Analytik von Nanobiosystemen mit Hilfe der Kraftmikroskopie
Who discovered the AFM?

The AFM was invented by Gerd Binning (see picture), Christoph Gerber, and Calvin F. Quate in the mid-eighties (Binning et al., Phys. Rev. Letters 1986), and is one of the scanning probe microscopes.
• Gallium nanoparticles on a silica substrate
AFM – examples

• Si 7x7 reconstruction - atomic imaging using AFM is possible
AFM – examples

- DNA plasmids imaged under water (2000 nm)
AFM – measurement basics
AFM – measurement basics

• Force is measured by watching the bending of a cantilever, as it approaches the surface – *contact mode*
AFM – measurement basics

- Force is measured by watching the bending of a cantilever, as it approaches the surface – *contact mode*

- Cantilever bending is measured using optical lever

- Feedback loop allows surface to be mapped
The atomic force microscope measures topography with a force probe. Cantilever touching a sample. Tube scanner measures 24 mm in diameter, while the cantilever is 100 µm long.
SPM scanners are made from a piezoelectric material that expands and contracts proportionally to an applied voltage. Whether they expand or contract depends upon the polarity of the applied voltage. Digital Instruments scanners have AC voltage ranges of +220 to -220V.

In some versions, the piezo tube moves the sample relative to the tip. In other models, the sample is stationary while the scanner moves the tip. AC signals applied to conductive areas of the tube create piezo movement along the three major axes.
Technische Universität Ilmenau, FG Nanotechnologie

Zentrum für Mikro- und Nanotechnologien

- mirror
- laser beam
- fluid cell
- O-ring
- x,y,z piezo translator
- sample
- fluid in
- fluid out
- photodiode
- SPM tip
- tipholder
- sample
- piezo translation or motor control
- in air and in buffer solutions

in air and in buffer solutions
AFM – physical basis

- Forces between atoms
  - Lennard-Jones potential: $-\frac{1}{r^6} + \frac{1}{r^{12}}$
  - More long-range than tunnelling current
- More problems with tip size
- Lower resolution
AFM: other modes

- **Tapping mode**
  - Uses less force on sample
  - Works on different part of Lennard-Jones potential
  - Less precise, as force is longer range
F0-parts of ATP-Synthase embedded in membranes of spinach chloroplasts
Phase in tapping-mode AFM
Poly(cyclohexylmethacrylate-co-methylmethacrylate-b-iso-octylacrylate-b-cyclohexylmethacrylate-co-methylmethacrylate)
Why study biological processes at the single-molecule level?

• Ensemble measurements $\Rightarrow$ average properties.
• Single-molecule methods can be used to study
  1. Detection of multiple kinetic paths
  2. Transient intermediate states
  3. Time trajectories
  4. Conformational changes
  5. Underlying fundamental mechanisms
• In general, they can lead to quantitave understanding of complex biological phenomena.
Single-Molecule Options

1. Visualization structures (e.g., folding, assembly, oligomeric structure)
2. Probing forces (internal tensions, pressure, driving forces)
3. Probing mechanical work (e.g., protein motors)
4. Probing motions and dynamics (e.g., frequency, folding-unfolding rates)
5. Probing diffusion coefficients (single-molecule diffusion in the cytosolic environment)
6. Probing interactions (e.g., binding affinities in protein-ligand interactions, protein-DNA interactions, protein-protein-interactions)
STM: scanning tunneling microscope
  tunneling of electrons between probe and surface

AFM: atomic force microscope
  measuring of the force on the probe

MFM: magnetic force microscope
  AFM with magnetic probe

In some cases can image individual molecules - Å level resolution.

Many artifacts are possible!
Some limitations on the types of samples that may be imaged.

Relatively inexpensive ($50K-$200K) & easy to learn… difficult to master.
AFM measurements of proteins

superhelical DNA plasmid

path of AFM tip

DNA double helix

negatively charged mica surface
AFM measurements of proteins
AFM Image of a Superhelical Plasmid
AFM: Image of DNA Strands
FORCES AT THE MOLECULAR SCALE

The smallest force on a molecule is set by the Langevin force $f_n$ due to thermal agitation. It sets the lower limit to force measurements and is due to the Brownian fluctuations (of energy $k_B T \sim 4 \times 10^{-21}$ J $\sim 0.6$ kcal/mol at room temperature) of the object of size $d$ (sensor, cell, membrane) anchored by the molecule. For a $d \sim 2$ m diameter bead or cell in water (viscosity $\eta \sim 10^{-3}$ poise), $f_n \sim (12p k_B T \eta d)^{1/2} \sim 10$ fN/Hz$^{1/2}$ (notice that this is a noise density, i.e. the faster the measurement, the more noisy it is). This can be compared to the typical weight of a cell: $\sim 10$ fN, i.e. every second a cell experiences a thermal knock equal to its weight!
Just above these forces lie the entropic forces that result from a reduction of the number of possible configurations of the system consisting of the molecule. The entropic forces are rather weak. Since the typical energies involved are of order $k_B T$ and the typical lengths are of the order of a nanometer, entropic forces are of order

$$k \frac{T}{\text{nm}} = 4 \text{ pN (4 \times 10^{-12} N)}.$$
Noncovalent (e.g. ligand/receptor) bonding forces are much stronger. They usually involve modifications of the molecular structure on a nanometer scale: breaking and rearrangement of many van der Waals, hydrogen, or ionic bonds and stretching of covalent bonds. The energies involved are typical bond energies, of the order of an electron-volt (1 eV ~ 1.6 × 10^{-19} J ~ 24 kcal/mol). The elastic forces are thus of order eV/nm ~ 160 pN.

Finally the strongest forces encountered at the molecular scale are those required to break a covalent bond of the order of 1 eV/A ~ 1600 pN.
Stress-Induced Structural Transitions

Extension of a single molecule caused by retraction of the piezoelectric positioner results in deflection of the cantilever. This deflection changes the angle of reflection of a laser beam striking the cantilever, which is measured as the change in output from a photodetector.
Forced unfolding of titin

The successive unfolding of titin domains gives rise to the corresponding peaks and valleys in the force-extension profile measured with the atomic force microscope.
Cartoon of titin I-band function. (Actual I-band contains 41 Ig domains (Rief et al., 1997).) Ig domains are explicitly shown, with the PEVK region depicted as a heavy black line. Horizontal gray arrows indicate the external force. (a) Titin I-band resting structure. (b) Titin I-band with PEVK region extended. (c) Titin I-band with Ig domain partially unfolded. This figure was created with VMD (Humphrey et al., 1996).
Scanning Near-Field Optical Microscopy – SNOM or NSOM
Tip held \(\sim 10\;\text{nm}\) from surface using AFM techniques.
Tip held ~10nm from surface using AFM techniques

Tip acts as an **antenna** to pick up E-fields from molecule
Tip held ~10nm from surface using AFM techniques
Tip acts as an antenna to pick up E-fields from molecule
Detect non-propagating fields – no diffraction limit
SNOM: fluorescence of single molecules in different orientations

Sample: DilC$_{18}$ in 10 nm PMMA film

Excitation occurs when molecular dipole matches direction of tip field

Color-coding indicates polarization of fluorescence

Apertureless SNOM – field enhancement at tip

Electric field of laser is enhanced by metallic tip

Potential resolution ~ tip radius – better than fibre tip SNOM

Can be difficult to interpret and model (!)
Imaging of J-aggregates of PIC dye in PVS film.

Two photon excitation via fs. laser – enhances selective excitation

Resolution ~15 nm

In use for single molecule fluorescence

Near-Field Fluorescence Microscopy Based on Two-Photon Excitation with Metal Tips