

## Veeco Multi-Mode AFM Training Guide

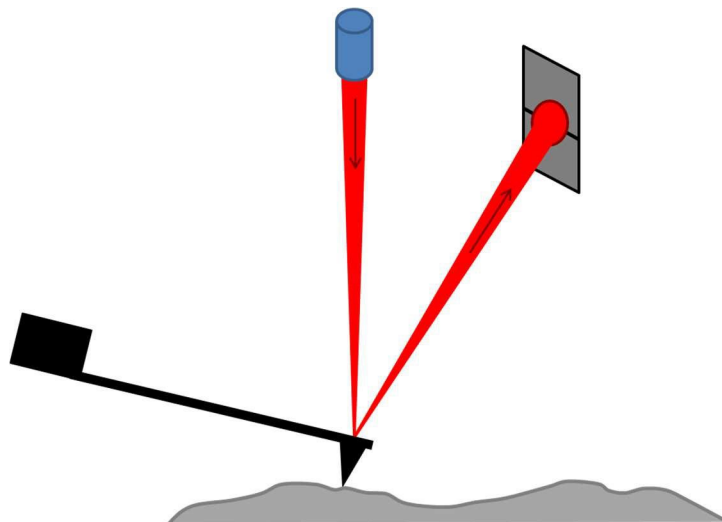
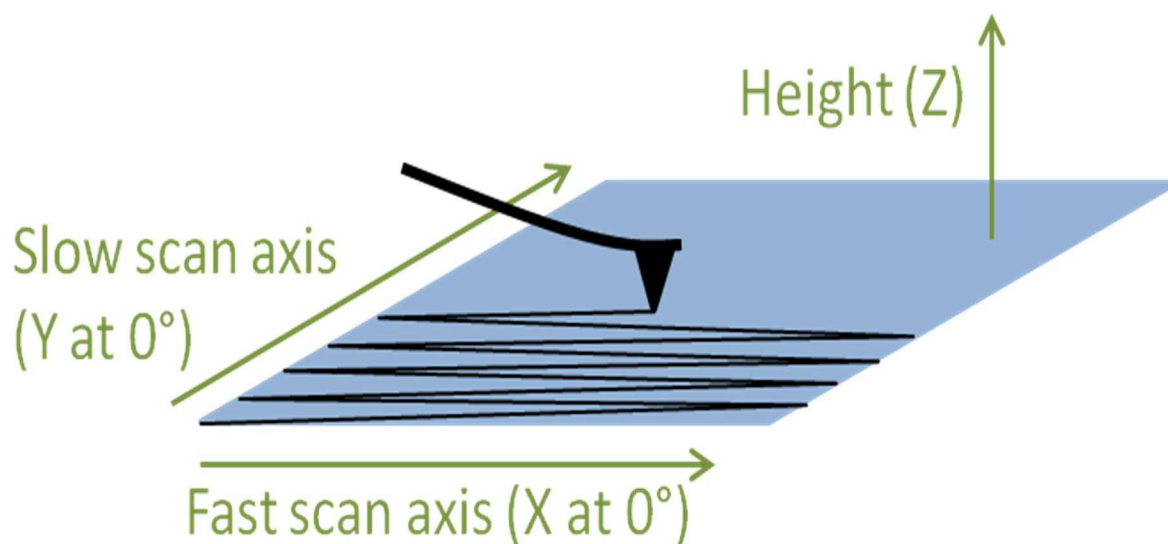


Figure 1: AFM probe scanning the sample.

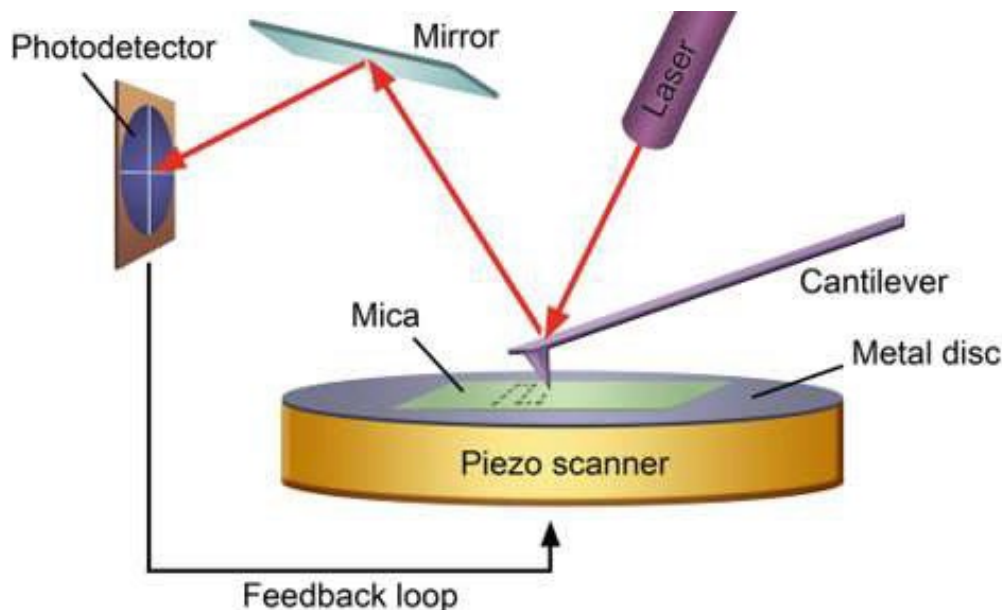


### **AFM Theory**

An Atomic Force Microscope (AFM) makes very fine-scale images of surfaces. A flexible probe with a sharp tip is scanned back and forth across the sample's surface. The mechanical interaction of the probe with the surface is used to generate a 3D map of the sample surface. The AFM is primarily run in one of two basic modes; contact and tapping. In contact mode the AFM tip is in continuous contact with the surface, whereas in tapping mode (also called intermittent contact mode) the AFM cantilever is oscillated near the sample surface, causing intermittent contact of the tip with the surface. This intermittent contact helps reduce shear forces associated with movement of the tip across the sample surface. Most AFM imaging is performed in the tapping mode, while contact mode AFM is only used for specific applications such as force curve measurements.

## ***Atomic Force Microscope Components:***

1. An atomic force microscope consists of (1) a flexible cantilever containing a sharp probe, (2) laser and photodiode detector, (3) piezoelectric scanner and (4) feedback electronics with several parts including (5) AFM Head, (6) photodiode array, (7) scanner, and (8) tipholder.



**Figure 2: Atomic Force Microscope Components**

### ***2. Cantilever and Tip***

The cantilever with a sharp tip (probe) at its end is used to scan the specimen surface. AFM tips are mainly silicon, Si, or silicon nitride, Si<sub>3</sub>N<sub>4</sub>. Depending on the application, these tips may be coated with Gold or Chromium. For contact mode of operation, the cantilever should be soft enough to be deflected by very small forces (i.e., small force constant) and has a high resonant frequency to not be susceptible to vibrational instabilities. For tapping mode operation, the tip and cantilever are an integrated assembly of single crystal silicon, these probes are stiffer than silicon nitride probes, resulting in higher force constants and resonant frequencies.

### ***3. Laser and Photodetector***

Laser light from a solid-state diode is reflected off the back of the cantilever. It is then reflected off the tip onto an adjustable mirror and is finally collected by a position sensitive quadrant photodetector consisting of four quadrants. By measuring where the laser is on the photodetector, the deflection of the cantilever can be detected (see below in details).

## 4. Piezoelectric Tube Scanner

AFM scanners are made from piezoelectric material, which expands and contracts proportionally to an applied voltage (within -100V - +100V). Piezoelectric Effect is **the ability of certain materials to generate an electric charge in response to applied mechanical stress**. Figure 2. Piezoelectric material working principle the scanner is constructed by combining three independently operated piezo electrodes for X, Y, and Z into a single tube. In MultiMode AFM, the scanner tube moves the sample relative to the stationary tip.

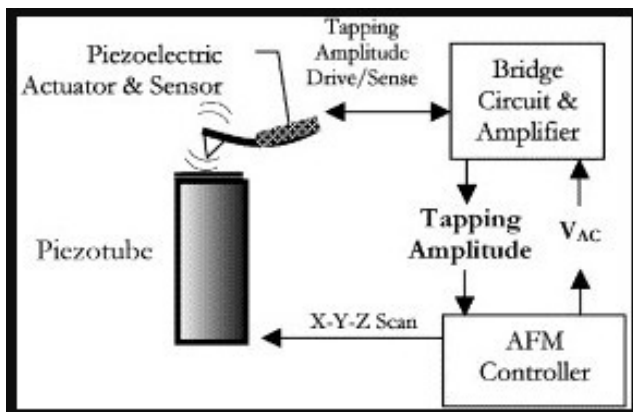


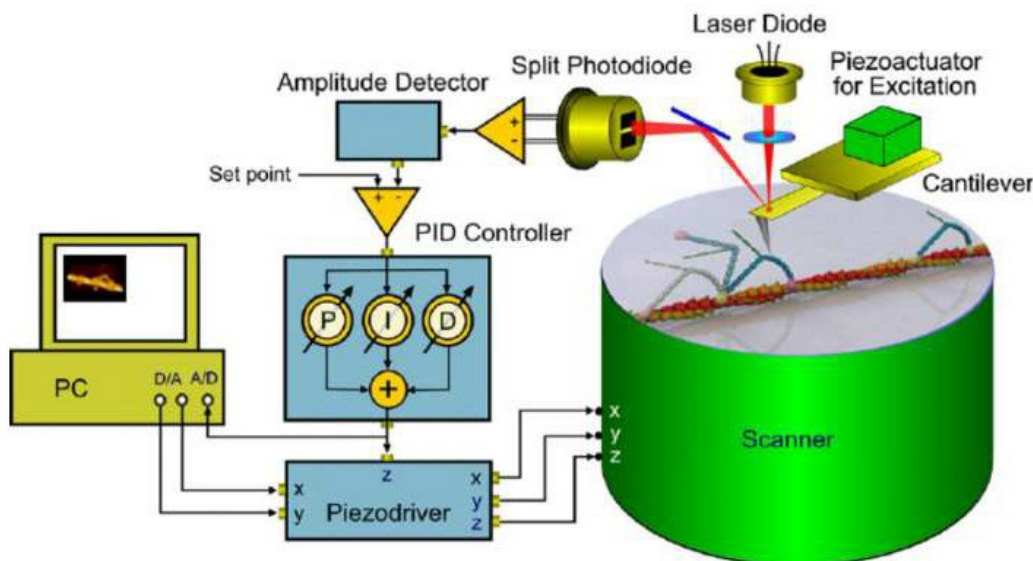
Figure 3: Self-sensing setup schematic. An AC voltage causes the cantilever to vibrate at or near its resonant frequency. The output of the bridge circuit is an AC voltage proportional to the oscillatory amplitude of the cantilever. This output is routed to the AFM controller through the signal access module. The microscope piezotube was used for *x-y-z* scanning.

## 5. Feedback Control

The final principle that is important to understanding AFM operation is that of feedback. Feedback and feedback parameters are ubiquitous in our life. For example, temperature is the feedback parameter in a thermostat. A thermostat is set to the desired temperature (setpoint). As the temperature in the environment changes, it is compared with the temperature setpoint so that the heater (or air conditioner) knows when to turn on and off to keep the temperature at the desired value.

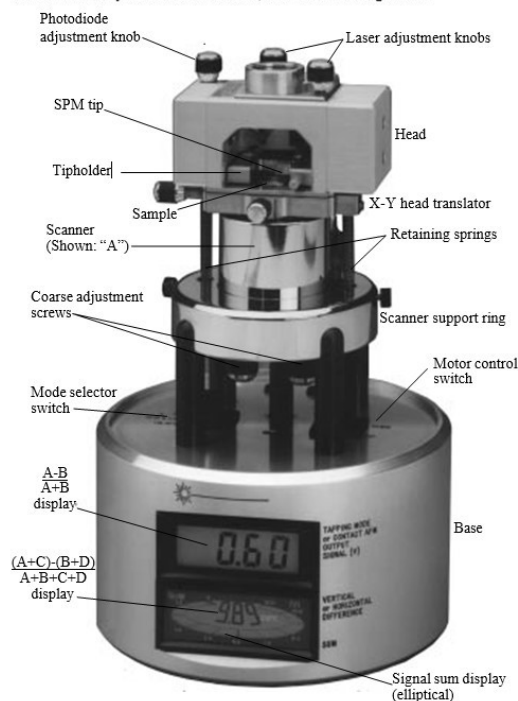
Similarly in atomic force microscopes, depending on the different modes, there is a parameter that serves as the setpoint. For example, in **static mode** (contact mode) the feedback parameter is cantilever deflection, while in the most common form of **tapping mode**, the **cantilever oscillation amplitude** is the feedback parameter. The instrument is trying to keep this feedback parameter constant at its setpoint value by adjusting the z-piezo to move the cantilever probe up and down. The resulting z-piezo movements provide the height information to create the surface topography.

Control of the feedback loop is done through the proportion-integral-derivative control, often referred to as the PID gains. These different gains refer to differences in how the feedback loop adjusts to deviations from the setpoint value, the error signal. For AFM operation, the **integral gain** is most important and can have a most dramatic effect on the image quality. The **proportional gain** might provide slight improvement after optimization of the integral gain. The **derivative gain** is mainly for samples with tall edges. If gains are set too low, the PID loop will not be able to keep the setpoint accurately. If the gains are chosen too high the result will be electrical noise in the image from interference from the feedback. The compensation for a deviation from the setpoint is larger than the error itself or noise gets amplified too strongly.

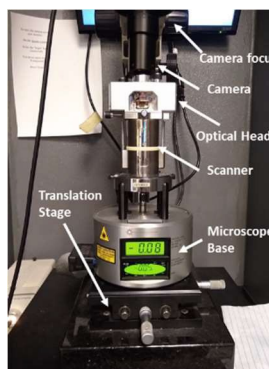


**Figure 4: Schematic presentation of tapping mode AFM system with PID feedback control**

The heart of the system is the SPM itself, shown below in figure 2.2.



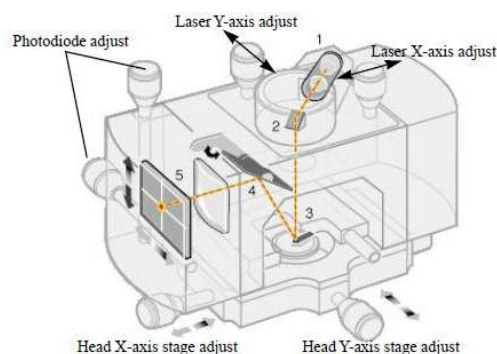
**Figure 5 MultiMode AFM**



## 6. AFM Head:

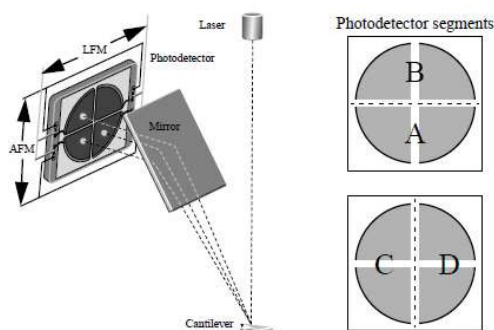
Figure 6 below shows a MM-AFM head with various adjustment knobs. The head and attached X-Y stage are kinematically mated to the scanner via three contact points. A pair of retaining springs hold down the head, allowing it to be raised and lowered using adjustment screws threaded through the scanner body. On older models, two screws are manually adjusted by the operator; the rearmost screw is motorized and under computer control. Newer, "vertical" scanners use single-screw adjustment

**Figure 6. MultiMode SPM head and major components: laser (1); mirror (2); cantilever (3); tilt mirror (4); photodetector (5).**



## 7. Photodiode array:

The four elements of the quad photodiode (position sensitive detector) are combined to provide different information depending on the operating mode. In all modes the four elements combine to form the SUM signal. The amplified differential signal between the top two elements and the two bottom elements provides a measure of the deflection of the cantilever. This differential signal is used directly in the contact AFM. It is fed into an RMS converter (or phase module if attached) for Tapping mode operation. Similarly, the amplified differential signal between the sum of two left photodiodes and the sum of the two right photodiodes provides a measure of the torsion in the cantilever and is used in Lateral Force Microscopy (**Image data type: Friction**). Figure 7 shows the arrangement of the photodiode elements in the MultiMode head.



**Figure 7. Quad photodiode arrangement. Different segments of the photodetector are used for generating AFM and LFM signals.**

## 8. Scanners:

Figure 8 below shows the various, interchangeable scanners. The maximum scan size and resolution of images depend upon the choice of scanner (see chart). Longer scanners, e.g., type "J," yield larger scan sizes; shorter scanners, e.g., type "A," offer smaller images down to the atomic scale. Smaller scanners tend to be more noise-free at acoustic frequencies because of their compact size and rigidity. Larger scanners offer wider scans, while requiring extra noise dampening precautions at smaller scan sizes of high resolution.



| Model                  | Scan Size                 | Vertical Range |
|------------------------|---------------------------|----------------|
| AS-0.5 ("A")           | 0.4 $\mu$ m x 0.4 $\mu$ m | 0.4 $\mu$ m    |
| AS-12 ("E")            | 10 $\mu$ m x 10 $\mu$ m   | 2.5 $\mu$ m    |
| AS-12V ("E" vertical)  | 10 $\mu$ m x 10 $\mu$ m   | 2.5 $\mu$ m    |
| AS-130 ("J")           | 125 $\mu$ m x 125 $\mu$ m | 5.0 $\mu$ m    |
| AS-130V ("J" vertical) | 125 $\mu$ m x 125 $\mu$ m | 5.0 $\mu$ m    |
| AS-200                 | 200 $\mu$ m x 200 $\mu$ m | 8.0 $\mu$ m    |

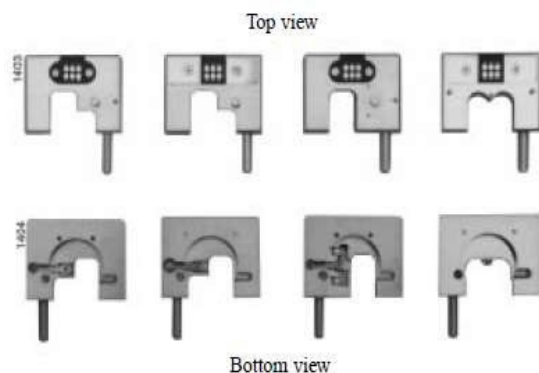
**Figure 8. Various scanners available with the MultiMode SPM. Left-to-right: AS-130V (vertical “J”); AS-200 (“K”); AS-130 (“J”);AS-0.5 (“A”). Not shown: AS-12 (“E”) All scanners are inter-changeable**

Because each scanner exhibits its own unique piezo properties, each has its own parameter file. When scanners are changed, the parameter file for the new scanner is changed along with it, ensuring maximum accuracy at any scan size. Loading new parameter files requires only a few seconds. The chart below describes the range capabilities of each MultiMode SPM scanner.

## 9. Tipholders:

The sample and mode of SPM to be performed dictate the choice of tip and tipholder. For example, if contact AFM is to be used for imaging, a silicon nitride cantilever mounted in a standard tipholder is the usual choice. If Tapping Mode is to be used for imaging a biological specimen in fluid, a special fluid cell is employed. STM utilizes a special tipholder, having a tiny tube holder adapted for holding wire tips.

Examples of each tipholder are shown in figure 9.



**Figure 9: Various types of tipholders**

## Appropriate Samples and Sample Prep

Samples must fit on a 15mm diameter disk and cannot be more than about 5mm thick. Samples must be flat since the microscope cannot measure features taller than 2μm. The maximum area that can be imaged with the microscope is 10μm by 10μm.

### 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  |

### Sample Preparation and related issues

- Sample size:** sample diameter is approximately 1 cm in diameter and a thickness of not more than 2 mm
- mount** your sample on the magnetic metallic disks with double-sticky tape, glue or epoxy
- if you need to clean surface, use isopropanol and dry it with the air gun gently
- Consider the size of the features you are interested to see versus the probe tip (this will affect your resolution of what you can see. Always make sure the probe is smaller than the feature of interest. If the surface has smaller features than the tip, the surface may scan the tip instead!)

## Mounting the probe and sample:

### Getting Started:

1. Log in to iLab account and start your experiment and then log into the PC.
2. Open the cabinet and make sure the switch on the left side of the microscope base is in either the middle (AFM & LFM) or forward (TM AFM) position. Switch turn on optical light source below the table (right side). The monitor will be on all the time (**never turn off the power of monitor**). The anti-vibration stage will be on automatically.
3. Align the optical light circle in the middle of the stage and the red laser light spot in the middle of the optical light circle, using micro movement stage controller at the bottom of AFM base.
4. **Put sample/holder onto scanner.** The strong magnetic force will hold it tightly. Focus the microscope on the sample surface. Using tweezers to move your sample until you find an area that you want to scan. If you need to **enlarge the gap**, push, and hold the switch on the right side of the microscope base in the **Up position** (Figure 10). Release the switch when the gap is large enough.

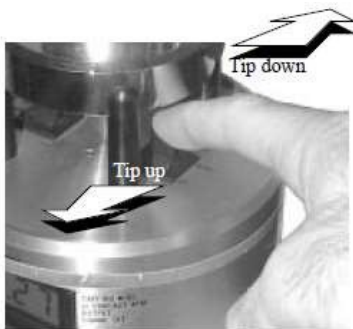


Figure 10. The tip is quickly raised and lowered using the Up / Down toggle switch on the MultiMode AFMM's base.



**Warning: if you hold the switch for too long you will lose the ability to raise and lower the stage and the microscope will require repair.**

5. Remove the cantilever holder from the optical head. Place your sample on the stage and mount your probe in the cantilever holder (see picture 11). Adjust sample surface position to level with the bottom line of the optical head window using **“UP”** toggle switch that is located below the tube scanner on the right side. This ensures a safety gap of about 2 mm between tip and sample surfaces.

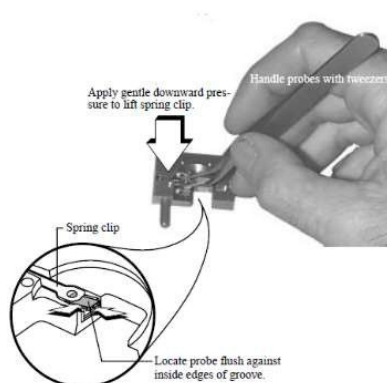
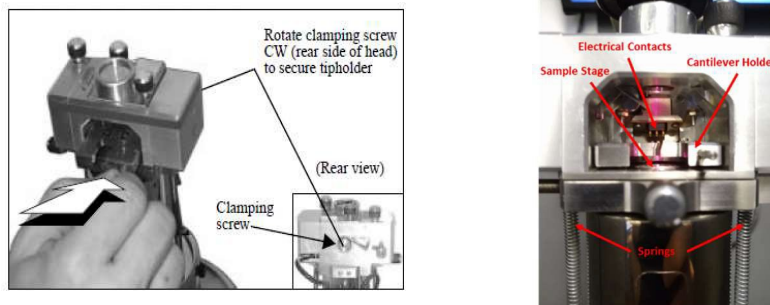


Figure 11. Slide the probe carefully into the tip holder's groove. Gentle downward pressure against the tip holder will lift the spring clip for probe insertion. (Shown: underside of AFM tip holder.)

- Put the cantilever holder upside-down back in the optical head and use the screw on the back of the optical head to lower the 6-pin (Figure 12) connector onto the electrical contacts on top of the cantilever holder ((do not over tighten).

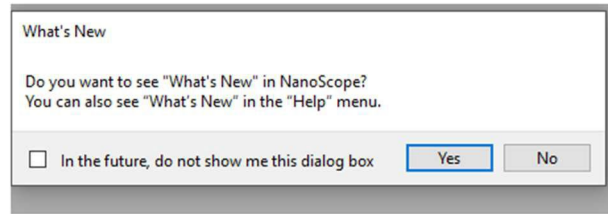
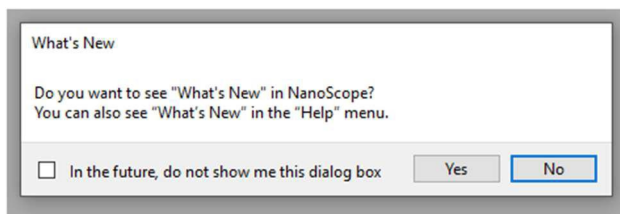
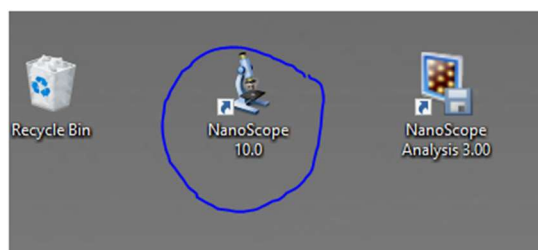


**Figure 12. Install tipholder in head without touching the sample. Secure tipholder using clamping screw at rear of head.**



**Warning: do not tighten this screw too hard or it will stop working!**

- Press and hold the switch on the right side of the microscope in the **Down** position to lower the probe tip to within a few mm of the sample surface. (**Tip 1:** focus on the sample then move it up until you see the probe slightly in focus.) Stop when you can see the tip nearly in focus. It is helpful to go up and check the focus of the cantilever from time to time to see how close it is to the sample surface. (**Tip 2:** If you are looking at a shiny or clear surface make sure you are not looking at the reflection or shadow of the tip, it is best to use the edge for focusing.).
- For new user, please check the sample with video screen (see below) and then put the probe holder.
- Launch the Nanoscope 10** software by double clicking the **Nanoscope 10.0** icon on the desktop



Select “NO” (this will not Bruker web site).

“YES” that will go to web page.

- NanoScope version 10 software is organized by experiment type. Click **EXPERIMENT > SELECT EXPERIMENT** or the **SELECT EXPERIMENT** icon in the top left of the NanoScope software window. This opens the **Select Experiment** window, shown below: **The select experiment dialog will launch**

Use of the **Select Experiment** window, shown below. Click Experiment > Select Experiment or the Select Experiment icon in the top left of the NanoScope software window. This opens the Select Experiment Form Select Experiment Form.

### The Experiment Window

Highlight the Experiment that you wish to select in this window.

### Description

A brief experiment Description is shown in this window.

### Load Experiment

Click LOAD EXPERIMENT to begin real-time microscope operation.

### Open Previous

Your previous experiment is saved in the Previous Experiment window. Click OPEN to start that experiment.

### Change Microscope

Click CHANGE MICROSCOPE to open the Microscope Select window, discussed in Section 2.5.1, that allows you to select a new microscope or new microscope features.

### Cancel

Click CANCEL to close the Select Experiment window and continue to use the existing Scan and Ramp Parameters.

Select Experiment: MultiMode 8

1 Select From:

☐ Use previous experiment  
TappingMode in Air - Standard - 05/24/23 | 14:22

Or

Choose an Experiment Category:

Scan Asyst Tapping Mode Contact Mode  
Electrical & Magnetic Mechanical Properties Other SPM

2 Select Experiment Group:

TappingMode in Air  
TappingMode in Fluid

3 Select Experiment:

TappingMode in Air - Soft Tapping  
TappingMode in Air - Standard  
Torsional Resonance (TR-Mode)

Microscope: MultiMode 8

>> Change Microscope Setup

Experiment Description

TappingMode is a standard Atomic Force Microscopy (AFM) technique where the sample topography (height) is measured by monitoring the amplitude of oscillation of the cantilever as the probe is scanned across the sample surface. A piezo stack excites the cantilever substrate vertically, causing the cantilever to move up and down with a constant sinusoidal amplitude at or below its fundamental resonant frequency. The feedback loop maintains a constant oscillation amplitude (typically 20 – 100 nm) by vertically moving the scanner at each (x,y) data point to maintain a constant "setpoint" amplitude. By keeping a constant oscillation amplitude, a constant tip-sample interaction is maintained during imaging.

TappingMode requires:

Cancel ☐ Ignore Probe Parameters Load Experiment

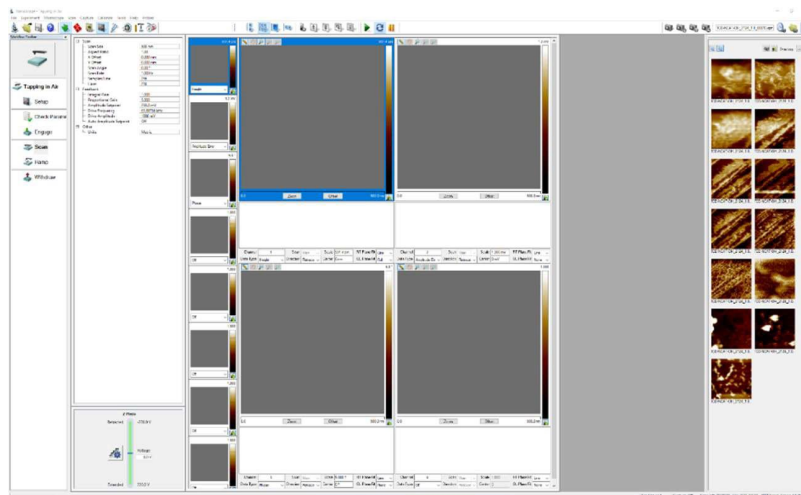
Use form to select a previously saved experiment configuration or by choose

① Experiment Category, ② Experiment Group & ③ Experiment to use one of the pre-loaded workspaces.

Choose **Tapping Mode: Tapping Mode in Air : Tapping Mode in Air – Standard**

Click **"Load Experiment"**

You will see this previous user display.

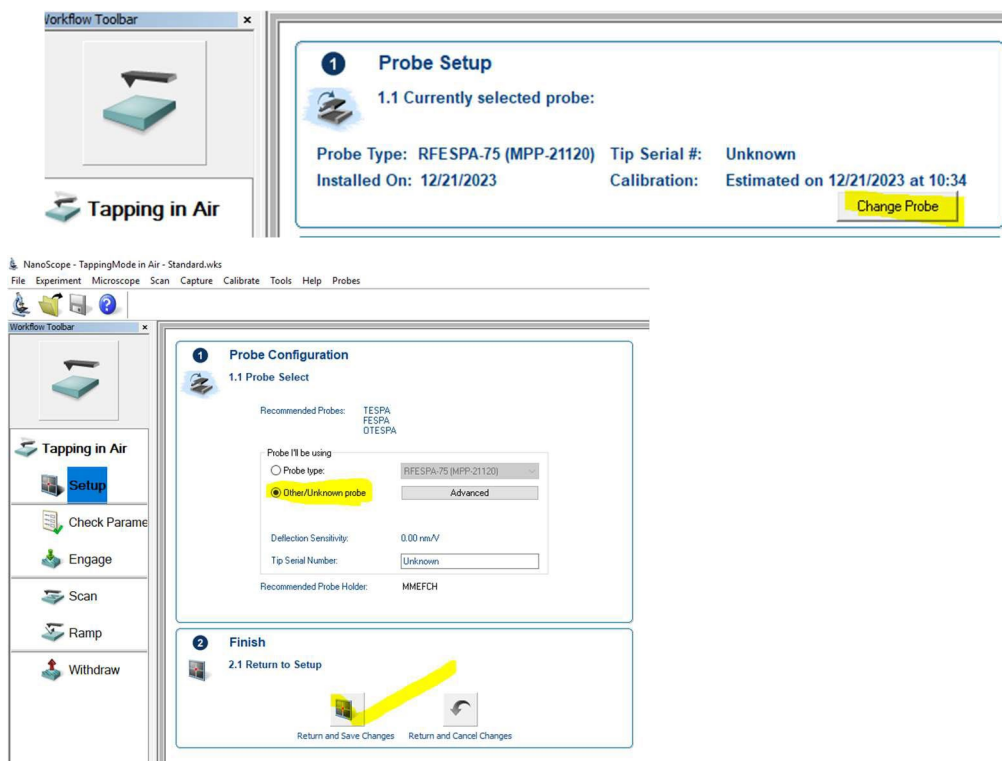
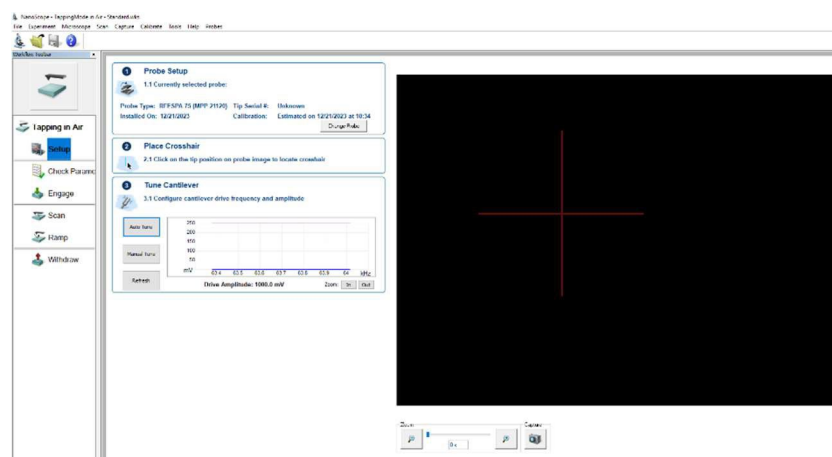


## 11. Align the Laser

To align the laser on the AFM probe, click the **Setup** button in the Workflow toolbar. Laser alignment can be completed using either the **shadow method** procedure or with the **assistance** of the alignment station.

The laser control knobs are located on the **top and side of the scan head**. A diagram printed on the scan head illustrates the movement direction of the laser when the control knobs rotated clockwise.

1. A window should appear with a video feed from the camera above the microscope (may be black at this point).
2. Probe setup: You can use either Bruker probe or other "as unknown" probe



3. Turn on the fiber light (if not yet) in the back left corner of the cabinet (see image below).
4. Move the camera focus down until you are focused on your sample surface. Use the large black knobs behind the camera to focus the camera on the tip

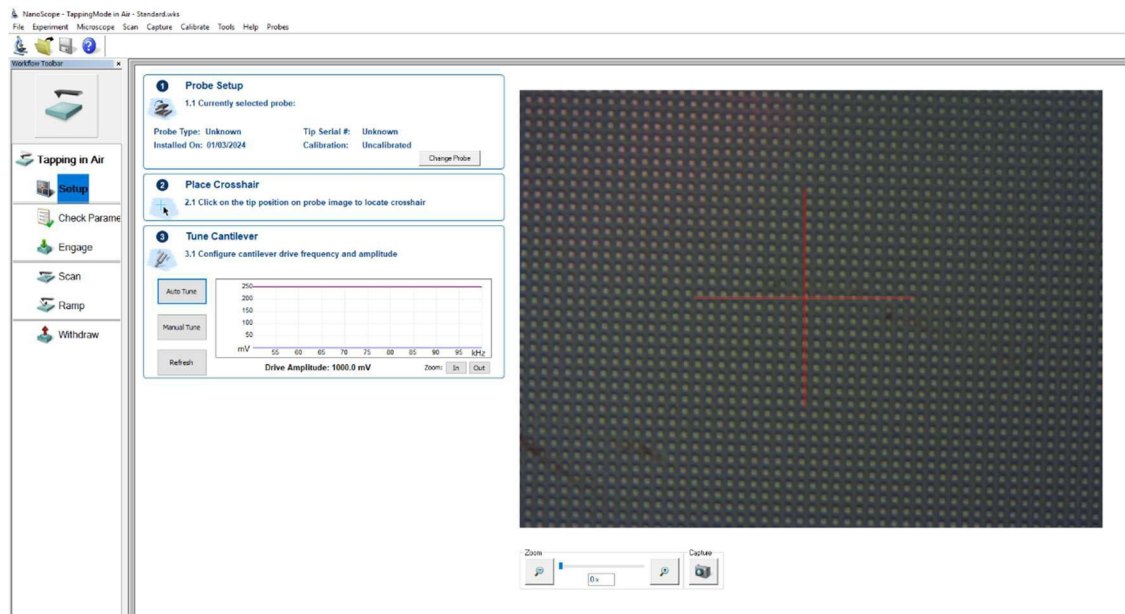


**Warning: turn the large black double knobs to focus the camera not the single black knob behind .**

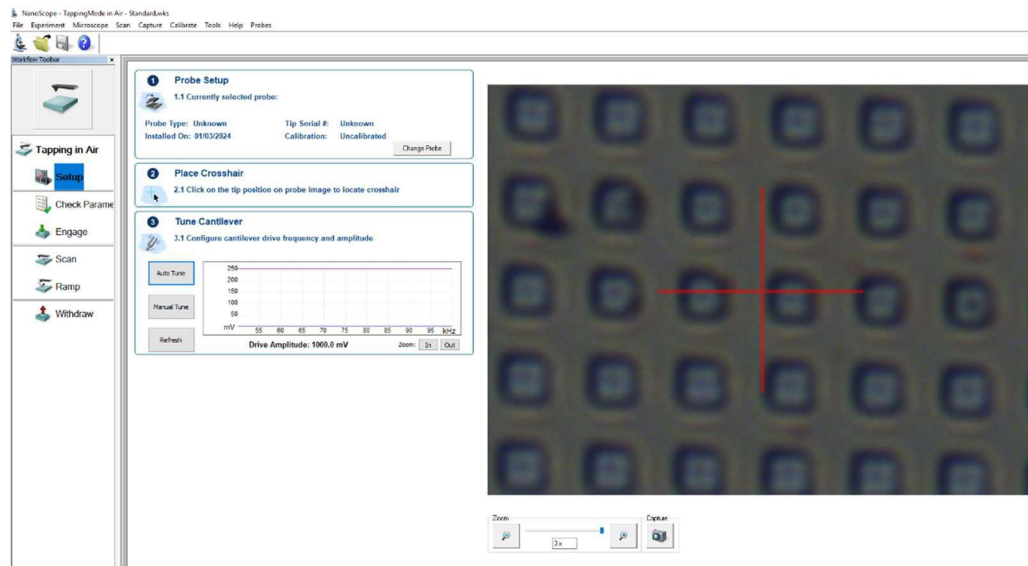


5. **Focus the sample:** Find the cantilever on the video image using the micrometers on the translation stage that the microscope is sitting on. Use the large black knobs behind the camera to focus the camera first on the sample (see image below) and then tip (by moving up) also. Then back to sample again and enlarge it .

6.



Move the slider to enlarge the picture

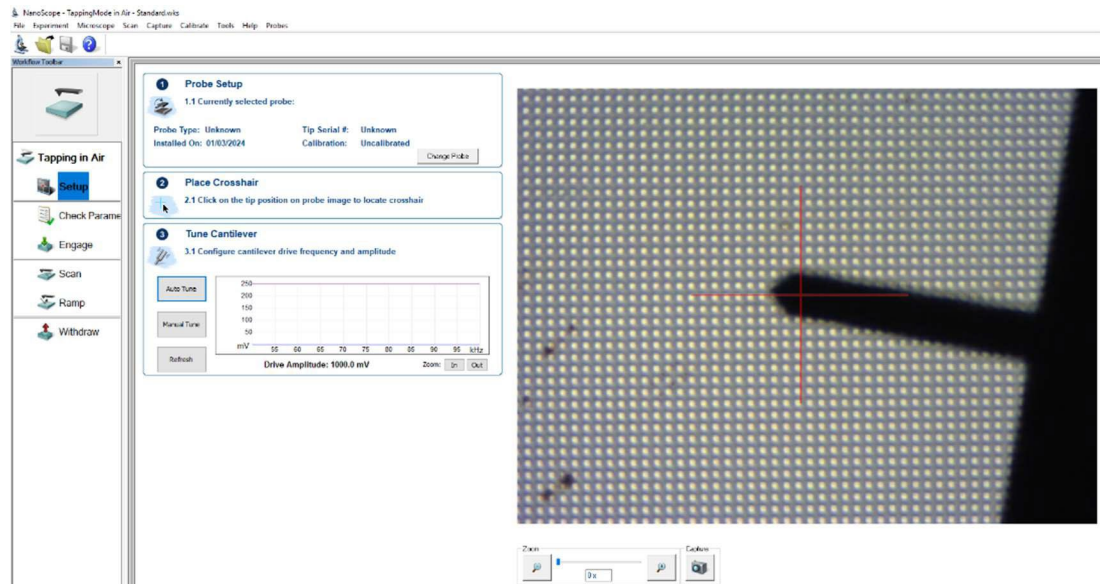


**Warning:** make sure you are focused on the tip and not its shadow, which appears below the plan of the sample.

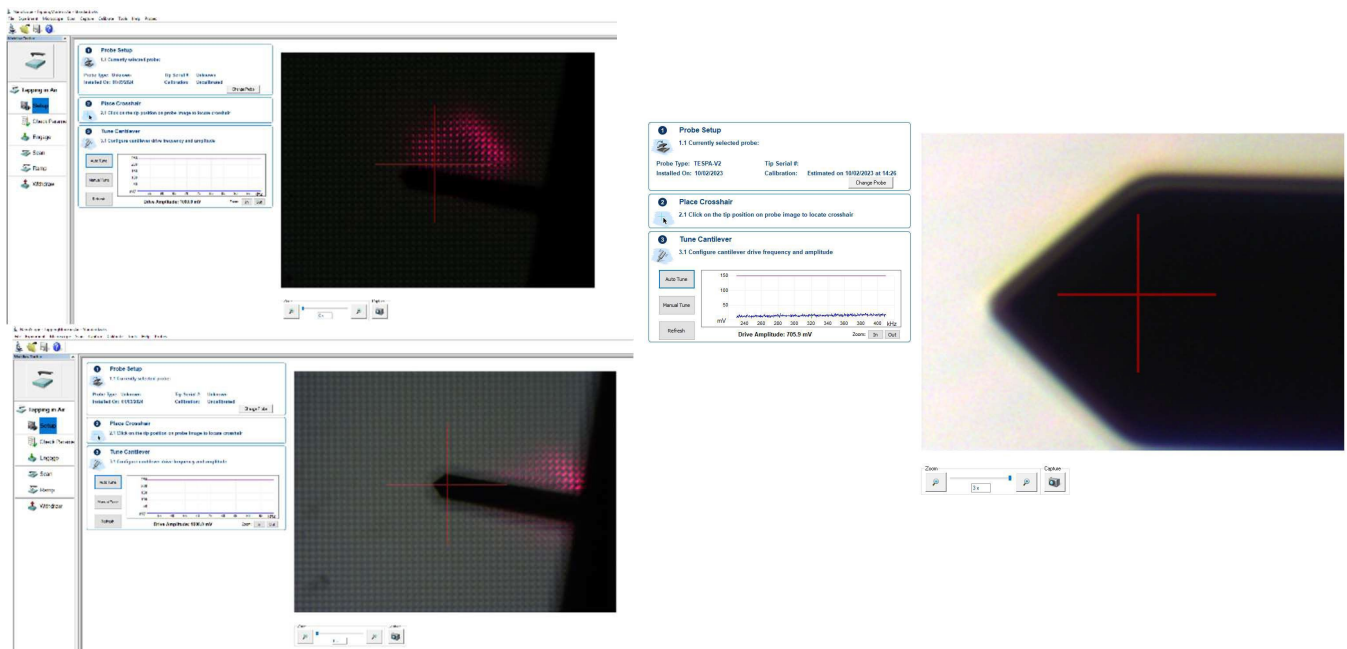
- Press and hold the switch on the right side of the microscope in the **Down** position. On the video image you should see the tip slowly coming into focus. Release the switch when the tip is **almost (80%) , but not quite**, in focus (see image above, last one).



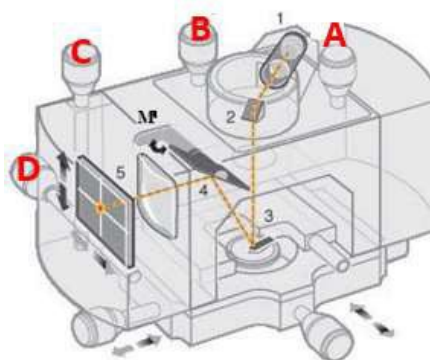
**Warning: if you are not actually focused on the sample surface, or if you move the tip down too far, you can crash the probe tip into the sample.**



- Find the laser light: and set on the probe. Use video image



9. Make sure that the mode switch, located below the tube scanner on the left side, is on the AFM&LFM or TM AFM. Align the laser beam at the end of the cantilever (use Figure as a

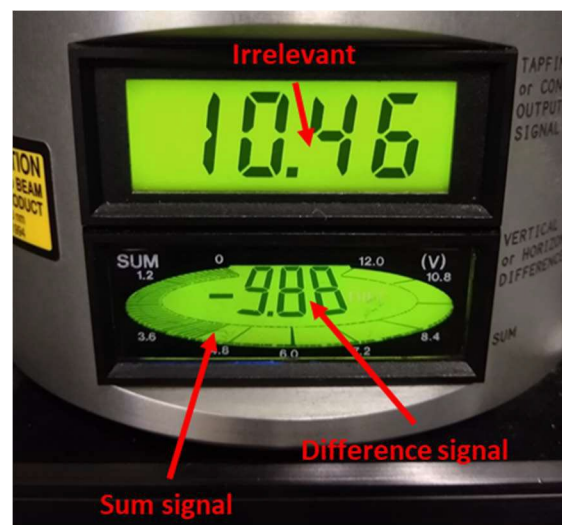


guide),

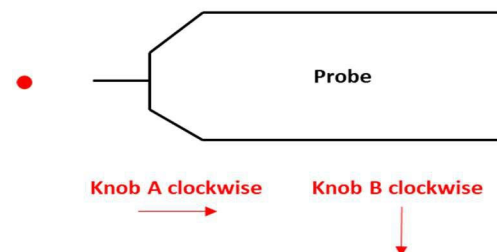
using the two positioning knobs on the **top right side (A & B of Figure)** of the optical head. Knob A moves the laser spot left to right on the video image while knob B moves the laser spot up and down. Check the Sum signal on the elliptical meter on the bottom LCD is at its maximum signal (3 – 6) - this value is dependent on the reflectivity (i.e.; the coating at the back of the cantilever) of the back of the tip. If the signal is low adjust the mirror (lever on back of optical head, **M** of Figure 1) until you achieve the maximum signal to center the laser reflection into the photodiode cavity. **Tip:** If you are unable to reach the maximum intensity of the laser on the elliptical meter, check if you are focused on the **reflection** of the cantilever instead of the cantilever itself.

10. When the laser is on the cantilever you will see the sum signal increase on the microscope's bottom display (see picture below). The sum signal is NOT the large number in the center of the display but is instead shown by the number of hash marks around the perimeter of the oval. Once you find the cantilever, turn knob B back and forth to move the laser over the cantilever until the sum signal is maximized.

11. Turn knob A counterclockwise to move the laser along the length of the cantilever until it drops off the end. You will see the sum signal drop. Turn knob A clockwise slightly to move the laser onto the tip of the cantilever where the sum signal is maximum. If necessary, slightly adjust knob B to maximize sum signal. The laser spot is now on the end of the cantilever.



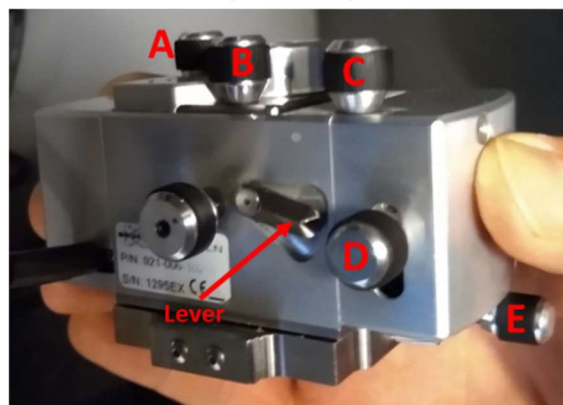
Laser Alignment  
(Top View)



## Zeroing the photodiode:

12. Tilt the small lever (mirror) on the back of the optical head up and down until sum signal is maximized (see picture).

Optical Head  
(Rear View)



13. Put the switch on the left side of the microscope in the middle position (**RED** light on). Turn knob D on the back left side of the optical head in while watching the difference value in the center of the microscope's bottom display. If the number passes through zero, turn the knob in the opposite direction until the difference value is near zero. If you turn the knob all the way in and it does not pass through zero, turn the knob the other way until it does. If you see the sum signal starting to go down, you are turning the knob the wrong way.

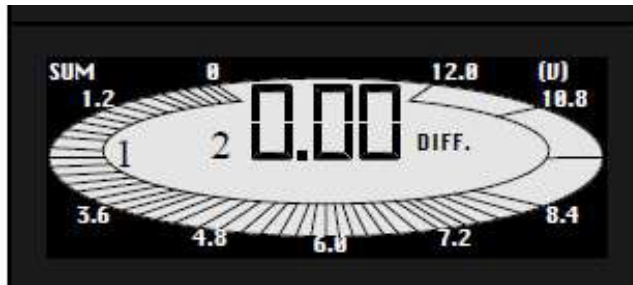


**Warning: if you turn a screw counterclockwise too much, it will come completely out of the optical head and repair will be required.**

**“+ve” value to “-ve value” (anti clockwise) and “-ve” value to “+ve” value (clockwise) for D and C.**

14. Move the switch on the left side of the microscope to the forward position. Repeat the zeroing process using knob C (**Green** light on) on top of the optical head.

15. Repeat steps 2 and 3 until the difference values are near zero with the switch in both positions.



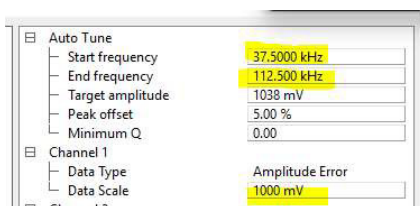
**VERTICAL  
or HORIZONTAL  
DIFFERENCE**

**SUM**

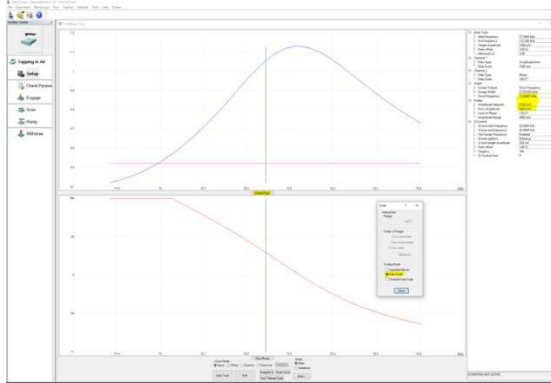
16. At this point you can use knobs E and F to move the cantilever around the sample surface to find your region of interest. You may need to adjust the micrometers on the translation stage to keep the tip in view.

## Tuning:

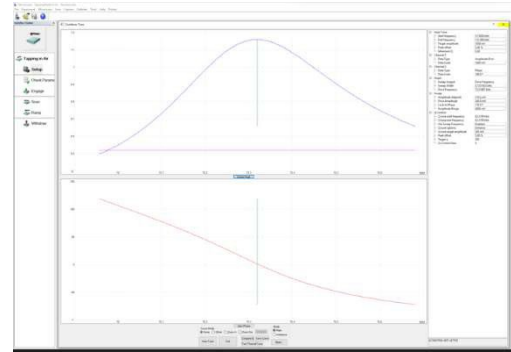
17. For unknow probe click manual tuning and change the parameters. In the *Start Frequency* and *End Frequency* boxes enter values that are 25% below and above your cantilever's expected resonant frequency (for example 50,000Hz and 100,000 Hz for 75kHz probe). Set the *Target amplitude* to **~1000mV** (1V).



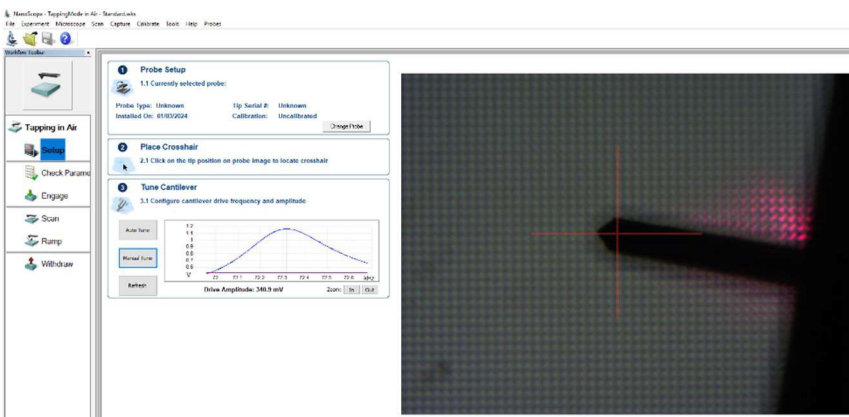
18. Click **Auto Tune**. The cantilever should tune to a frequency near its expected resonance. See figure below for typical tune output.



Right click  
and select  
auto scale



19. **Exit** the *Cantilever Tune* window.



20. Click **check parameter**: On the Menu Bar click *Acquire* → *Scan Control*. Enter your desired parameters in the *Main* tab. Set **Integral Gain to 0.5** and **Proportional Gain to 0.7**.  
Set up the scan parameters: use the following starting values and change as needed. Make sure the X and Y offsets and scan angles are set to 0.

Scan Controls:

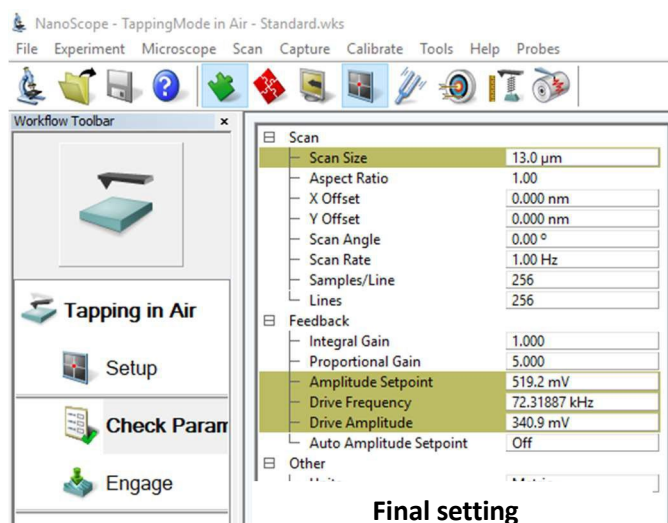
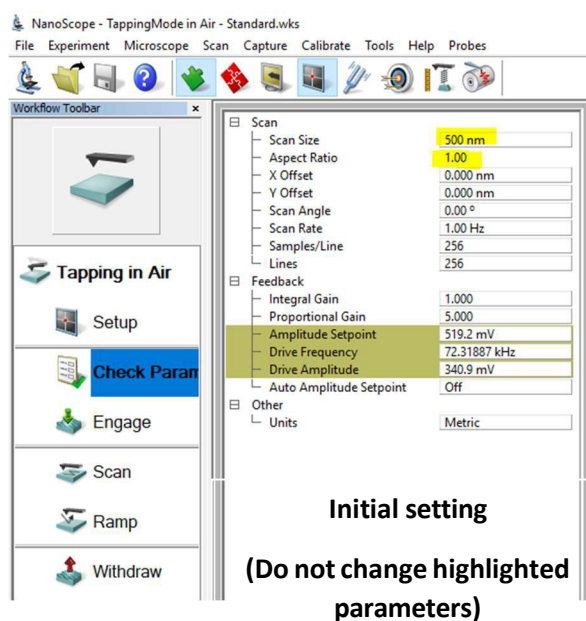
|                             |                                                  |
|-----------------------------|--------------------------------------------------|
| <b>Scan size:</b>           | <b>start with 1-13 <math>\mu\text{m}</math>;</b> |
| Scan rate:                  | start with 1-2Hz;                                |
| Samples/lines (resolution): | start with 256 Slow scan:                        |
|                             | Enabled                                          |

Feedback Controls

|                    |         |
|--------------------|---------|
| Integral Gain:     | ~0.4147 |
| Proportional Gain: | ~2.144  |

Channels 1-3

|             |                                    |
|-------------|------------------------------------|
| Data Type:  | at least height in Ch.1 Data Scale |
| (Z- range): | select for good contrast           |



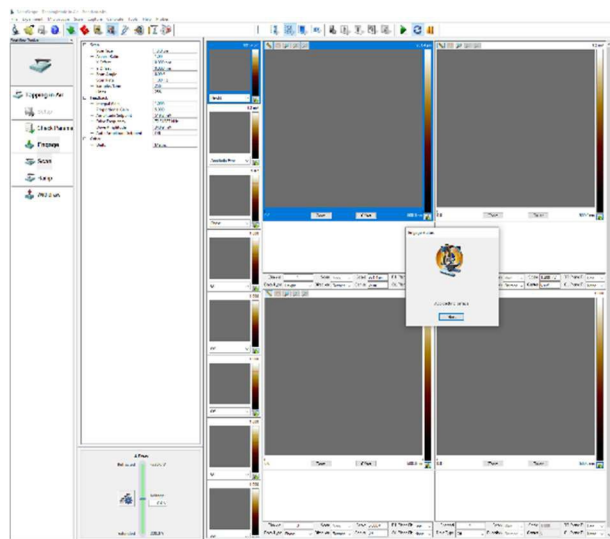
21. Open/click the video icon on the main tool bar.

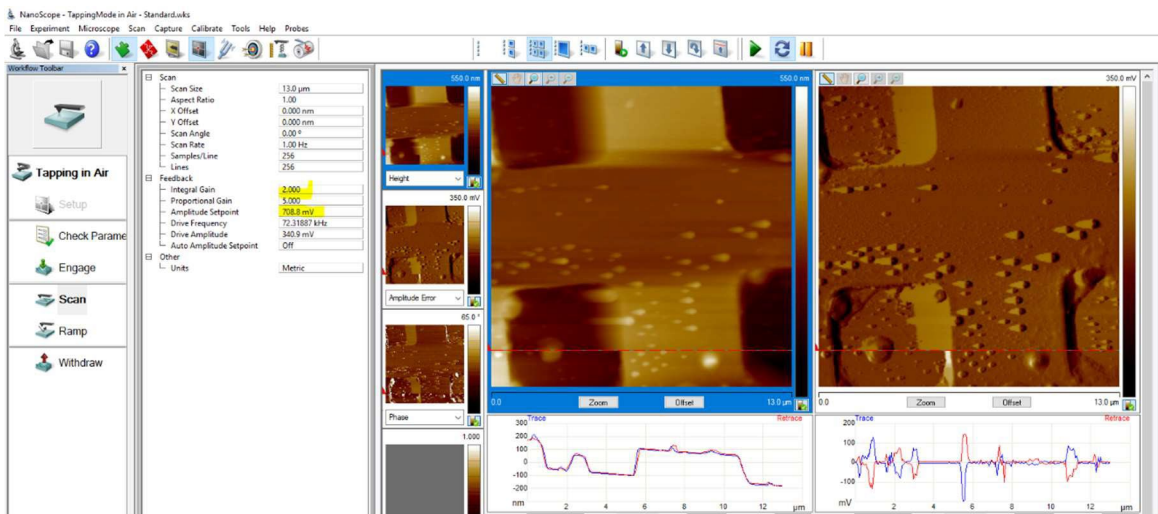
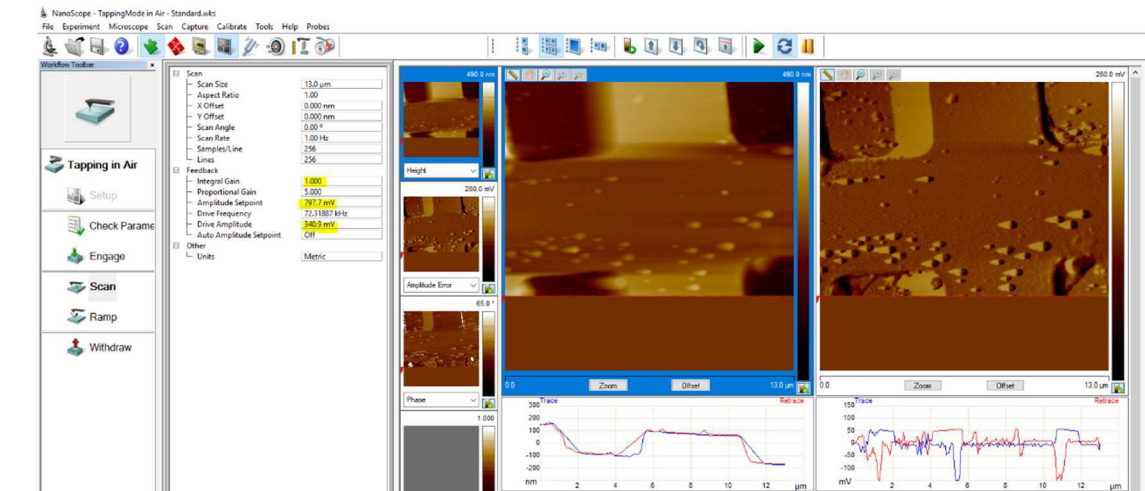
If necessary, focus the probe (make closer to sample using “Down” knob (figure 10). This will take less time during engagement time. Be careful-over closing can destroy the probe.



22. On the **workflow tool** bar click **Engage**. The microscope will move the tip down very slowly until it touches the surface. You should see the tip slowly come into focus on the video image. Once the tip is on the surface, the microscope will automatically begin acquiring an image. At this point it is often desirable to close the cabinet door.

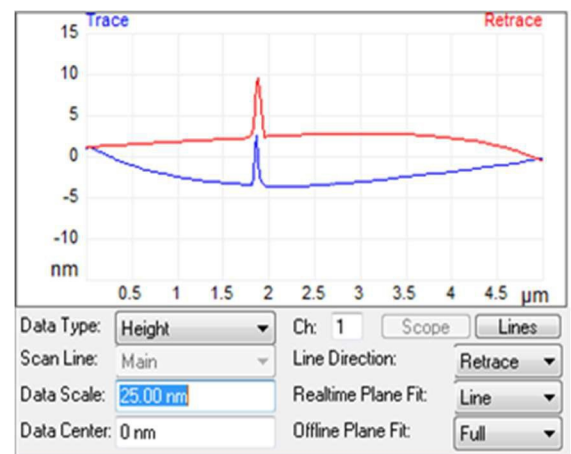
Double check if the Horizontal and Vertical Differences remain close to zero and that the laser intensity is at its maximum before you engage the tip. The AFM will go through some precautionary procedures and then it will engage on its own. Wait until the scanning image will appear on the right screen. Make sure the Vertical difference remains as close to zero as possible. If not, disengage the cantilever check the sum and difference signals, adjust it if necessary, and then engage again.





## Acquiring Data:

23. In the window, shrink or expand the *Data Scale* to suit your data. In the height data, you should see the trace and retrace lines tracking each other closely. Features should show up in both traces and there should not be a lot of noise on the baseline.
24. If the height data is a flat line at zero even when zoomed in to single digit nanometers, the probe tip is likely not touching the surface. In the *Scan Control* window, try reducing the *Amplitude Setpoint* 20mV at a time until the tip lands on the surface.



25. If the tip appears to be moving on/off the surface while scanning, for example you are seeing features in the trace but not the retrace, try increasing the *proportional gain* and/or *integral gain*. You may need to reduce the *scan rate* especially if you have tall, sharp features that the microscope has trouble tracking or if you are scanning a large area.

26. If the height data is very noisy try decreasing the gains.
27. Obtaining good images is often a matter of trial and error while adjusting parameters such as the gains, scan rate, and amplitude setpoint.

## 28. Set up Initial Operating Parameters

- SCAN SIZE – This parameter sets the length of the AFM scan. This also controls the magnification: shorter scan produces greater magnification. The scan size varies with the scanner used.
- SCAN RATE – this parameter sets the cycle time for the scan. The tip scan starts from the right side and scans across to the left end (TRACE) and then retraces its path back to the right (RETRACE) – as viewed on the video screen. This forward and backward path is one cycle. Thus, the scan rate is measured in complete cycles in one second (1 Hz=1 scan length in 0.5 sec).
- SAMPLES/LINE – This parameter sets the number of height samples per line length. This determines the resolution in pixels of the image.
- LINES – This parameter sets the number of line scans and is related to the SAMPLES/LINE through the ASPECT RATIO, which sets the shape of the image – either square (1:1), or rectangular i.e. 3:1 which is 3 times wider than high.
- Integral Gain – Sets the sensitivity and speed of the Z-piezo driver amplifier to vertical height changes as the tip scans the surface.
- Proportional Gain – This is also a sensitivity parameter, but it is less sensitive than the integral gain. The rule of thumb is that this parameter is to be set to twice the numeric size of the Integral Gain.
- Amplitude Setpoint – this is the value in mV of the tapping oscillation amplitude compared to the free-swinging cantilever amplitude of 500 mV as set during the cantilever tuning step. This represents how much of the free amplitude is left after the tip contacts the sample surface, and thus restricts the amplitude.

29. Use up/and down arrow to change the scanning directions



30. Select 1 to 4 windows, keep height and phase window



31. Set scanning parameters

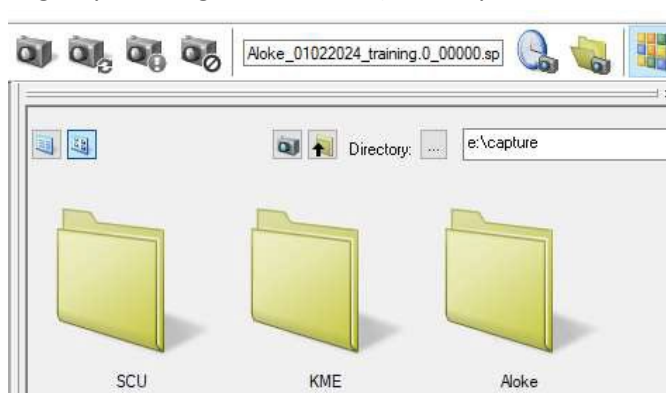
|               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Scan Size:    | The scan size varies with the scanner used. The J scanner currently mounted on the AFM has a maximum scan size of 125 um x 125um. The E scanner (suitable for fluid cell) has a maximum scan size of 10 um x 10 um.                                                                                                                                                                                                                                                                              |
| XY Offset:    | Set to 0                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Scan Angle:   | Set to 0 degree                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Scan Rate:    | Controls the rate at which the cantilever scans across the sample area Typically, 0.5 - 1.0 Hz (use lower scan rate to achieve better resolution). The scan rate must be decreased as the Scan Size is increased. <b>A slower scan rate generally leads to better images</b> , but not always. Decrease until you get the best image. Scan rate values that are too low as well as too high will cause poor images. You should try a range of values while in image mode to obtain the best one. |
| Samples/Line: | The number of pixels used to create the image. Set to 256 for parameter adjustment and 512 when recording images. <b>Increasing leads to better image quality</b> , but there is a trade-off with time                                                                                                                                                                                                                                                                                           |

## 32. Set controller parameters

|                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Setpoint:          | The value of the RMS of the cantilever vibration amplitude that the feedback loop maintains. (Setpoint is thus proportional to force applied to surface). The difference between the Vertical Deflection signal before engaging and the Setpoint is related to the force. A larger, more positive Setpoint voltage results in a larger contact force. You can adjust the voltage later during the measurement. <b>Reducing setpoint often leads to better quality images. Do this until the image improves.</b> |
| Drive Amplitude:   | The amplitude of the force at which the cantilever is driven. <b>Increasing drive amplitude often gives better phase data, up to a point.</b> Often times there will be a significant amount of strong noise. Increasing the Drive Amplitude will take care of this. Once you have a good picture, back off on Drive Amplitude as much as you can without coming off the surface.                                                                                                                               |
| Integral Gain:     | Determine how sensitive the feedback loop is to variations in the tip's amplitude of oscillation. <b>Increasing gains often helps obtain better images</b> (especially height) but only up to a point, above which high frequency noise is observed.                                                                                                                                                                                                                                                            |
| Proportional Gain: | Set to 2-5 X Of IG. Adjust the value if necessary                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

33. While the microscope is scanning you can click *Frame up*  or *Frame down*  to start a new image from the bottom or top, respectively. To capture an image, first go to Capture option on the top bar of Nanoscope Control window and choose Capture.

34. From the same column choose Capture “file name” and give your image some name (choose your folder in drive d). Start a clean scan from top or bottom of the scan area using the “up” or “down” icons. Then immediately press the camera icon to start capturing an image. On the bottom bar of the Nanoscope control window you will see the message “Capture: On”, time “on”. When it changes to the message “Capture: Done”, you will be able to find your image in analysis mode.



35. To designate the directory where your data will be saved, on the menu bar on the corner. Browse to your folder in the e:\capture\ directory. Enter your desired *Filename*. Consecutive saved images will have numbers appended after the filename.

36. Click *Capture*  to save the next image once it is completed. If you later decide you don't want to save the image, you can click *Abort Capture* . You can see whether or not capture is active by looking in the lower right corner of the software. If you want to save the image as it appears right now, click *Capture Now* .

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### 37. **Data Analysis (updating):**

1. To open an image, open nanoscope analysis icon, select your file and then click *OK*.
  - a. You can use the tools in the top left corner of each image  to zoom in on features and measure distances between features in the XY plane.
  - b. Right clicking on the color scale and selecting *Color Scale* brings up the *Color Scale* window. Here you can change the height scale in order to view/hide small features in the data.
  - c. You can export any of your images as pictures by right clicking it and selecting *Export* → *Screen Display*.
2. Flattening is used to correct common artifacts in the images such as streaking, curvature, and tilt. Click on the image you wish to flatten and click *Flatten* .
  - a. In the *Flatten* window choose appropriate *Thresholding Direction*: Use  $Z <$  for features that stick up from the substrate, Use  $Z \geq$  when features are pits/valleys in the substrate.
  - b. *Flatten Z Threshold %* should be set such that the substrate is flattened but not the features. Use the image height scale to determine a boundary percentage between substrate and features.
  - c. You can choose to define the threshold for the *whole image* or *line-by-line*. The latter is useful if there is a lot of height variations within the image.
  - d. You can vary the *Flatten Order* to see which gives the best results.
  - e. Click *Execute* when you are ready to flatten. If you don't like the results, you can click *Reload* and make adjustments. You can even flatten multiple times in succession using different parameters.
3. You can use the section tool to measure properties of features such as height/depth, size, and shape. You can also measure distances between features. To use the section tool click .
  - a. Click and drag on the image to create a line. To the right of the image you will see a graph showing the signal (often height) as a function of position along the line. The two crosshairs on the image are represented on this graph as dashed vertical lines. You can drag the dashed lines to move the crosshairs on the image along the line to features of interest.
  - b. The table below the image shows various measurements that are made between the two points on the line. To export this data you can right click the table and select *Copy Text*. You can then paste this text into another program such as notepad.
  - c. The above steps can be repeated two more times to create up to three different colored lines on the image.
  - d. The image including lines and crosshairs can be exported as a picture by right clicking the image and selecting *Export* → *Screen Display*. You can also export the data plot by right clicking it and selecting *Export* → *Graphic* or *Export* → *XZ Data*.

- 
4. You can see a 3D rendering of an image by clicking *3D Surface Plot* .
    - a. You can right click the Color Scale and select *Color Scale* to bring up the *Color Scale* window. Here you can adjust the *Data Scale* to zoom in the Z direction.
    - b. You can also click and drag the plot to change the viewing angle.
    - c. When you are finished setting up the image you can use the *Export* button to save it as a picture.
  5. You can use the roughness tool  to get various roughness parameters calculated from the image.
    - a. To export the parameters, right click on the parameter list and select *Copy Text*. The parameters and their values can then be pasted into another program such as notepad.

#### **When you are finished:**

1. To stop data acquisition click *Withdraw* in the *Scan Control* window → *Main* tab.
2. Use the switch on the right side of the microscope to raise the tip at least 1mm.
3. Remove the cantilever holder from the optical head and remove your probe.
4. Remove your sample from the stage (you may need to remove the optical head). Replace optical head if necessary.
5. Close the *Nanoscope* software.
6. **Do not turn off the monitor** in the cabinet, the light source, the anti-vibration stage, and the microscope controller.
7. Log out from your account only (not turn off the monitor).
8. Close the cabinet. Turn off the fiber light. Session out in iLab and sign the logbook

### **Trouble-Shooting**

#### *1) AutoTune will not work.*

- Make sure that the tip holder has been secured in the AFM.
- Make sure that your tip is not in contact with the sample. If so, do a tip up, and the tip may still be OK.

#### *2) Sample seems to move when lowering the tip.*

This probably means that you have placed the sample in such a way that it is encountering the tip holder. Rotate the sample so that any part of it that is hanging over the piezo tube is coming towards you (out of the microscope).

#### *3) Getting poor images.*

- Try optimizing parameters again.
- Realign the optics, making sure that you get the laser as close to the end of the cantilever

- 
- as possible and are getting as much light to the photodiode as possible
  - Be sure that the laser is as close to the end of the cantilever as possible and that you have a clear spot.
  - Try another area of the sample or change samples, especially if you are not seeing the kind of image you expected to see. Do not waste your time with a bad sample.
  - Change tips, but don't throw away old tips. The tip might be salvageable.

*4) Tip will not engage.*

- Make sure that something is not interfering with the tip reaching the sample. This impediment may be as small as a big ridge on your sample
- Check that you still have a cantilever. If you try to engage with no cantilever the AFM will simply drive what's left of your tip into the sample