

# EthoVision XT

Video tracking and analysis software



A solution by Noldus Information Technology

# AUTOMATED TRACKING AND ANALYSIS

EthoVision XT allows you to automate all standard behavioral tests such as the water maze, plus maze, and open field test.

EthoVision® XT is the solution for the automated tracking and analysis of animal activity and behavior, suited for everything from straightforward open field tests to high-throughput research.

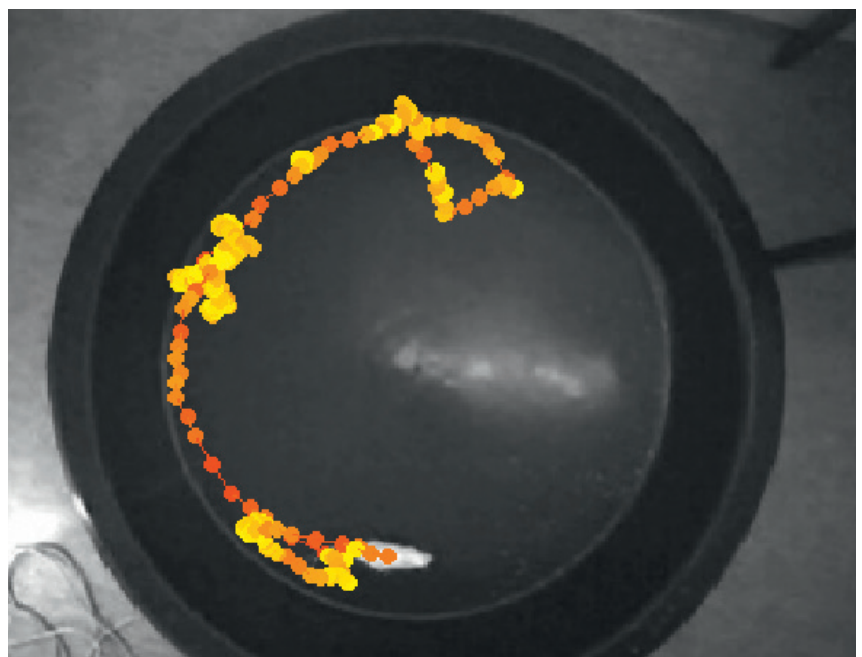
A versatile product in every sense of the word, EthoVision XT allows you to automate all standard behavioral tests such as the water maze, plus maze, and open field test.

## **ANY ANIMAL, ANY SETUP**

EthoVision XT is easily used to automate high-throughput experiments, such as monitoring zebrafish larvae activity in 96-well plates.

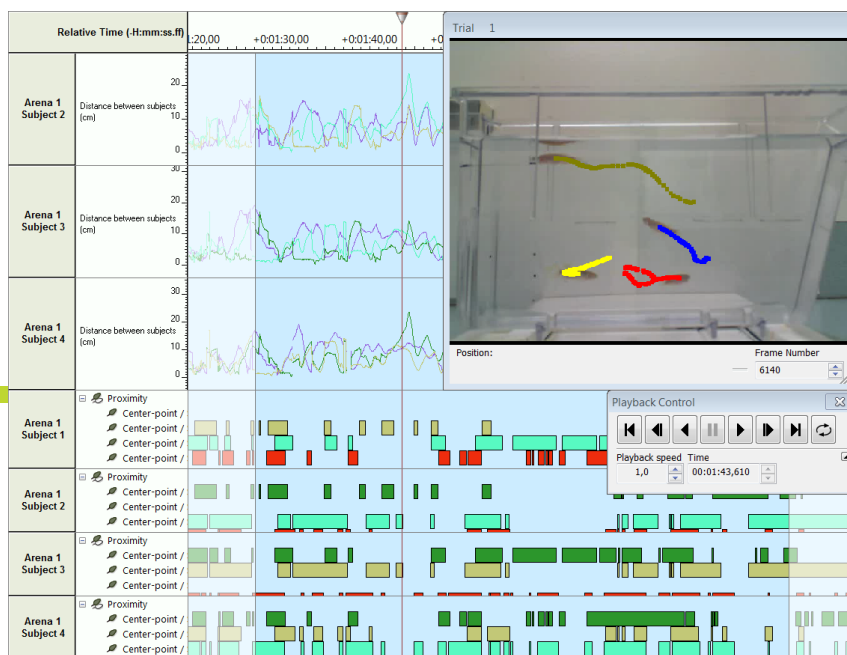
EthoVision XT can help in almost any step of your research. From setting up a trial list to the presentation of your results, templates and built-in tutorials make the program easy to use for first-time users, while extensive analysis options help you get most out of your data.

Analysis is made easy with EthoVision XT. You can link areas of interest within your arena to tracking parameters so you can easily answer questions such as:



*At the core of EthoVision XT:  
accurate tracking of any  
animal in any arena!*

*Integrated visualization shows your video, the tracking of the animals and a dynamic representation of your data in one view.*



What was the latency to enter the outer zone? How much time does the subject spend near the new object? Visualize your data in heatmaps, track plots, and graphs.

Dynamically plotted data streams are depicted alongside your video, which plays with superimposed tracking results to give you the best insight into your data.

In addition to video tracking, EthoVision XT also allows for activity detection, where frame-by-frame changes at the pixel level give you data on, for example, freezing behavior in rodents. Flip the analysis parameters to examine high levels of activity, such as those seen during seizure bouts.

# WHY USE ETHOVISION XT?

1

EthoVision XT is the industry standard with more than 7,500 peer reviewed publications and used at more than 2,000 sites worldwide

2

Automatic detection setting, superior detection methods, and powerful analysis

3

Cost effective solution

4

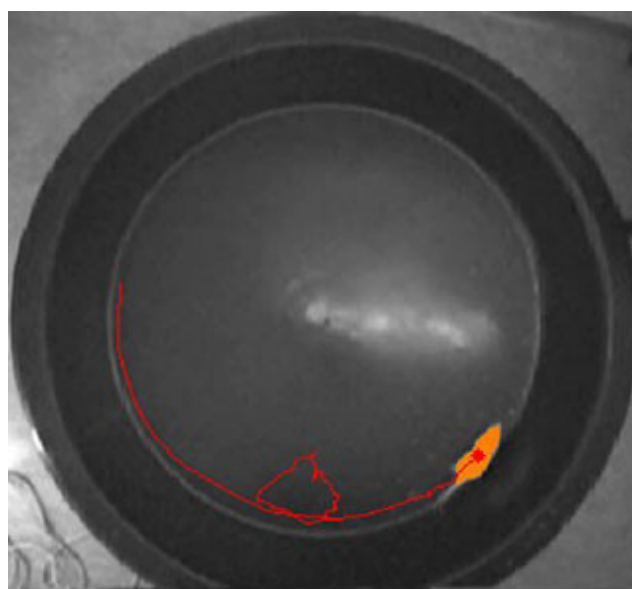
Flexible to fit your choice of hardware

5

Versatile to automate:  
AI standard behavioral tests and  
high-throughput research

# WHAT IS VIDEO TRACKING?

Video tracking means that EthoVision XT is able to detect an animal in a video file or from a live video feed, distinguish it from its background, and track its whereabouts and movements in x,y coordinates.



*Detection and tracking is at the core of EthoVision XT. The animal is detected, and from the tracking data, numerous parameters can be calculated.*

## ACTIVITY DETECTION

In addition to video tracking your animal, EthoVision XT can also detect activity. This means that the software detects changes at the pixel level from one frame to the next. This is useful to study specific behaviors such as freezing. You can use both a top and a side view camera position.

Another great advantage of activity detection is that it even works in situations where detection is difficult due to poor lighting or where there is little difference in contrast between the color of the animal and the background color (such as light subjects on a light background or dark subjects on a dark background).

# MANUAL EVENT RECORDER

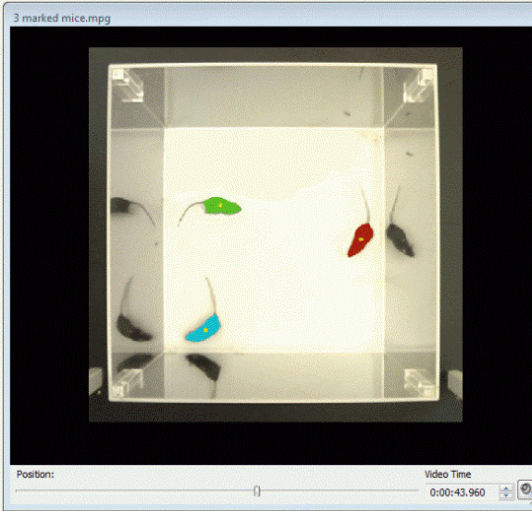
With the Manual Event Recorder (MER) you can:

- Score live and offline
- Edit scored events
- Score at reduced speed (frame by frame accuracy)
- Score point events (instances)
- Use scored behaviors for trial control

You can score behavior offline. This enhances scoring accuracy as you can slow down playback speed.

You can score behavior offline, which enhances scoring accuracy. This is because you can slow down playback speed. You can also edit manually scored events and change time-stamps simply by clicking and dragging the scored behavior across the timeline.

Scored behaviors can either have duration (start-stop behavior) or be point events. The latter is useful for behaviors that occur quickly (such as a bite or kick), or for when you only need to know the frequency of occurrence.



|                   | Arena 1   |           |           |
|-------------------|-----------|-----------|-----------|
|                   | Subject 1 | Subject 2 | Subject 3 |
| Trial start       | --:--:--  |           |           |
| Acquisition start | --:--:--  |           |           |
| Fill color        | Green     | Red       | Blue      |
| Play Behavior     |           |           |           |
| Pinning           | a         | b         | c         |
| Pouncing          | d         | e         | f         |
| Other             | g         | h         | i         |
| Grooming          | o/o       | p/p       | q/q       |
| Rearing           | v/v       | w/w       | x/x       |

# TEMPLATES AND TRIAL LISTS

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When starting your experiment in EthoVision XT, you can build it from scratch or use one of the predefined templates. These will save you a lot of time while setting up, for example while defining your arena(s).

Then the first step in your experiment is defining a trial list of all the test subjects. It will help you keep track of your experiment, and it is also useful later because you can use all the independent variables you define here (age, treatment group, gender, identification number, and more) in your data selection and analysis. You can copy this data from an existing database, such as Microsoft Excel, and enter it manually.

Build your experiment from scratch or save a lot of time using one of the predefined templates.

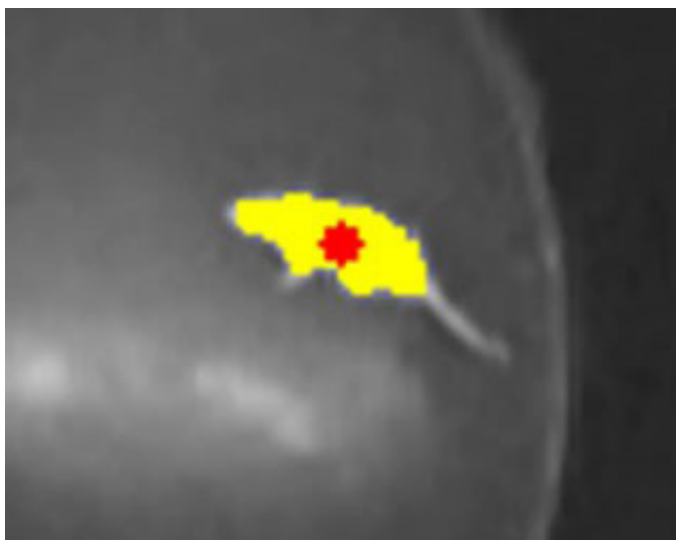


# DETECTION SETTINGS

Here is where we get to the core of EthoVision XT, because video tracking is only as powerful as the accuracy with which the animal is detected. The detection method is very important. There are several reasons why EthoVision XT detection is so powerful. One is that these settings are now completely automated. Another is that it is based on four different detection methods.



*Detection is recognizing the animal from its background.*



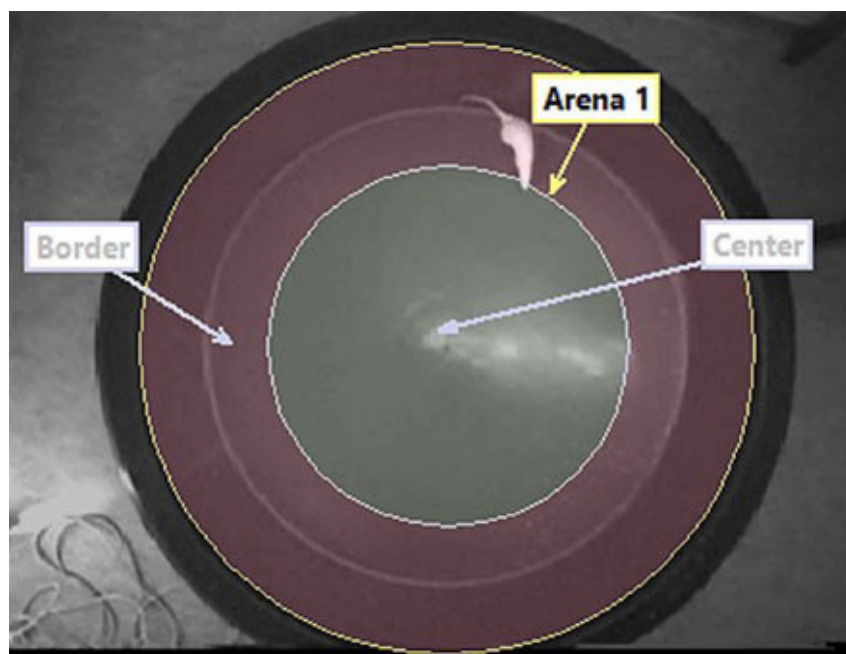
*EthoVision XT has very powerful detection methods and generates optimal tracking data.*



# ARENA DEFINITION

Use our intuitive drawing tools to indicate certain zones of interest, such as an inner and outer zone, where the shelter is, a feeding or drinking area, the arms of a maze, a novel object, etc.

EthoVision XT can track from a prerecorded video file or via live feed from a camera mounted above your arena. Of course, you want to define the area where the actual tracking should take place so that EthoVision XT is not picking up the hand of a researcher walking by, or a mouse in an adjacent cage. You also might want to indicate certain zones of interest, such as an inner and outer zone, where the shelter is, a feeding or drinking area, the arms of a maze, a novel object, etc. This is what we call arena definition, and with our intuitive drawing tools, this is easy to do. EthoVision XT uses all this information for data selection and analysis later on.



*Easily define the testing arena and zones of interest with the intuitive drawing tools.*

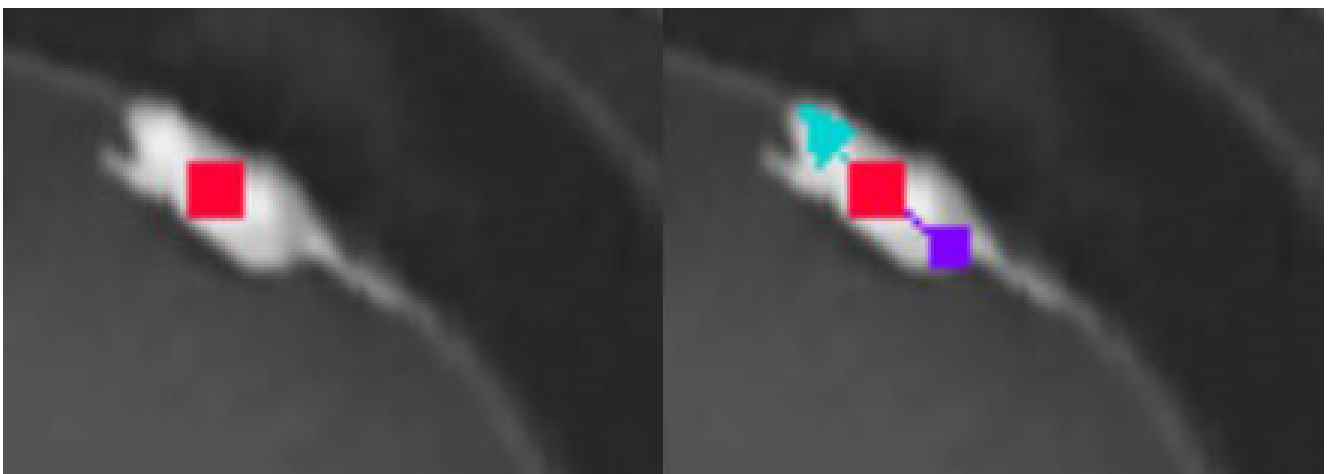
# METHODS OF TRACKING

EthoVision XT can track in two different ways: it either tracks

- the center of gravity of the animal, or
- the nose point, center point, and tail base of the animal.

The first method is suitable for any kind of animal, while the latter was developed for rats, mice, and zebrafish. The added accuracy of multiple body points tracking is useful in studies such as novel object recognition and social interaction.

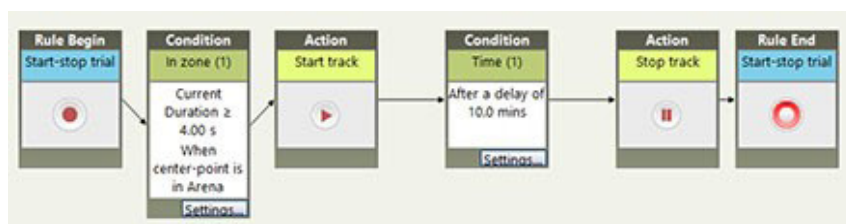
*Center point tracking (left) and multiple body points tracking (right). The added accuracy of the latter is useful in social interaction studies and novel object tests.*



# AUTOMATED START OF ACQUISITION

## TRIAL CONTROL

EthoVision XT starts tracking as soon as you click the button in the acquisition screen of the software. But what if you want the actual trial to start after certain conditions are met? This is what Trial Control is for; it gives you the option to set start and stop conditions based on the subject's behavior or on timing. For example, start tracking four seconds after the animal is detected in the arena, and stop 10 minutes afterwards.



*A straightforward example of trial control.*

## BATCH ACQUISITION

Batch acquisition means that you can acquire a series of trials at one push of a button. You can line up your video files and let EthoVision XT do the rest of the work for you. You don't have to come back and start each trial separately... you can even let it run overnight!

## SERIES OF TRIALS FROM A LIVE VIDEO FEED

When you are working with a live video feed, you don't have to start each trial manually, either. Use EthoVision XT to automatically acquire a series of trials, according to your predefined settings, from a live video feed.

## TRACK FROM UP TO 4 VIDEO SOURCES SIMULTANEOUSLY

With EthoVision XT, you can track in multiple arenas simultaneously. Using one camera, you can place arenas close together and define different arenas. You can also use a camera for each arena or cage and combine up to four cameras. The latter has the advantage that cages/arenas do not have to be placed close to each other and animals won't influence each other's behavior.

Line up video files and let EthoVision XT do the work: you can even let it run overnight.

# DATA SMOOTHING AND CORRECTION

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## SMOOTHING

In any tracking system, there are three sources of noise that potentially skew the values of dependent variables such as distance moved or velocity:

- system noise
- outliers
- small movements of the animal (body wobble)

EthoVision XT includes tools to smooth out your data.

## CORRECTION

In case of errors, such as tracking errors that may occur due to light reflections, there are several easy ways to correct your data. Because the results of the tracking are superimposed on the video, you can easily spot tracking errors and simply select and replace or delete them. You can also interpolate between data points.

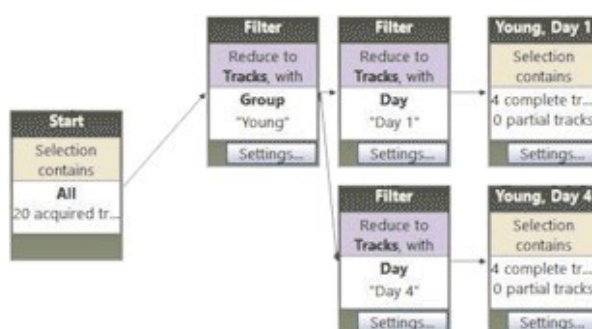


# DATA SELECTION AND ANALYSIS

## DATA SELECTION

EthoVision XT's data selection tools let you evaluate certain subsets of each trial. Selecting the data, for example, in which subjects received a specific treatment allows you to analyze only those data and ignore the rest. Or, you can nest your data on time, zone, or a behavioral or physiological state.

Data selection is very intuitive to set up, using blocks that, for example, contain “filters”. You can copy-paste and adjust these blocks as needed for an efficient workflow.



*In EthoVision XT you can copy-paste blocks to easily create protocols for data selection.*

In radial arm mazes, questions like “How long did it take the subject to get to a total of 5 correct arm entries?” are now easily answered using free interval selection. With this method you can also determine how long it took for your animal to learn that it has to stay in the correct arm for, let’s say, 20 seconds consecutively.

## DATA ANALYSIS

The number of parameters that can be used for analysis is substantial, including trial duration, in zone, distance to zone, distance to zone border, distance moved, velocity, heading, turn angle, angular velocity, mobility, proximity, rotations, elongation, body angle, acceleration, etc. Statistics include mean, variance, standard deviation, standard error, minimum, maximum, sum, total, number of samples, and more.

## FREE INTERVAL SELECTION

Free interval selection allows you to define your custom analysis. For example, in a (novel) object test, the main goal is to measure how much interest the subject has with a familiar and/or a novel object. EthoVision XT could already give you the time and frequency of ‘nose point in object zone,’ but maybe you are interested in how long it took, over the entire trial, for the subject to show a total of 10 seconds of cumulative interest in one or more objects. The new free interval selection makes this very easy to analyze.

Automatically measure behavior in more detail by combining manually or automatically scored behaviors.

## BUILD YOUR OWN PARAMETERS

Our customers often request to be able to define their own parameters within EthoVision XT. This would indeed be very useful, for example in novel object testing.

This test often involves using the “*proximity*” parameter, defined as proximity of the nose point to the objects. But maybe that parameter is not sufficient for object exploration; other parameters, such as animal movement and head direction, can be just as important.

In EthoVision XT, you can define a multi-condition variable. Continuing with the previous example, this means that you effectively create a new, customized parameter by combining “*heading to zone*,” “*subject is moving*,” and “*nose-point is in object zone*.”

In addition to using existing parameters, you can also combine manually or automatically scored behaviors. This allows for an effective way to automatically measure behavior in more detail.

Do you feel like you are missing a parameter? If the variables you are interested in are based on the x,y coordinates of the subject, they can be custom built for you and made available in your EthoVision XT software.

## AUTOMATIC AND BATCH ANALYSIS

In addition to batch acquisition, you can also perform automatic analysis and batch analysis. First, select which parts of your data need to be analyzed. Then, select the independent variables you are interested in. Now EthoVision XT will automatically analyze your data according to these settings right after the trial has ended or, in the case of batch analysis, after the selected trials have been acquired.

# VISUALIZE YOUR DATA

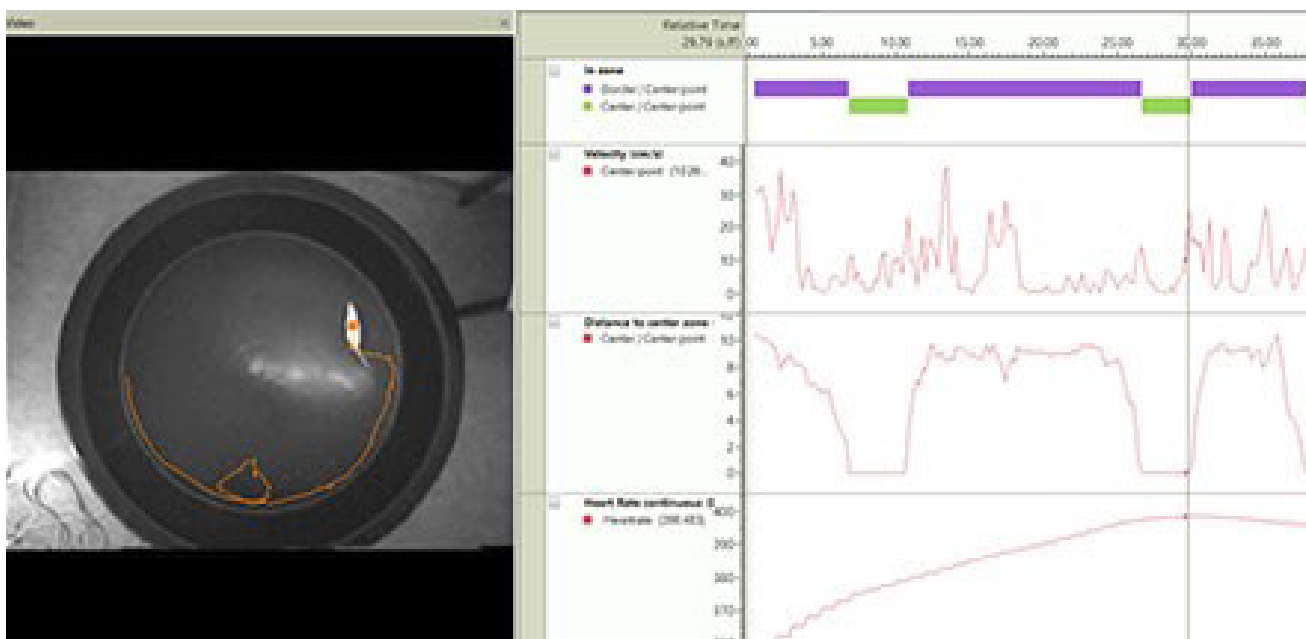
Visualizing your data is an important step in analysis, and can be done in several ways and at several points in time. While plotting your data in graphs is useful after selection and analysis, visualization of the tracks superimposed on the video file (integrated visualization) is helpful in the beginning steps of analysis, because it gives you a good feel for the data. Additionally, when you are interested in certain behaviors such as body elongation or mobility, you can use the visualization function to fine-tune the thresholds for these behaviors. You can also view all tracks side by side and even export these images at high resolution.

This part of the software also has a built-in screen recording tool, allowing you to make a video of the integrated visualization which you can use for presentations.

## GROUP STATISTICS AND CHARTS

With EthoVision XT you can easily compare the results between treatment groups, for example by using graphs. You can organize and adjust

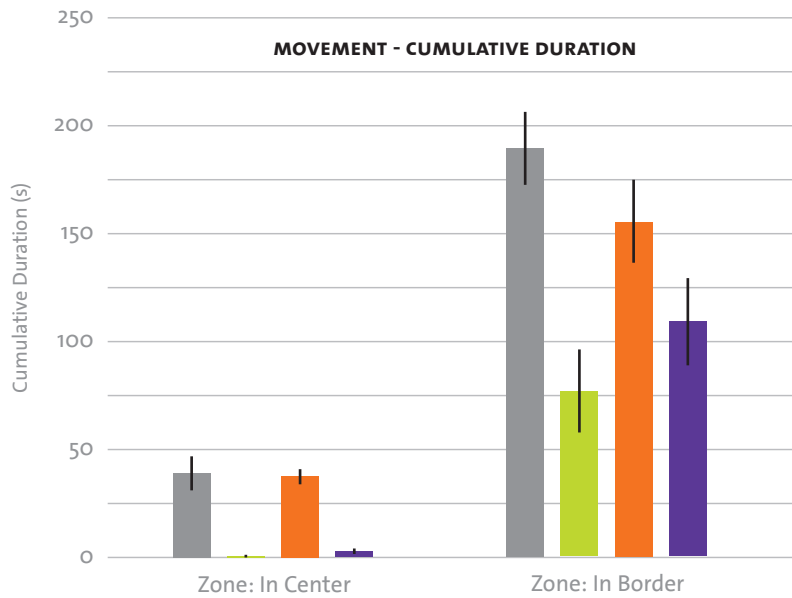
*Integrated visualization in EthoVision XT allows you to see the video, the track in overlay, and all your data (including external data) plotted in a dynamic graph.*



the designs of these graphs and export them in any standard format (PNG, JPG, BMP, GIF).

An example of a graph in EthoVision XT. Here movement data is compared between a control and a treatment group.

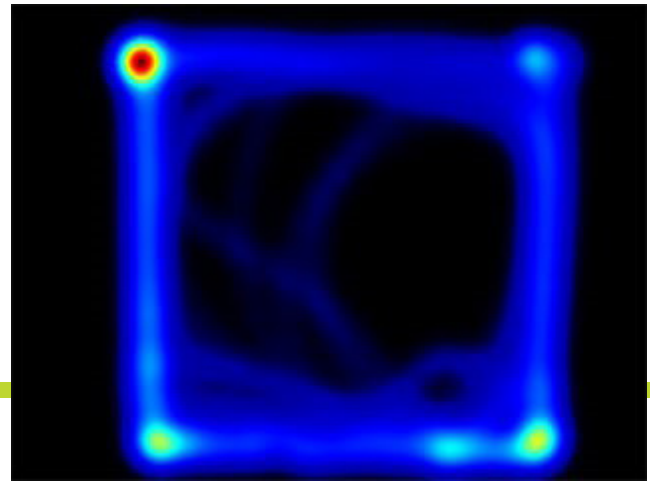
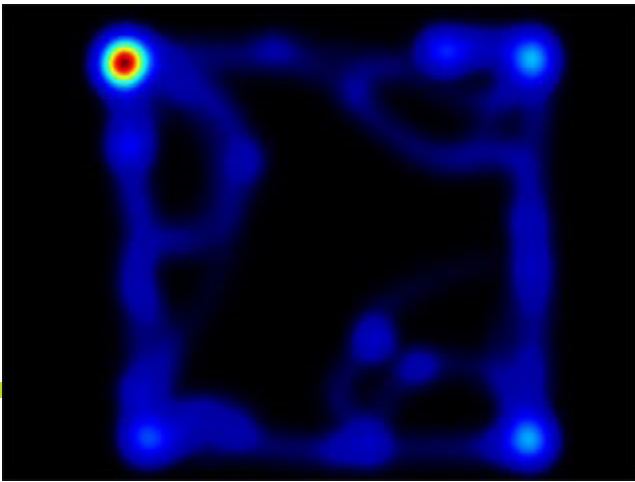
- control / moving / center-point ■
- control / not moving / center-point ■
- treatment / moving / center-point ■
- treatment / not moving / center-point ■



Results are also presented in tables, of which you can adjust the layout. This is ideal if your statistical program requires a certain set-up of the data, since you can organize and export your table accordingly.

|       | Trial    | Time         | Age | Distance moved |              | Velocity            | Movement              |                           |
|-------|----------|--------------|-----|----------------|--------------|---------------------|-----------------------|---------------------------|
|       |          |              |     | center-point   | center-point | center-point        | Moving / center-point | Not Moving / center-point |
|       |          |              |     | Total          | Mean         | Cumulative Duration | Cumulative Duration   |                           |
|       |          |              |     | cm             | cm/s         | s                   | s                     |                           |
| Aged  | Trial 1  | 00:01:59.819 | d1  | 802.5385       | 15.1704      | 109.7038            | 8.5800                |                           |
|       | Trial 2  | 00:01:59.686 | d1  | 287.7959       | 10.8630      | 96.9172             | 11.7004               |                           |
|       | Trial 5  | 00:02:00.620 | d1  |                |              |                     |                       |                           |
|       | Trial 6  | 00:01:59.886 | d1  |                |              |                     |                       |                           |
|       | Trial 9  | 00:02:01.285 | d4  |                |              |                     |                       |                           |
| Young | Trial 10 | 00:00:32.733 | d4  |                |              |                     |                       |                           |
|       | Trial 13 | 00:02:01.421 | d4  |                |              |                     |                       |                           |
|       | Trial 14 | 00:01:19.780 | d4  |                |              |                     |                       |                           |
|       | Trial 3  | 00:01:59.819 | d1  |                |              |                     |                       |                           |
|       | Trial 4  | 00:01:51.310 | d1  |                |              |                     |                       |                           |
|       | Trial 7  | 00:01:53.413 | d1  |                |              |                     |                       |                           |
|       | Trial 8  | 00:02:01.083 | d1  |                |              |                     |                       |                           |
|       | Trial 11 | 00:00:17.551 | d4  |                |              |                     |                       |                           |
|       | Trial 12 | 00:00:13.814 | d4  |                |              |                     |                       |                           |
|       | Trial 15 | 00:00:10.577 | d4  |                |              |                     |                       |                           |
|       | Trial 16 | 00:00:02.903 | d4  |                |              |                     |                       |                           |





*A heatmap can give you an instant feel for your data. Here a treated animal is compared to a control animal.*

### HEATMAPS

In addition to integrated visualization, a heatmap gives you an intuitive and unique view of your data. You can export these at high resolution for use in presentations and publications.

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If you are interested in video tracking for social interaction, automatic behavior detection, or incorporating and controlling equipment (e.g. operant conditioning, optogenetics, several stimuli), please check out [www.noldus.com/ethovision](http://www.noldus.com/ethovision) or contact us for more information.

### TRY IT OUT!

Free trial version available at [www.noldus.com/ethovision](http://www.noldus.com/ethovision)



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