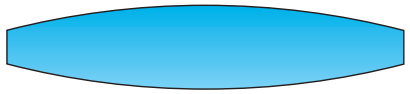
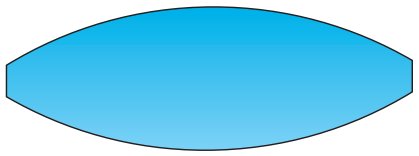
Resolution

It's all about the NA

f/# = focal_length / input_diameter

Lens Diameter	Focal Length	f/#	NA	Lens Shape	λ = 500 nm Resolution Lateral	Axial
25 mm	100 mm	4	0.12		2.1 μm	34 μm
25 mm	50 mm	2	0.24		1.04 μm	8.7 μm
25 mm	25 mm	1	0.44		0.56 μm	2.1 μm
12.7 mm	12.7 mm	1	0.44		0.56 μm	2.1 μm

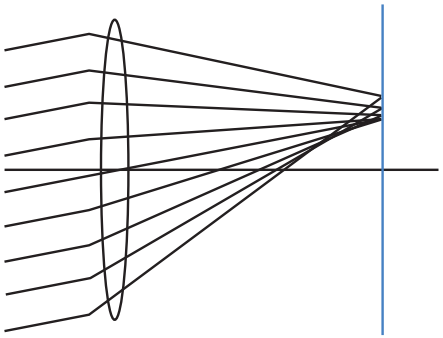
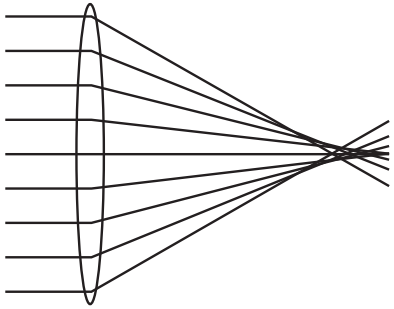
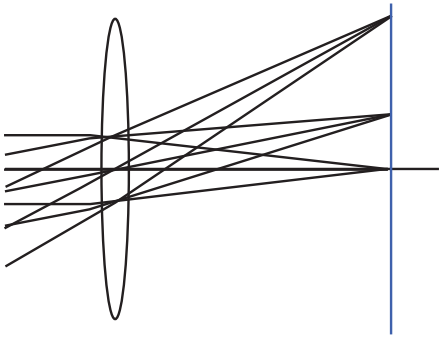
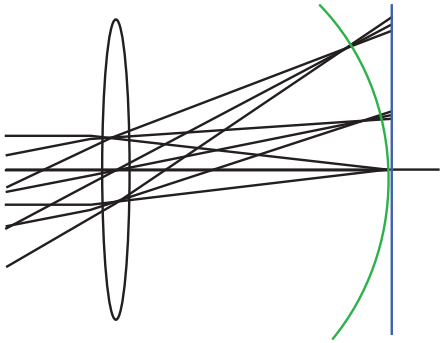
Optical Aberrations

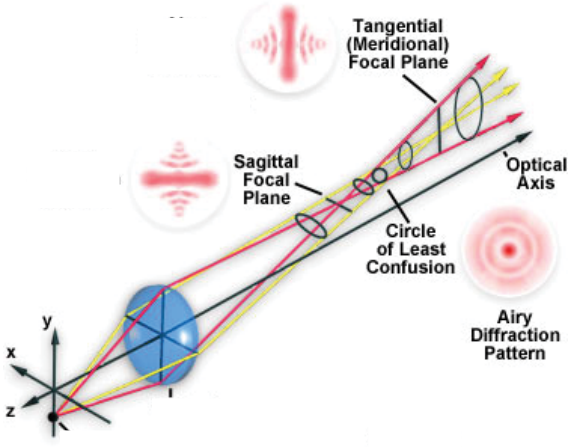
(Seidel abberations , aka third-order aberrations, monochromatic aberrations)

$$\sin(\theta) = \theta + \theta^3/(3!) - \theta^5/(5!) + \theta^7/(7!) - \theta^9/(9!) + \ldots.$$

$$\cos(\theta) = 1 - \theta^2/(2!) - \theta^4/(4!) + \theta^6/(6!) - \theta^8/(8!) + \ldots.$$

paraxial approximation

	Lateral	Axial
Focus Quality	 Coma	 Spherical Aberration
Focus Position	 Field Distortion (Barrel / Pincushion)	 Petzval Field Curvature



Astigmatism

*modified from MicroscopyU website

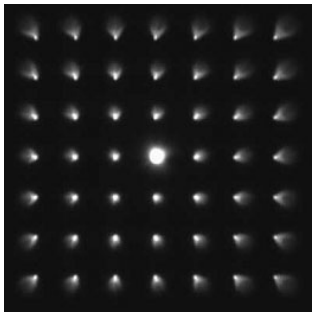
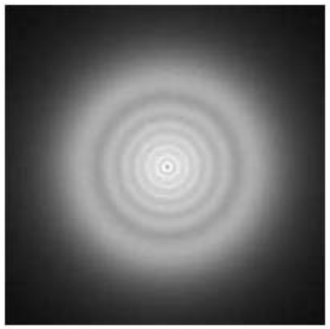
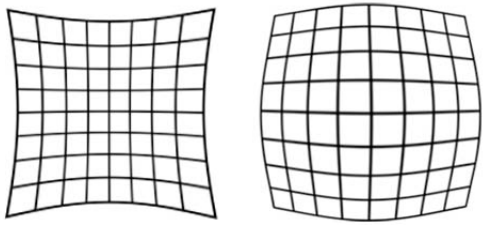
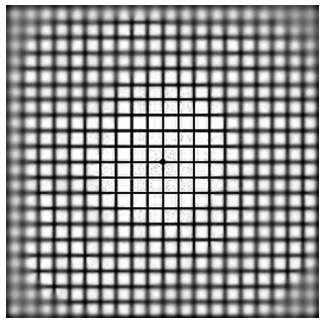
Optical Aberrations

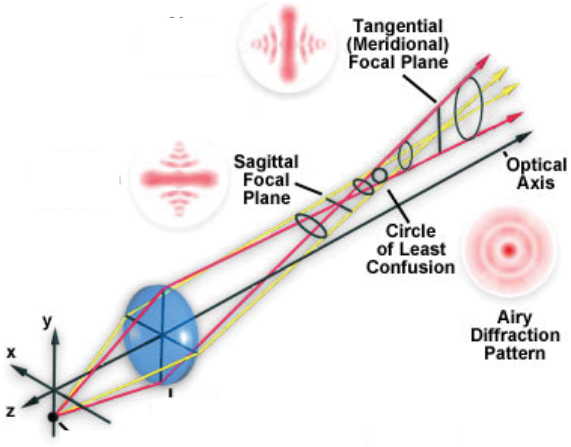
(Seidel abberations , aka third-order aberrations, monochromatic aberrations)

$$\sin(\theta) = \theta + \theta^3/(3!) - \theta^5/(5!) + \theta^7/(7!) - \theta^9/(9!) + \ldots.$$

$$\cos(\theta) = 1 - \theta^2/(2!) - \theta^4/(4!) + \theta^6/(6!) - \theta^8/(8!) + \ldots.$$

paraxial approximation

	Lateral	Axial
Focus Quality	 Coma	 Spherical Aberration
Focus Position	 Field Distortion (Barrel / Pincushion)	 Petzval Field Curvature

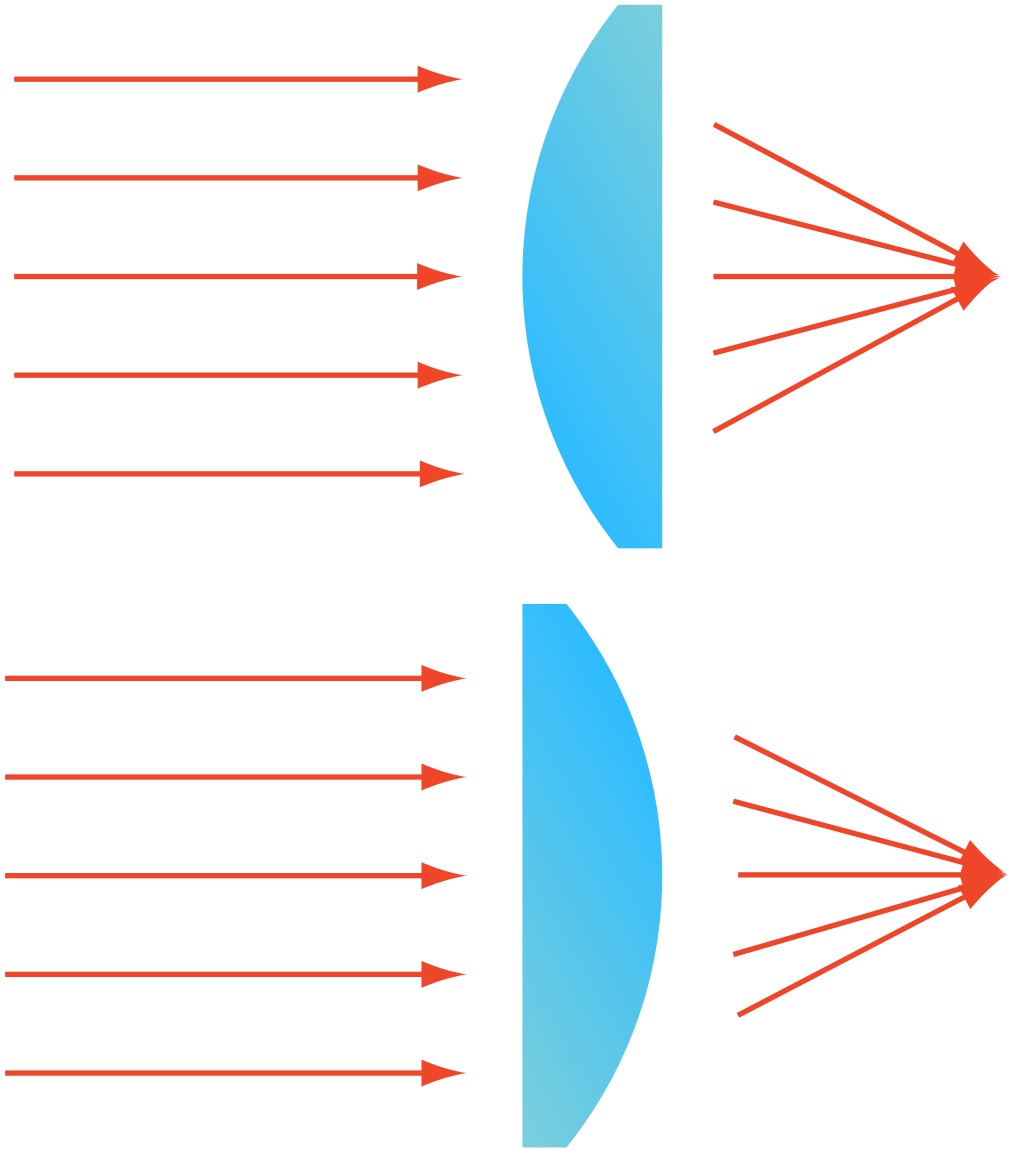


Astigmatism

*modified from MicroscopyU website

Minimizing Optical Aberrations

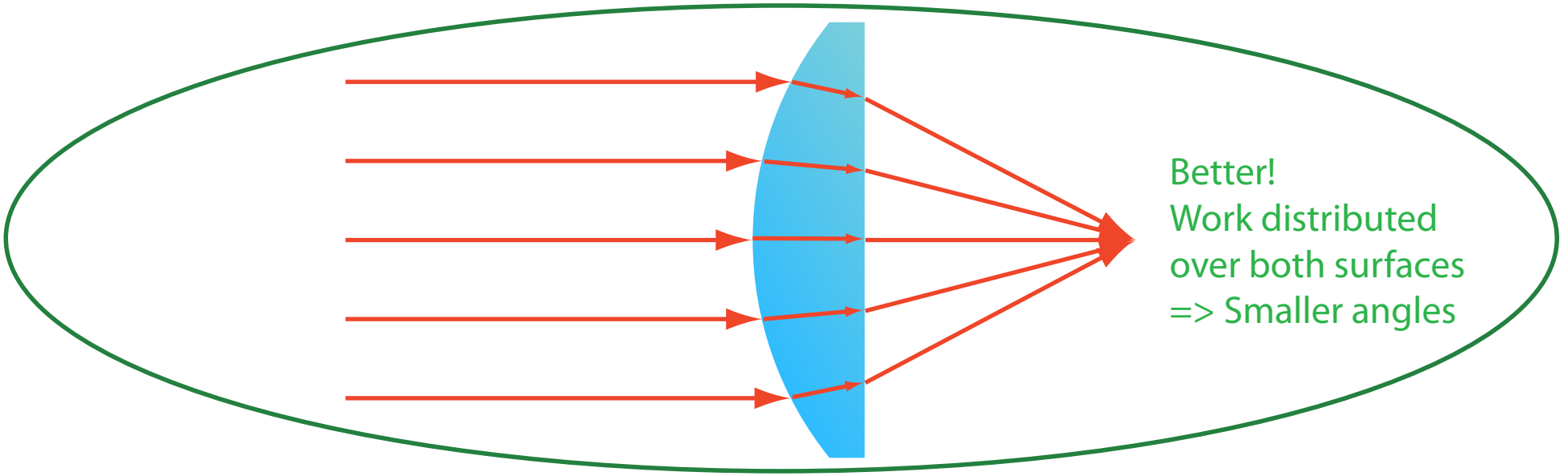
Which way should you insert this plano-convex lens ?

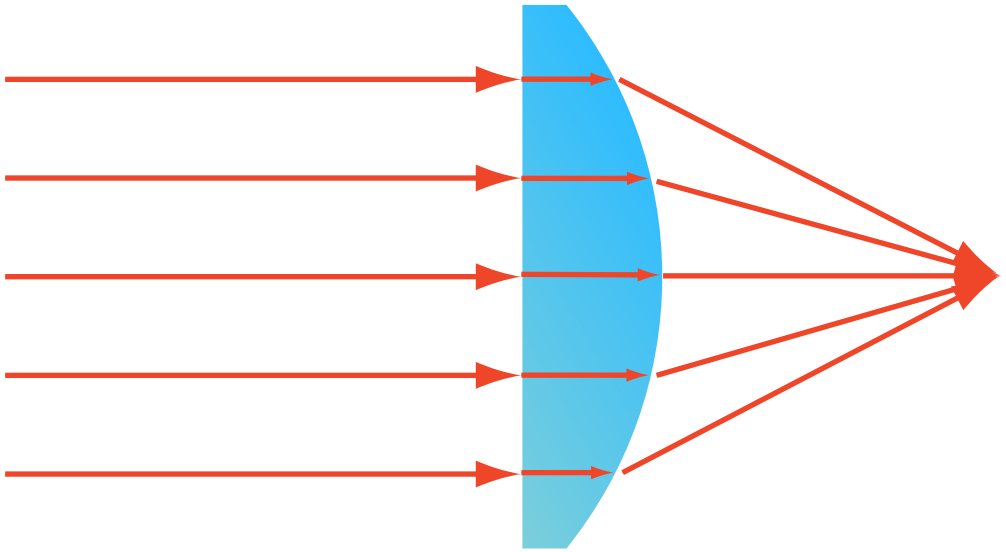


Minimizing Optical Aberrations

(Distribute optical power across multiple surfaces)

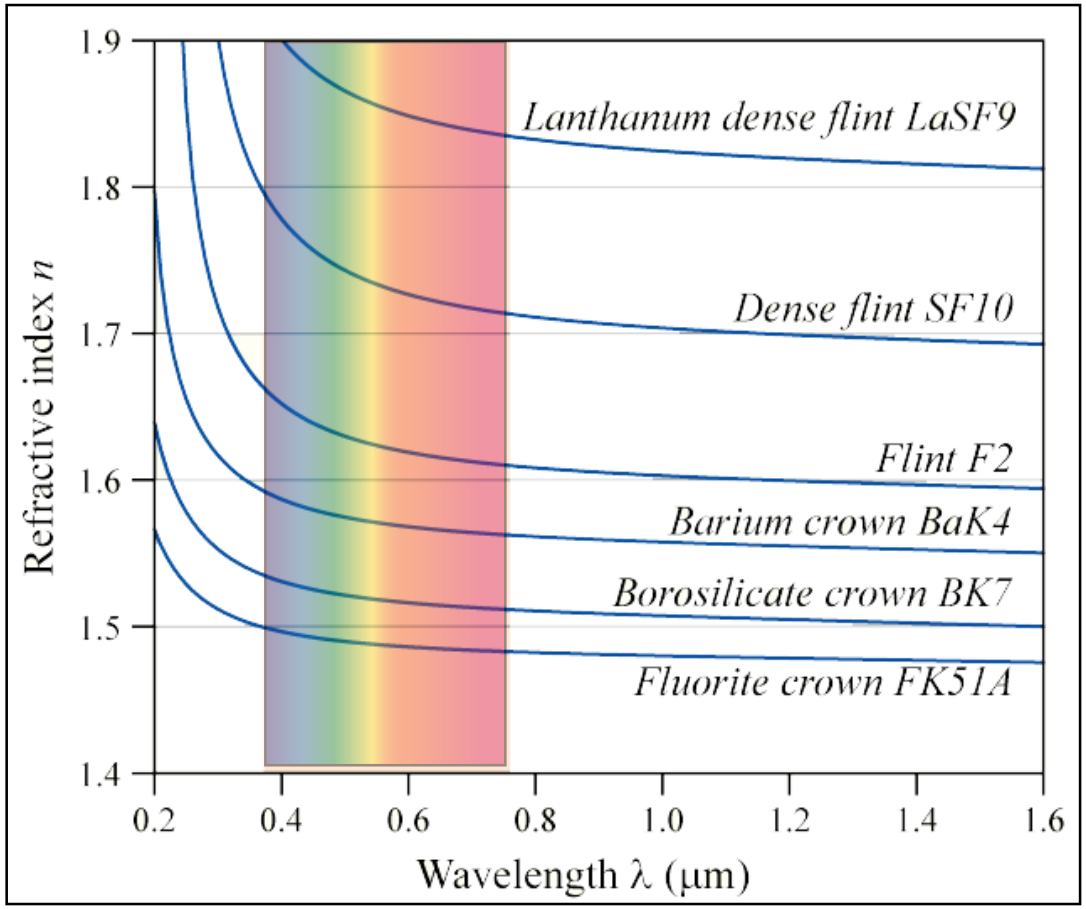
Which way should you insert this plano-convex lens ?

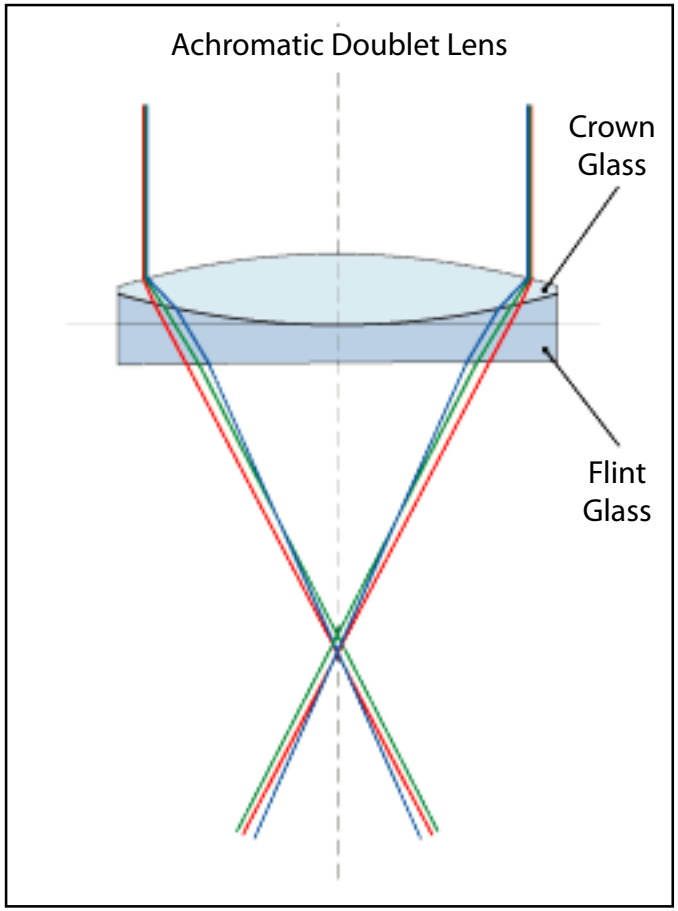




Chromatic Aberration

(Index of refraction is a function of wavelength)





Minimizing Optical Aberrations

(Distribute optical power across multiple surfaces)

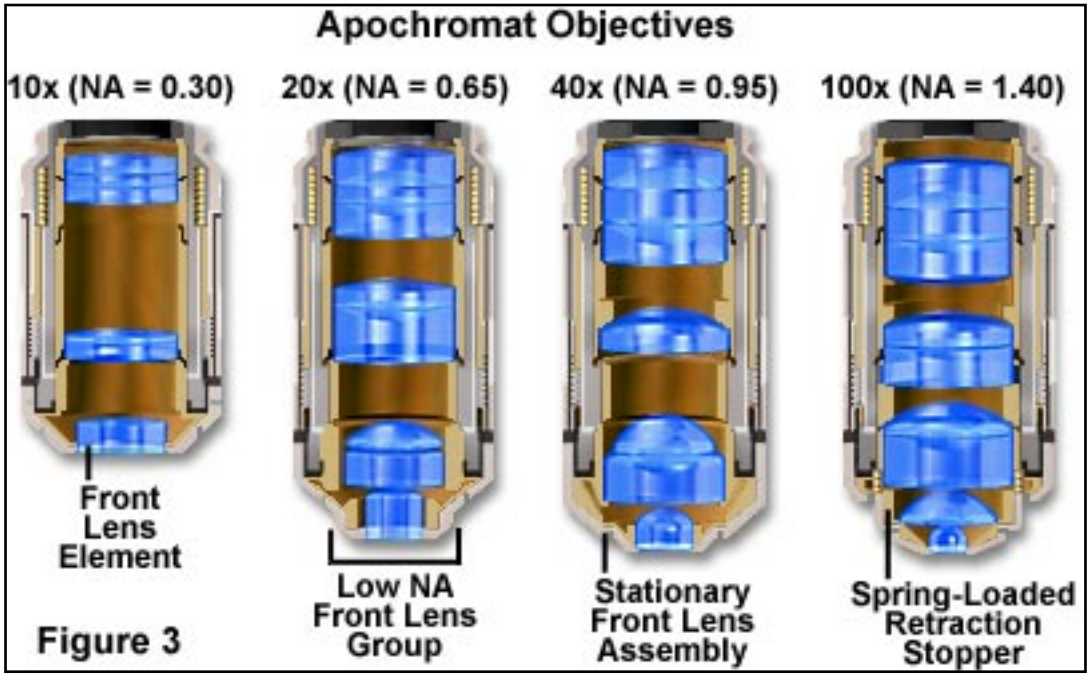
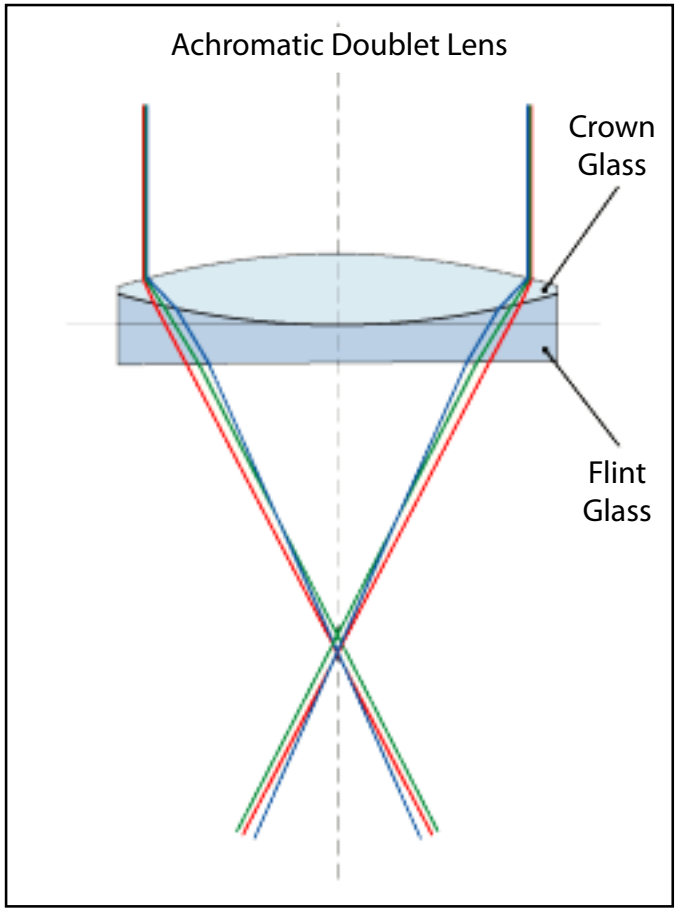


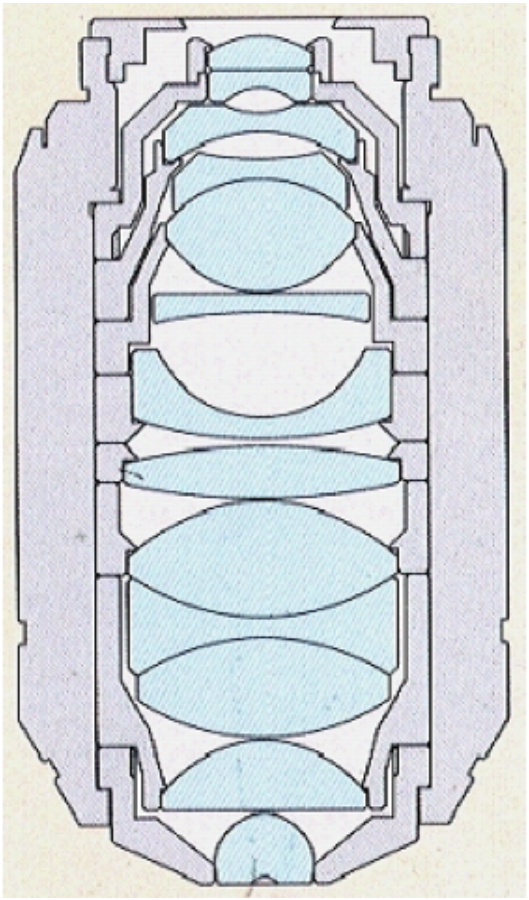
Image taken from Molecular Expressions website



Microscope Objectives

More $$$ = Better Aberration Correction

Figure 1



Microscope Objectives

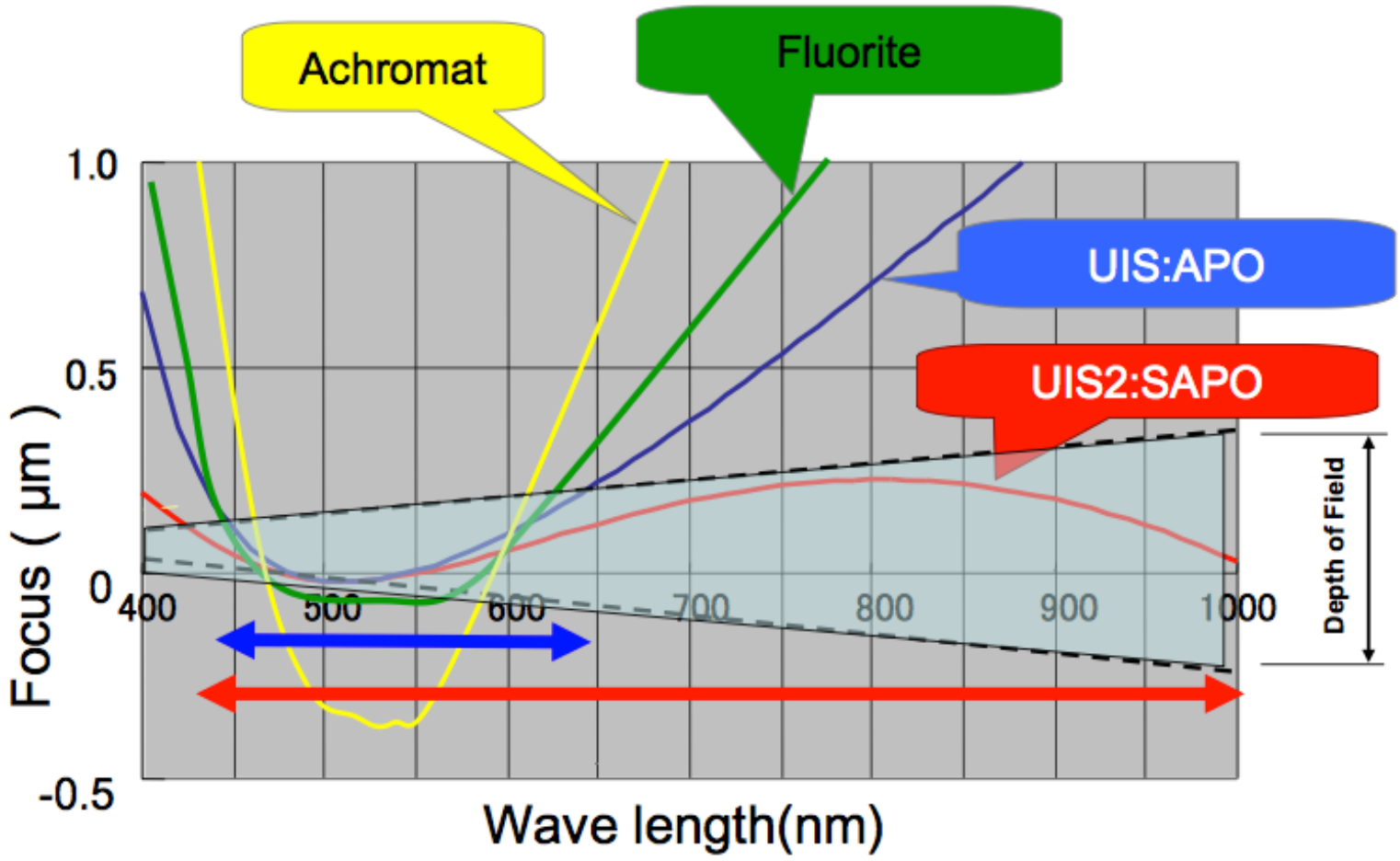
More $$$ = Better Aberration Correction

Specialized Objective Designations

Abbreviation	Type
Achro, Achromat	Achromatic aberration correction
Fluor, Fl, Fluar, Neofluar, Fluotar	Fluorite aberration correction
Apo	Apochromatic aberration correction
Plan, Pl, Achroplan, Plano	Flat Field optical correction
EF, Acroplan	Extended Field (field of view less than Plan)
N, NPL	Normal field of view plan
Plan Apo	Apochromatic and Flat Field correction
UPLAN	Olympus Universal Plan (Brightfield, Darkfield, DIC, and Polarized Light)
LU	Nikon Luminous Universal (Brightfield, Darkfield, DIC, and Polarized Light)
L, LL, LD, LWD	Long Working Distance
ELWD	Extra-Long Working Distance
SLWD	Super-Long Working Distance
ULWD	Ultra-Long Working Distance
Corr, W/Corr, CR	Correction Collar
I, Iris, W/Iris	Adjustable numerical aperture (with iris diaphragm)
Oil, Oel	Oil Immersion
Water, Wl, Wasser	Water Immersion
HI	Homogeneous Immersion
Gly	Glycerin Immersion
DIC, NIC	Differential or Nomarski Interference Contrast
CF, CFI	Chrome-Free, Chrome-Free Infinity-Corrected (Nikon)
ICS	Infinity Color-Corrected System (Zeiss)
RMS	Royal Microscopical Society objective thread size
RMS	Royal Microscopical Society objective thread size
M25, M32	Metric 25-mm objective thread; Metric 32-mm objective thread
Phase, PHACO, PC	Phase Contrast
Ph 1, 2, 3, etc.	Phase Condenser Annulus 1, 2, 3, etc.
DL, DM	Phase Contrast: dark low, dark medium
PLL, PL	Phase Contrast: positive low low, positive low
PM, PH	Phase Contrast: positive medium, positive high contrast (regions with higher refractive index appear darker)
NL, NM, NH	Phase Contrast: negative low, negative medium, negative high contrast (regions with higher refractive index appear lighter)
P, Po, Pol, SF	Strain-Free, Low Birefringence, for polarized light
U, UV, Universal	UV transmitting (down to approximately 340 nm) for UV-excited epifluorescence
M	Metallographic (no coverslip)
NC, NCG	No Coverslip
EPI	Oblique or Epi illumination
TL	Transmitted Light
BBD, HD, B/D	Bright or Dark Field (Hell, Dunkel)
D	Darkfield
H	For use with a heating stage
U, UT	For use with a universal stage
DI, MI, TI	Interferometry, Noncontact, Multiple Beam (Tolanski)

Microscope Objectives

Axial Chromatic Aberration



Comparison between 100x objectives

Slide courtesy of Nicholas George (Olympus Corp.)

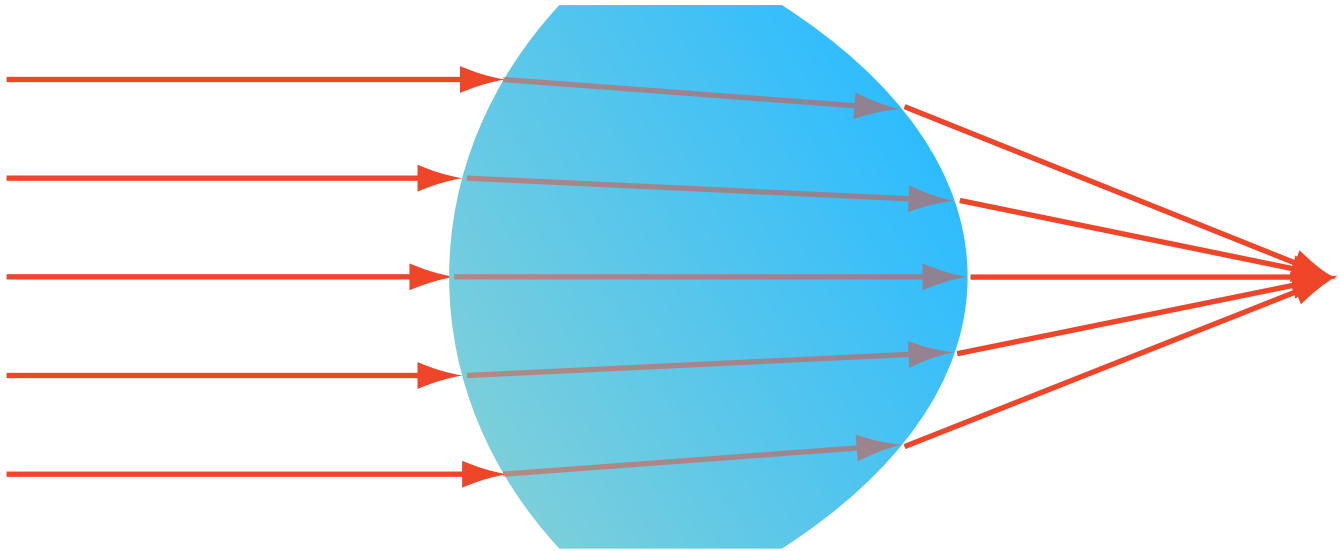
Approximations

Brutalizing optics into 3 limiting regimes

- Ray (Geometric Optics) : $\lambda \rightarrow 0$
- Paraxial Approximation : $\theta \ll \pi / 2$
- Thin Lens Approximation : lens thickness $\rightarrow 0$

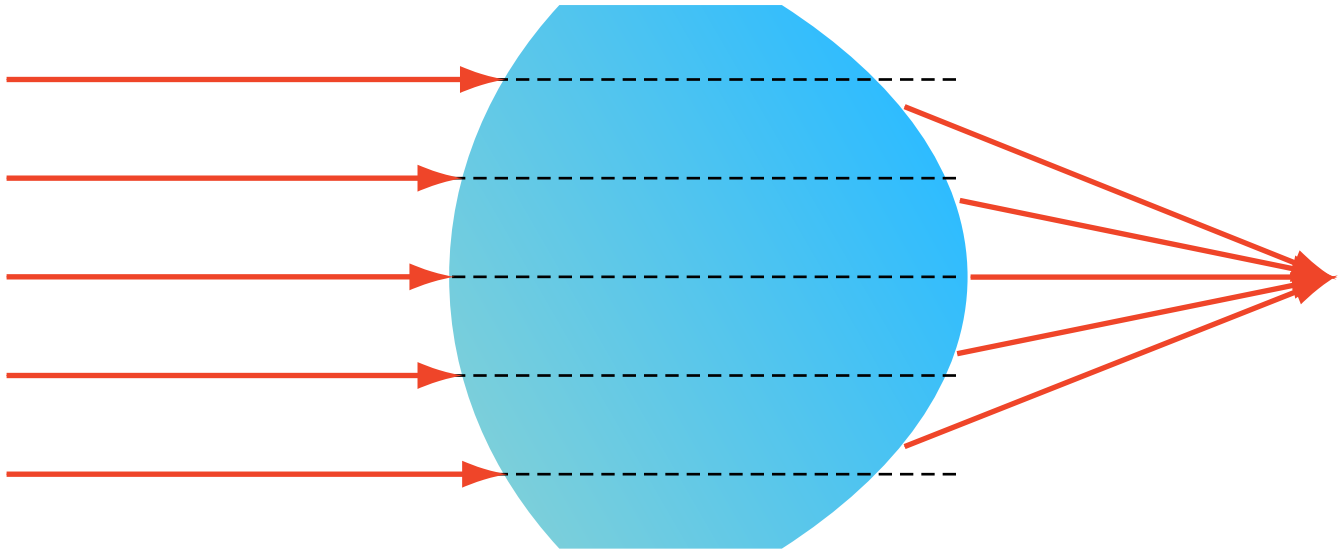
Thick Lenses

Focal length is measured from principal planes



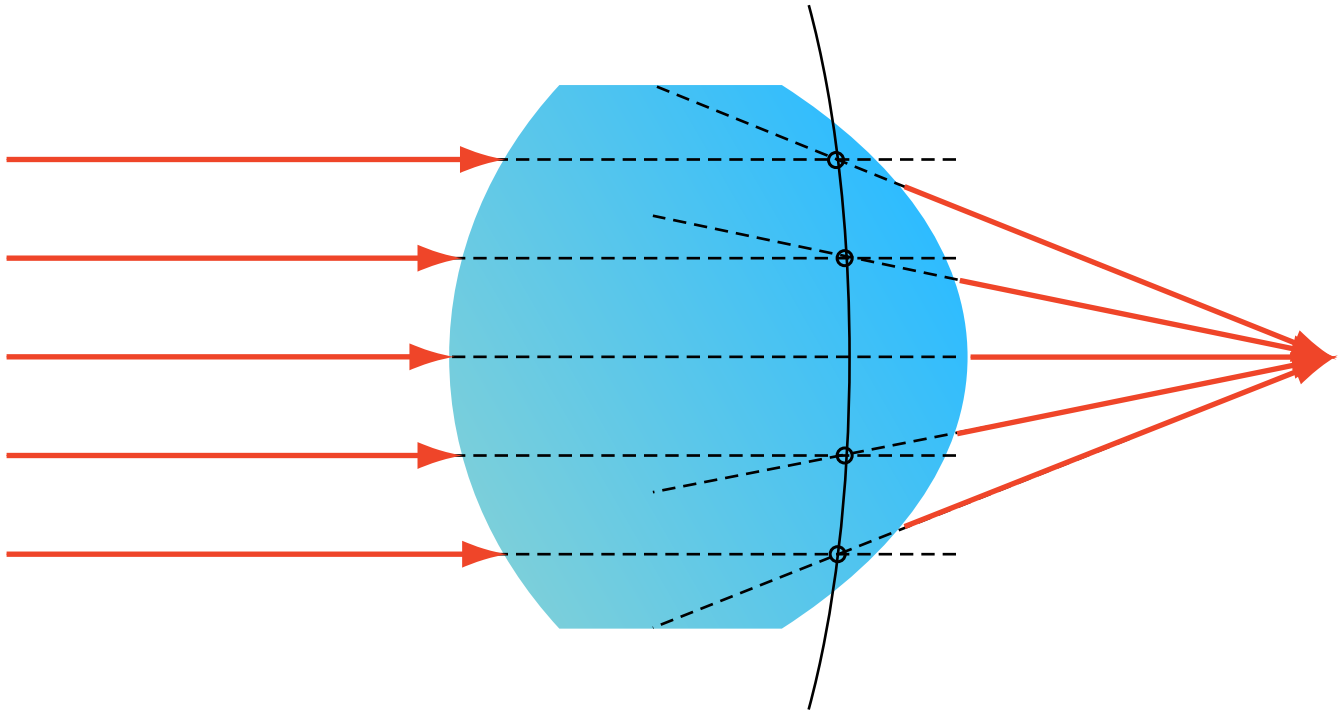
Thick Lenses

Focal length is measured from principal planes



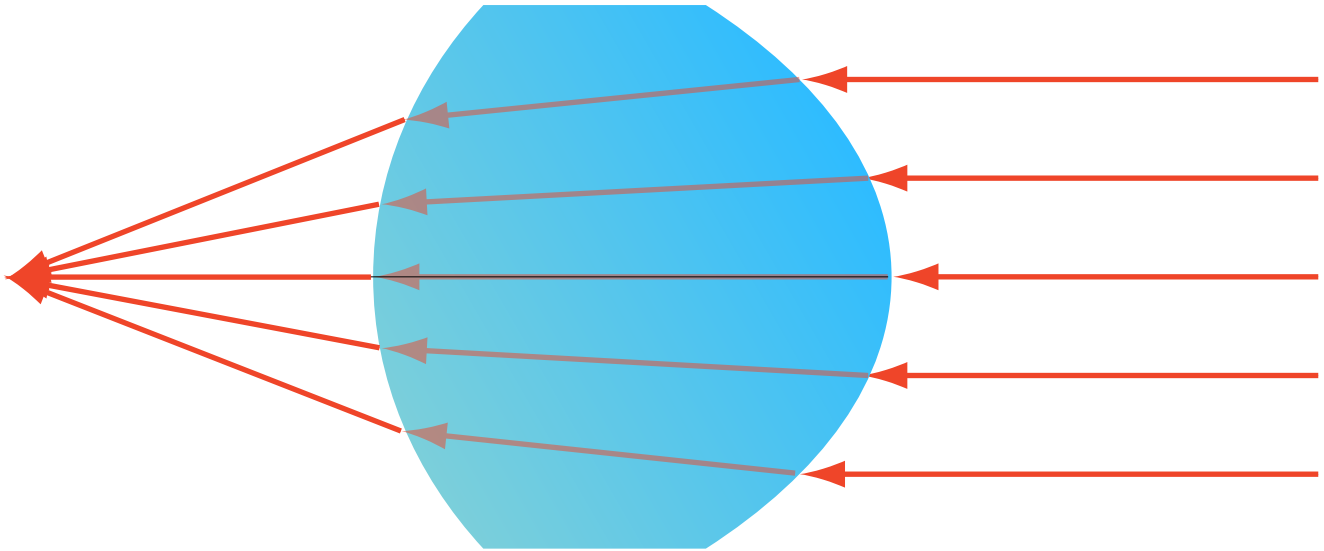
Thick Lenses

Focal length is measured from principal planes



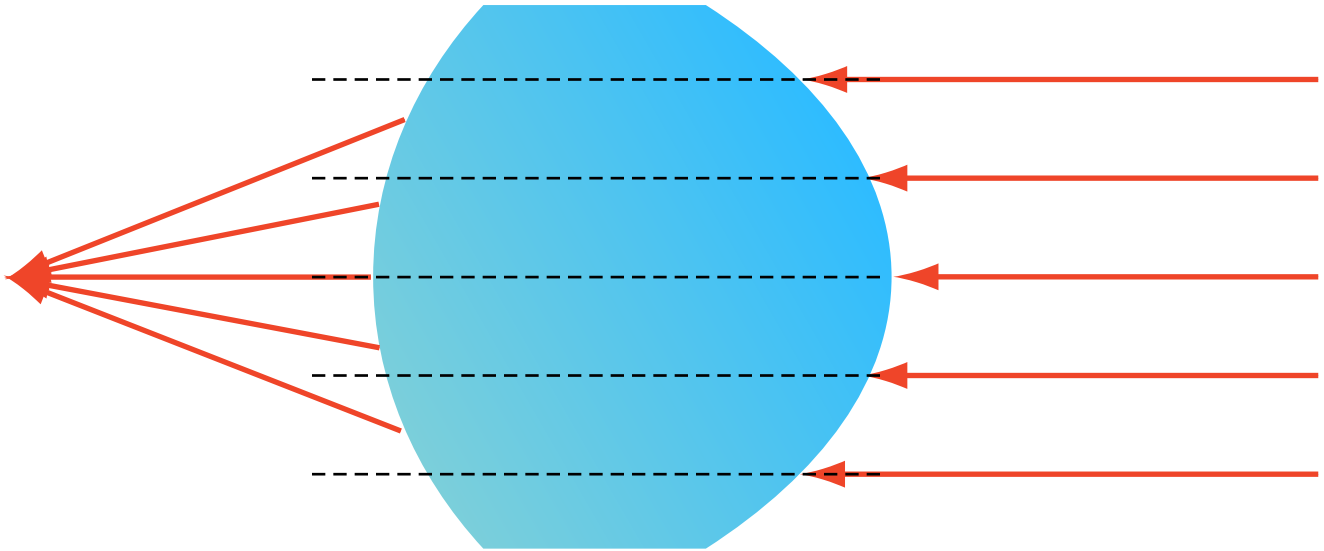
Thick Lenses

Focal length is measured from principal planes



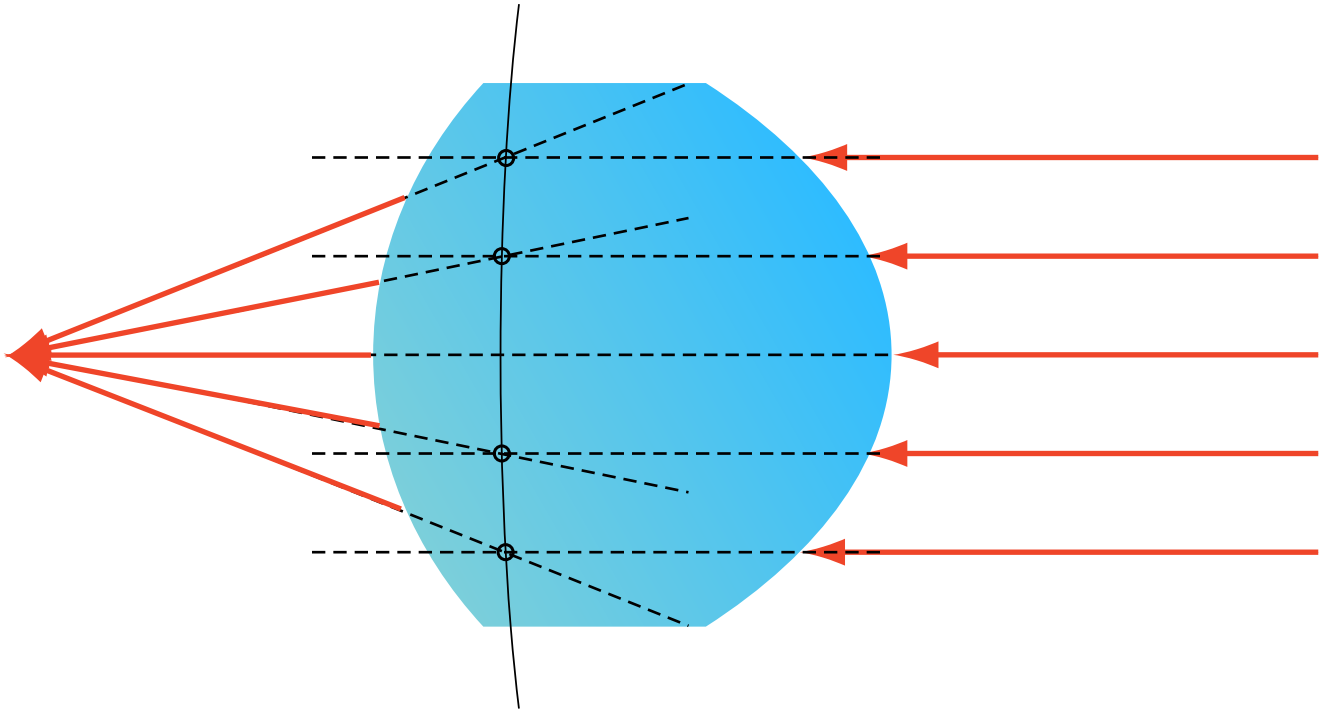
Thick Lenses

Focal length is measured from principal planes



Thick Lenses

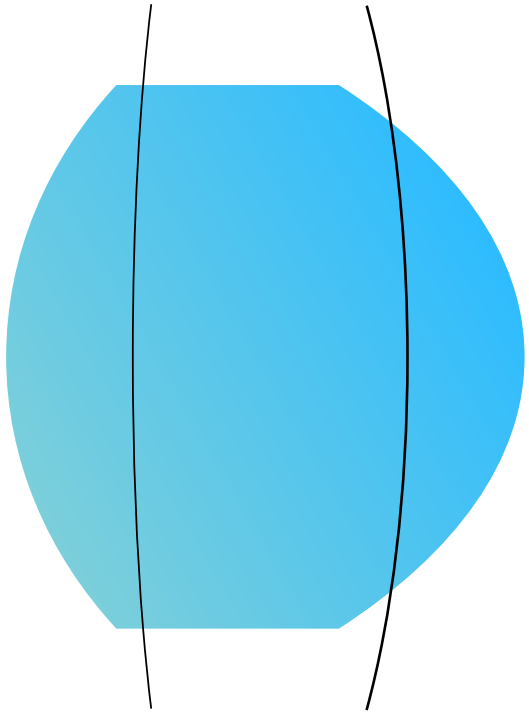
Focal length is measured from principal planes



Front
Principal
Plane

Thick Lenses

Focal length is measured from principal planes



Modern Microscope Components

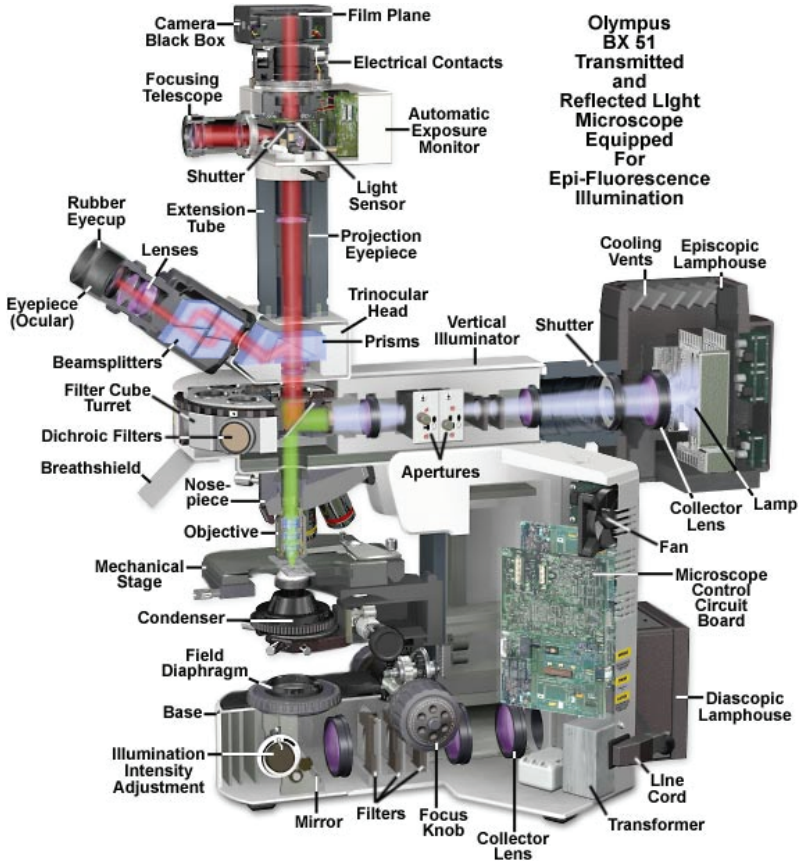


Image from Molecular Expressions webpage

Modern Microscope Components

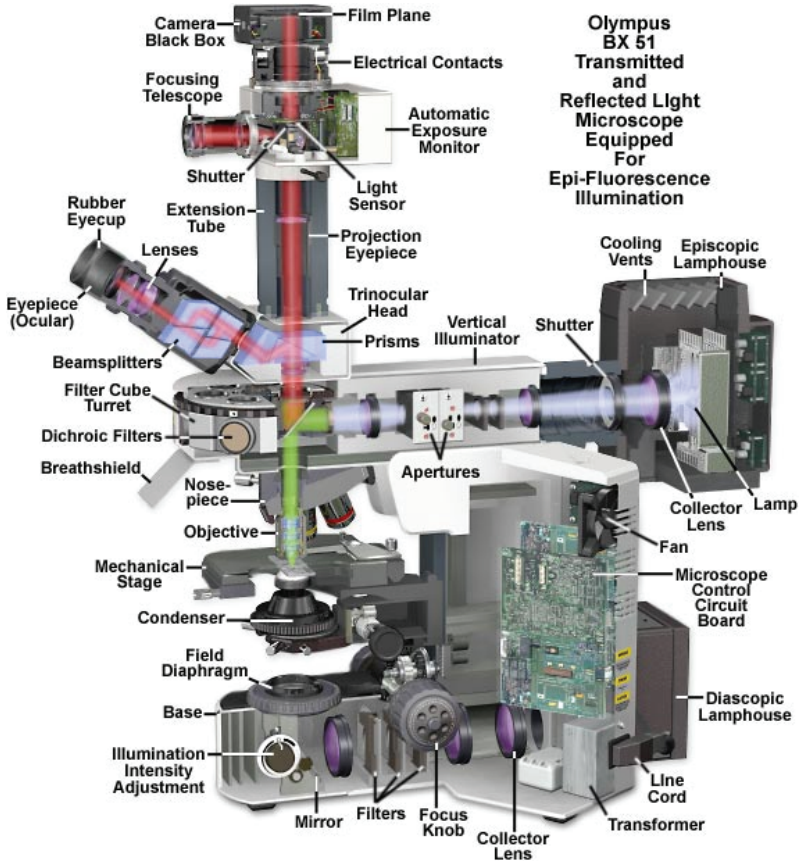


Image from Molecular Expressions webpage

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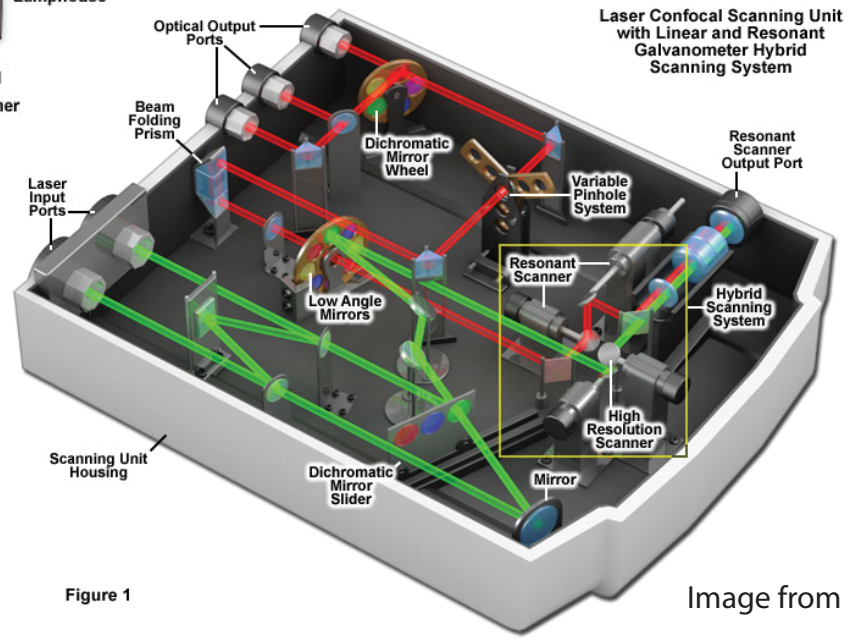


Figure 1

Image from MicroscopyU webpage

Modern Microscope Components

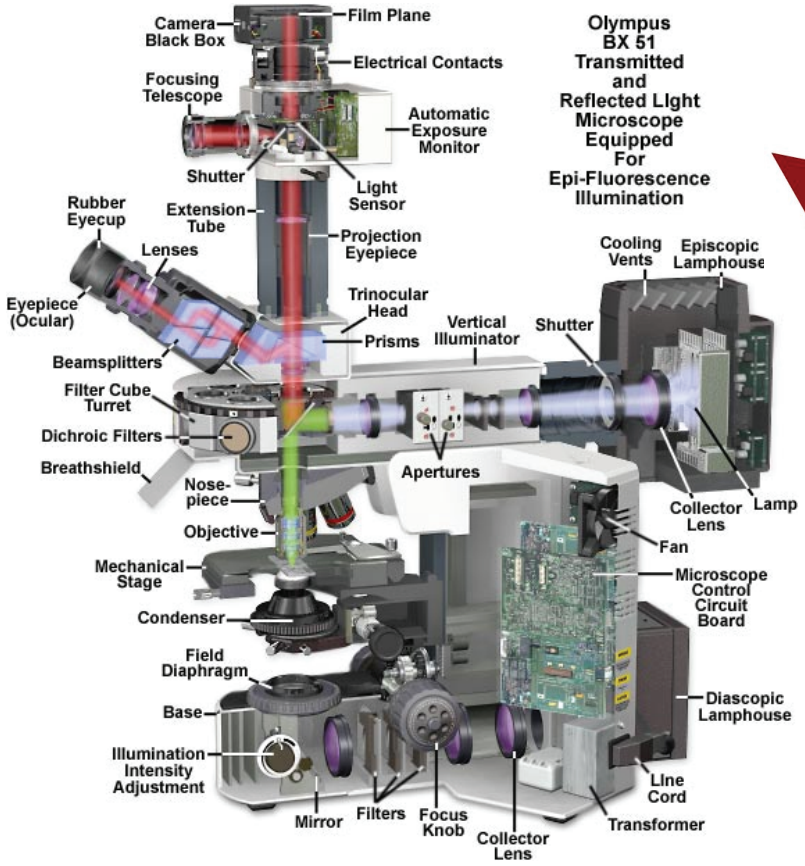
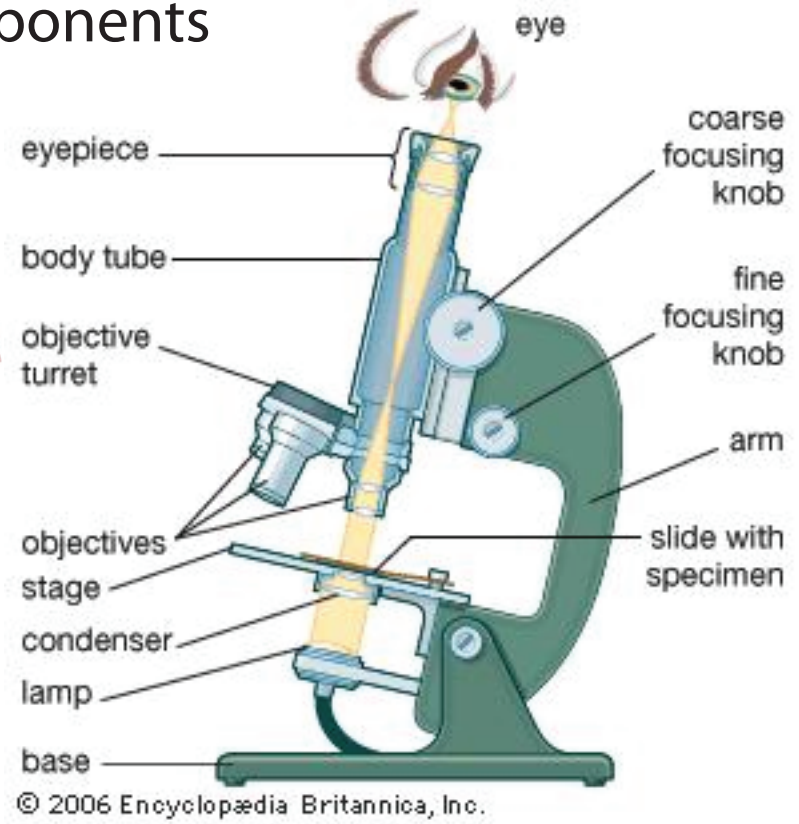


Image from Molecular Expressions webpage



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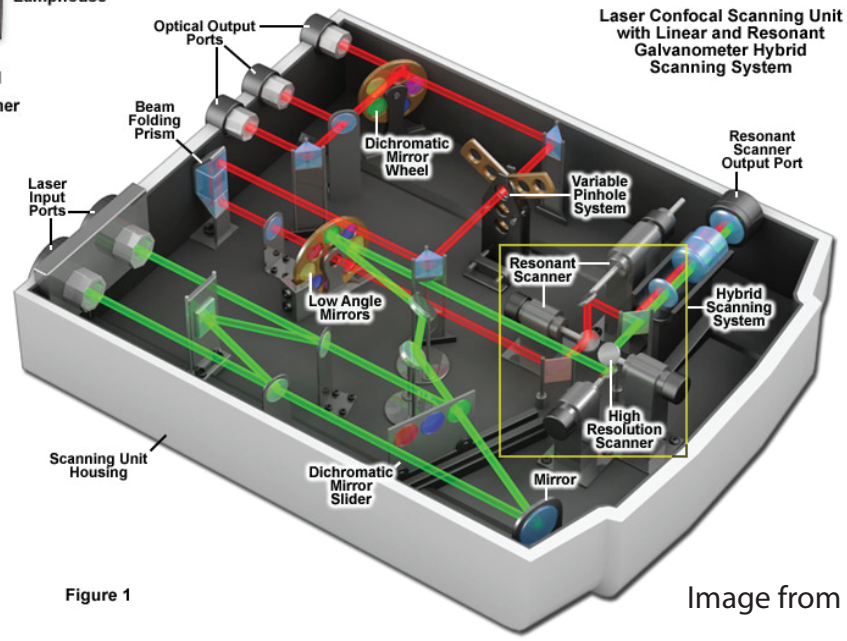
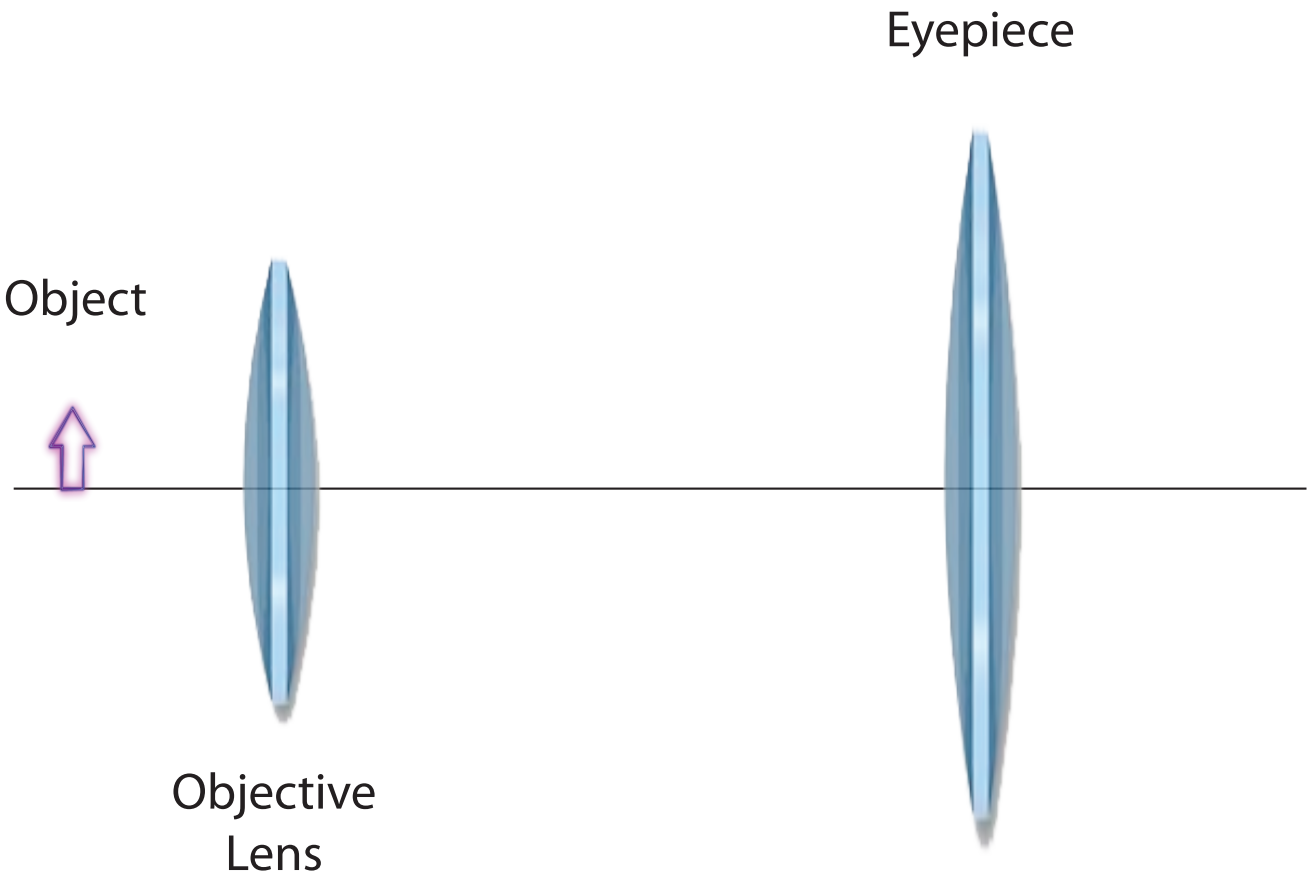


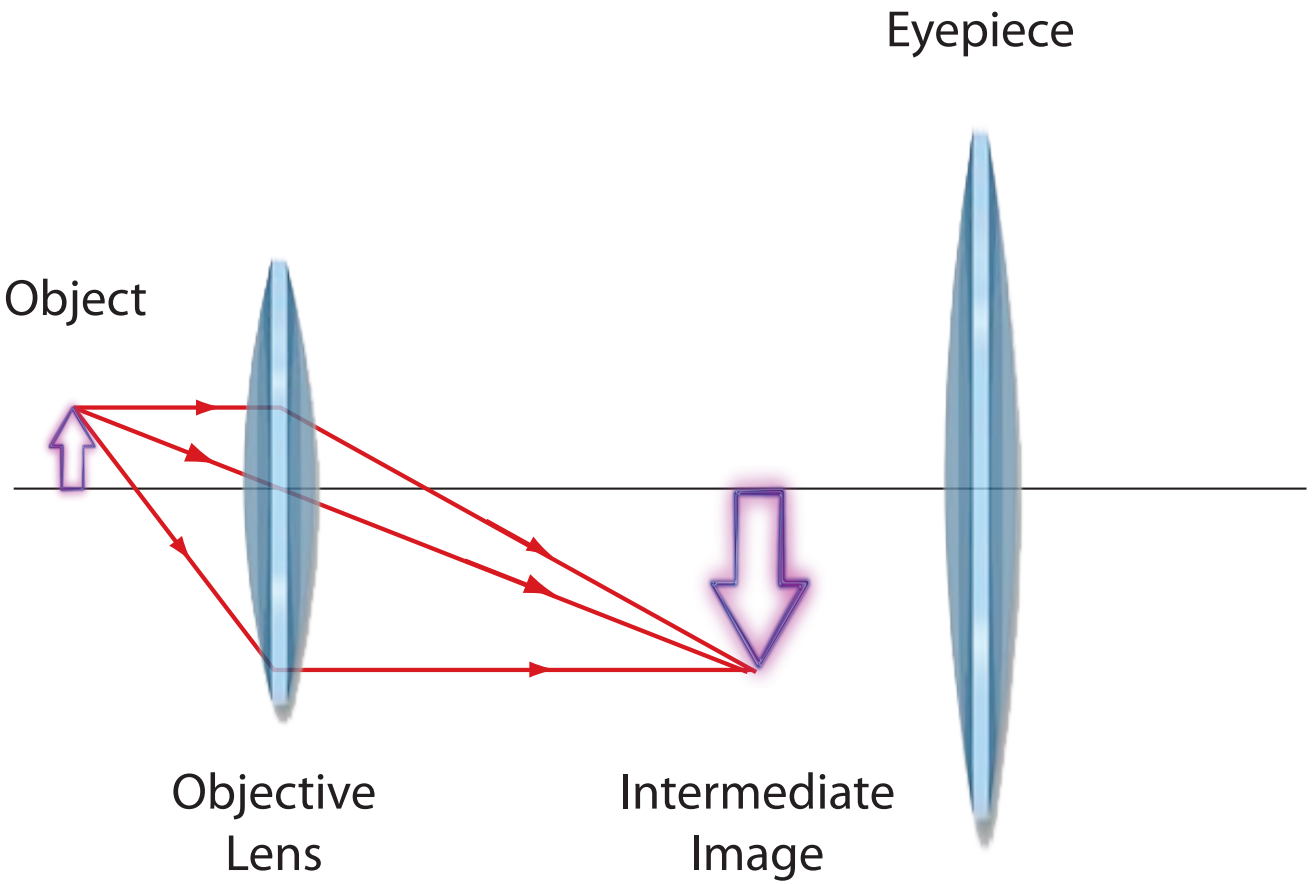
Figure 1

Image from MicroscopyU webpage

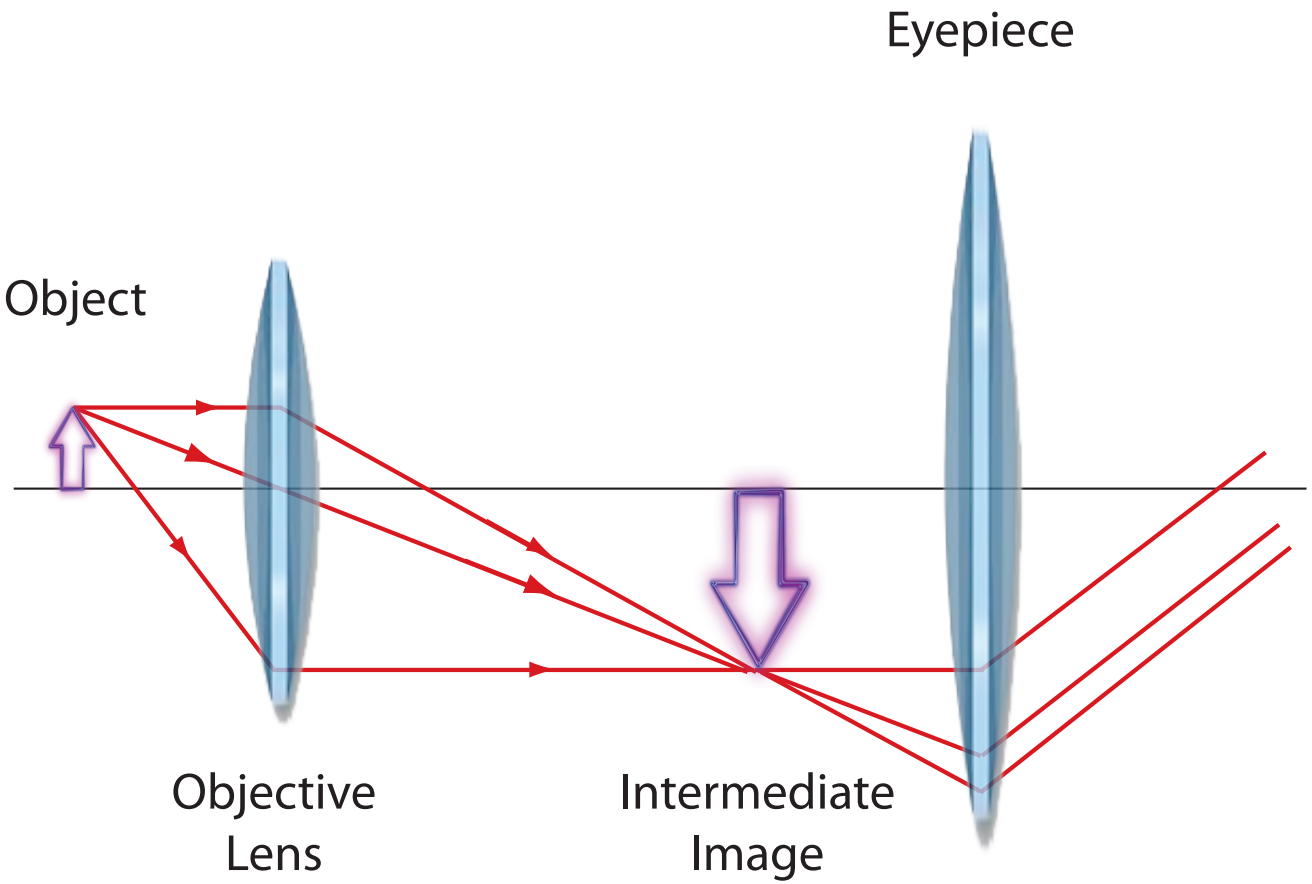
Basic Compound Microscope



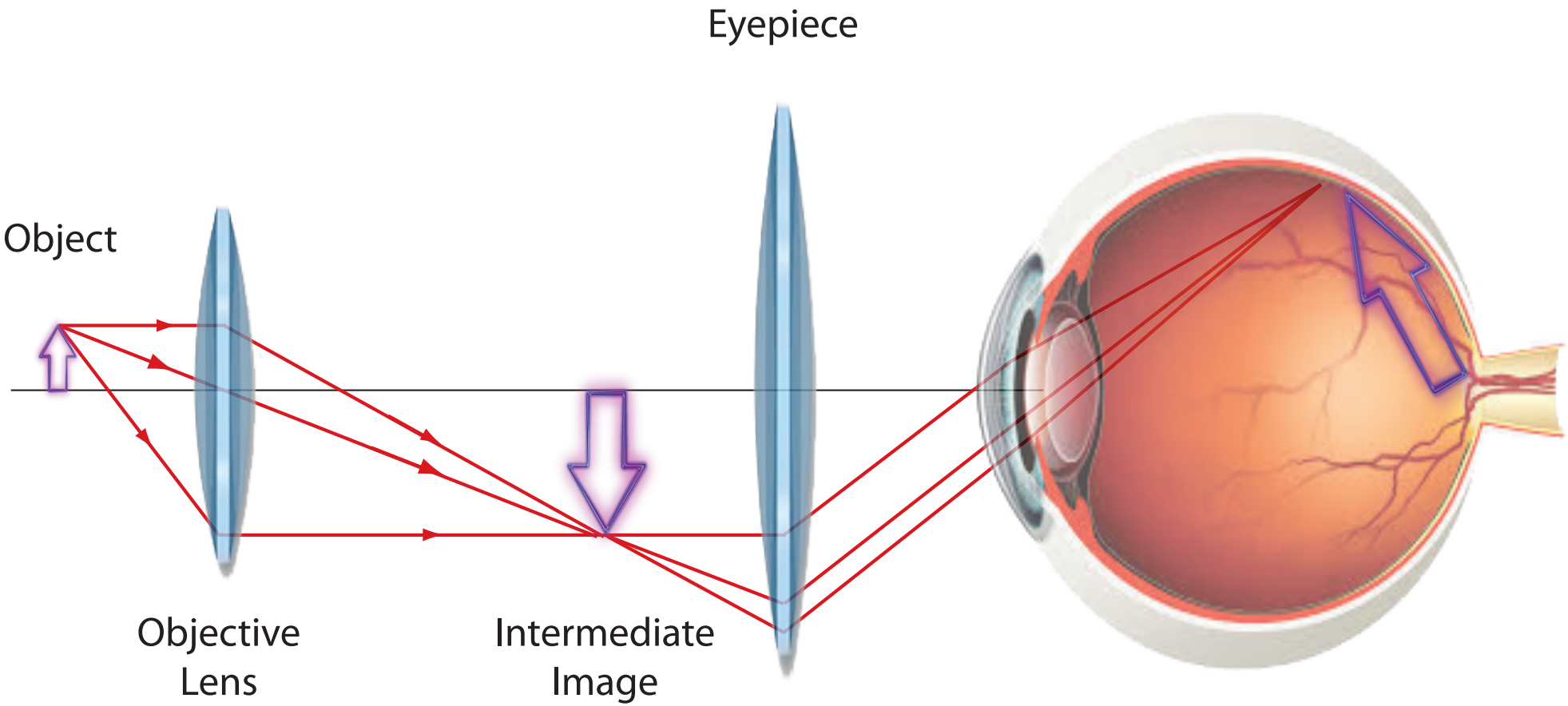
Basic Compound Microscope



Basic Compound Microscope

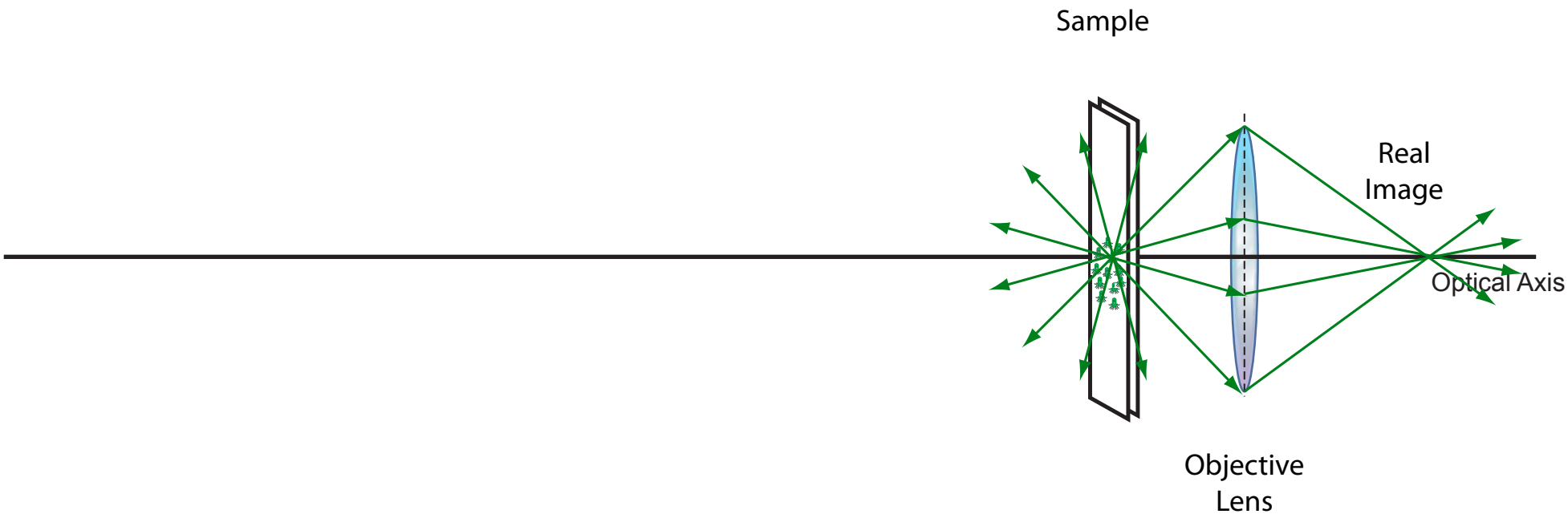


Basic Compound Microscope



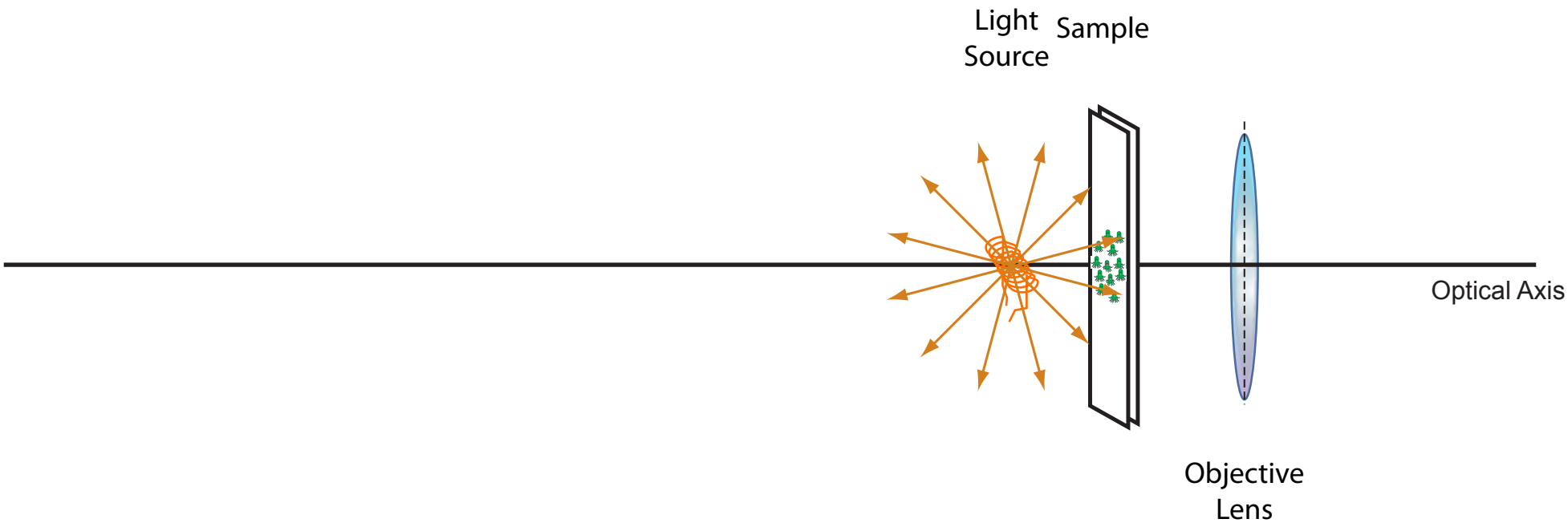
Glowing Sample

(No illumination required)



Non - Glowing Sample

How do we best illuminate the sample?



Advantages :

Bright. Large fraction of light source rays reach the sample.

Large Angles. Sample illuminated with many angles of light.

Disadvantages :

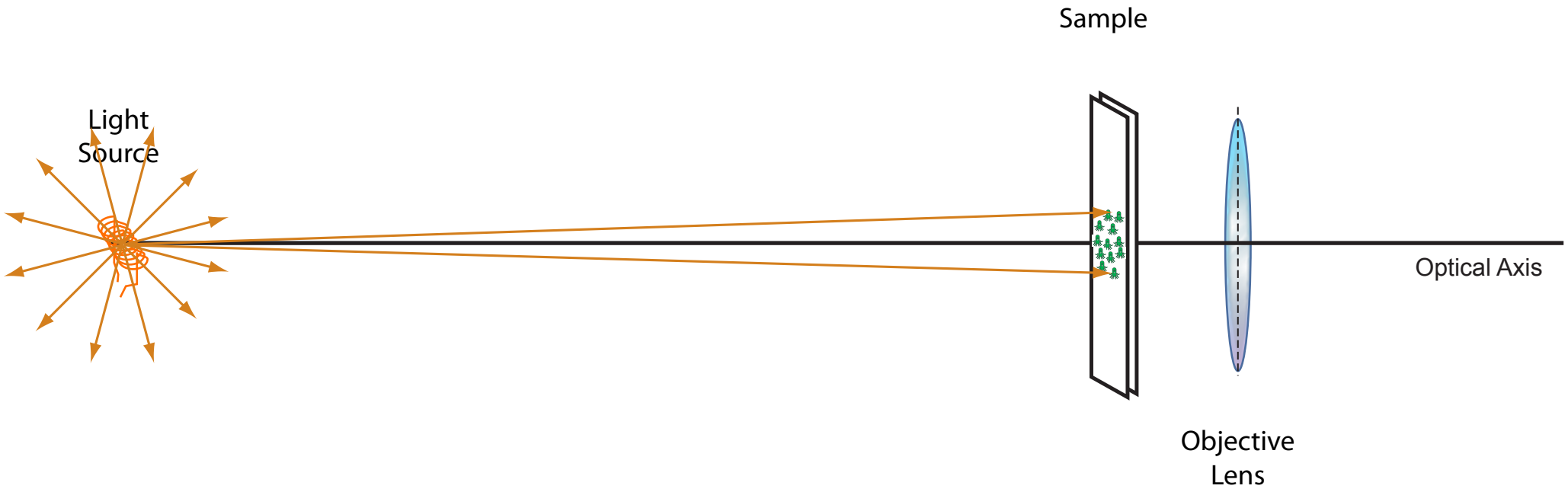
Light source is optically near to sample. Filament structure appears in sample.

No control of the field of illumination.

No control of the angles of illumination.

Non - Glowing Sample

How do we best illuminate the sample?



Advantages :

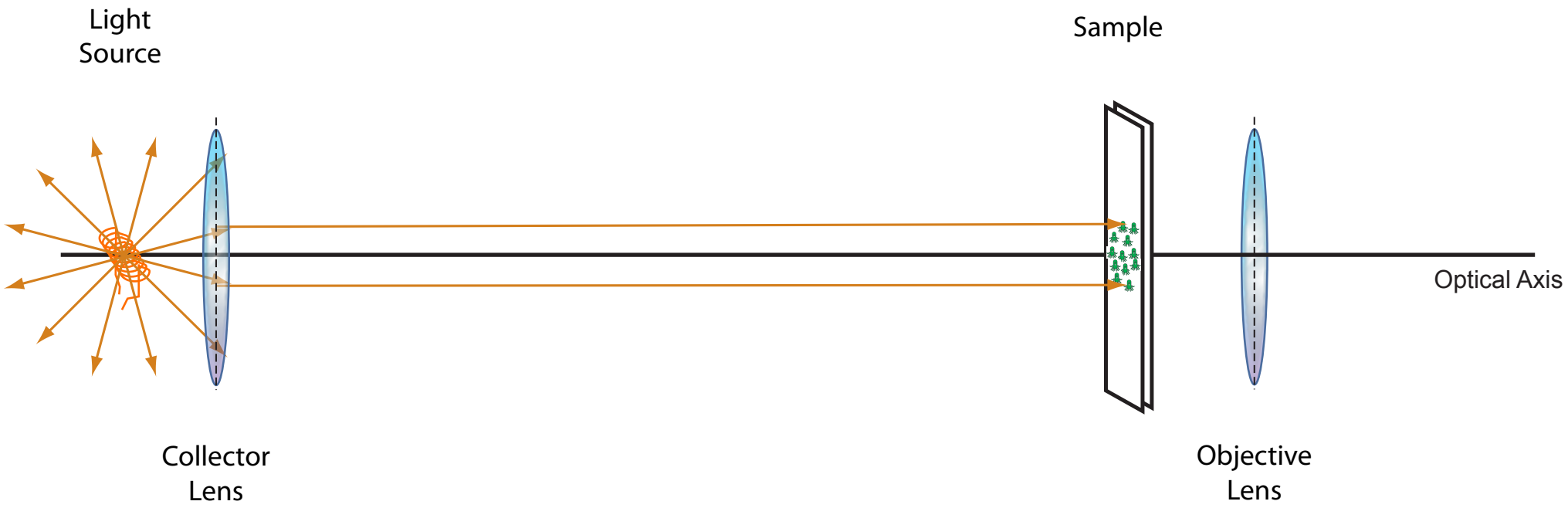
Light source is optically far from sample. Filament structure does not appear in sample.

Disadvantages :

Dim. Only small fraction of light source rays reach the sample.
Small Angles. Sample illuminated with only a few angles of light .
No control of the field of illumination.
No control of the angles of illumination.

Non - Glowing Sample

How do we best illuminate the sample?



Advantages :

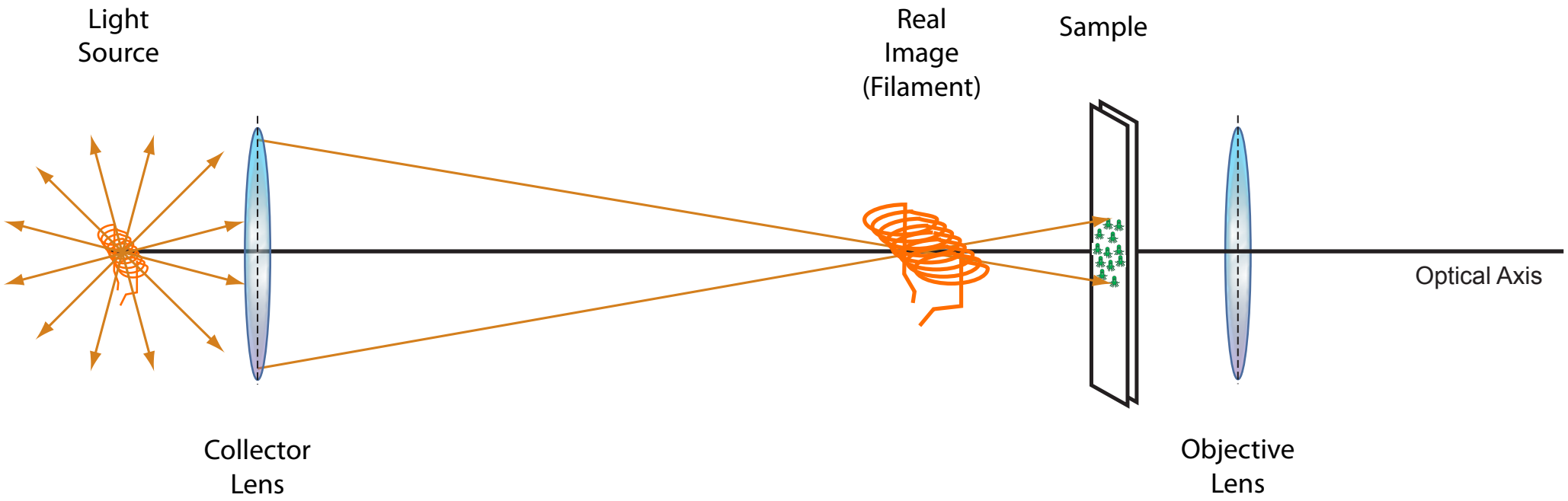
Light source is optically far from sample. Filament structure does not appear in sample.
Bright. Large fraction of light source rays reach the sample.

Disadvantages :

Small Angles. Sample illuminated with only a few angles of light .
No control of the field of illumination.
No control of the angles of illumination.

Non - Glowing Sample

How do we best illuminate the sample?



Advantages :

Bright. Large fraction of light source rays reach the sample.

Large Angles. Sample illuminated with many angles of light.

Have control of the angles of illumination.

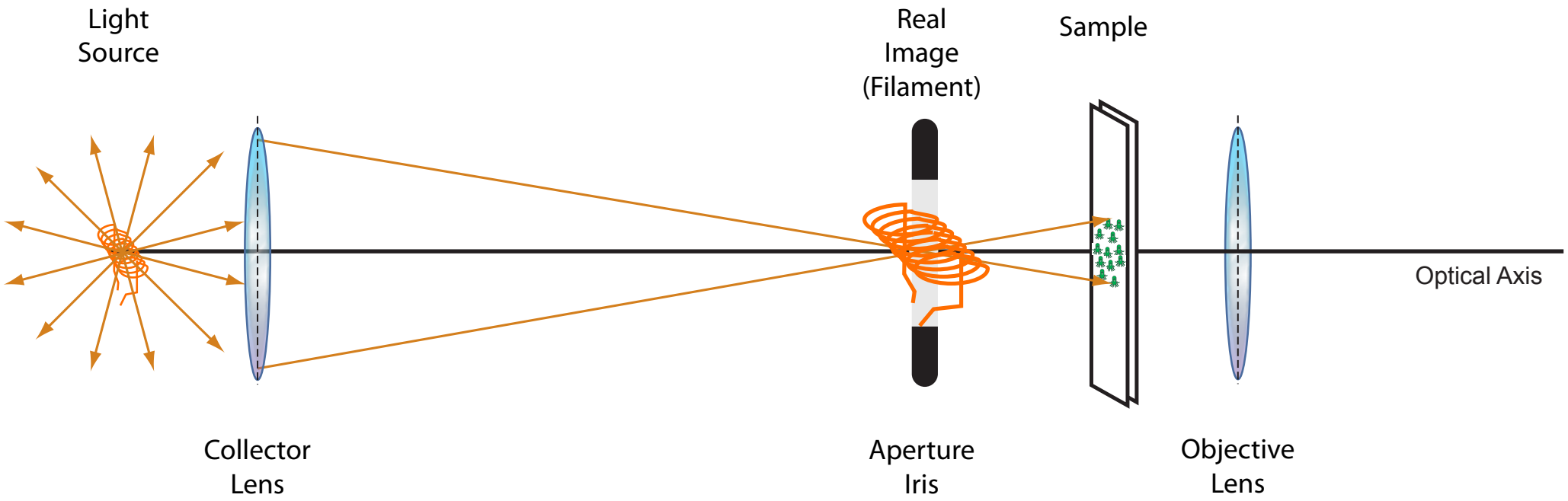
Disadvantages :

Light source is optically near to sample. Filament structure appears in sample.

No control of the field of illumination.

Non - Glowing Sample

How do we best illuminate the sample?



Advantages :

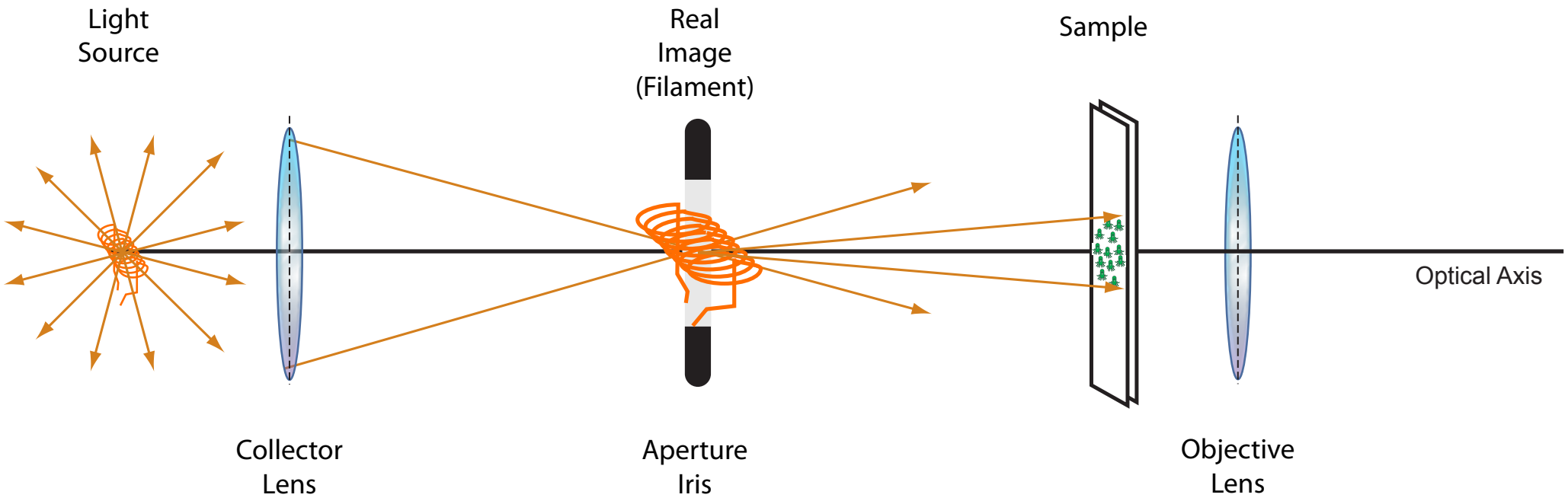
Bright. Large fraction of light source rays reach the sample.
Large Angles. Sample illuminated with many angles of light.
Have control of the angles of illumination.

Disadvantages :

Light source is optically near to sample. Filament structure appears in sample.
No control of the field of illumination.

Non - Glowing Sample

How do we best illuminate the sample?



Advantages :

Small Angles. Sample illuminated with only a few angles of light.

Light source is semi-far from sample. Filament structure does not appear in sample (much).

Have control of angles of illumination.

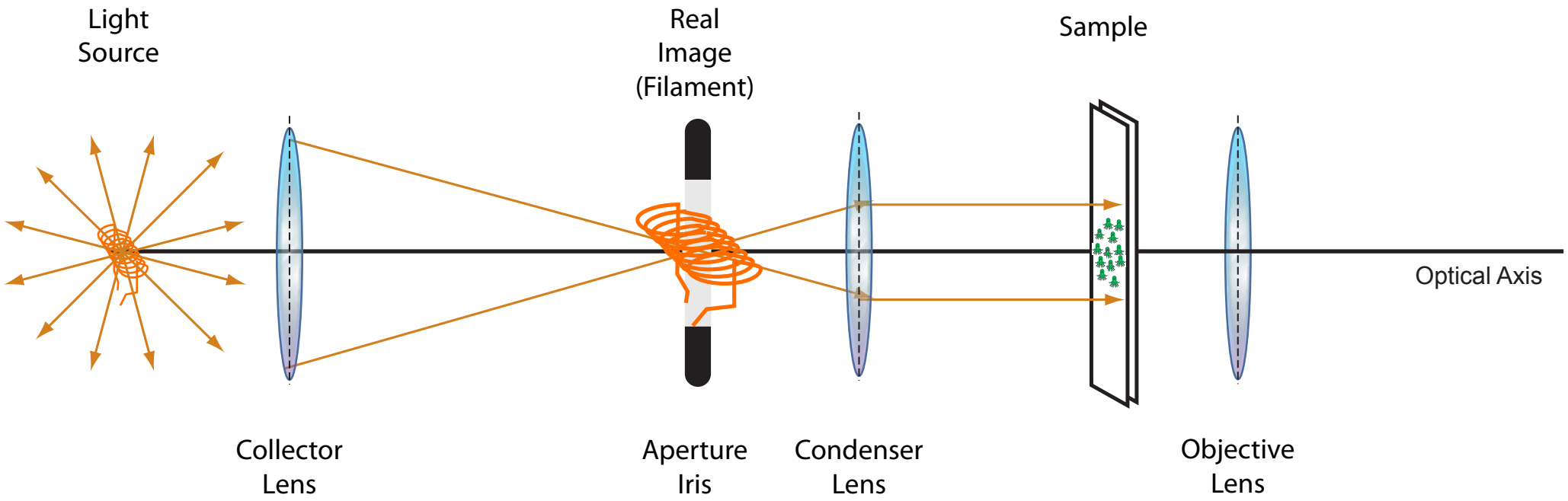
Disadvantages :

Dim. Small fraction of light source rays reach the sample.

No control of field of illumination.

Non - Glowing Sample

How do we best illuminate the sample?



Advantages :

Bright. Large fraction of light source rays reach the sample.

Large Angles. Sample illuminated with many angles of light.

Light source is optically far from sample. Filament structure does not appear in sample.

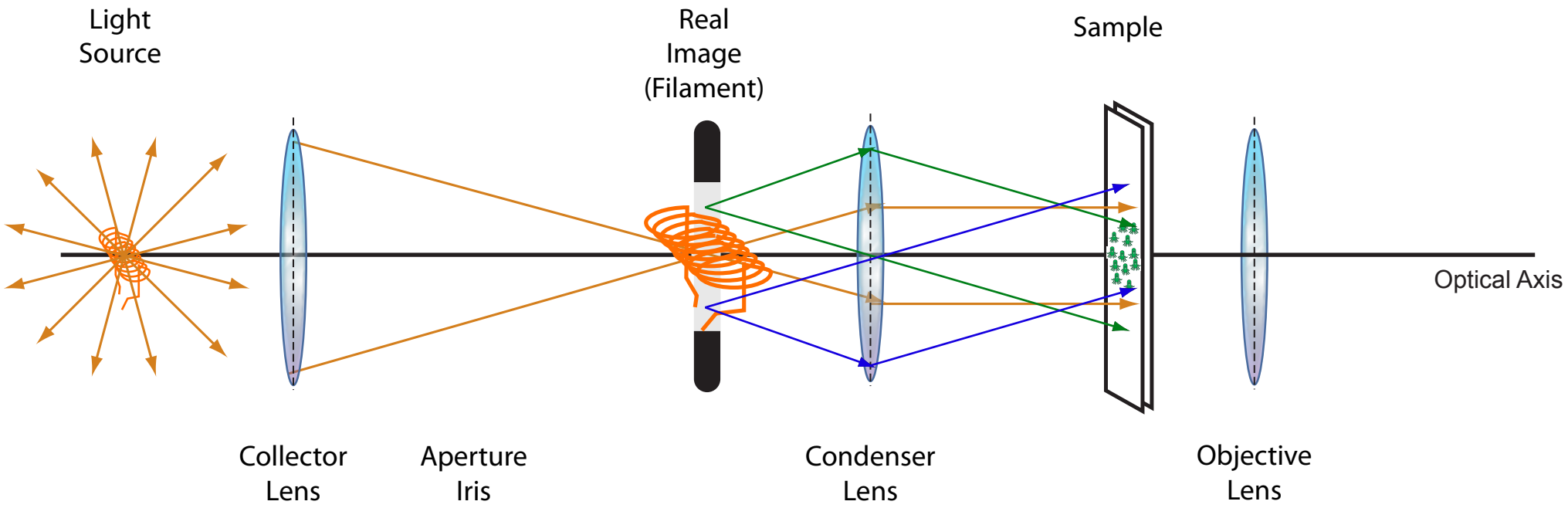
Have control of the angles of illumination.

Disadvantages :

No control of the field of illumination.

Non - Glowing Sample

How do we best illuminate the sample?



Advantages :

Bright. Large fraction of light source rays reach the sample.

Large Angles. Sample illuminated with many angles of light.

Light source is optically far from sample. Filament structure does not appear in sample.

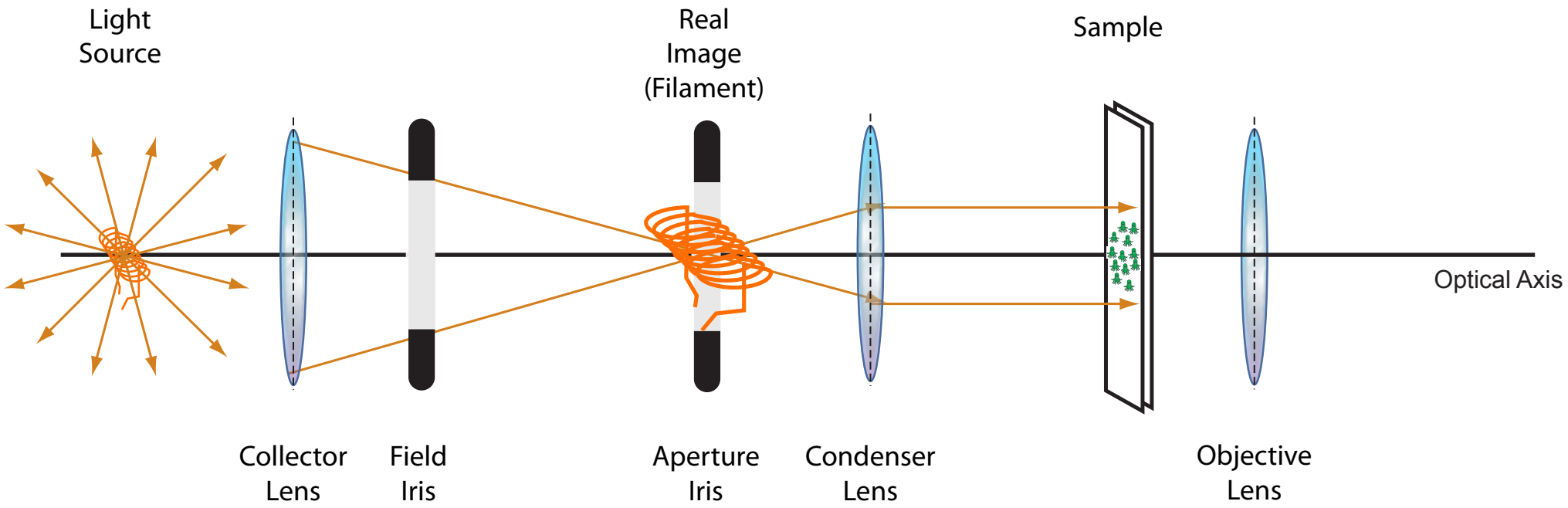
Have control of the angles of illumination.

Disadvantages :

No control of the field of illumination.

Non - Glowing Sample

How do we best illuminate the sample?



Advantages :

Bright. Large fraction of light source rays reach the sample.

Large Angles. Sample illuminated with many angles of light.

Light source is optically far from sample. Filament structure does not appear in sample.

Have control of the angles of illumination.

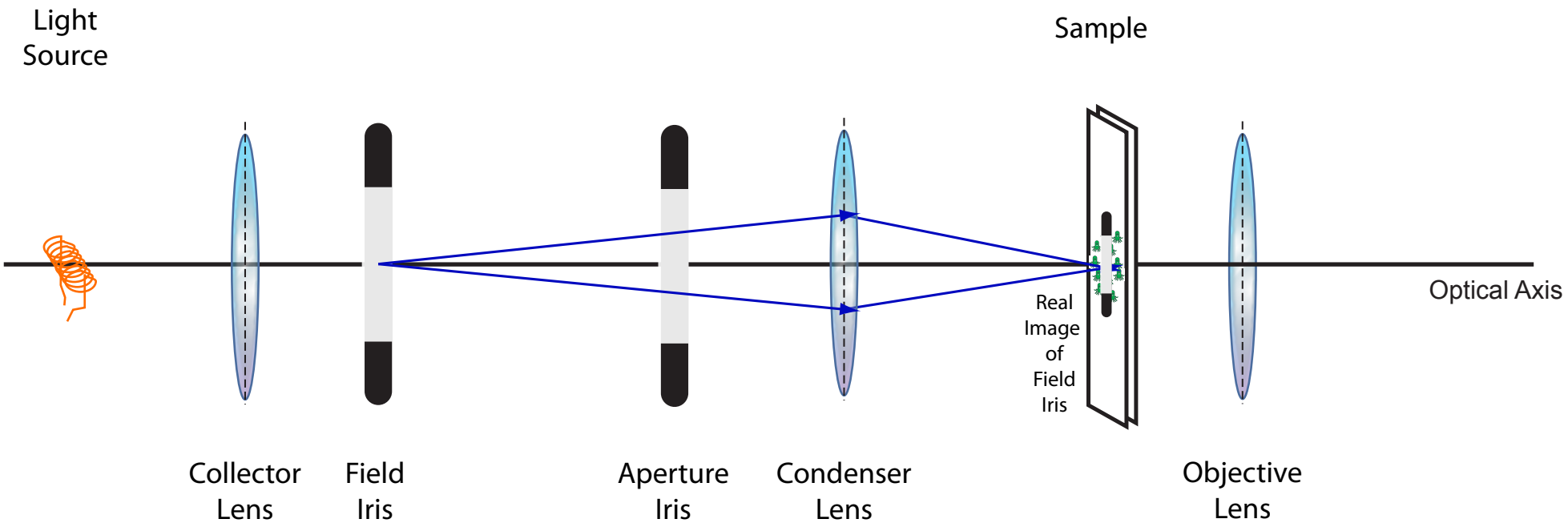
Have control of the field of illumination

Disadvantages :

None !

Kohler Illumination

How do we best illuminate the sample?



Advantages :

Bright. Large fraction of light source rays reach the sample.

Large Angles. Sample illuminated with many angles of light.

Light source is optically far from sample. Filament structure does not appear in sample.

Have control of the angles of illumination.

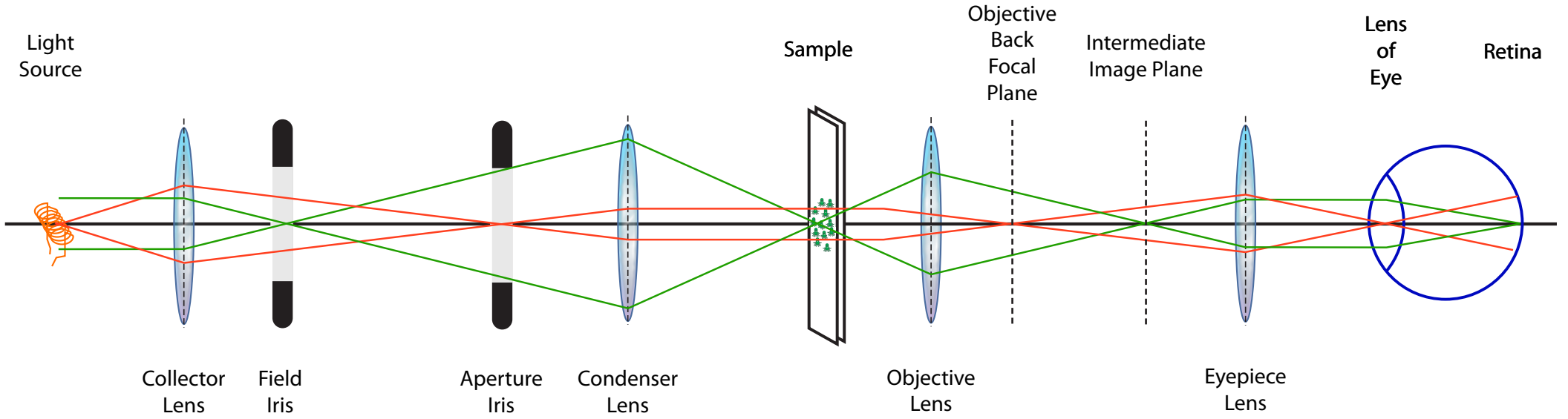
Have control of the field of illumination

Disadvantages :

None !

Kohler Illumination

Dual Light Paths



Illumination Conjugate Planes

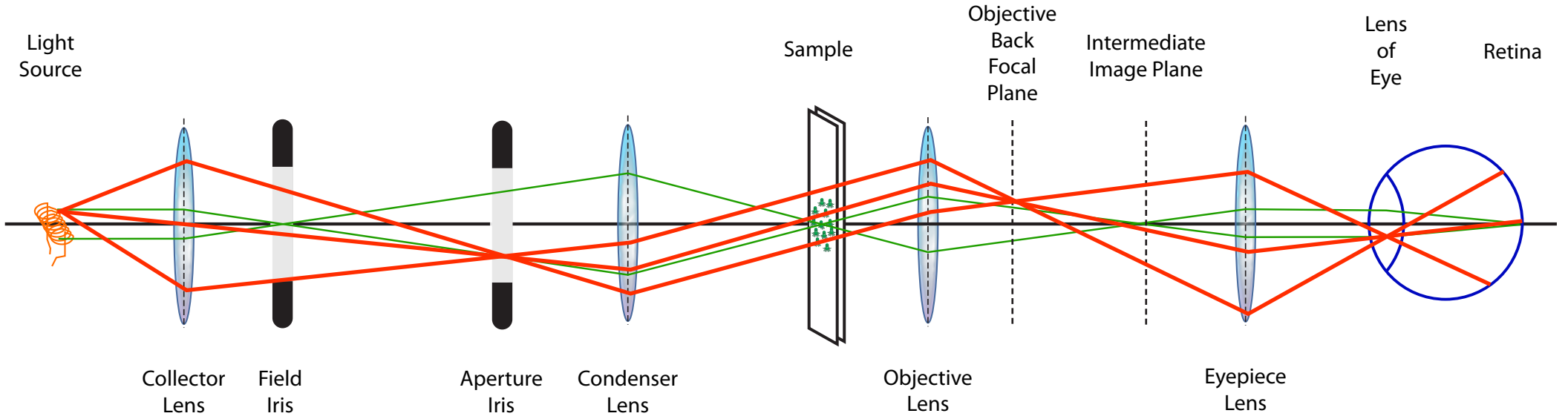
Light Source
Aperture Iris
Back focal plane of objective
Front lens of eye

Sample Image Conjugate Planes

Field Iris
Sample plane
Intermediate image plane
Retina

Kohler Illumination

Off-Axis Illumination Path



Illumination Conjugate Planes

Light Source

Aperture Iris

Back focal plane of objective

Front lens of eye

Sample Image Conjugate Planes

Field Iris

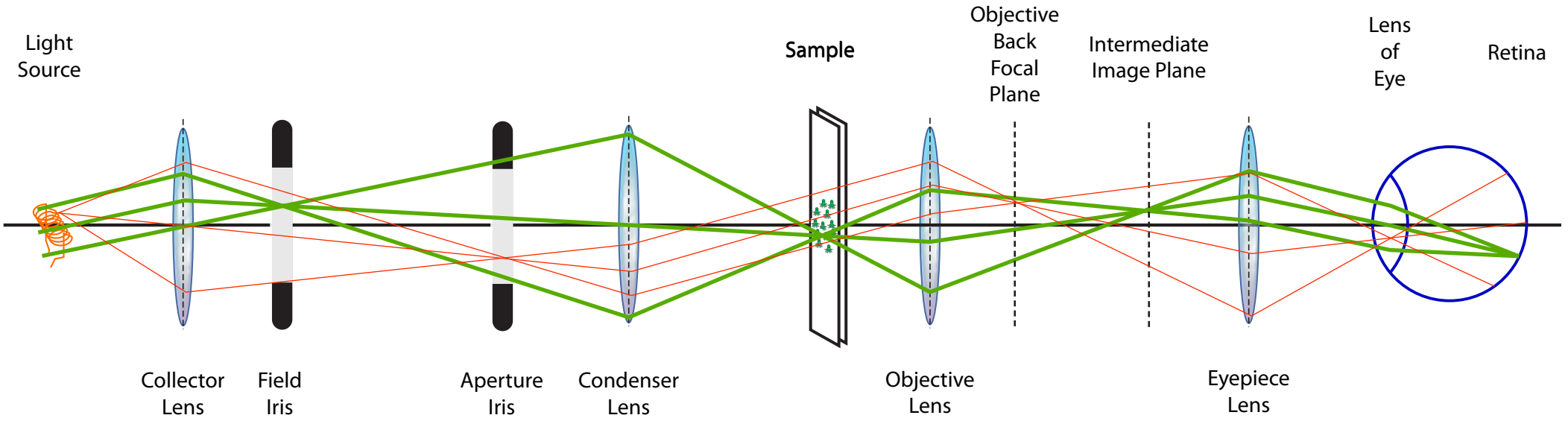
Sample plane

Intermediate image plane

Retina

Kohler Illumination

Off-Axis Sample Image Path



Illumination Conjugate Planes

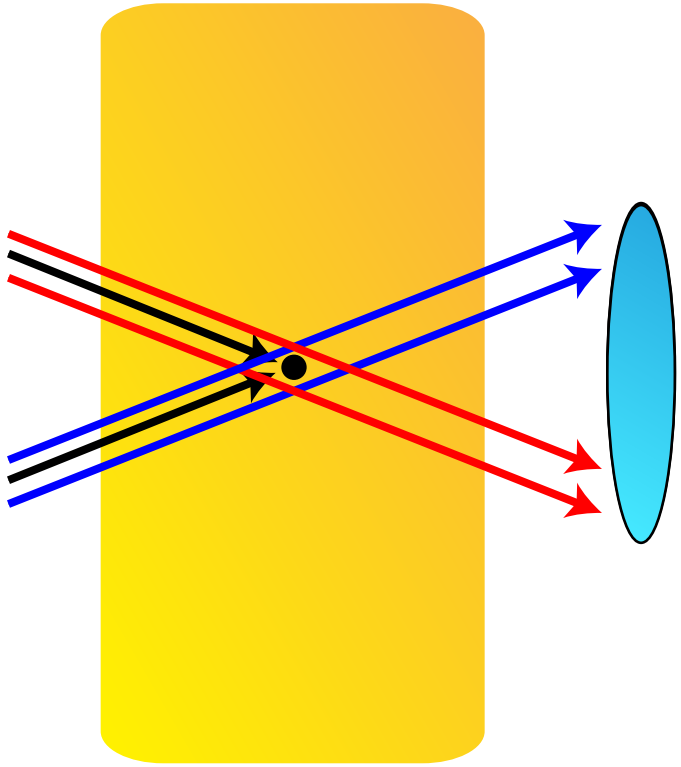
Light Source
Aperture Iris
Back focal plane of objective
Front lens of eye

Sample Image Conjugate Planes

Field Iris
Sample plane
Intermediate image plane
Retina

Kohler Illumination

Why is control of the illumination angles important ?

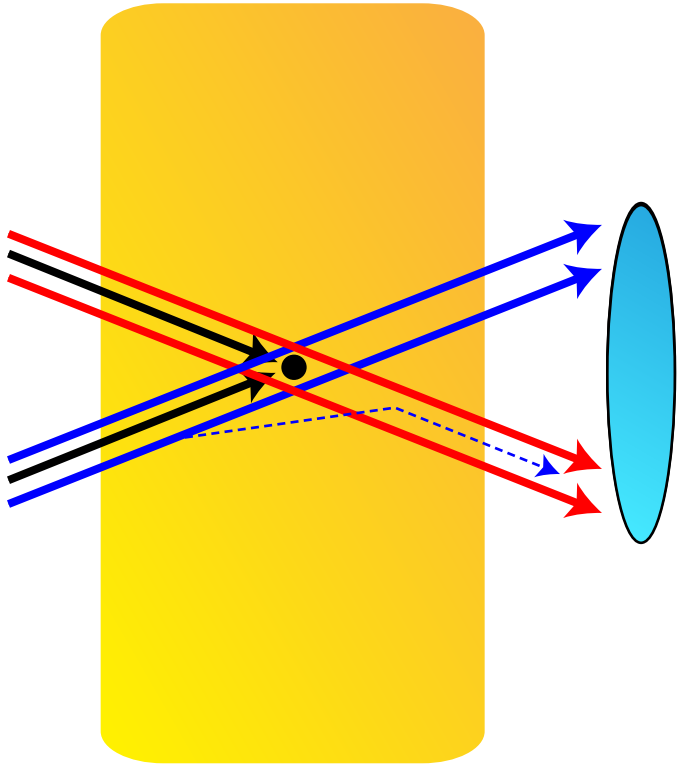


Scattering Sample

Objective Lens

Kohler Illumination

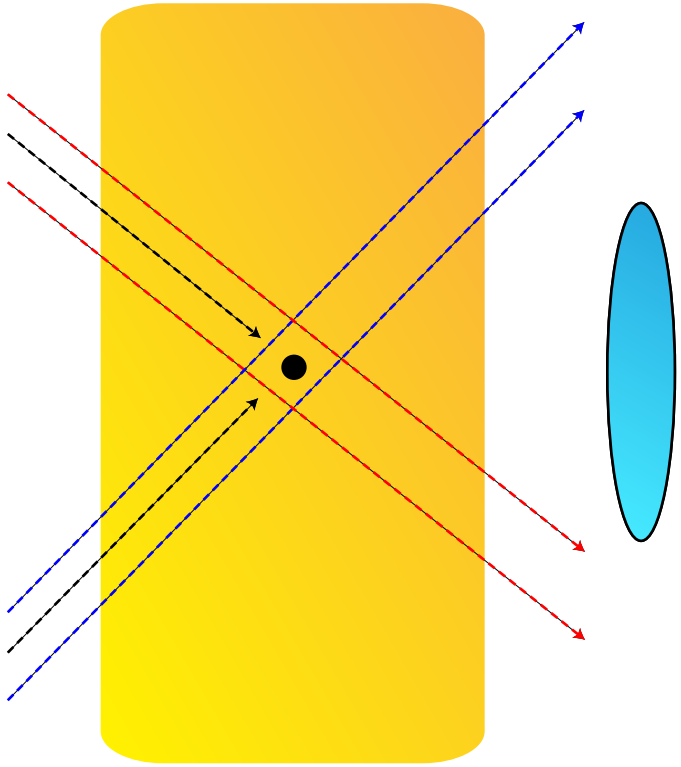
Why is control of the illumination angles important ?



Scattering can reduce contrast.
Light from the “bright” path scatters and appears to originate from the dark object.

Kohler Illumination

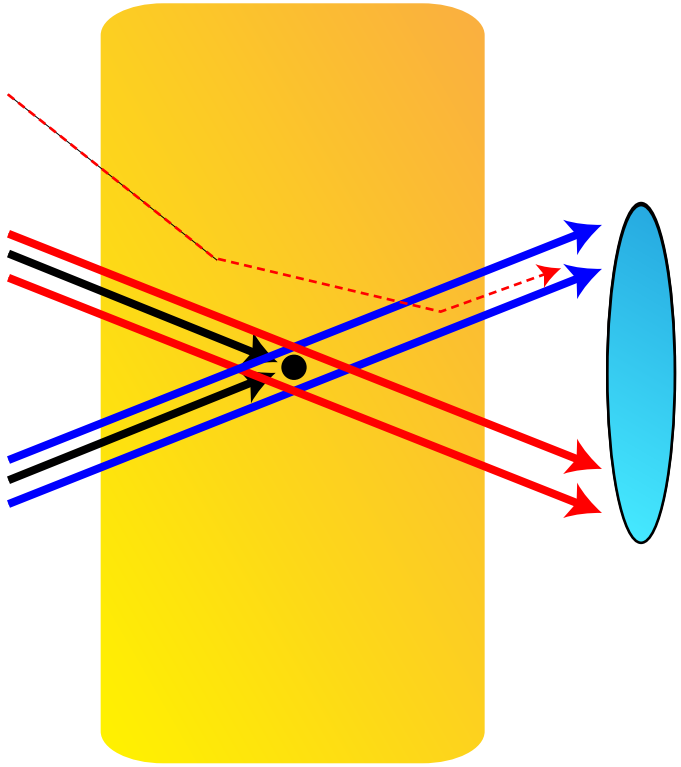
Why is control of the illumination angles important ?



Incoming light at too large of angles does not contribute to image formation.

Kohler Illumination

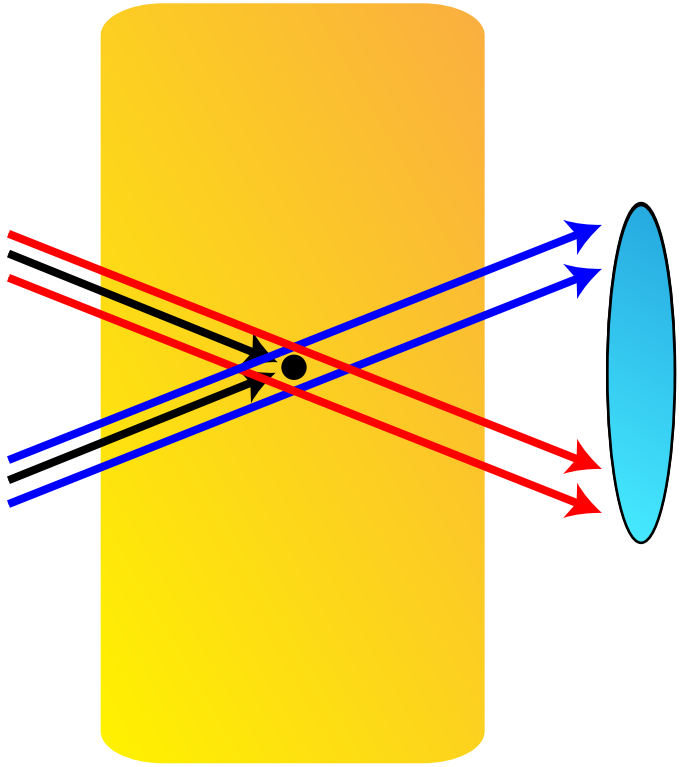
Why is control of the illumination angles important ?



Scattering can reduce contrast.
Light from a “bright path” at larger angles scatters and appears to originate from the dark object.

Kohler Illumination

Why is control of the illumination field important ?

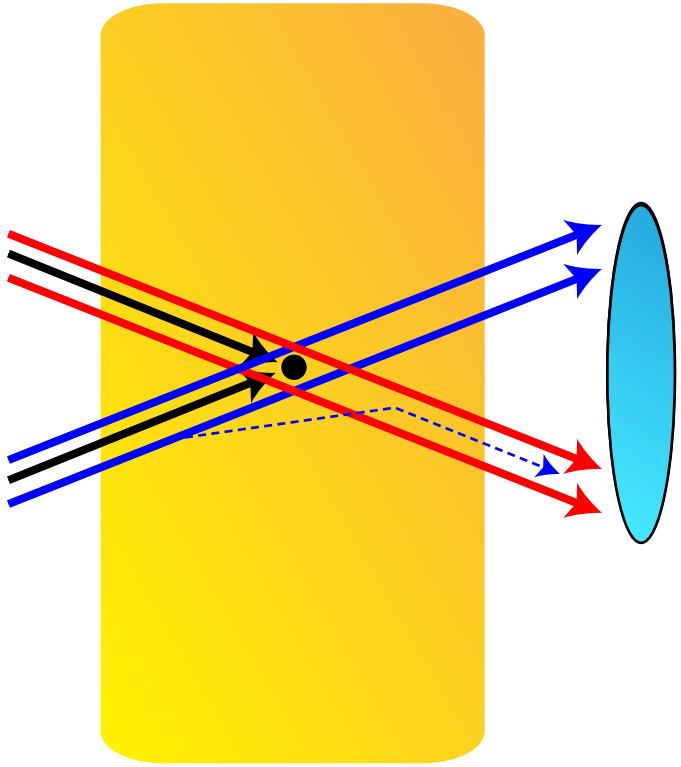


Scattering Sample

Objective Lens

Kohler Illumination

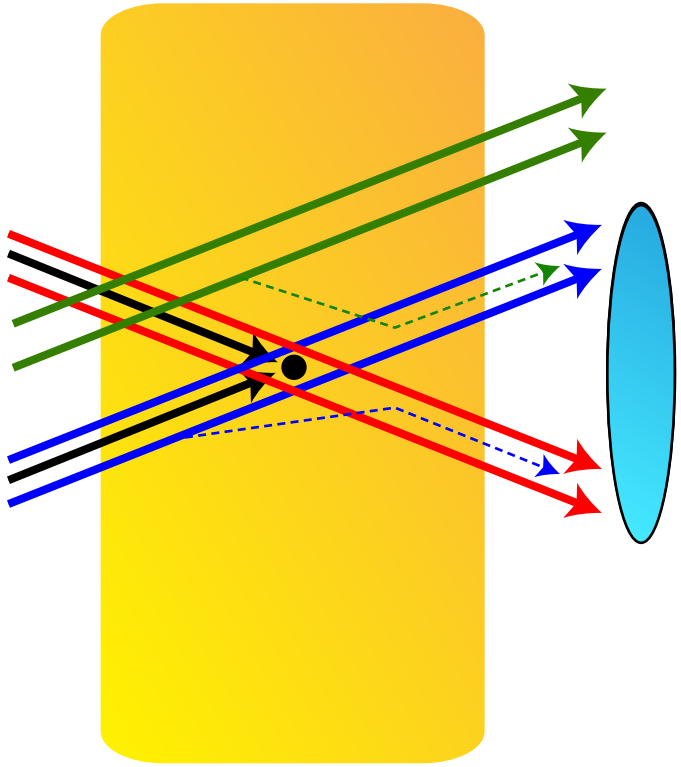
Why is control of the illumination field important ?



Scattering can reduce contrast.
Light from the “bright” path scatters and appears to originate from the dark object.

Kohler Illumination

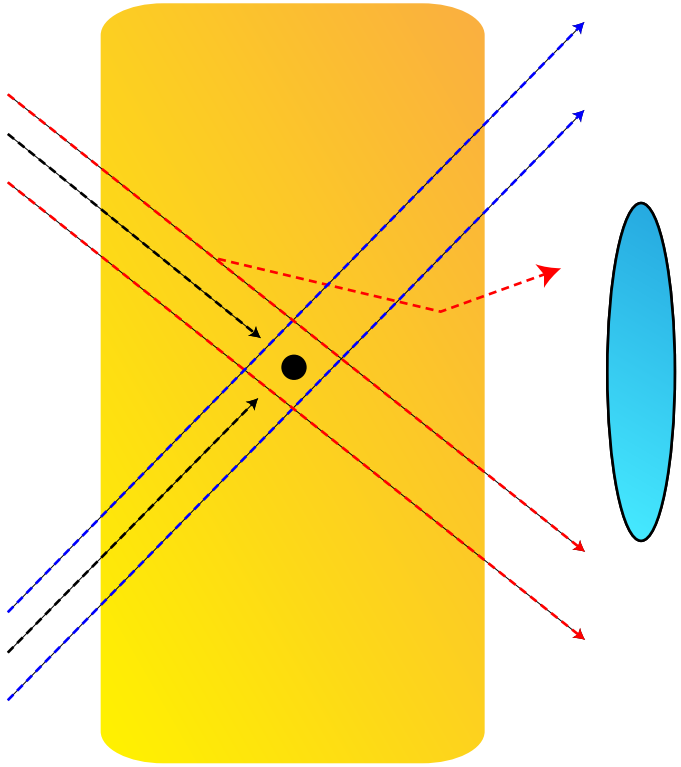
Why is control of the illumination field important ?



Illuminating too large of a field can reduce image contrast.
Light from outside the field of interest can scatter into the field.

Kohler Illumination

Why is control of the illumination angles important ?

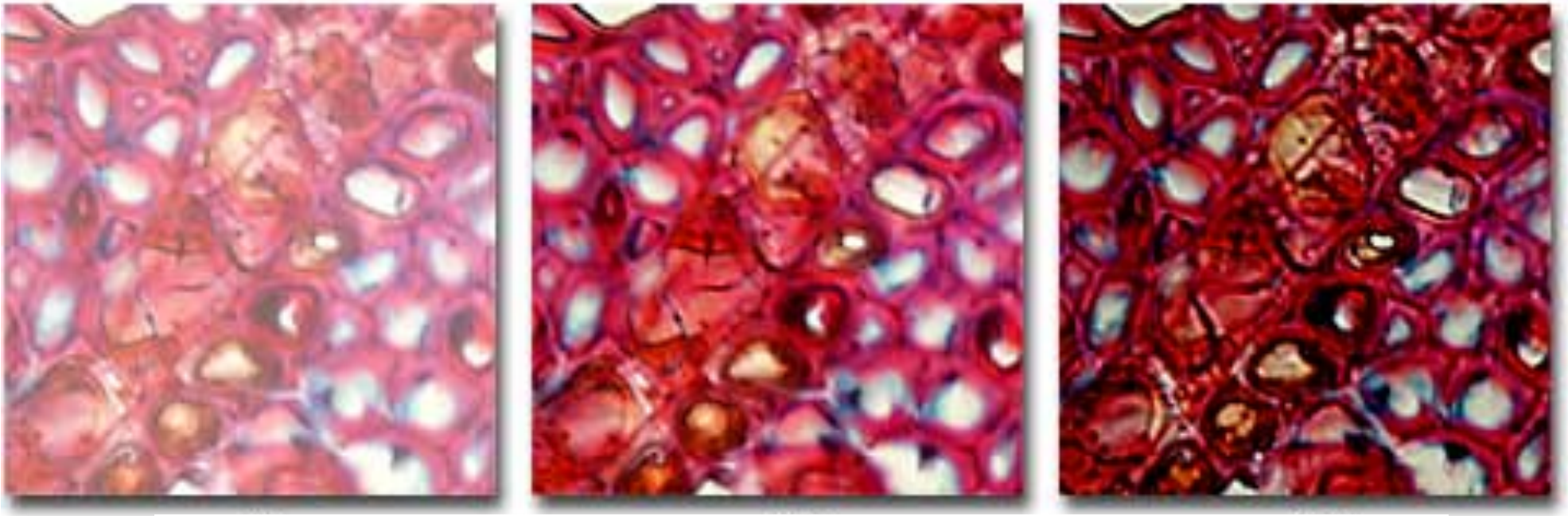


Scattering can reduce contrast.
Light from a “bright path” at larger angles scatters and appears to originate from the dark object.

Kohler Illumination

Effect of Aperture Diaphragm on Contrast and Resolution

Photomicrograph of Plum Tree Stem infected with Black Knot Fungush



Objective NA = 0.75
Condenser NA = 0.90

Objective NA = 0.75
Condenser NA = 0.54

Objective NA = 0.75
Condenser NA = 0.18

Image take from Olympus Microscopy Resource Center Website

Kohler Illumination

Light Pathways in an upright microscope

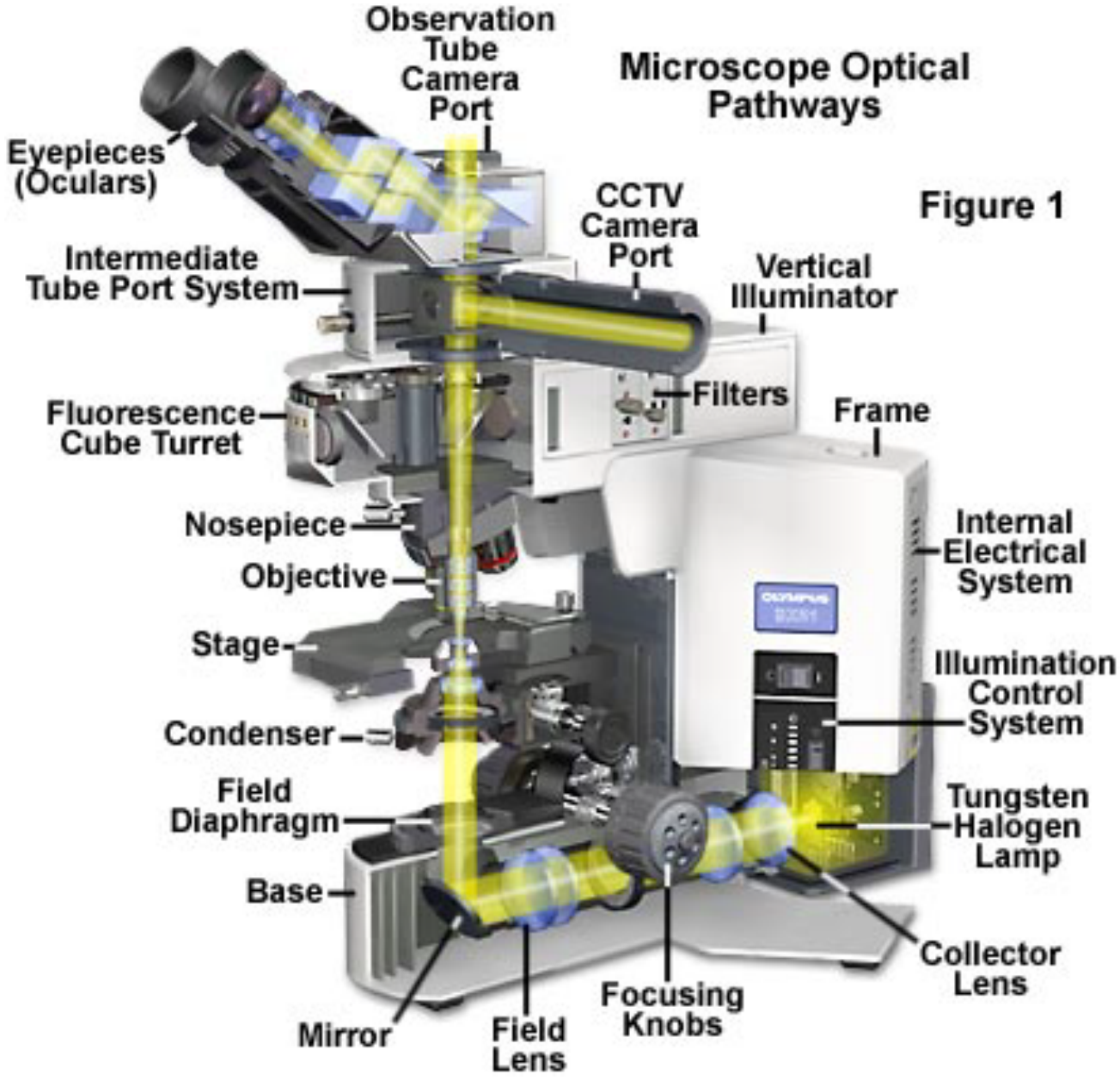


Image take from Olympus Microscopy Resource Center Website

Kohler Illumination

Light Pathways in an inverted microscope

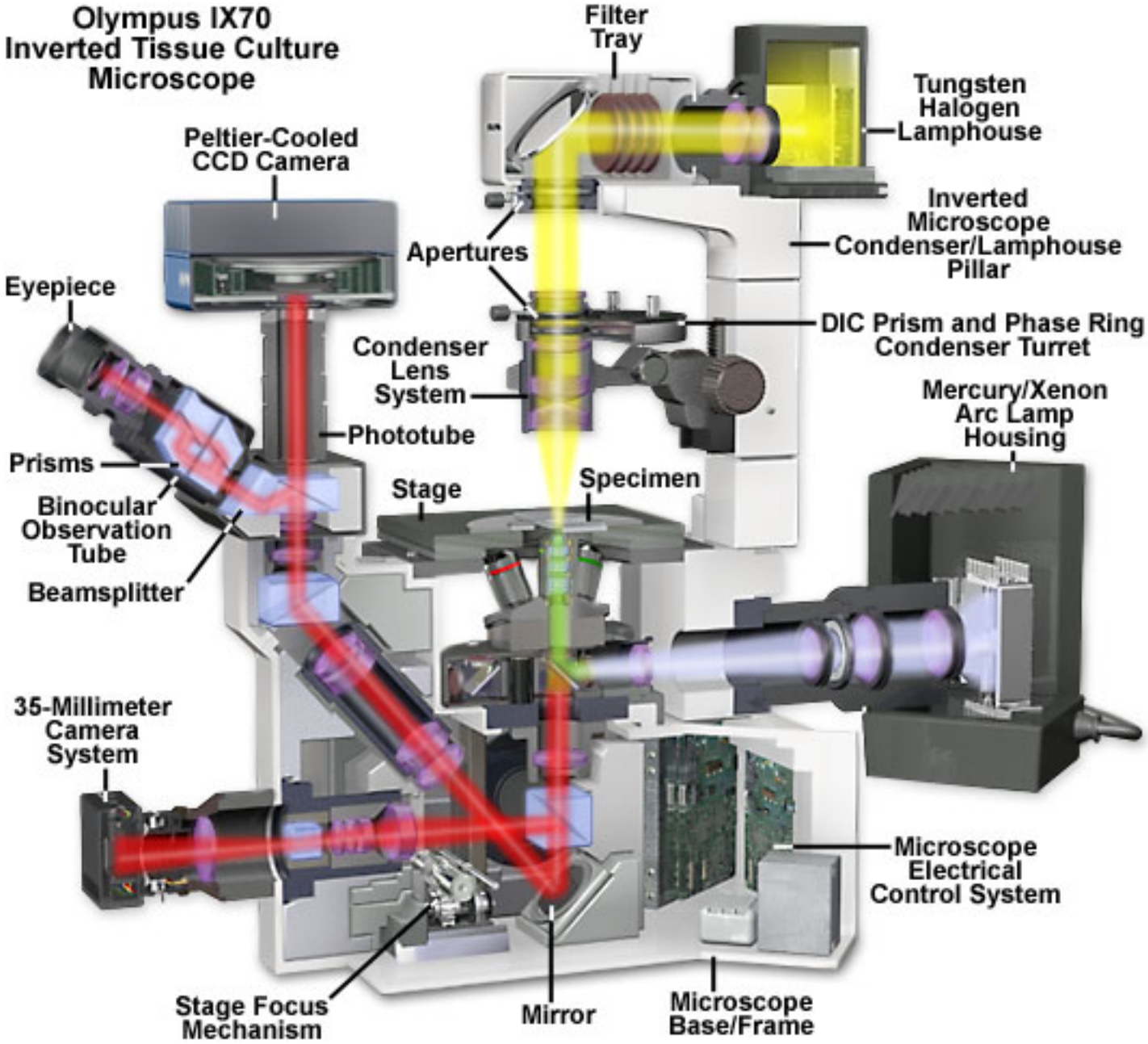
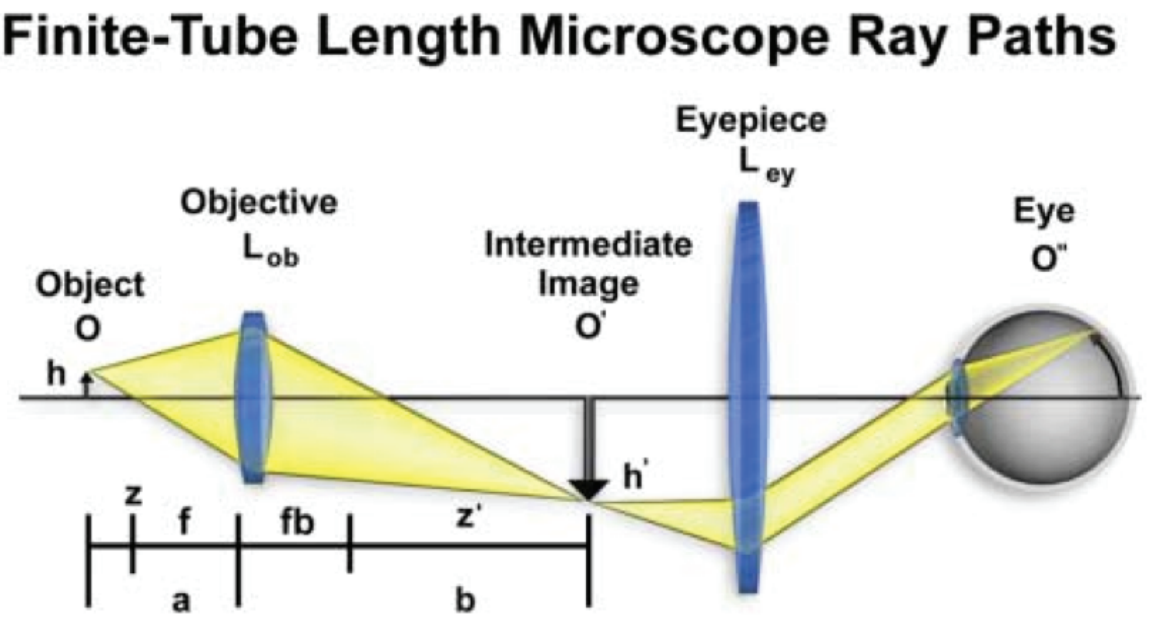
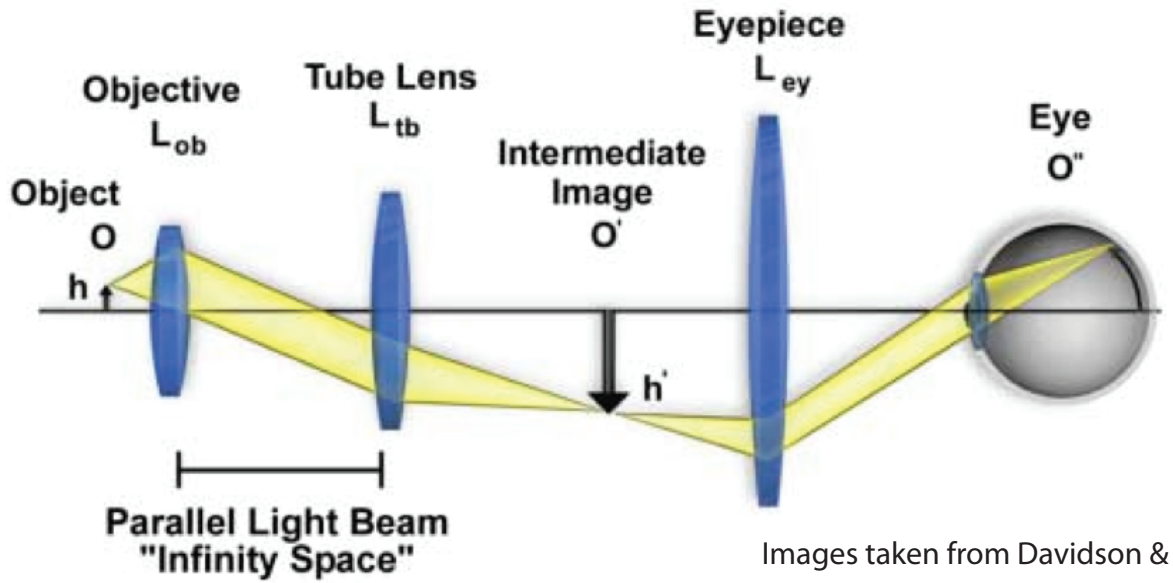


Image take from Olympus Microscopy Resource Center Website

Infinity-Conjugate vs. Finite-Conjugate Microscopes





Images taken from Davidson & Abramowitz, Optical Microscopy