Preparatory School to the Winter College on Optics: Advanced Optical Techniques for Bio-imaging

The Abdus Salam International Centre for Theoretical Physics

Geometrical Optics II: paraxial theory, microscope imaging and Kohler illumination, objectives and eyepieces

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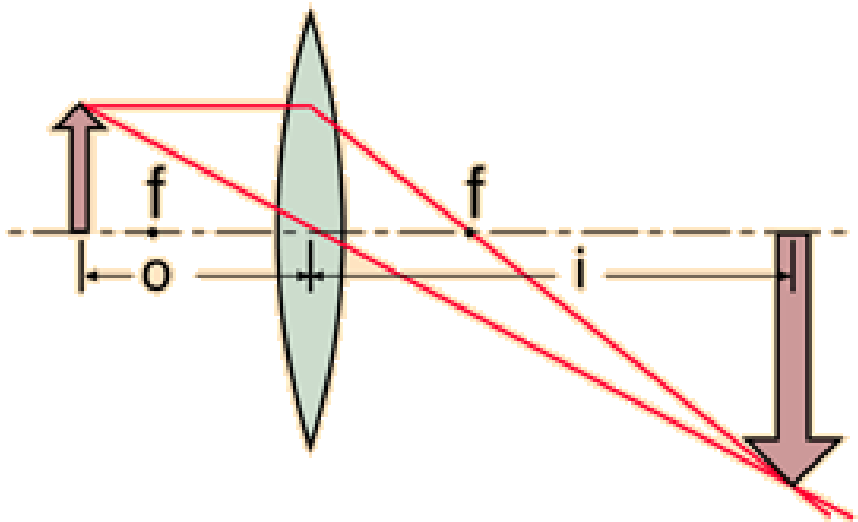
Imaging

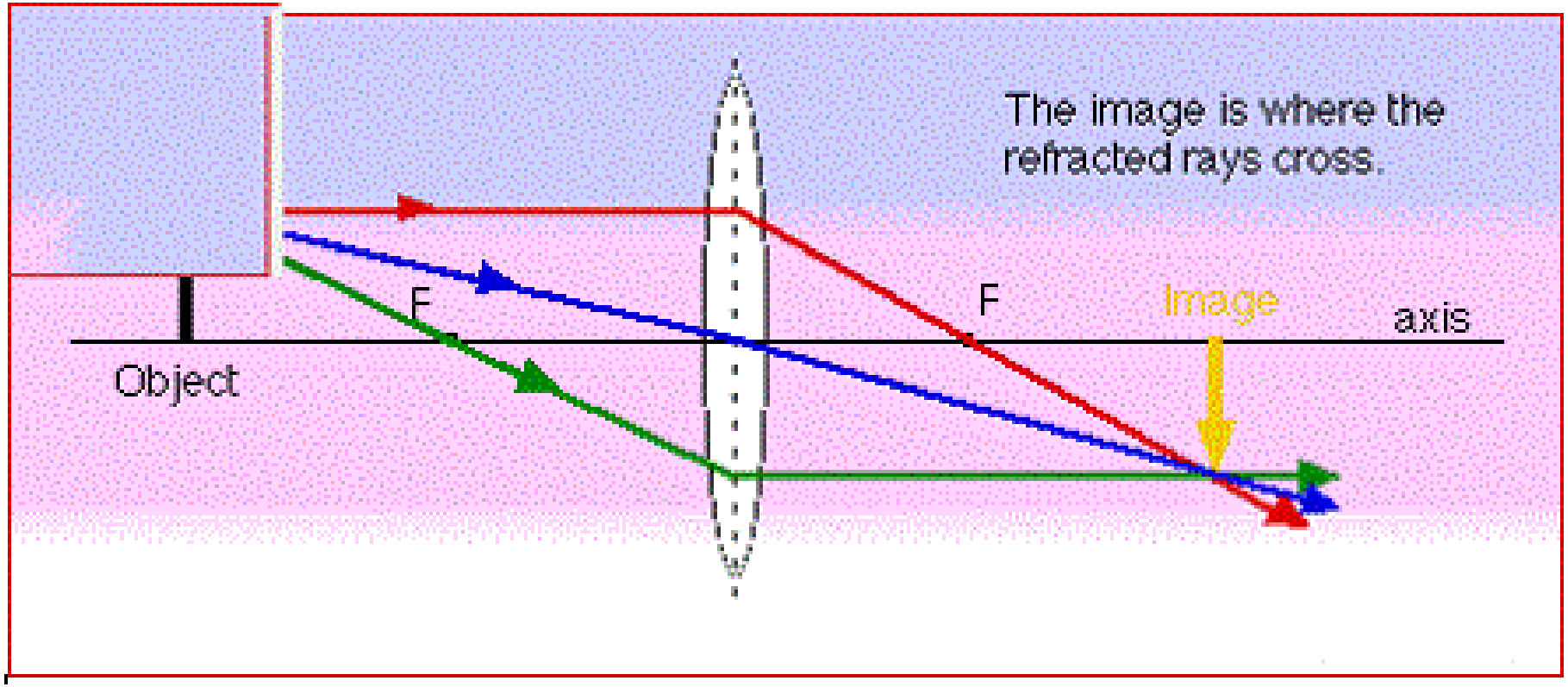


$$\frac{1}{o} + \frac{1}{i} = \frac{1}{f}$$ Lens equation

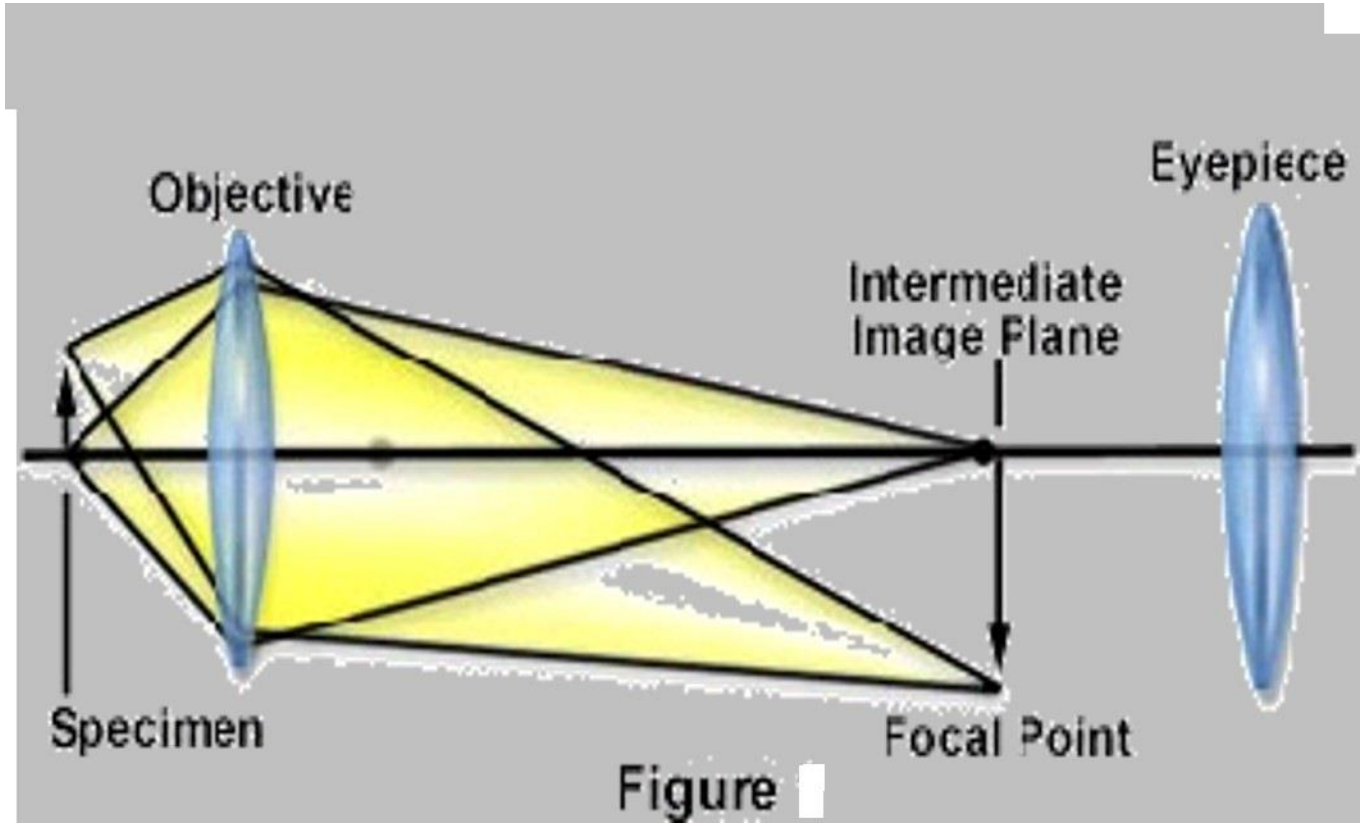
Linear magnification:

$$M = \frac{-i}{o} = \frac{h'}{h}$$

Imaging

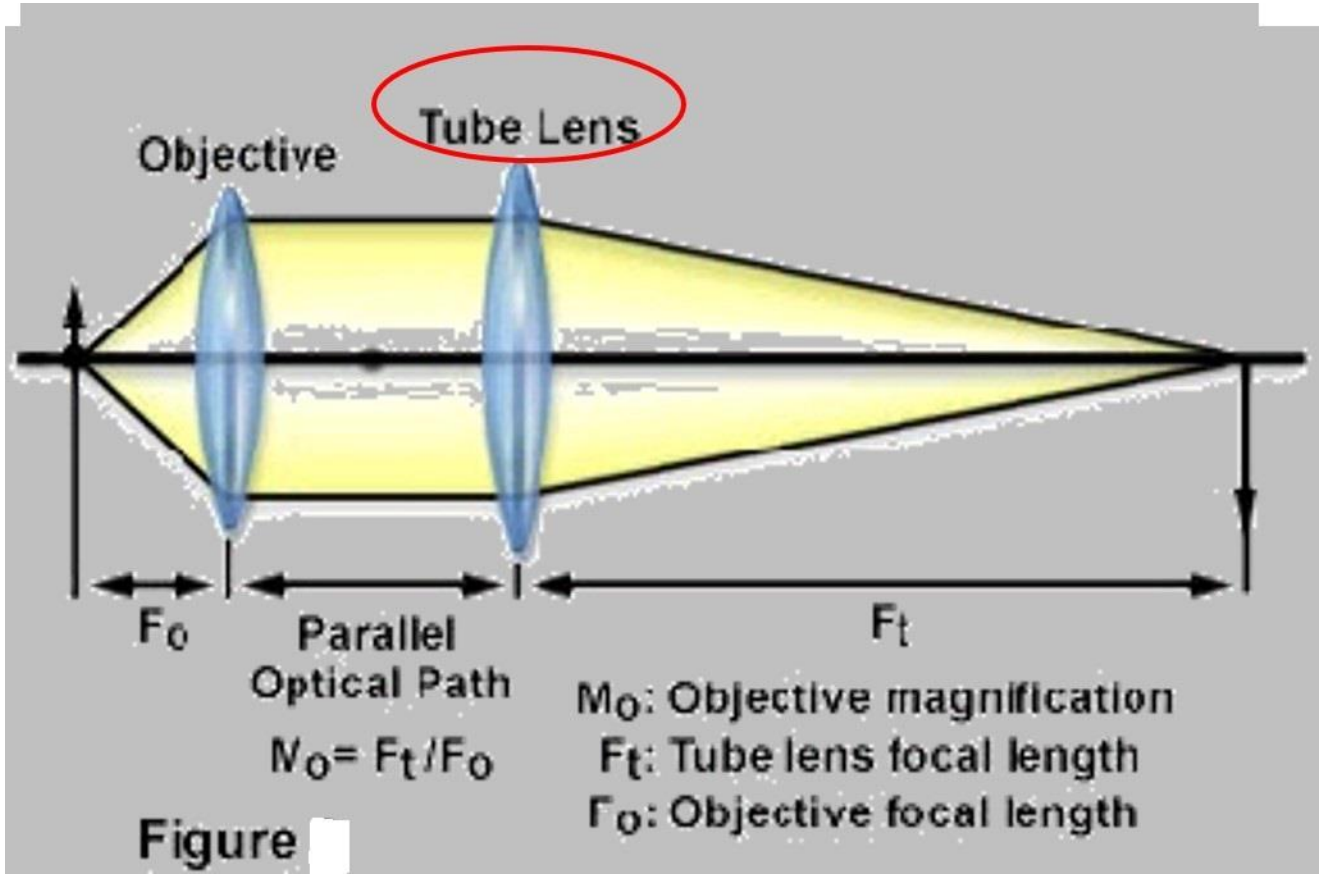

The image is where the refracted rays cross.
F
F
Image
axis
Object

Finite objetive lens



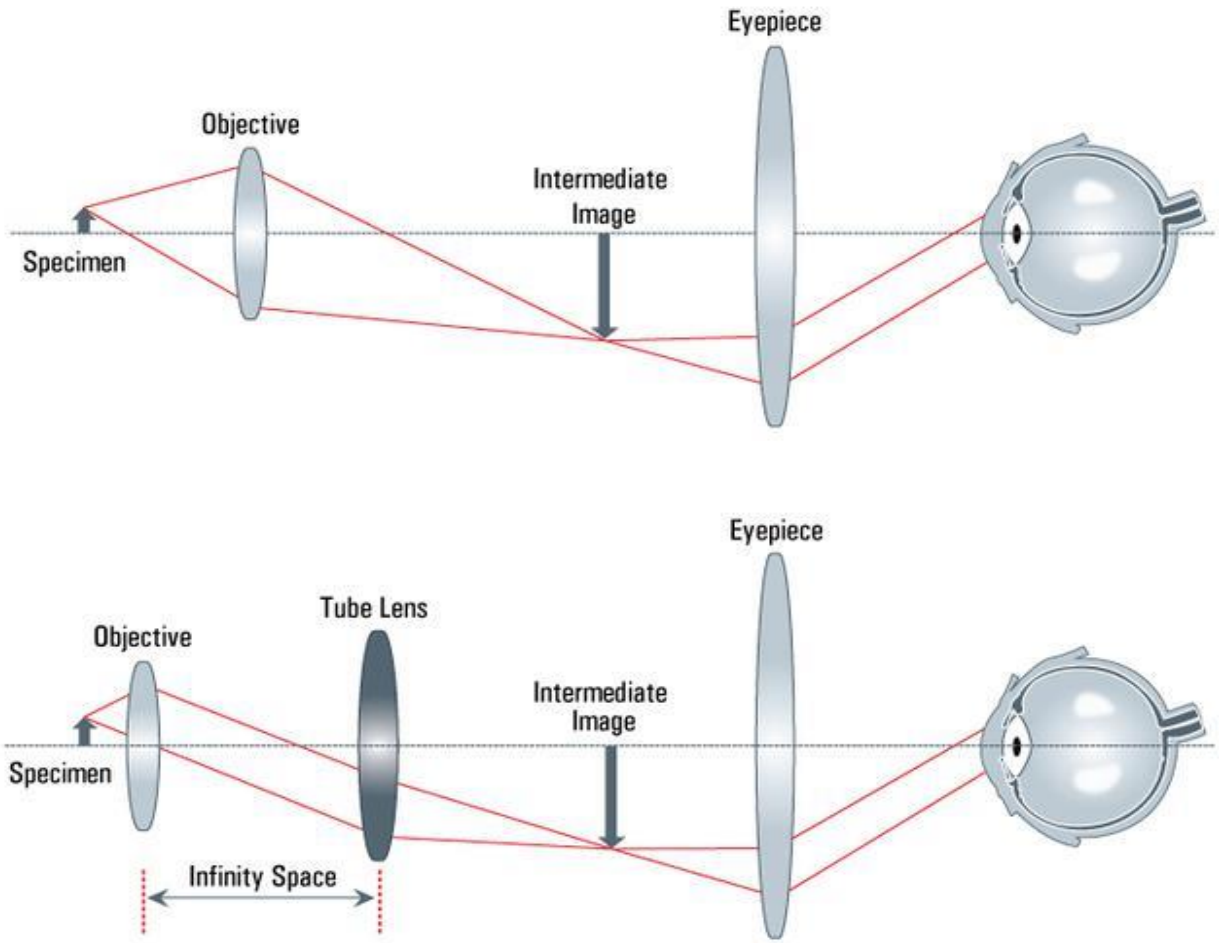
Figure

Infinite objetives work with tube lens to produce an image

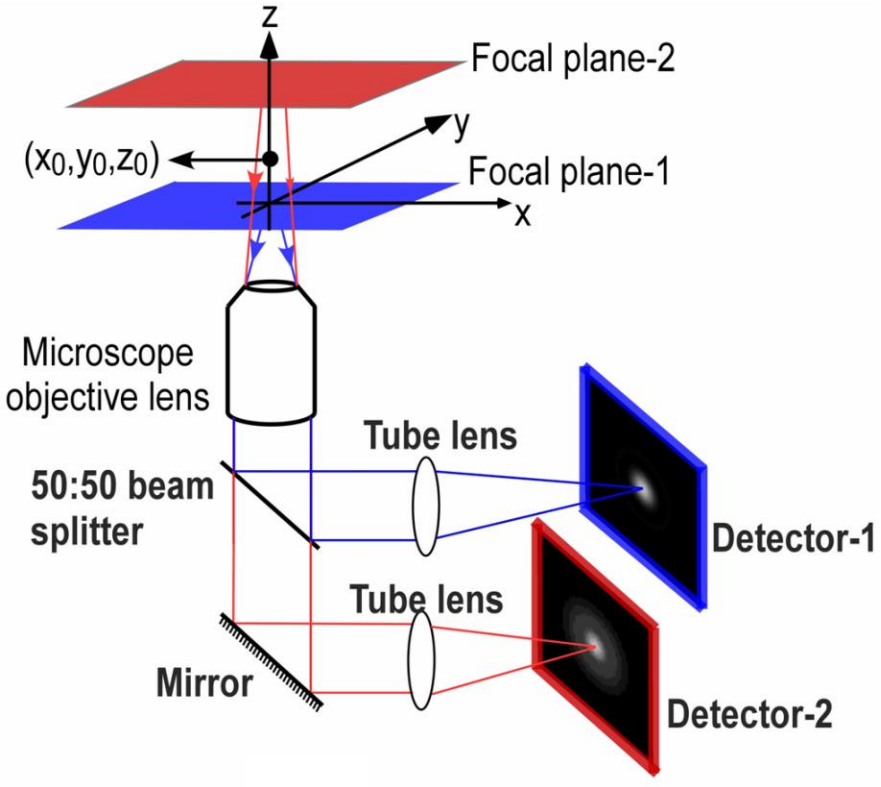


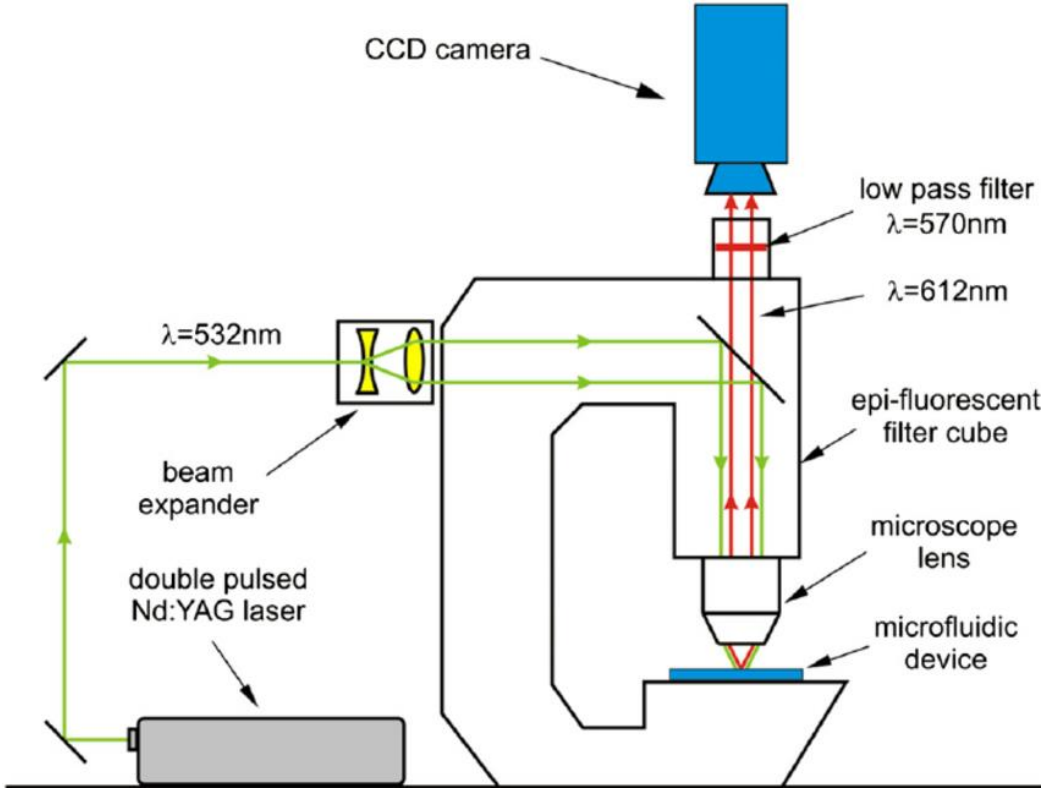
Figure

Projecting the image to your eyes

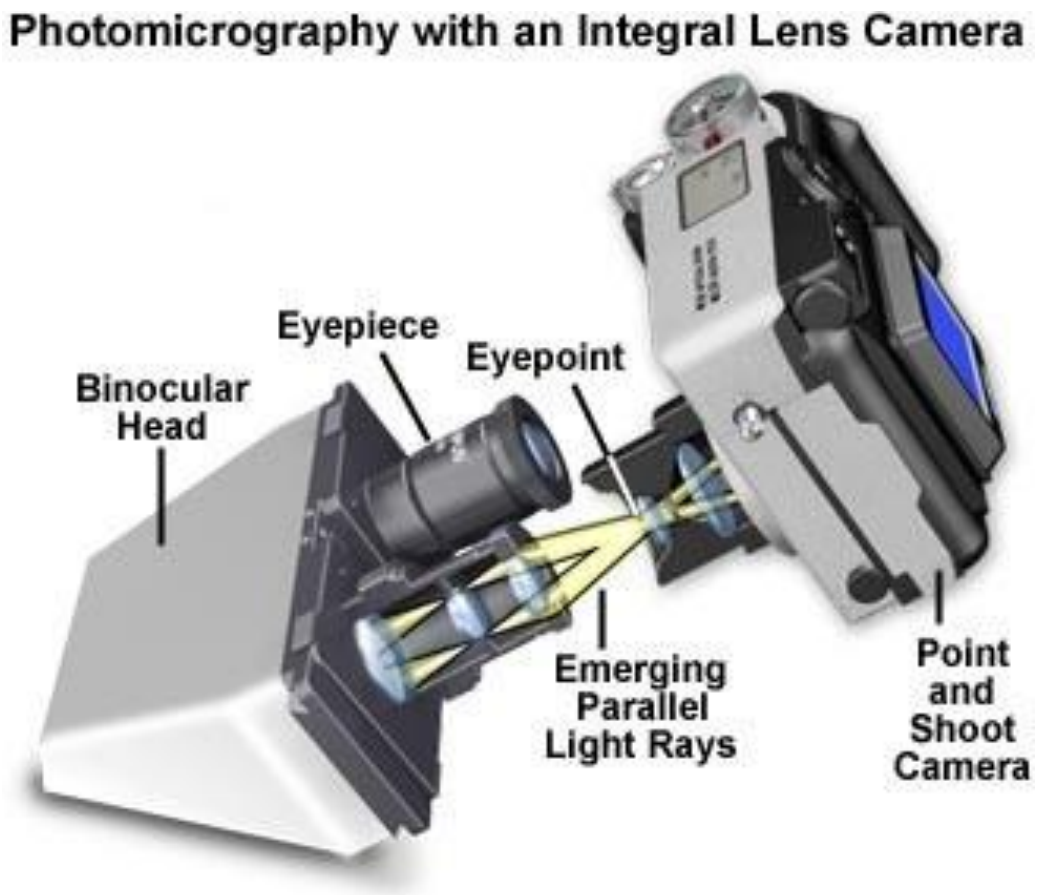


Imaging with a camera at the intermediate imaging plane

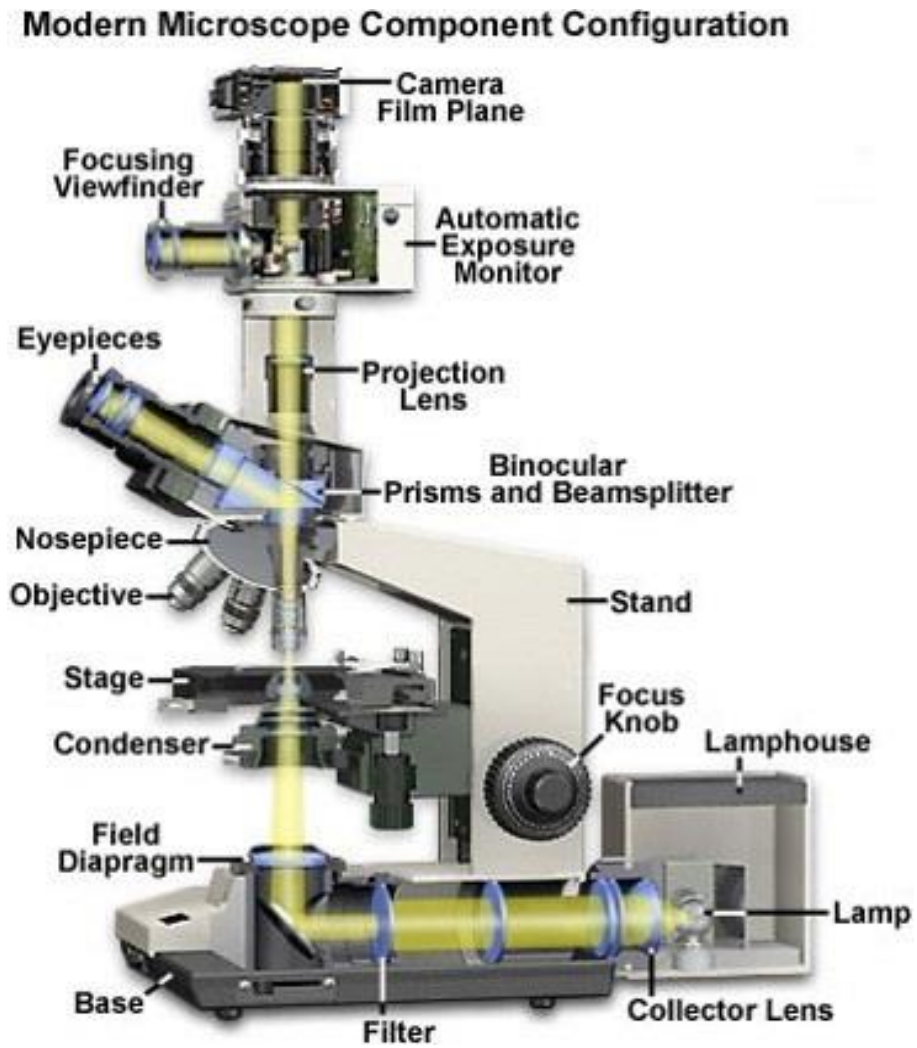




Imaging with a camera at the eyepiece imaging plane



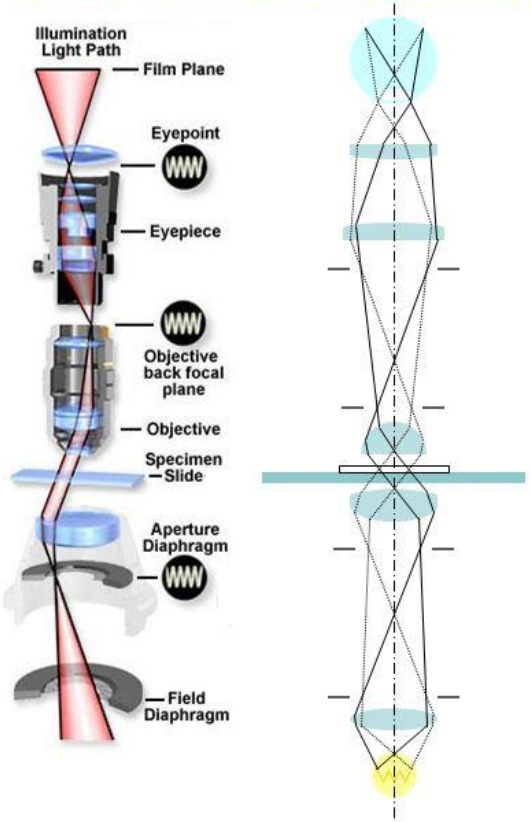
Illuminating the specimen



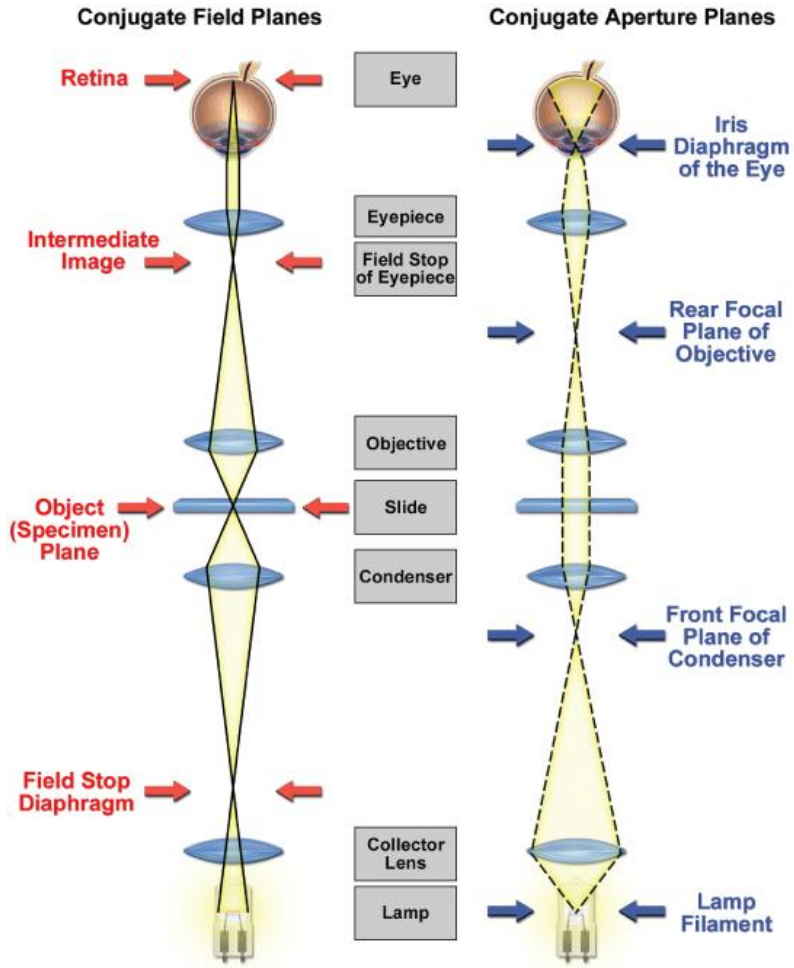
Kohler illumination

Köhler Illumination

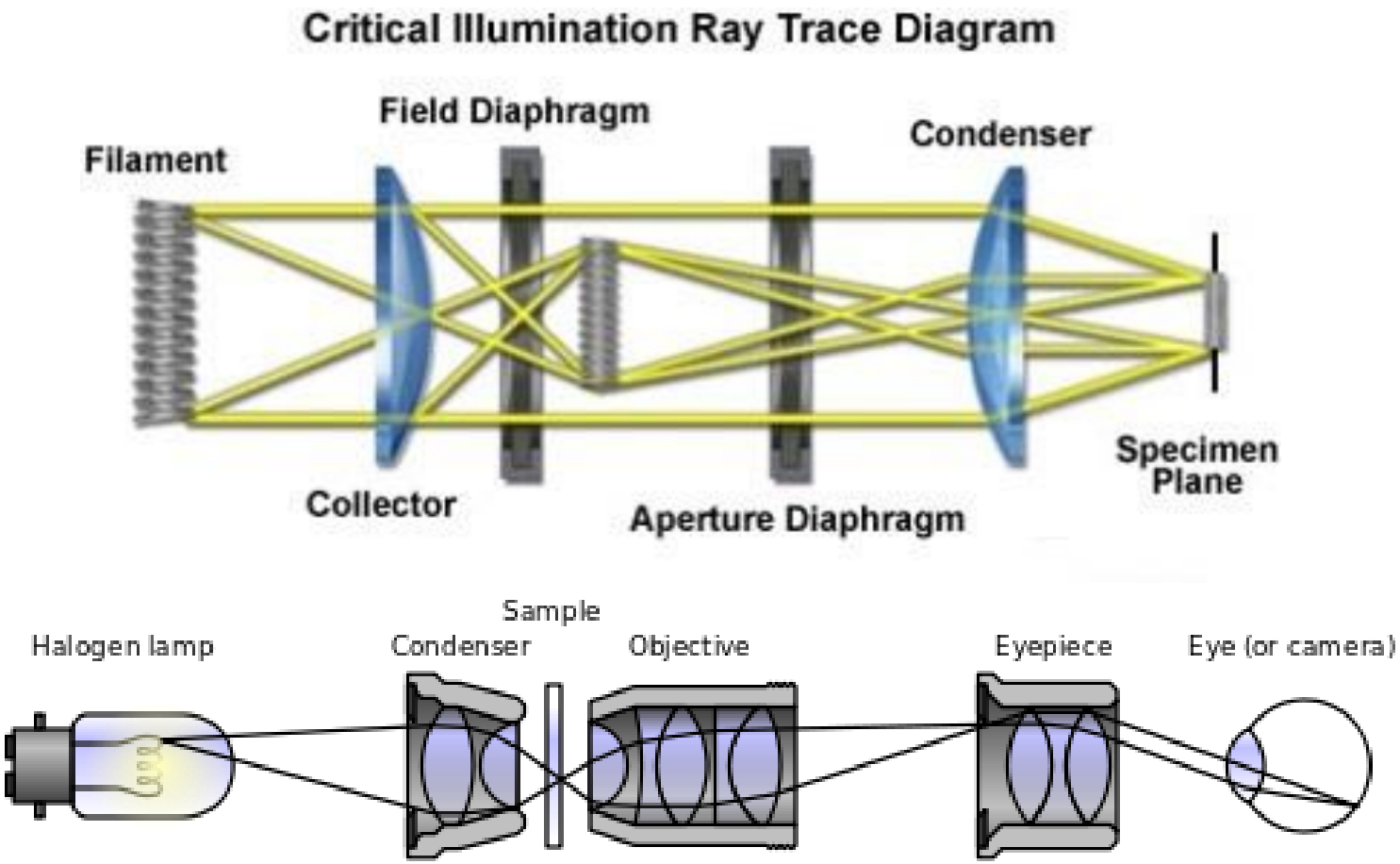
Köhler illumination (specimen illuminating light rays)



This technique is recommended by all manufactures of modern laboratory microscopes because it can produce specimen illumination that is uniformly bright and free from glare, allowing the user to realize the microscope's full potential.



Critical illumination



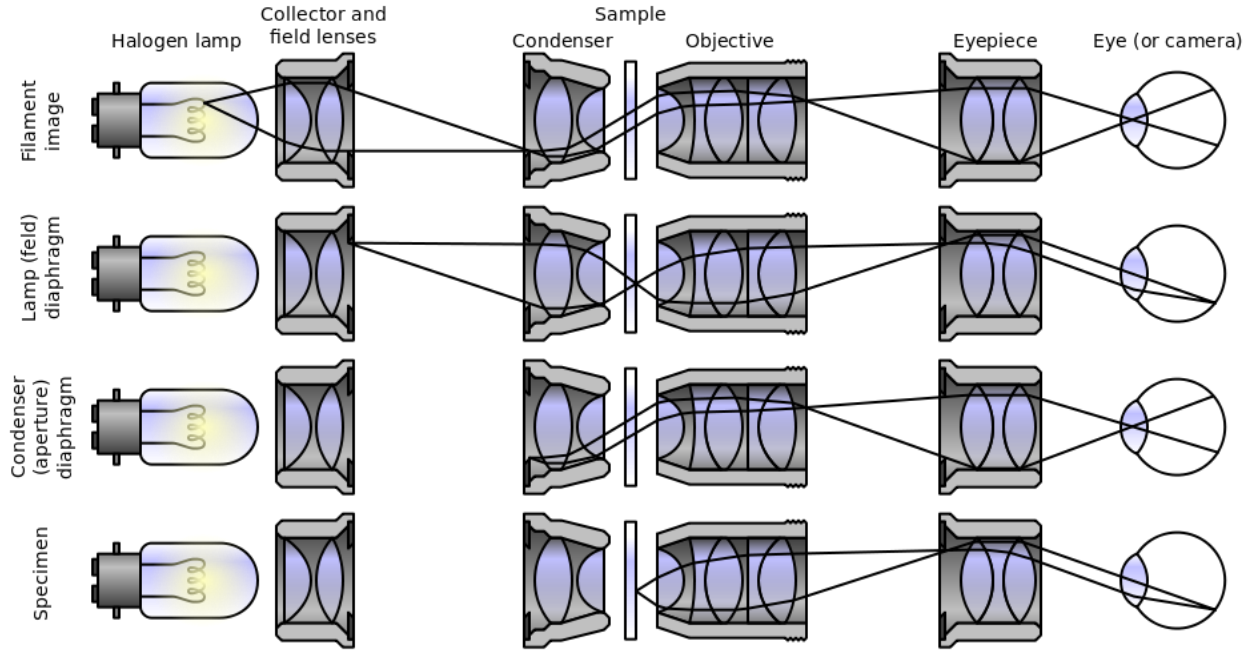
Kohler illumination versus critical illumination
why kohler illumination?

Light source image planes:

- Lamp filament
- Condenser diaphragm
- Back focal plane of the objective
- The eyepoint

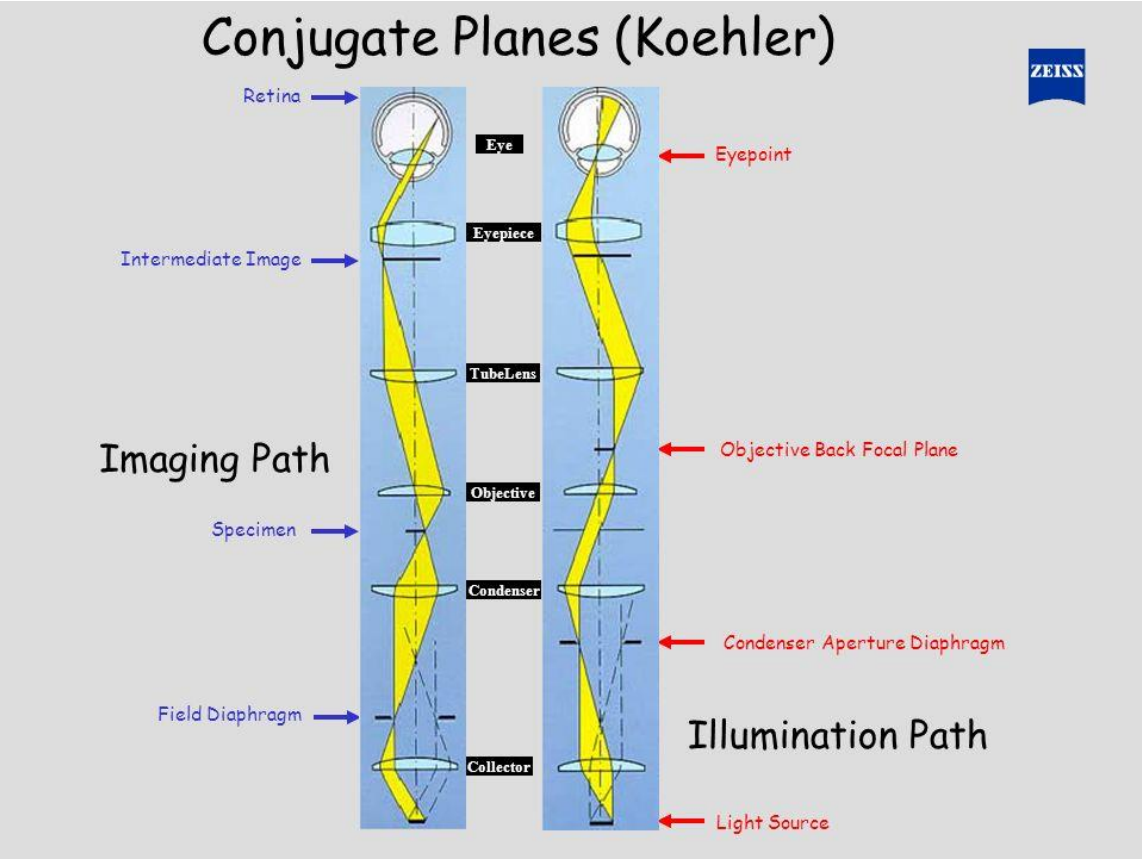
Specimen image planes:

- Field diaphragm
- Specimen
- Intermediate image plane (the eyepiece diaphragm)
- The eye retina or camera sensor

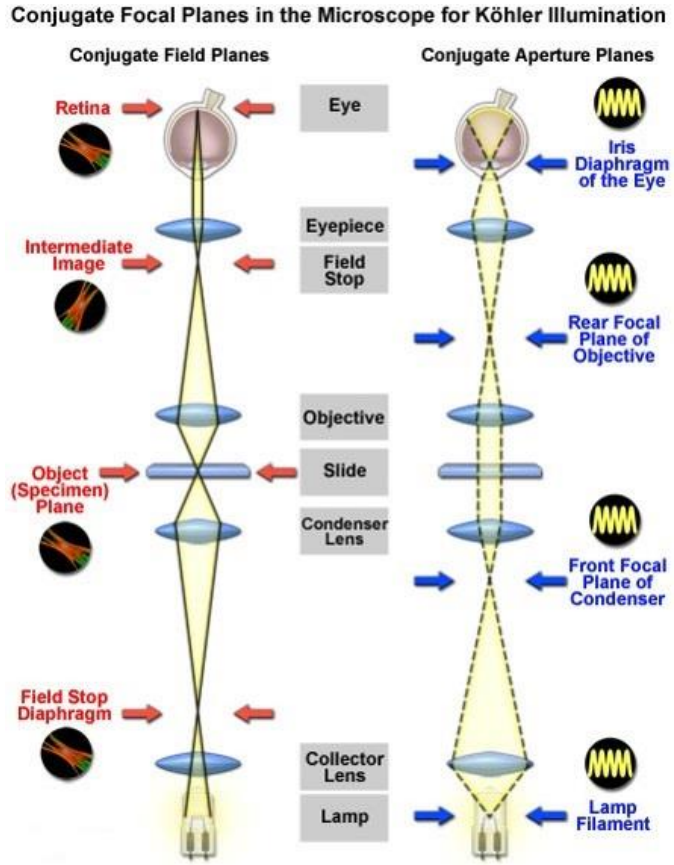


Conjugates planes

Infinite objetive



Finite objetive

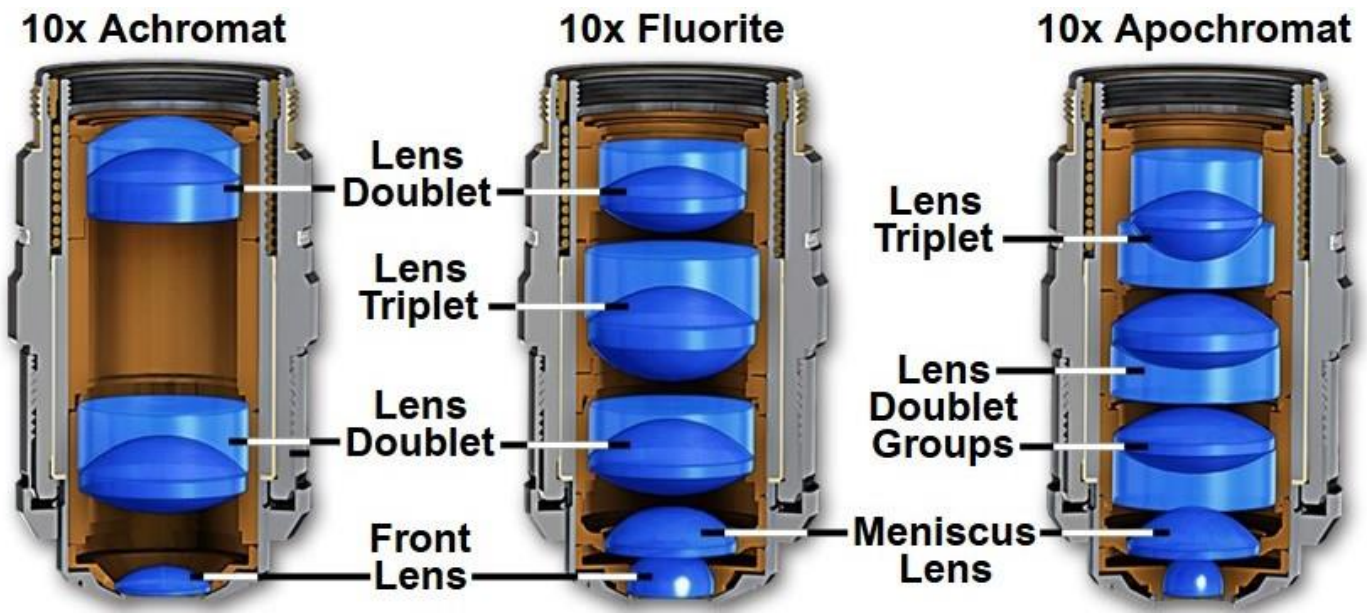


Conjugate planes are simultaneously in focus and appear superimposed when viewing through the microscope

Objective lenses and optical aberrations

There are many classes of objective lenses

Common objective optical correction factors



- ✓ Aberration correction
- ✓ Transmission
- ✓ Resolving power

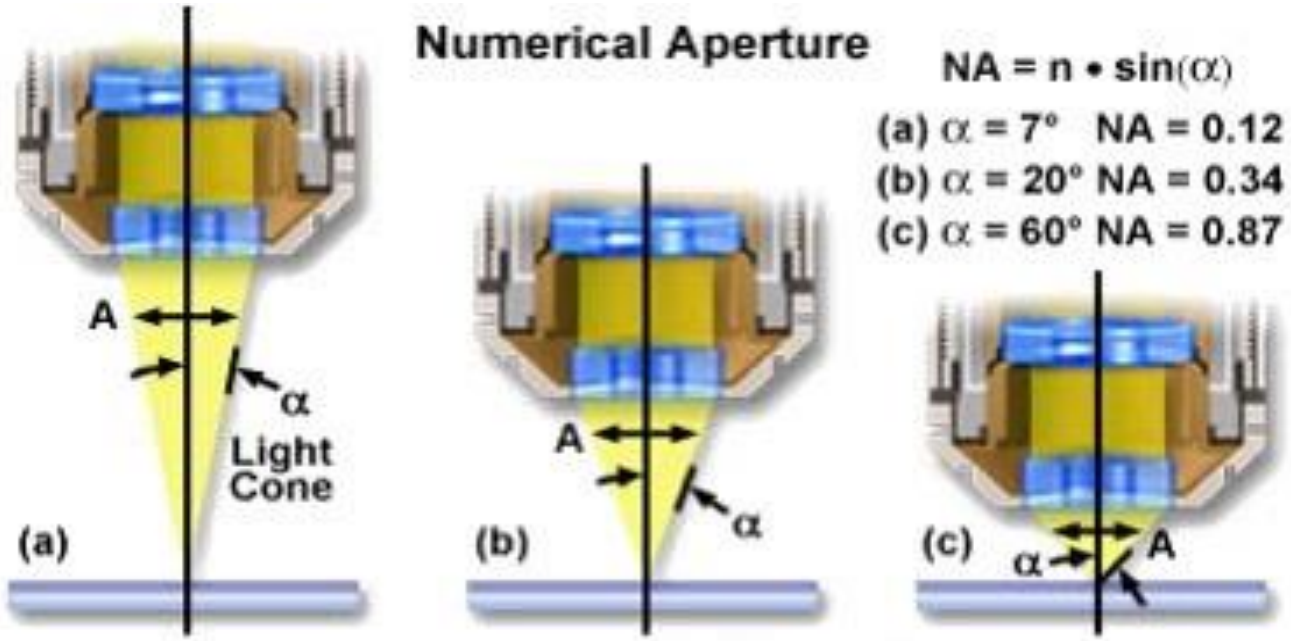
Aberration correction

- **Achromats**
- Axial - red and blue (656 nm-486 nm)
- Spherical - Green - 546 nm
- **Fluorites (Semi-Apo)**
- Axial -2 to 4 colors
- Spherical - 2 to 4 colors
- **Apochromats**
- Axial - 4 to 5 colors - violet, blue, green and red

All available in “Plan” versions

Numerical aperture

$$NA = n.\sin(\alpha)$$

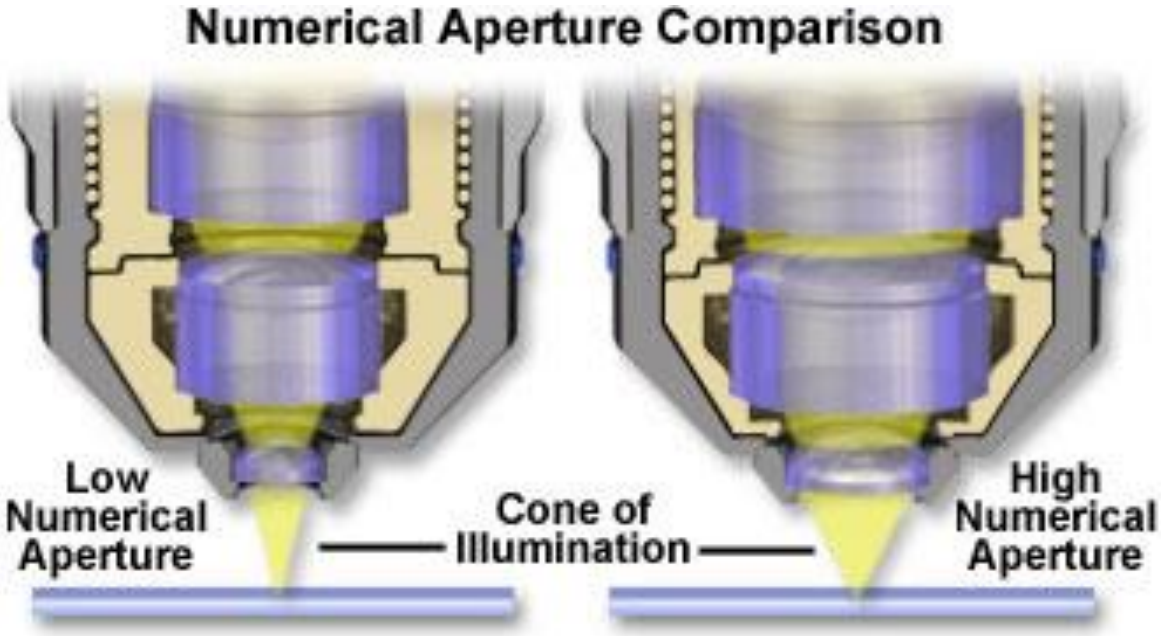


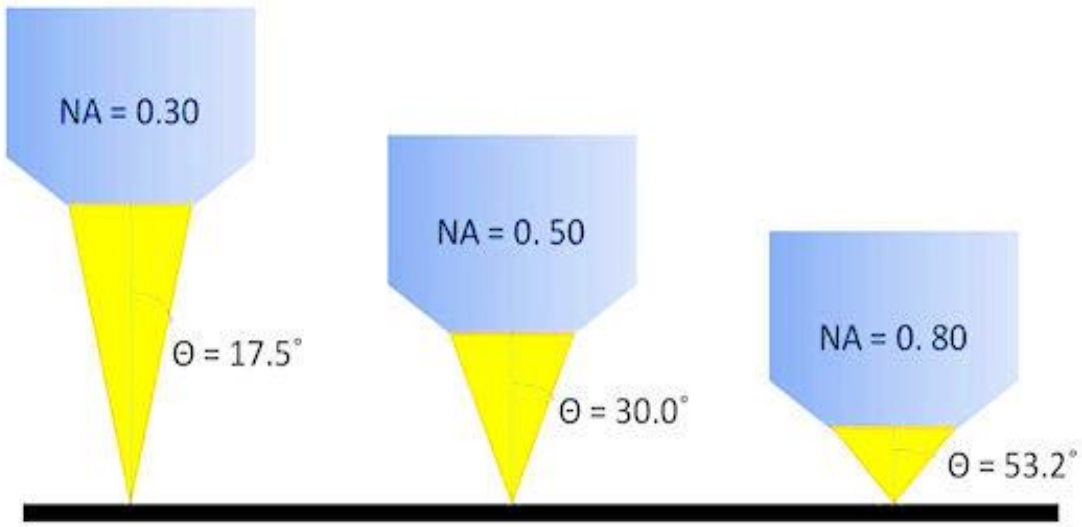
α: the half opening angle of the objective or angle of the cone of illumination

n: the refractive index of the immersion medium used between the objective and the object

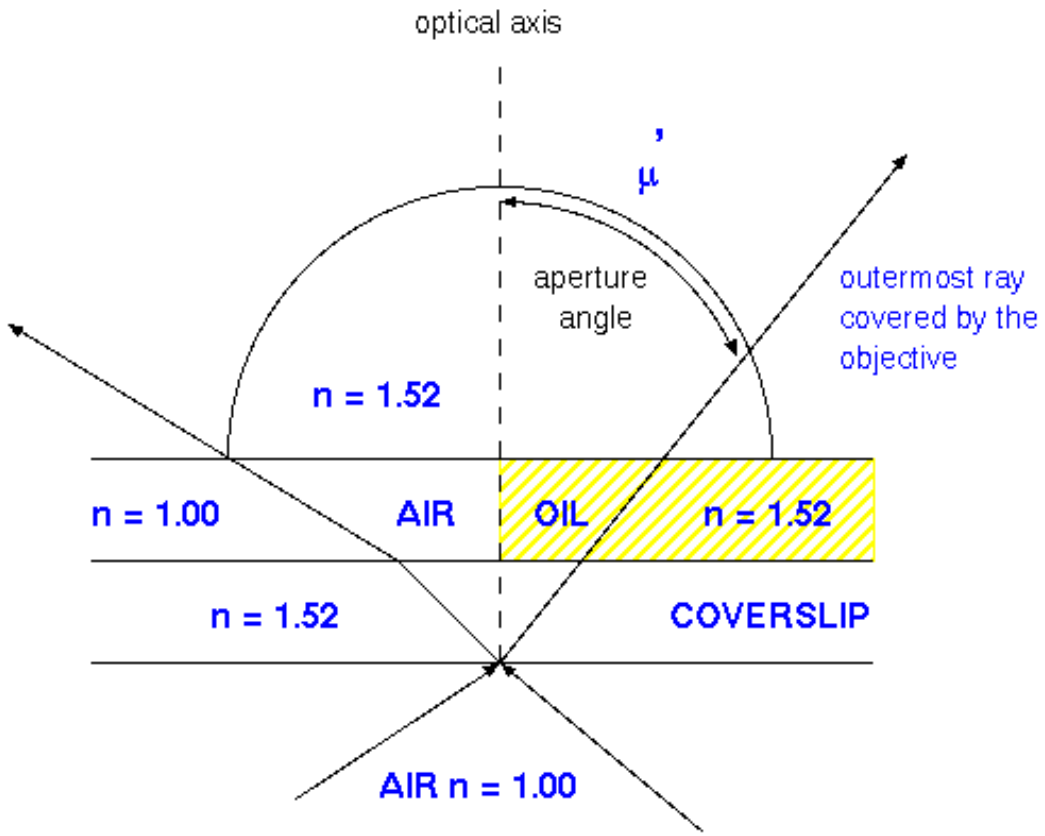
- Air n=1.0
- Water n=1.33
- Oil and glass n=1.5

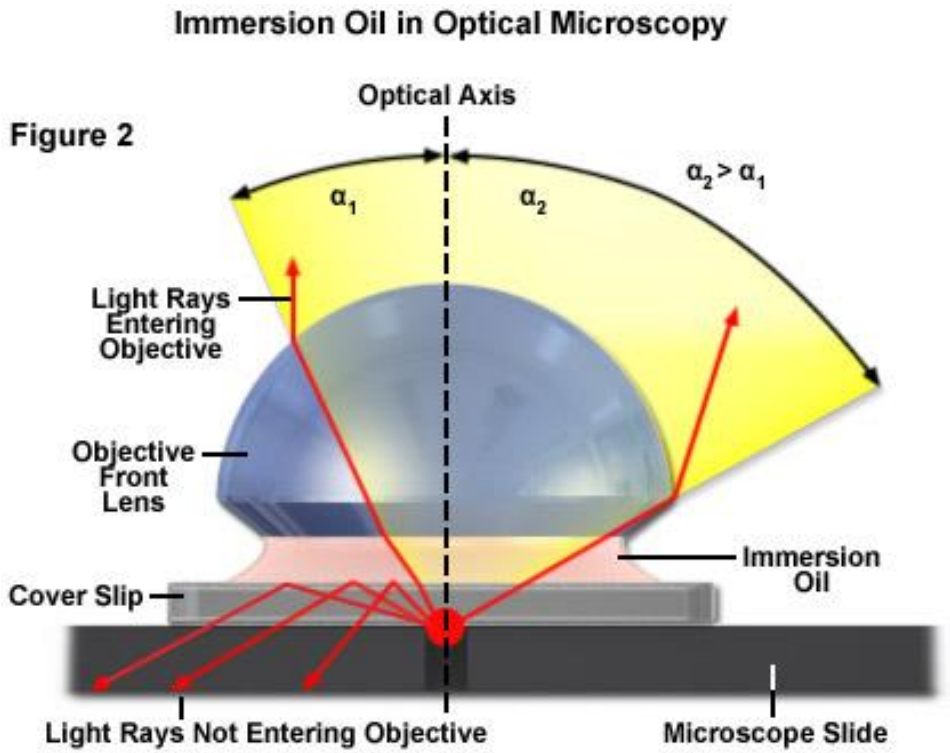
Numerical aperture





Why immersion media increases NA?





Maximum NA=1.49 in oil

and NA=1.27 in water

How immersion medium affects the true N.A. and consequently resolution

No immersion (dry)

- Max. Value for $\alpha = 90^\circ$ (sin = 1)
- Attainable: $\sin\alpha = 0.95$ ($\alpha = 72^\circ$)

- Actual angle α_1:

Snell's Law:

$n_1 \sin\beta_1 = n_2 \sin\beta_2$

$$\alpha_1 = \text{arcsine}\frac{NA}{n} = \text{arcsine}\frac{0.95}{1.52} = 39^o$$

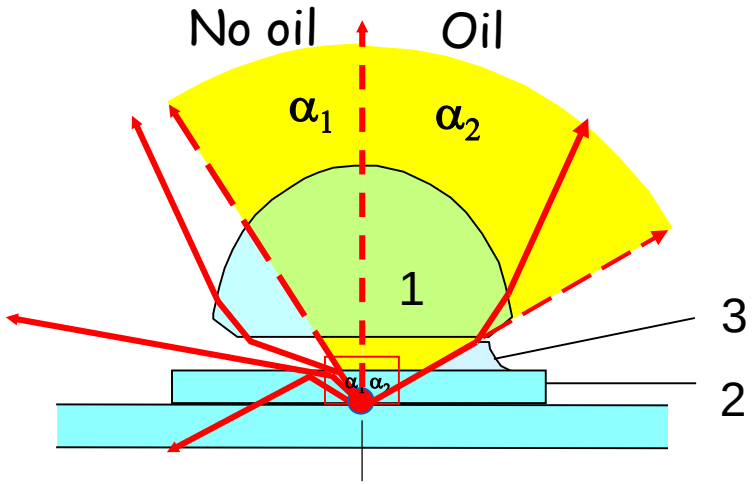
With immersion oil (3) n=1.518

- No Total Reflection
- Objective aperture fully usable

- $N.A._{max}$ = 1.45 > Actual angle α_2 :

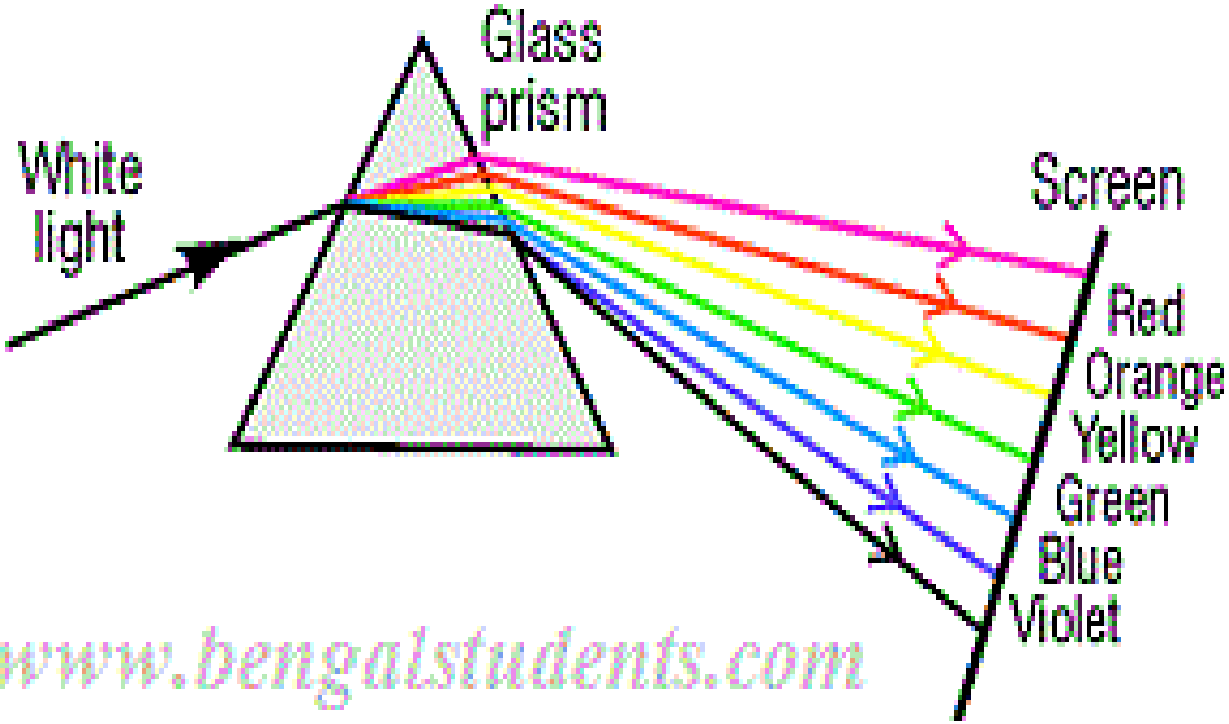
$$\alpha_2 = \text{arcsine}\frac{NA}{n} = \text{arcsine}\frac{1.45}{1.518} = 73^o$$

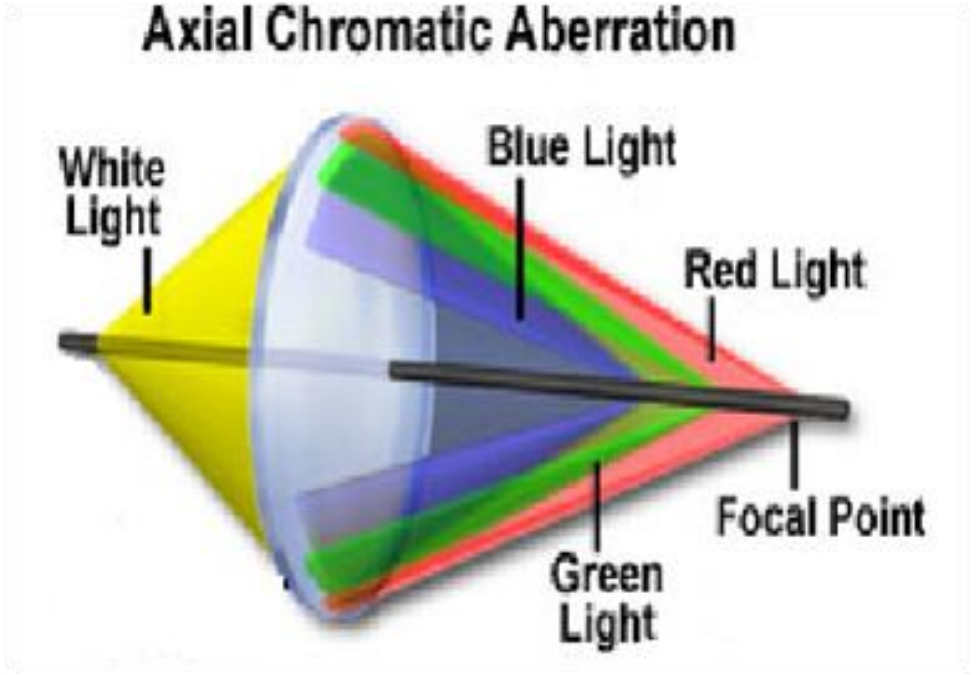
Beampath



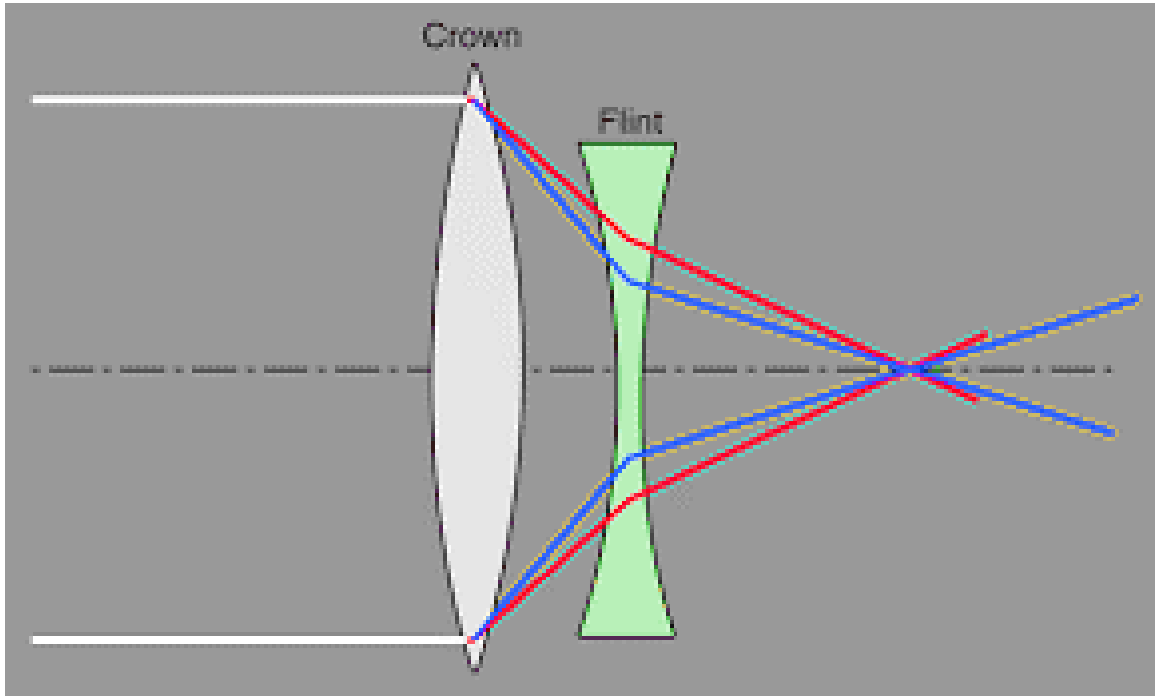
1) Objective
2) Cover Slip, on slide
3) Immersion Oil

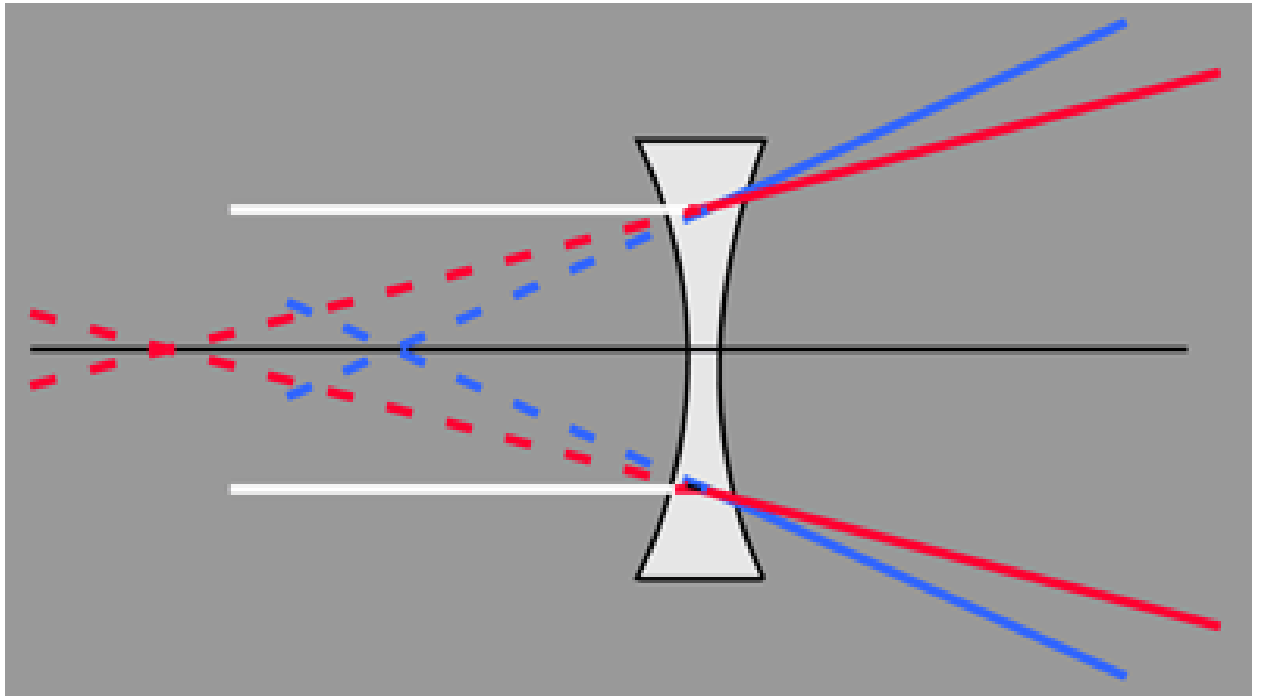
Chromatic aberrations



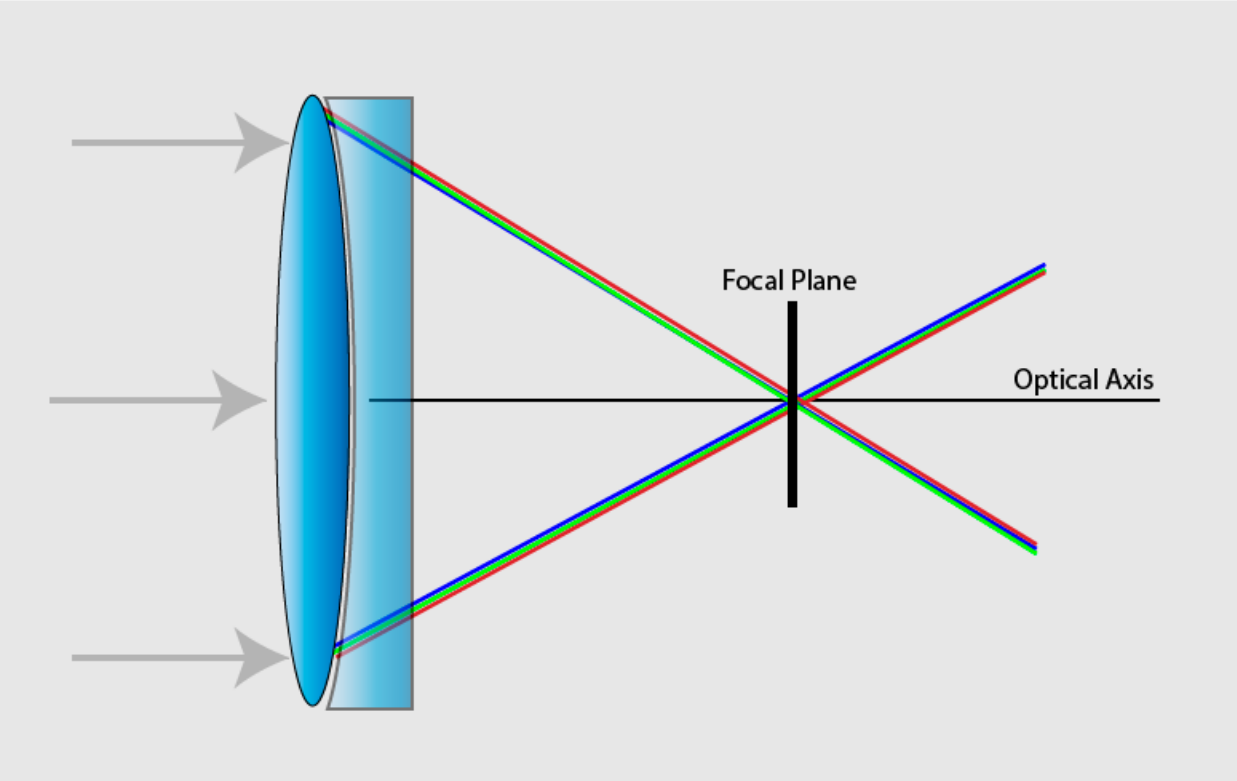


Chromatic aberrations correction

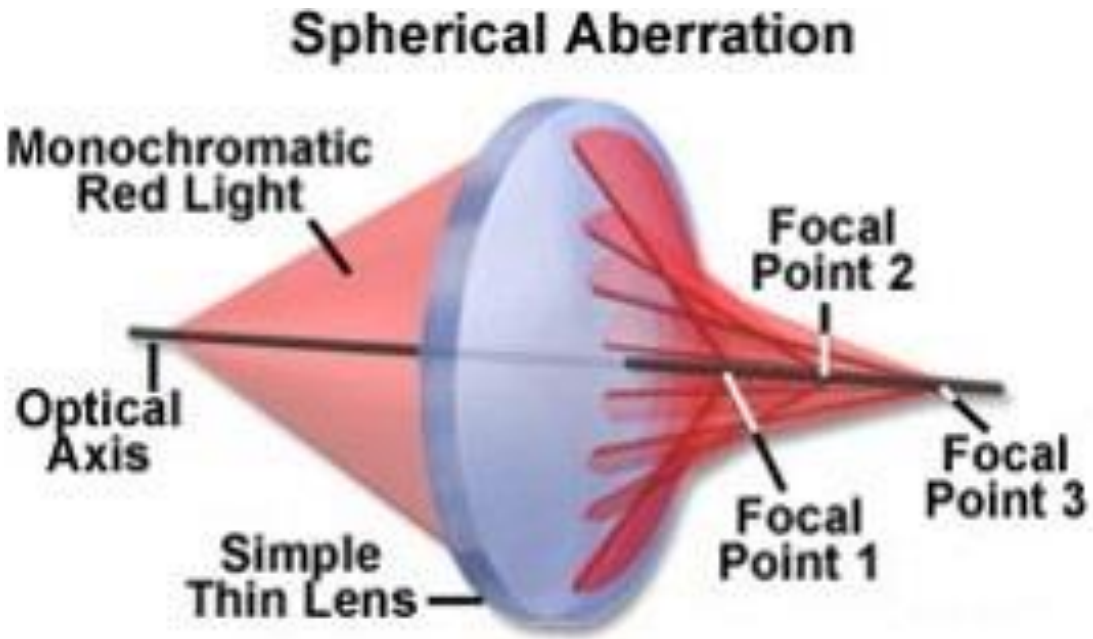


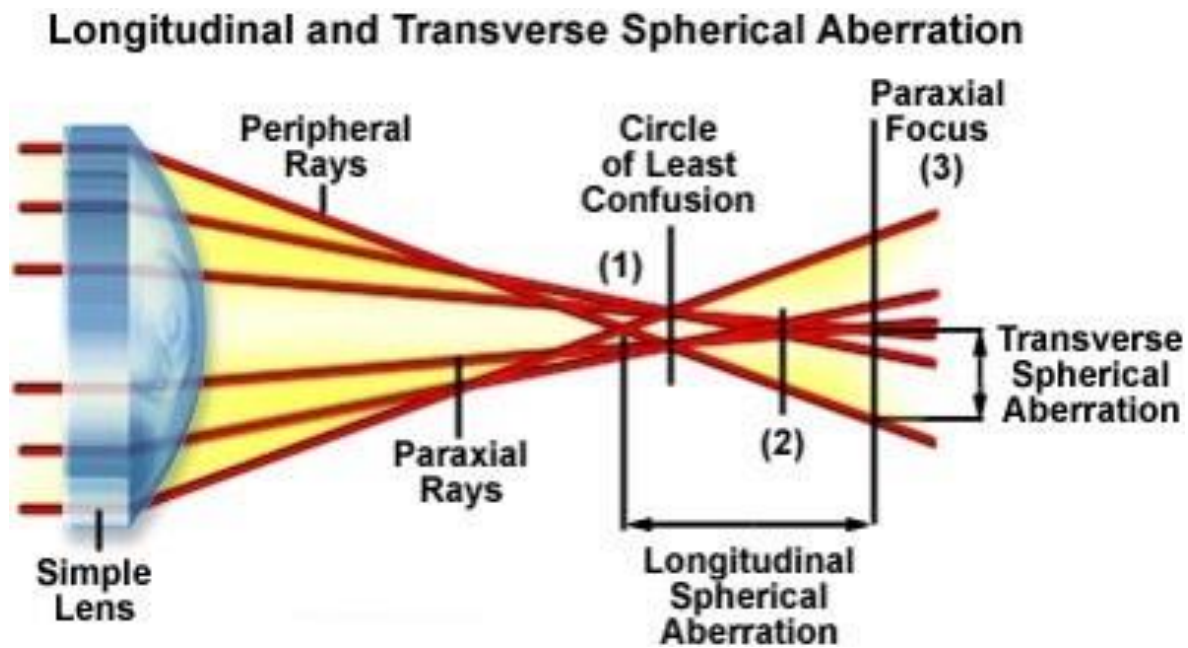


Chromatic aberrations correction



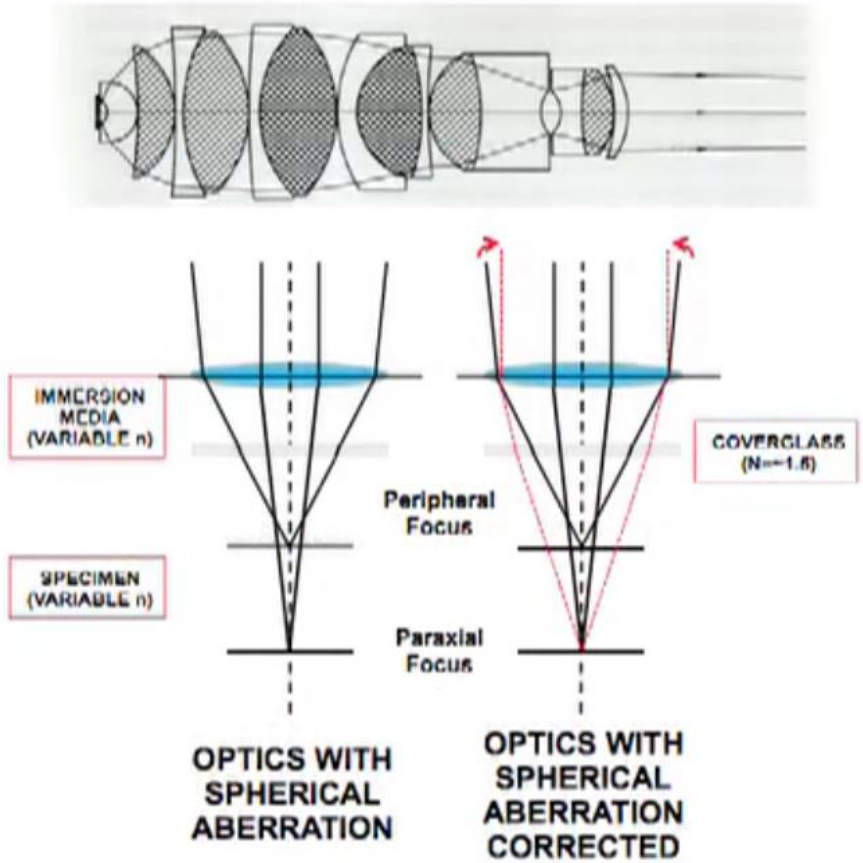
Spherical aberrations

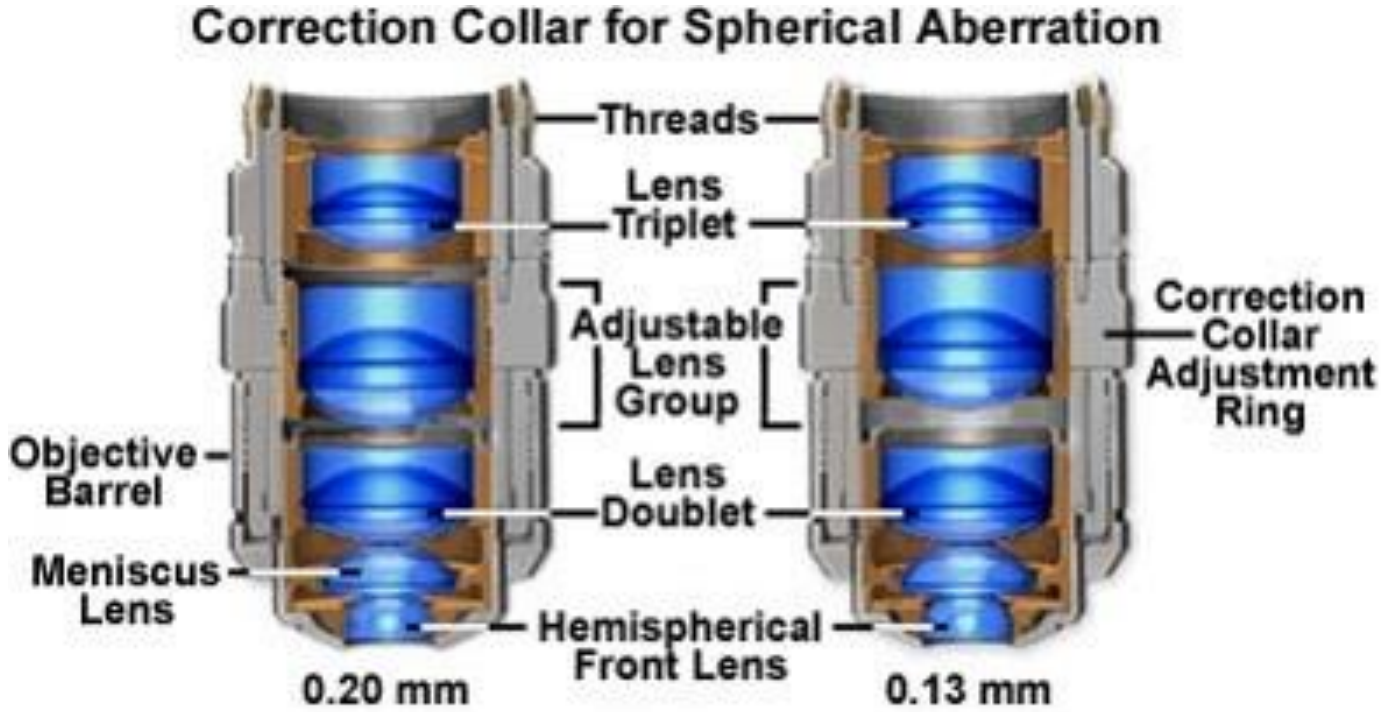




Spherical aberrations correction

How does a correction collar work??





Objective Magnification

Magnification of the Microscope

•M Microscope = M Objective X M Eyepiece X M Intermediate Factor

M = Magnification

Example: Objective = 60 x
Eyepiece = 10 x
Intermediate Factor = 1 x
Overall M = 600 x

Features of an objective

Thanks