

Cypher ES

Basic Training - Tapping Mode AFM (AC mode)

Standard Operating Procedure



Surface Analysis Center
Department of Chemistry and Biochemistry
A Maryland Nanocenter Partner Lab
University of Maryland College Park
9/13/2022

The images and text used in this SOP were adapted from the Cypher SPM user guide and Applications guide Version 18, Revision: A-2438 provided by Asylum Research, an Oxford Instruments company

SOP

Getting Started

1. Log in to the computer using your university ID and password.
2. Locate the shortcut to the software on the desktop and double-click the icon to start the software.
3. Turn the laser key on the ARC2 controller to the ON position. The red LED labeled “laser” at the front of the Cypher enclosure should be illuminated. Note that the Igor software needs to be open for the red LED to be illuminated on the front of the Cypher.
4. Wait while the software initializes. (do not press any keys during the initialization period)

Loading the Cantilever and Sample

This section covers sample and cantilever loading, as well as the coarse approach of the cantilever tip toward the sample.

Raise the cantilever holder:

- Rotate the ‘Engage Control Knob’ on the Cypher *clockwise* and hold until the cantilever holder is far from the sample or is at its upper limit of travel.

1. **Warning:** Pay attention! If you turn the knob the wrong way (counterclockwise), you will *lower* the cantilever holder instead of raising it. When you lower the cantilever holder, you can crash the cantilever holder into the sample and cause serious damage to the scanner.



Open enclosure:

2.
 - Lift the door latch and open the enclosure door.



3.

Unlock scanner:

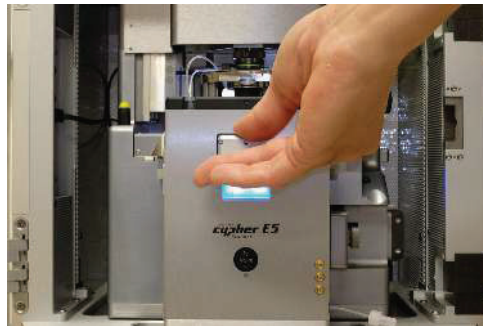
- Lift the lever located to the right of the scanner.



4.

Pull the scanner forward:

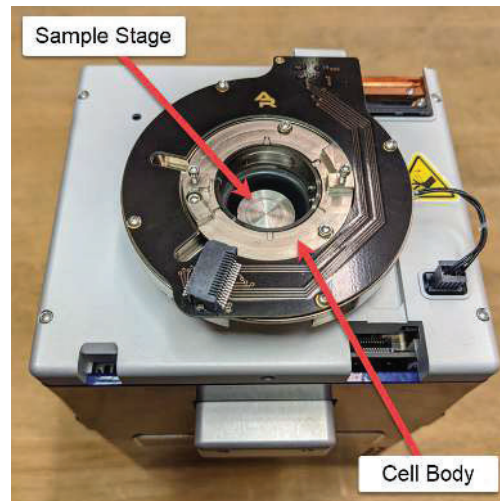
- Pull the scanner forward gently and stop when it is about halfway out. As you pull the scanner out, at some point you will feel resistance and should pull no farther.



5.

Familiarize yourself with the sample area:

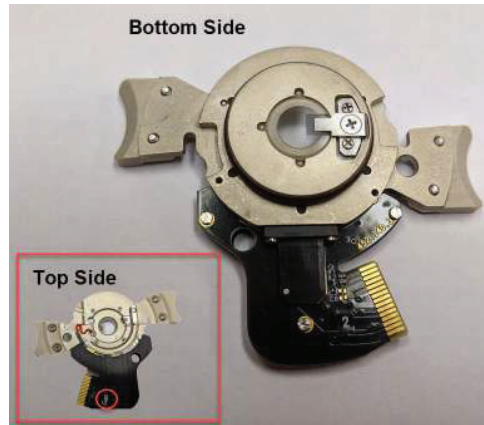
- While it may look solid, the scanner sample stage moves the sample in X, Y, and Z imperceptibly up to 40µm.



6.

Locate your cantilever holder:

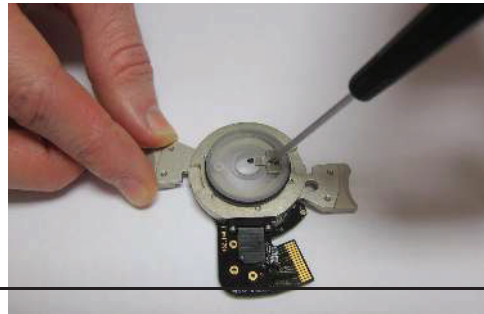
- Identify the appropriate cantilever holder. This tutorial requires the standard Gas cantilever holder.



7.

Loosen the cantilever clip:

- Unscrew the clamping screws by one quarter turn.
- Do not unthread the screw completely, this could result in damage to the cantilever holder. If you accidentally do, please refer to the chapter (8) in the user manual.



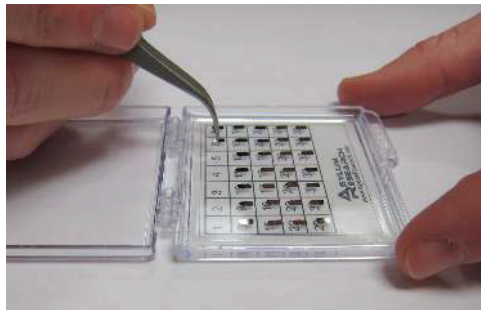
8.

Select new cantilever:

- Use tweezers to pick up the new cantilever.

Tip You may find it useful to first lay the chip down on a non-sticky surface and re-grip it before continuing.

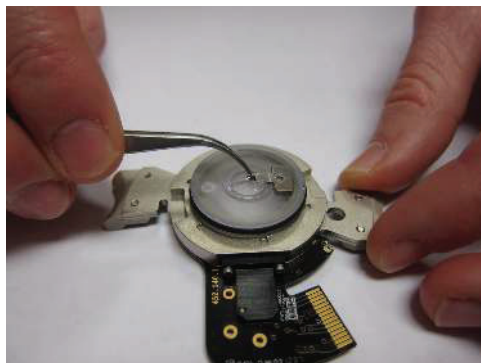
Practice with broken cantilevers at first



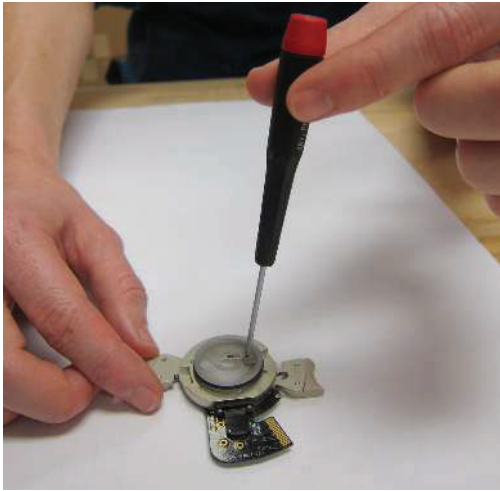
9.

Load new cantilever:

- Use tweezers to slide the new cantilever under the clip. You can “push” or “pull” the cantilever into place as you find comfortable.
- Position the cantilever so that the tip is approximately centered in the cantilever holder window.



10



Secure the new cantilever:

- Using *fingertip pressure only*, lightly tighten the screw securing the cantilever to the cantilever holder.
- The clip should be just tight enough to secure the cantilever chip when it is pushed from the side.

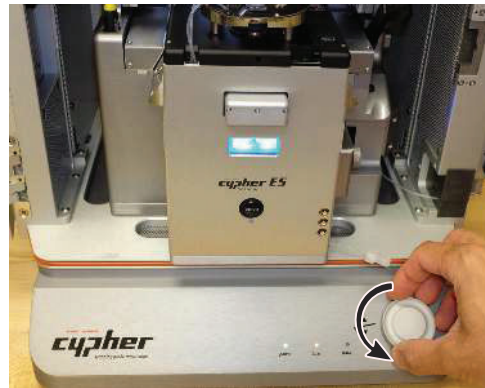
11.

Prepare scanner to load sample:

- Leave the cantilever holder on your workspace for now.

Note To load or unload a sample, you will need to lower the coarse engage stage so that you can access the sample stage.

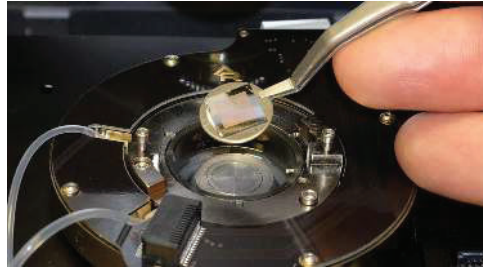
- Rotate the Engage Control Knob *counterclockwise* until it reaches its lower limit of travel.



12.

Load sample:

- Use “Platypus” tweezers to place new sample on sample stage.

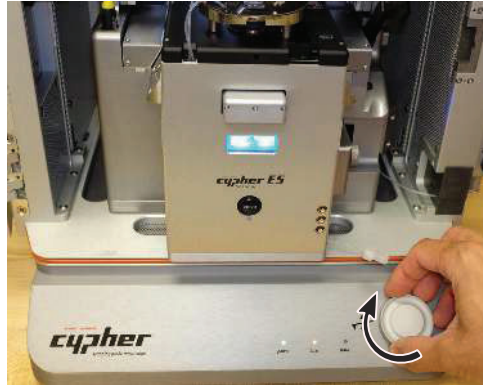


13.

Prepare scanner to load cantilever holder:

- RAISE THE COARSE ENGAGE STAGE by turning the Engage Control Knob *clockwise*. Raise the stage until it reaches its upper limit of travel.

Warning: Before loading the cantilever holder, raise the coarse engage stage. If you do not raise the coarse engage stage, you will crash the cantilever into the sample and ruin your cantilever, sample, and possibly even the cantilever holder!



14.

Place cantilever holder:

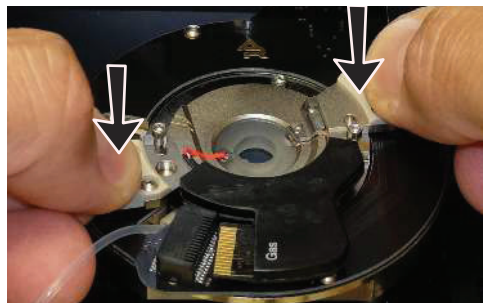
- Check to make sure that the coarse engage stage is raised to its highest position.
- Position the cantilever holder in the cell body, as shown in the image at right. The cantilever holder should sit level and be rotationally aligned so that the cantilever holder board is almost touching its mating connector.



15.

Press the cantilever holder down:

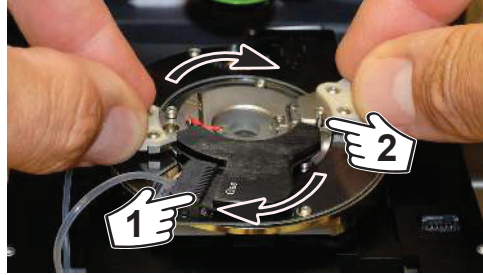
- Before starting this process, it is important that the cantilever holder is sitting level with respect to the top of the cell body.
- With fingers on both handles of the cantilever holder, use firm pressure to wiggle the cantilever down into the cell body. The O-ring will offer some resistance. When the cantilever holder is firmly seated, you will feel a hard stop as metal parts make contact.



16.

Rotate: Rotate the cantilever holder *clockwise* until you feel a hard stop.

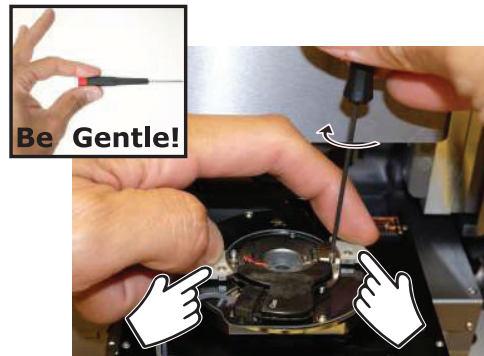
- 1 The cantilever holder contacts slip into the mating connector.
- 2 Notice the screws cause the hard stop as they slip into the matching metal cutouts.



17.

Tighten the screws:

- Place pressure on the cantilever holder handles as shown. Make sure it's firmly seated.
- Finger-tighten one of the screws and *stop as soon as you feel any resistance*.
- Do the same to the other screw.
- Release the pressure on the handles and tighten both screws a tiny bit more.
- At all times, only hold the screwdriver with your fingertips.



18.

Slide scanner into chassis:

- Slide the scanner back into the chassis. Use firm pressure until you feel a hard stop. Before you feel a hard stop you will feel a spring-like resistance. You are making thermal contact between the scanner and the chassis. This is necessary for best performance.
- Maintain pressure on scanner and press the lever at the right downward to lock the scanner into place.



19.

Check correction collar:

- Check that the green correction collar on the objective is set properly. For the Gas cantilever holder, set the correction collar to 1.5.



20.

Close enclosure door:

- Gently close the door and latch it.

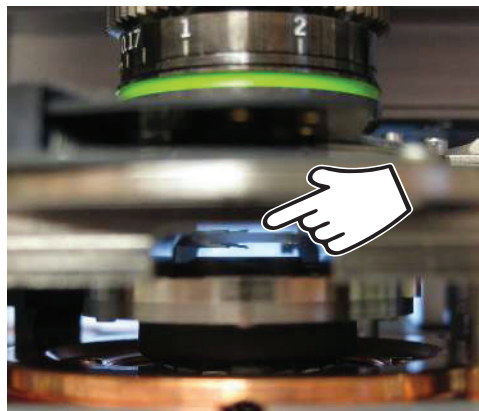


21.

Motor cantilever toward sample:

- Place your eyes level with the cantilever and sample, so you can clearly see the gap between cantilever and sample.
- Slowly turn the 'Engage Control Knob' on the AFM enclosure *counterclockwise*. This lowers the cantilever holder and objective toward the sample. The more you turn, the faster the stage moves.
- Close the gap between tip and sample to about 1 millimeter.

Warning: Nothing but your attentiveness will prevent the cantilever holder from crashing into the sample. If you crash the cantilever holder you may cause **serious** damage to your cantilever holder and scanner.




22. This concludes the manual interaction with Cypher. We next turn our attention to the computer.

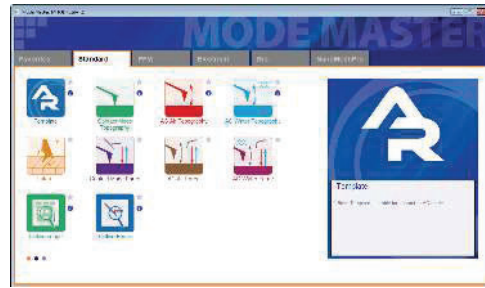
Engaging the Cantilever on the Sample

Bringing the Cantilever Close to the Sample

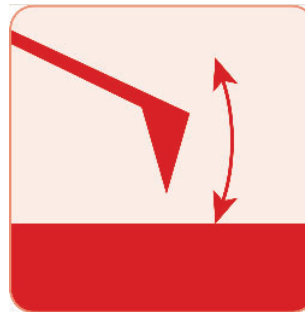
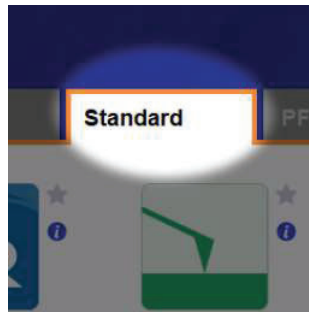
23.

The Mode Master window:

- The software should now be showing the Mode Master window.
- If not, click the Mode Master button at the bottom of the screen: .



24.



Select Mode:

- Select *Standard* tab > *AC Air Topography*
- The screen will now rearrange and present all the controls necessary for this type of AFM imaging.

25.

Setting video zoom and illumination:

- **IMPORTANT:** Slide the vertical slider (at the lower-left corner of the video window) all the way to the bottom. “Zoom 1.0” will be indicated just below.
- Illumination can be adjusted by moving the slider (at the bottom of the video window) to the right a quarter or third of its full range.



26. Familiarize yourself with the Approach tab on the Engage Panel as described next in Step .

Caution: Failure to understand the Approach controls may lead to serious damage to the Cypher.

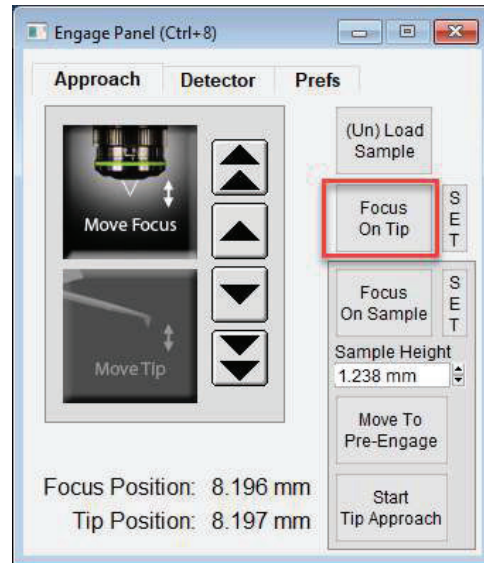
Go to last known Tip Focus:

- On the Engage panel, hit 'Focus on Tip'. This will move the objective lens to the last known position where the cantilever was in focus. *If you can see the cantilever proceed to step 29*

Notes

- Since the 'Focus on Tip' button only moves the objective to the *last known tip focus* and does not actually perform an auto-focus, the cantilever will most likely not be perfectly in focus after the motors are finished moving.
- Do not be alarmed if the cantilever is not at all visible. It most likely means that when you placed the cantilever chip in the holder, you put it in a place outside the ~1mm field of view of the objective.

27.



Locate cantilever in image (only if you did not locate the cantilever in the previous step):

- The goal of this step is just to get the cantilever into the field of view. Use the four arrows at the top left of the Video window to look for the edge of the cantilever chip and/or the cantilever. As mentioned in the previous step, most likely the cantilever will not be perfectly in focus.
- If you are oriented such that you are sitting directly in front of the Cypher microscope, hitting the left arrow will move the objective to your left, while hitting the top arrow will move the objective away from you.
- *If you see nothing at all in the field of view, most likely the cantilever chip is located to the left of the field of view.*
- Click the *left arrow* to move the objective towards the left and look for the cantilever chip edge.

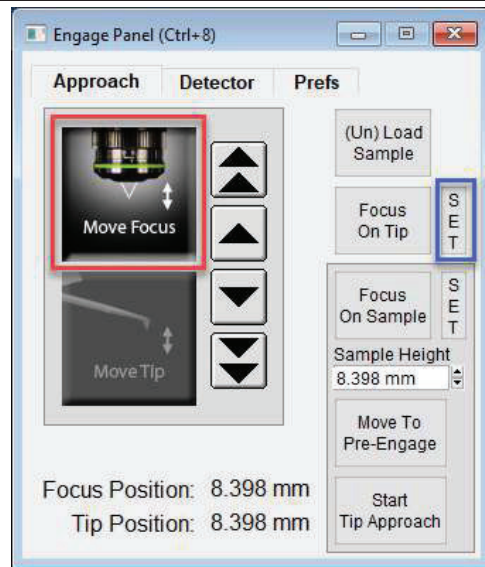
28.



29.

Set the tip focus position:

- Under the 'Approach' tab on the 'Engage Panel' select the 'Move Focus' large picture button (see red box at right).
- Use the on-screen arrow buttons until the end of the cantilever closest to the tip is in focus. Single arrows are slow; double arrows are fast.
- Click 'Set' next to 'Focus on Tip' button (see blue box at right).

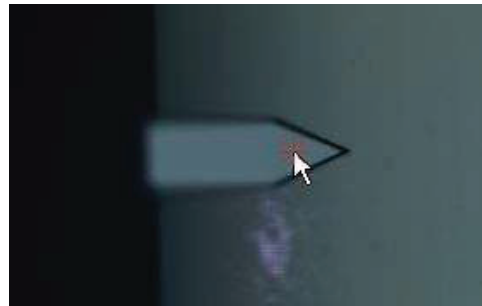


30.

Center laser spot on cantilever:

- Click the 'Spot On' button at the top left of the Video window. The mouse pointer acquires some small red lines.
- Then click the center of the cantilever (see figure at right).

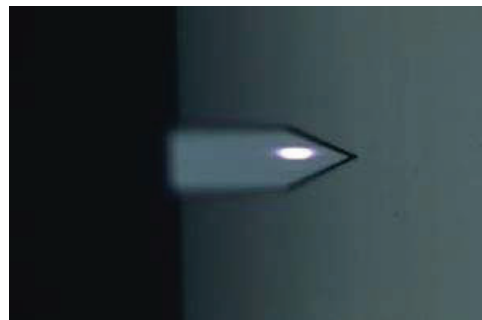
Note Alternately, right-click on the center of the cantilever and then select the 'Spot On' option.



31.

Observe spot on lever:

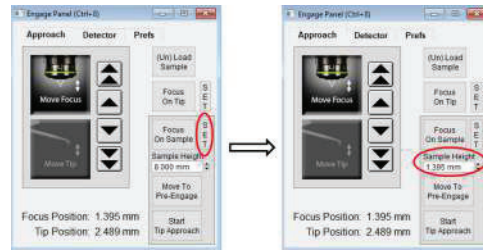
- The motors inside Cypher now move to bring the laser spot where you clicked.
- The spot position does not need to be perfect here, only roughly centered on the cantilever to produce a decent reflected beam (measured by the Sum and Deflection Panel).



32.

Locate sample surface optically:

- Under the 'Approach' tab on the 'Engage Panel' use the down-arrow keys to motor the microscope objective down toward the sample, until it comes in focus (see image in the next step). Use the up-arrows if you overshoot.
- Single arrows are slow, double arrows fast.
- Once in focus, click the 'SET' button next to the 'Focus on Sample' button. Note that the sample height value updates.

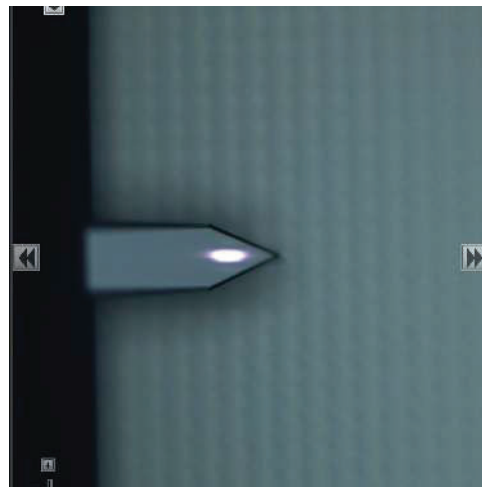


33.

Observe sample surface optically:

- Features may be hard to see at zoom = 1 you may need to use the zoom feature

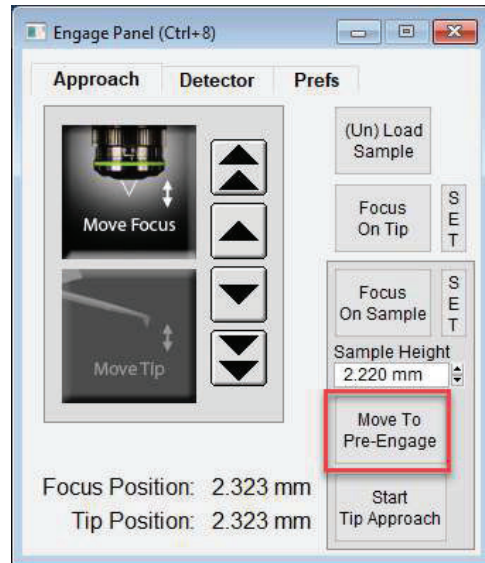
Fun Now that you have stored tip and sample position, you can repeatedly click the buttons 'Focus on Sample' and 'Focus on Tip' to go back and forth between tip and sample.



Prepare to land the tip:

- Click the 'Move to Pre-Engage' button. The motors automatically bring the tip to 50 μ m from the surface.

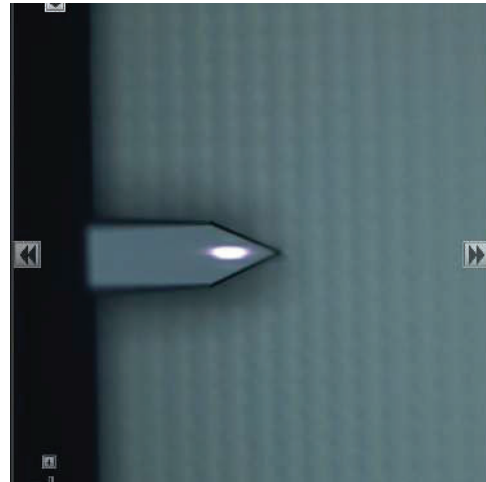
34. **WARNING** If you set a bad sample height and/or tip position, you may ram your cantilever into the sample and break it. A firmware safety feature immediately cuts motor power when the optical detector fails to measure reflected light from the broken lever. This prevents the cantilever holder from ramming the sample.



Fine tune spot position:

- At this point, you should adjust the spot position so that it is near the end of the cantilever using the controls in the upper left-hand corner of the Video panel.
- Note that a decrease in Sum Signal indicates that light is spilling off the cantilever. The latter is undesirable and should be avoided.

35. **HINT** For fine positioning, hold down the 'SHIFT' key on your keyboard while clicking the onscreen buttons which move the cantilever under the laser spot.



Center the laser on the photo detector:

- Click the red button at the center of the Video window motion controls and watch the Deflection Signal go to zero. This action causes small motors to steer the laser beam to the optimum position on Cypher's photo detector.

36.



Tuning the Cantilever and Setting Scan Parameters

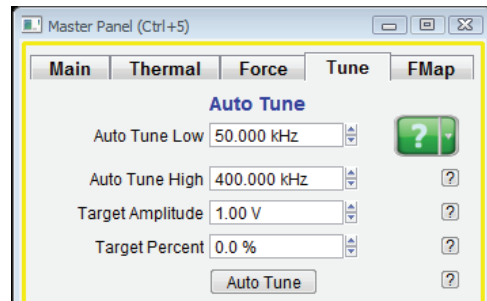
(assuming 1 V target amplitude with corresponding 800 mV set point)

Since this tutorial focuses on AC imaging, we will proceed to tune the cantilever.

37.

Initiate cantilever tune:

- Select the 'Tune' tab in the Master Panel.
- Set the four Auto Tune parameters (Low, High, Amplitude, Percent), as shown at right.
- Click the 'Auto Tune' button.

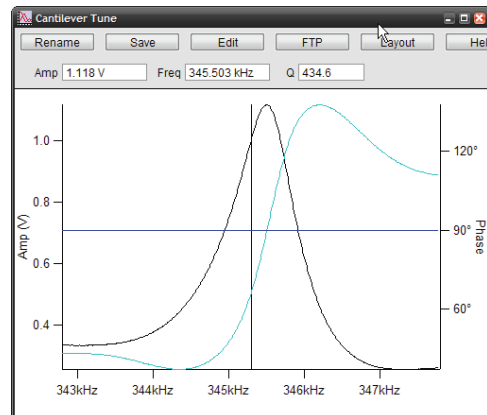


38.

Observe tune result:

- A graph will pop up with the tune result.
- The resonance curve should peak at around 300kHz.
- The relevant results are automatically stored. After inspecting that the amplitude and phase curves look "clean", you can close the graph.

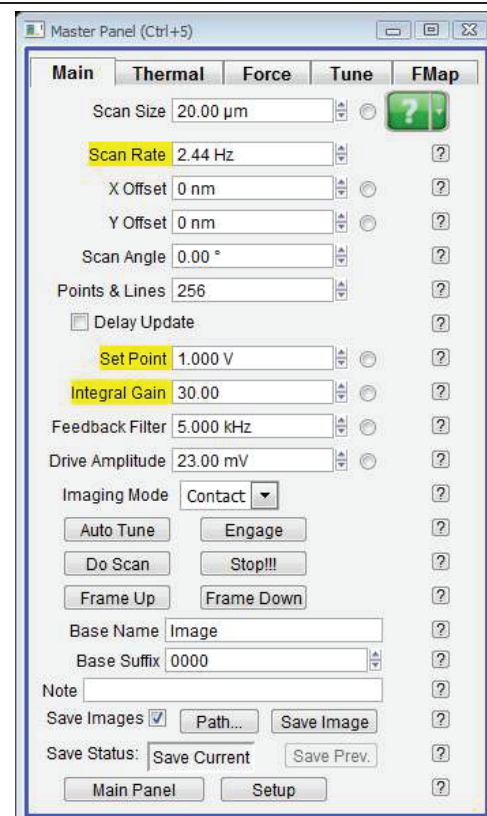
HINT Cleaner tunes can be obtained by blowing the cantilever holder with clean compressed air prior to loading cantilever to get rid of any leftover silicon/glass debris.

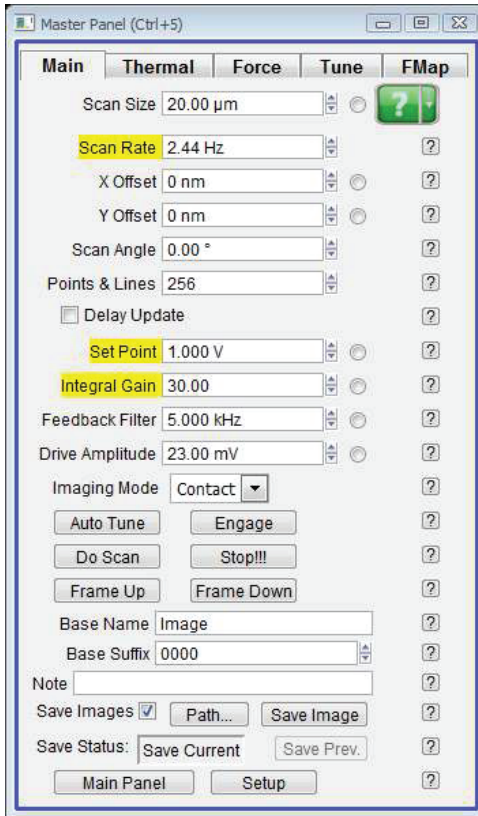


39.

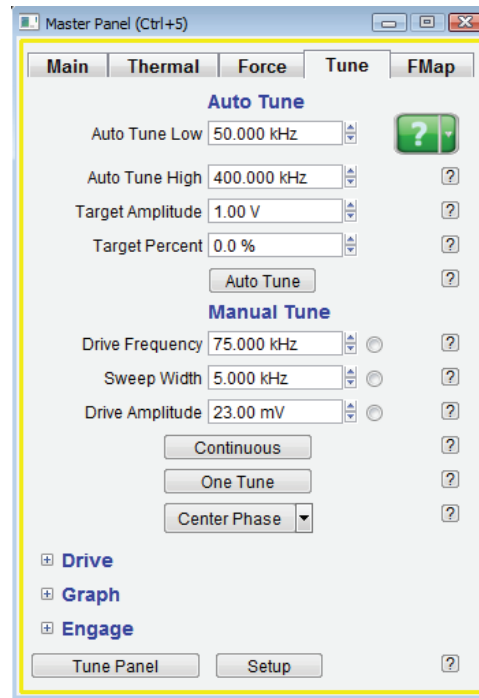
Set scan parameters:

- Select the 'Main' tab on the Master Panel .
- Set the Set Point value (the second item highlighted in yellow in Figure 7.4a on page 15) to 800mV .
- Make sure that all the other values and check boxes on your 'Main' tab panel are the same as 7.4a.





(a) Master Panel, Main tab.



(b) Master Panel, Tune tab.

Figure 7.4.: Master Panel: Main and Tune tabs.

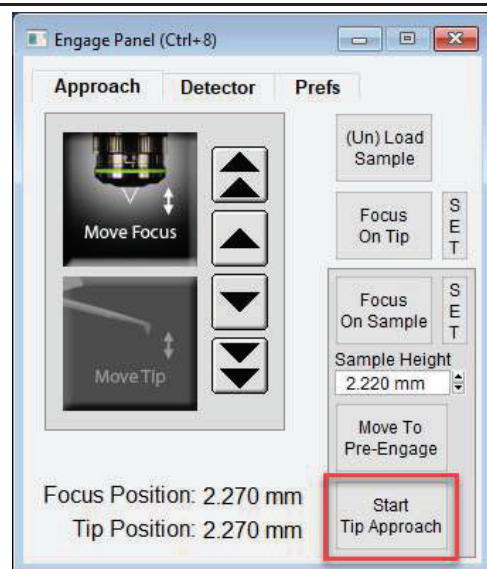
Landing the Tip

The preceding sections have left the tip vibrating at about 50 microns above the sample surface. It's time to land.

40.

Start final tip approach:

- Locate the Engage Panel.
- Click the 'Start Tip Approach' button.



41.

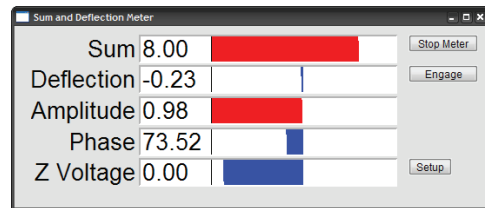
Wait for tip to reach sample:

- For the next minute or so, Cypher systematically moves the tip closer to the sample until the set point is reached.
- You can cancel the approach at any time by pressing and HOLDING the 'Esc' key on your computer keyboard.
- When the process is complete, the computer will beep, and the tip will be left at about half the Z range (about 3 microns) off the sample surface.

42.

Meter check:

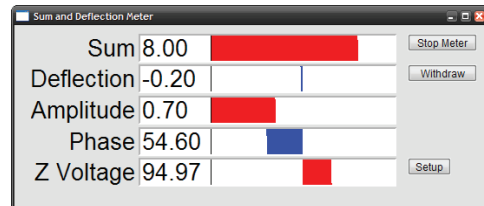
- Locate the Sum and Deflection Meter panel (Ctrl + 6).
- The values should be similar to the image at right and are typical for a cantilever that is a few microns off the sample surface.



43.

Engage:

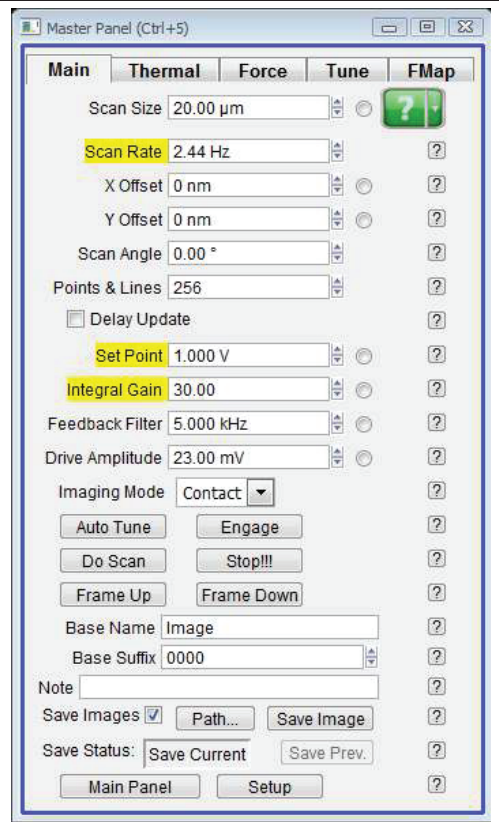
- Click the 'Engage' button on the Sum and Deflection Meter panel.
- The scanner will extend the sample into contact with the tip, and the Sum and Deflection Meter panel will look like the image at right.



Congratulations The tip is on the sample surface.

Question How do I know if my tip is firmly engaged on the sample surface?

Answer Type in a slightly lower set point (such as 750mV). If the Z voltage in the Sum and Deflection Meter panel does not change noticeably (e.g., more than a Volt), the tip will be firmly on the surface.



Question Why does the sample look out of focus when the tip is on the surface? How do I fix this?

Answer The laser and video image both pass through the same microscope objective. While performing AFM, the objective must remain focused on the back of the cantilever to keep the laser focused. Since the sample sits one tip height farther away, it will not be in focus. The fix is extra optics just before the video camera. Adjust the focus ring (at the center in the photo on the right) on the view system until the sample is in focus. Of course, the cantilever and laser spot will now appear blurred in the video image.

Note When it comes to focusing on the next cantilever, you must be sure to set the focus adjustment back to zero. Cypher includes a sensor to ensure that this occurs, and the software will warn you to zero the focus offset if necessary.



Imaging

This section gets you scanning and tracking the surface.

Set-Up and Initial Parameter Selection Based

on the previous section, it is assumed that:

- The cantilever tip is on the surface or was just disengaged from the surface.
- The laser is aligned on the cantilever, and the photo detector difference (deflection) signal has been zeroed.

44.

Set up Master Panel:

- In the 'Image' tab of the Master Panel, confirm that *AC Mode* is selected from the Imaging Mode pull-down menu.
- Integral gain (I): 24 to 30.
- Scan rate: 2.44 Hz.
- Scan angle, resolution (scan Points & Lines), and image size is up to you. These can be found on the Parm.s. tab (to the right the right of Imaging Mode).

Note: Setpoint should be set relative to the free air amplitude you tuned at, this value can be set to 70, 80 or 90% of the free air amplitude.
Hard engage = 80%
Soft engage = 90%

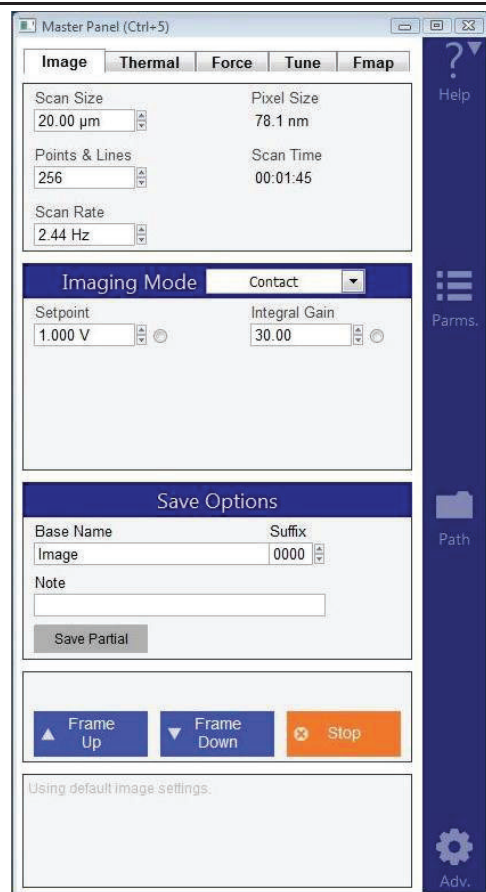
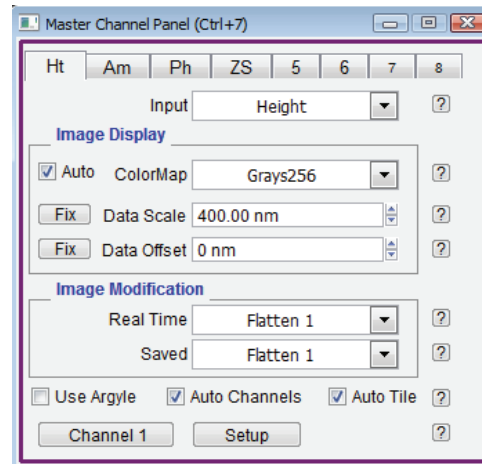


Image channel selection:

- 45.
- Go to the Master Channel Panel
 - Select the leftmost tab and confirm the default setting of 'Height' under the *Input* pull-down menu.
 - On the next two tabs, Am and Ph, do the same for 'Amplitude' and 'Phase'.
 - On the fourth tab, ZS, do the same for 'Zsensor'.

Note While not necessary, it's a good habit to activate the Z sensor channel when imaging, especially when sample features are larger than a few hundred nanometers; the LVDT sensors are more linear than the piezo actuators, and thus it is a more precise Z measurement.



46. Images are saved to disk automatically at the end of every image by leaving the 'Save Images' check box selected (lower left-hand corner of the Master Panel on page 17).

Start Imaging and Parameter Tuning

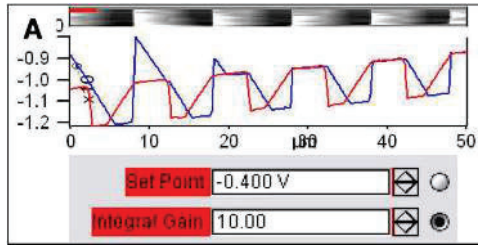
Start Imaging:

- 47.
- Click the 'Frame Up' button on the 'Image' tab of the Master Panel; imaging will begin after a moment. Scan initiation first moves the tip to the starting point of the image, then lowers the tip onto the surface, and then begins an endless series of image scans. The red cursor to the left of each image window indicates the scan line/location of the tip.



Tip

To enhance contrast on the image display, click, hold, and drag a box around the area of interest. Then right-click to select fix scale.



48.

Determining image quality:

- Start the learning process on a sample with a known topography, like the Asylum Research Calibration Grating being used in this tutorial.
- Look at the 'Scope Trace' below the image. This graph represents the most recent line of the image. Blue indicates the tip moving left to right (a.k.a. **trace**) and Red indicates tip returning from right to left (a.k.a. **retrace**).

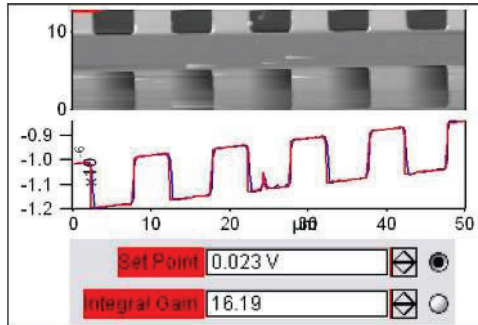
On most samples with relatively slowly changing features, trace and retrace should look the same. In other words, the landscape should look the same if you are flying the exact same route one way or the reverse. The image above shows the two as being quite different; this is an indication that imaging parameters need to be adjusted.

Nomenclature

In the previous image, the tip is not following the surface. As the blue trace shows (left to right), the tip seems to climb up out of the pits of the calibration grating quite nicely (the left edge of each pit is quite sharp), but then it descends back into the next pit along a relatively gentle slope. During this descent, the tip actually "flies through the air" while it is completely un-deflected, a bit like a hang glider running off a cliff. The lateral motion of the tip simply marches on as dictated by the XY scan pattern. The feedback control algorithm is simply not aggressive enough to bring the tip back down to the bottom of the pit. Such behavior is commonly called **parachuting** or **poor tracking**.

Hamster

The next steps go into details of strategies for tuning parameters in the main panel. Use the arrow clickers (to right of variable fields) to adjust parameters, rather than typing the values in. Alternatively, you can fine-tune the parameters using the 'Hamster' wheel on the front of the controller. Any parameter with a radio button next to it can be changed during a scan when it is activated (looks like black/ green dot in circle) with the 'Hamster'. The Hamster gives "digital control with analog feel". On the MFP-3D AFM controller, the toggle switch to the left of the 'Hamster' allows you to toggle between radio buttons in the panel. On the ARC2 SPM controller, the outer 'Hamster' ring performs this function. This tactile experience lets you concentrate on the image while tuning parameters.



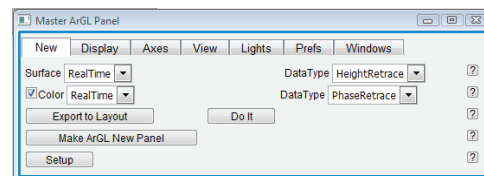
Adjusting Set-Point, i-gain, and scan speed:

- 49.
- The goal is to get trace and retrace to fall on top of each other (as shown above).
 - Increase the 'Integral Gain' or *i-gain* parameter (third highlighted item in Step 39 on page 14) and see what happens. The match between trace and retrace should improve. The feedback becomes more aggressive and the slope with which the tip “parachutes” down into the pits should become steeper.
 - Keep increasing the 'Integral Gain' until suddenly the trace and retrace start to oscillate wildly, a phenomenon called “ringing”. The feedback loop is now unstable, and the i-gain needs to be lowered a bit.
 - If increasing the i-gain does not seem to help, try to increase the 'Drive Amplitude' a bit. This will cause the cantilever to oscillate with greater amplitude.
 - Lowering the 'Set Point' can also improve tracking.
 - Decreasing the 'Scan Rate' control will also improve tip tracking.

Note The overall goal is usually to make a good compromise between imaging speed and tip tracking.

View Data in Real Time 3D:

- 50.
- Open the Master ArGL Panel by selecting from the menu bar *AFM Analysis > 3D Surface Plots*.
 - From the 'Surface' pull-down select 'Real time'
 - From the 'Data Type' pull-down to the right of it, select the desired channel in that image (usually *Height* or *Zsensor*).
 - Click the 'Do It' button.
 - You can click, hold, and drag the 3D data to change the view.



Moving the Sample Between Scans:

- Sometimes, it is desirable to move to another point of interest after some scans have been taken. For features within 12.5 microns, use the 'X offset' and 'y offset' fields. Note that a negative is to the left or below the initial area.
- For features that are further away, use the arrows toward the edges of the Video Panel. **The tip should be moved to the pre-engage position before moving the sample.** The single arrows are slow, and the double arrows are fast. It is also possible to click and hold the double arrows for faster, continuous movement.

Q When I make changes to scanning parameters, when do those changes take effect in the scanned image?
A Most parameters in the main tab of the main panel update as soon as you make a change. Note that changing points, lines, or scan rate, will take effect next frame.

If you check the 'Delay Update' box just above the 'Setpoint' parameter, any changes you make to parameters above that box will only update next frame. Until the image is complete, the changed variables are highlighted in blue.

You can always force a new image by clicking the 'Frame Up' or 'Frame Down' buttons. A nice way to see the effect of changing imaging parameters can be as follows:

- Check the 'Delay Update' box, as described above.
- Click 'Frame Up' and collect a dozen scan lines. Observe the image quality.
- Make some changes to the scan parameters (number of points, rate, gains, setpoint).
- Click 'Frame Up' again.
- Observe as the exact same scan region is "painted over" with new data taken with your new parameter choices.

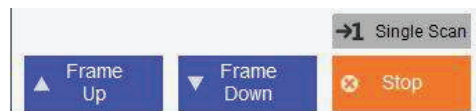
Image Refinement

To learn more about using the Asylum Research SPM software to refine your imaging parameters, please refer to the theory section of this SOP. Also consider watching this introductory video: [AC Mode Imaging in Air](#) (requires an internet connection).

Stopping Imaging

General Procedure for Stopping Imaging:

- 51.
- Once a scan has begun, a 'Continuous' button on the Master Panel will appear and can be changed to 'Single Scan'.
 - Clicking on 'Single Scan' will cause scanning to cease once the current scan has completed.



Note 'Last Scan' will change to 'Undo Last' if clicked. Clicking Undo Last will cause the software to keep imaging with current parameters.

Emergency Stopping Procedures:

- Clicking the 'Stop' button on the Master Panel will halt the scanning mid-image; this is a fairly abrupt way of halting scanning and should only be used if there is a problem. For instance, it would be appropriate to use this button if the cantilever were gouging holes in the sample.

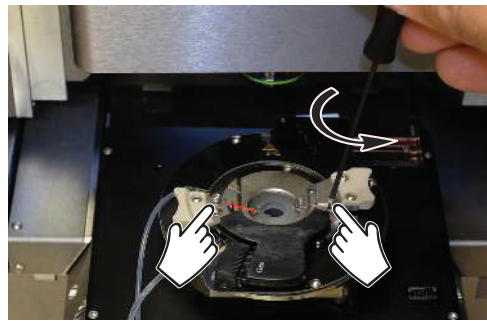
Shutting down the system and finishing up

52. Raw data is saved in the Raw data folder (backed up), the folder will be named with your university ID and a sub-folder with the current date, please do not do analysis in this folder, instead copy the data over to the user data folder (not backed up) and do Analysis there.
53. After stopping image use the (un)load sample button on the engage panel, this will sent the tip stage to the top and remove the cantilever holder.

54.

Release the cantilever holder:

- Use the tool to loosen the two screws clamping the cantilever holder. Three turns *counterclockwise* should be enough (do not completely unthread screws).



55.

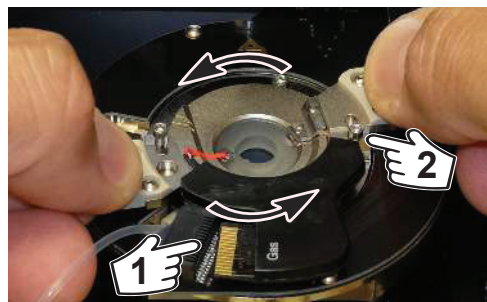
Rotate the cantilever holder:

- Place your fingers on the two side cantilever holder handles.
- Rotate *counterclockwise* a few degrees.

In the image at right:

1 The cantilever holder circuit board comes out of its mating connector. Stop when the board has cleared the connector.

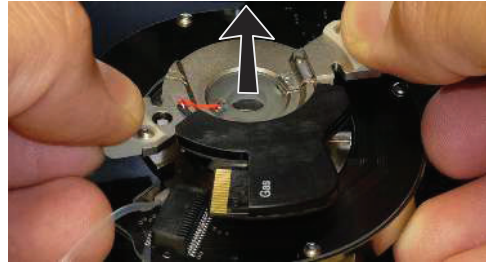
2 Notice the screws come away from the cutouts in the cantilever holder.



56.

Remove the cantilever holder:

- Once the board has cleared the connector, *carefully* wiggle the cantilever holder up and out.
- The resistance you feel as you remove the cantilever holder comes is the cantilever holder O-ring sliding out from the cell body. Once the O-ring clears the cell body, be ready for the resistance to drop suddenly.
- **Caution:** *Keep a firm grip on both handles* to keep from flinging the cantilever holder across the room!



57.

Remove cantilever:

- Set the cantilever holder on its handles with the cantilever facing up so that you do not crush the cantilever.
- Remove cantilever
- Place cantilever holder in storage box and leave on sample prep counter



58.

Remove old sample:

- **Lower sample stage**
- Use “Platypus” tweezers to remove the old sample from the sample stage.
- Wipe the sample stage clean with a kim wipe and ethanol. Any dust or grit will prevent the sample disc from seating properly.

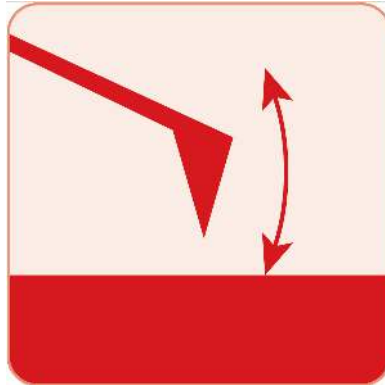


59. Turn off the laser key on the controller.
60. Close the door of the AFM instrument
61. Log out of your directory on the PC
62. Put all tweezers / screwdrivers etc. back in plastic box in sample prep area
63. Tidy up after your self.

END of SOP

Appendix A

Advanced tuning and image optimization



Chapter Contents

| | | |
|-------|--|----|
| 1 | Auto Tuning | 26 |
| 1.1 | Auto Tune Troubleshooting | 27 |
| 2 | Saving Tune Data | 28 |
| 3 | Optimizing Imaging Parameters | 28 |
| 4 | Net Attractive and Repulsive AC Modes | 28 |
| 4.1 | Net Repulsive Mode Imaging | 29 |
| 4.1.1 | Use a new cantilever | 29 |
| 4.2 | Net Attractive AC Mode | 29 |
| 4.2.1 | Chose the right probe | 29 |
| 4.2.2 | Gently drive the probe above resonance | 29 |
| 4.2.3 | Try Q control with positive Q gain | 29 |
| 4.3 | Preventing Mode Hopping | 30 |
| 4.3.1 | Make the Sample Less Attractive | 31 |
| 5 | Setpoint-Based Imaging Method | 31 |

Video Tutorial

Consider watching this introductory video tutorial: [AC \(Tapping\) Mode Imaging](#) (internet connection required).

1. Auto Tuning

The tutorials recommended above briefly touch upon the business of tuning the cantilever. Since this is at the heart of AC imaging, we'll talk about that now in a little more detail. If you just completed your AC mode imaging tutorial, you may be in a state of collecting an image. In this case, please halt the scan by clicking the 'Stop!!!' button in the *Main* tab of the Master Panel. This will retract the tip from the surface and stop the XY scanning of the sample. If you left your system not scanning, but with the tip simply engaged on the surface, click the 'Withdraw' Button on the Sum and Deflection Meter Panel. This pulls the tip from the sample.

Note These instructions are for repulsive mode AC imaging.

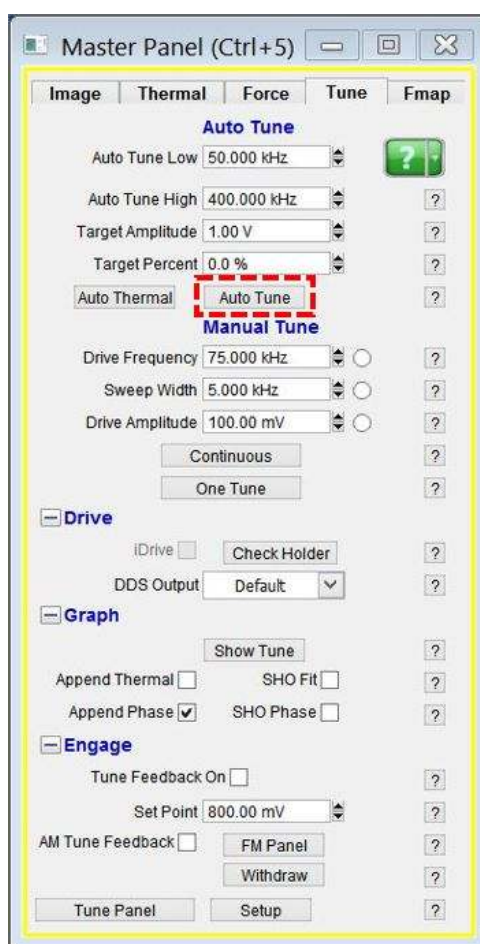


Figure 4.1.: Tune tab

1. Open the *Tune* tab of the Master Panel.
2. Choose a *Target Amplitude* of 1.0V. This will be the free air amplitude voltage. This is the peak-to-peak voltage of the oscillating cantilever.
3. Change the *Target Percent* to -5.0%. The minus sign indicates that the drive frequency will be on the left side of the resonant peak, which helps ensure the tip will remain in NET repulsive

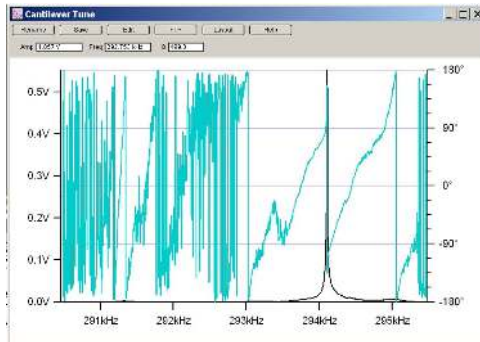
mode when engaged and scanning. For more detailed information about repulsive mode, see Section 5.3 on page 48.

4. The default *Auto Tune Low* and *Auto Tune High* values for air imaging are typically 50 kHz to 400 kHz, respectively, accommodating most fundamental drive frequencies for common commercially available AC Mode cantilevers.
5. Click the 'Auto Tune' button for the frequency sweep to commence. The shake piezo applies a frequency ramp through the Auto Tune low to high frequencies. The cantilever will give the greatest oscillation amplitude at its resonant frequency, allowing the tune algorithm to locate it and determine the Q factor (quality) of the peak.
6. The *Drive Frequency* value will automatically update in the *Tune* and *Main* tabs of the Master Panel, and the Q factor of the cantilever will be determined and displayed at the top of the Cantilever Tune graph.

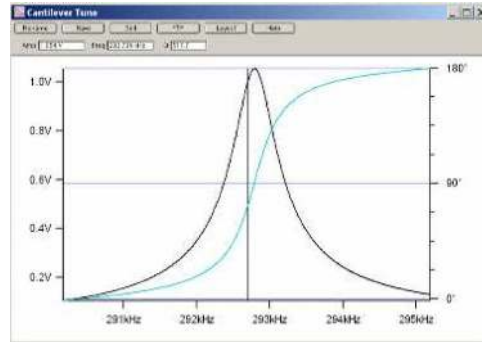
The Cantilever Tune graph, similar to the one in Figure 4.2 on page 37, will appear. The first tune will likely appear similar to Figure 4.2a on page 37, which then updates to one similar to Figure 4.2b on page 37.

The software automatically does the following:

- Picks a *Drive Frequency* at the specified *Target Percent* (default of -5%).
- Adjusts the *Drive Amplitude* applied to the shake piezo needed to make a 1.0 V (peak to peak) amplitude voltage on the photo diode, as displayed in the Sum and Deflection Meter.
- Adjusts the *Phase Offset* to have the phase signal at 90° on resonance.



(a) Early stages of Auto Tune



(b) Final result of Auto Tune

Figure 4.2.: Auto Tune of an Olympus AC160 Si ($f = 300\text{kHz}$; $k = 40\text{N/m}$; nominal values) cantilever in air

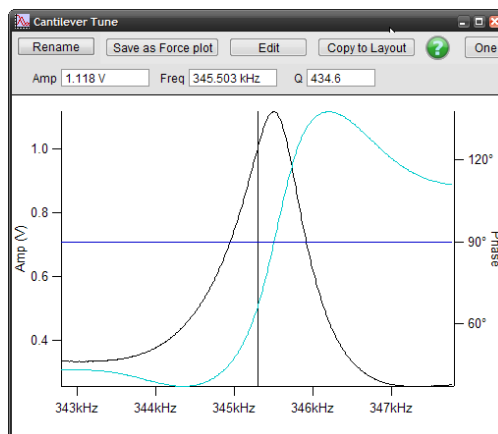
1.1. Auto Tune Troubleshooting

If you do not know the resonant frequency of your cantilever, see Chapter 21 on page 293 of the applications guide.

2. Saving Tune Data

Tip How to Save Tune plots?

- Click 'Save as Force Plot'. The saved tune can then be reviewed in the Master Force Panel.
- Click 'Rename'. The graph is saved in a separate window. Subsequent tunes can be overlaid on top of each other if they are given the same name or saved in a new graph when given a new name.
- Click 'FTP'. This saves the experiment on the computer in a Temp Folder. This allows you to upload the file to the Asylum Research FTP site for discussion with Asylum Research technical staff.
- Click 'Layout'. This appends the graph to a layout.



3. Optimizing Imaging Parameters

Please refer to the tutorial³. It has a very good explanation of how to get going in AC mode, adjust gains, interpret the images, and figure out what is going wrong. It may be written to be MFP3D centric, but it covers the basics. Even if you have a Cypher, a lot of what is in there is still very useful; you may only need to adjust the values of the gains and rates for your Cypher.

4. Net Attractive and Repulsive AC Modes

Repulsive AC mode is the most common AC mode. It is where the tip is in repulsive (hard) contact with the surface in some fraction of its oscillation. It is much easier to perform but is rougher on the tip and sample.

Attractive AC mode attempts to image while sensing only the very weak attractive forces by gently oscillating the tip just above the surface. In this way the tip will experience attractive forces over a broad range of its oscillation. This is much more difficult to do and cannot be done with all levers and samples. But it is much gentler on the samples and is sometimes required for soft biological materials.

4.1. Net Repulsive Mode Imaging

Imaging in Repulsive mode was used in the example tutorial from the *ARMFP3DUserGuide.pdf*. It is much easier and tends to give better phase contrast. You can tell you are in repulsive mode when your phase signal is $< 90^\circ$.

4.1.1. Use a new cantilever

If all else fails, the tip apex may be too blunt to stay in Repulsive Mode. (Yes, this is actually true.) Large radius, blunt tips can experience too much attractive force approaching the surface and get stuck in attractive mode. Put in a new cantilever and start over.

4.2. Net Attractive AC Mode

Imaging in Attractive mode is great for soft samples that can be damaged or situations where you want your AFM tip to stay sharp for a long time. You can tell you are in attractive mode when your phase signal is $> 90^\circ$.

As it can be tricky when you want attractive mode imaging to occur, some simple steps to help keep the tip in attractive mode are included here.

4.2.1. Chose the right probe

Softer, longer levers with relatively low resonance frequencies are a good choice. We always have good luck with the [AC240](#) or [AC160](#). Also see [Asylum Research Probe Store](#).

4.2.2. Gently drive the probe above resonance

Smaller amplitudes with drive frequencies above resonance improve Attractive mode imaging stability.

For an Olympus AC240 probe, the process is as follows:

1. In the *Tune* tab of the Main panel, set the *Target Amplitude* to 250mV.
2. Set the *Target Percent* to +10 to +20%; this will set the *Drive Frequency* on the higher (right) side of the cantilever's resonant peak, a position more likely to keep the tip in Attractive mode while imaging.
3. Click the 'Auto Tune' button. If this does not do well, try again with a *Target Amplitude* of 500 mV.

4.2.3. Try Q control with positive Q gain

Higher Q resonance peaks favor Attractive mode imaging. Q control gives some control of this.

Figure 4.3 on page 37 shows this process with an AC240 (~76kHz Si lever) with some common visuals to look for, although every lever shows different degrees of response relative to the amount of Q gain.

- Figure (a) shows the last tune of an Auto Tune; no Q gain is added.
- Figure (b) shows the response after a slight increase in Q gain. Notice the top of the peak is starting to look more round than sharp; this means the ringing is about to start.
- Figure (c) Ringing shows up to the right of the frequency peak; at this point, you should decrease the Q gain until the ringing goes away.
- Figure (d) is an example of it going even higher, showing even more ringing in the amplitude and phase data.
- Notice with additional Q gain, the amplitude response goes up. You may need to lower the *Drive Amplitude* to get back to the desired free air amplitude.

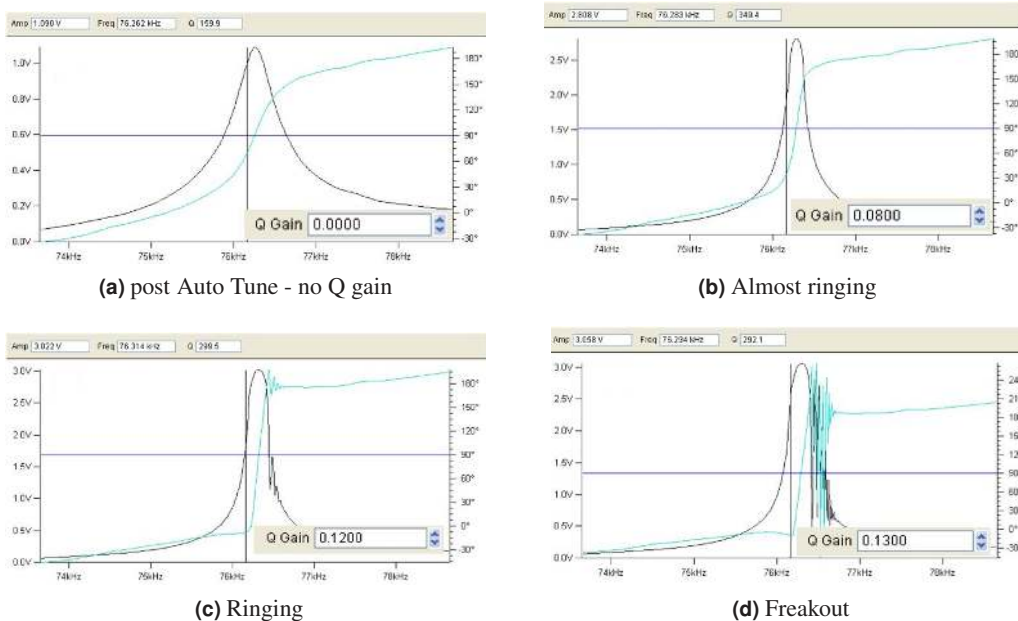


Figure 4.3.

4.3. Preventing Mode Hopping

Mode Hopping is where the tip will jump between Repulsive and Attractive mode while scanning. While it is good to stay in one mode or the other for imaging, the uncontrolled switching between them makes analysis difficult. The images in Figure 4.4 on page 38 show red regions, where phase is much smaller than 90° and blue, where phase is much larger than 90° (attractive behavior). During each scan line, the probe is switching back and forth between Attractive and Repulsive mode depending on what features of the sample it is interacting with.

The first thing to do to prevent Mode Hopping is to go more repulsive. Repulsive mode is easier to achieve. Decrease the *Drive Frequency*, increase the *Drive Amplitude*, and possibly decrease your setpoint.

If Mode Hopping is occurring and you want to be in Attractive mode, reduce the *Drive Amplitude* and then the *Set Point*. This should work most of the time, but if it does not, retune and select a *Drive Frequency* slightly more to the right of the peak.

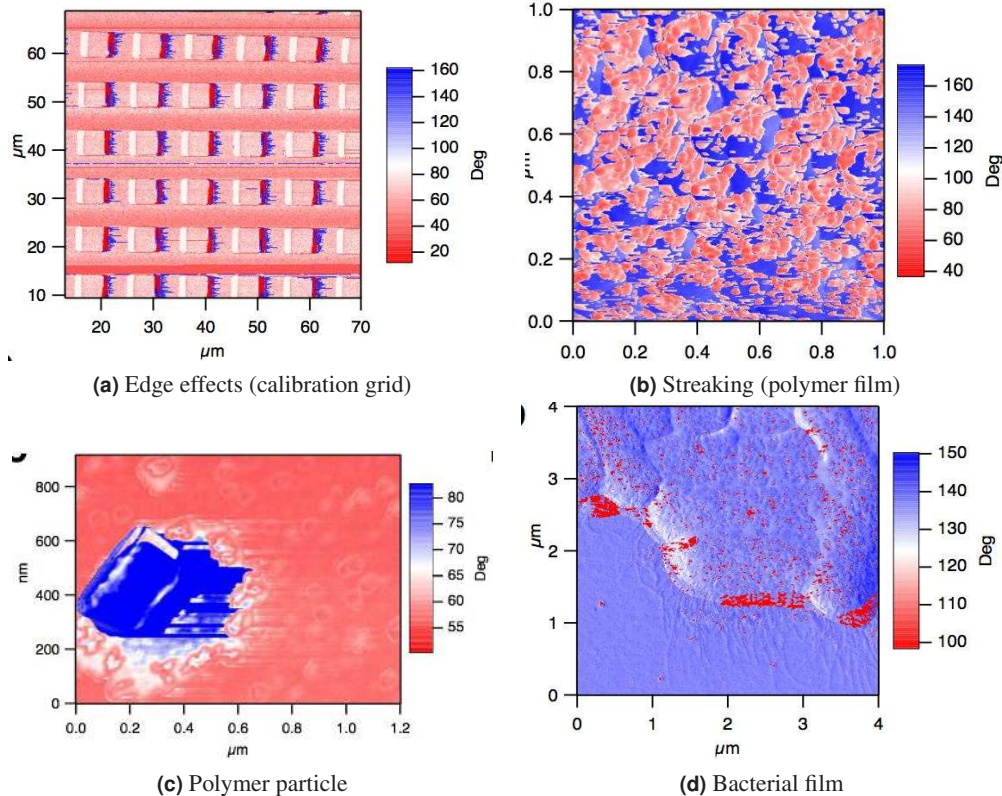


Figure 4.4.: Examples of mode hopping in the Phase Channel

4.3.1. Make the Sample Less Attractive

If Mode Hopping is still problematic, it could be due to the sample. The following situations may be contributing to it:

- Surface charges:
 - Use the Static Master device to ionize the air around the sample and make it slightly conductive; this dissipates surface charges.
 - Place the Static Master in the vicinity of the sample (fig. 4.5).
 - Sometimes the glass slide the sample is glued to is the culprit of the excess charge. In these cases, mounting the sample on a magnetic puck and placing it on a metal sample holder can help quell this charge.
- Tip sticking to the sample: Our findings have shown that a Pt-coated Si cantilever can sometimes work well with sticky samples. We use [Electrilevers](#) (Olympus AC 240s coated with Pt).

5. Setpoint-Based Imaging Method

Setpoint-based imaging is an iterative method of determining scanning parameters. Formerly, the procedure involved setting an arbitrary free air amplitude and adjusting the set point accordingly. In this alternative method, a setpoint is chosen based on the estimated roughness of the sample

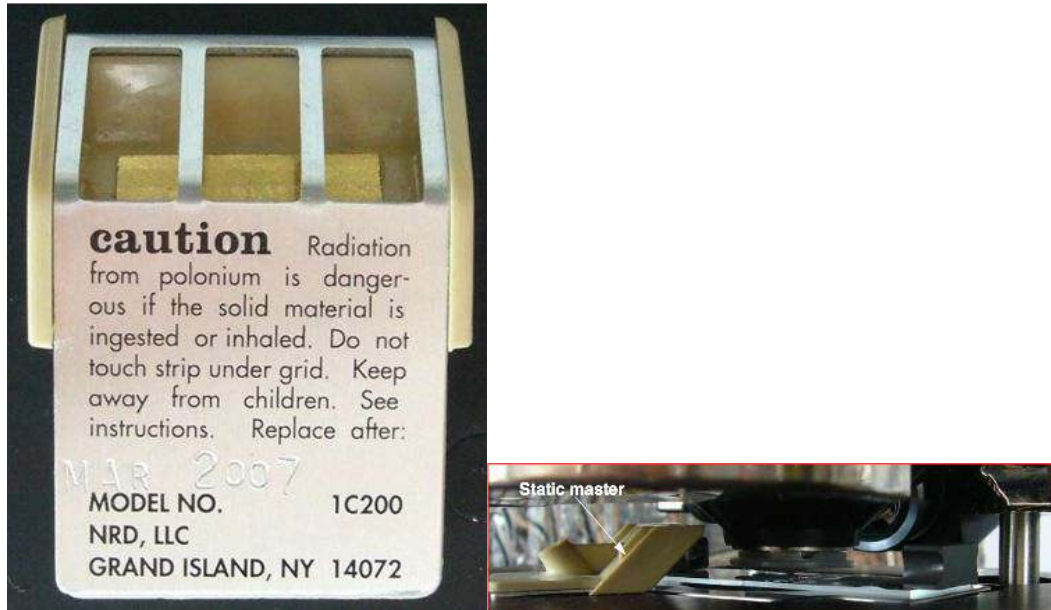


Figure 4.5.: Placing static master near sample on an MFP-3D AFM to dissipate charge

surface and the InvOLS of the cantilever. Once this has been determined, the setpoint is left as is, and the drive amplitude is instead adjusted to maintain either attractive or repulsive mode imaging. Overall, the goal is to establish the minimum setpoint to maintain a stable image.

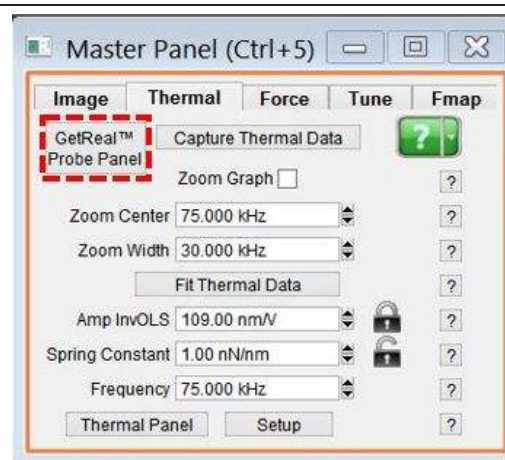
This is accomplished by first determining the optical sensitivity of the cantilever. In a physical sense, this refers to how much the laser point on the photodetector moves relative to the vertical motion of the cantilever.

To perform setpoint-based imaging:

1.

Getting started:

- Make sure the cantilever is engaged on the surface of the sample.
- Select the *Thermal* tab on the Master Panel (Ctrl+5).
- Click 'GetReal Probe Panel'. The panel opens.



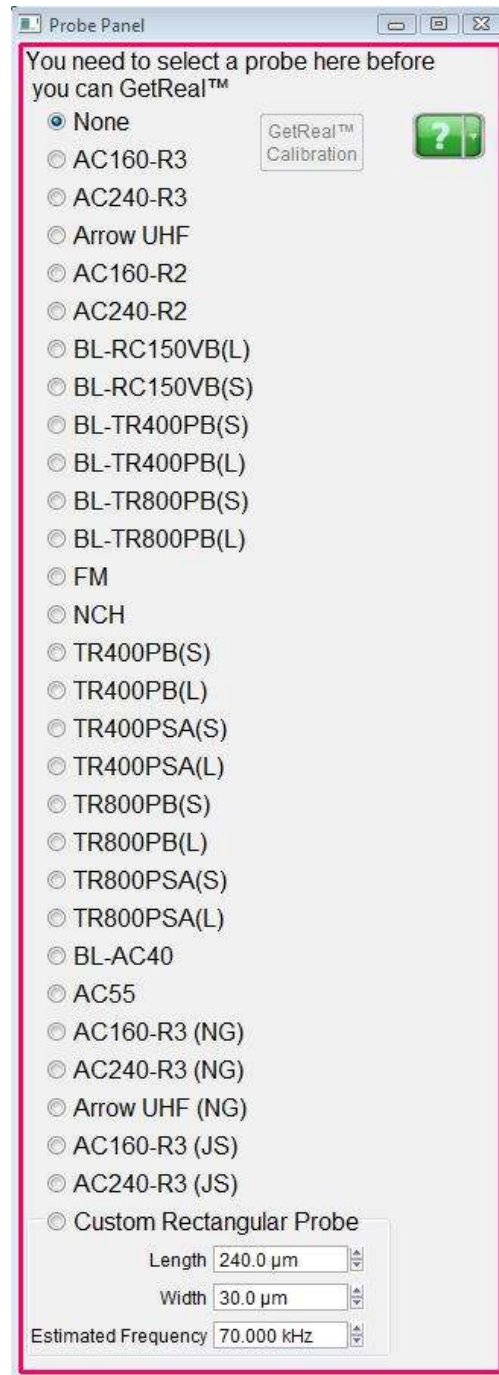
Initiating cantilever calibration:

- On the Probe Panel, you will see a list of different cantilevers. Select the cantilever you are using.

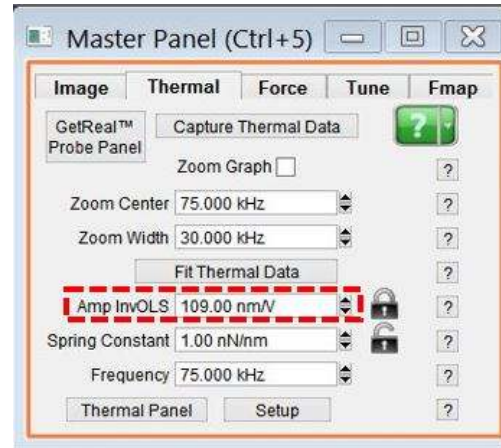
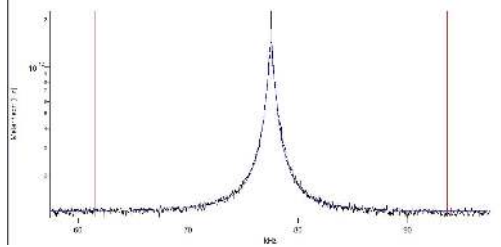
Notes

2.

- It is possible to select a similar cantilever, though the results will be slightly inaccurate.
- If you are using a cantilever not on the list, there is a link on the Asylum User Forum that allows you to request information about special cantilevers. It also includes instructions for performing your own calibrations (with reference to the John Sader paper on the subject).



3.



Calibration results:

- Click 'GetReal Calibration'. After a few moments, a graph of the calibration will display. It should resemble the figure shown, above left.
- Once the cantilever has been calibrated, a value appears in the *Amp InvOLS* field. This represents the amplitude in nm/V.

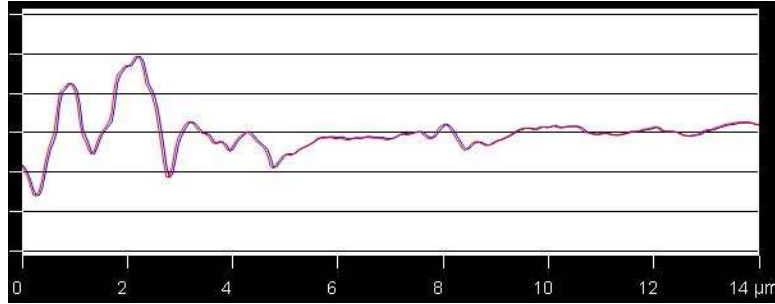
Note “InvOLS” stands for “Inverse Optical Lever Sensitivity.” However, the number is the reciprocal of the optical lever sensitivity rather than its inverse.

4.

Engaging on the surface:

- Choose a set point that is slightly higher than the free air amplitude. This will cause the cantilever to retract.
- Increase the *Drive Amplitude* until the tip is just on the surface. The Z voltage will stop changing at this point.
- Start imaging.

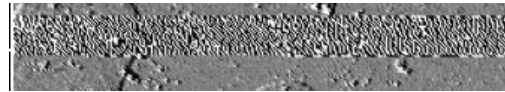
Note The set point can never be lower than the sample height. If the sample height is unknown, determine it before proceeding.



5. **Attractive or Repulsive Mode**

- For repulsive mode, increase the drive amplitude until the phase drops below 90 degrees. If this doesn't seem to work, it might be necessary to restart scanning with a higher set point.
- OR-
- For Attractive mode, keep the same set point but adjust the drive amplitude for closer tracking of the trace and retrace lines. See above image with an example of closely tracked trace and retrace lines.

Tip For better tracking in Attractive mode, increase the *Integral Gain*. Increasing the integral gain too much may cause feedback oscillations, pictured at right; aim for a gain that minimizes amplitude error without introducing feedback oscillations.



Appendix B

AC Mode Imaging: Scientific Background and Theory

Chapter Contents

| | | |
|-------|---|----|
| 1 | Feedback | 36 |
| 2 | Phase Image | 37 |
| 3 | Attractive and Repulsive Behavior | 38 |
| 3.1 | Selecting Attractive vs. Repulsive | 39 |
| 3.2 | Rules of Thumb for Attractive or Repulsive Mode Imaging | 41 |
| 3.2.1 | Free amplitude | 41 |
| 3.2.2 | Cantilever choice | 42 |
| 3.2.3 | Drive frequency above or below resonance | 43 |
| 3.2.4 | Q (Quality Factor) | 44 |
| 3.2.5 | Sample attractive forces | 45 |
| 3.2.6 | Tip sharpness | 49 |

1. Feedback

In AC mode, the cantilever is typically oscillated mechanically by a small piezo electric actuator very near the cantilever chip. Prior to imaging, the Drive Frequency is swept over a broad range to locate the first resonance of the cantilever. The Drive Frequency is then set at or near that Resonance Frequency. The name “AC mode” stems from the tip oscillation: Think of AC current vs. DC current. The optical detector senses the oscillatory motion of the cantilever, and the electronics inside the controller measure the amplitude of this oscillation and also the phase with respect to the drive signal.

When thinking about AC mode, it is a useful experiment to dissect an AC mode force plot. An AC mode force plot collects the cantilever amplitude as the oscillating tip is moved towards the surface and away again. This is shown in [Figure 5.3 on page 38](#). On the right side of the graph, the cantilever is oscillating 100 nm above a flat sample surface. In this force plot, the cantilever was oscillating at resonance with an amplitude of 60 nm. That means the tip swings sinusoidally from 60 nm below the rest to a position 60 nm above. Now we move the base of the lever (as well as the resting point of the lever) towards the surface, and we follow on the graph from right to left ([Figure 5.3 on page 38](#)). The amplitude remains constant until the resting position of the cantilever gets within 60 nm of the surface, then the bottom of the oscillation is just barely touching the surface. As we bring the cantilever closer to the surface, the tip oscillation must be reduced. You may think that the tip motion will now have the form of a truncated sine wave, but in reality, the equations of

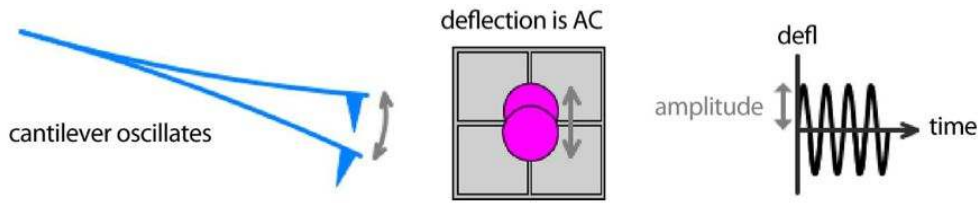


Figure 5.1.: An oscillating cantilever leads to an oscillating signal on the photodetector. Pause to consider the absolute magnitude of these oscillations. Picture a 12 μm tall cone. The absolute motion is twice the amplitude (120nm) or only 1% of the entire conical tip structure. Clearly, this is quite exaggerated as it shows an oscillation amplitude a thousand times larger than reality. Keep in mind that the oscillation amplitudes are quite small and that nearly any figure you see in any paper, book, or manual, will greatly exaggerate the amplitude of oscillation.

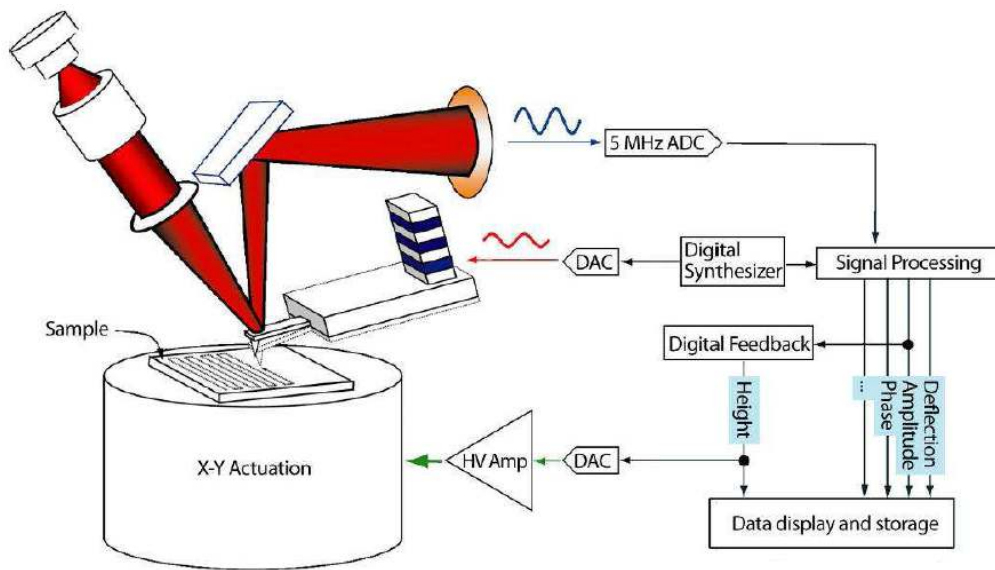


Figure 5.2.: Basic diagram of AC mode imaging.

motion produce something that is very nearly a sine wave with a reduced amplitude. You can see this in the plot; the amplitude is linearly decreasing as the surface inhibits the oscillation.

This linearly sloping amplitude with Z is well suited for feedback signal. In continuing our example, an amplitude setpoint of 40 nm will cause the feedback to reduce the average tip sample separation (z-position) to 40 nm. If, during scanning, the surface suddenly slopes up, the oscillation amplitude will decrease, and the feedback will drive the z piezo up until the amplitude is back at its setpoint.

2. Phase Image

A Phase image has a wealth of information; it is equivalent to a map of dissipation. Polymer samples may show little of interest in terms of topography but contain a lot of phase contrast in terms of nano variations in sample mechanical properties. While previous sections explain a lot

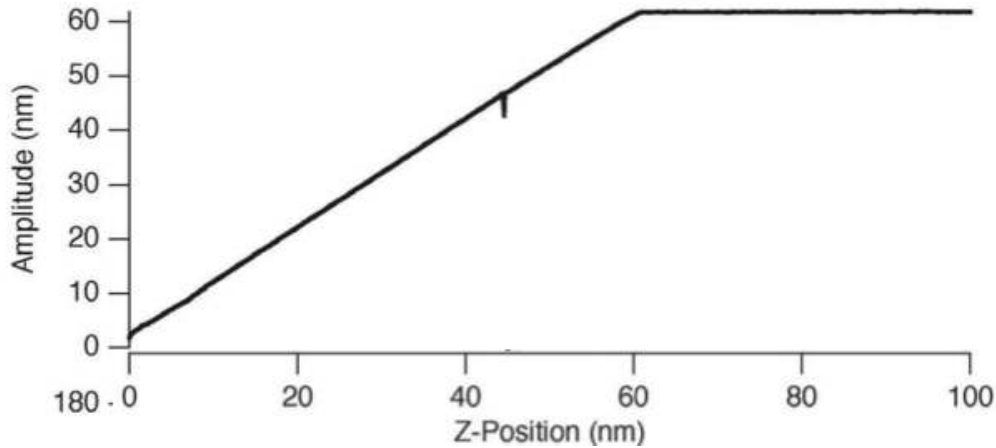


Figure 5.3.: Tip oscillation amplitude as it approaches the surface. The small glitch in amplitude is explained later. See 5.4.

about AC mode imaging in terms of amplitude, it is only half the story, as any driven oscillator has both amplitude *and* phase. Phase is expressed in degrees. A cantilever driven a good deal below resonance is said to be in phase (0°), i.e., while the base of the cantilever is slowly driven up, the entire lever moves up at the same speed. A cantilever driven a good deal above resonance will do the opposite at its tip and base. This condition is called “out of phase” (180°). At resonance, it is neither in-phase nor out, but in between. We can think about this with a human scale example, a swing. When swinging, you change the sign of the drive when the amplitude of the swing is at a maximum, meaning the drive signal is 90° behind the amplitude signal.

3. Attractive and Repulsive Behavior

Let’s revisit [Figure 5.4 on page 39](#). This time we will show the phase signal as well.

Again, we’ll start at the right side of the graph. The cantilever is driven nearly at resonance. The Amplitude is 60 nm, and the Z-Position is 100 nm. The tip does not touch the surface, even at its lowest point. The Phase is nearly at 90° , slightly larger, indicating that the cantilever is almost being driven at resonance.

As soon as Z-Position decreases to the point where the tip starts interacting with the surface, the Phase starts to grow larger than 90° . This behavior can be explained by the attractive force the tip experiences from the sample, during the brief time of each oscillatory cycle that it spends near the surface. The closer we move the cantilever to the sample, the more attractive force experienced, and the larger the phase grows. But as the tip gets closer to the surface, there is also repulsive forces, damping the oscillation. At some point during the decreasing of z-position, the repulsive forces are greater than the attractive forces, and the phase shifts to below 90° . The attractive forces are pulling down on the lever at the bottom of the cycle, and the repulsive forces are pushing at the bottom of the cycle. The attractive forces will shift the resonance frequency to lower frequencies, so if you started driving on resonance, the shift of the resonance to lower frequency means your phase is $> 90^\circ$. Then when the repulsive forces exceed the attractive forces, it is net repulsive. That repulsive pushing at the bottom of the oscillation shifts the resonance frequency to higher

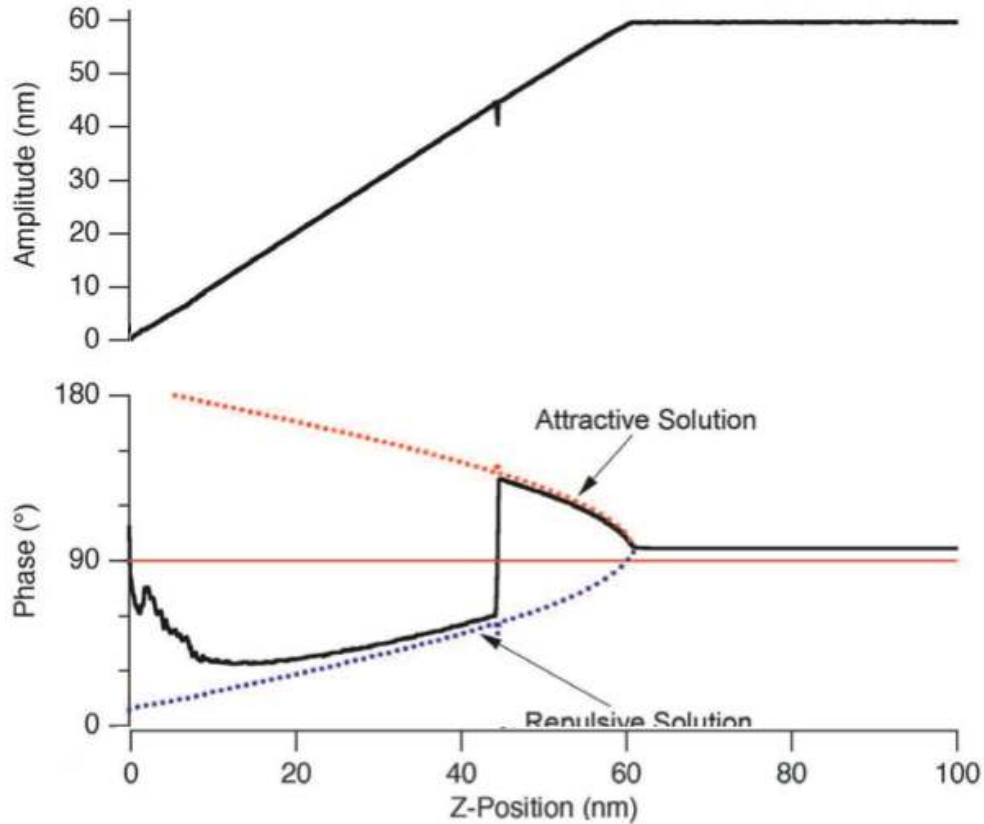


Figure 5.4.: Cantilever amplitude and phase as a function of average tip-sample separation

frequencies, meaning the drive frequency is below resonance, and you see a phase $< 90^\circ$. This shift in resonance frequency is further illustrated in Figure 5.6 on page 41.

Notice from Figure 5.6 on page 41 that if you choose a negative Target Percent amplitude during an Auto Tune (Section 4.3 on page 30), it helps keep the tip in Repulsive mode during AC mode imaging. Likewise, positive target percent amplitudes help the tip stay in Attractive mode

For more on this topic, see references^{1,2,3,4}.

3.1. Selecting Attractive vs. Repulsive

In practice, it can be quite difficult to select between Attractive mode and Repulsive mode imaging. For the specific case shown in Figure 5.5 on page 40, a setpoint chosen too close to 60nm will lead to unstable imaging. A setpoint chosen too close to 43 nm will cause the phase to constantly flip between attractive and repulsive. Luckily, we have not explored the option of choosing a different free amplitude. The 60 nm free amplitude used in the examples so far was arbitrary.

¹ J. Tamayo, R. Garcia, Appl. Phys. Lett., 1998 73(20), p2926.

² J.P. Cleveland, B. Anczykowski, A.E. Schmid, V.B. Elings, Appl. Phys. Lett., 1998 72(20), p2613

³ A. San Paulo, R. Garcia, Biophys. Journ., 2000 78, p1559.

⁴ R. Garcia, R. Perez, Surface Science Reports, 2002 47 p197-301.

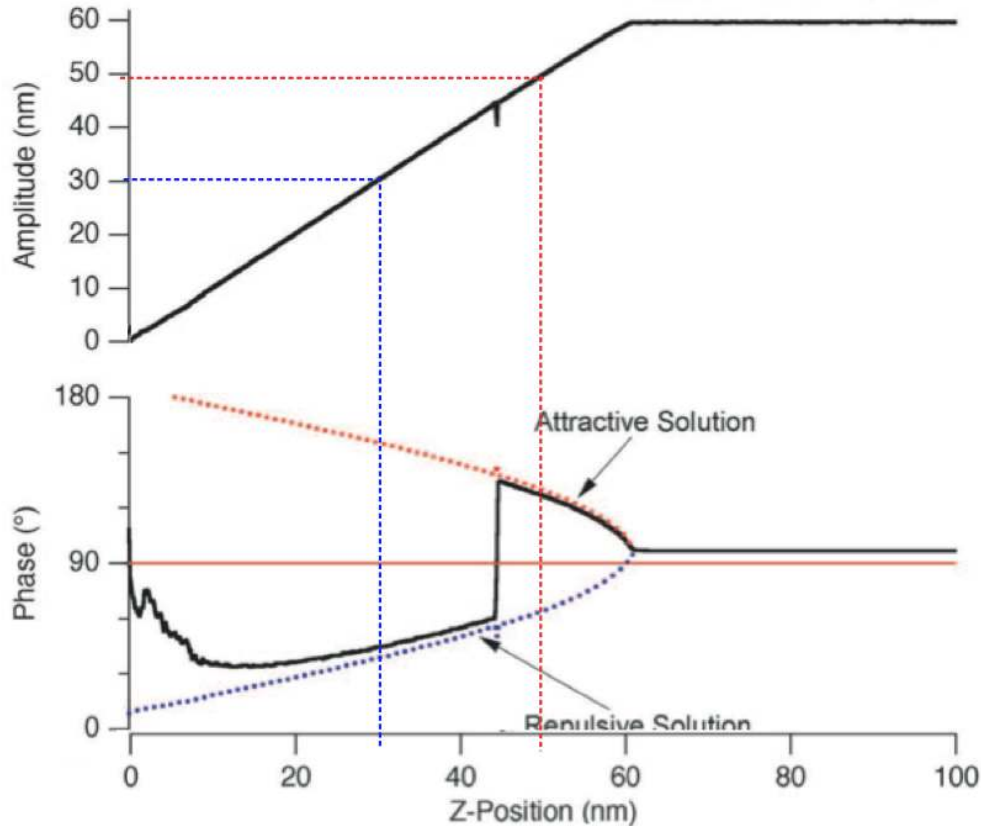


Figure 5.5.: AC mode feedback setpoint of 50nm (red dashed lines) gives attractive mode imaging, a setpoint of 30nm (blue dashed lines) gives repulsive mode imaging.

Take a look at [Figure 5.7 on page 42](#). This is a busy graph, but to get some frame of reference, the red curves on the graph are the same as we have been looking at in the few previous figures. The other curves are just repetitions but with higher or lower free amplitudes. For instance, for the top black curve, the cantilever is oscillating with an Amplitude of nearly 300 nm when it is well above the surface. Note that from the corresponding phase curve, the cantilever only oscillates in Attractive mode (phase $> 90^\circ$) for a very small range of setpoints very close to that free amplitude and then stays repulsive (phase $< 90^\circ$) for lower setpoints.

The green, blue, purple, and pink curves are for successively lower and lower cantilever drive amplitudes. The behavior is similar, but there is a growing trend of attractive behavior with setpoints near the free amplitude.

For the blue curve with a 50 nm free amplitude and the black curve with 20 nm free amplitude, there is a remarkable change of general behavior. No matter what setpoint you choose (below the free amplitude), the cantilever always oscillates in the attractive regime. Now we can make some fairly good rules of thumb for imaging in purely Attractive mode, purely Repulsive mode, or something prone to switching between the two.

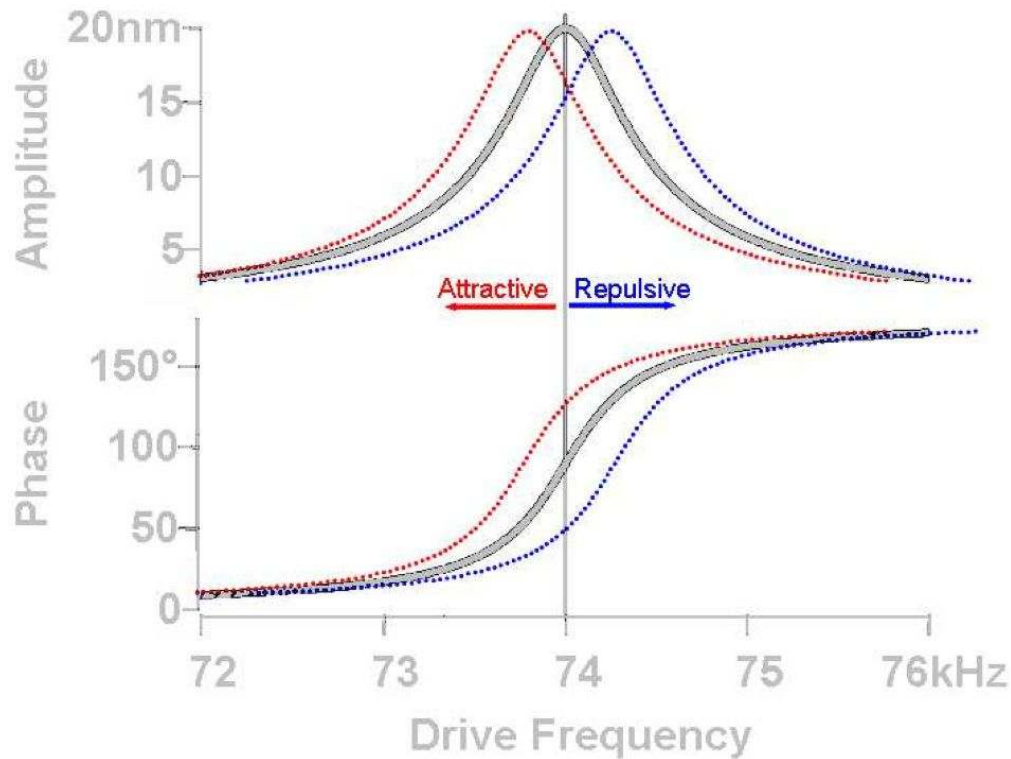


Figure 5.6.: A shift in resonance frequency, f_o (at constant amplitude) as the tip interacts with surface results in phase shift of the AC signal: Decrease in f_o results in phase increase (attractive mode), while increase in f_o results in phase decrease (repulsive mode). The colors represent the color of the phase on the Sum and Deflection Meter Panel.

3.2. Rules of Thumb for Attractive or Repulsive Mode Imaging

| Repulsive Mode More Likely | Attractive Mode More Likely |
|---------------------------------------|---------------------------------------|
| Bigger free amplitude | Smaller free amplitude |
| Stiffer cantilever | Softer cantilever |
| Drive frequency lower than resonance | Drive frequency higher than resonance |
| Lower Q, Negative Q gain | Higher Q, Positive Q gain |
| Sample with smaller attractive forces | Sample with bigger attractive forces |
| Sharp tip | Dull tip |

Table 5.1.: Attractive vs. Repulsive AC mode imaging “rules of thumb”

3.2.1. Free amplitude

Bigger or smaller free amplitude are relative terms. Only once you perform a series of AC force curves with a particular cantilever and a particular sample, you will be able to quantify what bigger and smaller mean. For instance, the experiments in Figure 5.7 on page 42 show for that cantilever and sample bigger means > 150 nm and smaller means < 50 nm.

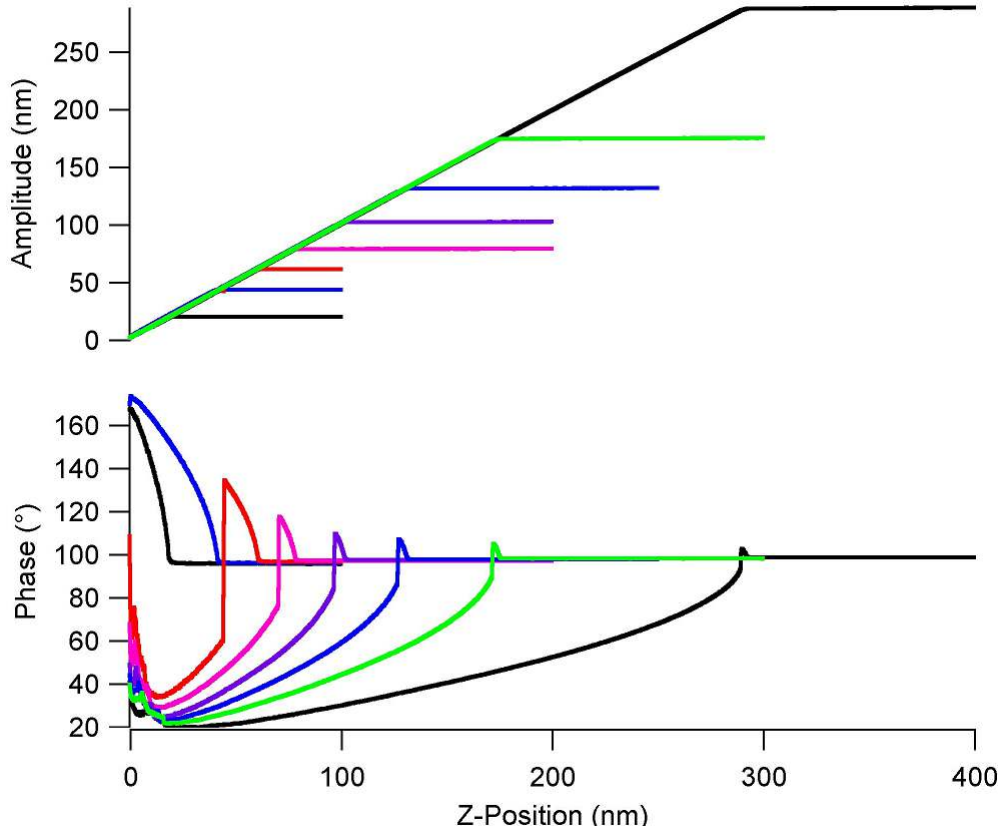


Figure 5.7.: A family of AC force curves for different free amplitudes

3.2.2. Cantilever choice

Stiffer and softer are relative terms, but for the sake of simplicity we will consider the two most common AC mode levers. On the [Asylum Research Probe Store](#) some general use AC mode probes are listed. The first choice for general AC mode imaging should probably be the Olympus AC160. With a spring constant of around 40 N/m it is considered a relatively stiff probe good for repulsive mode imaging. The next most commonly used AC mode lever is the AC240, it is 20 times softer, and considered a good probe for attractive or repulsive mode imaging. Note that the data presented in earlier in this section were for a probe similar to the AC240.

3.2.3. Drive frequency above or below resonance

In practice, the AR software makes it very easy to tune the cantilever slightly above or below resonance. Under the *Tune* tab of the Master Panel, you can select 'Target Percent'. A value of -10% is good to promote Repulsive mode imaging, and +10% helps promote Attractive mode imaging. Alternatively, you can right-click on the resonance curve after tuning and manually select the drive frequency.

3.2.4. Q (Quality Factor)

The Q factor of the cantilever is a result of both the lever and the environment (liquid or gas). If you have a relatively low Q (<30), you may find it difficult to stay in Attractive mode. In such cases, consider using the Q control feature of the AR SPM software. By means of digital processing in the SPM controller, it allows you to increase or decrease the Q. Y

3.2.5. Sample attractive forces

You typically don't have much control over sample attractive forces; but if you find it particularly difficult to achieve attractive or repulsive mode imaging, it may be due to your sample.

3.2.6. Tip sharpness

A dull tip has more contact area and is more strongly attracted to a surface than a sharp tip and increases the probability of Attractive mode imaging.