

Atomic Force Microscopy
Standard Operating Procedure
AC-mode (Tapping Mode)

1. Turn ON the power switch of control unit.
2. Turn the Key to switch ON the laser BEFORE the computer (otherwise camera doesn't work).
3. Turn the computer ON.
4. Open the software Asylum Research, in the program, close the cantilever window. You need the Master Panel and the Master Channel Panel open.
5. Check if the checkmark in the green MFP-3D box in the lower left corner of the program screen appears. If not, turn everything off and back on again in the right order.
6. Click the camera icon. If the camera works properly you should see video of gray noise. If you see a green screen in the video window, check the camera connections and if it is turned on, then restart the MFP-3D program.
7. If the tip needs to be changed, take the AFM assembly off the inverted microscope, set it upside down on the metal plate, place the tip head in its holder, use the small Philips screw driver to loosen the centre screw, remove old tip, take new tip from its box with tweezers and slide it under

the metal lid until the line on the cantilever lines up with the metal lid, without ever setting the tip down. Tighten centre screw until the tip appears spring loaded.

8. Take the tip head from the holder, place carefully in the AFM assembly, tilting it in at the pins first, and then pressing the button on the right to lock it in place (the end at the pins will slightly come up).
9. Flip AFM assembly and place it with its three legs in the wells.
10. First align the cantilever to the laser, then place your sample on the translation stage.
11. Find the tip on the camera, adjust the focus for the sharpest image by lowering the AFM assembly. Turn both wheels in the back (EVENLY!) counter clockwise, then lower the front by turning the wheel counter clockwise until the video image appears sharp.
12. In the Scan and Deflection Meter window, maximize the Sum by aligning the laser on the tip with the control wheels LDX and LDY at the AFM assembly. Aligning the laser in one direction will make the Sum reading go through a maximum with sharp drop-offs at both sides, find the maximum by moving right and left of it. Turning the other control wheel will increase the Sum reading slightly with a sharp drop off at the end.
13. In the Scan and Deflection Meter window, minimize the Deflection reading to 0 by turning the phase control wheel at the AFM assembly.
14. Now the laser is aligned.

15. Only the silver part of the microscope table is the translation stage and moves. Samples need to be held in place with two magnets on microscope slide to dampen noise.

16. To load a Sample

- a. Raise the back of the AFM assembly evenly (clockwise = up) until there is ample, clearance (~4mm is good) to slide in the sample that is attached to a microscope slide.
- b. Slide the sample slide in, hold the slide on one end and place a magnet on the other end to avoid tilting the slide and destroying the tip, repeat with the other magnet.
- c. Bring the back of the AFM assembly down to ~1mm above the sample; the front is still ~4mm above the sample.
- d. In the Master Panel window, click the tab Main, go to Imaging mode and choose AC mode (= tapping mode). Click the tab Tune and click Auto tune; this determines the natural tip frequency.
- e. Close the Auto tune window once a curve with a nice maximum is shown.
- f. Click the tab Main again, choose a scan size, depending on your feature size. The maximum scan size is 90um, at scan size 500nm images can be quite noisy.

- g. In the Scan and Deflection Meter window, click Engage (this starts the feedback loop). The Amplitude should be ~ 1 . Before there is physical contact with the sample established the Z-Voltage reads 150. A z-Voltage of ~ 70 is ideal for AC and contact mode operation.
- h. CAREFULLY lower the front of the AFM assembly by turning the wheel counter clockwise while watching the Z-Voltage reading and the physical distance of the tip from the sample. This approaches the tip to the surface.
- i. Once the Z-Voltage reading changes, turn the wheel VERY slowly until the z-Voltage reads ~ 70 . Do not run the tip into the sample, it will be destroyed (In the z-Voltage reading, red is good, blue is bad).
- j. Now the sample is loaded.

17. In the Master Panel window, check Save Images and select a path, type in a Base Name. This ensures that all images will be saved with a base file name and a number.

18. Turn the camera off to reduce noise. Also wait a couple of minutes after locking the vibration enclosure to let the air settle.

19. In the Master Panel window, click Do Scan. Three windows appear: phase, amplitude and height. In the height window, the blue and red traces should overlap. If they don't check your tip and sample (some samples repel tips).

20. Click Auto in the Master Channel Panel window to automatically zoom the z-direction in the false color image.

21. The program is set up so that it keeps repeating scans (and saving every single scan) until you tell it otherwise. To do so click Last Scan, then the current scan finishes and gets saved.

22. If the data acquisition needs to be stopped during a scan (e.g. because the data are crappy), click Stop. This automatically withdraws the tip, BUT the tip is still physically right at the surface. **Be aware that you need to remove the tip from the surface MANUALLY (about 6 to 7 cycles) to avoid damaging the tip!**

23. To change samples, click Withdraw, manually raise the front of the AFM assembly (~3- 4mm) and follow instructions of loading a sample.

Shut-off:

- 1) Manually move the tip head ~4mm above the sample.
- 2) Turn the camera off.
- 3) Turn the vibration table off.
- 4) Lock the vibration enclosure. (This keeps the dust out.)
- 5) Turn the program off.
- 6) Shut the computer down.
- 7) Turn the laser off. First the key, then the switch.

