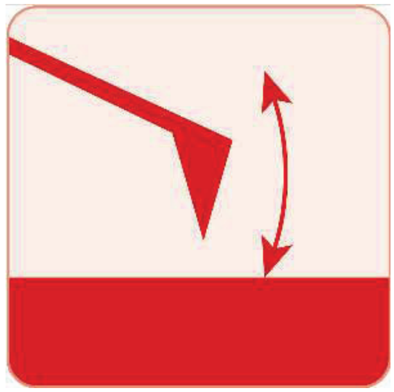
Advanced tuning and image optimization



Chapter Contents

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.

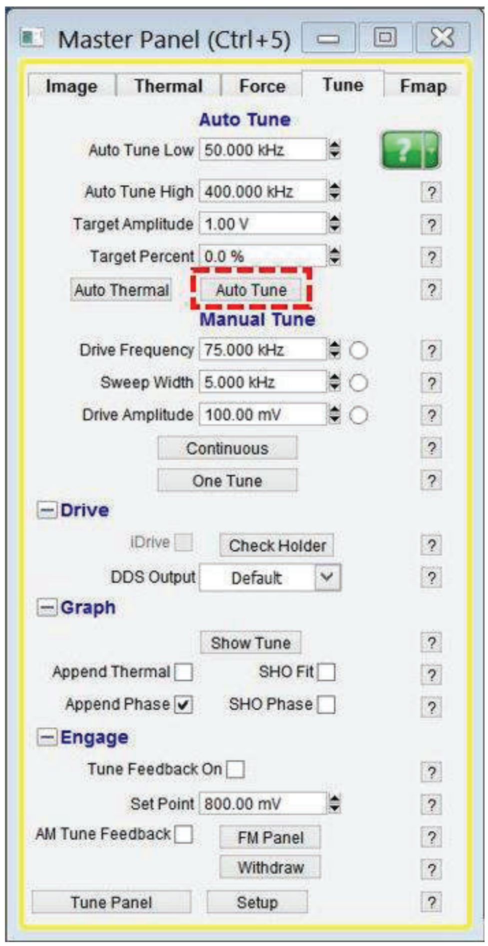


Figure 4.1.: Tune tab

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.

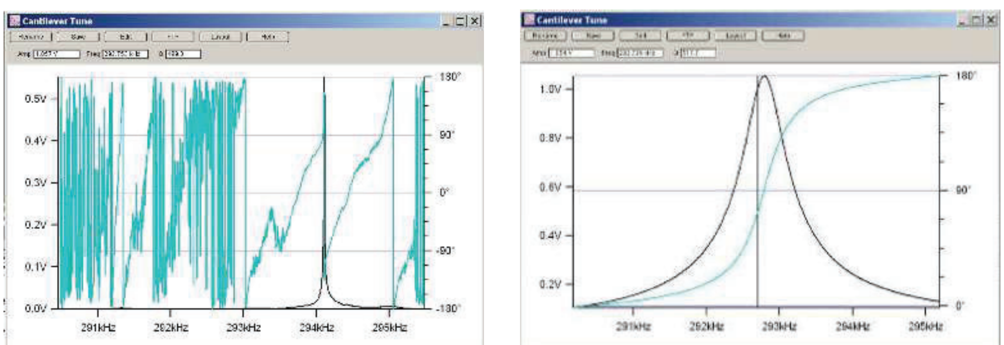
5. Click the 'Auto Tune' button for the frequency sweep to commence. The shake piezo applies a frequency ramp through the Auto Tune low to high frequencies. The cantilever will give the greatest oscillation amplitude at its resonant frequency, allowing the tune algorithm to locate it and determine the 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 300kHz; k= 40N/m; nominal values) cantilever in air

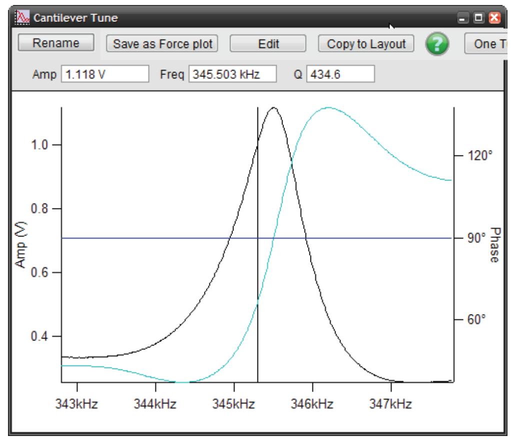
1.1. Auto Tune Troubleshooting

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 tutorial[3]. 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°.

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°.

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:

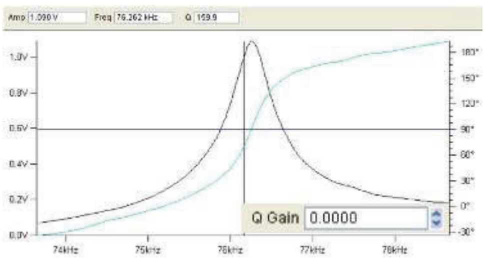
1. In the *Tune* tab of the Main panel, set the *Target Amplitude* to 250mV.
2. Set the *Target Percent* to +10 to +20%; this will set the *Drive Frequency* on the higher (right) side of the cantilever's resonant peak, a position more likely to keep the tip in Attractive mode while imaging.
3. Click 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

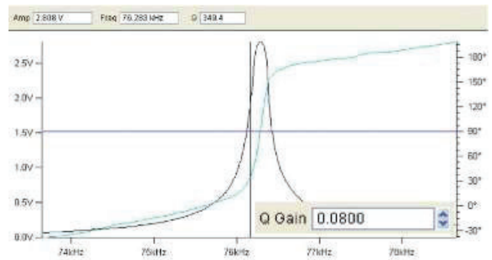
Higher Q resonance peaks favor Attractive mode imaging. Q control gives some control of this.

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.

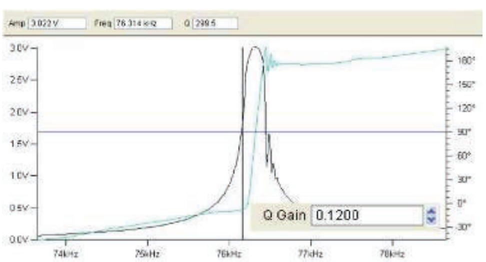
- Figure (a) shows the last tune of an Auto Tune; no Q gain is added.
- Figure (b) shows the response after a slight increase in Q gain. Notice the top of the peak is starting to look more round than sharp; this means the ringing is about to start.
- Figure (c) Ringing shows up to the right of the frequency 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.



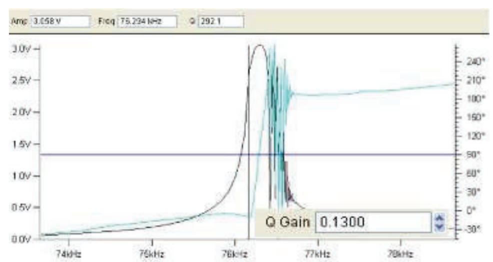
(a) post Auto Tune - no Q gain



(b) Almost ringing



(c) Ringing



(d) Freakout

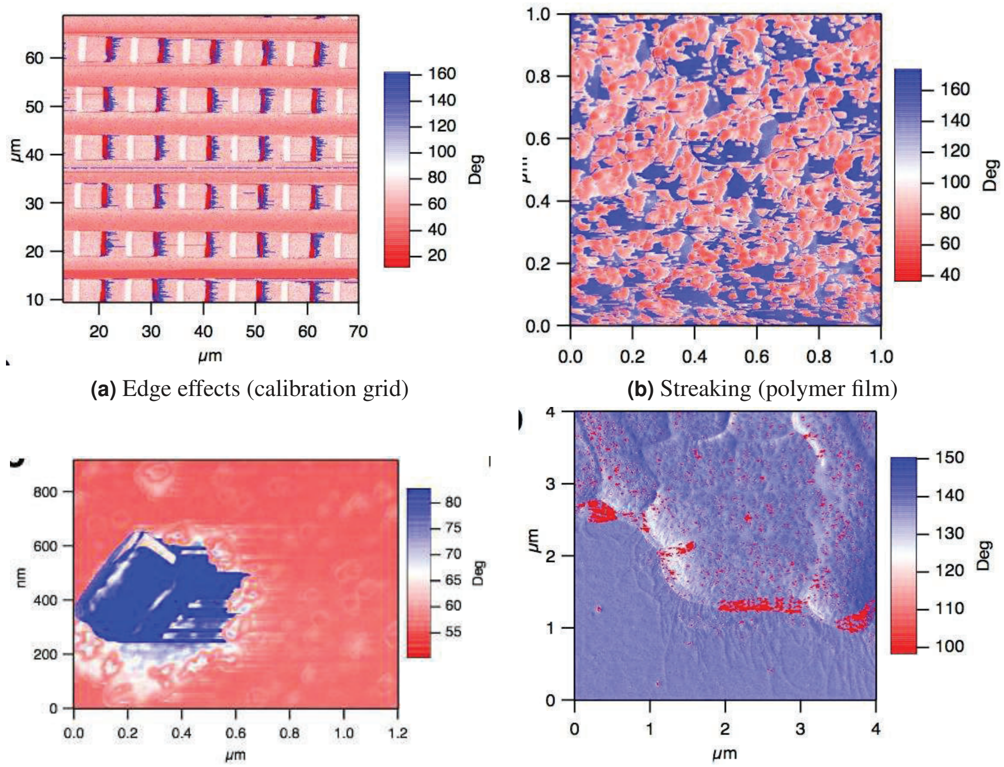
Figure 4.3.

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.



(a) Edge effects (calibration grid)

(b) Streaking (polymer film)

(c) Polymer particle

(d) Bacterial film

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.

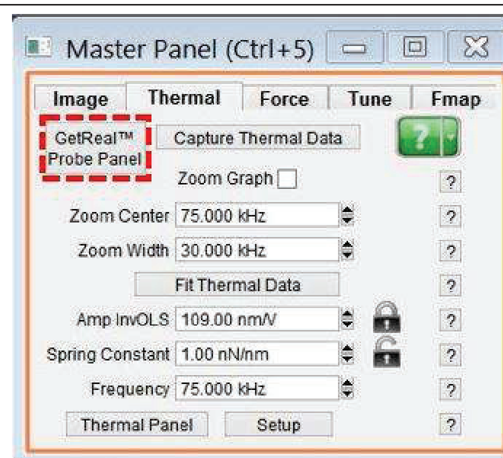
This is accomplished by first determining the optical sensitivity of the cantilever. In a physical sense, this refers to how much the laser point on the photodetector moves relative to the vertical motion of the cantilever.

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.



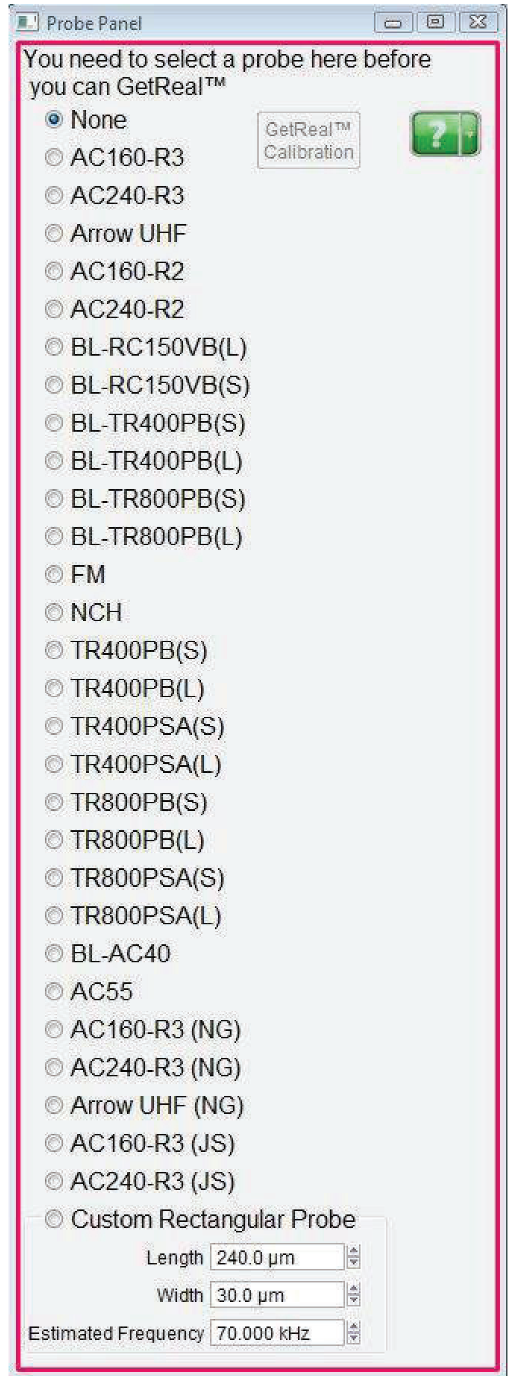
2.

Initiating cantilever calibration:

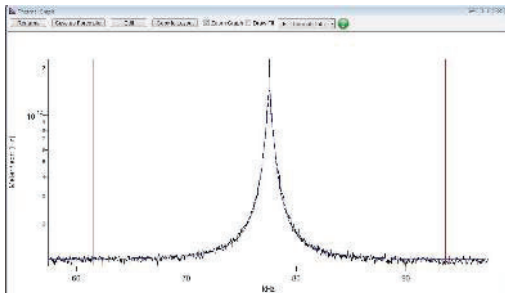
- On the Probe Panel, you will see a list of different cantilevers. Select the cantilever you are using.

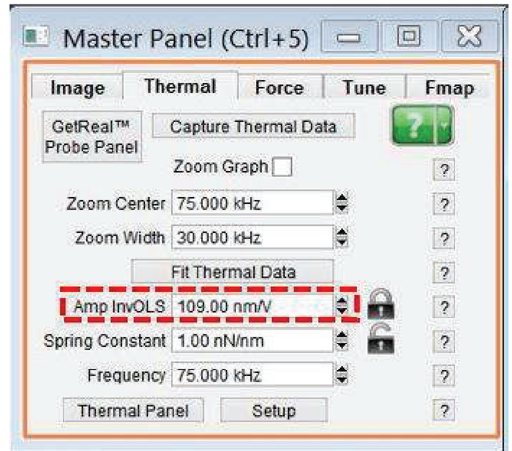
Notes

- 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.

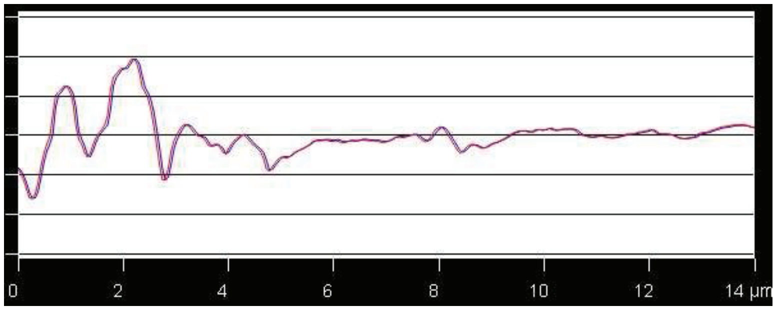
Note "InvOLS" stands for "Inverse Optical Lever Sensitivity." However, the number is the reciprocal of the optical lever sensitivity rather than its inverse.

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.