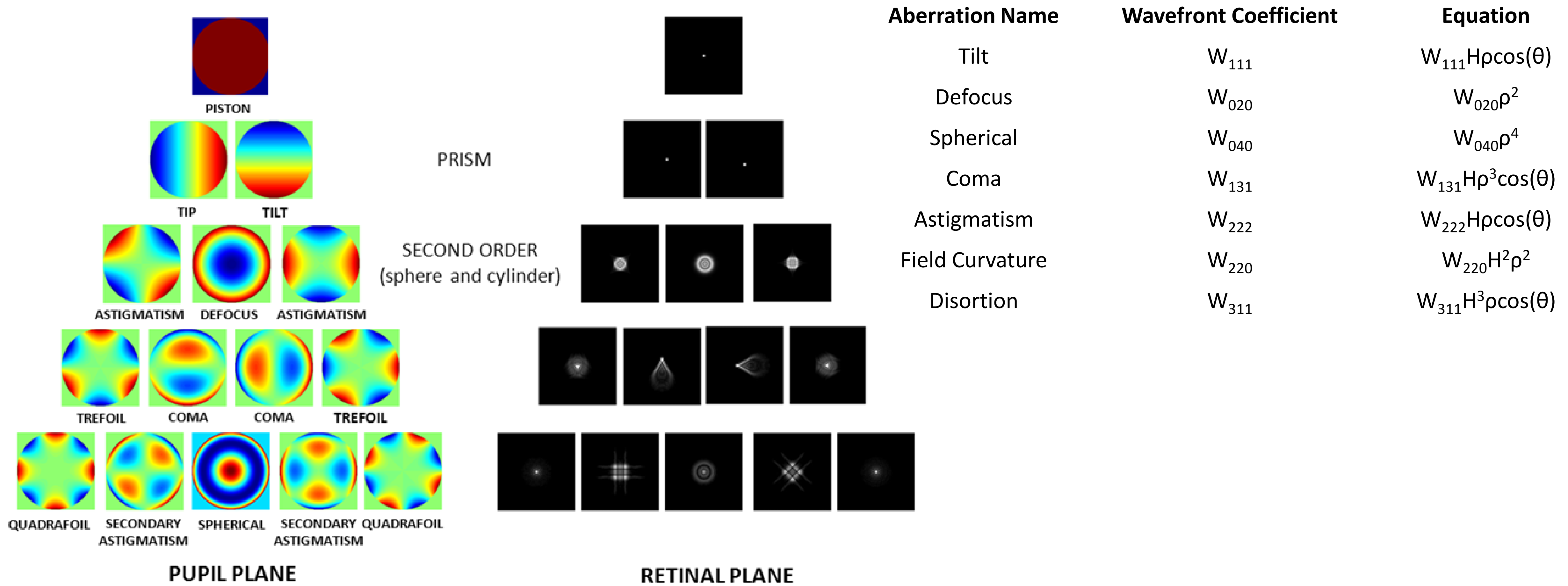
Aberrations and light sources

- Last class
  - Diffraction and imaging
  - Numerical aperture, diffraction limited spot, resolution limit
  - Aberrations
- This class
  - Finish aberrations
  - Light sources
  - Phase contrast imaging

Aberrations are well mapped

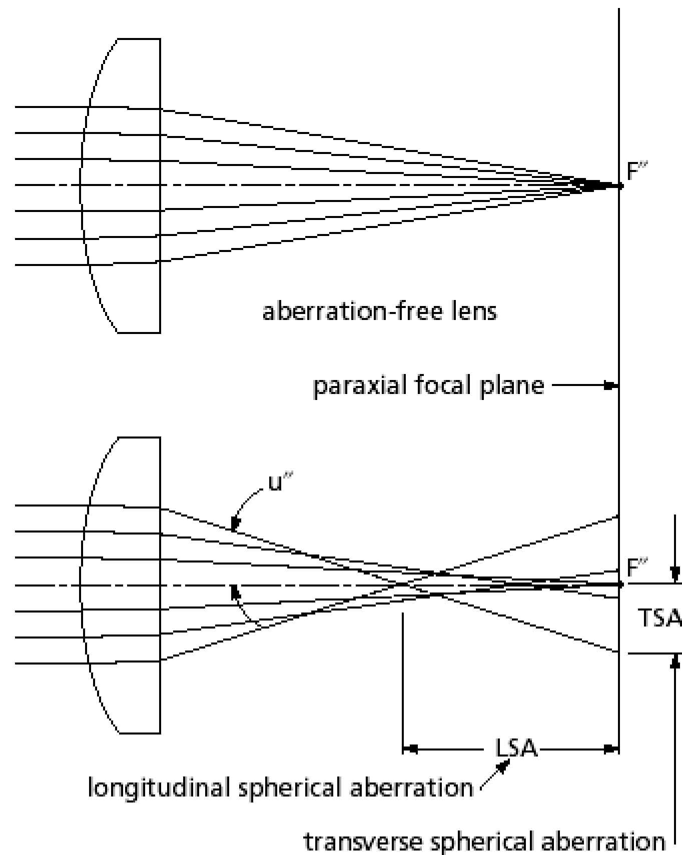


Aberration Name	Wavefront Coefficient	Equation
Tilt	W_{111}	$W_{111}H\rho\cos(\theta)$
Defocus	W_{020}	$W_{020}\rho^2$
Spherical	W_{040}	$W_{040}\rho^4$
Coma	W_{131}	$W_{131}H\rho^3\cos(\theta)$
Astigmatism	W_{222}	$W_{222}H\rho\cos(\theta)$
Field Curvature	W_{220}	$W_{220}H^2\rho^2$
Disortion	W_{311}	$W_{311}H^3\rho\cos(\theta)$

A

B

Aberrations are imperfections in collecting rays



Zernike polynomials

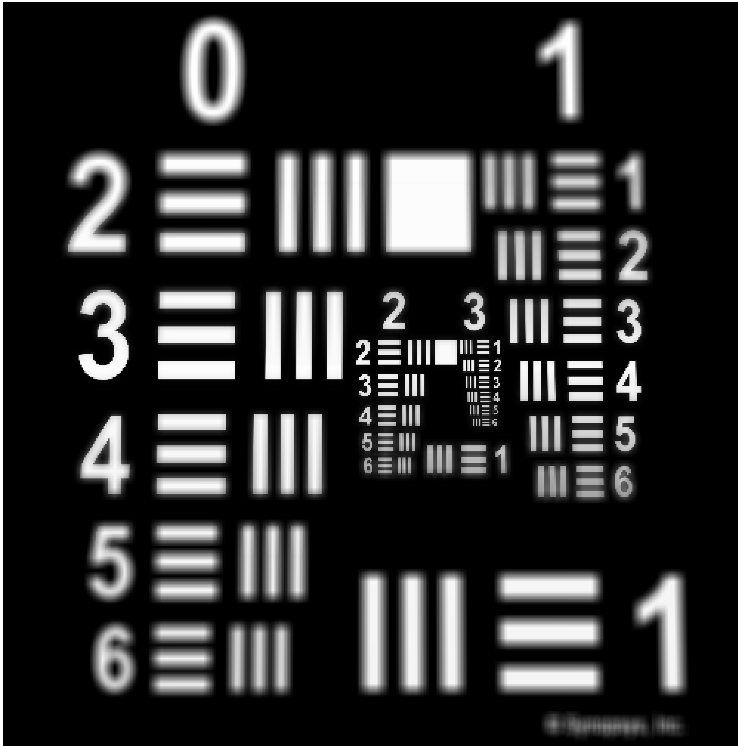
$$Z_n^m(\rho,\varphi) = R_n^m(\rho)\,\cos(m\,\varphi)$$

$$Z_n^{-m}(\rho,\varphi) = R_n^m(\rho)\,\sin(m\,\varphi),$$

$$R_n^m(\rho) = \sum_{k=0}^{\frac{n-m}{2}} \frac{(-1)^k\,(n-k)!}{k!\,\left(\frac{n+m}{2}-k\right)!\,\left(\frac{n-m}{2}-k\right)!}\,\rho^{n-2\,k}$$

Zernike polynomials have a complicated form, but don’t worry about those equations

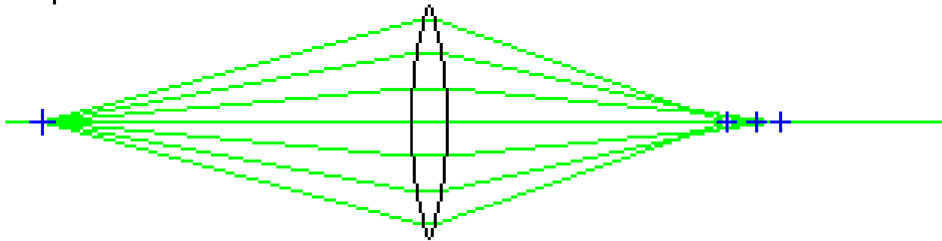
Tilt aberrations



First order aberration. Usually means your sample is not placed above the objective well.

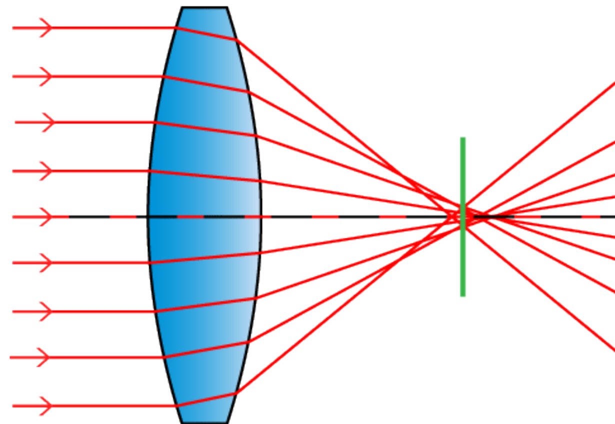
Completely correctable.

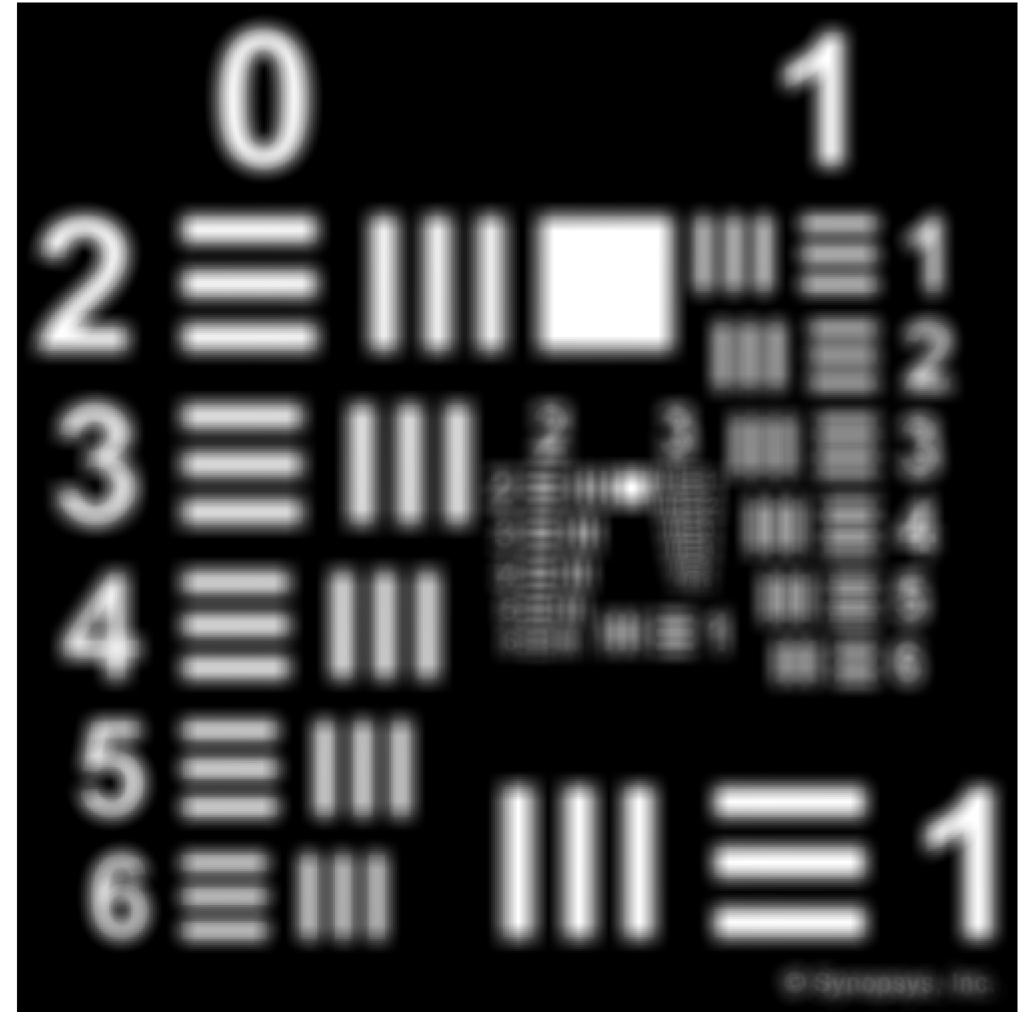
Spherical aberrations



Arise from the spherical curvature of the lens

Lens with
Spherical Aberration

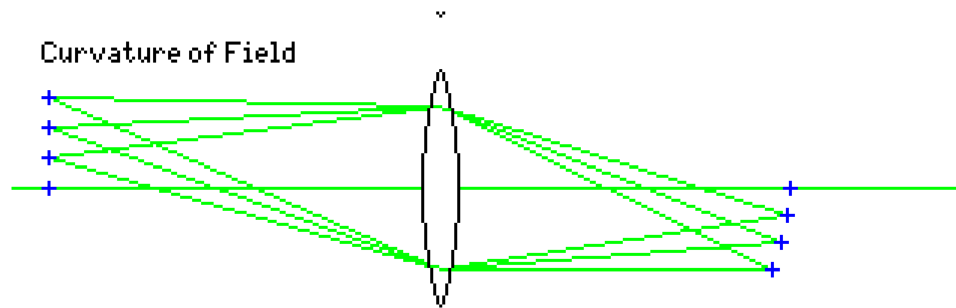


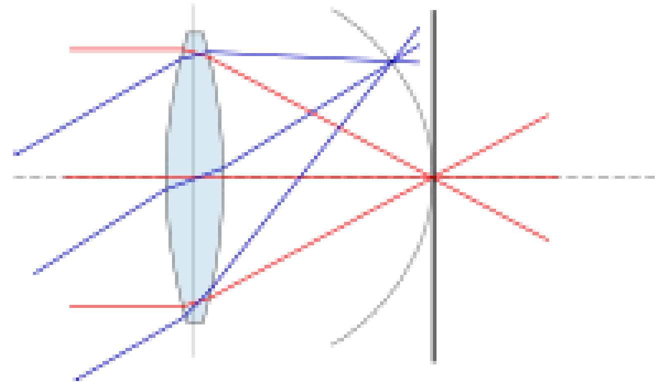


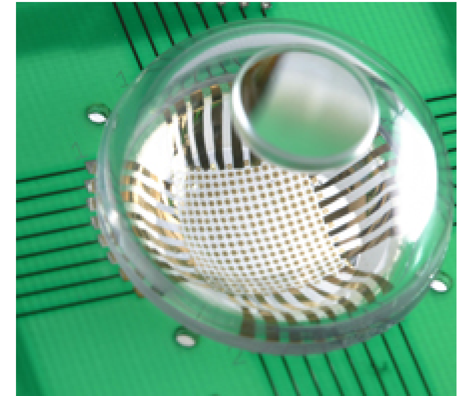
Corrections:

- Use multiple lenses with different indices
- Use aspheric lenses

Curvature of field







Arise because we focus onto a flat surface. The lens will ideally focus onto a spherical detector.

Cameras are hard to make as spheres.

Plan lenses correct for flat plane imaging

Limits overall field number (current limit 20-25 mm)

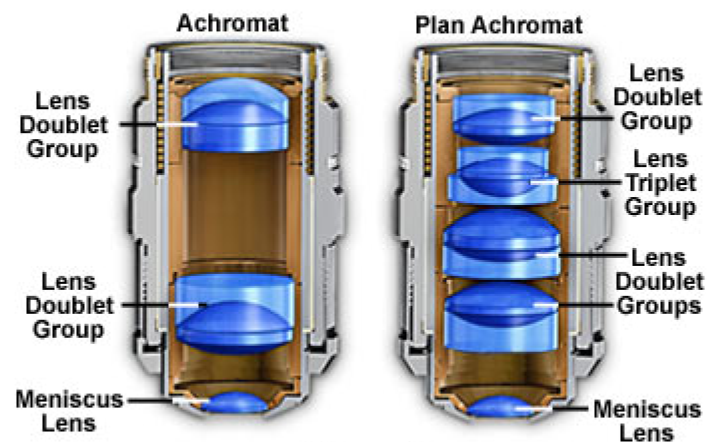


Figure 2

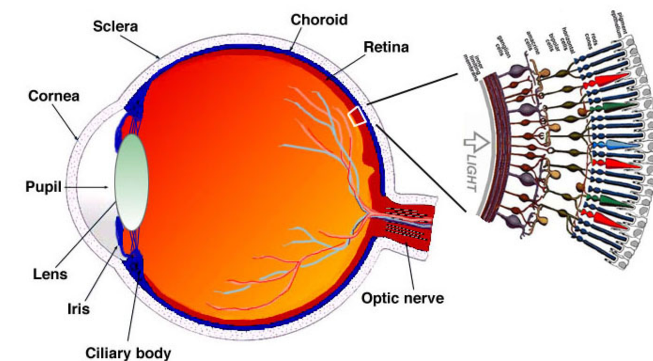
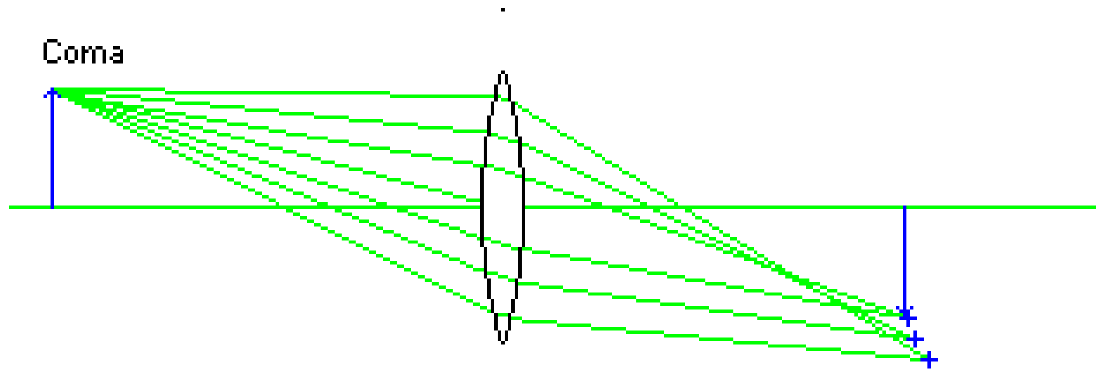
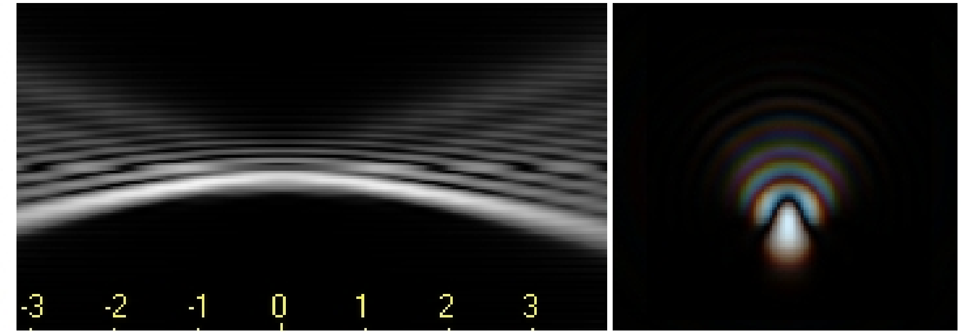
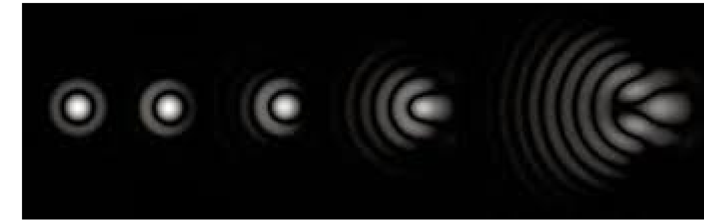


Fig. 1.1. A drawing of a section through the human eye with a schematic enlargement of the retina.

Coma



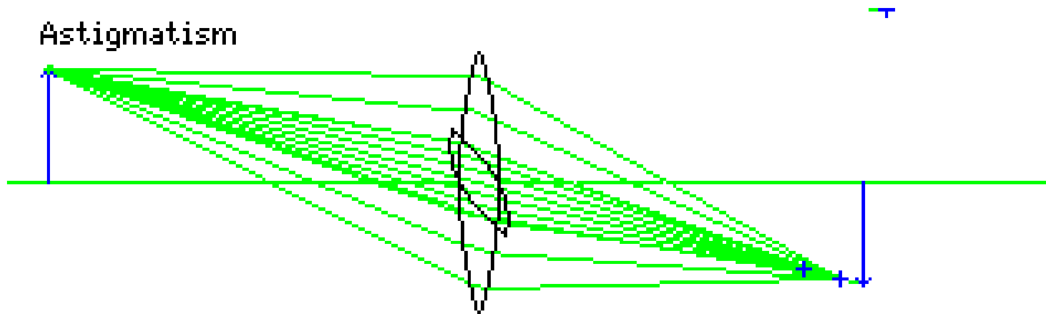


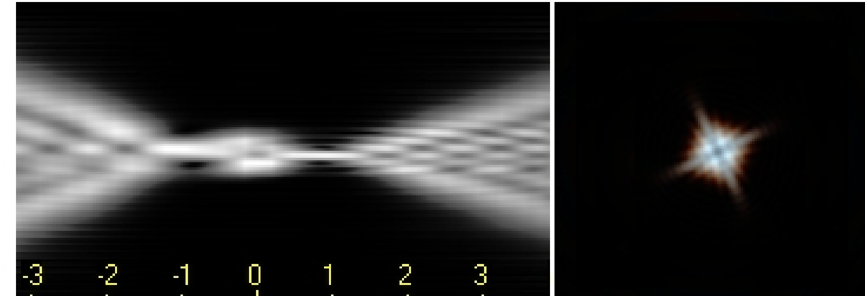


Dependent on lens shape.
Easily seen when a lens is tilted relative to the incoming light

Usually means that a lens is out of alignment

Astigmatism





Proportional to the diameter of optics

Changes from vertical to horizontal going in and out of focus

Another signature of mis-aligned optics

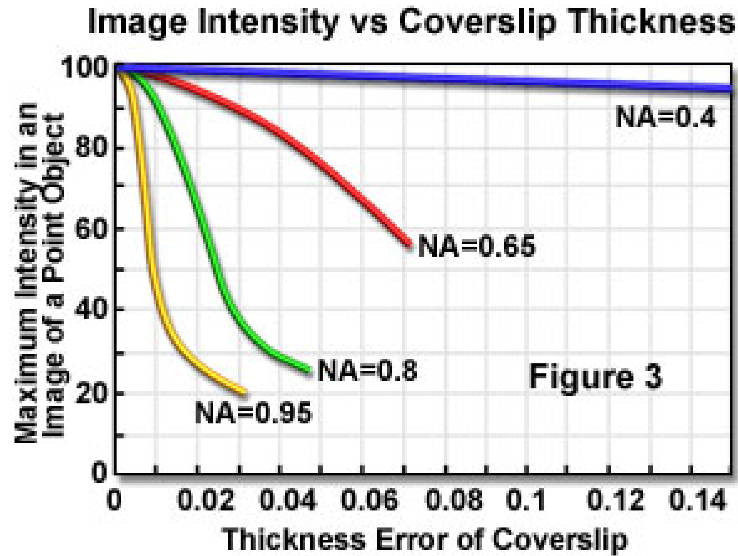
Original	Compromise
aio	aio
Horizontal Focus	Vertical Focus
aio	aio

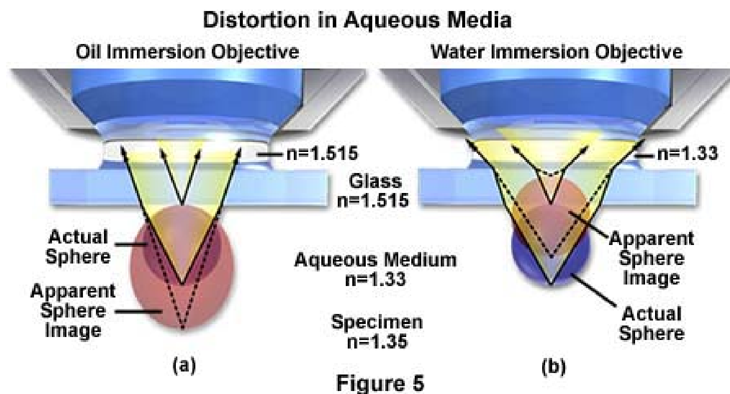
New objectives, coverslips

The transition from sample to coverslip to air/water to glass can cause serious distortions, especially at high NA

Most high NA objectives are made with correction collars that will adjust for this issue

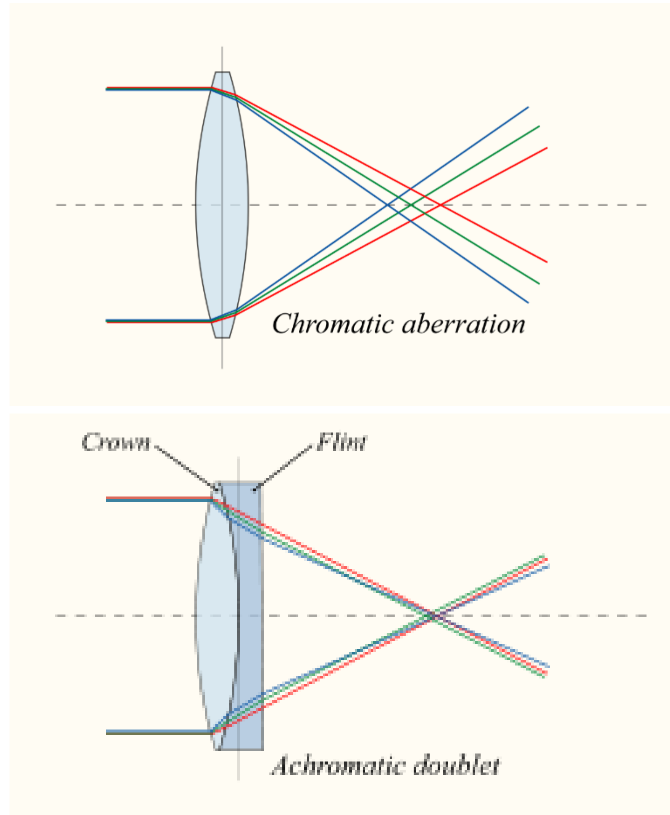
Make sure objective is coverslip corrected for inverted imaging!





- No. 0 – 0.085 to 0.13 mm thick
- No. 1 – 0.13 to 0.16 mm thick
- No. 1.5 – 0.16 to 0.19 mm thick
- No. 1.5H – 0.17 to 0.18 mm thick
- No. 2 – 0.19 to 0.23 mm thick
- No. 3 – 0.25 to 0.35 mm thick
- No. 4 – 0.43 to 0.64 mm thick

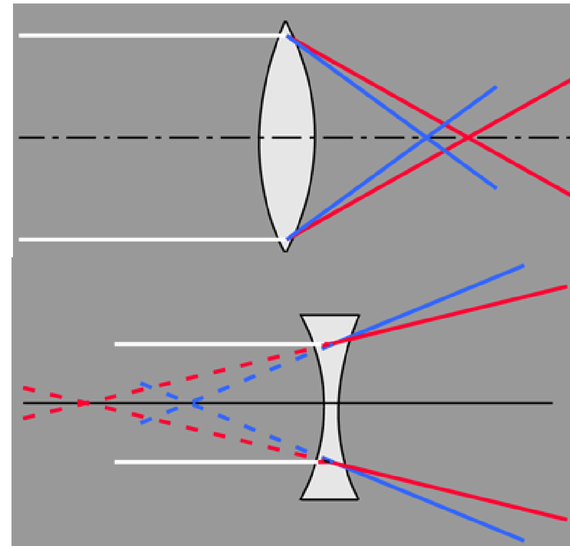
Chromatic aberrations

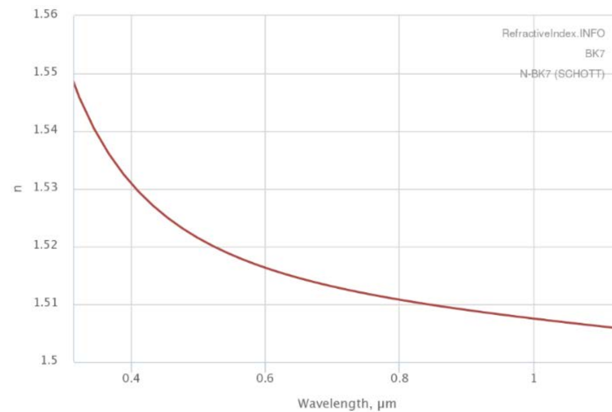


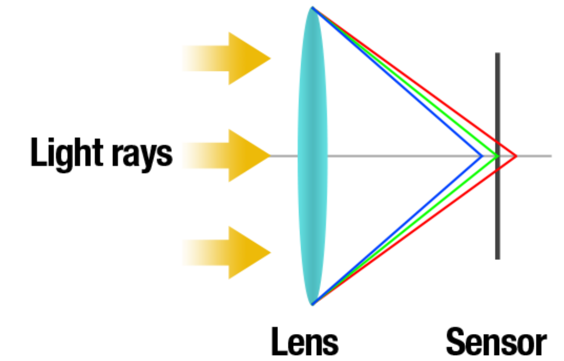
$$\frac{1}{f} = (n-1)\left[\frac{1}{R_1} - \frac{1}{R_2} + \frac{(n-1)d}{nR_1R_2}\right],$$

$$\frac{1}{f_{tot}} = \frac{1}{f_1} + \frac{1}{f_2}$$

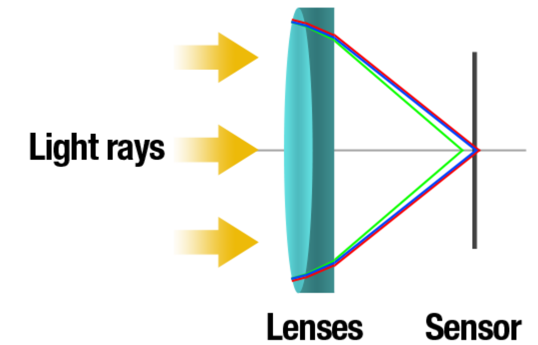
Use multiple lenses of varying indices.



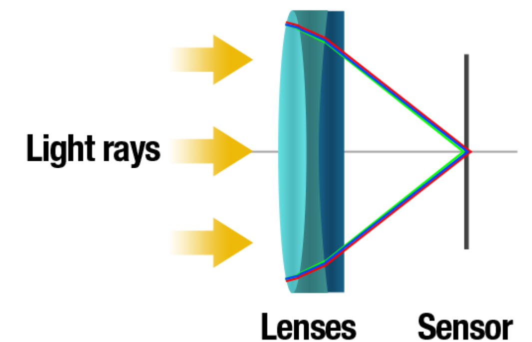




Achromatic lens design

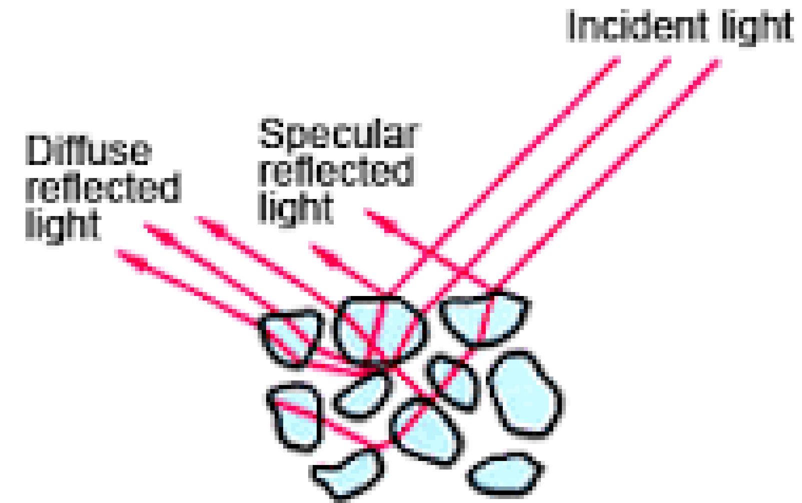


Apochromatic lens design



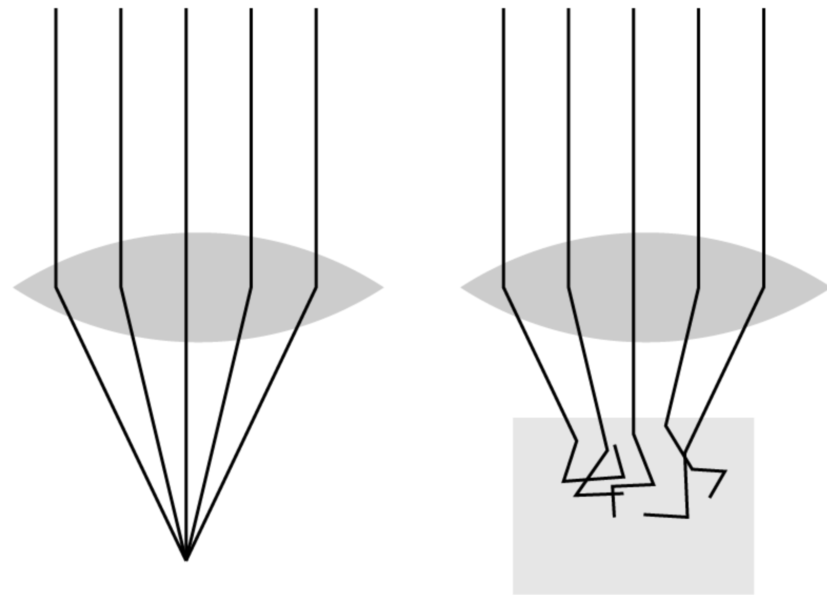
Scattering

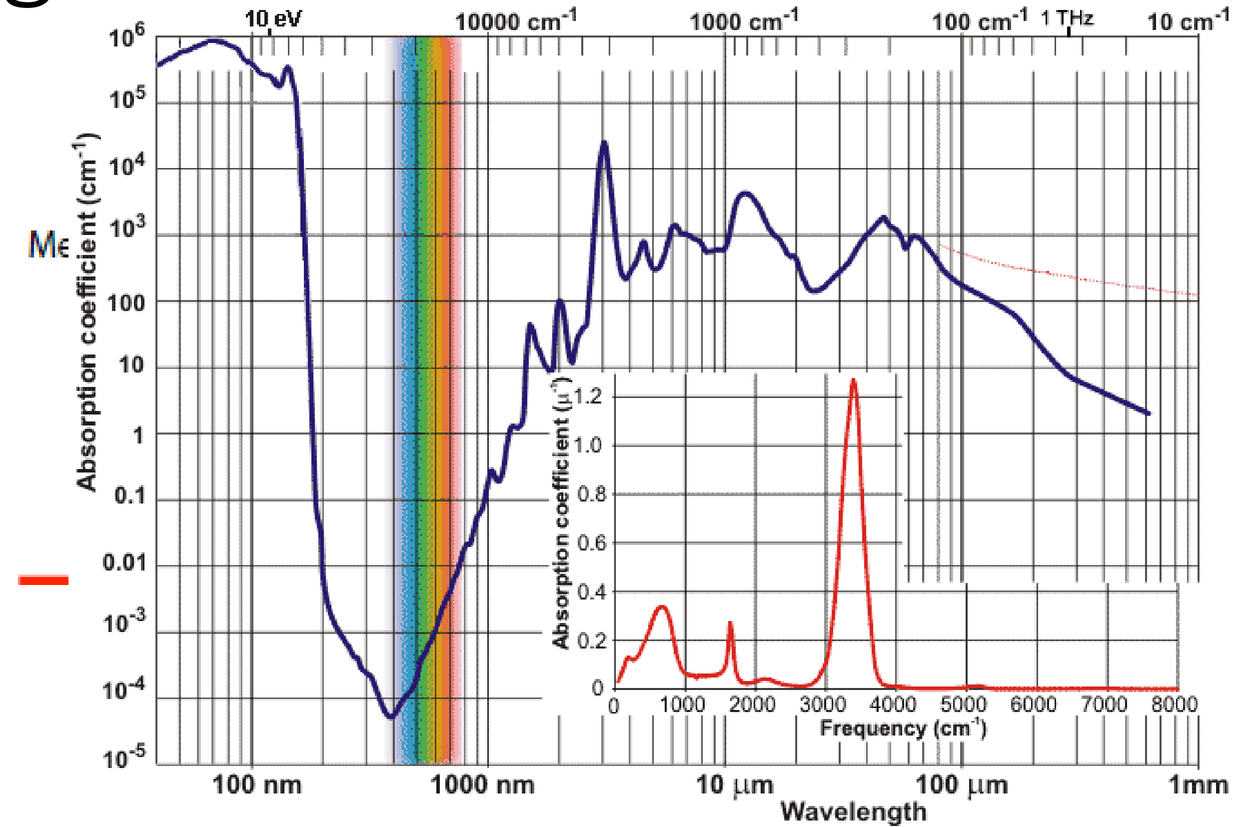
- Scattering is reflection, but off very small particles
- Wavelength in = wavelength out
- Light is redirected with angle probability $\cos^2\theta$
- Scattering in biology is mostly due to lipid bilayers (n ~ 1.5)



$$\sigma_s = \frac{2\pi^5}{3}\frac{d^6}{\lambda^4}\left(\frac{n^2-1}{n^2+2}\right)^2$$

Scattering changes the directions of light, makes imaging tough



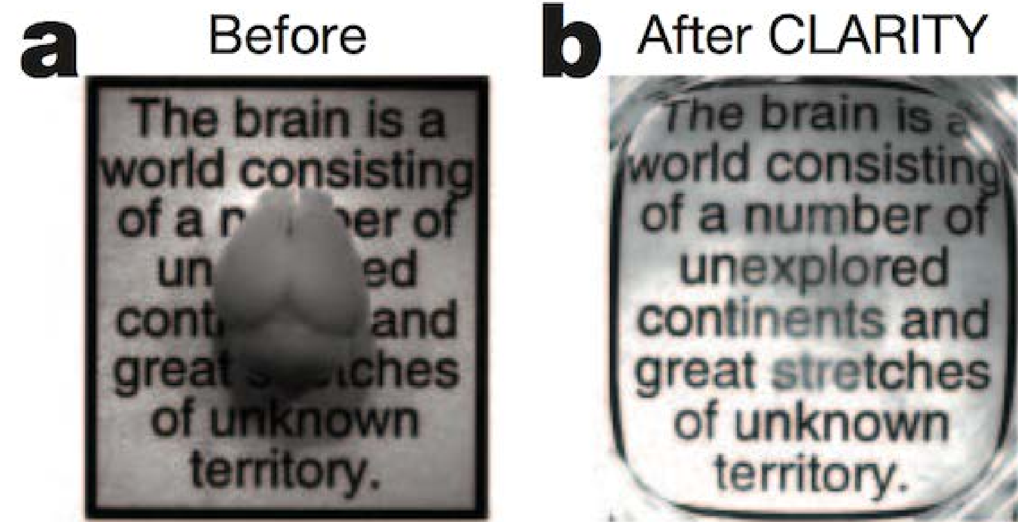


Mean free path ~100μm in biological tissue

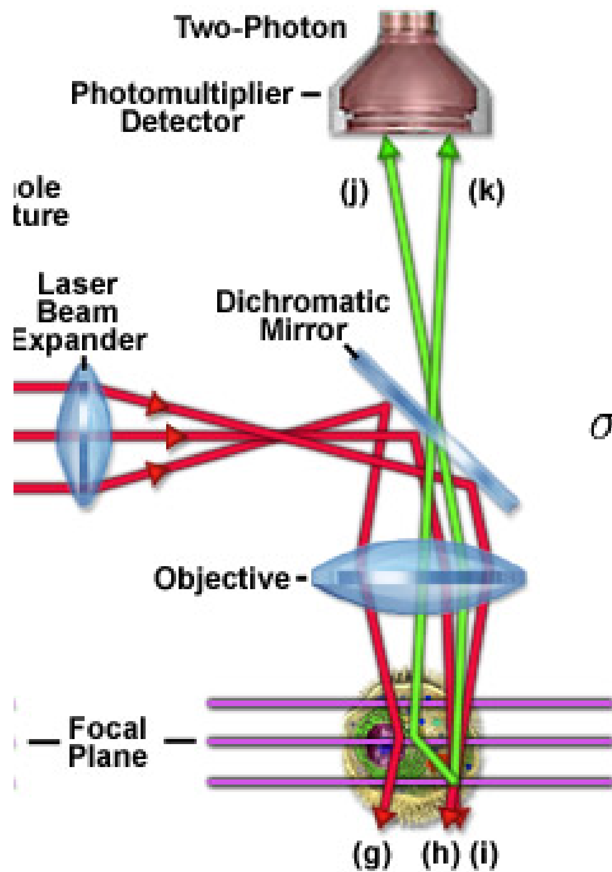
$$\sigma_s = \frac{2\pi^5}{3}\frac{d^6}{\lambda^4}\left(\frac{n^2-1}{n^2+2}\right)^2$$

Correcting scattering

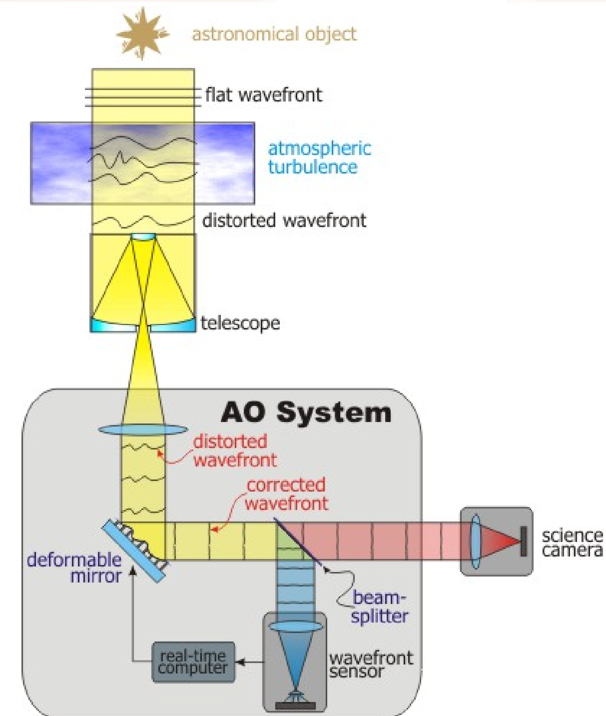
Remove lipids - Clarity



Use NIR light, 2-photon microscopy

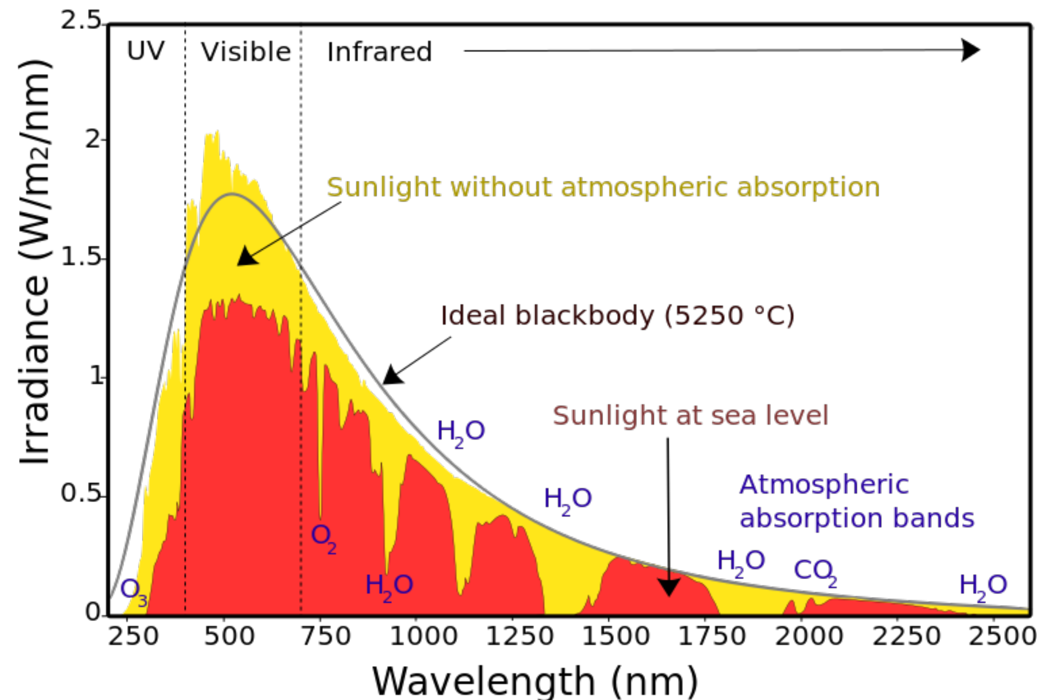


$$\sigma_s = \frac{2\pi^5}{3}\frac{d^6}{\lambda^4}\left(\frac{n^2-1}{n^2+2}\right)^2$$



Intensity calculations

- Reporting light values used, it is imperative to use an intensity
- Energy/(s*m^2)



I = total power/total area

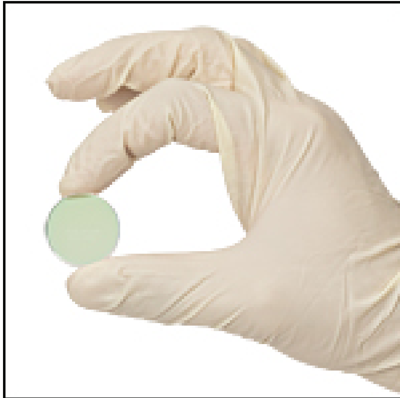
Shine 10 mW into 60x objective
Field number = 22

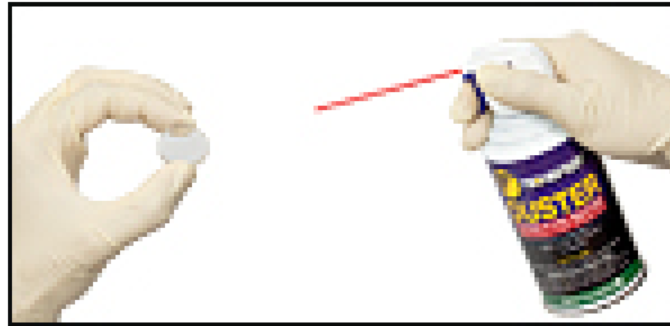
What is intensity?
How many photons?

Total solar intensity ~ 1000 W/m^2
On a bright day

~42% of that is visible light

Cleaning optical components





Use lens or lint free paper

If you see dirt on your microscope

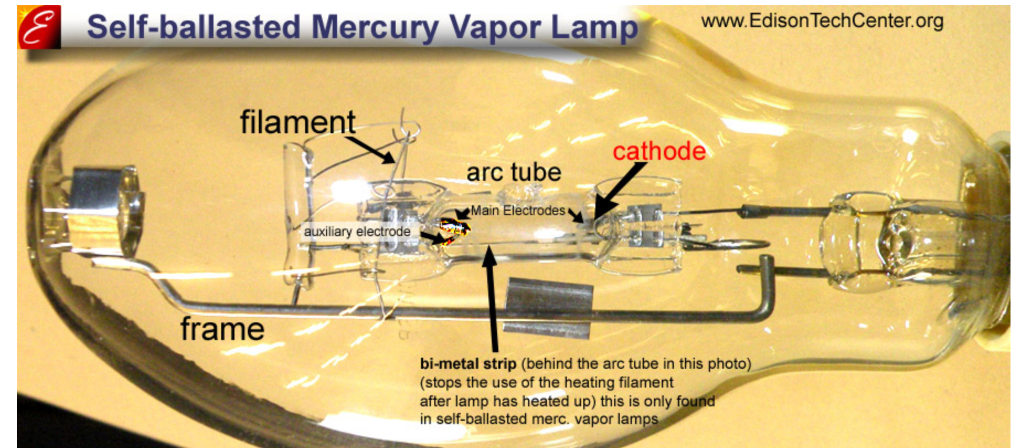
Try zooming up and down, is it before the sample, or after?

If it is imaged onto the camera, it must be somewhere close to an image plane

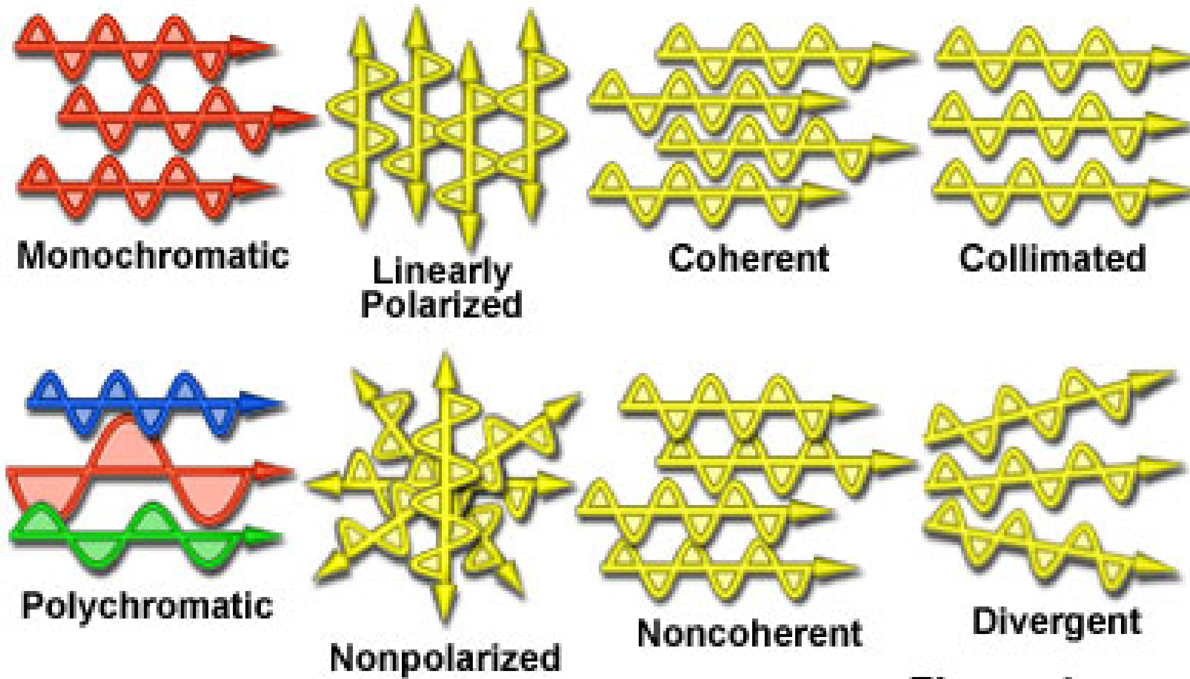
Light sources

Light source options

- Broadband sources (filaments)
- Light emitting diodes
- Lasers



Properties of light

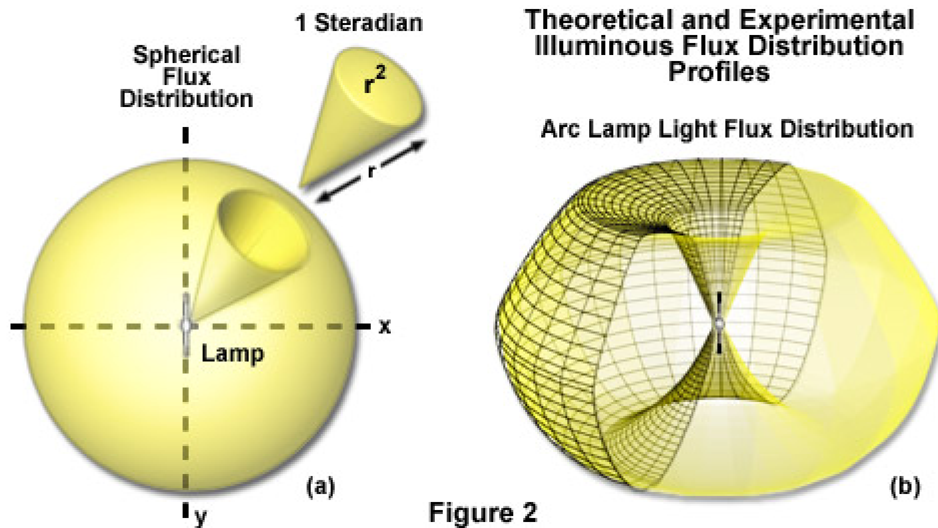


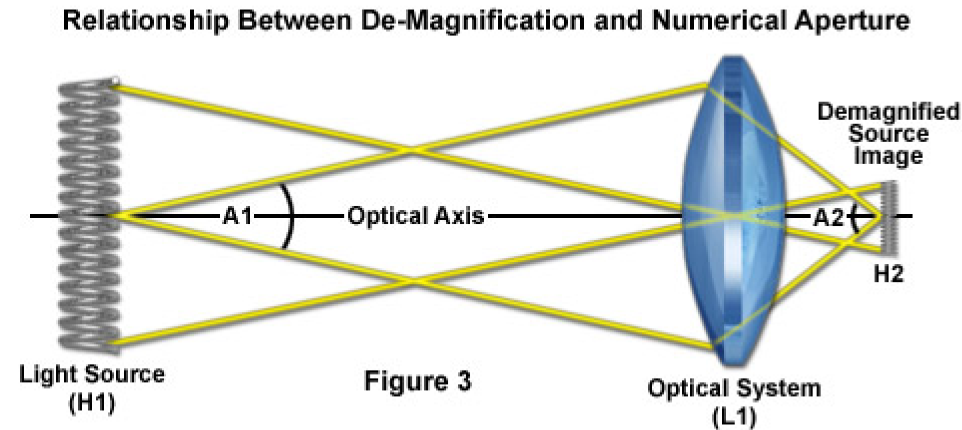
Other things to consider:

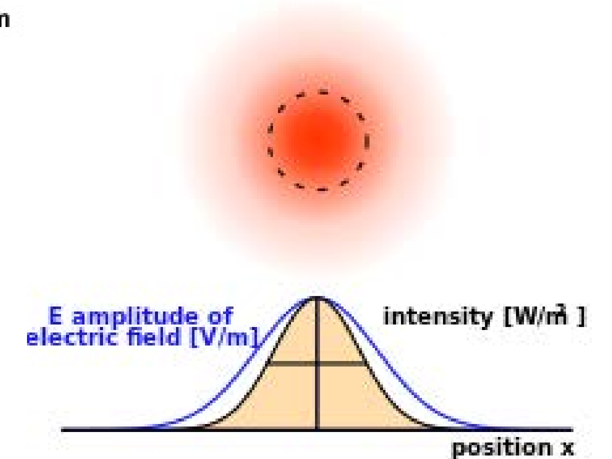
Intensity
Total cost
Compatibility with existing setup
Lifetime
Alignment

The goal of illumination (mostly)

- Uniform, stable illumination across the entire field of view
- Lamps necessarily emit in all directions
- Even lasers emit Gaussian profile beams

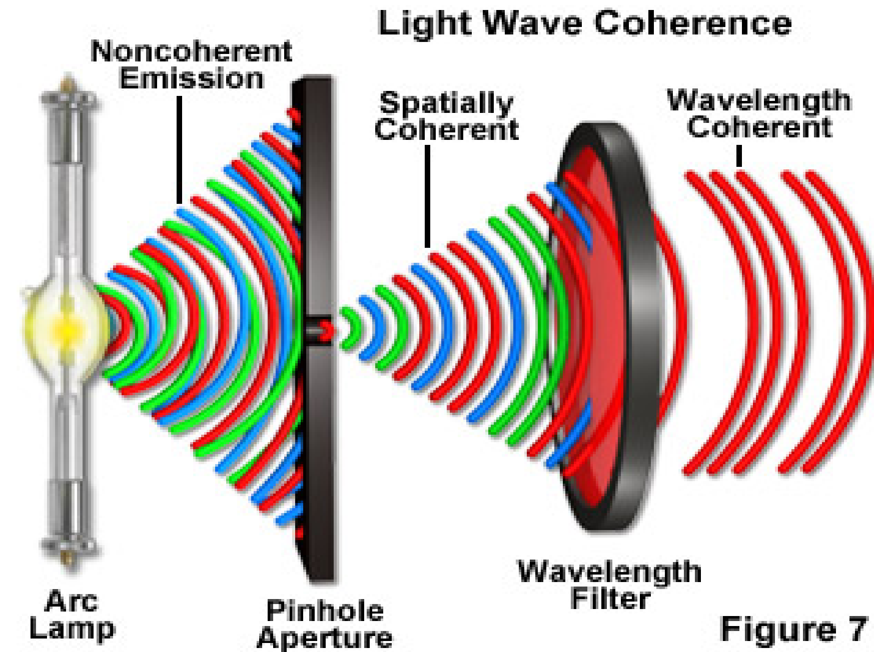


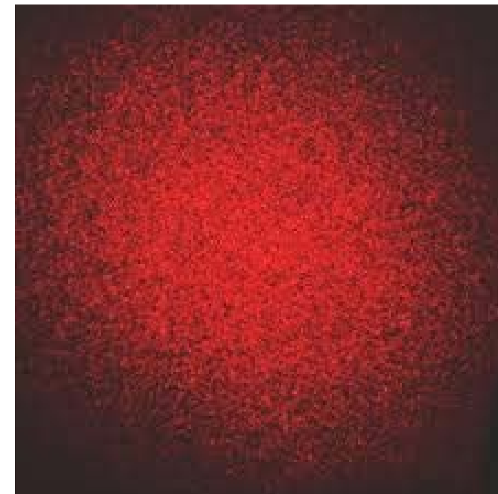




Importance of coherence

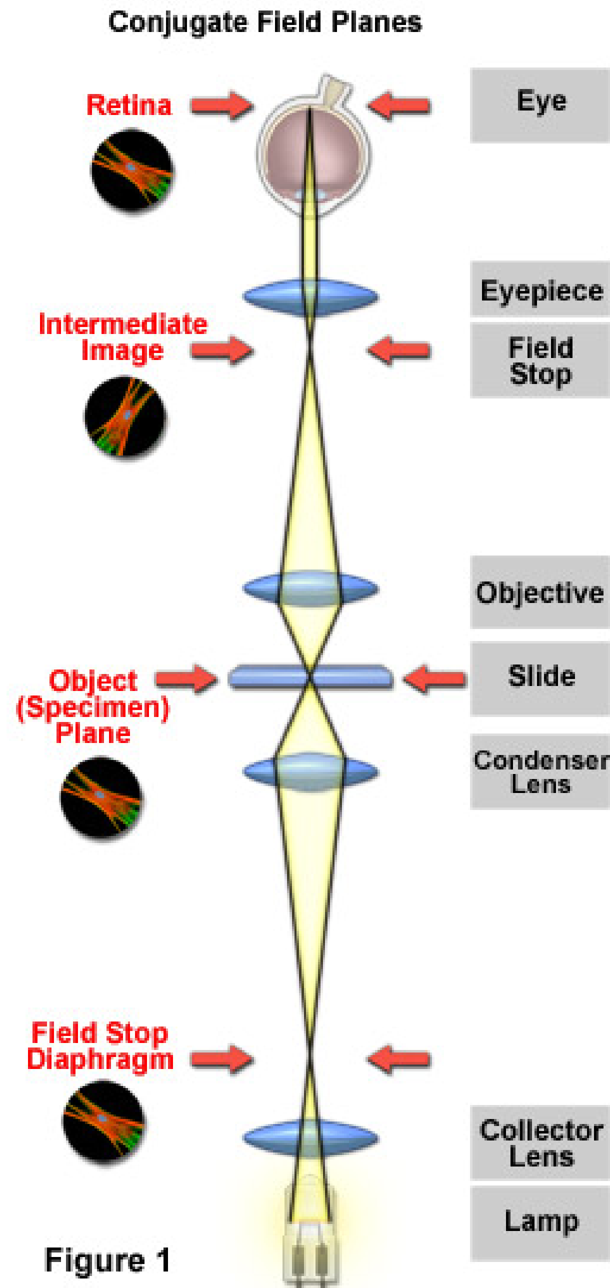
- Only coherent waves can interfere with each other
- Coherence has both a spatial and temporal scale
- Long spatial coherence will interfere of lenses, mirrors, specks of dust. We don't want that





Kohler illumination

- Ensure that sample is in infinity plane.
- Completely even illumination, don't have to worry about filament
- Form image of filament at focal plane of condenser
- As if it came from infinity



On to Matlab…