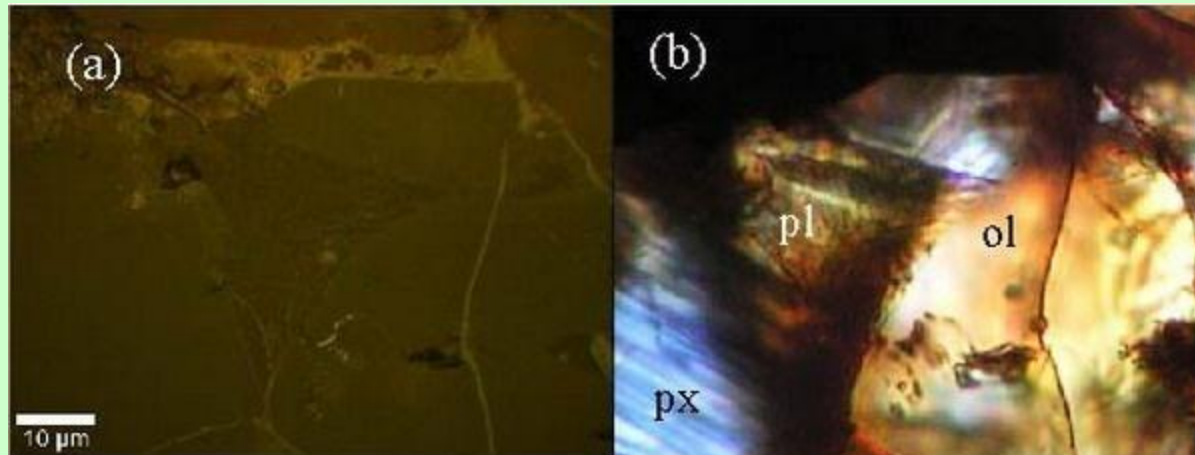


Optical Microscopy: Lecture 2

Magnification and Resolution in Optical Microscopy

Pavel Zinin

HIGP, University of Hawaii, Honolulu, USA



Lecture Overview

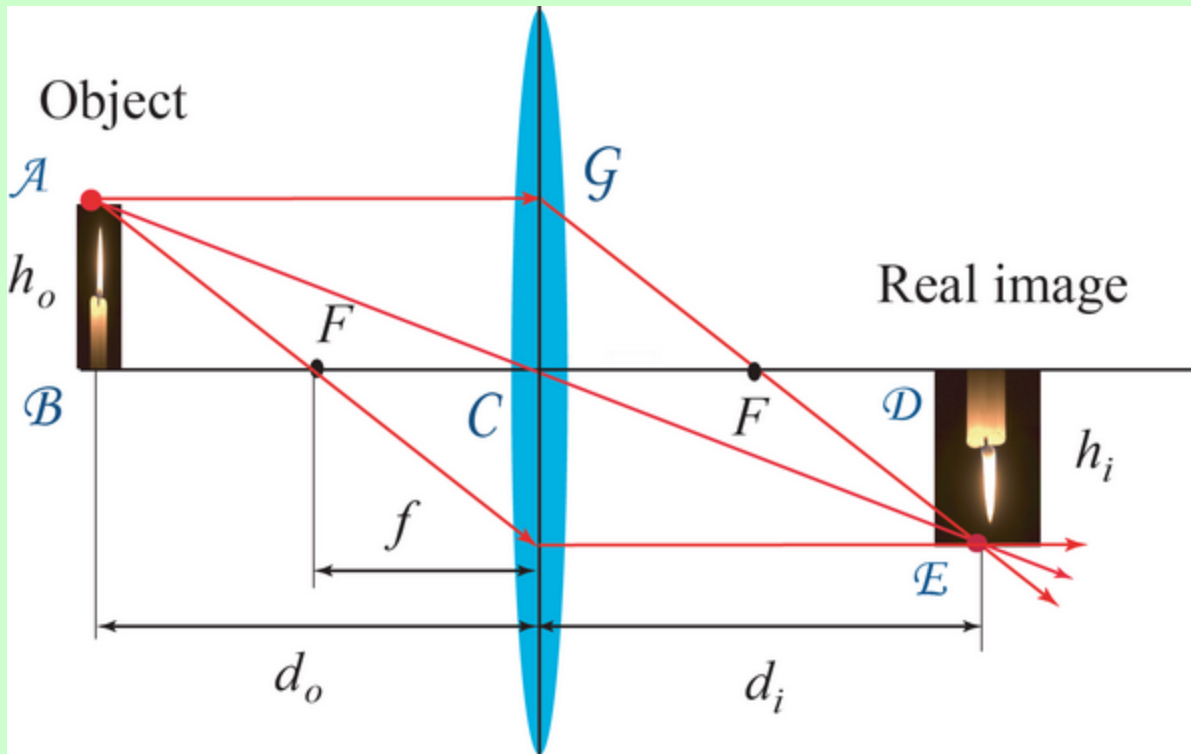
Simple and Compound Microscopes

1. Magnification of Compound Microscope
2. Concept of Plane Waves
3. Structure of the Electromagnetic Field at Focus
4. Lateral and Axial Resolutions of the Optical Microscope

Illumination in Light Microscopy

1. Abbe condenser
2. Kohler Illumination
3. Dark Field Microscopy

Deriving Thin Lens Equation



Using similarity of
ABC and CDE
triangles we can write

$$\frac{h_o}{d_o} = \frac{h_i}{d_i}$$

Triangles CFG (F is the right focal point) side of the is similar to triangle FDE. Then, we can write

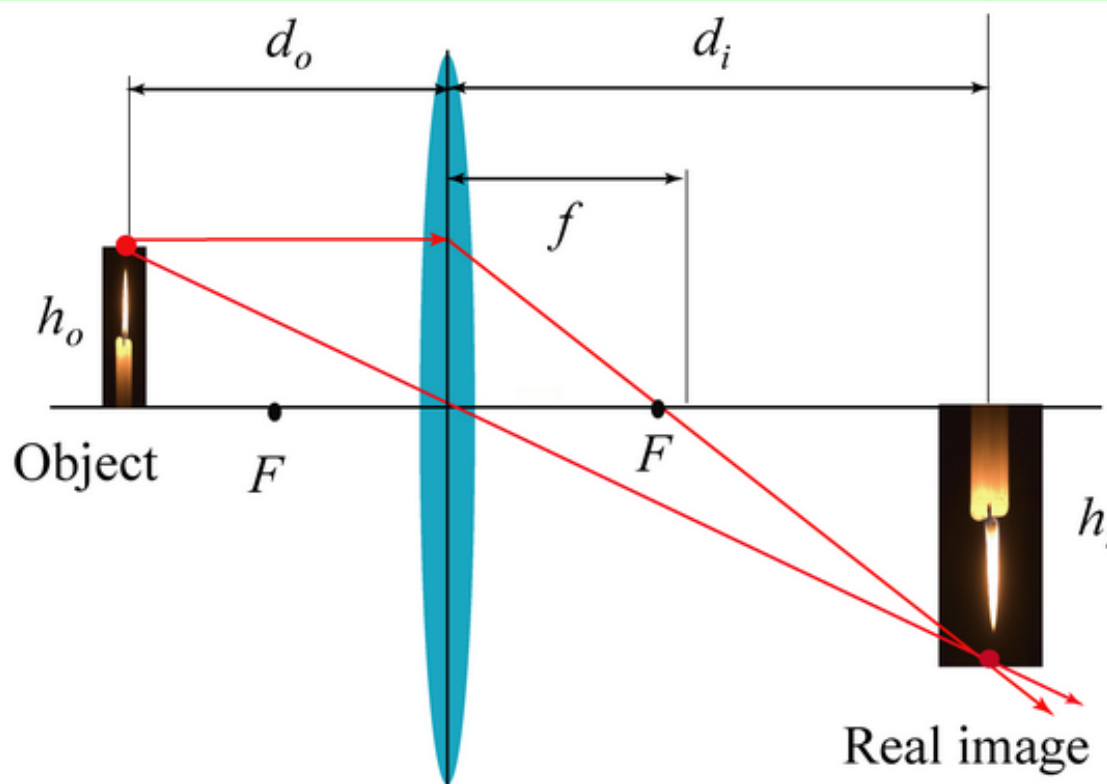
$$\frac{h_o}{f} = \frac{h_i}{d_i - f}$$

$$\frac{f}{d_o} = 1 - \frac{f}{d_i}$$



$$\frac{1}{d_o} + \frac{1}{d_i} = \frac{1}{f}$$

Thin Lenses Magnification



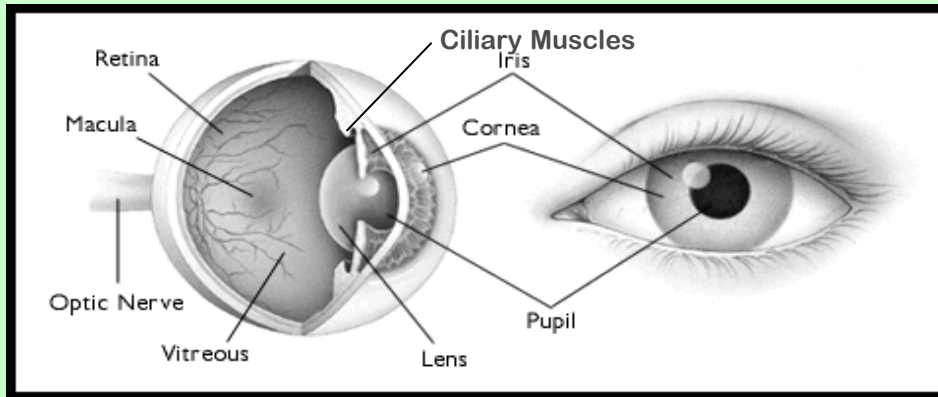
Thin lens equation

$$\frac{1}{d_o} + \frac{1}{d_i} = \frac{1}{f}$$

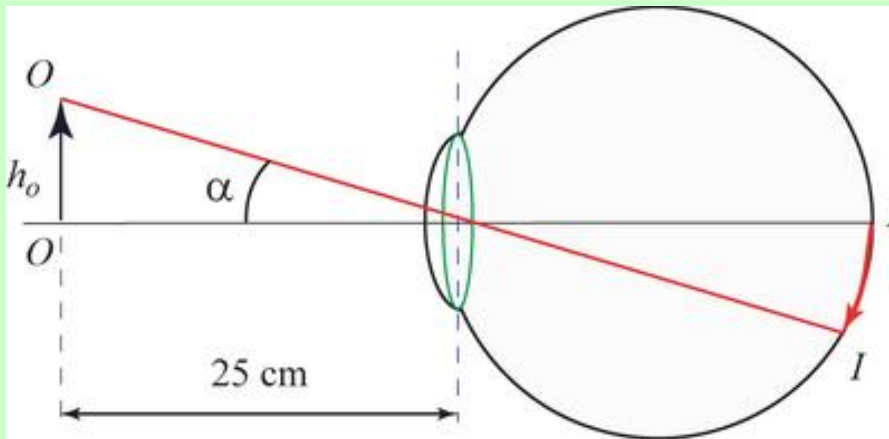
The linear magnification m is defined as the ratio of the image size h_i to the object size h_o :

$$m = \frac{h_i}{h_o} = -\frac{d_i}{d_o}$$

Magnification of the Human Eye

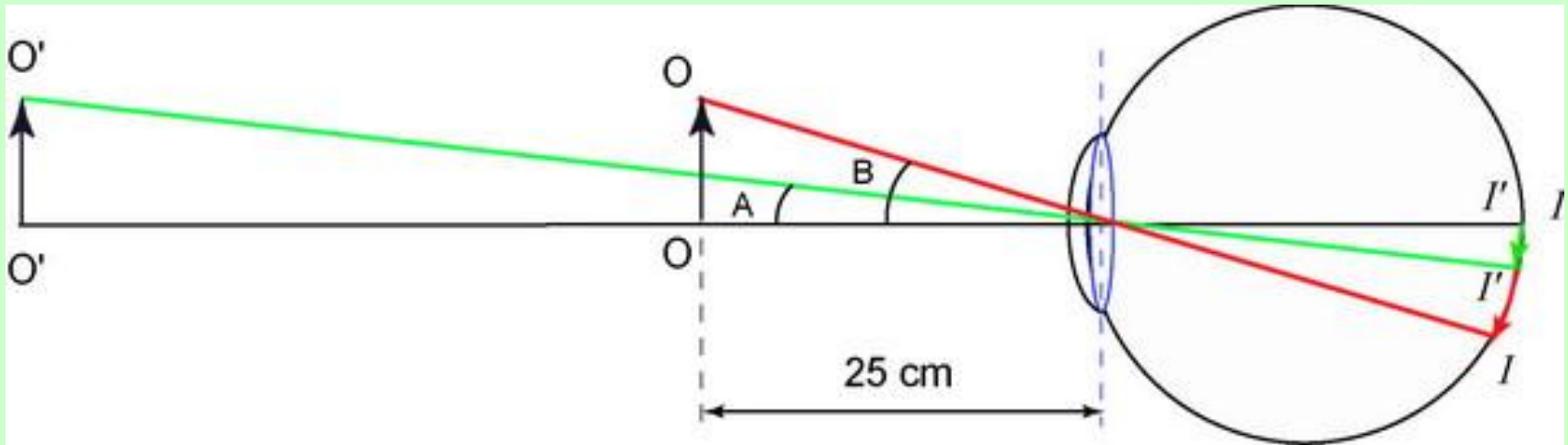


A person with normal eyesight can see clearly objects located anywhere from infinity to about 25 cm from the eye. The normal eye is unable to focus on an object closer than about 25 cm. Therefore, angle α in the figure is maximum at the near point.



The largest angular size of an object will be when it is held as close to the eye as possible. By the definition, the “closest distance for comfortable viewing “ is taken to be **25 cm**.

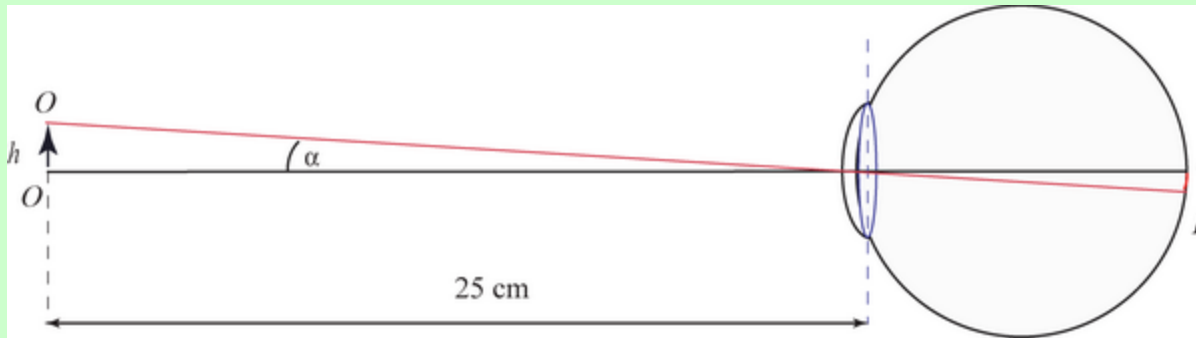
Magnification of the Human Eye



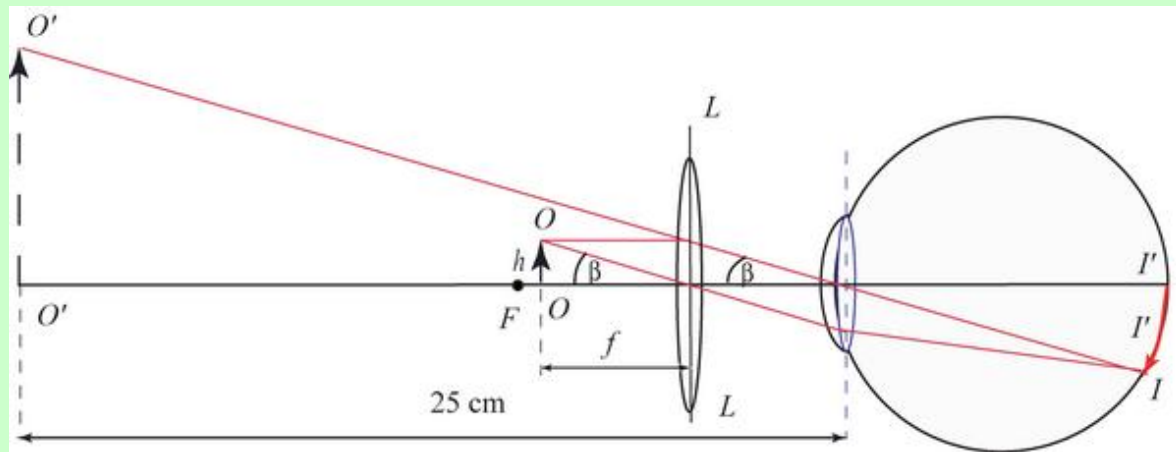
The maximum magnification of the eye: $d_i(\text{eye})=2$ cm, diameter, $d_o= 25$ cm.

$$m = \frac{d_i}{d_o} = \frac{2}{25} = 0.08$$

Magnifying Lens



α is the angle subtended by the object, O - O , with a height h when viewed from 25 cm by unaided eye.



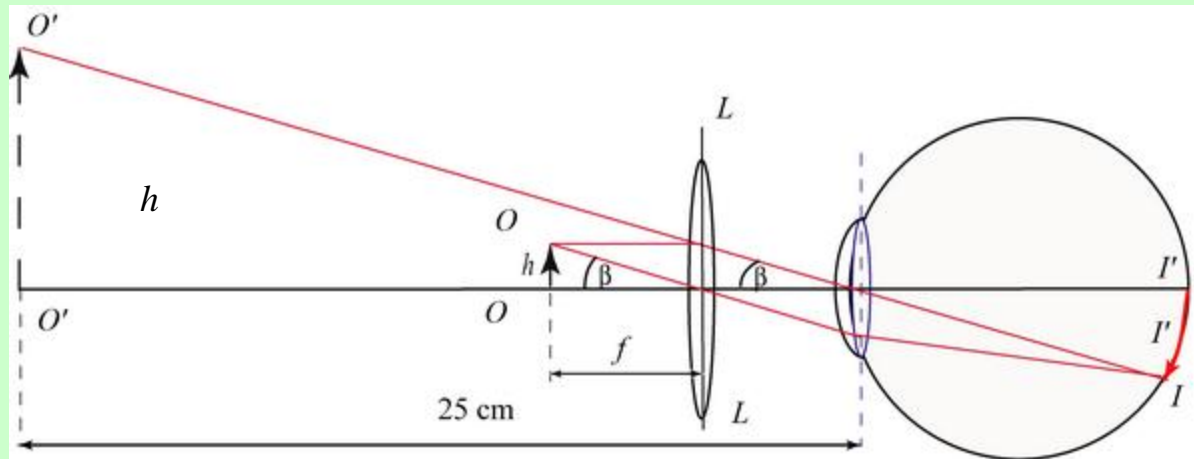
Now, let us use a lens and place the same object in front of the eye, and inside the focal point of the lens. At this position, the lens form a virtual, erect and enlarged image (see Fig.). The lens increases the angular size of the object.

We define the angular magnification as the ratio of the angle subtended by the image when using magnifier, β , to that subtended by the object when viewed from 25 cm by unaided eye, α .

Angular magnification

$$m_{\theta} = \frac{\beta}{\alpha}$$

Simple microscope



If the eye is relaxed when using the magnifying glass, the image is then at infinity, and the object is the precisely at focal point

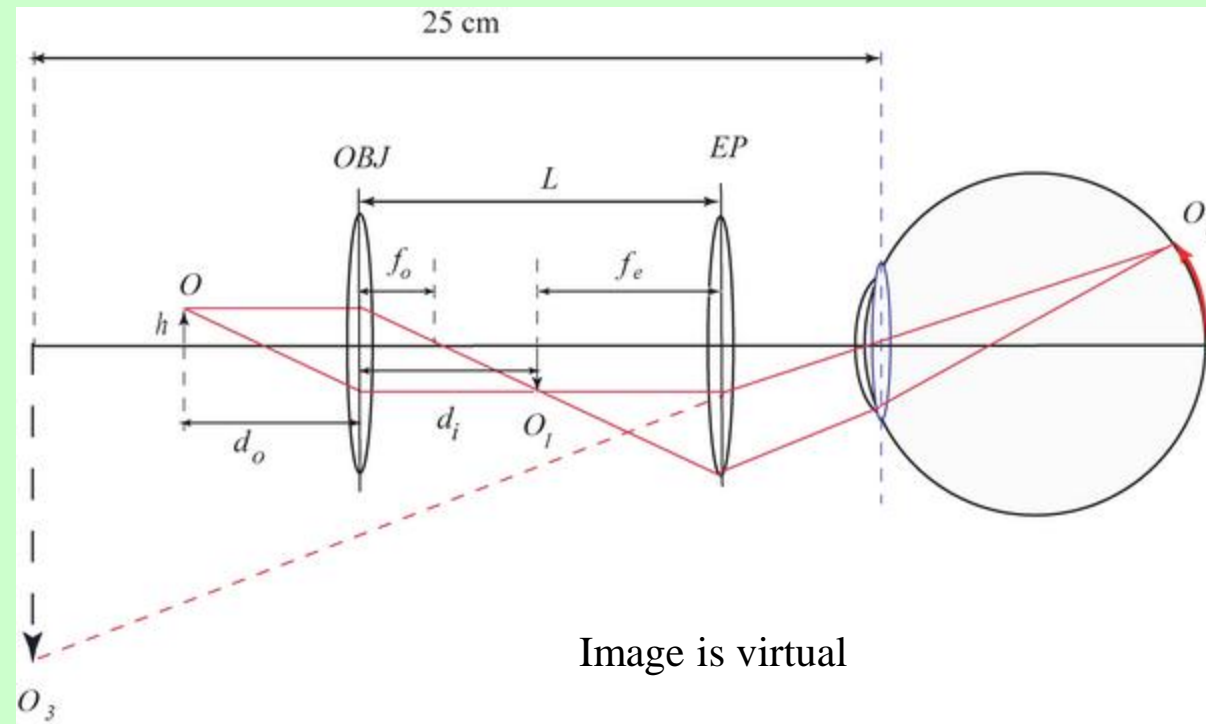
Angular magnification
of a simple microscope

$$m_{\theta} = \frac{25 \cdot \text{cm}}{f}$$



Antoni van Leeuwenhoek Microscope

Magnification in Compound Microscope



Let us place the object, O , outside the focal lens of the objective. The image O_1 is real and enlarged. The magnification of the first image is

$$M_o = d_i / d_o.$$

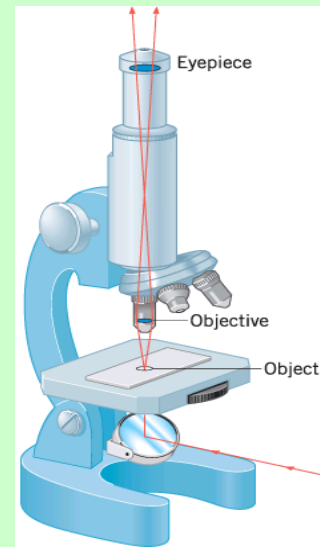
$$L \gg f_o, f_e$$

$$m_o = \frac{h_{o1}}{h_o} = \frac{L - f_o - f_e}{f_o} \approx \frac{L}{f_o}$$

Since magnification of the eye piece is

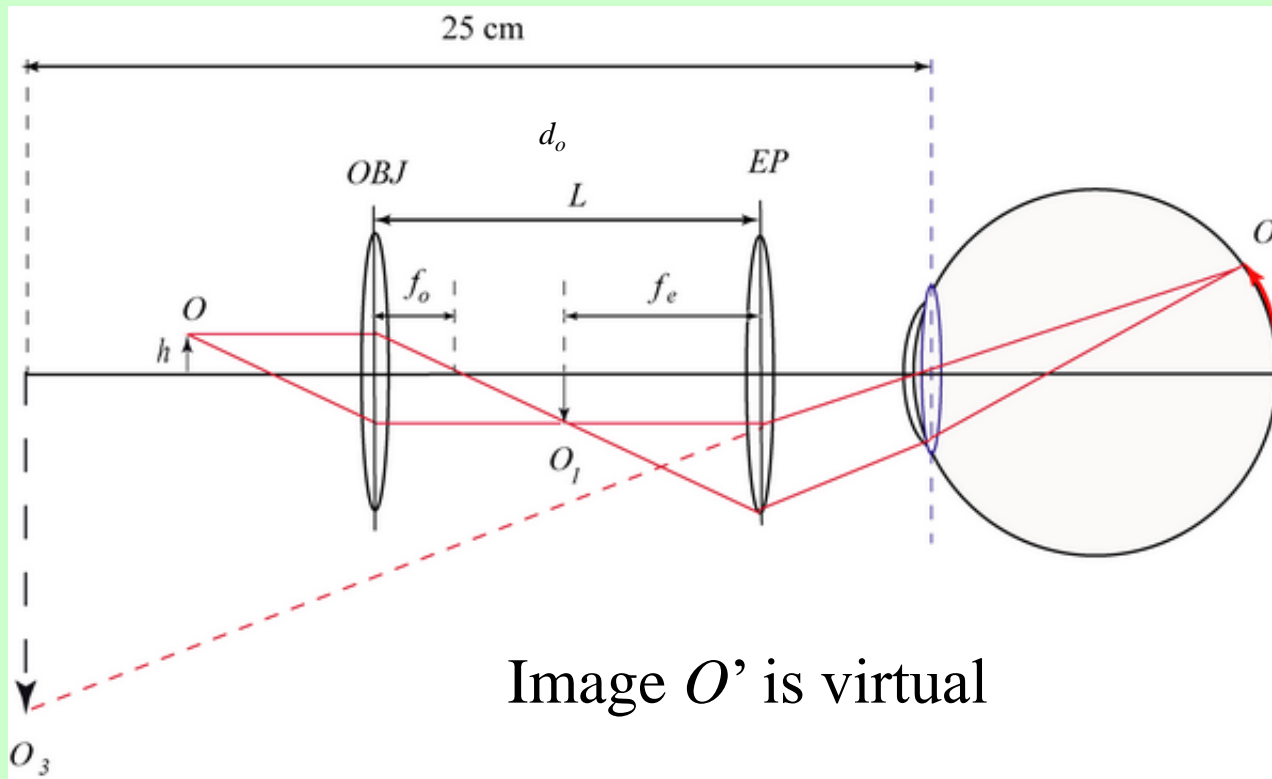
$$m_e = \frac{25\text{cm}}{f_e}$$

$$m = m_o \times m_e = \frac{L \times 25\text{cm}}{f_o \times f_e}$$



Compound Microscopes

Why in the previous sketch the object is placed out of focus? If the microscope is designed in such a way that the image O_1 of the object O is located at the focal point of the eye piece lens (EP) then



$$\frac{1}{d_o} + \frac{1}{L - f_e} = \frac{1}{f_o}$$

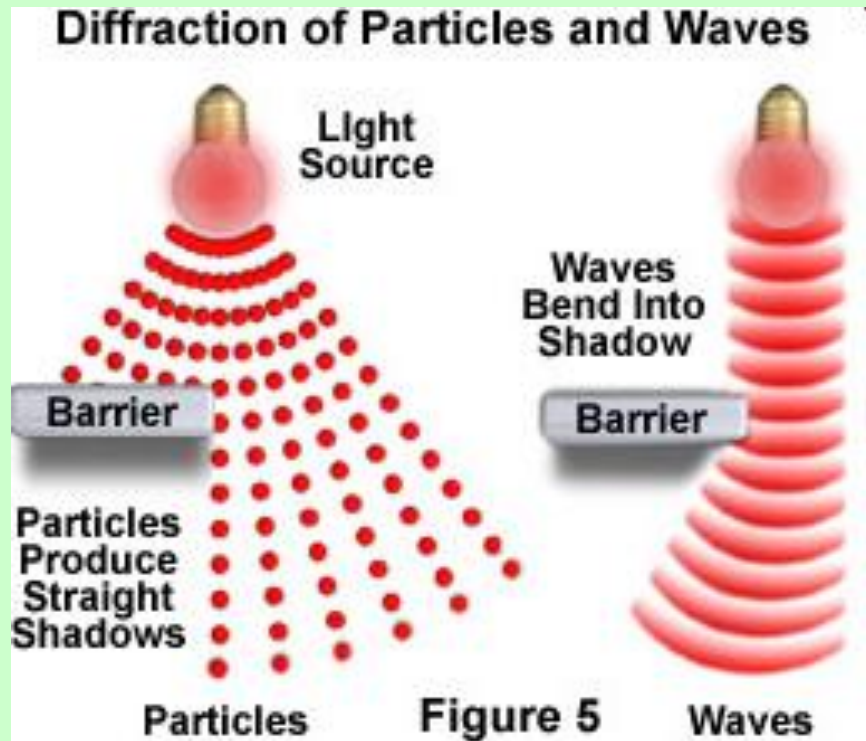
$$\begin{aligned} \frac{1}{d_o} &= \frac{1}{f_o} - \frac{1}{L - f_e} = \\ &= \frac{L - f_e - f_o}{f_o (L - f_e)} \approx \frac{L}{f_o L} \\ &\approx \frac{1}{f_o} \end{aligned}$$

Therefore, if $L \gg f_o, f_e \Rightarrow d_o \sim f_o$. Therefore the object should be placed at the focus of the objective lens

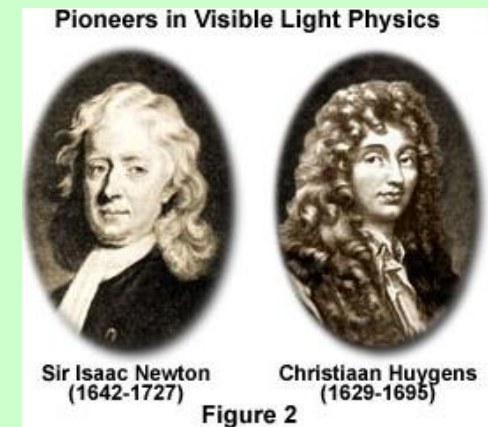
Resolution in Microscopy



Young's Double-Slit Experiment indicated light behaved as a wave

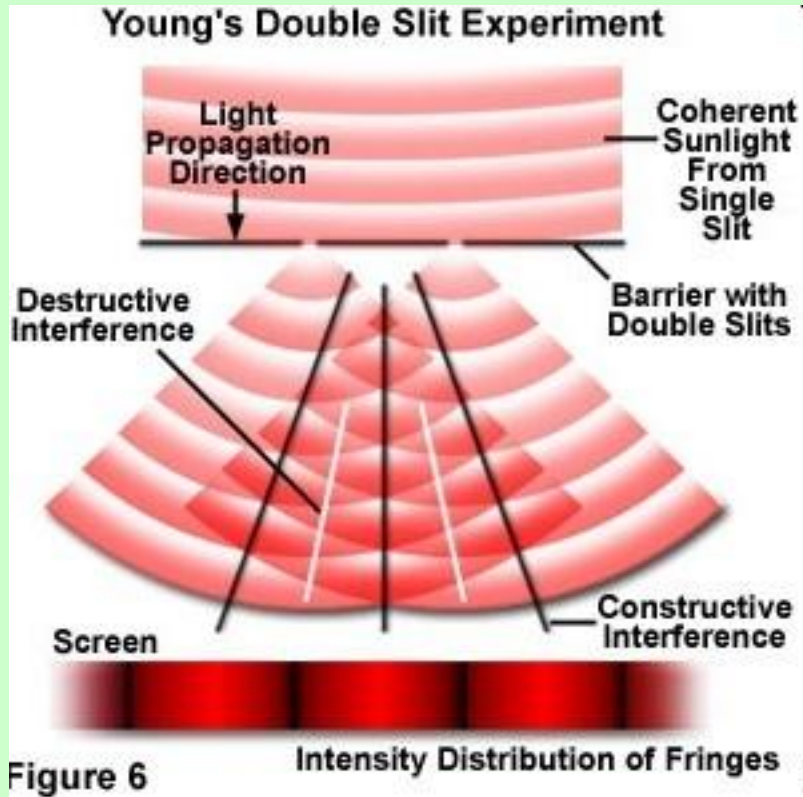


If the particles encounter the edge of a barrier, then they will cast a shadow because the particles not blocked by the barrier continue on in a straight line and cannot spread out behind the edge. On a macroscopic scale, this observation is almost correct, but it does not agree with the results obtained from light diffraction experiments on a much smaller scale.



Particles and waves should also behave differently when they encounter the edge of an object and form a shadow (Figure 5). Newton was quick to point out in his 1704 book *Opticks*, that "Light is never known to follow crooked passages nor to bend into the shadow". This concept is consistent with the particle theory, which proposes that light particles must always travel in straight lines.

Young's Double-Slit Experiment indicated light behaved as a wave



Young coined the term **interference fringes** to describe the bands and realized that these colored bands could only be produced if light were acting like a wave.

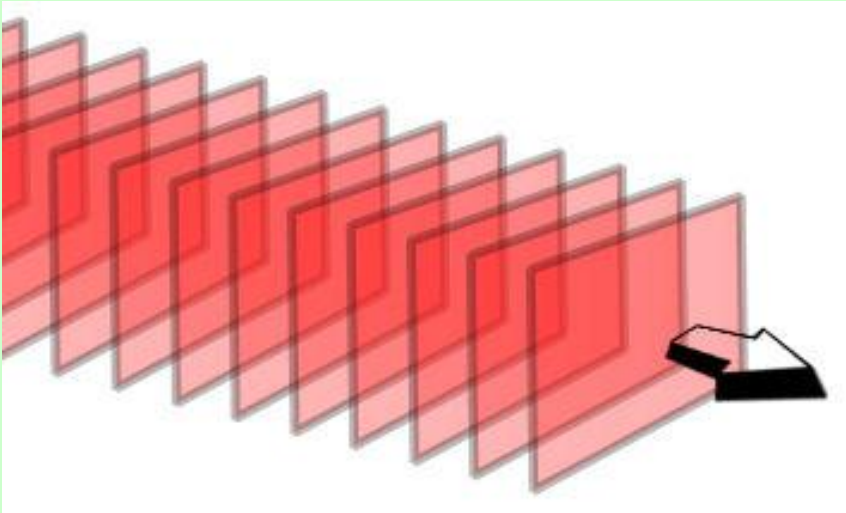
In 1801, an English physicist named Thomas Young performed an experiment that strongly inferred the wave-like nature of light. Because he believed that light was composed of waves, Young reasoned that some type of interaction would occur when two light waves met.

Young's experiment was based on the hypothesis that if light were wave-like in nature, then it should behave in a manner similar to ripples or waves on a pond of water. Where two opposing water waves meet, they should react in a specific manner to either reinforce or destroy each other.

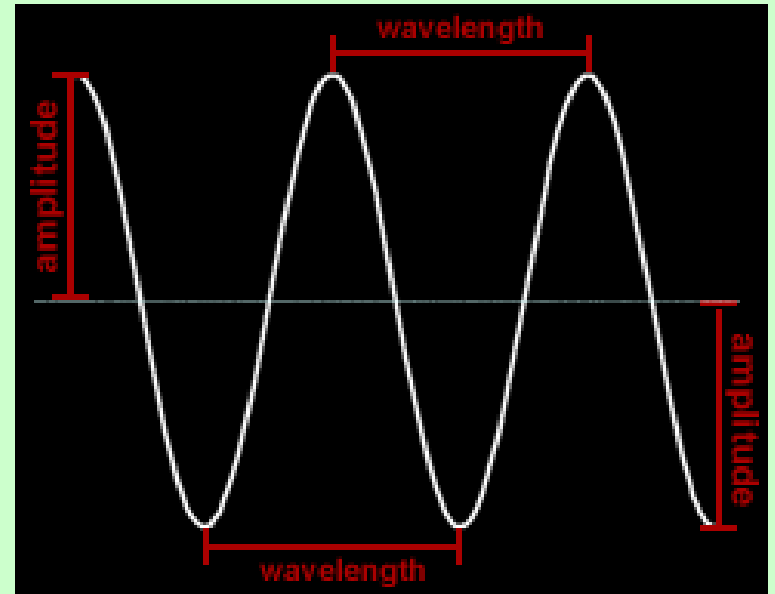
Young observed that when the slits were large, spaced far apart and close to the screen, then two overlapping patches of light formed on the screen. However, when he reduced the size of the slits and brought them closer together, the light passing through the slits and onto the screen produced distinct bands of color separated by dark regions in a serial order.

Plane Wave: Definition

Definition: In the physics of wave propagation, a plane wave is a constant-frequency wave whose wavefronts (surfaces of constant phase) are infinite parallel planes of constant amplitude normal to the phase velocity vector (Wikipedia, 2009).



The wavefronts of a plane wave traveling in 3-D space (Wikipedia, 2009).



Plane Wave: Parameters

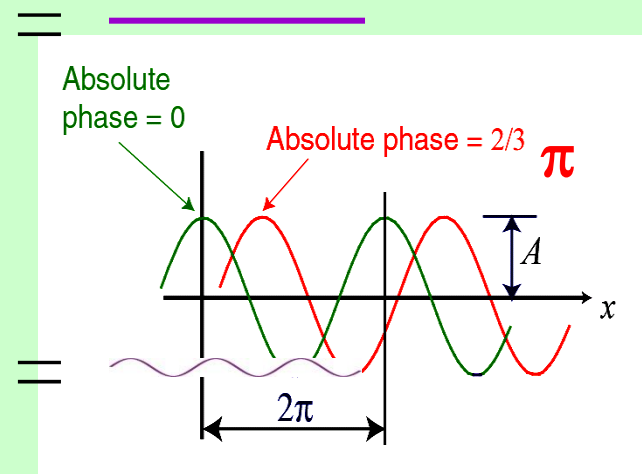
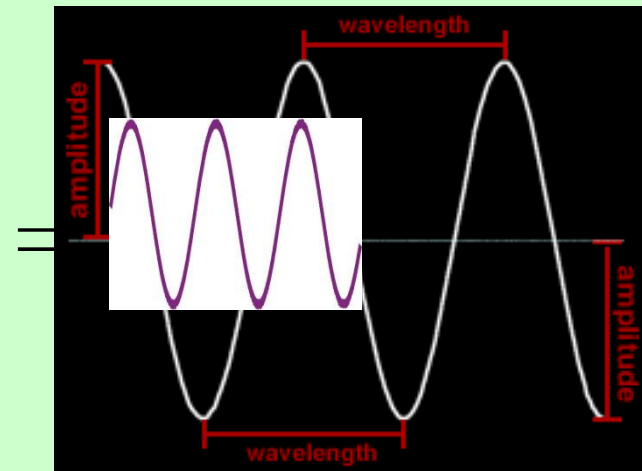
$$E = E_0 \cdot \cos(k \times x - 2\pi f \times t + \varphi)$$

Light waves are characterized by:

- Amplitude - E_0
- Frequency - f
- Wavenumber - $k = \omega/c$
- Wavelength - λ
- Phase - φ
- Velocity - c
- Angular frequency - $2\pi f$

$$\phi = Ae^{i(kz - \omega t)};$$

$$\exp^{i\alpha} = \cos \alpha + i \sin \alpha$$



The most convenient wave to write down introduce equation describing a plane wave is to use complex exponent e^{ia}

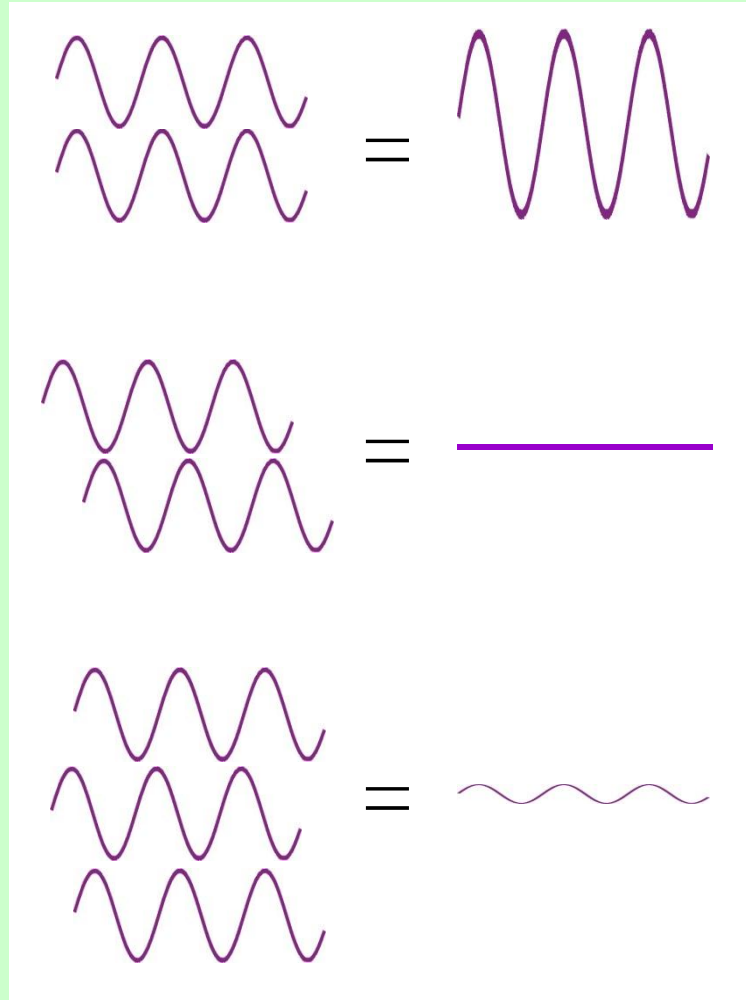
Plane Wave: Phase

Constructive vs. destructive interference;
Coherent vs. incoherent interference

Waves that combine **in phase** add up to relatively high irradiance.

Waves that combine **180° out of phase** cancel out and yield zero irradiance.

Waves that combine with **lots of different phases** nearly cancel out and yield very low irradiance.



Constructive
interference
(**coherent**)

Destructive
interference
(**coherent**)

Incoherent
addition

Plane Wave: Wavelength and Period

$$E = E_0 \cdot \cos(k \times x - 2\pi f \times t + \varphi)$$

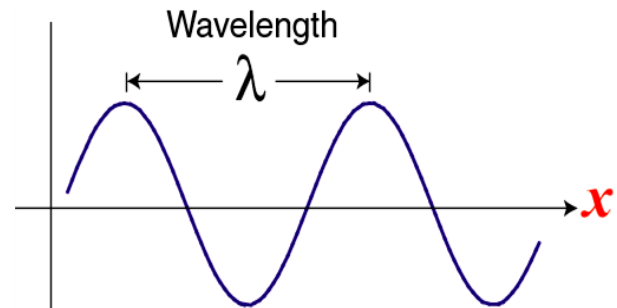


Surfer is sitting
on the wave



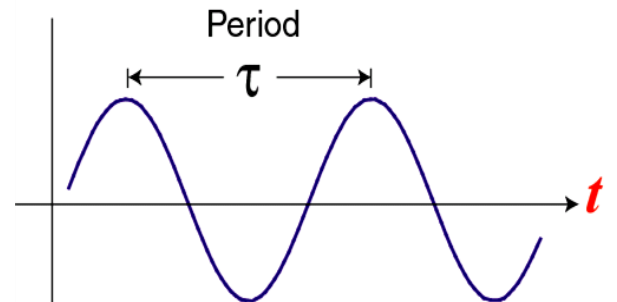
We are sitting
on the beach
and watching
waves

Spatial quantities:



The k-vector: $k = 2\pi/\lambda$

The wave number: $\kappa = 1/\lambda$

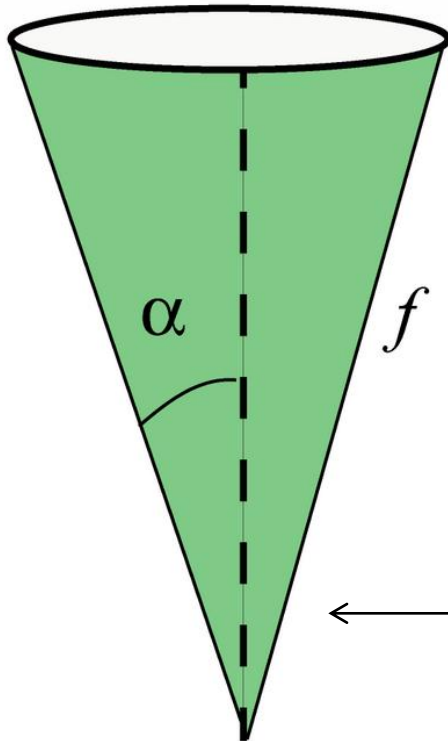


The angular frequency: $\omega = 2\pi/\tau$

The frequency: $\nu = 1/\tau$

Temporal quantities:

Numerical Aperture



Semi-aperture angle (α): angle between the normal ray and the furthest ray entering the system.

Numerical Aperture =

$$NA = n(\sin \alpha)$$

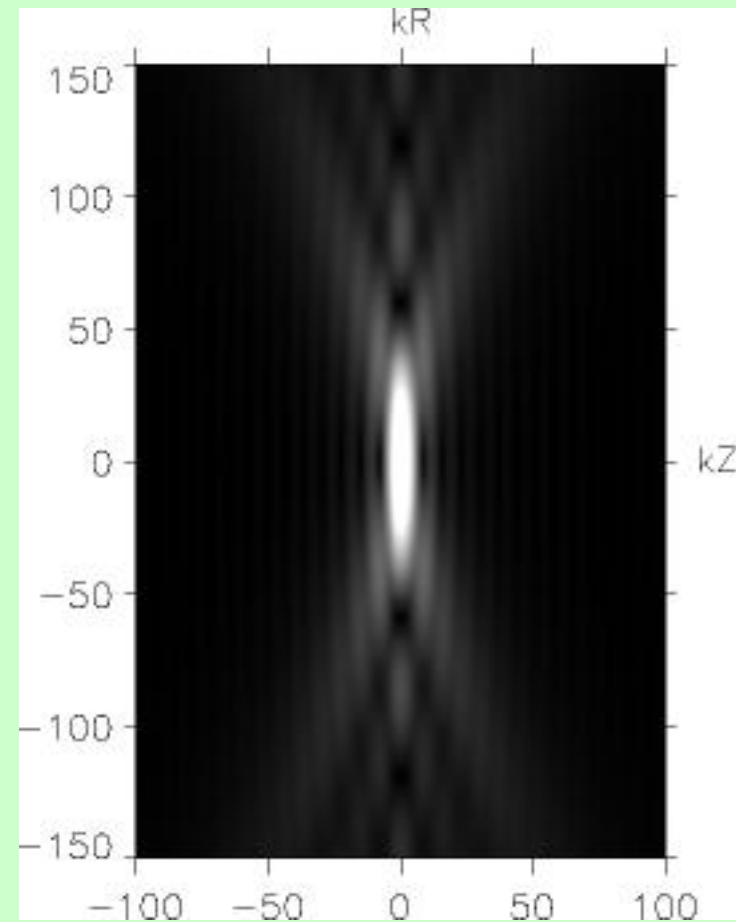
($n = \text{refractive index}$)

NA can exceed 1.0 by using other immersion liquids including water (1.333) or oil (1.51).

Structure of the focus

$$\Phi(r_t) = \frac{J_1(kr_t \sin \alpha)}{kr_t \sin \alpha}$$

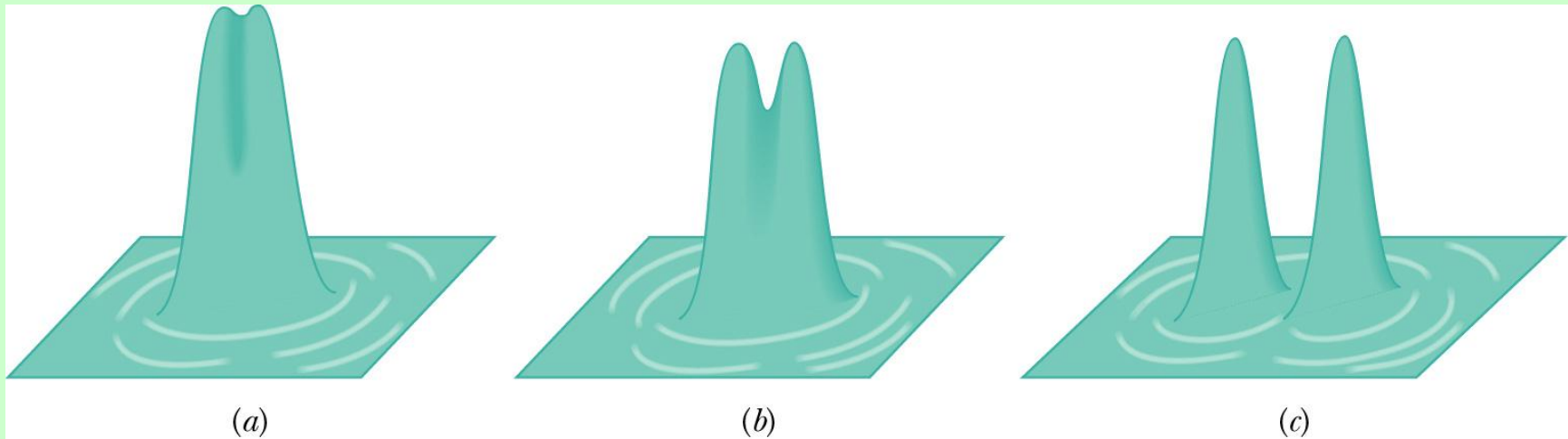
$$\Phi(z) = \frac{\sin[0.5kz(1 - \cos \alpha)]}{0.5kz(1 - \cos \alpha)}$$



Calculated acoustic field near the focus of an elementary acoustic microscope lens with semi-aperture angle $\alpha = 20^\circ$.

Resolution: Rayleigh criterion

If you view two point sources that are very close together, you may not be able to distinguish them.



An empirical diffraction limit is given by the Rayleigh criterion invented by *Lord Rayleigh*: The images of two different points are regarded as just resolved when the principal diffraction maximum of one image coincides with the first minimum of the other.

Axial and Lateral Resolutions

Lateral Resolution:

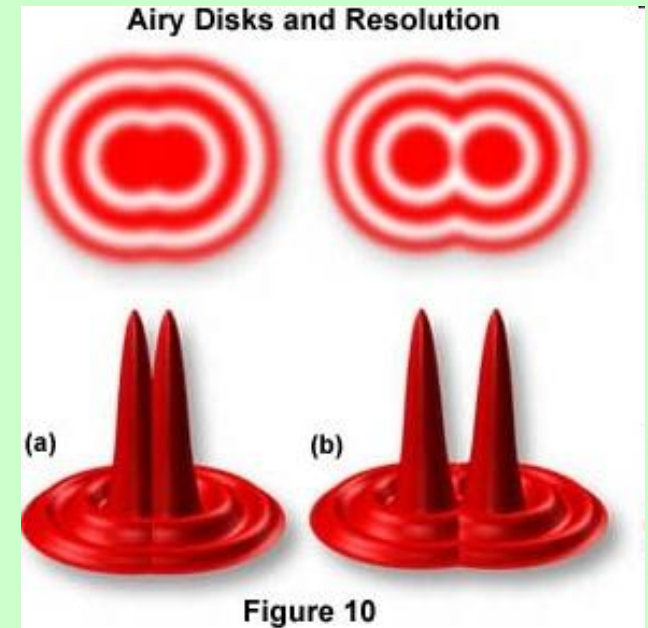
$$\Phi(r_t) = \frac{J_1(kr_t \sin \alpha)}{kr_t \sin \alpha} = 0$$

$$r_{Airy} = \frac{0.61\lambda}{\sin \alpha}$$

Axial Resolution:

$$\Phi(z) = \frac{\sin[0.5kz(1 - \cos \alpha)]}{0.5kz(1 - \cos \alpha)} = 0$$

$$z_{axial} = \frac{\lambda}{(1 - \cos \alpha)}$$

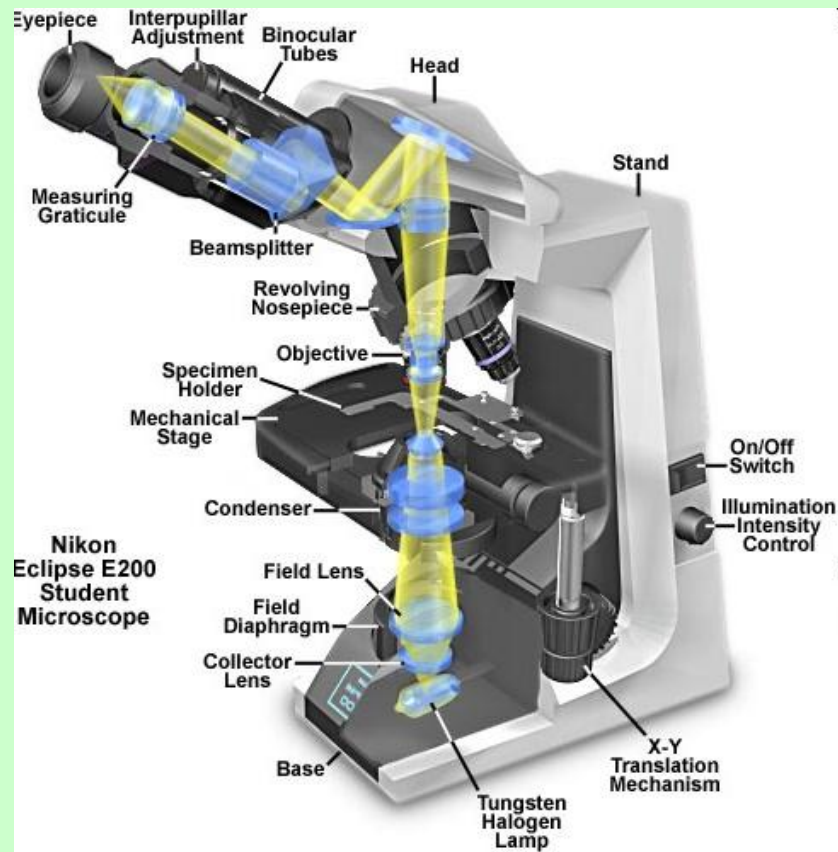


(<http://www.olympusmicro.com>).

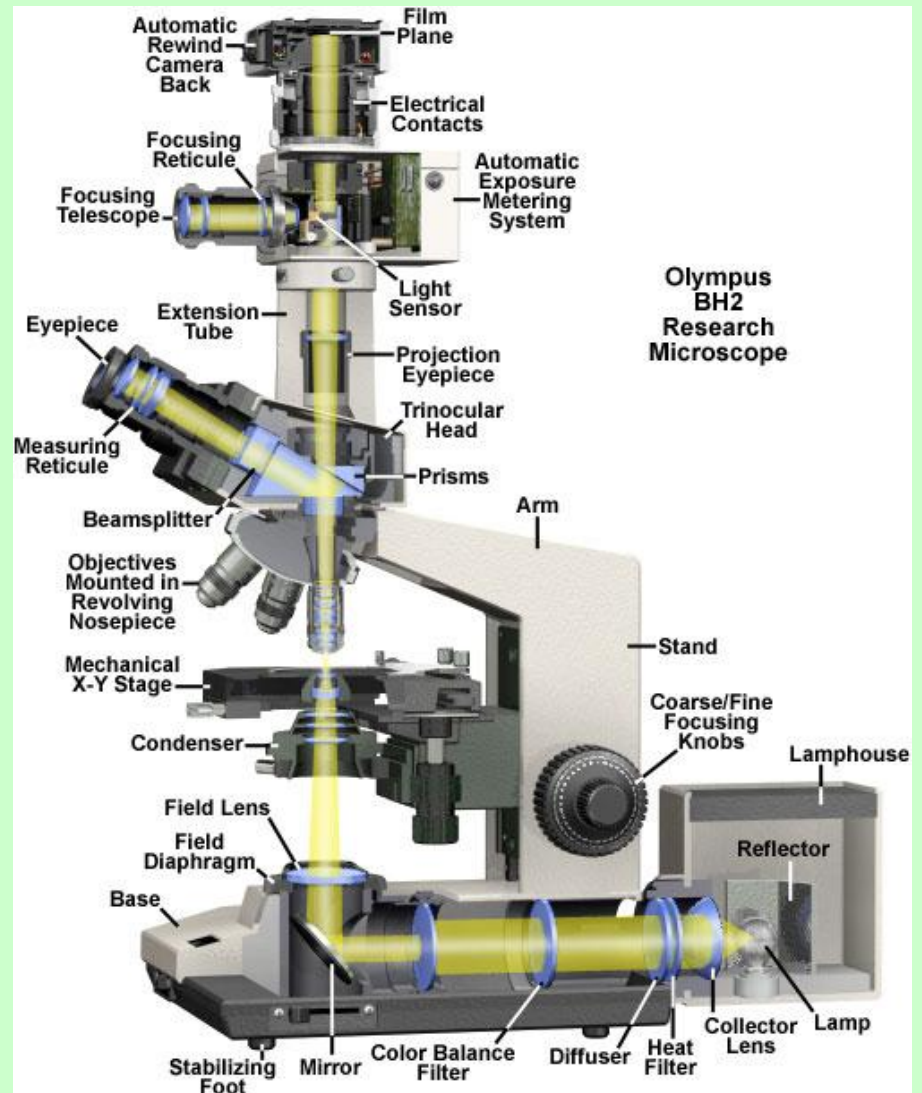
The images of two different points are regarded as just resolved when the principal diffraction maximum of one image coincides with the first minimum of the other.

Compound Microscope versus Research Microscope

Nikon Eclipse E200 Microscope Cutaway Diagram

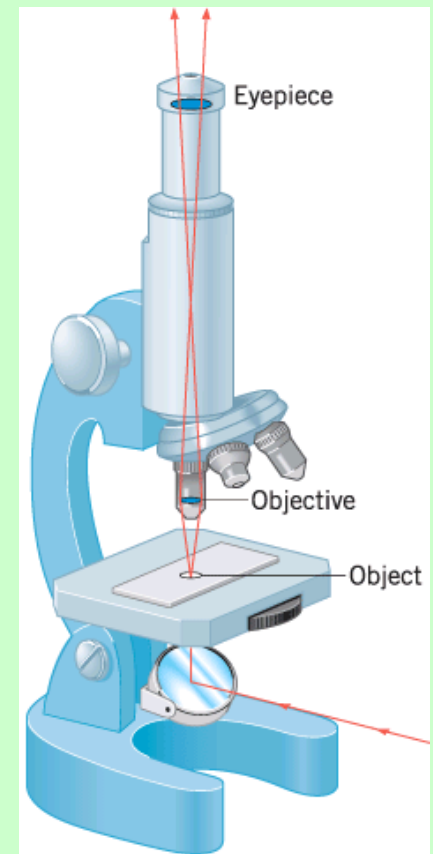
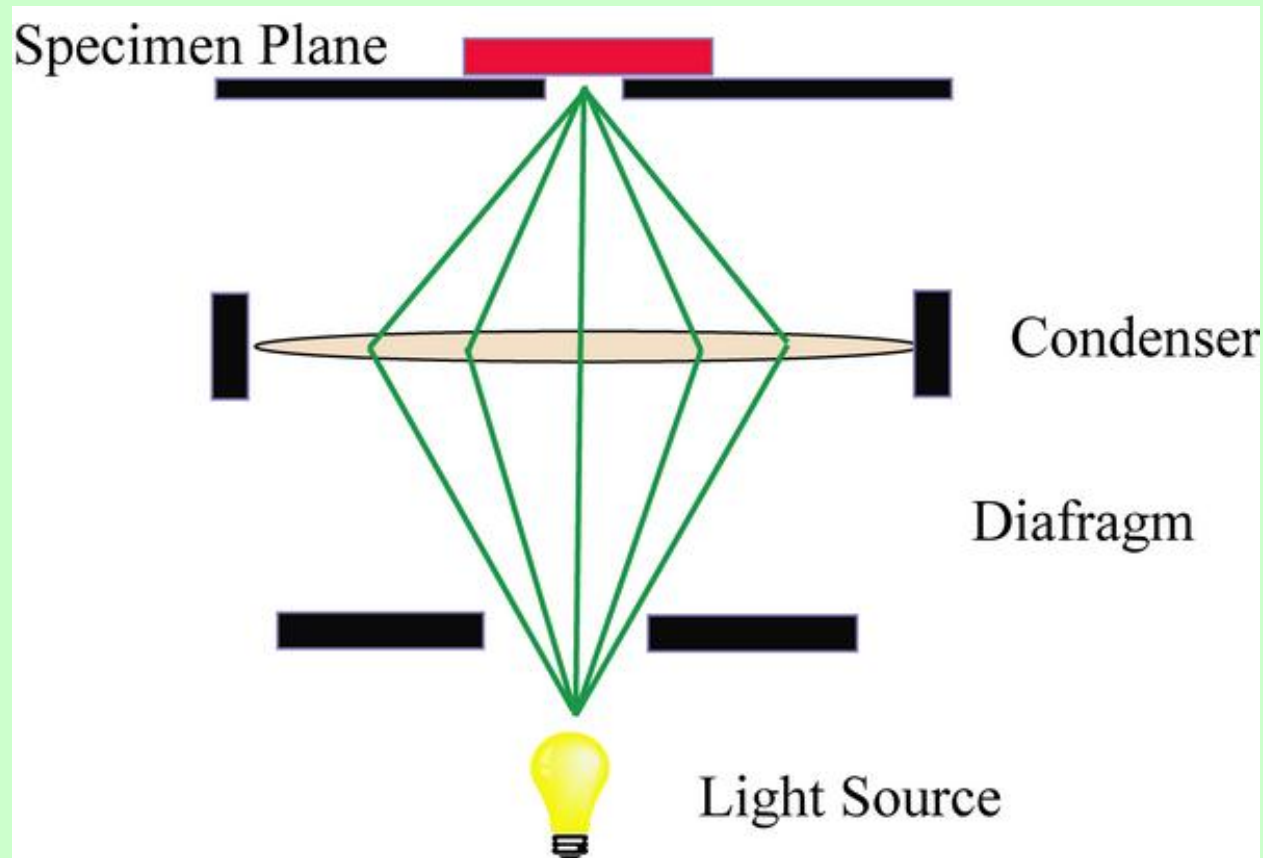


Olympus BH2 Research Microscope Cutaway Diagram



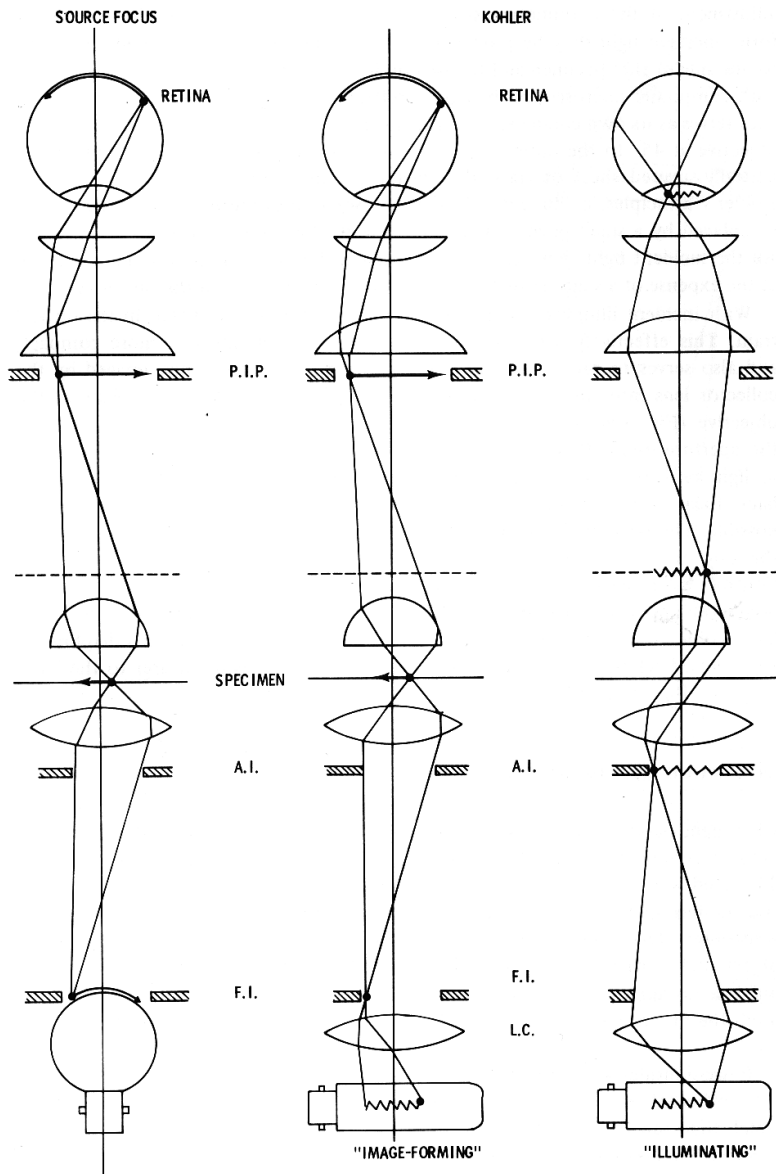
Specimen Illumination System

In 1869, Abbe's introduced a new patented illumination device - the **Abbe condenser**.



Old type illumination
(see Hooke's microscope)

Köhler Illumination

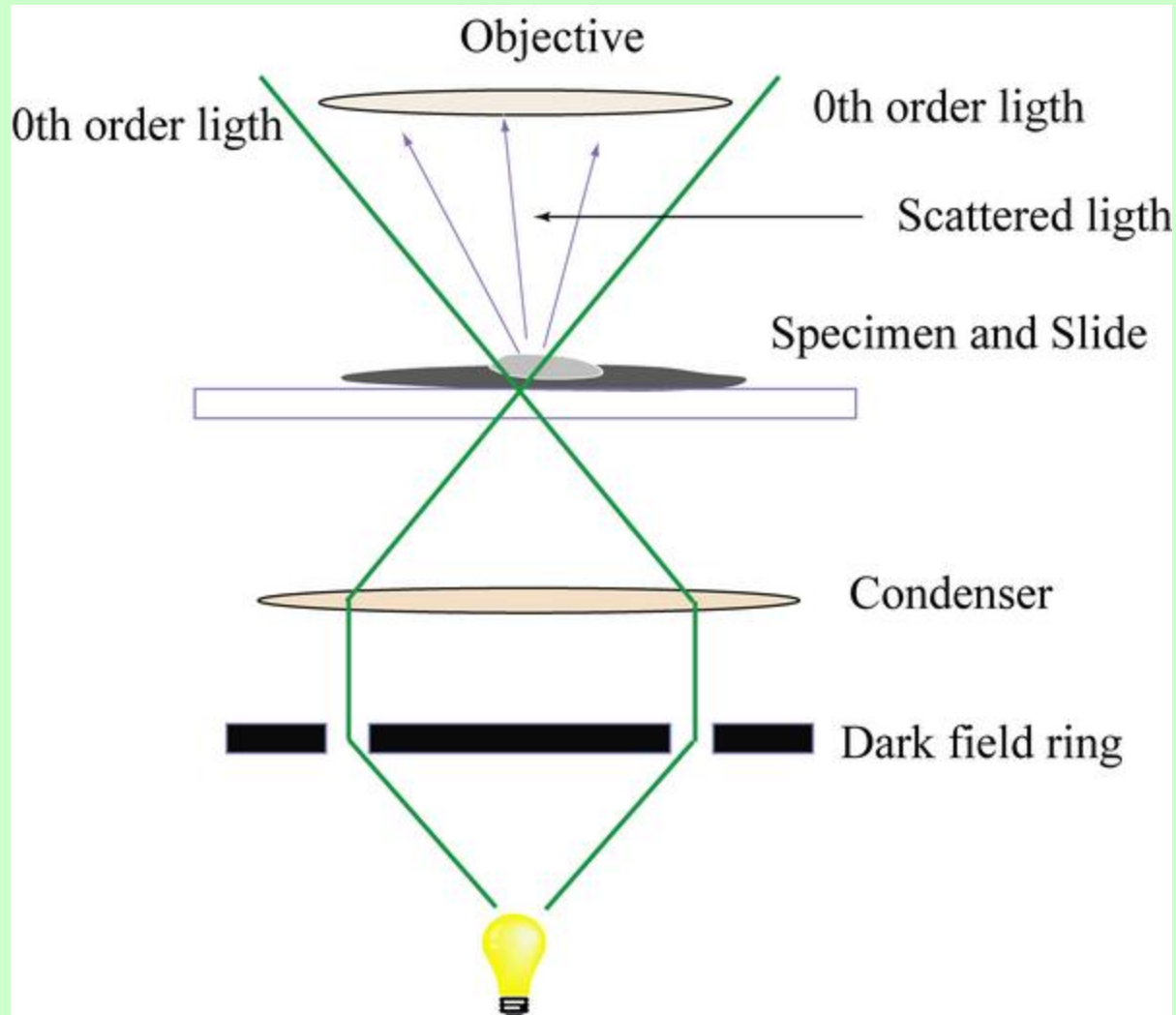


The older, rarely used today, is called 'source- focus'. A homogeneous lamp surface is imaged directly by the microscope condenser into the plane of the specimen.

Most microscopes with integral illuminators use a low-voltage tungsten filament lamp which is of high intensity but has a filament which possesses a marked structure. These cannot be used in the source-focus mode; instead the technique devised originally for photomicrography by Kohler must be used.

- The specimen is illuminated homogeneously
- The specimen and the images of the light source are in different planes

Darkfield Illumination

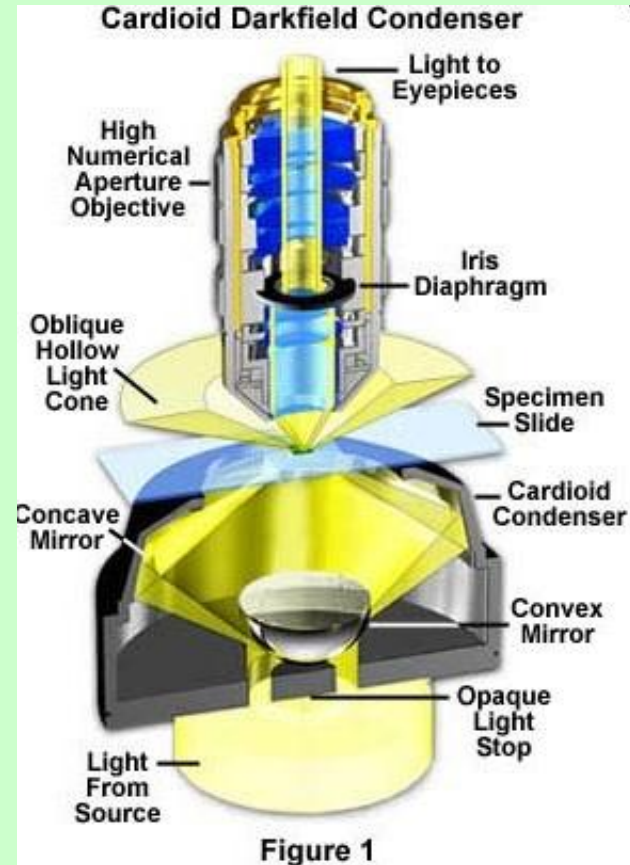
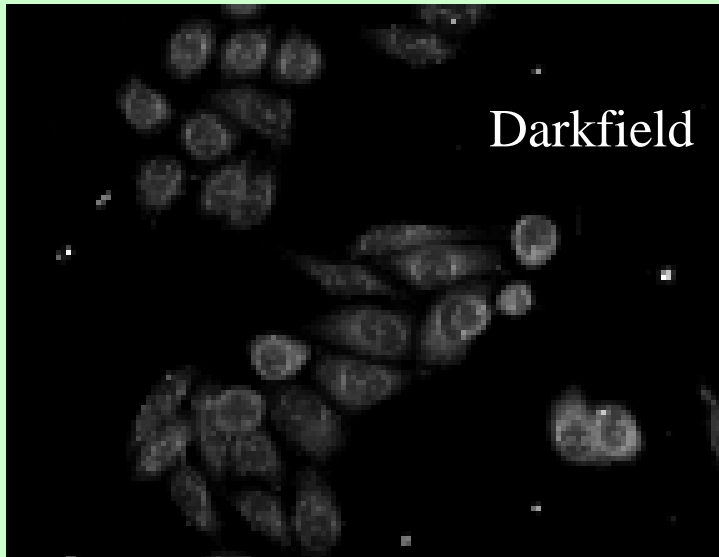


Allows for only the diffracted light to be collected by the objective lens.

Uses a large black Ring in the condenser to illuminate the sample. The cone of light produced is too large for the objective lens to collect.

Gives a black image with bright edges.

Darkfield Microscopy



- Blocking out the central light rays that otherwise pass through the specimen
- Allowing only oblique rays to illuminate the specimen.
- Suitable for unstained or shiny stained specimens

Home work

1. Introduce the concept of spherical waves and present mathematical equation describing a spherical wave.
2. Simulate lateral and axial resolutions as a function of aperture angle.
3. Describe the difference between magnification and resolution.