

# 1 Force Curves Acquisition

CHAPTER REV1874, DATED 05/06/2016, 19:33

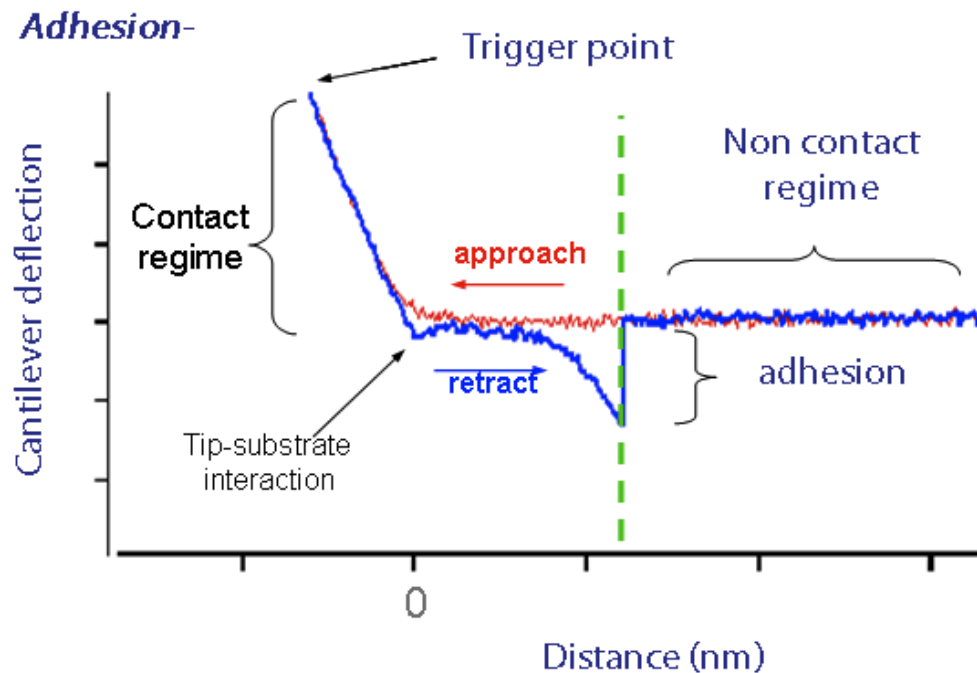
USER GUIDE REV1874, DATED 05/06/2016, 19:33

## 1.1 Introduction to the Force Spectroscopy

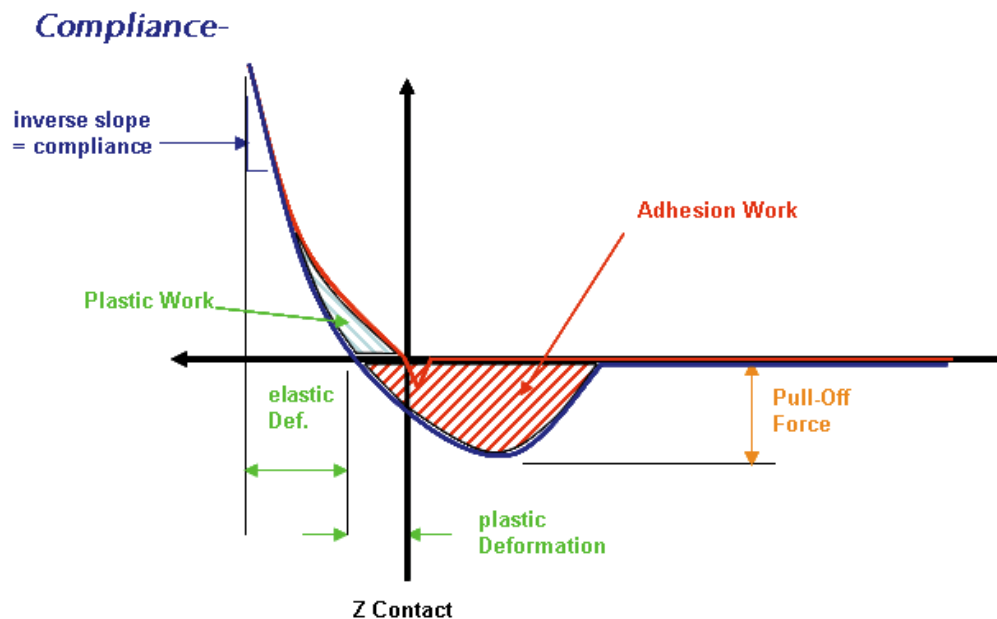
In brief, Force spectroscopy measures the deflection of the cantilever (with known spring constant) in the Z direction with the intent that an adhesion dissociation event between the tip and substrate will be measured, OR the compliance of a material under the 'tip' as it pushes into it. The process usually begins with the tip approaching the surface with some 'free air' (non-contact) deflection value, make contact to the sample for some period/ load until it reaches a user defined cantilever deflection (force), to then reverse direction for the retract cycle (although there are some exceptions).

There are two major classifications that most force spectroscopy experiments measure:

- A pulling event in which the tip interacts with the surface, and some adhesion dissociation event between the tip and surface is measured on the retract cycle (1.1).
- The tip pushes into the (material on the) surface to measure compliance (1.2).



**Figure 1.1:** Adhesion force spectroscopy classification. Pulling can yield adhesion forces.



**Figure 1.2:** Compliance force spectroscopy classification. Pushing can measure compliance forces.

This chapter is designed to help describe, and in some cases reveal, the versatility of the force spectroscopy capabilities with the Asylum Research AFMs, and also includes some empirical considerations.

Cantilever selection is very important in force spectroscopy: if a lever is too stiff, then small forces may not be recognized; if a lever is too floppy, perhaps not enough force can be applied during compliance measurements. Another way to state this is to say that for a lever that has too low a spring constant, the sample will appear to be infinitely stiff, and for a probe that is too stiff, a soft sample will appear to be infinitely soft, or will appear as no different from the noise in the measurement. Other considerations are the cantilever material regarding how well it will work in air/ fluid, and the ability to do chemical cross-linking to it.

There are some excellent force spectroscopy review papers in the literature; we list here a few:

- Butt, H.-J., Cappella, B., Kappl, M., 'Force measurements with the atomic force microscope: Technique, interpretation and applications', *Surface Science Reports*, 2005 59 1-152
- Cappella, B., Dieter, G., 'Force-Distance Curves by AFM', *Surface Science Reports*, 1999 34 1-104
- Heinz, W., Hoh, J. H., *Journal of Chemical Education*, 2005 82(5) 695-703.
- Heinz, W., Hoh, J. H., *Nanotechnology*, 1999 17 143-150.
- Dupres, V., Verbelen, C., Dufrêne, Y. F., 'Probing molecular recognition sites on biosurfaces using AFM', *Biomaterials* 2007 28 2393-2402.
- Reif, M., Grubmüller, H., 'Force Spectroscopy of Single Biomolecules', *ChemPhysChem* 2002 3 255-261

## 1.2 The Force Panel and Force Curve Acquisition

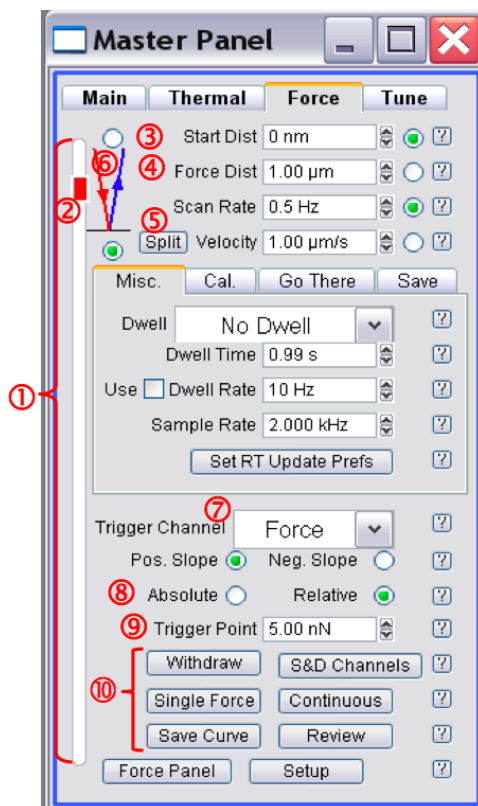


Figure 1.3: Force tab of the Master Panel.

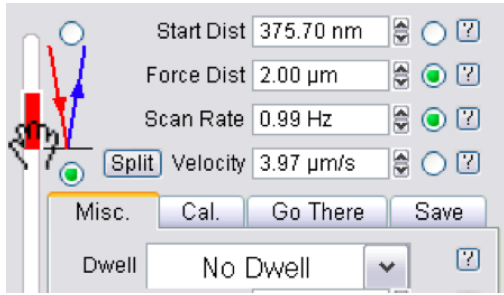
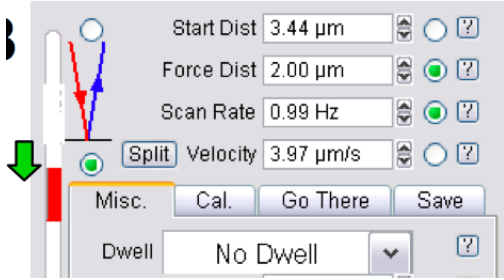
### 1.2.1 The Force tab of the Master Panel

The Force tab of the Master Panel is where most the basic force spectroscopy software control occurs. First we'll describe some of the general controls, and then more controls hidden under the additional tabs on this panel, also known as sub-tabs, as numbered in Figure 1.3 on page 3.

① **White Bar** Along the left of the panel represents the entire travel distance of the Z piezo; the top of the white bar represents the piezo as fully retracted; the bottom represents the piezo fully extended.

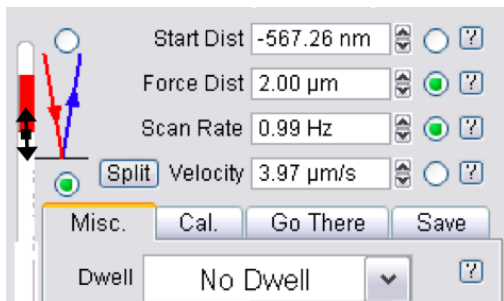
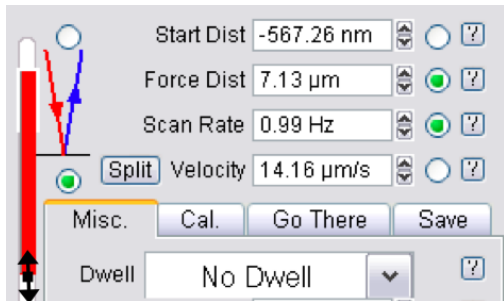
② **Red Bar** Within the white bar represents the 'Force Distance' of the force distance cycle, relative to the entire travel of the Z piezo (see below). Note that values in microns given are in reference to the piezo sensitivity. The captured and displayed force curve will show slightly different values as corrected by the z sensor.

③ **Start Distance** This parameter is the point at which the piezo begins its Z/ force distance cycle (i.e., the starting voltage applied to the piezo is another way to think about it). The red bar's upper position in the white slider bar qualitatively depicts this position. The red bar can be moved with the mouse cursor two different ways:

<p>1.</p> <p><b>Method 1</b></p> <ul style="list-style-type: none"> <li>Place cursor somewhere in red region</li> <li>Left mouse click until hand icon appears</li> <li>Drag red bar throughout the Z piezo range (white slider bar)</li> </ul>	
<p>2.</p> <p><b>Method 2</b></p> <ul style="list-style-type: none"> <li>Left click mouse in the white area to roughly scroll the red cursor up or down.</li> </ul>	

The Hamster wheel (on front of MFP controller) can also execute this movement in real time when the start distance radio button is activated.

④ **Force Distance** This parameter is how long the force-distance cycle will be, provided a trigger point isn't arrived at prior to that. Notice that as this setvar value is changed, the length of the red bar changes (i.e., the length of the red bar qualitatively depicts the force distance relative to the entire length of the Z piezo's distance (white slider bar). The length and position of the red bar can be manipulated with the mouse as follows:

<p>1.</p> <p><b>Step 1</b></p> <ul style="list-style-type: none"> <li>Place cursor at edge of red bar.</li> <li>Hold left mouse button down until double arrow appears</li> </ul>	
<p>2.</p> <p><b>Step 2</b></p> <ul style="list-style-type: none"> <li>Drag cursor to desired force distance value.</li> <li>Notice the change in the 'Force Dist' value in the box to the right.</li> </ul>	

⑤ **Scan Rate/Velocity** This is the estimated PIEZO velocity (NOT the tip velocity); changing the velocity, or scan rate, affects the other.

⑤\* **Sync:** Split / Synch Approach/ Retract velocities can be made different in 'Split' mode; for experiments that require constant velocities, turn on the closed loop in the Z axis (See Section 1.2.7

on page 15). See Section 1.2.8 on page 16 to select velocities that are not subject to hydrodynamic effects.

⑥ **Forward/Reverse pulls** These radio labels change whether the tip starts on or above the surface (Red mode/ Blue modes) :

**Forward pull (Red Mode):** Tip starts cycle in free air (non-contact) and approaches surface; then retracts; this is the default mode upon MFP3D software start up. This is the most commonly used mode for force spectroscopy experiments. See Figure 1.4a on page 5.

**Reverse pull (Blue mode)** Tip starts cycle at surface and travels (retracts) to free air (non-contact), then returns to surface; This mode is good for ‘Fishing’ , possibly Force Clamp (?? on page ??). See Figure 1.4b on page 5.

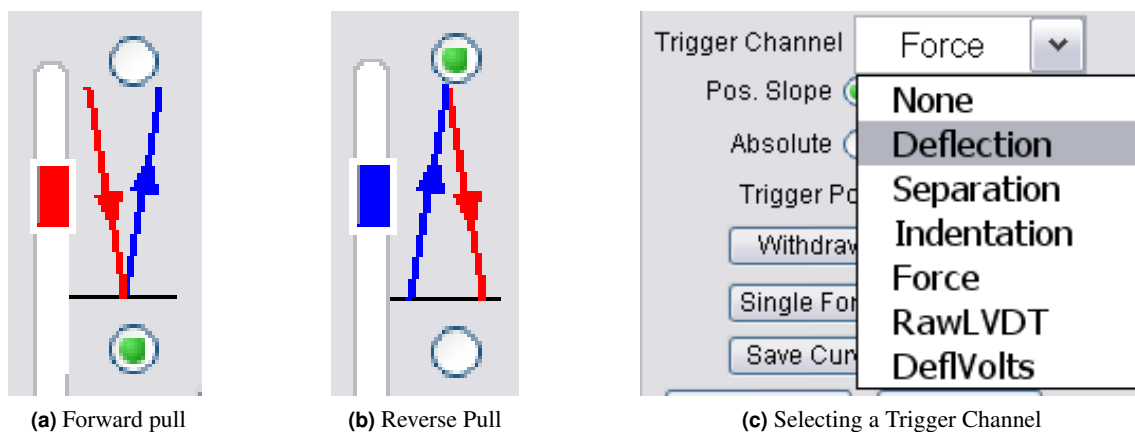


Figure 1.4

⑦ **Trigger Channel** This pull-down menu allows the user to define what units to measure for ‘triggering’- essentially a user defined Force that the tip applies before switching direction (Figure 1.4c on page 5).

**None** No trigger applied - The piezo will go through its force distance cycle regardless of either not making contact with surface (?? on page ??), or grinding tip into the surface.

**Deflection** Monitors the deflection (nm) on the photo-detector - Best used if the spring constant has been calibrated (See ?? on page ??).

**Separation** This is Deflection subtracted from the LVDT signal; puts contact regime on left side; makes the contact regime have a slope of infinity if the cantilever is pushing against an infinitely hard surface.

**Indentation** This is Deflection subtracted from the LVDT signal; puts the contact regime on right side; puts increasing indentation depth to right of start. (Force on Y axis)

**Force** Will monitor Force applied - value only accurate if the spring constant has been determined (See ?? on page ??).

**RawLVDT** Monitors the distance on the Z axis Closed loop sensor

**DefVolts** Monitors the amount of volts on the PSD- best used as trigger if the InvOLS has not been calibrated.

⑧ **Relative/Absolute Trigger** Check relative to trigger at the trigger trigger value relative to the start trigger value. For example, if the deflection at the start of the curve is 0.3V, and the trigger is 0.5V, the net deflection would be 0.5V, and the curve would trigger at 0.8V. If absolute was checked, then the net deflection would be 0.2V and the curve would trigger at 0.5V, ignoring the start value. Most deflection based force experiments use relative, and AC mode force experiments use absolute trigger.

**Slope** A positive slope will trigger on a rising trigger channel value. A negative slope will trigger on a falling trigger channel value. Deflection based force experiments typically use a positive slope, and AC mode force experiments typically use negative slopes. See Section 1.3.4 on page 27 for more on Dynamic (AC) Force Spectroscopy.

⑨ **Trigger Point** This setvar defines trigger point/load values. The 'Trigger point' is a user defined value detected on the Photo-detector that tells the piezo to switch directions even if the force (deflection) curve has not achieved the completed Force distance (deflection) value.

⑩ **Buttons**

**Withdraw** Withdraws the tip; puts Z piezo voltage at 0V (i.e. almost fully retracted).

**Channels** Brings up the 'Force Channel Panel'; see Section 1.2.6 on page 14.

**Single Force** Activates a single force cycle (Ctrl+3 also does this).

**Continuous** initiates force cycles to be continuously executed, until told to stop. The amount of continuous curves can be limited by clicking the 'Setup' button.

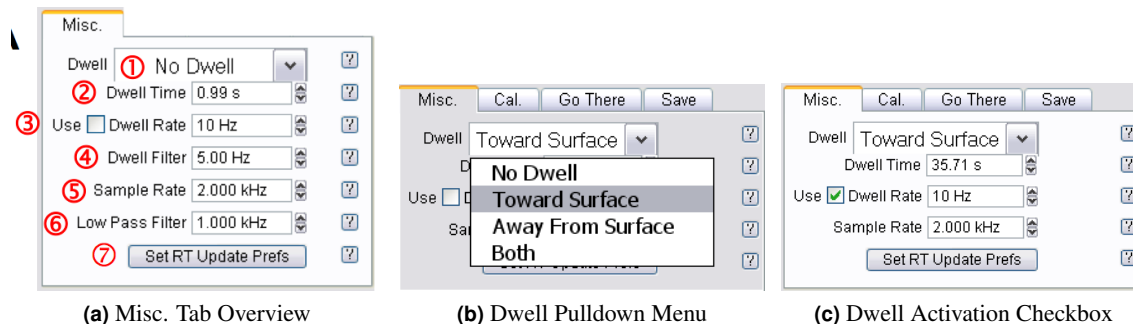
1. Activate the 'Limit Contin. To' checkbox. Stands for limit continuous to.
2. Click the 'Looks Good' button.
3. Enter the amount of curves needed to collect continuously in the Limit to Cont setvar.
4. Do some force spectroscopy.

**Save Curve** If the curve looks like it is something you want saved, and you do not have all curves being saved in the Save subtab, click this button.

**Review** Loads force curves for review/ analysis by bringing up the Force Display Panel.

### 1.2.2 The Misc. SubTab

This sub-tab controls dwell periods / sampling and sampling / bandwidth options. The MFP-3D software allows the user to define a period of 'Dwell' where the tip stops moving (or maintains some load) in the middle or end of a scan while continuing to collect data at some rate. This feature is great for data acquisition where perhaps a chemical reaction timescale needs to be expanded to accommodate some receptor-ligand event, or to assess some creep /compression event while applying/ maintaining a load to the sample with the 'tip'.



**Figure 1.5:** The Misc. Tab allows the user to define temporal and low pass filter settings for dwell and force curve collection.

### Dwell Periods and Sampling

① **indenter Ramp After Trigger** This will call whatever function has been developed in the indenter panel. The Indenter button brings up the indenter panel. Section 1.3.2 on page 20

① **Bias Ramp After Trigger** This will execute an IV curve using whatever settings are applied to the DoIV

① **Dwell Pull-down menu** Activate Dwell function here as shown in Figure 1.5b on page 7.

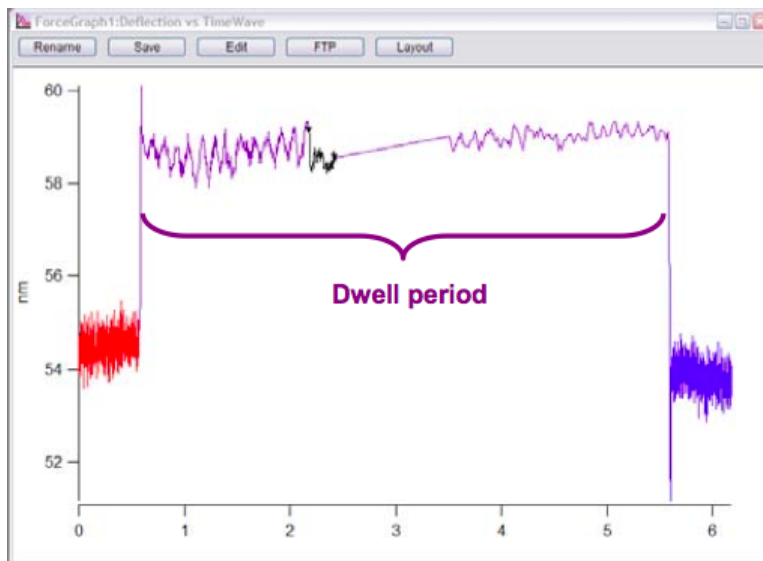
**None** No Dwell period is applied.

**Toward Surface** This is a Dwell period applied when the trigger point is reached. Bandwidth options during this segment can be chosen (See Figure 1.5c on page 7).

**Away from Surface** This occurs for reverse pull (see blue mode); a dwell is applied after the tip pulls away from the surface and before it approaches it again.

**Both** This applies a dwell when the tip retracts from surface, then again as the tip approaches the surface.

② **Dwell Time** This defines the length of Dwell. Click the 'Use' checkbox to increase Dwell rates. Maximum dwell time is 10 seconds. An example of a force distance curve (plotted as deflection vs. time) with a multi second dwell setting is seen in Figure 1.6 on page 8.



**Figure 1.6:** Example of a temporal force distance curve (plotted as cantilever deflection vs. time, with a multi second Dwell towards surface).

③ **Dwell Rate** This is the rate at which data is collected during a dwell- i.e., maybe the experiment calls for more or less points to be collected during this time. It must be activated with the 'Use' checkbox to the left of the setvar.

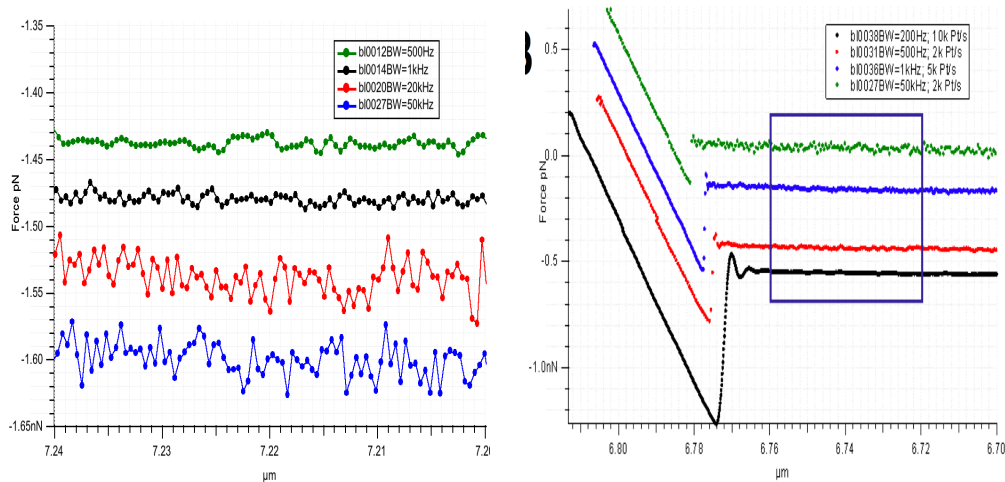
④ **Dwell Filter** This is a low pass filter that can clip from 1 Hz to 1kHz; often not a default parameter. Must be activated in the setup menu of the panel.

### Sampling and Bandwidth Options

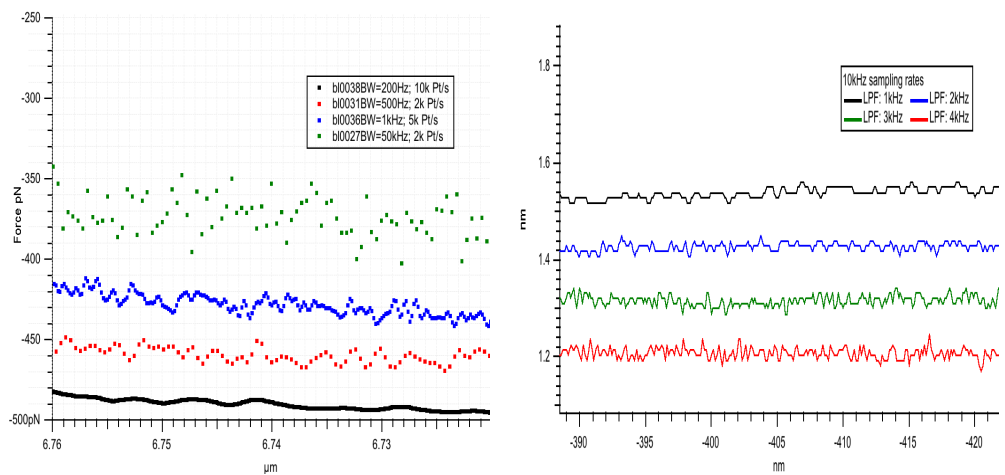
The band width (BW) options for the curve collection. Note, to view the BW options (in most version of Asylum Research software), they must be activated to be shown using the 'Setup' button. Setup can also be activated by right clicking anywhere on the panel.

⑤ **Sample Rate** This is essentially how many points per second that are collected during the force curve acquisition (See Figure 1.7a on page 9 for example).





(a) Various data streams from a free air portion of a force curve collected at 2kHz (i.e., 2,000 points/sec) with different low pass filter settings. Notice higher filter settings are more noisy.  
 (b) Examples of force curves collected at various sample Rates and low pass filter settings.



(c) Zoom of boxed area in Sub-Figure B to illustrate sampling point density  
 (d) Four force curves collected at 10kHz sampling rate with different low pass filter (LPF) band width settings. Notice at low LPF BW, the data is clipped sufficiently such that data fitting might not occur at desired point values (in Y).

Figure 1.7: Examples of filter settings for force curve acquisition

⑥ **Low Pass Filter** Sets the cut off value of the low pass filter applied to the incoming data. Lower values cut off more of the high frequency data. If measuring a high speed event, care must be taken to have this value high enough to see the event. For example, for an event that takes 2 milliseconds, you need a bandwidth of at least 500Hz (Figure 1.7a on page 9).

- Examples of varying Sample Rate and Low Pass Filter values can be seen in Figure 1.7b on page 9: this was acquired with an Olympus Biolever in water on a mica surface, but is shown to demonstrate using the Sample Rate and Low Pass Filter setvar settings to get the effect needed.

- An example of using high sample rates and lower LPF settings can be seen in Figure 1.7d on page 9, where all curves were collected at a sampling rate of 10kHz, while the various (lower) LPF settings were employed. Notice that at lower LPF settings, the data may be clipped too much for certain applications where deflection resolution with distance is important.

⑦ **Set RT Update Prefs button-** this brings up the UiMenuPanel (?? on page ??) allowing the user to choose how the Force graph gets updated:

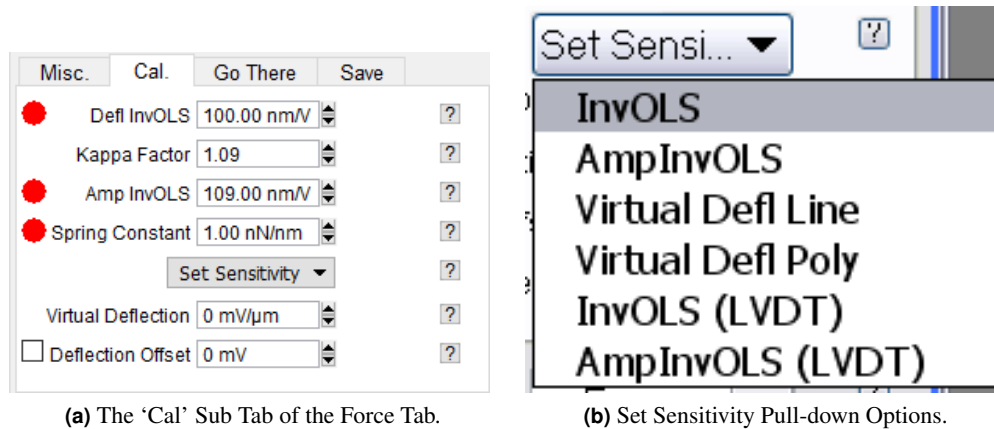
**Never** Force plots updates *after* the acquisition is complete.

**Auto** Will update with some time constraints within the acquisition parameters.

**Always** Force plot is updated as it is being collected; this will be evident by a black line that appears within the data stream in the force curve. See Figure 1.6 on page 8 for an example of this.

### 1.2.3 The Cal. Sub Tab

This sub-tab is where the calibrations are executed and displayed for spring constant determination (see ?? on page ?? for a complete description of that protocol).



**Figure 1.8**

**Defl InvOLS** Deflection inverse optical lever sensitivity- the slope of the contact regime (of a force distance curve on an infinitely hard surface) acquired in CONTACT mode, or using GetReal (R) in which the units are nm/V (i.e., amount of detected distance of cantilever deflection per voltage on the photodetector). The red circle indicates the system is still using the default value, and therefore the system is not calibrated.

**Kappa Factor** Coefficient between Defl InvOLS and AmpInvOLS; value is usually 1.09. This value attempts to compensate for the difference in the lever bending freely vs a lever bending under a load.

**Amp InvOLS** Amplitude inverse optical lever sensitivity- slope of the contact regime (of a force distance curve on an infinitely hard surface) acquired in AC mode, in which the units are also nm/V. Slightly different because it is an AC signal on the photodetector. The red circle indicates the system is still using the default value, and therefore the system is not calibrated.

**Spring Constant** The calibrated spring constant will be displayed here. See ?? on page ?? for protocol for determining spring constant  $k$  via the thermal or Sader methods. The red circle indicates the system is still using the default value, and therefore the system is not calibrated.

**Set Sensitivity Pull-Down Menu**

Set Sensitivity pull-down menu- this is where to select what is being calibrated (Figure 1.8b on page 10).

**InvOLS** Refers to Deflection InvOLS

**AmpInvOLS** See description above

**Virtual Defl Line** Fits the virtual deflection to a linear line

**Virtual Deflection Poly** Fits the virtual deflection to a 2nd order polynomial- use for Extended Z heads, which are identifiable by the horizontal blue strip around the head.

**InvOLS (LVDT)** Used data from LVDT closed loop sensor channel as the y-axis data

**AmplInvOLS (LVDT)** Used data from LVDT closed loop sensor channel as the y-axis data

**Amp2 InvOLS** Amp InvOLS for second frequency in DualAC™.

**Virtual Deflection** This is how to calibrate the slight aberrant deflection that occurs in the optical detection system using a very long force distance cycle. The free air often has several nm worth of deflection over the entire 15  $\mu\text{m}$  of Z travel. It must be calibrated to 'level' the free air part of the curve to allow for more accurate analysis/ determination of contact point; it is updated upon calibration.

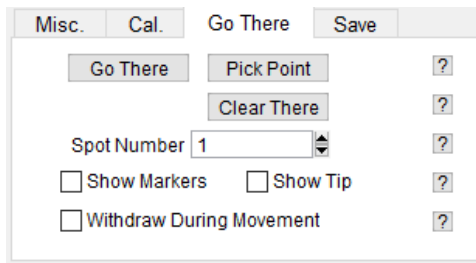
**Deflection Offset Checkbox** This will electronically set the Deflection voltage in the S&D meter to 0V; The offset the same magnitude is made to whatever the free air deflection is, this will electronically zero the deflection, and then an absolute trigger works like a relative trigger (see ?? on page ?? for more on Absolute and Relative triggers).

For example, if there was a -0.88V free air deflection in the S&D meter, typing 0.88V into the deflection offset setvar, and clicking the checkbox to activate it would electronically set the free air deflection to 0.0V.

**Virtual Defl. 2nd Term** This is for extended Z range heads that need a polynomial to fit the Virtual Deflection (i.e. using the Virtual Defl Poly fitting function described above) ; value is updated upon calibration.

For a more complete protocol description of spring constant calibration, see ?? on page ??.

### 1.2.4 The GoThere Sub Tab



**Figure 1.9:** The Go There subtab for Point and Click force curve acquisition.

The Go There subtab of Force tab in the Master panel allows the user to designate points on a previously acquired image for subsequent force curves. This is very useful when you have imaged the surface and want to immediately acquire force curves at specific points/features on your image. The MFP-3D's closed loop sensor accuracy makes this possible.

Once an area has been imaged for the (immediate) subsequent user defined force distance-curve (or I(V) curves) to be acquired, this tab is used for defining the location placement.

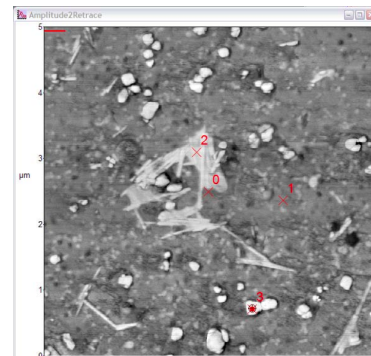
#### Procedure:

1. Acquire image in AC or Contact mode.
2. If the image was acquired in AC mode, but the desired force curve acquisition is to be in contact mode (i.e., not dynamic force spectroscopy), withdraw the tip, and change the imaging mode to 'Contact' from the imaging mode pull down menu in the Main tab of the Master panel.
3. Click the 'Show Tip'  checkbox- this will bring the location of the tip within the XY scan area, displayed as a red dot (by default).
4. Click the 'Pick Point' button. This will bring a  $\oplus$  cursor (on the all the open image channels) usually designated as '0', and will be in the center of the image (Figure 1.10a on page 13).
5. Using the left mouse button, drag the  $\oplus$  cursor to desired position on one of the image channels.
6. Click on the 'That's It!' button; which locks the point at that spot, meanwhile the Spot number index is advanced by one in anticipation of the next point location being chosen. Notice the user defined location/ spot is marked on the image with an 'X' and also labeled with the spot number (Figure 1.10b on page 13).
7. Also, notice the Spot Number base suffix will increase by one, expecting another point to be picked.
8. Repeat as necessary/ desired.
9. When ready to acquire the force curves, enter the Spot Number for the position on the surface you want to probe.
10. Click the 'Go There' button. Notice the red dot designating the tip position will move to that point (Figure 1.10b on page 13), generally at a rate the scan rate is set to in the Main tab of the Master Panel.

- Click the 'Single Force' button, 'Continuous' button, Ctrl+3, or downward function selector toggle (??) on the controller to acquire force curve(s).



**(a)** Drag the cursor from image center to desired location.

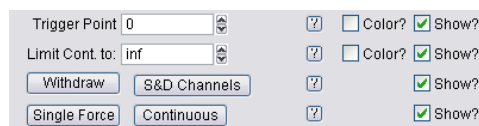


**(b)** Many locations selected; tip located at position 3, indicated by red dot. Data shown is a DualAC™ amplitude2 image of water based latex paint in air.

Sub-figures a and b in ?? on page ?? show an example of designating some user defined points on a DualAC™ second amplitude image of some water based paint (see ?? on page ??). In Figure 1.10a on page 13, the ⊕ cursor was moved from image center to designate first defined location (as seen in Figure 1.10b on page 13); In Figure 1.10b on page 13 three different points are selected at different contrasted image locations- Notice tip is at point three because of red dot marker.

To acquire multiple curves at a given point, click the 'Continuous' button, and designate the 'Limit to Cont' setvar to a desired value (see Section 1.2.1 on page 3).

Recall the 'Limit to Cont.' variable isn't shown in the default MFP-3D software, so it must be activated by clicking the 'Setup' button. If the value is set to 'inf' it means it will collect curves continually (infinitely) until told to stop.



**Figure 1.10**

The 'Go There' functions also work for the electrical characterizations techniques.

### 1.2.5 The Save Sub Tab

The Save sub-tab is where force curves get saved (Figure 1.11 on page 14). Use the same 17 character filename as with saving images; the filename can't start with a number or Igor lets you know its displeasure with your desire to attempt this.

As curves are acquired, the base suffix will increase by 1.

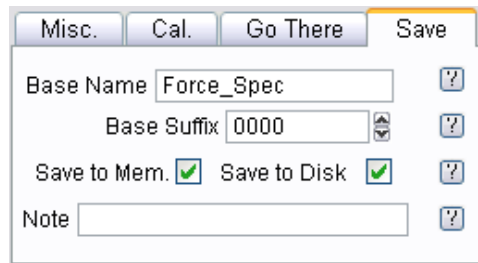


Figure 1.11: The Save Subtab.

### 1.2.6 Force Channel Panel

This panel assigns the data channels displayed and saved in Force Plots.

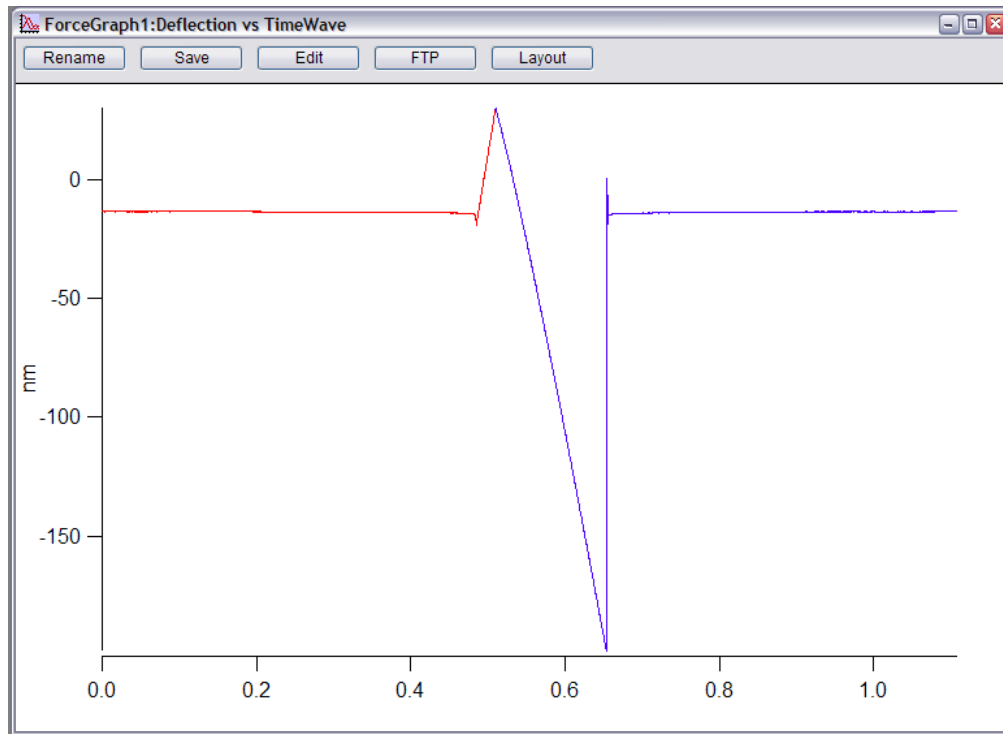


Figure 1.12: Force Channel Panel

The Green / Blue / Red circular dots to the left of the channels describe the settings of those channels as follows:

- Red = Channel is not on (no activation checkmarks)
- Blue = Channel is ONLY displayed (i.e., YDisp checked for a given Channel, but Save not checked)
- Green = Channel is saved (does not care if channel is displayed or not).
- The 'Channel' column gives all the possible data channels- note that depending on the experiment, some will give no real signal.
- The 'Save' column is what data is always saved - the channels checked here are what can be called up in the Force Display Panel in the Analysis section of this software (i.e., there is a pull-down menu that you can select the Y axis for).

- The 'Graphs' pull-down allows up to 5 channels to be plotted simultaneously. Depending on the number of graphs selected in the pull-down menu, that many X&Y columns will be displayed.
- Each Graph column (1,2,... 5) allows selection of two dimensions to plot the desired curve/ signal in. On each plot, multiple Y-axis dimensions can be plotted vs. a single X-axis.



**Figure 1.13: Time vs. Deflection Plot**

To display time as the X axis, activate the Time radio button towards the bottom of the Force Channel Panel. Figure 1.13 on page 15 shows an example of a deflection (Y) vs. Time (X) plot assigned via the Force Display Panel. The inset shows the X axis radio label selected to assign time as the X axis; Deflection would be checked in the Column tab.

### 1.2.7 Closed Loop (Z) Activation

To turn on the closed loop in force measurements that call for constant velocity or consistent loading. Note that it will have more noise associated with it than in open loop.

1. Go to the setup menu to activate it.

2. **Select Closed Loop**
  - From the pull-down menu, select Closed Loop.

3. Closed loop can also be activated when activating the Indenter checkbox in the Indenter Panel.

### 1.2.8 Empirical Considerations

#### Software Parameters:

##### Force Distance

1.
  - Choose a force distance that is large enough to have some of the 'Free Air' (non contact) portion in the curve, such that the contact point can be properly determined during the analysis.
  - Using force distances that are too long (i.e., mostly all free air in the curves) reduces the portion of the curve dedicated to the displacement by the surface, and therefore reduce the resolution of the curve.

##### InvOLS / k Changes

2.
  - Sometimes you can get different InvOLS values numbers before and after an experiment. There are a few possible causes of this. Contamination of the probe can change the sum, which can change the InvOLS. Thermal drift can change the superluminescent diode position over long periods of time (hours to days). degradation of the probe coating can also cause this. This can also manifest itself to some degree in a wider than expected force distribution results.
  - Floppy cantilevers can be electrostatically bent by the surface even when the tip is retracted several microns. This in turn compromises the thermal peak fit on the final step of the k determination. Wheel that tip several hundred microns up if possible, and the peak should be sharper (For an example, see ??).

#### Experimental

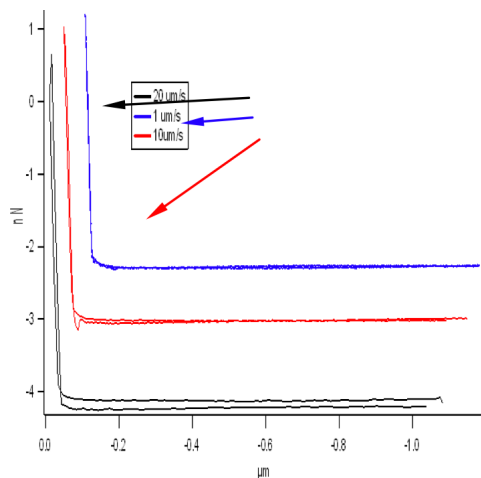
1. **InvOLS / k Changes** Choosing a cantilever that is appropriate for the measurement is important. In general, the spring constant of the probe should be close to the effective spring constant of the sample.
2. **Thermal Drift** It is common once a system is set up to let it equilibrate for 30 minutes or more. When operating in fluid, this process can take longer. This will be particularly common in labs that have a large variation in the room temperature.
3. **Sample Prep** It is important to have the substrate well affixed. Avoid two sided tape as it can introduce drift and creep into the experiment. This includes copper and carbon tape. An alternative to these that is removable is silicone vacuum grease, First Contact (R) from photonic cleaning solutions, and silver paint from Ted Pella.



### Thermal Equilibration

**Work in Fluid** \*\*If the sample allows – i.e., doesn't swell. Do this to avoid large adhesion (i.e., jump to contacts) which will make the point of contact determination even more subjective.

**IF Working in Fluid** When acquiring force curves in fluid (which is common) it is a good idea to let the system equilibrate for 30 min to a couple of hours. The most dramatic deflection drift will occur in the first 20 minutes, but drift will continue to occur as the thermal gradients approach equilibrium.



**Figure 1.14:** Hydrodynamic effects can occur in fluid with fast force curve acquisition cycles

## 1.3 Force Mapping

Force mapping is used to acquire an  $n \times n$  array of force curves to create an 'image' of the surface. It is essentially a 3D map of the tip-sample interactions.

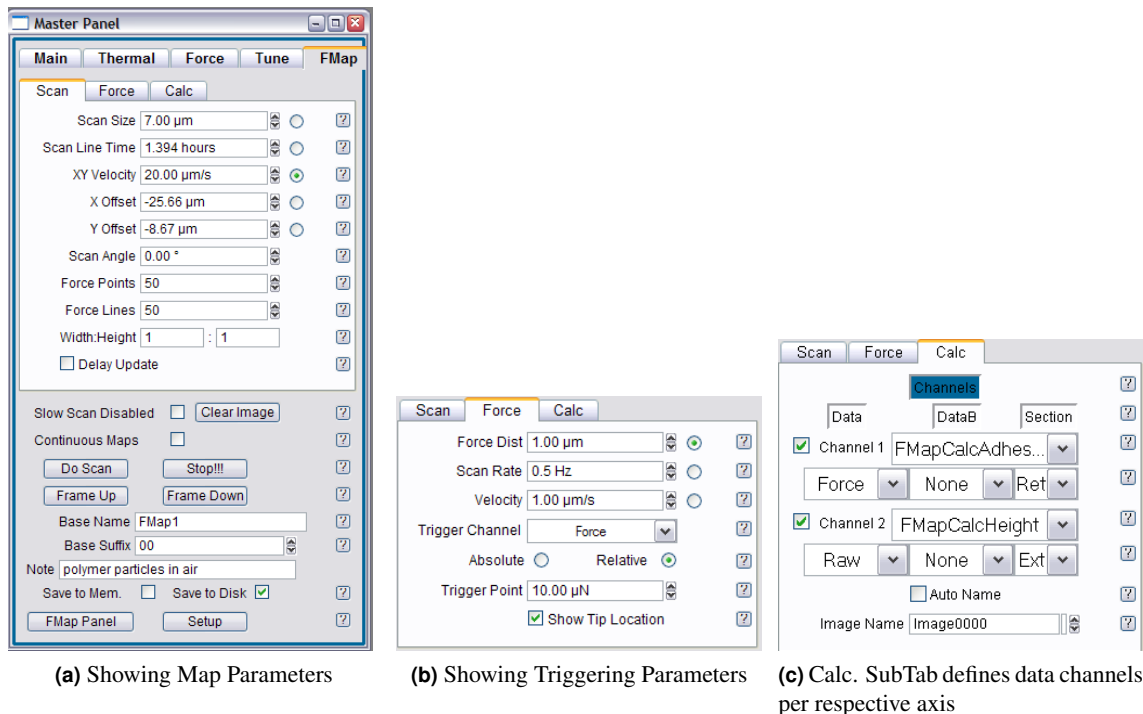


Figure 1.15: Force Map Tab

The fifth tab in the Master Panel called the FMap panel (Figure 1.15a on page 18) allows the acquisition of Force Maps on Asylum Research AFM's.

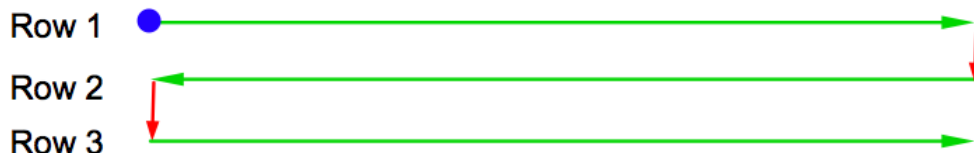


Figure 1.16: Curve Collection

As shown in Figure 1.16 on page 18, the curves are collected in a ‘Frame Down’ raster fashion (starting point blue dot) in an ‘across the fast scan, move to next slow scan line, across in fast scan’ iteration. The opposite is true is when doing a ‘Frame Up’ Force map.

- For the MatLab users out there, exporting is an important consideration for post processing due to the way the files are exported.

The FMap Panel has three subtabs that combine similar controls found for imaging in the main tab, and the force parameters in the Force Tab. The help menu defines these parameters pretty well:

- Scan subtab defines the XY scan area and operates similar to the Main tab (Figure 1.15a on page 18).
- Force subtab defines the piezo velocity and trigger points (Figure 1.15b on page 18).
- Calc subtab defines what channels are to be displayed (Figure 1.15c on page 18).

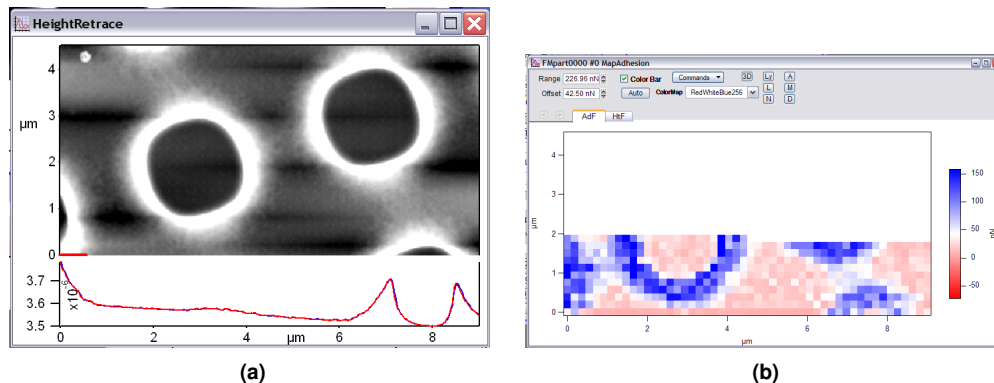
- Data Column displays / captures the X axis data.
- Data B Column displays / captures the Y axis data.
- Section Column gives option to extend or retract a portion of curve to be analyzed for the map.

The lower portion of the FMap tab is for capturing data, similar to the Main tab.

The Continuous Maps checkbox will collect force maps continuously in the same scan range region until told to stop.

### 1.3.1 Procedure for Force Map Acquisition

1. Define whether to perform Force distance acquisition in contact mode or AC mode.
2. Define the scan area for the Force Map. If acquiring a force map directly after imaging an area, the 'Scan Size' field will already have that area dialed in the 'Scan Size' field of the main tab of the master panel.
3. Define the velocity of the translation between points in XY. This is similar to scan rate.
4. Define a Scan Angle the tip will follow during the acquisition (i.e., if  $0^\circ$ , then the X axis will be parallel to the length of the cantilever;  $90^\circ$  will be normal to the length of the cantilever).
5. Define the number of points and lines. Note that high points and line values will result in long scan times, often hours long.
  - a) The 'Scan Line Time' setvar will tell how long it will take to acquire the Force Map based on the parameters defined (all the XY translations and time to acquire each force curve).
  - b) The Width:Height is the aspect ratio of the scan area, just as in imaging.
6. Define trigger points and tip velocity in the Force sub-tab (Figure 1.15b on page 18); they will be refreshed in the Force tab and vice versa.
7. To acquire a Force Map in real time, the Calc subtab needs to have the proper data inputs defined to display them as the Force Map is being acquired. Something that looks like an MFP-3D 'Display Window' appears during acquisition, with tabs that correlate to what Channels that are selected in the Calc. subtab (See Figure 1.17 on page 20).
8. Click 'Do Scan', 'Frame Up' or 'Frame Down' to begin Force Map Acquisition.
9. Go get a sandwich, or take a nap.



**Figure 1.17:** Screen shots of real time force map acquisition. Pixels are updated after each subsequent acquisition.

### 1.3.2 Cantilever Based Indentation

Cantilever based indentation probes compliance of materials, but it can be subject to error due to the ploughing effect of the probe as the piezo pushes the probe tip into the surface. The error can be reduced by using small indentation values.

This section will introduce:

- Most basic concepts of nanoindentation of materials.
- Empirical considerations regarding the cantilever/ tip selection.
- How to navigate the AR indenter panel software.
- Some examples of various materials from the AR user base.
- The tip material is important because it must have a much larger modulus than the material to be indented.
  - Silicon tips can be used for many biological and polymeric materials, but may not be effective on harder materials.
  - Diamond (solid or coated) and silicon nitride tips are widely available. Spheres or various modulus can be glued to levers for using Hertzian contact mechanics (also see Section 1.3.3 on page 24).

It is also important to mention the delicate interplay between elastic and plastic deformation in the material being indented (elastic will recover from the removed load, while plastic does not). The indenting probe pushes down into a material; reaches some trigger point and retracts. The primary difference between a simple force curve and an indentation is the use of the indentation panel (AFM Controls→Indenter), which gives better control of the indentation. Indenting on a plastic material leaves an indent depth ( $h_f$ ) in the material smaller than the total indent depth ( $h$ ). This can be described as a simple power law relation:

$$P = a(h - h_f)^m$$

Where:

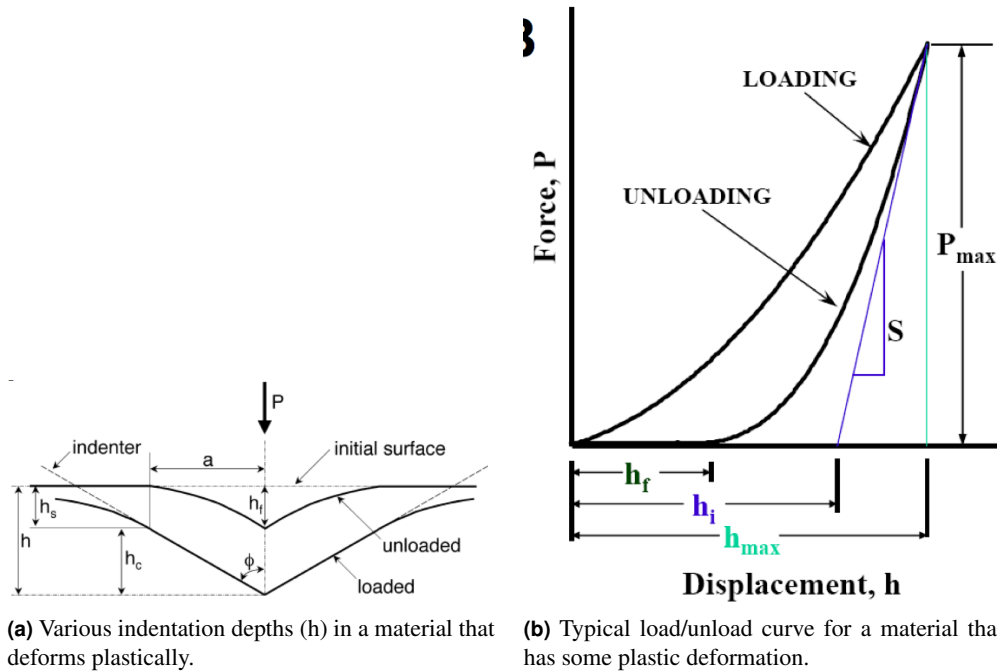
**P** is Load; typically in the force dimension (N).

**a** and **m** are empirically determined fitting parameters associated with indenter shape.

**h** is the total indentation depth

**h<sub>f</sub>** is the final displacement after complete unloading, and also determined by fitting.

There are various other **h**'s depending on the fitting model being used.



**Figure 1.18:** Qualitative schematic commonly used in nanoindentation to illustrate difference between maximum indentation (**h**) and indentation depth after load is removed (**h<sub>f</sub>**). Figure adapted from Oliver, W.C., Pharr, G.M., J. Mater. Res. 2004, 19 (1) p3-20.

General qualitative responses of various materials under applied load can be seen in ?? on page ??.

Panel A shows a fully elastic system; there is no hysteresis between the load and unload components, indicating the material fully recovered while / as the load was removed. Panels B and C Show various amounts of plastic deformation (less and more, respectively), based on the degree of hysteresis between load and unload components. Panel D shows some material response/rearrangement event occurred during the unload cycle; Panel E shows some material response/rearrangement event occurred during the load cycle. Panel F shows the that material is moving significantly at the initial unload, indicative by the negative slope of the unload component.

**Note** To obtain curves like this with the MFP-3D, the indenter Panel must be used (Section 1.3.2 on page 22).

References:

- Vertical indentation-
  - Oliver, W.C., Pharr, G.M., “Measurement of hardness and elastic modulus by instrumented indentation: Advances in understanding and refinements to methodology”, J. Mater. Res. 2004, 19 (1) p3-20. (REVIEW)

- Gong, J., Miao, H., Peng, Z., “Analysis of the nanoindentation data measured with a Berkovich indenter for brittle materials: effect of the residual contact stress “, *Acta Materialia* 52 (2004) 785–793.
  - Mencik, J., “Determination of mechanical properties by instrumented indentation“, *Meccanica* (2007) 42:19–29. (REVIEW)
  - W.C. Oliver and G.M. Pharr, *J. Mater. Res.* 7, 1564 (1992)
  - VanLandingham, M.R., “Review of Instrumented Indentation”, *J. Res. Natl. Inst. Stand. Technol.* (2003), 108, p249-265. (REVIEW)
  - Schuh, C.A., “Nanoindentation Studies of Materials“, *Materials Today* 2006 9 (5) p32-39.
  - VanLandingham, M.R., Villarrubia, J.S., Guthrie, W.F., Meyers, G.F., “Nanoindentation of Polymers: An Overview”, *Macromol. Symp.* 2001, 167, p15-43. (REVIEW)
  - Fischer-Cripps, A.C., “A review of analysis methods for sub-micron indentation testing”, *Vacuum* 58 (2000) 569-585. (REVIEW)
  - Fischer-Cripps, A.C., “Critical review of analysis and interpretation of nanoindentation test data”, *Surface & Coatings Technology* 200 (2006) 4153 – 4165. (REVIEW)
  - Mosesonm A.J., Basu, S., Barsoum, M.W., “Determination of the effective zero point of contact for spherical nanoindentation”, *J. Mater. Res.* (2009) 23 (1) p204-209.
- Cantilever based:
    - Lin, D.C., Dimitriadis, E.K., Horkay, F., *J. Biomech. Eng.* ”Robust Strategies for Automated AFM Force Curve Analysis—I. Non-adhesive Indentation of Soft, Inhomogeneous Materials”, (2007) 129, p430-440.
    - Lin, D.C., Dimitriadis, E.K., Horkay, F., *J. Biomech. Eng.* ” Robust Strategies for Automated AFM Force Curve Analysis—II: Adhesion-Influenced Indentation of Soft, Elastic Materials”, (2007) 129, p904-912.
    - Lin, D.C., Horkay, F., “Nanomechanics of polymer gels and biological tissues: A critical review of analytical approaches in the Hertzian regime and beyond”, *Soft Matter* 2008 4 p669-682. (REVIEW)
    - Masterson, V.M., Cao, X., “Evaluating particle hardness of pharmaceutical solids using AFM nanoindentation “ *International Journal of Pharmaceutics* 2008 362 p163–171.
  - Some useful web sites that provided some understanding:
    - [http://www.its.caltech.edu/~ae244/Lecture10\\_110107.pdf](http://www.its.caltech.edu/~ae244/Lecture10_110107.pdf)
    - [www.csm-instruments.com/en/webfm\\_send/42/1](http://www.csm-instruments.com/en/webfm_send/42/1)
    - <http://www.microstartech.com/>

### Indenting Procedure

Before Indenting, it is assumed that the following steps have occurred.

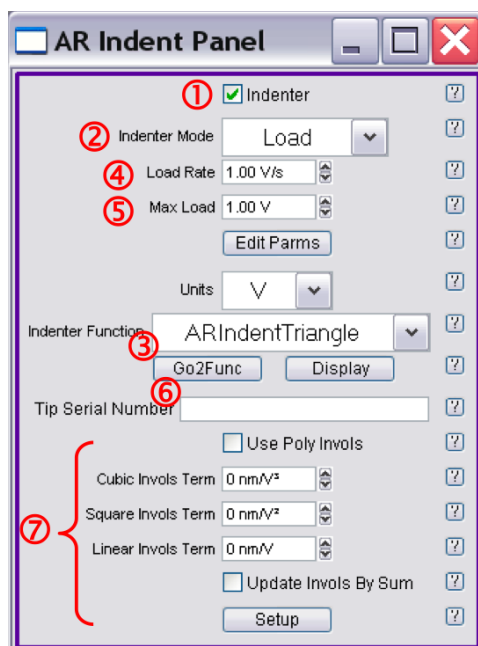
1. Load tip, align SLD on cantilever, zero PSD.

2. Bring the probe into piezo range of the sample.
3. Confirm the imaging mode is set to *Contact*.
4. Find InvOLS and cantilever spring constant. This can be done with the GetReal software, or with a force curve and the thermal method. (1.3.5)

Software Operation: There are two ways to approach cantilever based indentation in the MFP3D software.

1. Use the Force tab of the Master Panel exclusively; this is exactly the same as standard force curves. Trigger points can be used as loads; Dwells can be used as holds or creep studies.
2. Use the Force Tab in conjunction with the indenter panel. With this approach the force tab can be used to apply a very small trigger point (i.e., 1 nm deflection trigger), then the cycle defined in the activated indenter panel applies the load until it reaches its end and completes the Retract curve.

The AR Indenter Panel



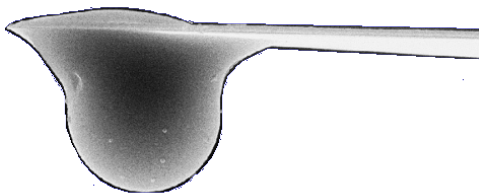
**Figure 1.19:** AR Indent Panel. Numbers correspond with steps below.

1. When the indenter check box is activated, this panel uses Closed loop Z sensor to apply the load (after the initial force trigger used to find the surface).
2. The indenter mode pull-down menu gives important options regarding the feedback:
  - a) *Load*: Uses force, or deflection feedback to control the load.
  - b) *Displacement*: The motion of the Z sensor signal, or how far the probe chip (the part you pick up with tweezers) moves during the indentation.
  - c) *Indentation*: The Z sensor signal minus the deflection. This indicates how far the probe tip pushes into the sample surface
3. The 'Units' pulldown lets the user choose between Volts, distance (m) or Force (N).

4. Loading Rate can now be chosen in a comfortable dimension.
5. Maximum load can also be selected.
6. Indenter function pulldown lets you choose between triangle (voltage/loading) waves, add a dwell (for creep) or use the AR Function editor for custom routines - very flexible - AC and DC modes. The pull-down menu options are:
  - a) *ARINdentTriangle*: Linear loading and unloading at the same rates.
  - b) *ARINdentTriangle2*: Linear loading and unloading at different rates.
  - c) *ARINdentTriangleDwell*: Linear loading and unloading at the same rates, with a hold (dwell) in between
  - d) *Function Editor*: Allows customer waves to be built, including static and dynamic load and hold applications; see Section 1.3.4 on page 27 for more about the function editor.
7. Disregard these. The tip serial number and poly InvOLS is for the vertical nanoindenter flexure module accessory option.

### 1.3.3 Colloidal Probe Microscopy

Colloidal probe microscopy is a force spectroscopy technique in which a spherical probe is mounted onto a cantilever, and used (in most cases) to push on a soft material. The sphere allows Hertzian contact mechanics to be applied to the analysis.



**Figure 1.20:** Colloid Lever Side View

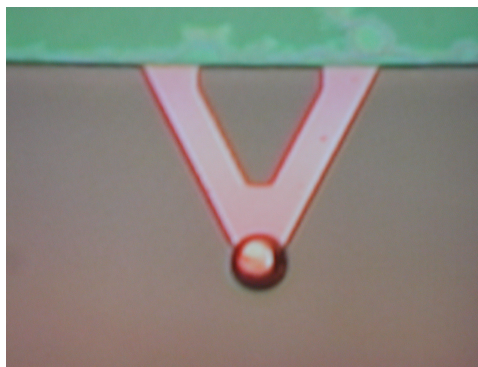
Some reviews and useful papers regarding colloidal probe microscopy include:

- Bonaccorso, E., Kappl, M., Butt, H.-J., *Current Opinion in Colloidal & Interface Science*, 2008 13 107-119.
- Vezenov, D., Noy, A., Ashby, P., *J. Adhesion Sci. Technology* 2005 19 (3-5) p313 – 364.
- Leite, F.L., Hermann, P.S.P., *J. Adhesion Sci. Technology* 2005 19 (3-5) p365 – 405.
- Christendat, D., Abraham, T., Xu, Z., Masliyah, J., *J. Adhesion Sci Technology* 2005 19 (3-5) p149-163.
- Tormoen, G.W., Drelich, J., *J. Adhesion Sci Technology* 2005 19 (3-5) p181 – 198.
- Kappl, M., Butt, H.-J., *Particle & Particle Systems Characterization* 2002 19 (3) p 129-143.
- Lin, D.C., Horkay, F., “Nanomechanics of polymer gels and biological tissues: A critical review of analytical approaches in the Hertzian regime and beyond”, *Soft Matter* 2008 4 p669-682. (REVIEW)



Some assumptions for acquisition and Hertzian analysis include:

- Load  $\propto \delta(\text{depth})^m$ , where  $\delta$  is a function of the geometry & the elastic properties (Young's modulus and Poisson ratio) and  $m$  represents the shape of the pressure distribution
- Is fully elastic
- Pressure distribution same as spherical indenter shape
- Radius of spherical indenter < radius of sphere
- Frictionless
- Bodies large compared to volume under contact
- Isotropic
- Spherical indenter contacting flat surface
- Cantilever spring constant is NOT similar to material being pushed - *want cantilever  $k$  much larger*
- Elastic indenter much stiffer than surface



**Figure 1.21:** Colloid Lever Top View

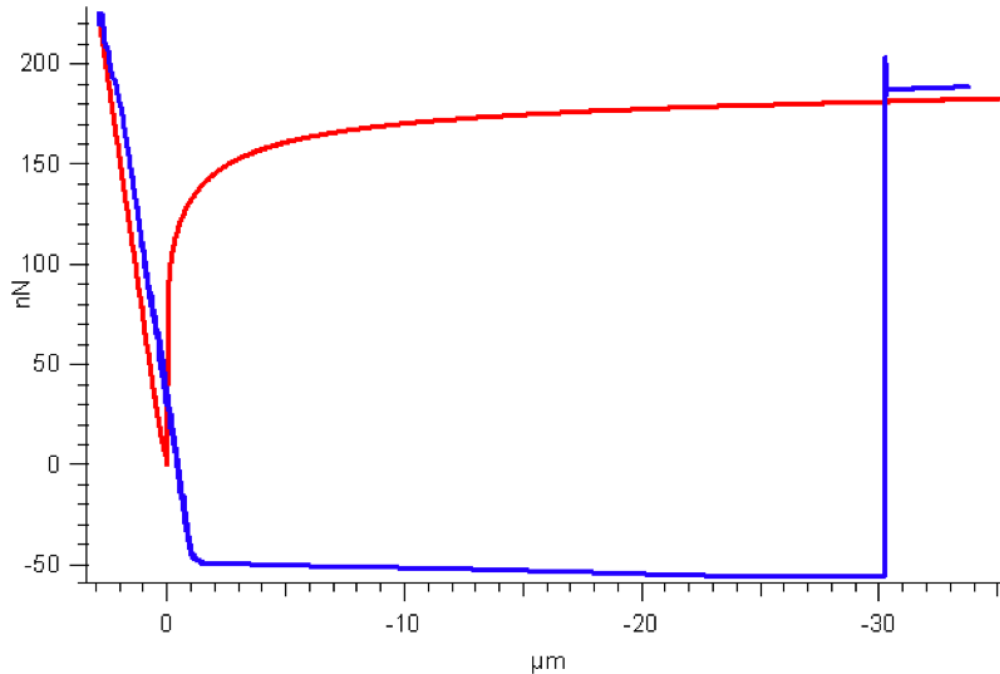
**Note** On cantilever calibration with spheres: it is usually more challenging to calibrate the cantilever spring constant (via thermal method) with an affixed sphere due to the increased Surface Area (especially in air). An example of large surface forces acting on a sphere can be seen in Figure 1.22 on page 26 - notice large jump to contact and adhesion to surface before snapping back to free air.

There are two ways to avoid large attractive forces that will affect the virtual deflection calibration:

- *If working in air* Calibrate virtual deflection and the thermal tune far off the surface (several hundred microns) to avoid long range charges between the sphere and surface that premature the deflection cantilever.
- *Work in fluid* Although the much lower  $Q$  of cantilever can make fitting the curve from thermal method more challenging.

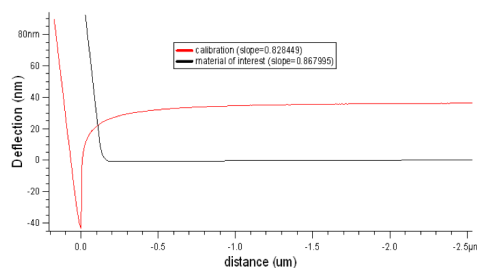
For acquisition, some considerations:

- Do not indent more than a quarter of the diameter of the sphere affixed to the cantilever - this is for the Hertzian mechanics based analysis.

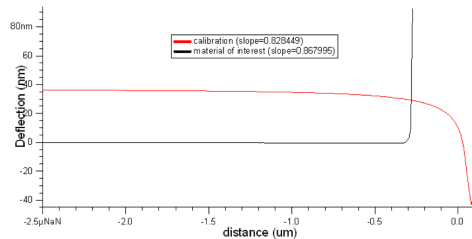


**Figure 1.22:** Large surface forces can act on a sphere affixed to cantilever (in air;  $k$  ?)

- Choose the cantilever spring constant so that it can apply a load to the sample, rather than (fully) deflect with the load. This can be determined from the Force vs. Indentation plot : If the contact regime has a slope that goes to infinity, then the lever is likely fully deflecting under the load, rather than deforming the sample. Figure 1.23 on page 26 shows an example of this. In Panel A), it is not really clear that the lever is fully deflecting. However, when force is plotted against indentation (or separation), the slope looks like a right angle to the free air (non contact) portion of the curve.



**(a)** Force vs. distance



**(b)** Force vs. Indentation (deflection (PSD signal) is subtracted from the LVDT signal).

**Figure 1.23**

Colloidal probes are available two configurations:

- Commercially available from NovaScan (Iowa), sQube (Germany) and probably some other vendors.
- Can be (home) made in the lab, especially if your AFM has some sort of bottom view optics.

**Note** Tipless levers are also available from most vendors.

Regarding sphere materials, things to consider are:

- Material's thermal expansion coefficient is an important consideration (Borosilicate has a favorable one)
- Material's modulus (do not want it too soft to act as another spring in the system)
- Mono/poly dispersity (i.e., as close to sphere shape as possible)
- Material charge - most polymers and glass products are charged, which can contribute to attractive/ repulsive forces.

### 1.3.4 Dynamic (AC) Force Spectroscopy

Dynamic force spectroscopy acquires force curves while in AC mode. Dynamic force spectroscopy is done by ramping the z piezo while oscillating the AFM probe. AC mode force curves show the interaction of the oscillating probe as it approaches, begins tapping, and often deflects against the surface of the sample (see ?? on page ??).

As the drive amplitude is varied, the degree of net attractive and net repulsive interaction the tip has with the surface can change. Figure 1.24 on page 27 shows various AC force curves acquired on mica at varying drive amplitudes. Notice the amount of net repulsive and net attraction the tip has with the surfaces varies with distance from the surface.

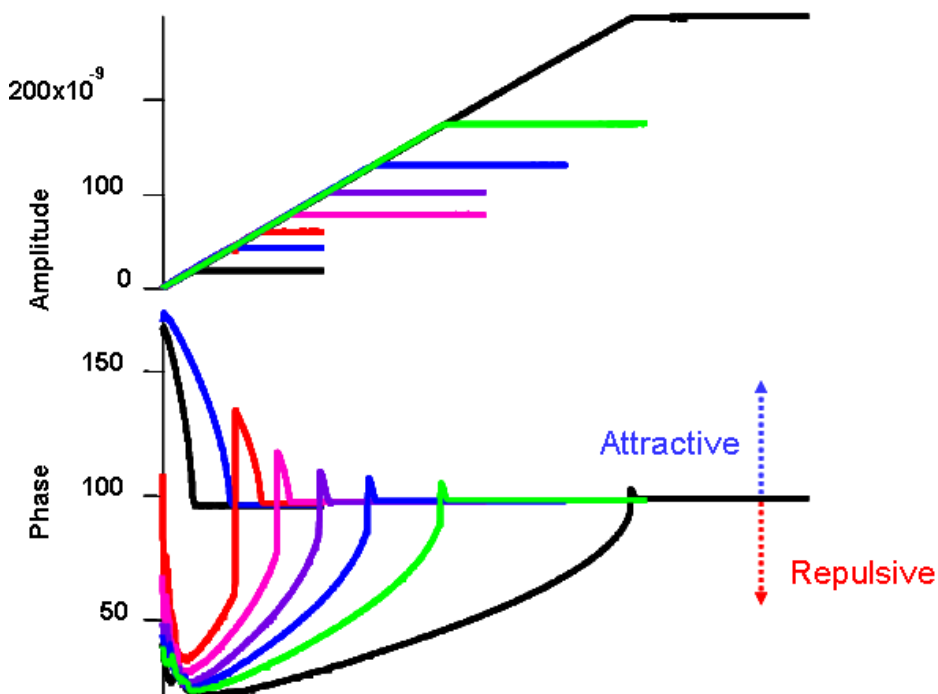
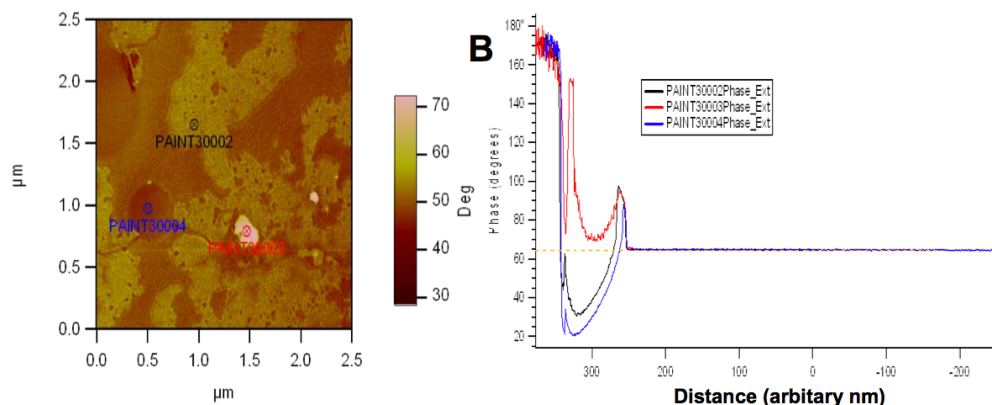


Figure 1.24: AC force spectroscopy.

Figure 1.25 on page 28 shows some 'point and click' AC force curves defined from a phase image of water based latex paint (Sherwin Williams).

- The sample was imaged with an Olympus AC 240 ( $k \sim 1.6 \text{ N/m}$ ;  $\sim 70 \text{ kHz}$ ) in air in repulsive mode; the free air phase was  $\sim 64^\circ$  (depicted by orange dashed line).
- The force curves were acquired at constant (drive) amplitude (same as the image) and the trigger point was 5 nm to reduce the amount of Phase bi-stability (mode hopping) under applied load.
- There are clear differences on the surface- notice that points 2 & 4 (black and blue, respectively) are similar during the approach (extension) curves, there is an initial attraction to the surface, then repulsion; at some point, the tip becomes closer to the surface, it flipped back to attractive mode.
- Point 3 (red) never goes into the repulsive regime.

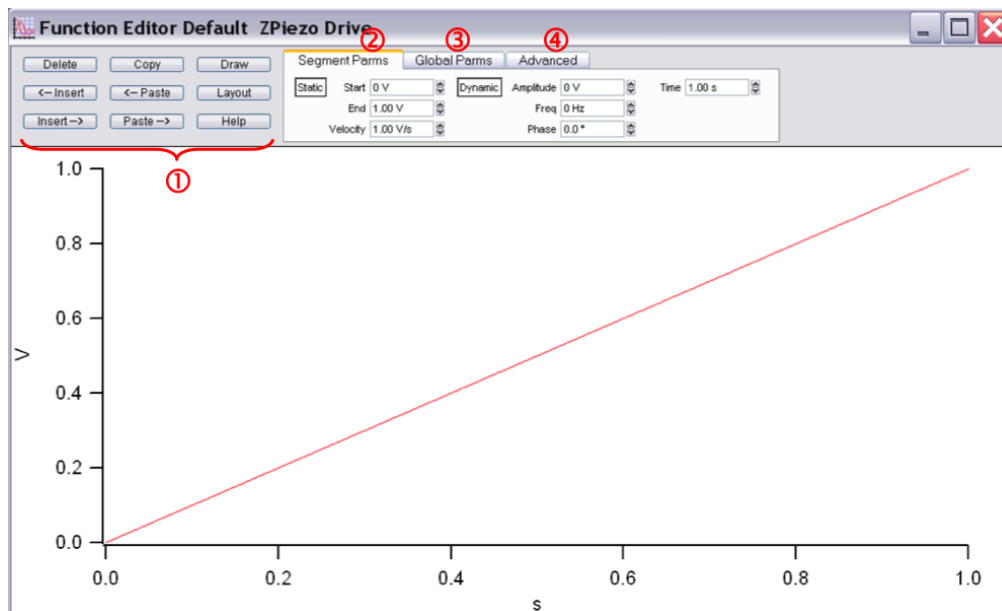


**Figure 1.25:** AC point and click Force curves on heterogeneous sample: A) Phase image of paint sample with location of user defined force curves; B) individual AC force curve Phase vs. distance (Z)-orange dash line indicates Free Air Phase values of  $\sim 64$  degrees. (yes, that is the di color table on the 3D)

### 1.3.5 The MFP-3D Function Editor

The MFP-3D software has a ‘Function Editor’, which is very useful for applying custom made waveforms for force spectroscopy, indentation, and various electrical techniques.

To open, click the Function Editor option in the indenter panel. A panel like the one shown in below will appear.



**Figure 1.26:** The AR function editor allows custom wave forms to be applied to the Z piezos, or tip sample biases, for advanced force spectroscopy experiments.

1. The buttons to the right of the panel allow the segments to be copied then pasted to the left or right of the copied segment.

**Insert** Will insert a segment to the left or right of the forward-most (red) segment, having the same constant values as the red segment its attached to.

**Paste** Paste a copied segment; will have the same parameters as copied segment.

**Copy** Copy a segment. Segments must in activated (red) with mouse click to copy them

**Draw** Allows 'free hand' line segments that are strung together.

**Layout** This dumps image of function to an Igor Layout (which are awesome!).

Whichever segment is displayed as red is the forward most segment to be manipulated via the buttons or setvars.

2. Segment Params Subtab:

**Static** This is for DC voltages applied to the piezo (stack); the 'Start' and 'End' Piezo voltages, and velocity can be user defined.

**Dynamic** This is for AC voltages applied to the piezo (shake); the amplitude, Frequency and Phase setvars can be adjusted accordingly. This is useful for oscillating the tip for viscoelastic materials.

3. Global Params Subtab:

This scales and offsets the signals in the function generator.

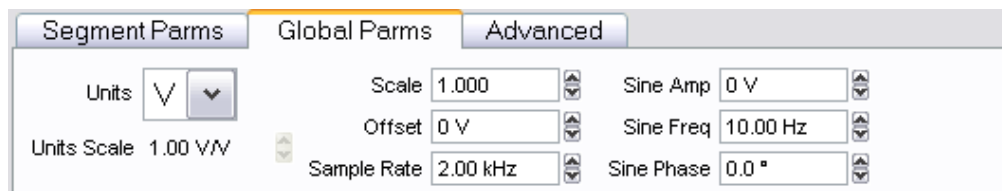


Figure 1.27: Global Params Subtab

**Units** Pull-down allows choice between Volts (applied to piezo) or meters (accurate with calibrated InvOLS). This can also be useful if using the AR Function Editor for applying electrical biases to tip or sample if doing an electrical technique (not described here).

**Units Scale** Will give the sensitivity (InvOLS) displayed if meters selected for Units.

**Scale** Allows the generated function to be scale by some setvar factor; axes will rescale.

**Offset** Will offset the signal on the Y axis.

**Sample Rate** What frequency (# of pts/sec) the data is collected at.

**Sin Amp** Allows the sinusoidal amplitude to be scaled.

**Sine Freq** Allows the frequency of the Sine signal to be scaled.

**Sine Phase** Offsets the Phase from 0 to some other user defined value.

Advanced:

This tab allows the user to save functions for future use, and to define what the function will drive.

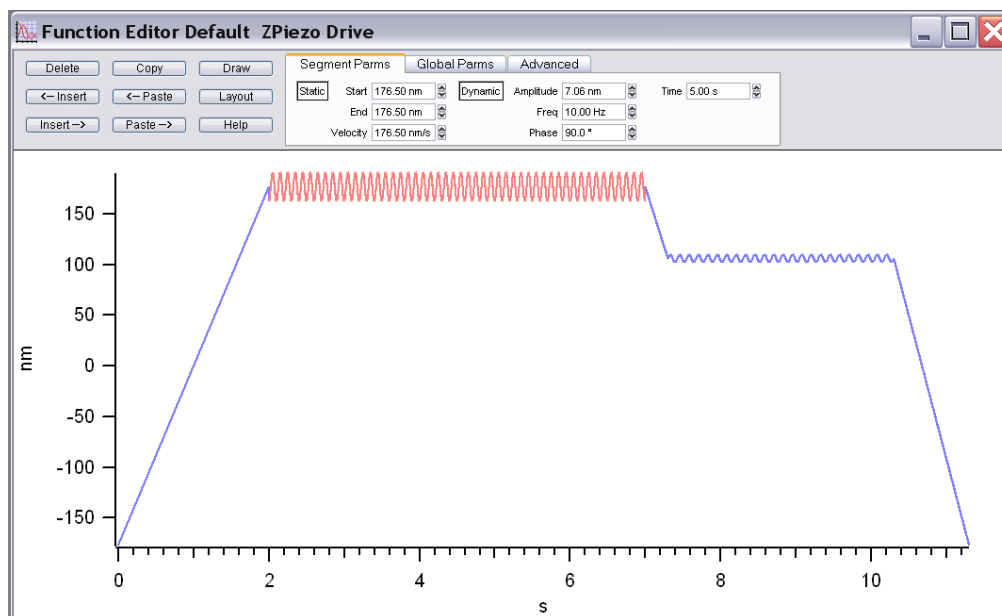


Figure 1.28: An Example Custom Waveform

Figure 1.28 on page 30 shows a simple function that was generated in the Editor. Many segments were added and set to tell the cantilever to deflect to 176 nm, hold for 5 seconds with an oscillation of 10 Hz with 7.06nm peak to peak amplitude at 90° phase, pull back a bit and hold again for 3 s at 8 Hz with 31.52 nm peak to peak amplitude 0°, then return to a negative deflection.