General 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.



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- Direct towards the control box and turn the switch up towards where it says power.
- 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.

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



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

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- 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.



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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

visible to everyone by flipping it.

Observation Chamber down in the room while running experiments. In addition, there is a sign on the door to the 1 Holding room, prompting people that there is currently Chamber an experiment in progress; please fill out the information on the sign and ensure that it is

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 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.

 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.





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

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.

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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

- 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.

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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.

/hich video source will y ● From video file	you use?	n	new experiment	
O Live tracking (and saving vic	deo files)	b d	template experiment default experiment	
Track pre-recorded video files. L arena and detection setup. Browse	Browse to select a video for the	pr t	F @ video tutorial the Noldus website	
			help topics	
1 of 5	< Previous	Next > Cancel		
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		EthoVision XT W EthoVision XT	Details	
		EthoVision XT W EthoVision XT Action Vrdee source	Details Euresys PCOLO U4 H.264 Nor0 - VD1, NTSC, Ngh resolution	
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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 to do this manually, you must specifically tell ethovision the paramaters of the animal you wish to track. Eg.size, color, how many pixels, ect.

/hich subjects will you tra Selecting an animal type allows I ORodents Fish Arthropods Other Other Zebrafish larvae Zebrafish larvae Goldfish Other	Vision XT to generate better setup defaults.	new experiment template experiment default experiment ore video tutorial the Noldus website help topics
2 of 6	< Previous Next > Ca	ncel

			new experiment
 Center-point Color marker 			default experiment
Track the subject's center-point.			pre
The animal is Darker Brighter Darker Brighter and darke	than the background er	Tracked center-point	the Noldus website
4 of 6	< Previous	Next > Cancel	

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.

low is the arena co	nfigured?		new experiment
Arena template:	Open field, square	\sim	template experiment
Zone template:	No zone template	~	default experiment
Number of Arenas:	1 ~		ore
Rows and columns:	1 × X 1		the Noldus website
			help topics
3 of 6	< Previous	Next > Cancel	1

5) The next step will be choosing a sample rate. This is important because if you choose too high of a sample rate, the software will over-track movements, but if the sample rate is too low, it will miss movements. 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.

Setting a correct If it is too high, n low, movement w	sample rate is important, oise will be tracked giving an ill be missed.	overestimation of e. <u>c</u>	n. distance moved. If it is t	new experiment template experiment default experiment	
The recommende your animal. The the tracked point moved per second Recommended: Applied:	d value is based on the most of optimal value also depends of and the magnification (i.e. nu t). 5 samples/sec 5.00 \$ samples/sec	iommon setup for n the velocity of mber of pixels	Original path Acquired track O Acquired sample	video tutorial the Noldus website help topics	
5 of 6		< Previous	Next > Ca	ancel	

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.

lew Expe	iment	New template experiment
Name:	1	New default experiment
ocation:	C:\Evneriments	Brausa
		Learn more
Experimen	t will be created as:	View video tutorial
		Visit the Noldus website
	OK	Cancel ? View help topics
	Restore backup	
	Latest news	
	A new EthoVision XT update is	available
	This fixes a few issues, in differe	int parts of the application.

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.)

Experiment Inform	ation		
Location:		C: \Experiments \Ethovision Template Predator-Prey \	
Description:		1	
Video Source:			
O From video file			
Live tracking	Video source:	Euresys PICOLO U4 H.264 No/0 - VID1 VID1 VISC, High (640x480)	Edit
	Saving source:	Euresys PICOLO U4 H. 264 No/0 - VID2 V	
Arenas Number of Arenas:		1 ~	
Tracked Features			
Center-point detecti	ion		
◯ Color marker trackin	g (treat marker a	as center-point)	
Non-tracking Feat ☑ Activity analysis	ures	6	
Units			
Unit of distance:		cm 🗸	
Unit of time:		s v	
Unit of rotation:		deg v	

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.

Manual Scoring Se	ettings				
🚰 Add Behavior.	. 🐋 Validate				
Behaviors					Keys
Behavior Name	Туре		Description	Initially Active	Subject 1
Add Beh Mutuali new be	avior y exclusive groups havior stops the a	s always h	nave an active behavior, and sc	× oring a	
to score E E	e the stop of the b lehavior name: lehavior type: lehavior group:	Start - S	Stop V	u nave	
to score	e the stop of the b lehavior name: lehavior type: lehavior group: Description:	Start - S	itop V		
to score	e the stop of the b lehavior name: lehavior type: lehavior group: Description: Initially active	Start - S	stop ~) ave	

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 seperately.



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.

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.

If multiple distances are used, the calibration is based on the average value OK	j Add <u>Z</u> one Group	🀋 Duplicate Complete <u>A</u> rena
	t Additidade Tenes Crown	
	1 yaa Elaasu zous elonb	코코 Multiple Arena Setup
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	Delete Zone Group	‡i Adjust Aspect Ratio
		📝 Validate Arena Settings
		Color View Lock
_	Background	 ⊡
	Calibration	
	📄 🔡 Arena 1	⊡
	IIII Zone Group 1	



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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.

Add Z	one Group	🚽 Du					
T Add H	idden Zone Group	atat Mu					
Add C	umulative Zone	🔕 Gra	ab <u>B</u> ackgr	ound In	nage		
Delete	Zone Group	‡∄ Ad					
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1			Color	View	Lock		
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🛓 🖓 🖓 🗛	rena 1						
	Predator Zone						
	Quadrante				-		

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.



An example of defined zones (including predator zone) within the arena. Note, each zone has a defined name.

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 left left-clicking on Conditions on the left-land side of the screen under "Components". Conditions can be 'Time', 'In Zone' or 'Movement' and vary by experiment.



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rial Control Settings

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 condition s 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.

me Condition			×
Name			
Condition name: Time (2)]	
Condition is met:			
After a delay of:	0.0	secs	\sim
O After a random delay between:	0.0	secs	\sim
and;	0.0	secs	\sim
○ After clock time (hh:mm:ss):	00:00:00	ŧ	
O Infinite delay (condition never me	t)		
			\sim
	<i>c</i>	Cano	el

Below is an example of a sequence 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.)

🚾 EthoVision XT - SOP										_	o x
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<u>File Edit View Setup Acquisition</u>	A <u>n</u> alysis <u>H</u> elp										
Experiment Explorer		• × Dete	ection Settings								
😹 Setup	Video window										😹 Show/Hide
Experiment Settings Manual Scoring Settings Arena Settings (1) Arena Settings 1 Trial Control Settings (1) Trial Control Settings (1)		Detection Sett	tings: Detection Settings 1								
Detection Settings (1)		Method			[***]	Detection	1		Ē		
Detection Settings 1			Grav scaling	~	-0000	Reference i	mage: s	ttings	×00000		
Trial List			Use scan window	v							
Acquisition						Select range	e: 🖬	255	40 - to 255 -		
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						Subject S Minimum: Maximum:	ize 0 Sul 125000	oject Average size	Status Edit		
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		Sample rat	te: 29.9700 ♀ per sec	Smoothing		Contour	r erosion: 1	 pixels pixels Erode firm Save 	t, then dilate V Changes Undo Changes		
		X + Du	Trial Status Dependent Van Arena Arena 1 5	īables Manua Subject Subject 1	I Scoring Tria	al start	Acquisition sta	rt Time acquiring	Missed samples 0.8% (4 samples)	Subject	ct not found samples)
		and Scori.									

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.)

Method		
	Static subtraction	~
	Gray scaling	
	Dynamic subtraction	
	Differencing	

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.

	-		Subject Size		×
etection Settings: De	etection Settings 1				
Method					
	Static subtraction	~			
	Use scan window				
			Minimum subject size:		0 pixels
			Maximum subject size:		125000 🜩 pixels
Video					OK Cancel
Sample rate: 29	.9700 🔶 per sec	Smoothing	Contour erosion:	1 pixels	
Image			Contour dilation:	1 🔹 pixels	Erode first, then dilate $\qquad \lor$
					Save Changes Undo Changes

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.

Video pixel smoothing: Track noise reduction: On Video Subject Contour Sample rate: 29.9700 ¢ per sec Smoothing Image Istatus Dependent Variables Manual Scoring	Video pixel smoothing: None Image: Imag	Method	Dynamic subtr	action Indow Smoothing Smoothing settings	Detection Reference imag Subject is	ie: Settings Brighter		than background
Video Subject Contour Sample rate: 29.9700 per sec Image Tage Smoothing Contour diation: 1 pixels Erode first, then dilate Save Changes Undo Changes Undo Changes Undo Changes I Status Dependent Variables Manual Scoring	Video Subject Contour Sample rate: 29.9700 + per sec Smoothing Image Image Image Image Image Image Itatus Dependent Variables Manual Scoring Arena Subject Trial start Acquisition start Time acquiring Missed samples Subject not fi			Video pixel smoothing: Track noise reduction:		None v (i) On v (i) Close	ge size	1 Status Edt
I Status Dependent Variables Manual Scoring	I Status Dependent Variables Manual Scoring Arena Subject Trial start Acquisition start Time acquiring Missed samples Subject not fi	Video	29.9700 🔶 per se	Smoothing	Subject Conta Contour ero Contour dia	our osion: 1 pixel ation: 1 pixel	s Erode firs	st, then dilate V
Arena Subject Trial start Acquisition start Time acquiring Missed samples Subject not for	Archa Subject Indistant Acquisition start Inne acquiring Missed samples Subject not i	Image					Save	Changes Undo Changes

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.

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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.

How many Trials do you want to add?	1
OK	Cancel

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 trials to you	r experime	nt.			
Trial List					
🛃 Add Trials 🔄 Add Variable					📑 🗟 Show/Hic
		System	System	System	System
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Description		The current status of acquisition per arena	The arena settings used for acquisition	The trial control settings used for acquisition	The detection setting used for acquisition
Туре					
Format					
Predefined Values					
Scope		Arena	Trial	Trial	Trial
Trial Arena S	Subject No.				

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.

Video window Trial 1 Ready for start 00:0 :00	Acquisiti © Live O Sav O Trai Se 1	on Settings : tracking e video only, track later ck next saved video file ck all saved video files ttings rial:	Save video O postponed trials Trial 1	
		video: rial Control settings: rial Control settings: Detection settings: Show Independent Variables	Live video Arena Settings 1 Trial Control Settin Detection Settings riables after acquisition	gs 1 v 1 v Redo Trial
Start trial				
Arena Subject Trial start	Acquisition start	Time acquiring	Missed samples	Subject not found
Arena 1 Subject 1 80:00:00	00:00:00	00:00:00	0.5% (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.

Components	* X	Data Profiles	
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Video start time			
Detection settings			
Trial Control settings			
Sync status			
Missed samples			
Subject not found			
Nesting			
Movement			
In zone			
Time			
Mobility state			
Trial Control state			
Latency to zone			
Common elements			
Result			

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.

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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.

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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.

Raw Data Export
Data to Export
Track & dependent variables
Manual scoring log
Trial Control log
Export Settings
Destination folder: C:\Experiments\180615\Export Files Browse
File type: Excel V Delimiter:
Start export

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