

## **Protocol for Running Automatic Trials**

*Last updated: April 14th*

### **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. **Make 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 **powerstrips** (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. Recheck syringes to make sure they’re 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, name as ‘none’ and proceed with what’s working. E.g. when maze 1 isn’t working, we put the fake rat in there and run trials with 2, 3, and 4.

#### **Running trials:**

7. Load EthoVisionXT and select correct **experiment**:
  - a. Right now it is labeled as “**Automatic trials**” and “**Automatic trials females**”
  - 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”**
8. Go to “**Trial List**”

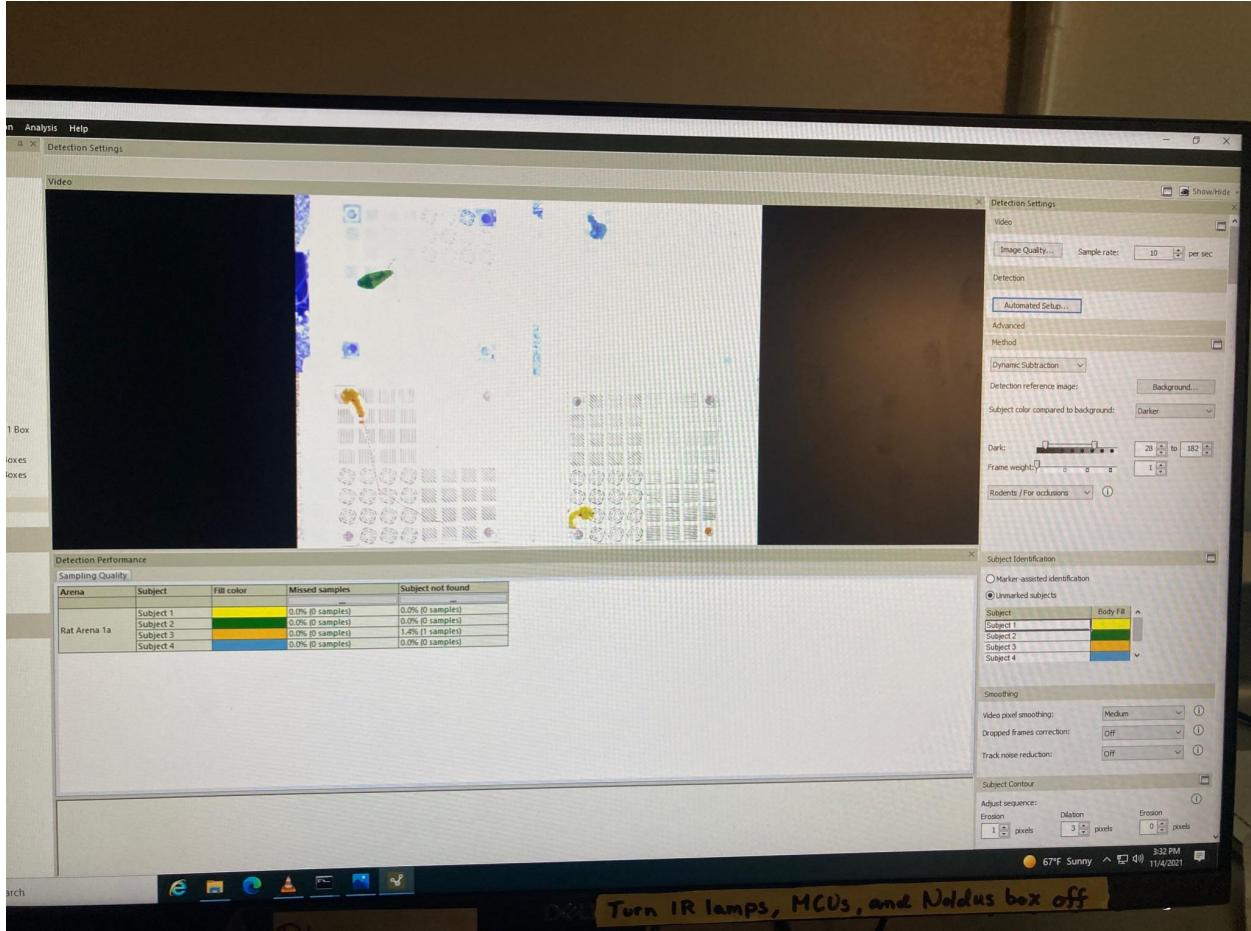
- Create/check the sequence of trials that you're going to run. Enter the TC Settings, Animal Names and User-Defined Feeder (1-4) and Light Level (0-3) variables. Check reward amounts and lux values per maze, by feeder (all variables to the right of the trial list table). Make sure that everything is filled out and matches.

**Note:** *The versions of the TC settings will change over time as we modify our task. This, for instance, is the trial list we were running last november - and, if you compare it to ours now, you'll see that we're using a different phase with a lot more variables.*

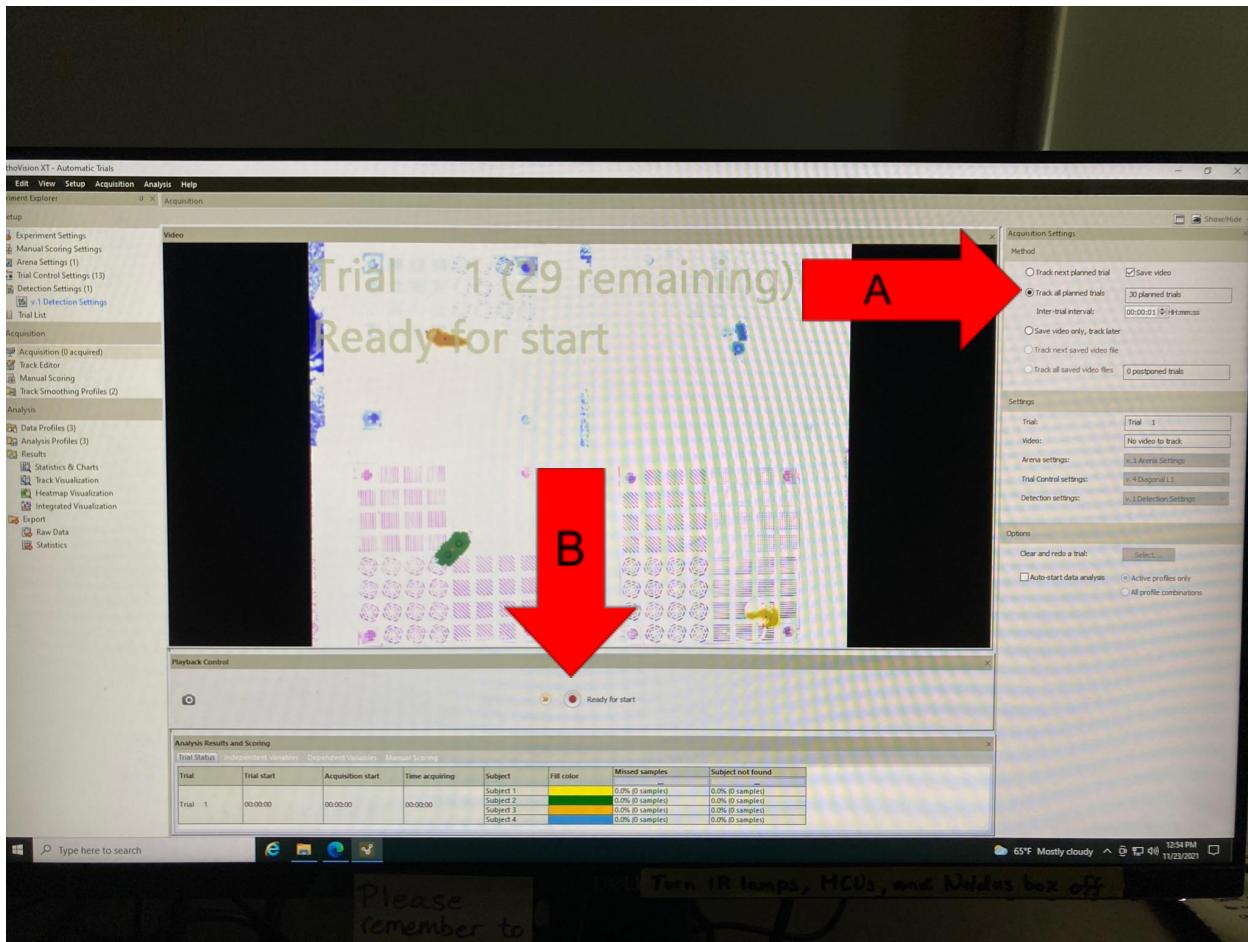
The screenshot shows the eVision.XT software interface with the 'Trial List' window open. The window contains a detailed table of experimental trials. The columns include Trial, Arena, Subject, No., Acquisition status, Arena settings, Trial Control settings, Detection settings, and various user-defined variables like Animal ID, Treatment, Novel Object, Familiar object, Feeder, and Light Level. The table shows 12 trials across Rat Arenas 1a and 1b, with subjects numbered 1 through 48. A cursor is visible over the table.

- Create/check 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. Check reward amounts and lux values per maze, by feeder (all variables to the right of the trial list table). Make sure that everything is filled out and matches.
- 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:
  - 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.

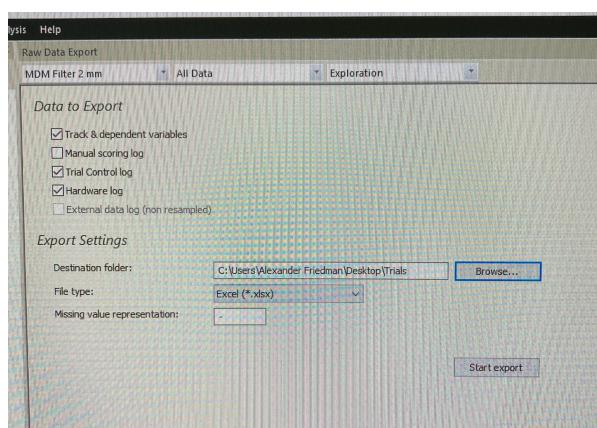


11. Go to “**Acquisition**” and, using the image as a reference, follow these steps:
- a. Be sure to select “**track all-planned trials**” in the menu on the right-hand side.



- 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.
12. At the end of the trial, a pop-up window will say “trial run finished”. Click “ok”.
13. Go to “Analysis”, click on “export” → “raw data”

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



- b. Export as an excel file to the 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).
14. 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 (e.g. if you bump a maze and need to redo arena and detection settings after moving it back) 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** (wet a paper towel with alcohol and wipe down mazes).
2. Turn off **cameras** (power strips underneath the table behind the Noldus I/O box)
3. Turn off **MCUs** (usb hub).
4. Unplug **Noldus/IO box**. Black box with black ethernet cord looking-things, take the semi-clear plug out from the back (which will be facing you).
5. Turn off **infrared lamps** (two power strips on the wall by the PCB box stand).
6. Close **ethovision**.
7. Turn off **all lights**.

### **Shut-Down for the Last Session of the Day**

1. **Complete steps 1-7 as you normally would.**
2. Suck the sucrose out of the syringe holders on the valve stands (using big syringes found in the sucrose beaker tray). Dump all left over sucrose into sink and let beakers air dry overnight.
3. At the end of every Friday, run 20mL alcohol through valves using PuTTY. Followed by 20mL of warm water.
4. Biweekly: change pads.