

# ASYLUM MFP-3D ATOMIC FORCE MICROSCOPE (AFM) SOP

Revised April 2020

**Purpose of this Instrument:** 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.

**WARNING:** Directly looking into the laser beam can cause permanent eye damage or blindness. Always use a piece of paper if you want to view the beam image.

**NOTE:** The purpose of this manual is for general measurements with the AFM. For more advanced measurements or measurements with accessories, please contact MNTC staff members or refer to the Asylum MFP-3D operation manual, which is located by the tool.

## START UP

1. Log in your session on the FOM.
2. Check the AFM controller box at the bottom of the computer cart to ensure that it is powered up (the green LED above the Power switch is ON).
3. Open the MFP-3D software by clicking on the icon on the computer desktop. Wait until **MFP-3D Ready** appears at the bottom of the MFP-3D software tray (Figure 1).

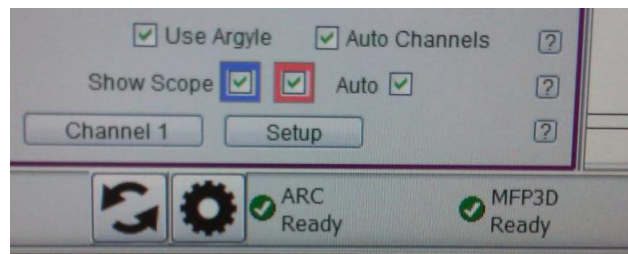


Figure 1. MFP-3D software tray

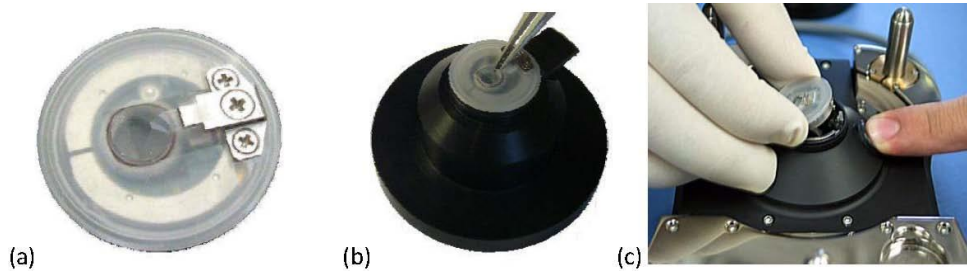
4. In the Master Panel, click on the Main tab and check the **Imaging Mode** at lower section of the tab. Select **AC Mode** (tapping mode) if it is not already shown.

## PROBE MOUNTING

1. Find the tip holder assembly on the loading pedestal (Figure 2a, b) and go to step 4. Otherwise, go to next step.
2. If you could not find holder assembly on the loading pedestal, it should be attached to the AFM head. Remove the AFM head from the sample stage and lay it upside down next to the sample stage.

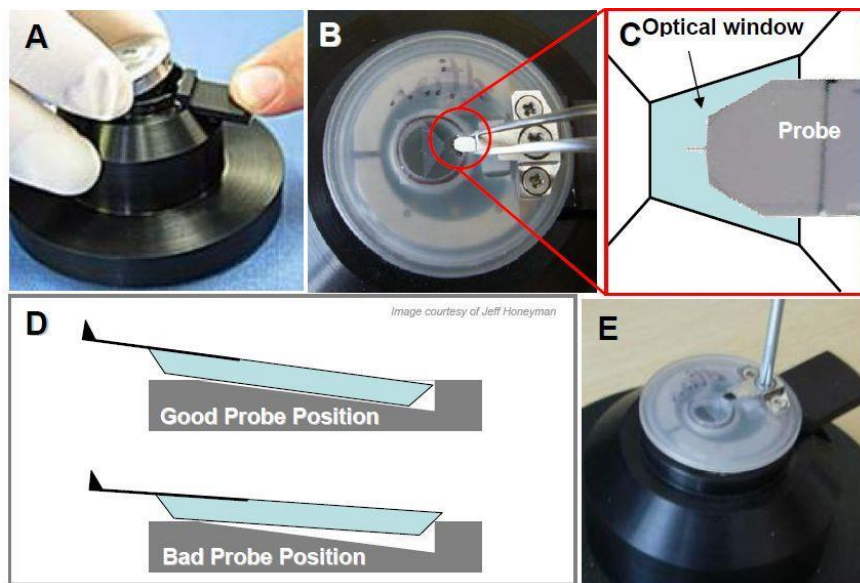
**NOTE:** Do not stretch the cable attached to the AFM head

3. 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).



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

4. Loosen the clamp screw (the middle one, Figure 3b) with a screwdriver.



**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.

**Figure 3.** Proper probe loading procedure.

5. Check the AFM probe against the light to ensure that the cantilever is not damaged.
6. 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.
7. Position the probe at the center of the clear trapezoidal shaped quartz optical window (Figure 3c).
8. Tighten the clamp screw with the screwdriver **until finger tight**. Do **NOT** over tighten the screw (Figure 3e).
9. Remove the tip holder assembly from the loading apparatus and put it back into the MFP-3D head (Figure 2c).

## SAMPLE MOUNTING

1. Attach your sample to a glass slide with sticky tab, or epoxy.
2. Place the sample at the center of the sample stage.
3. Secure the slide to the stage with the two small magnet blocks on both sides (Figure 4).

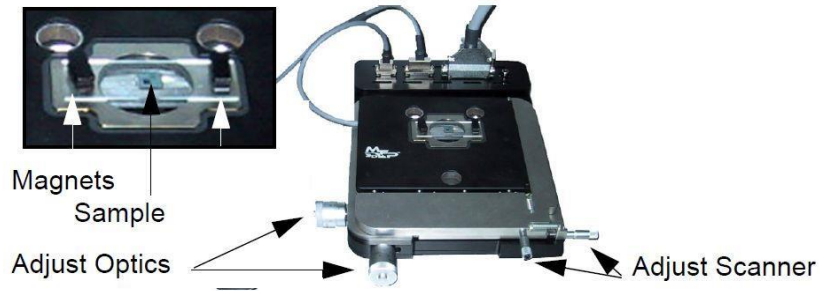


Figure 4. Sample stage and sample positioning.

## 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 5a).

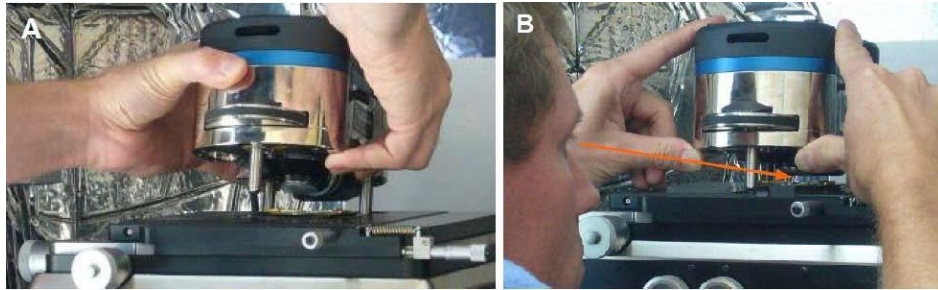


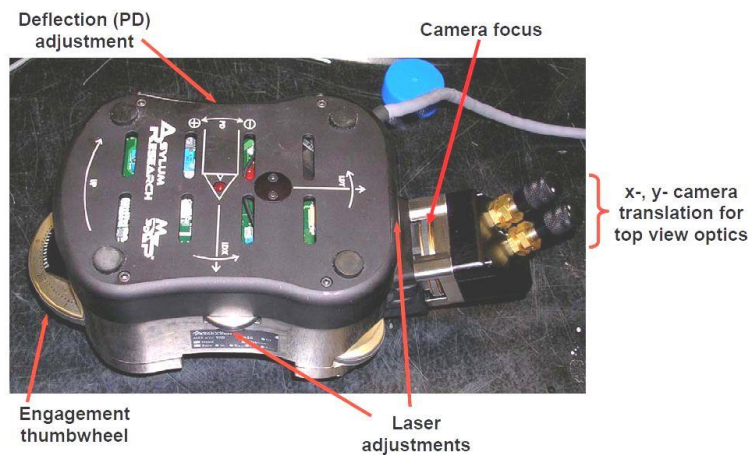
Figure 5. Mount the AFM head onto the base plate.

2. Move thumbs under the front of the AFM head (Figure 5b). Slowly lower the AFM head down towards the stage while **continually monitoring the tip-sample separation** to avoid the tip crashing into the sample (Figure 5b).
3. If the tip is too close to the sample, lift the head back and adjust the front leg height to increase the tip-sample separation. Repeat the process.
4. Adjust sample position with respect to the cantilever tip with the adjustment knobs on the right side of the base plate (Figure 4).

## ALIGNMENT CHECK WITH VIDEO CAMERA

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.
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.

3. Adjust the image brightness with the light source switch knob.
4. Adjust the two camera translation knobs at the back of the AFM head (Figure 6) to position the AFM cantilever probe in the middle of the video screen.
5. Focus the camera on the cantilever probe with the camera focus ring (Figure 6) at the back of the AFM head. Turn the ring clockwise to focus down and counter-clockwise to focus up.
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.
7. Adjust the focus ring clockwise to focus on the sample surface.
8. Find the area of interest on sample surface by adjusting the scanner adjustment knobs on the right side of the sample stage (Figure 4).
9. Position the area of interest under the AFM probe.



**Figure 6.** AFM head with various alignment thumbwheels labeled.

## LASER ALIGNMENT

1. Flip the AFM head to its normal position and settle it on the table surface.

**NOTE:** Do not stretch the cable attached to the AFM head

2. Turn on the laser by turning the key on the front of the AFM controller 90° clockwise to ON position.
3. **Optional:** Place the Newport infrared sensor card under the tip holder. You should see a laser spot on the card if the laser is not being blocked by anything.
4. If you can't see the laser spot, turn the thumbwheel on the right side of the AF head (labeled **LDX**, Figure 4) counter clockwise to move the laser spot outward along the cantilever length until the spot appears. **If no spot can be seen on the IR card, charge the IR card for a few seconds under the fluorescent light.**
5. Turn the laser adjustment thumbwheel on the right side (labeled **LDX**, Figure 6) clockwise until the laser spot disappears (moves back onto the AFM chip) (Figure 7, step #1). Note that the laser spot will also not be visible on the IR card if it is outside the transparent quartz window.

- Turn the **LDX** thumbwheel counter clockwise until the laser spot re-appear (Figure 7, step #2).
- Turn the laser adjustment thumbwheel on the backside of the AFM head (labeled **LDY**, Figure 6) (Figure 7 step #3) while monitoring the laser spot on the IR card. The laser spot intensity will be temporarily reduced when it crosses the cantilever beam and the **SUM** reading on the Signal and Deflection Meter (Figure 8) will increase significantly. Reverse the direction if the laser spot completely disappears (goes beyond the transparent window).

**NOTE:** Keep turning the thumbwheel forward beyond the limit will damage the LDY thumbwheel.

- Maximize the “**SUM**” reading by adjusting both **LDX** and **LDY** thumbwheels (Figure 7, step #4).

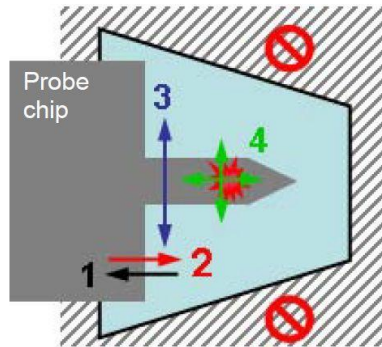


Figure 7. Laser alignment pattern.

## PHOTODECTOR (PD) ALIGNMENT

- Check the **Deflection** voltage signal in the Signal and Deflection (S&D) meter (Figure 8). It may show Red (positive) or Blue (negative) if the voltage is not zero.
- If the Deflection value is negative (blue), turn the PD adjustment thumbwheel (Figure 6) counter clock-wise (CCW) to get to around 0V. If the Deflection value is red (positive), turn the PD thumbwheel clockwise (CW) to get around 0V.

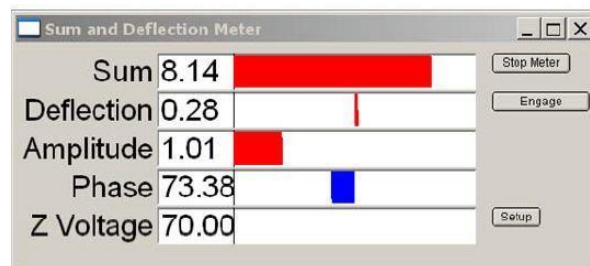


Figure 8. Signal and Deflection meter.

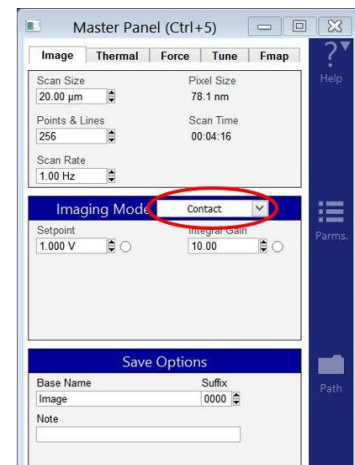
## CONTACT MODE

1. We assume that:
  - a. The cantilever is loaded into the cantilever holder and the cantilever holder is installed on the head.
  - b. The cantilever is close enough to the surface that the Z piezo actuator can bring the tip and the sample into contact.
  - c. The laser is aligned on the cantilever and the photodetector difference (**deflection**) signal has been zeroed.

**NOTE:** For more details, check the MFP-3D Operation Guide and Tutorials. This will familiarize you with the basics of loading the cantilever, sample, and such.

2. Open the AR Software, it will open the **Mode Master window**. If not, click the Mode Master button at the bottom of the screen.
3. Select Standard tab, then **Contact Mode Topography**. The screen will now re-arrange and present all the controls necessary for this type of AFM imaging.
4. **Set up Master Panel:** In the Main tab of the Master Panel, select Contact from the Imaging Mode pull-down menu.

- **Setpoint:** 1.0 V.
- **Integral gain (I):** 8 - 10
- **Scan rate:** 1 Hz; for softer samples, choose a slower scan rate, perhaps 0.5 or 0.2 Hz.
- **Scan angle, resolution** (scan points & lines), and **image size** is up to you. These are found in the Params tab to the right of the Master Panel.



**NOTE:** Parameters highlighted in orange will be mainly used to fine tune imaging parameters when scanning.

5. To start collecting data, click the **'Frame Up'** or **'Frame Down'** button on the Image Tab. After a brief moment, imaging will begin.

Tune the parameters with **Set Point**, **Integral Gain**, and **Scan Rate**. Use the arrow clickers to right of setvar windows to adjust parameters.

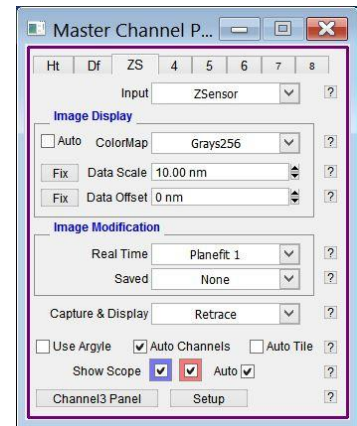
Alternately, you can fine-tune the parameters using the **Hamster Wheel** on the front of the controller. Any parameter with a **Radio button** next to it can be changed during a scan when it is activated by the Hamster Wheel. The Hamster gives **"digital control with analog feel."**

### A BRIEF SUMMARY OF IMAGE/DATA CHANNELS:

**Height:** The voltage applied to the Z piezo to maintain defined positive deflection per X, Y scan point. This signal is a linear approximation to a non-linear signal, meaning it is inaccurate at larger scales; for larger scales, you should use the Z sensor. The advantage of the height channel is that it has less noise than the Z sensor channel.

**Deflection:** The error signal of the feedback loop used to maintain the user setpoint deflection.

**Z sensor:** The movement of the optical lever detection assembly as monitored by a closed loop sensor. It is recommended to always activate the Z sensor channel when imaging, especially when sample features are larger than a few tens of nanometers; the Z LVDT sensors are more linear than the piezos, and thus give a more precise Z measurement.

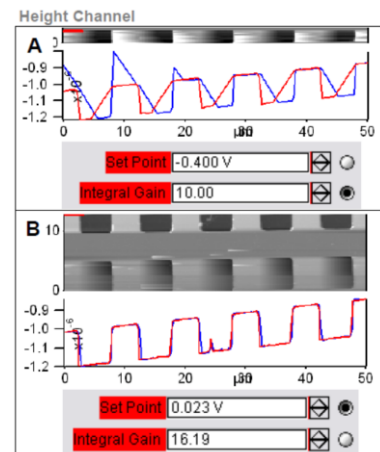


6. Determining Image Quality. Image quality can be monitored by image resolution and by the amount of noise in the line traces.

**Blue** indicates the tip moving left to right (**trace**) and **Red** indicates tip returning from right to left (**retrace**).

On most samples with relatively slowly changing features, trace and retrace should look the same. If the feedback control algorithm is simply not aggressive enough to bring the tip back down to the bottom of the pit. Such behavior is commonly called **parachuting or poor tracking**.

- a. The Setpoint voltage and Integral gain should first be adjusted to achieve good tracking. Don't be alarmed if you have to crank up the Integral gain when using long floppy cantilevers - the gain is related to the optical lever sensitivity (more gain for less sensitivity).
- b. The second parameters to adjust are the Scan Rate and Scan Angle. Having a vague idea of the orientation of the tip on the cantilever can make scanning at 90 degrees more advantageous than 0 degrees because of the shape of the tip at the end of the cantilever.
- c. The Delay Update checkbox (can be found by clicking on the Params) allows the user to change the parameters during a scan, which will take effect at the end of that scan. During the period before update, the parameters changed will be highlighted in an orange color.



## AC or TAPPING MODE

1. We assume that:
  - The cantilever is loaded into the cantilever holder and the cantilever holder is installed on the head.
  - The cantilever is close enough to the surface that the Z piezo actuator can bring the tip and the sample into contact.
  - The laser is aligned on the cantilever and the photodetector difference (deflection) signal has been zeroed.

**NOTE:** For more details, check the MFP-3D Operation Guide and Tutorials. This will familiarize you with the basics of loading the cantilever, sample, and such.

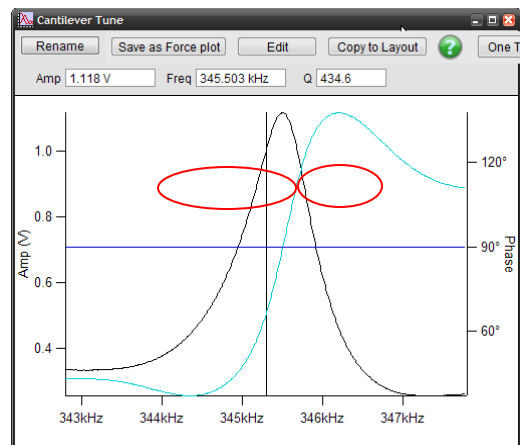
2. Open the AR Software, it will open the **Mode Master window**. If not, click the Mode Master button at the bottom of the screen.

3. Select Standard tab, then **AC Air Topography**. The screen will now re-arrange and present all the controls necessary for this type of AFM imaging.

**NOTE:** Consider watching introductory video tutorial: YouTube video [AC \(Tapping\) Mode Imaging](#)

## AUTO TUNING

4. Open the Tune tab of the Master Panel.
5. Choose a **Target Amplitude of 1.0V**; this will be the free air amplitude voltage. This is the peak to peak voltage of the oscillating cantilever.
6. Change the **Target Percent to -5.0%**. The minus sign indicates that the drive frequency will be on the left side of the resonant peak, which helps ensure the tip will remain in NET repulsive mode when engaged & scanning.
7. The **default Auto Tune Low and High** values for air imaging are typically **50 kHz to 400 kHz**, respectively, accommodating most fundamental drive frequencies for common commercially available AC Mode cantilevers.
8. Click the **Auto Tune** button for the frequency sweep to commence. The shake piezo applies a frequency ramp through the Auto Tune low to high frequencies. The cantilever will give the greatest oscillation amplitude at its resonant frequency, allowing the tune algorithm to locate it and determine the **Q factor** (quality) of the peak.
9. The **Drive Frequency** value will automatically update in the 'Tune' and 'Main' tabs of the Master Panel, and the **Q factor** of the cantilever will be determined and displayed at the top of the cantilever tune graph.



## NET ATTRACTIVE & REPULSIVE AC MODE

**Repulsive AC mode (when your phase signal is  $< 90^\circ$ )** is the most common AC mode. It is where the tip is in repulsive (hard) contact with the surface in some fraction of its oscillation. It is much easier to perform, but it is rougher on the tip and sample.

Repulsive Mode More Likely	Attractive Mode More Likely
Bigger free amplitude	Smaller free amplitude
Stiffer cantilever	Softer cantilever
Drive frequency lower than resonance	Drive frequency higher than resonance
Lower Q, Negative Q gain	Higher Q, Positive Q gain
Sample with smaller attractive forces	Sample with bigger attractive forces
Sharp Tip	Dull Tip

**Attractive AC mode (when your phase signal is  $> 90^\circ$ )** to image while sensing only the very weak attractive forces by gently oscillating the tip just above the surface. In this way the tip will experience attractive forces over a large range of its oscillation. This is much more difficult to do



and cannot be done with all levers and samples. But it is much gentler on the samples and is sometimes required for soft biological materials.

10. Establish the minimum set point to maintain a stable image. It is accomplished by first determining the optical sensitivity of the cantilever. In a physical sense, this refers to how much the laser point on the photodetector moves relative to the vertical motion of the cantilever.

Make sure the cantilever is engaged on the surface of the sample. Click on the Thermal tab of the main control panel, and then click **GetReal Probe** Panel.

11. Select the cantilever you are using from the list of different cantilevers or select Custom Rectangular Probe and enter the cantilever's Estimated Frequency, Length and Width.
12. Click **GetReal Calibration**. After some time, a graph of the calibration will display. Once the cantilever has been calibrated, a value will appear in the **Amp InvOLS** field. This represents the amplitude in nm/V.

**NOTE:** "InvOLS" stands for "Inverse optical lever sensitivity." However, the number is the reciprocal of the optical lever sensitivity rather than its inverse.

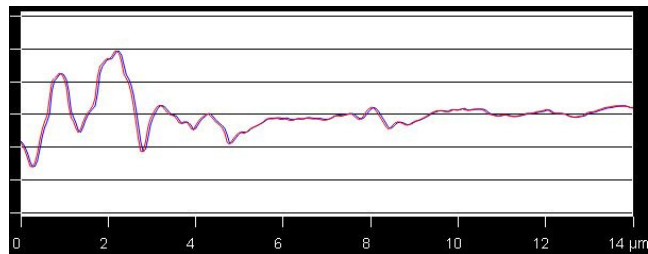
### 13. Engaging on the Surface

Choose a set point that is slightly higher than the free air amplitude. This will cause the cantilever to retract. Increase the drive amplitude until the tip is just on the surface. The Z - voltage will stop changing at this point.

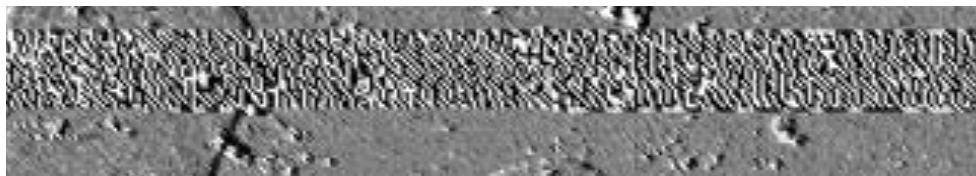
### 14. Attractive or Repulsive Mode

**For repulsive mode**, increase the drive amplitude until the phase drops below 90 degrees. If this doesn't seem to work, it might be necessary to restart scanning with a higher set point.

**For attractive mode**, keep the same set point but adjust the drive amplitude for closer tracking of the trace and retrace lines.



**Tip Better Tracking:** For better tracking in attractive mode, increase the integral gain. Increasing the integral gain too much may cause feedback oscillations (see picture below); aim for a gain that minimizes amplitude error without introducing feedback oscillations.



## 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** the laser by switching the key on the AFM controller counter clockwise to OFF position.
4. Remove the AFM head from sample stage. Set it upside down on the table.

**NOTE:** Do not stretch the cable attached to the AFM head

5. Remove the tip holder assembly from the AFM head and place it into the loading apparatus.
6. Loosen the middle screw and remove AFM tip from the holder. Tighten the middle screw until finger tight. Leave the holder assembly on the loading apparatus.
7. Remove sample from the sample stage.
8. Sign out on the FOM and sign out on the log book.