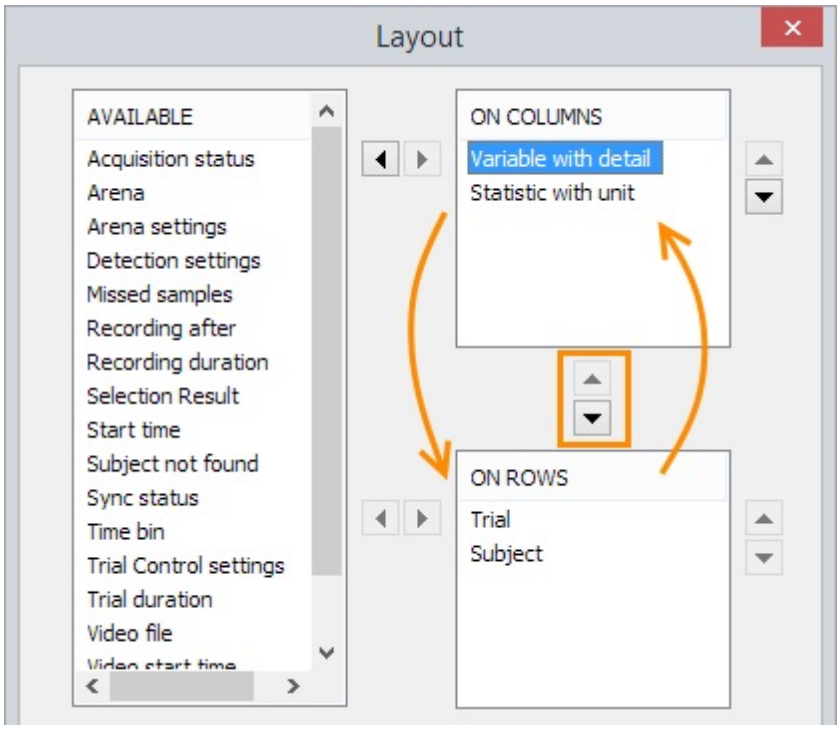
**EXAMPLE** The dependent variables (Velocity, In zone, etc.) are placed in columns. Move them to the rows, so that each row represents one trial*dependent variable combination.

1. Click the **Layout** button on the toolbar.
2. In the **On Columns** box, click **Variable with detail**.
3. Click the arrow button which points to the **On Rows** box.



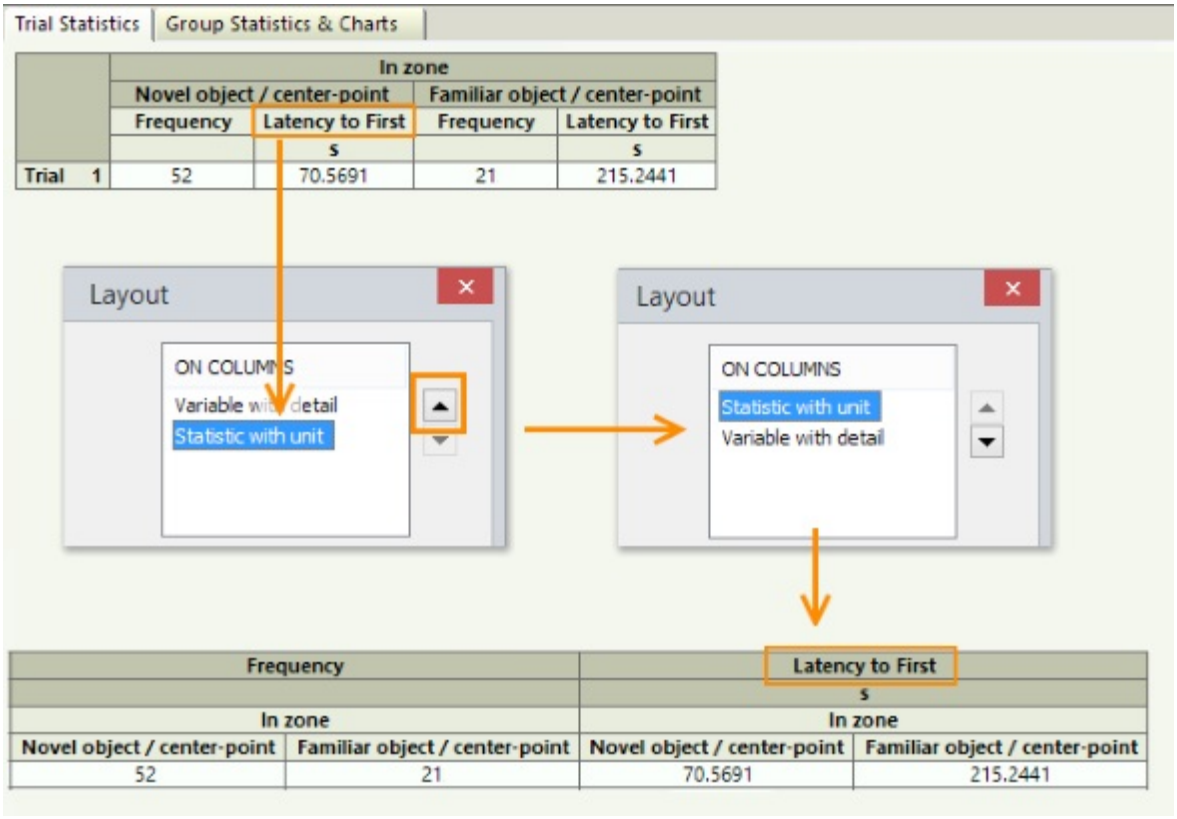

## Swap rows and columns

Select each item under **On Columns** and move it to **On Rows**. Next, select the items originally under **On Rows** and move them to **On Columns**.




## Modify the table hierarchy

To place a header to a higher level in the table, click the element in the **On Columns** or **On Rows** box, and then click the **Up** button.

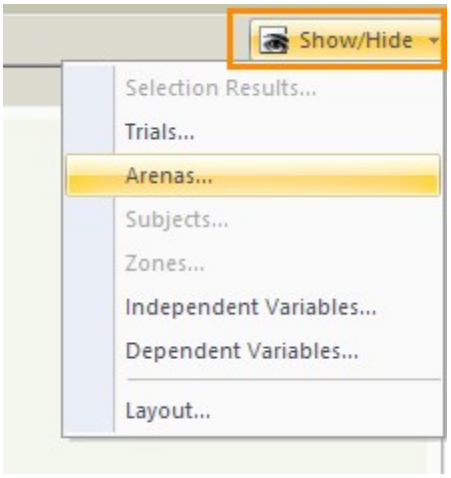



To place an element lower in the table hierarchy, click the element in the **On Columns** or **On Rows** box, and then click the **Down** button.

**NOTE** It is not possible to put Time bin above the Selection Result.

## Show and hide part of the table

1. Click the **Show/Hide** button at the top-right corner and select the corresponding element.



2. In the window that appears, select the trials, arenas, subjects, et. that you want to have in the table.
3. Click **OK**.

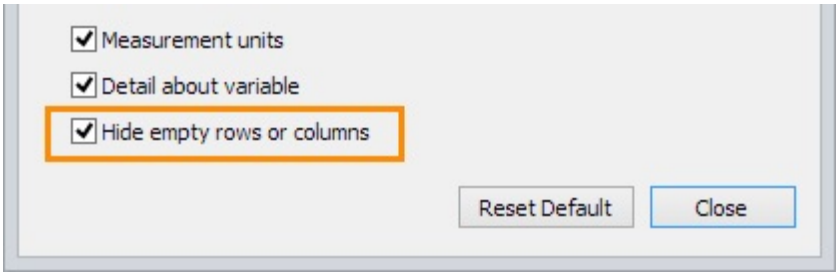
## Remove empty rows or columns

**TIP** Do this if you have many rows or columns that are empty, and you want to export only the rows and columns with significant results.

| | Latency to First |
|---|---|
| | s |
| | In zone |
| | Novel object / center-point |
| Trial 1 | - |
| Trial 2 | - |
| Trial 3 | 70.5691 |
| Trial 4 | - |

| | Latency to First |
|---|---|
| | s |
| | In zone |
| | Novel object / center-point |
| Trial 2 | 70.5691 |

In the Layout window, select **Hide empty rows or columns**.




**NOTE** Rows and columns are not removed if at least one dependent variable is calculated for that row or column.

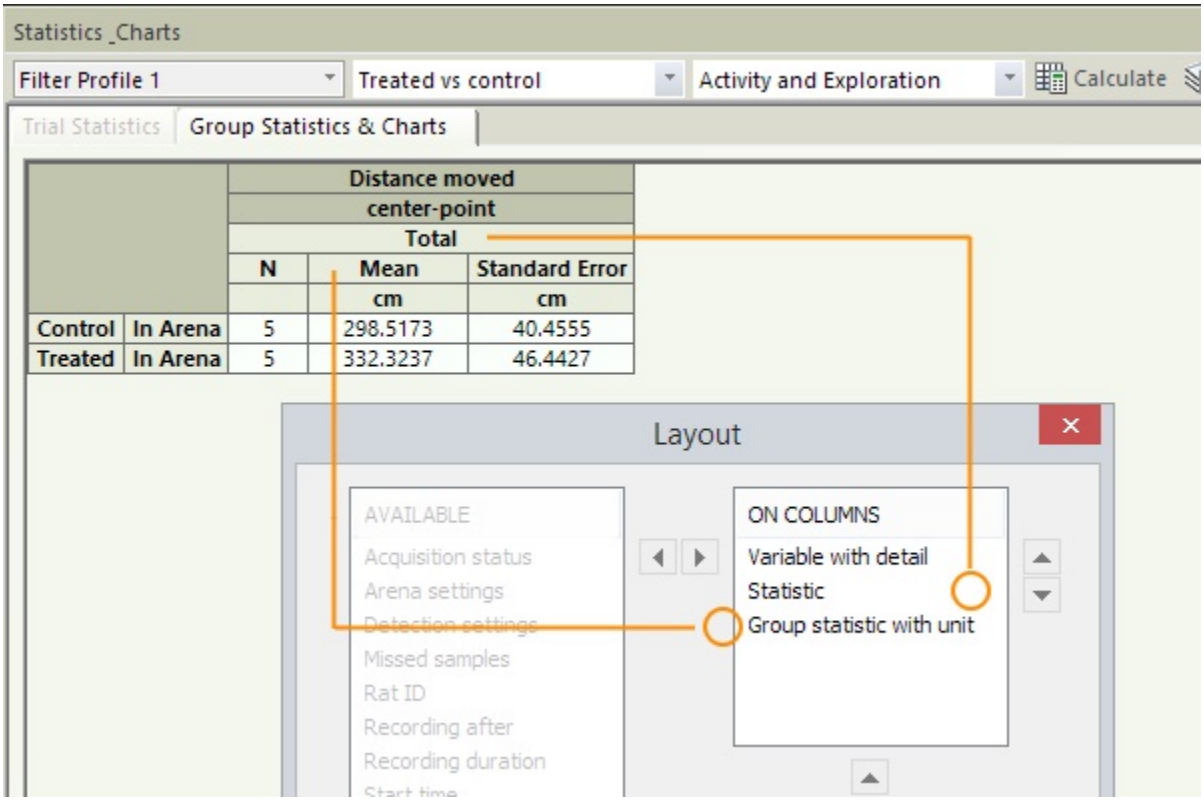
## Reset the table layout

To return to the default layout, click the **Layout** button and in the Layout window click the **Reset Default** button.

## Move the headers in the Group statistics results table

The results of group statistics shows the group statistic under the trial statistic. In the example below, you find the group statistic **Mean** under the trial statistic **Total** for distance moved. This means that the result is the mean value of the total distance moved calculated for each trial in that group.

When you click the **Layout** button, the item **Group Statistic with units** is selected under **On Columns**.




You can move the Group statistics headers and the units to the table rows or move them to a higher level in the table.

- Move a header between rows and columns
- Modify the table hierarchy
- **TIP** To have the track groups in different columns, not rows, select **Selection Result** and move it to **On Columns**.

## Special headers in the Layout window

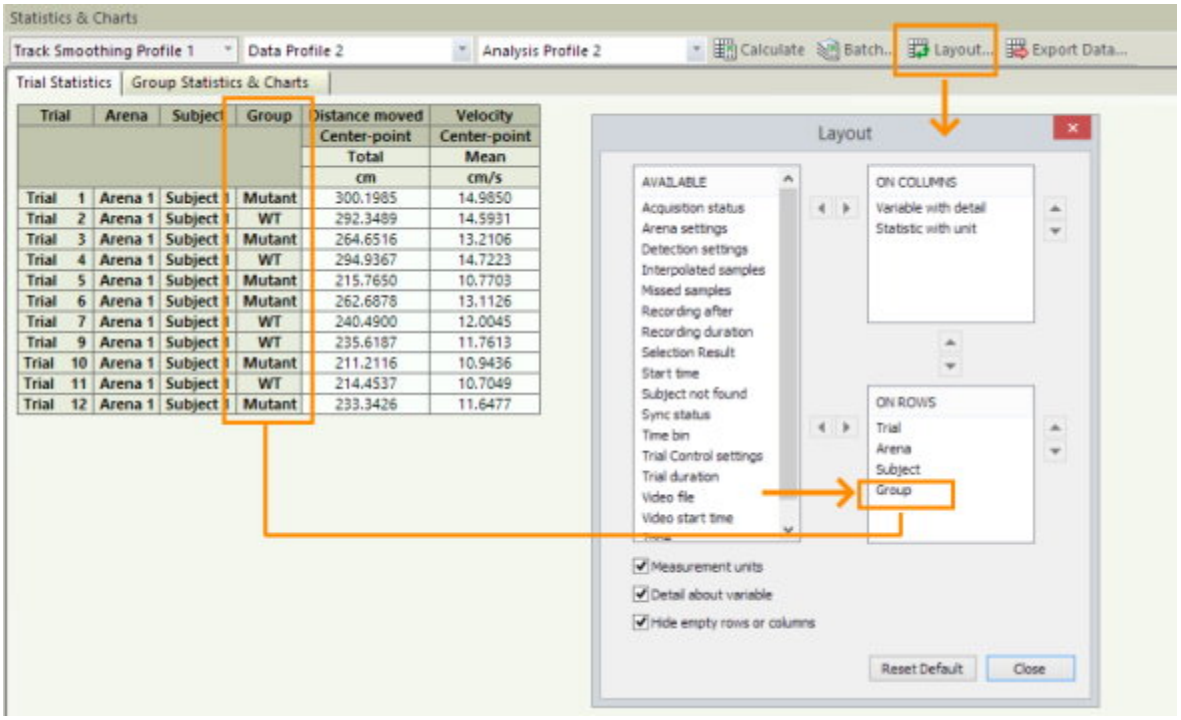
- **Statistic with unit** and **Group statistic with unit** show the names of the trial statistics and group statistic selected in the Analysis profile, respectively. If you move those headers in the table, the measurement units are moved to the same location.
- **Selection Result** refers to the dataset specified in the Results boxes of the Data profile. If the Data profiles includes two or more Result boxes, for example to group tracks, the headers for the corresponding boxes are automatically added to the table.

  **NOTE** If you remove **Selection Result** from the rows and columns, the results for different groups are still kept separate, because the Data profile

that specifies those groups is still active; only the headers disappear. If you want to merge results, use another Data profile.

- **Time bin** refers to the times bins defined in the **Results** box of your Data profile. If you do not define time bins in the Data profile, and select Time bin in the Layout window, the headers **Start-End** are added; no time bin is created. See Results per time bin
- **Zone** refers to the zones specified in the **Results** box of your Data profile. If zones are not specified, analysis is done for the whole arena and the table shows **In Arena**. See Calculate the results per zone
- The independent variables that you have defined are not shown in the table by default. To export the table with the independent variable values as an analysis factor, click the **Layout** button and move that variable to **On Rows** or **On Columns** depending on how your statistics program treats analysis factors.

  **EXAMPLE** An independent variable Group with possible values Mutant and WT has been defined in the Trial List. To have the values of this independent variable displayed for each trial, click **Layout**, select the variable *Group* on the left and move it to the **On Rows** box.




- **Missed samples**. This is the percentage of samples that EthoVision XT could not analyze. To reduce this percentage, see Factors affecting the occurrence of missing samples in Sample rate

- **Subject not found**. This is the percentage of the analyzed samples in which EthoVision XT could not find the subject. A lower value indicates high reliability of data. To reduce this value further, optimize the lighting conditions and adjust the Detection Settings to improve detection rate.
- If you select track segments with Nesting, there is no special header for them. However, remember that the results are calculated over the track segments defined, not the entire tracks.

*See also*

- The statistics result
- Trial Statistics result
- Group Statistics result

# Sort the results table

## Aim

- To sort the results table based on the row headers.
- To sort the results table based on the values in a column.
- To change the order of and group the dependent variables in the results table.

## Prerequisites

- You have run analysis (**Analysis** > **Results** > **Statistics and Charts**).

## Procedure

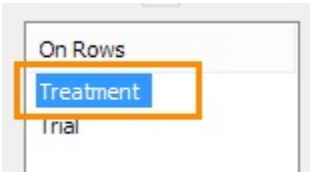
### *Sort the table rows based on their headers*

**EXAMPLE** Sort the results table based on the header *Treatment*, an independent variable with values *Drug* and *Vehicle*.

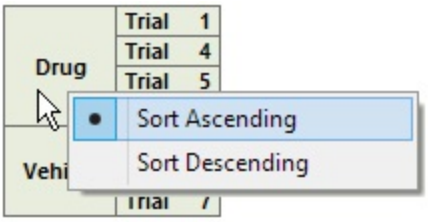
| | |
|---|---|
| Trial 1 | Drug |
| Trial 2 | Vehicle |
| Trial 3 | Vehicle |
| Trial 4 | Drug |
| Trial 5 | Drug |
| Trial 6 | Drug |
| Trial 7 | Vehicle |

| | |
|---|---|
| Drug | Trial 1 |
| | Trial 4 |
| | Trial 5 |
| | Trial 6 |
| Vehicle | Trial 2 |
| | Trial 3 |
| | Trial 7 |

1. Click the **Layout** button and under **On Rows** move Treatment to the highest position in the list. See Modify the layout of the results table




2. Right-click one of the headers you want to sort, and select **Sort Ascending** or **Sort Descending**.




**NOTE** If you sort the rows based on the headers on the second, third, etc. column, the rows are sorted only within that level, not globally. For example:

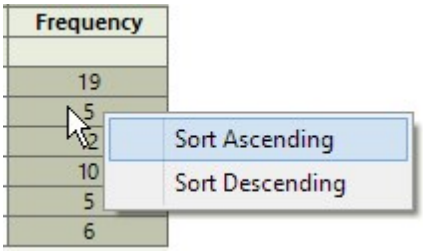
| | | |
|---|---|---|
| Trial 1 | Subject 1 | |
| | Subject 2 | |
| | Subject 3 | |
| | Subject 4 | |
| | Subject 5 | |
| Trial 2 | Subject 1 | |
| | Subject 2 | |
| | Subject 3 | |
| | Subject 4 | |
| | Subject 5 | |

To have all rows marked with Subject 1 at the top of the table, then all rows with Subject 2, etc., click the **Layout** button and move **Subject**s to the top of the list under **On Rows**, then sort the subject headers.

### *Sort the table rows based on the cell values*

**EXAMPLE** Sort the table based on the values of *Frequency* of zone entries.

Right-click one of the cells containing the values you want to sort, and select one of the two sorting options.



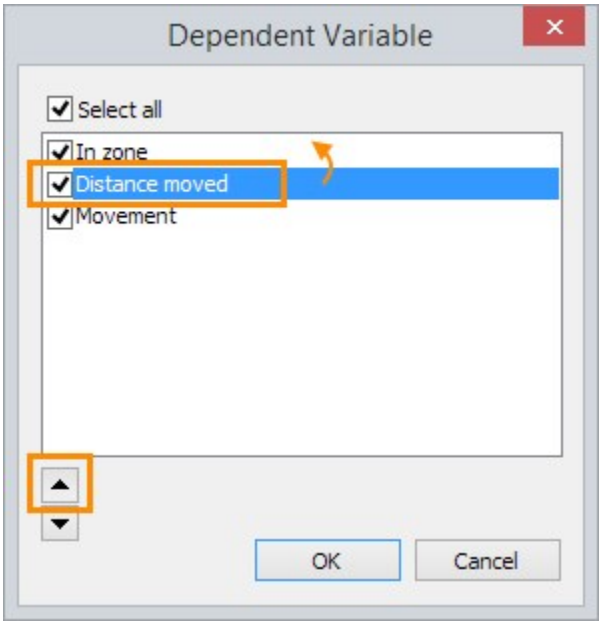

### *Sort the dependent variables in the results table*

**EXAMPLE** Sort the results table by the dependent variables; first, Distance moved, then Movement and then In zone.

| In zone | Distance moved | Movement |
|---|---|---|
| Novel object / center-point | center-point | Moving / center-point |
| Frequency | Total | Cumulative Duration |
| | cm | s |

| Distance moved | Movement | In zone |
|---|---|---|
| center-point | Moving / center-point | Novel object / center-point |
| Total | Cumulative Duration | Frequency |
| cm | s | |

1. Click the **Show/Hide** button at the top-right corner and select **Dependent Variables**.
2. In the window that appears, select a dependent variable and click the **Up** and **Down** buttons to move a variable to a higher/lower position in the table.




Result: The dependent variable at the top of the list in that window is shown in the first row or column in the result table.

# Group charts

## Learn about

- Group charts

## What do you want to do?

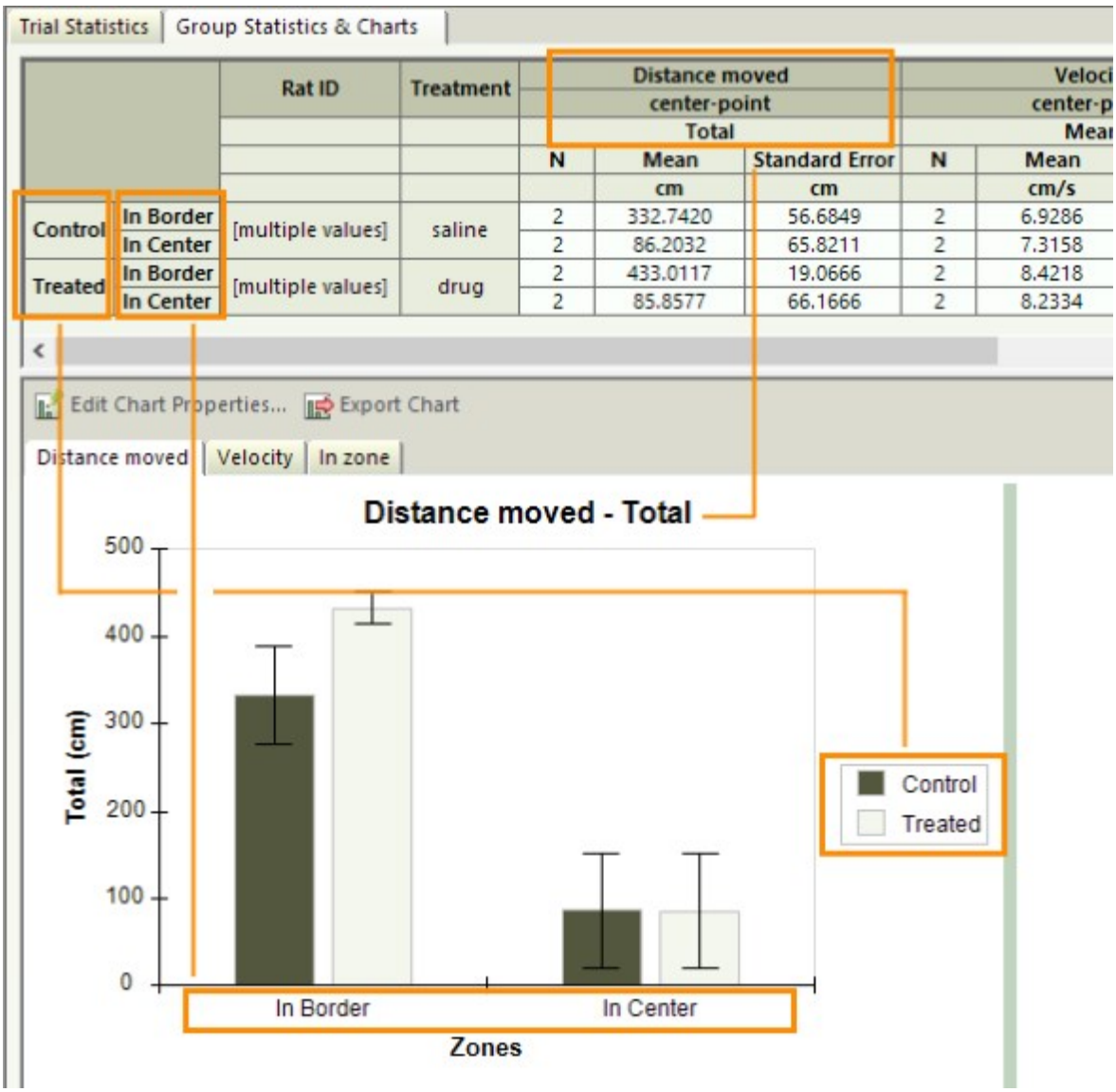
- Customize the group charts

# Group charts

## How to read the group charts

Charts are generated automatically from the results in the Group Statistics & Charts table.

In this example, charts are generated for the dependent variables *Distance moved* (here shown on top), *Velocity* and *In zone* (on the other two tabbed pages). The horizontal axis shows the zones (In Border and In Center). Two data series (from the Result containers Control and Treated) are plotted.



## Chart tabs

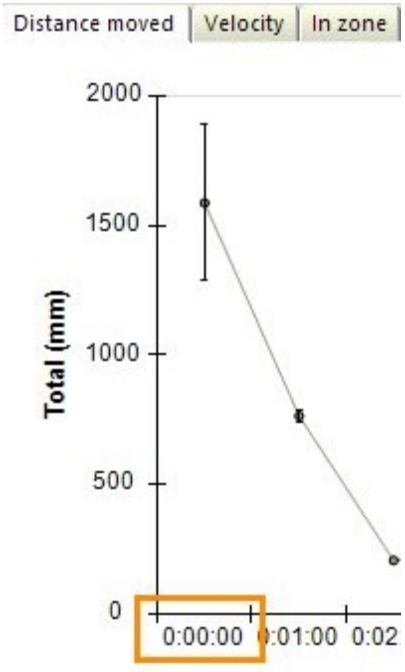
Each tab refers to a dependent variable defined in the Analysis Profile.

A tab may contain one or more charts, one for each Trial Statistic specified for that dependent variable.

## Notes

- A chart is not created for the Trial statistics Standard Deviation, Standard Error and Variance.
- Charts in EthoVision XT have limited formatting options and are mainly meant for inspection purposes. If you want to make a chart for a publication, export the statistics table to Excel or a statistics program.
- If you delete a dependent variable and subsequently add it again to the Analysis profile, the properties of the new chart are set to default.
- Chart settings are stored for each combination of data profile and analysis profile. If you make an exact copy of a data/analysis profile, the resulting charts have default settings.
- Effect of Data selection: **Results per time bin**

  If in the Data profile you specify Results per time bin, time bins are always shown on the horizontal axis. The first value is 0:00:00 and represents the results calculated in the first time bin (for example from 0 s to one minute).



- Effect of Data selection: **Results per zone**

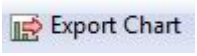
  If in the Data profile you specify to Calculate the results per zone, you can show the zones on the horizontal axis or as data series. See Customize the group charts

- If you specify both time bins and results per zone, time bins are displayed on the horizontal axis and zone statistics as data series.
- If you specify more than 1000 time bins in your Data profile, the group charts are not displayed.

## To export group charts

You can export one chart at a time. To export a chart as an image file, do one of the following:

- Right-click the chart and select **Export Chart**.
- Click the chart and then click the **Export Chart** button just above the chart.




The default file name is the same as the chart title.

Four formats are available: Portable Network Graphics (*.png), JPEG file (*.jpg), Windows bitmap (*.bmp), and Graphics Interchange Format (*.gif).

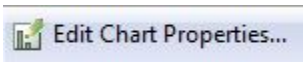
To copy the chart to an external program, right-click the chart and select **Copy**, then paste it to the other program.

# Customize the group charts

## How to access these options

Do one of the following:

- Double-click the chart.
- Right-click the chart and select **Chart Properties**.
- Click the **Edit Chart Properties** button (if there are more charts in the same tab, the first one is selected by default. To customize another one, first click the chart you want to edit).




**TIP** To apply the same properties to all charts in the same tab, make the changes in one chart and then click **Apply to All**.

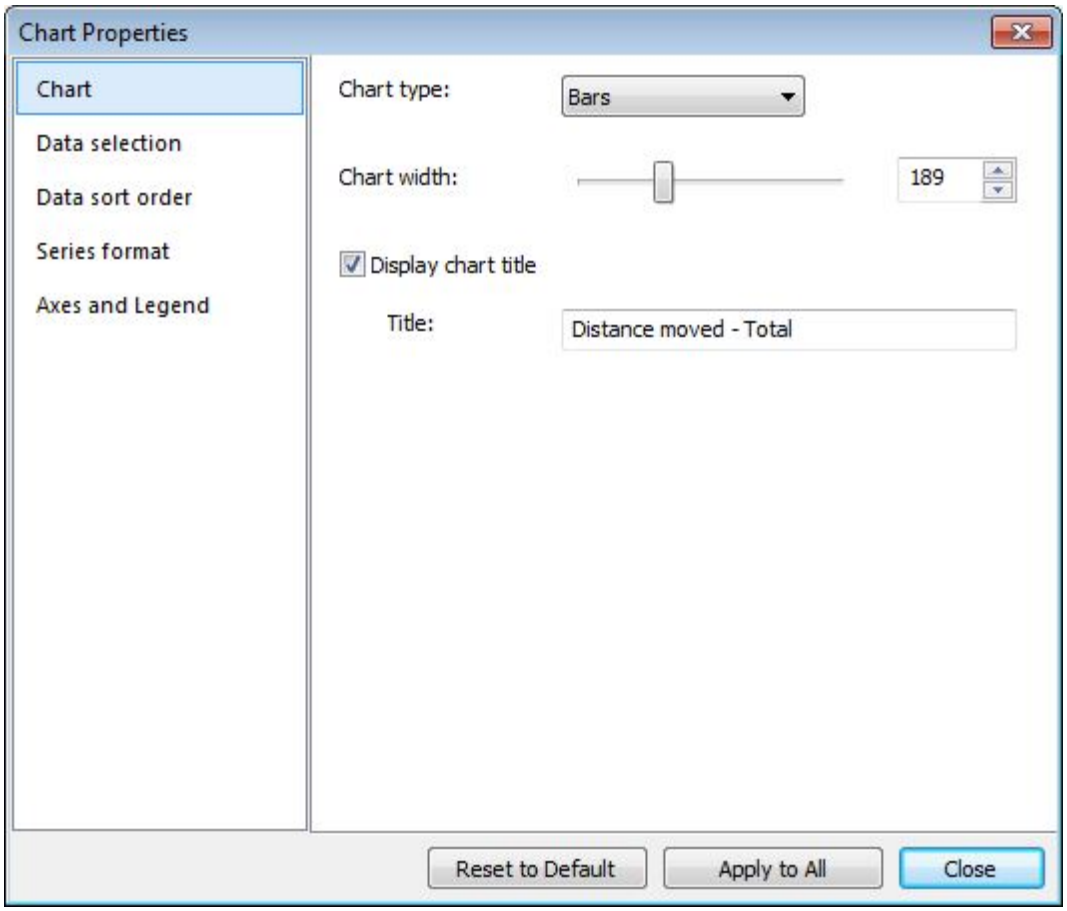
## What do you want to do?

- Change chart type, size and title
- Arrange the data in the chart
- Show the error bars
- Sort the element of a chart
- Change colors, fill patterns, dot size and bar width
- Edit axes, gridlines, and legend
- Apply the changes to other charts
- Reset to default charts

**TIP** If you want to make a Box and whiskers chart, you must first specify **5 number summary** as a group statistic for the variable of interest. You can do this in two ways: (1) in the Analysis profile, open the variable settings window and locate the Group Statistics tab. Or (2) in the statistics result table, right-click the variable name and choose **Properties** then locate the Group Statistics tab.

## Change chart type, size and title

Click **Chart** in the Chart Properties window.



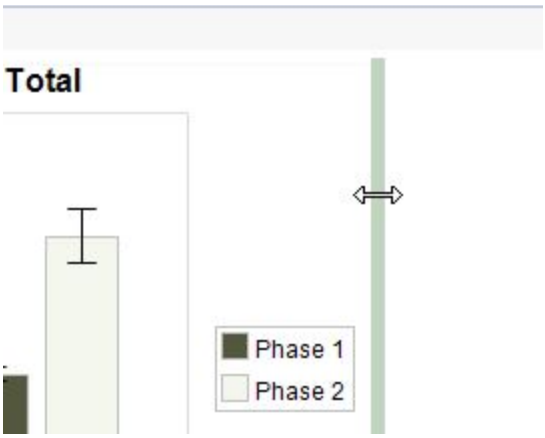

- **Chart type**. Choose between **Bars**, **Lines**, **Box and whiskers**, and **Box, whiskers and data points**.

  By default, a Bars chart is created. However, if you define time bins, a line chart is created; if you specify 5-number summary in Group Statistics, a box and whiskers chart is created. Select a different type from the list (when applicable).

  Choose **Box, whiskers and data points** if you also want to plot the data points that generated the charts. Note that each dot represents the value of a variable calculated for a specific trial.

- **Chart width**. Move the slider or click the arrow buttons to select the required width (range 50-500).

  **TIP** To change the width of a chart, you can also drag the vertical line at the right of the chart.




To change the height of the chart, drag the upper margin of the chart window.

- **Display chart title**. Enter the title you require. The default is [name of dependent variable] - [name of statistic].

## Arrange the data in the chart

Click **Data selection** in the Chart Properties window.

- **Specifiers**. These are elements that can create independent data series. What you see under **Specifiers** much depends on the variable you want to analyze. They can be single body points (e.g. center-point for the variable Distance moved), subjects, variable states (e.g. Mobile, Immobile and Highly Mobile for Mobility), manually-scored behaviors (Grooming and Not Grooming for the behavior Grooming), or combinations of elements, for example Proximity of Nose point to another subject's center-point.

  Select the specifier that you require.

- **Data Series**. Choose the categories that you want to appear in the legend.

  If you select **Specifiers**, for each category on the horizontal axis you get as many data series as the specifiers selected. Note that if you only have one item in the Specifiers box (see above), **Specifiers** is not in this list.

  **EXAMPLE 1** You have the two subjects Control and Treated and selected Relative movement in the Analysis profile and selected the body point Nose point for the actor and Tail base for the receiver. The **Specifiers** of the Chart for Relative movement are Moving to / Nose point / Control - Tail base; Moving from / Nose point / Control - Tail base; No movement / Nose point / Control - Tail base; etc.

  **EXAMPLE 2** You track the center-point of one animal, specified the zones Center and Border, and selected the dependent variable In zone in the

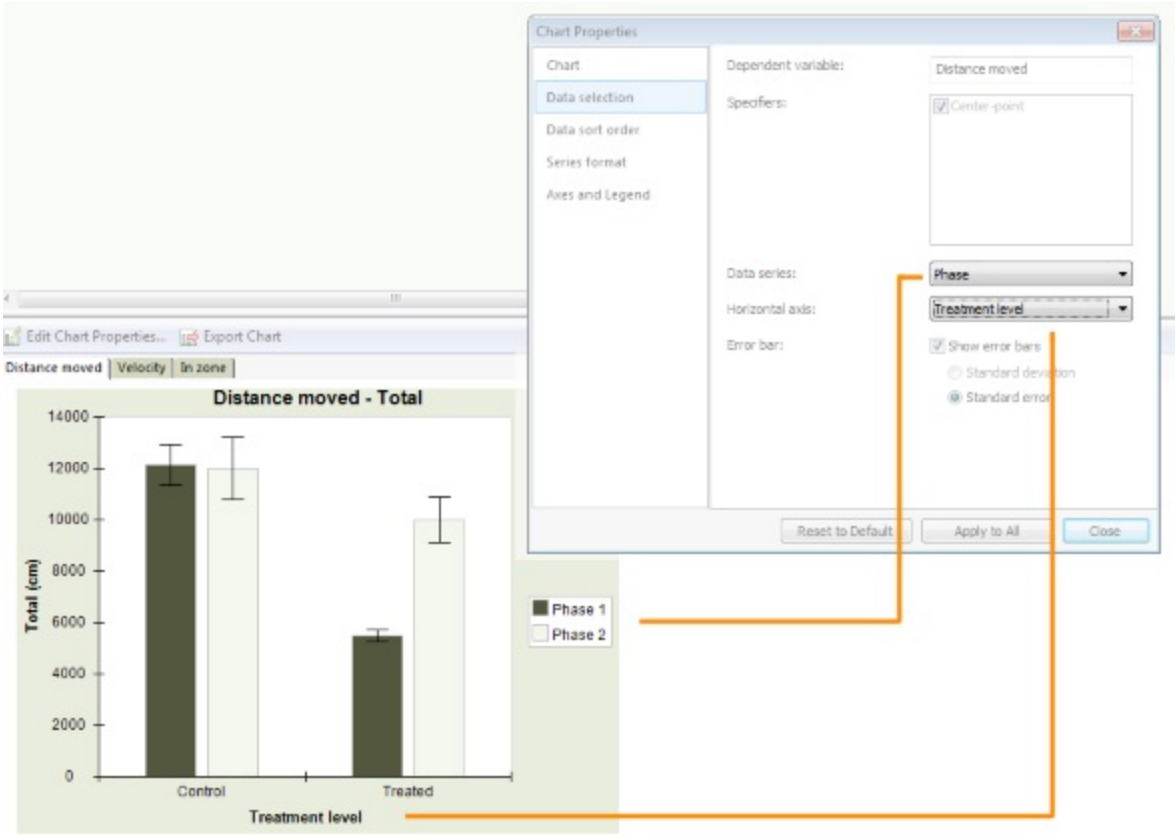
Analysis Profile. The **Specifiers** of the Chart for In zone are Border / Center-point and Center / Center-point.

- If you select **Results**, you get as many data series as the combinations [Result containers]*[Specifiers]. The Results are the names of the Result containers of your Data Profile.
- If you select **Results/Zones**, you get as many series as the combinations [Result containers]*[Zones selected in Results per zone in the Data profile]*[Specifiers].
- If you select **Zones**, you get as many series as combinations [Zones selected in Results per zone in the Data profile]*[Specifiers].
- If you select an independent variable, you get as many data series as combinations [independent variable values]*[Specifiers].

**IMPORTANT** A category or independent variable is listed only when it identifies each group uniquely. For example, if tracks in a group (selection in the Result container of your Data Profile) have two or more different values of Treatment level, this variable is not shown in the list. In this case, the Group Statistics table shows **[multiple values]** in the header.

- **Horizontal axis**. Select what you want to have as the categories on the horizontal axis.
  - **Results**. To have the groups defined in your Data Profile as the categories.
  - [Independent variable name]. To have for example treatment levels or Dosage values as the categories (but see the note above).
  - **Zones**, or **Results/Zones**, (when applicable).
  - **Time bins** is selected by default when you defined time bins in your Data Profile.

**TIP** To combine two independent variables, choose one as **Data series** and the other as **Horizontal axis** (see the figure below).




## Show the error bars

Click **Data selection** in the Chart Properties window. Select **Show error bars** when you want to plot the standard deviation/standard error as error bars.

The option is only available when the statistic is selected under **Group Statistics** for that dependent variable. To add the statistic, right-click the header of the dependent variable in the **Group statistics and Charts** tab and click **Properties**. Click **Group Statistics** and select the statistic you require.

## Sort the element of a chart

1. In the Chart Properties window, click **Data sort order**.
2. Here you can change the sort order of the categories for both horizontal axis and data series. Choose **Alphabetical** (**Ascending** or **Descending**) or **Manual** (click a category and then click the arrow buttons to move it up/ down).

## Change colors, fill patterns, dot size and bar width

1. In the Chart Properties window, click **Series format**.

2. Select either **Color** or **Pattern**.
3. Under **Elements**, choose the color or pattern for each item.

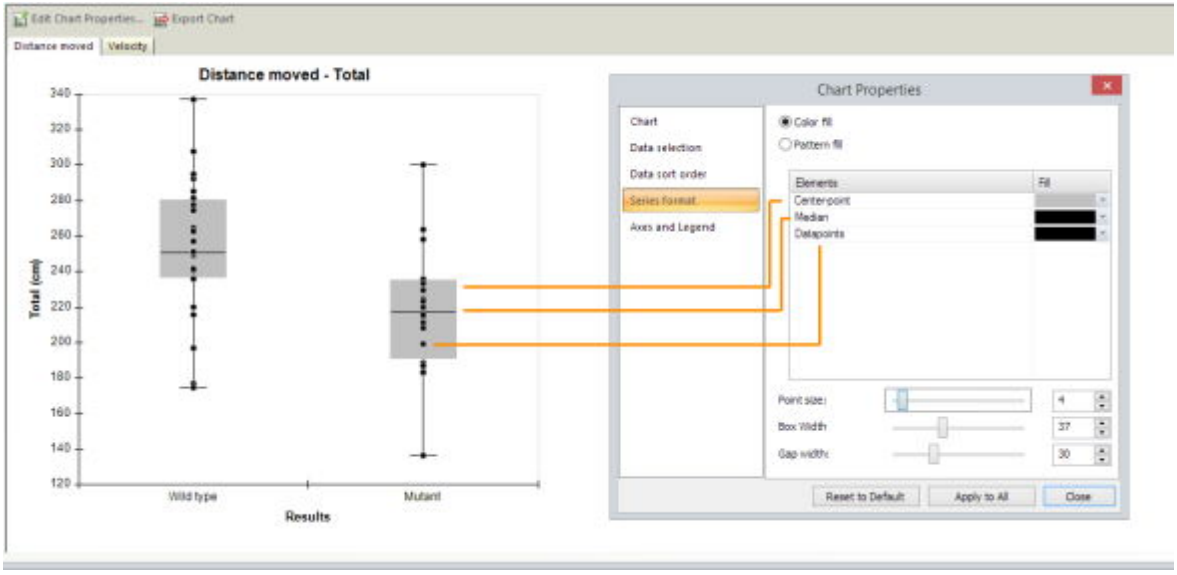
   These element may include, depending on the type of chart:

   - Body points.
   - Subjects.
   - The type of behavior (e.g. **Moving** vs. **Not Moving**).
   - The **Median** (when you choose a Box and whiskers chart) and the **Datapoints** (when you choose Box, whiskers and data points chart).

   **NOTE** Pattern fill is only available when you choose a **Bars** or **Box and whiskers** chart.

4. The following options are available for some chart types:

   - **Dot size**. Select the size of the dots. This applies to Box, whiskers and data points. **NOTE** All dots lie on the vertical middle line of the box plot. It is likely that individual points overlap and are therefore visible.
   - **Bar width / Box width**. Select the bar/box width (range 0-100). Note that for Box and whiskers charts, this also changes the width of the whiskers.
   - **Gap between bars**. Move the slider or click the arrow buttons to select the required gap between adjacent bars (range 0-100).



## Edit axes, gridlines, and legend

In the Chart Properties window, click **Axes and Legend**.

For the **Vertical axis**:

- Select **Display vertical axis title** and enter the new title. The default title is [name of trial statistic] [unit].
- Next to **Scale min** and **Scale max**, set minimum and maximum value of the vertical axis automatically (**Auto**), or select **Fixed** and enter the values you require.
- Select or deselect **Display gridlines**.

For the **Horizontal axis**:

- Select **Display horizontal axis title** and enter the new title. The default title is the name of the category selected as Horizontal axis.

For the **Legend**:

- Select **Display legend**.

  The legend lists the possible combinations of specifiers and the item selected under Data series, separated by "/".

## Apply the changes to other charts

Click **Apply to All** at the bottom of the Chart Properties window. This copies the settings to all charts on that tab, not in other tabs.

## Reset to default charts

Click **Reset to Default** at the bottom of the Chart Properties window. This resets the current chart to default, not other charts.

## Notes

- In Box and whiskers charts, the box includes from 25% (first quartile) to 75% (third quartile) of the data points; the range of data is also named interquartile range. The whiskers include the whole range of the data, including potential outliers.
- If you deselect an element from the **Show/Hide** menu, this element is removed from the statistics table, not from the chart.
- To remove a statistic from the chart, right-click the header of the dependent variable in the table, and click **Properties**. Click **Group Statistics**, and deselect that statistic.

# Batch statistics calculation

## What to you want to do?

- Run Batch calculation

*See also*

- Auto-start data analysis

# Batch calculation

## Aim

Batch calculation helps you perform calculations in multiple runs, each with a particular combination of track smoothing settings, data profiles and analysis profiles. It is handy when you have large data sets that take hours to analyze. In that case you can start batch calculation at the end of the day, and return the next day to collect the results.

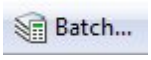
Each analysis run is based on a combination of three profiles:

- Track Smoothing profile (for smoothing tracks and filtering noise).
- Data profile (the trials and samples subject to analysis).
- Analysis profile (the dependent variables and their statistics).

**TIP** When you carry out batch data acquisition, it is also useful to couple this with batch statistics calculations, so that analysis is started automatically after acquisition of the last trial has ended. See Auto-start data analysis.

## To run batch calculation

1. Make sure that all Track Smoothing profiles, Data profiles and Analysis profile that you want to use are defined in the Experiment Explorer.
2. Choose **Analysis** > **Results** > **Statistics & Charts**, or in the Experiment Explorer, under **Results**, click **Statistics & Charts**.
3. Click the **Batch** button.

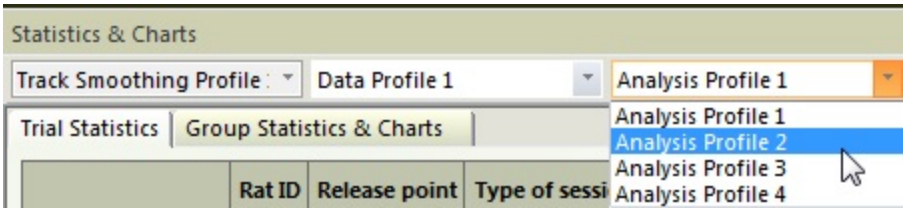
   
   

4. In the Batch Calculation window, do one of the following:
   - If you want to carry out batch calculation for all the combinations of Track smoothing profiles, Data profiles and Analysis profiles, click Add all.
   - If you are interested in specific combinations of profiles, select the combination of profiles that you want to use, and click Add. Repeat this step for all the combinations you require.

   **TIP** To remove a profile combination, click that combination in the list and then click **Delete**. To select multiple combinations, click each combination while holding the **Ctrl** key down.

**TIP** To minimize processing time and disk space occupied by the results, make sure that when you select **Add all**, the Track smoothing, Data and Analysis profiles in your experiment are those you want to use for analysis. Remove any duplicate or non-interesting profiles.

5. The Batch Calculation window lists the combinations selected for calculation.
6. Click **Calculate** when you are ready. To retrieve the results for a particular combination of profiles, select this combination from the lists on the toolbar.




7. Export the results. Note: You can export the result for one combination of profiles at a time. See Export the statistics result.

## Note

- Batch statistics calculations create a number of temporary files. For this reason, they may require large disk space, especially when you analyze large amount of data. These files may also make backup files excessively large. Before backing up an experiment, make sure to remove unnecessary files to create a smaller backup file. For more information, see Back up an experiment.

# Dependent Variables in Detail

## Main topics and tasks

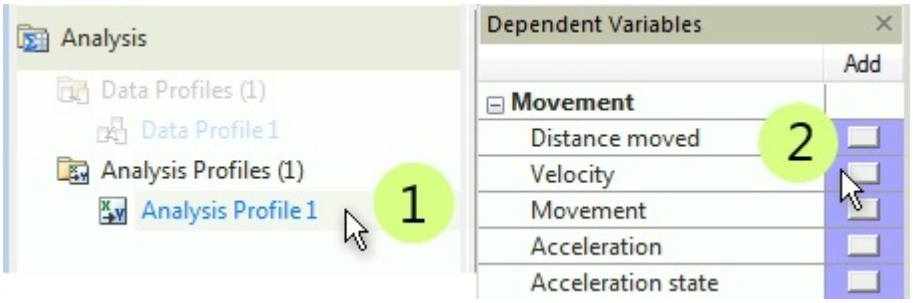


## Notes

- You can use dependent variables:
  - In Trial Control. For example to start tracking when a specific behavior or event occurs.
  - In the Data profile. For example, to select the intervals when the subject was moving.
  - In the Analysis profile. For example, to calculate statistics of distance, velocity or specific behaviors.

- Some dependent variables are only available if you have one of the add-on modules installed: Trial and Hardware Control, Social Interaction, Rat/ Mouse Behavior Recognition and Live Mouse Tracker.
- If none of the dependent variables cannot help quantify behavior of your subjects, please contact Noldus, so we can discuss your analysis requirements.

# Movement




- Distance moved 982
- Velocity 984
- Movement 988
- Acceleration 991
- Acceleration state 993

# Distance moved

## Definition

The distance traveled by the center, nose or tail-base point of the subject from the previous sample to the current one. It is calculated as:

$$DM_k = \sqrt{(X_k - X_{k-1})^2 + (Y_k - Y_{k-1})^2}$$

where:

- $DM_k$ = Distance moved from sample *k–1* to sample *k*
- $X_{k-1}$, $Y_{k-1}$ = X,Y coordinates of the center, nose or tail-base point at sample *k-1*,
- $X_k$, $Y_k$ = X,Y coordinates of the center, nose or tail-base point at sample *k-1*.


## How to specify Distance moved

1. Click **Add** next to **Distance moved**.
2. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the Body points tab and select the body points for which you want to calculate the distance.
- Because it is based on change in X,Y coordinates, Distance moved needs two valid (non-missing) samples. A missing sample in your track results in missing Distance moved values in that and in the next sample. Make sure that the proportion of missing samples is low (less than 1%).
- Sample rate influences the values of Distance moved. When tracking at too low a sample rate, parts of the actual path are cut off, resulting in an

underestimation of per-sample (and total) Distance moved. If, on the other hand, the sample rate is too high, EthoVision XT catches the wobbling of the body's center point of the walking animal, causing extra apparent movement, and therefore an overestimation of per-sample (and total) Distance moved. See Track Smoothing for a description of how to filter out small movements.

## Applications

Distance moved is often used to give a general measure of activity. It is also used as the basis for calculating other parameters such as velocity (see below).

In rodent models of aging, the total and maximum distance moved are used to calculate the frailty index. See Parks et al. (2012) *J. Gerontol. A* 67(3): 217-227.

# Velocity

## Definition

The distance moved by the center, nose or tail-base point of the subject per unit time.

Velocity is obtained by dividing Distance moved by the time difference between a sample and the previous one:

$$V_k = \frac{DM_k}{t_k - t_{k-1}}$$

where $V_k$ = velocity at sample *k* (expressed in the unit you have defined in the Experiment Settings) and $DM_k$ = Distance moved at sample *k*.


## How to specify Velocity

1. Click the **Add** button next to **Velocity**.
2. Under **Outlier filter**, specify the **Averaging interval**. Leave 1 if you want to keep the raw data; enter a value to smooth the values using a running average method. See Averaging interval
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the **Body points** tab and select the body points for which you want to calculate the velocity.
- Sample rate influences the calculation of Distance moved, and therefore Velocity. When tracking at too low a sample rate, parts of the actual path are cut off, resulting in an underestimation of per-sample Velocity. If, on the other hand, the sample rate is too high, EthoVision XT catches all random

movements and wobbling of the body point of the walking subject, causing extra apparent displacement, therefore an overestimation of Velocity.

See Track Smoothing for a description of how to filter out small movements.

- Because it is based on change in distance moved, Velocity needs two valid (non-missing) samples. A missing sample in your track results in missing Velocity values in that and in the next sample. Make sure that the proportion of missing samples is low (less than 1%).

  You can view the proportion of missing samples as one of the System Variables in the Trial list.

## Application

- Apart from the obvious applications of this dependent variable, the mean velocity is sometimes used as a measure of general activity (for example, Nilsson *et al*. 1993, *J. Exp. Biol.* **180**, 153-162; Winberg *et al.* 1993, *J. Exp. Biol.* **179**, 213-232).
- **TIP** Define Velocity and Movement in the same Analysis profile to find out which averaging interval results in the best match between movement bouts and video.
- In rodent models of aging, the average velocity is used to calculate the frailty index. See Parks et al. (2012) *J. Gerontol. A* 67(3): 217-227.

# Averaging interval

## Aim

To smooth the values of a dependent variable.

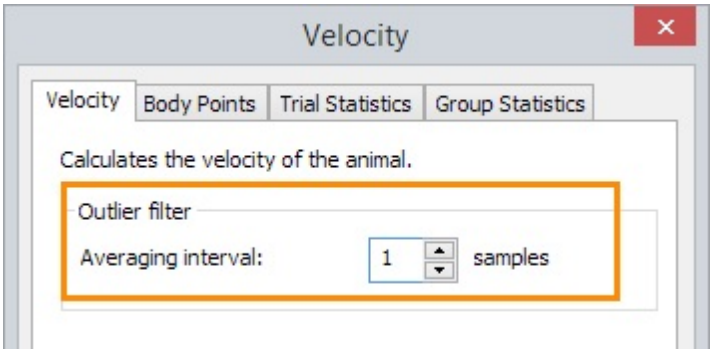
## This topic applies to

Dependent variables: Acceleration state, Activity, Activity state, Body angle state, Body elongation, Body elongation state, External data (resampled), External data - state, Mobility, Mobility state, Movement, Velocity.

The dependent variable can be in a condition defined in the Trial Control Settings, in the Analysis profile and in the Data profile. Setting the Averaging interval in one part of EthoVision XT does not influence the value of the same variable in the others.

## How to access this option

In the Trial Control Settings, in the Analysis profile or in the Data profile select the dependent variable and locate **Outlier filter**.




## How Averaging interval works

- When Averaging interval is 1, the outlier filter is off, thus the values of the variable are not smoothed.
- When Averaging interval is 2 or larger, EthoVision XT replaces the per-sample value of the dependent variable with the average calculated over the number of samples specified by the interval.

The table below shows how EthoVision XT re-calculates a variable V in a few samples when Averaging interval is set to 2. Note how the average (avg) is obtained when values of the dependent variable are missing. Remember that velocity V at time t is only calculated when there are valid samples at time t and t-1. See Velocity

| **Sample** | **Valid** (•) **or missing** (-) | **Original value** | **Smoothed value** when averaging interval = 2 |
|---|---|---|---|
| 1 | • | $V_1$ | $V_1$ |
| 2 | • | $V_2$ | avg ($V_1$, $V_2$) |
| 3 | • | $V_3$ | avg ($V_2$, $V_3$) |
| 4 | - | not calculated | avg ($V_3$, [no value]) = $V_3$ |
| 5 | - | not calculated | avg ([no value], [no value]) = [no value] |
| 6 | • | not calculated | avg ([no value], [no value]) = [no value] |
| 7 | • | $V_7$ | avg ([no value], $V_7$) = $V_7$ |

## Notes

- Note the difference between Track Smoothing and the Outlier filter (this topic):
  - With **Track Smoothing**, you smooth the *raw x,y coordinates*. This has also an effect on the dependent variables calculated based on those coordinates, for example *Distance moved*. See Smooth the Tracks
  - With **Outlier filter**, you smooth the values of the *dependent variable*, for example velocity or mobility, after they are calculated from the raw data. The Outlier filter is useful when you want to calculate state variables. Apply the Outlier filter, for example, when you want to smooth *Velocity* to calculate the *Movement* states, which are based on velocity; or smooth *Mobility* when you want to calculate *Mobility state*.
- If you combine Track Smoothing with Outlier filter, the dependent variable is calculated with the Outlier filter *after* the raw coordinates are smoothed with Track Smoothing.

# Movement

## Definition

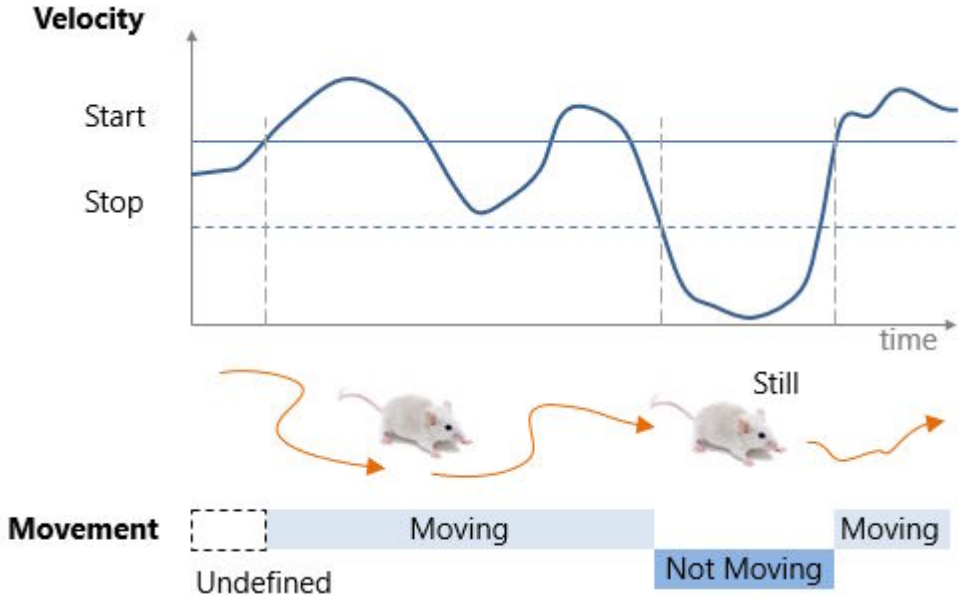
A discrete (state event) variable, based on one of the body points, with two possible states, *Moving* and *Not moving*:

- The state is *Moving* if the running average velocity of the body point exceeds the user-defined Start velocity.
- The state remains *Moving* until the running average velocity drops below the user-defined Stop velocity.
- The state then becomes *Not moving* until the running average velocity reaches the Start velocity again.

In order to reduce the sensitivity of this dependent variable to brief changes in velocity, the data can be smoothed by taking the running average of the last *k* samples. This number is referred to as the averaging interval.

When a body point is missing for more than three samples, the current Movement state ends and the remaining missing samples are ignored.

In the following example, the velocity initially lies between the Stop velocity and the Start velocity. Therefore, the Movement state is undefined. When velocity exceeds the Start value, Movement is given the value *Moving*. When velocity drops below the Stop value, Movement is given the value *Not moving*.



## How to specify Movement

1. Click the **Add** button next to **Movement**.
2. In the **Movement** tab, enter the following:
   - **Averaging interval:** The number of samples over which the running average velocity is based. The default value is 1, that is, velocity is not smoothed before calculating the Movement variable.
   - **Start velocity**: The velocity above which the subject is considered to be moving.
   - **Stop velocity**: The velocity below which displacements of the subject's body points are no longer attributed to locomotion but to system noise, body wobble or pivoting on the spot.

   **NOTE** The default values are an example and may not apply to your experiment. The threshold values also vary between species. If the subject is very slow, like a walking tick, you must reduce the two thresholds to detect true movement. See also a note below.
3. Under **Calculate statistics for**, select either one of them, or both.
   - **Moving**: To calculate statistics for when the subject is moving.
   - **Not moving**: To calculate statistics for when the subject is not moving.
4. Complete the procedure to add the variable See Calculate statistics: procedure.

## Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the **Body points** tab and select the body points for which you want to calculate movement.
- To find the optimal **Start velocity**, plot the values of Velocity and take note of the values when the animal moves in the video. Do this for a couple of videos. The Start velocity should be just below those values. Similarly, to find the optimal **Stop velocity**, take note of the values of velocity when the animal sits still. The Stop velocity should be just above those values. Obviously, a more objective evaluation could come from a statistical approach (e.g. to find cutoff values that discriminate between different behaviors, moving vs not moving).
- By increasing the averaging interval, you can increase the reliability of movement detection. A running average velocity based on more samples diminishes the effect of random changes in velocity due to noise. However, a drawback of increasing the averaging interval is that it causes a delay in

the determination of a state transition, proportional to the length of the interval. See Averaging interval

- Values of velocity between Start velocity and Stop velocity result in no change in the current state of the subject (moving or not moving). The smaller the difference between the two threshold velocities, the more likely that transitions between the states *Moving* and *Not moving* are scored. By defining such a buffer, you prevent overestimation of transition rates because of a velocity joggling just around the movement threshold.

## Application

Like Velocity, *Movement* provides information on the subject's locomotor activity.

In rodent models of aging, the total duration of *Moving*, both in seconds and as percentage of the trial time, are used to calculate the frailty index. See Parks et al. (2012) *J. Gerontol. A* 67(3): 217-227.

# Acceleration

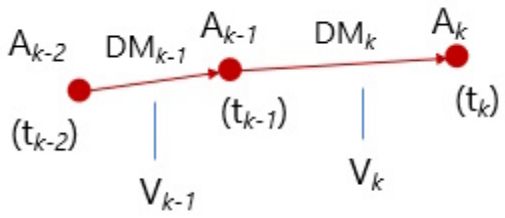
## Definition

Acceleration at sample *k* is obtained by dividing the difference in Velocity, by the time difference between that sample and the previous one:

$$A_k = \frac{V_k - V_{k-1}}{t_k - t_{k-1}}$$

Where:

- $A_k$ = Acceleration at sample *k*,
- $V_k$ = Velocity at sample *k*.




## How to specify Acceleration

1. Click the **Add** button next to **Acceleration**.
2. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the **Body points** tab and select the body points for which you want to calculate acceleration.
- Because it is based on change in velocity, Acceleration needs three valid consecutive samples. A missing sample in your track results in missing Acceleration values in that and in the next two samples. See also the notes under Velocity.
- Acceleration is sensitive to random changes in velocity between consecutive samples due to noise. Check the track and velocity plot to see if

changes in velocity represents true acceleration. If necessary, apply Lowess smoothing to the tracks.

# Acceleration state

## Definition

A discrete (state event) variable, related to one of the body points, with two possible states, *High acceleration* and *Low acceleration*. At any sample time:

- The state is *High acceleration* when the running average acceleration exceeds the High acceleration above threshold.
- The state is *Low acceleration* when the running average acceleration is below the High acceleration above t threshold.

The running average acceleration is calculated according to the formula for Acceleration for each sample, over the number of samples specified by the Averaging interval.

## How to specify Acceleration state

1. Click the **Add** button next to **Acceleration state**.
2. In the **Acceleration State** tab, enter the following:
    - **Averaging interval**. This is the number of samples over which the running average acceleration is based. The default value is 1, that is, acceleration is not smoothed before calculating the Acceleration state variable.
    - **High acceleration above**. The acceleration above which the animal must be considered in the High acceleration state.
3. Under **Calculate statistics for**, select the state you want to analyze, **High acceleration** and/or **Low acceleration**.
4. Under **State duration threshold**, next to **Exclude instances shorter than**, enter the minimum duration of the Acceleration state (see the note below).
5. In the **Body Points** tab (if present), select the body points for which you want to calculate Acceleration state.
6. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- Enter zero as **High acceleration above** to distinguish between positive and negative acceleration.
- To find the optimal **High acceleration above** threshold, run a few test trials and in the Integrated Visualization plot the values of Acceleration and

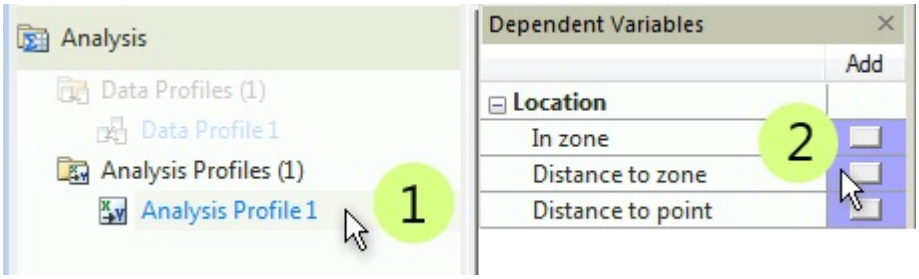
Acceleration state. Adjust the threshold value in such a way that the state High acceleration is scored only when the animal shows bursts of rapid movement in the video.

- We advise you to use an Averaging interval greater than 1 to remove the effect of random changes in velocity between consecutive samples that would result in false transitions between Low and High acceleration. However, the greater the Averaging interval, the longer the delay in the determination of a state transition. See Averaging interval
- The **State duration threshold** is the minimum duration of the set of consecutive samples with Acceleration above (or below) the **High acceleration above** threshold required in order to be scored as the corresponding state. If the set of samples passes the threshold, the samples are scored as the state. If the set of samples does not pass the threshold, the previous state ends, but no new state is defined. Use this option to filter out brief transitions between High and Low acceleration caused by body-point jitter or noise detection.

## Applications

Use *Acceleration state* to mark bursts of rapid movement, like swimming bursts in fish.

# Location




- In zone 996
- Distance to zone 1001
- Distance to point 1004

# In zone

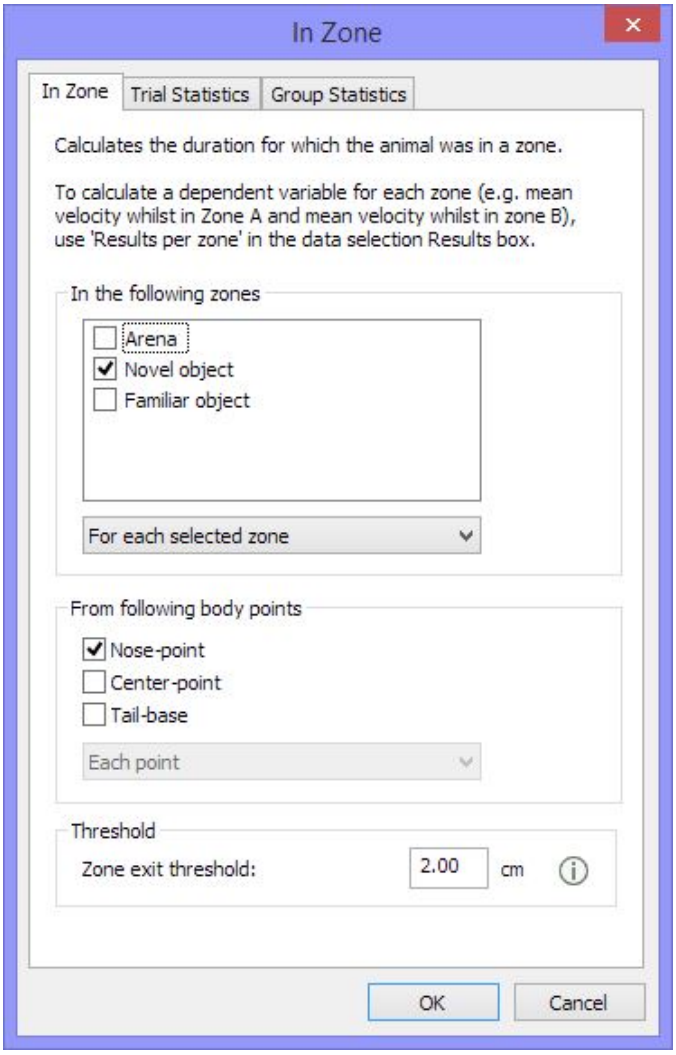
## Definition

A discrete (state event) variable with two possible states, *In zone* and *Not in zone*, depending on whether the body point chosen is within a zone (or group of zones).

The state for a specified zone is determined for each sample by comparing the coordinates of the chosen body point with the coordinates that make up the zone of interest.

## How to specify In zone

1. Click the **Add** button next to In zone and click the **In zone** tab in the window that appears.



2. Under **In the following zones**, select the zones you want to analyze. For example, if you want to calculate the total time the animal was in Zone 1, select Zone 1.

   If you have chosen two or more zones, select how body points should be analyzed:

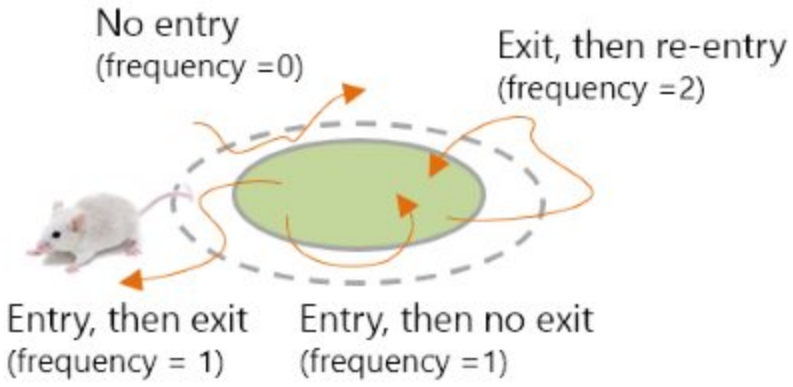
   - **For each selected zone**: The body points are analyzed in each zone separately.
   - **When in any of the selected zones**: The body points are analyzed when in any of the selected zones.
   - **When in all selected zones**: The body points are analyzed when in all those zones simultaneously.
   - **When not in any of the zones**: The body points are analyzed that are in none of the selected zones.

3. Under **From following body points**, select the points you want to consider for the calculation. For example, select **Nose-point** if you want to calculate the statistics of the time the nose point was in a specific zone. By default, **Center-point** is selected.

   If you have chosen two or more body points, select one of the following from the list:

   - **For each selected point**: Statistics are calculated for each point separately.
   - **If any point is in zone**: Statistics are calculated for when any of the selected points is in the zone.
   - **When all points are in zone**: Statistics are calculated for when all the selected points are in a zone simultaneously.

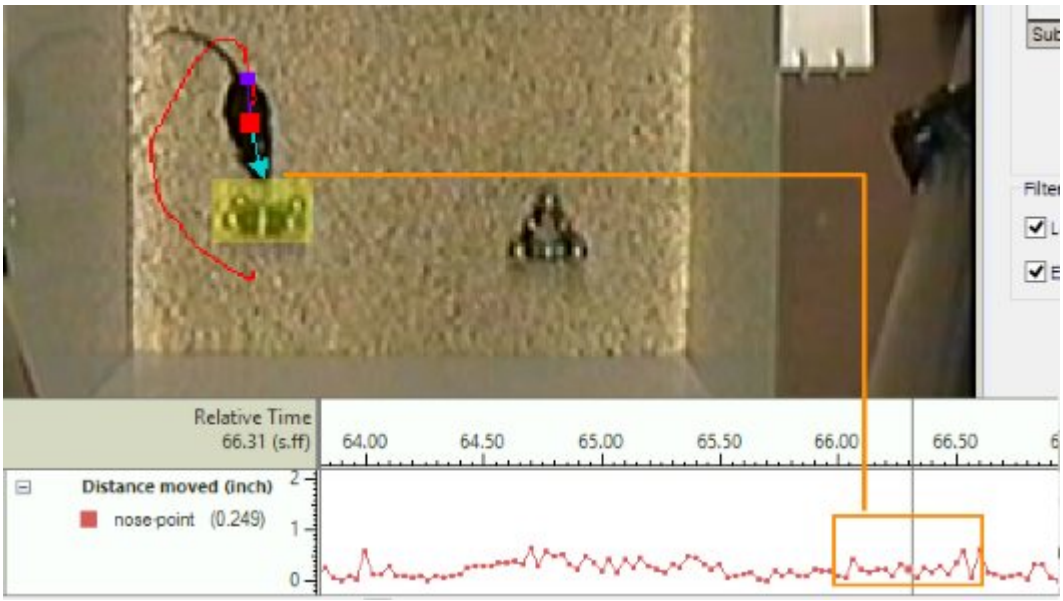
4. Under **Threshold**, enter the **Zone exit threshold**. That is, once the animal's body point is detected in the zone, the animal is considered to be in the zone until its distance from the zone border (when outside the zone) exceeds that threshold. Default: 0 cm. Use this option to remove false re-entries resulting from random movements of the body point around the zone border. The following example shows the effect of the threshold for different trajectories. The frequency is shown for *In zone*.



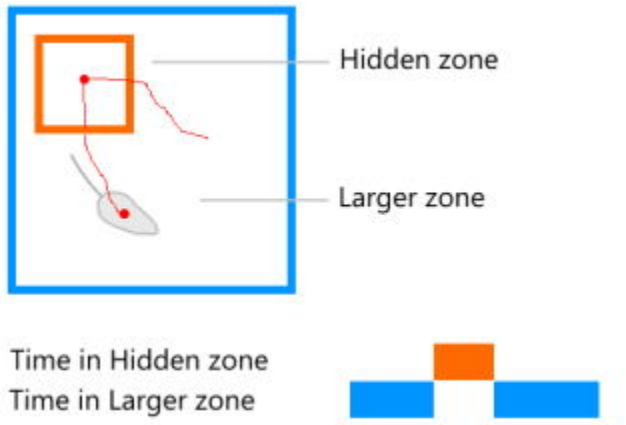

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- If your experiment is set to Only center-point detection or Color marker tracking, the *In zone* variable is calculated for the center-point.
- To find a good Zone exit threshold for *In zone*, plot the values of Distance moved for the body point you are interested in. For instance, in a Novel object test, plot the distance moved for the nose-point while the subject explores an object, that is, while its nose-point is within the border of the zone "object". Take note of the variation in the per-sample distance moved and set the Zone exit threshold higher than this range.



- If two zones are adjacent, that is, not overlapping, the subject's body point is supposed to be either in one or the other zone. However, when the zone exit threshold is greater than zero, the subject's body point may not yet be outside zone 1, and (by definition) be already in zone 2. To prevent this, re-define the two zones in such a way there is a gap between the two, with its width at least the same as the threshold.
- When you export *In zone* as raw data, the values are exported for each sample time, with possible values 0 (*Not in zone*), 1 (*In zone*), or "-" (unknown). See Export the raw data (track and dependent variables)
- When a body point is missing for more than three consecutive samples, the *In zone* state ends and the remaining missing samples are not assigned to any state (neither *In zone* or *Not in zone*).
- If you analyze the time that the subject spent in a zone that includes a hidden zone, the results are only given for when the subject is visible (and therefore detected) in the larger zone. If you want to calculate the total time in that zone including the time spent in the hidden zone, select both zones in the *In zone* settings (step 2 above), and sum up the time values for the two zones reported in the results table.




## Application

*In zone* is a standard variable for any study involving the usage of space by animals. For example:

- Open field: How much time did the animal spend by the walls, and how long did it take to cross the open center? (for example, Berendsen et al. 1994, *Behav. Pharm.* **5** (Suppl. 1): 81).
- Maze studies: How many errors did the animal make? (for example, Ploeger G.E. 1995, PhD thesis, Utrecht University) How long did it take to get to the target (Ploeger et al. 1994, *Behav. Neurosci.* **108**, 927-934) How many times

did the animal enter the open arms in a plus maze? (Law et al. 2003. *J. Neurosci.* **23**: 10419-10432).

- Four-way olfactometer: How much time did the animal spend in the treated odor field? When did it first enter one of the arms? (Kaiser and de Jong 1994, *Behav. Proc.* **30**: 175-184).
- Water-maze: How much time does the animal spend in an 18-cm wide path (Whishaw's corridor) from the starting location to the platform, designated as the correct route? If a rat deviated from this route, it received a maximum of one error on that trial (Whishaw's error, Whishaw 1985, *Behav. Neurosci.* **99**(5): 979-1005), indicating that it did not show a direct swim path.

# Distance to zone

## Definition

The shortest distance between a subject's body point (or selection of points) and a zone (or group of zones). You can calculate the distance regardless of where the body point is, or assuming that the point is always outside the zone (in the latter case, the distance is set to zero when the body point enters the zone).

The calculation of this variable is performed in two steps:

1. The coordinates of the point on the zone border that is closest to the coordinates of the body point for the current sample are found.
2. The distance in a straight line between the two coordinates is calculated.

## How to specify Distance to zone

1. Click the **Add** button next to **Distance to zone** and click the **Distance to zone** tab.
2. Under **To the following zones**, select the zones you want to consider for the calculation. For example, if you want to calculate the mean distance to Zone 1, select Zone 1. By default, **Arena** is selected.
   If you have chosen two or more zones, select how the zones should be analyzed:
   - **For each of the selected zones**. Zones are analyzed separately.
   - **Shortest distance to any zone**. For each sample, EthoVision XT chooses the zone that is currently closest to the point(s) you have chosen, and uses the resulting distances for calculating the statistics.
3. Select the **Include if in zone** option if you want to calculate the distance to the border of a zone of interest, regardless of whether the subject is outside or inside the zone. If you want to calculate the distance to the border of the zone when the subjects is outside the zone, leave this option cleared. See one of the Notes below.
4. Under **From following body points**, select the points you want to consider for the calculation. For example, select **Nose-point** if you want to analyze the distance between the nose-point and a specific zone. By default, **Center-point** is selected.

   If you have chosen two or more body points, select one of the following from the list:
   - **For each selected point**. Statistics are calculated for each point separately.

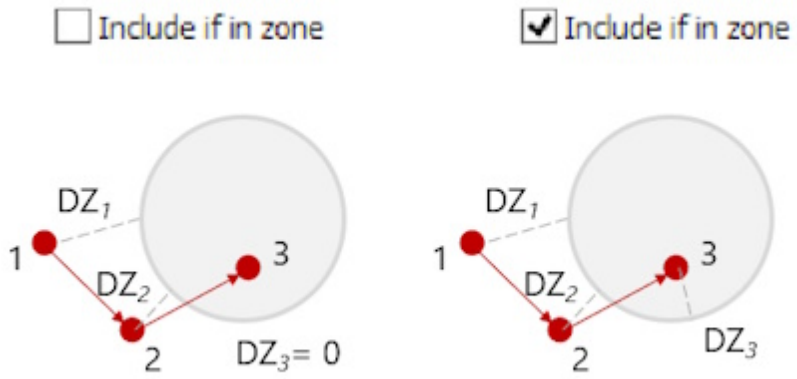
- **Shortest distance to any point**: For each sample, EthoVision XT chooses the body point that is currently closest to the zone, and uses the resulting distances for calculating the statistics.

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- If you have selected Include if in zone in the **Distance to zone** tab of the variable's properties window, and the body point's coordinates lie inside the zone, the Distance to zone is greater than 0.

  **EXAMPLE** Effect of the option **Include if in zone** on the distance to zone (DZ, dotted lines) for three consecutive samples 1, 2 and 3. The zone is shown in gray. When the option is not selected, and the sample 3 is within the zone, DZ for that sample is zero. When the option is selected, DZ for sample 3 has a value larger than zero.




- If your experiment is set to **Only center-point detection** or **Color marker tracking**, the body point options are not available. Calculations are based on the center point.

## Application

Two examples of how you can use *Distance to zone* with **Include if in zone** not selected:

- In a Morris water maze test with the hidden platform defined as a zone, Distance to zone can measure the animal's progress towards the platform. You can select the Total statistic to give a measure of the cumulative distance to zone (for training trials) and the Mean statistic to give a measure of average proximity (for probe trials; Gallagher *et al.* 1993. *Behav.*

*Neurosci.* **107**: 618-626). See also the chapter The Morris water maze test in the EthoVision XT 18 - Application Manual for the exact procedure. You can also divide the maze into quadrants to give a more fine-grained analysis of behavior during the trial.

- In a study of territorial behavior, the resident's territory could be defined as a zone. You can then measure how close the intruder comes to that area.

One example of how you can use *Distance to zone* with **Include if in zone** selected is the following:

- In a study of anxiety, one could define an entire open field as a zone, and then use *Distance to zone* to measure to what extent animals dare to move away from the wall. More generally, if you are interested in the distance between a subject and the edge of an open field or the border of an Elevated plus maze, you can select the complete arena as a zone.

# Distance to point

## Definition

The shortest distance between a subject's body point and one or more points.

The calculation of this variable is performed in two steps:

1. The coordinates of the defined point(s) and the body point(s) for the current sample are found.
2. The distance in a straight line between the coordinates is calculated.

If your experiment is set to Only center-point detection or Color marker tracking, the body point options are not available. Calculations are based on the center point.

## How to specify Distance to point

1. Click the **Add** button next to **Distance to point** and click the **Distance to point** tab.
2. Under **To following points**, select the points you want to consider for the calculation. For example, if you want to calculate the mean distance to Cue 1, select Cue 1. By default, **Arena** (that is, the center of the Arena) is selected.

   If you have chosen two or more points, select how the points should be analyzed:

   - **For each of the selected points**: Points are analyzed separately.
   - **Shortest distance to any points**: For each sample, EthoVision XT chooses the point that is currently closest to the body point(s) you have chosen, and uses the resulting distances for calculating the statistics.
3. Under **From following body points**, select the points you want to consider for the calculation. For example, select **Nose-point** if you want to analyze the distance between the nose-point and a specific point. By default, **Center-point** is selected.

   If you have chosen two or more body points, select one of the following from the list:

   - **For each of the selected points**: Statistics are calculated for each point separately.
   - **Shortest distance to any point**: For each sample, EthoVision XT chooses the body point that is currently closest to the point, and uses the resulting distances for calculating the statistics.

4. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

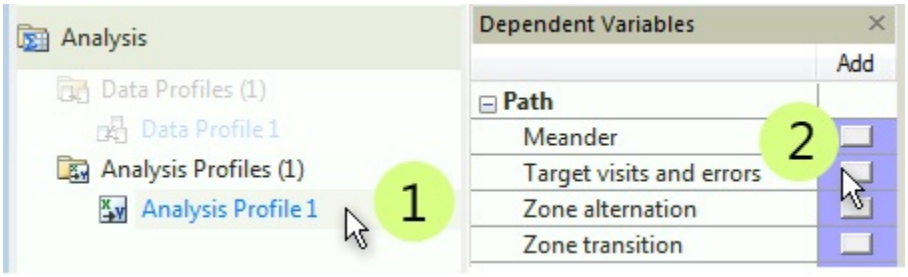
- If your experiment is set to Only center-point detection or Color marker tracking, the body point options are not available. Calculations are based on the center-point.
- The center point of a zone can lie outside the zone itself. This occurs when the zone is ring-shaped or very asymmetrical.

## Applications

Below are two examples of how *Distance to point* is of particular use in studies of spatial orientation:

- When analyzing the flight behavior of an insect in an odor plume, the plume itself can be defined as a zone, while the upwind odor source is regarded as a point. Using *Distance to point*, you can measure the insect's progress towards the source at any moment in time.
- In an open field test, using the center point of the central area of the arena, *Distance to point* can be used to measure how far the animal ventured into the central area.

# Path



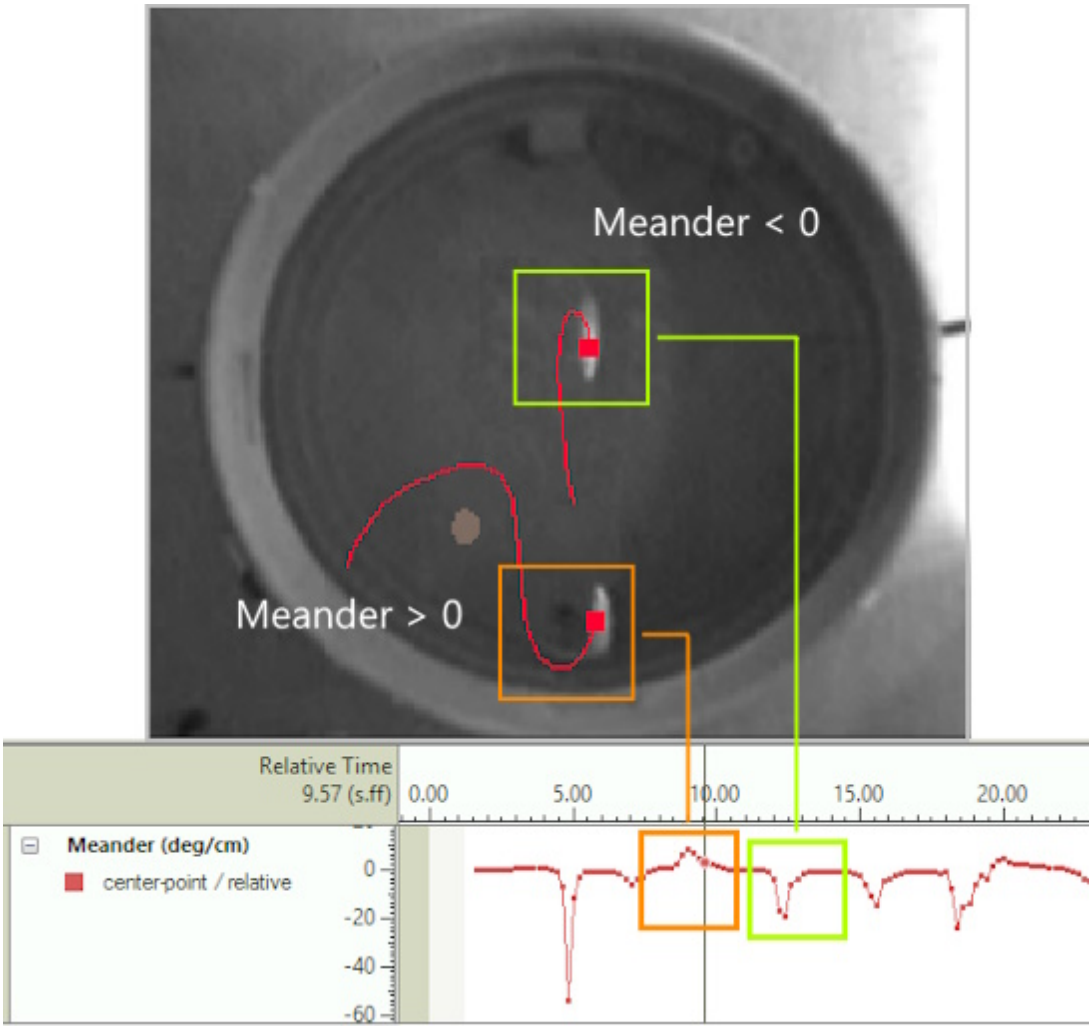

- Meander 1007
- Target visits and errors 1010
- Zone alternation 1013
- Zone transition 1016

# Meander

## Definition

*Meander* is the change in direction of movement of a subject relative to the distance moved by that subject. It provides an indication of how convoluted the subject's trajectory is. *Meander* can be relative or absolute:

- *Relative Meander*: The change in direction is signed. With a default position of the Calibration axes (x-axis pointing to the right; y-axis pointing upward), a clockwise turn is scored as negative value because turn angle is negative; a counterclockwise turn is scored as positive. With other orientations of the x- and y-axes, the same turn may have different sign, depending on the relative turn angle. See Turn angle for how turns are given positive vs. sign.



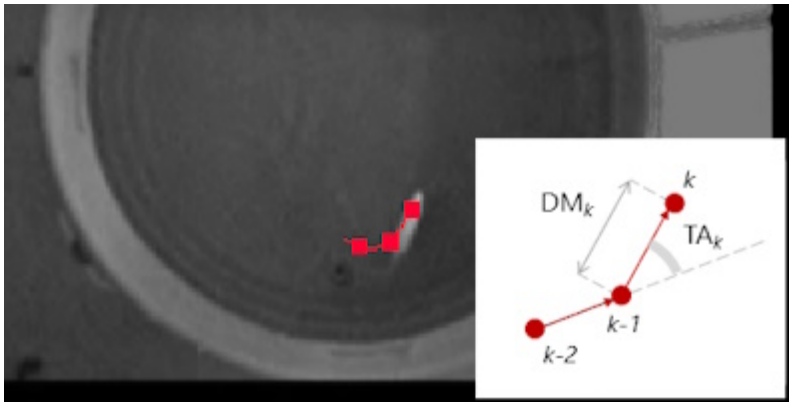

- *Absolute Meander*: The turn angle that measures the change in direction is unsigned. Meander is therefore always positive.

## Calculation

$$RM_k = \frac{RTA_k}{DM_k}$$

Where $RM_k$ is *Relative Meander*, $RTA_k$ is *the Relative Turn angle* and $DM_k$ is the Distance moved at sample *k*.

Absolute Meander is the absolute value of the Relative Meander.




You can calculate *Meander* in two ways:

- Based on body points: *Meander* is calculated from the turn angle and the distance moved of the specified body point, as shown in the figure above.
- Based on Head direction: *Meander* is calculated from the turn angle based on the Head direction line and the distance moved by the nose point.

## Range

Given the formula above, Relative Meander can range from -∞°/cm to +∞°/cm, while Absolute Meander from 0°/cm to +∞°/cm. When DM is very small, Meander can get high, unrealistic values. One case when DM is very small is when you use a high sample rate and the subject does not move significantly.

**TIP** Before running analysis, make sure you use Track Smoothing to remove very small values of distance moved from your tracks.

See also Troubleshooting: Statistics > I get unrealistic values of path shape and direction

## How to specify Meander

1. Click the **Add** button next to **Meander** and click the **Meander** tab. Select **Absolute** or **Relative**.
2. Select Head direction meander (body point is ignored) if you want to calculate meander based on the head direction line.
3. Click the **Body points** tab and select the body points for which you want to calculate meander. By default, **Center-point** is selected.
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- If your experiment is set to Only center-point detection or Color marker tracking, the Body points tab is absent. Calculations are based on the center point.
- *Meander* is very sensitive to small, random movements of the body points. When the animal sits still, *Meander* can get very high, unrealistic values. To remove such small movements from your data, Smooth the Tracks, then run analysis.

## Application

- The *Relative Meander* is a measure for the direction of turning per unit distance. This dependent variable can be of additional value to other turn bias variables, such as relative Turn angle and relative Angular velocity, since the turn bias is 'corrected' for the distance moved. For instance, if two individuals move at different speeds, the two can have very different values for the mean relative Turn angle, but at the same time have identical values for the mean relative meander.
- The *Absolute Meander* is often used in combination with the dependent variables absolute Turn angle and absolute Angular velocity to study turning rates. Bell (1991) reports that in most studies, when plotting the values, absolute Meander generates a smoother curve than absolute Angular velocity. This is caused by the fact that the latter dependent variable is influenced both by speed as well as by real turning rate. See Bell (1991). *Searching Behaviour: The Behavioural Ecology of Finding Resources*. Chapman & Hall, London.
- In rodent models of aging, the average meander is used to calculate the frailty index. See Parks et al. (2012) *J. Gerontol. A* 67(3): 217-227.

# Target visits and errors

## Definition

An event marking the time when the animal visits a zone defined as target, or non-target (error) zone.

## How to specify Target visits and errors

1. Click the **Add** button next to **Target visits and errors**.
2. In the **Target Visits and Errors** tab, under **Settings**, choose the **Target zones** and the **Non-target zones**.

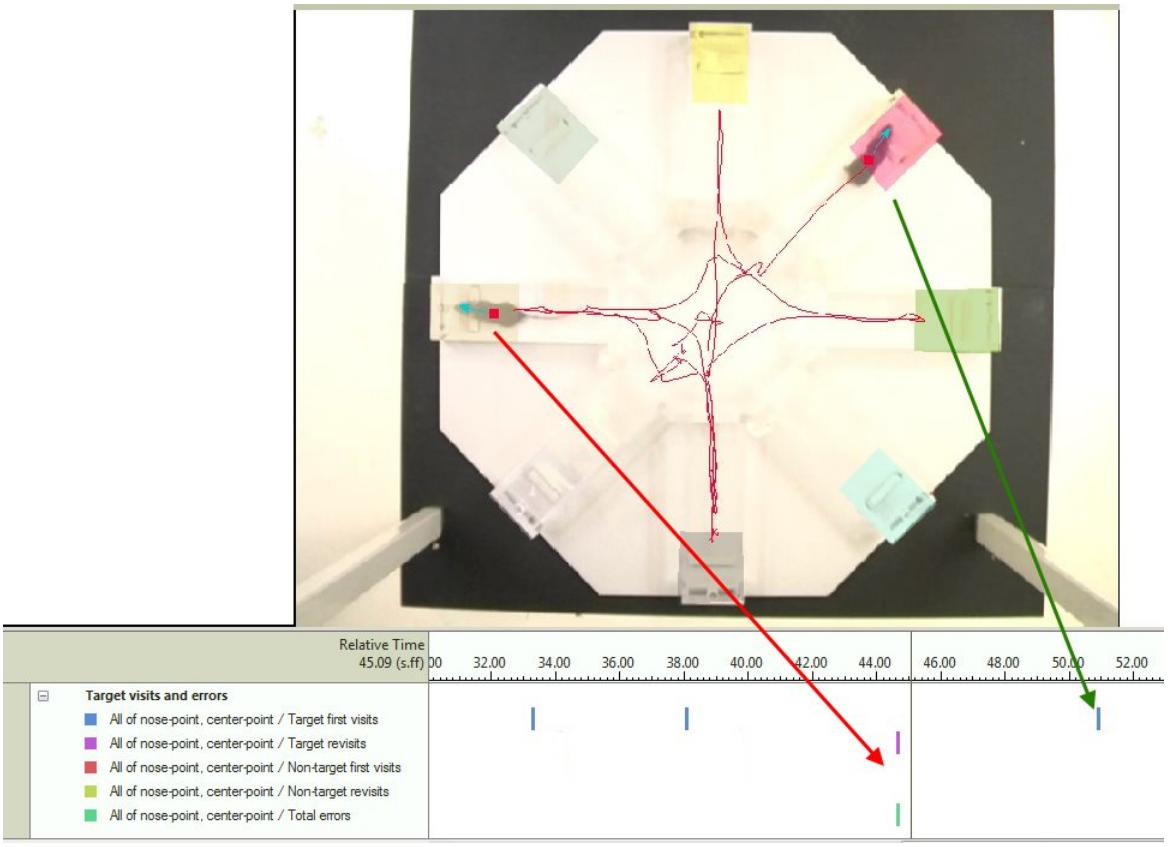
   Under **Threshold**, enter the **Zone exit threshold**. That is, once the animal is detected in the zone, the animal is considered to be in the zone until its distance from the zone border exceeds that threshold. Use this option to remove false re-entries resulting from random movements of the body point around the zone border. Click ⓘ for an example.
3. Under **Calculate Statistics for**, select the options you require:
   - **Target first visits**: Visits to zones defined as targets.
   - **Target revisits**: Revisits to the target zones (to analyze working memory).
   - **Non-target first visits**: First visits to non-target zones.
   - **Non-target revisits**: Revisits to non-target zones (to analyze reference memory).
   - **Total errors**: The total number of non-target zone visits and target zone revisits.
4. In the **Body points** tab, select the body point(s) you want to use for calculation.

   If you have chosen two or three body points, select one of the following from the list:

   - **Each point**: Visits are scored for each point separately. This results, for example, in one value of Successes for Center-point, and one value of Successes for Nose-point.
   - **Any selected point**: Visits are scored no matter which selected point enters a zone.
   - **All selected points**: Visits are scored when all the selected points are in a zone simultaneously.

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

Below: Visualization of Target visits and errors scores in a radial-arm maze. Left: the mouse visits an arm for the second time. This is scored as **Target revisit**; **Total errors** is also scored. Right: the mouse visits an arm for the first time. This is scored as a **Target first visit**.




## Application

- In a Barnes maze experiment, use *Target visits and errors* to analyze spatial reference memory. Calculate the number of successes and errors and their latencies, and compare those figures between probe trials (with escape hole being closed) and training trials. **TIP**: Select **Nose-point** to estimate nose pokes.
- In a Radial-arm maze experiment, use *Target visits and errors* to analyze working memory. Define the baited ends of the maze as targets. Calculate the number of target first visits and target revisits. To calculate the time needed to visit all arms, choose the Trial Statistic **Latency to Last** and in the results locate this statistics in the **Target first visits** column.

**NOTE** Latency is always calculated from the start of the track, even when you define time bins and nesting intervals.

- See also the EthoVision XT 18 - Application Manual for more information on the Radial-arm maze test.

# Zone alternation

## Definition

An event scored when the animal visits specific zones in:

- Alternations: Multiple entries into different zones, with no reentries, in overlapping sets of zone entries. For example, for a Y maze with three zones A, B and C, the sequence of zone entries ABC is an alternation.
- Revisits: Multiple entries into the same zone. They can be direct (for example, AA) or indirect (for example, ABA).

**EXAMPLE**

Consider, the zone entry sequence in a Y-maze: ABCBACBCAB. The eight overlapping 3-zone entry sequences are: ABC, BCB, CBA, BAC, ACB, CBC, BCA, CAB.

Of these, six are alternations (underlined): ABC, BCB, CBA, BAC, ACB, CBC, BCA, CAB

Alternation is often calculated together with the maximum possible number of alternations for the given sequence (that is, the total number of zones entries minus 2). In this example, it is 10-2 = 8.

The sequence above results in zero direct revisits, and two indirect revisits:

ABC, BCB, CBA, BAC, ACB, CBC, BCA, CAB

## How to specify Zone Alternation

1. Click the **Add** button next to **Zone alternation**.
2. In the **Zone Alternation** tab, under **Settings**, choose the zones that define the alternation.
3. Under **Threshold**, enter the **Zone exit threshold.** That is, once the animal is detected in a zone, the animal is considered to be in the zone until its distance from the zone border exceeds that threshold. Use this option to remove false re-entries resulting from random movements of the body point around the zone border. Click ⓘ for an example.
4. Under **Calculate Statistics for**, select the options you require:
   - **Alternations**: To calculate statistics of alternations (ABC, ACB, etc.).
   - **Max alternations**: To count the maximum possible alternations given the sequence of zone entries in your data (see a note below).
   - **Direct revisits**: To calculate statistics of the direct revisits (AA, BB, etc.).

- **Indirect revisits**: To calculate statistics of the indirect revisits (ABA, ACA, etc.).

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Applications

In a T-maze or Y-maze experiment, you can use *Zone alternation* to analyze Spontaneous Alternation. See Hughes (2004) *Neuroscience & Biobehavioral Reviews*, **28**(5), 497-505.

- In the **Zone alternation** tab, select the arm zones. If the arm zones are separated by a Center zone in the same zone group, do not include the Center zone.
- Also select **Max alternations**. The ratio Alternations/Max alternations expressed in percentage gives the spontaneous alternation index. An alternation index around 50% indicates random arm selection.

## Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the Body points tab and select the body points you want to use for calculation.

  If you have chosen two or three body points, select one of the following from the list:

  - **Each point**: Alternations and revisits are scored for each point separately.
  - **Any selected point**: Alternations and revisits are scored no matter which selected point enters a zone.
  - **All selected points**: Alternations and revisits are scored when all the selected points are in a zone simultaneously.
- **Max alternations** measures the maximum number of alternations that are possible with the actual data and given that number of zones considered. It is calculated in the following way:

  Max Alternation = Total visits - (Number of zones -1)

  - Where *Total visits* is the total number of zone visits;
  - *Number of zones* is the number of zones chosen under Settings; for the Y maze this is 3, for the plus maze it is 4.

  **EXAMPLE** You have a sequence of 22 visits to zones A, B, and C. Max alternations is 22 - (3 zones -1) = 22 - 2 = 20. The number of visits is

diminished by two to account for the fact that the last two visits in the sequence are not part of complete alternations, because the remaining visits are unknown. A value of 20 means that if the animal had visited each zone after visiting the other two in the previous two visits (ABCABC, etc.), it would have made 20 consecutive alternations (ABC, BCA, CAB; see the definition above). An example of the procedure can be found in Ragozzino and Gold (1994), *Journal of Neuroscience* **14**(12), 7478-7485.

- In Integrated Visualization, a **Max alternations** event is scored from completion of the first set of entries and for each new zone entry from that point. In the Statistics results, the total number of those events is shown under **Frequency**.
- **Direct revisits** are evaluated in 2-zone entry sequences, also ignoring entries in zones not selected in step 2 above. For example, the sequence ADA results in one direct revisit (AA) when D is not selected.
- **Indirect revisits** are evaluated in 3-zone entry sequences, also ignoring entries in zones not selected in step 2 above. For example, the sequence ABDAB results in the indirect revisits ABA and BAB when D is not selected.
- **Direct revisits** and **indirect revisits** are calculated when you select at least two and three zones, respectively.

# Zone transition

## Definition

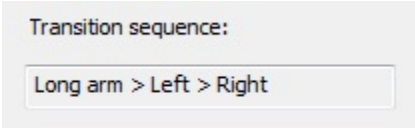
This is the number of times an animal visits two or more zones in a sequence. For example, in a T-maze study, the transition Long Arm > Left Arm > Right Arm.

A *Zone transition* is scored when the animal enters the last zone of the sequence.

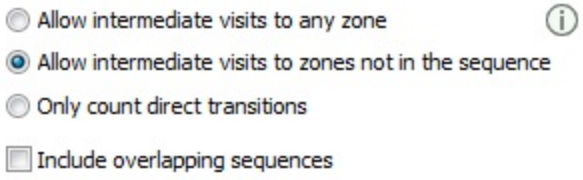
## How to specify Zone transition

1. Click the **Add** button next to **Zone transition**.
2. In the **Zone transition** tab, to add a sequence click the **Add** button.
3. Select a zone and click » or double-click a zone to include it in the sequence you want to define. Repeat this step to add more zones and complete the sequence.

   Under **Zone sequence** you can view the current selection.

   If the sequence is not correct, click ✕ and select the correct zones.
4. When ready click **Add**. Repeat steps 2-3 to add more sequences.
5. Depending on which option under **Settings** you select, you can get different results. See the notes below.

6. Under **Threshold**, enter the **Zone exit threshold**. That is, once the animal is detected in a zone, the animal is considered to be in the zone until its distance from the zone border exceeds that threshold. Use this option to remove false re-entries resulting from random movements of the body point around the zone border.

   Click ⓘ for an example.

7. Click the **Body points** tab, select the body point(s) you want to use for calculation.
8. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Zone transition counting options

For all options below, the zone transitions found in the data are marked in green. Zone transitions ignored are marked in red. The numbers in blue show the statistic Total number.

- **Allow intermediate visits to any zone**

  With this option the sequence ABC is found in the zone visit data ABDC, where D represents any zone defined.

  In the following example, the zone sequence CAB has been defined. A total of four transitions have been found.


- **Allow intermediate visits to zones not in the sequence**

  With this option the sequence ABC is found in the zone visit data ABDC, just like in the example above, because D does not belong to the sequence defined. However, the same sequence ABC is not found in the zone visit data ABAC, because the second A is defined in the sequence.

  In the following example, two transitions CAB have been found. The last two sequences are ignored.


- **Only count direct transitions**

  With this option, sequences with intermediate visits to any zone are ignored.

Do not use this option if your focal zones are not adjacent (for example, zones for a novel object and a familiar object), the arena includes multiple zone groups. In that case, direct transitions from the focal zones may not be counted if the subject crosses a third zone in another zone group. However, if your arena only includes one zone group with the non-adjacent zones, the results are reliable.

- **Include overlapping sequences**

  You can apply this additional option to any of the options above. Overlapping sequences may occur if the first zone of the sequence defined is visited again before the previous instance is completed. Consider the following zone visit data:

  ...BABAB...

  When looking for the sequence BAB, two overlapping sequences BAB are found:

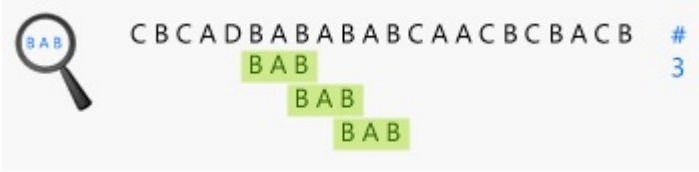
  ...BABAB...

  ...BABAB...

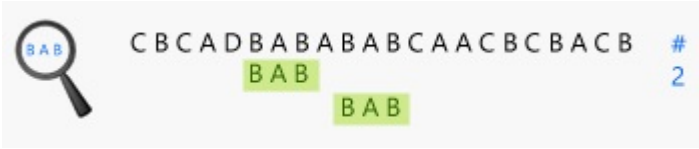
  Consider the following zone visit data:

  ...BABABAB...

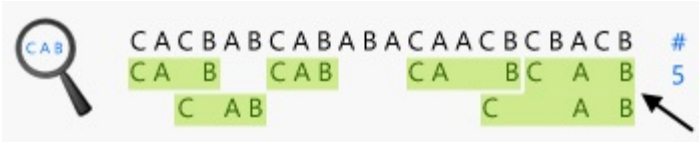
  If the option **Include overlapping sequences** is selected, the sequence BAB is found three times.




  If the same option is not selected, the sequence BAB is found two times.




  However, overlapping sequences that are completed at the same time point (that is, the same zone visit) are counted as one. In the following example, the sequence CAB is defined, and the method **Allow intermediate visits to any zone** is used. The two sequences CAB ending at the same visit to B are scored as one. Note that transitions are scored at the end of the last zone entry.



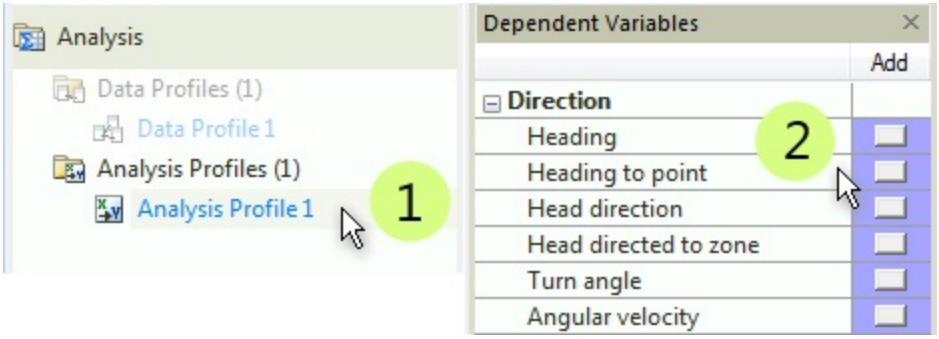

## Notes

- The **Body Points** tab is only available if your experiment is set to Center-point, nose-point and tail-base detection.
- When you visualize the *Zone transition* variable, only the end of the transitions (which equals the last zone entry) is marked on the time plot. EthoVision does not calculate the duration of the transition.
- To delete a sequence, select that sequence under Settings and click the **Delete** button.
- *Effect of missing samples*. A transition from Zone 1 to Zone 2 is also counted when the subject enters Zone 1, then it becomes missing before exiting the zone, it is found again outside Zone 1 and finally enters Zone 2.

## Applications

- In a Novel object test, calculate the number of transitions from the zone Familiar object to the zone Novel object, and from Novel object to Novel object. Or in a PhenoTyper or home cage test, calculate the transitions between the different corner visits. When the focal zones are not adjacent, select Allow intermediate zone visits.
- For Y-maze tests, see Zone alternation.

# Direction




- Heading 1021
- Heading to point 1024
- Head direction 1027
- Head directed to zone 1031
- Turn angle 1034
- Angular velocity 1040

# Heading

## Definition

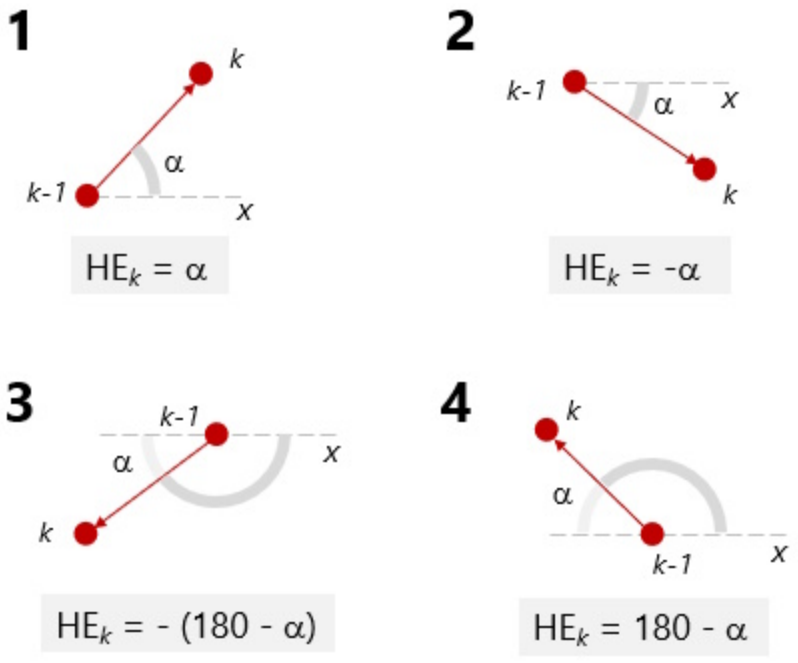
The direction of movement of the nose, center or tail-base point of the current sample relative to a line parallel to the x-axis in the coordinate system. 'Compass heading' and 'compass angle' are synonyms for *Heading*. See also Heading to point

## Calculation

Heading is calculated in three steps:

1. The smallest angle $\alpha$ is found between the reference line and the vector connecting the samples *k–1* and *k*.

   The figure below shows the relationship between the angle $\alpha$ formed by the segment joining samples *k-1* and *k*, the horizontal line parallel to the x-axis, and the dependent variable Heading (HE). Four cases are illustrated, corresponding to the possible directions an animal's body point can be moving relative to the x-axis. Here, it is assumed the x-axis is horizontal and pointing to the right, and the y-axis is pointing upward.

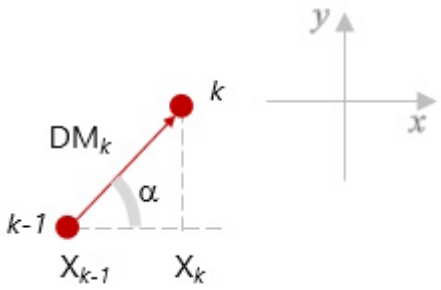



2. The value of $\alpha$ is calculated according to the formula:

$$\alpha = \text{acos}\frac{|X_k - X_{k-1}|}{DM_k}$$

Where:

- $DM_k$ is the distance moved at sample *k*.
- $X_k$ and $X_{k-1}$ the x-coordinates of the center, nose or tail-base point at sample *k* and *k–1*, respectively.




Because the way $\alpha$ is defined, it can only range from 0° to 90°.

3. How to convert $\alpha$ to Heading depends on the direction of movement between samples *k–1* and *k*. The relation between *Heading* and $\alpha$ is determined by the following rules (see step 1 above).

   - If $\Delta X > 0$ and $\Delta Y \geq 0$, then *Heading* = $\alpha$.

   - If $\Delta X \geq 0$ and $\Delta Y < 0$, then *Heading* = - $\alpha$.

   - If $\Delta X < 0$ and $\Delta Y \leq 0$, then *Heading* = -(180 - $\alpha$).

   - If $\Delta X \leq 0$ and $\Delta Y > 0$, then *Heading* = 180 - $\alpha$.

   Where $\Delta X = X_k - X_{k-1}$ and $\Delta Y = Y_k - Y_{k-1}$.

## Range

*Heading* ranges from -180° to +180°.

## How to specify Heading

1. Click the **Add** button next to **Heading**.
2. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the **Body points** tab and select the body points for which you want to calculate heading.
- The mean, standard deviation and variance are calculated with circular statistics. See Statistics available.
- Heading is calculated relative to the orientation of the x-axis you have chosen in the Arena Settings used for that trial. By default, the x-axis is horizontal and pointing to the right. If the x-axis is not horizontal or is pointing to another direction, Heading is calculated based on that axis direction.

## Application

Heading is used in studies of spatial orientation. For example, you can use it to determine the direction of flight of a moth relative to the direction of the air flow in a wind tunnel. In a Morris water maze test, you can use this variable to measure initial heading of the path after releasing the animal in the basin.

# Heading to point

## Definition

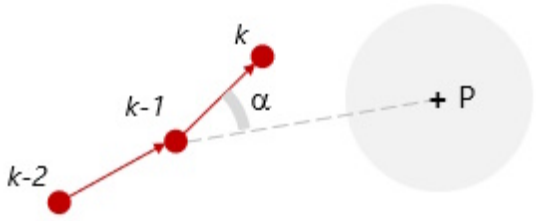
The direction of movement of the nose, center or tail-base point of the current sample relative to a point of interest. See Define zones and Draw a point

If your experiment is set to **Center-point detection** or **Color marker tracking**, calculations are based on the center point.

## Calculation

*Heading to point* is calculated in a way similar to Heading. The difference is that for *Heading to point* the reference line is the line that connects the previous sample and the point of interest. For Heading, the reference line is the line parallel to the x-axis.

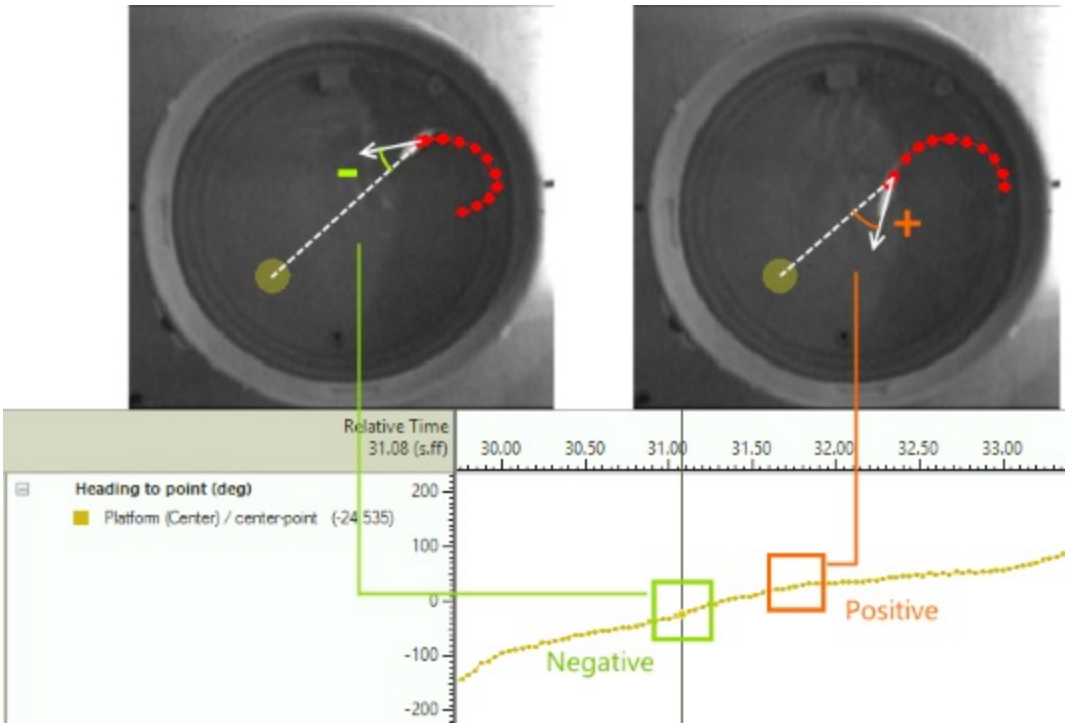
*Heading to point* for sample *k* is the angle $\alpha$ formed by the segment joining the samples *k-1* and *k*, with the line connecting sample *k-1* and the point P (a point of interest or the center of a zone).




## Range

*Heading to point* ranges from -180° to +180°. The closer to zero *Heading to point* is, the straighter the subject moves toward the point. Negative values occur when the subject moves to the right of the point; positive values in the other case.

Below: Example of Heading to point with positive and negative values in a water maze test. The platform has been specified as a target point. The chart shows *Heading to point* in the Integrated visualization (**Analysis** > **Results** > **Integrated Visualization**).




## How to specify Heading to point

1. Click the **Add** button next to **Heading to point**.
2. From the **Point of interest** list, select the center of the Arena, center of gravity (COG) of a zone or a point you defined in the Arena Settings.
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- If your experiment is set to Center-point, nose-point and tail-base detection, click the **Body points** tab and select the body points for which you want to calculate heading to point.
- The mean, standard deviation and variance are calculated with circular statistics. See Statistics available

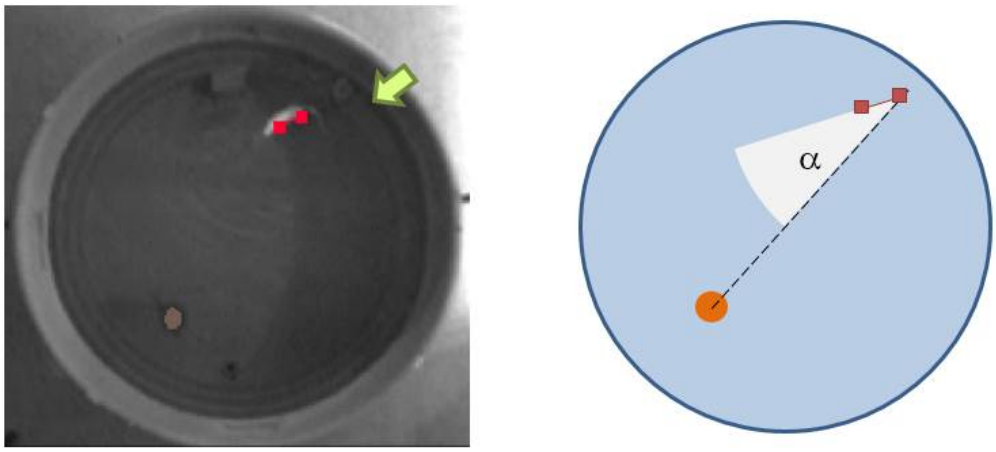
## Applications

Use *Heading to point* to measure the subject's orientation relative to a point of interest.

- *Morris water maze*. Use *Heading to point* to determine the Heading angle error. The Heading angle error is usually determined after the animal has

traveled a minimum distance, or after the first few seconds of each track. The Heading angle error at this point is the deviation from a direct line from starting point to center of the platform.

Below: The Heading angle error (α) in a water maze. The first two samples of the track are displayed. The arrow indicates the release point.




*How?* First, in the Data profile under **Nesting** choose **Time**, and select for example from 0 to 2 seconds. This means that analysis is done on the data points of the first two seconds of the track. Next, in the Analysis profile choose **Heading to point** and select the platform as point of interest. As Trial Statistic, choose **Mean**. For other water maze output variables, see also the EthoVision XT 18 - Application Manual.

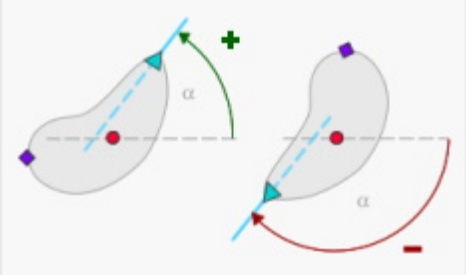
- *Novel object test*. Use *Heading to point* to determine the subject's movement relative to an object. The assumption is that an animal is interested in a novel object when it is heading towards the center of the object.

# Head direction

## Definition

The smallest angle formed by the Head direction line of the current sample relative to a line parallel to the x-axis in the coordinate system. See also Head directed to zone

The Head direction line is determined for each sample, based on the contour of the detected subject. It originates from the nose-point but it does not necessarily pass through the center-point. The angle $\alpha$ is assigned to the dependent variable *Head direction* for that sample.




**NOTE** *Head direction* is not available if your experiment is set to:

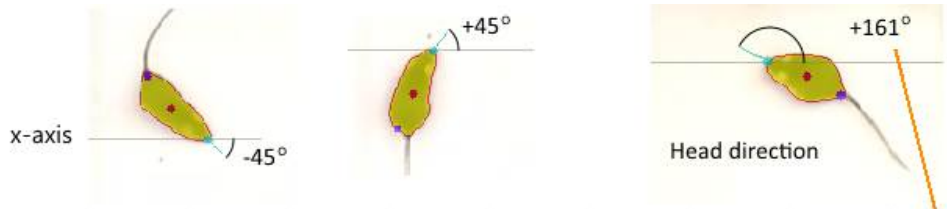
- Center-point detection.
- Color marker tracking.
- Live Mouse Tracker.
- Center-point, nose-point and tail-base detection, with two subjects per arena tracked with Deep learning.

## Examples

Below: An example of *Head direction* when exported in the raw data file (**Analysis** > **Export** > **Raw Data**; locate the **Head direction** column).

The angle is negative (-180°, 0] when the Head direction line departing from the nose-point lies left to the line parallel to the x-axis. The angle is positive [0, +180°] in the opposite case (in this example the x-axis is in the default position; it is horizontal and points to the right). The top-left picture shows the Head direction at the sample highlighted below.

**IMPORTANT** Note that the value of *Head direction* depends on the orientation of the x-axis set in the Arena Settings.




| Trial time | X center | Y center | X nose | Y nose | X tail | Y tail | Area | Areachang | Elongatio | Head direction |
|---|---|---|---|---|---|---|---|---|---|---|
| s | cm | cm | cm | cm | cm | cm | cm² | cm² | | deg |
| 28.8 | 5.67234 | 13.4769 | 3.54792 | 14.3752 | 7.26182 | 12.3656 | 8.36306 | 3.49318 | 0.503501 | 158.119 |
| 28.88 | 5.36834 | 13.5889 | 3.05876 | 14.2998 | 7.02722 | 12.7162 | 8.40166 | 1.62115 | 0.546821 | 161.036 |
| 28.96 | 5.18908 | 13.6375 | 2.96613 | 14.2082 | 7.0215 | 12.909 | 8.43382 | 1.02287 | 0.524414 | 161.753 |
| 29.04 | 5.28841 | 13.6406 | 3.09116 | 14.1343 | 7.07325 | 13.046 | 8.1186 | 0.817006 | 0.515517 | 164.261 |
| 29.12 | 5.57874 | 13.5124 | 3.54817 | 14.0439 | 7.27878 | 12.8036 | 7.62968 | 1.63401 | 0.458652 | 165.256 |
| 29.2 | 5.72202 | 13.376 | 3.87971 | 14.1019 | 7.34167 | 12.6858 | 7.28873 | 1.20299 | 0.414854 | 154.753 |
| 29.28 | 5.69759 | 13.3465 | 3.65675 | 13.8059 | 7.36981 | 12.762 | 7.37879 | 0.41172 | 0.450797 | 164.645 |
| 29.36 | 5.6517 | 13.3683 | 3.60968 | 13.9266 | 7.35515 | 12.7376 | 7.48172 | 0.30879 | 0.443904 | 162.502 |
| 29.44 | 5.60349 | 13.3928 | 3.53746 | 13.9413 | 7.29646 | 12.7079 | 7.54605 | 0.321656 | 0.463216 | 164.544 |
| 29.52 | 5.58964 | 13.3942 | 3.54929 | 13.8701 | 7.24315 | 12.7085 | 7.56535 | 0.096497 | 0.467954 | 165.522 |

## Range

*Head direction* ranges from -180° to +180°. Therefore, values like -179 and +179 represent very similar orientations (head pointing to the left). Take this into account when interpreting the raw data.

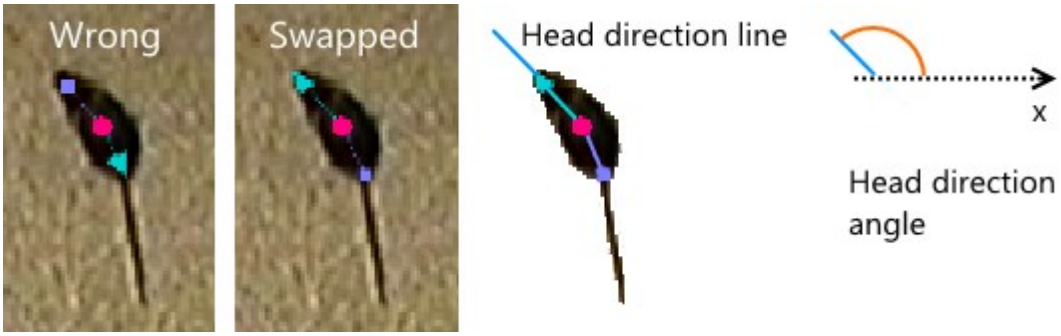
## How to specify Head direction

1. In the Analysis profile, click the **Add** button next to **Head direction**.
2. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- *Head direction* is a very different thing than the *Turn angle* based on the nose point. The latter variable measures the direction of movement, not the orientation of the head. See Turn angle
- You can check the head direction line during data acquisition. Click the **Show/Hide** button on the toolbar, select **Track Features** and make sure **Head direction** is selected. Next, let the animal move in the arena or play the video file. The real time *Head direction* values are shown in the Analysis Results and Scoring pane.
- The mean, standard deviation and variance are calculated with circular statistics. The mean *Head direction* represents the average orientation of the animal relative to the x-axis. See Statistics available

- *Head direction* is calculated relative to the orientation of the x-axis you have chosen in the Arena Settings used for that trial. By default, the x-axis is horizontal and pointing to the right. If the x-axis is not horizontal or is pointing to another direction, Head direction is calculated based on that axis direction.
- **NOTE** If you swap nose- and tail-base points, or interpolate those two points, the Head direction line is recalculated as the angle formed by the segment joining the nose-point and the center-point and the x-axis (see the figure below).

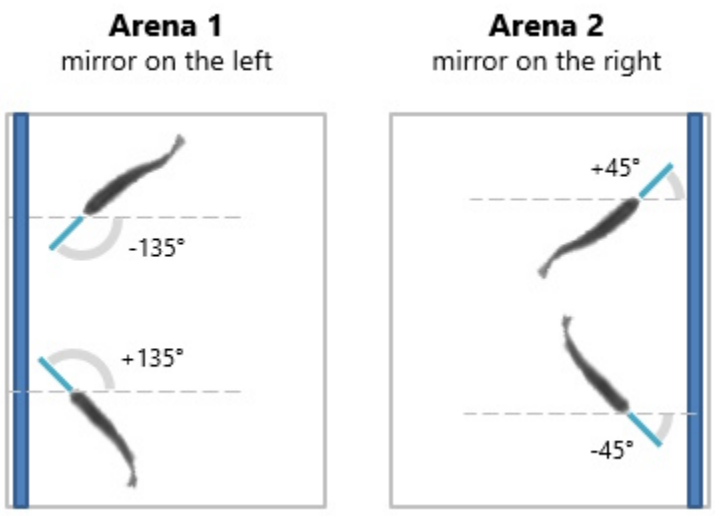



- If you edit the position of the nose-point or center point, the value of *Head direction* does not change.
- Be careful when interpreting values of Head direction relative to objects, for example two zones, or values extracted from different arenas.

  **EXAMPLE** In a mirror test, we want to measure the head direction of the fish, and compare the data between two arenas. The mirror is placed at opposite sides in Arena 1 and Arena 2. You can see that the same orientation relative to the mirror is measured with different angles:

  - For a negative angle in Arena 1: Angle in Arena 2 = Angle in Arena 1 + 180° (see the figure below, top).
  - For a positive angle in Arena 1: Angle in Arena 2 = Angle in Arena 1 - 180° (bottom).

  To compare the results between arenas, convert angles from Arena 2 to Arena 1 (or vice versa).




## Applications

*Head direction* is useful for studies of spatial orientation and searching behavior. For this you can, for example, use the average and variation in Head direction.

# Head directed to zone

## Definition

A discrete (state event) variable that is scored when the subject's head is directed towards a zone or a circular area around a point. See Define zones and Draw a point

**NOTE** *Head directed to zone* is not available if your experiment is set to:

- Center-point detection.
- Color marker tracking.
- Live Mouse Tracker.
- Center-point, nose-point and tail-base detection, with two subjects per arena tracked with Deep learning.

## Calculation

1. The Head direction line is calculated. See Head direction
2. If the Head direction line crosses the zone of interest (or a circular area around a point), the value of Head directed to zone for that sample is set to 1. The samples with 1 are used to calculate the duration of *Head directed to zone*.

The figure below shows Head directed to zone in the exported data file (**Analysis** > **Export** > **Raw Data**). Two samples are highlighted, one when the head is directed to the zone (circle), the other when it is not.

| Trial time | Recording | X center | Y center | X nose | Y nose | X tail | Y tail | Area | Head directed to zone |
|---|---|---|---|---|---|---|---|---|---|
| s | s | cm | cm | cm | cm | cm | cm | cm² | |
| 46.6 | 43.28 | -5.84058 | 2.56229 | -3.46564 | 1.01683 | -7.53956 | 3.82622 | 8.3169 | 1 |
| 46.64 | 43.32 | -5.85224 | 2.55667 | -3.51999 | 1.03485 | -7.5393 | 3.80314 | 8.31045 | 1 |
| 46.68 | 43.36 | -5.85779 | 2.53407 | -3.56009 | 1.03801 | -7.53588 | 3.81381 | 8.30399 | 1 |
| 46.72 | 43.4 | -5.85221 | 2.53974 | -3.53758 | 1.03839 | -7.50461 | 3.7968 | 8.26525 | 1 |
| 46.76 | 43.44 | -5.85186 | 2.53836 | -3.55301 | 1.04348 | -7.51435 | 3.80143 | 8.2717 | 1 |
| 46.8 | 43.48 | -5.86282 | 2.54036 | -3.53942 | 1.02826 | -7.50757 | 3.73823 | 8.26525 | 1 |
| 46.84 | 43.52 | -5.92391 | 2.53241 | -3.53901 | 1.38838 | -7.70024 | 3.6174 | 8.15547 | 1 |
| 46.88 | 43.56 | -6.09571 | 2.62004 | -3.7141 | 2.62832 | -7.82563 | 3.38175 | 7.66472 | 0 |
| 46.92 | 43.6 | -6.26682 | 2.90734 | -4.18754 | 3.71406 | -8.10907 | 3.27414 | 7.39352 | 0 |
| 46.96 | 43.64 | -6.20333 | 3.2555 | -4.35793 | 4.67708 | -8.12662 | 3.04698 | 7.39352 | 0 |
| 47 | 43.68 | -6.20619 | 3.52617 | -4.36414 | 5.35946 | -8.11241 | 2.87681 | 8.05216 | 0 |
| 47.04 | 43.72 | -6.15497 | 3.71558 | -4.87806 | 5.81607 | -7.82363 | 2.57624 | 8.14256 | 0 |
| 47.08 | 43.76 | -6.12407 | 3.83484 | -5.46088 | 5.92743 | -7.35383 | 2.51496 | 7.57432 | 0 |

## How to specify Head directed to zone

1. Click the **Add** button next to **Head directed to zone**.
2. In the **Head directed to zone** tab, under **Zone of interest** select a **Zone** or a **Point**. For the latter, you can select either a point you defined in the Arena Settings or the center of a zone.

   Because a point has an infinitely small surface area, you need to define a circular zone around the point. The default **radius** is 0.1 cm. The smaller the radius around a point, the less likely it is that the animal's head is exactly directed at this point.

3. Next, you can specify when *Head directed to zone* should be calculated, depending on the location of the animal:
   - **Calculate when**: From this list you select the body point that should be in the zone selected in the In list. If you select **All detected body points**, Head directed to zone is only calculated when all three body points are in the zone selected in the In list.
   - **In**: Select one of the zones from this list.
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- *Head directed to zone* is based on the *Head direction* variable, which in turn depends on which method is used to track the nose-point. See Head direction
- *Head directed to zone* is not available if your experiment is set to Only center-point detection or Color marker tracking.
- If you swap the nose-point and the tail-base point in the Track Editor, the Head direction line is updated, and it is calculated as the prolongation of the segment joining the nose-point and the center-point. *Head directed to zone* is calculated based on the new angle.
- If you edit the position of the nose-point or the center-point in the Track Editor, the value of *Head directed to zone* does not change.

## Applications

Head directed to zone is especially designed for use in the Novel object test. Exploration of a novel object is normally defined as directly attending to the object when the head is within a 2 cm radius of the object (Ennaceur and Delacour (1988) *Behavioural Brain Research*, **31**, 47-59). In EthoVision XT, you calculate *Head directed to zone* when the Nose-point of the animal is within a 2 cm radius from the center of the zone you have drawn around the novel object.

# Turn angle

## Definition

The change in direction of the nose, center, tail-base point or Head direction line between two consecutive samples.

## Calculation

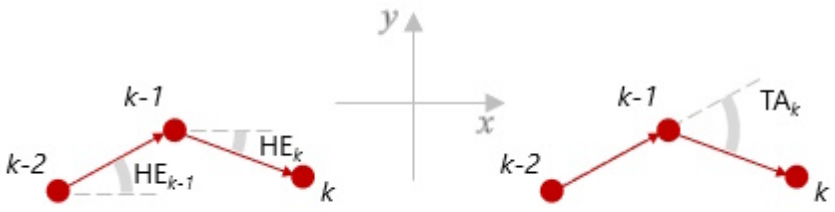
You can calculate Turn angle in two ways, based on body points or the Head direction line.

### *Based on body points*

Turn angle is calculated as the difference between two subsequent values for Heading of the specified body point:

- $\Delta$Heading = $\text{Heading}_k$ - $\text{Heading}_{k-1}$
- If $\Delta$Heading < -180°, then Relative Turn angle = $\Delta$Heading +360°.
- If $\Delta$Heading ≥ +180°, then Relative Turn angle = $\Delta$Heading -360°.
- Else Relative Turn angle = $\Delta$Heading.

In the example below, there is a turn to the right (clockwise) at sample *k*. The heading $HE_{k-1}$ is positive, while $HE_k$ is negative. As a result, the difference $HE_k$ - $HE_{k-1}$ is negative. For the default axis orientation shown in the figure below, a clockwise turn corresponds to a negative Turn angle. A counter-clockwise turn corresponds to a positive Turn angle.



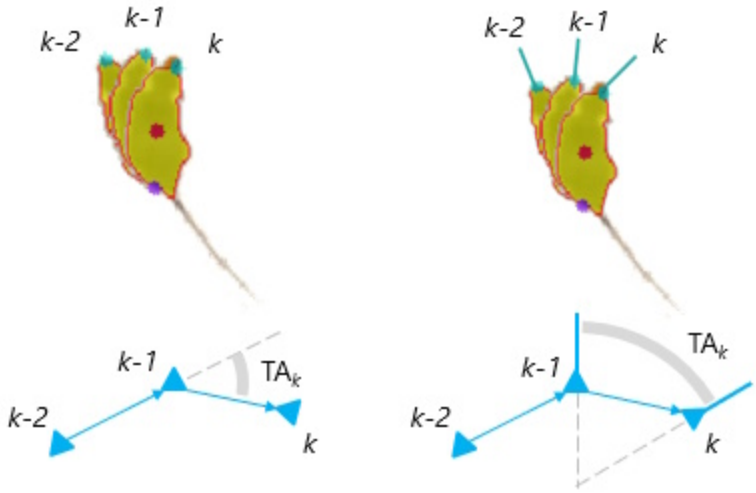

### *Based on Head direction*

*Turn angle* is calculated as the difference between two subsequent values for Head direction (see the figure below, left). This value is independent of the position of the body points:

- $\Delta$Head direction = $\text{Head direction}_k$ - $\text{Head direction}_{k-1}$

- If ΔHead direction < -180° then relative Turn angle = ΔHead direction +360°.
- If ΔHead direction ≥ +180° then relative Turn angle = ΔHead direction -360°.
- Else relative Turn angle = ΔHead direction.

The *Turn angle* based on head direction depends only on the samples *k–1* and *k*, while the Turn angle based on a body point depends on the last three samples (*k–2*, *k–1* and *k*. See the example below.



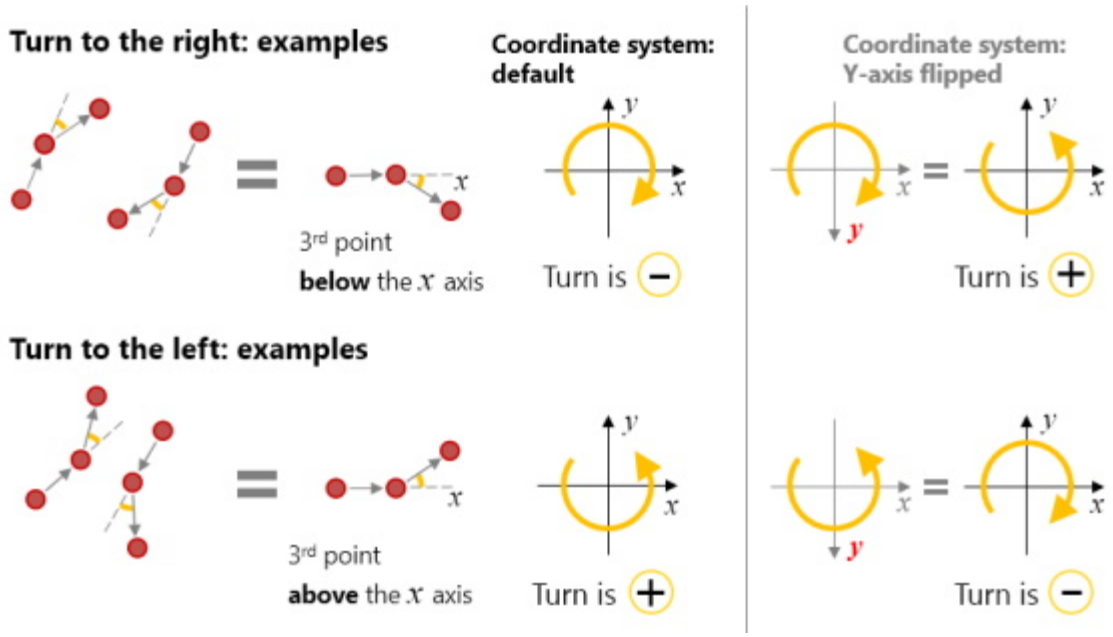

**NOTE** The Head direction line is calculated using the subject's contour. If you swap nose- and tail-base points, or interpolate those two points, the Head direction line is not recalculated using the contour, instead as the prolongation of the segment joining the nose-point and the center-point. *Turn angle* is then calculated based on those head direction lines.

## Absolute vs. Relative Turn angles

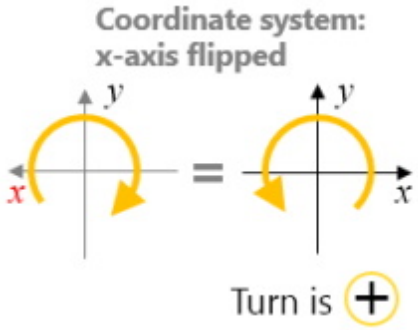
- Absolute Turn angle: The difference in direction is unsigned. Absolute Turn angle ranges from 0° to +180°.
- Relative Turn angle: The difference in direction is signed. With a default axis orientation, a clockwise turn is signed negative, and a counterclockwise is signed positive. Therefore, the Relative turn angle ranges from -180° to +180°. Relative angles help you distinguish between clockwise turns and counterclockwise turns.

Absolute and Relative turn angles result in different averages. Consider for example two angles, -10° and +40°. The average relative turn angle is (-10°+40°)/2 = 15°. The average absolute turn angle is (10+40)/2 = 25°.

**TIP** Use the picture below to know the sign of a turn. With the default axis orientation, a clockwise (right) turn means a negative turn angle. A counter-clockwise t (left) turn means a positive angle.




Whenever you use a coordinate system other the default one, rotate the image to know the sign of the turn. For example, a right turn on a coordinate system with the x-axis flipped gets a positive value:




## How to specify Turn angle

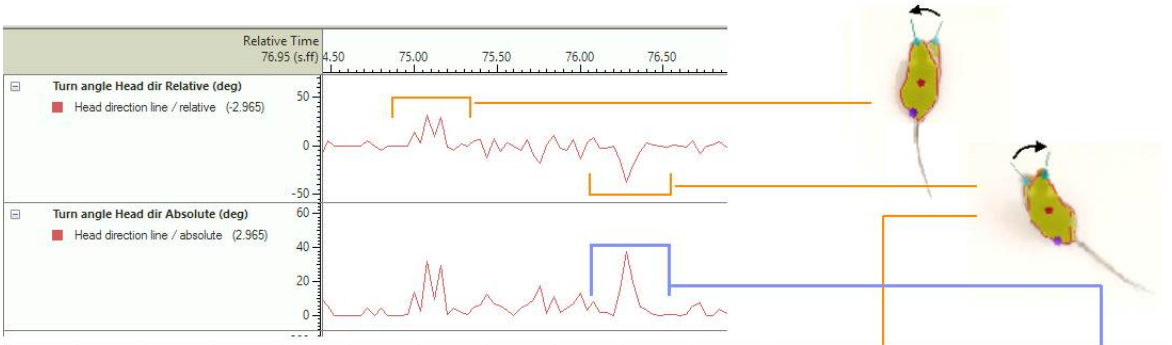
1. Click the **Add** button next to **Turn angle** and click the **Turn angle** tab. Select **Absolute** or **Relative**.
2. Select **Head direction turn angle (body point is ignored)** if you want to calculate turn angle based on the Head direction line.
3. Click the **Body points** tab. Select the body points for which you want to calculate the turn angle. By default, **Center-point** is selected.

If your experiment is set to Only center-point detection or Color marker tracking, this tab is absent. Calculations are based on the center point.

This tab is not available if you choose the option in step 2.

4. Complete the procedure to add the variable. See Calculate statistics: procedure.

Below: Relative and absolute turn angle based on the change in direction of the Head direction line. Top: dependent variables visualized in Integrated visualization (**Analysis** > **Results** > **Integrated Visualization**). First chart: Relative turn angle, showing positive and negative values for left and right turns of the head, respectively. Second chart: Absolute turn angle. Bottom: dependent variables when exported in the raw data file (**Analysis** > **Export** > **Raw Data**).

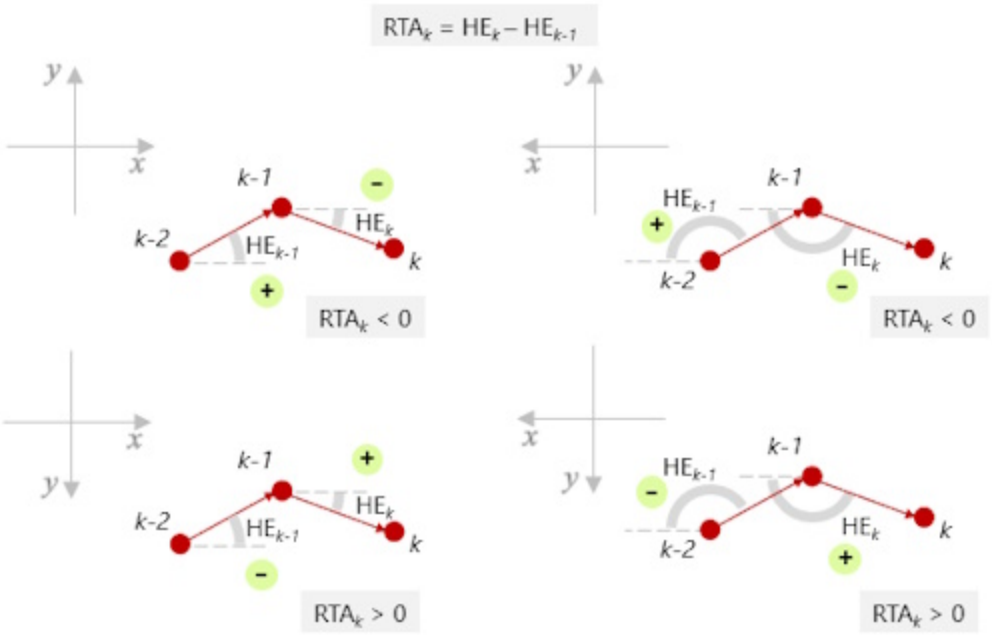



| Trial time | Recording | X center | Y center | X nose | Y nose | X tail | Y tail | Area | Areachan | Elongation | Turn angle Head dir Relative | Turn angle Head dir Absolute |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| s | s | cm | cm | cm | cm | cm | cm | cm² | cm² | | deg | deg |
| 76.12 | 72.8 | -4.69277 | 12.9647 | -6.16325 | 14.8386 | -3.73155 | 11.4638 | 6.41202 | 0.632808 | 0.712455 | -2.15755 | 2.15755 |
| 76.16 | 72.84 | -4.57086 | 12.7682 | -6.06 | 14.5669 | -3.58641 | 11.2306 | 6.46368 | 0.904011 | 0.698685 | -2.21293 | 2.21293 |
| 76.2 | 72.88 | -4.37916 | 12.5123 | -5.76443 | 14.3138 | -3.46479 | 11.0093 | 6.82529 | 1.42059 | 0.625341 | 0 | 0 |
| 76.24 | 72.92 | -4.2125 | 12.3624 | -5.11016 | 14.3268 | -3.43191 | 10.7354 | 6.98026 | 1.44642 | 0.602182 | -16.0206 | 16.0206 |
| 76.28 | 72.96 | -4.1184 | 12.3474 | -4.21323 | 14.4278 | -3.72923 | 10.5568 | 7.11586 | 1.65951 | 0.602396 | -37.9114 | 37.9114 |
| 76.32 | 73 | -4.00977 | 12.4823 | -3.3787 | 14.6753 | -4.10372 | 10.7673 | 7.45809 | 1.78865 | 0.614253 | -19.7169 | 19.7169 |
| 76.36 | 73.04 | -3.87578 | 12.5424 | -2.95828 | 14.8195 | -3.88297 | 10.4509 | 7.40644 | 1.65305 | 0.639846 | -5.48586 | 5.48586 |
| 76.4 | 73.08 | -3.7804 | 12.5099 | -2.69805 | 14.5994 | -4.20982 | 10.5692 | 7.25792 | 1.34956 | 0.644398 | 3.44655 | 3.44655 |
| 76.44 | 73.12 | -3.59883 | 12.6059 | -2.34342 | 14.7765 | -4.18953 | 10.6248 | 7.64535 | 1.44642 | 0.671256 | 1.10465 | 1.10465 |

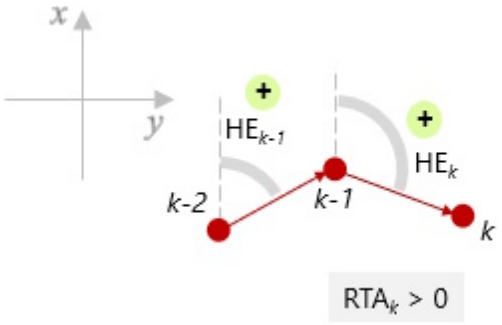
## Relative Turn angle and axis orientation

*Relative Turn angle* depends on the orientation of the x- and y- axes in the Arena Settings used for that trial. By default, the x-axis is horizontal and pointing to the right. A clockwise turn is scored as a negative value, and a counterclockwise is scored as a positive value. However, if the x-axis is pointing to the left, a clockwise turn is scored as a positive value, and a counterclockwise turn is scored as a negative value.

Below: The sign of the *Relative Turn angle* (RTA) depends on the orientation of the x- and y-axes. The example illustrates the effect of four different axis orientations on a clockwise turn. The sign inside the circles indicates the sign of Heading (HE) values. When the difference $HE_k$ - $HE_{k-1}$ is larger than +180° or smaller than -180° (examples on the right), the rules described in Heading apply.




If the axes are swapped, the reference line is now vertical. the Relative Turn angle is calculated relative to the vertical axis. Compare the following figure with the example at the top-left corner of the figure above.




## Notes

- The mean, standard deviation and variance are calculated with linear statistics.
- Turn angle is calculated in a way different from that in EthoVision 3. This is because Turn angle is based on Heading, which is set consistent with Head direction.
- *Turn angle* is very sensitive to small, random movements of the body points. When the animal sits still, *Turn angle* can get very high, unrealistic values. To

remove such small movements from your data, Smooth the Tracks, then run analysis.

- *Turn angle* should not be confused with turn bias or turning rate. These are actually synonyms for the dependent variables *Relative Angular velocity* and *Absolute Angular velocity*, respectively.

## Application

Assessing turn angles can be helpful for detecting stereotypic movements. In this case, consecutive turn angles tend to have large values (for example, in circling behavior of rodents), or show repeating patterns (for example, rocking or waving). For better quantification of biases in the left/right direction, first filter the data with the Minimal Distance Moved method, to ignore points where the animal does not move significantly.

Cumulative Turn angles are used to calculate rotations. See Rotation

# Angular velocity

## Definition

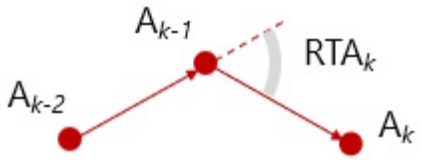
The change in direction of movement of the nose, center, tail-base point or Head direction line between two consecutive samples, calculated per unit time. *Angular velocity* is expressed in degrees/second (°/s) and can be either positive or negative (see below).

## Calculation

$$RAV_k = \frac{RTA_k}{t_k - t_{k-1}}$$

Where:

- $RAV_k$ is the *Relative Angular velocity* for sample *k*.
- $RTA_k$ is the *Relative Turn Angle* for sample *k*.
- $t_k$-$t_{k-1}$ is the time difference between the current and the previous sample.




*Angular velocity* can be calculated based on body points or the Head direction line:

- Based on body points, as in the figure above. The angular velocity is calculated from the turn angle of the specified body point (nose, center or tail-base; see below).
- Based on Head direction. The angular velocity is calculated from the turn angle based on Head direction.

**IMPORTANT** Note that calculating *Angular velocity* from Head direction is not the same as calculating it from the nose-point. This is because Turn angle (and therefore Angular velocity) at sample *k* based on a body point depends on the last three samples *k–2*, *k–1* and *k*, while Turn angle based on Head direction depends only on the samples k–1 and k.

## Range

The minimum and maximum angular velocity depend on the time between two samples. For example, if the sample rate is 25, the maximum attainable turn (+180°, see Turn angle) results in an angular velocity of 180/0.04= 4500°/s.

## Absolute vs. Relative Angular velocity

- Absolute Angular velocity: The rate of change in direction is unsigned. Also known as Turning rate.
- Relative Angular velocity: The rate of change in direction is signed. Depending on the orientation of the x- and y-axes, a clockwise turn is scored as positive or negative value, a counterclockwise turn is scored with opposite sign (see the relative Turn angle). Turn bias (degrees/s) and circling tendency are synonyms for this variable.

The difference between relative and absolute *Angular velocity* is best explained by looking at the mean values of the two dependent variables in the following example:

| Time | Absolute Ang. vel. | Relative Ang. vel. |
|---|---|---|
| 0.04 | 10 | -10 |
| 0.08 | 45 | 45 |
| 0.12 | 35 | -35 |
| **Mean** | **30** | **0** |

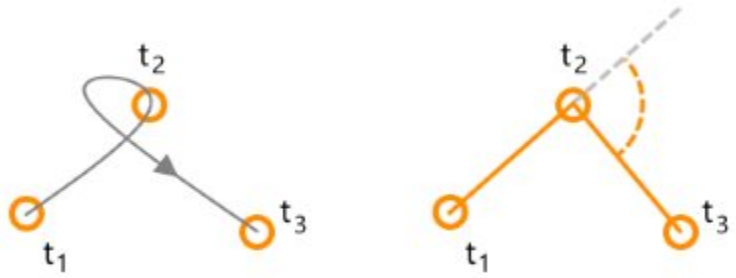
The mean *absolute Angular velocity* reflects the amount of turning, whereas the mean *relative Angular velocity* gives a better indication of the overall direction of turning.

## How to specify Angular velocity

1. Click the **Add** button next to **Angular velocity** and click the **Angular velocity** tab. Select **Absolute** or **Relative**.
2. Select **Head direction angular velocity (body point is ignored)** if you want to calculate angular velocity based on head direction.
3. Click the **Body points** tab. Select the body points for which you want to calculate the velocity. By default, **Center**-**point** is selected.
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- If your experiment is set to Only center-point detection or Color marker tracking, the Body points tab is absent. Calculations are based on the center point.
- If the body point turns more than 180° between one sample and the next, the direction of turning is calculated incorrectly. In the example below, the subject makes a fast counterclockwise turn of 270° between time 2 and 3 (left). The program interprets this as a 90º clockwise turn (right).




  As a result, *Angular velocity* gets a value smaller than expected, and in case of relative Angular velocity a false sign. This kind of error occurs when the sample rate is too low and the subject makes very fast turns. To prevent this kind of error, set the sample rate at such a level that it is practically impossible for the subject to make a turn more than 180° between two subsequent samples.

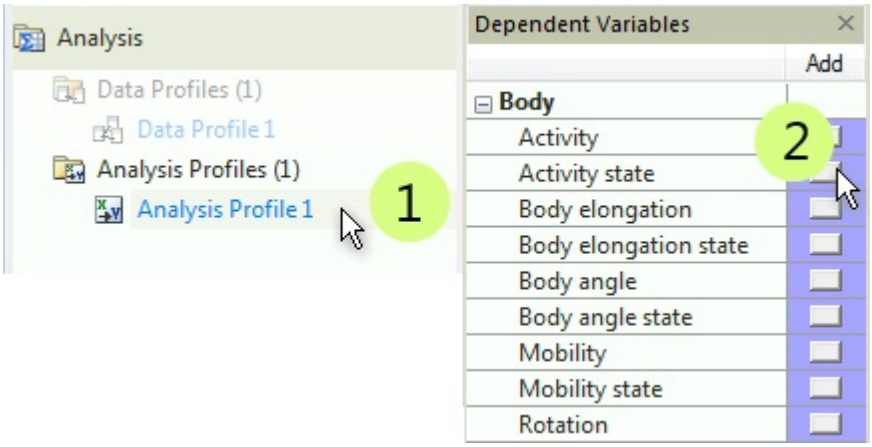
- The Head direction line is calculated using the subject's contour. However, if you swap nose- and tail-base points, or interpolate those two points, the Head direction line is not recalculated using the contour, instead as the prolongation of the segment joining the nose-point and the center-point. *Angular velocity* is then calculated based on those head direction lines.

## Applications

- The *absolute Angular velocity* is used to express the amount of turning per unit time. Generally, a high value of this dependent variable is associated with local search, for instance in response to non-volatile semiochemicals (Bell (1991) *Searching Behaviour: The Behavioural Ecology of Finding Resources*. London: Chapman & Hall).
- The *relative Angular velocity* measures the speed of change in direction of movement. The mean of this dependent variable can be used to assess turn bias or circular tendency, the tendency of a subject to turn to a specific direction. Studying this helps detecting peculiarities or abnormalities of

behavior (for instance, stereotypic movements, reaction to toxic substances, etc.).

# Body




- Activity  1045
- Activity state  1046
- Body elongation  1050
- Body elongation state  1052
- Body angle  1056
- Body angle state  1058
- Mobility  1060
- Mobility state  1063
- Rotation  1069

# Activity

## Definition

The dependent variable *Activity* is only available when you selected **Activity analysis** in the Experiment Settings and you track only one subject per arena. Activity gives the percentage changed pixels in the entire arena between current sample and previous sample.

## Calculation

See step 1 and 2 in Activity state for information on how this percentage of changed pixels is calculated. With the dependent variable Activity state, you can calculate how much time and how often your subject was inactive, moderately active and active and highly active. With the dependent variable Activity, you can calculate the average activity of your subject.

## How to specify Activity

1. Click the **Add** button next to **Activity**.
2. Under **Outlier filter**, select the **Averaging interval**. This is the number of samples over which the running average mobility is based. The default value is 1, that is, the current value of Activity is only based on the current image. See Averaging interval

   See Activity state for more information.
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

# Activity state

The dependent variable *Activity state* is only available when you selected Activity analysis in the Experiment Settings and you track only one subject per arena. With Activity state you can calculate how long and how frequent your subject has been in different activity states. These states depend on the total pixel change within the arena between a sample and the previous sample. The number of states and their thresholds are user-defined.

When the subject goes missing for more than three samples, the current *Activity state* is ended and the remaining missing samples are ignored.

## Calculation

### *Step 1 - Calculation of proportion of pixel change*

All pixel coordinates in the arena, so not only of the detected subject, are determined immediately after they have been detected. The gray scale values of all pixels are compared with the previous sample to determine the number of changed pixels in the arena between the two. The formula for Activity is simply the number of changed pixels for the current sample *k* divided by the total number of pixels in the arena:

$$Activity = \frac{CP_k}{P_k} \times 100$$

*Activity* is calculated by taking every pixel and comparing it between the current image and the previous one. If all the pixels are the same, there is zero activity. If all the pixels are different, there is 100% activity. If the animal is moving and increases its velocity (whilst keeping the same shape) there will be an increase in activity, because the pixels belonging to the animal are increasingly different as it moves faster.

### *Step 2 - Running average*

To smooth the values of Activity, an Averaging interval is used. This gives you the option to specify the number of samples for calculating a running average of Activity. The Activity percentage is summed over the number of samples that you specify, and divided by the number of samples. This way, sudden changes in surface area caused by such factors as the animal entering a shadowed area, or a reflection, are smoothed out.

**NOTE** When you export the dependent variable, each row in the export file contains the Activity value, but not the averaging interval.

*Step 3 - Calculation of the Activity state dependent variable*

The *Activity state* variable is established for each sample, according to the value of running average Activity relative to the thresholds. You can define between two and four states, varying from *Inactive* (below the lowest threshold) to *Highly active* (above the highest threshold).

## How to specify Activity state

1. Click the **Add** button next to **Activity State**.
2. Enter the following:

   **Averaging Interval**: The number of samples over which the running average mobility is based. The default value is **1**, that is, the current value of Elongation is only based on the current image.

3. Under **Number of states**, select the number of activity levels you are interested in. The following options are available, dependent on the number of states:

   **2 states**: Highly active above and Inactive below.

   **3 states**: Highly active above, Moderately active between and Inactive below.

   **4 states**: Highly active above, Active between, Moderately active between and Inactive below.

4. Under **Thresholds**, specify thresholds for the states defined in step 3.

   You can enter a number with up to two decimals.

   - Under **State duration threshold**, in the field next to E**xclude instances shorter than**, enter how long a state must last before it is scored as one of the four Activity states. If the duration passes this threshold, the samples in this time length are scored as one of the four states according to the criteria above. If the duration does not pass the threshold, the previous state ends, but no new state is defined. When you visualize *Activity state* in the Integrated Visualization, this is displayed as a gap between two adjacent color bars, which represent the scored activity states.
   - Under **Calculate statistics for**, select at least one of the states defined in step 3.

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

To find the optimal activity thresholds, run a few test trials and check in the Analysis Results and Scoring pane the values of Activity when the animal shows such

behavior. These values are calculated real-time during acquisition. See an example in Fear conditioning: view Activity state.

## Notes

- **IMPORTANT** The Activity detection thresholds set in Acquisition are not used in analysis. When you specify *Activity* in your Analysis profile, enter the new values in the appropriate fields.
- In some cases the number of samples available for smoothing can be less than the averaging interval entered. For example, when there are missing samples or at the beginning of the track. In such cases EthoVision XT uses the samples available in the specified interval. For example, the value of Activity for the first sample of the track is always calculated over one sample.
- You set the thresholds during acquisition, but you can override them when calculating statistics to produce new values for Activity state. To see what the original values of Activity state thresholds were (unless you have changed them while acquiring data), open the Acquisition module and click the button under **Activity** in the Analysis results and Scoring pane.

## Frequently asked questions about Activity

1. *What is the difference between Activity and Mobility?*

   Activity is the percentage change in all pixels in the arena between the current sample and the previous sample. This is independent of the detected subject.

   Mobility is the percentage pixel change between the current sample and the previous sample in the detected subject only. See Mobility

2. *When should I use Activity and when should I use Mobility?*

   Use Activity when the dependent variable Mobility does not give satisfying results, or if detection of your subject is difficult. This can be the case when bars of the shock grid floor in the background complicate detection of the animal's surface area, or when the animal is very large compared to the arena size.

3. *Does Activity detection depend on the size of the subject relative to the arena?*

   Yes. Activity is calculated as the number of changed pixels divided by the total number of pixels in the arena. The smaller the subject relative to the arena, the smaller the activity will be. When your animal is very large in comparison to the arena size, for example when you have a rat in a small cage, activity detection is generally more suitable than mobility detection.

4. *Does Activity detection depend on the video resolution?*

   Yes. The higher the video resolution, the greater the number of pixels that change when the subject moves. Therefore, when you have a high video resolution, it is less likely that a small movement of the subject results in an abrupt change in activity.

   However, this effect is present at very low resolution (for example, when the subject is less than 100 pixels large). We advise you not to compare values of Activity between videos of different resolutions.

5. *Does Activity detection depend on sample rate?*

   Yes. Since Activity is detected from the change in pixels between two samples, and the pixel change depends on how frequently the area is acquired (that is, the sample rate), Activity depends on the sample rate. All being equal, the higher the sample rate, the smaller the pixel change. See also the effect of the sample rate on Mobility in Frequently asked questions about Mobility.

## Applications

*Activity* can be used to determine freezing behavior in rodents. Also, it may be used to assess inactivity of rodents in a Porsolt Swim Test. Furthermore, the startle response of zebrafish larvae can automatically be detected with (in)activity.

# Body elongation

## Definition

*Body elongation* measures how much the subject's detected shape differs from a circle.

**NOTE** *Body elongation* is not available if your experiment is set to:

- Center-point detection.
- Color marker tracking.
- Live Mouse Tracker (but see Stretch attend posture).
- Center-point, nose-point and tail-base detection, with two subjects per arena tracked with Deep learning.

## Calculation

1. The *Body elongation* measure E is calculated as follows:

$$E = \frac{\sqrt{\left(\sum_x (x-x_c)^2 - \sum_y (y-y_c)^2\right)^2 - 4\left[\sum_{x,y} (x-x_c)\cdot(y-y_c)\right]^2}}{\sum_x (x-x_c)^2 - \sum_y (y-y_c)^2}$$

   Where:

   - x,y are the coordinates of the pixels of the subject's contour in the current sample.
   - $x_c$ and $y_c$ are the coordinates of the center of the subject.

2. The Body elongation value is smoothed at each sample using the optional Averaging interval.

*Body elongation* is expressed as a percentage and ranges from 0% (when the subject's shape is perfectly circular) to 100% (when the subject's shape is a line).

## How to specify Body elongation

1. Click the **Add** button next to **Body elongation** and click the **Body Elongation** tab.
2. Enter the **Averaging interval**. This is the number of samples over which the running average elongation is based. The default value is 1, that is, the

variable is not smoothed using the values of neighboring samples. See Averaging interval

3. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- Body elongation is not calculated with Live Mouse Tracker data.

# Body elongation state

## Definition

A discrete (state event) variable with three possible states: Stretched, Normal and Contracted, depending on where the running average elongation measure (E) of the subject's contour calculated for the current sample lays relative to two user-defined thresholds (see Calculation below).

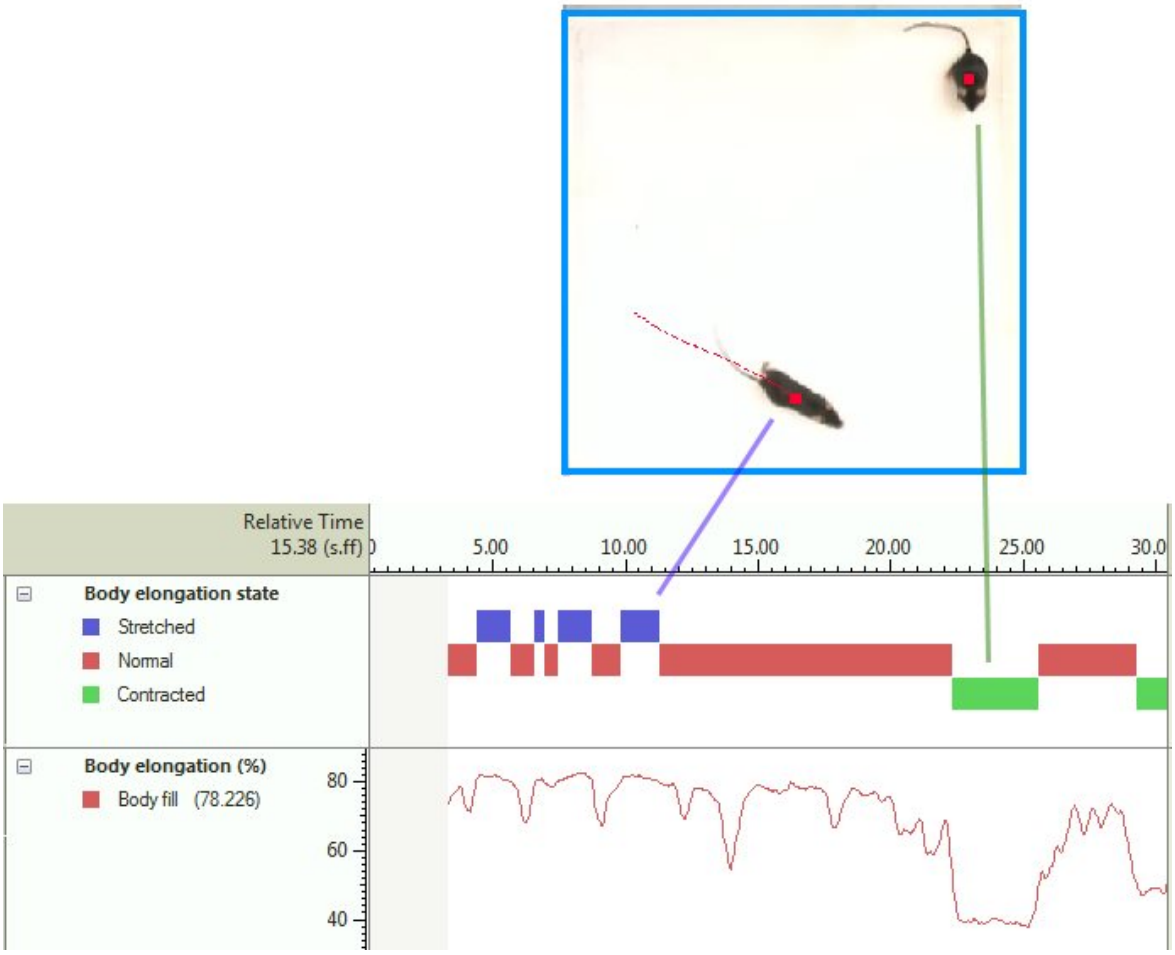
**NOTE** *Body elongation state* is not available if your experiment is set to:

- Center-point detection.
- Color marker tracking.
- Live Mouse Tracker (but see Stretch attend posture).
- Center-point, nose-point and tail-base detection, with two subjects per arena tracked with Deep learning.

## Calculation

1. The *Body elongation* is calculated for each sample. See Body elongation
2. The running average of Body elongation is calculated for each sample using the number of samples specified by the Averaging interval.
3. The *Body elongation state* variable is determined for each sample:
    - If the running average elongation percentage is greater than the Stretched above value, the state is *Stretched*.
    - If the running average elongation percentage is smaller than the Contracted below value, the state is *Contracted*.
    - In all the other cases, the state is *Normal*.

Below: Visualization of *Body elongation state* (top) and *Body elongation* (bottom). The two variables have been set with the same Averaging interval (10 samples). *Stretched* is scored when *Body elongation* is above 80%. *Contracted* is scored when the resampled variable is below 50%.




## How to specify Body elongation state

1. Click the **Add** button next to **Body elongation state** and click the **Body Elongation State** tab.
2. Enter the following:
   - **Averaging interval**: The number of samples over which the running average elongation is based. The default value is 1, that is, the elongation measure is not smoothed before calculating *Body Elongation state*.
   - **Stretched above**: The elongation measure above which the subject is considered to be *Stretched*.
   - **Contracted below**: The elongation measure below which the subject is considered to be *Contracted*.

   To find the optimal Stretched above and Contracted below, run a few test trials and check in the Analysis Results and Scoring pane the values of Body

elongation state when the animal shows such behavior. These values are calculated real-time during acquisition.

Values of elongation percentage between Stretched above and Contracted below result in the subject being scored as *Normal*.

3. Under **Calculate statistics** for, select which state you want to calculate statistic for: **Stretched**, **Normal** o **Contracted**. Select at least one state.
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- The elongation measure is independent of video size and the subject's position and orientation.
- When a body point is missing for more than three samples, the current Body elongation state ends and the remaining missing samples are ignored.
- The Body elongation thresholds set in Acquisition are not used in analysis. When you specify the Body elongation dependent variable in your Analysis profile, enter the new values in the appropriate fields.
- By increasing the averaging interval, you can increase the reliability of detection of stretching and contracting. A running average elongation based on more samples diminishes the effect of random changes in elongation measure between consecutive samples that would be detected as state transitions. However, a drawback of increasing the Averaging interval is that it causes a delay in the determination of a state transition, proportional to the length of the interval. See Averaging interval
- In some cases the number of samples available for smoothing can be less than the averaging interval entered. For example, when there are missing samples or at the beginning of the track. In such cases EthoVision XT uses the samples available in the specified interval. For example, the value of Elongation for the first sample of the track is always calculated over one sample.
- In the Detection Settings, under **Subject Contour**, select Erosion and then Dilation to remove the animal's tail from the detected image of the subjects. This way you get a more accurate measurement of Body elongation.
- Body elongation is not calculated with Live Mouse Tracker data.

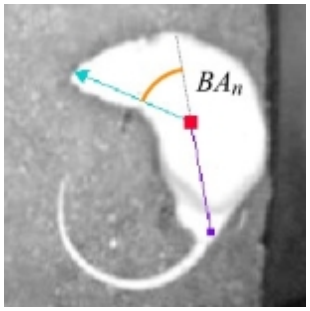
## Application

The *Body elongation state* variable can help you assess the frequency and cumulative duration of stretch attend postures in a more objective way.

# Body angle

## Definition

The angle formed by the prolongation of the segment joining the tail-base and the center-point, and the segment joining the center-point and the nose-point, calculated at the current sample.



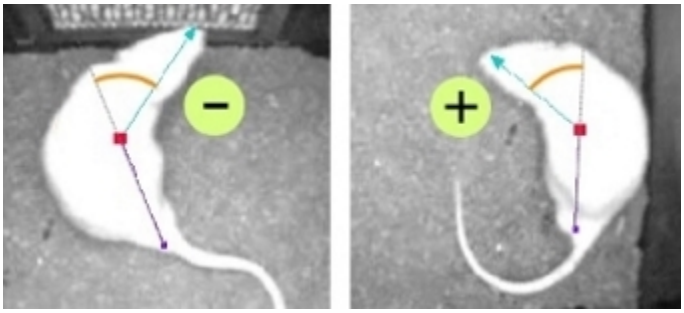

### *Range*

*Absolute Body angle* ranges from 0º to 180º. *Relative Body angle* ranges from -180º to +180º.

0º occurs when the nose point, center point and tail base point are aligned. Values around ±180º are only hypothetical, because they would imply that the animal is completely curled up in such a way that the nose points to the tail base. Values higher than 100º often occur with wrong detection of the body points.

## How to specify Body angle

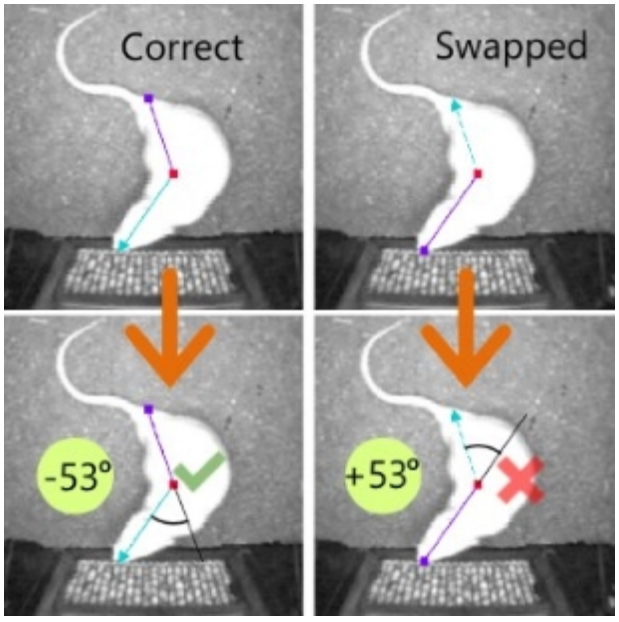
1. Click the **Add** button next to **Body angle**.
2. In the **Body Angle** tab, specify whether you want to calculate:
   - **Absolute angle**: The unsigned angle.
   - **Relative angle**: The signed angle. It is negative when the body is bent to the right (clockwise); positive when the body is bent to the left (counterclockwise).




3. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Note

When the nose-point and the tail-base point are swapped, and these are not properly corrected in the Track Editor, the value of *relative* body angle is opposite to what it should be. For example, if the actual body angle is +10º, EthoVision XT gives -10º. However, *absolute* angle remains correct. Therefore, if your track contains nose-tail base swaps, either correct them in the Track Editor, or use Absolute angle.



# Body angle state

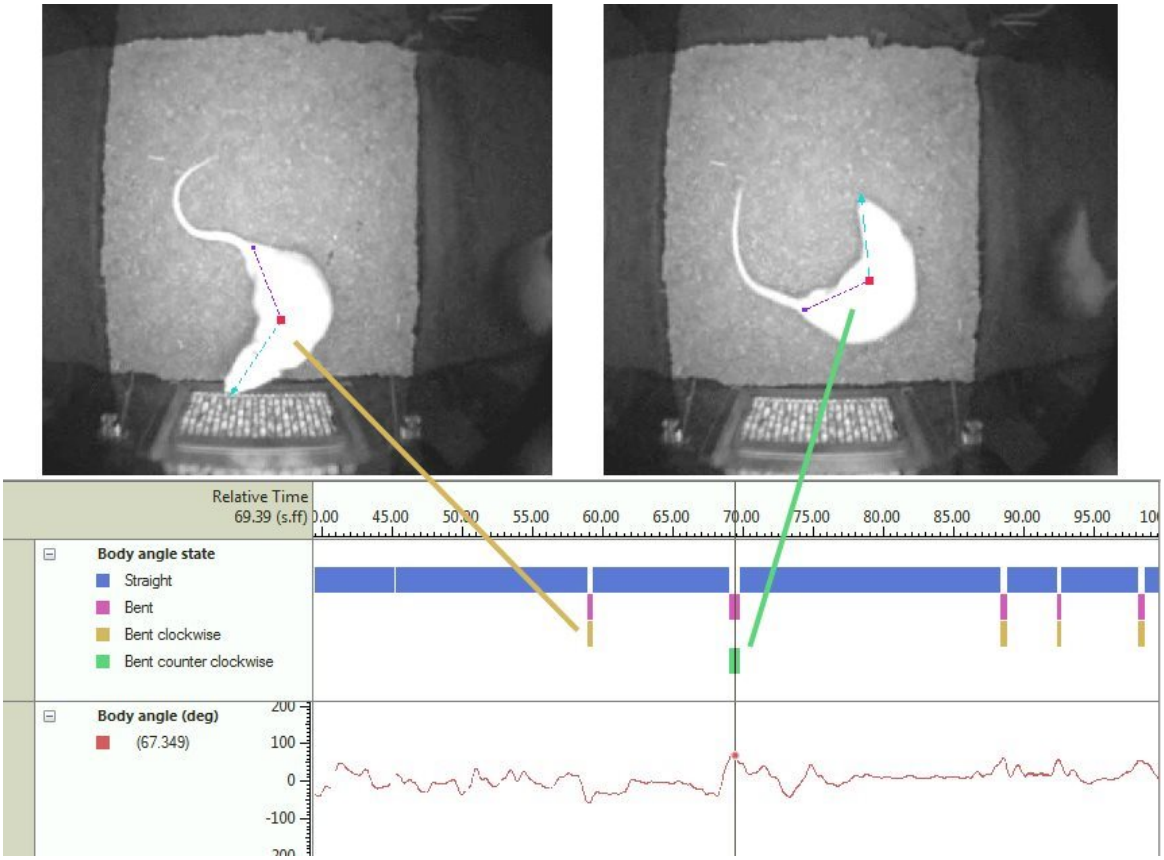
## Definition

A discrete (state event) variable with two possible states: *Straight* and *Bent*, depending on where the running average body angle (see Body angle for its definition) of the subject calculated for the current sample lays relative to a user-defined threshold:

- If the running average body angle is greater than the threshold value, the state is Bent. This state can be further split in *Bent counterclockwise* and *Bent clockwise* depending on the sign of the angle.
- If the running average body angle is smaller than the threshold value, the state is Straight.

The running average body angle is calculated for each sample, over the number of samples specified by the Averaging interval.

Below: Visualization of *Body angle state* (top chart) and *Body angle* (bottom).Body angle state was based on the Body angle values smoothed with averaging interval of 5.



## How to specify Body angle state

1. Click the **Add** button next to **Body angle state** and click the **Body Angle State** tab.
2. Enter the following:
   - **Averaging interval**: The number of samples over which the running average body angle is based.
   - **Body bent above**: The angle above which the subject is considered to be bent. This value is unsigned and applied to both negative and positive Body angles.
3. Under **Calculate statistics for**, select which state you want to calculate statistic for: **Straight**, **Bent**, **Bent counterclockwise** (that is, when the running average body angle is positive; see Body angle for its definition), and **Bent clockwise** (when the running average body angle is negative).
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- When a body point is missing for more than three samples, the current Body angle state ends and the remaining missing samples are ignored.
- **Averaging interval**. The default value is 1. In that case the body angle state is based on the value of *Body angle* at the current sample. If you select a number k higher than 1, EthoVision takes the body angle of the last k samples (including the current sample) and replaces the current value with the average. See Averaging interval

# Mobility

## Definition

The percentage changed pixels of the detected subject between current sample and previous sample.

**NOTE** *Mobility* is not available if your experiment is set to:

- Live Mouse Tracker (but see Stretch attend posture).
- Center-point, nose-point and tail-base detection, when you track two subjects per arena with Deep learning.

## Calculation

### *Step 1 - Calculation of the changed area*

All pixel coordinates of the subjects are determined immediately after they have been detected. Those coordinates are compared with the previous sample to determine the number of changed pixels between the two. The changed pixels are

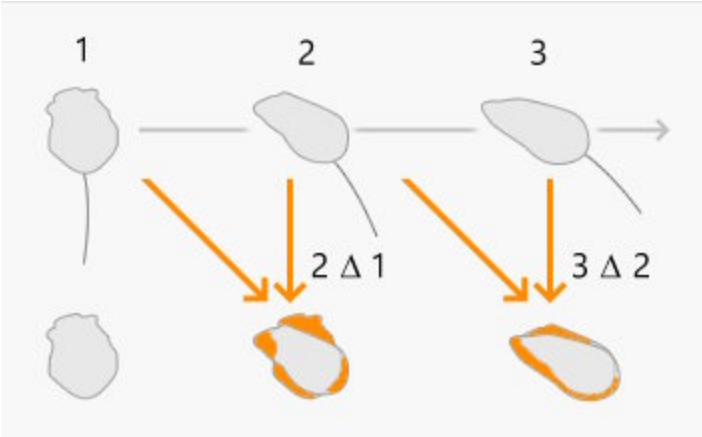
- The subject pixels found in the current sample but not in the previous sample AND.
- The subject pixels found for the previous sample but not for the current sample.

This can be expressed in the following formula:

$CA_k = (A_k - A_{k-1}) + (A_{k-1} - A_k)$

Where $CA_k$ is the changed area for the current sample $k$, $A_k$ is the area for the sample $k$, and $A_{k-1}$ the area for the sample $k–1$.

In the example below, CA is the sum of the orange areas when comparing the contour of the subject with that at the previous sample. The sum equals the symmetric difference between sets of points known in mathematics and is generally indicated with delta ($\Delta$). The changed area is shown for samples 2 and 3.




The changed area (in number of pixels) is available when you export your raw data. Open the export file and locate the column named **Areachange**. Note that **Areachange** is not the same as the difference in **Area** between the current and the previous sample!

Mobility is calculated by taking every pixel identified as the subject and comparing it between the current image and the previous one. Note that Mobility also includes movement in space (e.g. walking).

*Step 2 - Calculation of Mobility*

The formula for Mobility is the changed area for the current sample *k* divided by the sum of the current area and the previous area:

$$Mobility = \frac{CA_k}{A_{k-1} + A_k} \times 100$$

*Step 3 - Running average*

To smooth the Mobility parameter, an Averaging interval is used. This gives you the option to specify the number of samples for calculating a running average of Mobility. The Mobility percentage calculated as above is averaged over the number of samples that you specify. This way, sudden changes in surface area caused by such factors as the animal entering a shadowed area and not being identified correctly, or a reflection being identified momentarily as the animal, are smoothed out. See also Averaging interval

When you export data, you do not export any information about which averaging interval was used.

## Range

*Mobility* ranges from 0% to 100%.

- If all the pixels of the detected subject are equal in value between sample *k-1* and sample *k*, for example when the subject is completely still, Mobility at sample *k* is 0.
- If the animal moves and increases its velocity, whilst keeping the same shape, there will be an increase in mobility, because the pixels belonging to the animal are increasingly different as it moves faster.
- when the subject is so fast that its contour at consecutive samples does not overlap anymore, then all the pixels are different. CA is maximal and equals the sum of the two contours. Therefore, Mobility is 100%.

## How to specify Mobility

1. Open an Analysis profile and in the Dependent Variables panel, under **Body**, click the **Add** button next to **Mobility**.
2. Select the **Averaging interval**. See above Step 3 - Running average for more information on the Averaging interval.
3. Click the **Trial Statistics** tab and select the statistics you want to calculate.

## Notes

- When the experiment is set to **Color marker tracking**, that is, when EthoVision only tracks the markers and ignore the body contour of the subject, Mobility is calculated based on the surface area of the marker.

*See also*

- Mobility state
- Frequently asked questions about Mobility

# Mobility state

## Definition

A discrete (state event) variable with three possible states: Highly mobile, Mobile and Immobile, depending on where the changed pixels of the detected subject between current sample and previous sample (referred to as changed area) lay relative to two user-defined thresholds.

- For the difference between Mobility and Movement, see Frequently asked questions about Mobility.
- For the difference between Mobility and Activity, see Frequently asked questions about Activity

**NOTE** *Mobility state* is not available if your experiment is set to:

- Live Mouse Tracker.
- Center-point, nose-point and tail-base detection, when you track two subjects per arena with Deep learning.

## Calculation

The *Mobility state* variable is calculated for each sample according to the value of the running average Mobility relative to the thresholds:

- Below the Immobile threshold, the state is *Immobile*.
- Between the Immobile threshold and the Highly mobile threshold, the state is *Mobile*.
- Above the Highly mobile threshold, the state is *Highly mobile*.

When the subject goes missing for more than three samples, the current Mobility state is ended and the remaining missing samples are ignored.

See also Frequently asked questions about Mobility

## How to specify Mobility state

1. Open an Analysis profile and in the Dependent Variables panel, under **Body**, click the **Add** button next to **Mobility state** and click the **Mobility State** tab.
2. Enter the following:
    - **Averaging Interval**: The number of samples over which the running average mobility is based. The default value is 1, that is, the mobility

measure is not smoothed before determining the *Mobility state* variable.

- **Highly mobile threshold**: The percentage of change in body area above which the subject is considered to be *Highly mobile*.
- **Immobile threshold**: The percentage of change in body area below which the subject is considered *Immobile*. You can enter a number with up to two decimals.

3. Under **Calculate statistics for**, select at least one of the three following options:
   - **Highly mobile**: Statistics are calculated for when the subject is considered *Highly mobile*.
   - **Mobile**: Statistics are calculated for when the subject is considered *Mobile*.
   - **Immobile**: Statistics are calculated for when the subject is considered *Immobile*.
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- To find the optimal Highly mobile and Immobile threshold, run a few test trials and check in the Analysis Results and Scoring pane the values of Mobility when the animal shows such behavior. These values are calculated real-time during acquisition. See an example in Porsolt swim test: view the Mobility state variable.
- Since Mobility is calculated on the detected subject, the gray-scale threshold values used in detection also have an influence on the mobility variable. If your detection settings are such that only part of the animal is detected, then only the mobility for that part is calculated.
- In some cases the number of samples available for smoothing can be less than the averaging interval entered. For example, when there are missing samples or at the beginning of the track. In such cases EthoVision XT uses the samples available in the specified interval. For example, the value of *Mobility* for the first sample of the track is always calculated over one sample. See Averaging interval
- You set the thresholds during acquisition, but you can override them when calculating statistics to produce new values for *Mobility*. To see what the original values of Mobility thresholds were (unless you have changed them while acquiring data), open the Acquisition module and click the button next to **Mobility** in the Analysis results and Scoring pane.

## Applications

- *Mobility* can be used to assess general activity, and changes in behaviors in specific paradigms. For example, in Porsolt swim tests (for example, Russig *et al*. 2003, *Behav. Pharm.* **14**: 1-18) it allows to detect changes in behavior, for example from swimming to floating, more objectively than when observing directly.
- You can also use *Mobility* to detect freezing behavior in which case you need to set a very low value of Immobile threshold. Mobility can also be used to quantify movement of zebra fish embryos within their eggs in a 24-well plate with back-lighting.

*See also*

- Frequently asked questions about Mobility

# Frequently asked questions about Mobility

## What is the difference between Mobility and Movement?

Mobility can be defined as the degree of movement of an animal's body independent of spatial displacement of the center or any other body point, which is measured by Movement. 'Independent' does not mean that mobility is corrected for the center-point position, it means that the calculation does not use the x,y coordinates of the animal (they are not in the equation used to calculate it). Mobility is calculated 100% independent of movement of the coordinates identified as the center-point (or the nose/tail point). That means that the center-point can have zero movement but high mobility.

For example, imagine that you are tracking a rat in an open field. When the rat stands still and grooms, the center of gravity does not move, therefore there is no spatial displacement of the subject (the current state for the Movement variable is Not moving), however the rat's head and forelimbs move, resulting in changes in the surface area. Although there is no spatial displacement of the body, the current state of the Mobility variable is Mobile (depending on the threshold value).

## Does Mobility depend on the size of the subject vs. arena?

No. It is dependent on the size of the subject only. Keeping resolution constant, the smaller the subject, the smaller the number of pixels that form its image, the more likely that any small movement results in a change in area that is detected as Mobility.

## Does Mobility depend on the video resolution?

Yes. The higher the video resolution, the greater the number of pixels that form the image of the subject. Therefore, the less likely that a small movement of the subject results in an abrupt change in area.
However, this effect is present at very low resolution (for example, when the subject is less than 100 pixels large). At resolutions provided by video files, this effect is negligible.

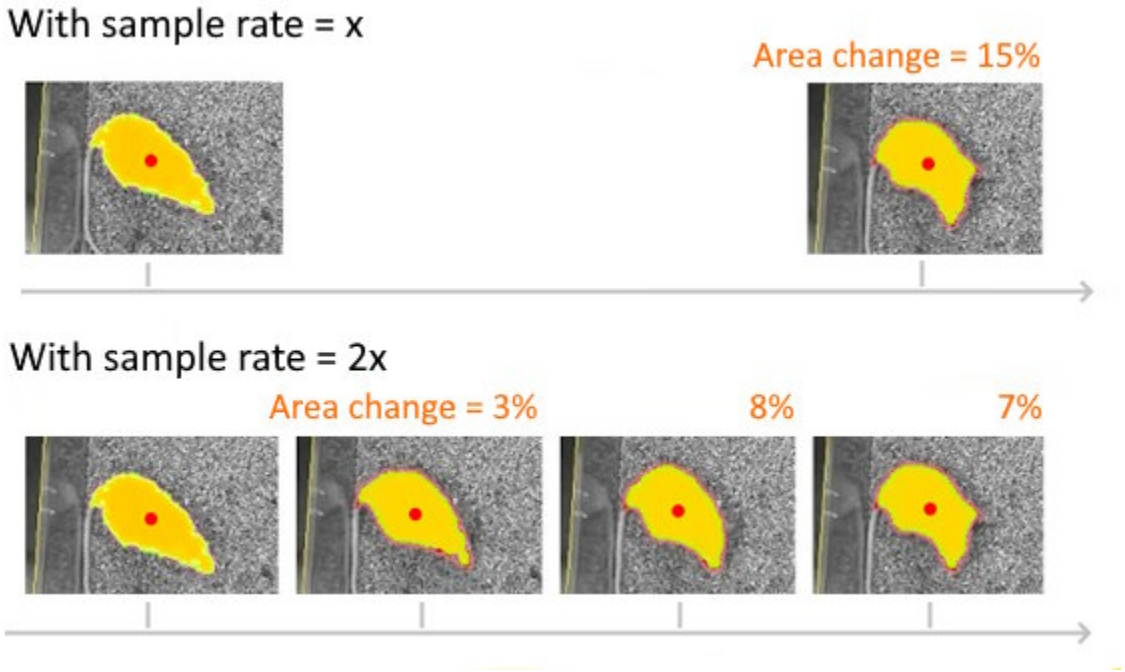
## Does Mobility depend on the sample rate?

Yes. Since Mobility is detected from the change in area of the subject between two samples, and the change in area depends on how frequently the subject area is acquired (that is, the sample rate), Mobility depends on the sample rate. All being equal, the higher the sample rate, the smaller the change in subject area.

In the example below, the Immobile threshold was set to 2%, and the Highly mobile threshold was set to 10%. A certain movement pattern detected with a sample rate x determines a change in area of around 15%, which causes EthoVision consider the subject Highly mobile.

If the sample rate doubles, more samples are captured in the same time interval, therefore the same movement results to a smaller change in area between samples. For the intermediate samples, EthoVision XT considers the subject Mobile since the change in area is smaller than 10%. The proportion of samples where the subject is considered Mobile increases relative to the proportion of samples where the subject is considered Highly mobile.

As a general rule, the higher the sample rate, the lower the Immobile and Highly mobile thresholds must be.




## Does Mobility depend on track smoothing?

The results of Mobility may differ depending on whether you set a **Minimum Distance Moved** filter (see The Minimal Distance Moved smoothing method). When you apply this filter, a sample in the track that is very close to that at the previous time stamp is given the same coordinates as the previous one. When that occurs, the **Area change** is also set to zero for that sample. This results in a lower value of the average Mobility.

In contrast, other track smoothing methods do not influence Mobility. See Smooth the Tracks

If you want to preserve the values of Mobility when the animal is almost completely still, instead of using the Minimal Distance Moved filter select the data based on *Movement*. In the Data profile, choose **Nesting** > **Movement**. Under **Calculate nesting for**, select **Not moving** and set the threshold velocities in such a way the animal is considered *Not moving* when its velocity is lower than the **Stop velocity** value. See Nesting over Movement. Next, calculate Mobility.

*See also*

- Movement
- Mobility

# Rotation

## Definition

This is the number of turns, either clockwise or counterclockwise, of 360° (if not otherwise defined).

A rotation can be based on the movement of a body point or on the change in direction of a body axis.

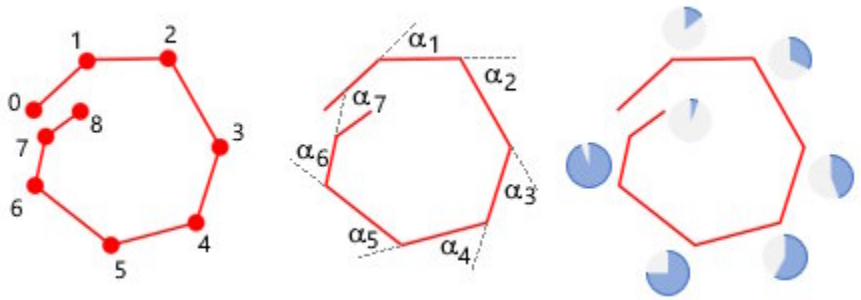
## Calculation

### *Body point rotation*

The software scores a rotation event when the cumulative turn angle of the chosen body point exceeds the threshold value (default: a complete rotation, that is, 360°).

Consider the simplified example in the figure below. Left: the original track, starting from the sample a time 0. Here we consider the center point but the calculation is the same of the nose-point and the tail-base point. Middle: The turn angles ($\alpha$) calculated for the body point of samples 1, 2, 3, etc. Right: cumulative turn angle. For details about turn angles, see the dependent variable Turn angle.

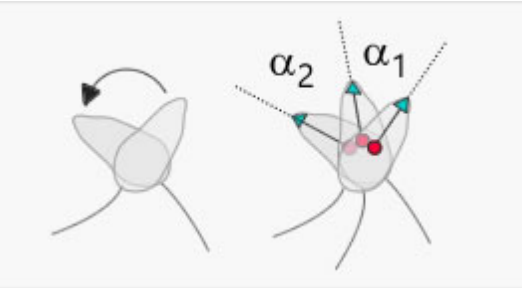
At sample 7, the cumulative turn angle exceeds 360°. Therefore, a rotation event is scored. The cumulative turn angle is reset and consecutive turn angles are summed until the cumulative Turn angle, again, exceeds the rotation threshold.




### *Body axis rotation*

This method is based on the turn angle formed between the body axis at two consecutive sample times (*k-1* and *k*). As a body axis, you can choose the segment joining the center-point and the nose-point, or the segment between the tail-base and the center-point.

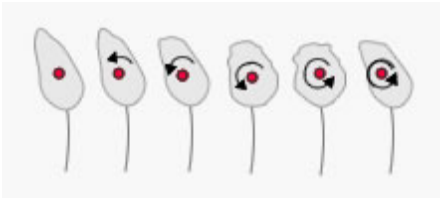
The software scores a rotation event when the cumulative turn angle of the chosen body axis $\alpha_1+\alpha_2+...$ exceeds the threshold value (default: a complete rotation, that is, 360°). Note that the angles depend only on the orientation of the body axis, not the position of the body center.




**NOTE** This rotation method is only available if your experiment is set to Center-point, nose-point and tail-base detection.

## Which method shall I choose?

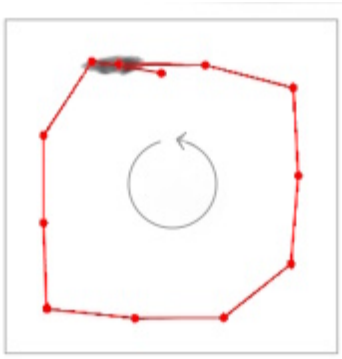
- Choose **Body point rotation** if the track clearly shows the rotation movement that you want to quantify. For example, a mouse walking along the walls of a cage, or a fish larva swimming in a well. In that case the trajectory of the center point is sufficient to characterize the behavior.
- Choose **Body axis rotation** if the subject is spinning around its own axis and you want to quantify exactly that behavior.
- Choose **Body axis rotation** if the subject is performing *micro-rotations* and you do not want to count them. Micro-rotations occur when the subject makes small repetitive movements of the body that cause the center point to rotate, while the subject itself does not walk along a circle. Such rotations are hardly noticeable in the track plots. That could occur for example while a rodent does body grooming. Note that the center point rotates while the body axis does not.

  If micro-rotations are a problem in your trials and you do not want them included in the results, choose this Body axis rotation.

- **TIP** To eliminate or reduce the detection of micro-rotations when using **Body-point rotation**, apply a track-smoothing filter based on minimum distance moved. See The Minimal Distance Moved smoothing method

- More generally, you can use the Minimum Distance Moved filter to rectify the trajectory of the subject, so that the software ignores the moments when the subject is still or moves little. In this example a MDM of 25 cm was used to make the larger-scale rotations more visible:



## How to specify Rotation

1. Click the **Add** button next to **Rotation**.
2. Under **Select**, choose one of the two methods **Body points rotation** or **Body axis rotation**. If you choose Body axis rotation, select which axis you want to use to count rotation: **Axis: Center-point to Nose-point** or the **Axis: Tail-base to Center-point**.
3. Under **Settings**, select whether EthoVision XT should count rotations in the **Clockwise** direction or in the **Counter-clockwise** direction.
4. In the **Count every … rotation** list, specify how rotations should be counted.

   The default value of 1 means that every 360° turn in the specified direction counted as one rotation. If you select 0.5 in this list, every 180° turn is counted as one rotation.

5. By entering a threshold angle in the **Threshold** box, you can compensate for turns in the direction opposite to the one you selected in step 2.

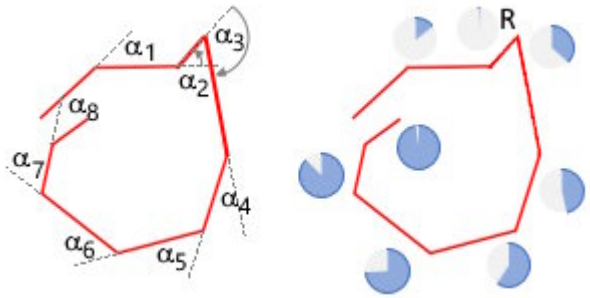
   **TIP** Visualize the rotation events in the Integrated Visualization to validate your choice of the threshold value.

6. Enter a value of **Minimum Distance Moved** if you want to remove the samples for which the distance from the last sample selected in the track is too short to represent actual movement. By default this is 2 cm/0.78 inch. Turn angles and Rotation are re-calculated according to this filter. If you do not want to apply any filter, enter 0.

7. If you track three body points (center-point, nose-point and tail-base point), and you select **Body point rotations**, in the **Body Points** tab select the body points you want to use to calculate the rotations.
8. Complete the procedure to add the variable. See Calculate statistics: procedure.

## The role of the threshold angle

Suppose that in figure shown at the top of this page the subject makes a counterclockwise turn between sample 2 and sample 3 ($\alpha_2$), then it makes a new turn in the clockwise direction (($\alpha_3$) before completing a rotation.




The threshold angle allows you to compensate for turns in the opposite direction. In this example we are interested in clockwise rotations. Depending on the value of the turn angle $\alpha_2$ in the counterclockwise direction, one of the following occurs:

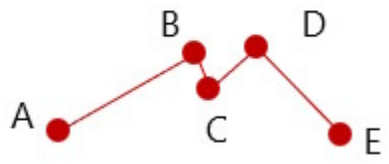
- The turn angle $\alpha_2$ is smaller than or equal to the threshold angle. EthoVision XT continues to calculate the cumulative turn angle until it exceeds the corresponding rotation count. A turn in the opposite direction means that the cumulative angle is reduced at that sample.
- The turn angle $\alpha_2$ is larger than the threshold angle. EthoVision XT resets the cumulative turn angle (R in the figure below) and uses a new series of samples to calculate the cumulative turn angle until the latter exceeds the corresponding rotation count.

The rationale for applying a threshold angle is that if the animal makes large turns in the opposite direction, the trajectory can no longer be described as a circle-like pattern, and the subject is likely to have changed its behavior. The counting of cumulative turn angle should therefore be reset.

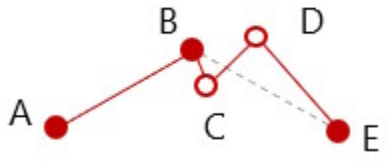
## Notes

- When you apply a Minimum Distance Moved filter, samples are filtered with the Direct method for Minimal Distance Moved. This filter changes the

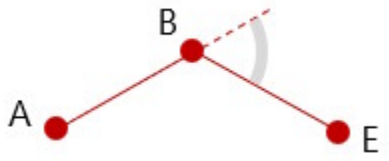
values of turn angle at a specific sample. Consider the following example (movement is from A to B):




The filter sets samples C and D equal to B since their distance from B is shorter than the threshold entered.




The turn angle for sample E is the angle formed with A and B (=C, D). See Turn angle

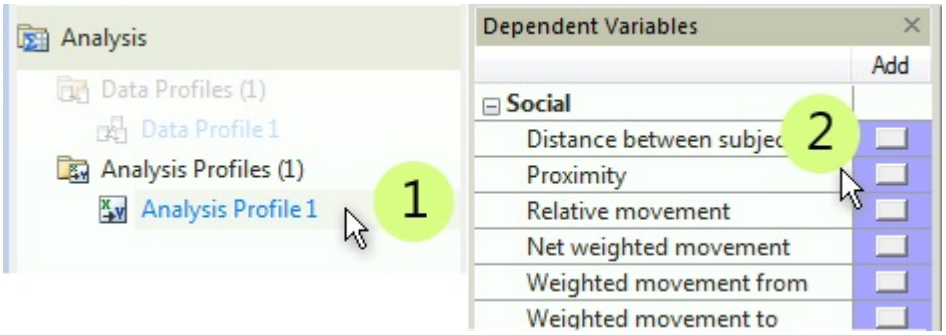



- To count both clockwise and counter-clockwise rotations, repeat the steps from step 1 and specify the alternative option. As a result, you have two instances of the variable *Rotation*, one for clockwise rotations and the other for counter-clockwise rotations.

## Applications

Circling or rotational behavior is used in rats as an indicator of cerebral asymmetry. For example, striatal asymmetries in dopamine characteristics, such as dopamine levels, metabolites, release and uptake, have been functionally related to an increase in rotational behavior (e.g., Carlson and Click, 1989; Schirmer et al., 2007). Amphetamine, a dopamine releaser, induces rotations in animals with striatal asymmetries, which can be blocked by haloperidol. The animal usually turns away from the side of higher dopaminergic activity.

# Social




- Distance between subjects 1075
- Proximity 1077
- Body contact 1080
- Relative movement 1083
- Net weighted movement 1088
- Weighted movement from 1090
- Weighted movement to 1093
- Side by side 1096
- Train 1098

# Distance between subjects

## Definition

The distance between a body point of a subject and a body point of another subject.

Distance is calculated for each Subject (*Actor*) relative to other subjects (*Receivers*).

## Calculation

Formula:

$$DS_k = \sqrt{(X_{a,k} - X_{r,k})^2 + (Y_{a,k} - Y_{r,k})^2}$$

Where $DS_k$ is the distance between Actor and Receiver at sample *k*, $X_{a,k}$ and $Y_{a,k}$ the X,Y coordinates of the selected body point of the Actor at sample *k*, and $X_{r,k}$ and $Y_{r,k}$ the X,Y coordinates of the selected body point of the Receiver at sample *k*.

## How to specify Distance between subjects

1. Click the **Add** button next to **Distance between subjects**.
2. Click the **Body points** tab and select the body points of the focal subject (Actor) you want to use to calculate distance.
3. Click the **Receivers** tab. Here, you specify the other subjects (Receivers).
   - Under **Select**, choose the subjects you want to calculate the distance from.
   - Under **Body points**, select the body points of the subjects selected above.
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

Distance is calculated for each combination of Actor's body points and Receiver's body points. Each row of the result table shows the results for an Actor. For example: The row heading shows Subject 1. The column heading **Nose point / Subject 2 / Center point**. This can be read as 'the cell contains the distance between the nose point of Subject 1 and the center point of Subject 2'.

## Applications

This parameter forms the basis for the Proximity dependent variable used in studies of social or aggressive behavior. In zebrafish, shoaling behavior is analyzed with Distance between subjects; see Green *et al.* (2012) *J. Neurosc. Methods*, **210**, 266–271.

# Proximity

## Definition

A discrete (state event) variable with two possible states, *In proximity* and *Not in proximity*:

- The state is *In proximity* when the distance between the selected body points of the focal subject (*Actor*) and the body points of another subject (*Receiver*) is lower than a user-defined In proximity threshold.
- The state is *Not in proximity* when the distance between the selected body points of the *Actor* and the body points of another subject *Receiver* is greater than a user-defined Not in proximity threshold.
- The state does not change from the previous sample when the distance stays between the two thresholds.

If at least one of two subjects' selected body points is missing for more than three samples, the current *Proximity* state ends and the remaining missing samples are ignored.

## Calculation

For each sample, the program calculates first the Distance between subjects, then it compares this value with the In proximity and Not in proximity thresholds to establish the state at that sample.

## How to specify Proximity

1. Click the **Add** button next to **Proximity**.
2. In the **Proximity** tab, enter the **In proximity** and **Not in proximity** distance values that specify when the two subjects are considered in proximity to each other.
3. Under **Calculate statistics for**, select the state you want to analyze.

   For example, select **In proximity** if you want to know how often or how long the subjects were close to each other. Select **Not in proximity** if you want to analyze when the animals were far from each other.

4. Click the **Body points** tab. Select the body points of the focal subject (Actor) you want to use to calculate proximity.

   If you select two or three points, a drop-down list becomes available. Choose:

- **All selected points**: A state is assigned only when all selected points are in that state relative to the Receiver (*Proximity* or *Not in proximity*). If body points are in different states, that sample is not used in analysis.
- **Any selected point**: A state is assigned when at least one selected body point is in that state relative to the Receiver (*Proximity* or *Not in proximity*).
- **Each point**: A state is defined for each point of the Actor. Results are displayed for each point separately.

5. Click the **Receivers** tab. Here, you specify the other subjects (Receivers).

   Under **Select**, choose the subjects you want to calculate the distance from. If you select two or more subjects, select one of the available options from the list:

   - **All selected subjects:** A state is assigned only when the Actor is in that state for all selected Receiver (*In proximity / Not in proximity*). If the Actor is in different states relative to different Receivers (for example, Subject 1 *In proximity* of Subject 2 and *Not in proximity* of Subject 3), that sample is not used in analysis.
   - **Any selected subject**: A state is assigned when the Actor is in that state for at least one Receiver (*In proximity/Not in proximity*).
   - **Each subject**: A state is assigned to each combination Actor*Receiver. Results are displayed for each Receiver.

6. Under **Body points**, select the body points of the Receivers you want to use to define proximity. If you select two or three points, select one of the available options:

   - **All selected points**: A state is assigned only when all selected points are in that state relative to the Receiver (*in proximity/not in proximity*). If different body points are in different states, the sample is not used in the analysis.
   - **Any selected point**: A state is assigned when at least one selected body point is in that state relative to the Receiver (*in proximity/not in proximity*).
   - **Each point**: A state is defined for each point of the Actor. Results are shown for each point separately.

7. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- If the experiment is set to Only center-point detection or Color marker tracking, the **Body points** tab is absent. Calculations are based on the center point.
- **Any selected points**: At any sample time, it is possible that the Actor's body points are in different states relative to the body points of the Receiver. For example, the Actor's nose point being In proximity of the Receiver's center point, and the Actor's center point *Not in proximity* of the Receiver's center point. In such cases when you select **Any selected point** (step 4 and 5 above), multiple states can be assigned to that sample.
- **Any selected Subjects**: At any sample time, it is possible that the Actor is in different states relative to different Receivers. For example, Subject 1 being *In proximity* of Subject 2 and *Not in proximity* of Subject 3. In such cases when you select **Any selected Subject** (step 5 above), multiple states can be assigned to that sample. You can check multiple states occurring at one sample time when exporting the raw data (see Export the raw data (track and dependent variables). At a specific sample time, the value of the variable is 1 in more than one column, depending on which subject is in proximity of the Actor.

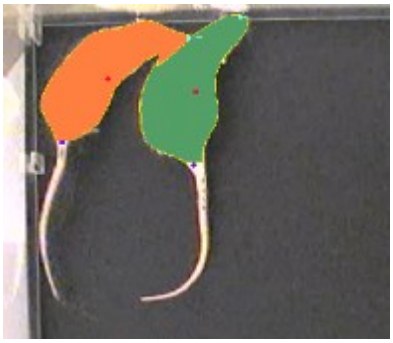
## Application

Proximity can be used to study the behavioral interactions between individual animals, for instance the effects of individual housing vs. group housing on social behavior of rats (Spruijt *et al*. 1992. *Physiology & Behavior* **51**: 747-752), or social isolation as a symptom of schizophrenia (Sams-Dodd, 1995. *J. Neurosc. Methods* **59**: 157-167).

# Body contact

## Definition

A discrete (state event) variable with two possible states, *In contact* and *Not in contact*, based on whether the subject's body contour is in contact with the body contour of any of the other subjects.



**NOTE** *Body contact* is not available or does not give results if:

- The tracks were recorded in EthoVision XT 11 or earlier.
- Your experiment is set to:
  - Color marker tracking.
  - Live Mouse Tracker - But see Raw Live Mouse Tracker data
  - Center-point, nose-point and tail-base detection, with two subjects per arena tracked with Deep learning.

## How to specify Body contact

1. Click the **Add** button next to **Body contact**.
2. In the **Body contact** tab, select the state you want to analyze.

   For example, select **Body Contact** if you want to know how often or how long the subjects were in contact during the test.
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- An animal's contour is determined by computer vision, which is unlike human vision. This is exemplified when dark animals are tracked: often their shadow on the background will be considered part of the animal. Thus, the

detected contour is regularly a bit bigger than the human eye would observe.

- *Body contact* is not subject-specific. That is, you cannot isolate instances of contact for a specific combination of subjects (for example, Subject 1 in contact with Subject 3). Instead, use *Proximity* and specify the two subjects.
- *Body contact* is based on the subject's contour, while *Proximity* is based on the distance between subject's body points. For example, two subjects next to each other may be in proximity, but not in body contact.
- Body contact is affected by the track smoothing methods The Minimal Distance Moved smoothing method and The Maximum Distance Moved smoothing method. When you apply one of those methods, Body contact is recalculated and may be slightly different with respect to when the method is not applied. The difference is usually limited to one or a few samples.

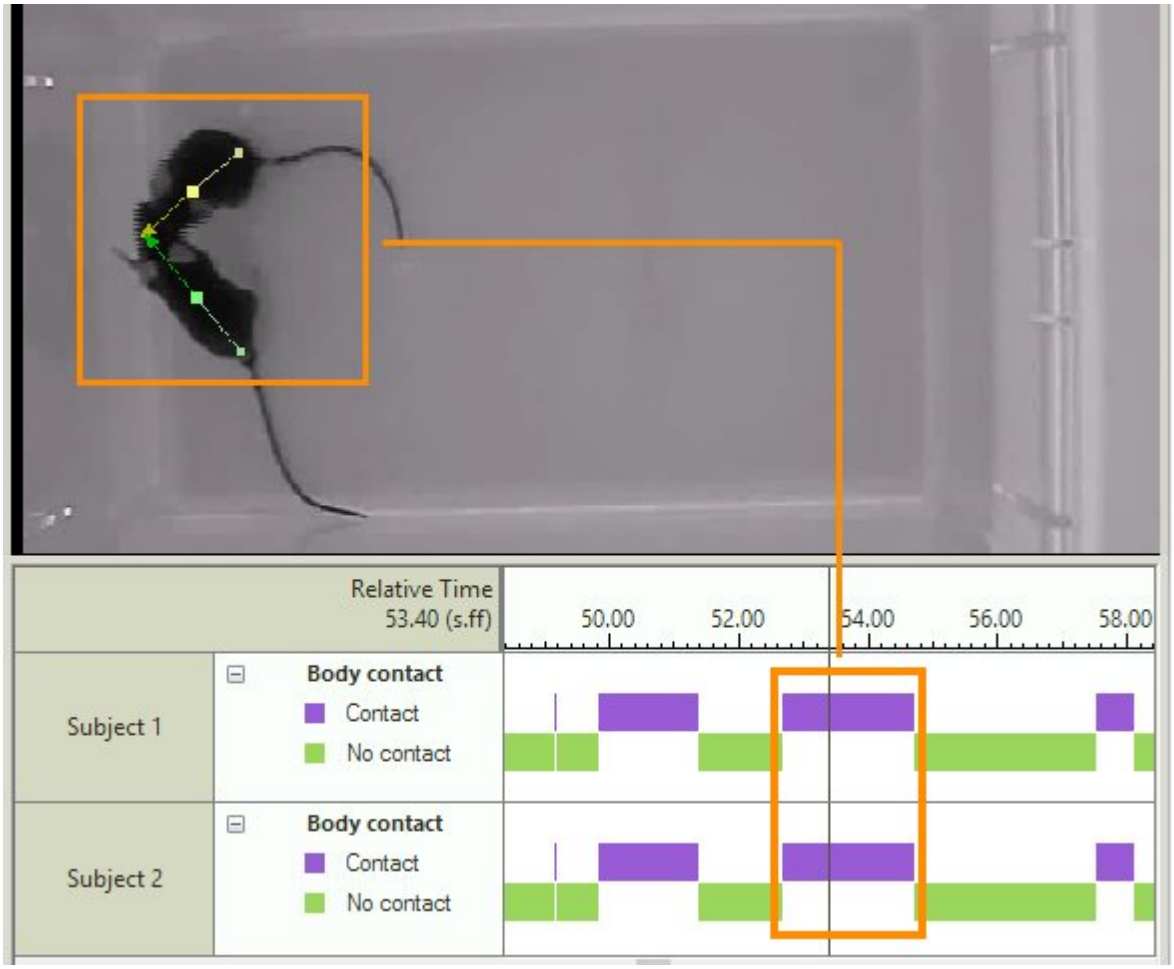
## Applications

*Body contact* can be used in any social interaction tests:

- To quantify the level of interaction between subjects. Add **Body contact** in the Analysis profile (see above).
- To exclude instances of body contact when these result in unreliable tracking. In this case, instead of adding *Body contact* in the Analysis profile, in the Data profile choose Nesting over Body contact and select **Not in contact**.

Below: Integrated Visualization of *Body contact* in a 2-subjects test.

**TIP** In Integrated Visualization, the body contour is not visible. To view the body contour, open **Acquisition**, click the **Show Hide** button, choose **Detection Features** and select **Body contour**.


Relative Time
53.40 (s.ff)
50.00
52.00
54.00
56.00
58.00
Subject 1
Body contact
Contact
No contact
Subject 2
Body contact
Contact
No contact

# Relative movement

## Definition

A discrete (state) variable with four possible states: *Moving to*, *Moving from*, *No relative movement*, and *No interaction*.

- The state is *Moving to* when the focal subject (*Actor*) is moving towards another subject (*Receiver*).

  **NOTE 1** An *Actor* is the subject listed on the rows in the statistics table, or in the Integrated Visualization at the left side of each chart. Other subjects in the same arena are *Receivers*.

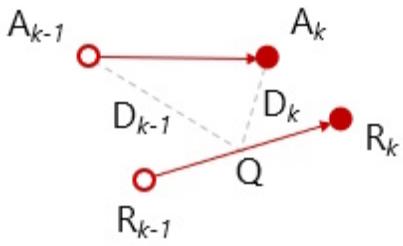
  **NOTE 2** The term *moving* refers to a subject moving faster then a specified minimum velocity.

- The state is *Moving from* when the Actor is moving away from the Receiver.
- The state is *No relative movement* when two subjects are not moving relative to each other.
- The state is *No interaction* when the distance between subjects is great enough that they can be considered as not interacting.

## Calculation

1. To calculate the Relative Movement for sample *k*, the body points of the Actor and the Receiver must be known for sample *k* and *k-1*.
2. The middle point Q is determined on the segment that joins the body points of the Receiver for samples *k–1* and *k*.

   In this example, A is the body point of the Actor, R of the Receiver. Q is the interpolated position of the Receiver's body point.

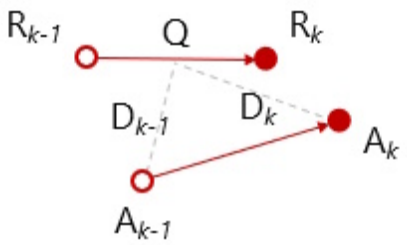



3. The distance between the Actor's body point and Q is calculated for samples: $D_k$ and $D_{k-1}$, respectively.
4. The state of Relative movement is determined:

- If $D_k > D_{k-1}$: *Moving from*
- If $D_k < D_{k-1}$: *Moving to*
- If $D_k = D_{k-1}$: *No relative movement*
- If $D_n$ > Maximum interaction distance (user defined): *No interaction*.
- If the velocity of the Actor is lower than the Minimum velocity (user defined): *No relative movement*.

From the figure above, one can see that the outcome depends on which animal is considered as Actor and which as Receiver.

Now suppose the other subject is the actor.




The point Q is defined in the middle of the segment joining the receiver's points $R_{k-1}$-$R_k$. Note that $D_k$ is longer than $D_{k-1}$. Therefore, this subject, when considered as the Actor, is *Moving from* the other subject.

## How to specify Relative movement

1. Click the **Add** button next to **Relative movement**.
2. In the **Relative Movement** tab:
   - Next to **Maximum interaction distance**, enter the distance above which you do not want to consider the subjects as interacting (Default: 50 cm/16.69 inches).
   - Next to **Minimum velocity**, enter the minimum velocity of the actor (A in the figures above) for it to be considered as moving from or moving to the other subject. Note that the velocity of other subjects does not count here.
   - Under **Calculate statistics for**, select the states you want to consider. By default, all states are selected.
3. Click the **Body points** tab. Select the body points of the focal subject (Actor) you want to use to calculate relative movement.

If you select two or three points, a drop-down list becomes available. Choose:

- **All selected points**: A state is assigned only when all selected points are in that state relative to the Receiver (moving to/from/no movement/no interaction). If body points are in different states, the state is not assigned to that sample.
- **Any selected point**: A state is assigned when at least one selected body point is in that state relative to the Receiver (moving to/from/no movement/no interaction). See the note below.
- **Each point**: A state is defined for each point of the Actor. Results are shown for each point separately.

4. Click the **Receivers** tab. Here, you specify the other subjects (Receivers).

   Under **Select**, choose the subjects. If you select two or more subjects, select one of the available options from the list:

   - **All selected subjects**: A state is assigned only when the Actor is in that state for all selected Receiver (moving to/from/no movement/no interaction). If the Actor is in different states relative to different subjects (for example, Subject 1 *Moving to* Subject 2 and *Moving from* Subject 3), the state is not assigned to that sample.
   - **Any selected subject**: A state is assigned when the Actor is in that state for at least one Receiver (moving to/from/no movement/no interaction).
   - **Each Subject**: A state is assigned to each selected subject as a separate Receiver. Results are shown for each Receiver.

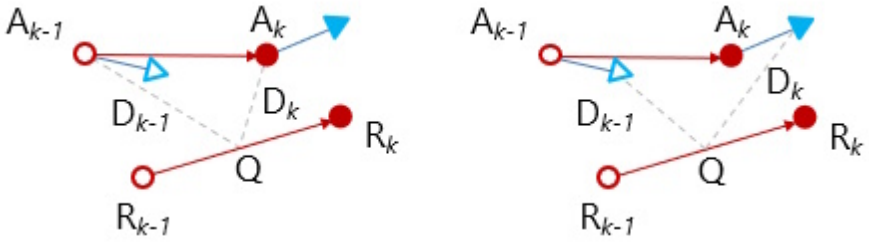
   Under **Body points**, select the body points of the subjects selected above. If you select two or three points, select one of the available options:

   - **All selected points**: A state is assigned when the Actor is in that state relative to all selected points of the Receivers (moving to/from/no movement/no interaction). If the Actor is in different states relative to different Receiver's body points, the state is not assigned to that sample.
   - **Any selected point**: A state is assigned when the Actor is in that state relative to at least one selected body point (moving to/from/no movement/no interaction).
   - **Each point**: A state is defined for each body point of the Receiver. Results are shown for each Receiver body point separately.

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- **All / Any selected points**. At any sample, body points of one subject can be in different states relative to another subject's point. Consider the example of the figure below. The center point of the Actor A (circles) is moving to the Receiver R, while the nose point of A (triangles) is moving from the Receiver. For clarity, the Receiver is represented by the center point only. Left: The Actor's center point is moving to R ($D_k$ is shorter than $D_{k-1}$). Right: The Actor's nose-point is moving from ($D_k$ is longer than $D_{k-1}$).
  - Selecting **All selected points** gives no unique state for Relative movement at sample *k*.
  - Selecting **Any selected points** gives the state *Moving to* for the center point and *Moving from* for the nose point at sample *k*.



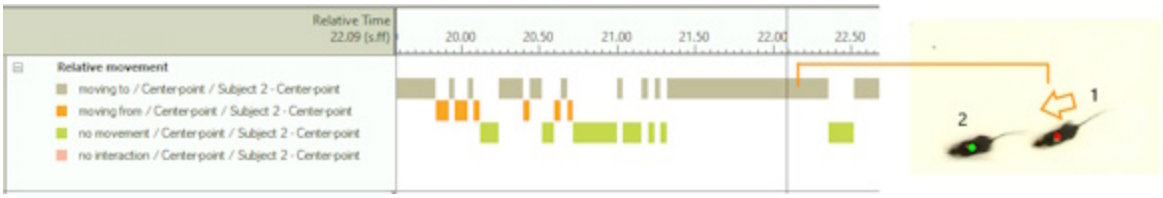

- **Any selected subject**: At any sample time, it is possible that the Actor is in different states relative to different Receivers. For example, Subject 1 *Moving to* Subject 2 and *Moving from* Subject 3. In such cases when you select **Any selected Subject**, multiple states are assigned to that sample. You can check multiple states being assigned to one sample time when exporting the dependent variable. At a specific sample time, the value of the variable is 1 in more than one column of the export file. In the example above, the columns for moving to and moving from will both show 1 in the corresponding sample row.
- If your experiment is set to Only center-point detection or Color marker tracking, the body point options are absent. Calculations are based on the center point.

## Application

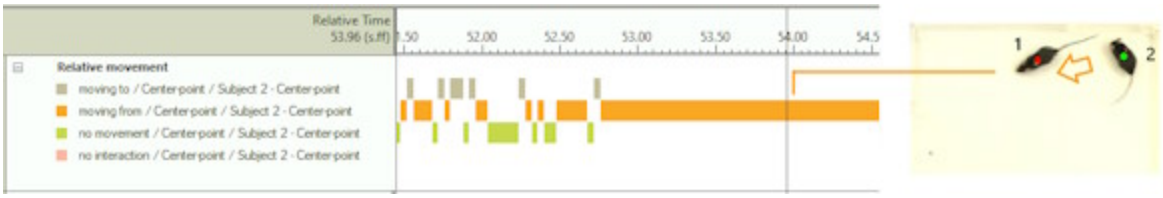
Relative movement can be used to study the effects of individual housing vs. group housing on the social behavior of rats (Spruijt *et al*. 1992. *Physiology & Behavior* **51**: 747-752; Hol *et al*. 1999. *Behavioural Brain Research* **100**: 91-97), or for studying the behavioral interactions between individually recognized animals.

The following examples show the behavior of subject 1 (red dot) relative to subject 2 (green dot).

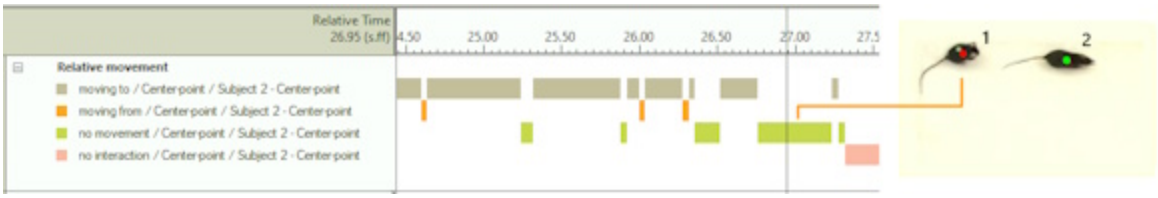
*Moving to*:



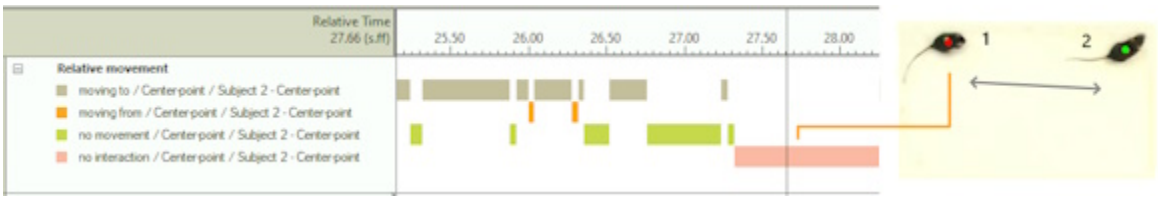

*Moving from*:




*No movement* (Subject 1's velocity is under the Minimum velocity threshold):




*No interaction* (Subject 1's distance from Subject 2 is greater than the Maximum interaction distance):




Note that the *No interaction* state is active even when Subject 1's velocity exceeds the threshold, until the distance between the two subjects goes below the Maximum interaction distance.

# Net weighted movement

## Definition

The signed, distance-weighted change in distance between two subjects from one sample to the next.

*Net weighted movement* is weighted by the distance between two subjects. Changes in positions of subjects which are at a large distance from each other have a lower weight, so they can be distinguished from movements at close distance, which have a different biological meaning.

Unlike *Relative movement*, this is a continuous variable (in distance units). The Net weighted movement is positive if the subject (*Actor*) is getting closer to another subject (*Receiver*), negative in the other case.

## Calculation

Formula:

$NWM_n = (D_{k-1} - D_k) * abs (DS_k - DS_{k-1}) / (max (DS_k, DS_{k-1}))$

Where:

$D_{k-1}$, $D_k$ is the distance between the Actor's body point and the interpolated point of the Receiver calculated for two consecutive samples.

$DS_{k-1}$, $DS_k$ is the distance between subjects for two samples.

In the example of the first figure in the topic Relative movement, the value of the variable depends on which subject is considered as Actor and which as Receiver. Similarly, Net weighted movement of subject A is positive relative to R. If the other subject was the Actor (see the second figure in the topic), the point Q would be defined for the other subject in the middle of the $R_{k-1}$-$R_k$ segment. In that case $D_k$ would be longer than $D_{k-1}$, thus Net weighted movement would be negative relative to R.

## How to specify Net weighted movement

1. Click the **Add** button next to **Net weighted movement**.
2. In the **Net Weighted Movement** tab, under **Maximum interaction distance**, enter the distance above which you do not want to consider the subjects as interacting. (Default: 50 cm/16.69 inches)

3. Click the **Body points** tab. Select the body points of the focal subject (*Actor*) you want to use to calculate net weighted movement.

   If you select two or three points, results are calculated for each point separately.

4. Click the **Receivers** tab.
   - Under **Select**, choose the subjects you want to consider as Receivers.
   - Under **Body points**, select the body points of the subjects selected above. If you select two or more subjects and points, results are calculated for each combination separately.
5. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- *Net weighted movement* is calculated for all the subjects selected in the Data profile. Each subject displayed on the rows of your result table is considered as Actor. The subjects displayed on the columns are the Receivers.
- If your experiment is set to Only center-point detection or Color marker tracking, the body point options are absent. Calculations are based on the center point.

## Application

*Net weighted movement* can be used as an objective measure for the intensity of approach and avoidance behavior (Spruijt *et al*. 1992. *Physiology & Behavior* **51**, 747-752). The advantage of this variable relative to Weighted movement to/from is that it integrates both. This means that you can analyze, for instance, the movement of subjects regardless of the direction towards or away from each other.

# Weighted movement from

## Definition

The distance-weighted change in distance between subjects, when a subject (*Actor*) moves away from another subject (*Receiver*).

*Weighted movement from* (WMF) is a continuous variable, and is always positive. It is calculated only when the state of the Actor is moving from the Receiver (see also Relative movement).

## Calculation

Formula:

- If $D_k - D_{k-1} > 0$, then

  $WMF_k = (D_k - D_{k-1}) * abs(DS_k - DS_{k-1})/(max (DS_k, DS_{k-1}))$

- If $D_k - D_{k-1} \leq 0$, or $DS_k = 0$, then

  $WMF_k$ = missing value

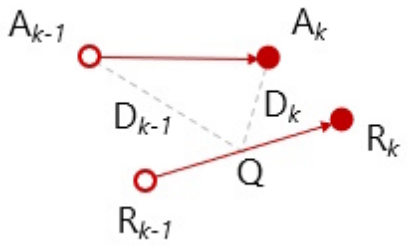
- If $DS_{k-1}$ > Maximum interaction distance, then

  $WMF_k$ = missing value

Where:

- $D_{k-1}$, $D_k$ is the distance between the Actor's body point and the interpolated point of the Receiver calculated for two consecutive samples.
- $DS_{k-1}$, $DS_k$ is the distance between the subjects' body points for two samples.

In the figure below, A is the body point of the Actor, R of the Receiver. Q is the interpolated position between the Receiver's body points at samples *k* and *k-1*.



## Notes

- *Weighted movement from* is equal to the absolute value of Net weighted movement, taken for those samples in which the value of NWM is negative (the focal subject moves away from another subject).
- *Weighted movement from* is weighted by the distance between two subjects. Changes in positions of subjects which are at a large distance from each other have a lower weight, so they can be distinguished from movements at close distance, which have a different biological meaning.
- From the figure above one can see that the outcome depends on which of the two interacting subjects is considered as Actor and which as Receiver. This is because formula takes possible differences in speed of approach of the subjects into account. In that case, the difference $D_k$-$D_{k-1}$ is positive, therefore *Weighted movement from* R is calculated. If R was the Actor, the point Q would be defined for the other subject in the middle of the $A_{k-1}$-$A_k$ segment. In that case $D_k$ would be shorter than $D_{k-1}$, thus $D_k$ - $D_{k-1}$ < 0, and the dependent variable would not be calculated.
- This dependent variable is not a speed, as time is not involved in its calculation. However, the parameter is quadratically sensitive to movement of the subject.

## How to specify Weighted movement from

1. Click the **Add** button next to **Weighted movement from**.
2. In the **Weighted movement From** tab, under **Maximum interaction distance**, enter the distance above which you do not want to consider the subjects as interacting. (Default: 50 cm/16.69 inches)
3. Click the **Body points** tab. Select the body points of the focal subject (*Actor*) you want to use to calculate the dependent variable. If you select two or three points, results are calculated for each point separately.
4. Click the **Receivers** tab.
   - Under **Select**, choose the subjects you want to consider as Receivers.
   - Under **Body points**, select the body points of the subjects selected above. If you select two or more subjects and points, results are calculated for each combination separately.
5. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- *Weighted movement from* is calculated for all the subjects selected in the Data profile. Each subject displayed on the rows of your result table is considered as Actor. The subjects displayed on the columns are the Receivers.
- If your experiment is set to Only center-point detection or Color marker tracking, the body point options are absent. Calculations are based on the center point.

## Application

*Weighted movement from* can be used as an objective measure for the intensity of avoidance (Spruijt *et al*. 1992. *Physiology & Behavior* **51**, 747-752).

# Weighted movement to

## Definition

The distance-weighted change in distance between subjects, when a subject (*Actor*) moves towards another subject (*Receiver*).

*Weighted movement to* (WMT) is a continuous variable, and is always positive. It is calculated only when the state of the Actor is moving to the Receiver (see also Relative movement).

## Calculation

Formula:

- If $D_k$ - $D_{k-1}$ < 0:

  $WMT_k$= ($D_{k-1}$ - $D_k$) * abs($DS_k$ - $DS_{k-1}$)/(max ($DS_k$, $DS_{k-1}$))

- If $D_k$-$D_{k-1}$ ≥ 0, or $DS_k$ = 0:

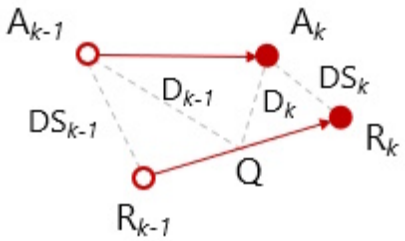
  $WMT_k$ = missing value

- If $DS_k$ = 0, and $DS_k$ > Maximum interaction distance, then

  $WMT_k$ = missing value

Where:

- $D_{k-1}$, $D_k$ is the distance between the Actor's body point and the interpolated point of the Receiver calculated for two consecutive samples *k-1* and *k*.
- $DS_{k-1}$, $DS_k$ is the distance between the subjects' body points for two samples (see Distance between subjects).



## Notes

- *Weighted movement to* is equal to the absolute value of Net weighted movement, taken for those samples in which the value of NWM is positive (the focal subject moves towards another subject).
- *Weighted movement to* is weighted by the distance between two subjects. Changes in positions of subjects which are at a large distance from each other have a lower weight, so they can be distinguished from movements at close distance, which have a different biological meaning.
- From the figure above one can see that the outcome depends on which of the two interacting subjects is considered as Actor and which as Receiver. If R was the Actor, the point Q would be defined for the other subject (A in the figure) in the middle of the segment joining the two consecutive body points. In that case $D_k$ would be longer than $D_{k-1}$, thus $D_k - D_{k-1} > 0$ (i.e., R moves away from A), and the *Weighted movement to* would not be calculated. Instead, Weighted movement from would be calculated.
- This dependent variable is not a speed, as time is not involved in its calculation. However, it is quadratically sensitive to movement of the subject.

## How to specify Weighted movement to

1. Click the **Add** button next to **Weighted movement to**.
2. In the **Weighted movement To** tab, under **Maximum interaction distance**, enter the distance above which you do not want to consider the subjects as interacting. (Default: 50 cm/16.69 inches)
3. Click the **Body points** tab. Select the body points of the focal subject (*Actor*) you want to use to calculate the dependent variable. If you select two or three points, results are calculated for each point separately.
4. Click the **Receivers** tab.
   - Under **Select**, choose the subjects you want to consider as Receivers.
   - Under **Body points**, select the body points of the subjects selected above. If you select two or more subjects and points, results are calculated for each combination separately.
5. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- *Weighted movement to* is calculated for all the subjects selected in the Data profile. Each subject displayed on the rows of your result table is considered as Actor. The subjects displayed on the columns are the Receivers.
- If your experiment is set to Only center-point detection or Color marker tracking, the body point options are absent. Calculations are based on the center point.

## Application

*Weighted movement to* can be used as an objective measure for the intensity of avoidance (Spruijt *et al.* 1992. *Physiology & Behavior* **51**, 747-752).

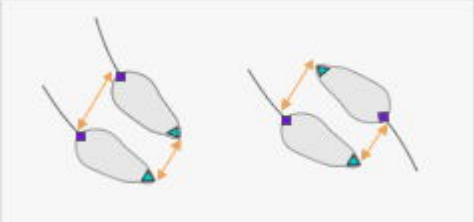
# Side by side

## Definition

The focal subject is side-by-side with another subject. The latter is named receiver in EthoVision XT.

Two categories are defined, based on how the subjects are oriented relative to one another.

- Same direction: the focal subject and the receiver are side by side and pointing to the same direction (in the figure below, left).
- Opposite direction: the focal subject and the receiver are side by side and pointing to the different directions (in the figure below, right).



This variable is available in experiments set to **Center-point, nose-point and tail-base detection**. See Tracked features

## Calculation

- For Side by side, same direction:
  - EthoVision XT finds the samples where both the distance between the nose-point of the focal subject and the nose-point of the receiver and the distance between the tail-base point of the focal subject and the tail-base point of the receiver are below the specified threshold (default: 5.26 cm).
  - If the two vectors formed by the tail-base point and the nose-point of each subjects point to the same directions (that is, the dot product of the two vectors is positive), then *Side by side, same direction* is scored for that sample.
- For Side by side, opposite direction:
  - EthoVision XT finds the samples where both the distance between the nose-point of the focal subject and the tail-base point of the receiver and the distance between the tail-base point of the focal subject and the nose-point of the receiver are below the specified threshold (default: 5.26 cm).

- If the two vectors formed by the tail-base point and the nose-point of each subjects point to opposite directions (that is, the dot product of the two vectors is negative), then *Side by side, opposite direction* is scored for that sample.

## How to specify Side by side

1. Open an Analysis profile and in the Dependent Variables panel, under **Social**, click the **Add** button next to **Side by side**.
2. Under **Threshold**, enter the maximal **In proximity distance** acceptable between the two subjects' body points (nose to nose or nose to tail-base).
3. Under **Calculate statistics for**, select the option **Side by side, same direction**, or **Side by side, opposite direction**, or both.
4. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Note

*Side by side* is similar to Proximity, however the latter cannot tell whether the subjects are oriented in the same or different directions.

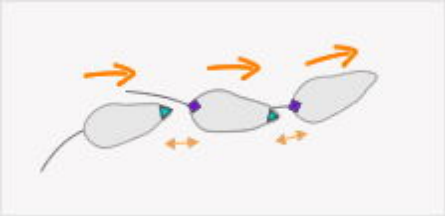
*See also*

- Proximity

# Train

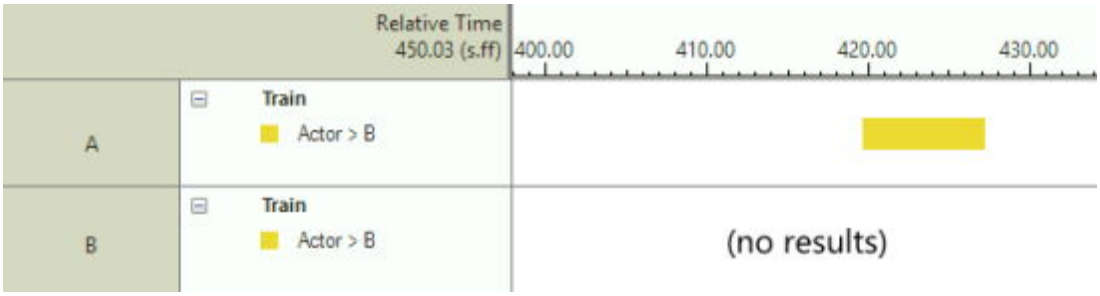
## Definition

A discrete (state event) variable that is scored when one subject follows (or is followed by) one or more subjects at close distance. Trains can be of two, three or four subjects.

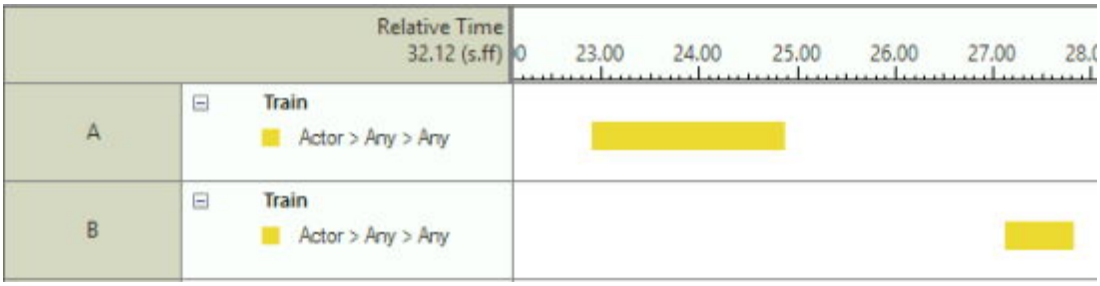


## Examples of trains

- **Actor** > **B**. EthoVision returns all instances when the actor (or focal subject) followed subject B. Here, **Actor** is the focal subject reported in the results table of in the plots of Integrated Visualization. For example, where subject A is the actor, the instances of the train A > B are found. Where subject B is the actor, no results are found since the train B > B makes no sense. Where subject C is the actor, the instances of the train C > B are found, etc.




- **Actor > Any > Any**. This is the predefined train when you create a new Train variable. All instances are found where the actor followed two other subjects, no matter who they were. For example, when subject A is the actor, trains like **A > B > C**, **A > C > B**, or **A > D > B** etc. are found. Similarly, where subject B is the actor, trains like B > A > C, B > C > D etc. are found. Statistics are given for all the combinations of "Any" subjects pooled together.




- **B > A**. This definition returns all the instances when subject B followed closely subject A.
- **A > Actor > Any**. This definition returns all the instances when the focal subject was followed by individual A and followed any other subject.

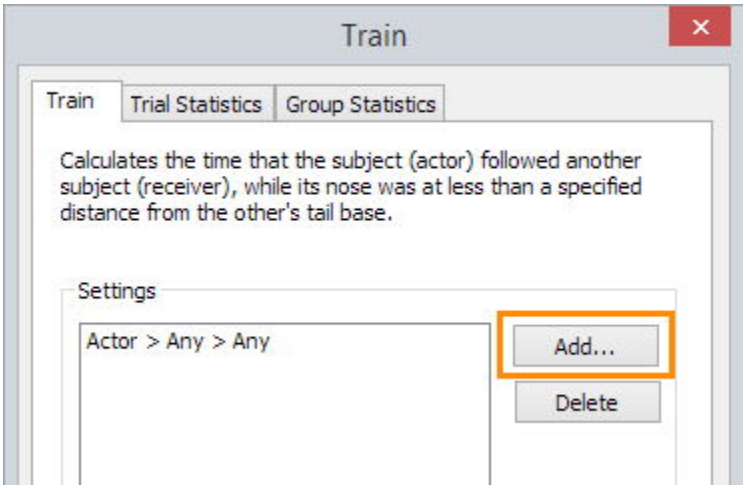
## Calculation

The state *Train* is active when:

- The speed of the subjects is higher than the minimum value specified (see below).
- The distance between the actor's nose point and another subject's tail base, or the distance between the actor's tail base point and another subject's nose point is less than the maximum value specified (see below).

## How to specify Train

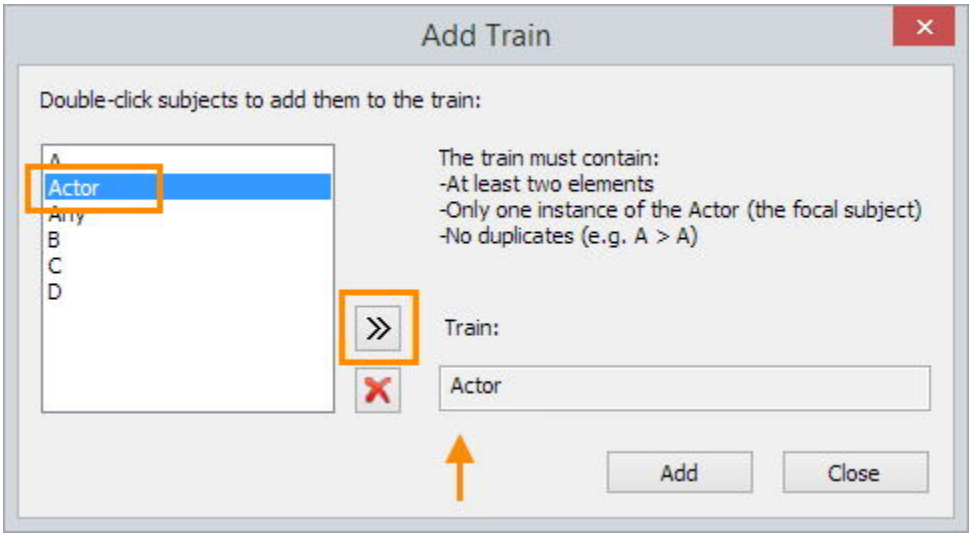
1. Open an Analysis profile and in the Dependent Variables panel, under **Social**, click the **Add** button next to **Train**.
2. By default, the variable Train contains a predefined train with three animals: **Actor** > **Any** > **Any**.
   - To define new trains, follow the steps below.
   - If you do not want to have the predefined train in this variable, select that train definition under **Settings** and click the **Delete** button.
3. Click the **Add** button to make a new definition of a train.



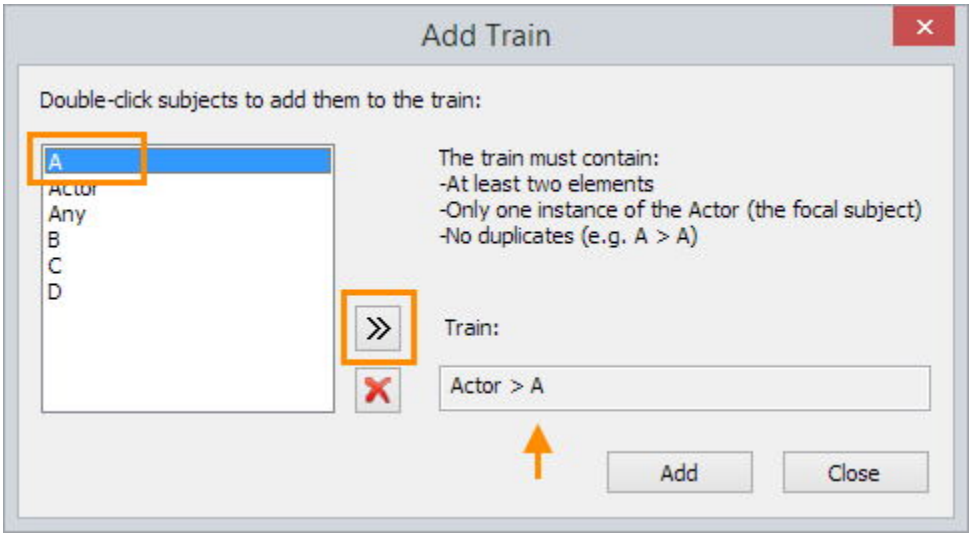

The Add Train window opens with the names of the subjects listed on the left.

4. Select the name of the first subject in the train, that is, the one that follows the other subjects. Next, either double-click the name or click the double-arrow button.

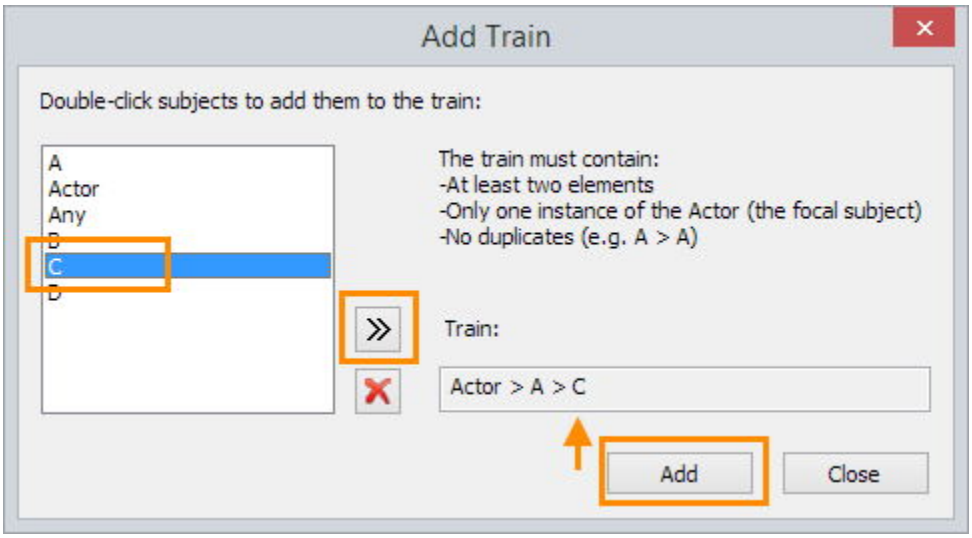
As a result, the subject's name is placed under **Train**.




5. Repeat the steps above for the second, third etc. subject that must be part of the train definition.




6. When ready, click the **Add** button.




7. As a Result, the new train definition, in this example **Actor > A > C**, is added under **Settings**.


8. Do one of the following:
   - To add more train definitions, repeat the steps above beginning from step 4.

- If you are ready with the definitions, click the **Close** button in the Add Train window.

9. Adjust the thresholds if necessary (see below for details).
10. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Notes

- **Maximum distance (nose - tail base)**. A train is defined only if the distance between subjects, measured between the nose-point of one subject and the tail-base point of another subject, is less than the value specified. This condition must be valid for all the subjects in the train definition.
- **Minimum velocity**. A train is defined only if the animals that follow each other are faster than the value specified.
- **Exclude instances shorter than**. You can use this setting to filter out instances that are too short to have biological meaning.
- To delete an existing train definition, select that definition under **Settings** and click **Delete**.
- To delete an element of the train, in the Add Train window click the **Delete** button.

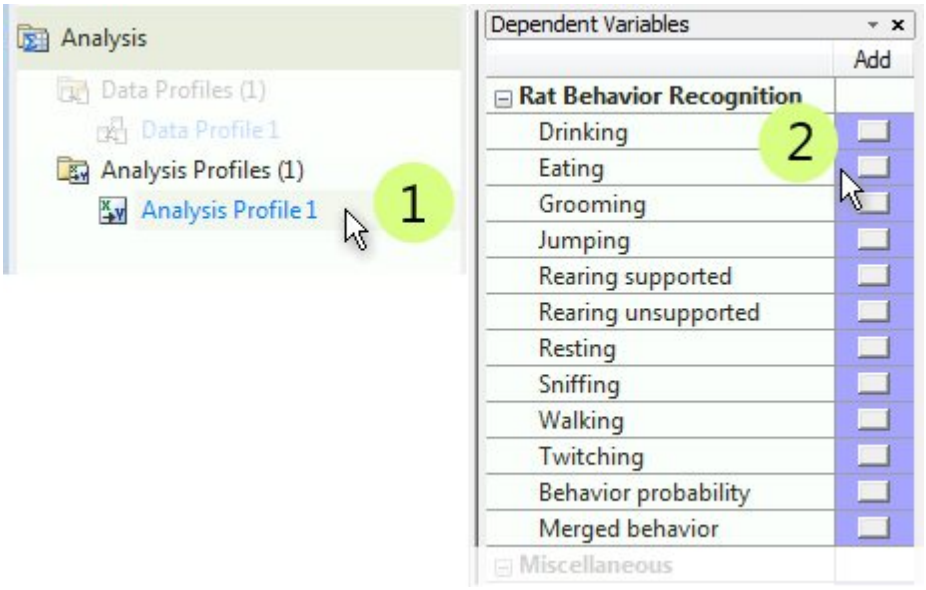
  

  Click multiple times until the element is deleted from the definition under **Train**. If necessary, add more elements to complete the train definition.

*See also*

- Dependent variables for Live Mouse Tracker

# Behavior Recognition




The Rat/Mouse Behavior Recognition group of dependent variables is only available when you have the Rat or Mouse Behavior Recognition add-on module of EthoVision XT, respectively.

- Behaviors detected with Behavior recognition  1104
- Behavior probability  1112
- Merged behavior  1113

# Behaviors detected with Behavior recognition

## Definitions

See also the reference in Notes.

### *Digging*

Rooting with the muzzle or digging with the front paws in the bedding material (for Mouse Behavior Recognition only).

### *Drinking*

The subject licks at the spout of the water bottle.

### *Eating*

The subject eats at the feeder or from the floor, or is eating while holding food in front paws.

### *Grooming*

The subject grooms snout, head, fur or genitals. Includes scratching and licking of paws during a grooming session.

### *Hopping*

The subjects moves forward with both hind limbs at the same time. Head goes first, followed by the rear. Unlike Jumping, Hopping is scored as a point event with no duration, and does not interrupt behavior states (for Mouse Behavior Recognition only).

### *Jumping*

The subject moves quickly forward with both hind limbs at the same time. Jumping interrupts other states like Walking (for Rat Behavior Recognition only).

#### *Rearing unsupported*

The subject stands in an upright posture, with front paws not in contact with any object. Includes the rise and descend.

*Rearing supported*

The subject stands in an upright posture, leaning with front paws against the cage-wall. Includes the rise and descend.

*Resting*

The subject rests with hardly any moving, either sits or is lying down. Includes sleeping. Apparently no interest in the environment.

*Sniffing*

The subject makes slight movements of the head, possibly with slight, discontinuous body displacement. Includes sniffing the air, the wall, the floor and other objects.

*Twitching*

The subject makes sudden and short movements of the body or head. Includes body shake and head shake. Twitching is scored as a point event with no duration, and does not interrupt behavior states (for Rat Behavior Recognition only).

*Twitching* is defined as a point event, that is, an event marking a point in the time line, but with no duration.

*Walking*

The subject moves to another place, and hind legs move as well.

Note the difference between *Walking* and *Movement*: Both dependent variables Walking and Movement are based on displacement of the subject's body point in the 2D space. However, Walking is based on many more video frames than Movement, and therefore Walking is a more accurate measure of walking behavior.

## How to specify a behavior for Behavior recognition

1. Click the **Add** button next to the behavior you want to analyze.
2. Under **Behavior decision method**, select:
    - **Default**: Statistics will be calculated by using all samples scored as that behavior (no filtering based on the behavior's probability).
    - **Probability greater than**: Statistics will be calculated by using only the samples for which the probability of that behavior is higher than a specific value. Select this value from the list (0-99%).
3. Under **Behavior duration threshold**, next to **Exclude instances shorter than**, enter how long the current state must last before it changes from the

previous state In seconds). If the bout length passes this threshold, the previous samples in the bout are included in the new state. If the bout length does not pass the threshold, the previous state ends, but no new state is defined.

4. Under **Calculate statistics for**, select which state you would like to calculate statistics for.
5. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Accuracy

According to our tests, the average recall rate, that is, the proportion of ground truth manually-scored behaviors that are correctly recognized, is around 70%. For more information, see the paper mentioned below.

- *Drinking* can be confused with *Sniffing* especially when the Drinking Spout Points are not defined.
- Make sure to draw the Feeder Zones in the Arena Settings to maximize the detection accuracy of *Eating*.
- To maximize accuracy of detection of *Rearing supported* and reduce false positives, carefully draw the Wall zone in the Arena Settings.

## Notes

- For *Twitching* and *Hopping*, which is an event with no duration, the Behavior duration threshold and the **Calculate statistics for** options are not available.
- For more information on Behavior recognition in EthoVision XT, see the following paper:

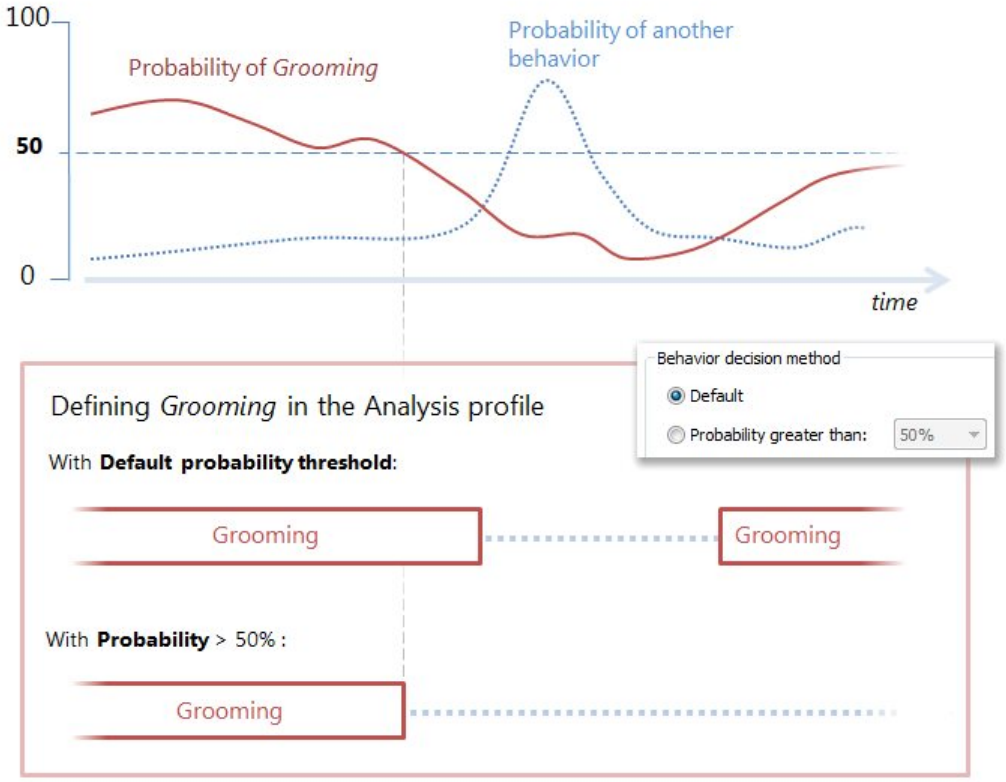
  van Dam, E., J.E. van der Harst, C.J.F. ter Braak, R.A.J. Tegelenbosch, B.M. Spruijt, L.P.J.J. Noldus (2013). An automated system for the recognition of various specific rat behaviours. *Journal of Neuroscience Methods* **218** (2), 214–224.

  http://dx.doi.org/10.1016/j.jneumeth.2013.05.012.

- The Behavior decision method acts as a filter to ignore samples for which the behavior is associated with a low probability, making it easier to extract more reliable data. By default, EthoVision XT uses the original scores (thus no filtering based on probabilities). See the figure below for an example.

  Top: The probability of *Grooming*, and another behavior plotted against time (for simplicity, other behaviors are ignored here). Bottom: When defining *Grooming* in the Analysis profile, consider the following options:

- With **Default** selected, Grooming occurs every time its probability exceeds the probability of any other behavior (default behavior recognition).
- With **Probability greater than 50%** selected, Grooming is limited to the samples for which its probability is higher than 50%. The higher the value, the more conservative the definition.

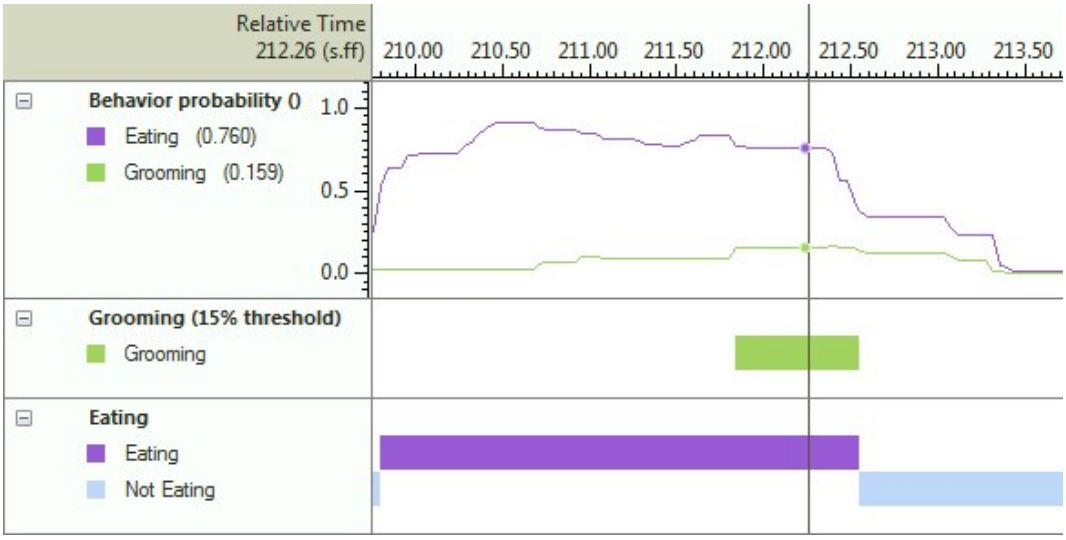



- The **Behavior duration threshold** acts as a filter to ignore short transitions between states. For example, if the behavior Grooming is scored for 0.8 s and you select a Behavior duration threshold of 1.0 s, the Grooming state does not exceed that threshold, and is therefore excluded from analysis.
- For all behaviors but *Twitching* and *Hopping*, the percentage statistics are calculated including *Unknown*. For example, when *Grooming* lasts 30 seconds, *Not Grooming* 1 min 30 s, and *Unknown* 30 s, then the Cumulative Duration within Track (%) for *Grooming* is 30/150 = 20%. If you want to exclude Unknown from the percentage, in the Data profile make two nesting intervals, one with [behavior name] and the other with Not [behavior name], then combine the two Nest boxes in parallel. Next, in the Analysis profile specify the behavior and its statistic, Cumulative Duration

within Nesting (%). In the example above, the result would be 30/120 = 25%.

- The duration of the behaviors may not add up to 100% of the track duration, also when including Unknown. This could result, for example, when you filter a behavior using a probability threshold. When visualizing behaviors, samples filtered out appear as gaps between behaviors.
- When you specify a Behavior probability threshold for one behavior, the other behaviors (which are mutually-exclusive by definition) are not re-calculated according to that setting. Depending on the value of the probability threshold, the behavior may become overlapping with other behaviors. For example, if you select a probability threshold of 15% for *Grooming*, some parts of the track may be scored as *Grooming* where also Eating was scored based on the default settings (see below). To prevent this from happening, either use Default or choose the same probability threshold for all behaviors.

  Below: Event plots of behaviors *Grooming* and *Eating*. Top: Behavior probability. Middle: *Grooming* is defined with a Behavior probability greater than 15%. Bottom: *Eating* is defined with Default probability settings, and is scored for most of the time due to the high probability (see the probability plot). As a result, *Grooming* is partially overlapping with *Eating*, although the two behaviors are supposed to exclude each other. With default settings, *Grooming* would not be scored.




## *See also*

- How behaviors are scored in Behavior recognition

# How behaviors are scored in Behavior recognition

## Basic rules

For each sample in your track, EthoVision XT calculates a probability value for each behavior (see Behaviors detected with Behavior recognition). The sum of probability values is 100%.

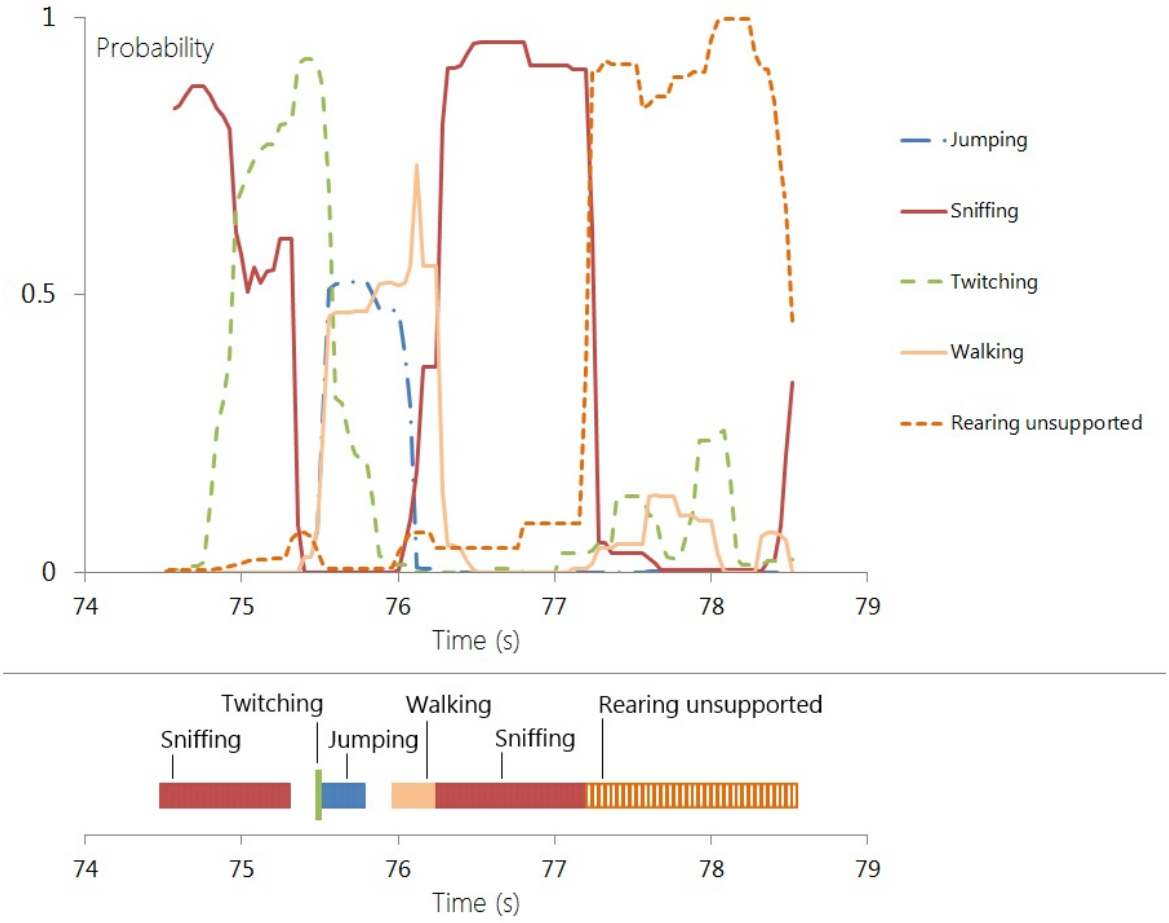
All behaviors but Twitching and Hopping are mutually-exclusive states, that is, only one can be assigned to a sample. A behavior is assigned to a sample based on a number of decision rules that take into account the probability of that behavior relative to that of others, and the behaviors assigned to adjacent samples. See also the figure below.

- *Twitching* and *Hopping* have their own probability, but are not compared with that of the others. As a result, Twitching and Hopping are scored independent of other behaviors. Each may be assigned to a sample also when one of the other behaviors is scored for that sample. For example, *Hopping* when *Walking* is active.
- When two or more behaviors (excluded *Twitching* and *Hopping*) have similar probability values, and the same behaviors are not scored in adjacent samples, or when all behaviors have probability values below 25%, then none of the behaviors are scored for that sample. This results in gaps between scored behaviors when you visualize them in the Integrated Visualization.

## Example

Below you see an example of how behaviors are scored based on their per-sample probability values. Top: X-Y chart of probability (here ranging from 0 to 1) of five behaviors (others are not shown for simplicity). Bottom: The resulting scored behaviors. In general, the behavior is assigned to a sample that has the highest probability (see for example *Sniffing* at the beginning of the time line). However, the state is smoothed based on the values in a number of samples before and after the current sample. For example, at 75 s *Sniffing* has a lower probability than *Twitching*, but the state *Sniffing* is still active. Note that *Twitching* is scored as a point event, with no duration, and is represented with a vertical segment.

The gap between scored states is caused by samples where two or more behaviors have similar probability values (for example at about 75.9 s, with *Jumping* and *Walking* having probabilities around 0.5) or the probability is below 0.25 for all behaviors. The example is taken from Rat Behavior Recognition data.




## Behavior, "Not" Behavior and "Unknown"

Each behavior type, except *Twitching* and *Hopping*, has three complementary, mutually-exclusive states. For example, *Grooming*, *Not Grooming* and *Unknown*.

At any sample, the "Not" behavior is automatically scored if the corresponding behavior is not scored, unless Unknown is scored for that sample (see below).

## Behavior "Unknown"

A behavior type is scored as *Unknown* in the following cases:

- At the beginning of the track if the Trial Control Settings rule does not include an additional Time condition that waits three seconds before the start of tracking, and in the last portion of the track (see Behavior Recognition: Data, performance and accuracy).
- When the nose and tail base points are not detected.

- In all cases when the subject is not found or samples are missed for more than 0.4 s.

Statistics of *Unknown* can be calculated for all behaviors except *Twitching*. The statistics are the same for all behaviors. For example, the duration of *Unknown* calculated for *Grooming* is equal to that of *Unknown* for *Rearing supported*.

# Behavior probability

Each behavior is associated with a probability, calculated per sample. Whether a behavior state is scored for a specific sample depends on a set of decision rules (for details see How behaviors are scored in Behavior recognition).

All behaviors but *Twitching* and *Hopping* are mutually-exclusive, therefore the sum of their probability values is 1. Twitching and Hopping have a probability too, but whether they are scored at a specific time does not depend on the probability of other behaviors.

## How to specify Behavior probability

1. Click the **Add** button next to Behavior probability.
2. In the **Behavior Probability** tab, select the state(s) you would like to calculate the probability for.
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Application

You may want to filter the occurrences of a behavior based on their probability values. If the probability of that behavior is lower than a threshold at a specific sample, that sample is left out and the behavior state is re-calculated (see an example in Behaviors detected with Behavior recognition).

1. In the Analysis profile:
   - Add the behavior you are interested in.
   - Add the Behavior probability variable and, in the window that appears, select the same behavior.
2. Plot the integrated data to view the behavior detected and its probability together with the video. Check how those states correspond with the subject's behavior in the video.
3. To filter data based on probability, in the Analysis profile click the behavior variable, and select a probability value for Behavior probability threshold.
4. Plot the data again to see the result of filtering.

# Merged behavior

You can define a behavior state that includes two or more of the behaviors detected automatically. For example, to calculate the overall frequency of Rearing = *Rearing supported* + *Rearing unsupported*.

## How to specify Merged behavior

1. Click the **Add** button next to **Merged Behavior**.
2. Under Select behaviors to merge, select which behaviors you would like to merge for analysis.

   For example, select Rearing supported and Rearing unsupported to analyze the total occurrences of Rearing.

3. Under **Behavior probability threshold**, select:
   - **Default**: Statistics will be calculated by using all samples scored as that behavior (no filtering based on the state's probability).
   - **Probability greater than**: Statistics will be calculated by using only the samples for which the probability of one of the merged behaviors is higher than a specific value. Select this value from the list. See a note below.
4. Under **Behavior duration threshold**, next to **Exclude behavior instances shorter than**, enter the minimal duration of the merged behavior to be considered for analysis.

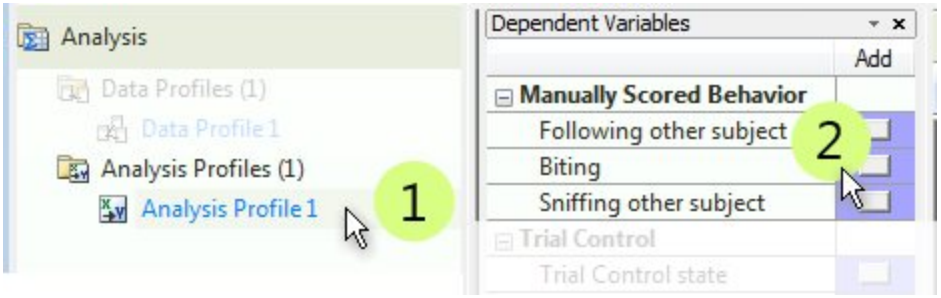
   **NOTE** The threshold is applied to the merged behavior, not the instances of single behaviors. For example, two instances of behaviors Rearing unsupported and Rearing supported follow each other in the time line. The first lasts one second, the other two seconds. When you merge the two behaviors and select a duration threshold of 3 seconds, the two behaviors are considered for analysis.

5. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Note

The Frequency of *Merged behavior* may not always be equal to the sum of the frequencies of the single behaviors. This can happen, for example, when an instance of *Rearing supported* is immediately followed by an instance of *Rearing unsupported*. Then the frequency of *Merged behavior* is 1, not 2.

# Manually scored behavior




## Definition

Behaviors defined in the Manual Scoring Settings are analyzed as dependent variables with discrete states, like *In zone*, *Movement* etc.

## How to specify a manually scored behavior

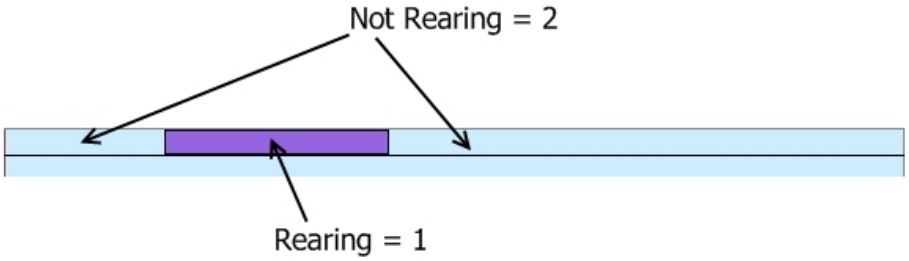
1. Click the **Add** button next to the name of the behavior (or behavior group) you want to calculate.
2. In the tab named as the behavior (or the behavior group), select the behavior you want to calculate statistics for, and clear the options for the behaviors you do not want to include in the table.
   - For *Mutually-exclusive behaviors*, the window lists all behaviors of the group selected by default. The analysis result will show the statistics for each behavior separately. Clear the option for the behavior you do not want to analyze.
   - For *Start-Stop behaviors*, the window lists the behavior (selected by default) and its opposite, indicated by **Not [behavior name]**. Selecting **Not [behavior name]** means that the program calculates the statistics for the time that the behavior was not active (see below).
3. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- **Not [behavior name]:** For example, if you define *Rearing* as a Start-Stop behavior and score it one time during the test (not at the start of the trial), then the results will be (see also the figure below):

  Frequency of *Rearing* = 1.

Frequency of *Not Rearing* = 2. The first occurrence of *Not Rearing* is at the start of the trial, and the second occurrence is after you press the Stop code for *Rearing*.

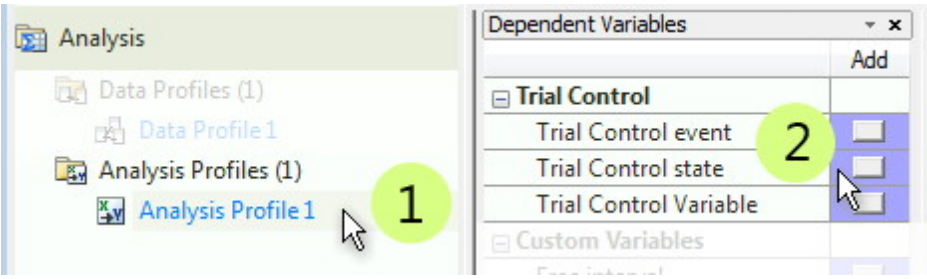



The cumulative duration of *Not [behavior name]* is the sum of all the periods of time that the behavior was not scored.

*See also*

- Export manually-scored behaviors

# Trial Control




- Trial Control event  1117
- Trial Control state  1119
- Trial Control variable  1123

*See also*

For **Hardware** variables related to devices like the Pellet dispenser, the Lickometer, PhenoWheel or the PhenoTyper Top Unit, see the **Analysis of Trial Control data** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual. To open this manual, choose **Apps** > **Noldus** > **EthoVision XT 18 Other Documentation**.

# Trial Control event

## Definition

A point event, with no duration, defined by an element of Trial and Hardware Control (condition, action, rule/sub-rule and reference).

A Trial Control event has no duration. For Trial Control events you can only calculate frequency and latency of first or last occurrence.

## How to specify Trial Control event

1. Click the **Add** button next to **Trial Control event**.
2. From the **Element** list select the Trial and Hardware Control element to be analyzed. For example, if you want to analyze the hardware-base action 'Drop pellet' select Action: Drop pellet.
3. From the **Event** list, select the state of the element. The options available depend on what you have chosen as **Element**.
4. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- Statistics of Cumulative duration and Latency can only be a multiple of the sample interval (=1/sample rate). For example, when you define a condition 'Subject in zone A for >= 3 s', this condition is met when the time elapsed from its activation exceeds 3 s. If the sample rate is 12.5 frames/s (thus the sample interval is 1/12.5= 0.08 s), the condition is met at the first multiple of 0.08 greater than 3 s, that is 3.04 s. This affects data analysis, for example the latency of the event 'Condition becomes true' is 3.04 s
- For Trial Control events based on Conditions, the sample time at which a Trial Control event is scored also depends on the statistic used in the condition:

  If you use Current to define the condition (for example: "when Current In zone= true"), the Trial Control event is scored at the expected sample time. For example, when the animal enters the zone (and thus Current= true for that sample). See also A in the figure in Trial Control state, where the start of the Trial Control state is like a Trial Control event.

  If you use any other statistic to define the condition (for example, "when Frequency of In zone >= 1), consider the following scenarios:

- - When the condition becomes true after the condition box is activated, the Trial Control event is scored at the expected sample time. See How the trial control instructions are executed for an explanation of the terms *true* and *active*.
- - When the condition is already true when the condition box is activated (for example, a condition “Frequency of In zone =1” is activated when the animal is already in the zone), such statistic is only evaluated at the next sample (or in the second next sample, in the case of Heading). In that case, the Trial Control event is scored one sample (or two) later than expected from the condition (see B in the figure in Trial Control state). However, for the consecutive frequencies of In zone =2, 3, etc, the condition is already active by definition; therefore the Trial Control event is scored at the expected time, when the condition becomes true.

*See also*

- The EthoVision XT 18 - Trial and Hardware Control - Reference Manual. To open this manual, choose **Apps** > **Noldus** > **EthoVision XT 18 Other Documentation**.

## Application

You can use *Trial control event* to test whether trial control works as expected. For example, visualize the Trial control event ‘condition true’ and plot it together with the video to check that the condition is met at the correct time. You can also define a *Trial control event* to calculate the number of occurrences of specific actions like dropping a food pellet in a learning experiment.

# Trial Control state

## Definition

A time interval specified by two events of Trial and Hardware Control occurred during the trial. The interval may also occur in two or more instances if the events that mark its start and end occur repeatedly during the trial.

If an interval occurs in more instances during a trial, you can choose to analyze either each occurrence or the sum up the results for all occurrences. See the Calculate statistics per interval option below.

## How to specify a Trial Control state

1. Click the **Add** button next to Trial Control state.
2. Next to **From**, from the **Element** list select the Trial and Hardware Control element that makes the criterion for the start of the interval. From the **Event** list, select the state of that element that makes the start of the interval.
3. Next to **To**, from the **Element** list select the Trial and Hardware Control element that makes the criterion for the end of the interval. From the **Event** list, select the state of that element that makes the end of the interval.

   Choose which occurrence of the ending event you want to consider.
4. Select **Ignore last interval if incomplete** to ignore the interval when the Trial Control event that defines the end of the state is not found. If you do not select this option, and the end criterion is *not* met, EthoVision XT defines an interval up to the end of the trial.

   **EXAMPLE** A Trial Control state is defined “from Trial start to when the condition “Subject in zone A” becomes true. If the subject never enters zone, then the end criterion is never met. If you select the Ignore option, the Trial Control state is not defined. If you do not select the option, the Trial Control state is defined from the start to the end of the trial.
5. An interval may occur several times in a trial. If your want to have statistics for each occurrence, select the **Calculate statistics per interval** option. Next to **For consecutive intervals**, choose the range of occurrences you want to have in the results.
6. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- Do not select **Calculate statistics per interval** when you want to sum up the results from the occurrences of the state interval in the trial. For example, to calculate the cumulative duration of the state From condition 'In Cue zone' becomes true To condition 'In Feeder zone' becomes true.
- Statistics of duration and latency can only be a multiple of the sample interval (=1/sample rate). For example, when you create a condition 'Subject in zone A for >= 3 s', this condition is met when the time elapsed from its activation exceeds 3 s. If the sample rate is 12.5 frames/s (thus the sample interval is 1/12.5= 0.08 s), the condition is met at the first multiple of 0.08 greater than 3 s, that is 3.04 s. This affects data analysis, for example the duration of the state 'From condition active to condition true' is 3.04 s.
- The Frequency of a Trial Control state is determined by the start of the state. This means that at the end of a trial, a Trial Control state is counted even if there is no stop event.

## Trial Control states based on Conditions

The sample time at which a Trial Control state starts (or ends) also depends on the statistic used in the condition in the Trial Control rule.

- If you use **Current** to define the condition (for example, "when Current In zone= true"), the Trial Control state starts (or stops) at the expected sample time. For example, when the animal actually enters the zone (and thus Current= true for that sample).
- If you use any other statistic to define the condition (for example, "when **Frequency** of *In zone* >= 1) consider the two scenarios:
  - When the condition becomes true after the condition box is activated (see How the trial control instructions are executed for an explanation of the terms *true* and *active*), the Trial Control State starts (or stops) at the expected sample time (see A in the figure below).
  - When the condition is already true when the condition box is activated (for example, a condition "Frequency of In zone =1" is activated when the animal is already in the zone), such statistic is only evaluated at the next sample (or in the second next sample, in the case of Heading). In that case, the Trial Control state starts (or stops) one sample (or two) later than expected from the condition (see B in the figure below). However, for the consecutive frequencies of In zone =2, 3, etc, the condition is already active by definition; therefore the Trial Control State starts at the expected time, when the condition becomes true.

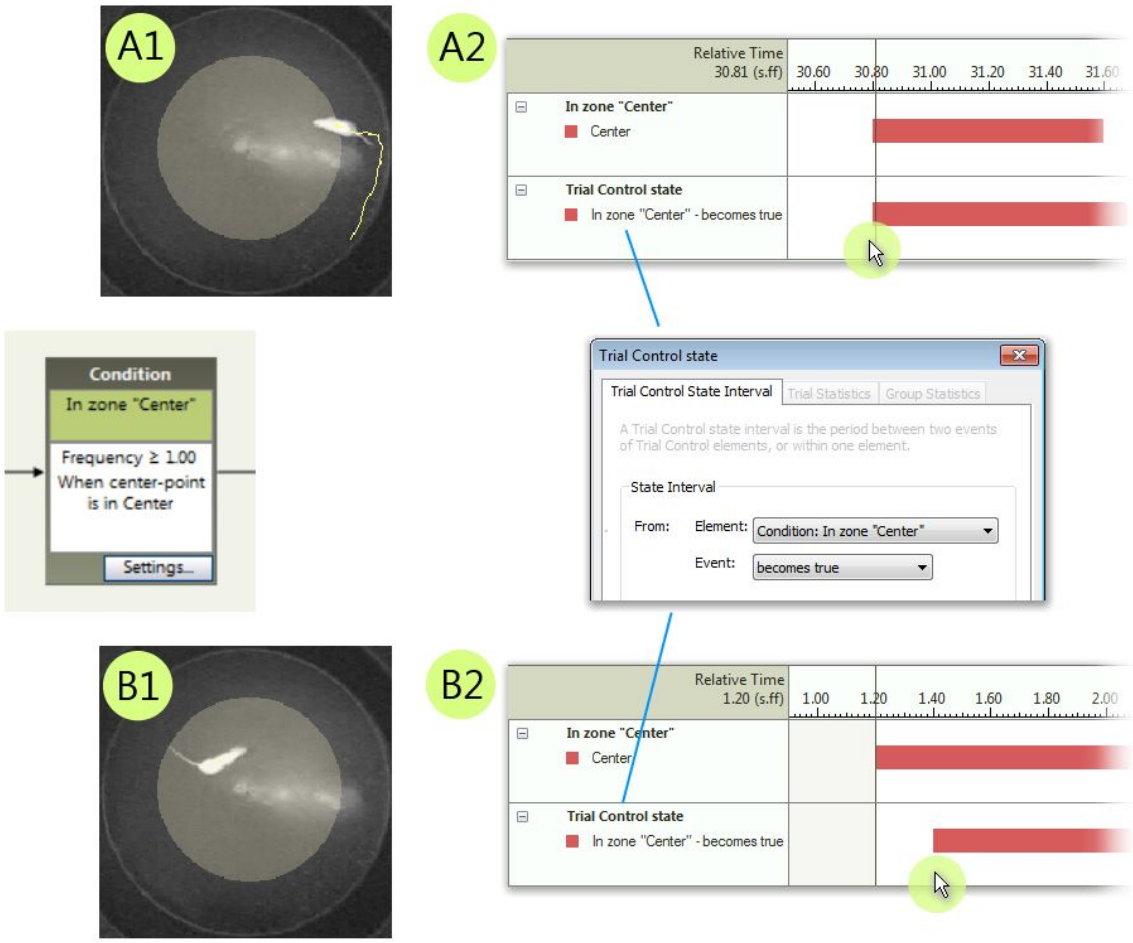
**EXAMPLE** A Trial Control condition has been defined in the Trial Control Settings, which checks that the statistic **Frequency** of the dependent variable *In zone* is >=1

for the Center of the open field. A Trial Control state has been defined in the Analysis profile, which starts when the condition “Frequency of In zone >=1” is true.

A1: The animal enters the Center zone (and therefore the condition becomes true) after the condition is activated. The Frequency statistic is evaluated at the same sample time. A2: Plot of the variables *In zone* for the Center and the Trial Control state. The Trial Control state starts at the expected sample time, that is, when the animal actually enters the zone.

B1: The animal is already in the zone when the condition is activated. Therefore, the condition becomes true at the same time that it is activated, but the Frequency statistic is evaluated at the next sample (here, sample rate = 5/s, thus after 0.2 s).

B2: Same plot as A2; Here, the Trial Control state starts o.2 s after the *In zone* state.



*See also*

- The EthoVision XT 18 - Trial and Hardware Control - Reference Manual. To open this manual, in the Apps screen under **Noldus** choose **EthoVision XT 18 Other Documentation**.

## Application

You can use Trial control states to test whether trial control works as expected, and for analyzing learning behavior. For example, calculate the duration of the Trial control state 'From Cue light ON to Subject in Feeder zone' to see whether this interval decreases during a trial.

# Trial Control variable

## Definition

A variable defined in the Trial Control settings. See Actions > To define a Trial Control variable

You may want to analyze or visualize the values of a Trial Control variable. For example, you defined a variable named *Counter*, and:

- You want to calculate the maximum value that the variable reached during different trials.
- You want to visualize its values plotted against time.

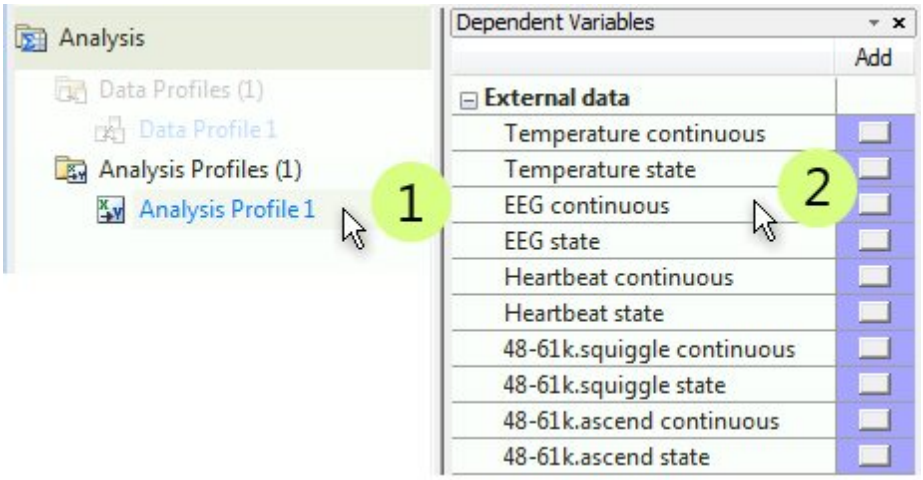
## How to specify a Trial Control variable

1. In the Analysis profile, click the **Add** button next to **Trial Control variable**.
2. From the **Element** list, select the variable you want to analyze.
3. Complete the procedure to add the variable. See Calculate statistics: procedure.
4. To visualize the variable on the timeline, choose the Integrated Visualization.

## Notes

- If you want to calculate the time that the Trial Control variable had a specific value, in the Analysis profile choose **Multi-condition** instead. There you can select the Trial Control variable and define the selection criteria. See Multi condition
- If you want to calculate the time based on a From - To criterion, choose **Free Interval** instead. There you can define, for example an interval *From* Trial Control variable = 10 *to* Trial Control Variable = 15. See Free interval

# External Data




- External data (resampled) 1125
- External data - state 1129

# External data (resampled)

## Definition

EthoVision XT only analyzes external data after *resampling*. That is, it does not analyze the original data, but a Dependent variable representing the imported signal, resampled to the same sample rate as the EthoVision XT sample rate. This is accomplished by combining upsampling and downsampling (see below).

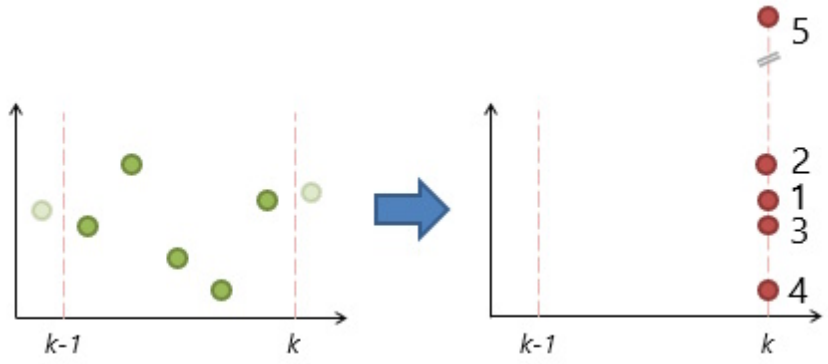
## Downsampling and Upsampling

### *Downsampling*

If the sample rate of the external data signal is higher than the EthoVision XT sample rate, the signal is downsampled. For each sample *k*, a new value is calculated from the values of the original signal in the interval (*k-1*; *k*] (or in multiple preceding sample intervals, depending on the Averaging interval chosen). The interval is half-closed, that is, all values of the signal between *k-1*, (not included) and *k* are considered. The new value gets the time stamp of the sample *k*.

Five methods are available to downsample the signal.

Below: Effect of downsampling of an external data signal. Left: original signal imported in EthoVision XT. Vertical hatched lines represent the EthoVision XT sample intervals. Right: Signal upsampled in EthoVision XT using the following five methods (for simplicity, only the values for sample *k* are shown). **1** Last value, **2** Maximum, **3** Mean, **4** Minimum, **5** Total value.

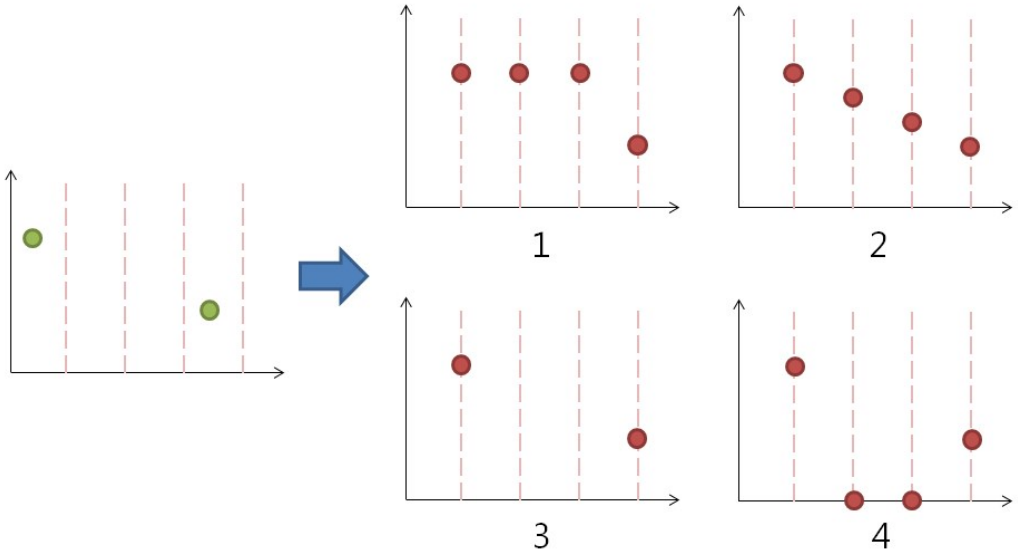



### *Upsampling*

If the sample rate of the external data signal is lower than the EthoVision XT sample rate, the signal is upsampled.

For each sample *k*, a new value is calculated from the values present in the sample interval (*k-1; k*] (or in multiple preceding sample intervals, depending on the Averaging interval chosen). If no value is found, A new value is interpolated using one of the methods available, or replaced by "missing sample" or a zero value.

Below: Effect of upsampling of an external data signal. Left: original signal imported in EthoVision XT. Vertical hatched lines represent the EthoVision XT sample intervals. Right: Signal upsampled in EthoVision XT in the following four methods: **1** Last value, **2** Linear interpolation, **3** Missing value, **4** Zero value.




## Averaging interval

With Averaging interval you can smooth the values of the converted signal. This has an effect on both resampled and state variables.

- With Averaging interval 1, the new value for sample *k* only depends on the original values of the signal in the interval (*k-1, k*].

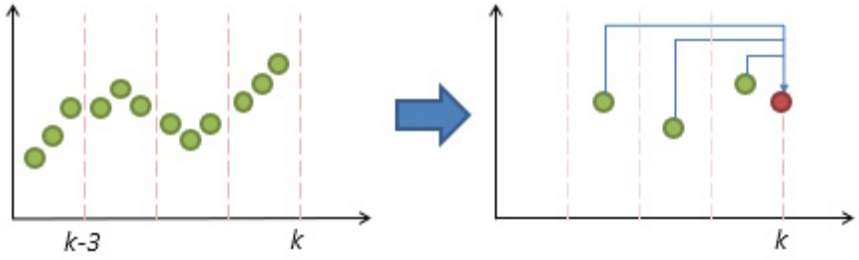
  **EXAMPLE** Downsampling a EEG signal with the Mean value method and the Averaging interval of 1. See **3** in the figure under Downsampling.

- With Averaging interval x (where x=2, 3,...), the new value for sample *k* depends on the original values of the signal in the interval (*k-x, k*].

  **EXAMPLE** Downsampling a EEG signal with the Mean value method and the Averaging interval of 3.

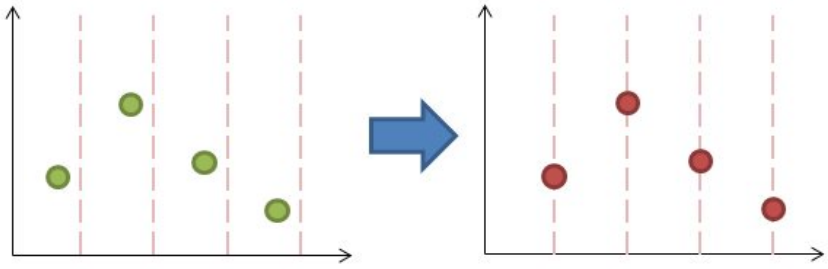
  Left: Original EEG signal. Right: Signal downsampled with Mean value and Averaging interval 3 (for clarity only sample *k* is shown). Green dots:

averages in the 3 sample intervals. Red dot: the average of the three averages.




## Special case: Resampling

If the sample rate of the external data signal is the same as the EthoVision XT sample rate, but with different time stamps, the signal is re-sampled to align its values to the EthoVision XT samples. Values are shifted to the right.



## How to specify an external data variable (resampled)

1. Click the **Add** button next to the [data set name].
2. Under **Select**, choose the **Downsampling method** (default: **Mean value**) and the **Upsampling method** (default **Last value**).
3. Under **Outlier filter**, select the **Averaging interval** (default: 1).
4. Complete the procedure to add the variable. See Calculate statistics: procedure.
5. Plot Integrated Data or calculate the statistics.

## Notes

- If you upsample a signal with a sample rate much lower than the EthoVision sample rate, and you choose **Missing value** as the **Upsampling method**, it

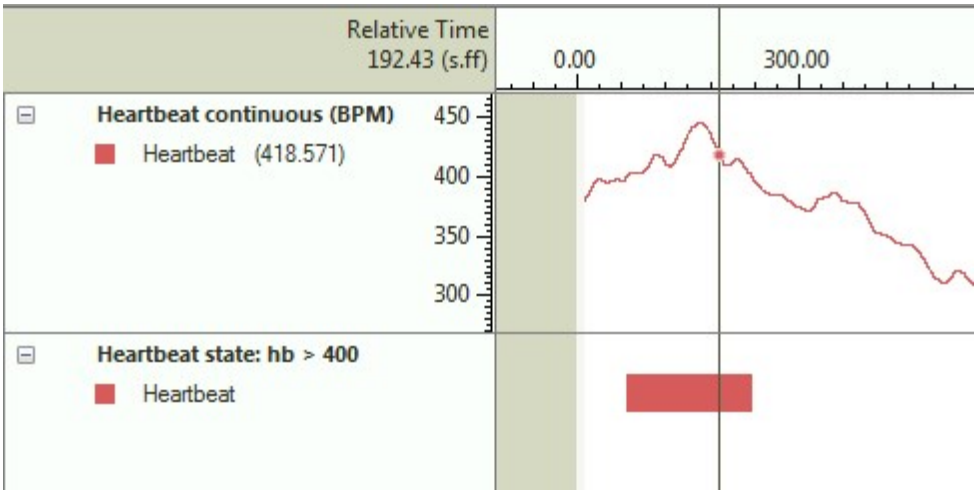
may be difficult to see the data points. In Integrated Visualization, choose **Show**/**Hide** > **Show Graph Data Points** and zoom in the plot.

- See also Averaging interval

# External data - state

## Definition

Dependent variable with two possible values (0 and 1) calculated from an imported data signal, resampled to the same sample rate as the EthoVision XT sample rate. The value of the state variable is calculated per sample from the values of the corresponding resampled variable, relative to one or two thresholds.




Top: Heartbeat. Bottom: Heartbeat state, defined in the Analysis profile as "Heartbeat > 400 bpm, with Downsampling= Mean value, Upsampling= Last value, Averaging interval =1". For clarity, it was renamed to **Heartbeat state: hb >400**.

## How to specify an external data variable "state"

1. Click the **Add** button next to the [data set name] state.
2. Under **Select**, choose the Downsampling method (default: Mean value) and the Upsampling method (default Last value).
3. Under **Outlier filter**, select the **Averaging interval** (default: 1). See Averaging interval in External data (resampled).
4. Under **State threshold**, specify that the state is scored when the **Signal is**:
   - above (>=) or below (<=) a threshold value x.
   - within a range (>= $x_1$ and <= $x_2$) or outside a range (<= $x_1$ and >= $x_2$).

   Enter the thresholds you require.
5. Complete the procedure to add the variable.
6. Plot Integrated Data, or calculate the statistics.

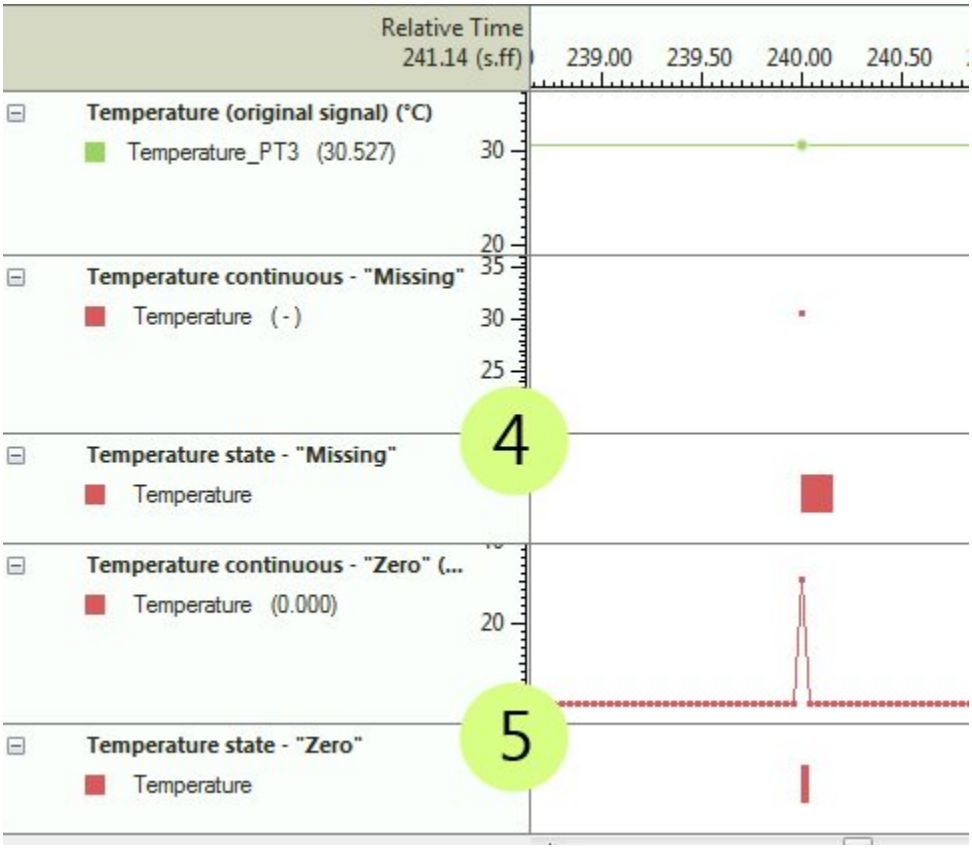
The resulting state (frequency and duration) is much dependent on what you choose as downsampling/upsampling methods.

Below: Effect of different upsampling methods on a state variable Temperature state. **1** Original temperature signal sampled every minute. **2**-**5** Variables resampled at 25 Hz. The state variable is scored when the resampled value is >= 30 ºC, using the methods Last value (**2**), Linear interpolation (**3**), Missing value (**4**), and Zero value (**5**). Note that Temperature state in cases 4 and 5 gives very short states (indicated by the arrows), because most of the data points in the corresponding Temperature resampled variable are missing or zeros, respectively. See the next picture for (**4**) and (**5**).


**NOTE** When calculating states, if the sample with a valid variable value is followed by missing samples, EthoVision XT keeps the calculated value of the state for the next three missing samples.

Below: Zoomed-in view of the previous picture for Temperature state upsampled with Missing value (**4**), and Temperature state upsampled with Zero value (**5**),

around 240 s. A state is scored when the resampled value is >= 30 ºC. For simplicity, Averaging interval = 1. When calculating states, if the value of the resampled variable is followed by missing values, like in Temperature - “Missing”, the calculated value of the state is kept for next 3 samples. This is the reason why the Temperature state - “Missing” around time 240 s lasts 4 samples (see **4**). In the case of upsampling with Zero value, the state is assigned only to the current sample.




## How to retrieve states from an imported signal

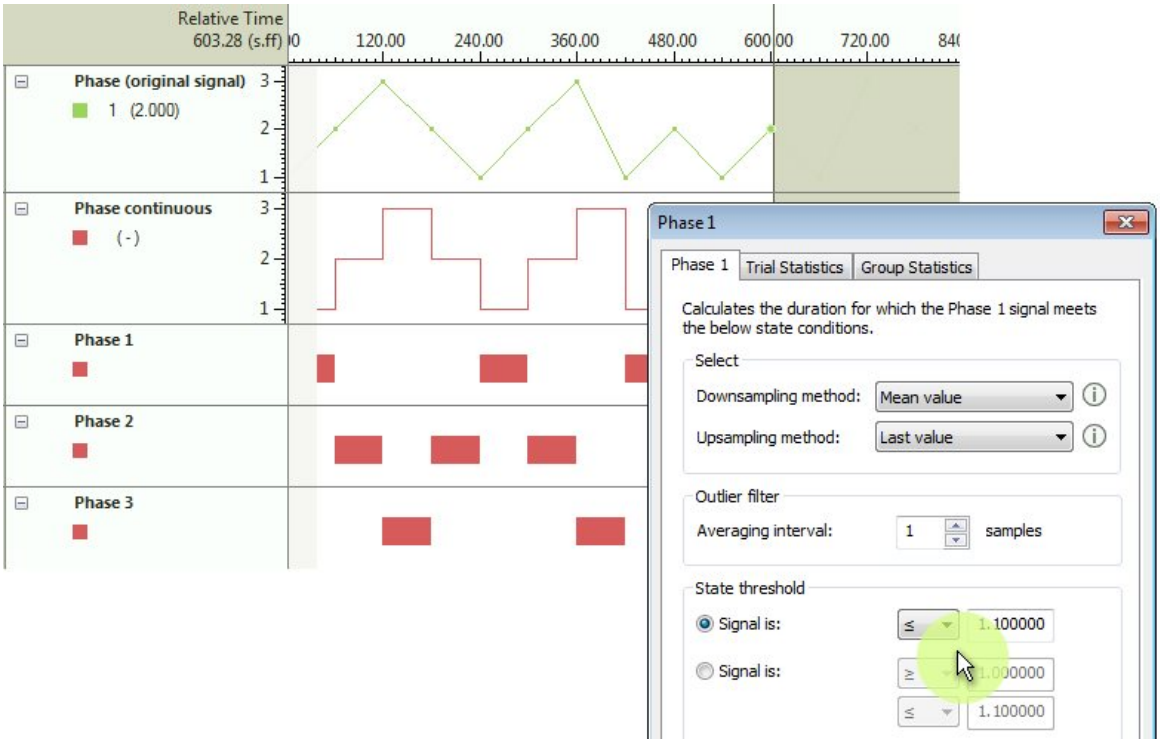
In the following example, the file containing the data set Phase with possible values 1, 2 and 3 is imported:

```
Start date; 12/18/2012
Start time; 11:31:55.5
Phase_PT5
time; Phase
s; Value
0;1
60;2
120;3
180;2
240;1
300;2
360;3
420;1
480;2
```

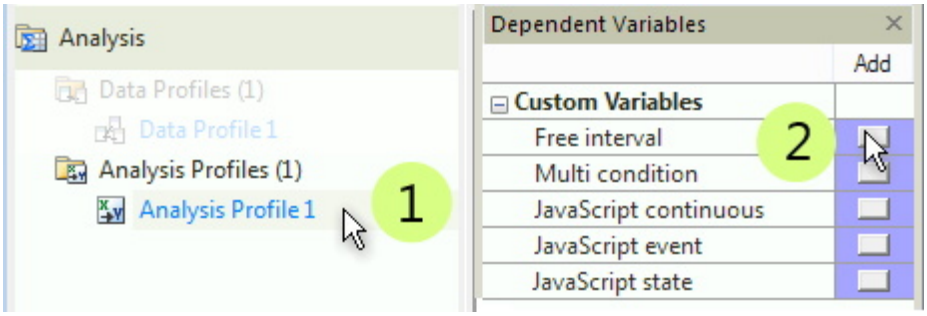
If you want to retrieve the original states with their duration:

1. In the Analysis profile, define three Phase state variables, and rename them to, for example, Phase 1, Phase 2, and Phase 3.
2. For each state variable, set the appropriate thresholds. For example, for Phase 1: **Signal is <=** 1.5.
3. Plot the variables to compare them with the original signal.

From top to bottom: original signal, resampled signal, states calculated using thresholds. Right: threshold for Phase 1.



# Custom Variables




- Free interval
- Multi condition
- JavaScript continuous
- JavaScript event
- JavaScript state

*See also*

- JavaScript custom variables
- Commands and functions for JavaScript variables

# Free interval

## Definition

A segment of track from sample A to sample B, where A and B are determined by a time, the value of another dependent variable, a Trial Control event, or the value of a signal from a hardware device.

## Use cases

Frequent cases:

- Group instances of behavior (e.g. Immobile) in a bout.
- Filter instances of behavior (e.g. Not moving) longer than one second.

See Examples of Free intervals in the Analysis profile

## Calculation

A Free interval is defined by a *Start criterion* and a *Stop criterion*. Depending on whether and how often the criteria are met within a track, a Free interval may result in zero, one or more *instances* of the interval within the same track.

**NOTE** Like all intervals defined with Nesting, a Free interval is left-closed [A, B). That is, the start sample A, not the stop sample B, is included in the analysis.

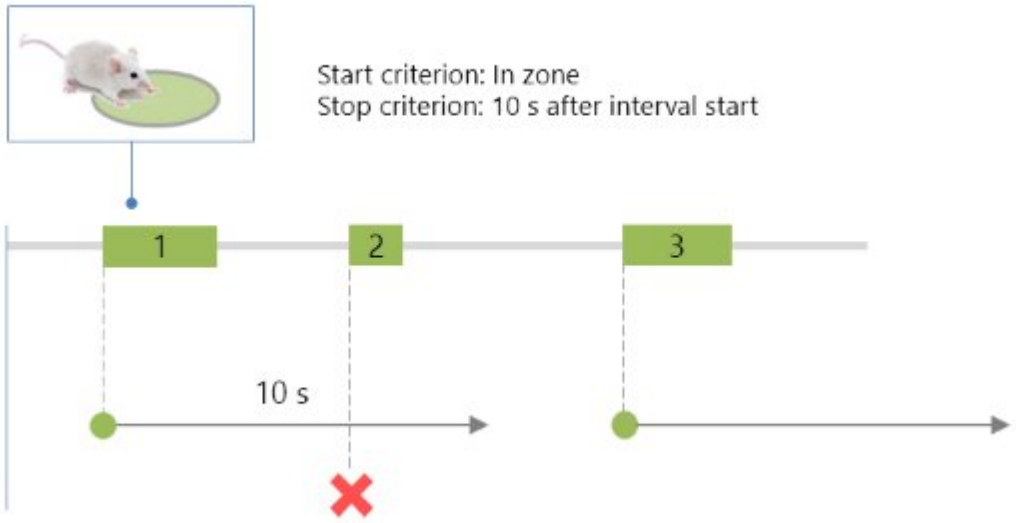
### *Multiple instances of the same interval definition*

When you define a Free interval, this may result in zero, one or more (up to 2000) instances of an interval depending on where the Start and Stop criteria are met.

An instance of a free interval starts at the sample time when the Start criterion is met, and stops at the sample time when the Stop criterion is met.

A new instance is defined if the Start criterion and Stop criterion are met again, after the time in the track that the last Stop criterion was met.

**NOTE** Multiple instances of the same free interval definition cannot overlap. An interval is NOT defined when its start criterion falls within a previous instance of the same interval definition. For example, you define a Free interval that starts when the subject enters zone A, to 10 seconds after that time. If a new instance of *In zone A* occurs before the end of the interval (see the green bar marked with 2 in the figure below), that instance of In zone is ignored.




Similarly, if you define a Free interval that goes from track start to when the external data *Heart rate* > 400, then one interval is defined, from Track start to the time when Heart rate reaches 400 bpm for the first time. This because the "Track start" criterion is met once in each track, no matter how many times Heart rate reaches 400 bpm.

### *Multiple free intervals in the Analysis profile*

You can define multiple intervals in the Analysis profile, by repeating the procedure below. The interval definitions are independent of each other, therefore the resulting intervals may be overlapping.

## To specify a Free interval

1. In the Analysis Profile, under **Custom Variables**, click the button next to **Free Interval**.
2. Under **Start criterion** select the starting point of the interval:
   - **Time**. To select a time. For example, **Track start** (that is, the first sample in a track) or an **Elapsed time** after the track start (format: H:mm:ss.fff). For example 0:01:00.00 for one minute after the start. **NOTE** Track start corresponds to the first sample in the track (Recording time = 0.0), not the Trial time. For multiple-subject setups, Track start is the first sample of the animal detected first.
   - **Dependent Variable**. To select the time that a dependent variable has a specific value. For example, *Distance moved* >= 1 m, or frequency of *In zone* >=10. This list also includes behaviors scored manually and external data. See Free interval based on a dependent variable

- **Trial Control**. To select the time that an event in your trial control procedure occurs. For example, when a condition becomes true, or a command to a hardware device is given. See Free interval based on a Trial Control event
- **Hardware**. To select the time that a Hardware-related variable has a specific value. For example, when the number of food pellets dropped by a Pellet Dispenser is greater than 10. This option is only available when hardware devices are defined in your experiment. See Free interval based on hardware

3. Under **Stop criterion** select the end point of the interval. See above for most of the options.

   If you choose **Time**, and under **Interval stop** you choose **Elapsed time**, you have two options: **after track start** to stop the interval at a specific time after the track start (that is, the first sample in the track); **after start event** to stop the interval a specific time after the event selected as start criterion. Note that this "start event" is not the same as the start time of the resulting interval, if you set some time before the event (for example, 10 s before the frequency of *In zone* becomes 1). The "start event" is when the Frequency of *In zone* becomes 1. However, the interval starts 10 s earlier.

4. Select **Ignore last interval if incomplete** to ignore the interval when the stop criterion is not met. If you do not select this option, and the stop criterion is not met, EthoVision XT defines an interval up to the end of the track.

5. A free interval may occur once or more in a track (see above). To analyze each instance separately, select **Calculate statistics per interval** and enter the number of intervals you want to view in the results (1 to 2000). To be sure you to view all intervals, enter a large number that exceeds a maximum possible number of instances per track. See Restrictions in EthoVision XT

   To analyze all instances as one, de-select **Calculate statistics per interval**.

6. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- Note the difference between Free Interval in the Analysis Profile (this topic) and Nesting over a Free interval in the Data Profile.
  - Define a Free Interval in an Analysis Profile when you want to analyze the interval itself, not other dependent variables within that interval. For example to calculate the duration of an interval.
  - Define a Free Interval in a Data Profile when you want to analyze any dependent variable *within the resulting track segment*. For example, to calculate the average velocity of the subject in the interval.

- If a start criterion is not met in a track, EthoVision XT does not define the Free intervals in that track.
- If a stop criterion is not met in a track, EthoVision XT does the following:
  - If you selected **Ignore last intervals if incomplete**, it does not define the interval.
  - If you did not select that option, it defines an interval up to the end of the track.
- *Free intervals in multiple-subjects setups*. A free interval is calculated for each subject separately, based on the data (dependent variables, behavior etc.) for that subject. See Free interval based on multiple subjects
- *Free intervals and missing samples*. A free interval is not interrupted if one, two or three consecutive missing samples occur. With four or more consecutive missing samples, the current interval is interrupted and a new one starts when the Start criterion is met.
- *Contiguous intervals*. When two instances of a Free interval are contiguous, for example when the Interval Start criterion is supposed to be met immediately after the Interval Stop criterion for the previous instance is met (see Example 3 in Examples of Free intervals in the Analysis profile, where a track is split in segments of equal path length), one sample is always excluded between the end of an instance and the start of the next instance. For example, the first instance goes from time 0 (included) to sample 100 (excluded), then from sample 101 (included) to sample 250 (excluded). Samples 100 and 250 are not included in the analysis. The reason is that the statistic used to find the start of the interval is updated one sample after the end of the previous interval.

*More details*

- Free interval based on a dependent variable
- Free interval based on a Trial Control event
- Free interval based on hardware
- Free interval based on multiple subjects
- Examples of Free intervals in the Analysis profile

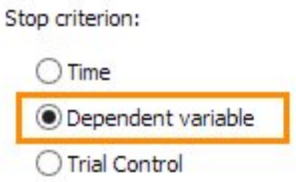
# Free interval based on a dependent variable

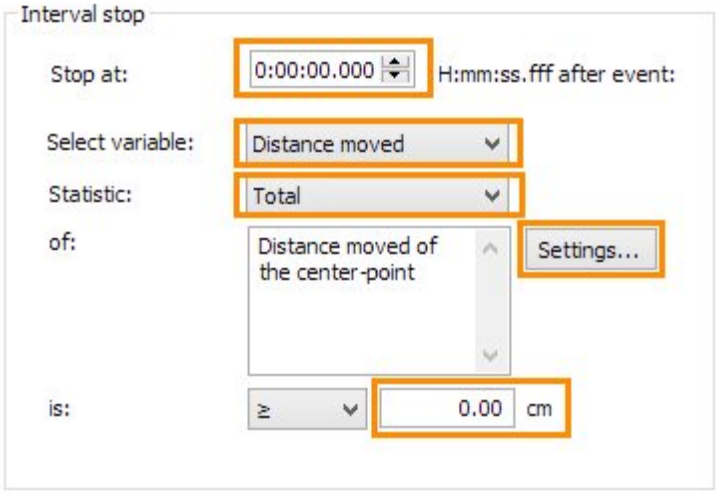
## Background

This topic contains details about defining a Free interval. For the main procedure, see Free interval.

**NOTE** Dependent variable here also includes external data and manually-scored behaviors, not behaviors of Rat and Mouse Behavior Recognition.

## To specify the dependent variable




In the Free Interval window, under **Start criterion** or **Stop criterion**, choose **Dependent variable**.

1. In the **Start at** / **Stop at** field, enter the time of the start (or stop) of the interval relative to the event described by the variable.

   **EXAMPLE 1** To start an interval 1 s before the animal enters a zone, next to **Start at** enter 0:00:01:000 **before event**.

**EXAMPLE 2** To stop an interval 10 s after the total distance moved reached 10 m, next to **Stop at** enter 0:00:10:000 **after event**.

2. From the **Select variable** list, choose the dependent variable.

   For example **In zone** if you want the interval to start or stop when the animal enters a zone for the nth time, or has spent a specific time in that zone.

3. From the **Statistic** list, choose the statistic of the dependent variable. For example:

   - **Frequency** if you want the interval to start/stop when the animal has entered a zone for the 10th time,
   - **Cumulative Duration** if you want the interval to start/stop when the animal has spent a specific total time in that zone.

   See Statistic: When to use Frequency, Current, or others

4. Click the **Settings** button to specify the dependent variable more in detail.

   For example, which zone the animal should enter to define the stop of the interval, or which body points should be in the zone. See Dependent Variables in Detail

5. Next to **is:**, specify which value the statistic should have to define the start/stop of the interval, using the operators available (**<=**, **>=** or **false**/**true**).

Return to To specify a Free interval

## Notes

- The following dependent variables are not available as start/stop criteria: *Activity state*, *Acceleration state*, and the behaviors of Rat or Mouse Behavior Recognition.
- A Free interval can only start at the time that, or *before* (not after) a variable reaches the specified value. Similarly, a Free interval can only end at the time that, or *after* (not before) a variable reaches the specified value.
- If you have imported external data in an experiment, you can define an interval based on the values of the resampled external data, not the original (imported) external data. Choose the external data from the **Select variable** list.

  See External data (resampled) and Examples of Free intervals in the Analysis profile

- If you want to define a free interval based on two values of external data, for example Heart rate between 300 and 400 bpm) then do not use Free

intervals. Instead, use the dependent variable under External data in the Analysis profile.

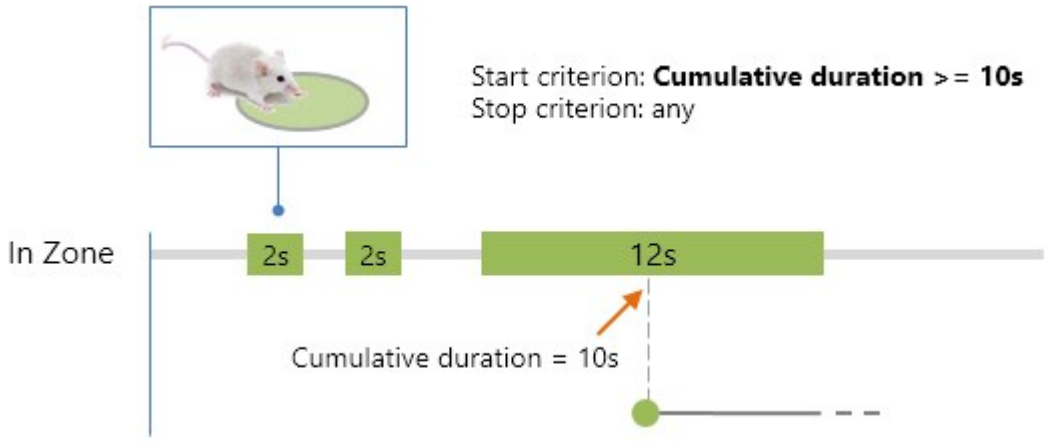
## Statistic: When to use Frequency, Current, or others

- **Frequency**. To define an interval that starts/stops when the animal has entered a zone or has shown certain behavior (*Moving*, *Highly mobile*, *Grooming*, etc.) a number of times.
- **Current**. To define an interval that starts/stops when a variable reaches a specific value. For discrete variables (state events or point events) like In zone, the Current statistic can be false or true.

  **EXAMPLES**

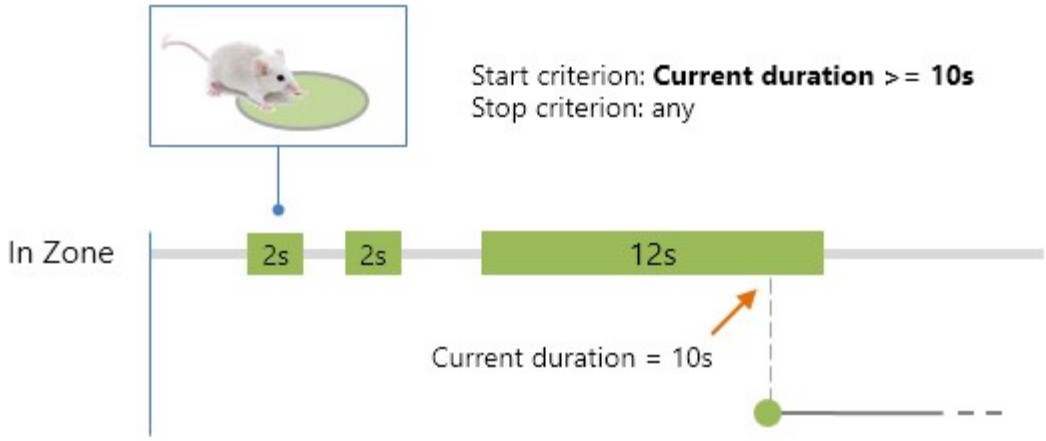
  - For *In zone*, Current= true means that the interval starts/stop as soon as the animal enters the selected zone. Current= false means that the interval starts/stops as soon as the animal is outside the selected zone.
  - For *Velocity*, Current >= 10 means that the interval starts/stops as soon as the animal's velocity is higher then or equal to 10.
- **Total**. This is used especially with *Distance moved*. This makes the interval starts/end when the animal has covered a specific distance since the start of the track or the start of the interval.
- **Cumulative Duration**. To define an interval that starts/stops when the animal has been in a certain state (that is, within a specific zone, or in the state *Highly mobile*, etc.) for a specific total time since the start of the track or the start of the interval. It does not matter if the subject's state was sometimes interrupted.

  **EXAMPLE** The interval starts when Cumulative duration of *In zone* >= 10 s. The green dot indicates where the interval starts.



- **Current Duration**. To define an interval that starts/stop when the animal has been in a certain state (within a specific zone, or in the state *Moving*, etc.) for a specific time without interruption.

  **EXAMPLE** The interval starts when Current duration of *In zone* = 10 s. The green dot indicates where the interval starts.




- **Latency to first**. To define an interval that starts/stops when the time to the first instance of an event (for example, the animal being in a certain state or within a zone) has reached a specific value. Note that latency is always calculated from the start of the track.

## How dependent variables are calculated to find the stop of an interval

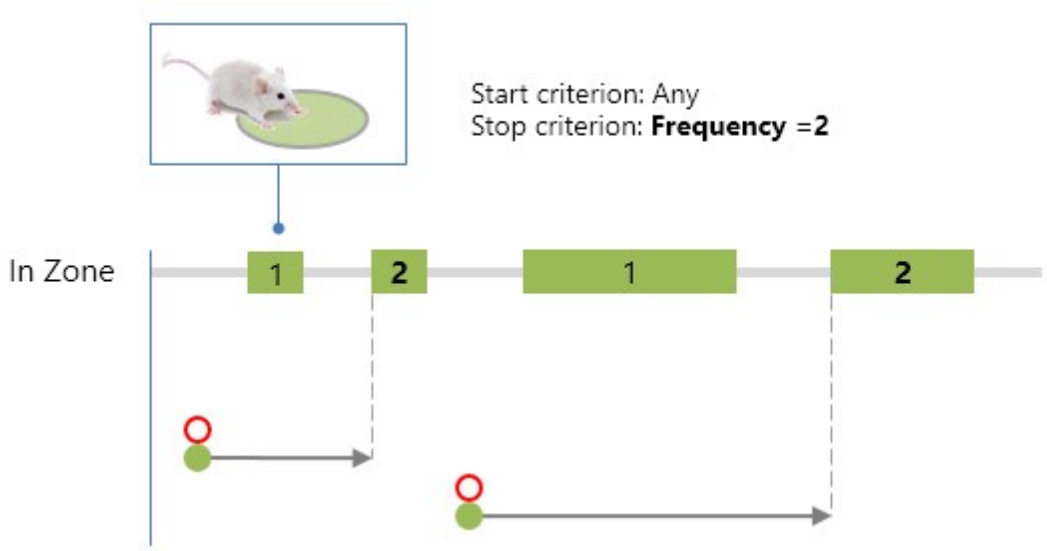
Where in the track an interval ends depends on the value of the statistic of the dependent variable used. The following information applies to the statistics Total, Frequency, Current Duration, Cumulative Duration, and Latency to first.

Once the start point of an interval is found, EthoVision XT resets the statistic at that point, and starting from the next sample time, updates its value until it matches the stop criterion.

- If the stop criterion is met, the interval is fully defined.
- If the value of the statistic does not match the stop criterion before the end of the track, the result depends on whether you select the option **Ignore last interval if incomplete**. See Free interval

**EXAMPLE 1** The statistic Frequency for the dependent variable In Zone. A free interval is defined that ends when the Frequency of *In zone* is equal to 2. In the figure below, the green bars represent the time when the subject is in the specified zone (numbers indicate frequency). The green dots indicate when the Start criterion

is met (for sake of simplicity, it can be any criterion). The arrows represent the free intervals defined. The red circles show when the statistic Frequency for *In zone* is reset to zero.




**EXAMPLE 2** The statistic Total for the dependent variable Distance moved. A recurring free interval is defined to split the track in segments of 100-inch distance moved.

- Interval start: Dependent variable Distance moved, Statistic Total >= 0.
- Interval stop: Dependent variable Distance moved, Statistic Total >= 100.

When the second, third, etc. interval starts, the total Distance moved is reset to zero (see the red circles) and updated, starting from the next sample time, until the Stop criterion is met.


*Other statistics*

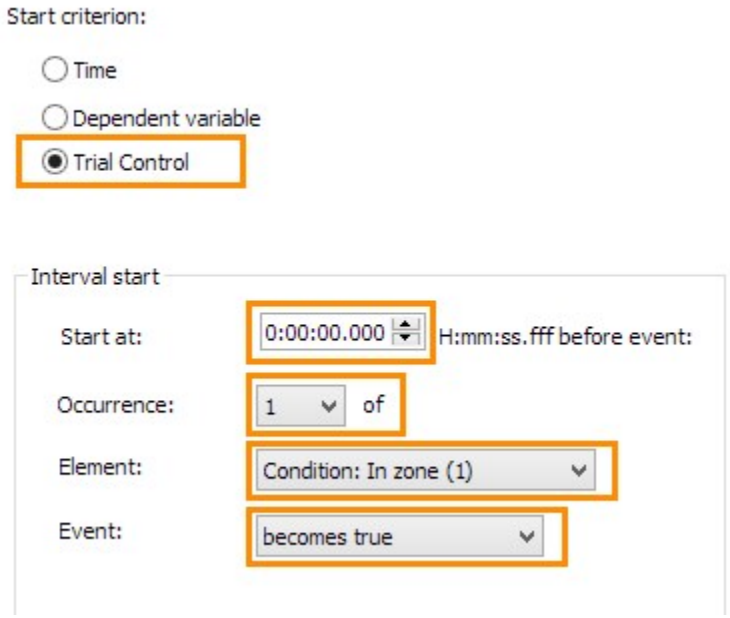
For other statistics like Current (for example In zone or Not in zone) EthoVision XT evaluates the value at the current sample, and checks that the Stop criterion is met.

# Free interval based on a Trial Control event

## Background

This topic contains details about defining a Free interval. For the main procedure, see Free interval.

## To specify the Trial Control event




In the Free Interval window, under **Start criterion** or **Stop criterion**, choose **Trial Control** and select the following:

1. In the **Start at** / **Stop at** field, enter the time of the start (or stop) of the interval relative to the Trial Control event specified in the next steps.

   **EXAMPLE 1** To start an interval 1 s before a Trial Control condition becomes true, next to **Start at** enter 0:00:01:000 **before event**.

   **EXAMPLE 2** To stop an interval 10 s after the Trial Control action "Drop pellet", next to **Stop at** enter 0:00:10:000 **after event**.

2. From the **Occurrence** list, select which occurrence should mark the start of the interval (1st to 9th).

Select **1st** if you want to define an interval from the first time that a trial control event occurs. Select **2nd**, etc. when the trial control event occurs repeatedly, for example in sub-rules.

3. From the **Element** list, select the trial control category (condition, action, sub-rule, etc.).
4. From the **Event** list, select the event. The options differ depending on the Element chosen.
   - For actions, sub-rules: **becomes active/inactive**.
   - For conditions: **becomes active/inactive**, and **becomes true/false**.
   - For sub-rule references: **becomes active/inactive**, and **makes sub-rule active/inactive**.

Return to To specify a Free interval

## Notes

- A Free interval can only start at the time that, or *before* (not after) a Trial Control event occurs. Similarly, a Free interval can only end at the time that, or *after* (not before) a Trial Control event occurs.

*See also*

- For information on the meaning of active/inactive, false/true, see How the trial control instructions are executed.
- For information on sub-rules and analysis of trial control data, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

## Difference between Free Intervals based on Trial Control and Nesting over a Trial Control state

With Free intervals you can select the same track segments that you can define with Nesting over a Trial Control state in the Data profile. The main differences are that:

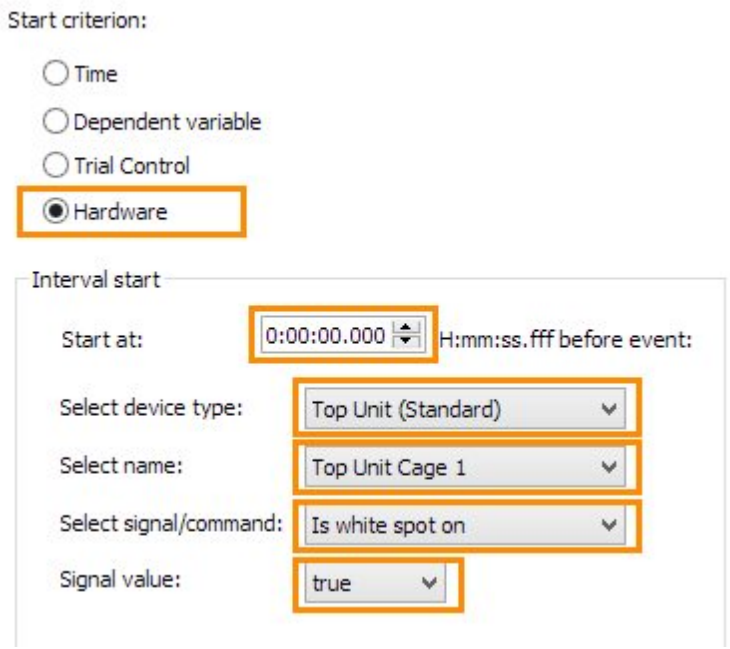
- With a Free interval, you can also specify the number of occurrences of an event in the Interval start criterion. For example, define an interval that starts when an action is carried out the 3rd time. With Trial Control state that is not possible. There, you can only specify the number of occurrences in the Interval stop criterion.
- With a Free interval, you can combine Trial Control events with other criteria (time, dependent variable values, hardware log values). For example, define an interval that goes from the time a light switches on, to 1 minute after that time.

# Free interval based on hardware

## Background

This topic contains details about defining a Free interval. For the main procedure, see Free interval.

## To specify the hardware state or value




In the Free Interval window, under **Interval start** or **Interval stop**, choose **Hardware** and select the following:

1. In the **Start at** / **Stop at** field, enter the time of the start (or stop) of the interval relative to the Hardware event specified in the next steps.

   **EXAMPLE 1** To start an interval 1 s before the PhenoTyper's white light switches on, next to **Start at** enter 0:00:01:000 **before event**.

   **EXAMPLE 2** To stop an interval 10 s after a pellet was dropped, next to **Stop at** enter 0:00:10:000 **after event**.

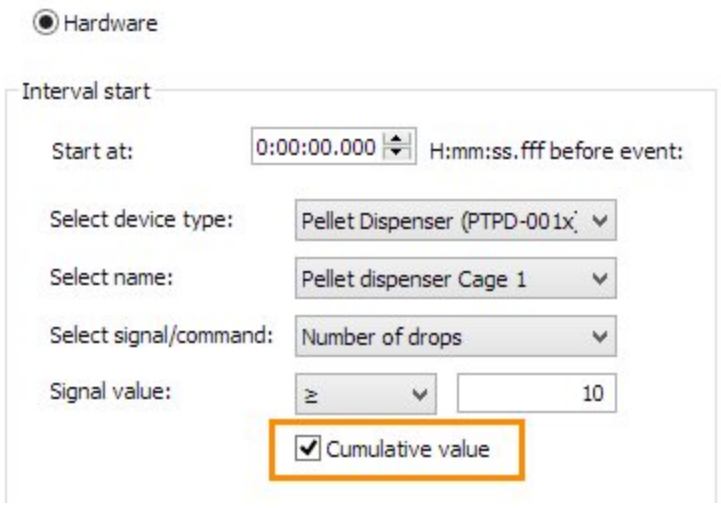
2. From the **Select device type** list, choose the type of device you want to use.
3. From the **Select name** list, select the name of the device of the chosen type. The names in this list have been assigned to that device type in the Arena - Hardware mapping window in the Arena Settings.

4. From the **Select signal/command** list, select the variable or the command you are interested in.
5. From the **Signal value** list, choose which value the signal should have to define the start/stop of the interval, using the operators available (**<=**, **>=** or **false/true**).

Return to To specify a Free interval

## Notes

- The **Cumulative value** option is available for numerical variables like Number of pellet drops, Number of licks, Duration of licks, or counts (for example, Input 1 L->H count).




  - When **Cumulative value** is selected, hardware events are summed up and EthoVision "remembers" that at any time. Keep this option selected if, for example, you want to start an interval when the **Signal value** is >= 10.
  - When **Cumulative value** is not selected, each hardware event is a state that lasts one sample time (for example, 0.04 s if the sample rate is 25). At any time, EthoVision does not "know" how many events have occurred up to that time. De-selecting **Cumulative value** provides results when the **Signal value** selected is **1**. De-select **Cumulative value** when you want to specify multiple intervals, for example for each time a pellet is dropped.
- The options under **Interval start** and **Interval stop** are based on the *Hardware state* dependent variable. For more information, see also **Hardware state** under **Analysis of Trial Control data** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

*See also*

- The EthoVision XT 18 - Trial and Hardware Control - Reference Manual. To open this manual, on the Windows **Apps** screen choose **Noldus** > **EthoVision XT 18 Other documentation**.

# Examples of Free intervals in the Analysis profile

For all the examples below, define the interval first, then choose **Analyze** > **Results** to calculate the statistics or visualize the results. See Free interval

**TIP** If you want to define a free interval, to further analyze the events within that interval, choose Nest over a Free interval in the Data profile.

## Examples

- Example 1: From the track start to when the subject is in a zone for a specific time
- Example 2: From the start of a conditioning routine to when 10 rewards are given to the subject
- Example 3: Split a track in segments of a specific path length
- Example 4: Detect bouts of a behavior
- Example 5: From the track start to when external (physiological) data reach a specific value
- Example 6: Filter the instances of a behavior longer than a certain time

## Example 1: From the track start to when the subject is in a zone for a specific time

### *Aim*

This Free interval can be used, for example:

- Exploratory behavior. Calculate the time that the subject takes to explore the novel object for at least 30 s (Cumulative duration).
- Learning. Calculate the time me that the animal takes to stay in a zone for 30 s uninterrupted (Current duration).

### *Solution*

Define a Free interval with:

- Start criterion: Track start

- Stop criterion: Dependent variable *In zone*. The Statistic depends on the type of question you want to answer.

  In the Novel object test, you want to stop the interval when the subject has explored the object for a *total* of 30 s. Choose then *Cumulative duration*.

  In the learning example, you want to stop the interval when the subject enters a zone and stays for 30 s without exiting. Choose then *Current duration*.

## Example 2: From the start of a conditioning routine to when 10 rewards are given to the subject

### *Aim*

In a conditioning experiment, the subject has to stay in a specific zone to obtain a food pellet. The action-reward sequence is repeated a number of times in a subrule. The researcher wants to know how long it took the subject to get 10 rewards.

### *Solution*

Create a Free interval with:

- Start criterion: Trial Control. Element: Subrule. Event: becomes active.
- Stop criterion: Hardware. Device type: Pellet dispenser. Select signal: Number of drops >=10. The option Cumulative is selected.

## Example 3: Split a track in segments of a specific path length

### *Aim*

Suppose you want to split your tracks in segments of a specific distance moved by the subject, for example 1 m, and you want to calculate the duration of each segment.

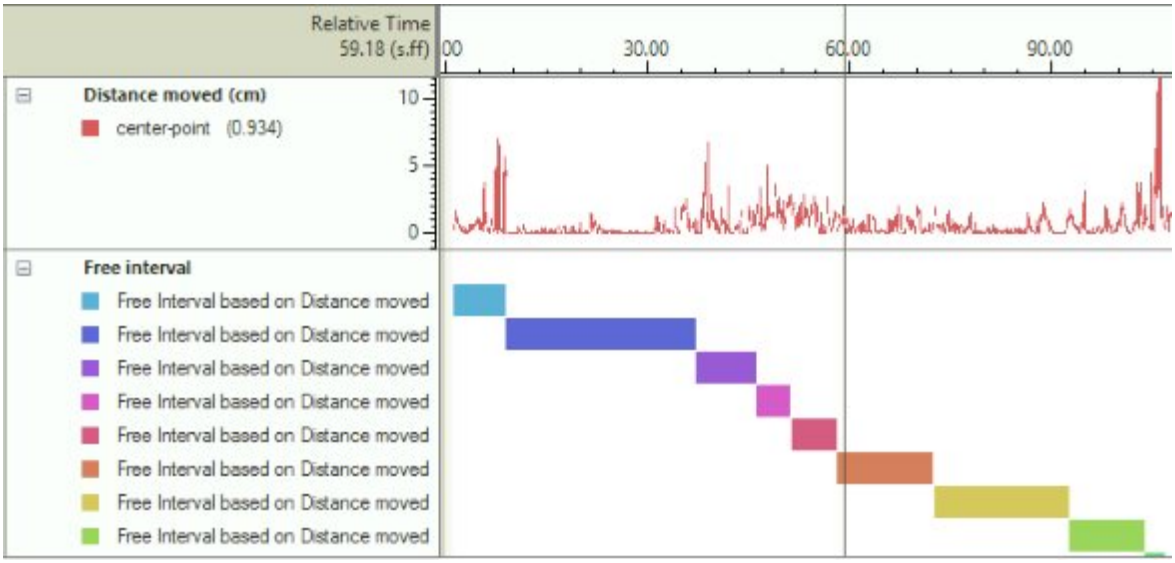
### *Solution*

Create a Free interval with:

- Start criterion: Dependent variable *Distance Moved*. Statistic: Current ≥ 0 cm.
- Stop criterion: Dependent variable *Distance Moved*. Statistic: Total ≥ 100 cm.

To calculate the duration of individual instances, select **Calculate statistics per interval** and enter a number that exceeds the maximum number of interval per track.

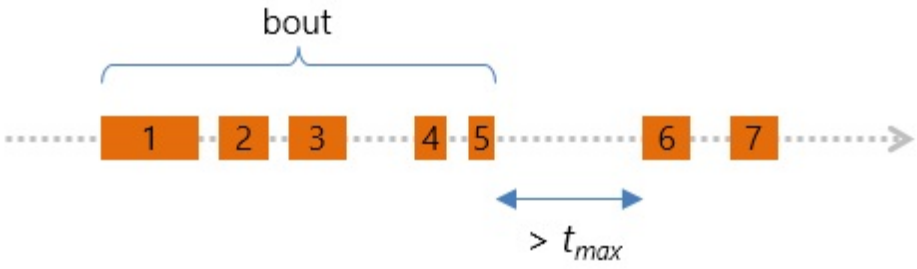
Below: Plot 1: Per-sample distance moved. Plots 2: Free interval that runs from Current Distance moved >=0 to Total Distance moved >=100 cm. Note that the faster the animal (i.e. the longer the per-sample distance moved), the shorter the resulting interval.




## Example 4: Detect bouts of a behavior

*Aim*

Calculate the number and duration of bouts of a behavior, for example *Movement* or *Activity*. The single instances of Movement being part of a bout may be separated by up to time $t_{max}$. In the example below, instances 1 to 5 are grouped in a bout.



*Solution*

Create a free interval with:

- Start criterion: Dependent variable *Movement* with state *Moving*. Statistic: Current.
- Stop criterion: Dependent variable *Movement* with state *Not moving*. Statistic: Current Duration ≥ 5 s ($t_{max}$). This ensures that the interval ends when the animal has not moved for five seconds without interruption.

In the Trial Statistics tab of the same window, select **Mean** if you want to know the average duration of a bout.


**NOTES**

- Because each free interval also includes $t_{max}$ (see the length of the orange bars in the figure above, which represent the free intervals), you have to subtract that time to obtain the true average duration.
- If the event which specifies the end of a bout (in this example, *Not moving* for at least 5 seconds) is never found, the result for that trial is that there is no bout at all. The result is one long bout if you de-select the option **Ignore last interval if incomplete**.
- For the same reason as described above, if the Stop criterion is not met, the last bout of a trial is not detected. For example, when the time between the start of *Not moving* and the end of the trial is less than $t_{max}$. You can have the last bout detected by selecting the option **Ignore last interval if incomplete**, but the duration of this bout is truncated.

## Example 5: From the track start to when external (physiological) data reach a specific value

*Aim*

To calculate the time that it takes for a physiological variable (e.g. heart rate) to reach a specific value.

*Prerequisite*

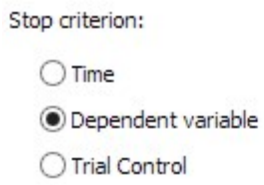
The physiological variable has been imported in the trial. See External Data

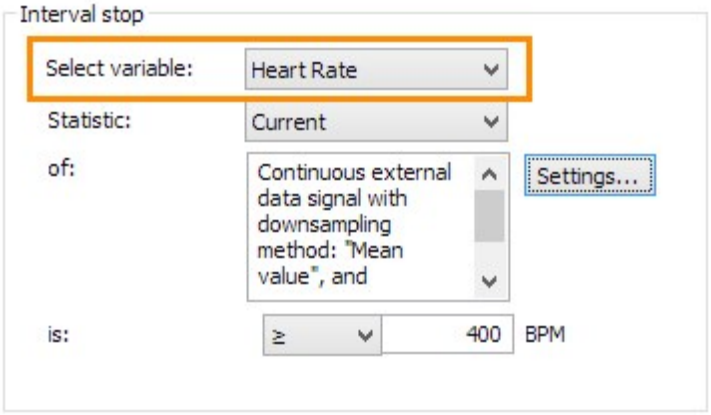
*Solution*

Create a Free interval with:

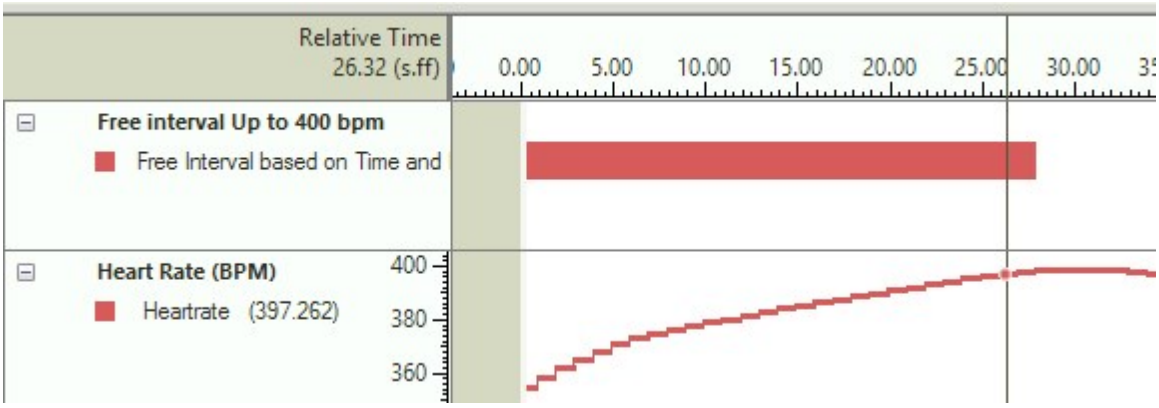
- Start criterion: Track start
- Stop criterion: Dependent variable *Hear rate*. The Statistic chosen is *Current*.

  If necessary, click Settings and choose how to re-sample the variable from the original signal. See External data (resampled)




The result is shown in the first plot below, together with the plot of Heart rate. Choose **Analysis** > **Results** > **Statistics and Charts** to obtain the length of the free interval.




**TIP** If you want to analyze the time between two values of external data, for example from Heart rate = 300 to Heart rate = 400, then in the Analysis profile choose External data (state) instead, not Free interval.

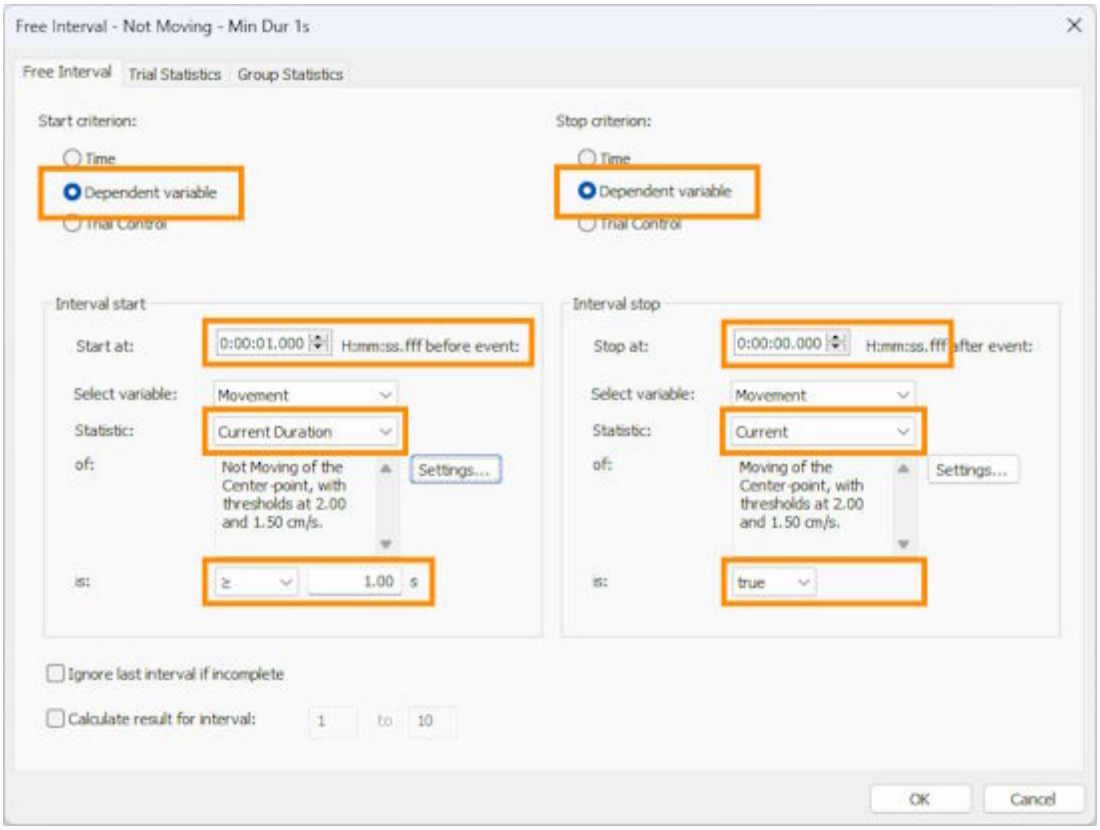
## Example 6: Filter the instances of a behavior longer than a certain time

### *Aim*

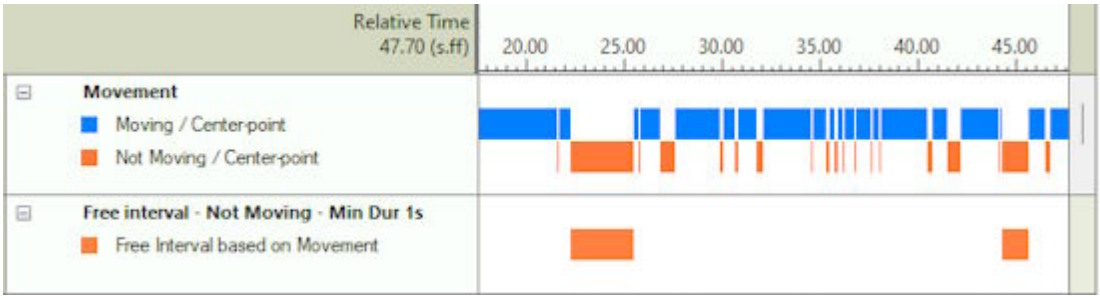
In this example we want to filter the behavioral states (e.g. Not Moving). We want to remove the instances of Not Moving that last less than 1 second.

### *Solution*

1. In the Analysis profile create a free interval with Movement as a criterion.
    - **Start Criterion**: Select **Dependent variable**.
    - **Start at**: Enter there the minimum time duration. In this example, 1 s.
    - **Select variable**: choose the behavior. In this example, Movement.
    - **Statistic**: **Current Duration**.
    - **Settings**: Define the behavior state. In this example, Not Moving is chosen, based on thresholds **Start velocity** 2.0 cm/s and **Stop velocity** 1.5 cm/s.
    - **Stop criterion**: The main difference from the Start criterion is that you must select the opposite of the behavior above. In this example, under **Settings**, **Moving** is selected with the same thresholds as above, and the **Statistic** is **Current**; then select **true**. Next to **Stop at**, leave 0.0 selected.




2. In the Integrated Visualization you can check if the free interval works as expected.
   - The first plot shows the typical Movement variable, with the two states **Moving** and **Not Moving**).
   - The second plot is that of the free interval defined above. It plots the instances of *Not Moving* longer than 1 s, while ignoring the shorter instances.




3. Calculate the statistics of the free interval.

**TIP** In the interval definition, select **Calculate results for interval 1 to ...** if you want results for each instance of the filtered behavior. On the **Trial Statistics** tab, choose the statistic that you need:

- Choose **Frequency** to know how many instances of the filtered behavior occur.
- Choose **Latency to first** to know at what time that behavior occurs, for each instance.

*See also*

- Free interval based on a dependent variable
- Free interval based on a Trial Control event
- Free interval based on hardware
- Free interval based on multiple subjects
- Analysis advisor

# Free interval based on multiple subjects

## Procedure

When working with multiple subjects per arena, you can define free intervals in two ways:

- Under Nesting, choose Free interval to define a Free interval for each subject separately. The interval is based on the behavior of that subject, not the other(s).

  As a result, for each subject a specific time period is selected.

- Under Nesting over Subjects, choose Free interval to define an interval based on a combination of conditions based on two or more subjects. The resulting interval is applied to all subjects.

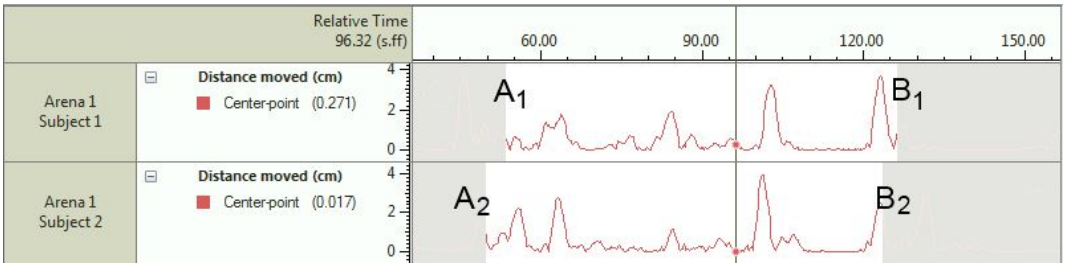
  As a result, the same time period is selected for all subjects.

## Example

Your arena contains two subjects. A free interval is defined that goes from Total Distance moved >= 100 to Total Distance moved >=200.

- If you choose **Free interval** from **Nesting**, the start/stop criteria are applied per subject, resulting in most cases in different time periods being analyzed for each subject.

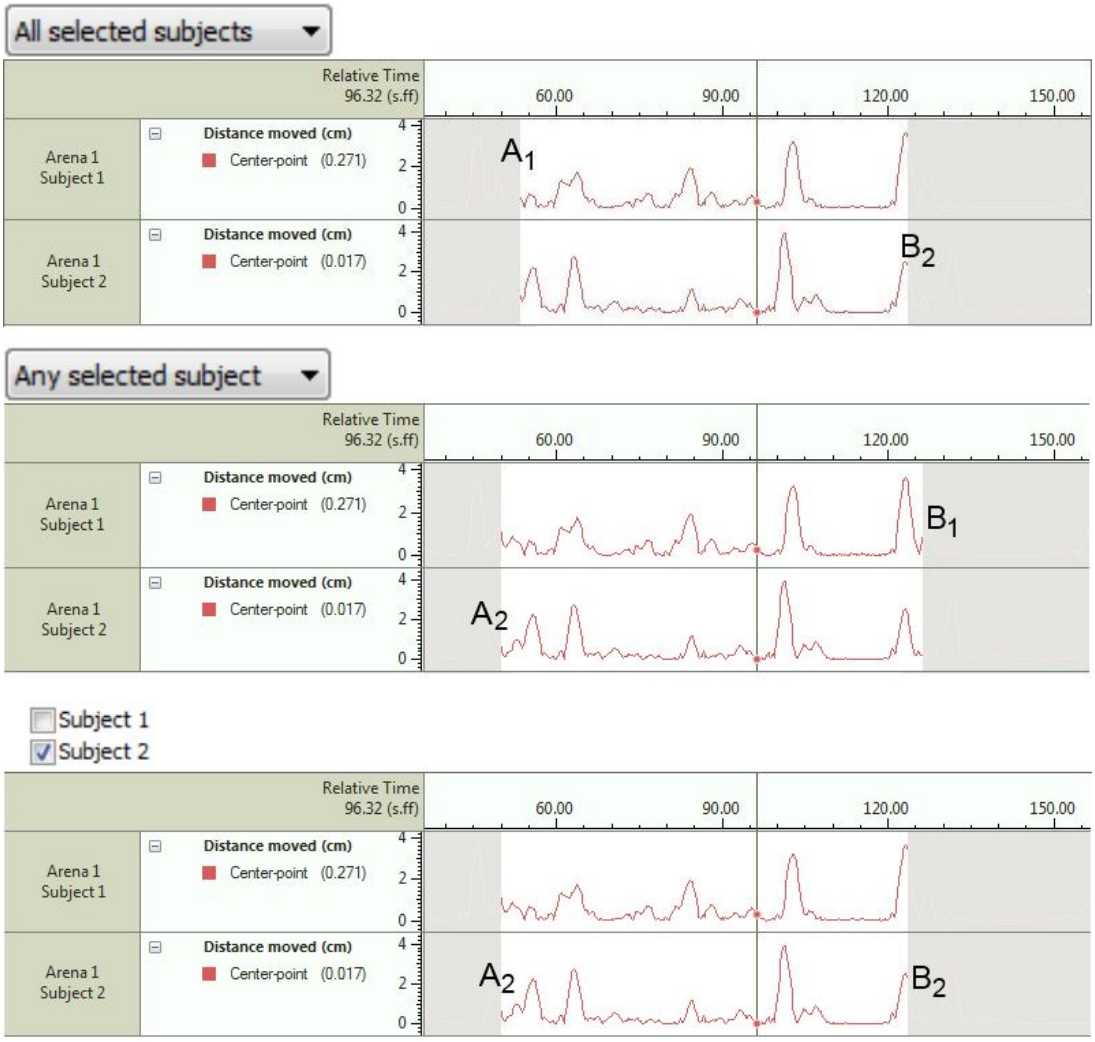
  Below: Time plot of a dependent variable (in this example, *Distance moved*) after defining the Free interval from Nesting. Different time periods are analyzed for Subject 1 (from A1 to B1) and Subject 2 (from A2 to B2).




- If you choose **Free Interval** from **Nesting over Subjects**, the start/stop criteria are applied per subject, but the interval are defined after combining the criteria based on what is selected in the **Actors** tab.

  Below: Time plots of a dependent variable (in this example distance moved) after defining a Free interval from **Nesting over Subjects** (compare with the previous figure). Depending on what is selected in the **Actors** tab:

- With **All selected subjects** (top). The time period shared between subjects (from A1 to B2).
- With **Any selected subject** (middle). The time period defined by at least one subject (from A2 to B1).
- With one subject selected (bottom). The time period defined by the criterion met by that subject (in this example, subject 2; therefore, from A2 to B2).



# Multi condition

## Definition

A segment of track that corresponds to the combination of the value of one, two or more dependent variables.

## Use cases

- *In Trial Control: Stop the trial when exploration time reaches a specified value*.

  In a Novel object test, stop the trial when the exploration of an object reaches 30 seconds. Exploration is defined as (a) Nose point in the zone "object sniffing zone", and (b) Head direction pointing to the "object sniffing zone". When the total time that (a) *and* (b) are simultaneously true reaches 30 s, stop the trial.

  **TIP** Define this condition in the Trial Control Settings.

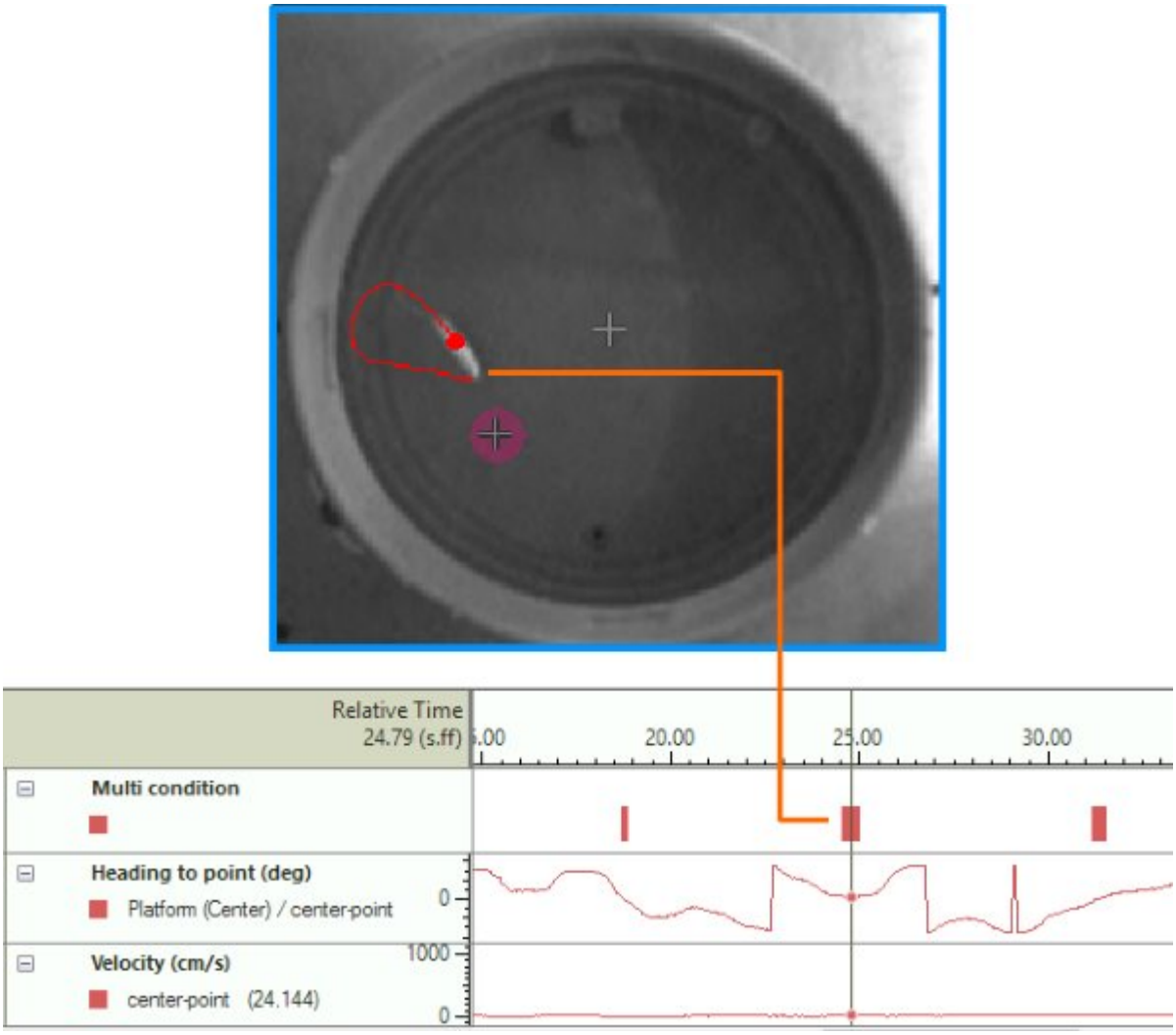
- *In Analysis*: *Calculate the time that the animal swims pointing to the platform*.

  In a probe trial of the Morris water maze test, the subject swims for a number of seconds around the platform. You want to know how long the animal points to the platform while it is swimming. This can be translated to (a) Heading to platform less than a user-define angle (for example 10 degrees) *and* (b) Velocity higher than a user-define value (for example 10 cm/s). **TIP** Define this in the Analysis profile.

## Calculation

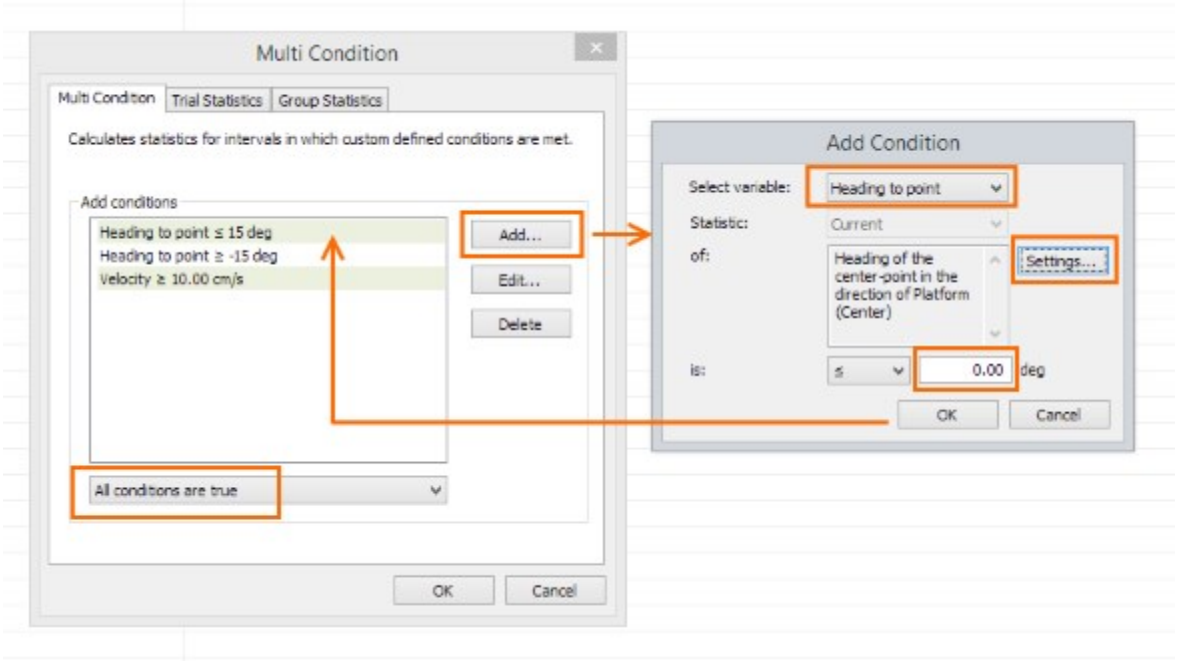
The track segments are defined for each condition independently. Then, the track segments are combined according to the logic specified (All conditions true = AND; Any condition true = OR).

Below: The variable *Multi condition* marks the time when the animal swims towards the platform with a certain speed. Here the multi condition is defined with three conditions: (1) Heading to platform less or equal than +15 degrees; (2) Heading to platform greater than -15 degrees; (3) Velocity greater than 10 cm/s. Conditions are combined with **All conditions true**. The first plot shows *Multi condition*; the second *Heading to point* (center of the platform), with both negative and positive values; the third plot *Velocity*.




## To specify a Multi-condition

1. In the Analysis profile, under **Custom Variables**, click the button next to **Multi condition**.
2. Click the **Add** button at the right of **Add conditions**.
3. Choose the variable from the **Select variable** list.
4. To specify further options, click **Settings**. Next, specify which values (or range of) make the condition true.
5. Click **OK**. The condition is added to the list.




6. Repeat the steps 2-5 to add more conditions.
7. Combine the conditions with **All conditions are true** (AND logic) or **Any condition is true** (OR logic).
8. Complete the procedure to add the variable. See Calculate statistics: procedure.

## Notes

- To define a Multi condition you can use continuous variables, like *Distance moved*, and state variables, like *In zone* (excluding *Activity state* and *Acceleration state*).
- Variables that are not available are: variables representing points in time, like *Rotation*, *Zone transition*, *Zone alternation*; behaviors of Behavior Recognition, and behaviors scored as point events are not available.
- To edit a condition, select that condition in the list and click **Edit**. To delete a condition, click that condition and click **Delete**.

*See also*

- For how to use a Multi condition in Trial Control, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.
- To analyze the track segments based on the Multi condition, see Nesting over a Multi condition in the Data profile.

# JavaScript custom variables

## Aim

To expand EthoVision XT's analysis capabilities by introducing calculations, custom expression, and search functions in JavaScript code embedded in EthoVision XT.

There is a wide range of questions that can be answered with custom JavaScript variables. Here we list a few examples:

- Calculate the average of coordinates x and y of a number of subjects.
- Calculate the number of subjects present in a zone.
- Find the body length of an animal by taking the distance between the nose-point and the tail-base point.
- Calculate the nearest neighbor distance in a school of fish.
- Recalculate the coordinates of a subject based on a reference point.
- Calculate the running average of a variable.
- Find the time when the animal reaches 80% of correct choices in a learning test.
- Create bins of velocity and assign each sample to one of them depending on the speed of the subject.

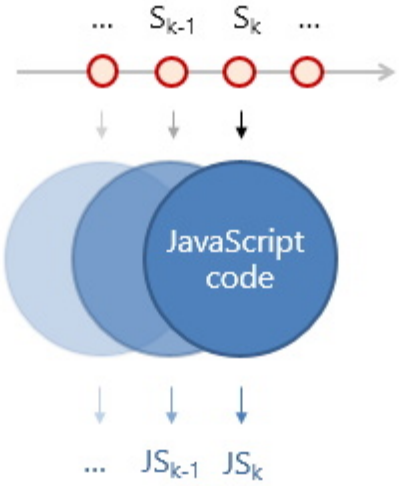
You can find JavaScript variables ready to use on the EthoVision XT 18 Installation package, under **Drivers and tools\JavaScript custom variables**. You can also find the variables on GitHub:

https://github.com/noldus/EthoVision-JavaScriptCustomAnalysis

Simply copy the code to the JavaScript window in an Analysis profile of EthoVision XT (see below). You can often customize the code by adjusting specific parameters.

## Calculation

When you define JavaScript code in EthoVision XT, the software considers the data available at sample $S_k$ and produces a value $JS_k$ (numeric, or boolean) based on the JavaScript code specified.




In the figure above, $S_k$ represents a generic sample, which contains not only the x,y coordinates of the subjects but also their body area, elongation and mobility at the current time. You can extract a lot of information and process it in the code.

The output values can be in one of three formats:

- A rational number, like the average of speed of three subjects. This is an example of JavaScript continuous variable.
- A point event, like the event that occurs when a certain variable reaches the threshold of 80%. This is an example of a JavaScript event variable.
- A state event, like the time that a variable stays above a certain threshold. This is an example of a JavaScript state variable.

Remember that the JavaScript code outputs one or more values (depending on the code) per sample. Those values are used to calculate Trial statistics and Group statistics just like any other dependent variable; for example, the average and the standard deviation.

## Procedure

1. Open your Analysis profile.
2. Choose of the three options: JavaScript continuous, JavaScript event, JavaScript state.
3. Insert the code in the window that appears.
4. Run analysis as usual.

# JavaScript continuous

## Definition

A custom variable that processes raw data (for example, the subject coordinates, or the distance moved) and produces a numeric value for each sample of the track, based on an algorithm.

The JavaScript continuous variable outputs a rational number; other solutions are possible with JavaScript state (with a duration) and JavaScript event (with no duration). See Commands and functions for JavaScript variables

## Use Cases

- *Subject counter*. Count the number of subjects currently present in a zone. For example: How many fish swim at the top of the tank? How many fruit flies enter each side of the T-maze?
- *Subject counter*. Calculate the ratio between the number of subjects in zone A to the number of subjects in zone B.
- *Body length*. Calculate the body length as the distance of the nose-point to the tail-base point.
- *Tail beat detector*. Calculate the ratio between the distance moved by the tail-base and the distance moved by the center-point. This helps quantify tail beats in fish.
- **TIP** Browse to my.noldus.com, log in or register and choose **Downloads** > **EthoVision XT** > **Drivers and tools**. There you can download examples of JavaScript continuous variables. Simply copy the code to the JavaScript Continuous window in EthoVision XT (see below).

## To specify a JavaScript continuous variable

1. In the Analysis profile, under **Custom Variables**, click the button next to **JavaScript continuous**.
2. In the JavaScript Continuous window, enter the code you require.

   **IMPORTANT** Always specify the following functions: Start(), Stop() and Process(), even when they do not include code lines. See the figure above as an example.

3. Click **Check** to see if there are syntax errors. Note that this check is not thorough and does not guarantee that the code provides meaningful data.

4. Complete the procedure to add the variable. See Calculate statistics: procedure

## Notes

- When you use functions like GetCenter, the coordinates are given in millimeters no matter what your measurement units is selected in the Experiment Settings. Therefore, all calculations based on coordinates are given in millimeters. If your calibration is done in cm, divide the results by ten; similarly, values of subject area obtained with the function GetArea are in $mm^2$.
- Select a **Number of outputs** other than 1 if the JavaScript variable is made of two or more outputs. For example, suppose your code creates three state outputs for b1, b2, b3:

  SetOutput(0, b1);

  SetOutput(1, b2);

  SetOutput(2, b3);

  Then select 3 as **Number of outputs**. The value of each output is updated at each sample.
- JavaScript is not the same thing as Java. Although the two programming languages may show similarities in the syntax, there are profound differences, for example in the rules that govern how variables and functions work.
- EthoVision XT uses the Internet Explorer 11-compatible JavaScript engine. This engine does not support the following features:
  - Strict equality operators, for example '===' or '!=='.
  - Class expression (classes are only available through function expressions).
  - Arrow function expression.
  - Fill for arrays.
  - the Math.hypot function.

*See also*

- JavaScript event
- JavaScript state
- Commands and functions for JavaScript variables

# JavaScript event

## Definition

A custom variable that processes raw data (for example, the subject coordinates, or the distance moved) and produces a point event (i.e., with no duration) for each sample of the track, based on an algorithm.

## Use cases

- *Zone visits detector*. For example, detect when the subject has visited 80% of the zones. Use the event to calculate the latency of that event.
- **TIP** Browse to my.noldus.com, log in or register and choose **Downloads** > **EthoVision XT** > **Drivers and tools**. There you can download an example of a JavaScript event variable that detects when a subject has visited a specific percentage of zones. Simply copy the code to the JavaScript Event window and follow the instructions (see below).

## Calculation

The main difference with the JavaScript continuous variable is that the value specified in SetOutput() is either 0 or 1 and has no duration.

See JavaScript continuous > Calculation

## How to specify a JavaScript event variable

1. In the Analysis profile, under **Custom Variables**, click the button next to **JavaScript event**.
2. Follow the rest of the procedure in JavaScript continuous > To specify a JavaScript continuous variable.

## Notes

- In the Trial Statistics tab, select **Latency to First** to calculate the latency of the event. Latency of a dependent variable is calculated from the start of the track, even when you define time bins and nesting intervals.

*See also*

- JavaScript continuous
- JavaScript state

- Commands and functions for JavaScript variables

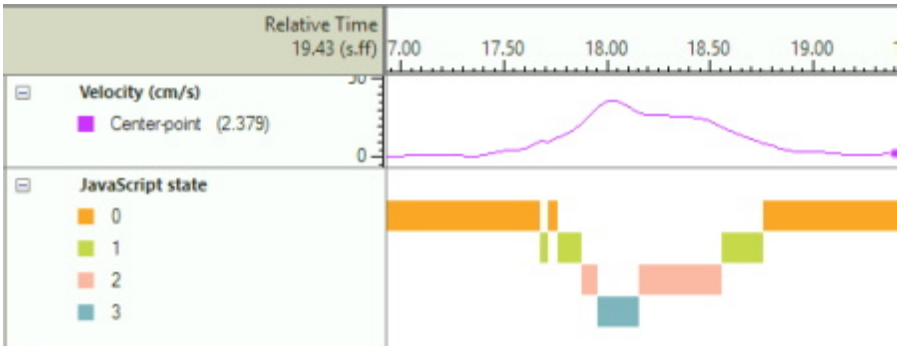
# JavaScript state

## Definition

A custom variable that processes raw data (for example, the subject coordinates, or the distance moved) and produces a state (on/off) value for each sample of the track, based on an algorithm.

## Use cases

- *Velocity bins*. Get the per-sample values of velocity and create velocity bins, for example from 0 to 100 mm/s, from 100 to 200 mm/s etc.




- *Aggregation detector*. Calculate the number of subjects present in a zone and set the output of the variable to 1 if the number of subjects exceeds a certain threshold. Use this variable for example to calculate the time that more than 50% of the subjects cluster in one zone.
- **TIP** Browse to my.noldus.com, log in or register and choose **Downloads** > **EthoVision XT** > **Drivers and tools**. There you can download examples of JavaScript state variables. Simply copy the code to the JavaScript State window and follow the instructions (see below).

## Calculation

The main difference with the JavaScript continuous variable is that the value calculated per sample and specified in SetOutput() is either 0 or 1. The variable has a duration based on the total number of 1s in the track or selected track segment.

See JavaScript continuous > Calculation

## How to specify a JavaScript state variable

1. In the Analysis profile, under **Custom Variables**, click the button next to **JavaScript state**.

2. Enter the code in the JavaScript State window.
3. Next to **Number of outputs**, select the number of possible states of the variable. For example, in the Velocity example described above, select 4.
4. Follow the rest of the procedure in JavaScript continuous > To specify a JavaScript continuous variable.

## Notes

- In the Trial Statistics tab, select **Cumulative Duration** to calculate the total time that the state variable is “on”.

*See also*

- JavaScript continuous
- JavaScript event
- Commands and functions for JavaScript variables

# Commands and functions for JavaScript variables

## Aim

In this topic you find the commands and functions that you can use to extract process tracking data with JavaScript code.

## Get started

We assume that you have basic knowledge about JavaScript. To get started with JavaScript we recommend this online compiler:

https://repl.it/languages/javascript

Many courses on the internet concern JavaScript for web page developers. An excellent course more focused on JavaScript as a data processing tool can be found here:

https://www.youtube.com/watch?v=Bv_5Zv5c-Ts

## Extract subject features

Use the functions below to extract the position and other features of your subjects.

- GetCenter, GetNose, GetTail
- GetSubjectCenter, GetSubjectNose, GetSubjectTail
- GetArea, GetChangedArea
- GetSubjectArea, GetSubjectChangedArea
- GetElongation
- GetSubjectElongation
- GetViewDirection
- GetSubjectViewDirection

## Extract other data

- GetSampleTime
- GetPixelChange
- GetDistanceToZone

- GetDistanceToPoi
- GetBehaviorEvent
- GetCommand
- GetSignal
- new Point
- GetPointPoi

## Query data

Use the functions below to ask simple questions, like *Is the subject in the central zone?* The software gives a boolean answer (true/false).

- IsInZone (discontinued)
- IsSubjectCenterInZone, IsSubjectNoseInZone, IsSubjectTailInZone
- IsPointInZone
- Point.Equals
- IsSubjectActor

## Calculate

- Distance
- Point.Distance
- TurnAngle
- Heading
- SetOutput
- SetOutputMissing

## GetCenter, GetNose, GetTail

Returns the x,y coordinates of the center-point, nose-point or tail-base point of the subjects in a single-subject trial, respectively. Use this function in a single-subject experiment, or in a multi-subject experiment to extract the body points of the focal subject.

- **SYNTAX**

  ```
  GetCenter()
  GetNose()
  ```

```
GetTail()
```

- **EXAMPLE 1**

  In the following example, the code verifies if the center point of the subject, when found, is in the zone named Zone 1.

```
const g_Zone = "Zone 1";
(...)
function Process()
{
   var ptC = GetCenter();
   if (ptC)
   {
      g_bInZone = IsInZone(g_Zone, ptC);
   }
```

- **EXAMPLE 2**

  In the following example, we calculate the average x coordinate of the center point and the nose point.

```
var ptC = GetCenter();
var ptN = GetNose();
var x_avg = ptC.x + ptN.x;
```

## GetSubjectCenter, GetSubjectNose, GetSubjectTail

Returns the x, y coordinates of a body point of a specific subject in a multi-subject experiment. You can use this function to compare the position of different subjects in the same JavaScript variable.

See also the sample experiment Subject Counter with JavaScript code XT160, which you can find on my.noldus.com, under **Downloads** > **EthoVision XT** > **Drivers and tools**.

- **SYNTAX**

```
GetSubjectCenter(Subject name)
GetSubjectNose(Subject name)
GetSubjectTail(Subject name)
```

- **ARGUMENTS**

  Subject name: the name of one of the subjects as defined in the Experiment Settings.

- **EXAMPLE**

  In the following loop (not shown entirely), the center point of each subject is loaded in the variable pt.

```
const g_aSubjects = ["Subject 1", "Subject 2", "Subject 3", ... ]
(...)
for (i = 0; i < g_aSubjects.length; ++i)
{
   var pt = GetSubjectCenter(g_aSubjects[i]);
(...)
```

Note that g_aSubject.length function gets the length of the array variable g_aSubjects which contains the names of the subjects. The variable g_aSubjects is defined at the top of the script.

## GetArea, GetChangedArea

Returns the current value of the subject's area (in practice, the yellow blob that you see during tracking), and the area that has changed from the previous sample, respectively. Use these functions for

- The subject in a single-subject experiment.
- The focal subject in a multi-subject experiment. Here "focal" refers to the subject for which the statistics are calculated.

Areas are expressed in internal units ($mm^2$), independent of the units selected in the Experiment Settings. For information on the Changed area, see Mobility.

- **SYNTAX**

  ```
  GetArea()
  GetChangedArea()
  ```

- **EXAMPLE**

  ```
  var a = GetArea();
  var ca = GetChangedArea();
  ```

- **NOTES**

  GetArea and GetChangedArea are not available when using Deep learning with two subjects per arena, because this method does not record the subjects' body contour.

## GetSubjectArea, GetSubjectChangedArea

GetSubjectArea returns the current value of the area of a specified subject in a multi-subject experiment.

GetSubjectChangedArea returns the area of the subject in a multi-subject experiment that has changed from the previous sample. For information on the Changed area, see Mobility.

The subject's area is the area of the yellow blob that you see during tracking. It is expressed in internal units ($mm^2$), independent of the units selected in the Experiment Settings.

- **SYNTAX**

  ```
  GetSubjectArea("subject name")
  GetChangedArea("subject name")
  ```

  where Subject name is the name of the subject specified in the Experiment settings.

- **EXAMPLE 1**

  ```
  var a = GetSubjectArea("Subject 1");
  ```

- **EXAMPLE 2**

  ```
  // This for loop extracts the area for a number of subjects

  for (i = 0; i < g_aSubjects.length; ++i)
  {
     var AreaSubj = GetSubjectArea(g_aSubjects[i]);
     ...
  }
  ```

- **NOTES**

  GetSubjectArea and GetSubjectChangedArea are not available when using Deep learning with two subjects per arena, because this method does not record the subjects' body contour.

## GetElongation

GetElongation returns the current value of Body elongation for

- The subject in a single-subject experiment.
- The focal subject in a multi-subject experiment. Here "focal" refers to the subject for which the statistics are calculated.

It is expressed as a number between 0 and 1. To obtain the same as the dependent variable Body elongation, multiply it by 100.

- **SYNTAX**

  ```
  GetElongation()
  ```

- **EXAMPLE**

  ```
  var y = GetElongation();
  if (y != null)
  ```

```
{
   SetOutput(y*100);
}
else
{
   SetOutputMissing();
}
```

- **NOTES**

  GetElongation is not available when using Deep learning with two subjects per arena, because this method does not record the subjects' body contour.

## GetSubjectElongation

Returns the current value of Body elongation for a specified subject in a multi-subject experiment. It is expressed as a number between 0 and 1. To obtain the same as the dependent variable Body elongation, multiply it by 100.

- **SYNTAX**

  ```
  GetSubjectElongation("Subject name")
  ```

  where Subject name is the name of the subject specified in the Experiment settings.

- **EXAMPLE**

  ```
  // This for loop extracts the body elongation for a number of subjects
  for (i = 0; i < g_aSubjects.length; ++i)
  {

     var EloSubj = GetSubjectElongation(g_aSubjects[i]);
  ...
  }
  ```

- **NOTES**

  GetSubjectElongation is not available when using Deep learning with two subjects per arena, because this method does not record the subjects' body contour.

## GetViewDirection

Returns the current value of the Head direction variable for

- The subject in a single-subject experiment.

- The focal subject in a multi-subject experiment. Here “focal” refers to the subject for which the statistics are calculated.

It is expressed in radians. Note that the Head direction dependent variable provided by EthoVision XT is expressed in degrees. To convert your JavaScript variable to degrees, see the example below.

- **SYNTAX**

  ```
  GetViewDirection()
  ```

- **EXAMPLE**

  ```
  var dir = GetViewDirection();
  ```

  If you want to extract the head direction in degrees, convert the value of head direction to degrees with the following formula:

  ```
  function toDegrees(angle)
  {
    return angle * (180 / Math.PI);
  }
  (...)
  var dir = GetViewDirection();
  angle = toDegrees(dir);
  ```

- **NOTES**

  GetViewDirection is not available when using Deep learning with two subjects per arena, because this method does not record the subjects’ head direction.

## GetSubjectViewDirection

Returns the value of the Head direction of a specified subject in a multi-subject experiment, for the current sample. The value is given in radians. Note that the Head direction dependent variable provided by EthoVision XT is expressed in degrees. To convert your JavaScript variable to degrees, see the example in GetViewDirection.

- **SYNTAX**

  ```
  GetSubjectViewDirection(“subject name”)
  ```

  where Subject name is the name of the subject specified in the Experiment settings.

- **EXAMPLE**

  ```
  // This for loop extracts the Head direction in radians for a number of subjects
  for (i = 0; i < g_aSubjects.length; ++i)
  ```

```
{
   var HDirSubj = GetSubjectViewDirection(g_aSubjects[i]);
...
}
```

- **NOTES**

  GetSubjectViewDirection is not available when using Deep learning with two subjects per arena, because this method does not record the subjects' head direction.

## GetSampleTime

Returns the sample time (in seconds) of the sample currently processed. You can use this function to calculate the latency of an event that you cannot obtain through the other dependent variables.

**NOTE** The sample time in this function is the track time, that is, the time elapsed from the start of the track (the first sample), not the start of the trial.

- **SYNTAX**

  ```
  GetSampleTime()
  ```

- **EXAMPLE**

  The following example outputs the value of the current sample time. If it is not found, a missing value is assigned with the SetOutputMissing command (see below).

  ```
  function Process()
  {
     var st = GetSampleTime();
     if (st!= null)
     {
        SetOutput(st);
     }
     else
     {
        SetOutputMissing();
     }
  ```

## GetPixelChange

Returns the current proportion of pixels in the arena that have changed intensity above the specified Activity threshold from the previous video frame. This proportion can be between 0 (no pixel in the arena has changed intensity value

above the threshold) and 1 (all pixels in the arena have changed intensity value above the threshold). A pixel change value of 0.156 (expressed as proportion) corresponds to an Activity value without smoothing of 15.6%.

To use this variable, you must select Activity analysis in the Experiment Settings and define the threshold value in Activity settings in the Detection settings. For information on the pixel change, see Activity.

- **SYNTAX**

  ```
  GetPixelChange()
  ```

- **EXAMPLE**

  ```
  var a = GetPixelChange();
  ```

## GetDistanceToZone

Returns the distance of a (body) point from a zone. The distance is zero if the body point is within the zone. Distances are expressed in internal units (mm), independent of the units selected in the Experiment Settings.

- **SYNTAX**

  ```
  GetDistanceToZone(Zone, Point)
  ```

- **ARGUMENTS**

  Zone: the name of the zone as it appears in the Arena Settings; e.g. “Platform”.

  Point: the name of the point; usually a body point.

- **EXAMPLE**

  ```
  const g_strZone = "Object";
  (...)
  function Process()
  {
     var ptNose = GetNose();
     var dist = GetDistanceToZone(g_strZone, ptNose);
  ```

## GetDistanceToPoi

This is similar to GetDistanceToZone; it returns the distance from the specified point of interest. Distances are expressed in internal units (mm), independent of the units selected in the Experiment Settings.

- **SYNTAX**

  ```
  GetDistanceToPoi(Point of interest, Point)
  ```

- **ARGUMENTS**

  Point of interest: the name of the point of interest as it is defined in the Arena Settings.

  Point: the name of the point to which distance is determined; usually a body point.

## GetBehaviorEvent

This command returns the value of an event scored manually using the Manual Scoring function. If you work with Live Mouse Tracker, this command also works with the events listed under **Raw Live Mouse Tracker data** in the Analysis profile.

- **SYNTAX**

  ```
  GetBehaviorEvent(behavior)
  GetBehaviorEvent(behavior, receiver)
  ```

- **ARGUMENTS**

  Behavior: The name of the behavior as in the Manual Scoring Settings or listed under **Raw Live Mouse Tracker data** in the Analysis profile.

  Receiver (optional): The name of the receiver, in case of multi-subject tracking.

- **RETURN VALUES**

  undefined: no behavior event

  1: start of a state event

  2: stop

  4: point event

- **EXAMPLE**

  The following code gets the values of the behavior *Sleep* that was scored. The function UpdateState at the top copies the values of *Sleep*, 1 or 2 to a new state variable g_bState with values true or false, respectively.

  ```
  var g_bState = false;


  function UpdateState(evt)
  {
     if (evt != undefined)
     {
        if (evt == 1)
        {
  ```

```
            g_bState = true;
        }
        else if (evt == 2)
        {
            g_bState = false;
        }
    }
}

function Process()
{
    var evt = GetBehaviorEvent("Sleep");

    UpdateState(evt);

    SetOutput(g_bState);
}
```

## GetCommand

Returns the event that represent a command to a hardware device.

- **SYNTAX**

  ```
  GetCommand(Device name, Command name)
  ```

- **ARGUMENTS**

  Device name: the name of the device specified in EthoVision XT. For example, “Device A”. To know the device name, in the Arena Settings click the **Arena-Hardware Mapping** button, under **Device name** click the cell of the device you require and press **Ctrl**+**C**. Paste the text into the JavaScript code as *Device name*.

  Command name: the name of the command used in the Trial Control Settings. For example, “Drop pellet”. To know the command name, open the Trial Control Settings, locate the Action box that contains that command and click **Settings**. Take note of the text near **Action to perform**. Enter this text in the JS code as *Command name*.

- **EXAMPLE**

  In the following example, JavaScript reads the command “Drop pellet” of a Pellet dispenser with name Device A and outputs the value for each sample of the track (1 = drop a pellet; missing value = no pellets dropped).

  ```
  function Process()
  ```

```
{
   var DropPellet = GetCommand("Device A", "Drop pellet");
   SetOutput(DropPellet);
}
```

## GetSignal

Returns the value of a hardware signal.

- **SYNTAX**

  ```
  GetSignal(Device name, Signal name)
  ```

- **ARGUMENTS**

  Device name: the name of the device specified in EthoVision XT. For example, “Device B”. To know the device name, in the Arena Settings click the **Arena-Hardware Mapping** button, under **Device name** click the cell of the device you require and press **Ctrl**+**C**. Paste the text into the JavaScript code as *Device name*.

  Signal name: the name of the signal that you want to acquire. For example, “Number of licks”. To know the signal name, open an Analysis profile, click the button next to **Hardware continuous**, select a **Device type**, **Device**, and **Signal**. Take note of the text near **Signal**, and enter this text in the JS code as *Signal name*.

- **EXAMPLE**

  In the following example, JavaScript reads the value of the signal “Number of licks” of a lickometer (Device B) and outputs it for each sample of the track.

  ```
  function Process()
  {
     var s = GetSignal("Device B", "Number of licks");
     SetOutput(s);
  }
  ```

## new Point

With the *new Point* statement you can define a point in space.

- **SYNTAX**

  ```
  new Point(x,y,z)
  ```

- **ARGUMENTS**

x, y, z (z is optional): The coordinates in mm based on the calibration axes and origin in the Arena Settings.

- **EXAMPLE**

```
pt1 = new Point(200, 300);
```

- **NOTE**

  You cannot visualize the position of points defined with JavaScript. To do so, define points in the Arena Settings.

## GetPointPoi

With this command you can extract the coordinates of a point of interest of the center of a zone or arena. Note that GetPointPoi returns the position of the point, then you have to extract the coordinates with additional commands (e.g. x = pt.x).

- **SYNTAX**

```
GetPointPoi(point)
```

- **ARGUMENTS**

  point: The name of the point or the zone or arena, of which you want to find the center.

- **EXAMPLE**

```
const g_zone = "Zone 1";
function Start()
{
}
function Stop()
{
}
function Process()
var pt = GetPointPoi(g_zone);
var xc = pt.x;
var xy = pt.y;
```

## IsInZone

This function has been replaced by other functions in EthoVision XT 18 (see below).

- For single-subject tracking: see IsPointInZone
- For multi-subject tracking: see IsSubjectCenterInZone, IsSubjectNoseInZone, IsSubjectTailInZone.

You can still use IsInZone in your code, but it won't work properly when analyzing data points in hidden zones.

## IsSubjectCenterInZone, IsSubjectNoseInZone, IsSubjectTailInZone

Use this function to determine if a body point of a subject is within a zone. The zone must be defined in the Arena Settings. The return value is boolean (true or false).

- **SYNTAX**

```
IsSubjectCenterInZone(Subject name, Zone name);
IsSubjectNoseInZone(Subject name, Zone name);
IsSubjectTailInZone(Subject name, Zone name);
```

- **ARGUMENTS**

  Subject name: the name of one of the subjects listed in the Experiment Settings.

  Zone name: the name of the zone of interest defined in the Arena Settings; for example, "Zone 1". You can specify the name directly ("Zone 1" with quotes), or a variable that contains that name. The zone can be a single zone, a cumulative zone, an entry zone, or a hidden zone.

- **EXAMPLE - ONE SUBJECT**

```
var bC = IsSubjectCenterInZone("Subject 1", "Zone 1");
var bN = IsSubjectNoseInZone("Subject 1", "Zone 1");
var bT = IsSubjectTailInZone("Subject 1", "Zone 1");
```

  or

```
const g_sZone = "Zone 1"
var bC = IsSubjectCenterInZone("Subject 1", g_sZone);
var bN = IsSubjectNoseInZone("Subject 1", g_sZone);
var bT = IsSubjectTailInZone("Subject 1", g_sZone);
```

- **EXAMPLE - MULTIPLE SUBJECTS**

```
var bC1 = IsSubjectCenterInZone("Subject 1", "Zone 1");
var bC2 = IsSubjectCenterInZone("Subject 2", "Zone 1");
```

## IsPointInZone

Use this function to determine if a point (a body point, or a point of interest) is within a zone. The zone must be defined in the Arena Settings. The return value is boolean (true or false).

- **SYNTAX**

```
IsPointInZone(Zone name, Point name)
```

- **ARGUMENTS**

  Zone name: the name of a zone defined in the Arena Settings; for example, "Zone 1".

  Point name: The name of a body point or a point of interest defined in the Arena Settings.

- **EXAMPLE**

```
pt = GetCenter();
var b1 = IsPointInZone(“Zone 1”, pt);
```

  or

```
pt = new Point (10, 10);
var b1 = IsPointInZone (“Zone 1”, pt);
```

## Point.Equals

Checks if two points are equal.

- **SYNTAX**

```
point1.Equals(point2).
```

- **ARGUMENTS**

  point 1: the first point

  point 2: the second point

- **EXAMPLE**

  The variable b gets the value 1 if the two points pt1 (the center of the subject) and pt2 (a point in space defined with the *new* statement) have the same coordinates.

```
var pt1 = GetCenter();
var pt2 = new Point(1.0, 2.0);
var b = pt1.Equals(pt2);
```

- **ERRORS**

  If one of the two points is null, the value is not calculated and an invalid reference is returned.

## IsSubjectActor

Determines if the subject specified (argument) is currently the actor (focal subject) of the track being analyzed. The return value is boolean (true or false). You can use this function to skip comparisons and calculations that do not make sense.

- **SYNTAX**

  ```
  IsSubjectActor(Subject name)
  ```

  Where Subject name is the name of one of the subjects listed in the Experiment Settings.

- **EXAMPLE**

  In this example a for loop scans the list of i subjects Subject 1, Subject 2, etc. contained in the array g_aSubjects[i] and calculates the distance between the focal subject (that is, the subject of the currently analyzed track) and the subject i.

  ```
  for (i = 0; i < g_aSubjects.length; ++i)
     var ptSubj = GetSubjectCenter(g_aSubjects[i]);
     (...)
     //invoke Distance function
     var Dist = Distance(ptFocal, ptSubj);
  ```

  Obviously at some point in this loop the focal subject and the subject i are the same individual. You can exclude the distance calculation focal-to-focal by inserting an extra check.

  ```
  var b = IsSubjectActor(g_aSubject[i]);
      if b = true
      {
          //invoke distance function
  ```

## Distance

To calculate the distance between two points, see Point.Distance. You can also use the code here below and invoke the function in your script. Distance is expressed in internal units (mm), independent of the units selected in the Experiment Settings.

- **FUNCTION**

  ```
  // Function to calculate distance between two points
  function Distance(pt1, pt2)
  {
      var dx = pt1.x - pt2.x;
      var dy = pt1.y - pt2.y;
      return Math.sqrt(dx * dx + dy * dy);
  ```

```
}
```

- **EXAMPLE OF FUNCTION CALL**

```
//Get the center point of subject 1 and subject 2
var cSubj1 = GetSubjectCenter(“Subject 1”);
var cSubj2 = GetSubjectCenter(“Subject 2”);
//call distance function
var Dist = Distance(cSubj1, cSubj2);
```

- **ERRORS**

  If one of the two points is null, the value is not calculated.

## Point.Distance

Calculates the distance between two points. Distance is expressed in internal units (mm), independent of the units selected in the Experiment Settings.

- **SYNTAX**

```
point1.Distance(point2)
```

- **ARGUMENTS**

  point1: the first point

  point2: the second point

- **EXAMPLE**

```
var pt1 = GetCenter();
var pt2 = GetNose();
var dist = pt1.Distance(pt2);
```

- **ERRORS**

  If one of the two points is null, the value is not calculated and an invalid reference is returned.

## TurnAngle

Returns the angle in radians between three points. The name suggests that this function is restricted to turn angles but you can use it to calculate any angle formed by three points in general. See also Turn angle

- **SYNTAX**

```
TurnAngle(Point 1, Point 2, Point 3)
```

- **ARGUMENTS**

  Point 1: the name of the begin point.

Point 2: the name of the middle point.

Point 3: the name of the end point. This is the point at the current sample time, while Point 1 and Point 2 are the points at two immediately preceding sample times.

- **EXAMPLE**

```
ta = TurnAngle(g_aPoints[0], g_aPoints[1], g_aPoints[2]);
```

where g_aPoints is an array variable of length 3.

Remember that TurnAngle is in radians. At the top of the code, define a function to obtain the value in degrees:

```
function toDegrees(angle)
{
  return angle * (180 / Math.PI);
}
(...)
var tadg = toDegrees(ta);
```

- **ERRORS**

If any of the three points is null, the value is not calculated.

## Heading

Returns the value of heading in radians based on two sample points. See Heading

- **SYNTAX**

```
Heading(Point 1, Point 2)
```

- **ARGUMENTS**

Point 1: Begin point.

Point 2: End point.

To calculate heading, Point 1 is the point at the previous sample time and Point 2 the point at the current sample time.

- **EXAMPLE**

```
var hd = Heading(g_aPoints[0], g_aPoints[1]);
```

where g_aPoints is an array variable of length 2.

At the top of the code, define a function to obtain the value in degrees:

```
function toDegrees(angle)
```

```
{
  return angle * (180 / Math.PI);
}
(...)
var hdg = toDegrees(hd);
```

- **ERRORS**

  If one of the two points is null, the value is not calculated.

## SetOutput

Assigns a value to the JavaScript variable for the current sample time.

- **SYNTAX**

  ```
  SetOutput(Value)
  SetOutput(Output, Value)
  ```

- **ARGUMENTS**

  Value: the value that you want to assign to the variable at the current sample time.

  - For JavaScript Continuous variables: a rational number.
  - For JavaScript State variables: true or false.
  - For JavaScript Event variables: 0 or 1.

  Output: Output number. When the variable has just one value per sample, like in most applications, then you do not need to specify Output. When the variable has multiple outputs, like in a state variable with 4 possible states, specify the output in each line (beginning from zero). For example

  ```
  SetOutput(0, bOutput1); //write first state
  SetOutput(1, bOutput2); //write second state
  SetOutput(2, bOutput3); //write third state
  SetOutput(3, bOutput4); //write fourth state
  ```

- **EXAMPLE**

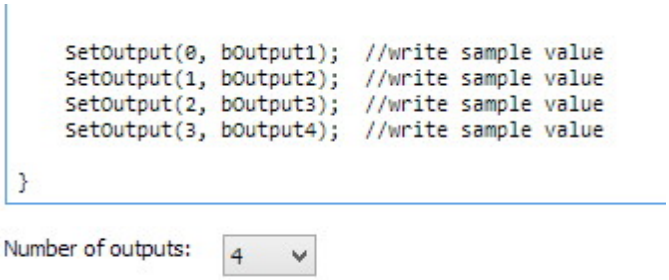
  ```
  if (x != null)
  {
     SetOutput(x);
  }
  ```

- **ERRORS**

  If the value is null or undefined, the output will be missing.

- **NOTES**

When the variable has multiple outputs, remember to specify the **Number of outputs** at the bottom of the JavaScript window.




## SetOutputMissing

Assigns a missing value to the JavaScript variable for the current sample time.

- **SYNTAX**

  `SetOutputMissing()`

- **EXAMPLE**

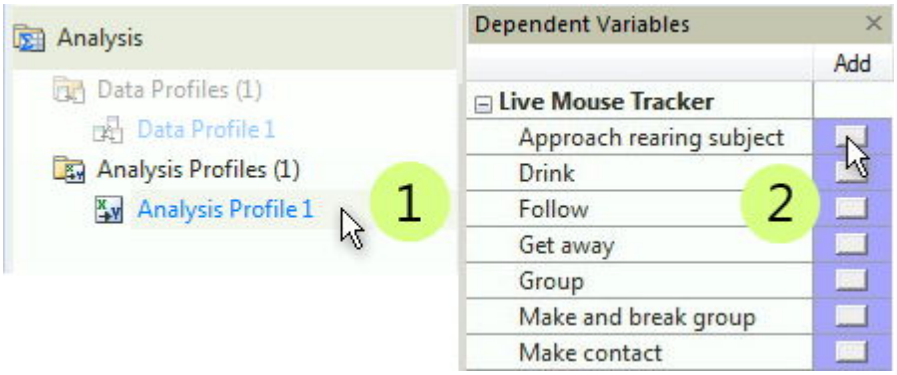
  In the following example, if the value of x is not null, it is assigned to the output of the JavaScript variable; otherwise it is set to missing.

```
if (x != null)
{
   SetOutput(x);
}
else
{
   SetOutputMissing();
}
```

*See also*

- JavaScript continuous
- JavaScript state
- JavaScript event

# Live Mouse Tracker




In the Analysis profile you find two groups of variables based on Live Mouse Tracker data.

Under **Raw Live Mouse Tracker data** you find basic events of Live Mouse Tracker, like Contact and Approach.

- Raw Live Mouse Tracker data

Under **Live Mouse Tracker** you find more complex variables:

- Approach rearing subject
- Drink
- Follow
- Get Away
- Group
- Make and break group
- Make contact
- Move
- Nest
- Nose contact
- Nose contact sequence
- Out of nest
- Rear
- Social approach
- Stretch attend posture
- Social escape

## Notes

- You can also use the basic events of Live Mouse Tracker (first group above) to carry out custom analysis. For example, analyze *Contact* events with JavaScript code. To extract Live Mouse Tracker events, use the function **GetBehaviorEvent**. See Commands and functions for JavaScript variables
- In the Analysis profile, under **Social**, you find variables derived from Live Mouse Tracker with extended options:
  - Side by side
  - Train
- In experiments based on Live Mouse Tracker data, some standard EthoVision XT variables are not available. See a note in the topic Live Mouse Tracker: Workflow

# Raw Live Mouse Tracker data

## Aim

You can use the following variables to analyze basic events in the Live Mouse Tracker datasets. For example, Contact events.

## Approach

### *Definition and calculation*

A discrete (state event) variable that is scored when the focal subject reduces its distance to another subject. *Approach* is based on the approach events detected by Live Mouse Tracker and imported in EthoVision XT. Approach events occur when:

- The speed of the focal subject is greater than the speed of the other subject.
- The distance between the two subjects at the sample *k-3*, that is, three video frames before the current one *k*, is greater than the distance at the sample *k+3*, that is, three video frames after the current one *k*.

Detection occurs during data acquisition, therefore outside EthoVision XT, and its settings cannot be modified in EthoVision XT.

## Contact

A discrete (state event) variable that is scored when the focal subject is in contact with another subject. *Contact* is based on the contact events detected by Live Mouse Tracker and imported in EthoVision XT. Contact events occur when the distance between the detected contours of the animals gets below a fixed threshold. Detection occurs during data acquisition, therefore outside EthoVision XT, and its settings cannot be modified in EthoVision XT. For more information, please see the Live Mouse Tracker documentation.

**NOTE** The dependent variable Body contact under Social behavior in the Analysis profile won't give results with Live Mouse Tracker data, because *Body contact* can only be based on EthoVision XT's own track data.

## Look

*Look* is a state event variable with possible values Look_up and Look_down.

- Look_down: The subject's head is pointing downward, for example when sniffing the substrate.

- Look_up: The subject's head is pointing upward, for example when rearing at the wall.

## Rearing

*Rearing* is a discrete (state event) variable with two possible values, *Rearing* and *Not rearing*. For how it is calculated, see Rear (you can find this variable under **Live Mouse tracker**).

The difference between *Rearing* and *Rear* is that *Rearing* includes all rearing events, instead of splitting them into two categories, *Rear in contact* and *Rear alone*.

## Speed

*Speed* is imported into EthoVision XT and shown in the Analysis profile under **Raw Live Mouse Tracker data**, but it is not used as an output variable.

## Stop

*Stop* is based on the stop events detected by Live Mouse Tracker and imported in EthoVision XT. Stop events occur when the speed of the subject gets below a fixed threshold (see Live Mouse Tracker > Stop). Detection of stop events occurs during data acquisition, therefore outside EthoVision XT, and its settings cannot be modified in EthoVision XT.

**TIP** To analyze stop events when the subject is in contact with its cage mates, see Stop under **Live Mouse Tracker data**.

## How to specify Raw Live Mouse Tracker data

1. Open an Analysis profile and in the Dependent Variables panel, under **Raw Live Mouse Tracker data**, click the **Add** button next to a category.
2. Under **Calculate statistics for**, select the options you require.
3. If the **Receivers** tab is available, there you can specify which subject the behavior is directed to. Keep all subjects selected for an overview of all the events directed to any individual (A, B, etc.).
4. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Notes

- You can also raw Live Mouse Tracker data within JavaScript code to create custom analysis variables. For this purpose, use the GetBehaviorEvent function. See Commands and functions for JavaScript variables

- Raw Live Mouse Tracker data are not affected by track smoothing. This is because the data are stored during acquisition, and not calculated after import in EthoVision XT. For example, no matter whether you apply Lowess smoothing, the statistics of *Contact* do not change. On the contrary, Live Mouse Tracker analysis variables are affected by track smoothing, just like any dependent variable in EthoVision XT.

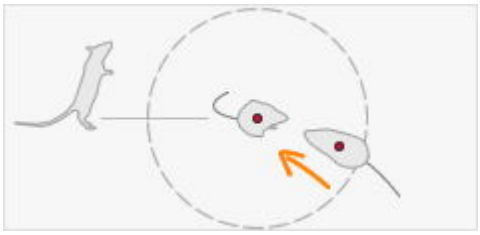
*See also*

- Dependent variables for Live Mouse Tracker

# Approach rearing subject

## Definition

A discrete (state event) variable that is scored when the focal subject approaches another subject in rearing posture.



## Calculation

This variable combines the variables Social approach and Rear in contact. The state *Approach rearing subject* is scored when:

- The focal subject is approaching the other, that is, the distance between the two shortens, and that distance is shorter than two times the average body length of the approached subject, calculated throughout the entire track.
- The speed of the focal subject is higher than that of the approached subject.
- The approached subject is in rearing posture, defined as any time that subject's nose point is at least 40 mm higher than its tail base. Rearing in contact occurs within 15 video frames (that is, 0.5 s for video at 30 fps) from the end of the Social approach event.

## How to specify Approach rearing subject

1. Open an Analysis profile and in the Dependent Variables panel, under **Social**, click the **Add** button next to **Approach rearing subject**.
2. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

*See also*

- Social approach
- Rear
- Live Mouse Tracker

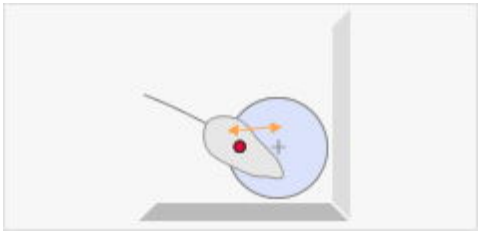
# Drink

## Definition

A discrete (state event) variable that estimates the time that a subject spent drinking.

*Drink* has two components:

- **In the water zone**: This marks the time that the focal subject is within a circular zone that includes the water source.
- **Drink**: The subject is within a smaller circular zone around the water source with a radius being half the radius as above. In addition, the subject is stopped for more than 2 seconds. Therefore, Drink is a more restrictive criterion to estimate the amount of time that the subject spent drinking.



## Calculation

Drink is essentially a variable like In zone, because it calculates the time that the subject's center point lies within a zone. In this case the zone is a circle that covers the water tank placed at the bottom-right corner of the apparatus.

- If the distance between the subject's center point and the center of the circle is less than the zone radius, then *In the water zone* is scored for that sample.
- A smaller zone concentric to the first zone is also considered. If the distance between the subject's center point and the center of the smaller zone is shorter than the zone radius, and if the subject is stopped (see Stop), then *Drink* is scored for that sample.

## How to specify Drink

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Drink**.
2. Under **Calculate Statistics for**, select **In the water zone**, **Drink** or both.

3. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

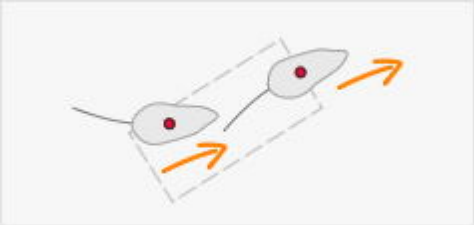
*See also*

- Dependent variables for Live Mouse Tracker

# Follow

## Definition

A discrete (state event) variable that is scored when the focal subject walks directly in the path of another subject.



## Calculation

Follow along path is scored when the following conditions are met:

- Both subjects move at a speed higher than 5 pixels per frame.
- The two subjects are not in contact with any other subject.
- The angle between their direction vectors is smaller than 45°.
- The focal subject's center point lies within a rectangular area drawn based on the position of the followed subject. This is checked beginning from the current video frame time to the 15th frame before the current one. So the software checks that the focal subject enters the path up to 0.5 s after the followed subject has been there.
- The distance between the two subjects' center points is smaller than two mean body lengths. This mean body length is calculated using all values of distance nose-tail base of the followed (i.e., non focal) subject within the track.

## How to specify Follow

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Follow**.
2. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Note

*Follow* looks similar to a train of two mice. However it depends on the definition of the train.

If subject A is following closely subject B, this is scored as *A Follows B* but also as a Train for subject A, when the distance between A and B is shorter than the threshold set in the Train settings, and when the train is defined as in the cases below:

- A > B.
- Actor > B.
- Actor > Any.

Note that:

- *Follow* is scored for the subject that follows another one. If subject A follows subject B, *Follow* is scored for subject A, not B.
- *Train* is scored for all the subject involved in the train definition. So when subject A follows subject B, an instance of *Train* defined as A > B is scored for subject A and for subject B.

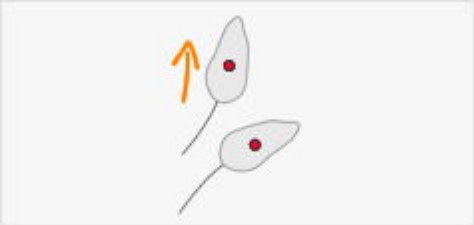
### *See also*

- Dependent variables for Live Mouse Tracker

# Get Away

## Definition

A discrete (state event) variable that is scored when the focal subject walks away from another subject.



## Calculation

The variable Get away is scored when:

- The speed of the focal subject is greater than or equal to the speed of the other subject
- The speed of the focal subject or the other subject is greater than the value set as threshold (5 cm/s). This threshold cannot be changed as it is hard-coded.
- The distance between the two subjects increases.

Note that if the other subject is sitting still, Get away is not scored for the focal subject, no matter what the latter does.

## How to specify Get away

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Get away**.
2. Click the **Receivers** tab.

   Under **Select**, choose the subjects that the focal subject moved away from. If you select two or more subjects, select one of the available options from the list:

   - **All selected subjects:** *Get away* is scored when the focal subject moved away from all the subjects selected above simultaneously.
   - **Any selected subject**: *Get away* is scored when the focal subject moved away from any of the subjects selected above.

- **Each subject**: *Get away* is scored when the focal subject moved away from one of the subjects selected above. The results are displayed for each Receiver.

3. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure.

## Notes

- To calculate this variable, EthoVision XT checks the subject's speed at time *t* based on the change in the X,Y coordinates between *t-1* and *t+1*, like in the original algorithm of Live Mouse Tracker. In EthoVision XT, the *Velocity* variable at time *t* is calculated based on the change in the X,Y coordinates between *t-1* and *t*. See Velocity

## Example

Subject A is moving to the left, away from subjects B and D. Accordingly, the state events **Get away** are scored for the two combinations **A-B** and **A-D**. The duration of the state events depends on when the conditions listed above are valid for each combination. Because subject C is still, *Get away* is not scored for the combination **A-C**.


In the Trial Statistics results table, with the default layout, the results of *Get away* are shown per subject (on the rows). The columns vary depending on what you chose in the **Receivers** tab (see above).

- If you chose **Each subject**, the columns contain the statistics of *Get away* from specific subjects (for example, **From A** to **D**).
- If you chose **All selected subjects** or **Any selected subject**, a column **From all of A, B, C, D** or **From any of A, B, C, D** contains cumulative statistics of *Get away* when the subject moved away from all the cage mates simultaneously or from any of them, respectively, depending on which option you chose.

*See also*

- Dependent variables for Live Mouse Tracker

# Group

## Definition

A discrete (state event) variable that is scored when the focal subject is in contact with one or more subjects.

## Calculation

EthoVision XT uses the events of type Contact stored in the Live Mouse Tracker database.

- Group size 2 is scored when the focal subject is contact with only one subject. If the animal is in contact with more than one animal the event is not scored.
- Group size 3 is scored when the focal subject is in contact with only two other subjects to form a group of three animals.
- Group size 4 is scored when the focal subject is in contact with three other subject to form a group of four animals.

EthoVision XT splits the results depending on who forms each group. For example A can form groups of 2 subjects, resulting in groups AB, AC and AD. The overall results (AC+AC+AD) are also shown.

## How to specify Group

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Group**.
2. Specify the **Group size**.
3. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure
4. If you wish to have the results for all group sizes in one table, repeat the steps above to add a total of three Group variables, one for each group size.

## Applications

You can use *Group* variable to quantify the tendency of subjects to form groups. For example, a mouse model of social deficit may show reduced time spent in groups.

### *See also*

- Dependent variables for Live Mouse Tracker

# Make and break group

## Definition

A discrete (point event) variable that is scored when the focal subject joins or leaves a group of conspecifics. Because it is a point event variable, it has no duration.

- *Make group* of size n (2 to 4). The subject joins a group of n-1 subjects to form a group of n subjects in contact with each other.
- *Break group* of size n (2 to 4). The subject leaves a group of n subjects. The group is now of size n-1, or does not exist anymore in the case of n=2.

## Calculation

EthoVision XT detect the make/break events based on the state of type Group.

- If the subject is not in contact with any subject one video frame before the start of the event Group, a **Make group** event is scored.
- If the subject is not in contact with any subject one video frame after the end of the event Group, a **Break group** event is scored.

**NOTE** In EthoVision XT, a point event is an event with no duration. In contrast, state events have a duration.

## How to specify Make and break group

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Make and break group**.
2. Specify the **Group size**. This is the final size when making a group, or the initial size when leaving a group. For example, enter 3 if you want to have the instances when a subject joined other subject to form a group of three subjects, or left a group of three, so the group became of two subjects.
3. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Applications

In a study of mouse model of ASD, Shank2 mutant mice observed in a social context displayed increased frequency of completing or breaking a group of three or four mice (de Chaumont et al. 2019. *Nat. Biomed. Eng.* 3: 930-942.

## Note

While Group is a state event that aims at quantifying the time that a subject was part of a group, *Make and break group* is a point event variable, that is, it tells you exactly when the subject joined or left a group.

*See also*

- Dependent variables for Live Mouse Tracker

# Make contact

## Definition

A discrete (state event) variable that is scored when the subject approaches another one to make contact with it.

## Calculation

*Make contact* is scored when the following conditions are met simultaneously:

- The distance between the focal subject and the other subject shortens (approach event).
- The speed of the focal subject is higher than that of the approached subject. If the speeds of the two animals involved in the contact are not different enough, *Make contact* is not computed.
- The contact event starts some time during the approach event or immediately after the end of the approach event. That is, the video frame immediately before a contact event must be within the timeline of the approach event.
- The contact event lasts more than three video frames. Instances of contact separated by one video frame are merged together.

An instance of *Make contact* starts at the start time of approach and ends at the video frame just before the contact event. It does not include the time of contact.

**NOTE** In Live Mouse Tracker, contact between subjects is detected when the distance between the contours gets below a fixed threshold. Contact is detected during data acquisition and its settings cannot be modified in EthoVision XT.

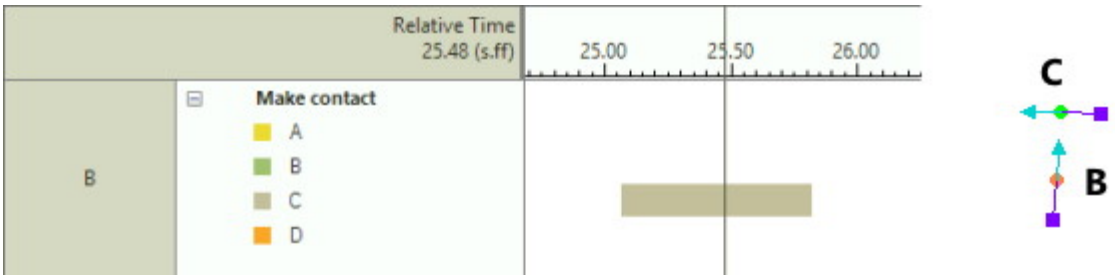
## How to specify Make contact

1. Open an Analysis profile.
2. In the Dependent Variables panel, under **Live Mouse Tracker** click the **Add** button next to **Make contact**.

3. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Example

Subject B has made contact with subject C.




Trial Statistics | Group Statistics & Charts

| Subject | Make contact | | | |
|---|---|---|---|---|
| | A | B | C | D |
| | Cumulative Duration | Cumulative Duration | Cumulative Duration | Cumulative Duration |
| | s | s | s | s |
| A | - | 31.9333 | 34.9333 | 32.1667 |
| B | 26.7000 | - | 27.5000 | 29.4333 |
| C | 28.3667 | 34.8333 | - | 28.9667 |
| D | 17.1000 | 14.2667 | 20.0000 | - |

*See also*

- Dependent variables for Live Mouse Tracker

# Move

## Definition

A discrete (state event) variable that marks the time that the focal subject makes some displacement in space. The subject may move alone (here below, left) or in contact with any other subject (right).

## Calculation

EthoVision XT considers the subject moving for the time between two subsequent events of type Stop. Events of type Stop are scored when the speed of the subject is lower then the threshold value (5.26 cm/s or 2 $^{5/64}$ in). This threshold is set in the Live Mouse Tracker algorithms and cannot be changed.

## How to specify Move

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Move**.
2. Under **Calculate Statistics for**, select **Move in contact**, **Move alone** or both.
3. Click the **Receivers** tab. These apply when you select **Move in contact**.

   Under **Select**, choose the subjects that were in contact with the focal subject while it was moving. If you select two or more subjects, select one of the available options from the list:

   - **All selected subjects:** *Move in contact* is scored only when the focal subject was in contact with all the subjects selected above.
   - **Any selected subject**: *Move in contact* is scored when the focal subject was in contact with any of the subjects selected above.
   - **Each subject**: *Move in contact* is scored when the focal subject was in contact with one of the subjects selected above. The results are displayed for each Receiver.

4. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Example

In the Trial Statistics results table, with the default layout, the results of *Move* are shown per subject (on the rows). The columns vary depending on what you chose in the **Receivers** tab (see above).

- If you chose **Each subject**, the columns contain the statistics of *Move* in contact with specific subjects (**With A** to **D**).

Trial Statistics | Group Statistics & Charts

| Trial | Subject | Move | | | | |
|---|---|---|---|---|---|---|
| | | With A | With B | With C | With D | Alone |
| | | Cumulative Duration | Cumulative Duration | Cumulative Duration | Cumulative Duration | Cumulative Duration |
| | | s | s | s | s | s |
| Trial 1 | A | - | 896.6666 | 1022.5666 | 913.4000 | 5163.8666 |
| | B | 921.3333 | - | 982.9334 | 952.4666 | 5035.5333 |
| | C | 1085.1667 | 1038.6667 | - | 1070.7667 | 6270.6000 |
| | D | 931.7000 | 968.5333 | 1046.3000 | - | 4474.9000 |

- If you chose **All selected subjects** or **Any selected subject**, a column **With all of A, B, C, D** or **With any of A, B, C, D** contains cumulative statistics of *Move* when the subject was in contact with all cage mates or any of them, respectively, depending on which option you chose.

## Notes

- Move may look similar to EthoVision XT's Movement, with its possible states *Moving* and *Not moving*. However, the results may not always overlap. Movement uses two adjustable speed thresholds, which filter the changes in velocity in a different way.

*See also*

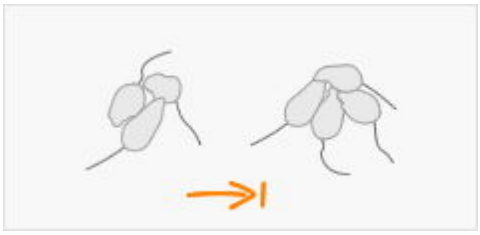
- Dependent variables for Live Mouse Tracker

# Nest

## Definition

A discrete (state event) variable that is scored when the subject is in contact with two or more subjects. All those subjects do not move.

Note that the variable *Nest* has nothing to do with nest-building behavior.



## Calculation

EthoVision XT considers the events of type Contact stored in the Live Mouse Tracker database, which overlap with the events of type Stopped. That means that *Nest* includes only instances when the focal subject is in contact with other subjects, like in *Group*, with the additional condition that none of those subjects moves.

Finally, instances of *Nest* shorter than two samples are removed and adjacent instances up to three samples apart are joined to form one instance.

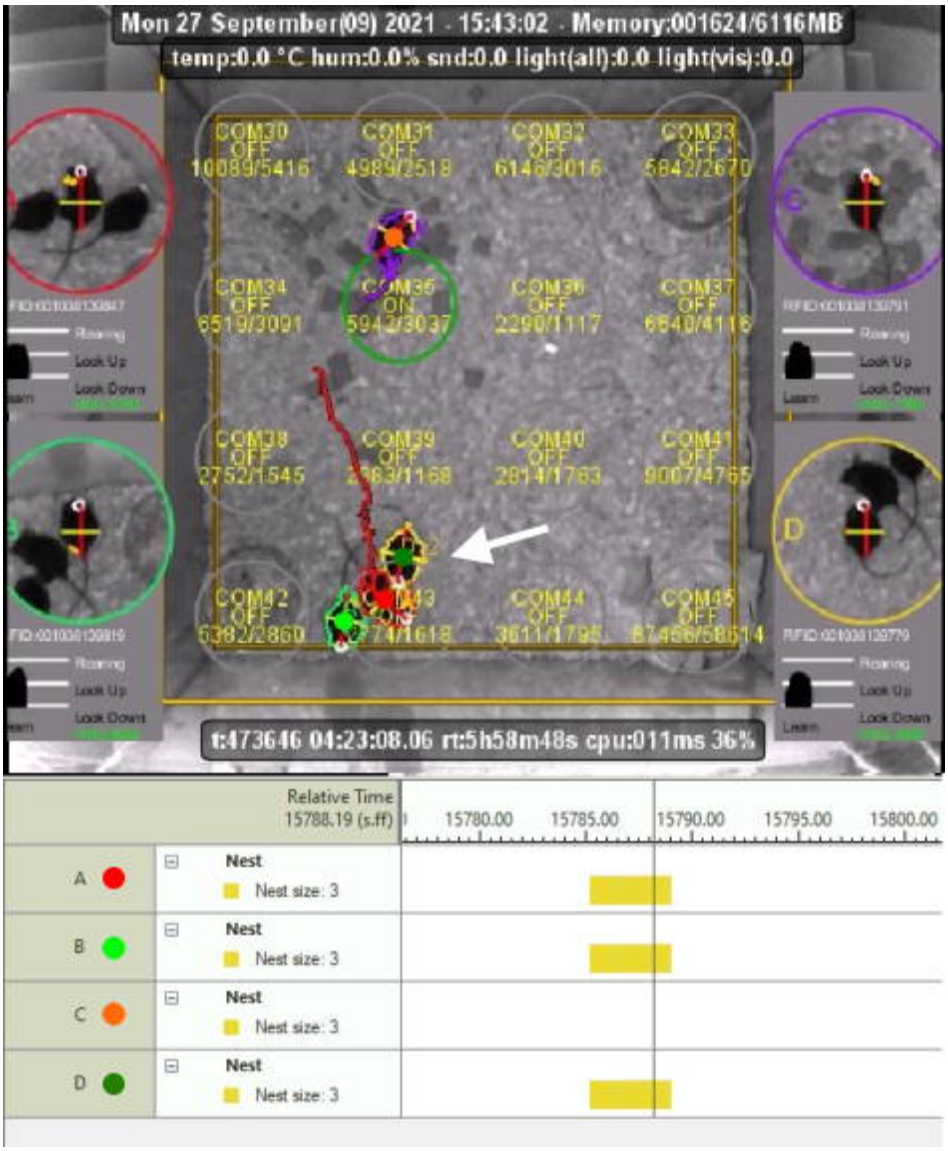
Contrary to *Group*, EthoVision XT does not split the results based on the identity of the subjects forming a *Nest*.

## How to specify Nest

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Nest**.
2. Specify the **Nest size**.
3. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure.
4. If you wish to have the results for all nest sizes in one table, repeat the steps above to add more *Nest* variables, one for each size.

## Example

Subject A (red dot), B (light green) and D (dark green) are forming a nest. Subject C (orange dot) walks around and is therefore not part of the nest.




In the Trial Statistics, Frequency is the number of instances of *Nest* (color bars on the time line as in the figure above) and Cumulative Duration is the sum of the duration of all the instances in the selected trial (or interval).

## Applications

You can use *Nest* to quantify the tendency of subjects to huddle together and form groups. Nest occurs especially in the sleep (light) phase.

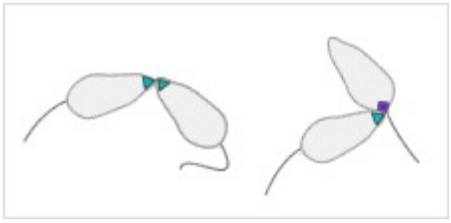
### *See also*

- Group
- Dependent variables for Live Mouse Tracker

# Nose contact

## Definition

A discrete (state event) variable that is scored when the focal subject's nose is near the nose point (see below, left) or the tail-base point (right) of another subject.



## Calculation

- **Nose-nose contact** is scored when the distance between the nose-point of the focal subject is at less than 15 pixels (26 mm for Live Mouse Tracker arenas) from the nose-point of another subject.
- **Nose-anogenital contact** is scored when the distance between the nose-point of the focal subject is at less than 15 pixels (26 mm for Live Mouse Tracker arenas) from the tail-base point of another subject.

## How to specify Nose contact

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Nose contact**.
2. Under **Calculate Statistics for**, select **Nose-nose contact**, **Nose-anogenital contact**, or both.
3. Click the **Receivers** tab.

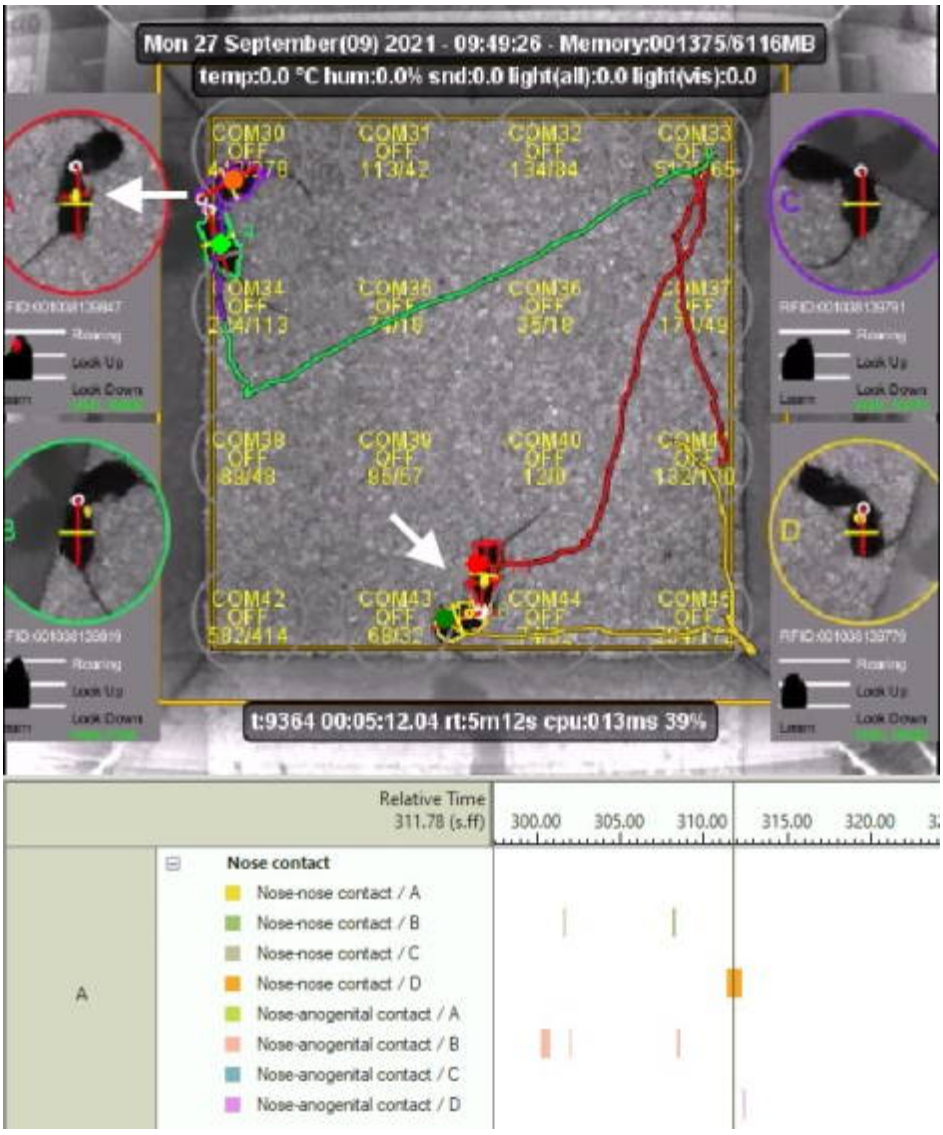
   Under **Select**, choose the subjects that the focal subject was in nose contact with. If you select two or more subjects, select one of the available options from the list:

   - **All selected subjects:** *Nose contact* is scored when the focal subject was in contact with all the subjects selected above.
   - **Any selected subject**: *Nose contact* is scored when the focal subject was in contact with any of the subjects selected above.
   - **Each subject**: *Nose contact* is scored when the focal subject was in contact with one of the subjects selected above. The results are displayed for each Receiver.

4. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Example

A Nose contact variable has been defined with both options **Nose-nose contact** and **Nose-anogenital contact** selected. Below you find the Integrated visualization of this variable. Note that the results are split based on the subject that the focal subject is in contact with. In the current video image, Subject A (red dot) is in nose contact with Subject D (dark green dot).




In the Trial Statistics results table, with the default layout, the results of *Nose contact* are shown per subject (on the rows). The columns vary depending on what you chose in the **Receivers** tab (see above).

- If you chose **Each subject**, the columns contain the statistics of *Nose contact* with specific subjects (for example, **Nose-nose contact / A** to **D**).
- If you chose **All selected subjects** or **Any selected subject**, a column **Nose-nose** (or **Nose-anogenital**) / **All of A, B, C, D** or **Nose-nose** (or **Nose-anogenital**) / **Any of A, B, C, D** contains cumulative statistics of *Nose contact* when the subject was in contact with all cage mates simultaneously or with any of them, respectively, depending on which option you chose.

## Notes

- For this variable, Live Mouse tracker takes the distance between the body points on the X,Y plane, and ignores their Z coordinates.
- In the Analysis profile under **Social**, you find Body contact. This is a variable that quantifies the level of generic body contact between subjects.

*See also*

- Nose contact sequence
- Social Behavior > Body contact
- Dependent variables for Live Mouse Tracker

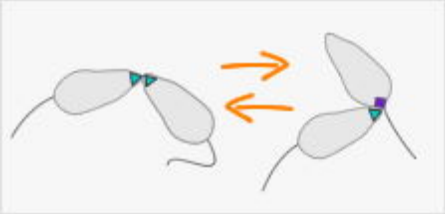
# Nose contact sequence

## Definition

A discrete (state event) variable that is scored from an instance of nose contact of one type to the following nose contact of opposite type, both performed by the focal subject.

In other words:

- From an instance of Nose-nose contact to the next instance of Nose-anogenital contact. Or
- From an instance of Nose-anogenital contact to the next instance of Nose-nose contact.



See also Nose contact

## Calculation

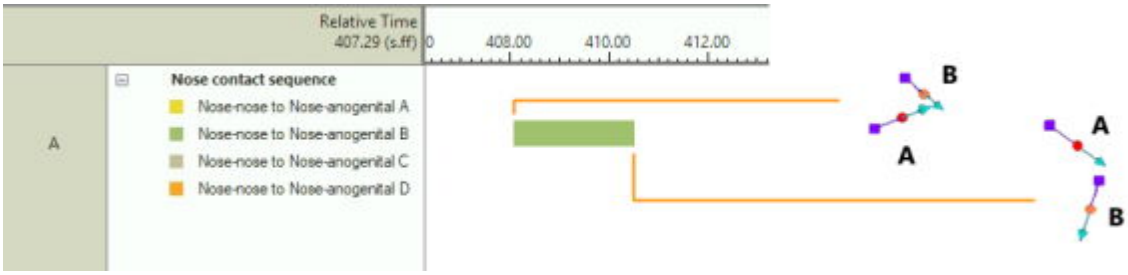
1. EthoVision XT selects all instances of type Nose-nose contact or Nose-anogenital contact (depending on what is chosen in the settings), and longer than 10 video frames (0.33 s for a video of 30 fps).
2. EthoVision XT selects all the instances of type of Nose-contact other than that selected in the previous step, and longer than 10 video frames (0.33 s).
3. If the second Nose-contact occurs within 60 video frames (2 seconds, for a video of 30 fps) from the end of the first one, a Nose contact sequence is found.

## How to specify Nose contact sequence

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Nose contact sequence**.
2. Under **Calculate Statistics for**, select **Nose-nose to Nose-anogenital**, **Nose-anogenital to Nose-nose**, or both.
3. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Example

The following example shows a sequence of type **Nose-nose contact** to **Nose-anogenital contact** of Subject A directed to subject B. The position and orientation of the subjects at the beginning and at the end of the sequence are shown on the right. Triangles represents the nose points and squares represent the tail-base points.



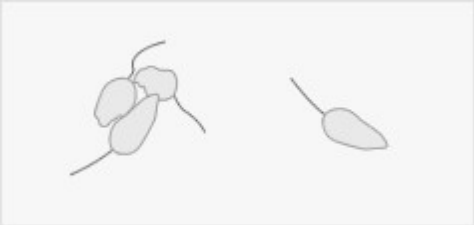

*See also*

- Nose contact
- Dependent variables for Live Mouse Tracker
- Social > Body contact

# Out of nest

## Definition

A discrete (state event) variable that is scored when the focal subject is away from the remaining subjects, which form a nest.



## Calculation

EthoVision XT finds all the instances of Nest 3, that is, when three subjects are in contact and do not move (see Nest). The state event **Out of nest** is scored for the remaining subject and lasts as long as the Nest 3 lasts.

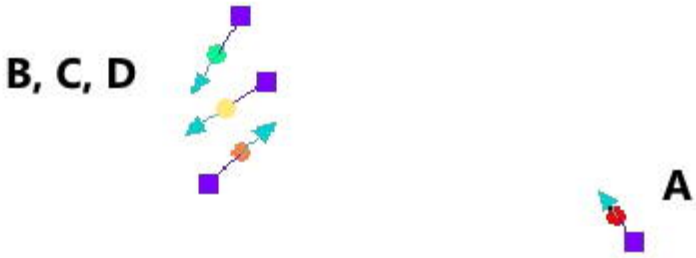
## How to specify Out of nest

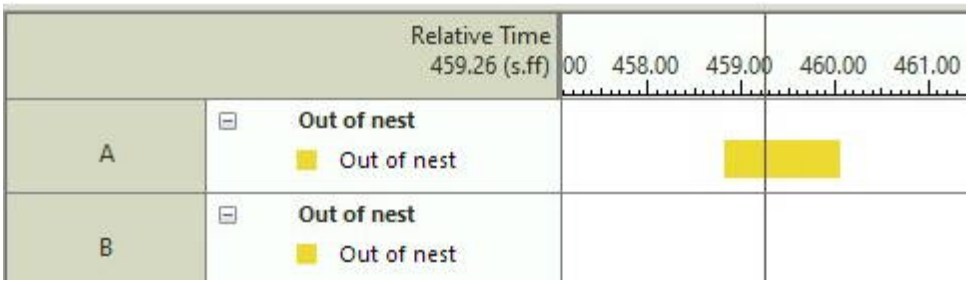
1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Out of nest**.
2. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Example

In the following figure, subject B is far from the remaining subjects B, C and D, which huddle together forming a nest.

The state event **Out of nest** has been scored for subject A and lasts until one of the subjects B, C, and D leaves the nest, or when A joins the others to form a nest of size 4.




*See also*

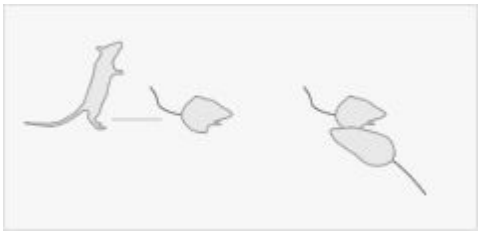
- Nest
- Dependent variables for Live Mouse Tracker

# Rear

## Definition

A discrete (state event) variable that is scored when the focal subject stands only on its hindlimbs and raises its head above its body. Two categories are defined:

- *Rear alone*: the subject is not in contact with any other subject.
- *Rear in contact*: the subject rears while in contact with another subject.



## Calculation

EthoVision XT considers the samples when the focal subject's nose point is at least 40 mm higher than its tail base.

- *Rear in contact* is scored when rearing overlaps with any event of type Contact scored for the focal subject.
- In all other cases, *Rear alone* is scored.

## How to specify Rear

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Rear**.
2. Under **Calculate Statistics for**, select **Rear alone**, **Rear in contact**, or both.
3. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Note

Note the differences between *Rear* in Live Mouse Tracker and *Rearing* in Behavior Recognition:

- *Rear* in Live Mouse Tracker is detected using 3D data obtained from the depth camera, while *Rearing* in Behavior Recognition is detected using features of the pose extracted from a series of 2D images.

- *Rear* in Live Mouse Tracker does not distinguish between rearing at the wall and rearing unsupported.
- *Rear* in Live Mouse Tracker with multiple subject in the arena, while *Rearing* in Behavior Recognition only works with one subject at a time.

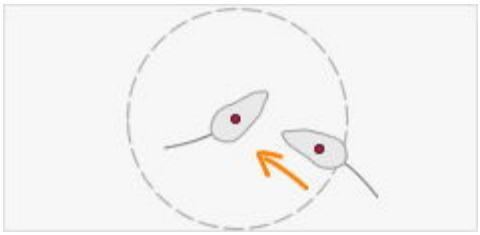
*See also*

- Setting up Behavior Recognition
- Behavior Recognition: Requirements
- Analysis of Behaviors detected with Behavior recognition
- Dependent variables for Live Mouse Tracker

# Social approach

## Definition

A discrete (state event) variable that is scored when the focal subject approaches another subject. The distance between the center points of the two subjects is within two mean body lengths of the approached subject. Social approach does not necessarily lead to contact.



## Calculation

1. EthoVision XT finds all the instances of the events *Approach* between two subjects in the Live Mouse Tracker database.
2. EthoVision XT calculates the average body length of the approached subject, measured as the distance between the nose-point and the tail-base point, throughout the entire track.
3. If the distance between the center point of the focal subject is within two times the average body length of the approached subject, *Social approach* is scored for that sample.

## How to specify Social approach

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Social approach**.
2. Click the **Receivers** tab.

   Under **Select**, choose the subjects that the focal subject approached. If you select two or more subjects, select one of the available options from the list:

   - **All selected subjects:** *Social approach* is scored when the focal subject approached all the subjects selected above simultaneously. For example, when the focal subject approached a group of three cage mates.
   - **Any selected subject**: *Social approach* is scored when the focal subject approached any of the subjects selected above.

- **Each subject**: *Social approach* is scored when the focal subject approached one of the subjects selected above. The results are displayed for each Receiver.

3. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Notes

In the Trial Statistics results table, with the default layout, the results of *Social approach* are shown per subject (on the rows). The columns vary depending on what you chose in the **Receivers** tab (see above).

- If you chose **Each subject**, the columns contain the statistics of *Social approach* directed to specific subjects (for example, **Social approach A** to **D**).
- If you chose **All selected subjects** or **Any selected subject**, a column **All of A, B, C, D** or **Any of A, B, C, D** contains cumulative statistics of *Social approach* when the subject was approached all the cage mates simultaneously or approached any of them, respectively, depending on which option you chose.

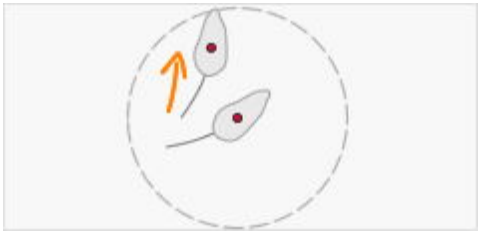
*See also*

- Approach rearing subject
- Social escape
- Social > Relative movement

# Social escape

## Definition

A discrete (state event) variable that is scored when the focal subject walks away from a conspecific (named *Receiver*). The distance between the center points of the two subjects is within two times the average body length of the receiver.



## Calculation

1. EthoVision XT finds all the events of type Get Away in the Live Mouse Tracker database.
2. EthoVision XT calculates the average body length of the subject which the focal subject is moving away from. The body length is measured as the distance between the nose-point and the tail-base point, and averaged throughout the entire track.
3. If the distance between the center point of the focal subject is within two times the average body length of the other subject, *Social escape* is scored for that sample.

## How to specify Social escape

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Social escape**.
2. Click the **Receivers** tab.

   Under **Select**, choose the subjects that the focal subject escaped from. If you select two or more subjects, select one of the available options from the list:

   - **All selected subjects:** *Social escape* is scored when the focal subject escaped from all the subjects selected above simultaneously. For example, when the focal subject left a group of three cage mates.
   - **Any selected subject**: *Social escape* is scored when the focal subject escaped from any of the subjects selected above.

- **Each subject**: *Social escape* is scored when the focal subject escaped from one of the subjects selected above. The results are displayed for each Receiver.

3. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Notes

In the Trial Statistics results table, with the default layout, the results of *Social escape* are shown per subject (on the rows). The columns vary depending on what you chose in the **Receivers** tab (see above).

- If you chose **Each subject**, the columns contain the statistics of *Social escape* from specific subjects (for example, **Social escape A** to **D**).
- If you chose **All selected subjects** or **Any selected subject**, a column **All of A, B, C, D** or **Any of A, B, C, D** contains cumulative statistics of *Social escape* when the subject escaped from all the cage mates simultaneously or from any of them, respectively, depending on which option you chose.

*See also*

- Social approach
- Social > Relative movement

# Stop

## Definition

A discrete (state event) variable that marks the time that the focal subject's center point moves little or not at all in space.

Two categories are defined, based on whether the subject is in contact with other subjects or not.

## Calculation

1. EthoVision XT finds all the events of type *Stop* in the Live Mouse Tracker database. These are scored when the speed of the subject is lower then the threshold value (5.26 cm/s or 2 $^{5/64}$ in). This threshold is set in the Live Mouse Tracker algorithms and cannot be changed.
2. EthoVision XT finds the events of type *Contact* in the Live Mouse Tracker database.
3. If the event Stop and Contact overlaps, then *Stop in contact* is scored for that sample. In all the other cases, *Stop alone* is scored.

## How to specify Stop

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Stop**.
2. Under **Calculate Statistics for**, select **Stop alone**, **Stop in contact**, or both.
3. Click the **Receivers** tab. This applies if you selected **Stop in contact** in the previous step.

   Under **Select**, choose the subjects that the focal subject was in contact with while it was not moving. If you select two or more subjects, select one of the available options from the list:

   - **All selected subjects:** *Stop in contact* is scored when the focal subject was in contact with all the subjects selected above simultaneously.

- **Any selected subject**: *Stop in contact* is scored when the focal subject was in contact with any of the subjects selected above.
- **Each subject**: *Stop in contact* is scored when the focal subject was in contact with one of the subjects selected above. The results are displayed for each Receiver.

4. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Notes

- The state *Not moving*, which you can find in the EthoVision XT dependent variable Movement, is similar to *Stop*. However, there are differences:
  - You can set the speed thresholds for *Not moving*, not for *Stop*.
  - Instances of *Not moving* cannot be split based on the events of contact with other subjects.
- In the Trial Statistics results table, with the default layout, the results of *Stop* are shown per subject (on the rows). When choosing *Stop in contact*, the columns vary depending on what you chose in the **Receivers** tab (see above).
  - If you chose **Each subject**, the columns contain the statistics of *Stop in contact* with specific subjects (for example, **Stop in contact with A** to **D**).
  - If you chose **All selected subjects** or **Any selected subject**, a column **With all of A, B, C, D** or **With any of A, B, C, D** contains cumulative statistics of *Stop in contact* when the subject was in contact with all the cage mates simultaneously or with any of them, respectively, depending on which option you chose.

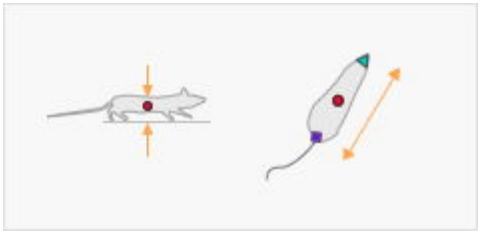
*See also*

- Live Mouse Tracker > Move
- Movement

# Stretch attend posture

## Definition

A discrete (state event) variable that is scored when the subject slows down and stretches its body forward. The subject's apparent body length (measured from the top view) increases and its height (measured with the depth camera) decreases.



## Calculation

1. EthoVision XT calculates the average and the standard deviation (SD) of the body length, measured as the distance between the nose-point and the tail-base point, throughout the entire track.
2. EthoVision XT calculates the median body height, where body height is measured using the z-coordinate of the subject's center point.
3. Stretch attend posture is scored when the following occur simultaneously:
   - The current body length is equal to or greater than the average + 1 SD of the body length.
   - The current body height is smaller than the median body height.
   - The current speed of the subject is lower than 5 pixels per video frame (at 30 fps, this is about 26 cm/s).

## How to specify Stretch attend posture

1. Open an Analysis profile and in the Dependent Variables panel, under **Live Mouse Tracker**, click the **Add** button next to **Stretch Attend Posture**.
2. Complete the procedure to add the variable to the Analysis profile. See Calculate statistics: procedure

## Note

Stretch attend posture is not detected if the nose-point or the tail-based point are not found at that time. The software needs both points to estimate the body length.

*See also*

- Dependent variables for Live Mouse Tracker

# File Management

## Main topics and tasks



**IMPORTANT** Manage your files and experiments EthoVision XT. If you delete, move or rename files using the Windows File Explorer, EthoVision XT may not be able to find the files again.

To move or copy data and experiments to another location, see Back up an experiment.

*See also*

- Using Digital Video
- File locations

# Manage your experiments

## Learn about

- File locations

## What do you want to do?

- Create a new experiment from scratch
- Save an experiment
- Save an experiment with a different name
- Open an existing experiment
- Delete an experiment
- Back up an experiment
- Import data from other experiments
- Manage Settings and profiles

# Create a new experiment from scratch

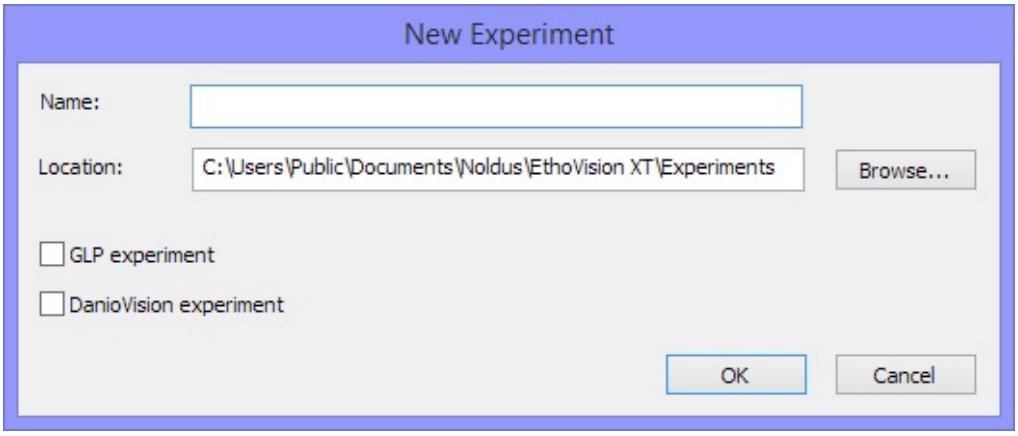
## Aim

To create an experiment without predefined settings. See below.

You can also create an experiment in EthoVision XT using a template, for example for a Morris Water Maze test, or a Radial Maze test, and for a specific animal, like the rat, mouse, or zebrafish. See Create an experiment from a predefined template

## Procedure

1. Choose **File** > **New**, or in the EthoVision XT Startup window, under **New experiment**, click **New**, or press **Ctrl**+**N**.
2. In the New Experiment window, enter a name for your experiment.




3. Browse to the location in which you want to store your experiment.

   **IMPORTANT** Do not create an experiment on a removable disc, like a USB Flash drive or an external hard disk.

4. Additional options:
   - Select **GLP Experiment** if you want EthoVision XT to support you in making a Quality Assurance-compliant experiment. For this, you need the Quality Assurance add-on module.
   - Select **DanioVision experiment** if your PC is connected to a DanioVision system and you want to create a DanioVision experiment.
   - Select **Live Mouse Tracker experiment** if you want to import Live Mouse Tracker data. For this, you need the Live Mouse Tracker add-on module.See Live Mouse Tracker: Workflow
5. Click **OK**.

## Notes

- You can find the experiment file (*.evxt) in a folder with the same name as the experiment. See File locations for the default experiment locations. You can specify the default experiment location during installation. You can change it any time in the Preferences for default folders.
- If you select **DanioVision experiment**, the EthoVision XT interface is adjusted for DanioVision. Unnecessary functions like the Manual Scoring Settings are not available. Detection settings are optimized for detection of zebrafish larvae and the arenas are detected automatically. for more information, see the DanioVision DVOC-0041 - Reference Manual.

# Save an experiment

## Procedure

Choose **File** > **Save** or press **Ctrl**+**S**.

## Notes

- You can also choose to save the data automatically at regular intervals. Choose **File** > **Preferences** > **Auto save**, select **Enable auto save** and enter the auto-save interval.
- When you make changes to your experiment, e.g., changes in settings, these are saved with save or auto-save.
- During data acquisition and track editing, data are saved to file immediately.

# Save an experiment with a different name

## Aim

To create a copy of an experiment, with a different name and saved in a different location (optional).

**IMPORTANT** Do not rename the experiment file *.evxt and any other files within the experiment. That would invalidate the data within the experiment. Instead, save the experiment with a different name.

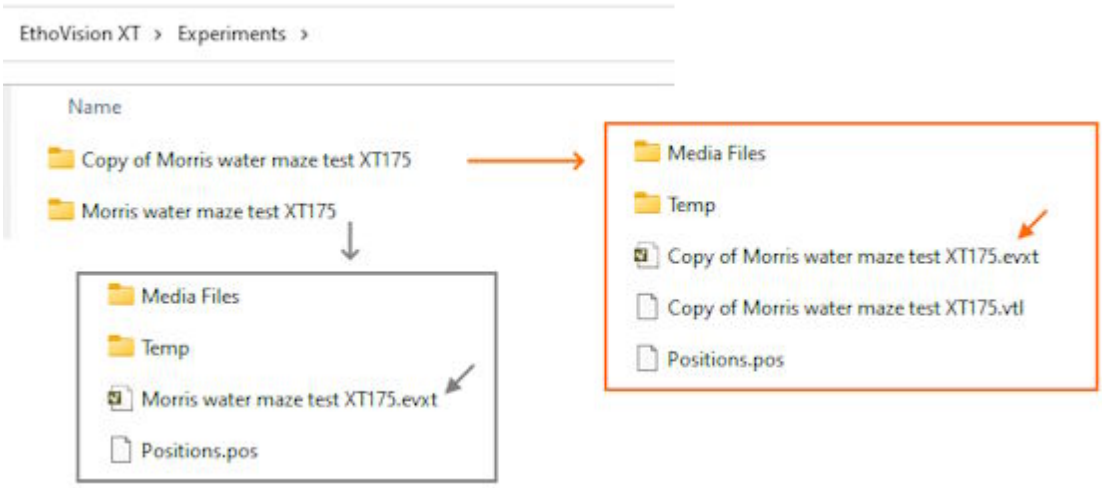
**IMPORTANT** Do not save experiments within the folder of another experiment!

## Procedure

1. Open the experiment in EthoVision XT.
2. Choose **File** > **Save As**.
3. Enter the new experiment name and choose the destination folder.

## Notes

- By default, the destination folder is the same folder as that of the original experiment.
- When you save an experiment with a different name, in the destination folder a new experiment folder is created with the same name as the experiment. For example:



# Open an existing experiment

## Procedure

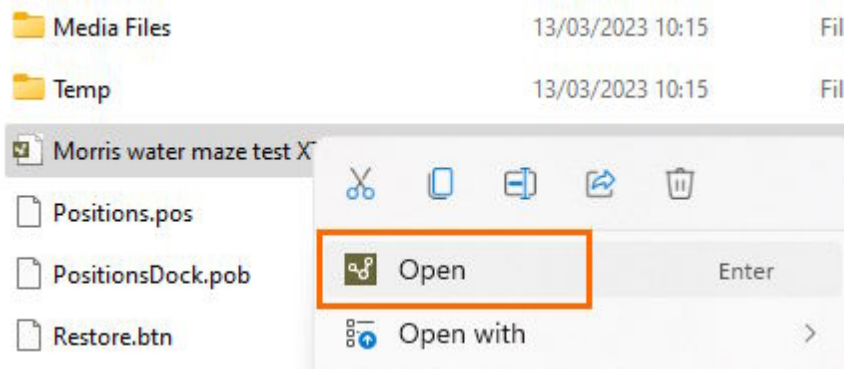
Choose one of the following:

*In EthoVision XT*

- In the EthoVision XT startup window, under **Open**, click one of the experiments in the recent experiment list. To open a different experiment, click **Browse** and open the folder where your experiment is stored. Select the file with the extension **evxt** and click **Open**.
- Press **Ctrl**+**O**. Browse to the location where your experiment is stored, select the file with the extension **evxt** and click **Open**.
- Choose **File** > **Open**. Browse to the location where your experiment is stored, select the file with the extension **evxt** and click **Open**.

*With the File Explorer*

- Using the Windows File Explorer, open the folder where your experiment is stored. Right-click the file with extension **evxt** and click **Open** or **Start experiment**.




## Notes

- You can open an experiment saved in older versions of EthoVision XT.
- You cannot open experiments made in newer versions of EthoVision XT. Use the newest version to open that experiment.
- GLP compliant experiments that have been made with EthoVision XT 12 or earlier cannot be opened in EthoVision XT 18.

# Delete an experiment

## Aim

To remove an experiment from your computer. If video files are included in the experiment folder, these files will be deleted.

## Procedure

1. In the Windows File Explorer, browse to the experiments folder.
2. Click the folder of the experiment that you want to delete and press **Delete**, or right-click that folder and select **Delete**.

# File locations

## Program folder

The default folder for EthoVision XT is

C:\Program Files\Noldus\EthoVision XT 18

## Experiments folder

The default folder for EthoVision XT experiments is

C:\Users\Public\Public Documents\Noldus\EthoVision XT\Experiments.

Each experiment has its own folder, the *experiment folder*. An experiment folder contains the following sub-folders:

- **Bitmap Files**. Contains the background images that were used in the Arena Settings and the reference images that were used in the Detection Settings.
- **Configuration Files**. Contains the configuration files that are created when you set the connection between EthoVision XT and hardware devices.
- **Data Files**. Contains the binary track files acquired during acquisition.
- **Export Files**. When you export your track data, manually scored behaviors, analysis results or the experiment's GLP log files, the export files are, by default, stored in this subfolder.
- **External**. When you import external data files, they are stored in this subfolder. In addition, the subfolder contains the sync-out files that were used for external data co-acquisition.
- **Intermediate**. This folder contains intermediate results.
- **Log**. Contains the log files if your experiment is set for Quality Assurance (GLP experiment).
- **Media Files**. This is the default location for media files that have been recorded during the experiment.
- **Temp**. This is a folder where temporary files are stored. It is usually empty.

## Import Profiles

The default folder for profiles for import of physiological data is

C:\ProgramData\Noldus\Common\Profiles.

## The log file for Technical Support

Locate the file in: C:\ProgramData\Noldus\EthoVision\XT 18\Log.

## The dump files for Technical Support

Our Technical Support department may also request the dump files **EthoVision XT [version number].dmp**.

You can find the dump files in the following locations:

- For *Noldus debug-mode* dump files: C:\Users\[user name]\AppData\Local\Temp.

  **TIP** To access this folder, in the Windows **Search** field type **%temp%**.

- For *user-mode* dump files: C:\Users\[user name]\AppData\Local\CrashDumps

  A user-mode dump is a dump created for a specific process, for example an application like EthoVision XT.

## Notes

- The AppData folder may be hidden on your computer. If so, in the File Explorer, from the **View** menu select **Options** and then **Change folder and search options**. Click the **View** tab and make sure that under **Hidden files and folders** the option **Show hidden files, folders and drivers** is selected.
- If EthoVision XT did not create a DMP file, do the following:

  1. Start EthoVision and use the system until it freezes. Let the software sit there frozen and proceed with the instructions below.

  2. Navigate to: C:\Windows\SysWOW64, double-click on a file called **Taskmgr.exe**. The Task Manager opens.

  3. On the Processes tab, right-click on EthoVision XT and select **Create dump file**.

  4. Wait until a message pops up telling you the location of the DMP file (likely here: C:\Users\<your name>\AppData\Local\Temp\EthoVision.DMP).

  5. Close EthoVision XT, copy the DMP file and send it to Technical Support.

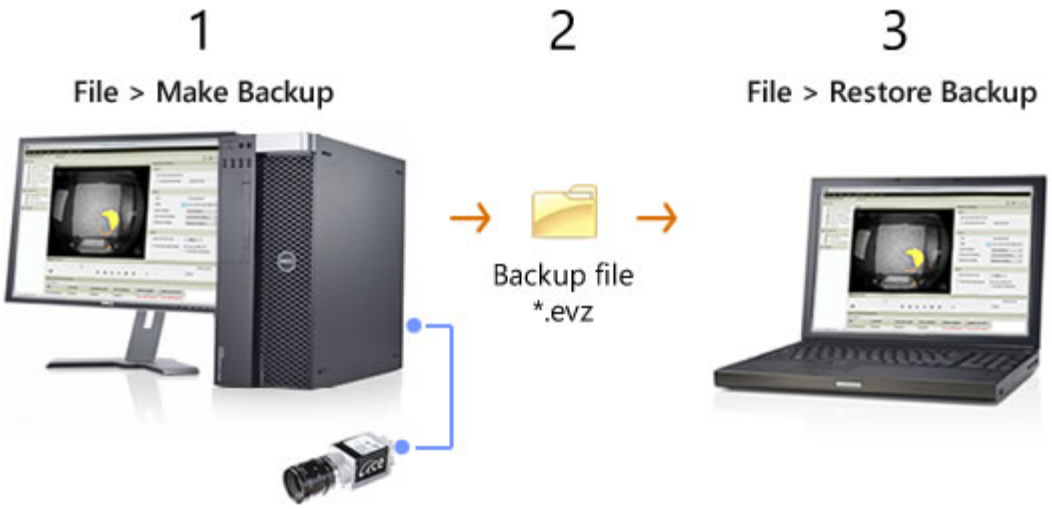
# Back up an experiment

## Aim

To keep a safe copy of your data, or transfer an experiment to a different computer.

## Prerequisites

- The second computer must have EthoVision XT installed, with a version equal or more recent than that in the first computer.
- Note that the hardware key is necessary to acquire the data, not create or restore the backup file.




## Procedure

1. Choose **File** > **Make Backup**.
   - In the Backup Experiment window, enter a name for your experiment backup file, and browse to the location in which you want to store your experiment backup file.
   - Choose the backup options (see below).
2. Copy or send the *.evz file to the final destination.
3. Restore the backup on the second computer (see below).

## Backup options

Select whether you want to:

- **Include Media Files**: These are the video files stored in the Media Files folder of that experiment. **IMPORTANT** Video files used in that experiment but stored in other locations are not copied to the backup file.
- **Include Export Files**: These are the result files and image files stored in the Export Files folder of that experiment.
- **Include External Data Files**: These are the physiological data files stored in the External folder of that experiment.
- **Make backup read-only**. When you select this option you get a warning in case you (accidentally) delete the backup file.

## Notes

- The backup file has the extension *.evz. The file name must not contain any of the following characters: / : * ? \ " < > | . ; .
- **IMPORTANT** Your backup files can get very big if you include your media files!
- If you have recently carried out batch statistics calculations, the backup file may be unnecessarily large. To reduce file size, for each Track Smoothing profile in your experiment click two times a Track smoothing option (see details below). Next, make a backup.
- Make sure that your media files, export files and external data files are within the experiment folder, otherwise they will not be included in the backup.
- You should store the backup file on a secure medium (preferably a network drive), in a separate location.
- When you carry out batch statistics calculations, EthoVision XT stores the results in a number of files, one for each combination of Track Smoothing profiles, Data profiles, Analysis profiles, dependent variable, track, etc. These files are stored in the Intermediate sub-folder in the experiment folder. A large number of result files can make the backup file exceedingly large especially when you deal with a large number of tracks and/or profiles. To remove the result files, open a Track Smoothing profile that you used for analysis and click an option two times, whether that option was selected or not:


Do this for all Track smoothing profiles that you used for analysis. Next, make a backup of your experiment.

To restore the batch calculation results, repeat the batch calculation procedure.

## To restore an experiment from the backup file

1. Choose **File** > **Restore Backup**, or in the EthoVision Startup window, under **Restore experiment**, click **Restore backup**.
2. Browse to the location where your backup file is stored, select it and click **Open**.
3. Browse to the location where you want to save the experiment and click **OK**.

The experiment folder and all associated files of the experiment are restored.

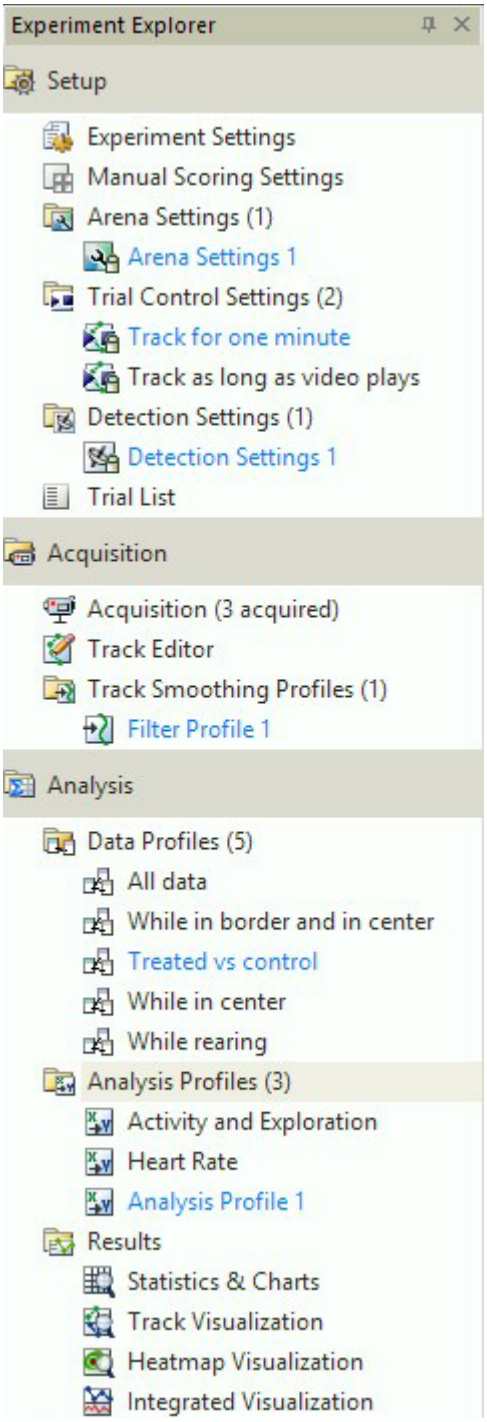
## To delete a backup file

Do one of the following:

- Choose **File** > **Restore Backup**. In the window that opens, click the backup file you want to delete and press the **Delete**. Click **Yes** to confirm.
- In Windows File Explorer, browse to the experiment backup file (*.evz) and delete it.

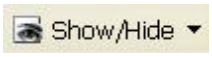
# Settings and profiles

You can store your favorite options in Settings and Profiles that apply to specific procedures. Settings and profiles are visible in the Experiment Explorer. Except for Experiment Settings and Manual Scoring Settings, you can store multiple settings for different situations, for example different Trial Control settings to acquire trials of different durations.



## To view the settings used in a trial

1. Choose **Setup** > **Trial List**.
2. Click the **Show/Hide** button on the toolbar and select **Variables**.




3. Choose the type of settings you want to view.
4. The settings column is added to your Trial List. For a specific trial, the column shows the name of the settings used to acquire the data.
5. In the Experiment Explorer, open that settings profile.

Profiles that have been used to acquire data are marked with a lock symbol (see below).

## To create a new settings/profile

1. Right-click the corresponding folder in the Experiment Explorer and select **New**.
2. Type the name for the new profile or accept the suggested name, then press Enter.

## To activate settings/profiles

In the Experiment Explorer, right-click the settings/profile you want to activate and select **Set as Current**.

Active profiles are highlighted in blue in the Experiment Explorer. Activating means that EthoVision XT uses that profile to carry out a procedure. For example, when acquiring the data it uses the Arena Settings active at that moment. When calculating statistics, it uses the active Track Smoothing profile.

## To open settings/profiles

Right-click the settings/profile you require under the corresponding folder in the Experiment Explorer and select **Open**.

Opening settings/profiles also means that those settings/profiles are activated.

## To edit settings/profiles

To edit settings/profiles, open them (see above) and then make the necessary changes.

When at least one trial has been carried out, the Arena Settings, Trial Control Settings and Detection Settings used for that trial are locked. Locked settings are marked by a lock symbol in the Experiment Explorer.

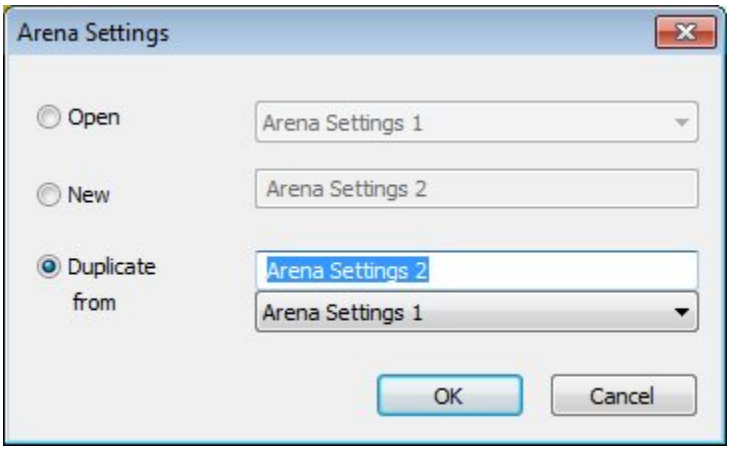

You cannot edit locked settings. If you want to make changes to those settings, make a copy (see below) and edit this. Then, make sure that you carry out the next trial using the new profile.

If you delete all trials acquired with specific settings, those settings are unlocked, so you can edit them.

## To copy settings/profiles

Do one of the following:

- Open the settings/profile folder in the Experiment Explorer. Right-click the name of the settings/profile you want to copy and select **Duplicate**.
- From the **Setup** or **Analyze** menu, select the type of settings/profile you want to copy. In the window that opens, select **Duplicate from**. Change the name of the new settings/profile if necessary. Next, select the settings/profile you want to copy from.




*Result*: A new item is added to the folder. Type a new name or accept the suggested one, then press Enter.

The suggested name for a new settings/profile is [**Settings/Profile] N**, where **N** is the first integer not yet used in the name of the other settings/profiles in that folder.

## To rename settings/profiles

1. In the Experiment Explorer, right-click the name of the settings/profile you want to rename and select **Rename**.
2. The profile name is highlighted. Type the name you require and press **Enter**.

You cannot rename settings/profiles that were used to acquire at least one trial.

## To delete settings/profiles

In the Experiment Explorer, right-click the name of the settings/profile you want to delete and select **Delete**.

## Notes

- You cannot delete settings/profiles that were used to acquire at least one trial. To delete those settings/profiles, you must first delete the trials (make sure you do not lose any important data!).
- You cannot delete settings/profiles if your experiment is set for Quality Assurance (GLP experiment) and you do not have specific rights.
- If the currently-active settings/profile is deleted, the first of the remaining settings/profiles folder is activated automatically.
- If you delete the only settings/profile in the folder, a new default settings/profile is created automatically.
- If you delete a Data profile or an Analysis profile used to create an analysis result open on your screen (track plot or statistics result), the result is updated according to the first available Data profile, or, if no other profiles are available, a newly-created default profile.

# Import data

## Aim

To import data from other programs to analyze tracks and other data combined.

## What do you want to do?

*Import track data from other experiments*

- Import data from other experiments

  **TIP** Choose this option to merge data from multiple experiments.

- Import Live Mouse Tracker data

*Import other types of data*

- Import UltraVox XT 3 call data
- Import Live Mouse Tracker data
- To import data acquired with a separate data acquisition (DAQ) system, see Import external data in EthoVision XT. For how to create an import profile see Create a new custom import profile.

**NOTE** You cannot import track files from one EthoVision XT experiment into another. This means that data which you want to analyze together in EthoVision must be acquired in the same experiment.

# Import UltraVox XT 3 call data

## Procedure

1. In EthoVision XT, open the Trial List.
2. Click the **Import External Data** button.
3. Under **Files of type**, select **UltraVox XT 3**.

   If you do not see this profile, install it from the UltraVox XT 3 installation installation package. Open the folder **...Extras\Import profiles**, and double-click **EthoVision XT 11 and Newer.bat**.
4. Select the *.etx files and click **Open**.
5. Drag the data lines from the Import External Data window to the trial, arena, or subject you require.
6. When ready, click **Import**.

## Notes

- Data for each call name (or call name*pattern label) is imported and resampled as a separate dependent variable.
- For more information on importing and analyzing UltraVox XT call data, and how to control UltraVox XT start and stop data recording, see the UltraVox XT 3 Help.

*See also*

- The import profile for UltraVox XT 3

# Export data

## Aim

To export EthoVision XT data to other programs to carry out further analysis.

## What do you want to do?

*Export data from EthoVision XT*

- Export the raw data (track and dependent variables)
- Export external data
- Export manually-scored behaviors
- Export Trial Control data

*Import EthoVision XT data in other applications*

- Import data into Excel
- Import manually scored data into The Observer XT

*See also*

- Export the statistics result

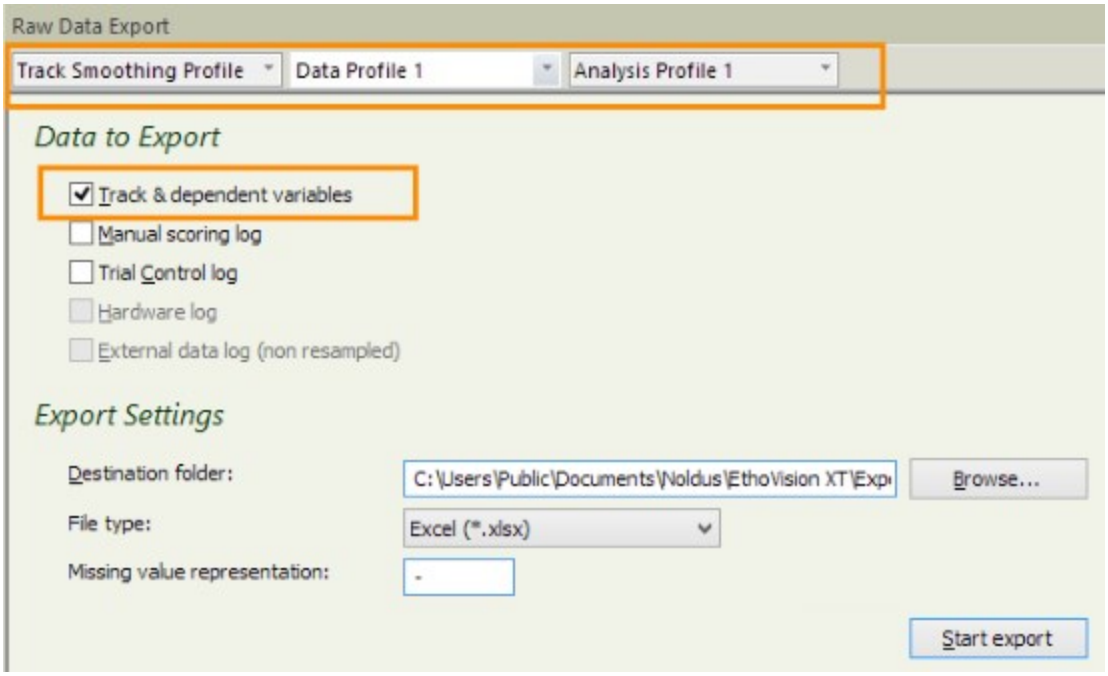
# Export the raw data (track and dependent variables)

## Aim

To export the x,y coordinates and the subject size for each sample together with the values of the dependent variables selected in the currently active analysis profile, including manually-scored behaviors and automatically recognized behaviors. The raw data file includes the independent variables that you defined in the Trial List.

## Procedure

1. Choose **Analysis** > **Export** > **Raw Data**, or in the Experiment Explorer under **Analysis** - **Export** click **Raw Data**.
2. In the Raw Data Export window, select the Track Smoothing profile, the Data profile and the Analysis profile that define the data to be exported.
3. Under **Data to Export**, select **Track & dependent variables.**




4. Click **Browse** next to **Destination folder** and navigate to the location where you want to save the export files.
5. Under **File Type**, select either **ANSI text** (*.txt), **Excel** (*.xlsx), or **Unicode text - default** (*.txt). For text export, enter the **Delimiter** character you want to use to separate columns in the exported file.

6. Next to **Missing value representation**, choose the character that you want to use to mark missing values.
7. Click **Start export**.

## Options

### *File type*

- If you export to text, data of each subject (track) is exported to a separate file. Each file name is unique, and is formed by, in the following order: Raw Data (or Manual Scoring, depending on what option you have chosen) - Experiment name - Trial number - Arena name - Subject name.

  For example Raw Data-Open Field-Trial 1-Arena 1-Subject 1.txt

- Choose **Unicode text** to export according to the Unicode character set. This supports most world languages, including Chinese. Choose **ANSI text** to export the data as text files according to the ANSI character set. Choose this option only if the application in which you will open the exported file cannot handle the Unicode character set.
- If you export to Excel, the file contains all tracks of a trial. The file name is unique and formed by the following elements: Raw data (independent of the type of export) - Experiment name - Trial number.

  For example Raw Data-Plus Maze-Trial 1.xlsx.

  Data of each subject is written in a separate tab of the worksheet, named for example Track-Arena 1-Subject 1.

  **IMPORTANT** If your tracks contain more than approximately one million samples, Excel is unable to load the exported file. With 30 samples per second, this happens just after 9 hours. To solve this, export the file to text and use the Excel macro **SplitTxtFiles.xlsm**. You can find this file on my.noldus.com, under **Downloads** > **EthoVision XT** > **Drivers and tools**.

### *Manual scoring log*

- If you want to export the manually-scored behaviors to The Observer XT, or in a format like for example, 5.12s Rearing, 5.88s Not Rearing, choose **Manual scoring log**.
- You can also export behaviors (both manually-scored and automatically recognized) in the same format as the other dependent variables, where a behavior is scored as 0 or 1 for each sample time, depending on whether it occurs or not at that time. Choose **Track & dependent variables**. Make sure that the Analysis profiles includes the behaviors to export.

### *Trial Control data*

- Choose **Track & dependent variables** also if you want to export Trial Control events and states defined in your Analysis profile. For example, to export the state ‘from Cue light on To Subject in Feeder zone’. The state is exported as a series of 0s and 1s (0= state not active, 1= state active) in the corresponding column of the export file.
- Choose **Trial Control log** if you want to export Trial Control events and states with their own time stamp, for example 6.8 s - Cue light on - Activated. For more information, see Export Trial Control data.

### *Hardware log*

Choose **Hardware log** if you want to export the complete log of events of hardware devices as a separate file. See the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

### *External data*

Choose **External data log (not resampled)** if you want to export the values of the physiological data streams. Note that these export file contain the original data, not the resampled data that appear in the EthoVision XT analysis profile. See External Data

### *Missing value representation*

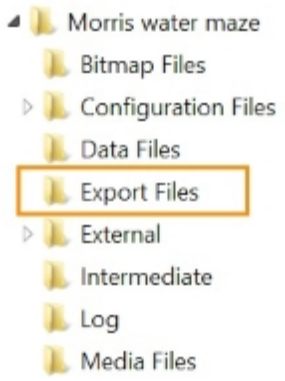
Enter the character for missing values. By default, missing values are exported as “-”. See also Export settings for specific applications

A missing value is exported in the following cases:

- The X,Y coordinates of a missing body point.
- The values of a dependent variable that could not be calculated for that sample. For example, the distance moved when the previous sample was missing.

## File names, folders, and data format

- If you export the same data multiple times to the same location, EthoVision XT attaches a number at the end of the file name: (1), (2), etc.
- The files' suggested location is the **Export Files** folder located in your experiment folder. See File locations




- The values exported have a variable number of decimals, depending on the number of integers. The total number of significant digits exported is always six (for example, 1.23456 or 1234.56), or seven if the unsigned number is smaller than one (0.123456).

## The raw data file

Below: Part of a raw data file, opened in Excel. Top: Headers with independent variables. Bottom: data lines with time, x,y coordinates, subject area and dependent variables (one line per sample).

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | Start time | 14-11-2006 14:49:56 | | | | | | | | | | | | |
| 14 | Trial Duration | 00:01:00 | | | | | | | | | | | | |
| 15 | Recording after | 00:00:02 | | | | | | | | | | | | |
| 16 | Recording duration | 00:00:58 | | | | | | | | | | | | |
| 17 | Track | Track filet0000a0000o0000_0001.trk | | | | | | | | | | | | |
| 18 | Tracking source | | | | | | | | | | | | | |
| 19 | Video file | C:\Documents and Settings\All Users\Documents\Noldus\EthoVision XT\Experiments\Open field XT 60\Media Files\Open field1.mpg | | | | | | | | | | | | |
| 20 | Video start time | 14-11-2006 14:49:56 | | | | | | | | | | | | |
| 21 | Trial status | | | | | | | | | | | | | |
| 22 | Acquisition status | Acquired | | | | | | | | | | | | |
| 23 | Track status | Acquired | | | | | | | | | | | | |
| 24 | Video file status | External | | | | | | | | | | | | |
| 25 | Sync status | Not available | | | | | | | | | | | | |
| 26 | Reference duration | | | | | | | | | | | | | |
| 27 | Reference time | | | | | | | | | | | | | |
| 28 | Sof file | | | | | | | | | | | | | |
| 29 | **External data Independent Variable** | | | | | | | | | | | | | |
| 30 | Heart rate | HR | | | | | | | | | | | | |
| 31 | **User-defined Independent Variable** | | | | | | | | | | | | | |
| 32 | Rat ID | 1 | | | | | | | | | | | | |
| 33 | Treatment | drug | | | | | | | | | | | | |
| 34 | | | | | | | | | | | | | | |
| 35 | **Trial time** | **Recording time** | **X center** | **Y center** | **Area** | **Areachan(** | **Elongatio(** | **Velocity** | **Distance r** | **In zone(b(** | **In zone(cc** | **Movemer** | **Distance t** | **Result 1** |
| 36 | 2 | 0 | -45.6744 | 12.24915 | 42.52506 | - | 0.566958 | - | - | 1 | 0 | - | 8.611514 | 1 |
| 37 | 2.2 | 0.2 | -46.1703 | 6.351053 | 43.40641 | 6.169439 | 0.628786 | 29.59456 | 5.918912 | 1 | 0 | 1 | 7.68348 | 1 |
| 38 | 2.4 | 0.4 | -46.0822 | 0.777878 | 46.49113 | 21.15236 | 0.856012 | 27.86935 | 5.573871 | 1 | 0 | 1 | 6.76849 | 1 |
| 39 | 2.6 | 0.6 | -45.4043 | -4.47473 | 52.2199 | 48.03349 | 0.834809 | 26.48089 | 5.296178 | 1 | 0 | 1 | 5.978689 | 1 |
| 40 | 2.8 | 0.8 | -44.2409 | -9.40688 | 50.67754 | 56.18596 | 0.810187 | 25.33758 | 5.067515 | 1 | 0 | 1 | 5.280633 | 1 |
| 41 | 3 | 1 | -42.6997 | -14.018 | 52.66057 | 53.10124 | 0.879901 | 24.30939 | 4.861877 | 1 | 0 | 1 | 4.582937 | 1 |
| 42 | 3.2 | 1.2 | -40.8594 | -18.3077 | 53.32158 | 50.4572 | 0.830969 | 23.33867 | 4.667735 | 1 | 0 | 1 | 4.03105 | 1 |
| 43 | 3.4 | 1.4 | -38.7709 | -22.275 | 55.30462 | 53.98259 | 0.847322 | 22.41744 | 4.483488 | 1 | 0 | 1 | 3.73625 | 1 |
| 44 | 3.6 | 1.6 | -36.4659 | -25.9189 | 52.66057 | 42.30473 | 0.81778 | 21.55847 | 4.311694 | 1 | 0 | 1 | 3.482803 | 1 |
| 45 | 3.8 | 1.8 | -33.9629 | -29.2372 | 51.99956 | 22.25405 | 0.860497 | 20.7822 | 4.15644 | 1 | 0 | 1 | 3.357782 | 1 |

*Reading the raw data file*

- The track file export takes into account your data selection for entire tracks, but not for within tracks. So, if you make a data selection such that you only have track 1 and only zone 1, EthoVision will export track 1, but the entire track. If you want to filter out zone 1 in your track data, you should include the zone as a dependent variable and then filter on that column in Excel. The result column in the export file has values 0 and 1 dependent on the fact if the data selection criteria were met (1) or not met (0).
- **Trial time** is the time elapsed from the start of the trial (basically, the moment when you clicked the green Start button). **Recording time** is the time elapsed from the start of the track. The difference between the two time values is also shown next to the header Recording after. For more information on trials and tracks, see Important terms in Trial Control.
- The values of the x,y coordinates and the subject size (**Area**) in the exported track files are expressed in calibrated units, (square) millimeters, (square) inches, etc., not in pixels.
- **Areachange** is the change in the pixels of the subject area from the previous sample, used to calculate Mobility. Note that this is not the same as the difference in **Area** between the current sample and the previous one.
- Samples that were *missing* (EthoVision XT could not analyze the video image) or *not found* (EthoVision XT could not find the subject) are indicated by "-" instead of the coordinate values. Note that sometimes the center point is found, not the nose point or the tail base (or neither of the two).

  To see the proportion of samples that were not found in a trial, in the Trial list click the **Show/Hide** button on the toolbar, choose **Variables** and select **Subject not found**. Check the value in the column **Subject not found** corresponding to that trial.
- Excel can import a limited number of rows and columns. For Excel 2007 and newer versions this is 16384 rows and 1048576 columns. Excel will only import the maximum number of rows. If necessary, you can import the exported files to another program like SPSS.

# Export external data

## Aim

To export data like physiological data that were imported into EthoVision XT, to another application for further analysis. You can export the original data or the resampled data.

## To export the original external data:

1. Choose **Analysis** > **Export** > **Raw Data**.
2. Select **External data log (non resampled)**.
3. Choose the **File type** and click **Start Export**.

*Notes*

- Each line in the exported data file represents one sample according to the original sample rate. For example, for Heartbeat sampled at 1 sample/s:

| 41 | Trial time | Recording time | External |
|---|---|---|---|
| 42 | s | s | BPM |
| 43 | 40 | 0.52 | 395.273 |
| 44 | 41 | 1.52 | 395.196 |
| 45 | 42 | 2.52 | 395.217 |
| 46 | 43 | 3.52 | 395.326 |
| 47 | 44 | 4.52 | 395.515 |
| 48 | 45 | 5.52 | 395.734 |
| 49 | 46 | 6.52 | 395.982 |
| 50 | 47 | 7.52 | 396.254 |
| 51 | 48 | 8.52 | 396.549 |

- **Trial time** and **Recording time** are the original time stamps relative to the start of the trial and the start of the track, respectively.
- *For text export*: each file contains the original signal combined with the current Data profile/Result container. The default file name is: External-[experiment name]-[Trial number]-[signal name]-[Data profile or Result container name]-[Arena name]-[Subject name].txt.
- *For Excel export*: each file contains the signal from one trial. The default file name is:

  Raw data-[experiment name]-[Trial number].xlsx.

  Within an Excel file, each sheet contains the signal combined with the current Data profile/Result container. Sheets are named in this way: [signal

name]-[Data profile or Result container name]-[Arena name]-[Subject name]. This name may be truncated.

- For more information on other options, see Export the raw data (track and dependent variables).

## To export the resampled external data:

1. Choose **Analysis** > **Export** > **Raw Data**.
2. Select **Track and dependent variables**.
3. From the list on the toolbar, select the Analysis profile that contains the resampled signals. See External data (resampled)
4. Choose the **File type** and click **Start Export**.

- In the exported data file, each line is one EthoVision XT sample. Each resampled signal is written in a column. The Data profile column indicates whether a sample is included (1) or not (o) in the Data profile.

| 41 | Trial time | Recording time | Heartbeat continuous | Heartbeat state | Data profile 1 |
|---|---|---|---|---|---|
| 42 | s | s | | | |
| 43 | 39.48 | 0 | 395.37 | 0 | 1 |
| 44 | 39.52 | 0.04 | 395.362 | 0 | 1 |
| 45 | 39.56 | 0.08 | 395.355 | 0 | 1 |
| 46 | 39.6 | 0.12 | 395.347 | 0 | 1 |
| 47 | 39.64 | 0.16 | 395.34 | 0 | 1 |
| 48 | 39.68 | 0.2 | 395.333 | 0 | 1 |
| 49 | 39.72 | 0.24 | 395.325 | 0 | 1 |
| 50 | 39.76 | 0.28 | 395.318 | 0 | 1 |

- Export files are named like Raw data files. For more information on other options, see Export the raw data (track and dependent variables).
- To export a specific selection of resampled external data, first edit the Data profile.

## To export the signal statistics

Make sure that the signal is selected in the Analysis profile, with the Trial Statistics that you require. Next, choose **Analysis** > **Export** > **Statistics**. See Export the statistics result

# Export manually-scored behaviors

## Aim

To export the behaviors scored manually, depending on the file structure you require.

- Export as dependent variable. The behaviors are exported as 0s or 1s, depending on whether the behavior is active (1) or not (0) at a specific sample time. Note that the export file contains a header. For example:

  **Time** **Grooming head**

  10.00 0

  10.04 1

  ... ...

  12.00 0

- As a Manual Scoring log. The behaviors are exported to a file where the start and stop events are written in sentences with the following structure:

  **Time** - **Subject** - **Behavior** - Event type (**State start** or **State stop**)

  For example:

  10.04 Subject 1 Grooming head State start

  12.00 Subject 1 Grooming head State stop

## Procedures

*To export manually-scored behaviors as dependent variables*

1. Make sure that the Analysis profiles includes the behaviors to export.
2. Choose **Analysis** > **Export** > **Raw Data**, and select **Track and dependent variables**. See also Export the raw data (track and dependent variables).

*To export manually-scored behaviors as a Manual Scoring log*

1. Choose **Analysis** > **Export** > **Raw Data**, or in the Experiment Explorer under **Analysis** - **Export** click **Raw Data**.
2. Select **Manual scoring log**. Select the export format. If you export to The Observer XT, select a **Text** option and select semicolon (;) as **Delimiter**.

3. Click **Start export**.

For more options see Export the raw data (track and dependent variables).

## Notes

- See Import manually scored data into The Observer XT.
- If you export to Excel, the manually scored data are stored in a separate tab of the Excel worksheet. The tab is named Manual scoring - [arena name].
- If you export to Text, data of different arenas are exported to different files.

# Export Trial Control data

Choose between two ways to export the Trial Control data, depending on the file structure you require.

- As dependent variables. Data are saved to additional columns of the track data file, and each sample point receives a “0” or “1” depending on whether a state (or event) is active at that time.
- As a Trial Control log. Data are written to a separate file, where all events occurred during the trial (also those not included in the Analysis Profile) are stored as lines each with a time stamp.

## To export Trial Control data as dependent variables

1. Define Trial Control events and states in the Analysis profile (see Trial Control in the Analysis profile).
2. Choose **Analysis** > **Export** > **Raw Data**. Select **Track & dependent variables** then click **Start export**.

Below: Part of a raw data file containing Trial Control data. The columns Frequency Light on and Duration Light on are dependent variables defined in an Analysis profile. Frequency Light on is a Trial Control event defined by the action “Light on”. Duration Light on is a state interval that goes as long as the light is on. At trial time 40.48, a light device switches on. This is marked by Frequency Light On which gets the value 1. From that moment, the variable Duration Light On gets value 1.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | Sync statu | Not available | | | | | | | | | | |
| 26 | Reference | + 0:02:12.1 | | | | | | | | | | |
| 27 | Reference | 56:02.0 | | | | | | | | | | |
| 28 | Sof file | | | | | | | | | | | |
| 29 | **User-defined Independent Variable** | | | | | | | | | | | |
| 30 | Animal ID | | | | | | | | | | | |
| 31 | Day | | | | | | | | | | | |
| 32 | Dose | | | | | | | | | | | |
| 33 | | | | | | | | | | | | |
| 34 | **Trial time** | **Recording** | **X center** | **Y center** | **X nose** | **Y nose** | **X tail** | **Y tail** | **Area** | **Frequency Light on** | **Duration Light on** | **Interva** |
| 536 | 40.08 | 40.08 | -39.1441 | -7.90048 | -42.475 | -7.1493 | -37.4353 | -13.0462 | 27.841 | 0 | 0 | |
| 537 | 40.16 | 40.16 | -39.1443 | -7.21036 | -42.475 | -7.1493 | -37.4353 | -13.0462 | 28.45053 | 0 | 0 | |
| 538 | 40.24 | 40.24 | -39.1196 | -6.359 | -42.475 | -7.1493 | -37.4353 | -13.0462 | 29.93849 | 0 | 0 | |
| 539 | 40.32 | 40.32 | -39.1171 | -5.26601 | -38.0493 | -1.01125 | -40.4126 | -9.18471 | 29.57994 | 0 | 0 | |
| 540 | 40.4 | 40.4 | -39.1235 | -4.30661 | -38.7624 | 1.013074 | -40.0025 | -7.88315 | 30.74521 | 0 | 0 | |
| 541 | 40.48 | 40.48 | -39.1372 | -3.35561 | -38.9161 | 2.922175 | -40.2511 | -6.17149 | 31.71328 | 1 | 1 | |
| 542 | 40.56 | 40.56 | -39.1508 | -2.05771 | -38.9161 | 2.922175 | -40.2511 | -6.17149 | 30.04605 | 0 | 1 | |
| 543 | 40.64 | 40.64 | -39.1889 | -0.37162 | -38.9161 | 2.922175 | -40.2511 | -6.17149 | 31.56987 | 0 | 1 | |
| 544 | 40.72 | 40.72 | -39.2659 | 0.434588 | -40.3744 | 6.698809 | -38.535 | -2.95356 | 34.29481 | 0 | 1 | |
| 545 | 40.8 | 40.8 | -39.4444 | 1.210378 | -40.3744 | 6.698809 | -38.535 | -2.95356 | 27.60795 | 0 | 1 | |

## To export Trial Control data as a Trial Control log

1. Choose **Analysis** > **Export** > **Raw Data**.

2. Select **Trial Control log** and click **Start export**.

Below: Part of a Trial Control log. Activation of conditions and actions is written as an event with a time stamp. The event is written in the Event column. The next columns describe which type of Trial Control element becomes true/false/active/inactive.

| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | Video file | D:\SDC\Media files\10 min C57Bl6 4 kooien mpg2.mpg | | | | | | | | | | |
| 20 | Video star | 53:54.0 | | | | | | | | | | |
| 21 | Trial status | | | | | | | | | | | |
| 22 | Acquisitio | Acquired | | | | | | | | | | |
| 23 | Track stat | Acquired | | | | | | | | | | |
| 24 | Video file | External | | | | | | | | | | |
| 25 | Sync statu | Not available | | | | | | | | | | |
| 26 | Reference | + 00:02:14.6 | | | | | | | | | | |
| 27 | Reference | 53:54.0 | | | | | | | | | | |
| 28 | Sof file | | | | | | | | | | | |
| 29 | | | | | | | | | | | | |
| 30 | **Trial time** | **Recording** | **Event** | **Rule** | **Condition** | **Action** | **Operator** | **Reference** | **Variable** | **Value** | **Reference1** | **Repeat1** |
| 31 | 1 | 0 | | | | | | | Var1 | 0 | | |
| 32 | 1 | 0 | becomes active | Start-stop trial | | | | | | | | |
| 33 | 1 | 0 | becomes active | | | Start track | | | | | | |
| 34 | 1 | 0 | becomes inactive | | | Start track | | | | | | |
| 35 | 1 | 0 | becomes active | Sub-rule (1) | | | | Reference (1) | | | | |
| 36 | 1 | 0 | makes sub-rule active | Sub-rule (1) | | | | Reference (1) | | | | |
| 37 | 1 | 0 | becomes active | Sub-rule (1) | | | | | | | Reference (1) | 0 |
| 38 | 1 | 0 | becomes active | | In zone (2) | | | | | | Reference (1) | 0 |
| 39 | 1 | 0 | becomes false | | In zone (2) | | | | | | Reference (1) | 0 |
| 40 | 1.04 | 0.04 | becomes true | | In zone (2) | | | | | | Reference (1) | 0 |
| 41 | 1.04 | 0.04 | becomes inactive | | In zone (2) | | | | | | Reference (1) | 0 |
| 42 | 1.04 | 0.04 | becomes active | | | Variable act (1) | | | | | Reference (1) | 0 |

The Trial Control log file contains the following columns:

- **Trial time** and **Recording time**. See Reading the raw data file for the difference.
- **Event**. The type of trial control event. For an explanation of the terms, see How the trial control instructions are executed.
- **Rule**, **Condition**, **Action**, **Operator**, **Reference**. The Trial control element that is involved in the event described in the corresponding **Event** cell.
- **Variable**, **Value**. The variable that is subject to an action, and the new value assigned to the variable.
- **Reference1**...**4**, **Repeat1**...**4**. Marks the time that a sub-rule is active. Repeat indicates the number of times that sub-rule has been repeated up to that time.

To export log files of the hardware devices operated during the trial, select also the **Hardware log** option.

For more information, see **Analysis of Trial Control data** in the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

# Import data into Excel

## To import analysis results

Analysis results here refers to the table in the Trial Statistics or Group Statistics tabs.

1. Export the statistics result, with Excel as format.
2. Open the file in Excel.

## To import EthoVision XT raw data and dependent variables

1. Export the raw data (track and dependent variables).
2. Open the file in Excel.

# Import manually scored data into The Observer XT

## Aim

To analyze the events scored manually in EthoVision XT in The Observer XT, for further analysis.

## Prerequisite

In EthoVision XT, export the data in the form of Manual scoring log (for details see Export manually-scored behaviors). Before clicking **Start Export**, select **Manual Scoring log**; select one of the **Text** options as export format, and select semicolon (;) as **Delimiter**.

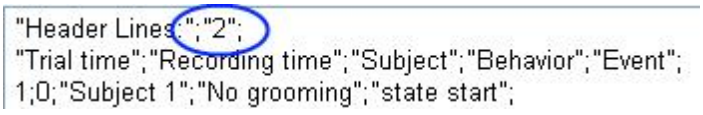
To export a subset of tracks or subjects, filter the data before exporting.

## Procedure

1. Open the export file in the Windows Notepad.
2. Remove the lines from **"Experiment"** to **""** so that the first line of the file contains **"Header Lines"** and the second **"Trial time"**.

   ```
   "Header Lines:";"30";
   "Trial time";"Recording time";"Subject";"Behavior";"Event";
   1;0;"Subject 1";"No grooming";"state start";
   13.36;12.36;"Subject 1";"No grooming";"state stop";
   ```

3. Change the number of header lines to **2**, then save and close the file.

4. In The Observer XT, create a project or open an existing one. Make sure that the project's coding scheme contains exactly the same behavior names and that these are organized in the same groups (mutually-exclusive or start-stop) as the corresponding behaviors scored in EthoVision XT. See Effects on the coding scheme below.
5. Choose **File** > **Import Observational Data**.
6. From the **Files of type** list, select E**thoVision XT Data File (*.txt)**.

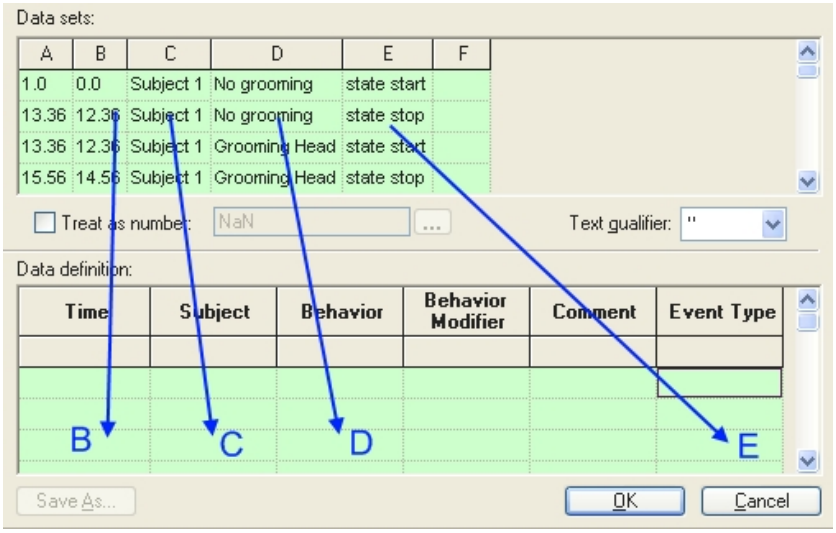
   EthoVision XT Data File is an import profile made for manual scoring logs exported from EthoVision XT. It is installed automatically when you install

EthoVision XT. If you do not have this import profile, click **Create New** and see below.

7. Select the file you want to import and click **Open**.
8. Repeat the procedure for the remaining export files (remember that each trial and arena results in a separate export file).
9. Choose **Setup** > **Independent Variables**. Define the independent variables and enter the values of those variables for the trials you have imported.

## To create an import profile in The Observer XT

In the Import Profile Definition window, drag the columns of the **Data Sets** box as shown here:




- *Time*. Drag the column **B** (Recording time) to the **Time** column. This is the time of manually scored behaviors relative to the time that tracking started. In the Select Time Format window, if the time matches one of the predefined formats, EthoVision XT automatically selects one. **Converted time** shows the converted time and Conversion is OK. Click **OK**.
- You can also define your own format by typing an 'H' for each number representing 'hour', an 'm' for 'minute', an 's' for second and a 'f' for each number representing millisecond (see the next picture). If Conversion is OK, then click **OK**.
- Under **Import Time**, select one of the options.
  - **Relative to time zero**. Suppose the first row in the imported external data set has time 00:00:05. When the option Relative to time zero is selected, the time stamp of the first row of the imported data set will remain 00:00:05.

    - **Relative to the time of the first data line**. Suppose the data set you want to import starts at 14:28:00 and sample every 5 seconds. With the option Relative to the time of the first data line, the first row of the imported data will get the time stamp 00:00:00. The second row will have time stamp 00:00:05. The options under **Import Time** are grayed out when your time stamps contain the date the file was created. In this case the option Relative to time zero is used.
- Do not drag the column **A** (Trial time) as this provides the same timing as column **B** when imported into The Observer.
- The predefined import profile EthoVision XT Data File uses the Recording time as time code for the manually scored behaviors.
- Event Type. Drag the E column and click OK in the windows that appears.
- If you want to import video in The Observer, you must keep the correct sync between events and video. See To import video in The Observer XT

## Effects on the coding scheme

If you import manually scored data without creating a coding scheme in advance, at import of the first file The Observer creates a coding scheme with all behaviors contained in that file. By default, all behaviors are put in a start-stop group named Behavior1. To re-arrange the coding scheme, delete the observation just imported, move the behaviors of Behavior1 to the groups they belong to, and re-import the data.

## Independent variables

The Observer does not import the independent variables from EthoVision XT. You must define those variables in the Observer's Independent Variables List and assign the values of those variables to each imported file. You can find those values in the headers of the original export files.

## Data from multiple arenas

If your setup includes multiple arenas, for each trial you import one file per arena. If you want to keep data from different arenas (in the same trial) in the same observation, you can import the files of the second, third, etc. arena in the same observation as that of the first arena. To so, do the following:

- Follow the procedure above for the first arena. Right-click the name of the resulting observation and select Properties. Edit the observation name by giving a name that specifies the trial only, not the arena (for example Open Field Trial 12).

- For the second, third, etc. arena file, instead of following step 6 of the procedure above, in the Project Explorer right-click the observation name and select Import Observational Data, then follow the rest of the procedure. The files are imported in the observation of the first arena as separate event logs. Rename each event log in such a way it clearly indicates the arena from which it was obtained.

## To import video in The Observer XT

1. Open the observation, and from the **Observe** menu select **Video**, then **Open in Current Observation** and select the video file.
2. Choose the option below that applies:
   - If the video file was obtained with programs other than EthoVision XT, or if you tracked from video previously recorded with EthoVision, take note of the difference between the **Start time** and the **Video start time** (you can find these in the headers of the raw data export file). This difference is 0 only if you start the trial from the first video frame. Also, take note of the value of **Recording after** (also available in the export file). Calculate the value of Offset = Start time - Video start time + Recording after.
   - If the video file was recorded with EthoVision while doing live tracking, take note of the value of Recording after (you can find this in the headers of the raw data export file). In your case Offset = Recording after.
3. In The Observer XT, click the **Offset** button.

4. Then choose **Numerical Offset** and locate the imported event log. In the corresponding cell, enter the **Offset** calculated as described above, and make sure that the sign stays positive (+).
5. Open the observation, click the start line of the event log. The video is positioned to the point that the tracking started.

*See also*

- Trial vs. track
- For more information on creating an import profile for The Observer XT, see Importing other observational data in The Observer XT Help.

# Camera Installation

## Main topics and tasks

# Choose your camera

## Learn about

- Types of cameras
- Cameras supported by EthoVision XT
- Using multiple GigE cameras

# Types of cameras

You should select the video camera most suitable for your needs:

## Color vs. monochrome

Monochrome cameras are cheaper, more compact and usually have greater light sensitivity. If you want to film in near darkness, you must use a monochrome (or specialist IR) camera. If you want to track multiple animals and distinguish them on the basis of color markers, you (of course) need a color camera.

*See also*

- Tips for color tracking
- Camera position and settings
- Lighting setup

## Digital vs. analog

The advantage of analog over digital cameras used to be that they can be placed at considerable distance from the computer and that they were more light sensitive. However, this is no longer true.

Furthermore, current digital cameras can be as light sensitive as analog cameras.

## GigE Vision digital cameras

Henceforth called GigE cameras.


Noldus can supply a GigE camera that is sensitive to 0.5 lux, and to near IR light. You can connect up to four GigE cameras to a desktop computer. See Install GigE cameras

Advantages of GigE cameras:

- You can use much longer cables than, for example, USB cameras. This way you can place the camera further away from the PC. When working with rodents, this solves the problem of ultrasound emissions from computers, which stress animals.

- You do not need an encoder board to convert the analog signal into digital video.
- Relative to analog cameras, you can use a higher frame rate and a greater video resolution. However, recording video with a GigE camera requires a lot of computer resources, which may potentially decreases the quality of tracking. Therefore, lower the frame rate at high resolution, and vice versa.

## USB 3.0 digital cameras


USB 3.0 cameras can create video of higher resolution and frame rate than analog cameras. However, the cable that connects the camera to the PC may up to 5 m long, unless you use an powered repeater cable. A 20-m powered repeater cable works good with EthoVision XT.

- EthoVision XT supports the use of one USB 3.0 camera. See USB 3.0 cameras
- Most USB 2.0 cameras are not accurate enough for video tracking with EthoVision XT. See USB 3.0 cameras

## FireWire (IEEE 1394 standard)

EthoVision XT does not support FireWire cameras. The Imaging Source FireWire camera of older DanioVision systems is no longer officially supported.

## Internet Protocol (IP) cameras

IP cameras are definitely cheaper than industrial GigE or USB 3.0 cameras, however they are less accurate in the timing of video frames sent out to the EthoVision XT computer. This can cause EthoVision XT to skip a number of video frames during tracking.

If you use IP cameras, for information open the Help of EthoVision XT 17 or earlier, which you can download from my.noldus.com.

## Webcams

EthoVision XT could in principle work with most webcams. However, we do not support and do not recommend to use webcams when timing of video frames is important. For example, you can use a webcam to film a mouse in an open field, with the aim of measuring the distance moved. However, do not use webcams to

track multiple animals in an apparatus, or in multiple apparatuses, or when you use the Behavior Recognition, or whenever precise timing of events and behavior is needed.

Furthermore, test if the webcam still works when other software needed to run during the trials is open, for example PowerPoint for stimulus presentation.

## Other cameras

If you want to use a camera model other than one of the models listed under Cameras supported by EthoVision XT, before starting an experiment make sure that the timing of the camera is accurate enough for a frame-level video analysis. To do so, create a live tracking experiment, and carry out a test trial with the video footage of a digital clock for some ten minutes. Just before stopping the trial, check that the trial duration shown on your screen does not deviate from that obtained with the clock time.

Also check the Noldus support center for more information. See Noldus Support

*See also*

- Cameras supported by EthoVision XT

# Cameras supported by EthoVision XT

## Overview

The maximum number of cameras that you can use depends on whether EthoVision XT is installed on a desktop or laptop computer. The figures reported in the following tables refers to the configurations tested.

For more details see Resolution, frame rate, and maximum trial duration

**IMPORTANT** You can use one, two, three, four, eight, 12 or 16 cameras simultaneously. Using for example 5 cameras is not supported. Furthermore, we do not support the use of a combination of different camera types.

| **Type** | **Brand/Model** | **Max number** | **Interface board (for Desktop)** | **Driver version** (a) | **Frame rate (fps)** |
|---|---|---|---|---|---|
| GigE | Basler acA1300-60gm (monochrome) | Desktop: 16<br>Laptop: not tested, not supported | CT1000 Pro (1 camera)<br>Adlink PCIe-GIE74 (1-4 cameras) (c)<br>Basler GigE Interface Card, 4 port PoE (1-4 cameras) (c)<br>FS X550AT2-2TP, 2-port (1-16 cameras) (c) | 7.5.0.15658 | 60 |
| GigE | Basler acA1920-40gc (color) (b) | Desktop: 4<br>Laptop: not tested, not supported | CT1000 Pro (1 camera)<br>Adlink PCIe-GIE74 (1-4 cameras) | 7.5.0.15658 | 40 |
| USB 3.0 | Basler acA1920-155um (monochrome) | Desktop: 1<br>Laptop: 1 | U3-PCIE1XG205 - 1S, U3X4-PCIE4XE111 | 7.5.0.15658 | 60 |
| Analog | PhenoTyper 1 (f) | Desktop: 16<br>Laptop: not tested, not supported | Picolo Alert (1-4 cameras) (g) | Multicam 6.15.1.3573 | 25 (PAL)<br>29.97 (NTSC) |

*Drivers*

- (a) You can find the drivers for the supported cameras on the full installation package, or on my.noldus.com, under **Downloads** > **EthoVision XT** > **Drivers and Tools**.

*GigE cameras*

- (b) Recommended for color tracking in low light (< 10 lux).
- (c) To connect multiple GigE cameras, see Using multiple GigE cameras and 16-camera configuration
- For resolution, frame rates etc. see GigE cameras

*USB 3.0 cameras*

- For resolution, frame rates etc. see USB 3.0 cameras

*Analog cameras*

- (f) In theory you could use any analog camera, in combination with the Picolo Alert encoder board. Test your camera thoroughly before running the actual trials.
- (g) One Picolo Alert board works with a maximum of 4 analog cameras, or 16 cameras in combination with Quad Unit Processors. See Connect analog cameras to the computer
- For resolution, frame rates etc. see Analog cameras.

*IP cameras and webcams*

- IP cameras and webcams are no longer tested and supported. If you wish to keep using those cameras, make sure you test them thoroughly before running the actual experiments. Make sure that the number of missing samples is not too high.

*See also*

- Resolution, frame rate, and maximum trial duration
- Restrictions in EthoVision XT
- Install GigE cameras

- Install USB 3.0 cameras
- Install analog cameras
- System requirements > Hardware

# Resolution, frame rate, and maximum trial duration

Click the link below for the camera type you are using. There you will find the main settings we used in our tests: the maximal resolution, frame rate and duration.

- In all tested configurations, EthoVision XT tracked the nose-, center- and tail-base point of **one subject** with the Contour-based method (that is, no Deep learning) and recorded video simultaneously. Video was recorded without audio. When using multiple cameras, one arena was defined for each camera. Each section reports which detection method worked with zero (or a low percentage of) missed samples.
- If you want to track the subjects using Deep learning, there are further restrictions. See Deep learning: Requirements.
- If a test was successful with Deep Learning (DL in the tables in the following sections), then it is assumed that Contour-based tracking (C) also works.
- The figures report the maximum time tested. Acquiring longer trials is possible in principle, but you must test your configuration thoroughly. Run a test trial with the desired resolution and frame rate, and check that the resulting video does not contain too many missing video frames, and check that the tracks obtained do not contain too many missing samples, for example less than 5%. See View the detection statistics
- **IMPORTANT** When tracking for more than 24 hours, always reboot the PC after every trial to refresh the PC resources, the RAM specifically. Failure to do so could result in crashing, which can corrupt the experiment, or the recorded video.
- **IMPORTANT** When you track many animals in multiple arenas, simultaneous tracking and video recording may go beyond the processor's capability. In those cases, choose **Save video, track later** in the Acquisition Settings window to be able to record at higher resolution without skipping video frames. See Record video, then acquire a trial
- **IMPORTANT** The test results reported here may differ from those you experience, even when using the same camera, PC and graphics card. For example depending on the lighting and the camera settings.

Click the camera type that applies:

- GigE cameras
- USB 3.0 cameras
- Analog cameras

# GigE cameras

## Basler acA1300-60gm

- Monochrome.
- Standard camera of PhenoTyper 2.
- Interface: Gigabit Ethernet (RJ45 connector, CAT5e or CAT6 (the latter recommended when using 16 cameras).
- Sensor: 1/1.8" Progressive Scan CMOS.
- Cable length: 10 m (standard) up to 100 m (not tested).
- Maximum number of cameras: 16.
- Recommended color space in EthoVision XT: Y800.
- This camera is also the standard camera in DanioVision and PhenoTyperversion 2.
- Tested on Operating system Windows 11 Pro, 23H2.
- Resolution: For configurations with two or more cameras, the resolution of the resulting video file is reported. The resolution of the single camera image is given in brackets.

  **EXAMPLE 1** 2560 x 2048 (1280 x 1024) means that four images 1280 x 1024 arranged 2x2 result in a video file with resolution 2560 x 2048. The resolution of each camera image in the video file is the same as the original value.

  **EXAMPLE 2** 1280 x 1024 (1280 x 1024) means that four images 1280 x 1024 arranged 2x2 result in a video file with the same resolution. That is, the resolution of the camera image in the video file is reduced to one half of the original value.

### *Tested configurations with 1 camera, desktop*

| Computer | Operating system | Resolution (W x H) | Frame rate (fps) | Duration (h) | DL or C |
|---|---|---|---|---|---|
| Dell Precision 3680 | W11 | 1280 x 1024 | 60 | 3 | DL |

**NOTE**

- Graphics card: NVIDIA T1000, 8 GB. Detection method: Dynamic Subtraction or Differencing. Missing samples: 0.1%.

### *Tested configurations with 2 cameras, desktop*

| Computer | Operating system | Resolution (W x H) | Frame rate (fps) | Duration (h) | DL or C |
|---|---|---|---|---|---|
| Dell Precision 3680 | W11 | 1920 x 540 (960 x 540) | 30 | 3 | DL |

**NOTES**

- Graphics card: NVIDIA T1000, 8 GB. Detection method: Dynamic Subtraction or Differencing. Missing samples: 0.0%.
- The cameras were connected to the network interface board FS X550AT2-2TP through a network switch FS 3100-16TMS. For this switch you need an adapter (SFP+ 10GBASE-T Copper 30m RJ-45 Transceiver module) attached to the Ethernet RJ45 connector. We recommend to use CAT6 network cables. Connect the cameras to the powered ports of the switch.
- See also Using multiple GigE cameras

### *Tested configurations with 4 cameras*

| Computer | Operating system | Resolution (W x H) | Frame rate (fps) | Duration (h) | DL or C |
|---|---|---|---|---|---|
| Dell Precision 3680 | W11 | 2560 x 2048 (1280 x 1024) | 25 | 3 | DL |
| Dell Precision 3680 | W11 | 2560 x 2048 (1280 x 1024) | 60 | 3 | C |

**NOTES**

- Graphics card: NVIDIA T1000, 8 GB. Detection method: Dynamic subtraction. Binning: 1 (see Binning). Missing samples: 0.0%.
- The cameras were connected to the network interface board FS X550AT2-2TP through a network switch FS 3100-16TMS. For this switch you need an adapter (SFP+ 10GBASE-T Copper 30m RJ-45 Transceiver module) attached

to the Ethernet RJ45 connector. Connect the cameras to the powered ports of the switch.

- See also Using multiple GigE cameras

### *Tested configurations with 16 cameras*

| Computer | Operating system | Resolution (W x H) | Frame rate (fps) | Duration (h) | DL or C |
|---|---|---|---|---|---|
| Dell Precision 3680 | W11 | 2560 x 1920 (640 x 480) | 30 | 72 | C |

**NOTES**

- Graphics card: NVIDIA T1000, 8 GB. Detection method: Dynamic Subtraction or Differencing. Missing samples: 0.0 %. Binning: 2 (see Binning).
- Body point detection technique: DL = Deep learning, C = Contour-based.
- For Deep learning, see also Deep learning: Requirements and GPUs tested and supported
- The cameras were connected to a network interface board FS X550AT2-2TP through a network switch FS 3100-16TMS. For this switch you need an adapter (SFP+ 10GBASE-T Copper 30m RJ-45 Transceiver module) attached to the Ethernet RJ45 connector. We recommend to use CAT6 network cables. With this switch, you need to connect eight cameras to eight PoE injectors because the switch has only eight powered ports. Instead, use a 16-powered ports switch (not tested).
- See also Using multiple GigE cameras and 16-camera configuration.

## Basler acA1920-40gc

- Color camera; recommended for tracking colors in dim light.
- Interface: Gigabit Ethernet (RJ45 connector, CAT5e or higher).
- Sensor: 1/1.2″ CMOS.
- Maximum number of cameras: 4.
- Recommended color space in EthoVision XT: **BYRG**.

- In the Basler pylon Viewer software the pixel format that corresponds to BYRG is **Bayer RG8**. See Settings for the color camera and Pixel format and color space
- Resolution: For configurations with two or more cameras, the resolution of the resulting video file is reported. The resolution of the single camera image is given in brackets.
- In brackets, the resolution of the single camera image is reported.

## *Tested configurations, desktop*

| Computer | Operating system | Nr. cameras | Resolution (WxH) | Frame rate (fps) | Duration (h) | DL or C |
|---|---|---|---|---|---|---|
| Dell Precision 3680 | W11 | 1 | 1920 x 1200 | 30 (DS, DF) | 3 | DL |
| Dell Precision 3680 | W11 | 4 | 3840 x 2400 (1920 x 1200) | 15 (DS) | 12 | DL |

**NOTES**

- Detection method tested: Dynamic subtraction (DS) or Differencing (DF). Missing samples 0.0%. For tracking monochrome video: color space Y800. For color-marker tracking: color space BYRG.
- Body point detection technique: DL = Deep learning, C = Contour-based. For Deep learning, see also Deep learning: Requirements
- Desktop: Dell Precision 3680, Graphics card NVIDIA T1000 8 GB, AdLink 4-port PCIe-GIE74 network interface board. For deep learning see also GPUs tested and supported.

## *See also*

- Cameras supported by EthoVision XT

# USB 3.0 cameras

## Basler acA1920-155um

- Monochrome.
- Interface: USB 3.0. A separate PCIe USB 3.0 interface board is required.
- Sensor: 1/1.2" CMOS.
- Cable length: 3 m (standard) or 20 m (with powered extension cable, not tested).
- Maximum number of cameras: 1.
- Recommended color space in EthoVision XT: Y800.
- See Install USB 3.0 cameras

### *Tested configurations with 1 camera*

| Computer | Operating system | Resolution (WxH) | Frame rate (fps) | Duration (h) | DL or C |
|---|---|---|---|---|---|
| Desktop Dell Precision 3680 | W11 | 1920 x 1200 | 60 (DS) | 12 | DL |
| Laptop Dell Precision 3591 | W11 | 1920 x 1200 | 25 (DS, DF) | 12 | DL |

**NOTES**

- Detection method used: Dynamic subtraction (DS) or Differencing (DF). Missing samples 0.0%.
- Body point detection technique: DL = Deep learning, C = Contour-based.
- Desktop: Dell Precision 3680, Graphics card NVIDIA T1000 8 GB. The camera was connected through a Basler USB 3.0 PCIe Interface card.
- Laptop: Dell Precision 3591, Graphics card NVIDIA RTX 1000. The camera was connected through a USB 3.0 port on the motherboard.
- For deep learning see also GPUs tested and supported.

### *See also*

- Resolution, frame rate, and maximum trial duration

# Analog cameras

## PhenoTyper 1

- TV Standard: PAL and NTSC.
- Cable length: up to 100 m (not tested).
- Maximum number of cameras: 16 when using one Picolo Alert encoder board and four video processors (Quad Units). See Install analog cameras
- Resolution: For configurations with two or more cameras, the resolution of the resulting video file is reported. In brackets, the resolution of the single camera image is reported.
- **IMPORTANT** When mixing 16 PhenoTyper 1 cameras, the resolution of a single camera image is 384 x 288 (PAL) or 320 x 240 (NTSC). This resolution is often too low for nose-tail tracking. If you want to use the Deep learning-based method to track subjects, work with up to four analog cameras (and four arenas).

### *Tested configurations with 4 cameras, desktop*

| Computer | Video | Resolution (WxH) | Frame rate (fps) | Duration (h) | DL or C |
|---|---|---|---|---|---|
| Dell Precision 3680 | PAL | 1536 x 1152 (768 x 576) | 25 | 3 | DL |
| Dell Precision 3680 | NTSC | 1280 x 960 (640 x 480) | 29.97 | 3 | DL |

### *Tested configurations with 16 cameras, desktop*

| Computer | Video | Resolution (WxH) | Frame rate (fps) | Duration (h) | DL or C |
|---|---|---|---|---|---|
| Dell Precision 3680 | PAL | 1536 x 1152 (384 x 288) | 25 | 72 | C |
| Dell Precision 3680 | NTSC | 1280 x 960 (320 x 240) | 29.97 | 72 | C |

**NOTES**

- Detection method used: Dynamic subtraction or Differencing. Missing samples: 0.0 %.

- Body point detection technique: DL = Deep learning, C = Contour-based.
- Desktop: Dell Precision 3680, Graphics card NVIDIA T1000 8 GB, Picolo Alert encoder board. For deep learning see also GPUs tested and supported
- Quad unit used for testing 16 camera: UHPPOTE 4CH Color Quad System.

*See also*

- Resolution, frame rate, and maximum trial duration
- Connect analog cameras to the computer
- The PhenoTyper - EthoVision XT 18 - Reference Manual. See Manuals

# Install GigE cameras

Together with the Basler GigE camera, you need a 1Gb Ethernet board installed in your computer. To connect the camera, you need at least cat-5e cables.

**IMPORTANT** To install the camera, follow each step below. Most information applies to the Basler GigE cameras.

## What do you want to do?

- Install an Ethernet board for GigE cameras
- Install the driver software for the digital cameras
- Assign IP addresses
- Disable the Windows firewall for the Ethernet board
- Connect the GigE camera to the PC
- Configure the digital camera
- Using multiple GigE cameras

*See also*

- Cameras supported by EthoVision XT

# Install an Ethernet board for GigE cameras

## Aim

To install a network board that receives video from one or more GigE cameras.

**NOTE** If you ordered a computer from Noldus Information Technology when you purchased EthoVision XT and Basler GigE cameras, the Ethernet interface board(s) has already been installed and tested. If you bought your computer somewhere else, you will have to install the board yourself. Follow the instructions below.

**IMPORTANT** The network interface board must support data transfer of 1 Gb/s.

This topic applies to Ethernet interface boards that have been tested with EthoVision XT.

- One-port board: the Intel PRO/1000 CT (or its successor), to connect one camera.

- Four-port board: the Adlink PCIe-GIE74 (A), or the Basler GigE Interface Card (B) to connect up to four cameras.


- Two-port board: FS X550AT2-2TP. This was combined with an optical switch FS 3100-16TMS to connect 16 GigE cameras. See 16-camera configuration

## Procedure

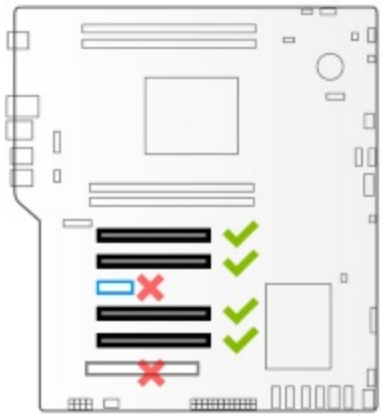
1. Turn off your computer and all connected peripherals, such as the monitor and printer. Make sure that the computer is unplugged.
2. Remove the PC's case according to the instructions provided in the PC's user manual.
3. Select a free PCIe expansion slot, and remove the corresponding extension cover.

   Different PCIe slots have different properties, resulting in different performances. When possible, choose the slot that gives maximum performance. To estimate performance, take note of the slot version (2, 3, etc.) and compare it with the following:

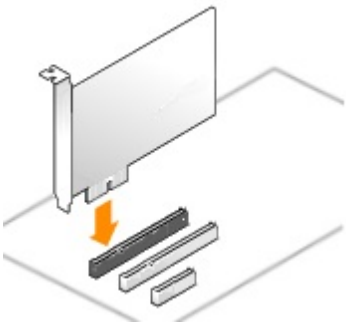
   - PCIe v1.x: 250 MB/s
   - PCIe v2.x: 500 MB/s
   - PCIe v3.0: ~1 GB/s
   - PCIe v4.0: ~2 GB/s
   - PCIe v5.0: ~4 GB/s
   - PCIe v6.0: ~8 GB/s

   Note that values are given per lane; they should not be multiplied by the number of lanes in a slot (e.g. x4) since the board has a 1x connector). For example, **SLOT1-PCIe3x4** written near the slot means version 3.0 with four lanes.

   **IMPORTANT** Do not insert the PCIe board in the blue or white slots.



4. Unpack the Ethernet board, place it into the slot, and press it carefully into position. If the board does not fit into place easily, remove it and repeat the operation.



**IMPORTANT** When touching the board, its electronic components can be damaged by static electricity. To avoid any such risk, make sure that you are grounded. You can ground yourself by putting on an earthing wristlet, and attaching its clip to the metal frame of the computer. If you do not have an earthing wristlet, hold the metal frame with one hand while holding the Ethernet board in your other hand. Ensure also that your clothing does not touch any components while handling the board.

5. This step is only for when installing a 4-port board.

   Connect the power connector on the board (Molex/IDE male 4-pin DC) to one of the power supplies inside the PC. See Power the Ethernet board for the GigE cameras

6. Fix the board to the chassis and re-fit the computer's cover.
7. **IMPORTANT** If you have not done yet so, turn off *Fast startup* in the Windows Power options. See Power options when using cameras
8. Next: Install the driver software for the digital cameras.

## Main settings of the Ethernet board

You may need to check these settings if the PC / camera are set correctly based on this Help and yet you get camera issues in EthoVision XT:

1. In Windows, open the **Device Manager**.
2. Under **Network adapters**, locate your Ethernet board, right-click on it and select **Properties**.
3. Click the **Power Management** tab.
4. Make sure that **Allow the computer to turn off this device to save power** is not selected.

## Notes

- Here is some information from Basler about network interface boards and which ones are supported with the GigE cameras:

  https://docs.baslerweb.com/hardware-installation-(gige-cameras)#basler-1gige-cameras

  See also the knowledge base of Basler:

  https://docs.baslerweb.com/knowledge-articles#gige

- **IMPORTANT** Turn off *Fast startup* in the Windows Power options. See Power options when using cameras

# Power the Ethernet board for the GigE cameras

## Aim

To power the GigE cameras without the PoE injectors.

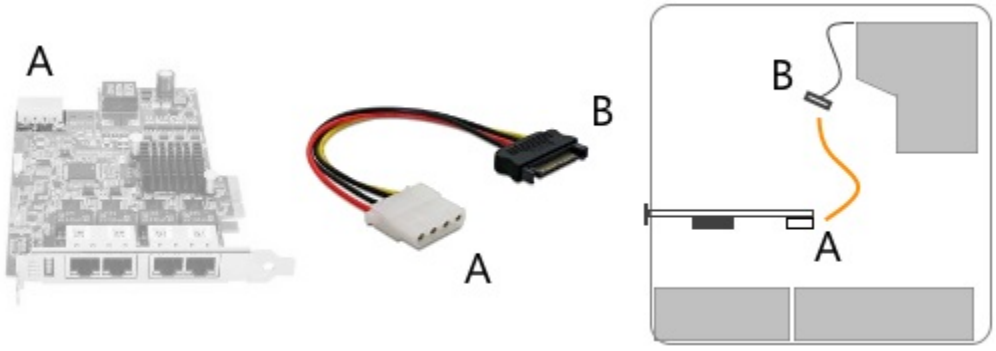
This topic applies when you have a 4-port Ethernet interface board to connect the GigE cameras to your EthoVision XT computer.

## Prerequisites

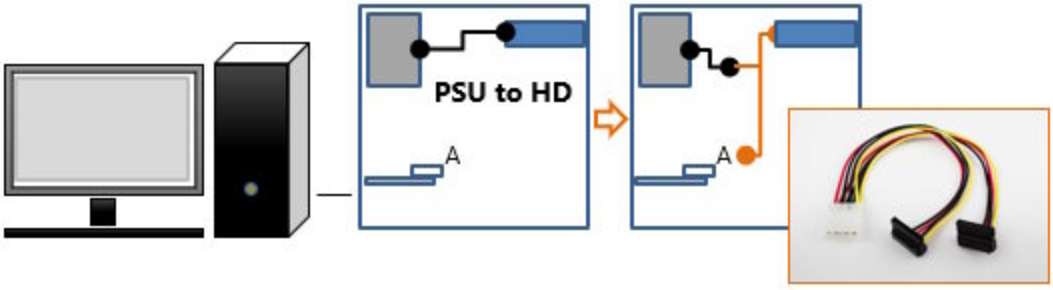
- You have installed a 4-port Ethernet board. See Install an Ethernet board for GigE cameras

## Procedure

Power cable and connector types vary between computers. Many computers have a SATA 15-pin power connector. Use an adapter cable with a 4-pin female Molex connector at one end and a SATA 15-pin connector at the other end.




If a separate cable is not available, you can disconnect the cable that from the power supply (PSU) goes to the hard disk (HD) and connect that end to a Y adapter cable, whose other two ends go to the hard disk and to the Ethernet board:




## Notes

- When your Ethernet board is not powered, you must power the camera using a Gigabit Power-over-Ethernet injector (PoE injector; one per camera). The data transfer rate of the PoE injector must be 1 Gbit/s. See Connect the GigE camera to the PC

# Install the driver software for the digital cameras

## Aim

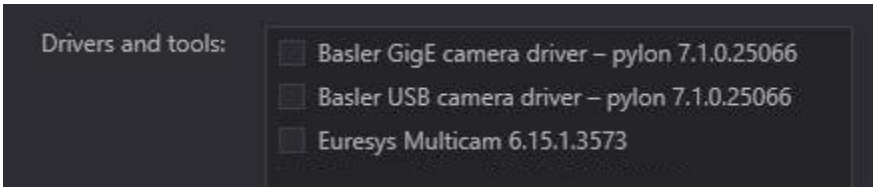
To install the software that enables you to configure the camera. For example, to set the video frame rate or the exposure time.

**IMPORTANT** Whenever possible, install the Basler driver software from the setup file of EthoVision XT, following the procedure below, not from the original Basler driver installation file **Basler_pylon_[version number].exe**. If, for any reason, you must install the driver from that file, follow the note at the end of this topic.

This topic applies to Basler GigE cameras (model name ending with **gm** or **gc**), and Basler USB cameras (model name ending with um).

## Procedure (recommended)

1. If you have not done so, download the EthoVision XT installation files from my.noldus.com.
2. Double-click **EthoVision XT - Setup - [version number].exe**. If you have already installed EthoVision XT, select **Modify**; otherwise select **Install**.
3. Under **Drivers and tools**, select**:**
   - **Basler GigE camera driver** if you have Basler GigE cameras.
   - **Basler USB camera driver** if you have Basler USB 3.0 cameras.




4. Click **Install/Update** and continue with installation.
5. Next: Assign IP addresses.

## Procedure (manual installation)

If you need to install the camera driver software from the original installation file, do the following:

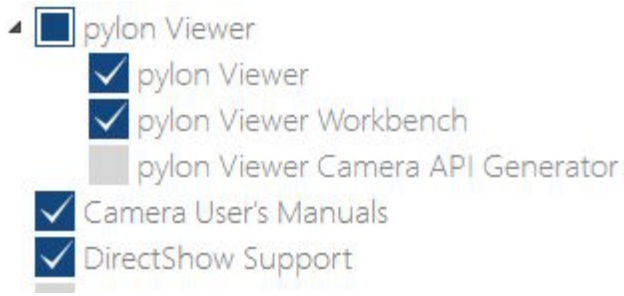
1. If you have the installation file already, make sure that its version is the version that EthoVision XT uses. For Basler cameras, this is Basler_pylon_**7.5.0**. Skip the next step and go to step 3.
2. To download the driver, log in to my.noldus.com. Choose **Downloads** > **EthoVision XT** > **Drivers and Tools**.

   For Basler cameras, choose **Basler Camera Driver**.

   Extract its content and copy everything to the local disk.
3. Double-click the exe file. Accept the Terms and Conditions, then click **Next**.
4. For the Basler driver: Under **Profiles**, select **Custom** and click **Next**.
5. For the Basler driver: Under **Features**, choose:
   - For GigE Vision cameras, **GigE Camera Support**.

     
     
   - For USB 3.0 cameras, **USB Camera Support**.

     
     
6. Also select:
   - **pylon Viewer** and
   - **DirectShow Support**.




7. Complete the installation.

## Notes

- Always install the drivers that you download from the Noldus web site. Do not install drivers from other sources, unless requested by Noldus Support.

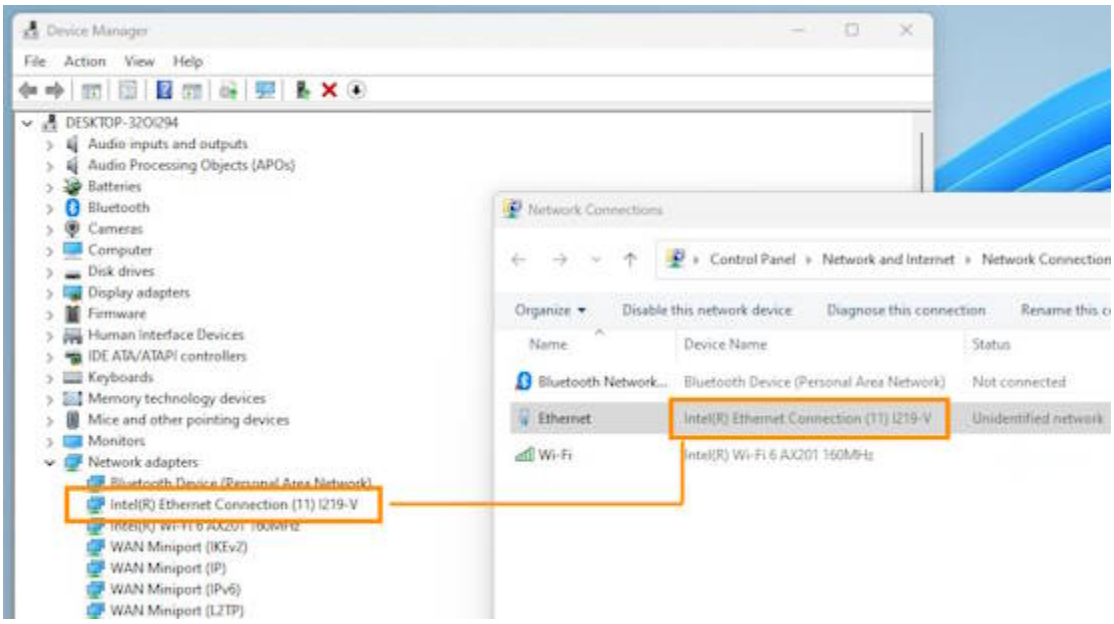
# Settings of the Ethernet board

## Aim

If camera is setup correctly based on this Help and you have checked all the System requirements and yet there are camera issues in EthoVision XT, e.g. dropped frames, it may be good to check the settings of the Ethernet interface board.

## Procedure

1. In the Windows search field, type in *device*. In the results list click **Device Manager**.
2. Open the **Network adapters** item.
3. Locate the item that has the same name as the network port connected to the camera.




4. Right-click that item in the Device Manager and select **Properties**, then click the **Advanced** tab.
5. Adjust the settings as here:
   - **Interrupt Moderation**: **Disabled**.
   - **Interrupt Moderation Rate**: **Off**.
   - **Receive Buffers**: **2048**.
   - **Jumbo Packet**: **9014 Bytes**.

6. Click the **Power Management** tab and de-select **Allow the computer to turn off this device to save power**.

# Connect the GigE camera to the PC

## Aim

To make sure that EthoVision XT receives the video signal from the GigE camera.

## Prerequisites

- You have followed the entire procedure in Install an Ethernet board for GigE cameras.
- No matter how many cameras you use, you need a 1-Gb Ethernet interface board (desktop computers only).
- When you use a 4-port Ethernet board, you can power the cameras with Power-over-Ethernet (PoE) injectors but you can also choose to connect the board to the power outlet inside the PC. See Power the Ethernet board for the GigE cameras
- You must use a 1-Gb (1 Gigabit/s) Power-over-Ethernet (PoE) injector:
  - When the Ethernet interface board is not powered. This is usually the case for 1- or 2- port boards. Also 4-port boards may not be powered - See Power the Ethernet board for the GigE cameras
  - When you use a network switch ant this has RJ45 ports that are not powered. In all cases, use one PoE injector per camera.

- Cables: Use Cat5e or Cat6e crossover cables.
- Do not use an Ethernet to USB adapter (in the picture below) to connect the camera to the PC. Always connect the camera and the PC directly using an Ethernet crossover cable.

## Procedure

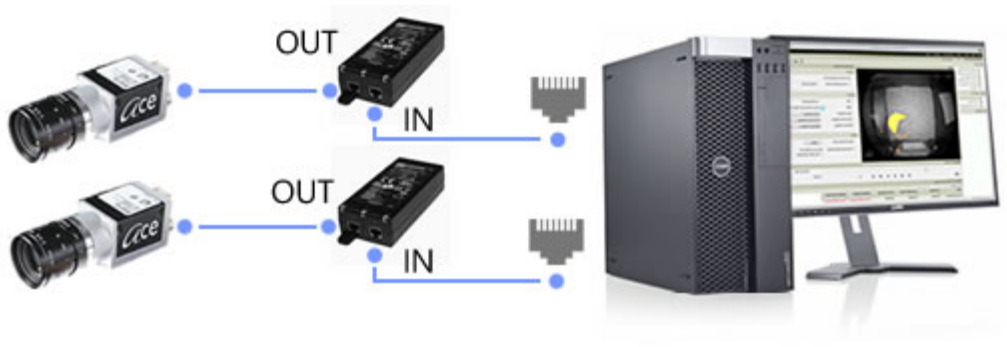
### *If you have a single or dual port Ethernet board*

To connect one camera, you need a single or dual port Ethernet interface board. To connect two cameras, you need a dual port Ethernet interface board. If the board is not powered, you need as many PoE injectors as cameras. Only use 1Gbit/s PoE injectors.

1. Using cross-network cables (cat-5e or newer), connect the Ethernet port/ board on the PC to the **IN** port of the PoE injector. Connect the camera to the **OUT** port. Use the short cable between PoE injector and PC. Use the long cable between PoE injector and camera.
2. Power up the PoE injector; make sure that the two **CONNECT** and **ON** green LEDs are on.

   If the green LEDs keep blinking, wait a few more seconds. If you see no change, disconnect the cables, then re-connect them.
3. Next: Configure the digital camera.

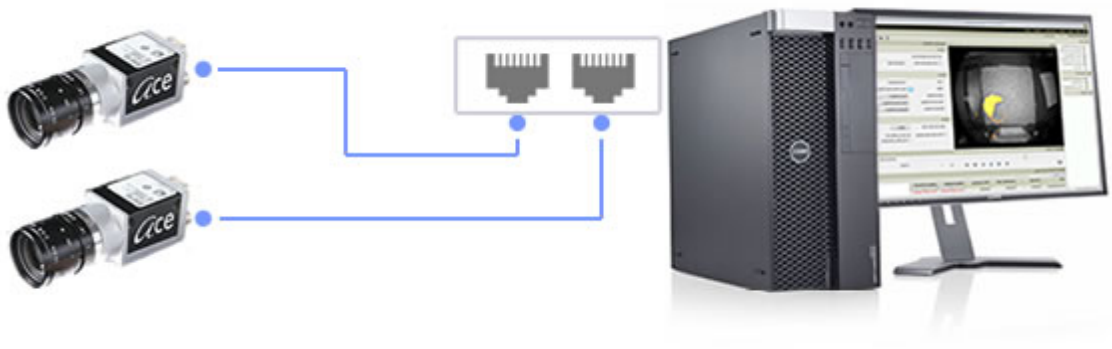

You can also connect two cameras to two single-port Ethernet boards:




*If you have a four-port Ethernet board*

When you use this board, the PoE injector is not required. However, **the Ethernet board must receive power from the PC**. See the connection scheme in Power the Ethernet board for the GigE cameras

1. Connect each camera (up to four) to one of the ports on the Ethernet board, as shown in the figure below.
2. Next: Assign IP addresses.



## Notes

- **TIP** Label the cameras and the Ethernet ports (for example Cam 1, Cam 2 etc.). Take note of the camera's serial numbers; these actions may be useful when you need to know which camera is connected to which port and assign or adjust the IP addresses.

- The Power over Ethernet (PoE) injector is a device that passes power along with data (in this case, video data from the camera) on twisted-pair Ethernet cabling.
- If you bought your camera elsewhere, make sure you bought the correct Ethernet cables. Cheap or incorrect cables will affect frame rate and result in missing samples. Always use Cat5e, Cat6 or Cat7.
    - Cat6 and Cat7 cables support higher network speeds (5 Gb/s or higher) than Cat5e (2.5 Gbps), but these are way above the network speeds required for a GigE camera and Ethernet board.
    - Cat5e cables are cheaper, lighter and thinner and may be the preferred choice for some applications due to ease of installation in tight spots. They are typically easier to terminate as well.

*See also*

- Using multiple GigE cameras
- Assign IP addresses
- Configure the digital camera

# Assign IP addresses

## Aim

To make sure that the IP address of the GigE camera and that Ethernet interface board physically connected to that camera match. This ensures reliable data transfer to and from the camera.

## What do you want to do?

- Assign the IP address to the Ethernet interface board
- Assign the IP address to the camera

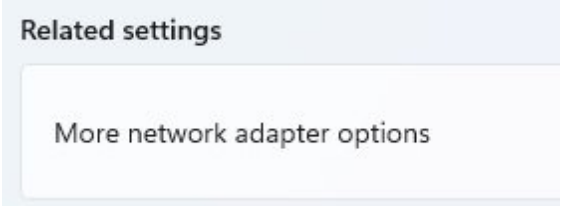
**TIP** For how to assign IP addresses and connect digital GigE Vision cameras, watch the video tutorial **Set Up the Cameras**. To open the tutorial, choose **Help** > **Video Tutorial**.

## Assign the IP address to the Ethernet interface board

This applies to Basler GigE cameras.

1. In Windows, search for **Network connections** or **Network computers**.


   Alternatively, in Windows 11, from the Start menu choose **Settings** > **Network and Internet** > **Advanced network settings** (at the bottom of the screen) > **More network adapter options**.

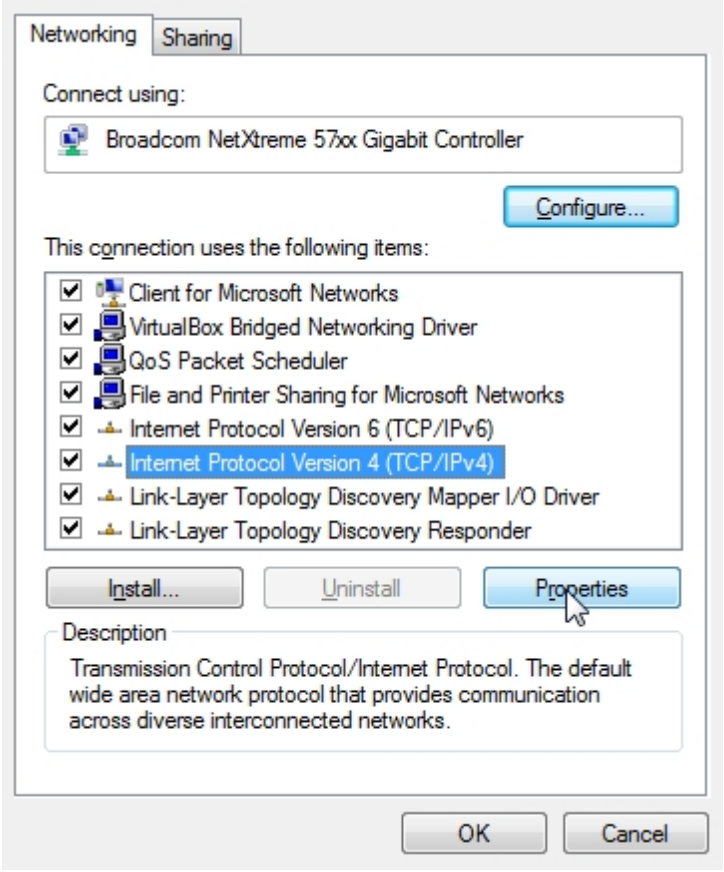



2. The Network Connections window opens. Right-click the item that correspond to your camera and click **Properties**.


**TIP** If your computer has more than one adapter item, unplug the camera and take note of which item shows **Unplugged**. That indicates the port that camera was connected to.

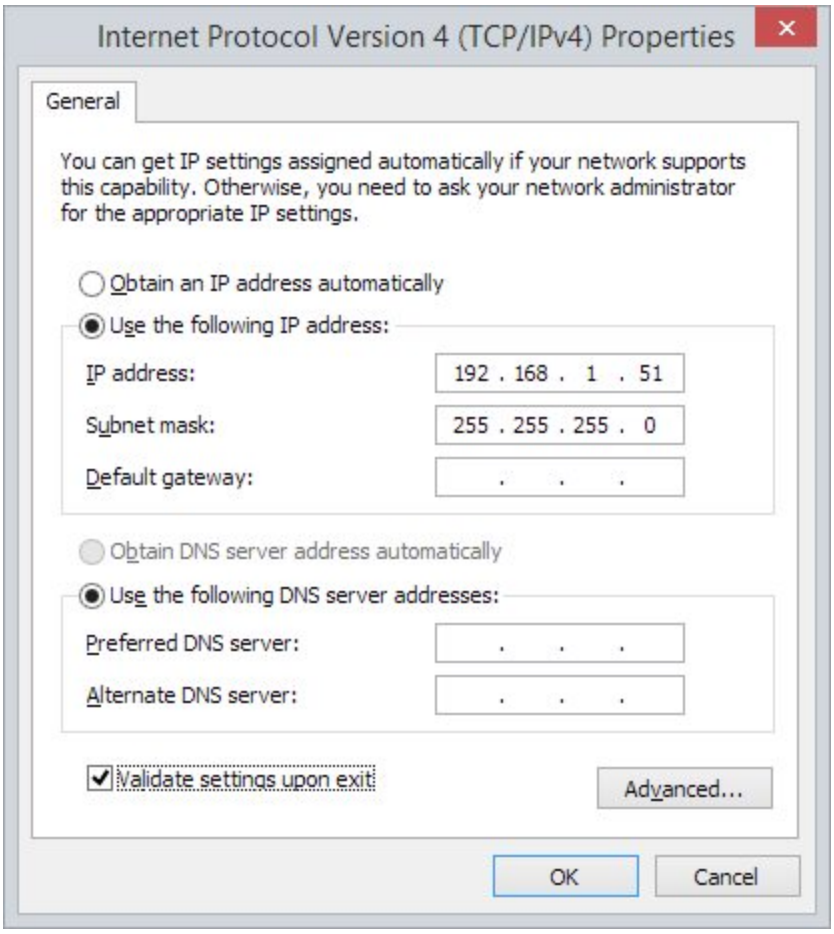
**NOTE** You may see one or more additional items named **Ethernet** or similar. This is the standard interface board that is connected to your local network and the internet. Do not use that port unless it is the only one available. If you use that port to connect the camera, you won't be able to connect the EthoVision XT computer to your local network and the internet.

3. Select Internet **Protocol Version 4 (TCP/IPv4)** and click **Properties**.




4. Select **Use the following IP address** and fill in the details as shown in the figure below. Also select the option **Validate settings upon exit**. When done, click **OK** and then **Close**.

For US-Canada users: please enter 192.168.200.101. In case of multiple Ethernet boards or one board with multiple ports, use 192.168.201.101 for the second board/port, 192.168.202.101 for the third, etc.



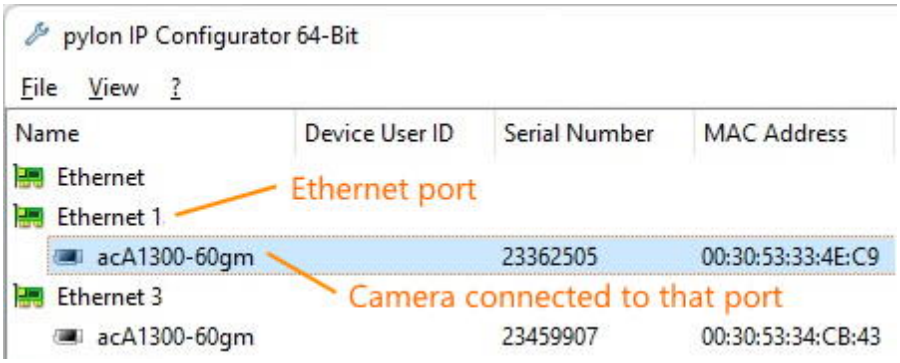

5. Close all windows. Repeat the steps for each port on the board connected to a camera. See also Using multiple GigE cameras

## Assign the IP address to the camera

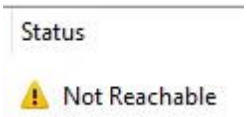
The procedure below is for Basler GigE cameras, not Basler USB 3.0 cameras.

1. If you have not done so yet, Connect the GigE camera to the PC.
2. In Windows, search for and start **pylon IP Configurator**.

3. Select the camera name that appears under the Ethernet port set in the previous procedure. This is the camera physically connected to that port.




Note that the camera status is **Not reachable**. This is normal since the camera cannot communicate yet with the Ethernet port.




4. Under **Static IP**, fill in the details in the **IP Address** and **Subnet Mask** fields as shown here.


If you have multiple cameras, the IP address must differ between cameras and match the IP address of the Ethernet port. See also Using multiple GigE cameras

For US-Canada users: Please enter 192.168.200.1 for camera IP address. For the second camera on same card, 192.168.200.2, etc. The first three numbers must match the address set for the Ethernet port the camera is connected to. For example, a camera connected to 192.168.201.101 can be designated as 192.168.201.1.

5. Click **Save** and then close pylon IP Configurator, or repeat the procedure for the next camera. See also Using multiple GigE cameras
6. Next: Configure the digital camera.

*See also*

- Connect the GigE camera to the PC
- Configure the digital camera
- Using multiple GigE cameras

# Disable the Windows firewall for the Ethernet board

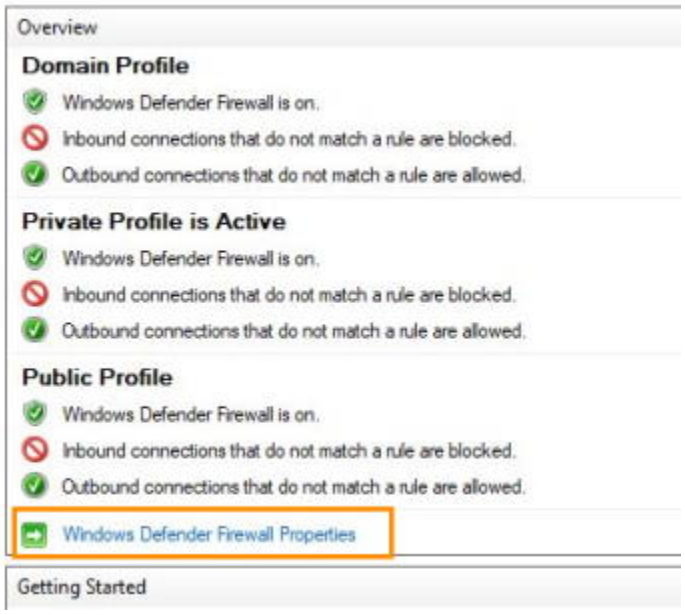
## Aim

To allow the camera network traffic through the firewall.

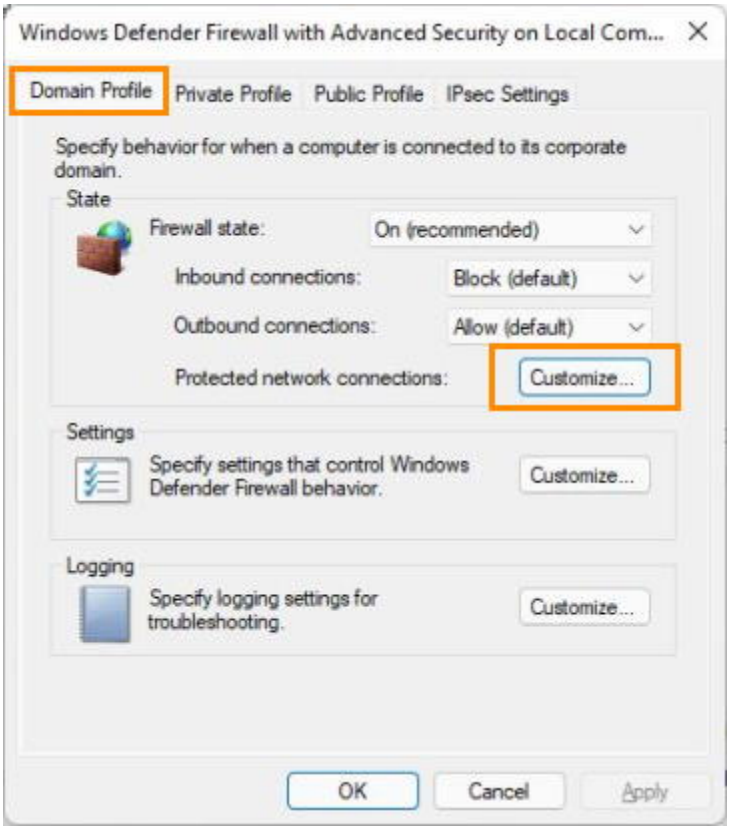
## Procedure

By following this procedure, you exempt the network adapter connected to the camera(s) from the firewall. You may need to do this if EthoVision XT does not show the live camera image, or EthoVision XT shows a message like **Device unplugged**.

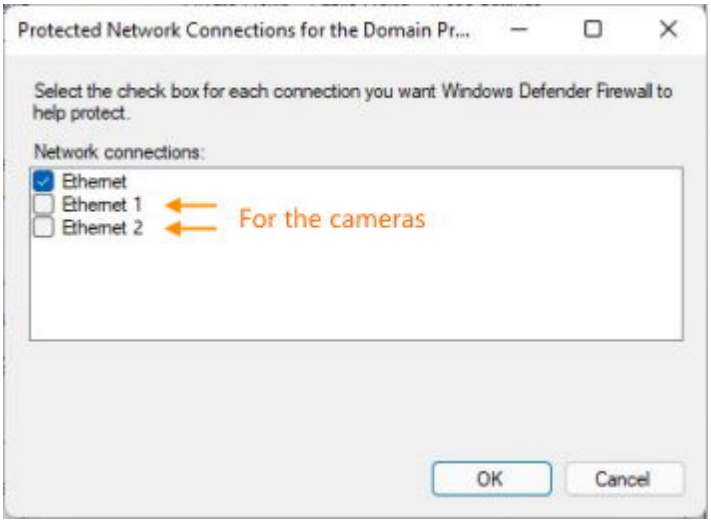
1. Do one of the following:
   - From the Windows Start menu, select **Settings**. Next, select **Privacy & Security** > **Windows Security** > **Firewall & Network Protection**, then click **Advanced Settings**.
   - In the Windows Control panel, select **Network and Internet** > **Network and Sharing Center** > **Windows Defender Firewall**, then click **Advanced Settings**.
2. At the bottom of the **Overview** middle panel click **Windows Defender Firewall Properties**.




3. In the new window that opens, click the **Domain Profile** tab.
4. Next to **Protected network connections**, click the **Customize** button.




5. De-select the network adapters that are used only for the camera(s), and click **OK**.




6. Repeat the steps 4-6 above on the **Private Profile** and **Public Profile** tabs.
7. Click **Close**.

*See also*

- Connect the GigE camera to the PC
- Configure the digital camera
- Using multiple GigE cameras

# Configure the digital camera

## Aim

To adjust settings in a Basler GigE camera or USB 3.0 camera, like the video frame rate, video frame resolution, and exposure time, within the camera software.

- You can also change most of these settings within EthoVision XT. See Adjust camera settings in EthoVision XT
- **IMPORTANT** Do not use pylon Viewer to record video! Always use EthoVision XT or MediaRecorder.

## Main actions

- Procedure (general)
- Set the video resolution
- Set the camera frame rate
- Center the camera view
- Set the pixel format
- Adjust the camera exposure time
- Adjust the camera gain
- Adjust the white balance
- Save the camera settings
- Do a factory reset on the Basler GigE camera

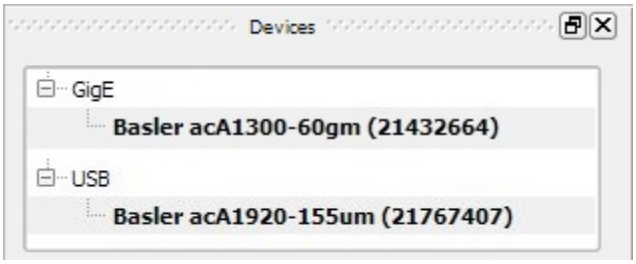
## Procedure (general)

1. Make sure EthoVision XT (and MediaRecorder, when present on the computer) are not running.
2. In the Apps screen, choose **Basler** > **pylon Viewer**.

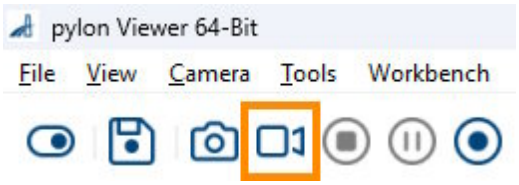
   pylon Viewer is installed only if you selected to install the Basler camera drivers during installation of EthoVision XT. See Install EthoVision XT
3. From the **Windows** menu of pylon Viewer select **Features - All**.

4. In the **Devices** panel, double-click the item Basler under **GigE** or **USB** depending on the camera you have.




   If an error message *Failed to download...* appears, it is probably caused by the mismatch between IP addresses of camera and Ethernet interface. Adjust the IP addresses and when ready click the **Refresh** button in pylon Viewer. See Assign IP addresses

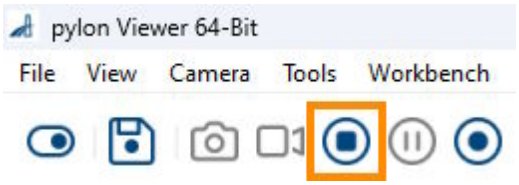
5. To preview the camera image, click the **Continuous Shot** button on the toolbar.




   **IMPORTANT** Do not use the Record button if you want to record video! This creates very large files. Always use EthoVision XT record video.

6. Under **Features - All**, expand the camera name and choose the settings category that you would like to adjust. See the instructions that apply in the following sections.

   **NOTE** To be able to adjust the settings, click the **Stop** button first.



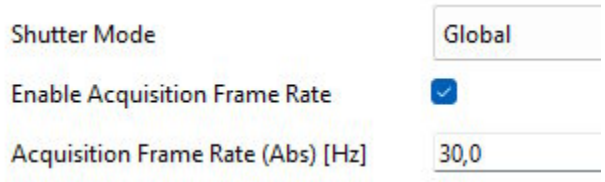

7. After you have adjusted the camera settings, save them under **Configuration Sets**. See Save the camera settings
8. When working with multiple cameras, repeat the procedure for each camera.

## Set the video resolution

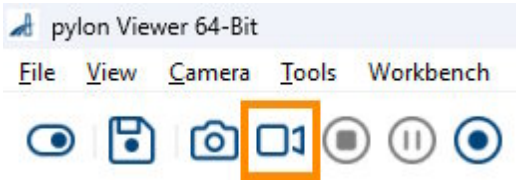
1. In the **Features** panel, open the Basler camera item and then the **AOI Controls** item (**Image Format Control** for USB 3.0 cameras).
2. You can set the resolution by adjusting the **Width** and the **Height**.
   - Recommended: 1280 x 960 or 1280 x 1024.
   - When using multiple cameras you may need to limit the resolution, also depending on the frame rate and the tracking methods you intend to use. See suggestions for GigE cameras and USB 3.0 cameras
3. Save the settings. See Save the camera settings

## Set the camera frame rate

1. In the **Features** panel, under **Category**, open the camera item and then click **Acquisition Controls**.
2. Select **Enable Acquisition Frame Rate**.
3. Next to **Acquisition Frame Rate (Abs) [Hz]**, enter the frame rate you require (frames per second).




4. To check that the frame rate is stable, click the **Continuous Shot** button on the toolbar.



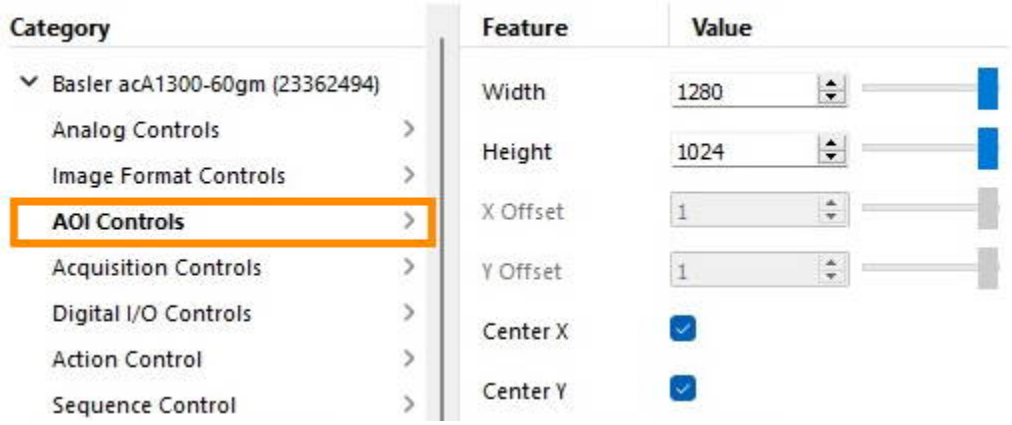

   Under the live video window, locate the resulting frame rate. This is the frame rate that the camera can sustain with the current settings. Check that this matches the value you have just set.

5. Save the camera settings.

## Center the camera view

Follow this procedure to make sure the camera view is centered. Alternatively, you can use part of the original camera image by operating the Offset controls.

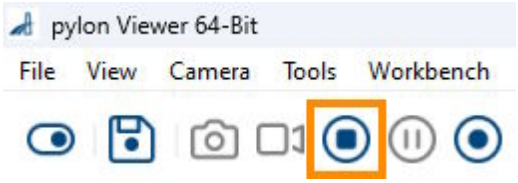
1. In the **Features** panel, open the Basler camera item and then the **AOI Controls** item (**Image Format Control** for USB 3.0 cameras).
2. Select both options **Center X** and **Center Y**.




3. Save the camera settings.

**NOTE** If you want to have the camera image off center, for example to focus on one region of interest, do the following in pylon Viewer:

1. Under **Features** > **AOI Controls** de-select **Center X** and **Center Y**.
2. Click the **Stop** button.

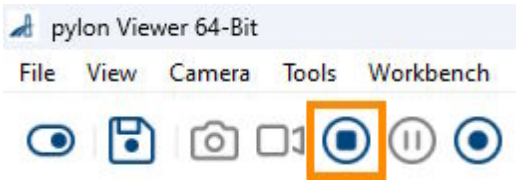



3. De-select **Center X** and/or **Center Y**.
4. If the **Width** and **Height** of the image are set to the maximum values, reduce those values so you have room for the offset adjustment (see below).
5. Move the **X Offset** and **Y Offset** sliders.
6. Click the **Continuous shot** button and check the live camera view.
7. When you are satisfied with the new off-center camera view, Save the camera settings. For more details, see the camera documentation.

## Set the pixel format

Pixel format is the format in which video data sent out by the camera sensor is represented and analyzed by EthoVision XT.

1. Click the **Stop** button.




2. In the **Features** panel, under **Category**, open the camera item and then click **Image Format Controls**.
3. Select the **Pixel Format**:
   - For monochrome cameras, choose **Mono 8**.
   - For color cameras, choose **Bayer RG 8**. See also Settings for the color camera

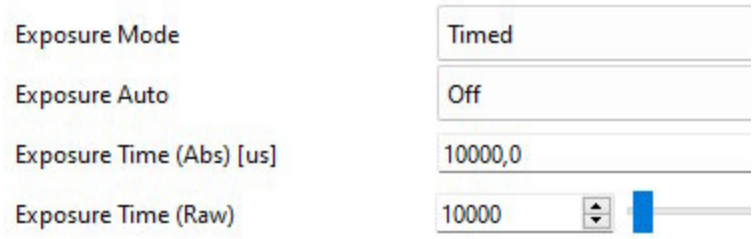
**IMPORTANT** Mono 8 corresponds to the option **Y800** in EthoVision XT. See Adjust camera settings in EthoVision XT > Format tab.

## Adjust the camera exposure time

The exposure time (or shutter time) is the time the camera shutter is kept open to let light in. The exposure time limits the maximum achievable frame rate. If the sum of the exposure time and the time needed for reading the chip (readout time) is greater than the time between consecutive video frames, the next frame may be dropped, resulting in a lower frame rate, and missed samples during tracking.

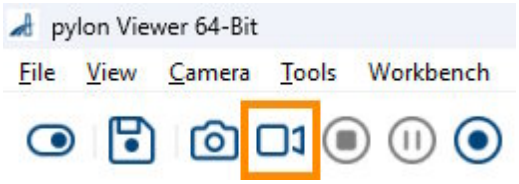
To adjust camera exposure that matches a frame rate:

1. In the **Features** panel, under **Category**, open the camera item and then click **Acquisition Controls**.
2. Make sure that **Exposure Mode** is **Timed**, and **Exposure Auto** is **Off**.
3. Next to **Exposure Time (Abs) [us]**, enter the exposure time you require, in microseconds.

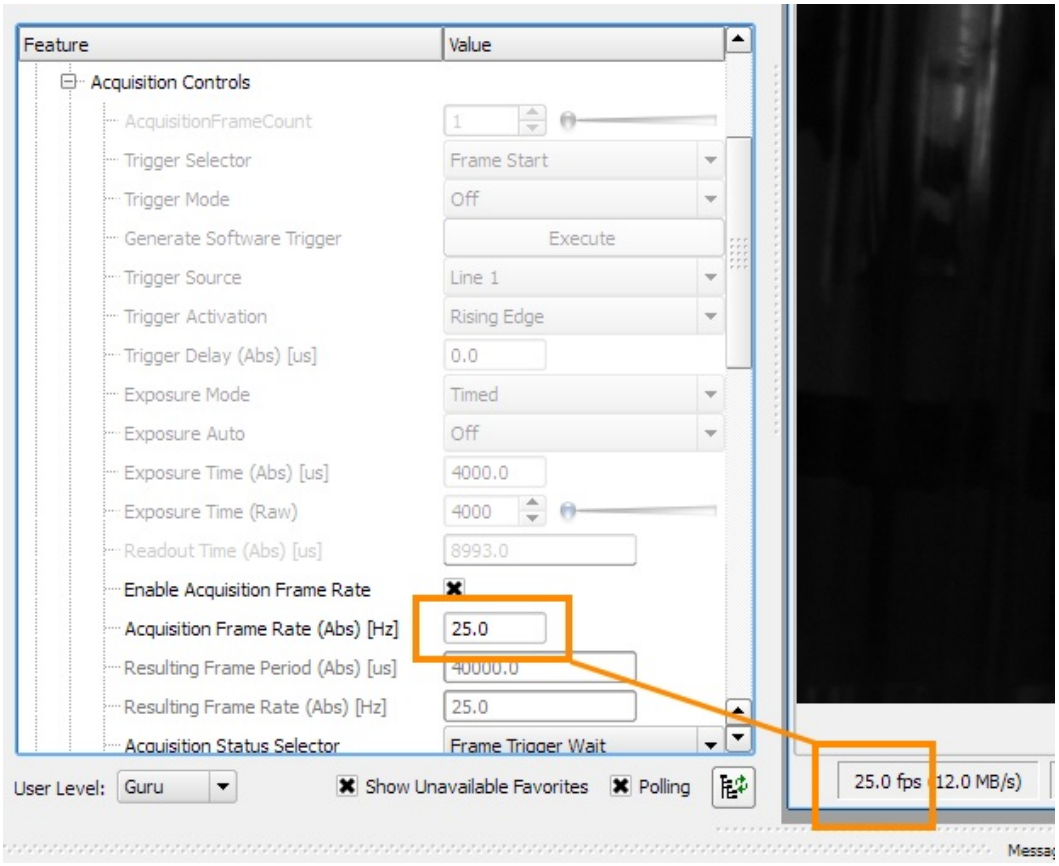



- For Zebrafish larvae, enter 4000 or 2000, depending on the video resolution chosen. If resolution in EthoVision is 1280 x 960 or similar, select 4000. If the resolution is 640 x 480 or similar, select 2000.
- In all other cases, you may choose a higher value. The ideal range of exposure will likely be anywhere from 2000 to 10,000.
- If lighting in the room is low, you will likely need a longer exposure time. Try 8000 up as high as 15000. When tracking under infrared light, you generally need to set high Exposure times, up to 20000.
- Values higher than 20000 are not recommended - increase illumination or set the Gain a bit higher. See Adjust the camera gain
- Note that a longer exposure time may cause motion blur. Faster animals like flying insects require shorter exposure times than other, slow-moving animals. In case of motion blur, reduce exposure time and compensate this by opening the lens aperture and/or increasing the amount of ambient light (the latter is preferred).

4. A longer exposure time may conflict with the time that the software needs to process an image, because both must fit within the time between two successive video images. In that case some video images may be dropped during acquisition. To check that the exposure time is compatible with the frame rate you have chosen, click the **Continuous Shot** button on the toolbar.

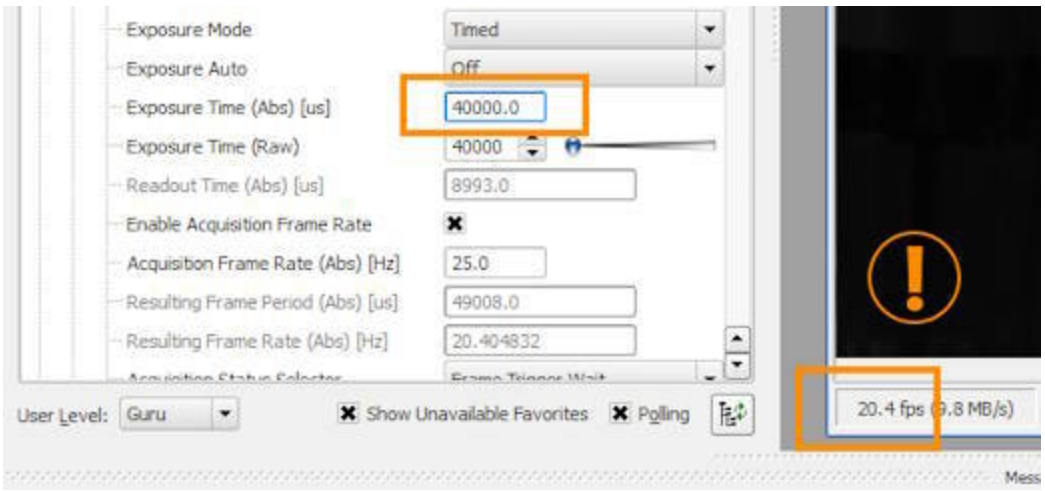



At the bottom of the live video window, locate the frame rate resulting with the new exposure time. If the camera can cope with the new exposure time, the current frame rate should be the same as that set next to **Acquisition Frame Rate (Abs) [Hz]**. This is usually 25, 30 or 60.




If the exposure time is very high, the resulting frame rate could be lower than the set frame rate **Acquisition Frame Rate (Abs) [Hz]**.

For example, setting an Exposure time of 40000 (40 ms) reduces the frame rate from 25 to 20:




To solve this, do one of the following:

- Under **Acquisition Controls**, lower the **Exposure Time (Raw)**. This makes the video image darker. To compensate for that, open the lens diaphragm so that the camera sensor receives more light. Note, however, that an open diaphragm reduces the depth of field, that is, the range of distances where the object appear sharp in the camera image.
- Under **AOI Controls**, lower the **Width** and **Height** (video resolution). Do this until you see the expected frame rate under the live view.

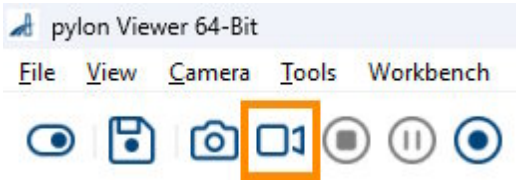
5. Save the camera settings.

## Adjust the camera gain

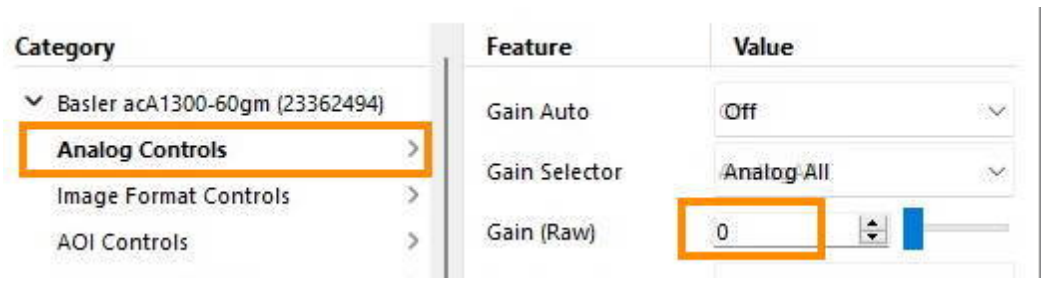
Camera gain amplifies the video signal. A higher value of gain results in a brighter image. That could be an option whenever the image is too dark for EthoVision XT to detect a subject, and you do not have other ways to correct for that. For example, when the lens of the camera is wide open and you cannot increase light intensity in the test room.

**TIP** Whenever possible, add lights instead of increasing camera gain. See Lighting setup and the video tutorial **Set up Your Test Environment** (Help > Video Tutorial).

1. Start the live view by clicking the **Continuous Shot** button.




2. In the **Features** panel, click **Analog Control**.
3. From the **Gain Auto** list, select **Off**.
4. Next to **Gain (Raw)**, select the value you require.



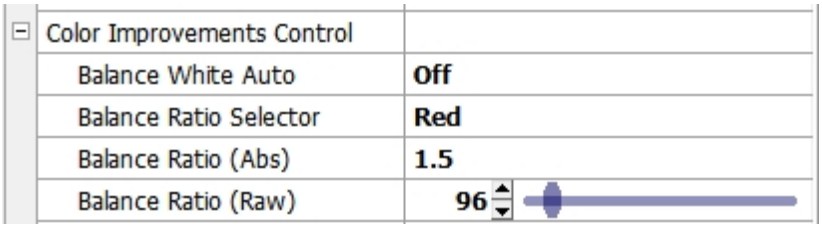

In normal situations values like 0 or 1 should suffice. Only increase the gain further if there is no other way to get a bright image (e.g. by increasing the exposure time, or improving the ambient light, or opening the aperture ring of the lens). However, increasing gain results in more image noise.

5. Check the live view and adjust the gain when necessary.
6. When ready, Save the camera settings.

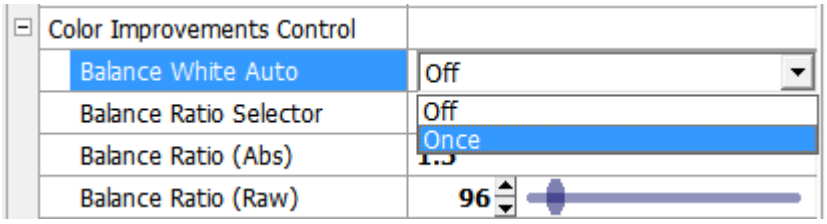
## Adjust the white balance

If you have a color camera, you also need to adjust its white balance.

1. Click **Color Improvements Control.**




2. Point the camera at a piece of white paper, so that the camera image looks entirely white. Click in the field next to **Balance White Auto** and select **Once** from the list.

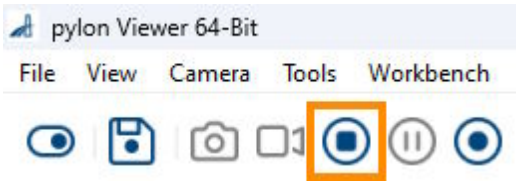



3. Save the settings (see below). See also Settings for the color camera

## Save the camera settings

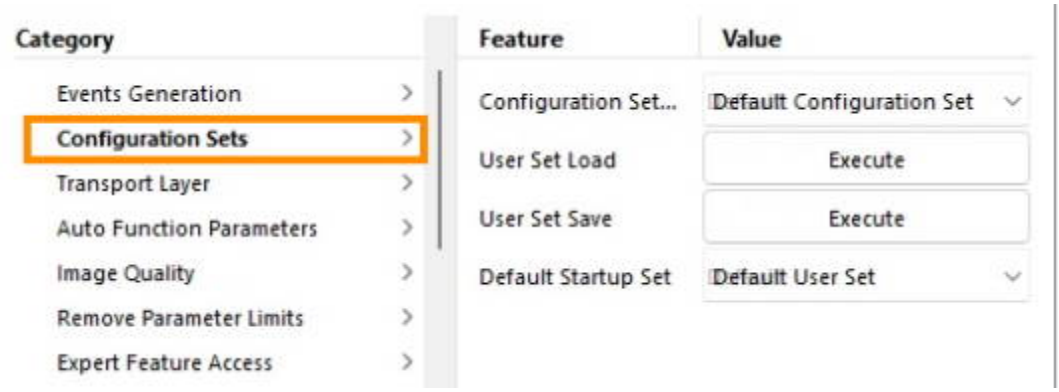
**IMPORTANT** If you have multiple GigE/USB 3.0 cameras, make sure that you save the settings for each camera. To select a camera, double-click its name in the Device panel in pylon Viewer, adjust the settings that you require and repeat the procedure to save the settings.

1. Click the **Stop** button.




2. In the **Features** panel, click **Configuration Sets** (**User Set Control** for USB 3.0 cameras).

3. From the **Configuration Set Selector** list (**User Set Selector** for USB 3.0 cameras), select **User Set 1**.
4. Next to **User Set Save**, click **Execute**. The camera settings are saved under **User Set 1**.
5. From the **Default Startup Set** list (**User Set Default** for USB 3.0 cameras), select **User Set 1**. This means that the camera always starts with the settings contained in User Set 1.




6. Close pylon Viewer.

These settings will be used each time you start up EthoVision XT.

**TIP** You can also save different settings for the same camera in User Set 2 and User Set 3. Under **Default Startup Set**/**User Set Default**, select which you want EthoVision to use.

## Do a factory reset on the Basler GigE camera

1. Open pylon Viewer and double-click the camera name. At the bottom left of the interface, click **All Features**.
2. In the Features window, expand **Configuration Sets**.
3. For **Configuration Set Selector**, select **Default Configuration Set**.
4. For **Default Startup Set**, select **Default User Set**.
5. For **User Set Save**, click the **Execute** button.
6. For **User Set Load**, click the **Execute** button.
7. The camera should now be reset to factory defaults.

At this point you need to set the resolution, frame rate, exposure and other settings while still in pylon Viewer. See the top of this topic. Finally, save the settings with a specific name (e.g. User Set 1) so you can always return to those settings (User Set Load) when needed.

# Using multiple GigE cameras

## Aim

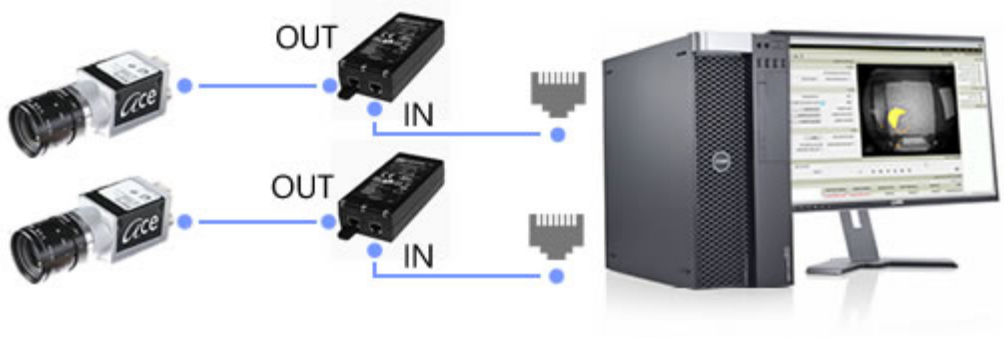
To track from two up to four GigE cameras. This topic applies to Basler GigE cameras only.

**IMPORTANT** You cannot use a combination of analog and digital cameras. Also, mixing video images of different camera types is not supported.

## Connect the cameras

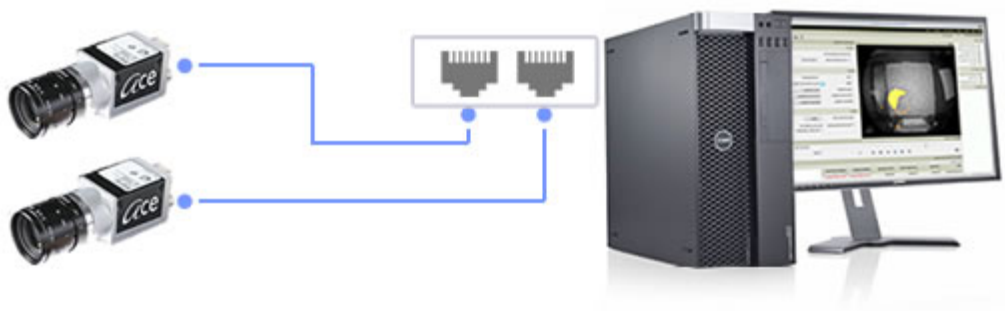
How you connect multiple GigE cameras depends on which Ethernet interface board you have installed.

- If you have multiple, 1-port Ethernet boards, connect each camera to a Ethernet board. If the boards are not powered, you must connect the cameras through PoE injectors to a power supply. Only use 1Gbit/s PoE injectors.



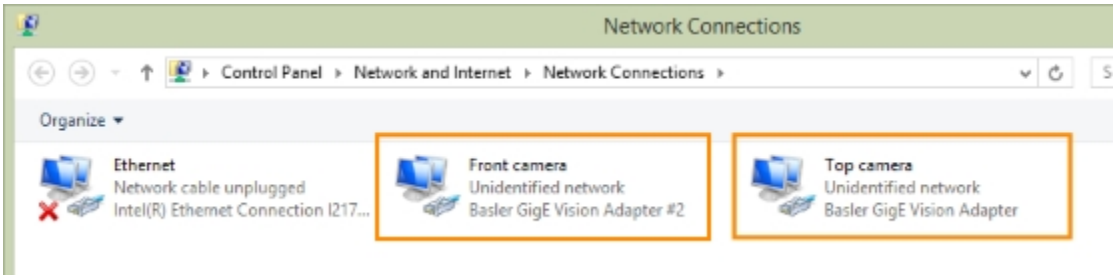

- If you have a 4-port Ethernet board, you can connect up to four cameras.

  **IMPORTANT** Make sure the Ethernet board also receives power from the PC. See the connection in Power the Ethernet board for the GigE cameras



- For a 16-camera configuration, see 16-camera configuration
- **IMPORTANT** Each camera must always be connected to the same port on the Ethernet board, because the IP address of the camera and that of the Ethernet port must match. In the case of a multi-port Ethernet board, each port is viewed as a network adapter, with its own IP address. The information of which port is linked is stored in the driver software of each camera. If you swap cameras, they will not be recognized. Therefore, always label the Ethernet ports and the cameras.

  **TIP** When using one Ethernet interface board per camera, rename the network adapters listed in the Windows Control Panel, for example *Front camera* and *Top camera*.




## Assign the IP addresses to multiple cameras

You can assign IP addresses with pylon IP Configurator.

1. Assign an IP address to each Ethernet port on your PC. See Assign IP addresses.
2. Assign an IP address to each camera. See Assign IP addresses

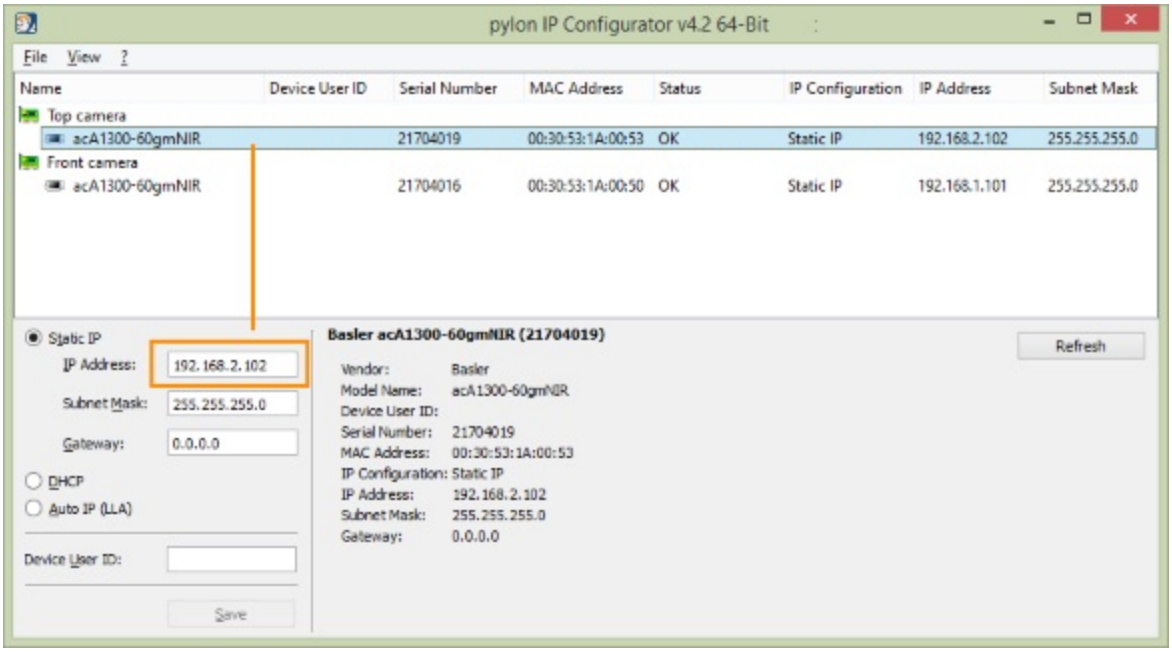
Here are the main rules:

- Each camera must have a unique IP address.
- Always set the first two numbers to 192.168.
- The third number must be the same for the camera and the Ethernet port the camera is connected with.

**EXAMPLE**

- Camera 1: IP address 192.168.**1**.101 connected to Ethernet port with IP address 192.168.**1**.51
- Camera 2: IP address 192.168.**2**.102 connected to Ethernet port with IP 192.168.**2**.52.

  In pylon IP configurator, the first camera looks like this.




## Video resolution

You can set the video resolution in EthoVision XT. See Select multiple GigE cameras in EthoVision XT.

*See also*

- 16-camera configuration
- GigE cameras
- Install an Ethernet board for GigE cameras
- Power the Ethernet board for the GigE cameras
- Assign IP addresses
- Configure the digital camera

# 16-camera configuration

**IMPORTANT** When using a 16-camera configuration, we recommend to use Contour-based tracking, not Deep learning. This because Deep learning is so resource-demanding that it is unlikely to work with 16 arenas on a typical PC.

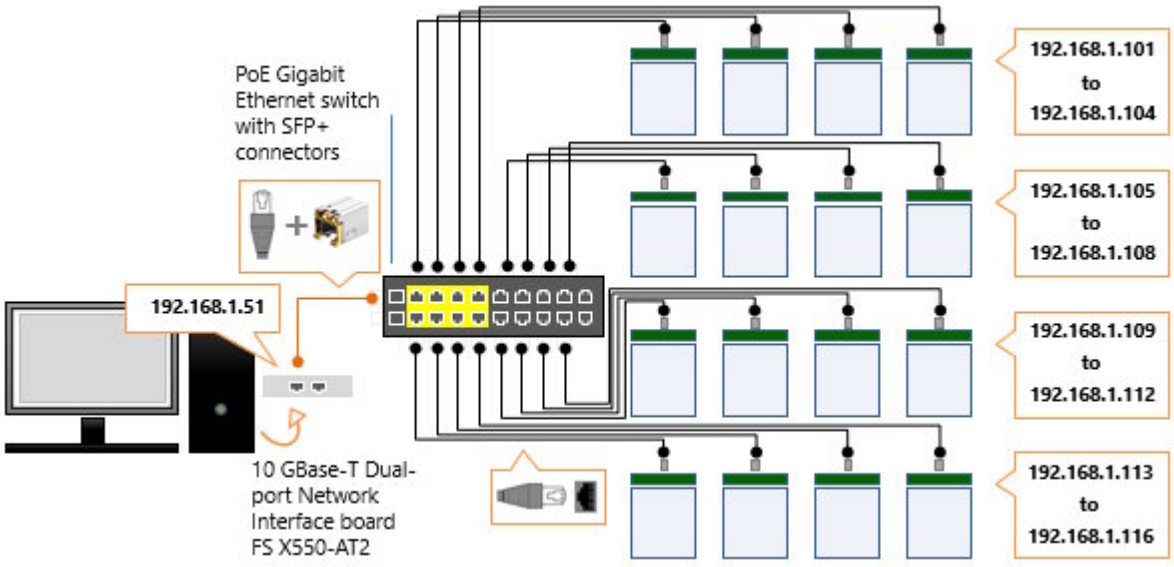
## Setup

### *Hardware and settings*

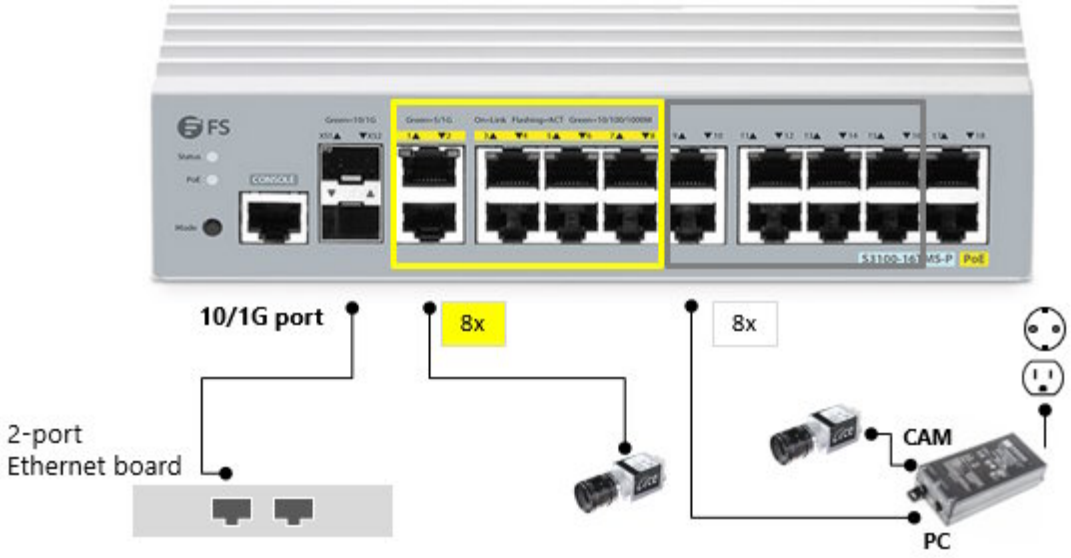
- PC: Dell Precision 3680, CPU i7-14700K 3.4 GHz, RAM memory 32GB, GPU NVIDIA T1000 8 GB; Windows 11 Pro 23H2.
- 16 Basler GigE cameras acA1300-60gm.
- PCIe Ethernet interface board: FS X550-AT2, 10GBase-T Dual-Port, PCIe 3.0 x4.
- Network switch: FS S3100-16TMS-P, 16-Port Gigabit Ethernet L2+ PoE+, 8x PoE+ Ports@125W, 2x 5Gb RJ45, with 2x 10Gb SFP+ Uplinks.
- For this switch you need an adapter (SFP+ 10GBASE-T Copper 30m RJ-45 Transceiver module) for the network cable that goes to the PC.
- We recommend to use CAT6+ network cables.
- Video resolution: single camera view 640 x 480, merged view in EthoVision XT 2560 x 1920.
- Image binning: 2. See Binning
- Video frame rate: 30 fps. Trial duration: 72 hours.
- Video source: Live tracking; Number of subjects per arena: 1; Tracked features: Center-point, nose-point and tail-base detection; Body Point Detection Technique: Contour-based. Detection method: Dynamic Subtraction or Differencing. Acquisition mode: Track + Save video.

### *Connection scheme*

Follow this scheme also for PhenoTyper 2. Note that in the S3100-16TMS-P network switch, the RJ45 ports marked in yellow are powered, so eight cameras do not need PoE injectors. Connect the remaining eight cameras through PoE injectors.




Connect the cameras as follows:



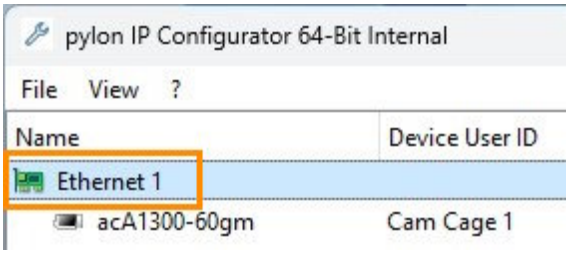

*Assign the IP addresses*

The figure above shows an example of IP addresses that worked. See also Assign IP addresses

Connect the cameras to the switch using the ports as in the figure above. For a good overview of the IP addresses we recommend to use **pylon IP Configurator**.

Start pylon IP Configurator.

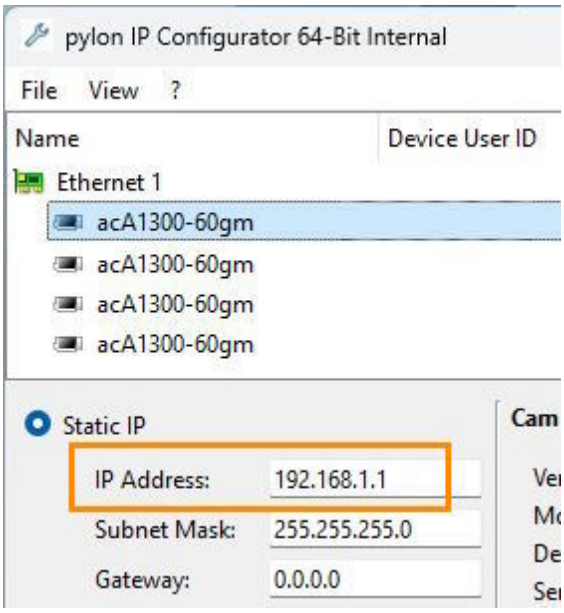
For the port of the Ethernet board of the PC connected to the switch, specify the address 192.168.**1**.51. To do so, locate the **Ethernet** row in IP Configurator, that is, the row with the board symbol connected to the cameras. Note that the name of the Ethernet row may vary between PCs.




Right-click and select **Properties**, then double-click **Internet Protocol Version 4**, select **Use the following IP address**, and enter the IP address in the **IP Address** field.

For the cameras (1 to 16), specify the following addresses: 192.168.**1**.**101** to 192.168.**1**.**116**.

Note that the third number must be the same as that for the Ethernet port connected to the network switch. To assign the IP addresses to a camera, select the camera row, then in the panel at the bottom of the screen choose **Static IP** and enter the IP address.




For all Ethernet ports and cameras, leave 255.255.255.0 as **Subnet Mask**.

For more schemes of multiple camera configurations, see also the PhenoTyper - EthoVision XT 18 - Reference Manual.

## Using network switches

- The network switches must be of type **PoE** (Power over Ethernet), that is, they must provide power to the cameras. Mind that the maximum power provided by a switch, measured in Watt (W), must be greater than that the total requirements of the cameras (one Basler camera requires about 2.5 W).
- The network switches must be able to handle large packets (known as jumbo packets or jumbo frames). With jumbo frame-capable devices, the maximum packet size can be up to 16 KB. For maximum network efficiency, your camera should be configured to use the largest packet size that your network adapters and switches can handle.
- Refer to the user guide of the network switch for how to set it up.

*See also*

- GigE cameras
- Install an Ethernet board for GigE cameras
- Power the Ethernet board for the GigE cameras
- Connect the GigE camera to the PC
- Assign IP addresses
- Configure the digital camera
- Select multiple GigE cameras in EthoVision XT

# Select multiple GigE cameras in EthoVision XT

## Aim

To have EthoVision XT mix the images from multiple cameras in a picture-by-picture fashion.

## Procedure

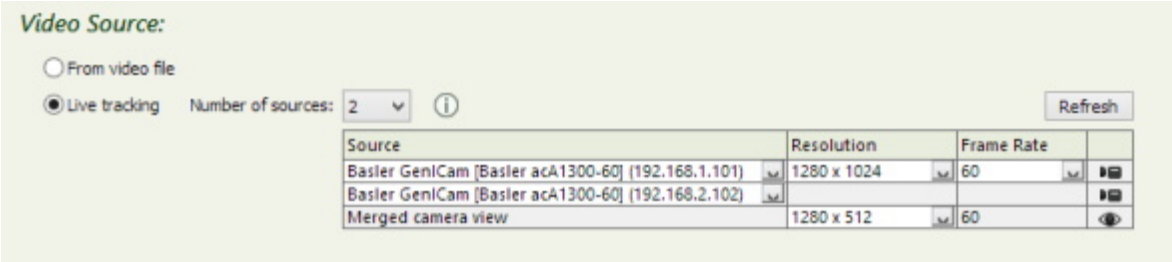
1. Connect your cameras.

   Connect each camera to the port of its Ethernet board. Each camera has its unique link with one of the Ethernet boards. This link is the IP address. If you swap cameras, EthoVision XT won't get the camera image. See Connect the GigE camera to the PC and Assign IP addresses

2. In EthoVision XT, choose **Setup** > **Experiment Settings** and select the number of cameras from the list next to **Number of sources**.
3. Select the cameras under **Source**. The camera names will show their own IP address in brackets at the end, which makes it possible to distinguish between the cameras.
4. Select the resolution and frame rate of the first camera. The resolution and frame rate of the other cameras is set automatically to the same values as for the first camera.
5. Next to **Merged camera view**, select whether the picture by picture mixed image should have the same resolution as the images from the individual cameras, of whether the resolution should be the sum of that of each camera image.

   **NOTE** The maximum total resolution depends on which cameras you have. For Basler monochrome cameras, the maximum resolution is 2560 x 2048 when mixing four camera images. However, each camera model has its own set of available resolutions, which also determine the maximum resolution when merging the camera images. See tested configurations for GigE cameras (including PhenoTyper 2).

6. Click the preview icon to get a preview of the merged camera images.

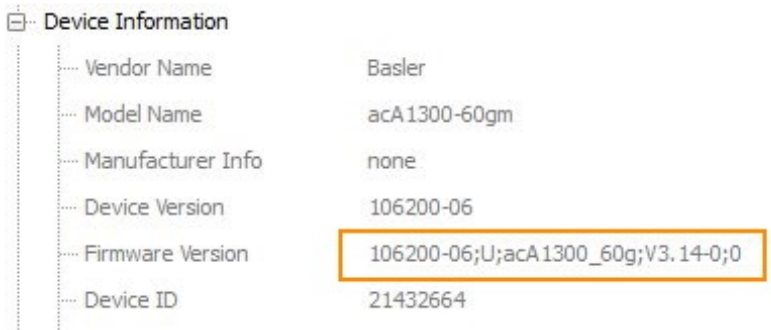



## Recommended video resolutions

- 4-camera configuration: Single camera view 1280 x 1024, merged camera view 2560 x 2048. See Using multiple GigE cameras
- 8-camera configuration: Single camera view 640 x 480, merged camera view 2560 x 960. See Using multiple GigE cameras
- 16-camera configuration: Single camera view 640 x 480, merged camera view 2560 x 1920. See 16-camera configuration
- See also Resolution, frame rate, and maximum trial duration

## Notes

- Noldus does not support video tracking from multiple GigE cameras connected to a laptop computer. Always connect multiple cameras to a desktop computer.
- When using multiple cameras, pay attention to the lens settings. These should be in such a way that the apparent size of the arenas is the same since you calibrate the distance for all the cameras (Shared calibration). If that is not possible, make one calibration for each arena (Unshared calibration). See Calibrate multiple arenas
- The cameras should have the same Firmware version. Check this in the pylon Viewer software. In the **Features** panel, under **Device Information**, check **Firmware Version**. If the two (or more) cameras do not have the same firmware, an upgrade/downgrade must be done.



*See also*

- Cameras supported by EthoVision XT
- For examples of frame rate and resolution when using multiple cameras: GigE cameras
- Connect the GigE camera to the PC
- Assign IP addresses
- To view the camera image Select multiple cameras
- PhenoTyper - EthoVision XT 18 - Reference Manual. See Manuals

# Install USB 3.0 cameras

To work with a USB 3.0 camera, if your PC does not have a USB 3.0 port, then you need to install a USB 3.0 interface board (for desktop computers).

## What do you want to do?

- Install the USB 3.0 interface board
- Install the USB 3.0 camera driver
- Connect the USB 3.0 camera to the PC
- Configure the digital camera

*See also*

- Cameras supported by EthoVision XT

# Install the USB 3.0 interface board

## Aim

To install an interface board that receives video from one or more USB 3.0 cameras.

We tested the following USB 3.0 interface boards:

- U3-PCIE1XG205-1S Ren 1HC, x1, 2 ports

- U3X4-PCIE4XE111 Fresco FL1100, 4HC, x4, 4 ports

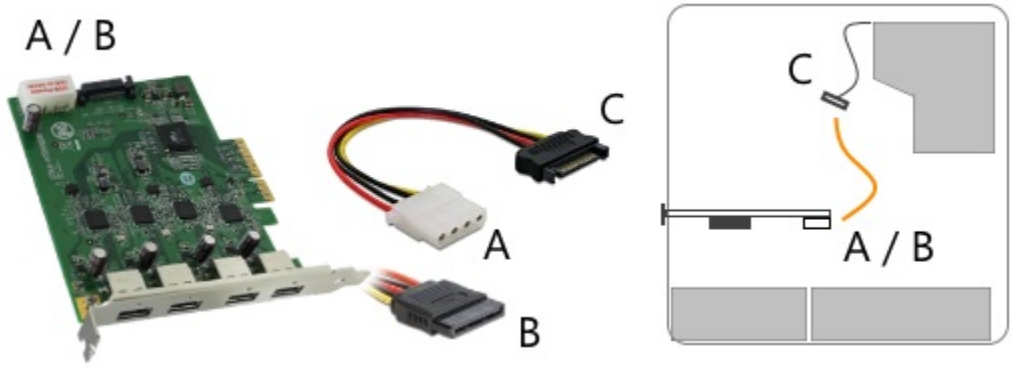
**IMPORTANT** Although these boards have multiple USB ports, always connect one camera only.

## Procedure

1. Turn off your computer and all connected peripherals, such as the monitor and printer. Make sure that the computer is unplugged.
2. Remove the PC's case according to the instructions provided in the PC's user manual.
3. Select a free **PCIe** expansion slot, and remove the corresponding extension cover. For details about PCIe slots, see Install an Ethernet board for GigE cameras.
4. Unpack the USB 3.0 interface board, place it into the slot, and press it carefully into position. If the board does not fit into place easily, remove it and repeat the operation.

5. Fix the board to the chassis and re-fit the computer's cover.

   If you have a 4-port Fresco Logic U3X4-PCIE4XE111 board, make sure that the board receives power from the PC. Connect the power connector on the board (Molex/IDE male 4-pin DC) to one of the power supplies inside the PC (C). Connector types vary between computers. Many computers have a SATA 15-pin power connector. Use an adapter cable with a 4-pin female Molex connector at one end and a SATA 15-pin connector at the other end.




6. Proceed with Install the USB 3.0 camera driver.

## Notes

- For the best performance, the USB 3.0 interface board should be installed in a PCIe Gen 2 compliant slot in the host computer. Most computers have PCIe Gen 2 (5.0 Gbps) throughput on x8, x16 or x 32 slots. A PCIe Gen 1 compliant slot reaches up to 2.5 Gbps throughput.
- **IMPORTANT** Turn off *Fast startup* in the Windows Power options. See Power options when using cameras

# Install the USB 3.0 camera driver

## Aim

To install the software that enables you to configure the camera. For example, to set the video frame rate or the exposure time.

## Prerequisite

- You have installed the USB 3.0 interface board. See Install the USB 3.0 interface board

## Procedure

1. Please follow the procedure in the topic

   Install the driver software for the digital cameras

   - If you have already installed EthoVision XT, on the installation screen choose **Modify** and under **Drivers and tools** choose **Basler USB camera driver**.
   - If you still have to install EthoVision XT, choose **Install**, then under **Drivers and tools** choose **Basler USB camera driver**.
2. Next: Connect the USB 3.0 camera to the PC

# Connect the USB 3.0 camera to the PC

## One USB 3.0 camera

Connect the camera to the USB 3.0 interface board of the EthoVision XT desktop computer, or to a USB 3.0 port on the EthoVision XT laptop computer.

- USB cables are usually 3 to 5 meter long. These are generally passive cables, meaning that quality of the signal degrades with cable length. Longer passive cables are not recommended. If you need cables to cover more than 5 m distance, use a active (powered) extension cable.
- Some USB 3.0 connectors and ports can be recognized by their blue color coding. Others show 'SS' (SuperSpeed) and the USB sign.


## Multiple USB 3.0 cameras

**IMPORTANT** EthoVision XT has been tested with one USB 3.0 camera. If you want to use multiple USB 3.0 cameras simultaneously, test them thoroughly before running your experiments. Install as many USB 3.0 PCIe interface boards as cameras, unless you have an USB 3.0 4-port interface board. See Install the USB 3.0 interface board

How you connect multiple USB 3.0 cameras depends on which USB 3.0 interface board you have installed.

- If you have multiple interface boards with 2 ports each, connect each camera to one of the ports on the USB 3.0 boards.

  **IMPORTANT** Do not connect two cameras to the same USB 3.0 interface board.

- If you have a 4-port interface board, you can connect up to four cameras. Connect each camera to one of the USB ports.

## Notes

- If you have one USB 3.0 interface board with two ports, always connect one camera. You can use the second port as a power source when using an extension cable.

*See also*

- Cameras supported by EthoVision XT

# Install analog cameras

## What do you want to do?

- Install the Picolo Alert encoder board

  **NOTE** the Picolo H.264 boards are no longer supported with EthoVision XT.

- Connect analog cameras to the computer
- Test an analog camera

*See also*

- Cameras supported by EthoVision XT > Analog cameras

# Install the Picolo Alert encoder board

## Aim

To install the encoder board Picolo Alert on your computer. You need this board to track and record video using analog cameras.

**NOTE** If you ordered a computer from Noldus Information Technology when you purchased EthoVision XT, the encoder board is already installed. Follow the procedure below if you bought your computer somewhere else.

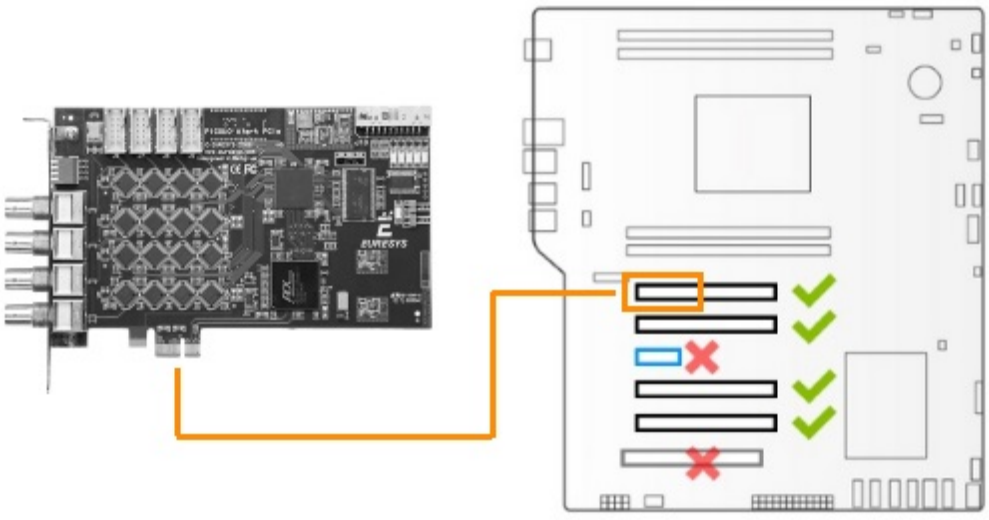
## Prerequisite

- **IMPORTANT** Turn off *Fast startup* in the Windows Power options. See Power options when using cameras

## Procedure

*Insert the Picolo Alert board in the PC*

1. Turn off the PC and disconnect the power cable.
2. Open the computer and gently but firmly insert the board into a free PCI express slot. Avoid touching the contacts or other metal parts of the board.

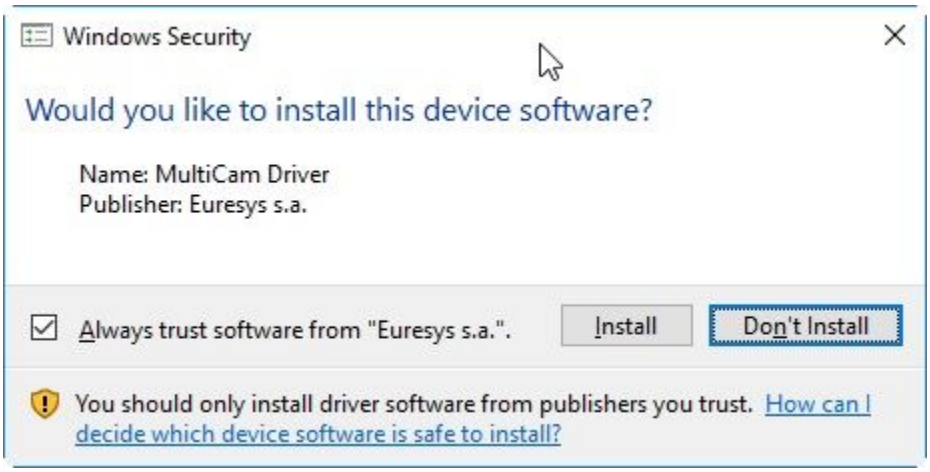


   For details about PCIe slots, see Install an Ethernet board for GigE cameras.
3. Close the PC.

4. Connect the power cable and turn the PC on.

*Install the driver for the Picolo Alert board*

1. Browse to my.noldus.com, log in or register, and select **Downloads** > **EthoVision XT** > **Drivers and Tools**.
2. Download and save the file **Euresys Multicam - Driver win10**.
3. Run the exe file and continue with installation.
4. When the following message appears:




5. Click **Install**.
6. At the end of installation, restart the PC.
7. Next, see Connect analog cameras to the computer.

## Notes

- Always install the drivers from my.noldus.com. Do not install drivers from other sources, unless requested by Noldus Support.

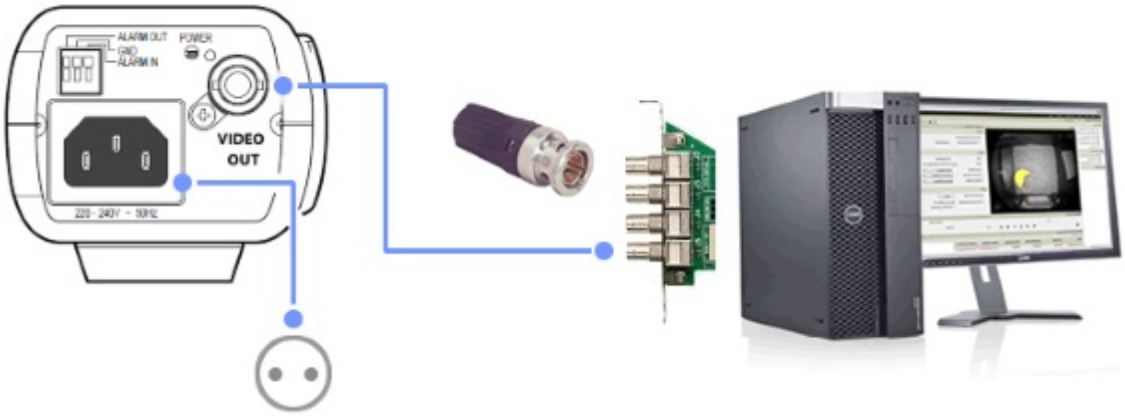
*See also*

- Analog cameras
- Install analog cameras

# Connect analog cameras to the computer

## Connect one to four cameras

Connect each camera to one of the inputs of the Picolo Alert encoder board.




Furthermore:

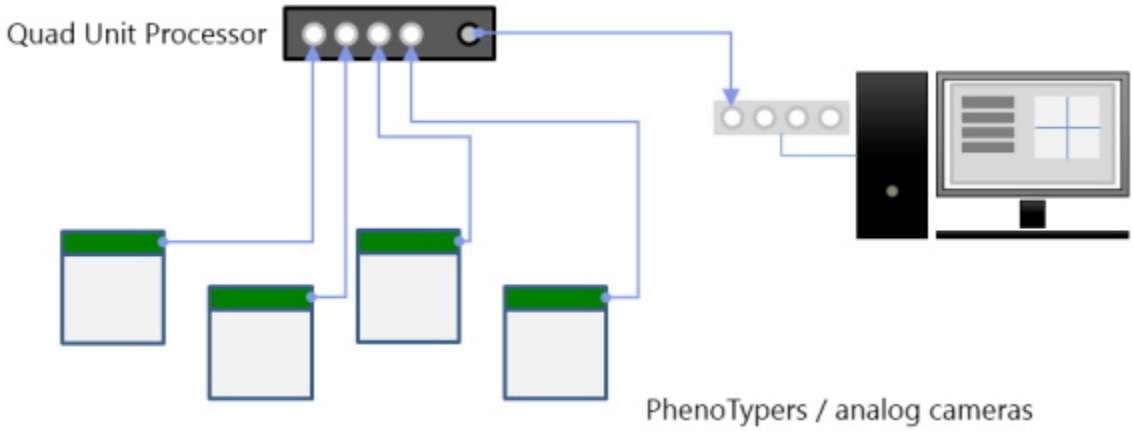
- Take note of which camera is connected to which input of the board.
- Make sure that all four switches on the board are in the position opposite to their labels.

- Create or open an experiment, choose **Setup** > **Experiment Settings**, then select **Live tracking** and under **Video Source** select the camera inputs. Next, view the mixed camera image. See Video source

## Connect up to 16 cameras

You can track from up to 16 analog cameras simultaneously if you connect Quad Unit Processors between each group of four cameras and the four inputs the Picolo Alert encoder board.

A Quad Unit Processor mixes the video inputs of up to four cameras. Connect the output of the Quad Unit Processor to one of the inputs of the Picolo Alert board, as shown in the figure below.




To connect 16 cameras, you need:

- Four Quad Unit Processors.
- One Picolo Alert board.

Connect the output of each Quad Unit Processor to one input of the Picolo Alert board.

## Notes

- The Picolo U4/U8 H.264 boards are no longer supported with EthoVision XT.

*See also*

- Install the Picolo Alert encoder board
- Experiment settings > Video source
- The PhenoTyper - EthoVision XT 18 - Reference Manual for more information about using multiple PhenoTypers. See Manuals

# Test an analog camera

## Aim

To check whether your analog camera and the Euresys Picolo Alert encoder board are functioning properly.

## Prerequisites

- When you install EthoVision XT you have also installed the Euresys Multicam driver for the Picolo Alert encoder board. See Install EthoVision XT. This software also include **Multicam Studio**.
- If Multicam Studio is not installed on your EthoVision XT computer, browse to my.noldus.com, log in or register, and select **Downloads** > **EthoVision XT** > **Drivers and Tools** and download and save the file **Euresys Multicam -Driver win10**. Run this file and install Multicam Studio.

## Procedure

1. Connect the camera to the EthoVision XT computer.
2. Power up the camera.
3. Double-click **Multicam Studio**.
4. Select the video input and check the camera image. When necessary, adjust the settings like the video standard.

*See also*

- Install analog cameras

# Power options when using cameras

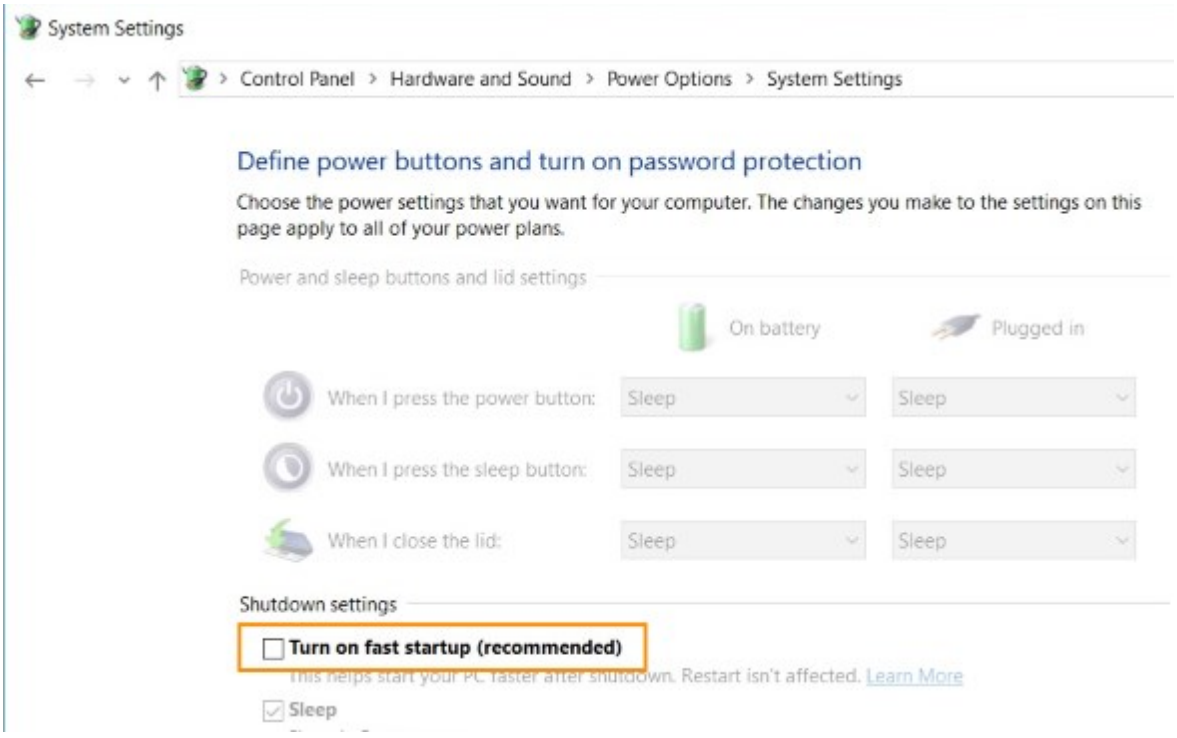
## Aim

To disable *Fast startup* on your computer. This prevents problems when recording videos from within EthoVision XT.

**NOTE** By default the computer has the Fast startup option enabled. This can sometimes cause EthoVision XT and MediaRecorder to stop responding when selecting an input of the frame grabber board or camera. That problem has been found when using encoder boards for analog cameras, but we recommend to follow the procedure below for any type of camera you have.

## Procedure

1. Open the Windows Control Panel, and select **System & Security**.
2. Under **Power Options** select **Choose what the power button does**. Click **Change settings that are currently unavailable**.
3. Under **Shutdown settings**, de-select **Turn on fast startup (recommended)**.



*See also*

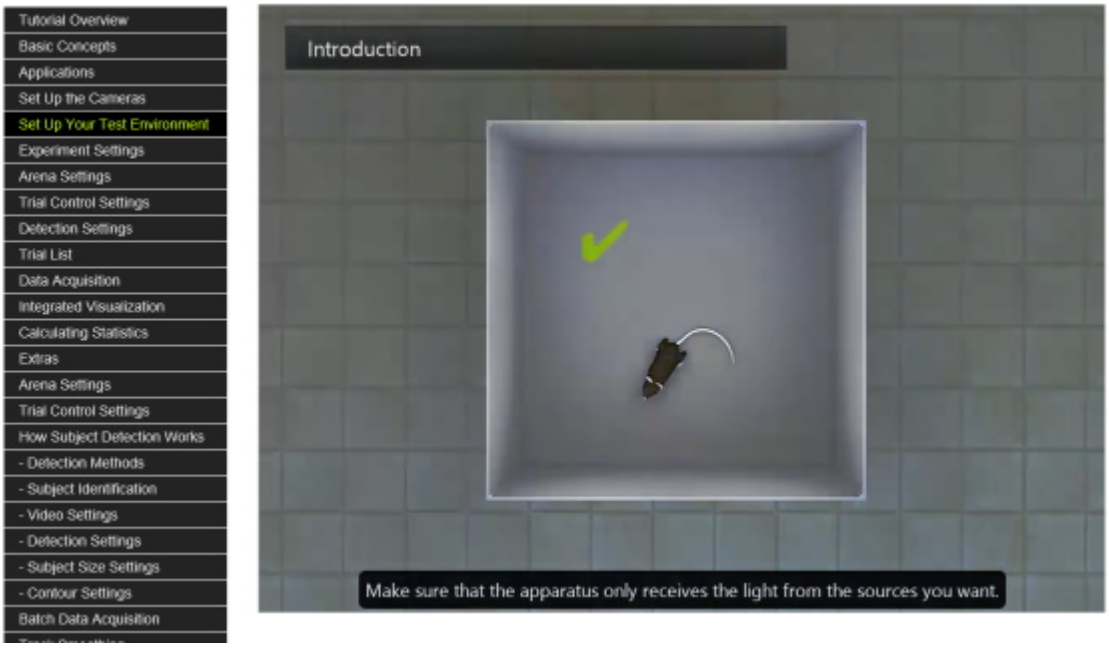
- Install GigE cameras
- Install USB 3.0 cameras
- Install analog cameras

# The test environment

## Learn about

- Camera position and settings
- Physical setup of an arena
- Lighting setup

**TIP** Watch the video **Set Up Your Test Environment**, which you can find in the main EthoVision XT video tutorial (**Help** > **Video Tutorial**).

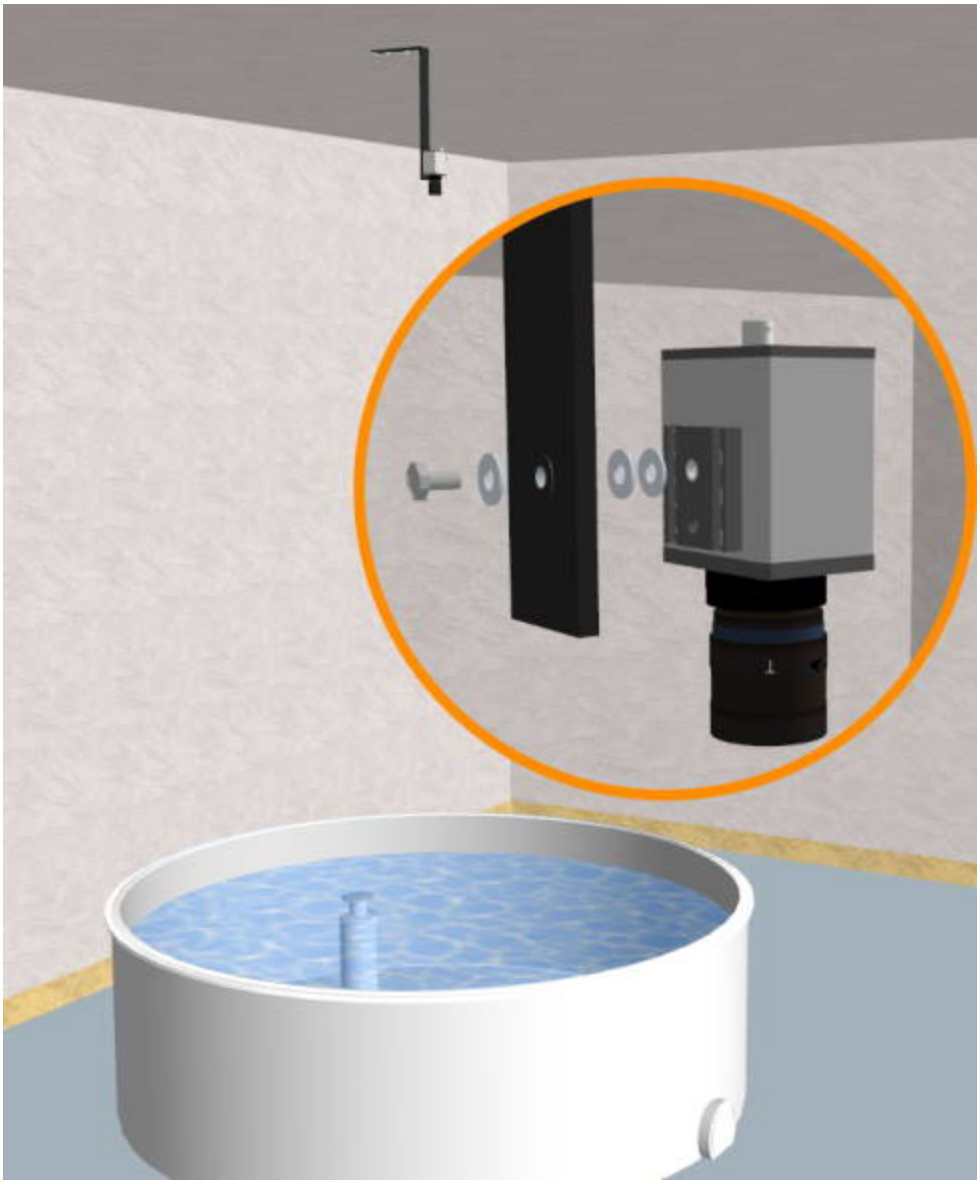


# Camera position and settings

**TIP** Watch the video **Set Up the Cameras**, in the EthoVision XT video tutorial (**Help** > **Video Tutorial**).

## Attach the cameras

*With the ceiling mount*



*With the Autopole*

For this configuration you need an Autopole, a SuperClamp and a camera adapter (all shown in the following figure).

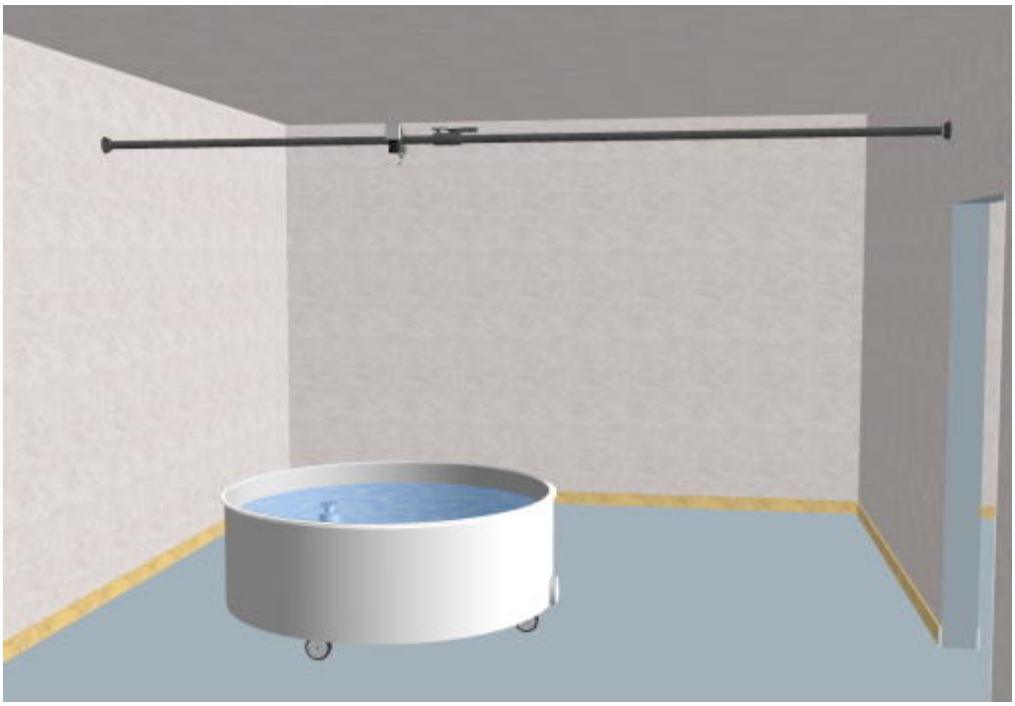
The Autopole range is 2.1-3.7m, or up to 5.7m with the extension (sold separately).

**NOTE** The elements shown here may slightly differ from those you have purchased.

How to attach the camera to the SuperClamp:

1. Connect the camera to the SuperClamp through the brass stud connector or the camera adapter.
2. The Autopole gets clamped between the jaws of the SuperClamp. Turn the handle to tighten the SuperClamp to the Autopole.

Horizontal installation is mainly for top view tracking. For this you need the Autopole and the SuperClamp.



Vertical installation is for top view tracking and side view tracking. For this you need the Autopole, the SuperClamp and a camera adapter (see above). For more flexibility you can use a variable friction arm attached between the SuperClamp and the camera adapter.

*Suspended ceilings*

If you need to attach the camera to suspended ceilings, you can get a special mount that has two parts which move like scissors. Move them to open up the mount and it will fit over the framework of the suspended ceiling, then close them to hold. Attach the camera adapter and then the camera.

This is a temporary mount, it is sturdy enough but you can always open the mount to remove it from the ceiling and place it elsewhere. If you have a suspended ceiling it's probably the best solution.

## Cable length

The maximal length of the cable between camera and computer depends on the type of camera you use.

- For GigE cameras and Analog cameras: 100 m.
- For USB 3.0 cameras: 5 m. You can increase this length by means of an active extension cable (not tested though). See Connect the USB 3.0 camera to the PC.

## Things to check

- Position the camera perpendicular to the plane in which the animal moves. If this is not the case, the shape of the arena and subject inside will appear distorted on the screen and calculated distances, velocities and spatial statistics will be incorrect.
- Anchor the entire setup. Secure the position of the arena, camera and illumination relative to each other, and fasten the camera zoom, focus control and aperture settings.
- Adjust the zoom setting (or select the appropriate lens) and focus the lens until the entire experimental arena is visible on the screen and is displayed in focus.
- When using multiple cameras, pay attention to the zoom settings. These should be in such a way that the apparent size of the arenas is the same since you calibrate the distance for all the cameras (Shared calibration). If that is not possible, make one calibration for each arena (Unshared calibration). See Calibrate multiple arenas
- Turn off all automatic camera settings: focus, gain, exposure (auto-iris), anti-shake.
- Adjust the camera aperture until the image shows maximal contrast. Depending on how bright your IR setup is, you might need to open the aperture on the lens all the way (do this first) and/or adjust the cameras **Gain** (Raw) and/or adjust the **Exposure time** in Pylon Viewer. See Configure the digital camera

See also Lighting setup

## View the camera image in EthoVision XT

1. **IMPORTANT** When using digital cameras, close the camera software Basler pylon Viewer before operating the camera in EthoVision XT.
2. Create a new experiment.
3. Choose **Setup** > **Experiment Settings**.
4. Set the Video source to **Live tracking**. For details, see Video source.
5. Click the camera button (for single cameras) or the eye button (for multiple cameras in merged view).

## Image magnification

If you need to get higher magnification images than is available with a standard lens (get 'closer'), then you can use one of the following techniques.

### *Macro lens*

A true macro lens, that is, a lens of a magnification of at least 1:1 (expressed as 'real world': CCD chip size) usually gives good quality images. However, it is often expensive and probably gives a lower magnification than either a close-up filter or an extension ring.

### *Extension rings*

An extension ring inserted between the camera body and the lens enables you to focus on subjects closer to the camera, and so provides a greater magnification. An extension ring is usually cheaper than a true macro lens, but more expensive than a close-up filter. It may give a reduction in optical quality, and it will reduce the light intensity of the signal, but you can use it to get very high magnifications.

### *Close-up filter*

This is a lens attached in front of the normal lens, to give greater magnification. It is also known as a close-up lens. It works by shortening the focal length of the lens while keeping the lens-to-camera distance constant, which increases the magnification of the lens. The strength of a close-up filter is measured in diopters, which is the fraction by which the lens' focal length is shortened. Thus, a +3 lens reduces the focal length of the lens to which it is attached by 1/3. A close-up filter is usually cheaper than a macro lens or extension ring, but it gives lower magnification, and poorer optical quality.

*Microscope*

EthoVision XT tracks well from cameras attached to microscopes. Use back-lighting to maximize contrast.

## Change color to monochrome

If you If you have a color camera, you can temporarily change it to monochrome by changing the Pixel format setting. For Basler cameras:

- In pylon Viewer, change the **Pixel format** to **Mono8**. See Set the pixel format
- In EthoVision XT, set the **Color space** to **Y800**. See Adjust camera settings in EthoVision XT > Color space

See also Settings for the color camera

# Physical setup of an arena

Think carefully about the kind of enclosure to use in your experiments. Consider both the background of the arena, as well as its size and position.

## Location

Place the computer and all electrical equipment other then the cameras in a room separate from the test room. Computers and electrical devices produce ultrasound, which may affect the behavior of the subjects.

For specific tests, see also the EthoVision XT 18 - Application Manual. See Manuals

**TIP** Watch the video **Set Up Your Test Environment**, which you can find in the EthoVision XT video tutorial (**Help** > **Video Tutorial**).

## Background

- The animals you are tracking should be able to move freely and naturally.
- The background must be fixed in relation to the camera. Make sure you firmly anchor the entire setup when it is completed. If the arena moves, you must update the background image.
- The background should be made of non-reflective surfaces. If this is unavoidable (e.g. the water in a Morris water maze) use indirect illumination (e.g. bounce light of the ceiling) and non-reflective surfaces (e.g. black pool sides). For details, see Lighting setup.
- There should be maximum contrast between the background and the animal. For a Morris water maze:
  - If you use dark animals, color the water with white tempera paint.
  - If you use white animals, add 300 g of tempera black nontoxic powdered paint to a 45 liter pool.

## Dimensions of the arena

- The arena should have no 'depth', because the camera can only see in two dimensions. If the animal can move towards or away from the camera, like when climbing on a shelter, its apparent size will vary. This may interfere with subject detection and tracking.
- If you want to track animals in a three-dimensional environment like an aquarium, then make sure this is as shallow as possible, or only calculate variables like In zone, not Distance moved or Distance to a zone. For true 3D movement analysis, contact your nearest Noldus office.

- The maximum arena size (in pixels) is limited by the image resolution (W x H) of the digital video file you are using, or the resolution of the live camera image set in the Experiment Settings. Your subject must be at least three pixels wide in order for EthoVision XT to distinguish it from system noise. Therefore, the ratio of your maximum subject size to the maximum arena size is given by the limiting resolution (usually H) divided by three. With digital; cameras and a vertical resolution of 1024 pixels, this is approximately about 300 times the animals' size. In practice the limit is lower, depending on various factors including the animal's contrast with the background. Especially when tracking small insects, for more robust tracking, make sure that the subject is always at least 10-20 pixels large.

## Maximum size of the arena

When you have video of resolution L × H (in pixels), and you want to track a subject of l × w (length x width, in mm), check the following:

- Calculate the ratio maximum subject size to maximum arena size = H/3.
- Calculate the maximum arena size = w × (H/3)

If the arena was bigger than that limit, the subject would often be less than three pixels wide and EthoVision XT would not be able to distinguish it from random noise.

## Maximum size of multiple arenas in one image

If you use multiple arenas, the calculation applies to the total distance across all arenas, thus if you have four arenas in a square, the maximum width of each one is half what it would be if you had only one arena.

# Lighting setup

## Ideal light conditions

A good lighting setup is vital to get a good image that EthoVision can use to accurately track your animal. This is particularly important when you want to do nose-tail tracking or track multiple animals with color markers. To produce a good image, the lighting must satisfy three criteria:

- It must be bright enough. Stand-alone video cameras (which we recommend) can sometimes work at 0.1 lux or darker. At higher light levels you can work with a smaller aperture. See also Using EthoVision XT in near darkness. Please note that poor lighting gives more noise in the image which enlarges the video file size.
- It must be even. That is, a diffuse light source, such as a shaded fluorescent tube or globe-type incandescent bulb. Spotlights can produce changing shadows and reflections.
- There must be no reflection of the light source visible in the image (see below).

**TIP** Watch the video **Set Up Your Test Environment**, which you can find in the EthoVision XT video tutorial (**Help** > **Video Tutorial**).

## Eliminating reflections

If you have reflections in your image, EthoVision may confuse those reflections with your subject, and track the reflections rather than your animal. Preventing reflections is particularly important when you want to do nose-tail tracking. There are a number of measures that you can take to reduce this problem:

- Surfaces within the arena should be (whenever possible) a dark color and matte texture.
- Lighting should be from a diffuse source, such as a shaded fluorescent tube or globe-type incandescent bulb.
- The light source should not be in direct line-of-sight of the camera (that is, it should be indirect lighting). You can achieve this by bouncing the light off the ceiling or walls (which should be a pale color and matte texture) and, if necessary, placing a shield in between the light source and lens.
- If your arena is transparent (for example, a glass olfactometer), you can use backlighting. This will eliminate reflections, but you must take special care that the lighting is even and diffuse. Place the light source underneath the setup and attach a sheet of white paper to the bottom to create diffuse

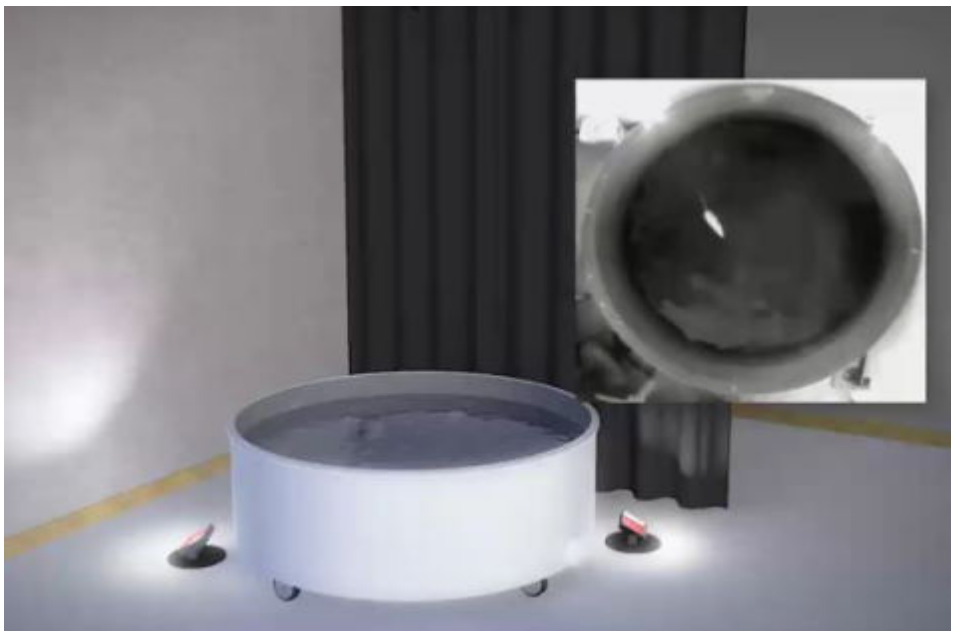
back light. It is important to avoid light coming from above as much as possible, otherwise there is no effect of back light.

- You could also try a polarizing filter in front of the camera lens. The filter will remove reflections that are incident to the reflective surface at 32° - 37°.
- If possible, draw your arena in such a way that bright reflecting rims of mazes are excluded.
- To remove reflections brighter or darker than your animal, set appropriate thresholds in the Detection Settings.
- To remove reflections larger than your animal, enter an appropriate value for the Maximum Subject size in the Detection Settings. Note that if your animal enters the area of the reflection, EthoVision might no longer be able to track it if you use this setting.

## Lighting a Morris water maze

Lighting a Morris water maze needs to be carried out particularly carefully because the water can give large and variable reflections. Some of these can be excluded by the software settings listed above, and the following lighting setup should give good results:

Place four (or more) bulbs round the pool, below the level of the water surface or at the ceiling. 'Globe' type bulbs are ideal (twice the diameter as standard incandescent light bulbs). They should be close enough to the pool wall so that there is no direct line of sight between the bulbs and the camera lens. The light is reflected off the walls and ceiling, so that it only reaches both the lens and water surface indirectly.



- Take special care when dealing with water and electricity in the same room. The lights should be placed so that water cannot splash on them, ideally they should be suitable for outdoor use (double-insulated) and if connected to an electrical outlet in the same room as the water maze they should be connected through a circuit-breaker.
- Ask a photographer for advice on the lighting of your water maze.

## Lighting water tanks

When using water tanks like in the Porsolt forced swim test, a problem is often that there is a lot of reflection, especially just above the water surface. Furthermore, detection of the animal can be hindered by the water's meniscus.

Try to make a set-up with as much contrast between animals and background as possible. For instance, when you have white rats, preferably use a completely black background. Back light (that is, light from behind the subjects so you only see the animals' contour) generally gives very good results. A solution to remove the water's meniscus in the video image is also to place the test cylinders in a separate tank with water (for example an aquarium) and then make recordings. The water level in the tank should be higher than in the cylinders.

For more information on the Porsolt forced swim test, see the EthoVision XT 18 - Application Manual.

## Using EthoVision XT in near darkness

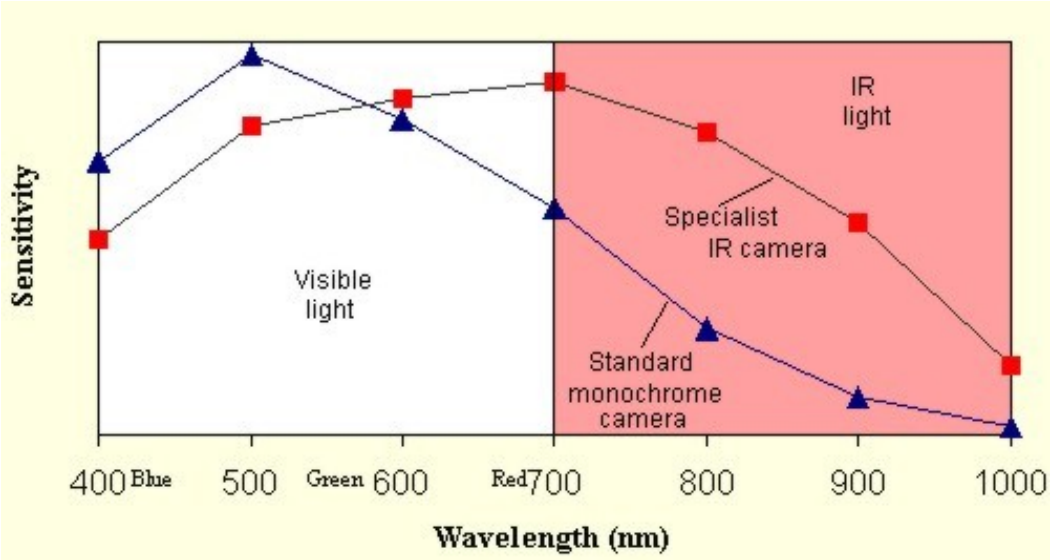
Nocturnal animals like rats and mice are active in the dark. It is, therefore, a logical choice to measure their behavior in the dark. You can use infrared (IR) lights to illuminate the arena and a camera that is sensitive in the infrared. Most animals, including rats and mice, do not see infrared (IR) or near-infrared (NIR) light. Infrared illumination is perceived by them (and also by humans) as being total darkness.

### *Camera*

Infrared light (IR) is light of a longer wavelength than visible light. Generally, to track with EthoVision XT, near-infrared light is used, with a wavelength of 700-900 nm (near the visible range of 400-700 nm). Color video cameras are insensitive to infrared but standard monochrome cameras have reasonable sensitivity to near IR.

In particular, the Basler monochrome GigE cameras are IR sensitive. Normally they do not require an IR-pass filter unless you want to track the animals in both dark and light conditions during the same trials, for example in multi-day trials. See Light in 24-hour experiments

Below: Sensitivity to infrared light of a standard monochrome camera and a specialist IR camera.




### *Optimizing image brightness*

Depending on how bright your IR setup is, you might need to:

- Open the aperture on the lens all the way (do this first).
- Adjust the cameras **Gain** and/or the **Exposure** setting in EthoVision XT.
  - A high Gain increases brightness but also noise in the image.
  - A longer Exposure time may not be compatible with the frame rate set in the camera. Check carefully in Adjust the camera exposure time

**TIP** Watch the video **Set Up the Cameras**, which you can find in the EthoVision XT video tutorial (**Help** > **Video Tutorial**).

### *Light source*

As your light source, you can use IR illuminators, which basically are LED lamps that produce infrared rather than visible light. Using an IR illuminator is just like using a lamp, except that neither you nor your animals can see its light. Check the image from your camera to see what the illumination actually looks like. Just as with visible light, you want uniform lighting throughout your arenas. You may find that objects that look light in visible light, appear dark in the IR image, and vice versa. Likewise, objects that appear transparent may be opaque, or opaque objects (especially some kinds of acrylic) may be transparent in the IR image. You can record through acrylic, unless it has been colored or coated with pigments that absorb IR light. You can use, for example, a sheet of acrylic to cover the top of a cage or arena. If you use an IR illuminator from overhead, you may gets spots of reflective glare, just as with normal lighting. Moving the illuminators to the sides, rather than overhead, can

eliminate the glare. To eliminate shadows, you may get the best results by using two or more illuminators.

- You can obtain IR LED illuminators from Noldus Information Technology.
- You can also use an IR lightbox as your light source. An IR lightbox provides a uniform source of IR illumination from beneath your animal. Unlike the case with an IR illuminator, the camera will see a dark silhouette of the animal against a light background, which is easy to track in EthoVision. An IR lightbox is also an excellent solution if your animals are highly variable or mottled in color, for example Long Evans rats, and eliminates any concern about glare.

## Light in 24-hour experiments

If you want to go from light to dark conditions (or vice versa) during a single trial (e.g., recording across a 24-hour circadian cycle), EthoVision needs consistent lighting. This can be accomplished by using an IR-pass filter on your camera and by preventing any variation in the amount of IR illumination.

Should you require an IR-pass filter, take note of the lens characteristics. The filter should match the diameter of the lens, and simply screws on to the front of the lens.

The filter prevents the camera from seeing visible light, only IR light gets through. The filter is not necessary under constant lighting conditions, but you need it if the visible light level changes during the trial. The IR illumination must remain on for the entire duration of the trial.

Some IR illuminators have the feature of automatically turning off when there is visible light (for example, IR illuminators designed for security applications usually turn off during the day). If your illuminator has this function, it needs to be disabled. Consult the documentation for your illuminator to see how this is done. If it is not possible to prevent the automatic turning off, you can cover the illuminator's light sensor with tape so that it will not detect light.

The normal light in the room should not affect the detection setting unless it also emits IR light. Many light fixtures will give off some IR illumination in addition to visible light. Incandescent and halogen lights especially will produce a great deal of infrared light. Preferably use fluorescent lights which produce much less infrared light. If there is an intensity adjustment on your IR illuminator, turn it up as high as possible. Increasing the amount of IR illumination will minimize the impact of any infrared light from your visible lights. Sunlight also has an IR component, so if you have windows, they should be covered. When watching the camera image, ideally you should see no change in the image when the visible lights turn on or off. It might be that your image is slightly out of focus, but that is no problem for tracking in EthoVision.

*Notes*

- If you have light colored animals, use the darkest non-reflective bedding possible for improved contrast. Be aware that some dark materials may appear white under infrared light. See also the section **Recommended materials** in the PhenoTyper - EthoVision XT 18 - Reference Manual. See Manuals
- If you have not eliminated all infrared light from the visible light in the test room, and you are using Static subtraction as your detection method, the entire arena may be detected as the animal after the visible lighting changes. Choose Dynamic subtraction and the animal will be detected after a few frames.

# Using Digital Video

## Main topics and tasks

# Video file formats

## Background information

One of the difficulties with digital video files is that the file *extension* does not indicate what the file *type* is. For example, the extension *.mpg can mean any one of different type of formats, and an MPEG-4 movie can have the extension mpg, mpeg, mp4, m4v or avi.

**IMPORTANT** If you are not sure whether the format of your video files is compatible with video tracking in EthoVision XT, test a few video files thoroughly and check that there are no or few missing video frames.

- Video file formats supported by EthoVision XT
- Video files created by EthoVision XT
- Video files created by Media Recorder
- Video formats not supported by EthoVision XT

## Video file formats supported by EthoVision XT

**IMPORTANT** When in doubt, test a video format thoroughly before carrying out experiments. Acquire test trials and check that there are no missing samples, and the video plays smooth in the various parts of the software.

If the video format is not compatible, convert video to H.264 MPEG-4. That should solve the issue.

### *MPEG-4*

This is a collection of formats, not all are supported. MPEG-4 can achieve a very high rate of compression with good quality, because it separately codes the background (which does not change much from frame to frame) from the moving parts of the video.

MPEG-4 (mp4v) in a MP42 container, with extension *.mp4, is the format of video files recorded with Media Recorder 4 and EthoVision XT 15.

### *H.264 AVC*

H.264 AVC is a type of MPEG-4 and is also known under the names H.264/AVC, AVC/H.264, H.264/MPEG-4 AVC, MPEG-4/H.264 AVC, MPEG-4 Part 10 or x.264. It creates good video quality and uses previously-encoded pictures as references in a much more flexible way than in other standards, allowing the use of up to 16 reference frames.

H264 AVC in MP42 container, with extension *.mp4, is the format of video created by EthoVision XT 16-18 and MediaRecorder 5 and 6. See Video files created by EthoVision XT

## Video files created by EthoVision XT

When you record video with EthoVision XT, video is encoded with the **H.264/ MPEG-4 AVC** *codec* and is stored in a container of **MP4-version 2** (MP42). The video file extension is **mp4**.

Note that a *container* is something different as the *codec*.

- The container is the structure that handles various streams such as video and audio streams. You can imagine the container as a box that contains your video frames.
- The codec is software that compresses the video stream data.

For information about the H.264 specification, you can find many internet pages that explain the process of video/audio compression. Here is one of them:

https://www.videoproc.com/resource/h264-codec.htm

## Video files created by Media Recorder

### *MediaRecorder 4.0*

- With analog cameras: H.264 AVC in an MPG container.
- With GigE cameras: MPEG-4 Video in an MP4-version 2 container.
- With IP cameras: H.264 stream in an MP4-version 2 container.

### *MediaRecorder 5.0*

- With all cameras: H.264 stream in an MP4-version 2 container.

### *MediaRecorder 6.5*

- With all cameras: H.264 stream in an MP4-version 2 container.

All video files have file extension **mp4**.

## Video formats not supported by EthoVision XT

**IMPORTANT** The term “not supported” means that the video formats listed below were not thoroughly tested by Noldus. It may be that some formats work fine. If you have old MPEG-2 videos and you want to re-track them with EthoVision XT, test

them and check whether (1) Video plays smooth and without artifacts (“blocks” in the image) and (2) Samples are not missed during tracking, even when selecting the **Detection Determines Speed** (DDS) option.

**IMPORTANT** When using camcorders or other encoding software to make video files, test those files in EthoVision XT before deciding to work with them or convert them to a supported format. See Video file formats supported by EthoVision XT

- MPEG-1.
- MPEG-2. This is a collection of formats, not all may work in EthoVision XT.
- DivX.
- Variants of MPEG-4. However, an AVI 1.0 file with codec MP43, and an OpenDML AVI file with codec MP4S-ISO MPEG-4 Video V1 may work.
- DV-AVI. This is uncompressed video from FireWire cameras. We do not recommend this format because of the large file size. DV-AVI is a special case of AVI. In general, EthoVision XT does not support video files of AVI format. The AVI format should not be confused with the file extension avi.
- WMV. However, an OpenDML AVI file with codec WMP v9 may work.
- Quick Time.
- FLV (Flash video, YouTube).
- XviD variants of the MPEG-4 format. However, an OpenDML AVI file with codec XviD 1.0 RC4 may work.

*See also*

- Record digital video

# Record digital video

## Background

Digital video requires a lot of storage capacity and always needs to be compressed before it can be stored on a disk medium. In order to compress a video recording you need an encoder, usually a plug-in board, which converts the video signal into a media file on disk at a fraction of the original size. The input signal can either be analog or digital.

## Prerequisites

- If you have analog cameras: EthoVision XT works with the Picolo Alert encoder board. You can use this board not only for live tracking, but also for recording video. See Install analog cameras
- If you have digital cameras:
  - Install GigE cameras
  - Install USB 3.0 cameras

Another possibility is to use the Noldus MediaRecorder software to make digital video files and track from these videos in EthoVision XT (offline tracking).

## Procedure

1. Create a new experiment or open an existing one.
2. In the Experiment Settings, select the camera(s). See Video source
3. In the Arena Settings, calibrate and draw the arena.
4. In the Acquisition screen, on the right-hand pane select **Save video only, track later**. With this option you record the live image to a video file without tracking. Select this option especially if your computer is not fast enough to do tracking and recording at the same time. For other options, see **Acquire Data** in this Help.
5. To start recording, click the **Start trial** button.

**TIP** Save this experiment as a template (**File** > **Save As**). You can then re-use the same settings in a new experiment when you Create a new experiment based on an existing experiment.

## Video file location

Your video files are saved in the **Media Files** subfolder of your experiment folder. By default in: C:\Users\Public\Public Documents\Noldus\EthoVision XT\Experiments\[experiment name]\Media Files.

## Video file size

Roughly speaking, the file size is given by:

File size = Bitrate x Duration x Compression ratio

Where:

- The Bitrate is the product of Frame size x Frame rate (frames per second)
- The Duration is the length of the video (in seconds).
- The Compression ratio tells how much space is needed to store similar frames that have fewer moving parts, like an empty open field.

You can use the following table as a reference for video created with EthoVision XT. Please note that the figures reported are only indicative. There are other factors which influence video file size, such as whether a variable bit rate is used or which color space is used. Also the amount of movement in the video influences file size. The more movement, the larger the resulting video file.

Therefore, the size of your video files may well differ from what reported here below, even when using a comparable setup.

| Resolution, frame rate | Camera | 10 minutes video | Duration per GB |
|---|---|---|---|
| 1280x1024, 30 fps | Basler GigE, monochrome | 75 -150 MB | 1-2 h |
| 1280x1024, 25 fps | Basler GigE, color | 175 MB | < 1 h |
| 2560x1024, 10 fps | Basler GigE, color | 100-160 MB | 1-2 h |
| 720 x 480, 30 fps | Analog cameras, monochrome | 60 MB | 16 h |
| DanioVision 800x600, 60 fps | Basler GigE, monochrome | 90-125 MB | 1-1.5 h |
| DanioVision 1280x960, 25 fps | Basler GigE, monochrome | 355 MB | < 30 min |

### *See also*

- Video file formats

# Tips for color tracking

## Main topics and tasks

# Lighting conditions

You need the information below when you track color-marked animals.

## General

Generally, for optimal tracking it is important that the contrast between the subject and the background is as good as possible. For marker tracking, EthoVision XT needs to be able to distinguish the different marker colors. For both issues, the lighting conditions play an important role.

- *It must be bright enough*. A low light intensity can result in a suboptimal contrast between the animal and the background and therefore tracking might not be optimal. Furthermore, a low light intensity makes it difficult to separate different colors. When it is not possible to use strong illumination in your setup, try using fluorescent marker colors with UV lighting.
- *It must be even*. The light source should be diffuse, for example, a shaded fluorescent tube or globe-type incandescent bulbs. This way, the contrast between the animal and the subject and the intensity of the marker color are the same throughout the arena.
- *Shadows must be avoided*. To avoid shadows, do not point the light directly to your animals, but, for example to the walls or ceiling. Use several lamps, to obtain an even illumination.

## Full-spectrum lights

For optimal color separation, illuminate your setup with lamps that approximate to day-light in frequency distribution of wavelengths, that is, have a full-spectrum range. There is no technical definition of “Full spectrum”, therefore it cannot be measured. However, to compare full-spectrum lights you can directly compare spectral distributions.

In practice, full-spectrum lights can be recognized from two parameters:

- High Color Temperature (measured in degrees Kelvin). Usually higher than 5000 ºK. Values of color temperature around 2500-3000 ºK are typical of “warm” color lights (yellowish), for example incandescent bulbs.
- High Color Rendering Index (expressed as percentage). Higher than 95. A value of 100 represents the ideal or natural light.

*See also*

- For color temperature: http://en.wikipedia.org/wiki/Color_temperature

- For color rendering index: http://en.wikipedia.org/wiki/Color_Rendering_Index

When buying LED lamps, make sure that the technical specifications report the required color temperature and CRI. A disadvantage of most LED lamps is that they cast light in one direction at a narrow angle compared to energy-saving fluorescent lights, creating more shadows and reflections.

We have tested the following lamps:

- True-Light Energy Saving Light 15 W. Color Temperature 5500 ºK, Power 15 W, Brightness 720 lumen.
- ProLite Helix Compact Fluorescent ES 15. Color Temperature 6400 ºK, Power 15 W, Brightness 795 lumen.

Both lamps have E27 fitting.

## Recommendations

- Choose a light source with color temperature ≥ 5500 °K and color rendering index > 95.
- Do not use halogen lamps; instead use LEDs, as halogen light is too “cold”.
- Use at least four lights and place them at equal distance around the arena, at least 1 m from the subjects.
- If the image of white animals looks overexposed, close the aperture of the camera lens. Too much light heavily reduces color discrimination.
- If light creates shadows in the arena, place white sheets in front of the lamps to obtain more diffuse light.
- Set the white balance of your camera, to obtain a wider color spectrum. Consult the manual of your camera for the exact procedure. Generally, you can set the camera to white balance the image automatically. To check whether this is done correctly, point the camera to a white wall and check whether the wall appears white in the video image. If this is not the case, search for the settings to set the white balance manually. Point the camera to a white wall and set the white balance. Make sure you do this every time the light changes.

## Useful web sites

Below you find the web address of full spectrum lights producers

- True-Light: http://www.true-light.eu/en/
- BlueMax Lighting: https://www.fullspectrumsolutions.com

- OSRAM: http://www.osram.com/
- Philips: https://www.lighting.philips.com/main/home
- Pro-Lite: http://www.prolite-lamps.co.uk
- Viva-Lite: http://www.viva-lite.nl/home.html

# Color markers

## Aim

You need the information below when you track color-marked animals.

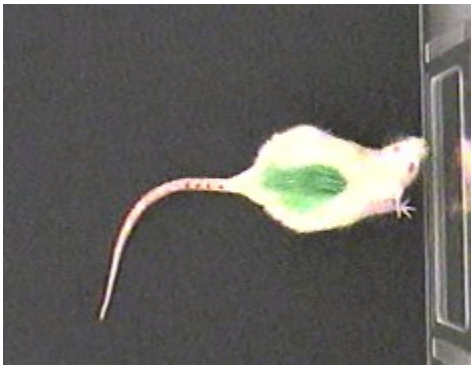
## General information

- Use a color scale (for example from a paint company) to find out which colors are most easily recognized by EthoVision XT in your setup and lighting conditions. Do this before applying color markers to your animals.
- Use colors that have different hue values. For example, use red and green and not red and orange.
- For birds and animals in general that perceive color in the red-green wavelength range, using red markers is not advisable as it looks like blood, and may influence the subjects' behavior. Rodents have dichromatic vision, that is, they perceive wavelengths around the blue and the green, so probably red is seen as gray or a mix of blue and green. Albino animals have heavily impaired vision, both in acuity and color perception.
- If you want to use red together with orange or pink, provide more light around (by means of additional lamps) and then close the aperture of the camera lens.
- If the color of your marker is also present elsewhere in the arena, you can get rid of it by using a minimal marker size.
- Marking your animals may stress them, and therefore affect their behavior. If necessary, ensure that you select a marking method that lasts for a longer period of time.
- To improve color discrimination in EthoVision XT, you can alter the Saturation settings of the video image (this only applies for live tracking). In the Detection Settings window, under Video click Image Quality. Alter the Saturation value and check in the video window if the markers are more distinguishable. However, before altering the Image Quality always try to optimize the lighting and camera aperture settings.

  Note that if you alter the Image Quality values you need to redefine the color detection (click Identification for each subject).

## Marker characteristics

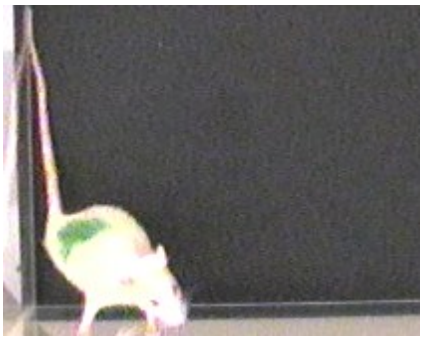
- Make sure that the marker is visible as much as possible; preferably place it on the center of the animals backbone.



- Make sure that the marker is as round as possible, this will ensure that the relative movement of the center of gravity of the marker is the same in all directions when the edges of the marker change due to posture changes or otherwise. For color marker tracking it will help to prevent the jitter of the marker.
- When you use marker assisted tracking, make sure the marker is not too big; the marker can interfere with proper detection of the body contour. For example, make sure that a dark marker on a white animal does not cover the complete width of the animal because it can cause the body to be split in two.

## Markers for rodents and other small mammals

There are several ways to color mark small mammals like rats and mice. Color mark as much of the head and back as possible. This way the mark is always visible, also when the animals rear or turn.



- Livestock markers are intended for large animals but can also be used on smaller animals. These markers come as paint sticks and spray cans, and are available in many colors including fluorescent ones. The markers are weather-resistant, non-toxic and they stay on for one or two days.
- Fluorescent tubes can be used for color-tracking in the dark. It is possible to mark rats with fluorescent markers and illuminate the arena with UV-light (350-400 nm, 2 lamps of 1.2 W each). The fluorescent spots on the rats

appear as patches of different color against a dark background. EthoVision XT can then effectively separate the two animals (source: J.P. Johansson and M.L. Carlsson, Institute of Clinical Neuroscience, Göteborg University, Göteborg, Sweden). See Tracking colors in the dark

- The fur of rats and mice can be marked with commercial products for dyeing human hair. Dark fur can be bleached (like dark human hair, to make it blonde) before coloring. Hair dyes are available in many bright colors, and provide long-lasting coloration. Schwarzkopf PolyColor Hair color Cream, for example, can be applied to rat's fur with a cotton swab.
- Permanent marker pens (for example, Edding or Marks-A-Lot) present a cheap and easy way to mark small mammals. Take care to make a large and uniform blob. This method may only work for a short period of time as many species may remove the mark by grooming.
- Chalk ink markers can be used to mark rodents. They are available in a variety of bright colors. Their main advantages is that they are not toxic, are readily available and inexpensive, and, in contrast with other marker types, can be applied directly to dark fur without bleaching it first. Coloration may last several hours.

  It is advised not to mark the animals just before you start a test, because the handling of the animals might affect their subsequent behavior. If you use a marker that stays on the animal for only a short period of time, you should habituate the animal to the marking procedure.

- Colored 'jackets' can be used to color-track mammals with colored fur like cats. Jackets cover most of the cat's back, and can be provided with Velcro strips, so one can place pieces of cloth of different colors to test optimal color combinations. Orange vests may give problems when animals are red-haired, so it should be replaced by either (bright) green, yellow or pink.

## Markers for large mammals

You can use livestock markers to mark large animals such as cows, sheep and pigs.

## Markers for birds

- The feathers of birds can be marked with commercial products for dyeing human hair or permanent marker pens (see 'Small mammals' above).
- Seabird ecologists often use picric acid (2,4,6-trinitrophenol, TNP) to mark their birds. You paint a dab of this yellow liquid on the feathers. It is permanent and very bright yellow, although it turns orange in the sun. To use it to mark animals you dissolve it in an excess of ethanol. If the acid (a powder) dries, it becomes explosive, so it is important to keep it dissolved

(Source: J. Blount, Division of Environmental and Evolutionary Biology, University of Glasgow, UK).

## Markers for fish

- Fish can be color marked by attaching plastic pearls. This technique has been used successfully in Mediterranean damsel fish (*Chromis chromis*, size: about 9 cm long) and trout (about 25 cm). Colored pearls can be bought from a toy store or a warehouse. Use bright colors that are as distinct as possible. For damsel fish pearls of 1 cm are used and for trout pears of 8 mm are used.

  *Damsel fish*. The pearls can be attached under the dorsal fin with blue non-resorbable surgery polyamide mono filament (for example, from B/Braun), using a surgical needle under anaesthesia. The fish can be anesthetized by placing them in a small tank with 2-phenoxy-ethanol (0.3 ml/l for the damsel fish) for five minutes, before attaching the pearl. The bottom and sides of the arena have to be very dark. The pearls do not significantly impair swimming in damsel fish (Source: M. Ylieff, Institut de Zoologie, Université de Liège, Belgium).

  *Trout*. The pearls can be attached at the base of the first ray of the dorsal fin. Use a curved needle and normal fishing-line to attach the pearls to the fin. X-ray images showed no injury after several weeks using this method. The fish can be anesthetized by placing them in a tank with 2-phenoxy-ethanol (0.3 ml/l), before attaching the pearl.

- Visible implant fluorescent elastomer (VIE) tags are produced by North west Marine Technologies (NMT). VIE material is implanted beneath transparent or translucent tissue (it may become difficult to detect it beneath pigmented tissue). The material is injected as a liquid and soon turns into a pliable, bio-compatible solid. VIE colors are well visible under normal lighting conditions and have greatly enhanced visibility under ultra-violet light or with other fluorescence enhancing techniques. You can test which colors work best in your own setup with a color test patch from NMT.

(Source: J. Merilä, Evolutionary Biology Centre, Uppsala University, Sweden and Delcourt *et al*. (2012). *Behavior Research Methods* **43**: 590–600).

## Markers for reptiles

Nail polish (available in many colors) can be used to mark lizards. The mark usually lasts 10 days in small (up to 10 cm) lizards in the field. (Source: M. Massot, Laboratoire d'Ecologie, Université Pierre et Marie Curie, Paris, France).

## Markers for amphibians

For amphibians you can use visible implant fluorescent elastomer (VIE) tags (see 'Fish' above). They work fine with small tadpoles.

## Markers for insects

- Acrylic paint (for example, Royal Talens Amsterdam deco) can be used to mark insects. The paint can be applied with a fine brush or thread. You have to hold the insect with forceps until the paint is dry (Source: G. Driessen, Institute of Evolutionary and Ecological Sciences, Leiden University, The Netherlands; S. Belmain, Natural Resources Institute, Chatham Maritime, UK).
- Some species of parasitic wasp develop a colored abdomen when you feed them honey with a non-toxic dye, as used for coloring food. The dyes can be obtained from a normal bakery. The advantage of this method over marking with paint is that you do not have to anesthetize the insects (which may influence their behavior and survival). The method will work best in insects with unpigmented abdomens (Source: G. Driessen, Institute of Evolutionary and Ecological Sciences, Leiden University, The Netherlands).
- Mosquitoes can be fed with a sugar solution in water containing fluorescent dyes. Blue-black light is used to illuminate the subjects. See the paper by Sarkar *et al*. 2017, *International Journal of Mosquito Research* **4**(6): 5-9 (animals were not video-tracked though). Other techniques using fluorescent powders or dyes are described by Verhulst *et al*. (2013), *Parasites & Vectors* **6**: 200-206.
- For insects you can also use visible implant fluorescent elastomer (VIE) tags (see 'Fish' above).
- Aphids have successfully been color marked with fluorescent powders. The powders that are fluorescent under ultraviolet light can be obtained from Day Glo, Cleveland, Ohio. For optimal detection, a high intensity UV long wave UV·366 nm lamp can be used. To apply the powder to the aphids, gently dust the powder with a powder insufflator. Make sure, each aphid

gets at least 10 grains of powder. For more information see Thomas *et al*. (1997). *Journal of Agricultural Entomology* **14**(2): 187-198.

- Ants can be color marked with a small drop of paint on the thorax or the abdomen. Anesthetize the ant with $CO_2$. To restrain the ant during painting, use a sponge with a single strand of hair attached to it. Place the ant under the hair and apply the color mark. Transfer the ant to a plastic tube and allow the paint to dry for approximately 20 minutes. After 24 hours, the ant can be used in experiments.
- For a review of the marking techniques see Hagler & Jackson (2001). *Annual Reviews of Entomology* **46**: 511–543.

## Markers for zooplankton

- Nanoparticles known as Quantum dots can have been applied to the carapace of crustaceans *Daphnia* sp. Like any other fluorescent molecule, they must be excited with light of one wavelength in order to emit fluorescence at a longer wavelength. Among the advantages of these markers, the great photostability (animals can be tracked for up to 24 hours after labeling, without loss of signal due to photo-bleaching. Note that some quantum dots may be toxic to animals.

  For more information see Lard *et al*. (2010). *PLoS ONE* **5**(10): e13516. doi:10.1371/journal.pone.0013516.

## Tracking colors in the dark

If you want to apply color markers and track the animals in the dark, you need lighting conditions that allow EthoVision XT to detect the marker and detect the contour of the animals.

To track colors in the dark, combine fluorescent markers with ultraviolet (UV) light. UV light makes the marker emit visible light, which is then detected by the camera. EthoVision will track the marker like it would normally do in daylight. See Markers for rodents and other small mammals

To track the contour of the animal, you must also use light sources other than UV. If you only use UV light, the contour of the animal won't be seen. A couple of solution that may work:

- Use visible light. Make sure that the contrast between animal and background is maximized.

- Use infrared (IR) light. Some good color cameras are also sensitive to IR light. You can then use IR light to enhance the contour of the animal, provided that the camera lens does not have a IR-block filter.

Note that fine-tuning this setup may take considerable effort and time.

## Useful web sites

- Carmel livestock markers: http://www.livestockmarkers.com.
- La-Co livestock markers: http://www.allweathermarker.com.
- Net-Tex livestock markers: http://www.net-tex.co.uk and search for "marker".
- Edding markers: http://www.edding.com.
- Marks-A-Lot markers: http://www.avery.com.
- MS Schippers (livestock or rodent markers): https://www.schippers.nl. See the product line "MS Merkstift" (in Dutch).
- Chalk Ink markers: http://www.chalkink.com. Choose a 15-mm broad tip marker.
- Schwarzkopf hair dyes: https://www.schwarzkopf.com
- Royal Talens acrylic paint: https://www.royaltalens.com/en/products/acrylic-colours/.
- B/Braun surgical sutures and needles for attaching pearls to fish: http://www.bbraun.com.
- Northwest Marine Technology fish markers: http://www.nmt.us/.
- Invitrogen quantum dots http://www.thermofisher.com/nl/en/home/brands/invitrogen.html.
- Fluorescent powders: https://www.dayglo.com/

# Settings for the color camera

## Aim

To make sure that the tracking of color markers in EthoVision XT is optimized.

This topic applies to the Basler color cameras acA1300-60gc and acA1920-40gc. Note that the Basler acA1300-60gc is no longer officially supported and therefore not tested by Noldus.

## Prerequisites

You have installed the camera, its driver software and EthoVision XT. See Install GigE cameras

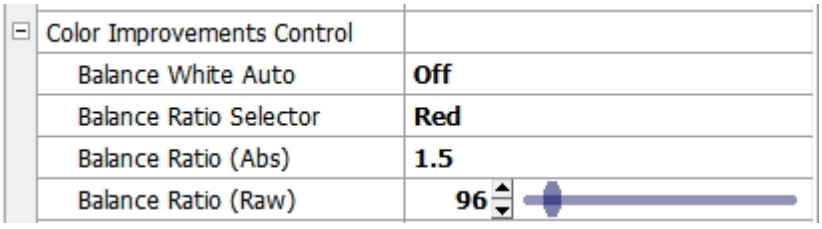
## Settings in pylon Viewer

The procedure below refers to the options available in the camera software. Most of the options are also available in EthoVision XT. See Adjust camera settings in EthoVision XT

1. Close EthoVision XT and open **pylon Viewer**.

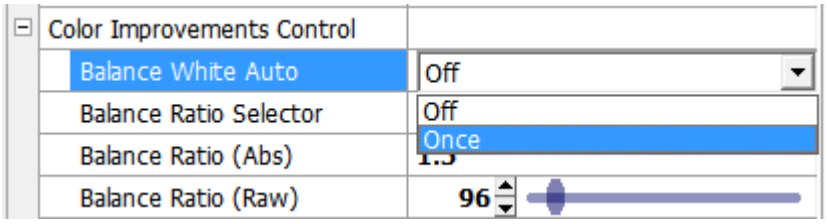
   **NOTE** The Basler pylon Viewer software is installed automatically when you install the camera drivers.

2. From the **Window** menu select **Features - All**.
3. In the **Devices** panel, double-click the item Basler under **GigE**.
4. Open the item **Image Format Control**. Next to **Pixel Format** select:
   - **YUV 422 Packed** for the camera acA1300-60gc. Note that this camera has not been tested with EthoVision XT 18.
   - **Bayer RG 8** for the camera acA1920-40gc.
5. Click **Color Improvements Control.**




6. Click the **Continuous Shot** button on the tool bar to view the live image.

7. Point the camera at a piece of white paper, so that the camera image is entirely white. Click in the field next to **Balance White Auto** and select **Once** from the list.




8. To save the settings, see Save the camera settings.
9. Close pylon Viewer and open EthoVision XT.
10. Open your experiment and in the Experiment Settings select **Live tracking** and the color camera.
11. Click the camera button and then in the window that appears click the Format tab. Choose the video resolution and frame rate.
12. Next to **Color space** select:
    - For the camera acA1300-60gc, **UYVY** or **YUV**.
    - For the camera acA1920-40gc, **BYRG**. If the image stays black and white, select UYVY or YUV, then select BYRG again.
13. Select the resolution and frame rate. See what has been tested for the Basler acA1920-40gc.
14. Check the **Current frame rate**. If this is significantly lower than the rate you set, choose a lower resolution.

## Settings for low light conditions

When tracking animals in low light, you can adjust the camera settings in such a way that the colors are more apparent. The settings below apply to the camera acA1920-40gc.

In pylon Viewer, do the following:

1. Under **Analog Controls**, set **Gain** to for example 240 or higher if necessary.
2. Next to **Gamma Selector**, choose **User**.
3. Next to **Gamma**, choose **1.0**.
4. Increase the **Digital Shift** for example to **1** or higher if necessary.
5. Under **Acquisition Controls**, set **Exposure Time** to for example **20000** µs. A higher value is not recommended.

For adjusting a specific color (usually not necessary if the white balance has been done correctly):

- Next to **Color adjust enable**, select the option.
- Next to **Color Adjust Saturation**, adjust color saturation (per color).

## Color to monochrome

If you have a color digital camera, you can temporarily change it to monochrome.

- For Basler cameras: see Change color to monochrome

**NOTE** This does not make your color camera infrared-sensitive. For that, you must either remove the infrared block filter (see below) from your color camera or use a monochrome GigE camera.

**TIP** Basler cameras have model numbers like this: acA1300-60xx, where

- xx = gm means monochrome.
- xx = gmNIR means Near Infrared, that is, monochrome with extra sensitivity to infrared light.
- xx = gc means color.

## Color to infrared-sensitive

Most color cameras are not infrared-sensitive. To make them more infrared (IR)-sensitive:

1. Set them to monochrome (see above).
2. Remove the infrared-block filter, if this is present. For how to do so, see below

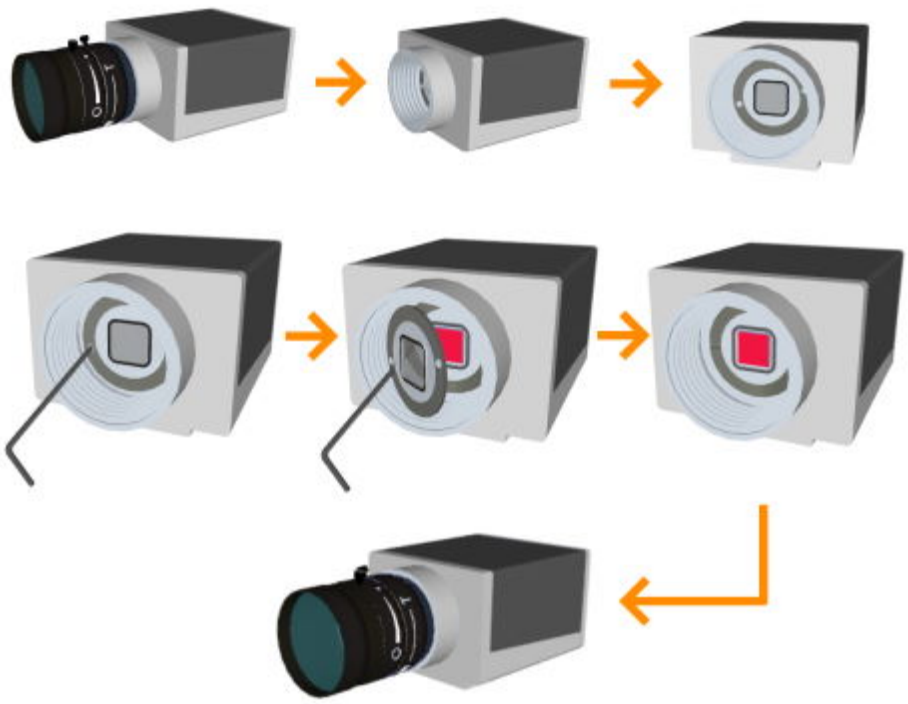
## Infrared-block filter

Some color cameras may have a built-in infrared block filter which prevent them to see some infrared light. If you bought a Color GigE camera from Noldus prior to July 2018, you can remove this filter to make your color camera IR sensitive.

**NOTE** Color GigE cameras of model acA1300-30gc purchased after July 2018 from Noldus had the IR block filter removed making the color camera IR sensitive like the monochrome version (model: acA1300-30gm). If you have such a camera you can skip this procedure.

1. Remove the lens from the camera.

2. Check that the filter is present: you should see two circular holes at the sides of the filter.
3. Use a small Allen key or a similar object to lift the filter.
4. Put the lens back on the camera.



*See also*

- Lighting setup

# Pixel format and color space

## Aim

To inform you about the relation between pixel format and color space used to create video.

## This topic applies to

- Basler digital cameras.

## Background

- A **Pixel format** is the format of the image data transmitted by the camera. For example, for monochrome cameras the default pixel format is Mono8. This means that the camera outputs 8 bits of data for each pixel in the image captured by the chip.

  **TIP** You find the **Pixel format** setting in the pylon Viewer software. See Configure the digital camera

- A **Color space** is a specific organization of colors. Selecting a color space in the camera driver software or in EthoVision XT determines the way color information is encoded in the video file.

  **TIP** You find the **Color space** setting in EthoVision XT. See Adjust camera settings in EthoVision XT

There is a relation between the pixel format and the color space. When you specify the color space in EthoVision XT, the camera software may automatically change the pixel format. Follow the tables below to find which pixel format is used in your video. This is usually the format that is optimal to convert color into the selected color space.

*If the starting pixel format is Mono8 or YUV422 (YUYV) Packed*

| When you set the color space to... | Then the pixel format changes to... |
|---|---|
| UYVY | YUV422 Packed |
| RGB24 | YUV422 (YUYV) Packed |
| RGB32 | YUV422 (YUYV) Packed |
| YV12 | YUV422 (YUYV) Packed |
| YUY2 | YUV422 (YUYV) Packed |
| Y800 | Mono8 |
| BYRG | Bayer RG8 |

*If the starting pixel format is YUV422 Packed*

| When you set the color space to... | Then the pixel format changes to... |
|---|---|
| UYVY | YUV422 Packed |
| RGB24 | YUV422 Packed |
| RGB32 | YUV422 Packed |
| YV12 | YUV422 Packed |
| YUY2 | YUV422 (YUYV) Packed |
| Y800 | Mono8 |
| BYRG | Bayer RG8 |

*If the starting pixel format is Bayer RG8, Bayer RG12 or Bayer GRG12 Packed*

| When you set the color space to... | Then the pixel format changes to... |
|---|---|
| UYVY | YUV422 Packed |
| RGB24 | Bayer RG8 |
| RGB32 | Bayer RG8 |
| YV12 | Bayer RG8 |
| YUY2 | YUV422 (YUYV) Packed |

| | |
|---|---|
| Y800 | Mono8 |
| BYRG | Bayer RG8 |

*See also*

- Adjust camera settings in EthoVision XT
- Configure the digital camera (in pylon Viewer)

# EthoVision XT and Quality Assurance

## Main topics and tasks



If you need a Certificate of Validation for EthoVision XT, please contact Noldus. Other contact information

**IMPORTANT** It is not possible to open a GLP experiment that was made with EthoVision XT 12 or earlier. If you upgrade, you can leave both versions of EthoVision XT on one computer, to be able to open the old experiments.

*See also*

- Troubleshooting: Quality Assurance (GLP)

# EthoVision XT and Quality Assurance

EthoVision XT offers functionality to support the user in fulfilling quality requirements in *in-vivo* studies. The Quality Assurance (QA) functions can also be used to help comply with Good Laboratory Practice (GLP) regulations.

## Quality Assurance functions

- EthoVision XT user management

  You can set up the program so that only authorized users are able to take particular actions, and that certain actions such as editing or deleting data are prevented.

- EthoVision XT logging

  All user actions which can result in changes to the acquired data (for instance altering settings) are logged and every time you leave a part of the program you are prompted to add a comment to the log file. This creates an audit trail suitable for a QA auditor to review.

**IMPORTANT** You need the EthoVision XT Quality Assurance module to use the Quality Assurance functions in EthoVision XT. See Modules of EthoVision XT

## Note about GLP

GLP (Good Laboratory Practice) is a set of rules for conducting non-clinical laboratory studies that support or are intended to support applications for research or marketing permits for products regulated by the U.S. Food and Drug Administration. These products include animal food additives, medical devices for human use, biological products and human and animal drugs. The principles of and regulations for GLP are described under the Code of Federal Regulations Title 21, Part 58 (CFR21/58).

For Europe, the GLP rules have been compiled and adapted by the Organization of Economic Cooperation and Development (OECD).

Subparts of the CFR21/58 GLP rules define and describe the following major points:

- Organization and personnel.
- Facilities.
- Equipment.
- Testing Facilities Operations.
- Test and Control Articles.
- Protocol for and Conduct of a Non-clinical Laboratory Study.

- Records and Reports.
- Disqualification of Testing Facilities.

All these items require to be managed in such a way that GLP-compliance is facilitated and ensured.

CFR21/11 more specifically describes guidelines for the use of electronic records.

**IMPORTANT** The Quality Assurance functions in EthoVision XT do not actively check that experiments made in EthoVision XT are GLP-compliant.

# EthoVision XT user management

With EthoVision XT User Management you can:

- Prevent non-GLP users to access EthoVision XT with the Quality Assurance module installed.
- Define which users may access which function in EthoVision XT (for example experiment design, data recording, editing etc.).

## Learn about

- How EthoVision XT user management works

## What do you want to do?

- Add a user
- Assign rights to users

# How EthoVision XT user management works

You can define a list of users that can access EthoVision XT. Furthermore, users can have different rights; these rights can only be assigned by a user with User Management rights in EthoVision XT.

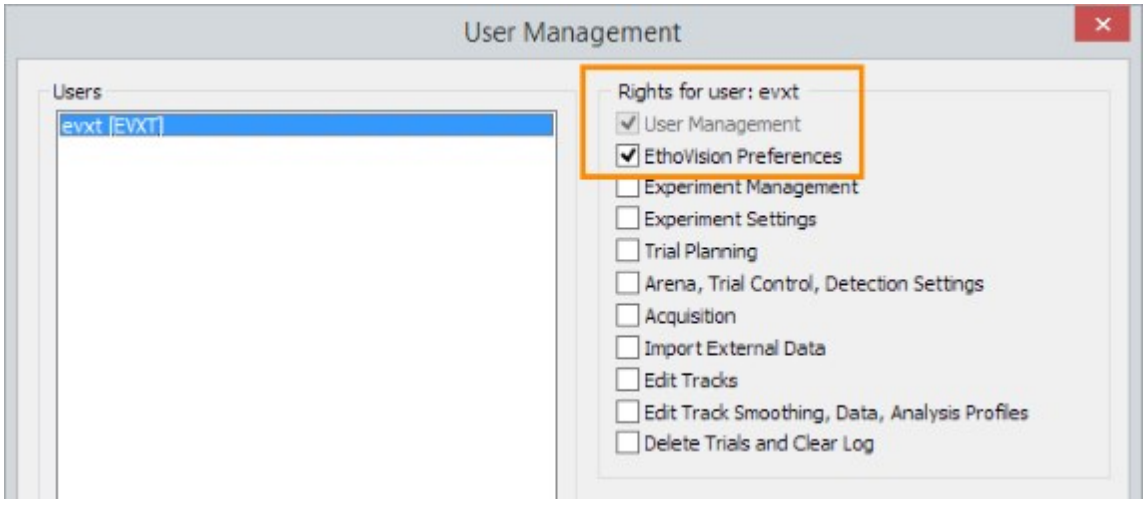
Initially, after installation, only one user has access to EthoVision XT. Which user this is depends on how EthoVision XT was installed:

- EthoVision XT came installed on a computer provided by Noldus Information Technology.
- EthoVision XT was installed by the user.

## EthoVision XT came installed on a computer

When you purchased both EthoVision XT and the EthoVision XT computer from Noldus Information Technology, EthoVision XT is already installed on that computer. In that case, a user account **EVXT** has been created to log on to Windows. This user EVXT, with administrator rights, initially is the only user who can access EthoVision XT on that computer.

Choose **File** > **Users**. User EVXT has, by default, the following rights: User Management and EthoVision Preferences.

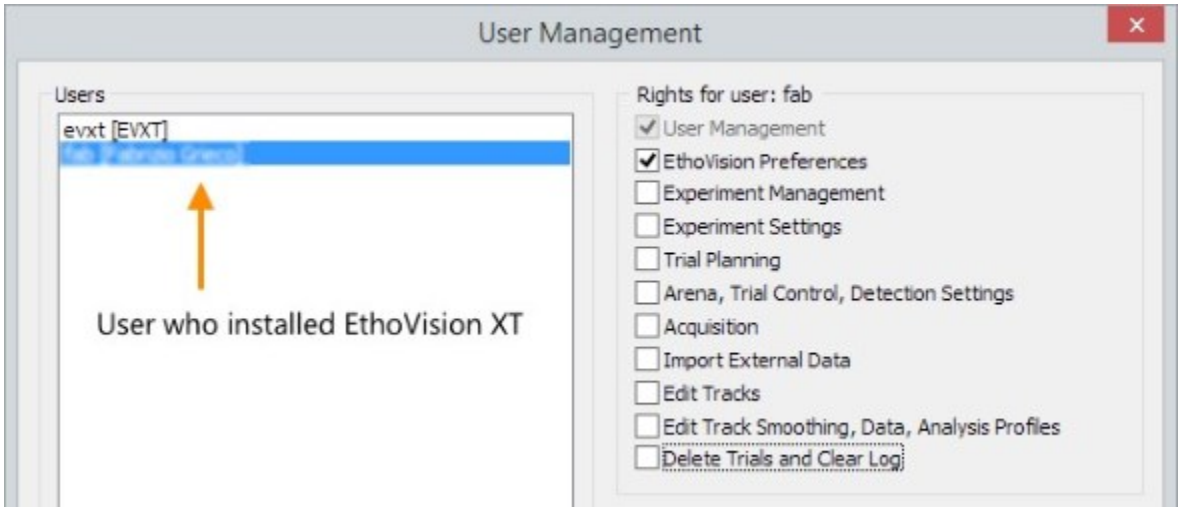



Use EVXT to add new users and assign them rights.

- Add a user

## EthoVision XT was installed by user

When a user with administrator rights installed EthoVision XT, this user is automatically added to the list of users in the User Management window in EthoVision XT.




Also, a user **EVXT** is automatically added to the list of users. With this standard user EVXT, any administrator can at all times create a user account EVXT through which EthoVision XT can be accessed.

Next, either the user that installed EthoVision XT or user EVXT can add new users and assign them rights (see below).

# Add a user

The procedure to add a user differs depending on whether EthoVision XT came installed on a computer, or was installed by the user, and on whether the computer is on a domain or not.

Follow the procedure according to your situation.

## EthoVision XT came installed on a computer that is on a domain

Make sure that user EVXT has also been added to the domain by your network administrator.

To add a new user in EthoVision XT:

1. Log on to Windows as user EVXT and open EthoVision XT.
2. Choose **File** > **Users**.
3. In the User Management window, click **Add**.
4. In the Add New User window, click **Network**.
5. A list of users on the domain appears. Select a user from the list.

   At this point, you have the option to select an initial role (Administrator, Researcher or Technician) for the new user. See Assign rights to users

6. Click **Add** to add a user to the list of users that can access EthoVision XT.

Next, assign rights to the new user.

## EthoVision XT came installed on a computer that is not on a domain

When EthoVision XT was installed by Noldus Information Technology on an EthoVision XT computer, initially there is only one user, EVXT, on that computer. Log on to Windows as this user. You can find the password in the welcome letter you received with the software.

You first need to define new users in Windows before you can add them to the list of authorized users in EthoVision XT. You must have a local computer administrator account to add a new user to the computer.

To add a new user in Windows, click the **Start** button, then select **Settings** > **Accounts** > **Other people**/**Other users**.

Next, add this user in EthoVision XT:

1. Open EthoVision XT and choose **File** > **Users**.
2. In the User Management window, click **Add**.
3. Local users on the computer are shown. Select one of the users in the list.

   At this point, you have the option to select an initial role (Administrator, Researcher or Technician) for the new user. See Assign rights to users
4. Click **Add** to add this user to the list of users that can access EthoVision XT.

Next, assign rights to the new user.

## EthoVision XT was installed by user on a computer that is on a domain

The user that installed EthoVision XT can add new users to the list of authorized users in EthoVision XT.

1. Open EthoVision XT and choose **File** > **Users**.
2. In the User Management window, click **Add**.
3. In the Add New User window, click **Network**.
4. A list of users on the domain appears. Select a user from the list.

   At this point, you have the option to select an initial role (Administrator, Researcher or Technician) for the new user. See Assign rights to users
5. Click **Add** to add a user to the list of users that can access EthoVision XT.

Next, assign rights to the new user.

## EthoVision XT was installed by user on a computer that is not on a domain

First, you might need to add local users to the computer (see Add a user for a description of how to do this. You must have a local computer administrator account to add a new user to the computer.

To add a new user in EthoVision XT:

1. Open EthoVision XT and choose **File** > **Users** > **Add**.
2. Local users on the computer are shown. Select one of the users in the list.

   At this point, you have the option to select an initial role (Administrator, Researcher or Technician) for the new user. See Assign rights to users

3. Click **Add** to add this user to the list of users that can access EthoVision XT.

*See also*

- Assign rights to users

# Assign rights to users

## Prerequisites

- You have been assigned User Management rights in EthoVision XT.
- There is no experiment open.

## User roles

When you add a new user, you have the option to select one of three predefined roles, each representing a set of rights. However, these rights can be changed at any time.

The three initial roles are:

- **Administrator**

  With default rights User Management and EthoVision Preferences.

- **Researcher**

  With default rights Experiment Management, Experiment Settings, Trial Control Settings, Arena / Trial Control / Detection Settings, Acquisition, Import External Data, Edit Tracks, Edit Data Profile / Analysis Profile.

- **Technician**

  With default rights Arena / Trial Control / Detection Settings, Acquisition, Import External Data.

## User rights

- **User Management**

  Gives you the right to add and remove users, and change user rights.

- **EthoVision Preferences**

  Gives you the right to change the default experiment folder, the warnings displayed and the autosave options.

- **Experiment Management**

  Gives the right to create new experiments, make and restore backups.

- **Experiment Settings**

  Gives the right to set and change Experiment Settings.

- **Trial Planning**

Gives the right to add trials and variables to the Trial list, and to skip trials or arenas within trials, and re-do trials.

- **Arena / Trial Control / Detection Settings**

  Gives you the right to create and or edit Arena Settings, Trial Control Settings and Detection Settings.

- **Acquisition**

  Gives the right to change Acquisition settings and carry out Acquisition.

- **Import External Data**

  Gives the right to import external data files.

- **Edit Tracks**

  Gives the right to edit tracks.

  **NOTE** It is likely that your Standard Operating Procedure forbids modifying acquired data. If this is the case, no user should have this right.

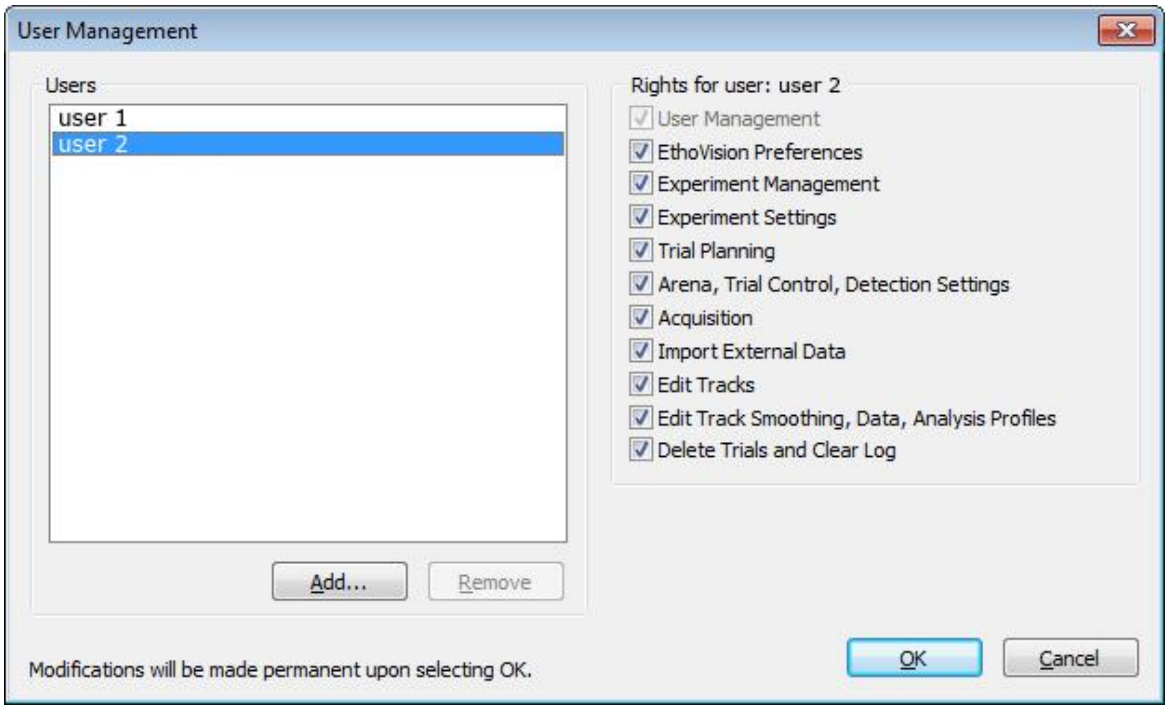
  The original data is always stored in your experiment.

- **Edit Track Smoothing, Data, Analysis Profiles**

  Gives the right to smooth tracks, create and edit Data profiles and Analysis profiles.

- **Delete Trials and Clear Log**

  Gives you the right to delete trials and delete the Experiment log file.




## Notes

- It is likely that your Standard Operating Procedure forbids modifying acquired data. If this is the case, no user should have this right. By default, this right is disabled for all users.
- The User Management right to delete trials and the Experiment log file is useful when you want to create a template experiment. This template experiment, for example, contains default settings as defined in a Standard Operating Procedure, but should not contain test trials and an already filled log file. Also see Creating an Experiment template. It is advisable to create a special user for this right. In normal operation under GLP no user should be able to perform these actions.

# EthoVision XT logging

## Aim

With the logging functionality in EthoVision XT, you enable auditing of (changes to) settings that were made during an experiment. EthoVision XT creates two types of log files:

- General log file

  In this Application log the usage of EthoVision XT by authorized users is registered. See The General log file

- Experiment log file

  One for each experiment, to register the settings made and actions carried out during that experiment. The user, date and time are logged for each setting and action. See The Experiment log file

## Notes

- The user can at any time add additional user comments to the Experiment log file.
- The log file is tamper-proof. As soon as someone edits it outside EthoVision XT, that is detected and it is logged that the file has been tampered with.

# The General log file

The General log file is created the moment EthoVision XT is installed. The following actions are logged:

- Start and stop of an experiment.
- Creation of a new experiment.
- Users that are added / user rights that are assigned / user rights that are modified.
- Errors that occurred when opening or closing an experiment.

For every action, the user name, date and time are logged.

## To view the General log file

1. Choose **File** > **GLP Log Files**.
2. In the GLP log window, select **General log file**.

## To export the General log file

1. Choose **File** > **GLP Log Files**, and click the **Export** button, or in the Experiment Explorer, under **Export**, click **GLP Log**.
2. Select **General log file**.
3. Browse to the folder where you want to export the log file to and click **OK**.

## Notes

- The export format is HTML (*.html). The name of the export file is **GLP-EthoVision(n)**, where n is a progressive number.
- You cannot delete the General log file.

# The Experiment log file

The Experiment log file is started at the moment a GLP experiment is created. Changes made by the user that affect the data and results of an experiment can be traced back in the log file.

These changes are logged from the moment you actually start acquiring data. At that moment a snapshot is taken of the various settings in your experiment and this snapshot is logged. Thereafter, all changes that can affect your data are logged.

When a users makes changes to settings (for example, in the Trial List or Trail Control Settings) and/or leaving a Setting, the user is prompted to add a comment to the Experiment log file. A new snapshot is then taken when new data are acquired. When the user cancels this automatic pop-up or does not enter a comment, a line appears in the Experiment log file with user name, date and time but without a comment.

## To add a comment to the Experiment log file

1. Choose **File** > **Add log comment** (**Ctrl+L**).
2. Type in comments and click **OK**.

## To view the experiment log file

Before you can view an experiment log file, you need to open the corresponding experiment. To view the experiment log file in EthoVision XT:

1. Choose **File** > **GLP Log Files**.
2. In the GLP log window, select the experiment log file radio button. If necessary, click on one of the html links to go to a specific part of the Experiment log file.

## To delete the Experiment log file

You can only clear the contents of the Experiment log file, not delete it. You can only clear the Experiment log file when you have the user management right to **Delete Trials** and **Clear Log**.

1. Choose **File** > **GLP Log Files**.
2. Select **Experiment log file** and click the **Clear Log** button.

## To export the experiment log file

1. Choose **File** > **GLP Log Files**, and click the **Export** button, or in the Experiment Explorer, under **Export**, click **GLP Log**.
2. Select **Experiment log file**.
3. Browse to the folder where you want to export the log file to and click **OK**.

## Notes

- In the case of large experiments, when the log file exceeds a size of 1 Mb, it is split in multiple log files.
- The export format is HTML (*.html). The name of the export file is GLP-[Experiment name](n), where n is a progressive number.
- Experiment Settings, Arena Settings, Trial list, Trial Control Settings and Detection Settings are not stored in the log file until the first data are acquired. After that, only changes are logged.
- Logged Arena Settings include a screenshot of Arenas and their Calibrations. It also contains a screenshot of each Zone Group and its Zones.
- If your current experiment is not set for Quality Assurance, there is no Experiment log file present.
- The suggested export location is the experiment's **Export Files** folder.

# Create a GLP experiment from a template

## Aim

Create a new experiment with all the right default settings, but without tracks and an Experiment log file, based on an existing GLP experiment.

## Prerequisites

- You must have the User Management right to delete trials and clear log. See Assign rights to users
- You have a GLP experiment that you want to use as a template. For example an experiment with specific arenas and zones, etc.

## Procedure

1. Choose **File** > **New template experiment** or press **Ctrl+T**.
2. In the Template option window, select **Use a custom template**.
3. Click **Browse** and select the existing GLP experiment you want to use as a template. Select the file with extension ***.evxt** and click **OK**.
4. Type in the name of the new experiment.
5. Select **GLP experiment** and click **OK**.

## Notes

- Create a backup of the template and store it in a safe place.
- To open a GLP experiment, you need an EthoVision XT license that includes the Quality Assurance module. Upgrade EthoVision XT
- In a GLP experiment you cannot change/edit the zones already used in Acquisition.
- If your license includes the Quality Assurance module, you can still create or open non-GLP experiments. In that case User Management does not apply and no log file is kept.
- Multiple copies of the same version of EthoVision XT installed on the same computer share the General log file. Different versions of EthoVision XT installed on the same computer each have their own General log file.

# Troubleshooting

## Main topics and tasks

# Troubleshooting: Installation and license

## Issues: Installation

- Error message (ID 10020
- Installation stops halfway
- Blue-screen crash during installation of Euresys Multicam
- The hardware key does not light up
- When I start EthoVision XT, I get the message: No license key found
- Installation of EthoVision XT fails, likely due to an error during installation of the Basler camera drivers
- I have realized that I installed the wrong camera driver software. What can I do?

## Issues: License

- My EthoVision XT license has expired. What should I do?
- I am trying to upgrade my license, but the upgrade key is not being accepted
- I cannot start EthoVision XT with a floating license
- I purchased an add-on module but I cannot use it
- I purchased an add-on module. How do I update my license?
- I entered a license key but an error message shows a different one
- I need to create a Firewall rule to access the Noldus license server. Which port do I need to access?
- I cannot deactivate a license. The menu item Deactivate license is greyed out
- EthoVision XT is in Analysis mode, but there is no way to work with a full license
- Message “Activation not complete”
- Message “License already activated...”

## Error message (ID 10020

This usually happens when you install EthoVision XT after logging in with a guest account, or an account with insufficient rights. In those cases EthoVision XT may not use various folders on the hard drive.

Ask your system administrator to create a user account with more (local) rights than guest accounts, or ask the administrator to log in and install EthoVision XT.

## Installation stops halfway

There may be a few reasons why installation fails. The log file may provide clues about the actual cause. To get this file, see The log file for Technical Support.

Do the following:

- Open the Windows Control Panel, and under **Programs and Features** check that an item named **Sentinel Runtime** is listed. If that is the case, remove it. Next, restart the PC, and install EthoVision XT (choose **Modify** on the installation screen).
- The issue could also be cased by the Basler Installer which tries to install its certificates on a Windows 10 version LTSC. If the procedure above does not solve the problem, you may first need to remove the root certificates from your PC, then install the Basler driver, then install EthoVision XT. You can remove root certificates with the app **mmc.exe** in Windows. Contact the Noldus Support for more information.
- Open the Windows Control Panel, and under **Programs and Features** check whether software like **Microsoft Visual C++ Redistributable** (version 14) is present. If that is the case, uninstall it. We know that version 14, not earlier, of this software has caused a couple of issues during installation of EthoVision XT. Next, install EthoVision XT. After that, you may want to re-install Visual C++ redistributable software.

## Blue-screen crash during installation of Euresys Multicam

### *For the Dell Precision 3650*

Solution: Disable the Secure Boot option in the PC's BIOS.

1. Start up the PC. Press the F12 key and once in the BIOS Setup menu, select **Boot Configuration**.
2. Set the **Enable Secure Boot** option to **(disabled)**.
3. Save and exit the BIOS Setup menu.

**NOTE** There may be different ways to open the BIOS Setup menu in different computers. In Windows 11, you can press **F2** while your PC is booting. Or, if the PC is running, hold the **Shift** key while selecting **Restart**. Go to **Troubleshoot** > **Advanced Options** > **UEFI Firmware Settings**. Then select **Restart**. The BIOS Setup opens.

*For the Dell Precision 3640*

A blue-screen crash in Dell 3640 has been reported after the update of the firmware of the BIOS (the firmware update is nowadays integrated in the Windows Update routine). The crash is triggered with firmware version 1.4.3 or later.

It is possible to roll back to an earlier version of the firmware.

1. Start up the PC. Press the F12 key and once in the BIOS menu, select **Maintenance > BIOS downgrade**.
2. Select the option **Allow BIOS downgrade**.
3. Save and exit the BIOS.

Note that firmware version 1.3.2 is tested OK. If you need this version, consult the Dell support web site or contact the Noldus Noldus Support.

## During installation, a message says that a newer version of the Sentinel Runtime is already installed.

1. Cancel installation of EthoVision XT.
2. Open the Windows Control Panel and choose Programs and Features. Look for Sentinel Runtime and uninstall it.
3. If necessary, download the latest version of the Sentinel Hasp Driver from my.noldus.com. Copy the setup file to your PC and run it.
4. Re-install EthoVision XT.

## The hardware key does not light up

1. Close EthoVision XT and remove the hardware key.
2. Run the installation file of EthoVision XT. Select **Custom**, then **Repair**. See Install EthoVision XT
3. Insert the hardware key into the computer and start EthoVision XT. The hardware key should now light up.

## When I start EthoVision XT, I get the message: No license key found

- If you work with a hardware license key, check that the hardware key is plugged in and its red LED is burning (see above).

  **NOTE** When the hardware key is not plugged in, you can open an existing experiment and analyze data already collected. However, you cannot acquire more data. See EthoVision XT in analysis mode

- See also Activate your EthoVision XT license

## Installation of EthoVision XT fails, likely due to an error during installation of the Basler camera drivers

Based on the Basler error log file, installation stops because the system fails to register a Basler ax file.

Solution:

1. Install EthoVision XT while keeping all **Basler camera driver** options de-selected. See Install EthoVision XT
2. Browse to my.noldus.com, log in or register, then select **Downloads** > **EthoVision XT** > **Drivers and tools** and download **Basler Pylon Camera Driver** to your PC. Run this file. During installation, choose the **Custom** option, and de-select **DirectShow Support**.

   **TIP** Remember to also select **pylon Viewer** and the option that applies to your camera (**USB** vs. **GigE**).

3. Continue with installation.
4. At the end of installation, double-click the exe file again, and choose **Modify the current installation**. Choose **Custom**, and this time keep **DirectShow Support** selected.

## I have realized that I installed the wrong camera driver software. What can I do?

1. Run the file **EthoVision XT - Setup -[version number].exe** (see Install EthoVision XT) and select **Modify**. Select the correct driver and continue installation.
2. If the action above does not help, open the Windows Control Panel and under **Programs and Features** select the driver software (for example for Basler camera there is an item named Basler Pylon), and select **Uninstall**. Next, run the setup file (step above) and select the correct driver. See also Install the driver software for the digital cameras

## I am trying to upgrade my license, but the upgrade key is not being accepted

This may occur when your hardware key (dongle) specifies an EthoVision XT version that is later than that specified in the Upgrade Key that you have received. For example, you have a license number **EV180**-... (this number you can view in **Help** > **About EthoVision XT** > **License Info**), and you are trying to enter an Upgrade Key for a (probably old) license number that starts with EV150 or the like.

Another possible cause of the error is that the version of the Upgrade Key is not the same as the EthoVision XT version.

Please contact Noldus to receive a matching Upgrade Key.

## My EthoVision XT license has expired. What should I do?

1. Contact Noldus to purchase a new license. See Noldus Support
2. Start EthoVision XT.
3. A message says *No License key found*. Choose **Activate software license key**.
4. Choose either **Floating** or **Computer-locked** depending on how you want to use the software. See Types of EthoVision XT license
5. Click OK and enter the new software activation key that you received from Noldus. See Activate your EthoVision XT license

## I purchased an add-on module but I cannot use it

Choose **Help** > **About EthoVision XT** > **License info** to view which modules are enabled on your EthoVision XT system.

See Upgrade EthoVision XT. If necessary, contact Noldus for help.

## I purchased an add-on module. How do I update my license?

This depends on whether you have a software license or a license stored on the USB hardware key. See Add new modules to your license

**TIP** To know which modules are included currently in your license, choose **Help** > **About EthoVision XT** > **License Info**. See also Modules of EthoVision XT

## I cannot start EthoVision XT with a floating license

This occurs when a floating license was not deactivated on one of the other computers before starting EthoVision XT on that computer. This could happen for example when EthoVision XT is closed on a computer that has a floating license but currently no internet connection. Restart EthoVision XT on that computer and make sure that the computer is connected to the internet, then close EthoVision XT. Then start EthoVision XT on the computer you want to work with.

**NOTE** With a floating license, all EthoVision XT computers must have an internet connection. See Types of EthoVision XT license. However, you can work offline with EthoVision XT under a floating license for a maximum of 90 days. After that, you must re-connect the EthoVision XT PC to the internet in order to continue to work with the software.

See also Software license - offline

## I entered a license key but an error message shows a different one

Contact Noldus for support. Contact the Noldus Support

## I need to create a Firewall rule to access the Noldus license server. Which port do I need to access?

You need port 443.

## I cannot deactivate a license. The menu item Deactivate license is greyed out

Close the experiment, then select **Help** > **Deactivate license**.

## EthoVision XT is in Analysis mode, but there is no way to work with a full license

After starting EthoVision XT, you can work in Analysis mode, but the dialog that offers to work with the full license does not show up.

1. Close the experiment (if open).
2. Choose **Help** > **Deactivate License**.
3. Click **Continue**. EthoVision XT will now close.
4. Insert the hardware key in the PC or have the license key ready.
5. Start EthoVision XT and choose how to activate the license.

## Message “Activation not complete”

This may be caused by the following reasons:

- A wrong or expired license key was entered in the previous step of the activation procedure. Click Back, and enter the license activation key that you received from Noldus.
- The computer may temporarily be disconnected from the internet. Check the internet connection and repeat the license activation procedure.‘
- The license may have been revoked, or is expired. Please contact your Noldus representative to purchase a new license.

## Message “License already activated...”

After trying to activate a license, EthoVision XT (or the Noldus page displayed on your smartphone) shows a message:

*The license [license number] has already been activated on a system called [computer name]*.

The license that you are trying to activate is still active on another computer.

1. Deactivate the license on the first computer. See Deactivate an EthoVision XT license
2. Activate the license on the second computer. See Activate your EthoVision XT license

It is possible that EthoVision XT was removed from the first computer, but the license is still active there. Please re-install EthoVision XT back in order to deactivate the license on the first computer. See Install EthoVision XT

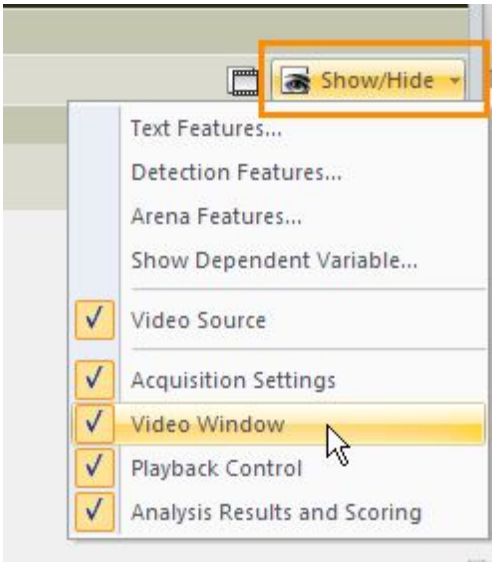
# Troubleshooting: General

## Issues

- Some items in the EthoVision XT screen are missing
- EthoVision XT does not start when I double-click the icon on the desktop
- I cannot open an experiment
- EthoVision XT crashes after opening an experiment
- EthoVision XT hangs after creating a new experiment
- I cannot create new experiments, or acquire data with EthoVision XT
- Video and data seem out of sync
- When I create a new DanioVision experiment with a template made with a previous EthoVision XT version, my trial control rule does not work
- I get a message that a dll file is missing
- I cannot work remotely with EthoVision XT
- I get a message that the backup file cannot be created
- After selecting Deep Learning in the Experiment Settings, the software becomes unresponsive
- EthoVision XT crashes after opening a GLP log
- The text and boxes look very small (Windows 11)

## Some items in the EthoVision XT screen are missing

Check that the item is selected in the **Show/Hide** menu at the top-right corner of the EthoVision XT screen.




Menu items, items in the Experiment explorer, icons, etc. may become unavailable due to memory limitations. Use the data selection functions to reduce the amount of data to analyze.

## EthoVision XT does not start when I double-click the icon on the desktop

This means that an instance of EthoVision XT is running in the background. This could be also due to removal of the dongle while EthoVision XT was running. Do one of the following:

- Press **Ctrl**+**Alt**+**Del** and then click **Task Manager**. Click the **Processes** tab, select each instance of **EthoVision XT** and click **End task**.
- Restart the computer and start EthoVision XT.

## I cannot open an experiment

*Experiment (in general)*

- This occurs when you try to open an experiment (**File** > **Open**) that is saved in a newer EthoVision XT version. Start that version and open the experiment.
- Another reason could be that the experiment contains files whose names include non-ASCII or special characters. Please use standard ASCII characters when creating backups and saving copies of experiments (**Save As**).

- It may be that the experiment repository file (*.evxt) is corrupt. First, delete that file (though not permanently - just leave it in the recycle bin). Locate the file **Restore.btn** and rename this file as the file you have just deleted, including the extension *.evxt. Next, in EthoVision XT, open that repository file (*.evxt).

If none of the actions above solve the issue, contact Noldus Support.

### *Experiment from a backup*

- You get a message like *Backup [name of backup file] could not be restored*.

  Probably the backup file is stored on a location for which you do not have write rights like a DVD or a network drive. Copy the backup to a location where you have full rights, and restore the experiment from that file.

- You get a message like *[name of backup file] is not a valid backup archive. (ID 10060)*.

  Probably the backup is still in a zip folder after downloading (for example, My PC > Downloads > [folder name].zip). Extract the files to another folder. The backup should be outside zip folders.

### *Experiment from MyDocuments or Windows Explorer*

Make sure that the MyDocuments is a mapped network drive.

If My Documents is on a non-mapped network drive (the file path shows no drive letter but \\computer\), you can open the experiment there by double-clicking its name (*.evxt), but it is not possible to open it by selecting **File** > **Open**.

## EthoVision XT crashes after opening an experiment

This may occur if you open an EthoVision XT experiment and UltraVox XT 3 is open on the same PC.

Always follow this sequence of actions when working with UltraVox XT 3 and EthoVision XT:

Close UltraVox XT > Start EthoVision XT > Open the EthoVision XT experiment > Start UltraVox XT.

This issue does not occur if you use UltraVox XT 4.

## EthoVision XT hangs after creating a new experiment

This may occur when the USB-IO box is connected to a USB port on a docking station or a USB hub. Some docks and hubs interfere with the signal between EthoVision and the hardware connected over USB.

Therefore, always connect the USB-IO box and other USB peripherals directly to the computer instead of through USB hubs or docking stations.

- If you use a laptop and encounter issues, connect the USB-IO box on the other side of the machine.
- If you use a desktop and encounter issues, connect the USB-IO box to the back side of the machine.

## Video and data seem out of sync

You can test a video source on timing. This can determine how accurate a camera is in combination with EthoVision XT and can in some cases explain synchronization problems.

To do so you can use the TestVideo utility software. Browse to my.noldus.com, log in or register, then select **Downloads** > **EthoVision XT** > **Drivers and tools** and download **TestVideo**. Copy the file **TestVideo [version number].exe** to a location on your EthoVision XT computer and run it.

**IMPORTANT** Only install and operate TestVideo when requested by Noldus Support.

## When I create a new DanioVision experiment with a template made with a previous EthoVision XT version, my trial control rule does not work

This happens if you change the Trial Control Hardware Settings in the Experiment Settings. We recommend to create a new experiment in EthoVision XT 18 and use this as a template for new ones.

## I get a message that a dll file is missing

When starting EthoVision the message appears: *The program can't start because api-ms-win-crt-heap-I1-1-0.dll is missing from your computer*.

To solve the issue, do a Windows Update.

## I cannot work remotely with EthoVision XT

You cannot control EthoVision XT from another computer through the Remote Desktop app. See Work with EthoVision XT remotely

See also Use a remote control to start and stop acquisition

## I get a message that the backup file cannot be created

The backup is created anyway. You can restore the backup as usual.

The issue occurs when some files in the experiment folder are still opened. For example, one or more Excel export files. Close all files before creating a backup of an experiment.

## After selecting Deep Learning in the Experiment Settings, the software becomes unresponsive

The first time the Deep learning function is selected, a model is created for the specific GPU installed on that computer. For some GPUs this process takes some time. During this time Ethovision will be unresponsive and appear to be crashed. If this is so in your case, wait until EthoVision will become responsive again. If the software no longer responds, contact Noldus.

## The text and boxes in EthoVision XT look very small (Windows 11)

This occurs sometimes in Windows 11 at very high screen resolutions.

Do the following:

1. Right-click the shortcut of EthoVision XT on the desktop and select **Properties**.
2. Click the **Compatibility** tab.
3. Click **Change high DPI settings**.
4. Under **High DPI scaling override**, check the option **Override high DPI scaling behavior** and select **System** or **System (Enhanced)** from the list below.

# Troubleshooting: pylon camera software

This section applies to the applications **pylon Viewer** and **pylon IP Configurator** for Basler cameras.

**NOTE** These two applications are installed automatically when you select to install the drivers for the Basler GigE camera or the Basler USB 3.0 camera. See Install EthoVision XT

## Issues

- Pylon IP Configurator is not seeing a camera
- Pylon IP Configurator can see my camera but the Status column says Not Reachable
- I cannot save my camera settings in pylon Viewer
- No camera image in pylon Viewer

## Pylon IP Configurator is not seeing a camera

1. Make sure that the camera is connected. See Connect the GigE camera to the PC.
2. If you use a Power injector or PoE switch, make sure that it is powered.
3. Restart the PC (if you didn't after installing the camera's driver).
4. If the issue persists, check the Ethernet board that is connected to the camera. See the following:
    - Install the driver software for the digital cameras'
    - Settings of the Ethernet board

**NOTE** An issue has been reported for Dell 3540 running Windows 10 Pro, when using a 4-port AdLink Ethernet interface board. All ports in the Device Manager show an exclamation mark (!) and the cameras are not detected by pylon IP configurator. The problem appears to be caused by the Fast startup feature in Windows. This feature is disabled by default by Noldus. To enable Fast startup on Window 10 systems, navigate to **Settings** > **System** > **Power & sleep** > **Additional power settings** > **Choose what the power buttons do** and click **Change the settings that are currently unavailable**. Select **Turn on fast startup** and save the changes.

## Pylon IP Configurator can see my camera but the Status column says Not Reachable

This may be caused by the IP address of the network card not matching that of the camera.

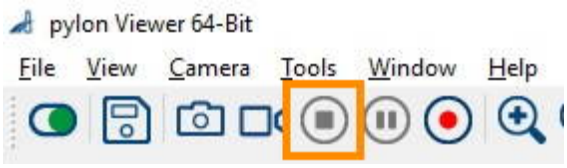
For example, the network card should be 192.168.1.51 and camera should be 192.168.1.101, that is, the first 3 numbers must be the same and the fourth number must be different.

Also verify that the subnet mask is 255.255.255.0.

See Install GigE cameras

## I cannot save my camera settings in pylon Viewer

1. In pylon Viewer, click the **Stop** button on the tool bar.




2. From the **Window** menu select **All Features**.
3. In the **Features** panel, locate **Configuration Sets**. Now you can save the current settings. See Save the camera settings

## No camera image in pylon Viewer

1. Check the issues above about IP Configurator.
2. Check that each Ethernet board has different networks in their IP addresses. For example 192.168.**200**.x for board 1, and 192.168.**201**.y for board 2.
3. If the video screen is completely back it may be that the camera has an IR-block filter and the light in the test room contains little infrared, or that the aperture of the lens is minimized.
4. Swap cables, cameras etc. to exclude the possibility that hardware is faulty.
5. Disable firewall and anti-virus software.
6. Check the Ethernet board settings and driver:
   - Settings of the Ethernet board
   - Make sure that the Ethernet board allows data transfer of 1 Gigabit/s.

# Troubleshooting: Camera

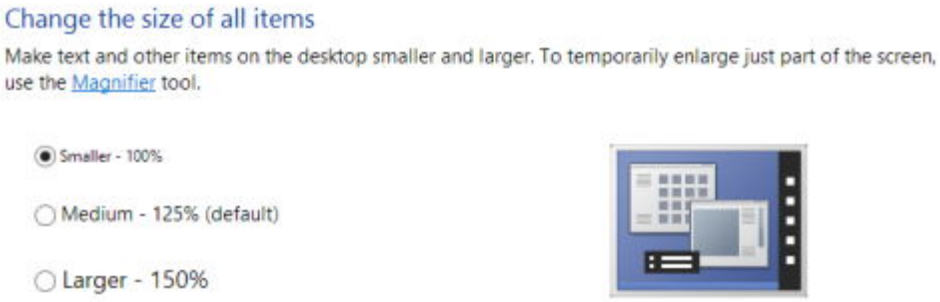
## Issues

- The camera name is partly hidden in the Experiment Settings
- I cannot see the camera image when I click on the Camera icon in the Experiment Settings
- When I click on the camera icon in the Experiment Settings, the Video Settings box says “No frames available”
- There is no video camera listed in the Experiment Settings
- One or more cameras are not listed in the Experiment Settings
- The settings for color space are not available
- I cannot select a specific combination of frame rate and color space
- EthoVision XT stops working when two or more digital cameras are connected
- I cannot select a high frame rate
- I notice that video frames are dropped. Reducing Exposure time did not help.
- After selecting the first GigE camera in Experiment Settings, when I select the second camera, EthoVision crashes
- When using multiple GigE cameras, if one of the camera signals is lost, the order of the cameras in EthoVision XT scrambles
- EthoVision XT freezes in live video mode
- Error message “Device unplugged”

## The camera name is partly hidden in the Experiment Settings

For example:


In the Windows Control Panel, choose **Display** and reduce the text size, for example select 100%.




## I cannot see the camera image when I click on the Camera icon in the Experiment Settings

1. Make sure that pylon Viewer is closed.
2. Make sure that the camera(s) is connected to the PC and powered prior to opening EthoVision XT.
3. Make sure that the aperture of the lens is fully open (lens ring all the way to **Open** or **O**).
4. Make sure the correct camera is selected in the **Video Source** list of the Experiment Settings. For example: Basler GenICam [Basler acA1300-60].
5. Make sure that the correct camera driver version **7.5.0** is installed. To find the version number, In the Windows Control Panel choose **Programs and Features** and locate the **pylon Camera Software Suite**.
6. Check the IP addresses of the Ethernet board ports and the cameras. See Assign IP addresses

## When I click on the camera icon in the Experiment Settings, the Video Settings box says “No frames available”

1. When using Windows 10: make sure you installed the camera driver from the installation files. If you installed it from an older installation disk, you could have installed an older driver (for example 4.2.1) which doesn’t support Windows 10. For the driver version that applies to your cameras, check the table in Cameras supported by EthoVision XT
2. If you changed the settings in pylon Viewer, check in that software under **Acquisition Controls** that the **Trigger mode** is set to **Off**.
3. Other causes are possible like installing Windows 10 on an older PC which isn’t compatible with Windows 10.
4. If none of these examples fit your situation, contact the Noldus Support for further assistance. Please also Make a PC report for Supports.

## There is no video camera listed in the Experiment Settings

- Make sure that the IP addresses are assigned to all cameras and ports in the Ethernet board, based on this topic: Assign IP addresses
- Check in the Windows Control Panel that the drivers for the camera are installed.
- Check the driver version. If the driver version differs from that reported in the table (see Cameras supported by EthoVision XT), uninstall the driver from the Control Panel. Install the correct driver, which you can find on the Noldus web site.

  **IMPORTANT** Always re-install the camera drivers from the EthoVision XT setup file, using the Modify option. If you need to install the Basler camera driver software from the original installation file **Basler_pylon_[version number].exe**, do the following:

  - Log in to my.noldus.com. Choose **Downloads** > **EthoVision XT** > **Drivers and Tools**. Download **Basler Camera Driver - Noldus - [version number]**. Extract its content and copy everything to the local disk.
  - Double-click the file **Basler_pylon_[version number].exe**. Accept the Terms and Conditions, then click **Next**.
  - Under **Profiles**, select **Custom** and click **Next**.
  - Under Features, choose **GigE Camera Support** for GigE Vision cameras, or **USB Camera Support** for USB 3.0 cameras. Make sure to select **pylon Viewer** and **DirectShow Support**.

- Complete the installation.

## One or more cameras are not listed in the Experiment Settings

- Check that you selected the correct number of cameras.
- Check that all cameras are connected and powered up. For digital cameras: close EthoVision XT and start pylon IP Configurator. There you should see which cameras are connected.
- Make sure that the IP addresses are assigned to all cameras and ports in the Ethernet board, based on this topic: Assign IP addresses
- Check whether that camera is already selected in a different input. For example you do not see a camera for input 4 because that camera was already selected for input 1.
- It may be that after adjusting the settings for the first camera, those settings are no longer compatible with those still applied to the other cameras.

  For example, suppose you select **Binning** for the first camera, then you decide to de-select it. When you return to the camera list, Binning is still selected for the remaining cameras. This makes the camera views of the other camera incompatible with that of the first one. The result is that the remaining cameras are removed from the list. To solve this, select a new camera from the list at the top, and clear the **Binning** option also for that camera. Click **OK**, and do the same for the remaining cameras.

## The settings for color space are not available

I have a color camera Basler AcA1920-40gc. When choosing the color space, the settings for the BYRG are not available.

Solution:

1. Select **BYRG** as color space.
2. Disconnect the camera, so it does not receive power.
3. Reconnect the camera.
4. Fine-tune the camera setup leaving the color space to BYRG.

## I cannot select a specific combination of frame rate and color space

This happens because some values of video frame rate and color space are not compatible in some cameras. Those frame rate * color space combinations have been removed in the software.

## EthoVision XT stops working when two or more digital cameras are connected

The problem occurs when the video resolution does match the values accepted by the software. Select for example 800 x 600, not 640x 426. See the tables in Cameras supported by EthoVision XT

*See also*

- Adjust camera settings in EthoVision XT
- Camera Installation

## I cannot select a high frame rate

For example, in the EthoVision XT Experiment Settings, when I set the resolution to something in the range of 1280x960, the maximum attainable frame rate is for example 7.5 frames per second.

1. Check that the **Exposure time** setting isn't too high. See Adjust camera settings in EthoVision XT > Camera/General tab
2. Make sure that the Power Injector is in fact a 1GB Power Injector.
3. Make sure that the network card is in fact a 1GB network card. If that is the case, check that it is set to **Auto-negotiate**. If it is, then the network card either needs a driver update or is defective.
4. Check the cables. Cheap, incorrect or defective Ethernet cables can also reduce the frame rate.
5. The PC may have insufficient resources, for example only has an on-board video card. Please contact the Noldus Support and Make a PC report for Support.

## I notice that video frames are dropped. Reducing Exposure time did not help.

See this page and check that the camera settings match those of the Ethernet board:

https://docs.baslerweb.com/knowledge/how-to-troubleshoot-lost-packets-or-frames-while-using-gige-cameras

## After selecting the first GigE camera in Experiment Settings, when I select the second camera, EthoVision crashes

In the Experiment Settings, next to each video source, select a standard resolution like 640x480. Avoid resolutions like 640x426.

## When using multiple GigE cameras, if one of the camera signals is lost, the order of the cameras in EthoVision XT scrambles

1. Always plug the cameras into the same ports. Label the ports and camera cables if needed to keep things in order.
2. Restore the camera signal.
3. In EthoVision XT, choose **Setup** > **Acquisition**, then choose **Setup** > **Experiment Settings**, and then go back to **Acquisition**. The camera order should now be restored to what you originally setup when first setting up this experiment. Despite the visual swapping of the cameras listed in Source table (via Experiment Settings), the order of the cameras should still load in the correct order matching the arenas.

## Error message “Device unplugged”

Check first the cable connections between cameras and PC.

Check the video resolution in the Experiment Settings screen. Probably the resolution of resulting merged view is too large. Reduce the resolution in such a way it is compatible with the software.

- For eight cameras, 2560 x 960.
- For sixteen cameras, 2560 x 1920.

# Troubleshooting: Video image

## Issues

- The camera image is not detected by EthoVision XT
- EthoVision XT shows a message “No video frames”
- I do not see the image of the video file
- The video image looks grainy
- The live image from the camera looks distorted
- The camera image is static/pixelated with red, blue and green pixels
- The camera image is -center
- The camera image is too narrow to display the entire arena
- The camera image is ok in the Experiment Settings, but in the Arena Settings I see a white background image
- The camera image is not shown in the Arena Settings or Integrated Visualization
- The camera image flickers
- The camera image doesn’t flicker but the overall image lightens and darkens
- There is motion blur in the camera image
- The camera image is not in focus, no matter what I do
- The camera image is dark, even when using a lot of infrared light
- My video file is not playing correctly
- Video is being processed poorly
- A black bar is displayed when zooming in to a video file
- Ethovision XT freezes when playing a video file
- EthoVision XT freezes in live video mode

## The camera image is not detected by EthoVision XT

Try the following:

- Make sure that the camera is powered and switched on, that the video cables are not broken and that there is no loose connection between

camera and computer. Try, for example, another video cable to see whether the image is then detected.

- If you have an analog camera, check that the camera is connected to the input of the encoder board that is selected under **Video source** in the Experiment Settings. If you have a digital camera, check that it is connected to the correct port on your PC.
- If you have a Basler digital camera, make sure to close the **pylon Viewer** software.
- Make sure that the lens cap is removed, and turn the camera's aperture ring to **Open**.
- Click the **Show/Hide** button on the toolbar and make sure that **Video Source** is selected.
- If the camera was accidentally disconnected, reconnect the camera, then in EthoVision XT choose **Setup** > **Experiment Settings** and click the **Refresh** button next to the camera name.
- If you have MediaRecorder 2.5 installed on the same PC, it could be that the problem occurs because you have installed MediaRecorder 2.5 after installing EthoVision XT. Note that this problem does not occur with MediaRecorder 4 to 6.

  To solve the issue, download the installation files (see Install EthoVision XT) and double-click **EthoVision XT - Setup - [version number].exe**. In the window that opens, select **Repair**. This re-installs the correct software for displaying the camera image.
- If the problem persists, re-install the drivers for the encoder board or digital camera. To do so, download the installation files (see Install EthoVision XT) and double-click **EthoVision XT - Setup - [version number].exe**. On the window that opens, select **Modify** and choose the appropriate driver from the list. See Install EthoVision XT

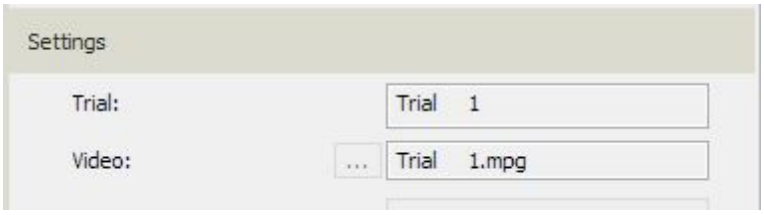
## EthoVision XT shows a message “No video frames”


The message is shown in the Experiment Settings > Video Settings window.

- See the issue above.
- Confirm that the PC monitor is plugged into the stand-alone graphics card (GPU) and not the motherboard.
- If you are using Windows 10, and you are using GigE Basler cameras, make sure you installed the driver from the installation files. But if you installed from an older installation drive, you might have installed an older driver (e.g. 4.2.1) which doesn’t support Windows 10.
- The message “No video frames” is also shown when there is something wrong with the camera or the power supply or the video cable. Please replace cables or connect the camera to another input channel of the board, or another computer.
- Disable the Firewall for the network ports connected to the cameras. Disable the Windows firewall for the Ethernet board

## I do not see the image of the video file

- Click the button next to **Video** in the Acquisition Settings window and select the file you require.




- Click the **Show/Hide** button on the toolbar and make sure that **Video Source** is selected.

- **TIP** Choose **File** > **Preferences** > **Default Folder**. Under **Alternative media location** enter the folder where your video files are stored. This way EthoVision XT looks in this folder if video files are not found in the Media Files folder of the experiment.

## The video image looks grainy

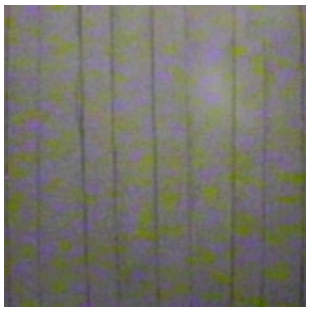
The problem occurs only when connecting the video cables from a T splitter to input 1 and 2 of the Picolo H.264 board. Splitting the video signal into two cabling was necessary to acquire the tracks and simultaneously save video. However, In EthoVision XT 12 and later versions this is no longer necessary. Just connect the camera to video input 1 of the board.

## The live image from the camera looks distorted

This may occur when you change the resolution of the camera image in the Experiment Settings (see Adjust camera settings in EthoVision XT), without updating the background image in the Arena Settings. To so, open the Arena Settings, and click the **Grab Background Image** button in the lower part of the Arena Settings window.

## The camera image is static/pixelated with red, blue and green pixels

For example:



Solution: Make sure that the camera is set to black and white mode (via the camera menu; see the camera user manual for how to access this menu).

- If you have a digital monochrome camera, in EthoVision XT, make sure that the **Color space** is set to **Y800**. If you set the color space in pylon Viewer, the correct option is **Mono 8**.
- If you have a digital color camera, see the camera type in Cameras supported by EthoVision XT for the recommended color space.

See also Adjust camera settings in EthoVision XT > Format tab

## The camera image is -center

This may occur if you select a lower resolution in the Experiment Settings, and the **Center X** and **Center Y** options are not checked in the camera settings.

To solve this, increase the resolution to the maximum value and the arena will be centered. See Adjust camera settings in EthoVision XT > Format tab

If you prefer a lower resolution, check the option **Center Image** in the Camera/ General tab. See Adjust camera settings in EthoVision XT > Camera/General tab

## The camera image is too narrow to display the entire arena

- This may occur when you select a low video resolution in the Experiment Settings. Rather than scaling the entire image down to the lower resolution, this camera displays a limited area of interest in order to maintain the full image quality. For the widest possible view, increase the resolution to the maximum value (typically 1280x1024).
- If you have a varifocal lens, you can also adjust the zoom setting on the lens. Note that adjusting the zoom will also change the focus, so you will need to adjust the focus as well in order to see the actual effect. Also note that you may need to loosen the lock screw on the side of the lens before the zoom setting may be adjusted.

  **TIP** Watch the video tutorial **Set Up Your Test Environment**. To open the tutorial, choose **Help** > **Video Tutorial**.

## The camera image is ok in the Experiment Settings, but in the Arena Settings I see a white background image

- The video resolution set in the Experiment Settings is either too high or an odd resolution is selected, like 640x426. Reduce the resolution and/or pick a standard resolution (like 640x480).
- A similar issue occurs when no drivers have been installed for the computer's graphics card. See below.

## The camera image is not shown in the Arena Settings or Integrated Visualization

**NOTE** This information is probably obsolete for EthoVision XT 18, since EthoVision XT no longer needs hardware acceleration. We keep it in the Help in the case you encounter this issue in EthoVision XT 17 or earlier.

This issue can occur in computers and laptops with two graphics cards, a primary (integrated) card and a secondary card (Graphics Processing Unit, or GPU), usually

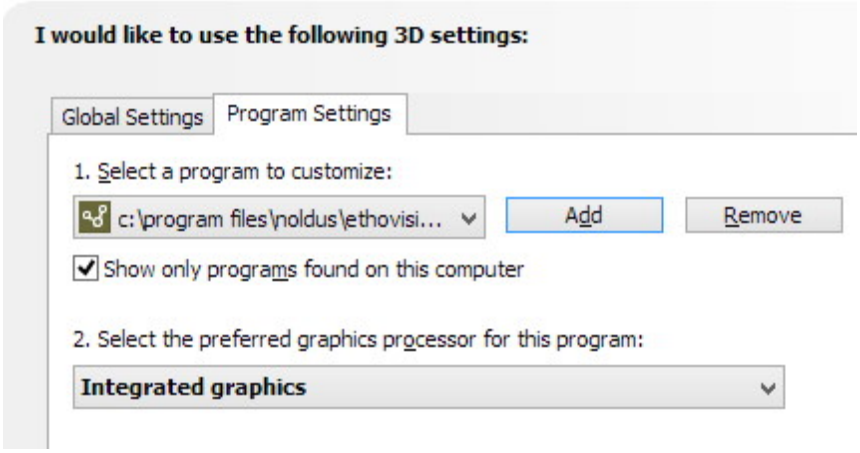
more advanced and powerful than the integrated one. The issue occurs when EthoVision XT uses a graphics card that is not given hardware acceleration.

Choose one of the two solutions. In either case the chosen card is given hardware acceleration.

**SOLUTION 1** Select the integrated (primary) card for EthoVision XT.

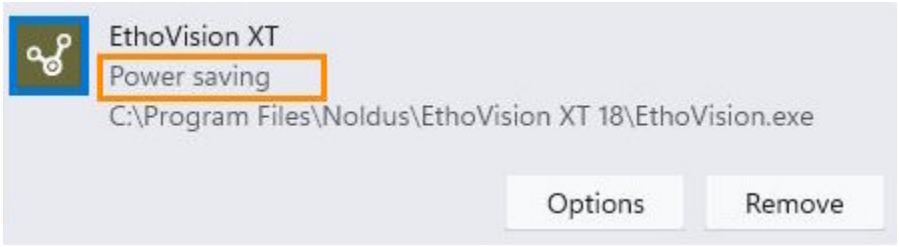
With this solution EthoVision XT uses the integrated card, which saves energy. You can choose that other programs use the secondary card, which requires more power.

- Method 1.
  - Open the settings for the secondary card. For example, **NVIDIA Control Panel**.
  - Under **Manage 3D Settings**, click **Program Settings** and click the **Add** button to add EthoVision XT.
  - From the list below **2. Select the preferred graphics processor for this program**, select **Integrated graphics**. If the option is not available, follow Method 2 below.




- Method 2.
  - In Windows choose **Settings**. Next to Search, type 'graphics' and press **Enter**. Choose **Graphics Settings**.
  - Click **Graphics**.
  - If EthoVision XT is not listed yet, under **Add an app** choose **Desktop app**, browse to the installation folder (default C:\Program Files\Noldus\EthoVision XT 18), select **EthoVision.exe** and click **Add**.
  - Click **Options** under **EthoVision XT**. In the Graphics Preference window, choose **Power saving**. Below this option you should see the

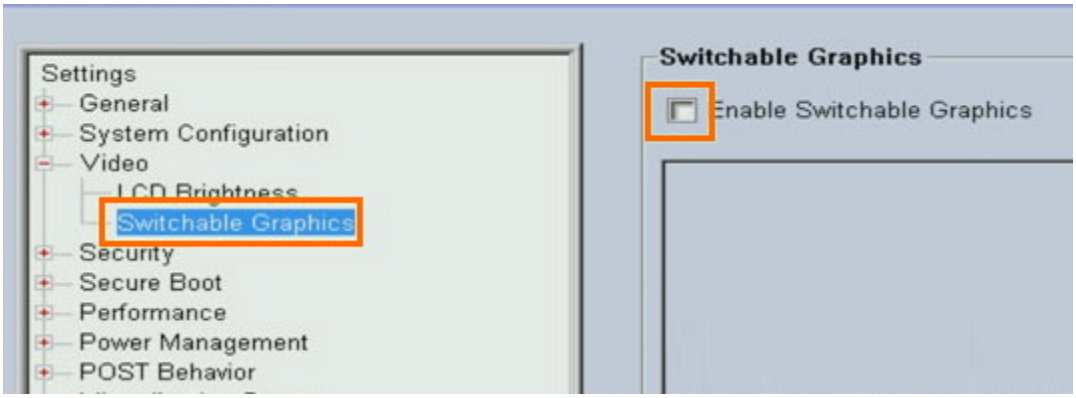
name of the integrated graphics card. EthoVision XT will use this card to play back video.




**SOLUTION 2** Disable the integrated card in the computer's BIOS.

This solution has the disadvantage that the computer won't use the integrated graphics card, which saves energy compared with the secondary card. EthoVision XT will always use the secondary graphics card.

1. Shut down the computer and start it again. Enter the BIOS. This is usually done by pressing the **F2**, **F12** or other keys when the first screen appears. See your PC's documentation for details.
2. Under **Video**, select **Switchable graphics** on the left, and de-select **Enable Switchable Graphics**, click **Apply** and exit the BIOS.




3. Now Windows only sees the secondary graphics card, which is given hardware acceleration. You can check that in the Windows Device Manager, under **Display adapters**.

## The camera image flickers

The underlying issue is that fluorescent and LED lighting cycles at a specific frequency, for example at 60 Hz in the US, and that frequency does not align with a frame rate of 25. Instead, use a frame rate of 30. Sometimes this is enough to resolve this issue.

If the problem persist, try the following:

1. The **Exposure Time** setting set in pylon Viewer or EthoVision XT is likely too low. To increase Exposure time, see Adjust camera settings in EthoVision XT. Try something in the 4000-8000 range. If lighting is low in the room, go for a higher exposure or open the aperture of the lens.
2. If none of the above resolve the problem, then was your PC purchased from Noldus? If not, it may be insufficient to run EthoVision as EthoVision is a very CPU intensive program. If you would like the Noldus Support to review your PC specs, please Make a PC report for Support.

## The camera image doesn't flicker but the overall image lightens and darkens

1. Check that in pylon Viewer under **Acquisition Controls**, **Exposure Auto** is set to **Off**.
2. Check that in pylon Viewer under **Analog Controls**, **Gain Auto** is set to **Off**.
3. Check that in pylon Viewer under **Color Improvement Control**, **White Balance Auto** is set to **Off**.
4. If none of the above resolve this, then check that your Exposure setting (in pylon Viewer under **Acquisition Controls**, or in EthoVision XT (Experiment Settings > click the camera button), **Exposure Time** is high enough (try 8000).

## There is motion blur in the camera image

The **Exposure Time** setting is likely too high, for example 20000 (microseconds) or higher.

1. See Adjust camera settings in EthoVision XT for how to reduce the camera's Exposure Time. The faster the subject, the lower the Exposure Time must be.

2. With a lower exposure time, the image becomes darker. You can compensate for this by increasing the lens' aperture or the camera Gain. For the latter, see Gain in Adjust camera settings in EthoVision XT.

## The camera image is not in focus, no matter what I do

It does not matter how high the camera is mounted or whether I zoom in then focus. Image from camera is always blurry.

The camera will not focus if the wrong type of lens is mounted:

- A C-mount lens on a CS-mount camera. The camera can be used but requires an adapter ring installed between the lens and camera.

- A CS-mount lens on a C-mount camera. In that case the camera and the lens are not compatible.

If the camera and lens have the same mount type, make sure this adapter ring has **not** been installed, as it will prevent correct focus.

If the solution above does not help, try resetting the camera. See Do a factory reset on the Basler GigE camera. When in doubt, contact Noldus Support.

## The camera image is dark, even when using a lot of infrared light

This may be due to a few reasons:

- The Aperture ring on the camera lens is closed all the way.
- The **Exposure** time of the camera is too low. See Adjust the camera exposure time
- The **Gain** of the camera is too low. Note that if you increase the camera gain, noise also increases. See Adjust the camera gain
- Some color cameras may have infrared block filters attached by default. In that case the IR light is blocked and the image looks dark. For Basler cameras, you can remove that filter. See Infrared-block filter

## My video file is not playing correctly

Download and use the Gspot software to check that the video file contains header information. If a video lacks header information, playback may not be smooth in EthoVision XT.

http://www.headbands.com/gspot/

We advise to convert the video file to another format.

- Some MPEG-2 videos play back irregularly in the Track Editor and in the Integrated Visualization. In this case too, convert the video to another format.
- The following formats are NOT supported in EthoVision XT: WMV, Quick Time, FLV (YouTube), Xvid variants of the MPEG-4 format. See Video file formats

## Video is being processed poorly

Set the hardware acceleration to maximum. You can do this in the graphics card control panel.

## A black bar is displayed when zooming in to a video file

This may happen in the Track Editor or in Integrated Visualization. When zooming in and the size or position of the video exceeds the total size of graphic display then on some computers the hardware acceleration needed for rendering the video will stop functioning, causing a (partially) black video. Contact Noldus Information Technology for a new video graphics card.

## Ethovision XT freezes when playing a video file

This may happen with MPEG-2 videos. Convert the video file to another format.

## EthoVision XT freezes in live video mode

This occurs with one or more digital cameras connected simultaneously. Experience tells that the software does not freeze only when you lower the resolution to e.g. 800x600.

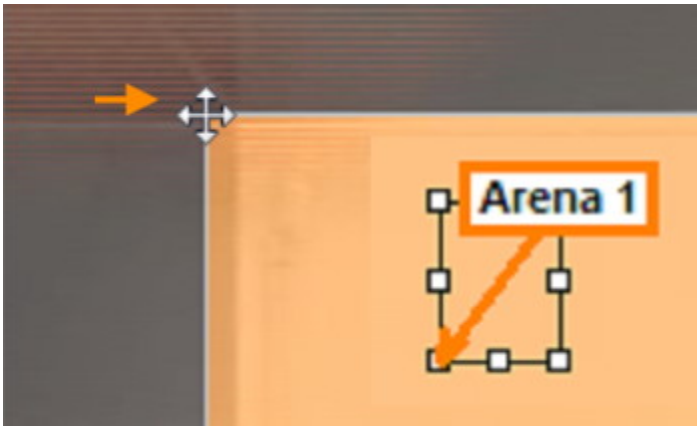
# Troubleshooting: Arenas and zones

## Issues

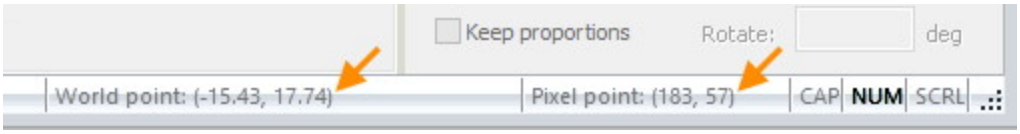
- How do I know the coordinates of the arenas, points and zones?
- Tracking does not start when the animal is inside the shelter
- The animal is in the hidden zone but the data show "missing sample"
- How do I view the analysis results for hidden zones?
- What if a hidden zone is part of a larger (overlapping) zone?
- I get a warning that two zones point to the same region, when in fact they don't
- I want to repeat calibration but I do not see the '+' cursor anymore

## How do I know the coordinates of the arenas, points and zones?

1. Open the Arena Settings.
2. Position the mouse pointer on one vertex of the arena/zone outline or on a point of interest.




3. Do not move the mouse. In the Status bar, locate the **World point** (for the coordinates in calibrated units, for example cm) or **Pixel point** (for the coordinates in pixels).



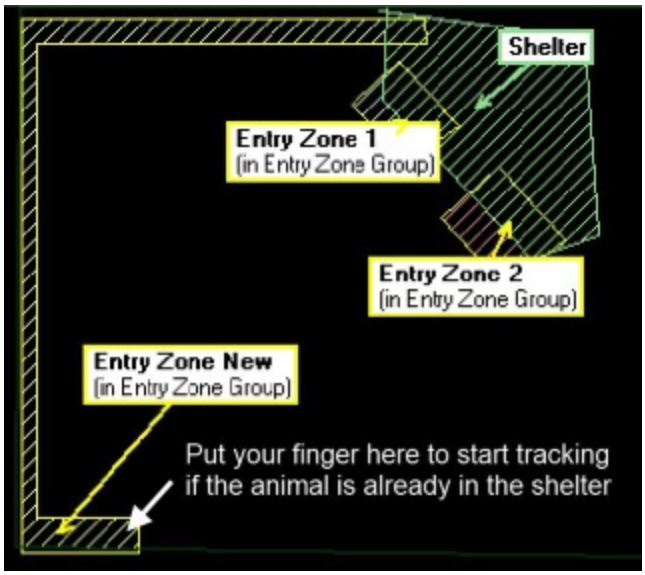
**TIPS**

- If you do not see the Status bar, choose **View** > **Status bar**.
- Zoom in the arena for a more precise positioning of the mouse pointer.
- You can use the point coordinates to calculate the coordinates of the subject relative to a point of interest. See an example in Troubleshooting: How can I merge heatmaps generated in different arenas?
- See also The x-y coordinate system in the arena

## Tracking does not start when the animal is inside the shelter

In an experiment with a hidden zone, for example a shelter, it is possible that the animal is already inside that zone when you start the trial. In that case, the trial will only start when the animal first exits the hidden zone. If you want the trial to start straight away, recording the animal inside the hidden zone, you can make a second entry zone, somewhere where you can put, for instance, your finger.

If you cannot get close to the shelter, you can draw a zone that runs along the arena side:




After starting the trial, put your finger in that zone in such a way that EthoVision detects it (so the trial can start), then remove it (but make sure that the finger only appears and disappears in this entry zone, not the rest of the arena. This way EthoVision assumes that the animal has entered the hidden zone).

## The animal is in the hidden zone but the data show “missing sample”

You may encounter this issue in the Track Editor or in the Track Visualization/ Integrated Visualization. Normally this happens when the last recorded position of the animal immediately before disappearing from view is outside the entry zone. There are at least two possible reasons for that to occur:

- The entry zone is too narrow and too near the hidden zone. So chance increases that the last data point occurs outside the entry zone, before the subject disappears from view.
- Another reason is that Track noise reduction is applied during tracking. In such a situation, the position of the subject at any time is smoothed using the last 12 samples. Especially when the subject moves fast, the data points may “lag behind” the image of the moving subject (you can check this in the Integrated Visualization). It can happen that the last smoothed position is calculated outside the entry zone.

In both cases there is some chance that the last data point before the subject goes missing is still outside the entry zone. For this reason, EthoVision XT considers the subject missing, not in the hidden zone.

To solve the issue, do at least one of the following:

- *Make the entry zone larger*. Duplicate the Arena Settings, enlarge the entry zone in such a way that the last position is likely to be inside that zone, and then use the new Arena Settings for the next trials.
- *Increase the sample rate*. Especially if the animals move fast, increase the sample rate. This increases the chance that at least one point position is determined inside the entry zone.
- *Disable Track noise reduction*. If you use Track noise reduction, duplicate the Detection Settings, under **Smoothing** set **Track noise reduction** to **Off**, and use the new Detection Settings to acquire the next trials.

## How do I view the analysis results for hidden zones?

To calculate the time that the subject spent in a hidden zone, choose In zone in the Analysis profile and select the hidden zone.

## What if a hidden zone is part of a larger (overlapping) zone?

- If the hidden zone is part of a larger zone, the results for that zone do not include the time spent in the hidden zone. This is because the time in the hidden zone is always calculated separately.

- If an entry zone is part of a larger zone, the time in the entry zone is also included in the time spent in the larger zone.

In practice: if a zone includes a hidden zone, in order to know the total time spent in that zone, including the time spent in the hidden zone, you must sum up the times shown in the results table. See a note under the topic In zone

## I get a warning that two zones point to the same region, when in fact they don't

This is caused by very sharp corners of one or more zones. Using the Point edit tool (press P), move single points so that sharp corners are removed. For instance by adding a point just next to the sharp corner. See Move, rotate and resize a shape

## I want to repeat calibration but I do not see the '+' cursor anymore

Click the **1. Draw Scale to Calibrate** button at the top-right corner of the screen.

# Troubleshooting: Detection

## Issues

- I cannot modify the Detection Settings
- The yellow blob changes its size periodically
- The percentage of Missed samples is very high
- The detected nose point is far from the animal's contour

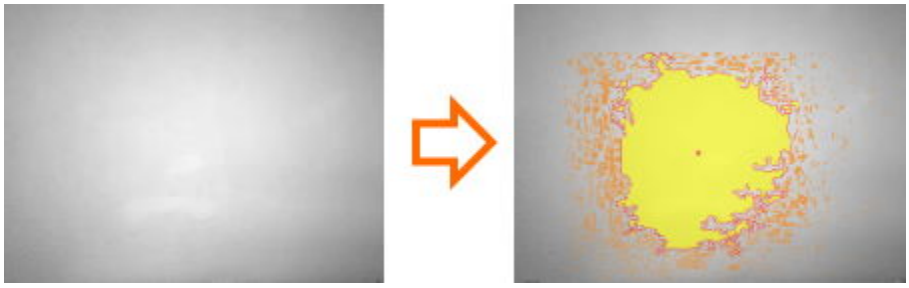
## I cannot modify the Detection Settings

Once data has been acquired in EthoVision using a specific profile (Arena Settings, Trial Control Settings, or Detection Settings), they become locked from changes. This ensures that EthoVision always preserves the settings you used to acquire your data, so it can be checked or replicated.

If you need to make changes, right-click your existing settings profile, and select **Duplicate**. The new profile will allow modifications.

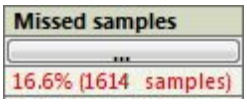
## The yellow blob changes its size periodically

The area considered as detected subject changes at regular intervals, as if the brightness of the image was not constant.



- Check that the auto iris function of the camera is not enabled.
- For digital cameras, increase the Exposure time. It may be that periodical changes in the intensity of the ambient light are picked by the camera sensor.

## The percentage of Missed samples is very high



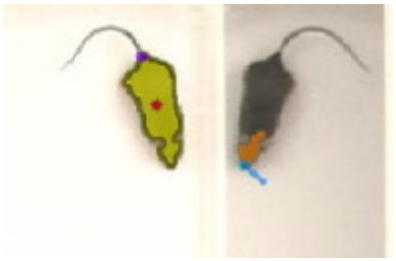

There may be a few reasons for this:

- The video resolution of the camera and/or the frame rate are set too high for the current detection method. EthoVision XT cannot acquire so much video information and at the same time track the subject. Reduce the frame rate and if necessary the video resolution in the Video source.
- The camera may not be sending the video frames at the expected rate. This occurs often with webcams or IP cameras. Select Dropped frames correction.
- The detection method currently selected is using too much processor time and that may not be necessary. For example, try a less demanding method like Static Subtraction instead of Dynamic Subtraction or Differencing.

See also Sample rate

## The detected nose point is far from the animal's contour

This may happen in some cases with detection based on Deep learning. For example, the nose is placed somewhere on an object or on the image of the subject reflected on a wall, as shown in this figure:



In the Detection Settings, under **Method**, click **Define** and reduce the size of the box around the animal.

# Troubleshooting: Data acquisition

## Video tracking

- The Start trial button is grayed out
- I track from the live camera image, and I get missing samples
- I track from video, yet I get missing samples
- Tracking from video is slow
- During tracking there is a significant lag
- The subject's trail (or the area where it was first detected) is detected as the subject
- The text on top of the video window during acquisition is too small
- Tracking does not start when the animal is inside the shelter
- I cannot make tracks of long duration
- I selected the DDS option but my video plays at normal speed. Why?
- I see spikes in the track
- Error: The trial duration does not correspond with the system time (Id: 12015)
- The subjects is detected in one arena, while in another arena I only see an orange blob

## Manual scoring

- I do not see a behavior category in the Manual Scoring screen
- I want to remove the center point of the subject while observing
- The body points of one or more subjects are not displayed in the Manual Scoring screen

## Other issues/questions

- I cannot start acquisition with a remote control
- I cannot import external data, or I get an error message "a gap was detected in the raw data"
- How can I randomize the start time of my trials?
- The Acquisition screen is not available in a Quality Assurance (GLP) experiment

- How do I start data acquisition on multiple PCs simultaneously?

## The Start trial button is grayed out

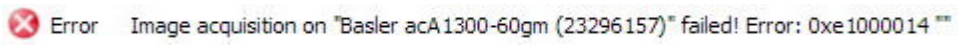
This may happens also when trials are planned. Adding trials in the Trial List does not help.

1. Make sure that, in the trial list, all the necessary settings are selected (Arena, Trial Control, Detection).
2. Make sure that the video selected in the Trial List is the same as that selected in the Acquisition screen (you find its name next to the Video button).
3. If you defined behaviors in the Manual Scoring Settings, make sure that there is one selected as initially active.

## I track from the live camera image, and I get missing samples

This could be due to a number of reasons.

- Tracking is too processor-demanding.
  - Try reduce the sample rate, or use another detection method (e.g. Dynamic subtraction instead of Differencing).
- Camera settings are not optimal.
  - Make sure that the Exposure of the camera does not reduce the frame rate of the camera. See Adjust the camera exposure time
  - Make sure in EthoVision XT that **Video Pixel Smoothing** is set to **None**. See Detection Settings > Video pixel smoothing.
  - In the Experiment Settings, click the camera button. Make sure that the color space is Y800 for monochrome cameras. See Adjust camera settings in EthoVision XT
- Communication between camera and computer not optimal.
  - In some circumstances the Ethernet board is not set properly, or the data transfer rate is too low for the frame size. You may see this message in pylon Viewer:

    Either reduce the video resolution and frame rate, or increase the packet size in pylon Viewer (in the **Feature** panel, locate **Transport Layer**). A packet size of 1500 should be fine. If this is not sufficient,

increase the packet size to the maximum. If it does not help, there may be something wrong with the Ethernet board. See also Settings of the Ethernet board

- Disable the firewall. See Disable the Windows firewall for the Ethernet board
- Disable your anti-virus software: check the settings in your anti-virus software or ask your local IT person to disable it.
- As a last resort, do a factory reset on the camera. See Do a factory reset on the Basler GigE camera
- If none of the above solves the issue please Make a PC report for Support.

*See also*

- Missed samples

## I track from video, yet I get missing samples

This is probably caused by the option **DDS** (Detection Determines Speed) not being selected. When DDS is selected, EthoVision XT takes the time necessary to process the images, and samples are not missed. See DDS (Detection determines speed)

If you work with multiple subjects per arena, and you do not want to select DDS, for example because you score behaviors live and you do not want video to be played back too fast, then in the Detection Settings (Subject size section), move the **Modeling effort** slider towards **Performance**. This reduces the likelihood that samples are missed. See Advanced detection settings: Subject size (multiple animals per arena)

Also make sure that the camera Exposure time does not conflict with the camera frame rate. See I cannot select a high frame rate

## Tracking from video is slow

Close other programs, including virus scanners. Check if the drivers of the video board (not the encoder board) are up to date.

## During tracking there is a significant lag

At times the system freezes a bit, then catches up and resumes.

1. Restart the PC daily to refresh PC's resources.
2. Assuming you are using a PC purchased from Noldus and the setup isn't too demanding (e.g. multiple arenas, deep learning), then this is likely to be

related to an incorrect exposure settings in pylon Viewer (see Adjust the camera exposure time).

3. Check that the video resolution is compatible with the frame rate; this may also need adjusting. See Adjust camera settings in EthoVision XT
4. Do also a check for missing samples. See Check that the camera is set up properly
5. If none of the above resolve your lag problem, then was your PC purchased from Noldus? If not, it may be insufficient to run EthoVision XT. If you would like Noldus Support to review your PC specifications, please Make a PC report for Support and submit a support case at my.noldus.com.

## The subject's trail (or the area where it was first detected) is detected as the subject

Make a reference image of the empty arena. Or if you use video, open the Advanced section in the Detection Settings pane and click **Background** next to **Detection reference Image**. Choose one of the options in the window that opens.

## The text on top of the video window during acquisition is too small

The size of the text is proportional to the screen resolution. The higher the resolution, the smaller the text. To solve this, choose a lower screen resolution.

## I cannot make tracks of long duration

Try the following:

- Switch off the Windows power-save options.
- Remove virus-scanners
- Switch off the automatic Windows Update. If that cannot be done, disconnect the computer from the internet and restart it before acquiring data.
- Make sure that you have enough disk space.

For information on the maximum trial duration tested with specific cameras, see Resolution, frame rate, and maximum trial duration.

To track or record video for time longer than that reported above, we recommend that you make a series of trials. See Split a multi-day test in multiple trials

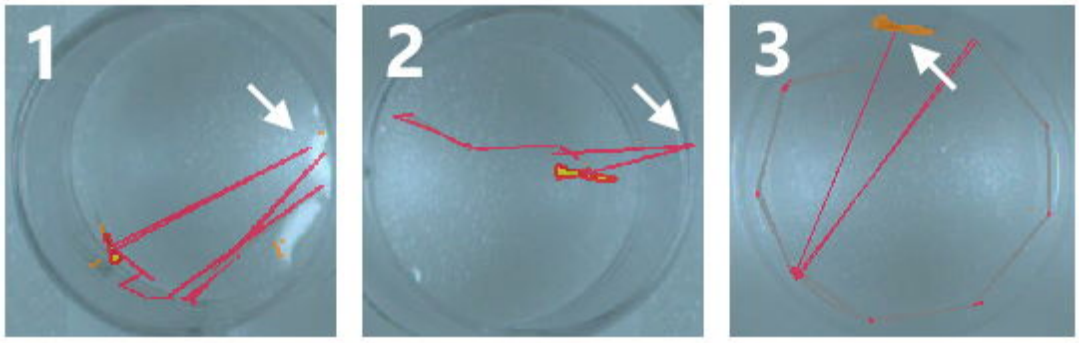
## I selected the DDS option but my video plays at normal speed. Why?

The cause could be related to the amount of data processing that EthoVision XT carries out. For example, the following options in the Detection Settings may be enabled: Track noise reduction and Dropped frames correction. To speed up data acquisition, make a copy of the Detection Settings, set the options above to Off and re-do the trial with those new settings. Or, accept the slow data acquisition if you need those settings to produce good data.

## I see spikes in the track

This may occur in the Detection Settings, during data acquisition or in Track Visualization, or in the Integrated Visualization. In most cases you need to correct the arena settings and the detection settings.

Here a few examples:




1. EthoVision XT tracks light reflections as the subject. Remove sources of reflections by using diffuse, indirect light. Watch the video chapter **Set Up the Test Environment** in **Help** > **Video Tutorial**.
2. EthoVision XT detects some noise at the margin of the well. You can do two things:
    - In the Arena Settings, reduce the size of the arenas, so they do not include the margin of the wall where the animal will never be. This should solve most of the issues.
    - In the Detection Settings, increase the **Minimum Subject Size** so small groups of pixels will not be detected as the subject. If you work with DanioVision, move the **Sensitivity** slider to the right. See Advanced detection settings: Subject size (one subject per arena)
3. EthoVision XT finds the subject but it considers it as noise (here in orange), while other objects are detected as the subject. In this case it may suffice to increase the **Maximum Subject Size** in the Detection Settings. The

**Maximum** value should always be higher than that under **Current** when the animal is detected correctly. See Advanced detection settings: Subject size (one subject per arena)

You can also remove spikes by editing the tracks (not in DanioVision though). See Edit the Tracks

## Error: The trial duration does not correspond with the system time (Id: 12015)

This could occur if the video camera provides an unreliable (i.e., fluctuating) frame rate. This may cause a mismatch between the system time and the tracking time. This halts data acquisition.

Industrial cameras such as the Basler cameras have reliable frame rates, while IP cameras and web cams often do not. If the camera is faulty or the driver is not installed properly, unreliable frame rates could occur.

## The subjects is detected in one arena, while in another arena I only see an orange blob

This may be caused by one of the following:

- The second animal is of a color that does not match the current detection settings.
- The reference image is not updated.
- The subject in the second arena is larger than the one in the first arena, and its exceeds the **Maximum subject size** currently set in the Detection Settings. See Advanced detection settings: Subject size (one subject per arena)
- The second arena is set to **To Skip**. Open the Trial List and set the **Acquisition Status** for that arena back to **Planned**.

## I do not see a behavior category in the Manual Scoring screen

This happens when you open the Manual Scoring screen, then open the Manual Scoring Settings to define a new behavior there, and then return to the Manual Scoring screen.

Solution: Close and reopen the experiment.

## I want to remove the center point of the subject while observing

In some cases the center point of a small animal makes it hardly visible.

Solution: To hide the center point, choose **Track Visualization** or **Integrated Visualization** or **Track Editor**, and de-select the body points on the right-hand pane. Next, close and re-open the experiment.

## The body points of one or more subjects are not displayed in the Manual Scoring screen

This happens when one or more subjects have been de-selected in the Track Editor or in one of the Visualization screens.

Solution:

1. Open the Track Editor or the Integrated Visualization.
2. In the Track Plot Settings, under **Subjects** select the items you are interested in.
3. Return to the Manual Scoring screen.

## I cannot start acquisition with a remote control

The remote control only works after opening the Acquisition screen and adding a new trial.

To activate the remote control, follow the instructions, see Use a remote control to start and stop acquisition.

Contact Noldus Information Technology to obtain a remote control.

**IMPORTANT** Before using the remote control, make sure that the Video window or the Playback Control window are on focus. To do so, click anywhere on the Video window or the Playback Control window. Then, operate the remote control.

## How can I randomize the start time of my trials?

For example, start a one-minute trial every N minutes chosen randomly between 60 and 120.

1. Create a batch file that performs a key press **Ctrl+F5**, which triggers the start of the trial. In this example, a batch (*.bat) file calls a VBS script (*.vbs) in Windows:

wscript "C:\vbsprogram.vbs"

2. The VBS script contains the following code:

   Set WshShell = WScript.CreateObject("WScript.Shell")

   WshShell.AppActivate "EthoVision"

   WScript.Sleep 1000

   WshShell.SendKeys "^{F5}"

3. In Windows, open the Task Scheduler and create a new task.
   - In the Triggers tab, specify when a trial should start (e.g. with a random delay).
   - In the Actions tab, select the batch file (*.bat) that triggers the key press **Ctrl+F5**.
   - See also Start and stop the trial at specific clock times

**NOTES**

- When the task is enabled, make sure that EthoVision XT Acquisition screen is open, and there are no other windows or applications open.
- The end of each trial should be set in the Trial Control Settings.
- In the Trial List, create a list of trials with the appropriate settings already assigned to each trial.
- The WScript.Sleep 1000 (1 s delay) ensures that the key press is given when EthoVision XT is in focus.

## The Acquisition screen is not available in a Quality Assurance (GLP) experiment

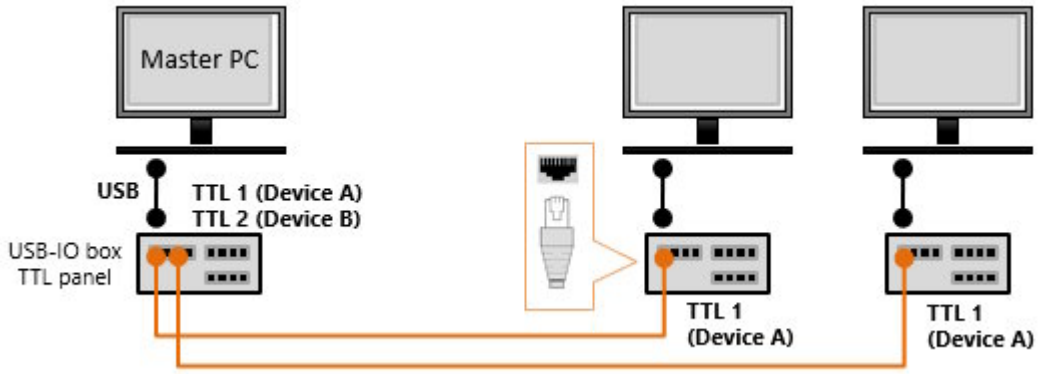
First, make sure that you have a valid EthoVision XT license that includes the Quality Assurance module. See Modules of EthoVision XT

This issue could occur if you use EthoVision XT 17 or earlier, and you try to open Acquisition with an EthoVision XT software license. In that case you need a license on a hardware key instead. See Types of EthoVision XT license Contact Noldus when needed.

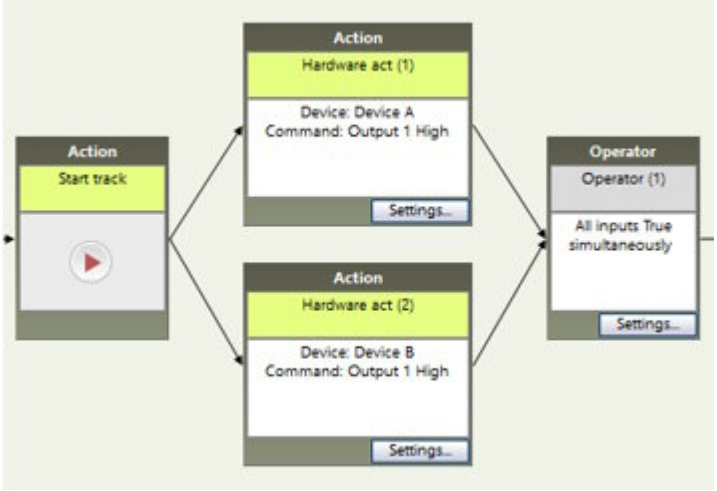
# How do I start data acquisition on multiple PCs simultaneously?

In the following example, we want to start data acquisition (that is, tracking) in three computers. For this you need three USB-IO boxes and EthoVision XT with the Trial and Hardware Control module in all PCs.

1. Connect the first PC (master) to the other two through the USB-IO boxes: TTL port 1 of the master PC to the TTL port 1 of the second PC, and TTL port 2 of the master PC to the TTL port 1 of the third PC.

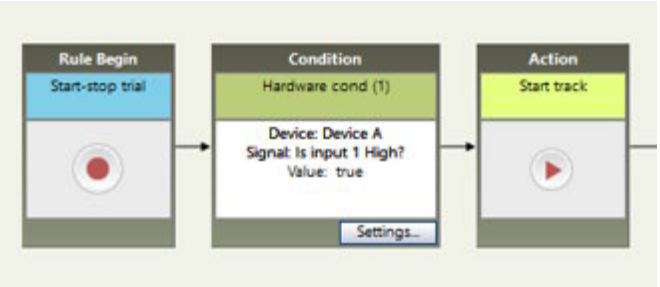



2. For each PC, configure the hardware in the Experiment Settings.
3. On the master PC, open the Trial Control Settings and modify the Start-Stop Trial rule in such a way that a TTL "high" signal is sent after the start of tracking to both ports TTL 1 and TTL 2 simultaneously. Make sure that the Action boxes and the Operator are at the right of the **Start track** box.




4. For each of the other PCs, open the Trial Control Settings and modify the Start-Stop Trial rule in such a way that tracking starts as soon as TTL "high"

signal is detected in TTL port 1. Make sure that you place the Condition box at the left of the **Start track** box.




5. To start acquisition in all the PCs, click the Start Trial button on the master PC.

As an alternative solution, you could devise a push button that sends TTL signals to each of the USB-IO boxes through UTP cables. Contact Noldus if you need this device.

# Troubleshooting: External data

## Issues

- I cannot import external data, or I get an error message “a gap was detected in the raw data”
- I imported the external data in EthoVision XT, but the values are missing
- The external signals look “stretched”, or “compressed” over the time line.

## I cannot import external data, or I get an error message “a gap was detected in the raw data”

Check carefully the prerequisites for importing external data. See Import external data: General information

**TIP** Open one of your data files with the Notepad, and check whether there are irregularities in the file. Move the cursor between the numbers.

```
# Col: Rat31.EEG,Rat31,3,EEG,mV
# Col: Rat31.EMG,Rat31,1,EMG,mV
0.0000,  2052.738,   -0.013,   -0.008
0.0040,  2052.738,   -0.010,    0.000
0.0080,  2052.738,    0.037,   -0.007
0.0120,  2012.452,    0.024,   -0.009
```

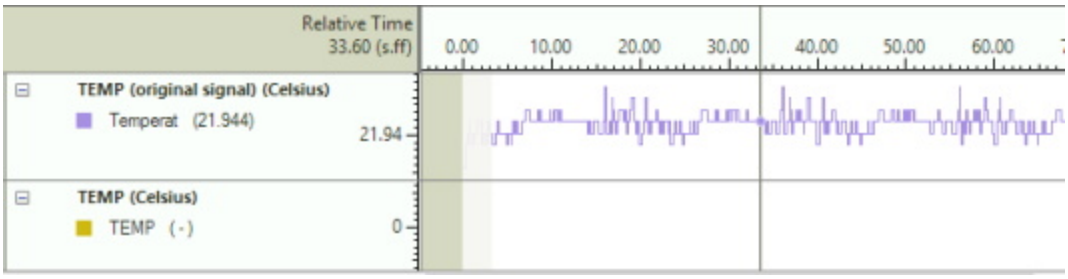
If there are blank spaces, not tabs between them, then in the import profile you must specify **Space** under **Select data delimiters**.

*See also*

- Create a new custom import profile
- Edit a custom import profile

## I imported the external data in EthoVision XT, but the values are missing

- **CASE 1** In the Integrated Visualization, you see the original signal, but not the downsampled signal as defined in the Analysis Profile:




In most cases this could be due to the fact that the original signal is not in sync with the track data. Check the synchronization and re-import the files, or change the set of the external data by editing the start/end time.

See Synchronize data manually after import and Synchronize data automatically

**CASE 2** The external data, both original and downsampled, are absent. When I try to re-synchronize the data, the Start date and time and the End date and time are identical.

This occurs when the original imported data (files with extension *.pbi) are not available, for example when they are stored on an external device. Make sure all files (experiment, and external data) are stored on the local drive.

## The external signals look “stretched”, or “compressed” over the time line.

This may occur if one of the TCAP pulse trains, which encode the time used for alignment, is truncated. EthoVision XT calculates the average set using 10 values of time, and if one or more of the train pulses are incorrect, the data may be out of sync, or their form may look compressed or stretched.

In this example the last pulse train is shorter than the others:


1. Remove the imported data from the trial.
2. Open the original data (text) file and remove the last pulse train.
3. Re-import the edited data file.

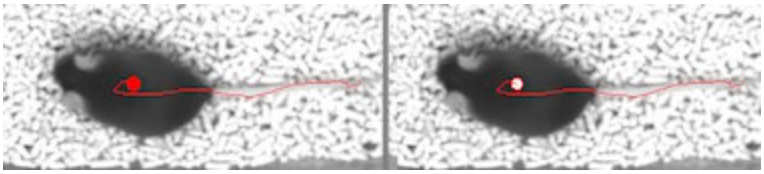
# Troubleshooting: Track editor

## Issues

- When I select a sample in the Sample List, the corresponding point in the track is not highlighted
- I try to move a point, but nothing happens. The software moves a point at an earlier time stamp
- When I select a group of samples of one subject, samples of another subject are also selected
- I no longer see the Track editor toolbar
- When I click to jump to another time, video update takes very long time

## When I select a sample in the Sample List, the corresponding point in the track is not highlighted

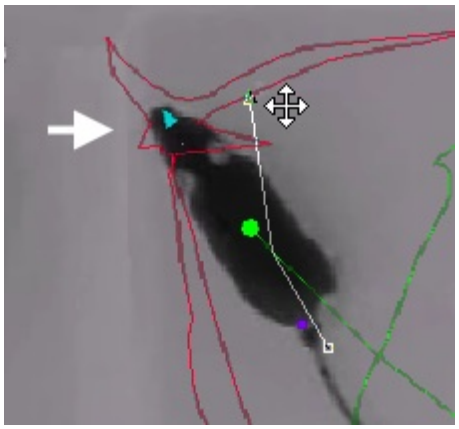
In other words, when double-clicking a row in the Sample List, the corresponding point stays as a filled symbol (left), while it should be highlighted (right).



This occurs when the trials were recorded with EthoVision XT 17 or an earlier version. If that is the case, and provided that you still have the associated video files, clear the trials and re-do them using the same video and settings. See Redo a trial

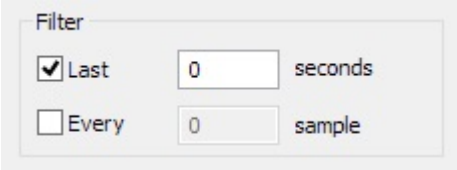
## I try to move a point, but nothing happens. The software moves a point at an earlier time stamp

You may have noted that when you click the point you want to move, the cursor on the Sample List (indicated with a green row) jumps to another time stamp. In the video image, the point is no longer highlighted (see an example with the nose-point in the following picture):

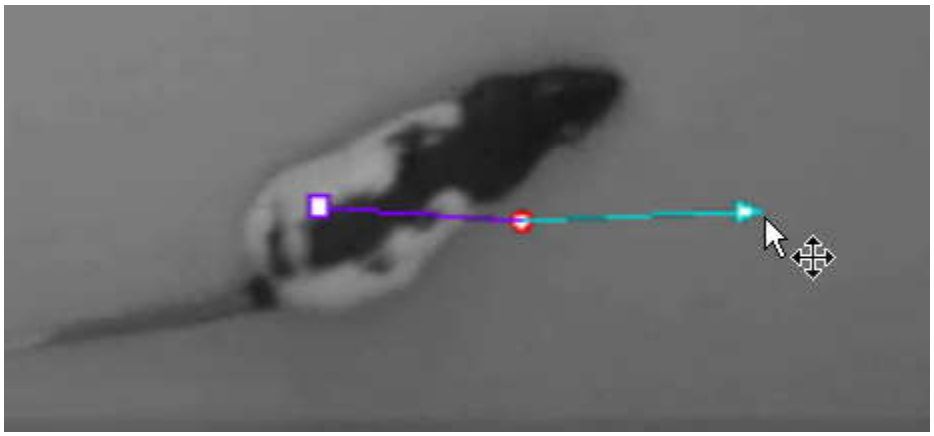


This happens because multiple samples overlap in the small portion of the image where you click. It is more likely to happen when you visualize the last few seconds of a track.

Solution: In the Track Plot Settings pane on the right, under **Filter** select **Last** and enter **0** next to **seconds**. This way, only the current sample, that is, the one highlighted in blue in the Samples List, is selected.




**TIP** To select the nose point, hover with the mouse until the tip of the mouse cursor is near the tip of the triangle. To select the tail-base point, hover the mouse next to the lower-right corner of the square. When the mouse cursor is near the point, a four-headed cross symbol appears. Drag now the point to the new position.



If the actions outlined above do not solve the issue, it may be that the trials were recorded with EthoVision XT 17 or an earlier version. If that is the case, and

provided that you still have the associated video files, clear the trials and re-do them using the same video and settings. See Redo a trial

## When I select a group of samples of one subject, samples of another subject are also selected

This occurs when you draw a box to select a group of samples, and the **Filter** option **Last ... seconds** is set to a value greater than zero. In that case EthoVision XT also select the samples of other subjects that lie within the box, and have a time stamp within the interval set in the **Last ... seconds** option. This makes it likely that you move or delete points of other subjects unintentionally.

Solution: Set the filter to **Last 0 seconds**.

If the problem still occurs it is because the body points of two subjects are very close to each other, and those subjects appear in the Samples List. To avoid this issue, de-select the subject you are not editing from the Samples List.

## I no longer see the Track editor toolbar

1. Close EthoVision XT.
2. In the experiment folder, locate two files with extension POS and POB, respectively. Delete both files.
3. Start EthoVision XT and open the experiment.

## When I click to jump to another time, video update takes very long time

Do one or more of the following until the problem is solved:

- From the Apps screen, under Windows System, click **Run**, type in **dxdiag** and click **OK**. After the diagnostic procedure has been completed, click **Exit**.
- Restart the PC and have the computer enter hibernation mode. Then resume the PC and start EthoVision XT.
- Upgrade the video graphics card drivers.
- Change the video graphics card. Contact Noldus Information Technology for information.
- See also System requirements: Hardware

# Troubleshooting: Statistics

## Issues

- A variable does not appear in my results
- I get unrealistic values of path shape and direction
- A subject shows rotational behavior. However, when I look at the stats of Angular velocity or Turn Angle, the values are lower than for a subject which does not show rotation. Why?
- I get different results in two EthoVision XT versions
- I get different results when I analyze the same video twice
- I want to validate angle variables like Heading, Head direction, and Heading to Point, but the average value I calculate in Excel does not match the results in EthoVision XT
- The total distance moved does not always match the mean velocity
- I want to analyze Activity to detect freezing in rodents. However, when I plot the data I get lots of Inactive states
- I get wrong statistics of manually-scored behaviors
- The statistics result table contains many empty rows or columns
- The statistics result table contains two or more values of a dependent variable for the same trial, arena or subject
- The statistics result table does not appear
- The Data Preparation Report says “Failed” for some trials
- The percentage of “Subject not found” does not match the time in the arena
- The results table contains “?” in many cells
- How do I know the time between the starting point of a video and the time that I started tracking?
- I get more zone entries than expected
- The Analysis profile does not list Mobility and Head direction

## A variable does not appear in my results

One or more of the following may the case:

- The experiment is not set to Nose-point, center-point and tail-base point tracking.
- You do not have the add-on module that allows to calculate those variables.
- For social behavior variables: double-click a variable in the Analysis Profile and define Receivers there.
- Your experiment is set to Live Mouse Tracker. This type of experiment does not contain the raw data needed to calculate variables like Mobility, Head direction and Body elongation.

*See also*

- Dependent variables in detail: Social

## I get unrealistic values of path shape and direction

Variables like *Meander* may have very high values (e.g. 5000 °/min) despite the fact that the animal moves approximately on a straight line. High values of the variable occur when body points for consecutive samples (that is, at times *t-1*, *t*, *t+1*...) lie near each other. For example, when the sample rate is high, and the subject does not move much, the distance moved between consecutive samples is very small, consequently *Meander* (which is by definition *Turn angle* divided by *Distance moved*) will be very high.

In this case it is useful to filter data using the Minimal Distance Moved method.

To choose the threshold, plot the data of *Distance moved* together with your variable. For example, if you see that values of *Distance moved* around 0.05 produce unrealistic values of the variable, set Minimal Distance Moved = 0.1.

If this does not fix the problem, try re-tracking from the same video, but with a lower sample rate.

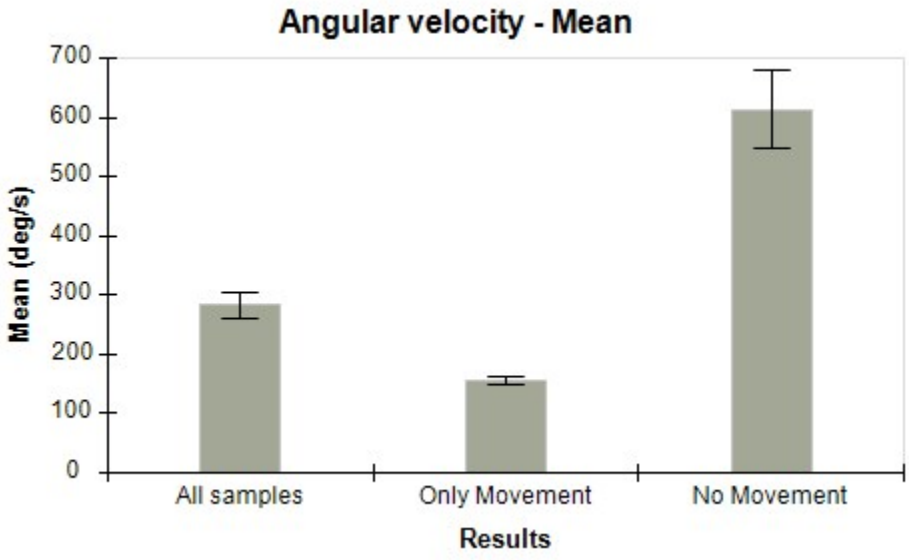
*See also*

- Dependent Variables in Detail > Path
- The Minimal Distance Moved smoothing method

## A subject shows rotational behavior. However, when I look at the stats of Angular velocity or Turn Angle, the values are lower than for a subject which does not show rotation. Why?

Angular velocity does not necessarily correlate with rotations. If the animal turns slowly, its *Angular velocity* is low. Furthermore, when the subject sits still, but its

body point oscillates in all directions due to random noise, high values of *Turn angle* (and therefore *Angular velocity*) will appear. To minimize this effect, either use the Minimal Distance Moved filter (see the previous issue), or nest over Movement to only select the data points when the animal is Moving. Then, calculate your variables. The figure below compares Angular velocity calculated for when the animal moves significantly, with when it does not move.




To quantify rotational behavior, also use Rotation.

## I get different results in two EthoVision XT versions

The possible causes of this issue are:

- You specified a sample rate that is lower than the maximum sample rate; in that case EthoVision XT analyzes every *n*th video frame (where *n* is the video frame rate/your sample rate; for example if you choose a sample rate of 12.5 for a video file of 25 frames per second, then *n* =2, that is, the software analyzes every two frames). In some previous versions, EthoVision XT always took the second frame in the video and every *n*th frame thereafter. The results like distance moved may be slightly different, but that is very unlikely to affect the outcome of an experiment.
- EthoVision XT 17.5 and later play video using the GPU by default. In contrast, EthoVision XT 17.0 and earlier used the CPU. This may result in slight differences in the appearance of the video image, for example, video looking brighter, which in turn could affect detection and the tracking results. You have two options:
  - Revert to playback with the CPU in the Preferences for video settings.

- Keep using the GPU and adjust the detection settings in the new version so they match the detection in the old version as much as possible.

## I get different results when I analyze the same video twice

There may be a few reasons for this to happen.

*Reason 1* - The effect of the reference image. When you use the detection methods Dynamic Subtraction or Differencing, the image used as reference may differ between trials even when you use exactly the same detection settings. The effect is more pronounced when you use Differencing.

- If you position the video file at different times (thus at different frames) then you go back to the beginning and start the trial, the reference image may be different in the two trials. This will cause the track to differ slightly between the trials.
- If you choose to use the **dynamic reference image** instead of the **saved reference image** at the start of each trial in a series (see The reference image) then the reference image with which trial 2 starts may not be exactly the same as the one at the start of trial 1. The difference may be of one or few more pixels brighter or darker, but still that difference has an effect.

To ensure that you use a consistent reference image, duplicate the Detection Settings and under **Advanced** select **Background** > under Acquisition Settings select **Use saved reference image** or **Use first frame of each trial**. This way at the start of each trial the same reference image is used. See The reference image

*Reason 2* - Missing samples can affect the track duration. This happens especially if the missing samples happen to occur just when trial control start or stop conditions are evaluated.

*Reason 3* - In multi-subject tracking doe with Deep learning, the results are not entirely repeatable due to the non-deterministic nature of the algorithm. Note that this occurs when you select to track two subjects in the arena, not just one. See Troubleshooting: Deep learning > The results of two-subject tracking are not consistent when using the same video

Concluding, to improve the consistency between trials:

- Start both trials at the beginning of the video file, without positioning the video on any frame in the middle.
- Improve the subjects' detection to reduce the number of missing samples.
- Use Gray Scaling or Static Subtraction (if possible). If you use Differencing or Dynamic Subtraction, choose to use a fixed reference image. See The reference image

- To smooth out the tracks and remove outliers, apply the Lowess smoothing to the tracks. See The Lowess smoothing method

## I want to validate angle variables like Heading, Head direction, and Heading to Point, but the average value I calculate in Excel does not match the results in EthoVision XT

If you use the ATAN formula, the value is wrong because ATAN uses another convention for the sign of the average sine and cosine of angles. Use instead ATAN2.

The formula for the average in a track is

=DEGREES (ATAN2(AverageCos, AverageSin))

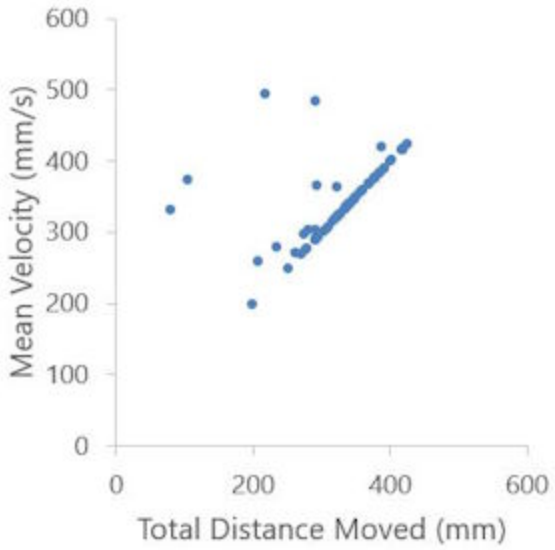
Where AverageCos is the average of Cos values (calculated as =COS(RADIANS(angle)), and AverageSin is the average of Sin values (calculated as =SIN(RADIANS(angle)), and angle is the per-sample value of Heading, Head direction, or Heading to point.

## The total distance moved does not always match the mean velocity

When plotting the average velocity and the total distance moved, there should be a linear relationship between the two. In particular:

Average Velocity = Total distance moved / T

Where T is the time interval (e.g. trial, or time bin) within which the samples are used to do the calculations. For example (here the time bin is 1 second):




In some cases the data points deviate from the linear relationship. The reason is that the total distance moved is calculated over the available samples. If some samples within an interval are missing, or in some samples the subject was not found or if the interval is incomplete (for example, the last time bin in a track), then the total distance is underestimated while the average velocity is unaffected. As a result, the mean velocity no longer lies on the straight line, rather, it lies above the line. The mean velocity is higher then expected from the current distance moved. This pattern is therefore an artifact of the variation in the number of samples available for each time interval. In principle, there is nothing wrong with the data, provided that the proportion of missing samples or samples with "subject not found" is low.

## I want to analyze Activity to detect freezing in rodents. However, when I plot the data I get lots of *Inactive* states

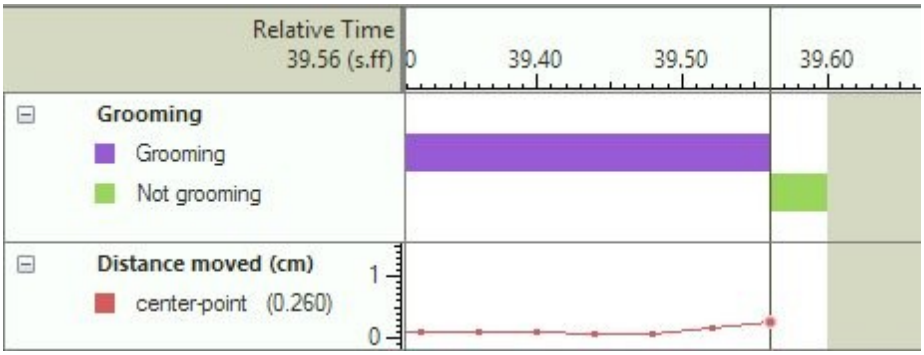
This usually happens when the Activity settings in the Detection Settings are too high. For example, the **Activity threshold** is 20, or **Background noise filter** is set to more than 3. In such cases the thresholds are so high that almost no video frame the subject is considered *Active*, even if in the Analysis profile the *Active* state was defined with low threshold values.

Solution: make a duplicate of your Detection Settings, reduce the **Activity threshold** and/or the **Background noise filter**. Check in the video image that purple pixels appear only when the subject moves. Then repeat the tracking with those Detection Settings.

## I get wrong statistics of manually-scored behaviors

Problem: I scored a behavior of type "Start-stop". In the Statistics and Charts page, the frequency of *Not [behavior name]* is X+1 when it should be X.

Explanation: This occurs when you score a Start-stop behavior until the end of the trial. At the last sample time, the behavior is automatically ended (see *Grooming* below), and its complementary *Not [behavior name]* is scored. This results in the additional occurrence of *Not [behavior name]* in the statistics. However, the statistics of the actual behavior are correct.




## The statistics result table contains many empty rows or columns

This occurs often when you make multiple data selections. For example, you make a Nest condition to select the time from 12 hours to 24 hours. Then you create time bins of one hour each. The results table also shows the “empty” bins from 0 to 12 hours:

| | Cumulative Duration |
|---|---|
| | % |
| 0:00:00-1:00:00 | - |
| 1:00:00-2:00:00 | - |
| 2:00:00-3:00:00 | - |
| 3:00:00-4:00:00 | - |
| 4:00:00-5:00:00 | - |
| 5:00:00-6:00:00 | - |
| 6:00:00-7:00:00 | - |
| 7:00:00-8:00:00 | - |
| 8:00:00-9:00:00 | - |
| 9:00:00-10:00:00 | - |
| 10:00:00-11:00:0 | - |
| 11:00:00-12:00:0 | - |
| 12:00:00-13:00:0 | 99.9056 |
| 13:00:00-14:00:0 | 99.4500 |
| 14:00:00-15:00:0 | 97.2222 |
| 15:00:00-16:00:0 | 90.4444 |
| 16:00:00-17:00:0 | 87.5167 |

To hide the empty rows/columns:

1. Click the **Layout** button on the toolbar (or click the **Show/Hide** button at the top-right corner of the screen and select **Layout**).

2. In the window that opens, select **Hide empty rows or columns**.

## The statistics result table contains two or more values of a dependent variable for the same trial, arena or subject

| | Distance moved |
|---|---|
| | center-point |
| | Total |
| | cm |
| Trial 1 | 98.9930 |
| | 672.1242 |

This may be due to the fact the results are calculated with a data profile containing two or more Results boxes, but the table does not show the corresponding headers. Click the **Layout** button and add **Selection Result**, or **Arena name**, or **Subject**. See Modify the layout of the results table

## The statistics result table does not show the measurement units

This occurs when in the Layout window you place the Variable with detail on the rows and the Statistic with unit on the columns, and vice versa. To solve the issues, place both items on the rows or on the columns. See Modify the layout of the results table

## The statistics result table does not appear

Problem: The results table in Trial Statistics or Group statistics and charts shows no headers and no results.

Explanation/Solution: This could happen for example when

- The experiment has no data yet.
- The data have been completely filtered out in the Data profile. Please check your data selection.
- For Group statistics: An independent variable was inserted in the table Layout, and at least one of those groups contains multiple values of that variable.

  **EXAMPLE** In the Data profile, you group tracks in two groups, *Treated* and *Control*. In the Group statistics and charts, you insert the independent variable *Rat ID* in the Layout, but each group contains many values of this variable (one per subject tested). Therefore, the grouped results cannot be listed properly.

To solve this, either remove the variable from the Layout (Modify the layout of the results table), or in the Data profile group your trials in a different way. See also Trial Statistics result

## The Data Preparation Report says “Failed” for some trials

This message occurs in experiments with Deep learning based body point detection. In some cases EthoVision XT fails to review the tracks and fix subject identity swaps. For example, when the two subjects spent most of the trial time in close contact, or the video file was too short to obtain a reliable trained neural network. See a note in Prepare the data in multi-subject trials

## The percentage of “Subject not found” does not match the time in the arena

Problem: “Subject not found” gives one value (e.g. 5%) and the total time the animal is in the arena (Cumulative duration% in the Arena) is 96%, while I expect them to be complementary.

Explanation/Solution. In the following example there is a gap of 25 frames of “subject not found”. With 25 frames per second (so 0.04 s per frame), that comes down to a missed sample time of 1 second, which may be for example 0.5% of the total time. That percentage you see in the Trial list. However, when the subject goes missing, EthoVision XT assumes that it is still in the arena in the next 3 frames, before considering it “not in the arena”. In this example, the gap is not 25 frames but 25-3 = 22 frames. Therefore, the total time “not in the arena” is 22*0.04 s = 0.88 s instead of 1 s. Thus, Subject not found and Cumulative duration% in Arena not always match.

## The results table contains “?” in many cells

This could indicate that one or more trials are corrupt. However, it is also possible that you edited the tracks and something went wrong with editing.

1. Click **Calculate** and take note of a trial that gives the “?” results.
2. Go to the Track Editor, select the trial and then click **Undo All**. Note that this action erases all your edits in that trial.
3. Re-do editing when necessary.
4. Run analysis.

## How do I know the time between the starting point of a video and the time that I started tracking?

This applies to cases when you positioned the video later than its starting point and then clicked **Start Trial**.

1. Export the raw data (Choose **Analysis** > **Export** > **Raw data**).
2. Open the exported file and look at the difference between the value next to **Start time** and the value next to **Video Start time**.

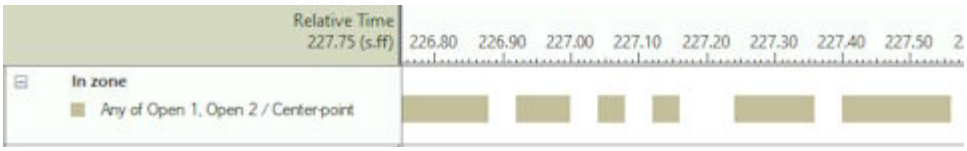
## I get more zone entries than expected

This applies to *In Zone* variables where the number of entries is higher than that expected. For example:

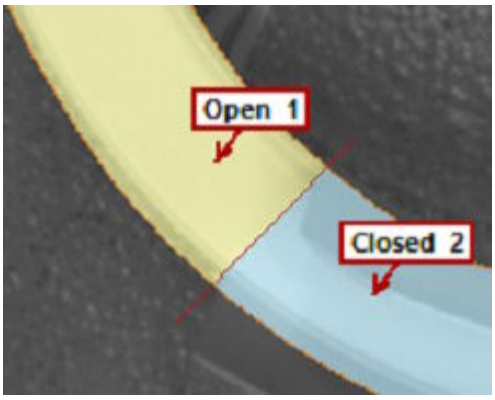
This could occur for different reasons:

- A common issue is that the center point of the subject moves back and forth around the boundary between zones when the animal moves just on that spot. This overestimates the number of true zone entries. To avoid that, use the **Zone exit threshold**, in the **In Zone** dependent variable settings, or use all the three body points to score a zone entry. See In zone
- Another reason for an overestimate of zone entries is that the center point is no longer detected in a zone, although the subject is still found. Consider for example this zero maze. The open and closed zones do not cover the arena completely.


The orange part in the figure above is the region of the arena outside the zones. When the center point is detected there, a zone exit is scored. A new zone entry is scored when the center point is found in the zone again. As a result, you could see a train of zone entries like this:




To solve this issue, re-draw the zones in such a way they overlap the entire region of interest within the arena. For example:




## The Analysis profile does not list Mobility and Head direction

This occurs when your experiment is set to Number of Subject = 2 and Body Point Detection Technique = Deep learning. In that case the software does not calculate the body contour and the Head direction line.

# Troubleshooting: Behavior recognition

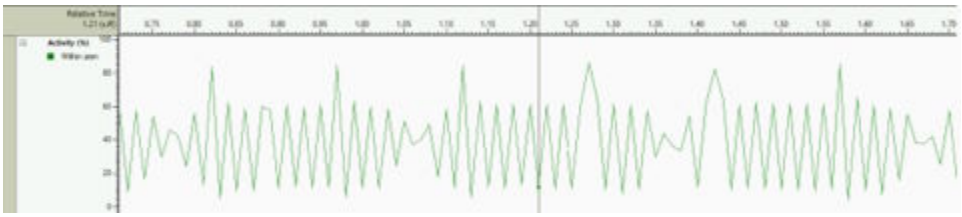
## Issues

- How do I check that the video file contains artifacts?
- EthoVision XT finds more instances of a behavior than expected
- EthoVision XT finds no instances of Grooming
- I get different results when I use Behavior Recognition in two EthoVision XT versions

## How do I check that the video file contains artifacts?

Artifacts due to video compression can reduce performance of Behavior Recognition.

1. Create an experiment and set it to tracking **From video file** and **Activity analysis**.
2. In the Detection Settings, open the Activity analysis. Reduce the Activity threshold to the minimum.
3. If the video contains artifacts, you should see purple pixels appearing and disappearing with a regular rhythm.
4. Just to be sure, do a test trial and plot the Activity values in the Integrated Visualization. A pattern resulting from video compression artifacts looks like this:



## EthoVision XT finds more instances of a behavior than expected

This may occur due to the low contrast of the subject with the background, and too little contrast within the subject area, that is, too little detail in the fur.

For example, too many *Grooming* instances are scored when the subject is asleep. This occurs when the image of the subject is overexposed. For example, white rats

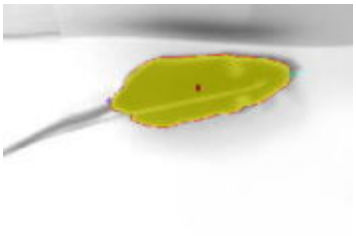
look pure white. In this case the inner part of the subject area is fairly constant in time, compared with the outline, and that increases the probability of detecting grooming. To solve the issue, close the lens' aperture or reduce the exposure time. See Adjust camera settings in EthoVision XT

## EthoVision XT finds no instances of Grooming

The subjects clearly displays bouts of grooming, however EthoVision XT does not detect it at all. This may happen when the regular shape of the subject viewed from above is more "round" than usual, as it occurs in certain strains or circumstances.

In that case, after clicking **Grab** in the Define Subject Properties dialog often results in a value of **Posture** being lower than the minimum required (70 for mice). This means that the contracted shape of the subject during grooming is more likely to be considered as "normal", therefore grooming is not detected.

Find a video frame where the subject walks and looks a bit stretched, preferably when one can see the contour of the hindlimbs. Then, click **Grab**. Repeat these steps until the value of **Posture** obtained is higher than the minimum required.



## I get different results when I use Behavior Recognition in two EthoVision XT versions

There may be differences in the scores of behaviors between EthoVision XT 18 and a previous version, even when using the same video file and the same Behavior Recognition settings. This may have to do with the different video decoding software that is used in the two versions. Small changes in the way video is displayed (e.g. the same pixel displayed with slightly different intensity value) may

result in changes in the probability of some behaviors, and therefore influence the automatic scores.

We tested a number of video files and found that the codec used to create the video file may affect the difference in the results between software versions. When the video was created with H.264 MPEG-4 AVC codec or the mp4v MPEG-4 codec, the average difference in the behavior scores (either frequency of occurrence or cumulative duration) expressed as a percentage of the scores obtained with the same video in EthoVision XT 15, is between 0 and 2% (averaged across all behavior categories).

The H.264 MPEG-4 AVC codec is used by EthoVision XT 16-18 and MediaRecorder 5-6. The mp4v MPEG-4 codec is used by EthoVision XT 15 and MediaRecorder 4.

For codecs like DivX, Xvid, MPEG 1 and MPEG 2, the average difference between EthoVision XT 15 and 16 is larger - up to 22%.

As general recommendations:

- Always use EthoVision XT or MediaRecorder to record video that you will analyze with Behavior Recognition.
- When you upgrade an experiment made with an older version of EthoVision XT to EthoVision XT 18, and you want to re-do Behavior Recognition, make sure you use exactly the same Arena Settings and Detection Settings that you used with that particular video file.
- In the Analysis profile, adjust the criteria based on the behavior probability to only consider the instances of behaviors that have a high probability, for example higher than 95%. That usually gives more robust results.

*See also*

- Behavior Recognition: Data, performance and accuracy
- How behaviors are scored in Behavior recognition
- Dependent variables: Behavior Recognition

# Troubleshooting: Deep learning

## Issues

- I see identity swaps during data acquisition
- The nose and tail base points are not stable even when the contour of the subject is detected well
- The results of two-subject tracking are not consistent when using the same video
- The Data Preparation Report says “Failed” for some trials
- A subject is labeled as Subject 1 in the first trial and Subject 2 in the second trial
- The results of two-subject tracking differ depending on the graphics card (GPU) used
- One of the subjects was not tracked at the start of the video

## I see identity swaps during data acquisition

This is normal when using the Deep learning technique in two-subject tracking. The identity swaps are corrected during the Data Set Preparation process. During that process, the track segments are sorted and put together based on the visual appearance of the subjects. This procedure greatly reduces identity swaps. See Prepare the data in multi-subject trials

## The nose and tail base points are not stable even when the contour of the subject is detected well

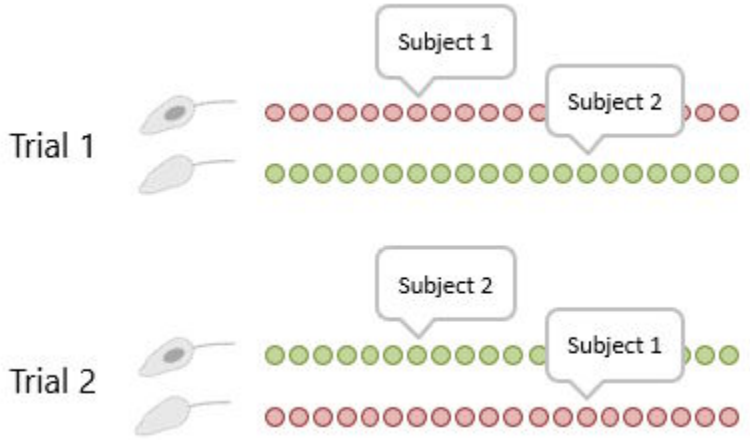
- With one subject per arena: In the Detection Settings, click the **Define** button, then **Automated**.
- With one or two subjects per arena: check that there is enough contrast between subjects and background.
- Furthermore, objects in the arena may reduce the accuracy of the position of the nose point.
- See also Deep learning: Requirements

## The results of two-subject tracking are not consistent when using the same video

This occurs because the individual recognition algorithm is not entirely deterministic. Probability plays a role in determining whether an animal in a certain video frame is labeled as Subject 1 or Subject 2.

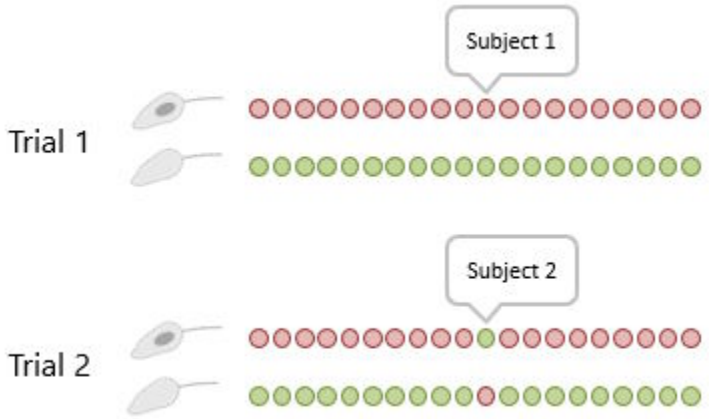
As a result, the statistics may differ between two trials, even when using the same video and tracking settings (Trial Control Settings, Arena Settings and Detection Settings).

- *Mirror labeling*. The first thing you may notice is that, when you acquire two trials from the same video, one individual (say that with the tail marks) is labeled as **Subject 1** in trial 1 (red dot; left), whilst as **Subject 2** in trial 2 (green dot; right). Conversely, its partner is labeled as **Subject 2** in trial 1, and as **Subject 1** in trial 2. Thus, the second trial is a sort of mirror image of the first one.




  If you want to keep consistency of labeling, for example to have the resident individual of a resident-intruder pair of mice always labeled as Subject 1, swap the subjects in the entire trial (see below).

- *Consistency of ID assignment*. Even when the same individual is labeled as **Subject 1** in both trials, there is a small chance that the results are not 100% consistent. Within short segments of the tracks, the identity labels may differ: for example, the marked mice is labeled as **Subject 1** in trial 1, and as **Subject 2** in trial 2.




Such segments last a few seconds and usually occur during situations of body contact. According to our tests carried out on videos provided by users, the probability that an individual at any time point in the video is labeled the same way in both trials, after correcting for the mirror labeling (see above), is 99.6% (range 83.5 to 99.9, n= 9). Low values occurred in videos where the animals were in contact most of the time, or videos of low resolution and with more noise.

In the Track Editor, review the video to evaluate the extent to which identity swaps occur. If that is not acceptable, swap the subjects where necessary, so that you get fully consistent results.

- *Consistency of general statistics*. The non-deterministic mechanisms of the multi-subject tracker based on deep learning also cause the statistics to be not 100% repeatable when tracking multiple times from the same video. When analyzing the Total Distance moved in trials acquired from the same video, the coefficient of variation is 0.4 % (range 0.1 - 2.5). In practice, a video where an animal walks 100 meters could result in some 40 cm difference in distance moved between trials. Higher-quality videos produce variability of the results lower than 1% of the mean.

  If this variability is not acceptable, open the Track Editor and swap the subjects where necessary.

## *See also*

- Prepare the data in multi-subject trials
- Deep learning: Requirements

## A subject is labeled as Subject 1 in the first trial and Subject 2 in the second trial

You may want to keep the identity labels consistent. For example, to assign the label **Subject 1** always to the resident individual, or the individual marked on the tail.

To make identity labeling consistent, swap the subjects for the entire trial.

1. Open the Track Editor (**Acquisition** > **Edit Tracks**).
2. Select the trial where you want to swap the subjects.
3. Click the header for the first subject (default: **Subject 1**) in the Sample List.

   You may have replaced **Subject 1** and **Subject 2** by other role names in the Experiment Settings; see Subjects per arena).
4. Press **Shift**, and click the header of the second subject (default: **Subject 2**).
5. As a result, the entire sample list is highlighted in green. See also Select samples manually
6. Click the **Swap subjects** button.


See also

- Swap subjects
- Select samples manually

## The results of two-subject tracking differ depending on the graphics card (GPU) used

The results of two-subject tracking could be slightly different based on which GPU was installed, everything else being equal (e.g., computer, arena settings, etc.

When the neural network model is not highly certain about the pose of the subject, small differences in handling of number with decimals could lead to noticeable differences, for example in the position of the nose at a certain sample time. Another reason could be that precision handling could differ between NVIDIA driver versions.

Tests carried out with supported GPUs show that the maximal difference in distance moved between GPUs is in the order of 0.1%.

## One of the subjects was not tracked at the start of the video

When you open the Integrated Visualization, there is one track in each arena, while you expect two tracks. One of the two subjects apparently was not detected. This usually occurs in the first few seconds of the video.

In reality both subjects were detected, but their tracks now overlap. You can check this by de-selecting one of the subjects in the **Show/Hide** pane.

To prevent this from happening.

- Create new Detection Settings and select the maximal sample rate, i.e. at least 25 samples per second, then re-do the trial. See Sample rate
- Make sure that the two animals are released at the same time, or one shortly after the other (e.g. less than 30 seconds). The video should not contain a long segment with just one animal in the arena.

# Troubleshooting: Data visualization

## Tracks and Integrated Visualization

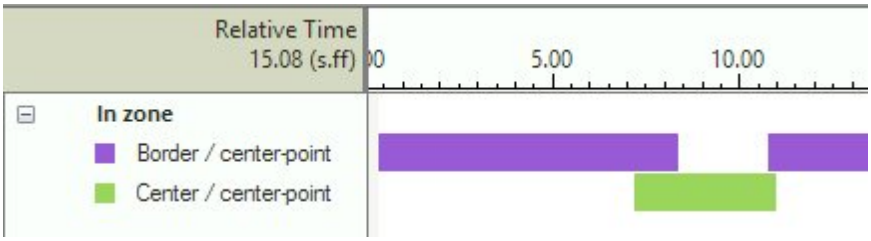
- I defined “In zone” for two adjacent zones. In the Integrated visualization, I see that the animal is in the two zones simultaneously
- I defined a free interval, and in the visualization I get an interval every other sample
- I plotted a variable in the Integrated visualization. I only get a few values on the timeline. Why?
- I interpolated my data, but some variables including Elongation and Mobility still show missing values
- Screen recording could not be started
- There seems to be a delay between video and track
- The video image is not shown
- Video is choppy or does not play smooth
- The points are too small (or too large) or the traces are too narrow (or too thick)
- When I import UltraVox XT data, some calls are visualized as 1, others as 0.5. Why?
- The Integrated Visualization shows “Not calculated”
- The Data Preparation Report says “Failed” for some trials
- I cannot visualize a trial
- The name of the trial is not fully visible

## Heatmaps

- A heatmap is plotted outside the arena, or merged with the heatmap of other arenas
- In the Heatmaps screen the Group Mean button is not available
- How can I merge heatmaps generated in different arenas?

## I defined “In zone” for two adjacent zones. In the Integrated visualization, I see that the animal is in the two zones simultaneously

This happens when a Zone exit threshold has been defined that is greater than 0, and the two zones *Center* and *Border* are adjacent. In such a case, when the animal moves from *Border* to *Center*, it is at some point considered to be in *Center*, but if it is not yet outside the zone exit threshold of *Border*, it is also considered to be in *Border*.



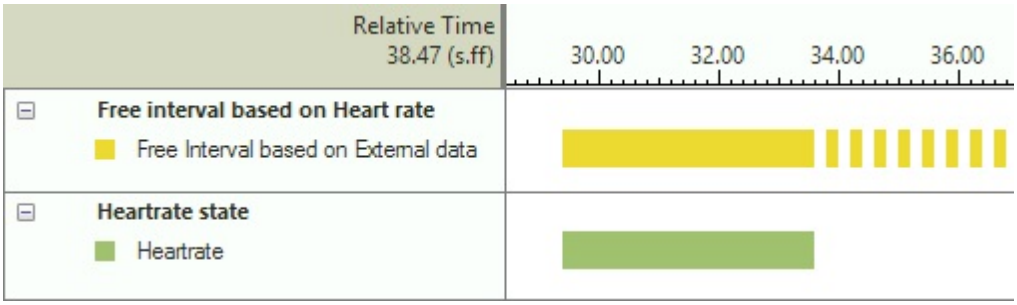

To prevent this, re-define the two zones in such a way there is a gap between the two, its width being at least the same as the zone exit threshold.

## I defined a free interval, and in the visualization I get an interval every other sample

Most probably you defined a free interval based on two values of external data or a hardware variable, for instance:

- From Heart rate >= 300 to Heart rate >= 400
- From Number of pellet drops >= 1, to Number of pellet drops >= 10

When the value of the (resampled) variable reaches the second (higher) threshold, the interval ends and EthoVision XT finds the next sample that matches the criterion defined in *From* (first threshold). If the current value is higher than the first threshold, a new free interval is defined. However, if the value at the next sample is also higher than the second threshold, the interval is ended, resulting in a free interval of one sample. The search is repeated in the next samples, generating the pattern shown in the first plot below.




In such cases, do not use a free interval. Instead, use a state variable that is based on the original external or hardware signal. You can find the state variable in the Analysis profile. For example, for the external data *Heart rate*, in the Analysis profile under **External data** choose **Heart rate state**. For a hardware signal, under **Hardware** choose **Hardware state**. There you can define the range of values you are interested in. In the example above about heart rate:


## I plotted a variable in the Integrated visualization. I only get a few values on the timeline. Why?

This may happen for variables which require two or more consecutive data points to be calculated. Consider the following examples:

- Your data contain missing samples. If one sample is missing, the variable is not calculated for one, two or more time points. For example, Distance moved is not calculated for two time points, and Turn Angle is not calculated for three time points.
- You may have applied Minimal Distance Moved smoothing method. When using this method, if the distance between body points for time *t* and *t+1* is lower then the Minimal Distance Moved, the body point at time *t+1* is "moved" to the same coordinates as the point as time *t*. In this case Distance moved gets the value 0 (zero), but any variable that quantifies change in direction has no meaning here. Therefore, is not calculated for that sample.

  If many samples in your track are filtered out that way, variables like Turn Angle, Meander and Heading only appear in short segments. Because the filter removes data points that do not represent movement, these few remaining segments are a more realistic measure of the subject's behavior.

*See also*

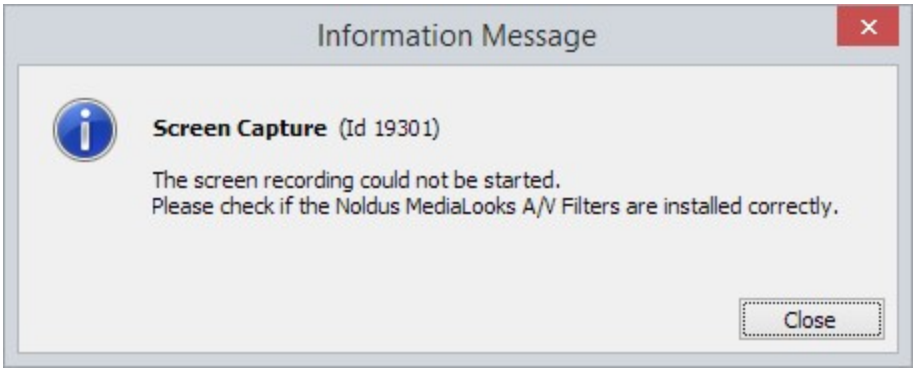
- How variables are calculated: Dependent Variables in Detail

## I interpolated my data, but some variables including Elongation and Mobility still show missing values

Interpolation is only applied to missing body points to obtain valid x,y coordinates. When the subject is not detected, the subject's contour and surface area remain unknown. This is why variables such as *Activity*, *Elongation* and *Mobility* still show missing values after interpolation.

## Screen recording could not be started

When trying to make a screen recording, you get the following message:

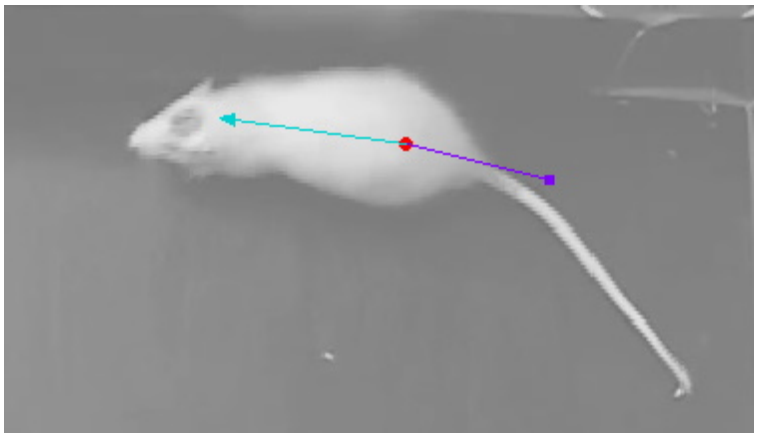



This may be caused by the fact that older versions of the Noldus MediaLooks A/V filters are still installed, but the newest version was not installed.

1. Close EthoVision XT.
2. In the Windows Control Panel, choose **Programs and Features**, and remove old versions of Noldus MediaLooks A/V Filter.
3. Download the full installation ZIP file (see Install EthoVision XT), extract and browse to **Drivers and tools** > **PreRequisites** > **MediaLooks**. Copy **Noldus Medialooks ScreenCapture 2.0 x64.msi** to your EthoVision XT computer and run that file.
4. Follow the installation, then start EthoVision XT.

## There seems to be a delay between video and track

The track is lagging behind the video or the other way round.



This issue can only occur when you track live and save video.

Solution:

1. Choose **Acquisition** > **Edit Tracks**.
2. Choose the trial from the list at the top.
3. Play the video until the subject and the track are well in view.

   **TIP** Choose a frame when the animal walks fast so you can see exactly in which sample the nose point overlaps with the subject's nose in the video.
4. In the Playback Control window, click the **Offset** button.

   This button is only available when you tracked live and saved video.
5. In the **Synchronize video** window that opens, enter a number of frames (negative or positive) to correct the discrepancy between track and video. Click OK when ready.

   For example, enter -1 to move the video one frame backward relative to the track.

## The video image is not shown

This issue can occur in computers with two graphics cards: a primary (integrated) card and a secondary card, usually more advanced and powerful than the integrated one. The issue is solved when you choose which card EthoVision XT uses.

See The camera image is not shown in the Arena Settings or Integrated Visualization

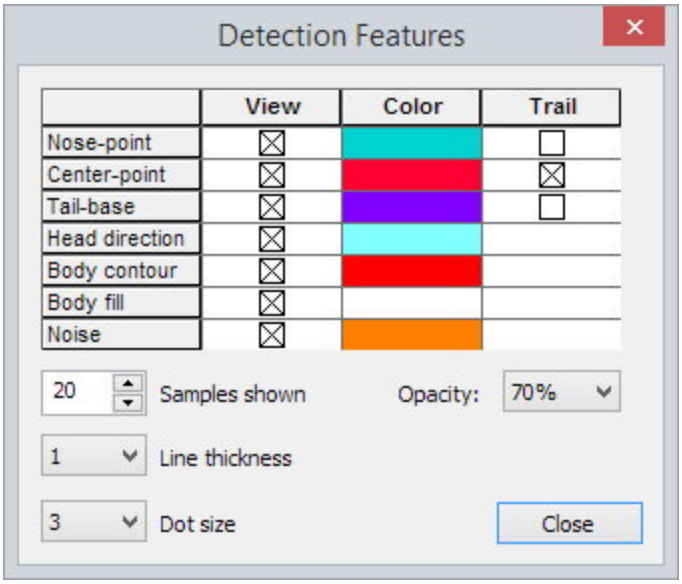
## Video is choppy or does not play smooth

This may occur if you play video at high playback speed like 16x in the Integrated Visualization. The effect is worse if the secondary graphics card (GPU) which takes care of the playback is less powerful.

## The points are too small (or too large) or the traces are too narrow (or too thick)

Solution: in various parts of EthoVision XT you can change the thickness of the lines and the size of the dots.

1. Click **Show/Hide** (at the top-right corner of the screen) and select **Detection Features**.
2. Adjust the **Line thickness** and **Dot size**.

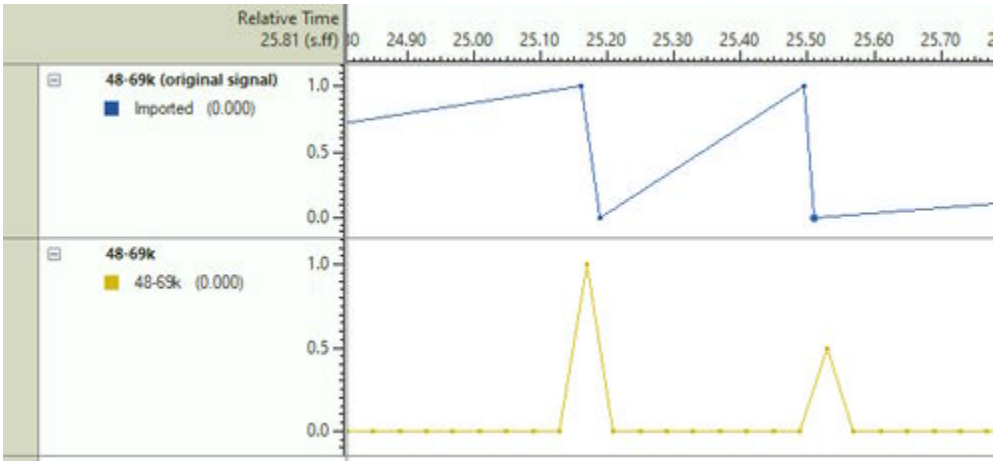



Note:

- What you select in the Detection Settings screen are also selected in the Acquisition screen, and the other way round.
- What you select in one of the following screens is also selected in the other screens: Track Editor, Track Visualization, Integrated Visualization, Manual Scoring screen
- The line thickness is also applied to the body contour in Detection Settings and Acquisition screen. It is not available in the Manual Scoring screen.

# When I import UltraVox XT data, some calls are visualized as 1, others as 0.5. Why?

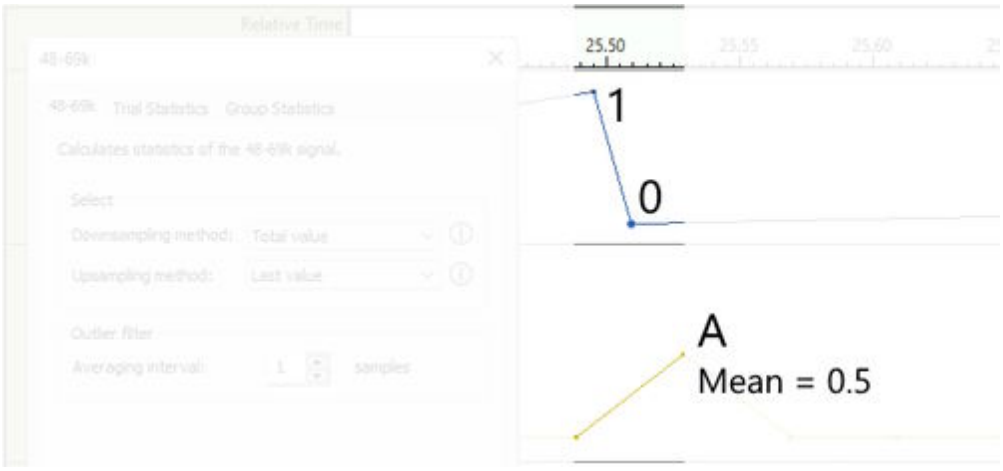
This is due to the type of resampling selected in the external variable of your Analysis profile. Consider the following example. The first plot shows the original signal imported from UltraVox XT and the second plot shows the same signal after resampling:



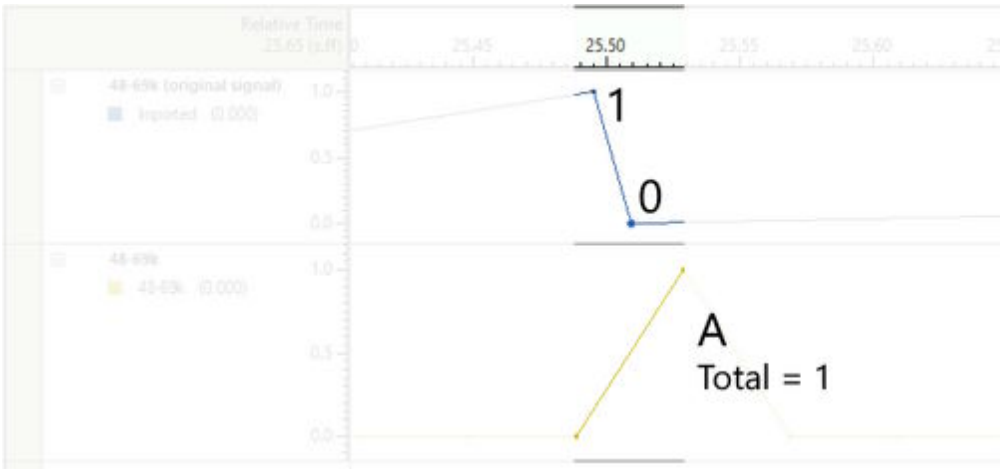

For the second call at 25.5, the time between the “1” an “0” in the original signal is so short that it fits in one sample interval.

Two points in the same sample interval result in downsampling. Now open the Analysis profile, and open the External data variable. See External Data

- If you choose **Mean** for downsampling, the value A in the resampled signal will be the average of 1 and 0, that is 0.5.




- If you choose **Total** for downsampling, the value in the resampled signal will be 1 + 0 = 1. After selecting Total, the signal is as follows:




So, choosing **Total** should solve the issue.

## The Integrated Visualization shows “Not calculated”

One or more trials are corrupt, or something went wrong with editing the tracks. See The results table contains “?” in many cells.

## The Data Preparation Report says “Failed” for some trials

This message occurs in experiments with Deep learning based body point detection. In some cases EthoVision XT fails to review the tracks and fix subject identity swaps. For example, when the two subjects spent most of the trial time in close contact. See a note in Prepare the data in multi-subject trials

## I cannot visualize a trial

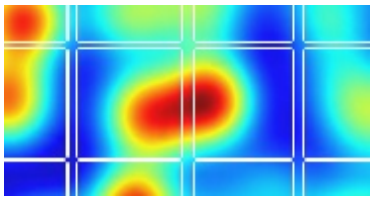
In most cases, this occurs when a Data profile is active that does not include that trial. Make a Data profile that includes that trial and visualize the data. See Filter tracks

## The name of the trial is not fully visible

The name of the trial on the toolbar of the Integrated Visualization screen is partially hidden. This may occur when the display is set to a high resolution. Reduce the screen resolution in Windows.

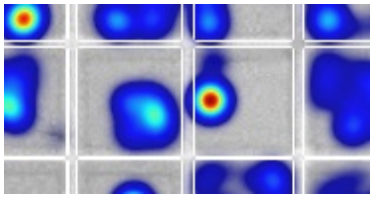
## A heatmap is plotted outside the arena, or merged with the heatmap of other arenas

For example:



Solution: In the Heatmap Settings pane, click **Colors** and under **Smoothing** select a lower value.

Result:



*See also*

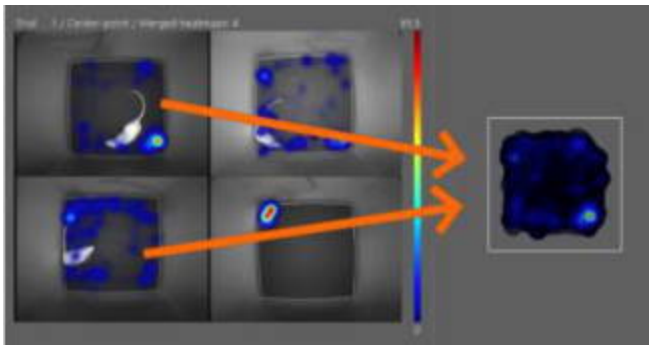
- Color smoothing

## In the Heatmaps screen the Group Mean button is not available

Probably you are working with a DanioVision experiment. The **Group Mean** button is disabled in the experiments set as DanioVision.

## How can I merge heatmaps generated in different arenas?

For example, you acquired tracks in Arena 1 and Arena 3 with subjects that belong to the same treatment group, and you would like to create one heatmap based on the data of both arenas.



Unfortunately merging heatmaps from different arenas is not possible within EthoVision ZXT. However, with simple JavaScript code you can re-calculate the coordinates in the tracks and export them to an external applications that creates heatmaps. Here below we describe a possible solution:

1. In the Arena Settings, define a Point of interest (see Draw a point) in each arena. For example, the south-west corner of the open field. This point will be the new reference point of the coordinates, so it must represent the same physical location in all arenas.

   If the arenas includes already a zone or point, like the shelter of PhenoTyper, and the zone has the same position and orientation in all arenas, then you do not need to define a Point of interest.

2. In the Analysis profile, add two JavaScript continuous variables. See JavaScript continuous

   The first variable is for recalculating the x-coordinate. Add the following text (replace “Point” with the name of you point or zone).

```
const g_zone = "Point";
function Start()
{
}
function Stop()
{
}
function Process()
{
   var pt1 = GetCenter();
   var pt2 = GetPointPoi(g_zone);
   if (pt1)
   {
      var xc = pt1.x - pt2.x;
      SetOutput(xc);
```

```
    }
    else
    {
        SetOutputMissing();
    }
}
```

3. For the second variable, repeat the step above, this time replace x with y.
4. Export the two variables (**Analysis** > **Export** > **Raw Data**). These contain the x,y coordinates expressed relative to the point in the arena.
5. Import the data in an application that creates heatmaps, like Excel Power Maps, R or MatLab.

# Troubleshooting: Control of hardware devices

## Issues

- EthoVision XT does not record the pellet drops
- The USB-IO box was accidentally disconnected, or is not found
- EthoVision XT does not trigger the LED in an optogenetics setup
- I cannot select devices from the second USB-IO box
- Some inputs of the Fear Conditioning System do not work
- How can I send data through a COM port?

## EthoVision XT does not record the pellet drops

The problem occurs also when the Pellet dispenser works just fine.

1. Check that all USB-IO boxes are powered up. If the USB-IO box is not powered up, it may work half way and that is rarely noticed.
2. If many devices are connected or there is much USB activity, EthoVision XT cannot read the pellet drops in the necessary time. The Pellet dispenser sends out an approximately 100 ms pulse to the USB-IO box and then EthoVision XT; if it takes longer to read the pulse, EthoVision XT misses the read out. Try reduce USB devices that work simultaneously.

See also the EthoVision XT 18 - Trial and Hardware Control - Reference Manual for limitations in the number of devices that can be controlled.

## The USB-IO box was accidentally disconnected, or is not found

The message appears *At least one USB-IO box was not found*.

1. Reconnect the USB-IO box to the EthoVision XT PC. Check that the USB-IO box is powered.
2. Click the **Retry** button (when this button is available).
3. Establishing connection requires some time. Wait until a message is given that the USB-IO box is detected again.

If the message of re-established connection does not appears, close and then restart EthoVision XT.

## EthoVision XT does not trigger the LED in an optogenetics setup

In an optogenetics experimental setup, EthoVision XT controls one or more Pulsers through the USB-IO box. The Pulser and the LED can be triggered manually or with the Pulser software, but not through EthoVision XT. When the trigger command is sent out from EthoVision XT, the LED in the USB-IO box does not switch on.

Solution: Review all the cable connections as in the chapter **Optogenetics experiments** in the EthoVision XT 18 - Application Manual. Check that on the LED controller box, the switch at the back is set to **Ext**. Also in the Device Configuration window of EthoVision XT, make sure that for the ports TTL1, 2, etc. **Custom hardware** is selected. For details on this step, see the EthoVision XT 18 - Trial and Hardware Control - Reference Manual.

## I cannot select devices from the second USB-IO box

A number of devices are connected to the EthoVision XT system through two USB-IO boxes. When one tries to select devices in the Device Configuration window, the drop-down menu under Devices is grayed-out.

Solution:

1. Close EthoVision XT, and uninstall the Noldus HardwareInterface software. You can find this in the Windows **Control Panel** > **Programs and Features**.
2. Download the full installation file (see Install EthoVision XT), and browse to **Drivers and tools** > **PreRequisites** > **HardwareInterfaceIobox**. Run the installation file HardwareInterface_Iobox_x64 [version number].msi.

## Some inputs of the Fear Conditioning System do not work

Check that the USB-IO box is powered. Review the cable connections as described in the section of the EthoVision XT 18 - Application Manual which describes the Ugo Basile Fear Conditioning System.

## How can I send data through a COM port?

Use case: Send a string or an integer to an Arduino board via COM.

To do so, you must first install a DLL interface component. Please contact Noldus for further details. Once this component is installed:

1. In the Experiment Settings, declare the COM port as you would when using the Noldus USB-IO box and other devices.

2. In the Arena-Hardware mapping window, map the COM port onto the arena.
3. In the Trial Control Settings, add an action based on a COM port.

Note that:

- Data (string or long integers) is sent out as a string. For example, if you specify 1234, the string “1234” is sent out.
- A string can also be hexadecimal, for example, 0x6261. Two bytes are sent out, first 0x61, then 0x62.

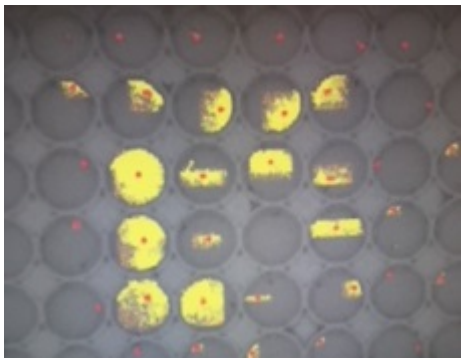
# Troubleshooting: DanioVision

## Issues

- The quality of detection deteriorates with time
- I cannot specify the white light fade duration in minutes and hours
- There are spikes in the tracks
- The DanioVision White light does not work when I skip some arenas

## The quality of detection deteriorates with time

After a few trials, the subjects are no longer correctly detected. For example:



The issue likely occurs because of condensation on the Fresnel lens.

1. Set the anti-condensation mechanism to permanent **On** (see the DanioVision DVOC-0041 - Reference Manual for details). This bypasses the automatic control.
2. If condensation disappears after a while, the temperature sensor and/or the sensor circuit may be defective. If condensation remain, the glass heater may be defective. Contact Noldus Support for more information.

## I cannot specify the white light fade duration in minutes and hours

Probably you have an experiment created with a previous version of EthoVision XT. There you can only select fade duration of the white light in seconds.

## There are spikes in the tracks

See the topic Troubleshooting: Data acquisition > I see spikes in the track

## The DanioVision White light does not work when I skip some arenas

Normally the White light device is associated with Arena 1 (A1 in most well plates). If you set that arena to **To Skip**, once you start the trial EthoVision XT does not send any command to the White light device because the corresponding arena is not active.

To solve this issue, do one of the following.

- In the Trial List, set Arena 1 back to **Planned**. EthoVision XT collects data for all the planned arenas, including Arena 1. If you are not interested in the data of Arena 1, you can filter it out in the Data profile (**Filter** option).
- In the Trial List, keep Arena 1 set to **To Skip**. Open the Arena Settings and in the Arena-Hardware Mapping window select **No device allocated** under Arena 1 and select **White Light Stimulus** under one of the arenas that you do not skip.

  The second option is only available when your experiment does not contains tracks yet.

# Troubleshooting: Live Mouse Tracker

## Issues

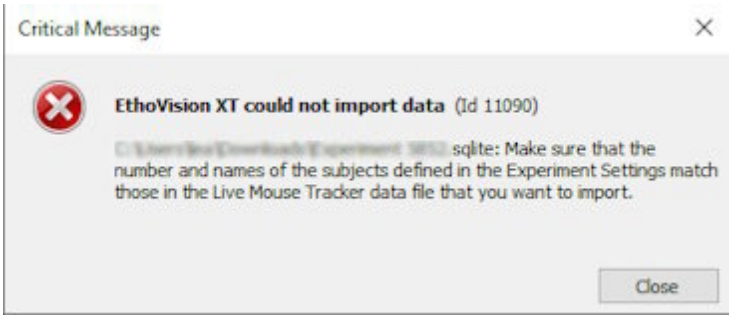
- My results differ from what I obtain using the original Python scripts
- Number and names of subject do not match those in the LMT database file
- The LMT database file is of compatible format

## My results differ from what I obtain using the original Python scripts

This may occur when you use Python scripts that were modified after the release of EthoVision XT. In that case we cannot guarantee that EthoVision XT gives exactly the same results. EthoVision XT is based on the Live Mouse Tracker scripts of version 1.0.3 (October 4, 2022).

## Number and names of subject do not match those in the LMT database file

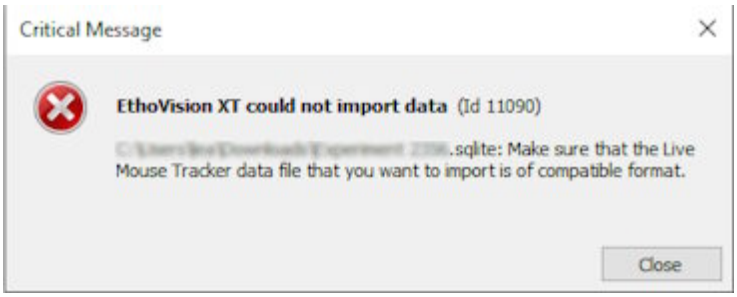
After you have selected a LMT database (*.sqlite) for import, the following error message opens:




1. Click **Close** and open the Experiment Settings.
2. Next to **Number of Subjects per Arena**, enter the number of individuals that interacted in the LMT experiment.
3. If necessary, rename the subject to **A**, **B**, **C**, etc. Do not enter other names. See Subjects per arena
4. Repeat the import data procedure. See Import Live Mouse Tracker data

## The LMT database file is of compatible format

After you have selected a LMT database (*.sqlite) for import, the following error message opens:




This could be caused by the experiment file being empty or without of a definition of the subjects. It is probably corrupt. Try to export the LMT data again.

# Troubleshooting: Import trials

## Error message ID10107: The source experiment has different hardware

This occurs when the **Device ID** of the hardware devices declared in the experiment settings (Device Configuration window) differ between the experiments. Or, if the names are the same but the devices ID have been mapped to different arenas. For example, when Lickometer 1 was assigned to Arena 1 in the current experiment, and in Arena 2 in the experiment you want to import the trials from.

Unfortunately there is no way to re-assign Devices ID post-acquisition. Always make sure to use the same names and mapping criteria when creating new experiments that you want to merge.

# Troubleshooting: Quality Assurance (GLP)

Issues

- I cannot start EthoVision XT (GLP restriction)
- I cannot create new experiments, or acquire data with EthoVision XT
- EthoVision XT crashes after opening a GLP log

## I cannot start EthoVision XT (GLP restriction)

A message appears:

*You are not registered as a GLP user, so you cannot use EthoVision XT. Please ask a EthoVision XT user manager for help.*

This issue could be caused by the fact that the user that has started EthoVision XT is not known as GLP user.

1. Restart the computer and ask the system administrator or someone with an administrator account to log in.
2. Start EthoVision XT. Add users as GLP users. Make sure to add all the persons that are going to use EthoVision XT. See EthoVision XT user management
3. The administrator logs out.
4. Log in with your account.
5. Start EthoVision XT.

Alternatively, ask your system administrator to add your user account to the administrator group.

## I cannot create new experiments, or acquire data with EthoVision XT

The **File** > **New** menu items and the **Acquisition** menu are disabled. When using the Quality Assurance module, it seems that users have been removed as authorized GLP users.

This may occur for two reasons:

- The hardware key is not plugged in.
- You reinstalled or repaired the application.

In the latter case the Quality Assurance user list is deleted. Ask your Quality Assurance user manager to add your user ID and give you the appropriate rights.

## EthoVision XT crashes after opening a GLP log

This occurs when using Quality Assurance experiments, after you click within the GLP log or when you click the **Close** button.

Do the following:

1. Open the Windows Notepad and enter the following text:

   ```
   Windows Registry Editor Version 5.00
   [HKEY_CURRENT_USER\SOFTWARE\Microsoft\Internet
   Explorer\Main\FeatureControl\FEATURE_USE_LEGACY_JSCRIPT]
   "EthoVision.exe"=dword:00000001
   ```

2. Save the file as [file name].reg on the EthoVision XT computer.
3. Run the file.
4. Click **OK** in the message that appears.

**TIP** Files with extension *.reg are blocked by Windows and mail servers. Do not run REG files stored on network drives. If you need to send a REG file by email, enclose it in a ZIP file.

# Buttons and Keyboard Shortcuts

Keyboard shortcuts are only available when the corresponding menu or window is active.

- Windows – General
- Experiment Explorer
- EthoVision XT – General
- Select and Edit
- Trial List and other grids
- Row-select grids
- Playback Control, Acquisition and Visualization
- Arena Settings
- Manual Scoring Settings
- Trial Control
- Detection Settings
- Track Editor
- Analysis Profiles
- Statistics and Charts

## Windows – General

Some of these shortcut keys can also be used in the Experiment Explorer, the Show/Hide menu, the Arena Settings window, the Trial Control window, and the Detection Settings window.

| | |
|---|---|
| **Esc** | Cancel action |
| **F1** | Help |
| **F2** | Rename/ edit |
| **F3** | Find next |
| **Shift**+**F3** | Find previous |
| **F4** | Expand pull down list |
| **Ctrl**+**F4** | Close active window (does not apply to EthoVision XT) |
| **Alt**+**F4** | Close application |
| **Alt**+**Enter** | Open properties of selected item (e.g. Settings dialog of dependent variable) |
| **Shift**+**F10** or context menu key | Open context menu of selected item |
| **Alt**+**Spacebar** | Open shortcut menu of selected window |
| **Ctrl**+**Esc** | Display Start menu |
| **Alt**+underlined letter | Application: open corresponding main menu |
| | Dialog: carry out corresponding command |
| **F10** | Activate main menu bar |
| Main-menu: **down** arrow key | Open menu-item/ cycle thru sub-items |
| Main-menu: **up** arrow key | Open menu-item/ cycle thru sub-items |
| Main-menu: **right** arrow key | Open sub-items/ Cycle thru items |
| Main-menu: **left** arrow key | Close sub-items/ Cycle thru items |
| **Spacebar** | Select/ clear checkbox if active option is a checkbox |
| Arrow keys | Move/ nudge cursor (highlighted item in grid) or selected item |
| **Alt**+**Tab** | Switch between open applications |
| **Alt**+**Esc** | Cycle through applications in order they were opened |

## Experiment Explorer

| | |
|---|---|
| **End** | Display the bottom of the active window |
| **Home** | Display the top of the active window |
| ***** on numeric keypad | Display all subfolders under the selected folder |
| **+** on numeric keypad | Display the contents of the selected folder |
| **-** on numeric keypad | Collapse the selected folder |
| **Left** arrow key | Collapse current selection if it's expanded, or select parent folder |
| **Right** arrow key | Display current selection if it's collapsed, or select first subfolder |
| **Up** arrow key | Previous item |
| **Down** arrow key | Next item |

## EthoVision XT – General

### *Shortcuts from the menu*

Use the keyboard to activate all the functions in EthoVision XT that are on the menus. Press **Alt** plus the letter underlined in the menu and then select the desired function by scrolling down to the function or by pressing the underlined letter. For example, to go to Preferences on the **File** menu, press **Alt**+**F**, **f**.

### *Main shortcuts*

| | |
|---|---|
| **Ctrl**+**N** | New default experiment |
| **Ctrl**+**O** | Open (experiment or other file type) |
| **Ctrl**+**S** | Save experiment |
| **Ctrl**+**Shift**+**S** | Save experiment as... |
| **Ctrl**+**T** | New template experiment |

## Select and Edit

| | |
|---|---|
| **Ctrl**+**X** | Cut |
| **Ctrl**+**C** | Copy |
| **Ctrl**+**V** | Paste |
| **Ctrl**+**Delete** or **Delete** | Delete |
| **Ctrl**+drag item | Copy selected item |
| **Shift**+drag item | Move selected item |
| **Shift**+arrow keys | Expand/ contract block selection in direction of arrow key |

## Trial List and other grids

| | |
|---|---|
| **Ctrl**+**left** arrow key | Go to first cell of row |
| **Ctrl**+**right** arrow key | Go to last cell of row |
| **Ctrl**+**up** arrow key | Go to first cell of column |
| **Ctrl**+**down** arrow key | Go to last cell of column |
| Arrow keys | Move highlight in direction of key |
| **F2** | Rename/ edit cell content (see Windows - general) |
| **Tab** | Go to next cell in row. If last cell, do nothing |
| **Shift+Tab** | Go to previous cell in row. If first cell, do nothing |
| **Enter** | (Accept entry and) Go to same column, next row. If last row, go to first cell last row |
| **Home** | Go to the first character within the cell (edit mode, only for user-defined variables) |
| **End** | Go to the last character within the cell (edit mode, only for user-defined variables) |
| **Page Up** | Go to row - no. of rows visible in window and highlight same location (and de-select) |
| **Page Down** | Go to row + no. of rows visible in window and highlight same location (and de-select) |
| **Ctrl**+**Home** | Go to first cell of first column |
| **Ctrl**+**End** | Go to last cell of last column |
| **Ctrl+Alt+T** | Add trials to the Trial List |
| **Ctrl+Alt+V** | Add an independent variable to the Trial List |

| | |
|---|---|
| **Ctrl+Alt+L** | Add videos to the Trial List |
| **Ctrl+Alt+I** | Import external data into the Trial List |
| **Ctrl+Alt+E** | Import trials into the Trial List |

## Row-select grids

An example of a row-select grid is the Components pane in the Data Profile screen and Analysis Profile screen. There you can select rows, not individual cells.

| | |
|---|---|
| **Up** arrow key | Step one row up |
| **Down** arrow key | Step one row down |
| **Enter** | Open the settings window of the selected row in the Analysis profile |

## Playback Control, Acquisition and Visualization

| | | |
|---|---|---|
| **Ctrl**+**up** arrow key |  | Jump to begin |
| **Ctrl**+**down** arrow key |  | Jump to end |
| **Ctrl**+**left** arrow key |  | Step 1 sample (or frame) backward |
| **Ctrl**+**right** arrow key |  | Step 1 sample (or frame) forward |
| (no shortcut) |  | Loops the video (only for Integrated Visualization) |
| **Ctrl**+**9** |  | Play forward at the speed selected. When playing back the tracks, one step forward corresponds to the time interval for the sample rate used for tracking. For example, 0.04 seconds if the sample rate is 25/s. |
| **Ctrl**+**Shift**+**4** |  | Play backward. When playing back the tracks, one step backward corresponds to the time interval for the sample rate used for tracking. |
| **Ctrl**+**0** |  | Pause / stop |

| | | |
|---|---|---|
| **Ctrl**+**.** |  | Zoom x2 (Track Visualization, Heatmaps and variable plots in Integrated Visualization) |
| **Ctrl**+**,** |  | Zoom x1/2 (Track Visualization, Heatmaps and variable plots in Integrated Visualization) |
| **Ctrl**+**/** |  | Fit all (Track Visualization only) |
| **Ctrl**+**'** | | Play forward at speed 0.01x (Visualization only)<br>Note: This is the key left to the key **1** on the international keyboard |
| **Ctrl**+**1** | | Play forward at speed 0.04x (Visualization only) |
| **Ctrl**+**2** | | Play forward at speed 0.2x (Visualization only) |
| **Ctrl**+**3** | | Play forward at speed 0.5x (Visualization only) |
| **Ctrl**+**4** | | Play forward at speed 1x (Visualization only) |
| **Ctrl**+**5** | | Play forward at speed 2x (Visualization only) |
| **Ctrl**+**6** | | Play forward at speed 4x (Visualization only) |
| **Ctrl**+**7** | | Play forward at speed 8x (Visualization only) |
| **Ctrl**+**8** | | Play forward at speed 16x (Visualization only) |
| **Ctrl**+**Shift**+**'** | | Play backward at speed 0.01x (Visualization only)<br>Note: This is the key left to the key **1** on the international keyboard |
| **Ctrl**+**Shift**+**1** | | Play backward at speed 0.04x (Visualization only) |
| **Ctrl**+**Shift**+**2** | | Play backward at speed 0.2x (Visualization only) |
| **Ctrl**+**Shift**+**3** | | Play backward at speed 0.5x (Visualization only) |
| **Ctrl**+**Shift**+**4** | | Play backward at speed 1x (Visualization only) |
| **Ctrl**+**Shift**+**5** | | Play backward at speed 2x (Visualization only) |
| **Ctrl**+**Shift**+**6** | | Play backward at speed 4x (Visualization only) |
| **Ctrl**+**Shift**+**7** | | Play backward at speed 8x (Visualization only) |
| **Ctrl**+**Shift**+**8** | | Play backward at speed 16x (Visualization only) |
| **Ctrl**+**F3** |  | New trial (Acquisition only) |

| | | |
|---|---|---|
| **Ctrl**+**F5** |  | Start trial (Acquisition only) |
| **Ctrl**+**F6** |  | Stop trial (Acquisition only) |
| **Ctrl** + **+** |  | Playback speed one step up. The current speed is shown next to this button.<br>Note: It does not work with the key **+** on the numerical keyboard. |
| **Ctrl** + **-** |  | Playback speed one step down<br>Note: It does not work with the key **-** on the numerical keyboard. |
| (no shortcut) |  | Reset the background (grabs the current video frame and uses it as background) |
| (no shortcut) |  | Skip trial |
| (no shortcut) |   | Optimize tracking speed<br>See DDS (Detection determines speed) |
| (no shortcut) |  | Starts screen recording (Integrated Visualization only) |

## Arena Settings

See also Windows – General and Normal Grids shortcut keys.

| | | |
|---|---|---|
| **Del** | | Delete selected element |
| **Ctrl**+**J** | | Validate the Arena Settings |
| **V** |  | Normal (arrow) mode |
| **R** |  | Rotation mode |
| **P** |  | Point edit mode |
| **L** |  | Draw lines |
| **S** |  | Draw rectangle |

| | | |
|---|---|---|
| **O** |  | Draw open polyline |
| **Y** |  | Draw closed polyline |
| **E** |  | Ellipse |
| **T** |  | 3-Point circle |
| **I** |  | Subdivided circle |
| **U** |  | Subdivided rectangle |
| **Z** | | Add zone label |
| **A** | | Auto label zone |
| **C** | | Add point |
| **Up** arrow key | | Nudge selected element smallest resolution unit up |
| **Down** arrow key | | Nudge selected element smallest resolution unit down |
| **Left** arrow key | | Nudge selected element smallest resolution unit left |
| **Right** arrow key | | Nudge selected element smallest resolution unit right |
| **Shift**+arrow key | | Nudge 10x |
| **Ctrl**+**.** |  | Zoom in the background image and arena elements |
| **Ctrl**+**,** |  | Zoom out the background image and arena elements |

## Manual Scoring Settings

| | |
|---|---|
| **Ctrl+B** | Add a behavior category |
| **Ctrl+V** | Validate the current coding scheme |

## Trial Control

See Windows – General and Row-select Grids shortcut keys.

## Detection Settings

See Windows – General and Row-select Grids shortcut keys. The following shortcut keys for playing back the video file work in the Detection Settings window.

| | |
|---|---|
| **Ctrl**+**up** arrow key | Jump to begin |
| **Ctrl**+**down** arrow key | Jump to end |
| **Ctrl**+**left** arrow key | Step -1 sample |
| **Ctrl**+**right** arrow key | Step +1 sample |
| **Ctrl**+**9** | Play forward |
| **Ctrl**+**0** | Pause / stop |

## Track Editor

| | |
|---|---|
| **Ctrl**+**E** | Open the Auto Select window |
| **W** | Swap nose and tail in selection |
| **Ctrl**+**F** | Open the Fix Nose-Tail swaps window |
| **D** | Swap subjects in selection |
| **Ctrl**+**Del** | Set selection to missing |
| **Ctrl**+**I** | Interpolate selection |
| **Ctrl**+**Shift**+**I** | Interpolate only center-points |
| **Ctrl**+**M** | Set tail-base points to missing |
| **Ctrl**+**Shift**+**M** | Set nose points to missing |

The shortcut keys for playing back the video files, listed in Playback Control, Acquisition and Visualization also work in the Track Editor screen.

## Analysis Profiles

- For the Components pane: see Row-select grids
- For the Dependent Variables list: see Trial List and other grids

## Statistics and Charts

| | |
|---|---|
| **C** | Refresh the statistics results table |
| **B** | Start batch analysis |
| **E** | Export the statistics results table |