

MultiMode 8 SOP

PeakForce Tapping/ScanAsyst, Tapping, and Contact Mode Imaging

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Procedure

- 1) Turn on the MultiMode 8 and enable the active vibration isolation table (Herzan TS-140).
 - a) Turn on the MultiMode AFM using the power strip located at the bottom right rear of the instrument (Figure 1, below left).
 - b) Turn on the power to the Herzan TS-140 vibration isolation table by flipping the power switch to the on position as shown at below right in Figure 1. The switch will illuminate green.
 - c) Enable active vibration isolation by pushing the circle E button (Figure 1, below right). The red "Isolation On" indicator light will illuminate. **Do not lean or push on the vibration isolation table once active isolation is enabled.**

WARNING: Do not power cycle the computer with the AFM and Nanoscope V controller turned on, as the resulting voltage spike could cause severe damage to the piezos.

Note: Allow laser to warm up for ~30 minutes prior to imaging if your experiment requires very precise measurements.

MultiMode 8 Power Strip



Vibration Isolation Table Controls



Figure 1. Power strip for Multimode (left) and controls for Herzan active vibration isolation table (right).

- 2) Double click the "Sign In" icon on the computer desktop; sign in on the login spreadsheet.

- 3) Open the AFM control software by double clicking the Nanoscope V8.15 icon (Figure 2).



Figure 2. Bruker Nanoscope AFM control software icon.

- 4) Select the appropriate Experiment Category, Experiment Group, and Experiment based on the AFM mode best suited to your sample and desired information, then click “Load Experiment” (see Figure 3).

Note: If you select the ScanAsyst Experiment Category, you will be asked to choose between ScanAsyst and ScanAsyst-HR. ScanAsyst-HR acquires force curves at a sampling rate of up to 8 kHz (versus 2 kHz for standard ScanAsyst mode) in tip drive (as opposed to sample drive) mode. As a result, it *requires* use of the HR (“High Rate”) probe holder pictured later in this SOP. ScanAsyst-HR enables acquisition of high resolution images at a faster scan rate than ScanAsyst, but the ScanAsyst-HR probe holder is not compatible with fluid imaging. In addition, while it is possible to toggle between ScanAsyst-HR and PeakForce QNM (for nanomechanical mapping), the latter can only be carried out at the slower 2 kHz sampling rate utilizing sample drive. Normal ScanAsyst can be carried out using *either* the ScanAsyst-HR probe holder or the normal ScanAsyst probe holder pictured later in this SOP, as well as with the fluid cell.

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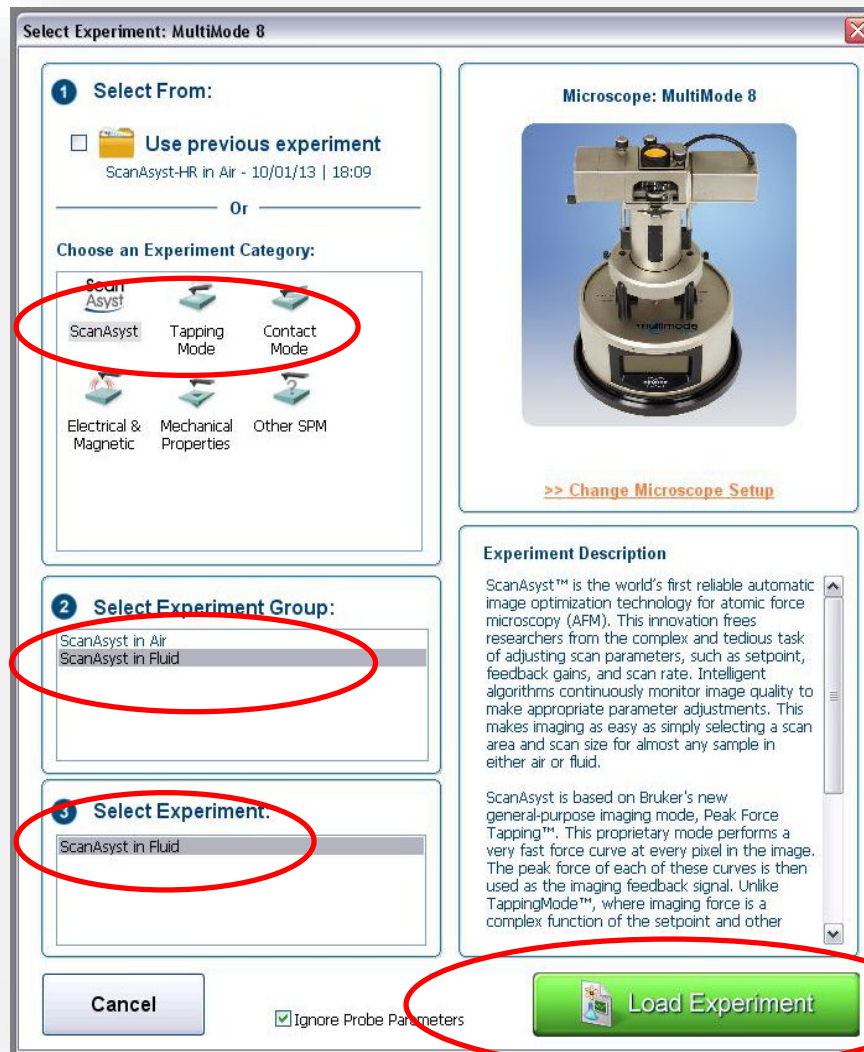
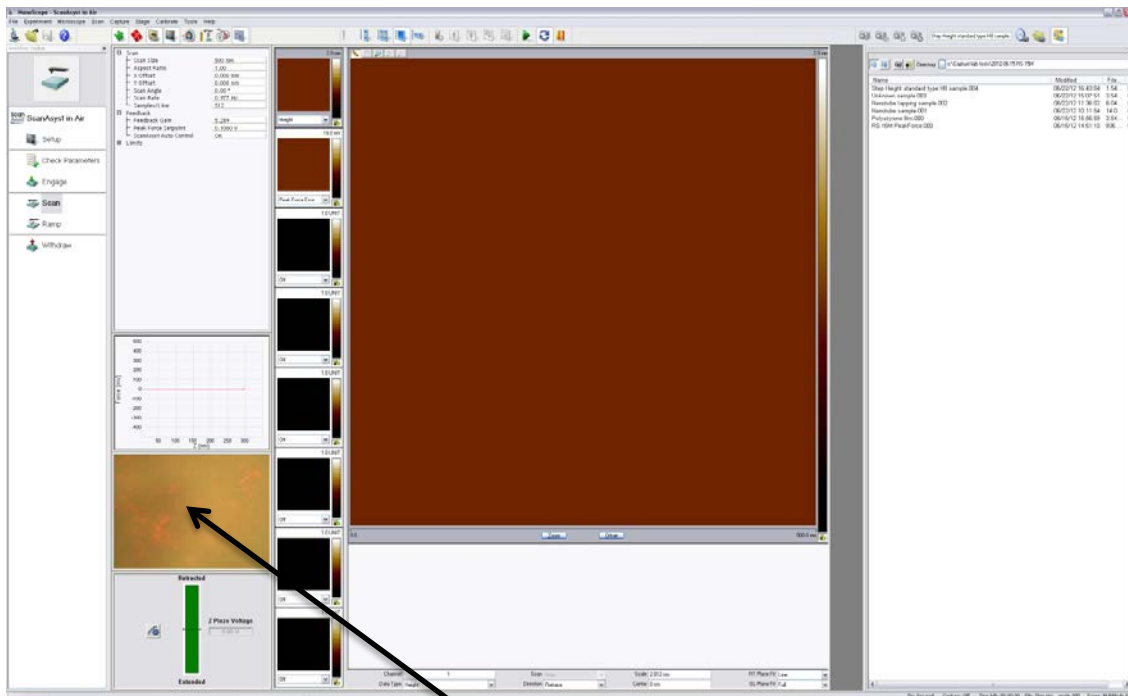
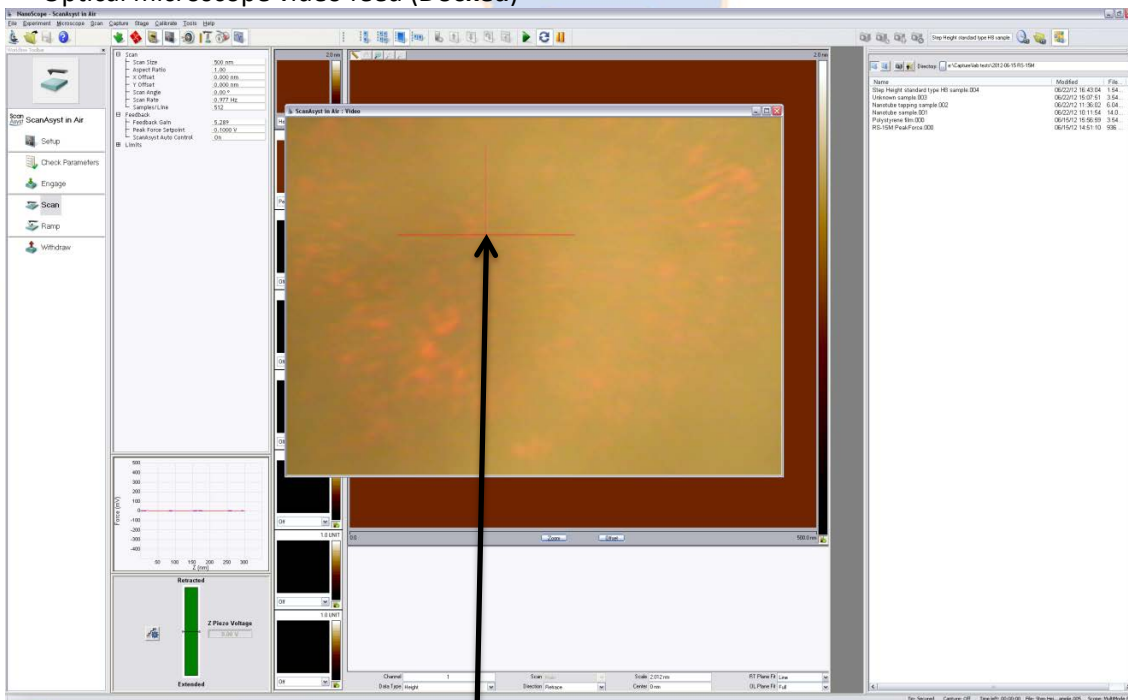


Figure 3. Initial Nanoscope software interface. Select the desired Experiment Category, Experiment Group, and Experiment, then click “Load Experiment” (green button circled in red).

- 5) Right click on the optical microscope video feed window and select “Dock/Undock” to view the video as a larger screen to aid alignment of the laser on the AFM probe (see Figure 4).



Optical microscope video feed (Docked)



Optical microscope video feed (Undocked)

Figure 4. Optical microscope video feed window in the Nanoscope AFM control software, docked (upper image) and undocked (lower image).

- 6) Choose the appropriate probe for imaging your sample based on the AFM mode you selected. (Consult the probe selection guides found in the probe locker cabinet if necessary.) Examples of probes commonly used with the Multimode are shown in Figure 5.

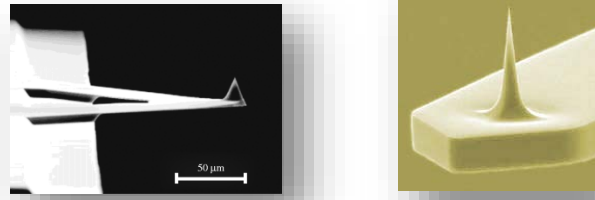


Figure 5. SEM images of ScanAsyst-Air (left) and ScanAsyst-Air-HR (right) probes.

- 7) Select the appropriate probe holder (ScanAsyst or ScanAsyst-HR, see Figure 6 below) and load the selected probe.

WARNING: Located within each probe holder is a drive/dither piezo. In addition, the HR holder contains a Z piezo for carrying out high frequency (8 kHz) force curves. These piezos are extremely fragile and very expensive to replace. Do not drop, bang, compress, or wet the probe holder, as these actions could damage the piezos and necessitate replacement.

For ScanAsyst holders:

- Turn the probe holder upside down with the groove and spring clip facing up as shown in the left hand image of Figure 6.
- Gently press downward to apply pressure against the plunger on the opposite surface (middle image of Figure 6), thereby lifting the spring clip.*
- With the spring clip lifted, carefully slide the probe into the probe holder's groove until the probe is located parallel to the groove edges.
- Lower the spring clip by gradually releasing pressure against the plunger. This will ensure the probe is held securely in the probe holder's groove.*
- Use the optical microscope on the table along the west wall of the SSL to ensure the probe is parallel with the sides of the holder and not touching the rear. Gently manipulate the probe with a pair of tweezers as necessary to achieve optimal alignment in the probe holder.

***Note:** For ScanAsyst-HR probe holders, the clip that holds the probe is locked/released by a lever.

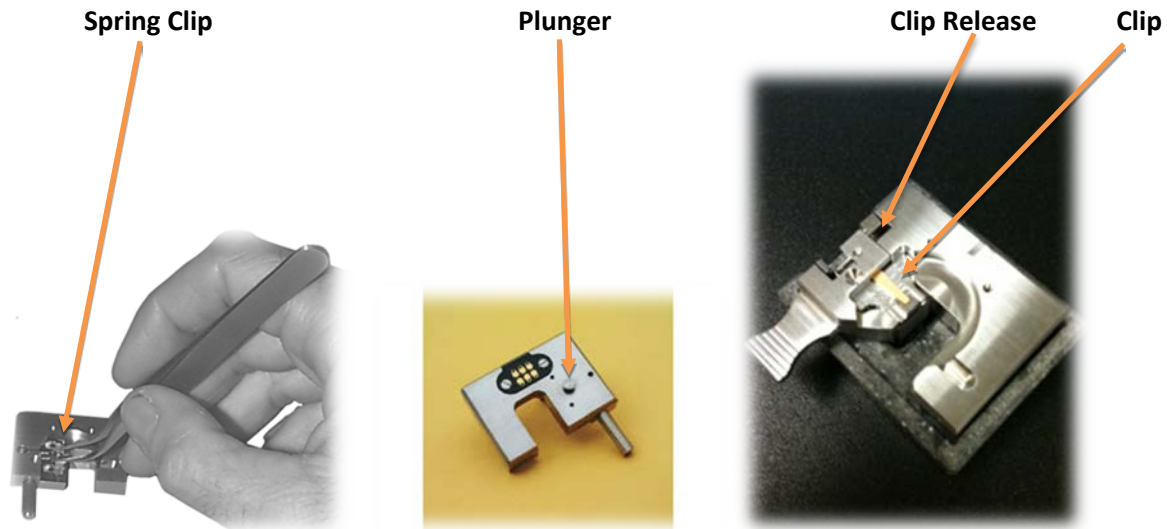


Figure 6. ScanAsyst (left and middle images) and ScanAsyst-HR (right image) probe holders.

- 8) Load the metal sample puck onto the scanner tube (see Figure 7). An internal magnet will secure the sample to the scanner tube. **Do not lean or push on the vibration isolation table while doing this!** If the red “Isolation On” light starts blinking (indicating the active vibration isolation is attempting to compensate for a disturbance), wait for it to stop blinking before continuing.

Note: Due to its use of a magnetic stage rather than a vacuum chuck, all samples for imaging on the MultiMode must be mounted on a metal puck to properly secure the sample to the stage.

WARNING: Prior to inserting the probe holder in the MultiMode, use the stage height adjustment switch (“Tip Up”) to move the stage and sample below the top of the ball pillars that support the probe holder. This will ensure that the probe cantilever and tip are not damaged prior to engaging the sample surface.

Note: Because the tip is in fact stationary, “Tip Up” actually lowers the sample.

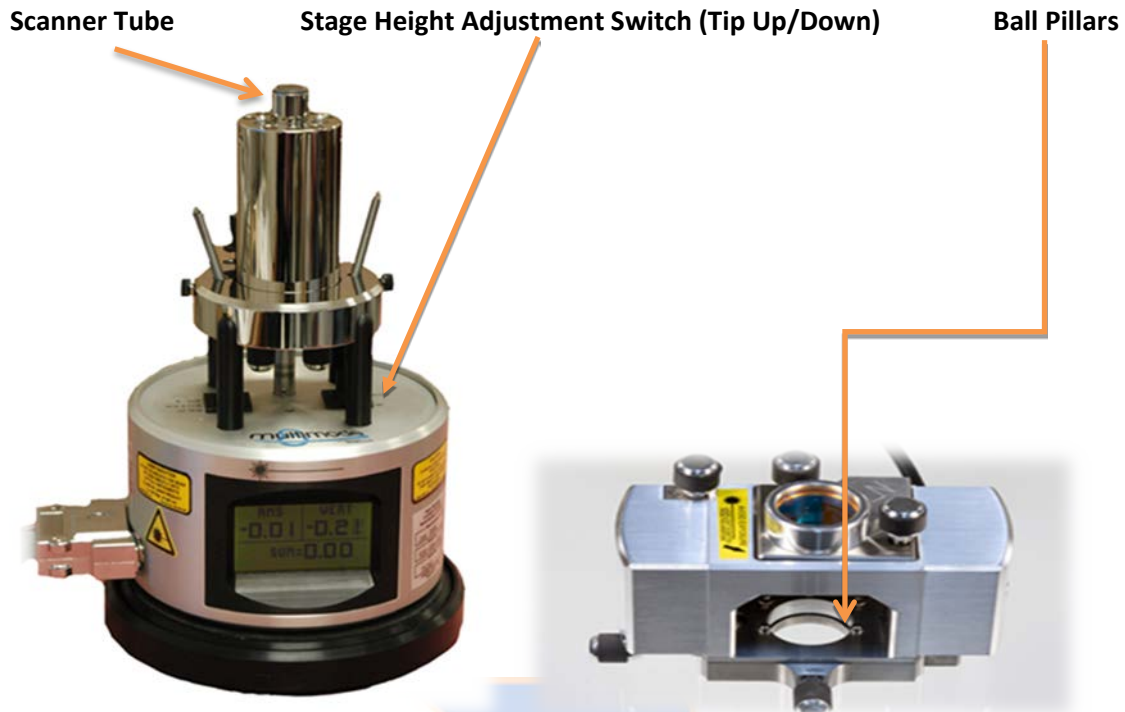


Figure 7. MultiMode scanner tube and stage height adjustment switch (left). Sample chamber with ball pillars (right).

- 9) Load the probe holder and probe in the Multimode (Figure 8, left panel) and hand tighten the set screw on the center back of the MM8 head to secure the probe holder in place (Figure 8, right panel).

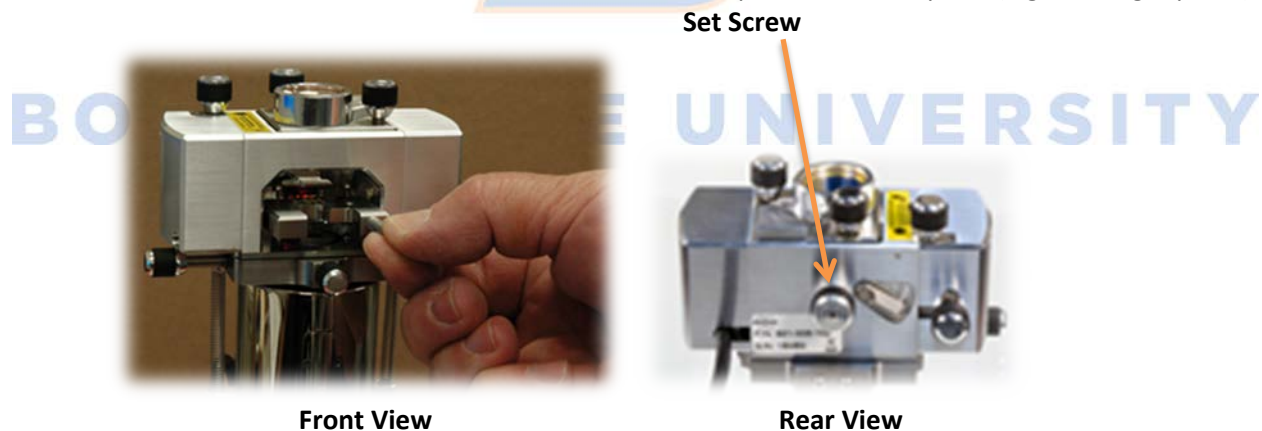


Figure 8. MultiMode head showing probe holder installation (left) and probe holder set screw (right).

- 10) Now that the probe and probe holder are in place, gradually lower the probe tip toward the sample surface using the up/down toggle switch (“Tip Down”, Figure 7).

Note: To properly align the laser on the probe cantilever, you must first bring the tip and cantilever within ~ 1 mm of the sample surface. Directions to achieve this are below.

WARNING: Make sure to approach the surface slowly to ensure you don’t crash the tip, potentially damaging the probe, sample, and/or piezos (very expensive and delicate).

- a) Focus on the sample surface using the microscope focus adjustment knob (Figure 9). It may be helpful to find a crack in the mica surface to focus on; otherwise you may mistakenly focus on the underlying steel puck and crash the probe. Once the surface appears focused, back off slightly until the surface just begins to appear blurry.

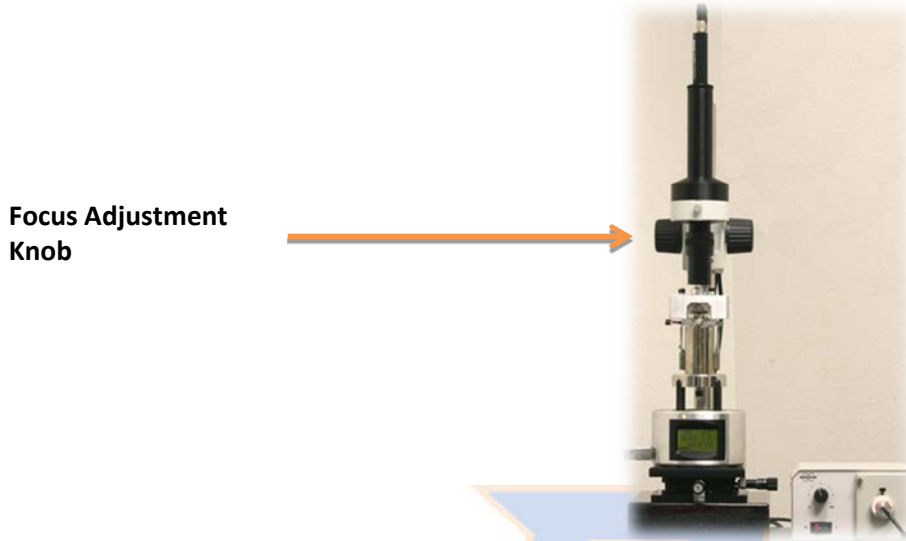


Figure 9. MultiMode microscope with focus adjustment knob labeled.

- b) Use the Tip Up/Down switch (stage height adjustment switch, Figure 7 above) to **slowly** bring the tip down toward the surface until the tip and cantilever appear in focus in the optics video feed. (Note that because the tip is in fact stationary, “Tip Down” actually raises the sample up towards the tip.)
- c) The tip is very close to the surface now and it is possible to focus on the sample surface. As mentioned above, it is very useful to find a defect or crack on the sample surface so as not to accidentally focus on the mica beneath a very clear sample. If both the probe cantilever and its reflection are visible and slightly overlapping as shown in Figure 10 below, the tip is within ~ 1 nm of the surface. If not, repeat steps a and b.

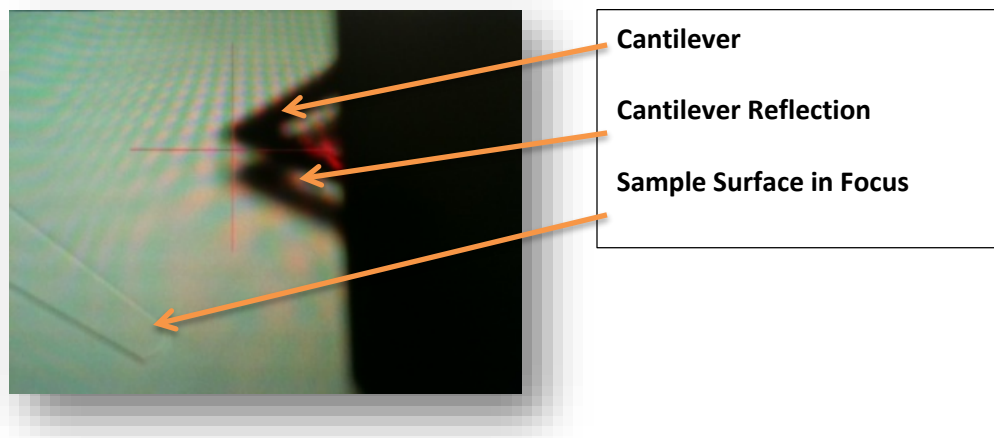


Figure 10. Optics focused on sample surface, with cantilever image and its reflection both visible and overlapping.

- 11) Now that the surface is in focus and the probe is $\sim 1\text{mm}$ away from the sample surface, align the laser onto the probe cantilever and center it on the quad detector (position sensitive photodiode).
- Bring the probe cantilever into focus on the enlarged optics video feed screen by adjusting the microscope optics adjustment knob (Figure 9).
 - Using the X and Y axis adjustments (Figure 12), bring the laser into view on the enlarged optics video feed screen (Figure 4). In very rare cases it may become necessary to manipulate the micrometers located at the base of the AFM to re-center the laser and AFM/sample in the microscope optics.

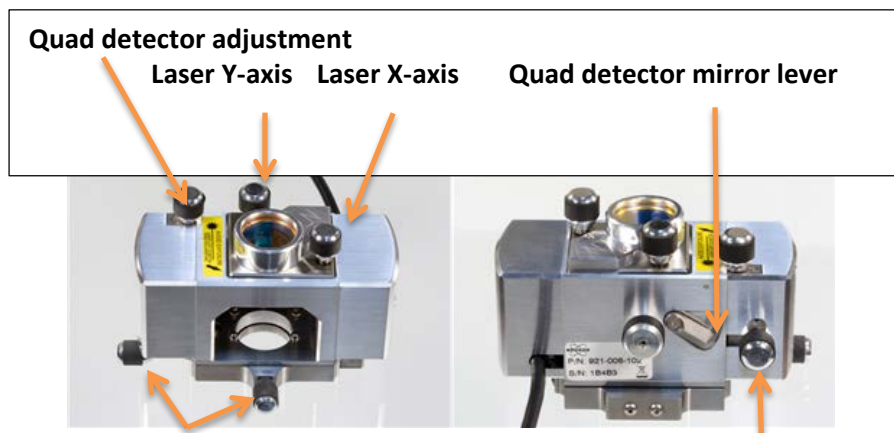
Note: Before making any adjustments to the micrometers, take care to note their current settings so that the stage can be brought back to the original location if necessary.

- Based on the optics video feed, visually align the red laser spot on the cantilever and note the photodiode SUM displayed on the base of the MultiMode (Figure 11). An acceptable value for the SUM is $\sim 8.0\text{-}9.0\text{V}$.



Figure 11. Quad detector (position sensitive photodiode) output display.

- Continue adjusting the laser X and Y axes until the maximum SUM voltage is obtained. Check that the laser is indeed at the end of the cantilever by adjusting the X and Y axes. If the SUM drops off rapidly in all directions, the laser is at the end of the cantilever over the tip. Take care not to align the laser on the cantilever substrate as this will cause an inaccurate SUM and may potentially cause the probe to crash.
- Adjust the quad detector knobs until the "VERT" and "HORZ" displays read zero within $\pm 0.01\text{ V}$.
- Once the VERT and HORZ outputs read approximately zero, verify the SUM is still near its max value. If the SUM has decreased significantly, repeat steps d and e as needed.



**Stage Adjustment (X & Y)
(Front top view) 2nd Quad detector adjustment
(Rear view)**

Figure 12. Sample stage position, laser beam steering, and quad detector alignment adjustments knobs and levers.

Note: Moving the stage position via the marked/indexed micrometers at the base of the AFM moves the probe relative to the microscope optics. In contrast, the micrometers at the base of the stage move the sample relative to the probe. You may need to adjust both the stage position and the micrometers together (not while engaged) at the same time to image new areas of the sample.

12) Check Engage Parameters:

ScanAsyst or ScanAsyst-HR (Peak Force Tapping in Air)

WARNING: Before selecting the Engage icon, review the following parameters to ensure the tip and sample will not be damaged.

Parameter	Initial Value
Scan Size	0 μm
Scan Rate	1 Hz
X Offset	0
Y Offset	0
Sample/Line	512
ScanAsyst Auto Control	ON
Feedback Gain	Auto
Engage Setpoint	0.1500 V

- a) Scan Asyst Auto Control will attune the Feedback gain and Setpoint for the user dynamically and automatically. Leave ScanAsyst Auto Control **ON** while engaging so that the system can safely engage.
- i) Select ScanAsyst Auto Control **OFF** to manually control the peakforce setpoint and feedback gains
 - ii) Select ScanAsyst Auto Control **Individual** to allow ScanAsyst to control only certain user specified parameters.
- b) Select “Microscope” on the tool bar in the upper left corner of the screen. Scroll to “Engage Settings”. Ensure settings match the parameters listed below:

Note: The PeakForce Engage Setpoint should not exceed 0.20V. Higher setpoints will engage harder than lower setpoints.

Parameter	Initial Value
Sew Tip	Yes
Peak Force Engage Setpoint	0.10 V
Engage int. Gain	40.0

Note: Tuning the cantilever is *not* required for Peak Force Tapping. After ensuring all parameters are correct, proceed to step 14.

Contact Mode

WARNING: Before selecting the engage icon, review the following parameters to ensure the tip and sample are not damaged.

Parameter	Initial Value
Scan Size	0 μm
Scan Rate	2 Hz
X Offset	0
Y Offset	0
Sample/Line	256
Integral Gain	25
Proportional Gain	50
Deflection Set Point	2.0 V

- c) Select “Microscope” on the tool bar in the upper left corner of the screen. Scroll to “Engage Settings”.

Parameter	Initial Value
Sew Tip	Yes

Note: Tuning the cantilever is *not* required for contact mode. After ensuring all parameters are correct, proceed to step 14.

Tapping Mode

WARNING: Before selecting the engage icon, review the following parameters to ensure the tip and sample will not be damaged.

Parameter	Initial Value
Scan Size	0 μm
Scan Rate	1 Hz
X Offset	0
Y Offset	0
Sample/Line	256
Integral Gain	1
Proportional Gain	5
Amplitude Set Point	150.0 mV
Drive Frequency	320 kHz

- d) Select “Microscope” on the tool bar in the upper left corner of the screen. Scroll to “Engage Settings”.

Parameter	Initial Value
Sew Tip	Yes

Note: Tuning the cantilever is **required** for Tapping Mode. After ensuring all parameters are correct, proceed to step 13.

13) Tuning the Probe (see Figure 13 below): **Tapping Mode ONLY**

- a) Click the “Tune” icon on the tool bar located at the top of the screen.



- b) Set the “Start Frequency” to the low end of the recommended frequency range for the selected probe (located on the tip box).
- c) Set the “End Frequency” to the high end of the recommended frequency range for the selected probe (located on the tip box).
- d) Click the “Auto Tune” icon at the bottom of the screen. The probe will now be automatically tuned. The resulting resonant peak should be a single, relatively narrow Gaussian (see below in Figure 13), but may be somewhat asymmetric.
- e) Use the offset under ‘Cursor Mode’ to offset the frequency to the left of the natural resonance frequency to ensure the AFM engages in the repulsive regime (similar to PeakForce Tapping). Once the green cursor is placed as shown in Figure 13, click Execute.
- f) Increase the Amplitude Setpoint (pink line) to the position shown in Figure 13.

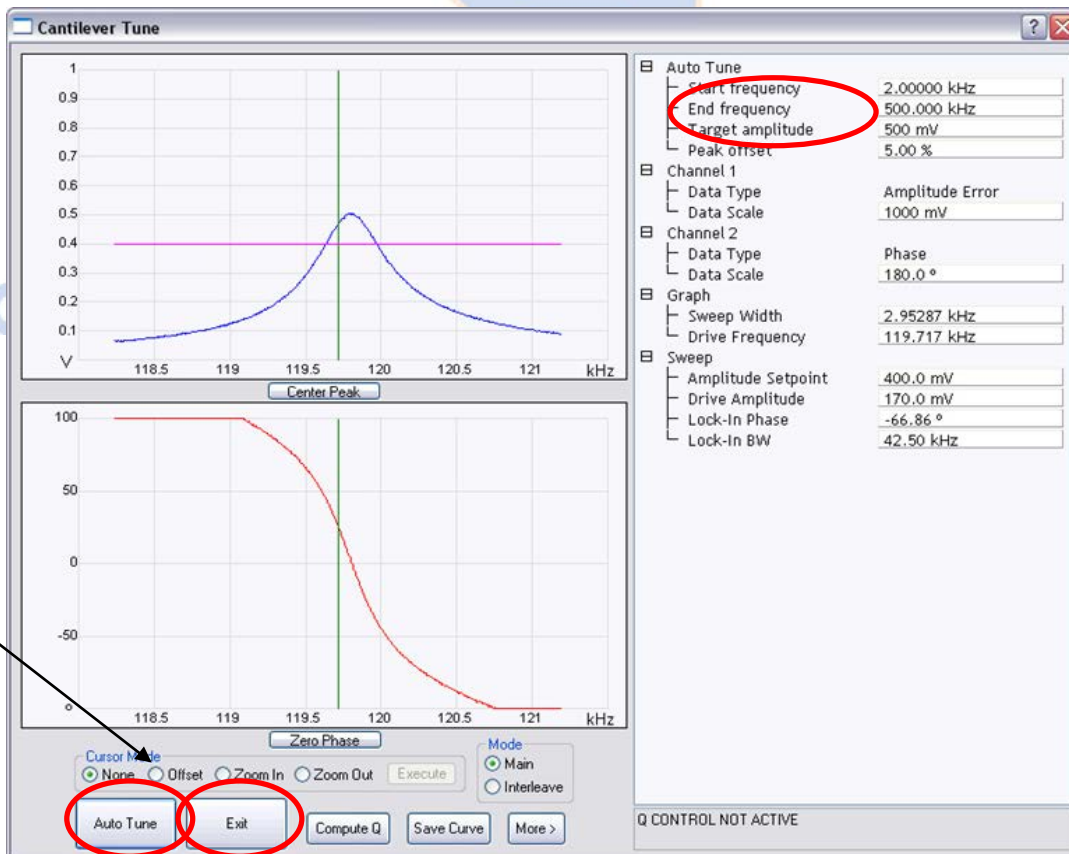


Figure 13. Cantilever Tune screen. Ideal tuning curve amplitude (Gaussian at top) and phase (lower sigmoidal curve) plots with Start and End frequencies, Auto Tune, and Exit circled in red.

g) Once tuning is complete, click the “Exit” button located at the bottom of the page. The tuning screen will close and open the Tapping Mode Configuration Screen.

14) Select “**ENGAGE**” on the Work Flow column (see Figure 14 below).

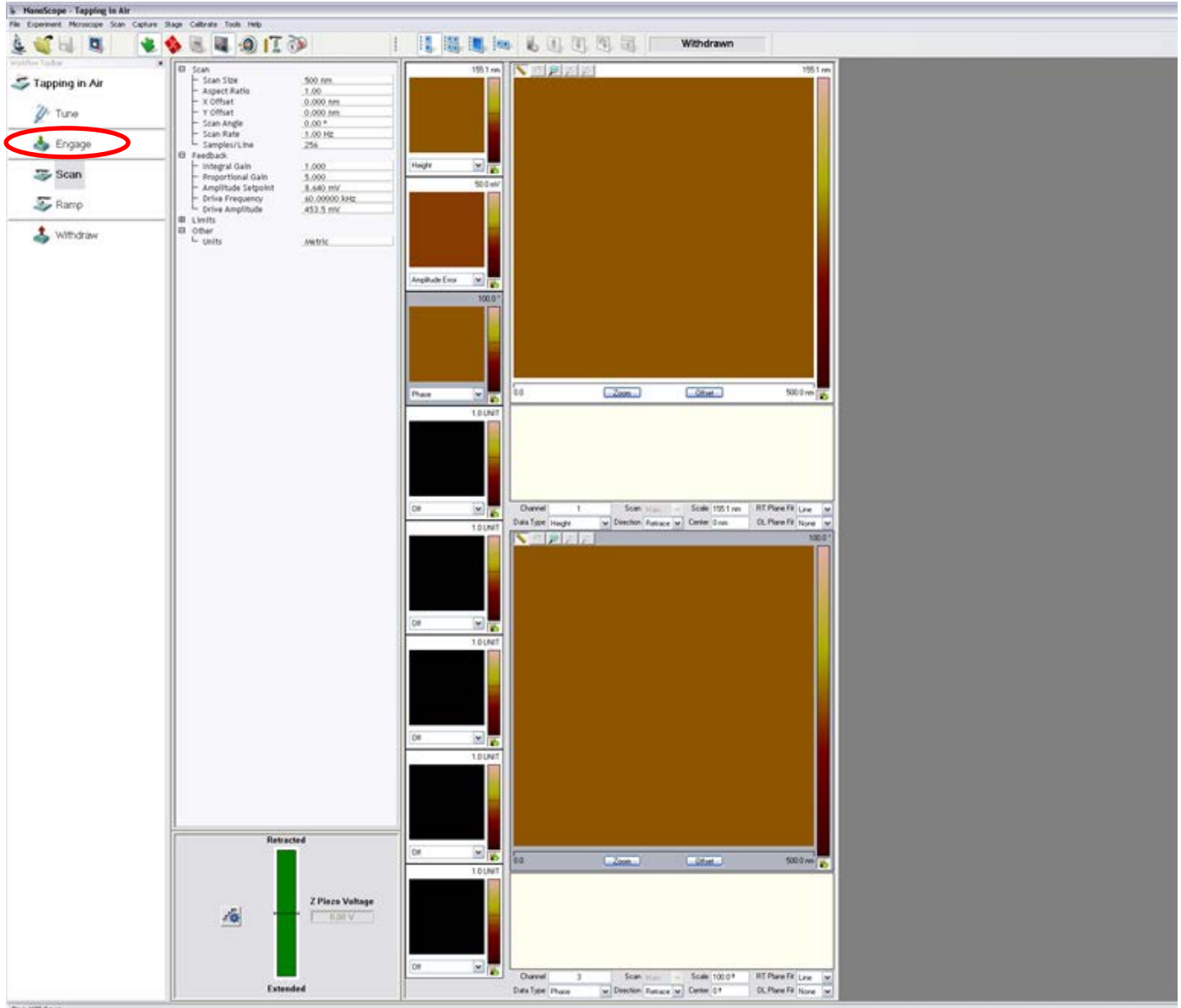


Figure 14. Work Flow page with Engage circled in red.

15) Improving Image Quality

a) PeakForce Setpoint

- The PeakForce Setpoint determines the maximum amount of force that the tip may impart/exert before retracting from the surface.
- The applied force is calculated using the vertical deflection voltage measured on the quad detector and is displayed in mV. This deflection can be converted to a force (in nN) using the relationship $F=-kx$, where k is the spring constant of the cantilever (found on the probe box or determined via a thermal tune), and x is the amount of distance the cantilever flexes (calculated based on the deflection sensitivity of the probe in question).
- A good starting value is ~ 0.02 V (20 mV equates to just under 1 nN of force for ScanAsyst-Air probes).
- This value can be increased if the tip is not tracking well, but should be used as one of the last resorts to improve the image/tracking, as higher forces are more likely to dull the tip and/or deform or damage the sample, resulting in inaccurate measurements on soft samples.

b) Feedback Gain

- The Feedback Gain is used to adjust how much the probe responds to changes in topography (the higher the gain, the greater the adjustment to a given change in sample height).
- As the Feedback Gain is increased, amplification of any electrical noise grows, but tracking of the sample surface also improves. Eventually the added noise outweighs the improvement in tracking. Noise and tracking quality can be assessed using the amplitude error channel.
- Increase the Feedback Gain incrementally (~ 5 -10 units at a time) until the noise grows substantially. Once this occurs, back off the Feedback Gain by one two increments (e.g., if the noise increases greatly at a Feedback Gain of 50, then drop the Feedback Gain to 40-45).
- Typical values for the Feedback Gain are 20-60.

c) LP Deflection BW

- The LP Deflection BW minimizes the high frequency noise (ringing) visible on the force curve.
- The lower the LP Deflection BW, the more frequencies are filtered and the lower the noise. This noise can be observed at the beginning of the force curve, prior to the probe making contact with the surface.
- A good value to start with is ~ 40 kHz. Try decreasing and increasing in increments of ~ 5 kHz until the noise is minimized without affecting the shape of the force curve during the time it is in contact with the sample (since this is used to calculate the sample's nanomechanical properties).

d) PeakForce Amplitude

- PeakForce Amplitude measures the midpoint height as the tip lifts off the surface during each force curve across the surface of the sample.
- The best value will vary based on the roughness of the sample surface and the amount of tip-sample adhesion. If the surface is rather smooth (e.g., DNA on mica), the PeakForce Amplitude can be < 10 nm, although you will likely be limited by the amount of tip-sample adhesion present. If the surface is rougher, such as an unpolished metal, the PeakForce Amplitude should be greater.

- A good rule of thumb is that the PeakForce Amplitude should be greater than the lowest valley or highest peak present so that the tip won't crash when encountering a large feature on the sample.
 - A good value to start with for a known rough surface would be ~100 nm. A good value to start with for a known smooth surface would be ~30 nm.
- e) PeakForce Frequency
- The PeakForce Frequency is the number of force curves acquired per second.
 - A higher rate can enable faster scanning and/or enhanced signal to noise, but at too high a frequency (relative to the probe's natural resonant frequency), the force curve will deteriorate due to excessive ringing. This is particularly true in fluid due to the increased viscosity relative to air of the medium through which the probe is traveling.
 - The choices available on the Multimode are 2 and 8 kHz (the latter only when using the special HR probe holder).
- f) Z limit
- The Z limit caps the range of motion of the Z piezo. Since the analog to digital converter has a fixed number of bits, the Z limit can be used to control the resolution of the height measurement: the lower the Z limit, the higher the height resolution.
 - However, be sure the Z limit is large enough so that all features on the sample can be imaged without damaging the Z piezo.
 - There are numerous parameters that can impact your choice of Z limit: surface roughness, sample tip and tilt, PeakForce Amplitude, and amount of thermal drift.
 - Surface roughness – the smoother the surface, the lower the Z limit can be. The rougher the surface, the higher the Z limit needs to be.
 - Sample tip and tilt – the sample is never perfectly perpendicular to the probe. The tip and tilt of the sample can be observed by changing the *RT Plane Fit* to *None* on the height channel. The change in height across the sample can be observed now by scanning up from the bottom and down from the top. This will typically scale with scan size.
 - PeakForce Amplitude – the lower the PF Amplitude, the lower the Z limit can be.
 - Thermal Drift – the more the piezo temperature changes as the electronics heat up over time (especially with the acoustic enclosure closed), the more the Z limit will need to be increased to compensate. Thermal drift will scale with scan time (and hence scan size and tip velocity). Although there will be more thermal drift if the acoustic enclosure surrounding the AFM is closed, if the door is open, acoustic noise appearing in the data may become an issue. This tradeoff should be thought through prior to engaging the surface and imaging.
 - A good value to start with for a small area scan on a smooth sample is 1 μm , with 500 nm being the minimum value recommended.
- g) Deflection Limit
- Use this parameter to change the maximum allowable deflection signal.
 - Lowering the deflection limit allows users to achieve higher resolution but when the deflection limit is too small, saturation of the deflection channel will occur.

16) Capture image(s).

- a) Ensure the image is saved in the proper file directory by clicking on the “Capture” tab. After the tab expands, click “Capture Filename”. In the dialog box, select the directory location where the images should be saved (Figure 15).



Figure 15. Tool Bar with the Capture Tab.

- b) Camera Icons and File Name (Figure 16, left to right):

- Capture: Will capture the next complete image, provided the scan parameters are not changed during the acquisition.
- Capture Continuous: Will sequentially capture each complete image acquired until directed by the user to stop doing so.
- Capture Now: Will capture the current image regardless of whether or not scan parameters were changed midway through (i.e., during) image acquisition.
- Cancel Capture: Will cancel the currently selected capture image function.
- Capture Withdraw: Will capture the next complete image and then withdraw the probe once complete. This feature is useful if you will not be in the lab when the scan finishes.
- File Name: Image filename for saving in the selected directory. By default, a counter of format “*.###” (i.e., *.000, *.001, etc.) will be appended to the filename; this will count the saved images.

Note: Always press the enter key after naming the image or the image name may revert back to the previous image name.



Figure 16. Capture Icons (Capture, Capture Continuous, Capture Now, Cancel Capture) and File Name

c) Line Scan Tool Bar (Figure 17).

- Arrow up will begin the scan at the bottom and move upward toward the top of the image area.
- Arrow down will begin the scan at the top of the image area and move downward.
- The arrow with the red line allows the user to designate a starting scan line and direction (up or down).



Figure 17. Scan Line Tool Bar

17) Withdraw tip and shut down the AFM.

- Once imaging is complete and all images have been captured and saved, select the Withdraw icon (work flow column) to stop imaging and retract the tip 50 μm from the sample.
- Move tip well away from the sample using Tip Up/Down switch ("Tip Up", Figure 7).
- Once tip is clear of sample, release the probe holder using the set screw at back (Figure 8) and remove the probe holder from the AFM. Remove the probe from the probe holder.
- Carefully remove the sample from the stage area.
- Turn off the power to the Herzan TS-140 vibration isolation table by flipping the power switch off.
- Turn the MultiMode power strip off.
- Close the Nanoscope software.
- Complete the log in sheet on the computer desktop with the correct hours, number of probes used, etc. Be sure to make careful note of any error messages or issues you encountered.

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