

Bruker Multi-mode 8 Atomic Force Microscope

Operation, Fundamentals, and Analysis

Rachel A. Letteri, April 2018

Bruker Multi-mode 8 AFM

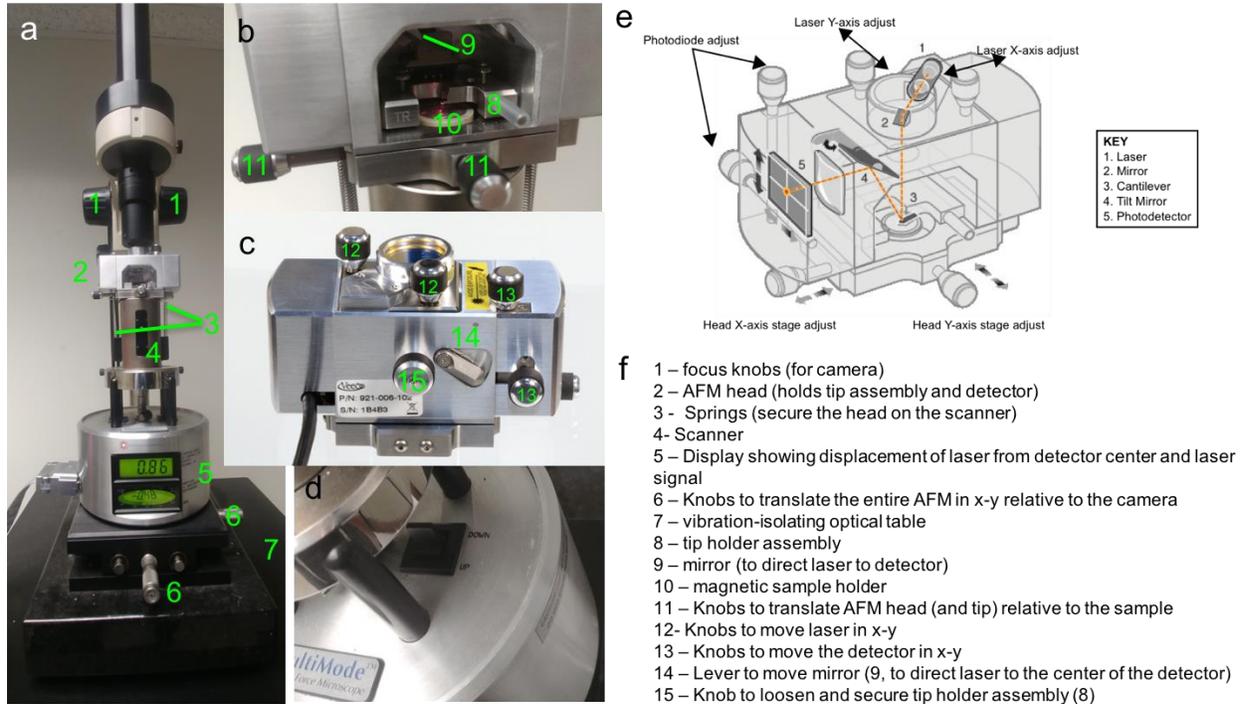


Figure 1. (a) Bruker Multimode 8 AFM: Zoomed in images of the (b) front and (c) back of the AFM head, (d) z-scanner motor switch (up and down directions indicate tip movements relative to the sample); (e) labeled transparent image of AFM head components; (f) names of AFM components labeled in green. Images (c) and (e) were taken and/or adapted from the Bruker AFM Multimode 8 user guide.¹

Sample preparation

- Note that sample preparation will vary based on sample and substrate composition, and many other factors. General guidelines are provided below, but for best results, optimize concentrations, substrates, and sample preparation procedures.
- For solid state measurements: Substrates must be first adhered to magnetic pucks, using sticky tabs (similar to double-sided tape) or carbon tape (for a stronger bond). Next, either (1) dropcast, (2) spincoat, or (3) dispense liquid solution on a substrate for 2-3 minutes to enable adsorption and wick away excess solvent.
 - **Dropcast:** While dropcasting and allowing samples to dry completely can produce undesired drying patterns, it is often the preferred method for materials that do not adhere easily to substrates (e.g., by electrostatic interactions). For neutral materials, e.g., polyethylene glycol, this is an especially valuable sample preparation method.
 - Prepare a 0.5-1 mg/mL solution of your polymer/nanostructure. Dropcast 2-4 μL of this solution and allow to dry.
 - **Spincoat:** This method can also be useful for coating substrates with materials that don't easily adhere. Prepare a 0.1-0.5 mg/mL solution of your polymer/nanostructure.

- On our spin-coater, this program is already in place, but if not, write one with a similar procedure: step 1 – 500 RPM for 10 s; step 2 – 3000 RPM for 2 min.
 - Place substrate-containing magnetic puck on the spincoater (vacuum will hold it in place).
 - Draw 20 μL of your solution in a pipette
 - Start the program, and during the first slow spinning step, add your solution onto the spinning substrate. This will encourage even distribution of the materials.
 - Dry substrates under a stream of $\text{N}_{2(g)}$ to remove any residual liquid.
 - Adjust sample concentration, or repeat depositions as needed.
 - Dispense solution, then wick away excess solution
 - For nanostructures that exhibit attractive interactions with the surface (e.g., electrostatic), this method is particularly useful, as it prevents any adverse drying features
 - Dropcast a 0.1-0.5 mg/mL solution such that it covers the entire substrate.
 - Allow to stand 1-3 min
 - Tilt substrate onto filter paper to wick away excess solution, then dry substrate under a stream of $\text{N}_{2(g)}$
- Solution-state imaging
 - Nanostructures must adhere to substrate, either *via* covalent interactions or attractive non-covalent interactions (e.g., electrostatics). As such, often sample preparation means tailoring the substrates to facilitate adsorption to nanostructures of interest.
 - Static solution imaging: prepare a 0.1-0.5 mg/mL solution of polymer/nanostructures. Place the substrate onto the magnetic sample holder, align tip, remove the tip assembly, add a drop or two onto the substrate, then replace tip holder assembly
 - Dynamic imaging under flow: Prepare a very dilute solution of nanostructures (since we're adding more volume – maybe 10-100x more dilute relative to static imaging), and pull into a syringe
- Substrate preparation
 - Mica: This is an anionic, layered substrate. After adhering to magnetic pucks, mica can be reused by cleaving off the top layer. Apply Scotch tape, then pull away. A mica disc should be visible on the piece of tape. Repeat until 2 almost whole discs are cleaved, and appear on the tape. This is a great substrate for solution-state imaging of cationic nanostructures.
 - Glass, silicon wafers: Anionic, non-layered/non-reusable substrates. Clean with soap and water, then wash with acetone and water to remove oily and water-soluble contaminants, respectively. Dry under $\text{N}_{2(g)}$.
 - Coating substrates to changes surface chemistry: Spincoat a 0.1 mg/mL solution of polylysine, or coat substrates with a silane (e.g., 3-aminopropyltriethoxysilane) to render the surface cationic. Consult the literature for best procedures, since both methods can generate aggregates (polylysine can aggregate upon drying to produce 2-4 nm aggregates, and water-induced cross-linking of silanes also produces aggregates). As such, be sure to image coated substrates to determine surface roughness prior to imaging nanostructures.

SOP

The tip holder should always be secured in the AFM head when not in use. Please do not store on the counter.

- Power on controller (back left of box, as shown in Figure 2)
- Power on TV and light, turning the dial up almost all the way to the right to get sufficient light intensity to allow visualization of the surface and tip
- Power on computer and monitor
 - Note that both the computer and monitor should be powered off when not in use
 - Username: nanoscope, password: none (just press enter)
- Click on Nanoscope 8.15
 - Note that the Nanoscope 8.15, Nanoscope Analysis, and Multimode manual shortcuts are located at the center of the desktop
- Select experiment category, e.g. 'ScanAsyst' (or Tapping Mode, Contact Mode, Quantitative Analysis, etc.), as shown in Figure 3
 - Select experiment group, e.g., Scanasyst in air (or in fluid)
 - Select experiment, e.g., Scanasyst in air (or in fluid)

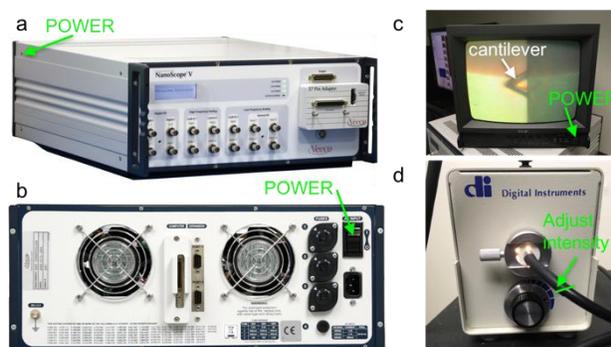


Figure 2. Power-on procedures: Photos of the (a) front and (b) back of the AFM controller, with the power button indicated in green; (c) photo of the TV showing an optical microscopy image of a cantilever; (d) light intensity adjustment on light source for the optical microscope. In the manual,¹ the optical microscope video set-up is abbreviated OMV.

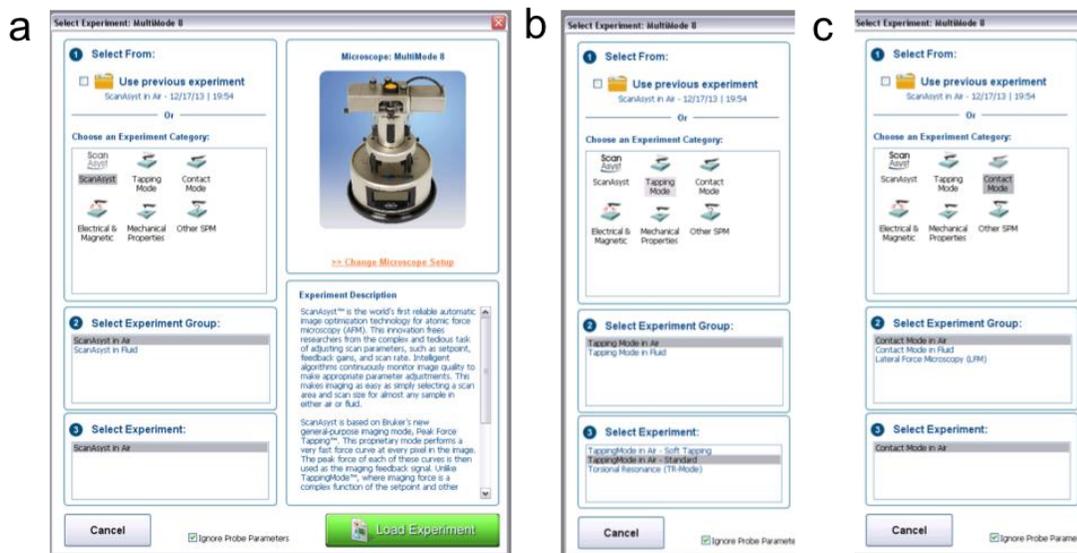


Figure 3. Experiment selection: Screenshots of experiment selection for (a) Scanasyst, (b) tapping, and (c) contact modes performed in air. For solution-state imaging, select the appropriate experiment group and experiment.

- **Load sample and probe:**

- Remove tip/probe holder assembly (part 8) from instrument by unscrewing knob (part 15) on back-center of AFM head, and place on a table with the tip facing up (Figure 4).
- Place sample (adhered to magnetic puck) in the center the magnetic platform, and push the lever at the base of the instrument (Figure 1d) in the up direction to move the sample sufficiently far down (tip up) so that the probe will not crash into the sample when the probe holder is placed in the AFM head

- The 'up' and 'down' labels on the switch in Figure 1d indicate the direction of tip motion relative to the sample, but the sample moves (*i.e.*, the up direction moves the stage down such that it appears that the tip is moving upwards away from the stage, and *vice versa*)

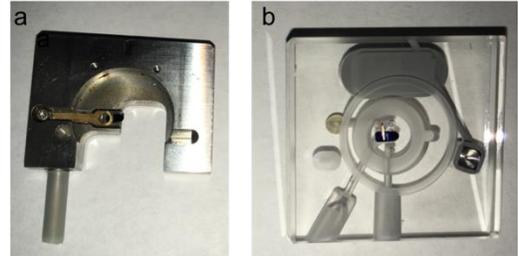


Figure 4. Tip holder assemblies for (a) solid-state and (b) solution-state imaging.

- For loading probes onto either solid or solution-state tip holder assemblies, push down *gently* on probe holder to open clip. Use forceps to carefully place tip on platform and then close clip
 - Note: it is strongly advised to practice placing broken tips into the probe holder *ca.* 2-3 times before handling good tips – even for experienced users (especially if you've had a lot of coffee!!)
 - *For solution-state imaging*, it's important that the metal clip secures the tip – it falls out easily if the clip is not perpendicular to the long axis of the cantilever. Test the security of the tip by inverting the tip holder after installing a broken tip.
- Insert probe holder with tip facing down into scope such that pins fit on the protruding pins of the AFM head (part 2), as shown in Figure 1b. Secure probe holder by tightening knob in the back-center of the AFM head (part 15) until the clip contacts the tip holder assembly
 - *Do not overtighten*, as long as the pins touch the probe holder, it will be secure
- *Solution-state imaging note: It's easiest to align the laser in air before adding solution.*

- **Align the laser:**

- Focus the camera on the tip by using the large black knobs (part 1), as shown in Figure 2c
 - If the cantilever is not visible, move the AFM relative to the camera into the illuminated light circle using the micrometer knobs at the bottom of the instrument (part 6) or move the AFM head assembly (and tip) relative to the sample and camera using the knobs on the AFM head (part 11)
- Look for the red laser spot, and if needed, move the laser close to the tip in the x and y directions using the knobs on the top of the AFM head (part 12)
- **Alignment method 1:** Raise camera using the focus knobs (part 1), and holding the AFM head carefully, unhook the springs (part 3), and hold head such that the laser spot is aimed on a sheet of paper (or your hand)
 - As shown in Figure 5, align laser such that the laser is on the tip using the two laser dials (one on right of head to move laser in x direction and one on the back to move in y direction)

- Replace head in microscope. Look at the sum reading (want this to be about 6)

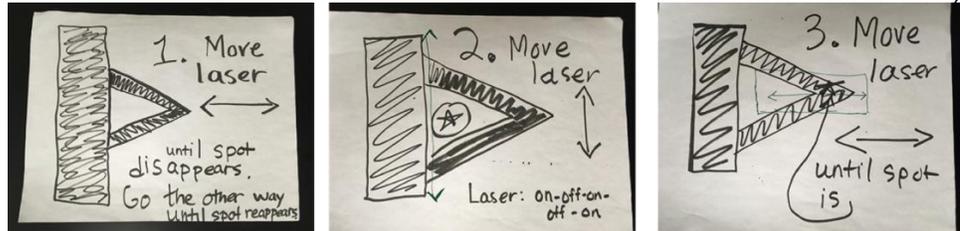


Figure 5. Tip alignment procedure, using method 1. In method 2, the same procedure can be used, except that the laser spot will be visible on the paper covering the detector when it's hitting the cantilever (whereas in method 1, the laser is not visible when it's hitting the cantilever).

- **Alignment method 2:** Slide a piece of paper in front of the detector such that when the laser bounces off the cantilever, it will be visible on the sheet of paper – as shown in **Figure 6**.

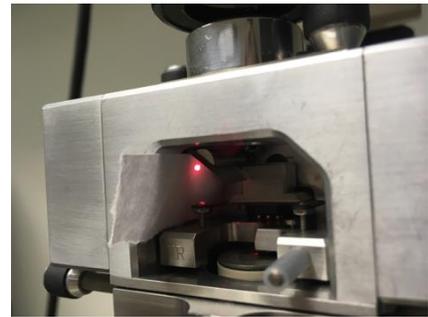


Figure 6. Photo of laser alignment using method 2

- Move the laser in x and y to center it on the back of the cantilever closest to the tip following a similar procedure as shown in **Figure 5**, however in this method, you will see a laser spot when it's hitting the cantilever, unlike in method 1 when the laser is hidden from the paper directly below it when it's reflected off the cantilever
- Ensure that once the spot is centered and hitting the piece of paper, that it is a round/Gaussian spot rather than elliptical or distorted (otherwise make small x-y adjustments using the knobs, [part 12](#)).
 - This last step allowing us to ensure good laser spot shape represents an advantage over method 1 (in addition to not having to remove the AFM head).

- Note that, if the laser is aligned and the sum (looks like a speedometer on the digital display, **Figure 7**) is still below 4-5, try adjusting the lever on back of the AFM head ([part 14](#)) to tilt the mirror, and thereby redirect laser toward the detector, to maximize the sum

- **Align the detector:** Move the detector such that the laser spot hits the center of the detector by adjusting the knobs on the left/top of the AFM head ([part 13](#)) one at a time. This will change the numbers in the displays in **Figure 7**. Adjust the position of the detector until one of the display values approaches 0, then adjust the other knob so that both numbers go to 0 (+/-0.1-0.3 is preferable since these numbers will change during imaging, but +/- 1 is fine)



Figure 7. Photo of the Multimode 8 displays, with the sum indicated by an arrow.

- If the numbers don't change immediately, it's totally fine – it just means the detector center is still far away from the laser spot. Turn one of the knobs all the way in one direction, then all the way in the other. At some point, a change should occur. If the detector is at the edge of its movement range and still needs to move more to bring the display numbers (indicative of the displacement of the laser spot from the center of the detector) to 0, adjust the mirror using the lever on the back of the AFM head (part 14) to direct the laser to a different spot.
- **Focus tip just above surface:**
 - First, focus far above the surface turning knobs (part 1), then slowly focus downwards. You will first pass through the tip, then the surface, and finally the tip reflection (which looks just like the actual tip, so you'll want to be sure you know you're focused above the surface on the tip before moving the tip).
 - Focus on the surface, then move the focus just above the surface (such that the surface looks blurry)
 - Use the 'tip down' switch (Figure 1d) to move the tip slowly towards the surface until it's in focus. Since the focus is just above the surface, when the tip comes into focus, it too will be just above the surface.
 - Once the tip is in place just above surface, turn off light (Figure 2d) to avoid interference with the laser light traveling to the detector
 - *Solution-state imaging note: As the tip is immersed in solution, the alignment/sum will decrease due to the different bending of light/refraction across the air/water interface. Move only the mirror (part 14) to direct the laser back toward the detector.*
- You're ready to image – see the sections below for procedures for imaging in ScanAsyst, contact, and tapping modes.

Imaging in PeakForce Tapping/ScanAsyst mode (most common)

PeakForce Tapping mode combines the benefits of contact and tapping mode, and adds tremendously beneficial automation, allowing users to obtain nice images irrespective of experience level. At each point along a line, the cantilever is moved relative to the sample until the 'peak force' is reached, and then withdrawn. As such, force-deflection curves are obtained at each point, enabling mechanical mapping in a less destructive way than contact mode.

- **Specify imaging parameters** in the software, as shown in Figure 8. Good initial parameters are:
 - Scan size = 1-5 μm is
 - Scan speed = 1 Hz
 - Aspect ratio = 1
 - Samples/per line = 512
 - While 512 points/line is best for publication, 256 is a great way to survey a larger area in half the time
 - The red button expands parameters, but this shouldn't be necessary in routine imaging
- **Engage** (this moves the tip into contact with the surface)

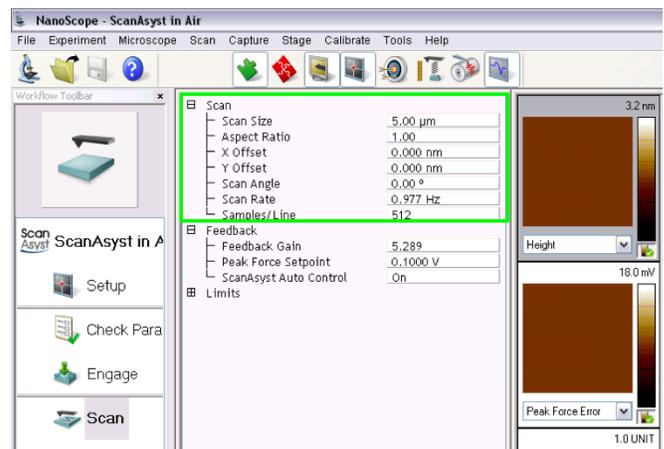


Figure 8. ScanAsyst parameters. Adjust those in the green box if desired and make sure ScanAsyst Auto Control is on; the others are adjusted automatically.

- The height and peak force error data are collected automatically
 - If you'd like both the trace and retrace data from each line, you'll have to collect this in separate channels (just open and select new channels)
- **Withdraw** the tip when imaging is complete, click several times

Contact Mode Imaging

In contact mode, the cantilever is pressed into the sample until it deflects to a specified set point. While this is the most traditional imaging mode and allows acquisition of mechanical information (i.e., force-distance curves), the constant contact with the sample can be destructive for soft/polymeric samples.

- **Set initial scan parameters**
 - Scan rate = 1 Hz
 - Scan size = 100 nm
 - Scan angle = 0°
 - Slow scan axis = enabled
 - Integral gain = 2; proportional gain = 5
 - Deflection setpoint = 0 V
 - Units = metric
 - Z limit, deflection limit = max (enter really large number and the software will convert it back to an acceptable value)
- **Engage**
- **Increase deflection setpoint by 0.5 V** at a time until you see an image, and the trace and retrace line/height profiles match well
 - This is telling the tip to press harder into the sample such that it deflects more
 - Ideally, you have the lowest deflection setpoint possible while still following the sample to avoid wear on the tip.
- Height and deflection error will be collected automatically in channels 1, 2. If not, select these. Note that it doesn't matter whether you collect trace or retrace data (i.e., that collected as each line of the sample is scanned and retraced)
- **Withdraw** when imaging is complete

Tapping Mode Imaging

Tapping mode minimizes sample damage by contacting the sample only periodically, while providing additional chemical information about the surface. The tip is oscillated near its resonance frequency, and then brought near the surface. As the tip approaches the surface, the amplitude/oscillation is dampened (slowed) by enhanced sample-tip interactions. In tapping mode, an amplitude setpoint is specified, and the sample moved up and down to maintain a constant amplitude, enabling height and 'phase' imaging. Phase imaging provides insight into tip-surface interactions, and is especially useful for block polymer films. Even if the films have a constant height, the morphology can be imaged by contrast between tip-sample interactions in different polymer domains.

- For tapping mode, it's suggested to use a silicon probe
- **Set scan parameters** – the following are good starting points:
 - Scan size = 1 μm
 - X, Y offsets = 0 nm
 - Scan angle = 0°
 - Scan rate = 1 Hz
 - Samples/line = 256-512

- Slow scan axis = enabled
- Integral gain = 4
- Proportional gain = 8 (note that but gains for tapping mode will be ca. 10% of those used in contact mode imaging)
- Amplitude set point = leave as is (this will be set by the software as the tip engages the surface)
- Drive frequency, drive amplitude = leave as is (this will be set during cantilever tuning)
- Limits: set to max by entering large numbers and allowing software to make the correction to the maximum possible value
- **Tune the cantilever**
This step measures the resonance frequency of the cantilever and adjusts the piezo voltage to oscillate the cantilever such that it vibrates at a frequency near resonance to ensure good amplitude. The tuning process involves application of a range of frequencies to cantilever, and the amplitude measured to determine the resonance frequency. The amplitude vs. frequency curve should be symmetrical and Gaussian, though some deviation is okay. If the peak shape looks bad/multimodal, change the probe.
 - Click on the tuning fork icon or select microscope -> cantilever tune
 - In the tune window (**Figure 9**), set mode = main
 - Start and end frequencies should be set so they are +/-20-50 Hz of the listed resonance frequency f_0 (found on the probe box)
 - Click auto tune
 - After tuning is complete, click center peak and zero phase.

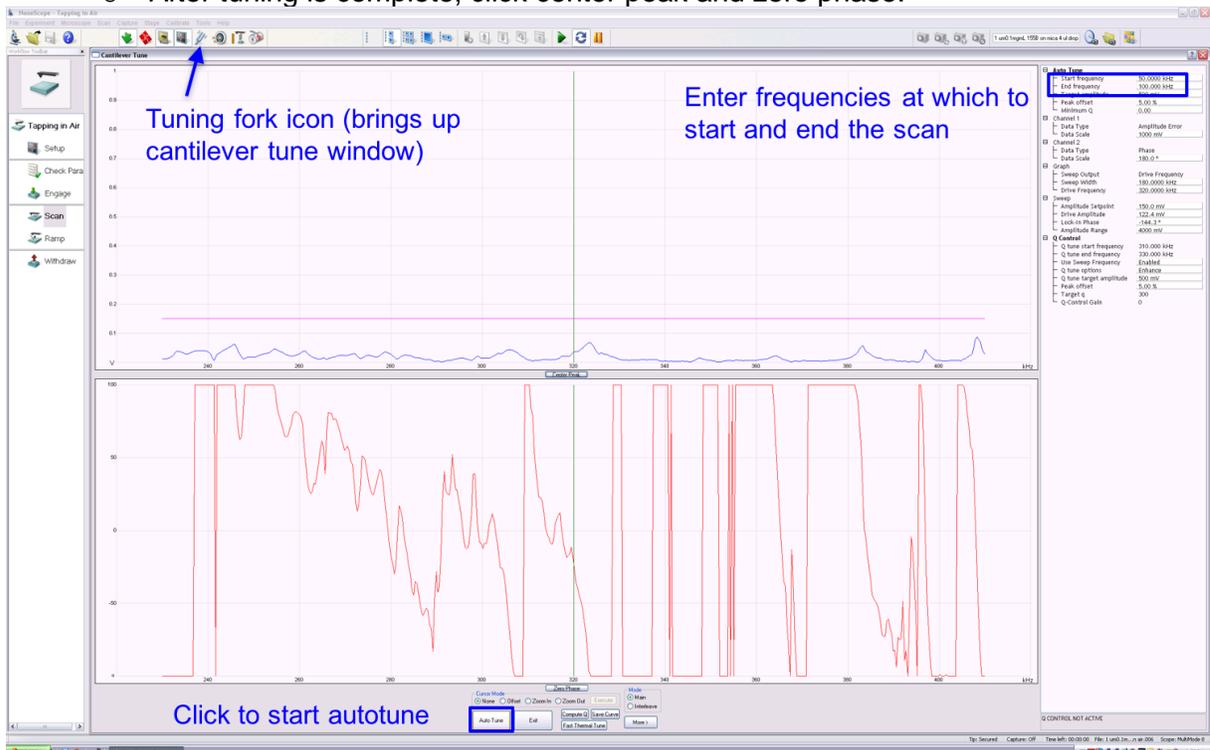


Figure 9. Cantilever tune window.

- **Reset gains: integral gain = 0.5; proportional gain = 5**
- Height, amplitude error, and phase should be automatically collected, but if not, add these channels. The line direction can either be trace or retrace.

- Engage
- Adjust the scan rate, gains, and amplitude set point to bring the trace and retrace lines as close together as possible.
 - Sometimes increasing drive amplitude can be helpful

Solution-state imaging (under flow)

- Align probe in air
- Wet a silicone thin ring and place in ring around tip platform (widest end should fit into the ring, and the narrow end of the ring extend upward. The water helps to hold the ring in place, even when the tip holder assembly is inverted).
- Bring the tip into focus just above the surface (as described above) – this helps to create a good seal between the substrate and the silicone ring prior to introduction of fluids.
- Prepare a very dilute solution of nanostructures (since we're adding more volume – maybe 10-100x more dilute relative to static imaging).
- Pull sample into a syringe and remove as much air as possible, then introduce using the inlets and outlets shown in **Figure 10**.
- Add a vial to collect solution flowing out of the cell.
- Very slowly introduce solution into cell – rapid flow may apply shear sufficient to break the tip.
- If there are no leaks, solution should flow out of the outlet as you introduce solution.
 - If leaks occur, remove and re-install silicone ring.

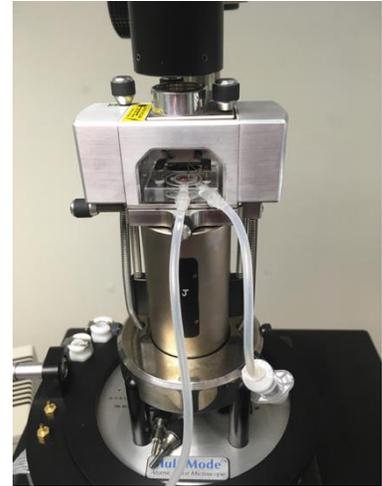


Figure 10. Solution-state imaging under flow

Image capture

- Select capture directory by clicking the file button next to the text box for file name entry (top right of software window) and selecting the folder in which you'd like your data to be saved
- Name file, and press enter (to save the name)
- Click capture (or continuous capture, which will keep capturing images as the sample is scanned)
 - The image will be captured once the scan (from top to bottom, or bottom to top) is complete – though you can always force capture by clicking capture again
 - If you wish to start a scan from the bottom click the button containing an up arrow (middle top of software window)
 - To start a scan from the top, click the button containing a down arrow
- The zoom button at the bottom of the height channel image allows you to draw a box, and then upon clicking execute, an image will be acquired in the place specified by the rectangle (or close to it – the zooming is not always exact)
- Click offset, then place the cursor at a point on the image. Upon clicking execute, a new image with the same dimensions will be acquired, this time centered on the point indicated.
- When done imaging, click withdraw several times to lift tip off of surface in a controlled manner to avoid breaking the tip, then use switch on the instrument to move tip faster away from surface

Image analysis

- Open Nanoscope analysis, and then open the image file you wish to analyze

- **Flatten** to correct systematic errors/features, such as substrate tilt, which can often be parabolic. Click the flatten icon, boxed in **Figure 11**. 2nd order flattening is a good starting point. Select no thresholding, the whole image. **Click execute**.

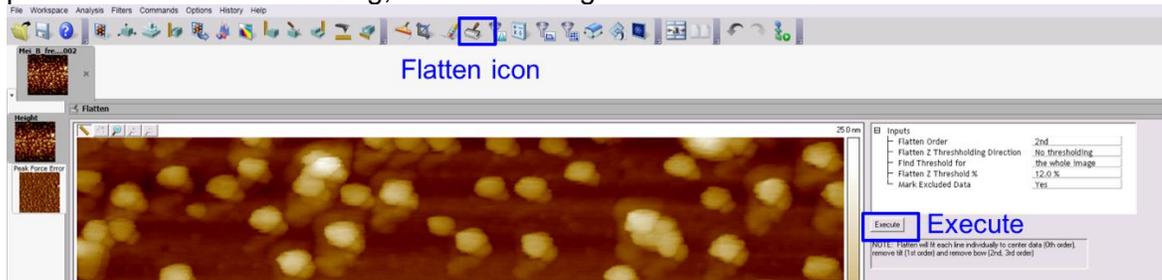


Figure 11. Flatten image to remove systematic variation in substrate curvature, etc. Click the boxed flatten icon to access the window shown above, then once inputs are as desired, click execute.

- **Set data scale** by right clicking on the image color bar, and click color scale
 - In the window that appears, click the **modify data scale tab** and set the max and min of each image. When comparing multiple samples, it's best that this the data scale be kept constant
- **Save image** as a jpg, by right clicking on the image, select export, then screen display
- **Section analysis:** Use this feature to obtain line profiles (e.g., height vs. x-y dimension).
 - As shown in **Figure 12**, click the section analysis icon and draw a line to generate a profile, which can be exported as txt files and plotted in origin. It's a good idea to also save an image with the section lines drawn, so you know at which point on the image you took the line profile.

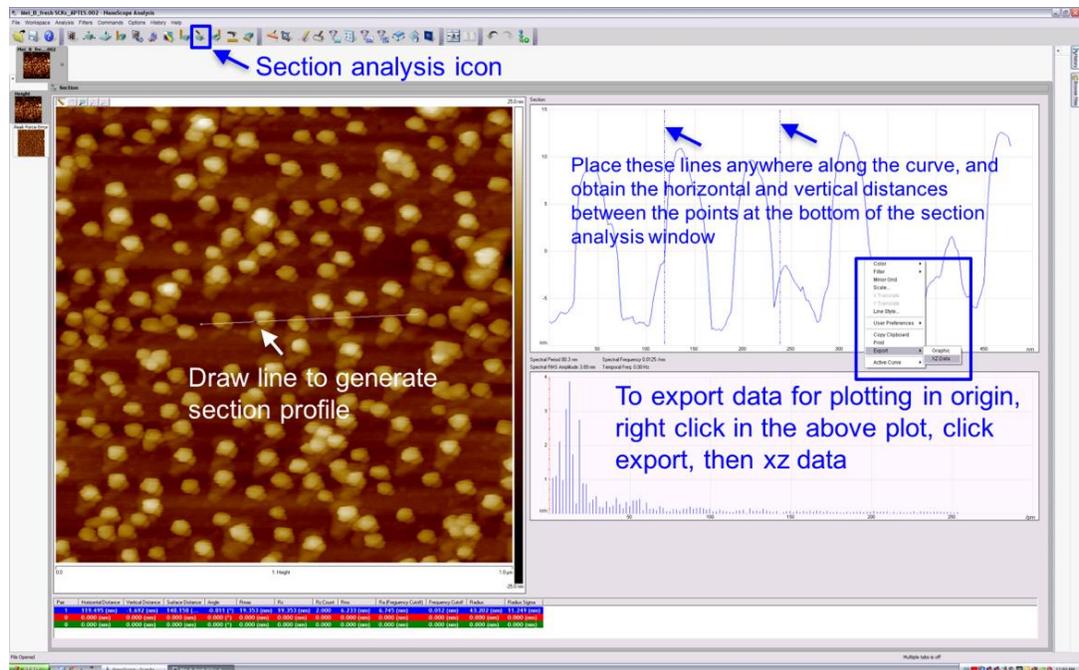


Figure 12. Section analysis, including instructions on how to bring up the window, draw a line to generate a line profile, and export x-z data for plotting in Origin or excel.

Nanomechanical measurements

(based on training by Cody Chalker, April 2018)

Since contact and PeakForce tapping modes generate force vs. displacement curves, these can be used to extract mechanical information about surfaces. In PeakForce mode, where force-deflection curves are acquired at each point, mechanical mapping is possible. However, since determination of mechanical properties (e.g., modulus) is highly dependent on probe geometry, probe spring constant, and sample/probe composition (as well as contamination), it's crucial that users exercise diligence in ensuring these measurements are conducted properly and reported data are representative. As such, mechanical properties should always be listed as a range – never as a particular value.

- **Adjust the load** on the table by moving the load adjust such that the vertical position indicator spot is at the line (not too high or low), as shown in **Figure 13a**, to minimize noise. If further noise reduction is desired, the AFM can be placed on a tripod, as shown in **Figure 13b**.
- **Calibrate deflection sensitivity.**
 - Use a hard sample (e.g., sapphire or silicon)
 - Check noise: In the quantitative nanomechanical analysis mode, set scan size = 0 and click engage.
 - Activate ramp mode, and click start single ramp (this will acquire a force-deflection curve in a single point).
 - On the resulting force distance curve, place the cursors at 2 points in the linear range closest to the surface (highest z value), and the software will calculate the slope.
 - This will bring up update deflection sensitivity window and it will ask you if it's OK. If you click OK, this will update the deflection sensitivity in the calibration window.
 - Repeat this test to make sure you obtain similar values.
 - Note that it's actually best to put silicon and sample on same tip, so as not to move tip after this deflection sensitivity measurement.
- **Calibrate the probe spring constant**
 - Spring constant values listed on the probe boxes are nominal, and vary from batch to batch, so calibration is necessary. To learn more, see the Veeco application note on spring constant calibration² and the papers by Sader and coworkers.³⁻⁴
 - Perform a **thermal tune**
 - Note that this measures the vibration as a function of frequency without excitation (*i.e.*, without shaking the cantilever with a piezo).
 - Fit the power spectra density curve, or power as a function of frequency to the simple harmonic oscillator equation. – damping is used for tapping mode since the oscillations are near the surface. Fit by adding the 2

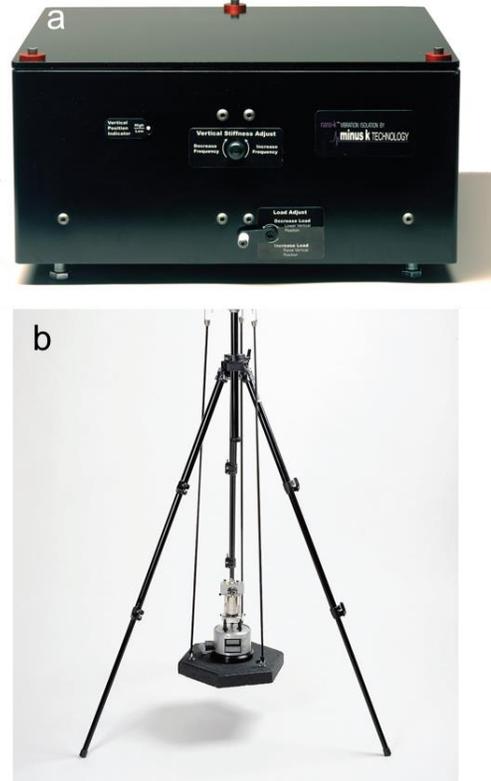


Figure 13. Minimize noise by leveling the vibration-reducing table (a) or placing the AFM on a tripod (b).

cursors on either side of the peak (just like a GPC peak), entering the temperature, and clicking fit. This will calculate the spring constant

- **Calibrate the tip radius**
 - Values listed on the probe boxes are nominal, and vary from batch to batch, so calibration is necessary. There are several methods for measuring probe radius.
 - TEM is a great way to calibrate tip radius, especially for small tips, however requires special probe holders.
 - Tip reconstruction
 - Input nominal tip radius
 - Engage nioprobe sample/standard using a scan area of 200 nm. Acquire several images, then calculate probe radius using algorithms in SPIP or Gwiddion to do 'blind tip reconstruction'.
 - Consider larger (30-50 nm) or beaded tips for force-distance curve acquisition in a more reliable way, and be sure to check how the tip radius and mechanical properties change over time.
- **Notes**
 - References and advice on calibration for mechanical measurements.²⁻⁵
 - For best results, clean AFM tips with UV ozone and perform mechanical measurements in argon or nitrogen atmospheres to minimize capillary forces
 - Consider storing tips and standard samples in a controlled humidity chamber or in a glove bag
 - Purchase new PS/LDPE QNM standards and use for ensuring measurements are conducted properly
 - JKR modulus models are best to use for mechanical measurements, rather than DMT modulus – all improvements upon Hertzian mechanical models

Recent review on AFM of polymer samples⁶

References

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