Physical methods in bio-molecular studies

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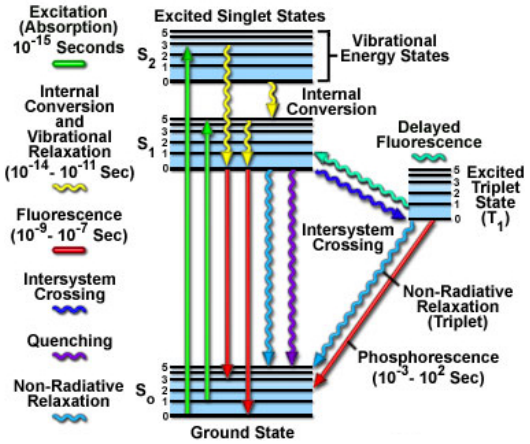
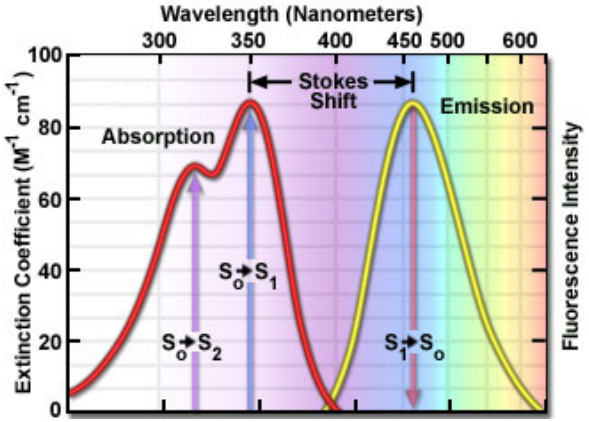
Light emission and absorption – Jablonski diagram

The Jablonski diagram is an energy diagram that illustrates the electronic states of a molecule and the transitions between them.

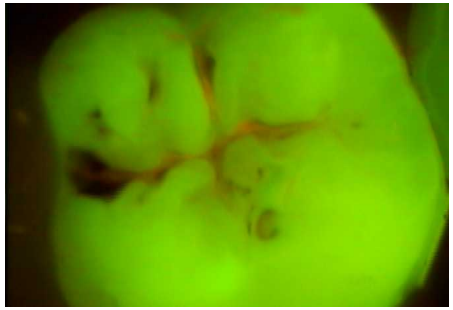


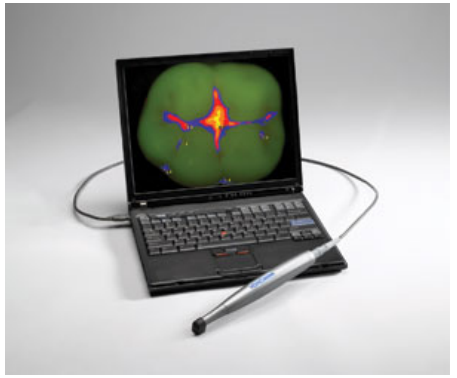
Light emission and absorption spectra

Stokes shift is the difference (in wavelength or frequency units) between the positions of the absorption and emission maxima.

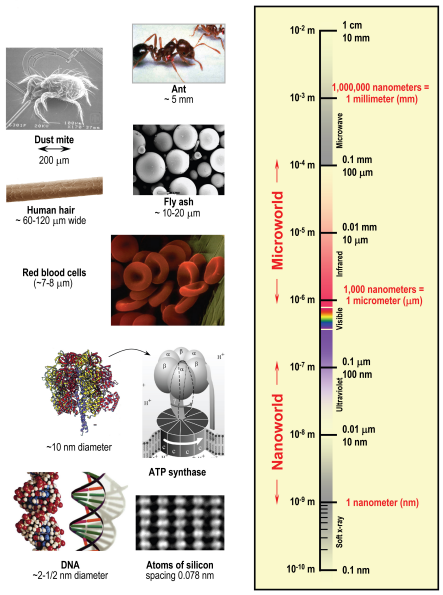


Quantitative Light-induced Fluorescence (QLF)



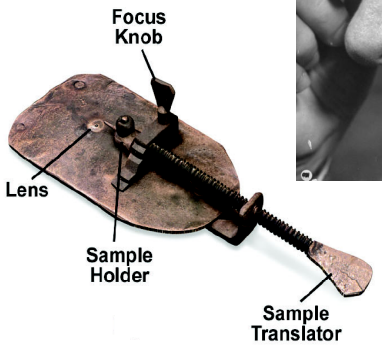


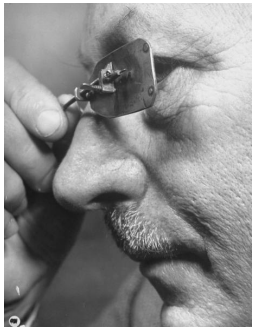
How big are things?



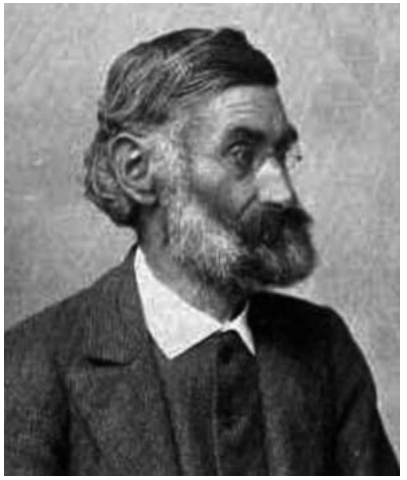
Hans Jansen and Zacharias Jansen construct a compound microscope in 1590

Antoni van Leeuwenhoek (Thonis Philipszoon) 1632-1723 constructs a simple microscope in 1674





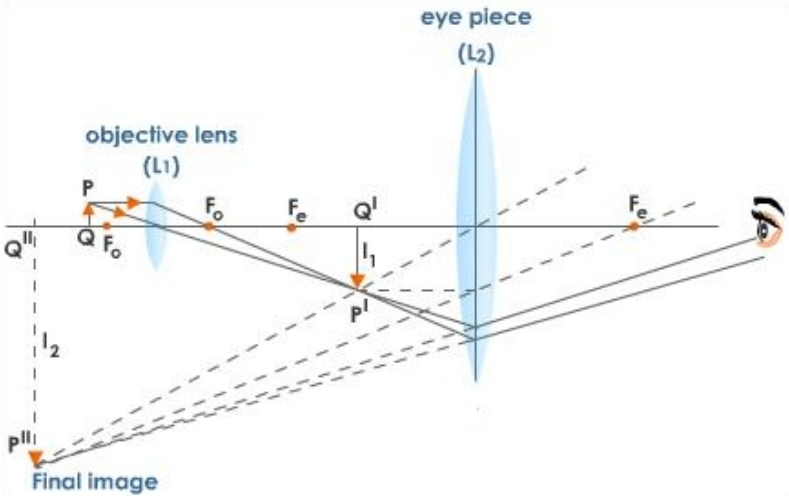
Ernst Karl Abbe (1840-1905)



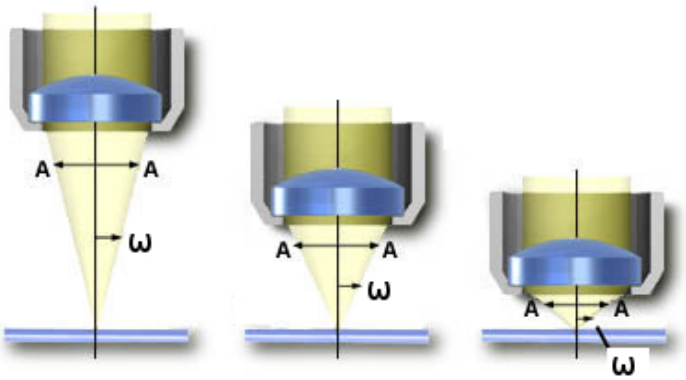
Physicist and social reformer

He put the production of optical devices on scientific bases.

Light path in the compound microscope



Numeric aperture

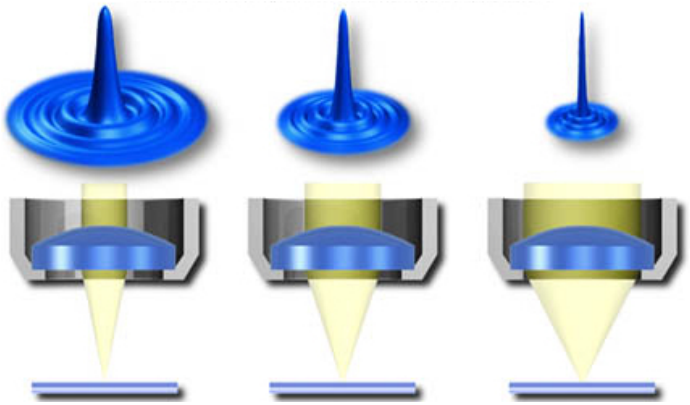


Point Spread Function (PSF)

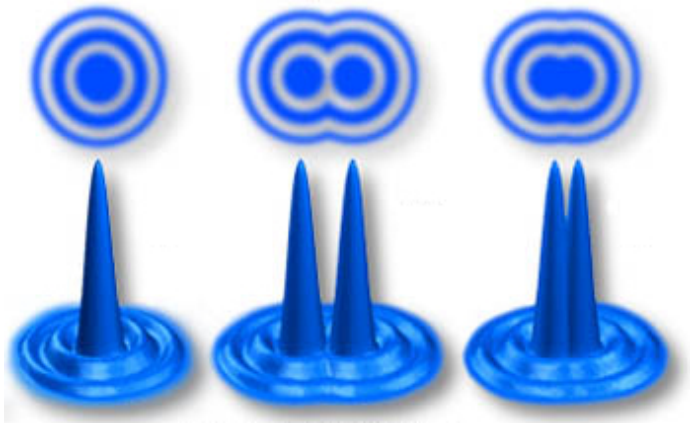
The image of a single point of an (fluorescent) object is not a point but a spot. This effect is the consequence of the wave nature of light.

The objective focuses light in a volume and not into one point.

The effect of the numeric aperture on the PSF



The effect of the wave nature of light on the image

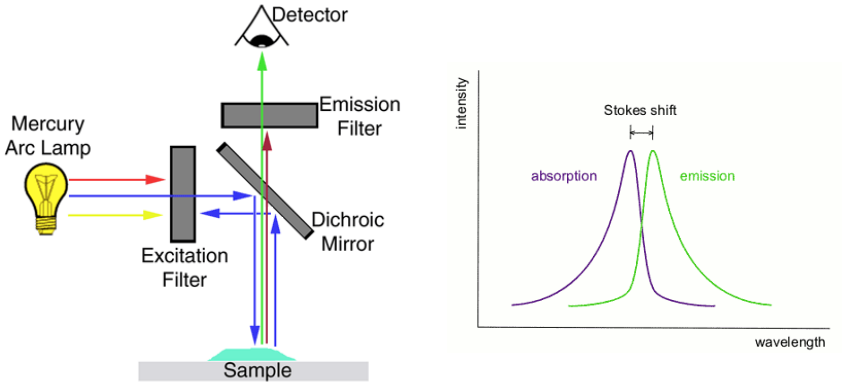


Abbe formula

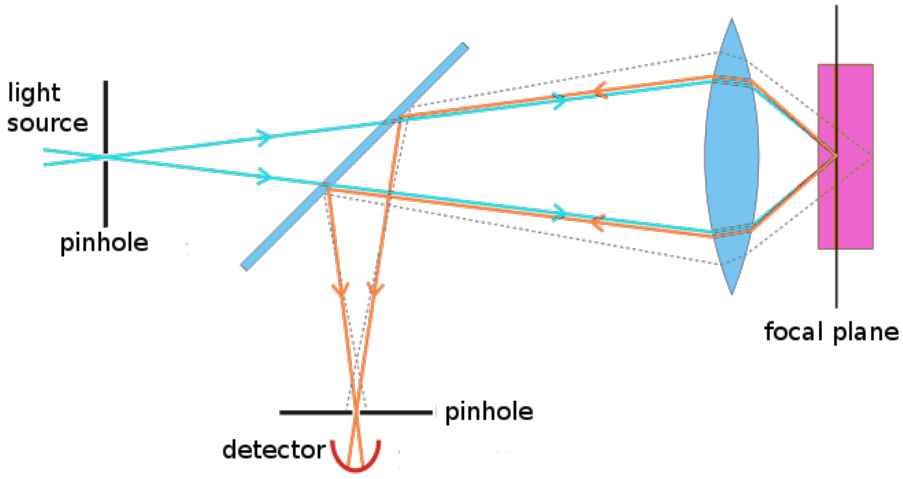
$\delta = 0{,}61 \cdot \lambda / (n \cdot \sin\omega)$

It was implicitly assumed that:
- we image the different parts of the sample at the same time
- we distinguish the different points of the sample by distinguishing the diffraction limited spots that belong to them in image

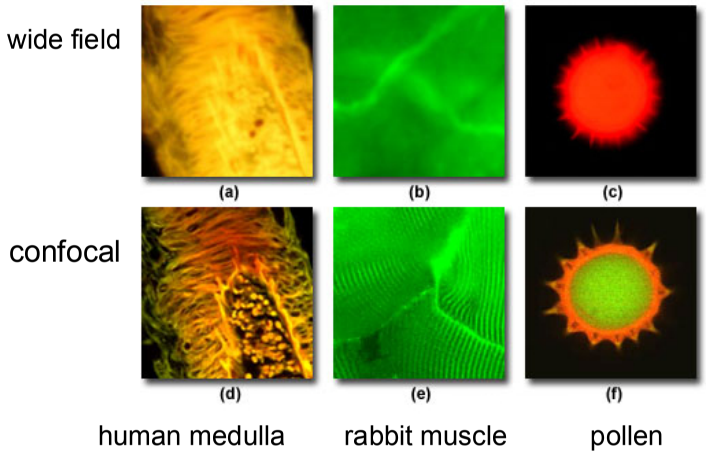
Fluorescence microscope



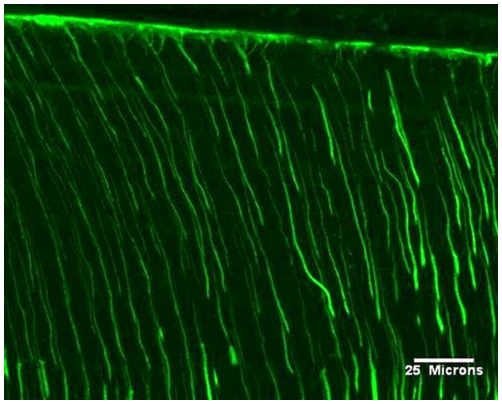
The working principle of the confocal microscope



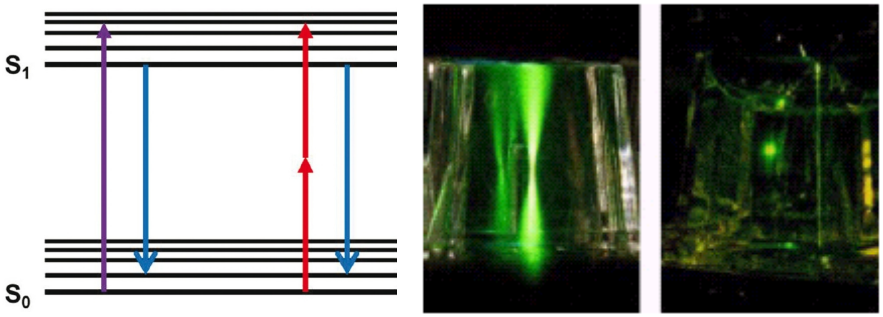
Comparing the wide field and confocal images



Dentinal tubules of intact human tooth



The working principle of the two-photon microscope



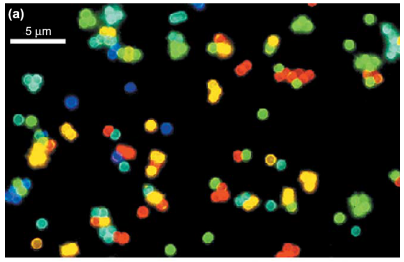
The ideal fluorophore

- small
- hydrophilic
- absorbs and emits in the visible region
- has large Stokes shift
- specific binding
  (biotin/avidin, His-tag/Ni, antibody/antigen, NH2, SH)
- bright (absorption*fluorescence efficiency)
- does not burn
- does not make photochemical reactions
- does not blink

Fluorescent quantum dots

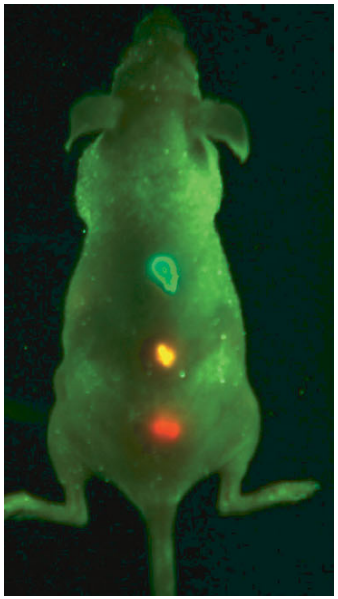
(a) fluorescence microscopic image of quantum dots made of CdSe with ZnS coating

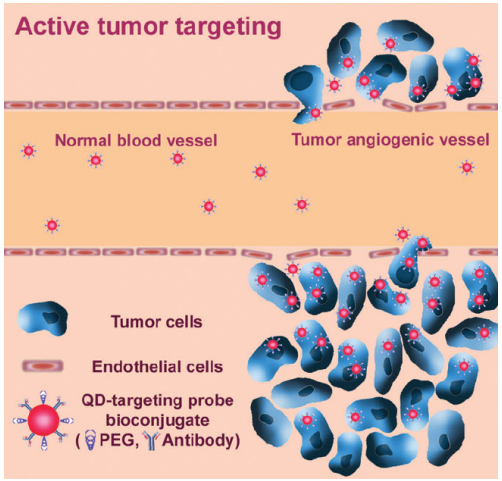
The size of the quantum dots determines their color.



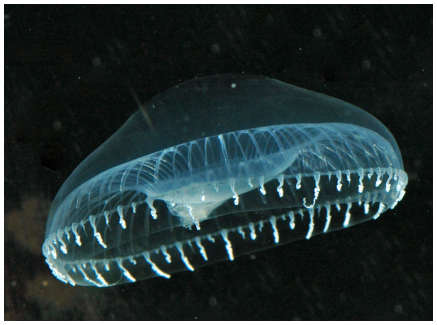
(b) ten solution containing CdSe/ZnS quantum dot of ten different sizes, thus fluorescing in ten different colors

Tumors labeled in vivo with fluorescent quantum dots

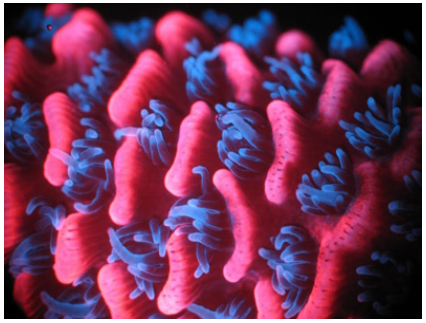




Fluorescent proteins



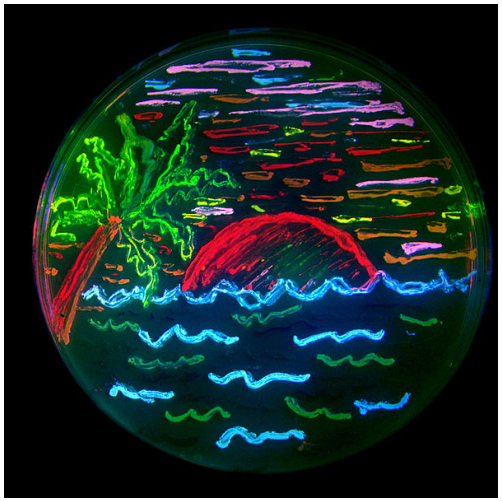
Aequorea victoria
(jellyfish)



Acropora millepora
(coral)

A large variety of fluorescent proteins is available

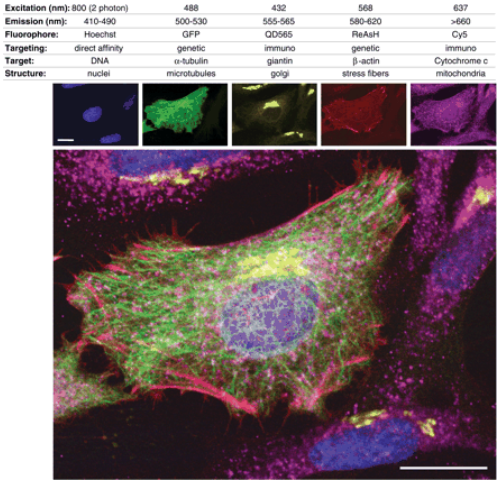
A picture painted entirely using bacteria expressing fluorescent proteins.



Parallel use of several fluorescent labels

HeLa cells stained with five different fluorescent dyes.

The bar is 20 μm long.



Study of single molecules

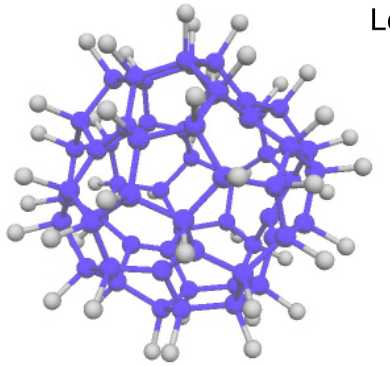
"Plenty of Room at the Bottom"

" The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom. It is not an attempt to violate any laws; it is something, in principle, that can be done; but in practice, it has not been done because we are too big."

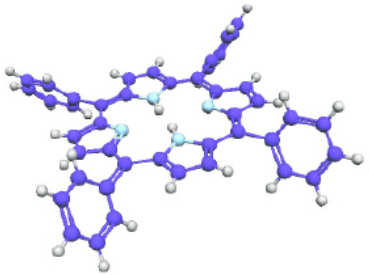
Richard Feynman, 1959

Wave-particle duality

Louis De Broglie: $\lambda = h/p$

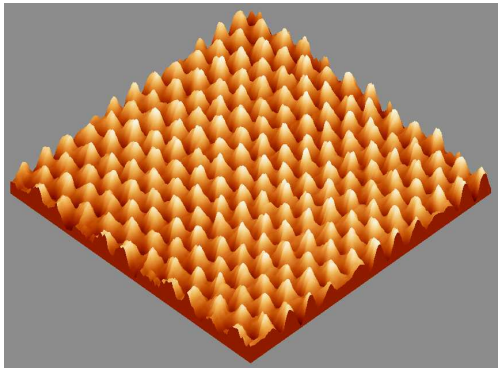


fluorofullerene $C_{60}F_{48}$
1632 Da



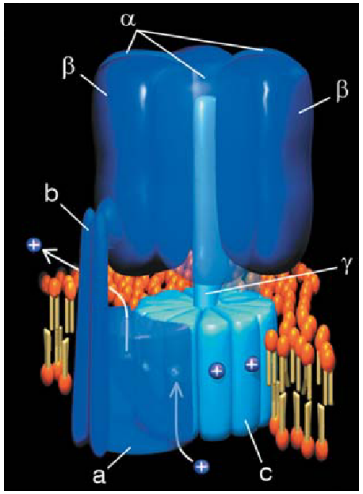
tetraphenilporphirin $C_{44}H_{30}N_4$

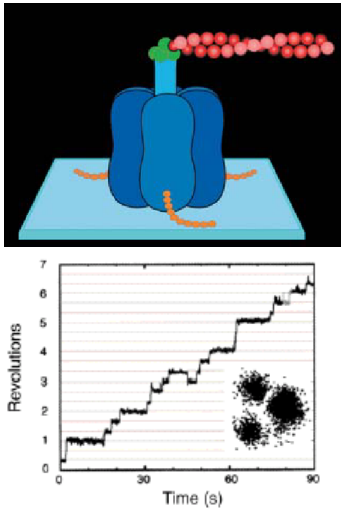
Wave-particle duality



Scanning Tunneling Microscope (STM) image of a graphite surface

Rotating movement of single ATP sintase molecules





Localization

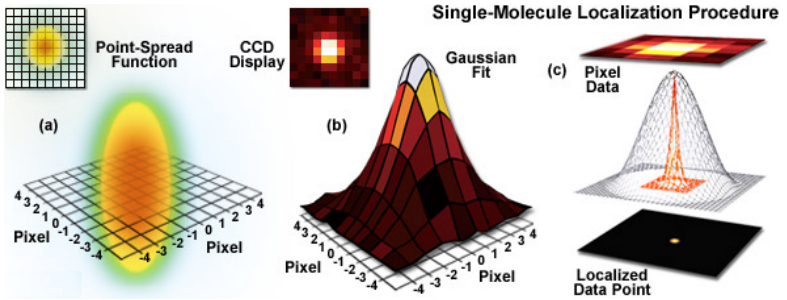
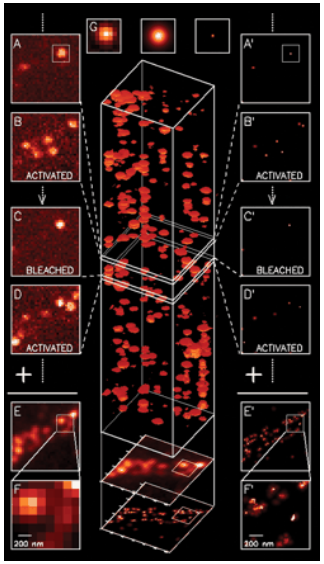


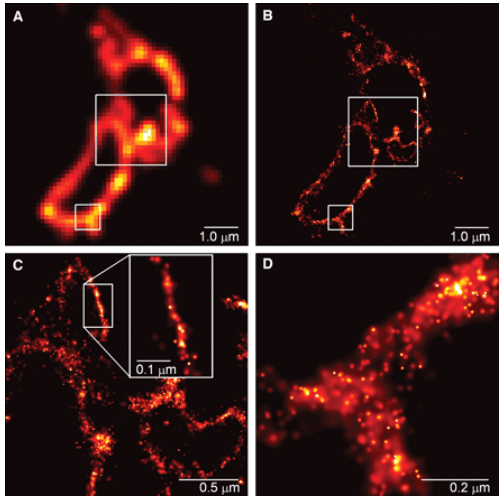
Photo-Activated Localization Microscopy (PALM)



Based on the technology developed by Eric Betzig and Harald Hess

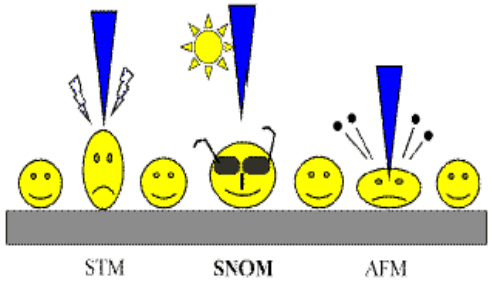
Photo-Activated Localization Microscopy (PALM)

CD63, lizosome transmembrane protein



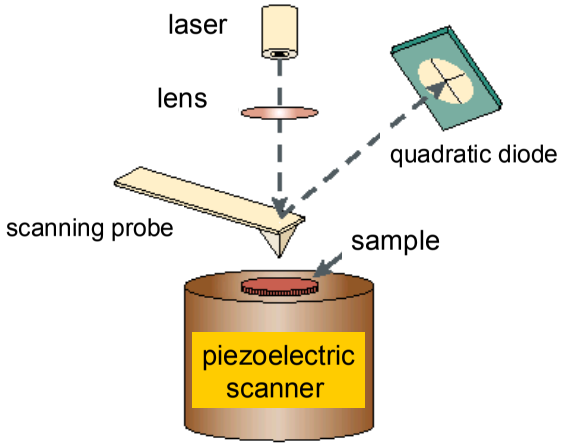
Scanning Probe Microscopy (SPM)

A topographic image of the surface of the sample is created by scanning the sample surface with a sharp probe and detecting interaction with the surface.



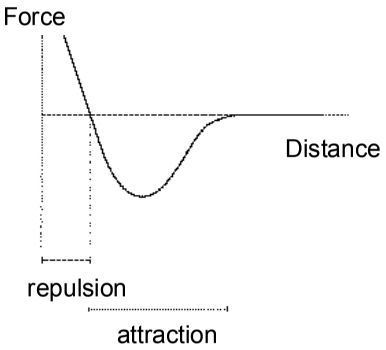
Atomic Force Microscopy - AFM

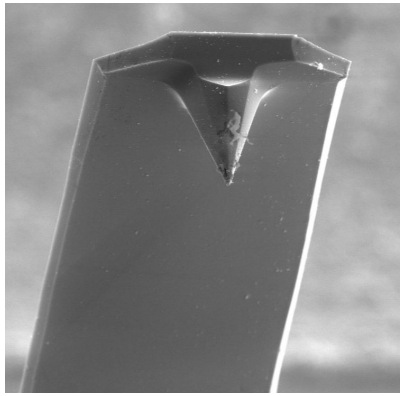
AFM: the detected interaction is the force between the sample surface and probe



Force between the probe tip and the sample

Properties of the probe:
- typically 100 μm long, 1μm thick, V shaped
- small spring constant
- high resonance frequency
- silicon (-oxide, -nitride)





Contact Mode AFM

The probe and the sample are is permanent contact.
Works in the repulsive region.
Keeps the interaction force constant and follows the surface.
The vertical deflection of the cantilever is detected.
Local force spectroscopy: record the interaction force as a function of displacement in one point of the surface.

Tapping Mode AFM

The probe oscillates with 20-100 nm amplitude touching the surface in every oscillation.
The oscillation frequency and amplitude and phase changes according to the topography of the surface.

Advantages and drawbacks

Contact Mode AFM

Advantage:
fast scanning
atomic resolution
good for hard surfaces

Drawback:
horizontal forces distort the image
water on the surface distorts the image
scratches soft biological samples

Tapping Mode AFM

Advantage:
large lateral resolution (1 – 5nm)
less damage to soft samples

Drawback:
slow scanning

AFM image of the extracellular connexon surface

Calcium induced conformational changes of the extracellular connexon surface.

Scale bars represent 250 Å