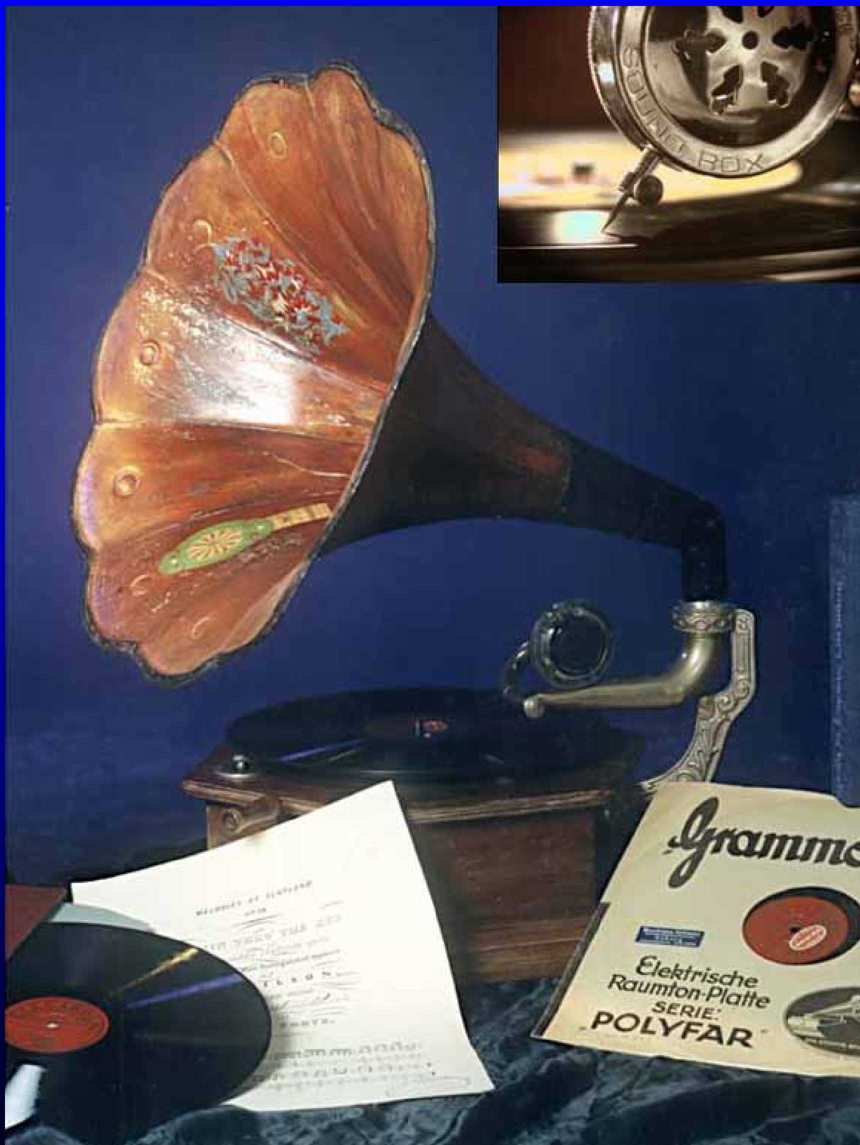
Methods of Biomaterials Testing
Lesson 3-5

Biochemical Methods

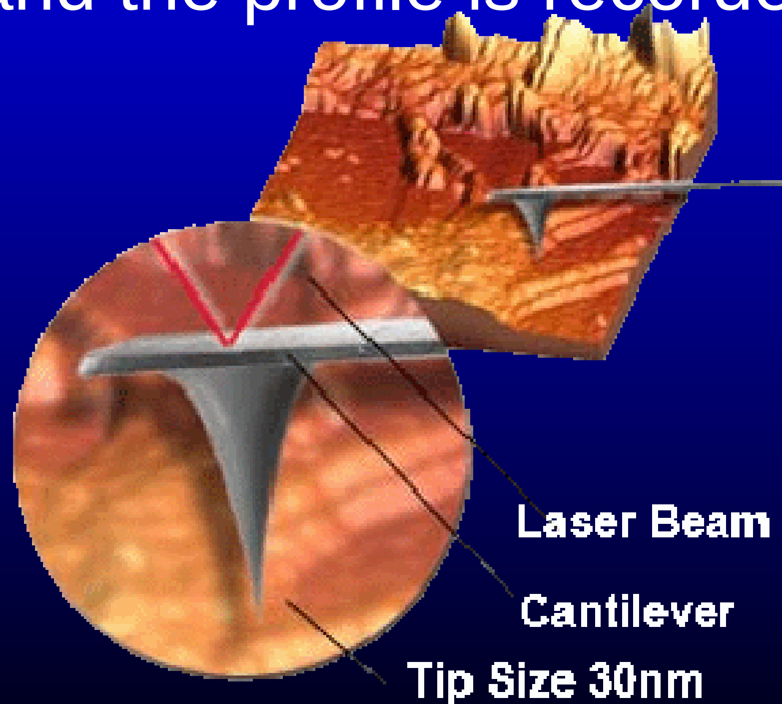
- Recent Physical Methods -

Atomic Force Microscopy

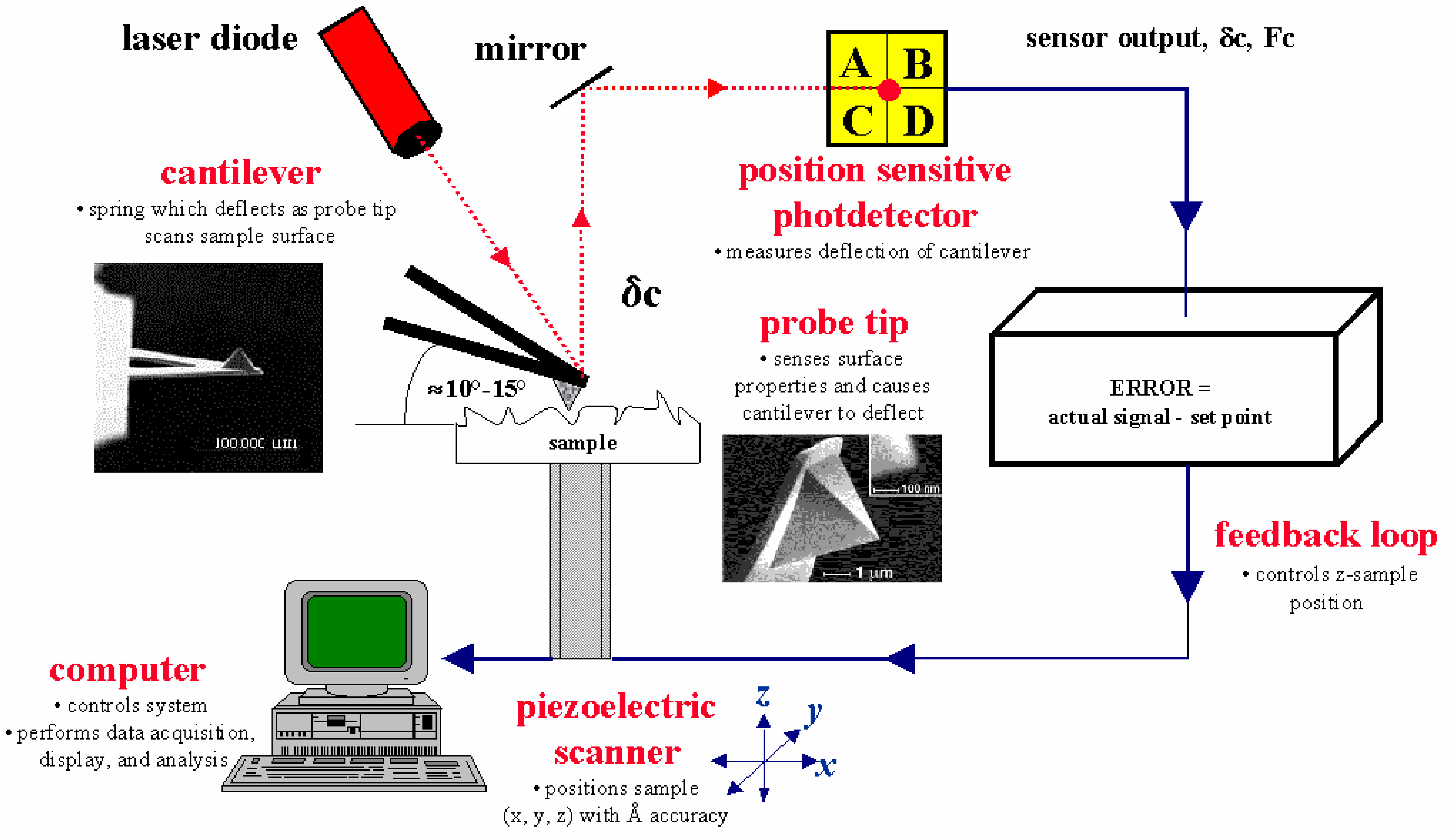
AFM Principle



The principle of an Atomic Force Microscope is very similar to an old-fashioned record player: A fine needle moves over the surface and the profile is recorded.



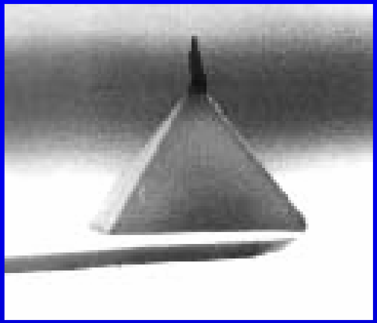
AFM – General Components

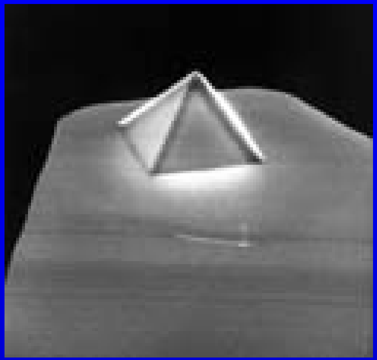


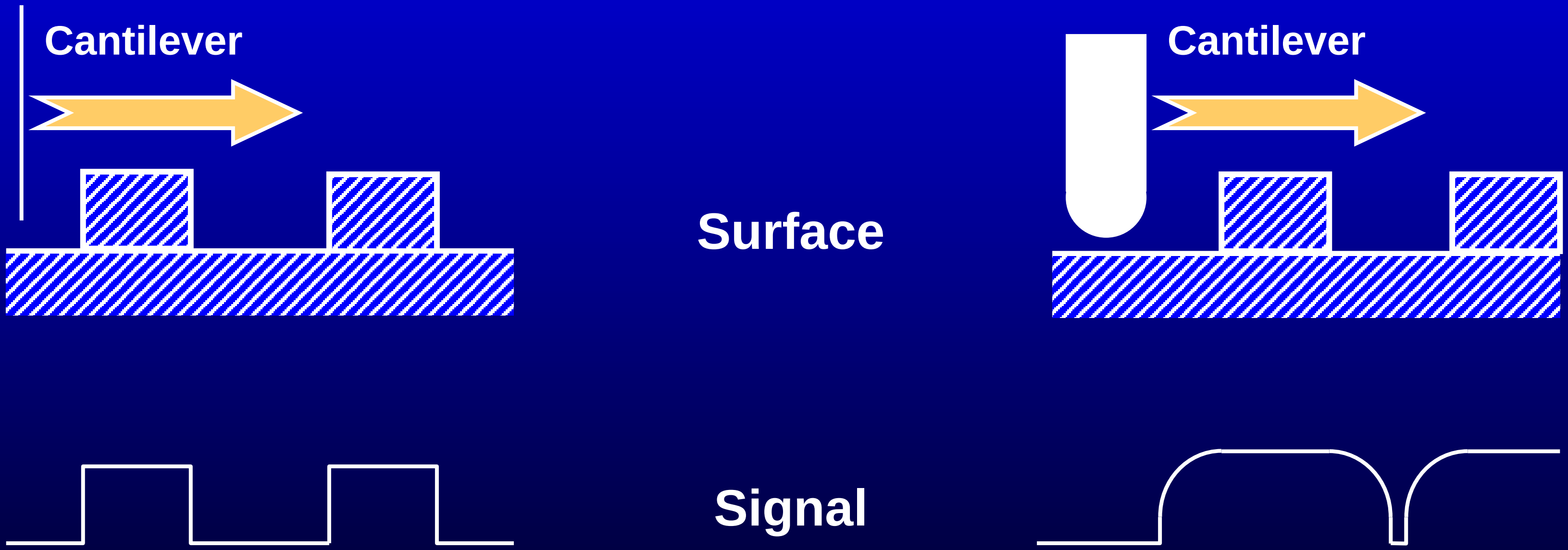
AFM Properties

- AFM applies forces at the sample (typically 0.1 – 0.2 nN). Given the low tip radius, this force results in high pressure (50 kN/m^2 = 0.5 bar)
- The AFM cantilever interacts with the surface mainly by van-der-Waals forces (weak interactions)
- Unlike SEM, AFM can work in vacuum, air and even under water
  - → Simple sample preparation
  - → Suitable for biological material
- Unlike SEM, AFM allows measurement in the z-axis
- Resolution of AFM can be similar to SEM, but images usually are less sharp
- Adsorbed water molecules at the tip and/or the samples can disturb the images; meniscus formation between both surface and tip can produce high attractive forces of 10-100nN

AFM Tips

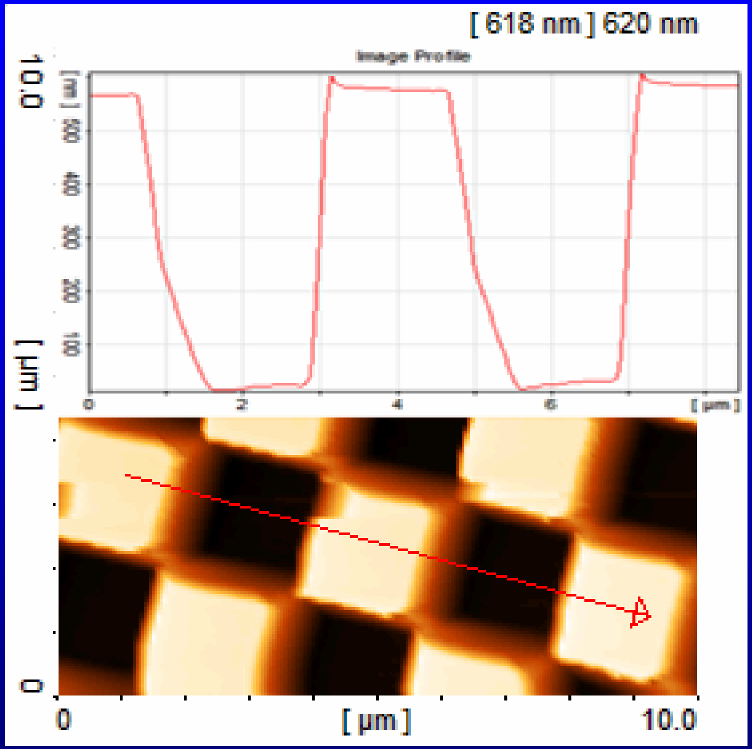


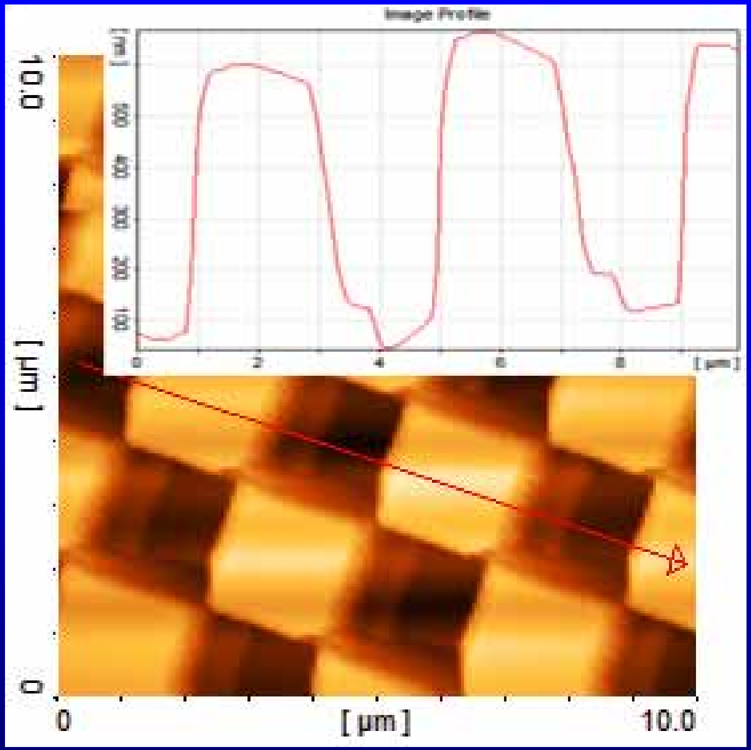




The shape of the ATM cantilever is superposed to the morphology of the scanned surface

Influence of Tip Geometry





AFM Modes

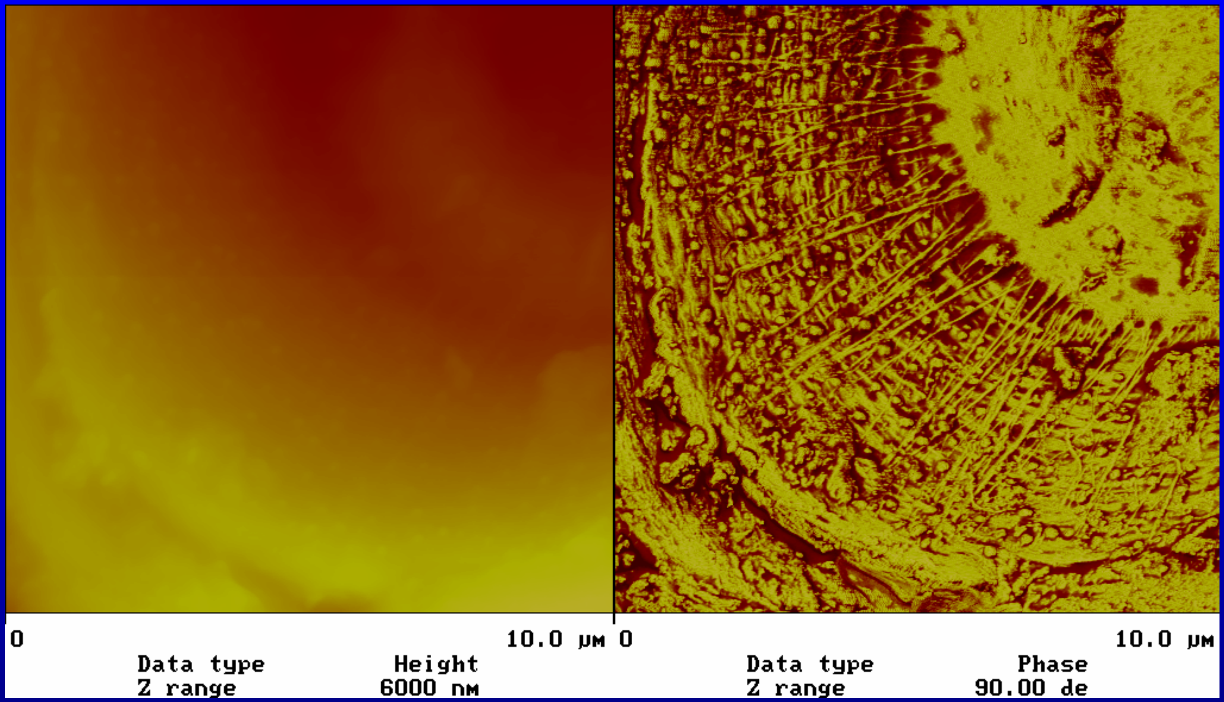
Contact mode

- Cantilever moves directly over the surfaces
- Problems with sharp edges
- Friction measurement
- Force measurement possible

Non-contact mode, tapping mode

- The cantilever oscillates (vertically) with a high frequency. Interaction with dipole and van-der-Waals forces of the surface dampens the oscillation
  - Dipole: between permanent dipoles: $F \propto 1/r^4$
  - Between a dipole and a polarizable group: $F \propto 1/r^5$
  - Between two induced dipoles: $F \propto 1/r^6$
  - Van-der Waals forces: $F \propto 1/r^{12}$
- Damping can induce a phase shift of the oscillation $\rightarrow$ additional information, which has information about the hardness (polymers, adsorbed proteins on hard biomaterials)

Phase Contrast Image

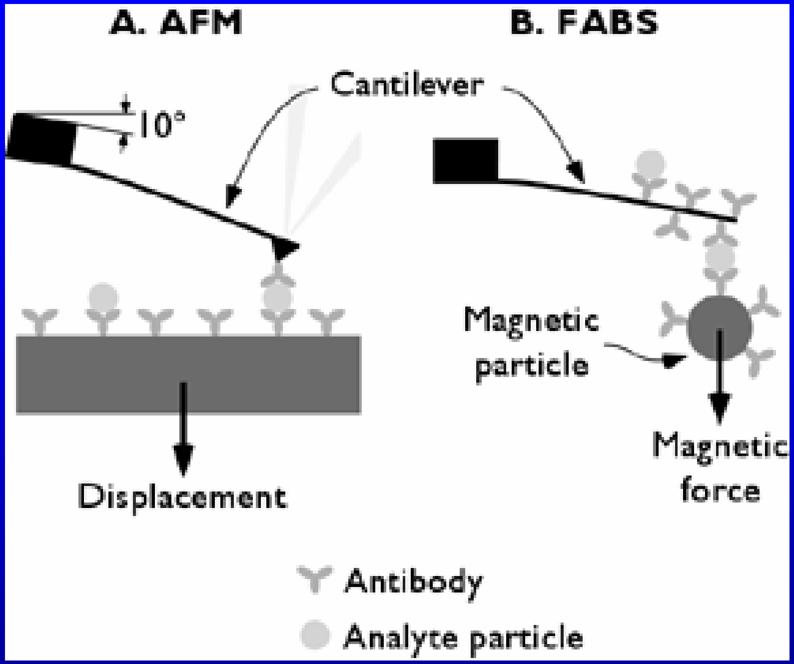


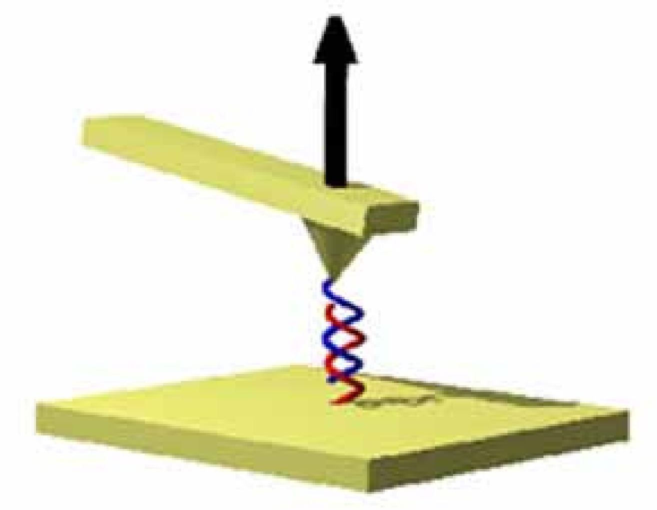
Topographic image of a wood particle

Phase contrast image of a wood particle

Dr. Mandla A. Tshabalala at the Forest Products Laboratory

Functionalized Tip
Chemical Force Microscopy



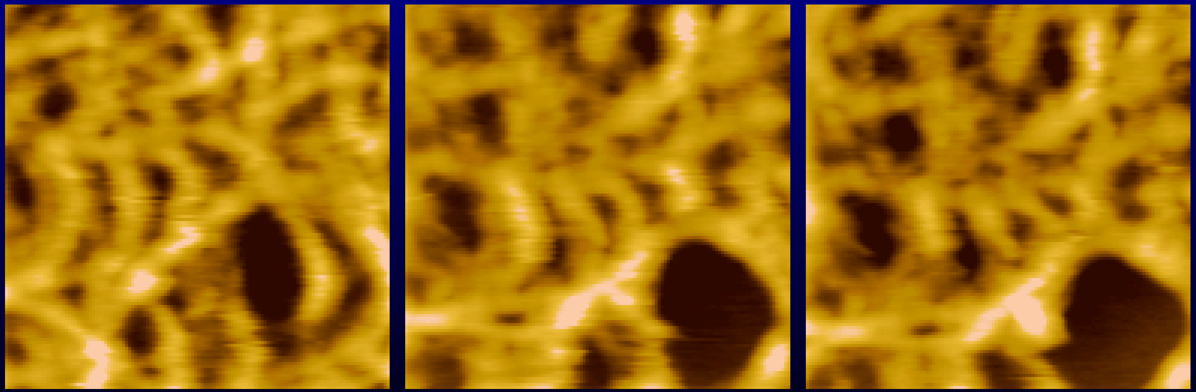


Antibodies or single strand DNA can be linked to the cantilever

- Measurement of binding forces
- Mapping of specific antigens/ markers

Difficulties of AFM in Biosciences

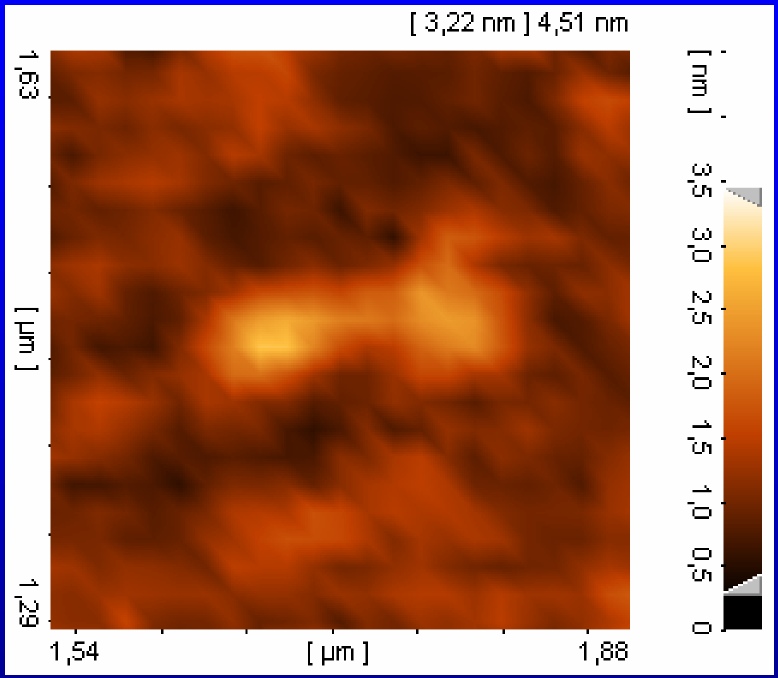
- Water strongly dampens the oscillation of the cantilever
  - Sensitivity in water is only 1 – 10% of that in air/ vacuum
- Mechanical effects
  - Forces by AFM are higher than adhesion forces of the biomolecule on its substrate
    - Molecule is shifted
    - Molecule attaches to the AFM tip



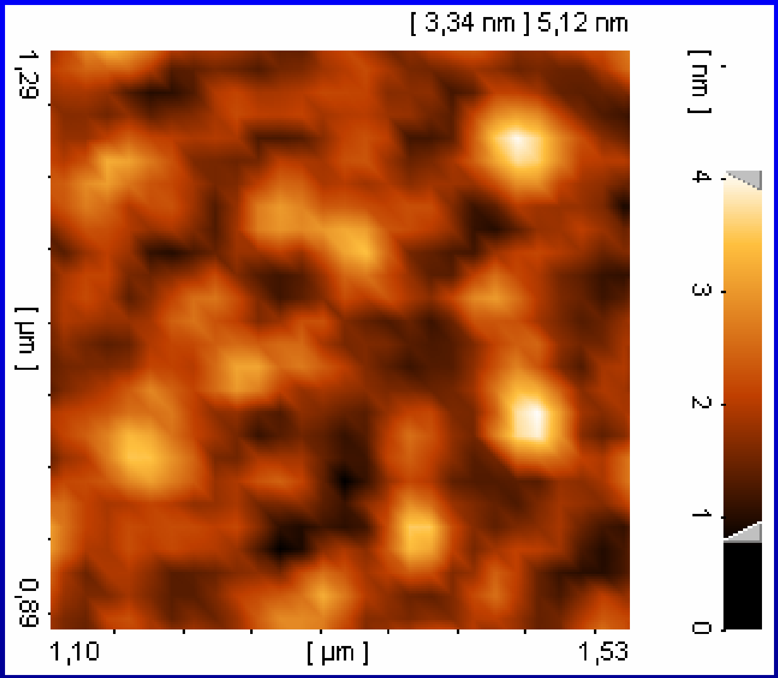
Repeated measurement of the same area in a collagen mesh (scan 1, 50, 100)

David Baselt, California Institute of Technology, 1993

AFM of Surface-adsorbed Fibrinogen



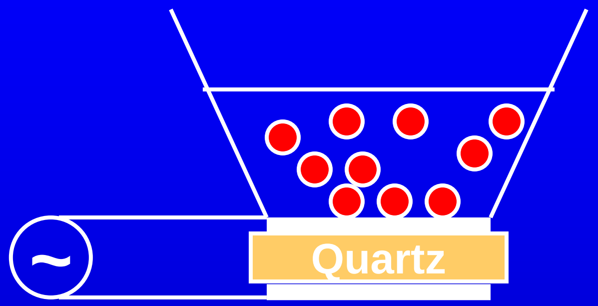
Fibrinogen on reduced Si: elongated structure



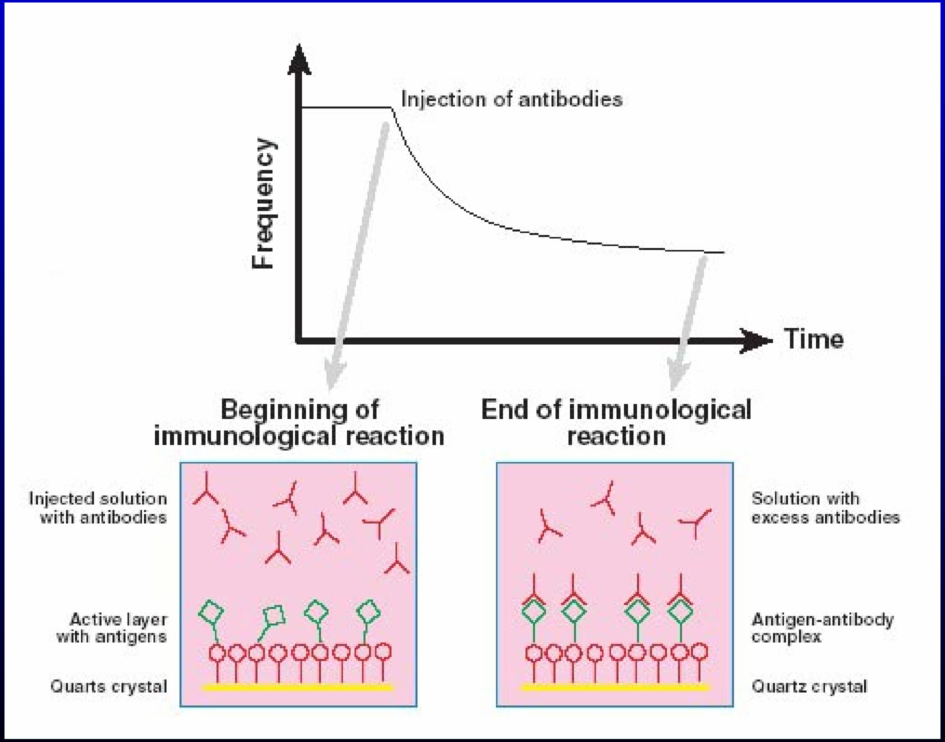
Fibrinogen on oxidized Si: globular structure/ aggregates

Quartz Crystal Microbalance (QCM)

Principle



Voltage at resonance frequency of the quartz



The resonance frequency of a quartz is dependent of the mass (Sauerbrey Equation)

$$\Delta f = - C_f \frac{\Delta m}{A}$$

Then the driving power is switched off, the voltage between the electrodes decays as a dampened sinusoidal function. This way the resonance frequency can be obtained.

The method can be used for very sensitive weight measurement (down to femto-gram range), also under water

Limitations

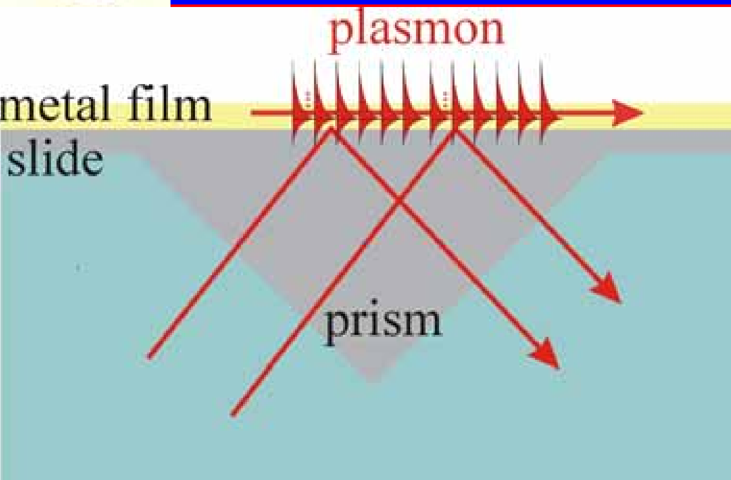
- Biomolecules often are flexible (internal friction in the adsorbed layer)
  - They may not perfectly follow the oscillations of the quartz

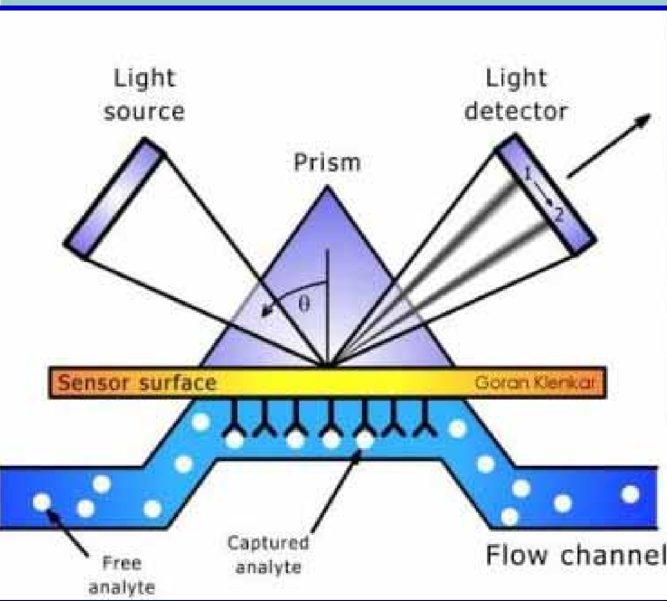
  →The observed mass is calculated too low
- Biomolecules frequently have water adsorbed on their surface
  - Adsorbed water
  - Water trapped in the protein layer
  - Hydrodynamically coupled water

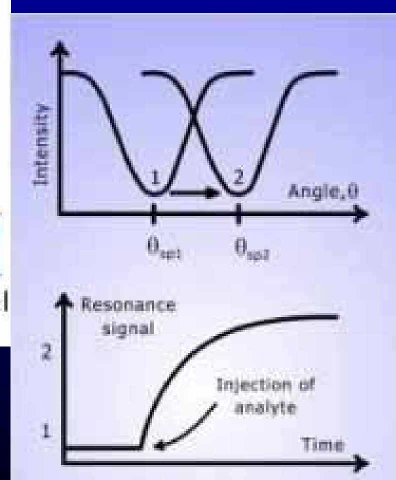
  →The adsorbed mass is calculated too high

Surface Plasmon Resonance

Principle







Light irradiation of a metal can interact with the electrons in the metal (resonance situation)

→ Formation of a electromagnetical surface wave ("Plasmon") with an exponential decay perpendicular to the surface (typical penetration ~200nm)

→ Plasmon formation is a very sharp function of the of the angle of incidence (or the wavelength) and the refractive index of the medium on the surface of the metal

Plasmon formation consumes a huge amount of energy of the light → Reflected light decreases (what is measured)

Specifities

- Requirements
  - Transparent substrate
  - Metal (gold) coating
- Characteristics
  - Surface adsorbed material has different diffractive index than the solvent (water) $\rightarrow$ detection well possible
  - High sensitivity (ng range)
  - Penetration depth fits well with the dimensions of adsorbed biological macromolecules
  - Trapped and protein-bound water is not measured
  - Good suitability for measurement under flow conditions