# Quick Start Guide

# EthoVision® XT
## Version 18.0

**Noldus**
Information Technology

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# Install EthoVision XT

*To install EthoVision XT*

1. Browse to https://my.noldus.com.

2. Log in or register using the registration code on your welcome letter.

3. Click **Downloads**, then **EthoVision XT**. Under **Versions**, download the **EthoVision XT 18 - Installation Package [version number]** zip file.

4. Unzip the file and save the content to your PC.

5. Double-click the file **EthoVision XT - Setup - [version number].exe**.

6. Under **Set Application Language**, select either **English (United States)** or **Chinese (Simplified)**. Under **Installation type**, select **Standard.**

*Notes*

- You can also change the language at any time in EthoVision XT. Choose **File** > **Preferences** > **Language**, then close and restart EthoVision XT.

- If you want to use video cameras with EthoVision XT, first install the appropriate camera interface boards and drivers on the EthoVision XT computer. For details, see **Camera Installation** in the EthoVision XT Help. To open the Help, press **F1** in the software.

- If you save the installation files on a network drive before installation, copy them to your PC before running the Setup file. Make sure that all files included in the installation zip file are copied to your PC!

# Activate your license

## WITH A USB HARDWARE KEY

If you have a hardware key, insert it into a USB port of the computer and start EthoVision XT.



If you upgrade your EthoVision XT license, start EthoVision XT and enter the Upgrade key in the window that opens.

## WITH A SOFTWARE LICENSE KEY

If you received a software license key, start EthoVision XT and activate your license. Choose between a Floating license or a Computer-locked license.

- **Floating** — The license can be used in different computers, though not simultaneously. Choose this solution if you want to be flexible on which computer you use EthoVision XT to acquire data, and your computers are connected to the internet.

- **Computer-locked** — The license is linked to one specific computer. To activate your license, that computer must have an internet connection, or you must have a smartphone with an internet connection.

If you want to use EthoVision XT on a second PC to analyze existing data, not acquire new data, you can run it on that PC in Analysis mode, which does not require a license.

# Your first EthoVision XT experiment

## CREATE AN EXPERIMENT

EthoVision XT creates an experiment based on the choices you make in the following guided procedure. For example, the type and color of the animals, the test enclosure, etc.

1.  In the EthoVision XT Startup window, under **New experiment**, click **New from template** or choose **File > New from Template**.

2.  Select **Apply a pre-defined template** and follow the instructions in the setup guide. Choose whether you track from video files or live, select the study species, the type of arena, etc.
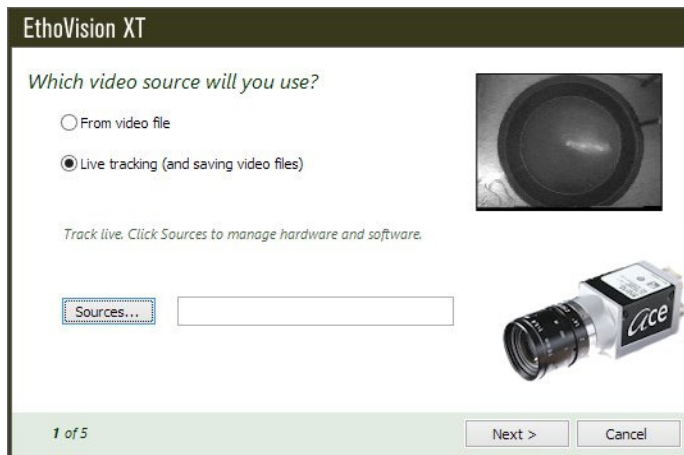


3.  In the New Experiment window, enter a **Name** for your experiment.

4.  Browse to the **Location** in which you want to store your experiment. Then click **OK**.

5.  You are now ready to complete setting up your experiment. You must complete the arena set-up and adjust the Detection Settings. You can optionally make a Trial List, Trial Control Settings, and Manual Scoring Settings.

*Notes*

- If your test setup is not among those included in the template list, choose **File > New**. This way you create an experiment without predefined settings.

- If you work with the DanioVision Observation Chamber, see the DanioVision DVOC-0041 - Reference Manual.

## WORKING WITH ETHOVISION XT EXPERIMENTS

An EthoVision XT experiment is the container of all information related to an experimental setup. The **Experiment Explorer** (Figure 1, left) lists all components of the experiment currently open.
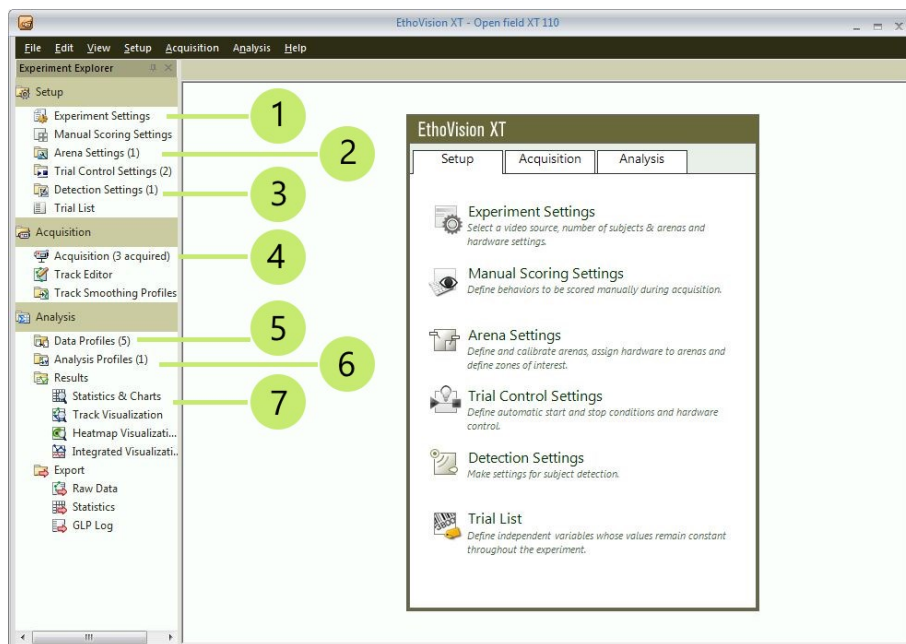


**Figure 1** *Important components in your experiment:* **1.** *Experiment Settings (basic properties that do not change throughout the experiment).* **2.** *Arena Settings to draw the arena and zones.* **3.** *Detection Settings to detect the subject in your camera image.* **4.** *Acquisition to record data and video.* **5.** *Data Profiles to select which part of your data to analyze.* **6.** *Analysis profiles to specify output variables (distance, speed etc.).* **7.** *Results options.*

By default, your experiment is located in:

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

All experiment files are stored in a folder with the same name as the experiment.

**NOTE** *Settings* and *Profiles* are collections of settings that belong to a specific function, for example the arena. You can define multiple settings and later choose which to use for a specific trial or group of trials. For example, in a water maze experiment, for a first set of trials, use Arena Settings 1 with the platform defined in a specific quadrant. For another set of trials, use Arena Settings 2 where the platform is defined in different quadrant.

# The experiment's basic properties

Choose **Setup** > **Experiment Settings**.

Specify the options listed below. Depending on your EthoVision XT license, some of the options may not be available. If you created a new template experiment, you already specified most properties of your experiment.

### Video Source

Choose whether you want to track **From video files** or from the live camera image (**Live tracking**). In the latter case, select the cameras that you are going to use. If your cameras are not listed, install the camera drivers. See **Camera Installation** in the EthoVision XT Help.

### Number of Arenas

An *arena* is a closed space where subjects move. Select the total number of arenas in your camera images. For example, select **1** for one water maze or open field; select **4** for four open fields, or four PhenoTyper cages.

### Number of Subjects per Arena

Specify how many individuals per arena you want to track. Note: Because this guide applies to the Base version of EthoVision XT, it assumes that you track one subject per arena.

### Tracked Features

Select which body points you want EthoVision XT to detect: the center-point only or the center-point with nose-point and tail-base.

### Body Point Detection Technique

If you track the nose-point and the tail-base point, select which technique should be used: the contour of the subject or a trained neural network (Deep learning). The latter option only applies to rodents, and with one or two subjects per arena.

### Analysis Options

With Activity analysis, you can have EthoVision XT detect behaviors like freezing in rodents and immobility in the Porsolt swim test. With Behavior recognition, EthoVision XT detects several rodent behaviors like grooming, sniffing and rearing.

### Units

Select the measurement units you prefer.

# Draw the arena and the zones

Tell EthoVision XT in what region in the video image the subject moves (the *arena*).

If you created a template experiment, the arena has already been drawn in the correct shape. Change the size and/or position of the arena to match it with your video image.



### *To obtain a background image*

1. If you use video cameras for live tracking, before starting EthoVision XT power up and connect the video camera to the PC.

2. In EthoVision XT, choose **Setup** > **Arena Settings**. Open the default **Arena Settings 1** or create a new one.

   If you do tracking from video files, click **Browse** and open the video file you want to use to draw the arena.

3. Click **Grab**. If possible, do so when the arena contains no animals.

### *To draw an arena*

1. Click **1. Draw Scale to Calibrate** in the **Arena Settings** window.

2. Draw a line between two points of the arena that are at a known distance from each other. In the window that appears, type in the real world distance between the two points and click **OK**. Optionally repeat this procedure a few times.
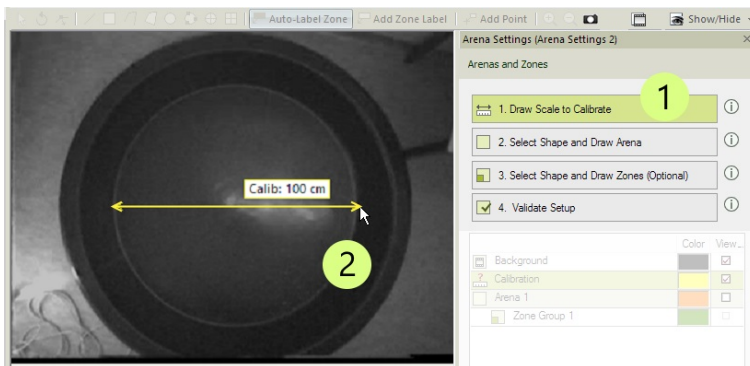
3. Click **2. Select Shape and Draw Arena** in the **Arena Settings** window.

4. Click one of the drawing tools and draw the outline of the region of the video image where the animal moves. Make sure that the label **Arena** points to inside this region.



### To draw a zone

A zone is a region within the arena that you may want to use for analysis or for starting/ stopping data acquisition. If you created a template experiment, zones are already defined. Change their size and/or position to fit the arena, delete zones or draw new ones.

1. Click **3. Select Shape and Draw Zones** in the **Arena Settings** window.

2. Select one of the drawing tools.

3. Draw the contour of each zone. In this example, the platform in a water maze.

4. Give the zone a name.



### Notes

- For more details, see **Arena Settings** in the EthoVision XT Help.

- For a DanioVision experiment, see the DanioVision DVOC-0041 - Reference Manual.

# Control the start and stop of data acquisition

Specify the rules that control the start and stop of data acquisition.

*Default settings*

Choose **Setup** > **Trial Control Settings**. Open the default **Trial Control Settings 1**.

- Each experiment has default Trial Control settings to start tracking when the subject is detected in the arena for one second and to stop tracking manually.

- If you created a template experiment, the Trial Control settings have been adjusted for that template. For instance, for the Morris water maze experiment, tracking stops either when the animal's center-point has been in the platform zone for more than five seconds, or when the animal has been swimming around for two minutes without finding the platform.

*To program the start of tracking*

1. In the second box, click **Settings**.

2. If you want to start tracking when the animal is in a zone, click **Settings**, deselect **Arena** and select that zone.

3. If necessary, change the criterion to be used (for instance, Frequency instead of Duration).
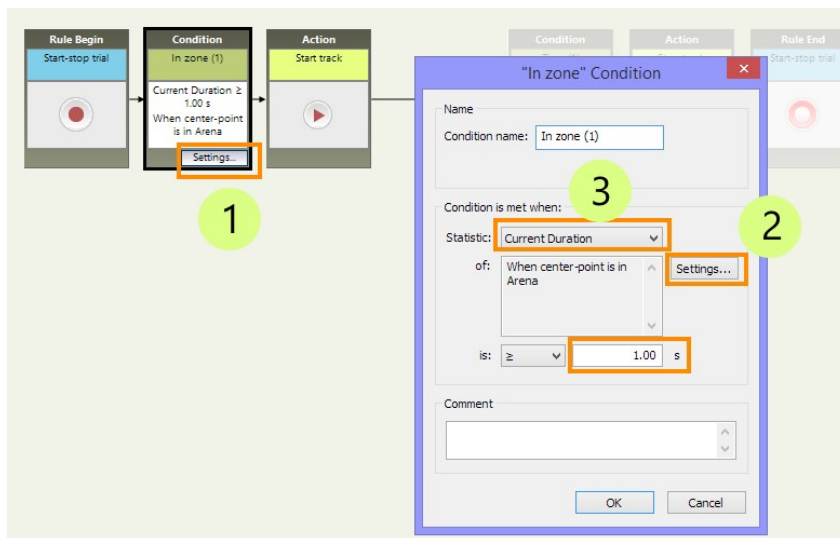
### To program the stop of tracking

1. In the box immediately before **Stop track**, click **Settings**.

2. If you want to stop tracking after a specific time, choose **After**, and enter the time needed.
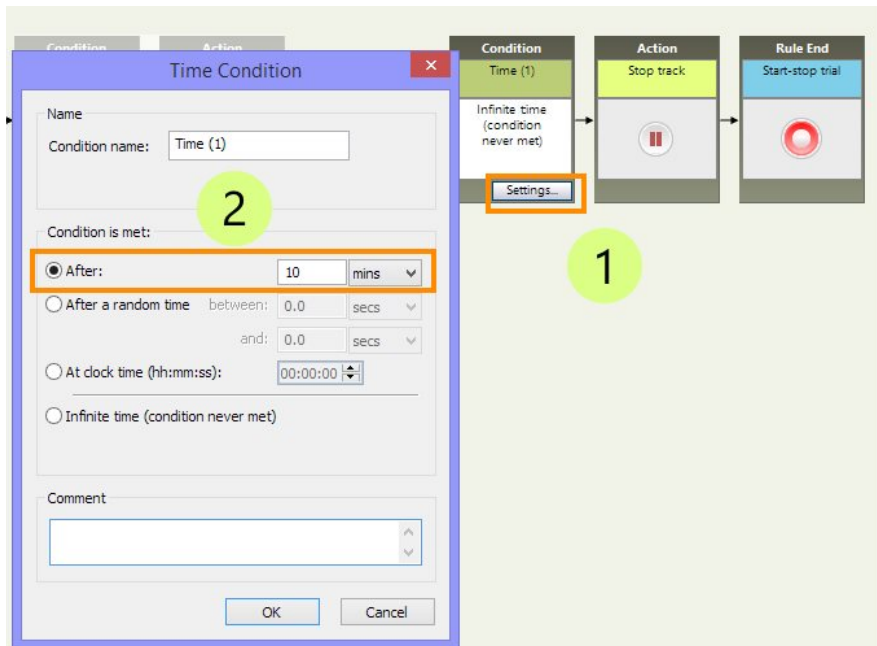


### Notes

- The first box, **Start Trial** marks the moment when you click the **Start Trial** button. The **Start track** box marks the time when EthoVision XT starts tracking.

- For more complex start-stop rules, like "stop when the subject is on the platform", replace the **Time** condition with an **In zone** condition, and specify the platform zone. For more examples, see **Trial Control** in the EthoVision XT Help.

- Make sure that the **Trial Control Settings** you want to use is selected in the **Acquisition settings** window before you start your data acquisition.

- With the Trial and Hardware Control module, you can control hardware (lights, a pellet dispenser, etc.). For instance, turn on a light when the animal enters its shelter. It also allows you to create routines, for example in conditioning experiments.

# Detect the subject

1. Choose **Setup** > **Detection Settings**. Open the default **Detection Settings 1**.

2. If you work with video files, under **Video** click **Select Video** and choose a video file. Play the video up to when the subject appears. If you work with cameras, release the animal in the arena.



3. Click **Automated Setup**. Choose the type of subject you test, and click **Next**.

4. Wait until the subject is free from contact with objects or walls and walks with a normal posture. When ready, draw a rectangle around the subject. Do this for all subjects in the arena.
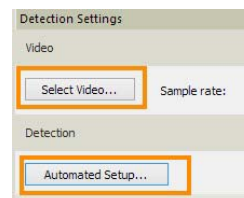
   **TIP** For rodents, try to leave the tail out of the rectangle. In all other cases, include the animal's tail.
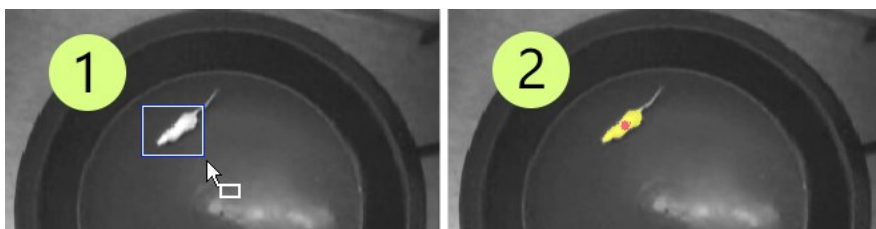


5. If the subject's body is well detected, click **Yes**. Otherwise, try moving the **Finetune** slider and check if the yellow blob covers the entire animal's body (excluding the tail).

If detection does not improve, try again a few times using another image from the video. If this does not help, click **No** and click **Advanced**. For details, see **Configure Detection Settings** > **Advanced Setup** in the EthoVision XT Help.

### Tracking the nose and tail-base

- In all cases, make sure that the body of the animal is well detected.

- If you use the Deep learning detection technique with one subject per arena, under **Method** click **Define** and select a box around the subject. Make sure that there is some space around the subject's body, like in this example.
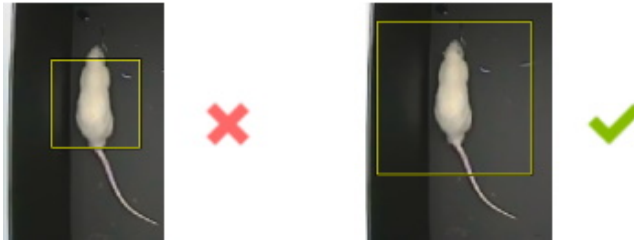


*Notes*

- Check that the subject is well detected in all parts of the arena. In the **Detection Performance** pane**,** check that the proportion of samples where the subject is not found is acceptable (for example less than 1%).
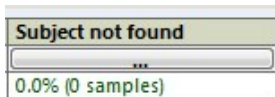


- If you selected **Activity analysis** in the **Experiment Settings** to detect freezing, click **Activity**. Adjust the settings until most of the purple pixels only appear when the animal moves. When the animal freezes, the number of purple pixels should be minimized.

# Prepare a list of trials (optional)

## THE TRIAL LIST

In EthoVision XT, a *Trial* is an uninterrupted recording session. The *Trial List* is a table listing the trials planned for an experiment. When you create a new experiment, a few planned trials are already present.

### To add more trials

1. Choose **Setup** > **Trial List**. Click the **Add Trials** button.

2. Type the number of trials you plan to carry out and click **OK**.

3. For each row:
   - Specify the independent variables (optional; see the next page).
   - Specify which video file you want to use (when tracking from video files).
   - Select **Arena Settings**, **Trial Control Settings**, and **Detection Settings** (optional). If those columns are not present in the Trial list, click the **Show/Hide** button, click **Variables** and select them.



*Figure 2* Planning ten trials in the Trial List.

### Notes

- Planning your trials in advance also allows you to acquire trials in batch mode. See **Trial List** in the EthoVision XT Help.

## INDEPENDENT VARIABLES

*What are Independent variables?*

Independent variables are either simple descriptive categories (such as the genotype or age of your rats or mice), or conditions manipulated by the researcher (such as the substance being tested and the dosage). You can use independent variables to create analysis groups or filter data. For example:

- Compare tracks of treated subjects with tracks of control subjects.

- Only analyze subjects of a specific age class.

**NOTE** If you created a template experiment, one or more independent variables have been predefined in your Trial List. For instance, *Animal ID* and *Treatment*.

*To define an independent variable*

1. Choose **Setup** > **Trial List**.

2. Click the **Add Variable** button. A new column appears on the right of the table.

3. Specify the **Label** (for example: Dose), **Description, Type** (for example, Text or Numerical), **Format, Predefined values** (for example, 0.01, 0.02 etc. mg/kg), and **Scope** (whether the values of the variable differ between subjects within the same arena, between arenas within the same trial, or between trials).
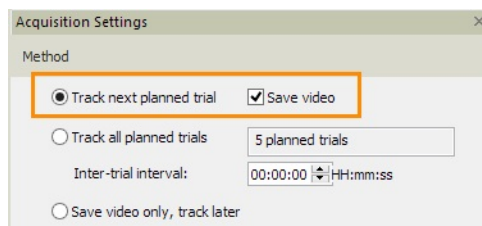
# Acquire the data

## METHODS

Choose **Acquisition** > **Open Acquisition**. Locate the Acquisition Settings window at the right side of the screen.

- If you track from pre-recorded video files, select **Track next planned trial**, click the button next to **Video** and open the video you want to use.
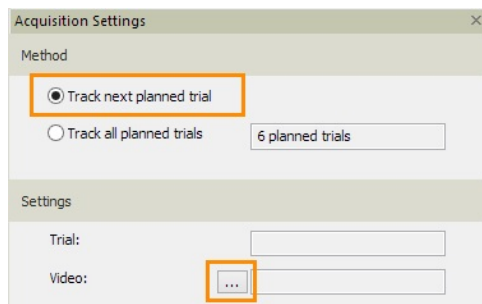


- If you track from the live camera image, select **Track next planned trial**. Select **Save video** if you want to save the camera footage to a video file.



For more acquisition methods, see **Acquire Data** in the EthoVision XT Help.

## PROCEDURE FOR ACQUIRING ONE TRIAL

1. Under **Settings**, choose the **Arena Settings**, **Trial Control Settings**, and **Detection Settings**, if they are not already selected in the Trial List.

2. If you track from video and do not score behaviors manually, make sure the **DDS** (Detection Determines Speed) checkbox is selected in the **Playback Control** window. With this option you ensure that every sample is analyzed.
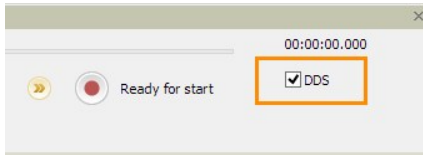
3. To start a trial, click the **Start trial** button in the **Playback Control** window.



4. Release the subject in the arena.

5. To stop the trial, click the **Stop trial** button.



6. Return the subject to its home cage or container.

7. Prepare the next subject, or load a new video file to acquire the next trial.

## MORE OPTIONS

- You can automatically start and stop your trial depending on the behavior or position of your subject. See page 10.

- You can also acquire data in batch mode. For details, see **Batch data acquisition** in the EthoVision XT Help.

- You can also score behaviors manually during tracking. If you saved the video during tracking, you can review the video and add or edit existing behavioral data. For details, browse the EthoVision XT Help:
  - To define behaviors, see **Set Up an Experiment** > **Manual Scoring Settings**.
  - To score behaviors, see **Acquire Data** > **Score behaviors manually**.

- If no planned trial is left, click the **Add Trial** button in the Playback Control window.



- Sometimes EthoVision XT tracks a reflection instead of the subject, it confuses the nose-point and the tail-base or swaps subjects when you track multiple animals simultaneously. Whenever that occurs, you can correct those errors. For more information, see **Edit the Tracks** in the EthoVision XT Help.

# Calculate the statistics

## PREPARE THE TRACKS

A *track* is the group of samples acquired for a subject. You can choose to *smooth* the tracks to get a more reliable measure of the animal's movement path and remove the effect of outliers (caused by accidental wrong detection), random noise and body wobble.

1. Choose **Acquisition** > **Track Smoothing Profile**. Select **Open** and click **OK**.

2. Select one or more of the options: **Smoothing (Lowess), Minimal Distance Moved** and **Maximum Distance Moved**.

For information on the smoothing options, see **Smooth the Tracks** in the EthoVision XT Help.

## ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile**. Select **New**, name the analysis profile and click **OK**.

*Distance moved* and *Velocity* are predefined as dependent variables. To add a variable to the Analysis profile, click the button next to that variable.

**NOTE** If you created the experiment from a template, it contains a few analysis profiles. For example, the Morris water maze template experiment with the platform zone template, contains four analysis profiles. One of them, **Latency to reach platform**, contains the variables for calculating the time taken to reach the platform.

## BASIC CALCULATIONS

### Total distance moved or average velocity

1. Open the Analysis profile (see also the picture on the next page).

2. If the variable is not yet listed under **Selected Dependent Variables**, click the button next to **Distance moved** or **Velocity**. In the **Trial Statistics** tab, select **Total** for Distance moved, or **Mean** for Velocity.

3. Choose **Analysis** > **Results** > **Statistics and Charts**.

### Time in a zone and number of zone entries

In step 2 above, click the button next to **In zone**. Specify the zone. In the **Trial Statistics** tab, select **Cumulative duration** and **Frequency**.
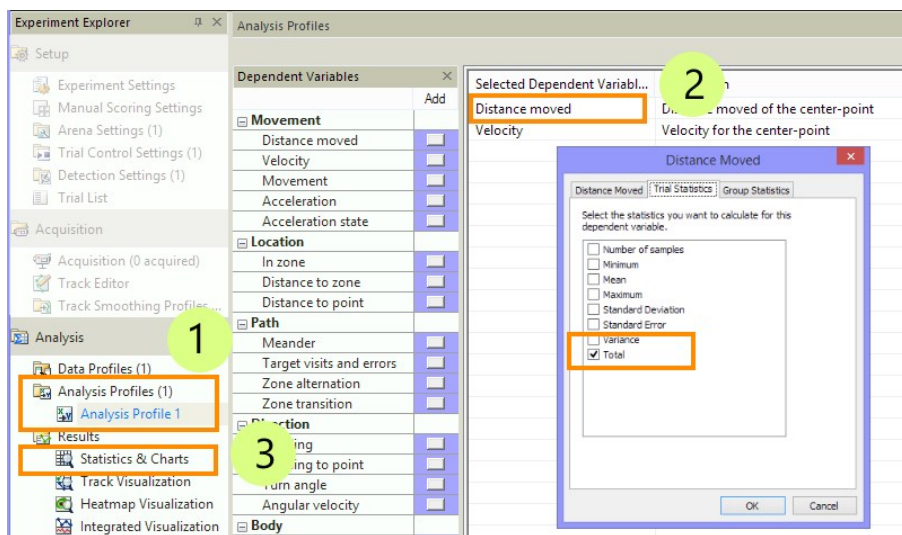
### Time before reaching a zone

In step 2 on the previous page, click the button next to **In zone**. Specify the zone you are interested in. In the **Trial Statistics** tab, select **Latency to first**.

### Distance or velocity in a zone

1.  Choose **Analysis** > **Data Profile** > **New** or open an existing Data profile. In the box **Results** click **Settings**, then select **Results per zone**. Next, choose the zones you want the results for.

2.  Open an Analysis profile. Choose the variable you want to calculate. For example, **Distance moved**. Next, choose **Analysis** > **Results** > **Statistics and Charts**.

### Notes

*   EthoVision XT offers a wide range of analysis variables and methods to select data. See **Other analysis options** (page 21).

*   In the results screen, the **Trial Statistics** tab shows the analysis results per trial, while the **Group Statistics & Charts** tab shows the results for all trials together, or for the groups of trials defined in your Data profile. To modify the layout of the results table, click the **Layout** button.

*   To carry out multiple calculations at once with different filters, data profiles or analysis profiles, click the **Batch** button.

# Visualize the data

## TRACK VISUALIZATION

Choose **Analysis** > **Results** > **Plot Tracks** to view the tracks that you selected. This allows you to visually compare tracks. You can play back your tracks to see how your subjects moved.

To display the entire track, under **Filter** deselect the **Last [ ] seconds** option, then click the **Jump to End** button in the **Playback Control** window.
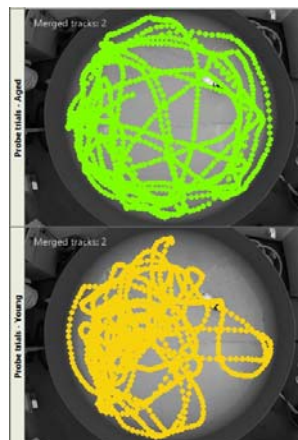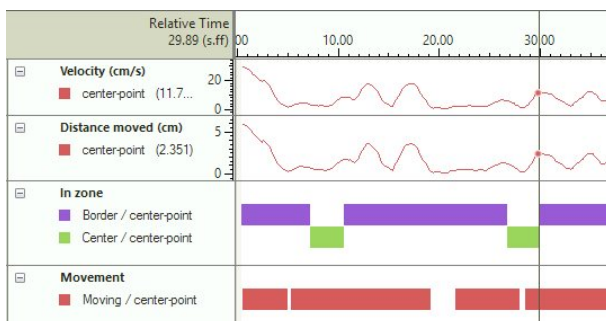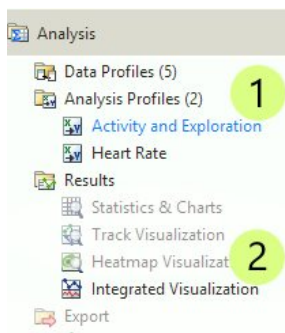


## INTEGRATED VISUALIZATION

Choose **Analysis** > **Results** > **Plot Integrated Data** to view the track with the corresponding video (when video was recorded) and the time plot of the dependent variables (velocity, etc.).
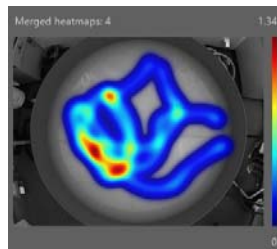
To plot a variable:

1. Open an Analysis profile and add the dependent variable you want to view.

2. Choose **Analysis** > **Results** > **Plot Integrated Data**.

## HEATMAPS



Choose **Analysis** > **Results** > **Plot Heatmaps** and click **Plot Heatmaps** on the toolbar. With heatmaps you get an immediate impression of which area the animal spent most time in.

# Other analysis options

## SELECT AND GROUP TRACKS

Choose **Analysis** > **Data Profile** > **New**.

### *To analyze some tracks, not others*

1. Choose one of the options under **Filter**. For example:
   - Choose **Trial Name** and select the trials you want to analyze.
   - Choose **Dose** (an independent variable previously defined; see page 15) and choose the values of the variable you want to use.

2. Insert the **Filter** box between **Start** and **Result 1**.
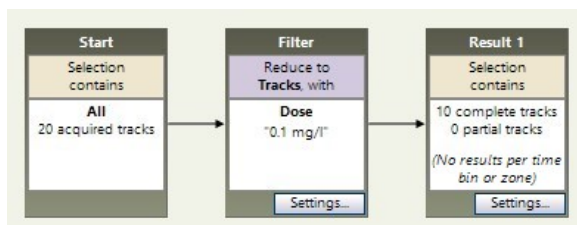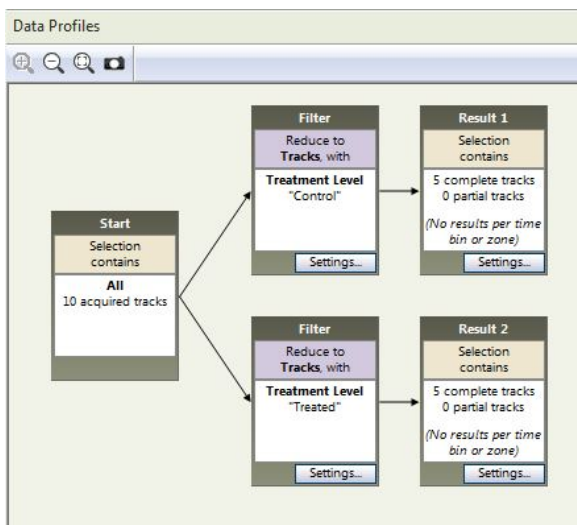


### *To analyze tracks in groups*

For example, you want to compare the results for subjects treated with a drug with those for control subjects.

1. In the Trial List, make sure that the independent variable that you want to use for grouping is defined, and that each trial is labeled with one of the possible values (e.g. *Treated* vs. *Control*).

2. Make a Filter for the first group based one of the values of the independent variable, and place the resulting box between the **Start** and the **Result 1** box (see above).

3. To create the second group, click the **Result** button under **Common elements**. Click and drag from the center of the **Start** box to the center of the new **Result** box. The two boxes are now connected with an arrow. Next, make a filter for the second group, and place the **Filter** box in the middle of the second branch.



4. Choose **Analysis** > **Results** > **Statistics and Charts**. Click the **Group Statistics and Charts** tab to view the results per group.

## ANALYZE TRACK SEGMENTS

For instance, visualize the data of the first five minutes of the tracks, or visualize all samples when the subject was moving or grooming.

Choose **Analysis** > **Data Profile** > **New**.

### Nesting

To select track segments, based on time, zones or states of the subject, click the appropriate button under **Nesting** and specify the options there. For example, to analyze the first five minutes, choose **Time**.

Insert the **Nest** box between **Start** and **Result 1**.
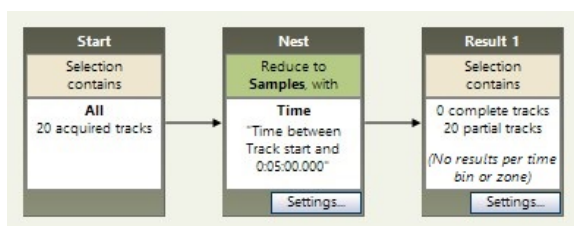
*Time bins*

To analyze the tracks split in regular time intervals, in the **Result** box click **Settings** and select **Results per time bin**. For example, define intervals of 1 minute each.



Then, choose **Analysis** > **Results** > **Statistics and Charts**.

For more information, see **Analyze Track Segments** in the EthoVision XT Help.

# Export data and results

### MAIN PROCEDURE

To find out if your independent variables (like treatment, dose, etc.) have a significant effect on the dependent variables, you can export your data to a statistical package.

- To export the averages, totals etc. per trial, or per group of trials, choose **Analysis** > **Export** > **Statistics**.

- To export the raw x,y coordinates and the surface area of the subject, or the per-sample values of the dependent variables, choose **Analysis** > **Export** > **Raw Data**.

For details, see **File Management** > **Export data** in the EthoVision XT Help.

### COPY ENTIRE EXPERIMENTS

To make a copy of an entire experiment, choose **File** > **Make Backup**. Choose the options you require and select **OK**.

The backup file (*.evz) contains all your settings and data, and optionally media files, exported files and physiological data. You can copy this file to another computer where EthoVision XT is installed. To open the experiment on that computer, choose **File** > **Restore Backup**.
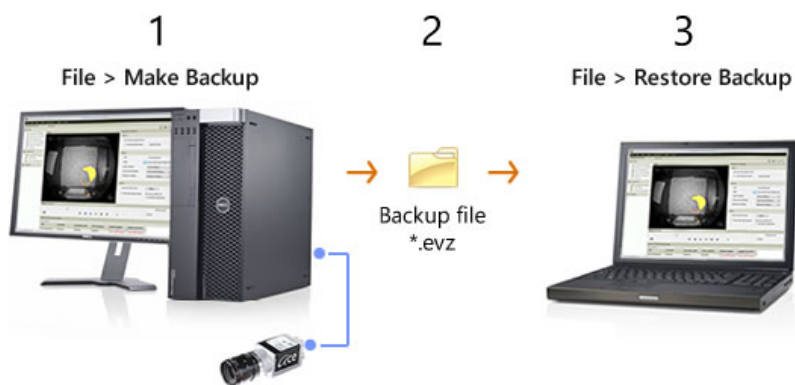


**IMPORTANT** Backup your data to a network drive or external hard disk at least once per day.

# For more information

*Help and other manuals*

For more details, in EthoVision XT, press **F1** to open the Help. See also other manuals stored on your computer. On the Windows **Start** menu, choose **All Apps** > **Noldus** > **EthoVision XT 18 Other Documentation**.

*Sample experiments*

Choose **File** > **Restore Backup** and browse to C:\Users\Public\Documents\Noldus\ EthoVision XT\Experiments\Sample Experiments. You find the sample experiment **Morris water maze test XT180.evz**.

For more sample experiments, browse to my.noldus.com. Log in or register using the code that you have received. Next, choose **Downloads** > **EthoVision XT** > **Sample Experiments**. Each experiment focuses on a specific apparatus, or on a feature of EthoVision XT, for example, trial control.

*Video tutorial*

Choose **Help** > **Video Tutorial**. Watch the tutorial to learn how to set up a video tracking study in EthoVision XT.

There are also mini video tutorials available in several parts of the program. Click the video button on the tool bar to view the tutorials.



*Remote training courses*

Noldus offers training courses to help you get the most out of our software/hardware. In addition to on-site courses at your lab and in-house courses at our headquarters in Wageningen (The Netherlands) and Leesburg (VA, USA), we offer remote training courses. Remote trainings are available in the same range of languages as on-site courses. Please contact your sales representative for more information about remote trainings.

*Technical support*

If you encounter problems, see my.noldus.com to search the support knowledgebase or contact the help desk.