

Scanning Probe and Electron Microscopy

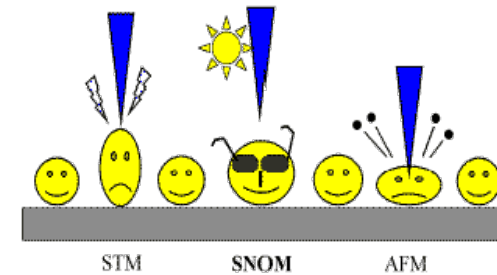
Szabolcs Osváth

Semmelweis University

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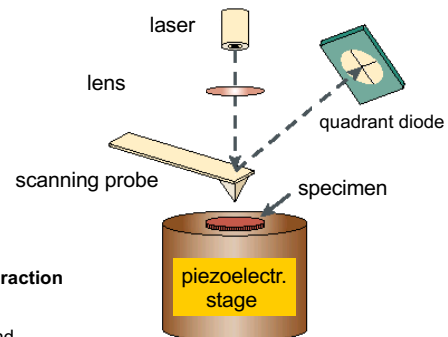
Scanning Probe Microscopy (SPM)

- Surface imaging by scanning a **sharp tip**.
- Scanning Tunneling Microscope (STM) invented by Heinrich Rohrer and Gerd Binnig in 1981. Awarded Nobel prize in 1986.



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Atomic Force Microscopy (AFM)



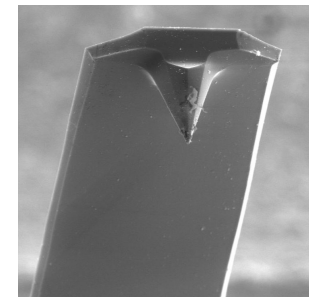
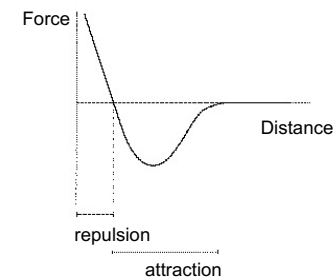
- Measures **mechanical interaction** between tip and surface.
- Sensitive to **topography** and **mechanical properties**.

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Force-Distance Relationship

The probe:

- Typically, 100 μm long, 1 μm thick, V shaped
- Small spring constant
- Large resonance frequency
- Silicon (-oxide, -nitride)



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Contact Mode AFM

- Tip always in **contact** with surface.
- Works in the repulsive range.
- Keeps the force constant: follows the topography of surface.
- **Local Force Spectroscopy**: The force / displacement function can be recorded at a given point on the surface.

Tapping Mode AFM

- Tip **oscillates** with an amplitude of 20-100 nm and touches the surface at each vibration.
- The amplitude and phase of the vibration change as the probe passes above hills and wells of the surface.

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Contact vs. Tapping Mode

Contact Mode AFM

Advantages:

- Fast
- Atomic resolution
- Good for rough surfaces

Disadvantages:

- Horizontal forces distort the image
- Distortion due to water on the surface
- Can scratch soft biological samples

Tapping Mode AFM

Advantages:

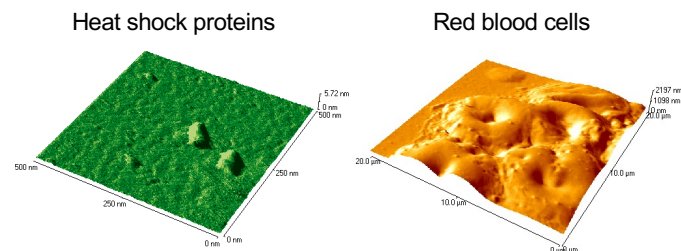
- Higher lateral resolution
- Damaging less the soft samples

Disadvantages:

- Slower scanning

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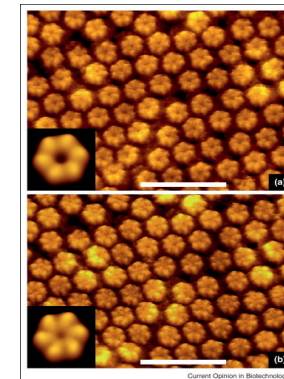
AFM Images of Biological Samples



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AFM Image of Extracellular Connexon

- Calcium-induced conformational changes in the extra-cellular connexon surface.
- The scalebar is 23 nm long.



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Electrons as Waves



Louis de Broglie:

$$\lambda = h / p$$

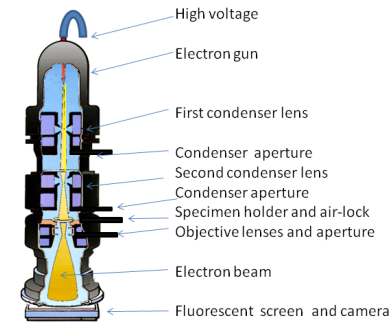
λ – wavelength of the electron
 h – Planck's constant
 p the momentum of the electron

Electrons are waves, enabling **high-resolution imaging**

Louis-Victor-Pierre-Raymond de Broglie
 the 7th duke of de Broglie

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Transmission Electron Microscope



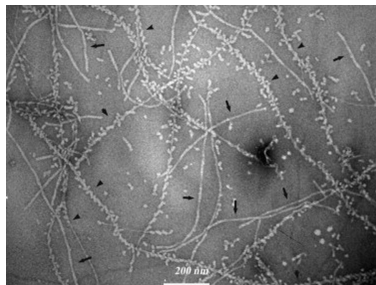
The microscope built
 by Ruska in 1933



- Built by Ernst August Friedrich **Ruska** and Max **Knoll** (1931).
- **Sub-nanometer resolution**
- Ruska received Nobel prize in 1986.

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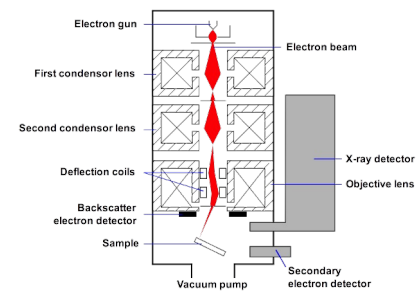
Transmission Electron Microscopy Example



- Imaging of **amyloid fibrils** and cholesterol binding

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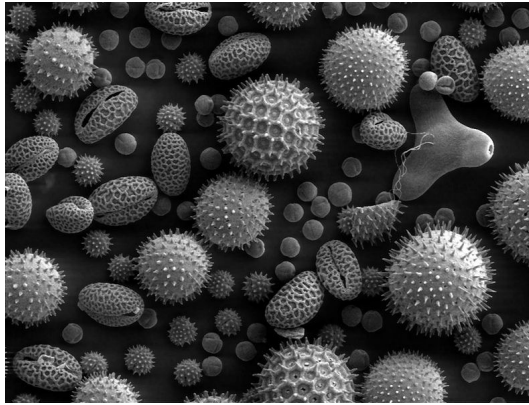
Scanning Electron Microscopy (SEM)



- Surface imaging: **high resolution** and **large depth of field**

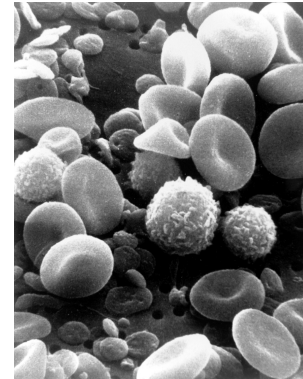
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SEM Image of Pollen Particles



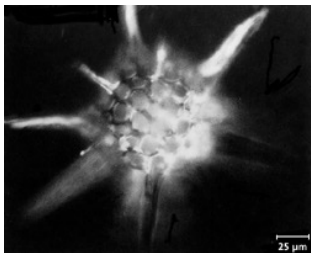
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SEM Image of Blood Cells

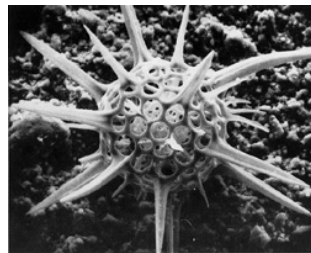


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Optical vs. Electron Microscopy



- small depth of field
- low resolution
- + live sample, life processes
- + at atmospheric pressure



- + large depth of field
- + high resolution
- fixed sample
- in a vacuum

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