Fundamentals of Microscopy Course
Light Properties and Optical Principles

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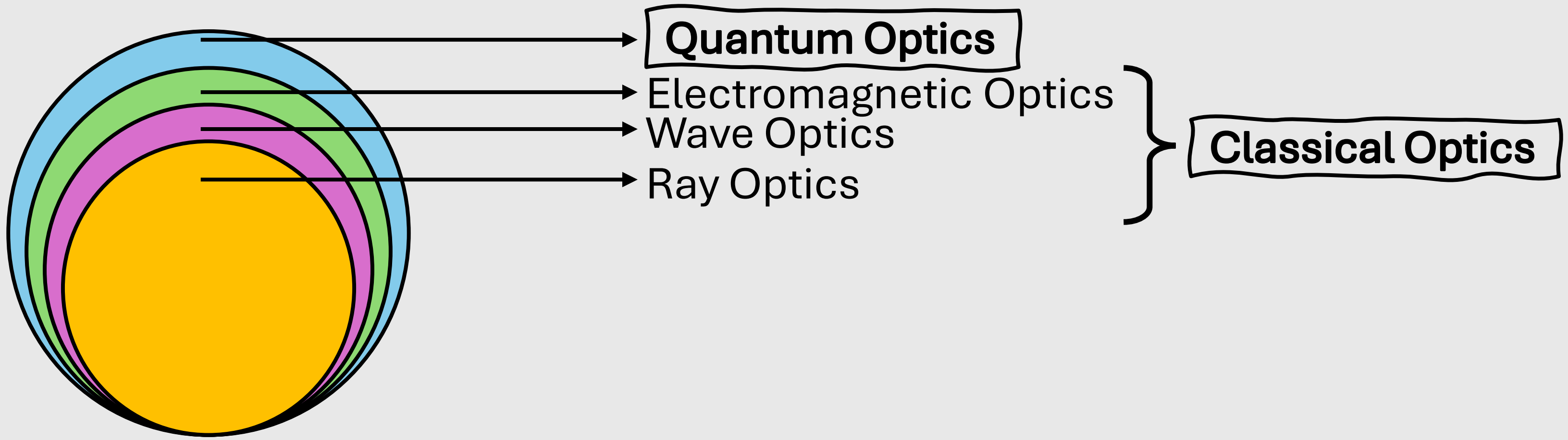
Outline

- Definitions
- Electromagnetic fields and spectrum
- EM field propagation (reflection, refraction)
- EM field interference and diffraction
- Manipulation of light rays
- Building a basic microscope
- Imaging, resolution, numerical aperture

Definition

- **Optics:** branch of Physics that studies the behavior and properties of light, its generation, propagation, interaction with matter, and detection.
- **Light:** electromagnetic radiation that can be detected by the human eye; shows both wave and particle characteristics (visible EM).
- **Photon:** the smallest discrete amount or quantum of electromagnetic radiation; has zero (rest) mass and travels at c = 299,792,458 m/s.



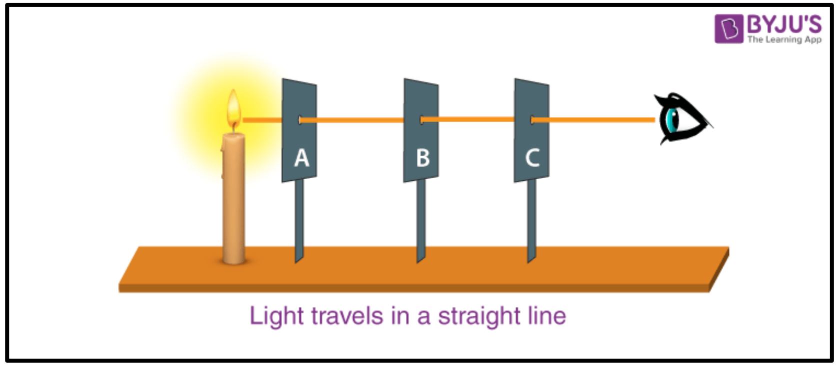
Photonics: science and technology of generating, controlling, and detecting photons (i.e., “particles” of light).
Biophotonics: optics with an emphasis on interaction of light with biological tissues
Biomedical Optics: design and application of advanced optical techniques to solve problems in medicine and biology

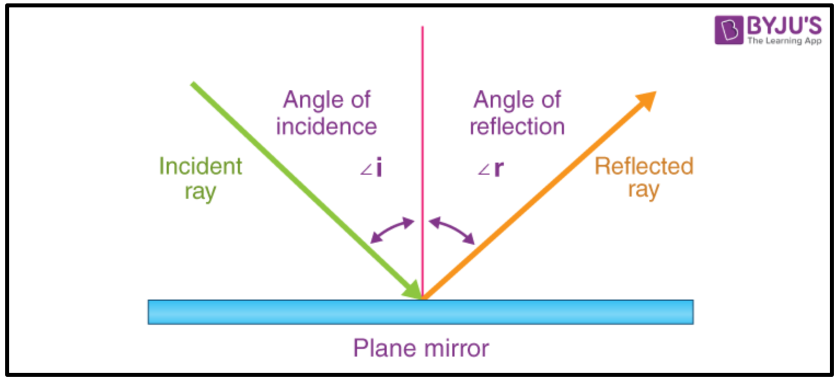
Basis of geometrical optics

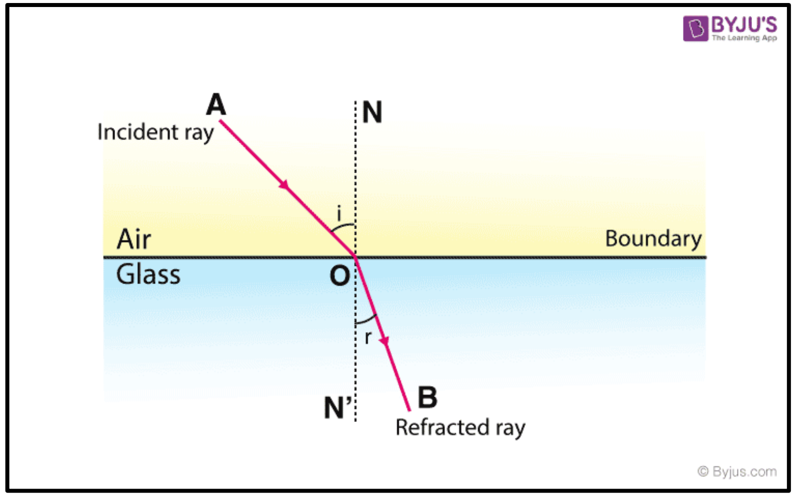
Rectilinear propagation of light rays
Law of reflection,
(Euclid ~ 300 BC)

shortest path,
(Hero's principle ~100 A.D.)

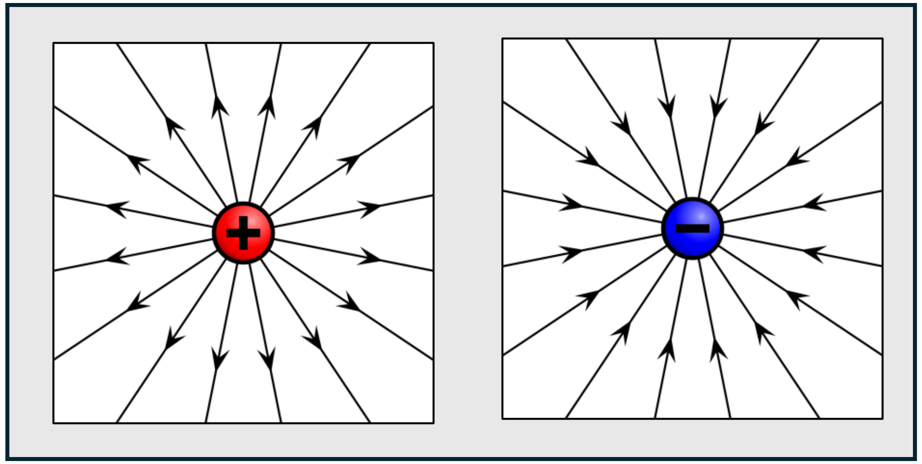
law of refraction,
dates to Ptolemy (~170 A.D.)

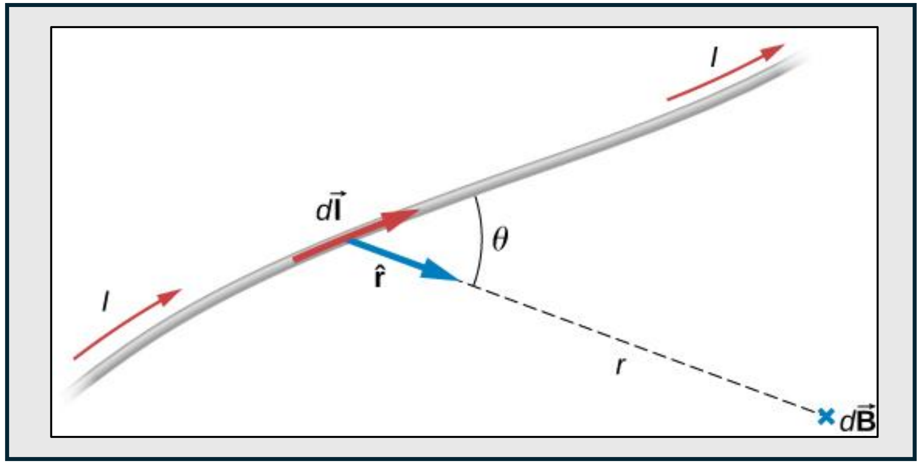






Electric and magnetic fields





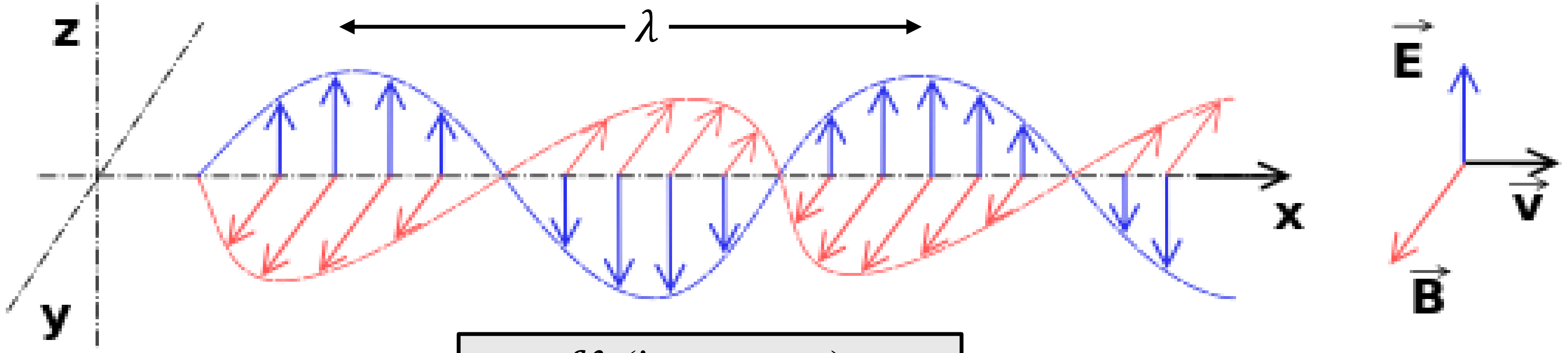
$$\overrightarrow{E_q} = \frac{1}{4\pi\epsilon_0}\left(\frac{q}{r^2}\hat{r}\right)$$

Electric field
stationary electric charge q,
at distance r
in vacuum (ϵ_0)
$\epsilon_0 = 8.854187 \times 10^{-12}\ F\ m^{-1}$
(vacuum electric permittivity)

$$\vec{B} = \frac{\mu_0}{4\pi}\int_{wire}\frac{I\ d\vec{l} \times \hat{r}}{r^2}$$

Magnetic field
wire carrying current I,
at position r
in vacuum (μ_0)
$\mu_0 = 4\pi \times 10^{-7}\ H/m$
(vacuum magnetic permeability)

Electromagnetic fields



$c = f\lambda$ (in vacuum)
$E = hf = h\frac{c}{\lambda}$

c = 299,792,458 m/s

Vector Wave Equation
(second-order linear PDE)

$$\nabla^2 \vec{E} = \mu_0 \varepsilon_0 \frac{\partial^2 \vec{E}}{\partial t^2}$$

$c = \frac{1}{\sqrt{\epsilon_0 \mu_0}} = 3 \times 10^8 \left(\frac{m}{s}\right)$ speed of light in vacuum

$v = \frac{1}{\sqrt{\epsilon\,\mu}}$ speed of light in medium

$n = \frac{c}{v} = \sqrt{\frac{\mu\epsilon}{\mu_0 \epsilon_0}}$ refractive index

Maxwell's differential equations (in vacuum)
(linear, first-order partial differential equations (PDEs) in terms of field)

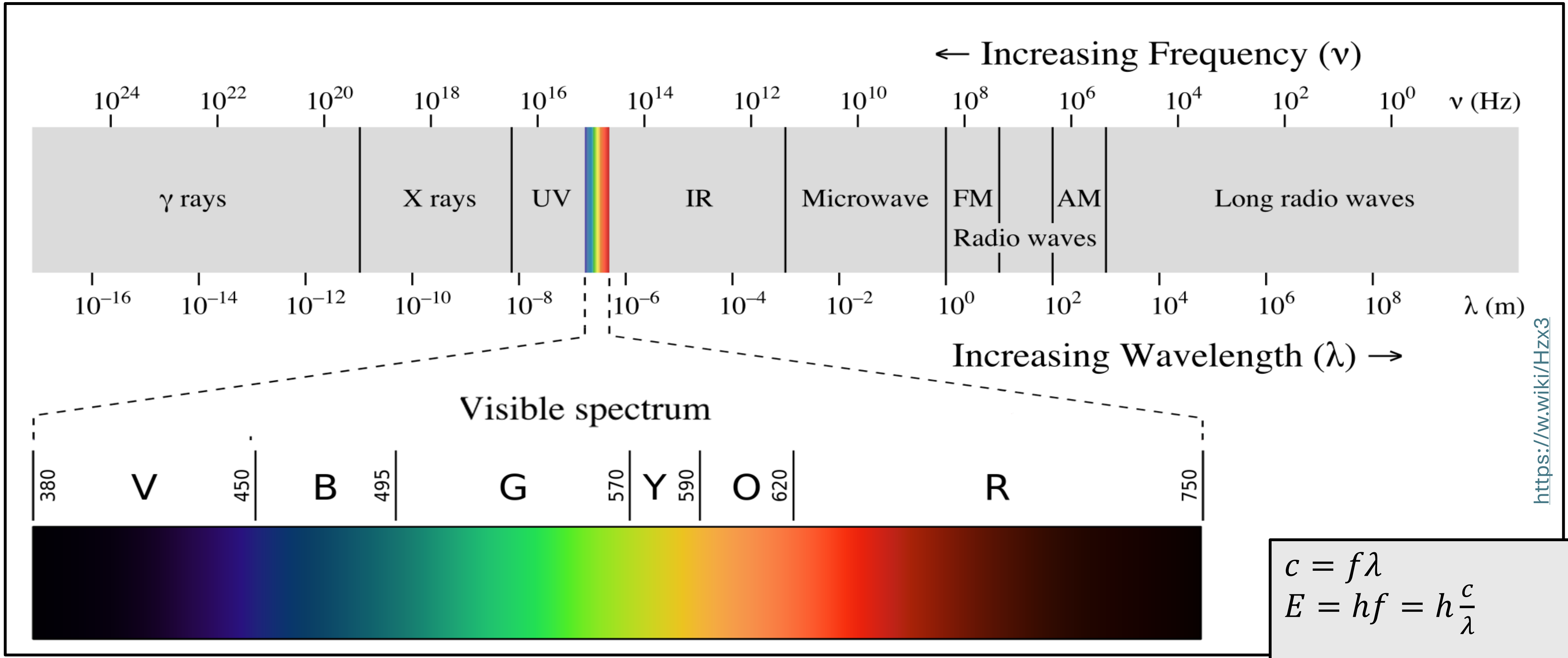
$\nabla \cdot \vec{E} = \frac{\rho}{\epsilon_0}$: Guass's law (electric field)

$\nabla \cdot \vec{B} = 0$: Guass's law (Magnetic field)

$\nabla \times \vec{E} = -\frac{\partial \vec{B}}{\partial t}$: Faraday's law

$\nabla \times \vec{B} = \mu_0 \left(\vec{J} + \frac{\partial \vec{E}}{\partial t}\right)$: Ampere's law

Electromagnetic spectrum

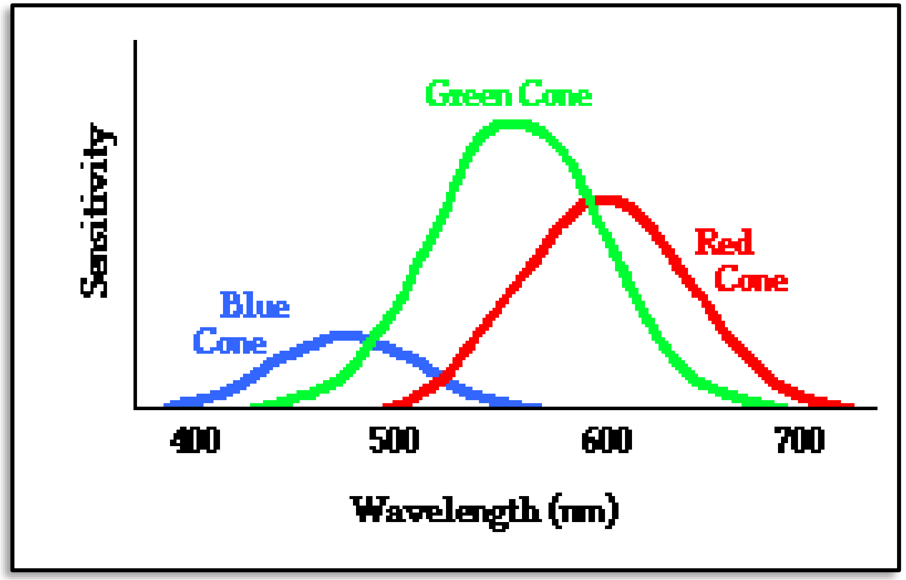


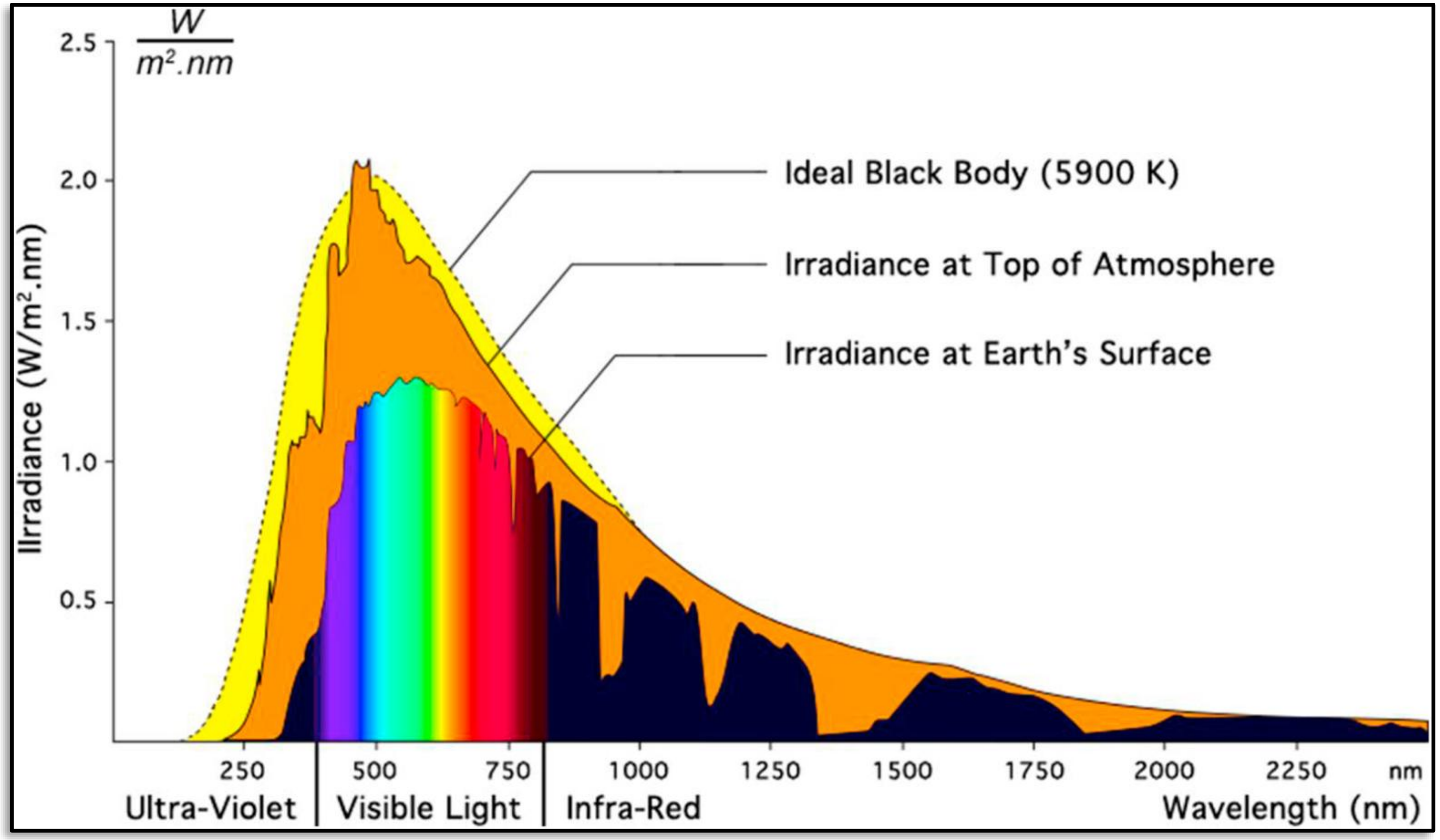
$$c = f\lambda$$
$$E = hf = h\frac{c}{\lambda}$$

c = 299,792,458 m/s

Electromagnetic spectrum

human eye cone sensitivity curve

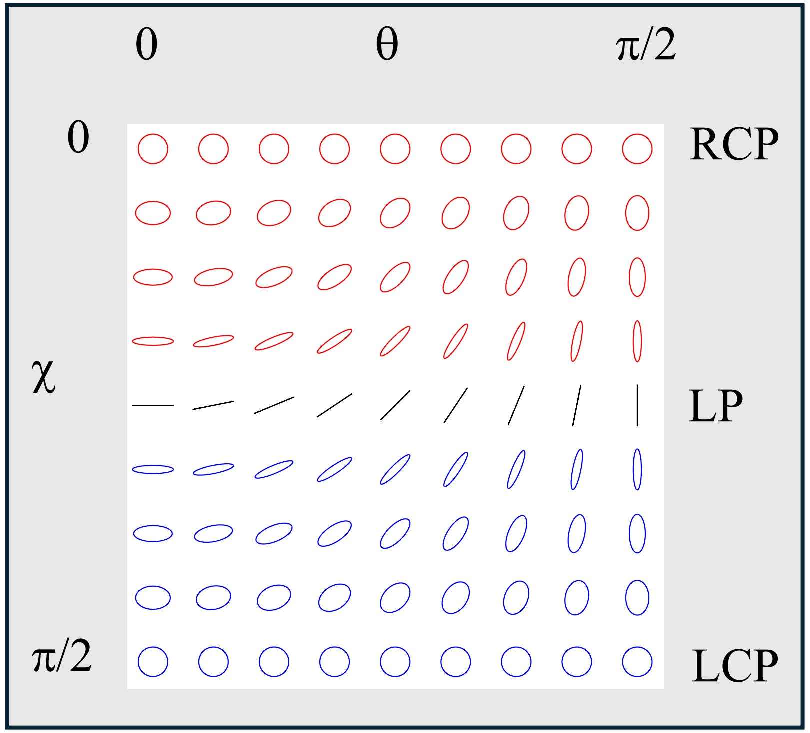


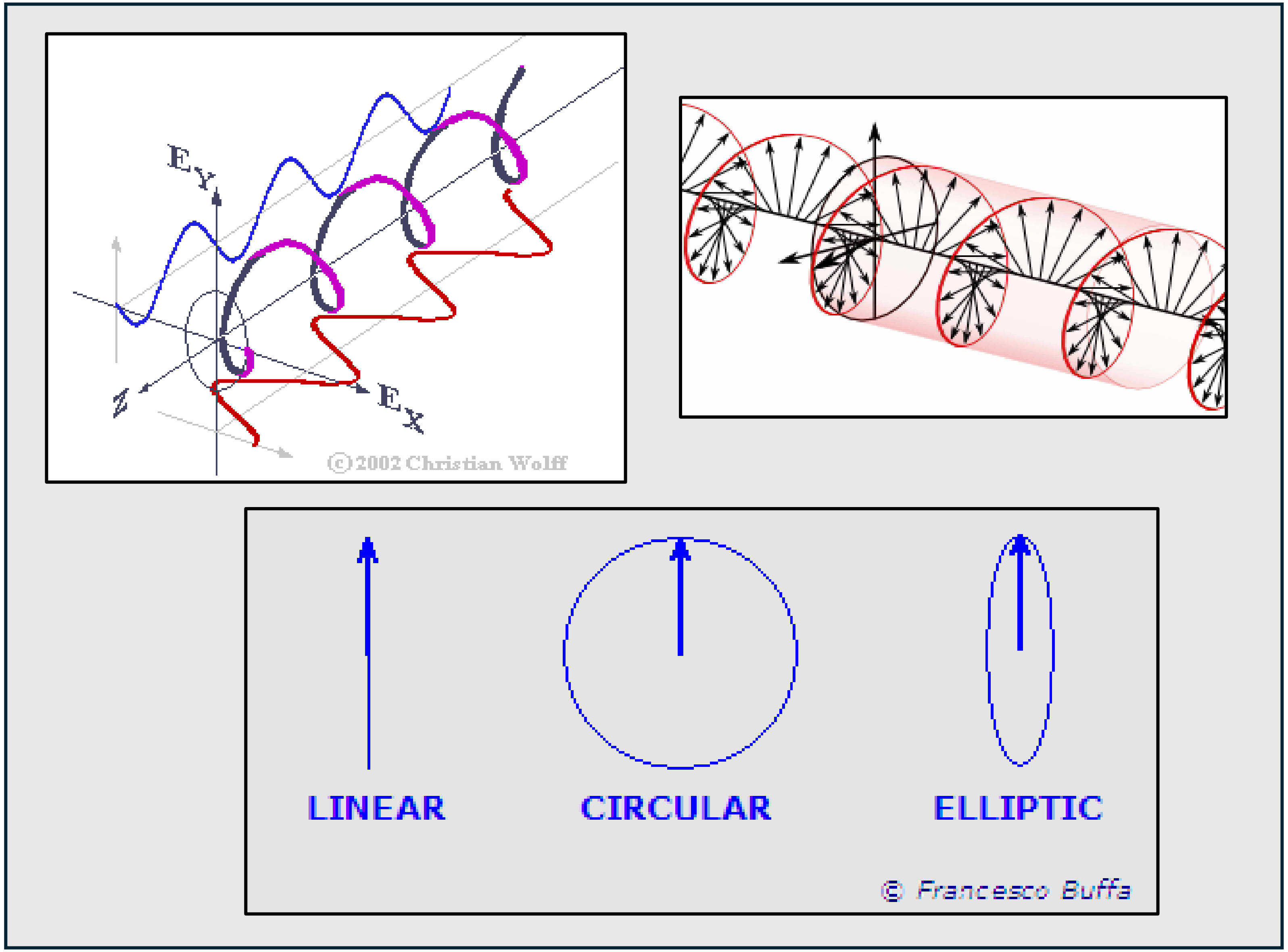


EM field - Polarization

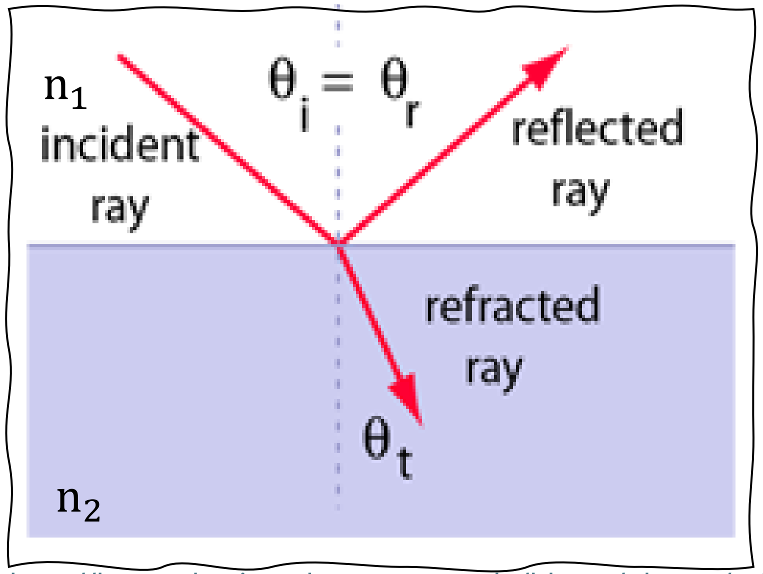
EM wave is a **transverse** wave.
Electric field vector **oscillations** determines the **polarization:**

Linear, Circular, Elliptical





EM field - propagation

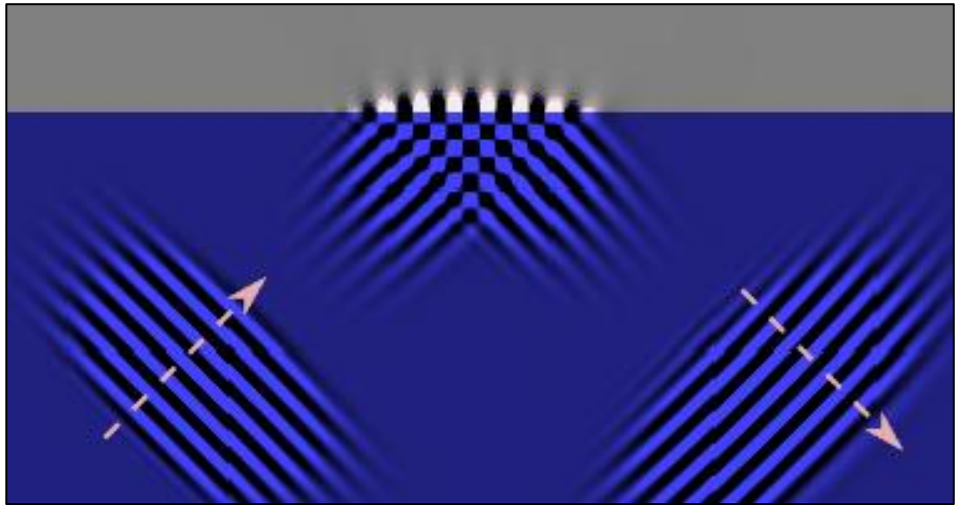


http://hyperphysics.phy-astr.gsu.edu/hbase/phyopt/reflectcon.html#c1

Reflection

$\theta_i = \theta_r$

Reflection



https://commons.wikimedia.org/wiki/File:Internal-reflection.gif

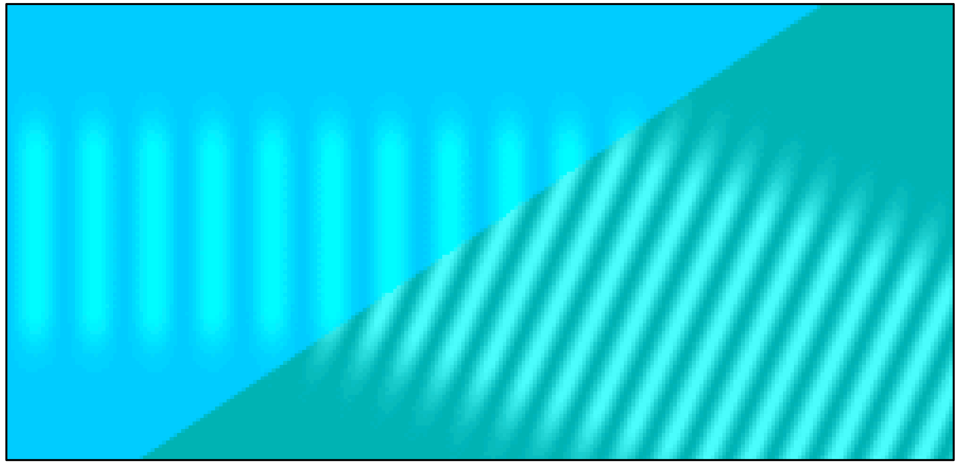
n: Refractive index
is the parameter with which light slows down while propagating in a medium. The ration speed of light in vacuum with respect to medium.

In vacuum: $c = \lambda f_{vacuum}$
In medium: $V = c/n$, n>1

Refraction: Snell's law

$$n_1 \sin \theta_i = n_2 \sin \theta_t$$

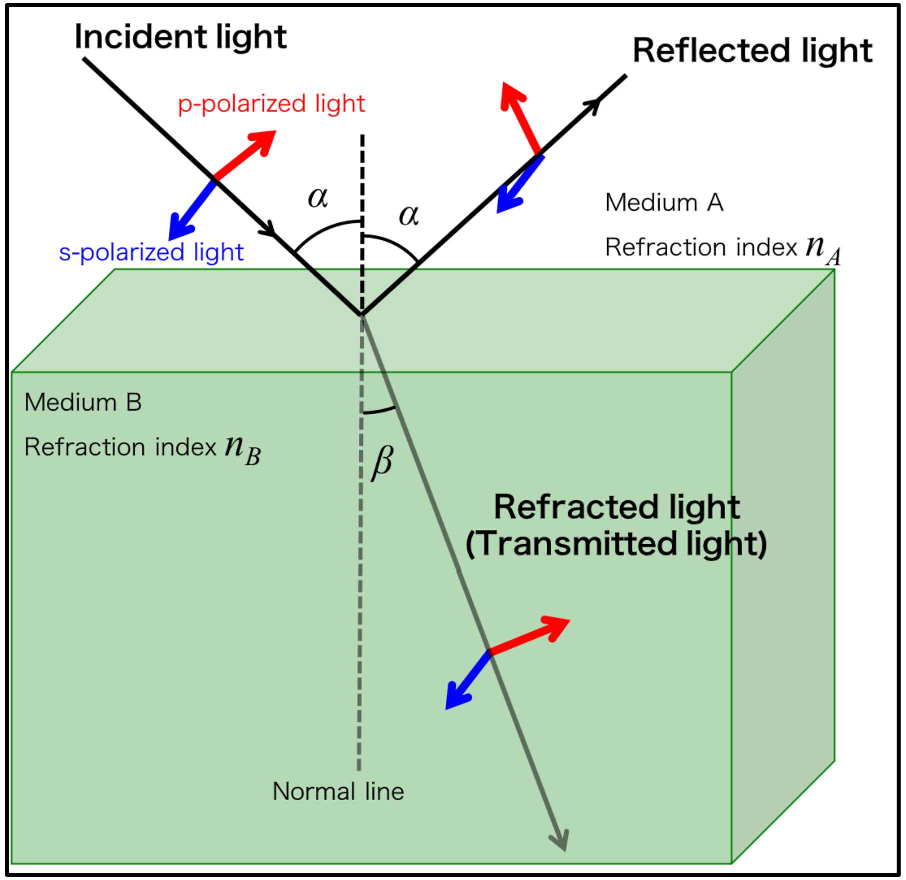
Refraction



https://en.wikipedia.org/wiki/Refraction

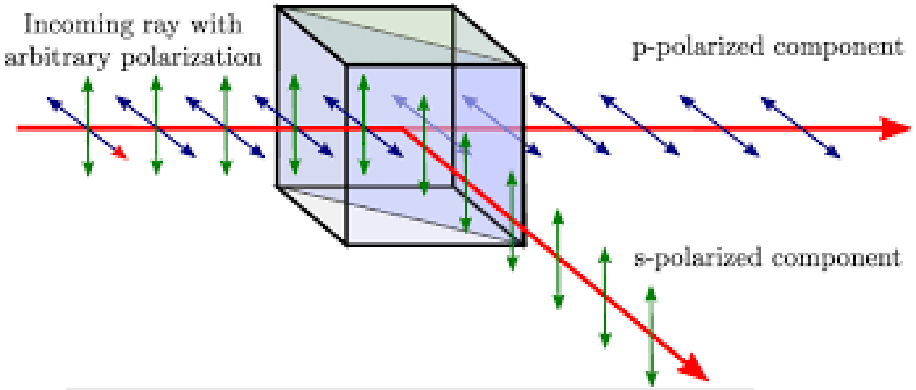
EM field - propagation

Polarization effect in Reflection and Refraction



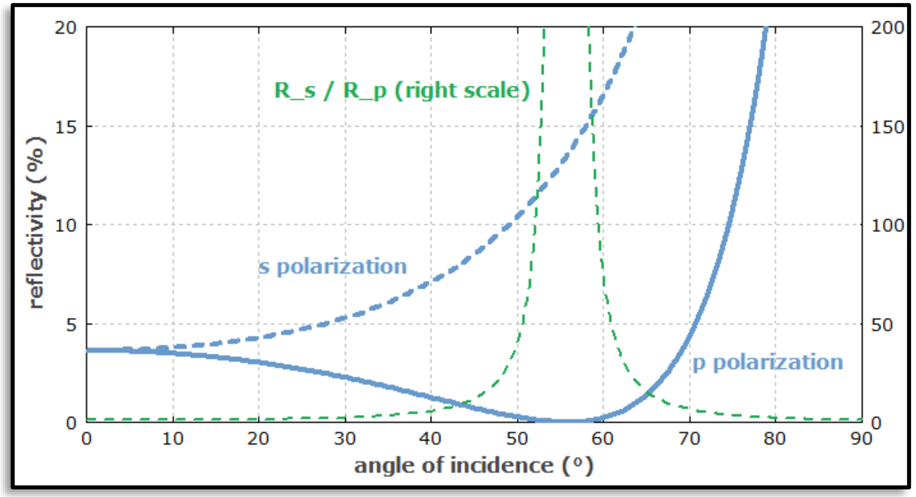
physics.thick.jp

Polarizing BS in microscopes



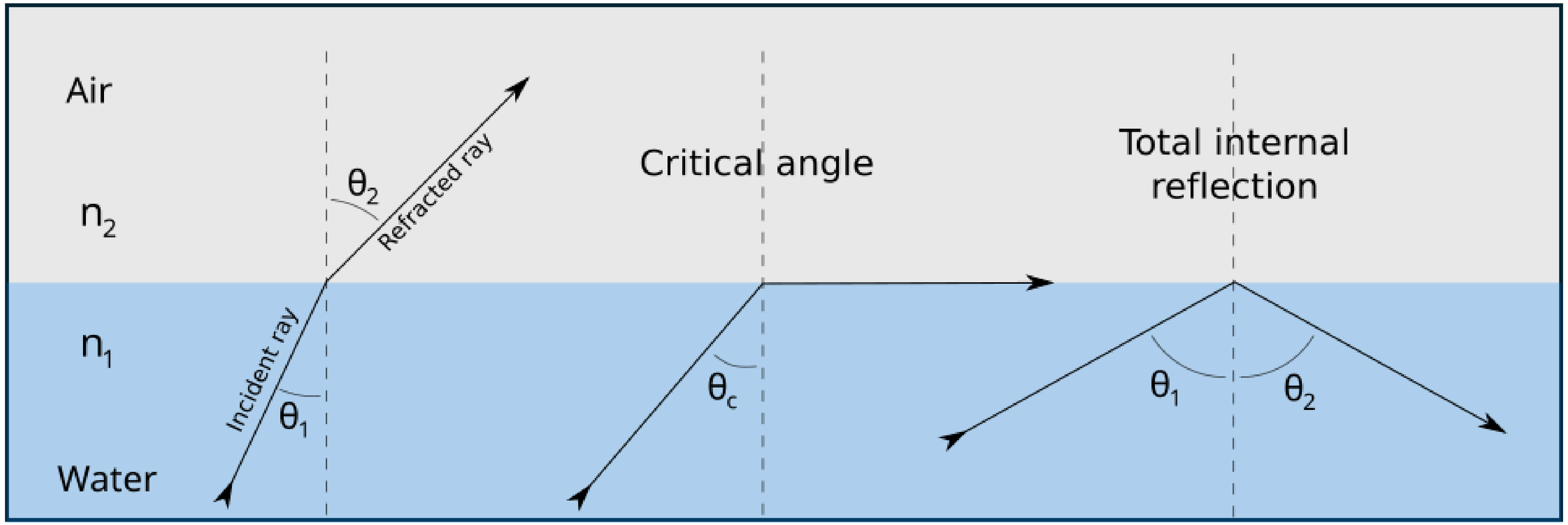
Refraction simulation

Fresnel equations



EM field - propagation

Refractive Index and Total Internal Reflection



https://en.wikipedia.org/wiki/Reflection_(physics)#Laws_of_reflection

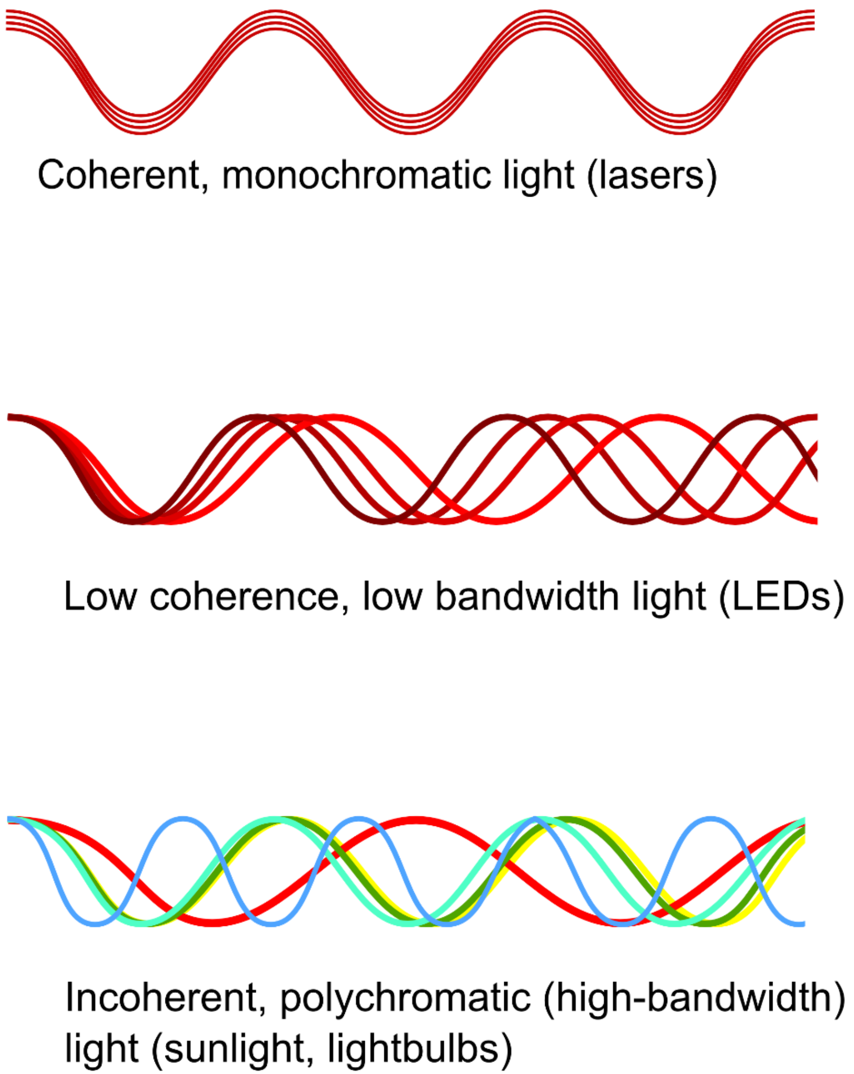
Material	n
air	1
water	1.33
PBS	1.37
Immersion oil	1.52
glass	1.46 - 1.8

Refractive index of most biological specimen is around 1.4

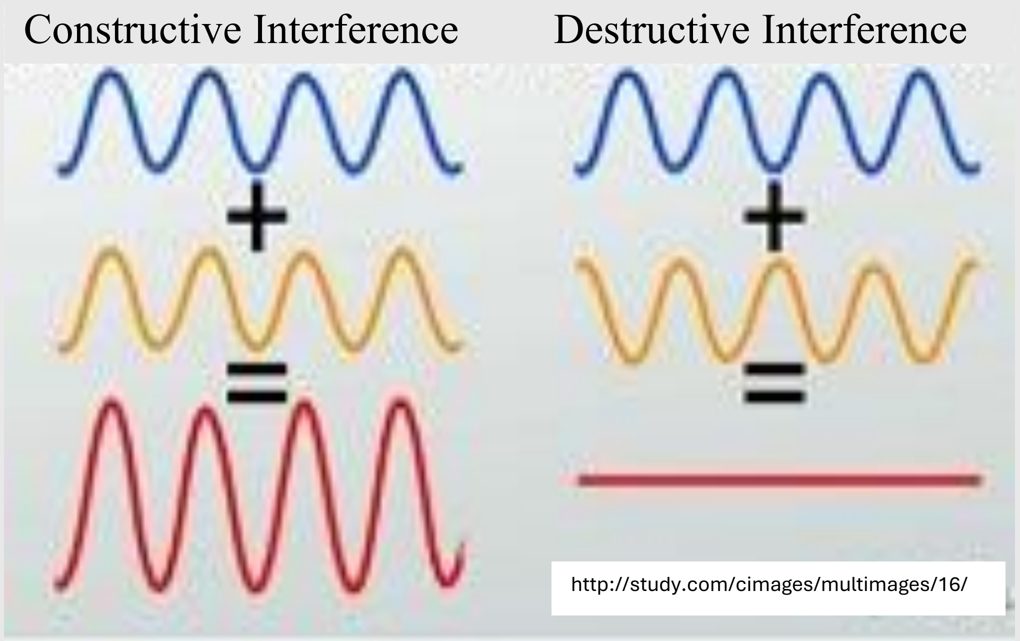
In vacuum: $c = \lambda f_{vacuum}$
In medium: $V = c/n$
$f_{vacuum} = f_{medium}$ (function of source)
$E_{photon} = hf$ (Energy of light photon)
The color that our eyes perceive: Energy → frequency

Refractive index, $n = \frac{c}{v}$ is a function of the wavelength

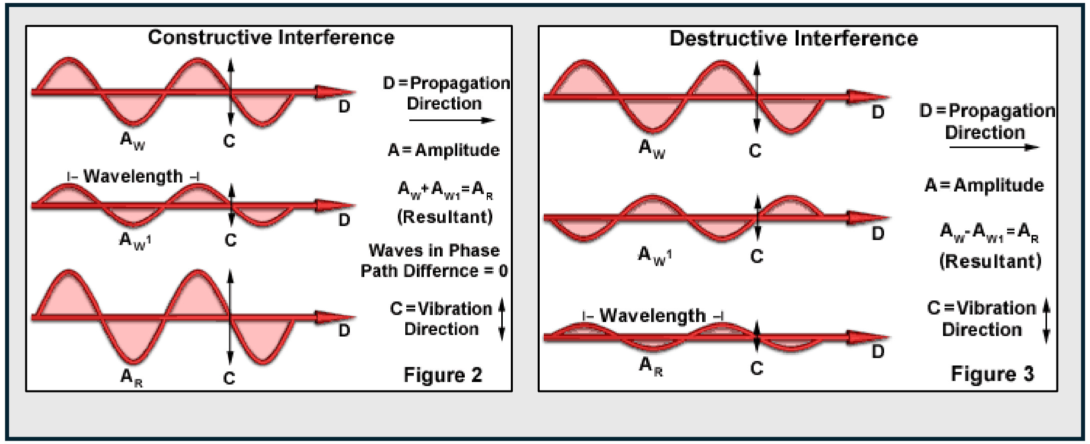
EM field - Coherence and Interference



- Coherent light: well-defined relationship in phase at different points in space (wavelength dependent)
- Generates constructive and destructive interference- useful for looking at path-length differences and moving light-scatterers

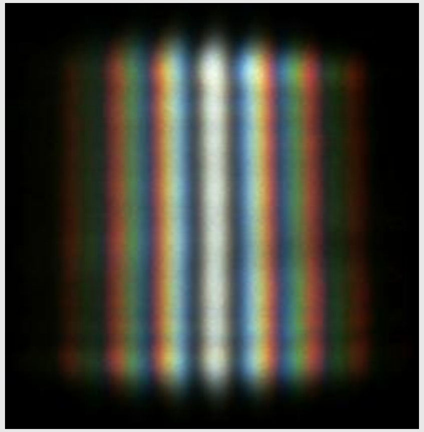


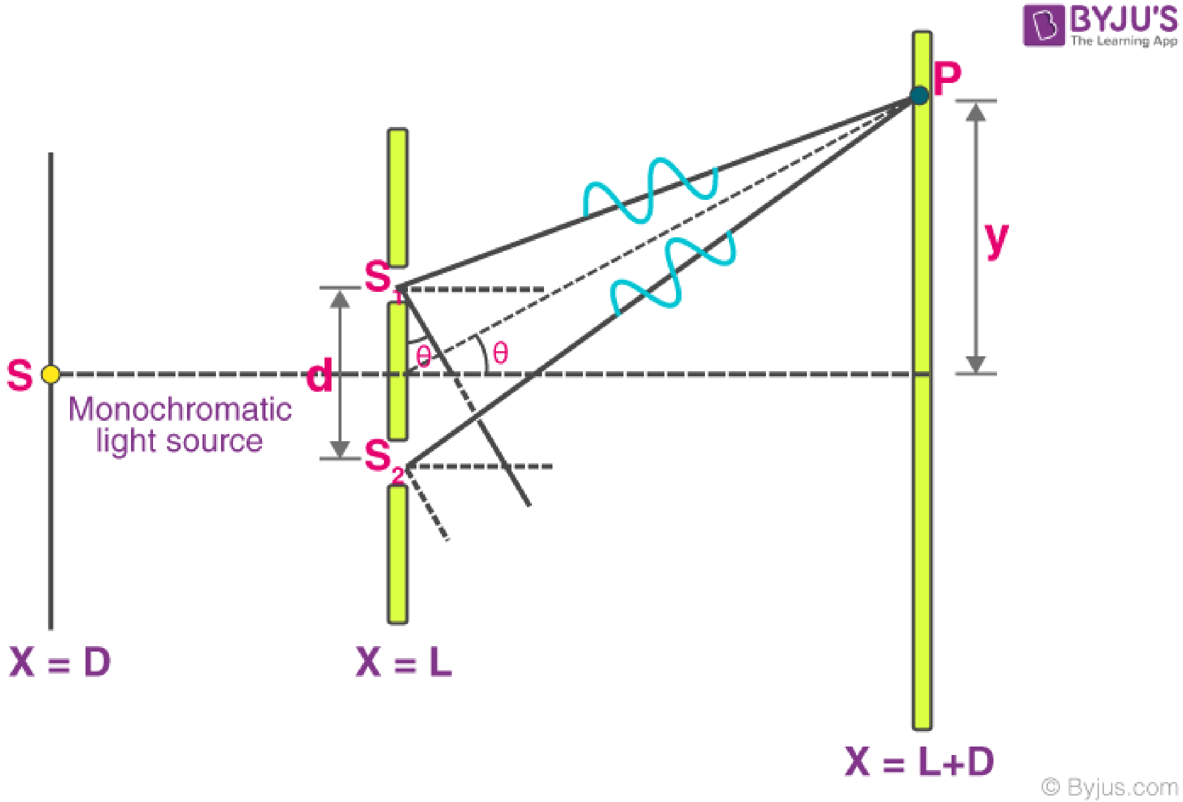
Interference and Young's double slit experiment



https://evidentscientific.com/en/microscope-resource/knowledge-hub/lightandcolor/interference

double slit interference of sunlight



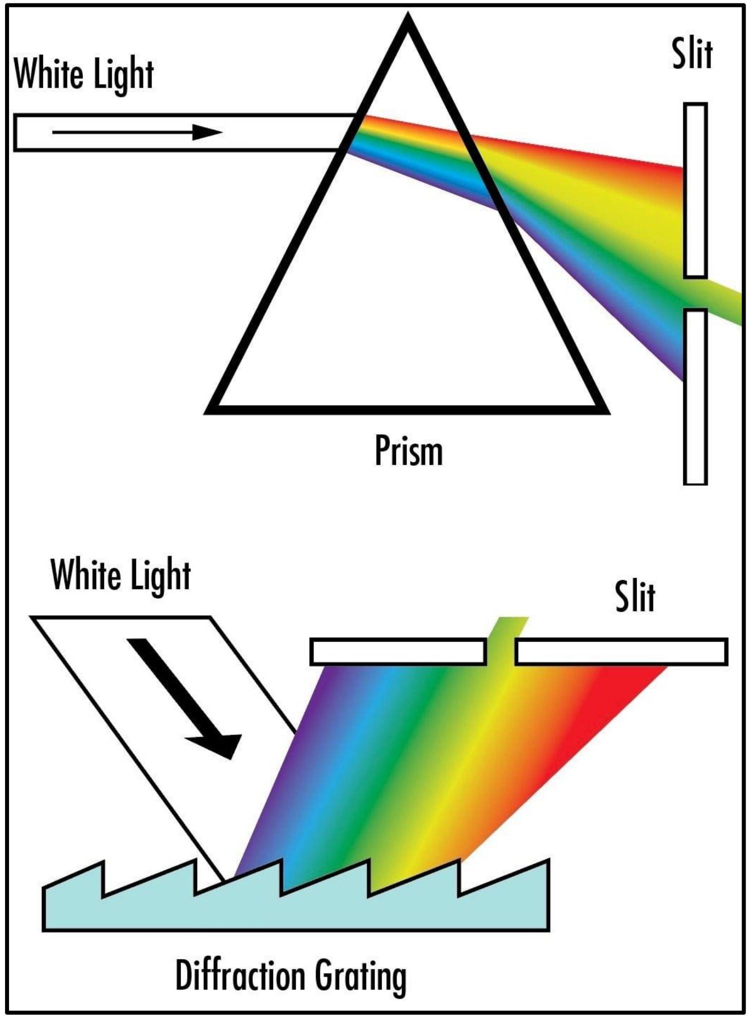


For small θ: $\tan\theta \approx \theta \approx \sin\theta \approx \frac{\lambda}{d}$

EM field - Diffraction

Superposition of many interferences from an aperture (wavelength dependent)

MATLAB app

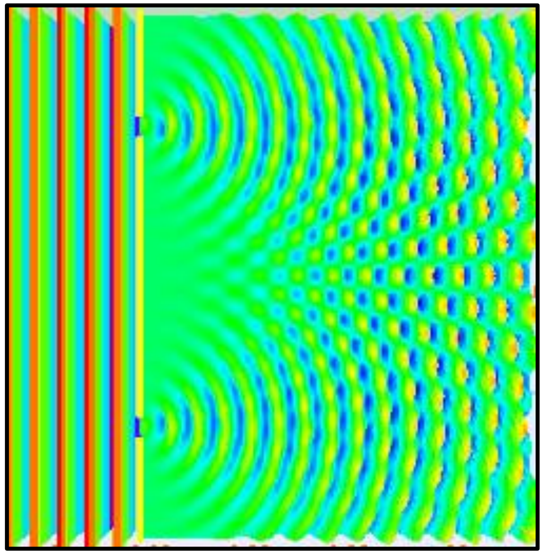


dispersion prisms separate wavelengths through refraction (top)

diffraction gratings separates wavelengths through diffraction (bottom)

EdmundOptics

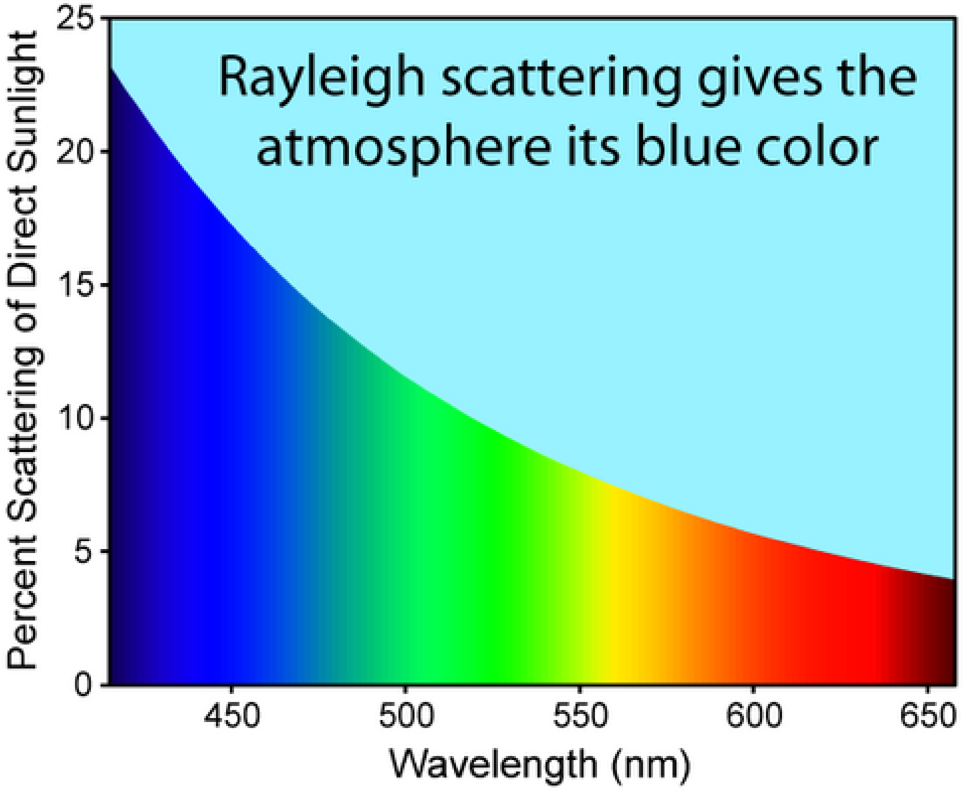
Double slit simulation

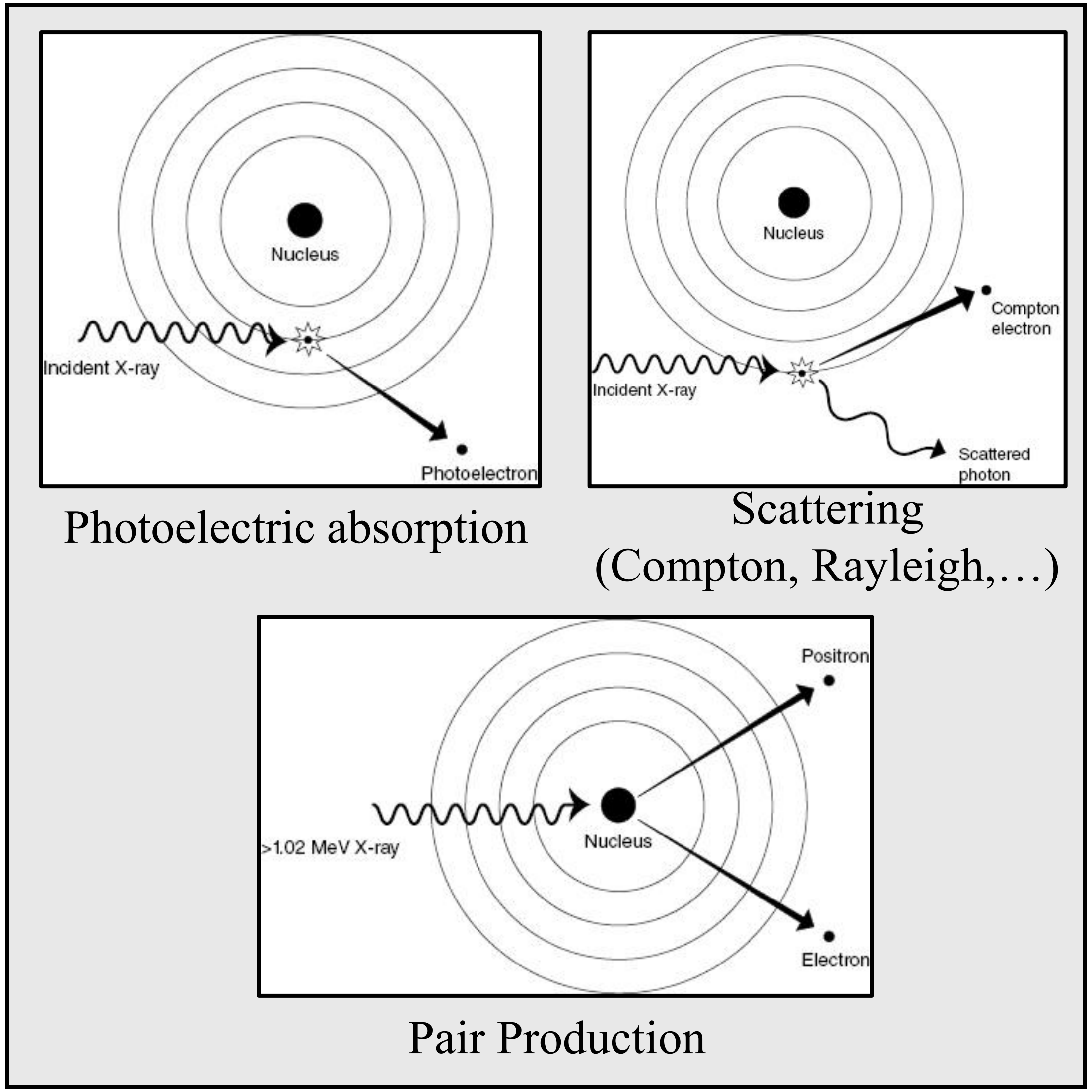


[Most accurate approximation]
Rayleigh-Sommerfeld
Fresnel
Fraunhofer
[Least accurate approximation].

Light Interaction with Matter

Longer Wavelength : Rayleigh scattering ↓



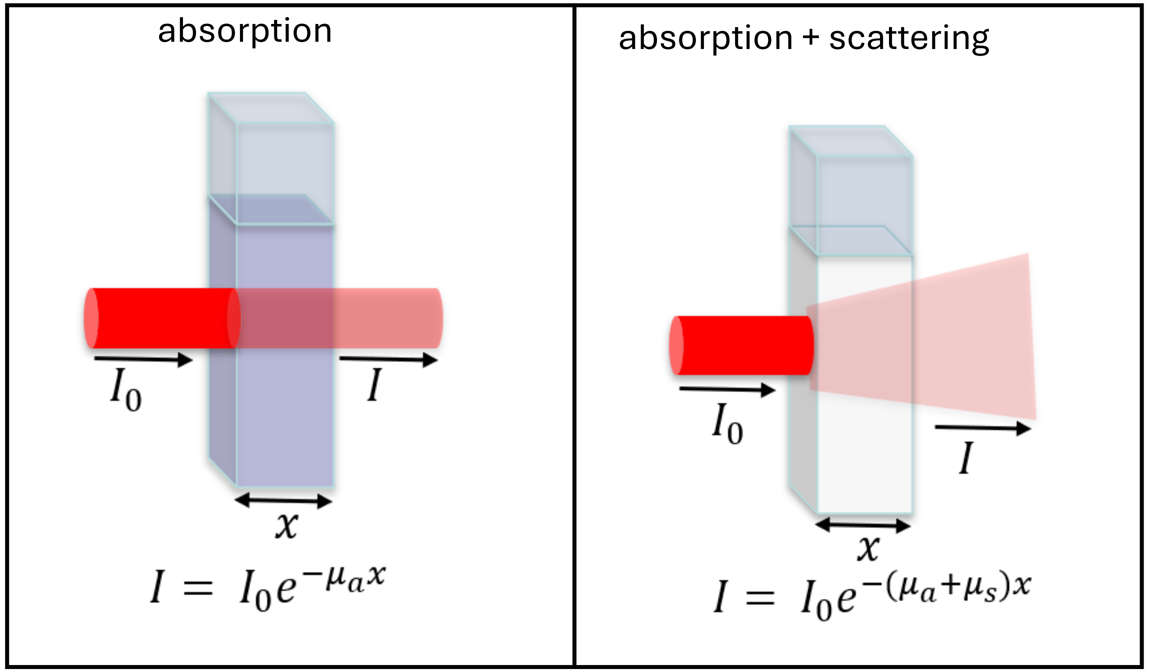


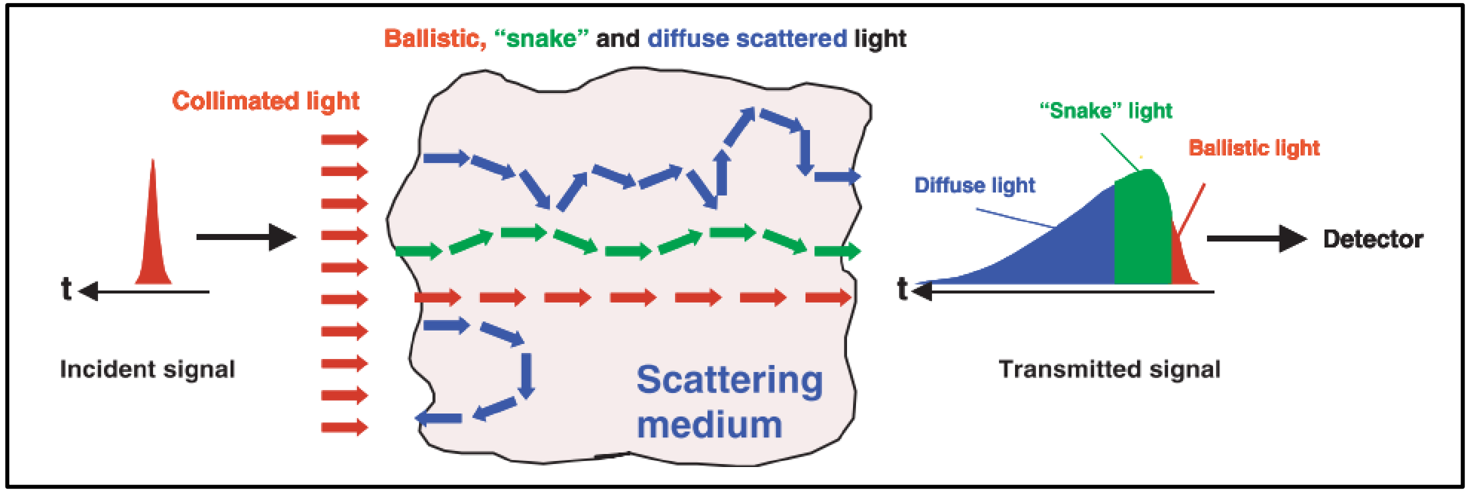
https://radiologykey.com/4-radiation-interactions-with-tissue/

Light Interaction with Matter

Light Propagation in Tissue

EM wave (in material)
https://emanim.szialab.org/index.html

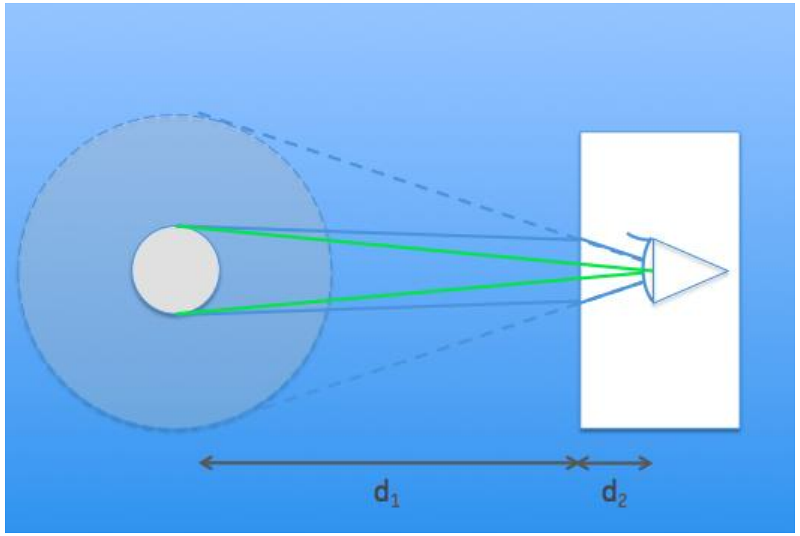


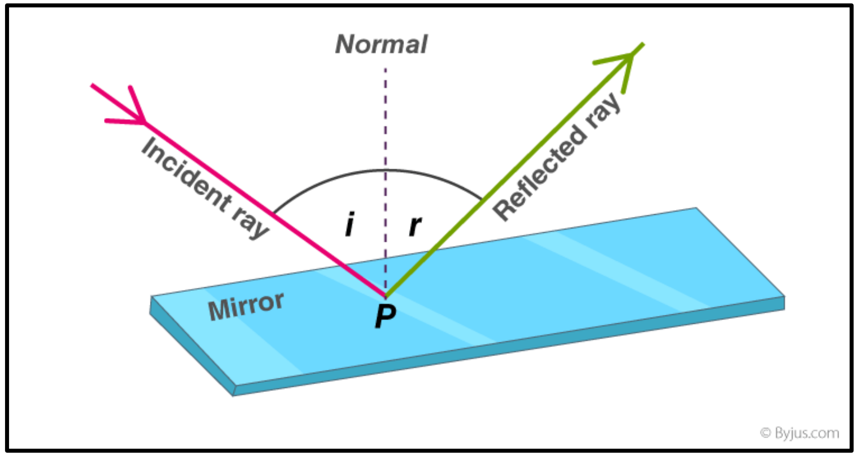


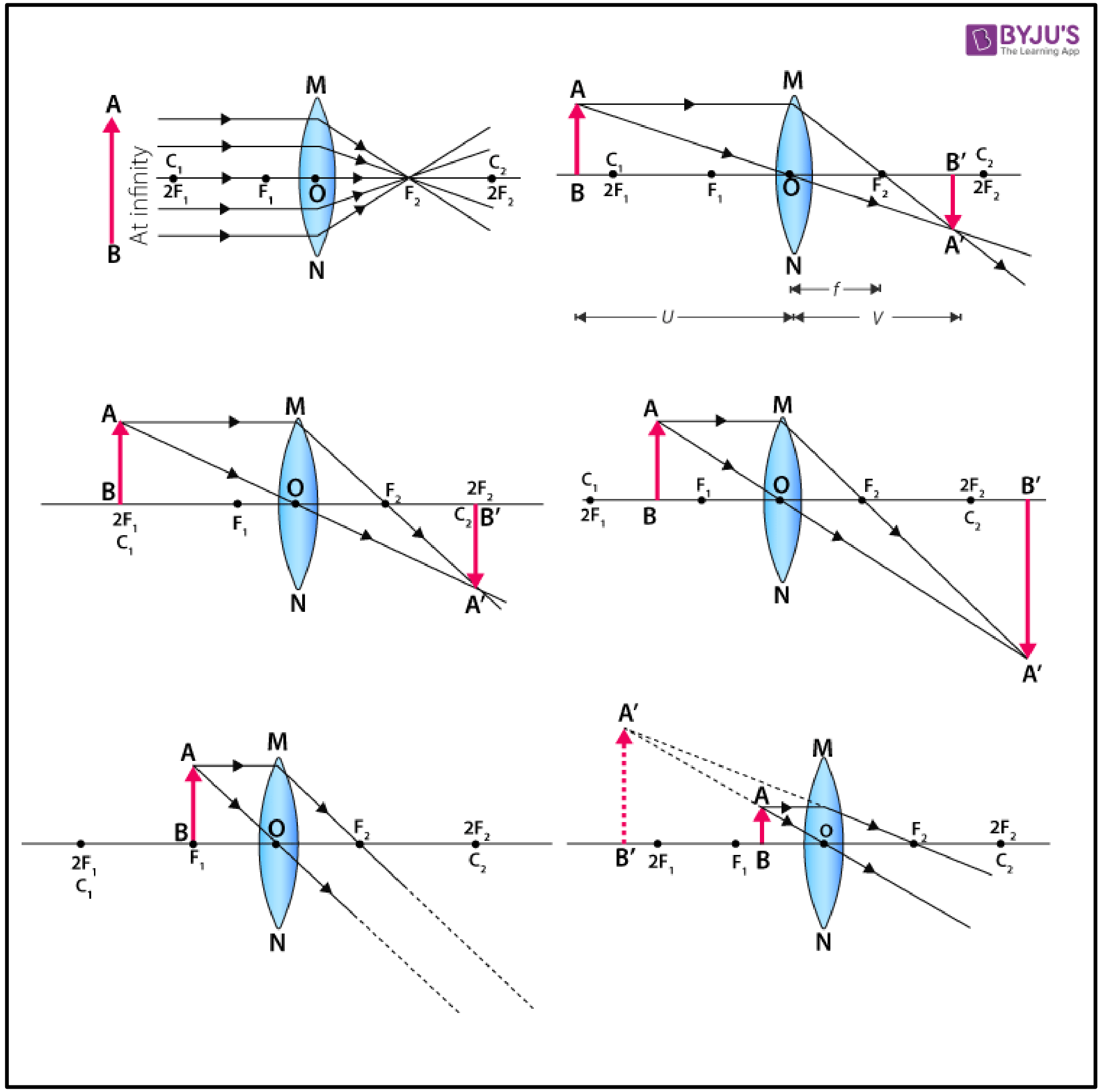
C Dunsby and P M W French 2003 J. Phys. D: Appl. Phys. 36 R207

light ray manipulation

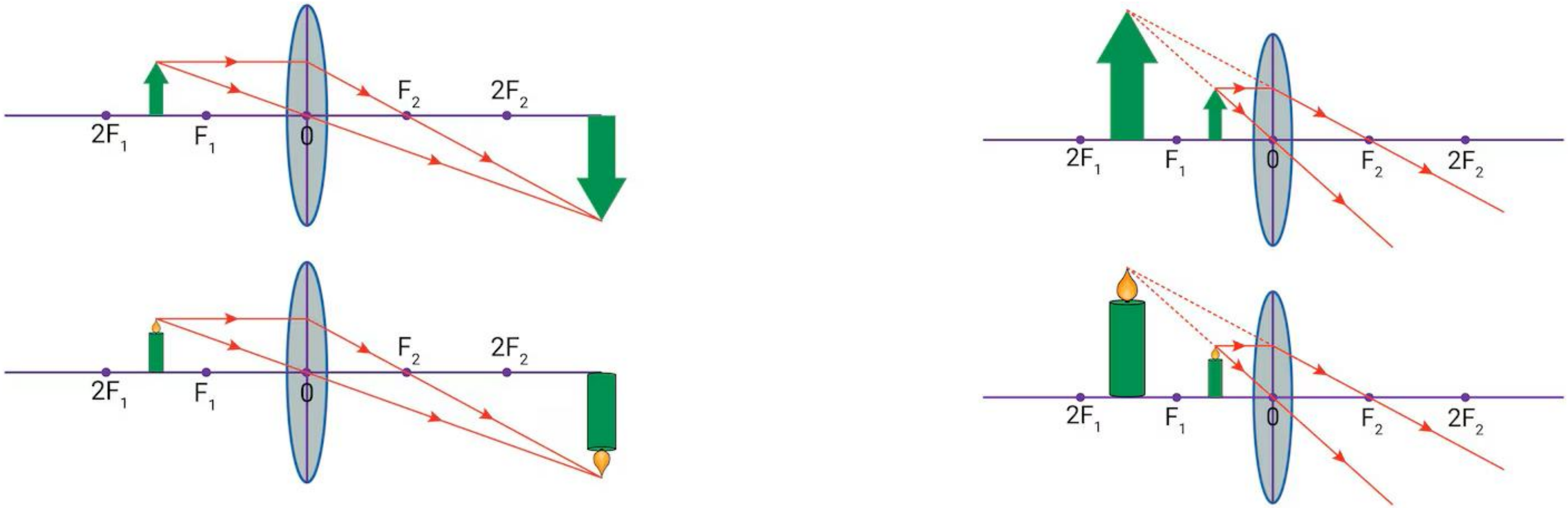
Refraction, Reflection, Image formation, (De)Magnification





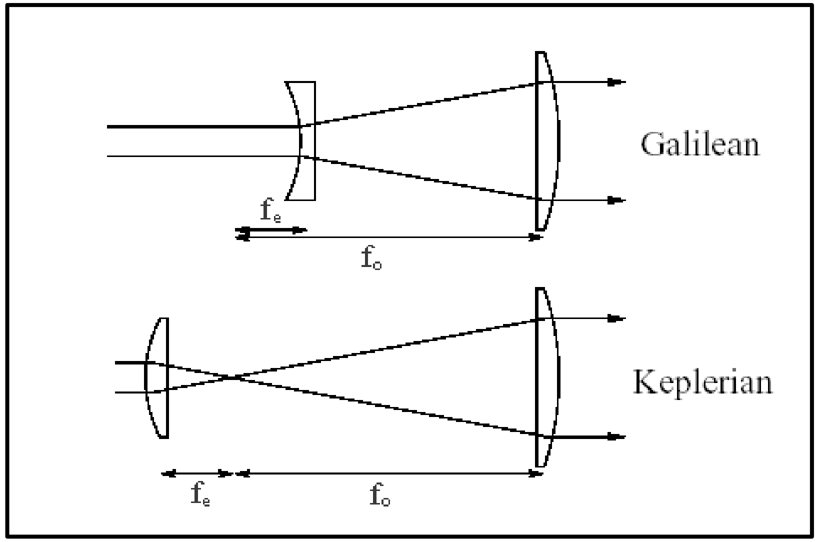


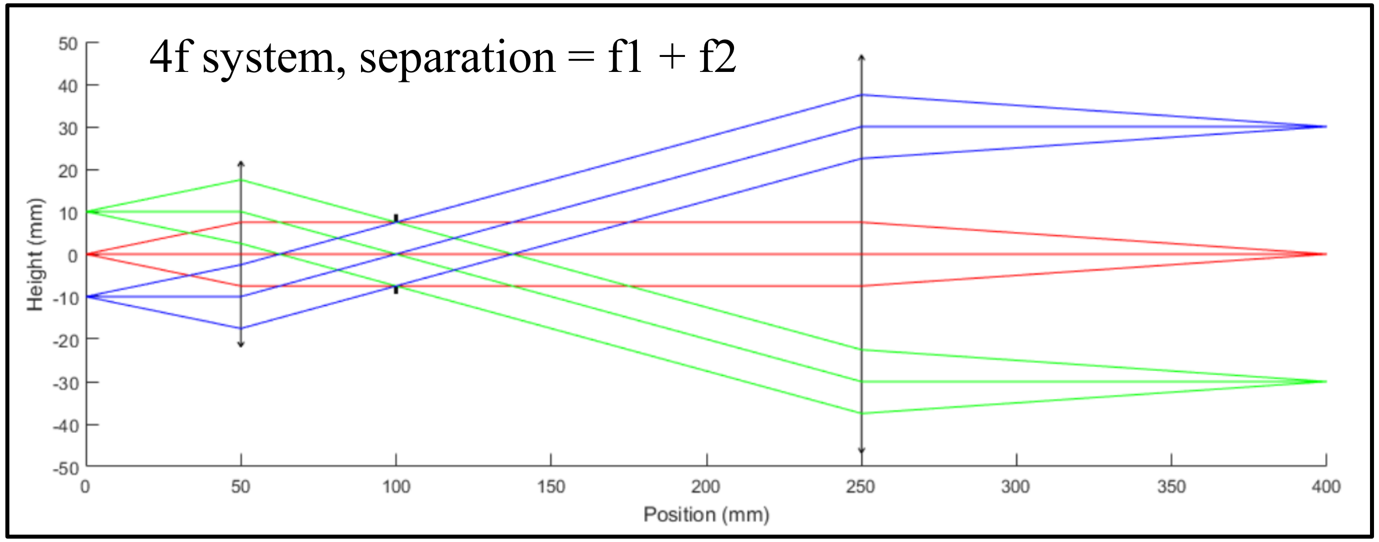
Ray diagram for a converging lens



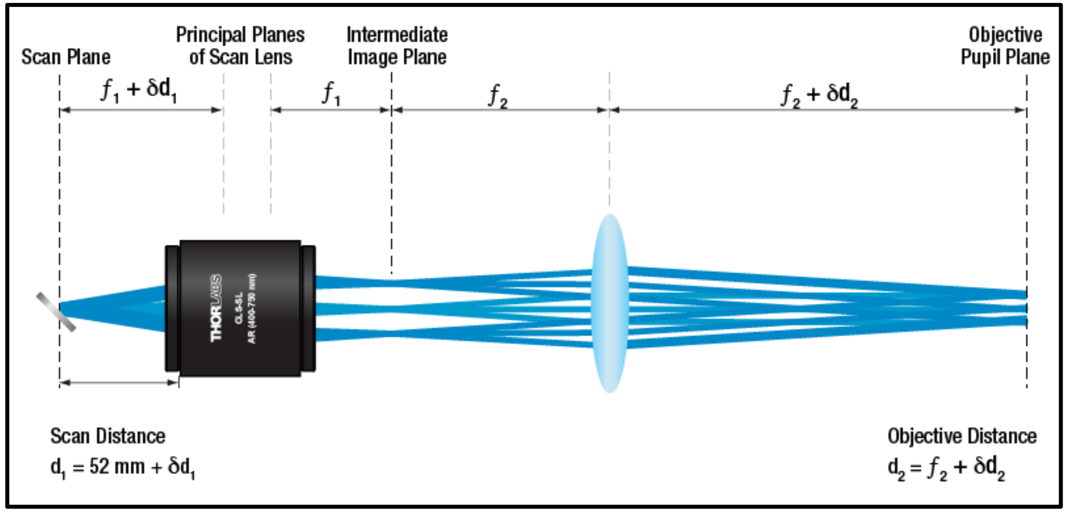
1. Rays parallel to symmetry axis (optical axis) get focused (focal point).
2. Rays crossing the center point of the lens are not refracted.
3. Rays crossing the focal point, refract parallel to the optical axis.

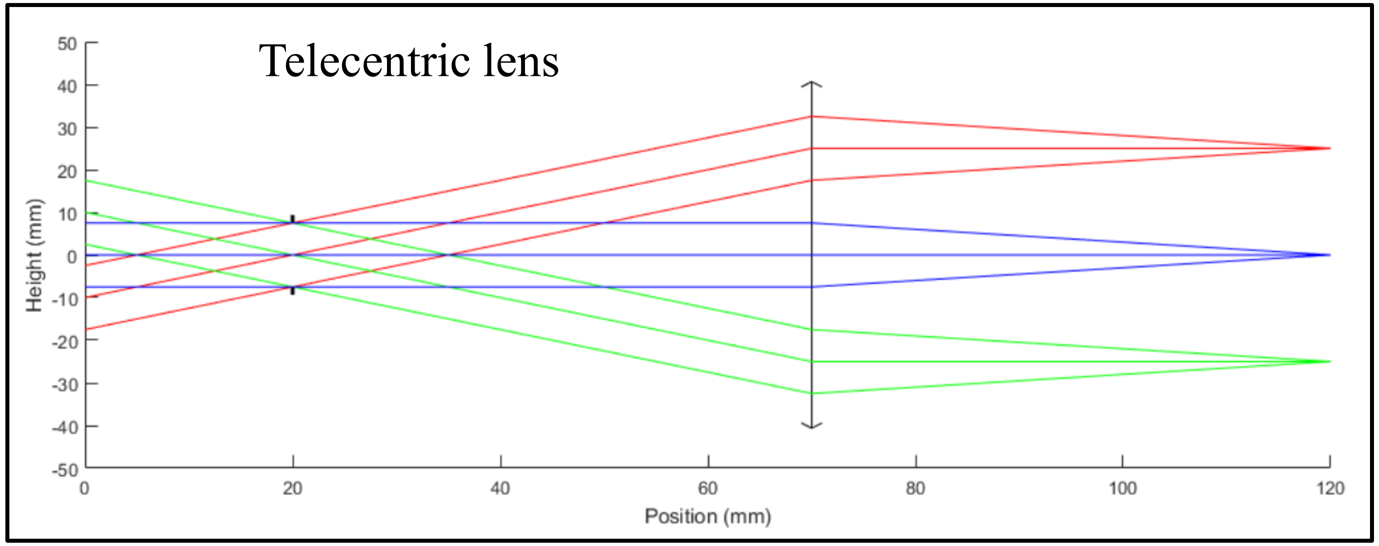
light ray manipulation – 4f system, telescopes, telecentric scan, etc.



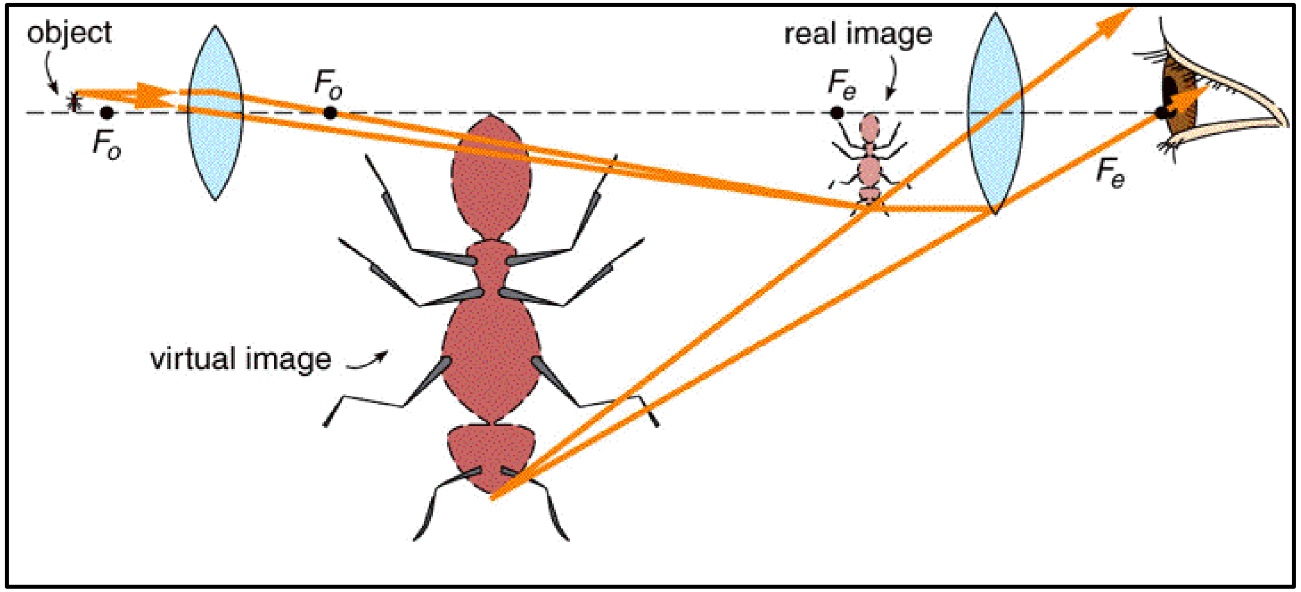


www.thepulsar.be

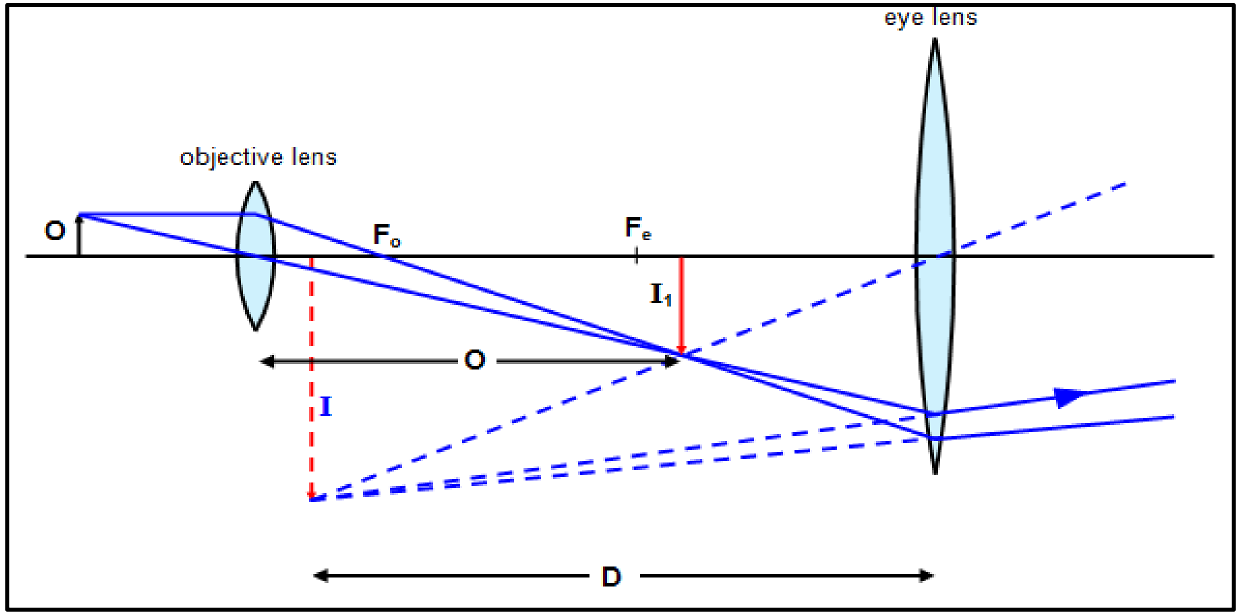




light ray manipulation – building microscope

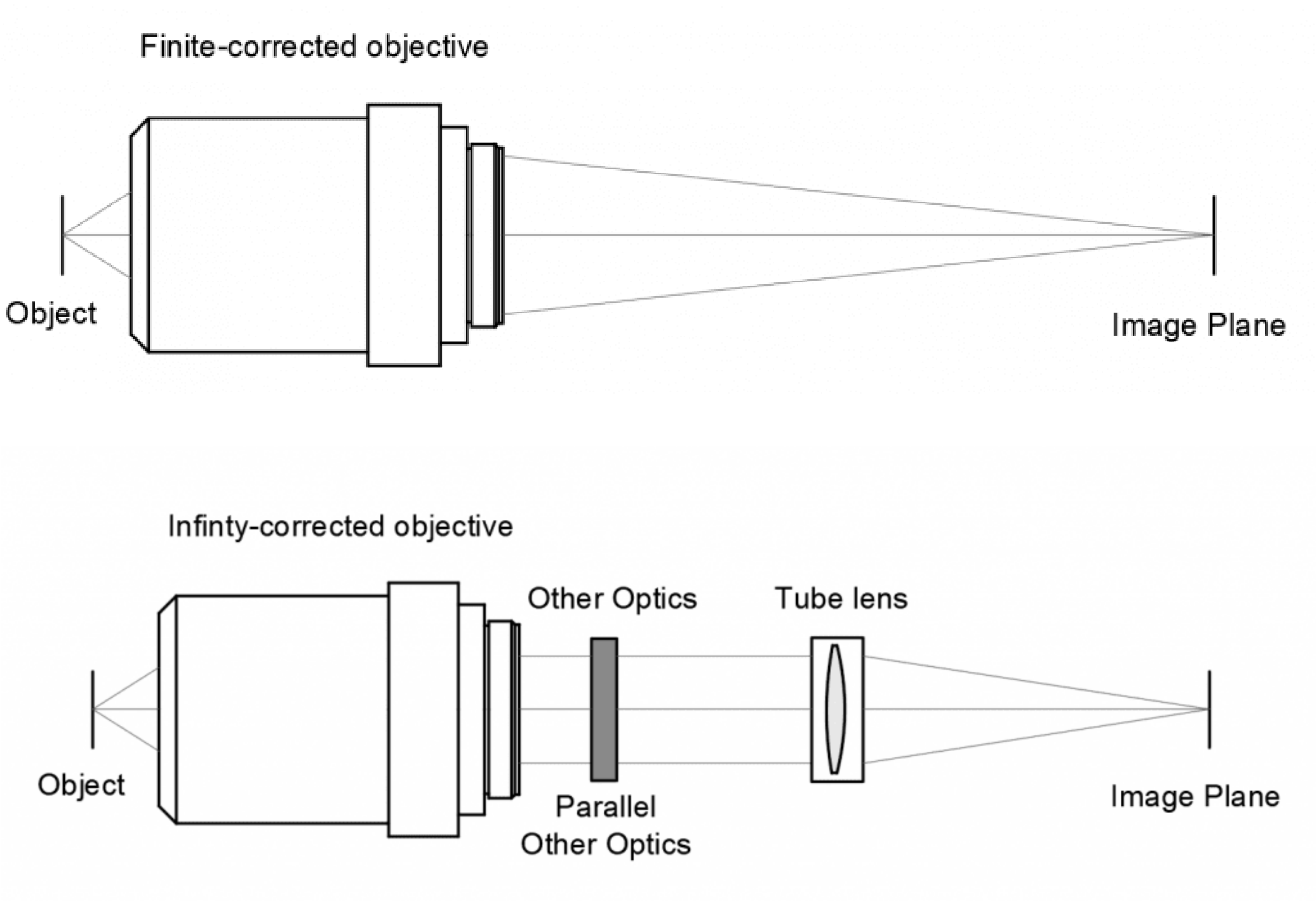


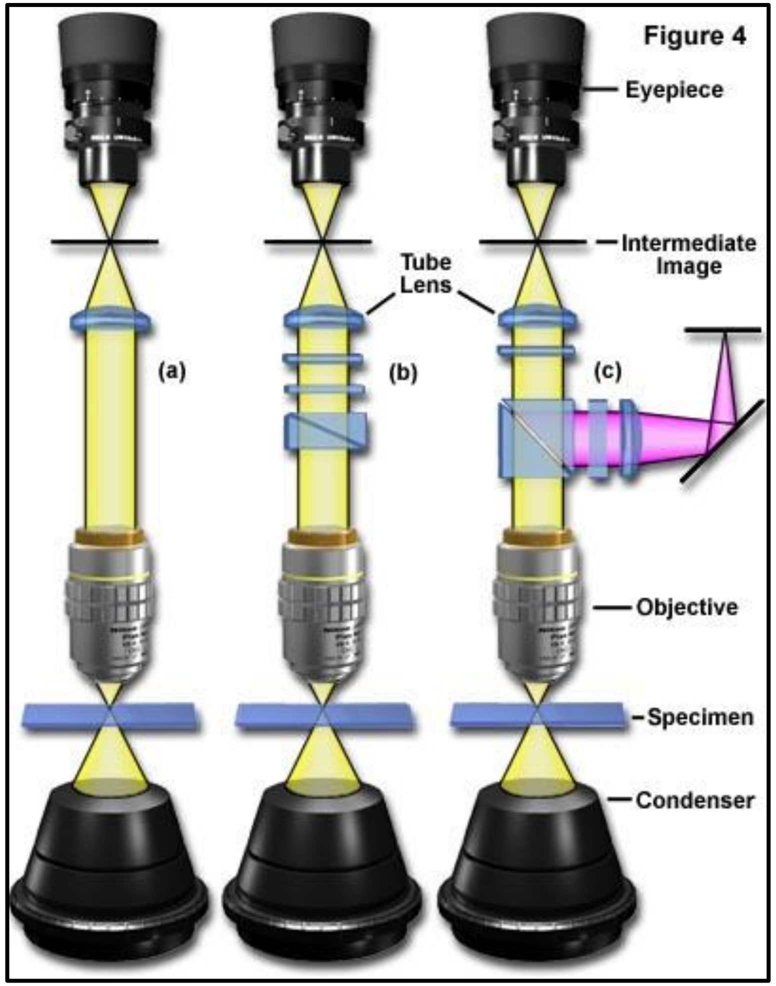
https://www.school physics.co.uk/



https://physics.sta ckexchange.com/

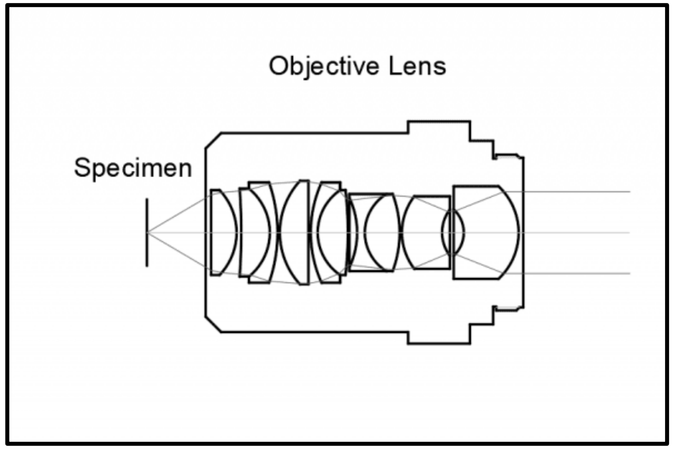
light ray manipulation – building microscope



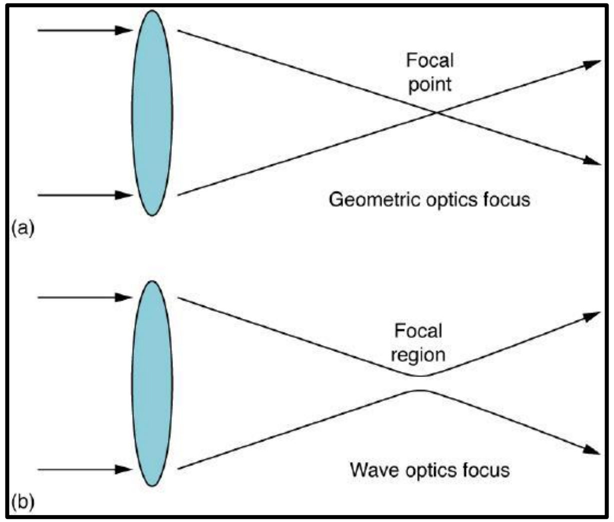


https://micro.magnet.fsu.edu/

Objectives come in various designs.
Dry, water, oil, NA, working distance, aberrations, etc.



Resolution and NA dependence

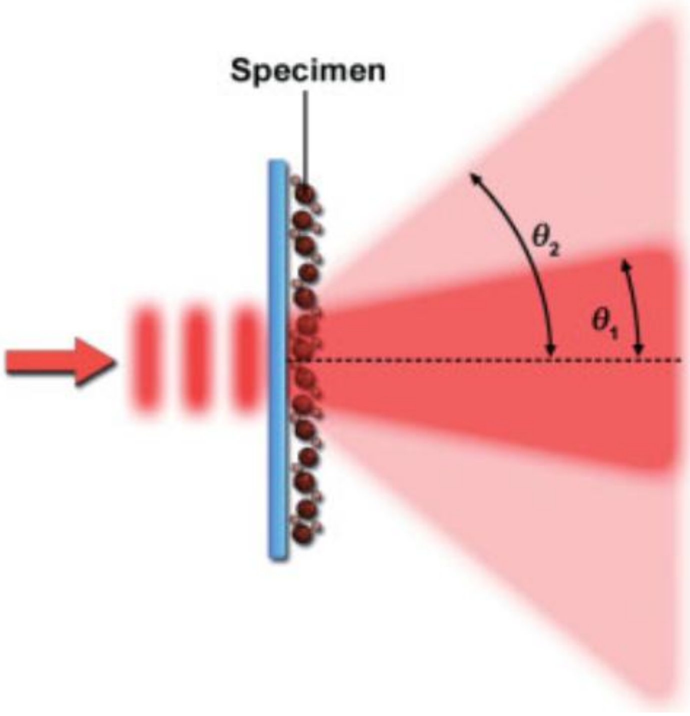


https://phys.libretexts.org/

$$\tan\theta \uparrow \approx \sin\theta \uparrow \approx \theta \uparrow \approx \frac{\lambda}{d \downarrow}$$

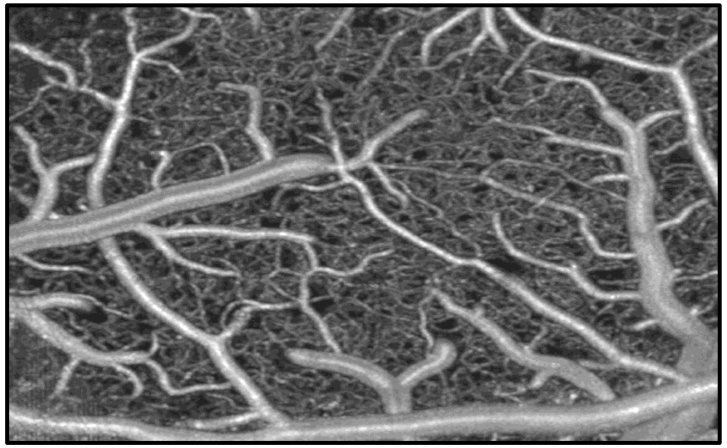
The angle of spreading (θ) is inversely proportional to the size of the particles

finer periodic structures leaves the sample at a higher angle.



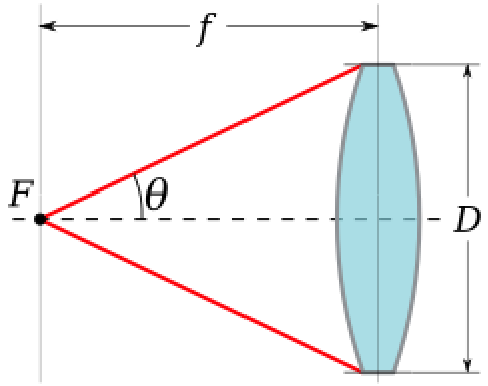
Douglas B. Murphy Michael W. Davidson, Fundamentals of Light Microscopy..

Diffraction gratings Demo



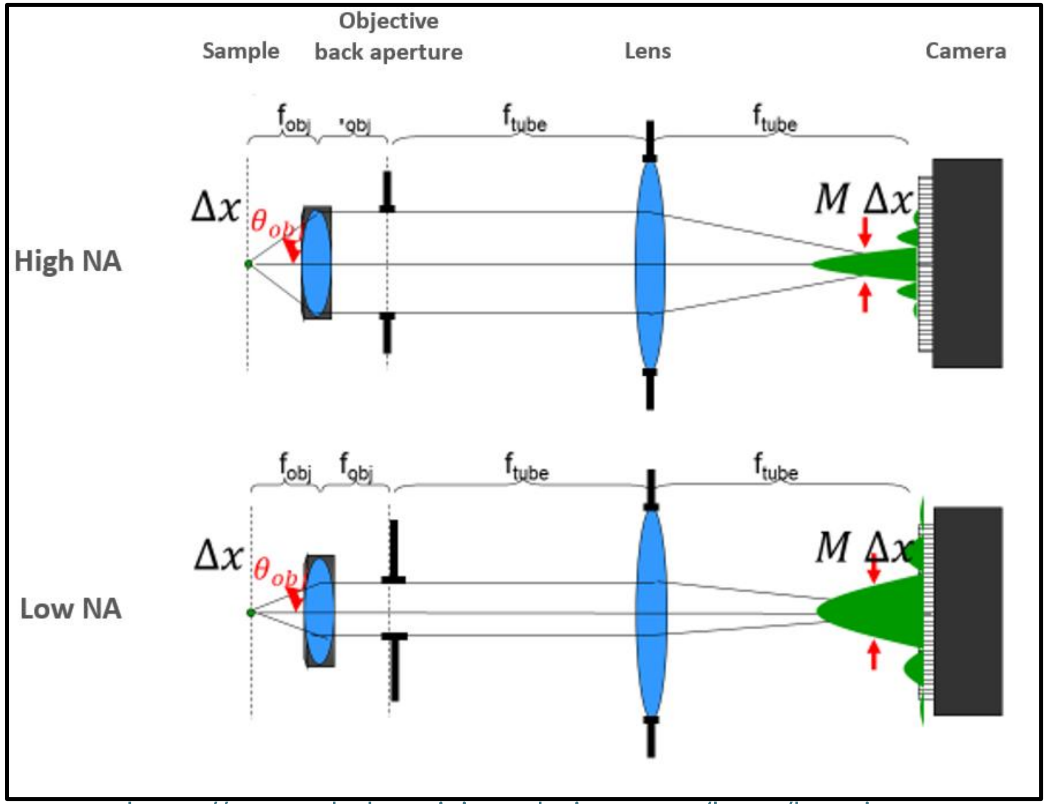
Resolution and NA dependence

Numerical Aperture: NA $= n \sin\theta$



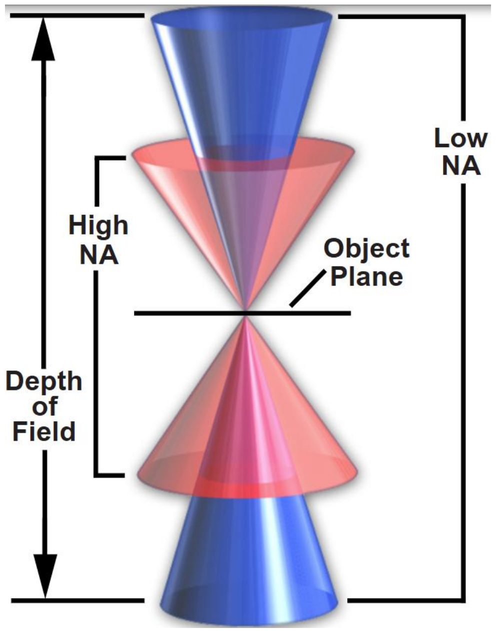
n: refractive index
θ: is the half-angle of the maximum cone of light that can enter or exit the lens

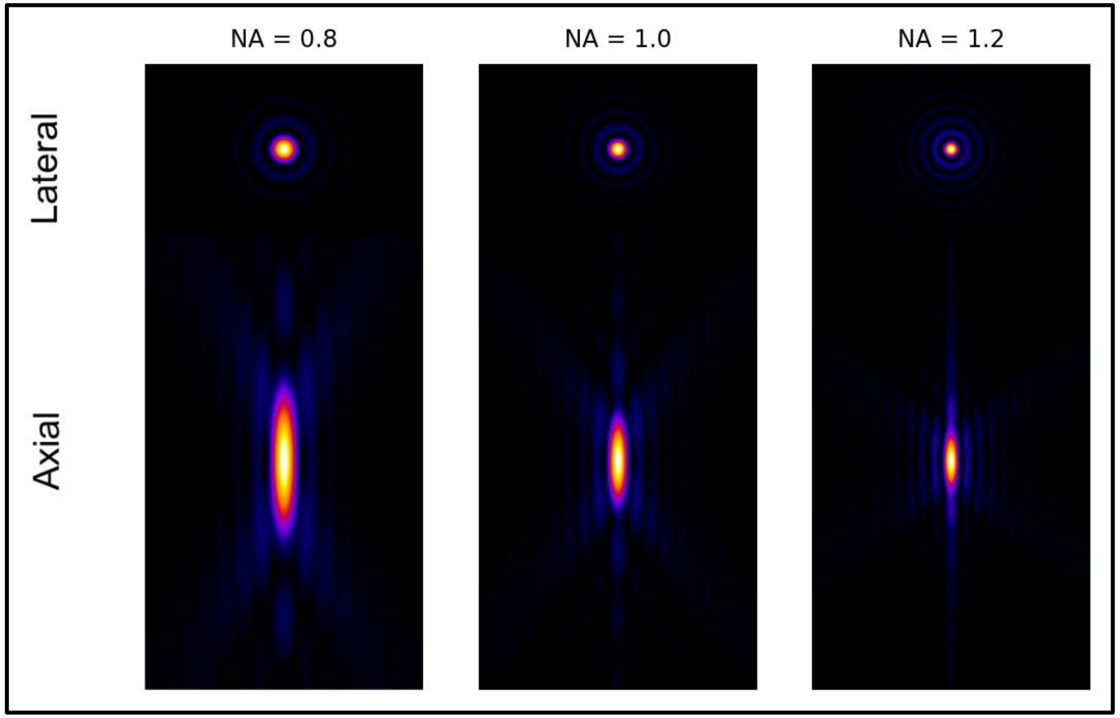
The numerical aperture of a microscope objective is a measure of its ability to gather light and resolve fine specimen detail at a fixed object distance.



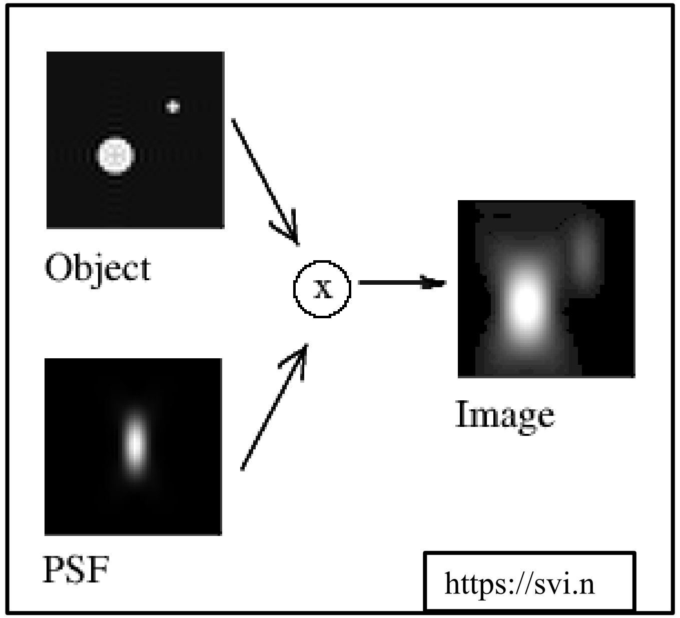
https://www.teledynevisionsolutions.com/learn/learning-center/scientific-imaging/resolution-and-numerical-aperture/

Image formation, PSF, and Resolution





https://bioimagebook.github.io/



$$g = h * f$$

The image (g) = the convolution of the object (f) and the PSF (h)

PSF: Point Spread Function (how a point-like object appears)

$$\text{Abbe Lateral Res} \sim \frac{\lambda}{2\,n \sin\alpha} = \frac{\lambda}{2\,NA}$$

$$\text{Abbe Axial Res} \sim \frac{2\lambda}{(NA)^2}$$

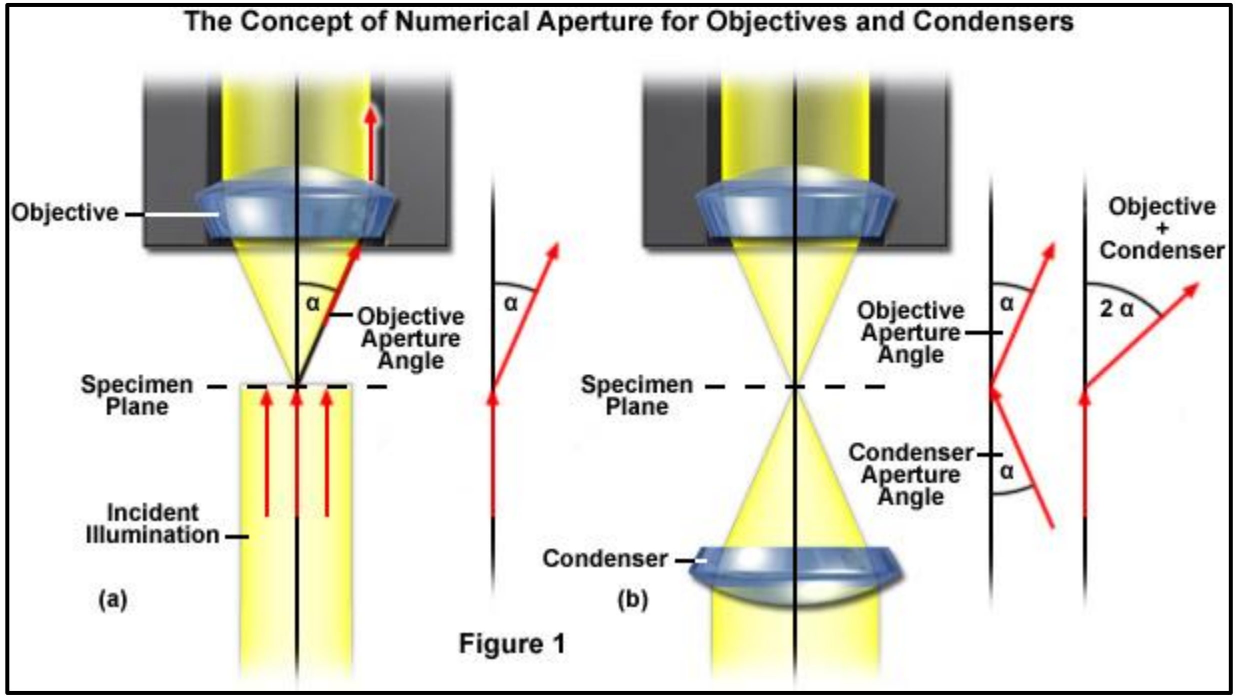
Resolution and NA dependence

$$\text{Lateral Res} \sim \frac{\lambda}{2\,n\sin\alpha} = \frac{\lambda}{2\,NA}$$

$$\text{Axial Res} \sim \frac{2\lambda}{(NA)^2}$$

If $NA_{cond} \leq NA_{obj}$:

$$\text{Lateral Res} \sim \frac{1.22\,\lambda}{NA_{obj}+NA_{cond}} \text{ (Abbe formula)}$$

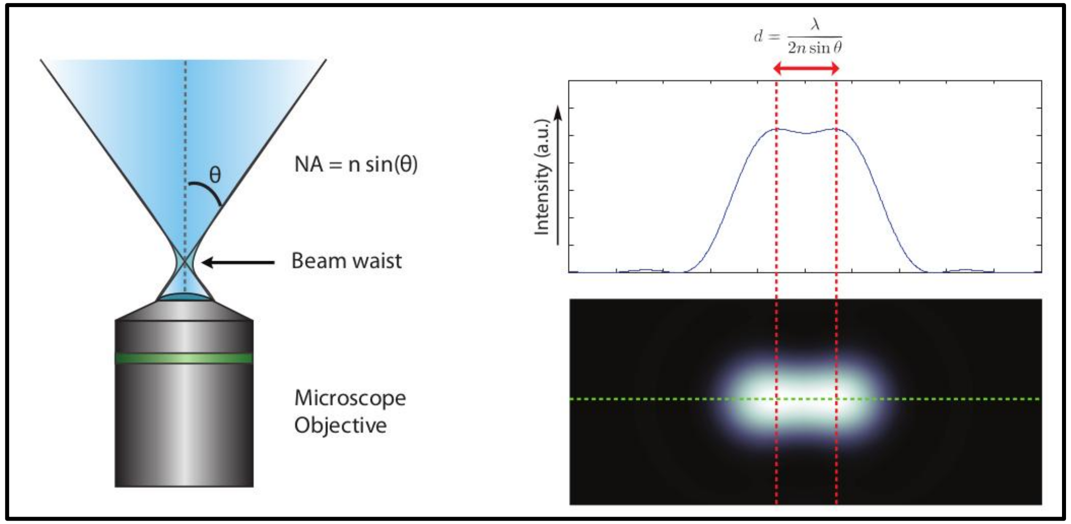


https://zeiss-campus.magnet.fsu.edu/

In epi FL microscopes the objective acts as the condenser:
Lateral resolution: only objective NA

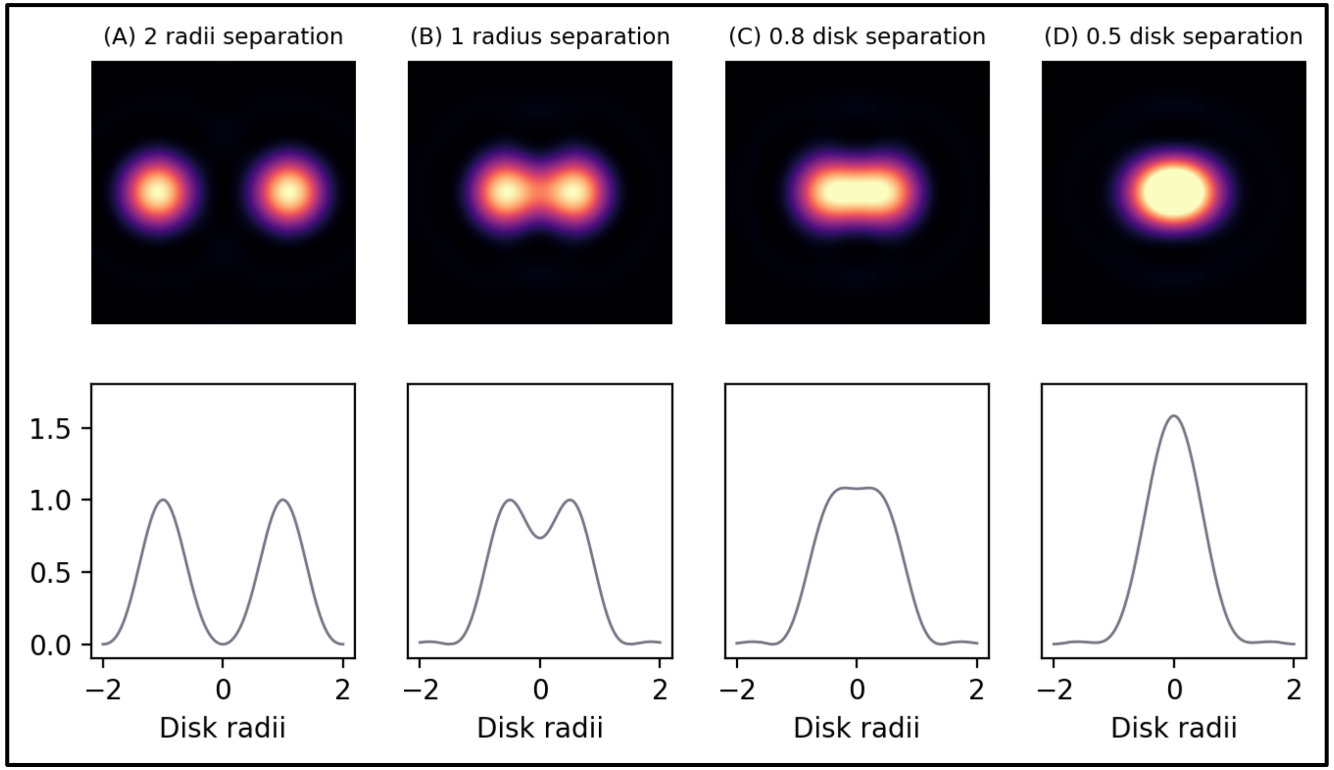
Diffraction limited resolution

Resolution Limit Imposed by **Wave Nature** of Light



Rayleigh Lateral Res $n \sin\theta = \frac{\lambda}{2d}$

$$d = 0.61 \frac{\lambda}{NA}$$

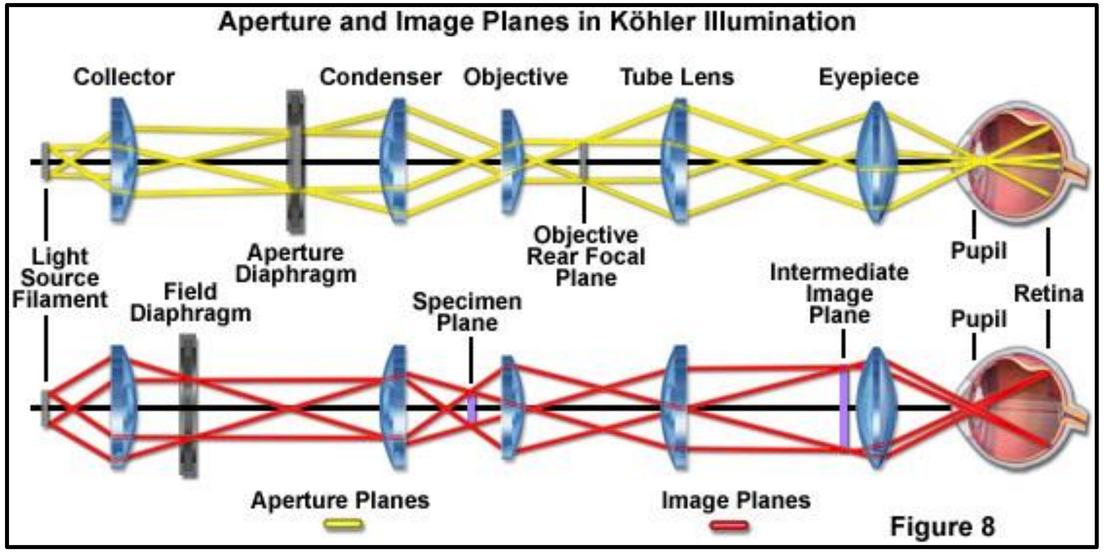


https://bioimagebook.github.io/

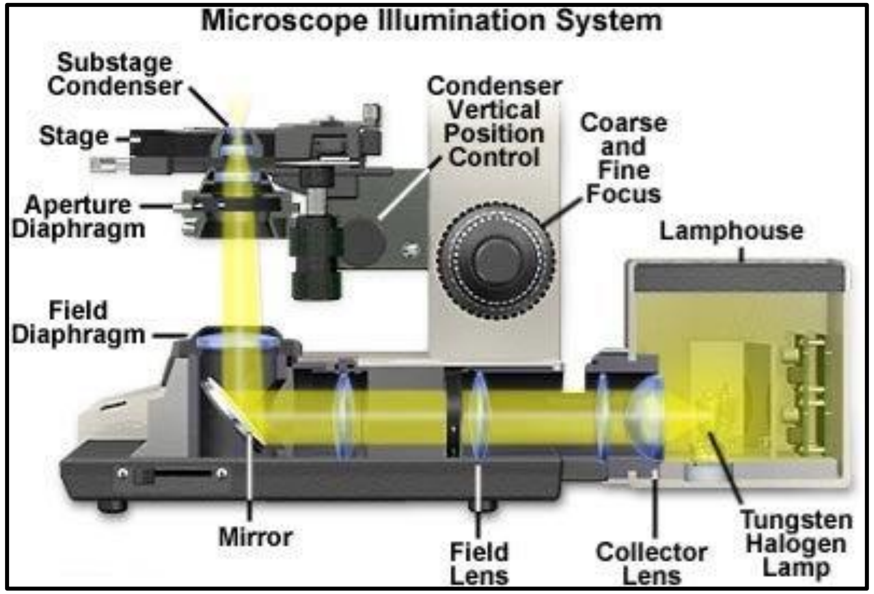
The Rayleigh criterion : the minimum resolvable detail.
"The first diffraction minimum of the image of one source point coincides with the maximum of another."

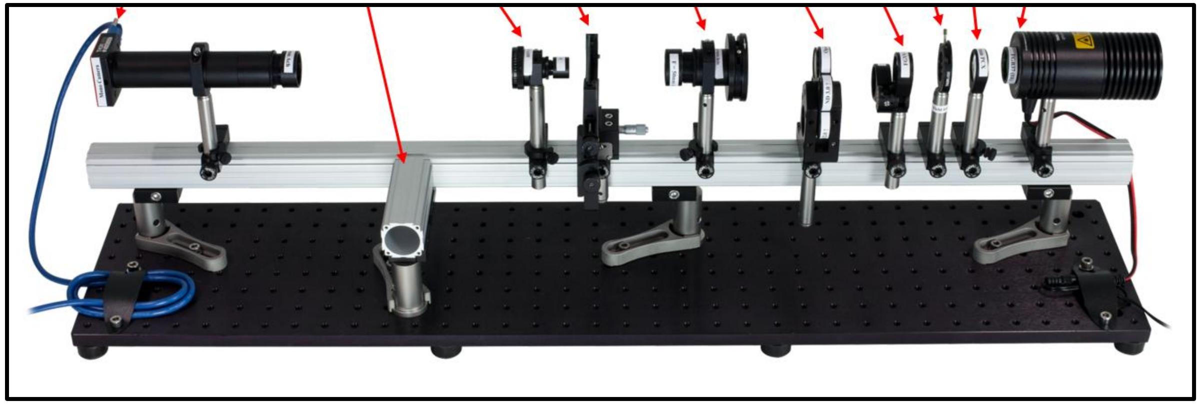
light ray manipulation – Köhler illumination

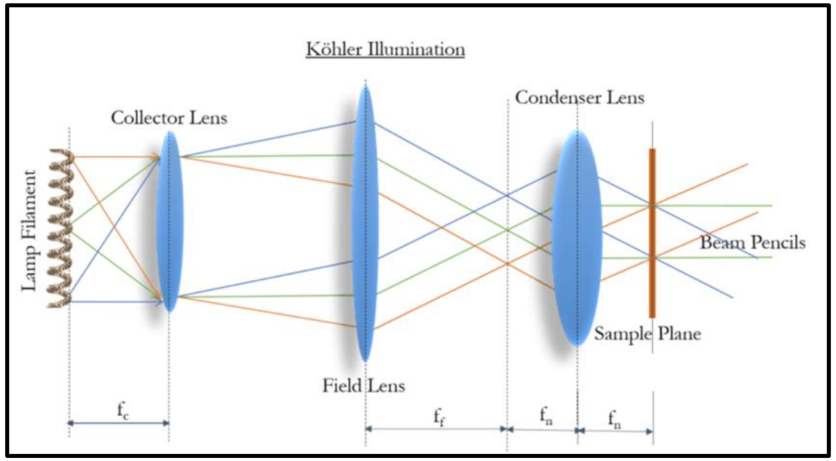
Uniform illumination using several lenses



https://zeiss-campus.magnet.fsu.edu/







www.teledynevisionsolutions.com/

Microscope main components

Illumination Sources

Optical Components

Detectors

Data Acquisition Components

Microscope main components – Illumination sources

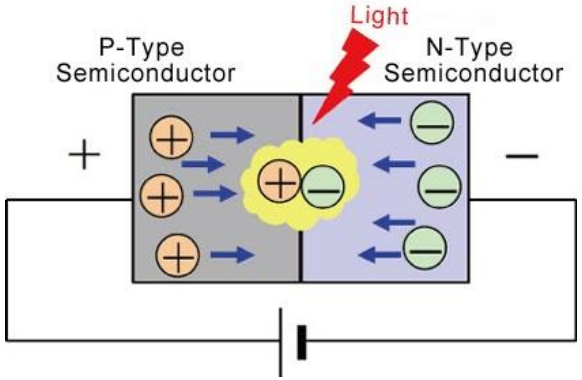
Lowest coherency

Lamps

Broad spectral excitation

LEDs

Cheap, Long-lasting, Poor spectral purity,
Hard to focus into fibers, Portable systems



Laser Diodes

High-power, Better coherence, Stable, Easy to couple w/ fibers,
Delicate – easy to burn down, More expensive

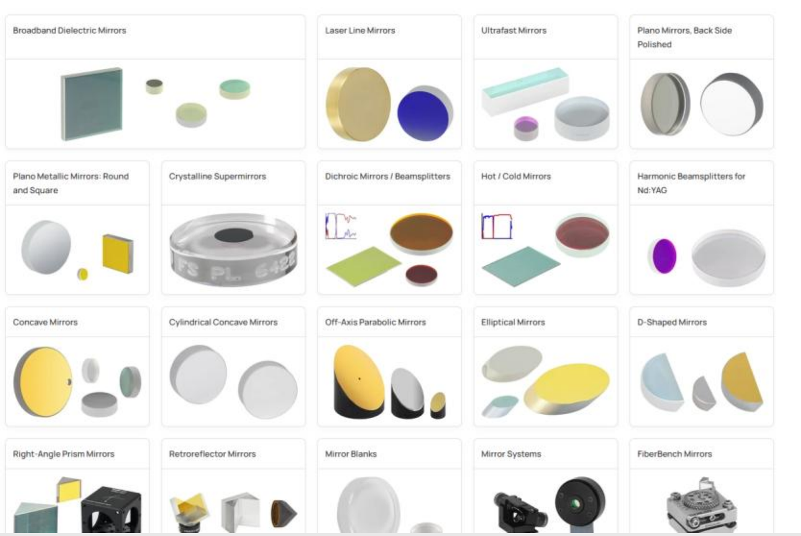
Laser Systems

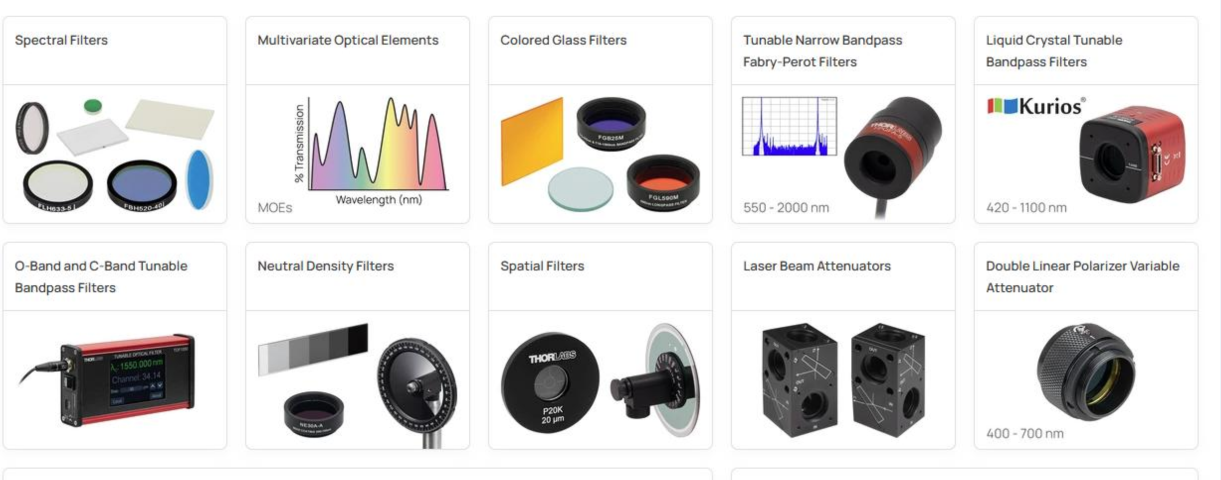
Desired bandwidth, wavelength, stability, frequency,
high coherency, Most expensive

Highest coherency

Microscope main components - Optics

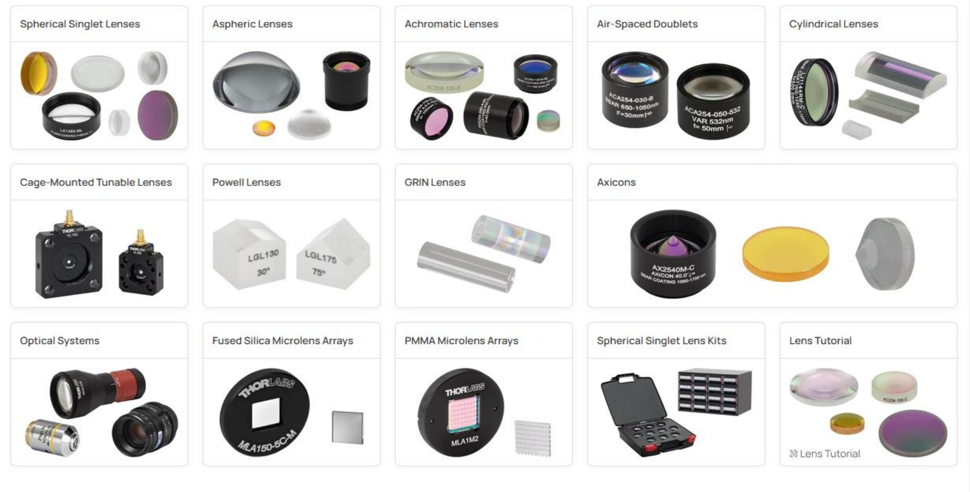
Mirrors: Broadband dielectric, gold, cylindrical, Concave,

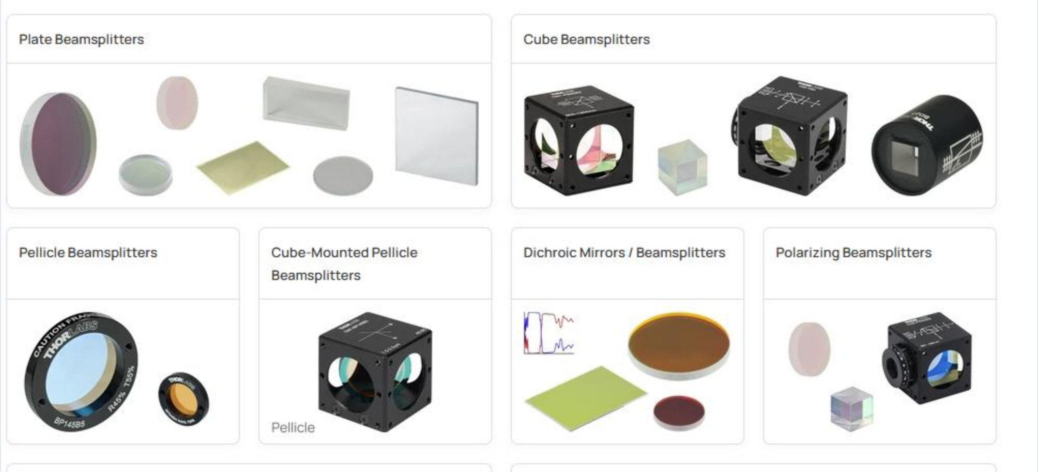




Filters: Intensity (ND), bandpass, long/short pass, polarization, …

Lenses: Aspheric, Achromatic, Convex, Concave, Doublet, Powell, Cylindrical, …



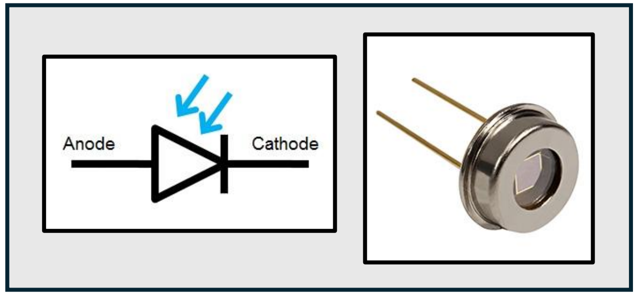


Beamsplitters: X/Y (50/50), Dichromatic, Polarizing,…

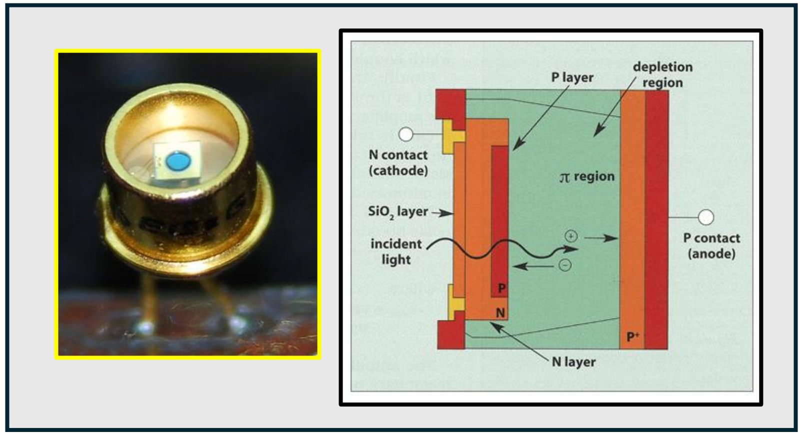
Microscope main components - Detectors

Photodiodes

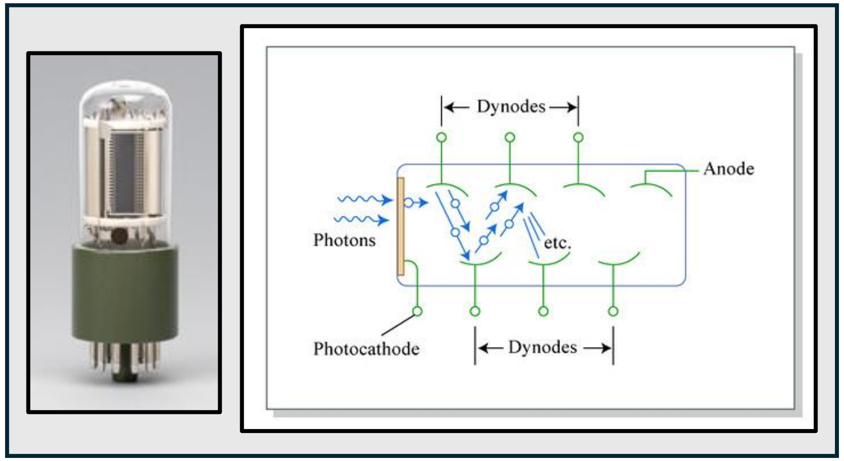
(PN junction diode in reverse bias)
cheap, high speed, moderate sensitivity



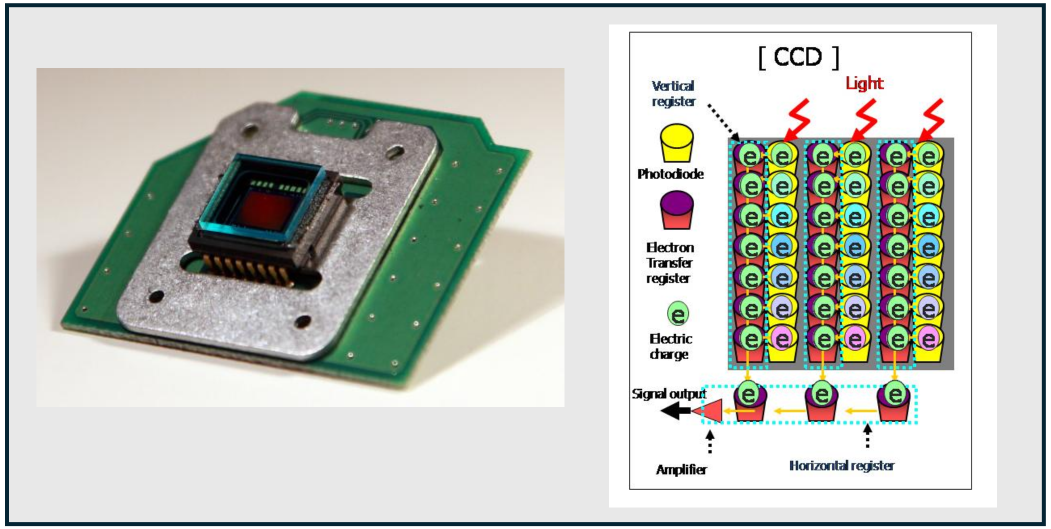
Avalanche Photo Diodes (APD)
high gain/sensitivity, higher cost,
more delicate



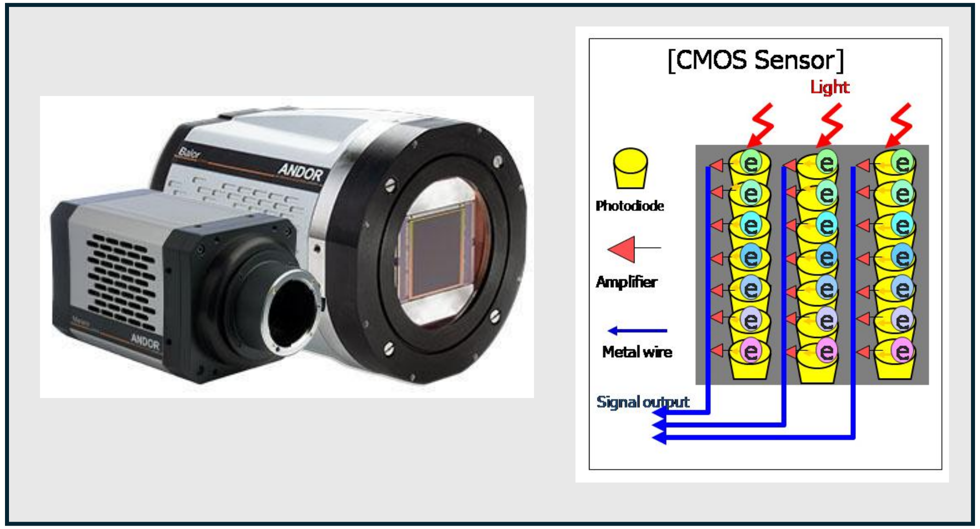
Photomultiplier tubes (PMT)
very high gain/sensitivity,
require high voltage,
prone to damage,
limited near-IR reach (< 850 nm)



Charge coupled devices (CCD)
- Analog
- Linked capacitors transfer charges
- Cheap



Complementary metal–oxide–semiconductor (CMOS)
- Digital
- High speed
- Low sensitivity



sCMOS (Scientific CMOS)
- Very low noise

Microscope main components - DAQ components

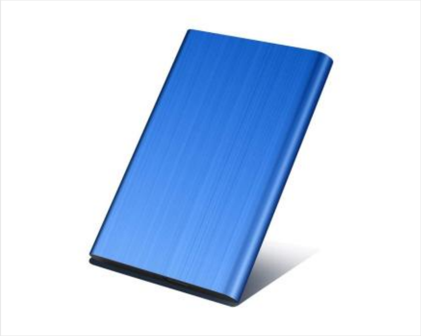
Analog to Digital Card (Digitizer)

- Converts the analog signal pulses to digital ones
- Transfers data …

Camera (Detector array) Frame Grabber

- Converts the signal pulses from camera (detector array) to digital ones
- Transfers data …

High Speed Data Storage drive



- Up to several GB/s data transfer speed

Microscope main components - DAQ components

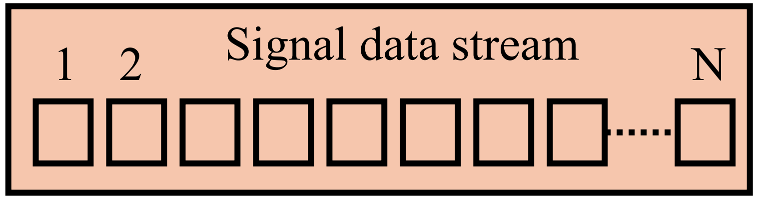
Multifunction I/O Modules, BNC cables, fibers, etc.

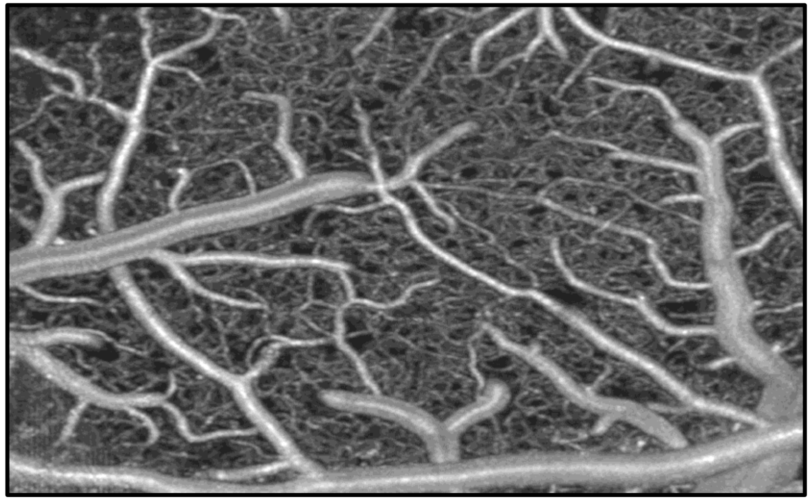





Software (Acquisition & Processing)

Single detector

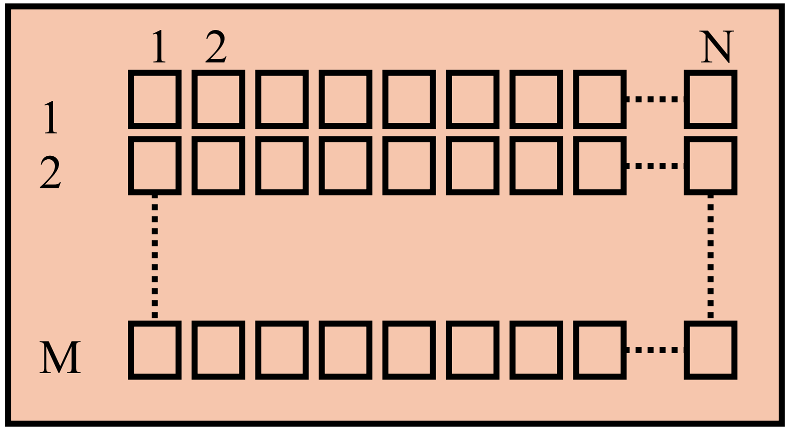




Detector array

Camera frame width: N

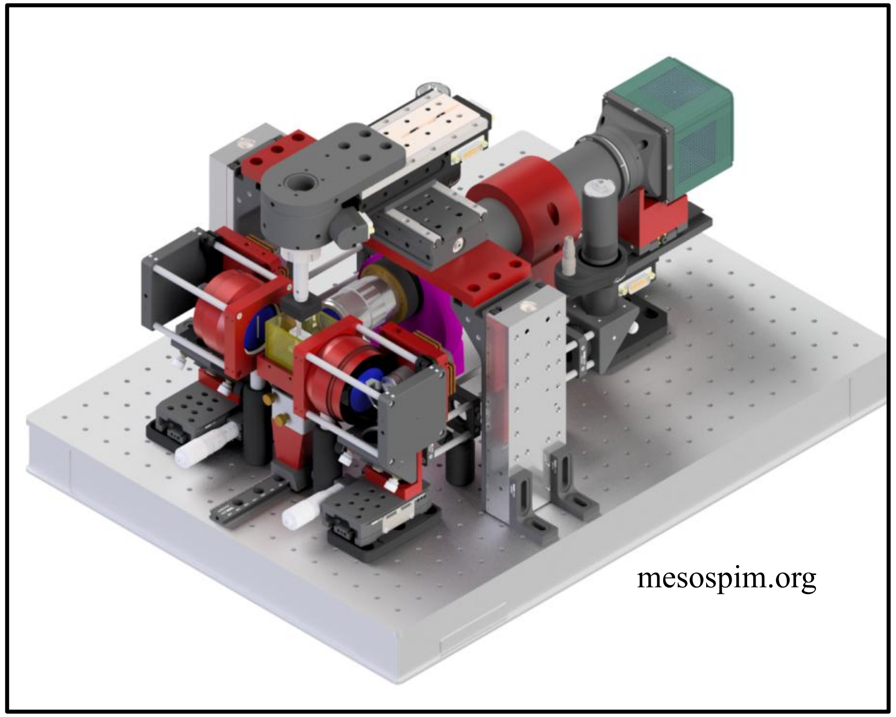
Camera frame height: M



Processing procedure could be composed of: Averaging, filtering/Noise removing methods, deconvolution, etc.

(Explained in second session of the course)

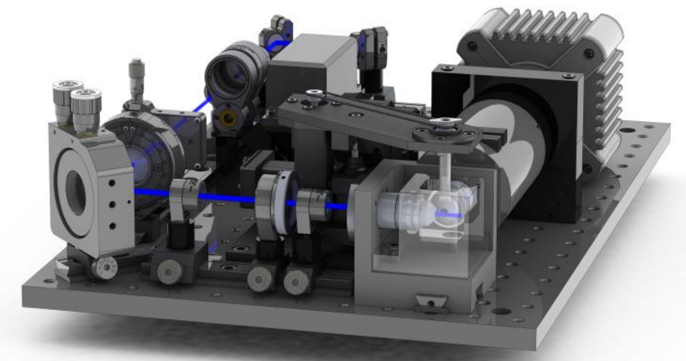
Examples of noncommercial microscopes



mesospim.org

aicjanelia.org/mosaic

openspim.org



Thanks

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