AFM Standard Operating Procedure

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1.0 Hardware

This section describes the MultiMode Atomic Force Microscope (MM-AFM) and accompanying hardware. The MM-AMF consists of five primary components: AFM head, AFM base, controller, computer, and video optical microscope, as shown in the following images.

Figure 1 MM-AFM system hardware.

Figure 2 OMV system components (Camera, Camera light, and Monitor)
1.1 MultiMode Atomic Force Microscope

Figure 3 shows the AFM itself and its components.
1.2 AFM Cantilever

The central component of an AFM is a cantilever probe on the order of several microns in length. The cantilever is fashioned out of a flexible material with a sharp tip functionalized near one end. The tip of the cantilever is used to probe a sample by physically “feeling” the morphology of a surface. Measurements of surface height, composition and local forces can be used to form images, often with sub-nanometer resolution.

![Silicon Nitride and Etched Crystal Silicon](image)

**Figure 7** Scanning electron microscope images of a silicon nitride probe and an etch crystal silicon probe

**Tapping AFM - Etched crystal silicon probes**
During tapping AFM, a cantilever is vertically oscillated at its resonant frequency as it is raster scanned over a sample. Changes in oscillation amplitude are measured to form surface images based solely on variations in height. Phase changes of the cantilever oscillation can also be monitored to give information about the degree of interaction between the tip and surface. An etched crystal silicon cantilever probe is most commonly used for tapping AFM. It is a relatively stiff material that oscillates at approximately 300kHz. Oscillating a cantilever at a high frequency such as this prevents it from being capture by any surface tension forces on a sample. The high rigidity also makes the silicon probes more brittle than others and is highly delicate.

**Contact AFM - Silicon nitride probes**
In contact AFM, the cantilever is scanned in close proximity over a sample without any applied oscillations. As the tip is scanned, Van der Waals forces or surface roughness causes the cantilever to flex and produce a measurable displacement. Most contact AFM is conducted with probes composed of silicon nitride. These probes are very flexible (low spring constant) which makes them easier to use and more “forgiving” than stiffer etched crystal silicon cantilevers. Silicon nitride probes are offered in a variety of sizes and coatings to accommodate the sample being imaged. A concern with silicon nitride tips is that they are easily captured by the sample’s surface tension (capillary) properties; that is, trapped within a microscopic layer of condensed atmospheric water vapor or a similar substance on the sample surface. This surface tension exerts
considerable force at the probe’s tip. In some cases silicon nitride tips are used for tapping mode, while in liquids.

1.3 AFM Cantilever holders

A cantilevers holder is used to position a probe inside the AFM head and to induce oscillatory motion if needed. The holder can apply an alternating current to the probe which causes the cantilever to vibrate through the piezoelectric effect.

The sample and type of measurement to be performed dictates the choice of cantilever holder. Tapping and contact AFM use the standard probe holder shown in figure 6. Special fluid cell can be employed if a sample is immersed in liquid. Scanning tunneling microscopy can also be performed with this instrument but requires a specialized probe holder.

![Figure 6 Probe holder for the MM-AFM](image)

1.4 AFM Head

Figure 4 illustrates the MM-AFM head along with the laser detection system and various adjustment knobs. To measure and quantify the motion a cantilever, a laser is directed at a reflective coating on the top of the cantilever. Reflected light is then detected by a position sensitive photodiode array.

The head and attached X-Y stage are kinematically mated to the piezo scanner via three contact points. The X-Y stage is used for position the cantilever over a desired location of the sample while the piezo controls the raster scanning motion. A second X-Y stage is located at the base of the AFM allows the position of the video optical microscope to be adjusted. A pair of retaining springs secures the head, allowing the sample to be raised and lowered using the Z translation switch.
Photodiode array—Figure 5 shows the photodiode arrangement in the AFM head. The four elements of the photodiode provide different information depending on the operating mode. In all modes, the combined signal from all four elements is shown on the elliptical SUM display. The differential signal between the top two elements (A) and the two bottom elements (B) provides a measure of the vertical deflection of the cantilever during contact and tapping AFM. Similarly, the differential signal between the sum of two left photodiodes (C) and the sum of the two right photodiodes (D) provides a measure of the torsional deflection of the cantilever (lateral force microscopy) during contact mode.

2 Instrument and Sample Preparation

2.1 Powering up the system

- Turn on the PC and PC monitor if not already on.
- Turn on the Controller by switching switch 1 shown in Figure 2.
- Turn on the camera monitor by switching switch 2 shown in Figure 2.
- Turn on the microscope camera light to medium intensity (Figure 2).
2.2 Prepare the Sample

Verify that your sample will fit atop the scanner tube and is less than 8mm thick. If it is your first time operating the microscope, we recommend that you image the calibration sample provided with the instrument (usually a 1μm-pitch platinum grid of 100nm step height). The calibration sample or other small samples is placed on a 15mm diameter steel disk (sample puck). These disks are attached to the magnetic sample holder, located atop the piezo scanner.

1. Obtain a steel sample puck and place a piece of 2-sided adhesive tape on one side. This leaves a patch of the two sided adhesive on the steel sample disk, which will hold the sample in place.

2. Using tweezers, place your sample firmly on the sticky tape (see Figure 8) so the area of interest is close to the center of the puck (the sample can only be manipulated 2mm in the x and y directions once is on the stage). Be sure the sample is as flat as possible and that is does not protrude off the sides.

![Figure 8 Gently Press the Sample onto the tape until secured](image)

2.3 Load the Sample

Mount the sample puck containing your sample (or calibration standard) on the scanner tube. An internal magnet holds the puck in place (Figure 9). At this point, take note of the three ball bearing in a triangle formation outside of the sample stage. These ball bearings will be used to hold the probe holder in place as describes in step 2.2.d.
2.4 Load Probe in Probe holder

While using tweezers, carefully examine the chosen AFM probe and locate the following features. One side of the probe has a reflective coating (usually gold) used to reflect the laser beam towards the detector. On one edge of the probe, the cantilever (barely visible to the naked eye) protrudes horizontally.

1. Next, obtain the probe-holder shown in figure 6 and turn it upside down with the groove facing up as shown in figure 10. Apply gentle downward pressure against the probe-holder to depress the button on the opposite side and lift the spring clip.

2. With the gold platted surface of the probe facing downwards and the cantilever facing away from the spring clip, carefully slide the probe into the probe holder's groove under the spring clip until it is positioned squarely against the innermost edges.
3. Finally, lower the spring clip by releasing pressure against the probe-holder. Check that the probe's edges are flush with the back of the groove and flat against one side (this keeps the probe's cantilever oriented in the correct direction).

2.5 Install the Probe holder

After the probe-holder has been loaded, the holder is placed inside the AFM head and clamped into position using the clamping screw behind the AFM head.

1. Before installing the probe holder, verify that the AFM head is sufficiently raised so the sample does not come in contact with the tip while the holder is loaded. Even the slightest contact is likely to damaging or break the highly delicate tip. Avoiding this by raising the AFM head with the Z translation switch (see Figure 11).

![Figure 11 Tip up/down switch on the MM-AFM base](image)

2. Carefully insert the loaded AFM probe holder into the AFM head with the groove facing downwards. Move the holder to the back of the cavity while keeping the handle raised slightly.

3. Gently lower the probe-holder into position and allow it to come to rest on the three ball bearing located on the bottom of the sample cavity (see figure 12).

4. Locate the clamping screw on the back side of the AFM head (figure 12 bottom left). Rotate the knob counter-clockwise to secure the probe-holder in place. The screw does not need to be tight. The procedure for loading probe holders into the AFM head has been summarized in figure 13.
2.6 Use of the video optical microscope

The video optical microscope is used not only for observing the surface of a sample but also for alignment of the AFM laser detection system. Three focal planes can be brought into focus: the cantilever, the sample surface and the shadow of the cantilever.

1. Locate the focus adjustment knobs above the AFM (see figure 2). The inner knob adjusts the fine focus of the image while the larger outer knob adjusts the coarse focus.

2. With the coarse focus knob, find the three focal planes shown in figure 13 A, B and C. The image of the cantilever and cantilever shadow are highly similar and can be easily confused. Differentiating them can be achieved by observing the physical height of the optical microscope. The cantilever image plane is higher than the shadow plane. The height of the microscope will match these heights.

   In the following step, the cantilever is lowered until it is several hundred microns above the sample surface. Much care should be taken during this step, as lowering the cantilever too far will cause the tip to crash into the surface.

3. With the sample surface in focus, lower the cantilever using the Z translation switch. Do this until the cantilever is in the image and slightly out of focus, similar to figure 13B.
Figure 13 Image planes available to the video optical microscope. A) cantilever, B) sample surface, C) cantilever shadow, D) cantilever shadow with illumination turned off.

2.7 Laser Alignment

Figure 4 shows the functionality of the adjustment knobs on the AFM head. Using the laser alignment knobs, direct the laser onto the end (as far to the right as possible) of the cantilever. This step may take several attempts and will require practice. A misaligned laser can produce reflections off various surfaces which cause confusion due to multiple beams in the sample cavity. A helpful technique is to use the cantilever shadow for alignment with the illumination set to low intensity or turned off (figure 13D). A clear image of the laser position relative to cantilever can be seen with these settings.

2.4 Maximizing the SUM Signal and Verifying laser Alignment
While looking at the signal sum display, adjust the mirror tilt lever on the back of the AFM head (Figure 16). Maximize the SUM reading signal (BE CAREFUL! Avoid moving the head while adjusting the tilt mirror). The SUM signal should be approximately 5.0-9.0 Volts for silicon nitride cantilevers and 3.0-8.0 for etched silicon cantilevers. The intensity of the signal varies with many factors, especially with the coating material. It is important to note that it is possible to see a large response on the elliptical bar graph without having the laser beam on the cantilever (example: reflecting on a shiny sample surface), so it is important to visually verify that the laser beam is on the cantilever and not rely on the elliptical bar graph alone. Attempting to engage with the laser beam improperly aligned will usually destroy the cantilever.

A quick way of checking laser alignment is to place a piece of paper in front of the photodetector (Figure 16) to ensure the laser reflection is solid. Look for a small, symmetric laser spot. (Note: Because the slip of paper prevents light from reaching the photodetector, the sum signal cannot be monitored while using this method.) After verifying the laser position, remove the piece of paper from the head and continue to maximized the SUM display with the laser alignment knobs until maximum signal is achieved.
2.5 AFM Voltmeters

The elliptical bar meter indicates the total voltage generated by the position sensitive photodetector. This value is displayed in all modes. The bottom numerical meter (Figure 18) reads differences in voltages between various segments of the photodetector. With the mode selection switch on the AFM base (see Figure 3), toggle to AFM & LFM (contact mode). The bottom numerical display now indicates the voltage difference between the left and right segments (C-D). The top display indicates the voltage difference between the top and bottom segments (A-B). Next, change the mode to TM AFM (Tapping Mode). The bottom numerical display now indicates the top-bottom difference and the top display indicates the RMS output signal of the AFM.

![Figure 18 SPM voltage display.](image)

2.6 Photodetector Signal Setting

- **Contact Mode**: Toggle the mode selector switch to **AFM & LFM** (the LED on the face of the AFM base should turn red). Use the photodetector horizontal adjustment knob (Figure 16) to bring the displayed value to **zero** (bottom display). Use the vertical adjustment knob to make the top signal signal approximately **-2V** for (SiNi cantilevers) or **-0.5** and **-1.0** (Si cantilevers).

- **Tapping Mode**: Toggle the mode selector switch to **TM AFM** (the LED on the face of the AFM base should turn green). Use the top knob to make the vertical difference signal **zero** (bottom display) and allow the software to adjust the RMS amplitude.

3 AFM Imaging

**Start the Microscope Program**

1. Log in into the computer:
**Username:** nanoscope user  
**Password:** nanoscope

2. To start the NanoScope software, double-click the NanoScope startup icon on the computer desktop. The NanoScope software window will span two monitor displays. This large window will contain all the areas and panels you use to control the microscope and analyze your results.

3. Click on the icon to select real-time window.

At this point you may want to check the interactive **AFM Model, Driven Oscillator** and **Probe Simulator** programs available on the computer desktop to understand the effect of the scan parameters.

### 3.1 Contact mode AFM

**Review of General Operating Concepts for Contact Mode**

The AFM system is comprised of the scanner and the AFM detection system. The scanner houses the piezoelectric transducer. The piezo element physically moves the sample in the X, Y and Z direction. The detection system consists of a laser which generates a spot of light that is reflected off of a microfabricated cantilever onto a mirror and finally into a photodetector (see Figure 19). The position of the spot is determined by circuitry which generates a voltage from the difference between the photodiode segments (A - B). The circuit outputs a voltage ranging from +10V to -10V depending on the position of the spot on the two photodiodes. The AFM system maintains the tip at the end of the cantilever in contact with the sample surface. The sample is scanned under the tip in X and Y. Features on the sample surface deflect the cantilever, which in turn change the position of the laser spot on the photodiodes. This position change is read by the feedback loop. The feedback loop moves the sample in Z to restore the spot to its original position.

1. A flat portion of the sample surface is scanned beneath the tip left-to-right, maintaining the laser beam at the center of the photodiode array.

2. As the tip encounters a raised feature, the cantilever is pushed up and the laser beam is
deflected onto the “A” portion of the array. With the “A” photodiode receiving an increased portion of the laser light, its voltage increases while portion “B’s” decreases (A > B).

3. The Vertical Deflection (A-B) voltage differential is sensed by the feedback electronics, causing a dropped voltage to the Z piezo crystal—the piezo retracts. As the Z piezo retracts, the cantilever recenters the laser beam onto the photodiode array (A = B).

4. As the tip encounters a decline in the sample topology, the tip drops. This directs more of the beam onto the “B” portion of the photodiode array. With the “B” photodiode receiving an increased portion of the laser light, its voltage increases while portion “A’s” decreases (A < B).

5. Again, the Vertical Deflection (A-B) voltage differential is sensed by the feedback electronics, increasing voltage to the Z piezo crystal—the piezo extends. As the Z piezo extends, the tip is pushed down until the laser beam recenters on the photodiode array (A = B).

The AFM always first engages in the repulsive region of its operating range. In other words, the cantilever exerts a positive pressure on the sample surface. The AFM block diagram shows the relationship between the cantilever movement and the laser spot on the photodiode array. The diagram shows that the spot moves onto “A” when the cantilever is pushed up. The initial setup is to have the Vertical Deflection (A-B) voltage about 2-3V more negative than the Setpoint voltage. Veeco recommends starting with the Setpoint voltage set to 0V and the Vertical Deflection (A-B) set to -2V. The reason for this is that 0V is the middle of the control range. The indication of a good engagement is a distinct jump of about 1V from the Vertical Deflection (A-B) voltage to the Setpoint voltage.

The displayed height image is an average of the corrections made to Z in a given display period (number of samples menu item). The two gains are set to values to effectively “tune” the feedback response to the particular sample topology. This will set response time of the system so that there is no difference between the SPM's signal and the setpoint reference during scanning.
Proportional gain—The computer multiplies the value read from the comparison circuit each time the A/D converter is read by this number. It is the high frequency feedback control.

Integral gain—The average of A/D readings is multiplied by this number. This is the low frequency feedback control. One of the easiest ways to set the gains properly is to view the input of the feedback loop in scope mode. This means displaying the STM current, the AFM deflection, or the TMAFM amplitude signal. Then raise the gain values until the input of the feedback is minimized. Note that this will result in an image that shows only large transitions in Z; this is normal. There will always be a time lag between the input and the output (height data) of the feedback loop. Usually, the Integral gain is the most sensitive control. Raise the gains together until the input signal (current, deflection or amplitude) is minimum. Don't set them so high as to cause oscillations in the image. Oscillations are an indication of too much feedback correction voltage sent to Z. This is generally known as feedback oscillation. The Proportional gain can usually be set about 20 percent higher than the Integral gain, but it is not required.

3.1.a Suggested Initial Control Settings

Enter the initial settings as shown in Figure 19.

Control details:

X Offset, Y Offset: Translates the scan area in X and Y directions without changing the scan size.
**Scan Angle:** Controls the angle of the fast scan direction of the scanner with respect to the X-axis.

**Samples/lines:** Determines the number of data points or pixels in X and Y (128, 256, or 512).

**Scan rate:** Controls the rate at which the cantilever scans across the sample area.

**Data Scale:** Controls the vertical scale of the full height of the display and color bar. This parameter does not affect the real-time operation of the microscope, only the expansion and contraction of the color scale in image mode or the window height in scope mode.

**Deflection setpoint:** In contact mode AFM, increased Set point yields higher tip-sample forces. The Set point can be adjusted to increase or decrease the cantilever deflection and, therefore, the contact force of the tip on the sample.

### 3.1.b Initiate the Engage Command

Select **Motor > Engage** (or click the **Engage** icon).

A pre-engage check, followed by Z-stage motor sound should be observed. If for any reason it does not engage when the tip has moved for more than 100μm abort engagement and Engage again. Assuming the tip is better positioned before engaging again, an image should begin to appear on the image monitor.

When the probe engages the surface, the screen on the right changes and shows the image scan. Change the Deflection Setpoint to press a little harder on the surface. Watch the Z Center Position bar on the right monitor. If the Z Center Position Voltage changes by more than a few volts, you have a false Engage. Left click on the Withdraw icon. Left click on the Green ‘Engage’ icon and try again.

If the Z center is fluctuating, then the image will be unstable. This happens when you have not adjusted either your setpoint offset properly (disengage-minus-setpoint, initially set to -2), misalign the laser or the cantilever has picked up some dust on it.

After good engagement is obtained, gradually reduce the setpoint offset to give a smaller contact force. A large offset between the setpoint and disengaged signal is recommended during a first try, because sometimes there are difficulties in engagement that can be reduced with a larger offset. On subsequent engagements, try smaller signal offsets as confidence in use of the instrument is gained.
3.2 Tapping mode AFM

3.2.a Suggested Initial Control Settings

Enter the initial settings as shown in Figure 20. Note that:

- The **Amplitud Setpoint** is initially set by the software during engagement. It may be adjusted up or down afterward.
- The **Drive frequency** varies according to the length and stiffness of each cantilever. TappingMode cantilevers are shipped in boxes stamped with their resonant frequency (200–400 kHz). The **Drive frequency** should be set close to (± 50 kHz) this value during cantilever tune up.

![Figure 21 Initial settings in Tapping Mode AFM](image)

3.2.b Cantilever Tune

Click on View/Cantilever Tune (or on the Cantilever Tune icon). To Auto tune the cantilever (i.e., allow the software to automatically find the resonant frequency), make sure the Start Frequency and End Frequency cover a range over the expected resonant frequency for your tips (usually listed on the box). For our tips, the resonant frequency is usually around 300Hz and
typically Auto tune is performed with a Start Frequency of 100Hz and an End Frequency of 500 kHz. Target Amplitude should be 2-3 volts.

Click on “Auto Tune” bottom. A “Tuning...” message should appear and then disappear once Auto Tune is done. The right hand monitor will display a plot of amplitude vs. frequency (the white curve) while the Auto tune is working. You will see the software choose the center of the largest peak for the drive frequency. Auto tune will also set the Drive Amplitude to reach the Target Amplitude. You can view the shape of the resonant peak by changing the frequency sweep width (the range on the x-axis of the graph). A good tip usually has one large mostly symmetric peak.

Click on Image mode when finished tuning. The top number on the front of the AFM base displays now shows the amplitude of the resonating tapping mode cantilever and should be close to the target amplitude chosen in the Auto Tune control box.

3.2.c Initiate the Engage Command

Select Motor > Engage (or click the Engage icon).

4 Optimize the image by adjusting the scan parameters

Select View/Scope Mode (or click on the Scope Mode icon). Check to see if the Trace and Retrace lines are tracking each other well (i.e. look similar). If they are tracking, the lines should look the same, but they will not necessarily overlap each other, either horizontally or vertically. If they are tracking well, then your tip is scanning on the sample surface. You may want to try keeping a minimum force between the tip and sample by manually adjusting the Setpoint. Click inside the Deflection Setpoint box and use the left or right arrow keys to adjust it. You want to pull the tip off the surface by moving the Deflection Setpoint towards the starting value.

CONTACT: The vertical difference started at –2, and moved to 0 when the tip touched the surface. To minimize the force on the sample, use the left arrow key to decrease the Deflection Setpoint (head towards –2).

TAPPING: The resonant amplitude started between 2-3V and was damped (reduced) when the tip touched the surface. To minimize the force on the sample, use the right arrow key to increase the Deflection Setpoint (head towards 2-3V).

When the tip pulls off the surface, trace and retrace will no longer track each other. If you keep going, trace and retrace will look completely flat, and the tip position indicator will show the tip is fully retracted.
Reverse direction to manually approach the surface (i.e. contact – increase the Deflection Setpoint; tapping– decrease the Deflection Setpoint) until the Trace and Retrace follow each other again. To make sure that the tip will continue to track the surface, it is best do one or two arrow clicks more. Then choose View/Image Mode (or click on the Image Mode icon ) to view the image.

If trace and retrace are not tracking well, adjust the Scan Rate, Gains and/or Deflection Setpoint to improve the tracking. Try reducing the Scan Rate to the lowest speed with which you feel comfortable. For scan sizes of 1-3μm try scanning at 2Hz; for 5-10μm, try 1Hz; and for large scans, try 0.5-1.0Hz.

Next, try increasing the Integral Gain using the right arrow key. As you increase the Integral Gain, increase the Proportional Gain as well (Proportional Gain can usually be 30-100% more than Integral Gain). The tracking should improve as the gains increase, although you will reach a value beyond which the noise will increase as the feedback loop starts to oscillate. If this happens, reduce the gains until the noise goes away.

TAPPING: The Drive Amplitude could be adjusted after engagement. Often times there will be a significant amount of strong noise. Increasing the Drive Amplitude will take care of this (typical value 50mV, but it could vary from 30mV to 1V)

If Trace and Retrace still do not track satisfactorily, the last thing to try is to change the Deflection Setpoint to push the tip harder onto the surface.

Once the tip is tracking the surface, choose View/Image Mode (or click on the Image Mode icon ) to view the image.

4.1 Set Desired Scan Size, Scan Angle, and Offsets

Once the scan parameters are optimized, scan size and other features may be adjusted for capturing images for analysis. When changing the scan size value, keep in mind that the scan rate will need to be lowered for larger scan sizes.

5 Saving Images

5.1 Capture the image

To record and capture an image, click on the camera icon (or select Capture/Capture). The status line on the bottom of the control monitor will read “Capture: On”. If any parameters are changed as the image ids being captured the status line will change to read
“Capture: Next”. This means that it will scan to the end of the current image and then capture the following image. If the current image is required then the capture can be forced by clicking on the camera icon again. The status line will read “Capture: Forced”

A captured image will automatically be given a name (date and time of capture) and will be save in the !:\ directory (with the extension.001).

Remember to disengage the tip before manipulating images.

5.2 Image manipulation

To get the image manipulation part of the program, click on the off-line mode icon on the right hand side of the NanoScope Control Window. Images must be move to your own folder on the c:\afm-data\username directory before proceeding. Images that are left on the !:\ directory may be deleted at any time.

- Go to c:\afm-data\
- Under file drop-down menu create a directory “your name”
- Go back to !:\ directory and highlight your files.
- Under file drop-down menu move files to c:\afm-data\”your name” directory.

The image can be then manipulated in many ways and a lot of useful information can be derived from the initial image. The nature of the manipulations depends on the project, so this should be discussed with a member of the technical staff before proceeding.

6 Imaging Problems

6.1 Contact and Tapping Mode Problems:

Streaks appear on the trailing edge of surface features Streaks are an indication of the tip not tracking the surface due to:

1. the scan rate is too fast
2. the gain settings are too low
3. there is not enough imaging force It can also be any combination of these things. Try one of the following to try and eliminate the condition.
1. Reduce the scan rate. Typically it should be around 1-3 Hz.
2. Increase the integral and proportional gains. This will speed up the response time of the Piezo transducer.
3. Increase the amount of tapping force on the surface. This will probably be the most effective. However be careful when doing this on soft samples, the sample surface can be disturbed even though the force is very small. (contact: increase setpoint; tapping: decrease setpoint voltage)
Multiple or repeating patterns

The tip is probably chipped. This is usually caused by too much imaging force on the surface.

1. Change the tip.
2. Operate with a minimum force between the tip and the sample.

6.2 Tapping Mode Problems:

(Both problems can be fixed by following the same 5 step solution at the end.)

Line across the image

Lines in the X scan direction may be caused by the tip sticking to the surface, this condition can be remedied by increasing the RMS voltage. Working with a larger RMS voltage has the effect of giving the tip more energy to pull off the sample surface.

There are rings around features on the surface

This effect might also be described as the image looking as though it’s half way under water. This is caused by operating with a drive frequency a little too close to the cantilever resonance.

1. Set the Setpoint voltage to a greater value than the initial RMS amount was set prior to engagement. This will cause the feedback loop to retract the sample away from the tip. The Z center position will go to the Retracted end of its range. The image on the display monitor will also disappear. The reason for doing this is that the initial cantilever tune frequency and the RMS voltage were chosen when the cantilever was relatively far away from the sample surface. The frequency is sometimes affected by forces between the tip and the sample surface and needs to be fine-tuned.

2. Use the arrow keys to increment the drive frequency while watching the RMS meter. Adjust the drive frequency until the RMS voltage is a maximum.

3. Use the arrow keys to lower the drive frequency until the RMS voltage reduces by about 0.2V. This ensures that you are operating on the low side of the cantilevers actual resonance. We do this primarily because it seems to decrease the sticking of the tip to the surface.
4. Adjust the Drive Amplitude menu item to the desired RMS amount. Usually 3-4 volts RMS is good. The RMS voltage is displayed on the upper meter of the base of the TM AFM.

5. Set the Setpoint Voltage menu item equal to the RMS voltage. Use the arrow keys to increment the Setpoint Voltage lower until the image appears on the display monitor. You will notice that the first thing that the display shows will be an undefined or blurred image. Continue to lower the setpoint voltage until the image “pops” into clarity. At this point the amount of tapping force is strong enough to push the tip through the fluid layer to actually touch the sample surface. The tapping force is increased by further reducing the setpoint voltage.

7 Ending the session

1. Stop scanning.
   - Click on the disengage icon.
   - Push UP on the Motor Control Switch on the SPM and raise the tip at least 4mm above the sample.
2. Remove probe holder.
3. Remove the probe (tip) from probe holder and place the probe in a safe place to be reused in future experiments.
4. Turn off Controller
5. Turn off Camera light
6. Turn off OMV monitor
7. Close Nanoscope Program