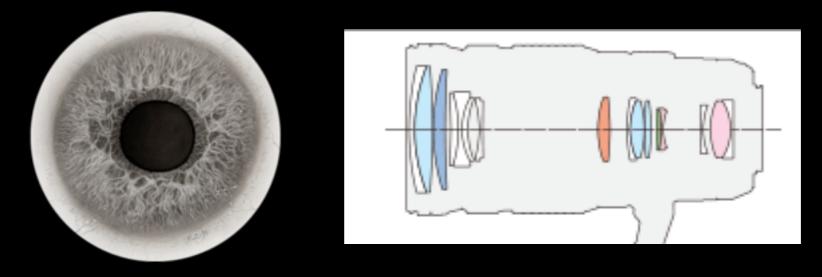
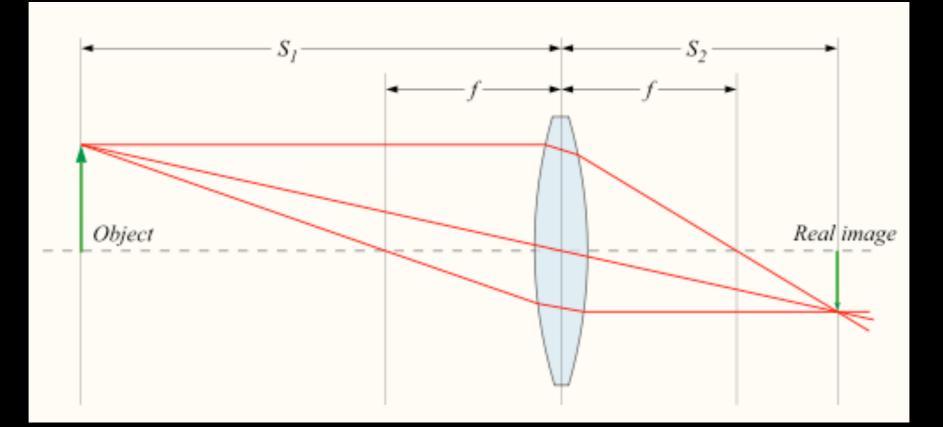
Recap from Tuesday: Imaging with a "single lens"





Brief note on: Optical lens design stuff

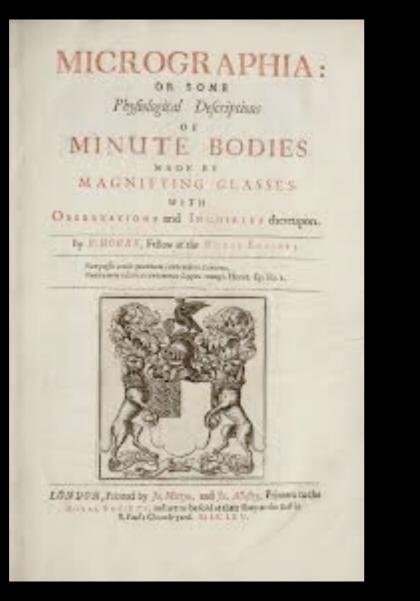
- If you are interested in this, there are commercial week-long classes from the optical design softwares Zemax and Code V
- I have taken two Zemax classes, they were pretty useless. A lady I met - who designs riflescopes recommended the Code V classes instead.
- Jobs in optical systems design in Silicon Valley are according to my friends there available in various illumination design appliations

Microscopy



Anton van Leeuwenhoek (1632-1723)

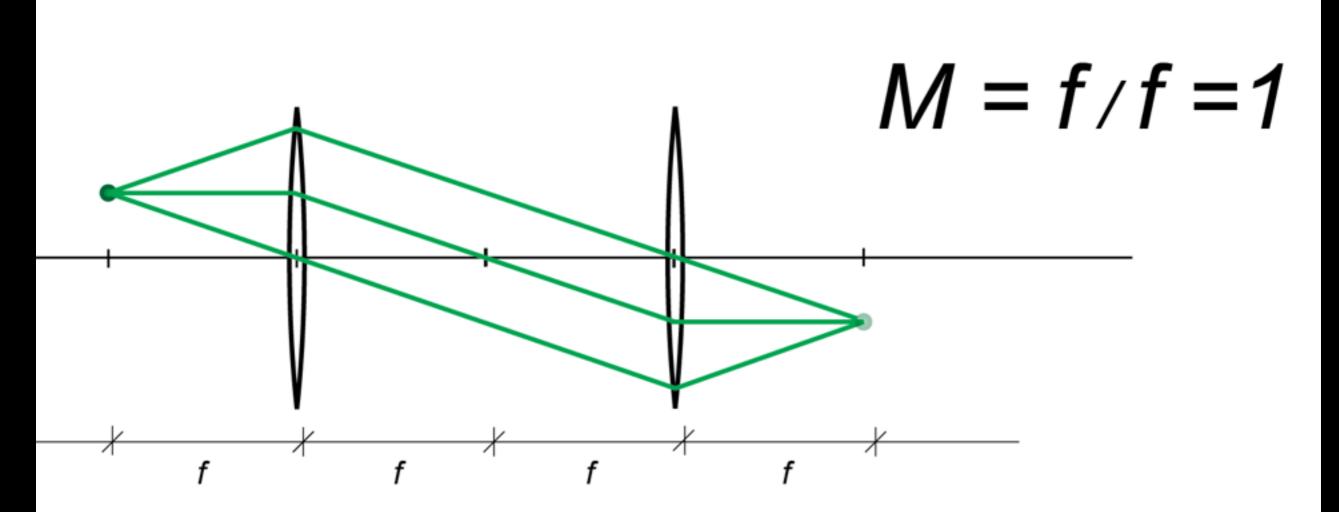
Micrographia



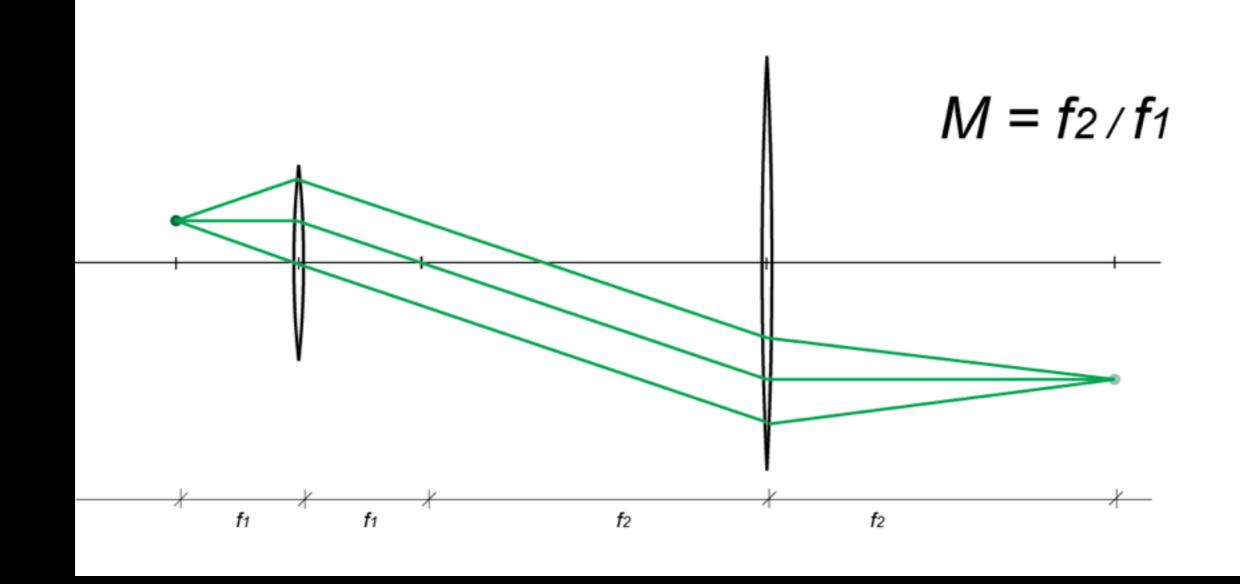


Robert Hooke published Micrographia in 1665. Stunning illustrations

Imaging with two lenses

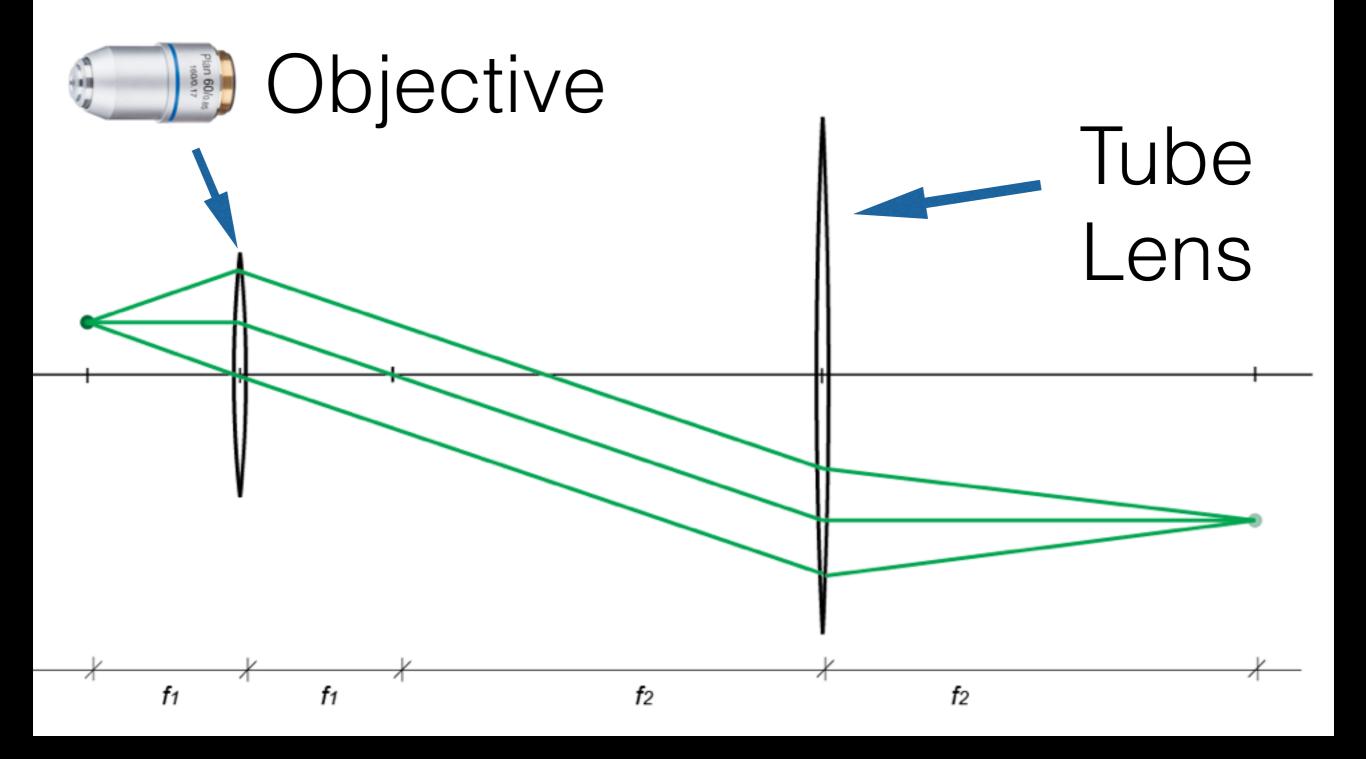


Magnify!



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

Microscope:

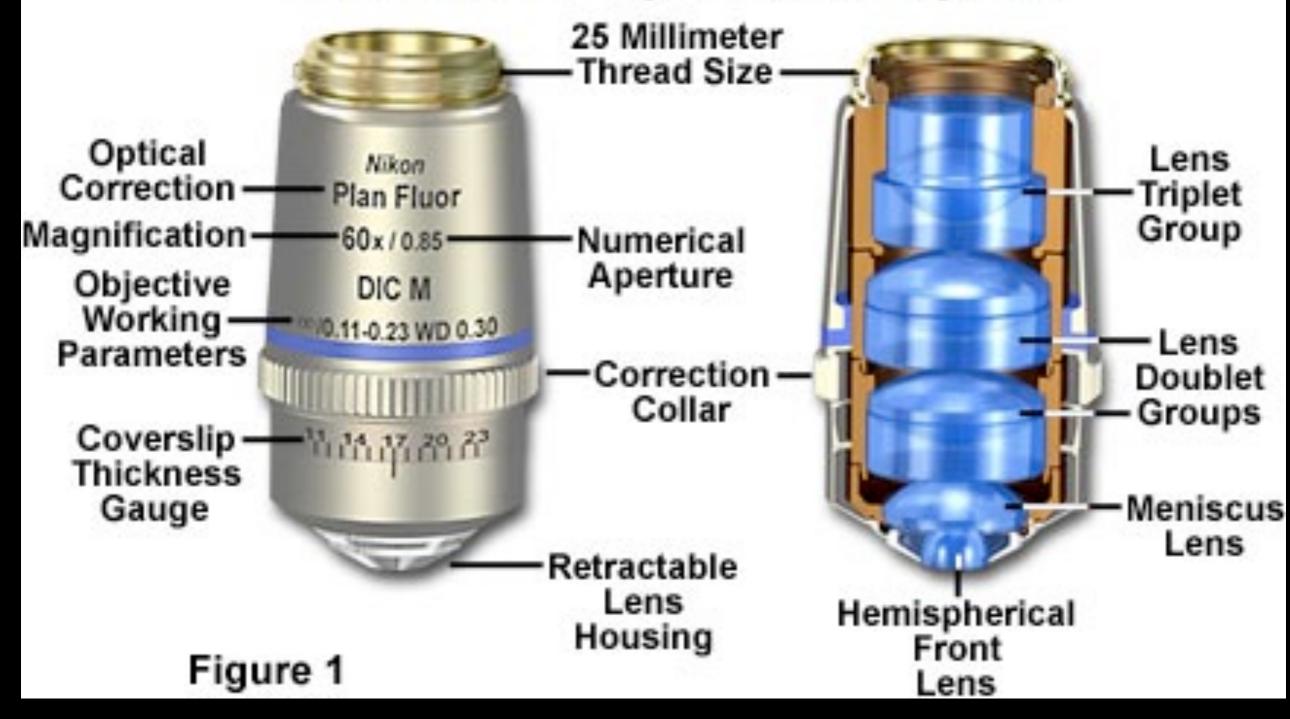


Tube Lens ~ Objective $M = f_2/f_1$

- 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* T.L. = 200 mm =>
 f objective = 200mm/100 = 2mm

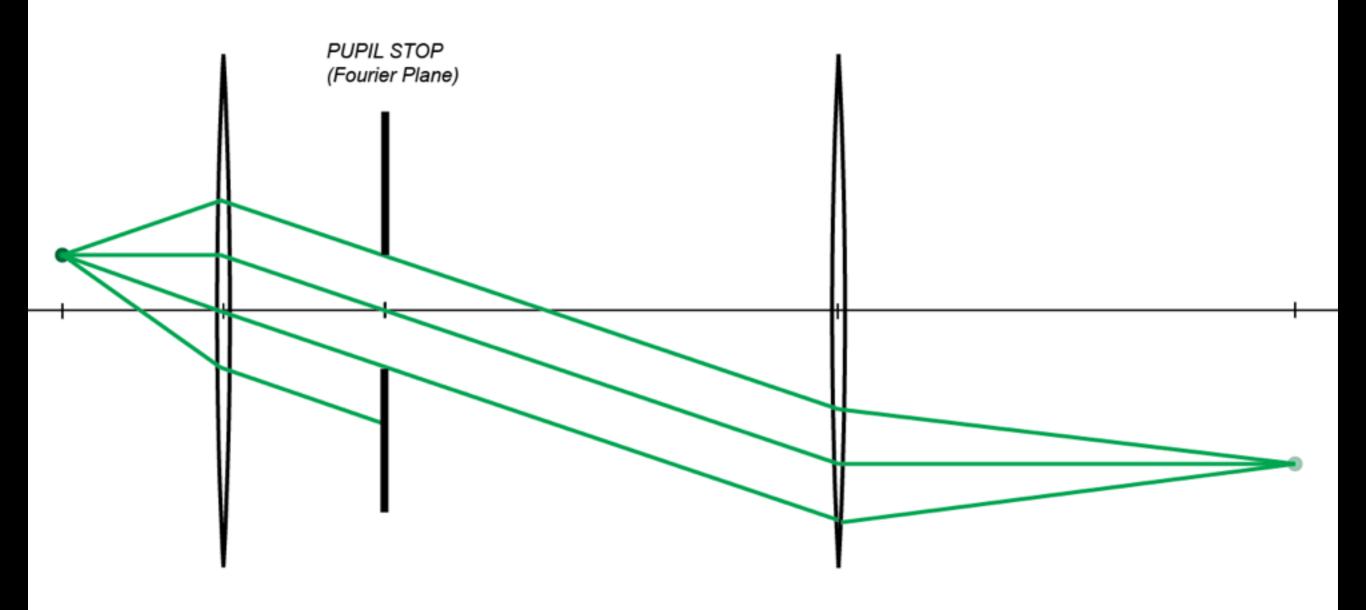
The Objective

Nikon CFI60 Infinity-Corrected Objective



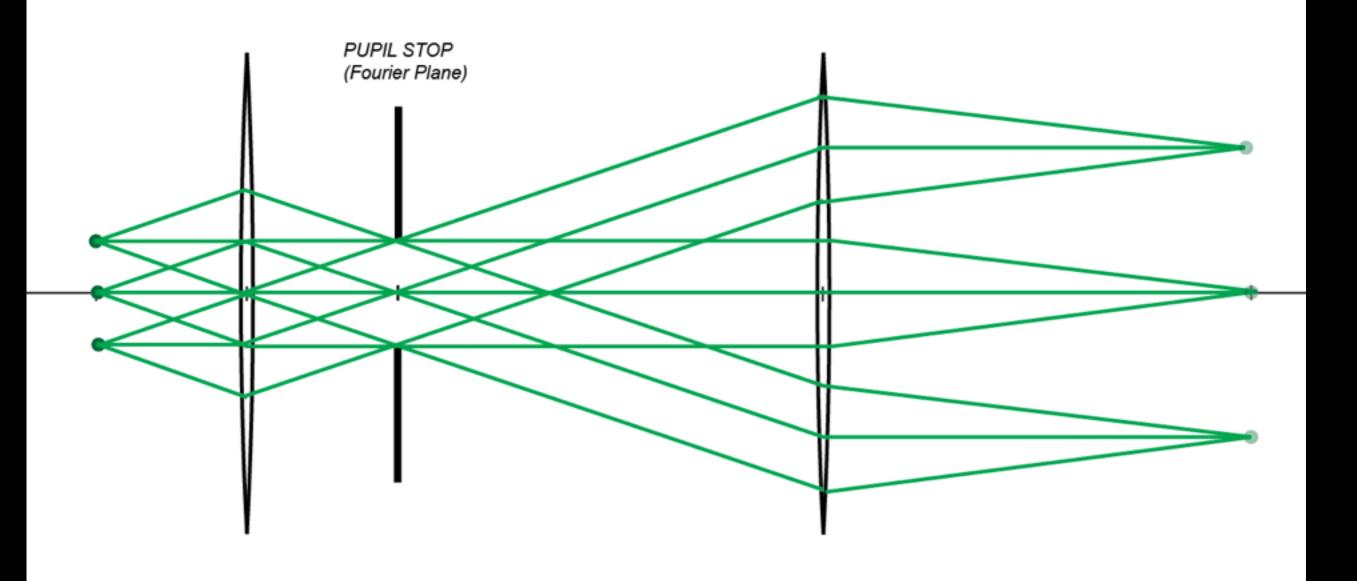


Pupil Stop



Pupil stop determines Numerical Aperture (NA)

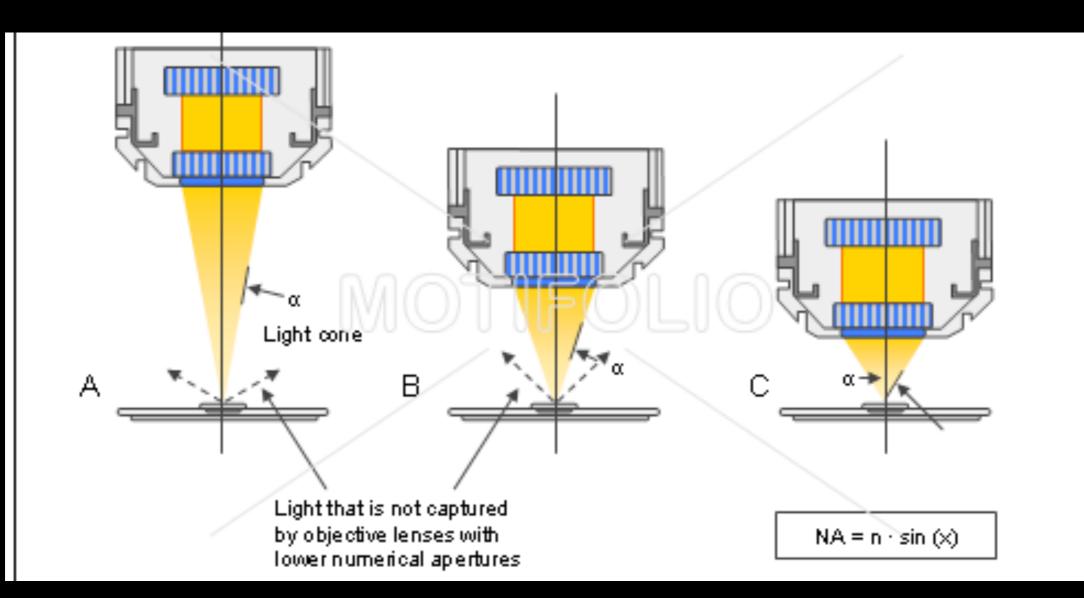
Lets add some rays



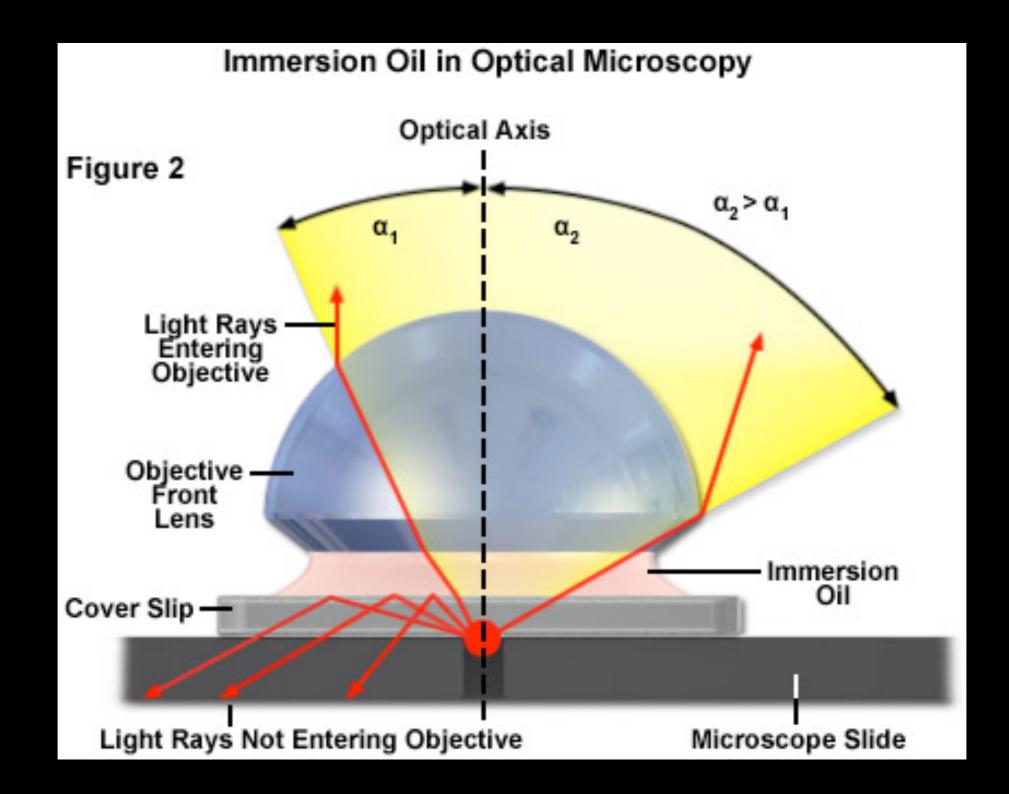
Now we start seeing the special properties of the Fourier plane

NA = n sin(a)

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

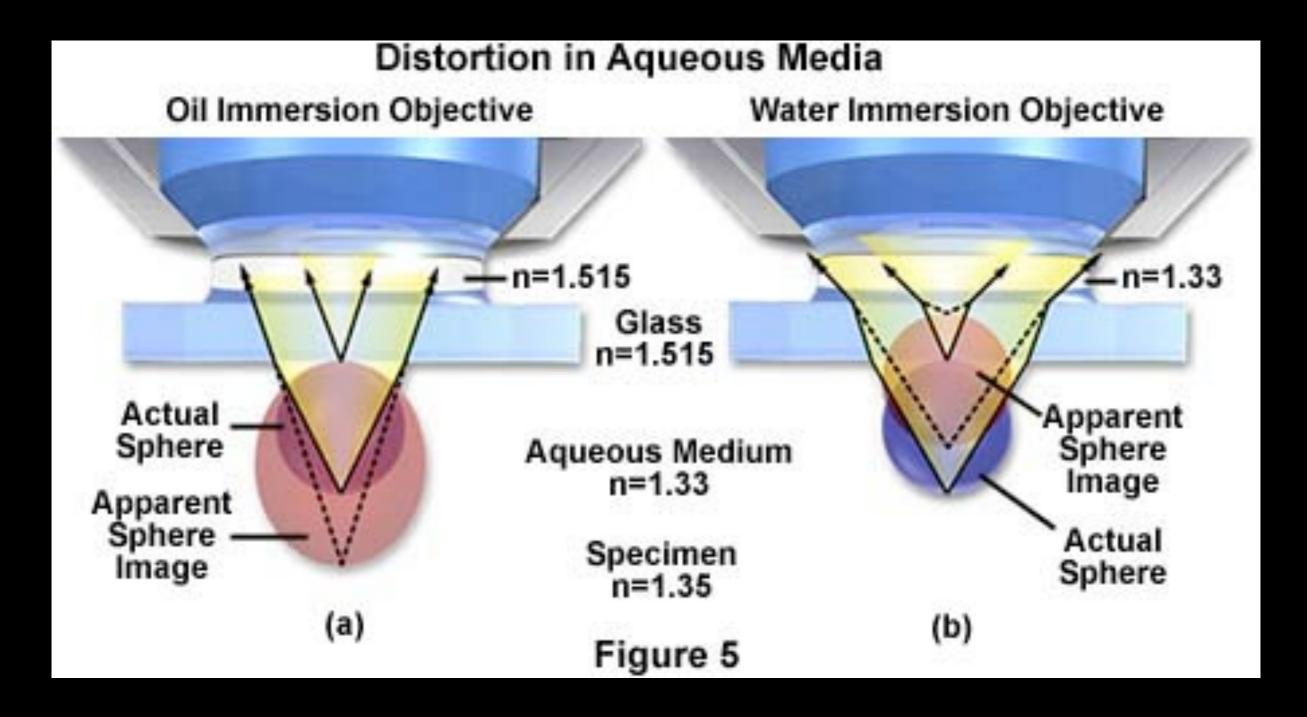


Immersion Medium

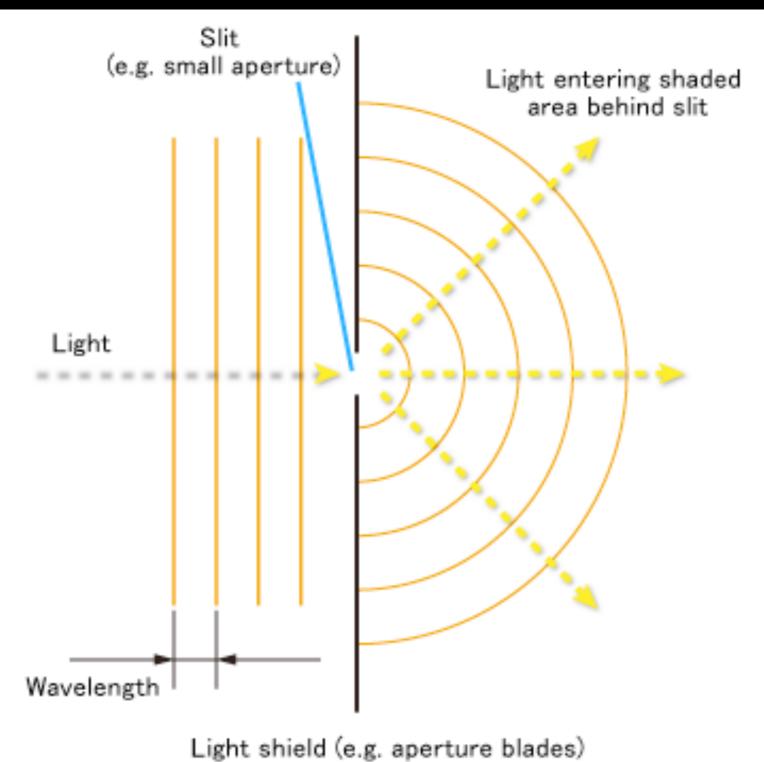


Match to Specimen!

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



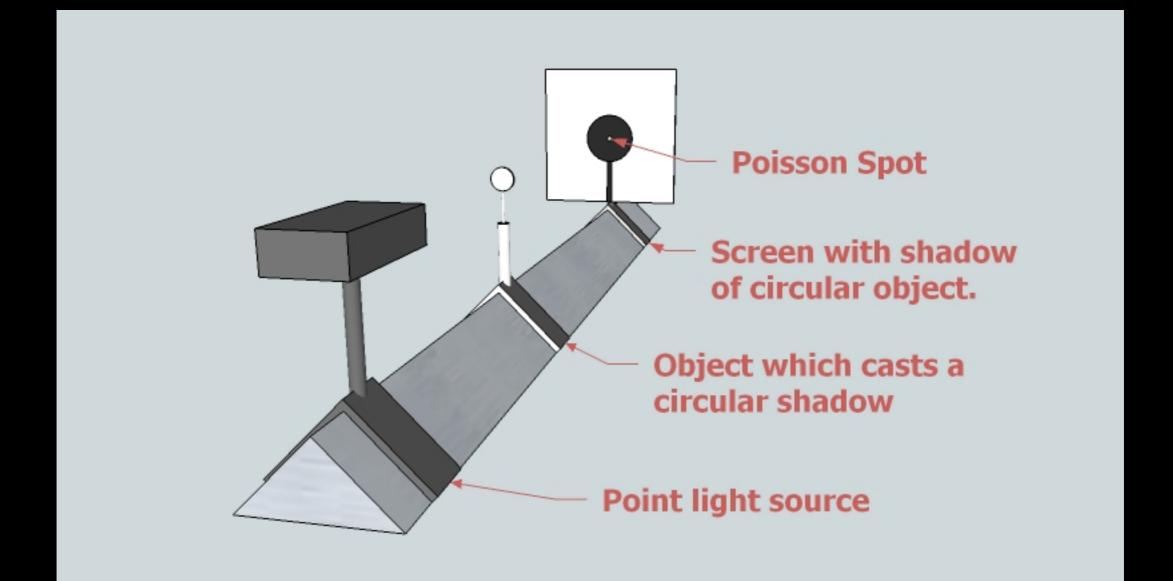
Diffraction



Ripple Tank

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

Poisson's spot



Light ripples and bends around edges too

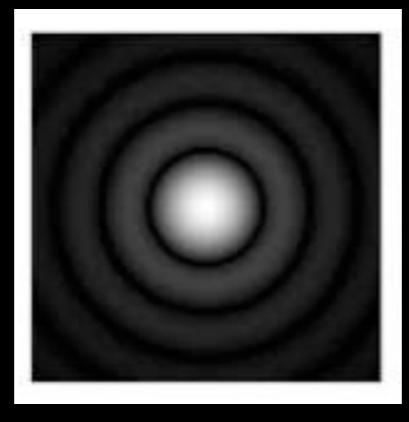
Fresnel Diffraction

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.

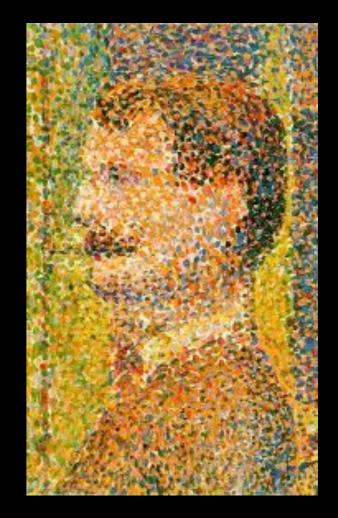
The Airy Disc



Arises from diffraction when we image through a circular aperture

Imaging Point Sources

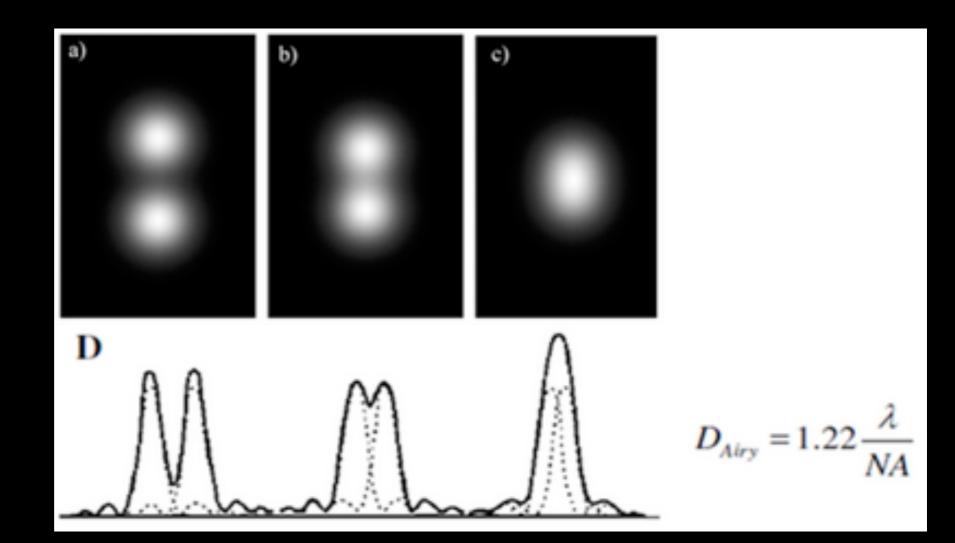
- The object can be modeled as a number of point sources
- The points are blurred by the imaging system (The response function of a point imaged through a circular aperture is the Airy disc)



(from Seurat's *the Circus*)

Resolution criteria:

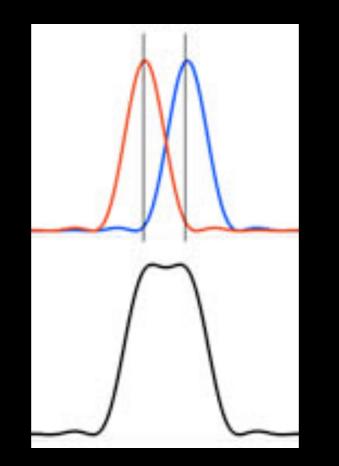
- Raleigh
- Sparrow
- Airy
- Abbe



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

Assumptions

- Limited NA
- Uniform Illumination
- Linearity



Resolution

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High resolution

Low resolution

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Contrast

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High contrast

Low contrast

- a yellowish wax-like substance unders ing slow combustion at ordinary to peratures & hence appearing luminous produce, the dark; ~us neeros'is (colloq, phoney jaw), gangrone of jawbone due to ~us fumes esp. in match-making. Hence or nonetic · mm.; cogn. phospho'ric, ~ous, aa., ~iss(b) n. (path.). [L. = morning star, f. Gk phosan by 110 B., phoros (phös light + -phoros -bringing)] DNO- --phöss'y. See PHOSPHORUS.
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Modulation Transfer Function

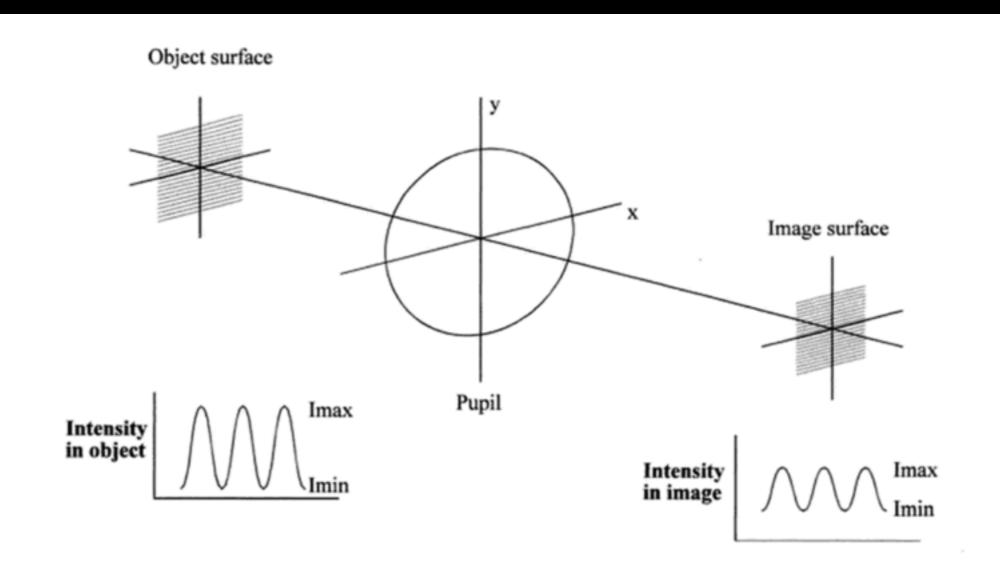


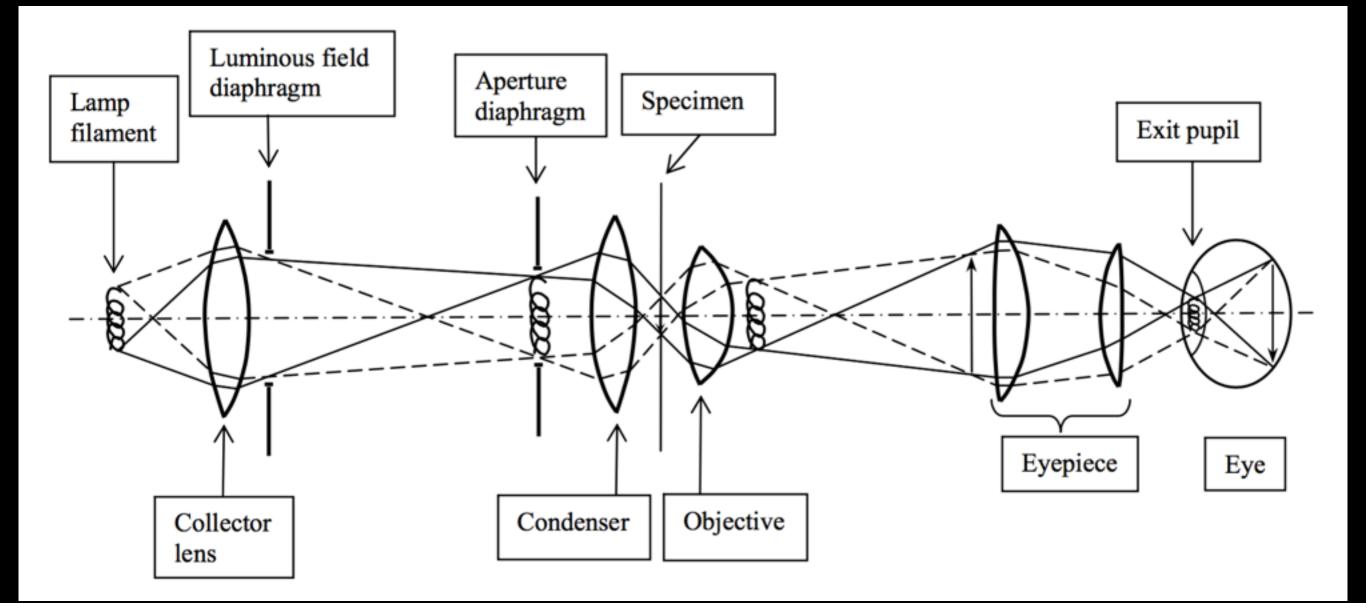
Figure 4.23. Object and image contrast.

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

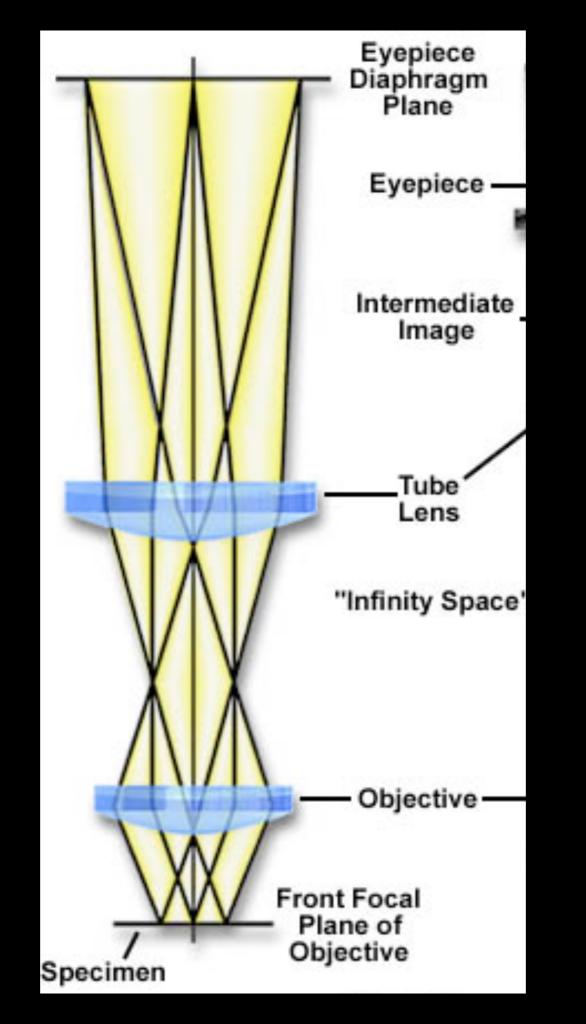
Field Stop limits the Field of View

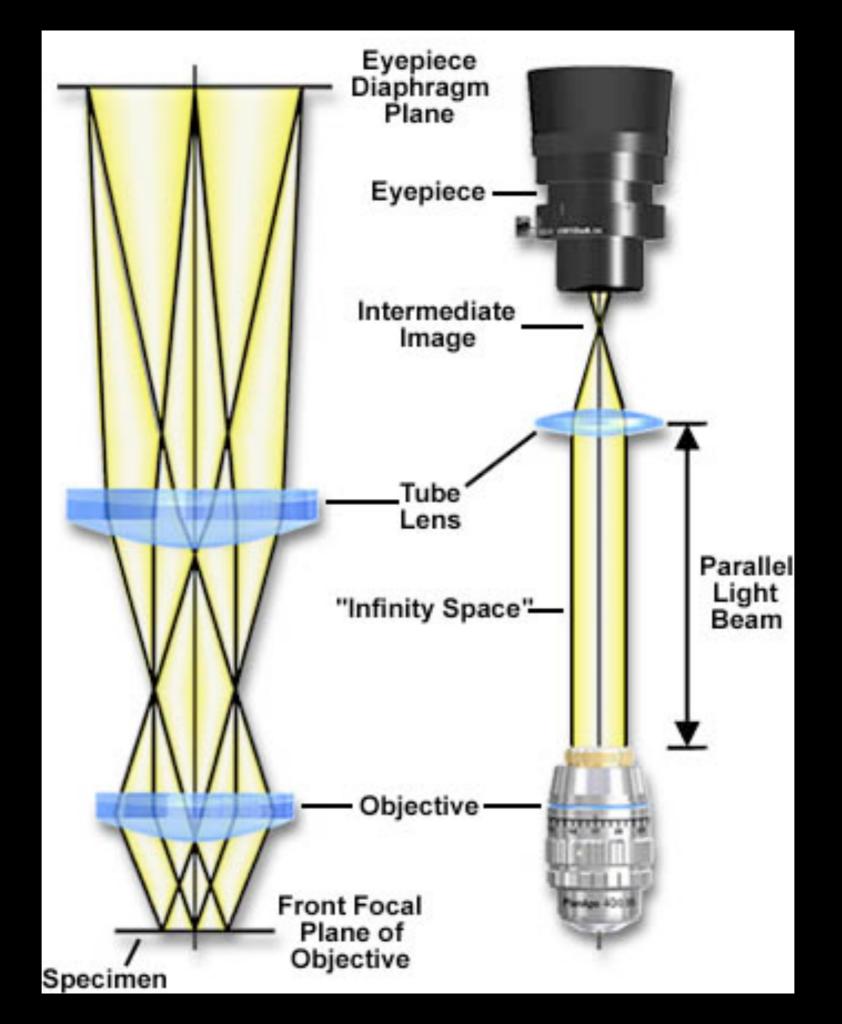
Pupil Stop limits the angle of illumination, which effectively limits the NA

• Draw out a microscope illumination system

Summary:

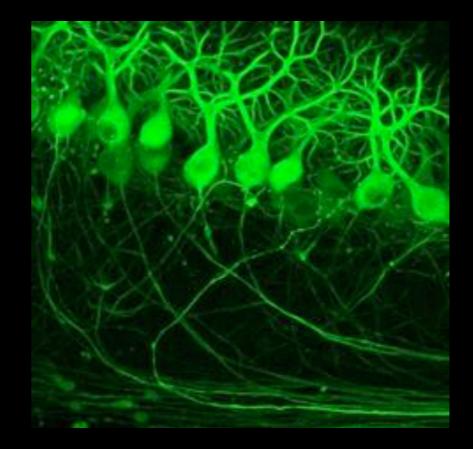
- Modern microscopes work with two lenses:
 - Objective
 - Tube Lens





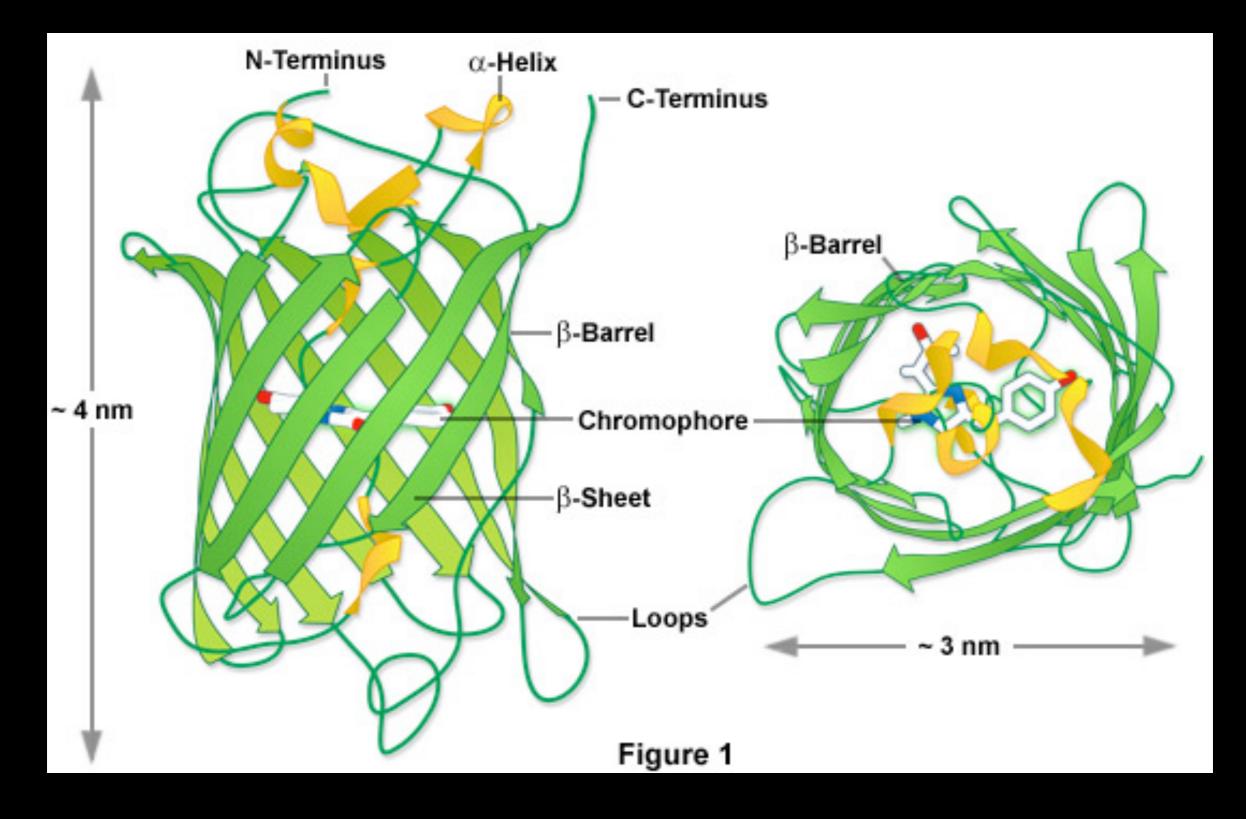
Fluorescence Microscopy



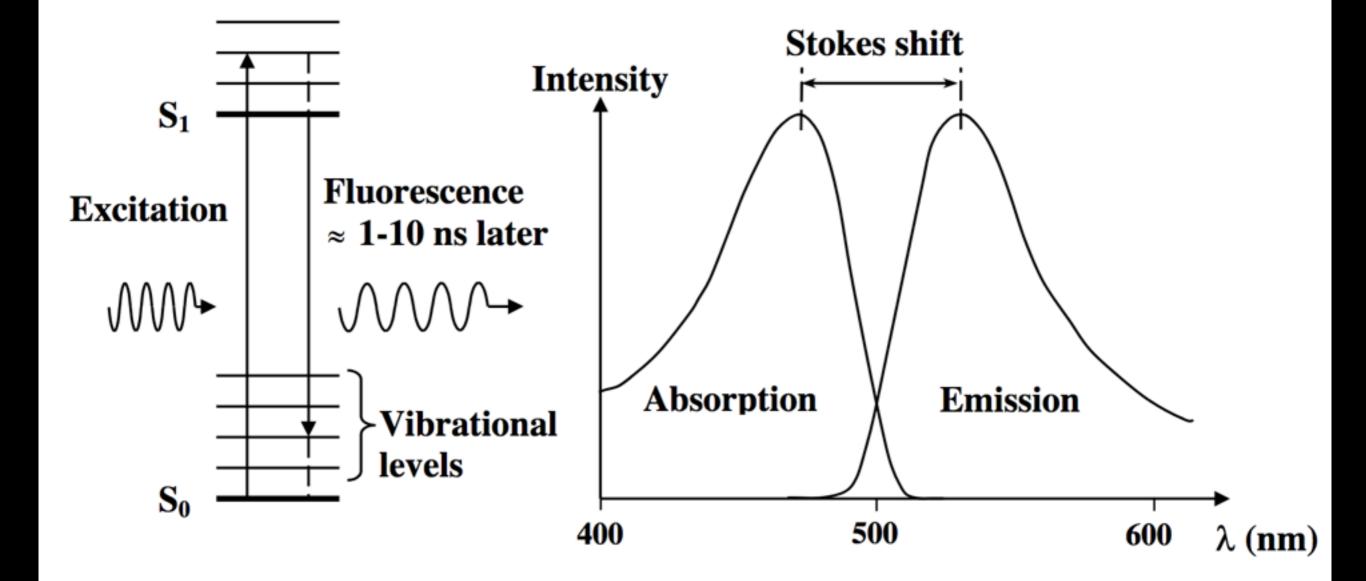


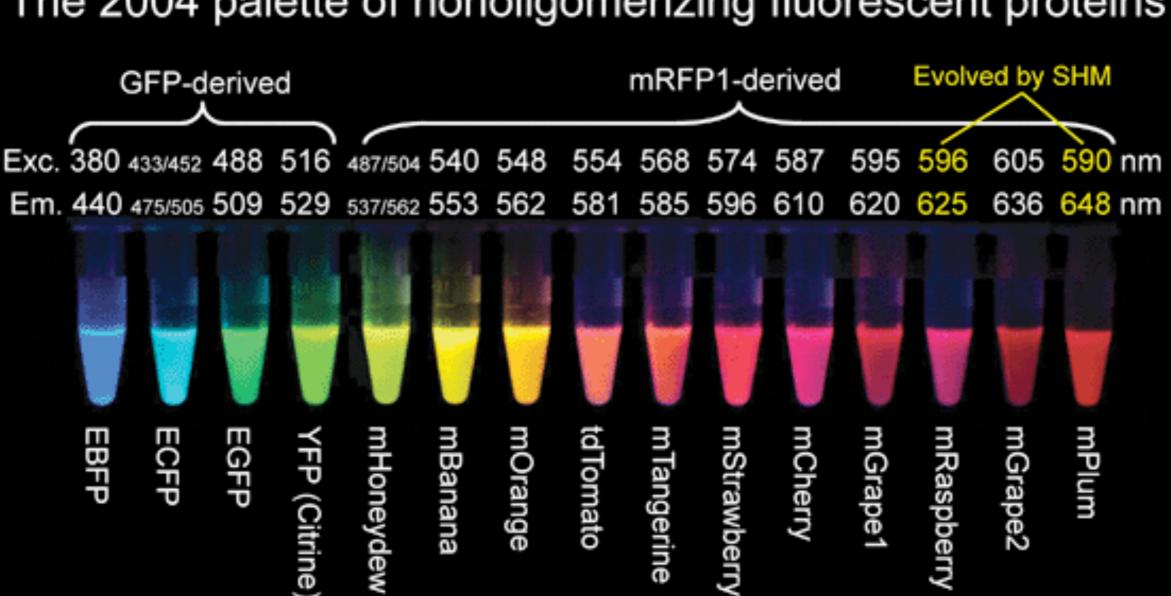
Green Fluorescent Protein

Green Fluorescent Protein



Fluorescence Excitation

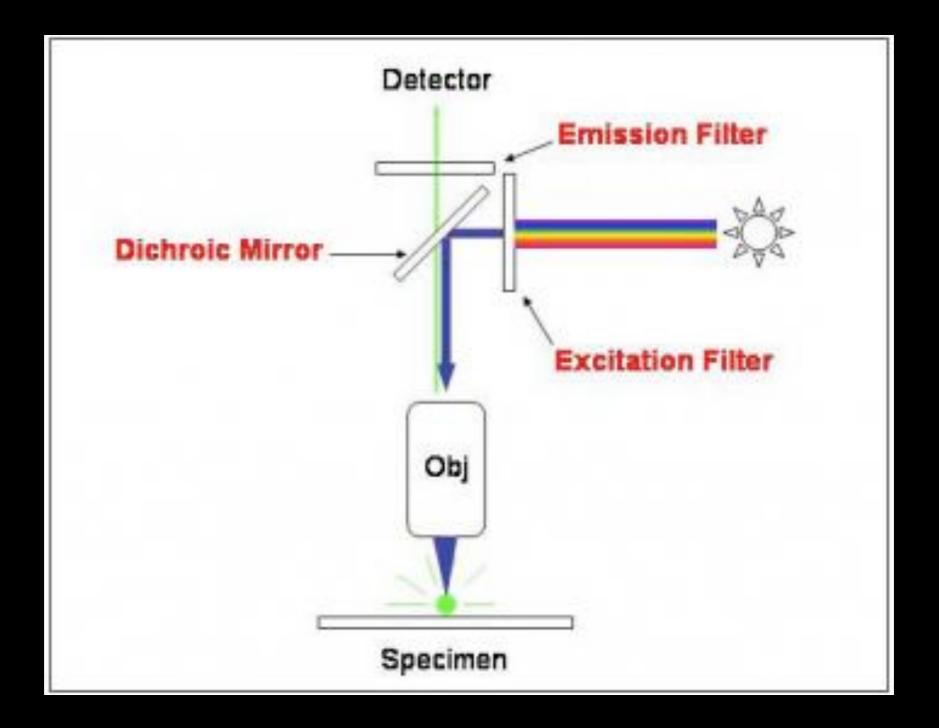


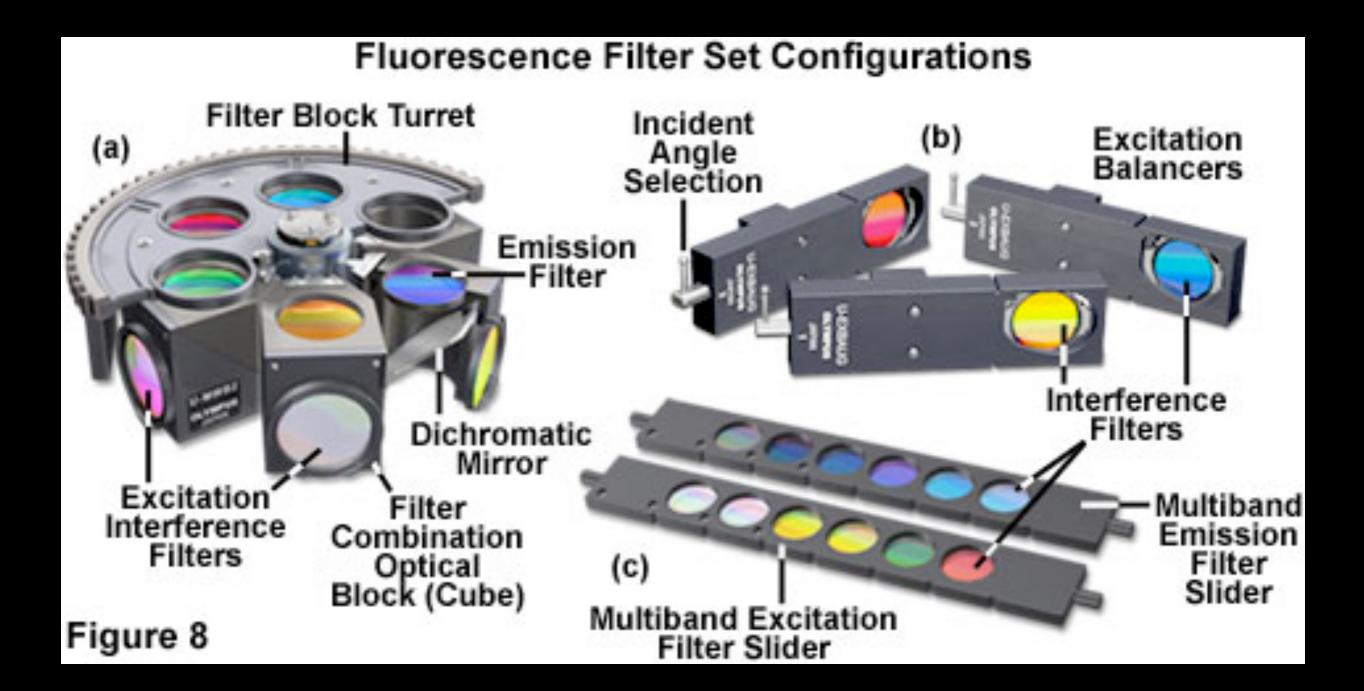


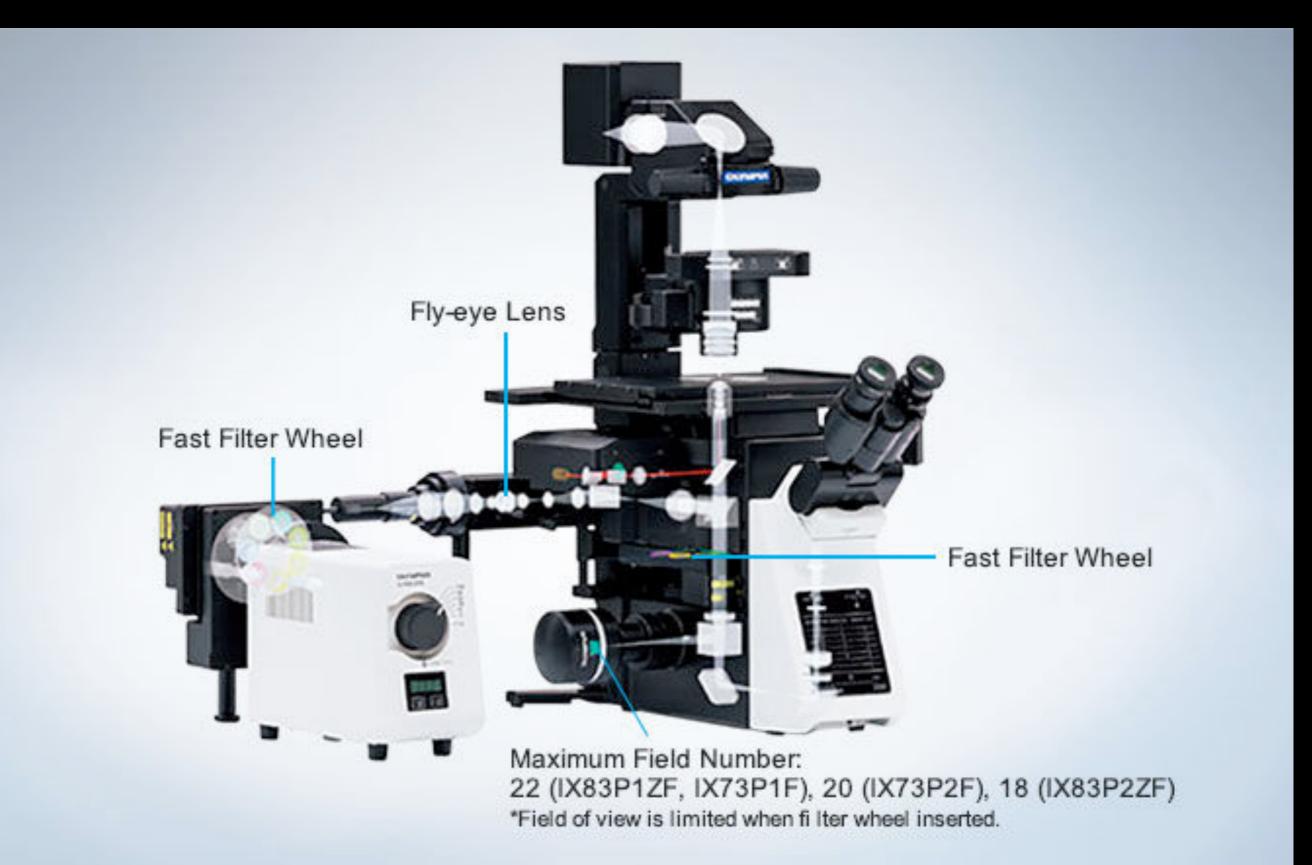
The 2004 palette of nonoligomerizing fluorescent proteins

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





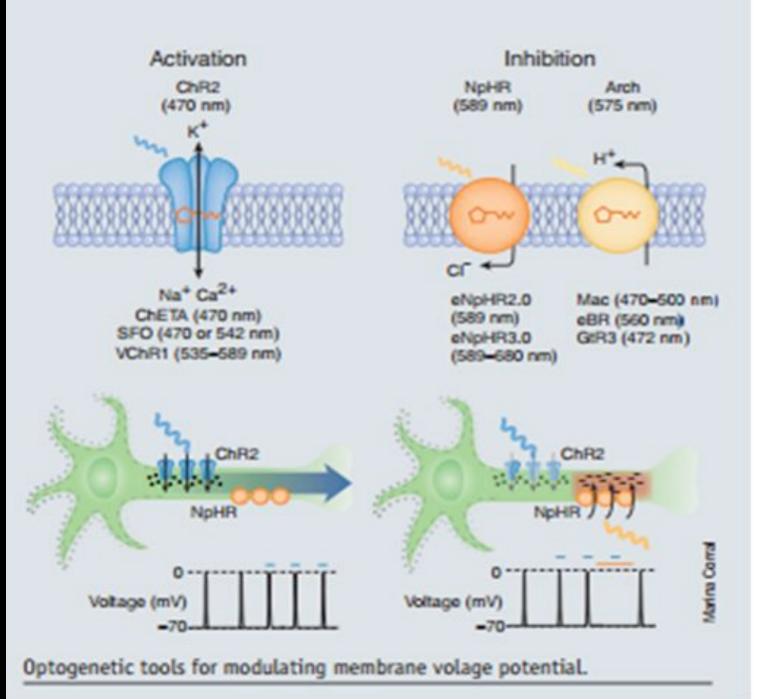


 <u>https://www.chroma.com/products/sets/89000-</u> <u>et-sedat-quad</u>

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⁺ 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⁻ which hyperpolarizes the cell and inhibits the neuron
- Acts as the Off switch

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

