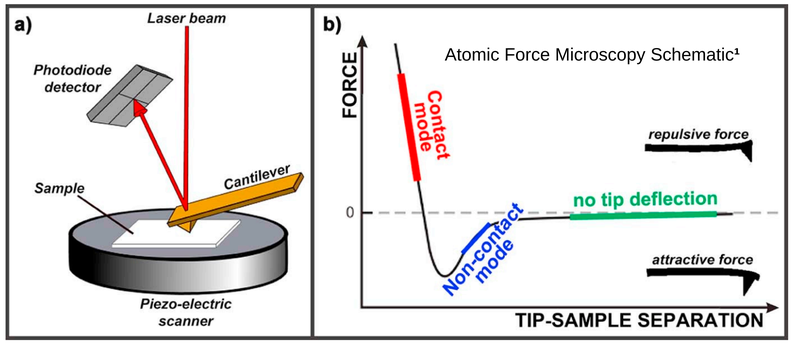
Advanced Atomic Force Miroscopy for BioMaterials Research

Jesse Schimpf,[1] Michael Abend,[2] Conner Patricelli,[3] Paul H. Davis.[1*]
Gunes Uzer,[2] Daniel Fologea,[3] and Elton Graugnard[1]
[1]Micron School of Materials Science & Engineering, [2]Department of Mechanical & Biomedical Engineering,
[3]Department of Physics, *Mentor

BOISE STATE UNIVERSITY
College of Engineering
Materials Science and Engineering

Atomic Force Microscopy (AFM)

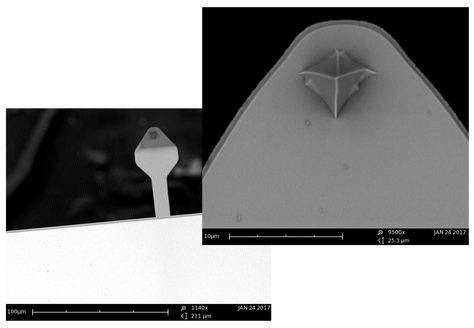
AFM is a surface characterization technique that can generate high resolution maps of sample topography and surface properties such as adhesion or modulus at very small length scales (~1 nm – 100 µm). To achieve this, a sharp probe is brought in contact (or near-contact) with a sample and rastered across the surface.



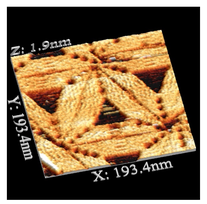
To tracks changes in the probe deflection as it encounters changes in topography or tip-sample interaction strength, a laser reflects off the back of the probe to a 4 quadrant photodetector. This data is then used to create a topographical image of the surface or force-distance curves.

Bio AFM

- Sample Types
  - DNA
  - Proteins
  - Lipid bilayers
  - Live or stained cells
- Physiologically relevant
  - Fluid environment
    - Buffer (pH control)
    - Salt concentration (osmotic pressure/ionic strength)
  - Temperature control
- Applications
  - High resolution topography
  - Video rate imaging = dynamics/kinetics
  - Nanomechanics



Above: SEM micrograph of a Bruker ScanAsyst-Air-HR probe with an ~2 nm radius of curvature.

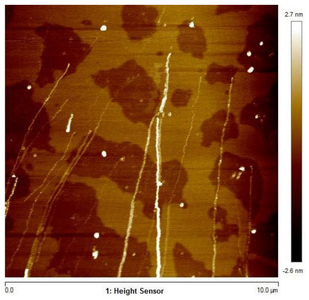


Left: High resolution fluid AFM image of DNA origami sharp triangles.[2]

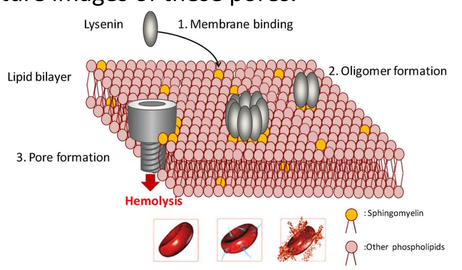
High Resolution Fluid Imaging

Proteins and Lipid Bilayers

Cytoxic bacteria such as anthrax and cholera produce proteins that insert into the phospholipid bilayers of cell membranes, forming tiny pores that allow essential ions to leak out. These pores are a few nanometers in diameter, making their characterization extremely difficult. However, AFM probes can be made sharp enough to discover and capture images of these pores.



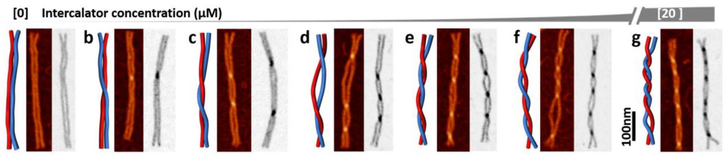
AFM image of 500 pm tall lipid rafts on mica.



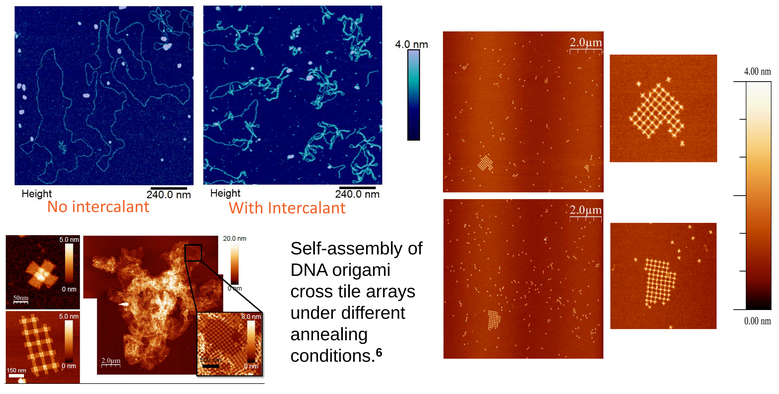
Pore forming protein insertion into phospholipid bilayer.[3]

DNA

High resolution fluid AFM allows effective characterization of individual DNA strands and DNA origami structures.



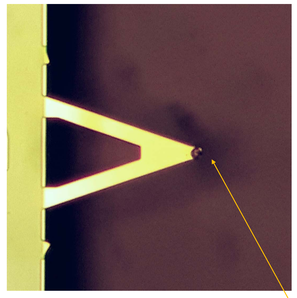
Effect of ethidium bromide (EtBr), an intercalating agent, on the tertiary structure of DNA origami nanotube pairs (above) and circular DNA (below left).[4-5]



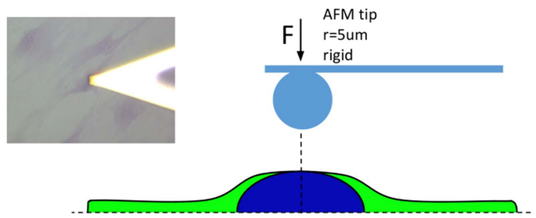
Self-assembly of DNA origami cross tile arrays under different annealing conditions.[6]

Nanomechanics

When probe cantilever properties such as the spring constant and deflection sensitivity are known, changes in laser deflection can be translated into precise force measurements when pushing on a sample. This can in turn be used to measure various mechanical properties of the sample, such as its elastic modulus.

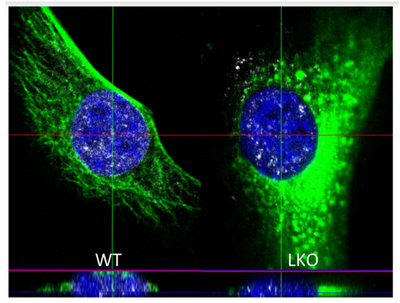


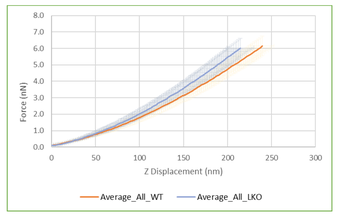
AFM probe with 10 µm glass bead.

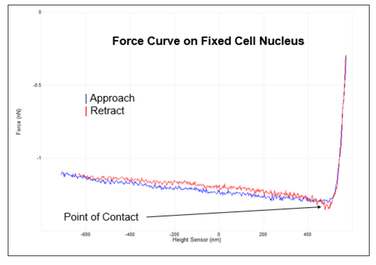


Tissue Culture Plastic, E=100MPa

Traditional AFM probes are sharp, but cells/nuclei are too soft to be measured this way. Instead, a 10 µm diameter glass bead is attached to the cantilever, providing relevant force measurements without puncturing the cell membrane.



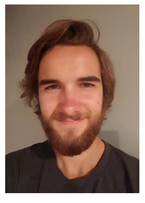




Above: Force response data from wild type (WT) versus lamin knock out (LKO) fixed mesenchymal stem cells (MSCs) showing differences in cell nucleus stiffness.[7]

Left: Typical force distance curve obtained from the nucleus of a fixed MSC.

Author:
Jesse Schimpf
Materials Science & Engineering
Undergraduate Research Assistant
Surface Science Laboratory,
Micron School of Materials Science & Engineering
jesseschimpf@u.boisestate.edu



Co-Authors
Michael Abend, michaelabend@u.boisestate.edu
Conner Patricelli, connerpatricelli@u.boisestate.edu
Paul H. Davis (mentor), pauldavis2@boisestate.edu
Gunes Uzer, gunesuzer@boisestate.edu
Elton Graugnard, eltongraugnard@boisestate.edu
Daniel Fologea, danielfologea@boisestate.edu

References & Acknowledgements

1. U. Maver, T. Velnar, M. Gaberšček, O. Planinšek, and M. Finšgar, *TrAC, Trends Anal. Chem.* **80**: 96-111 (2016).
2. Image courtesy of Paul Davis and Brett Ward.
3. N. Sukamwang and K. Umezawa, *Toxins* **5** (2013).
4. R. M. Zadegan, E. G. Lindau, W. P. Klein, C. Green, E. Graugnard, B. Yurke, W. Kuang, & W. L. Hughes *Sci. Rep.* **accepted** (2017).
5. E. Krueger, J. Shim, A. Fathizadeh, A. Chang, B. Subei, K. Yocham, P. Davis, E. Graugnard, F. Khalili-Araghi, R. Bashir, D. Estrada, D. Fologea *ACS Nano* **10**: 8910-8917 (2016).
6. C. M. Green, K. Schutt, N. Morris, R. Zadegan, W. L. Hughes, W. Kuang, & E. Graugnard *Nanoscale* (2017).
7. Data and images courtesy of Jesse Schimpf, Michael Abend, and Gunes Uzer.