Atomic Force Microscopy
- Basics and Applications

Summer School June 2006
„Complex Materials: Cooperative Projects of the Natural, Engineering and Biosciences“
Outline

• Scanning Probe Microscopy

• Atomic Force Microscopy
  – General set-up & operation modes
  – Sample preparation

• Applications in life science
  – Imaging mode
  – Force-distance mode

• Conclusion
Scanning Probe Microscopy (SPM)

~1600 Light Microscope

1938: Transmission Electron Microscope

1964: Scanning Electron Microscope

1982: Scanning Tunneling Microscope

1984: Scanning Near-field Optical Microscope

1986: Atomic Force Microscope
- magnetic force, lateral force, chemical force...
Scanning Probe Microscopy

- Creates images of surfaces using a probe.
- Probe is moved (scanned) over the sample.
- Sample-probe interaction is monitored as function of location.

+ Image resolution limited by probe-sample interaction volume - not by diffraction.
+ Interaction can modify surface - nanolithography possible.
- Scanning technique quite slow.
- Limited maximum image size.
Atomic Force Microscopy

Molecular interaction:
\[ E = F \Delta s \]
\[ E \sim \text{eV}; \quad \Delta s \sim \text{Å} \]
\[ F \sim 2 \cdot 10^{-9} \text{N} \]

Typical AFM resolution:
- x-y: 1nm; z: 0.1nm

Detection:
- sub-Å deflection
- pN forces
General AFM set-up

Moving tip / moving sample:
Use U=+/- 220 V
x-, y-axis: 1 ... 125 µm
z-axis: 1 ... 20 µm
closed / open loop control
Basic AFM modi

- Imaging mode
  - contact mode
  - non contact mode
  - intermittent / tapping mode

- Force-distance mode
  - force spectroscopy
  - combined imaging & force spectroscopy
Static AFM modi

• Contact mode:
  - tip in continuous contact with sample
  - preferably used for hard samples
  - imaging in air and liquid
  - high resolution
  detect: deflection

• Force spectroscopy mode:
  - consecutive cycles of tip approach and retract
  - interaction forces between tip and sample are recorded

\[ F = -k_{\text{spring}} \cdot \Delta x \]
Dynamic AFM modi

• Intermittent/tapping mode:
  – oscillating cantilever, tip touching surface gently and frequently
  – often used for biological samples
  – imaging in air and liquid
  – good resolution

• Non contact mode:
  – oscillating cantilever, tip not in contact with sample
  – used for soft samples
  – imaging in vacuum
  – distance range 50Å - 150Å

\[ \omega = \sqrt{\frac{k}{m_{\text{eff}}}} \]

detect: amplitude
phase
deflection

http://www.jpk.com/tutorial/tutorial1.htm
Microfabricated AFM cantilevers

Typical cantilevers:
1µm thick, 100s µm long
\( k_{\text{spring}} \sim 0.01 \ldots 20\text{N/m} \)
\( f_{\text{res}} \sim 4 \ldots 400\text{kHz} \)
\( r_{\text{tip}} \sim 1 \ldots 20\text{nm} \)
reflective backside coating:
- better signal

spring constant:
- force resolution
- resonance frequency

tip radius:
- lateral resolution

tip aspect ratio:
- "depth" resolution

\[ k_{\text{spring}} = \frac{E \cdot w \cdot t^3}{4 \cdot l^3} \]
Cantilever calibration and signal-to-noise ratio

- Thermal noise:
  Equipartition theorem
  \[ k_{\text{spring}} \cdot \langle \Delta x^2 \rangle = k_B T \]

- Comparison:
  small – large cantilever
  \[ k_{\text{small}} = k_{\text{large}} \]

- thermal noise spreads over larger frequency range

- small cantilever:
  - better signal-to-noise
  - faster measurements

Viani et al, *APL* 1999
Imaging artefacts

- **Appearance of objects**
  - tip radius
  - aspect-ratio
  - double tip
  - blunt tip

- **Blurred images**
  - contamination
  - feedback adjustment
  - interference
  - noise
  - thermal drift
  - static charging
Sample preparation

- Suitable substrate
  flat and rigid
  - mica (atomically flat, hydrophilic)
  - SiO$_2$, glass (nm roughness, hydrophobic)
  - ultraflat gold (stripped gold)

- Immobilisation of sample

- Typical sample size
  - Scanning surface: $\sim 1$cm$^2$
  - Scanning tip: $\sim$ Petri dish
  - Liquid sample: 1 ... 100µl
Surface-/ Tip-Functionalisation

- Surface modification
  self assembling monolayers (SAM)
  - silanes on glass- and Si-surfaces
  - thioles on Au-surfaces

- Tip modification
  - Adsorption of molecules from solution e.g. proteins
  - Decrease AFM tip radius with attachment of molecules or nanotubes
  - Attachment of linker molecules e.g. PEG linker for antibodies, crosslinker for SH-, NH-groups
Applications

Lifescience
- Actin filaments
- Proteins
- Erythrocytes
- Bacteria
- Linearised DNA

Materials and Surface Science
- Organic film
- Transistor
- Ferroelectric domains
- Triblock copolymer film
- Polymer

Nanolithography & Nanomanipulation
- Polymer film engraving
- Anodic oxidation
- DNA on mica

http://www.jpk.com/
http://www.veeco.com/nanotheatre/
http://www.ntmdt.ru/Scan-gallery/
Imaging of isolated molecules in air

Biomolecular structure

Imaging DNA-protein complexes on aminoterminated mica

λ-DNA restriction enzyme complex (Hae III restriction endonuclease induces bending at GGCC)

Supramolecular structure of aggregation factors of marine sponges (Microciona prolifera) (circular proteoglycans)

MAFp3: self-interaction between MAF
MAFp4: binds cell surface receptors

Imaging of 2d crystals in liquid

Conformational changes

Extracellular connexon surface.
contact mode measurements in buffer solution

Without Ca$^{2+}$ open channel 1.5nm

With Ca$^{2+}$ closed channel 1.5nm

Extracellular aquaporin surface.
contact mode measurements in buffer solution
Change of conformation of tetramers

- c) Minimal force
- d) Maximal force

Müller et al., EMBO J. 2002
Scheuring et al., Single Mol. 2001
Imaging of cells and long term processes

Time-lapse AFM for imaging growth of amyloid fibrils (synthetic human amylin)

Goldsbury et al., J. Mol. Biol. 1999

Measuring the heartbeat of single cells (chicken cardiomyocytes)

Radmacher, Uni Bremen
Forces in molecular biology

- covalent bond (1000 pN)
- dextran bond flip (500 pN)
- extraction of bacteriorhodopsin (100 pN)
- DNA B-S transition (50 pN)
- unzipping DNA (10 pN)
- antigen-antibody (1 pN)
- biotin-avidin
- selectins
- rupture forces typ. at 0.1 - 5 nN/sec
- motor proteins
- active forces
- optical tweezers
- RNA polymerase
- extracting lipids
- extracting forces
- elasticities
- optical tweezers
Interpretation of force-distance curves

- Pressing on surface
- Long range repulsion
- Penetration of layer
- Repulsive forces
- Approach
- Retract
- Rupture force
- Attractive forces
- Molecular structure; elasticity
- Relative distance / nm
- Force / pN

0
Rupture forces

biotin - avidin interaction

MD simulation

experimental data

Grubmüller et al., *Science* 1996
Fritz at al., *J.Str.Biol.* (1997)
Molecular elasticities

Experimental data

- BS transition
  - $F \sim 80$ pN

- Spring-like elasticity

- Entropic elasticity

Modelling data

- Force-distance curve = fingerprint for polymers

Persistance lengths:
- ss DNA: $p \sim 1$ nm
- ds DNA: $p \sim 50$ nm
- Polypeptide: $p \sim 0.4$ nm

References:
- Rief et al. 1999
- Janshoff et al., 2000
Connection of rupture force to biochemical data

Chemical reaction: \( A + B \rightleftharpoons AB \)

\[
K_d = \frac{k_{\text{off}}}{k_{\text{on}}}
\]

\[
\Delta G_{\text{bind}} = RT \ln(K_d)
\]

- Rupture force influenced by pulling velocity
- Force decreases energy barrier for unfolding
- \( k_{\text{off}}(F) = k_{\text{off}}^0 \cdot e^{\frac{-F \cdot x}{k_B T}} \)
  - Off-rate increased by force
  - Rupture / unfolding forces correlate with \( k_{\text{off}} \)

Schwesinger et al., PNAS 2000
Rief et al., PRL 1998
Unfolding proteins

- Gain new insights in protein folding
- Novel design strategies for glue

29 nm $\sim$ 89aa $\times$ 0.4nm

Rief et al., Science 1997
AFM @ IUB

- Investigation of DNA - protein interaction
  S. Maurer

- Investigation of proteins & liposomes
  A. Kronenberger

- Investigation of thin organic films
  Prof. V. Wagner

- Investigation on cantilever sensor arrays
  Prof. J. Fritz
Conclusion

AFM is a versatile tool to investigate

- topography of surfaces
- properties of surfaces
- properties of single molecules
- forces within molecules

But: always consider experimental conditions and artefacts on measurements!
Thanks!