Formation of Biological Structures

Szabolcs Osváth

Semmelweis University

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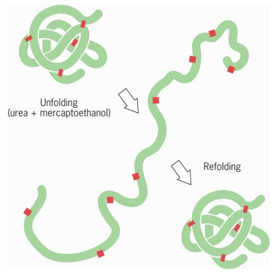
Anfinsen's Dogma



Refolding of Ribonuclease A

Christian B. Anfinsen

The information of the 3D protein structure is encoded in the 1 D AA sequence.

2

Importance of the Protein Folding Problem

One of the most important questions of molecular biophysics.

We sequence genomes, we build databases, but we can't predict protein structure and function based on the genetic information.

There are roughly two dozen conformational diseases:

Misfolded proteins and deposition of amyloid plaques was observed in various diseases (pl. Creutzfeld-Jakob disease, Alzheimer disease, Parkinson disease).

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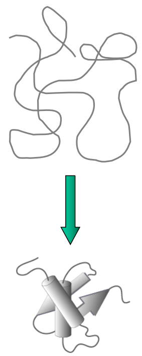
In vitro and *in vivo* Folding

in vitro



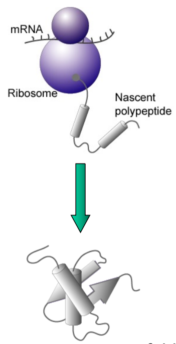
unfolded protein

folded protein

differences:

- entire sequence / co-translational folding
- crowded intracellular environment

in vivo



folded protein

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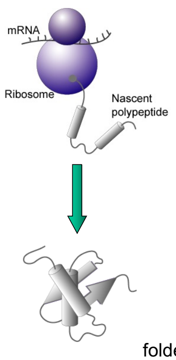
Co-Translational Folding

in vivo

The N terminal of the nascent polypeptide chain starts to fold before completion of the translation.

20-30 AAs of the C terminal are protected within the ribosome.



folded protein

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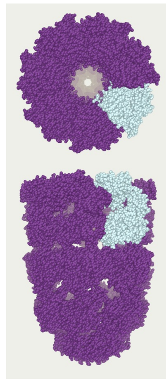
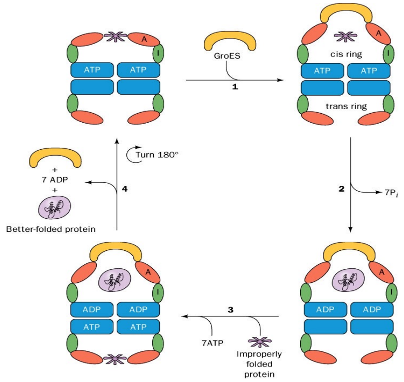
GroEL/ES Chaperon Cycle





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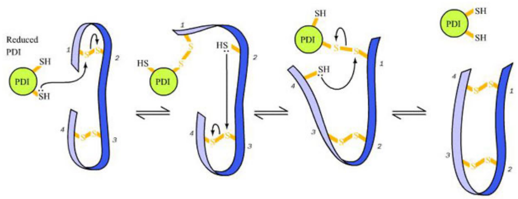
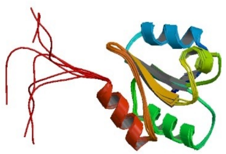
Protein Disulfide Isomerase Function



structure of the human protein disulfide isomerase



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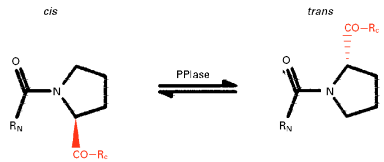
Proline Cis/Trans Isomerase

Due to the activation barrier between the cis and trans prolines, the presence of cis prolines in the native structure:
- speeds up early folding steps
- slows down the final formation of the native structure.

PPIase (peptidyl-prolyl isomerase)



PPIase structure

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Fate of the Protein in Eukaryotic Cells

cytosol .... protein synthesis and folding,
extracellular volume .... export of folded protein
mitochondrion .... limited protein synthesis
chloroplast .... limited protein synthesis
endoplasmic reticulum .... import of unfolded protein
peroxisome .... import of folded protein
nucleus .... import of folded protein
lysosome .... import of unfolded protein

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Levinthal's Paradox - Calculation

Cyrus Levinthal

Consider a protein of 151 AAs. Assume all the 150 bonds connecting them have only two possible conformations. Assume that a reorientation of the bonds happens in 10^{-13}s.

A random search through the phase space would last:
$2^{150} \cdot 10^{-13}$s $= 4.6 \cdot 10^{24}$ years.

Age of Earth: $4.6 \cdot 10^9$ years
Age of the Universe: $13.7 \cdot 10^9$ years
Proteins typically fold on the ms to s timescale.

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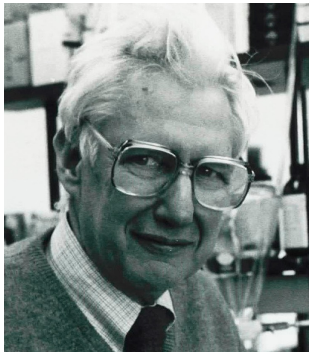
Levinthal's Paradox - Conclusion

The phase space of a protein is way too big to find the native structure by random search.

Cyrus Levinthal
1922 - 1990



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Kinetic Pathways and Intermediate States

All proteins have a most stable conformation.

The protein can find this conformation by following a kinetic pathway and adopting specific intermediate states.

In vivo, trapping of the protein in intermediate states is prevented by protein disulfide isomerases, peptidyl prolyl isomerases and chaperones.

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Energy Landscape Models

At constant pressure and temperature every thermodynamic system tends to minimize Free enthalpy (Gibbs free energy).

A free enthalpy (Gibbs free energy) value is associated to every conformation of the protein.

The protein does not search through the entire phasespace, but starts to "flow" towards lower free enthalpies.

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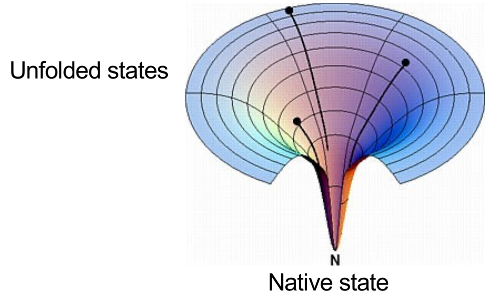
Smooth Funnel



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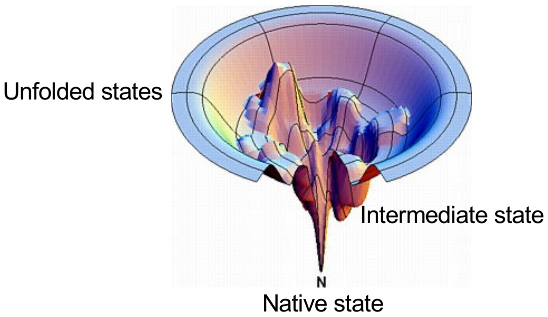
Rugged Funnel



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Comparison of the Two Folding Models

Pathways	Landscape
Given pathways	Energy landscape
Well distinguished intermediates	Multitude of intermediates
Consecutive steps	Parallel folding routes
Classical chemical kinetics applied to protein folding	Statistical physics developed to understand spin glasses

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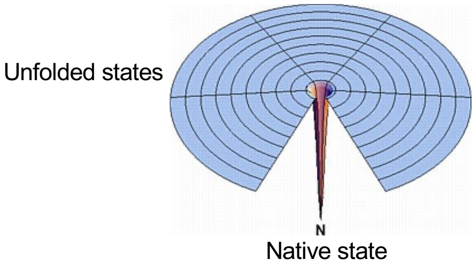
Energy Landscape View of Levinthal's Paradox



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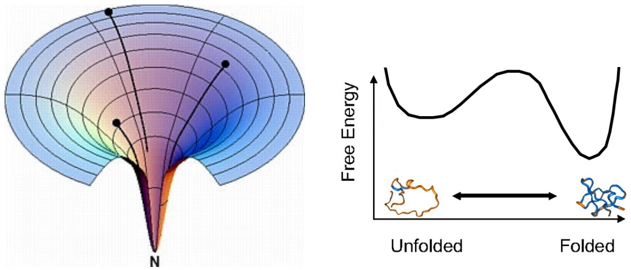
Averaging Less Important Coordinates



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Super-Resolution Microscopy Techniques

Szabolcs Osváth

Semmelweis University

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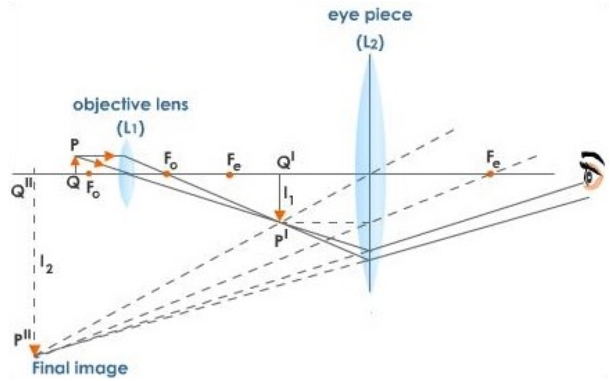
Diagram of the Compound Microscope



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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.

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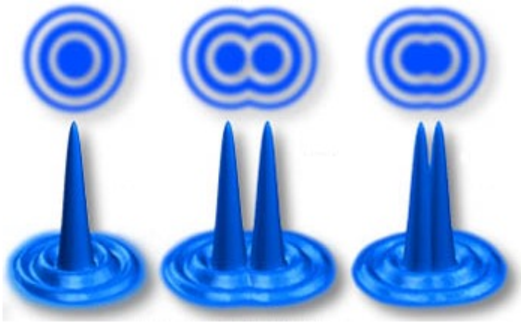
The Effect of the Wave Character of Light on the Image



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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.

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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.

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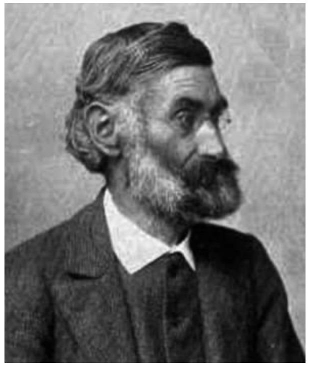
Ernst Karl Abbe (1840-1905)



Physicist and social reformer

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

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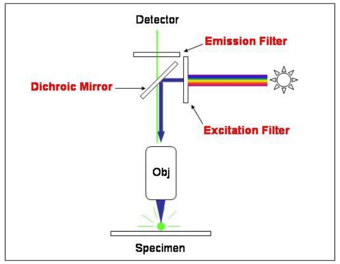
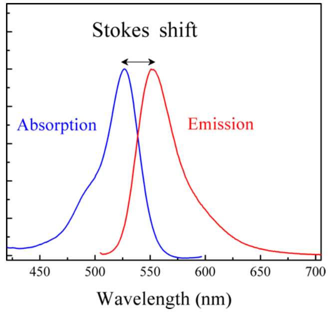
Fluorescence microscope





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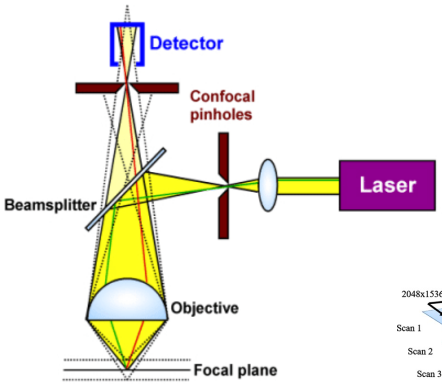
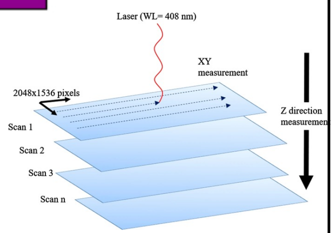
The confocal fluorescence microscope





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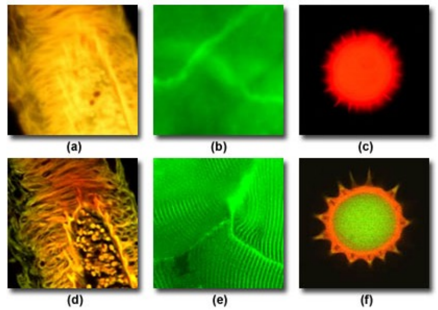
Comparison of the Epifluorescent and Confocal Microscopes

epifluorescent

confocal



human medulla rabbit muscle pollen

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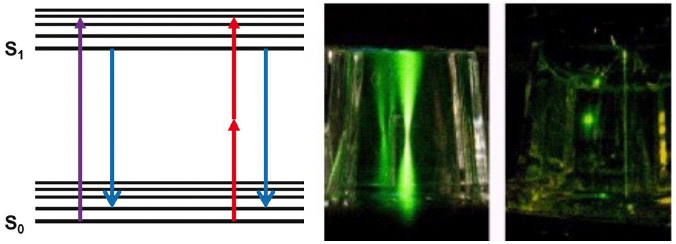
Two photon microscope



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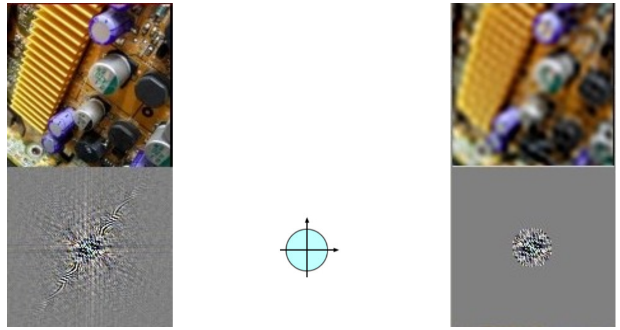
Abbe's Principle in the Wavenumber Representation



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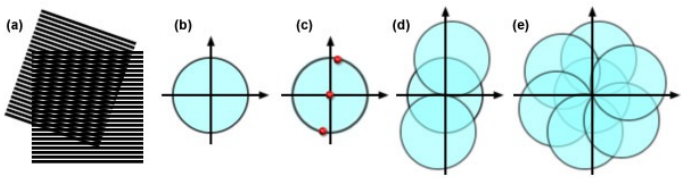
Structured illumination microscope



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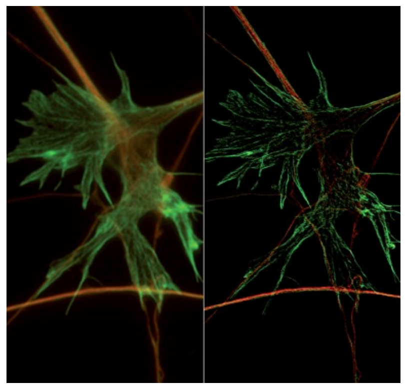
Structured Illumination Microscope



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

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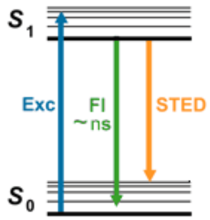
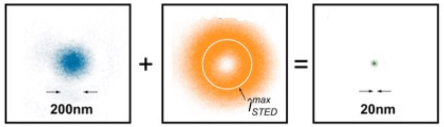
STimulated Emission Depletion (STED) Microscope



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

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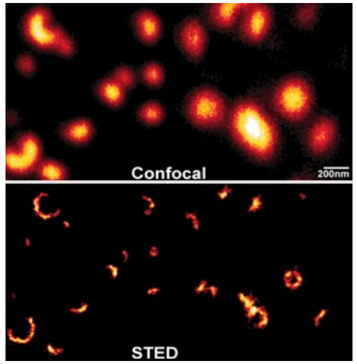
STimulated Emission Depletion (STED) Microscope



Organization of synaptolysin in reused synaptic vesicles.

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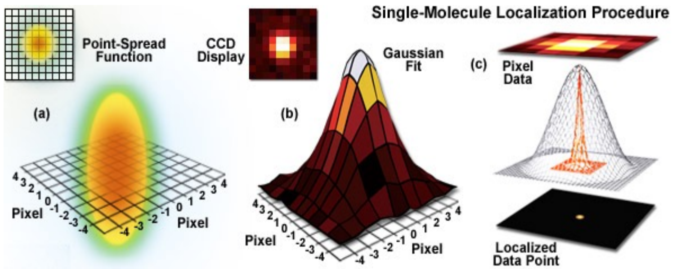
Localization



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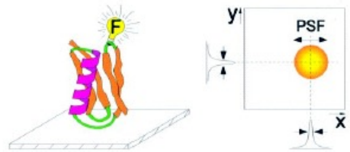
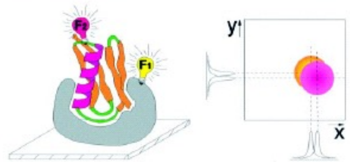
Localization and Co-Localization



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



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

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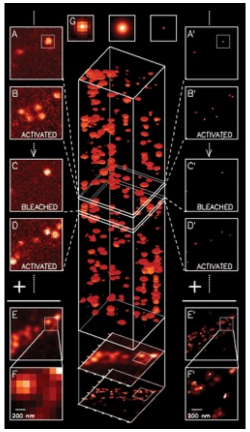
Photo-Activated Localization Microscopy (PALM)



Invented by Eric Betzig and Harald Hess

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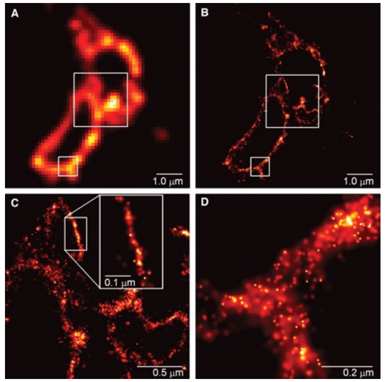
Photo-Activated Localization Microscopy (PALM)

CD63, lysosome transmembrane protein



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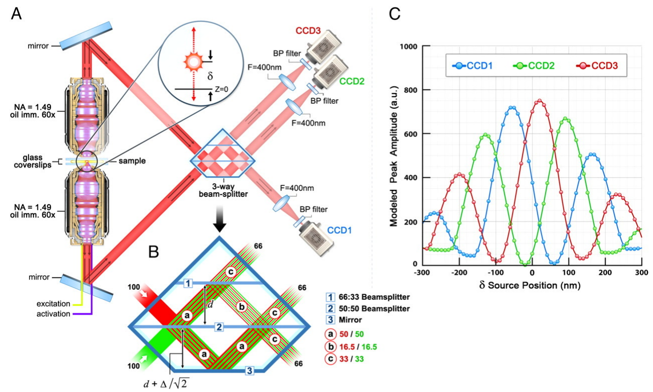
Interferometric Photo-Activated Localization Microscopy (iPALM)



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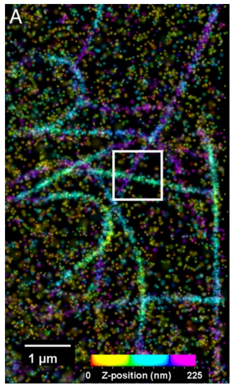
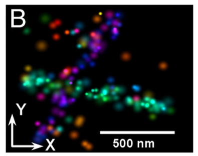
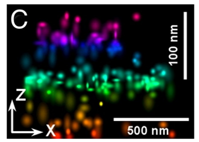
Interferometric Photo-Activated Localization Microscopy (iPALM)



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





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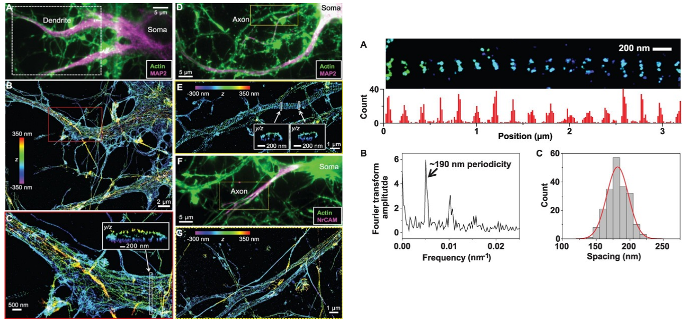
Cytoskeletal Structure of Axons



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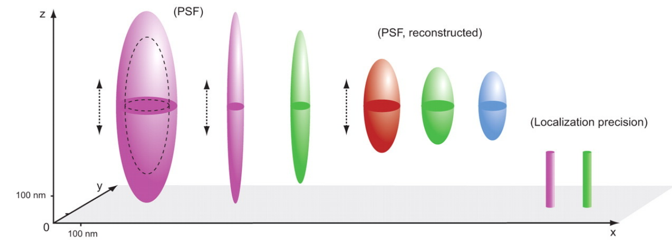
Comparison of Different Super-Resolution Techniques

	CLSM	STED	CW-STED	3D-SIM			PALM/STORM	
$\sim\lambda_{em}$ [nm]	460-670	670	520	620	520	460	670	520



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

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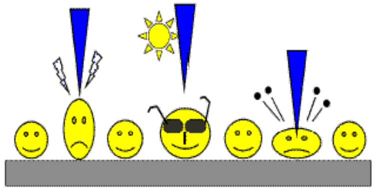
Scanning Probe Microscopy (SPM)

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.



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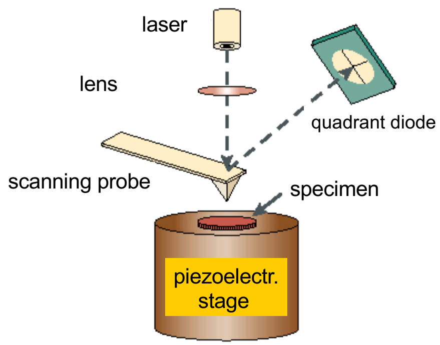
Atomic Force Microscopy (AFM)

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



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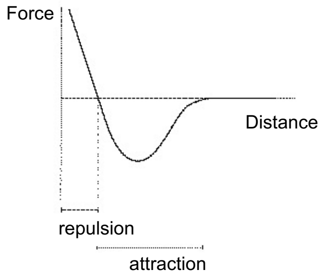
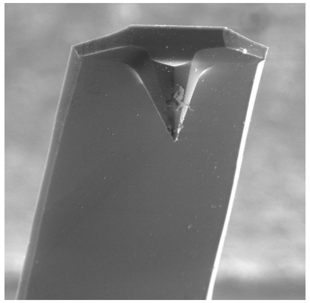
Force Between the Probe Tip and Specimen

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





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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.

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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

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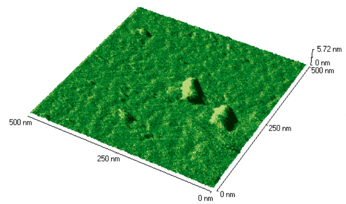
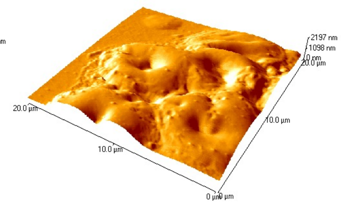
AFM Images of Biological Samples

Heat shock proteins



Red blood cells



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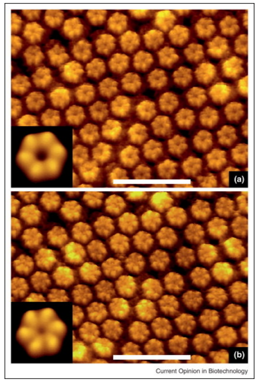
AFM Image of Extra-Cellular Connexon

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

The line is 23 nm long.



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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

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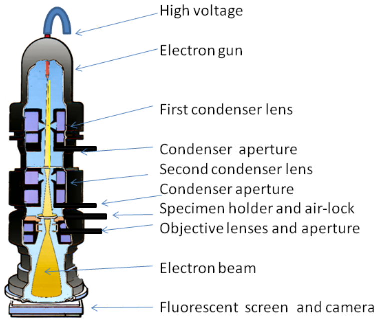
Transmission Electron Microscope



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.

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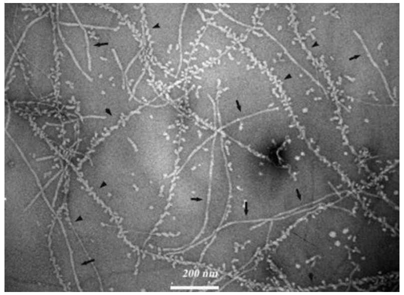
Amyloid Fibrils in Transmission Electron Microscope



Binding of cholesterol to amyloid fibrils.

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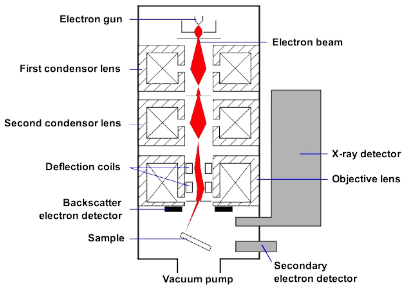
SEM



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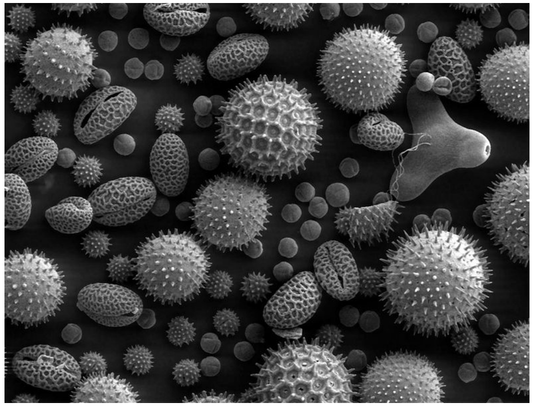
SEM Image of Pollen Particles



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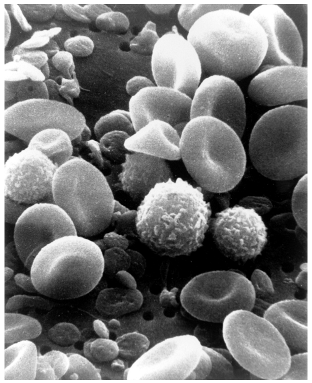
SEM Image of Blood Cells



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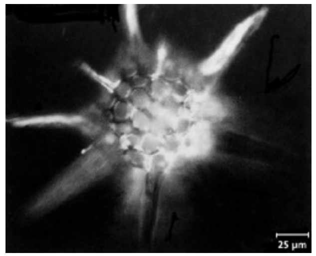
Comparison of the Optical and the Electron Microscope



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

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

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