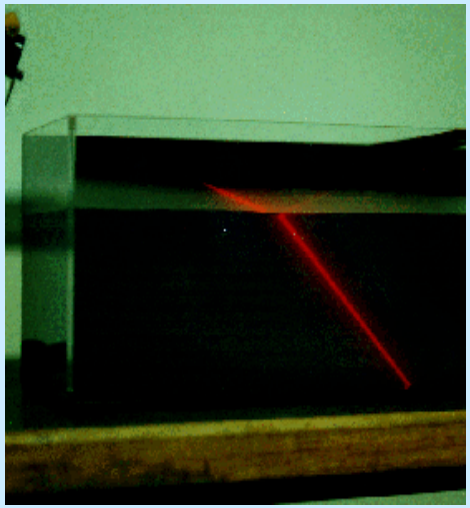
Interactions of light 1

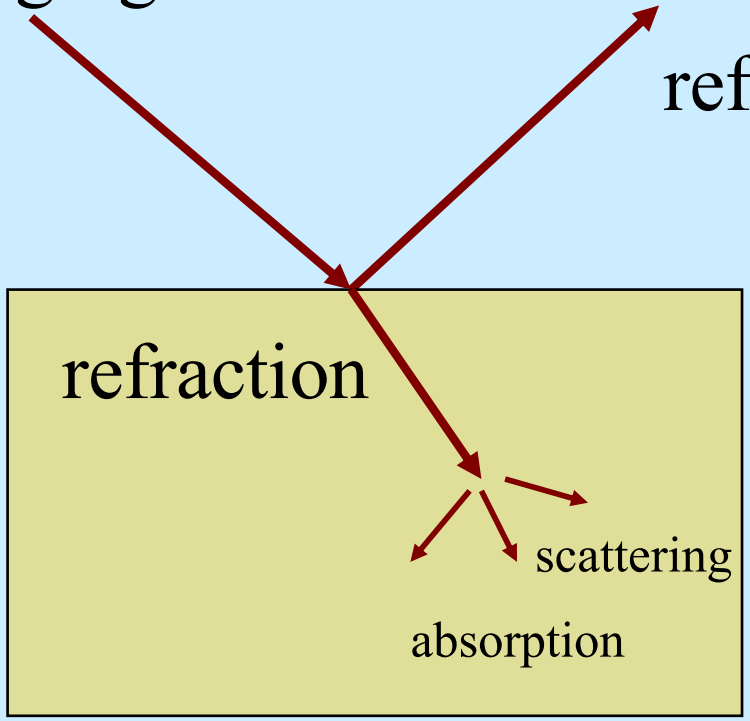
refraction, optical fiber, light microscope, electron microscope

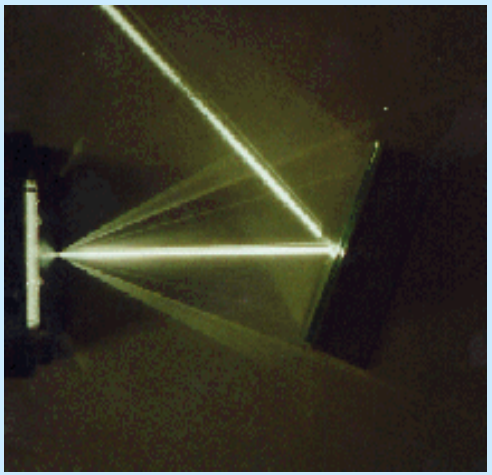
28-09-2020

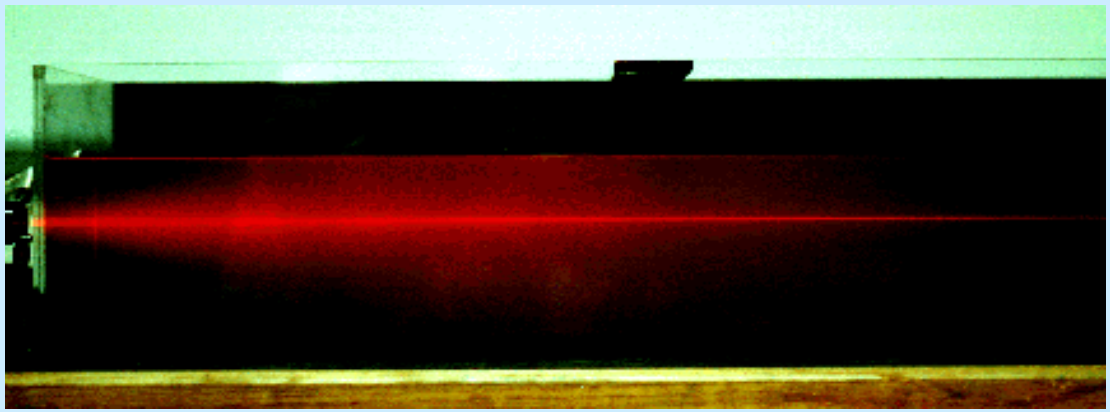
Liliom Károly

Interactions of light with matter





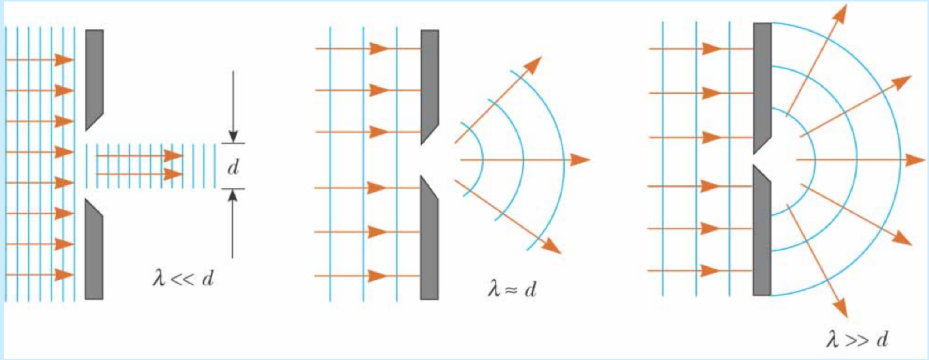




Geometric optics

Remember: light propagates as a wave➔

In homogeneous and isotropic medium
light travels a straight line➔ **light ray**



Light travels with velocity c = $3x10^8$ m/s (2,99792458...$x10^8$ m/s) – only in vacuum!

– in optically dense medium it slows down: $c_1 < c$ (as $E=hf$ and $c=\lambda f$, thus $\lambda_1<\lambda$)

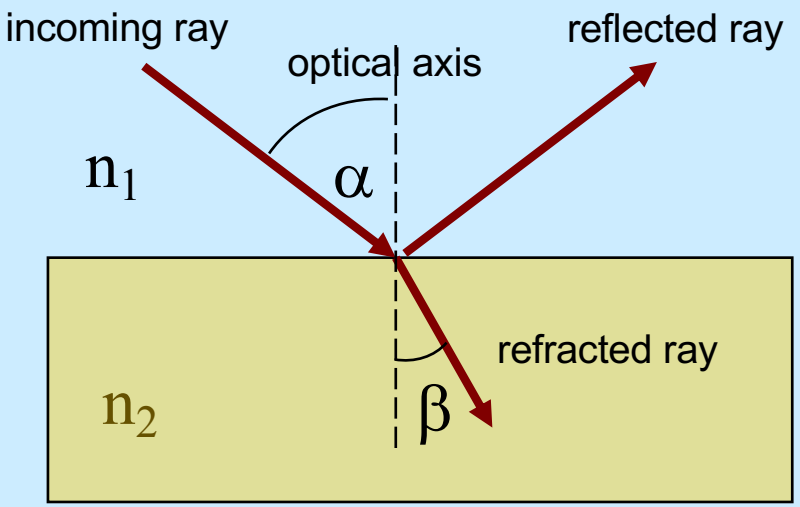
– absolute index of refraction is the ratio of light speed in vacuum to the medium: $n_1=c/c_1$

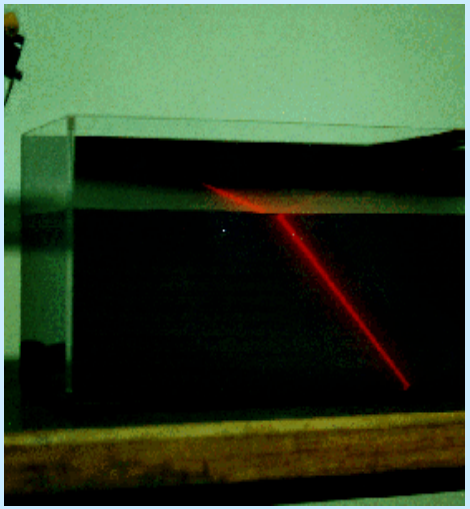
– relative index of refraction of two media: $n_{2,1} = n_2 / n_1$

– light ray is an arrow, arrowhead showing the propagation of energy
reversibility principle: the opposite direction is possible as well

– **Fermat's principle:**
light travels the path between points that needs the least time to take

Refraction



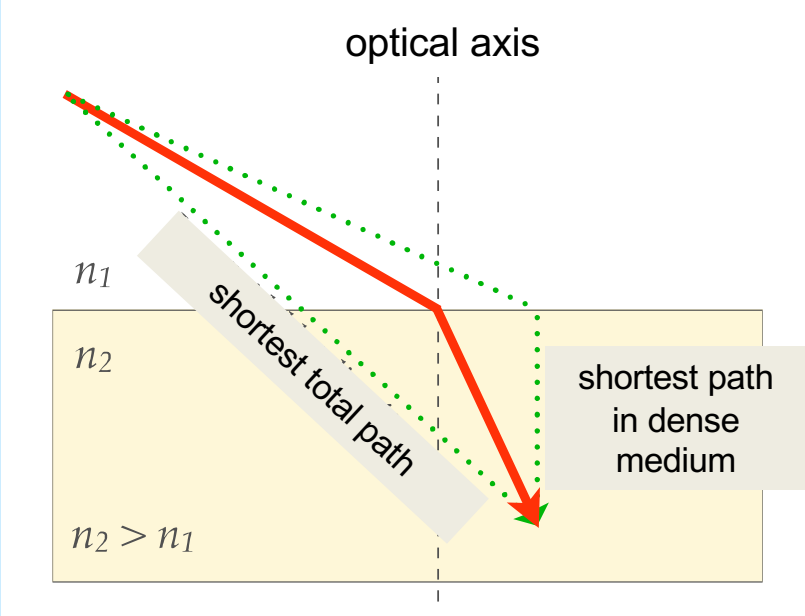


$$n_1 < n_2$$

$$\alpha > \beta$$

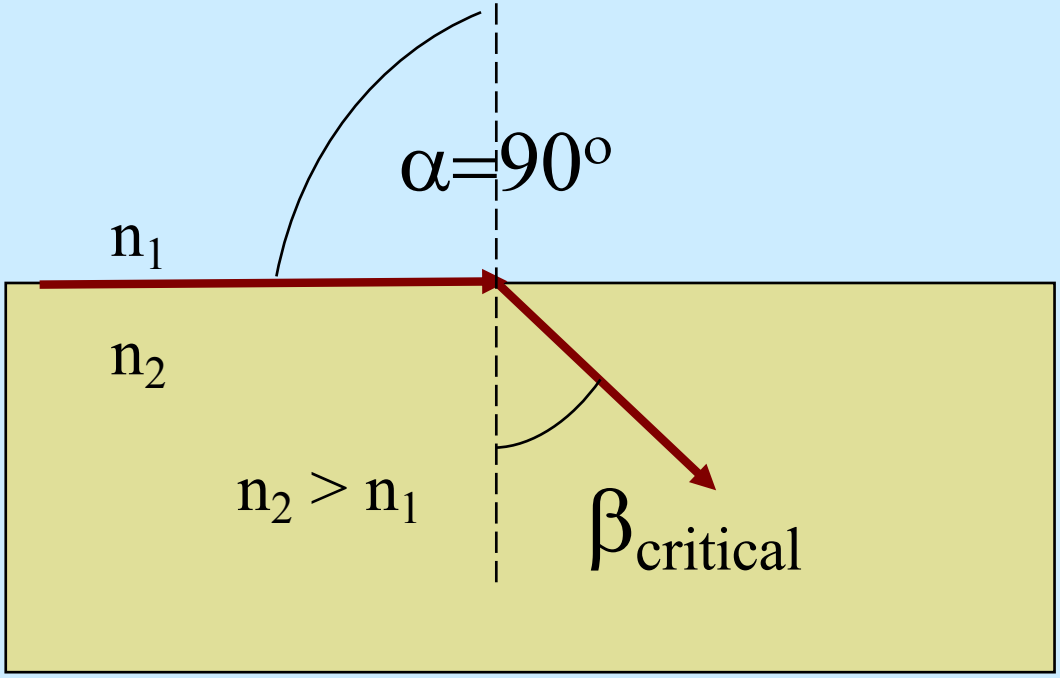
Snell's law:

$$\frac{\sin\alpha}{\sin\beta} = \frac{c_1}{c_2} = \frac{n_2}{n_1} = n_{21}$$



Refraction can be explained by the Fermat's principle

Limiting (critical) angle of refraction

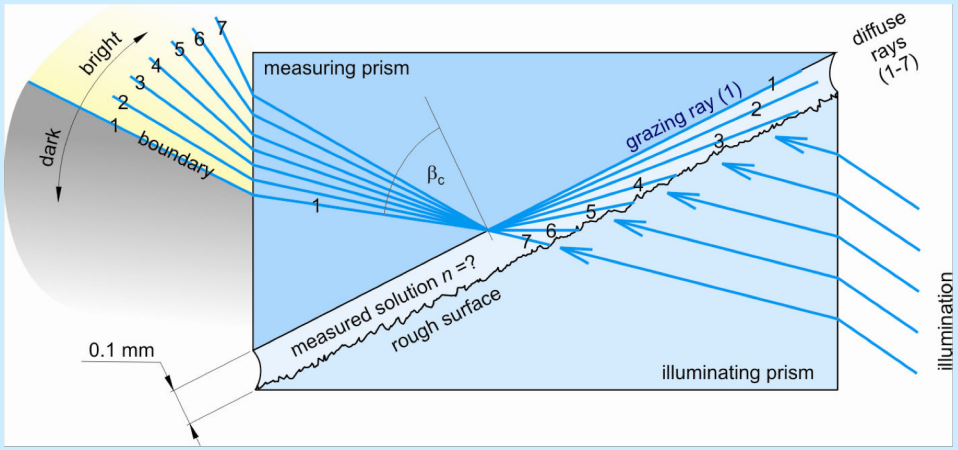


$n_2/n_1 = \sin 90° / \sin \beta_{critical} = 1/\sin \beta_{critical} \rightarrow n_1 = n_2 \times \sin \beta_{critical}$

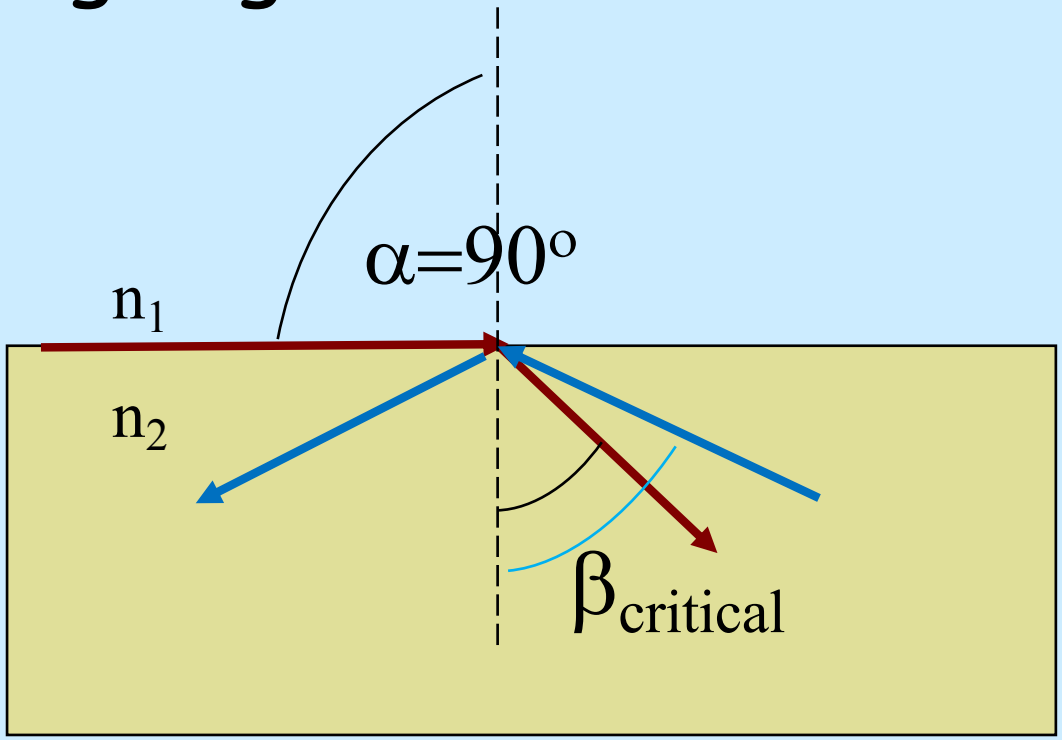
Analytical application:

the index of refraction of diluted solutions depends linearly on their concentration (c): $n_1 = n_0 + k*c$

if $n_1 < n_{measuring\ prism}$



Limiting angle and total internal reflection

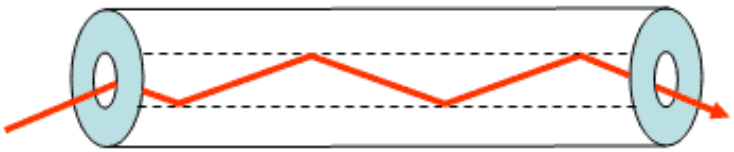


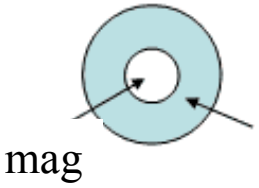
$\beta > \beta_{critical}$

application:

optical fiber

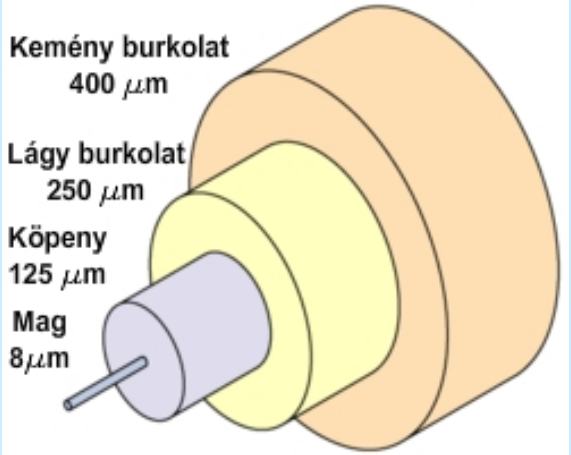
optical light guidance





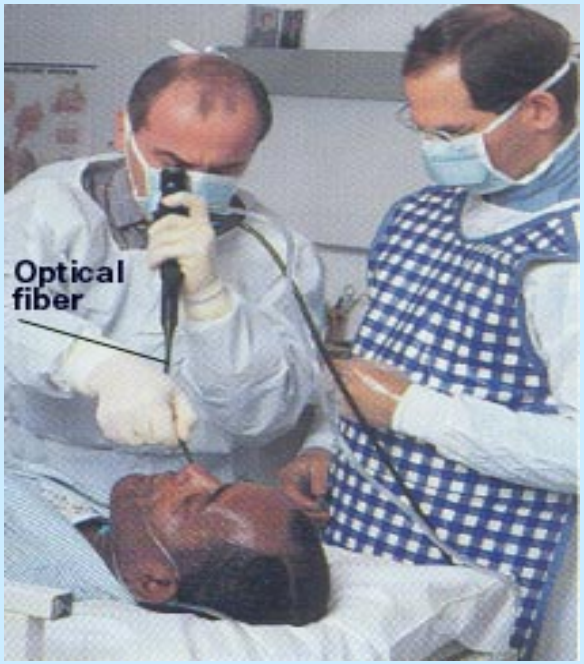
$n_{mag}=1,458$

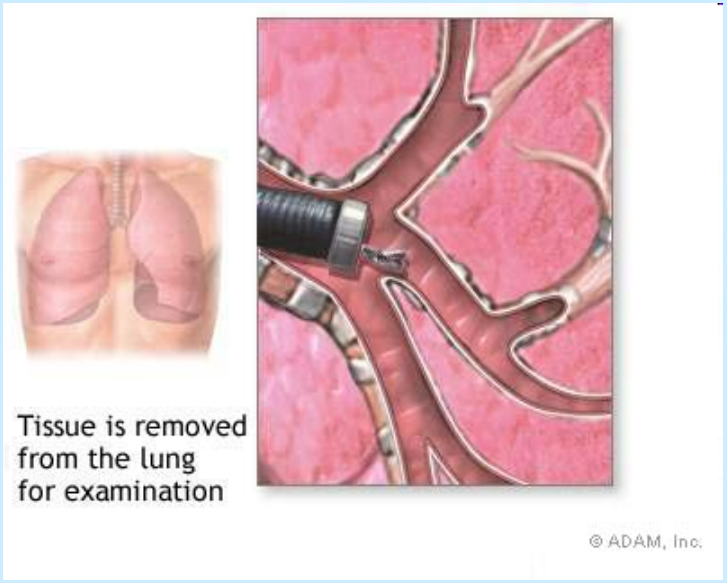
$n_{köpeny}=1,440$



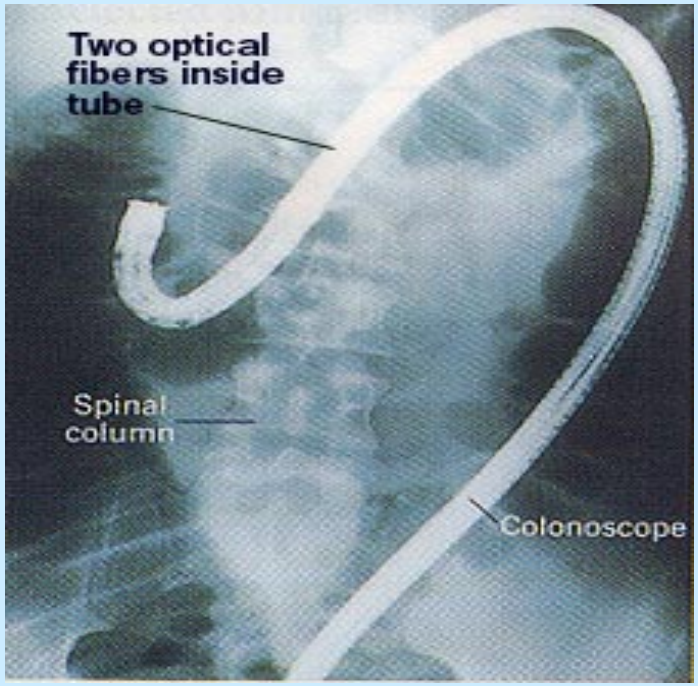
Applications in medicine:

– diagnostics: local inspection, biopsy,...
– therapy: surgery,...



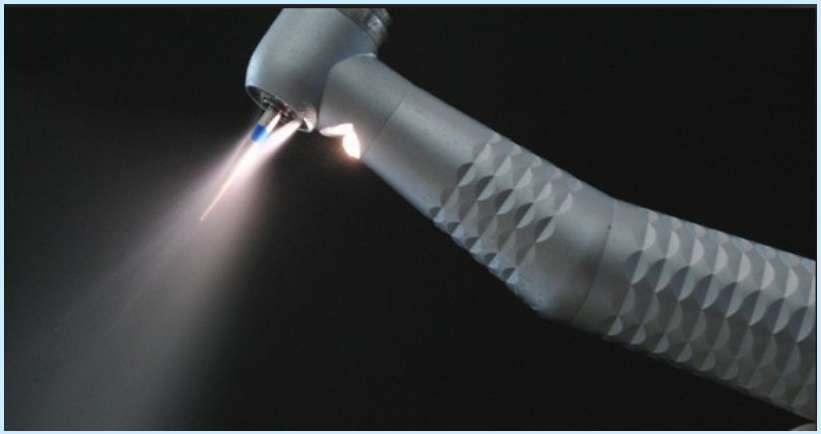


Bronchoscopy



Colonoscopy

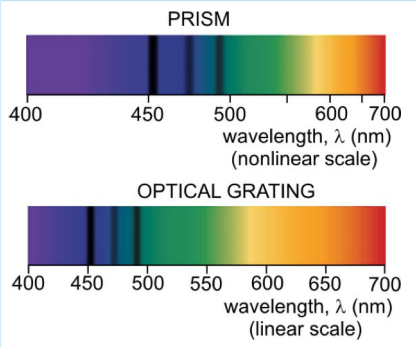
Examples of applications in dentistry:



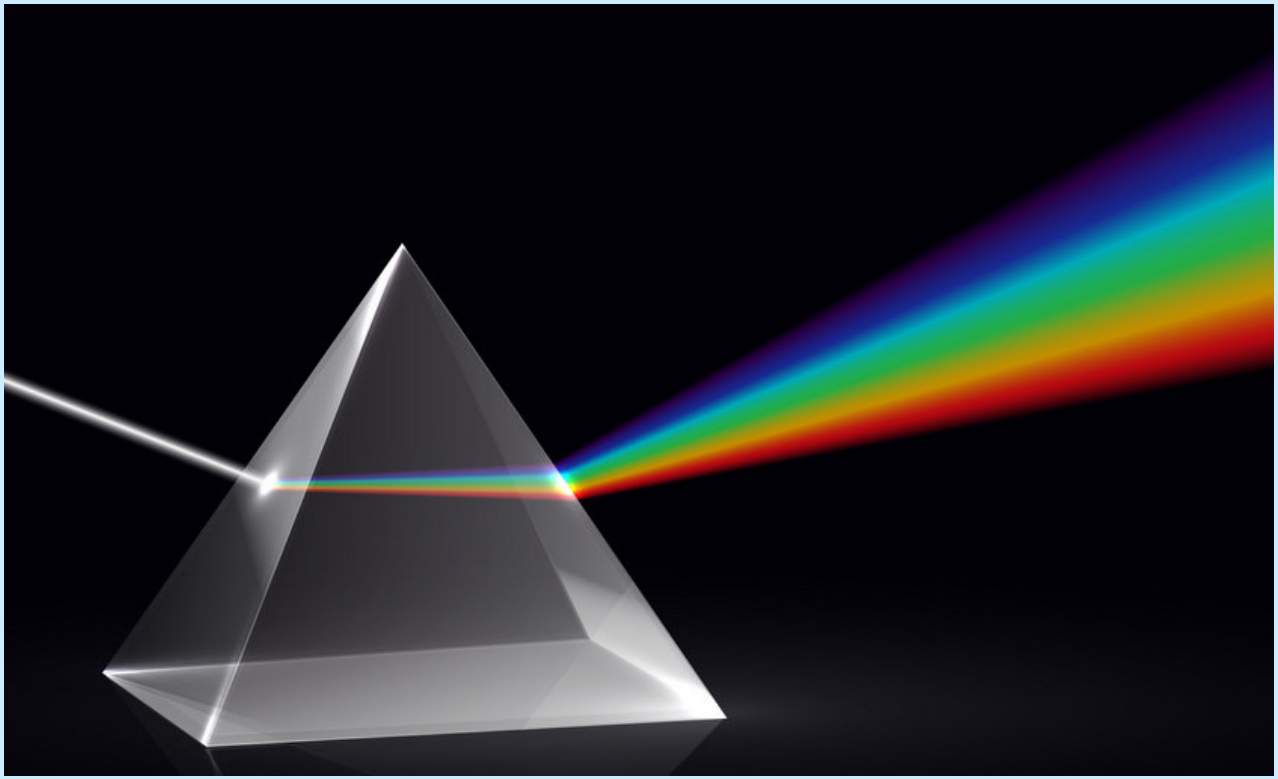
Dispersion

Index of refraction depends on the frequency of light:
increased frequency = increased index of refraction ➔

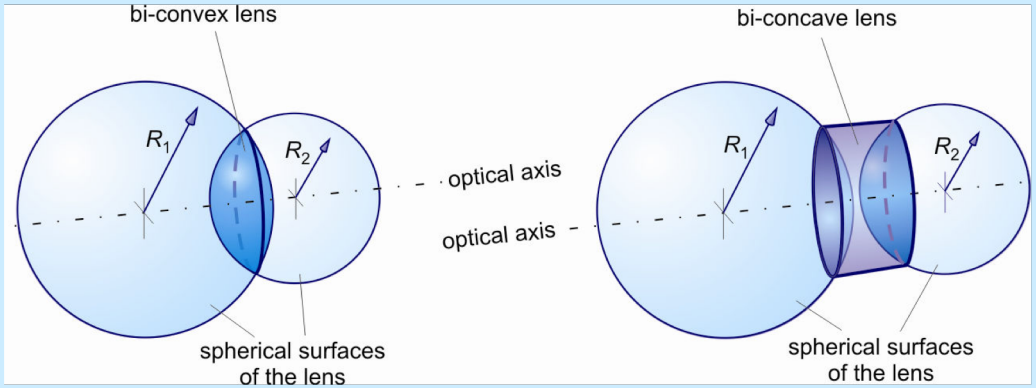
spectral decomposition of mixed (white) light is possible:



spectrum of a prism is non-linear, whereas spectrum made by an optical grating is linear

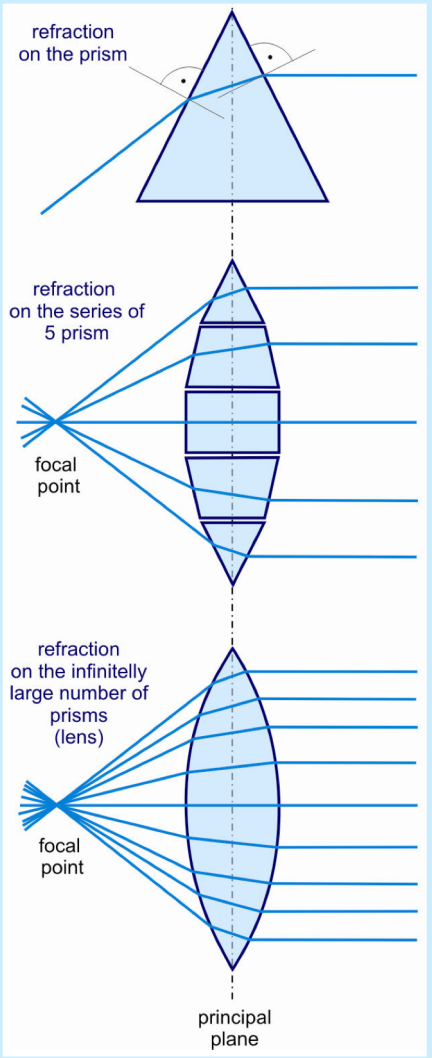


Refraction on curved surfaces



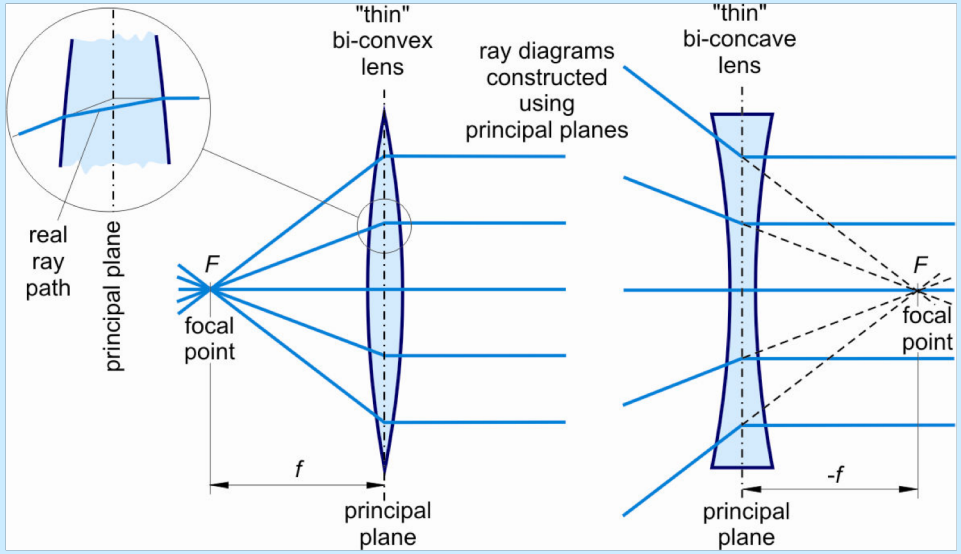
Power of a lens (diopter, 1/m)

$$D = \frac{1}{f} = (n_{21} - 1)\left(\frac{1}{R_1} + \frac{1}{R_2}\right)$$



– rays travel parallel with the optical axis focus in one point (converging lens), or diverge as they are emanated from one point (diverging lens)

– simplification for thin lenses: if only one refraction would happen in principal plane!



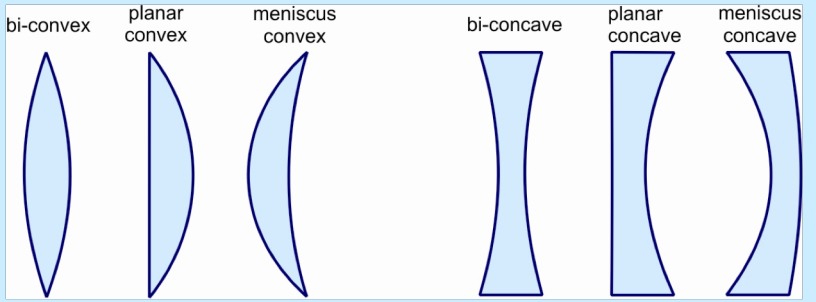
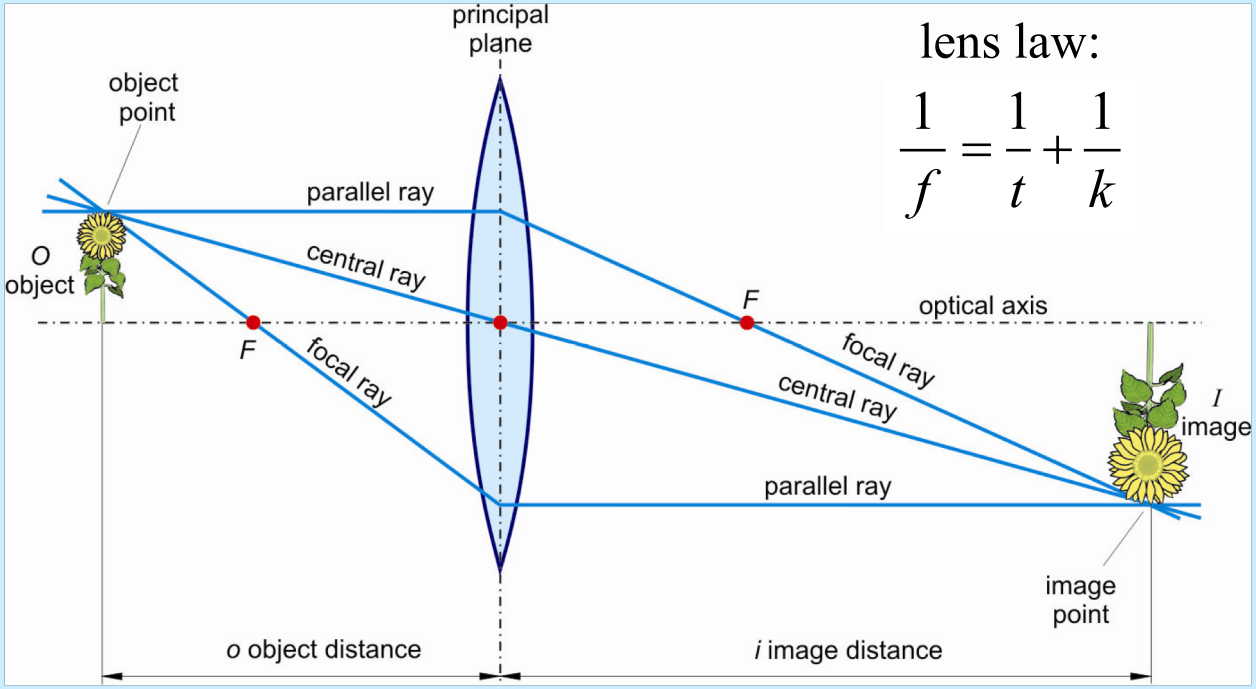


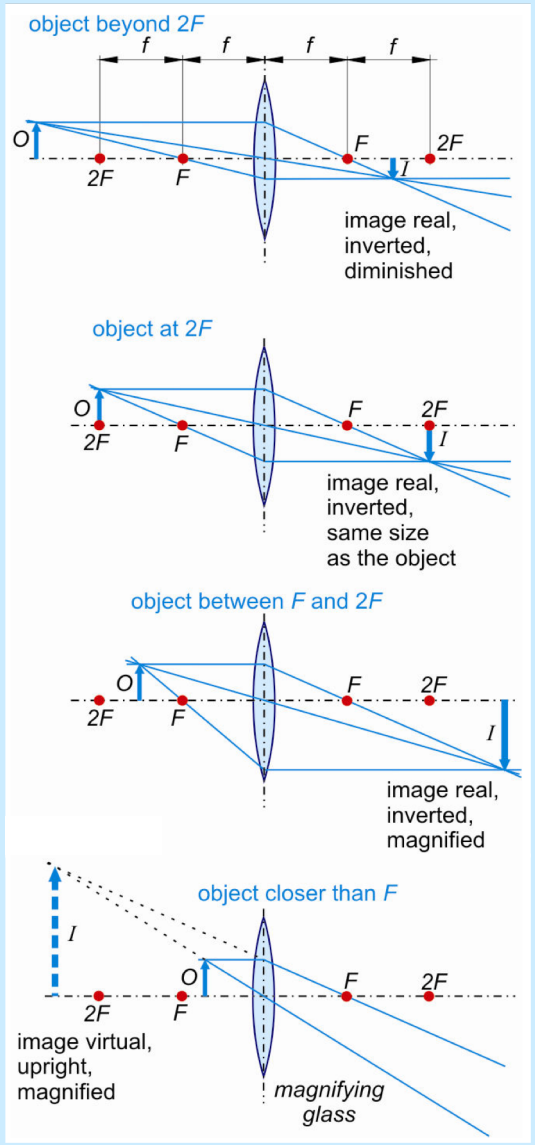
Image formation by thin spherical lenses – principal rays

Image is formed if rays emanating from a point re-unite:



lens law:

$$\frac{1}{f} = \frac{1}{t} + \frac{1}{k}$$



Linear magnification: ratio of image size to object size

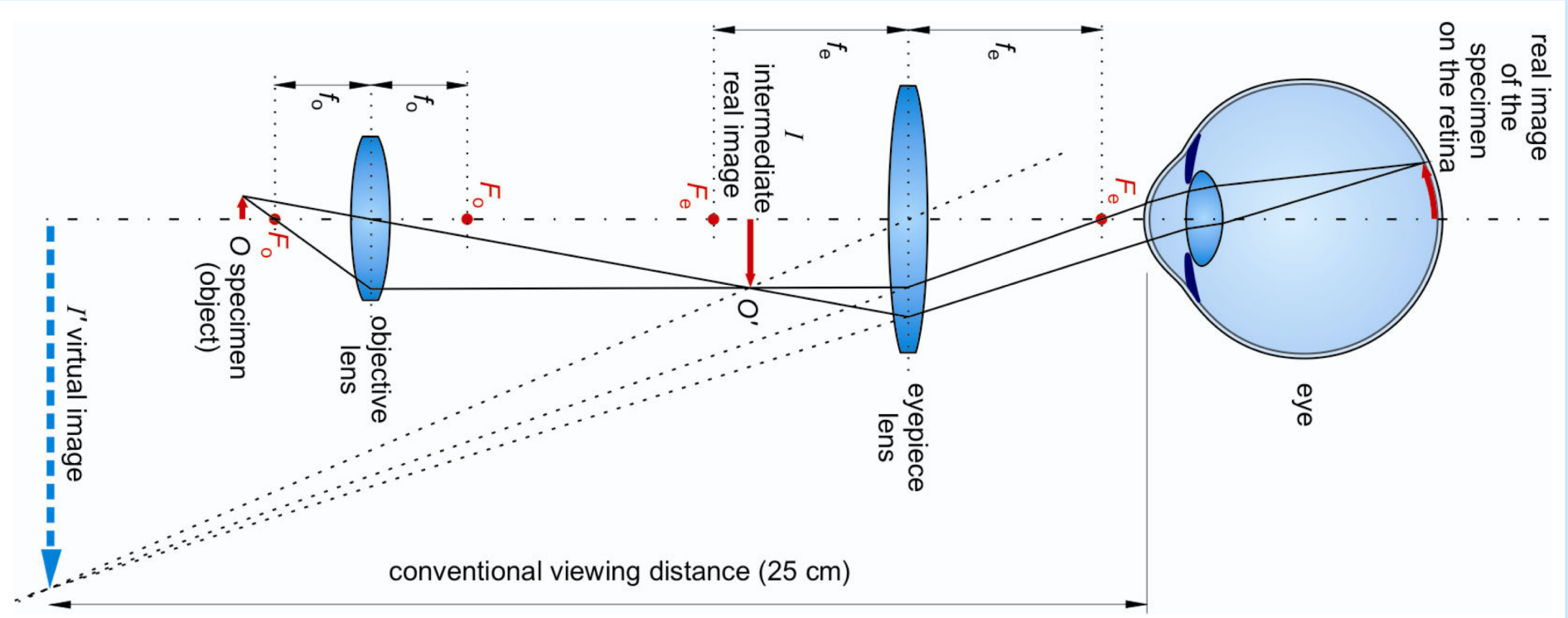
N = K / T (= k/ t)

– real image: can be directly monitored or stored

– virtual image: a real image can be formed by another lens

Image formation by a microscope

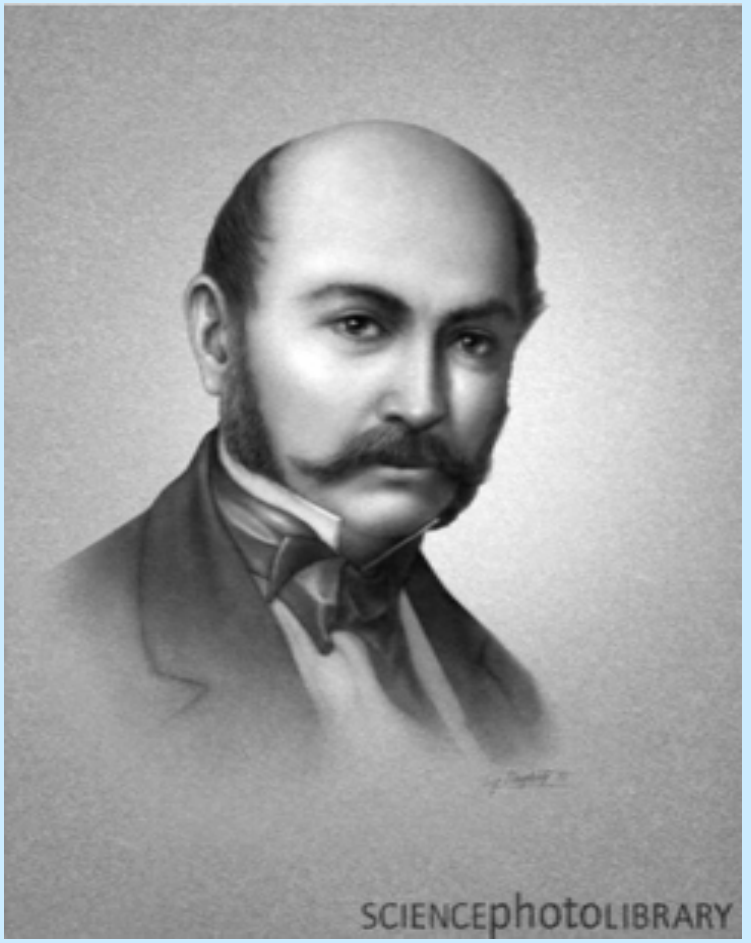
– based on geometrical optics



The final image is magnified, inverted and virtual!

$$N = N_1 * N_2 = (T'/T) * (K'/T') = K'/T$$

What is the limit on magnification?



Magnification

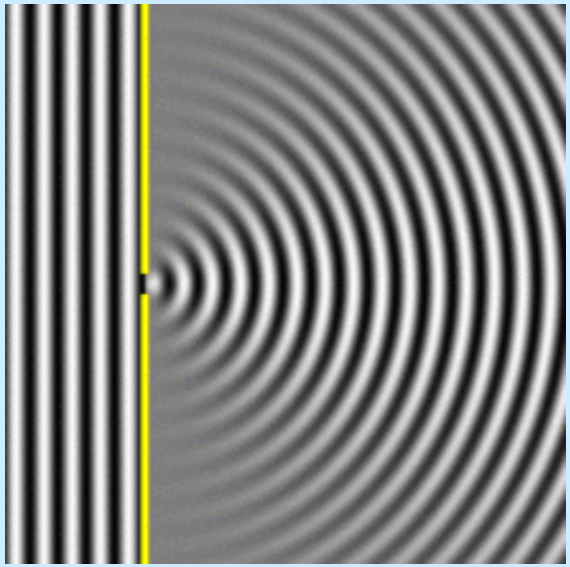
Resolution

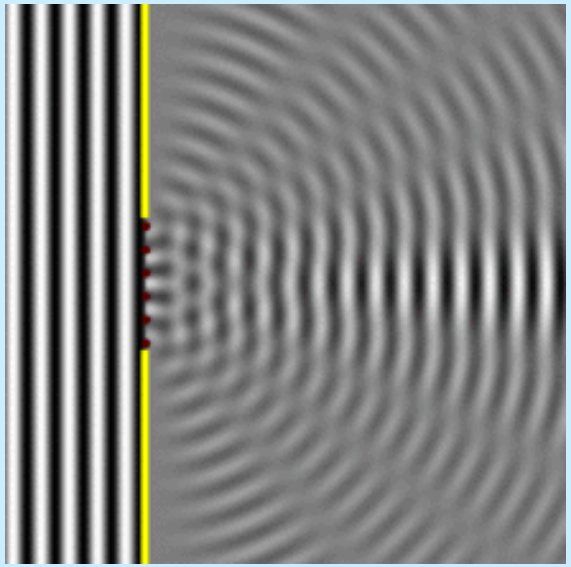
What is the smallest distinguishable detail in the image?

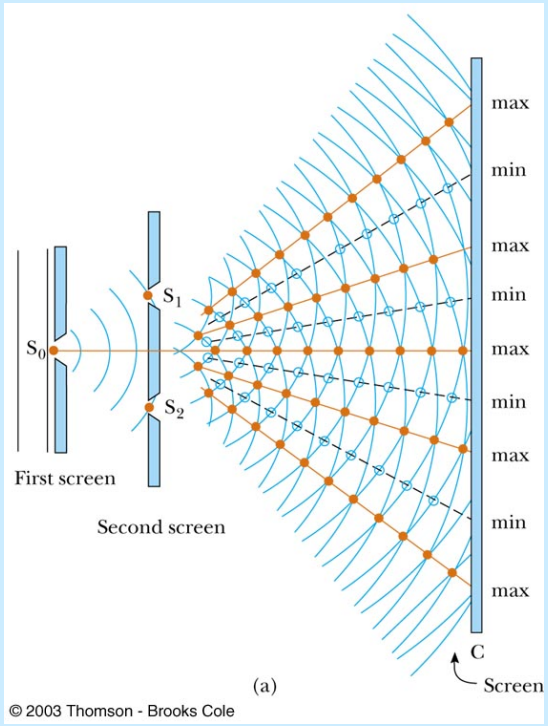
Image formation by a microscope

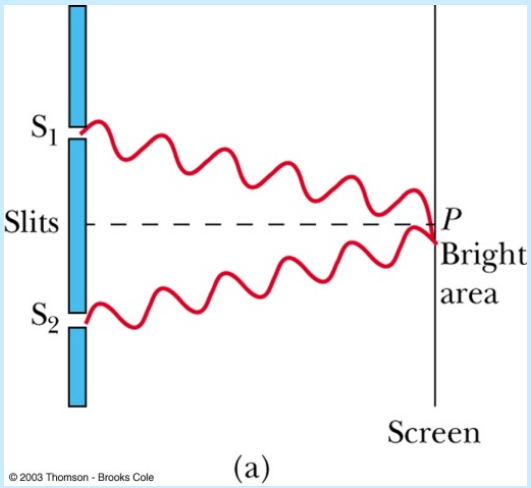
– based on wave optics

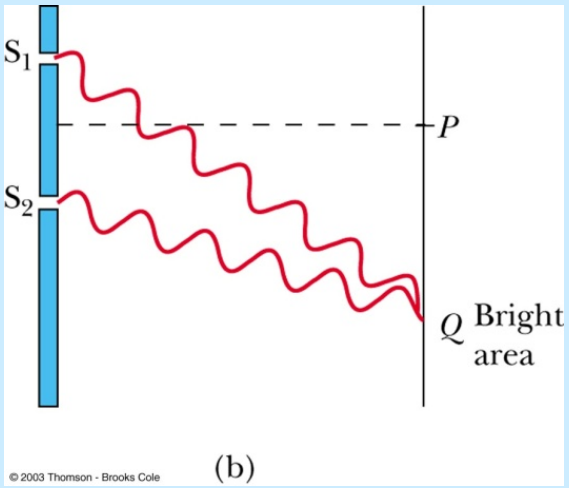
Any object comparable in size with the wavelength evokes diffraction!









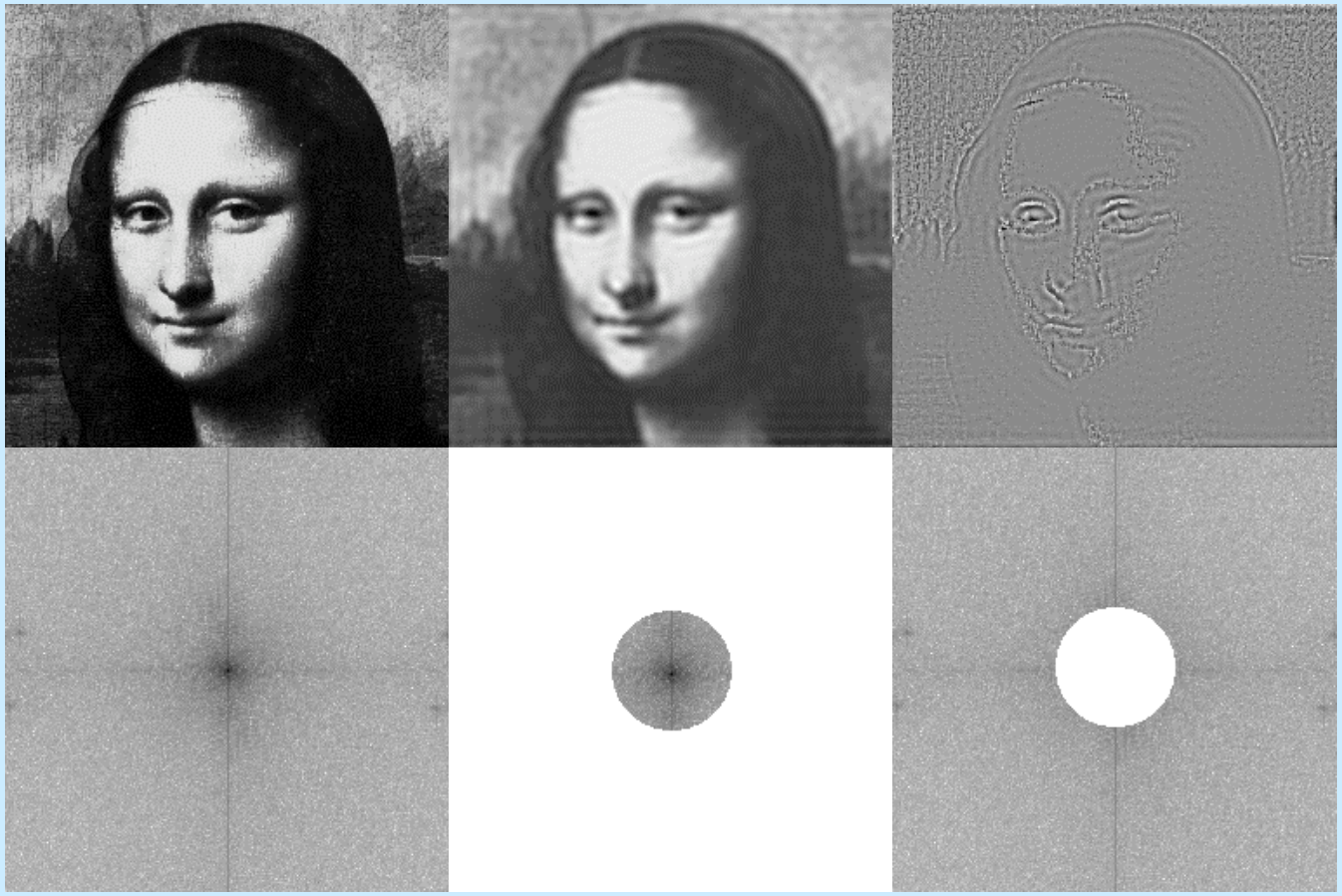


Resolution is limited by interference of light emanating from different parts of the object

Wave optics basis of image formation

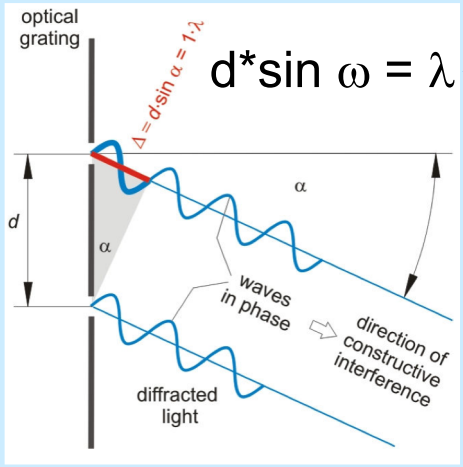
Image formed by the lens

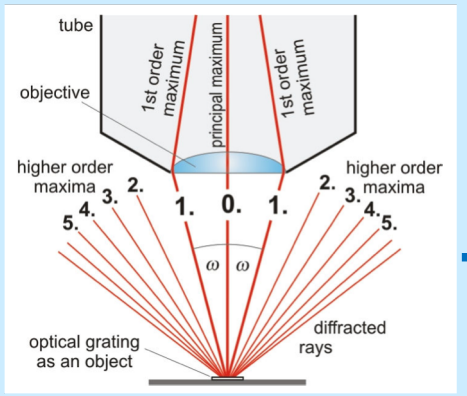
Diffraction pattern of the above image

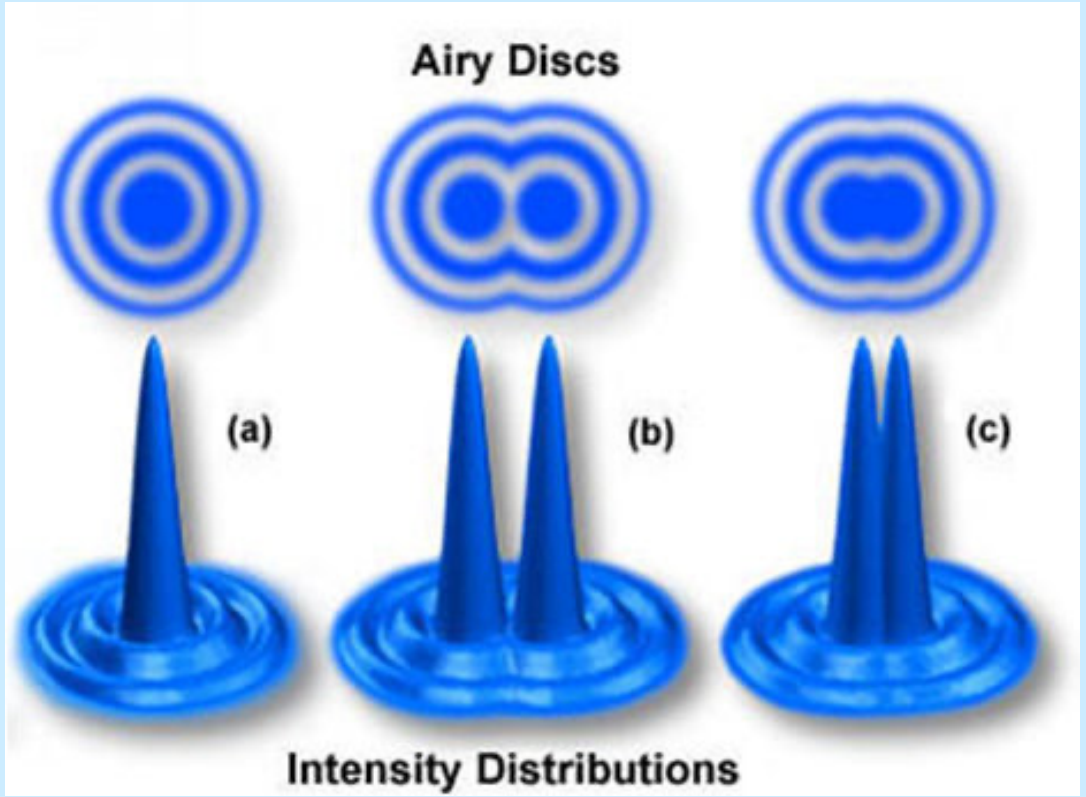


full diffraction retained | higher orders excluded | zero order excluded

Wave optics basis of image formation



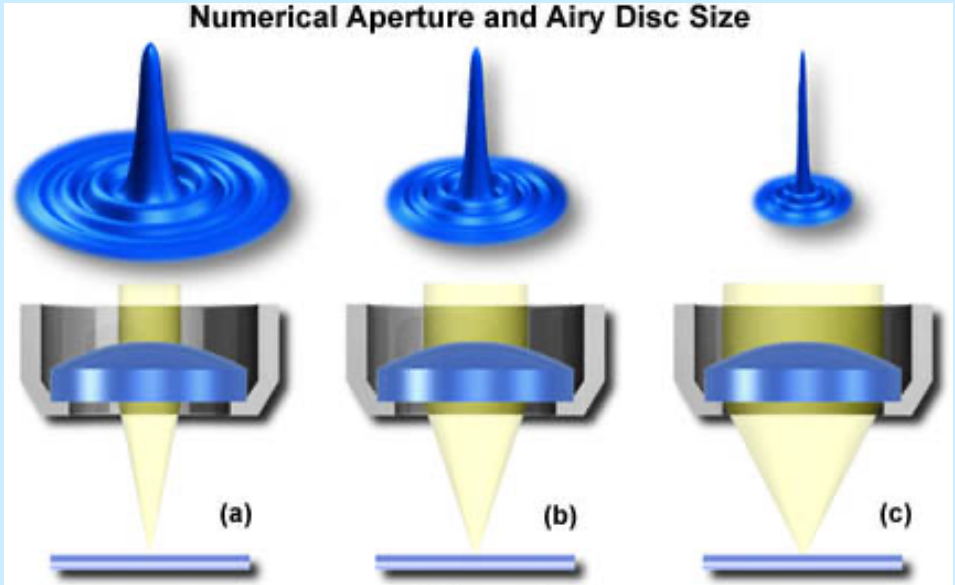




$$d = 0{,}61*\lambda/(n*\sin\omega) = 0{,}61*\lambda / NA$$

Abbe's principle: an image to form, at least the first order maxima should enter the objective! The resolution is determined by the wavelength of illumination and the numeric aperture of the objective – not by the magnification!

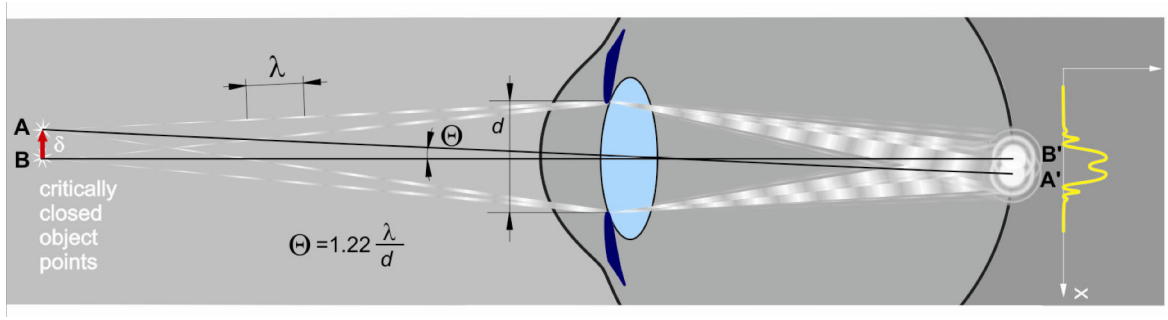
Wave optics basis of image formation



Magnification	N.A	Resolution (µm)	N.A	Resolution (µm)	N.A	Resolution (µm)
4x	0.10	2.75	0.13	2.12	0.20	1.375
10x	0.25	1.10	0.30	0.92	0.45	0.61
20x	0.40	0.69	0.50	0.55	0.75	0.37
40x	0.65	0.42	0.75	0.37	0.95	0.29
60x	0.75	0.37	0.85	0.32	0.95	0.29
100x	1.25	0.22	1.30	0.21	1.40	0.20

The best resolution with blue illumination and high (expensive) numeric aperture objective is around **200 nm**! Smaller objects can not be resolved in details in conventional light microscope!

Image formation by human eye



Limiting angle of view: the smallest angle for two points being distinguished α_L=1.22 λ/d, for average wavelength (550 nm) and pupil diameter (4 mm) α_L=0.6'

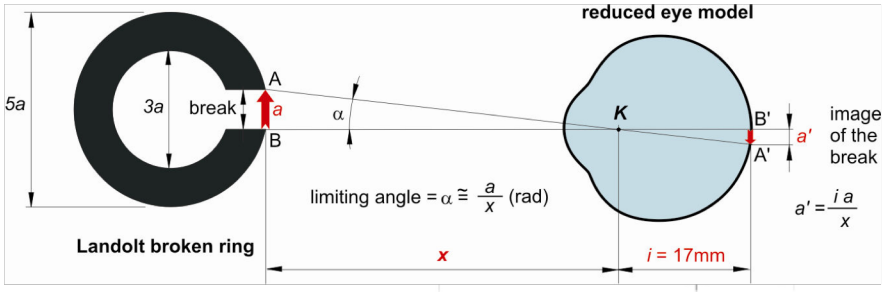
Object	Image on receptors	Visual sensation
●●	~2μm	●
●●		●
● ●		●●

biological limit of resolution: density of receptor cells

To recognise two points being separated minimum one receptor cell must remain inactive between two activated ones.

visual acuity (visus):
= 1(') / α(') *100 (%)

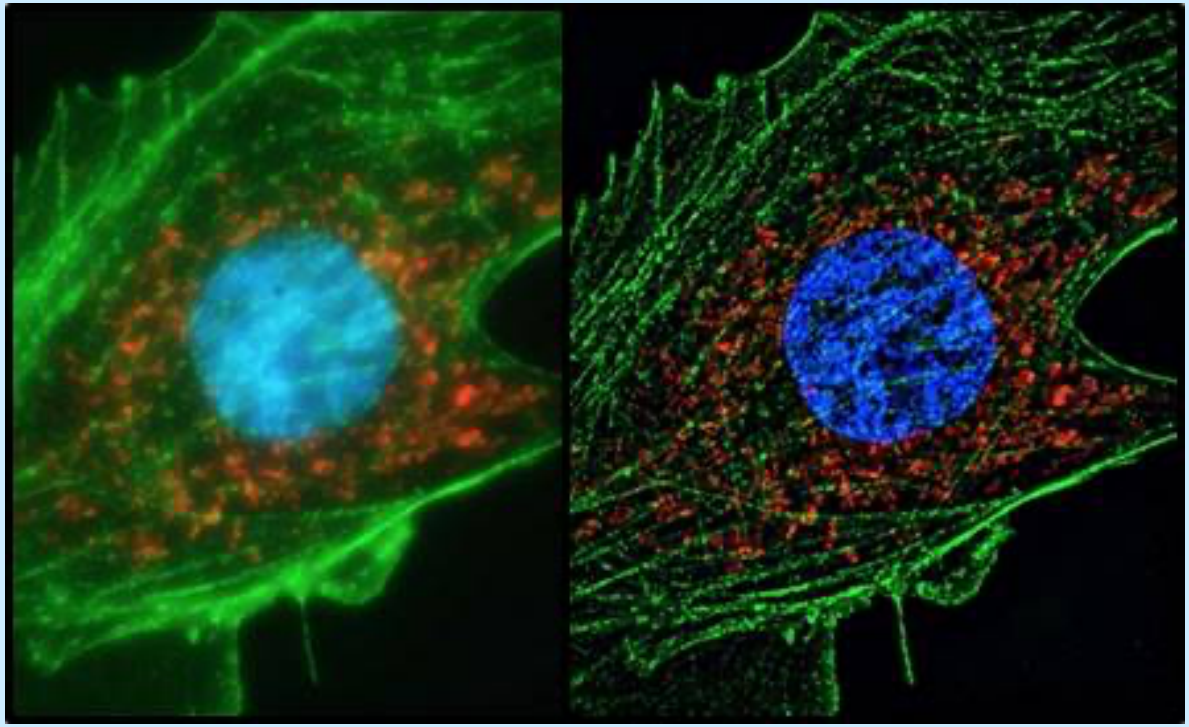
normally α = 1'



Can Abbe's formula be overcome?

Super resolution microscopy...

(in 5 weeks ☺)



Abbe's formula – any place for improvement?

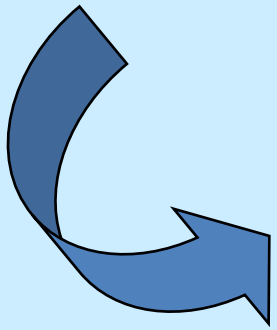
$$d = 0{,}61\frac{\lambda}{n \sin \Theta}$$

limit of resolution for blue light ~ 200 nm

To improve resolution ➔ shorter wavelength needed

➔ electron beams**?!**

$$\lambda = h / m_e v$$



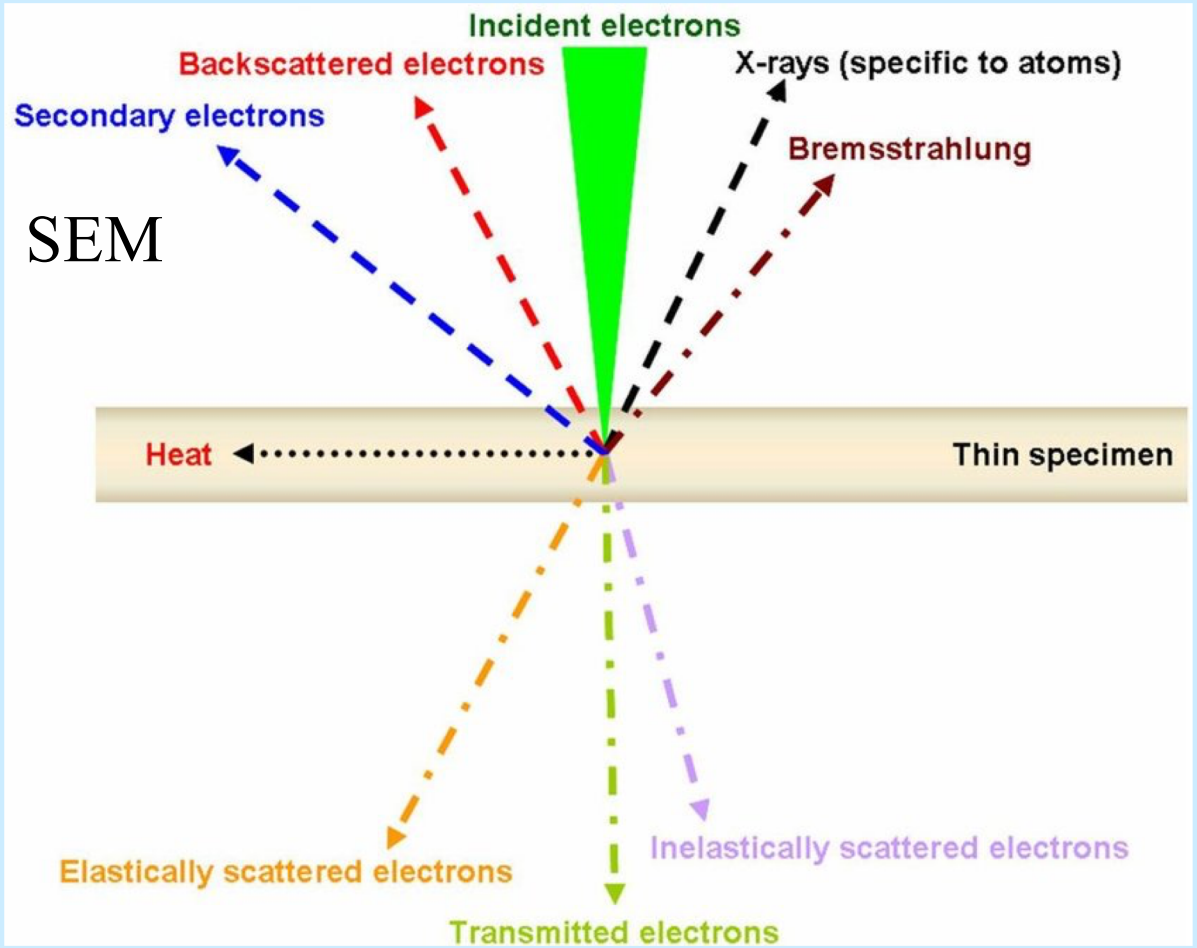
$$v = \sqrt{\frac{2eU}{m_e}}$$

U = 100 kV

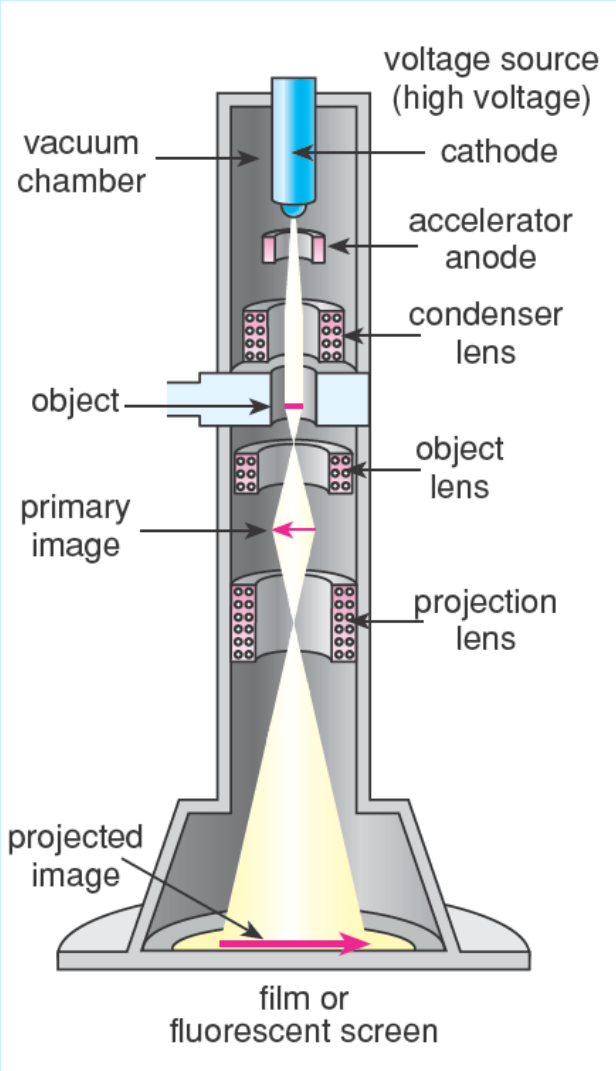
➔ $\lambda \sim 2$ pm

➔ let's make an electron microscope!

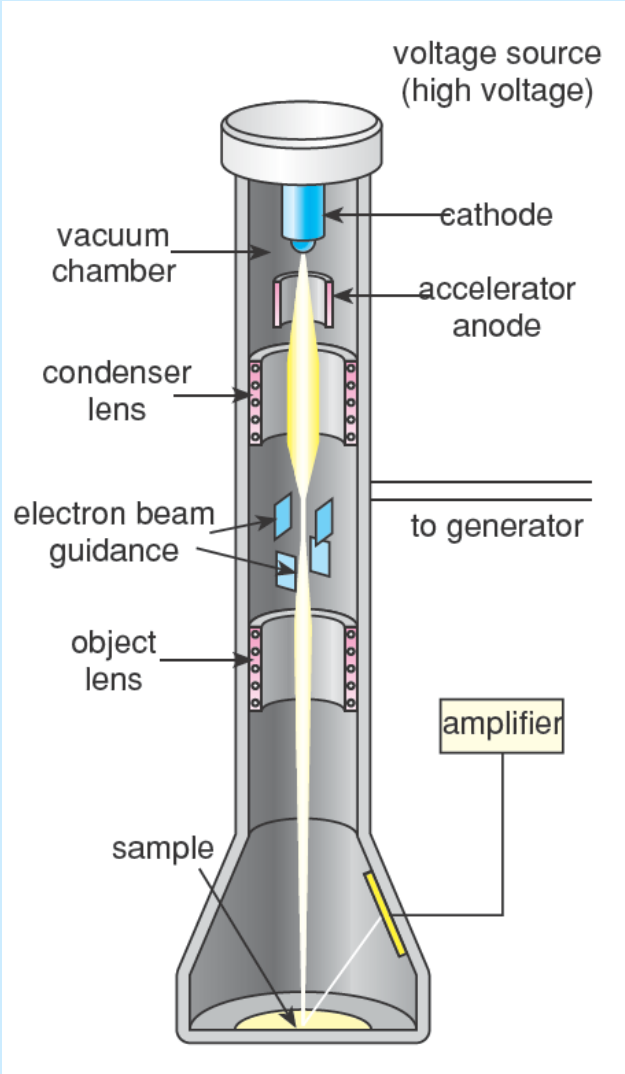
Electron beam's interactions with sample



Construction of Electron Microscope

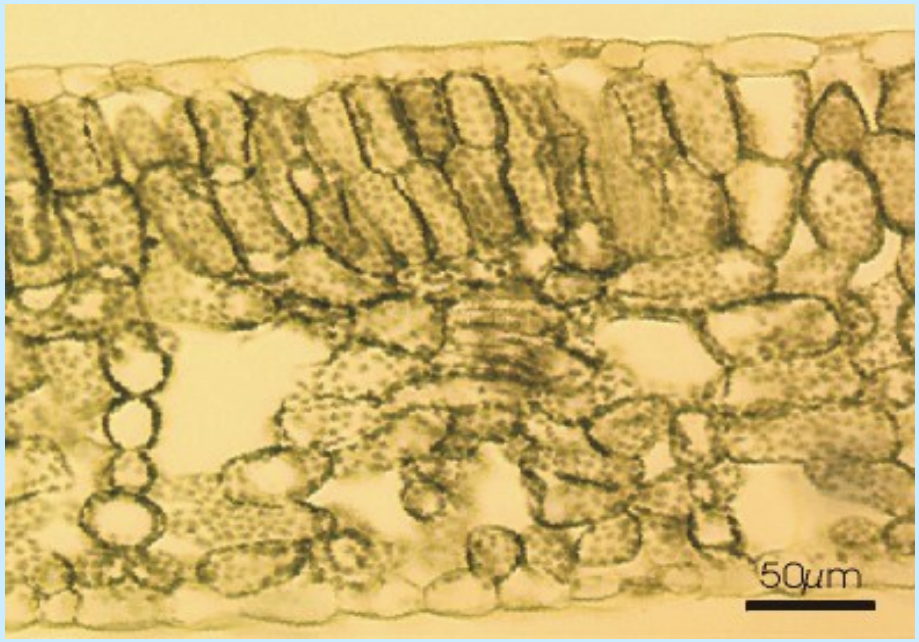


Transmission electron microscope
TEM

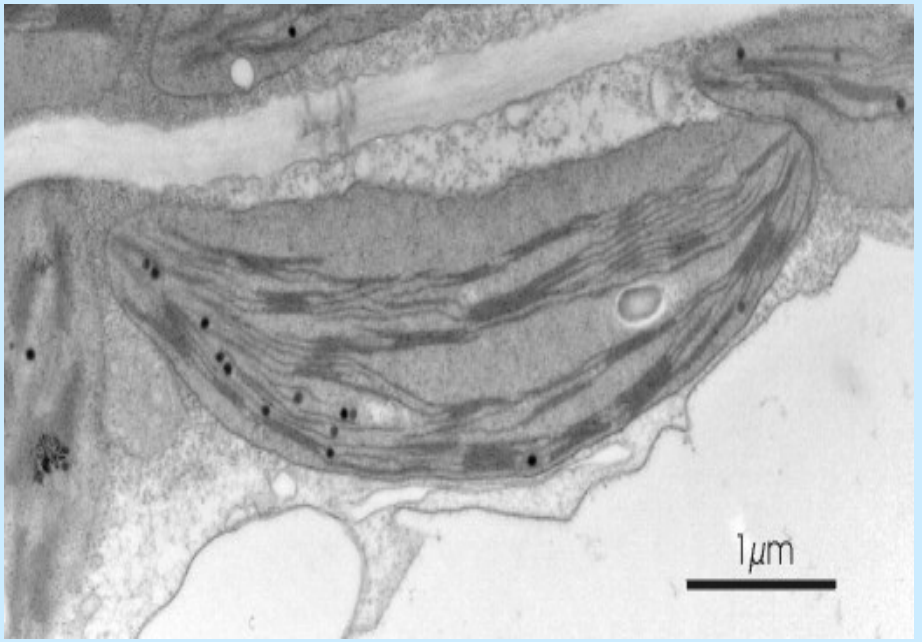


Scanning electron microscope
SEM

Light microscope vs Electron microscope



spinach leaf, semi-thin section in light microscope



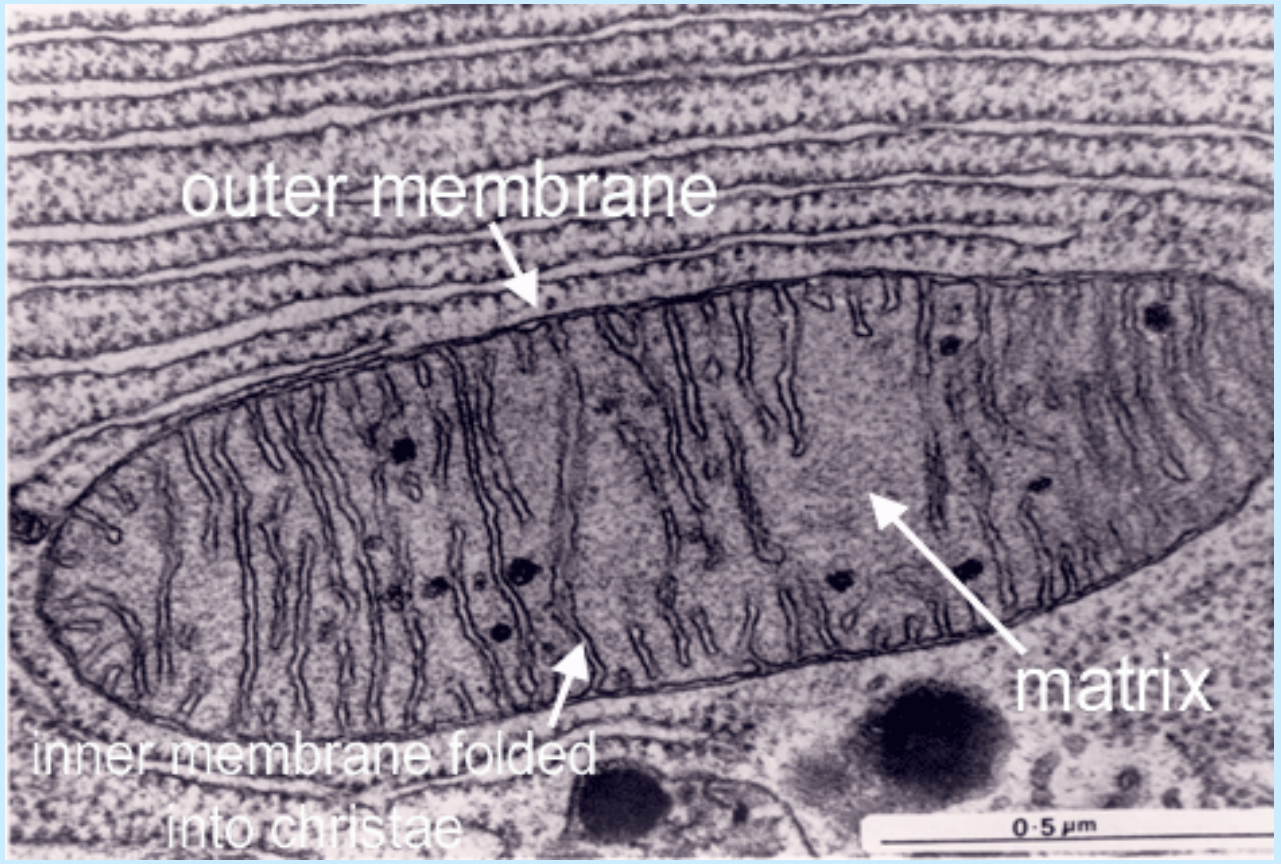
chloroplast in spinach leaf cell, ultra-thin section, in electron microscope

limit of resolution

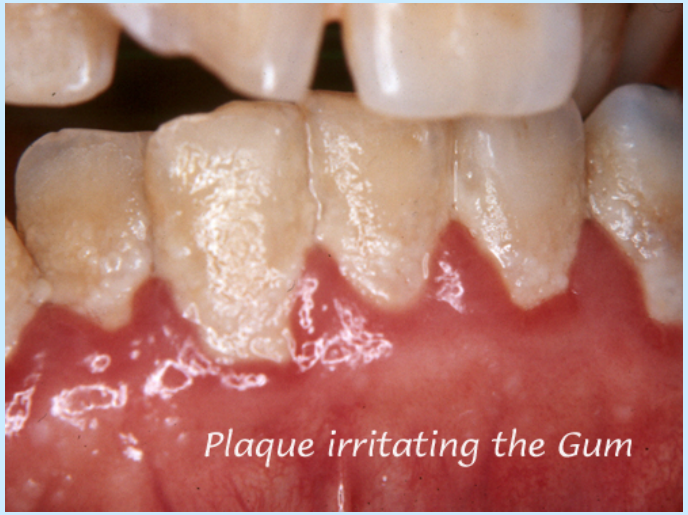
λ ~ 400 nm
d = 0,61*λ/NA
NA ~ 1,54
d ~ 200 nm

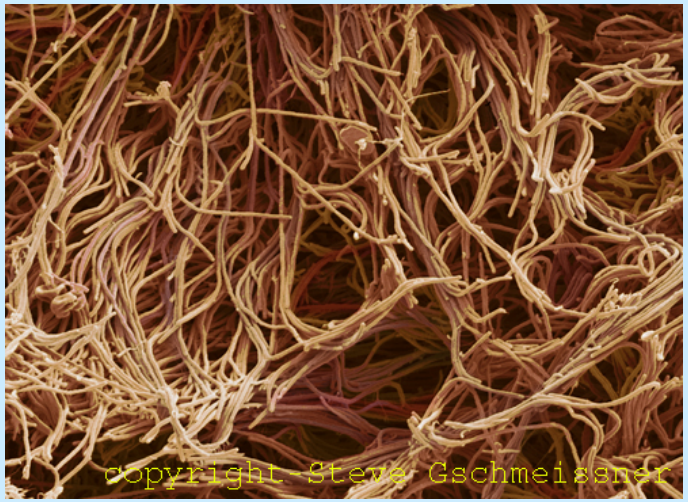
λ ~ 2 pm
d = λ/NA
NA ~ 0,01
d ~ 0,2-0,5 nm

TEM



Electron micrograph showing a mitochondrium and endoplasmic reticulum

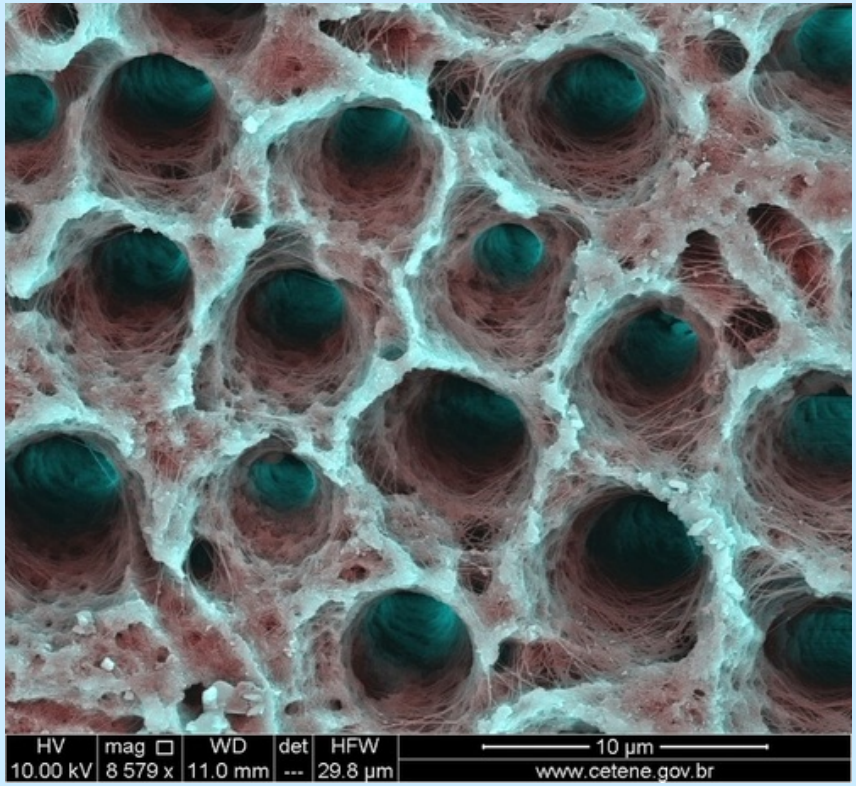




SEM

Scanning Electron Microscope image of bacteria in dental plaque

Magnification: 30000X



Question of the day ☺

Electron microscopy image of dentinal tubules in teeths made of dentin. Which method was applied? (SEM or TEM)?

Checklist:

Snell's law

Fermat's principle

limiting angle – total internal reflection

spectrum by prism

image formation by thin lenses – principal rays

lens equation, lens-makers' equation

image formation by light microscope

resolution limit of light microscope – Abbe's principle

electron microscope

wavelength of an electron beam

Related chapters:

Damjanovich, Fidy, Szöllősi: Medical Biophysics

I.1.
- 1.1.2
- 1.1.3

II. 1.1.
- 1.1.1

II. 2. 1.
- 2.1.1
- 2.1.2
- 2.1.3
- 2.1.4
- 2.1.5
- 2.1.8

VI. 2.
- 2.1.
- 2.2.

X.5.