Fluid Imaging Tips & Trick

Suggestions for Successful Fluid AFM Sample Prep and Imaging

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Sample Preparation

General Guidelines

The following suggestions are distilled from numerous discussions with Bruker applications and development engineers regarding their experiences attempting to acquire high resolution fluid images, in particular trying to resolve the major and minor groove of the DNA double helix. More information can be found in *Bruker Application Note #142*, "Imaging of the DNA Double Helix with PeakForceTapping Mode Atomic Force Microscopy" and the literature references cited therein, including:

Pyne, et al. *Small* **10:** 3257-61 (2014).

Ido, et al. *ACS Nano* **7:** 1817-22 (2013).

Leung, et al. *Nano Lett* **12:** 3846-50 (2012).

Lyubchenko *Micron* **42:** 196-206 (2011).

AFM Probe and Probe Holder Cleaning

- AFM Probes
  - Soak in 50:50 IPA/EtOH mixture for 2-3 days before using for imaging
- FastScan Z scanner
  - Using tweezers, carefully remove visible silicon debris from the probe channel and laser window, taking care not to scratch the window's AR coating
  - After mounting in the polypropylene Z scanner cleaning cell and ensuring a leak-tight seal against the O-rings, use a soft toothbrush to gently scrub the scanner using soap (lemon Joy liquid dishwashing detergent is recommended) and water
  - Rinse successively with tap water, DI water, and ultrapure water
  - Dry with a gentle stream of UHP N_2
  - May need to repeat this process 2-3 times
- MultiMode Fluid Cell/Probe Holder
  - Sonicate gently (preferably in ultrapure water)
  - Clean with soap (lemon Joy liquid dishwashing detergent is recommended) and water using a soft toothbrush
  - Rinse successively with tap water, DI water, and ultrapure water
  - Soak in a 50:50 IPA/EtOH mixture for ~2 hours (or store in solution, taking care to prevent it from completely evaporating and leaving solvent residue)

- Dry with a gentle stream of UHP N_2
- May need to repeat this process 2-3 times

Sample Puck and Substrate

- Use V1 (highest quality) mica
- Use epoxy (5 minute epoxy is fine), not superglue (cyanoacrylates), for mounting as the latter can lead to contamination due to leaching into solution
- Consider mounting a hydrophobic PTFE (Teflon) ring between the sample puck and the mica to cause the fluid droplet to bead up and stay on the mica surface, thereby minimizing wicking of contaminants from the steel puck onto the sample surface

Buffers and DNA Solutions

- Ensure all glassware or other containers used are rigorously clean and free of dust
- Filter all buffer solutions through a 0.2 µm (200 nm) syringe filter immediately prior to use
- A commonly used alternative to TAE and TBE is HEPES; TA and TB (no EDTA) are also options
- Typical sample volumes are 5 µL of DNA solution mixed with 20-80 µL of 0.5-1x buffer spiked with 1-50 mM $MgCl_2$ (weaker DNA binding) or $NiCl_2$ (stronger DNA binding)
    - Mixing of solutions can be encouraged/sped up by aspirating with a micropipette (shaking or vortexing can damage the DNA origami)
    - The buffer/salt solution can be added first and allowed to incubate (1-3 minutes) to promote creation of a uniform positively charged surface prior to addition of the DNA solution
    - 1-15 nM DNA and 5-15 mM divalent cation concentrations are most common
    - DNA concentration on the mica surface will usually increase with incubation time
    - To prevent evaporation during incubation, samples can be placed in a covered Petri dish, and buffer solution (or a buffer-soaked tissue) can be placed in the dish to create a saturated atmosphere
    - Rinsing of the sample by adding 25-75 µL aliquots of buffer followed by aspiration (5-10x) and subsequent removal of the aliquot volume can be used to remove loosely bound or unbound DNA
- Tighter binding of the DNA (i.e., using higher salt concentrations and/or Ni^{2+} versus Mg^{2+}) will result in less movement and more bound DNA, but may distort the DNA structure and/or lead to undesirable multilayers of DNA or salt crystal aggregates on the surface
- A volume of 25-50 µL between the probe and the probe holder is usually sufficient to fully wet the laser window

DNA Origami Sharp Triangles

DNA origami sharp triangles can serve as a good test sample for AFM training or evaluating a new imaging mode or probe. Following is a suggested sample preparation method that should yield a mica surface covered with a monolayer close packed array of sharp triangles. It is based in large part on the following literature reference from Paul Rothemund's group at CalTech: Gopinath and Rothemund *ACS Nano* **8 (12):** 12030-12040 (2014).

- Combine a 5 µL aliquot of 1-15 nM DNA in 1x TAE solution with a 20 µL aliquot of 1x TAE spiked with 10-15 mM $MgCl_2$ in an ultracentrifuge tube to yield a final DNA concentration of ~0.2-3 nM.
    - This should provide sufficient mixing of the two solutions without resorting to pipette mixing, shaking, or vortexing, which could damage the DNA origami structures.
    - $MgCl_2$ is preferable to $NiCl_2$ in this case because Ni^{2+} binds the DNA origami more tightly to the mica surface and tends to cause stacking (multilayers) of the structures. $MgCl_2$ concentrations of up to ~50 mM can probably be used.
- Deposit the pre-mixed DNA/TAE/$MgCl_2$ solution on a freshly cleaved mica substrate and let incubate for 2-5 minutes.
    - For high resolution fluid imaging, high quality V1 mica is best.
    - To minimize contamination, all fluid samples should use epoxy rather than superglue (cyanoacrylates) to adhere the mica to the sample puck.
    - It is often advisable to glue a circle of PTFE (Teflon) between the metal puck and the mica to serve as a barrier to fluid overflow.
    - If the 2-5 minute incubation period appears to be insufficient, you can use longer incubation times. To prevent evaporation, the sample can be placed in a Petri dish supplied with a tissue soaked in buffer solution and sealed with Saran wrap.
- After completion of the incubation period, add a 60 µL aliquot of the 1x TAE spiked with 10-15 mM $MgCl_2$ solution to the sample. Pipette mix 3-5 times before removing and disposing of this additional 60 µL of solution. Repeat 8 times.
    - This rinsing procedure is intended to clean the sample by removing any loosely bound or unbound DNA floating in solution to prevent it from interfering with imaging.
- Add a final 60 µL aliquot of the 1x TAE spiked with 10-15 mM $MgCl_2$ solution to the sample (for a total sample volume of 85 µL).
- Carefully pipette a 40 µL aliquot of the 1x TAE spiked with 10-15 mM $MgCl_2$ solution between the AFM probe and the probe holder so that it completely covers the beam bounce laser's optical path.
- Merge the sample and probe fluid droplets, engage the surface, and image. The final DNA concentration should now be ≤ 0.04-0.6 nM (i.e., 40-600 pM).

Imaging

- AFM Probes
    - Because the side angles of AFM probes are generally symmetric, but the front and back angles often aren't, it can be good to scan at 90° to obtain identical trace and retrace curves
    - Good options include FastScan B, C, and D for tapping mode on the FastScan, ScanAsyst-Fluid+ and ScanAsyst-Fluid-HiRes (still in beta testing) for PeakForce Tapping on the FastScan or MultiMode 8, and SNL (A, B, C, or D) for tapping mode on the MultiMode 8
    - Unless otherwise stated, nominal natural resonance frequencies (f_0 values) on probe boxes are in air; the corresponding value in fluid will be ~2-3x less (i.e., ½ to 1/3 the value in air)

- PeakForce Tapping Mode
    - Once you're scanning, decrease the engage setpoint to equal the PF Tapping Setpoint to minimize forces experienced by the probe (and hence the potential for dulling the tip) when changing parameters, carrying out an AutoConfig, etc.
    - Very low setpoints (10s-100s of pN) can be used, with ~70 pN ideal for imaging the major and minor grooves of the DNA double helix
- Tapping Mode
    - The precise frequency chosen relative to the natural resonance frequency (as determined from a thermal tune) is less important in fluid than in air
    - Image quality is more sensitive to tapping amplitude in fluid, so it is important to choose a frequency near the natural resonance frequency where the probe is especially responsive
        - Choose Manual Tune, then carry out a Fast Thermal Tune
        - Select a Drive Amplitude that delivers a tapping amplitude of ~1-3 nm at a frequency near the peak of the Thermal Tune where the cantilever has a strong response
            - For optimal stability, it's best to avoid sharp peaks and instead choose shallow valleys (good cantilever response) between peaks near the Thermal Tune maximum
            - Typical Drive Amplitudes are 1-2 V for a 1-2 nm tapping amplitude
    - Decrease the Engage Step Size from its default of ~1 µm to ~500 nm
    - Cary out a surface tune at a lift height/offset of ~50 nm after engaging the surface
    - Typical I and P Gain values are 4 and 8, respectively