Standard Operating Procedure of 3D Bio Atomic Force Microscope (Asylum Research MFP-3D™ AFM)

To obtain 3D surface topography at sub-nanometer scale resolution, measure contact and friction forces between surfaces in contact, measure sample conductivity and magnetic property in nano-scale region and image samples in liquid environment.
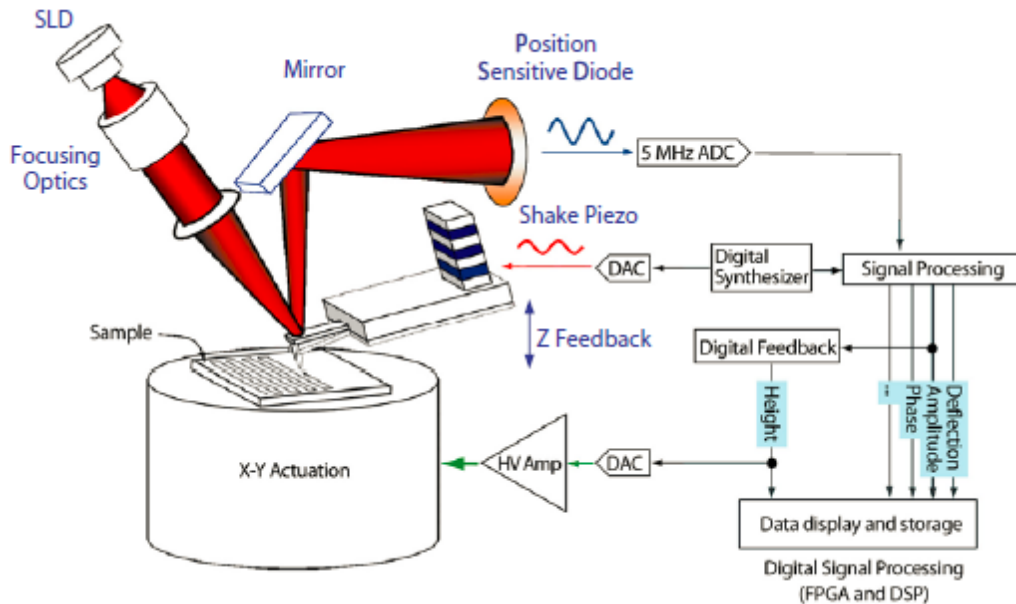

Figure 1: Simplified schematic of MFP-3D system.

I Loading the cantilever

➢ Find the tip holder assembly on the loading pedestal (Figure 2a,b)

➢ Remove the tip holder assembly from the AFM head by pressing the round button on the side (Figure 2c) and mount it into the loading pedestal apparatus (Figure 3A).

➢ Loosen the clamp screw (the middle one, Figure 3B) with a screwdriver.

➢ Check the AFM probe against the light to ensure that the cantilever is not damaged.

➢ Slide the AFM probe under the tongue clamp (Figure 3B). You may need to gently lift the tongue clamp with a pair of sharp tweezers to accommodate thicker probe chips.

➢ Position the probe to the center of the clear trapezoidal shaped quartz optical window (Figure 3C).

➢ Tighten the clamp screw with the screwdriver until finger tight. Do **NOT** over tighten the screw (Figure 3E).

➢ Remove the tip holder assembly from the loading apparatus and put it back into the MFP-3D head (Figure 2c).

Figure 2: (a) AFM tip holder assembly; (b) holder assembly in the loading pedestal; (c) Removing tip holder assembly from AFM head.
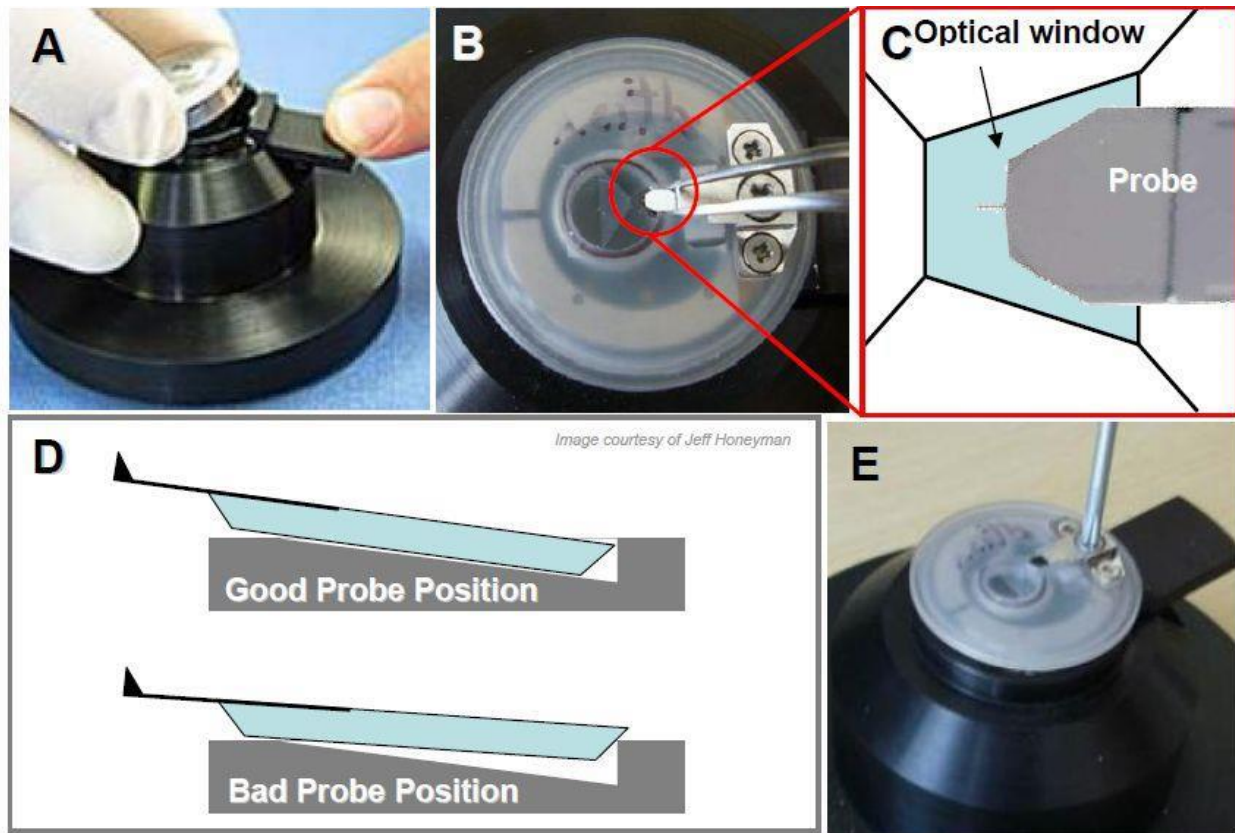

Figure 3.1: Proper probe loading: A) loading the cantilever holder into the cantilever holder stand. B) use tweezers to position cantilever in middle of polished quartz window. C) proper position of probe in quartz window. D) Proper position of probe in pocket; if too far back, probe slides on back ledge of pocket compromising deflection signal; E) Screw tongue clamp- *no more than finger tight* - with a Phillips (00x40) screwdriver.

II Sample mounting

1. Attach your sample to a piece of glass slide with sticky tab, super glue, epoxy or vacuum grease.

2. Mount the sample slide at the center of the sample stage.

3. Secure the sample slide to the stage with the two small magnet blocks on both sides.

III AFM head mounting

1. Lift the AFM head with two hands. Place the two back legs into the divots on the AFM baseplates first (Figure 4A).

2. Move thumbs under the front of the AFM head (Figure 9B). Slowly lower the AFM head down towards the stage while **continually monitoring the tip-sample separation** to avoid tip crash (Figure 4B).

3. If the tip appears to crash to sample, lift the head back and adjust the front leg height to increase the tip-sample separation. Repeat the process.
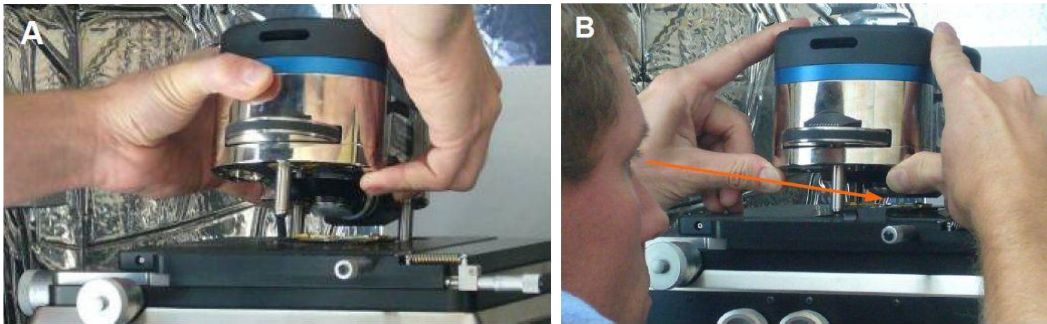

Figure 4. Mount the AFM head onto the base plate
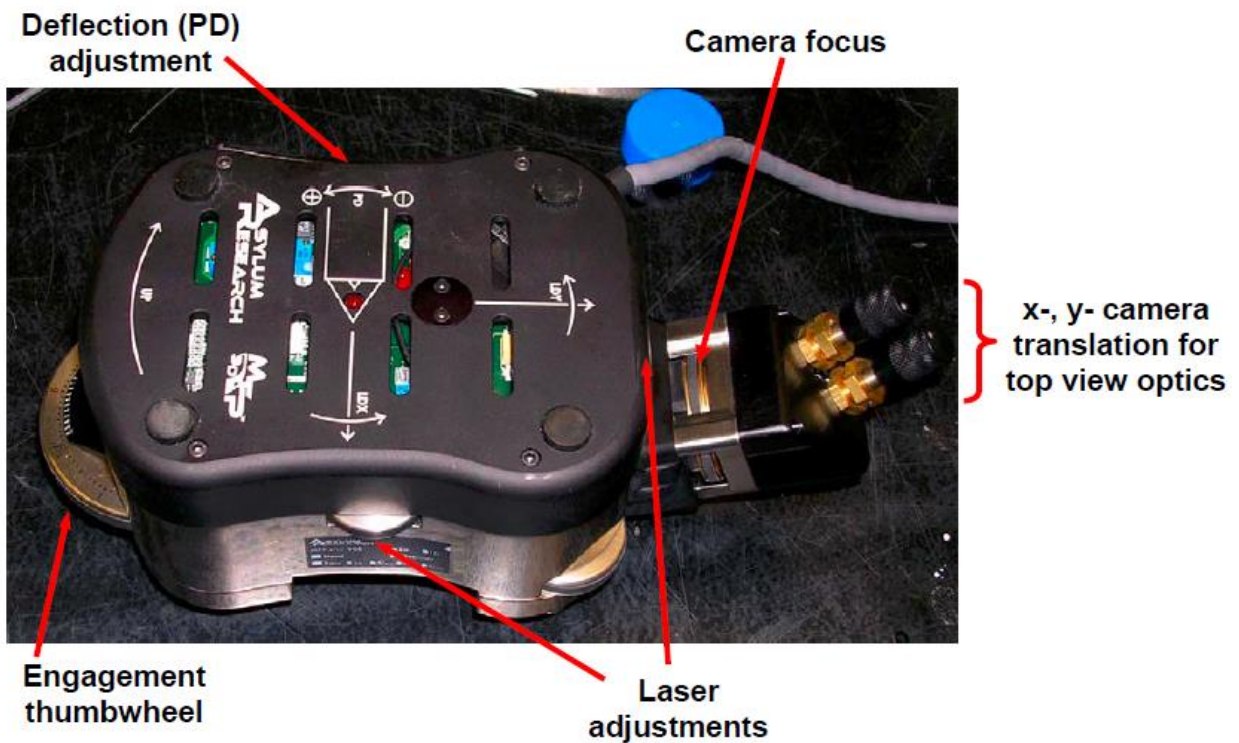
IV Laser Alignment

Figure 5: MFP-3D head and its thumbwheel locations (*top down view model shown*).

1 Lens Alignment via top view optics CCD
1.1 Turn on the light source for the video camera. The black switch knob is located on the right corner of the AFM computer cart.
1.2 Load the video camera window by clicking the camera icon at the bottom of the AFM software window. Select the *S-video* input in the Source pull-down menu in the video window.
1.3 Adjust the image brightness with the light source switch knob.
1.4 Adjust the two camera translation knobs at the back of the AFM head (Figure 5) to position the AFM cantilever probe in the middle of the video screen.
1.5 Focus the camera on the cantilever probe with the camera focus ring (Figure 5) at the back of the AFM head. Turn the ring clockwise to focus down and counter-clockwise to focus up.
1.6 Check and ensure that the laser spot is positioned on the cantilever beam. If not, adjust the LDX, LDY laser positioning thumbwheels to position the laser spot onto the cantilever beam and maximize the SUM reading (Figure 6).
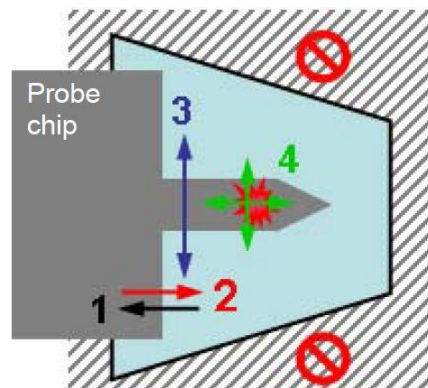

Figure 6. Laser alignment pattern

2 Adjust Photodector (PD)
- Use the thumbwheel on the left side of the head (Figure 5) to adjust the PD, whose value is seen in the Sum & Deflection meter (Figure 7).
- For contact mode imaging, the deflection should be slightly negative (slightly blue); for AC mode, it should be zero (no color).
- At any point, clicking the 'Withdraw' button will disengage the tip, retracting the piezo to a reading of 0.0V (mostly blue on the meter).
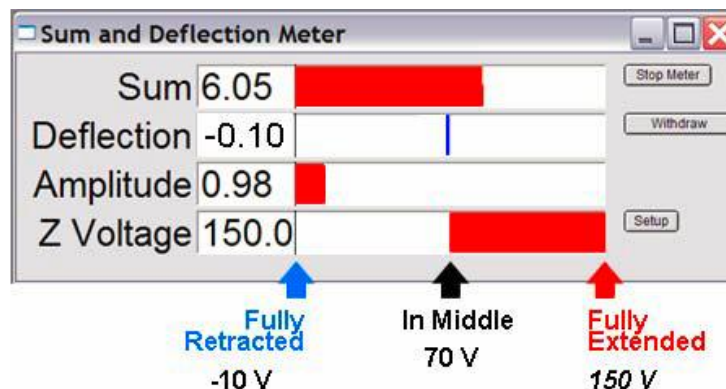

Figure 7. Signal and Deflection meter

V Probe Engagement (Hard Engage)

1. Click the 'Main' tab in Master Panel (Figure 8).

2. Select a Set Point voltage that is around 20% off the free air amplitude voltage. (*E.g.*, if the free air amplitude is 1.0 V, the Set Point voltage should be 0.8 V).

3. Choose an Integral Gain of 8 to 10.

4. Choose a Scan Rate of 1 Hz.

5. Click the "Engage" button on the S&D meter. Monitor the Z Voltage reading, which should be 150 V.

6. Slowly turn the thumbwheel on the front of the AFM head counterclockwise to lower the tip towards sample surface. When the Amplitude Voltage decrease to the selected Set

Point voltage, there is a concomitant chime sound (if the speaker is on).

7. Further turn the thumbwheel and monitor the Z Voltage, which should decrease. Stop when the Z Voltage drops to 70V.

8. The AFM tip is now stationary on the surface (not scanning).
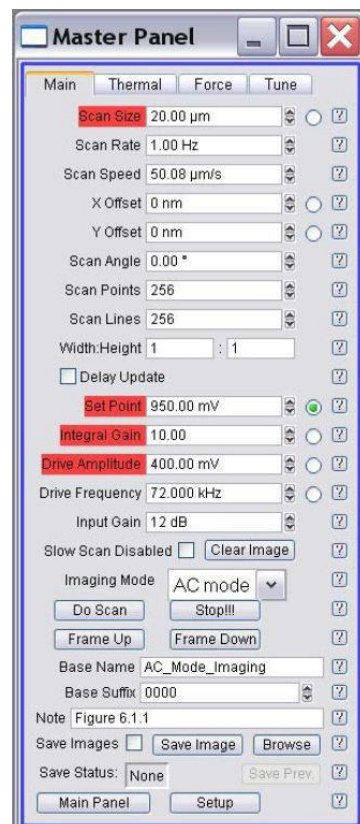
VI DETERMINATION of SPRING CONSTANT

The determination of spring constant (k) is a 2-step procedure: 1) determine the slope of contact region from a force curve to determine the sensitivity of the lever (in nm/V); 2) perform a thermal tune to determine resonant frequency of your cantilever. An algorithm computes the spring constant using the equi-partition theorem.

Step 1: Engage in CONTACT MODE on a *clean, hard* surface.
  ➢ Select 'Contact' from the Imaging Mode pull-down menu on the *Main* Tab of the Master panel.
  ➢ Choose a set point force: low force is slightly larger than the value of the Deflection value (volts) in the S&D meter; (I usually choose 0.2 to 0.5 V)
  ➢ Integral gain = 5 to 10 (integral gain) is usually sufficient.
  ➢ Click the '**Simple Engage**' button on the S&D meter; Engage tip by turning the thumbwheel until you here a bell sound; the *z*-voltage goes from railed in the red (150 V), to some other lower value.
  ➢ Slowly adjust this down to around 70 V (no color in z- voltage meter).
  ➢ Click on the Force Tab of the Master Panel (Figure 9): This panel is set up so the user can define the specific distance the piezo travels during the force-distance cycle (called 'Force distance' in Force tab).

Initial Parameter adjustment
  ➢ Choose a 'Force' distance (0.5 to 1 μm).
  ➢ Activate the 'Trigger' from the Trigger channel pull-down menu- choose the 'DeflVolts' channel,
  ➢ Enter a 'trigger' point value of 0.2 to 0.4 V.
  ➢ Choose a start distance with the red bar towards the beginning of its travel (top). Activate the 'relative' trigger radio button,
  ➢ Click the '**Single Force**' button. This makes the software choose a proper start distance on its own, and you should have a force distance curve that looks similar to the one in Figure 10A.

- ➢ In the *Force* tab, click on 'Deflection' in the 'Set Sens' pulldown menu. The 'Defl InvOLS' (deflection inverse optical lever sensitivity) value will automatically be updated in the force panel.
- ➢ Disengage tip by clicking the 'Stop!!!' button on the bottom of the 'Main' tab of the master panel, or by clicking the 'Stop' button to the right of the deflection meter on the Sum and Deflection Meter window, or by clicking the 'Withdraw' button in the Force tab.

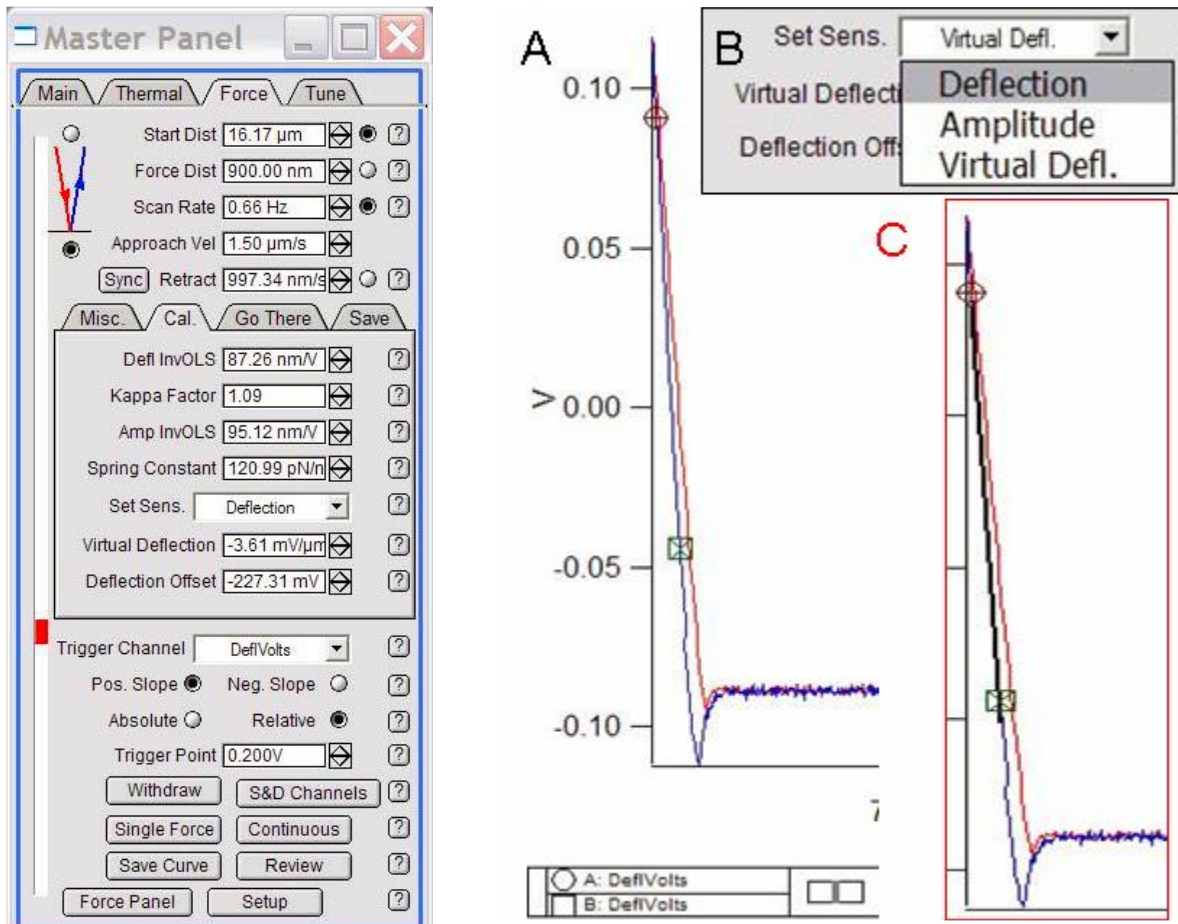**Figure 10**: Force distance curve on a hard surface.

Figure 9: Force Tab of Master Panel

Step 2: Click on the *Thermal* tab on the Master Panel (Figure 11)

- ➢ Adjust the deflection on the AFM head so it reads zero deflection in the Sum & Deflection meter.
- ➢ Click the 'Do Thermal' button at bottom of panel. This detects the natural resonant frequency of the cantilever by doing an iterative series of frequency sweeps and averaging them. A real-time amplitude *vs*. frequency (power spectrum) plot comes up like the one seen in Figure 12. Let it go through many cycles (the more you collect, the more it filters out noise).
- ➢ Hit 'Stop Thermal' to cease sampling when plot looks like it has definition enough to fit a curve to it. On the power spectrum plot (Figure 12A), expand the first large peak (red dash line). Do this by holding down the mouse button and creating rectangle around peak, then right or left clicking the mouse button;
- ➢ Click on the 'Show Fit □' checkbox; then the '**Fit Guess**' button (this brings up a blue Gaussian shaped curve very near to the thermal resonant peak); Click on the '**Try Fit**' button- a blue curve

will fit to this resonant peak (Figure 12B). The software automatically calculates the spring constant and updates the resonant frequency, seen in Thermal and Main tab. At this point the software has determined the cantilever's spring constant by the equi-partition method. This is very useful when performing force distance curves or needing to know how much force the set point is applying to the tip. This value will be store in any saved data parameter file.
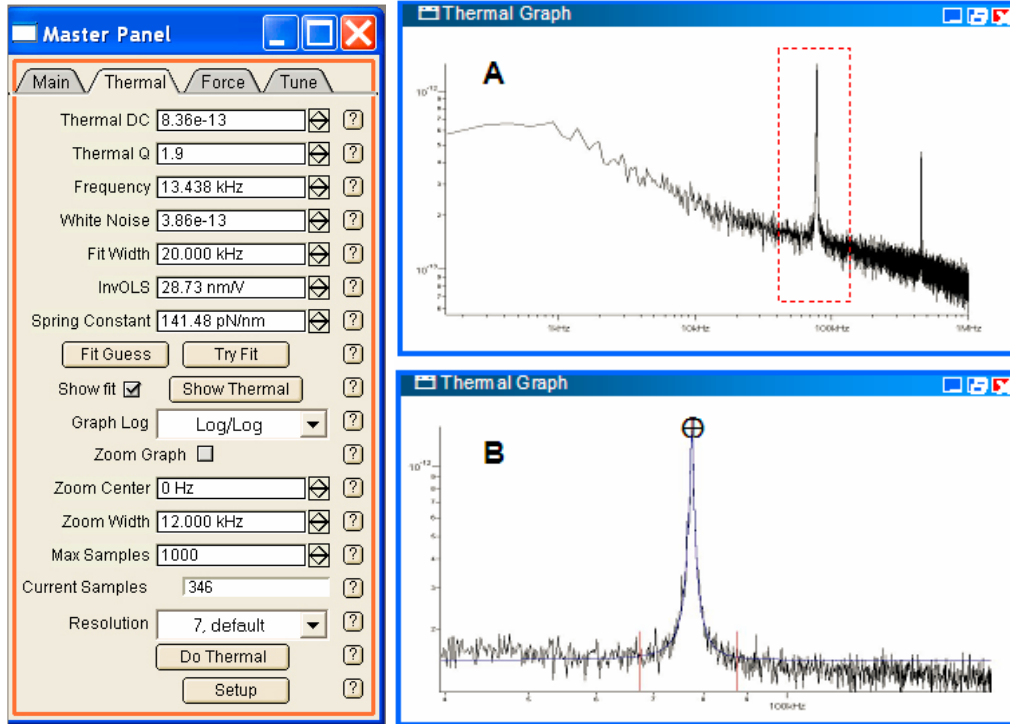

Figure 11: Thermal Tab

Figure 12: Frequency vs. amplitude plot from a thermal.tune (taken in air). A) full-scale spectrum. B) zoomed and fitted (blue line).

VII Scanning of Interested Area

1 Adjust the focus ring clockwise to focus on the sample surface.
2 Find the area of interest on sample surface by adjusting the scanner adjustment knobs on the right side of the sample stage.
3 Click the '**Do Scan**' button on the Main tab of the Mater Panel to start scanning.
4. Set proper channel for image observation: Ht (Height) Am (Amplitude), Ph (Phase), ZS (Z sensor).
5. Check the Trace and Retrace fast scan lines below each image channel to see if they follow each other well.
6. Slightly increase the **Drive Amplitude** in the Main tab and monitor the improvement.
7. Adjust the **Integral Gain** in the Main tab to better track the surface. Increase the Integral Gain until high frequency noise shows up in the trace/retrace lines. Decrease the Integral Gain until the noise disappears.
8. Select a proper **Scan Rate** for image acquisition. After adjusting the Scan Rate, click '**Frame Up**' or '**Frame Down'** buttons to initiate the newly entered scan rate.

VIII Saving Images

1. Select proper **Scan Points** and **Scan Lines** in the Main tab of the Master Panel. Use 128x128 or 256x256 for rough scan, 512x512 for data acquisition.
2. Adjust Image Z scale: Click **Auto** check box in top left corner of the image channel tabs. Click the '**Fix**' button on the left of the Data Scale section.
3. Select Real Time Flattening by choose the 'Line' or 'Masked Line' in the **Live Flatten** pull-down menu.
4. Give a proper filename for you image in the '**Base Name'** section at the bottom of the Master Panel.
5. Click the '**Save Images'** checkbox. Use Browse to choose an image folder.
6. Check the **Save Status** section to see if it will save current scan.
7. If you want to save a screen shot of one of the windows, you can make it the front window, and go to menu *File -> save Graphics…* Select the desirable format and size.

IX Image Analysis

1. In top menu, select *AFM Analysis > Browse Saved Data* to locate the saved images.
2. Open the chosen image file by placing the cursor over the image thumbnail and double clicking the mouse button. The image will open in the Display Window.

Flattening/PlaneFit
1. Click the '**M**' button on the upper right corner of the image display window to open the Modify Panel.
2. Select the '**Flatten**' Tab (Figure 12). Set a Flatten Order to 0 in the pull-down menu, and select the '**All**' radio button underneath.
3. Click the '**Flatten**' button and the image will be flattened.
4. Select the '**Planefit**' Tab (Figure 12).
5. Set the '**Planefit Order**' to 1 in the pull-down menu. Select the '**All**' radio button.
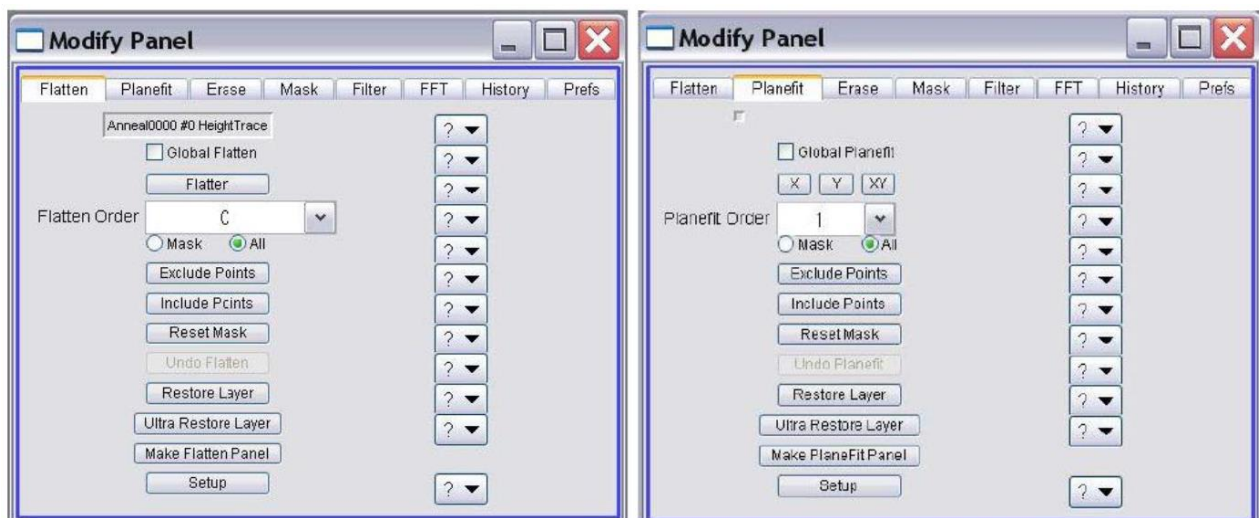6. Click the '**XY**' button to planefit the image in the X-Y plane with respect to Z.

Figure 12. (left) Flatten tab and (right) Planefit tab [2].

Roughness
1. Click the '**A**' button on the top right corner of the Displaywindow to open the Analyze Panel.
2. Open the **Roughness** tab on the Analyze Panel (Figure 13).
3. The data present in the Roughness tab is the most recent image.
4. Click the '**Export Roughness'** button at the bottom to create a text file of the data.

Section Analysis
1. Click the '**A**' button in the upper right corner of the Display
window to open the Analyze Panel.
2. Open the **Section** tab in Analyze Panel.
3. Select '**Line'** from the Mode pull-down menu.
4. Click the '**Draw**' button.
5. Move cursor over to image and draw a line on the feature of interest. A section plot will appear.
6. If needed, place cursors on the curve to measure points on the line.
7. If the curve is not leveled, perform a 1st order XY plane fit. Refer to Section 12.5.2B in operation manual for leveling the line sections.
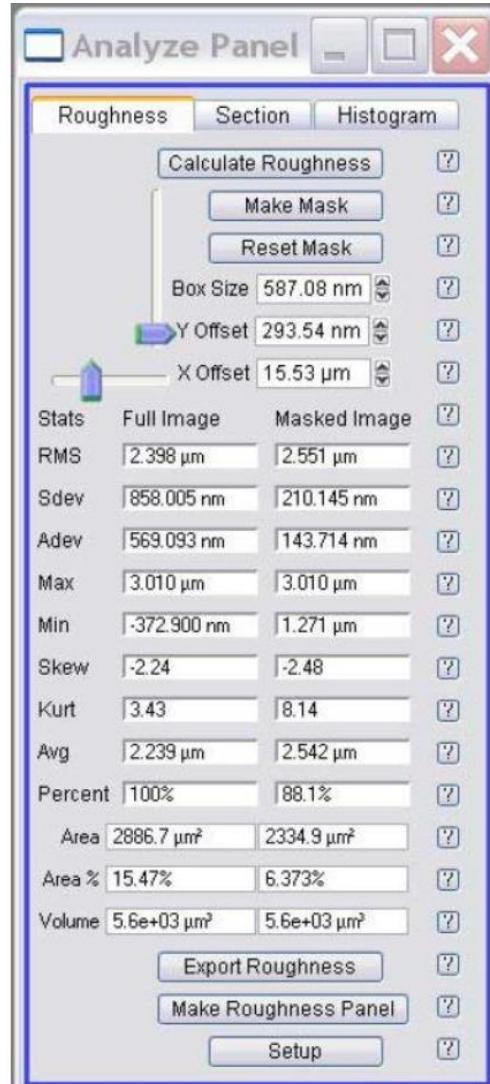8. Click the **'Export Roughness'** button at the bottom to create a text file of the data.

Figure 13. Roughness tab

X Shut Down

1. Click the '**Stop!!!**' button in the Master Panel to withdraw the tip. The S&D meter Z Voltage will read 0.0 V.
2. Turn the front thumbwheel **clockwise** to retract the tip from sample surface. Turning the thumbwheel in the wrong direction will cause tip and sample damage.
3. Turn off camera fiber optics light (switch on the AFM).
4. Remove the AFM head from sample stage. Set it upside down on the table. NOTE: Do NOT stretch the cable.