

Super-Resolution Microscopy Techniques

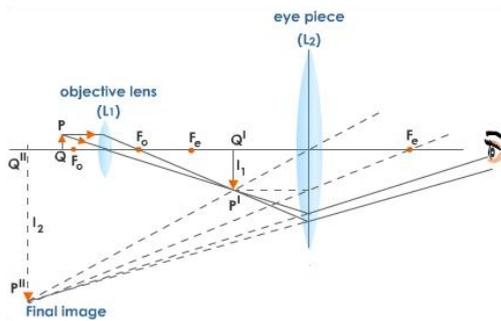
Szabolcs Osváth

Semmelweis University

Hans Jansen and Zacharias Jansen
Build a Compound Microscope in 1590



Diagram of the Compound Microscope

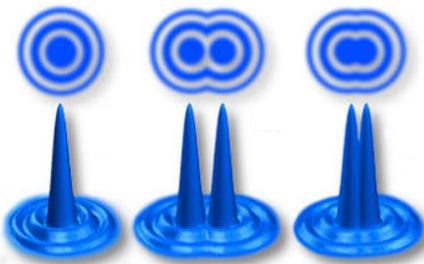


Point Spread Function (PSF)

The PSF is the transfer function (impulse response) of the microscope.

As a consequence of the wave character of light, the image of a point of the object is not a point, but an extended blob.

The Effect of the Wave Character of Light on the Image



Abbe's Principle

The smaller the detailed structure of the object, the wider the angle of diffraction.

Each spatial frequency component in the object produces diffraction at a specific angle dependent upon the wavelength of light.

Two points can be resolved in the microscope if and only if at least the first order diffracted beams are combined in the image.

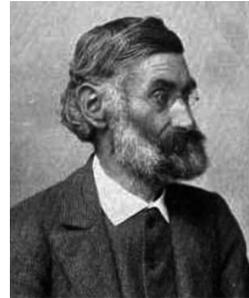
Abbe's Formula

$$\delta = 0,61 \cdot \lambda / (n \cdot \sin\alpha)$$

Tacit assumptions:

- different parts of the object are imaged simultaneously
- details of the object are distinguished by the fact that the light coming from them give distinctive image patches.

Ernst Karl Abbe (1840-1905)



Physicist and social reformer

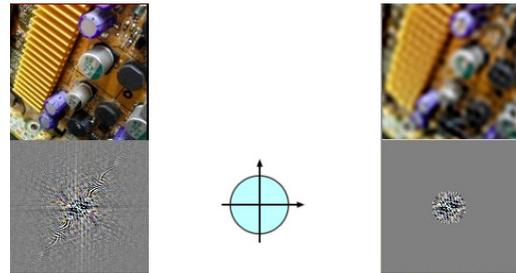
He placed the production of optical devices on a scientific basis.

Super-Resolution Microscopy

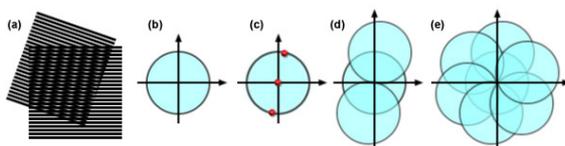
2014 Nobel prize in chemistry:

- Eric Betzig
- Stefan W. Hell
- William E. Moerner

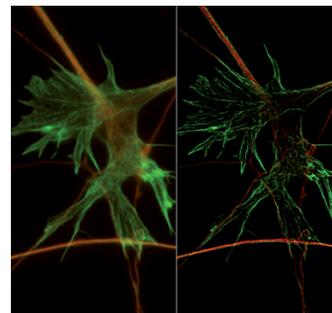
Abbe's Principle in the Wavenumber Representation



Structured illumination microscope

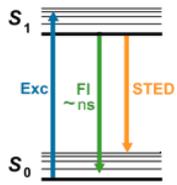


Structured Illumination Microscope

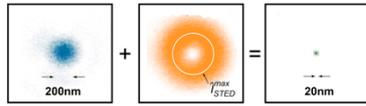


Traditional (left) and structured illumination microscope image (right) of neural cells.

STimulated Emission Depletion (STED) Microscope



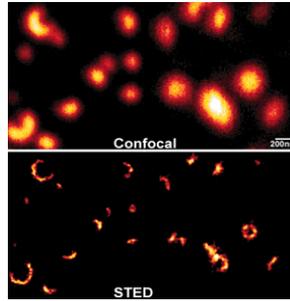
pioneered by Stefan Hell



$$\Delta r \approx \frac{\Delta}{\sqrt{1 + I_{max}/I_s}}$$

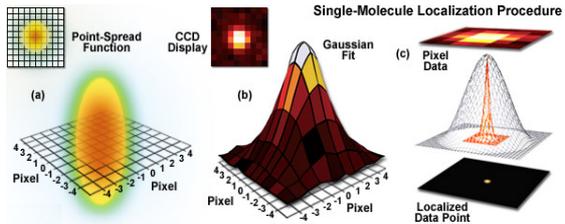
I_{max} the maximal used STED intensity
 I_s the STED saturating intensity

STimulated Emission Depletion (STED) Microscope

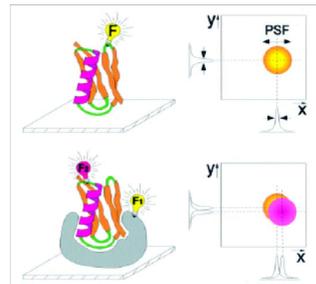


Organization of synaptophysin in reused synaptic vesicles.

Localization



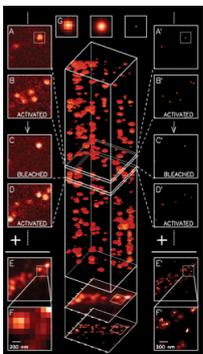
Localization and Co-Localization



The macromolecule can be localised with nm precision by fitting the PSF.

Co-localization of two molecules does not imply interaction between them.

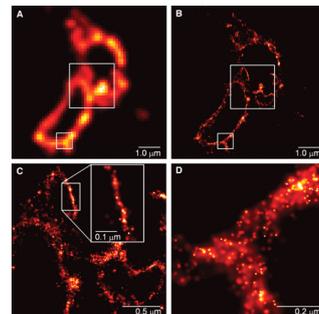
Photo-Activated Localization Microscopy (PALM)



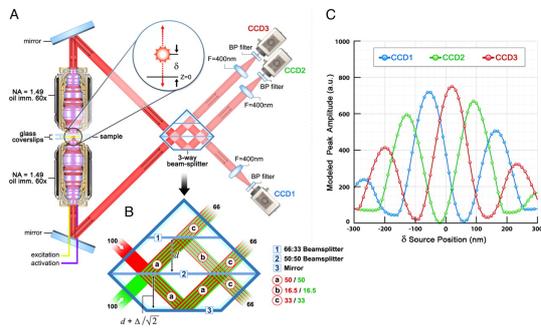
Invented by Eric Betzig and Harald Hess

Photo-Activated Localization Microscopy (PALM)

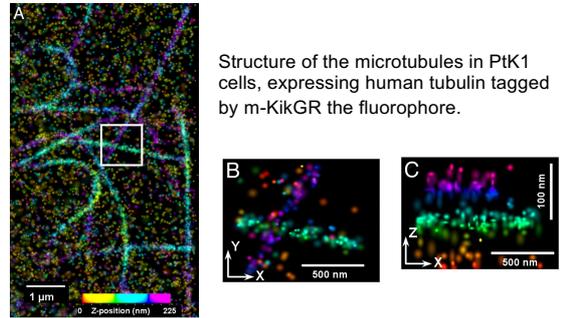
CD63, lysosome transmembrane protein



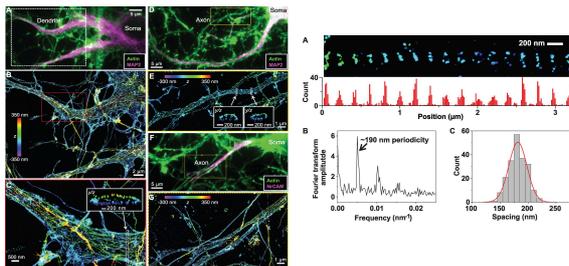
Interferometric Photo-Activated Localization Microscopy (iPALM)



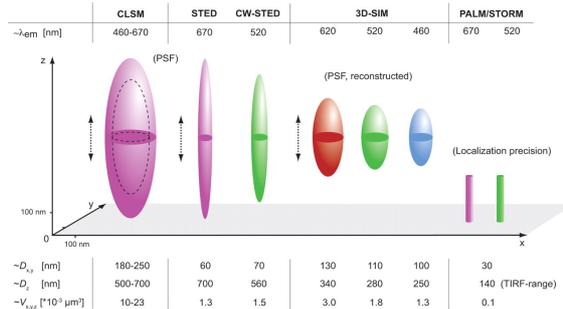
Interferometric Photo-Activated Localization Microscopy (iPALM)



Cytoskeletal Structure of Axons



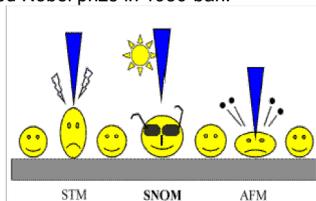
Comparison of Different Super-Resolution Techniques



Scanning Probe Microscopy (SPM)

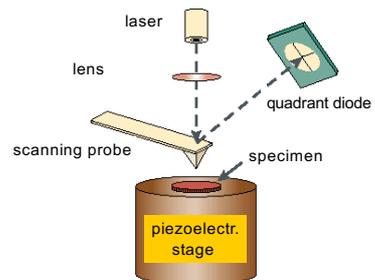
This family of microscopes creates a topographic image of the sample surface by scanning it with a pointed needle and measuring the probe-specimen interaction.

The first SPM, the Scanning Tunneling Microscope (STM) Was invented by Heinrich Rohrer and Gerd Binnig in 1981. They received Nobel prize in 1986-ban.



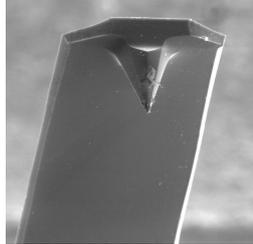
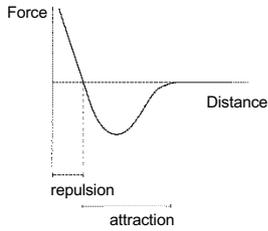
Atomic Force Microscopy (AFM)

The measured interaction is the mechanical force between the probe tip and specimen surface



Force Between the Probe Tip and Specimen

- The probe:
- typically 100 μm long, 1 μm thick, V shaped
 - Small spring constant
 - large resonance frequency
 - silicon (-oxide, -nitride)



Contact Mode AFM

The needle and specimen are in constant contact. It works in the repulsive range. It keeps the force constant: follows the topography of surface. The vertical deformation of the probe is detected. Local Force Spectroscopy: The force / displacement function can be recorded at a given point on the surface.

Tapping Mode AFM

The needle vibrates 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.

Comparison of the Contact and Tapping Mode

Contact Mode AFM

Advantages:
quick scan
atomic resolution
good for rough surfaces

Disadvantages:
horizontal forces distort the image
distortion due to water on the surface
can scratch soft biological samples

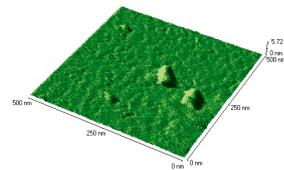
Tapping Mode AFM

Advantages:
higher lateral resolution
damaging less soft samples

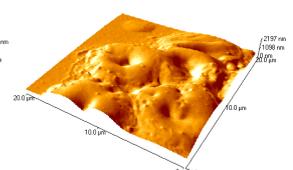
Disadvantages:
slower scanning

AFM Images of Biological Samples

Heat shock proteins



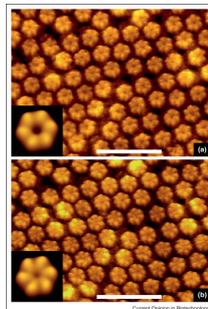
Red blood cells



AFM Image of Extra-Cellular Connexion

Calcium-induced conformational changes in the extra-cellular connexion surface.

The line is 23 nm long.



The Electron as a Wave



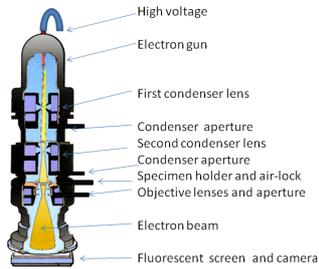
Louis de Broglie:

$$\lambda = h / p$$

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

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

Transmission Electron Microscope

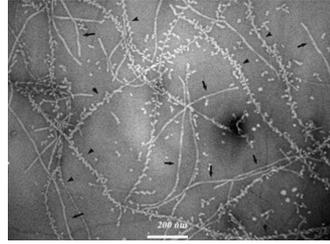


The microscope built by Ruska in 1933



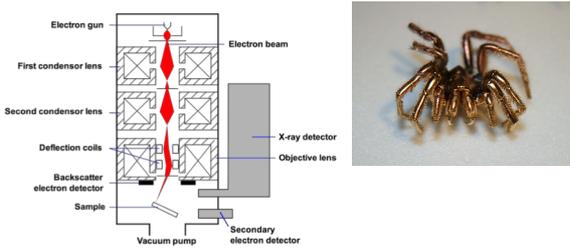
Ernst August Friedrich Ruska and Max Knoll built the first electron microscope in 1931. Ruska received Nobel prize in 1986.

Amyloid Fibrils in Transmission Electron Microscope

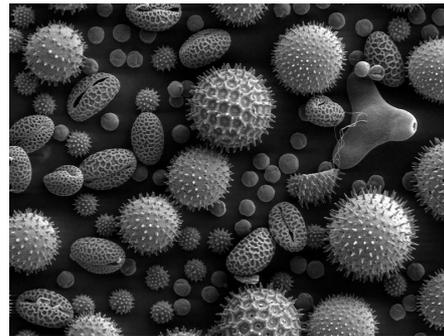


Binding of cholesterol to amyloid fibrils.

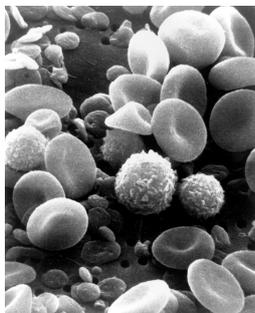
SEM



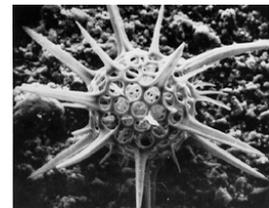
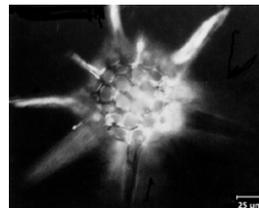
SEM Image of Pollen Particles



SEM Image of Blood Cells



Comparison of the Optical and the Electron Microscope



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

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