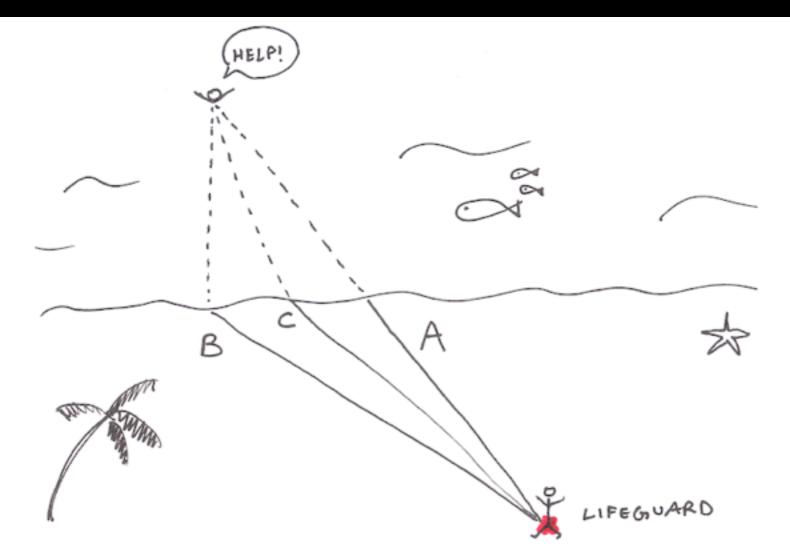
# Review

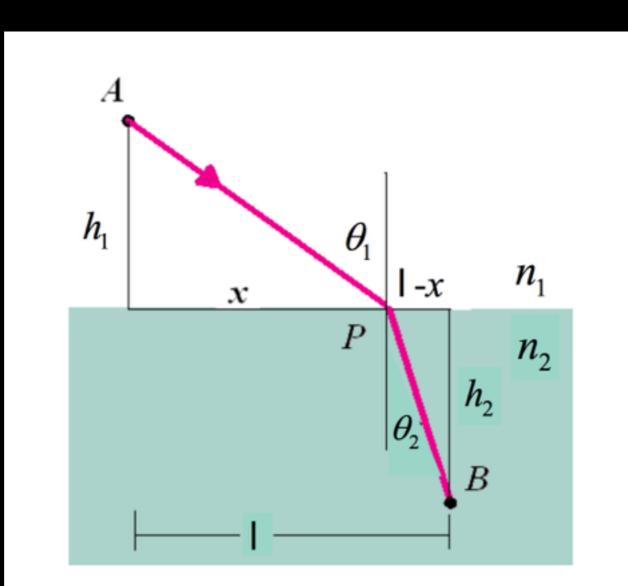
# Fermat's Principle

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

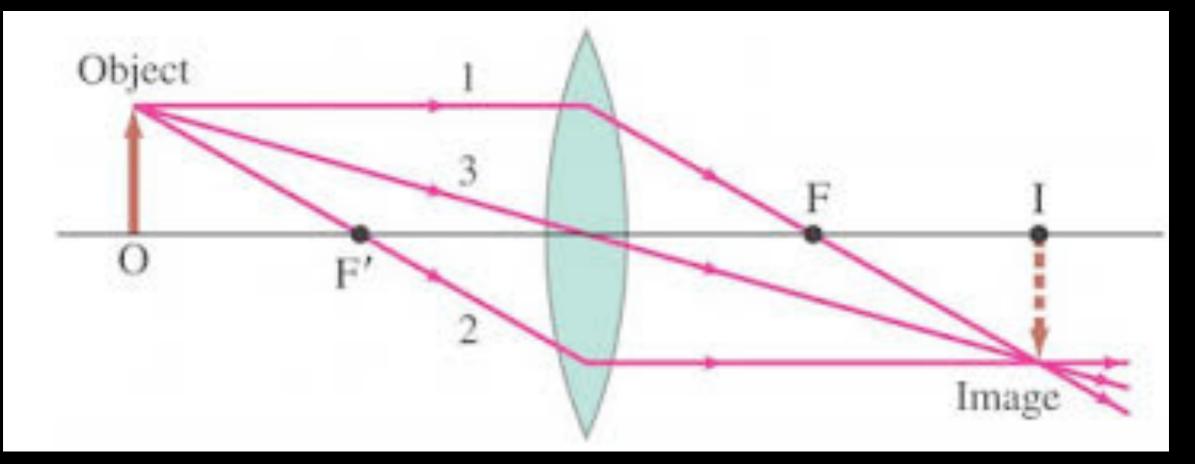


# Total Internal Reflection

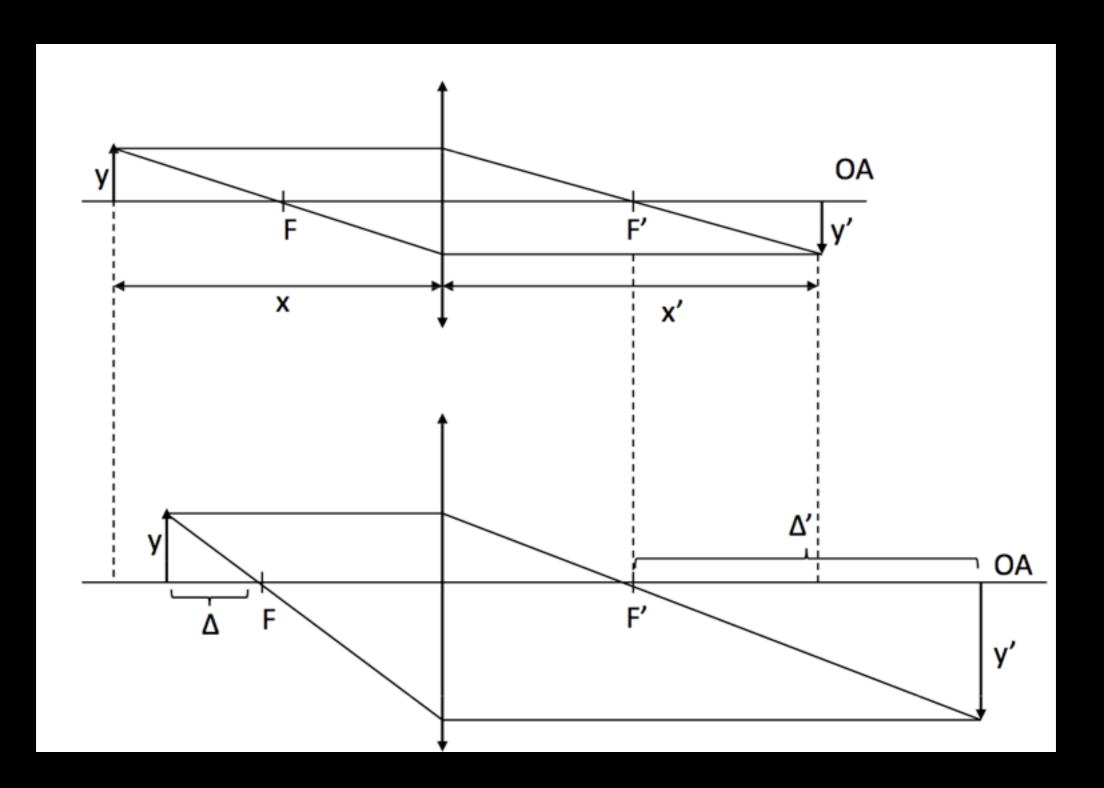
#### $n_1 \sin \theta_c = n_2$ Critical Angle

# Geometrical Ray Optics

#### Three Principal Rays:

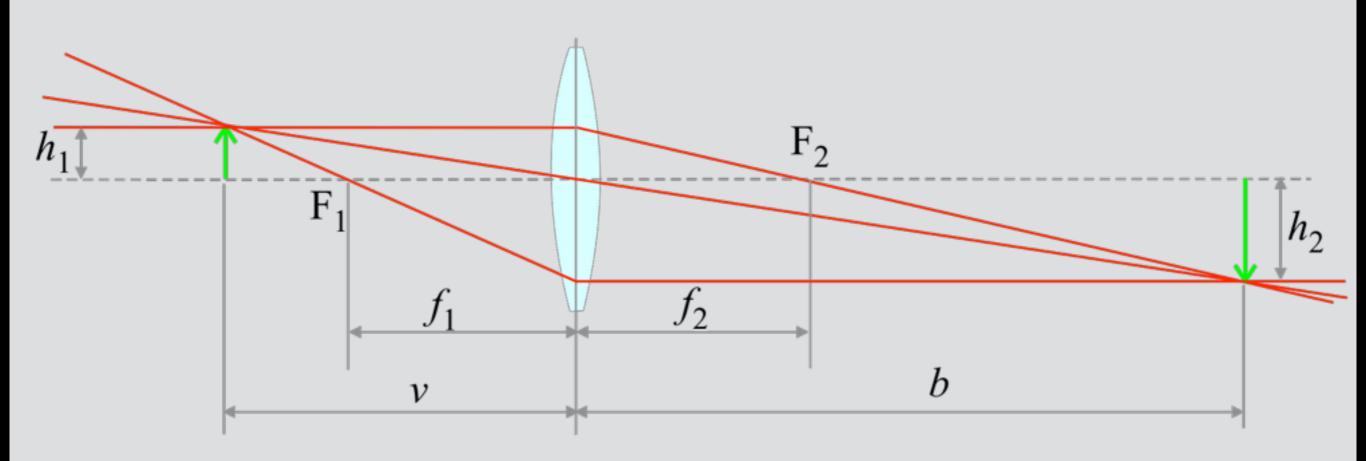


# "4f" with single lens

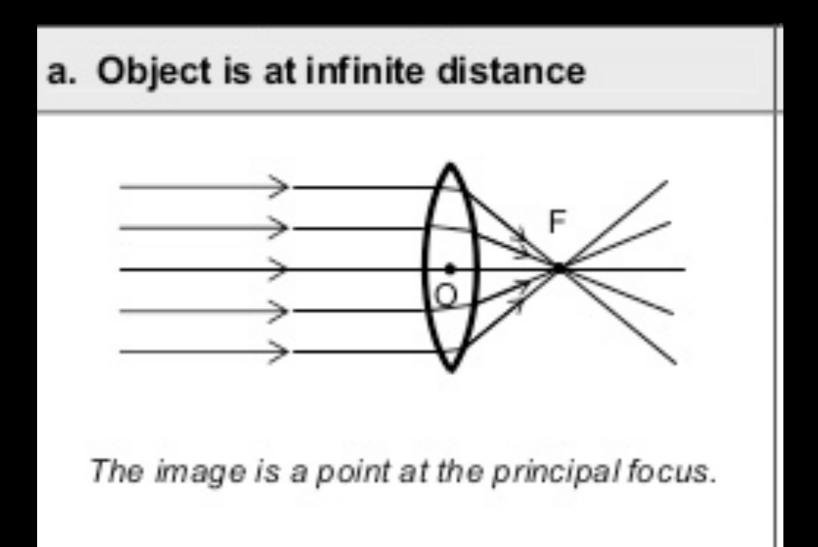


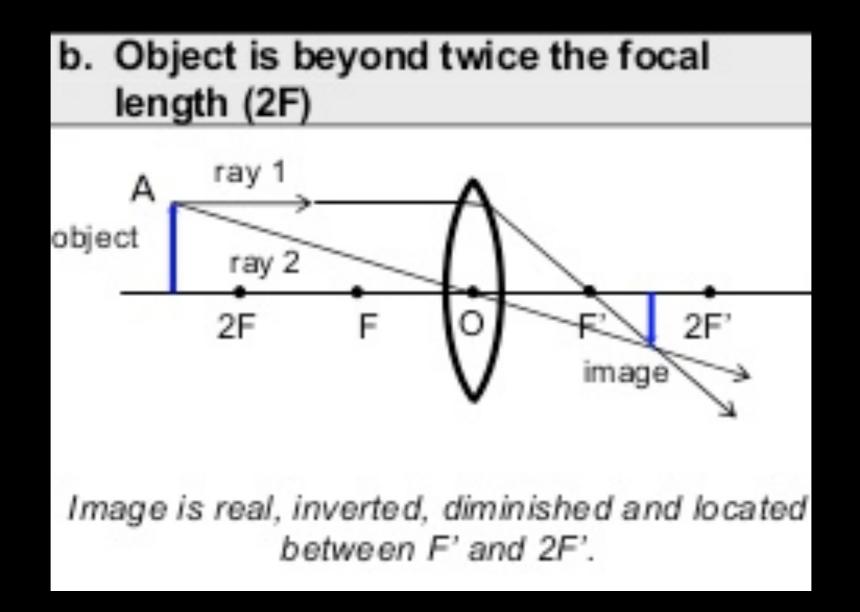
# Magnification

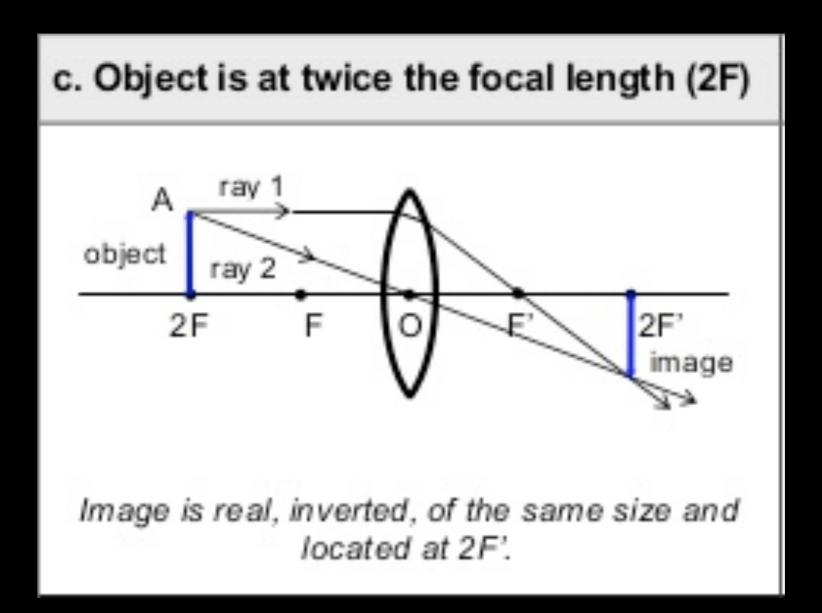
Simple geometrical proof: Two triangles

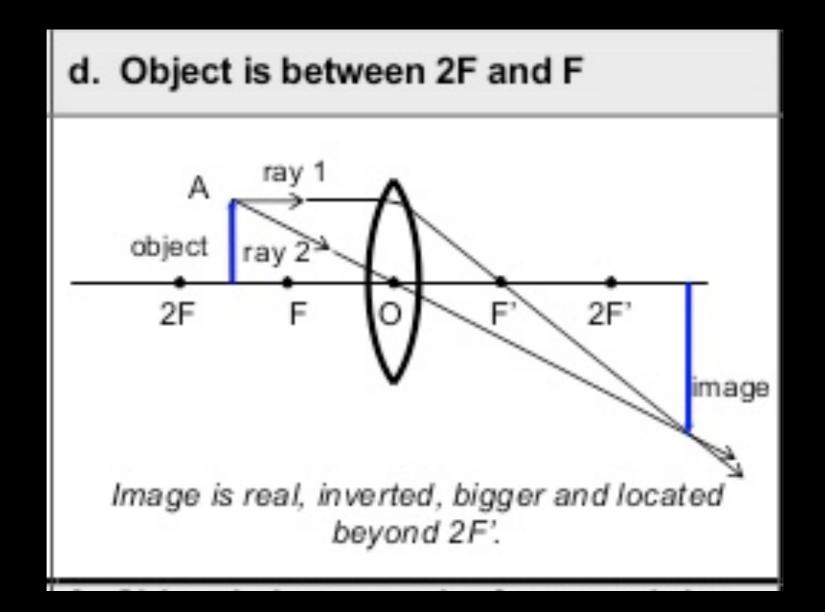


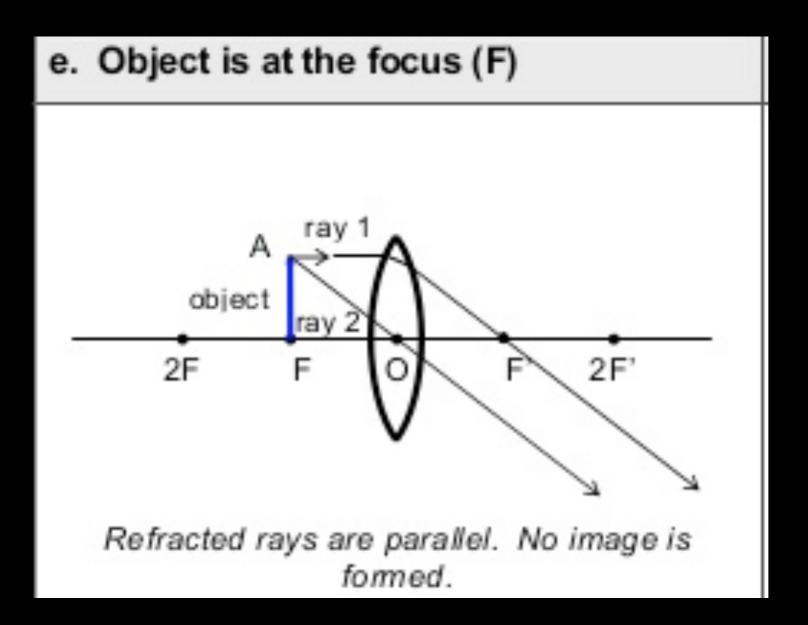
M = f / (f - v)

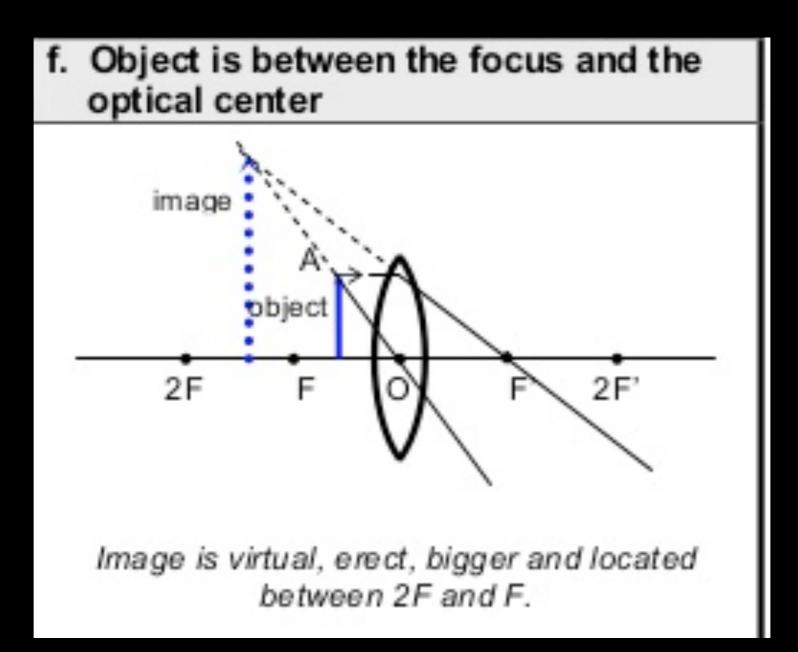




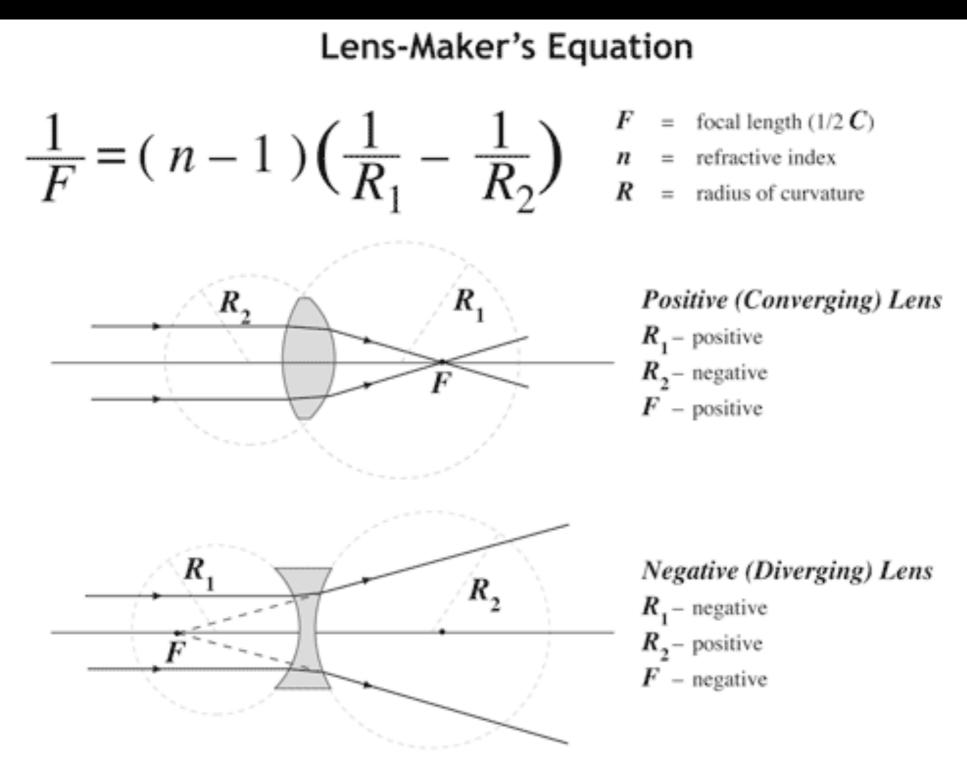




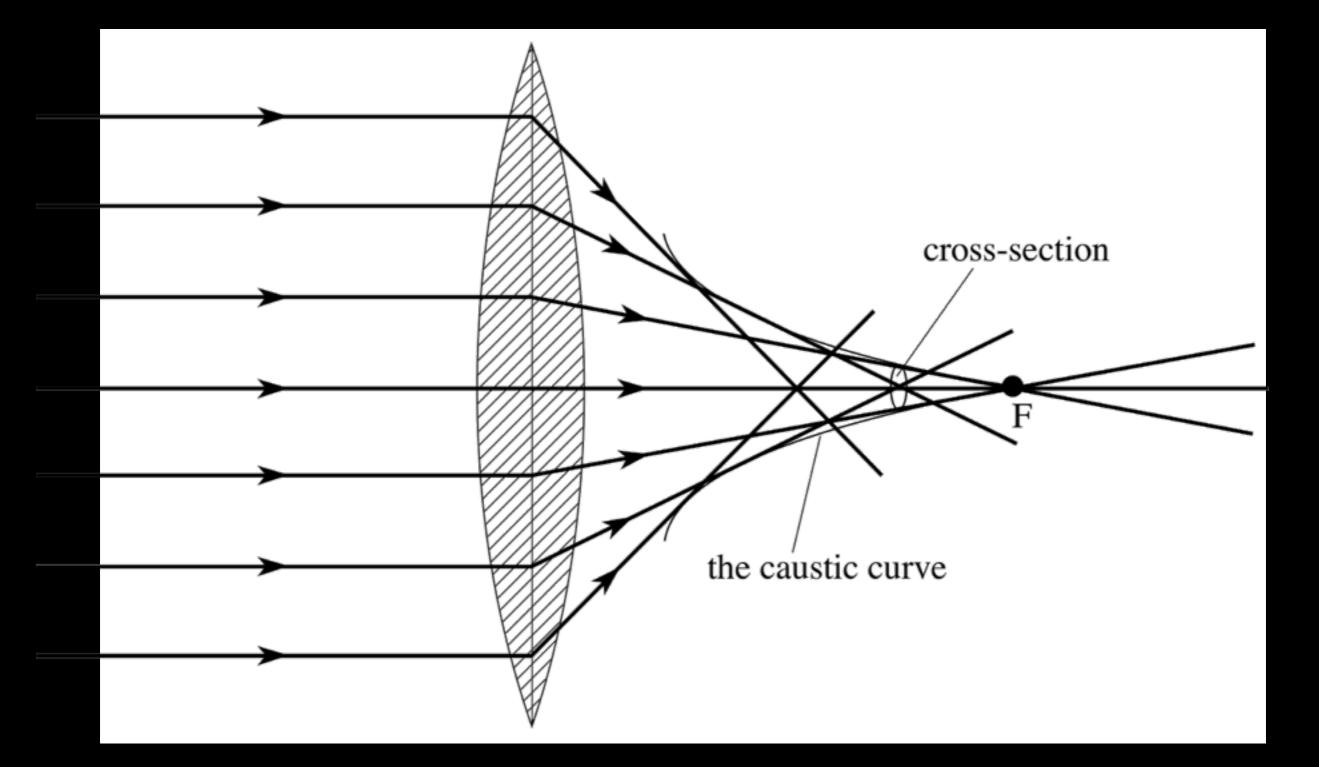




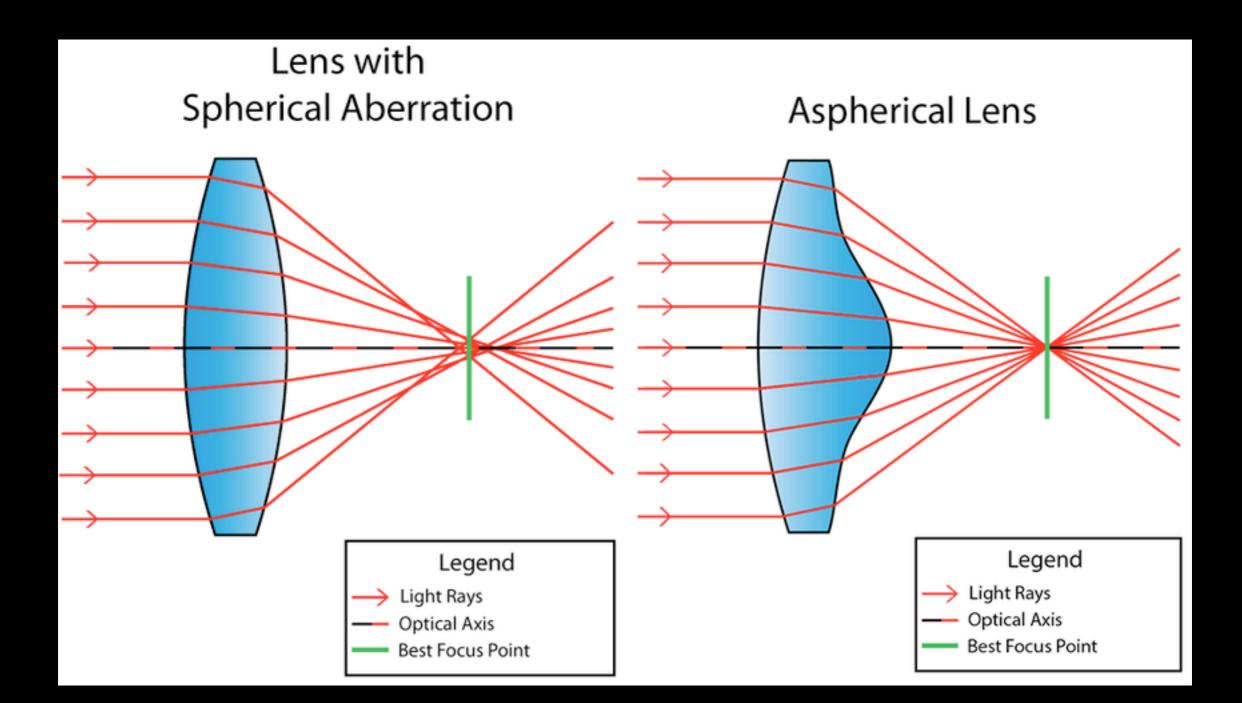
#### You don't need to memorize this I just want you to have seen it:



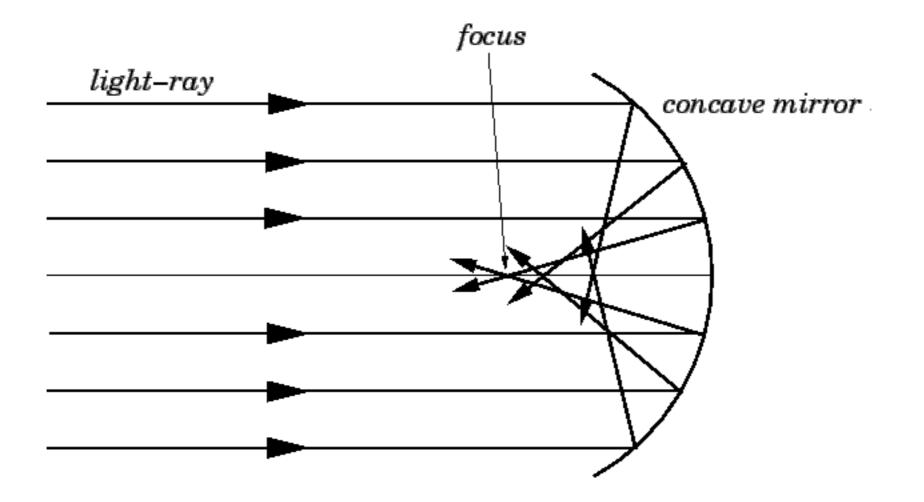
# Spherical Aberration



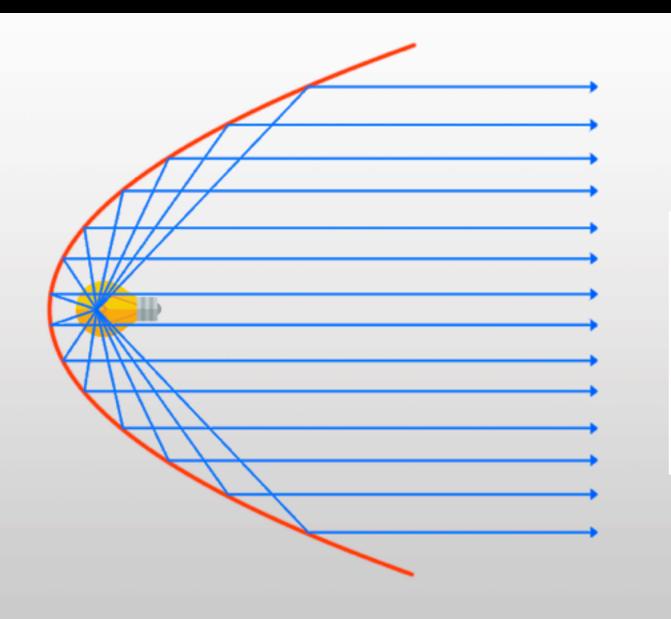
# Spherical Aberration



# Spherical mirror



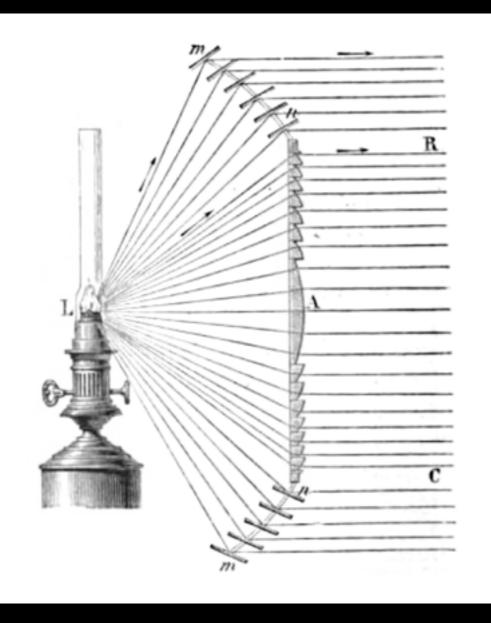
# Parabolic Reflector





Parabolic Reflector

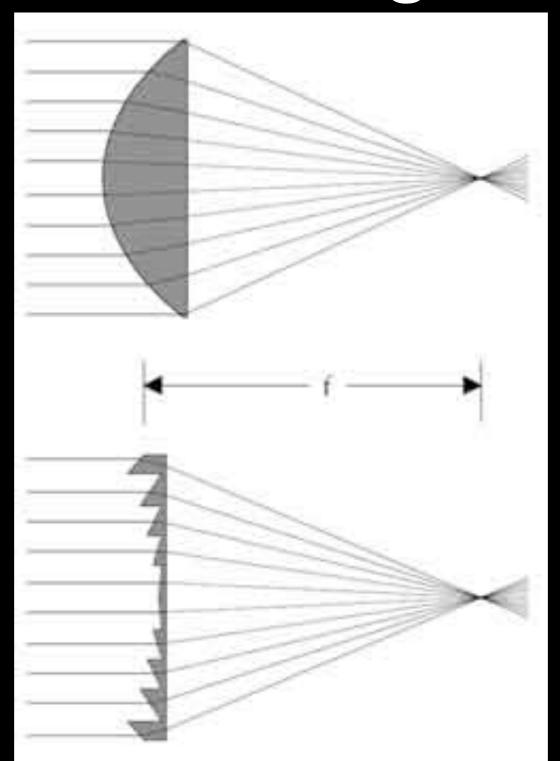
#### Fresnel Lens



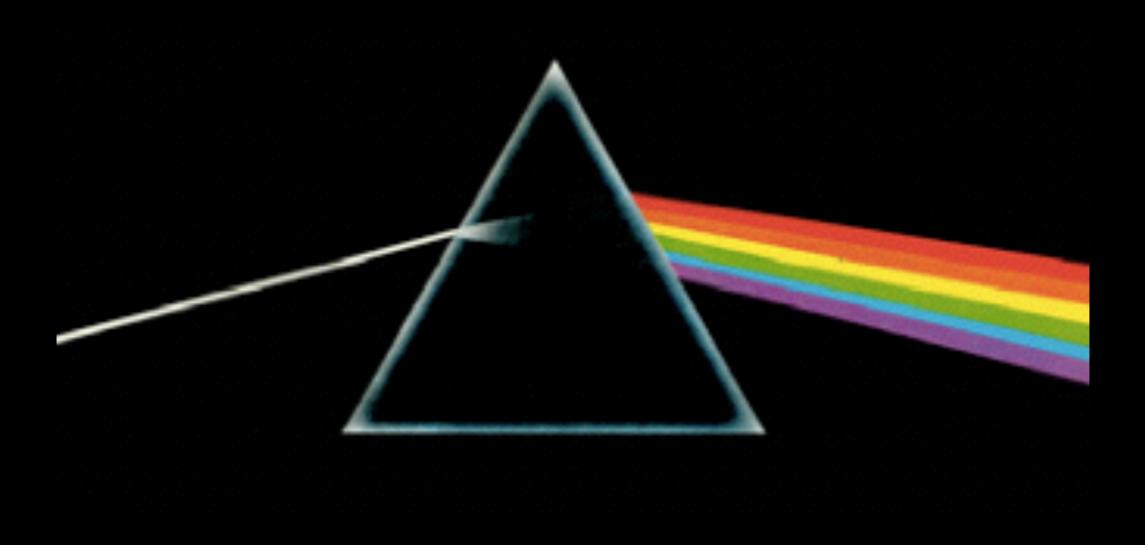


Fresnel lens in lighthouse

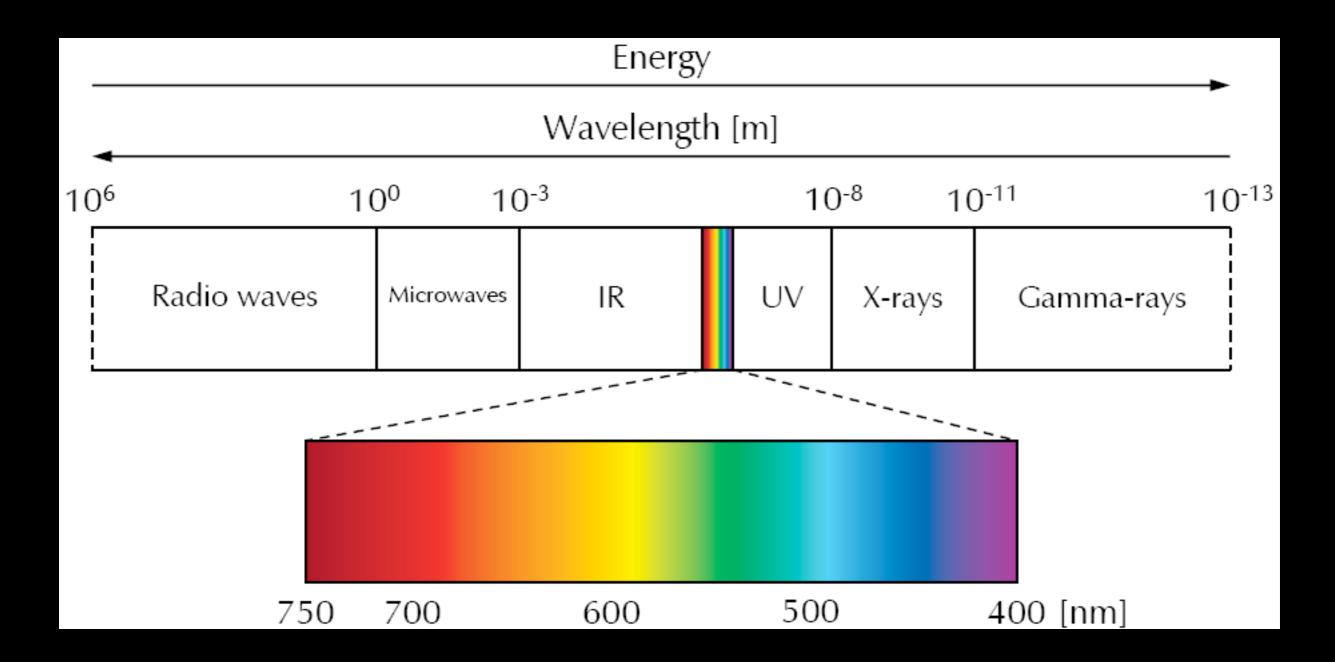
# The Fresnel Lens saves bulk/weight



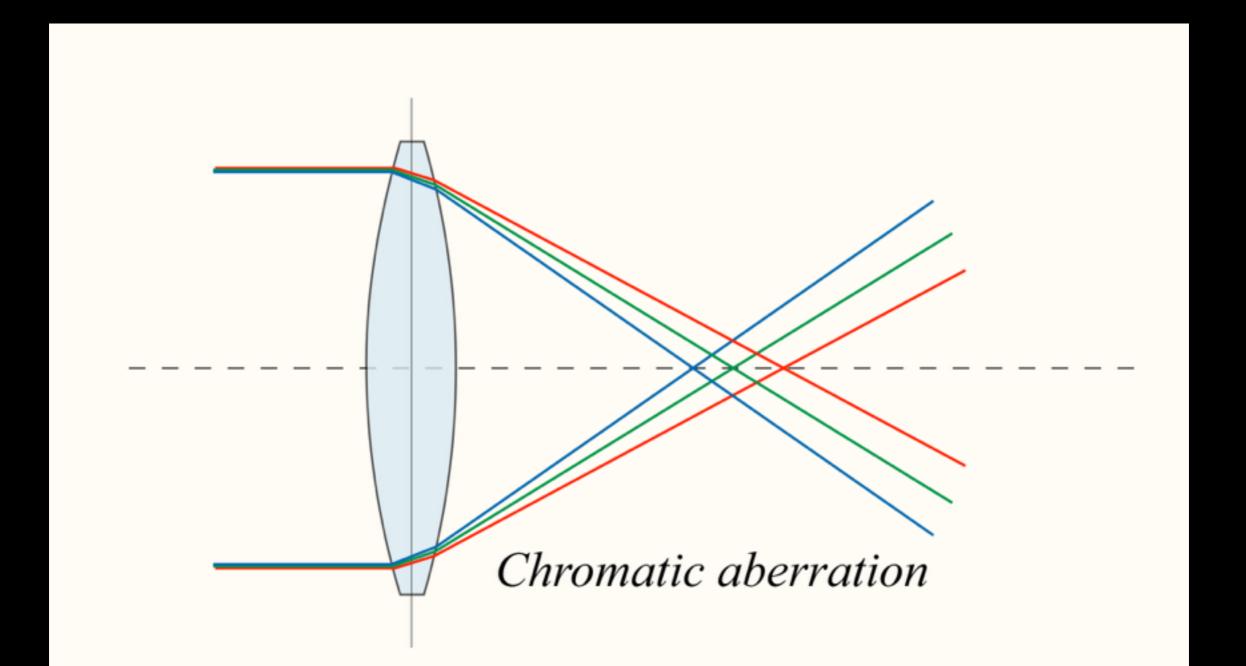
# Refraction in a Prism



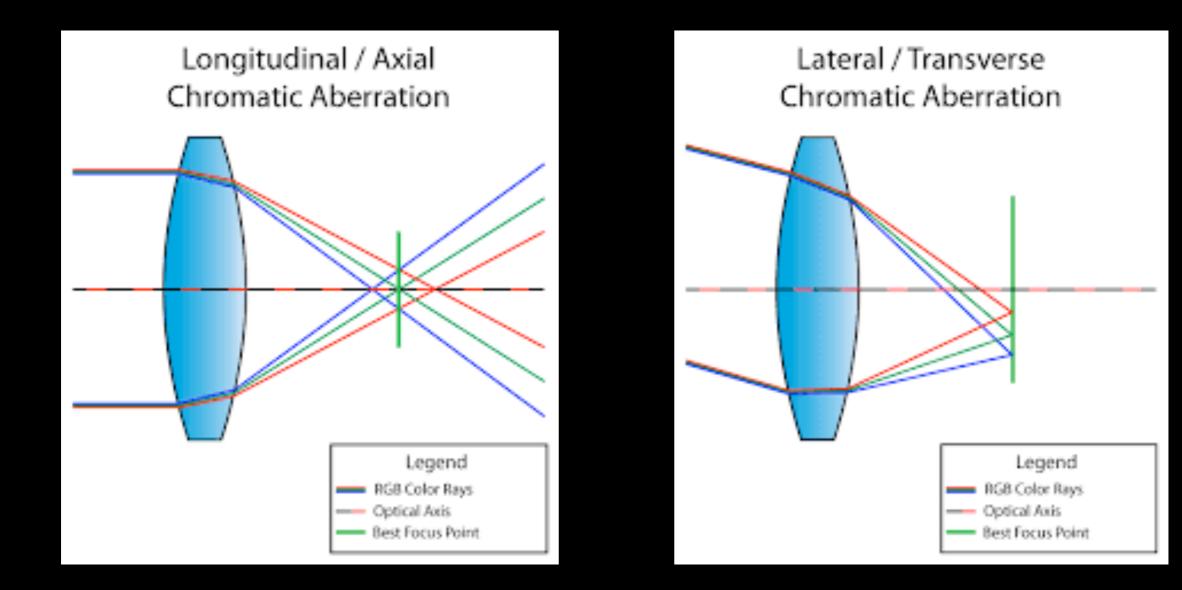
#### Visible Light Wavelength Spectrum



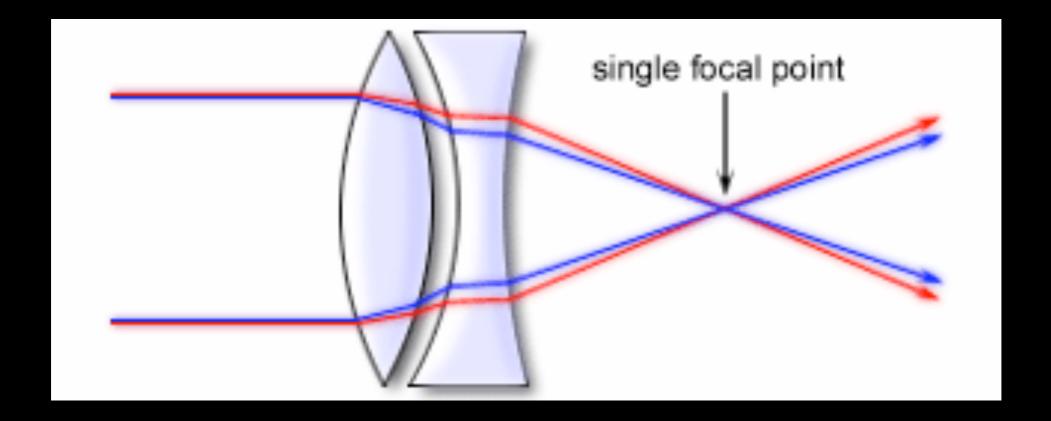
#### Refractive index ~ wavelength



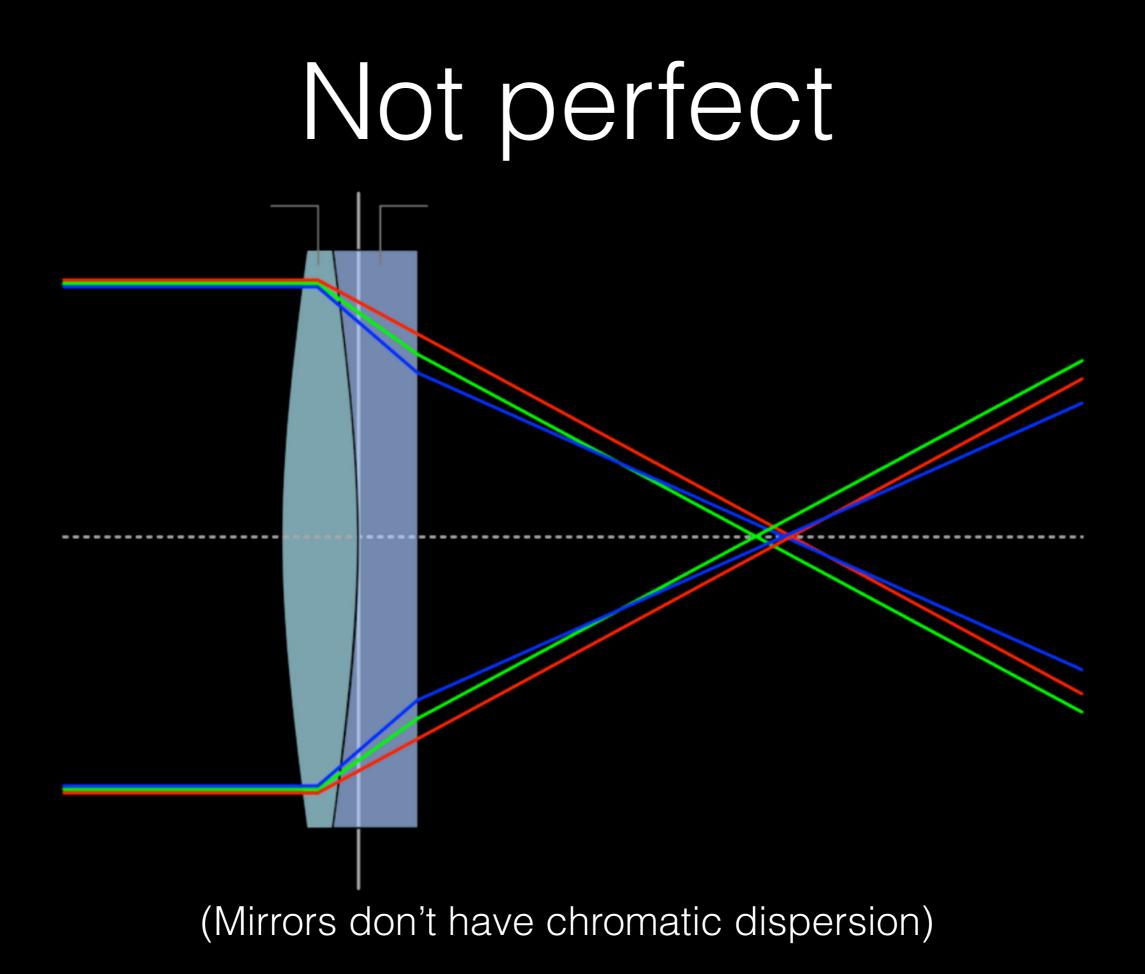
# Chromatic Aberration



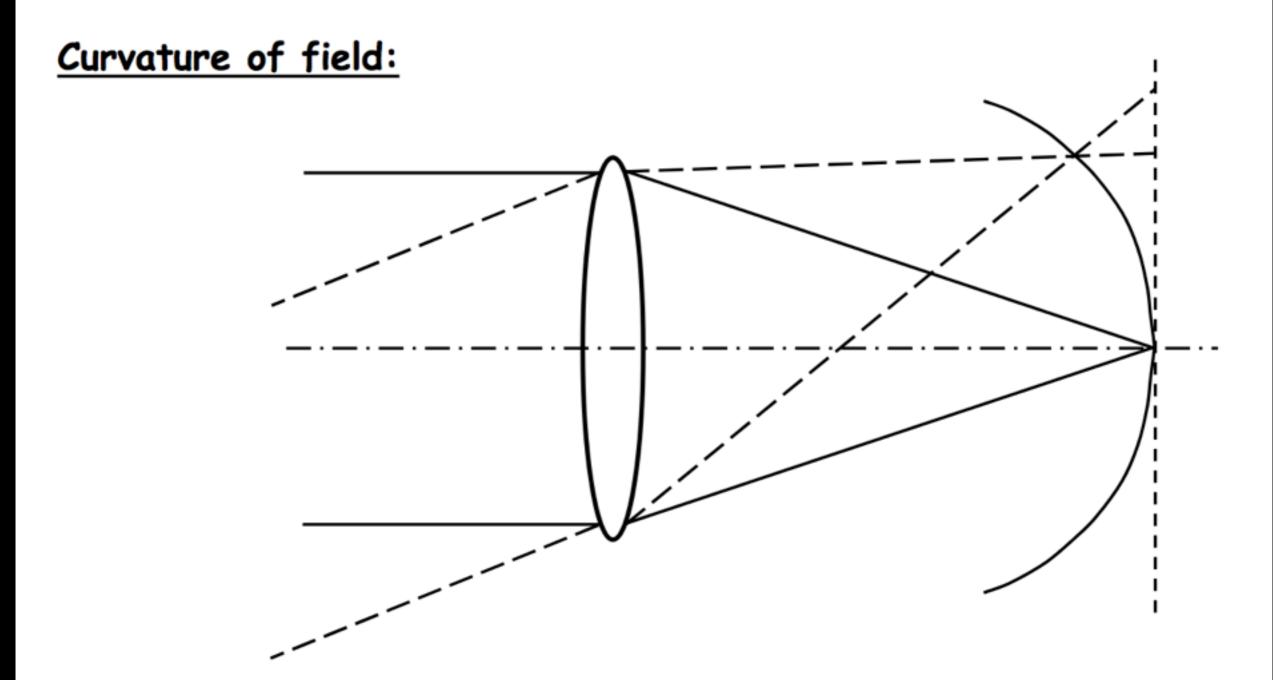
# Achromatic Doublet



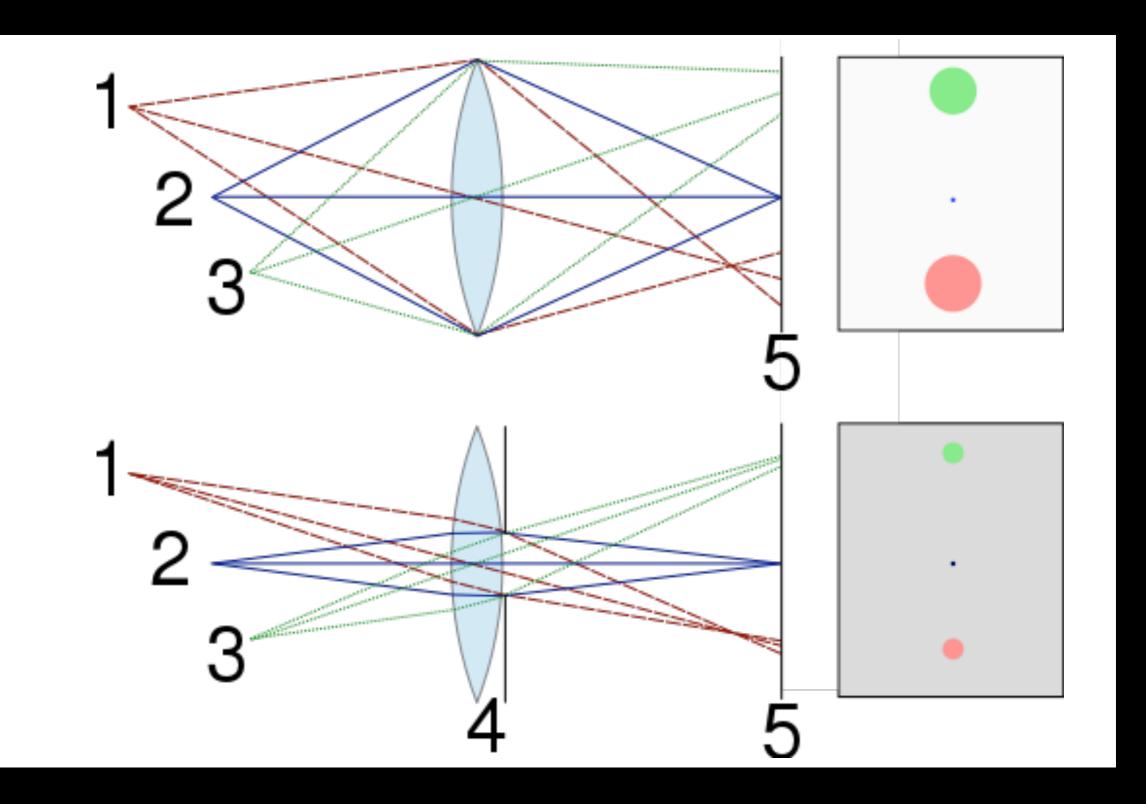
First lens: Crown glass Second lens: Flint glass



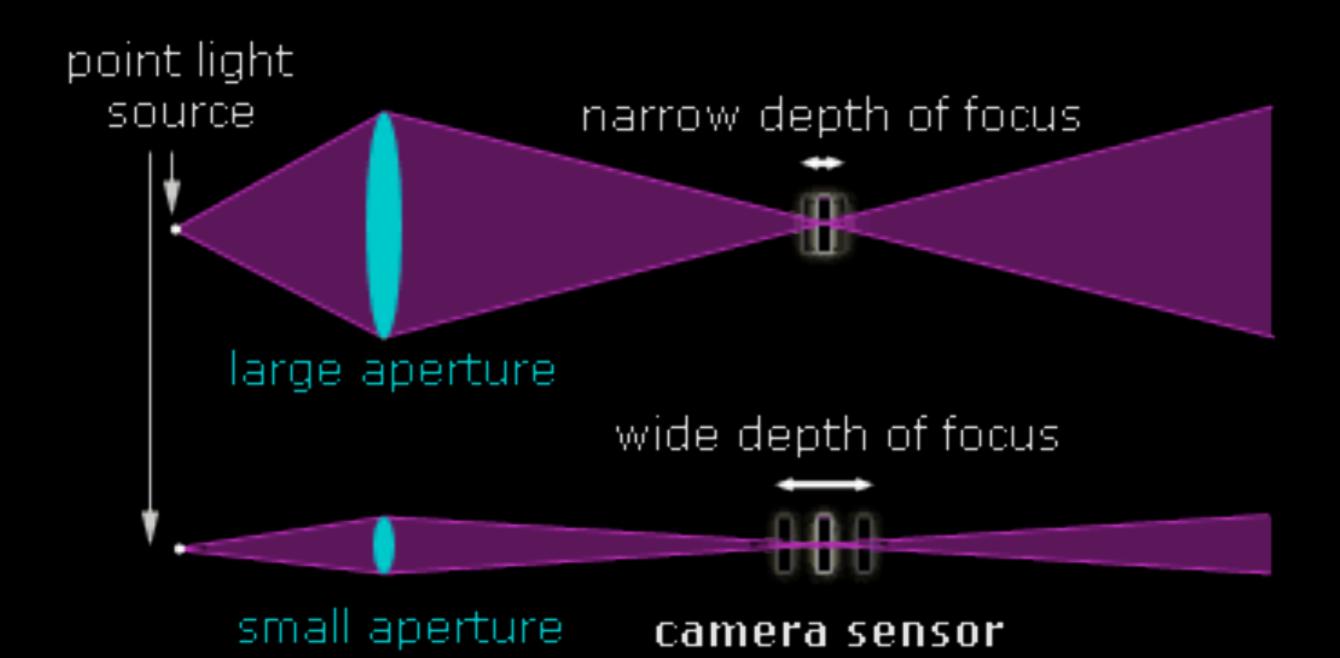
# Field Curvature



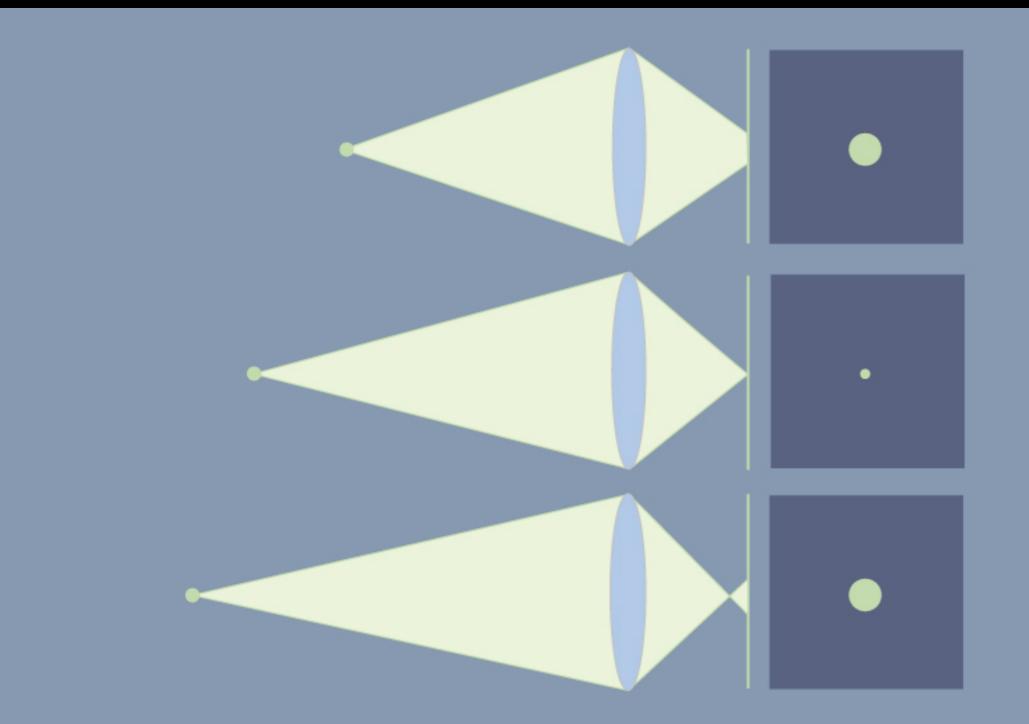
### Large aperture = less DOF



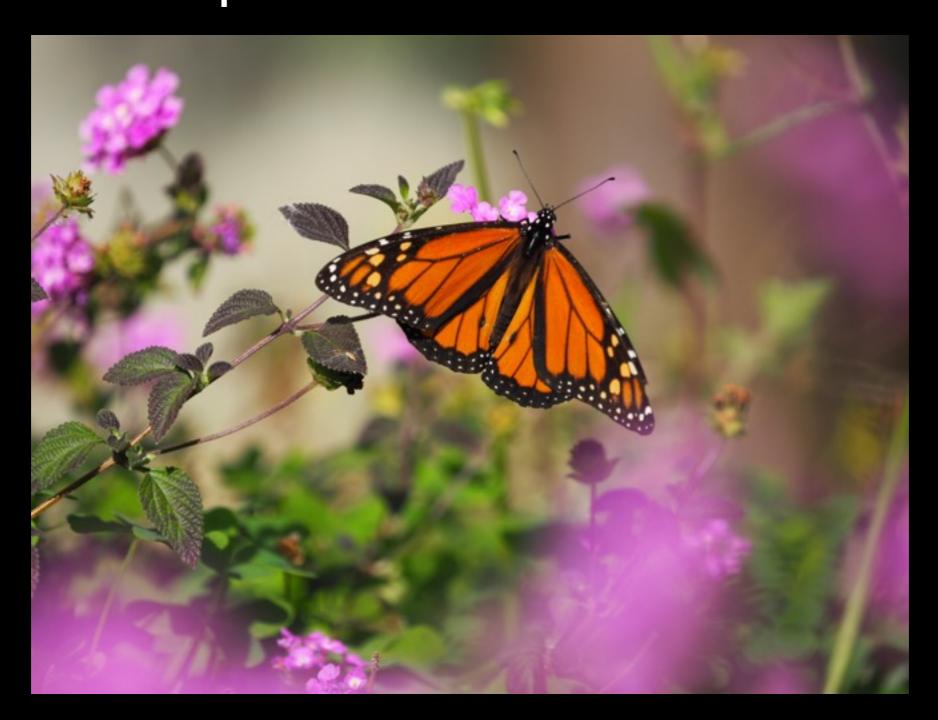
#### Small aperture = more DOF



# Circle of Confusion



# Did I use a small or large aperture here?

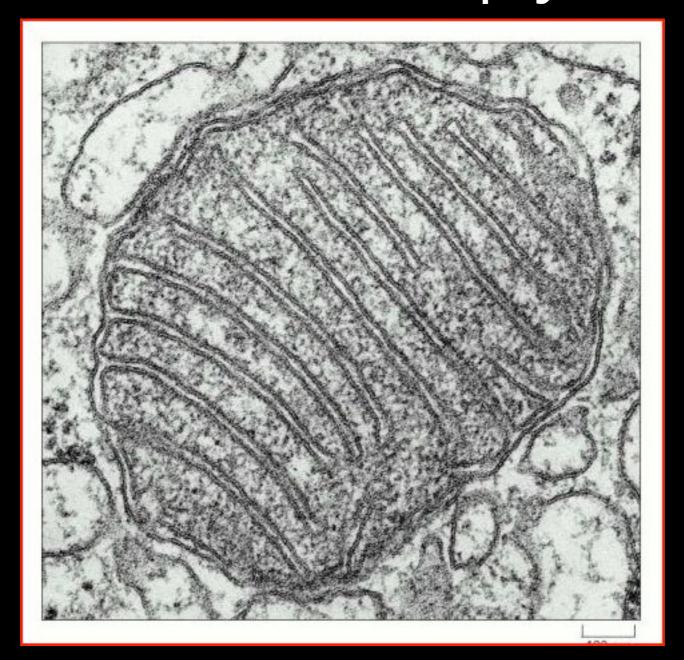


Monarch butterfly (Danaus plexippus) on its favorite food, the milkweed plant

# Straight Photography

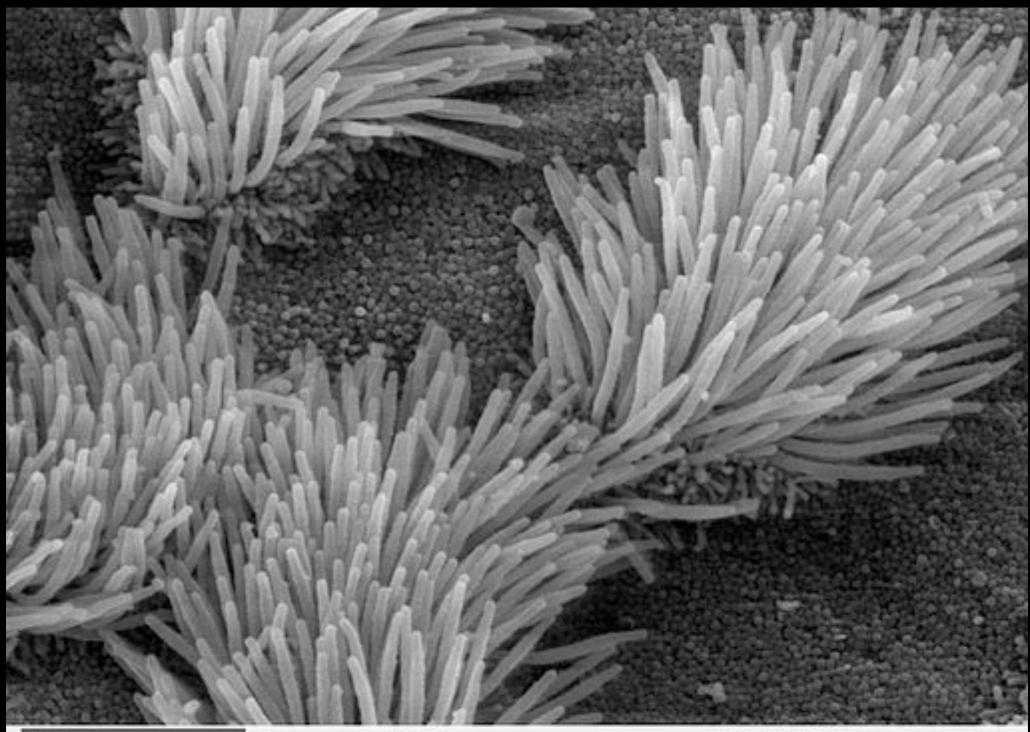


#### Transmission Electron Microscopy

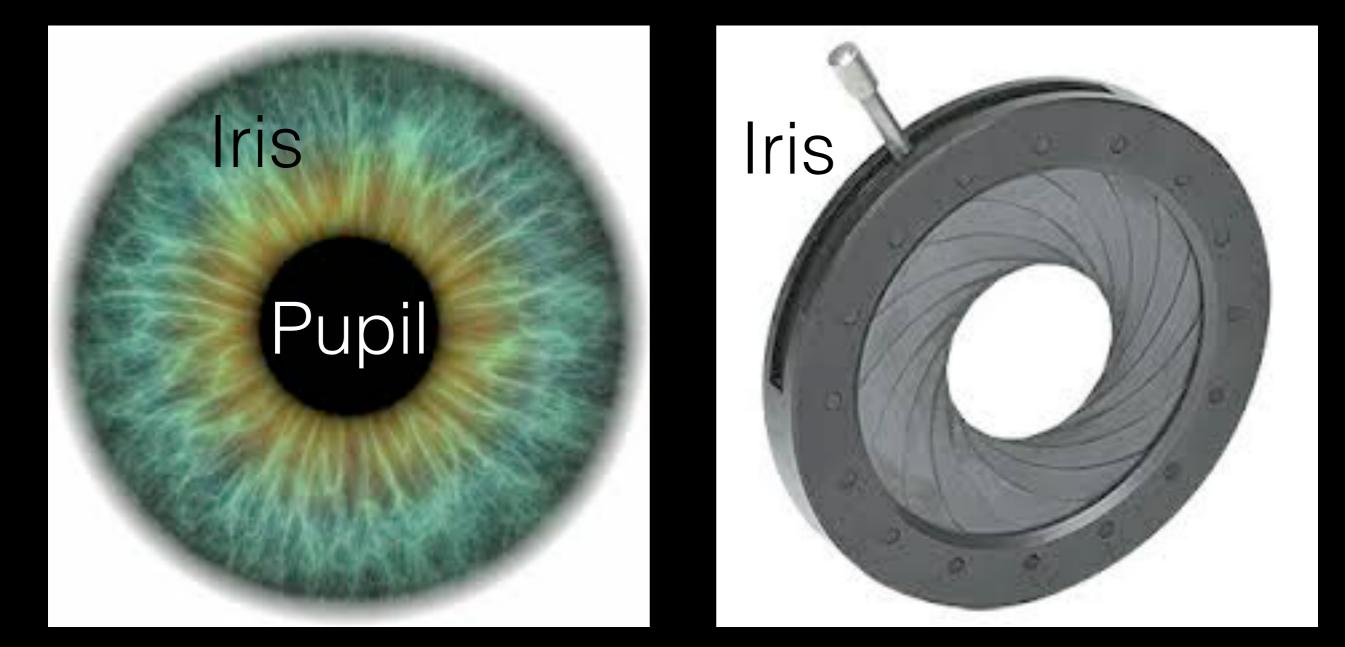


#### 100nm

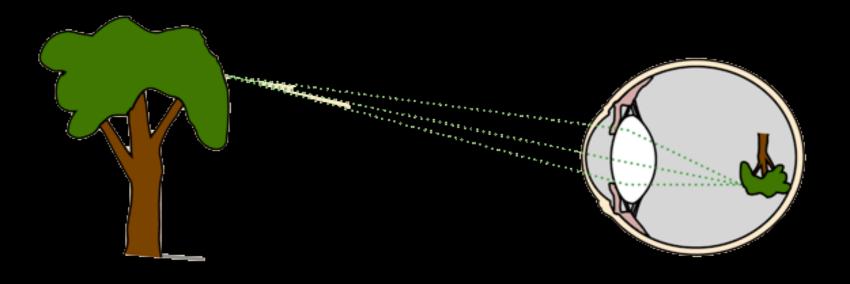
#### Scanning Electron Micrograph

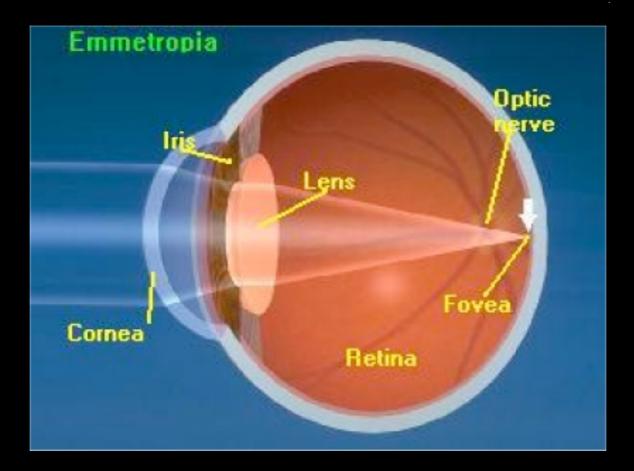


# lris vs. pupil

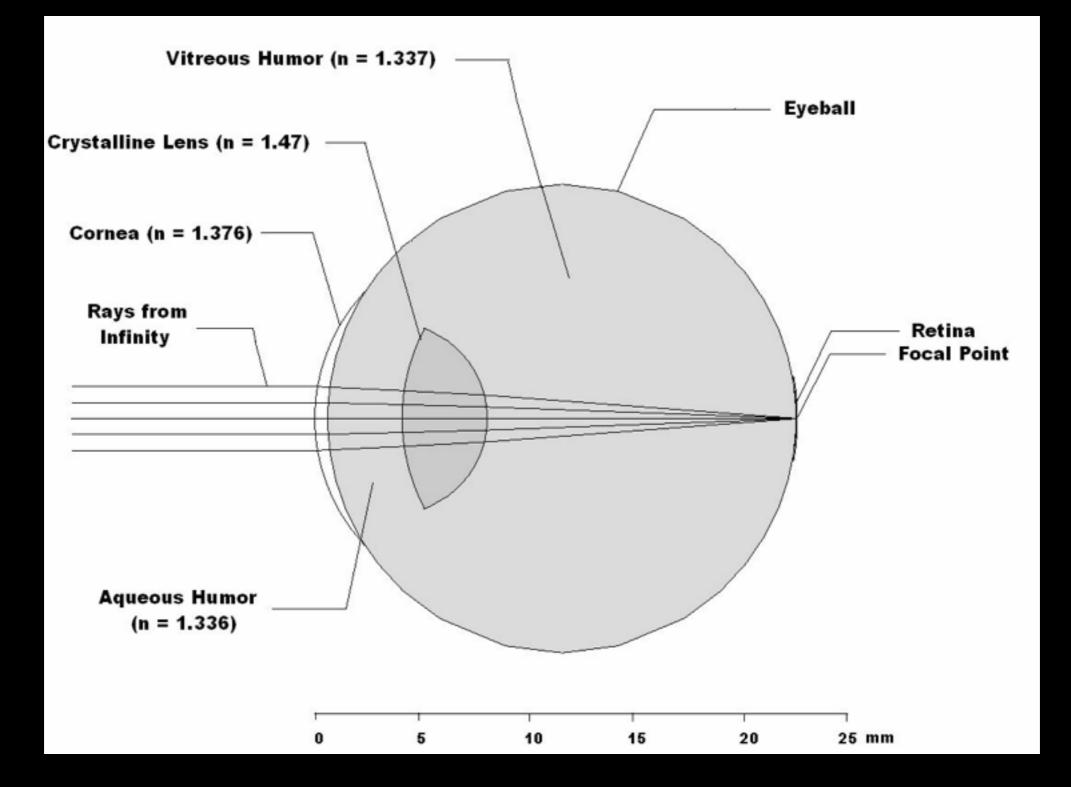


#### Image Formation in the Eye





### Refractive Index map





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

This is necessary to correct for optical aberrations

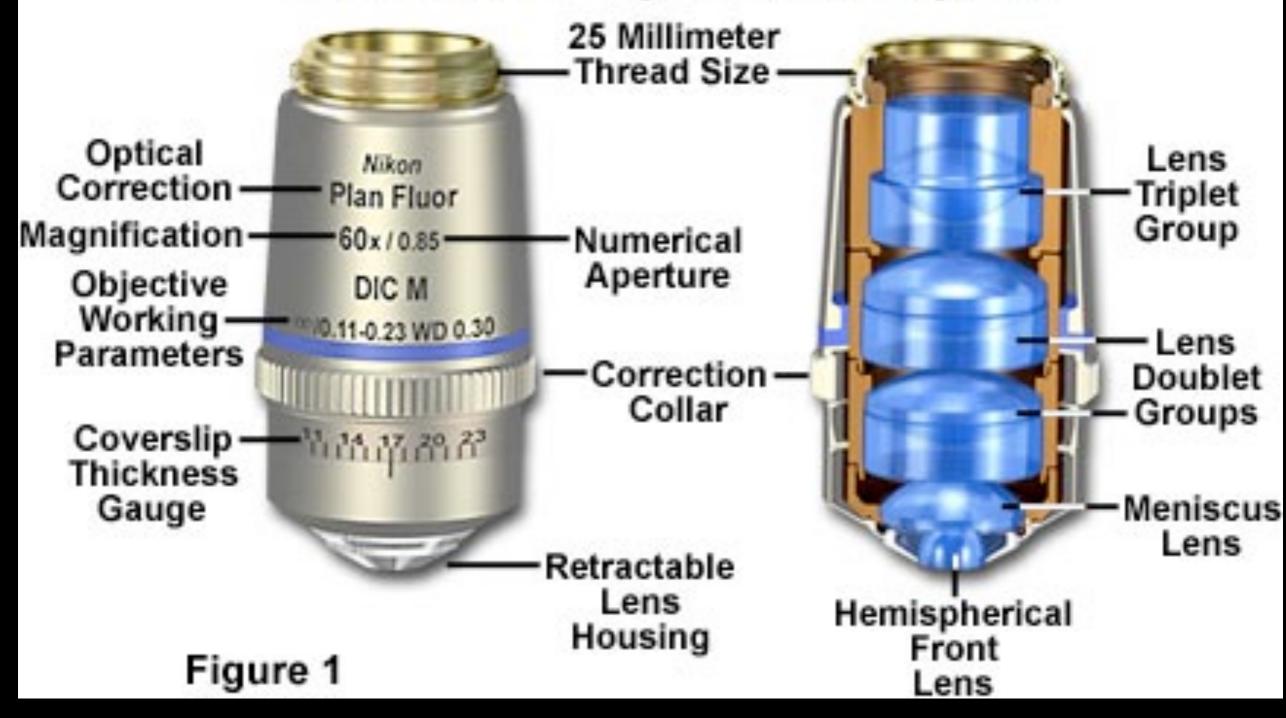
# Optical Aberrations

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

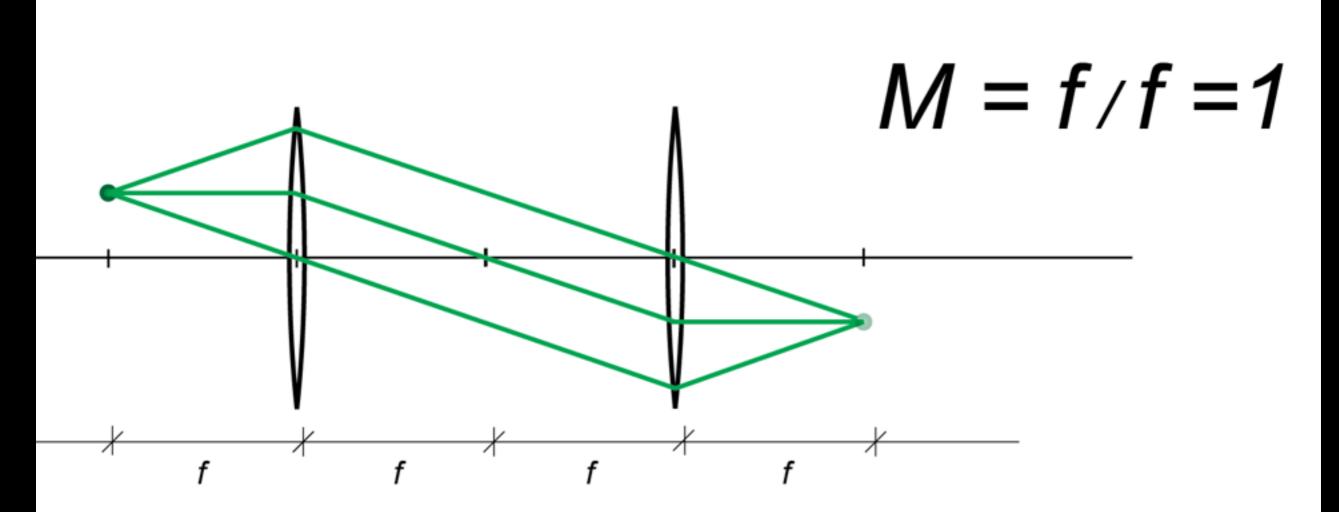


# Microscope Objective

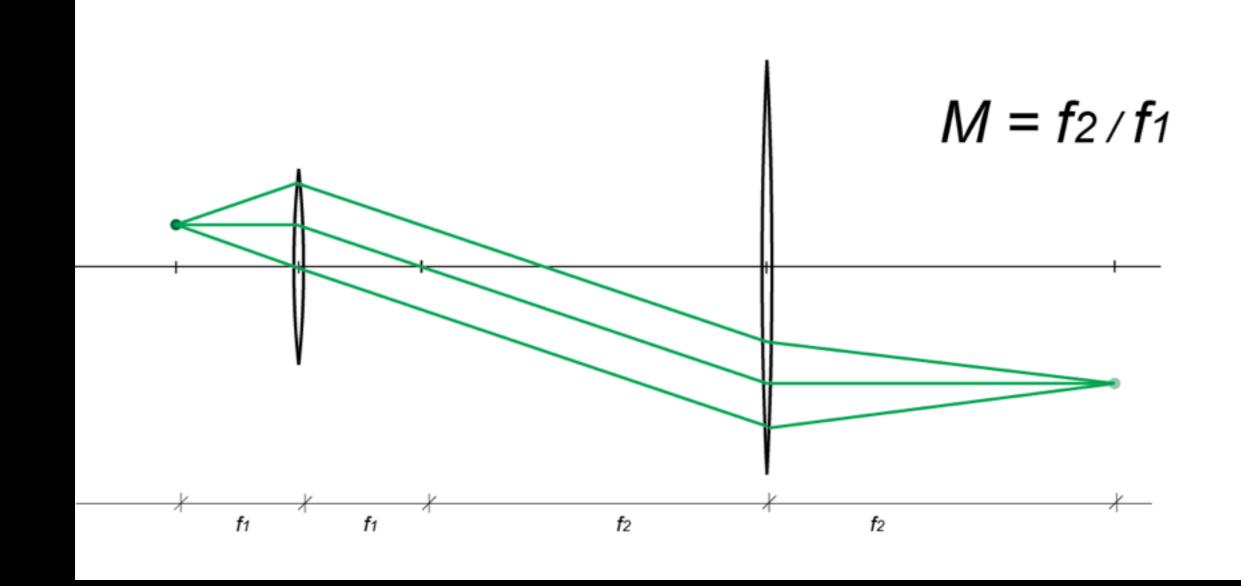
#### Nikon CFI60 Infinity-Corrected Objective



# Imaging with two lenses

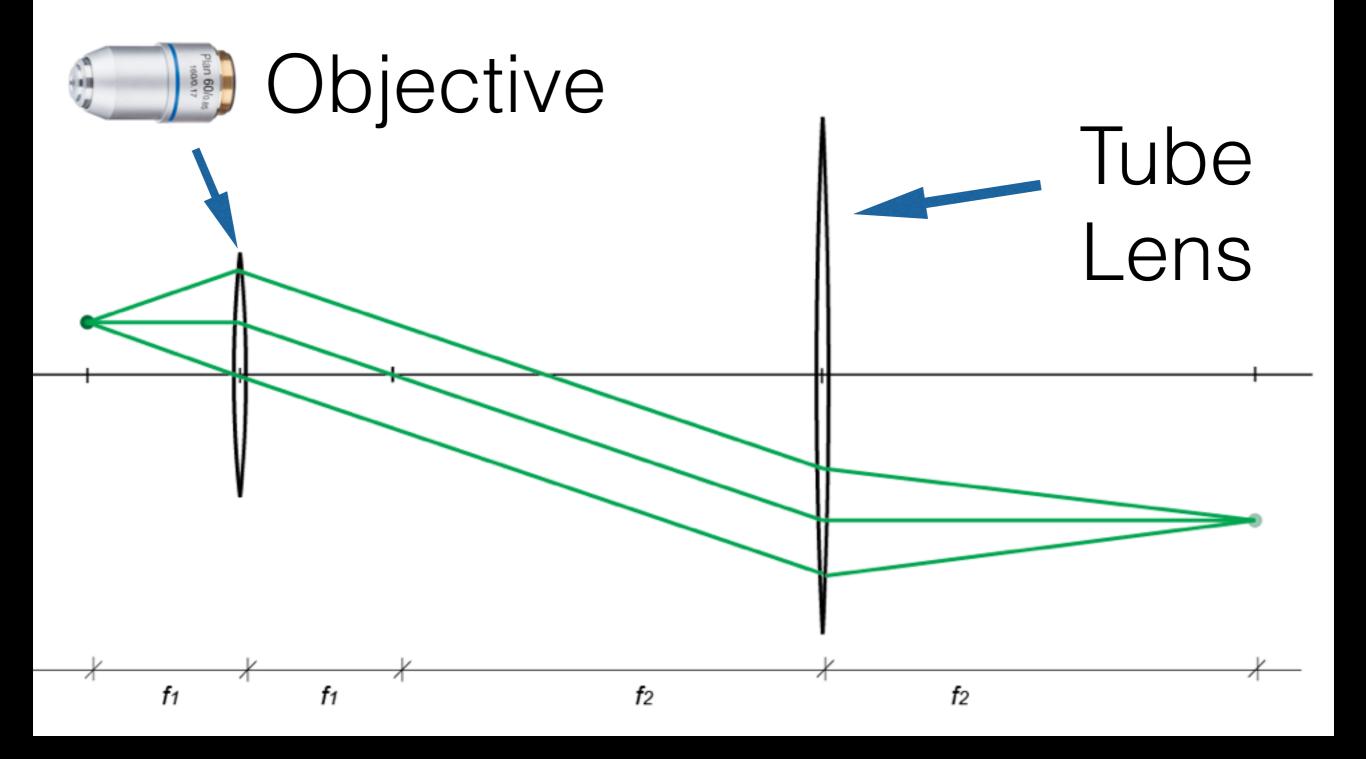


# Magnification



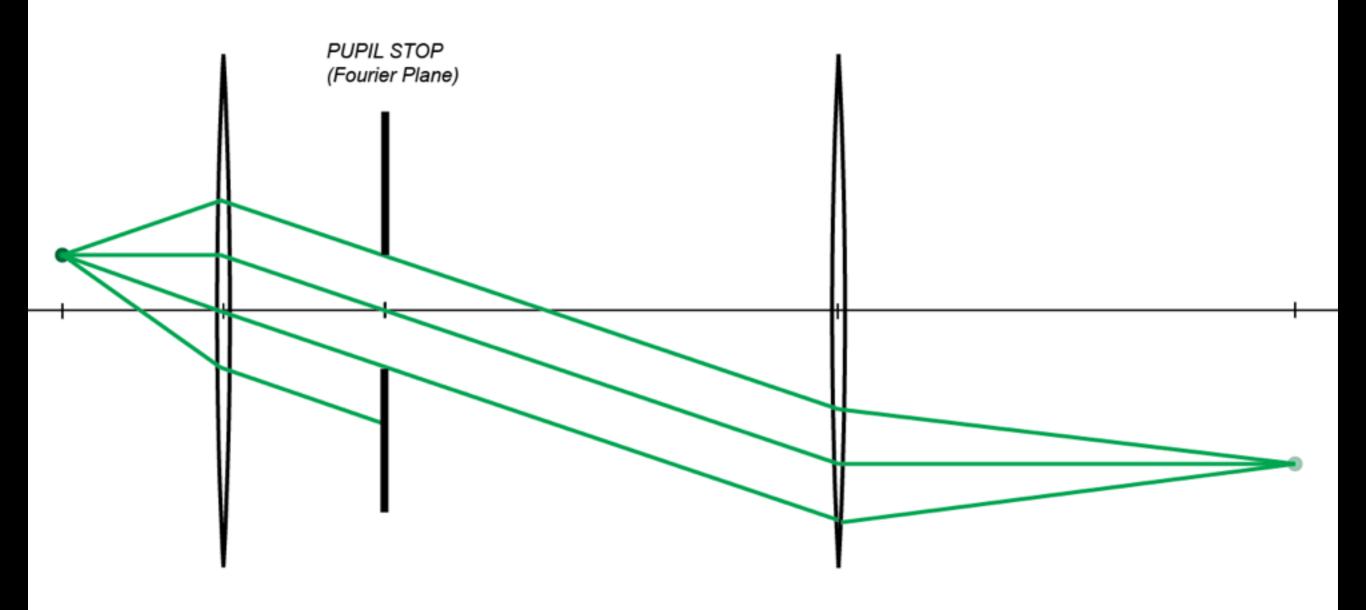
Simple geometrical proof that this is M with like-sided triangles

### Microscope:



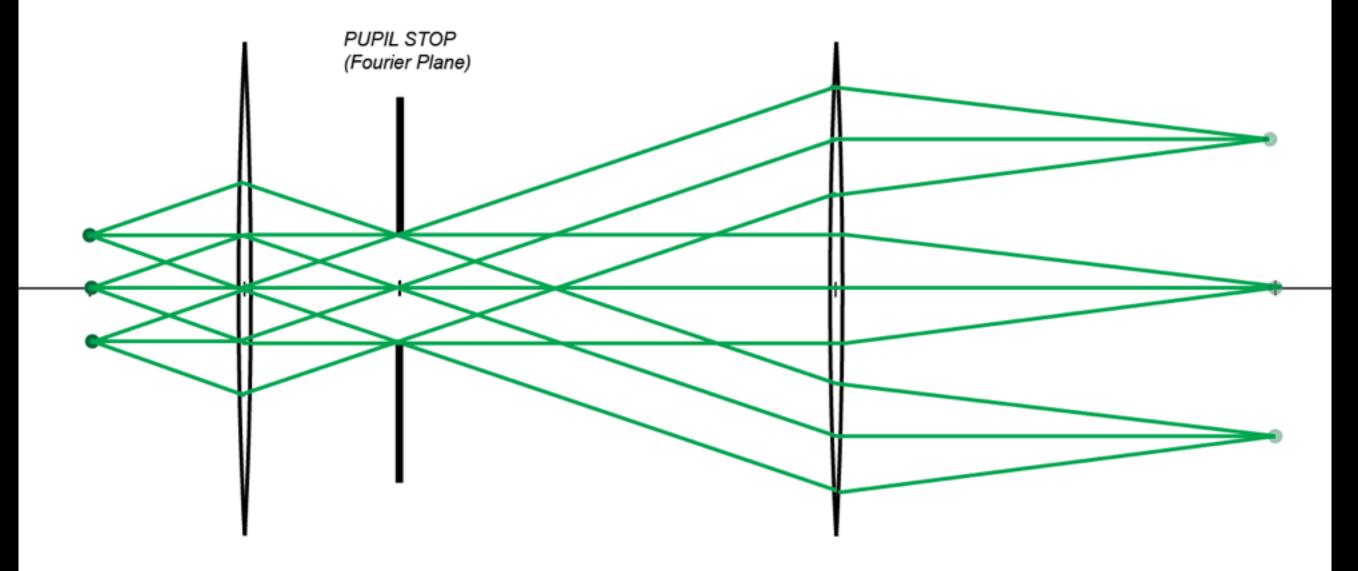


# Pupil Stop



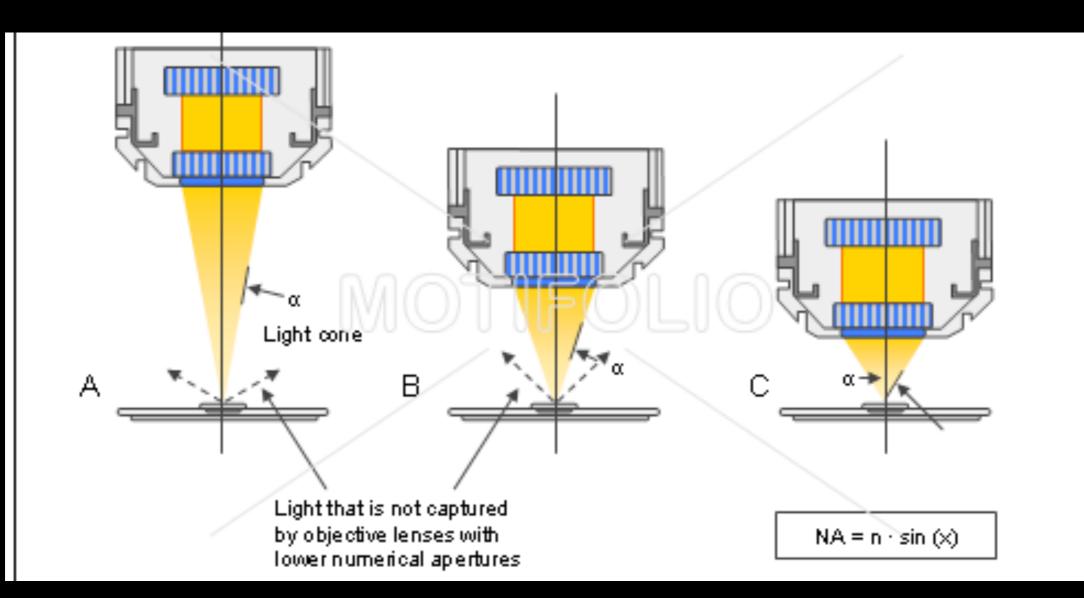
#### Pupil stop determines Numerical Aperture (NA)

# Angle vs. Position

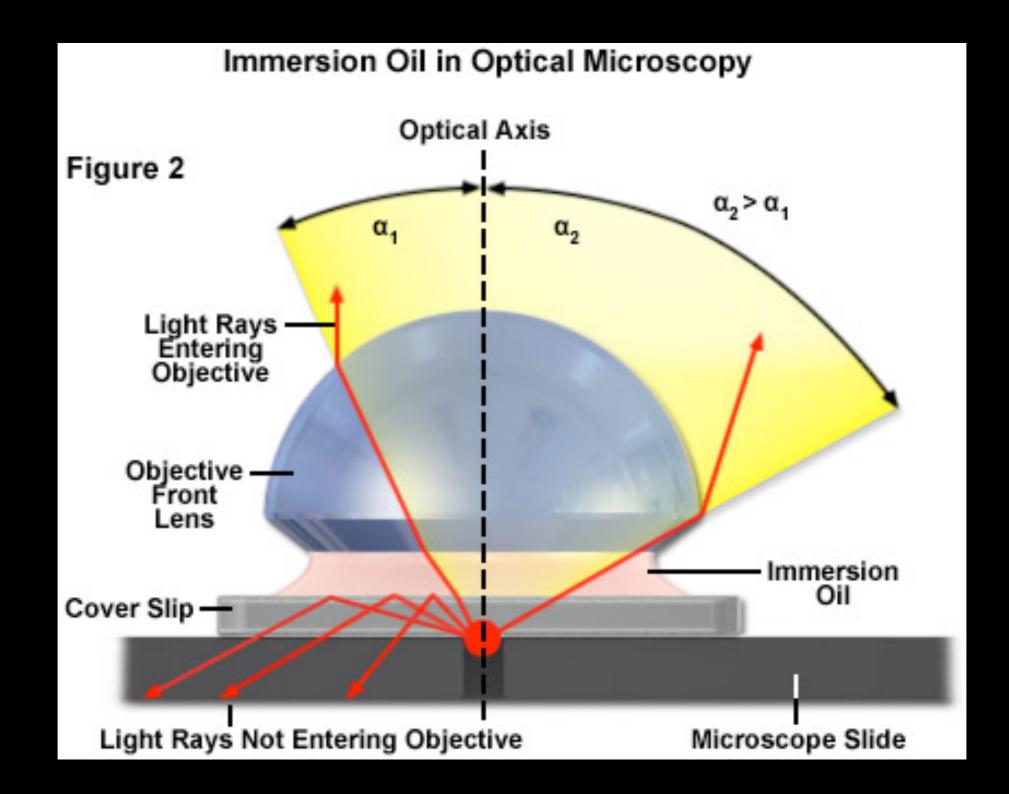


# NA = n sin(a)

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

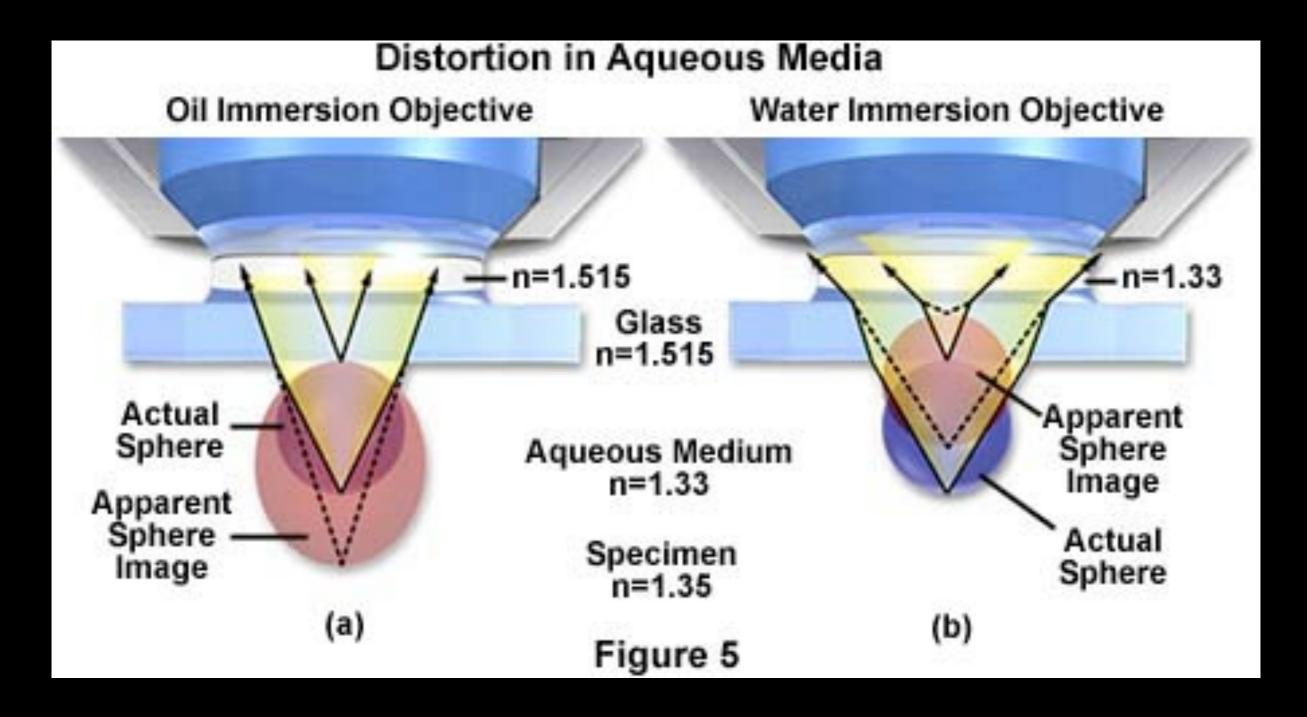


### Immersion Medium



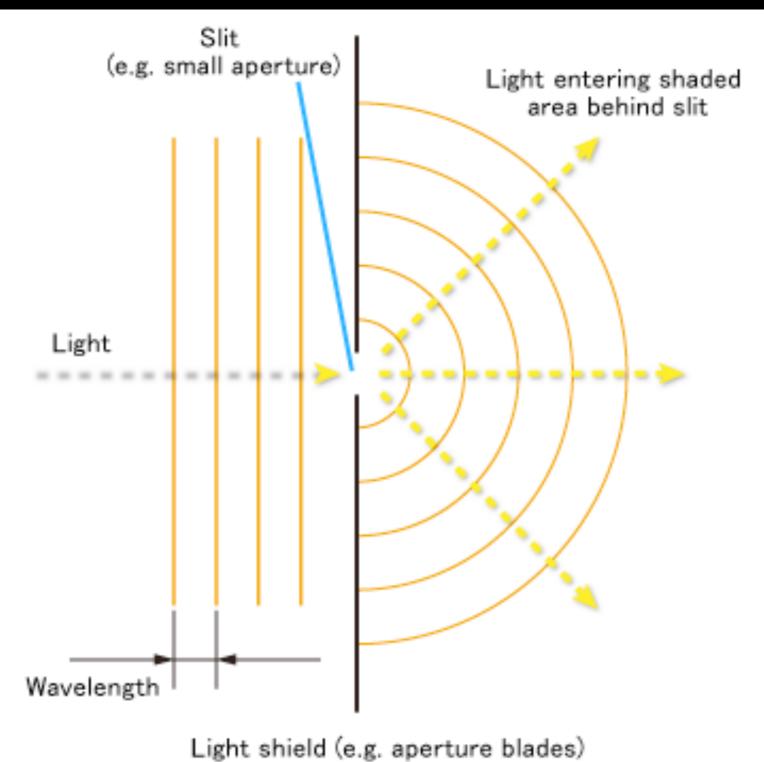
# Match to Specimen!

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

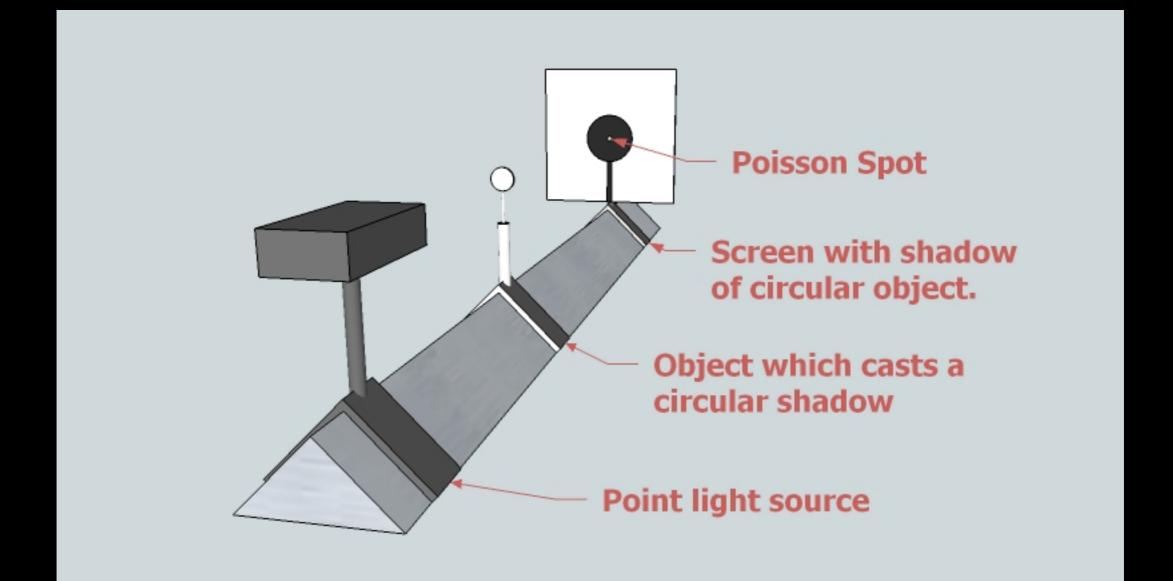


#### EXAMPLES AND QUESTIONS

### Diffraction

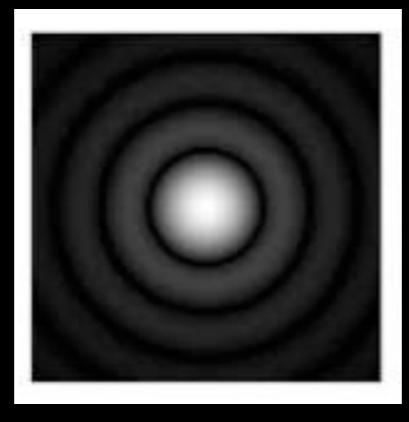


### Poisson's spot



Light ripples and bends around edges too

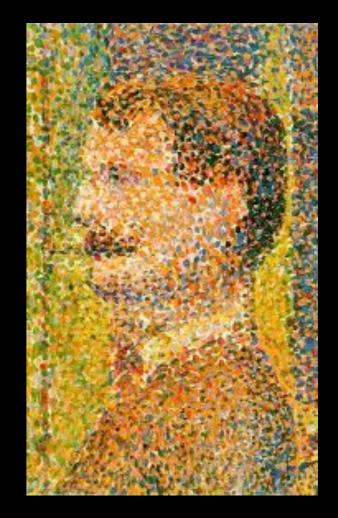
# The Airy Disc



Arises from diffraction when we image through a circular aperture

# Imaging Point Sources

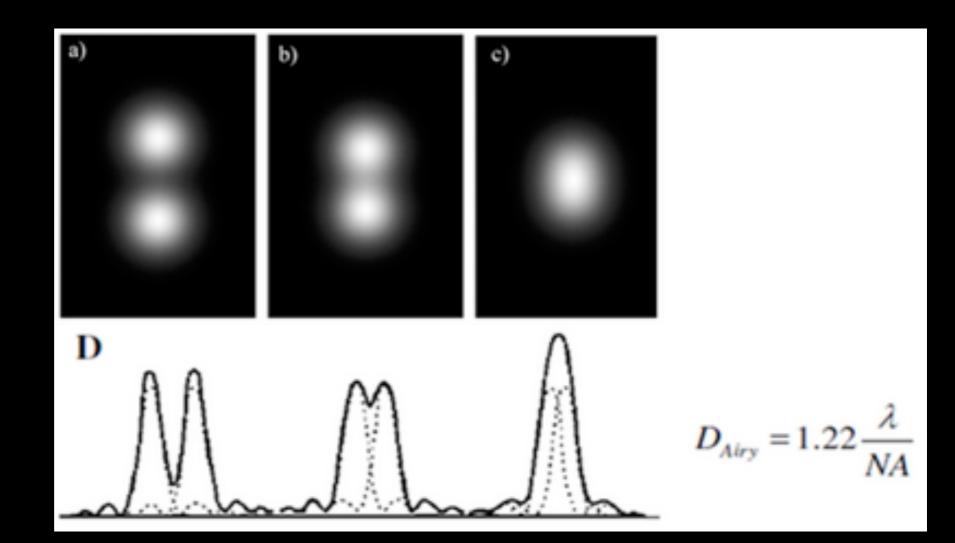
- The object can be modeled as a number of point sources
- The points are blurred by the imaging system (The response function of a point imaged through a circular aperture is the Airy disc)



(from Seurat's *the Circus*)

### Resolution criteria:

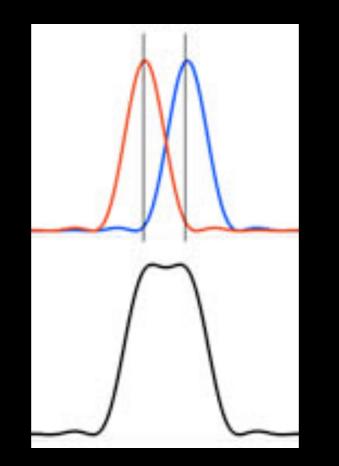
- Raleigh
- Sparrow
- Airy
- Abbe



# 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:  $d = (550 \text{ nm}) / (2 \times 1.4) \approx 200 \text{ nm}$ 

## Assumptions

- Limited NA
- Uniform Illumination
- Linearity



#### Resolution

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coloured lights. phos'phor us, n. A non-metallic element, a yellowish wax-like substance underga-ing slow combustion at ordinary temthe dark; ~us neeros'is (collog. phoses jaw), gangrone of jawbone due to ~ue fumes esp. in match-making. Hence or cogn. phospho'ric, ~ous, aa., ~iss(5) a. (path.). [L, = morning star, f. Gk phosphoros (phos light + -phoros -bringing)) phoss'y. See PHOSPHORUS.

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shine f. phös phötos light, see -ISM)] ous,

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#### High resolution

#### Low resolution

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High contrast

#### Low contrast

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- ous, shine f. phös phötos light, see -ISM)]
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### Depth of field

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- of electrons from solid, liquid, or gaseou  $V^{-}$
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#### Large depth of field

#### Small depth of field

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#### **Modulation Transfer Function**

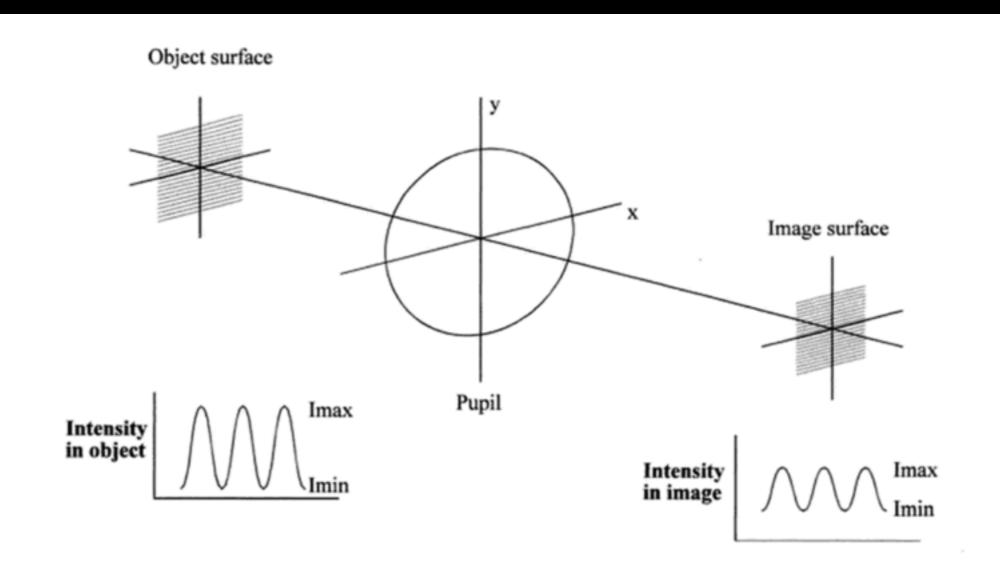
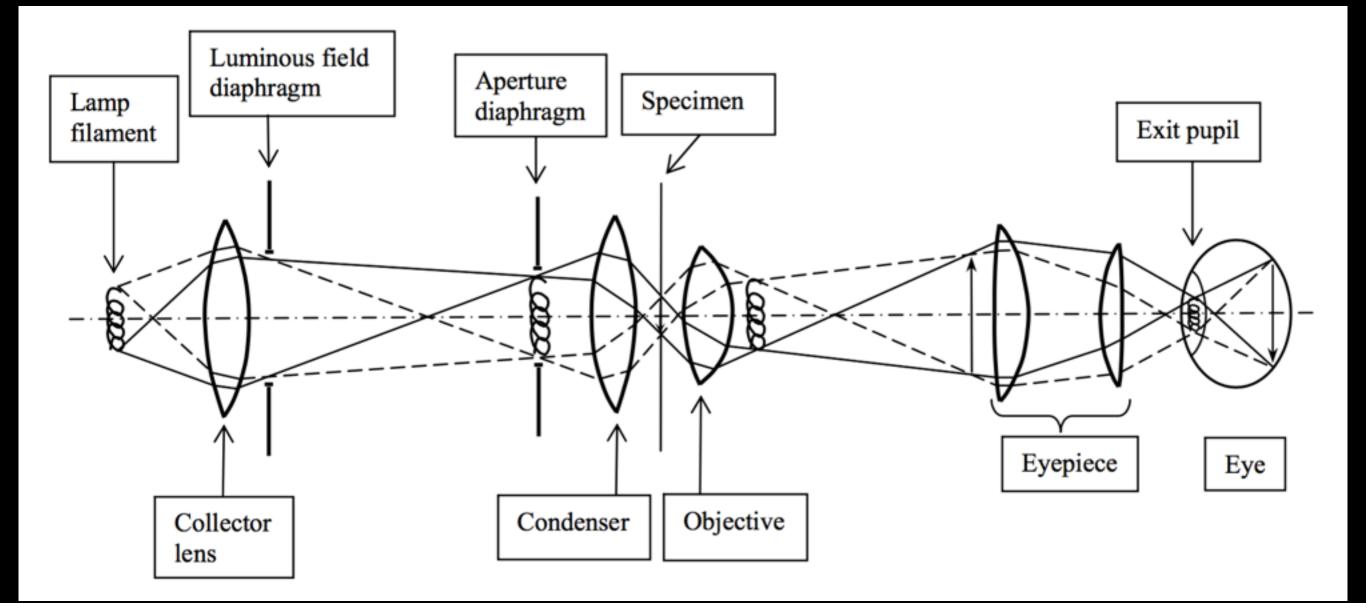


Figure 4.23. Object and image contrast.

### 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

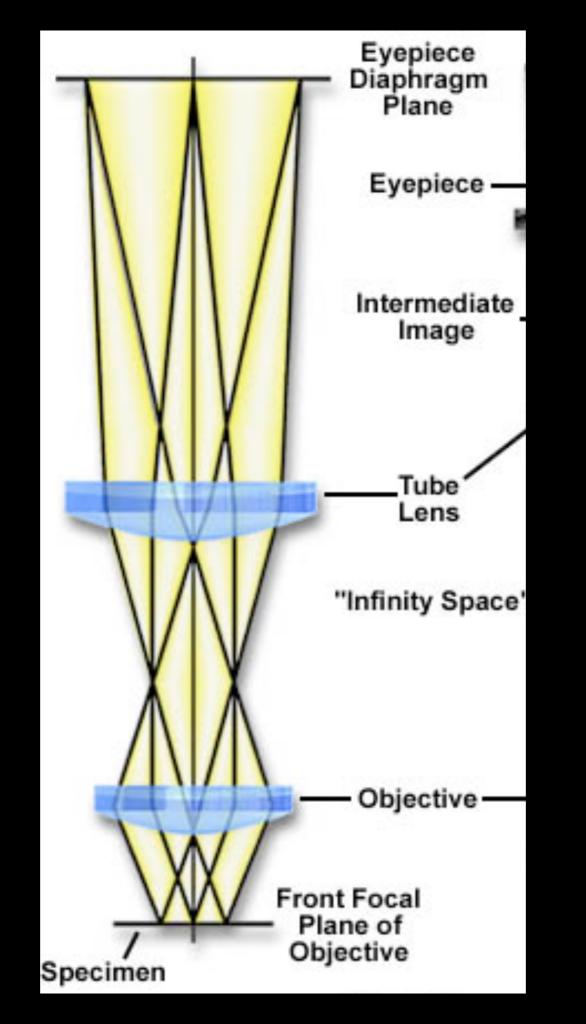
Pupil Stop limits the angle of illumination, which effectively limits the NA

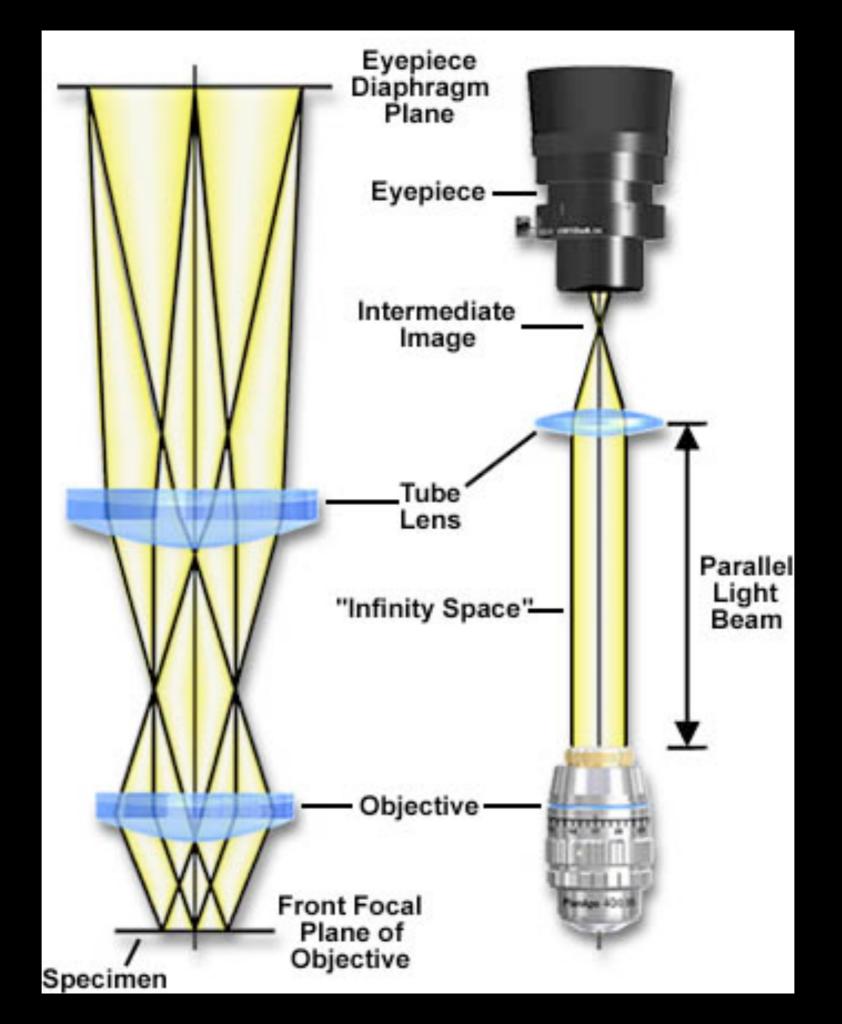
### Advice for the exam:

 Be able todo this: Draw out a Köhler illumination system

## Summary:

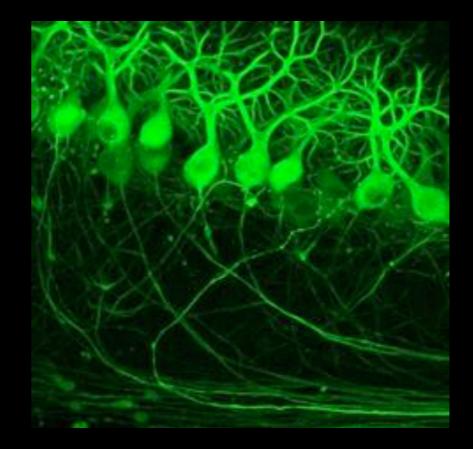
- Modern microscopes work with two lenses:
  - Objective
  - Tube Lens





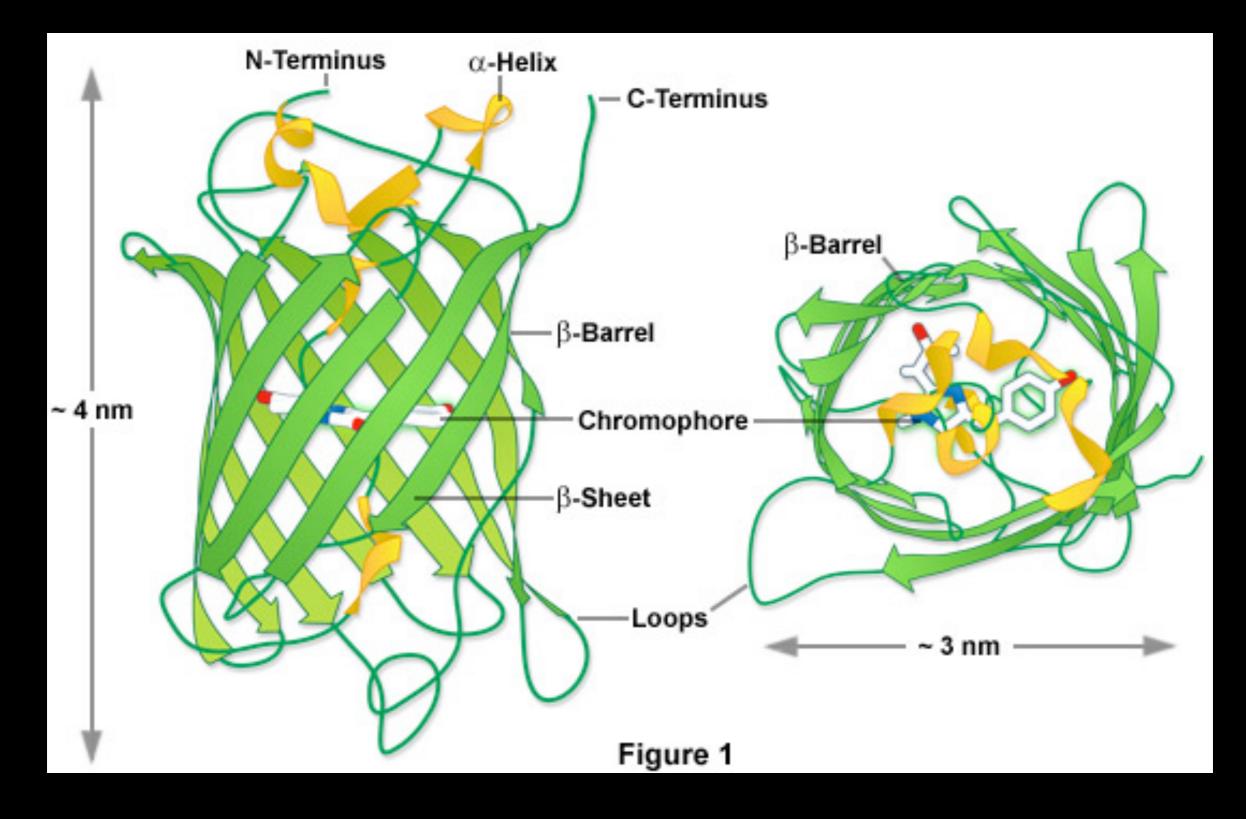
### Fluorescence Microscopy



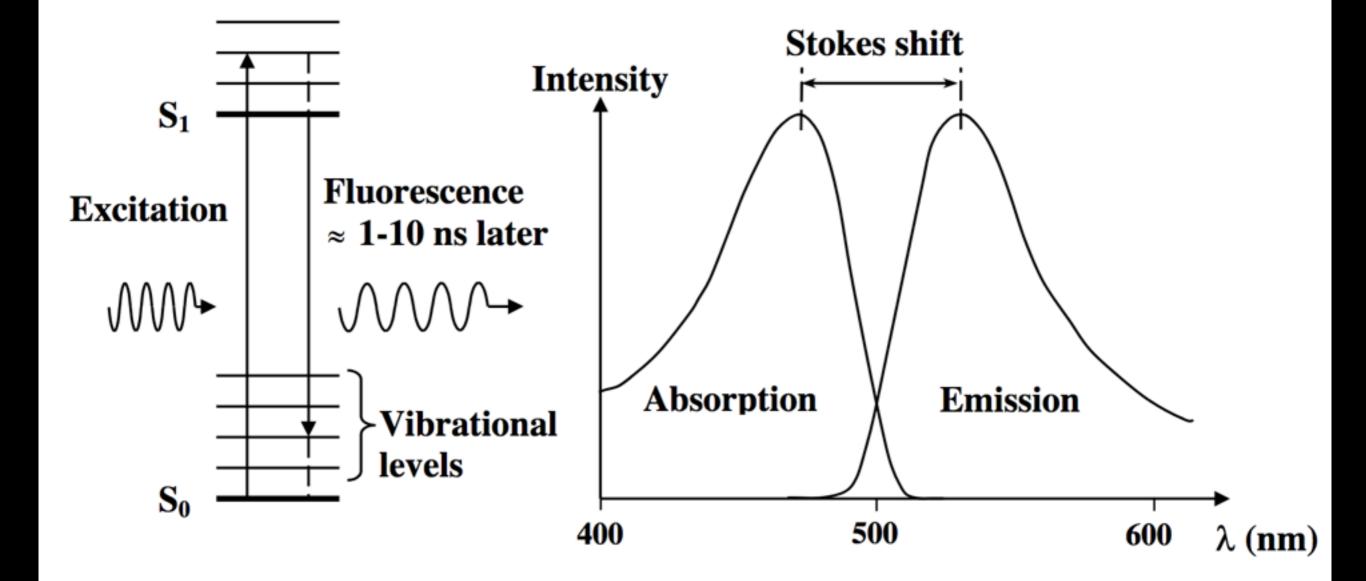


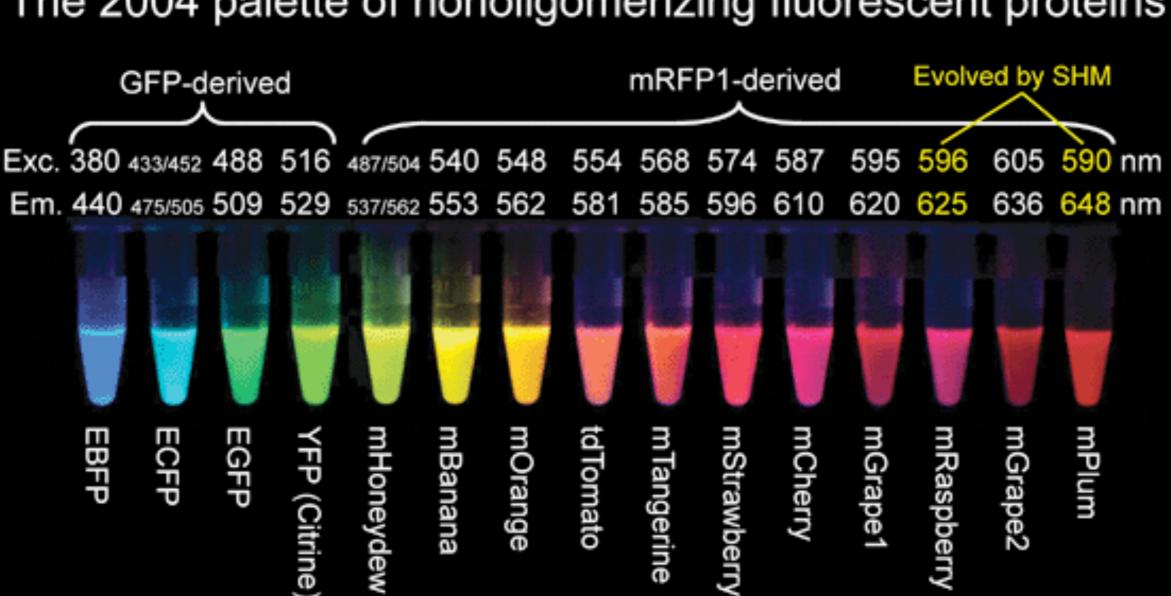
#### Green Fluorescent Protein

#### Green Fluorescent Protein



### Fluorescence Excitation

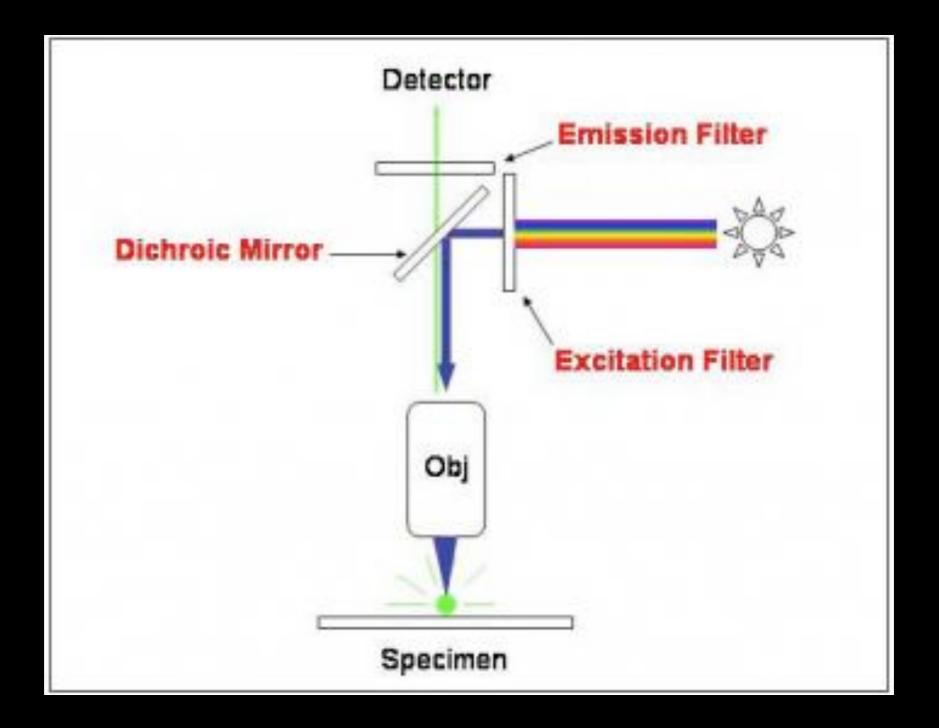


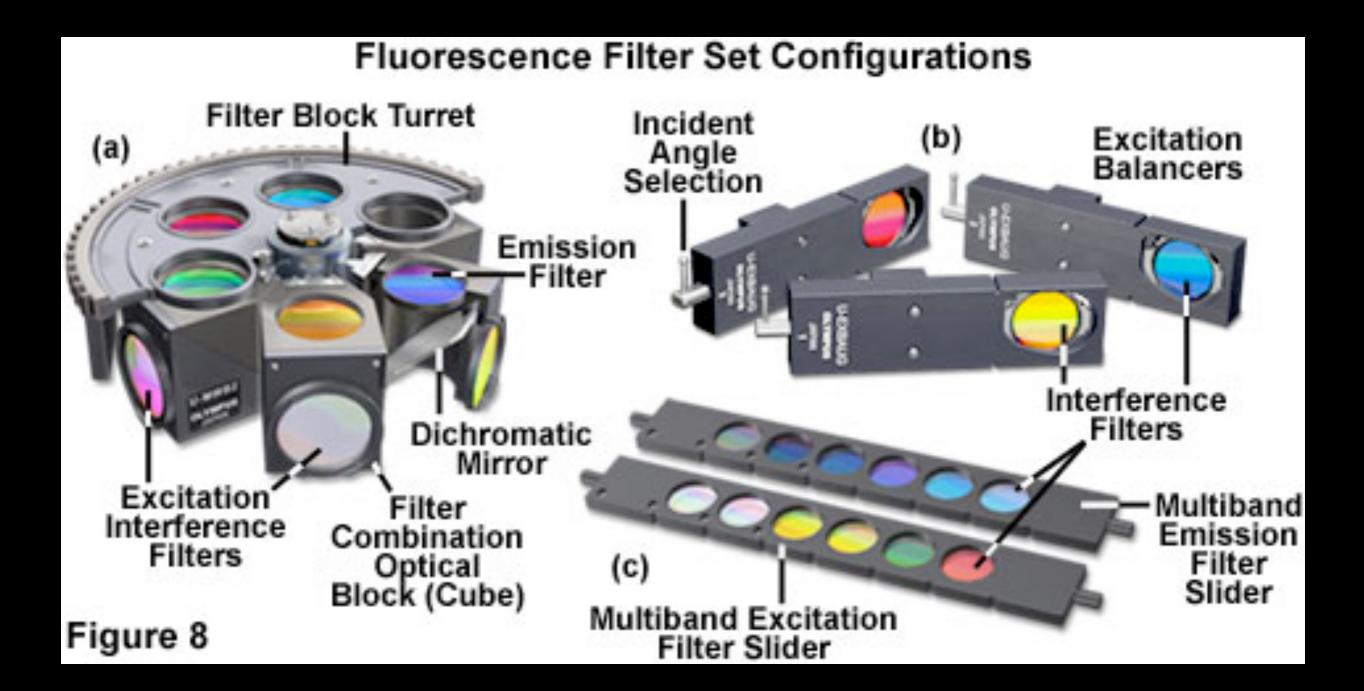


The 2004 palette of nonoligomerizing fluorescent proteins

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

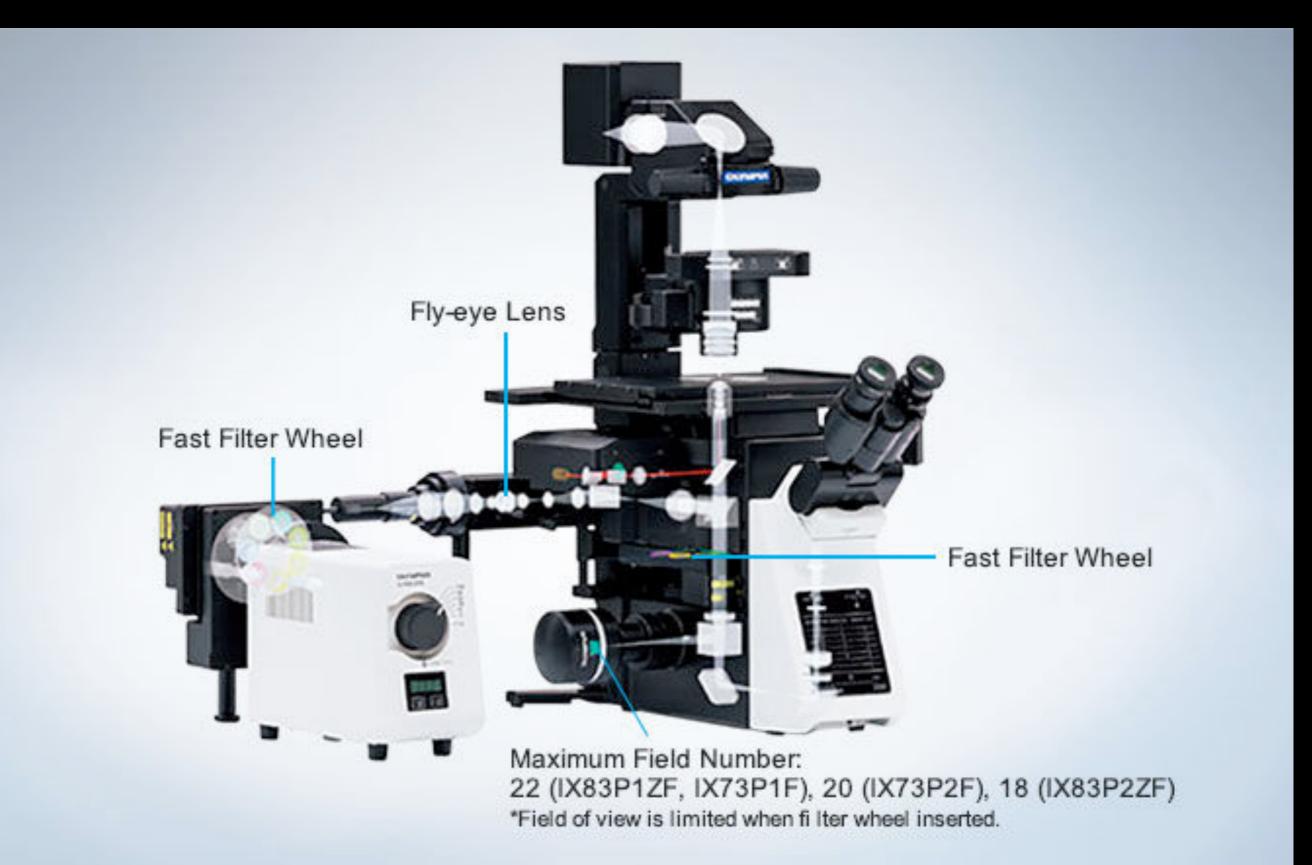




### Advice for the exam:

• Be able todo this:

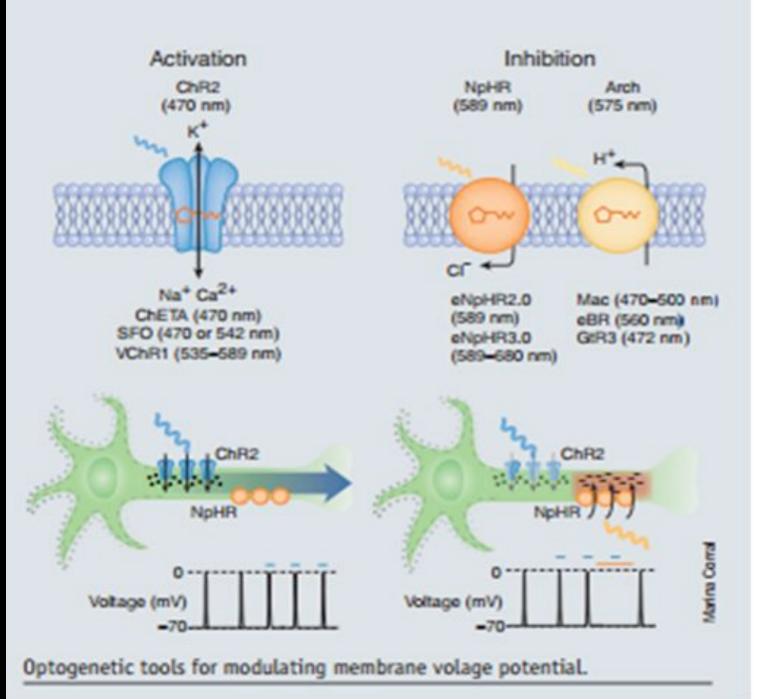
Draw out how the excitation illumination light and fluorescent light go through a dichroic mirror/filter set



## 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)



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

#### Channelrhodopsin

- Cation channel
- •Activated by blue light (470nm)
- •Allows Na<sup>+</sup> 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 CI<sup>-</sup> which hyperpolarizes the cell and inhibits the neuron
- Acts as the Off switch