The Alchemist's Experiment takes Fire
Heindrick Heerschop

Imaging in Liquids with AFM

W Travis Johnson, PhD
Agilent Technologies
Why Image In Liquids?

Native Environment for Biological Molecules

Mimic Physiological Environment
• Aqueous Conditions
• Physiological pH
• Maintain Correct Ionic Concentration
• Keep Cells Alive in Growth Media
• etc

Maintain Std Conformations/Properties
• Nucleic Acids
• Proteins
• Cell Membranes
• Molecular Complexes
• Other Biological Structures

Liquid Keeps Cell Physio Processes Functioning Properly
AFM Basic Configuration
Agilent 5500 Liquid Cell

Unassembled liquid cell components

Liquid cell on sample plate

5500 AFM/ILM
10x Optical Image
Human Lung Cancer Cells
Contact Mode AFM Image
Living Human Lung Cancer Cell

Conditions
Grown for 4 days on glass cover slip
Contact mode
Room temperature
Imaged in PBS buffer (pH 7.4, 137 mM NaCl).
2 Main AFM Imaging Modes

Contact Mode AFM
- Dynamic in x and y.
- Tip is in contact or near contact with the surface.
- Small vertical force, but the probe dragged over the surface exerting lateral force.
- Weakly bound or soft samples move easily.
  ➔ Low lateral resolution.

AC Mode AFM
- Dynamic in x, y, and z.
- Intermittent contact.
- Impact is predominately vertical, therefore large vertical force, but small lateral force.
- Soft surfaces are stiffened by viscoelastic response
  ➔ Higher lateral resolution.
AC Mode Data Channels (typical)

- **Topography** – Image derived from voltage applied to Z piezo required to keep oscillation amplitude constant
- **Amplitude** – Image from “error signal” = the change in amplitude
- **Phase** – Image derived from phase lag of cantilever response with respect to drive signal

Image courtesy of Nanotechnology Center of Tsinghua University

Image of inner surface of blood vessel in buffer
How AC Imaging Works

**Drive Signal**

- Before contact

**Detected Signal**

- Contact with hard material dampens amplitude but has little effect on phase

- Contact with soft material effects phase and amplitude

  - Phase lag related to material stiffness

Topography image of block copolymer

Phase image of SEBS block copolymer
AFM Probe-Surface Interactions 1

Electrostatic Interactions (aka ionic or charge-charge)

Long Range Attractive or Repulsive

For Example:
- Silicon AFM Probes: SiO\textsuperscript{-}
- Silicon Nitride AFM Probes: SiO\textsuperscript{-} and NH\textsubscript{3}\textsuperscript{+}
- Mica: SiO\textsuperscript{-}
- Samples: variety of charges (eg, proteins + & -)

Short Range VDW Interactions Between Uncharged Molecules

- Attractive: Induced Dipole
  - Induced & Fluctuating Dipoles; aka Dispersion or London Forces
- Attractive: Dipole-Dipole (permanent dipoles)
- Repulsion: Approaching VDW radius

Coulombic Forces

- VERY VERY short range
- Repulsion
- The electron shells of the tip and the sample are in “contact”
- Applying more force can cause the tip and/or the sample to be deformed.
**VDW Interactions: AFM Probe ↔ Sample**

- **Contact**
  - Cantilever Deflection in Force (N) or Voltage (V)

- **Non-contact**
  - VDW radius

**VDW Repulsion**

**Dipole-Dipole Attractions**

Adhesion greatly exaggerated
AFM Probe-Surface Interactions 2

Hydrophobic Interactions
- Short Range Nonpolar interactions
- Decay exponentially with distance
- Not really “bonds”

Fluid Surface Tension (STRONG ~ 1-10nN)
- Meniscus forces
- Capillary forces
- “Squeezing” water between tip and sample
- Occur only in ambient conditions (e.g., in air)

Fluid Film Dampening
- AC Mode in Air
- Oscillating probe “squeezes” air between the probe and the surface
- Alternating increasing & decreasing air pressure “dampens” oscillation

Atoms Near End of Probe vs Atoms Further Away
- Complex Mixing of Attraction - Repulsion

Can Modify Ionic, VDW, Hydrophobic Properties by Modifying Chemistries on AFM Probe/Substrate
- For example, coat with hydrophobic or charged silanes
AC Mode: Resonance Frequency is Affected By Short Range Interactions (Attraction/Repulsion)

Attractive gradient is equivalent to additional spring tension on probe, which reduces resonance frequency of cantilever.

Repulsive gradient is equivalent to additional spring compression on probe, which increases resonance frequency of cantilever.
Additional Forces in Liquid 1

H Bonds (RO···H, RS···H, RN···H)

Ionic Interactions Change (salt bridges)

Hydrodynamic Forces in Fluid
- Viscous damping
  - Viscosity is influenced by probe geometry
    ➔ Increase in effective mass
    ➔ Res freq reduction & peak broadening
- Compression ➔ Repulsive force
  - Increase in viscosity in confined areas (viscoelasticity)

Liquid lowers ‘Q factor’
- Measure of AFM probe sensitivity & responsiveness
- Higher Q ➔ Higher sensitivity
- Q ~ 100 Air ➔ Q ~ 2-3 Liquid
- Lower Q ➔ More damage to soft samples
- Q further decreases as tip gets closer to surface
Oscillatory Forces in Liquid \( (F_{\text{liquid}}) \)

Probe Tip Size and Shape Influence \( F_{\text{liquid}} \)
- Smooth Probe Tips/Substrate Surfaces
  - Increase Order/Packing of Liquid Molecules
  - Increases \( F_{\text{liquid}} \)
- Rough Surfaces
  - Decrease order \( \Rightarrow \) asymmetry \( \Rightarrow \) \( < F_{\text{liquid}} \)
  - Variations in \( F_{\text{liquid}} \)
  - Artifacts

Liquid Effects Thermal Fluctuations of Lever
- Liquid Dampening Decreases Thermal Noise

Liquid Dampening and AC Mode:
- Liquid Lowers Resonant Frequency
- Broadens Resonant Peak
- New Osc Frequencies Arise in AAC Mode
AC AFM in Liquid

AAC Mode

- A solenoid applies an AC field to piezoelectric transducer (sonic wave)
- Transducer shakes the cantilever holder
- Reduction of amplitude signals contact

Liquid Lowers Res Freq
Causes Numerous Peaks
Peak Broadening

MAC Mode®

- Cantilever is coated with magnetic film
- Magnetic field specifically drives cantilever ONLY

Liquid also Lowers Res Freq
Cleaner Oscillation in Liquids
Single Broad Peak
Oscillatory Forces & Viscosity

Force curve showing increase in viscosity of confined liquid between tip and sample. As tip approaches surface, viscosity increases.

AFM tip surface roughness can disrupt tip-liquid-sample surface interface. Roughness introduces disorder, reduces packing and helps to minimize oscillatory forces.

Size: Smaller solvent molecules also increase averaging.

For high res imaging, force between tip-sample ($F_{image}$) should be small.

AFM Probe ↔ Sample Interactions vs Distance

Fluid Film Dampening (10 µm)

Electrostatic (Ionic) Interactions (100nm - 1µm)

Fluid Surface Tension (10nm - 200nm)

Attractive VDW Interactions (2Å – 100Å)

Repulsive VDW Interactions (<2Å)

Coulombic Forces down to 10^{-16} m or ~1/10 diameter of atomic nuclei

Hydrophobic Interactions (<Å – 10nm)

Hydrogen Bonds (~2Å)

Fluid Surface Tension (long range; capillary or meniscus forces)
Biological Samples Must be Immobilized Prior to Imaging in Liquid
Immobilizing Live Cells:
BCE Cells on Collagen Coated Glass
Immobilizing Charged Small Molecules

Silanol groups deprotonate in aqueous solutions to
immobilized on mica using divalent cations
immobilized on mica at neutral pH

Mica

lys residues on surface of protein

Positively charged proteins immobilized on mica at neutral pH

DNA/RNA (PO₄⁻)

Negatively charged molecules (DNA, RNA, viruses, etc)
immobilized on mica using divalent cations
(Mg++, Ni++, Co++, Zn++, Cu++)

Agilent Technologies
AFM Image of lambda phage DNA
attached to mica by Mg++/Ni++
imaged in aqueous buffer
Covalent Immobilization of Proteins on Mica-APTES-Gluteraldehyde Substrate
Flow through liquid cell
• Exchange buffers/reagents without removing substrate
• Permits *in situ* expts
• Add blocking agents to isolate SB from NSB
Chromatin Immobilized on Mica-APTES-Glutaraldehyde

AFM Image of Chromatin Covalently Immobilized on Mica-APTES-Glut

Why Image In Liquids?

Mimic Physiological Environments

Maintain Correct Conformations/Properties

Keep Cells Alive and Functioning Properly

But Also:

• Lower Thermal Noise
• Eliminate Meniscus/Capillary Forces
• Eliminate Fluid Film Dampening
• Promote Hydrogen Bonding
• Perform Studies in situ
Thank You!

Questions?

W Travis Johnson
w-travis_johnson@agilent.com