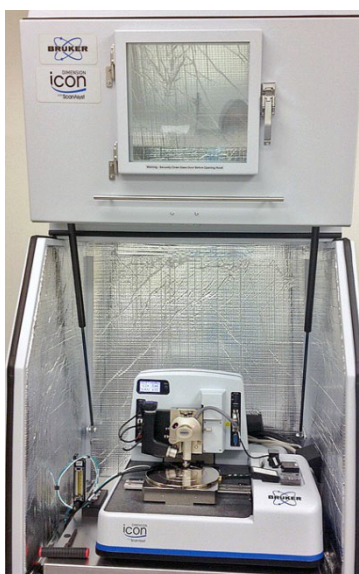


Using the Bruker Icon Dimension Icon AFM

Tapping Mode AFM (Updated by Aloke on May 2025)



Bruker Dimension Icon® Atomic Force Microscope is equipped with ScanAsyst® automatic image optimization mode based on PeakForce Tapping technology, which enables users to obtain consistent high-quality results easier and faster. This system is capable of many SPM applications, contact mode, tapping mode, ScanAsyst peakforce mode, AFM in fluid, Phase imaging, piezoresponse, and many others.

Appropriate Samples and Sample Prep

You can image anything that can fit on the stage. Samples must be flat since the microscope cannot measure features taller than 2 μ m. The maximum area that can be imaged with the microscope is 100 μ m by 100 μ m.

Stuff to Buy

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

For additional information please refer to the basic training videos provided by Bruker. (Icon Probe Load & Laser Align Video, Icon Basic Contact Video, Icon Basic Tapping Video, Scanasyst PFT Video).

<https://www.youtube.com/watch?v=4PwN71cnHPs&t=66s>
(for Icon Probe Load & Laser Align Video)

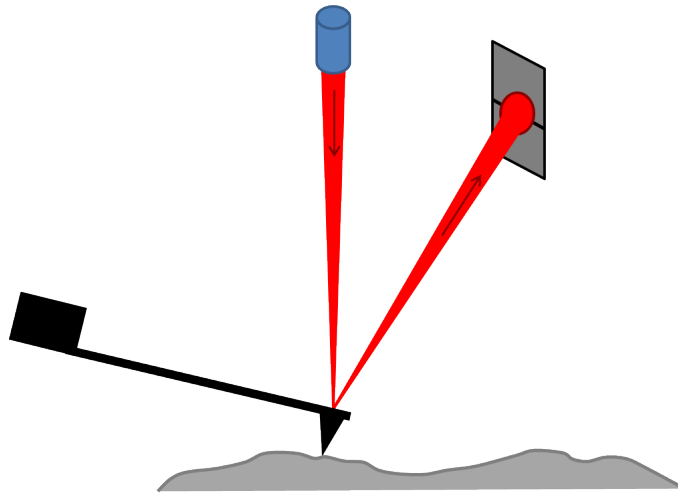
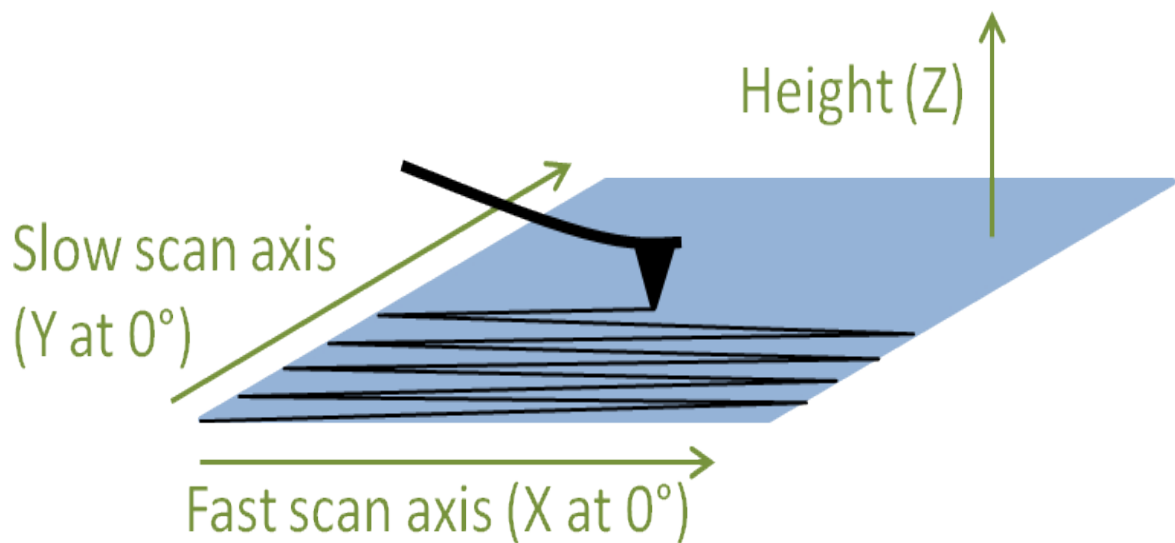
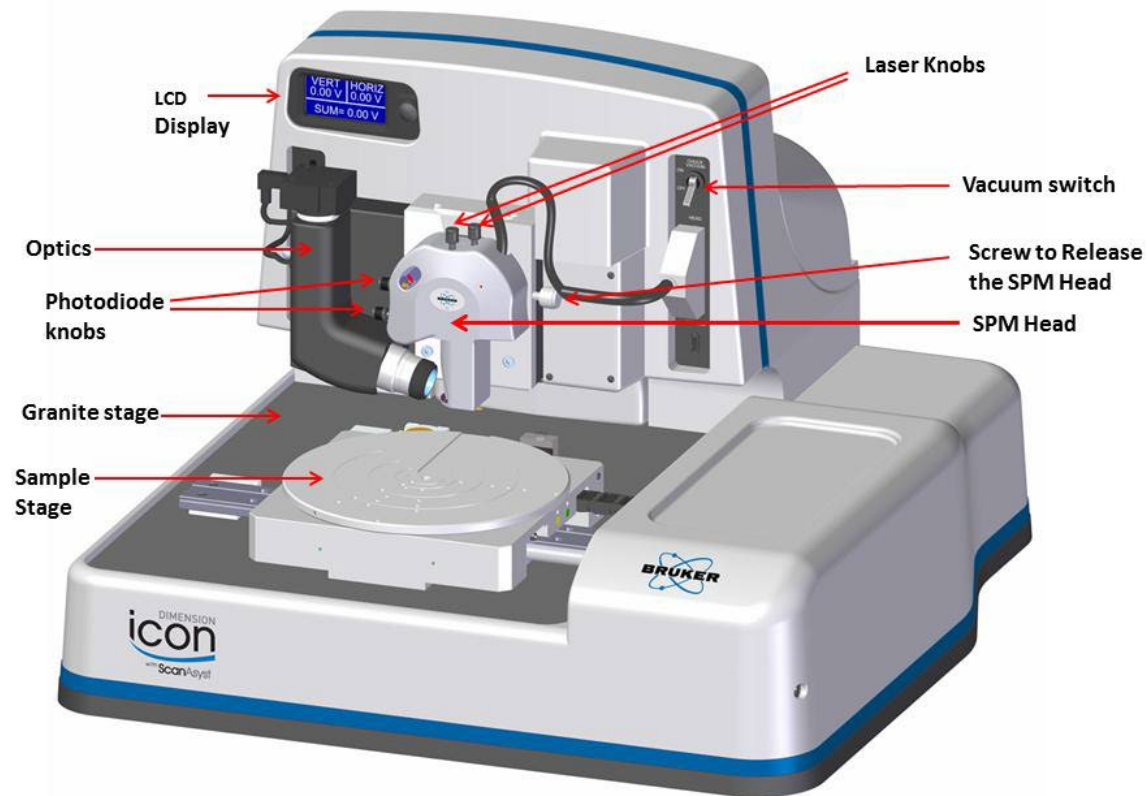


Figure: AFM probe scanning the sample.

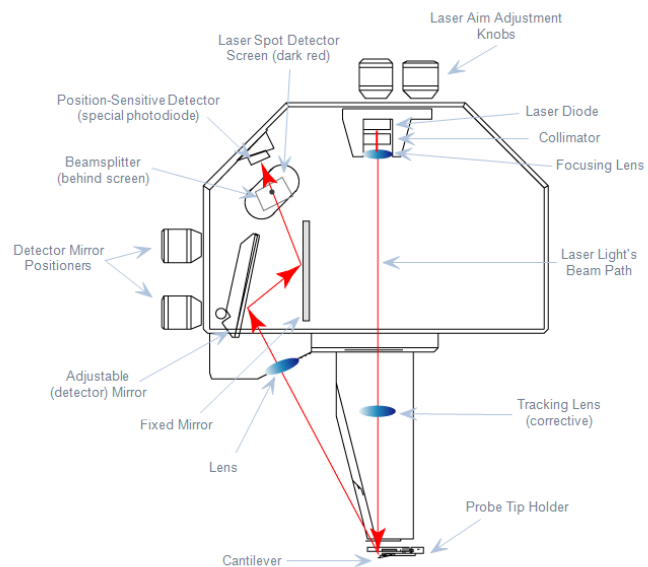


AFM Theory

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.



AFM Stage & Head assembly



Optical path of the laser beam inside a scanner.

Tapping Mode AFM

In general AFM operation is a process of several sequential procedures. Nanoscope control software provides a logical workflow which helps facilitates proper procedure execution. The general order of procedures follows below.

1. Session starts in iLab and sign logbook
2. Prepare and load the probe into holder
3. Install Probe holder on the SPM scanner head
4. Login in computer
5. Launch the Nanoscope 10.0
6. Load experiment-tapping mode
7. Laser alignment (shadow method)
8. Probe and laser set up and tuning
9. Sample surface/Navigator
10. Scan parameter
11. Engage, scan, optimization
12. Scanning and parameters setting tips
13. Capturing Image (Folder, file name & capture)
14. Withdraw: Changing sample location or put new sample
15. Data analysis
16. **Shutdown (9 steps)**

Getting Started

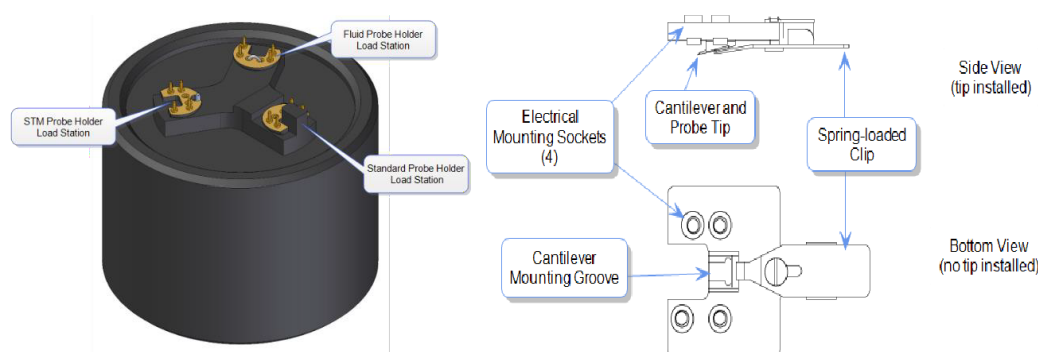
1. **Log in to iLab account** and start your today's experiment in RIC. **Sign logbook.**

2. Loading the Tip

- **Open the door of the instrument. Get the probe holder.**
- **Place the cantilever holder onto the cantilever holder stand (it is there).**
- **Press down and pull the probe clip all the way to the back.**
- **Place the AFM probe into the cantilever holder groove.**
- **Press down and gently push forward the tip holder clip to hold the cantilever.**

Using sharp tweezers, grab the probe substrate firmly by the sides and lift it free from the wafer or gel-pack. (In gel packs the tip ends are pointing upward and do not need to be turned over before being placed onto cantilever holder)

Dropping the AFM probe damages or destroys the cantilever. Do not use damaged probes.

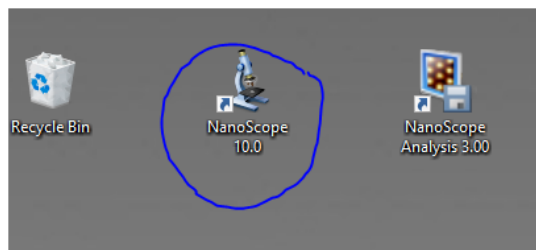


3. Install the Probe Holder onto the SPM Scanner

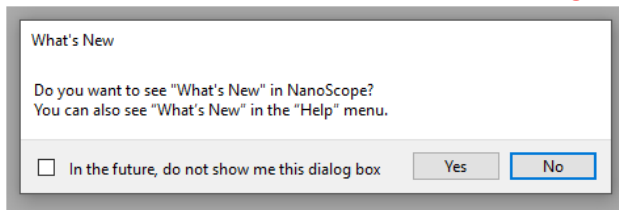
- Verify that adequate space exists to safely load the without touching the sample surface
Tip to sample distance can be increased by selecting Microscope > Withdraw several times or by selecting Navigate > **Focus Surface and raising the Z-stage upward.**
- Unlock and lift the scan head out of the dovetail groove
Turn the dovetail release screw located on the right side of the scanner dovetail clockwise to unlock the scan head.
- Carefully slide the scan head up and out of the dovetail groove (\$25,000 is the cost of the SPM scanner)..
- Mate the probe holder sockets to the pins on the scan head.
Verify the tip points down and away from the scanner The end of the cantilever must point in the direction of the optics assembly
Verify the AFM probe holder mounts flat against each pin on the end of the scan head.
- Replace and secure the scan head into the dovetail groove.
- Carefully slide the scanner down into the dovetail groove. Make sure to guide the scan head to the bottom of the groove. Do not drop or mechanically shock the scan head!
- Tighten dovetail release screws (turn counter-

4. Turn on the AFM computer (in general it is already on) and log in to the computer account.

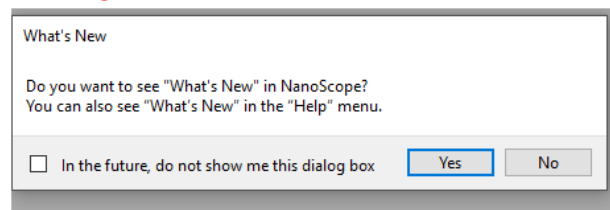
5. Launch the Nanoscope software by double clicking the Nanoscope 10.0 icon on the desktop.



Nothing on Granite Stage



Select "NO" if you want to visit internet then select "YES" that will go to Dimension icon web page



6. Load Experiment- 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 (Figure right side)

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

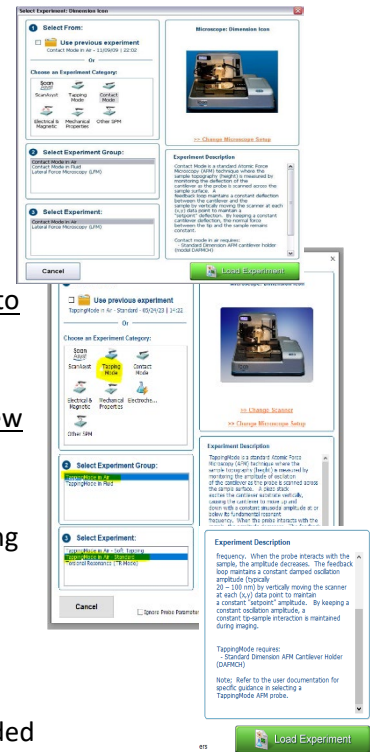
Description

- 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.

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

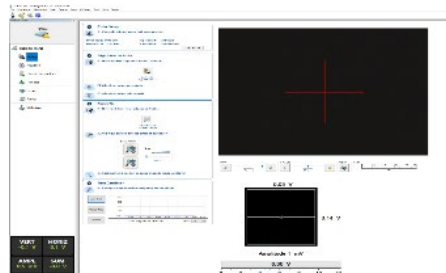
① Experiment Category, ② Experiment Group & ③ Experiment to use one of the pre-loaded workspaces.

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



Click "Load Experiment"

You will see this kind of window

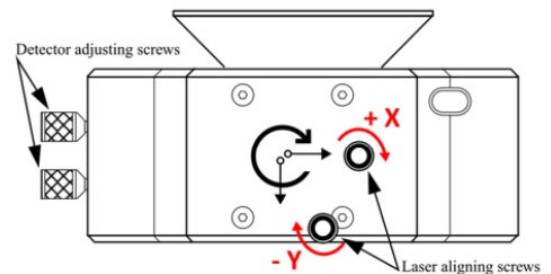


7. **Laser alignment:** Laser alignment can be completed using first by the **shadow method** procedure then with the **assistance** of the alignment station (discuss later in step 8).

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

Shadow Alignment Procedure:

To align the laser on the cantilever, follow these three steps while observing the laser spot on the granite surface (or a piece of white paper) below the scan head. When the laser is not hitting the cantilever or the probe substrate, it will appear as a bright red spot on the surface below. When the laser is properly aligned on the cantilever, a shadow will appear beneath it.



Step A: Horizontal Alignment

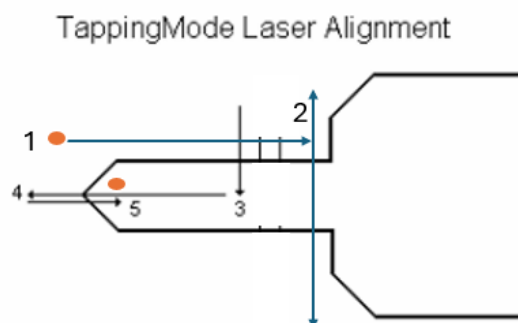
- I. If the laser spot becomes completely invisible, it is typically on the substrate. Redirect it into -X direction (counterclockwise) until it becomes visible. Start by moving the laser horizontally (Clockwise) while watching the laser spot on the surface below.
- II. If the laser is off the probe substrate, the red spot will appear clearly on the surface.
- III. Slowly move the laser until you find the edge of the probe substrate —once it strikes the front edge of the substrate the beam splits into two, one fraction becomes visible. Move little bit (anticlockwise).

Step B: Vertical Alignment

- I. Once the laser is positioned near the edge of the substrate, adjust the laser vertically.
- II. Carefully move the laser spot until it is positioned at the center of the desired cantilever (move both clockwise and anticlockwise until you find a spot for less laser spot on paper and also in the display window).

Step C: Free End Positioning

- I. After centering the laser on the cantilever, adjust the spot so it is positioned near the free end of the cantilever.
- II. You should see less light on the surface below and bright light on the display, confirming that the laser is aligned with the cantilever.

**8. Probe and laser Setup:**

To change the probe and align the laser on the AFM probe, click the **Setup** button in the **Workflow toolbar**.

A: Probe setup: Click **Change probe** and select the tip

- you want to use in the database or other/unknown cantilever if it is not in the database and **save**.

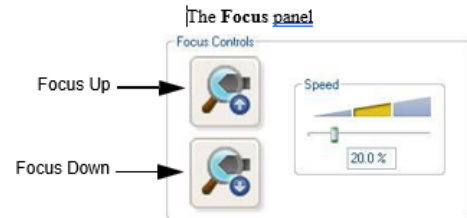
B: Align laser light:

- It is easier to align the laser on the tip in the **ALIGNMENT STATION**.

- Click **MOVE TO THE ALIGNMENT STATION** icon in the **Align** window. click “Yes” when prompted. Zoom out as far as possible (you should see the laser light if you done the alignment in step #4) in case you cannot see the laser spot as well as the tip in the optical field of view. Align the laser using the laser control knobs.



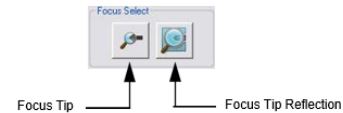
- This is not necessary (When stage movement is complete, use the focus control buttons to bring the cantilever and laser spot into focus. Click **FOCUS UP** or **FOCUS DOWN** The focus Speed is controlled by the sliding bar or by typing a value from 0 to 100).



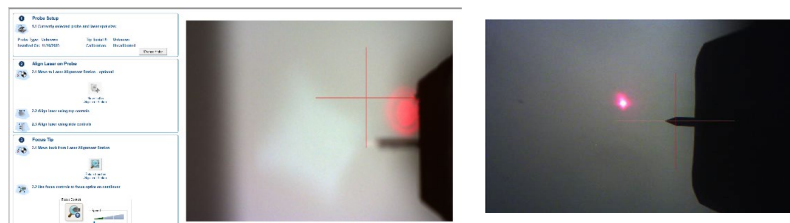
- Adjust the illumination for a better view.
- Move crosshair on the tip. Use zooms to see the tip.
- Laser alignment can be completed using first by the **shadow method** procedure then with the **assistance** of the alignment station.

- (optional) Select the Tip Reflection button to focus on reflection. This is particularly useful when scanning samples.

The Focus Select panel in the Align window

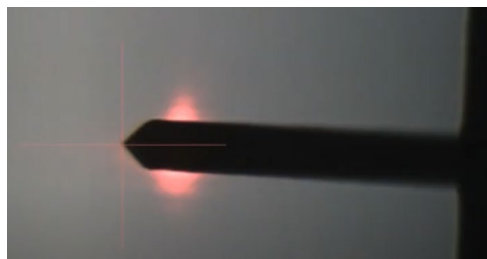


the tip
reflective



You will see these two pictures (laser light not on the tip) if you did not finish the Shadow Alignment Procedure in step #4.

- Using the video image as a guide, adjust the laser control two knobs on the top of the Dimension head to place the laser spot near (not at end) the probe tip. to maximize the **SUM** value (>3) on the monitor.

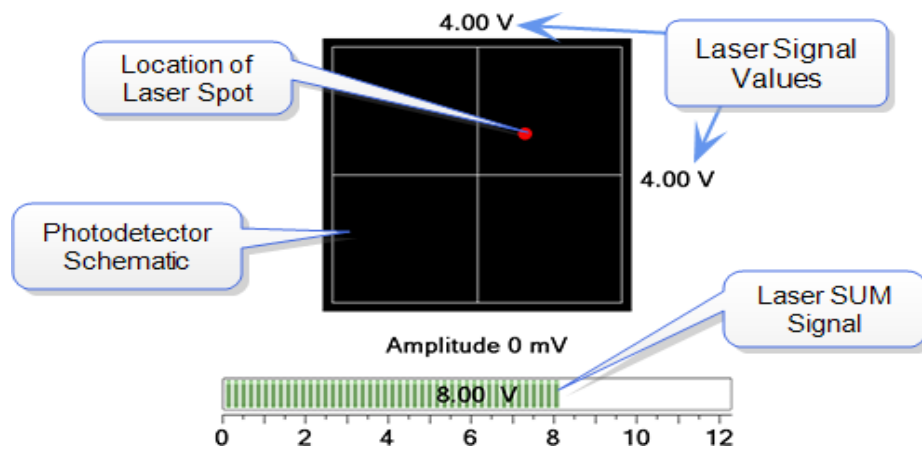


After this Click **Return from the Alignment Station** and click "Yes" when prompted (this step is very important).

C: Adjust the Photodetector

Once the laser is in place on the cantilever, adjust the photodetector position to obtain an adequate sum signal (greater than 3V) and properly orient the laser spot on detector quadrants.

Adjust the photodetector using the two photodetector knobs so that the red dot is at the center of the



Dimension head filter screen. Do fine adjustment to attain (0,0) for vertical (behind or top knob), and horizontal (front or bottom knob), Clockwise if see (+ve) values and counter clockwise for (-ve) values deflections respectively.

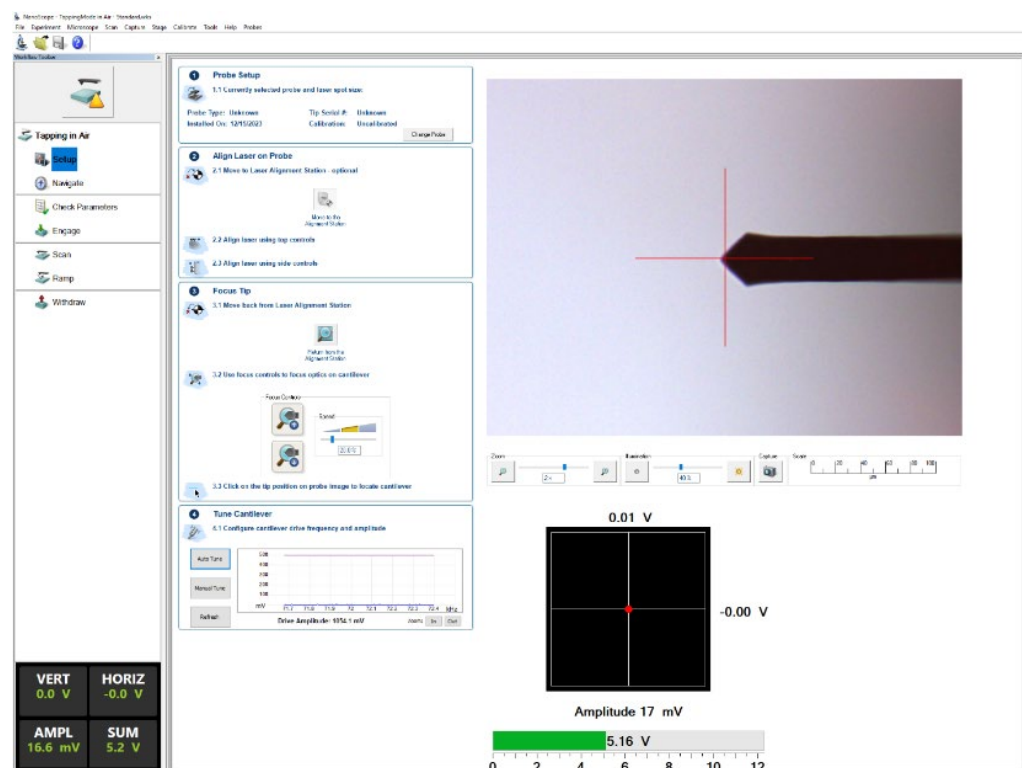
After this Click **Return from the Alignment Station** and click “Yes” when prompted (this step is very important).



D: Focus tip

In this section, the tip position is determined using the integrated optical microscope. The tip position (Z height) is located using optical focal distance measurements.

- Zoom out as far as possible (Zoom level 0) using the Zoom Out button
- Using either the trackball or the on-screen focus controls move the optics **up or down to focus** on the cantilever.
- On the video panel, click on the tip location to place the **crosshair over the end** of the cantilever where the probe tip is located.



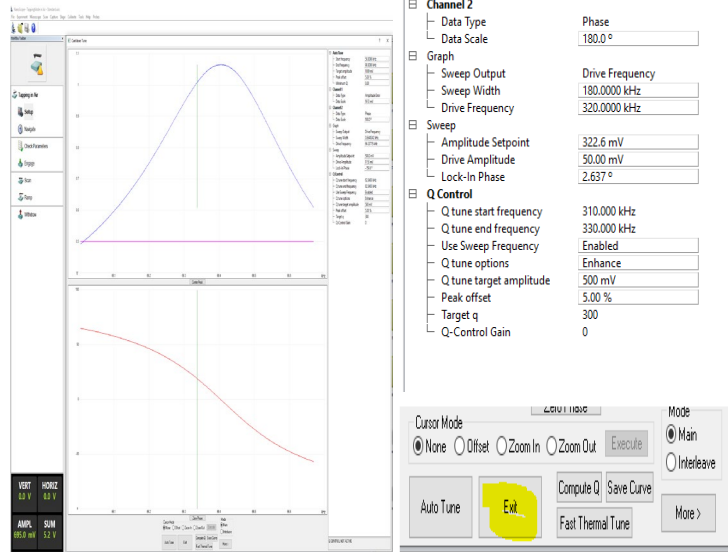
E: Tune the Cantilever (TappingMode AFM)

In TappingMode the cantilever is driven near a resonance frequency with a constant amplitude. The tune procedure optimizes the frequency, amplitude, and phase of the driving of the signal.

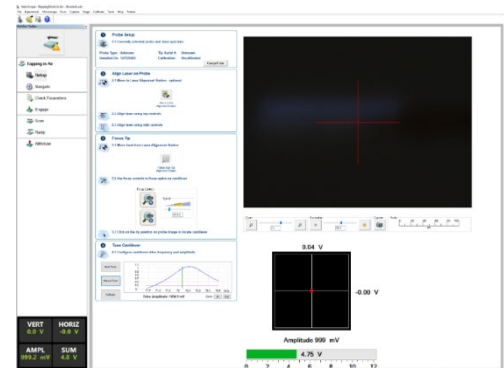
Manual Tune button for Unknown probe

If the appropriate probe was selected from the probe database during the experiment setup procedure, click the Autotune button located on the Tune Cantilever Panel. Otherwise, or if Autotune fails,

- click the **Manual Tune button** to open the Cantilever Tune window.
- Start frequency & End frequency about **30 % lower and higher** respectively than the listed nominal cantilever frequency. Target amplitude 300 mV default, Peak offset 5%.
- Click the **Auto Tune** button control to initiate the peak search and tuning.
- Use **auto scale** for better view by selecting scale using right mouse.
- If the Auto tune search has returned a satisfactory operating condition, then click the **Exit** button to save the drive parameters and return to the setup.



EXIT now from auto tune and you will see this window (with sum signal and tune curve)

**9. Navigation/ Navigate: Moving the sample and scanner Focus on the Sample Surface**

In this step the Z axis is moved to focus on the sample surface.

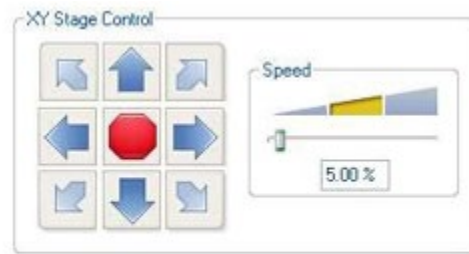
The Z axis moves both the optics and the scan head; **Care should be taken to avoid overshooting the focus and crashing the tip into the sample.**

When working with thin / transparent or low contrast samples it may be beneficial to focus on the sample edge.

For certain sample types it may be helpful to focus the tip reflection instead of the sample surface. The focus target can be toggle with controls in the Navigate view.

- Click the **Navigate icon in the Workflow Toolbar, Workflow Toolbar > Navigation**

- Move the X-Y stage to align the desired location on the sample under the crosshairs either by using the trackball without holding down any buttons or by using the XY Stage Control arrows, shown in below, in the Navigate View.



XY Stage Controls

- To focus on the sample SURFACE (normal operation). For samples which are difficult to bring into focus, move to an edge of the sample, which is easy to find in the optical image, and bring the top of the edge into focus.
-
- **Focus on the surface** by clicking the Sample icon in the Focus Sample panel of the Navigate view Focus on the sample surface using either the Focus: Z motor arrow controls or by rolling the trackball up or down

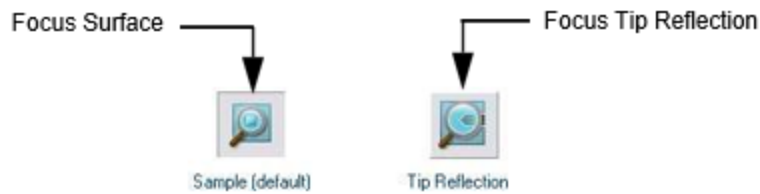


Focus: Z Motor Controls

while pressing the bottom-left button.

- Use XY Control buttons or trackball to position the probe above the location of interest.
- Clicking the **Nagivation** button activates the trackball to move the sample stage in X and Y and move the scanner in Z.
- Spinning the trackball up/down moves the sample front-to-back. Spinning it left/right moves the sample left/right/
- Holding focus down while spinning up/down moves the scanner (and tip!) up and down.
- Hold lock down allows continuous motion of the stage/scanner without continuous spinning.
- To focus on the sample SURFACE (normal operation) or the TIP REFLECTION (for extremely clean samples), change the Focus Select parameter accordingly.

The Navigate Focus Select Panel

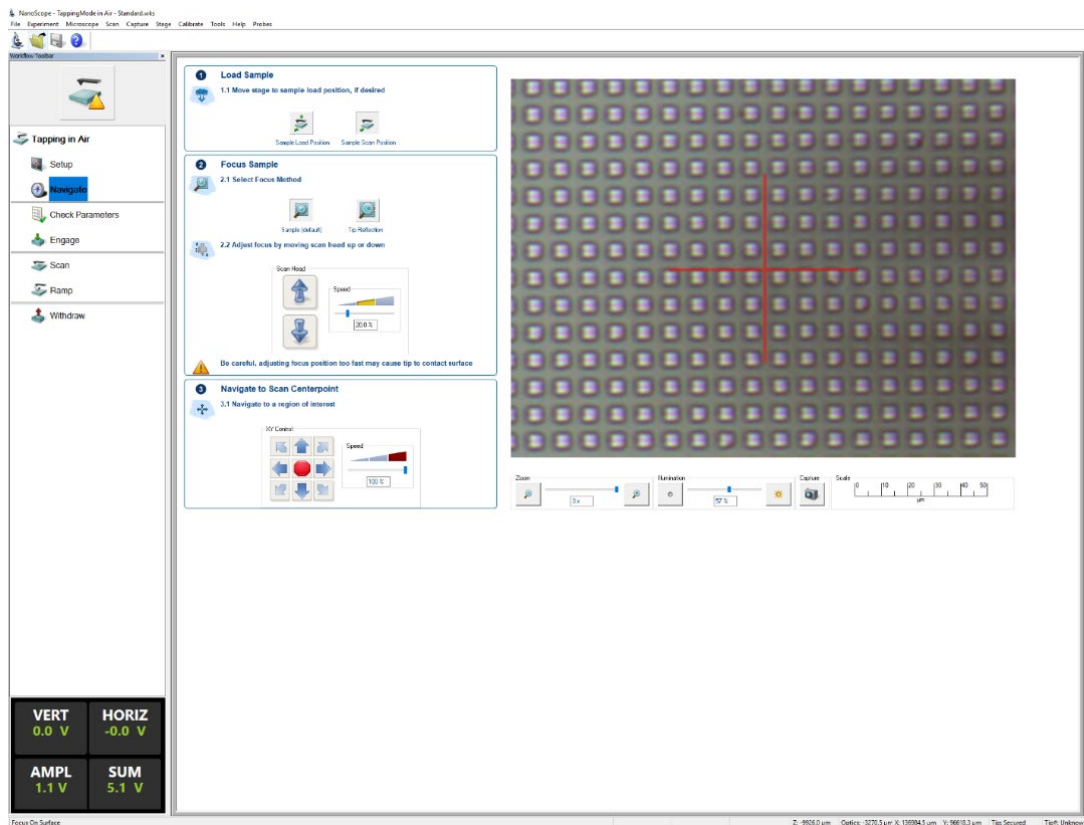


Note: For reflective or semi-reflective samples, the tip reflection is easier to bring into focus than the surface, especially if the sample is very flat or clean.



CAUTION:

When moving the SPM stage up and down, it is possible to crash the tip into the surface. To prevent a crash while focusing on the surface, watch the optical image **and** tip-to-sample proximity. The sample should be in focus when the tip is 1mm (1000µm) above the surface.



10. Check Initial Scan Parameters

The Check Parameter view highlights an important subset of experiment parameters, and it provides a simple interface for adjusting default values prior to engaging the tip. If using a pre-defined experiment, changes from the default values are **highlighted in yellow**. It is typically recommended to use Scan size around 1 µm prior to first engagement.

For additional details about initial parameter setting, refer to information given for the specific imaging modes.

Select **500nm scan size** (and later increase to higher values).

11. Engage, Scan & Withdraw

Now that the tip is focused, the laser is aligned, the cantilever is tuned, and the surface is focused, it is time to engage the tip to the surface.

To engage select Microscope Engage or **click the Engage Icon** in the Workflow Toolbar

There are two types of engage process. Engage mode can be changed in the Engage Settings window.

Microscope  Engage Setting

- Engage: available for all probe types; this is the default setting.
- Smart Engage: available for certain probes types operating in air.
 - Choose an appropriate amplitude setpoint in the Feedback Controls menu. For
 - 0.5 V free amplitude, a setpoint of 0.3 V is pretty good.
 - Select **Engage**. You will see the cantilever make a rough approach to the surface and you can hear the stepper motor advance until the tip reaches the surface. Upon making contact, you will hear a beep.

12. Scanning and Scan Parameters

Next, you set scan parameters and scan the sample.

A. In the Scan Parameters window, use the following initial parameter settings in the Scan tab. These values may already be set; they are handy starting values.

- Scan size: 1-100 μm
- Aspect Ratio: 1.00
- Scan Angle: 0.00 °
- Scan Rate: 1Hz
- To collect 5K points of data per line, set the following parameters in the Scan panel:
- Aspect Ratio: 8.00 (4.00 if Lines is 1024; 1.00 if Lines is
- 5120) and you want square pixels
- Samples/Line: 256, 512
- Lines: 640, 1280 or 5120

Note: You can use the mouse to adjust the value in many parameter fields. Click on the value and drag the mouse left to decrease the value or right to increase the value.

The Aspect Ratio controls the X:Y ratio of the pixels in the displayed image. Because there is an 8:1 ratio between 5120 samples/line and 640 lines, using an Aspect Ratio of 8 causes the pixels displayed in the image to be square.

Note: Some microscopes, e.g. Dimension Icon-PI, are limited to 1K (1024) lines and 1K points per line.

For TappingMode, use the following initial parameter settings in the Feedback panel:

SPM feedback: Amplitude

Integral gain: 0.5

Proportional gain: 5.0

B. Click the ENGAGE icon in the Workflow Toolbar. Scan lines appear in the Image Windows

C. once the tip engages and scanning begins.

D. Check to see whether the trace and retrace lines in the Scope window are tracking each other well. They should have a similar shape, but they may not overlap each other horizontally or

- E. vertically. Adjust the Scan Rate, Integral Gain, Proportional Gain, and/or Setpoint (that is, Amplitude Setpoint for TappingMode and Deflection Setpoint for Contact Mode) parameters. Once the trace and retrace are tracking well, your tip is scanning the sample surface.
- F. At this point, you may want to adjust the Scan Size, X offset, Y offset, and Scan Angle parameters to locate the scan over features of interest. If you increase the Scan Size, remember that the Scan Rate should be lowered.
- G. Note: You can zoom in on the scan image by selecting the Zoom button below the image. Then, use your mouse to drag a box outline over the area you want to zoom in on.
- H. Click Offset to offset the center position of your scan.
- I. With a large image, it may be useful to zoom in on the scan without changing the Scan Size. Select the data ZOOM button above the image. Use your mouse to drag a box outline on the image (begin by clicking where you want the center of the box to be). When you release the left button, you will be zoomed in (scan size of image display will change) but the scanner will remain scanning the original scan size. You may also choose to physically change the scan size or X/Y offsets by using the Zoom or Offset buttons below the image.

12. Scanning:

1. Look at the line traces of height or height sensor images. Confirm that the Trace and Retrace lines look similar. If they are tracking each other, they should look the same, but they will not necessarily **overlap** each other, either horizontally or vertically.
2. You may want to try adjusting the Setpoint, by using the right arrow key to adjust the setpoint value gradually until the tip lifts off the surface (the Trace and Retrace lines no longer track each other). Then, decrease the Setpoint with the left arrow key until the Trace and Retrace lines follow each other. Decrease the Setpoint one or two more clicks more with the left arrow key to ensure the tip will continue to track the surface.
3. If they are not tracking, adjust the Scan Rate, Gains and/or Setpoint to improve the tracking. First try decreasing the Setpoint until they exhibit common features. Then, reduce the Scan Rate. Next, try increasing the Integral Gain using the right arrow key. As you increase the Integral Gain, increase the Proportional Gain as well. The tracking should improve as the gains increase. If Trace and Retrace still do not track satisfactorily after following the above steps, try reducing the Setpoint. The default value of the Integral Gain is 0.5, which is too low for most images, usually it should be at least 2.
4. Scanning parameters are dependent upon the type of sample. If you are having trouble collecting good images, ask someone who is scanning similar samples for assistance in scanning those sample types.

A: Set up Initial Operating Parameters

- a) 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.
- b) 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).
- c) SAMPLES/LINE – This parameter sets the number of height samples per line length. This determines the resolution in pixels of the image.
- d) 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.
- e) Integral Gain – Sets the sensitivity and speed of the Z-piezo driver amplifier to vertical height changes as the tip scans the surface.
- f) 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.

- g) 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.

Use up/and down arrow to change the scanning directions
Select 1 to 4 windows, keep height and phase window



B: Set scanning parameters

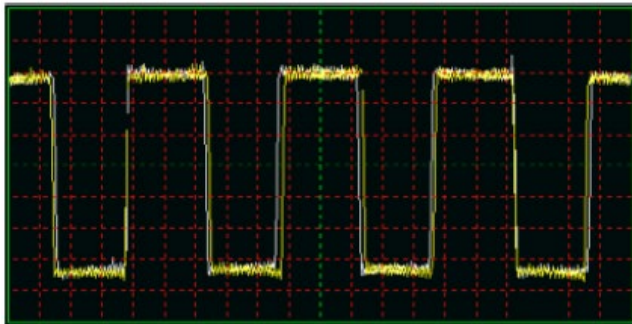
Scan Size:	The scan size varies with the scanner used. The J scanner currently mounted on the AFM has a maximum scan size of 125 um x 125um. The E scanner (suitable for fluid cell) has a maximum scan size of 10 um x 10 um.
XY Offset:	Set to 0
Scan Angle:	Set to 0 degree
Scan Rate:	Controls the rate at 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. A slower scan rate generally leads to better images , 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. Increasing leads to better image quality , but there is a trade-off with time

C: Set controller parameters

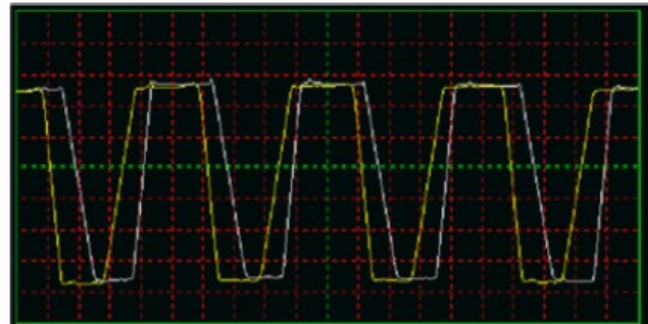
Setpoint:	The value of the RMS of the cantilever vibration amplitude that the feedback loop maintains. (Setpoint is thus proportional to force applied to surface). The difference between the Vertical Deflection signal before engaging and the Setpoint is related to the force. A larger, more positive Setpoint voltage results in a larger contact force. You can adjust the voltage later during the measurement. Reducing setpoint often leads to better quality images. Do this until the image improves.
Drive Amplitude:	The drive amplitude of the force at which the cantilever is driven. Increasing drive amplitude often gives better phase data, up to a point. 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. Increasing gains often helps obtain better images (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



Set point too high



Integral gain too high



Integral gain too low

13. Capturing an Image

Once you have adjusted the scan parameters, you can capture a scanned image. Perform these steps once a scan you want to capture is in progress.

1. You can capture a scan in any one of four ways:
 - Click the **CAPTURE** icon in the toolbar.
 - Click the **CAPTURE NOW** icon in the toolbar.
 - From the menu bar, select Capture > Capture.
 - From the menu bar, select Capture > Capture Now.

The scan will continue. Notice that the status bar at the bottom of the NanoScope window.

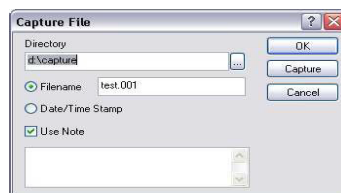
Optics: -2998.5 um X: 0.0 um Y: 0.0 um Tip: Engaged Tip#: Capture: On File: Date/Time Scope: Dimension 4000

When the current scan is complete, the image will be stored automatically in the Capture Directory with the file name indicated in the status bar.

Capture Status

The file name and directory can be changed by selecting Capture > Capture Filename from the menu bar

Change Filename:



You may also select the capture filename by clicking the SELECT CAPTURE DIRECTORY icon and/ or the DATE/TIME STAMP, shown in below, icons on the menu bar.

Figure 2.5r Date/Time Stamp menu bar menu



Capture Now, and Capture Last save as much of the image buffer as possible including parts of it that were generated with different parameters such as gains, setpoints, etc., so some of the information in the header may be incorrect for some parts of the saved image.

2. In the Image Browser area (see Figure below), check to see if you are looking at the Capture Directory. If not, select the Capture Directory icon, shown at left, (or click the  button and select the Capture Directory, which is usually d:\capture). If you don't see the Image Browser, click the SHOW/HIDE BROWSE icon on the toolbar.

NanoScope Image Browse Window



14. Withdraw and changing location

When imaging is complete, withdraw the tip by selecting Microscope > Withdraw, or the click the Withdraw icon in the Workflow Toolbar. This typically raises the tip 1 mm above the sample.

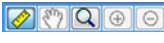
Although the 1 mm withdraw distance provides enough clearance to loosen the dovetail and remove the SPM scanner, if further distance is desired perform one of the following:

- Select Microscope > Withdraw to raise the Z-stage with the trackball.
- Execute Withdraw from the Workflow Toolbar multiple time
 - Withdraw the tip by clicking on the Withdraw button on the left-hand menu.
 - Click this button several times only if sample shape demands it.
 - Click navigator
 - Use the stage controls to move to a new spot on the same sample surface. Bring the tip closer to the surface and engage as described above.

15. Data Analysis:

Most people use Gwyddion (<https://gwyddion.net/>). Better to check analysis with here.

A. 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 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*.

B. 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.

C. 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*.

D. 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.

E. 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.

F. 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.

G. 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*.

H. 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.

16. Shutdown (Overview)

1. Withdraw the tip twice (more times if needed).
2. Roll the stage toward you and remove your sample from the stage and clean sample chuck.
3. Move the stage back so that scanner is on the stage near a (press) sticker. .
4. Remove the tip holder from the head, then your tip from the tip holder, and save the tip for future scans (if not damaged).
5. Move your saved data from the capture directory to pen drive or email or account.
6. Close the program, exit Nanoscope software.
7. Log out your session
8. Sign the logbook
9. Session out in iLab