AFM Water Matrix 2: Matrix Changes and Automation

# Introduction and Matrix 1

In Matrix 1, the experimental procedures developed in the trials of Matrix 0 (which does not have adequate quality for use in paper results) were used with high consistency to get reasonably repeatable data. This data is summarized in the Matrix 1 write up. Through the experiment, a major time consuming item was the visual analysis of FD curve data, at the same time as data collection. After an FD plot would show up in the ezAFM software, I would visually determine a baseline (non-contact) force value and the minimum (retract curve dip) force value, and record the difference between the two in mV. Then, in 4 curves at the beginning and end of each experiment, which were always done on polished SiC (for its hardness and smoothness), I would use the software to determine a laser sensitivity (actually inverse sensitivity) value (nm/mV). Then in an excel spreadsheet containing all of the above data I would multiply the average force (mV) by average sensitivity on SiC (nm/mV) to get probe tip deflection (nm). Finally, I would use the probe’s spring constant (nN/nm), given on its packaging, and multiply it by the probe tip deflection to get an adhesion force (nN). While this gave reasonable results, there were a few issues with the procedure:

* Entering measured force values (mV) into excel was time-consuming and the visual determination of the force was inconsistent in terms of sometimes picking minimum and sometimes what appeared to be minimum, and same for non-contact force, sometimes a maximum and sometimes an apparent average of lf noise.
* Visual determination of the force made the experimental part take longer than doing it automatically.
* Part of the procedure required verifying microsphere on optical microscope. The optical microscope was situated far from the AFM, necessitating a brief walk every time, along with disconnecting the cable on the AFM head (that connector seems to be a source of electronic noise and is not very robust).
* It turned out, from discussions with Buket, that the FD curves y-axis actually use V as a unit rather than mV. The inverse sensitivity measurement is still ok as (nm/mV) but the adhesive forces are 3 orders of magnitude higher than in the original calculation. The Matrix 1 spreadsheet has been adjusted to show this, but experiment (E103-E113) spreadsheets have not.
* The glass samples and controls showed very similar adhesion throughout and thus seemed to be repetitive. Most minerals also appeared very rough under optical microscope (see E109) and thus using that data in the paper is questionable. After completing Matrix 1 I made a list of the highest standard deviation materials (least repeatable) and these rough materials were at the top of the list. I decided to remove these materials from subsequent matrix trials, to both increase repeatability and decrease measurement time.

# Changes from Matrix 1

As outlined above, the updated matrix (Matrix 2) has fewer substrate materials and relies on a computational analysis rather than visual analysis during the experiment. This allows to both increase number of physical measurement points per sample from 6 earlier to 8 now (still at 4 ‘valid’ curves per point), and to reduce overall experiment time to about half of previous, now completing 4 samples per hour. In addition to these changes, optimizing the procedure (including moving optical microscope closer to AFM and performing multiple steps at once while loading new sample) shaves off a few minutes consistently leading to faster data collection. The updated procedure is outline in detail below:

## Detailed Procedure

1. Turn on AFM to warm up (with laser powered on to 96.2% and camera on), turn on DI fan over uncovered samples and new probe in its cover placed in air stream. Put water from glass container into syringe, rinse once, and put in water again – approximately 1.5 mL.
2. Turn off laser with checkbox. Remove old AFM probe and check its serial inscription before putting away; keep AFM on its stand and laser turned back on while doing so to avoid thermal gradient from table
3. After 10 minutes under DI fan check inscription of new AFM probe and load into AFM. Turn off DI fan. Begin AFM measurements after at least 30 minutes total warm up time.
4. Place AFM head under optical microscope, and confirm presence of microsphere. Load new sample into AFM and check centering with blue triangular centering tool. Check that the old sample was correctly identified. Turn on temperature/humidity meter. In case of hydrophobic sample, place 1-2 drops of water on sample. Place 3-4 drops of water on AFM probe, and place AFM head on stand above sample.
5. Turn on laser with checkbox and verify that ‘Cantilever Ready’ green message shows up. Check laser spot location in ‘Other’ tab, it should be visible and close to center. Make sure camera is active. Click ‘auto land’ to begin landing. Meanwhile write down the microsphere confirmation, new sample loaded, and conditions from temp/hum sensor. Turn off temp/hum sensor.
6. When landed record time and take snapshot in experiment’s directory. (sample z height is already recorded in sample list, no need to record again unless there is large deviation over 0.1 mm) If landed on scratch or contamination area, lift up and move to better area (take another snapshot if desired). Move probe off sample slightly and if non-contact force reading abs(FN)>0.003 click ‘Null FN’ three times. Then auto-land again.
7. Click approach button once, to get probe height in level of -500 to -1500 nm or so. Begin with an offset of -500 nm on the displayed probe height, and set this as the lowest point in the FD curve (FD curve mode is distance, not offset). Total distance covered should be 500 nm so highest point in FD curve is offset by -1000 nm from displayed probe height. FD curve settings are 1000 nm/sec, 2000 samples, 1 sec wait time. Begin collecting data, adjusting minimum FD height to maintain a maximum applied force (max downward contact force minus leftmost non-contact force on y-axis) in the region between 0.0100 V and 0.0200 V (scale shows mV which is incorrect, readings are in V).
8. Once four curves are collected which are consistent in approach and retract non-contact force (no divergent noise) and have applied force in the above range, stop FD curve collection, save all curves as a numeric formatted filename, and record filename as well as any comments about the curves. If four curves cannot be easily collected, try another spot and record this in the comments of the experiment log.
9. Raise probe by clicking retract 4-5 times, then move the sample in 100um increments using the motor control dialog. A single 100um increment is to be used when possible, exceptions to be made when avoiding contaminants or cracks and when finding special areas (such as in fluorite coated sample which is not 100% covered). Ensure that FN is still within 0.003 V of zero, if not then click ‘Null FN’ three times. Auto-land on new position and continue from step 7 until 8 total FD files are saved (corresponding to 8 physical points and 8\*4=32 virtual points).
10. Retract to the height of next sample to be loaded + 1 mm. Then turn off AFM laser, flip AFM head over, and use paper wipe to dry AFM head and sample. Continue to step 4 to verify microsphere presence and load new sample, until all samples are done. SiC is to be tested as first and last sample, both because of its hardness (ability to verify sensitivity and adhesion consistency) and its large height requiring the AFM to be left in state ready for large samples.
11. If finishing experiment, turn off AFM laser and cover samples. If switching probe, continue to step 1.

## Analysis Procedure

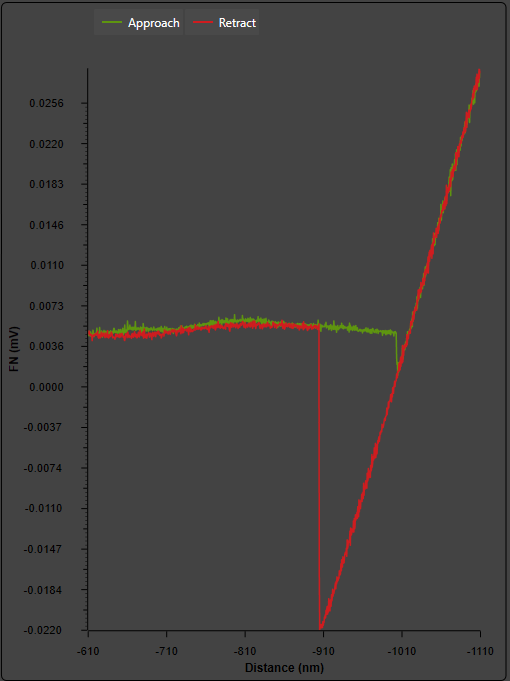
1. In matlab script main.m under automation folder, set probe name and sample name, and numbers corresponding to FD files saved above. Run script, which will copy data into clipboard. The clipboard data can be then pasted into excel spreadsheet.
2. In spreadsheet containing results of all experiments (it will contain both force in V and sensitivity in mV/nm), add measure of probe spring constant from package to calculate force in (N) units.

# Automation Approach

The matlab script is designed to do mostly the same thing that I would do visually with each plot.

The function ImportFD.m is a simple import method that opens same plots that would have been visible in ezAFM software, except it has a filter to only open plots that contain both 1000 approach points and 1000 retract points. The function saves an approach and retract curve at each z-height, meaning that either approach or retract curve may be off by 500 nm / 1000 pts = 0.5 nm from an ‘absolute’ value, which is deemed acceptable here.

The function AnalyzeFD.m does analysis much as I would do visually. First it determines a non-contact force by averaging the first 150 points (75 nm) of both the approach and retract curves. These two force values are compared in main.m to ensure no divergent noise occurs. The function also calculates standard deviation of the 150 non-contact force values to serve as a reference noise level or minimum resolvable force. Second it determines a loss of contact point at which the approach and retract curves transition from a linear slope (in the engaged region) to a dip as the sphere is pulled farther from the surface. From this loss of contact point to the end of the curve, it determines a slope value which is the probe sensitivity – now the sensitivity can be obtained from each FD curve individually rather than just from the SiC samples as before. Third, it determines maximum applied force (for controlling applied force in main.m), and minimum force (for calculating adhesion force in main.m), and also integral and sum-of-squares values for the curves (which may be useful in main.m as lower-noise alternatives to adhesive force). The physical relevance of these calculations is shown on the figure below.



Non-contact force average

Non-contact force stdev (noise level)

For approach and retract

Min force

For approach and retract

Max force

For approach and retract

Contact slope (sensitivity)

For approach and retract

Work of adhesion integral

Delta2 integral

## Noise Immune Parameters

In addition to the previously used parameter of adhesion force (the difference between non-contact and minimum retract curve force), I designed two parameters with the goal of having the parameters be immune to the observed noise – including LF and HF noise – in a way that the adhesion force is not. Both involve an integral over the entire curve rather than point measurements.

### Work of Adhesion

The work of adhesion is a physically relevant parameter, which integrates the adhesive force over distance, (units of N\*m). In the case of the FD curve, force F=(retract(V)-approach(V))\*(sensitivity (m/V)\*spring constant (N/m)), resulting in a force in Newtons. The last two factors are constants of the probe and setup, and can be removed from inside the integral. Thus which can be calculated numerically from the saved plot data. The numerical implementation is of the sum-of-rectangle-areas form:

Carrying out the integral in this fashion is expected to make the measurement immune to LF and HF noise, since LF noise will affect both approach and return voltage and thus result in zero contribution, while HF noise contribute randomly to both approach and retract curve, resulting in a zero-sum contribution.

### Delta-Squared

A similar integral to the above is designed to have a preference towards large difference in the approach and retract curves, by measuring squared difference so that larger differences have bigger contributions.

The numerical implementation is of the sum-of-rectangle-areas form:

The above formula ensures that dividing into finer z increments results in the same answer.

The reported result is the sqrt-squared-delta,

The integral formulation of this parameter is . The units of delta-squared are then (V2\*m), and units of delta are (V\*√m).

This parameter should be immune to LF noise since it will affect both the approach and return curves. HF noise may be problematic since it will contribute to the integral due to the squaring action changing the initial zero-sum difference. With very low adhesive force the HF noise will dominate, making this parameter questionable. I keep it out of curiosity.

### Theoretical Effect of Sample Motion

The above ‘noise immune parameters’ will not be immune to noise from the sample moving upwards during measurement. That is one noise source which adhesion force is immune to. In case of significant sample movement, the slope of the approach and retract engaged curves will be noticeably different. Then, the integral will have a negative contribution from the difference between these sloped linear sections, but a positive contribution from the longer dip section.

## Performance of Parameters

The performance of the new and old parameters is evaluated with the automated script. The following are considered figures of merit:

* Mean value over all valid plots divided by standard deviation over all valid plots (inverse spread) – a high value of this parameter means the underlying dataset has low ‘dispersion’ about the mean. This only applies to positive number experimental values, since standard deviation is taken as a fraction of the mean.
  + “Valid plots” refers to all plots within an experiment with same probe and substrate that have passed above criteria (noise and applied force) to be considered in analysis.
  + This is evaluated for adhesive force, work of adhesion, and delta-squared
  + The best performing parameter by this metric displays a consistent measurement throughout the sample and low changes between points – it exhibits less geometric variation than the worse performing parameters. Perhaps this means the parameter is better at picking out data from noise, if one assumes the underlying measurement is actually consistent. But if noise contribution is significant then this metric will be flawed as the parameter least sensitive to underlying data will appear more stable.
* RMS standard deviation at each individual point divided by standard deviation over all valid plots – a high value of this parameter means that expected variation in a point measurement is same as over multiple points. This may be particularly applicable to sample characterization if different probes consistently have similar values.
  + The best performing parameter by this metric performs similarly when measuring a single point or measuring multiple points on the sample. Then either the parameter or the sample (or both) are particularly uniform.
  + On the other hand, a high value of this metric might mean that the parameter performs as poorly at a single point as on the rest of the sample, or is even entirely independent of the sample. Thus caution should be used as with the first metric.

In terms of the first metric (mean value/stdev) the sqrt(delta^2) parameter has generally best performance, followed by min force, followed by work of adhesion – surprising!

For second metric, all samples exhibit single point measurement spread that is about 10% of the many-points spread – this should be quantified, but is very likely due to actual geometric variation since forces are not well controlled for either single or many points. The laser is always powered on for a single sample so the only differences between single and multi-point are raising/lowering head, moving sample in XY, potentially zeroing FN, and starting new FD curve request, none of which seem to be reasonable for affecting the FD curve. In addition to actual stickiness variations as function of sample position, there are also variations in sample reflectivity and presence of bubbles/scratches that may reflect the laser at odd angles, resulting in increased noise or perhaps a bias of force measurement lower or higher also as a function of position.

# Processed Data

With processed data it was seen that the adhesive force outperformed both work of adhesion and delta-squared parameters in terms of both metrics defined above. This was a bit surprising as I expected the adhesive force to be most susceptible to noise. Ultimately it was decided that the adhesive force will continue to be used as the measure of interest, with a slight modification. It was observed that shifts in laser power will lead to: a shift in probe offset required to achieve a consistent applied force, a shift in non-contact FN read out in software, and a shift in sensitivity (larger/smaller recorded V values for same cantilever movement). Luckily, checking AFM z-axis calibration by scanning over a calibration sample (with 113 nm step height) showed that the z-axis is accurately calibrated assuming the correct calibration settings are loaded on experiment start-up (which should be evidenced by a correct stage coarse z-height). So, we can account for the shifting sensitivity’s effect on actual adhesive force by processing each plot and multiplying the adhesive force (V) by the inverse slope (nm/V) during contact, to get the adhesive motion (nm). The automation algorithm was adjusted to calculate a linear fit to the points in the contact section for an accurate slope determination (previously it was based on just two points at beginning and automatically selected ‘end’ of contact region). For each plot, the adhesive motion is calculated, and these are then averaged over multiple plots and files. The underlying deflection (V) is not recorded as it is seen to always be relative to the sensitivity (nm/V). Conversion from the adhesive motion to actual adhesive force is easy: multiply the adhesive motion (nm) by the cantilever spring constant or stiffness (N/m) to get the adhesive force (N or nN). This data is recorded as Sensitivity-Adjusted Matrix 1 vs 2 excel file.

## Reproducibility with Matrix 1 data

Is it ok?

# Continuation

What to do next, keep same procedures?