AFM Operating Protocol – Dimension 3100 (Tapping Mode)
* note: here 'probe' refers to the whole object, and "cantilever" refers to the small projection on which the AFM tip is located.
** always GROUND YOURSELF when handling the instrument**

1) Choose appropriate tapping mode cantilever (NSC-15 or TESP).  
2) Mounting the AFM probe.  
   a) Position new AFM probe in metal slot, with markings facing up.  
   b) Make sure the probe is centered (not touching either side) and resting against rear of the slot.  
   c) Carefully slide clamp over probe and lower gently down to hold probe in place.  
   d) Carefully side probe holder back onto the foot of the scanner housing. Apply even pressure on both sides of the cantilever side of the AFM holder to mount on scanner.  

***************DO NOT BEND THE PINS!***************
***************DO NOT DROP THE SCANNER!***************

3) Open software version 5.31 and press the microscope button to load instrument controls.  
4) Center laser on the tip of the cantilever by adjusting dials on top of the scanner housing.  
   a) Adjust dials until you get a signal, as evidenced by a spot of laser light appearing in the window on the side of the housing, as well as an increase in the “Sum” bar value on the right-hand screen. The presence of the signal means that the laser is reflecting off of the probe, not necessarily that it is reflecting off of the cantilever.  
   b) The dials on top of the housing are directional. To locate the forward edge of the probe, move the laser to the left until the signal disappears, then just to the right to regain the signal.  
   c) Move the laser a bit more to the left (about a quarter of a turn) to move it off of the body of the probe and into the space occupied only by the cantilever. You should be able to see the laser spot on the sample holder directly under the probe.  
   d) To align the laser with the cantilever, first move the laser up and down looking for the appearance of the laser spot in the window on the side of the scanner housing.  
   e) To fine tune laser location on the cantilever move the laser up and down, then left and right, scanning to locate the tip of the cantilever – when the laser is on the tip, the slightest deviation up and down will result in loss of signal.  
   f) Maximize the signal by using the same dials, while observing the effect on the “Sum” bar value – the maximum value for common tips is around 4-6. The reflectivity of the material the cantilever is made of will determine the specific value.
5) Align the laser with the detector by adjusting the dials on the side of the housing.  
   a) Position new AFM probe in metal slot, with markings facing up.  
   b) Turn on vacuum to secure the sample and make sure that even pressure on both sides of the cantilever side of the AFM holder to mount on scanner.  

***************DO NOT BEND THE PINS!***************  
***************DO NOT DROP THE SCANNER!***************

6) Locate and focus on the cantilever (using the Visual Window.)  
   a) Click on the yellow magnifying glass over a cantilever “Locate Tip” button (control bar), and, looking at the visual window on the right screen, adjust the illumination so that you can clearly see the tip.  
   b) Adjust “Zoom” and “Focus” by holding down the appropriate button and rolling the trackball up and down, until the cantilever edges are clearly in focus in the visual window.  
7) Center the camera on the tip of the cantilever.  
   Looking at the visual window, center the crosshairs on the tip of the cantilever (halfway width-wise, with the same margin all around) by adjusting the two dials on the camera housing.  
8) Place your sample on the sample plate over the hole in the center of 4 depressions. Turn on vacuum to secure the sample and make sure that something is covering the hole in the center of the plate. Rotate sample plate so that your sample is located directly under the probe.  
9) Focus the camera on the surface of your sample.  
   a) Click on the magnifying glass button over a red surface (control bar).  
   b) Using low illumination, depressing the “Focus” button on the mouse, roll the trackball toward you to manually cause the cantilever to approach the surface of the sample. If the cantilever is too far above the sample to begin with (a few mm or more,) you can eyeball the apparatus instead of the visual window and roll the ball quickly to cover most of the distance. Then, when the cantilever is sufficiently close, direct your attention to the visual window for fine adjustment.  

Eventually, the surface will come into sharp focus. *note: as the cantilever comes within about 200μm, you will likely begin to see the laser reflection on the edge of the body of the cantilever in the visual window – this should alert you to proceed with caution and avoid colliding with the surface which could potentially damage the tip and/or sample. Also, there will likely be features present on the surface large enough that you can use them to focus on (dust particle, etc.) Scan in the plane of the surface using the roll ball without depressing any buttons to find such features and select imaging area.  
10) Close the lid and lock it securely in place.  
11) Tune your cantilever.  
   a) Press the tuning fork button (control bar).  
   b) Select “Auto Tune” and wait for the instrument to finish optimizing the tuning parameters. The final tuning curve should look mostly Gaussian and slightly off center. The drive amplitude value should not exceed 250 mV (if it exceeds 250 mV check that the probe holder is pressed firmly against the scan head and realign laser on the tip of the cantilever).  
   c) Select “Zero Phase”  
   d) Press “Eye” button to return to imaging.
12) Engage the tip (Downward green arrow button in the control bar.)  
13) Name your file. Using the Capture menu bar, select “Capture Filename” and name your file with initials date sample name (you can use the “note” feature to create a short description of your sample.)  
14) Make the final adjustments to maximize the quality of your image. The “Integral and Proportional Gains” serve as directive parameters that optimize the signal to noise ratio. The “Amplitude Setpoint” value determines how hard the probe is interacting with the sample, and also affects the quality of the image – larger the Setpoint, the less force on your surface; but if the Amplitude Setpoint is too large you will have trouble tracking the surface.  
   a) Disable the Slow Scan Axis. This allows the cantilever to repeatedly scan a single line, which enables you to more clearly observe the effects of your adjustments on image quality.  
   b) Click on the sine wave button (control bar) to switch to oscilloscope mode.  
   c) Adjust the “Amplitude Setpoint” (in the Feedback Controls window) and observe the Scope Trace response. The Scope Trace represents the particular line (2D topography) that is being scanned at that moment. Increase the “Amplitude Setpoint” until you see a flatline/no line (indicating no contact between the cantilever and surface,) then reverse direction to optimize – stop adjustment when only a very small change in the relationship between the forward (white line) and reverse (yellow line) scan is observed. The goal is to contact the surface as lightly as possible (as large a setpoint as possible) and just hard enough to register feature detail.  
   d) Adjust integral and proportional gains. Start by increasing the I gain until noise is introduced to the line scan, and then decrease until noise is no longer present. Set the P gain to = (or up to 1.5x) I gain.  
   e) In the Channels windows on the left-hand screen, choose either “trace” or “retrace” for the computer to display your image, depending on which function in the Scope Trace window more accurately represents the topography of your sample. Also, set the image height scale as appropriate for you image.  
   f) Click on the eye button to return to your image and ENABLE the slow scan axis.  
15) Scan and save image(s).  
   a) Reposition the probe at the top or bottom of your scan area using the appropriate menu bar buttons (small blue arrows going up or down).  
   b) Click “Capture Image” (camera button) to save this particular scan. Data is saved from the last point at which parameters other than “Data Scale” were changed and an image is completed.  
16) Once imaging is complete stop the scan by pressing the red up arrow button on the control bar. Move the scan head away from the sample by rolling the trackball away from you with the “Focus on Surface” window open in the software. Remove sample from stage and TURN OFF THE VACUUM. Finally, exit the software on the computer.