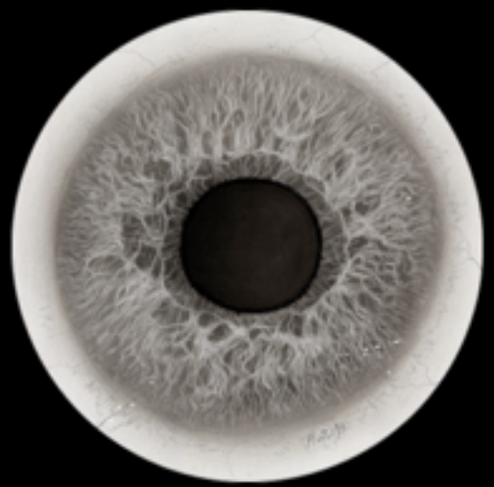
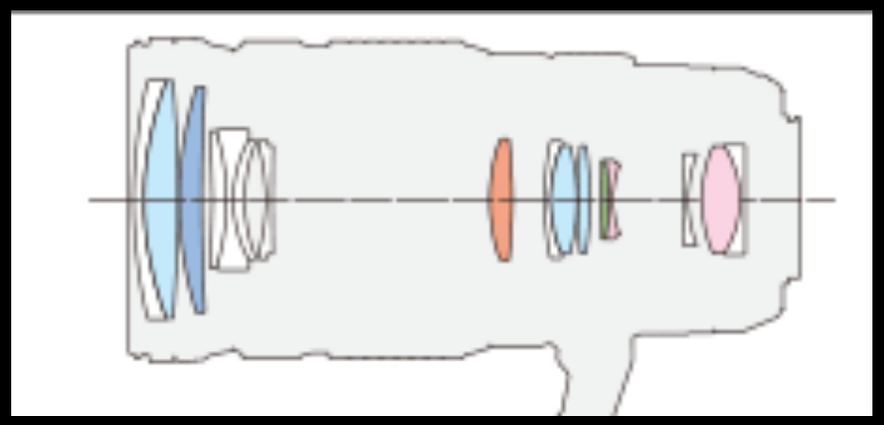
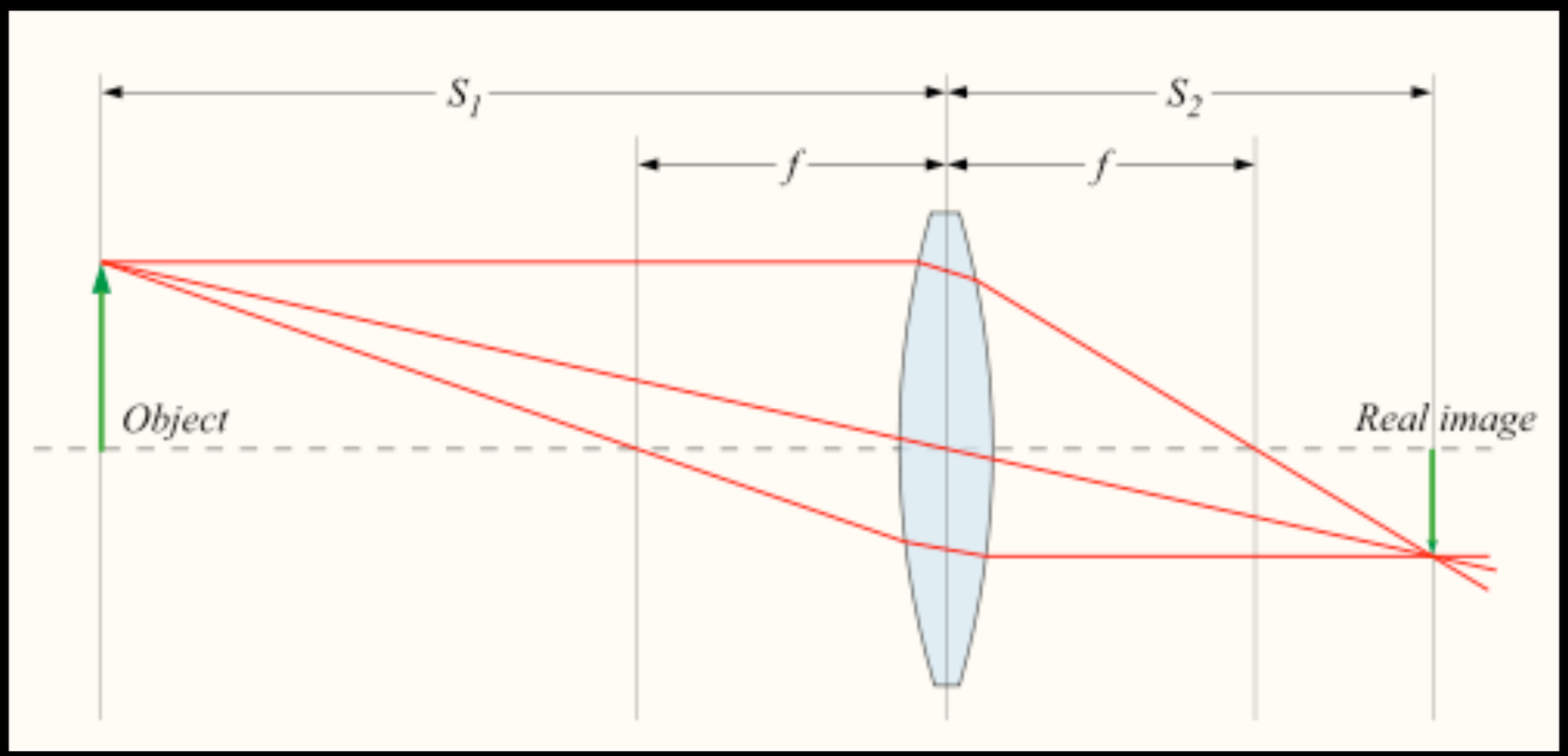
Recap from Tuesday: Imaging with a “single lens”







Brief note on:

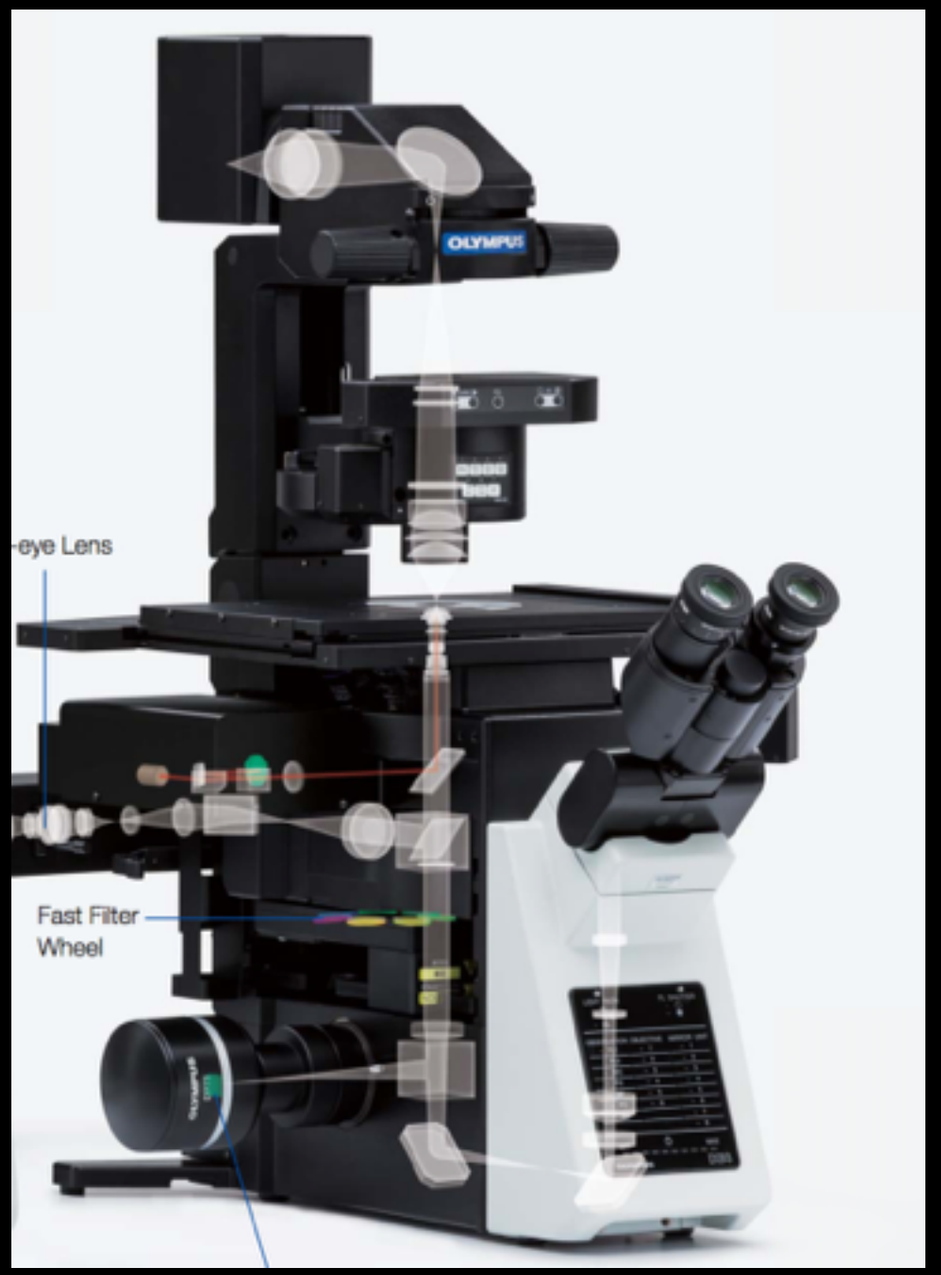
Optical lens design stuff

- If you are interested in this, there are commercial week-long classes from the optical design softwares Zemax and Code V

- I have taken two Zemax classes, they were pretty useless. A lady I met - who designs riflescopes - recommended the **Code V** classes instead.

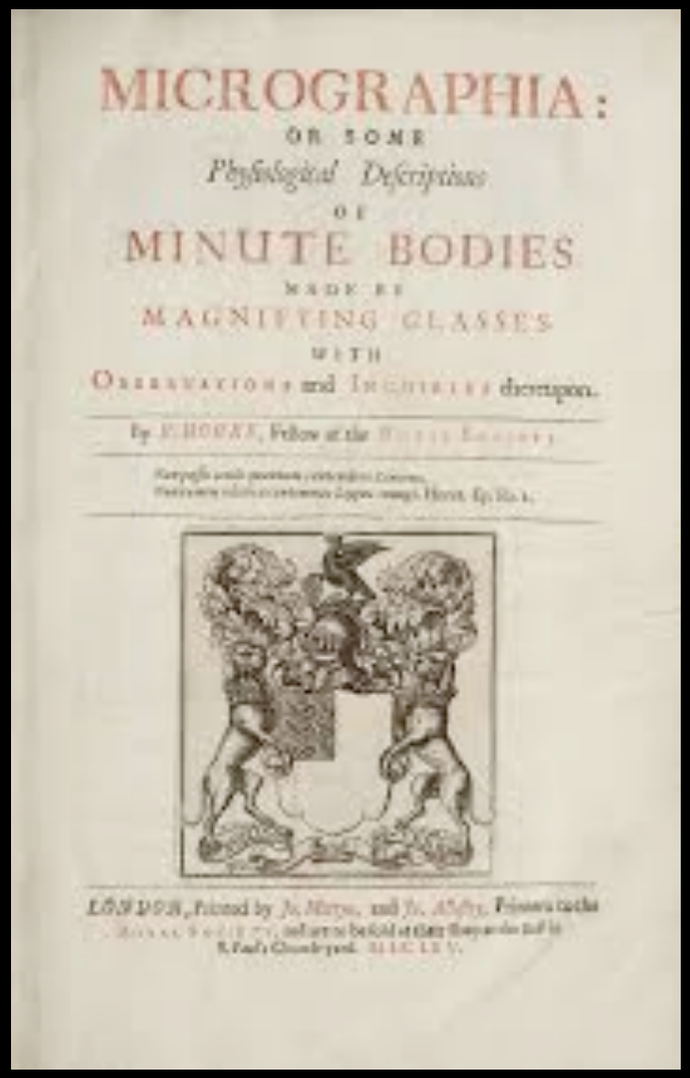
- Jobs in optical systems design in Silicon Valley are according to my friends there available in various **illumination design appliations**

Microscopy



Anton van Leeuwenhoek (1632-1723)

Micrographia



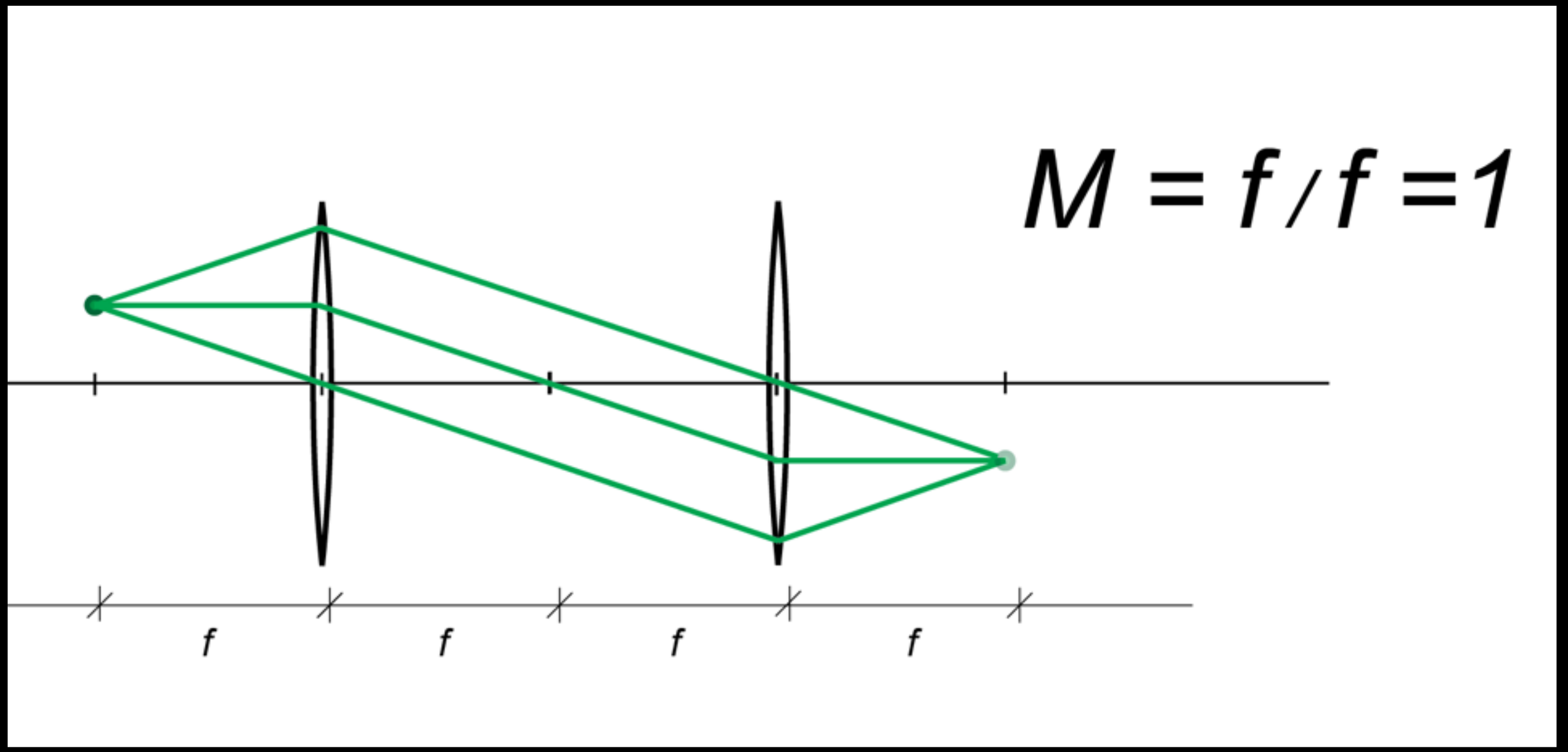
MICROGRAPHIA:
OR SOME
Physiological Descriptions
OF
MINUTE BODIES
MADE BY
MAGNIFYING GLASSES
WITH
OBSERVATIONS and INQUIRIES thereupon.

By R. HOOKE, Fellow of the ROYAL SOCIETY

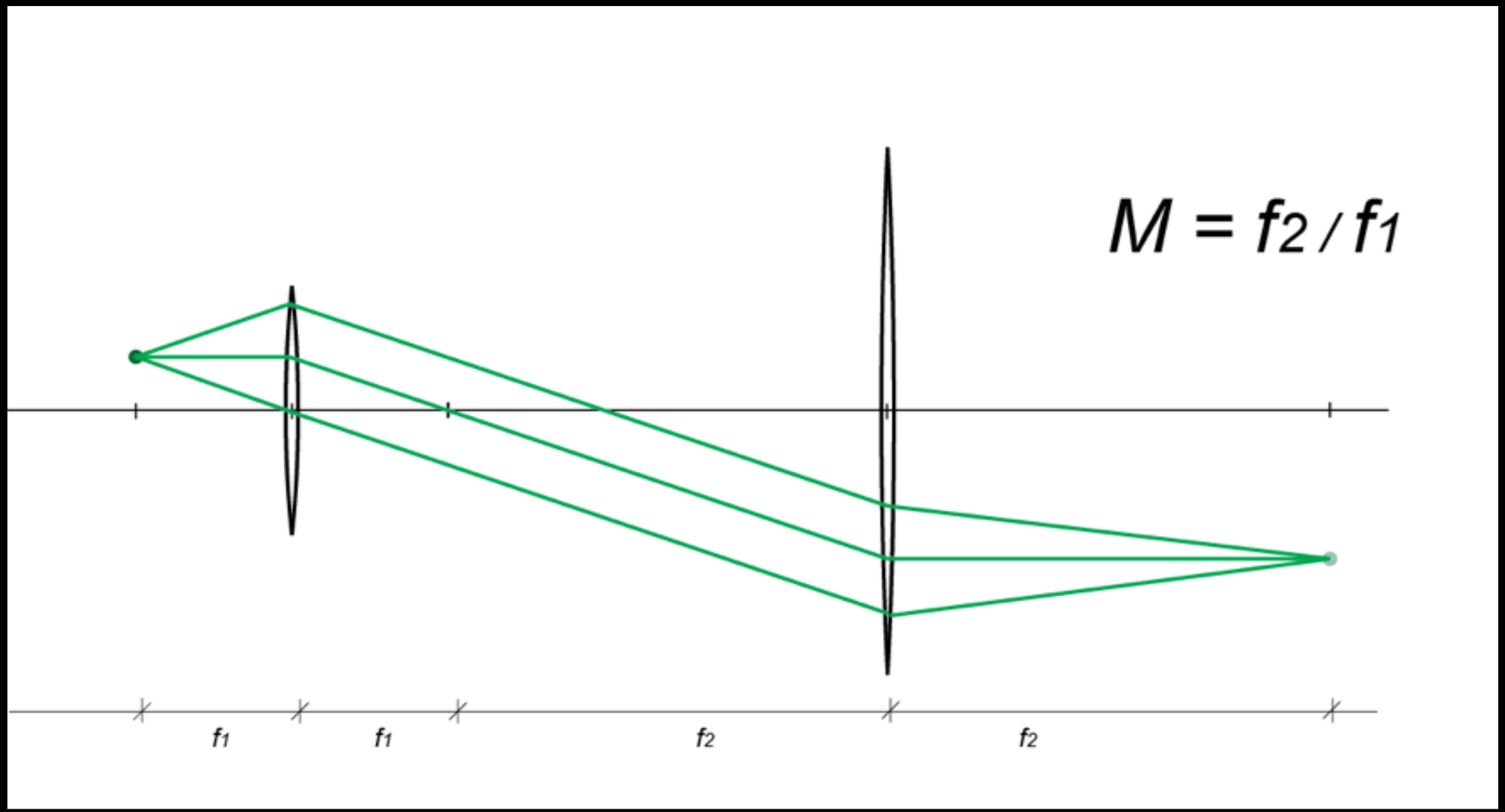
LONDON, Printed by Jo. Martyn, and Ja. Allestry, Printers to the ROYAL SOCIETY, and are to be sold at their Shop under the Inside of S. Paul's Church-yard. MDCLXV.

Robert Hooke published Micrographia in 1665. Stunning illustrations

Imaging with two lenses



Magnify!



Simple geometrical proof that this is M with like-sided triangles

Microscope:

Objective

Tube Lens

f_1 f_1 f_2 f_2

Tube Lens ~ Objective

$$M = f_2 / f_1$$

- Different brands (Olympus, Nikon, Zeiss, Leica) have different Tube Length (=tube lens focal length)

- Example: You want a 100x objective.
  Your Tube Lens has $f_{\text{T.L.}}$ = 200 mm =>
  $f_{\text{objective}}$ = 200mm/100 = **2mm**

The Objective

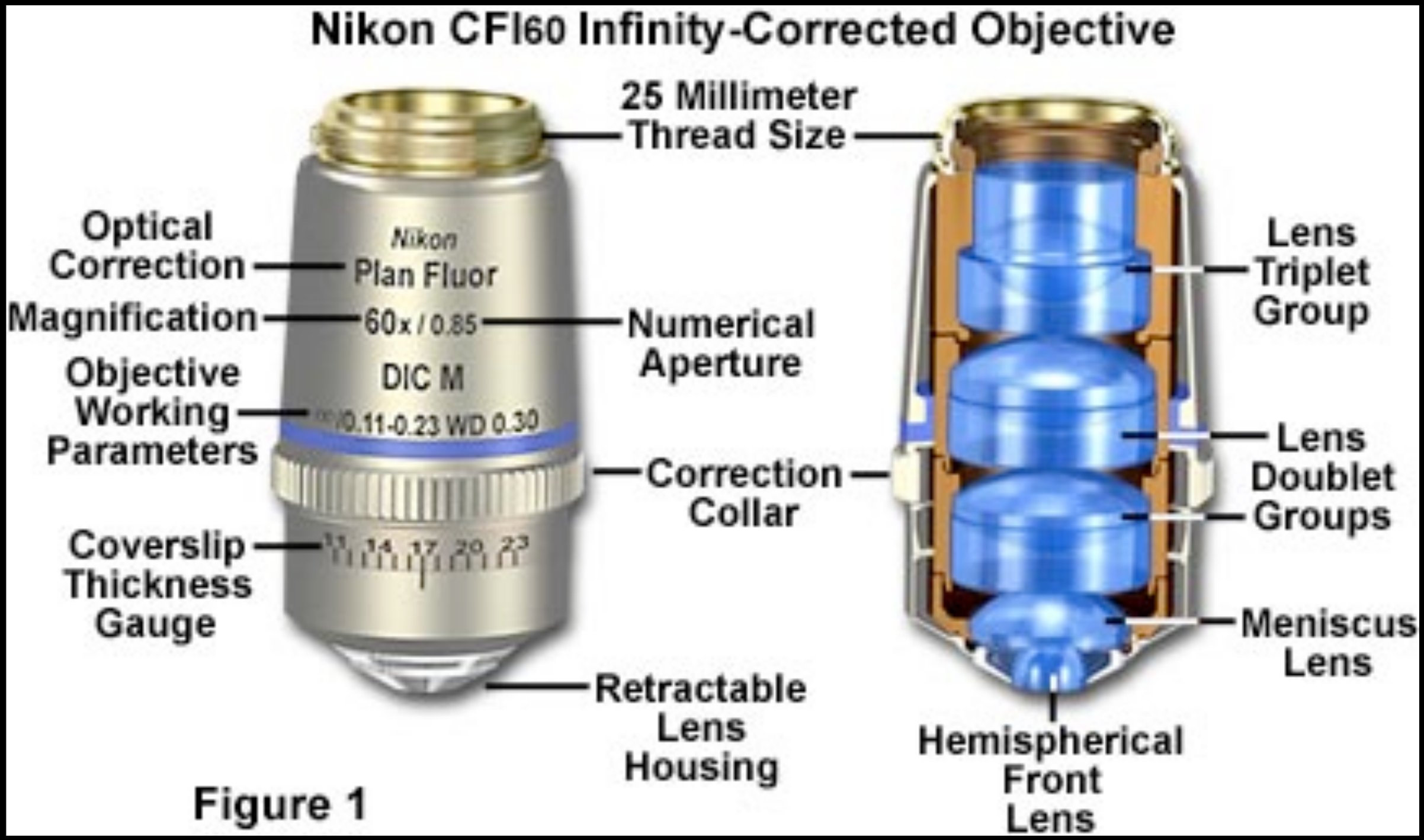
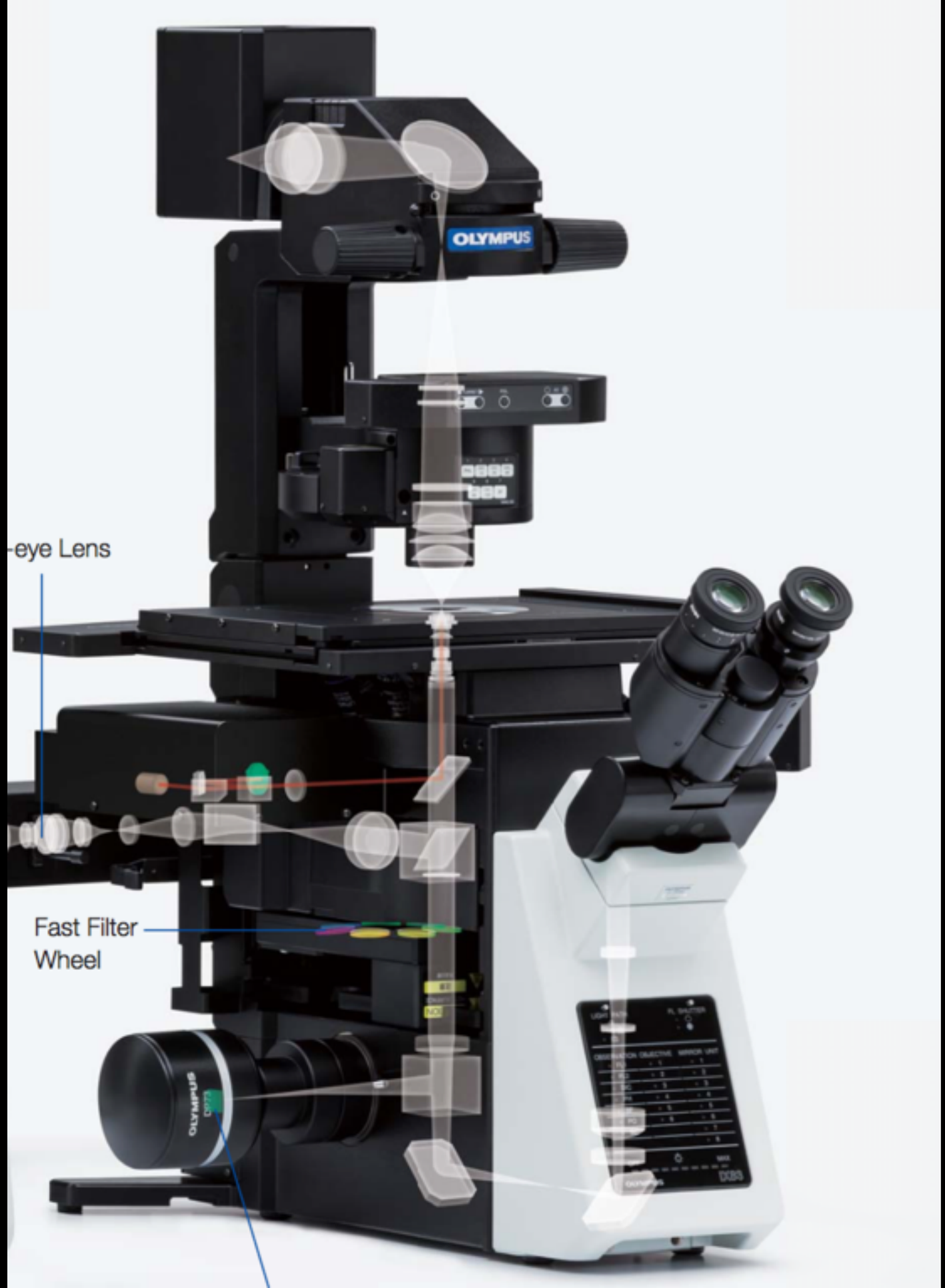
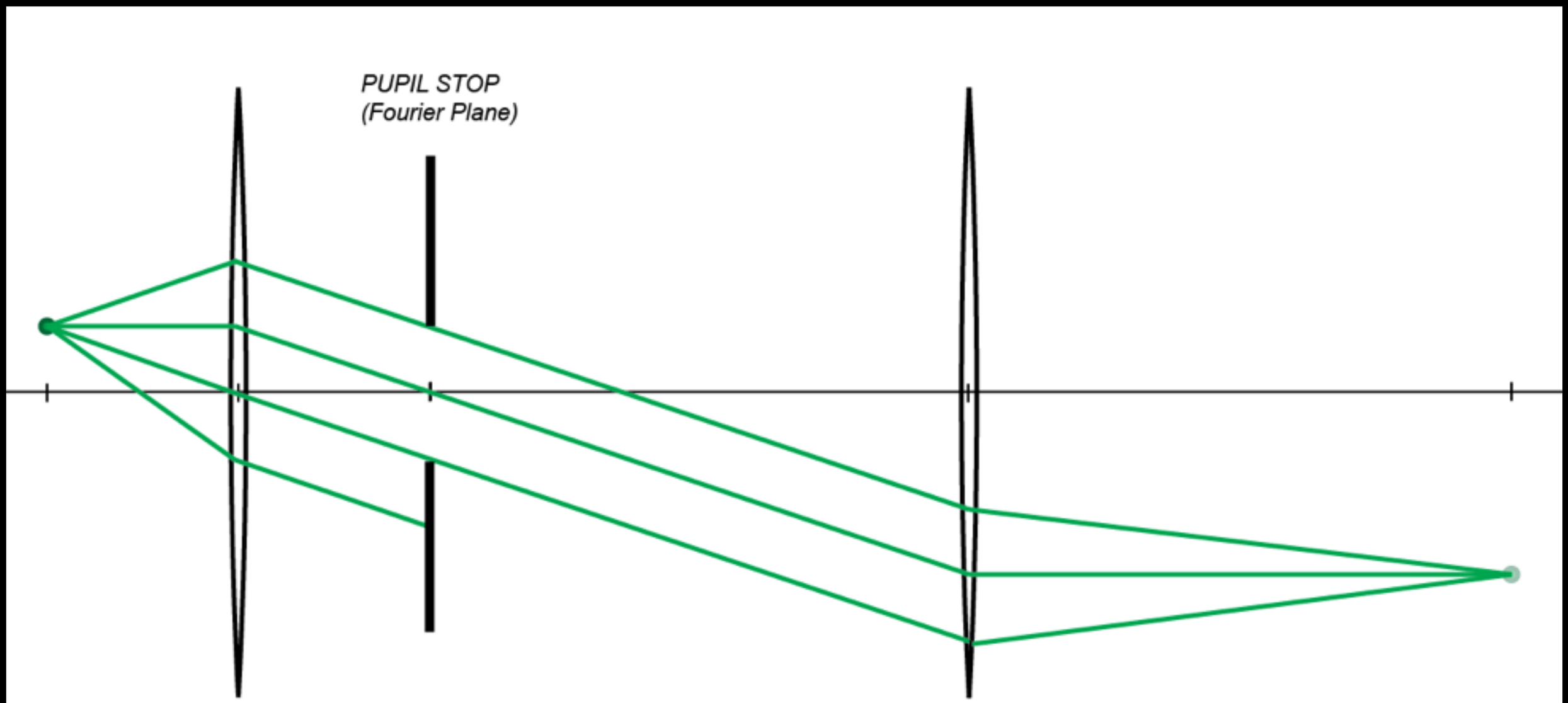


Figure 1

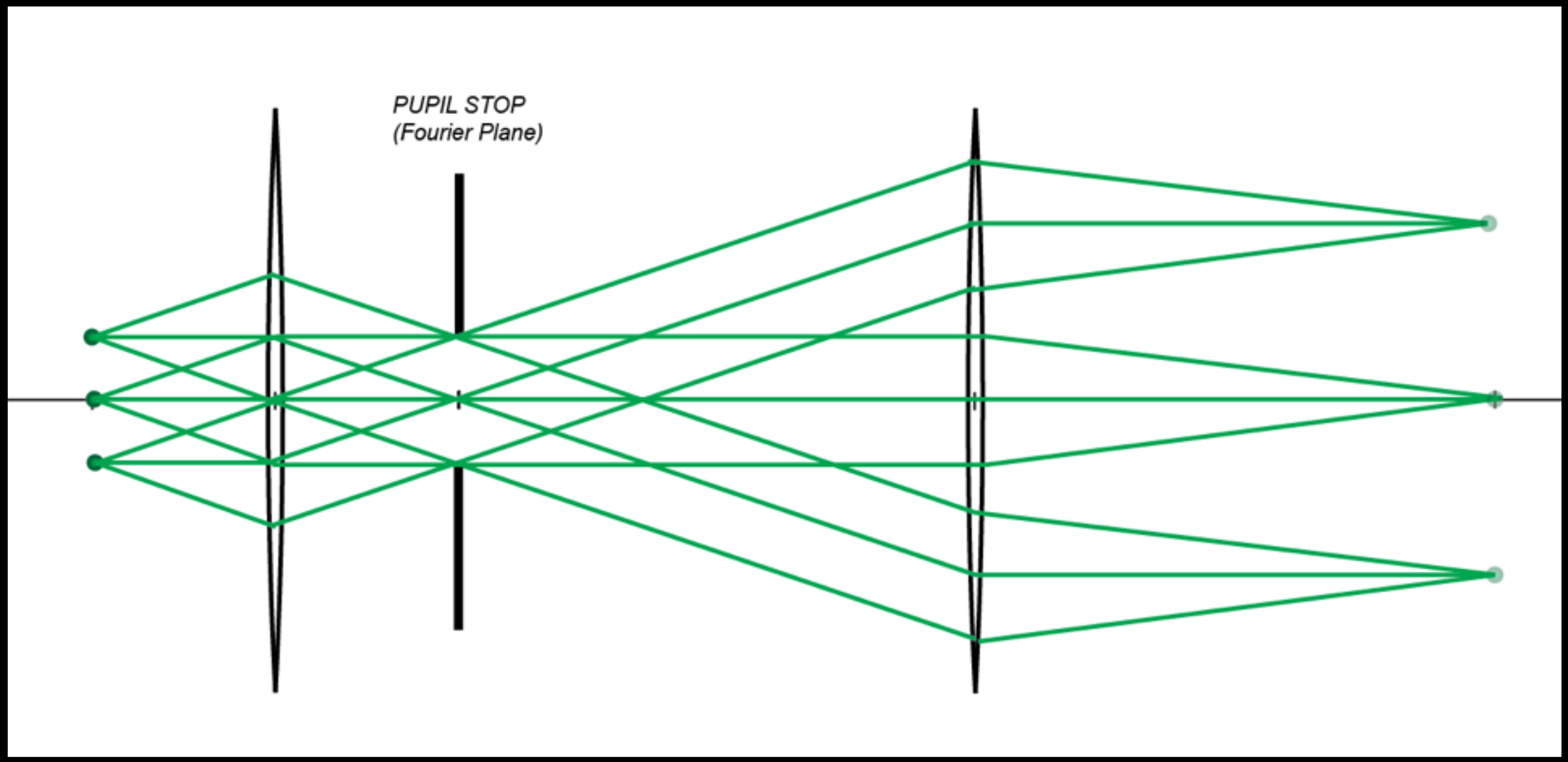

-eye Lens
Fast Filter
Wheel
OLYMPUS

Pupil Stop



Pupil stop determines Numerical Aperture (NA)

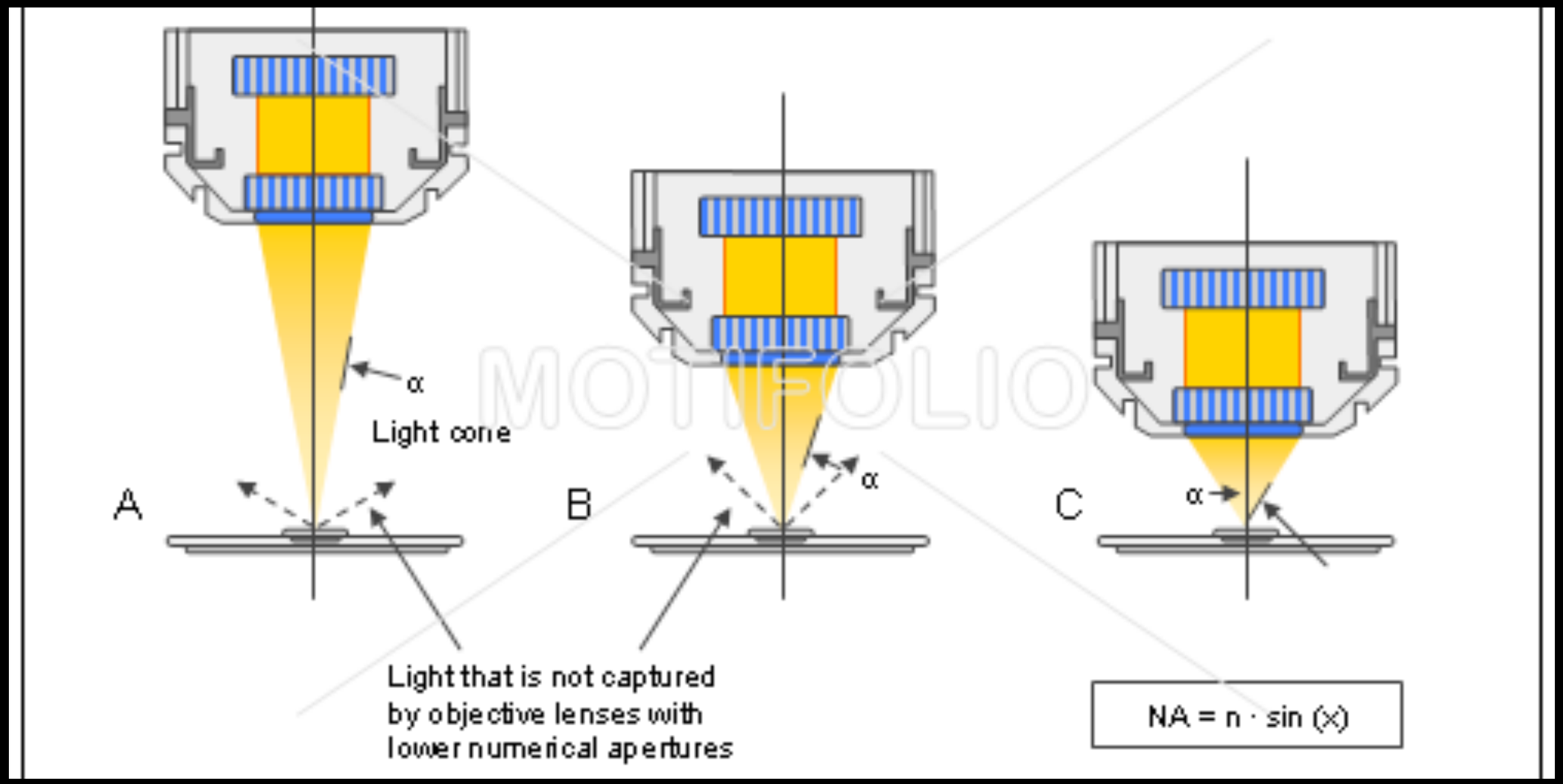
Lets add some rays



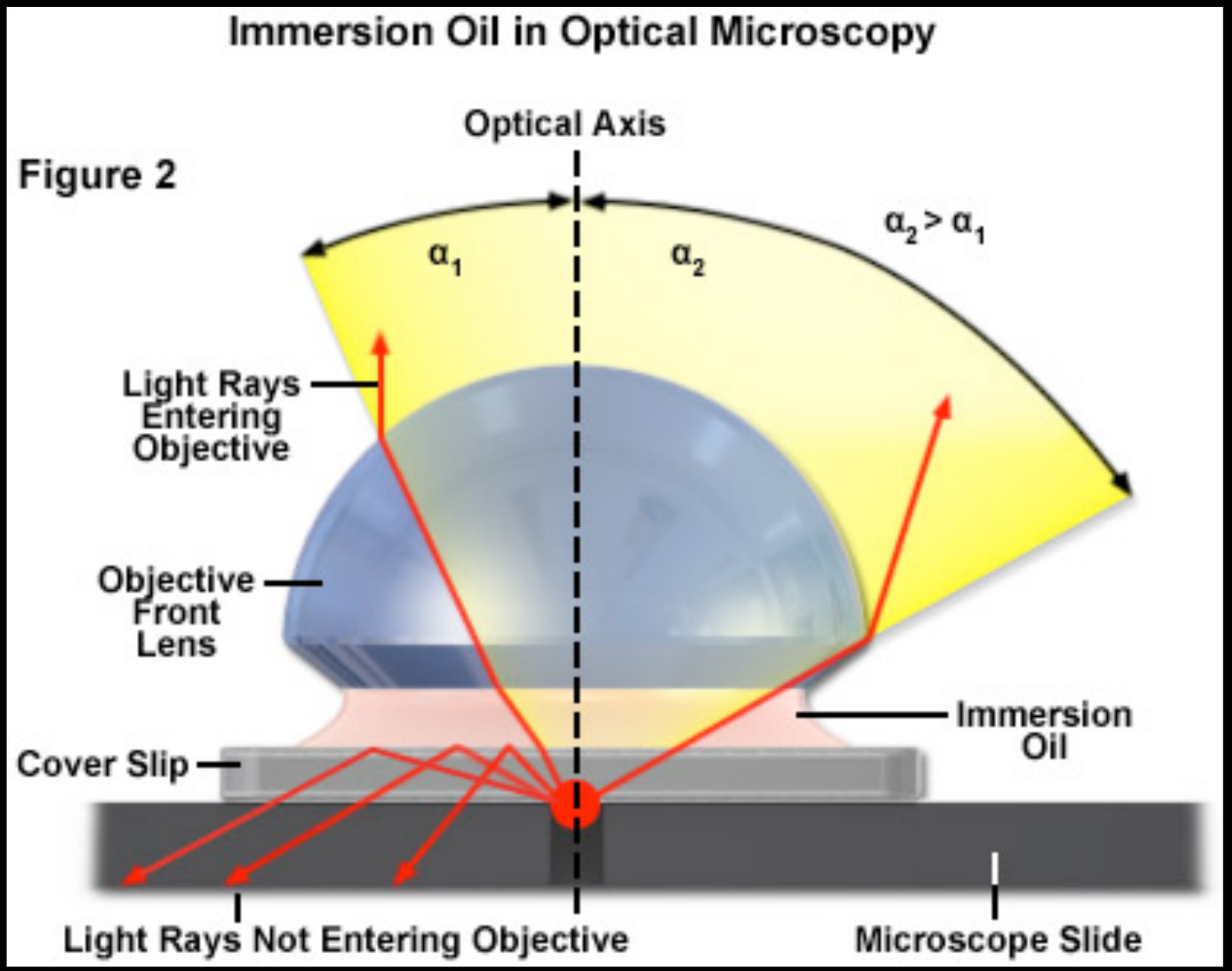
Now we start seeing the special properties of the Fourier plane

NA = n sin(a)

- n = refractive index of immersion medium
- a = half angle of light acceptance angle



Immersion Medium



Match to Specimen!

- Air: n = 1
- Water: n = 1.33
- High refractive index Oil: n = 1.515
- Glycerol / Silicon oil n = 1.4

Distortion in Aqueous Media

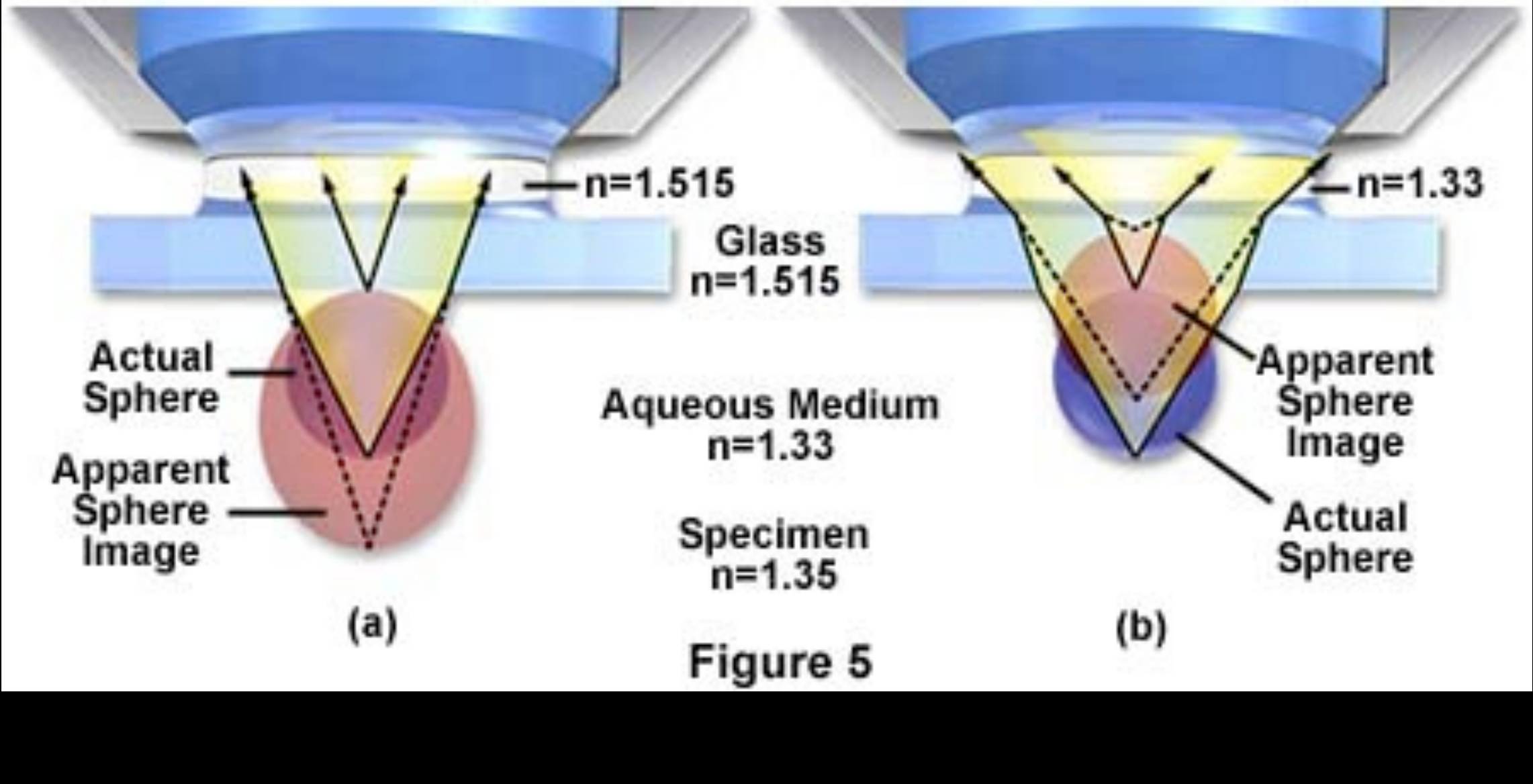
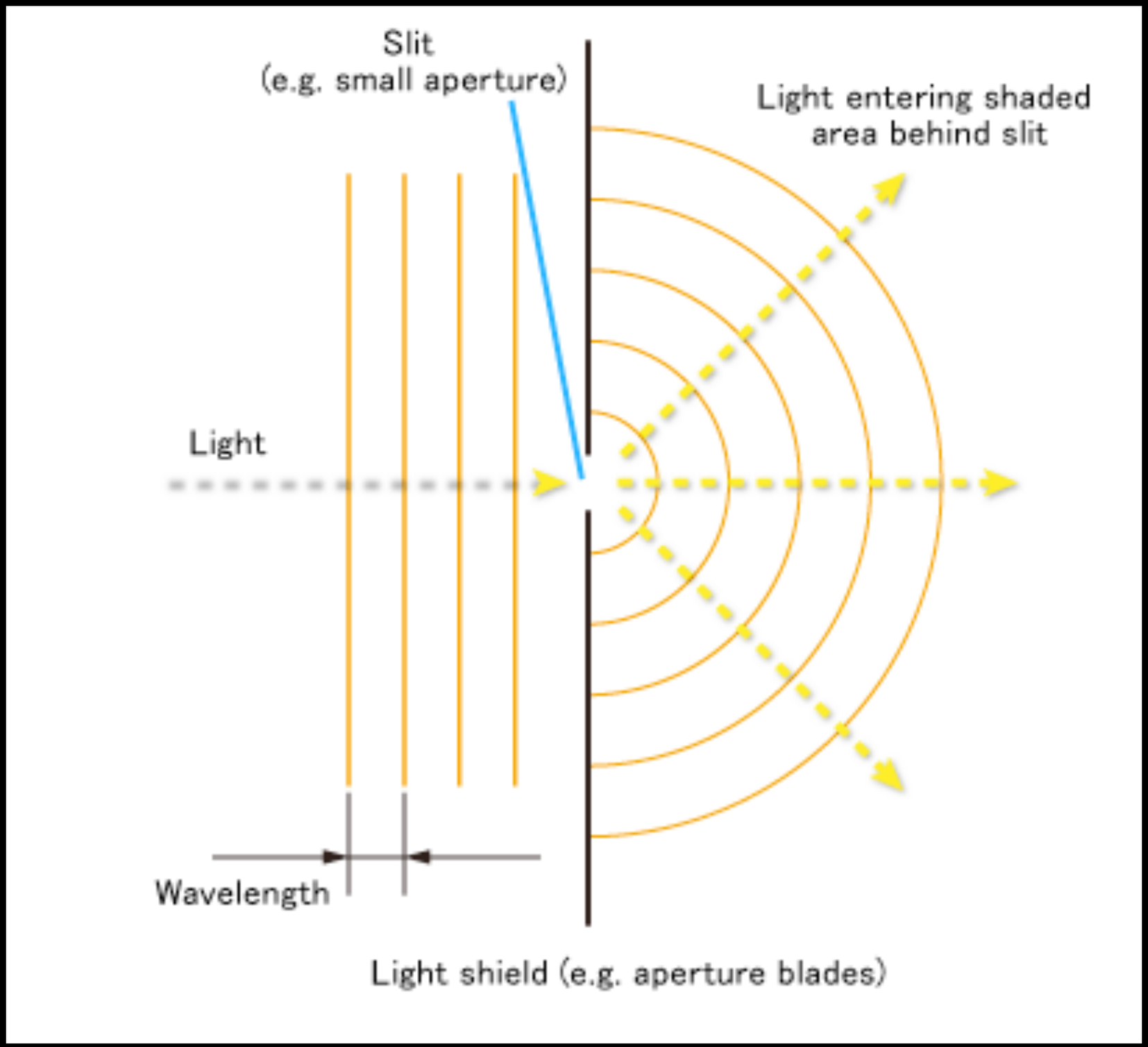


Figure 5

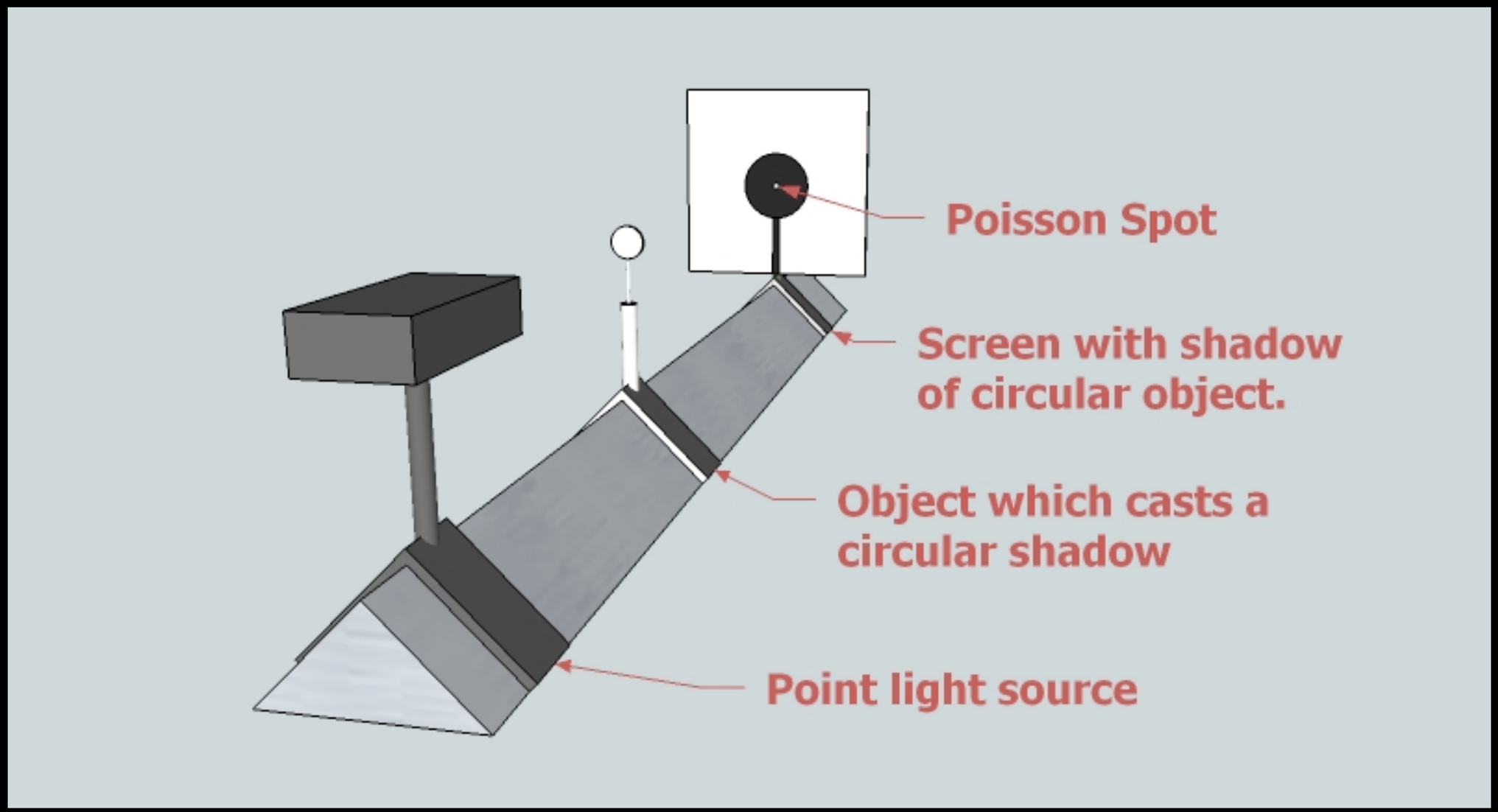
Diffraction



Ripple Tank

https://www.youtube.com/watch?v=-8a61G8Hvi0

Poisson's spot



Light ripples and bends around edges too

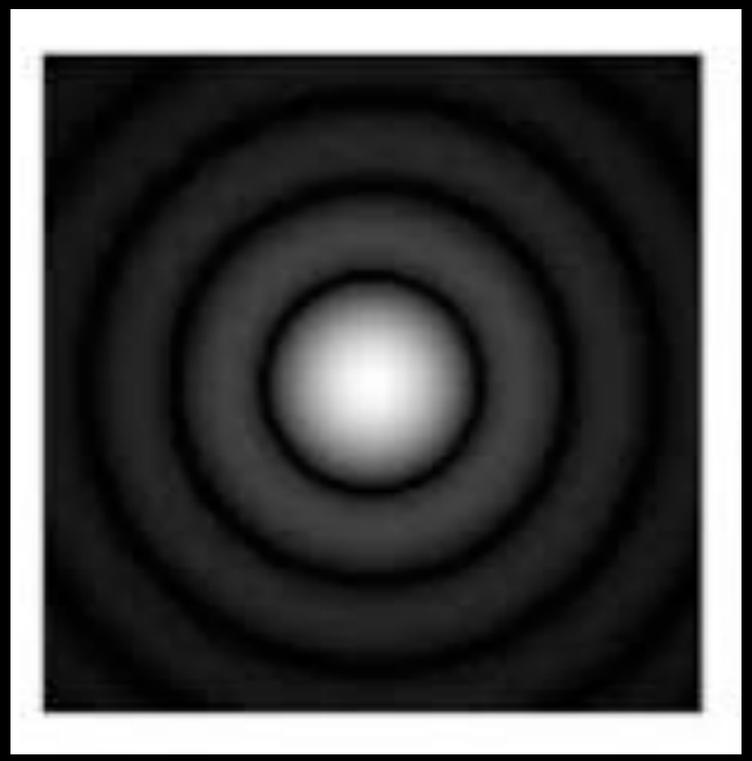
Fresnel Diffraction

Huygens' principle models that every point in the plane of the obstacle acts as a new point source of light.

The light coming from points on the circumference of the obstacle and going to the center of the shadow travels exactly the same distance, so all the light passing close by the object arrives at the screen in phase and constructively interferes.

This results in a bright spot at the shadow's center, where geometrical optics and particle theories of light predict that there should be no light at all.

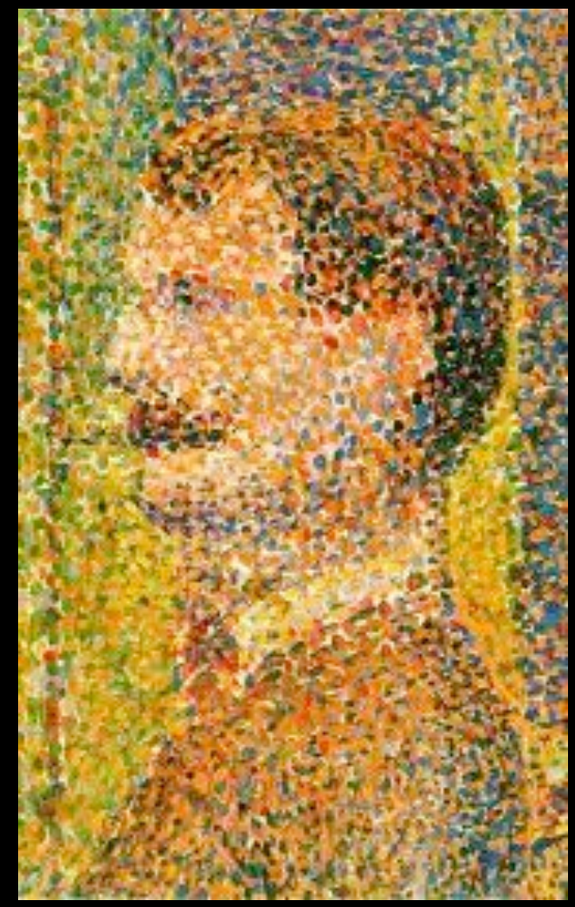
The Airy Disc



Arises from diffraction when we image through a circular aperture

Imaging Point Sources

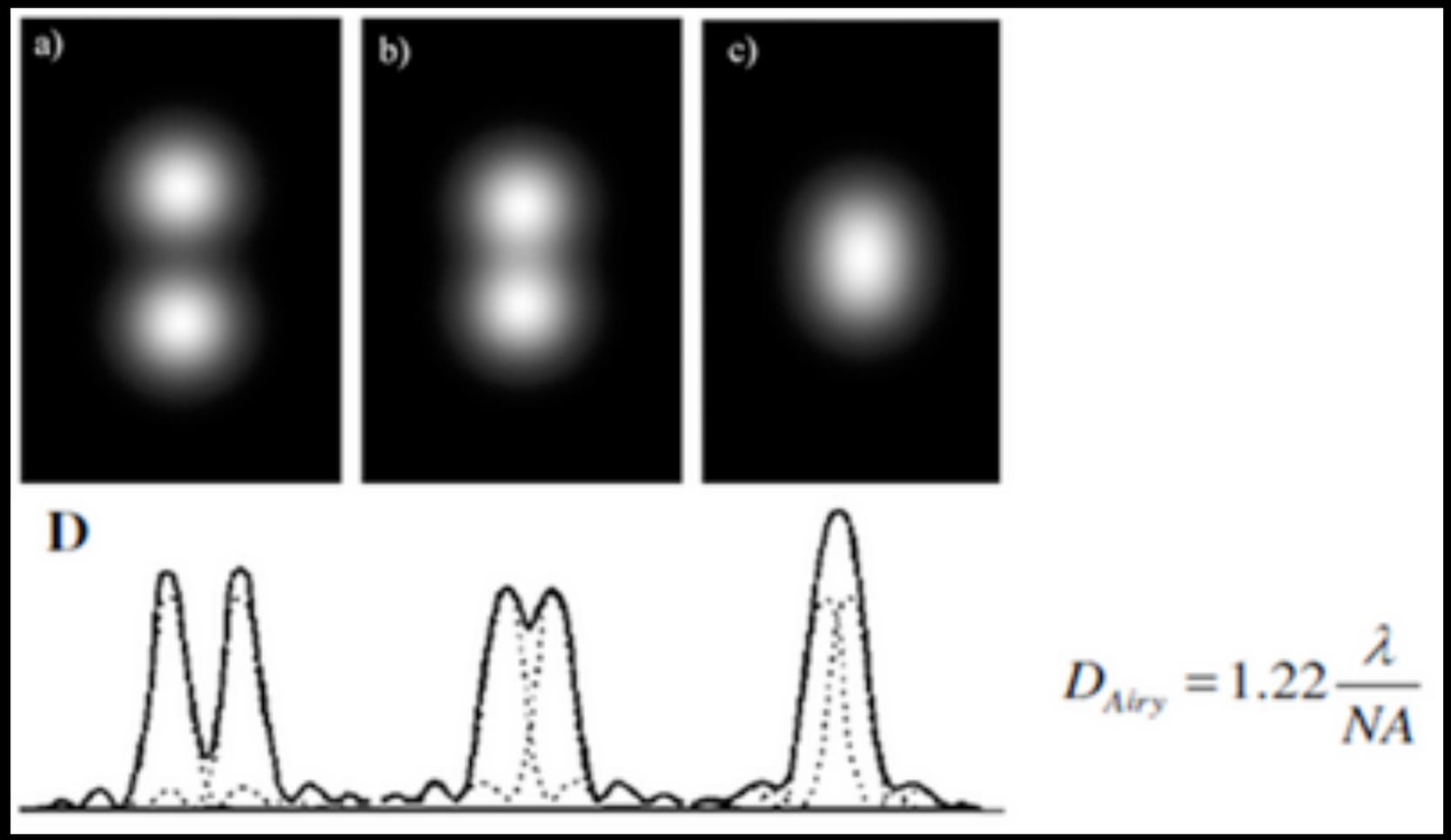
- The object can be modeled as a number of point sources
- The points are blurred by the imaging system
  (The response function of a point imaged through a circular aperture is the Airy disc)



(from Seurat's *the Circus*)

Resolution criteria:

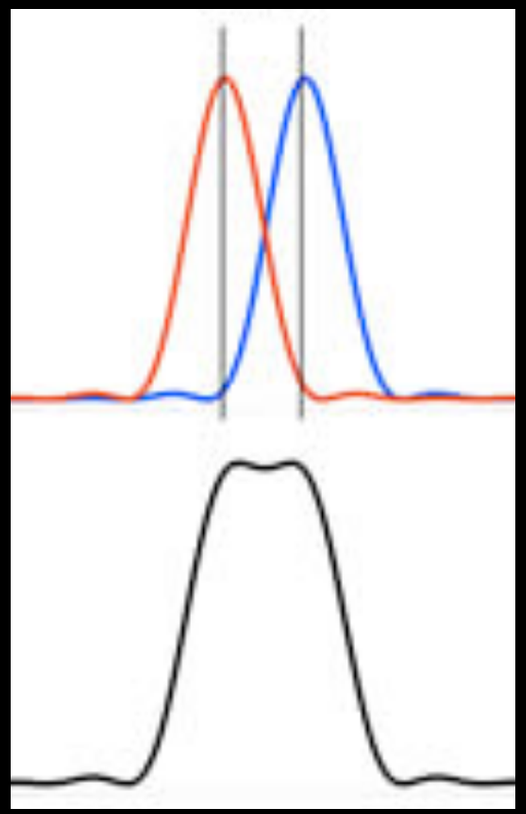
- Raleigh
- Sparrow
- Airy
- Abbe



Abbe Limit of Resolution

$$d = \lambda / (2 \times NA)$$

Lateral resolution is classically limited by diffraction to ~200nm
(determined by Numerical Aperture NA and wavelength)



Example for green light with high NA objective: $d = (550\ nm) / (2 \times 1.4) \approx 200\ nm$

Assumptions

- Limited NA
- Uniform Illumination
- Linearity

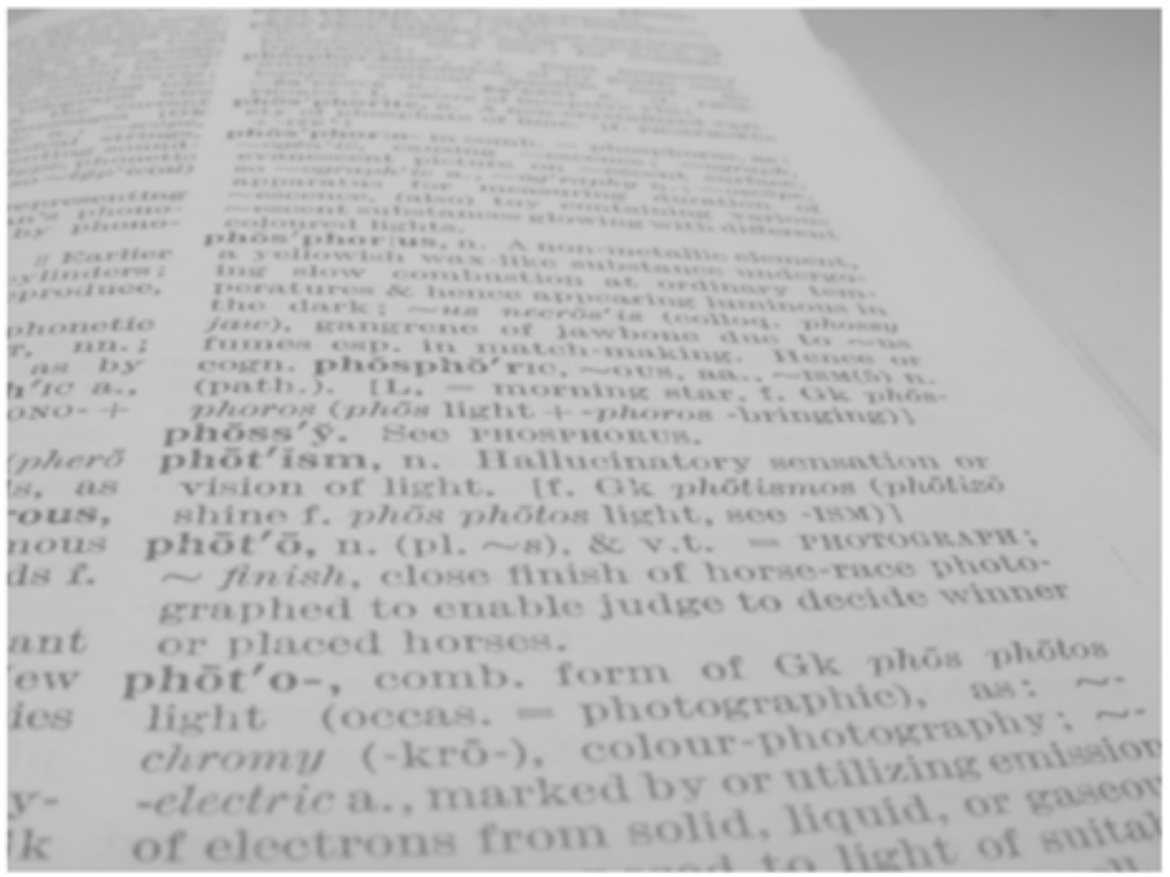
Resolution

High resolution

Low resolution

Contrast

High contrast



Low contrast

Modulation Transfer Function

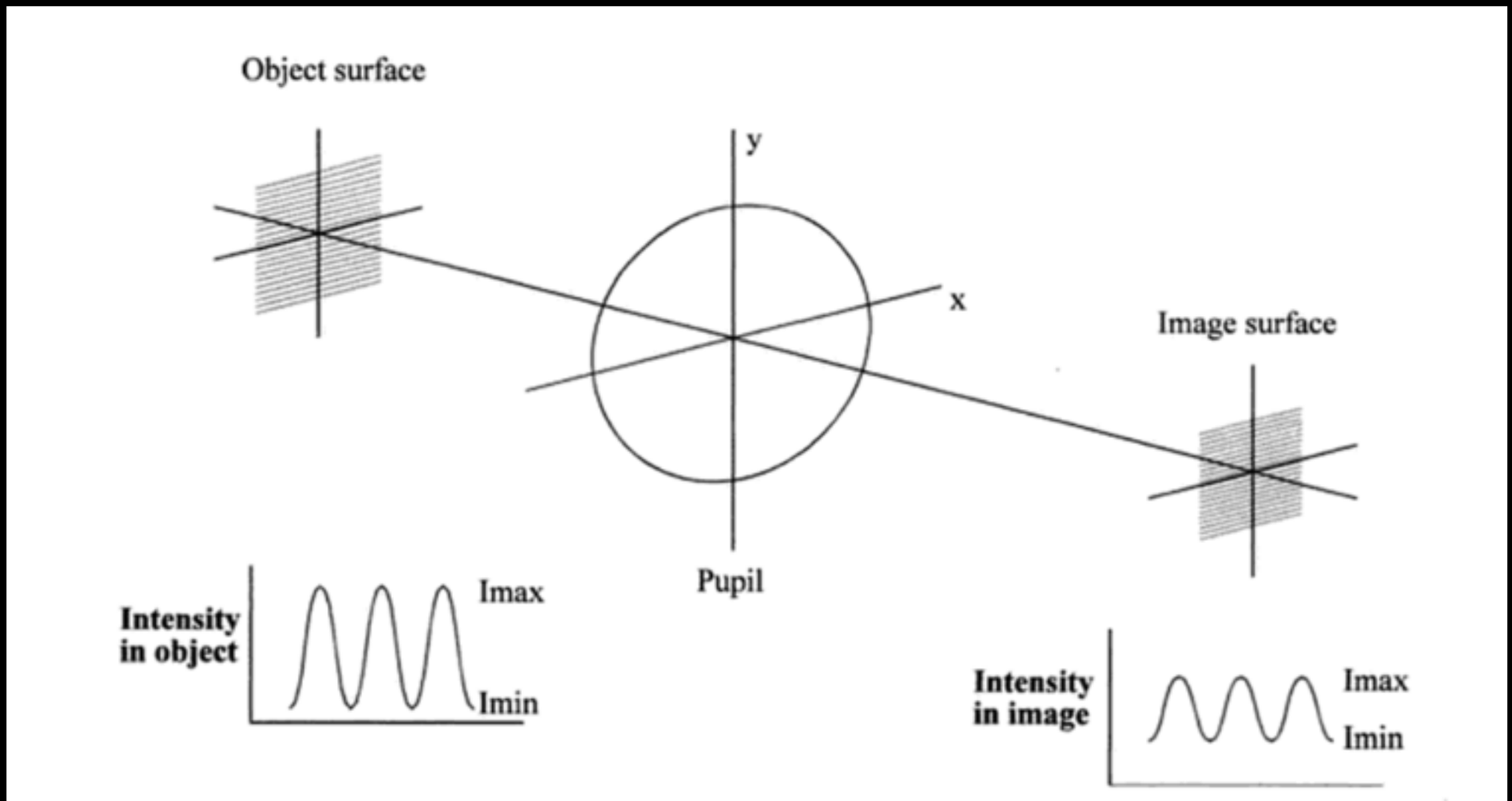


Figure 4.23. Object and image contrast.

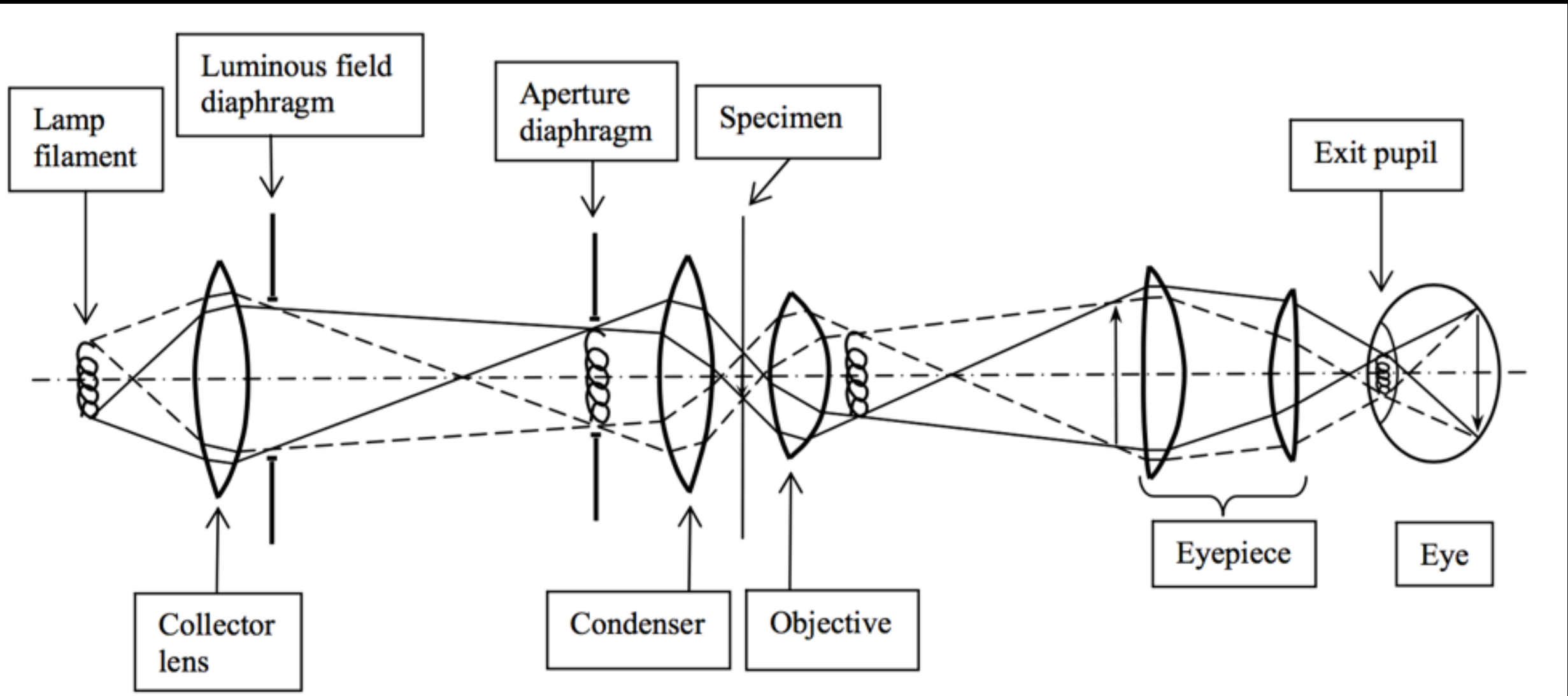
Break Time

Fluorescence *vs.* not

- Shining light onto / through a specimen to see the effects of absorption, reflection, diffraction etc

- Shining light onto a fluorescent protein and imaging the light emanating from it

Köhler Illumination



Two conjugate planes exist here: the Pupil Stop and the Field Stop

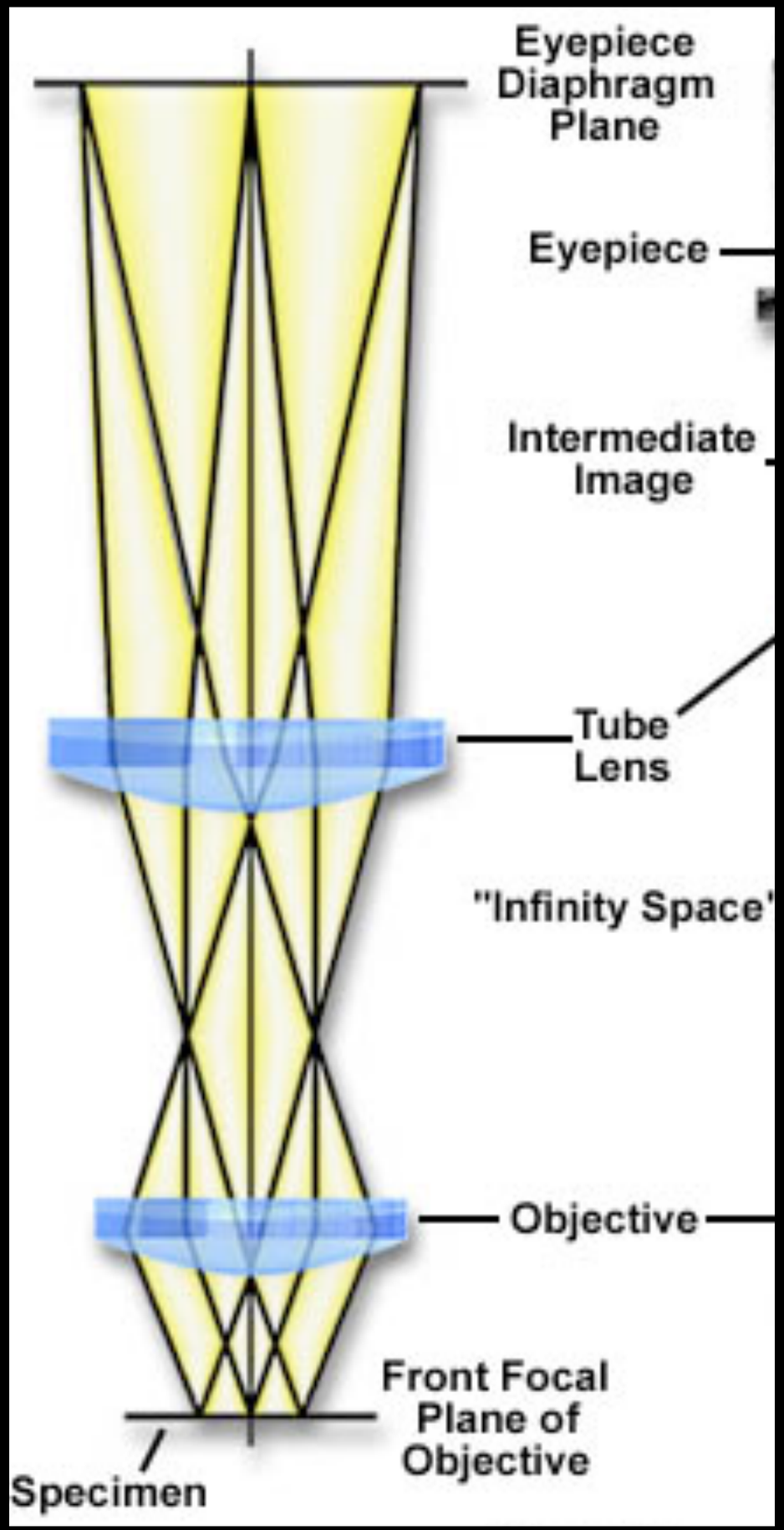
Field Stop limits the Field of View

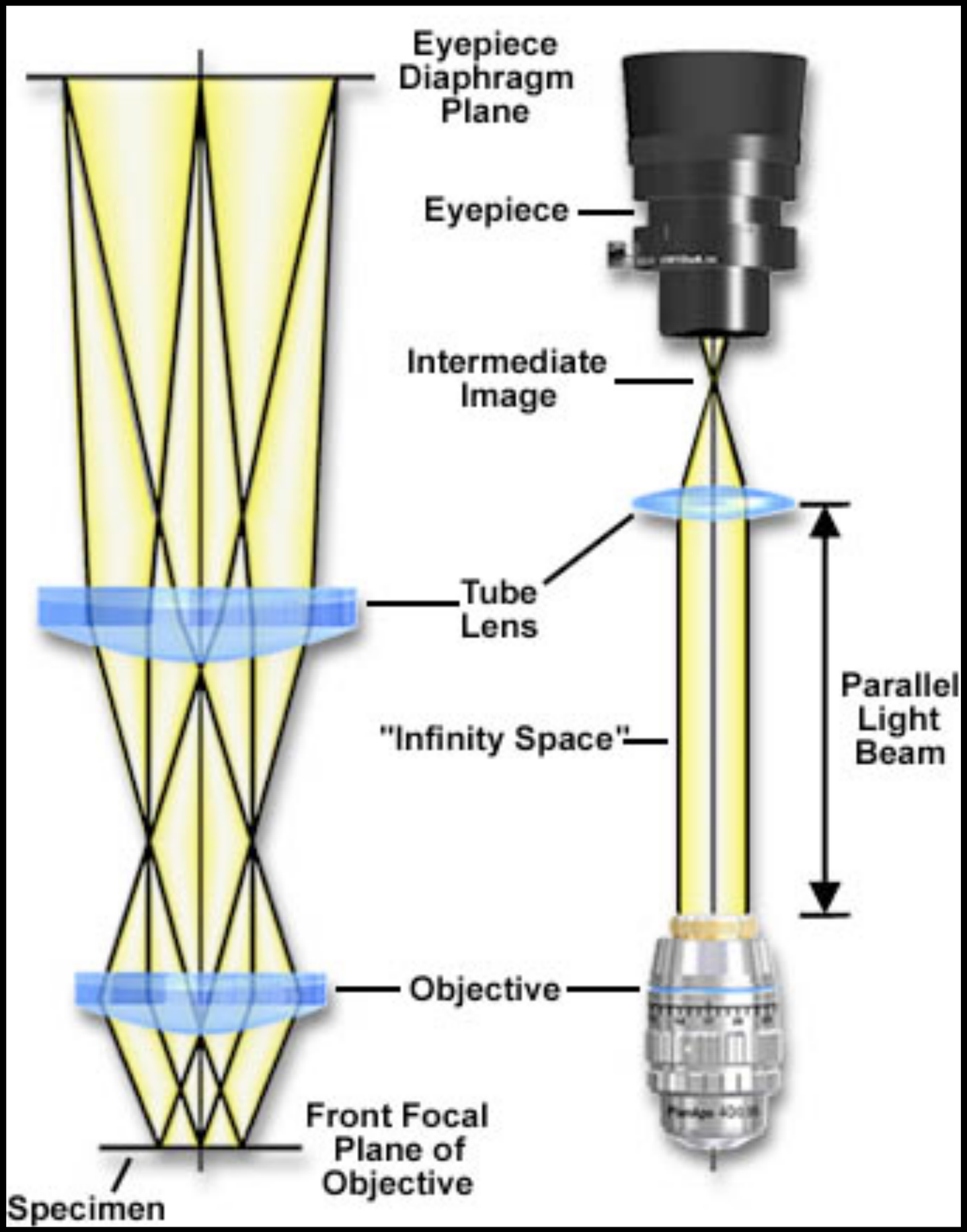
Pupil Stop limits the angle of illumination, which effectively limits the NA

- Draw out a microscope illumination system

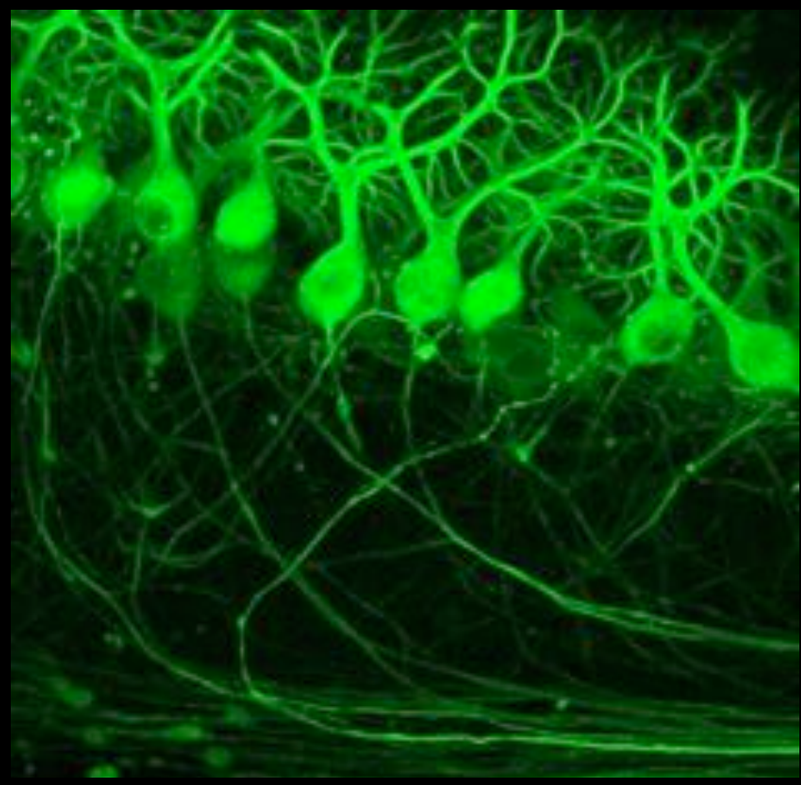
Summary:

- Modern microscopes work with two lenses:
  - Objective
  - Tube Lens


Eyepiece
Diaphragm
Plane
Eyepiece
Intermediate
Image
Tube
Lens
"Infinity Space"
Objective
Front Focal
Plane of
Objective
Specimen


Eyepiece
Diaphragm
Plane
Eyepiece
Intermediate
Image
Tube
Lens
"Infinity Space"
Parallel
Light
Beam
Objective
Front Focal
Plane of
Objective
Specimen

Fluorescence Microscopy



Green Fluorescent Protein

Green Fluorescent Protein

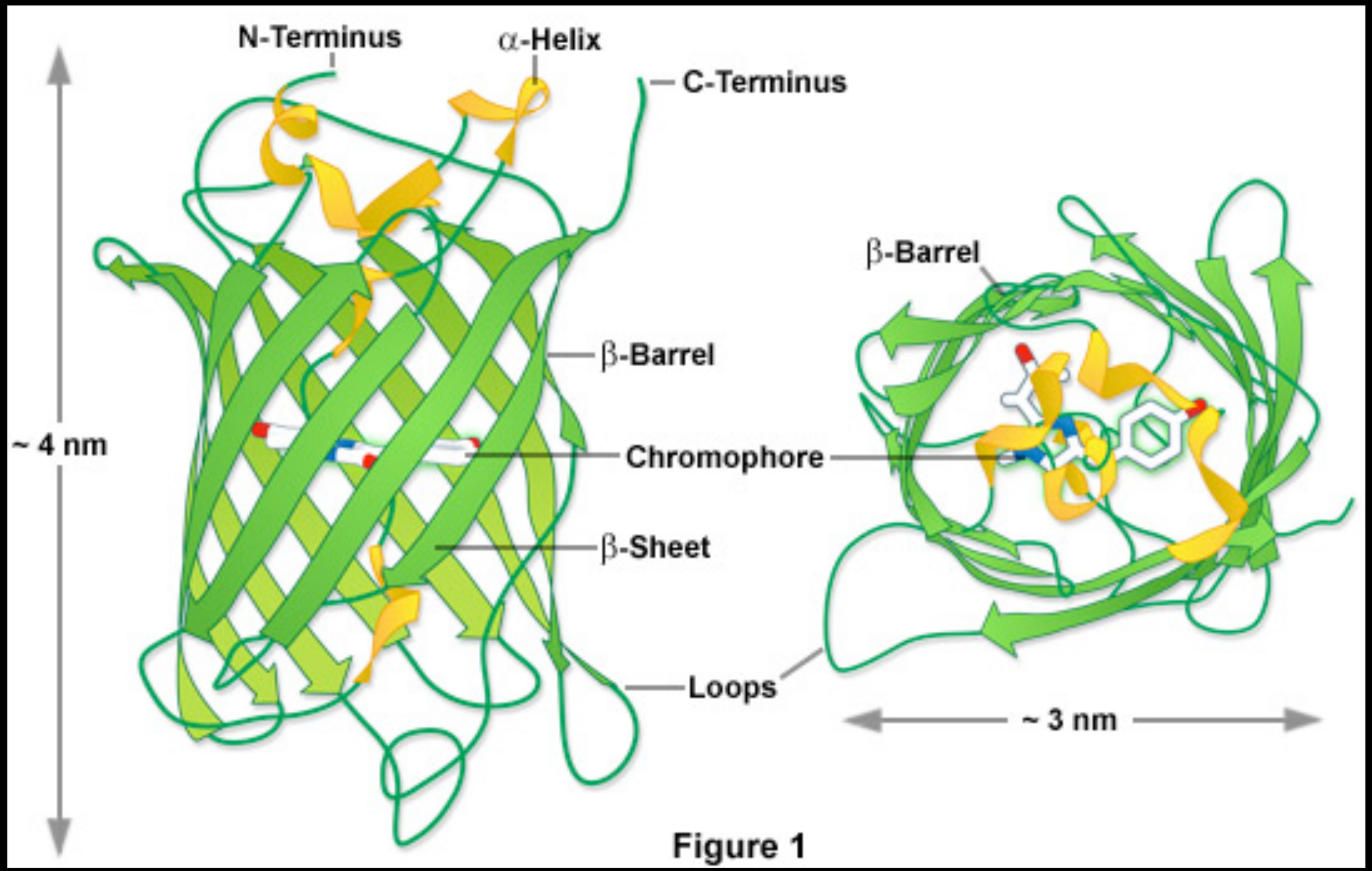
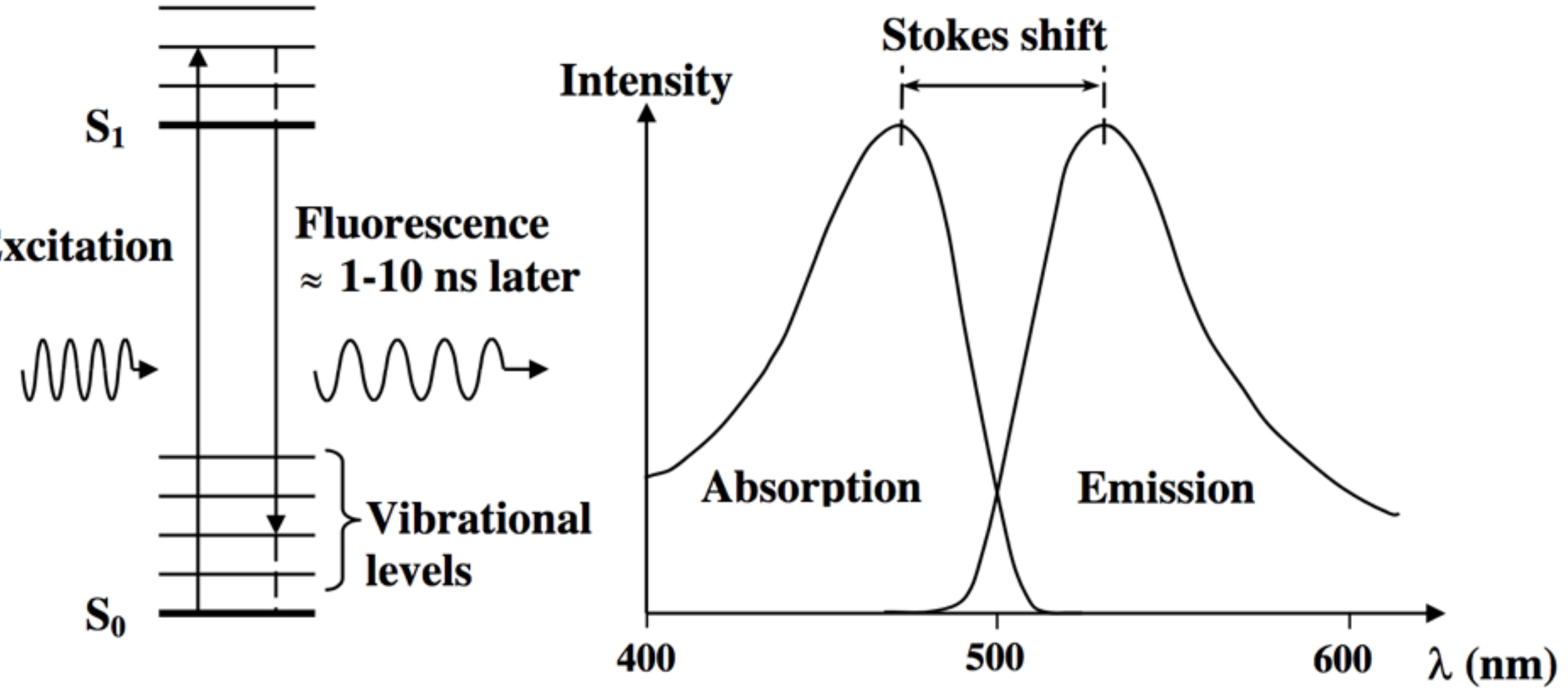
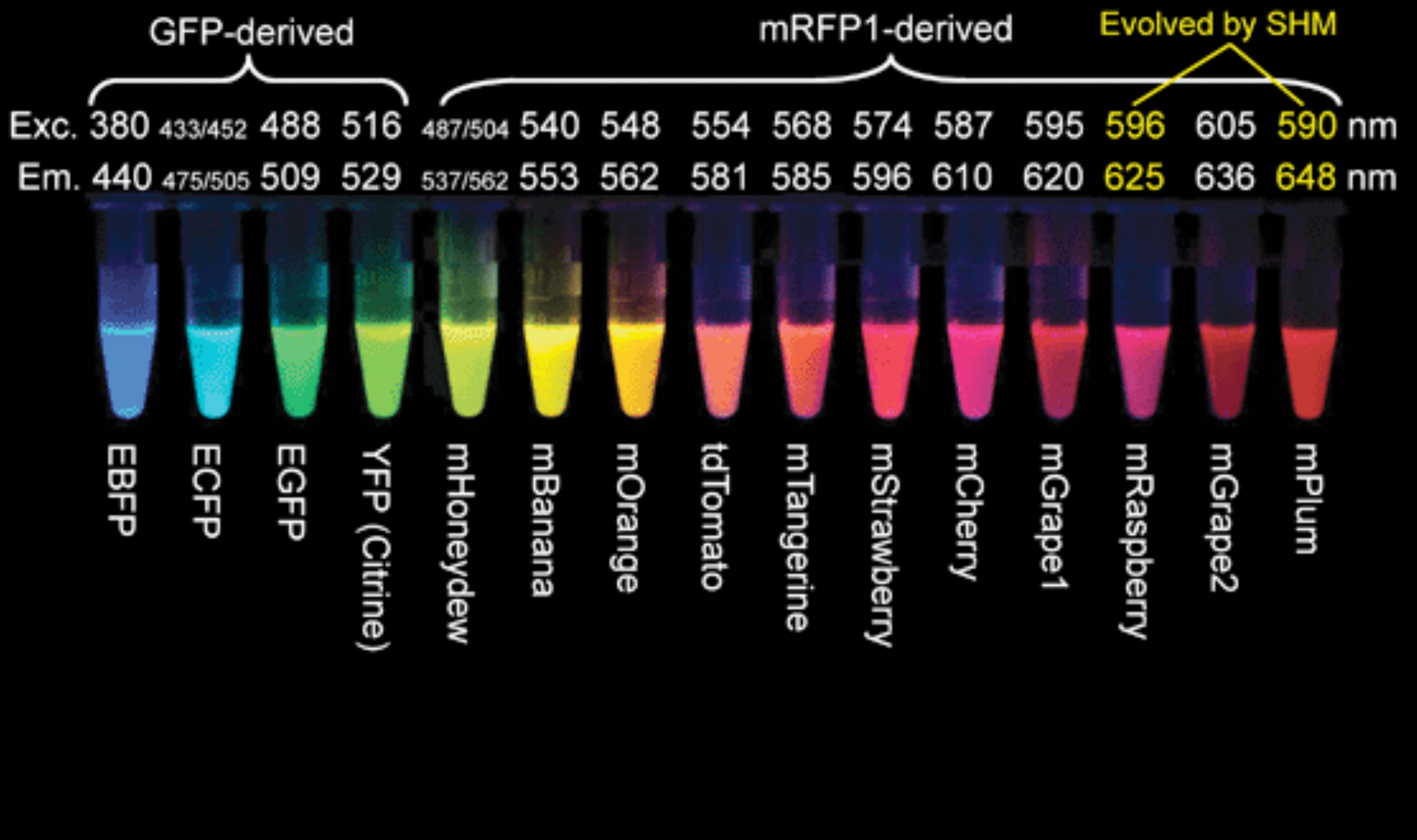


Figure 1

Fluorescence Excitation

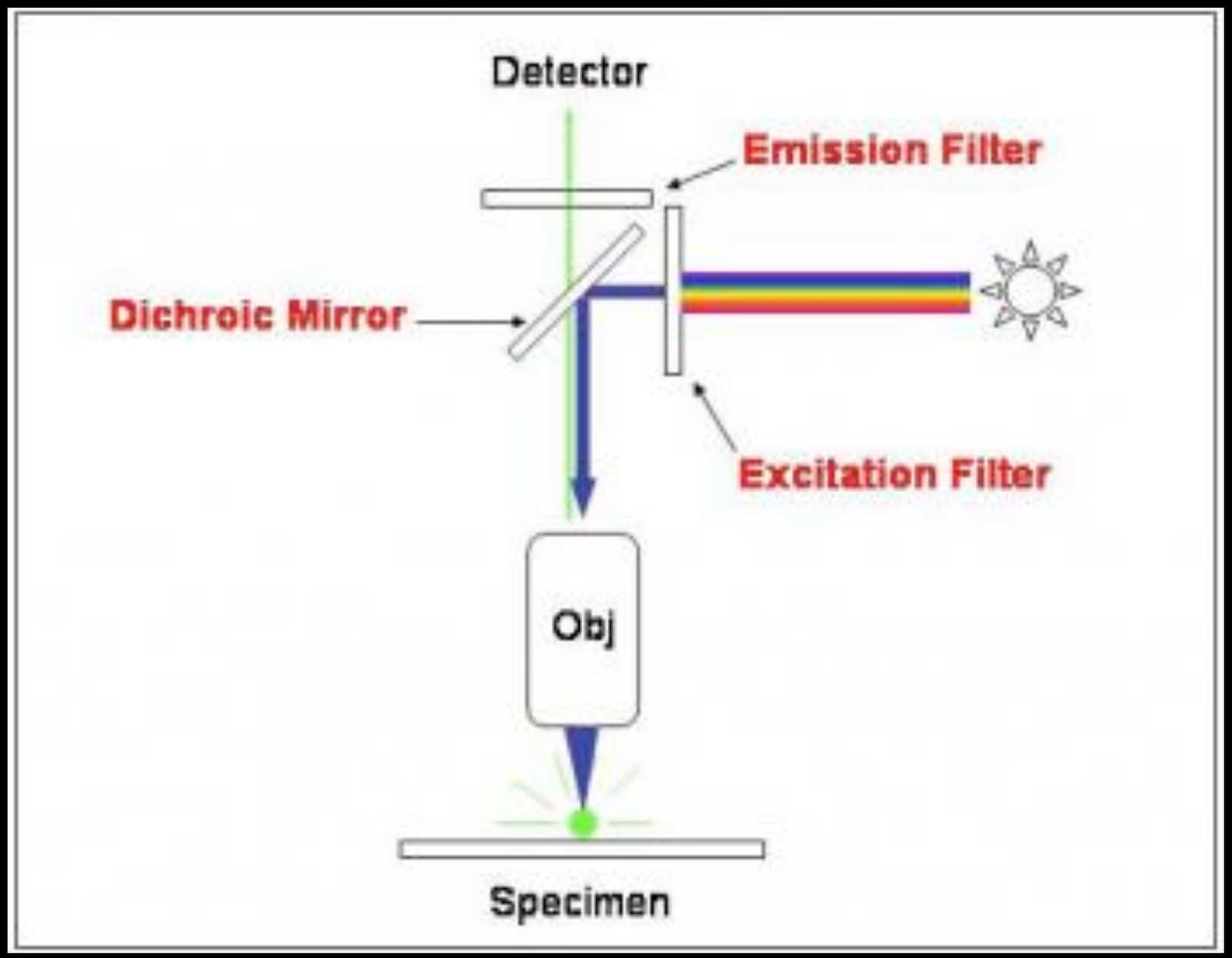


The 2004 palette of nonoligomerizing fluorescent proteins



Nathan Shaner et al (2004) *Nature Biotech.* **22**: 1567-1572
Lei Wang et al (2004) *Proc. Natl. Acad. Sci. USA* **101**: 16745-16749

Filters and Dichroic Mirrors



Fluorescence Filter Set Configurations

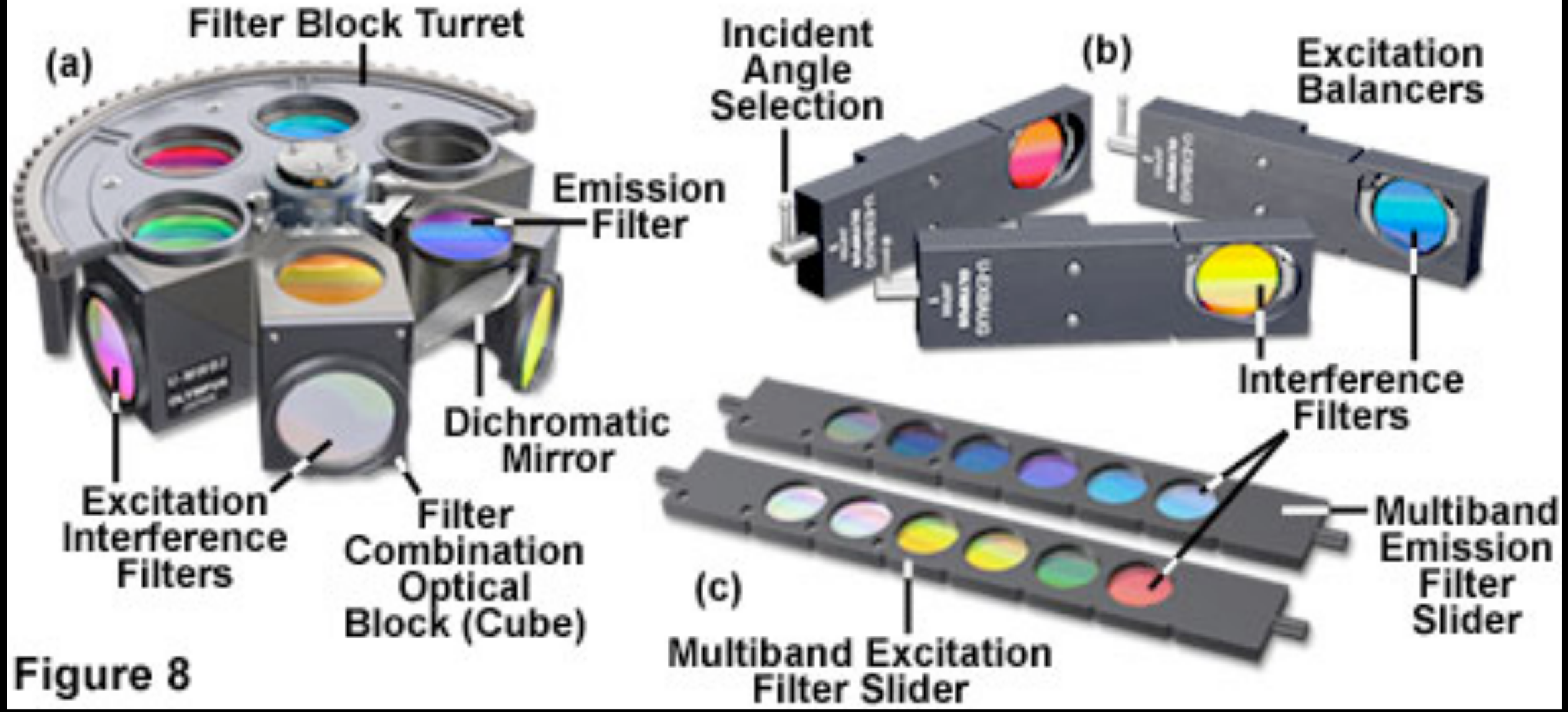
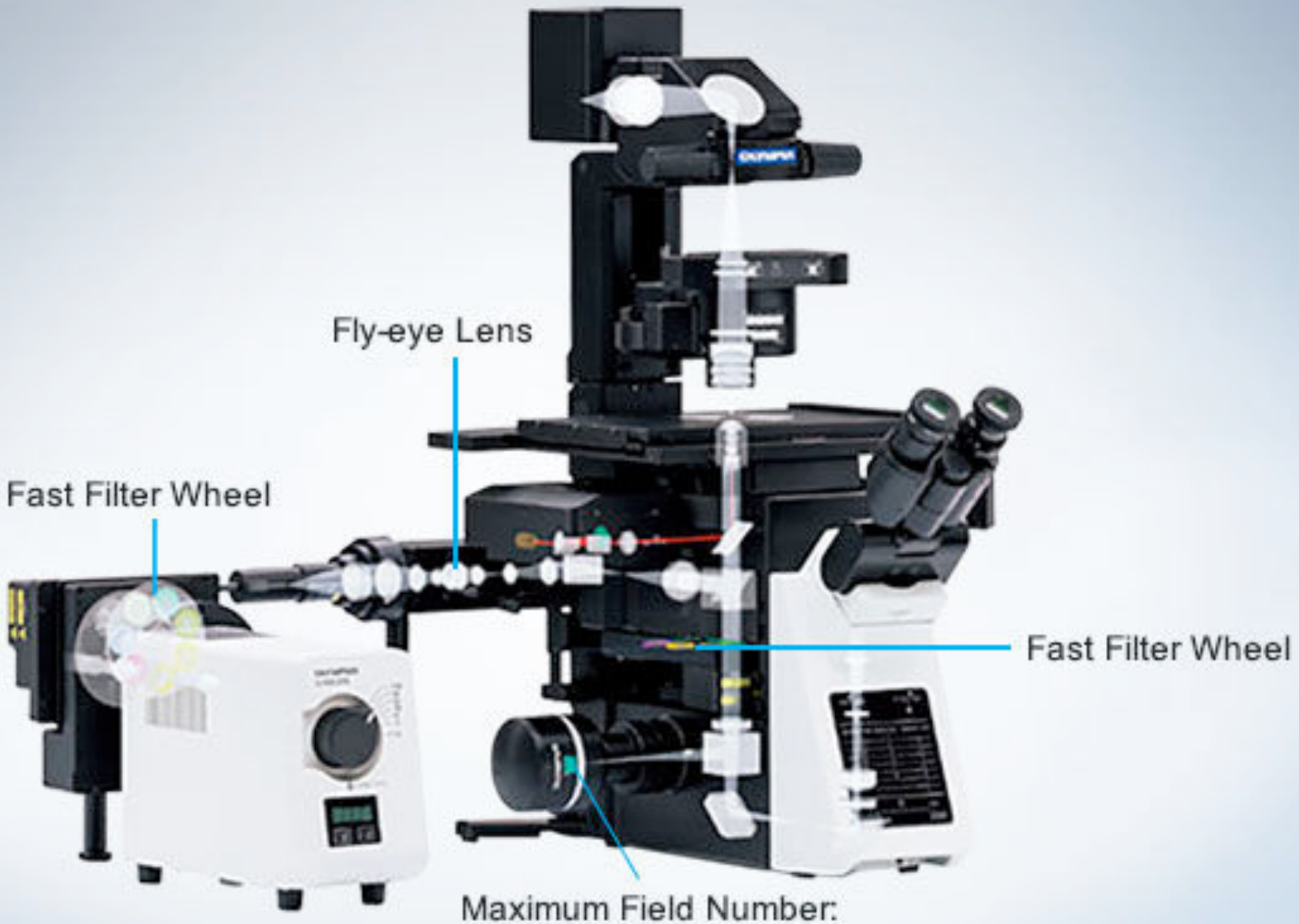


Figure 8

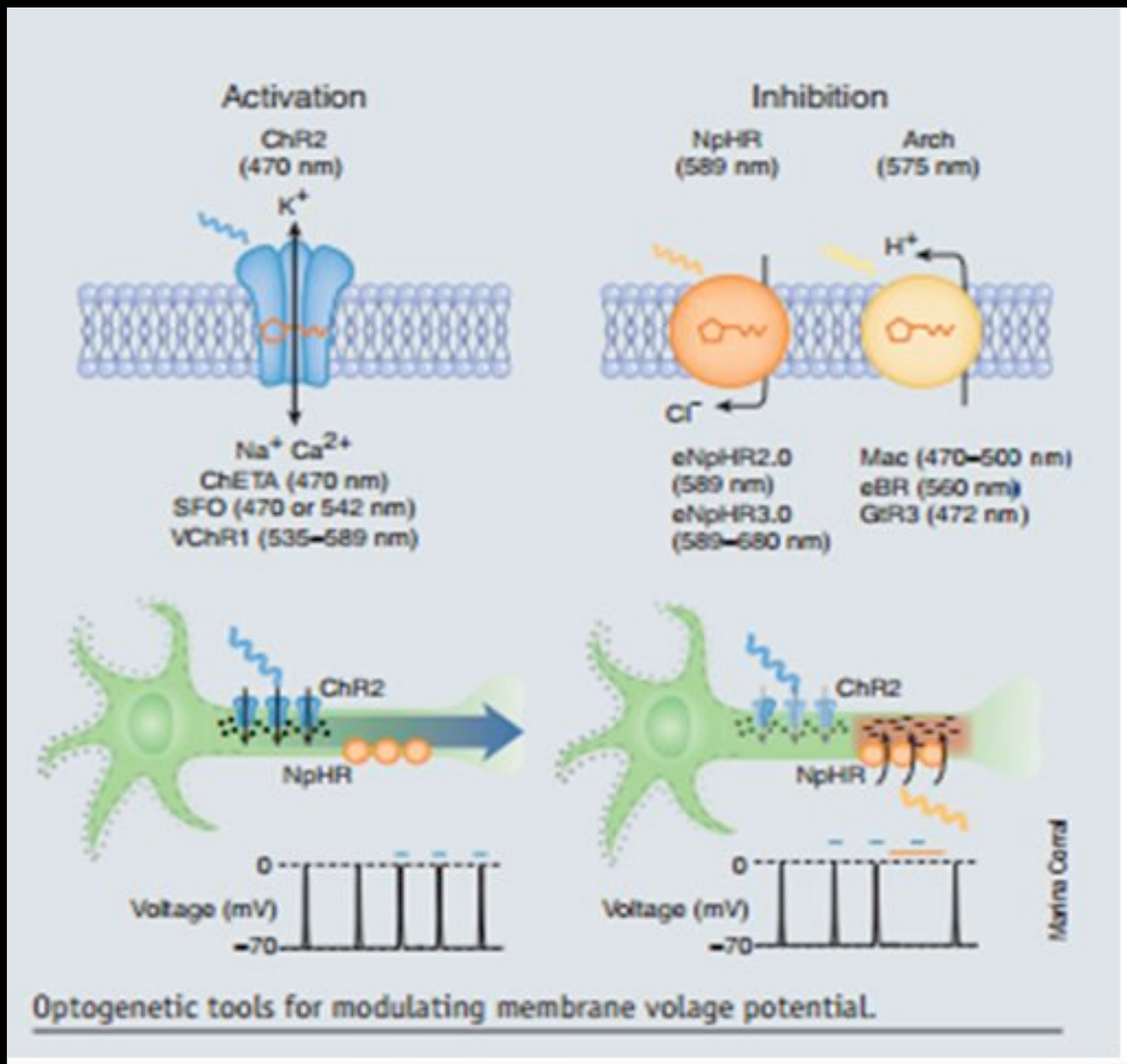

Fly-eye Lens
Fast Filter Wheel
Fast Filter Wheel
Maximum Field Number:
22 (IX83P1ZF, IX73P1F), 20 (IX73P2F), 18 (IX83P2ZF)
*Field of view is limited when fi lter wheel inserted.

- https://www.chroma.com/products/sets/89000-et-sedat-quad

Functionalized FP:s

- Use chemistry and spectroscopic methods to probe the environment inside a living specimen such as:
  - Voltage
  - pH
  - Calcium concentration

Optogenetics (Laser mind control)



Optogenetic tools for modulating membrane volage potential.

Channelrhodopsin

- Cation channel
- Activated by blue light (470nm)
- Allows Na^+ influx across the membrane and depolarizes the neuron, thus activating it
- Acts as the on switch

Halorhodopsin

- Chloride pump
- Activated by yellow light (580 nm)
- Triggers influx of Cl^- which hyperpolarizes the cell and inhibits the neuron
- Acts as the Off switch

Pastrana, E. (2011). Optogenetics: Controlling cell function with light. *Nature Methods, 8(1),* 24-25. DOI:10.1038/nmeth.f.323

The first rule of Microscopy is...don't break the microscope.