Turning on QLIPP Microscope, microscope controls and using software (NIS elements)

1.) Turn on microscope by switching on one button on the bottom right next to the table

2.) If using live cells, turn on temperature underneath microscope – **we recommend turning this on the day before** because it can take several hours to get to the correct temperature so PLAN AHEAD

3.) The screen on the microscope tells you:

- The height of the stage (in microns)
- The objective you are currently using

4.) The buttons to control the microscope are:

- **The escape button**
  - This will lower the stage to a SAFE level to change objectives and to put on your samples (500um)
  - **Remember to ALWAYS hit escape any time you want to switch objectives and when you are putting on or taking off your samples**
  - If you are finding it difficult to focus on your sample, you likely have pressed the escape button and need to cancel out of it by holding down the escape button and refocus button simultaneously
- **The refocus button**
  - After you find and focus on your sample, you can hit this button to remember the spot and return to this same height on the stage between samples
  - To REFRESH the refocus option you need to push the escape button and hold down the refocus button simultaneously
  - **PLEASE do this at the end of your session!**
- There are two focus knobs on each side of the scope
- Move the focus knob **TOWARDS** you to raise the objective towards your sample
- Move the focus knob **AWAY** from you to lower the objective away from your sample
- There are three focus speeds – coarse (this will focus the fastest to your sample), fine focus, and super fine focus
  - If you are having trouble focusing on your sample, you may have clicked the super fine focus and it will seem like you aren't moving the objective! Always remember to take a look at the screen in front of the microscope to see the height of the stage, where it is, and how much it is moving while you are turning the focus knob
- The **light path buttons** are on the front of the microscope and you can either have the light go to your eyes in the eyepieces or to the camera by clicking the left button

5.) To control the stage, there is a joystick to move in the X and Y positions

- To control the speed of the stage movement, turn the knob on the joystick

6.) Open NIS elements software and the Nano indenter software

7.) Turn on the two Nano indenter boxes labeled with numbers 1 and 2 (make sure you turn them on in this order!) [insert image here]
8.) The Nano indenter software will ask you to 'home the stage'

- Click YES to home the stage
- MAKE SURE THERE IS NO PROBE OR SAMPLE ON STAGE BEFORE YOU HOME IT
- Homing the stage is important to calibrate the Nano indenter spatially and set it to an XY position of '0'

Calibrating the Nano indenter

1.) Nano indenters are underneath the microscope that vary in stiffness

- Choose which nano indenter you want to use for your experiment

2.) Clean the probe!

- First, put stage insert inside scope to hold a 60mm dish
- Clean the plastic 60mm dish and fill with diH20 – put on stage insert inside microscope – this is what you will calibrate the probe in
- Open the probe plastic container carefully
- Pick up the black end with the probe with your left hand and feed the green end of probe to your left hand
- Hold probe over a plastic petri dish and wash with 70% isopropanol first and then diH20 after
- Once probe is clean, put probe in holder inside the microscope
- Carefully insert the black probe in to holder on the nano indenter arm
- Feed the green end of the probe towards the right hand side and have the metal feet facing the computer to insert in place – it will click in

3.) Carefully lower probe by twisting knob on the arm and immersing the probe in the water (Make sure the probe is fully coated in the water or calibration will NOT be accurate)
4.) Find probe on the camera

- Focus on the bottom of the glass or plastic dish that is mounted on the stage
- Try to move the stage so that the objective is underneath where the probe is
- Use the X and Y stage controls on the Chairo software to move the probe so that it is over the objective and it is in focus on the camera screen

5.) Begin calibration

- Open the calibration menu by clicking the initialize button on the top section of the software interface
- Input the probe parameters which can be found on the front of the probe plastic box (keep calibration depth set to 3000um)

- Click scan wavelength button
- If no error is reported, you can move on to the next step
- Click the find surface button
- Confirm that the probe is in contact with the surface by pressing the probe stage up or down in 1um steps (do this only once)
- You should see a small fluctuation of the green signal in the live window
- Click calibrate in the initialize menu to start the last step
- When calibration is complete, a new geo factor is given
- The calibration factor should be ~1.33 times lower in the medium than it is in air, which is given on the box of the probe
    - e.g. if the number on the box is 3.2, then the geo factor in the medium should be 3.2/1.33 = 2.4 (there can be about <5% margin of error in the new geo factor number)

6.) Once you have finished calibrating the probe, carefully take out the probe and put it back in the plastic box while you put in your sample on the microscope stage (cells, matrix, etc.)

7.) Carefully, put probe back on the cantilever arm and begin experiment set up

<u>Cleaning Nano indenter and shut down</u>

1.) Once you have completed your data collection, raise cantilever arm and remove nano indenter

2.) Clean nano indenter probe first using diH2O and then 70% isopropanol

3.) Carefully wipe off excess liquid on probe using a kim wipe moving from bottom to top of probe

4.) Put probe back in plastic container and put underneath microscope

5.) Remove all samples from the microscope stage and hit escape to return the objective back to safe distance

6.) Shut down NIS elements software and Chairo software

7.) Turn off Chairo nano indenter boxes (#1 and 2)

8.) Turn of incubator if using for live cells and turn off microscope