Title: Make your own DNA or microtubule AFM samples
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Abstract:
Atomic force microscopes (AFMs) are commonplace in research laboratories and have recently been priced for educational use. We have designed several biophysical experiments for these educational AFMs that could be completed in a 3-hour undergraduate laboratory. Many of these experiments require students to image either microtubules or DNA and determine their polymer properties or the stretching force on the molecules. In this workshop, we will show you how to make DNA samples and how to perform the biophysical experiments.

Related Materials: L. M. Devenica, C. Contee, R. Cabrejo, M. Kurek, E. D. Deveney, and A. R. Carter, "Biophysical measurements of cells, microtubules, and DNA with an atomic force microscope." (under review at *AJP*).

DNA sample preparation
To prepare DNA samples, we first used double-sided scotch tape to adhere a mica coverslip (Ted-Pella, 10-mm-diameter) to a metal specimen disk (Nanomagnetics, 28-mm-diameter). The metal disk allows the sample to be magnetically attached to a sample holder. We then pressed a piece of single-sided scotch tape to the mica and quickly removed the tape, leaving an atomically flat, clean layer. This procedure was repeated if the tape did not remove an even layer. Next, we diluted double-stranded DNA from bacteriophage λ (New England Biolabs, 48,502 base pairs or 16.4 μm, 500 μg/mL) to 1 μg/mL in a solution of 1 mM magnesium acetate (Sigma). (All salt solutions and buffers are reagent grade and are filtered with a 0.2 μm filter.) We then immediately pipetted 20 μL of the DNA-magnesium acetate solution onto the mica coverslip and waited 5 min before rinsing with 500 μL of filtered deionized water. The positively charged magnesium coats the negatively charged mica surface and is thought to trap the negatively charged DNA in a salt layer. If the surface is not rinsed properly before drying, the salt layer can build up, obscuring the DNA adhered to the surface. During rinsing, excess water can be dripped into the sink or can be absorbed with filter paper, before air drying. To prepare λ phage DNA samples in the presence of a flow, we pipetted 500 μL of the DNA-magnesium acetate solution onto the mica cover slip while holding the sample at an angle. We then waited 5 min and rinsed with deionized water as before. The flow creates a force on the DNA as it adheres to the surface, stretching the DNA along the flow direction. Total prep time is ~10 minutes and samples can be kept at room temperature for months. To make new samples, just remove the top few layers of mica with single-sided scotch tape and begin again.

Microtubule sample preparation

To prepare samples with microtubules, we first prepared the mica coverslips as before. Then, we added 5 μL of 1 mg/mL poly-lysine (0.1%, molecular weight >300,000 u, Sigma) to the mica by spreading it on the surface with a rectangular glass cover slip before allowing the surface to air dry. The poly-lysine is positively charged and serves the same purpose as the magnesium acetate. However, the poly-lysine layer creates a surface roughness of about 3-5 nm peak-to-peak. Next, we diluted a stock of 5 mg/mL microtubules to 0.1 mg/mL in PEM buffer (made with 100 mM PIPES, 2 mM EGTA, and 1 mM $MgSO_4$ from Sigma) and either 1% or 4% glutaraldehyde (Sigma) under a fume hood. Finally, we immediately added 10 μL of the solution to the poly-lysine coated mica and let sit for 5 min before rinsing with 1 mL of filtered deionized water and air drying. Taxol-stabilized microtubules were given to us by colleagues, but microtubule kits are available for purchase (Cytoskeleton, Inc). The glutaraldehyde and poly-lysine should be kept on ice during the procedure, while the microtubules should be kept at room temperature. Prep time is 15-20 minutes and samples will remain intact for several months or longer at room temperature.