

Atomic Force Microscope

Standard Operating Procedures

Setup & Scan

- 1) Login to computer using Nanoscope login. (.\\nanoscope with password: **nanoscope**)
- 2) Turn on AFM power at surge protector strip (attached to left leg of table).
- 3) Turn on coaxial illuminator at white box to left side.
- 4) Push “E” button on the vibration isolation unit.
- 5) Set the AFM head upright in the Easy Align cradle.
- 6) Remove the cantilever holder from the head.
- 7) Place the cantilever holder onto mounting stand (black disk).
- 8) Load AFM tip on the cantilever holder. Tips are to be provided by the research group.
- 9) Load cantilever holder onto the AFM head.
- 10) Gently set AFM head down flat into the Easy Align cradle.
- 11) Start NanoScope software.
 - a. Choose experiment type.
 - b. Click “**Load**”.
 - c. If there is no laser sum on the detector the stage will not initialize, and you will need to initialize it after getting the laser beam on the cantilever.
- 12) Turn on EasyAlign power.
- 13) Adjust laser position to the tip of the cantilever (knobs on right).
- 14) Adjust the horizontal (H) and vertical (V) detector positions to 0.00 (± 0.1) (knobs on left).
- 15) Turn off the EasyAlign power.
- 16) If the stage did not initialize in step 11, go to “**Stage>Initialize**” in the software and initialize the stage. **This is important – if the stage is not initialized it may be “low” with respect to your sample, and the weight of the head can crush the \$3000 tip holder against your sample. This happened to a student. Double check!**
- 17) Click on “**Setup**” button.
- 18) Mount your sample on the microscope stage.
 - a. Place a white magnetic holder around your sample.
- 19) Check to be sure that z is high enough to clear your sample. Raise z if it is not (mouse on up arrow in setup/navigate panels).
- 20) Place the AFM head on the microscope stage, taking care to place the 3 actuator posts in the holes on the stage.
 - a. You must tilt the condenser (top light tower on microscope) back out of the way if it is up.
- 21) Lift the black microscope optics up and turn 90° to the right so they are directly over your sample.
- 22) Find the top focal plane of your sample. You can see the image on the computer setup screen.
 - a. Move the stage in x-y as needed to find a point of interest (computer mouse on x-y arrows).
 - b. Focus the image focal plane slightly above your sample for AFM cantilever placement. (Blur your sample image by turning focus knob slightly back).
- 23) Lower z until the cantilever comes into focus on the camera image OR you are about 200 microns above your estimated z value.
- 24) If you are using Tapping Mode, click “**Tune**”.
 - a. Choose “**Autotune**”.

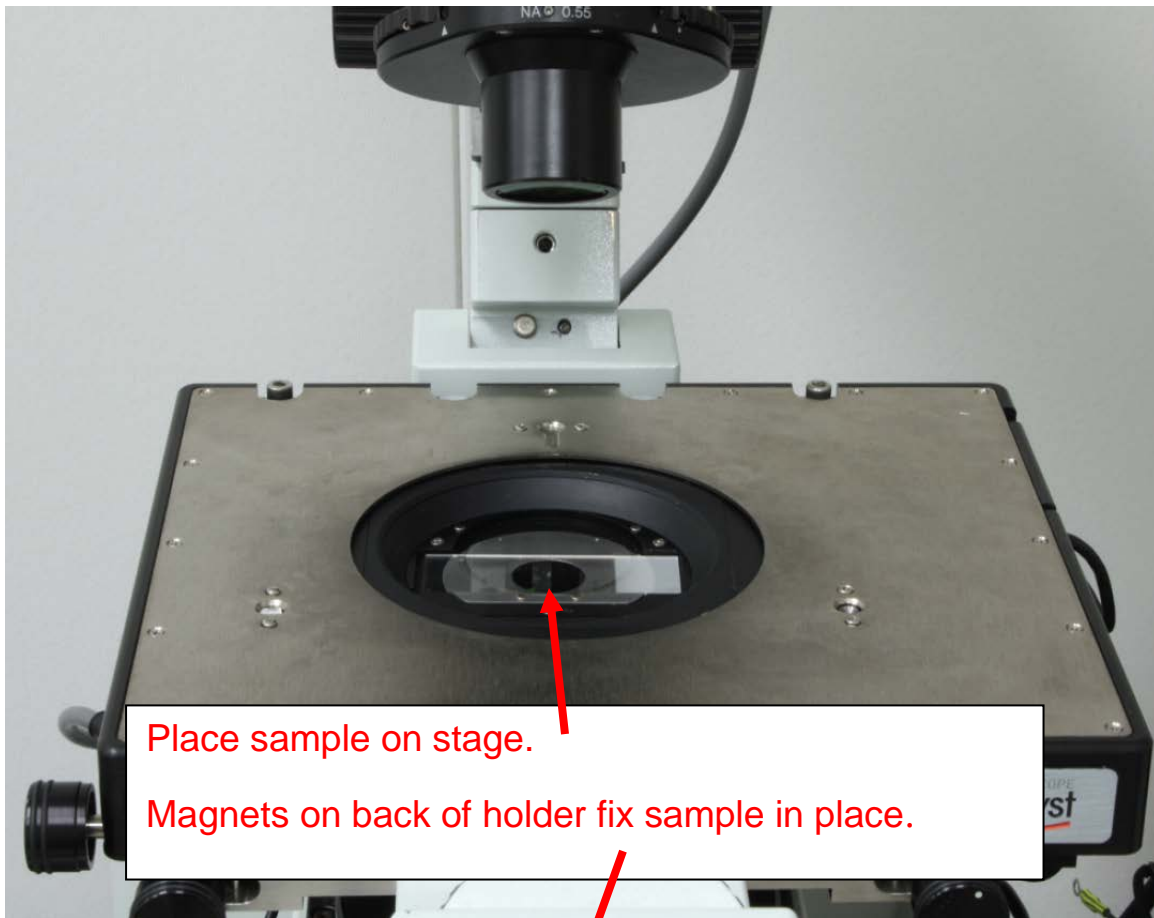
- b. If tune fails, refer to Bruker instruction manual for details. Hit manual tune and ensure that the frequency range encompasses your tips specified range. Make the upper and lower 100 Hz away from your tip's specified frequency.
- 25) Click "**Engage**" button.
- 26) If you are using ScanAssist, the software will adjust the parameters.
- 27) If you are in other modes, you will need to adjust parameters as follows.
 - a. Set point (deflection, amplitude or peak force depending on the mode you are in). Lowest value with good signal. In tapping mode lowering value pushes *harder* on sample. In contact mode, lower value pushes *softer* on sample.
 - b. Scan rate – can be fast in contact mode. Use highest value with good signal.
 - c. Watch the "error" signal – want to minimize differences in trace/retrace. If gain too high this oscillates, turn gain down. This will be amplitude/ peak force/ deflection error depending on the mode.
 - d. Integral gain (corrects for small variations) and proportional gain (corrects for large variations). Use highest values that give good signals. IG is usually about ½ the value of PG. Trace/retrace of height signal should match.
- 28) General points for AFM scans:
 - a. If you withdraw and reengage, it is best to make the scan size small again (e.g. 1µ or less).
 - b. A "survey" scan that is large, fast and low res can be used to find areas of interest. E.g. **size 150µ, 1+Hz, 128 samples/line, 64 lines.**
 - c. When you find an area that you want to image with b above, use the zoom feature to move to that spot and set the resolution back to **256 samples/line and line or higher.**
 - d. For long high resolution scans there is a "capture withdraw" feature in the software that will capture the image and withdraw the cantilever. You can set this and leave. "**Capture> Capture Withdraw**"

Shutdown

- 1) Click "**withdraw**". Use the setup window to **move Z up a few millimeters.**
- 2) Move the top microscope back to side if over sample.
- 3) Lift the AFM head, placing it upright in the EasyAlign cradle.
- 4) Remove cantilever holder from the AFM head.
- 5) Remove AFM tip from the cantilever holder.
- 6) Replace the cantilever holder on the AFM head.
- 7) Place the AFM head **flat in the EasyAlign cradle (so it doesn't get knocked over and damaged).**
- 8) Remove your sample from the stage.
- 9) Close NanoScope software.
- 10) Logoff computer.
- 11) Turn off AFM power at surge protector strip.
- 12) Tidy area for the next user.

Setting/Mode	Contact	Tapping	Noncontact
Set point deflection/amplitude setpt	Lowest with good signal (higher # pushes harder)	Highest with good signal (lower # pushes harder)	Highest with good signal (lower # pushes harder)
Integral & Proportional gains	Highest with good signal	Highest with good signal	Highest with good signal
Scan rate	Highest with good signal can be >1	Highest with good signal	Highest with good signal. Low helps tracking .1 or .2 Hz

Microscope stage.



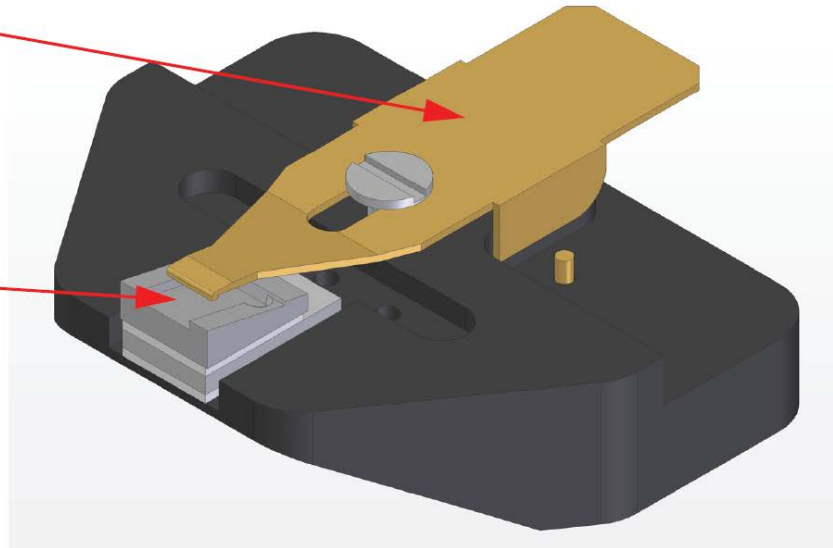
Mounting stand for cantilever holder.



Cantilever holder.

Spring Release Clip -
Push down and pull
away from the probe
to release

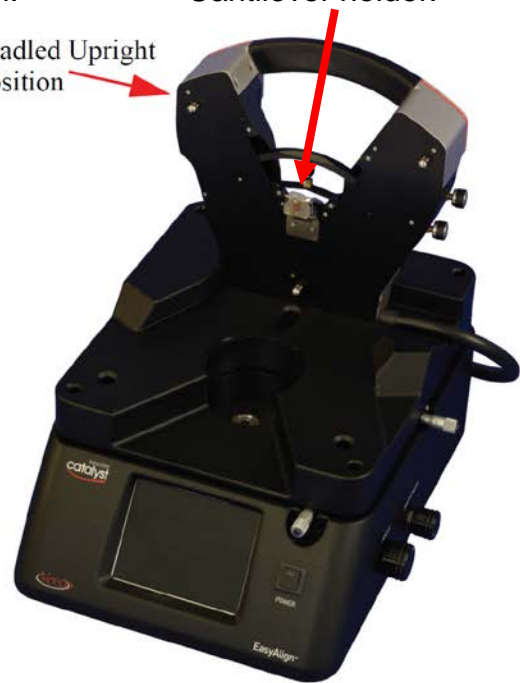
Recessed Probe Area



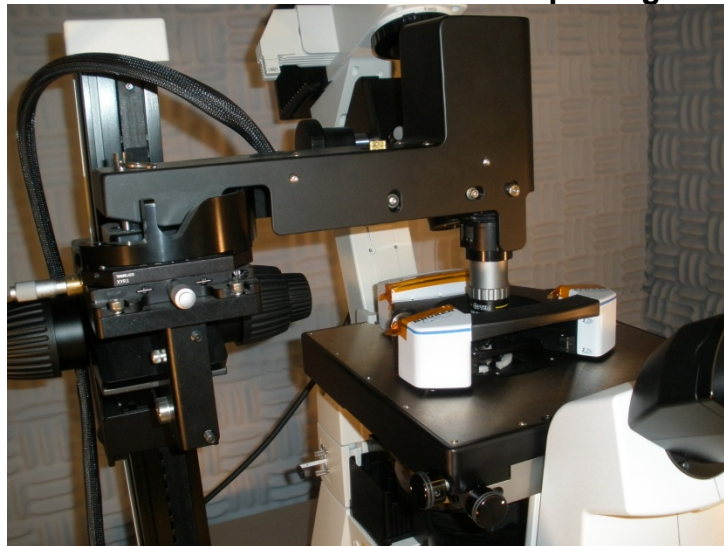
Adjust H and V.
EasyAlign stage.
Adjust laser position.



Cantilever holder.
Cradled Upright Position



AFM Head mounted on microscope stage.



Instrument Hazards and Best Practices: AFM

This document will cover the inherent hazards when utilizing this piece of equipment as well as the best practices and procedures to avoid danger.

Lab coats are to be provided by the user.

Hazards:

- Chemical exposure
- Ultraviolet light exposure

1. Required PPE

Appropriate laboratory attire is required at all times in the AMSEC laboratories. Whenever chemicals are being used, an additional requirement of a lab coat is required. Lab coats are to be provided by the user.

Anytime liquid chemicals are present in the same room as the user without a direct barrier, all users in the lab must wear chemical splash goggles. Splash goggles must be approved by State of Washington Administrative Code (WAC 296-155-215).

If chemicals being used are considered toxic, caustic, corrosive, flammable solvents, carcinogenic, mutagenic, or teratogenic, a minimum of disposable nitrile gloves is required. Avoid chemical transfer by taking off gloves when using anything other than the chemical(s).

2. Ultraviolet Light Exposure

The AFM contains an ultraviolet light (UV) sources used for fluorescence imaging. During the common operations that our users carry out, this source is not on. If this source is switched on, please take note of the following. Every effort is taken to reduce the risk of ultraviolet light exposure, however it is impossible to eliminate all possibilities.

It is recommended to avoid eye contact with the light source at all times unless looking through the eyepieces or camera as they are shielded from UV light.