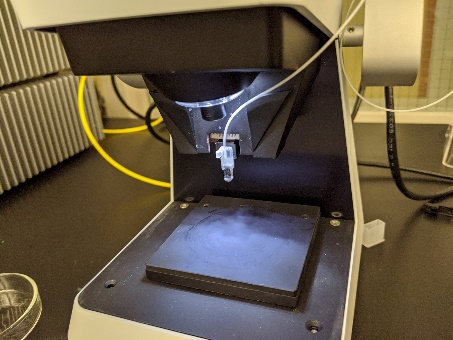
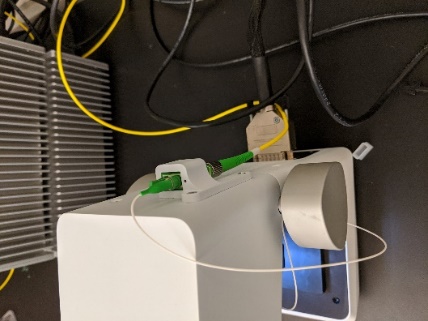
PIUMA Nanoindenter Operating Procedure

1. Sign into iLabs/Kiosk
2. Make sure the laptop computer is on.
3. Turn on the OP1550 interferometer (the silver box with the display screen). Note: it will come up in the “Measure” tab, with a yellow “not calibrated” message in the bottom right corner.
4. Turn on the Controller box (the silver box with just a power button).
5. Remove the clear plastic cover and ensure the probe holder is manually moved to its highest z-position using the gray knobs on either side of the instrument.
6. Start the Piuma Nanoindenter program by double-clicking the Piuma software icon on the desktop.
7. When dialog box pops up asking if you want to Home the Stages, Click “Yes.” (Stages will move to (0,0)). Make sure any tip is far from the stage/sample when you do this.



1. Load a tip: The glass/white side goes into the port in the front center of the instrument, and the green end plugs into the port at the top back of the instrument.
2. Confirm/enter the Probe Parameters: Click the ‘Probe’ button at the top of the screen. Ensure that the cantilever spring constant, k (N/m) and tip radius (μm) match the packaging for the installed probe.
3. Place an empty petri dish under the tip. Using a pipet, pre-wet the probe by applying a drop or two of your solution to the probe at the junction between the 3D printed white plastic piece and the clear glass portion. The drop(s) will run down the face of the probe and become suspended hanging off the probe tip. (Often I clean the tip with water, ethanol, water, before wetting the tip with the solution to be used in the experiment.)

Note: Once the tip has been wetted with something other than water, it is important to keep a drop of solution on the tip until it can be rinsed with deionized water. If your solution is allowed to dry on the tip, the residual material in the solution could cause functional issues.

1. Place a glass petri dish filled with the fluid you would like to make your measurements in on the stage. There should be 2-3 mm of fluid covering the bottom of the dish.
2. Lower the z-position using the gray knobs until the tip is just above the surface of the liquid. Using the software Stage Controls, enter 300 m into the Stage Controls Travel box and press the z-down arrow until the drop is submerged in the fluid. Once submerged, you may want to press the z-down arrow 1-2 more times to make sure it is well below the surface.



1. Optical Calibration: Click the ‘Calib’ button at the top of the screen to enter the calibration menu.
   1. Select ‘Scan OP1550 Wavelength’ to start the optical calibration. The Calibration Menu window will close automatically; then you can observe the wavelength scan process. You’ll see a curve that looks somewhat like a sine wave in the real time display in the bottom right portion of the software.
   2. The interferometer will automatically switch over to the “Demodulation” screen. When the optical calibration is complete, the interferometer screen will display a white dot/short line drifting near a red circle.
   3. On the interferometer, if you see a “Quadrature scan failed” message, press OK to continue and make sure your tip is well submerged below the surface of the liquid. If you did not see the sine wave in the real time display, the tip may be stuck. Pull it out of the liquid and resubmerge it a few times and try the wavelength calibration again. Make sure that the green end of the tip is fully plugged into it’s port.
2. Find Surface: After optical calibration, you will use the Piuma’s automated find-surface approach procedure. Click the ‘Calib’ button and select ‘Find Surface.’

Note: The Find Surface in the Calibration menu will leave the tip in contact with the surface. The other Find Surface at the bottom left corner of the software will find the surface and then pull off a certain distance.

1. Calibrate: Now that the probe is in contact with the glass, click the ‘Calib’ button and select ‘Calibrate’ to initiate geo factor determination.
   1. Click OK to confirm that the probe is in contact with a stiff surface.
   2. After the procedure is finished, the program will ask you whether to use the newly calculated factor or retain the old one. By pressing ‘Yes’ the software automatically saves the new calibration factor in the probe configuration menu. The geo factor in solution will be less than what is reported on the box in air. (A tip with a geo factor in air of 3.1 had a geo factor of ~2.1 in aqueous solutions.)
   3. On the interferometer you should see the white dot on the red circle.
2. Calibration Complete!
   1. Press the “z up” button to raise the probe to its highest z position. If it will be a while before you have a sample ready, reapply a drop of solution to keep the tip wet.
   2. Use the grey knobs to raise the probe to its highest z-position.
   3. Remove the glass petri dish.
3. Prepare Specimen for Piuma Testing
   1. Fill your sample petri dish with 2-3 mm of solution. The liquid layer should be deep enough that you can confidently move the probe below the surface of the liquid without crashing the tip into the sample.
   2. With the probe at its highest z-position, place the sample on the stage.
   3. Using the grey knobs, **slowly** lower the probe towards the specimen. Ensure that the probe and probe assembly won’t hit the sides of your dish when it moves down in z. Adjust the x,y position of the sample on the stage (either manually or with the stage controls) if necessary. Stop lowering the probe when it is just above the surface of the liquid.
   4. Use the stage controls to move z down in steps (300μm steps are recommended), until you see the probe tip enter the liquid. Then lower the probe another 1-2 steps to ensure it is deep enough in the liquid to avoid the effects of surface tension or optical interference.

Note: Often, the drop of liquid on the probe tip will “pull off” when it touches the fluid in the sample dish. Continue to lower the probe in steps and do not try to reapply a drop to the tip.

* 1. Press the Find Surface button, and the tip will slowly approach the surface of the sample, and then pull off the surface a set amount. (This distance can be adjusted in the Options menu at the top of the screen.)

1. Configure the Single Indentation or Matrix Experiment:
   1. Click the Configure Experiment button at the top of the screen.
   2. Set the “Experiment path” field by navigating to the folder icon, and either selecting an existing program, or creating a new file name for a program. We save our data and programs with the file structure described below.

C:\Data\YourAdvisor’sFolder\YourFolder\

* 1. If creating a new program, you’ll need to press the Add button to add a step, and then click on the Select pull down to choose the type of experiment (Indentation or Matrix Scan).
     1. In the General tab, make sure the path to save your data and the file name are correct.
     2. In the Profile tab, choose the type of experiment you want to do (Displacement Control, Load Control, or Indent Control), and then write a program by adjusting the displacement, load, or indent parameters and times in the boxes on the right. Below the graph are controls for approach/retract speed and the contact threshold for load and indent control modes. Smaller values of the contact threshold make it more sensitive to contact. A good contact threshold to start with is 0.005.
     3. If you are doing a Matrix indent, you’ll also want to adjust the parameters in the Matrix Scan tab including: number of points, spacing between points, height to pull off the sample while moving in x and y, and height to pull off the sample after finding the surface. You can press the Use Stage Pos button to use the current stage position as your starting position.
  2. Save the experiment configuration. Click OK to replace the existing experiment (or choose to Save As a new experiment).
  3. Confirm/enter the Options Settings: Click the “Options” button and in the Options tab, verify that the values for *Poisson’s Ratio* (typically 0.5 for silicone elastomers) and *% of Pmax for fit* – Hertzian contact model are set as desired. The choice of these values will affect the curve fit calculations and the Young’s Modulus results that are reported by the software. These values can be adjusted in DataViewer during post-processing if necessary.
  4. Under the Options button, and the Find Surface tab, you can set the *Z above surf.* distance. This is the distance that the instrument will lift the tip after it does a *Find Surf*.

Note: With each new specimen, it is a good idea to run a single indentation experiment first to make sure the PIUMA is finding the surface and reporting reasonable results. Then you can collect multiple modulus readings for the specimen using a matrix experiment.

1. Perform Piuma Testing
   1. Click the Run Experiment button in the lower, left-hand portion of the screen. It is best not to lean on the table during a measurement.
   2. During the experiment run, you can observe the z-position bar and z (μm) read-out change as the probe approaches the surface. Then you will see the real time movement of the piezo bar and readout during the actual indent. The graph in the bottom right will also display real time piezo and tip data. As each indentation is completed, the Indentation Result/Graphs in the top of the software will update.
   3. If the Scan Result graph does not indicate cantilever bending and/or if the Indentation Result graph appears irregular, here are a few things to consider:
      1. If the Force versus Indentation curve only bends up (there is no horizontal approach section), your tip may have already been in contact with the sample. In the options menu, increase the *Z above surf* distance.
      2. If it doesn’t look like it ever pressed on a surface – check to make sure that the Find Surface actually found the surface. Look at your sample from the side. Does the probe look close to the surface? Try Find Surface again.
      3. If the slope of the curve is too steep or too shallow, you may need to change the stiffness of the probe that you’re using.
      4. If you see nonsense – circles or curlicues, try making a measurement in a new location. I’ve seen some of these features when the tip I was using was too soft for the sample. Contact Carrie, and she can help troubleshoot.
   4. If testing at more than one position on the same sample, BE SURE to RAISE the PROBE above the surface using the z up arrow before using the x-y position motors to adjust the sample position.

Remember: Once the tip has been wetted with solution, it is important to keep a drop of solution on the tip until it can be rinsed with deionized water. If solution is allowed to dry on the tip, the residual materials could cause functional issues.

1. Changing Samples
   1. Click the ‘Z up’ button to raise the tip to its highest z piezo position.
   2. Manually raise the probe to its highest z-position (using the gray knobs).
   3. Apply a drop of solution to the probe tip to keep it hydrated.
   4. Carefully remove the old sample, replace it with a new sample, and go back to step 17.
2. Shut Down
   1. When you are finished for the day, rinse your tip with at least DI water before storing it. I like to use DI water, ethanol, and a final rinse with DI water. Tips must be stored in the plastic box that they came in (to keep track of the tip parameters).
   2. On the instrument, replace the black plug where you unplugged the tip, and put the clear plastic cover back in place.
   3. Turn off the controller.
   4. Turn off the interferometer.
   5. Clean up the petri dishes you used.
   6. Close the software.
   7. Sign out of iLabs/Kiosk