Description of sample experiments

EthoVision® XT

version 19

Noldus

Information Technology

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EthoVision XT sample experiments

sample experiments available

All EthoVision XT sample experiments are available on my.noldus.com.

- Morris water maze XT190

  This experiment is installed by default when you install EthoVision XT. You can find it in C:\Users\Public\Documents\Noldus\EthoVision XT\Experiments\Sample Experiments.

  If you do not find this file, do one of the following:
  - If you have the installation files of EthoVision XT, run the Setup file (*.exe), select **Modify** and choose to install the sample experiment.
  - Go to my.noldus.com and download the sample experiment **Morris water maze test XT190.evz** and its documentation **EthoVision XT 19 - Description of sample experiment - Morris water maze.pdf**.

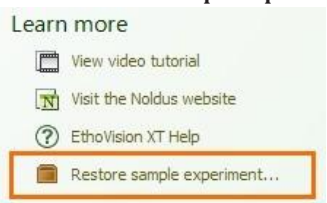
Note Not all experiments may be available at the time you have downloaded this document. You can, however, download and use older versions (e.g. Open Field test XT175). Check our web site regularly for more sample experiments.

For more information

The EthoVision XT 19 - Application Manual.pdf contains useful information on many of the tests listed above. You can find this manual in **All Apps** > **Noldus** > **EthoVision XT 19 Other Documentation**. You can also find this manual on the my.noldus web portal.

HOW TO USE THE SAMPLE EXPERIMENTS

1. To download the sample experiments, browse to my.noldus.com, log in (or create an account first), open the **Downloads** page and under **EthoVision XT** choose **Sample experiments.** Always copy the backup files (*.evz) to your computer first.
2. In EthoVision XT, choose **File** > **Restore Backup**, or in the EthoVision Startup window, choose **Restore sample experiment**.



3. Browse to the backup file that you want to restore and click **Open**.
4. In the next step you must specify where to save the experiment. The default experiment location is:
   - If you purchased a PC with EthoVision XT:
     D:\Noldus\EthoVision XT\Experiments.
   - In other cases:
     C:\Users\Public\Documents\Noldus\EthoVision XT\Experiments.

   Click **OK**. The experiment is restored and opens automatically on your screen.

Notes

- The video files are stored in the **Media Files** subfolder of the experiment's folder.
- Some sample experiments are based on Deep learning. This technique can only work with a recent graphics card (GPU) and with up-to-date drivers installed on your PC. If your GPU is not compatible, you can still explore the data already acquired.

Open Field test XT190

OVERVIEW

The Open field test is an experimental test used to assay general locomotor activity levels, anxiety, and willingness to explore in rodents. In this experiment, EthoVision XT is set to track the nose, center and tail-base points of mice of different colors: in Trial 1, a dark (C57BL/ 6J) mouse; in Trial 2, a white (ICR) mouse.

Media Files

Dark mouse.mpg, White mouse.mpg.

ARENA SETTINGS

In the **Arena Settings**, the open field is divided into two zones, **Center** and **Border**. Rodents typically prefer to spend time in enclosed spaces and will therefore tend to spend more time in the Border (safe zone) versus the Center (less safe zone).

An additional zone is defined for the **Wall** of the open field. You can use this zone to estimate:

- Thigmotaxis, when the mouse's center point is at short distance from the walls. For this purpose, use the Analysis profile **Distance from Wall zone**.
- Sniffing/Rearing at the walls, when the mouse's nose is detected within the **Wall** zone. For this purpose, use the Analysis profile Nose over Wall zone.

TRIAL CONTROL SETTINGS

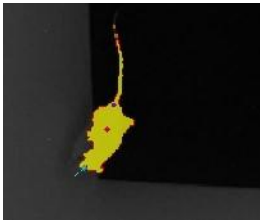
The Trial Control Settings **Trial Control Settings 1** has been defined so that tracking starts after the mouse has been detected for 1 s in the arena, and lasts until the end of the video file.

DETECTION SETTINGS

The experiment contains two Detection Settings profiles:

- **Dark mouse**. Uses Dynamic subtraction as a method for finding the mouse of a white background. Use this profile to track from the video file Dark mouse.

- **White mouse**. This Detection Settings profiles uses **Differencing** as a method to find the mouse. The reason is that other detection methods would not be able to find the mouse's head when the animal rears at the wall, because of the low level of contrast with the white wall. However, Differencing can pick very subtle changes in the image and in most cases can find the whole contour of the mouse.



DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The experiment has three Data profiles:

- **All data**. This is a default Data profile to analyze all tracks together, without any filtering or nesting criteria.
- **With time bins**. Data are split in one-minute time bins. Use this Data profile to view temporal changes in the behavior of the subject.
- **Groups**. This Data profile is an example of how you can group tracks, for example to all trials with the dark mouse vs. all trials with the white mouse. Use this Data profile when you track multiple times from the same video file. Start tracking at different times of the video to create some within-group variation in the results.

ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile** > **Open**. The experiment has three Data profiles:

- **Distance and velocity**. This profile contains variables to quantify locomotor behavior, for example to calculate the mean velocity and the total distance moved of the animal's center-point.
- **JS body length**. This is an example of a custom variable created with JavaScript code. It uses EthoVision XT raw data to calculate the length of the mouse (from nose to tail-base) at each sample time. Plot this variable with Integrated Visualization to see how this variable varies with behavior (walking vs. sitting/grooming, etc.).

With JavaScript custom variables, you can extract a wealth of additional information on the behavior of your subjects. For more details, see the EthoVision XT Help.

- **Crossings**. This profile contains a variable of type *In zone* that measures how often the mouse crosses the center of the open field.
- **Distance from walls**. This profile contains a variable of type *Distance to zone* for calculating the average distance of the mouse to the walls.
- **Nose over Wall zone**. This profile contains a variable of type In zone, which measures the time that the mouse's nose is within the wall zone. This variable estimates the time that the subject rears at the walls.

  tip With the Mouse/Rat Behavior Recognition module you can detect rearing behavior automatically.

TRIAL STATISTICS

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

Select other Data profiles and Analysis profiles from the lists on the toolbar to view more results.

GROUP STATISTICS AND CHARTS

Choose **Results** > **Statistics and Charts**, then click the **Group Statistics and Charts** tab. Select the Data profile **Groups** from the list on the toolbar.

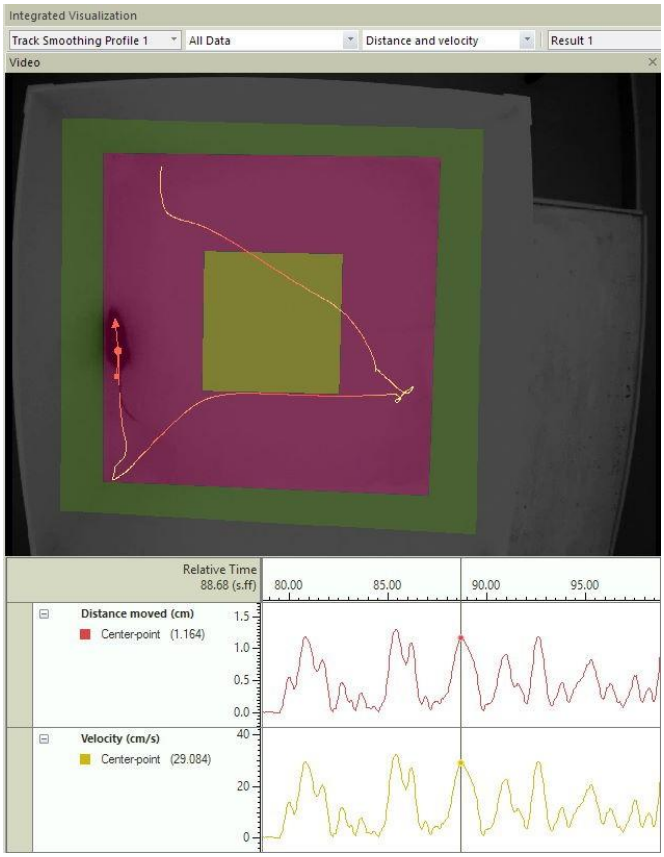
TIP Acquire more trials using the two video files and the corresponding detection settings. Start tracking at various points in the video to create some variation in the groups. Then open the Group Statistics and Charts.

INTEGRATED VISUALIZATION

Choose **Results** > **Integrated Visualization**, then choose the Data profile and the analysis profile from the lists on the toolbar to select the variables you want to plot.

Choose **Distance and velocity** from the Analysis profile list on the toolbar.

Next, on the Track Plot Settings pane click the **Colors** tab. Under **Level** choose **Sample** and then **Velocity (Center point)** as the **Variable** to visualize. When you play the tracks, you see the data points in different colors based on the mouse's speed.

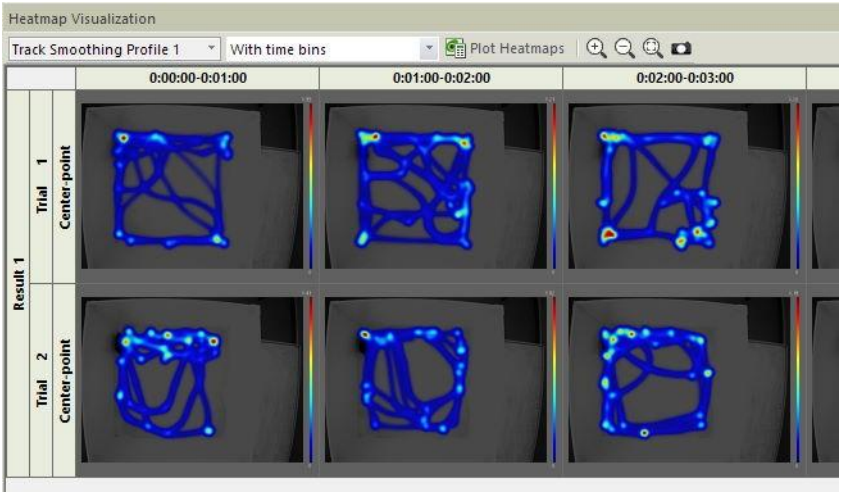


TIP Click the **Jump to end** button to view the entire tracks.

HEATMAP VISUALIZATION

Choose **Results > Heatmap Visualization**. Select the Data profile **With time bins** from the list on the toolbar, then click the **Time bins** button on the right under **Layout presets**.

There you see where the mouse spent time in each of the one-minute time bins.



TIP Click the **Fit all** button on the toolbar to view all time bins in one row.


ACKNOWLEDGEMENTS

Video files were kindly provided by Dr. Lior Bikovski, director of the Myers Neuro-Behavioral Core Facility, Sackler School of Medicine, Tel Aviv University, Tel-Aviv, Israel; and School of Behavioral Sciences, Netanya academic college, Netanya, Israel.

Elevated plus maze XT190

OVERVIEW

This is an example of a plus maze experiment with a mouse. We used nose-tail tracking to calculate the mouse's head dips over the edge of the open arms of the maze.

Media file

Elevated plus maze mouse.avi

ARENA SETTINGS

Arena Settings 1

The North and South arms of the maze are open, the other two (West and East) are closed. The North and South arms are defined as the cumulative group **Open arms** in the Arena Settings. The West and East arms are defined as the cumulative group **Closed arms**. We used nose-tail tracking to investigate how much time the mouse spent in the open arms. We also defined the zones **Outside North open arm** and **Outside South open arm**, which together form the cumulative zone **Outside open arms**. We used this cumulative zone to investigate how often the mouse showed head dips over the edge of the open arms.

TRIAL CONTROL SETTINGS

Trial Control Settings 1

In the **Trial Control Settings** we defined a condition to start the track two seconds after the mouse was first detected in the arena.

DETECTION SETTINGS

Detection Settings 1

Dynamic subtraction is the detection method used. A maximum body size of 1300 pixels is used. This is to prevent EthoVision from tracking the arm of the experimenter who puts the animal in the arena. To prevent EthoVision from tracking noise, a minimum body size of 100 pixels is used. Contour dilation is used to compensate for low contrast in some parts of the arena, which makes the rodent's shape less consistent.

TRACK SMOOTHING PROFILES

The experiment has two Track Smoothing profiles:

- **Smoothing off**. Track smoothing is not enabled.

- **Filter based on Minimal Distance Moved**. A filter based on a minimal distance moved from one sample to the next is activated. Keep this profile active especially when calculating distance moved, since it filters out false movements caused by noise when the mouse is sitting still.

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The experiment has three Data profiles:

- **All data.** The default Data profile without any filtering or nesting criteria.
- **Results in 30-s bins**. This is a Data profile that splits the tracks in 30-s intervals called bins. Note that the last bit of the track is excluded from analysis because it is shorter than 30 s.
- **Results in open/closed arms** - With results per zone, and the center point of the mouse and the zones open arms and closed arms selected. This divides the tracks into segments in which the center point of the mouse is in either the open arms or the closed arms.

ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile** > **Open**. The experiment has four Analysis profiles:

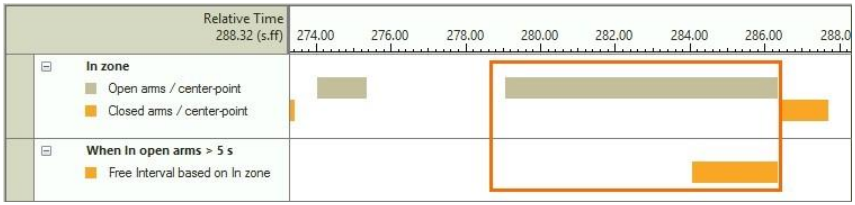
- **Velocity and distance moved.** To calculate the (mean) velocity and (total) distance moved of the animal's center-point.
- **Time in open/closed arms**. Contains the following variables:
- **In Zone**. To calculate the total time spent in open arms and closed arms separately, also as percentage of the total recording time. For frequency of arm entries, see the Analysis profile **Arm entries** below.
- **When in open arms > 5 s.** This variable is an example of how you can mark events based on the duration of a behavior. Here, we want to find the number of times that the animal spent more than a specific time, 5 seconds, in either of the open arms.

  To determine the instances of zone visits that last longer than a specific time, a Free Interval is used based on the dependent variable *In Zone*. The start condition is defined as the moment the animal's center-point is in one of the open arms for more

than 5 s. The stop condition was defined as the moment the animal's center-point was no longer in the open arm.

Double-click this variable to see how it was defined. For more information, see Free intervals in the EthoVision XT Help.

When you plot this variable in the Integrated Visualization (**Analyze** > **Results** > **Plot Integrated data**), you see that only the visits to the open arms that lasted longer than 5 second are plotted.



Note that the interval starts five seconds (or the time specified in the start condition of the Free Interval Settings) after the animal actually enters the zone.

- **Nose over edge open arms**. To calculate the frequency and the duration of head dips over the edges of the open arms. To do so, the number of times and the duration the nose point was present in the cumulative zone *Outside open arms* was assessed.
- **Arm entries**. This profile contains two *Zone transition* variables. Entries to Open arms counts the transitions from the Center zone to either open arm. Entries to Closed arms counts the transitions from the Center zone to either closed arm. *Zone transitions* are generally more reliable indicators of true zone entries than *In zone* variables, especially when the body points jitter around the zone borders.
- **Body Length with JavaScript**. This profile contains a custom variable written with JavaScript code. You can use JavaScript to extract more information from the track data. In this example, the JavaScript code defines the body length as the sum of the distance between the nose and the center and between the center and the tail base point of the mouse. For more examples of variables with JavaScript, see **JavaScript custom variables** under **Drivers and tools/Utilities** on the EthoVision XT installation USB stick.

TRIAL STATISTICS

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

From the lists on the toolbar, choose the Data profile **All Data** and the Analysis profile **Time in open/closed arms**. With this combination you calculate the time that the subject spent in the open arms and in the closed arms. This time is expressed in seconds and in percentage of the total time.

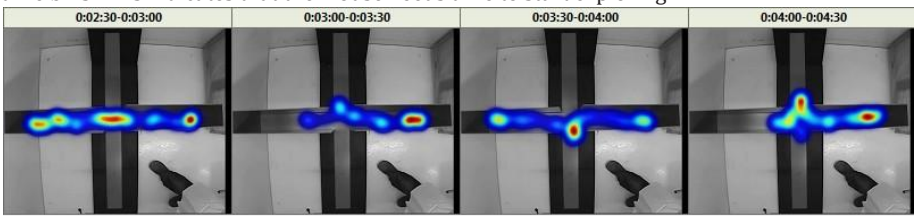
Combine the Data profile **Results in open/closed arms** with the Analysis profile **Velocity and distance moved**. There you have the velocity and distance moved in the open and closed arms. The subject explored the closed arms far more than open arms. The velocity in the open arms was slightly lower than in the closed arms.

Combine the Data profile **All Data** with the Analysis profile **Arm entries**. There you compare the number of entries between open arms and closed arms.

HEATMAPS

Choose **Analysis** > **Results** > **Plot Heatmaps**.

Choose the Data profile **Results in 30-s bins** from the list at the top. Next, click the **Time bins** button on the right-hand pane. You see that the mouse enters the open arms only in the last two time bins. This indicates that the mouse needs time to start exploring.



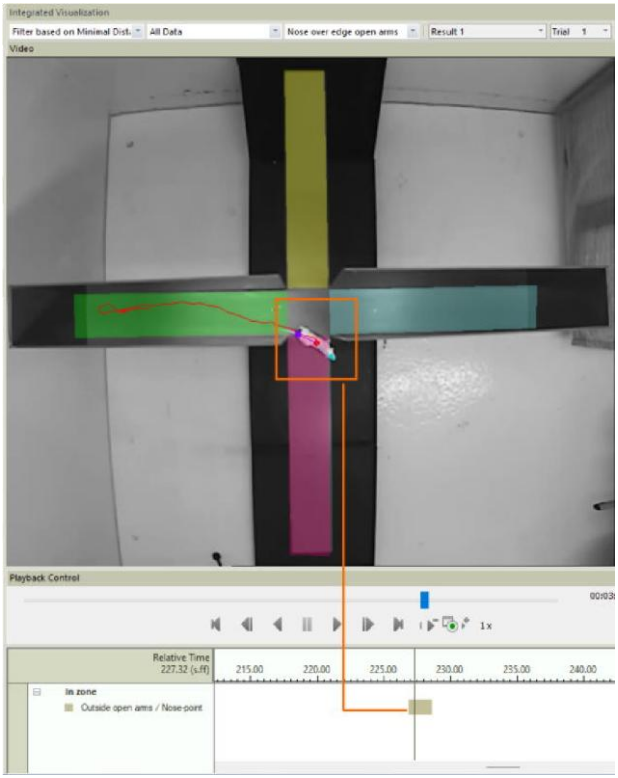
TIP Click the **Fit all** button on the toolbar to view all time bins in one row.

INTEGRATED VISUALIZATION

Choose **Analysis** > **Results** > **Plot Integrated data.** Choose the Analysis Profile and Data Profile from the lists on the toolbar.

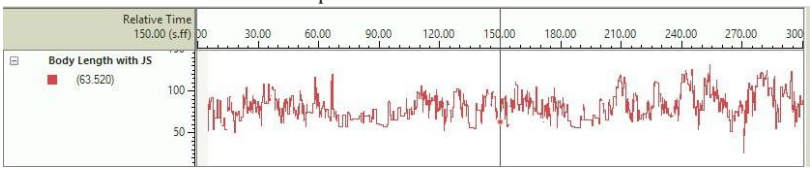
Head dipping

Combine the Data profile **All Data** and the Analysis profile **Nose over edge open arms** to display the head dips in a time plot.



Body length

Choose the Data profile **All data** with the Analysis profile **Body Length with JavaScript** to plot the body length against time. There you see an increase in body length at the end of the video, each time the mouse enters the open arms.



ACKNOWLEDGEMENT

The video was provided by Niek van Stipdonk from Delta Phenomics BV, who is acknowledged.

Novel Object Recognition test with Deep Learning XT190

OVERVIEW

In the Novel Object Recognition (NOR) test, the researcher evaluates the recognition memory of the animal. A mouse is presented with two similar objects during the a session (*Familiarization*), and then one of the two objects is replaced by a new object during a following session (*Test*). The amount of time taken to explore the new object provides an index of recognition memory.

In this sample experiment, the software tracks the mouse's nose to quantify its exploratory behavior. Three trials have been acquired:

- Trial 1 (Habituation). The mouse is free to explore the open field for 5 minutes.
- Trial 2 (Familiarization). The mouse explores two identical objects, Familiar object 1 and Familiar object 2.
- Trial 3 (Test). The mouse explores one familiar object, the same as Familiar object 2, and one novel object, which replaced Familiar object 1.

NOTE This experiment uses the **Deep learning** method for tracking the subject's nose point. This method requires recent CUDA software and a recent graphics card (GPU). These requirements may not be met in some computers. In that case, you can visualize the existing track but you cannot acquire new data. See the EthoVision XT Help for more information.

Media files

- NOR test video no objects.mpg
- NOR test video two identical objects.mpg.
- NOR test video familiar and novel object.mpg

Reference

Leger *et al*. (2013). Object recognition test in mice. *Nature Protocols* **8**: 2531–2537.

EXPERIMENT SETTINGS

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

To track the subject's nose, under **Tracked Features** the option **Center-point, nose-point and tail-base point detection** is selected.

Under **Body Point Detection Technique** you see that EthoVision XT uses **Deep learning** to find the body points of the mouse.

ARENA SETTINGS

Three Arena Settings are defined:

- **With no objects**. Use this profile when tracking from the video file named NOR test video no objects.mpg.
- **With identical objects**. Use this profile when tracking from the video file NOR test video two identical objects.mpg.
- **With novel object**. Use this profile when tracking from the video file NOR test video familiar and novel object.mpg.

In the last two Arena Settings profiles, the zone group **Objects** contains zones covering the two objects and boundary zones around each object. The zones are slightly different in the two Arena Settings profiles because the objects slightly differ in size and position.

You can use the boundary zone to define the properties of the dependent variable **Head directed to zone**.

For all the Arena Settings profiles, two zones Floor and Wall are defined in a separate group **Floor and Wall** which you can use for additional analyses.

TRIAL CONTROL SETTINGS

Choose **Setup** > **Trial Control Settings** > **Open**. The experiment contains the profile **Start after 5 seconds**. This profile contains a condition to wait five seconds before starting tracking. You can use this profile to avoid faulty tracking at the beginning of the video when a change in the lighting occurs.

DETECTION SETTINGS

Choose **Setup** > **Detection Settings** > **Open**. The experiment contains three profiles, one for each video. The main difference between the three profiles lies in the background image that must match the image of the video (without the animal in the arena), with or without the objects. Make sure that you use the correct Detection Settings profile when you track from one of the three video files.

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The experiment contains three profiles:

- **All Data**. This is the default Data profile.
- **Contrast object exploration**. This Data profile contains two result boxes. The aim of this profile is to compare exploration of the two objects when they are identical versus when one of them is novel. The two selections are based on the type of Arena Settings used.
- **Time bins**. You can use this profile to analyze exploration per time interval.

ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile** > **Open**. The experiment contains two profiles:

- **Exploration**. Here a few variables have been defined to measure exploratory behavior.
- **Nose within object zone** measures the time that the subject's nose touches objects.
- **Distance to objects** returns the distance of the subject's nose to the objects.
- The variables of type **Head directed to [object name]** measure the time that the subject's head is directed to the zone, while its center point is in the boundary zone. The results are similar to those obtained with Nose within object zone, however this adds the condition that the head is directed toward an object, even if the nose is not precisely inside the object zone. The measure is expected to quantify the time that the mouse's attention is directed toward the object.
- **Locomotion**. In this profile the variables Distance moved and Velocity are defined.

STATISTICS

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

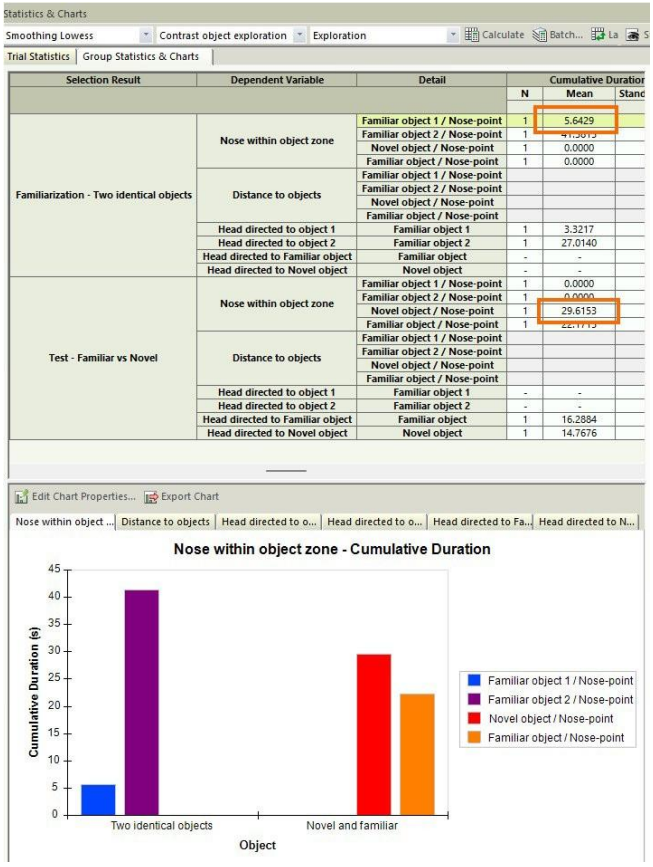
- From the Data profile on the toolbar, choose **All Data** and from the Analysis profile list choose **Exploration**.

Under **Trial Statistics**, the results are shown per trial. The table gives you the statistics per zone. Note that some zones give result "-" or zero; those results refer to zones that were not in the Arena Settings used to acquire that trial. For example, for trial 2, the zones Familiar object 1 and Familiar object 2 were used. For trial 3, the zones Familiar object and Novel object were used.

- From the Data profile on the toolbar, choose **Contrast object exploration** and from the Analysis profile list choose **Exploration**. Choose then **Group Statistics and Charts**.

  The table shows two groups, one for the Familiarization phase where Familiar object 1 and Familiar object 2 are compared, and one for the Test phase where the Novel object replaced Familiar object 1.

  In the following example, in the columns under **Cumulative Duration**, the Familiar object 1 was explored for about 5 seconds, while the Novel object was explored for about 30 seconds. The Standard Error is not calculated since only one trial is included per group.



Selection Result	Dependent Variable	Detail	Cumulative Duration N	Mean	Stand
Familiarization - Two identical objects	Nose within object zone	Familiar object 1 / Nose-point	1	5.6429	
		Familiar object 2 / Nose-point	1	41.3073	
		Novel object / Nose-point	1	0.0000	
		Familiar object / Nose-point	1	0.0000	
	Distance to objects	Familiar object 1 / Nose-point			
		Familiar object 2 / Nose-point			
		Novel object / Nose-point			
		Familiar object / Nose-point			
	Head directed to object 1	Familiar object 1	1	3.3217	
	Head directed to object 2	Familiar object 2	1	27.0140	
	Head directed to Familiar object	Familiar object	-	-	
	Head directed to Novel object	Novel object	-	-	
Test - Familiar vs Novel	Nose within object zone	Familiar object 1 / Nose-point	1	0.0000	
		Familiar object 2 / Nose-point	1	0.0000	
		Novel object / Nose-point	1	29.6153	
		Familiar object / Nose-point	1	22.1715	
	Distance to objects	Familiar object 1 / Nose-point			
		Familiar object 2 / Nose-point			
		Novel object / Nose-point			
		Familiar object / Nose-point			
	Head directed to object 1	Familiar object 1	-	-	
	Head directed to object 2	Familiar object 2	-	-	
	Head directed to Familiar object	Familiar object	1	16.2884	
	Head directed to Novel object	Novel object	1	14.7676	

INTEGRATED VISUALIZATION

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

From the lists on the toolbar, select the Data profile, the Analysis profile and the trial number that you want to visualize.

HEATMAP VISUALIZATION

Choose **Analysis** > **Results** > **Plot Heatmaps**.

From the lists on the toolbar, select the Data profile **Contrast object exploration**.

Heatmaps give you an immediate sense of where the animal spent most of the time during a trial. The following heatmap shows the position of the nose point in Trial 2 (Familiarization; left) and Trial 3 (Test; right). In the Test phase, the positions of the nose point are far more dense around the novel object than those at the same location during the familiarization phase.

ACKNOWLEDGEMENTS

Video files were kindly provided by Dr. Lior Bikovski, director of the Myers Neuro-Behavioral Core Facility, Sackler School of Medicine, Tel Aviv University, Tel-Aviv, Israel; and School of Behavioral Sciences, Netanya academic college, Netanya, Israel.

Porsolt forced swim test XT190

OVERVIEW

This experiment shows a Porsolt forced swim test performed on two subjects, each being tracked in its own arena. Note that you need the **Multiple Arenas** Module to be able to track subjects in two arenas simultaneously.

Media file

Porsolt Swim Test.mp4

ARENA SETTINGS

In the **Arena Settings**, an arena has been defined over each of the two water tanks in the video image. No zone has been defined as in this experiment the researcher was not interested in the exact location of the subject in the tank.

TRIAL CONTROL SETTINGS

A **Trial Control Settings** profile **Five minutes track** was defined to stop tracking 5 minutes after the start of tracking. These settings were used to acquire Trial 1. You can create a duplicate of this profile and modify the **Time** condition to acquire shorter or longer tracks.

DETECTION SETTINGS

In the **Detection Settings**, the **Static Subtraction** is selected as the detection method. The video file was tracked at 12.5 samples per second. Note that the sample rate affects the results of Mobility: the higher the sample rate, the smaller are the changes between two consecutive video frames, and the lower are the values of per-sample Mobility. For details, see **Mobility** in the EthoVision XT Help.

TRIAL LIST

In the **Trial List**, the variable **Session** has been defined, with two possible values, Test and Pretest. Each trial is assigned to either value. Trial 1, a Test, has been acquired with Animal ID 1 and 2. Other variables are **Treatment**, a variable with values Drug and Saline, **Animal ID**and

Dose, a numerical variable with values 0, 2 and 5 mg/l. You can use those variables to filter trials and tracks and group subjects. Note that each trial will contain two tracks.

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The experiment has two Data profiles.

- **All Data.** Contains all data in the experiment. Use this Data profile to visualize statistics calculated per trial.
- **Drug vs. Saline**. With two groups based on the independent variable *Treatment*. Use this Data profile to compare statistics and charts of the variables per group.

ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile** > **Open**.

Two Analysis profiles include the *Mobility* dependent variables. Each profile contains two variables, **Mobility continuous** and **Mobility state**. Mobility continuous returns the value of body mobility based on the change in body area between subsequent samples. Mobility states marks the behavior of the subjects with one of three possible scores (*Immobile*, *Mobile*, *Highly mobile*) based on the value of Mobility relative to user-set thresholds. Doubleclick the name of the variable to view those settings.

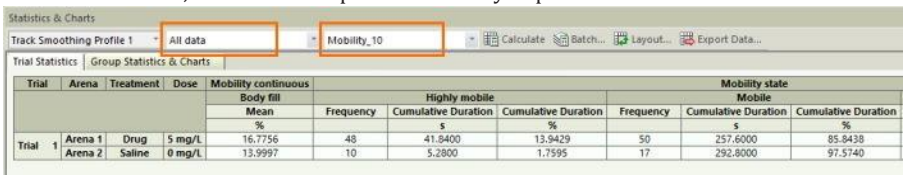
- **Mobility_1**. Mobility is calculated with an **Averaging interval** of 1, that is, the value is obtained directly from the per-sample change in the subject's apparent body area. However, random changes in the body area around the thresholds, due for example to changes in the orientation of the animal independent of its swimming behavior, can influence the value of Mobility, resulting in many transitions between Mobility scores (e.g. *Mobile* > *Highly mobile* > *Mobile* > *Highly mobile*, etc.). For this reason we also defined a smoothed Mobility variable (see below).
- **Mobility_10**. The values of Mobility are smoothed out using an **Averaging interval** larger than 1. In this example, the interval is 10 samples wide. The effect of random changes in body area is minimized, and that results in fewer transitions between Mobility scores.

NOTE For all variables, the following thresholds were used: **Immobile below** 6.5%, **Highly mobile above** 22%. Feel free to edit those values and open the Integrated Visualization. The resulting Mobility scores should match the behavior of the rats in the video as much as possible.

TRIAL STATISTICS

Choose **Analysis** > **Results** > **Statistics & Charts**.

To view the results, select the Data profile and Analysis profile from the list on the toolbar.

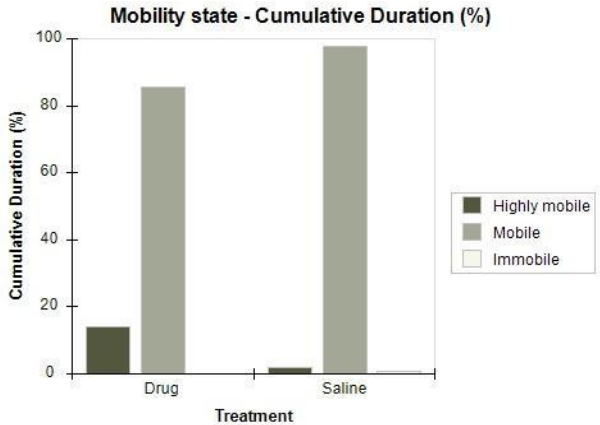


Trial		Arena	Treatment	Dose	Mobility continuous	Mobility state					
					Body fill	Highly mobile			Mobile		
					Mean	Frequency	Cumulative Duration	Cumulative Duration	Frequency	Cumulative Duration	Cumulative Duration
					%		s	%		s	%
Trial	1	Arena 1	Drug	5 mg/L	16.7756	48	41.8400	13.9429	50	257.6000	85.8438
		Arena 2	Saline	0 mg/L	13.9997	10	5.2800	1.7595	17	292.8000	97.5740

GROUP STATISTICS AND CHARTS

Choose **Analysis** > **Results** > **Statistics & Charts** and open the tab **Group Statistics and Charts**. Select the Data Profile and Analysis Profile from the lists on the toolbar.

Below you see one of the charts that appear when you select the Data profile **Drug vs. Saline** and the Analysis profile **Mobility_10**. Click the **Mobility state** tab just above the charts.



ACKNOWLEDGEMENT

The experiment was provided by Dr. Sanna Lemming Kjær, National Research Centre for the Working Environment, Copenhagen, Denmark.

Social interaction test in PhenoTyper with Deep Learning XT190

OVERVIEW

This is an example of how you can analyze the interaction between two or more animals in the same arena. Note that you need the **Social Interaction** add-on module to track two animals per arena.

The two mice in the accompanying video are tracked with the Deep learning method. One of the two animals is partially shaved on its back. EthoVision XT uses the difference in visual appearance between the two animals to track their identities. Note that this process is completed after tracking. For more information, see the topic **Prepare the data in multisubject trials** in the EthoVision XT Help.

note This experiment uses the **Deep learning** method for tracking the subject's nose point. This method requires recent CUDA software and a recent graphics card (GPU). These requirements may not be met in some computers. In that case, you can visualize the existing track but you cannot acquire new data. See the EthoVision XT Help for more information.

Media file

Two mice in PhenoTyper v2.mp4. The footage was recorded with EthoVision XT and a Basler digital camera mounted on a PhenoTyper version 2 top unit.

EXPERIMENT SETTINGS

In the Experiment Settings, we set the **Number of Subjects per Arena** to 2; the **Tracked features** to **Center-point, nose-point and tail-base detection**, and the **Body Point Detection Technique** to **Deep learning**. The **Subject roles** are **Subject 1** and **Subject 2**.

ARENA SETTINGS

In the **Arena Settings**, the **Zone Group 1** includes two zones (the feeding zone and the area covering the top of the shelter) and one point (Drinking spout).

- To create and edit an Arena Settings profile with the same zones, right-click **Arena Settings 1** and select **Duplicate**.
- Alternatively, to create an Arena Settings profile from scratch, using the same video file as background image, choose **Setup** > **Arena Settings > New**, and load the video file.

TRIAL CONTROL SETTINGS

A Trial Control Settings profile **Trial Control Settings 1** has been defined. The condition placed before **Start track** ensures that tracking starts when at least one subject is detected in the cage for one second. In this video both animals are present since the start, so tracking starts exactly one second from the first video frame. The condition placed immediately before **Stop track** ensures that tracking stops at the end of the video file.

DETECTION SETTINGS

When using Deep Learning in two-subjects experiments, the **Detection settings** under **Advanced** are greatly simplified. In most cases you do not need to adjust the remaining options. You are ready to acquire the data! For details about Deep learning-based tracking in EthoVision XT, see the EthoVision XT Help.

TRIAL LIST

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

Besides other "classic" variable such as mouse strain, treatment level, or age, you may want to know which mouse is given the label **Subject 1** or **Subject 2**. This can be useful, for example if you want to check whether there are biases in the behavior of animals marked on the back. Because of the way the Deep learning method works, which Subject label is given to a marked mouse can differ between trials, even when using repeatedly the same video, and cannot be established at the start of each trial.

Click the **Add Variable** button. In the column that appears:

- In the **Label** cell (the first cell of the new column), enter *Marked mouse*.
- In the **Scope** cell select **Subject**.
- Double- click the **Predefined Values** cell in the same column and under **Predefined Value** enter *Yes* and click **Add**, next enter *No* and click **Add**.

You can now label each track that you collect based on whether either **Subject 1** or **Subject 2** was marked on the back. For each trial acquired, select **Yes** or **No** in the corresponding cell of the **Marked mouse** column. Check the tracks in the Integrated Visualization (see below) to know which mouse received which label.

DATA ACQUISITION

To start tracking, click the **Start Trial** button.

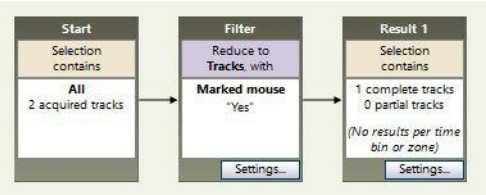
You may note that the software occasionally swaps the identity of the two animals. That's normal during acquisition.

Once you start analysis, EthoVision XT reviews the tracks and sorts them based on the information on the visual appearance of the mice collected during the tracking phase. The software re-assigns the labels **Subject 1** and **Subject 2** to segments of tracks where an accidental swap occurred. This phase is called *Data Set Preparation*. For more details, see the EthoVision XT 18 Help.

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The experiment contains the default data profile with all data.

If you have added a variable for the marked animal (see Trial List above), to analyze the behaviors of the marked animal, under **Filter** click the button next to Marked mouse, select **Yes** and insert the box in the sequence between **Start and Result 1**.



ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile** > **Open**. The experiment contains four Analysis profiles.

- **Distance and Velocity**, which contains variables typically used to quantify each subject's activity: Distance moved and Velocity.
- **Social behavior measures - S1** and **Social behavior measures - S2**. These analysis profiles are meant to examine the social behavior of Subject 1 and Subject 2. The reason why there are separate profiles for Subject 1 and Subject 2 is that they contain JavaScript custom variables, each defined for one subject or the other.

  The JavaScript variables analyze the movement and the position of the two subjects and output various states of each subject: *Approach*, *Follow* (the other subject) and *Social contact*. You can customize the code in the variable settings to fine tune the criteria for scoring those behaviors. For example, adjust the speed that define movement, or adjust the distance under which we speak of "interaction".

For more examples of JavaScript custom variables, download the **JavaScript custom variables** from my.noldus.com (click **downloads**, then under **EthoVision XT** choose **drivers and tools**).

These analysis profiles also contain typical social behavior variables such as *Distance between subjects* and *Proximity*.

- **Feeding and Drinking**. This profile calculates the frequency, duration and latency of the events "nose in feeding zone" and "nose in drinking zone".

TRIAL STATISTICS

Choose **Analysis** > **Results** > **Statistics & Charts**.

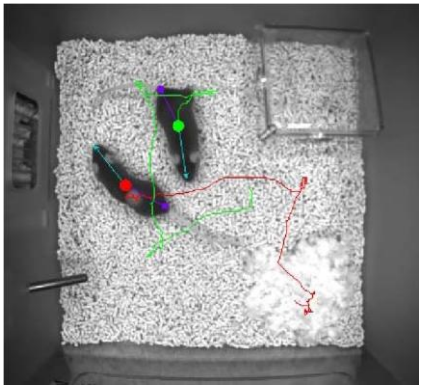
To view the results of the behavior of **S1** (Subject 1) towards **S2** (Subject 2), locate the row **Subject 1**, then locate the column of a behavior of **S1**.

Similarly, to view the variables of the behavior of **S2** towards **S1**, start locating the row **Subject 2** and locate the column of a behavior of **S2**.

INTEGRATED VISUALIZATION

Choose **Analysis** > **Results** > **Plot integrated data**.

In the Integrated Visualization screen, you can check how consistent the identity was assigned to the two mice. Play the video and view the color of the center point. If identification worked correctly, the mouse with a mark on its back should always (or almost always) have the same color tag (either green or red). You can correct the remaining identity swaps in the Track Editor.



Social interaction test in Social Box with Deep Learning XT190

OVERVIEW

This is an example of how you can analyze the interaction between two or more animals in the same arena. Note that you need the **Social Interaction** add-on module to track two animals per arena.

The two mice in the accompanying video are tracked with the Deep learning method. One of the two animals was marked on its tail. EthoVision XT uses the difference in visual appearance between the two animals to track their identities. Note that this process is completed after tracking. For more information, see the topic **Prepare the data in multisubject trials** in the EthoVision XT Help.

NOTE This experiment uses the **Deep learning** method for tracking the subject's nose point. This method requires recent CUDA software and a recent graphics card (GPU). These requirements may not be met in some computers. In that case, you can visualize the existing track but you cannot acquire new data. See the EthoVision XT Help for more information.

Media file

SocialBox_2animals_PORT.avi.

Video was recorded at the Group of Astrocyte Biology, Łukasiewicz - PORT, Wrocław. Credit: Bartosz Zglinicki, Patrycja Ziuzia, Michał Ślęzak.

EXPERIMENT SETTINGS

In the Experiment Settings, we set the **Number of Subjects per Arena** to 2; the **Tracked features** to **Center-point, nose-point and tail-base detection**, and the **Body Point Detection Technique** to **Deep learning**. The **Subject roles** are **Subject 1** and **Subject 2**.

ARENA SETTINGS

In the **Arena Settings**, the **Zone Group 1** does not contain zones. Add a zone if for example you want to know how much time each subject spends in the center of the arena, or near the feeder.

- To create an Arena Settings profile from scratch, using the same video file as background image, choose **Setup** > **Arena Settings > New**, and load the video file.

TRIAL CONTROL SETTINGS

A Trial Control Settings profile **Trial Control Settings 1** has been defined. The condition placed before **Start track** ensures that tracking starts when at least one subject is detected in the cage for one second. In this video both animals are present since the start, so tracking starts exactly one second from the first video frame. The condition placed immediately before **Stop track** ensures that tracking stops at the end of the video file.

DETECTION SETTINGS

When using Deep Learning in two-subjects experiments, the **Detection settings** under **Advanced** are greatly simplified. In most cases you do not need to adjust the remaining options. You are ready to acquire the data! For details about Deep learning-based tracking in EthoVision XT, see the EthoVision XT Help.

TRIAL LIST

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

Besides other "classic" variable such as mouse strain, treatment level, or age, you may want to know which mouse is given the label **Subject 1** or **Subject 2**. This can be useful, for example if you want to check whether there are biases in the behavior of the animals marked on their tail. Because of the way the Deep learning method works, which Subject label is given to a marked mouse can differ between trials, even when using repeatedly the same video, and cannot be established at the start of each trial.

Click the **Add Variable** button. In the column that appears:

- In the **Label** cell (the first cell of the new column), enter *Marked mouse*.
- In the **Scope** cell select **Subject**.
- Double- click the **Predefined Values** cell in the same column and under **Predefined Value** enter *Yes* and click **Add**, next enter *No* and click **Add**.

You can now label each track that you collect based on whether either **Subject 1** or **Subject 2** was marked on its tail. For each trial acquired, select **Yes** or **No** in the corresponding cell of the **Marked mouse** column. Check the tracks in the Integrated Visualization (see below) to know which mouse received which label. Next, fill in the cells under **Marked mouse** in the Trial List.

DATA ACQUISITION

To start tracking, click the **Start Trial** button.

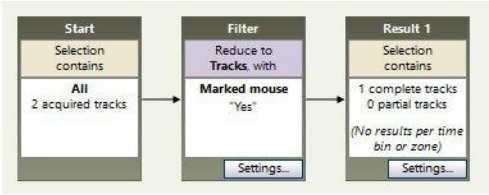
You may note that the software occasionally swaps the identity of the two animals. That's normal during acquisition.

Once you start analysis, EthoVision XT reviews the tracks and sorts them based on the information on the visual appearance of the mice collected during the tracking phase. The software re-assigns the labels **Subject 1** and **Subject 2** to segments of tracks where an accidental swap occurred. This phase is called *Data Set Preparation*. For more details, see the EthoVision XT 18 Help.

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The experiment contains the default data profile with all data.

If you have added a variable for the marked animal (see Trial List above), to analyze the behaviors of the marked animal, under **Filter** click the button next to Marked mouse, select **Yes** and insert the box in the sequence between **Start and Result 1**.



ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile** > **Open**. The experiment contains four Analysis profiles.

- **Distance and Velocity**, which contains variables typically used to quantify each subject's activity: Distance moved and Velocity.
- **Social Behavior**. These analysis profiles are meant to examine the social behavior of Subject 1 and Subject 2. They contain typical output variables like Train, Side by side, and Proximity, but also an example of JavaScript custom variables, each defined for one subject or the other.

  The JavaScript variable analyzes the movement and the position of the two subjects and is scored when one subject approaches the other. You can customize the code in the

variable settings to fine tune the criteria for scoring that behavior. For example, adjust the speed that define movement, or adjust the distance under which we speak of "approaching".

For more examples of JavaScript custom variables, download the **JavaScript custom variables** from my.noldus.com (click **downloads**, then under **EthoVision XT** choose **drivers and tools**).

TRIAL STATISTICS

Choose **Analysis** > **Results** > **Statistics & Charts**.

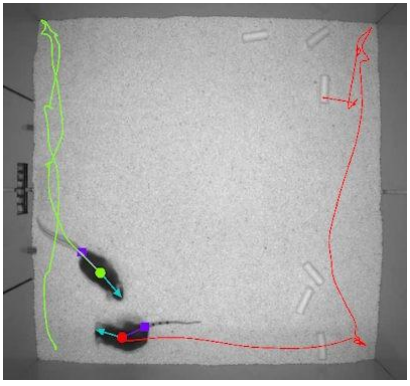
To view the results of the behavior of **Subject 1** towards **Subject 2**, locate the row **Subject 1**, then locate the column of a behavior that starts with **Subject 1**.

Similarly, to view the variables of the behavior of **Subject 2** towards **Subject 1**, locate first the row **Subject 2** and locate the column of a behavior that starts with **Subject 2**.

INTEGRATED VISUALIZATION

Choose **Analysis** > **Results** > **Plot integrated data**.

In the Integrated Visualization screen, you can check how consistent the identity was assigned to the two mice. Play the video and view the color of the center point. If identification worked correctly, the mouse with a mark on its tail should always (or almost always) have the same color tag (either green or red). You can correct the remaining identity swaps in the Track Editor.



Social Interaction test with color markers XT190

OVERVIEW

This is an example of how you can analyze the interaction between two or more animals in the same arena. Note that you need the **Social Interaction** add-on module to track more than one animal per arena.

Media file

Social Interaction test.mp4.

EXPERIMENT SETTINGS

In the Experiment Settings, we set the **Number of Subjects per Arena** to 2 and the **Tracked features** to **Center-point, nose-point and tail-base detection**. The **Subject roles** are **Control** and **Treated**.

ARENA SETTINGS

In the **Arena Settings**, the **Quadrants** zone group includes four quadrants to be able to analyze the location of the subject during the test.

- To create a new Arena Settings profile with pre-defined arena and zones, right-click **Arena Settings 1** and select **Duplicate**.
- Alternatively, to create an Arena Settings profile from scratch, using the same video file as background image, choose **Setup > Arena Settings > New**, and load the video file. Make sure to adjust the video aspect ratio. To do so, click the **Adjust Aspect Ratio** button at the bottom of the Arena Settings window.

In the window that appears, choose **Custom** and enter **768** and **576** as video image size. This adjusts the video image size to the original proportions.

TRIAL CONTROL SETTINGS

A Trial Control Settings profile **One-minute track** has been defined. Tracking starts when both subjects are detected in the cage for two seconds. With this Trial Control profile you ensure that the subjects are tracked for exactly the same time. The other condition placed immediately before “Stop track” ensures that tracking stops after one minute.

DETECTION SETTINGS

In the **Detection settings**, we selected **Dynamic subtraction** with **Marker-assisted identification**.

When you select **Center-point, nose-point and tail-base detection** in the **Experiment settings** for multiple animals, EthoVision XT automatically selects the **Rodents/For occlusions** as nose-tail tracking method.

Because the animals frequently come in close contact, in the advanced **Subject Size** settings, the **Modelling effort optimized for** slider is moved to **Modelling** and the **Shape stability optimized for** slider is moved to **Occlusions**. For details about those options, see **Advanced detection settings: Subject size (multiple animals per arena)** in the EthoVision XT Help.

TRIAL LIST

Choose **Setup** > **Trial List**. User-defined variables **Dose**, **Day** and **Marker color** are defined. You can use the Marker color variable to specify Subject role * Marker color combinations. In the acquired trial, the Treated subject has a yellow marker.

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The experiment contains the default data profile with all data.

To analyze the behaviors of the animals in the four quadrants separately, click the **Settings** button on the Results box, select **Results per zone** and select the four quadrants. Then redo the analysis.

ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile** > **Open**. The experiment contains two Analysis profiles.

The first is **Individual behavior**, which contains variables typically used to quantify each subject's activity: distance moved, velocity and the state variable Movement (Moving vs Not Moving).

The second profile is **Social behavior** with the following dependent variables:

- **Distance nose point to tail base Control**. This calculates the mean distance from the nose-point of both animals to the tail-base of the Control animal.

  In the statistics table, look up the value for the Treated animal, to obtain the distance from the nose point of the treated animal to the tail base of the control animal.

- **Proximity nose point to tail base Control**. This calculates the mean duration of the states **In proximity** and **Not in proximity** based on the distance from the nose-point of both animals to the tail-base of the Control animal. The states In proximity and Not in proximity have with a lower and upper threshold of 5 and 6 cm, respectively.

  In the statistics table, look up the value for the Treated animal, to see whether the nose point of the treated animal is in proximity to the tail base of the control animal.

- **Distance nose point to tail base of Treated**. This calculates the mean distance from the nose-point of both animals to the tail-base of the Treated animal.

  In the statistics table, look up the value for the Control animal, to obtain the distance from the nose point of the control animal to the tail base of the treated animal.

- **Proximity nose point to tail base of Treated**. This calculates the mean duration of the states **In proximity** and **Not in proximity** based on the distance from the nose-point of both animals to the tail-base of the Treated animal. The states In proximity and Not in proximity have with a lower and upper threshold of 5 and 6 cm, respectively.

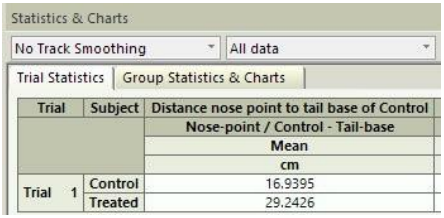
  In the statistics table, look up the value for the Control animal, to see whether the nose point of the control animal is in proximity to the tail base of the treated animal.

- **Body Contact**. This calculates whether the animals are in contact with each other or not.

TRIAL STATISTICS

Choose **Analysis** > **Results** > **Statistics & Charts**.

For variables of distance and proximity of the rat **Treated** to the rat **Control**, locate the column **Distance (Proximity) nose point to tail base of Control**, then locate the row for **Treated**. For example, the average distance of the nose point of Treated to the tail base of Control was about 29 cm.

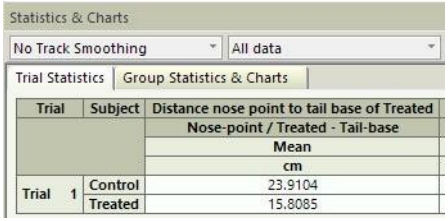

Statistics & Charts

No Track Smoothing | All data

Trial Statistics | Group Statistics & Charts

Trial	Subject	Distance nose point to tail base of Control
		Nose-point / Control - Tail-base
		Mean
		cm
Trial 1	Control	16.9395
	Treated	29.2426

Similarly, for the variables of distance and proximity of **Control** to **Treated**, locate the column **Distance (Proximity) nose point to tail base of Treated** and locate the row for **Control**. The average distance of the nose of Control to the tail-base of Treated was about 24 cm.


Statistics & Charts

No Track Smoothing | All data

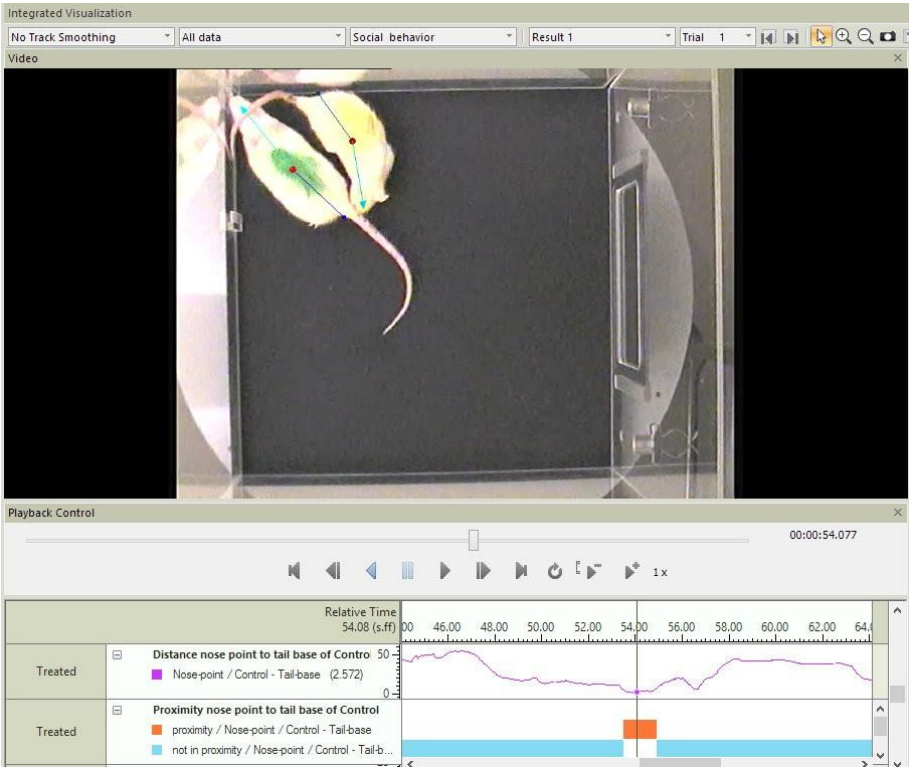
Trial Statistics | Group Statistics & Charts

Trial	Subject	Distance nose point to tail base of Treated
		Nose-point / Treated - Tail-base
		Mean
		cm
Trial 1	Control	23.9104
	Treated	15.8085

INTEGRATED VISUALIZATION

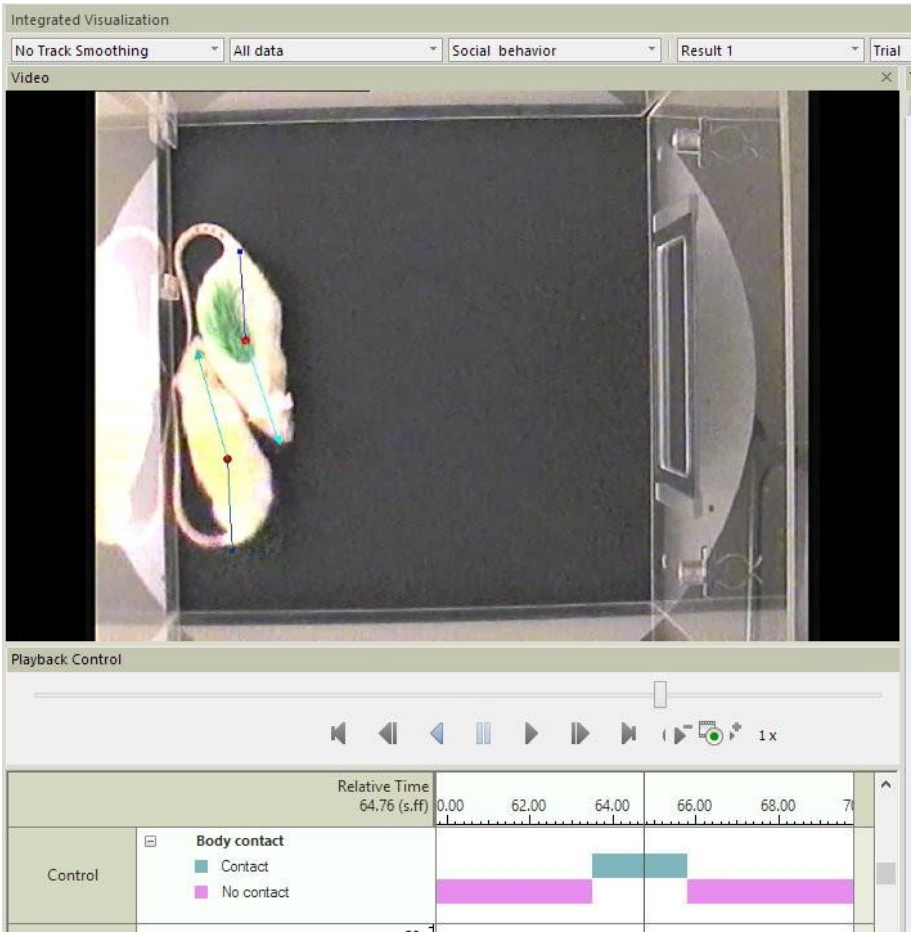
Choose **Analysis** > **Results** > **Plot integrated data**.

The figure below shows an example of the variable *In proximity* based on the nose-point of Treated and the tail-base of Control.



Please note that the plots for the subject **Control** displaying Distance/Proximity of nose point to the tail base of **Control**, that is, the distance between body points of the same animal, do not contain useful information. The same holds for the plots of the subject **Treated** displaying Distance/Proximity nose point to the tail base of **Treated**.

The next figure shows the plot of the variable *Body contact*. Body contact is scored when the animals were in physical contact with any part of their bodies, not just their nose points or tail bases.



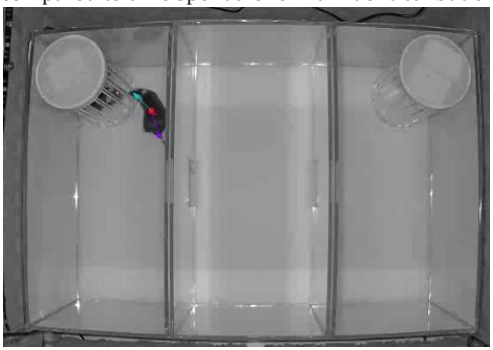
ACKNOWLEDGEMENT

Video was recorded with the help of Niek van Stipdonk and Raymond de Heer (Delta Phenomics, The Netherlands).

Social approach test with Deep Learning XT190

OVERVIEW

This sample experiment uses a method based on trained neural networks, also known as **Deep learning**, to track the nose of the test subject. In the Social approach test, the subject is free to explore an apparatus divided in three compartments (Left, Center, Right). A conspecific is placed under a wire cage in one of the sides of the apparatus (either Left or Right; social zone), while an empty wire cage (control zone) is placed at the opposite side. The aim of the test is to measure the "Sociability", that is the propensity to spend time with another subject, as compared to time spent alone in an identical but empty chamber.



- The videos include the image of two arenas, so you need the Multiple Arena module to do tracking in both arenas simultaneously. If you do not have the Multiple Arena Module, you can create Arena Settings with one arena and track from that arena.
- In order to acquire data with this experiment, you need a graphics card (GPU, or secondary graphics card) which supports a recent version of CUDA software. Make sure that the driver for that card is up to date.

Media files

- Social approach test Trial 1.mp4: The social zone is in the left compartment.
- Social approach test Trial 2.mp4: The social zone is in the right compartment.

EXPERIMENT SETTINGS

In the Experiment Settings, we set the **Number of Arenas** to 2 and the **Number of Subjects per Arena** to 1 because we are going to track only one subject, that is, the test subject, not the subject under the wire cage.

Under **Tracked Features**, **Center-point, nose-point and tail-base detection** is selected. The **Body Point Detection Technique** is set to **Deep learning**.

note If the driver of the GPU is not compatible with the CUDA version needed to use Deep learning, a message appears next to this option. You can only visualize the data already acquired (Trials 1 and 2). Another option is to install this experiment on a PC with a more recent graphics card. For more information, see the topic **Deep learning: Requirements** in the EthoVision XT Help.

ARENA SETTINGS

Two Arena Settings are defined, one for each video file, depending on in which compartment they placed the wire cage with the second mouse (left or right).

- **Social zone left**. Use this profile when tracking from the video file Social approach test Trial 1.mp4, where the social zone is in the left compartment.
- **Social zone right**. Use this profile when tracking from the video file Social approach test Trial 2.mp4, where the social zone is in the right compartment.

In both Arena Settings, and for both arenas, each wire cage is defined as a zone (either Social zone or Control zone, depending on whether they contain a mouse). Furthermore, a larger zone (Outer social zone and Outer control zone), is defined for analysis purposes (see below).

Note that the arena does not include the part of the image where the mouse inside the wire cage is visible. This has been done to avoid that the software tracks that mouse.

Before you start tracking, make sure that you choose the correct Arena Settings for that video!

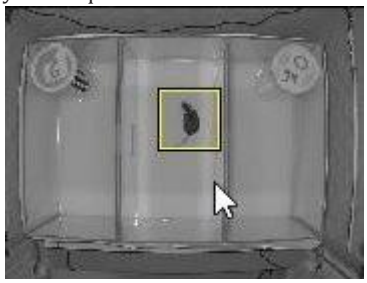
TRIAL CONTROL SETTINGS

In the beginning of the video, the operator removes the doors that divide the compartments in both arenas. That marks the start of the test. The Trial Control Settings includes a condition “Wait 6 seconds after detecting the mouse”. This has been done to ensure that tracking starts after the arms of the operator are no longer visible in the video.

DETECTION SETTINGS

The method Dynamic subtraction has been used to detect the contour of the test mouse. **Deep learning** is used to find the nose-point and the tail-base point.

In case you create new Detection Settings, under **Method** click **Define** and make sure that the yellow square box includes the entire body of the mouse in Arena 2.



DATA PROFILES

The experiment includes two Data profiles:

- **All data**. Use this data profile for an OVERVIEW of all tracks.
- **Groups KO vs WT**. Two fictitious groups, WT and KO, have been created, each containing two tracks. You can view which track belongs to which group in the **Type** column of the Trial List. Use this data profile to compare groups of test subjects.

ANALYSIS PROFILES

The experiment includes three Analysis profiles:

- **Time spent in compartments**. This profile includes a variable of type **In zone** to quantify the time that the mouse spent in the three compartments (Left, Center and Right). It is based on the detection of the mouse's center point in each zone.
- **Exploration of social vs control zones**. This profile is based on the detection of the nose point and the head direction, and aims at quantifying exploration more in detail.
- A variable of type **In zone** tells when the nose of the mouse is within the social zone and the control zone. The variable is smoothed out using a Zone exit threshold.
- Two variables of type **Head directed to zone**, one for the social zone and the other for the control zone, measure the time that the animal's head was directed to a zone (social/control) while its body center was in the outer zone (Outer social/control

zone). This extra condition acts as a filter to exclude the instances when the animal was pointing to the zones of interest but was still far from them.

- **Locomotory activity**. This profile includes **Distance moved** and **Velocity**, to quantify the level of activity of the mouse.

TRIAL STATISTICS

Choose **Analysis** > **Results** > **Statistics and Charts**. Choose a Data profile and an Analysis profile from the toolbar to visualize the results.

GROUP STATISTICS

Choose **Analysis** > **Results** > **Statistics and Charts** then click **Group Statistics and Charts**. Select the Data profile **Groups KO vs WT** to view the statistics for two groups of tracks.

INTEGRATED VISUALIZATION

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

HEATMAPS

Choose **Analysis** > **Results** > **Plot Heatmaps**. Select the Data profile **Groups KO vs WT** and click **Adjacent** in the panel on the right. The heatmaps are based on the nose point, so you see exactly where exploration was directed to.

ACKNOWLEDGEMENTS

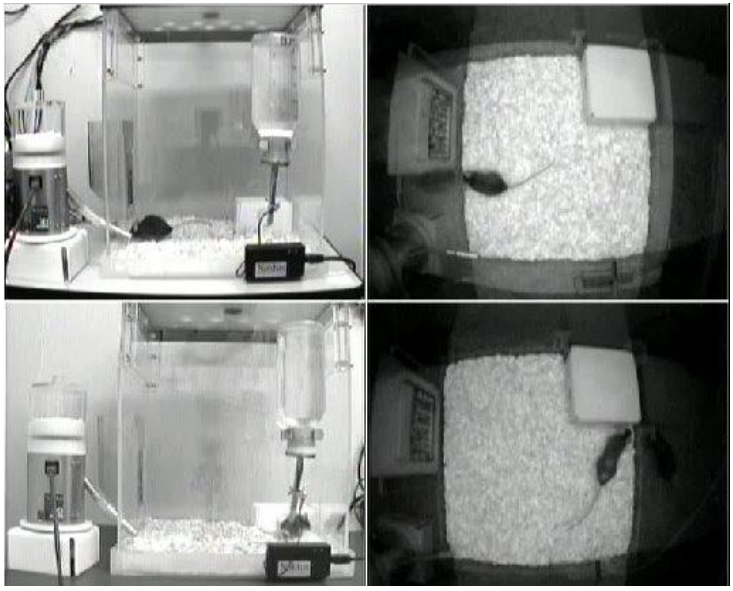
We thank Dr. Giorgio Bergamini, Idorsia Pharmaceuticals, Allschwil, Switzerland, who kindly provided the video files.

PhenoTyper hardware XT190

OVERVIEW

This is an example of how you can use Trial Control to carry out a conditioning experiment in a PhenoTyper cage.

The aim of this experiment is to teach a mouse to go from the pellet feeder on top of the shelter to receive a reward (a food pellet). The start of the conditioning session ("pellet session") is indicated by a yellow light cue from the PhenoTyper top unit (see also **trial control settings**). In the video file, video recordings of two PhenoTyper cages have been combined. The top row shows Arena 1, the bottom row Arena 2. The videos on the left are the recordings with a normal camera, which enable you to see the yellow light come on at the start of the 'pellet session'. The videos on the right show the recordings of the PhenoTyper top unit IR-camera. This view is used for video-tracking.



Media file

PhenoTyper hardware.mp4. Note that although the video shows two lickometers placed near the PhenoTypers, lickometer data are not included in this experiment.

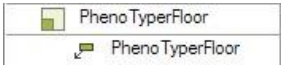
EXPERIMENT SETTINGS

The **Video Source** was originally set to **Live tracking** to acquire data, record video and control the PhenoTyper devices. It was then set to **From video file** to allow acquisition of new trials from the existing video file. Under **Trial Control Hardware**, **Use of Trial Control hardware** is selected.

ARENA SETTINGS

In the Arena Settings, two arenas have been defined, **Arena 1** (top-right) and Arena 2 (bottom-right).

Four zone groups have been defined:



- **PhenoTyperFloor**. Includes the zone **PhenoTyperFloor**. Use this zone to calculate, for instance, distance moved in a horizontal plane (so, excluding vertical movement of the mouse climbing on the shelter).
- **OnShelter**. Includes the zone **OnShelter**, to calculate the time spent on top of the shelter.
- **PelletZone**. Includes the zone **PelletZone**. This zone is used to trigger the hardware action *Drop Pellet* (see **trial control settings** below) when the animal moves from this zone on top of the shelter.
- **Shelter** (hidden zone group). This is a special group of zone, named Hidden zone group. It includes a hidden zone named **Shelter** and its entry zone named **Entry Zone 1**. A hidden zone, together with its entry zone (or zones), allows to calculate the time spent inside the shelter. For more information on hidden zones, see the EthoVision XT Help.

Under **Arena - Hardware mapping**, a PhenoTyper top unit and a Pellet dispenser have been assigned to each arena.

If you wish to create a new Arena Settings profile, after selecting the video file as a background image adjust the Aspect ratio (728x576).

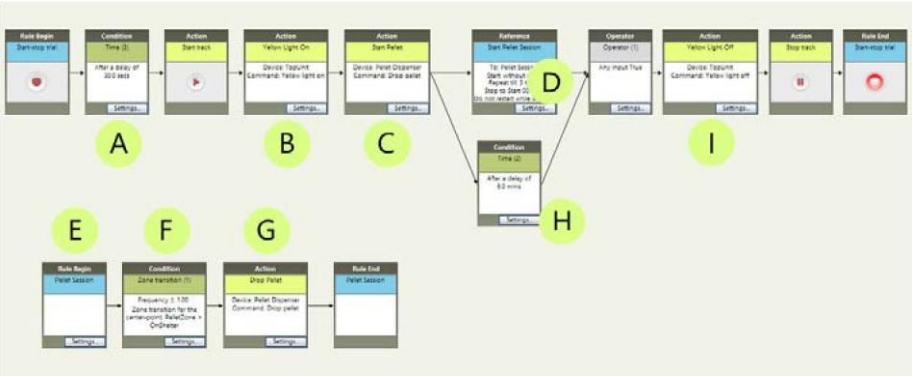
DETECTION SETTINGS

To detect the mice, the method named Dynamic Subtraction was used to compensate for the temporal changes in the background, caused primarily by the movement of the bedding material during the test.

TRIAL CONTROL SETTINGS

In the Trial Control Settings, the following procedure has been programmed. To view the details of actions and conditions, click the **Settings** buttons within the Trial Control boxes.

1. After a set time of 30 seconds (box A), tracking starts, at which moment the yellow light of the PhenoTyper top unit is turned on (box B) as a signal that a 'Pellet session' starts. A pellet is dropped that marks the start of the task ("Start Pellet"; box C). Next, the Reference 'Start Pellet Session' (box D) is activated. This starts the sub-rule 'Pellet Session' (box E). This sub-rule is repeated 3 times, according to what is specified in the Reference box.
2. In the sub-rule 'Pellet Session', every time the animal moved from PelletZone to the OnShelter zone (box F), a pellet is dropped (box G).
3. When this has occurred 3 times, or when 5 minutes have passed since the start of the track (box H), the yellow light in the top unit is switched off (box I) and tracking stops.



DATA ACQUISITION

Choose **Acquisition** > **Open Acquisition**. Ignore the message **There is no IO-box connected** and click **Continue**. You can acquire a new trial using the same video file **PhenoTyper hardware.mp4**. To start acquisition, click the **Start Trial** button.

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The sample project contains the following Data profiles:

- **All data**. The default Data profile that selects all tracks in the experiment.
- **All data - Results per zone**. When this Data profile is active, the dependent variables are calculated for each selected zone separately.

ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile** > **Open**. The sample project contains the following analysis profiles:

- **In and On shelter and in pellet zone**. This profile contains the variables **Inside shelter**, **On shelter** and **In pellet zone**. With these variables you can calculate the frequency and duration of the time spent in each zone and also the latency of entering each zone. The fourth variable, **Drop pellet**, is a variable of type *Hardware command*. With this variable you can visualize and calculate the number of pellets dropped by the Pellet dispenser (see also the figure under **integrated visualization**).
- **Total distance moved and mean velocity**. This profile contains the variables **Distance moved** and **Velocity**. With this Data profile the mean distance moved and mean velocity is calculated.
- **Time to complete the task**. Consider the following variables:
- **From first to third Drop Pellet**. This is a Trial Control state based on the events "Drop pellet" in the subrule. You can use it for example to visualize and calculate the time from the first and the third Drop pellet event.
- **Time to complete the task**. Unlike the variable above, this is a free interval, which starts from the first event "Start Pellet" to the last "Drop Pellet" of the Pellet session (subrule). It is a measure of the time to complete the task.

ANALYSIS

Choose **Analysis** > **Results** > **Statistics and Charts**. Select the Analysis profile **In and On shelter and in pellet zone** and the Data profile **All data**. Locate the columns:

- **Inside shelter**. Here you find how often and how long the mice were in the shelter.
- **Drop pellet**. Here you can see how many commands "Drop a pellet" have been given for each subject.
- **On Shelter**. Under **Latency to Last**, you find the time that the subject reached the top of the shelter for the last time. This is a measure of the time taken to complete the task. you can see that it took the animal in Arena 1 119.6 s to get its last reward, whereas the

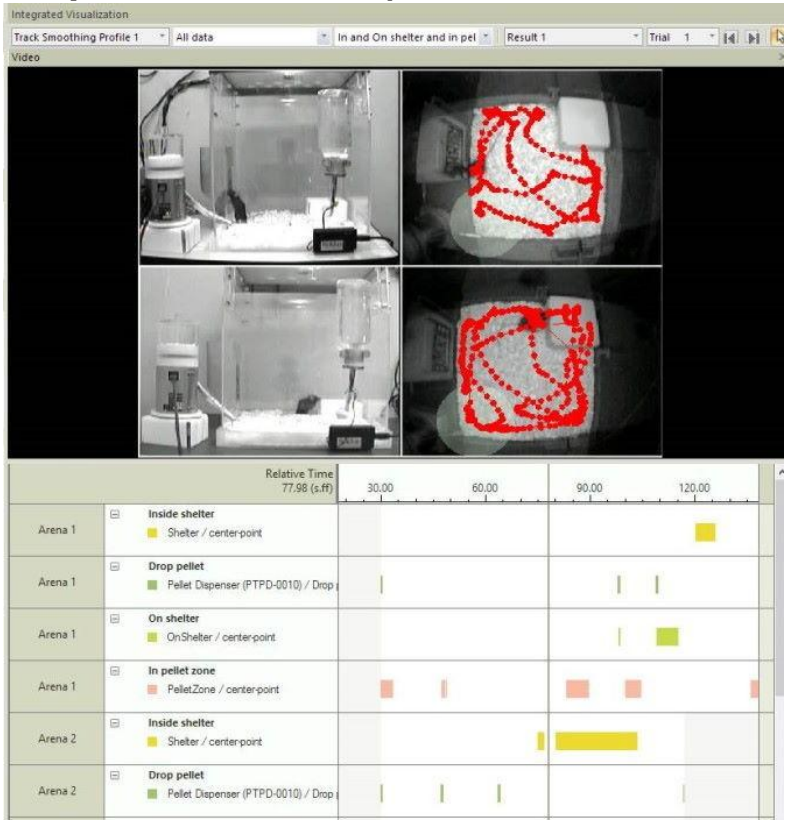
animal in Arena 2 needed 86.9 s. Hence, the animal in Arena 1 was slower than the animal in Arena 2 at getting the three rewards.

- **In pellet zone**. Locate this column in the analysis results table, and note the difference between the subjects in Arena 1 and Arena 2 in the time spent in the pellet zone.

Now select the Analysis profile **Total distance moved and mean velocity** from the toolbar. The results table shows the total distance moved and the mean velocity of the two mice. When you select the Data profile **Results per Zone** from the toolbar, the table is updated and shows the total distance moved and the velocity in each of the zones.

INTEGRATED VISUALIZATION

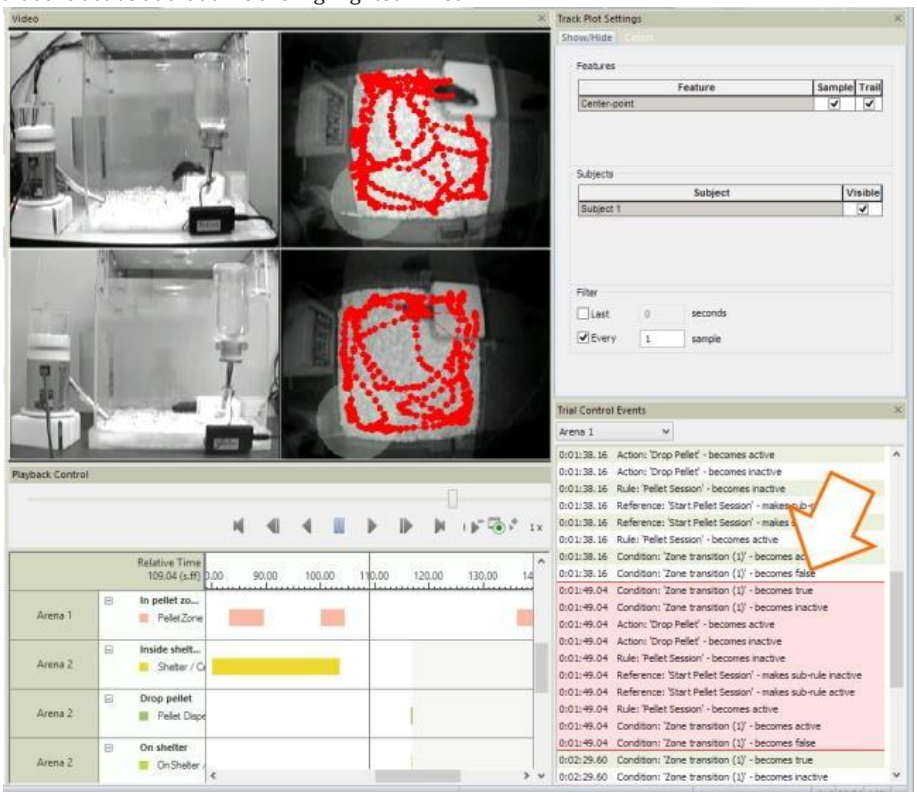
Choose **Analysis** > **Results** > **Plot Integrated Data**. Choose the Data Profile and Analysis Profile from the lists on the toolbar. The visualization of Analysis profile **In and On shelter and in pellet zone** is shown in the next picture.



At the bottom-right corner of the screen the **Trial Control Events** panel shows you when the trial control events took place, for example when a condition became true or a pellet drop was performed.

If you do not see this list, at the top-right corner click **Show/Hide** and select **Trial Control Events**.

Double-click any row in the Trial Control Events panel. The events (conditions and actions) that are active at that time are highlighted in red.



ACKNOWLEDGEMENT

The experiment was made by Coen van Kaam with the help of Raymond de Heer (Delta Phenomics).

DanioVision with 96 wells XT190

OVERVIEW

This is an example of an experiment carried out with a DanioVision system. In this experiment, 96 zebrafish larvae were tracked in a well-plate. One minute after the start of tracking, using trial and hardware control, a light stimulus was turned on for 1 minute. After this minute, the light was turned off again for 1 minute and then tracking stopped. During live acquisition, the Trial Control protocol White Light Routine (see page 49) ensures that the stimuli are given at the right time. You can then compare several behavioral endpoints between the three phases of the trial (between light; during light; after light). To assess very rapid movements of the larvae, a camera frame rate of 60 frames per second (fps) was used.

Media file

DanioVision 96 wells.avi

EXPERIMENT SETTINGS

In the original experiment, the **Video Source** was **Live tracking** and **Use of Trial Control hardware** was selected. The experiment was then set to track **From video file** so you can acquire your trials from the video file, using your own Arena, Trial Control and Detection settings.

NOTE If you open Acquisition, a message appears saying that EthoVision XT cannot find the DanioVision connected. Click **Continue** to go on with data acquisition.

TRIAL LIST

At the top-right corner, click **Show/Hide** and select **Variables**. Choose to view:

- **Treatment** was created as a user-defined variable with the predefined values **Treated** and **Control**.
- **Subject not found** and **Missed samples**. These are system variables. **Subject not found** is almost always 0% for Trial 1, which means that the subject was always found in each well, and for each frame of the video. The **Missed samples** is always 0%, which indicates that no sample was skipped.

ARENA SETTINGS

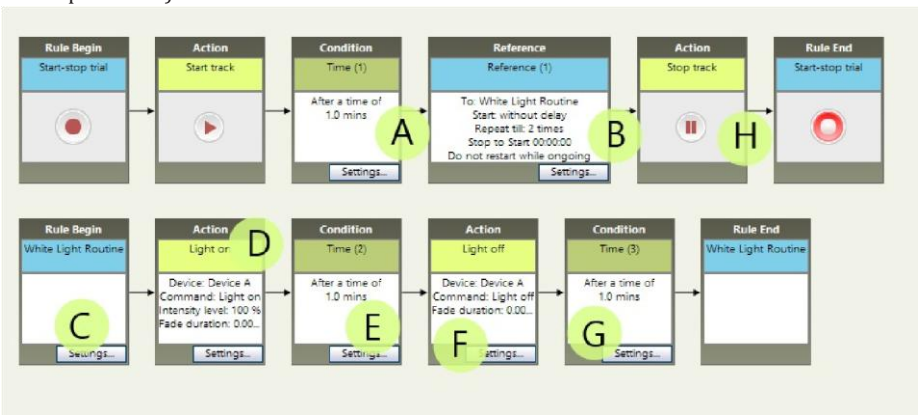
Ninety-six arenas were created for the well-plates. In the **Arena - Hardware Mapping** window, the DanioVision White Light was specified.

NOTE Creating a zebrafish experiment is facilitated by the automatic detection of the wells. See the DanioVision DVOC-0041 - Reference Manual.pdf for details.

TRIAL CONTROL SETTINGS

This sample experiment contains two Trial Control settings:

- **Default**. This is the default Start-Stop trial control rule. You can use this to acquire data without controlling the DanioVision White Light.
- **White Light Routine**. This was used for acquiring Trial 1. The following procedure was programmed:
- After a time of 1 minute (**Condition** box "Time (1)", see A in the figure below), a Reference (see the **Reference** box, B) activated the sub-rule **White light routine** (**Rule Begin** box, see C).
- In this sub-rule, the white light was turned on to 100% of its maximum intensity in 5 seconds (**Action** box "White Light On", see D) and was on for 1 minute (**Condition** box "Time (2)", see E).
- Next, the white light was turned off from 100% to 0% in 1 second (Action box "White Light Off", F).
- After a time of 1 minute (**Condition** box "Time (3)", G) tracking was stopped (**Action** box "Stop track" H).



TIP Click the **Settings** buttons to see the details of a Trial Control box.

DETECTION SETTINGS

The **DanioVision** method was used. A sample rate of 60 samples per second was used.

DATA ACQUISITION

Choose **Acquisition** > **Open Acquisition**. Ignore the message **There is no IO-box connected** and click **Continue**. You can acquire a new trial using the video **DanioVision 96 wells.avi**. To start acquisition, click the **Start Trial** button.

TRACK SMOOTHING PROFILES

Choose **Acquisition** > **Track Smoothing Profiles** > **Open**.

A track smoothing profile **MDM 0.2 mm** has been defined in which a **Minimal Distance Moved** (MDM) filter is used. This filter removes sample-to sample distances shorter than 0.2 mm. For the DanioVision set-up a Minimal Distance Moved filter of 0.2 mm is recommended for a good estimate of distance moved (swim path length). For details, see the DanioVision DVOC-0041 - Reference Manual.

If you want to analyze data without this filter, choose **Acquisition** > **Track Smoothing Profile** > **New**. In the new profile **Track Smoothing Profile 1**, all filters are disabled.

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The project contains the following Data profiles:

- **All data, Treated vs. Control**. Contains entire tracks, but split in two groups based on the value of the **Treatment** independent variable, *Treated* or *Control*.
- **Intervals based on White Light state**. This contains three separate **Result** boxes are defined. Using the Nesting function **Trial Control State**, the tracks are split in three intervals (each represented by a **Result** box):
  - **1. Before White light goes on**. This interval goes from the start of the track to the moment that the White Light On command was activated (box D in the figure).
  - **2. White light on**. This interval goes from when the command "White Light On" was activated (box D in the figure) to when "White Light Off" was activated (box F).
  - **3. After white light goes off**. This interval goes from when "White Light Off" was activated (box F) to the stop of the track (box H).

  In this Data profile, Treated and Control subjects are analyzed together.

- **Before vs. after White light on**. This is an example of how you can use the **Free Interval** function to select a segment of the tracks depending on a combination of time and events. With this Data profile you compare the two seconds immediately before the white light goes on and the two seconds immediately after it goes on. The Data profile also splits the data further based on control vs. treated subjects.

ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile** > **Open**. The project contains the following analysis profiles:

- **Distance moved and Velocity**. With the mean and total *Distance moved* and the mean *Velocity*.
- **Movement**. In this Analysis profile, the frequency, duration and latency of the behavioral state *Movement* are calculated for each subject, which is a measure of their locomotor activity. Use the Data profile **Intervals based on White Light state** to see in which time period the light was on (see the figure under **integrated visualization** below).
- **Rotation**. The variable CW Rotation counts the number of clockwise rotations, and CCW Rotation calculates the number of counter-clockwise rotations.
- **Turn angle**. To quantify the amount of turning (in degrees) per sample.

INTEGRATED VISUALIZATION

IMPORTANT Visualizing the video and analysis results for 96 subjects is very processor intensive. Close all other software running on your computer before taking this step.

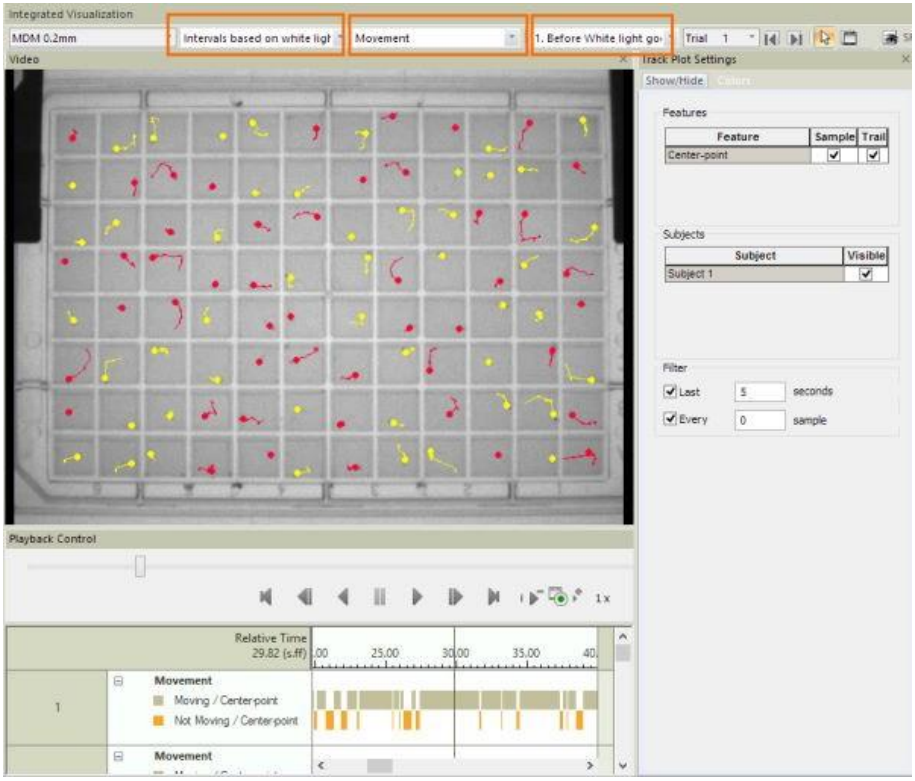
Choose **Analysis** > **Results** > **Plot Integrated Data**. Choose the Data Profile, Analysis Profile and Result from the lists on the toolbar.

The figure below shows the tracks and the variables when you select:

- The Data profile **Intervals based on white light state**.
- The Analysis profile **Movement**.
- The Result **1. Before White Light goes on**.

Yellow and red tracks are of Treated and Control subjects, respectively.

Choose another item from the third list to view the results for another interval. Note that the third list is only available if your Data profile contains two or more **Result** boxes.



STATISTICS

Choose **Analysis** > **Results** > **Statistics & Charts**.

When you open the **Group Statistics & Charts** tab and select the Data profile **Interval based on white light state** and the Analysis profile **Distance moved and Velocity**, the table shows the statistics of distance moved and velocity for each interval *1. Before white light on*, *2. White Light on*, and *3. After white light off*. The decrease in distance moved (swim path length) and velocity in the interval *2. White Light on* suggests that the white light reduced the activity levels of the larvae. A similar result can be seen when you select the Data profile **Before vs. after White light goes on**.

Furthermore:

- Select the Data profile **Intervals based on white light state** and the Analysis profile **Rotation** or **Movement**. There you see that rotation and frequency of movement

significantly decreased after turning on the white light, and strongly increased after turning it off again.

- Select the Data profile **All data, Treated vs. Control** and the Analysis profile **Rotation** or **Turn Angle**. You see some difference between *Treated* and *Control* subjects in the average values of rotation (clockwise and counterclockwise) and turn angle, although this difference is probably not statistically significant.

ACKNOWLEDGEMENT

M. ter Veld (The Aquaculture and Fisheries Group, Animal Sciences Group, Wageningen UR, The Netherlands) is gratefully acknowledged for supplying us with zebrafish larvae.

FOR MORE INFORMATION

See the DanioVision DVOC-0041 - Reference Manual.pdf. You can find this manual in **All Apps** > **Noldus** > **EthoVision XT 19 Other Documentation**.

DanioVision with camera zoomed into four wells XT190

OVERVIEW

This is an example of an experiment carried out with a DanioVision system using a camera with a zoom lens. The image of a 96 well plate was zoomed in to four wells.

In this experiment, four zebrafish larvae (two controls, two treated) were tracked for about ten minutes. The aim of the experiment was to compare activity and movement parameters (including rotation frequency) in the two experimental groups.

Media file

DanioVision four wells.avi

EXPERIMENT SETTINGS

The **Video Source** was set to **From video file**. Because the four wells are independent replicates, the number of **Arenas** was set to **4** and the number of **Subjects** per Arena was **1**.

TRIAL LIST

Choose **Setup** > **Trial List**. A user defined variable *Treatment* is defined with the predefined values *Treated* and *Control*. Each subject is assigned either value.

ARENA SETTINGS

Four arenas were created for the well-plates. Each arena was divided into a border zone and a center zone.

TIP In EthoVision XT, creating a zebrafish experiment is facilitated by pre-defined templates in which settings, including arena settings, have already been made to enable you to set up an experiment more quickly. Also, adjusting arenas is easy with the Multiple Arena Setup.
See the EthoVision XT Help for details.

TRIAL CONTROL SETTINGS

In the Trial Control Settings profile named **Default**, tracking was set to start as soon as the trial was started, that is, when one clicks the **Start trial** button. Tracking lasts until the end of the video file.

DETECTION SETTINGS

In the Detection Settings named **Detection Settings 1**, the **DanioVision** method was selected. To remove some noise the subjects' contour was filtered using the options under **Subject Contour: Erosion (1 pixel)** followed by **Dilation (1 pixel)**.

TRACK SMOOTHING PROFILES

Under Track Smoothing Profiles, besides a profile named **No Smoothing** with no smoothing selected, a second profile, **MDM 0.2 mm** is defined (MDM = Minimal Distance Moved). When you use this track smoothing profile, data points with a distance from the previous point of less than 0.2 mm are set to the previous position. This removes a large part of the false movements of the subject's center point which do not correspond to actual movement of the fish. We recommend using this filter for the DanioVision set-up.

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The experiment has the following Data profiles:

- **All Data**. The default Data profile with all the data.
- **Treated vs. Control vs. All data**, **with time bins**. To compare treated and control animals in 1-minute intervals.
- **Treated vs. Control vs. All data, no time bins**. To compare treated and control animals throughout the whole test duration.

For both **Treated vs. Control** Data profiles, the **Result** box **All data** allows you to view the results when pooling the data of the two treatment levels.

ANALYSIS PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The experiment contains four Analysis profiles:

- **Distance, Time & Movement**. In this Analysis profile, the total Distance Moved and the mean Velocity are calculated. Also, the frequency, duration, percentage of time spent and latency of the behavioral state Movement are calculated for each zebrafish larvae, which is a measure for their activity.
- **Path shape**. In this Analysis profile, the path shape of the larvae is determined by calculation of the mean relative Turn Angle and the mean relative Angular Velocity.
- **Rotation**. In this Analysis profile, the variable CW Rotation counts the number of clockwise rotations, CCW Rotation calculates the number of counter-clockwise rotations.
- **In zone** - In this Analysis profile, the time until the first visit and the frequency, duration and percentage of time spent are calculated for the center zone and border zone.

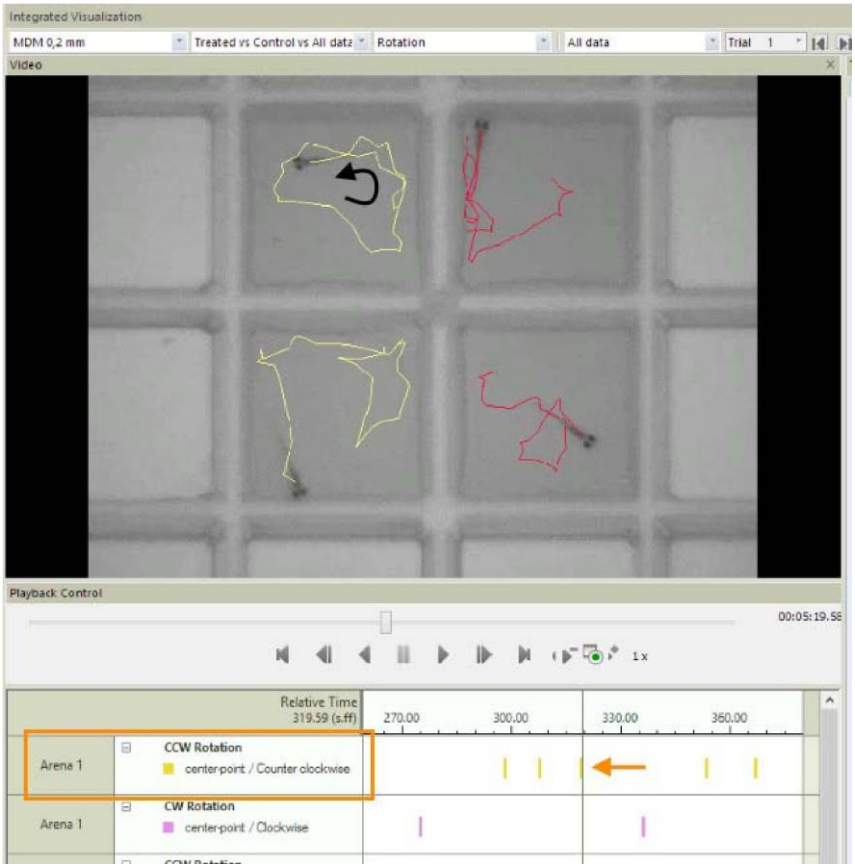
INTEGRATED VISUALIZATION

Choose **Analysis** > **Results** > **Plot Integrated data**.

On the toolbar you can choose which of the treatments to display (*Treatment*, *Control* or *All data*). You can also choose other Analysis profiles, Data profiles or track smoothing profiles from the lists on the toolbar.

The next figure shows the visualization of the Analysis profile **Rotation**. The animal in Arena 1 (top left in the video) has just completed a counter-clock wise (CCW) rotation. This is marked with the vertical segment in the first plot, **CCW Rotation**.

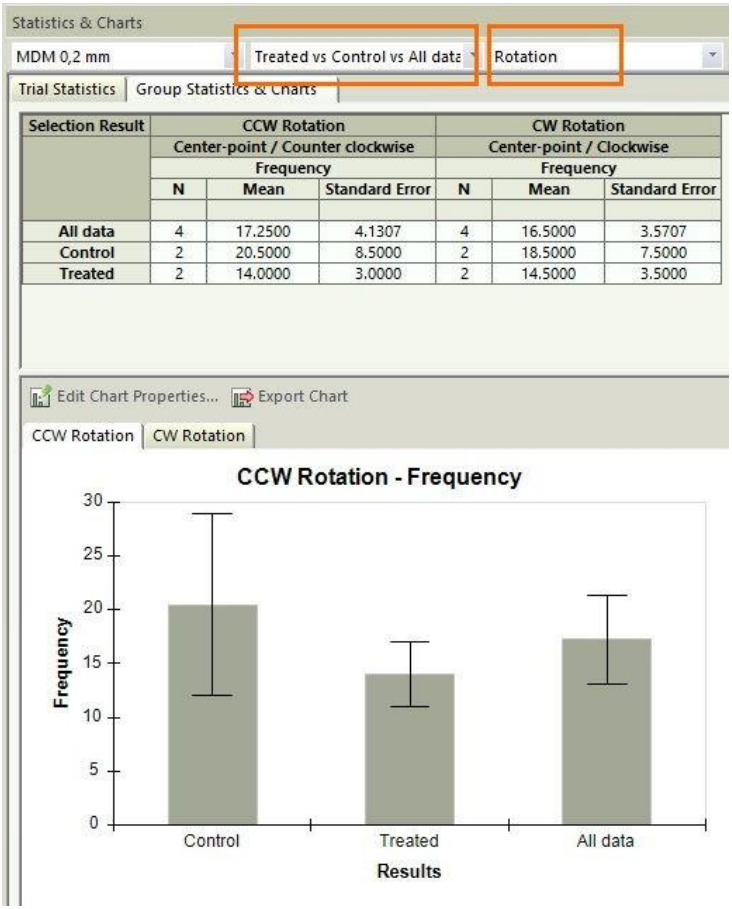
Tracks of Control and Treated subjects are visualized in different colors. To change colors, click the Colors tab on the right-hand panel.



ANALYSIS

Choose **Analysis** > **Results** > **Statistics & Charts**, then click the **Grouped Statistics and Charts** tab. Select the Data profile and the Analysis profile you want to use from the toolbar.

For example, select the Data profile **Treated vs. Control vs. All data no time bins**, and the Analysis profile **Rotation**. The counts of rotations are shown for the two treatment groups separately and for all the trials together.



Selection Result	CCW Rotation			CW Rotation		
	Center-point / Counter clockwise			Center-point / Clockwise		
	Frequency			Frequency		
	N	Mean	Standard Error	N	Mean	Standard Error
All data	4	17.2500	4.1307	4	16.5000	3.5707
Control	2	20.5000	8.5000	2	18.5000	7.5000
Treated	2	14.0000	3.0000	2	14.5000	3.5000

ACKNOWLEDGEMENT

M. ter Veld (The Aquaculture and Fisheries Group, Animal Sciences Group, Wageningen UR, The Netherlands) is gratefully acknowledged for supplying us with zebrafish larvae.

FOR MORE INFORMATION

See the DanioVision DVOC-0041 - Reference Manual.pdf. You can find this manual in **All Apps > Noldus > EthoVision XT 19 Other Documentation**.

Barnes Maze test with Deep Learning XT190

OVERVIEW

This sample experiment shows you how you can use the dependent variable **Target visits and errors**, to analyze the animal's path in a memory test paradigm where certain zones are defined as targets. For more information, see the EthoVision XT Help.

In a Barnes maze experiment, a mouse or rat tends to escape from the brightly lit surface of the maze. One of the holes gives entrance to an escape box, which is a dark shelter under the hole board. The animal is trained to find the escape box. During this *training* phase, the number of visits to other holes should decrease rapidly, which is a measure for learning ability. In the *probe* phase, the escape box is removed. The time to reach the hole where the escape box previously was is determined, together with the number of visits to other holes and the total distance moved.

In this example, three white mice were tracked during training trials. EthoVision XT uses neural networks to find the nose and the tail base of the subjects. In this experiment you will see that the nose point is found correctly even when part of the body of the subject is not detected as the mouse walks near the white patches.

Media files

- Trial 1.mpg
- Trial 2.mpg • Trial 3.mpg

EXPERIMENT SETTINGS

- The **Video Source** was set to **From video file**.
- As **Tracked Features**, **Center-point**, **Nose-point**, **and Tail-base detection** is selected.
- Under **Body Point Detection Technique**, **Deep learning** is selected.

  NOTE In order to use Deep learning, your PC must have a compatible graphics card (GPU) and its drivers must be up-to-date. Consul the EthoVision XT Help and contact Noldus to purchase a compatible graphics card.

ARENA SETTINGS

Since the position of the holes is slightly different in the three videos, the project contains separate arena settings for each video. Each arena settings contains:

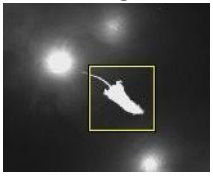
- A zone group called **Holes**, with zones **H1** to **H18**. These are zones that cover the holes. H1 to H17 are non-target holes. H18 is the zone of the escape box. The zone H18 is used for analysis of the time that the nose point was inside the zone, just like for H1 to H17.
- **Non-target holes**. This is a cumulative zone with the zones H1 to H17. You can use this zone to calculate and visualize the total time spent exploring all non-target holes.
- A hidden zone group with the zone **Target hole**. This is a hidden zone that covers the hole with the escape box. It covers the same area as zone H18, but it is used during acquisition. When the mouse enters the entry zone, and then disappears, EthoVision XT considers it to be in the hidden zone and stops the trial. The entry zone is a ring around the target hole. See the EthoVision XT Help for more information about hidden zones.

TRIAL CONTROL SETTINGS

The experiment contains trial control settings *Max. track duration 3 min*. The track starts when the mouse was detected in the arena for two seconds. It ends either after three minutes, or when the mouse has been in the hidden zone *Target hole* for one second.

DETECTION SETTINGS

Under **Advanced**, **Dynamic Subtraction** is used with the appropriate contrast (Subject color **Brighter than background**) to detect a mouse with white fur. Tn the Deep learning settings, the bounding box should include some space between the mouse and its edges. For example:



TRIAL LIST

The trial list contains the following user-defined variables:

- **Treatment**. This variable indicates which experimental group the mouse belongs to. It has the predefined values *Treated* and *Control*.
- **Animal ID**. This numerical variable identifies the mice, for example 1, 2, 3, etc.
- **Type of Trial**. This variable indicates the type of trials and has the predefined values *Training* and *Probe*.

TRACK SMOOTHING PROFILES

The experiment contains two profiles:

- **No smoothing applied**. Select this profile to view the unfiltered data points.
- **Lowess smoothing On**. With this profile, the data points are filtered using the Lowess method (recommended; for details. see the EthoVision XT Help).

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The project contains two Data profiles.

- **All data**. This is the default Data profile that contains all tracks.
- **Treated vs. Control**. The trials are grouped, based on the independent variable *Treatment*. Two groups are created:
- **Treated**, with one trial.
- **Control**, with two trials.

ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile** > **Open**. The project contains two analysis profiles.

- **Distance & Velocity**. with the total distance moved and the mean velocity.
- **Hole visits**. This profile contains two variables.
- **Target visits and errors**. Double-click this variable to see how it is set up.

  Under **Target zones**, the **Target hole** is selected.

  Under **Non-target zones**, the holes **H1** to **H17** are selected (note: do not select **Other holes together**. You can use this zone in the variable **Hole exploration time**; see below).

  On the **Body points** tab, **Nose-point** is selected, that is, a visit is calculated when the nose point is detected within the corresponding zone.

With this variable you can calculate (1) the number of visits to the targets (including revisits), (2) the number of visits to non-target holes, (3) the latency to the first visit to the target holes. In probe trials, revisits to target holes and all visits to non-target holes are considered as errors.

Note that this variable also includes a **Zone exit threshold**. That means that, when the nose-point is just outside one of the hole zones, but within the threshold distance, the nose-point is still considered in the zone. Therefore, the zone visit only ends when the nose-point is found further than the threshold distance. You can use the Zone exit threshold to avoid that multiple nose pokes are considered as separate visits.

- **Hole exploration time**. This is an instance of the dependent variable In zone. You can use this variable to calculate the total time that the animal's nose was detected inside each hole, or all holes together. For this purpose, the cumulative zone **Other holes together** is also selected in the variable's settings (see under **In the following zones**).

INTEGRATED VISUALIZATION

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

On the toolbar you can choose which of the trial types to display (**Treated vs. Control** or **All data**). You can also choose Analysis profiles, or Track Smoothing profiles from the lists on the toolbar. The first plot shows the target visits and errors. The second plot shows the hole exploration time.

TRIAL STATISTICS

Choose **Analysis** > **Results** > **Statistics & Charts**. Select the Data profile and the Analysis profile you want to use from the toolbar.

For example, when selecting the Data profile **Treated vs. Control** and the Analysis profile **Hole visits**, you can compare the number of errors made by treated and control mice.

When you select the analysis profile **Distance & Velocity**, you can compare the distance moved and the speed of the subjects.

ACKNOWLEDGEMENT

Video files were kindly provided by Dr. Lior Bikovski, director of the Myers Neuro-Behavioral Core Facility, Sackler School of Medicine, Tel Aviv University, Tel-Aviv, Israel; and School of Behavioral Sciences, Netanya academic college, Netanya, Israel.

Mouse Behavior Recognition in Open Field XT190

OVERVIEW

The aim of this sample experiment is to demonstrate how EthoVision XT automatically detects various behaviors of mice, like rearing, walking and sniffing.

Media file

Mouse Behavior Recognition in Open Field.mpg.

Note

To work with the Behavior Recognition function, you need the **Mouse Behavior Recognition** Module. For information about performance and the limitations of the Behavior Recognition function, see **Behavior Recognition** in the EthoVision XT Help. See also the paper by van Dam et al. (2013). An automated system for the recognition of various specific rat behaviours. *Journal of Neuroscience Methods* **218**,(2), 214–224.

EXPERIMENT SETTINGS

Under **Analysis Options**, **Behavior Recognition** is selected. The options are selected that apply to the video, thus **Arena walls** is selected, not **Feeder** and **Drinking bottle**, which are not present in the cage. For the same reason, the additional options available when clicking **Settings** are not selected.

ARENA SETTINGS

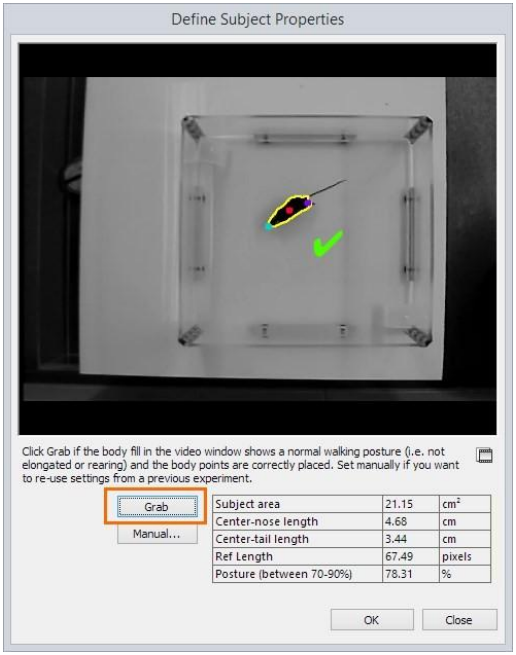
In the **Arena Settings**, two zone groups are defined:

- **Zone Group 1**, with two zones, Center and Border.
- **Wall Zones**, with a zone called **Wall Zone 1**. This zone is important for automatic detection of rearing. It is defined by drawing a polyline shape around the open field floor, excluding the Plexiglas blocks at two of the corners. Note that the zone label is placed outside the shape, and points to the walls.

DETECTION SETTINGS

A **Detection Settings** profile has been defined:

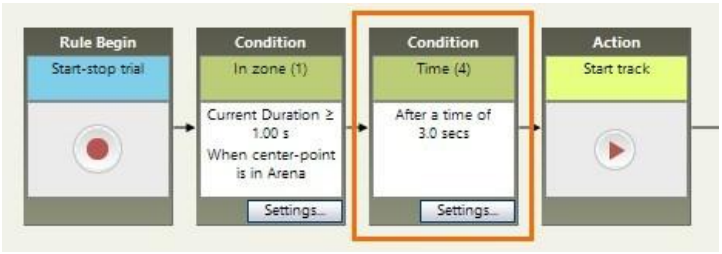
- Under **Video**, the **Sample rate** is set to 29.97, the maximum for that video. Behavior Recognition works with a sample rate of 25 samples/s or higher.
- Under **Advanced**, **Contour erosion/dilation** is set to the minimum. If you create your own detection settings, we advise you not to increase contour erosion/dilation as this might affect recognition of subtle behaviors, like sniffing.
- Behavior settings are set under **Behavior Recognition**. If you create your own settings, click the **Define Subject Properties** button, play the video up to when the animal walks with a normal posture and average speed, and click **Grab**.



For details, see **Detection Settings for Behavior Recognition** in the EthoVision XT Help.

TRIAL CONTROL SETTINGS

The settings in **Trial Control Settings 1** specify that EthoVision XT waits three seconds after the mouse has been detected in the arena, before it starts actual tracking. This is because the Behavior Recognition function needs a few seconds of data before being able to detect behaviors.



Without the additional Time condition, the first three seconds of the track would not be scored.

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The experiment contains three Data profiles.

- **All data**. The default Data profile without any filtering or nesting criteria. Use this profile to analyze and visualize the whole data set.
- **When rearing**, **When sniffing**. These two Data profiles select the track segments in which *Rearing* (*supported* or *unsupported*) and *Sniffing* were detected, respectively. Use these Data profiles to produce heatmaps. The resulting heatmap shows where that behavior occurred.

HEATMAPS

Choose **Analysis** > **Results** > **Plot Heatmaps**. Select the Data profile **When grooming** or **When sniffing**. The heatmap indicates where that behavior took place.

ANALYSIS PROFILES

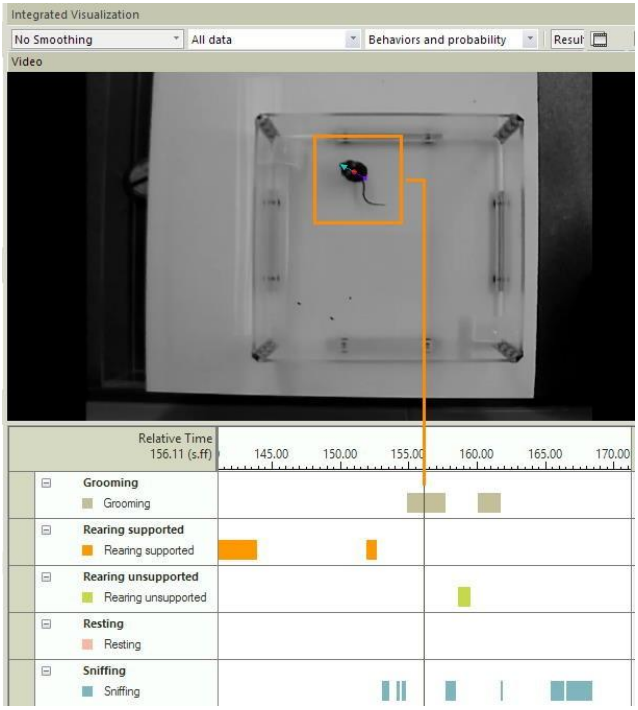
Choose **Analysis** > **Analysis Profile** > **Open**. The experiment contains two Analysis profiles.

- **Behaviors and probability**. Specifies all ten behaviors that can be detected. Behaviors are analyzed with the default settings. The last item in the list is **Behavior probability** of the various behaviors. Note that the behavior scored at a certain time is the one with the highest probability. You can modify the Analysis profile in such a way that a behavior is only scored when its probability exceeds a specific value, for example 90%.
- **Rearing (merged)**. This is an example of how you can analyze two behaviors as one. *Rearing supported* and *Rearing unsupported* are merged in one behavior, renamed *Rearing (merged)*. The *Behavior probability* of the two Rearing behaviors is also specified.

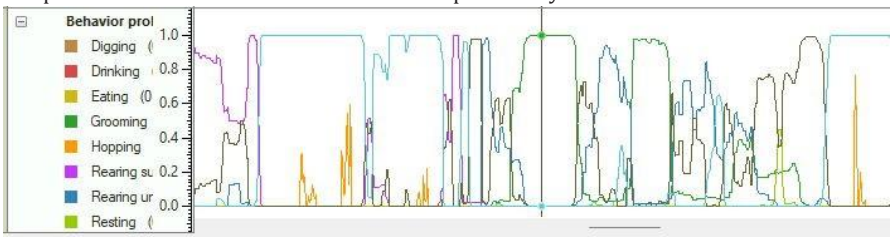
INTEGRATED VISUALIZATION

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

- Select the Analysis profile **Behaviors and probability**. There you can see the instances of the behaviors detected.



The plot at the bottom of the screen shows the probability of the behaviors.



TIP Use Behavior probability to check whether some instances of behavior have low probability. To remove those instances, in the Analysis profile double-click that behavior and under **Behavior decision method** specify **Probability greater than**.

- Select the Analysis profile **Rearing (merged behaviors)** to see how different behaviors are merged in one category.

ACKNOWLEGEMENT

Video was provided by Dr. T. van Groen, Dept. Cell Biology, University of Alabama, Birmingham, Alabama, USA.

Mouse Behavior Recognition in Social Discrimination XT190

OVERVIEW

The aim of this sample experiment is to demonstrate how EthoVision XT can automatically detect various behaviors of a mouse, like sniffing, digging and grooming.

Media file

Mouse Behavior Recognition in Social Discrimination.mp4.

Note

To work with the Behavior Recognition function, you need the **Mouse Behavior Recognition** Module. For information about performance and the limitations of the Behavior Recognition function, see **Behavior Recognition** in the EthoVision XT Help. See also the paper by van Dam et al. (2013). An automated system for the recognition of various specific rat behaviours. *Journal of Neuroscience Methods* **218**,(2), 214–224.

EXPERIMENT SETTINGS

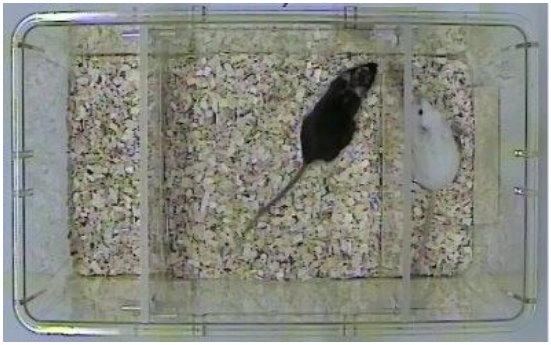
Although the video shows two mice in the same cage, only one was tracked. Therefore, under **Number of Subjects**, 1 is selected.

Under **Analysis Options**, **Behavior Recognition** is selected. The options are selected that apply to the video, thus **Arena walls** is selected, not **Feeder** and **Drinking bottle** which are not present in the cage. When clicking the **Settings** button, the additional option **Bedding present** is selected.

ARENA SETTINGS

The test cage has been divided in three compartments: a central compartment where the focal male mouse is confined, and two side compartments. In the right compartment a female mouse is placed to serve as a social stimulus.

In the Arena Settings, only the central compartment of the home cage has been defined as Arena, because that is where the focal subject is going to be moving. The other subject, confined in the right compartment, is not tracked.



A zone group **Wall Zones** is defined. It contains one zone called **Wall Zone 1**. This zone is important for automatic detection of rearing. It is defined by drawing a polyline shape around the open field floor. Note that the zone label is placed outside the shape, and points to the walls.

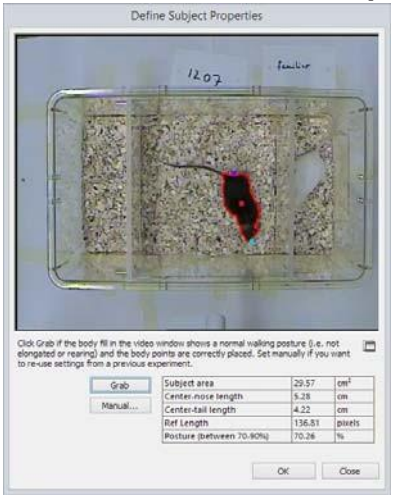
A zone group is also defined with two zones, **Social side** (right) and **Non-social side** (left). This way you can split your results based on the position of the nose point in one of the two zones. For example, to calculate the total duration of *Sniffing* when the mouse's nose was in the zone "Social".

NOTE If you have a similar experiment/apparatus and you want to exchange the social with the non-social sides, by placing the social mouse in the left compartment, you do not need to re-create the arena and zones. First, duplicate the Arena settings. Then, in the new Arena Settings, swap the labels of the two zones. Use the new Arena Settings for the trials when the social mouse is placed in the left compartment.

DETECTION SETTINGS

A **Detection Settings** profile has been defined:

- Under **Video**, the **Sample rate** is set to 25, the maximum for that video. This is compulsory for behavior recognition.
- Under **Advanced**, both **Contour erosion** and **Contour dilation** are set to 2. If you create your own detection settings, we advise you not to increase contour erosion/dilation as this might affect recognition of subtle behaviors, like sniffing.
- Under **Behavior Recognition**, you find the settings for the subject size and posture. If you create your own settings, click the **Define Subject Properties** button, play the video up to when the animal walks with a normal posture (e.g. 3:06.760), and click **Grab**.



For details, see **Detection Settings for Behavior Recognition** in the EthoVision XT Help.

TRIAL CONTROL SETTINGS

The settings in **Trial Control Settings 1** specify that EthoVision XT waits three seconds after the mouse has been detected in the arena, before it starts actual tracking. This is because the Behavior Recognition function needs a few seconds of data before being able to detect behaviors. Without the additional Time condition, the first three seconds of the track would not be scored.

ACQUISITION

During data acquisition, you can view live statistics of events and behaviors of the subjects. In the Acquisition screen, after starting a trial, click the Dependent Variables tab. There you can view how many times and for how long the mouse's nose was detected in the zone **Social side** (highlighted on the video window). To view more dependent variables, at the topright corner of the screen click **Show/Hide** > **Show Dependent Variable**.

DATA PROFILES

Choose **Analysis** > **Data Profile** > **Open**. The experiment contains three Data profiles.

- **All data**. The default Data profile without any filtering or nesting criteria. Use this profile to analyze and visualize the whole data set.
- **When sniffing**, **When digging**. These two Data profiles select the track segments in which *Sniffing* and *Digging* were detected, respectively. Use these Data profiles to produce heatmaps. The resulting heatmap shows where that behavior occurred. The Data profile **When sniffing** is further divided into two result boxes, one for each side of the cage. This means that you can analyze sniffing when the nose point was in one of the sides, Social vs. Non social.

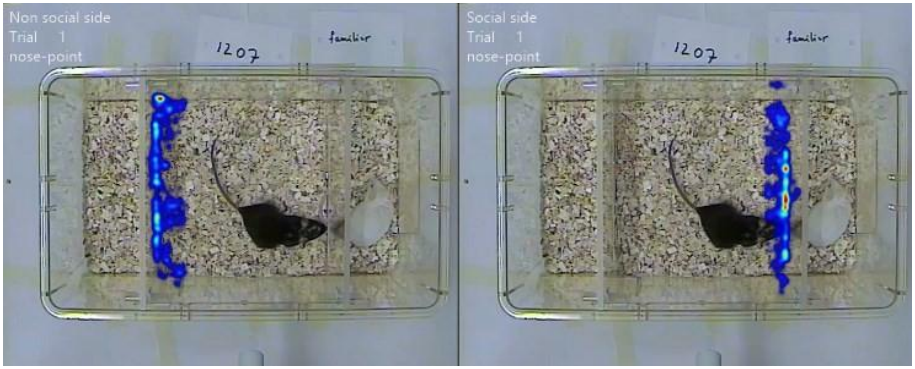
ANALYSIS PROFILES

Choose **Analysis** > **Analysis Profile** > **Open**. The experiment contains three Analysis profiles.

- **Behaviors and probability**. Specifies the behaviors that can be detected automatically. Behaviors are analyzed with the default settings (that is, using the criteria of the behavior recognition algorithm). In this sample experiment we focus on behaviors like *Digging*, *Grooming* and *Sniffing*. **Behavior probability** is defined for those behaviors but you can also select it for others. This gives you an idea of the level of confidence that EthoVision XT has when scoring a particular instance of one of those behaviors. Doubleclick this item and specify to view the probability of the remaining behaviors. The last item in the list is for when no behavior was scored; usually this is at the end of the video; to check this, see **integrated visualization**.
- **Sniffing**. This is an example of how you can filter instances of a behavior based on their probability and duration. It contains the same behavior, *Sniffing*, evaluated with two criteria: default and with probability higher than 75%. The second means that *Sniffing* is scored if the probability of *Sniffing* is higher than 75%. You can set the threshold probability in the variable settings, under **Behavior Decision Method**. The second filter is specified under **Behavior Duration Threshold**. There you specify to include the instances of *Sniffing* longer than a certain threshold (here **0.20 s**). The third variable in this profile is the probability of *Sniffing* calculated for each sample.
- **Sitting still with nose in social zone**. This is an example of a *Multi condition* variable. It combines two dependent variables, *In zone* (Nose point in social zone) and *Movement* (Not moving). The thresholds for *Moving* and *Not moving* are 4.00 and 3.75 cm/s respectively. The analysis profile also contains the dependent variable *Velocity of center point*. If you plot the dependent variables in an integrated visualization, you can use the values of *Velocity* to fine tune the settings for *Movement*.

HEATMAPS

Choose **Analysis** > **Results** > **Plot Heatmaps**. Select the Data profile **When sniffing**. Since that profile only selects the samples when the animal was scored as *Sniffing*, the heatmap indicates where sniffing took place.



INTEGRATED VISUALIZATION

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

- Select the Analysis profile **Behaviors and probability**. There you can see the instances of the behaviors detected. The plot **Behavior probability** shows how the probability of a behavior changes with time. See the definition of probability in the **analysis profiles**.

  TIP Use this chart to check whether some instances of behavior have low probability (= high uncertainty). To remove those instances, in the Analysis profile double-click that behavior and under **Behavior decision method** specify **Probability greater than**.

- Select the Data profile **All data** and the Analysis profile **Sniffing** to see the effect of filtering instances of *Sniffing* based on probability.

- Select the Data profile **All data** and the Analysis profile **Sitting still with nose in social zone** to see when the animal was exploring the social zone without moving.

ACKNOWLEDGEMENT

Video was provided by Prof. Martien Kas, Department of Neurobiology, University of Groningen, The Netherlands.

Rat Behavior Recognition in PhenoTyper XT190

OVERVIEW

The aim of this sample experiment is to demonstrate how EthoVision XT can automatically detect various behaviors of a rat, like rearing, grooming, sniffing, drinking and eating, in a home-cage environment.

Media file

Rat Behavior Recognition in PhenoTyper.mpg.

Note

To work with the Behavior Recognition function, you need the **Rat Behavior Recognition** Module. For information about performance and the limitations of the Behavior Recognition function, see **Behavior Recognition** in the EthoVision XT Help. See also the paper by van Dam et al. (2013). An automated system for the recognition of various specific rat behaviours. *Journal of Neuroscience Methods* **218**,(2), 214–224.

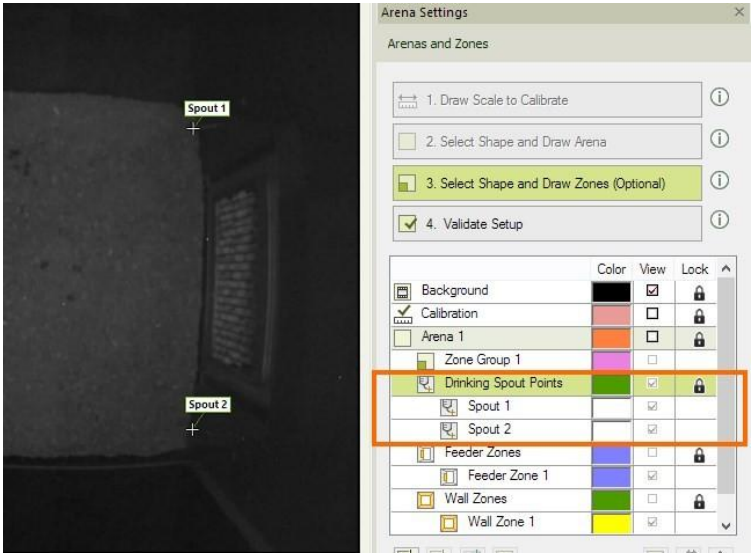
EXPERIMENT SETTINGS

Under **Analysis Options**, **Behavior Recognition** is selected. All options for Rat Behavior Recognition are selected: **Feeder**, **Drinking bottle** and **Arena walls**.

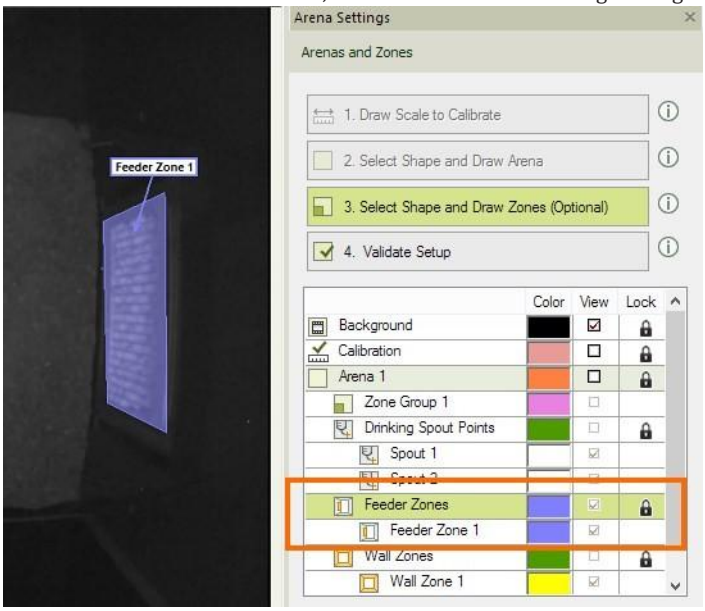
ARENA SETTINGS

In the **Arena Settings**, four zone groups are defined:

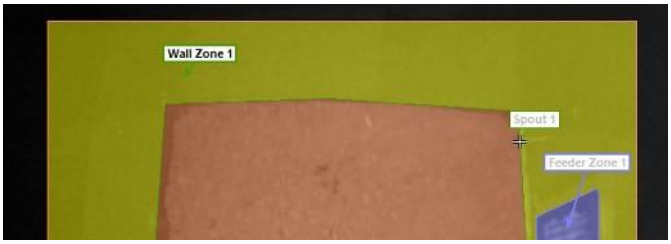
- **Zone Group 1**, with no zones defined. You can use this layer to define for example the Center and Border zones of PhenoTyper.
- **Drinking Spout Points**. Because PhenoTyper for rats has two drinking bottles, two drinking spout points are defined for detection of drinking, **Spout 1** and **Spout 2**.



- **Feeder Zones**. Includes one zone, **Feeder Zone 1** for detecting feeding behavior.



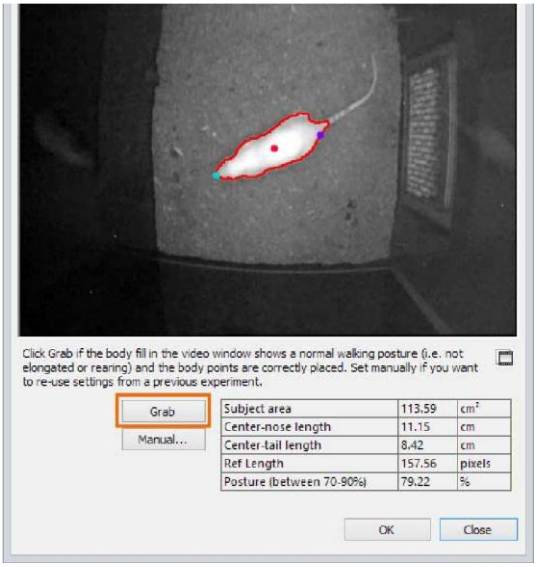
- **Wall Zones**. It includes one zone, **Wall Zone 1**. This zone is important for automatic detection of rearing at the walls. It is defined by drawing a polyline shape around the PhenoTyper's floor. Note that the zone label is placed outside the shape, and points to the walls.



DETECTION SETTINGS

A **Detection Settings** profile has been defined:

- Under **Video**, the **Sample rate** is set to 25, the maximum for that video. This is compulsory for behavior recognition.
- Behavior settings are set under **Behavior Recognition**. If you create your own settings, click the **Define Subject Properties** button, play the video up to when the animal walks with a normal posture, and click **Grab**.

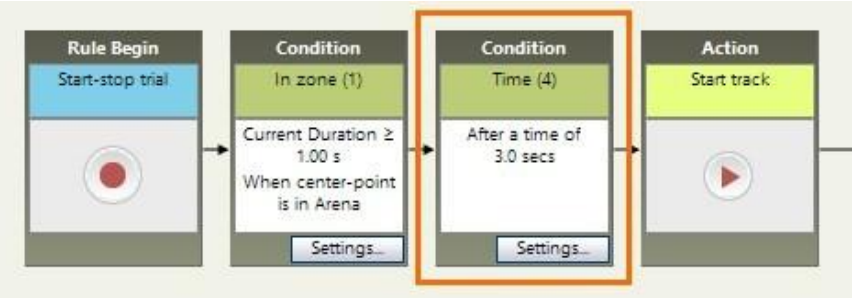


- Under **Advanced**, **Contour erosion/dilation** is set to the minimum. If you create your own detection settings, we advise you not to increase contour erosion/dilation as this might affect recognition of subtle behaviors, like sniffing.

For details, see **Detection Settings for Behavior Recognition** in the EthoVision XT Help.

TRIAL CONTROL SETTINGS

The settings in **Trial Control Settings 1** specify that EthoVision XT waits three seconds after the rat has been detected in the arena, before it starts actual tracking. This is because the Behavior Recognition function needs a few seconds of data before being able to detect behaviors. Without the additional Time condition, the first three seconds of the track would not be scored.



DATA PROFILES

Choose **Analysis** > **Data profile** > **Open**. The experiment contains two Data profiles.

- **All data**. The default Data profile without any filtering or nesting criteria. Use this profile to analyze and visualize the whole data set.
- **When sniffing**. This Data profile selects the track segments in which *Sniffing* was detected for more than half second (click the **Settings** button in the **Nest** box to view the settings). With this profile activated, you can create a heatmap to show where *Sniffing* occurred.

  TIP Choose **Analysis** > **Data Profile** > **New** and create a similar Data profile for when the rat was grooming or rearing.

ANALYSIS PROFILES

Choose **Analysis** > **Analysis profile** > **Open**. The experiment contains two Analysis profiles:

- **Behaviors and probability**. This Analysis profile contains all ten behaviors that can be detected, plus their probability (last item in the list). Some behaviors are analyzed with the default settings (that is, using the criteria of the behavior recognition algorithm), others with more restrictive criteria, for example when the behavior instance is longer than a certain time, or its probability is higher than 90%. Modify those settings and see what changes in the behavior visualization.

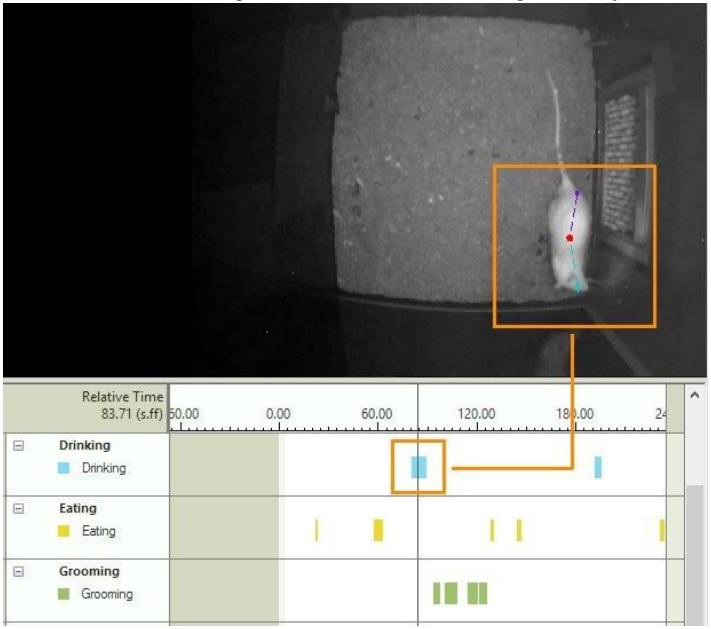
  TIP Click the item **Merged behavior** in the list of variables under **Behavior Recognition** and analyze two or more behavioral categories as one.

- **Distance and Velocity**. This profile contains two dependent variables, Distance moved and Velocity to quantify the overall activity of the subject.

INTEGRATED VISUALIZATION

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

Select the Analysis profile **Behaviors and probability**. There you can see the instances of the behaviors detected. The plot at the bottom shows the probability of the single behaviors.



TIP Use the time plot to check whether some instances of a behavior have low probability. You can specify to have the software ignore those instances as they contain some uncertainty of classification. To do so, in the Analysis profile double-click that behavior and under **Behavior decision method** specify **Probability greater than** and enter your threshold value (e.g. 90%).

HEATMAPS

Choose **Analysis** > **Results** > **Plot Heatmaps**. Select the Data profile **When sniffing**. The heatmap indicate where that behavior took place.

ACKNOWLEDGEMENT

Video was provided by Dr. Johanneke van der Harst, Delta Phenomics, The Netherlands.

Shoaling behavior with JavaScript XT190

OVERVIEW

This sample experiment provides an example of the capabilities of JavaScript code embedded in EthoVision XT, which you can use to further analyze your raw data. In this sample experiment a group of five zebrafish is tracked and the researcher wants to count how many fish are in each quadrant at any time, and quantify the time that all the fish swim together. For this, JavaScript code processes the raw data (position of each fish) and calculates the output variables (see Analysis profiles below).

Video file

Zebrafish shoal 5 fish.mp4

experiment settings

The experiment is set to offline tracking. Under **Video Source**, the option **From video file** is selected.

The center point of each fish is tracked, therefore under **Tracked Features**, the option **Centerpoint detection** is selected.

ARENA SETTINGS

The arena has been divided in four quadrants, named Zone 1 to Zone 4.

DETECTION SETTINGS

The method **Dynamic Subtraction** is used for body detection. The fish are not individually marked, so under **Subject Identification** the option **Unmarked subjects** is selected.

DATA PROFILES

- **All subjects**. This is the default Data profile containing all tracks.
- **One subject selected**. This profile only contains the data for the subject labeled **Subject 1**. Since the statistics of subject counts are applied to each subjects, and therefore generate

redundant results (for example, the mean number of subjects in zone 1 is 1.58 for Subject 1, 1.58 for Subject 2, 1.58 for Subject 3 etc.), this profile makes the results table simpler. Therefore, when you want to visualize the statistics of the number of subjects in the various zones, activate this Data profile.

ANALYSIS PROFILES

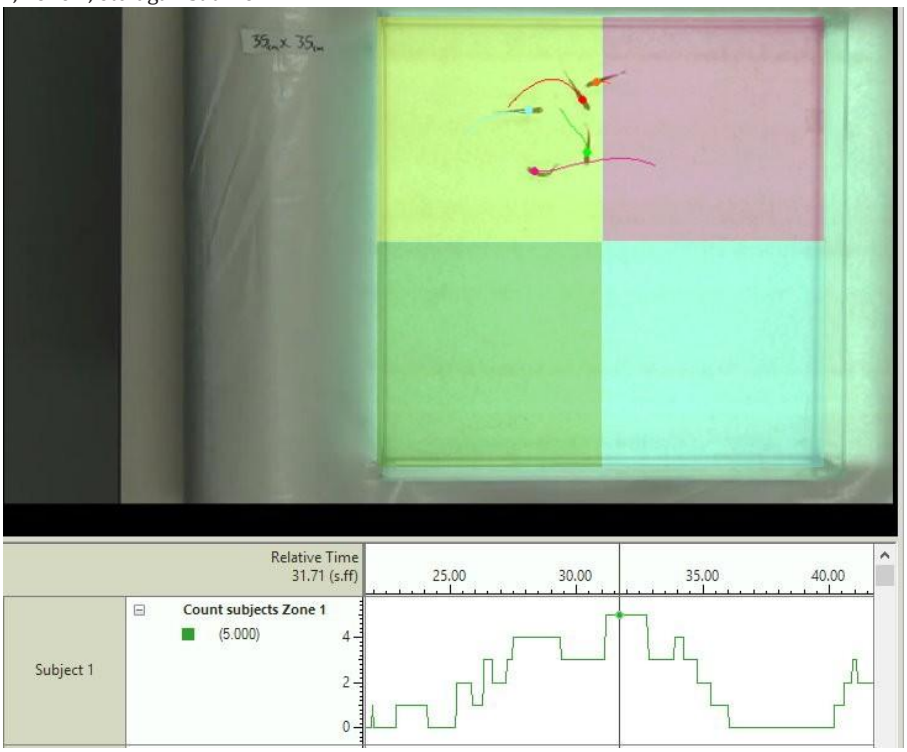
Besides the default Analysis profile with distance and velocity, four profiles have been created.

- **Distance and velocity**. Use this profile to calculate the total Distance moved and the average speed of each fish.
- **JS Continuous - Interindividual Distance**. This profile contains one JavaScript continuous variable that calculates the average distance of each subject from all the others. Results are shown per subject.
- **JS Continuous - Number of subjects in 4 quadrants**. This profile contains four JavaScript continuous variables. Here "continuous" means that the number of subjects in each zone is updated at each sample.
- **JS Continuous - Subject coordinates**. This is another example of the use of JavaScript code. The variables contain the x, y coordinates of the subject, respectively. Using JavaScript functions and commands you can create additional variables which process that data.
- **JS States - All fish in one quadrant**. The variables in this profiles are state variables, meaning that the value can be 0 of 1. Use state variables to calculate the time that the fish are all in a particular zone, or any zone, or the latency of the first occurrence of the event "all fish in a zone".
- **JS Continuous - Ratio of Subjects**. This is another example of how you can extract additional information from data like "subject in zone". A JavaScript variable calculates the ratio of the number of subjects swimming in Zone 1 to the total number of subjects. Because this is a JavaScript continuous variable, the ratio is updated at each sample.
- **JS Continuous - Nearest Neighbor Distance**. This profile contains two variables: (a) **Distance between subjects**, which outputs the distance of each fish from each of its mates, at every sample; and (b) **JavaScript Continuous - NND**, a JavaScript continuous variable that calculates the shortest distance from each subject to all the others, and for each sample.

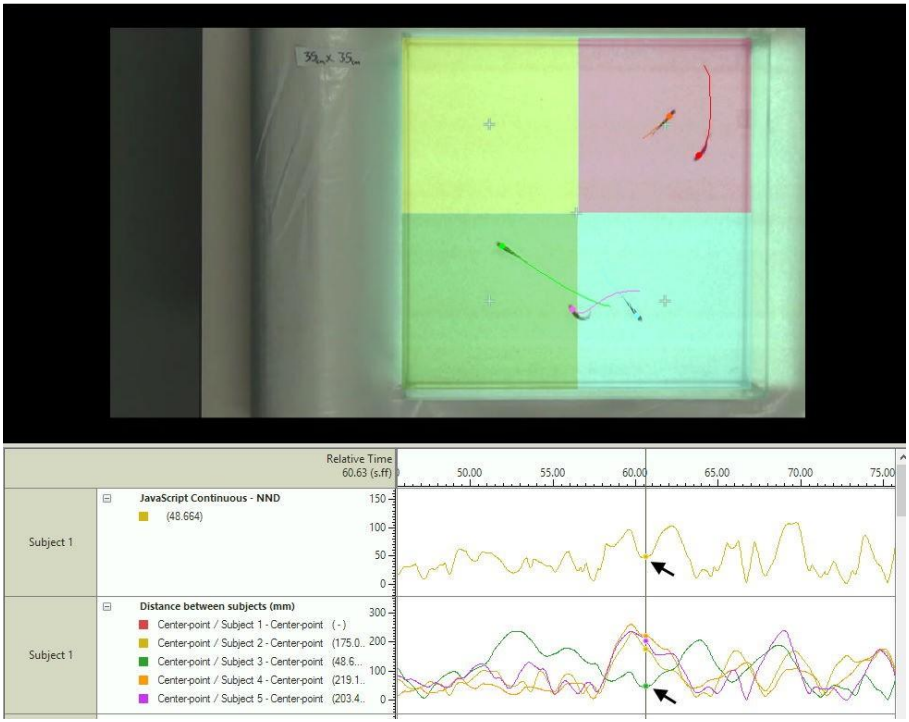
INTEGRATED VISUALIZATION

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

Select from the list on the toolbar: Data profile **One subject selected** and Analysis profile **JS Continuous - Number of subjects in 4 quadrants**. The plots show the number of fish in Zone 1, Zone 2, etc. against time.



Now select from the list on the toolbar: Data profile **All subjects** and Analysis profile **JS Continuous - Nearest Neighbor Distance**. For each subject, one plot, **Distance between subjects (mm)**, shows the distance of that fish from each of the other fish, plotted against time. The other plot, **JavaScript Continuous - NND**, shows the Nearest Neighbor Distance of that subject plotted against time. You can see that the value of **JavaScript Continuous - NND** at any time is always equal to the lowest value among those plotted in **Distance between subjects**.



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