CNE 2018 Sept
Updated 2022-12-07

Summit box guide

Box assembly

-Laser cut box panels from ¼" black acrylic

-You will need the following hardware to assemble the box:

Item	Qty	Supplier	Catalog #	Purpose
T-slotted railing	6 ft	McMaster-Carr	47065T101	To support panels (1)
Acrylic - 1/4" black	2.5	McMaster-Carr	8505K758	For back, 2x sides, top and bottom panels (2.5)
Acrylic - 1/8" black	0.5	McMaster-Carr	8505K741	For front door panel (0.5)
T-Slotted Framing, Compact-Head End-Feed Fastener, 1/4"-20 Thread	24	McMaster-Carr	47065T139	Attach all door panels, hinges, magnet to rails (24)
Handle	1	80-20	2888	Door (1)
Hinges	2	Global Industrial	T9FB797477	Attach door to rails (2)
12 - 24 screws	8	McMaster-Carr	91251A452	Attach base and top panels to rails after tapping (8)
1/4-20 1" screws	8	McMaster Carr	92196A542	Screwing on hinge to rail (4), mounting diffuser (4)
8-32 screws (3/8")	1	McMaster-Carr	90272A192	Holding magnet to rail (1-2)
End-feed fastener, 8-32	1	Thor	47065T139	Holding magnet to rail (1-2)
Magnetic latch	1	80-20	9315	Hold door closed (1)
5/16-18 1/2" screw	6	McMaster Carr	93615A450	Screwing on handle (2), screwing on hinge to door (4)
5/16-18 hex nut	4	McMaster Carr	90499A030	Attaching hinge to door (4)
4-40 1/2" screw	1	McMaster-Carr	91735A106	Hold magnet on door (1)
4-40 hex nut	1	McMaster-Carr	90730A005	Hold magnet on door (1)

-Use bandsaw to cut one 6' rail into 4 x 18" pieces and clean off lubricant/aluminum shards. Make sure ends are smooth and not sharp.

<u>Initial assembly:</u>

-Secure some newspaper or paper towels and place under area where you will assemble box so you can catch oil from tapping the rails

-Secure end-fasteners to back and side pieces using ¼"-20 ½" screws (ones that came with end-nut fasteners are fine for this).

-Slide one side's end-fasteners into railing, making sure top of side comes into flush contact with floor (to ensure evenness). Secure at least one screw.

WARNING - Engraved markings (SIDE, BACK) should be upside down - you will FLIP the box after this step.

-Slide another rail into the other side, making sure it sits flush with floor.

-Continue assembly until you have both side panels and one back panel together. The bottoms the sides and back piece should sit flush with the floor.

-Tap railings with 12-24 tap.

-Screw on bottom piece of box, making sure 8-32 hole is situated at the opening of the box.

-Flip the box 180 degrees such that the bottom panel is now on the floor and the free ends of the rail are facing up.

-Drop end-nut fastener with ¼"-20 1" screw into each of the rail slots that face the middle of the box. These will be used to support the panel holding the flies.

-Attach hinges to the front door by situating so the wider part sits flush behind the 5/32 holes. The hinge should sit snugly in the space next to the holes, it should not sit behind the gap. Hinges can be attached using 5/32 ½" screws and hex nuts in any orientation. I think it's prettier to have the hex nut on the outside of the box.

-Attach end-nut fasteners to other panel of each hinge using ¼"-20 ½" screws (ones that came with end-nut fasteners are fine for this).

-Carefully slide the door into place by sliding end-fasteners into rail. This is usually a bit clunky, having to jiggle the piece to get it to slide down. Stop when top of door is flush with top of other pieces and tighten.

-Attach magnetic catch (the thin metal part) to the door with the head of the screw on the outside of the box, end of screw and hex nut facing in (the screw will stick out. That's ok).

-Drop magnetic catch attached to end-nut fastener with 8-32 ⅜" screw into the railing that sits immediately facing the railing holding the screw. Find a reasonable place to secure latch (where magnet catches but doesn't catch so strong that you risk breaking door).

-Secure end-fasteners to stability bar using ¼"-20 ½" screws (ones that came with end-nut fasteners are fine for this).

-Slide stability bar above door with the end with the larger gap between screw hole and edge facing up (if you insert in the reverse orientation, you won't be able to have the top piece sit flush with the box).

-Loosely tighten 4x drop in screws that will hold mount board at ~2.5". Use level and empty mountboard to make sure the mountboard sits flat on these screws.

-Screw on lid, making sure orientation is correct (the distance between posts will guide you).

-Adjust height of sides, back, door and stability bar so that they sit flush with the top panel.

Adding electronic components

-It's important to check that everything is working BEFORE you put the box on the shelf, where it will be less accessible for modifications.

-Attach PCB using nylon screws to the top panel.

-Situate camera through camera hole and secure with tape.

-Adjust camera focus to capture all behavior chambers in field of view.

Finalizing box

-Replace front left corner (your right when on the shelf) with ¾" 12-24 screw inserted into plastic hinge.

-Attach 4" post with 8-32 hex-head screw.

-Assemble magnet base, stick base on shelf and slide rod into base.

CAREFUL - If you slide the rod too far, the rod will stick to the magnet at the bottom of the base.

-Adjust the height of the rod such that the box is angled at 30 degrees (using digital angle measuring tool).

Computer preparation

1. Change power settings to
    i. Monitor turn off after 10 min (or whatever you want)
    ii. PC sleep NEVER (important to not fall asleep during experiment)
2. Install Ourlink wireless adapter from USB (copied over install files from CD onto USB CNE Lab II)
    i. Go to Windows > Setup and open file
3. Download and install Matlab
4. Download Arduino IDE (v1.8.6) and install
5. Download Teensyduino installer and install
6. Install Generic Image Acquisition tool in Matlab (full name: Image Acquisition Toolbox Support Package for OS Generic Video Interface):
    i. Go to "Add-Ons"
    ii. Click "Get Add-Ons" to open Add On Explorer
    iii. Search for "image acquisition" > should see generic add on in results

b. Install

7. Install **MATLAB Support Package for USB Webcams** following steps outlined in 13)..

8. Upload circadian script to Teensy
9. Download Margo from https://github.com/de-Bivort-Lab/margo.
10. Move directory “Circadian” from “examples” to “experiments”.
11. Open margo > make sure light board and camera are both detected.
12. Close margo and disconnect camera via

    >> imaqreset

    Closing margo alone will not properly release camera for usage by other progams (i.e. you won’t be able to access it because Matlab will think that Margo is still using it).

Optional: Setup image calibration to correct camera fisheye

1. Enter cameraCalibration in Matlab (Margo should not be running).

a. If you get an error that camera is in use, enter imaqreset to reset imaging devices.

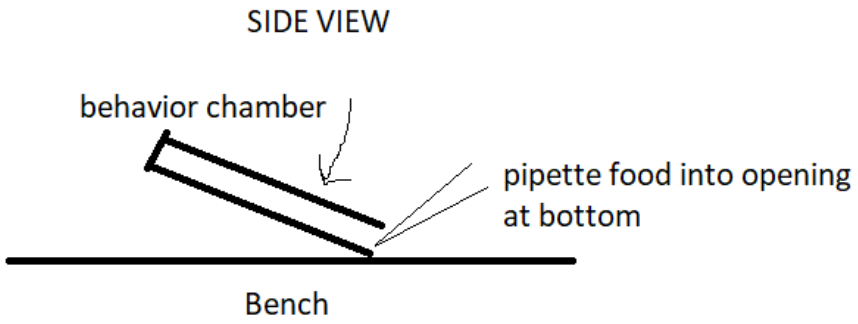
2. Select “Images from camera”.
3. Adjust camera resolution to the same resolution you will use for tracking.
4. Change options to take 1 image per second and take 60 images.
5. Have your checkerboard ready to go (printed and mounted on something flat and sturdy), with your fingers out of the way.
    a. https://markhedleyjones.com/projects/calibration-checkerboard-collection
6. Press start to start capturing images. Move the checkerboard around the box in the sameish plane that your flies will be tracked in, trying to take a picture of the checkerboard at each point in the box. Slightly tilt the checkerboard back and forth as you do this.
7. Once images have been captured, the images will save and be processed. There will then be a box that pops up and asks you how big each square is, in millimeters. Enter this value and hit enter.
8. You will now need to exit out of image capture. This will return you to the main calibration window.
9. Click “Calibrate” to use the images you just captured to run the calibration. The images will be listed on the left-hand side of the screen as thumbnails.
10. After calibration runs, you will see two graphs on the right hand side of the screen: the top one indicates how many pixels differ between expected checkerboard and actual checkerboard in each of the images you took, the bottom one recreates the position of the checkerboard in each of the images you took. The latter can be useful in helping you figure out which images are helpful and which are not.
11. Pull the red line of the top graph down to where you’d like the cutoff point to be for keeping images - images with errors below the line will be kept, above will be marked to discard.
12. Right click on the thumbnails of your images (left-hand side) and click “Remove and recalibrate” to redo the calibration with your best cohort of images.
13. Check the corrected image by clicking on the checker sphere icon up top (labeled “Corrected image”, or something similar) to see what the image looks like with the calculation correction.
14. If all looks good, click on “Export calibration parameters” and close the calibration toolbox. You don’t need to save your session.

15. In Matlab, you will see the variable cameraParameters is now in your workspace. Right click on this variable and click “Save as”, then save cam_params.mat in “.../margo/hardwared/camera_calibration/”. If you don’t save to the correct path, Margo will not be able to load this file.
16. You should now be able to load Margo and select “Hardware > Camera > Use Calibration” to apply your correction.

Assaying behavior of flies (running an experiment)

Prepare behavior boards

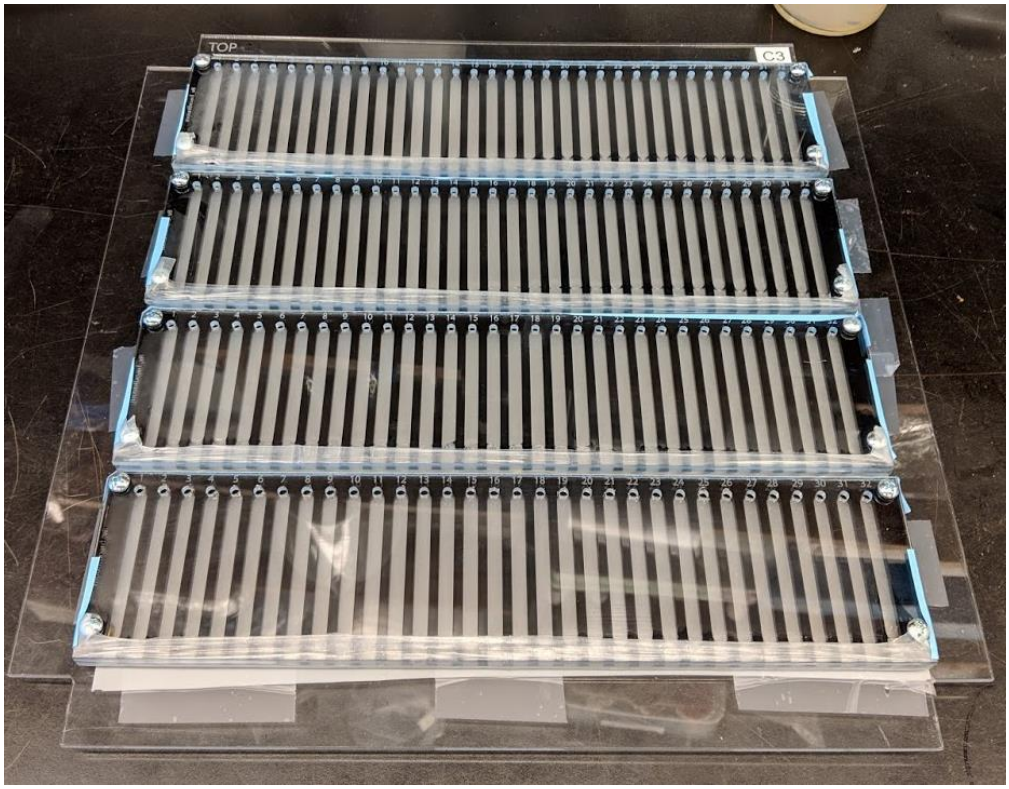
a. Wipe bench with 70% ethanol before beginning. Make sure you are wearing a glove on the hand that will come into direct contact with the side of the well where food goes.
b. Screw together 4 sets of rows by lining up notched left-hand corners.
    i. I usually just hand-tighten the screws/nuts, but you can also use a power drill for this.
    ii. Each row consists of one base (sanded clear), one set of walls (black) and one lid (clear with tiny holes).
c. Melt desired solid diet using the microwave.
    i. CNE uses 5% sucrose, 1.5% agar in autoclaved milliQ water (called 5AS MQ).
d. Use P1000 pipette and non-sterile tips to dispense between 0.5 and 1 cm in each well.
    i. This works best if slightly angle the row such that the top of the row is a few cms higher than the bottom (where the openings are). Angling in this way prevents the food from running into the top of the chamber - it keeps the food pooled at the bottom. If the food is very hot (i.e., very runny) you will need a shallower angle than when the food has started to cool (i.e., has become more viscous). It’s also best if you pipette the food against a surface (lid or sides) as opposed to just pipetting into the open space of the well. This way, the food can wick along the surface of the plastic.



    ii. After pipetting in the food, I usually rest the top of the chamber on a petri dish (rather than laying the row flat) to make sure the food cools and sets at the bottom of the well.
    iii. You will get a meniscus (severity dependent on how hot the food was when you dispensed it) at the bottom of each well which you can “patch” (add additional

food to to have it lay flat) after the first food has cooled. An easy way to do this is place the row upside down on the bench so that the bottoms of the row is facing you and run a pipette full of 5AS MQ along the wells to fill the small gap that formed during cooling.

e. Use kimwipe to remove any food stuck to back of chamber.
f. Cover bottom in two strips of parafilm (I usually have a stash of precut ~2cm strips), then use ½" labeling tape to secure parafilm on both sides to keep from sagging/coming loose.
g. Place a strip of ½" tape over the top of the row to close up any potential fly-sized gaps in the middle of the row.
    i. As you reuse these pieces, they will begin to bow in the middle, so this step is essential.
h. Attach four rows onto one mountboard using nuts. If you are not going to use mountboards on the same day as they are prepared, store in fridge to mitigate food evaporation.
    i. CNE has found that prepared trays can last about a week before desiccating, but you should confirm with your particular diet. Desiccated boards should be remade rather than trying to "patch" with additional food as the original food will have become overly stiff.
    ii. Boards should be warmed to room temperature before loading with flies.



This is a prepared "mountboard" or "tray" - it has 4 "rows" of summiting chambers containing 5AS MQ.

Load flies.

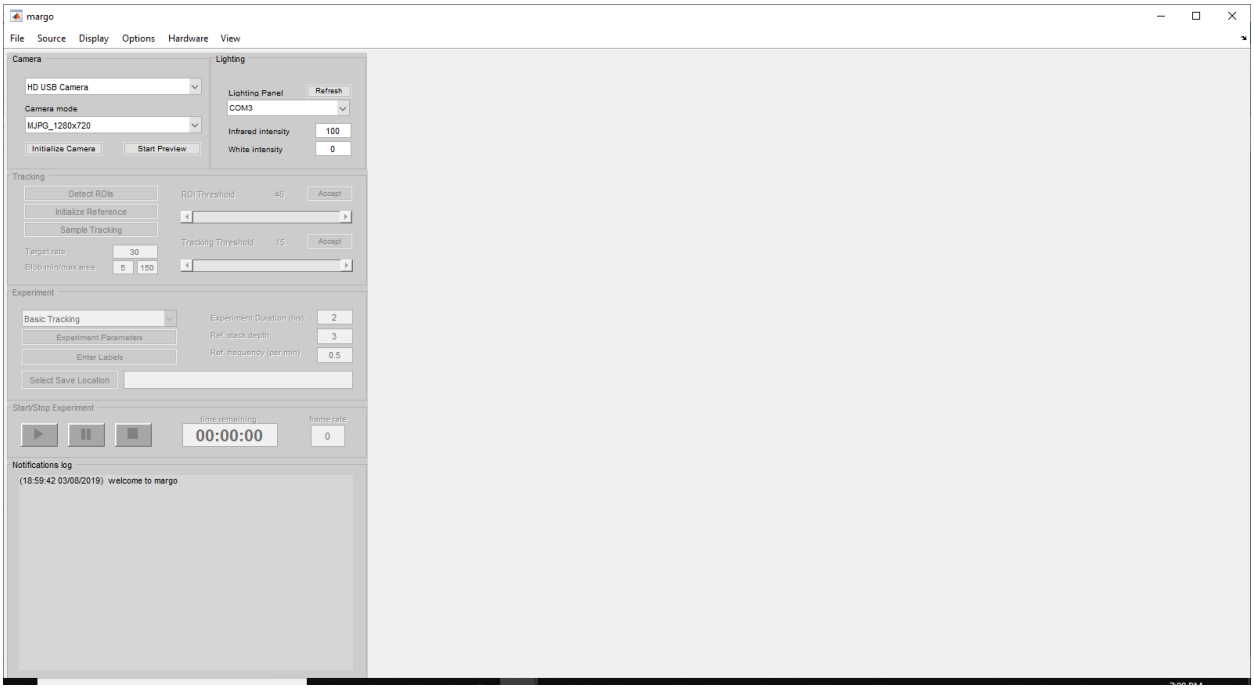
i. Anesthetize experimental flies with CO2, sex or sort by phenotype as desired and load into behavior chamber as follows:
    i. Use paintbrush to pick up single fly and place in entry hole. You can repeat this for up to 15 more flies before moving on to capping, depending on how long flies have been anesthetized.
        1. For best results, avoid using small flies or flies that have damage to wings or legs.
    ii. Angle board so flies are at the top and gently tap down to make sure all flies move away from entry hole.
    iii. Cap each filled well with one piece of size 3 dental cotton (https://richmonddental.net/products/cotton-pellets/).
    iv. Repeat until board is filled.
    v. Return board to incubator until ready to track.

Track flies.

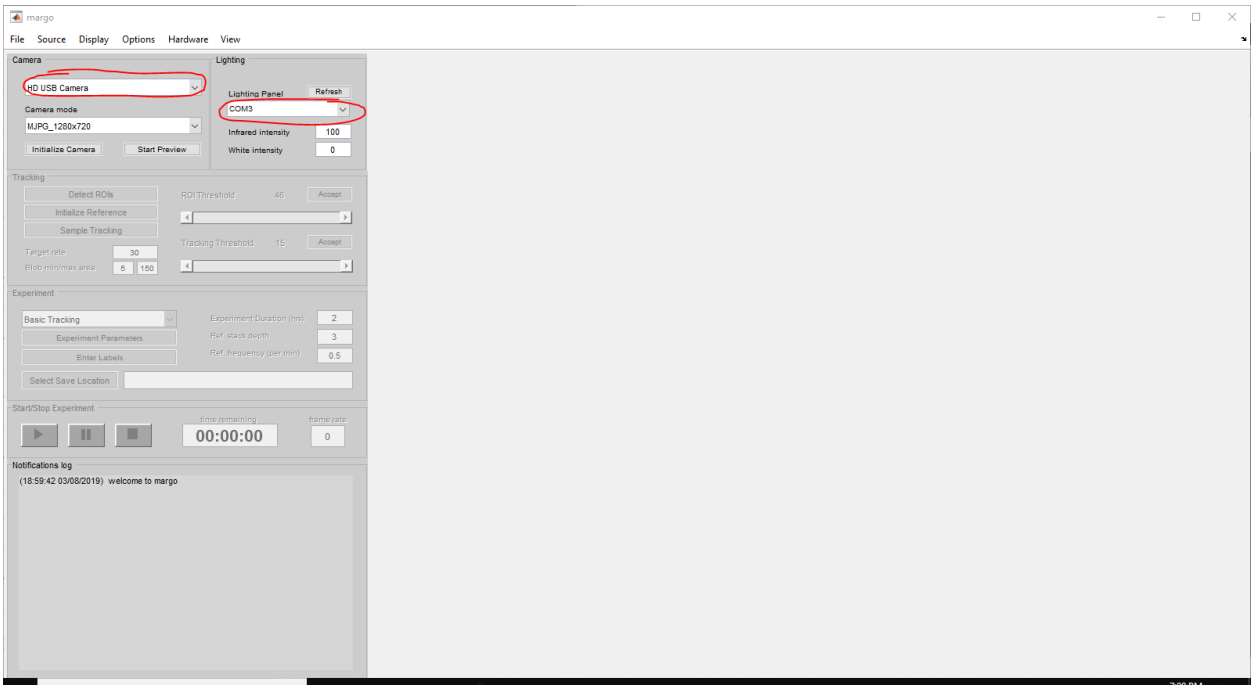
Full documentation for Margo can be found at https://github.com/de-Bivort-Lab/margo/wiki.

j. Place mountboard in behavior box on top of resting screws with the food pointing away from you.
    i. You will need to tilt the board so the right side is higher than the left while you load (to avoid hitting the magnetic catch).
    ii. Make sure the board is sitting flat on all four screws once you place it down.

k. If Matlab isn't already open, launch Matlab, type in "margo" once it's fully loaded and press enter. If Matlab is open and Margo is running, double-check tracking settings and skip down to step o. If Margo is not already running, type in "margo" and press enter. The Margo window that pops up looks like this:



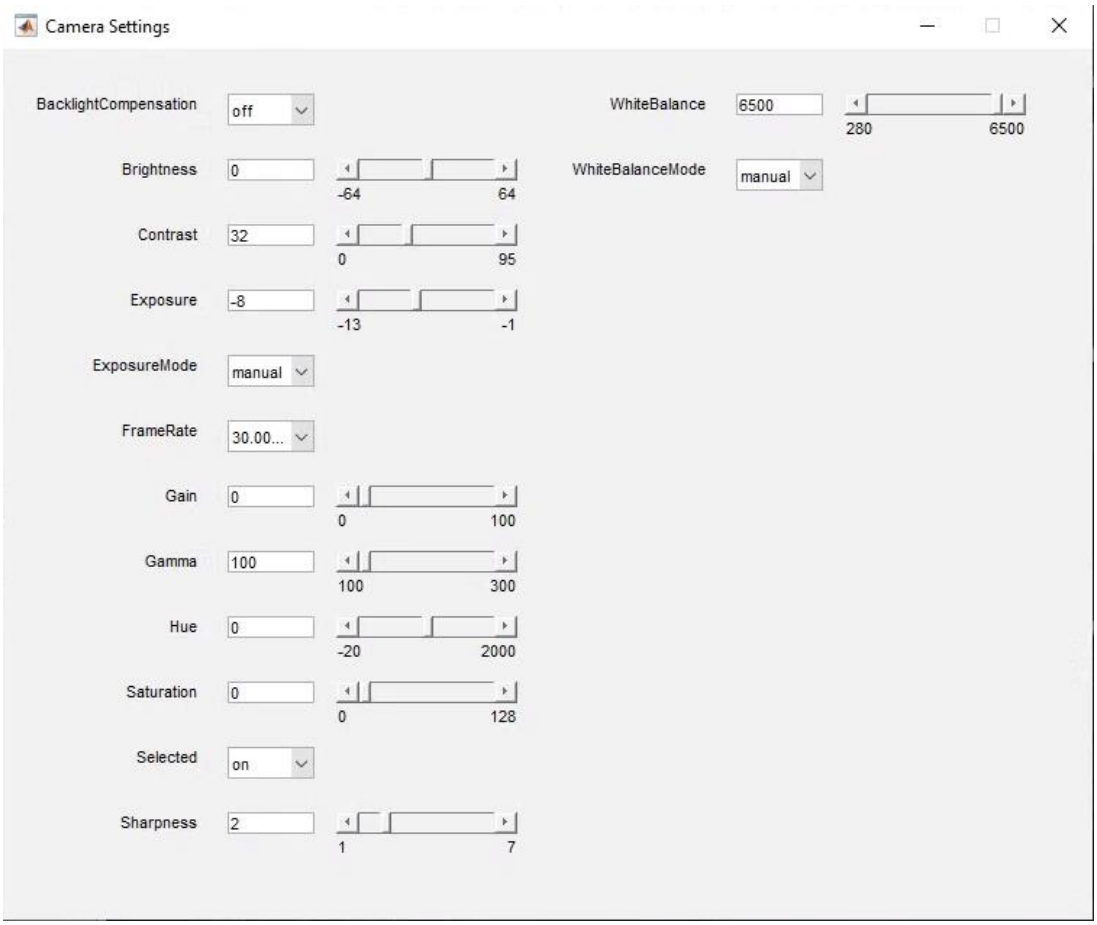
l. Check that the camera and the light controller (COM) are both detected.



i. If no camera and/or no COM is listed, try quitting and reopening the GUI by closing the window then re-entering >> margo.
ii. If this doesn't work, double-check that your camera and printed circuit board are properly connected to the computer via USB. If everything appears connected properly but is not showing up in Matlab, you may have a bad cable.

d. If you've used Margo previously and have saved a preset, you can load this by going to "File" > "Load saved preset" and selecting your preset file. You don't need a preset to use Margo, but it can save you some time in setting up your experiment if you use consistent parameters.

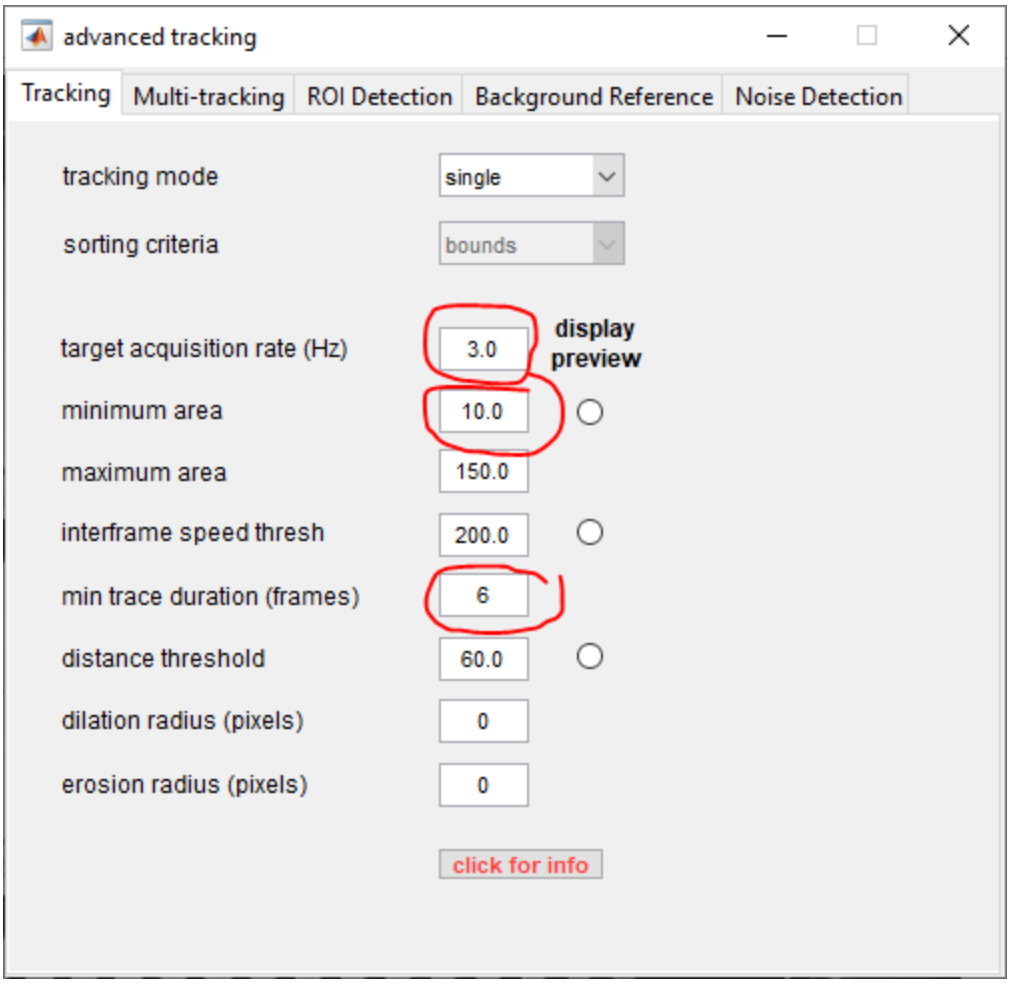
e. Adjust your camera settings so that you have decent contrast between board and flies by going to "Hardware" > "Camera" > "Camera settings". The precise settings for this depend on your specific make of camera.
i. Generally, you want to disable any automatic image correction (e.g. white balance, backlight compensation, exposure etc).
ii. If you have a color camera, you will want to remove color information (e.g. set saturation to 0). Margo only uses color information from the green channel, so if you skip this step you can get wonky results.
iii. Adjust exposure such that contrast is optimal - you want your flies to really stand out on the white background. If you can easily see them by eye, your camera can too.
iv. Here's an example of my camera settings:



f. Once you've adjusted your camera settings, select the desired resolution for your camera and click "Initialize camera".
    i. If you decide you need to alter any camera settings going forwards, you will need to click the "Initialize camera" button again after each change.
g. You should see something like this if you haven't performed camera calibration:

Check that the light settings are adequate. White should be at 50; IR should be between 50-100, depending on what setting gives you good contrast. The contrast in the image below is what you're aiming for (image shows camera with calibration):

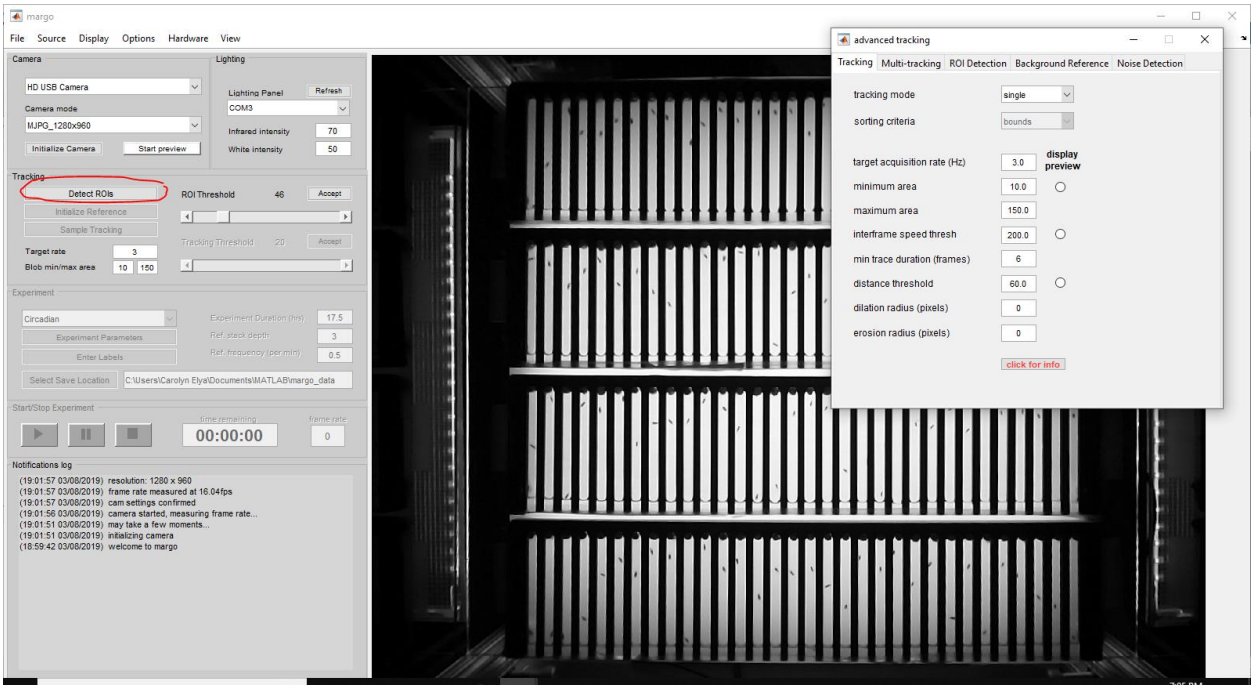
h. Double-check that your tracking settings are correct by going to "Options" > "Tracking" and adjust your tracking settings as desired - this will likely require some trial and error until you find parameters that suit your experiment. I use the following settings:



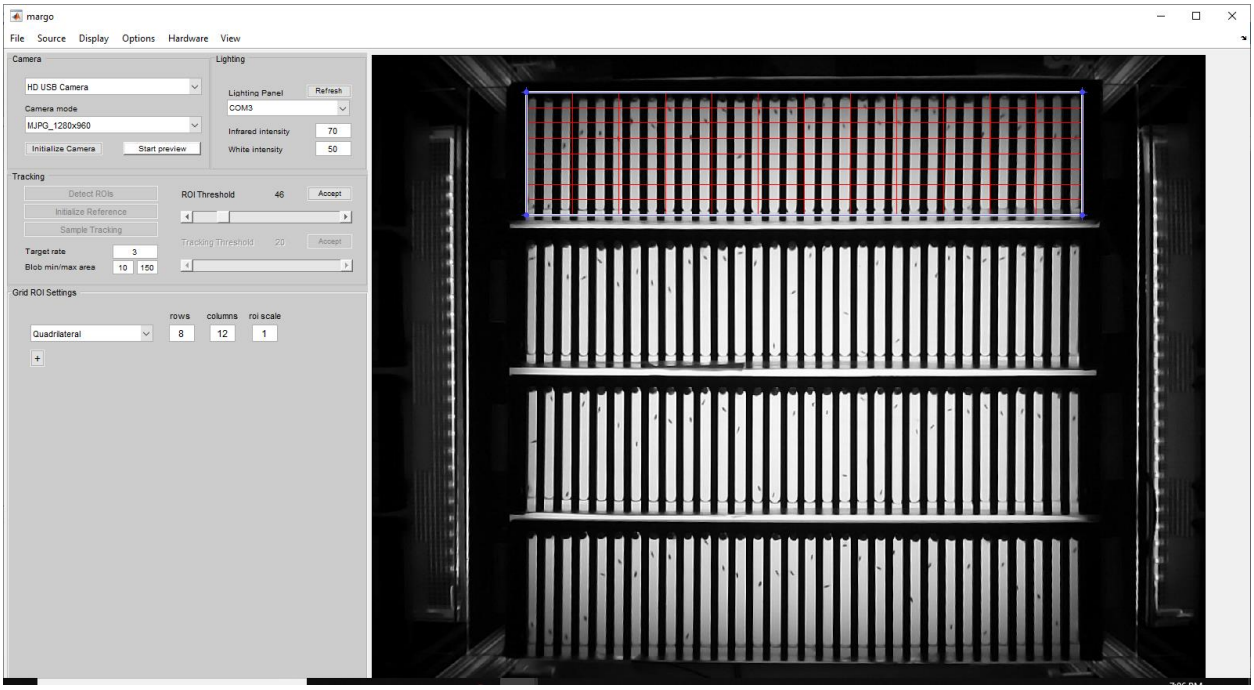
The circled values are the ones that are different from Margo's default settings.

i. **SUPER IMPORTANT!!! If Margo was already opened and configured when you went to track, it's very important to double-check the connection between the computer and the lightboard (the connection can drop over time, and if that happens during an experiment it can cause us to lose our data). All you need to do is click the "Refresh" button next to the text that says "Lighting Panel". This should cause the white light to turn on to 50% intensity, which is an added bonus - tracking will turn out best if the white light is on during setup.**

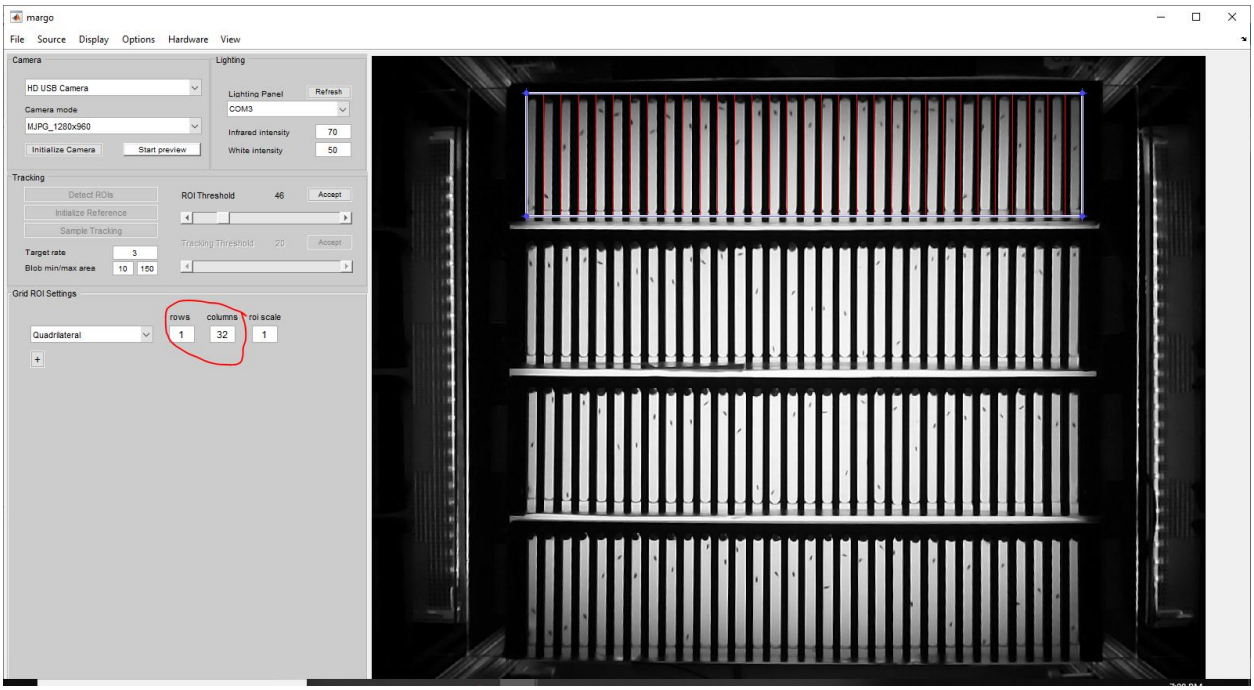
j. You are now ready to begin setting up tracking. Click the "Detect ROIs" button.



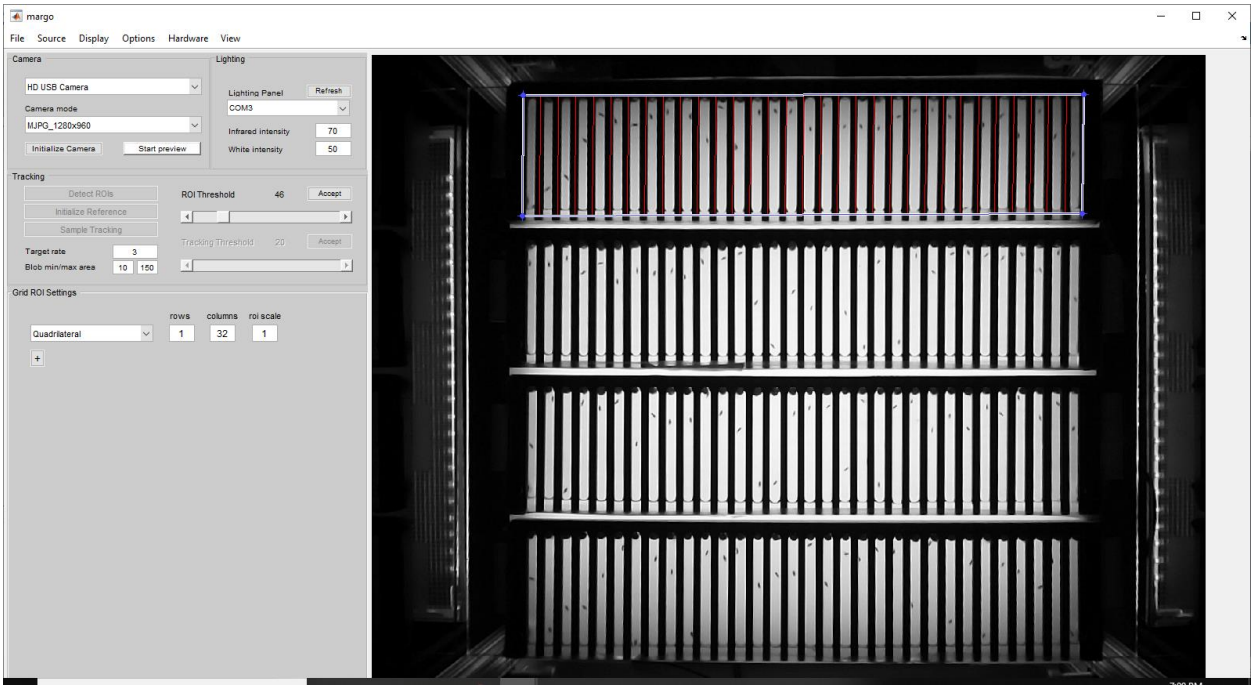
k. Using the grid tool, draw 4x 1 x 32 well grids that line up with the rig. The first box you draw (if Margo wasn't already open when you began) will look like this:



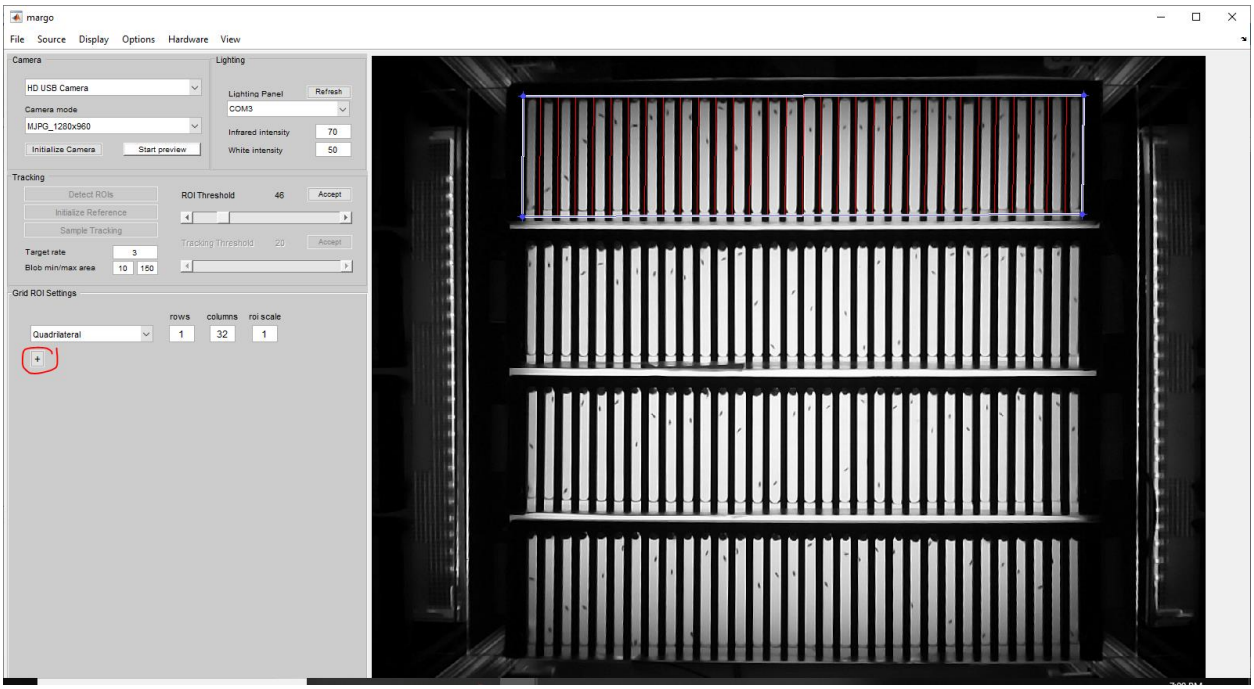
l. Change the number of rows to 1 and the number of columns to 32 to make the grid match our behavior trays.



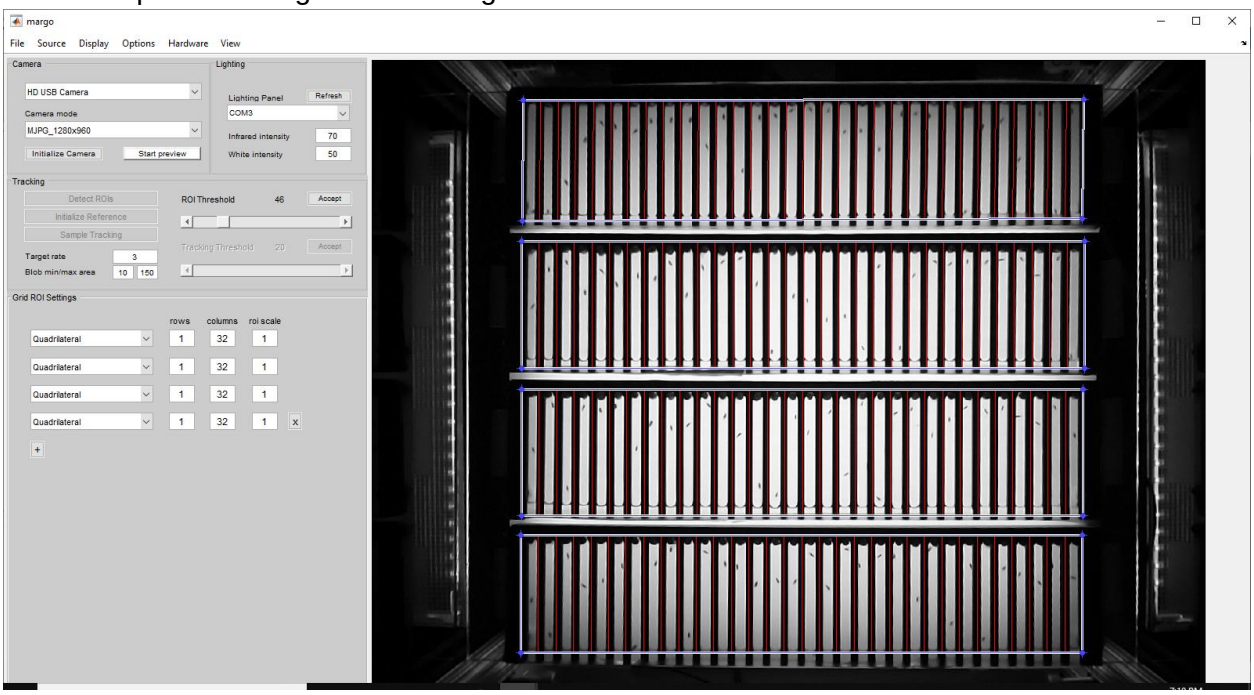
m. Adjust the corners of the box to make sure to get the entire well in each box (you can include part of the food and all of the cotton). Here's an example:



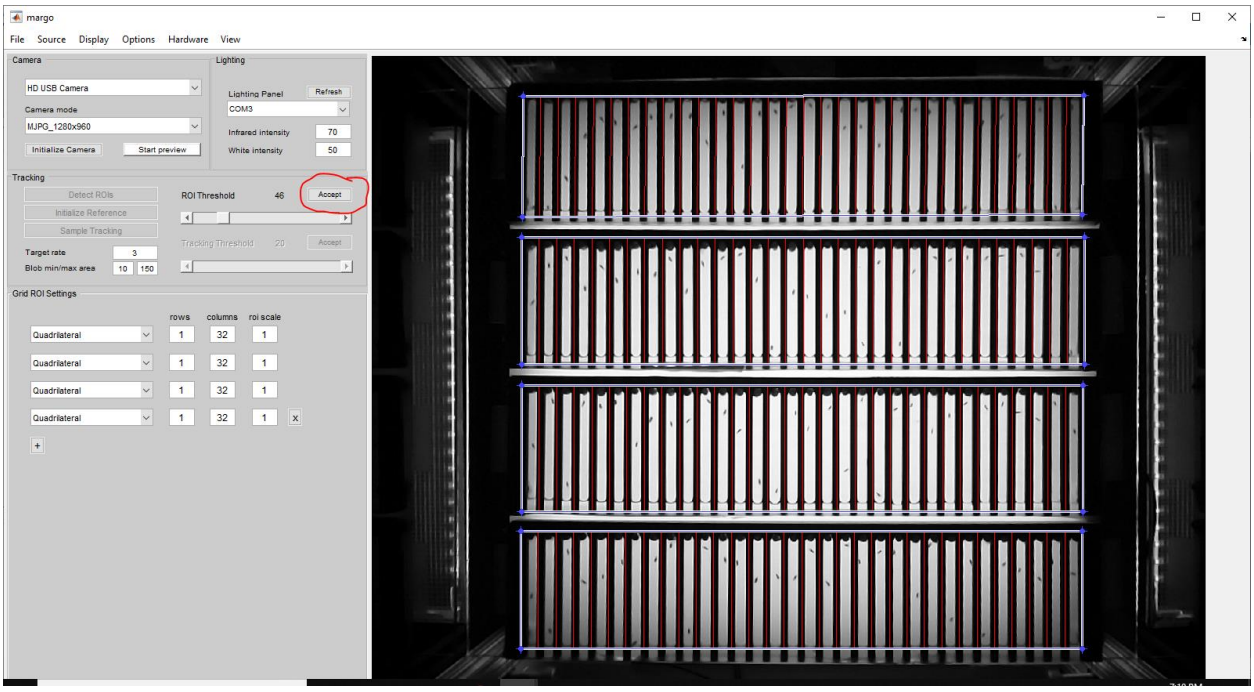
n. Use the "+" button to add another row



o. Repeat outlining the remaining 3 rows. Your screen should look similar to this:

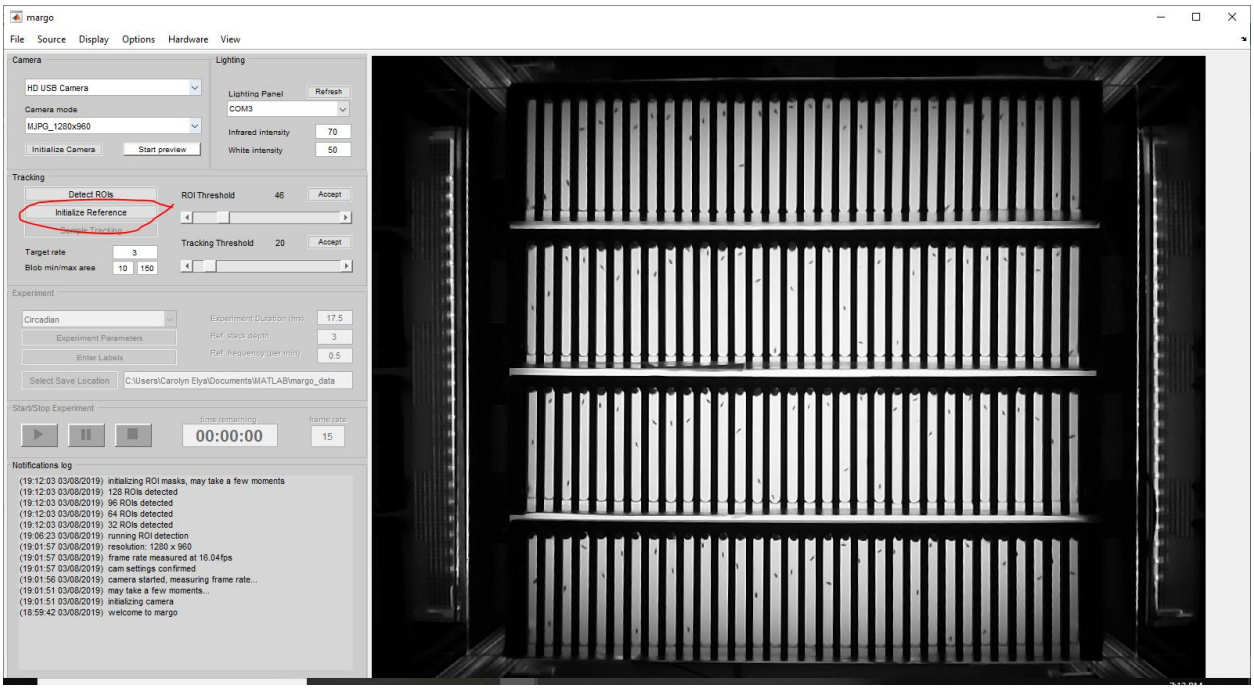


p. When things look okay, click on the “Accept” button.

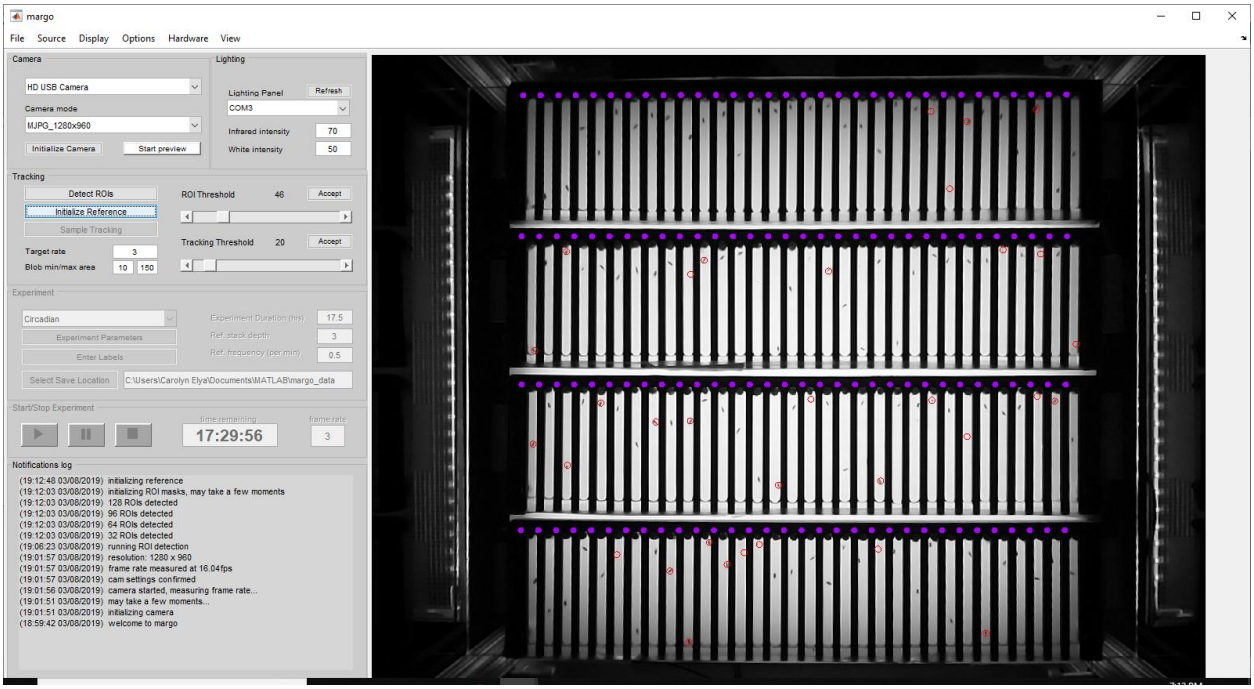


q. It will take a second or two before you can continue to the next step. You'll know margo's ready when the buttons are no longer grayed-out.

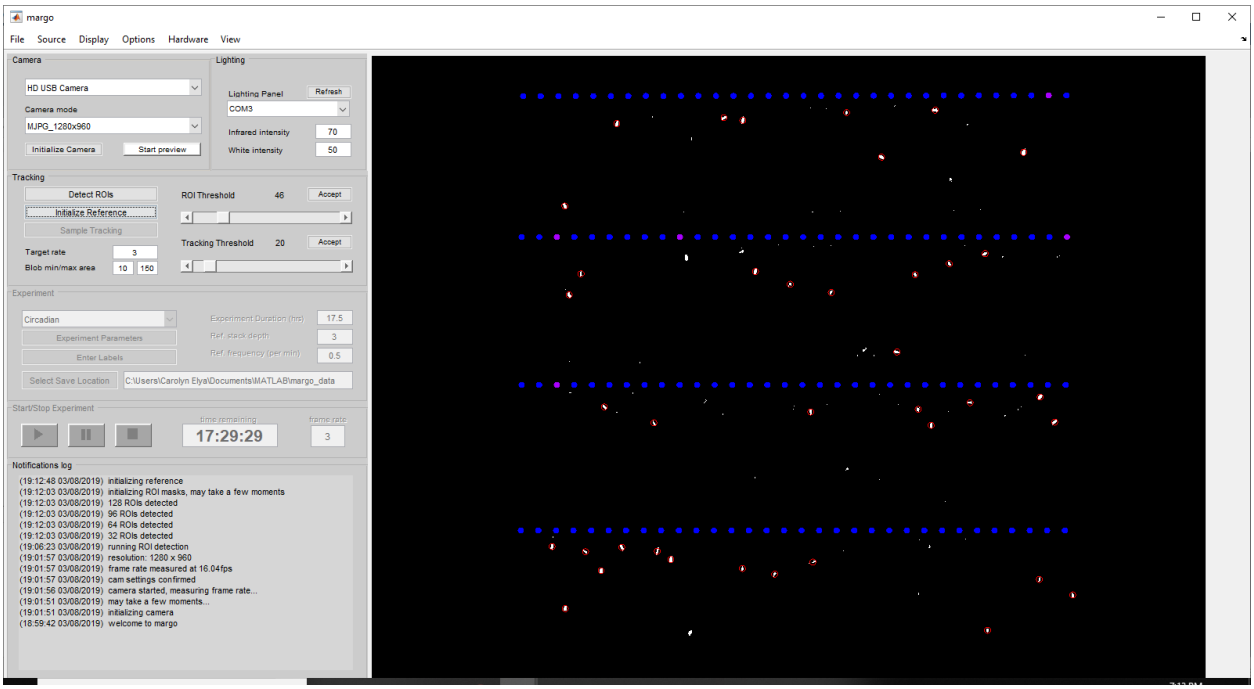
r. Now click the "Initialize Reference" button.



s. You will see red circles pop up surrounding the moving flies in the video preview., like this:

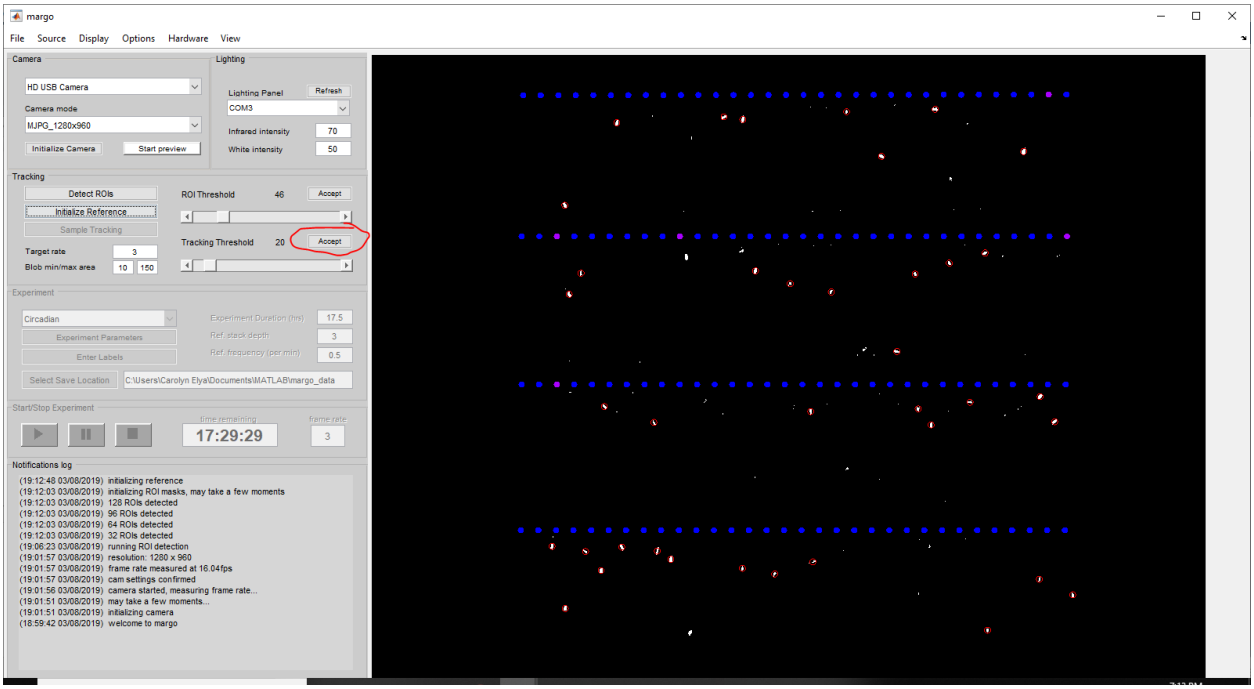


t. Switch views by going to “Display mode” > “Tracking threshold”. You should now see something like this:

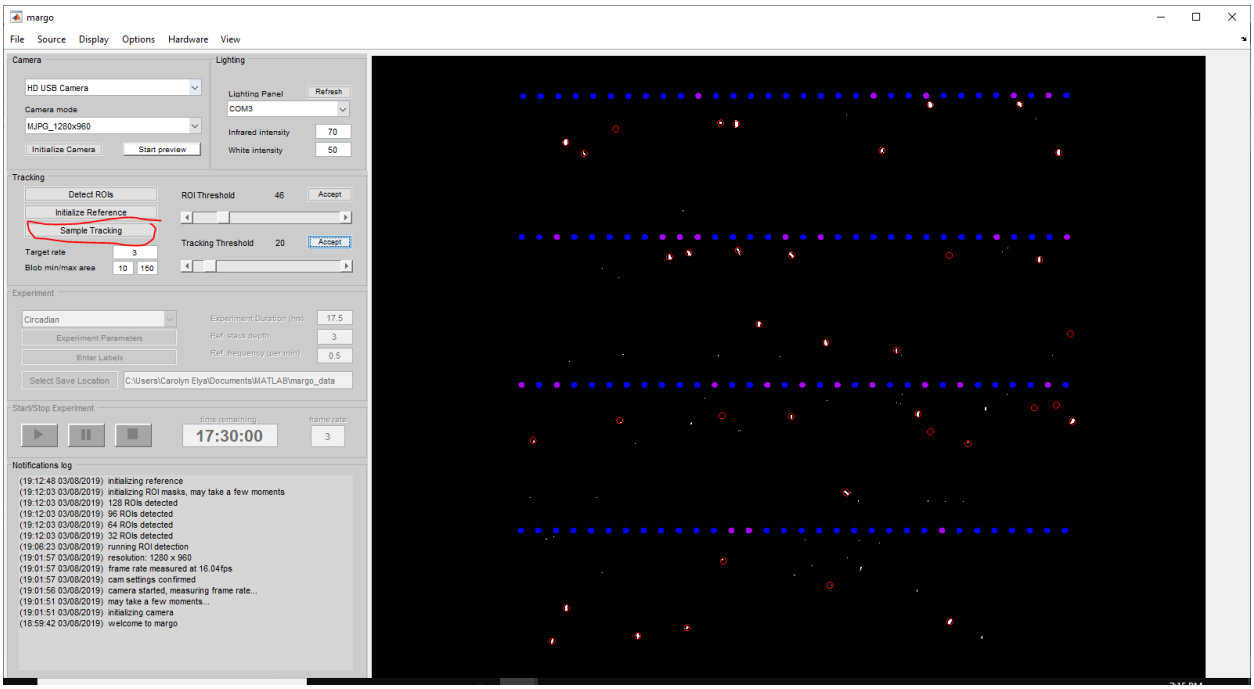


u. **SUPER IMPORTANT!! Margo always tries to set the Tracking Threshold too high!** For my experiments, I lower it to 18 and check that the majority of the white you see are fly “blobs” moving up and down, and not individual pixels/obvious non-fly noise.

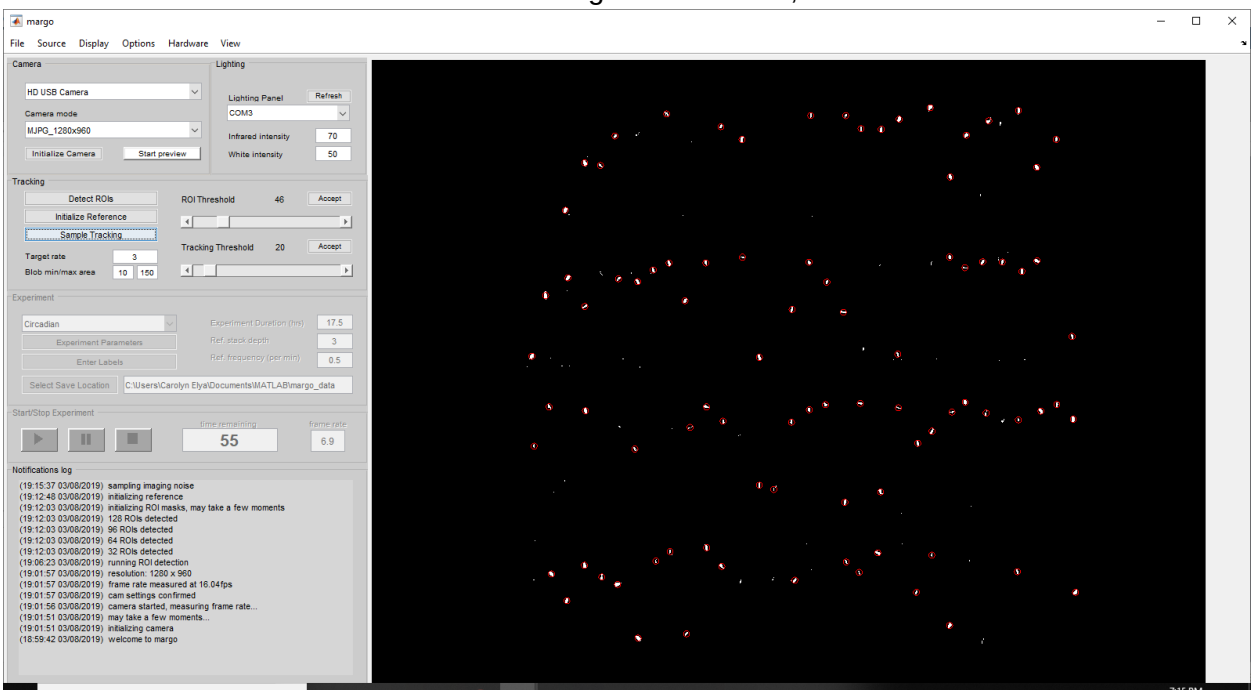
v. Once your threshold is set, click on the “Accept” button.



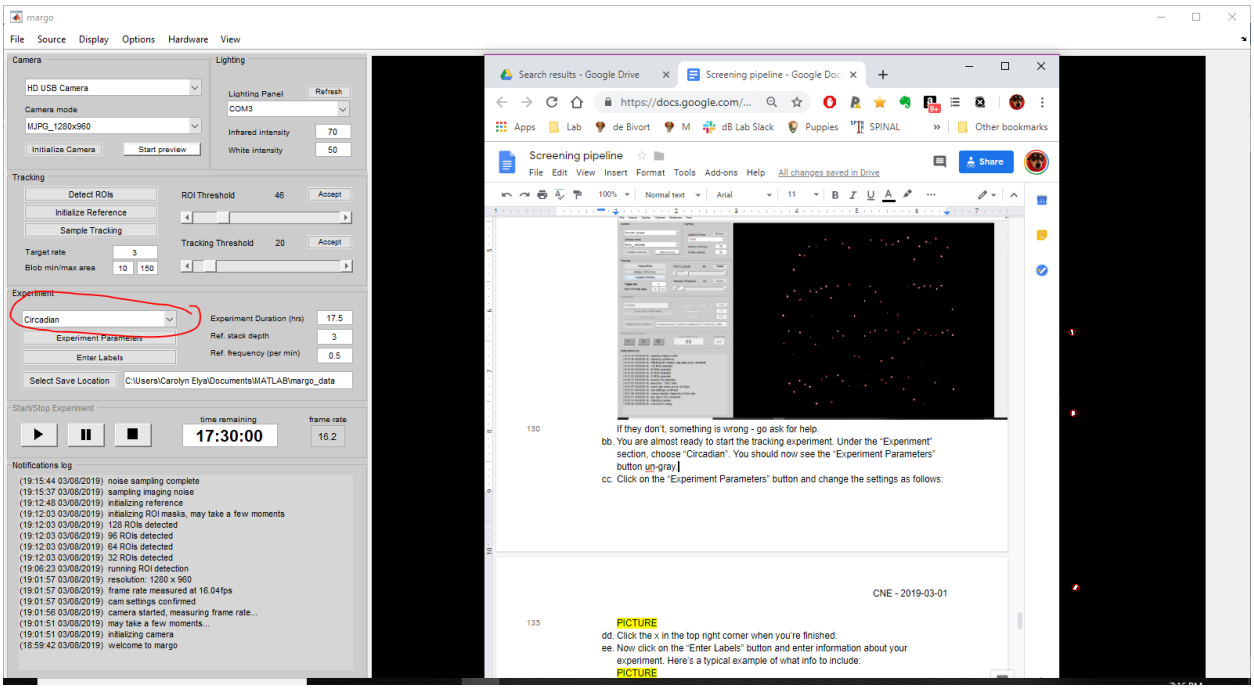
w. Finally, click on the “Sample tracking” button to show a preview of tracking the flies.



x. Watch to make sure the red circles align with the flies, like this:



y. You are almost ready to start the tracking experiment. Under the “Experiment” section, choose “Circadian”. You should now see the “Experiment Parameters” button un-gray.
z. Click on the “Experiment Parameters” button



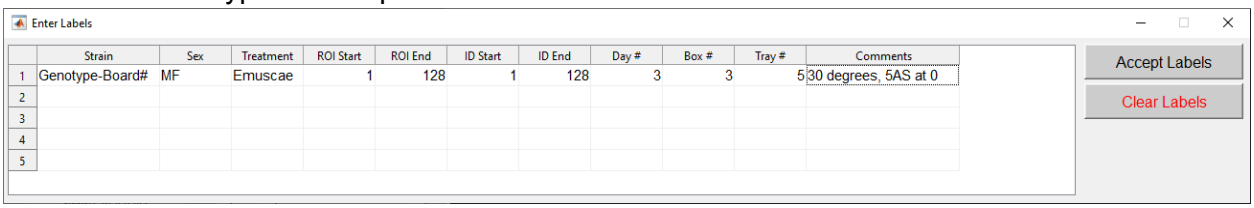
aa. **Beware of daylight savings time!** Enter the lights on and lights off time for the light, as desired.

bb. Set ramping time to “1” if you want a sharp light transition (this will turn lights on or off over course of 1 minute). You cannot use the value 0 here otherwise your experiment will crash when Margo throws an error.

cc. Set “Trial number” to 0. This is leftover from an experiment Zach did with a motor crown.
   i. The “Test” button does nothing.

dd. Click the **x** in the top right corner when you’re finished.

ee. Now click on the “Enter Labels” button and enter information about your experiment. Here’s a typical example of what info I include:



ff. Select a save location > on most computers this should be “...\MATLAB\Margo-data\”.

gg. You’re now ready to start tracking! Click on the play button.