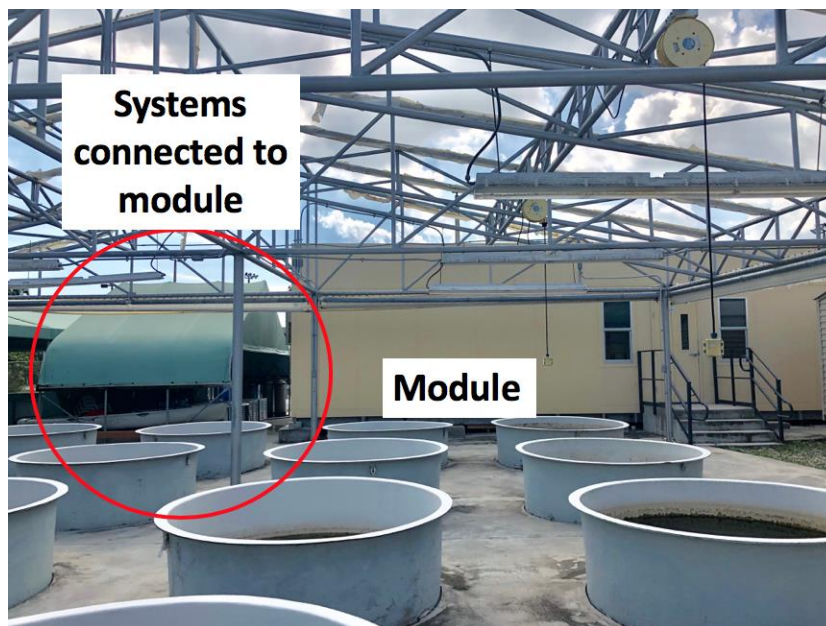


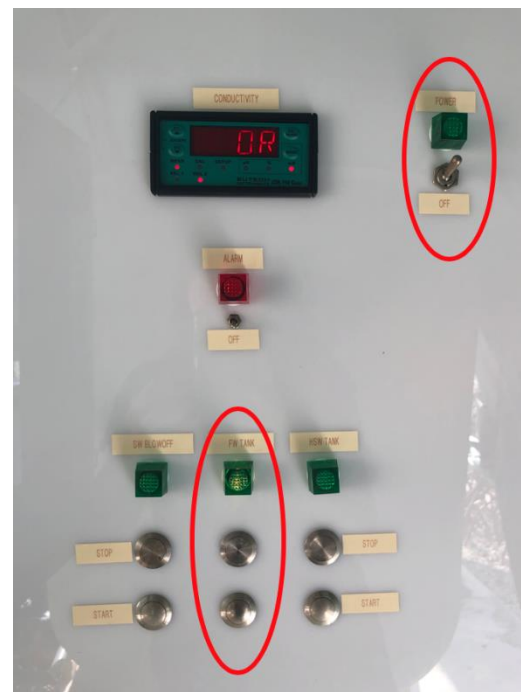
## Predator-Prey Behavior Analysis Protocol

### Experimental Set-Up

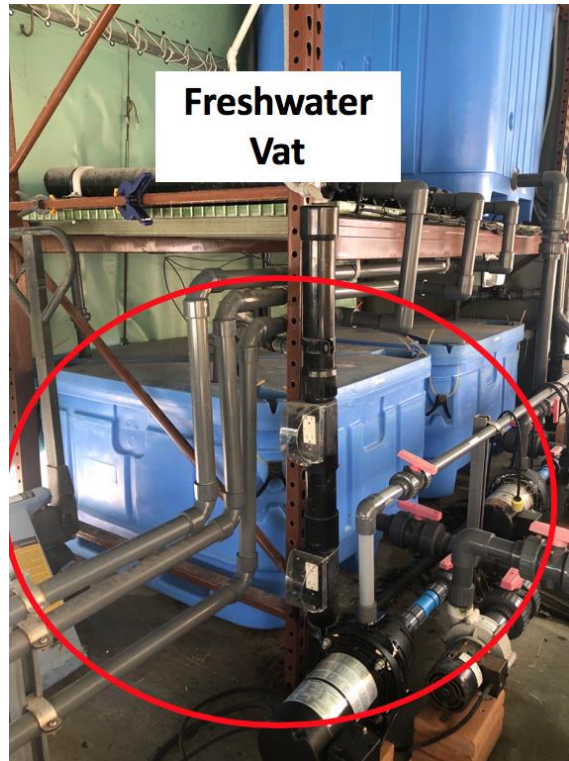
- 1) In order to set up the experiment, you will first need to fill up the vat with water. The vat will store the water that gets brought into the module building
- 2) To do this, look at the picture below and go to the systems under the green cover, to the left of the module building.



- 3) Direct towards the control box and turn the switch up towards where it says power.
- 4) Once it's on, press start under the label that says FW Tank.



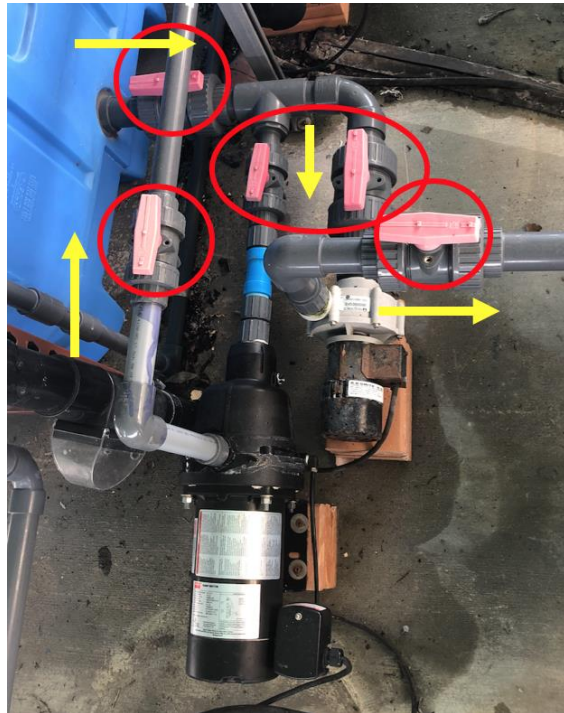
- 5) Head towards the freshwater vat, located to the far left, see picture below, check if it's filled with water all the way to the top. If it is, skip to step #6. If it is not, read step #5.



- 6) Turn these valves vertically, so they start pumping freshwater into the freshwater vat. Wait until the vat is completely full.



- 7) Make sure all these valves are open. If one of these is not open, it could cause the pump to run dry.



- 8) Once all valves are open, connect the 2 pumps and the heater cords to the electricity outlets.

The yellow cord belongs to the big pressure pump that will push the water inside of the module building.

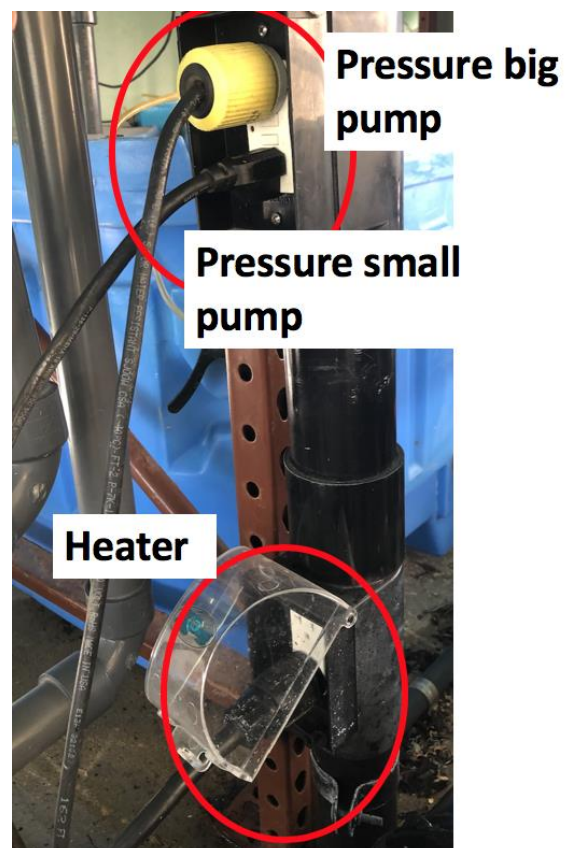
The thin black cord belongs to the small pressure pump that collects the water from the vat and pushes it to the heater.

The thick black cord belongs to the heater.

**\*\* IMPORTANT \*\***

**Once you are done filling the tank inside of the module. Immediately:**

- a) Disconnect the electrical cords,**
- b) Flip the switch from the control box towards off,**
- c) And turned valves from step 5, horizontally.**





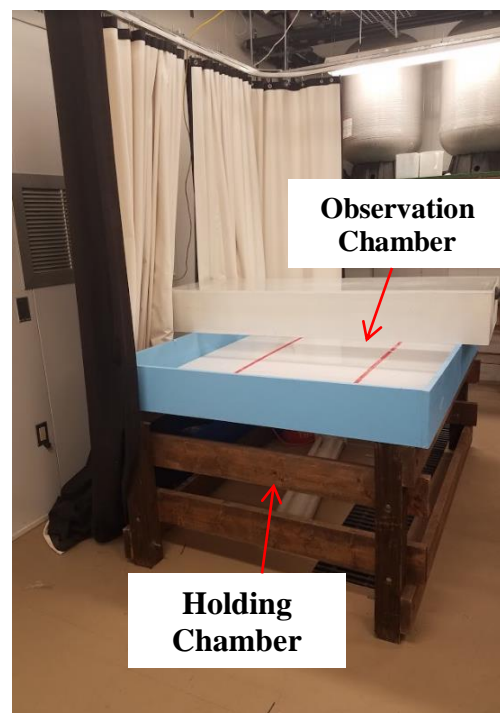
- 9) Inside of the module building, you'll need to turn this valve vertically to fill up the gray tank. Once you do this, it is very important that you hold the hose with the two hands because the water comes in with a lot of pressure. I recommend filling up the gray tank first, and placing a heater and air stones into it, as this ensures the water within the tank is always kept oxygenated and at the temperature needed for each experiment.



- 10) From here on out, you can use this water for everything in your experimental set up, including rinsing the chambers, water changes etc.

### Experimental Chamber Set-up

- 1) First start by setting up the blue holding chamber. This chamber will be filled with water and two heaters to regulate the temperature within the observation chamber. This holding chamber is always completely surrounded by the blackout curtains, which will blackout any outside activity, but not noise, so it is important to keep the noise level down in the room while running experiments.



In addition, there is a sign on the door to the room, prompting people that there is currently an experiment in progress; please fill out the information on the sign and ensure that it is visible to everyone by flipping it.

- 2) Place the observational chamber in the middle of the holding chamber. It is recommended to mark the exact location with a waterproof marker or colored tape, so the chamber is always put in



the exact same spot throughout the experiment. In the image to the right, the markings were made with red tape.

- 3) Begin filling experimental chamber with your heated, filtered water. It is recommended starting by filling the observational chamber first. This prevents the observational chamber from floating away from its designated location once you begin filling the blue holding chamber. Once you are done, both the holding chamber and observational chamber should be filled with water. The amount of water needed is dependent on the specific experiment and the size of the aquatic organism used. The larger the organism, the more water is needed.

- a. Dependent on the size of the organism being used, the size of the observational chamber can be modified by adding and removing partitions. In the above image you can see the experimental chamber without any partitions. The image on the right is an example of an experimental set-up including partitions.



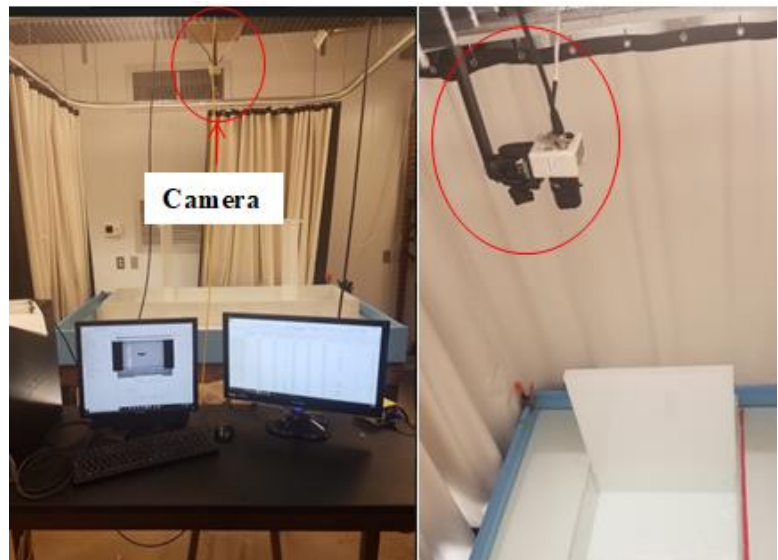
- b. The partitions can be placed anywhere in the observational chamber by placing plastic brackets on the sides with the use of aquarium safe silicon. These brackets are to be removed at the end of the study for the next user.



- 4) Above the experimental chamber, there is a black & white camera mounted with brackets.

**Do not touch this camera!  
Ever!**

It is placed in a way that the entire experimental chamber is always captured and moving the camera would require setting up the location of the chambers and recalibrating the software.



- 5) Once the experimental chamber is set up, you need to choose a source of lighting. For this, there are two default options within the behavior analysis room.

- a. The first option is the default lights in the room hanging directly above.



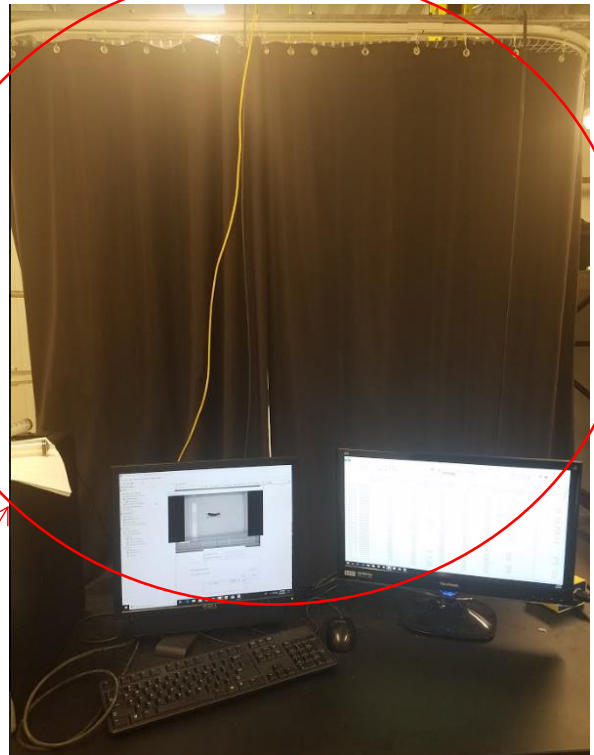
- b. The second option is the 40 watt, 4ft. led light located underneath the experimental set-up. This light option will provide light from underneath the chamber, reducing any glares which can occur when using the overhead lights.



- 6) By now, the holding chamber and observational chamber should be filled with aerated, heated water, the camera set and the source of light turned on. Next, enclose the experimental set-up with the blackout curtain and secure using a clip.



**Blackout curtains when they are open, prior to the beginning of an experiment.**

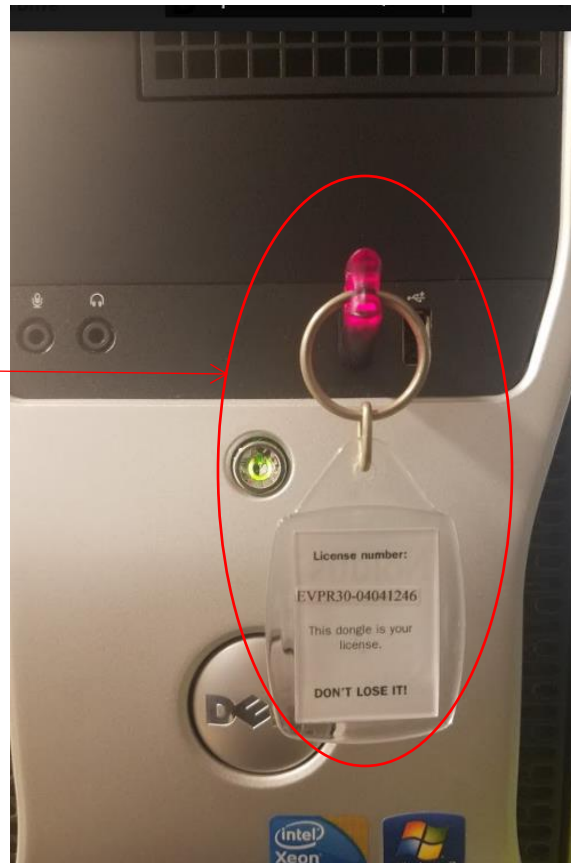
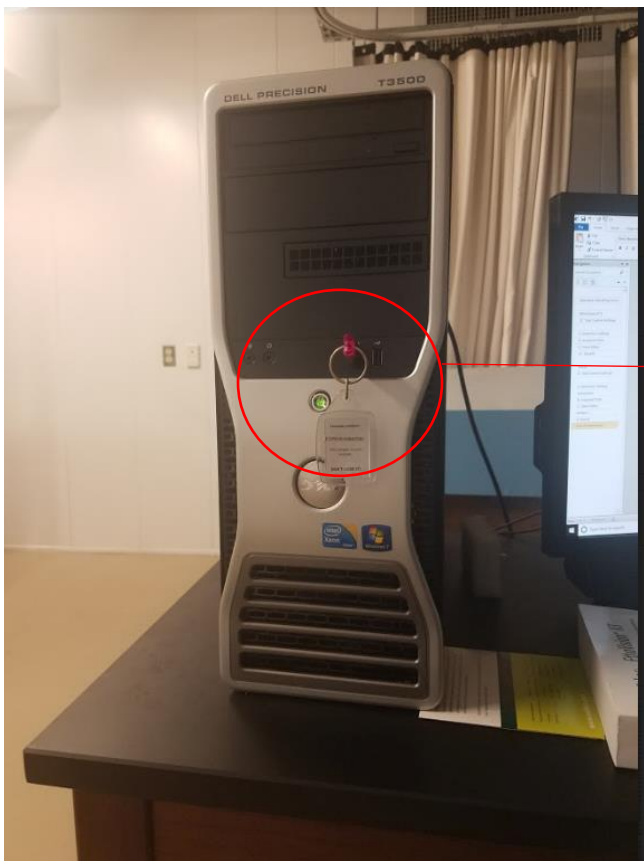


**Blackout curtains when they are closed, while an experiment is in progress.**



**Important! There is a USB dongle inserted into the computer. This dongle must never be removed from the computer! This dongle is the license key for the software. Without this dongle, the software will not open on the computer.**

**DO NOT REMOVE!**



**Now you are ready to begin setting up the software component for your experiment!**



## **Ethovision Behavior Software Set-up**

### **EthoVision XT 9**

#### **1. SETUP**

##### **1.1 Running EthoVision**

##### **1.2 Experiment Settings**

##### **1.3 Manual Scoring Settings**

##### **1.4 Arena Settings**

##### **1.5 Trial Control Settings**

##### **1.6 Detection Settings**

##### **1.7 Trial List**

#### **2. ACQUISITION**

##### **2.1 Acquisition**

##### **2.2 Acquired Trials**

##### **2.3 Track Editor**

#### **3. ANALYSIS**

##### **3.1 Data Profiles**

##### **3.2 Analysis Profiles**


##### **3.3 Results**

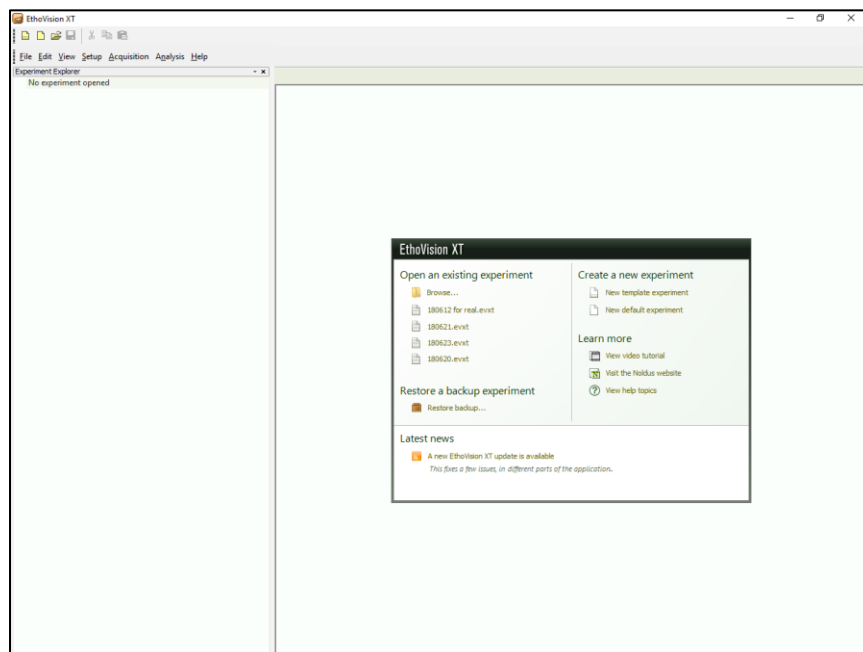
##### **3.4 Exports**

# 1. Setup

## 1.1

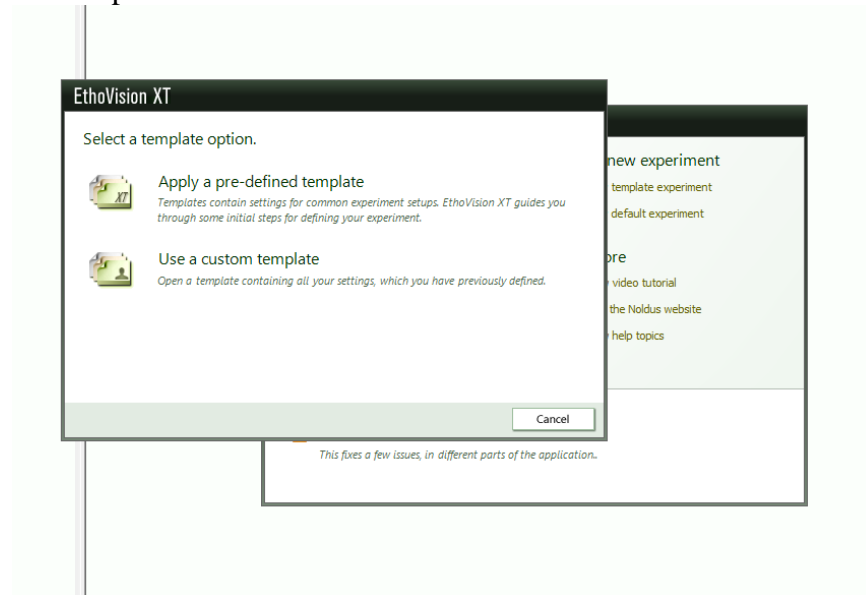
### Running EthoVision

- 1) Open EthoVision XT 9 by double clicking the EthoVision XT 9 Icon on Desktop or Search for EthoVision XT 9 in the Search bar at the bottom right corner of the screen. 
- 2) Home Screen will open, prompting the user to either open an existing experiment or to create a new experiment, using either a new template experiment or new default experiment. If using for more than one trial, it is recommended to create a template with your settings saved into it.

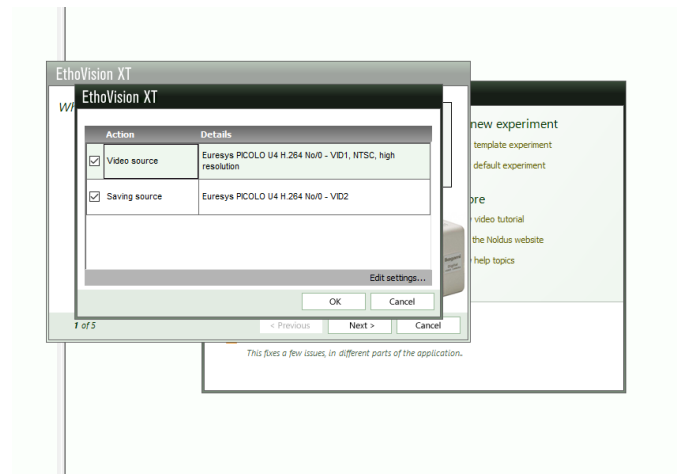
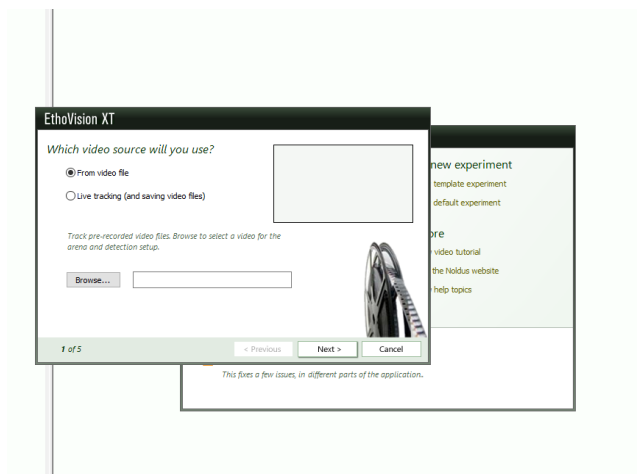


## Setting up your template

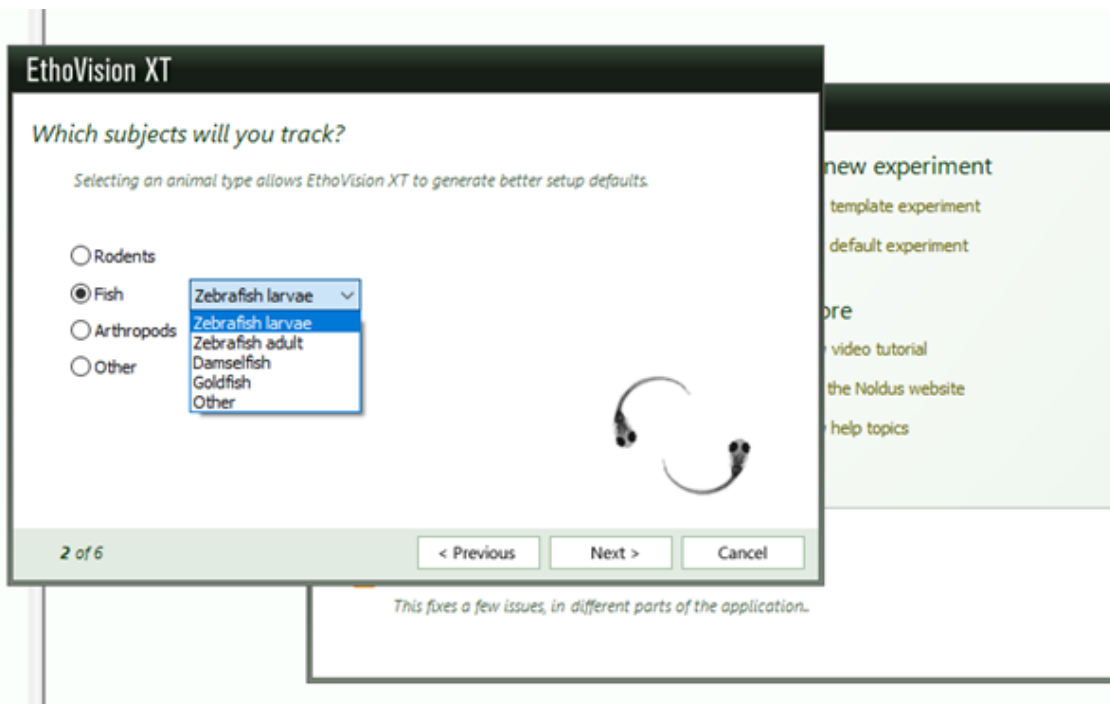
1) To begin setting up your template select 'New template experiment'. Here you will be able to choose between using a pre-defined experiment and a custom template. It is suggested to begin with a predefined template if using model species.



2) Then you will select your video source.

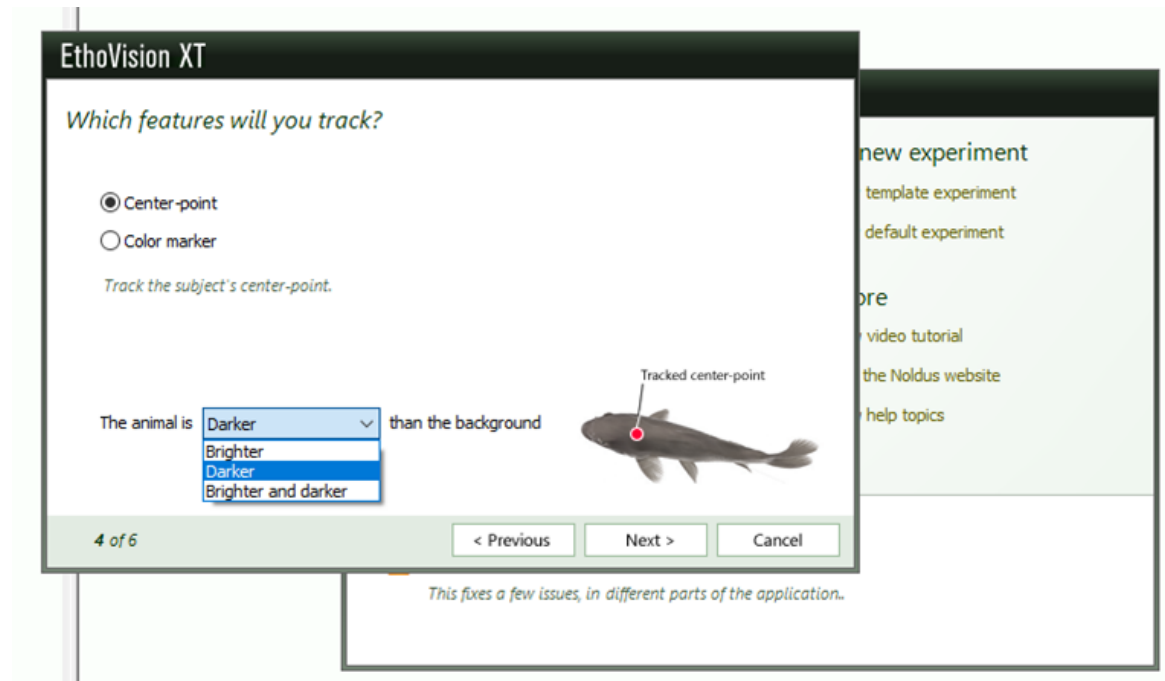


3) In the next step, you will select which subject you are trying to track. Ethovision provides you with the basic settings for model species. Select which type of animal you are tracking and then specify which species. If you choose 'other' for any species not listed, you must specifically tell ethovision the parameters of the animal you wish to track (see, section 1.6 Detection settings for details).

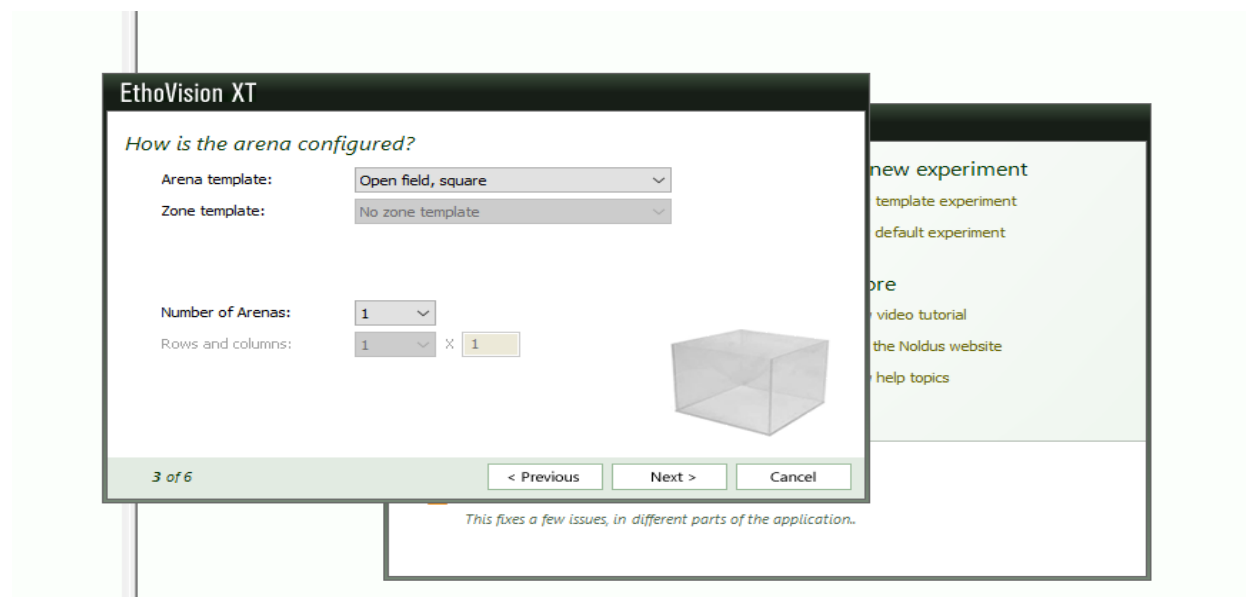


Next, you will see a window to choose which features you wish to track. With this camera, you can only use center-point tracking because it is a black and white camera. In this specific case, the animal is darker than the background since our arena is white. This varies by type of arena.



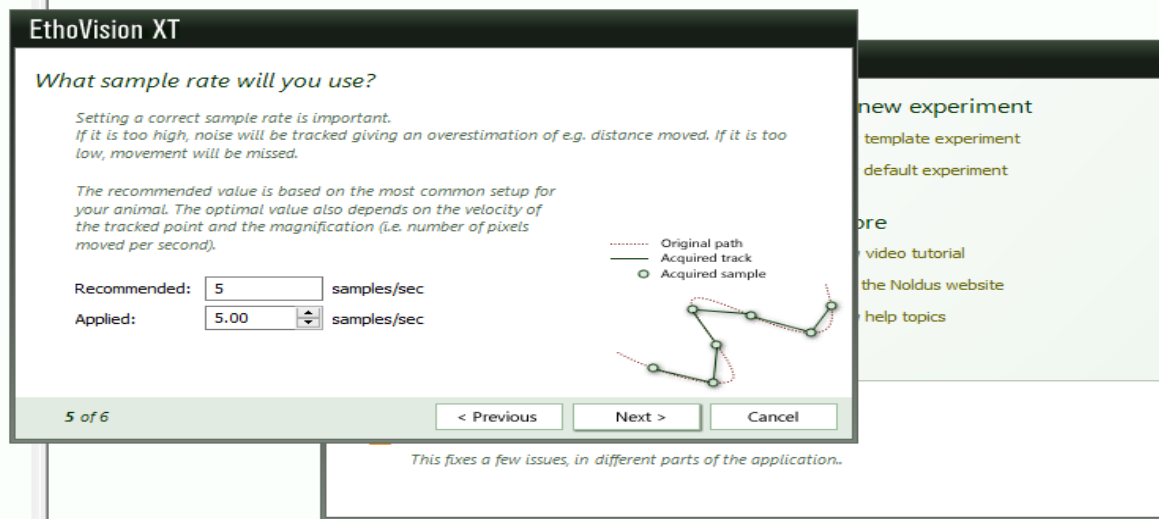


4) Next will be arena settings, simply tell ethovision how many arenas you would like the template to have. You can create multiple arenas, so long as there is no overlap between them or your organism cannot move between them. Eg. Two separate tanks that do not connect.

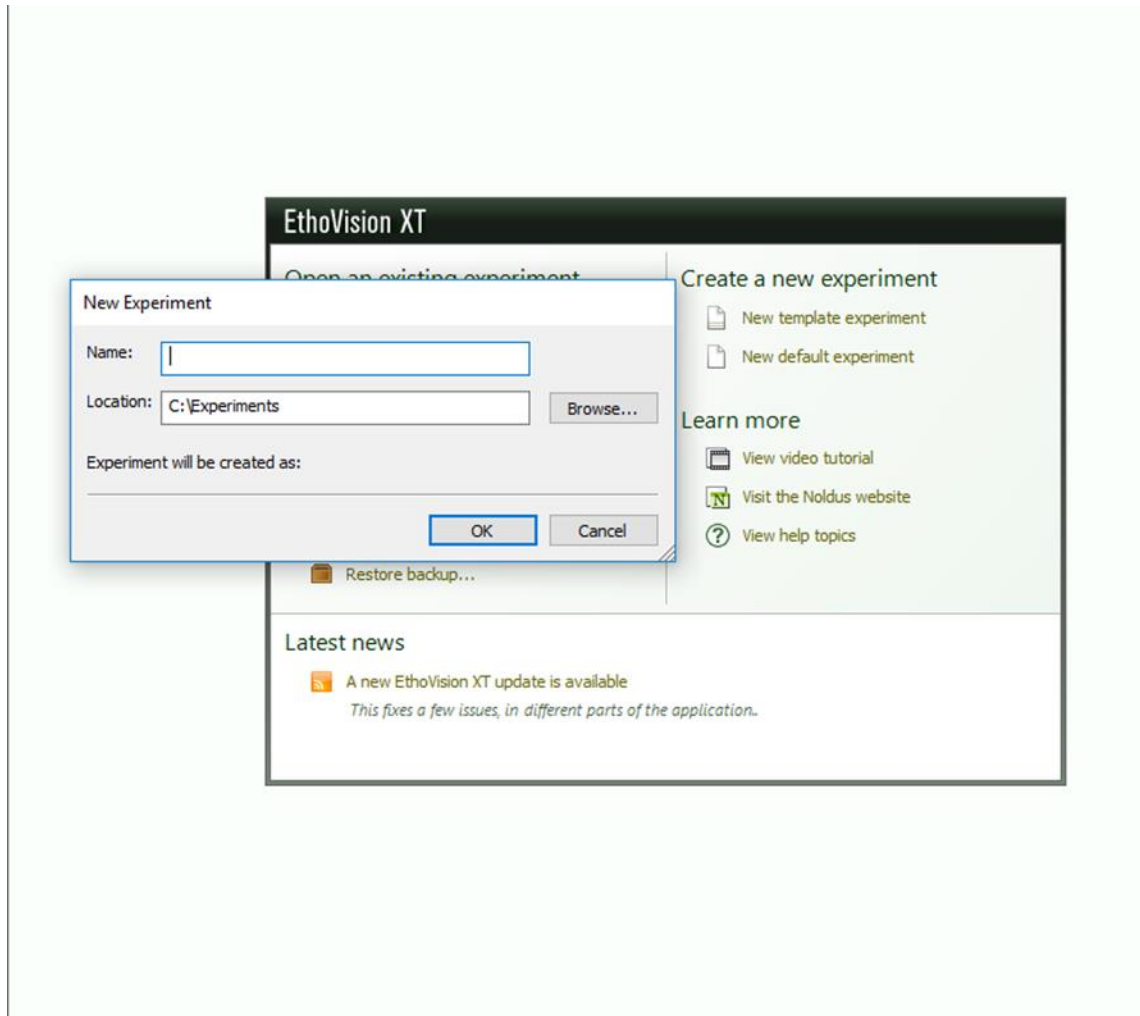


5) The next step will be choosing a sample rate; the sample rate is the number of movements tracked per second. A movement can be fish swimming along the arena, but it can also be a small tail movement without the fish moving forward.

Selecting the appropriate sample rate is important because if you choose too high of a sample rate, the software will over-track movements (it will track small movements such as the fish moving its tail to maintain buoyancy) but if the sample rate is too low, it will miss movements (not tracking while it is swimming). It is recommended to use the sample rates recommended by ethovision for a model species similar to yours. If the animal is a bit larger or smaller, you can simply adjust it accordingly.



6) When creating a new experiment, a screen will pop up directing you to assign a name and location for this experiment. Input the name and location default will be C:\Experiments. Click OK.



7) Now the home screen for the experiment will open. From here you can use the side controls to navigate to all of your settings such as arena settings, experiment settings etc. You will start by setting up the first option “Experiment Settings”.

## 1.2. Experiment Settings

1) *Experiment information*: Experiment settings will show the location to which the experiment is being saved and will usually look like this: C:\Experiments\**Experiment Name**\

2) *Video Source*: Can be used to determine whether the source for the data will come from a previously acquired video or a live tracking feed. You need to select “From video file” or “Live tracking”. Do not change the video or saving sources. (We only have one camera and this option is only modified when you have multiple cameras.)

The screenshot shows the 'Experiment Information' dialog box. It has several sections: 'Location' with a text field containing 'C:\Experiments\Ethovision Template Predator-Prey\'; 'Description' with a large text area; 'Video Source' with radio buttons for 'From video file' and 'Live tracking' (selected), and dropdown menus for 'Video source' and 'Saving source' both set to 'Euresys PICOLO U4 H.264 No/I0 - VID1' and 'VID2' respectively, with an 'Edit' button; 'Arenas' with a 'Number of Arenas' dropdown set to '1'; 'Tracked Features' with radio buttons for 'Center-point detection' (selected) and 'Color marker tracking (treat marker as center-point)'; 'Non-tracking Features' with a checked checkbox for 'Activity analysis' and a small icon; and 'Units' with dropdowns for 'Unit of distance' (cm), 'Unit of time' (s), and 'Unit of rotation' (deg).

3) *Arenas*: Will be used for assigning how many arenas will be present in the experiment. The default is **one arena**.

4) *Tracked Features*: Will be used for determining which is the best method for tracking. Center-point detection is the suggested for single-subject experiments. Color marker tracking is used when a color camera is being used and the individual subjects all vary by color. **Default will be Center-point detection.**

5) *Non-tracking features*: Activity analysis is used to detect movement from your subject, when it is not moving from its location. E.g. Movement of tail when a subject is immobile. It tracks the movement by the variation in pixels surrounding the subject. **If you wish to use this feature, click the box now. Once the experiment begins you cannot go back and select this. It is recommended not to select this feature when working with fish; by selecting this feature, the software will recognize fish as moving when they are in place only moving the posterior part of the body due to the movement of pixels.**

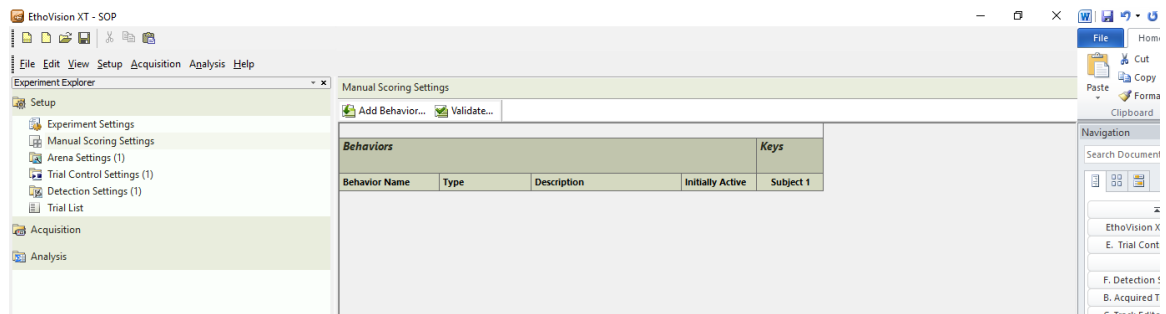


6) *Units*: Unit of distance, time and rotation can be specified. The default unit of **distance will be cm, unit of time s and unit of rotation deg.**

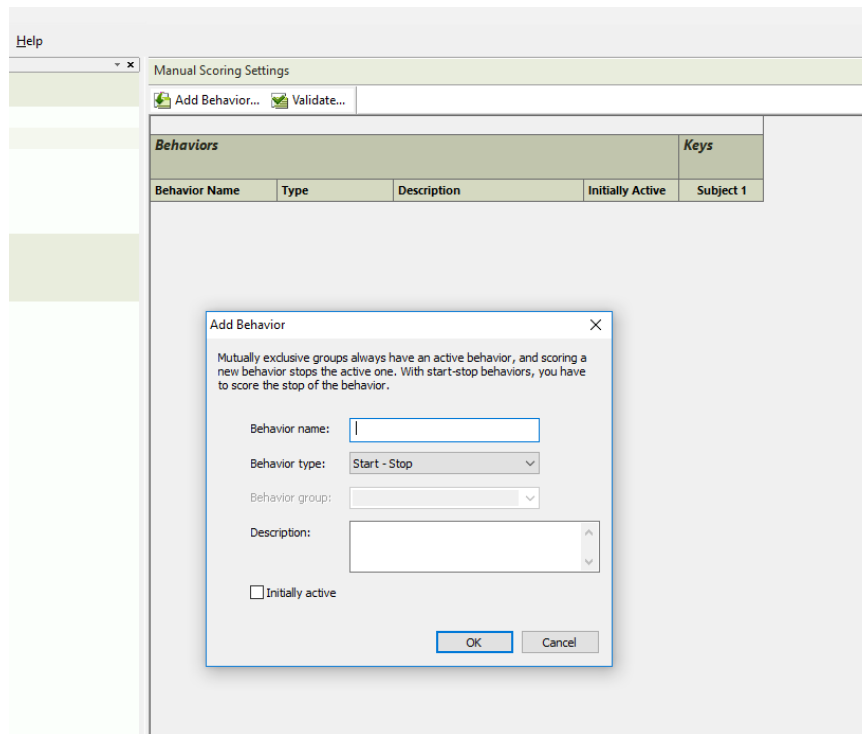
You have finished setting up “Experiment Settings”. Next, you will setup “Manual Scoring Settings” if needed. Manual scoring is used for very specific behavioral endpoints. These behaviors must be manually scored; i.e. you must be present during acquisition and manually tell the software when to start and stop acquiring each behavioral endpoint. If manual scoring settings are not set up, you will still acquire behavioral data (e.g. speed, movement, mobility, duration etc.). If not using manual scoring settings, skip this step and move on to “Arena Settings”.

### 1.3 Manual Scoring Settings

1) Manual Scoring is used to define behavior that you want to record manually. To do so, you would click on ‘Add Behavior’



2) An action screen will pop-up prompting you to name the behavior, select the behavior type, default will be Start-Stop and give a description of the behavior. Important note, for manual scoring you must be present at the computer to manually tell it what type of behavior you want it to look for. Eg. Approaching a novel object.



## 1.4 Arena Settings

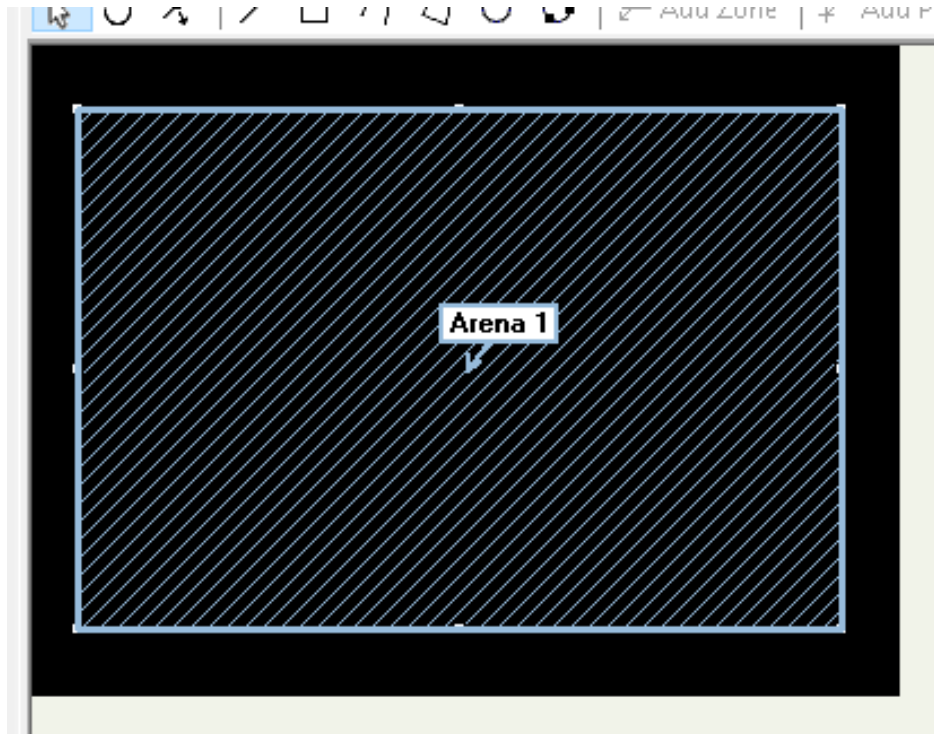
1) Arena settings allow you to define the region on a screen in which EthoVision XT can detect subjects. It is important to define the Arena the subject will be in because if the subject moves outside of that designated Arena, tracking will be disrupted.

a) To begin an experiment use the “Grab image from camera” function to grab a still shot of your experimental set-up. Once you have grabbed the background image, you can assign zones, calibrate the arena and add zone groups.

2) *Defining an Arena:* Select the shape and size of your arena in which you want to track the behavior of your subject.

To do so, you will see “Arena 1” and you can drag the corners to select the size of the arena of interest to you. Your arena will be shaded with diagonal lines; this will indicate the area of your arena. Ensure that the entirety of your arena is selected.

*You can create multiple arenas, so long as there is no overlap between them or your organism cannot move between them. Eg. Two separate tanks that do not connect. It is also recommended to calibrate each individual arena separately.*



3) *Calibration*: Calibrate the arena size by clicking on box at the top of the screen that reads '*Calibrate Scale*'. By calibrating the scale, it allows the software to give you real-time accurate data to calculate end-points such as speed, velocity, distance traveled, etc., by using the values from the calibration as a reference point. There are two steps in the calibration.

**Length: 78.74 cm**

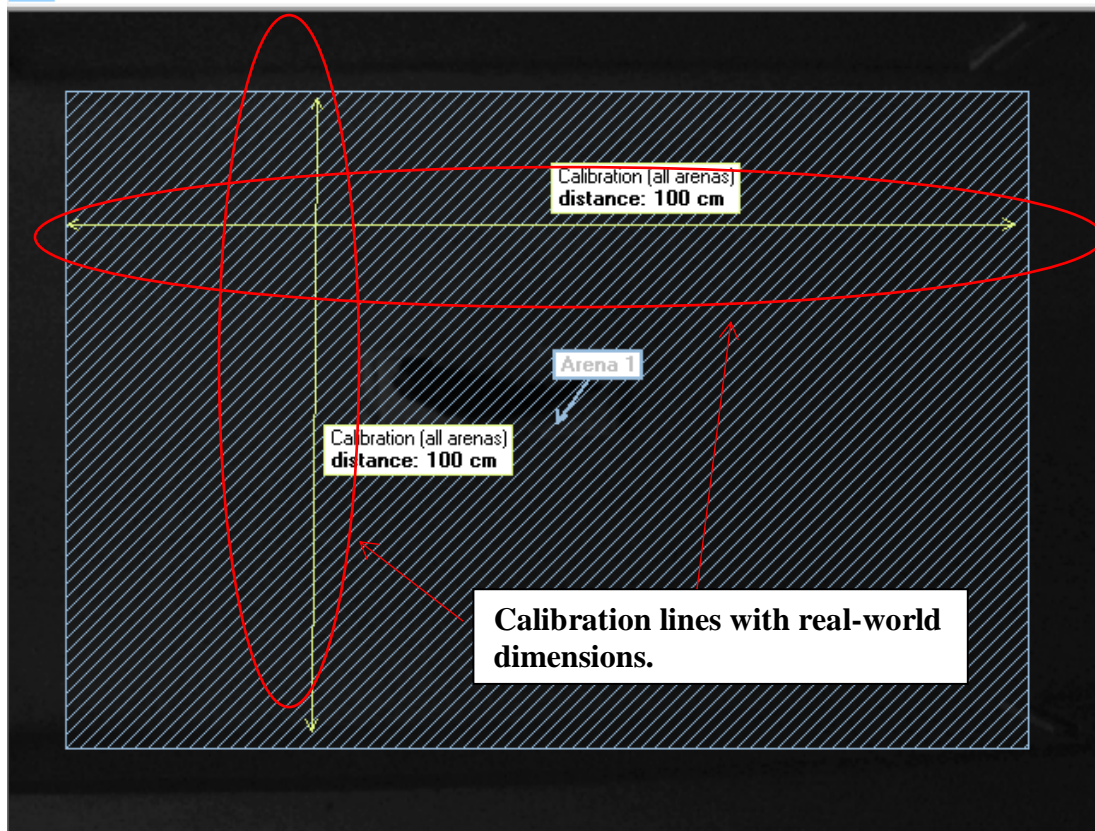
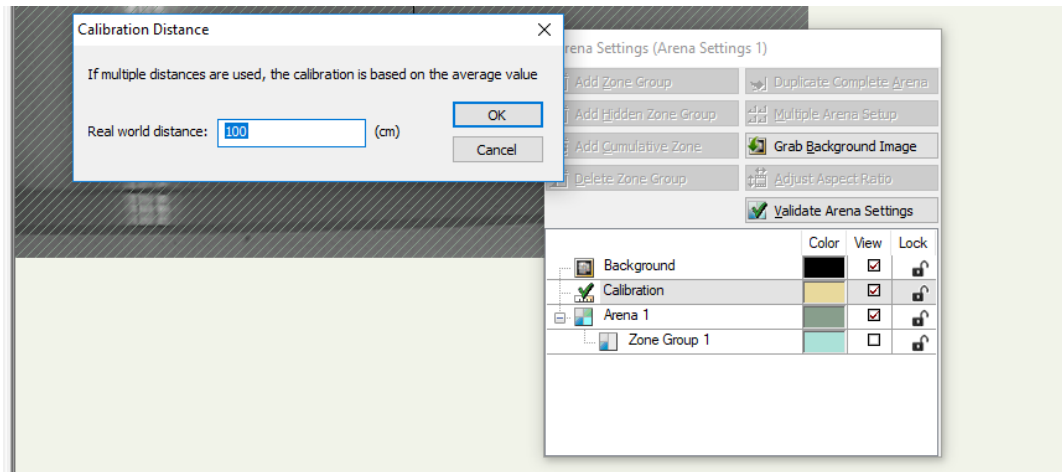
**Width: 52.58 cm**

3.1) Measure the length and width of the actual physical observation chamber and keep these values on hand for part b.

3.2) Once you click on calibrate scale, click on the edge of your arena and pull it all the way to the opposite end. Once you release the button, a calibration distance window will pop-up prompting you to input your actual measured dimensions in step a. Do this both horizontally and vertically. If multiple distances are used, the software will calculate the average value and use that.

By right-clicking a zone border, the zone can be subdivided into smaller quadrants. Arena is subdivided **6x4 giving us 24 quadrants** in total.

Another zone is then added, in which the **predator zone** is defined in the center of the arena. Use the brackets in the arena as a guide to what you should select as the predator zone.





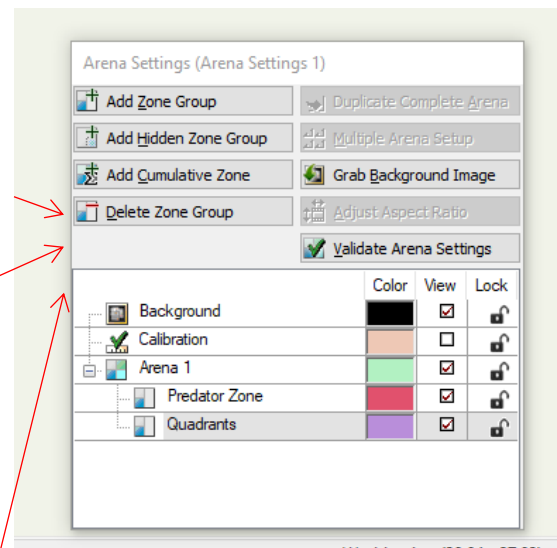
4) *Zone Groups*: Zones are areas of interest defined within an Arena. Zones are added by clicking on the 'Add Zone Group' button at the top-left corner of the 'Arena Settings' Box to the right of the screen.

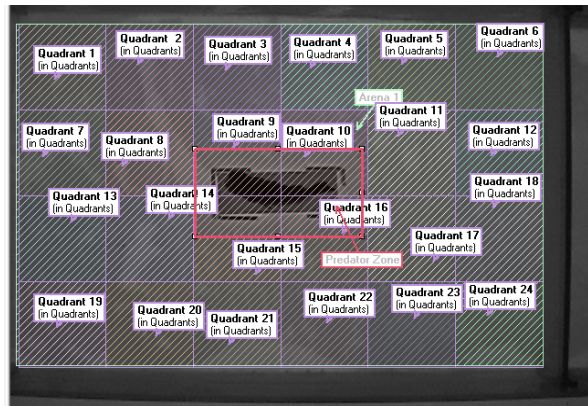
*Hidden Zone Group*: is a hidden zone, such as a cave or shelter used in an experiment. This zone is added by clicking on "Add Hidden Zone Group" and designating where in the arena it would be.

*Cumulative Zones*: you can add several zones together to create a cumulative zone so when different zones are analyzed, they are treated as a single zone.

*Delete Zone Group*: this allows you to delete any zone or zone group you have created.

By right-clicking a zone border, the zone can be subdivided into smaller quadrants. In the menu at the top of the screen, there will be a button called Add zone, click on Add zone and then click on the different quadrants to name them. This would be done if you had a predator zone for example.





For this experiment we will have 24 quadrants and a designated predator zone in the center.

The “Grab Background Image” serves for when you already have a previously set up template for your experiment and now you would select “Grab Background Image” to add the live feed (current camera image) of the observation chamber.

Duplicate Complete Arena, Multiple Arena Setup and Adjust Aspect Ratio will be grayed out. These features cannot be used with only one camera.

7) Select “Validate Arena Settings” and your arena will be calibrated and ready to begin the experiment.

**Once you are done, you can leave this screen, as the changes save automatically.**

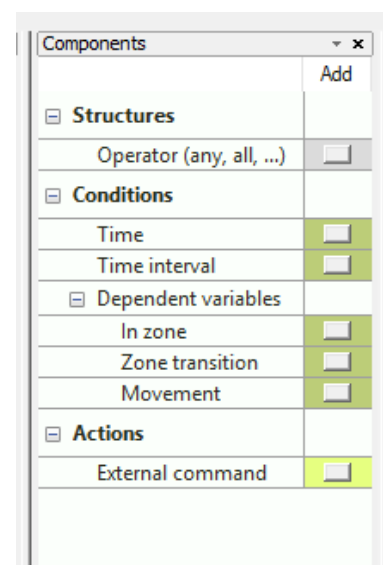
You have finished setting up “Arena Settings”. Next, you will setup “Trial Control Settings”.

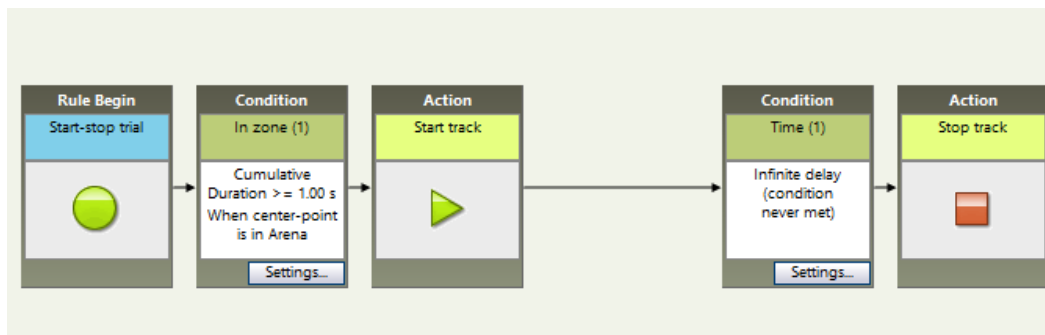
### 1.5 Trial Control Settings

1) Trial control settings are used to automate your experiment. For example: if you want to set a maximum duration for your trials or you want to automate the start and/or stop of data acquisition.

2) A trial control setting profile will open with default settings. The default settings will always be 5 boxes: *Rule Begin – Condition—Action – Condition – Rule End*. These can be deleted or edited to the conditions and actions of the experiment.

3) Conditions are added by left-clicking on Conditions on the left-hand side of the screen under “Components”. Conditions can be ‘Time’, ‘In Zone’ or ‘Movement’ and vary by experiment.

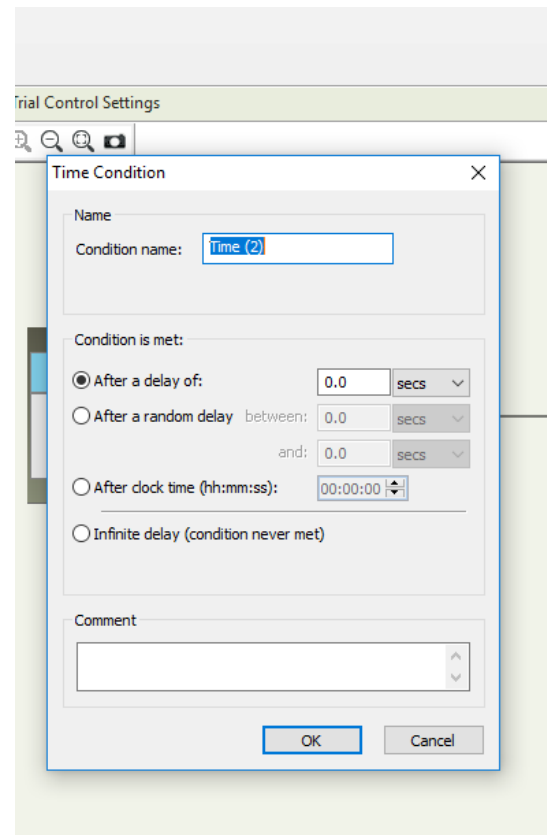




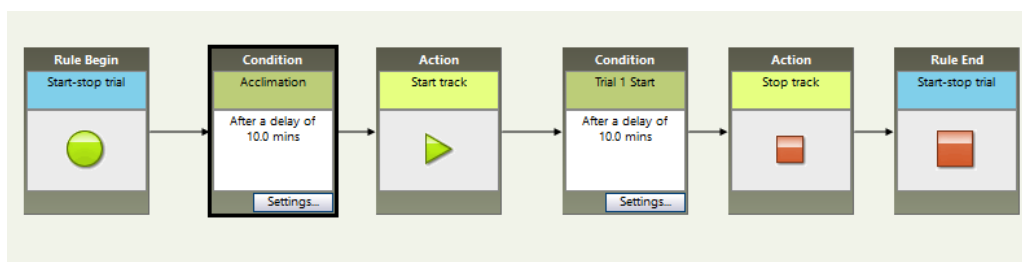
3.1) For example, if you wanted your trial to begin after a 10-minute acclimation period, you would add a Time Condition and specify that the conditions met after a delay of 10 minutes.

4) Define the conditions that, when met during your trial, trigger specific actions.

A condition is a statement that Ethovision evaluates. An action is a command that is executed when the condition is met. For example, if you want your trial to start once your organism approaches an object in the arena. The action is *start tracking* and the condition is *approaching the object*. These conditions and actions can be sequenced and arranged however you would like.



Below is the sequence used for predator-prey interactions starting the acquisition of data after a 10 minute acclimation period and then stopping the tracking after another 10 minutes.



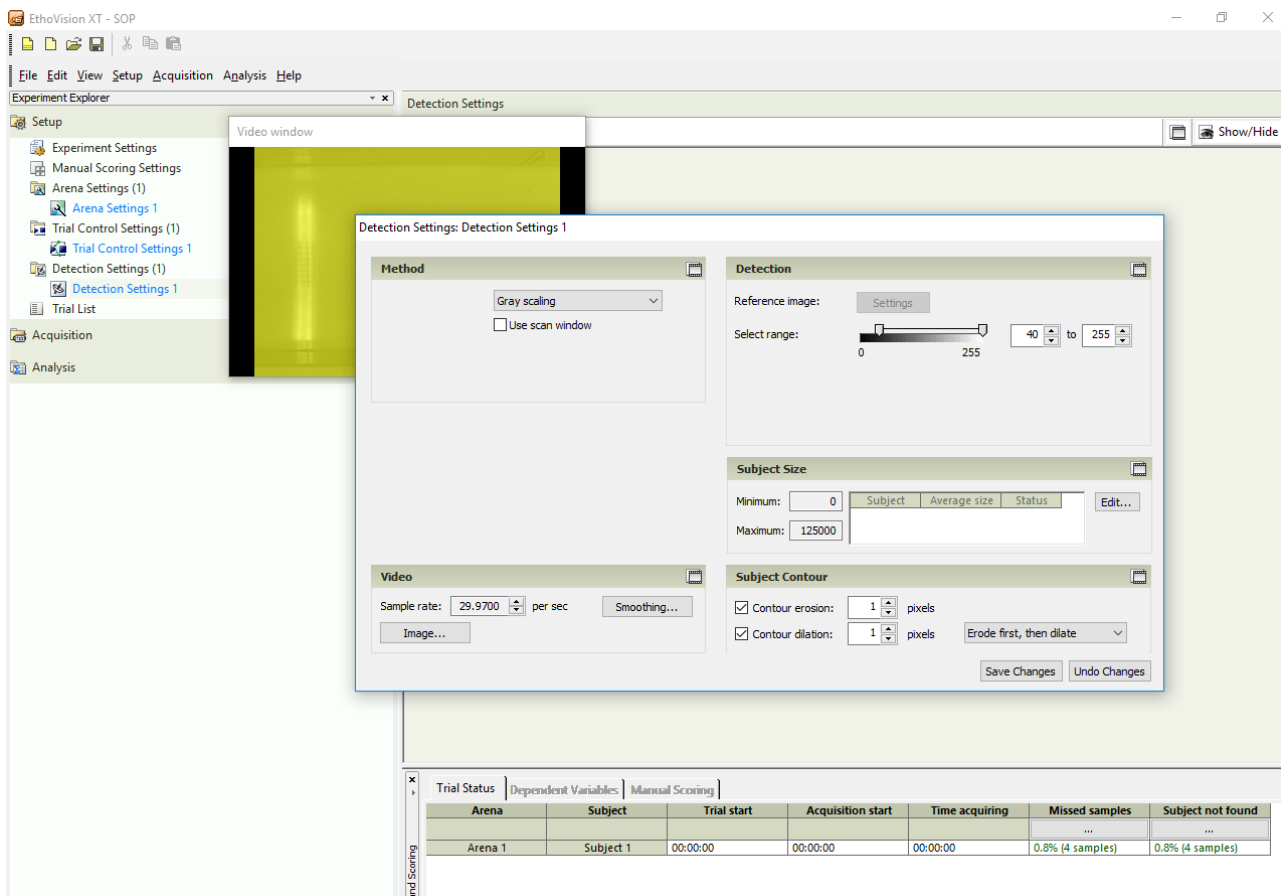
**Once you are done, you can leave this screen, as the changes save automatically.**

You have finished setting up “Trial Control Settings”. Next, you will setup “Detection Settings”.

## 1.6 Detection Settings

1.6.1) Detection settings are used to specify the criteria needed to track moving subjects. Separate detection profiles can be set-up, it is recommended to do this if there will be multiple trials that are not identical, for example, if something will be introduced to the arena during a separate trial. (Such as a predator or another fish)

1.6.2) Detection settings are used to let Ethovision know how different the subject is from the background. (Note: it is recommended to have as much contrast between the subject and the background as possible, as this will make it easier for Ethovision to track the subject.)



2) *Method*: This section contains the methods for subject detection. Your four options are Gray scaling, static subtraction, dynamic subtraction and differencing.

a) Use **Gray scaling** when:

- The animal's grayness differs from the background in the entire arena
- The background does not change during a trial (e.g., no water or substrate is present)
- The lighting is completely even

*Example: tracking a white rat against a uniform solid black background.*

b) Use **Static Subtraction** when:

- You have objects in the arena similar to the animal's color. (e.g., a shelter)
- There are unavoidable reflections or bright items in the arena

*Example: tracking a white rat in an open field with reflections and bright objects.*

c) Use **Dynamic Subtraction** when:

- Light conditions change gradually during the trial
- The background changes during the trial

*Example: tracking a rat in an arena with bedding that gets kicked around.*

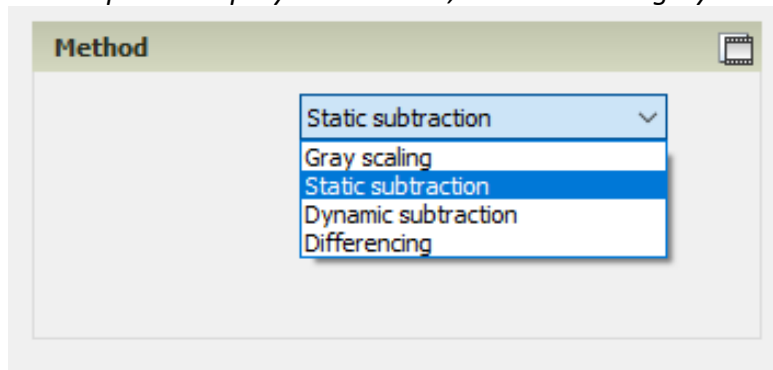
***Note: This is the better option when working with aquatic organisms due to water constantly moving and causing changes in light reflections.***

d) Use **Differencing** when:

- There is a lot of variation in contrast between a subject and the background

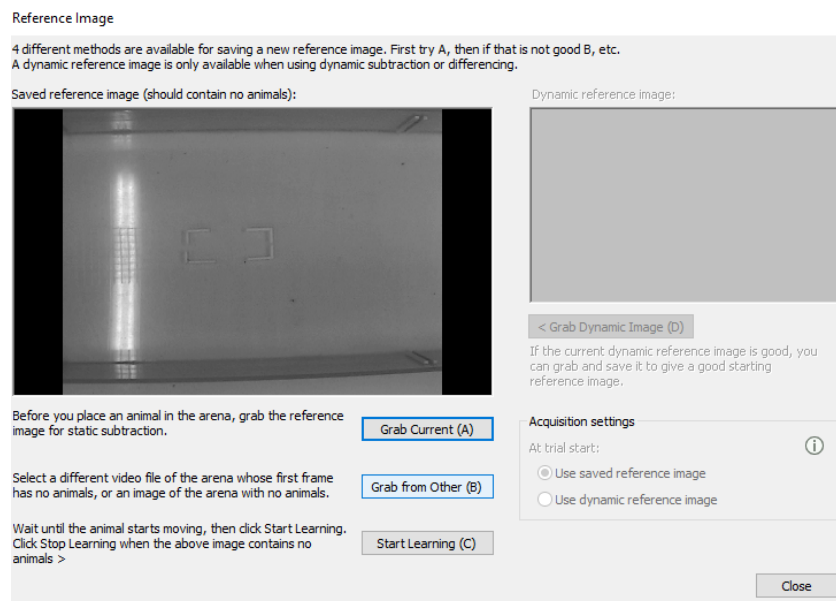
*Example: Tracking an organism with varying body shades (e.g. multi-colored fish, hooded rats etc.)*

*For the predator-prey interactions, we will be using Dynamic Subtraction.*



3) *Detection*: In this section you configure the Subject Detection Settings.

3.1) Reference image refers to the image that you are using to track the subject. When you click on settings, a window will pop up. This window will give you three options on how to track your subject.



Grab Current (A): Use this option before you place an animal into the arena. The software will use static subtraction to capture anything that was not in the previous image.

Grab from Other (B): Use this option when you have already taken an image of the arena with no animals. Image taken during step 1.4.2.

Start Learning (C): Use this option when you have not gotten a previous image. Wait until the subject begins moving and click "start learning" and wait until the animal has moved significantly from its previous location, and then click "stop learning". The software will use the movements to track the subject.

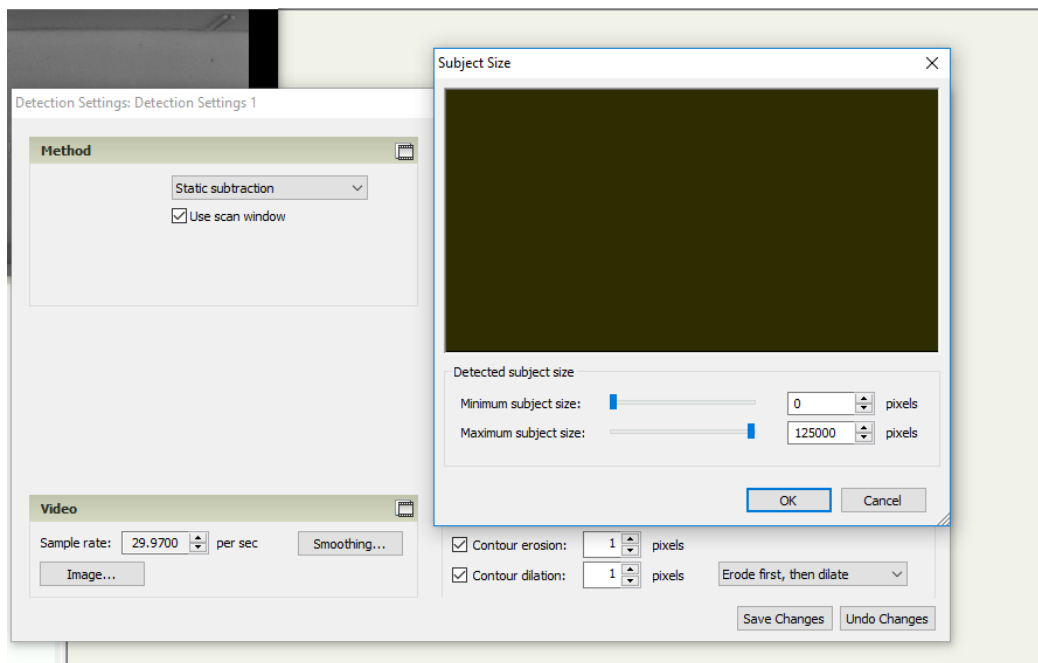
**Once you are done. Click close. The changes will save automatically.**

3.2) Subject is: select whether the subject is brighter or darker than the background.



3.3) Bright contrast: This varies by subject, simply try different variances until you find the appropriate setting of contrast between the subject and the background.

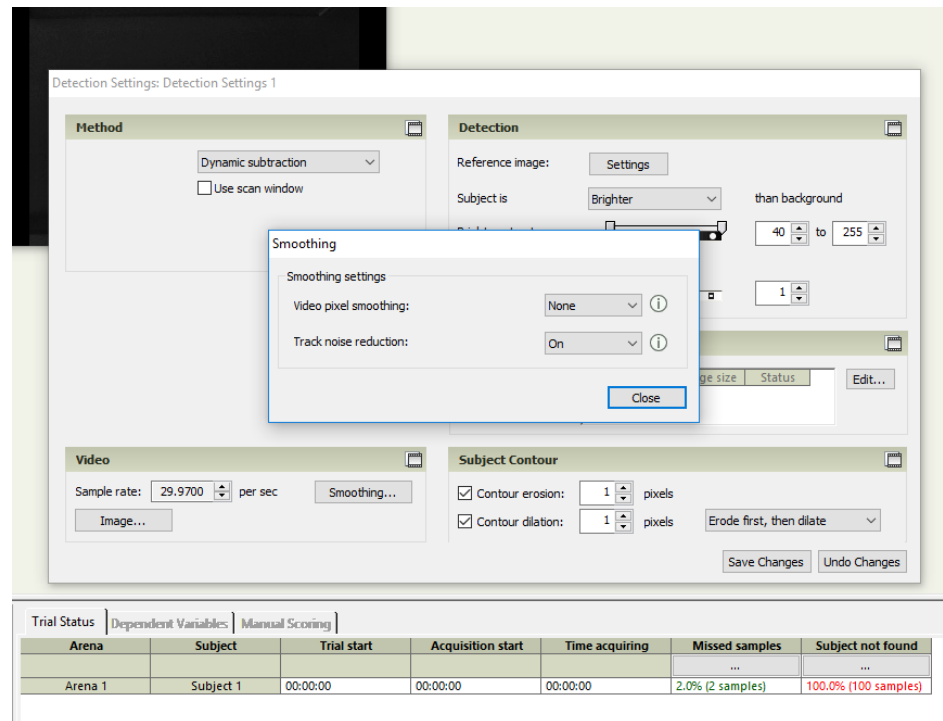
4) *Subject Size*: Left-click edit. A subject size window will pop up. By inputting minimum and maximum values for the subject size, the software knows to exclude anything larger, or smaller than those values for tracking. These values are calculated in pixels and vary depending on the size of your subject. It is recommended to use the live feed as a reference to determine the size of your organism in the “Subject Size” window.



5) *Video*: In this section you can set the sample rate (frames per second) and pixel smoothing.

5.1) The default is 29.9700 frames per second.

5.2) Smoothing is used when there is a lot of static or noise in the background, for example, when using water, the movements of the water can cause noise. The settings will vary by experiment, but it is recommended to track noise reduction due to water movement.



6) *Subject contour*: Contour erosion and dilation is used to fine tune the edges of your subject, for example, if it is thinner or thicker throughout different parts of the body, it is recommended to increase the pixels. The default is 1. With the live feed, you can adjust the pixels until the entire subject is selected.

7) You can create a separate detection settings profile by right-clicking on the “Detection Settings” tab on the left-hand side under “Experiment Explorer” and clicking “New”. Repeat steps 1-6 for this profile. If not, move on to section 1.7

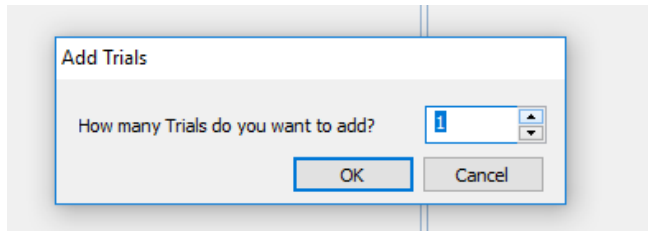
**Note:** Separate profiles are created when the detection settings will vary by trial. (e.g. you are adding a predator or prey in the second trial that you do not wish to track.

**Click Save Changes. This window will not close. It always remains open in Detection Settings.**

## 1.7 Trial List

Trial List allows you to inform the software of how many trials you would like each experiment to have. For example, different trials could be different behavioral treatments of interest (e.g. presenting a predator to a prey, adding food to your subject or introducing any other novel object (such as a shelter)).

By left-clicking 'Add trials' at the top-left hand side of the screen you can choose how many trials you would like to add. Select the amount of trials and click OK.

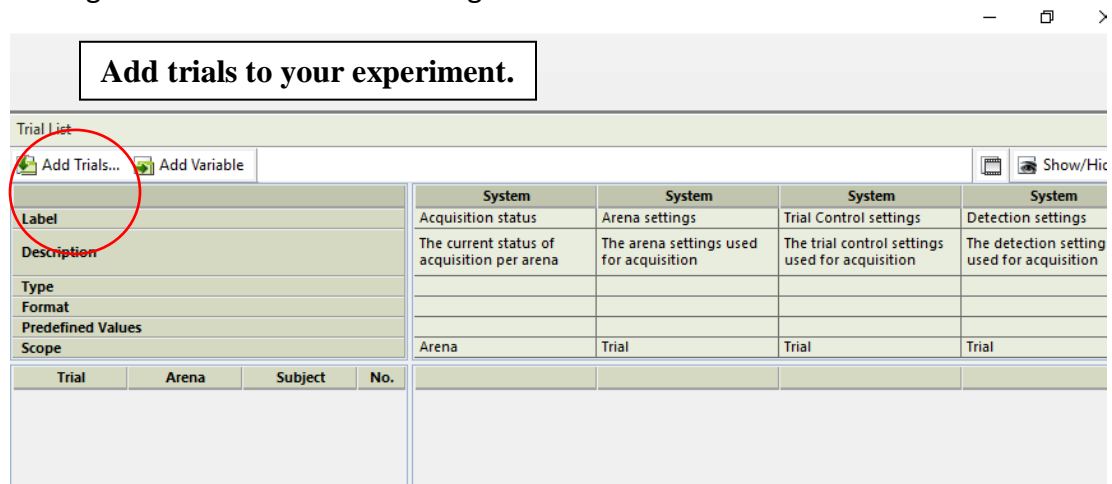


From here you can also designate which arena, trial control and detection settings each trial will be using. This is particularly useful when you have different detection and trial control settings for each trial. This was set up in step 1.6.7.

Add two trials to the list by left-clicking 'Add trials' at the top of the screen and selecting two.

Trial 1: Acquisition Status: Planned. Arena Settings: Arena Settings 1. Trial Control Settings: Acclimation + No predator. Detection settings: No predator

Trial 2: Acquisition Status: Planned. Arena Settings: Arena Settings 1. Trial Control Settings: Predator. Detection settings: Predator



**Once you are done, you can leave the page. Changes save automatically.**

## 2. Acquisition

### 2.1 Acquisition

By left-clicking acquisition, a window will open displaying video window, Acquisition settings and Acquisition Control.

1) *Video Window*: Use it to verify that the detection settings are tracking your subject. Your subject will be yellow with a red dot indicating the center-point. The window will read: Trial # ready for start 00:00:00 and will track the duration of the trial.

2) *Acquisition Settings*: Select Live Tracking, as we will be tracking the subject using a live video and make sure 'save video' is selected, so the video will be saved to the folder we selected in step 1.2.1, in case you need to go back and look at the data at a later time.

3) *Acquisition Control*: Press the green circle to begin the trial. These controls can be used to begin a trial, stop a trial and skip a trial.

At the bottom of the screen, there is a table called *Analysis results and Scoring*. This table displays live data from the trial as it is being recorded. By selecting dependent variables one can see the velocity, movement, distance etc. being recorded in real-time. **Note: All this data will still be acquired by the end of the trial regardless of whether you're tracking it live or not.** E.g. If you leave the room and let the trials run, the system will still be tracking that data and will be visible once exported.

The screenshot displays the software interface for trial acquisition. It includes a Video window showing a trial ready for start, an Acquisition Settings panel with options for Live tracking and Save video, an Acquisition Control panel with a green circle button, and an Analysis results and Scoring table at the bottom.

**Acquisition Settings**

- ☒ Live tracking
- ☐ Save video only, track later
- ☐ Track next saved video file
- ☐ Track all saved video files
- ☒ Save video
- 0 postponed trials

**Settings**

- Trial: Trial 1
- Video: Live video
- Arena settings: Arena Settings 1
- Trial Control settings: Trial Control Settings 1
- Detection settings: Detection Settings 1

**Acquisition Control**

Start trial

**Analysis results and Scoring**

Trial Status	Dependent Variables	Manual Scoring					
Arena	Subject	Trial start	Acquisition start	Time acquiring	Missed samples	Subject not found	
Arena 1	Subject 1	00:00:00	00:00:00	00:00:00	0.3% (2 samples)	100.0% (695 samples)	

Dependent variables

## 2.2 Acquired Trials

By clicking on Acquired trials, the system displays the information of the trials acquired so far.

## 2.3 Track editor

Track editor allows you to playback the video feed of each trial.

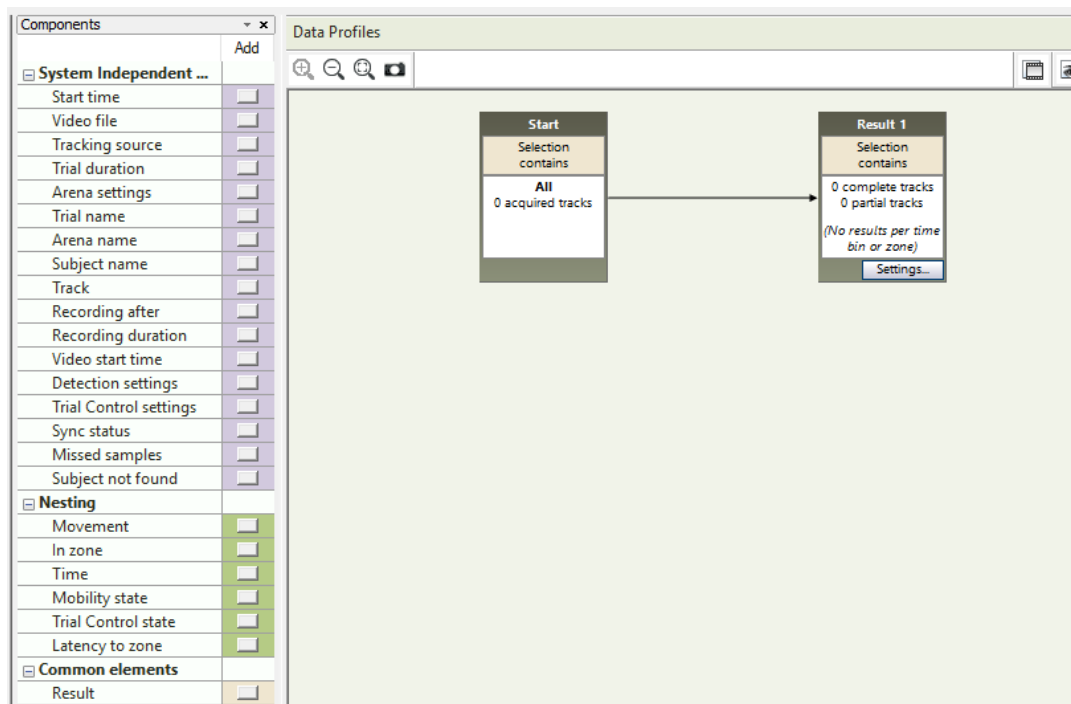
## 2.4 Track smoothing profiles

Track smoothing profiles are used to filter out system noise and outliers and small movements of the animal (body wobble). These are defined in step 1.5.2

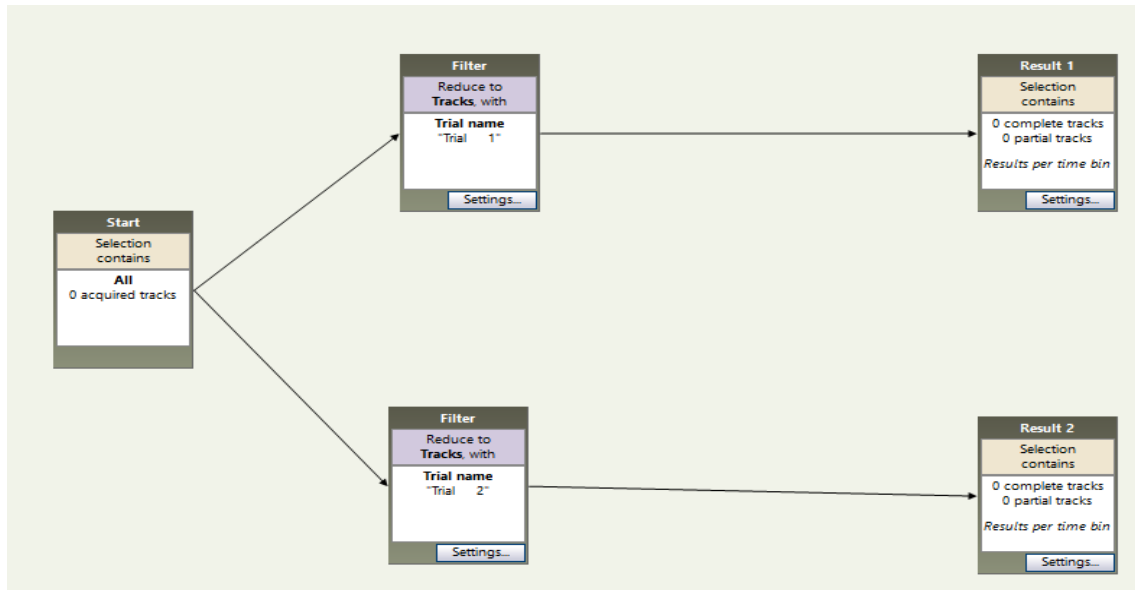
# 3. Analysis

## 3.1 Data Profiles

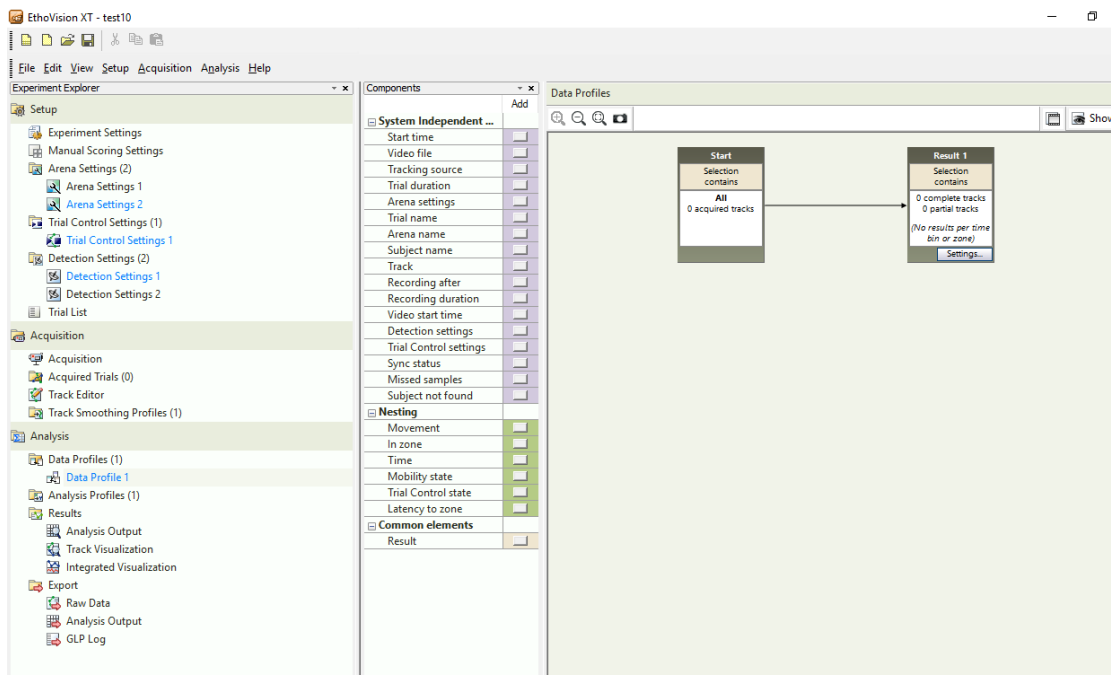
1) Data profiles are used to define what endpoints we want the system to track.



2) By creating multiple data profiles, the software allows us to directly compare the results between trials. Below is an example of two data profiles, one for each trial.

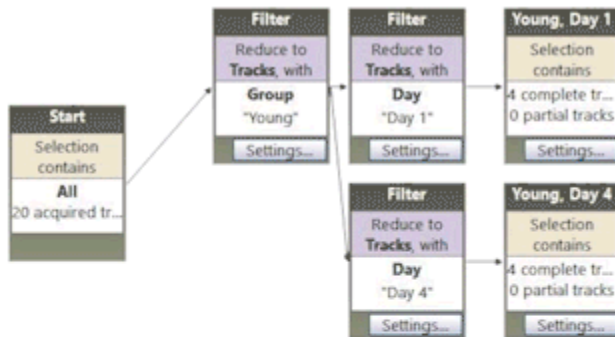


3) Data selection tools let you evaluate certain subsets of each trial. For example, selecting the data in which animals received a specific treatment allows you to analyze only those data sets and ignore the rest. In addition, you can also nest your data by time, zone, speed or any other behavioral endpoint. If you wish to analyze all of the data, simply create a filter for each individual trial and link it between “Start” and “Result”. Each trial will require its own “Result” bin. You can add this by clicking “Result” at the bottom of the “Components” list.





4) Data selection is very intuitive to set up, using blocks that, for example, contain "filters". You can add these filters to select data before analyzing your data. This is done for example, if you only wish to look at the data for a certain trial. Filters are added by left-clicking on each individual box and dragging them to your line up.



### 3.2 Analysis Profiles

1) The Analysis Profile is used to specify what data we want displayed when we export the data. Here you can choose which endpoints you want to look at. Note: You can always adjust this after you acquired your trials, for example, if you only wanted the data for the total distance moved within the trial.

2) 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.

Dependent Variables

Add

Analysis Profiles

Show/Hide

Selected Dependent Variable

Description

Distance moved

Distance moved of the center-point

Velocity

Velocity for the center-point

In zone

When center-point is in any of Arena, Quadrant 1, Quadrant 2, Quadrant 3, Quadrant 4, Quadrant 5, Quadrant 6, ...

Mobility state

Immobile, Mobile and Highly mobile, averaged over 30 samples, with thresholds at 50.00 and 10.00%

Activity state

Inactive, Moderately active, Active and Highly active with thresholds at 10.00, 15.00 and 50.00%

Distance to zone

Distance from center-point to the border of each of Arena, Predator Zone

Distance to point

Distance from center-point to each of Arena (Center), Predator Zone (Center)

Heading to point

Heading of the center-point in the direction of Predator Zone (Center)

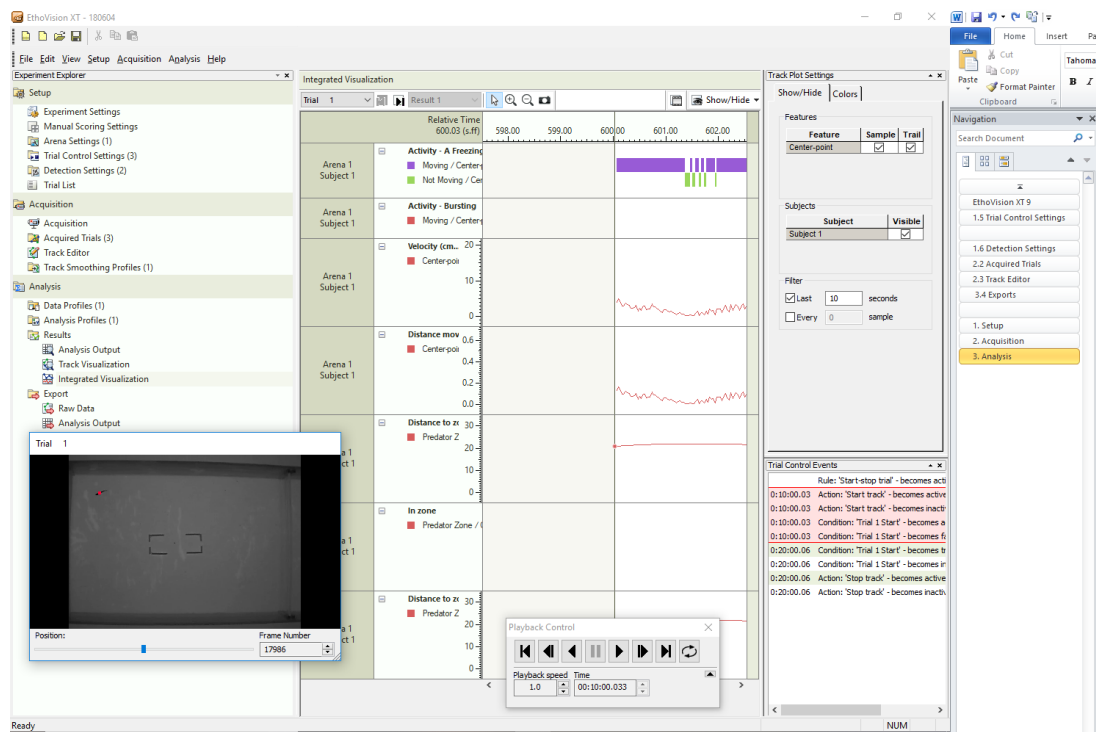
**Data profile set up for predator-prey interactions, tracking information such as velocity, distance moved, time in predator zone, distance to predator zone, time spent immobile and mobile.**

### 3.3 Results

1) Analysis Output: This is where the data acquired previously in the experiment will be displayed. By clicking 'Calculate' at the top left-hand corner of the screen a table with all of the numerical inputs will appear on the screen. Results will be displayed in numerical order, allowing us to directly compare the trials. Next to calculate, there is a button to 'Export', by clicking export we can export this data sheet and open it in a software, such as Excel, for further calculations.

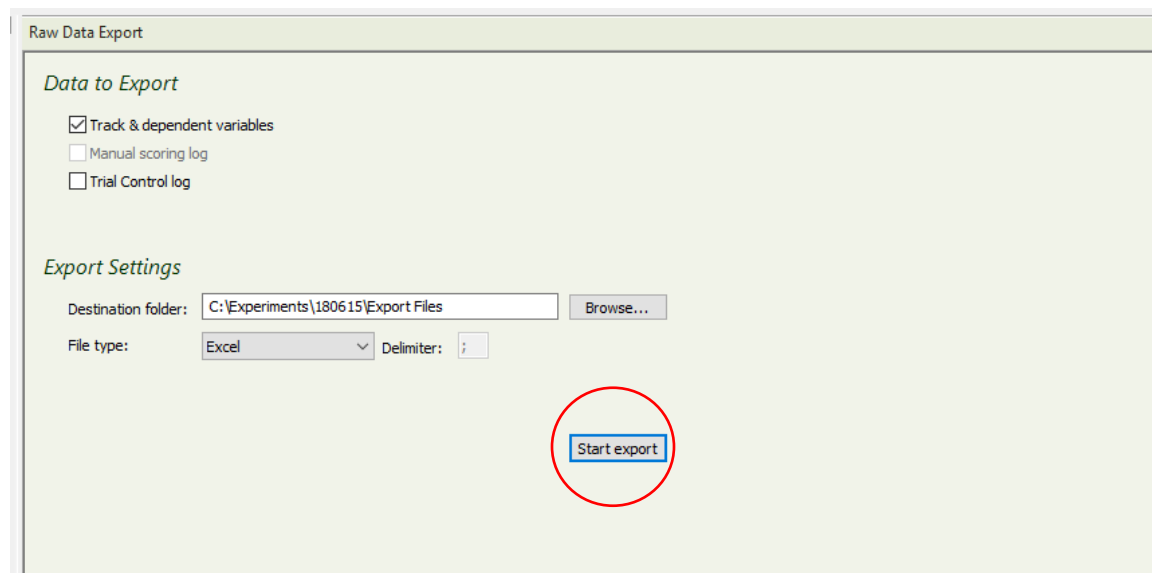
2) Track Visualization: Allows you to visualize the data by using the video feed and displaying the numerical values simultaneously.

3) Integrated Visualization: Allows you to visualize the tracks superimposed on the video file. It allows you to view the plots for each endpoint alongside the video footage. This is particularly helpful if you wish to compare the video footage with the results of the analysis output. For example, you can compare if your organism was not moving for the duration the software told you it was not moving.



### 3.4 Export

- 1) Raw Data: Data to export should have *Track and dependent variables* box clicked.
- 2) *Export Settings*: Destination folder will be C:\Experiments\**Experiment Name**\Export Files. File type will be Excel. By clicking *start export*, it will begin exporting the raw data into an Excel document.
- 3) *Analysis Output*: Destination folder will be C:\Experiments\**Experiment Name**\Export Files. File type will be Excel. By clicking *start export*, it will begin exporting the analysis output data into an Excel document.



### End of Experiment

Save your experiment as the experiment name: Last two digits of the year/ Month / # Of Experiment in that month. Example: 180601, 180602 etc.

Click the X at the top-right corner of the screen and close EthoVision.

**4) Save exported data to your own personal hard-drive or flash-drive. This is a shared facility and computer space is limited.**