

Operating Asylum Cypher AFM

Appropriate Samples and Sample Prep

Samples must fit on a 15mm diameter disk and cannot be more than about 1cm thick. Samples must be flat since the microscope cannot measure features taller than 2.5 μ m. The maximum area that can be imaged with the microscope is 10 μ m by 10 μ m.

Stuff to Buy:

Description	Vendor	Part no.
Magnetic disks for sample mounting	Ted Pella	16218
Adhesive tabs	Ted Pella	16079
10mm mica disks	Ted Pella	50
Tweezers for handling probes	Ted Pella	5374-NM



11.2. 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.

Note Although it is not required, for safety reasons we recommend making motor moves with the door closed. Beware of pinch points (Figure 20.1 on page 230).

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.



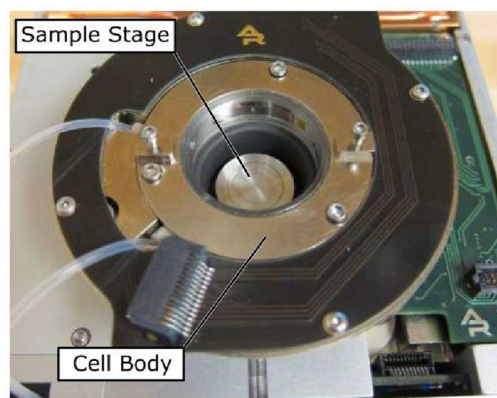
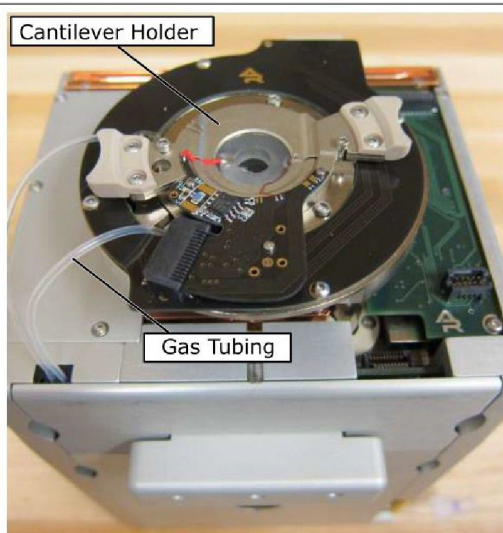
3. **Unlock scanner:**
- Lift the lever to the right of the scanner.



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.

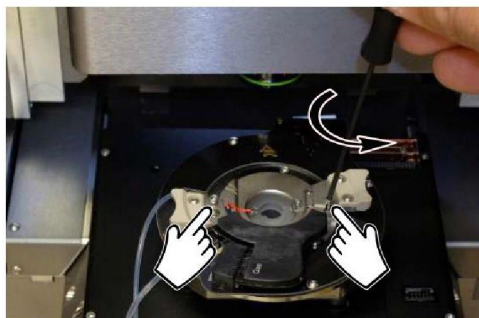


Familiarize yourself with the sample area:

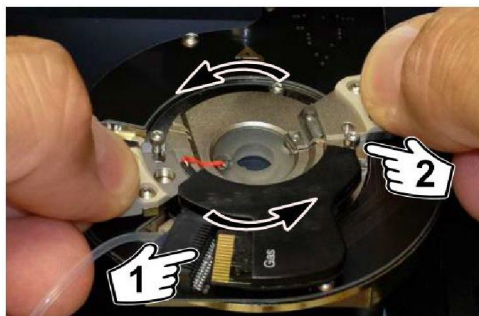
- While it may look solid, the scanner sample stage moves the sample in X, Y, and Z imperceptibly up to 40 μ m.

Release the cantilever holder:

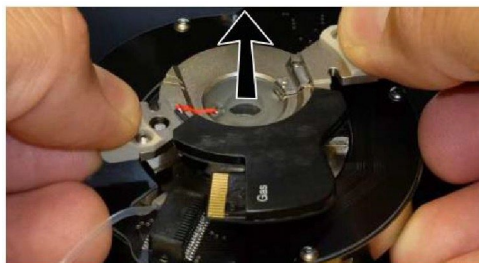
- 6.
- Locate the tool with yellow tip in the chassis to the left of the scanner.
 - Use tool to loosen the two screws clamping the cantilever holder. One turn *counterclockwise* should be enough (do not completely unthread screws).
 - Replace the tool.

**Rotate the cantilever holder:**

- 7.
- Place your fingers on the two side cantilever holder handles.
 - Rotate *counterclockwise* a few degrees.
- 1 the cantilever holder circuit board come 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.

**Remove the cantilever holder:**

- 8.
- 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. *Keep a firm grip on both handles* to keep from flinging the cantilever holder across the room!



9.

Set aside the cantilever holder:

- Set the cantilever holder on its handles with the cantilever facing up so that you do not crush the cantilever.



10.

Locate your cantilever holder:

- Identify the appropriate cantilever holder. This tutorial requires the standard Gas cantilever holder, Asylum Part# 901.758.
-

Note To learn more about cantilever holders for the Environmental Scanner, please refer to Chapter 12 on page 141.



11.

Prepare cantilever mounting workspace:

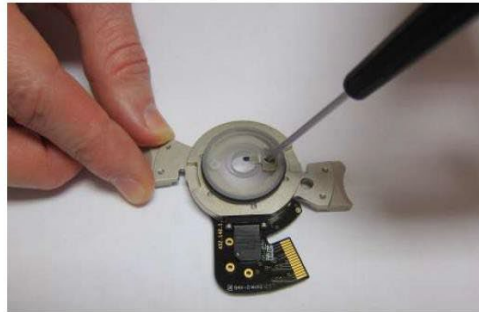
- You will need the following items:
 - a) Tweezer, curved (Asylum Part #290.102)
 - b) 300 Philips Screwdriver (Asylum Part #290.106)
 - c) Box of AC160TS cantilevers
 - d) Gas cantilever holder
- A low power binocular dissection stereoscope with light source can be useful for some of the following steps.
- Cleaning the tweezer tips with alcohol improves the handling of the cantilevers.



12.

Loosen the cantilever clip:

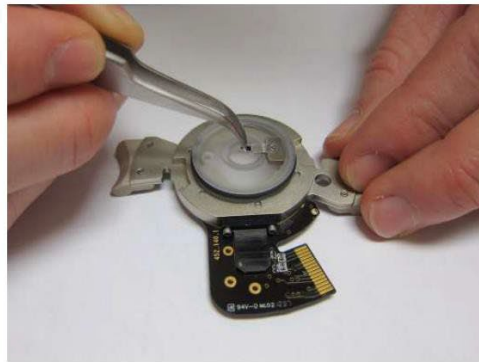
- Unscrew the clamping screws by one half turn.
- Do not unthread the screw completely. If you accidentally do, please refer to the cantilever holder chapter.



13.

Remove the old cantilever:

- If a cantilever has already been loaded, use tweezers to remove it from the cantilever holder.



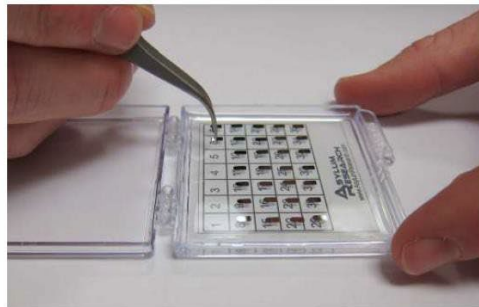
14.

Select new cantilever:

- Use tweezers to pick up new cantilever.
- Close the box! Ruining \$1k of levers by putting your hand on an open box is not unheard of.

Note If your lab saves some old cantilevers, consider practicing with a “dummy” cantilever.

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

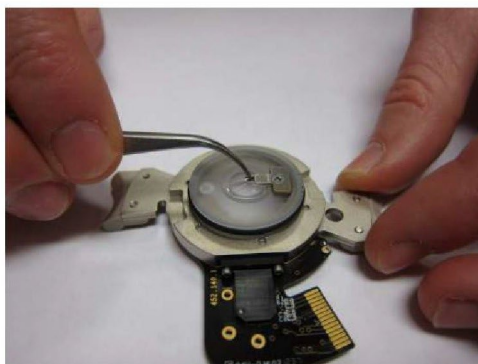


15.

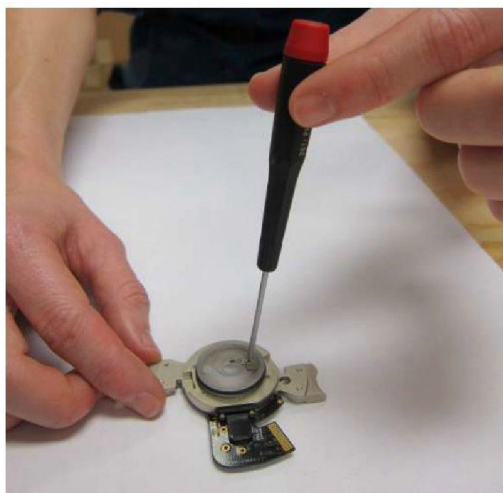
Load new cantilever:

- 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.

Note You may nudge the cantilever chip from the side to align it, but do NOT nudge the end of the chip, as you risk damaging the cantilever. It helps to do this at least once under a binocular stereo microscope.



16.

**Secure the new cantilever:**

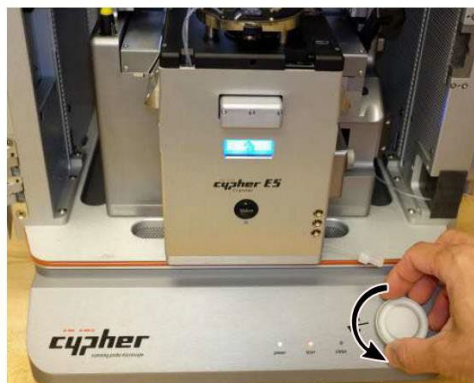
- *Using fingertip pressure only*, lightly tighten the screw securing the cantilever to the cantilever holder.
- The clip should be just tight enough to secure the cantilever chip when it is pushed from the side.

Prepare scanner to remove old sample:

- Leave the cantilever holder on your workspace for now.

17. **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.

**Remove old sample:**

- 18.
- Use “Platypus” tweezers (Asylum Part # 114.721) to remove the old sample from the sample stage.
 - Wipe the sample stage clean with a cloth or use compressed air. Any dust or grit will prevent the sample disc from seating properly.

**Replace with new sample:**

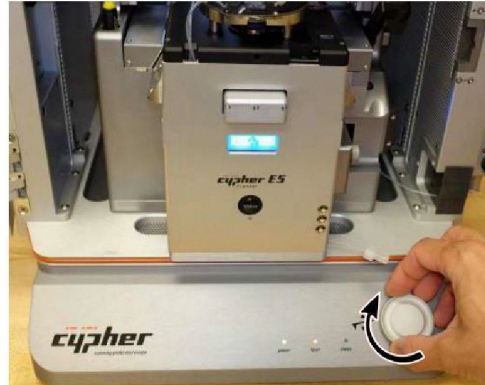
- 19.
- Use “Platypus” tweezers (Asylum Part # 290.165) to place new sample on sample stage. For this tutorial, use the Asylum Research calibration grating sample, part # 900.237. It will attach magnetically.



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.

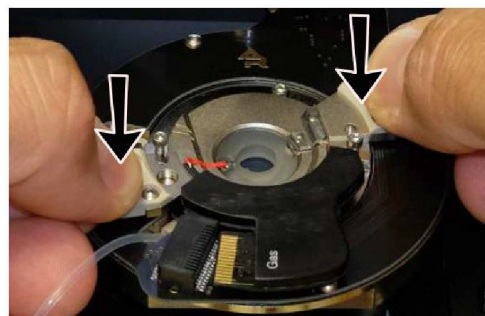
20. **Warning:** Before loading the cantilever holder, raise the coarse engage stage. If you do not raise the coarse engage stage, you will crash the cantilever into the sample and ruin your cantilever, sample, and possibly even the cantilever holder!

**Place cantilever holder:**

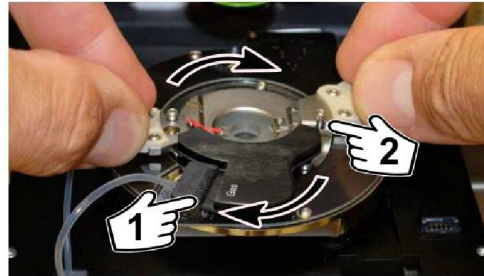
21.
 - Check to make sure that the coarse engage stage is raised to its highest position.
 - Position the cantilever holder in the cell body as shown in the image to the right. The cantilever holder should sit level and be rotationally aligned so that the cantilever holder board is almost touching its mating connector.

**Press the cantilever holder down:**

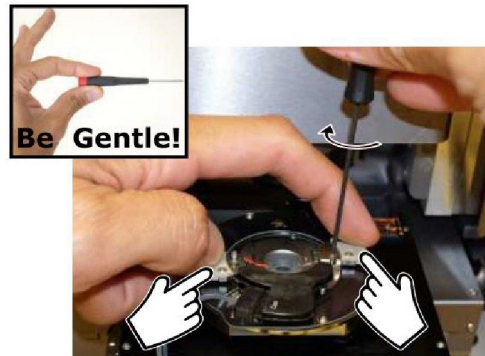
22.
 - Before starting this process it is important that the cantilever holder is sitting level with respect to the top of the cell body.
 - With fingers on both handles of the cantilever holder, use firm pressure to wiggle the cantilever down into the cell body. The O-ring will offer some resistance. When the cantilever holder is firmly seated, you will feel a hard stop as metal parts make contact.



23. **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.



24. **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.



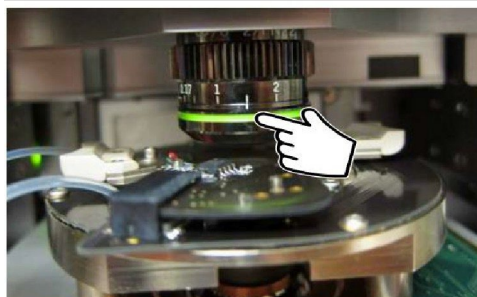
25. **Slide scanner into chassis:**
- Slide the scanner back into the chassis. Use firm pressure until you feel a hard stop. Before you feel a hard stop you will feel a spring-like resistance. You are making thermal contact between the scanner and the chassis. This is necessary for best performance.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



26.

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.



27.

Close enclosure door:

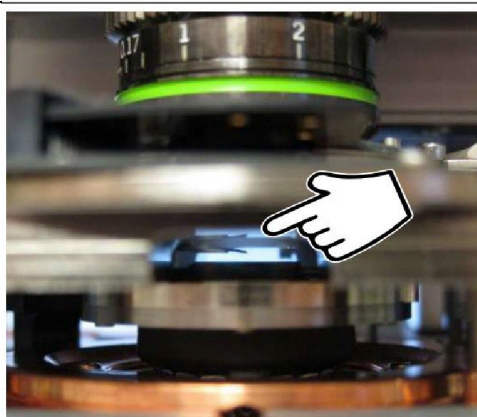
- *Gently* close the door and latch it.



28.

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 will lower 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.

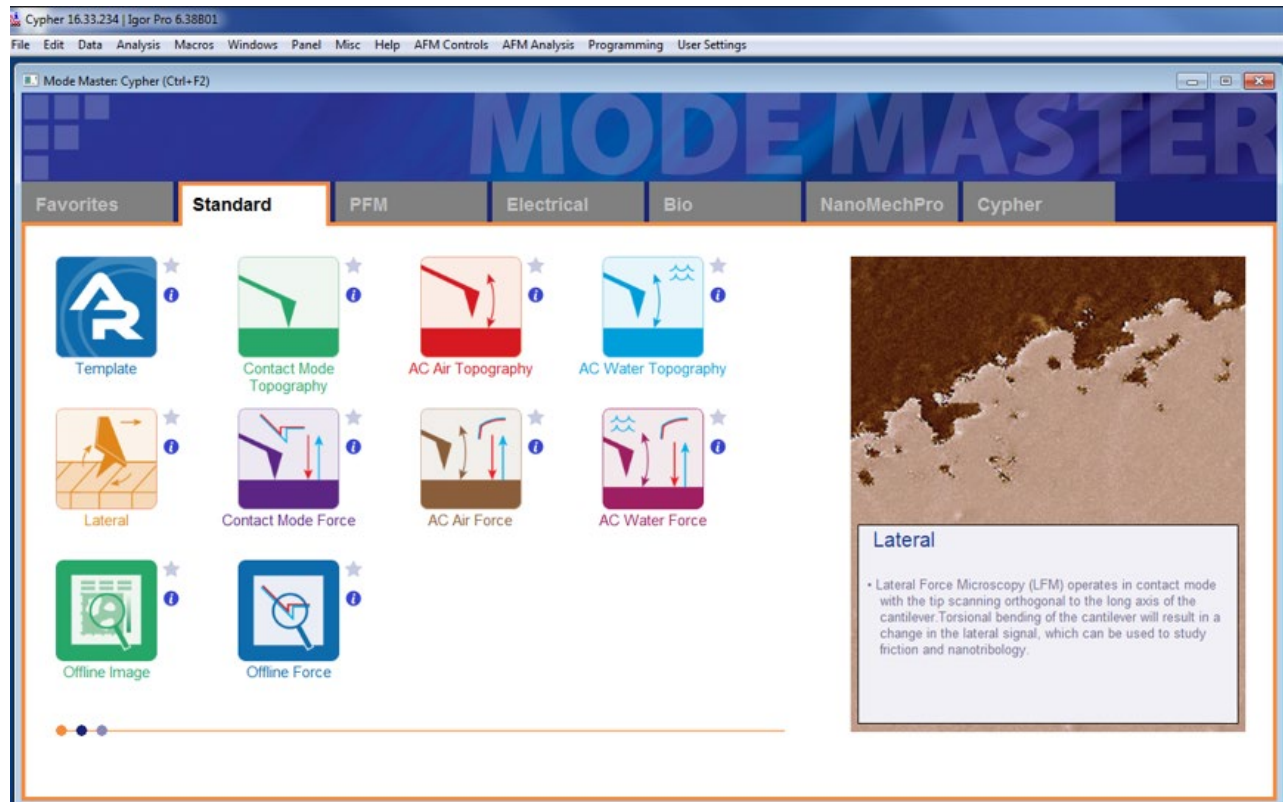


Warning: Nothing but your attentiveness will prevent the cantilever holder from crashing into the sample. If you crash the cantilever holder you may cause *SERIOUS* damage to your cantilever holder and scanner.

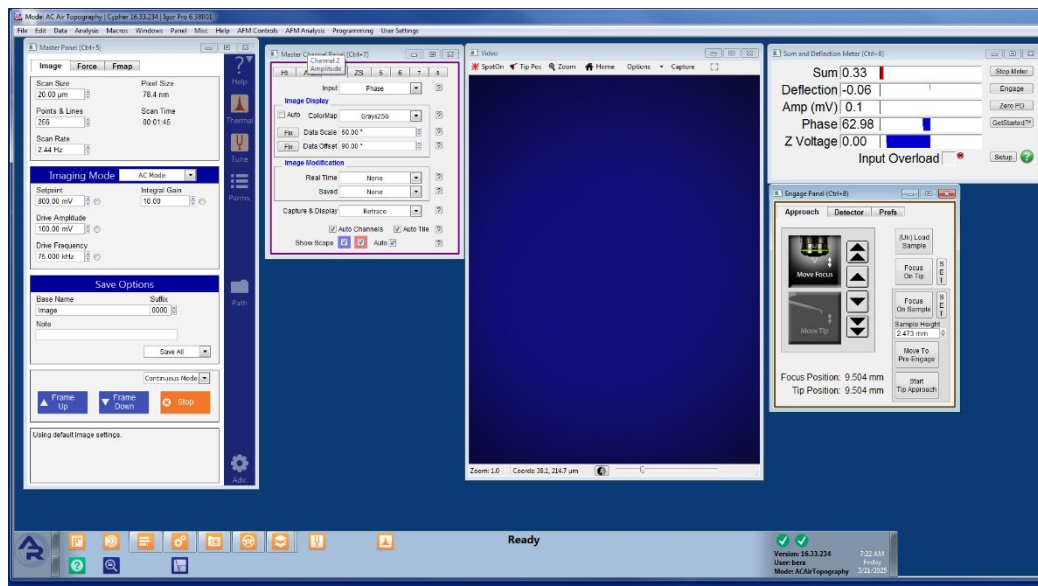
29. This concludes the manual interaction with Cypher. We next turn our attention to the computer. Please jump to [Section 4.3](#) on page 26.

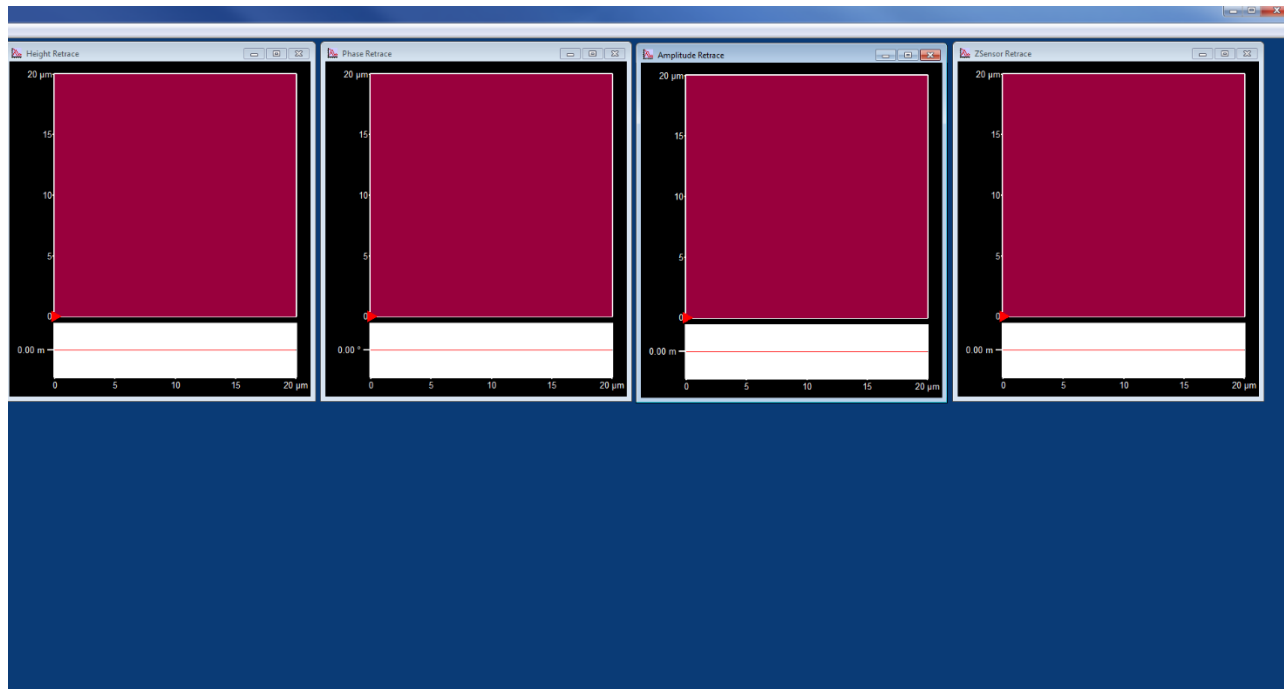
Engaging the Cantilever on the Sample

1. Log in to the computer and start AR 16.33.234 software



2. From Mode Master select Standard > AC Air Topography and you will see this window and make this following arrangement:

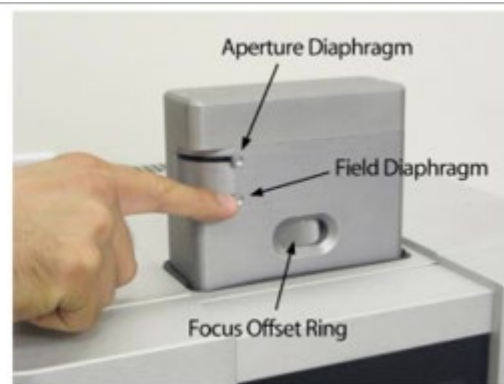




Right monitor.

Prepare the view module:

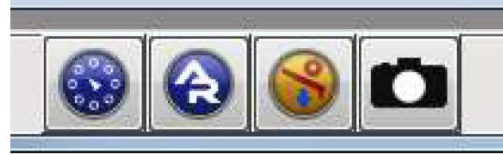
- Turn the **focus offset ring** until the white dot is centered on the alignment mark. There will be a gentle click when this occurs.
- Fully open the **field and aperture diaphragms** by pushing both levers toward the front as shown.



3.

Open video window (If necessary):

- 4.
- In the software, click on the camera icon  on the bottom status bar.
 - This will open the video window (or highlight it in case it was already open), which displays an optical view of the cantilever and sample.

**Setting video zoom and illumination:**

- 5.
- **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.
 - Turn up the illumination by moving the slider (on the bottom of the video window) to the right a quarter or third of its full range.



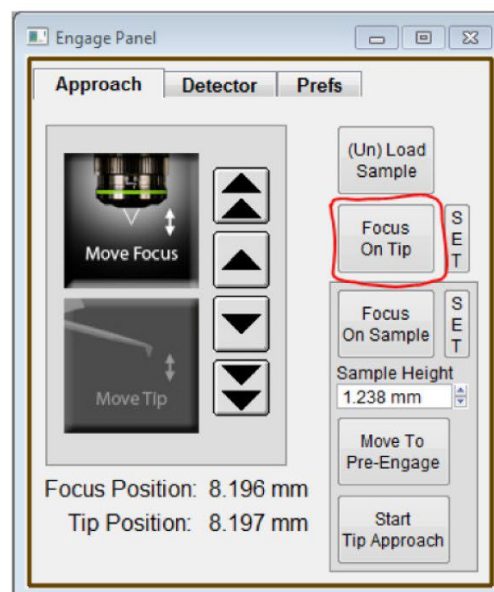
6. Familiarize yourself with the Approach tab on the Engage Panel as described next in [Step 7 on page 27](#). *Failure to understand the Approach controls may lead to serious damage to the Cypher.*

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.

Notes

- 7.
- 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. (Cantilever chips have varying thicknesses and how the cantilever chip gets positioned in the holder will affect the sample position.)
 - Don't be alarmed if the cantilever is not visible at all. 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. This will get addressed in the next step.
 - If you hit the 'Focus on Tip' button and nothing happens (i.e. the motors do not move), it just means that the objective is already at the tip focus point. Note that after the motors are homed, the objective is moved automatically to the tip focus point.



Locate cantilever in image:

- 8.
- 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. Hit the left arrow to move the objective towards the left and look for the cantilever chip edge.*

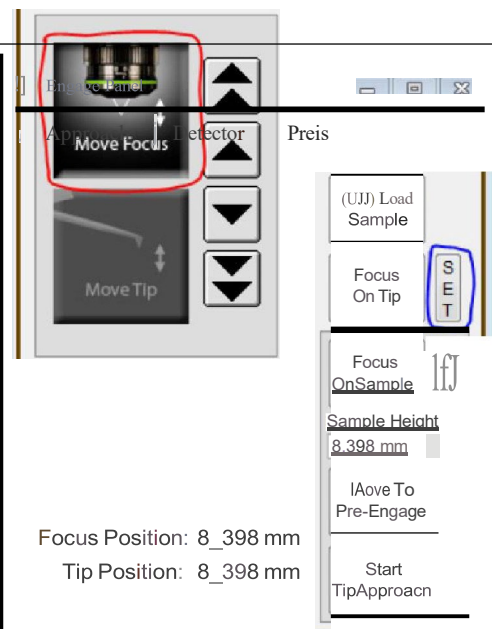


See Figure 4.1 on page 34 for example images.

Set the tip focus position:

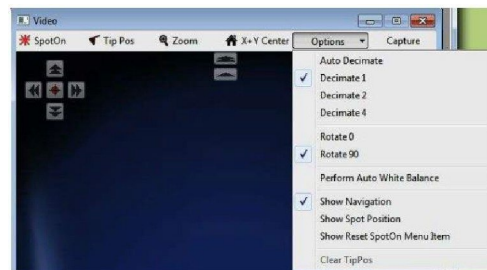
- 9.
- Under the 'Approach' tab on the 'Engage Panel' select the 'Move Focus' (on the left, red) large picture button.
 - Use the on-screen arrow button until cantilever is in focus. Single arrows are slow; double arrows are fast.
 - Click 'Set' next to 'Focus on Tip' button (on the right, blue).

Important The cantilever is at an 11° angle and the whole lever cannot be in focus at once. Bring the end of the cantilever closest to the tip in focus.



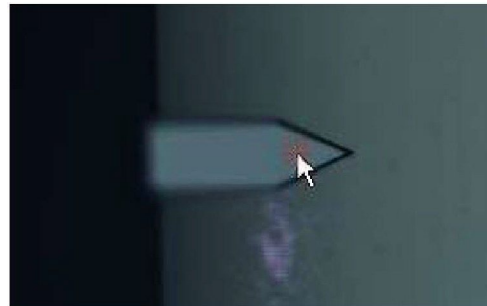
Optional image enhancement and zoom, particularly useful for small cantilevers:

- 10.
- If you want to see the image with more resolution, select *Decimate 1* from the *Options* pull-down menu. This brings all the pixels down from the video camera but will slow the screen update rate.
 - To the left of the *Options* menu is a 'Zoom' button. This button, once clicked, will change the cursor into a magnifying glass. Click on the cantilever to get an enlarged view.
 - Both of these items may improve your ability to focus from the previous step. If you do refocus, be sure to click 'Set' next to the 'Focus on Tip' button.



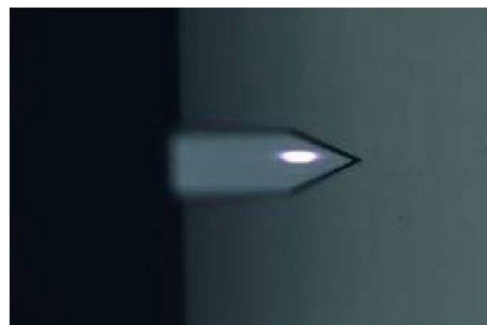
Center laser spot on cantilever:

- 11.
- Click on the 'Spot On' button at the top left of the video window. The mouse pointer will acquire some small red lines.
 - Now click on the center of the cantilever (see figure to right).
 - Alternately, right-click on the center of the cantilever and then select the 'Spot On' option.



Observe spot on lever:

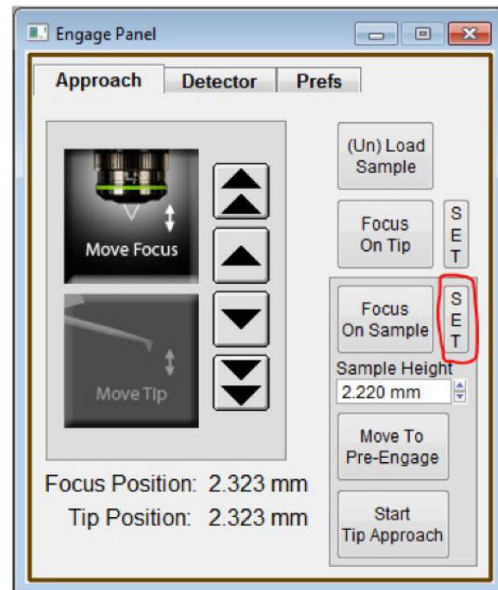
- 12.
- Motors inside Cypher will now move to bring the laser spot where you clicked.
 - The spot position does not need to be perfect here, only roughly centered on the cantilever to produce a decent reflected beam (measured by the Sum signal in the Sum and Deflection Panel).
 - If needed, the spot position will be fine tuned in a later step.



13.

Locate sample surface optically:

- Under the 'Approach' tab on the 'Engage Panel' use the downward 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, hit the 'Set' button next to the 'Focus on Sample' button. Note that the sample height value updates.

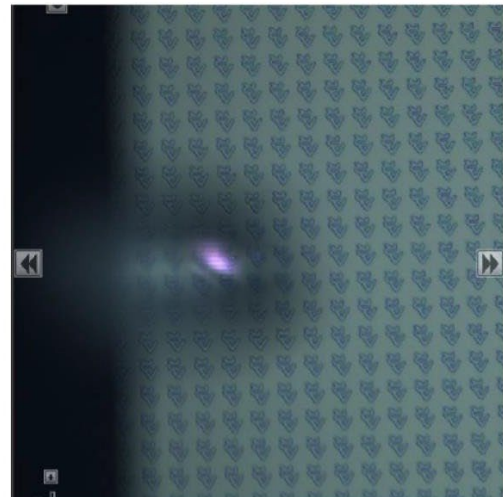


14.

Observe sample surface optically:

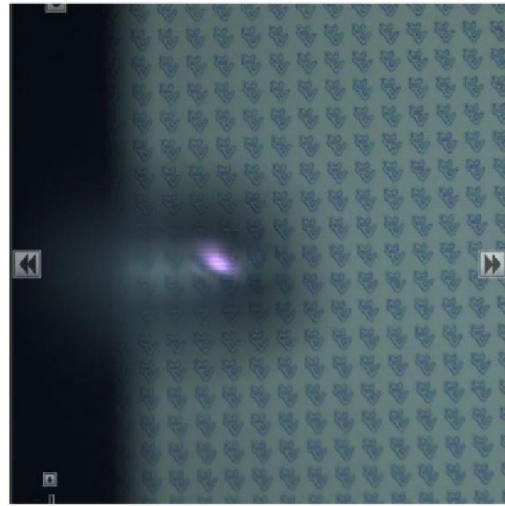
- Features may be hard to see at zoom = 1 and auto decimation turned on. For instance, in the image to the right decimation = 1 and zoom = 2. See [Step 10 on page 29](#) for more information.

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



Question I cannot seem to focus on the surface of my rough sample.

Answer The objective can only motor down a few millimeters before it meets up with the cantilever. If you did not manually move the cantilever close enough you will never get a focused image (such as the one on the right) of the sample. In this case hit the "Focus on Tip" button in the engage panel and repeat Step 22 on page 25.



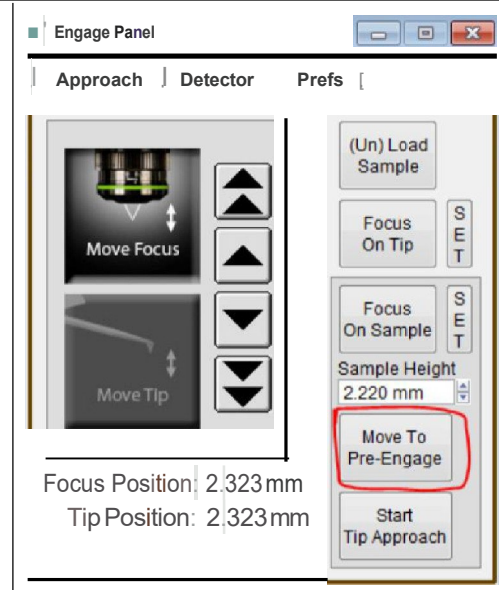
Question I cannot seem to focus on the surface of my smooth sample.

Answer Perfectly reflecting samples may not offer enough feature to allow the focus to be determined. In this case move the Field Diaphragm lever (marked with an F) on the view system until you see a dark circle on the screen. As you motor down this circle will become sharper. When the ring is in focus, as in the image to the right, so is the sample.

Prepare to land the tip:

- Click the 'Move to Pre-Engage' button. Motors automatically bring the tip to 50µm from the surface.

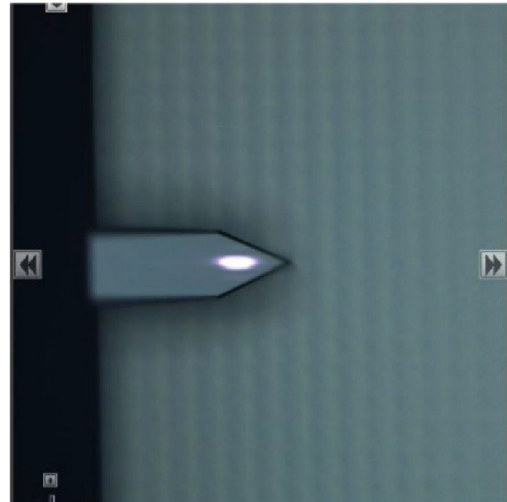
15. WARNING: If you set a bad sample height and/or tip position you may ram your cantilever into the sample and break it. A firmware safety feature immediately cuts motor power when the optical detector fails to measure reflected light from the broken lever. This prevents the cantilever holder from ramming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



Fine tune spot position:

- 16.
- At this point you should adjust the spot position so that it is near the end of the cantilever using the control in the upper left hand corner of the video panel. Note that a decrease in Sum Signal indicates that light is pulling off the cantilever. The latter is undesirable and should be avoided.

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 (See Step 8 on page 28).

**Center the laser on the photo detector:**

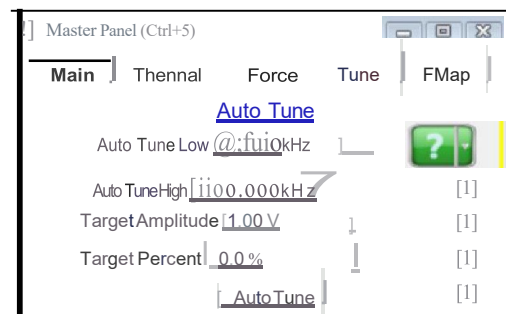
- 17.
- Hit the red button at the center of the video window motion controls and watch the Deflection Signal go to zero. This action causes small motor to steer the laser beam to the optimum position on Cypher's photo detector.

**4.3.2. Tuning the Cantilever and Setting Scan Parameters**

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

Initiate cantilever tune:

- 1.
- Select the tune tab in the master panel.
 - Set the four auto tune parameters (Low, High, Amplitude, Percent) as shown to the right.
 - Click the 'Auto Tune' button.

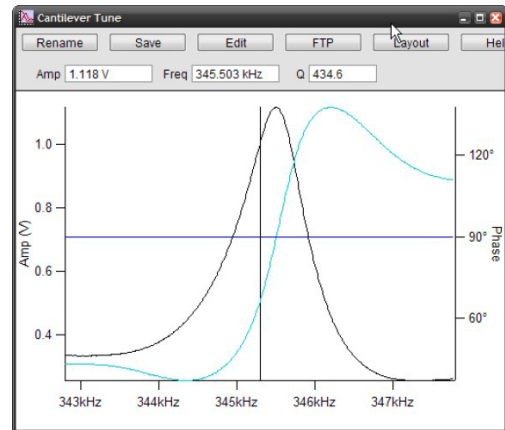


2.

Observe tune result:

- A graph will pop up with the tune result.
- The resonance curve should peak 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 left over silicon/glass debris.



3. **Set scan parameters:**

- Click the 'Main' tab on the 'Master Panel'
- Set the set point value to 700mV, the second item highlighted in yellow in Figure 4.4a on page 37.
- Check that all the other values and check boxes of your 'Main' tab panel are the same as 4.4a.

(a) Master Panel, Main Tab.

(b) Master Panel, Tune Tab.

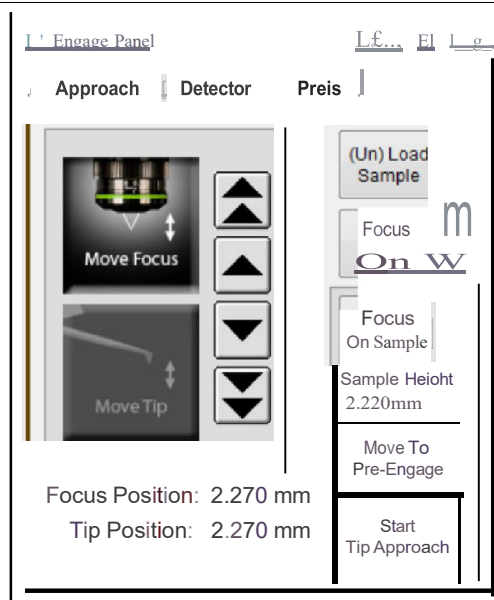
Figure 4.4.

4.3.3. Landing the Tip

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

Start final tip approach:

1.
 - Locate the Engage Panel.
 - Click on the 'Start Tip Approach' button.

**Wait for tip to reach sample:**

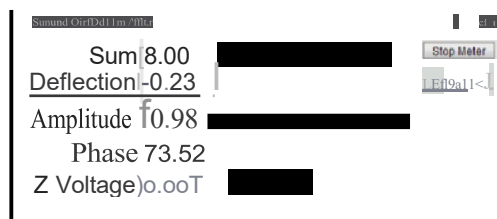
2.
 - For the next minute or so Cypher will systematically move the tip closer to the sample until the set point is reached.
 - You can cancel the approach at any time by pressing AND HOLDING the 'Esc' key on your computer keyboard.
 - When the process is complete, the computer will beep and the tip will be left about half the Z range (about 3 microns) off the sample surface.

Q What's going on during the tip approach?

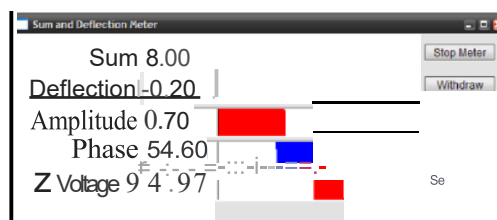
A Cypher executes a series of repeated steps. First the scanner fully extends the sample toward the tip while monitoring the cantilever amplitude. If the amplitude reaches the set point, the process stops. If not, the sample is fully retracted again and motors move the cantilever down by one extension length. The process is repeated until the sample is close enough to the vibrating cantilever to reduce its amplitude to the set point. One final time the sample is fully retracted and the cantilever is motored down just enough so that when the sample is brought back up it will trip the tip amplitude set point at half the scanner's vertical extension range.

Meter check:

- 3.
- Locate the 'Sum and Deflection Panel'(Ctrl + 6).
 - The values should be similar to the figure on the right, typical for a cantilever a few microns off the sample surface.

**Engage:**

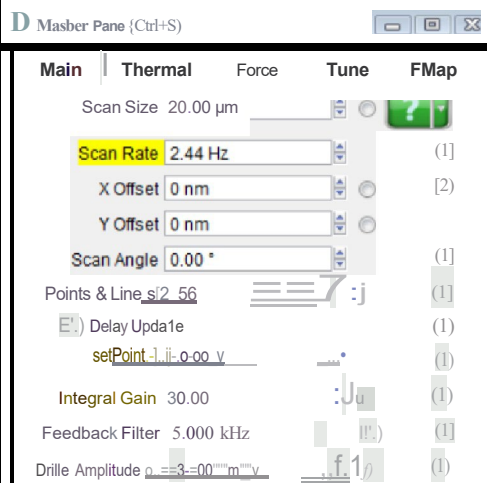
- 4.
- Hit the engage button on the 'Sum and Deflection' meter panel.
 - The scanner will extend the sample into contact with the tip and the 'Sum and Deflection' panel will look like the figure to the right.



Congratulations The tip is on the sample surface.

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

Answer Try to slightly lower the set point (such as 650mV). If the Z voltage in the 'Sum and Deflection' meter panel does not change noticeably (*i.e.* more than a Volt) the tip is 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 ([Step 9 on page 29](#)) you must be sure to set the focus adjustment back to zero, as in [Step 3 on page 26](#). Cypher includes a sensor to see that this has occurred and the software will warn you to zero the focus offset when necessary.



4.4. Imaging

This section will get you scanning and tracking the surface.

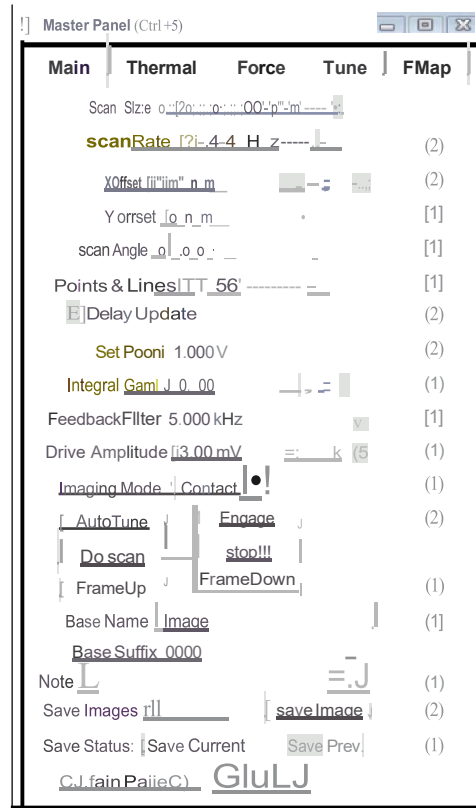
4.4.1. Set-Up and Initial Parameter Selection

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.

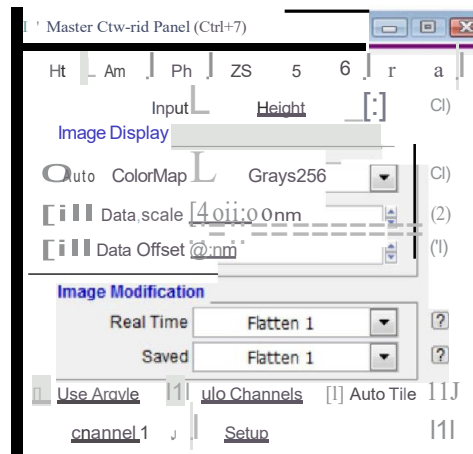
Set up Master Panel:

1.
 - In the 'Main' 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 (can point & lines) and image size is up to you. The figure to the right gives typical values.

**Image channel selection:**

2.
 - Go to the 'Master Channel' panel
 - Select the leftmost tab and confirm the default setting of 'Height' under the *Input* pull-down menu.
 - For the next two tabs do the same for 'Amplitude' and 'Phase'.
 - For the fourth tab, 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 nanometer ; the LVDT sensors are more linear than the piezo actuators, and thus it's a more precise Z measurement.



3. Images are saved to disk automatically at the end of every image if you leave the 'Save Images' check box selected, near the lower left hand corner of the Master Panel in Step 1 on page 40.

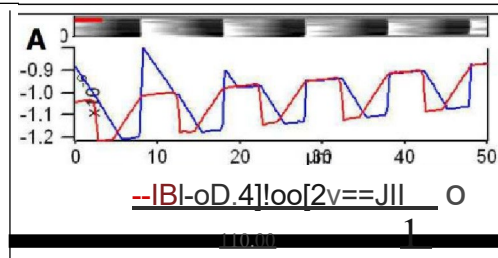
4.4.2. Start Imaging and Parameter Tuning

1.

- Click the 'Do Scan' button on the 'Main' tab of the 'Master Panel', and 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.

Do Scan**Stop!!!****Frame Up****Frame Down****Tip**

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



2.

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**).

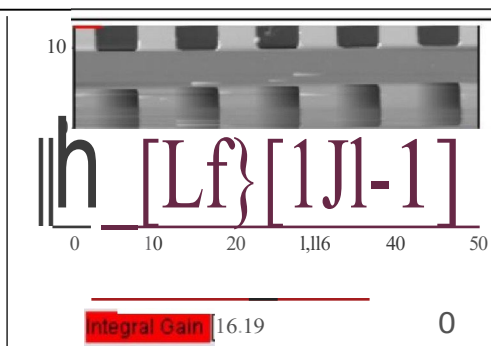
On most samples with relatively slowly changing features, trace and retrace should look the same. In other words, the landscape should look the same if you are flying the exact same route one way or the reverse. The image above shows the two as being quite different; this is an indication that imaging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

3.

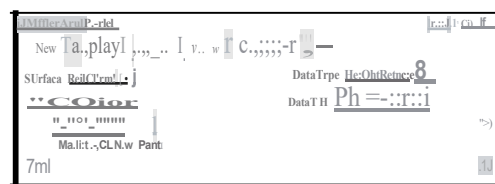
- The goal is to get trace and retrace to fall on top of each other (as shown above).
- Increase the 'Integral Gain' or *i-gain* parameter (second highlighted item in Step 1 on page 40) and see what happens. The match between trace and retrace should improve. The feedback becomes more aggressive and the slope with which the tip "parachutes" down into the pit 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:

4.

- Open the 'Master ArGL Panel' by electing 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 and drag on 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 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. See [Figure 4.5 on page 46](#) for an example of sample movement.

Note These buttons move the sample rather than the cantilever, and so the laser and objective stay in alignment. Be sure to avoid accidentally moving the tip, and remember that the smaller arrow buttons in the upper left hand corner of the Video Panel are set to the cantilever rather than to the sample.

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 (See 1) will 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, then 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 'Frame Up' or 'Frame Down'. A nice way to see the effect of changing imaging parameters can be as follows:

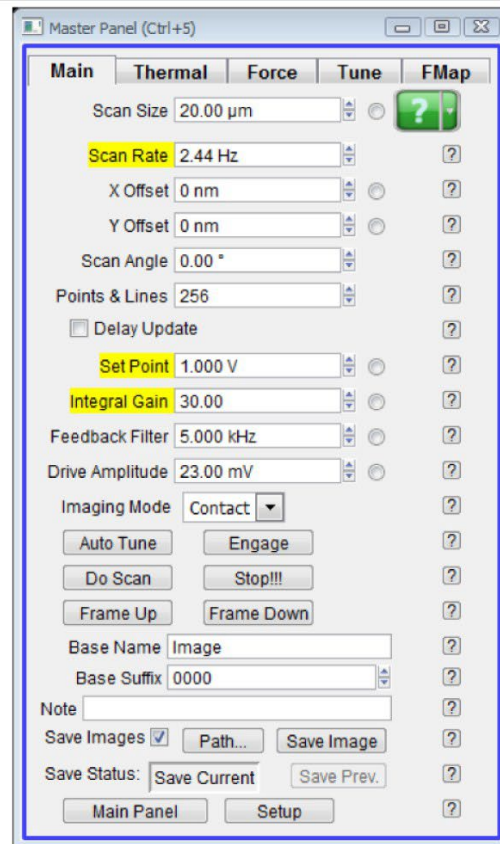
- Check the 'Delay Update' box as described above.
- Click 'Frame Up' and collect a dozen scan lines. Observe the image quality
- Make some changes to the scan parameters (number of points, rate, gains, setpoint).
- Click 'Frame Up' again.
- Observe as the exact same scan region is "painted over" with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

4.5. Stopping Imaging

General Procedure for Stopping Imaging

1.
 - Once a scan has begun, the 'Do Scan' button on the Master Panel will change to 'Last Scan'.
 - Clicking on 'Last Scan' will cause scanning to cease once the current scan has completed.

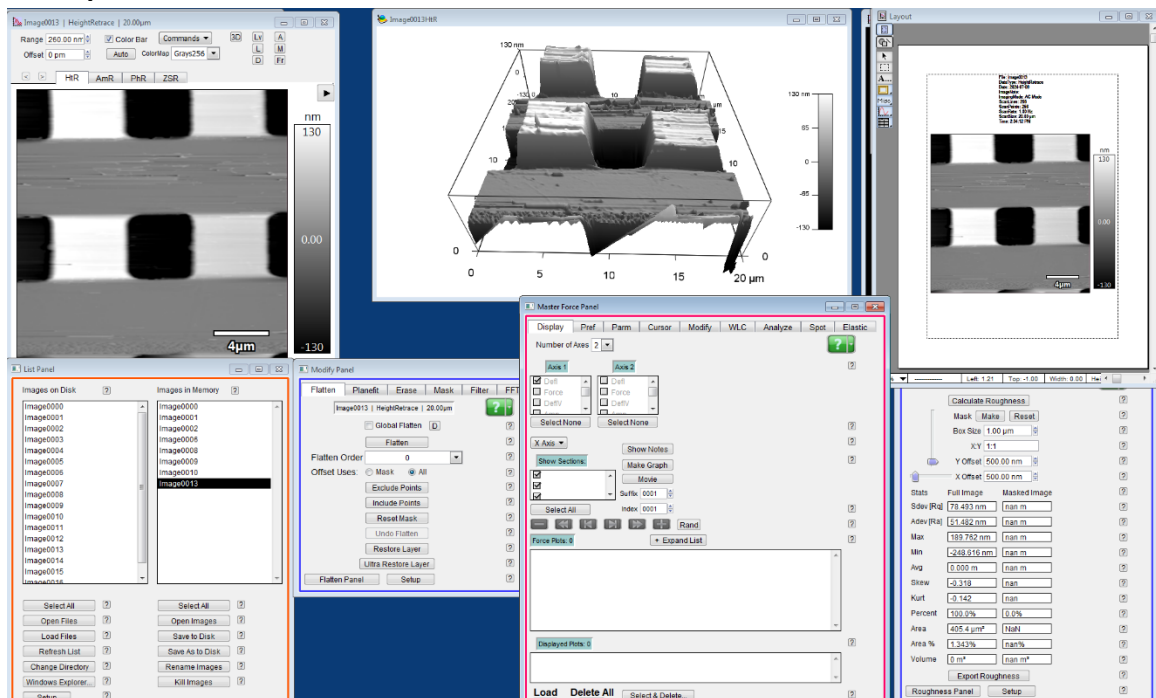
Note 'Last Scan' will change to 'Undo Last' if clicked. Clicking Undo Last will cause the software to keep imaging with current parameters.



Emergency Stopping Procedures

2.
 - 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.
 - Measures such as closing the software, turning off the controller, or unplugging the microscope will stop scanning, but are not recommended except in extreme circumstances because of the complications and the risk of tip, sample, or hardware damage.

Example:



Data Analysis

1. To open saved data, on the menu bar click *AFM Analysis* → *Browse Saved Data* and double click the file to open. The appearance of the data can be changed using the display window.
2. On the display window click *M* to open the *Modify panel*
 - a. On the *Mask tab*, click in the histogram and drag the blue line to create a mask that highlights the features but not the background
 - b. On the *Flatten tab*, check the box that says *Offset Uses: Mask*. Experiment by flattening with the different polynomial orders to improve the appearance of the data. The *Undo Flatten* button can be used to undo the previous flatten operation.
 - c. On the display window click *Commands* → *Export to Layout* to add the image to the layout for saving/printing. You can also click *Commands* → *Export Image as Tif* to create a tif image file of the data.
3. On the display window click *A* to open the *Analyze panel*
 - a. On the *Section tab* click *Draw*. Click and drag on the image to draw a line through features of interest. Drag the two cursors on the *Section plot* and they will appear along the line on the image. Drag the cursors to features of interest to make measurements such as the distance between the two features. You can copy the section plot and associated data to a layout for saving/printing

- b. For this step to work you must have a mask active as described in 2a above. On the *Particle Analysis* tab click *Analyze Particles*. Click *Find Particles* button to find particles that meet certain criteria. *Particle Stats* button gives count and stats of located particles.
4. Click menu bar → *File* → *Save Experiment As* to save your acquisition settings and analysis operations
5. Click menu bar → *File* → *Print Layout* to save your layout as a pdf

When you are finished

1. If you are scanning, follow the procedure under “Stopping Imaging” above.
2. Click *Master Panel* → *Main tab* → *Withdraw*
3. Click *Engage Panel* → *Approach tab* → *Move to Pre-Engage* to remove the tip from the surface. While looking into the microscope to monitor the distance between the tip and the sample, rotate the engage control knob clockwise to raise the tip several mm above the sample surface.



Caution: If you turn the engage control knob the wrong direction, you will crash the probe into the sample!

4. Open the microscope and slide the scanner forward enough that you can remove the cantilever holder by loosening the two screws using the screwdriver stored inside the microscope and turning the cantilever holder counter clockwise.



Caution: If you slide the scanner too far forward it will fall out of the microscope!

5. Rotate the engage control knob counter clockwise to raise the sample stage to its highest position.
6. Remove your sample from the stage.
7. Rotate the engage control knob clockwise to lower the stage to its lowest position.



Caution: If you don't lower the stage far enough, the stage will interfere with the cantilever holder during the next step.

8. Remove your probe from the cantilever holder and replace the cantilever holder on the scanner.
9. Slide the scanner back into the microscope and close the door.
10. Exit the software and log out of the PC