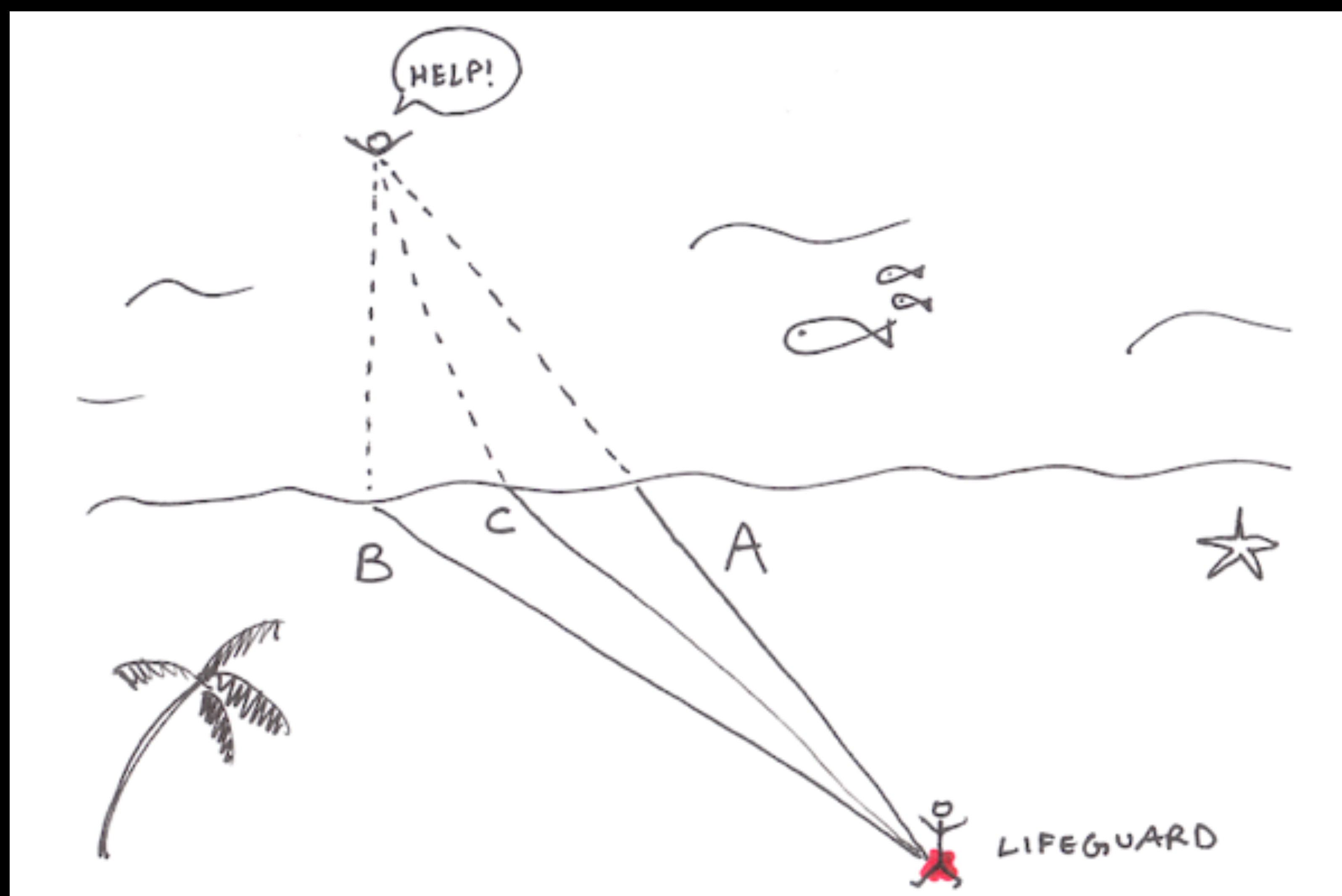
# Review

# Fermat's Principle

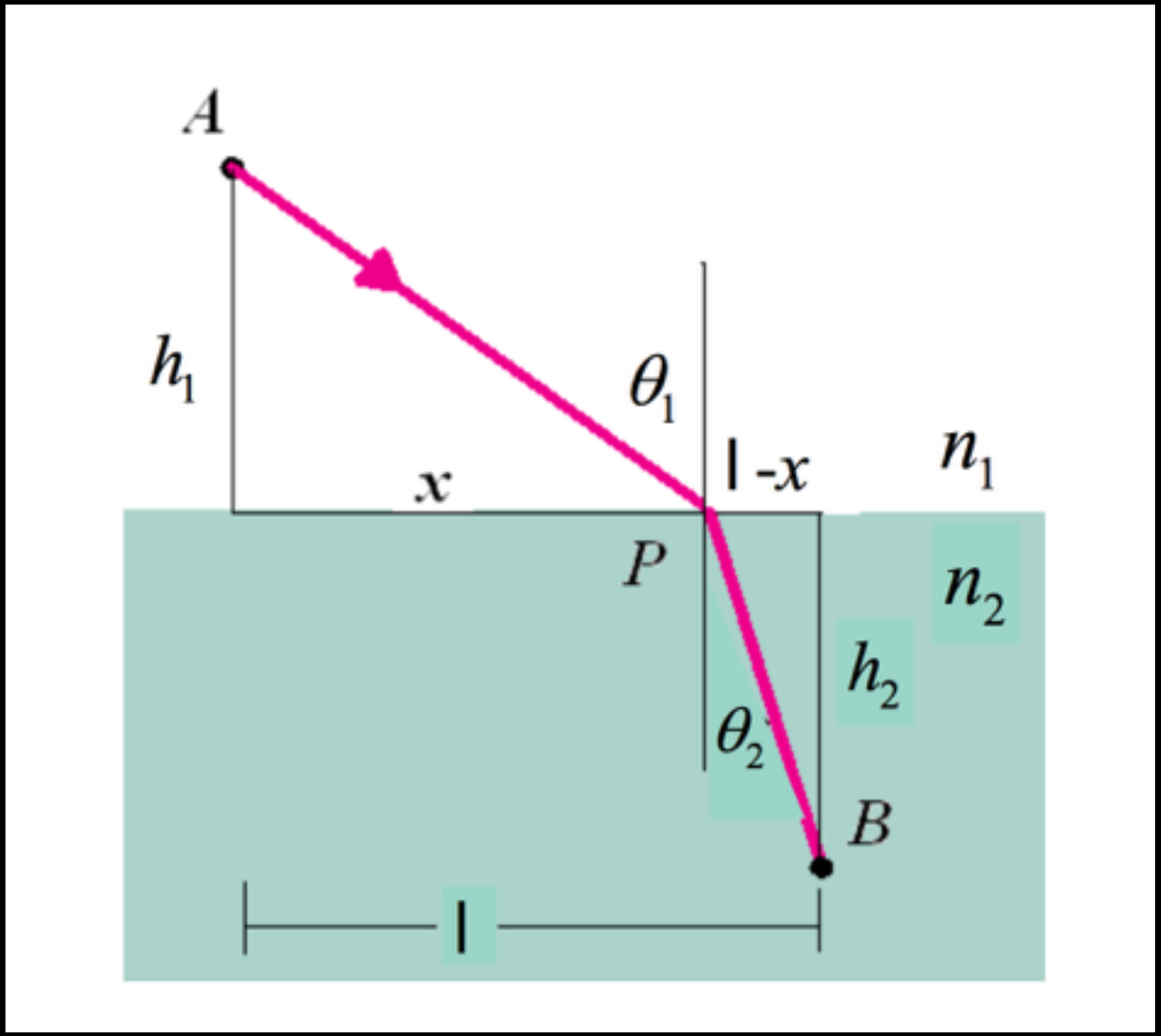
Light travels between two points along the path
that requires the least time,
as compared to other nearby paths




(Fermat = French mathematician, 1600s)

# Snell's Law (Refraction)

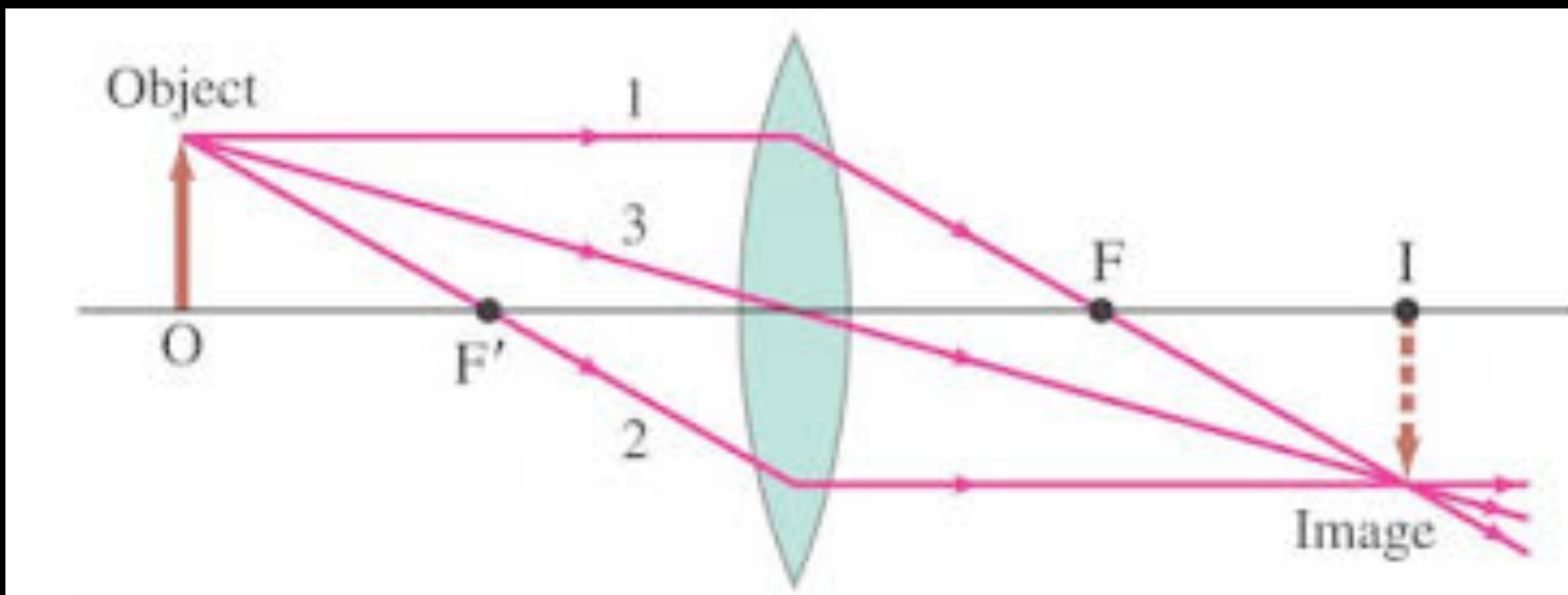
$$n_1 \sin \theta_1 = n_2 \sin \theta_2$$



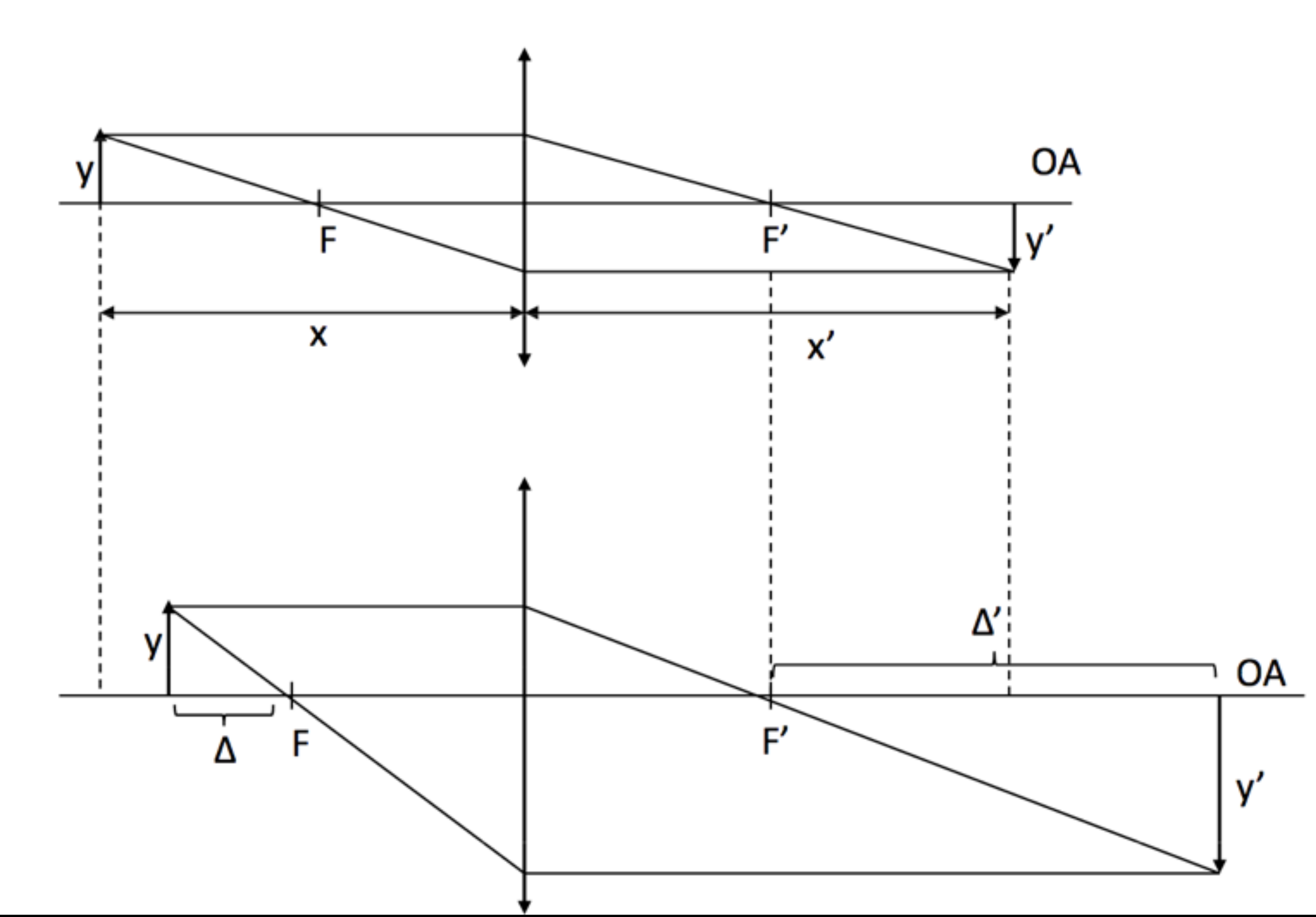
# Total Internal Reflection

# Geometrical Ray Optics

## Three Principal Rays:

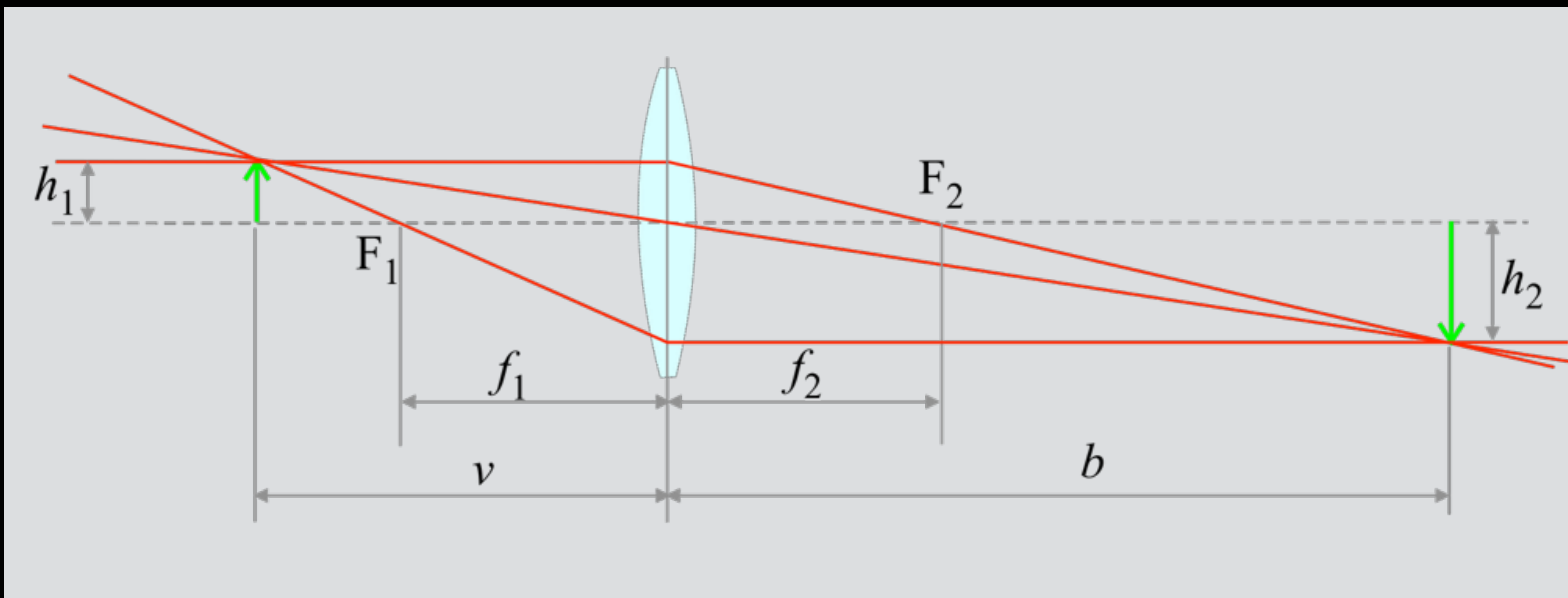


# “4f” with single lens



# Magnification

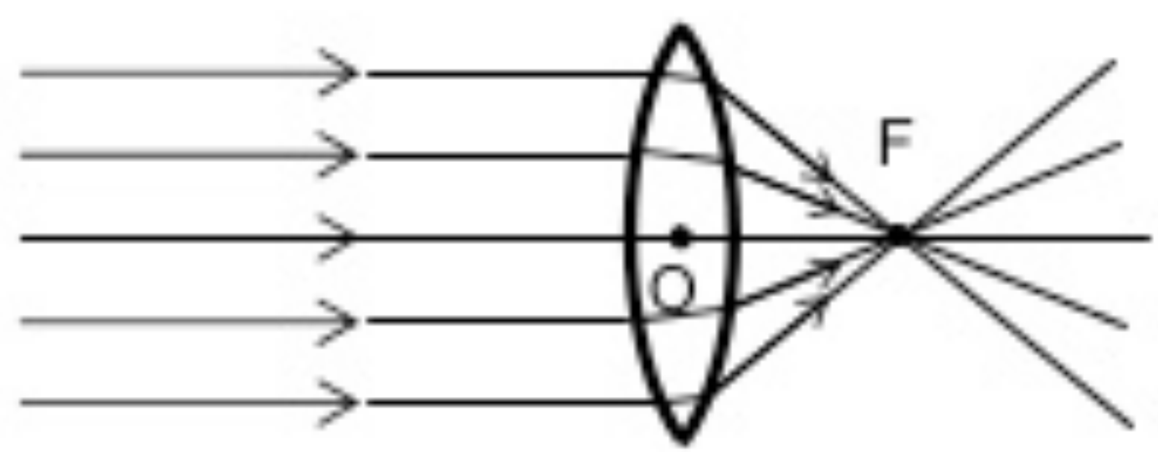
Simple geometrical proof: Two triangles




$$M = f / (f - v)$$

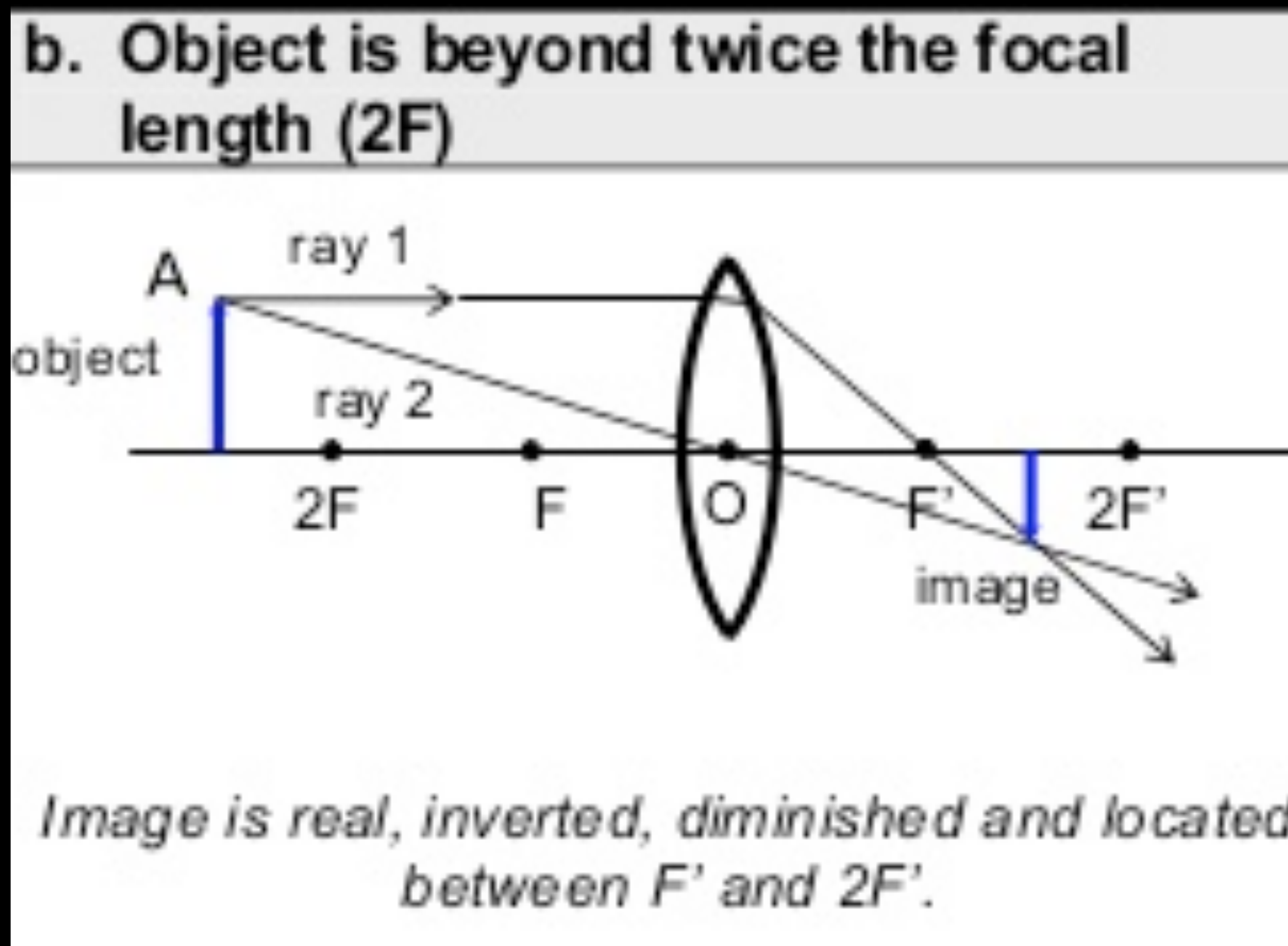
# Imaging at different focal positions

**a. Object is at infinite distance**




*The image is a point at the principal focus.*

# Imaging at different focal positions



# Imaging at different focal positions

**c. Object is at twice the focal length (2F)**

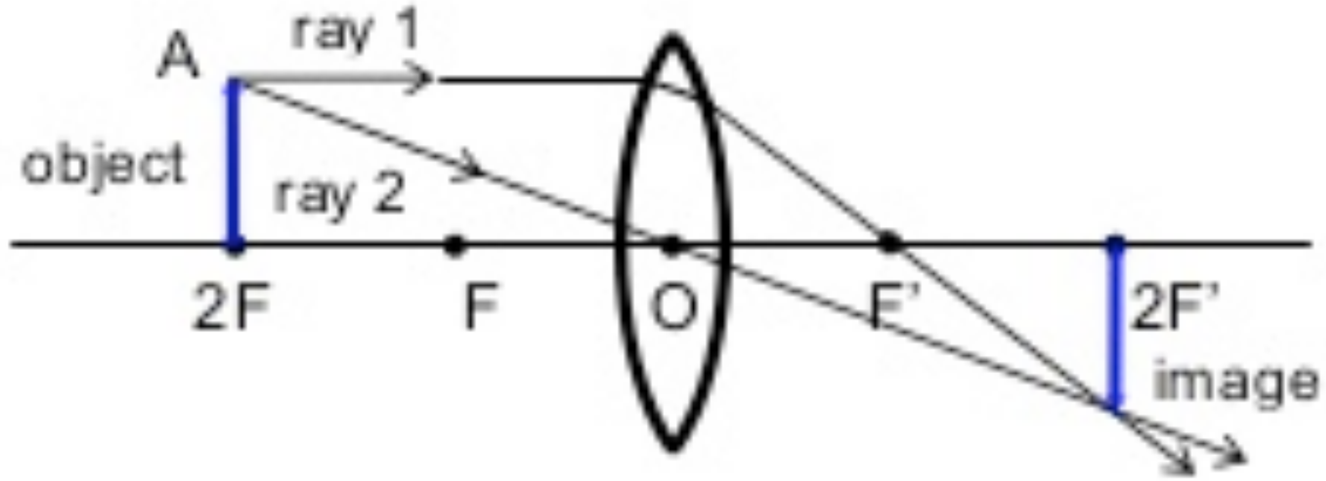
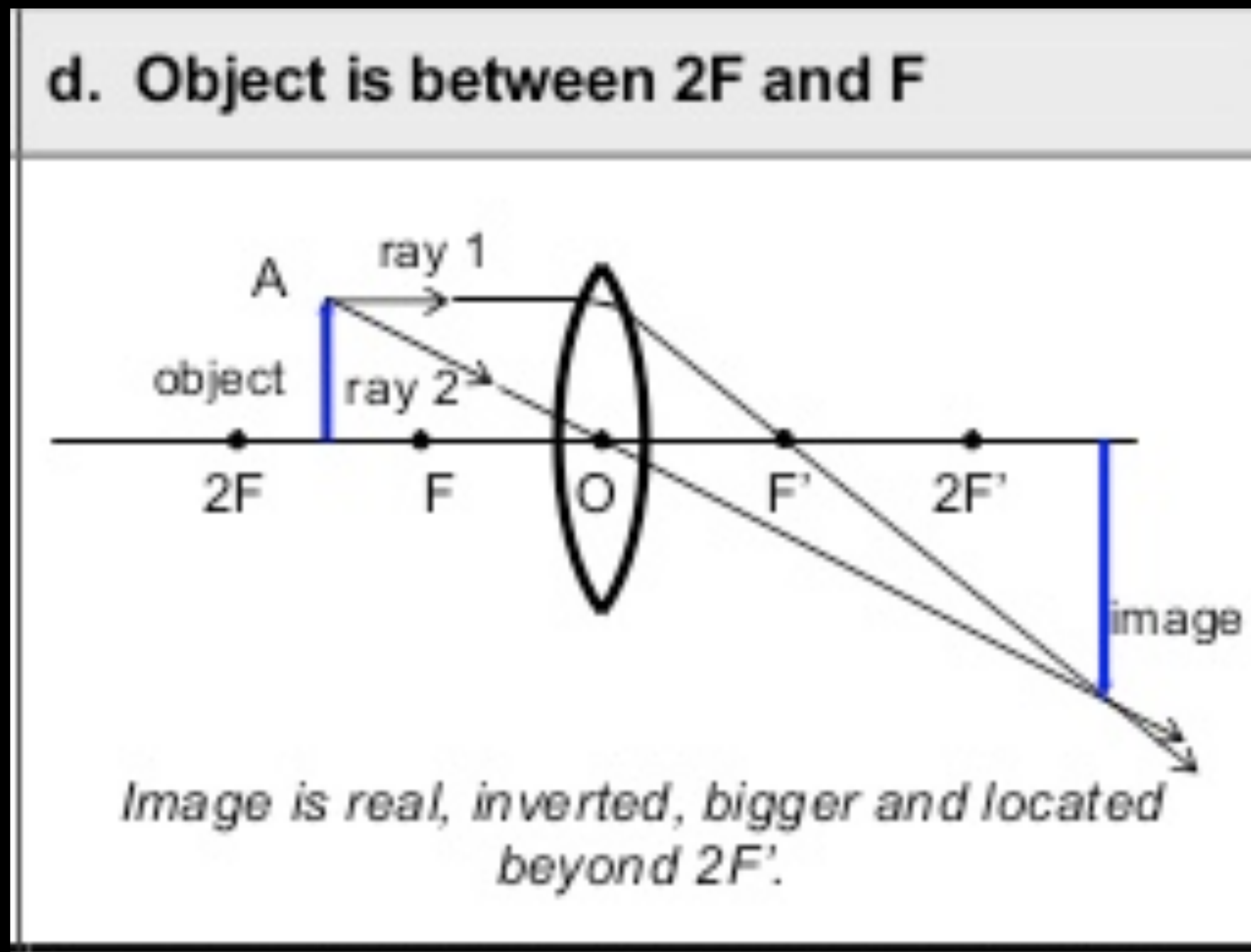
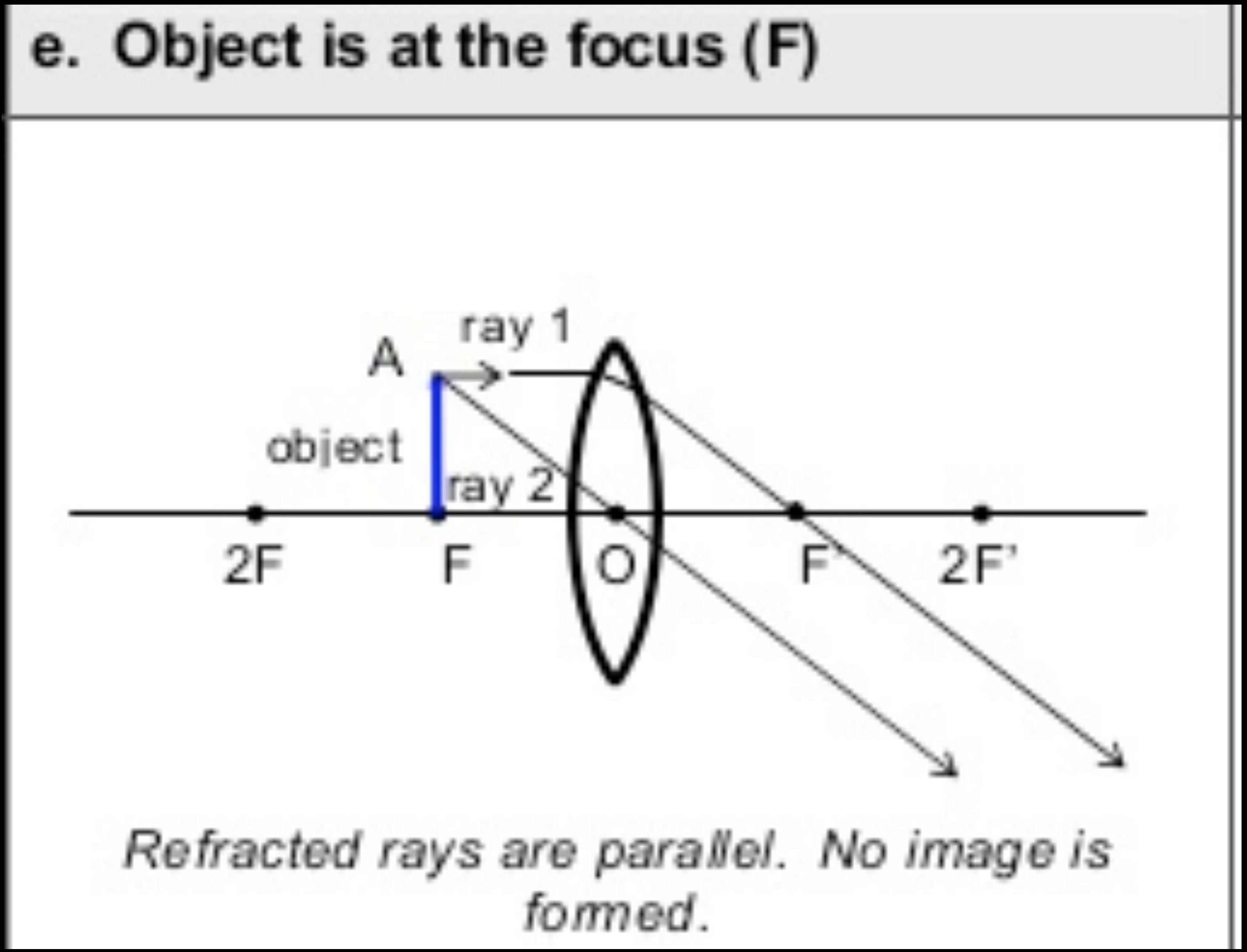



*Image is real, inverted, of the same size and located at 2F'.*

# Imaging at different focal positions



# Imaging at different focal positions



# Imaging at different focal positions

**f. Object is between the focus and the optical center**

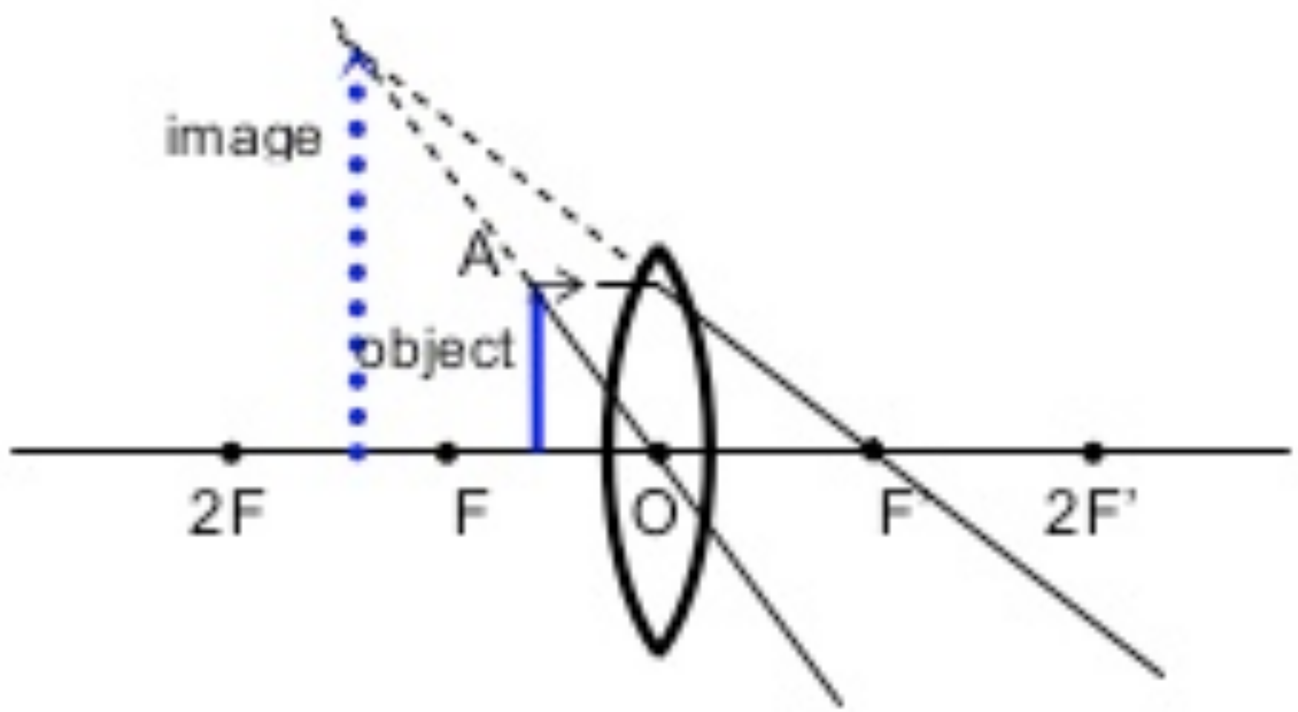


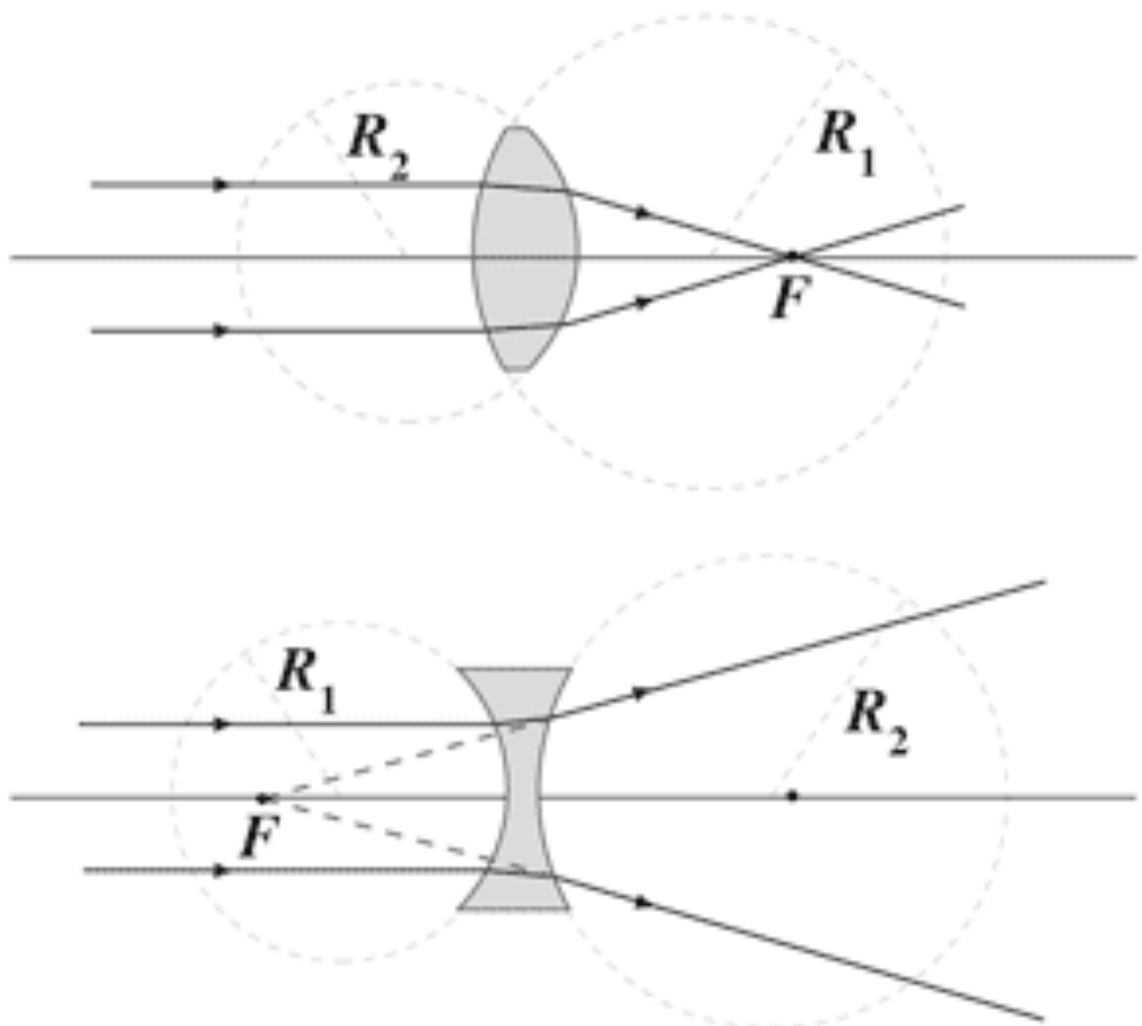

*Image is virtual, erect, bigger and located between 2F and F.*

# You don't need to memorize this I just want you to have seen it:

## Lens-Maker's Equation

$$\frac{1}{F} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$$

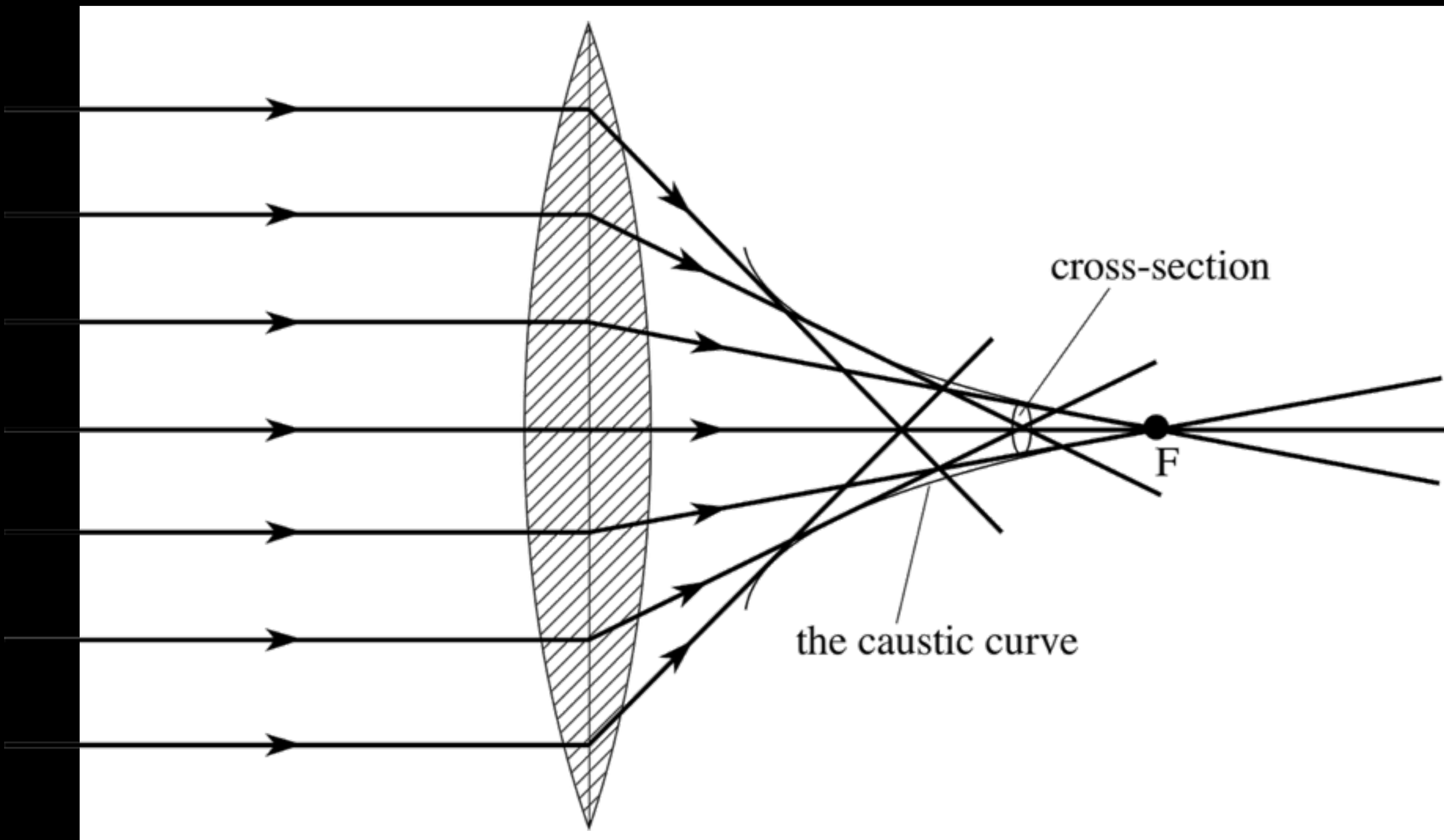
$F$ = focal length (1/2 $C$)
$n$ = refractive index
$R$ = radius of curvature



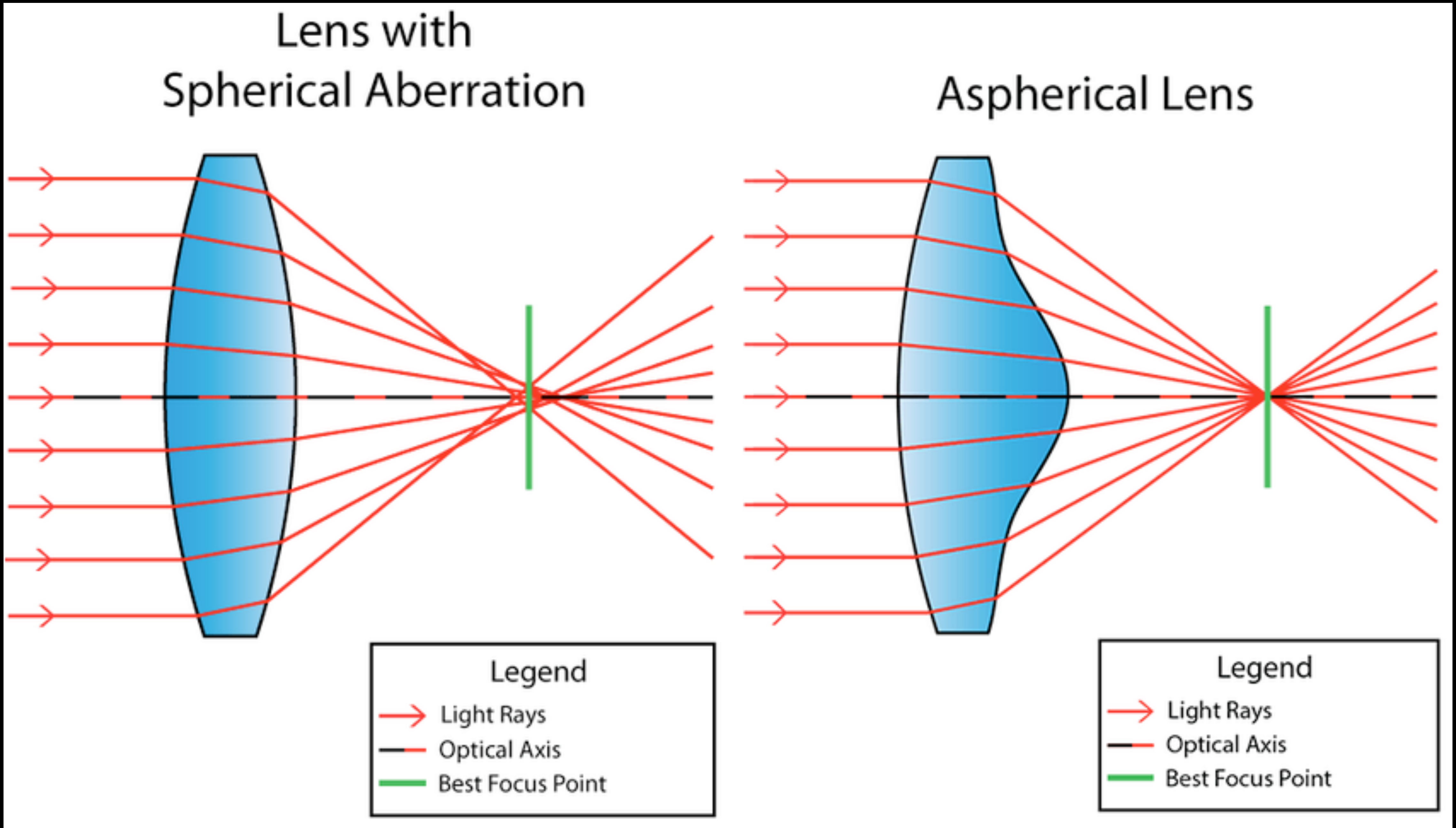

**Positive (Converging) Lens**
$R_1$ – positive
$R_2$ – negative
$F$ – positive

**Negative (Diverging) Lens**
$R_1$ – negative
$R_2$ – positive
$F$ – negative

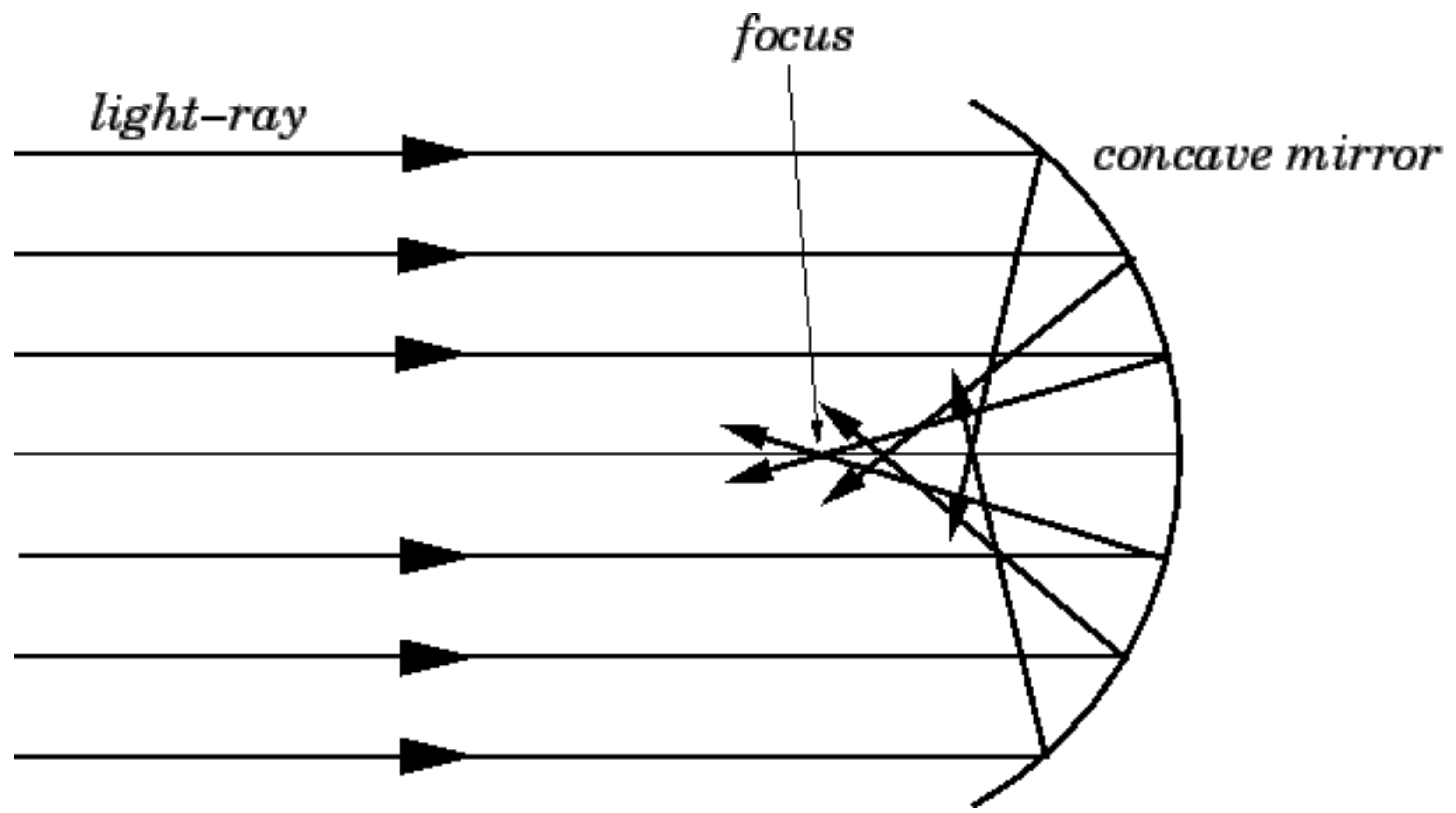
# Spherical Aberration



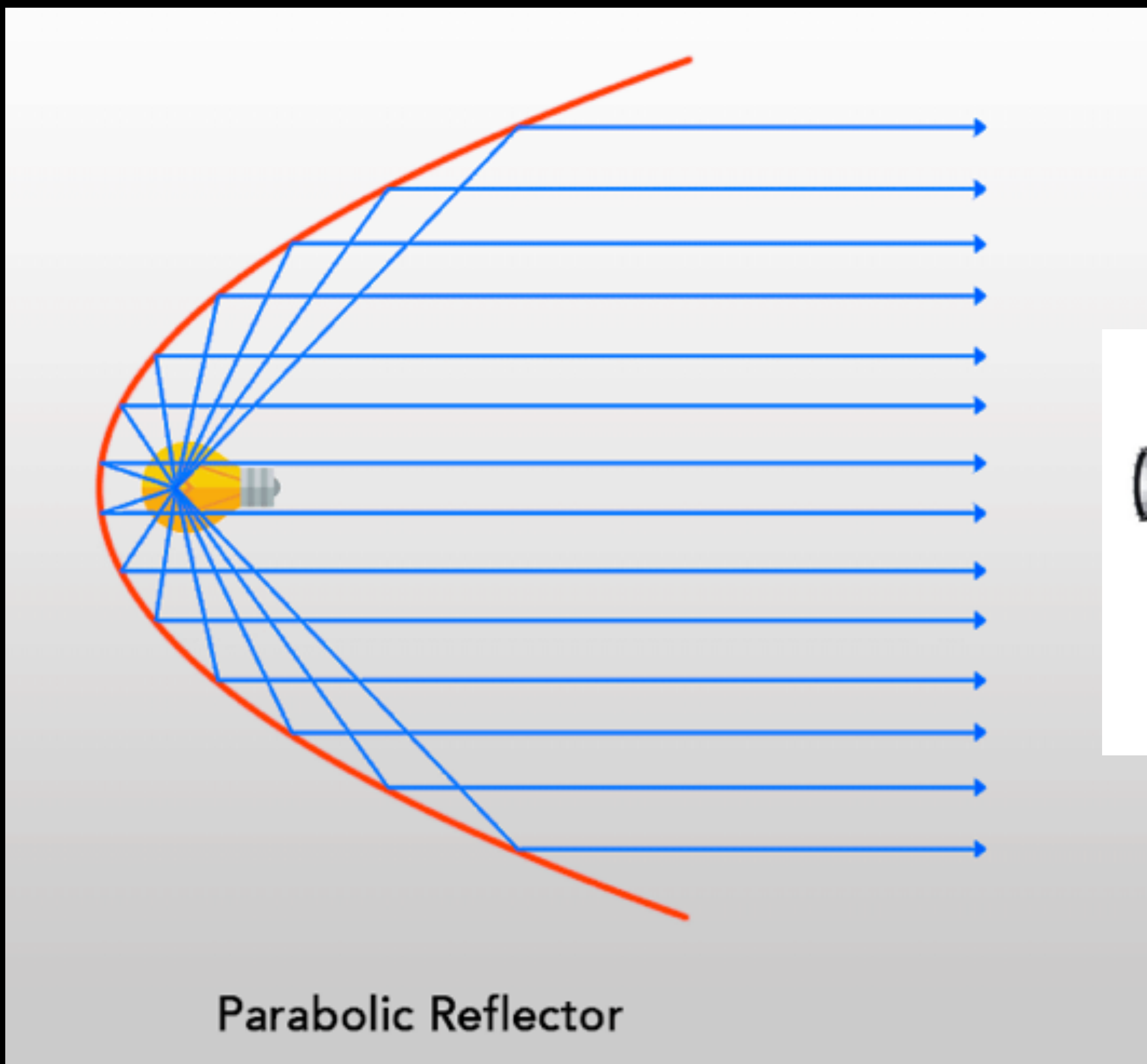
# Spherical Aberration



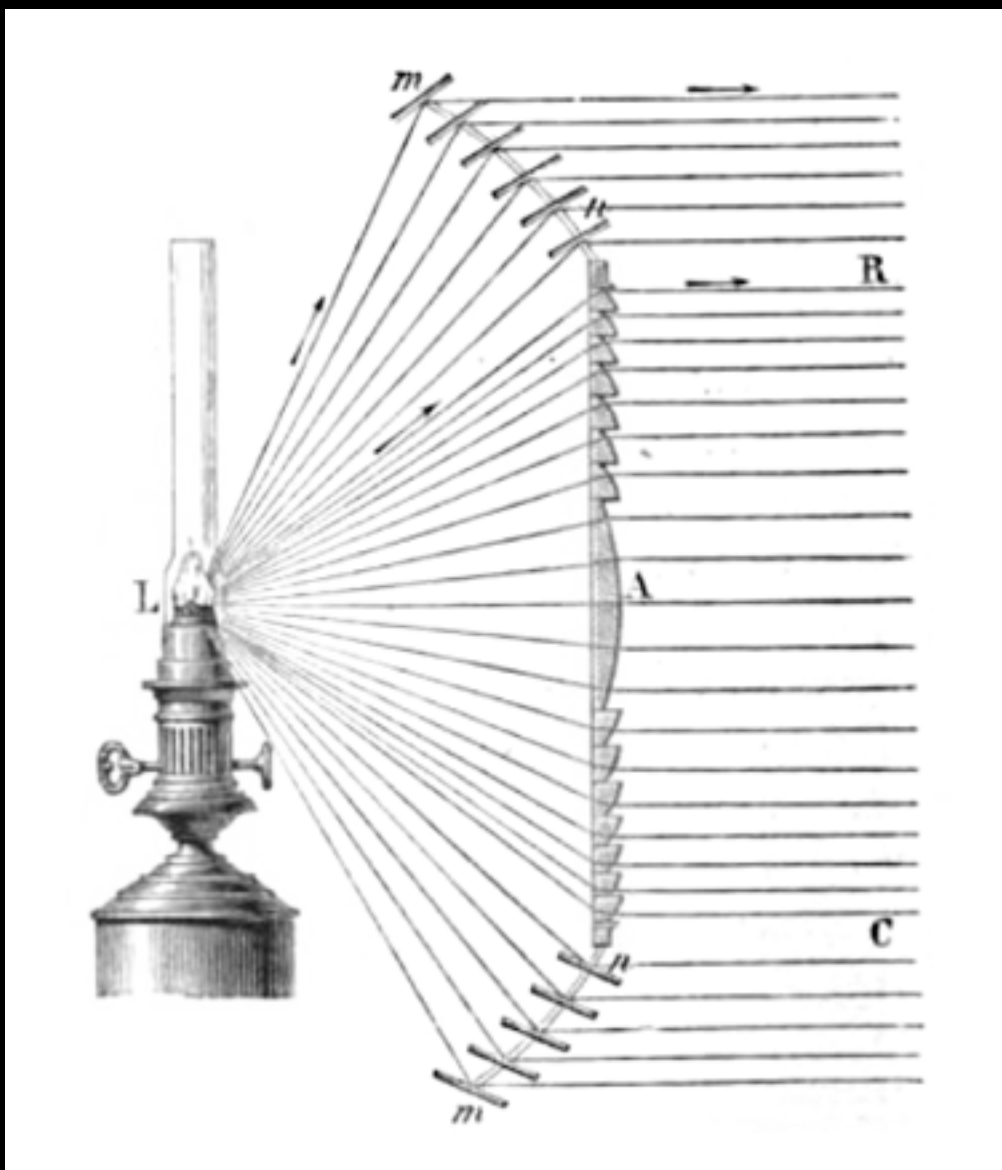
# Spherical mirror



# Parabolic Reflector

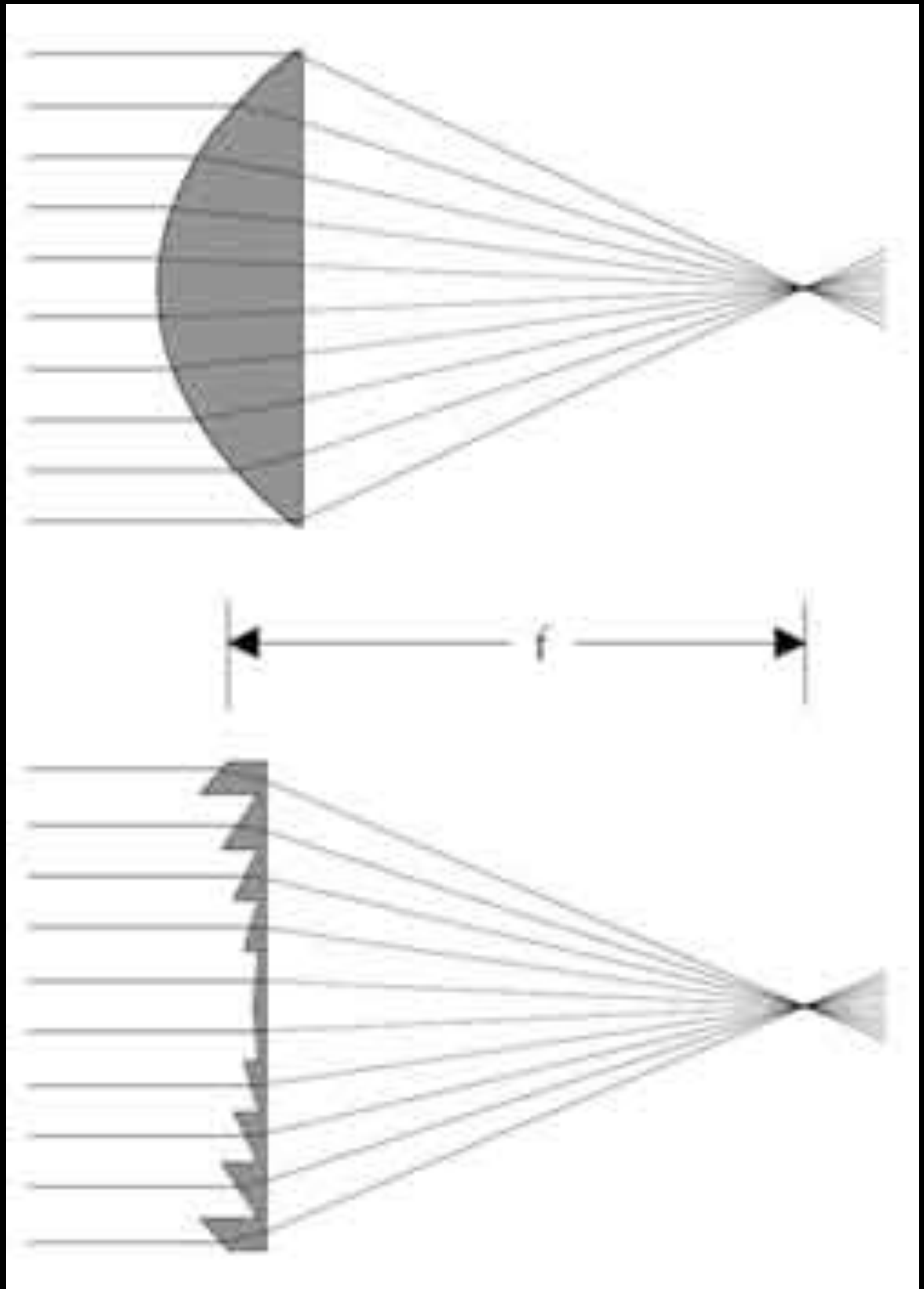



# Fresnel Lens



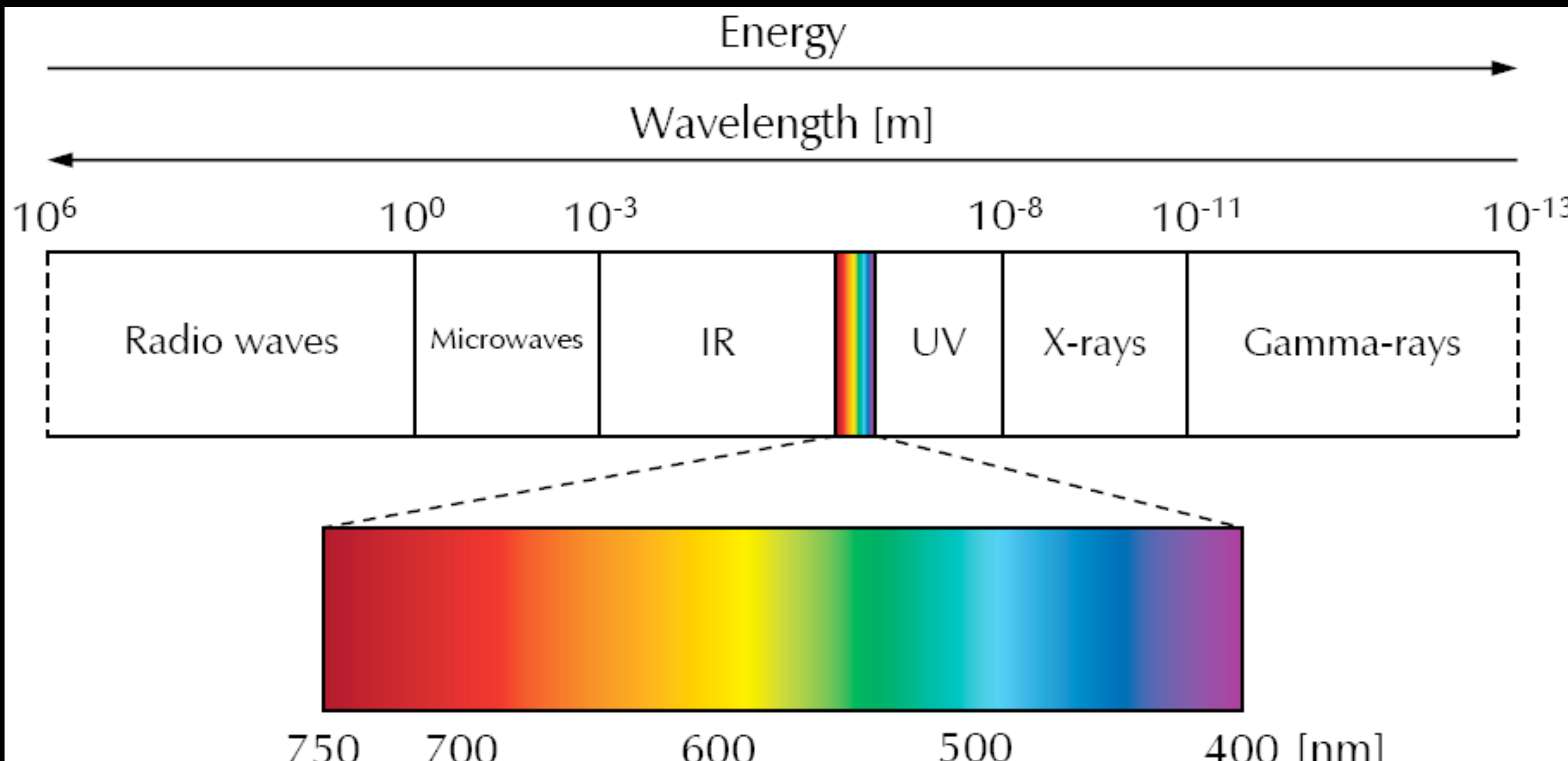

Fresnel lens in lighthouse

# The Fresnel Lens saves bulk/weight

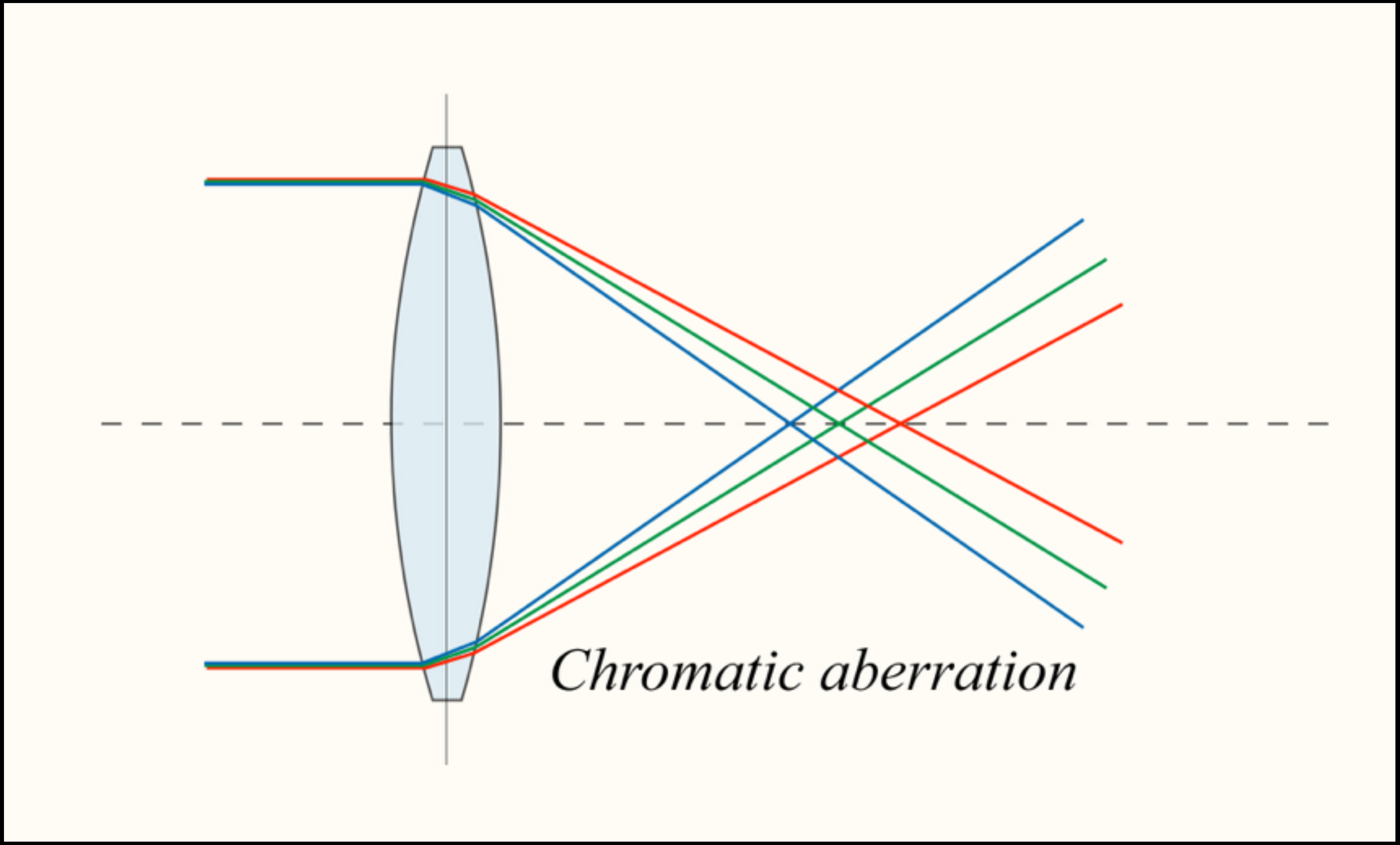


# Refraction in a Prism

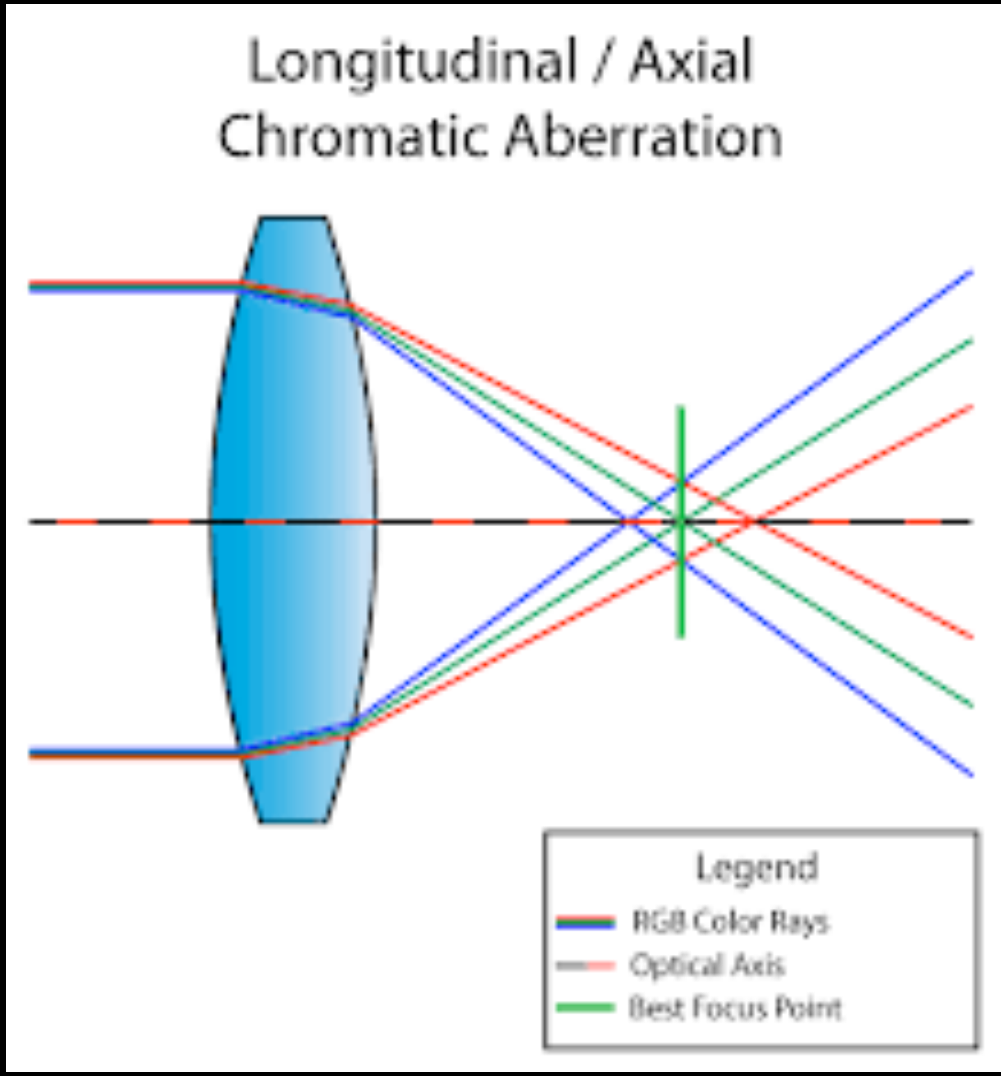
# Visible Light Wavelength Spectrum

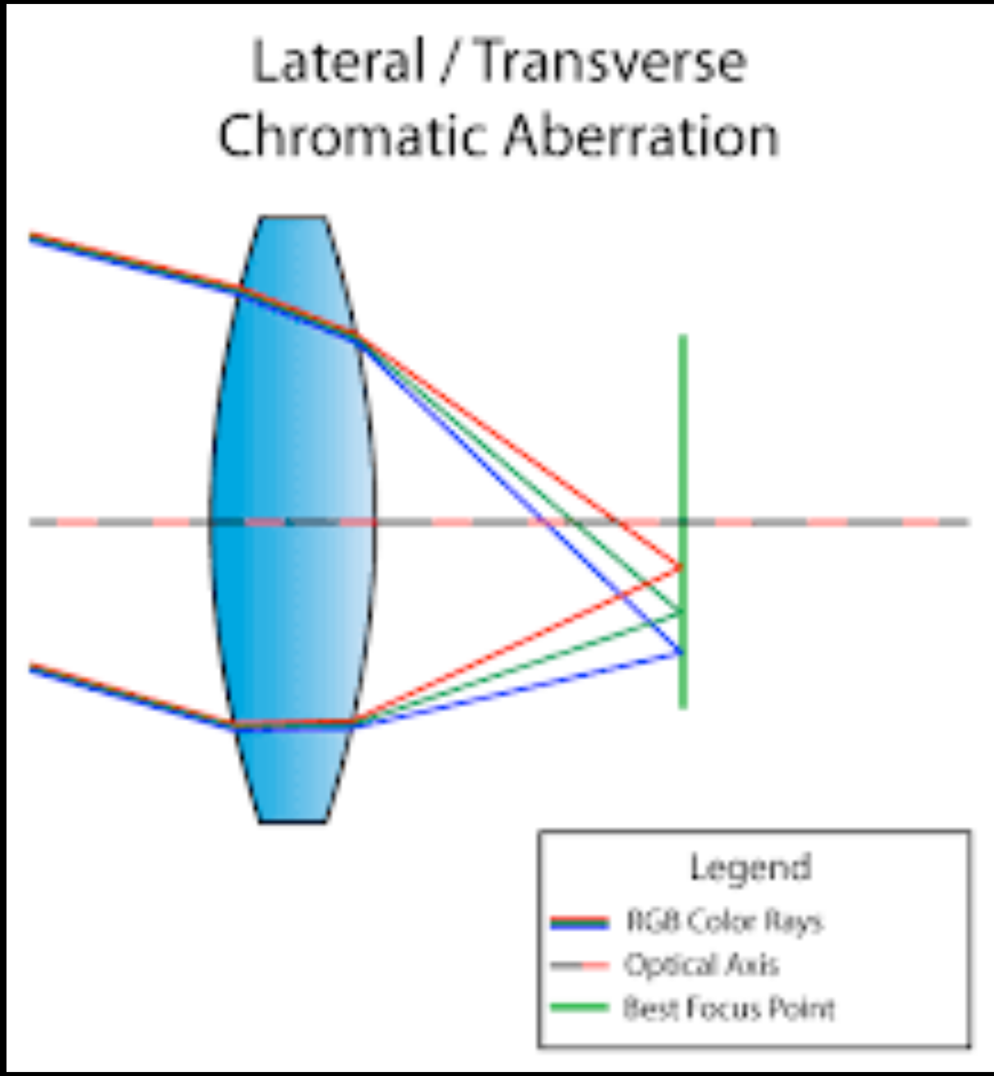


# Refractive index ~ wavelength

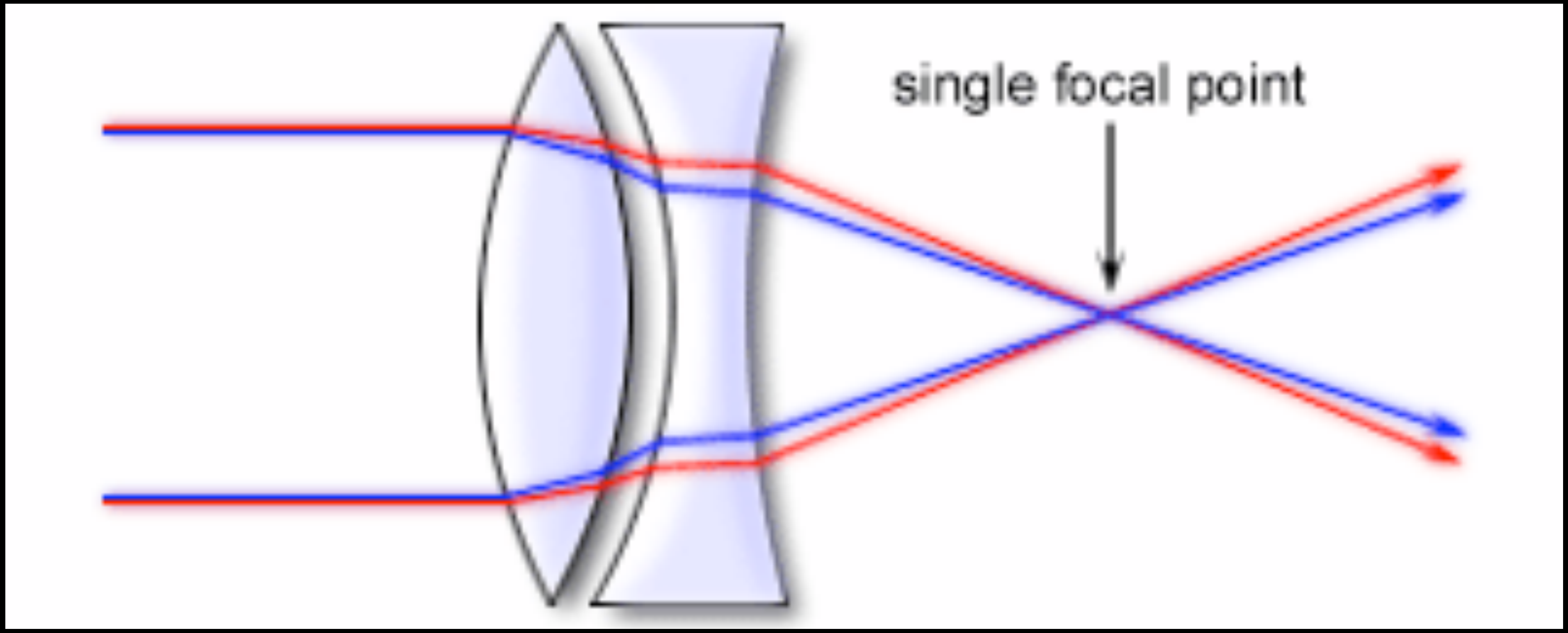


# Chromatic Aberration



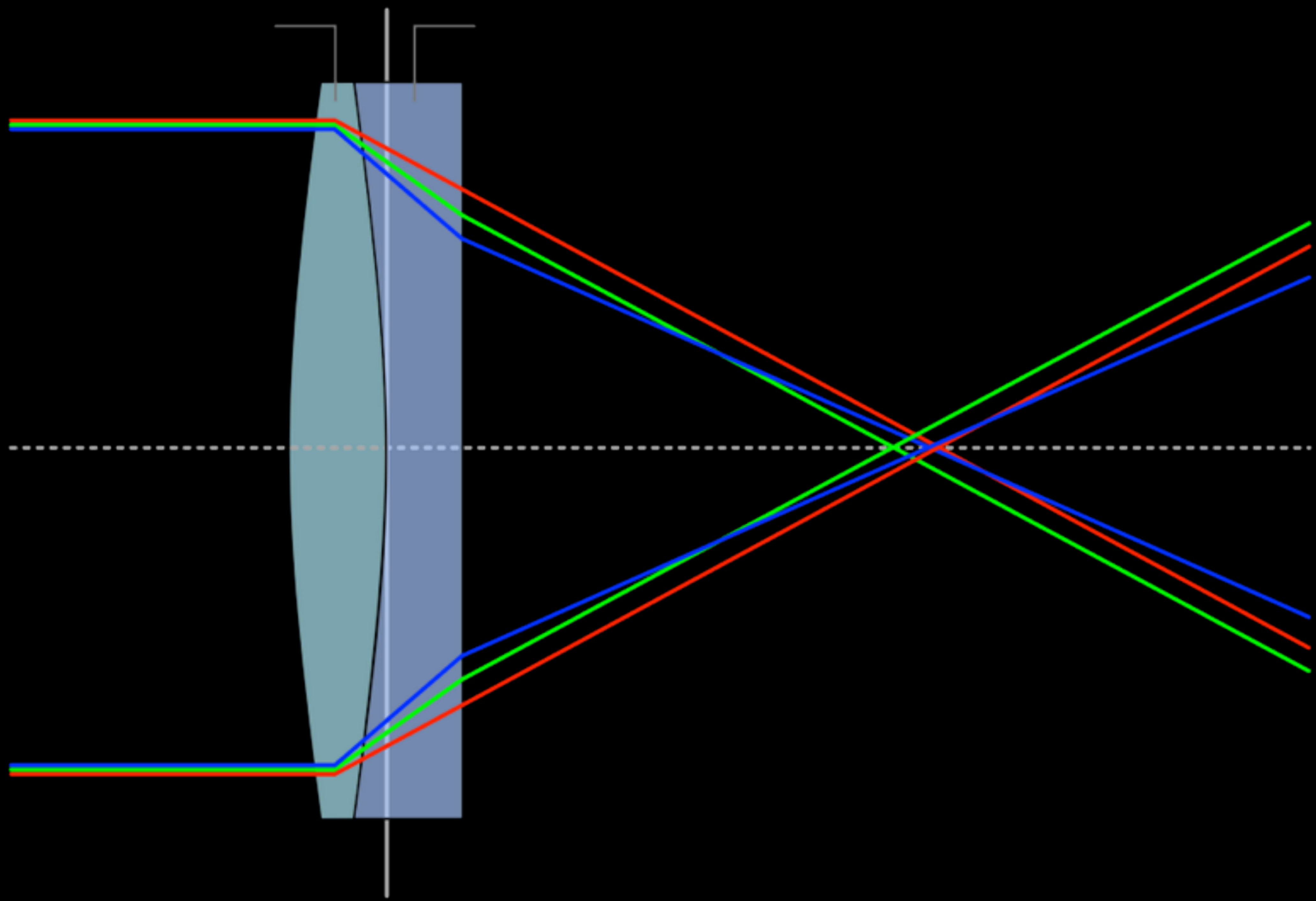



# Achromatic Doublet



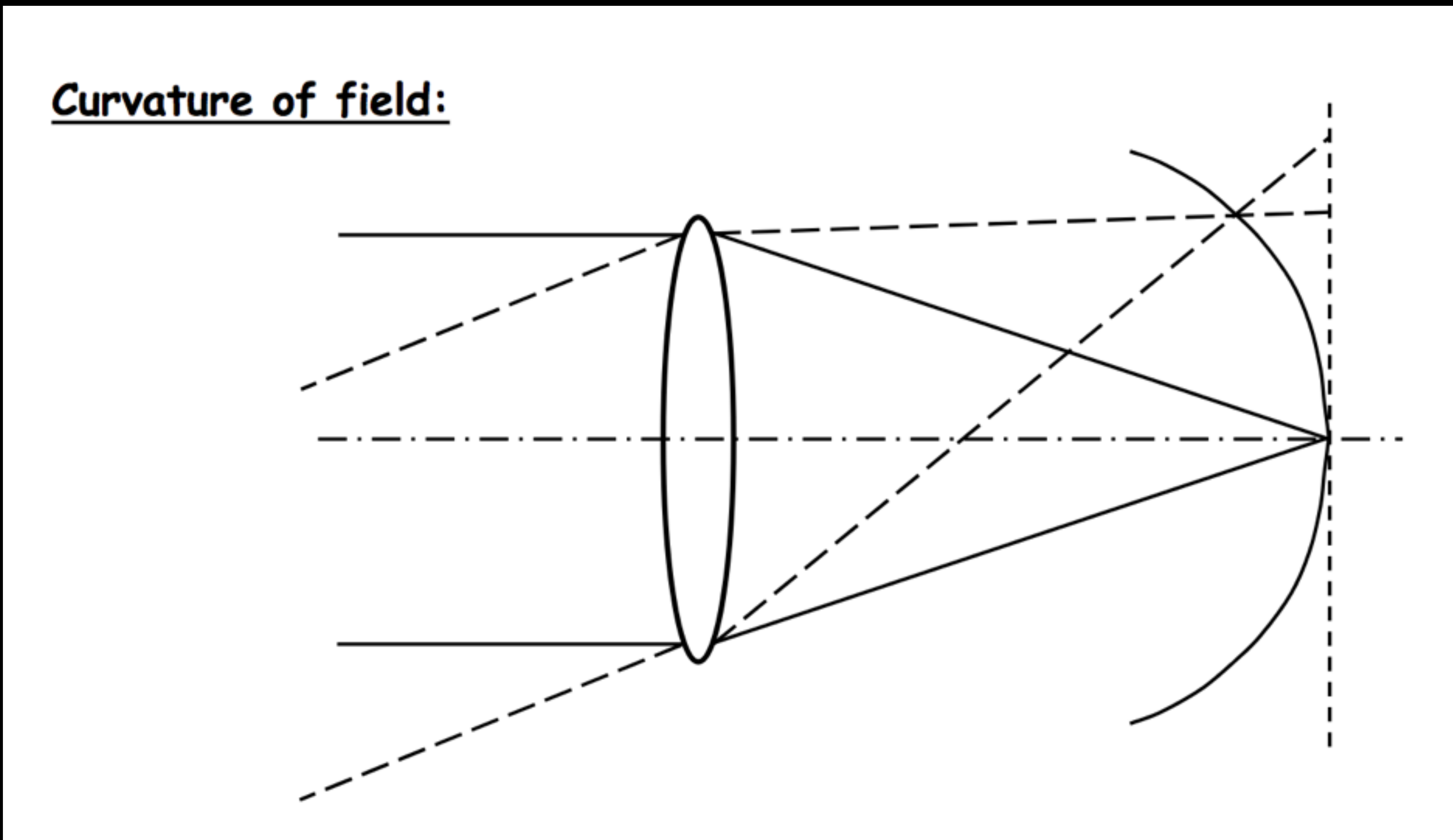

First lens: Crown glass    Second lens: Flint glass

# Not perfect

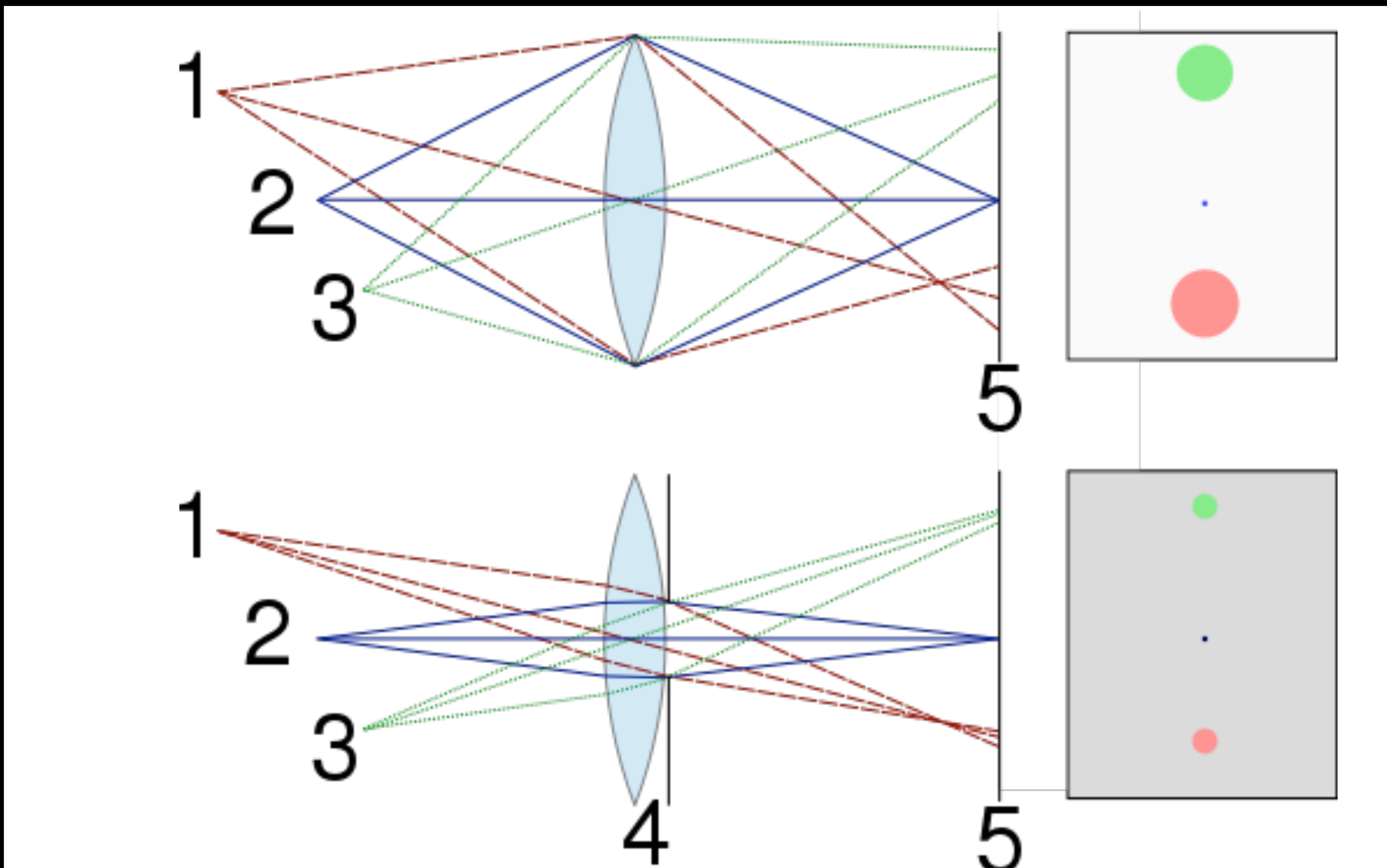


(Mirrors don’t have chromatic dispersion)

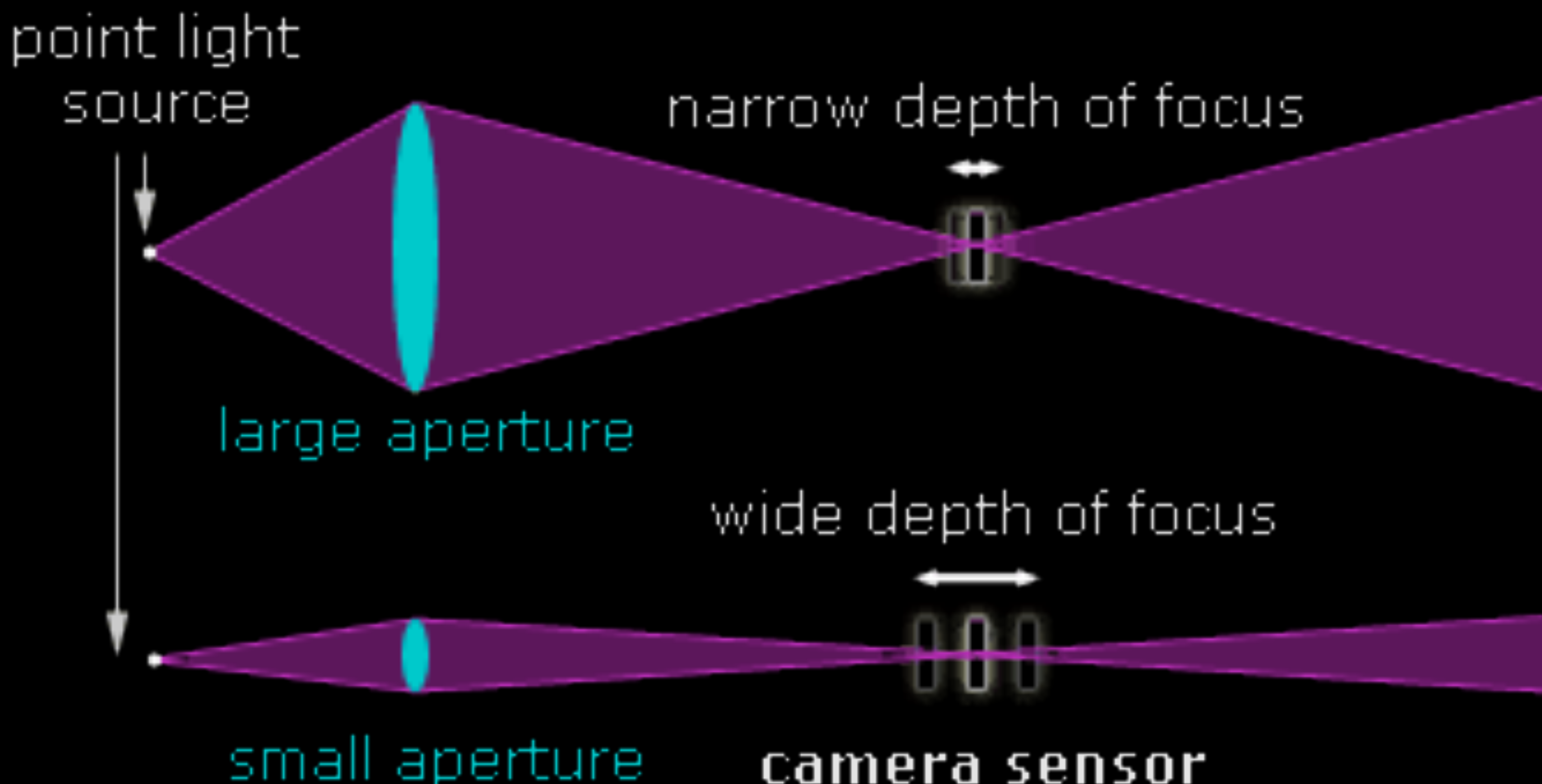
# Field Curvature



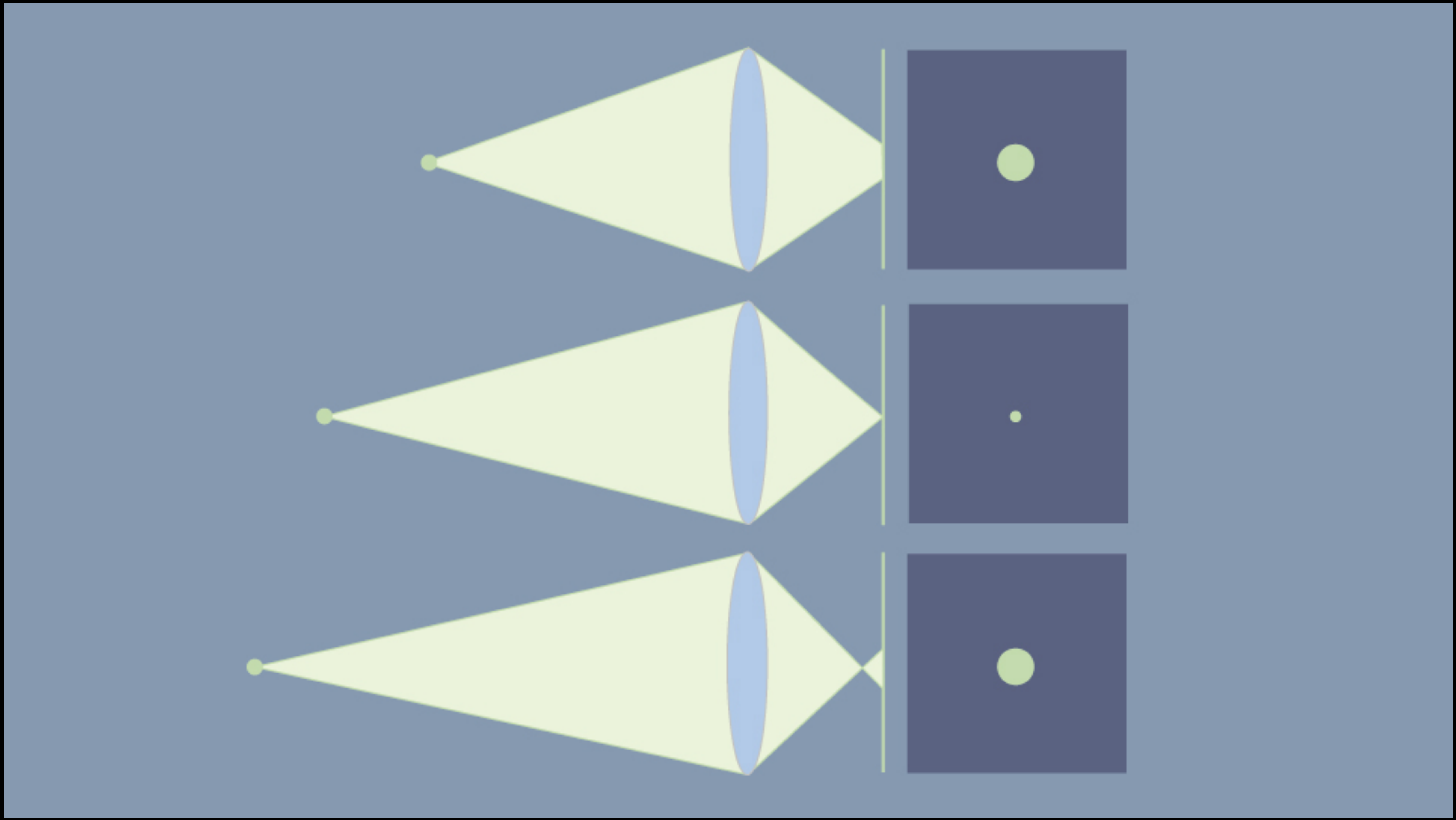
# Large aperture = less DOF



# Small aperture = more DOF



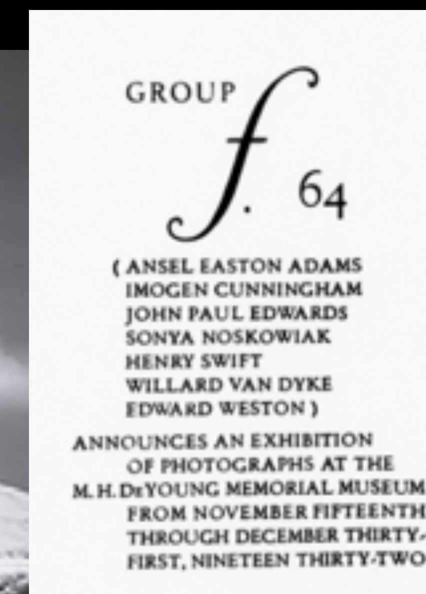
# Circle of Confusion



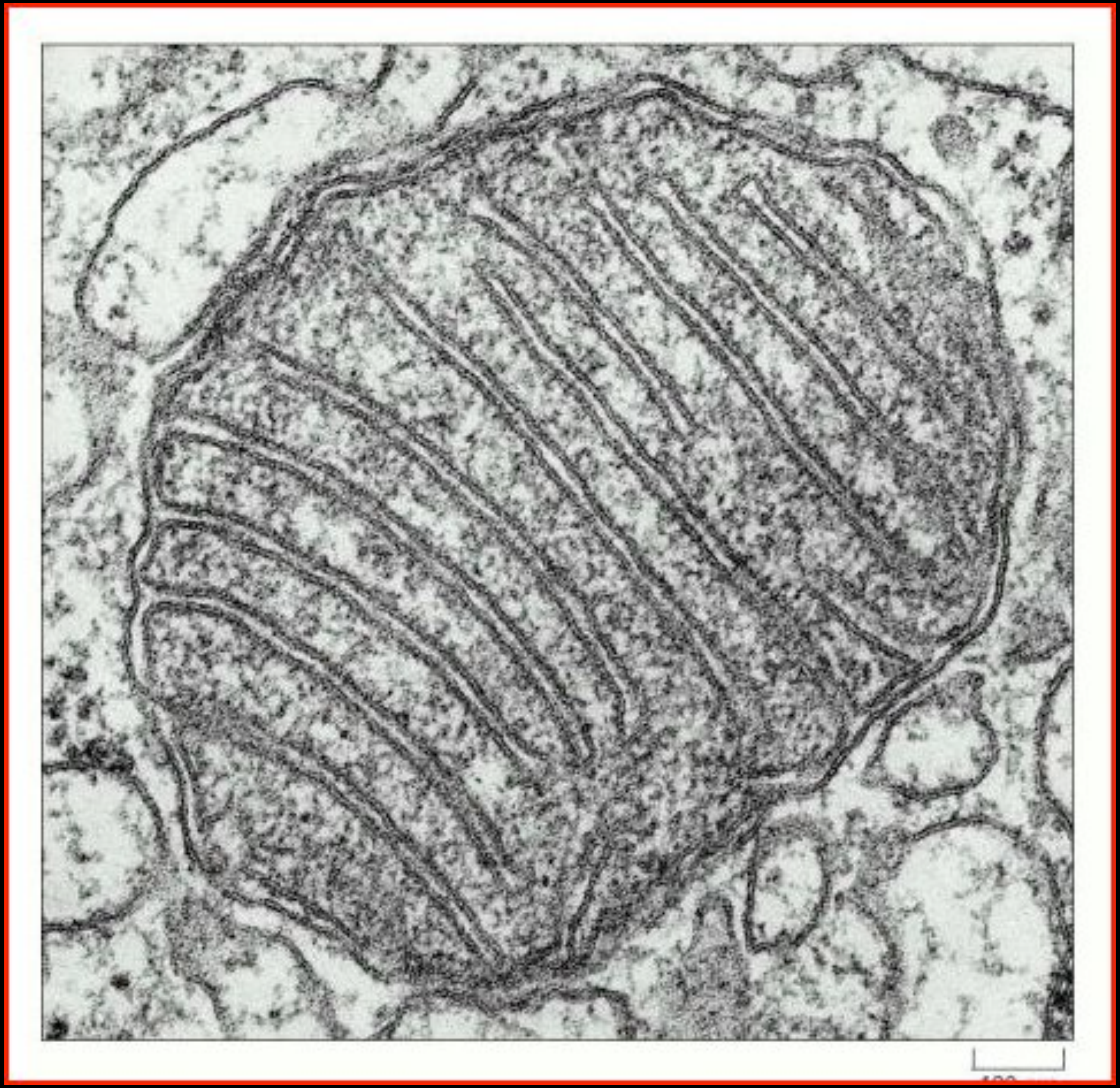
# Did I use a small or large aperture here?

Monarch butterfly (*Danaus plexippus*) on its favorite food, the milkweed plant

# Straight Photography

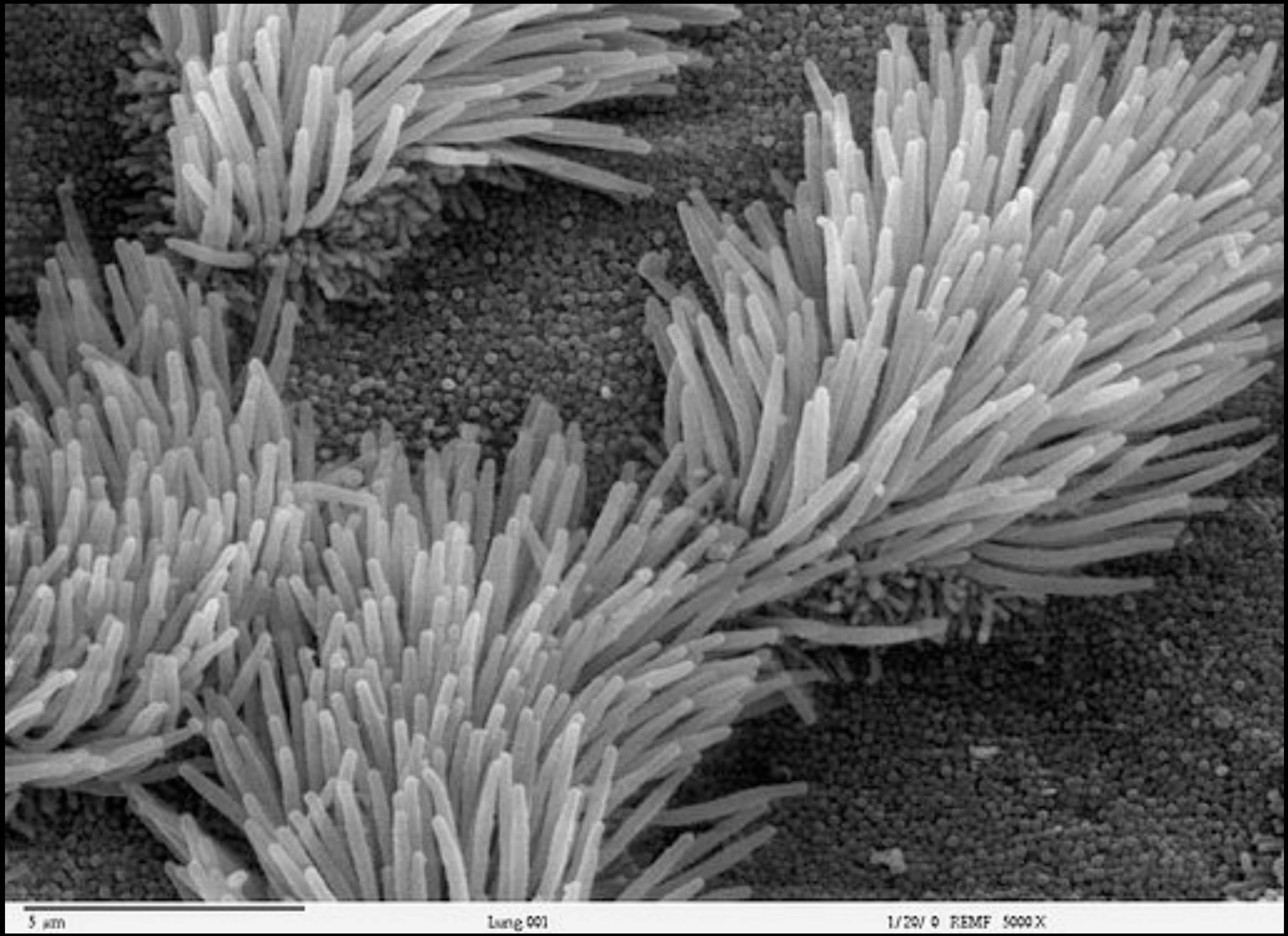


# Transmission Electron Microscopy

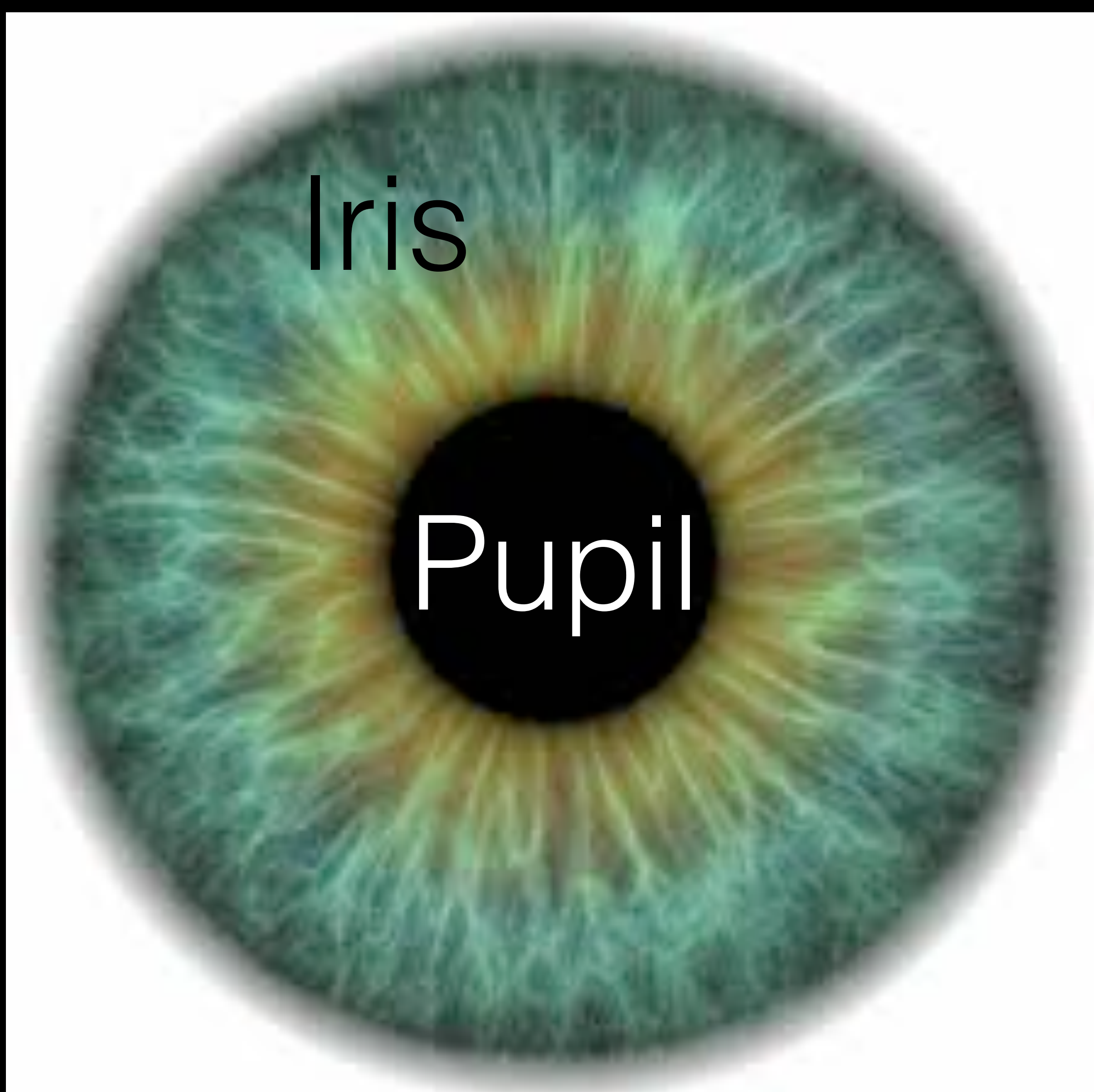


100nm

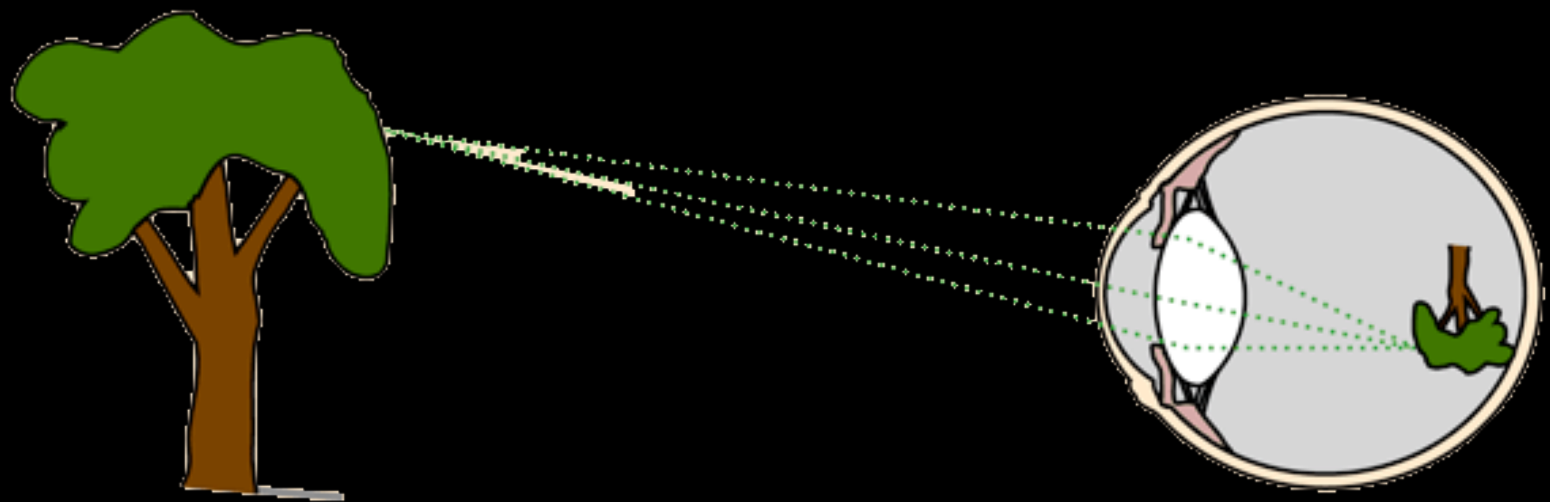
# Scanning Electron Micrograph

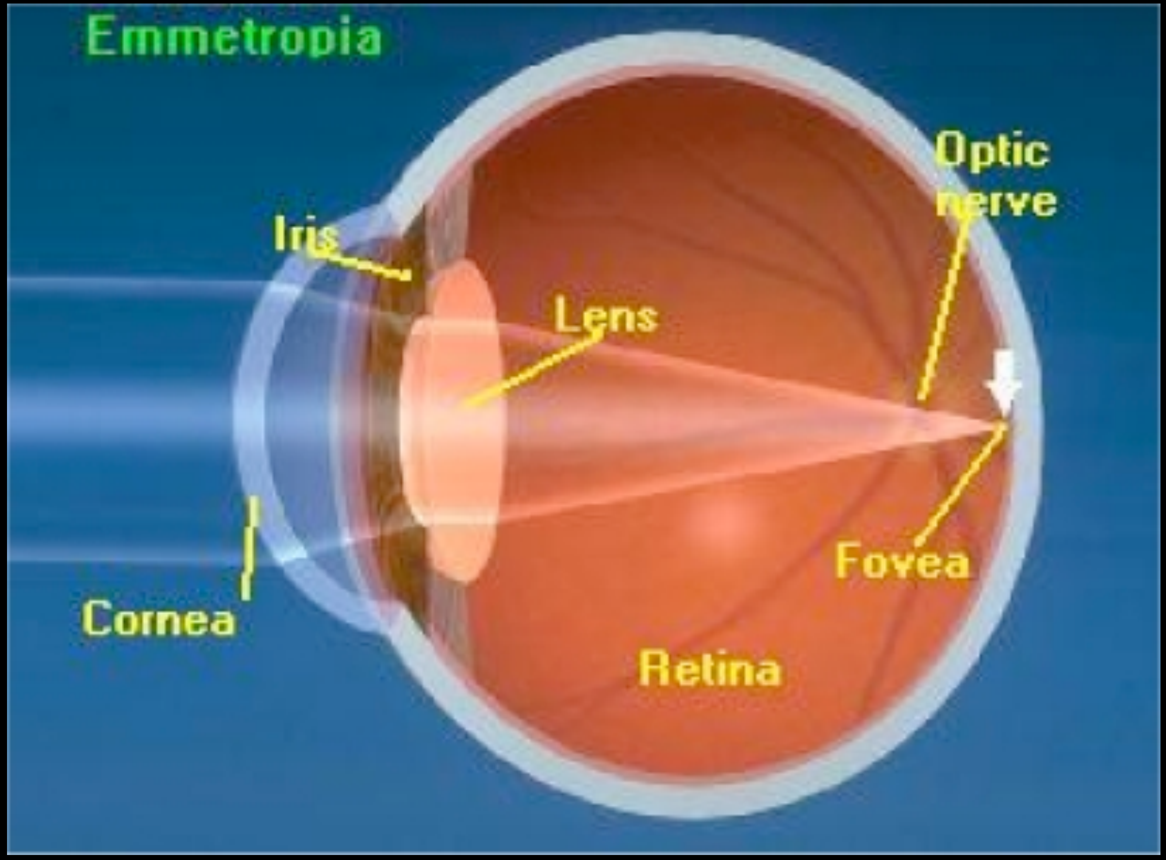


# Iris *vs.* pupil

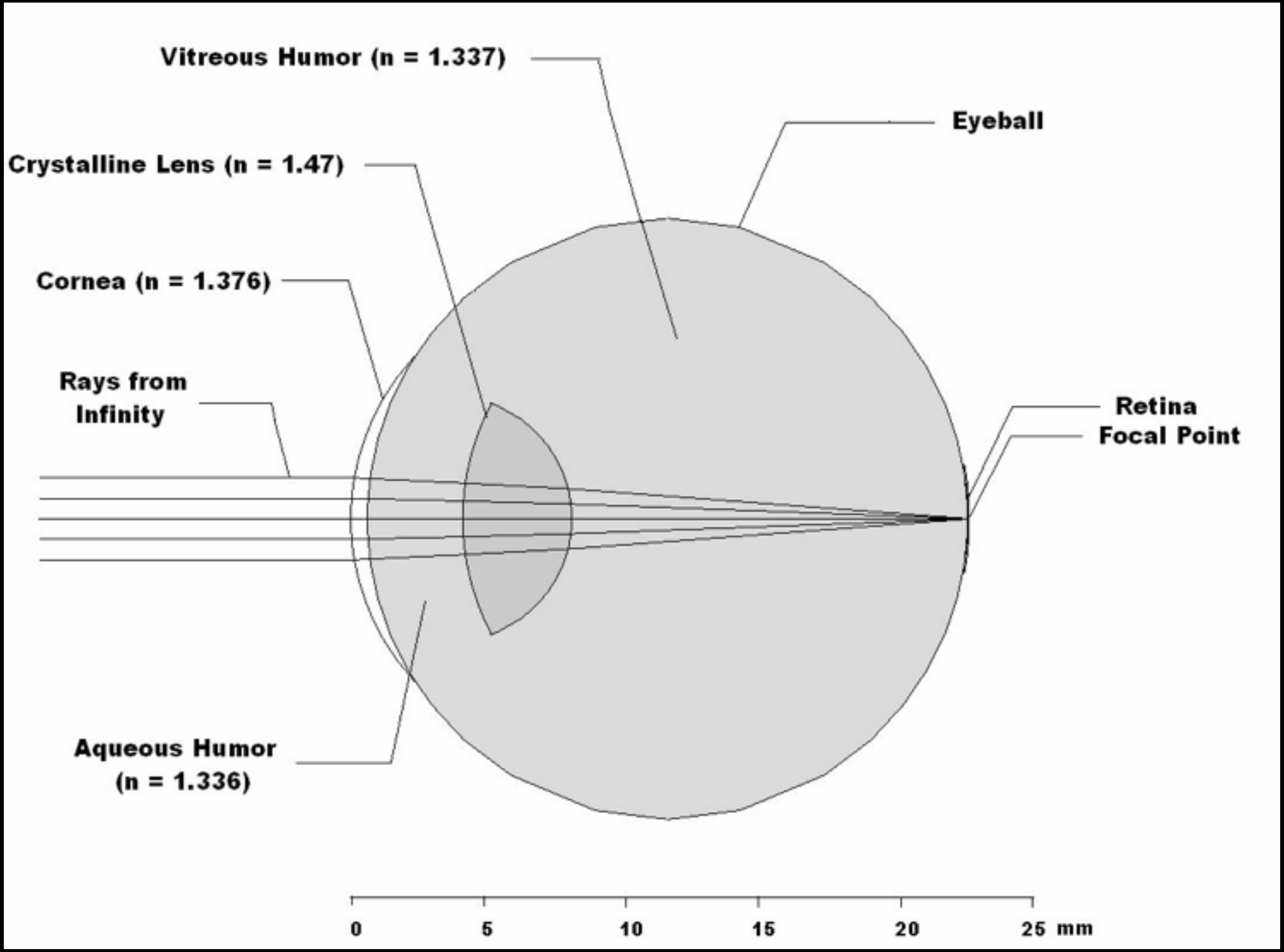



# Image Formation in the Eye

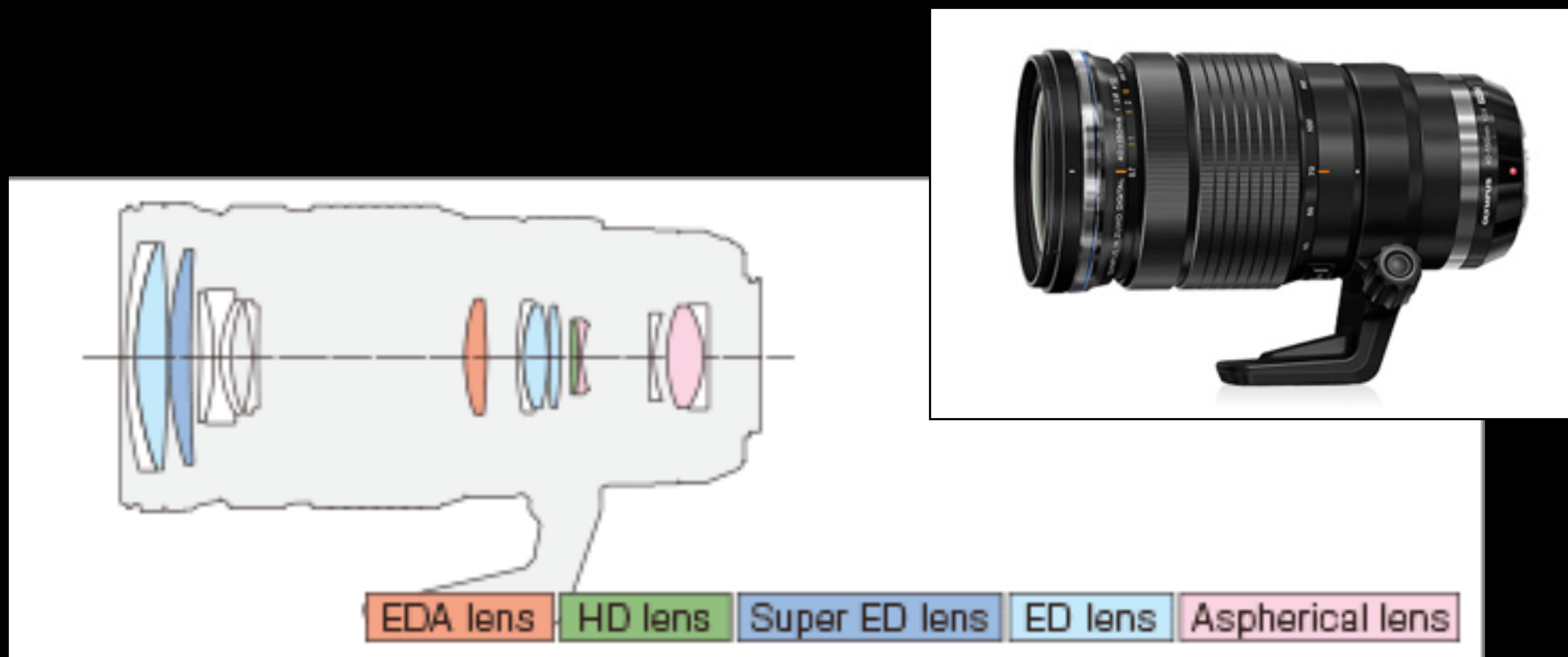




# Refractive Index map



# Camera Objective




Camera objectives contain many different lenses that act together as a single next-to-perfect lens

This is necessary to correct for <u>optical aberrations</u>

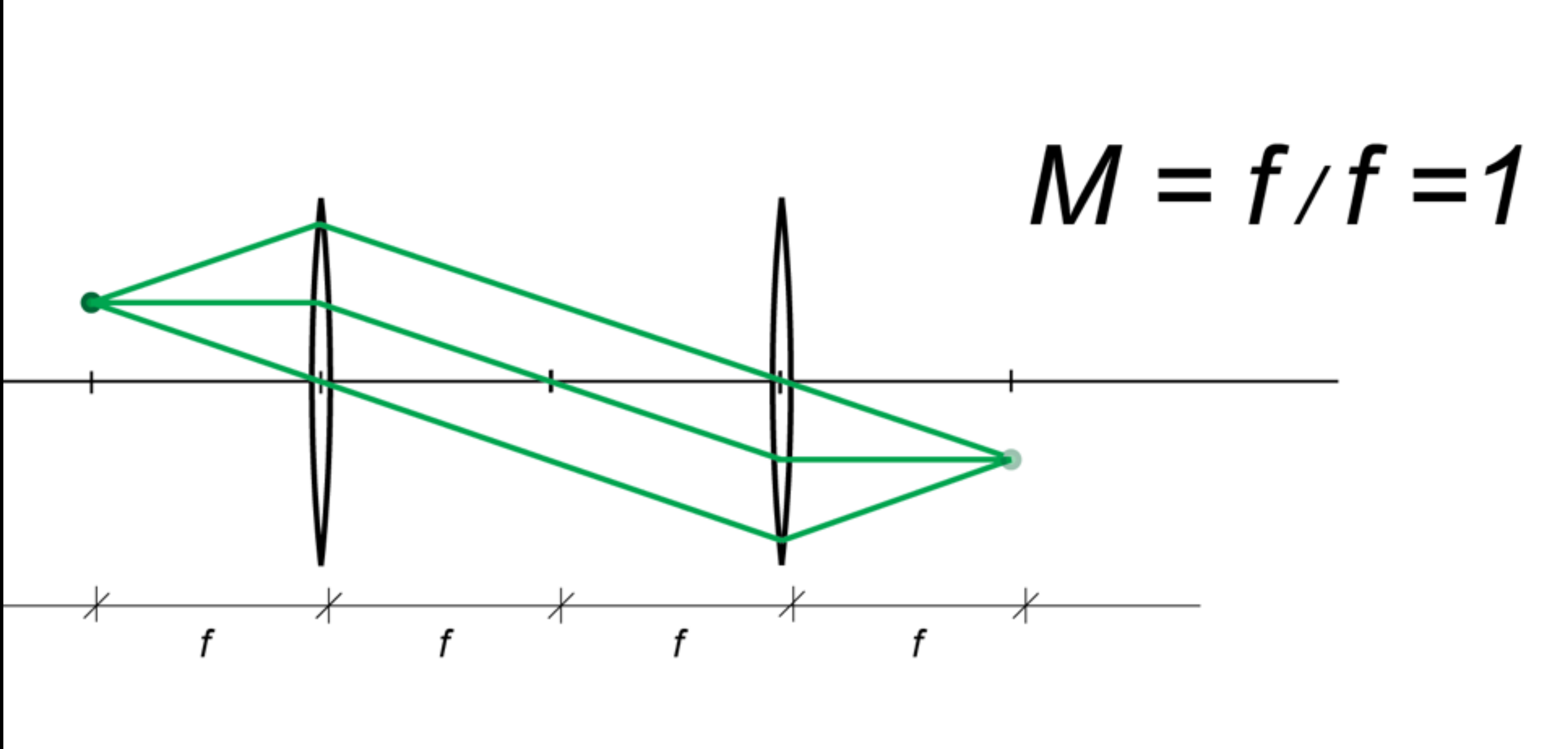
# Optical Aberrations

- Aberrations deteriorate image quality. Lens systems are designed to mimic a single, ideal, infinitely thin lens.

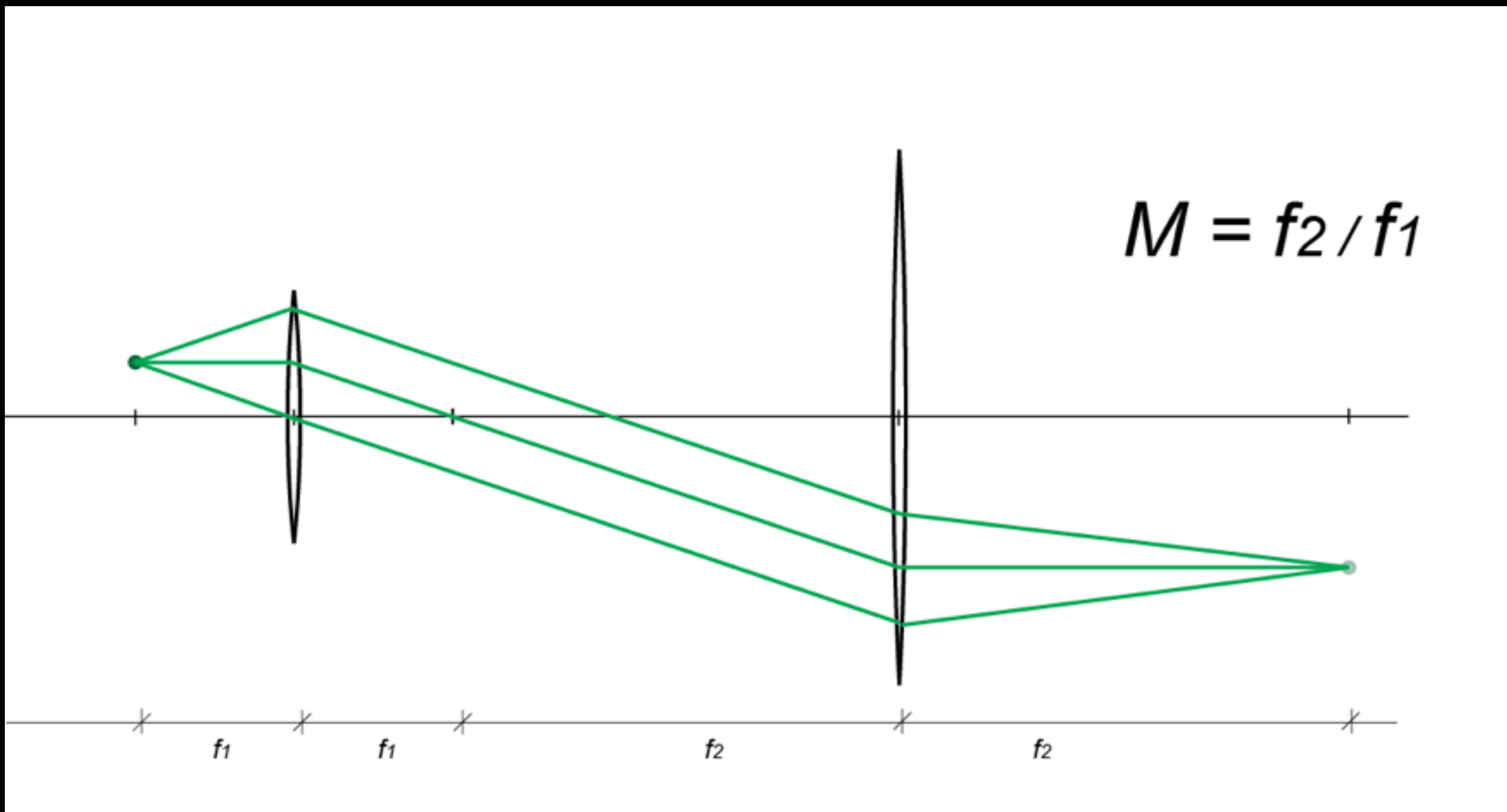
# Microscope Objective

Figure 1

# Imaging with two lenses

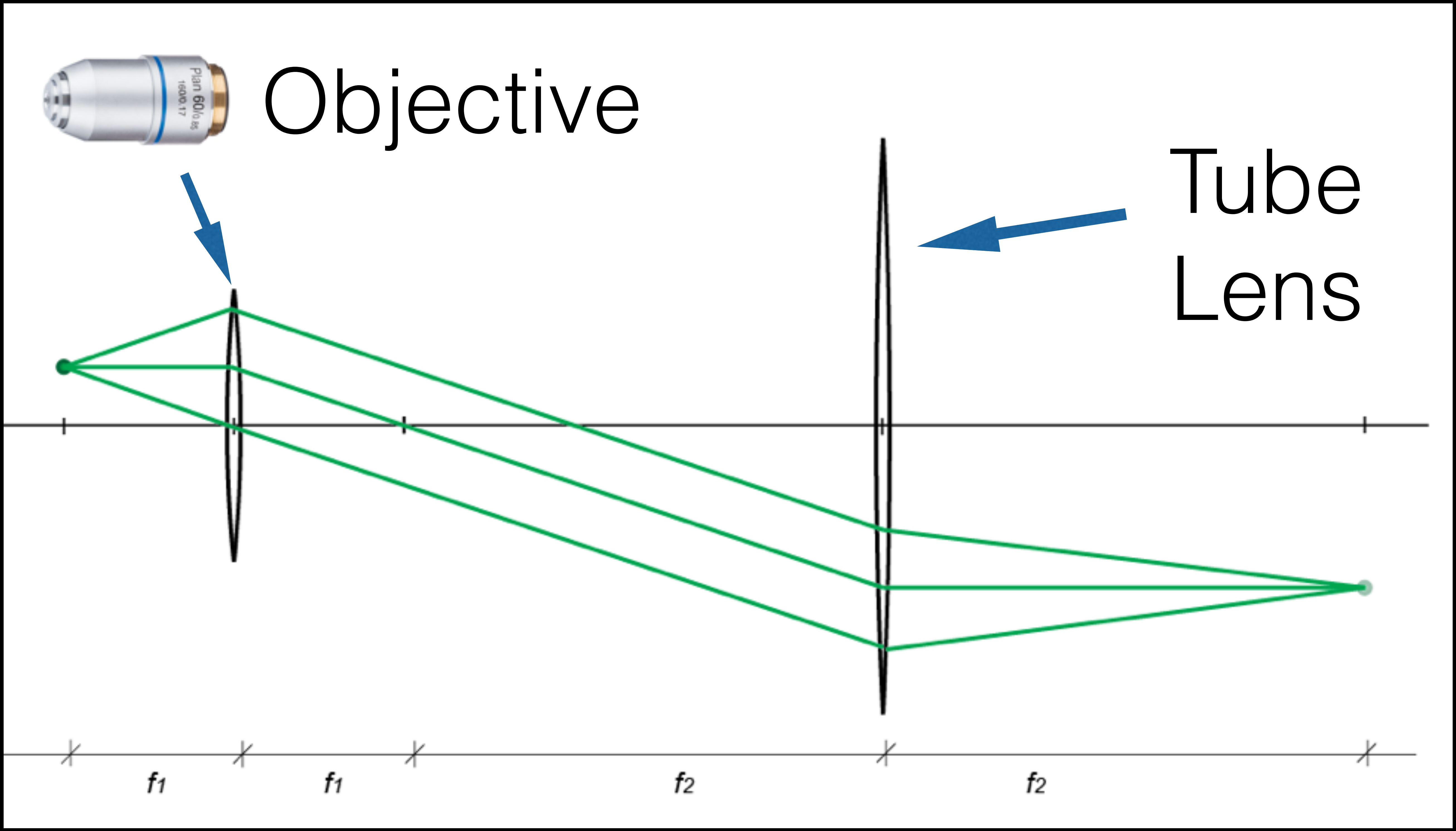


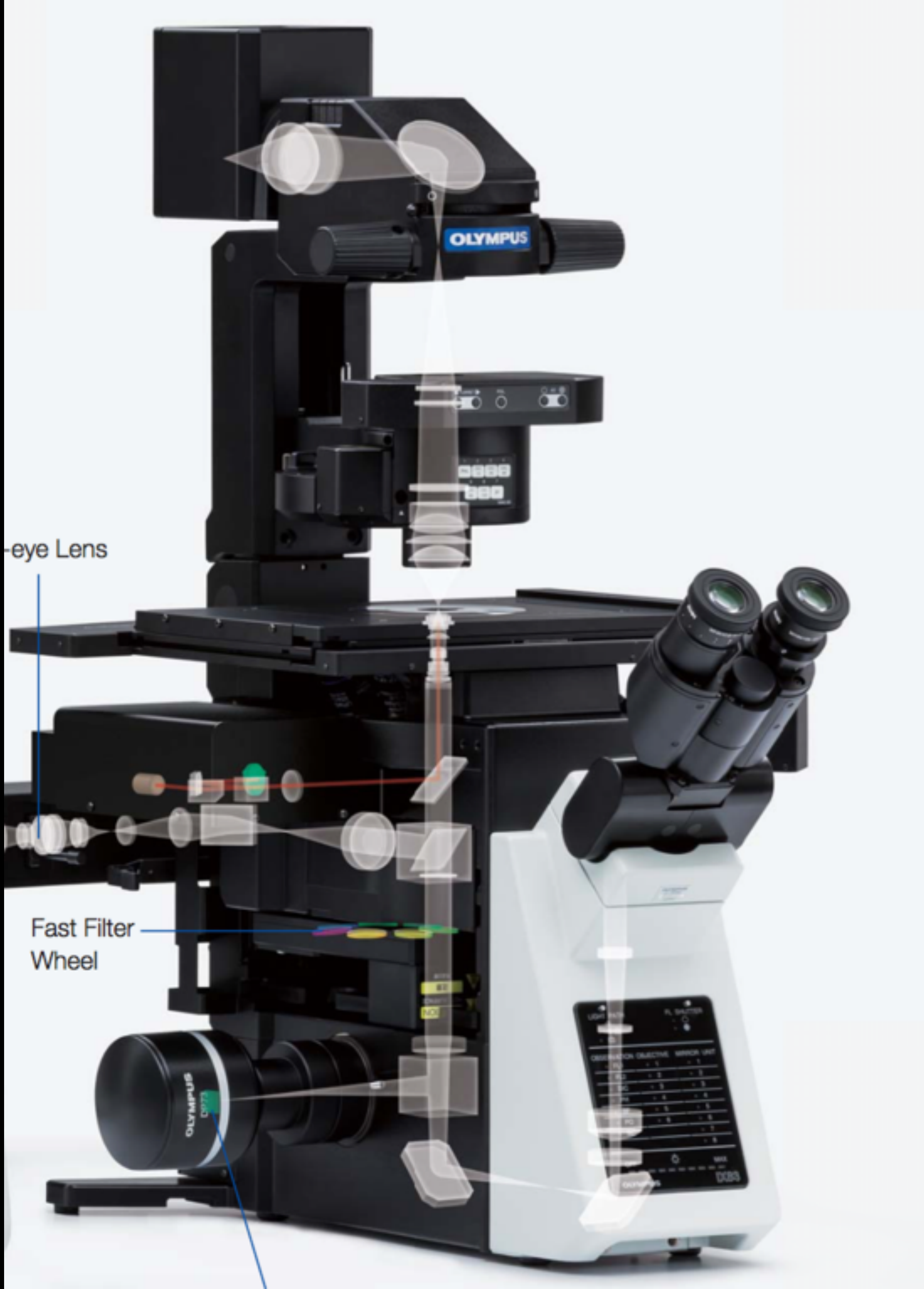
# Magnification




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

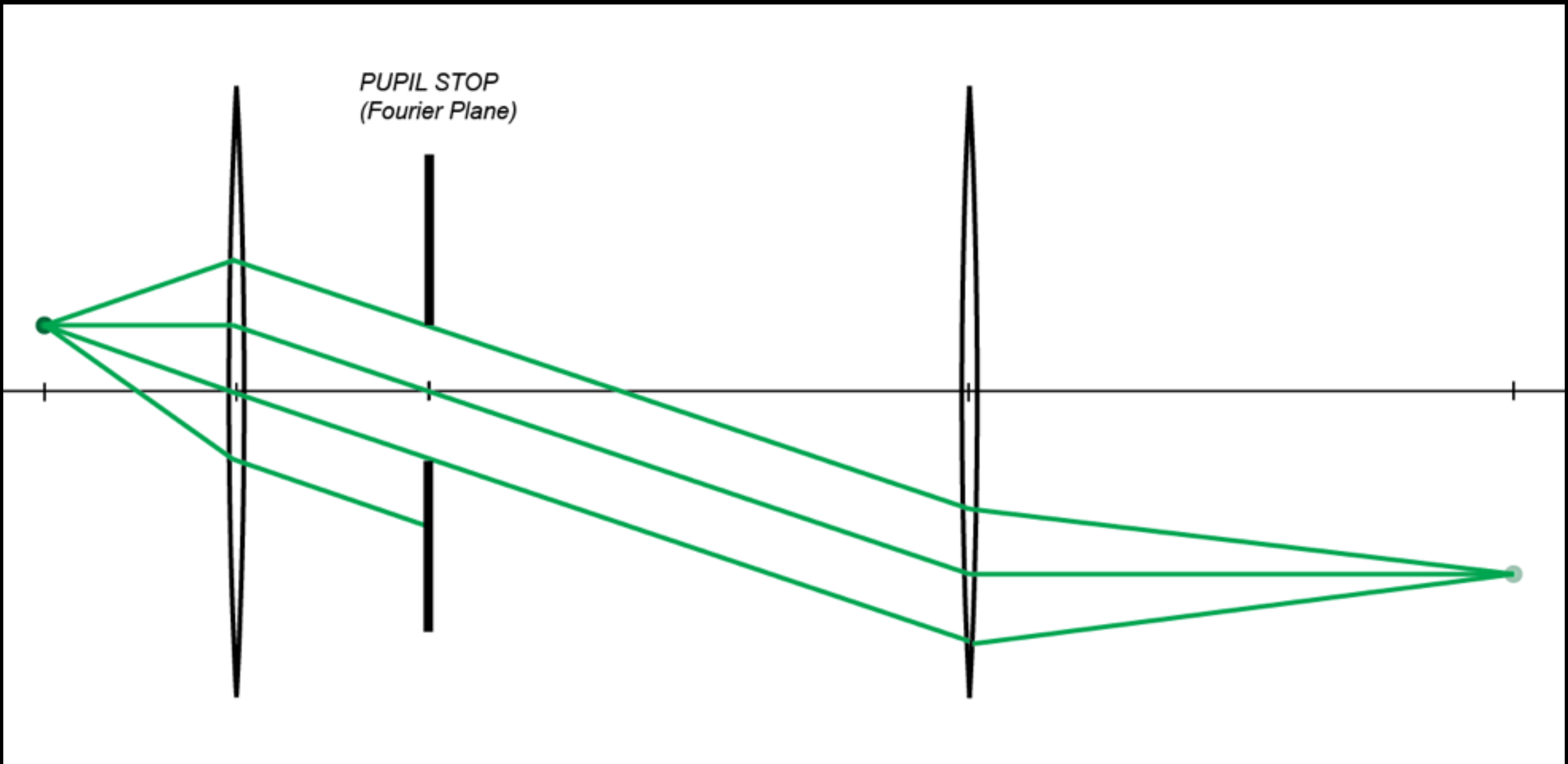
# Microscope:





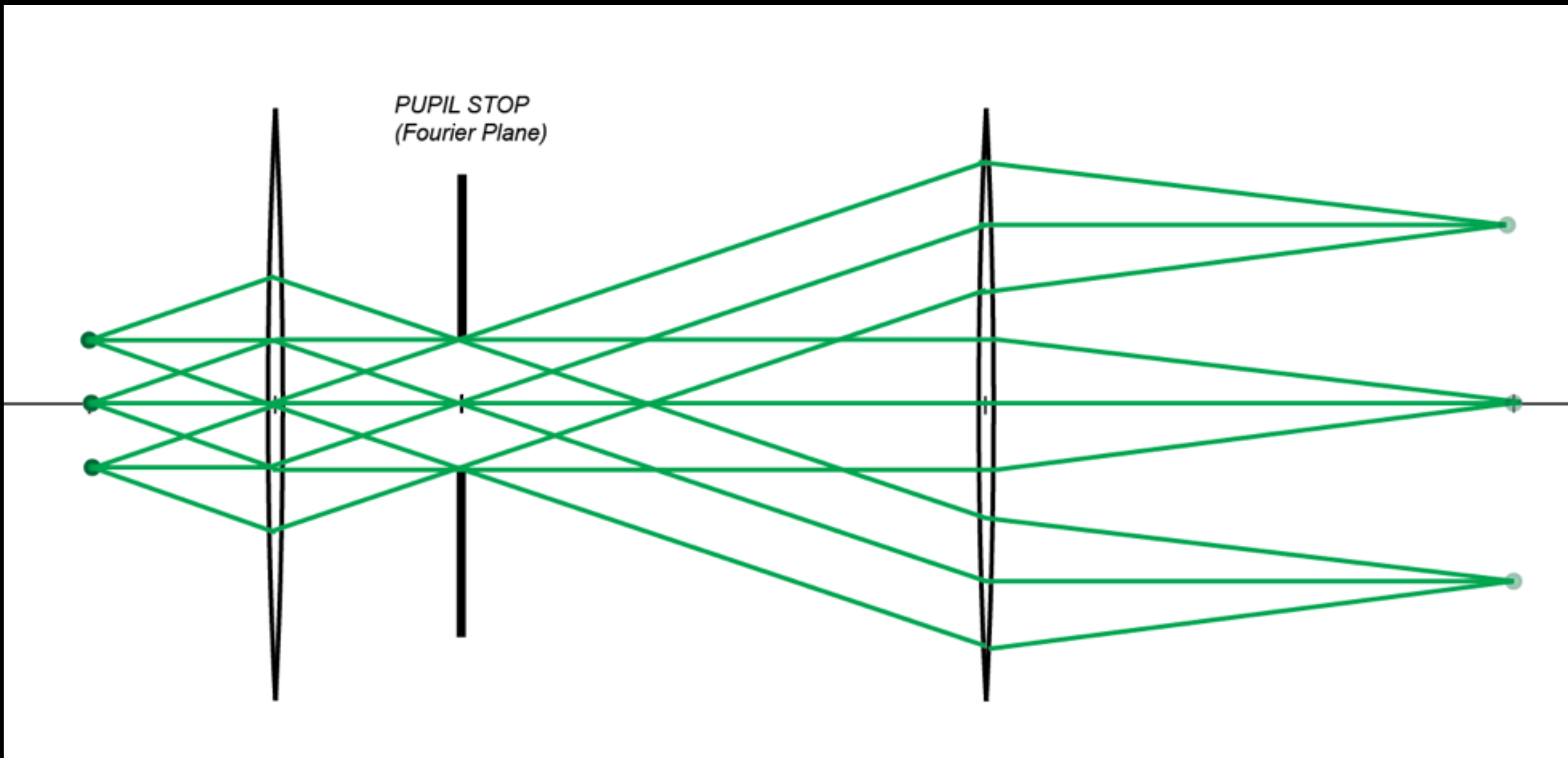
-eye Lens
Fast Filter
Wheel
OLYMPUS

# Pupil Stop



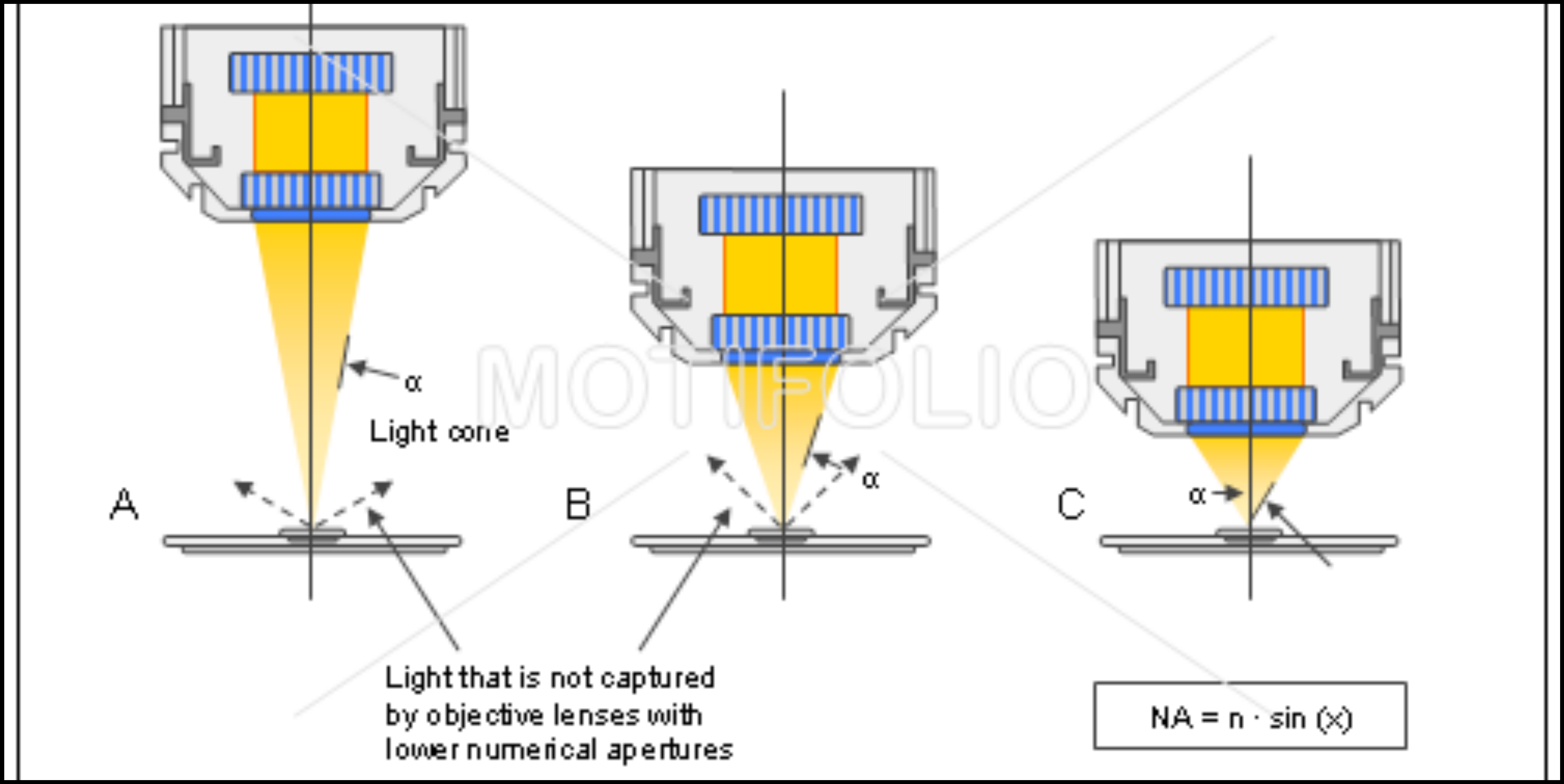

Pupil stop determines Numerical Aperture (NA)

# Angle *vs.* Position

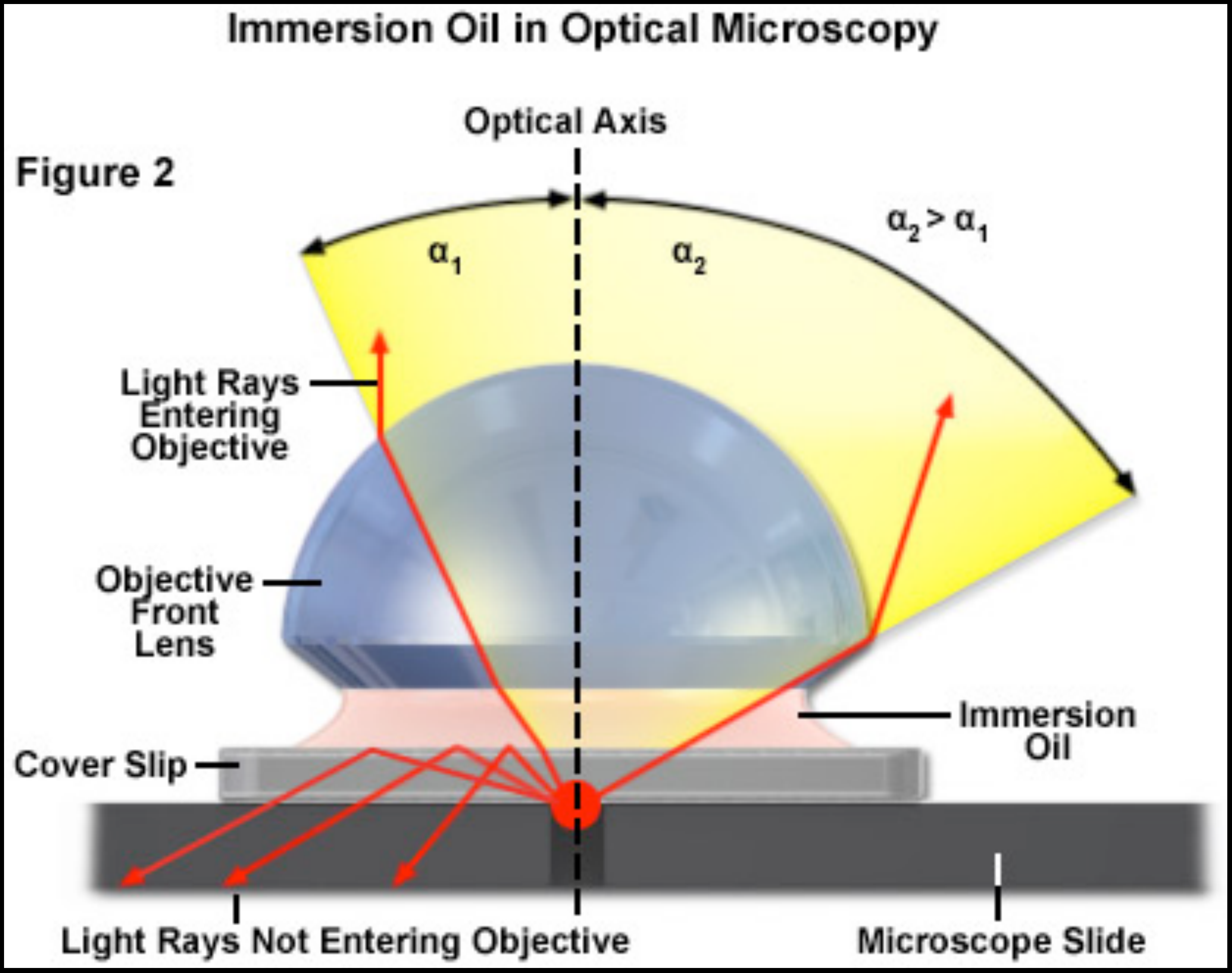


# NA = n sin(a)

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

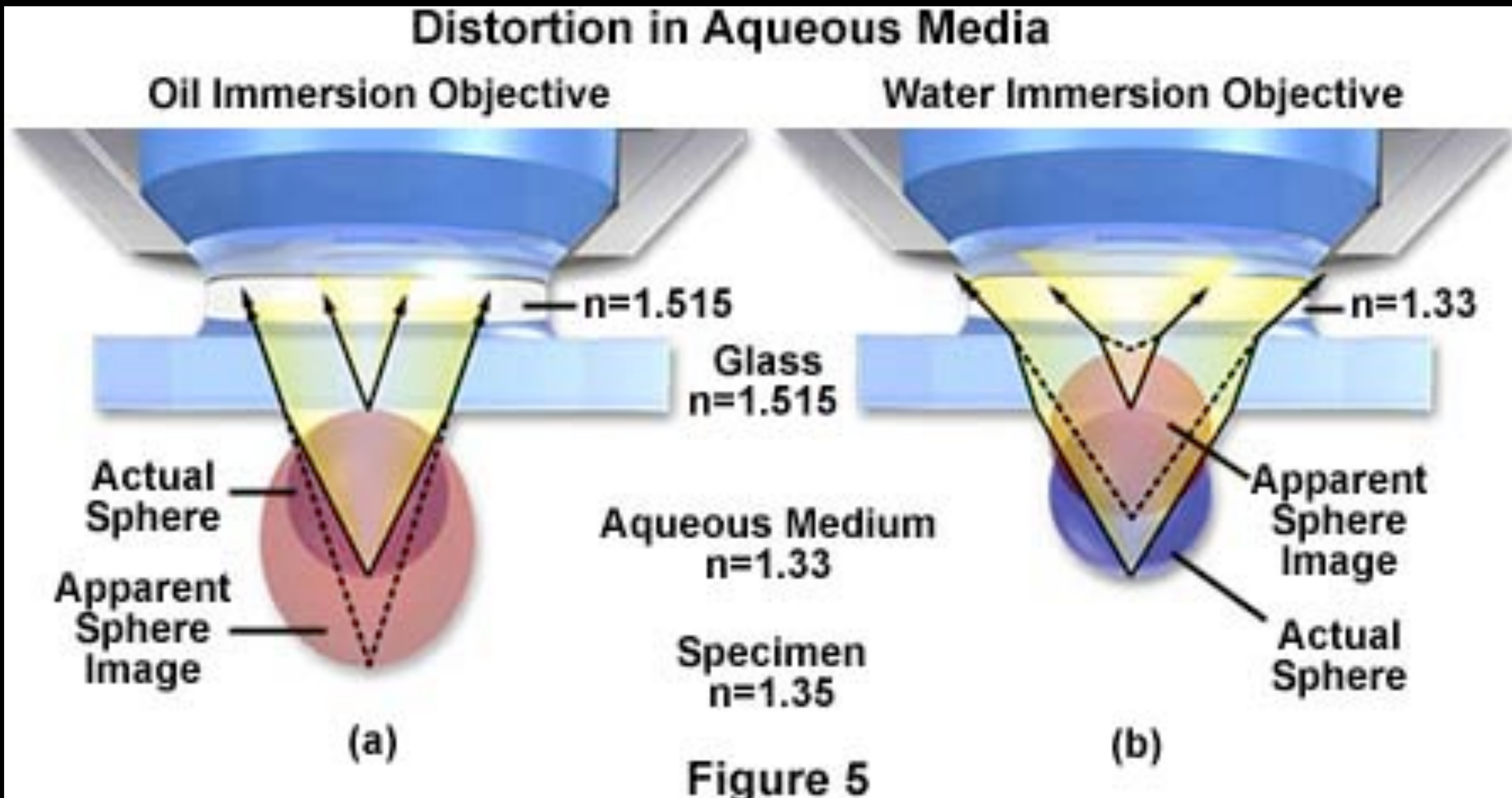


# Immersion Medium



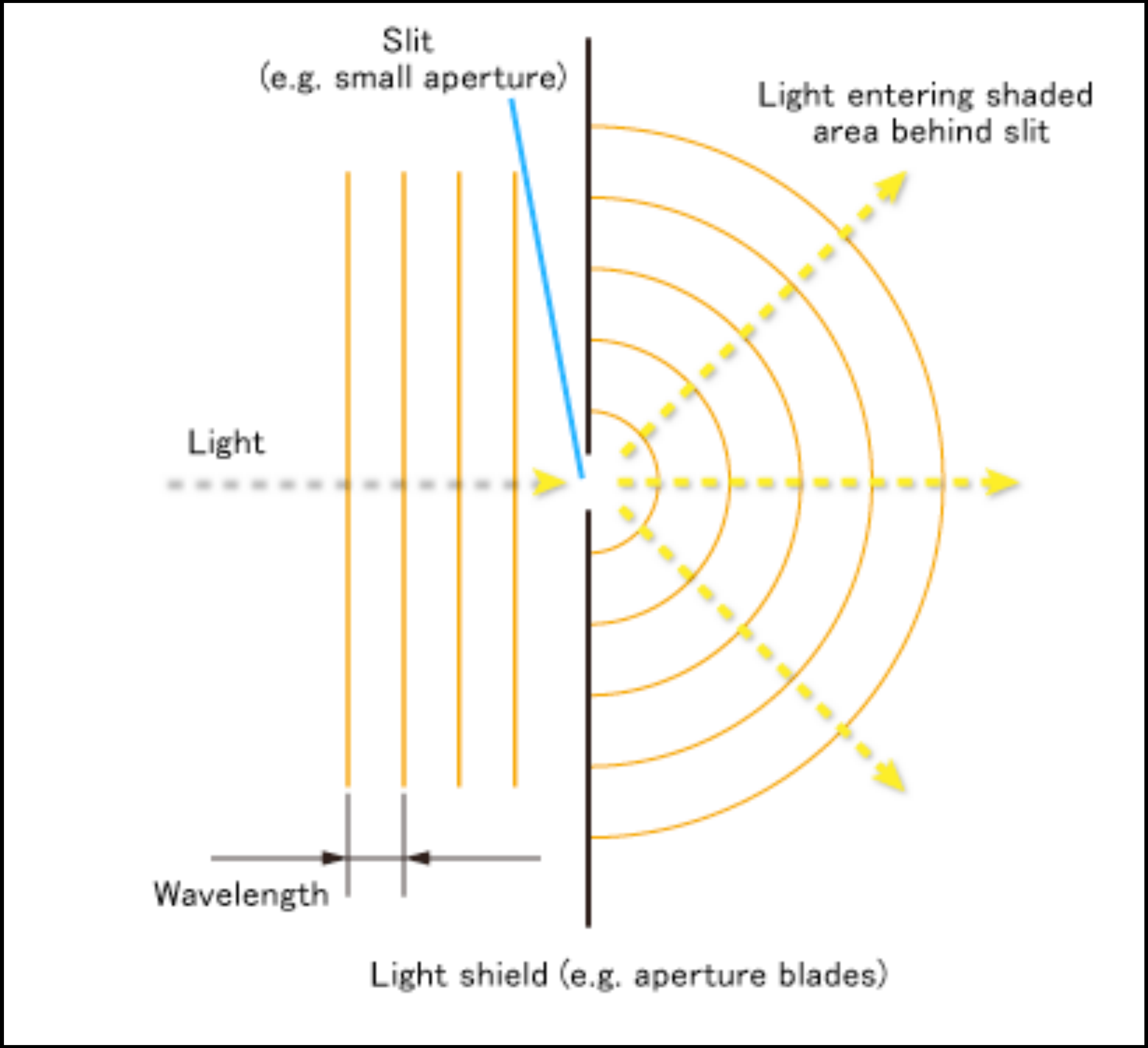
# Match to Specimen!

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

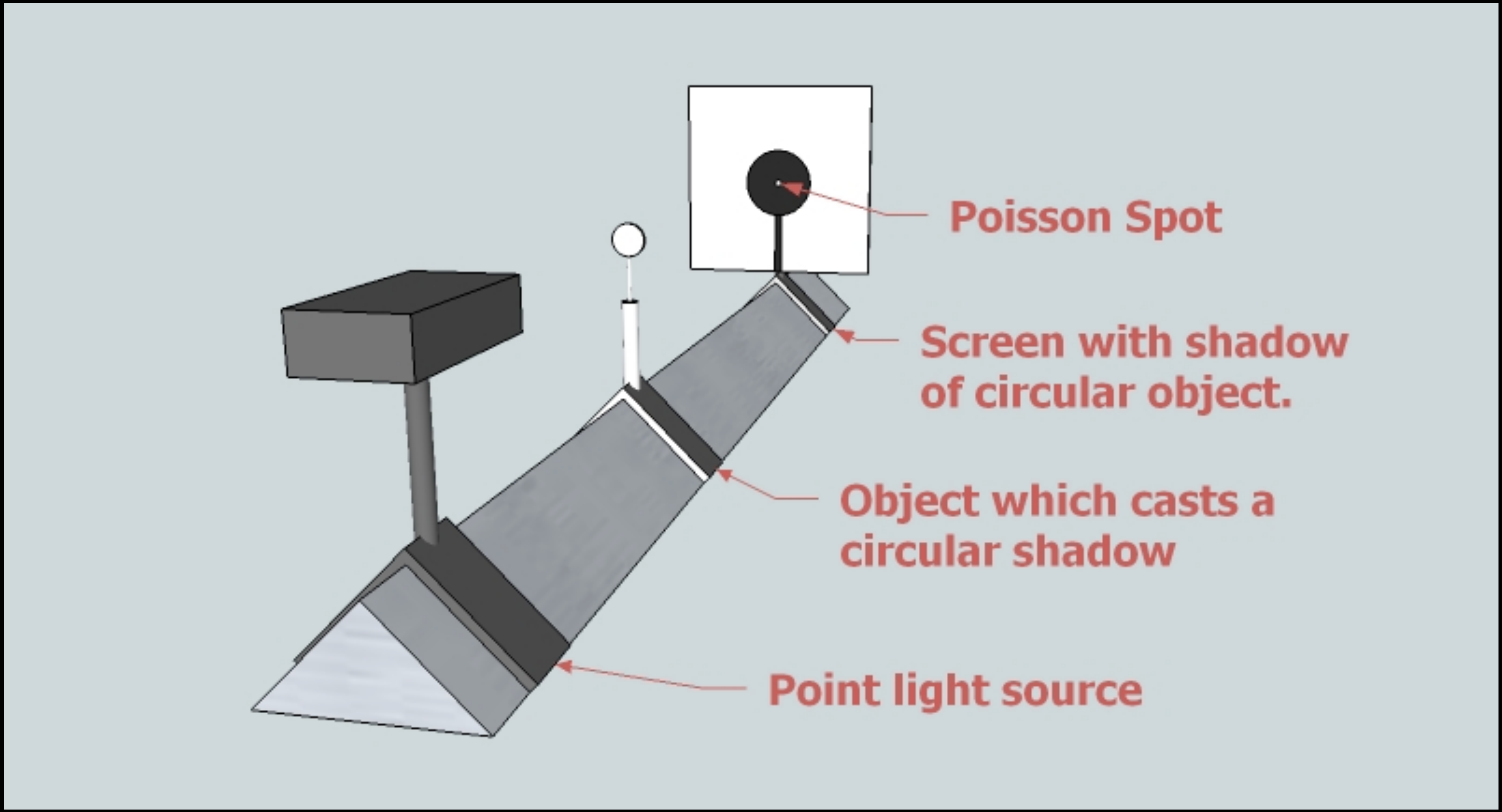

Distortion in Aqueous Media
Oil Immersion Objective
Water Immersion Objective
n=1.515
n=1.33
Glass
n=1.515
Actual
Sphere
Apparent
Sphere
Image
Aqueous Medium
n=1.33
Specimen
n=1.35
Apparent
Sphere
Image
Actual
Sphere
(a)
(b)
Figure 5

# EXAMPLES AND QUESTIONS

# Diffraction

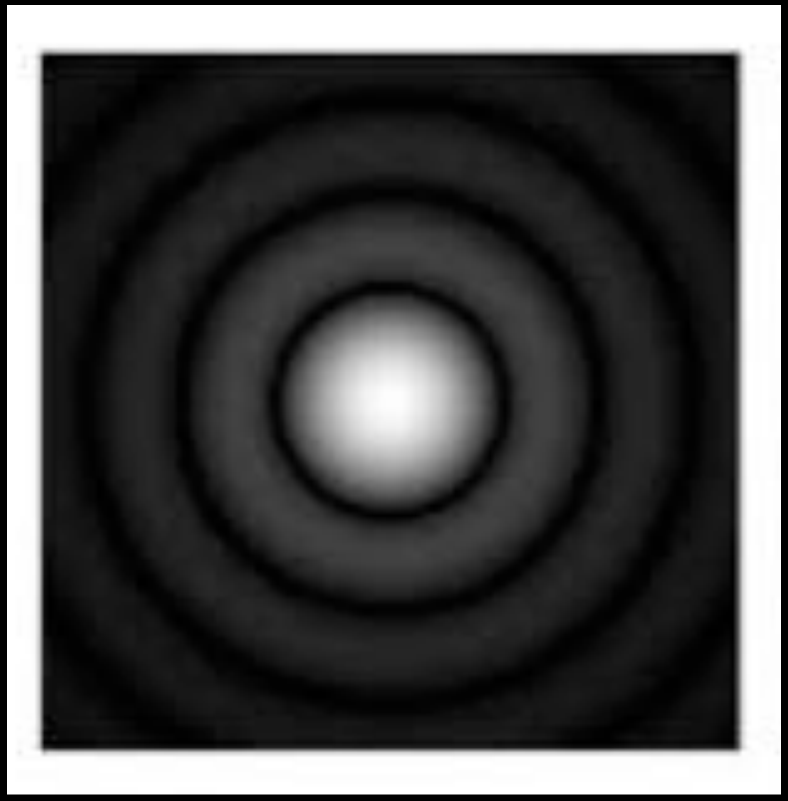


# Poisson’s spot




Light ripples and bends around edges too

# The Airy Disc

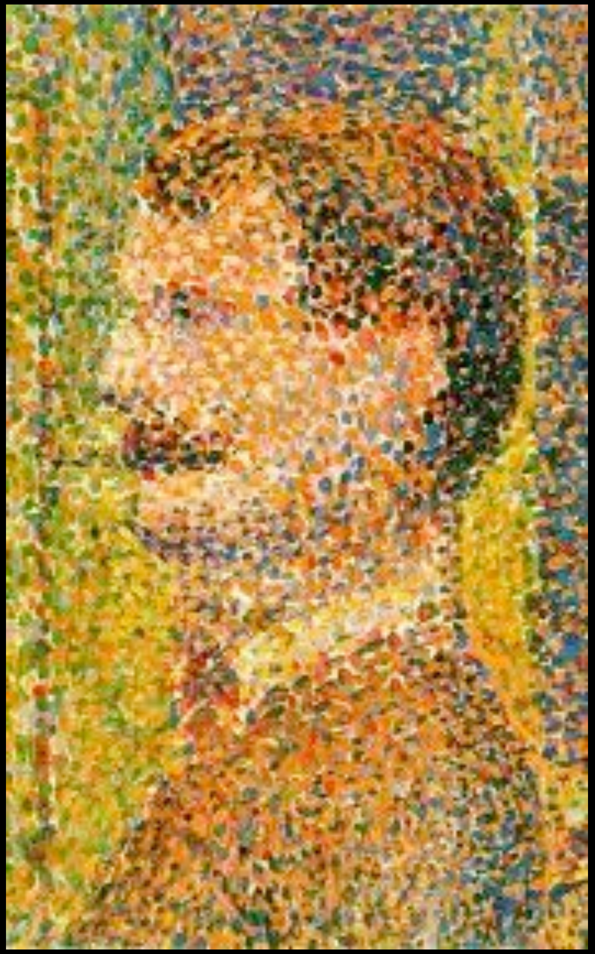


Arises from diffraction when we image through a circular aperture

# Imaging Point Sources

- The object can be modeled as a number of point sources

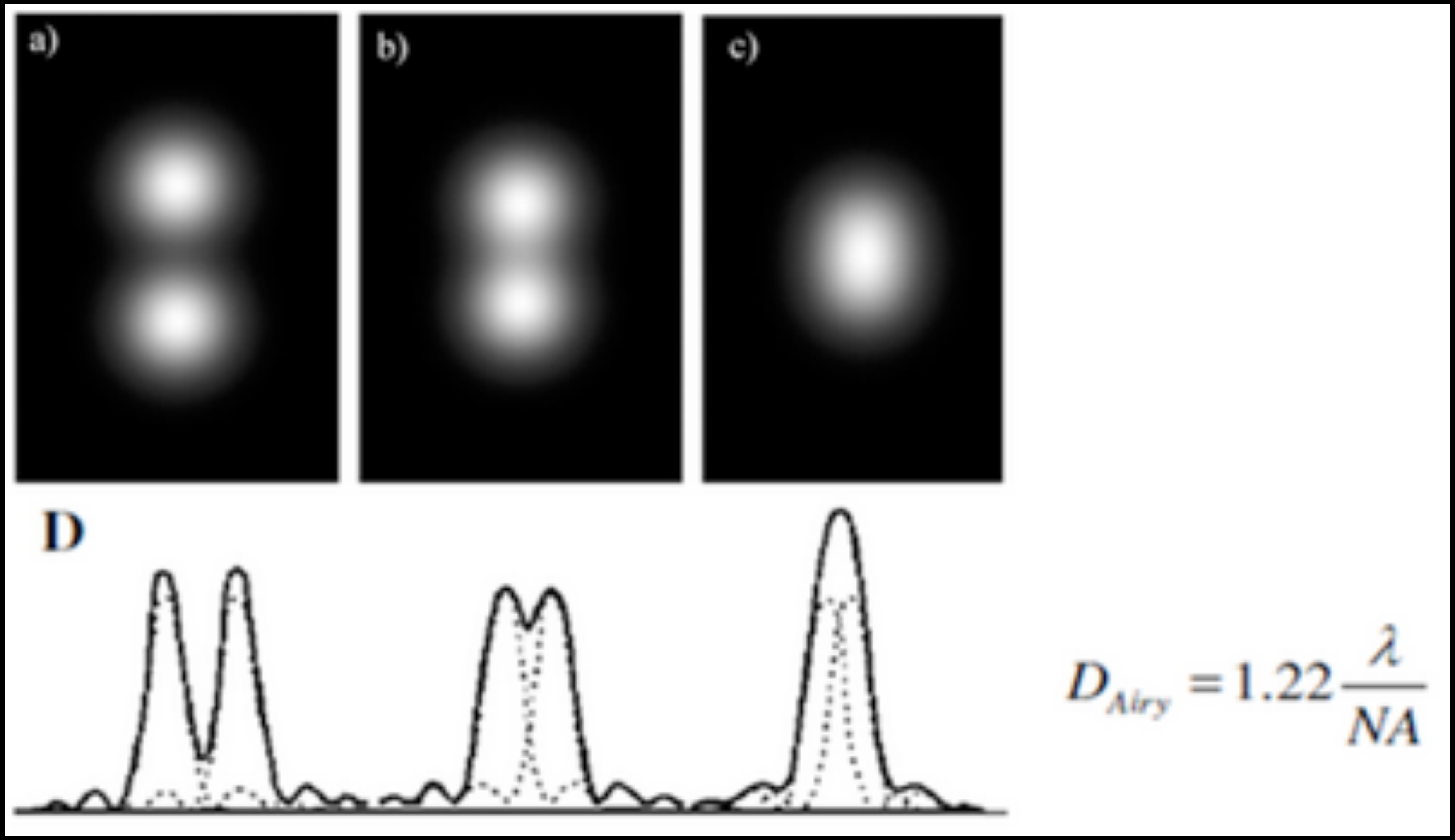
- The points are blurred by the imaging system
  (The response function of a point imaged through a circular aperture is the Airy disc)



(from Seurat's *the Circus*)

# Resolution criteria:

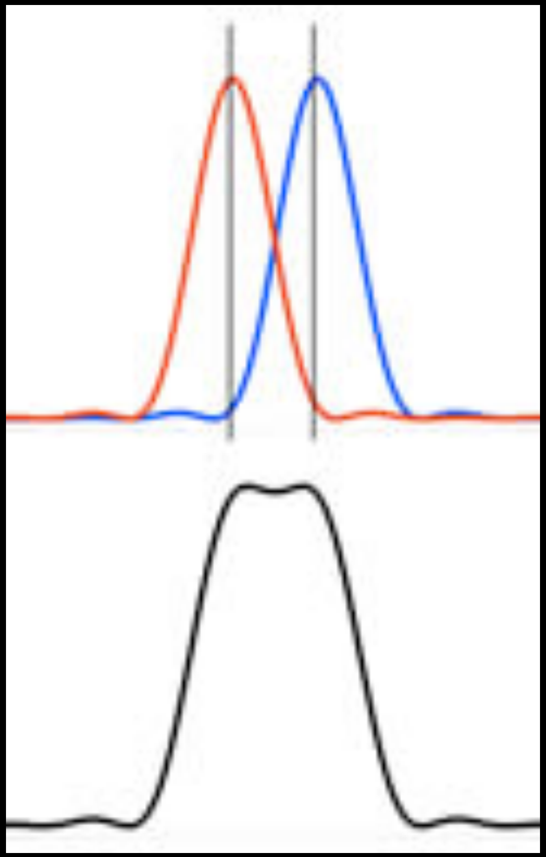
- Raleigh
- Sparrow
- Airy
- Abbe



# 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

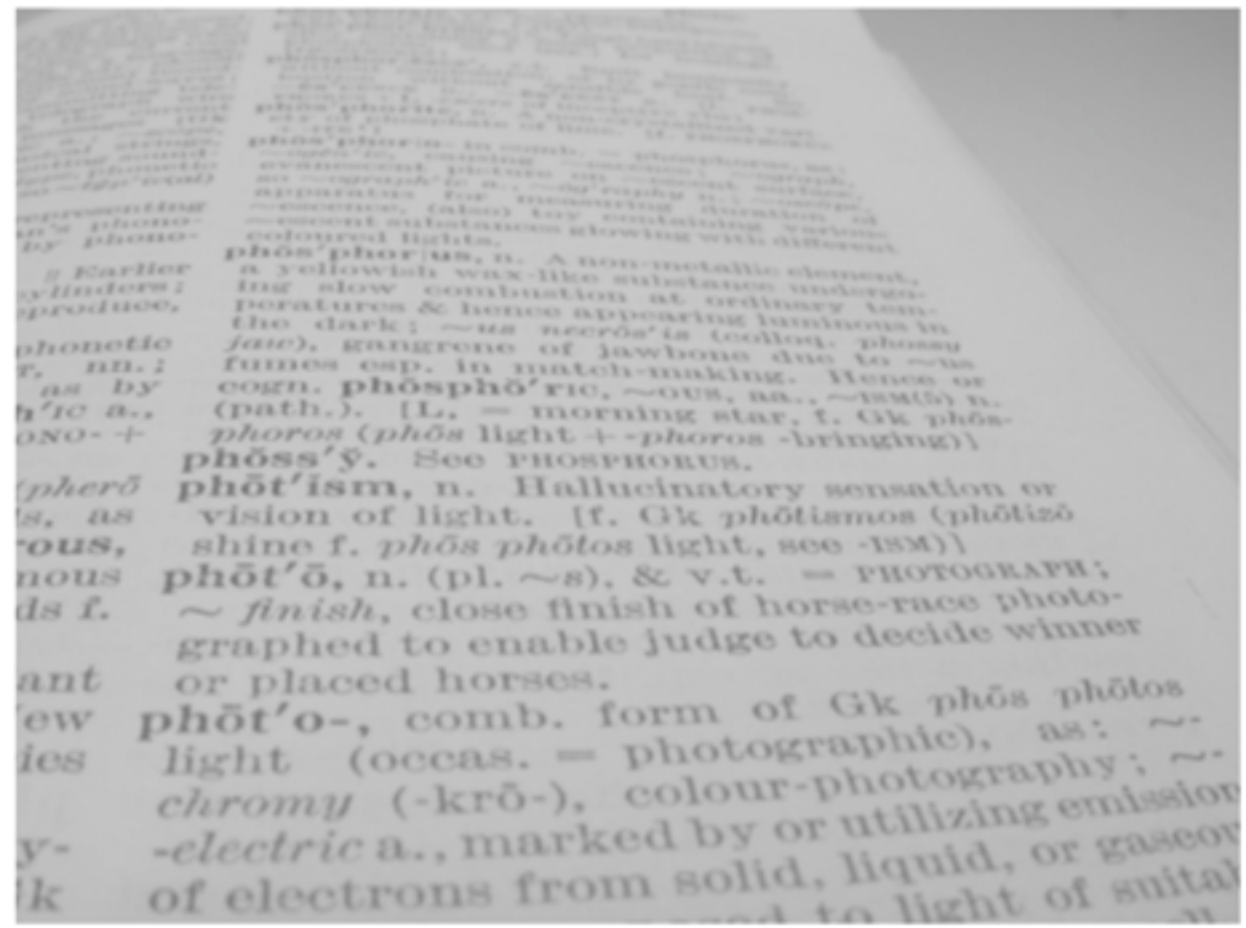
# Resolution

High resolution

Low resolution

# Contrast


High contrast




Low contrast

# Depth of field


Large depth of field


Small depth of field

# Modulation Transfer Function

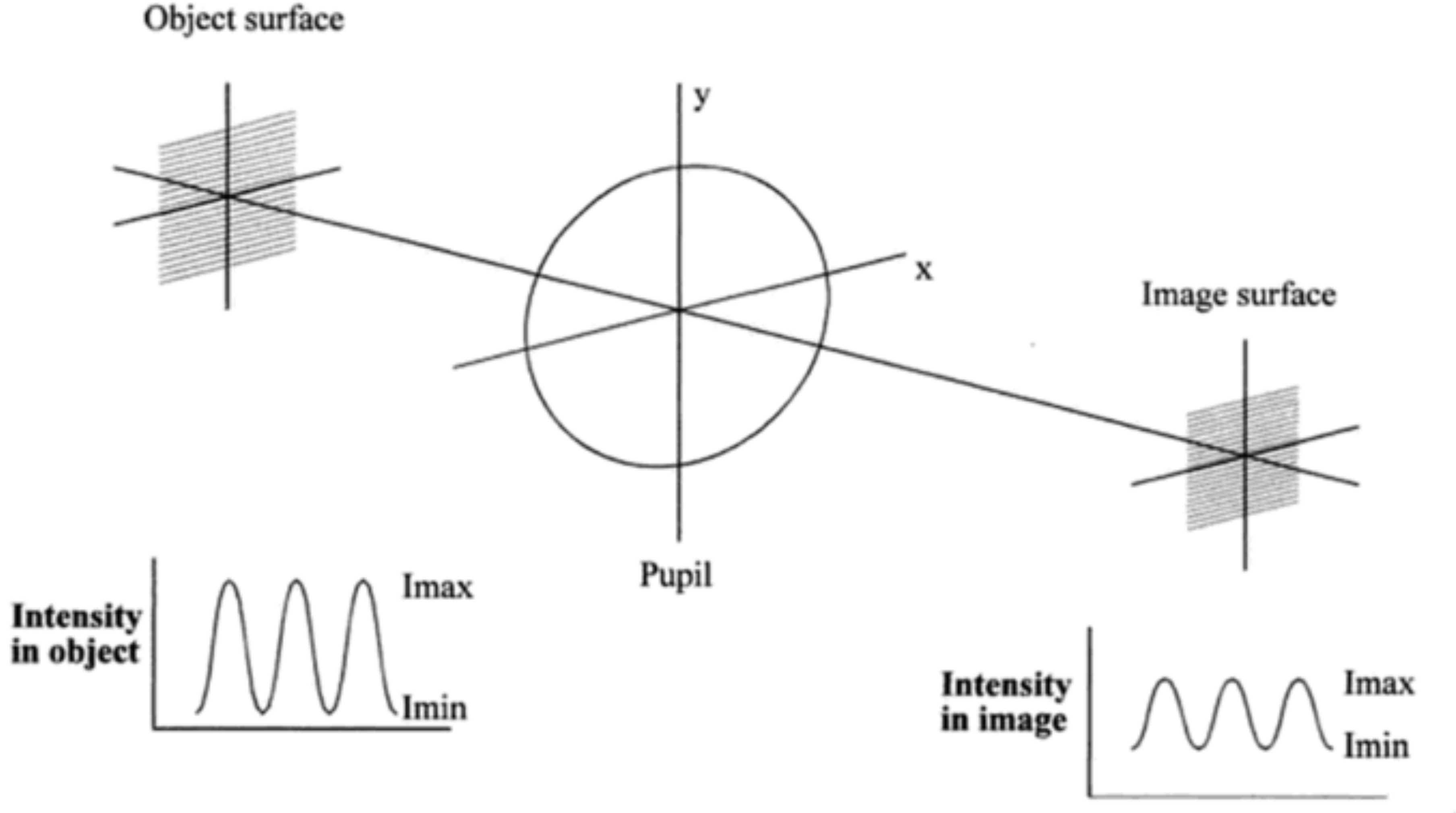
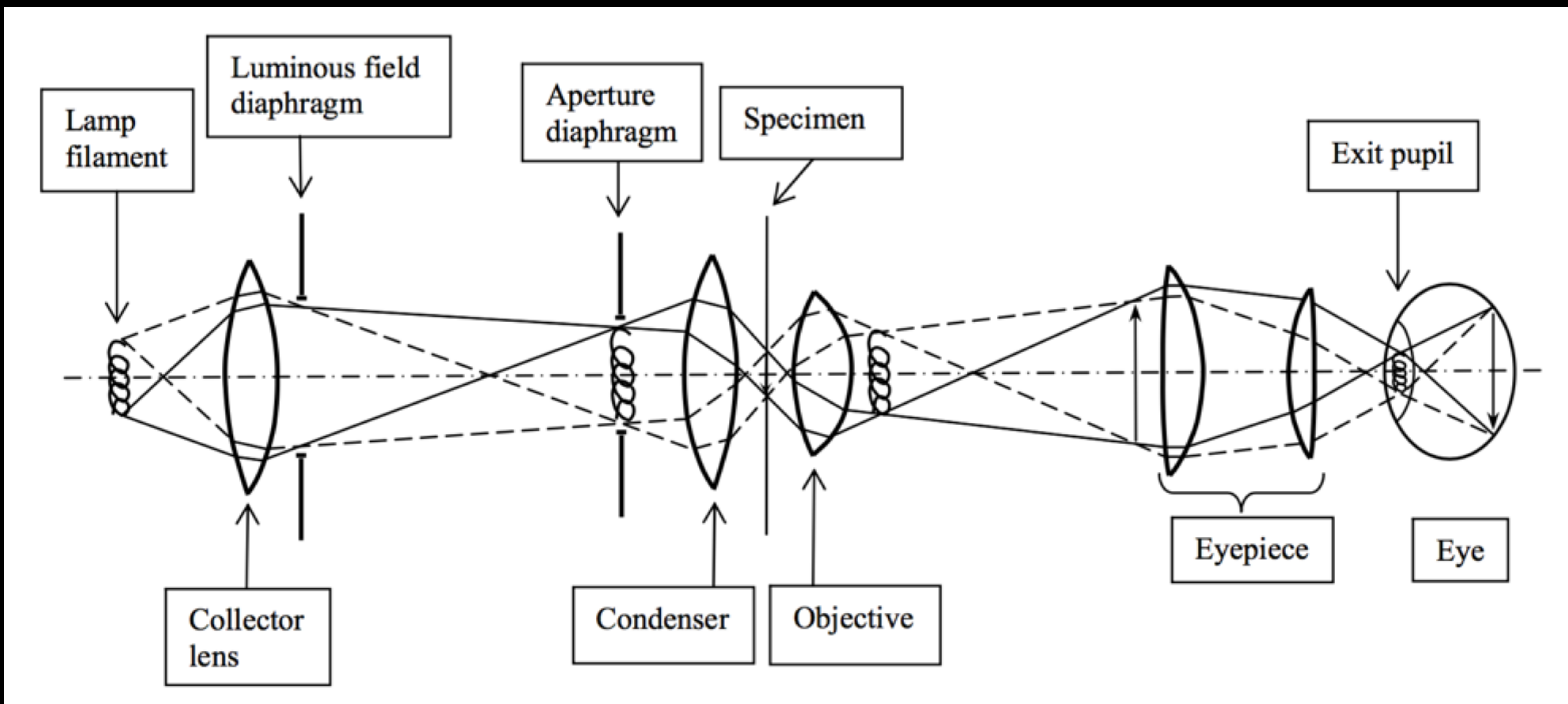



**Figure 4.23.** Object and image contrast.

# 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: the Pupil Stop (=aperture stop) and the Field Stop

Field Stop limits the Field of View

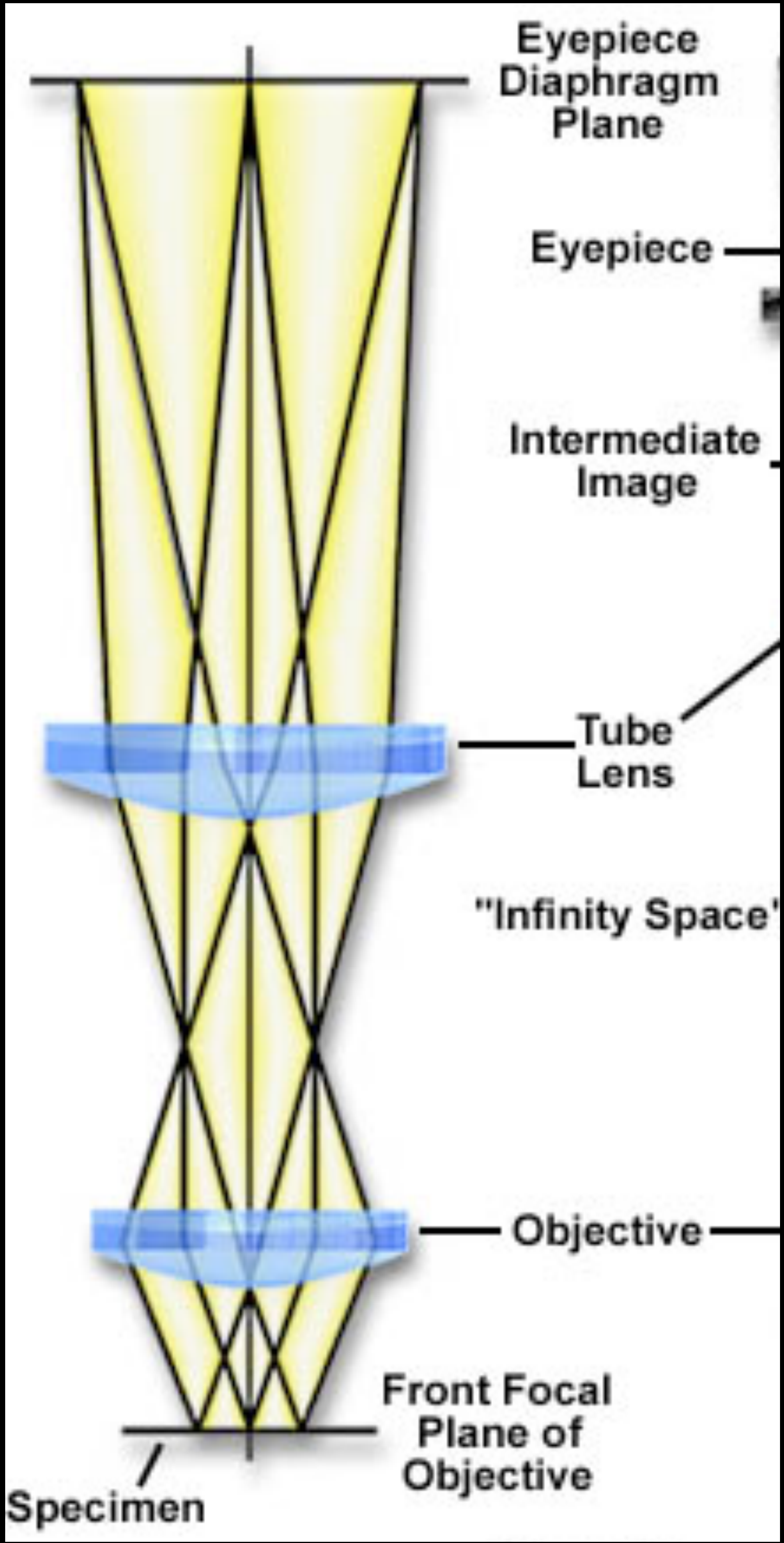
Pupil Stop limits the angle of illumination, which effectively limits the NA

# Advice for the exam:

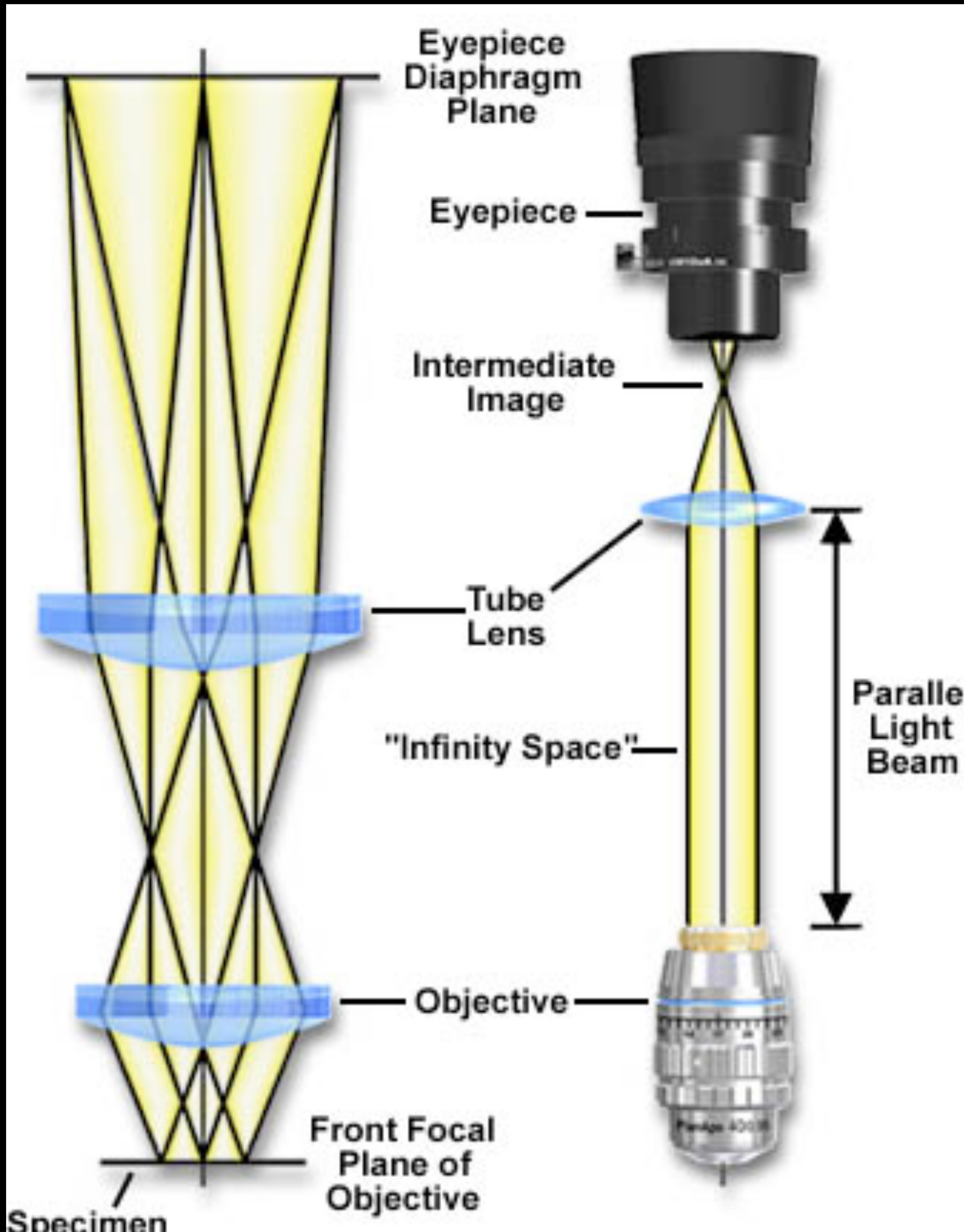
- Be able todo this:
  Draw out a Köhler illumination system

# Summary:

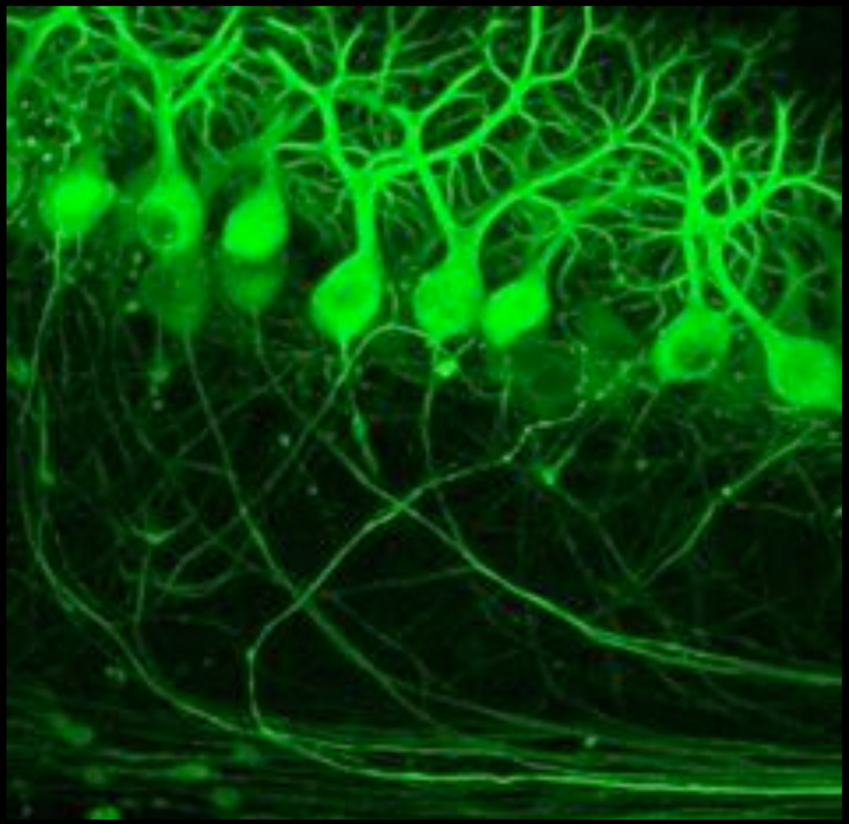
- Modern microscopes work with two lenses:
    - Objective
    - Tube Lens



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
Parallel
Light
Beam
"Infinity Space"
Objective
Front Focal
Plane of
Objective
Specimen

# Fluorescence Microscopy



Green Fluorescent Protein

# Green Fluorescent Protein

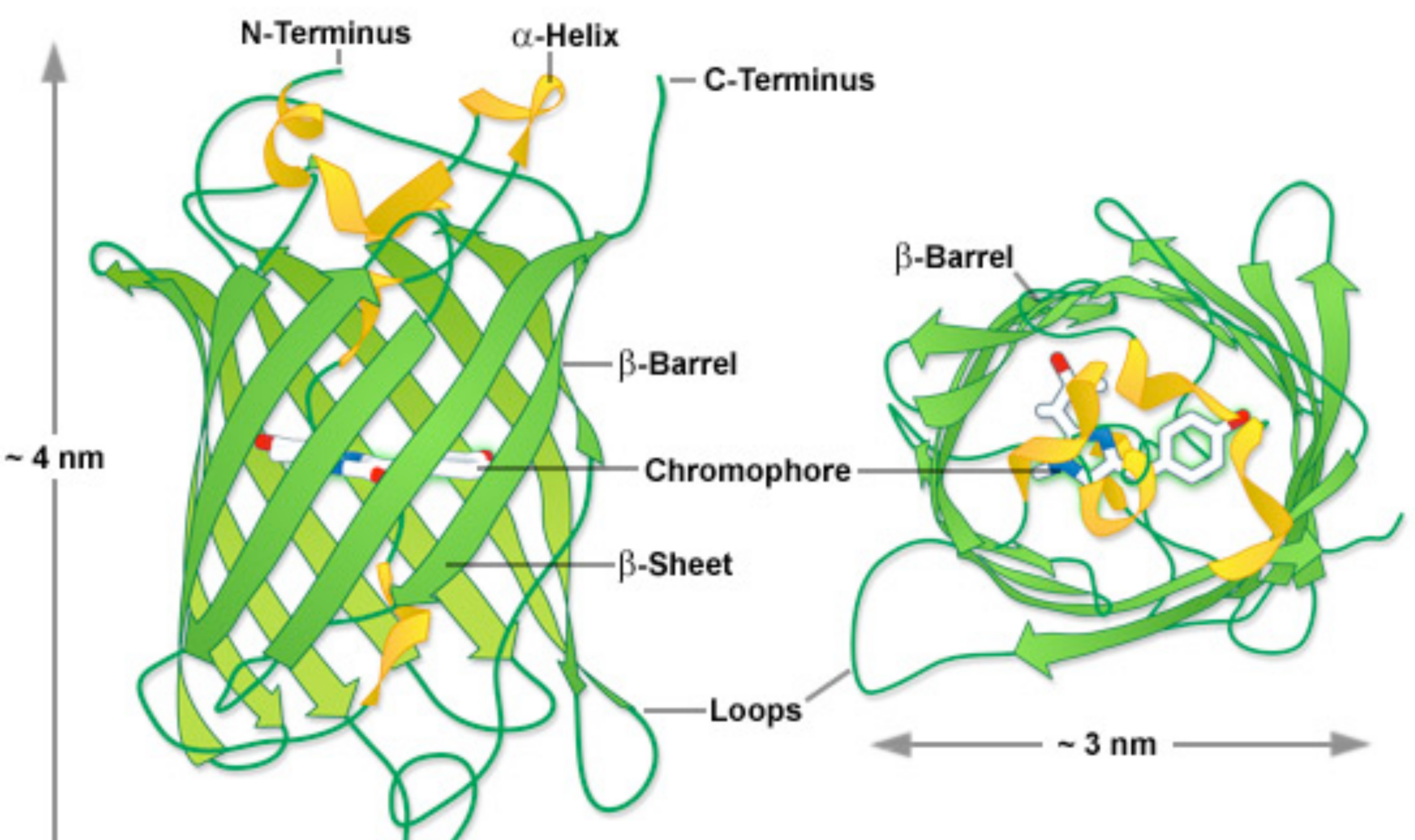
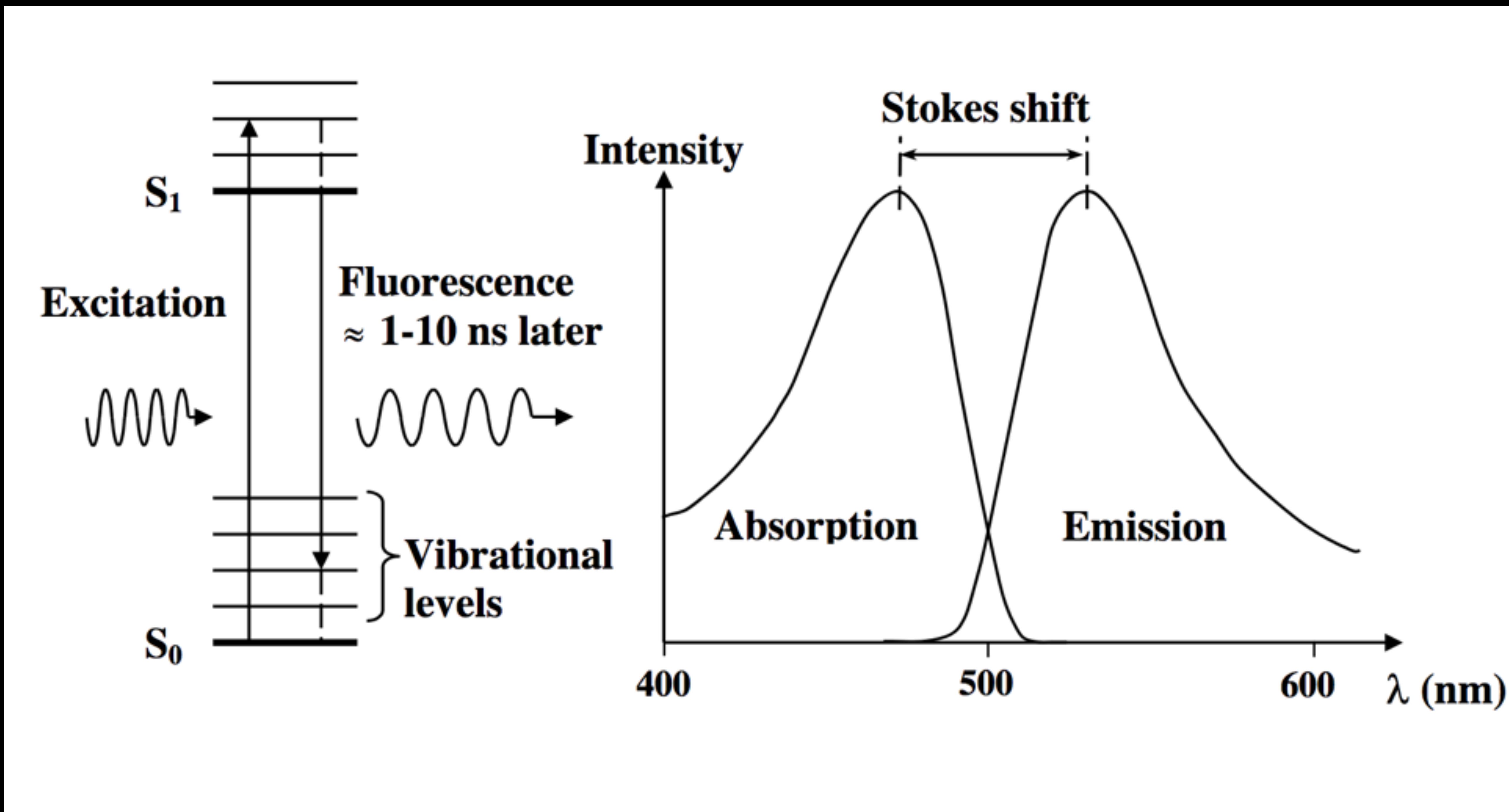
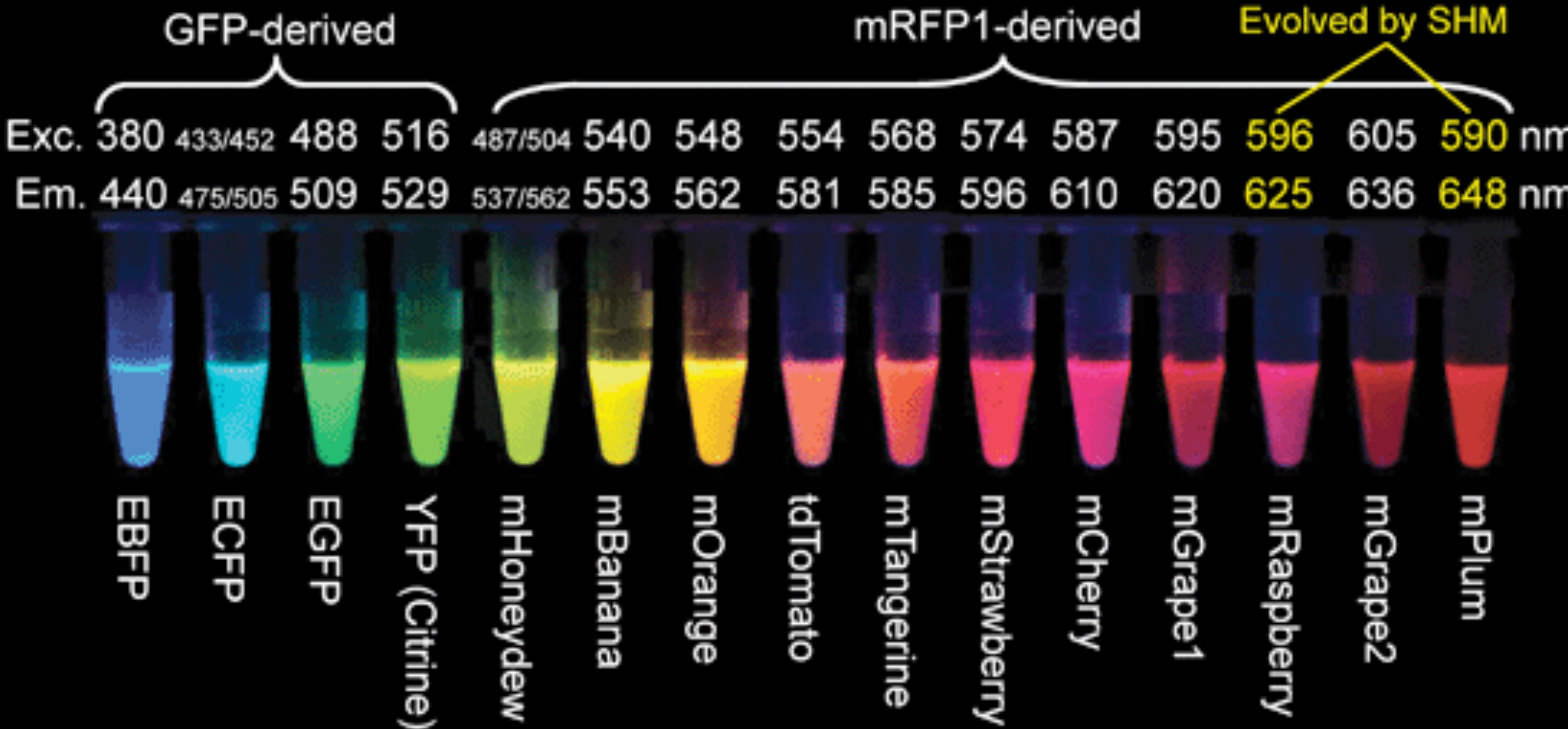



Figure 1

# Fluorescence Excitation

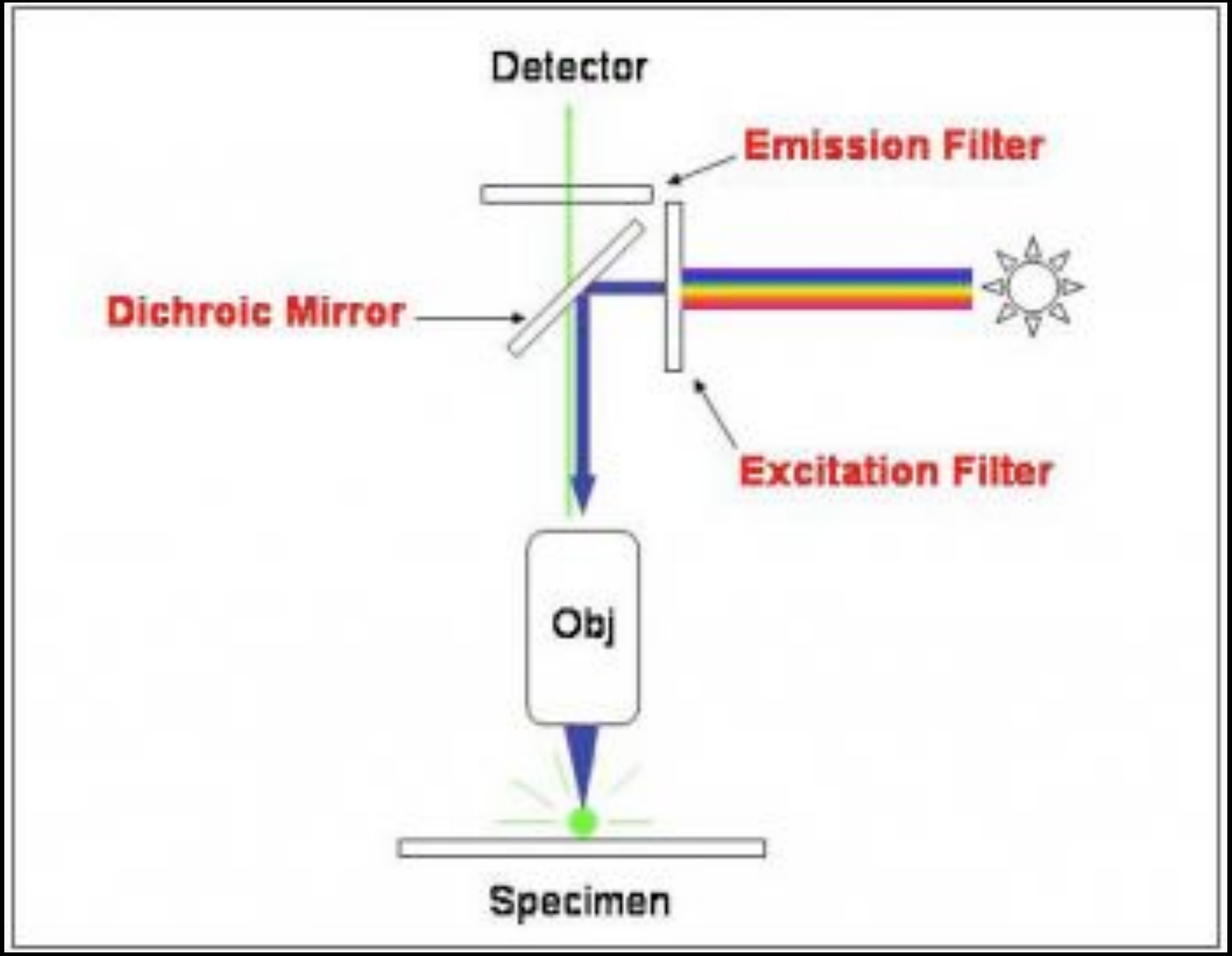


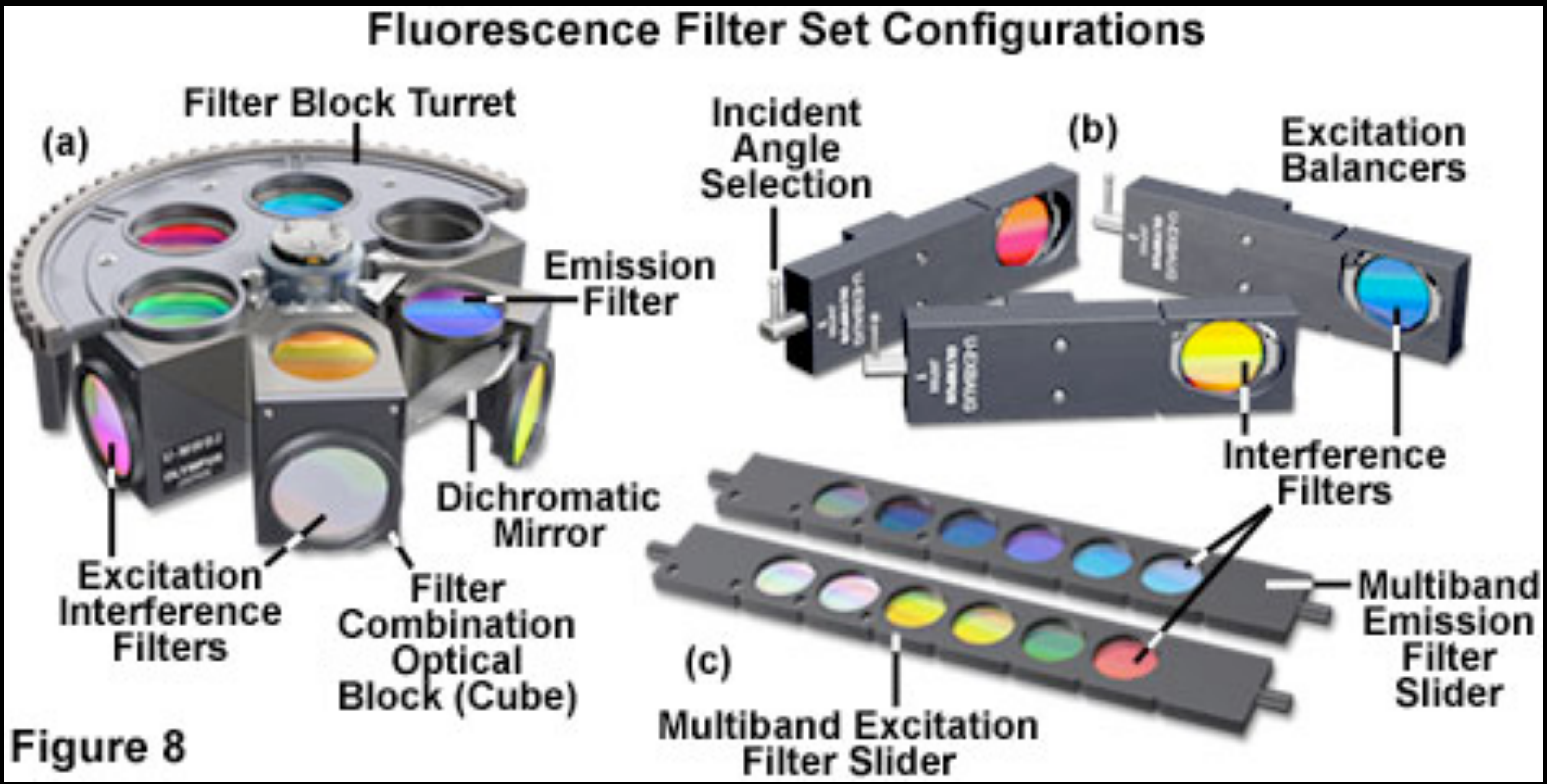
# The 2004 palette of nonoligomerizing fluorescent proteins




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

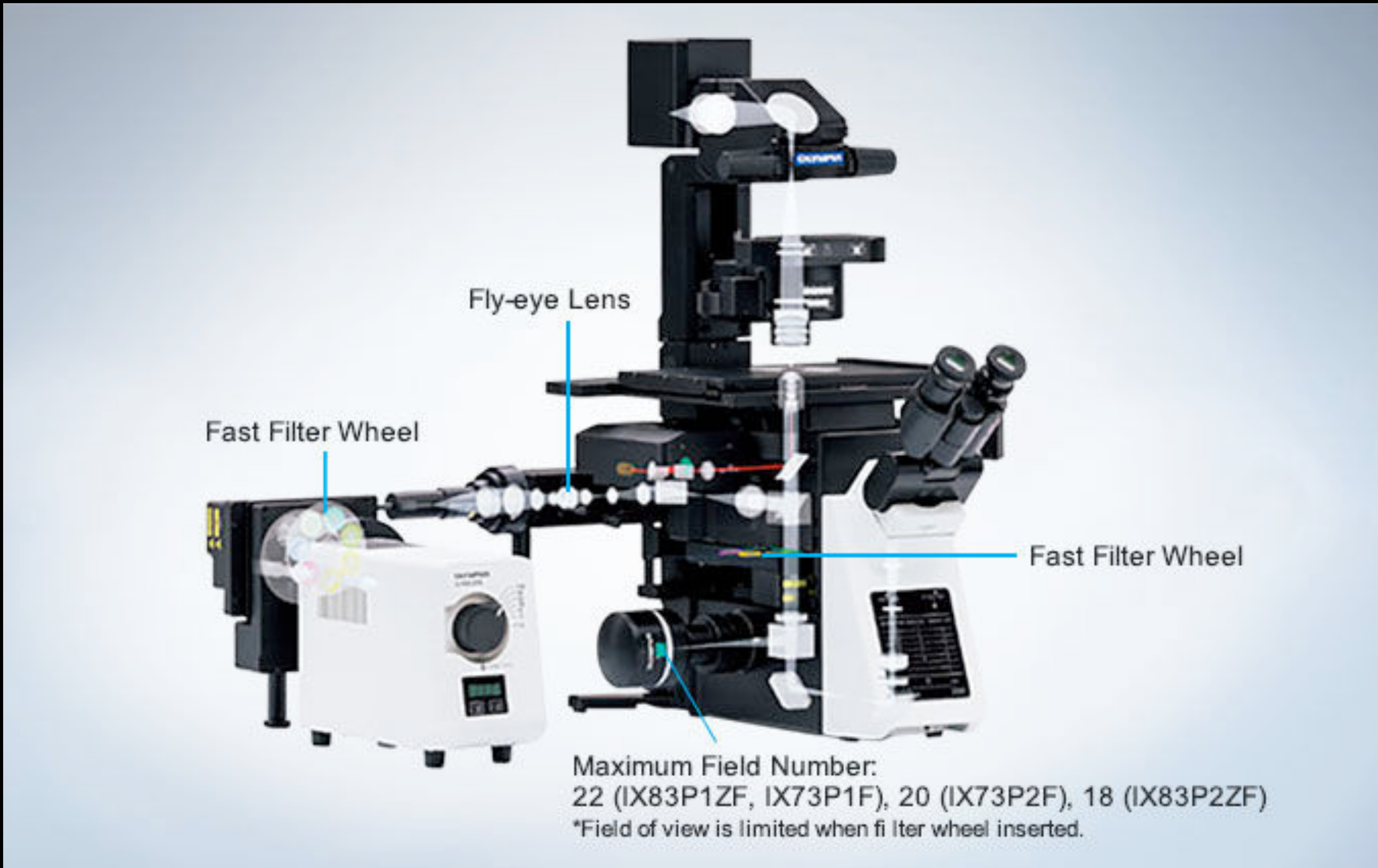
# Filters and Dichroic Mirrors




Fluorescence Filter Set Configurations
(a)
Filter Block Turret
Emission Filter
Dichromatic Mirror
Excitation Interference Filters
Filter Combination Optical Block (Cube)
(b)
Incident Angle Selection
Excitation Balancers
Interference Filters
(c)
Multiband Emission Filter Slider
Multiband Excitation Filter Slider
Figure 8

# Advice for the exam:

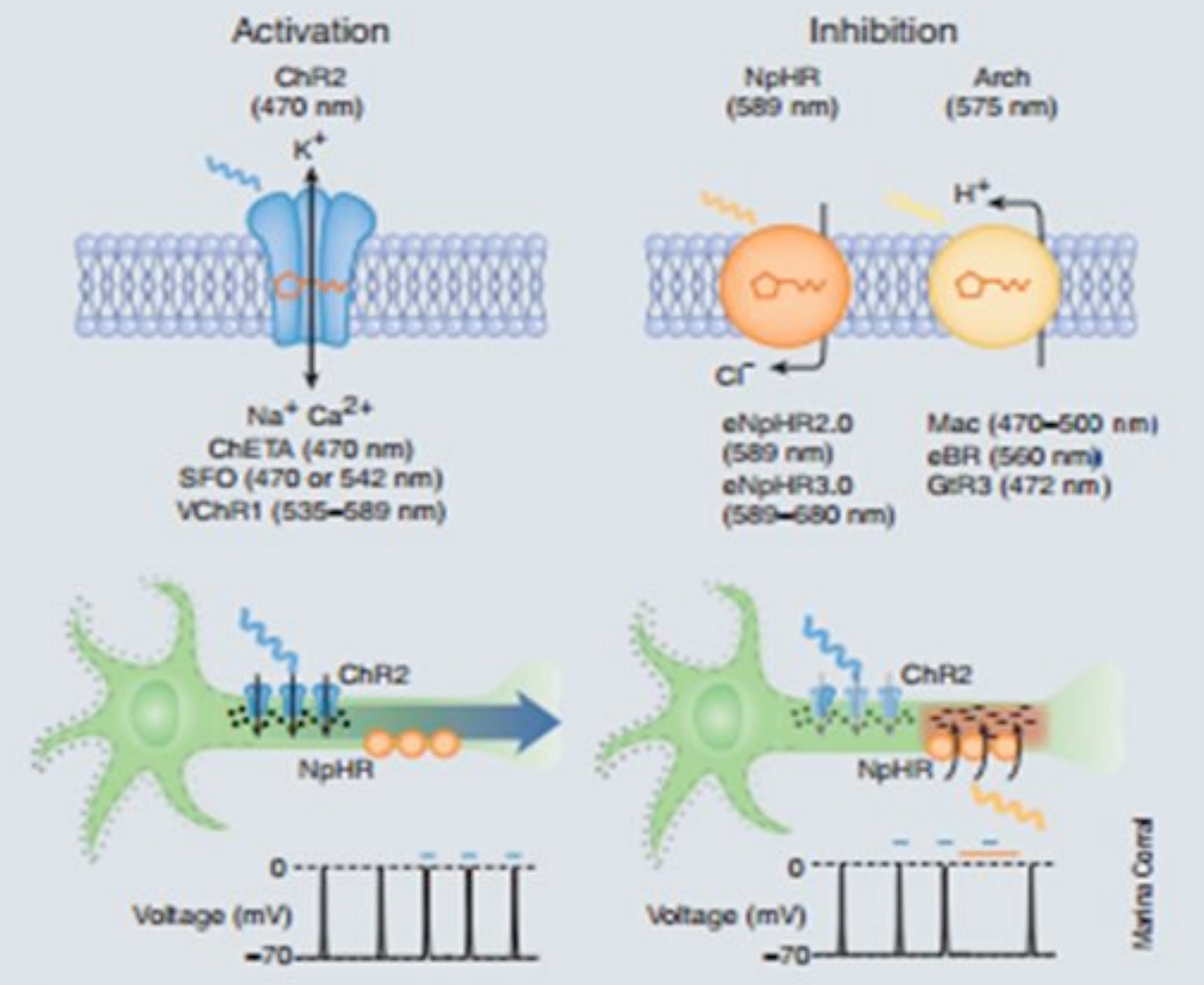
- Be able todo this:
  Draw out how the excitation illumination light and fluorescent light go through a dichroic mirror/filter set


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.

# 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