Super-Resolution Microscopy Techniques

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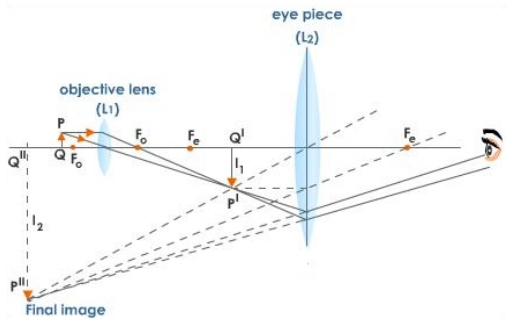
Hans Jansen and Zacharias Jansen Build a Compound Microscope in 1590

Diagram of the Compound Microscope

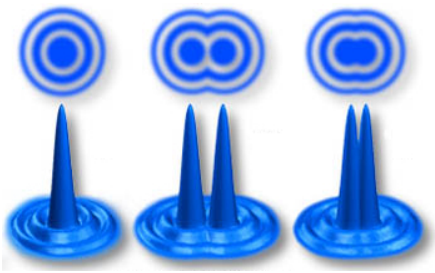


Point Spread Function (PSF)

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

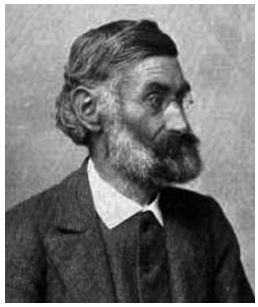
$\delta = 0{,}61 \cdot \lambda / (n \cdot \sin\omega)$

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

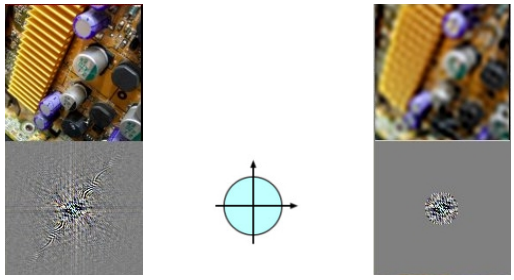
He placed the production of optical devices on a scientific basis.

Super-Resolution Microscopy

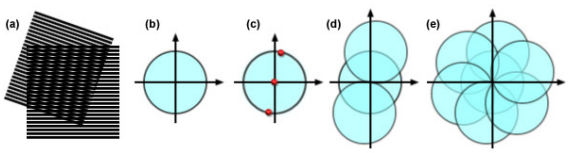
2014 Nobel prize in chemistry:

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

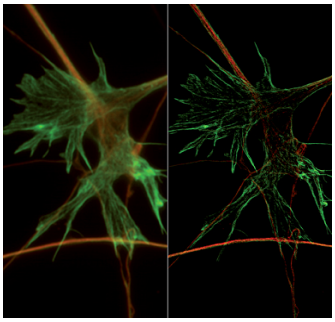
Abbe's Principle in the Wavenumber Representation



Structured illumination microscope

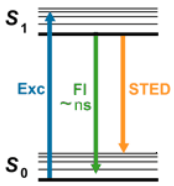


Structured Illumination Microscope

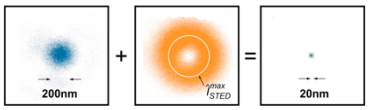


Traditional (left) and structured illumination microscope image (right) of neural cells.

STimulated Emission Depletion (STED) Microscope



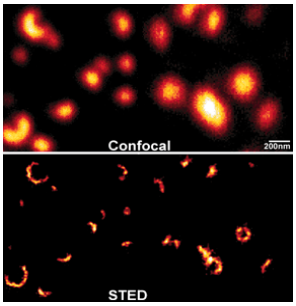
pioneered by Stefan Hell



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

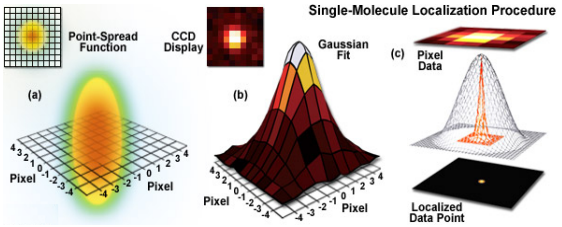
I_{max} the maximal used STED intensity
I_s the STED saturating intensity

STimulated Emission Depletion (STED) Microscope

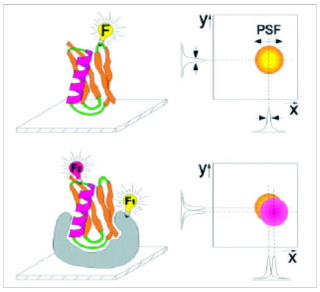


Organization of synaptolysin in reused synaptic vesicles.

Localization



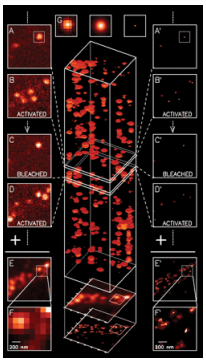
Localization and Co-Localization



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

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

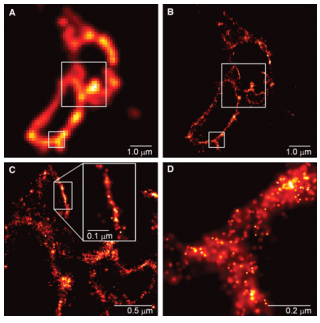
Photo-Activated Localization Microscopy (PALM)



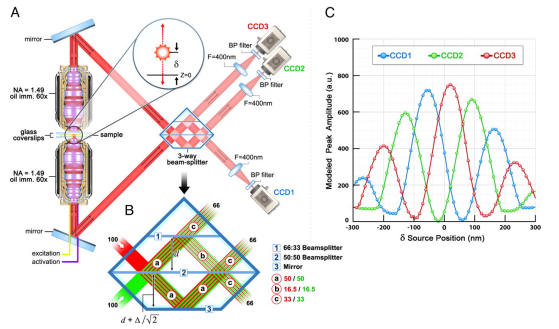
Invented by Eric Betzig and Harald Hess

Photo-Activated Localization Microscopy (PALM)

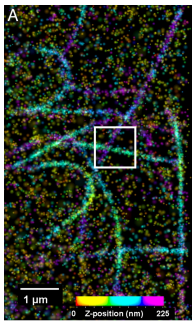
CD63, lysosome transmembrane protein



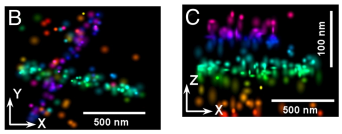
Interferometric Photo-Activated Localization Microscopy (iPALM)



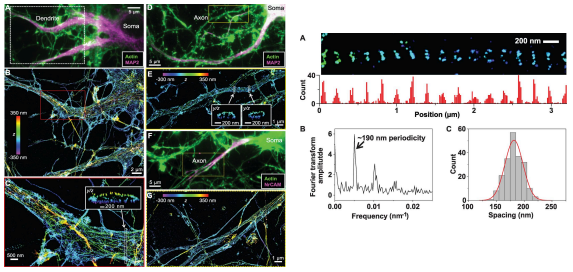
Interferometric Photo-Activated Localization Microscopy (iPALM)



Structure of the microtubules in PtK1 cells, expressing human tubulin tagged by m-KikGR the fluorophore.

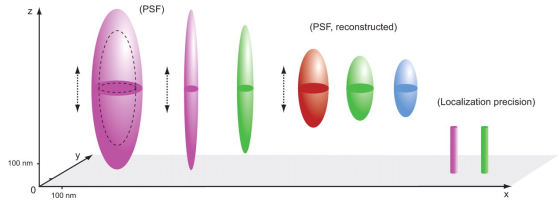


Cytoskeletal Structure of Axons



Comparison of Different Super-Resolution Techniques

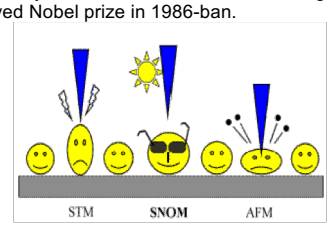
	CLSM	STED	CW-STED	3D-SIM			PALM/STORM	
$\sim\lambda_{em}$ [nm]	460-670	670	520	620	520	460	670	520
$\sim D_{x,y}$ [nm]	180-250	60	70	130	110	100	30	
$\sim D_z$ [nm]	500-700	700	560	340	280	250	140	(TIRF-range)
$\sim V_{x,y,z}$ [$*10^{-3}$ µm³]	10-23	1.3	1.5	3.0	1.8	1.3	0.1	



Scanning Probe Microscopy (SPM)

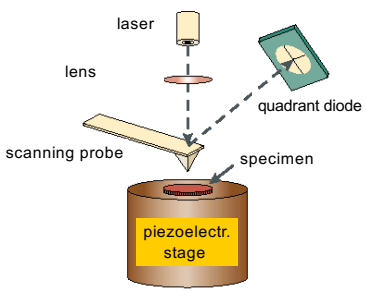
This family of microscopes creates a topographic image of the sample surface by scanning it with a pointed needle and measuring the probe-specimen interaction.

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.



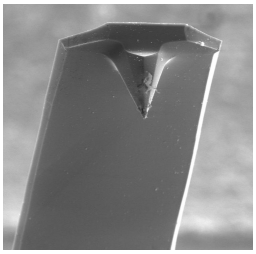
Atomic Force Microscopy (AFM)

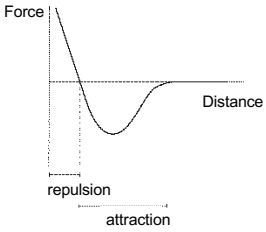
The measured interaction is the mechanical force between the probe tip and specimen surface



Force Between the Probe Tip and Specimen

The probe:
- typically 100 μm long, 1μm 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

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

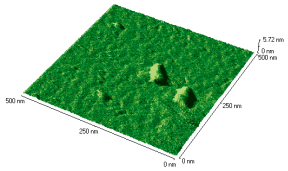
Tapping Mode AFM

Advantages:
higher lateral resolution
damaging less soft samples

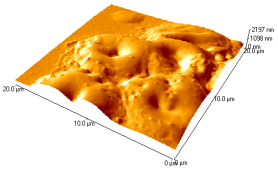
Disadvantages:
slower scanning

AFM Images of Biological Samples

Heat shock proteins



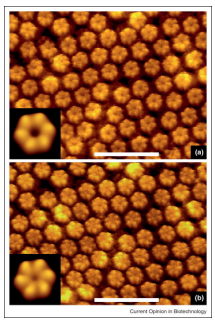
Red blood cells



AFM Image of Extra-Cellular Connexon

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

The line is 23 nm long.



The Electron as a Wave

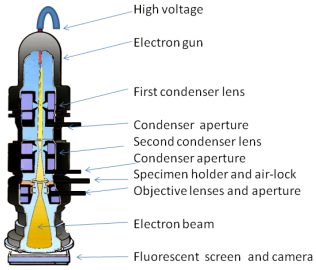
Louis de Broglie:

$\lambda = h / p$

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

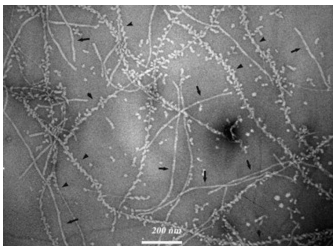
Transmission Electron Microscope



The microscope built by Ruska in 1933

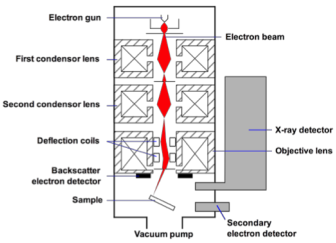
Ernst August Friedrich Ruska and Max Knoll built the first electron microscope in 1931. Ruska received Nobel prize in 1986.

Amyloid Fibrils in Transmission Electron Microscope

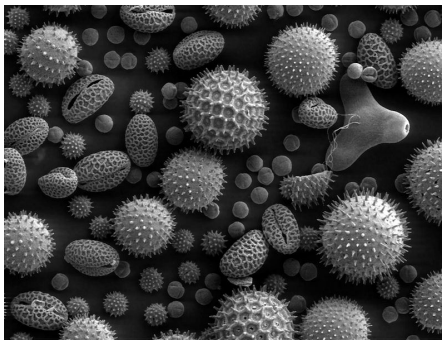


Binding of cholesterol to amyloid fibrils.

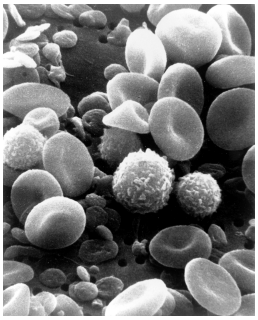
SEM



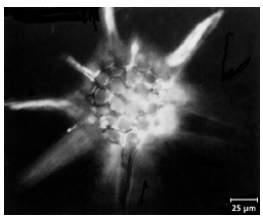
SEM Image of Pollen Particles



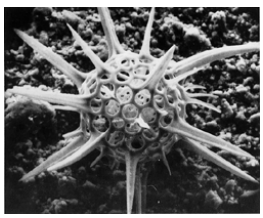
SEM Image of Blood Cells



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