

Protocol for Running Automatic Trials

Last updated: November 24th

Set-up & Running Experimental Trials

***Note:** Don't get animals from the holding room until you're ready to put them in for detection. settings (Steps 10 & 11). First, run the **system set-up** below.

System set-up:

1. **Check solutions** for all arenas and make any if necessary (each syringe should be at least half full after the system check, before any given session).
2. Turn on **powerstrip** (underneath table).
3. Plug in the **noldus box** (back of Noldus box, bottom right, labeled "in").
4. Turn on **MCUs** (usb hub, right button → blue lights will turn on around the MCU usb in use).
5. Turn on **infrared lamps** (one is *by itself* next to the **Noldus Box** and the others are connected to two surge protectors to the *left of arenas 1 and 3*).
6. Make sure *lights and feeders* are working by going to **Documents → Noldus → MCU Commands → File → Open Windows Powershell**
 - a. Then type “**./system_check FR2355**” [or 2355_2, 2355_3, 2355_4] The program will then run a valve and light check sequence. Be sure water is coming out from all feeders, and all lights are turning on for each maze.
 - i. Hit Ctrl C to terminate the batch job and Y to confirm, then you can rerun the “**./system_check FR[ID#]**”
 - b. Hit enter and the program will run, checking valves and feeder lights. Do this for all four mazes.
 - c. Make sure syringes are full after running system checks for each arena.

Important: If only 2-3 mazes are working that day then put fake rats in mazes that don't work and proceed with what's working → be sure to make a note for the guys in serendipity that only 2-3 mazes were used → see data export under step 16b.

7. To use ‘randi (4, 30)’ in MATlab to generate a trial list to run for the session.

Note* you can just use the sequences saved in the note on the desktop, next to the “Trials” data folder.

- a. ‘randi (#of different TC settings, # of trials you're going to run)’
- b. Use the random number sequence generated by MATlab to create a **trial list** in EthoVision (to be used in step 10, below).

Example of ‘randi(#,#)’ command used for 4 different trial types and 10 trials

The screenshot shows the MATLAB interface with the Command Window open. The window displays the command `>> randi(4,10)` and its output, which is a 10x4 matrix of integers ranging from 1 to 4. The output is divided into two parts: 'Columns 1 through 7' and 'Columns 8 through 10'. The matrix is as follows:

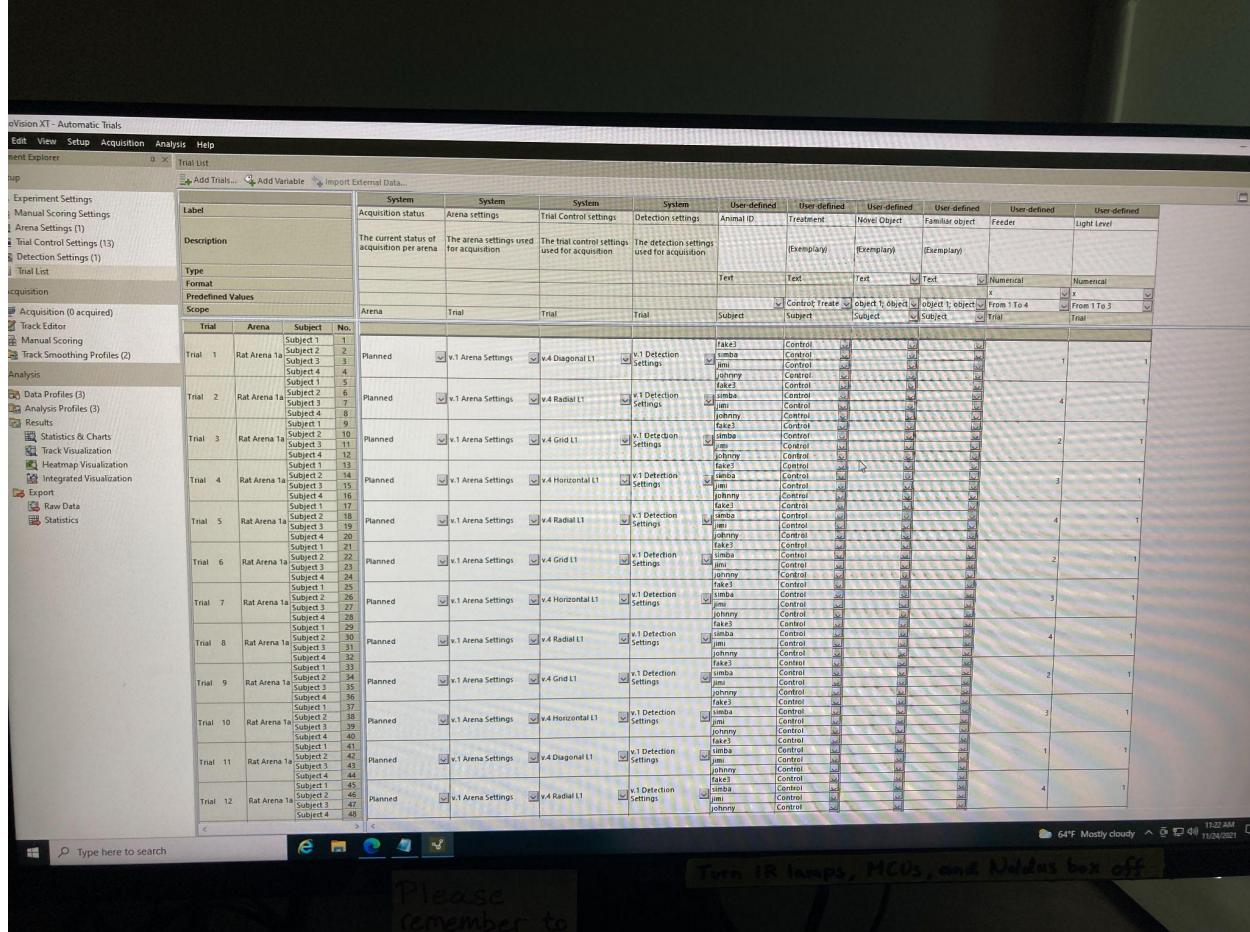
Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
4	1	3	3	2	2	4	2	4	1
4	4	1	1	2	3	2	4	1	1
1	4	4	2	4	3	3	3	4	3
4	2	4	1	4	1	3	1	2	4
3	4	3	1	1	1	4	3	1	2
1	1	4	4	2	2	4	1	3	3
3	2	3	3	2	4	3	2	1	4
2	2	3	3	2	4	3	1	2	4
3	4	2	2	3	2	1	3	4	3
4	4	3	4	3	3	1	2	1	4
4	4	1	1	4	1	2	4	3	2

When the system is checked and you've come up with a trial list move on to ethovision.

Running trials:

8. Load EthoVisionXT and select correct experiment:
 - a. Right now it is labeled as “**automatic trials**”
 - b. **Close other programs** (e.g. MATLAB).
 - c. **Make sure you go to task manager (ctrl→ alt → delete) and set EthoVision’s priority to “realtime”**
 9. Go to “**Trial List**”
 - a. Create the sequence of trials that you’re going to run. Enter the TC Settings, Animal Names and User-Defined Feeder (1-4) and Light Level (0-3) variables
Example of trial list set using our current settings.

Note: The versions of the TC settings will change over time as we modify our task

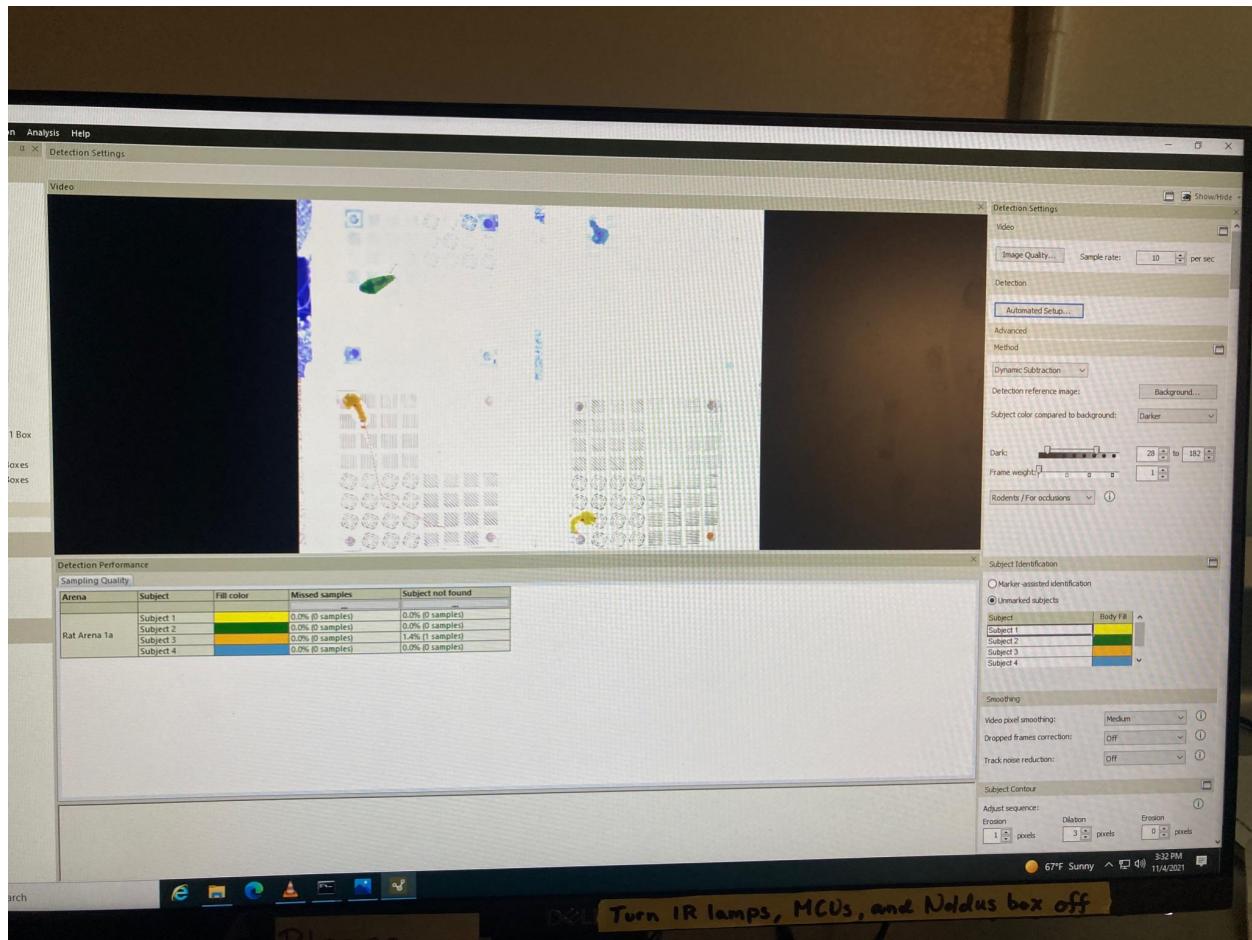


10. Get animals and put them into the arenas (cage 1 into arena 1 and 2, and cage 2 into arena 3 and 4).

11. Select Detection Settings by going to “Detection Settings” selecting “v.1 Detection Settings”. This should automatically detect the animals with our pre-saved settings.

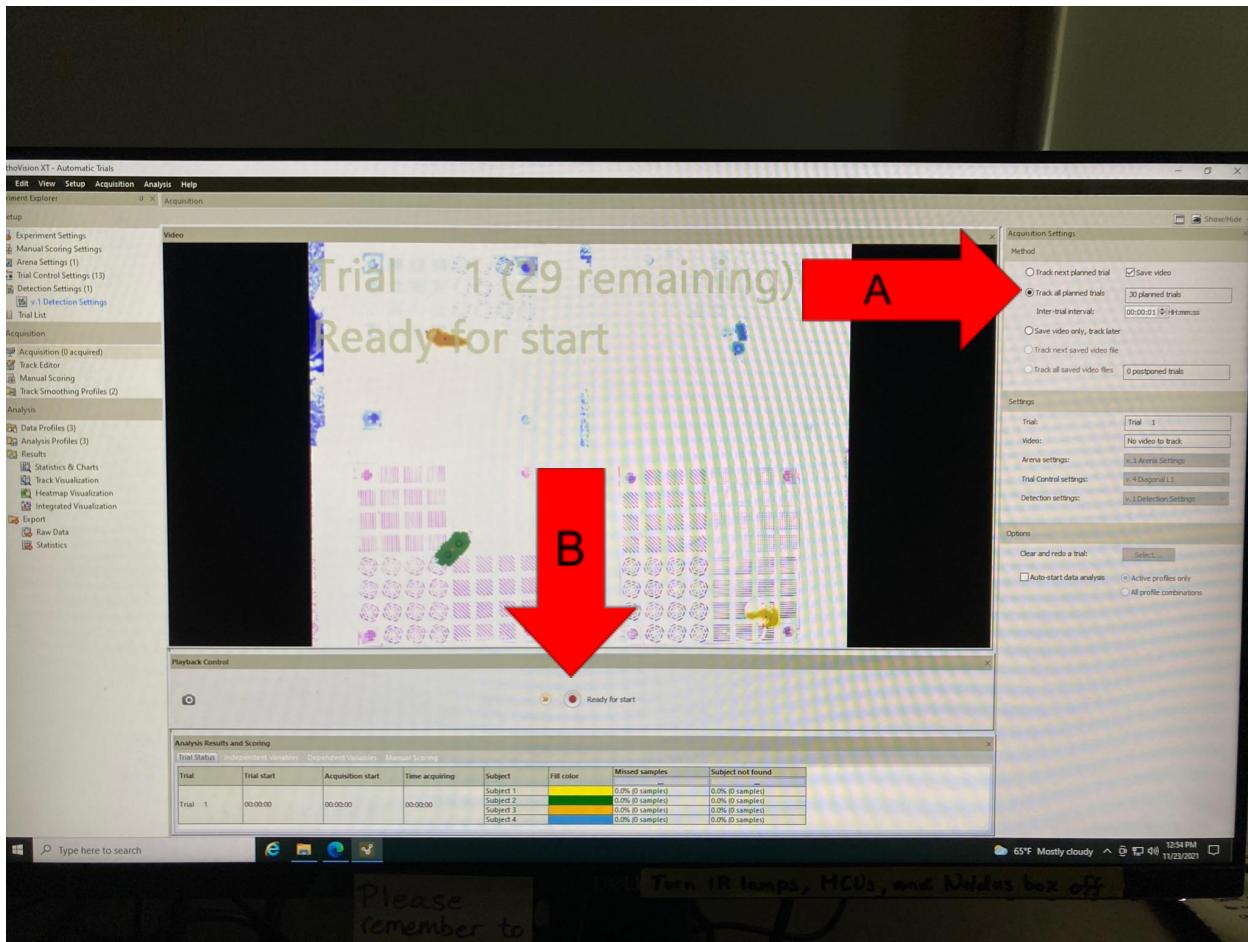
If you ever need to re-do them, then follow these steps:

- a. Place your animals in one-by one, and click “**Automated Setup**”.
- b. Then drag boxes around your four animals, finetune your detection results if necessary and click ok.
- c. If you need to, mess with advanced settings under “Method, Smoothing, & Subject Contour” and make sure that you have “unmarked subjects” selected.



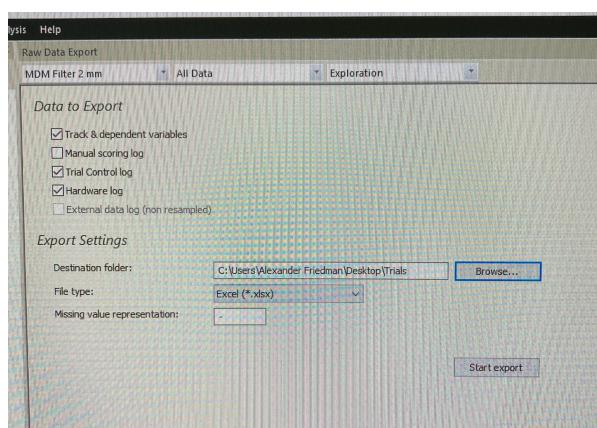
12. Go to “Acquisition” and, using the image as a reference, follow these steps:

- a. Be sure to select “**track all-planned trials**” in the menu on the right-hand side.



- b. Click “Ready for Start” button in middle of screen.
 - c. Let program run, various code (command line) windows will pop up, run, and close themselves.
13. At the end of the trial, a pop-up window will say “trial run finished”. Click “ok”.
14. Go to “Analysis”, click on “export” → “raw data”

- a. Make sure you have the following checkboxes selected:



- b. Export as an excel file to desktop folder: “**Trials**”.
 - i. Then go to ‘**Trials**’ folder on the desktop and create/add to a folder by date and sessions (see examples - for how to format - within Trials folder).
Note: eventually David and Omar will have a program/app for the exportation of data → much like serendipity for animal habituations.
For example: go to 11/24 folder and create a sub-folder titled “**s4 Scar, Simba, Jimi, Johnny**” which you would export data to for that session.
15. Running trials will lock experimental settings (arena, trial control and detection). To unlock and make changes after exporting data and before starting a new session, go to options on the right side and click the box that says “**select**” next to “**Clear and Redo Trial**”, select clear tracks and video click ok.

Shut-down

After trials have been run, data exported, and animals put away, do the following:

1. **Clean mazes** (wipe down floor pieces and clean valves+feeders) and **sanitize** any necessary **surfaces** (e.g. table if you were touching it after handling animals).
2. Turn off **cameras**.
3. Turn off **MCUs** (usb hub).
4. Unplug **Noldus box**.
5. Turn off **infrared lamps**. Don’t forget the one on the Noldus computer table.
6. Close **ethovision**.
7. Turn off **all lights**.