**On the computer – there’s no order how to open this.**

* Open Kinesis (Thorlab’s software for linear stage)
  + check if all 3 stages are connected properly. If yes, then close the software and proceed to next step.
  + If not, click connect and manually select all stages that are not connect and try connecting. If successful, then close the software and proceed.
  + If any stage cannot be connected after manual connection, follow instruction in Troubleshoot. (If linear stage can be opened properly in Kinesis, it should be controlled properly in Python as well)
* Open **Spyder** (Python)
  + have to run the program in its entirey first, to import the modules. and then close the zoom lens after that.
  + WE ARE IMAGING In WATER, so focal length (config\_f\_mm) should be set to 148.54. Focal length=197.556/RI
  + Go into Spyder, change this in the code for zoom\_lens.py; and line ~150ish
* Open **ThorCam** – for the brightfield
* Open **USB 4D stage**
  + click on “Go To Reference” to position the stage in the midpoint of the field
* Open **Matlab**
  + Go to “Set\_voltage” script and run it
* Open **Micromanager**.
  + Hardware config is camera.cfg. Click Okay. If you see an error, it’s because camera is not on, and needs to be turned on.
* Open **Cobolt Monitor** for the laser.

**Starting the microscope**

* Make sure the brightfield mirror is in
* In Spyder, go to zoomlens.py, and run
* Prep sample by adding water
* Manually raise the labjack to dip the objective.
  + Make sure there is no bubble trapped with the dipping objective. If so, raise, wipe with lens paper; lower again.
* Turn LED brightfield ON
* Go to ThorCam. Select the only available camera. Click Run.
* Use Picard stage software to move.
  + Its Z, Y and Z axis is different from the conventional definition. Y corresponding to up/down
* Change the default exposure on the Zyla camera. Then Click “Live” in MicroManager
  + To do this, go to Micromanager. Devices > Device Property Browser > Andor sCMOS Camera Exposure…. Change to 75ms for example. . This should look a little funky; in that it’ll be focused on only one parts.

**3D image acquisition**

* Turn on the laser using Cobolt software
* Open the shutter.
  + Won’t see 375nm (outside of visible)
* Make sure MicroManager is closed before using MATLAB for 3D acquisition
* Following comments in MATLAB code to enter the proper parameters
  + Use Filenames with an Underscore at the end so that the numbering happens automatically.

**Shutdown Procedure**

* IN MATLAB command line type in: write(dq,0)
  + This is to set the galvo corresponding to zero, in case of power shut down. This is just to be cautious, so that when power comes back, it doesn’t see a very large signal.
* Close Matlab
* In Spyder, run: zoom\_lens.close()
  + To make sure the zoom lens does not collide.
* Close Spyder
* Close ThorCam
* Lower labjack to the lowest point
* Remove sample
* Wipe O1 with washing solution or 70% ethanol on the lens paper. Swab gently.
* Put turning mirror back in.
* Turn off the camera
* Picard stage – set to Home for X, Y, Z. Shut down.

# Troubleshoot

Thorlabs software to operate this is Kinesis

Enable one fo the 50 mm stages; Test to see if it holds position, to figure out which unit is being enabled.

Zoom stages do not coordinate with Kinesis order; 50 mm stages are lens 1 and 3 (labelled DD050 on the screen).

ON ZOOM LENS 1, (): set to home. then set to 50

Enable Zoom lens 2 (100 mm stage): set to home; move to 70

On Zoom Lens 3 (50 mm stage): set to home, set to 0

On Zoom Lens 2 (100 mm stage AGAIN): set back to 0 (this procedure avoids crashes)

On Zoom lens 1: set this to 20

SO FINAL POSITION: lens 1: 20mm; Lens 2: 0mm; Lens 3: 0mm – this is the CLOSE POSITION.

Close Kinesis