Department of Medical and Biological Physics

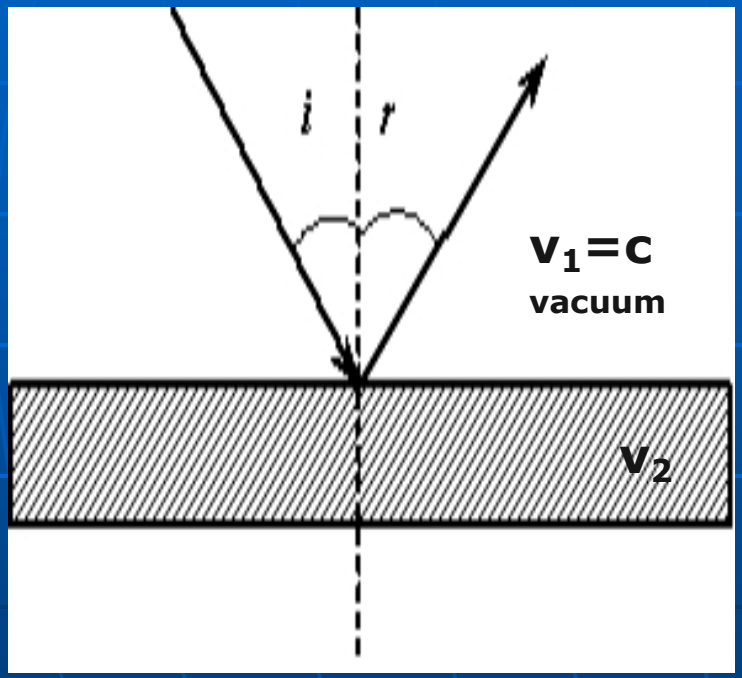
Geometrical and physical optics. Microscopy

Lecture 5

Overview

- Geometrical optics. Optical fibers.
- Microscopes. Biophysics of the vision.
- Light dispersion.
- Interference of light.
- Diffraction of light. Diffraction grating.
- Polarization of light.
- Polarimetry. Optical activity.

Geometrical optics: Law of reflection

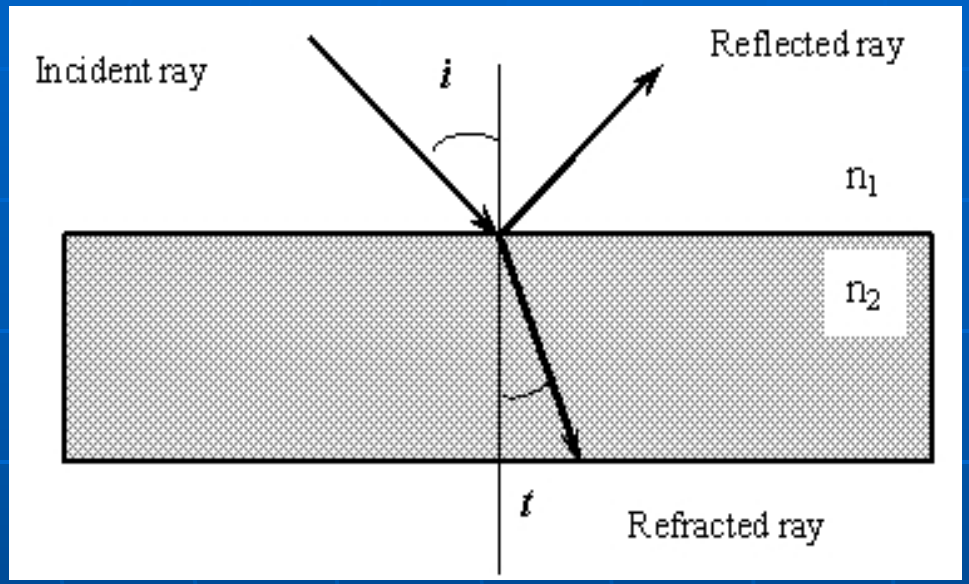


1) The incident ray, the normal, and the reflected ray all lie in the same plane.
2) *i=r*

the **absolute refractive index**

$$n = \frac{c}{v_2}$$

Geometrical optics: Law of refraction



1) The incident ray, the normal, and the refracted ray all lie in the same plane

2)

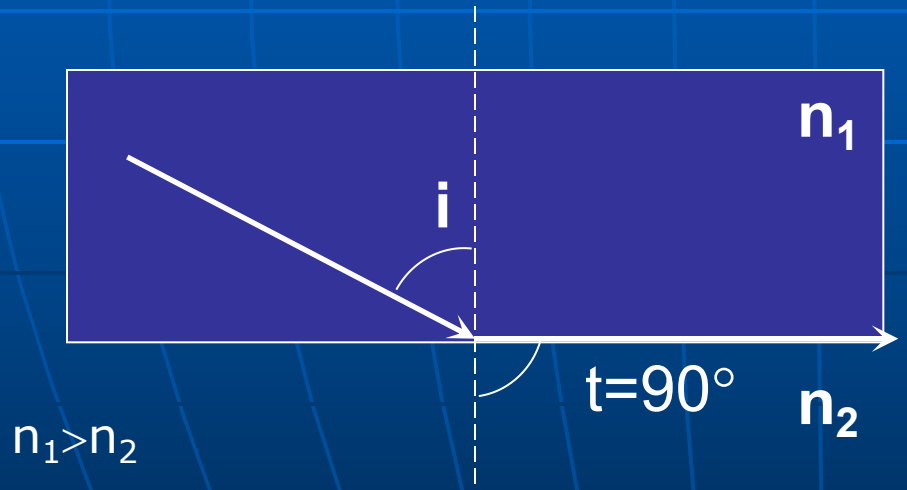
$$\frac{\sin i}{\sin t} = \frac{v_1}{v_2}$$

Where v_1 / v_2 is the **relative index of refraction** of medium 2 with respect to medium 1:

$$\frac{v_1}{v_2} = n_{21} = \frac{n_2}{n_1}$$

Geometrical optics: Total internal reflection

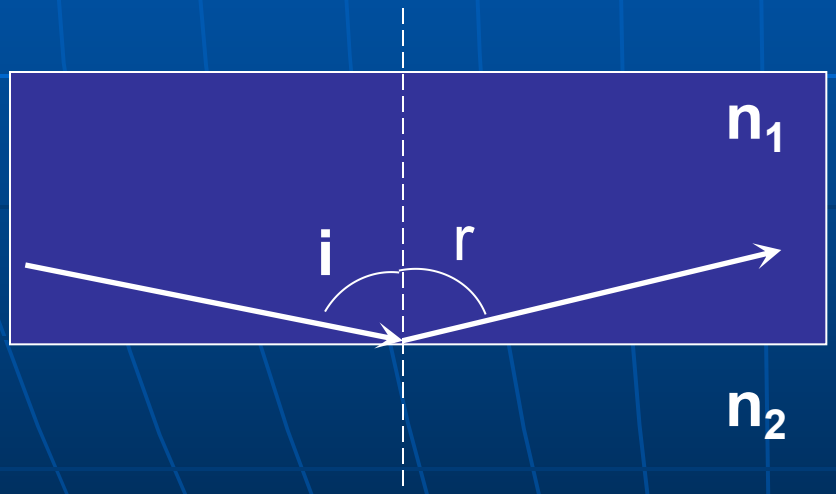
- The angle of incidence that causes the refracted ray to bend through 90° is called the **critical angle of incidence**.



$$\frac{\sin i_{critical}}{1} = \frac{n_2}{n_1}$$

Geometrical optics: Total internal reflection

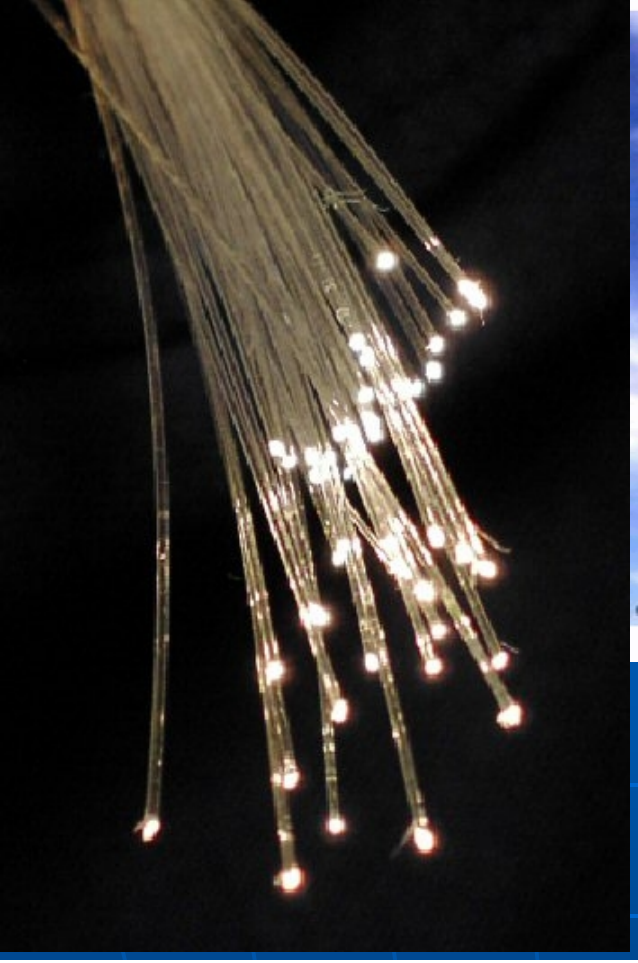
- When the incident angle becomes greater than the critical angle, no refraction occurs, all the light is reflected. This condition is called a **total internal reflection**.

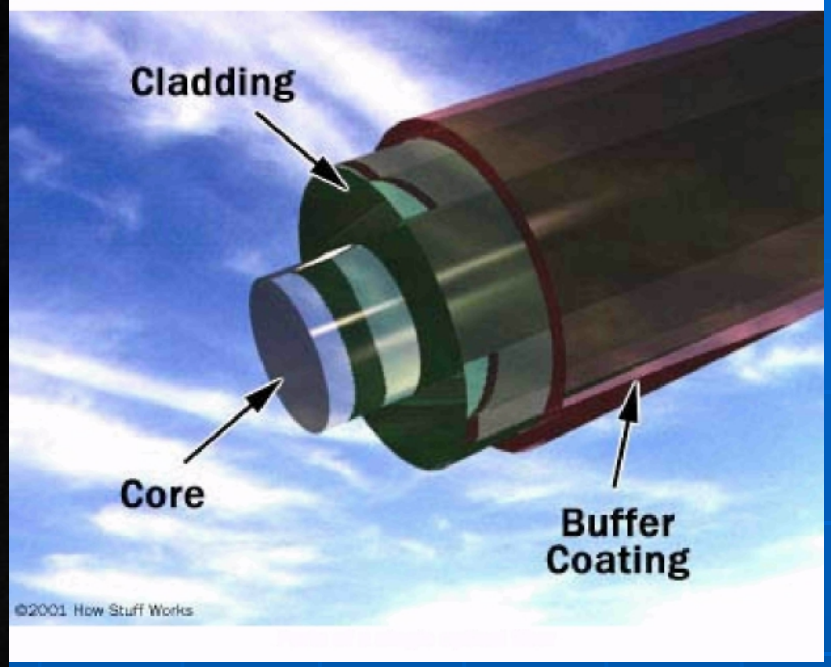


Total internal refraction can only occurs when light travels from a denser medium to a rarer medium:

$n_1 > n_2$

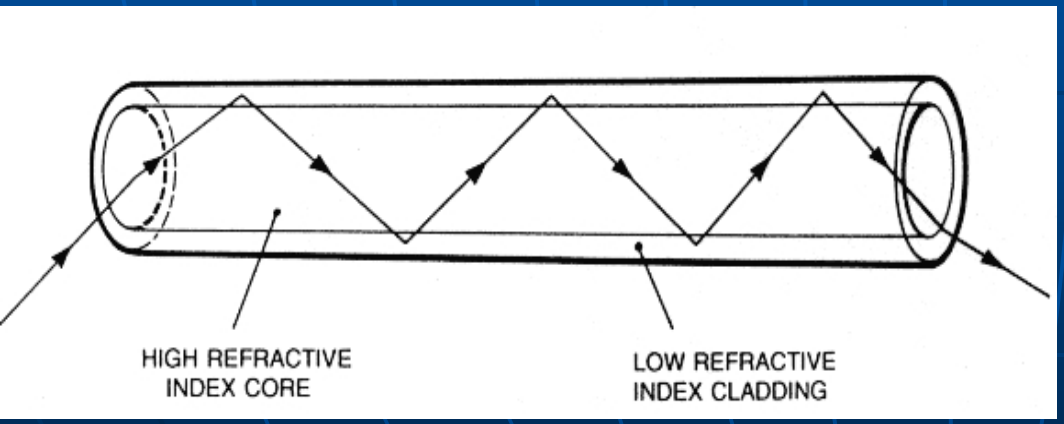
Optical fibers





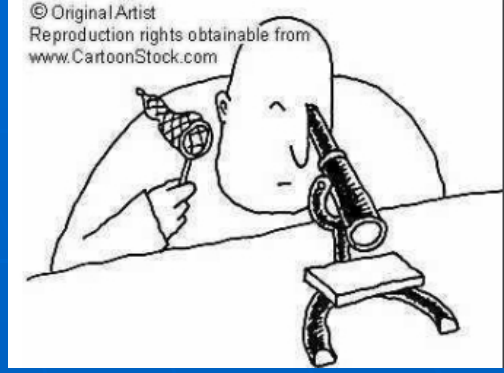
An **optical fiber** is a thin fiber of glass or plastic that can carry light from one end to the other

1) no significant energy losses
2) total internal reflection
3) used in medical imaging in bronchoscopes, endoscopes, laparoscopes.

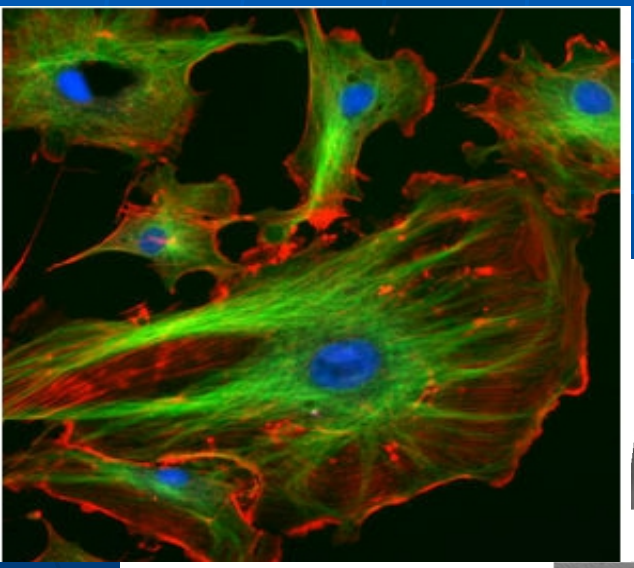


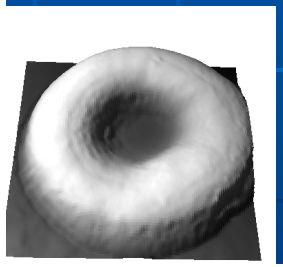
Microscope

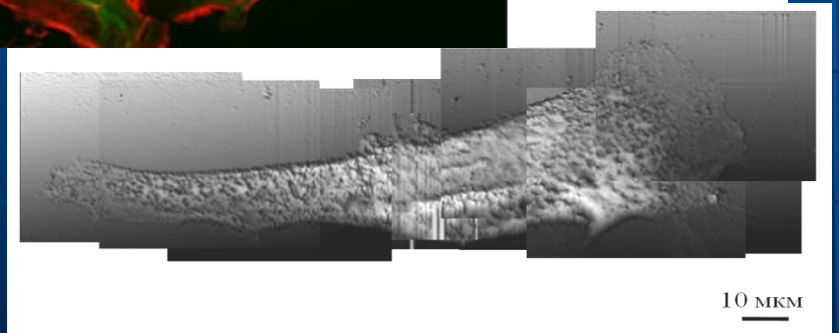
- **A microscope is an instrument used to produce enlarged images of small objects.**

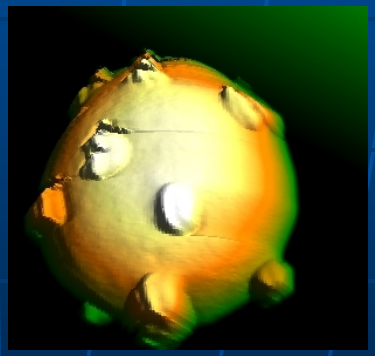


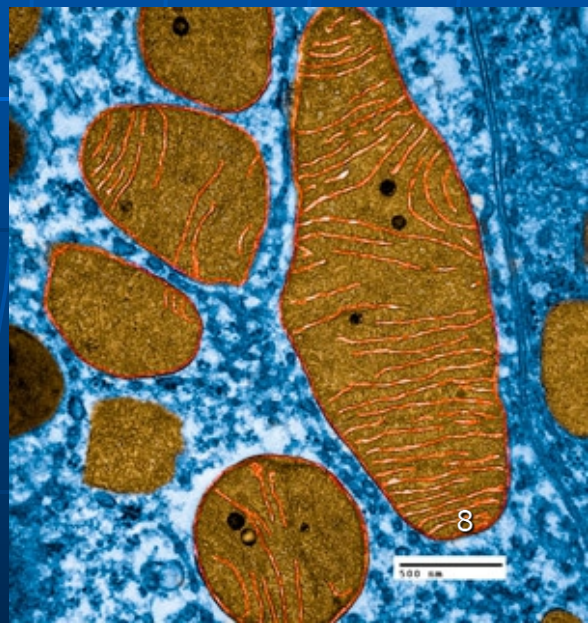
The most common kinds of microscope are optical microscope, electron microscope, scanning probe microscope (atomic force microscope).









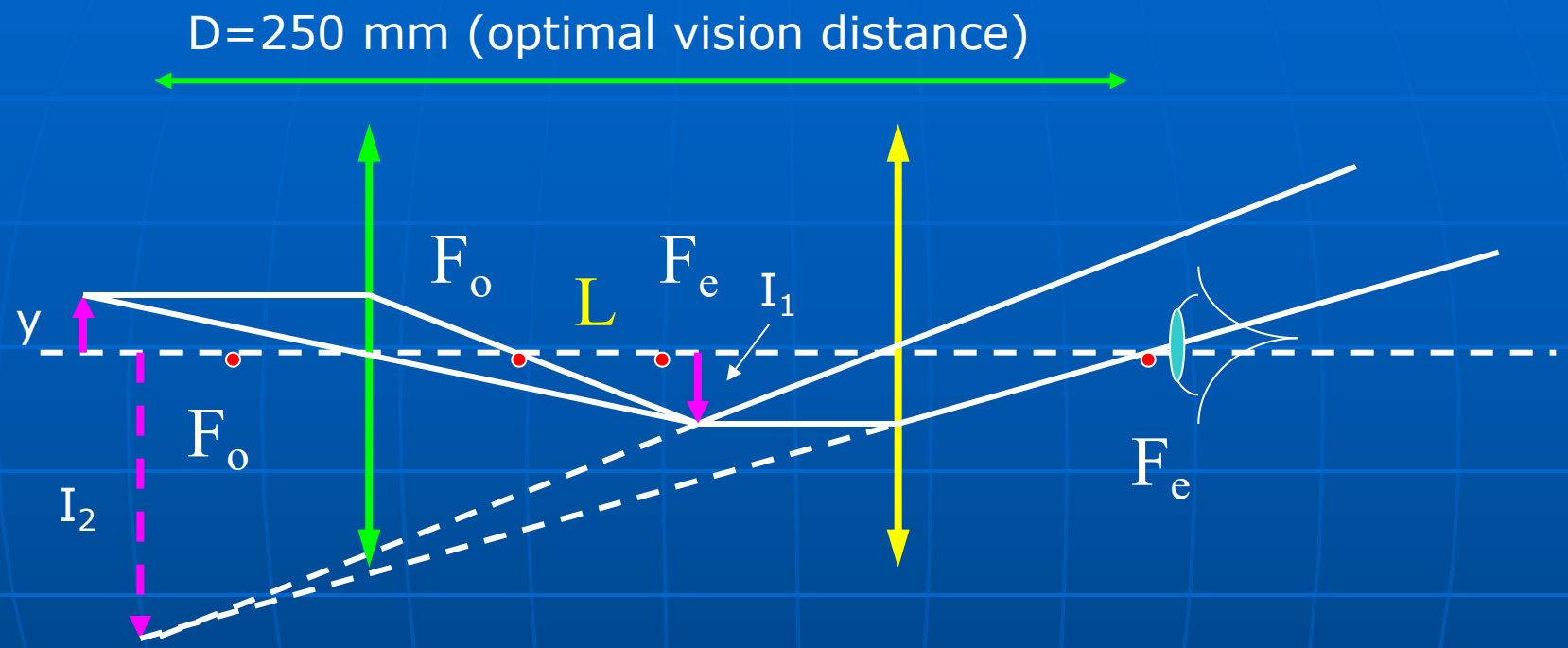


Optical compound microscope

- Ocular (eyepiece)
- Objective

- Condenser

Magnification of compound microscope

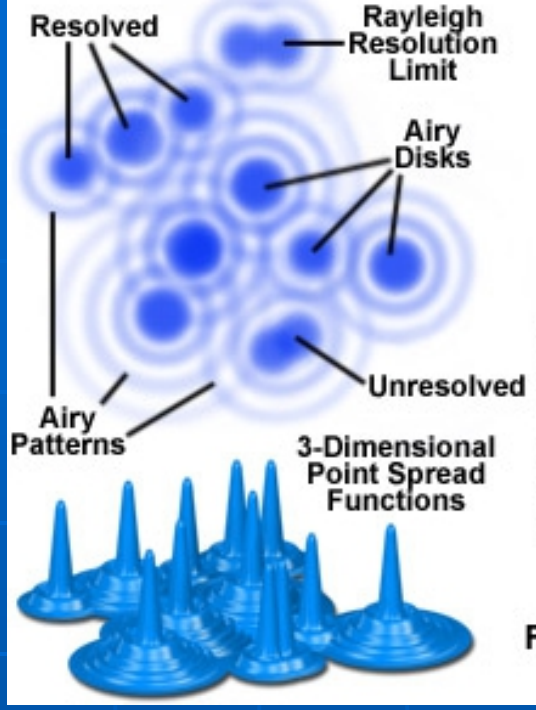


$$M = \frac{I_2}{y} = \frac{I_2}{I_1}\ \frac{I_1}{y} = M_e\ M_o = \frac{250}{F_e}\ \frac{L}{F_o}$$

L is a tube length, the distance from a focus F_o to a focus F_e.

Resolution of microscope

- Resolution limit is the least distance between two points or lines at which they are seen as two separate objects rather than a single blur.
- The resolution limit of microscope is determined primarily by the resolution limit of the objective:



$$z = \frac{1.22 \ \lambda}{2 \ n \ \sin u} = \frac{0.61 \ \lambda}{NA}$$

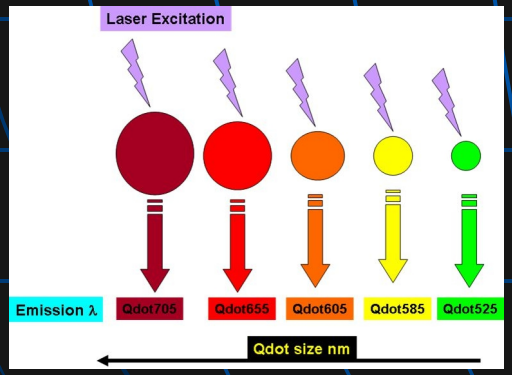
where u is the semi-angle of the cone of rays collected by the objective, λ is wavelength. The product ***n*** and ***sinu*** is called the numerical aperture (NA)

To decrease the resolving limit we can:
1) decrease λ (UV and electron microscopy)
2) increase NA (immersion systems)
3) focus on a very thin layer (confocal microscopy)
4) reject lenses (ptychography)
5) use the fluorescent dyes and specificity of their response to excitation (fluorescence microscopy of super-resolution)

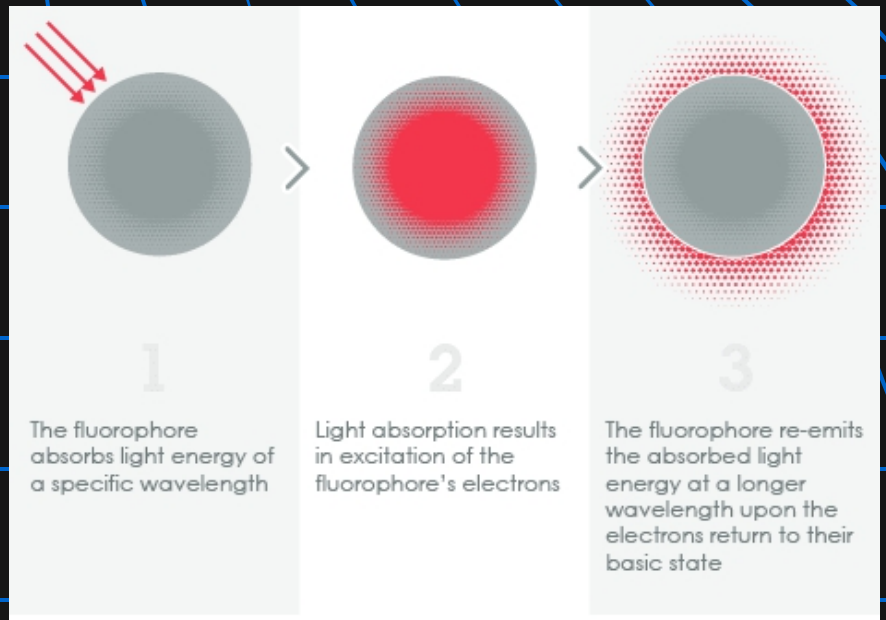
Fluorescence microscopy

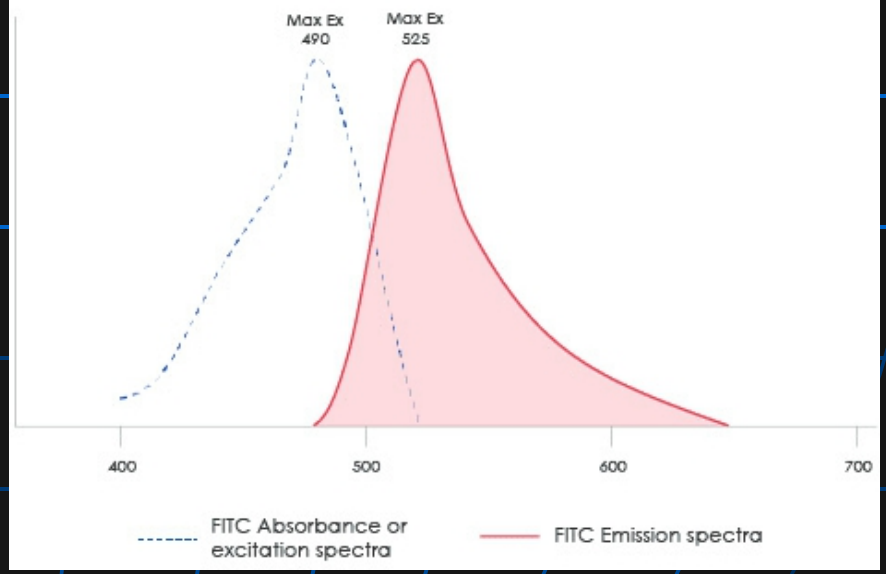
A fluorophore (or fluorochrome) is a fluorescent chemical compound that can reemit light upon light excitation.

Green fluorescent protein (*GFP*) is the protein first isolated from the jellyfish Aequorea victoria that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range.



Quantum dots

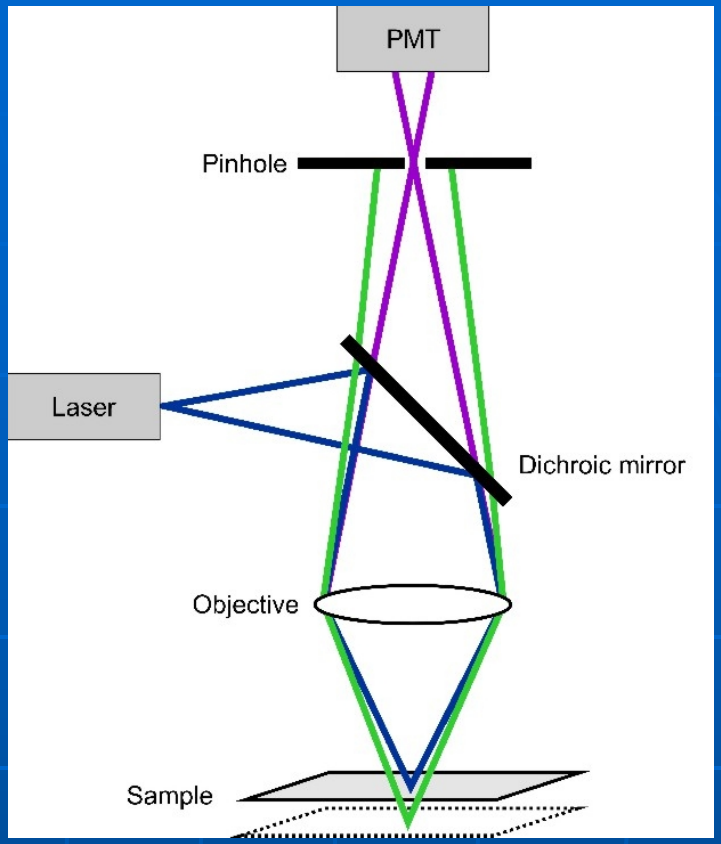


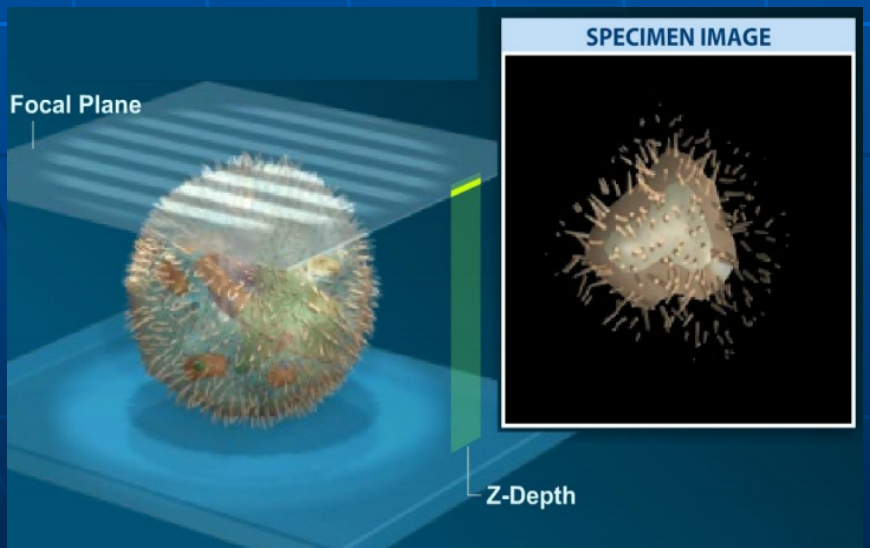


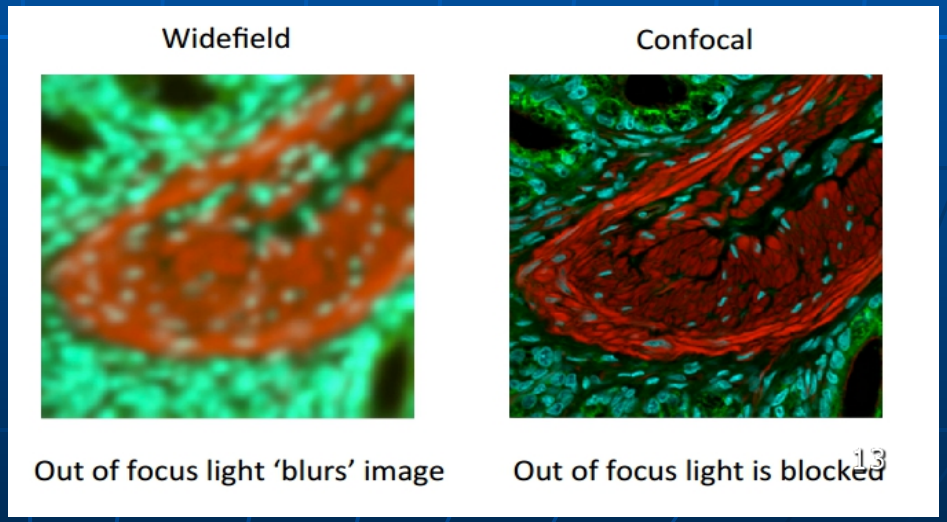
Qdots of the size of 10-20nm diameter are made up of a semiconductor core, shell of zinc sulphide, polymer coating and a layer of biomolecules-fluoriphores.

Confocal fluoresence microscopy

Confocal laser scanning microscopy (CLSM) is an optical imaging technique for increasing optical resolution and contrast of a micrograph by means of using a spatial pinhole to block out-of-focus light in image formation and allowing three-dimensional reconstructions of topologically complex objects.





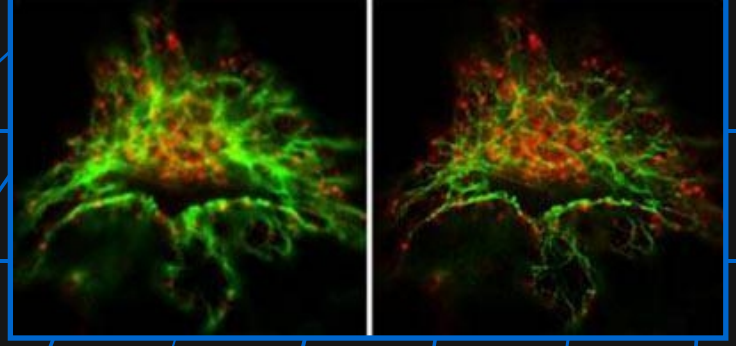


There are two major groups of methods for functional super-resolution light microscopy:

Deterministic super-resolution: The most commonly used emitters in biological microscopy, fluorophores, show a nonlinear response to excitation, and this nonlinear response can be exploited to enhance resolution. These methods include STED, GSD, RESOLFT and SSIM.

Stochastic super-resolution: The chemical complexity of many molecular light sources gives them a complex temporal behavior, which can be used to make several close-by fluorophores emit light at separate times and thereby become resolvable in time. These methods include Super-resolution optical fluctuation imaging (SOFI) and all single-molecule localization methods (SMLM) such as SPDM, SPDMphymod, PALM, FPALM, STORM and dSTORM.

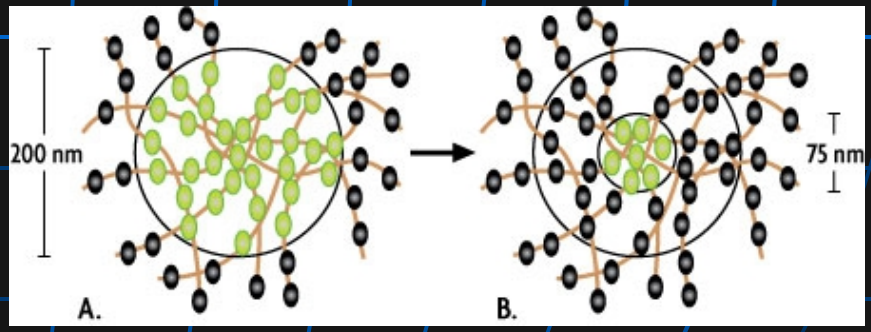
On October 8, 2014, the Nobel Prize in Chemistry was awarded to Eric Betzig, W.E. Moerner and Stefan Hell for "the development of super-resolved fluorescence microscopy," which brings "optical microscopy into the nanodimension".



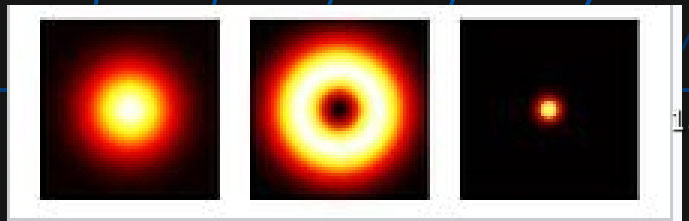
With the **STED** system, subcellular details below 80 nm can be visualized.
In figure, vimentin and clathrin were visualized by immunohistological co-staining. The image on the left was prepared using a confocal microscope, while that on the right was produced using a STED microscope.

Stimulated emission depletion (STED) microscopy

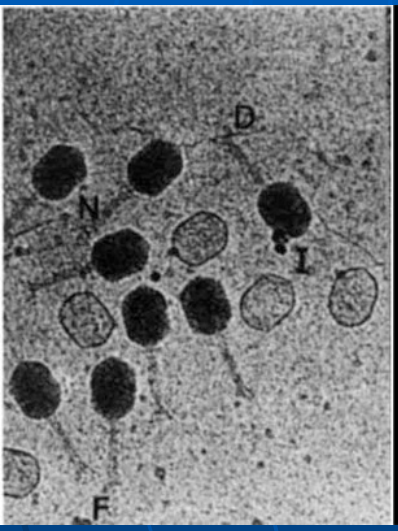
Principle of STED microscopy. Illustration of a 200 nm excitation spot of a classical confocal microscope (A) or the downsized emitting spot (~75 nm) created by a STED microscope (B, inner ring). The spheres represent individual dye molecules in fluorescent (green) or "off" mode (black).

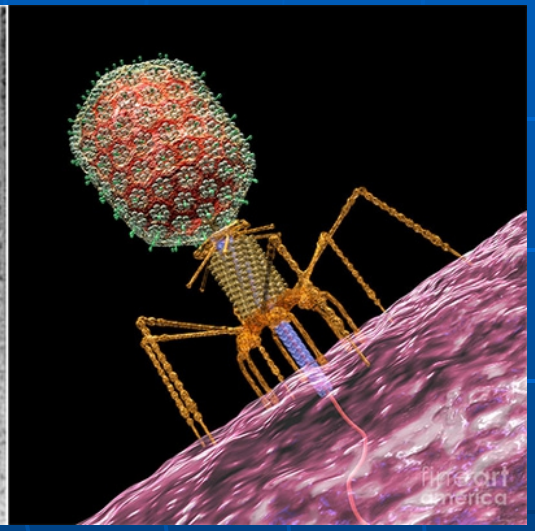


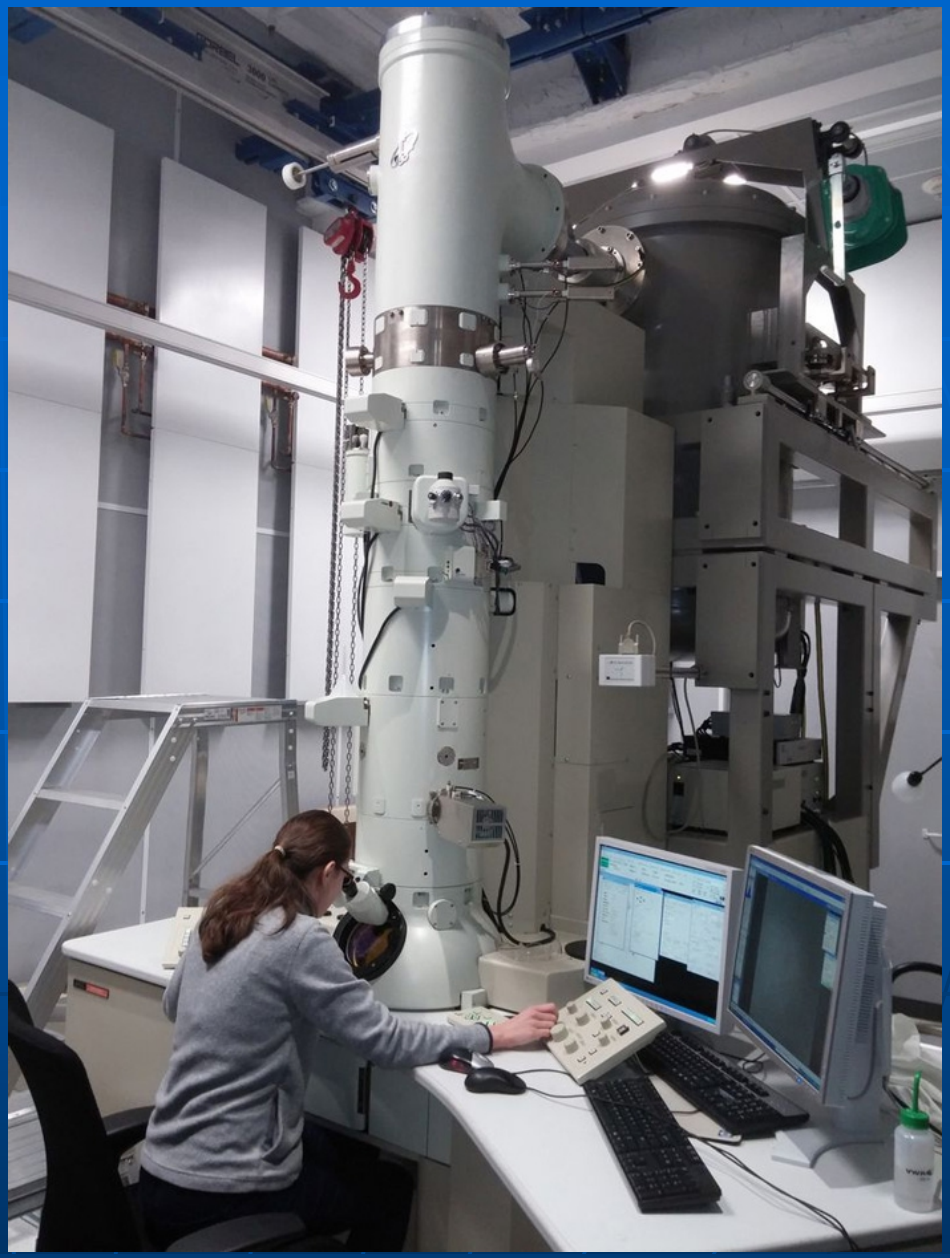
The basis of STED microscopy is the coupling of the excitation laser with the STED depletion laser, resulting in the doughnut-shaped depletion. The two perfectly aligned laser systems minimize the size of the fluorescence spot, overcoming the resolution-limiting effects of diffraction.



ELECTRON MICROSCOPY

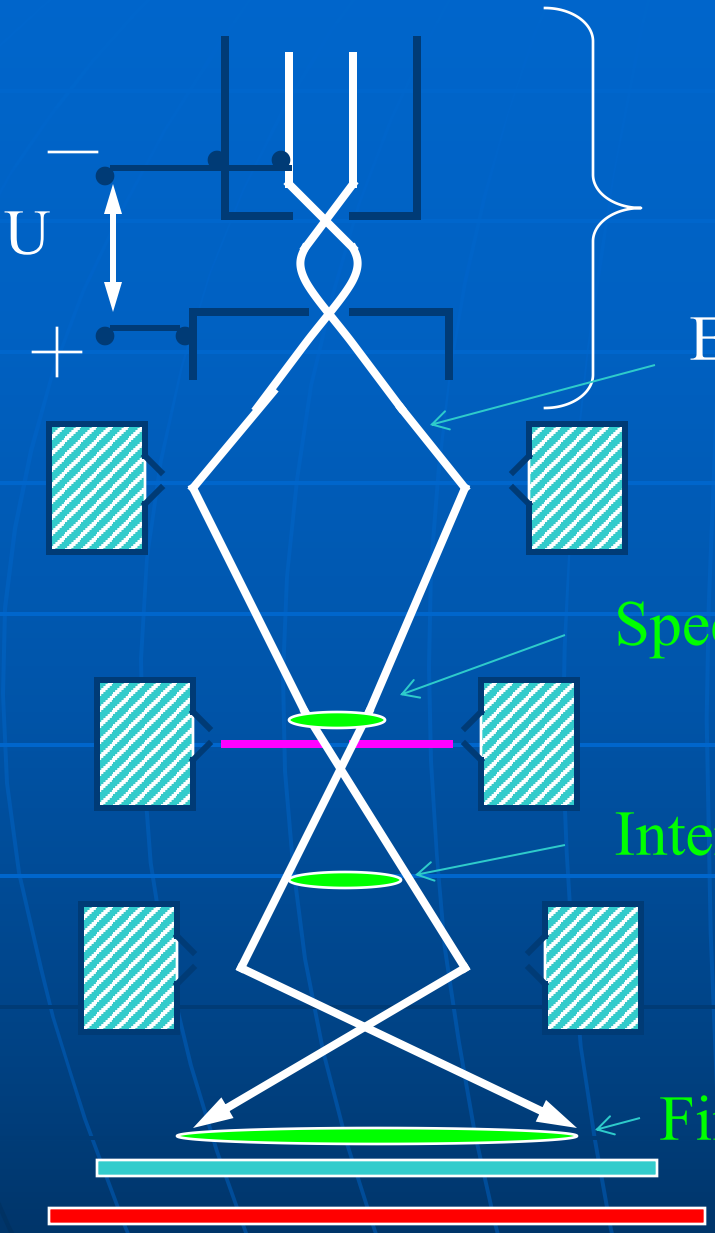






Electron microscopes uses electrons instead of photons, because electrons have a much shorter wavelength than photons and so allows you to observe matter with atomic resolution.

Electron microscope



Electron gun

Electron beams

Condenser lens (Electromagnets)

Specimen

Objective lens

Intermediate image

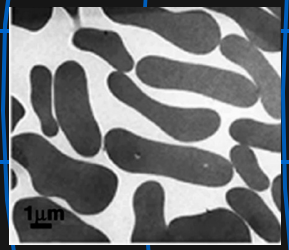
Projection lens

Final image

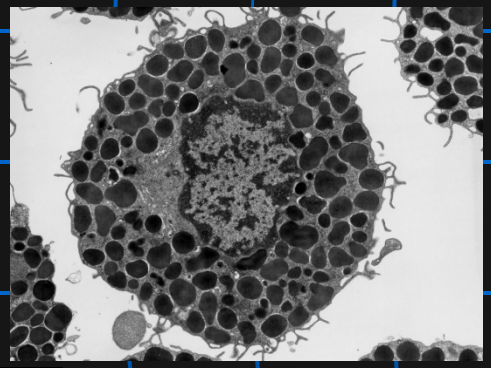
Luminescent screen or photographic plate

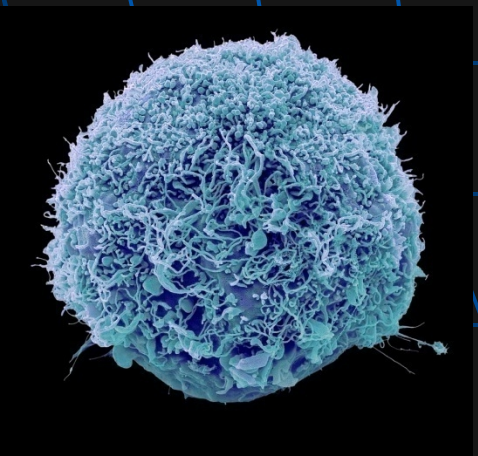
Types of electron microscope

1) Scanning Electron Microscope (SEM) that scans an electron beam over the surface of an object and measures how many electrons are scattered back.
2) Transmission Electron Microscope (TEM) that shoots electrons through the sample and measures how the electron beam changes because it is scattered in the sample.



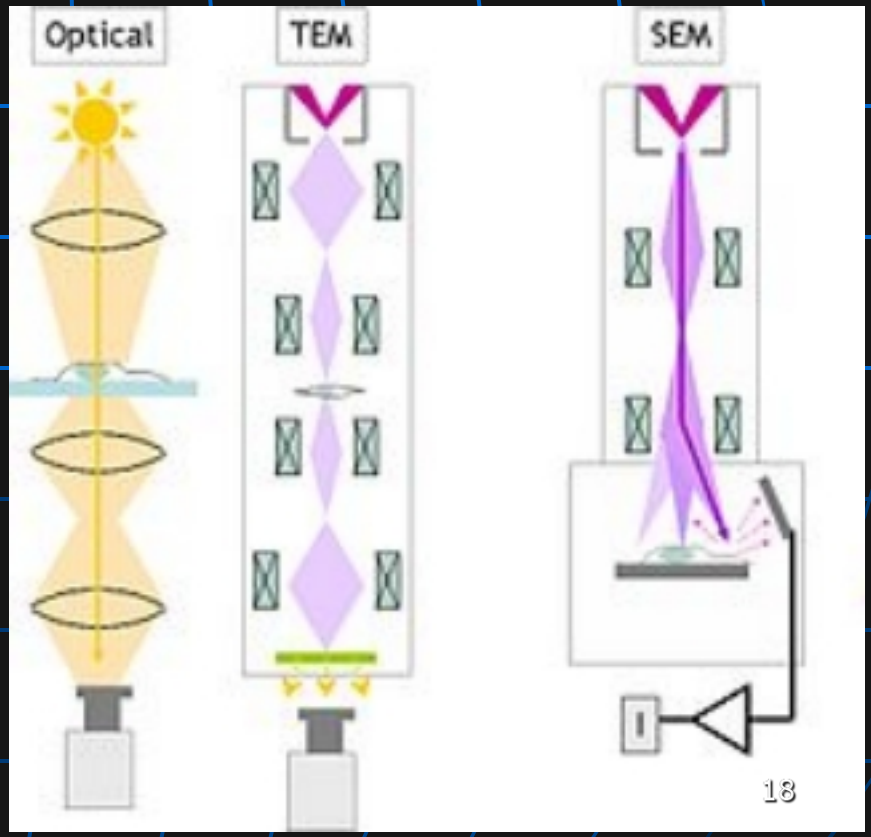
Erythrocytes
like dumbbells



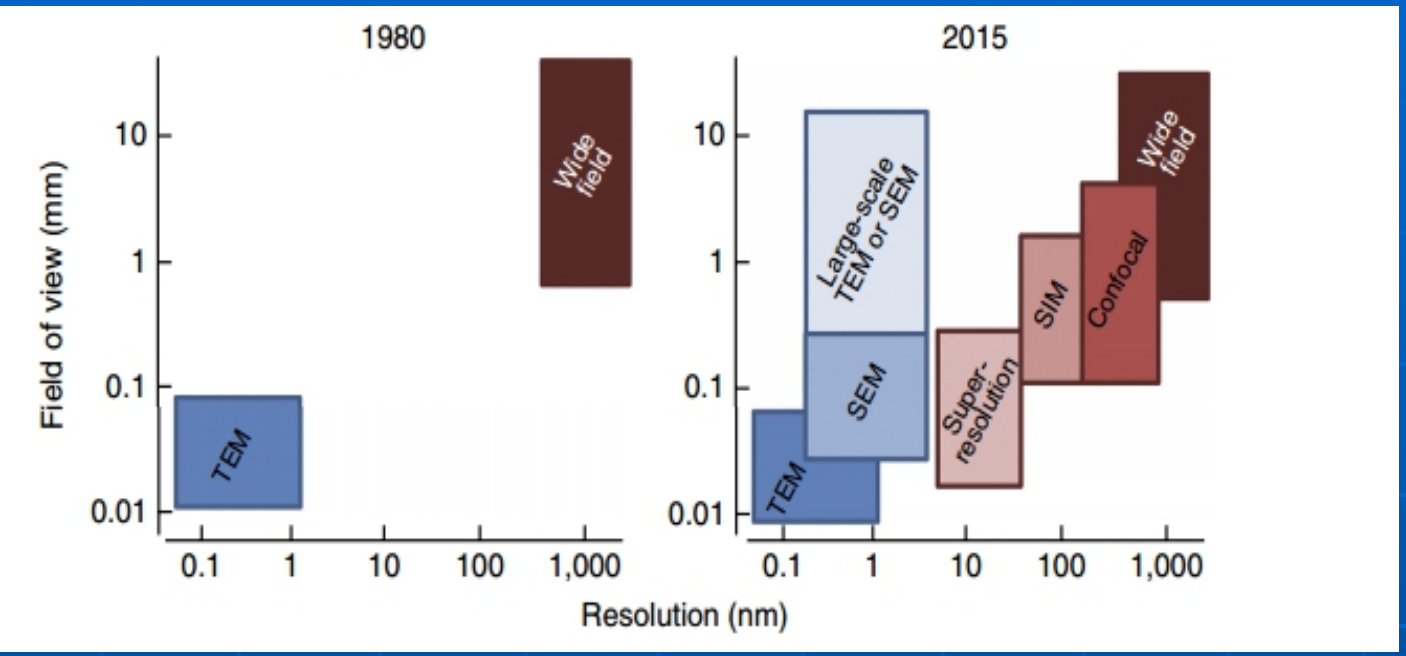


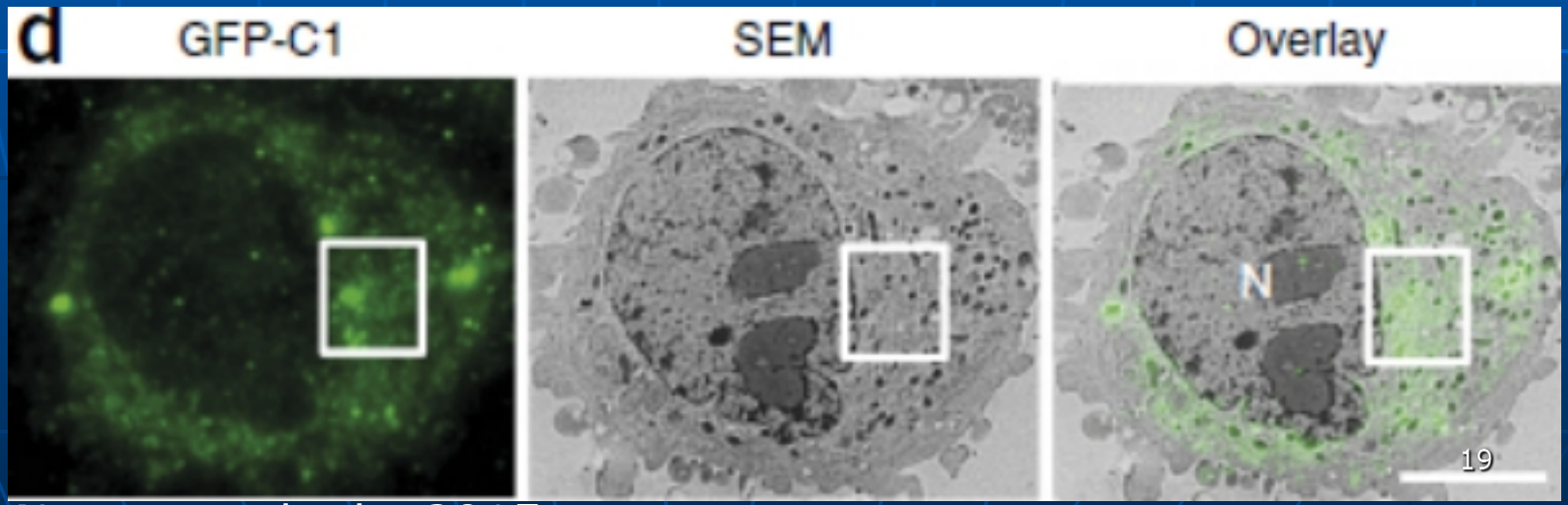
Mast cell

Human mesenchymal stem cell



Optical and electron microscopy

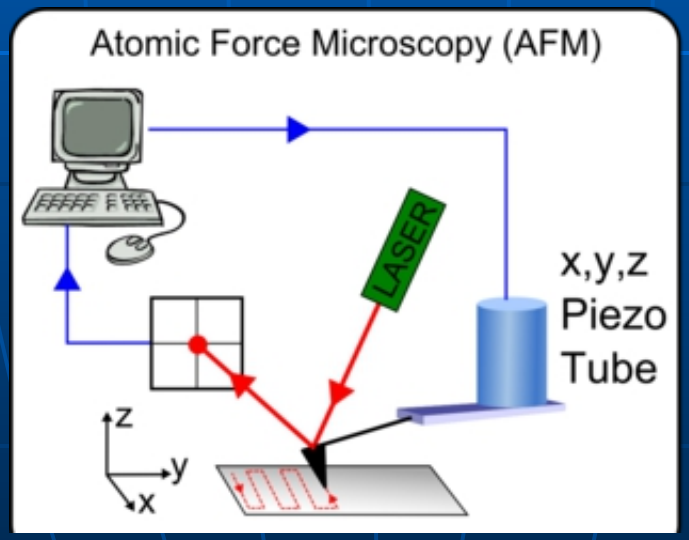




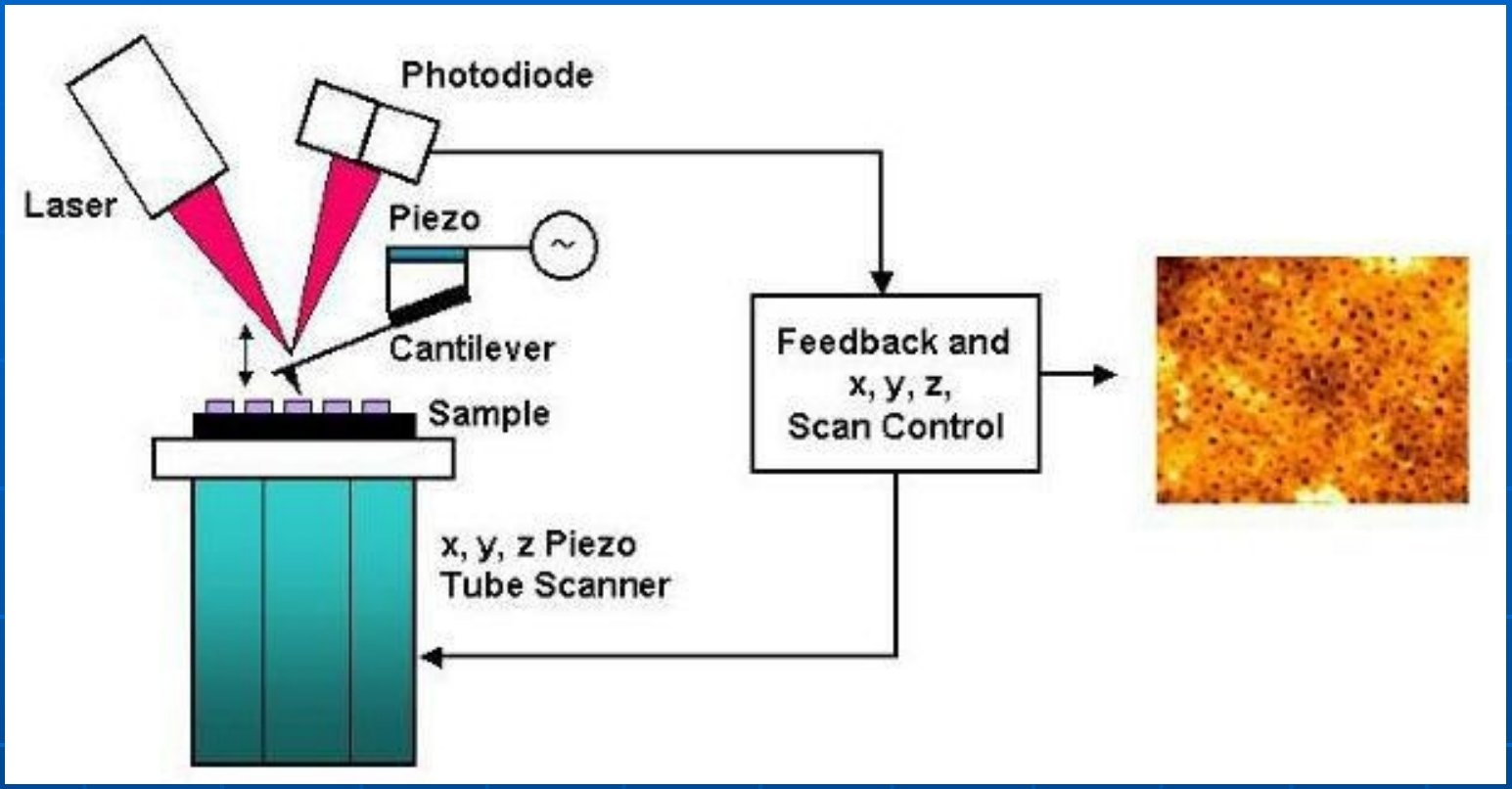
de Boer, Nature methods, 2015

Scanning probe microscopy: atomic force microscopy

The **atomic force microscope** (AFM) or scanning force microscope (SFM) scans the object surface with very sharp tip to produce 3D-image of surface.
AFM allows to study both geometrical and mechanical properties of objects.



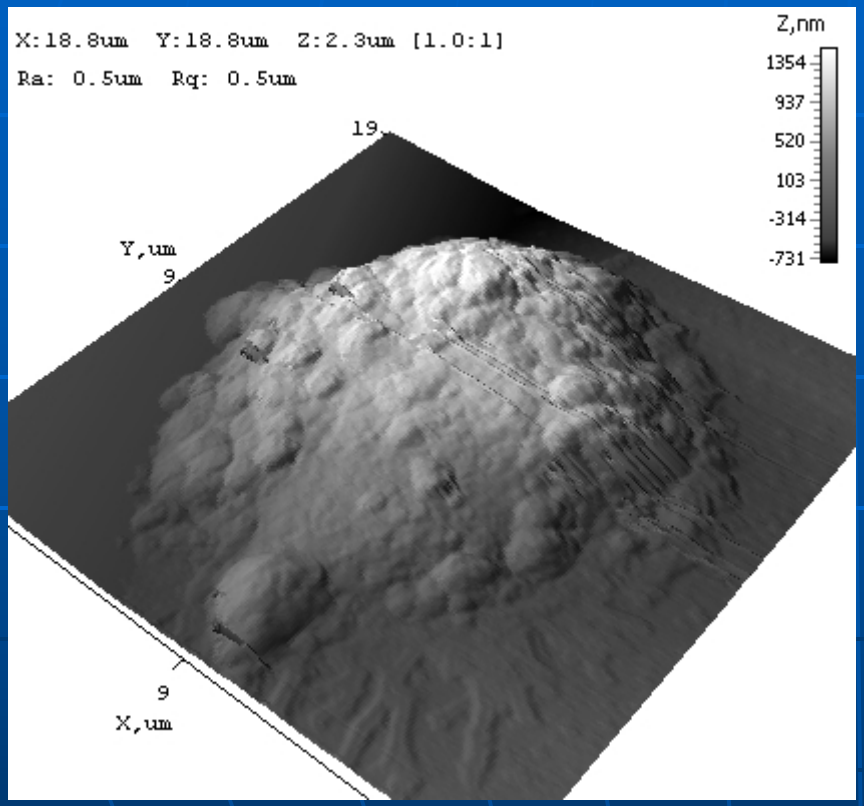
A scheme of an atomic force microscope



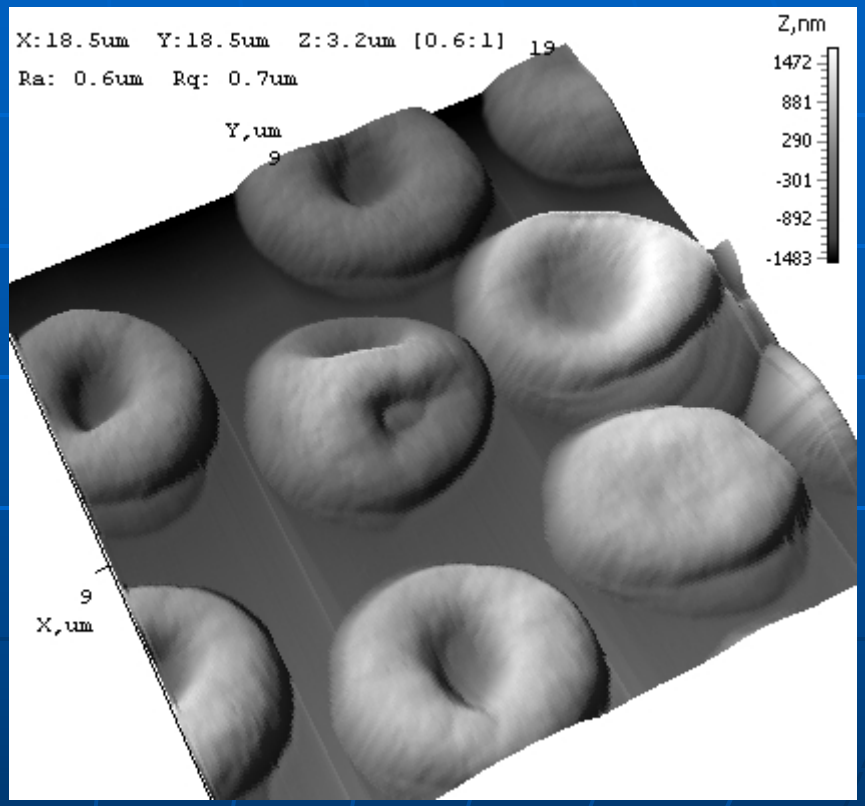
The AFM probes the sample surface with a sharp tip (cantilever).

Forces between the tip and the surface cause the cantilever to bend or deflect.

AFM picture of human blood cells



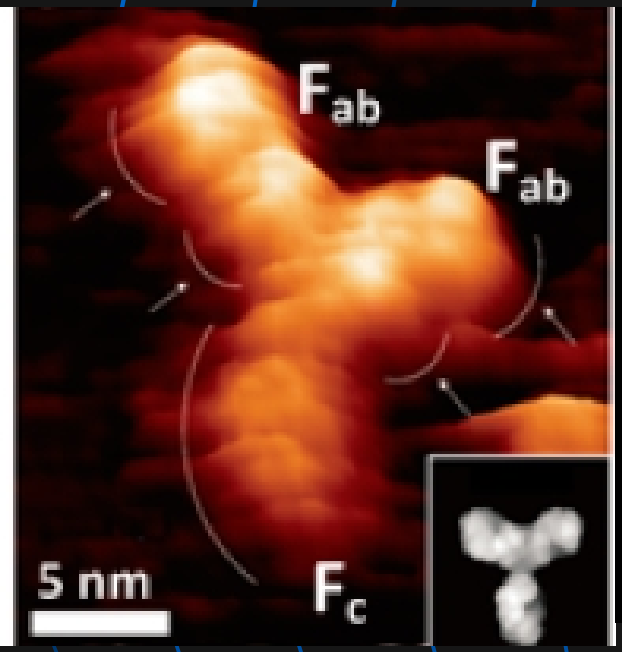
neutrophil

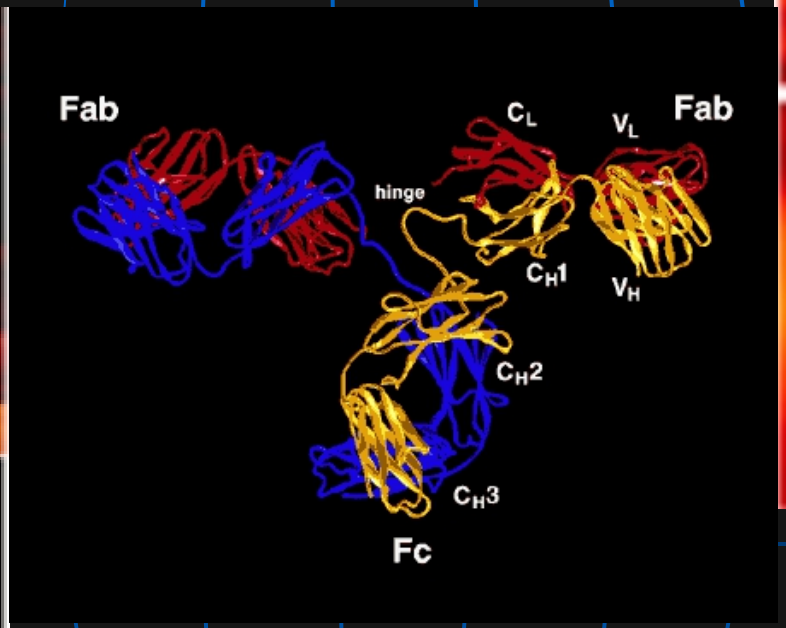


erythrocytes

Molecular Imaging

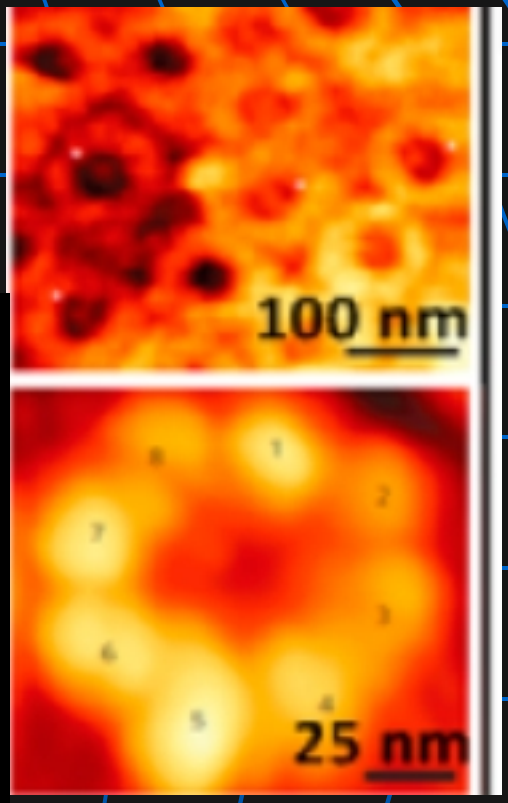
- Imaging the topography of single native biological molecules under physiological conditions is one of the most important applications of AFM

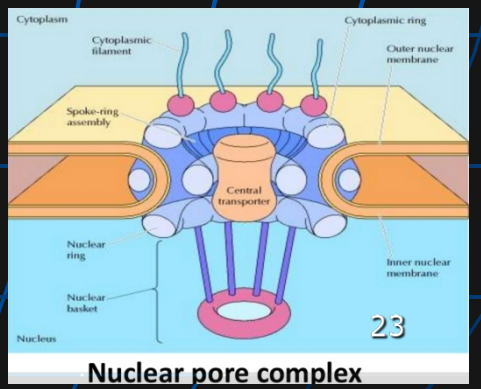




IgG antibody molecule. Ido, 2014

Nuclear pore complexes
Sakiyama, 2016



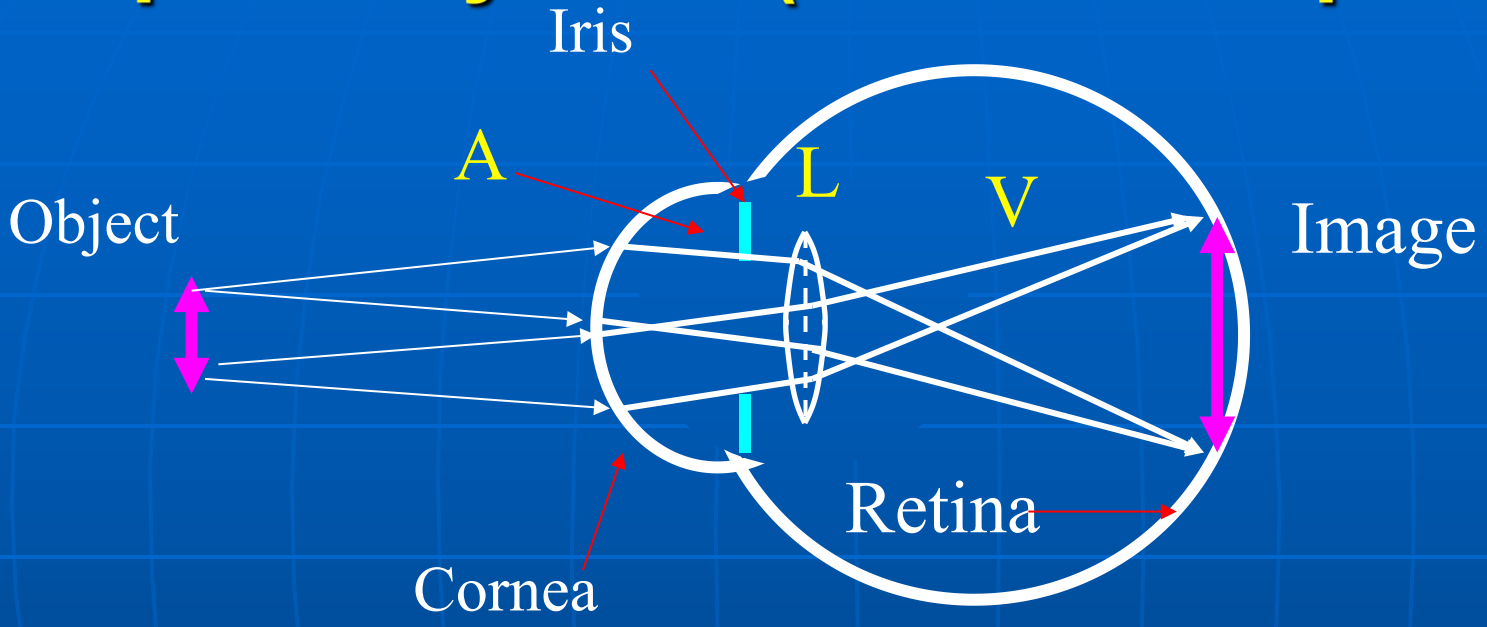


Nuclear pore complex

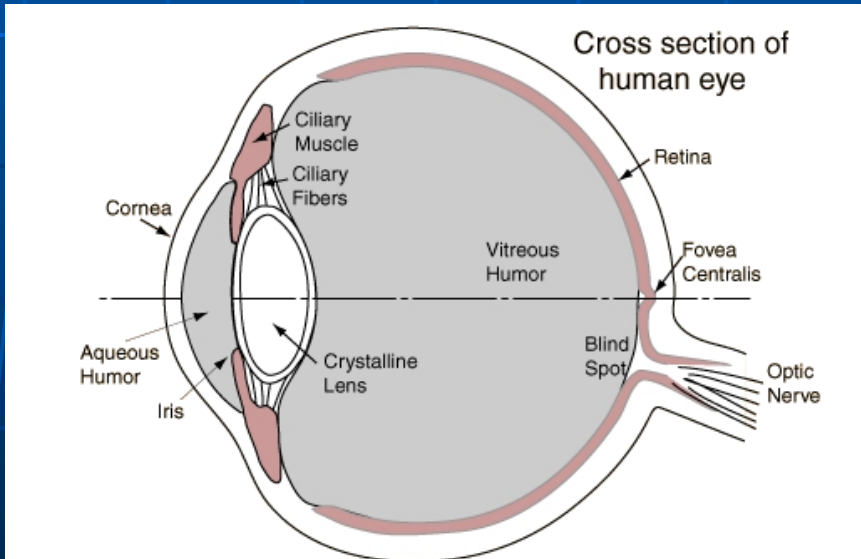
Comparison between optical microscope, electron microscopes and SPM

Type	Magnification	Image dimension	Probe	Mechanism
Optical microscope	1000×	2D	Light	Based on wave properties: diffraction, deflection, scattering
Electron microscope	100000×	2D	Electron beam	
Scanning probe microscope (AFM)	1000000 ×	3D	Very sharp tip	Based on force interaction between tip and sample surface

The human eye: optical system (4 lenses +aperture)



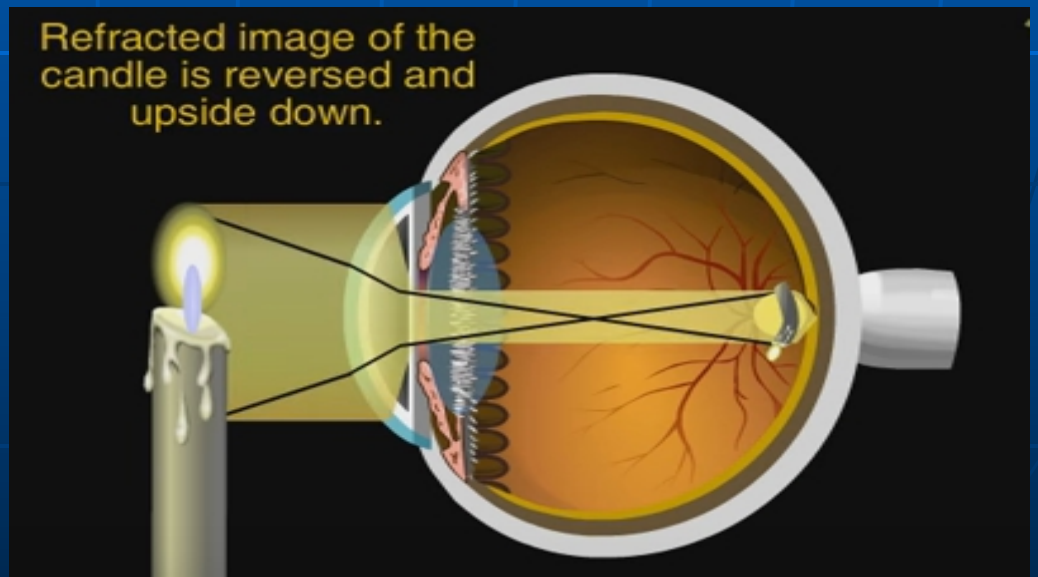
1) The cornea
2) The region A (an aqueous humour, n=1.336).
3) The lens (n=1.413).
4) The region V (a vitreous humour)
5) The circular aperture of iris is called the pupil (2-8 mm diameter)

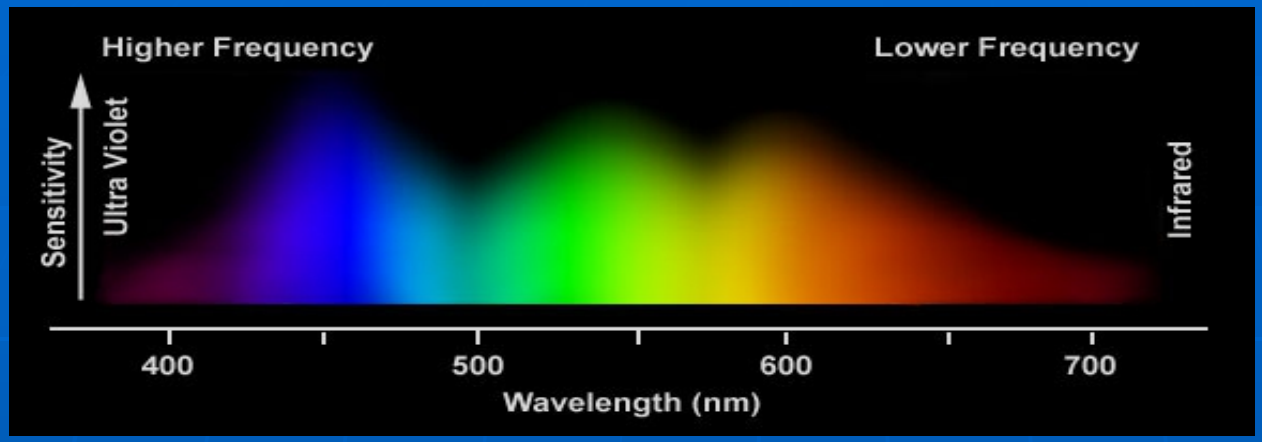


Biophysical bases of the image formation by the eye

- Four lenses deviate the incident light and form the image of an object on the retina.
- Light photon energy transforms into electric pulse energy by special cell's elements (rods and cones) in the retina.
- The electrical pulses

are transmitted to
the brain through
the optic nerve
and cause
the sensation
of vision

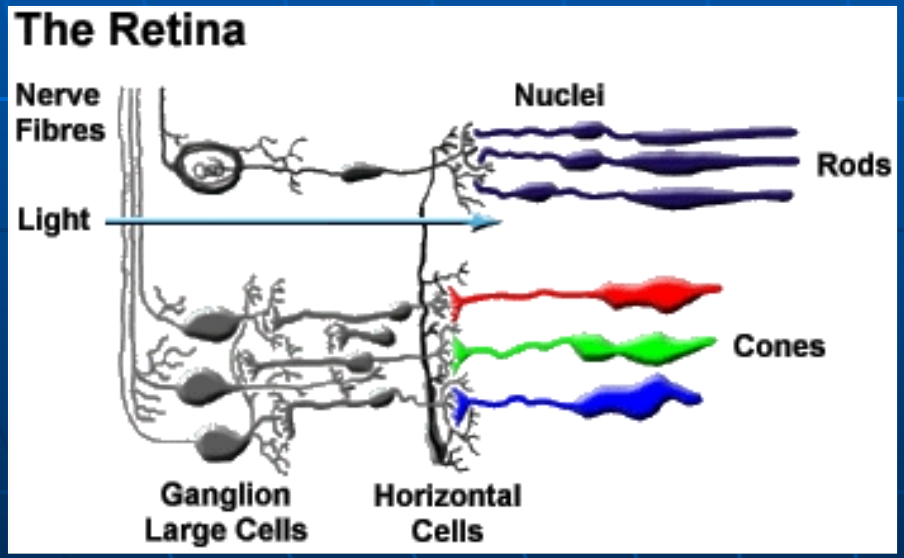


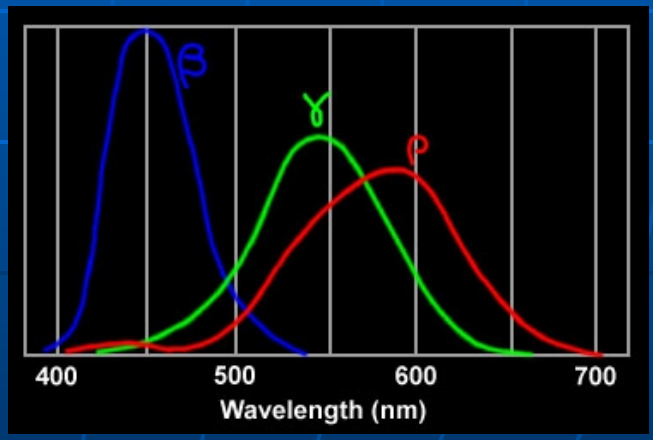


The human eye is able to detect from about 390 to 780 nanometers, defining the *visual spectrum*.

Photosensitive cells: rods and cones

Cones (red, green and blue) are about 5% of the retinal cells
Rods are responsible for twilight vision

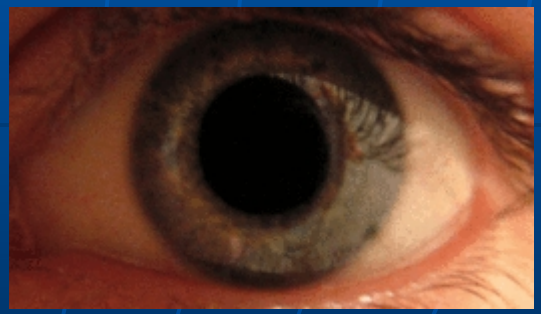




Photosensitive enzymes are rhodopsin and iodopsin

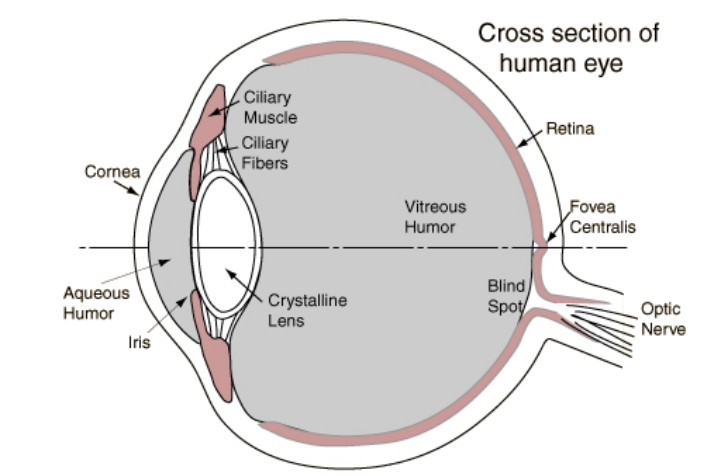
Adaptation

- Adaptation is a process of controlling the intensity of light falling on the retina with pupil



Accommodation

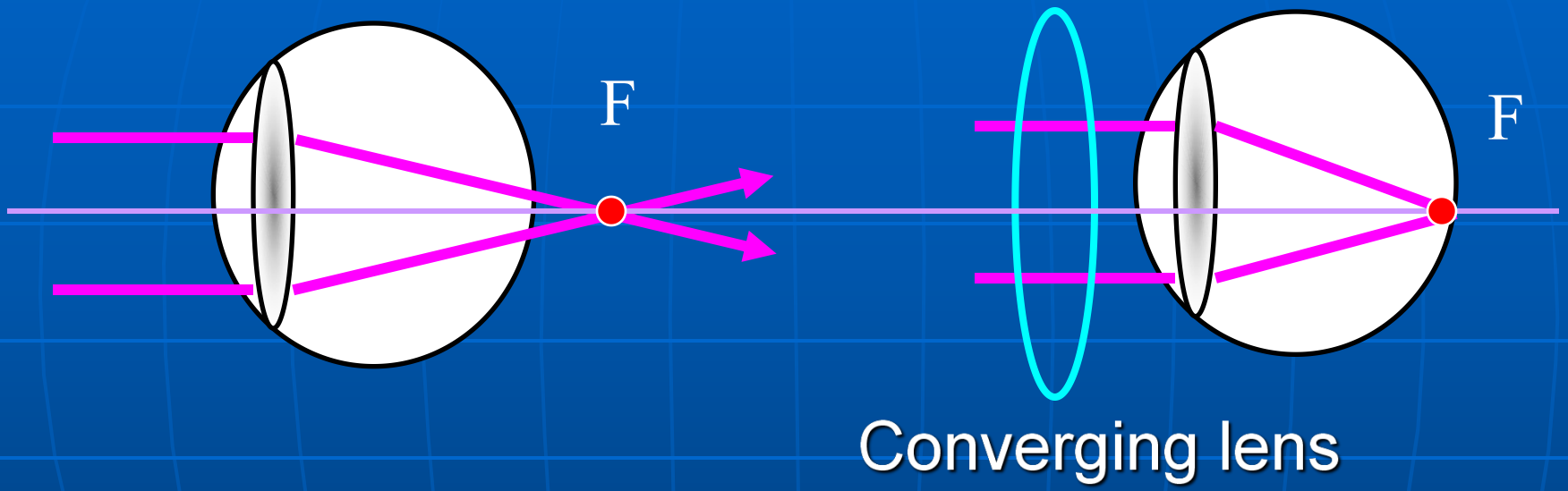
- Accommodation is a process of changing the radius of curvature of the lens surfaces (the focal length) with the ciliary muscles



Optical defects of the eye

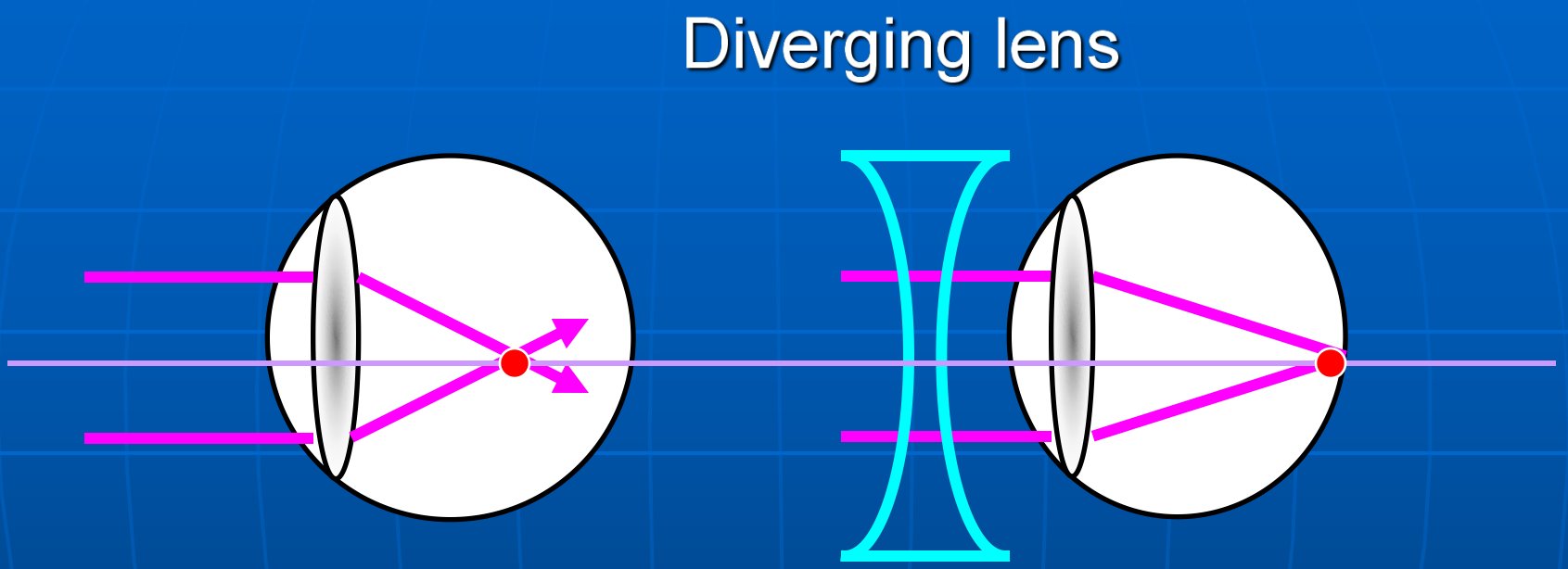
- Hyperopia
- Myopia
- Astigmatism

Hyperopia
(farsightedness, longsightedness, long sight)



- The image is formed behind the retina
- The defect is easily remedied by placing a converging lens in front of the eye

Myopia
(nearsightedness, short sight)



- The image is formed in front of the retina
- Placing a diverging lens in front of the eye remedies this defect

Focal power of a lens

- For a curved surface

$$P = \frac{n}{f}$$

where the unit of P is the dioptre

and f (focal length) is in meters

1 dioptre is the focal power of the system with f=1meter in air

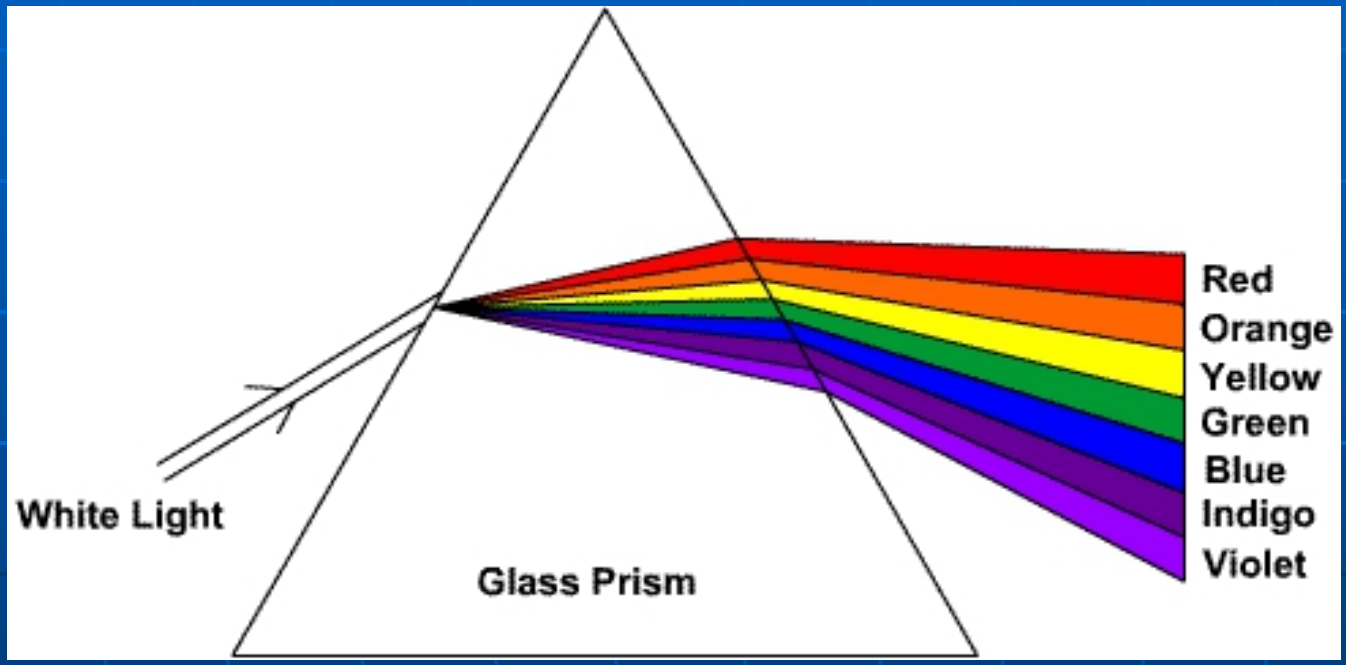
- The converging lens has positive focal power but the diverging lens has negative focal power
- The corrective lens for myopia has -P and one for hyperopia has +P

Astigmatism

- Astigmatism occurs in some people, because their eyes are not completely spherical.
- That is, the radius of curvature of the eye in the vertical direction is not the same as the radius of curvature in the horizontal direction. Hence, vertical rays do not converge to the same position as horizontal rays.
- This defect is usually corrected with lens with cylindrical curvature.

Light dispersion

Light dispersion is a separation of visible light into its different colors due to the dependence of the refractive index on wavelength (or frequency).



- Blue light (434 nm) n=1.528
- Yellow light (550 nm) n=1.517
- Red light (700 nm) n=1.510

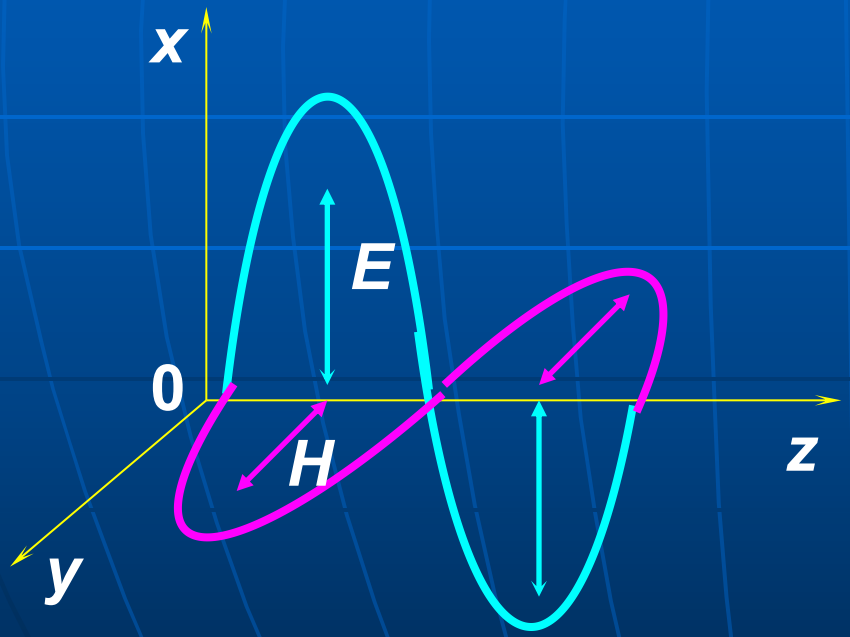
- The spectrum of visible light contains the colors red, orange, yellow, green, indigo, blue and violet bands

Dispersive elements

- There are two basic light dispersive elements (the apparatus which splits the white light into spectrum).
- They are **prism and diffraction grating**

Light as electromagnetic wave

An electromagnetic wave is a transverse wave that has both an electric *E* and a magnetic *H* components

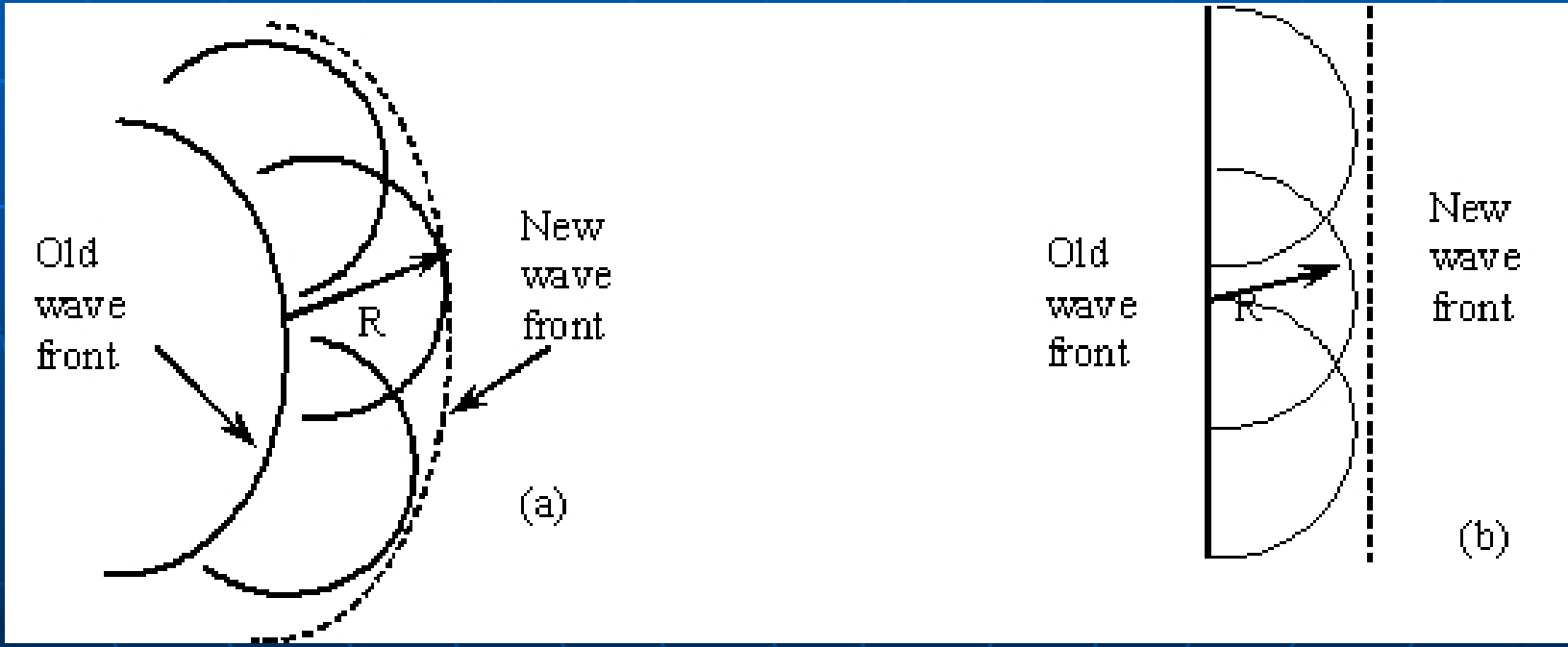


The relation between the wavelength λ, frequency ***f***, and speed ***c*** of the light is given by the fundamental equation of wave propagation as:

$$\lambda f = c$$

Huygens' principle

Each point on a wavefront serves as a source of coherent wavelets which then spread forward at the same speed, interact and create the new wavefront that can be found by drawing the tangent to secondary wavelets at the later time



Wavefront represents a surface of identical phase

Interference of waves

The interference phenomenon is a superposition of coherent waves

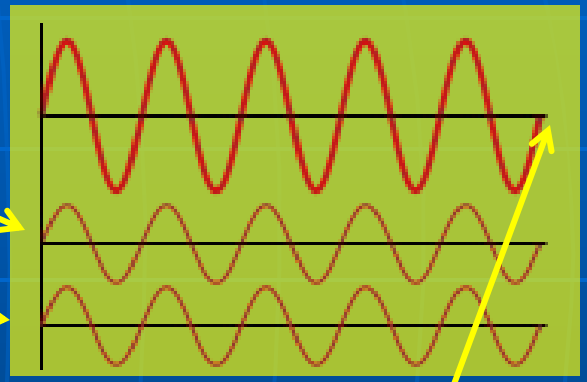
Coherent waves are monochromatic waves that phase difference $\Delta\phi=\phi_2-\phi_1$ remains constant in space and time.

Interference of light

a) If $\phi_1 = \phi_2 = 0$ and $A_1 = A_2 = A$, therefore:

$$y_1 = A\sin(kx - \omega t)$$

$$y_2 = A\sin(kx - \omega t)$$



$$y_1 + y_2 = A\sin(kx - \omega t) + A\sin(kx - \omega t) =$$

$$= 2A\sin(kx - \omega t)$$

k is the spatial angular frequency (wavenumber) of the wave, $k=2\pi/\lambda$.

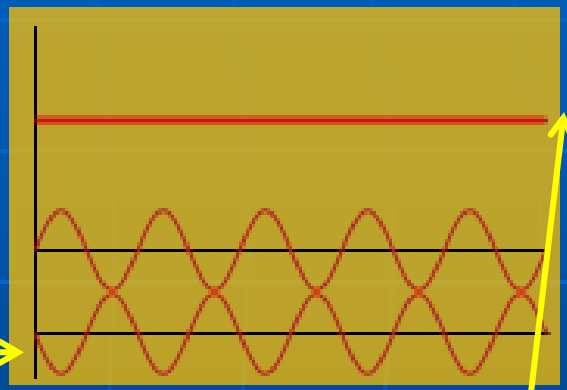
Interference of light

b) If $\phi_1 = 0^\circ$ $\phi_2 = 180^\circ$ and $A_1 = A_2 = A$

therefore

$$y_1 = A\sin(kx - \omega t - 0)$$

$$y_2 = A\sin(kx - \omega t - 180)$$



$$y_1 + y_2 = A\sin(kx - \omega t) + A\sin(kx - \omega t - 180) =$$

$$= A\sin(kx - \omega t) - A\sin(kx - \omega t) = 0$$

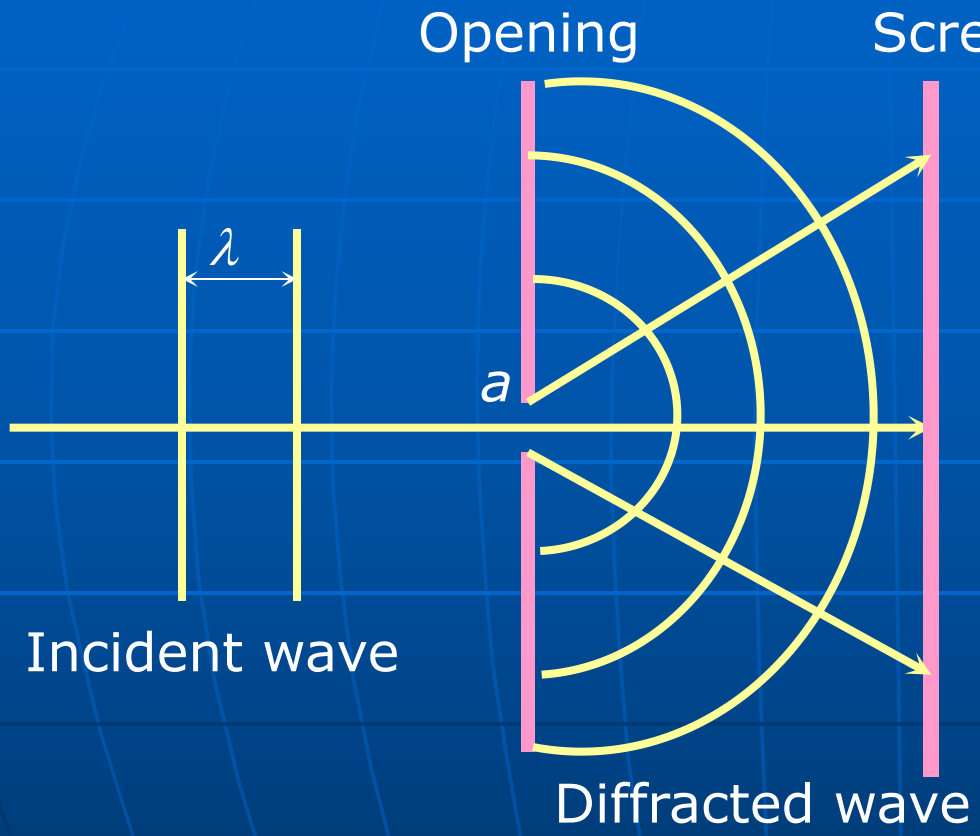
Conditions of constructive and destructive interference

$$d \sin\theta = m\lambda$$ max

$$d \sin\theta = (2m-1)\frac{\lambda}{2}$$ min

where $d \sin\theta$ is a path length difference, m is the order of spectrum.

Diffraction

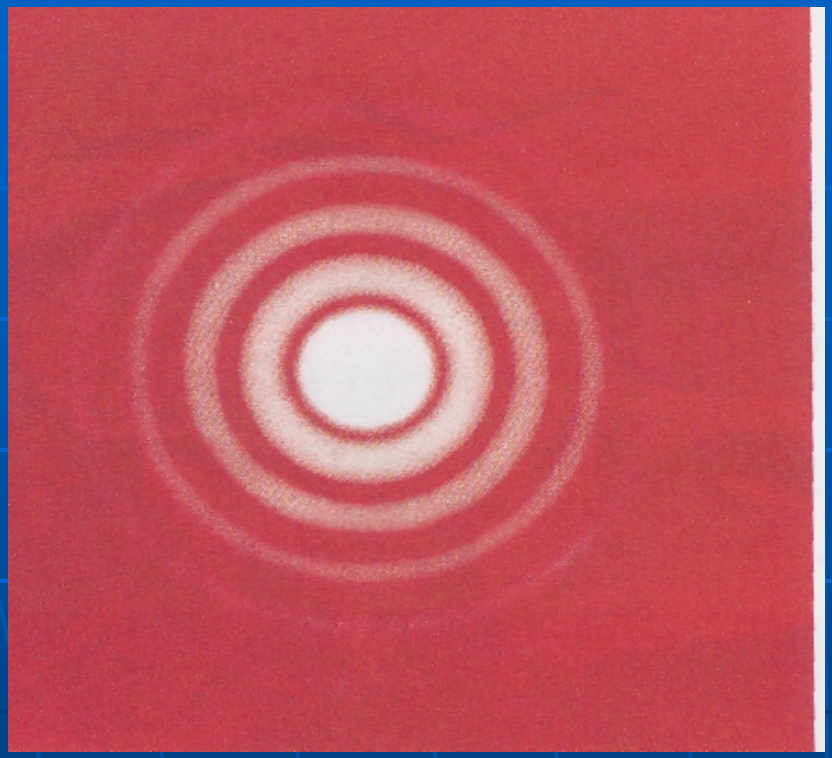


Diffraction by single slit

The bending of light around obstacle (or opening), into region that should be a shadow area is called

a diffraction phenomenon.

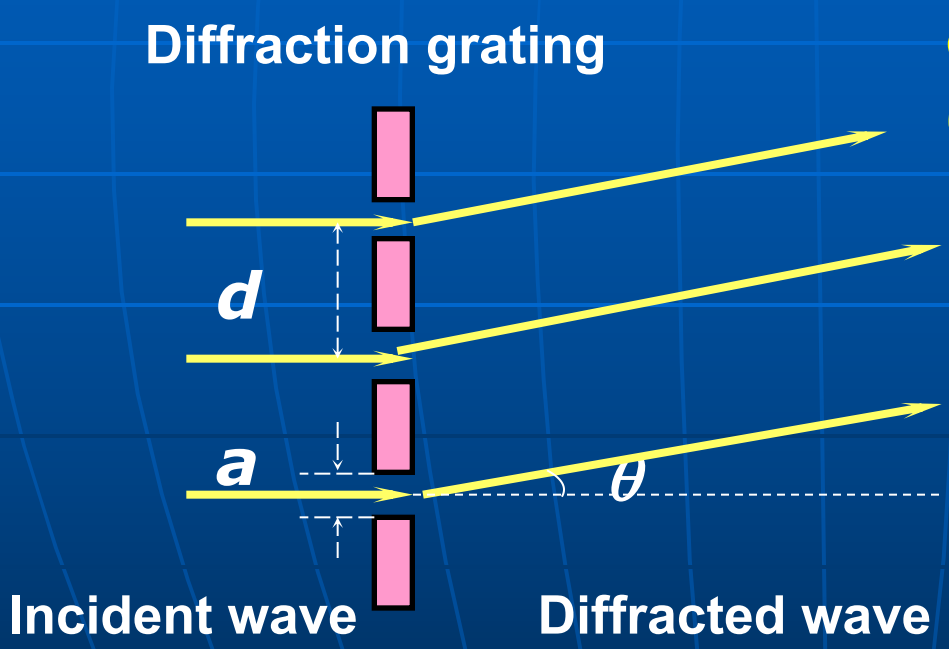
Diffraction



The diffraction pattern of a circular aperture.

Diffraction grating

Several parallel slits of equal width *a*, equally spaced a distance *d* apart are called a diffraction grating.



Condition for the formation of a bright fringe (diffraction maximum)

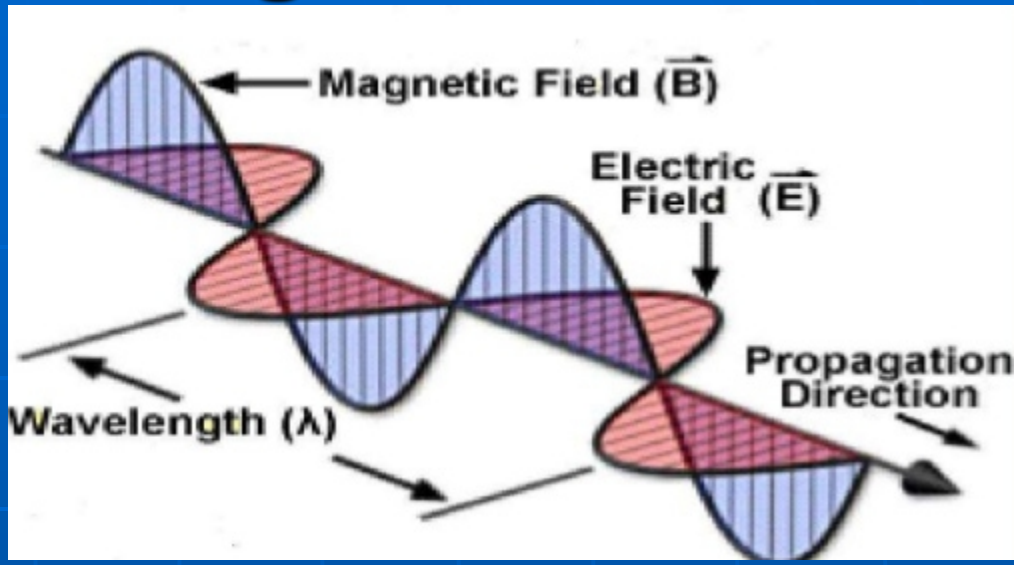
$$d \sin\theta = m\lambda$$

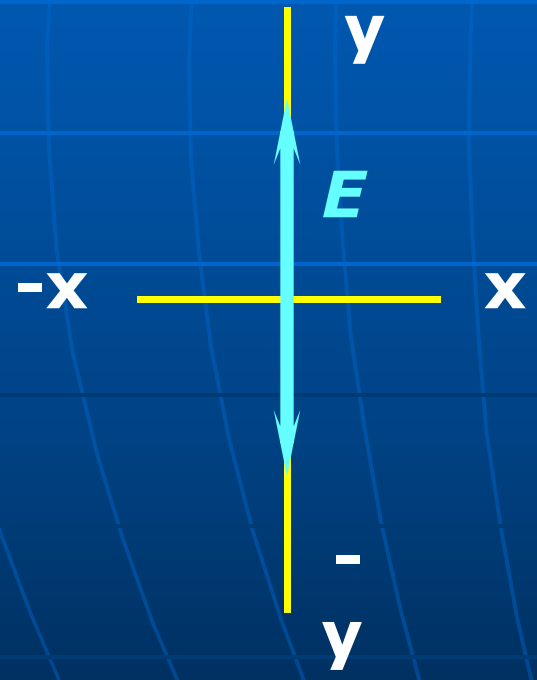
m = 0,1,2,…

Polarization of light

Polarized light

- Single electromagnetic wave is **plane-polarized.**



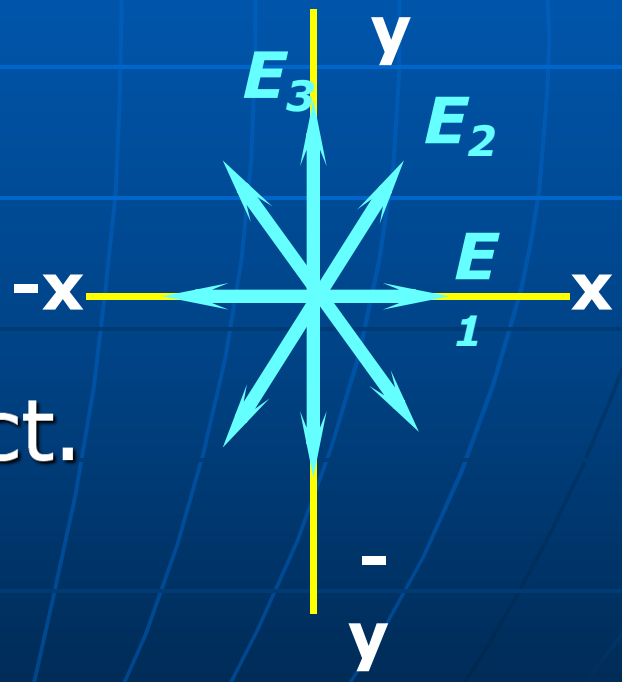


Representation of a polarized light by diagrammatically, where *E* is the projection of vibration of electric vector to plane XOY

Unpolarized light (natural)

- **Unpolarized light** is the superposition of many beams of light, in the same direction of propagation, but each with random polarization

- Sources of un-polarized light are sun, lamp and ect.



Light polarization is a process of thrans-forming unpolarized light into polarized one.

Methods of light polarization

1. Polarization by transmission
2. Polarization by reflection
3. Polarization by refraction

A polarizer is an optical filter that lets light waves of a specific polarization pass and blocks light waves of other polarizations.

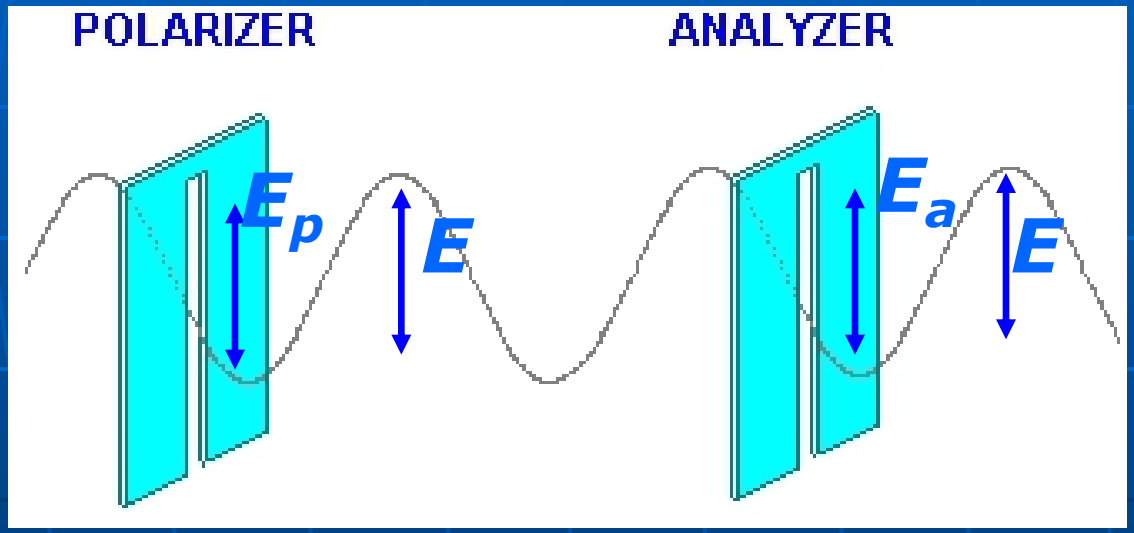
- Polarizer is called an analyzer if it serves for an analysis of polarized light

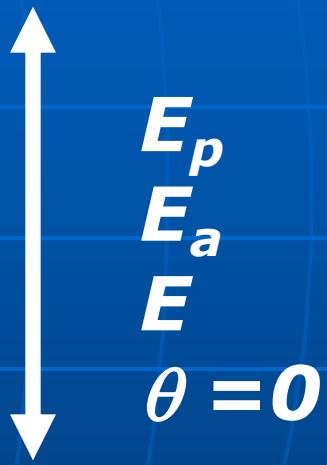
Polarization by transmission

- **Polaroid** is a thin sheet of plastic in which the large molecules are oriented in a particular direction.
- When the electric vector *E* of incident light is parallel to the direction of molecular orientation it is absorbed and when it is perpendicular to the molecular orientation it is transmitted (this is a polarization axis).
- Preferential absorption of polarized light is called a **dichroism**.

Polarization by transmission

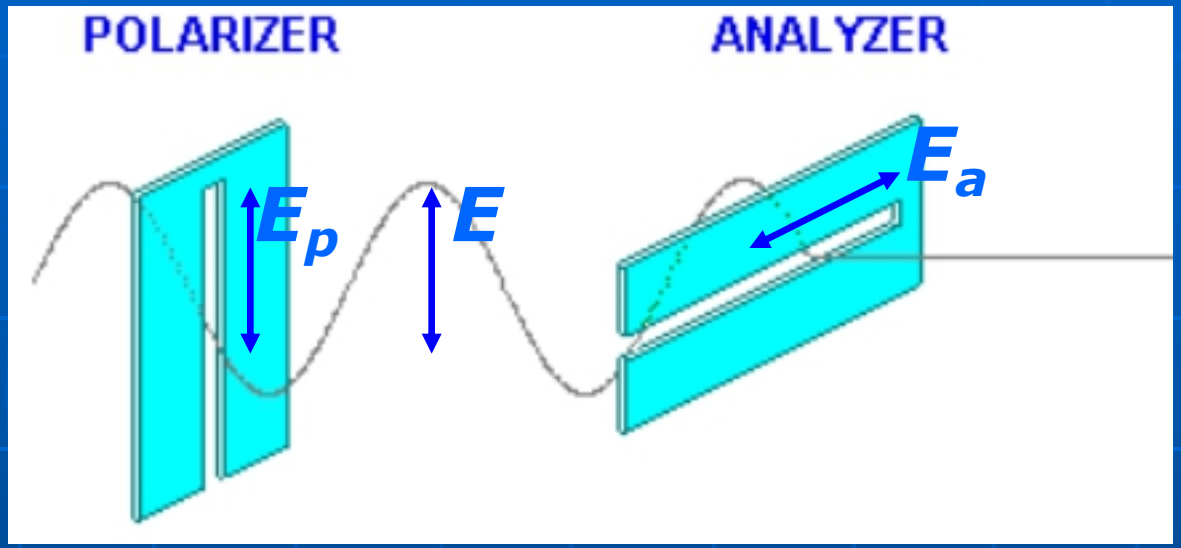
Intensity of the transmitted light depends upon the angle θ between the polarization axis of polarizer E_p and the polarization axis of analyzer E_a.

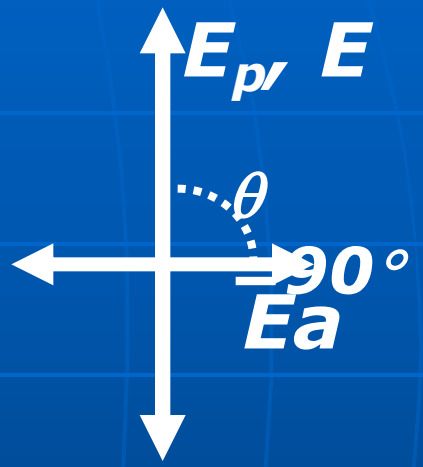




When an angle $\theta = 0°$ the transmitted light intensity I is maximum and an analyser is parallel to a polarizer.

Polarization by transmission





When an angle $\theta = 90^\circ$ the transmitted light intensity I is zero.

- Position, when an analyser is crossed with a polarizer is called **the extinction position**.

Malus law

Intensity of transmitted light is:

$$I = I_0 \cos^2\theta,$$

where:

I is an intensity of transmitted light after analyzer,
I_0 is an intensity of polarized light before analyzer,
θ is angle between the polarization axises of polarizer and analyzer

2.Polarization by reflection

- Un-polarized incident light is polarized to a certain degree when it is reflected from an insulating surface. In this case, light waves that have the electric field vectors parallel to the surface are reflected to a greater degree.

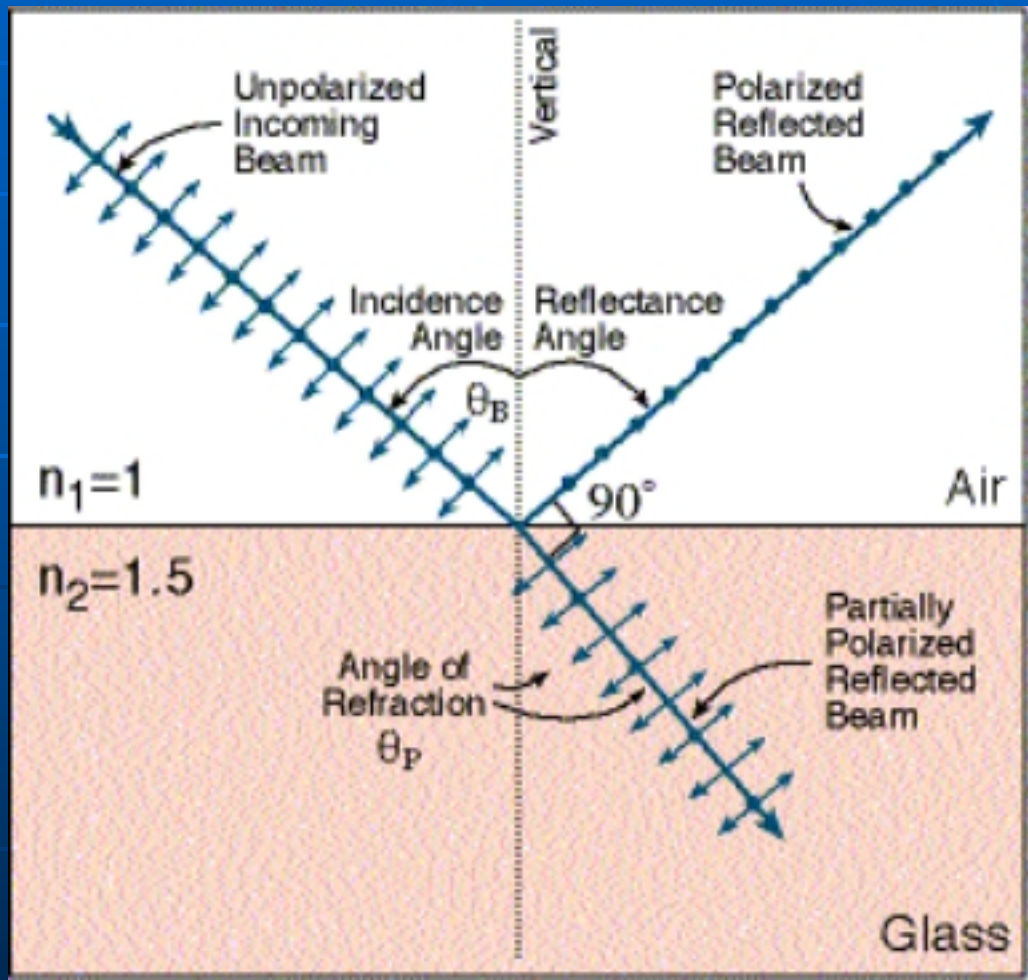
Brewster's law

Incident angle inducing a maximum polarization is known as the Brewster angle given by the expression:

$n_{21} = \tan(i)$

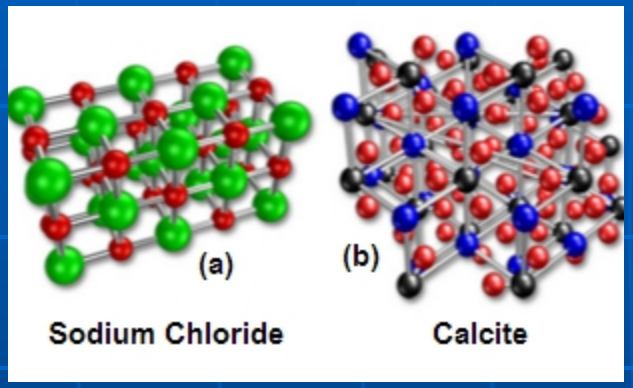
where $n_{21}=n_2/n_1$ is the refractive index,

i is the angle of incidence.



3.Polarization by refraction

- Crystals having different optical properties (it is dependent upon the orientation of the crystalline lattice) in different directions are called **anisotropic crystals**.

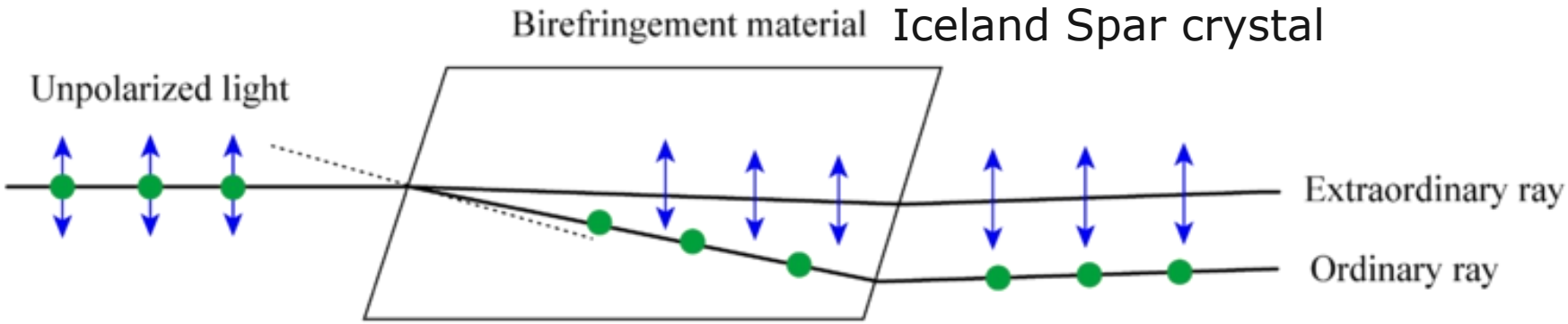


- isotropic – sodium chloride,
- anisotropic - calcite

- Refraction of incident light into two polarized rays with perpendicular orientation of electric vectors inside of anisotropic crystal is termed **double refraction or birefringence**.

3.Polarization by refraction

- One ray is called **an ordinary ray (o)**. Its behavior is described by laws of geometrical optics.
- Other ray is called **an extraordinary ray (e)**. Its behavior is not described by laws of geometrical optics

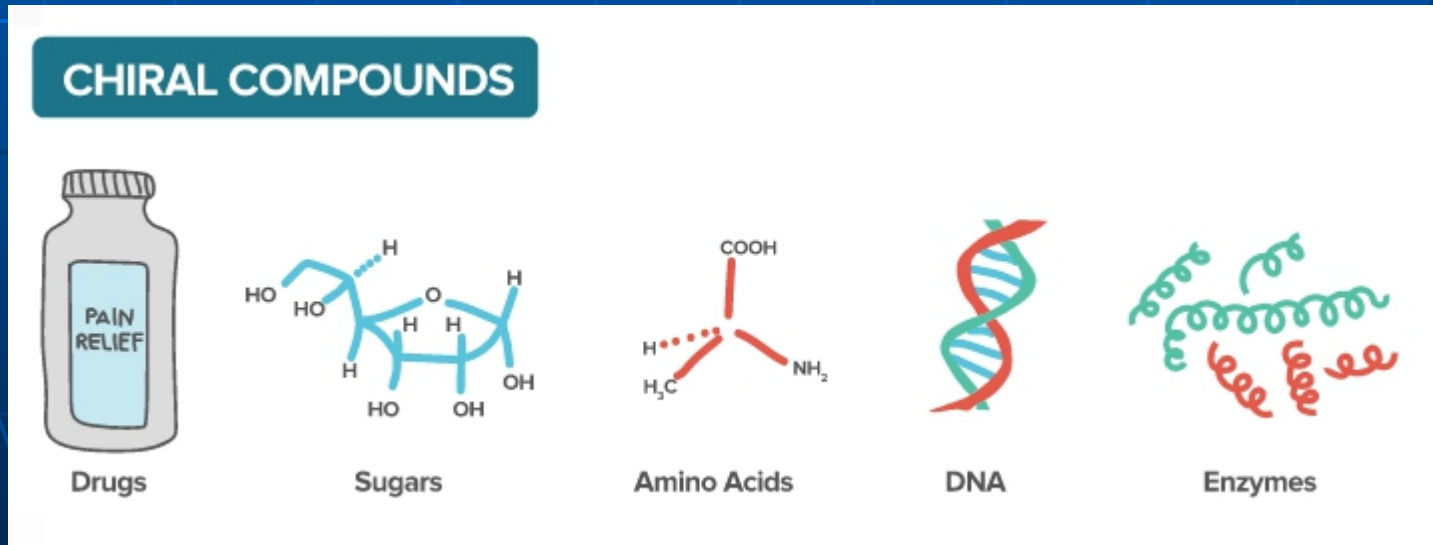


Optical activity is

- An ability of a chemical substance to rotate the plane of polarization of plane-polarized light.

Substances which *rotates the plane of polarized light* when passed through them are called optically active substances.

Optical activity of molecule is caused by its non-symmetrical structure (chirality).

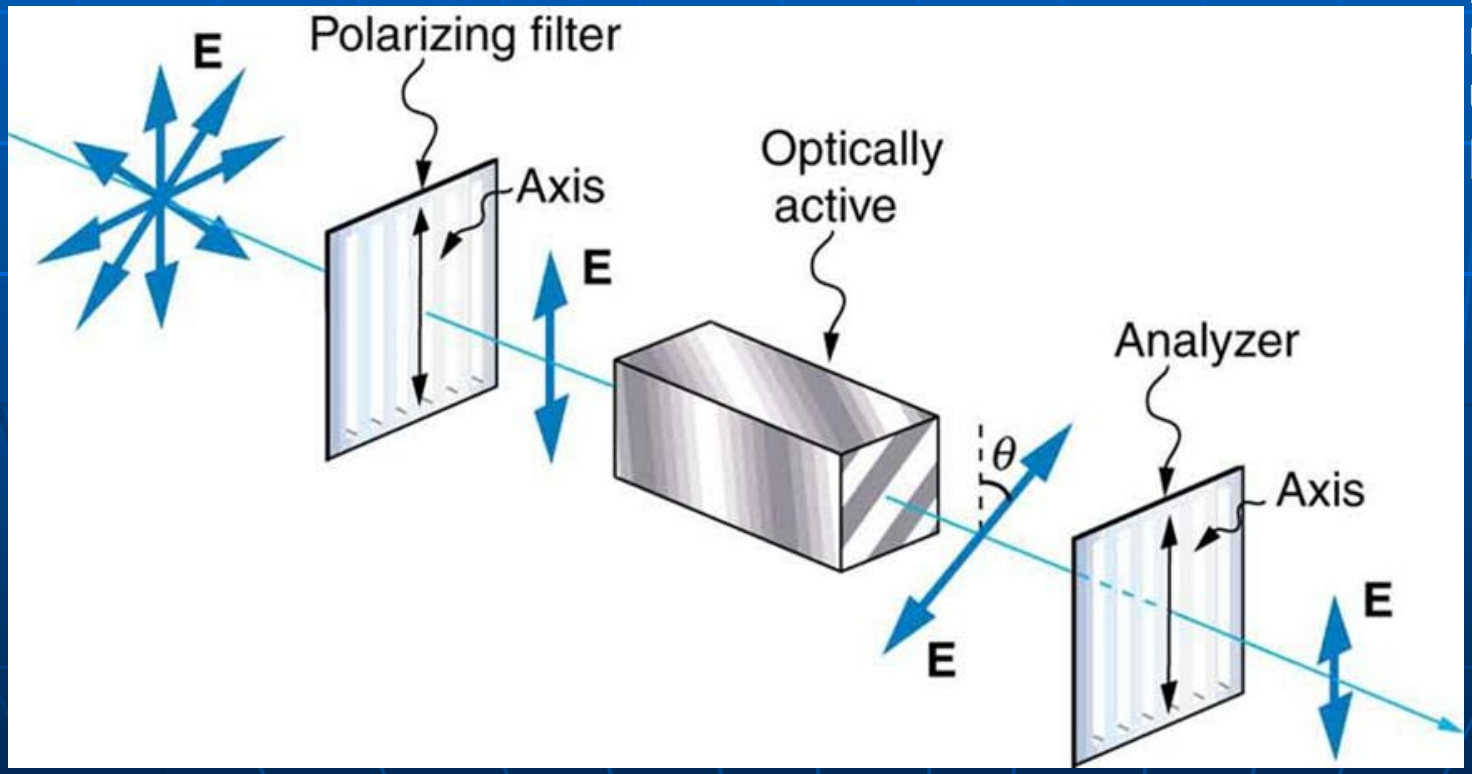


Optical activity

- For optically active solutions a rotation angle α is:

$$\alpha = \alpha(T, \lambda) L c$$

where $\alpha(T,\lambda)$ is a specific rotation for a particular wavelength λ and temperature T, L is an optical path length through the solution and c is a concentration.



The optical rotation can be measured using a polarimeter

Polarimetry is used for the quantitative analysis of solutions (composition and concentration)