

Technical Note

Merging heatmaps from different arenas

in EthoVision[®] XT 18

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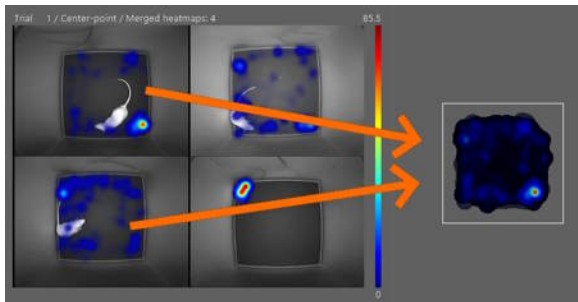
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Why this technical note

This technical note describes how you can merge heatmaps into one, when the data have been collected in different arenas/apparatuses.

USE CASE EXAMPLE

An EthoVision XT experiment has subjects being grouped based on the treatment level (or genetic background, etc.). Subjects are tested in four separate arenas. Because of the randomized design, each arena will be used for all treatment levels/groups. For example, Arena 1 will receive a subject of treatment level A in trial 1, then a subject of level B in trial 2 etc. As a result, one arena will not contain data of all trials with a specific group. The researcher would like to create a heatmap with all the trials that belong to that treatment level, and plot it on one arena layout, not four. EthoVision XT cannot do that with the current functionality.



IMPORTANT TERMS

Reference point

A point in the 2D space defined in the EthoVision XT Arena Settings, which serves as a reference point for re-calculation of the x,y

coordinates of the subjects. The reference point must be defined in all the areas.

JavaScript variable

A custom variable defined in the EthoVision XT Analysis profile based on JavaScript code. In this note, JavaScript variables are defined to convert the original x,y coordinates to a new set of coordinates relative to the point of reference.

Merging heatmaps with data from different arenas

WHAT YOU NEED

- EthoVision XT 16 or later. If you have EthoVision XT 15, let us know. You can still work with JavaScript by adding a registry key.
- Microsoft Excel.
- Power Map for Microsoft Excel, or software for creating heatmaps, for example R, MatLab, or OpenJump HORAE (featured in this note).

ASSUMPTIONS

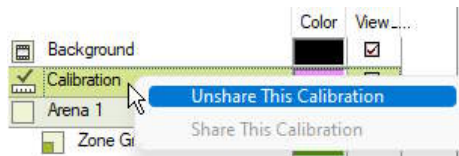
You have an experiment with two or more arenas oriented in the same direction.

Choose what applies:

- You still have to track the subjects. Go to Step 1a.
- You have already acquired the tracks. Go to Step 1b.

STEP 1A - DRAW THE AXIS ORIGIN FOR EACH ARENA

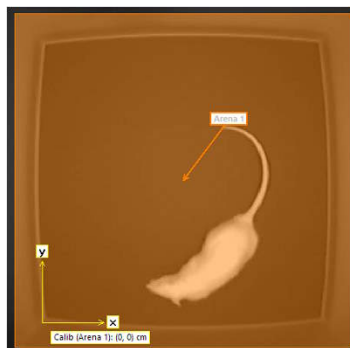
1. Open the Arena Settings that you want to use. Draw the arenas, the zones, and do the calibration as usual.
2. Choose a point in the arenas that you want to use. For example, a corner of the cages.
3. Right-click the **Calibration** layer and select **Unshare this Calibration**.



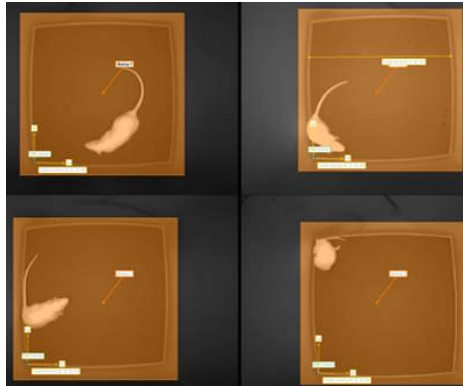
4. On the panel at the right hand side, click one of the Arena layers (e.g. **Arena 1**).
5. Click the **Calibration Axes** button below the layers list.



6. Leave the coordinates of the origin to 0,0 and click **OK**.
7. Move the Axes object in such a way that the origin of the x,y axes is over the reference point.

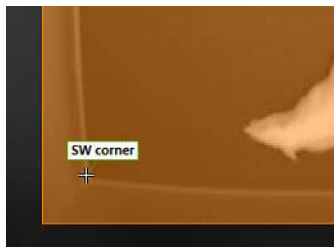


8. Repeat the steps above (4-7) for each arena.
9. At this point, each arena should have its own Axes object positioned over the reference point and oriented the same way. You can now acquire the data. Next, go to Step 2.
















STEP 1B – ADD A REFERENCE POINT TO EACH ARENA

1. Open the Arena Settings that you used to acquire the data.
2. Under the layer Arena 1, click the layer named Zone Group 1 (or any other zone layer).
3. From the tool bar choose Add point.
4. Click over a specific location and give the point a name.



- Repeat the steps above (2-4) for the remaining arenas. Choose to use the same point name (here: SW corner).
- Each arena should have its own reference point. Make sure that the points are listed under the corresponding arena layer!

	Color	View	Lock
Background		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Arena 1		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Calibration		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Zone Group 1		<input checked="" type="checkbox"/>	<input type="checkbox"/>
SW corner		<input checked="" type="checkbox"/>	
Arena 2		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Calibration		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Zone Group 1		<input checked="" type="checkbox"/>	<input type="checkbox"/>
SW corner		<input checked="" type="checkbox"/>	
Arena 3		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Calibration		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Zone Group 1		<input checked="" type="checkbox"/>	<input type="checkbox"/>
SW corner		<input checked="" type="checkbox"/>	
Arena 4		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Calibration		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Zone Group 1		<input checked="" type="checkbox"/>	<input type="checkbox"/>
SW corner		<input checked="" type="checkbox"/>	

- Create or open an Analysis profile.
- Under **Custom Variables** select **JavaScript Continuous**.

Custom Variables	
Free interval	<input type="checkbox"/>
Multi condition	<input type="checkbox"/>
JavaScript continuous	<input checked="" type="checkbox"/>
JavaScript event	<input type="checkbox"/>
JavaScript state	<input type="checkbox"/>

- In the window that opens, delete the text already present. Copy the text under **JavaScript code for x** (replace the “SW corner” with the name of your reference point) and paste it in the window. Next, click **OK**.
- Under **Custom Variables** select **JavaScript Continuous** again. In the window that opens, delete the text. Copy the text under **JavaScript code for y** (replace the “SW corner” with the name of your reference point) and paste it in the window. Next, click **OK**.

11. At this point you should have two JavaScript Continuous in your Data profile. Rename the variables to **X** and **Y**, respectively. To rename a variable, right-click its name select **Rename**).

Selected Dependent Variables	Description
X	JavaScript for continuous data
Y	JavaScript for continuous data

12. **OPTIONAL** Visualize the variables in Integrated Visualization to check they are correct. The values displayed should be the coordinates x and y of each subject relative to the reference point.

NOTE The new coordinates are in mm, no matter what you have selected as Measurement Units in EthoVision XT.

13. Go now to Step 2.

JavaScript code for x

```
// This code returns the x coordinate relative to a point
// Make sure you define this point in all the arenas
const g_zone = "SW corner";
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();
    }
}

```

JavaScript code for y

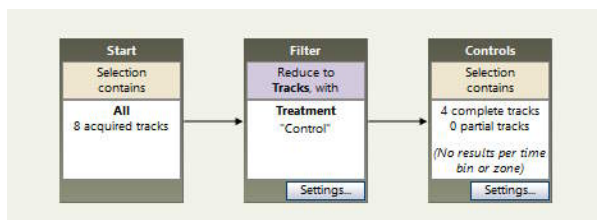
```

// This code returns the y coordinate relative to a point
// Make sure you define this point in all the arenas
const g_zone = "SW corner";
function Start()
{
}
function Stop()
{
}
function Process()
{
    var pt1 = GetCenter();
    var pt2 = GetPointPoi(g_zone);
    if (pt1)
    {
        var yc = pt1.y - pt2.y;
        SetOutput(yc);
    }
    else
    {
        SetOutputMissing();
    }
}

```

STEP 2 - EXPORT THE TRACKS THAT BELONG TO A SPECIFIC GROUP/TREATMENT LEVEL

1. In EthoVision XT, make a Data profile and use the Filter function to select the tracks of one group/treatment level (e.g., control subjects).



2. Choose **Analysis > Export > Raw Data**. Choose Excel as export format.
3. Repeat the steps 1 and 2 to create different datasets, one for each treatment level. For each treatment level, make a Data profile and export the data. You will create a heatmap for each dataset.

STEP 3 – PREPARE THE EXCEL FILES

If you are going to use Microsoft Excel 3D Maps, see the section on the next page.

Procedure for creating one file per subject

1. Each Excel file corresponds to one trial and contains one, two or more tabs depending on which arenas contain data for a specific group/treatment level. If you have two or more tabs, you must save them as separate Excel files (move a tab at the front and select Save As; repeat this for all the tabs).
2. Open each Excel file and delete the rows up to the headers. Also delete the row with the units, immediately above the data.

The file should look like this: row 1 with the headers, row 2- with the data.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Trial time	Recording	X center	Y center	X nose	Y nose	X tail	Y tail	Area	Area chan	Elongation	Direction	X	Y	Result 1
2	0	0	26.282	18.7065	33.0982	14.1811	22.8794	24.1105	66.9301	0	0.68955	-23.3772	24.9363	68.7092	1
3	0.04	0.04	26.282	18.7065	33.0982	14.1811	22.8794	24.1105	66.9301	0	0.68955	-23.3772	24.9363	68.7092	1
4	0.08	0.08	26.282	18.7065	33.0982	14.1811	22.8794	24.1105	66.9301	0	0.68955	-23.3772	24.9363	68.7092	1
5	0.12	0.12	26.282	18.7065	33.0982	14.1811	22.8794	24.1105	66.9301	0	0.68955	-23.3772	24.9363	68.7092	1
6	0.16	0.16	26.282	18.7065	33.0982	14.1811	22.8794	24.1105	66.9301	0	0.68955	-23.3772	24.9363	68.7092	1
7	0.2	0.2	26.282	18.7065	33.0982	14.1811	22.8794	24.1105	66.9301	0	0.68955	-23.3772	24.9363	68.7092	1
8	0.24	0.24	27.3823	18.4058	35.466	15.5393	23.893	23.9944	70.1846	8.55508	0.706811	-4.50905	35.94	65.7018	1
9	0.28	0.28	27.3823	18.4058	35.466	15.5393	23.893	23.9944	70.1846	8.55508	0.706811	-4.50905	35.94	65.7018	1

3. Save each file in the format that your software for generating heatmaps requires. If you use OpenJump HORAE or R, the format **CSV (MS-DOS)** should work fine

Procedure for creating one global file for 3D Maps

1. Open the Excel files and copy the X and Y coordinates of each of them to one global file. Append the rows instead of creating multiple columns. There should be no empty rows in between.

	X	Y
1		
2	3.25211984	-3.958211793
3	3.25211984	-3.958211793
4	3.25211984	-3.958211793
5	3.25211984	-3.958211793

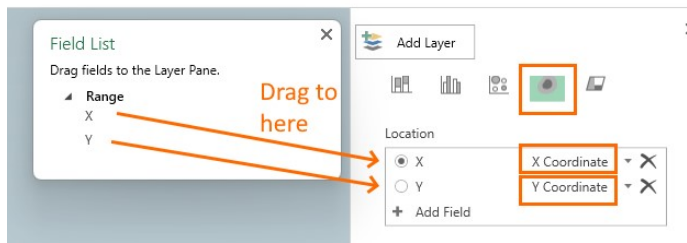
2. **OPTIONAL** Add an extra column which specifies the subject name. You can use this column to show the heatmap in different colors based on the subject identity.

X	Y	SubjectName
258.297	85.2355	1
258.297	85.2355	1
258.297	85.2355	1
258.297	85.2355	1
258.297	85.2355	1
258.297	85.2355	1
258.297	85.2355	1
258.297	85.2355	1
245.027	79.4237	2
245.027	79.4237	2
245.027	79.4237	2
245.027	79.4237	2
245.027	79.4237	2

3. Save the new file. This file should contain all the X,Y coordinates of subjects in one group, in just two columns.
4. Repeat the steps above for each group/treatment level.

STEP 4A - CREATE THE HEATMAP IN 3D MAPS

1. Open a “global” file for a specific group/treatment level.
2. Right-click the image of the Earth and select **Change Map Type**.
3. Select **New Custom Map**. Leave the selection as it is and tilt up the default background.
4. In the **Field List**, drag **X** and **Y** to the **Location** box and specify **X Coordinate** for **X** and **Y Coordinate** for **Y**.



5. On the **Layers** pane, choose **Change visualization to Heat Map**.
6. Click on **Layer Options** and adjust the settings when necessary, but keep **Visual Aggregation** set to **Sum**.

STEP 4B - CREATE THE HEATMAP IN OPENJUMP HORAE

OpenJump HoRAE is free software for analysis and visualization of spatial behavior. You can import a global file for all subjects or separate files, one per subject. Separate files can be visualized in separate layers and contribute to a global heatmap.

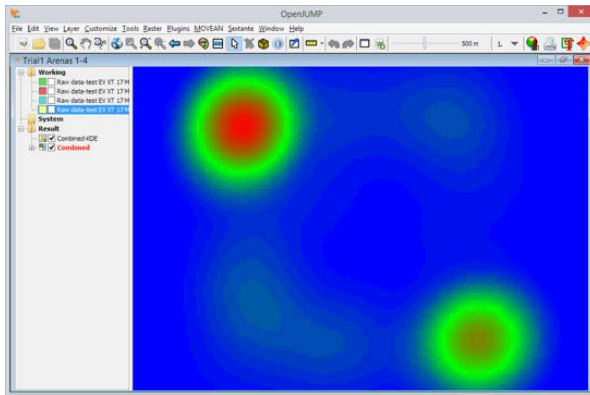
Install the software

1. Download from
https://www.researchgate.net/publication/349224514_OpenJUMP_HoRAE_Software
2. Extract the files from the zip file and copy them to your PC.
3. Run the file .../bin/OpenJump.exe.

Import the CSV files into OpenJump HoRAE

1. Choose **File > Open Files**.
2. Select the CSV files (**Ctrl + click** each file) and click **Next > Finish**. The tracks are imported as layers.
3. Save the Project with a name (.jmp).
4. Select all layers, right-click **Combine Selected Layers**.
5. Save each new layer! Right-click and select **Save Dataset as**. Save layers in the same folder as the project.
6. To make the heatmap, select the **Combined** layer and from the **MOVEAN** menu choose **HRE > Point KDE > Point Kernel Density**.
7. The most important setting is the **Distance h** (smoothing factor) which is similar to the Smoothing value in EthoVision XT. You can have the software calculate it for you or you can enter a value in the same units as the coordinates. The value **Cell Size** is the size of each pixel in the heatmap: the greater the value, the grainier the image.
8. To change colors, select the layer **Combined-KDE** and from the **Raster** menu choose **Raster Color Editor**.

9. To hide the original data points, de-select all the layers except **Combined-KDE**.



Extras

To create probability contours:

1. Select the layer **Combined-KDE** and from the **MOVEAN** menu choose **HR Analysis > Create Probability Contours from Raster**.
2. Enter the probability value (e.g. 50%) and click **OK**.
3. De-select the other layers to view the contours.

