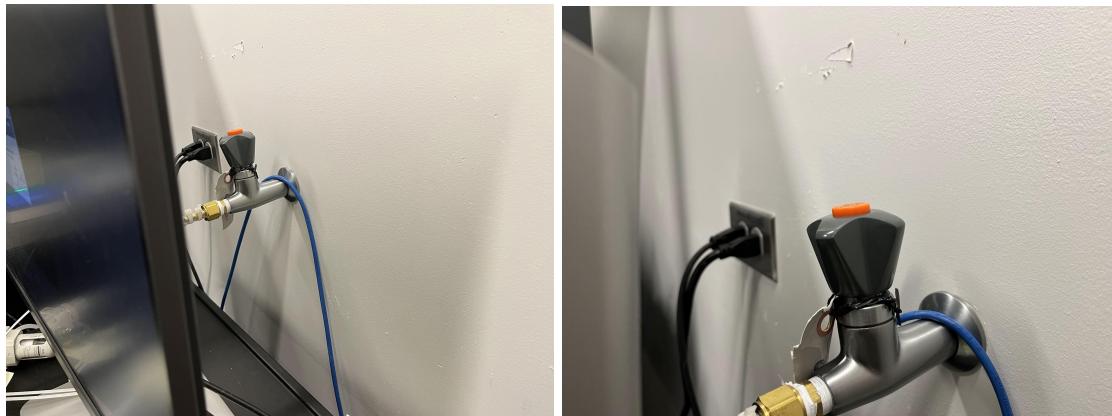


## **Needed Materials:**

- Proper clothing (especially if using with cells)
  - Lab coat
  - Gloves
  - Laser protective goggles
- Samples
- Baseline sample (suspension liquid)

## **TURNING ON THE LASER MICROSCOPE:**

1. Enter the room and get laser protective goggles ready.
2. Turn on the lights but dim to lowest level.
3. Turn on the air for the self-leveling table.



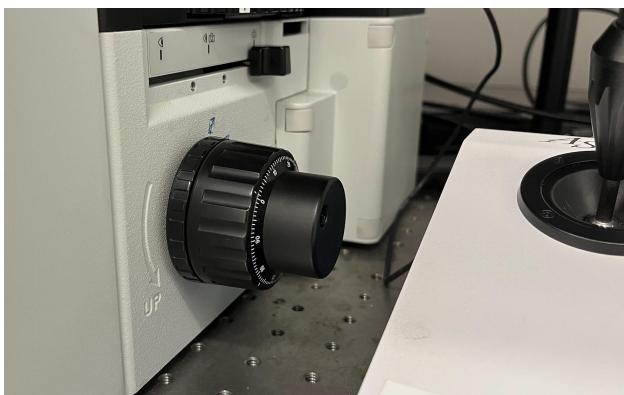
4. Turn on the laser filtering box (black box at left-most of table).



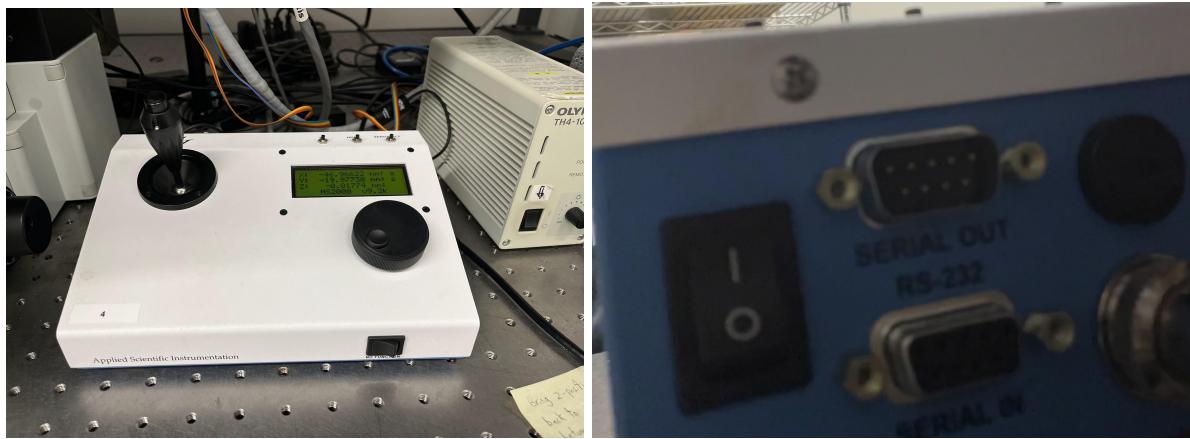
5. Turn on the 730 nm laser controller and set the strength to its max (10).



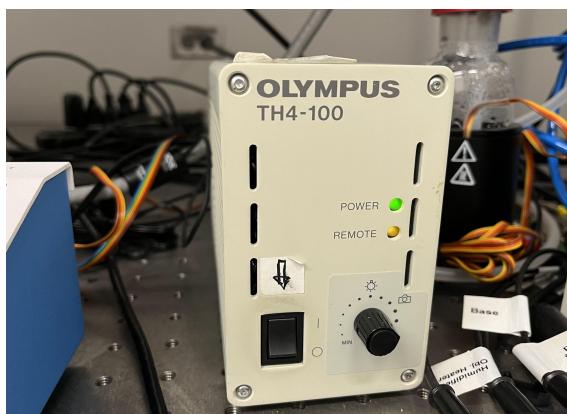
6. Move the objective lens stage to the lowest position.



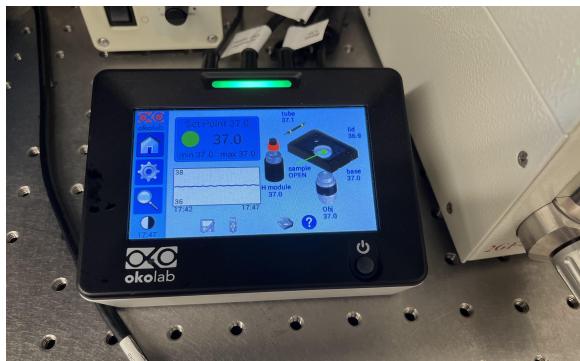
7. Turn on the stage controller. (Switch located on the back)



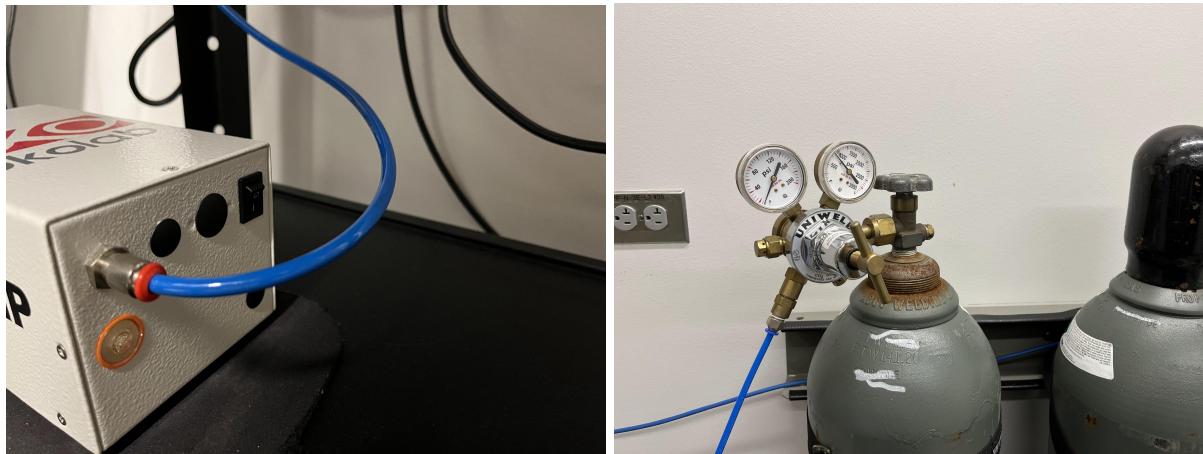
8. Turn on the white light.



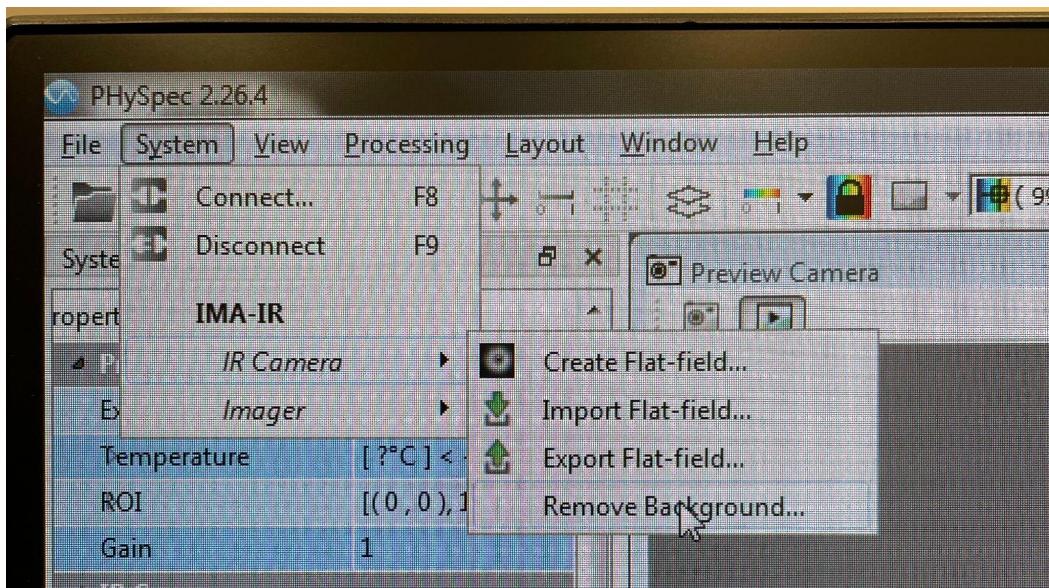
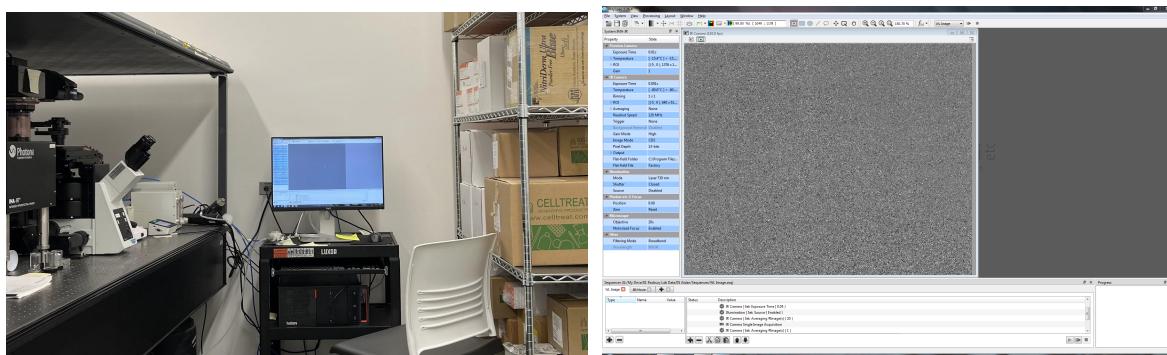
#### **9. Turn on the stage incubator controller.**



10. Turn on the Air pump and open the CO<sub>2</sub> canister valve. (Only if the sample is being left for longer than 15 mins out of the incubator.)

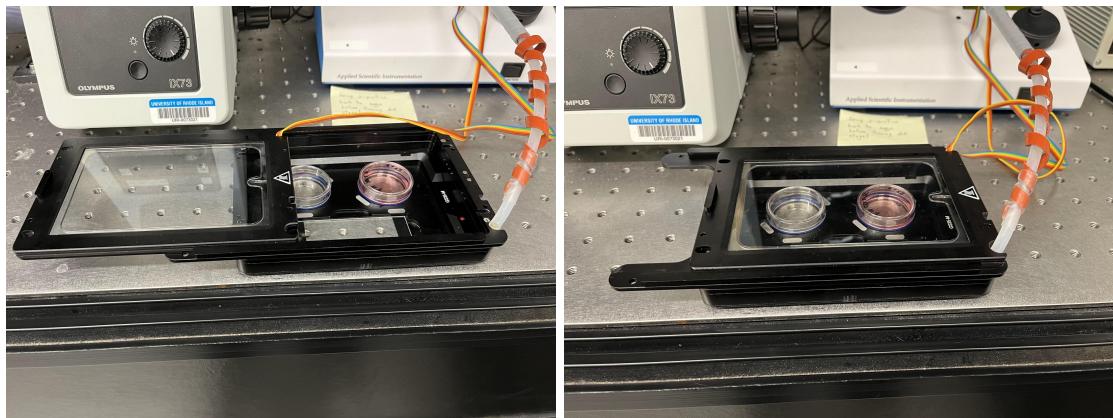


11. Ensure the computer is ready. (disconnect and connect IR Camera; remove background at desired exposure time eg. 0.05sec if imaging at that setting)



## USING THE LASER MICROSCOPE:

1. Use an appropriate sample plate and load the sample into it.



2. Load the sample plate onto the stage.

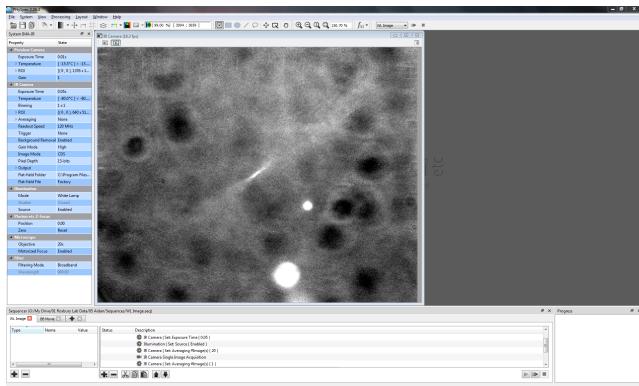


3. Move the stage using the controller to be viewing the sample.

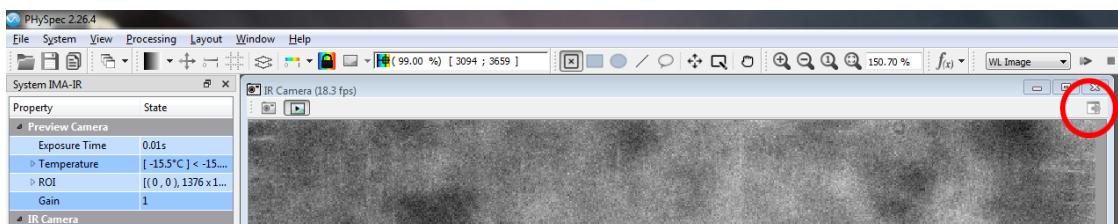
- o To view the sample on the computer make sure live view is turned on (To turn this on click the camera icon on the viewer window - use IR preview rather than general preview).

4. Focus the white light so that proper contrast is achieved.

- o While doing this ensure that the white light is toggled on in the computer software.
- o To do this on the left toolbar under illumination, click "White light".
- o Then in the same section on the computer enable the illumination.



5. Put on the laser protective goggles.
6. Take pictures of sample with white light, with laser, and a cube image.
  - o Change exposure time for laser depending on the sample.
  - o Make sure to take images of a blank which only has the suspension liquid.
  - o For manual cube images: “Processing” -> “Wavelength Rectification”
  - o For manual BB images: turn off preview and click Export (far corner of preview window)
  - o otherwise, use premade “sequences” saved in GoogleDrive or make own



7. Save to a proper folder on Google Drive.
8. Turn off the Air pump and CO2 canister.
9. Remove sample plate from stage.
10. Remove samples and return them to the actual incubator if cells as soon as possible.



## **TURNING OFF THE LASER MICROSCOPE:**

1. Turn off the air for the self-leveling table.
2. Turn off the Air pump and CO<sub>2</sub> canister if not already done.
3. Turn off the stage incubator controller.
4. Turn off the white light.
5. Turn off the stage controller. (Make sure z component dial is centered at zero.)
6. Set the strength of the 730 nm laser controller to 0 and then turn it off.
7. Turn off the power supply for the laser (black box at left-most of table).
8. Leave the camera turned on - takes a while to shut down and start up so just leave on.
9. Remove goggles and clean before storing.
10. Turn off lights before leaving.

## **IF USING STAGE INCUBATOR:**

(ie if imaging live cells)

on OKOlab box - adjust CO<sub>2</sub> levels to 0.03 and air levels to 0.6



Monitor throughout imaging process as levels may fall and need readjusting  
make sure to seal off all holes in stage bottom plate (ie with additional dishes/slides)

**shutter/filter settings (see pictures below)**

red arrows: 660 and 730 laser aperture (top and bottom respectively)

blue arrows: top filter (??) and bottom filter( =camera output marked with blue arrow (8 IR preview, 1 oculars?)

bottom switch (green arrow) (won't work on the camera/ocular duel middle setting) for between ocular and preview camera on computer

