**AFM Operating Protocol – Agilent (Tapping Mode)**

*note: here “probe” refers to the whole object, and “cantilever” refers to the small projection which oscillates during operation.*

1) Choose appropriate tapping mode tip (NSC15 or TESP).
2) Choose appropriate AFM scanner: smaller scanner with a maximum scan area of 10 microns or closed loop large scanner with a maximum scan area of 100 microns.
3) Place scanner head on stand and insert appropriate nose assembly (with black o-ring and 2 contact pins) assuming that the contact pins are aligned correctly. Manually push the nose assembly in place by applying even pressure to both sides of the nose assembly.

*******CAUTION: Do not bend contact pins!*********

4) Place cantilever on nose assembly.
   a) Using the provided “spring key,” rock back the retainer spring on the nose assembly while inserting the AFM probe.
   b) Place the probe between the guides such that the cantilever extends over the window (i.e. make sure the cantilever is in the center of the window and the probe is not touching the guides on either side).
   c) Gently lower the retainer spring in order to hold the cantilever in place.
   *Always store the spring key on the magnet on the scanner stand.*

5) Insert scanner and connect cables to AFM platform.
   a) Place scanner into microscope such that the frosted screen is facing upwards and towards the AFM box door.
   b) Tighten the knob on the lower right side of the microscope to hold the scanner in place. Do not turn the projecting silver peg!
   c) Plug the scanner into the microscope (blue cord on the right; red cord on the left).
   d) Plug in the light blue closed-loop cable to the back right corner of the scanner if needed.

6) Turn on the microscope and software.
   a) Turn on the laser on the control box beneath the AFM.
   b) Open the PicoView 1.6 software.
   c) Turn on the light source to the right of the AFM.

7) Center laser on the tip of the cantilever by adjusting dials on top of the scanner.
   a) Turn on laser switch on the AFM controller under the air table.
   b) Align the laser by visualizing the laser spot on the piece of paper below the microscope.
   c) Locate the forward edge of the cantilever, move the laser back until the spot disappears, and then back forward just until the spot reappears.
   d) To align the laser on the cantilever, first move the laser left and right looking for the disappearance and reappearance of the laser. The disappearance corresponds to the laser reflecting off of the cantilever. Then move the laser back just to the point where the spot is partially blocked.
   e) Fine tune laser alignment using the laser spot projected off the cantilever onto the frosted glass screen. Move the laser forward and backward and left and right till a nice oval appears on the screen.

8) Insert photodiode detector.
   a) Insert detector into the scanner by placing it under the frosted screen and plugging it into the front of the microscope.
   b) Use the knobs on the front and left side of the detector to move the photodiode to the laser spot location.
   c) Use the Laser Alignment box in PicoView to maximize the sum and center the spot over the green axis. The Deflection and Friction values should be $\pm 0.1$ V.
   d) If needed change all four gain switches on the back of the photodiode so that the sum signal on the detector is greater than 1 V but less than 10V.

9) Mount the sample.
   a) Secure sample on appropriate magnetic sample plate using magnet and specimen disc or carbon tape.
   b) Verify that there is enough room beneath the scanner to accommodate the sample without crashing into the AFM probe. (use “Open” on AFM controller to increase space between magnetic sample holder posts and AFM probe)
   c) Insert sample plate into microscope, by aligning one tab over the front alignment pin and the other tab over the alignment pin to the right.
   d) Allow the magnetic posts to gently engage the sample plate into place.

10) Close the distance between the sample and the tip.
    a) Using the “Close” switch on the control box beneath the AFM and Camera view in PicoView software bring the cantilever close to, but not touching the sample.
    b) Focus on the real image of the cantilever by first rotating the microscope focus knob (large black knob at the back of the microscope) away from you until all images of the cantilever and surface are out of focus. Then rotate the microscope focus knob towards you until the first image of the cantilever comes into focus.
    c) Once the cantilever is in focus, lower the focal plane of the microscope slightly below the cantilever by rotating the microscope focus knob toward you.
    d) Using the “Close” switch on the control box beneath the microscope and Camera view in PicoView, bring the surface into focus.

11) Locate desired surface area/features on the sample by adjusting the silver micrometers on the front corners of the AFM platform.
12) Under the Controls drop down menu select “AC Mode” to open the ACAFM control panel.
    a) Set drive mechanism to AAC and the gain should be 1x.
13) Auto tune the cantilever.
    a) In the servo window set the Setpoint to 0.
    b) In the Controls menu, select “AC Mode Tune” to open the AC Tune panel.
    c) Click "Auto Tune" in the ACMode Tune panel.
14) Check and set important parameters:
    - scan size = $\leq 10^2 \, \mu m$ or $100^2 \, \mu m$ (scanner dependent)
    - x- and y-offsets = 0
    - Speed = 1 ln/s
    - Setpoint = 1.0 V
    - I and P Gain = 10%
    - Range = maximum value (maximize by typing in 999)
15) Engage the tip (Downward red arrow button in the control bar.)
16) Make the final adjustments to maximize the quality of your image.
    a) In the Scan and Motor box, start the scan by clicking the blue up or down arrows.
    b) In the Realtime Images window, choose to display Topography (or any other mode you wish to observe). To add additional windows, click the "+" button in the upper left of the window.
    c) If the features in the images are not visible or look distorted, adjust the Display range value and choose an appropriate flattening technique (typically, "Flatten 1st Order").
    d) Adjust the Setpoint to enhance the image; a Setpoint that is too high will not accurately trace the surface topography, while a Setpoint that is too low will damage the surface. For soft tapping, increase the Setpoint until the tip is no longer in contact with the surface (indicator in the Servo window will turn red), then slowly decrease the Setpoint so that the tip is just in contact with the surface and then decrease the Setpoint ~0.1V from that value.
    f) Adjust the gains to improve image quality: first increase the I gain until noise is evident in the image or real time cross section, then decrease I gain until noise disappears. Set the P gain to ~ (or 1.5x) I gain.
17) Scan and save images(s).
    a) Reposition the probe at the top or bottom of your scan area using the appropriate buttons in the Scan and Motor box and choose to capture only 1 Frame. Once the image is complete choose File > Save As.
    b) Autosave will continuously save every completed image in numerical sequence until turned off.

18) Once imaging is complete, remove detector, scanner, nose assembly, and cantilever.
    a) Disengage the tip by pressing the upward red arrow button in the control bar.
    b) Use the Open lever on the control box beneath the AFM to move the sample away from the AFM probe and turn off the laser.
    c) Unplug detector and scanner components from AFM platform.
    d) Place the scanner in the stand and remove the cantilever using the spring key to pull up the retaining spring. Place used tips that are still good in box (aligned horizontally).
    e) Use the nose cone removal tool to remove the nose assembly from the scanner.
    f) Place detector, scanner, and nose assembly in the box for storage.

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