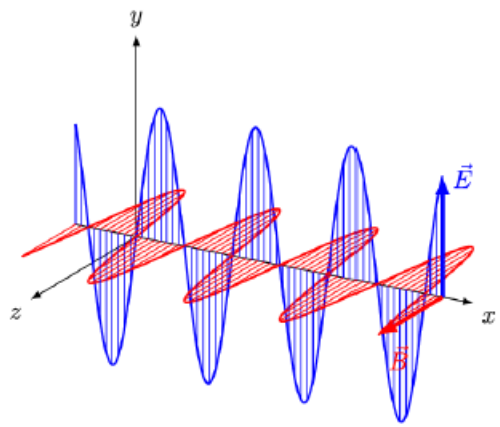
INTRODUCTION TO LIGHT MICROSCOPY

- Light and its properties
- A simple microscope
- The resolution limit

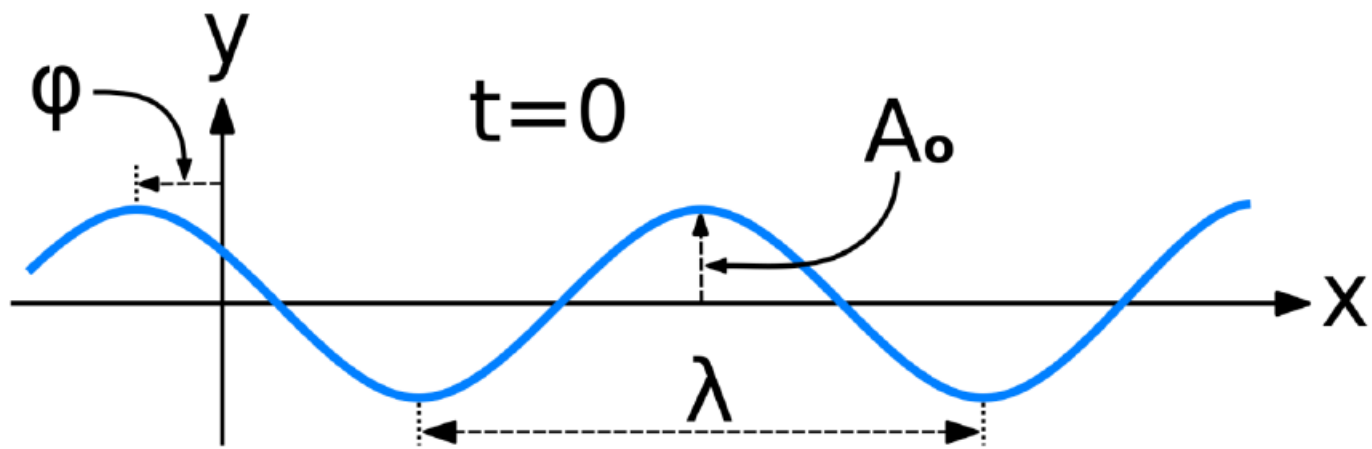
An electromagnetic wave



AND

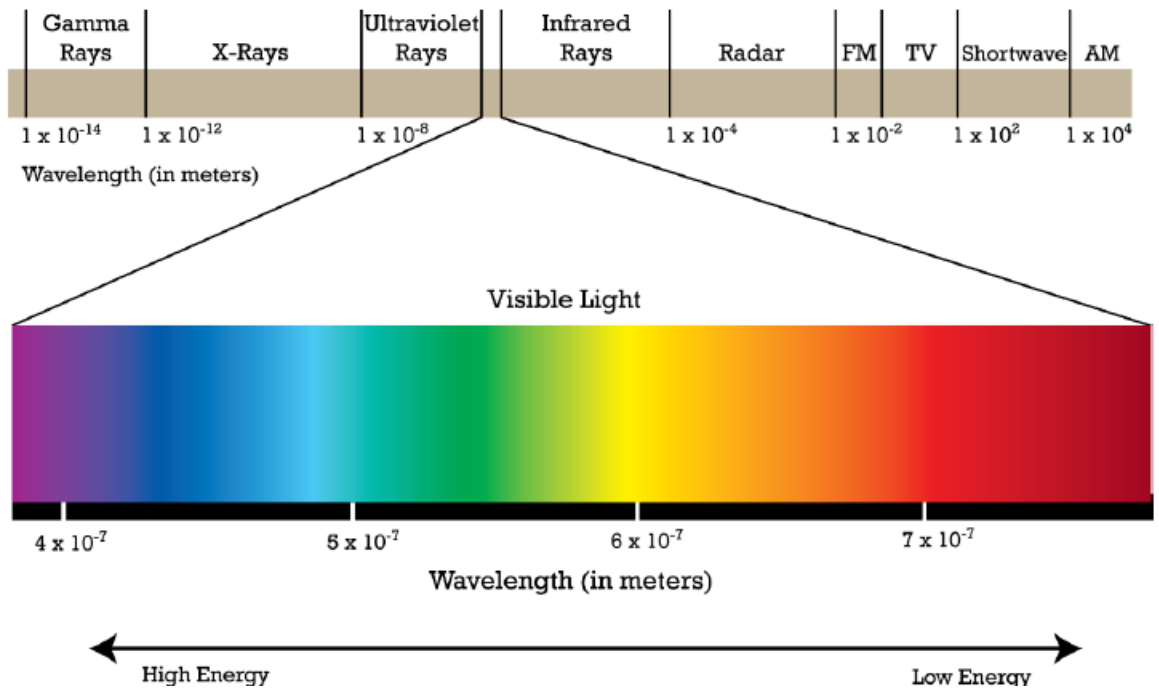
A massless particle

γ



Light waves are transverse waves – they oscillate orthogonally to the direction of propagation

Important properties of light: wavelength, frequency, speed, amplitude, phase, polarisation



$$E_{photon} = h\nu$$

$$c = \lambda\nu$$

E_{photon} = photon energy

h = Planck's constant

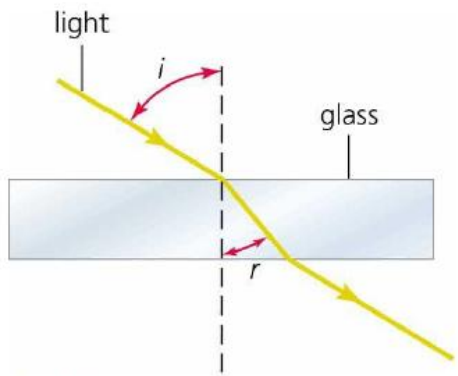
ν = frequency

c = speed of light

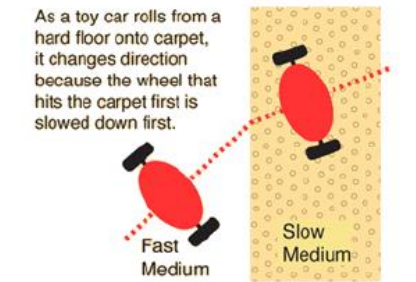
λ = wavelength

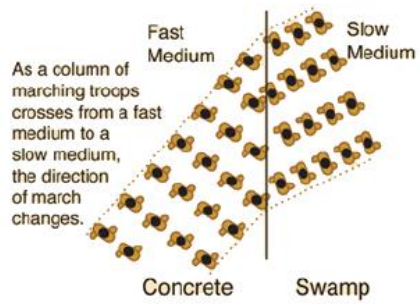
Light bends when it encounters a change in refractive index e.g. air to glass

www.thetastesf.com



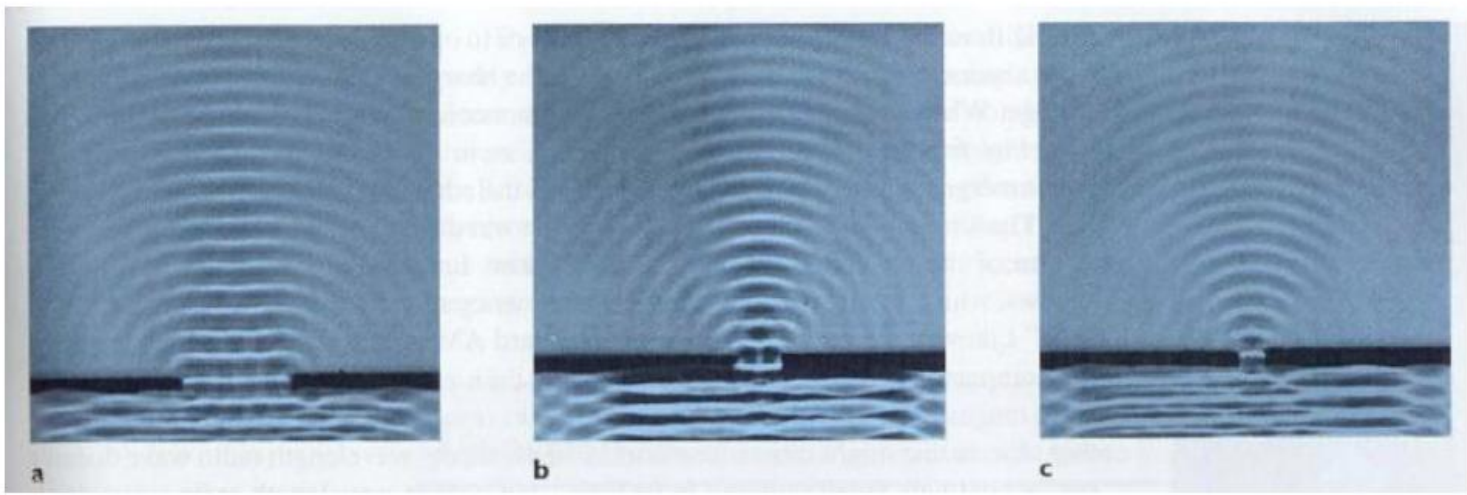
files.askiitians.com





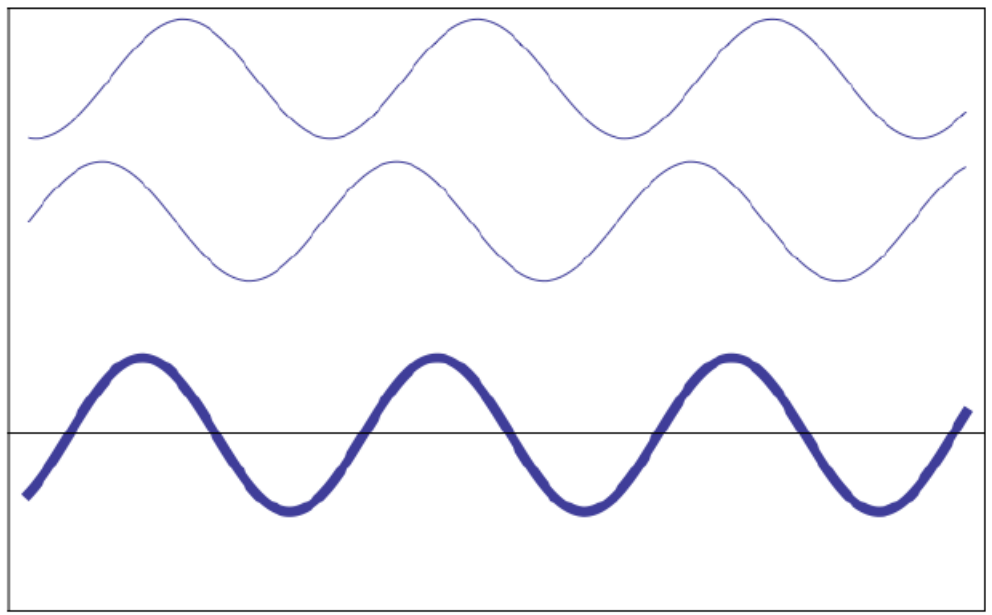
hyperphysics.phy-astr.gsu.edu/hbase/Sound/imgsou/refr.gif

Light waves spread out when they encounter an aperture.



electron6.phys.utk.edu/light/1/Diffraction.htm

The smaller the aperture, the larger the spread of light.



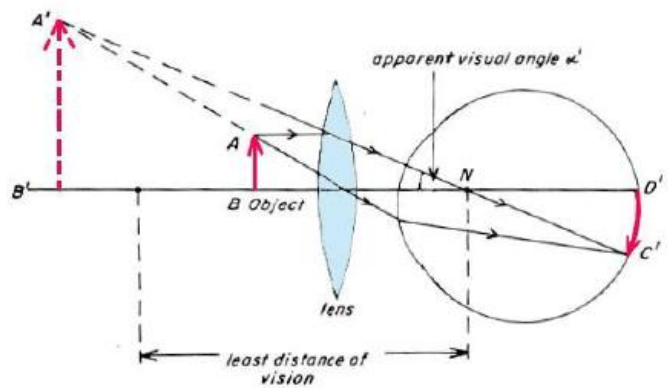
When waves overlap, they add together in a process called interference.

peak + peak = 2 x peak

trough + trough = 2 x trough

constructive

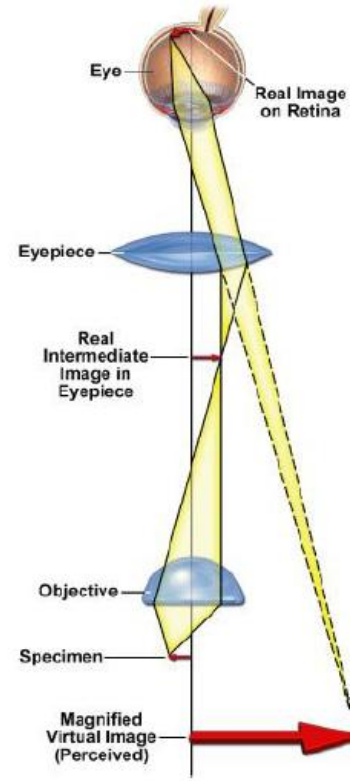
peak + trough = 0 → **destructive**



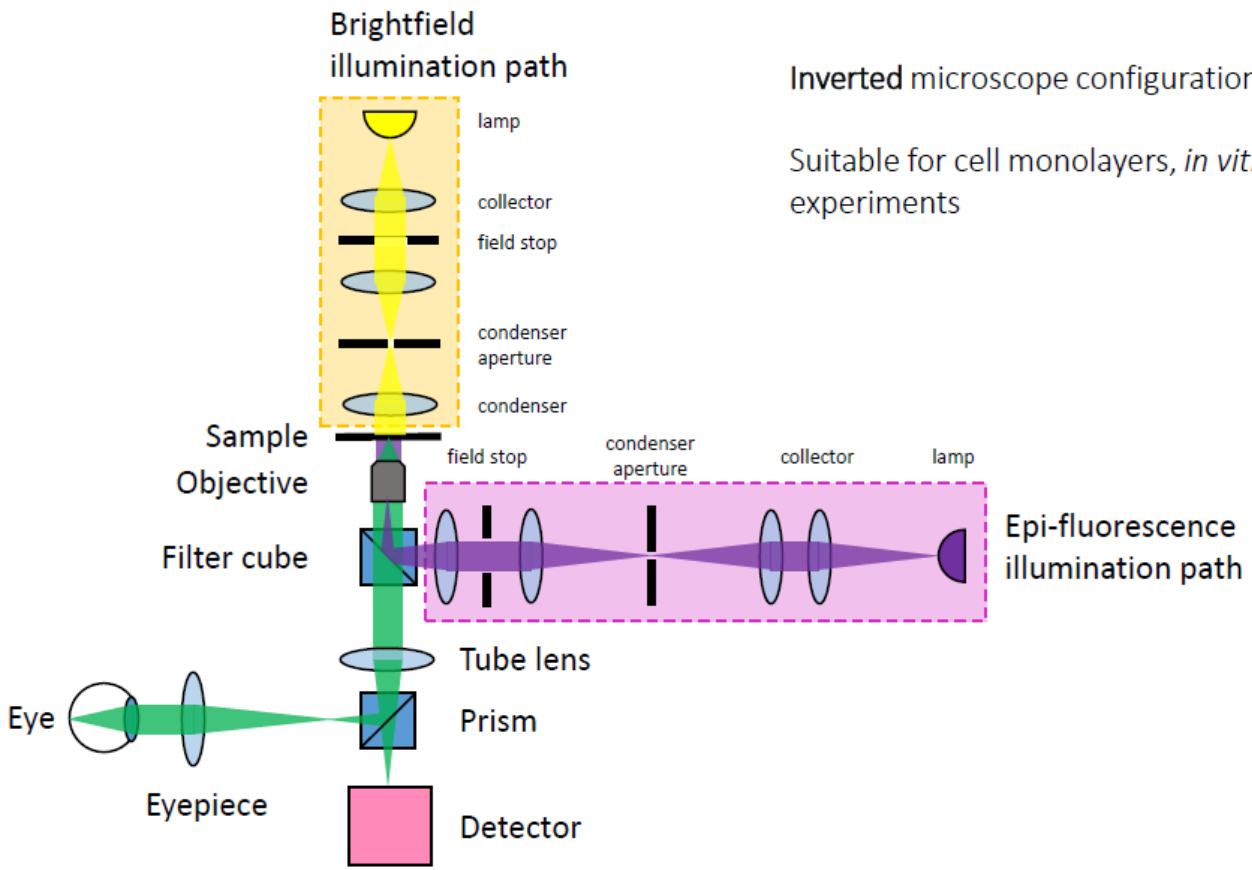
quekett.org

Magnifying glass – single lens to magnify an image

Compound microscope – a series of lenses creates a magnified image on the detector (eye/camera). Greater magnification possible with higher fidelity



Fundamentals of light microscopy and electronic im
and Davidson



Inverted microscope configuration

Suitable for cell monolayers, *in vitro* experiments

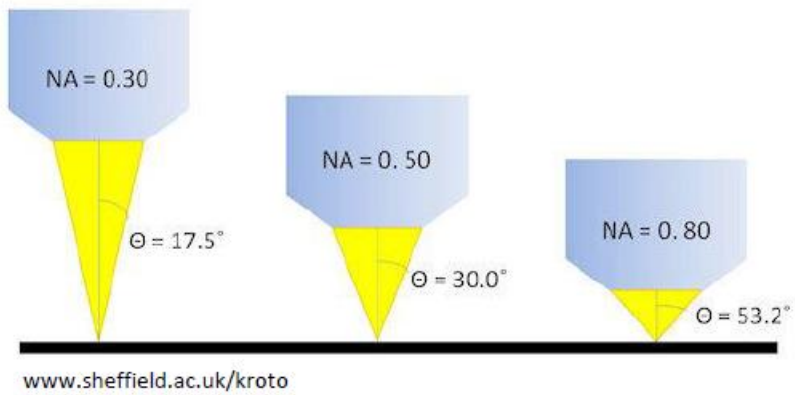
A series of lenses acting a single magnifying lens with superior optical characteristics

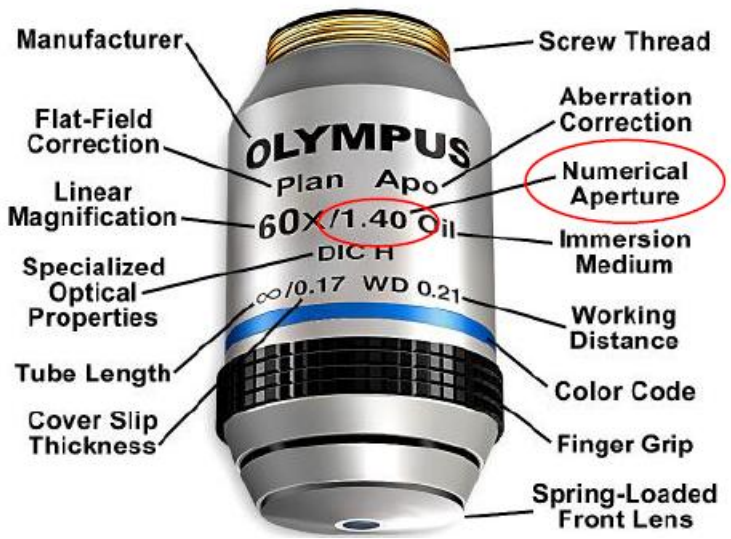
Microscopes typically have >1 objective to change total magnification

Figure 1

Mag.	1×	2×	4×	10×	20×	40×	50×	60×	100×
Code	Black	Gray	Red	Yellow	Green	Light Blue		Dark Blue	White
Imm. Med.	Oil		Water		Glycerin	Oil/Water/Glycerin			
Code	Black		White		Orange	Red			

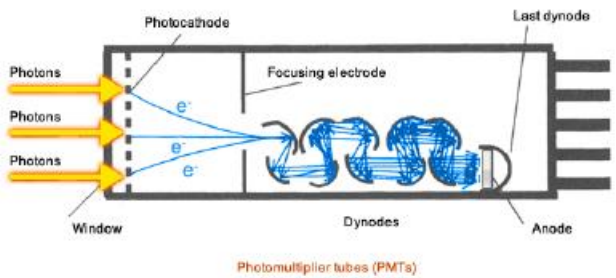
- Number defining the opening angle of an objective
- $NA = nsin\theta$ n = refractive index of immersion medium
- Typically between 0.3 – 1.46 for research microscopes





Turns photons into electrons!

Point detectors i.e. PMTs



Photomultiplier tubes (PMTs)

ammrf.org.au/myscope/confocal/confocal/lasers.php

Detector arrays i.e. cameras

- Arrays of individual sensors 5-20 µm in size
- Each pixel is a semi-conductor (doped silicon)
- Electrons are produced in proportion to the light intensity according to the photoelectric effect.

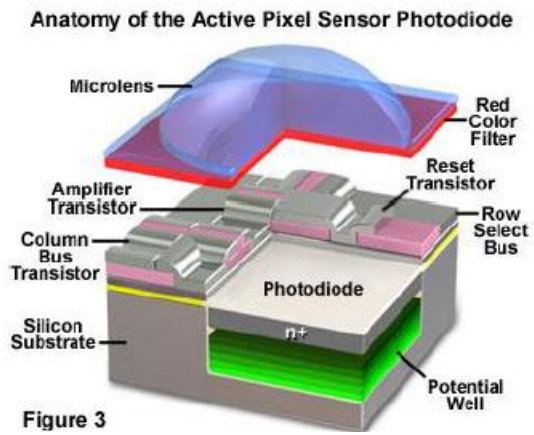


Figure 3

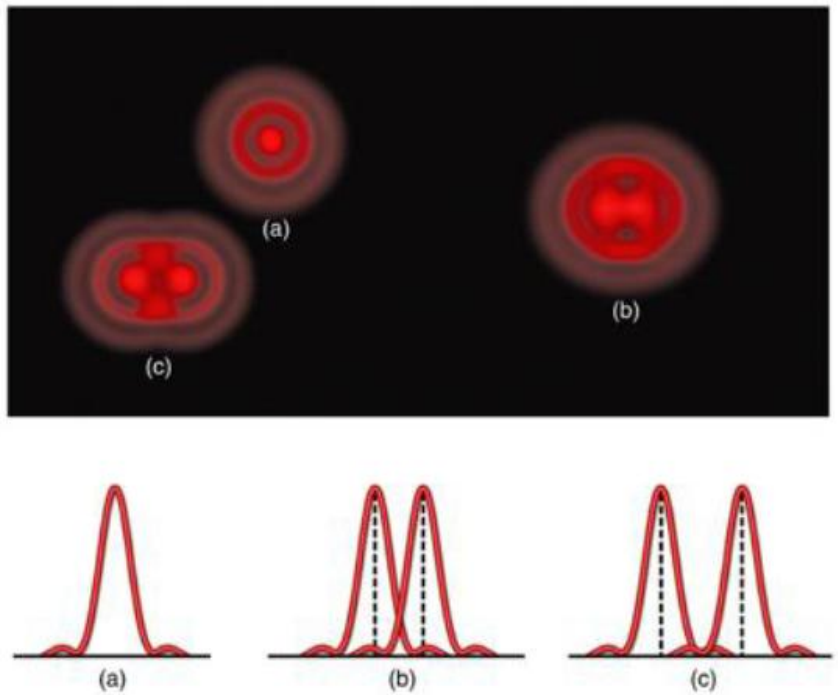


Figure 6.3

Rayleigh criterion for spatial resolution. (a) Profile of a single diffraction pattern: The bright Airy disk and 1st and 2nd order diffraction rings are visible. (b) Profile of two disks separated at the Rayleigh limit such that the maximum of a disk overlaps the first minimum of the other disk: The points are now just barely resolved. (c) Profile of two disks at a separation distance such that the maximum of each disk overlaps the second minimum of the other disk: The points are clearly resolved.

$$r = 0.61 \bullet \lambda \bullet NA^{-1}$$

r is the minimal distance of two closely spaced diffraction spots that can be resolved

2. Different types of light microscope

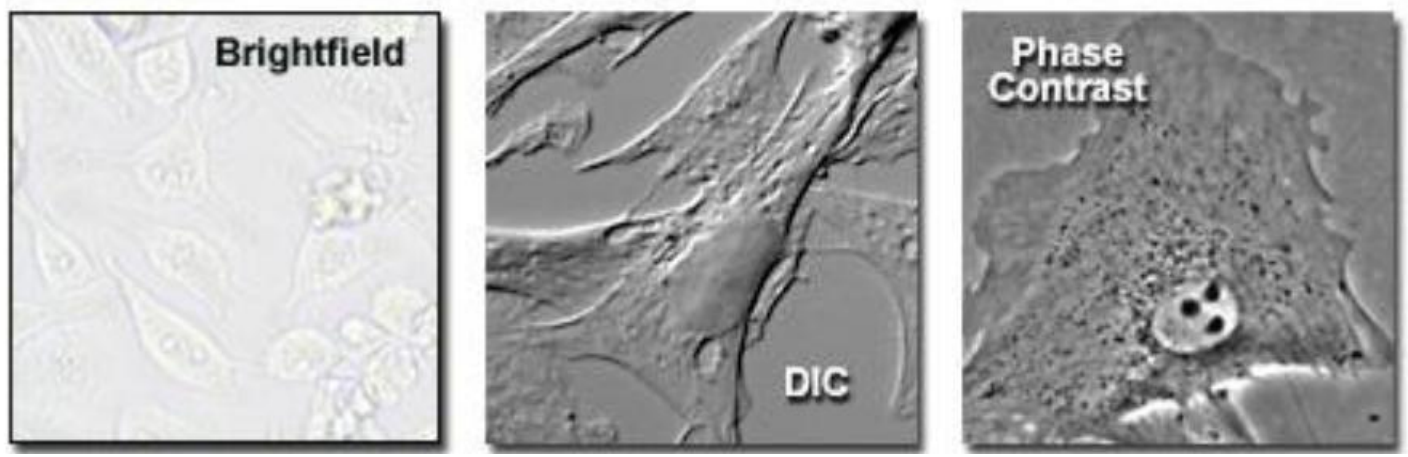
2.1 Brightfield

2.2 Fluorescence microscopy

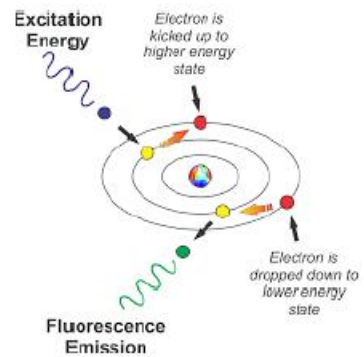
2.3 Volumetric microscopy

2.4 Super-resolution

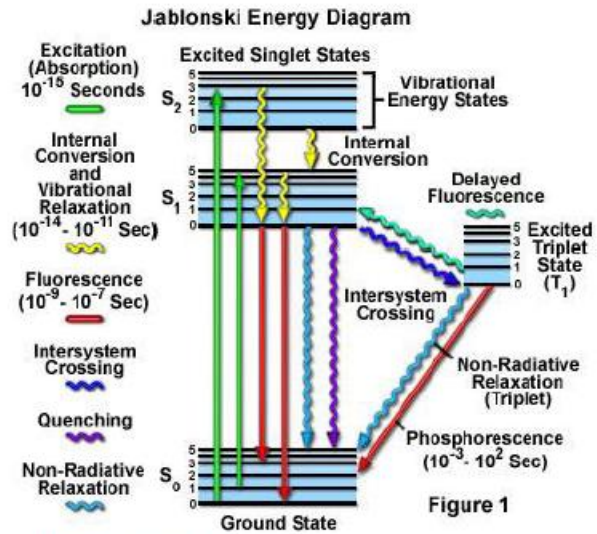
- Cells and other biological samples are quite transparent and difficult to see.
- Turning phase shifts into variations in intensity/amplitude
- Introduction to **phase contrast** and **differential interference contrast**.



An atom/molecule absorbs a photon and an electron is promoted to a higher energy level



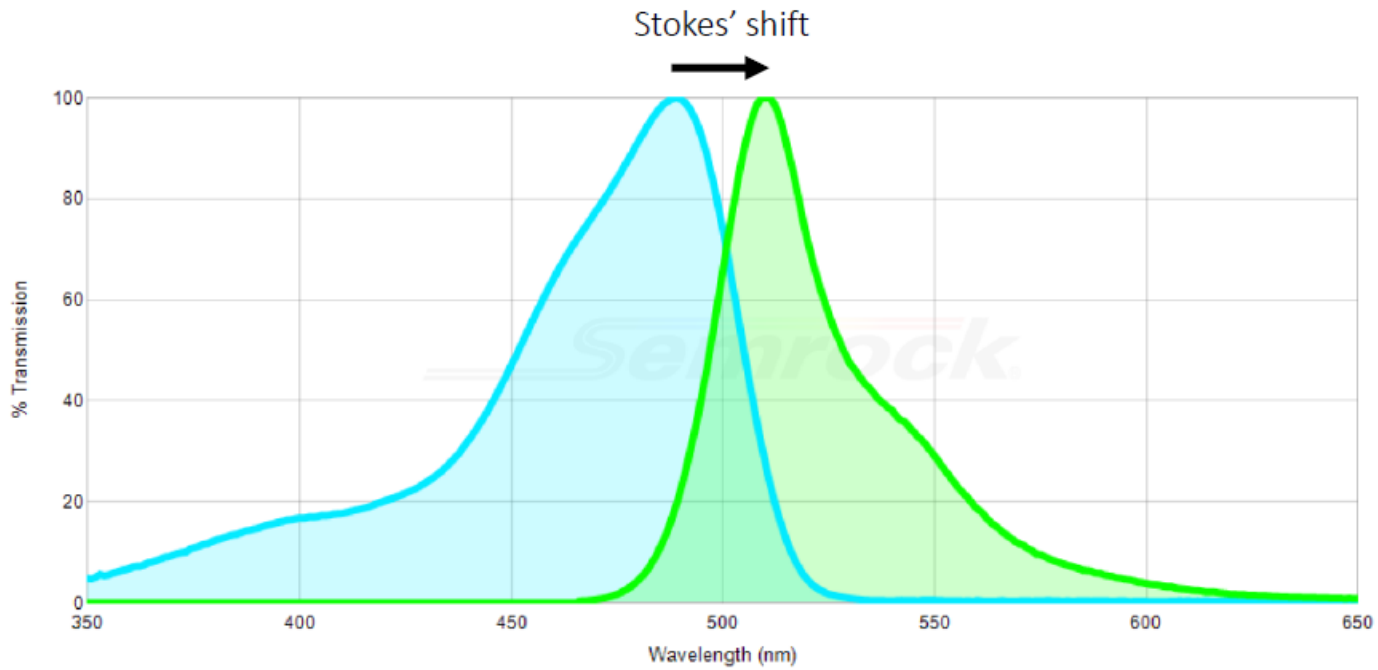
firedivegear.com/the-science-of-fluorescence



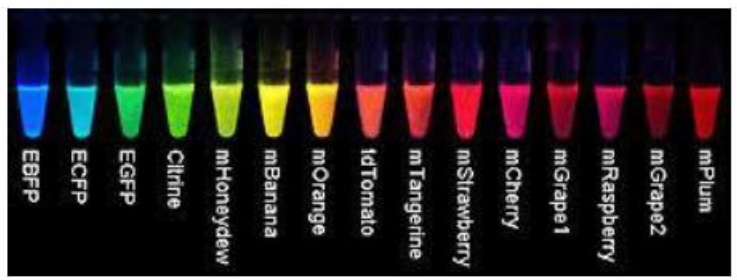
olympus-lifescience.com

Some energy is lost through molecular collisions (internal conversion)

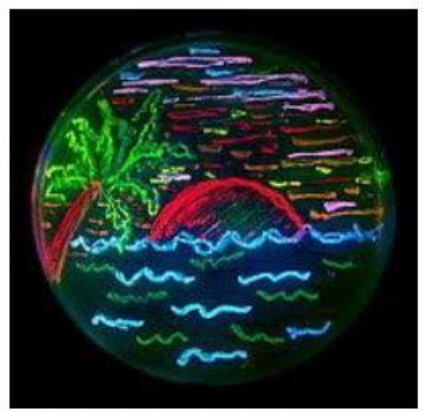
The electron moves back to the ground state, a lower energy (longer wavelength) photon is emitted



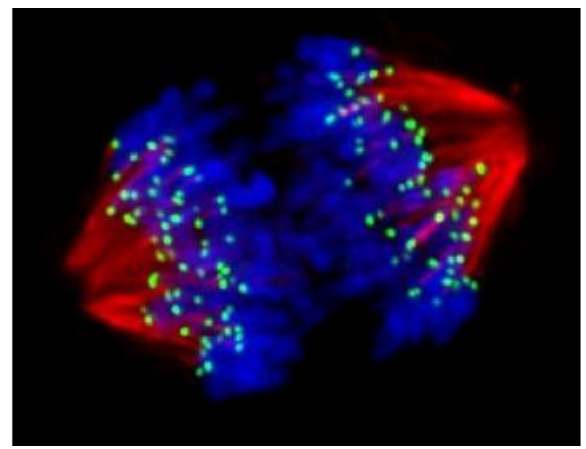
Excitation/emission spectrum of green fluorescent protein (GFP)



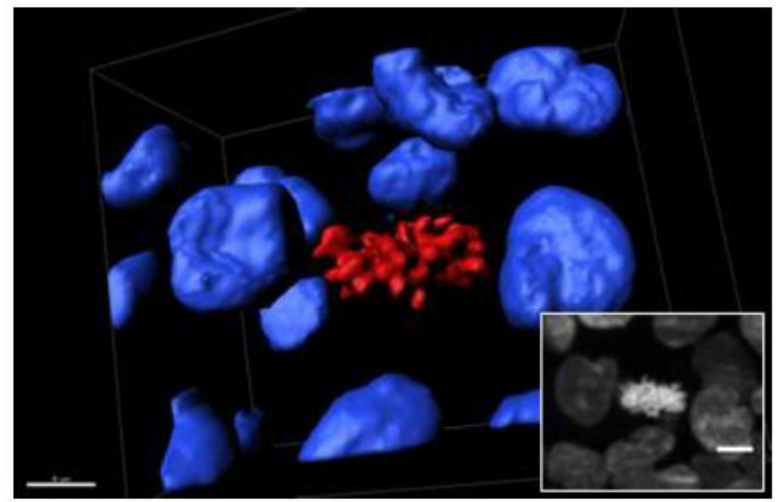
www.conncoll.edu



Wikipedia

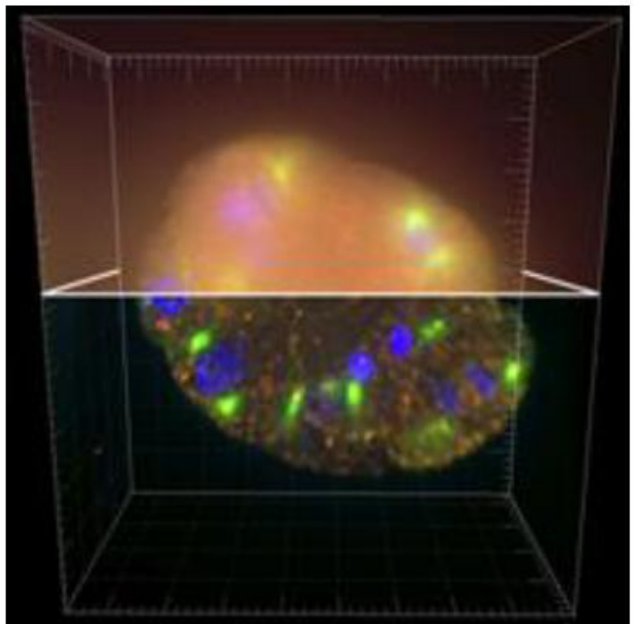


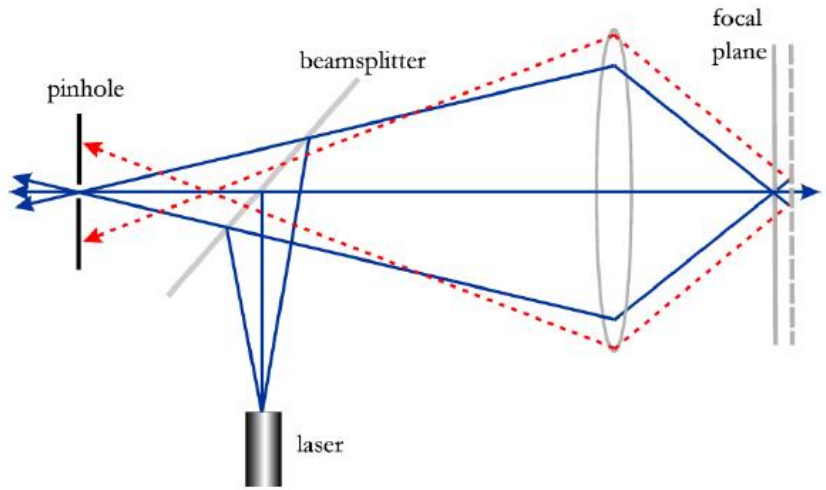
- Biology happens in 3D!
- Microscope objectives collect light from above and below the focal plane
- Better NA -> better sectioning
- Microtomes can be used for thick tissue
- We need optical methods to improve optical sectioning to build up volumetric information



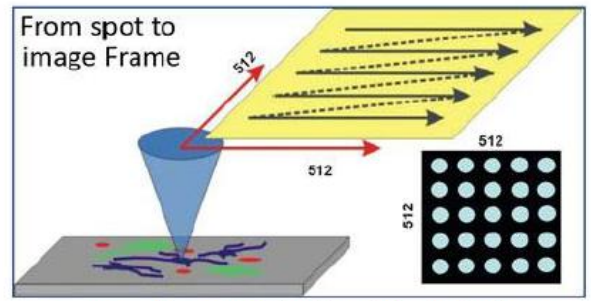
www.bitplane.com

- Computationally restore light back to the correct plane
- Improves SNR and axial resolution
- Several methods, the more computationally intensive, generally the better
- Can cause artifacts

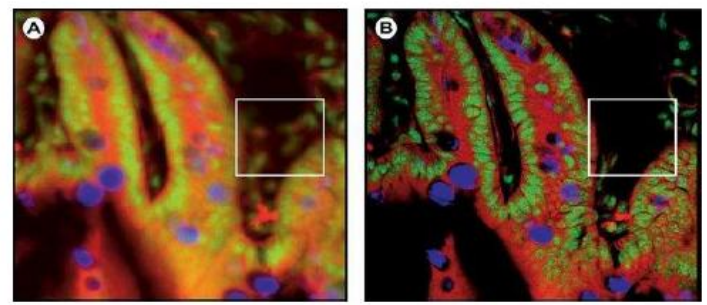




li155-94.members.linode.com/myscope/images/confocal/confoc1.png



microscopist.co.uk/wp-content/uploads/2017/04/confocal-working.jpg



Ishikawa-Ankerhold et al, Molecules 17(4):4047-132 (2012)

Several point-scanning confocals available through the SLS Imaging Suite