Atomic Force Microscopy (AFM) for Nanomedical Systems (cells and nanoparticles)

Helen A. McNally, PhD
Assistant Professor
Electrical and Computer Engineering Technology
Birck Nanotechnology Center and Bindley Bioscience Center
Purdue University
20 September 2007
Overview

Introduction to Scanning Probe Microscope and Atomic Force Microscopy

Cells and Nanoparticles Applications

Bindley Biological Atomic Force Microscopy Laboratory
Scanning Probe Microscopy (SPM)

• Scanning Tunneling Microscopy – Rohrer and Binnig 1982

• Atomic Force Microscopy (AFM/SFM) – Binnig et al 1986

Resolution:
Optical – 200nm
AFM  – atomic resolution possible
  – tip dimension, detection system,
    operating conditions & controls

Measurement Capabilities:
  Topography and
  Material Characteristics

Operating Conditions:
  Vacuum, air (gas), liquid

Principle of Operation
Atomic Force Microscope, G. Binnig, C.F. Quate, and C. Gerber,
Other SPM Techniques:

STM – Scanning Tunneling Microscopy

LFM – Lateral Force Microscopy

EFM – Electric Force Microscopy

MFM – Magnetic Force Microscopy

SCM – Scanning Capacitance Microscopy

FMM – Force Modulation Microscopy

SNOM – Scanning Near Field Optical Microscopy
Atomic Forces Involved

Attractive and Repulsive Forces

- Pauli exclusion principle – no two electrons in an atom can be at the same time in the same state or configuration
- van der Waals Force – dipoles of individual particles
- Electrostatic or Coulombic Forces – ionic bonds
- Capillary and Adhesive Forces – liquid meniscus and tip contamination
- Double Layer Forces – ionic atmosphere around a charged substrate in fluid
Equations of Interest

Hookes’ Law: \[ F = -kd \]
- \( F \) is the force applied to the sample
- \( k \) is the cantilever spring constant
- \( d \) is the tip displacement

Resonant Frequency: \[(2\pi f)^2 = \frac{k}{m}\]
- \( f \) is resonant frequency of cantilever
- \( k \) is the cantilever spring constant
- \( m \) is the mass on the cantilever
AFM System Configuration

AFM modes: contact, non-contact and tapping
AFM Head – the guts of the system
DNP Silicon Nitride Probes

- spring constants: 0.58, 0.32, 0.12, 0.06 N/m
- tip radius of curvature: 20-60nm
- cantilever length: 100 & 200μm
- reflective coating: gold
- shape of tip: square pyramidal
- tip half angle: 35°
AFM Image Acquisition and Analysis

Original image
- 40X40μm (variable)
- scale bar (variable)
- image parameters –
  - P&I gains
  - scan rate
  - set point
  - # samples/line
  - scan angle

Section analysis
  height and width measurements of interesting features
Image Types

- Height mode provides information on feature size. It gives detail of changes in height but does not provide actual numbers.

- Amplitude mode provides detailed changes in height but lacks actual numbers.
DNA intercalated with ethidium homodimer on mica entitled "NanoMan and Best Friend“
55nm scan, courtesy of Elizabeth D. Gadsby, Mark A. Poggi and Lawrence A. Bottomey,
Georgia Institute of Technology, College of Chemistry and Biochemistry, Atlanta, GA.

10nm colloidal gold particles co-adsorbed with Tobacco Mosaic Virus. 2µm scan.

Stefan W. Schneider; Kumudesh C. Sritharan; John P. Geibel; Hans Oberleithner; Bhanu P. Jena
Proceedings of the National Academy of Sciences of the United States of America,


Field of view 8.3 µm (left) and 4.5 µm (right)
AFM image of bacteria on a filter membrane.
This particular image demonstrates how AFM imaging can be used for quality assurance testing.
Field of view Mosaic of 10 Images taken each at 100µm x 100µm
Liquid AFM image of fibroblast-like cultured cells chemically fixed with glutaraldehyde on a glass cover slip. From this image one can see the cell-to-cell contacts, cell division, and the formation of stress fibers.
Image Courtesy of M. Drechler, LS Pharm Tech - FSU Jena, Germany

Living endothelial cells grown directly on a petri dish and imaged by AFM on a Digital Instruments BioScopeTM using contact mode in liquid. The image shows the interaction between multiple cells and between the cells and the substrate. Scan time was 35 min and scan size = 65µm.
Imaged by I. Revenko, M.D., Applications Scientist, Digital Instruments.
Sample courtesy of Georges Primbs, Miravant Inc.

Contact mode image of human red blood cells 15µm scan courtesy M. Miles and J. Ashmore, University of Bristol, U.K.
Preliminary Results: MCF-7 Breast Cancer Cells

50um scan of a single MCF-7 breast cancer cell (height image)
AFM Compared to Confocal Microscopy

H. McNally, B. Rajwa, and J. P. Robinson, accepted for publication in the Journal of Neuroscience Methods, April 2003
AFM Force Measurements

Pulling

Pushing
Title: The Beginning
Media: Xenon on Nickel (110)

Cell Death by AFM Probe

Change in Volume with Time

- Cell body
- Cytoplasm
- Total volume

Volume (μm³)

- 0
- 20
- 40
- 60
- 80
- 100
- 120
- 140
- 160
- 180
- 200

Time

- 2 min
- 5 min
- 5 min
Nanoparticles

Quantum Dots

Immunofluorescent images of human cancer cells labeled with green fluorescent dye.
Shuming Nie, Emory University

Functionalized Particles

DNA on particle and substrate w/ biotin-avidin link

Magnetic Particles

Systemic use of nanoparticles measures blood flow

Silicon Substrate

ds DNA
biotin
avidin
Devices

Head
Lungs
Heart
Lower Body

Magnetic sensor
Preliminary Results: Nanoparticle Imaging

Amino-Functionalized Quantum Dots (Invitrogen Qdot® 585 ITK™ amino (PEG) quantum dots)
3rd order flattened images (flattened using offline analysis software)
The Biological Atomic Force Microscopy (BioAFM) laboratory is a multiuser facility aimed at bringing the premiere tool of nanotechnology to the life sciences community.

- Veeco Bioscope II installed on an Olympus IX-71 inverted microscope with acoustic enclosure and vibration isolation
- 1st placed as a beta site in Nov 05, upgraded to a production instrument in Jan 07.
- located in Bindley Bioscience Center, room 122.
BioScope II - Overview

• SPM Performance
  – 10mmX10mm stage range
  – Three axis closed loop
  – >150μm X-Y scan range
  – >15μm Z scan range

• Complete Optical Integration
  – Olympus IX-71 Inverted Scope
  – IR deflection laser, 850nm
  – 0.55NA condenser
  – phase, DIC, brightfield
  – fluorescence, confocal, TIRF

• Biological Sample Compatibility
  – Coverslip
  – Microscope slide
  – 35mm petri dish
  – 60mm petri dish
  – 50mm glass petri
  – Coverslip on bottom of petri
<table>
<thead>
<tr>
<th>Project</th>
<th>College</th>
<th>Discipline</th>
<th>Faculty</th>
<th>Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular Membrane Structure</td>
<td>Science and Technology</td>
<td>Physics &amp; ECET</td>
<td>Ken Ritchie &amp; Helen McNally</td>
<td>Mirlea Mustata</td>
</tr>
<tr>
<td>Nanomedicine</td>
<td>Veterinary Medicine</td>
<td>Basic Medical Sciences &amp;BME</td>
<td>Jim Leary</td>
<td>Christy Cooper</td>
</tr>
<tr>
<td>Cellular Mechanics</td>
<td>Engineering and Technology</td>
<td>Mechanical Engineering &amp;ECET</td>
<td>Arvind Raman &amp; Helen McNally</td>
<td>Melanie Kemmerlin &amp; Matt Spletzer</td>
</tr>
<tr>
<td>Biofilms</td>
<td>Engineering</td>
<td>Civil Engineering</td>
<td>Kathy Banks</td>
<td>Zhen (Jen) Huang</td>
</tr>
<tr>
<td>Lilium Pollen Tubes</td>
<td>Agriculture</td>
<td>Agriculture and Biological Engineering</td>
<td>Marshall Porterfield</td>
<td>Mavash Zuberi</td>
</tr>
<tr>
<td>Dielectrophoretic Force Microscopy</td>
<td>Science</td>
<td>Chemistry</td>
<td>Garth Simpson</td>
<td>Kyle Jacobson</td>
</tr>
<tr>
<td>Plant Cuticles</td>
<td>Agriculture</td>
<td>Horticulture and Landscape Architecture</td>
<td>Matt Jenks &amp; Helen McNally</td>
<td>Dylan Kosma</td>
</tr>
<tr>
<td>Biofuels</td>
<td>Bindley</td>
<td>Bindley</td>
<td>Charles Buck</td>
<td>Elizabeth Ayres</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>Agriculture</td>
<td>Agriculture and Biological Engineering</td>
<td>Joseph Irudayaraj</td>
<td>Ali Shamsaie</td>
</tr>
</tbody>
</table>

**Current Projects**
References:


Questions