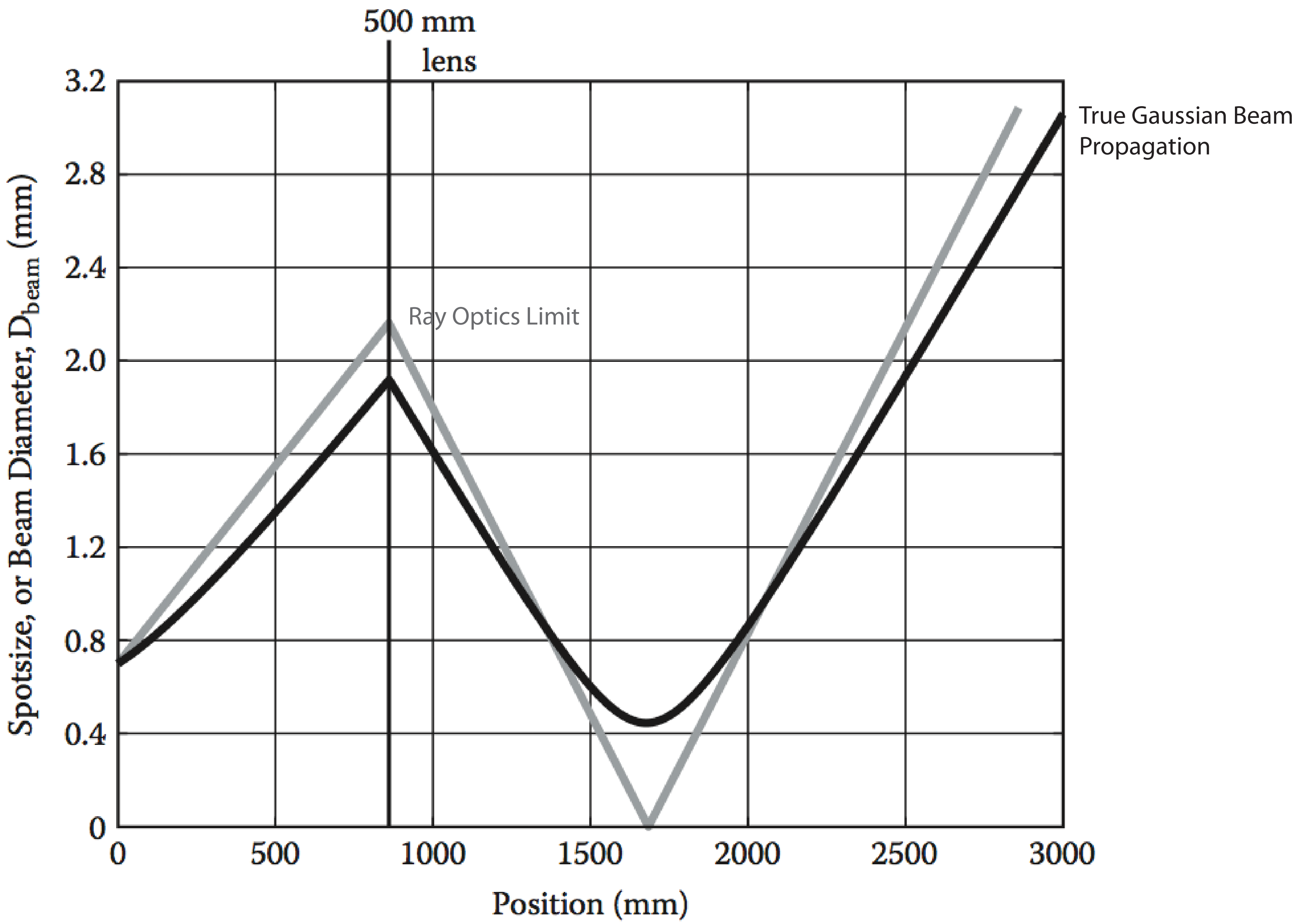
Approximations

Brutalizing optics into 4 limiting regimes

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

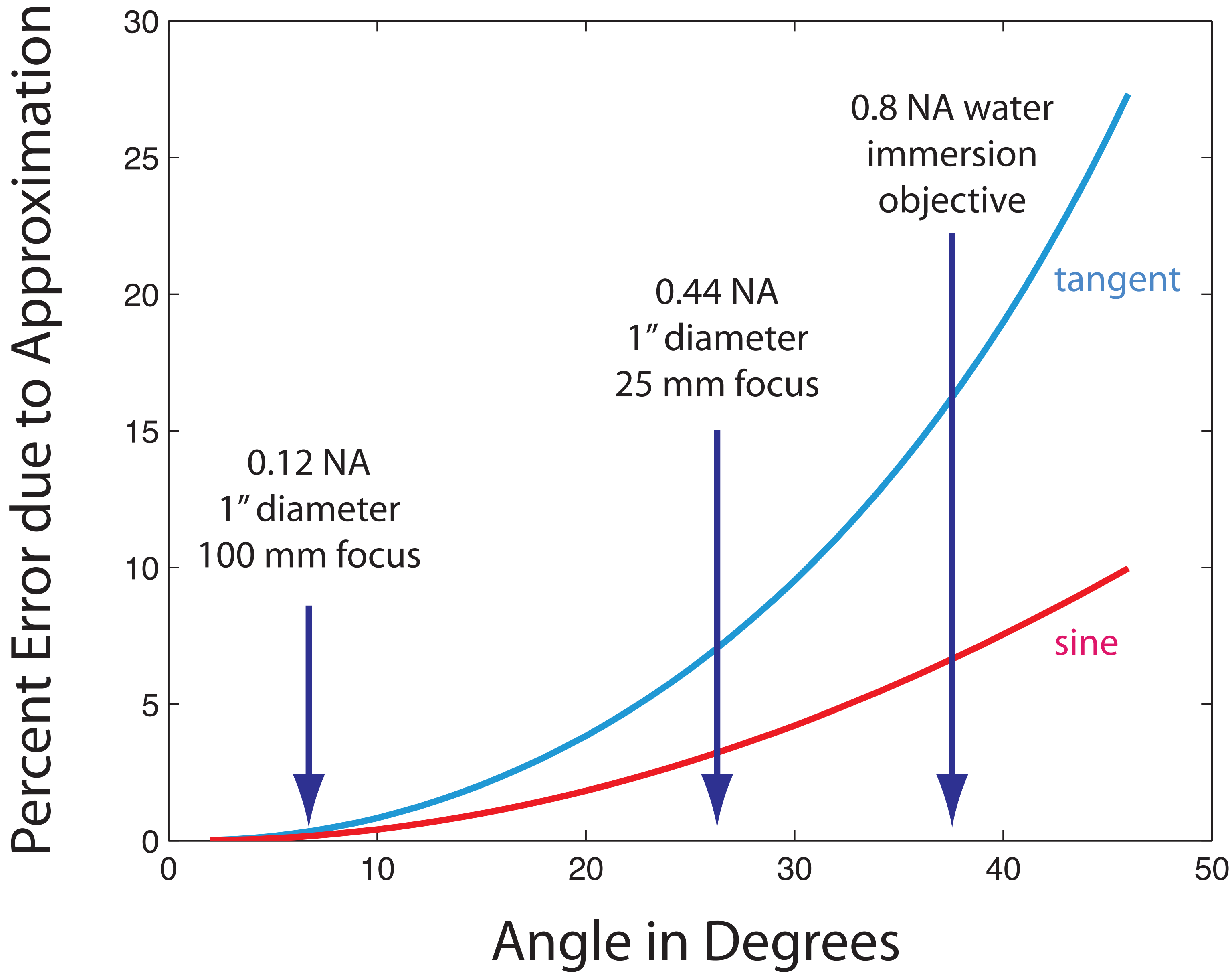
Ray (Geometric) Optics Approximation

wavelength, $\lambda \rightarrow 0$



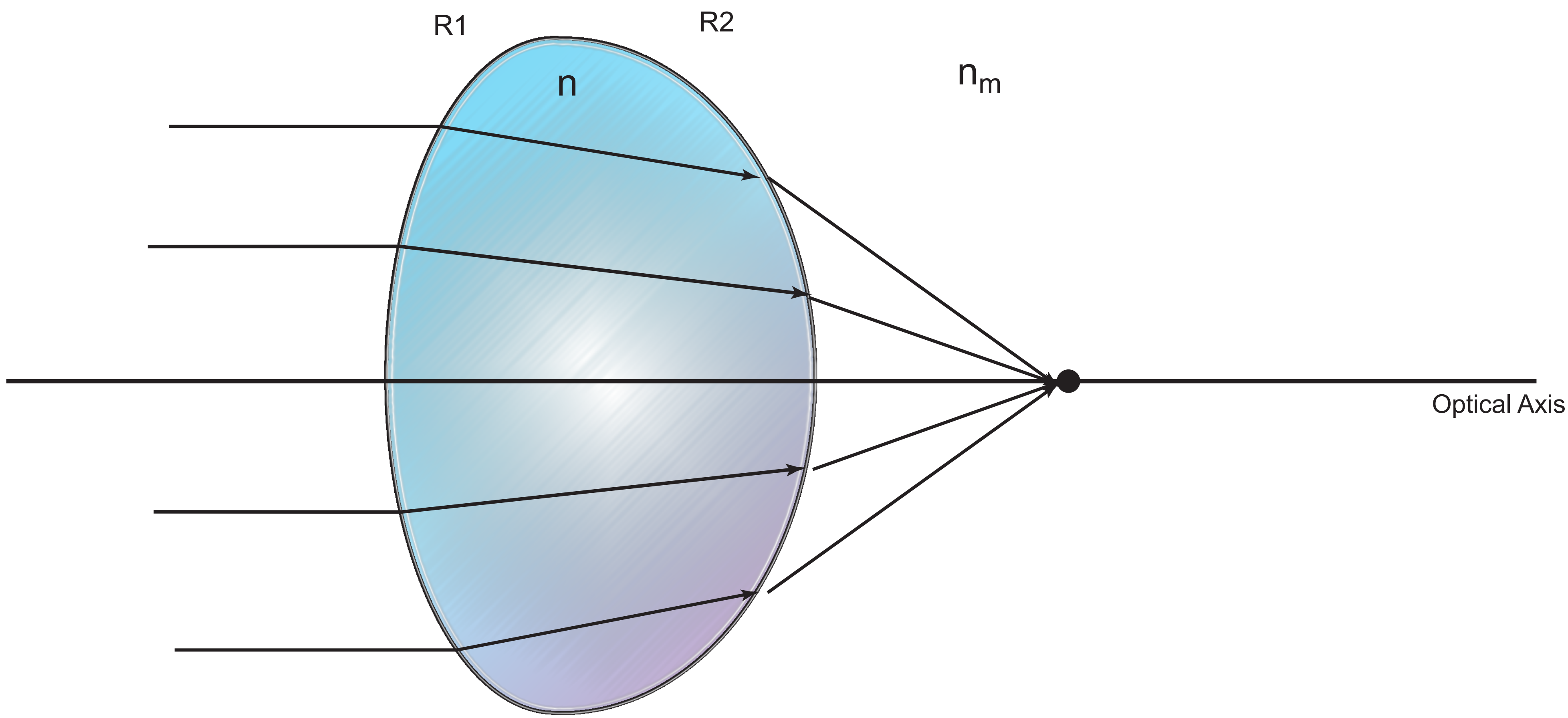
Paraxial Approximation

small angle approximation : $\sin(\theta) \approx \tan(\theta) \approx \theta$



Lensmaker Formula

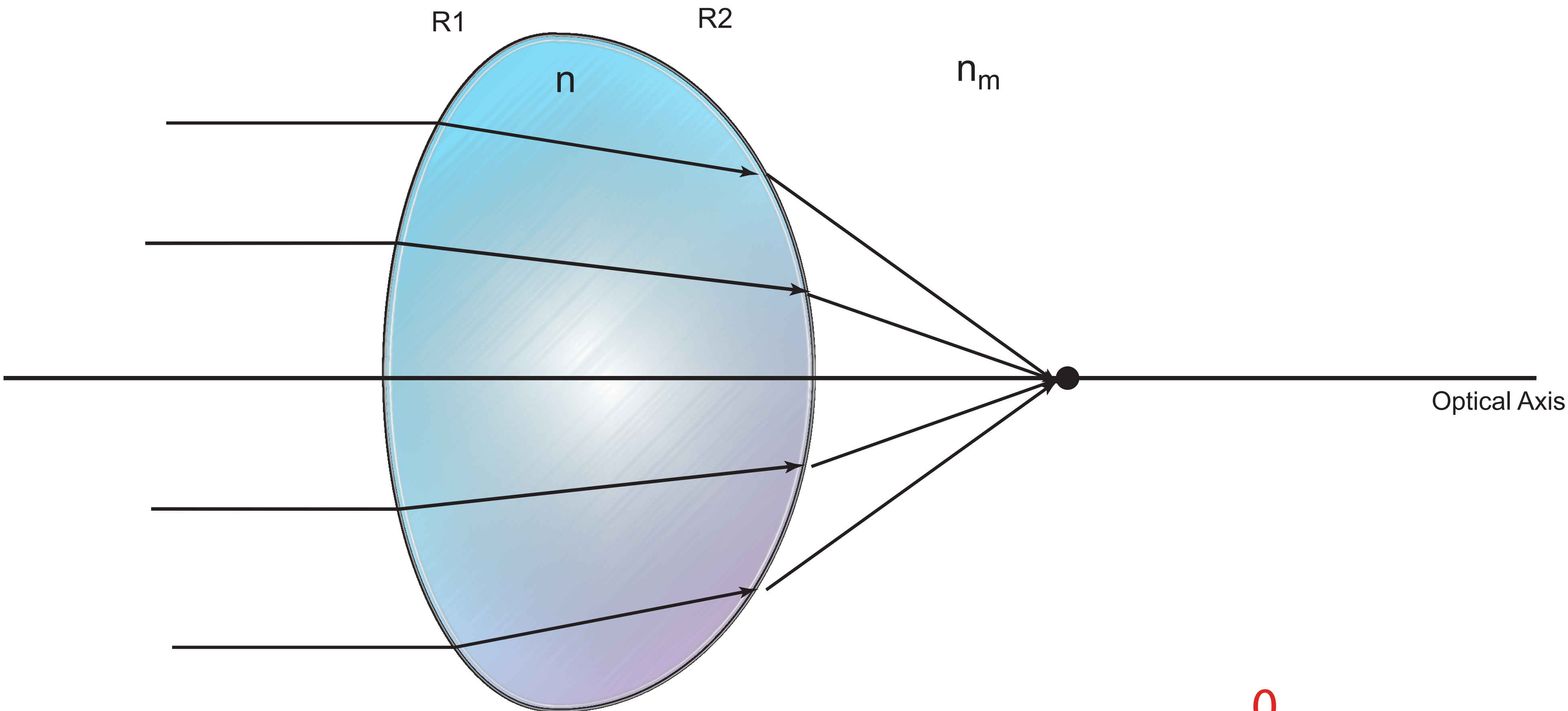
Thick Lens



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

Lensmaker Formula

Thick Lens



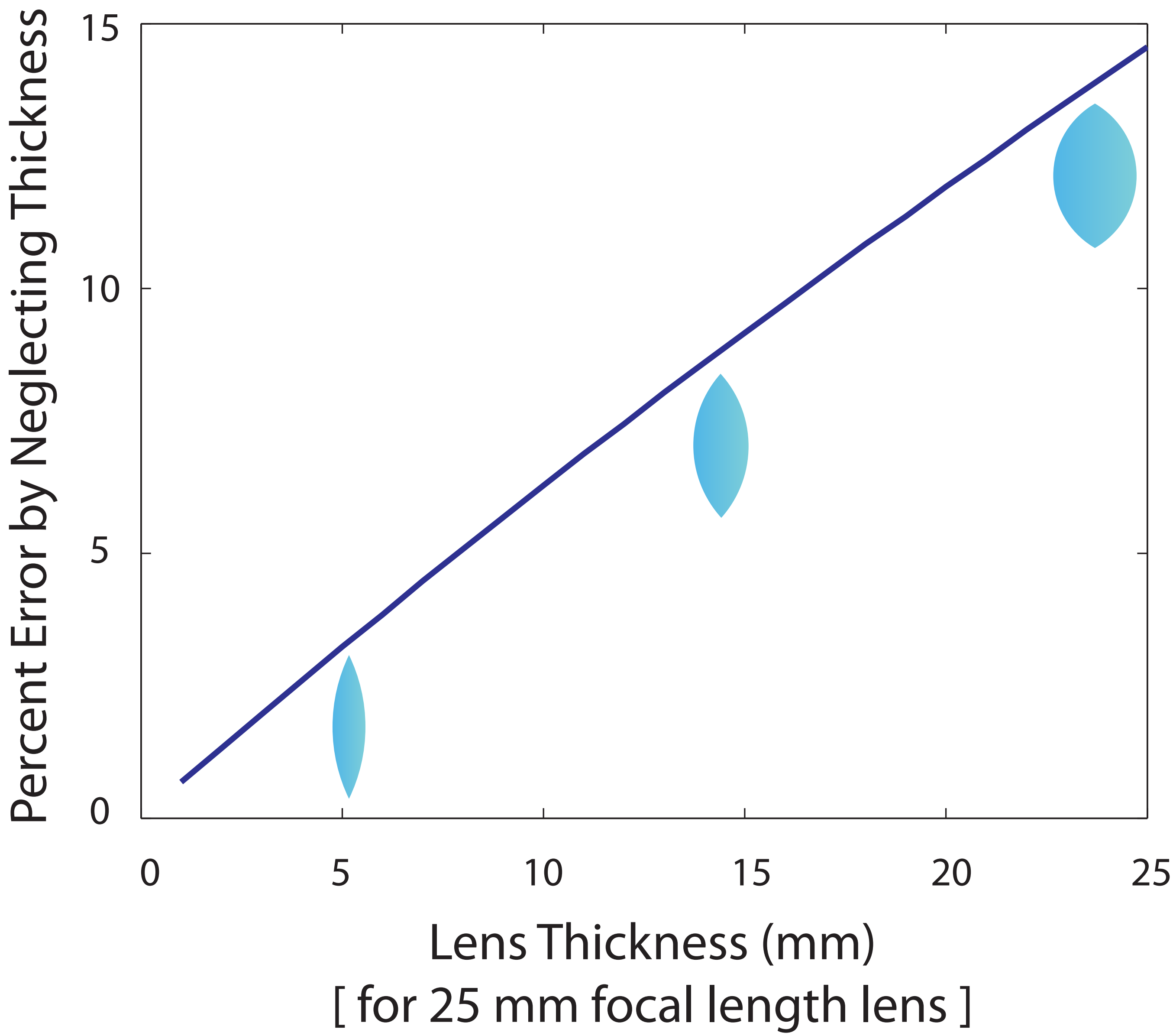
Lensmaker Formula : $$\frac{1}{f} = \left(\frac{n}{n_m} - 1\right)\left[\frac{1}{R_1} + \frac{1}{R_2} + \frac{(n-1)d}{nR_1R_2}\right],$$

0

Thin Lens Approximation

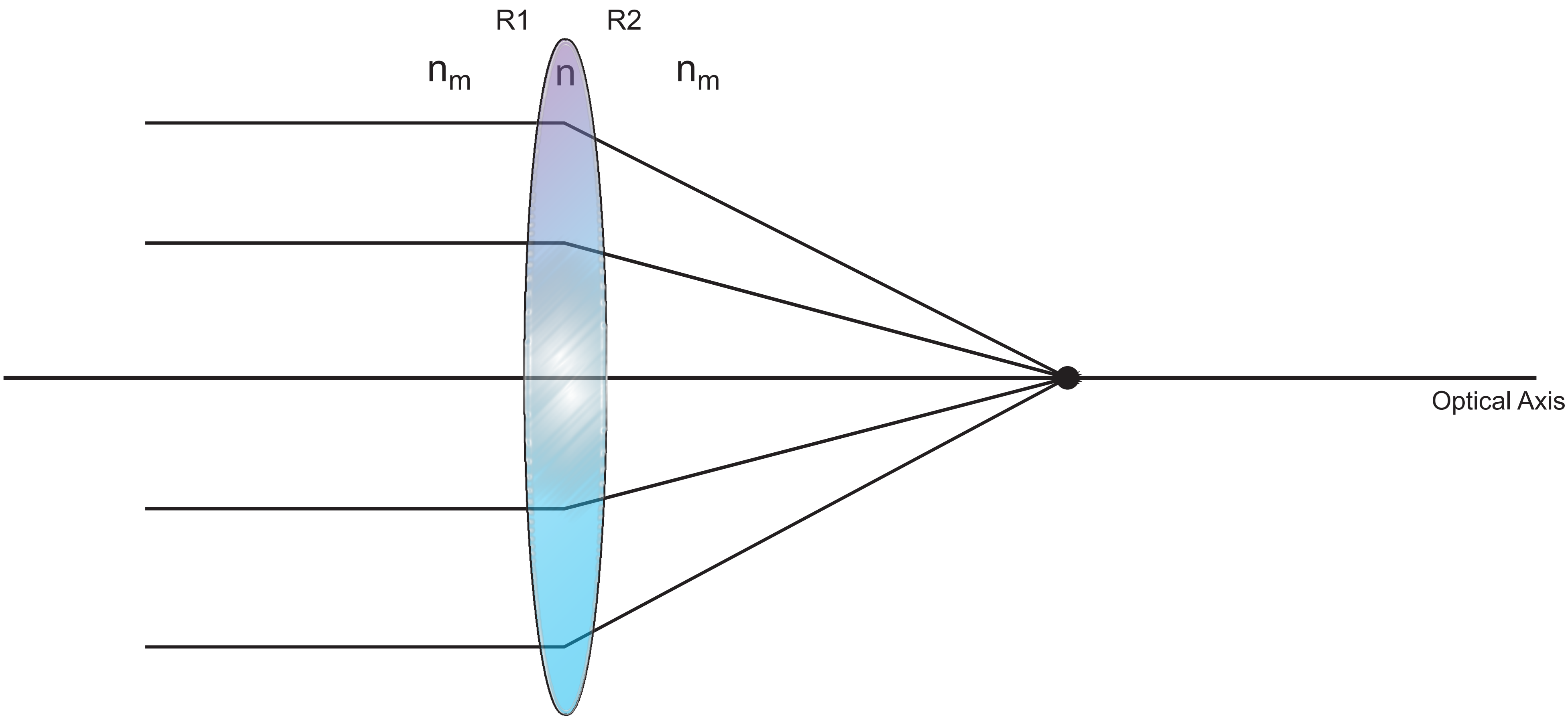
Thin Lens Approximation

neglect lens thickness in calculating focal length



Lensmaker Formula

Thin Lens



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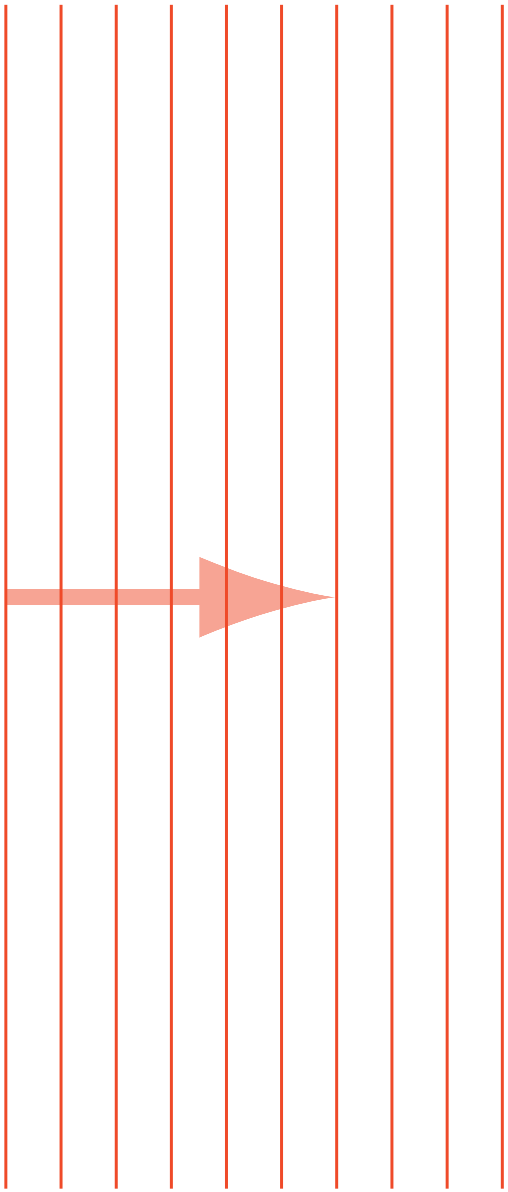
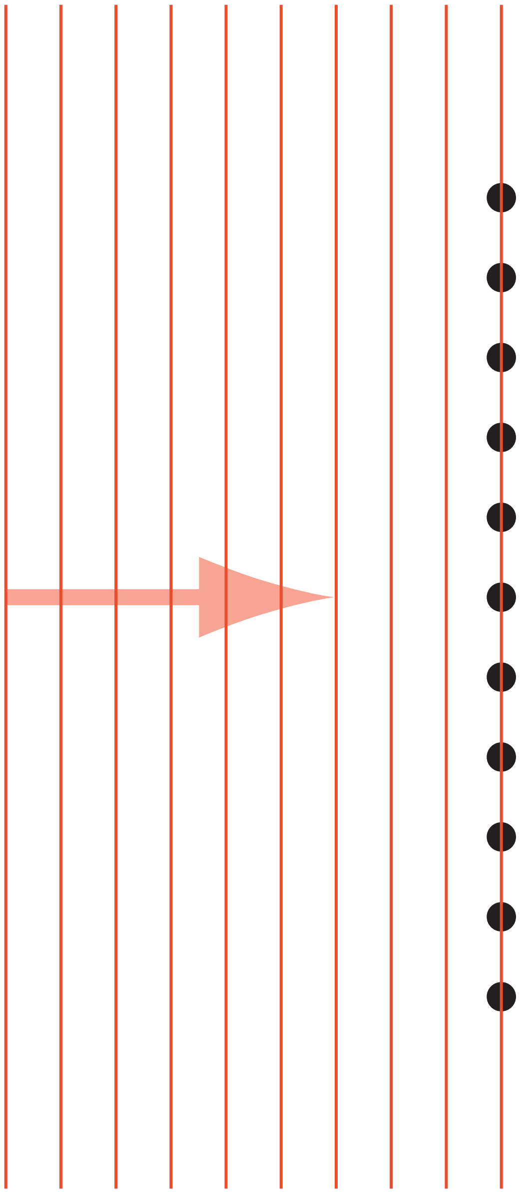
Huygen's Principle



Every point on a wave front acts a spherical source

Huygen's Principle



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Huygen's Principle

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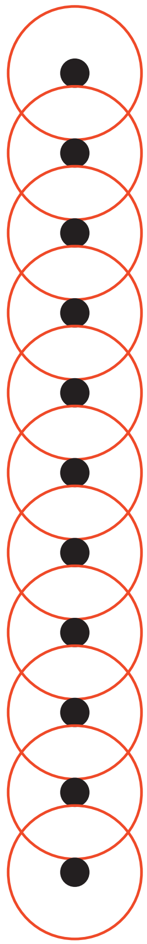
Huygen's Principle



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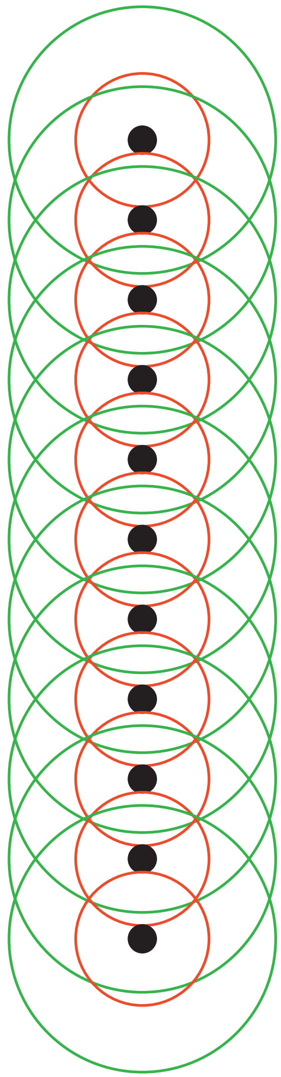

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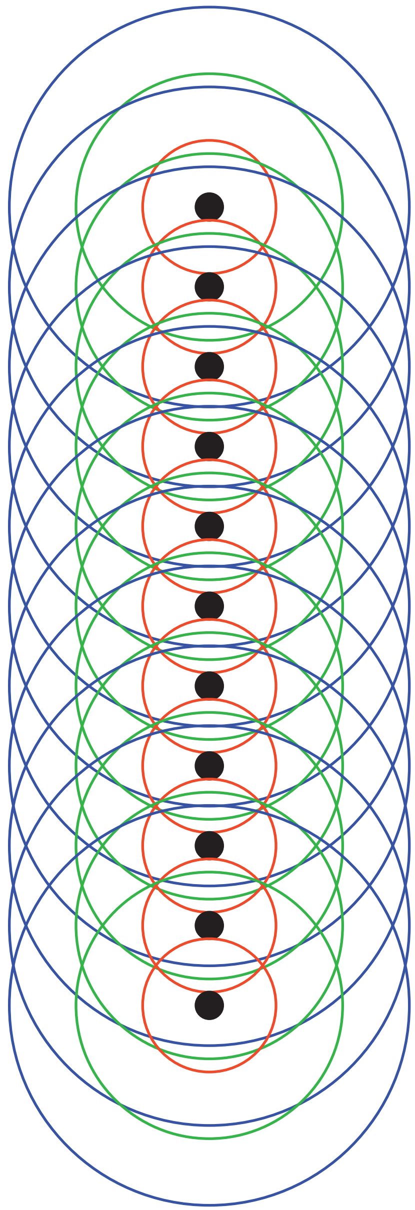

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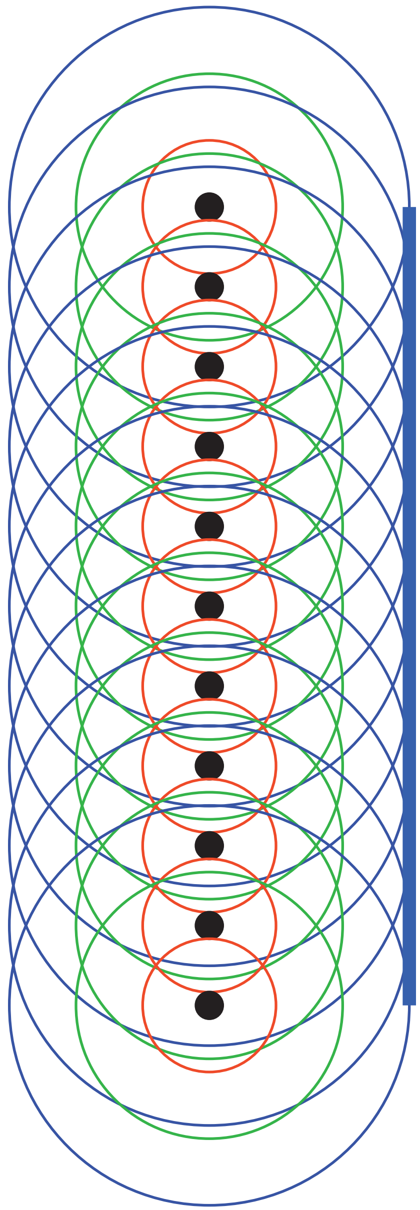

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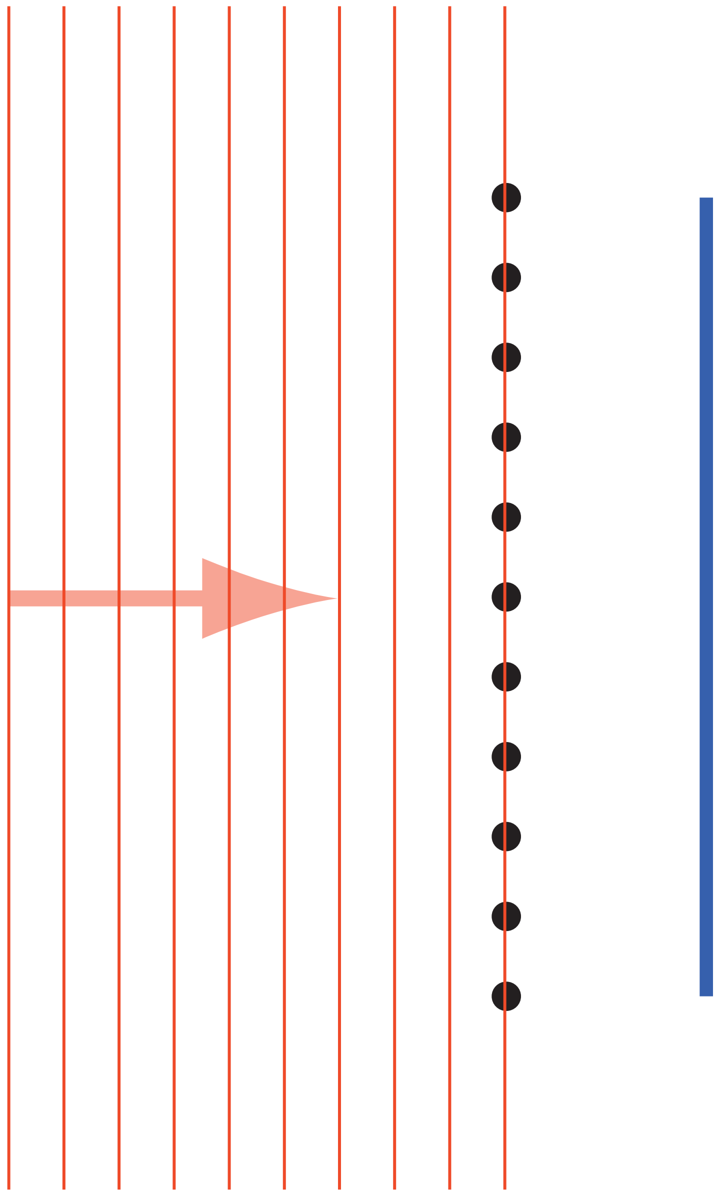

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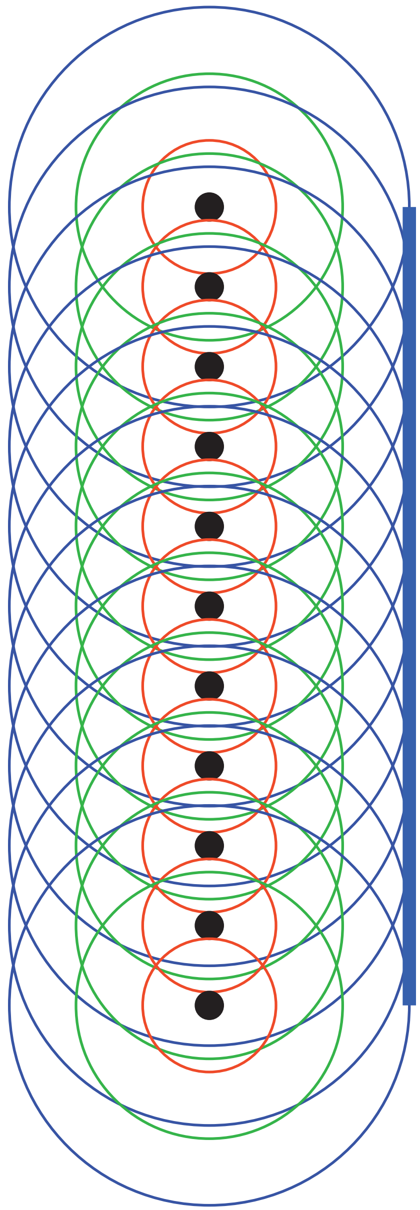

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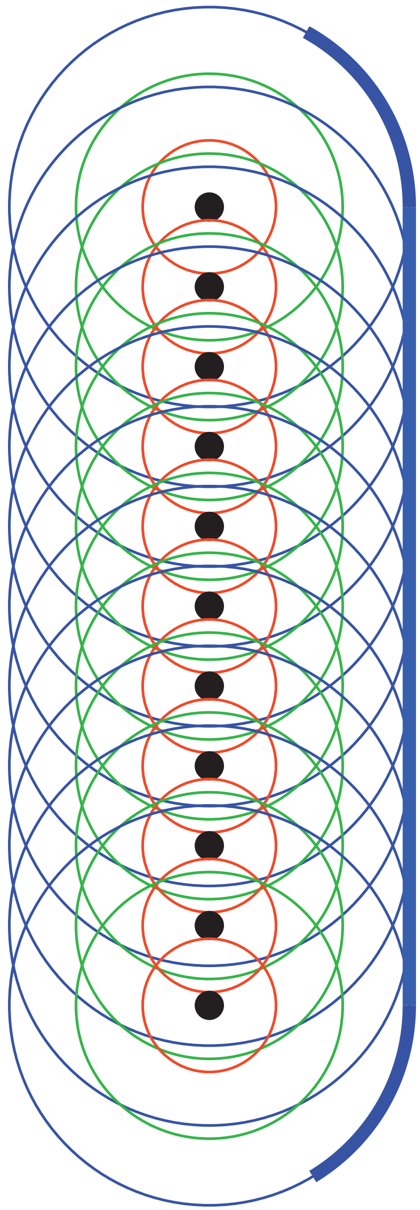

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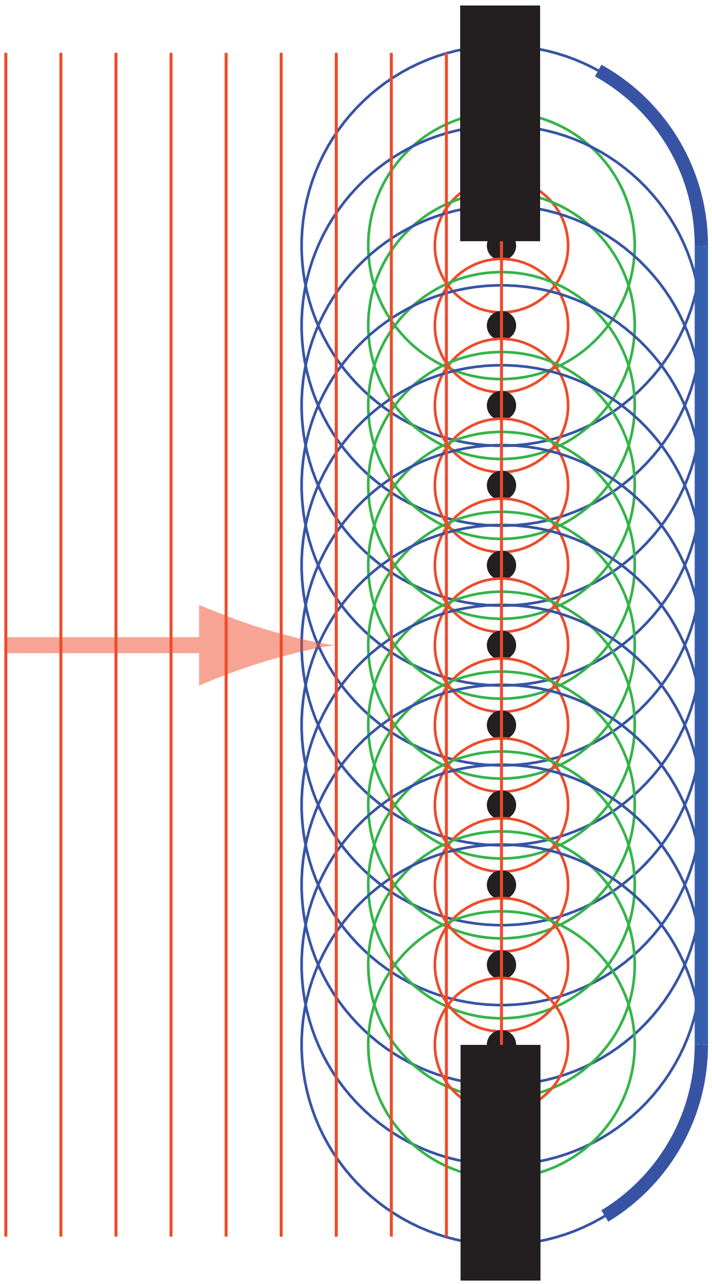

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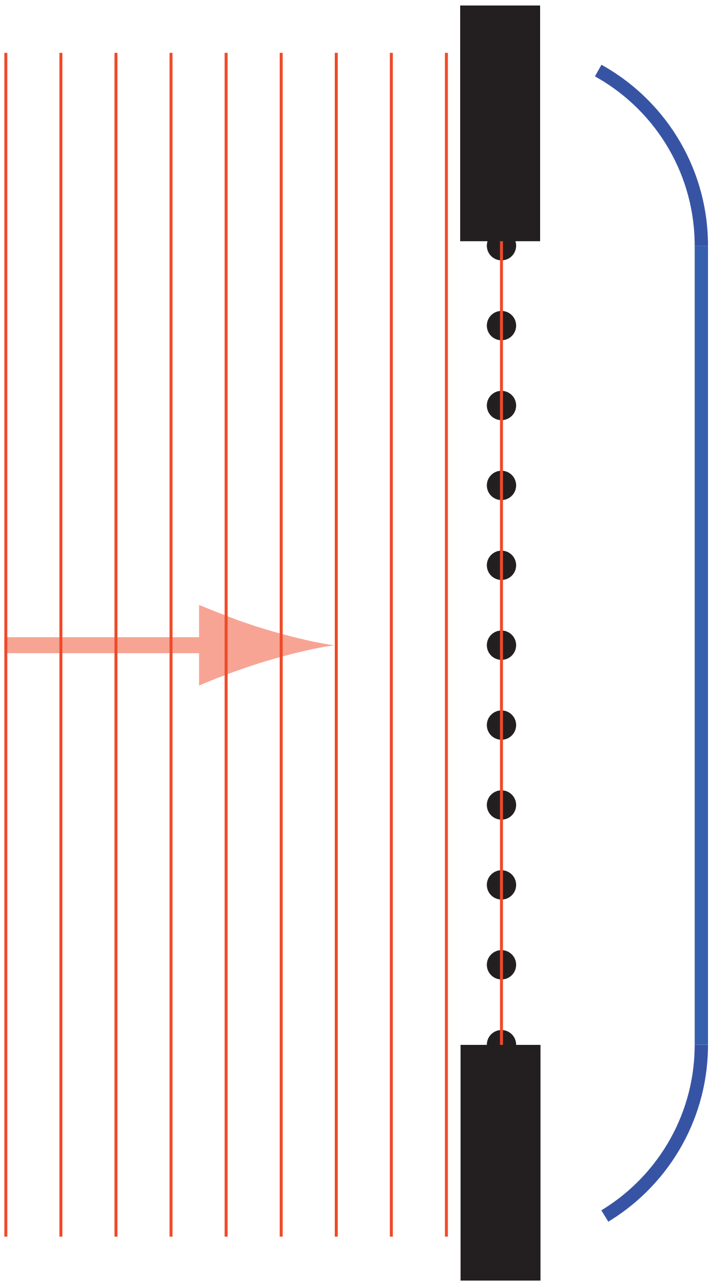
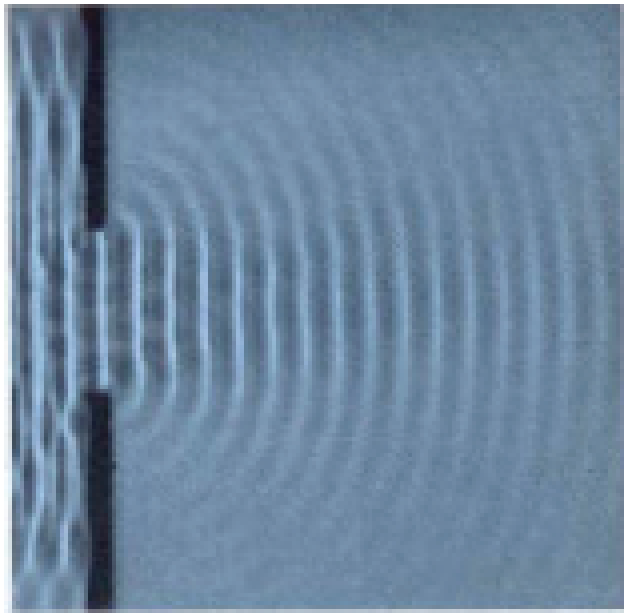


Every point on a wave front acts a spherical source

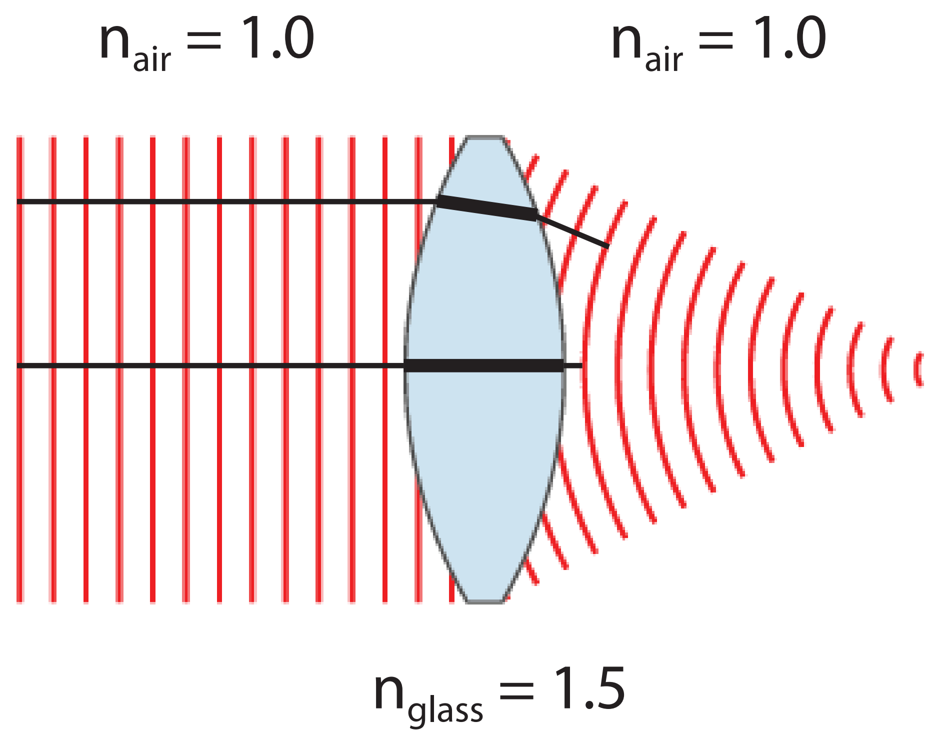
Single Slit Wave Diffraction



Imges adapted from : http://electron6.phys.utk.edu/light/1/Diffraction.htm

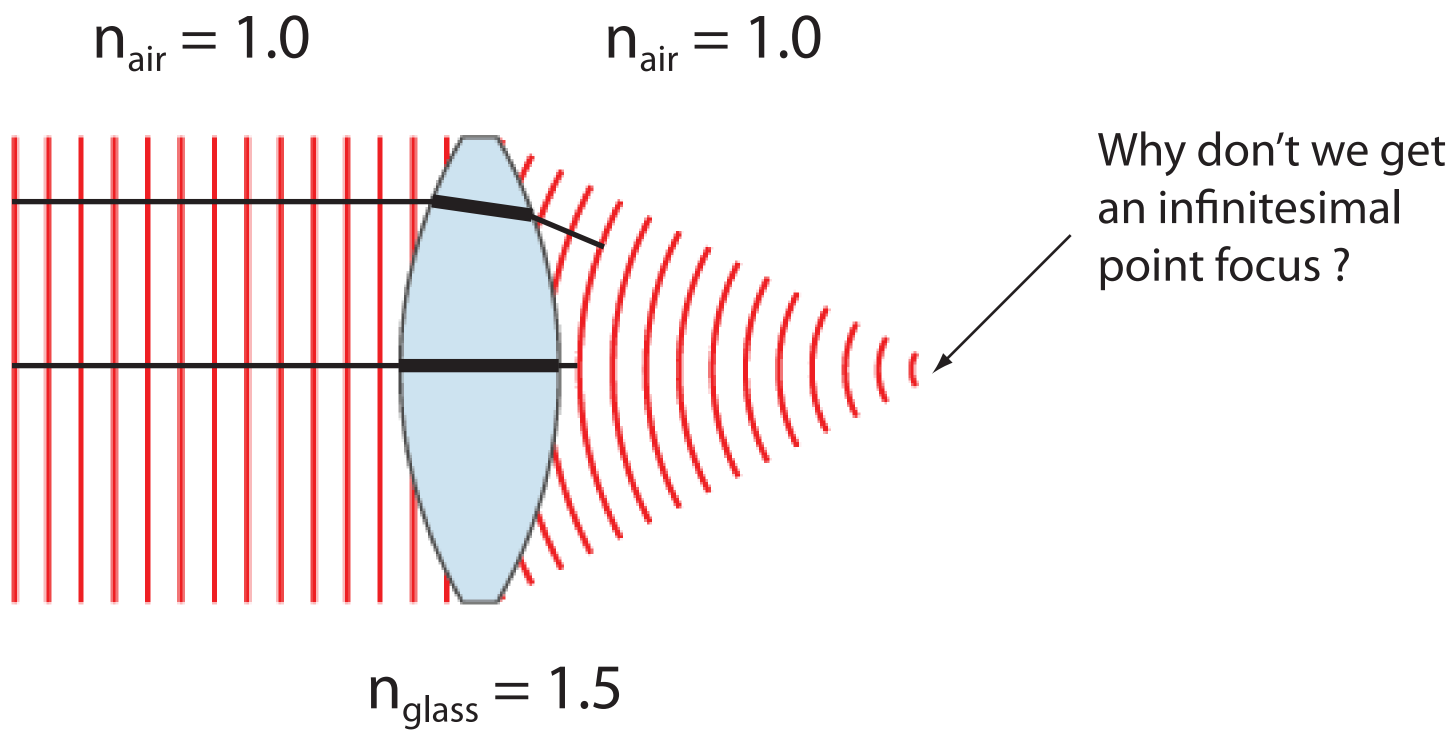
Water waves passing through a barrier exhibit diffraction.

Focusing by a Lens



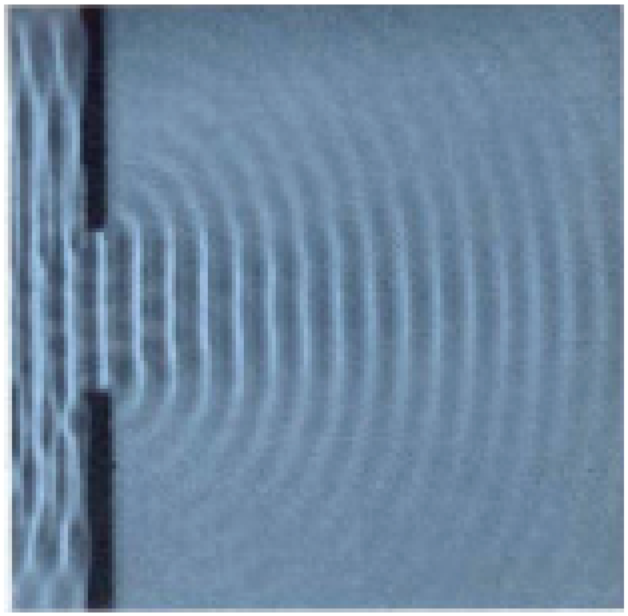
Optical Path Length (OPL) $= \Sigma\, n_i \cdot d_i$

Focusing by a Lens



$$\text{Optical Path Length (OPL)} = \Sigma\, n_i \cdot d_i$$

Single Slit Wave Diffraction



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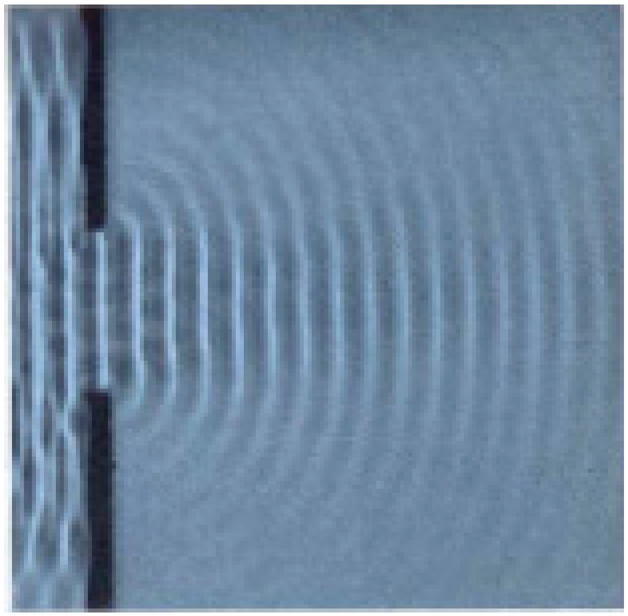

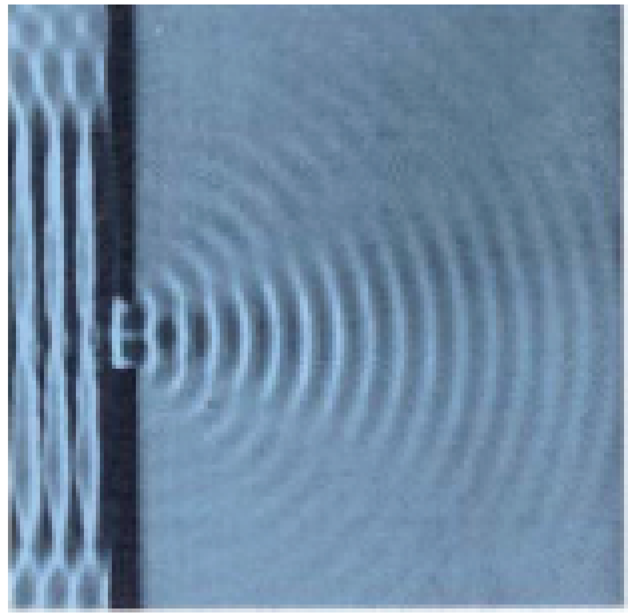

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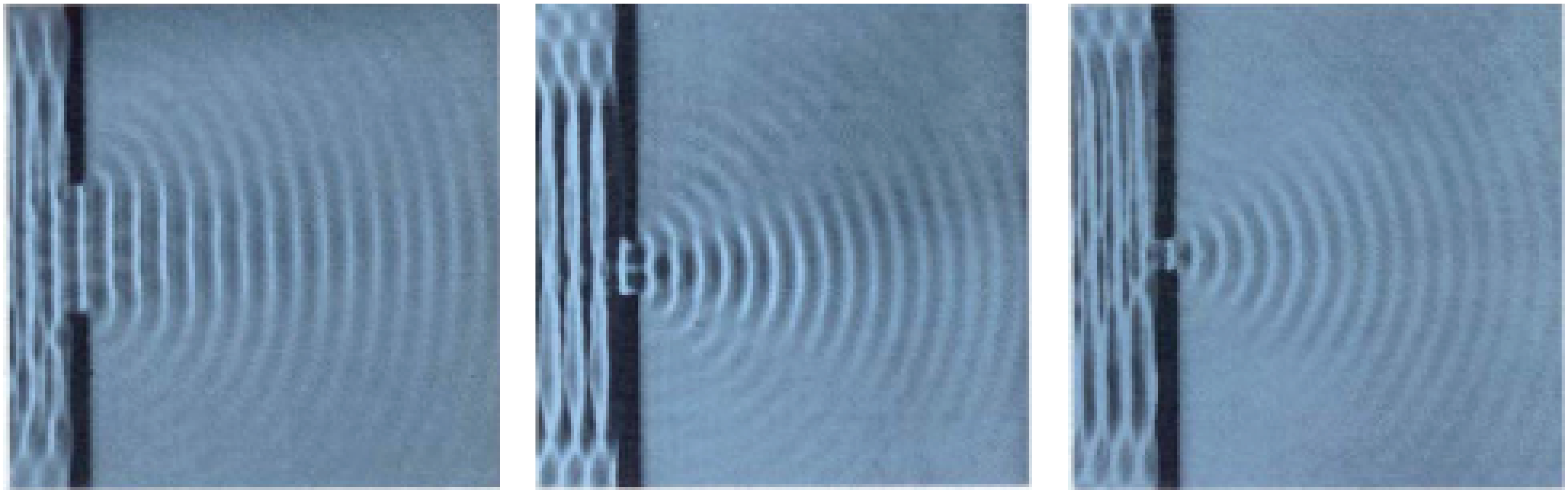

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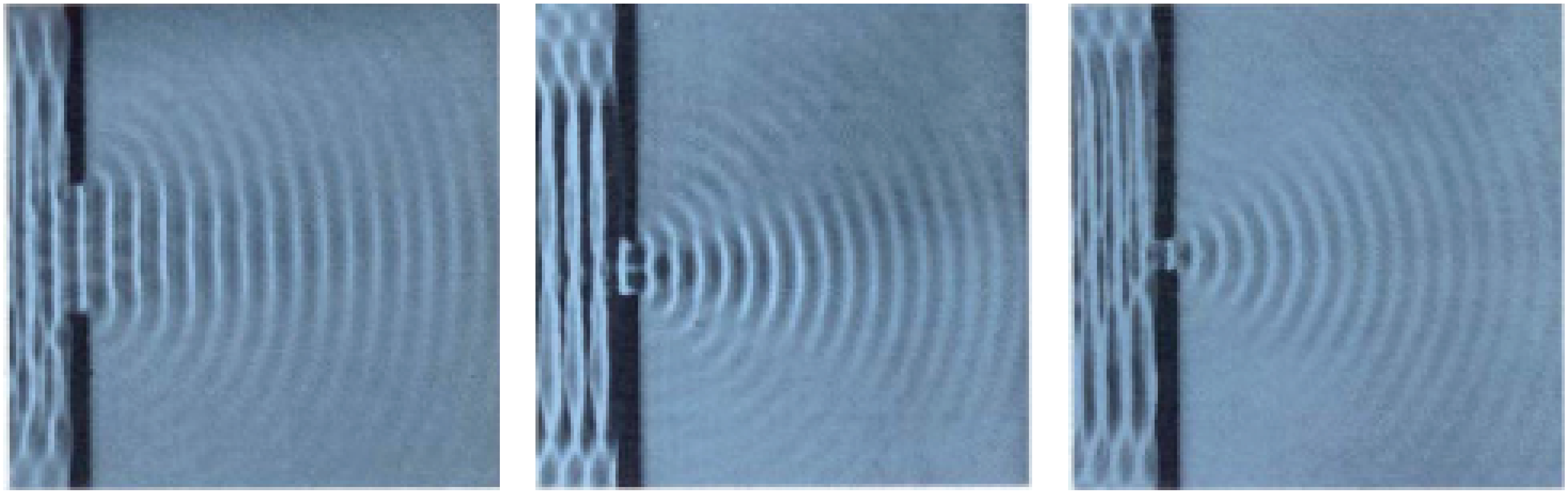

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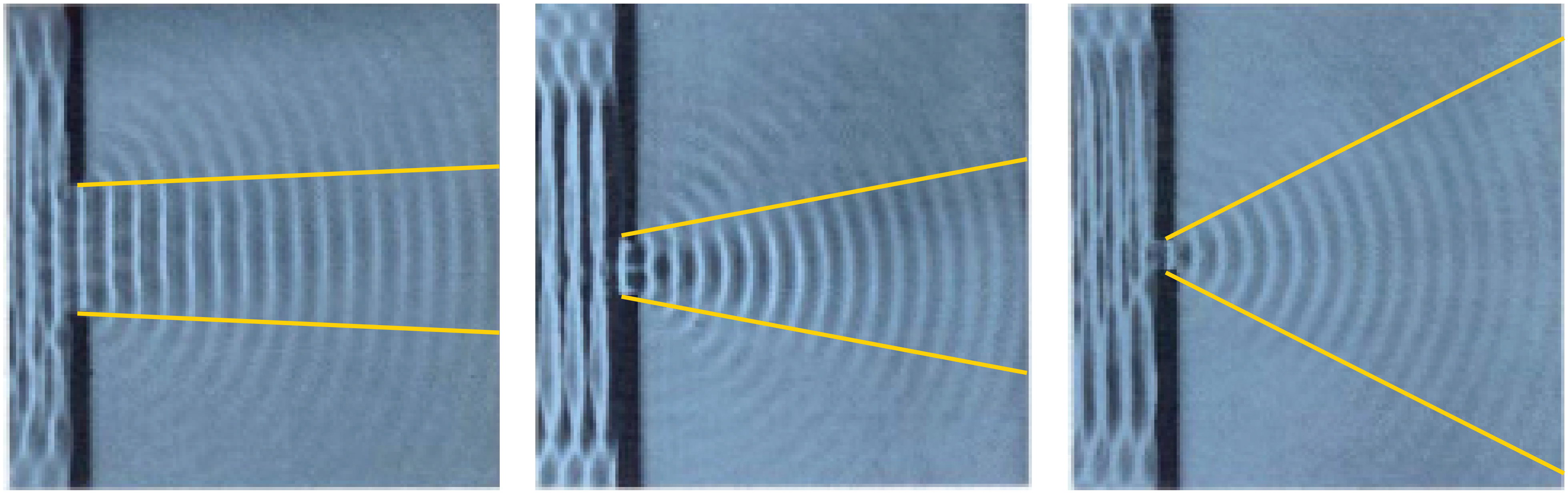
Water waves passing through a barrier exhibit diffraction. As the size of the hole decreases, the wave that passes the barriers goes from nearly planar to nearly spherical.

Single Slit Wave Diffraction



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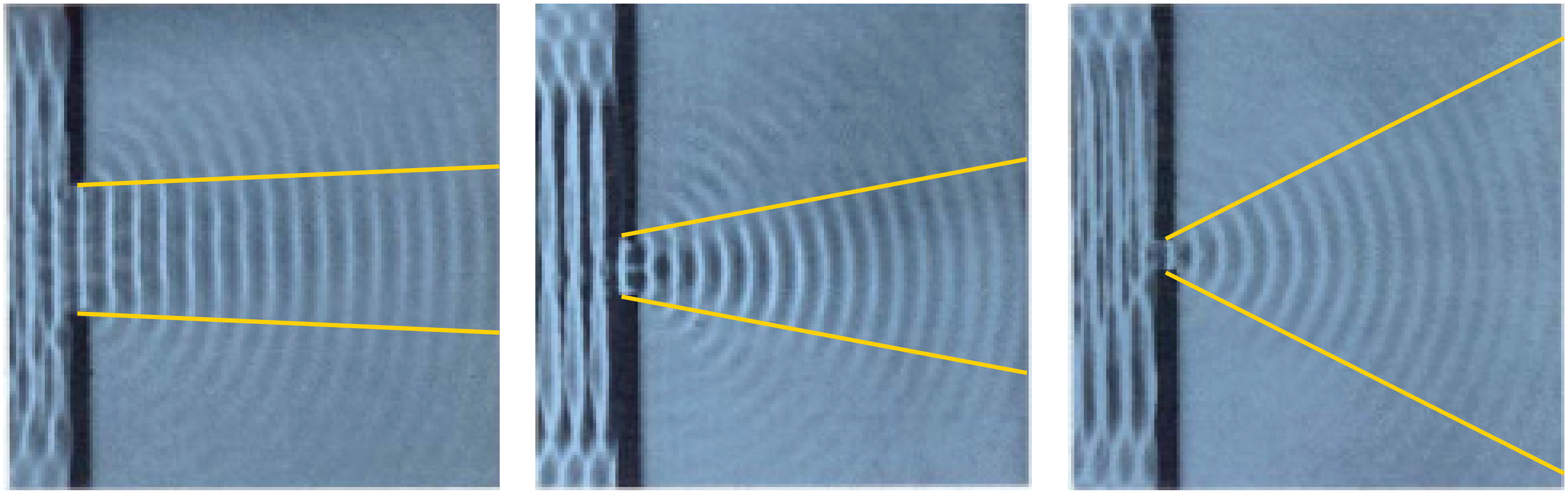

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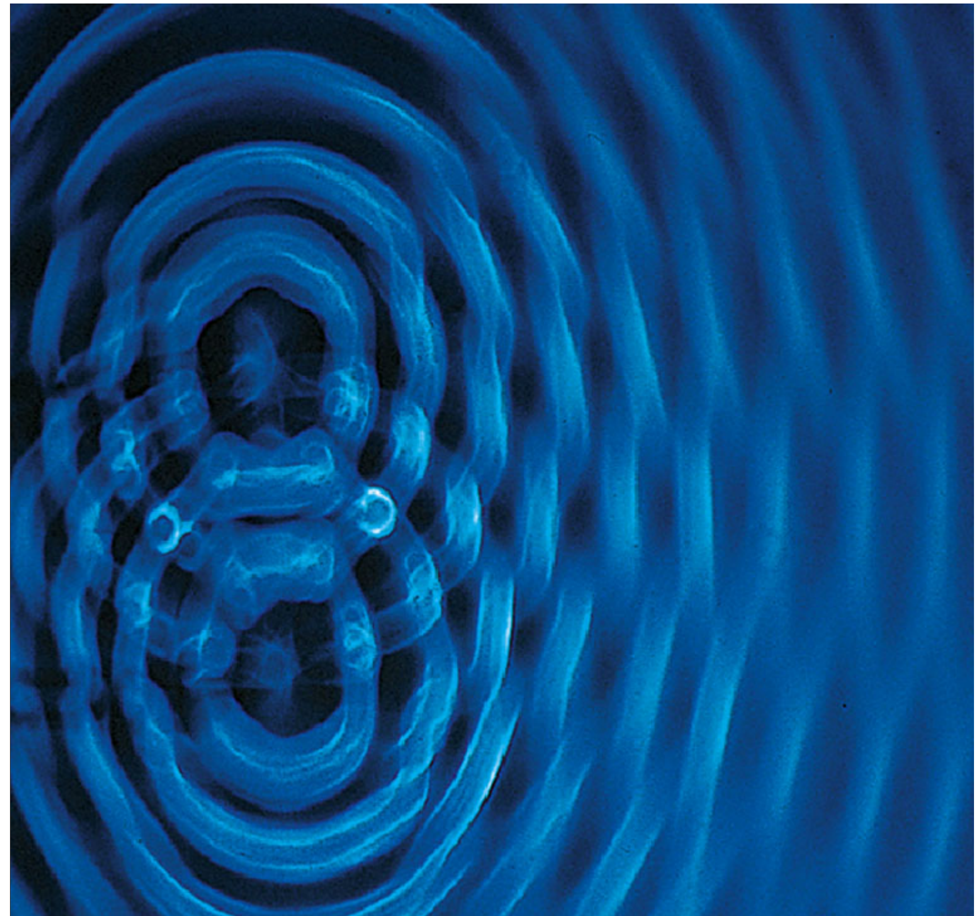
Water waves passing through a barrier exhibit diffraction. As the size of the hole decreases, the wave that passes the barriers goes from nearly planar to nearly spherical.

Smaller openings diffract waves to larger angles!

Wave Interference

In order to create
sustained interference
you need (at least)
two soures with
the same wavelength
that are **coherent**



Coherent sources are sources
that maintain a constant
phase between each other.

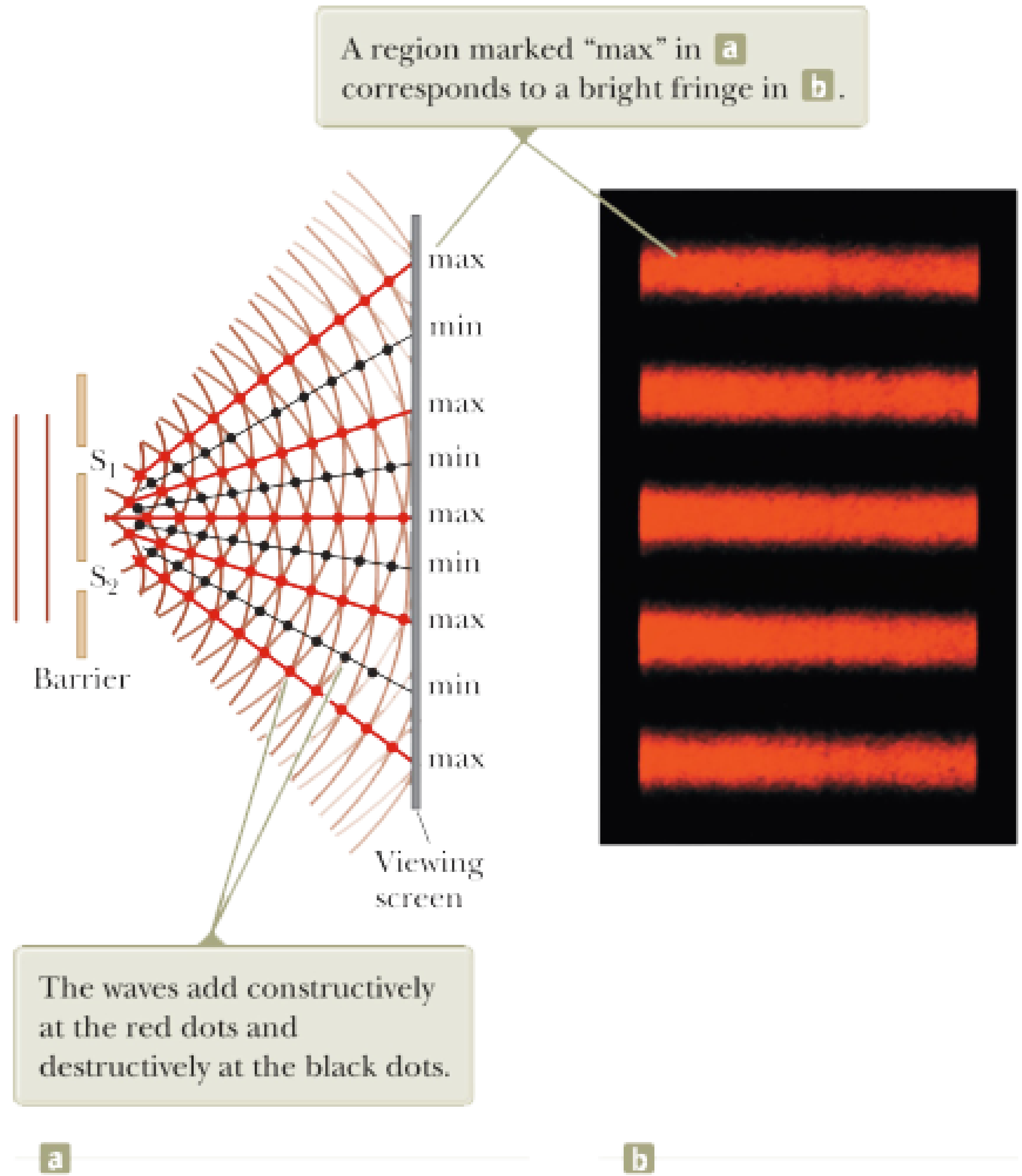
If all the waves have only a single wavelength, then we say
the waves are **monochromatic** ("one color", see Chapter 24)

Young's Double Slit Experiment

Two narrow slits act as coherent sources of monochromatic waves

Because the waves emerging on the right from the two slits are from the same wavefront (on the left), they are in phase with each other (coherent).

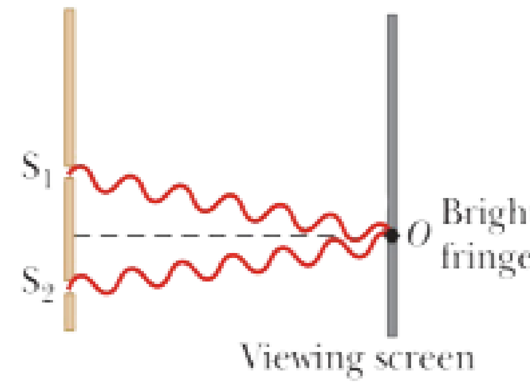
If a screen is placed to the right of the two slits, a pattern of bright and dark parallel bands (call **inteference fringes**) will appear on the screen



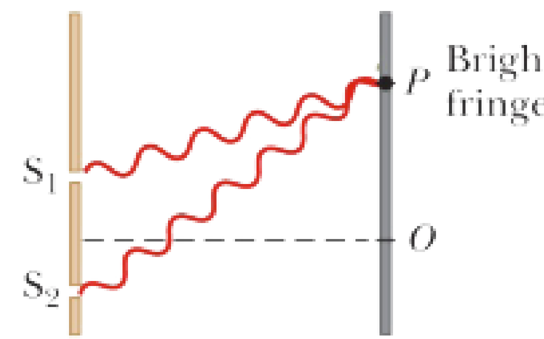
Young's Double Slit Experiment

Two narrow slits act as coherent sources of monochromatic waves

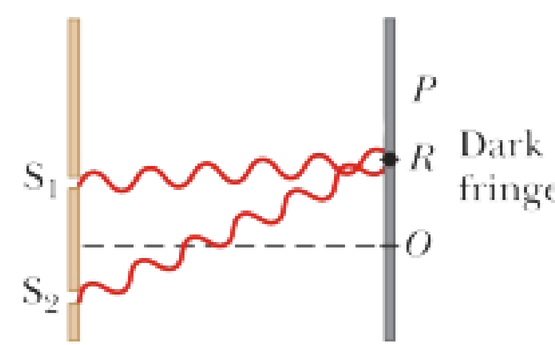
The bright and dark fringes are due to constructive and destructive inteference.



In the center of the screen both waves have traveled the same distance and so they arrive in phase.

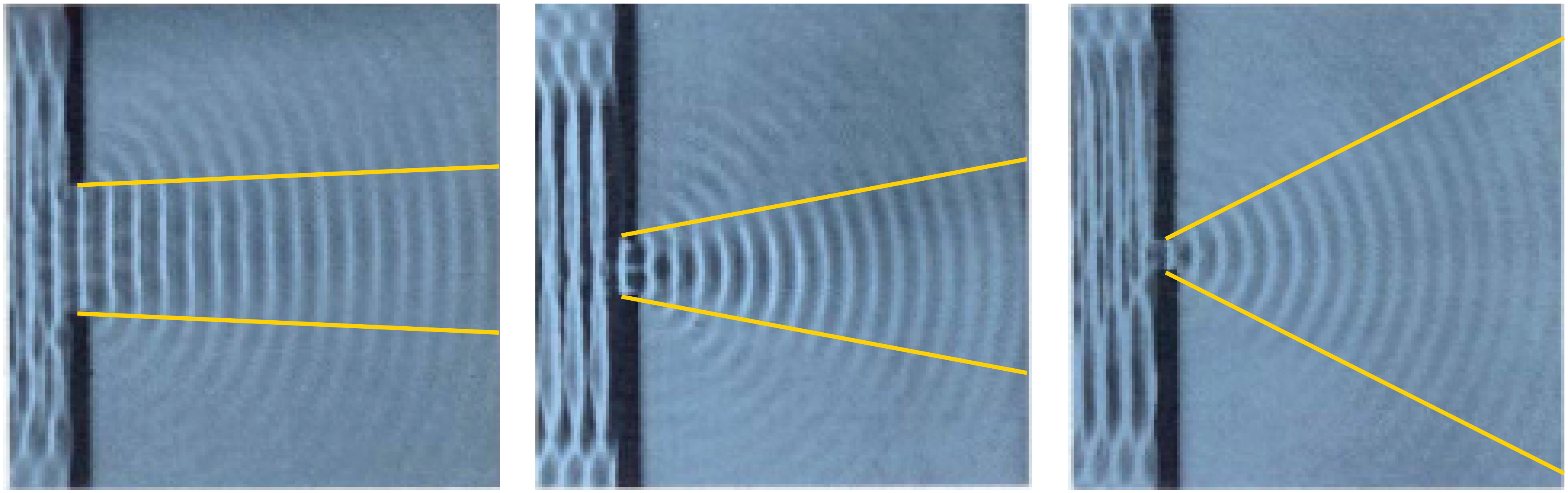


At the center of the first off-center bright fringe, the wave from the lower slit has traveled exactly one extra wavelength than the wave from the upper slit, and so they again arrive in phase.



In between these two bright fringes, there is a dark fringe where the path length difference is exactly half-a-wavelength and so the waves arrive exactly out of phase.

Single Slit Wave Diffraction



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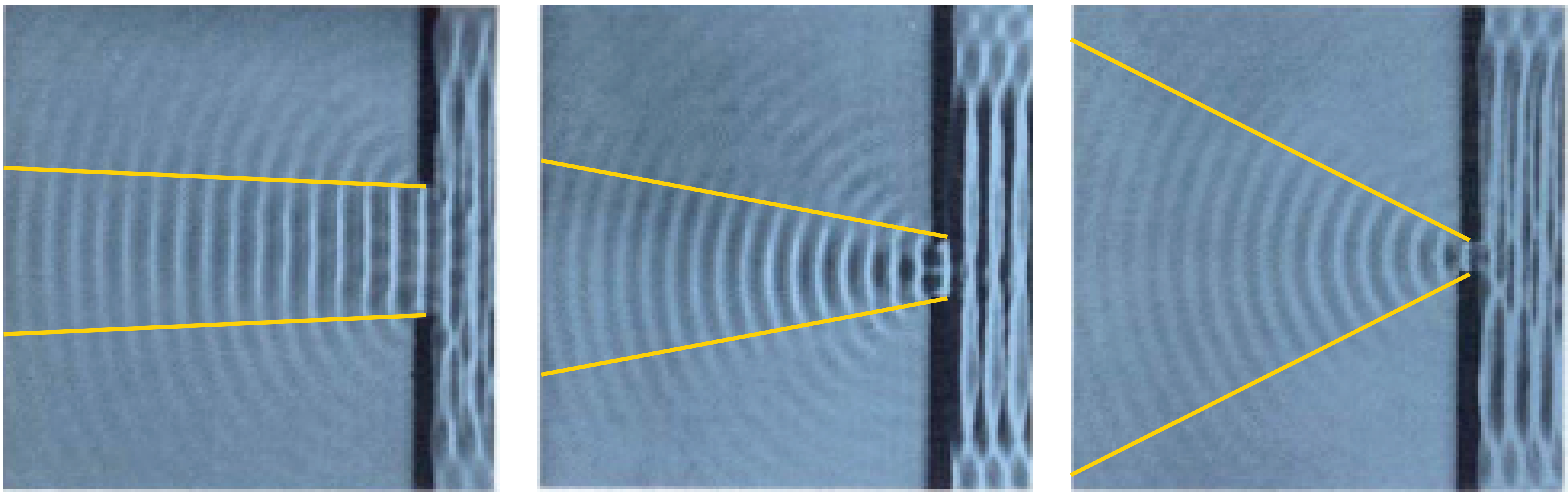


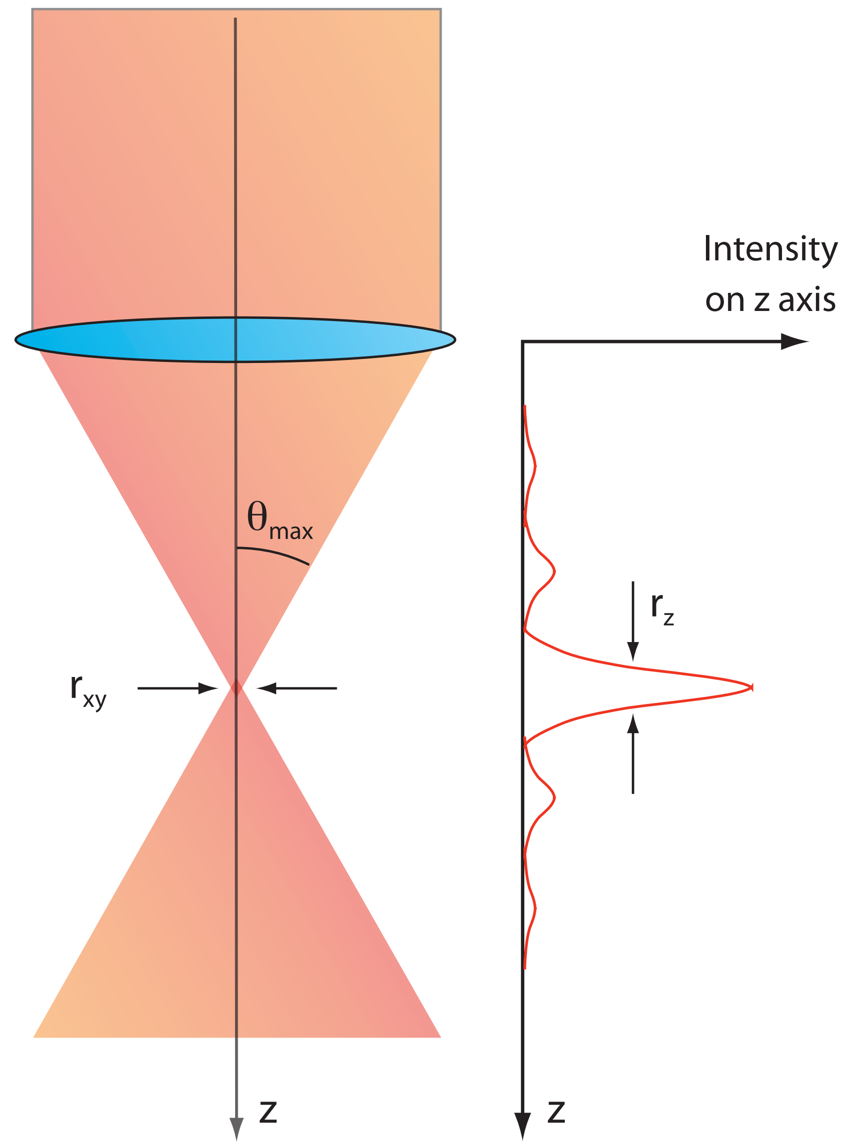
Resolution

It's all about the NA

$$\text{Numerical Aperture (NA)} = n \cdot \sin(\theta_{max})$$

n = index of refraction of media

θ_{max} = maximum angle of incidence

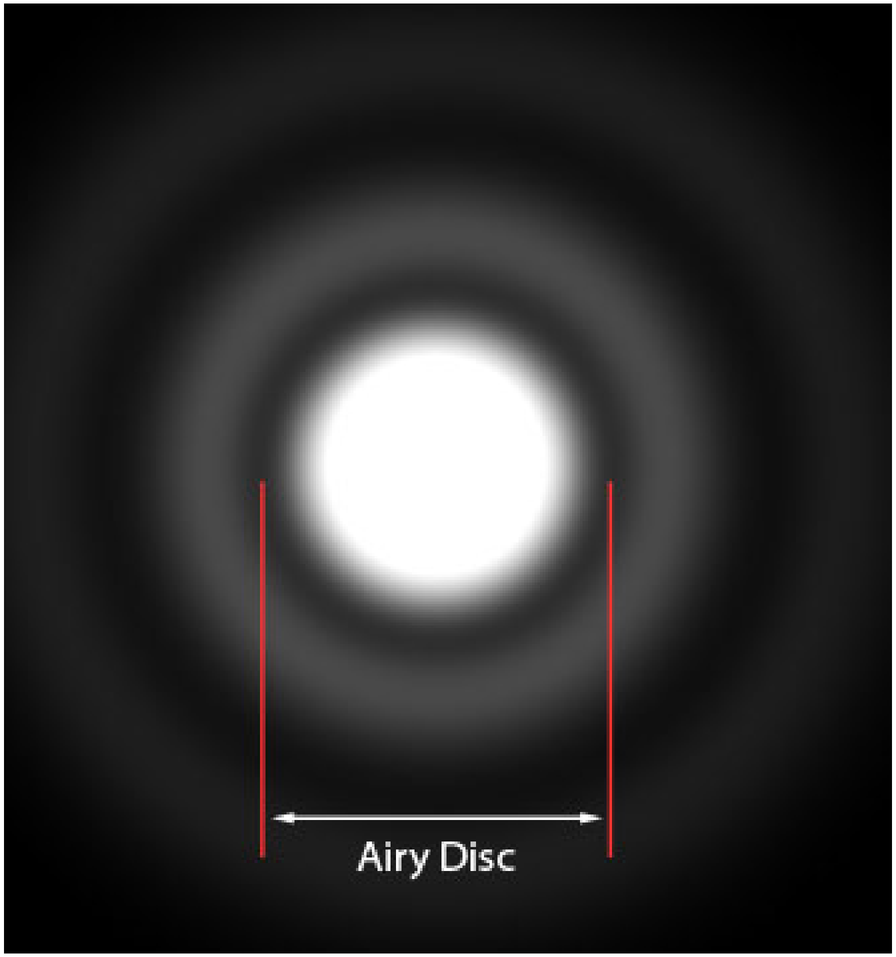


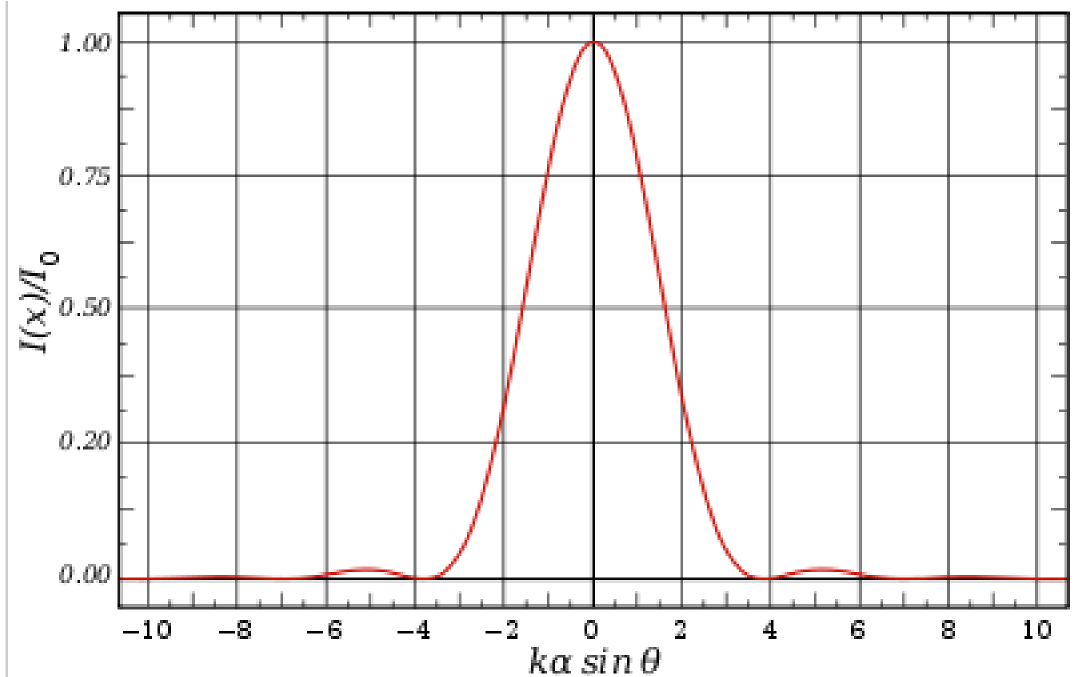
$$\text{Abbe resolution Limit}: \quad r_{xy} = \frac{\lambda_o}{2 \cdot NA}$$

$$r_z = \frac{n \cdot \lambda_o}{NA^2}$$

Airy Disc

Diffraction through a round aperture





$$I(\theta) = I_o \left(\frac{2 \bullet J_1(x)}{x} \right)^2$$

I = Intensity

$x = k \cdot a \cdot \sin(\theta)$

$k = 2 \cdot \pi / \lambda$

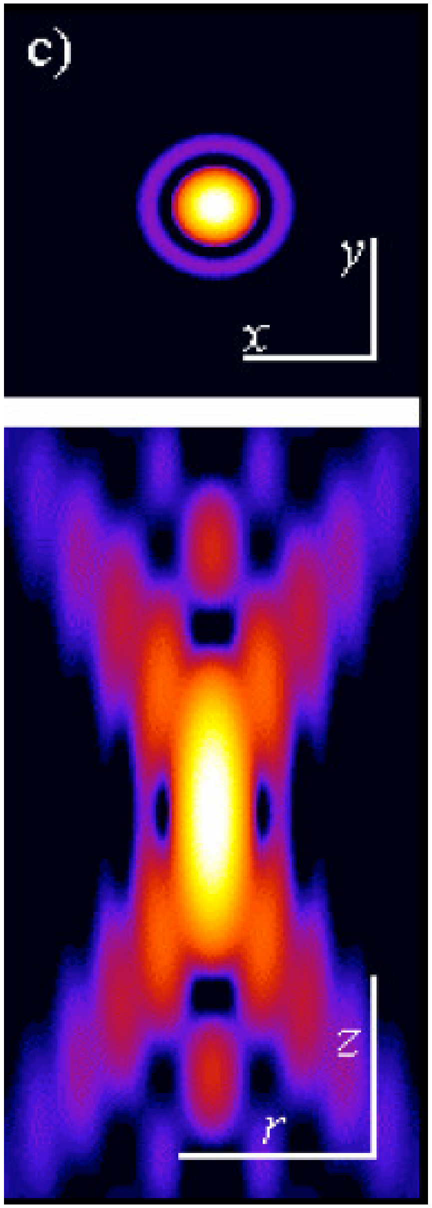
a = aperture radius

θ = angle from center of aperture to evaluation point

J_1 = Bessel function of the first kind, first order

Point Spread Functions

Measuring the resolution of your microscope



Diaspro et al. BioMedical Engineering OnLine 2006

Abbe resolution Limit : $r_{xy} = \dfrac{\lambda_0}{2 \cdot NA}$

$$r_z = \frac{n \cdot \lambda_0}{NA^2}$$

Point Spread Functions

Measuring the resolution of your microscope

Abbe resolution Limit : $r_{xy} = \dfrac{\lambda_o}{2 \cdot NA}$

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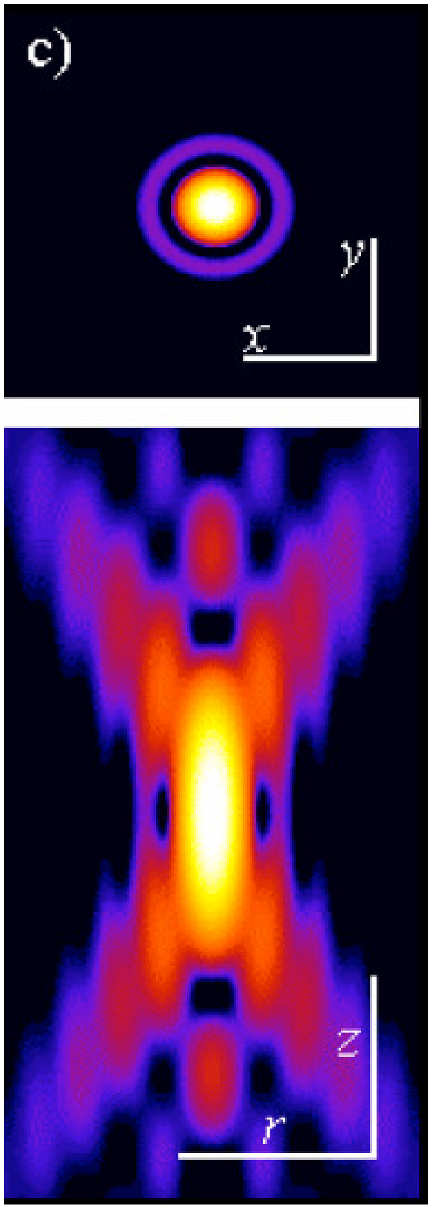
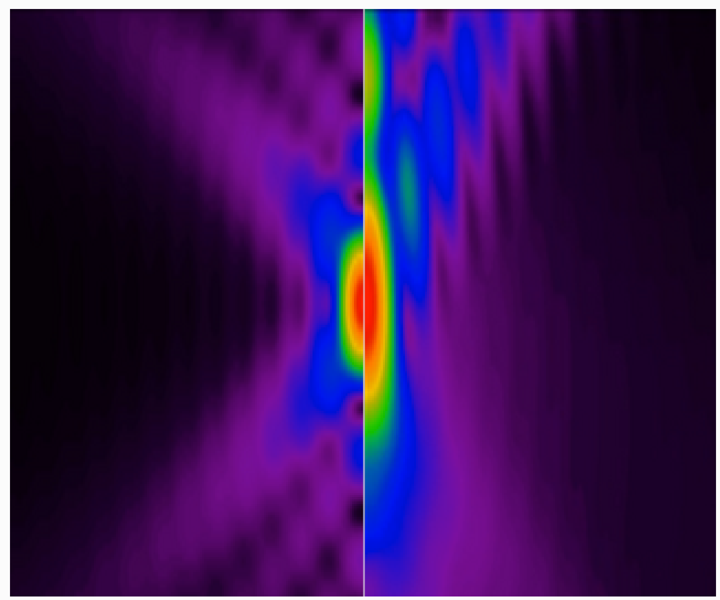


Diaspro et al. BioMedical Engineering OnLine 2006

Unaberrated Aberrated



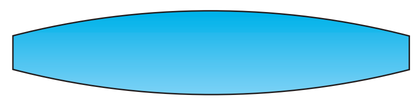
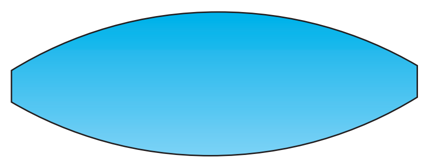
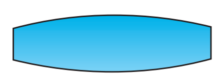
XZ (optical axis vertical) slice through the focus distribution of an Numerical Aperture = 1.3 lens. Left: no spherical aberration; right: imaging into a medium with refractive index 1.4 at a depth of 10 micron.

Taken from SVI website

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	λ = 500 nm Resolution 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

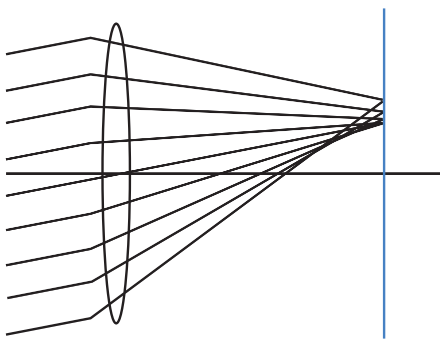
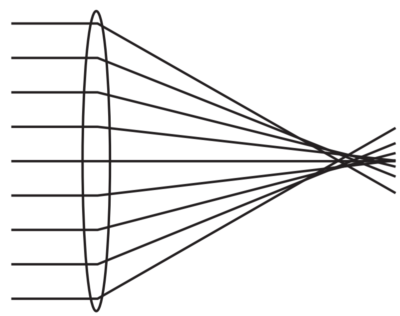
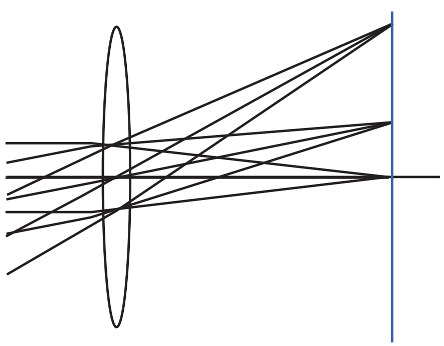
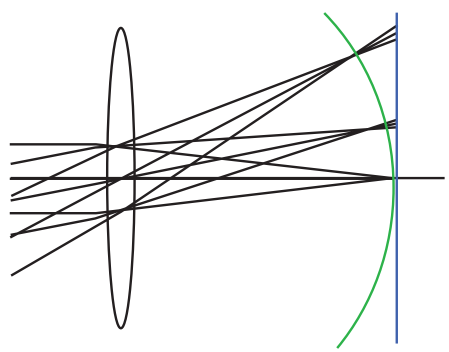
Optical Aberrations

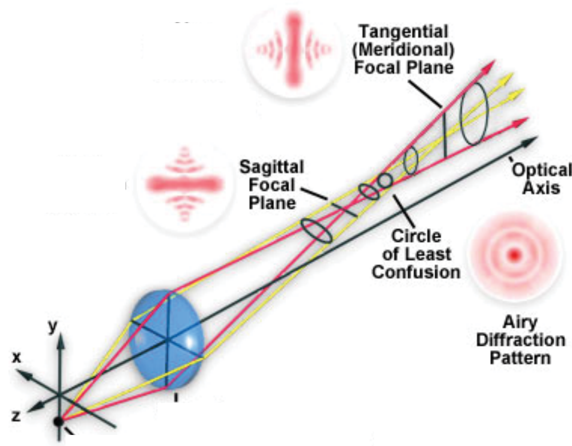
(Seidel abberations , aka third-order aberrations, monochromatic aberrations)

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

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

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

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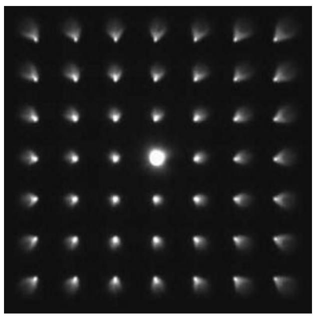
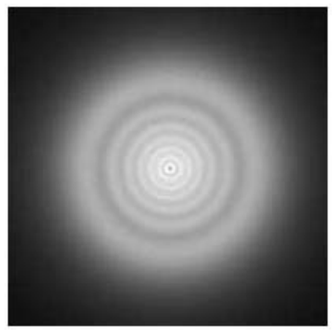
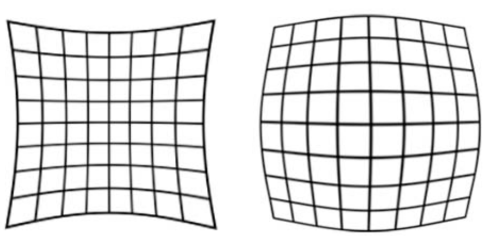
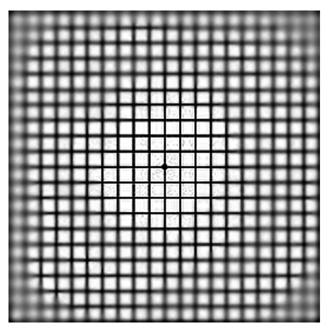
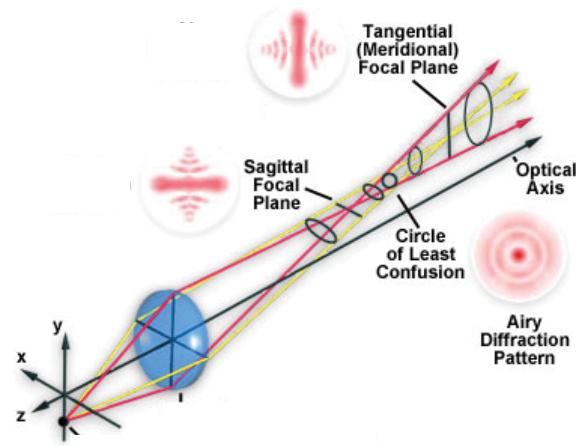
paraxial approximation

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

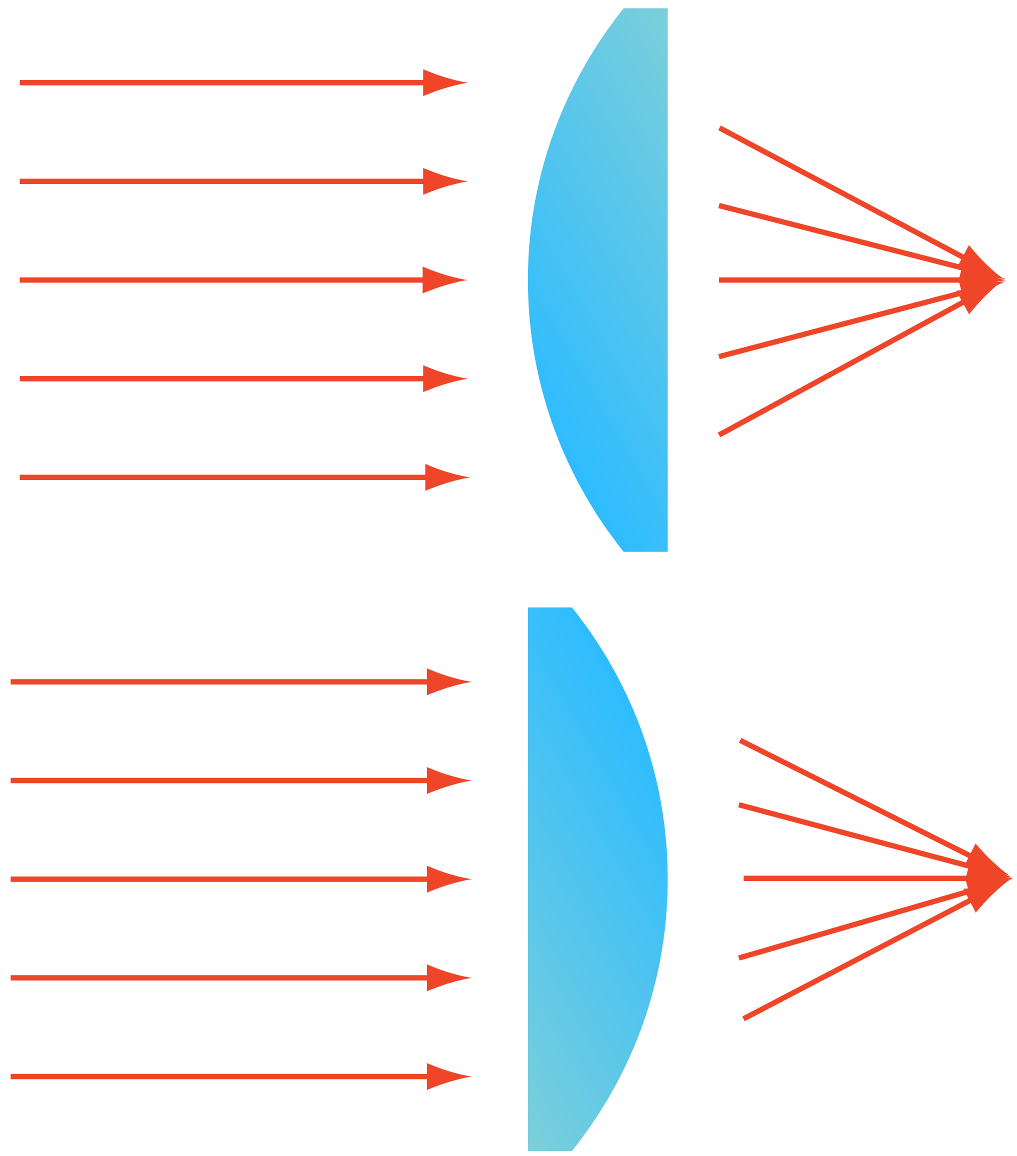


Astigmatism

*modified from MicroscopyU website

Minimizing Optical Aberrations

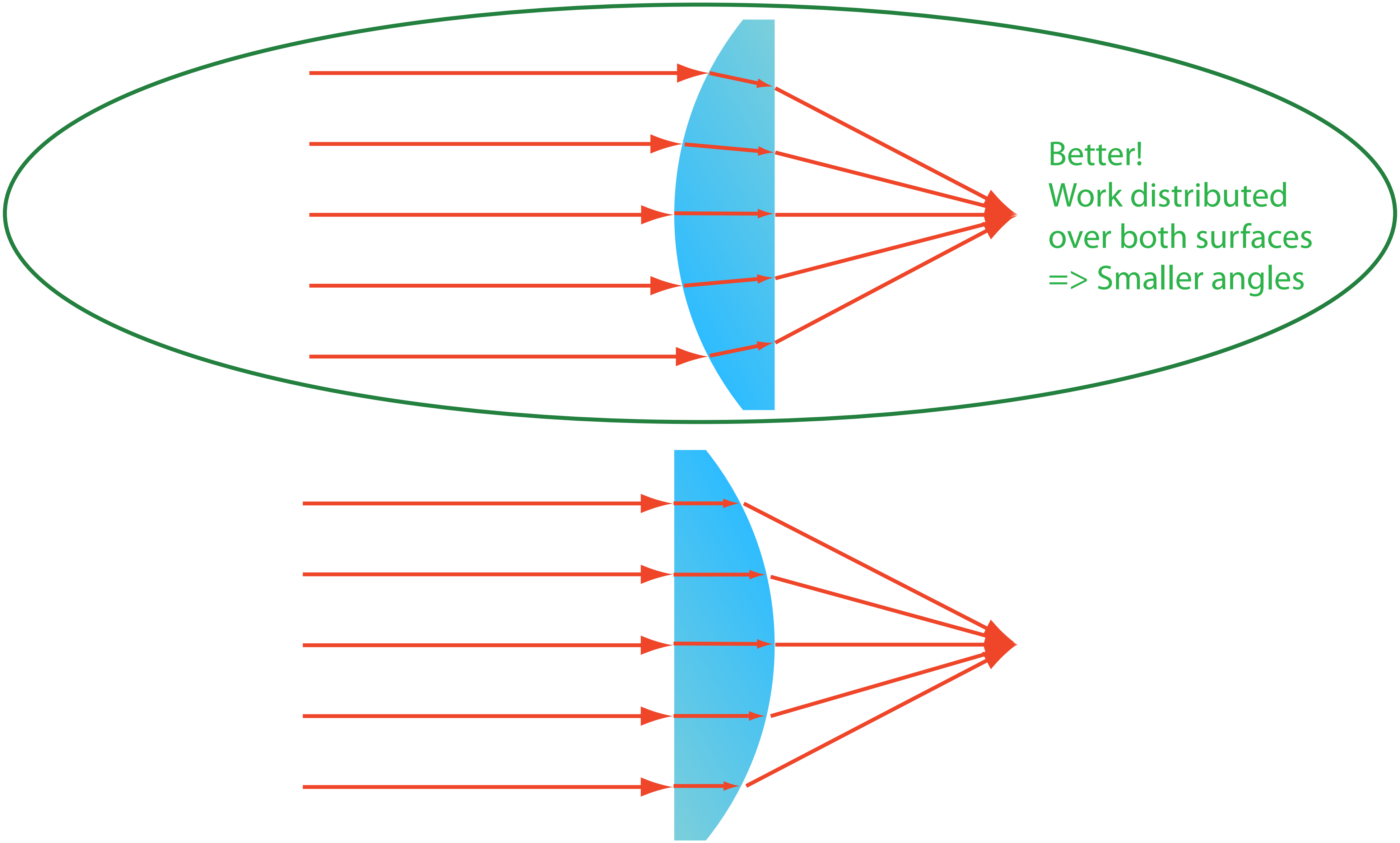
Which way should you insert this plano-convex lens ?



Minimizing Optical Aberrations

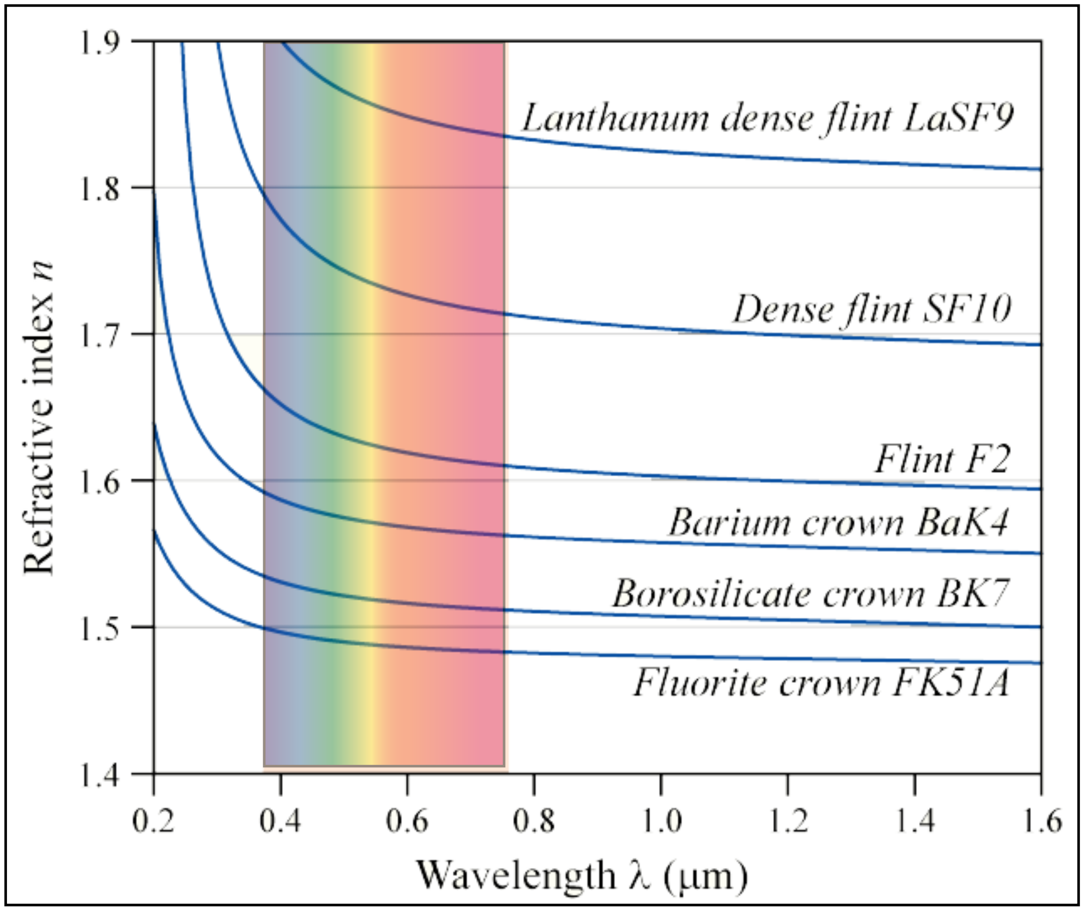
(Distribute optical power across multiple surfaces)

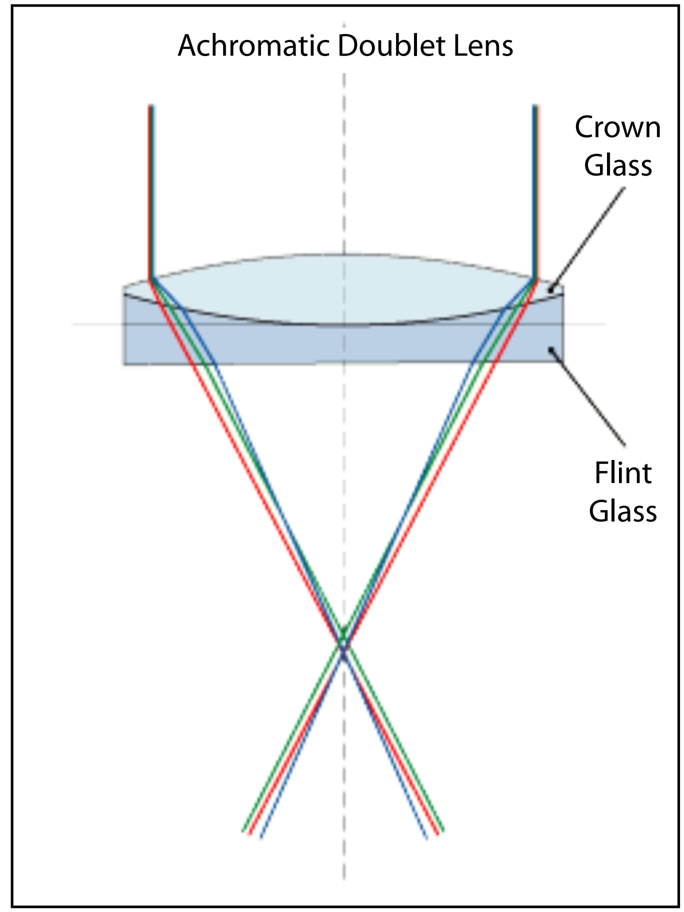
Which way should you insert this plano-convex lens ?



Chromatic Aberration

(Index of refraction is a function of wavelength)





Minimizing Optical Aberrations

(Distribute optical power across multiple surfaces)

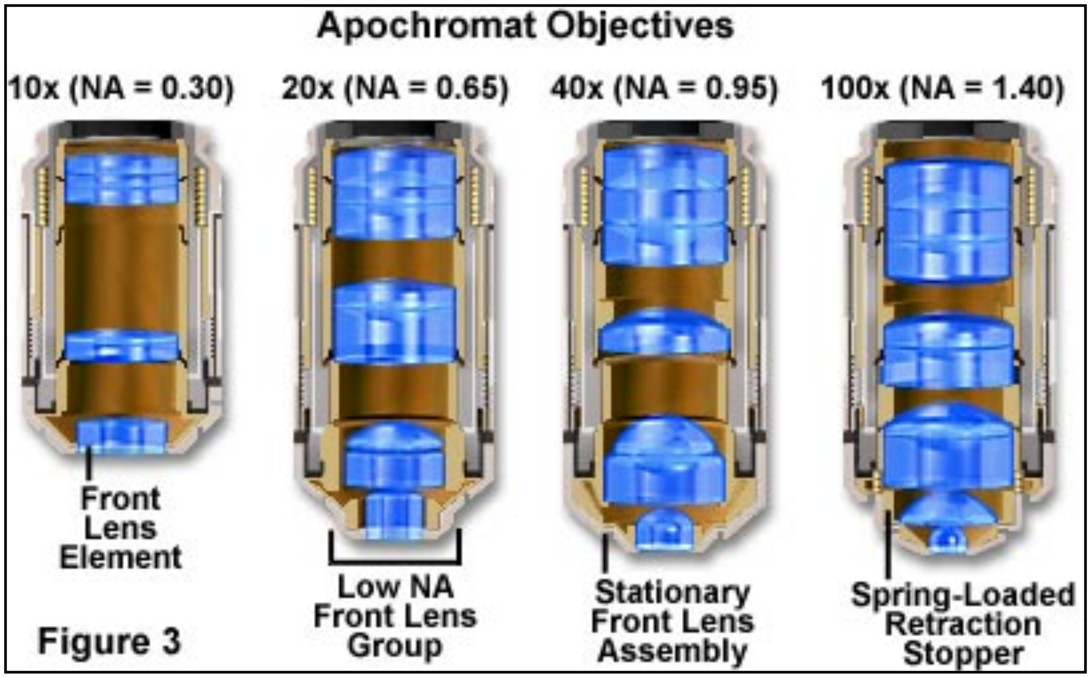
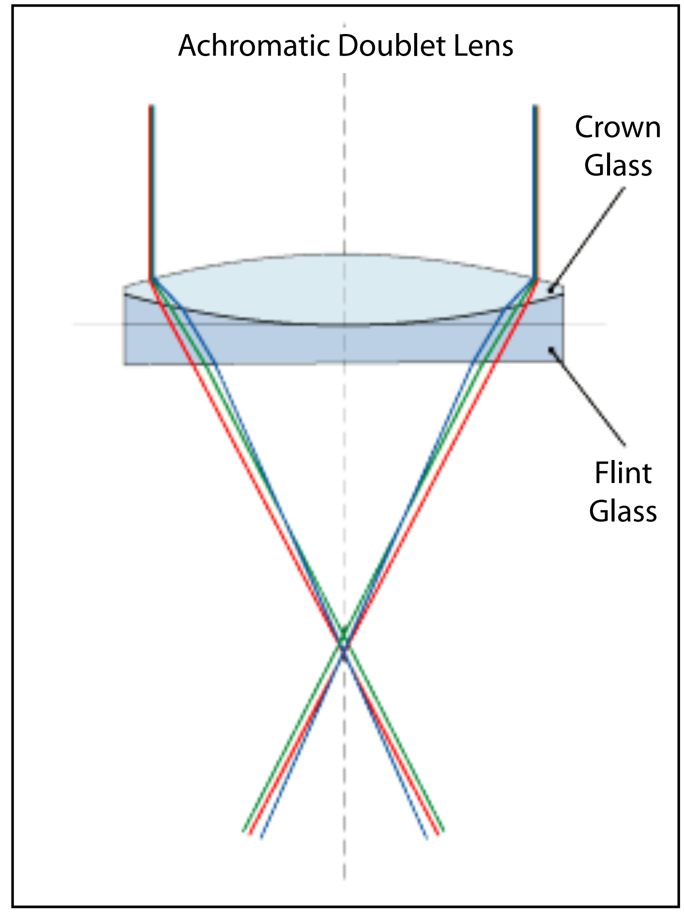


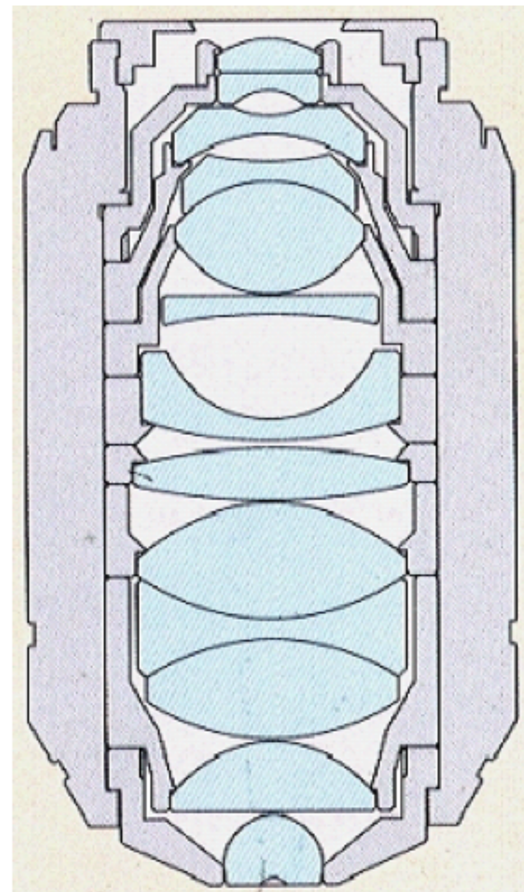
Image taken from Molecular Expressions website



Microscope Objectives

More $$$ = Better Aberration Correction

Figure 1



Microscope Objectives

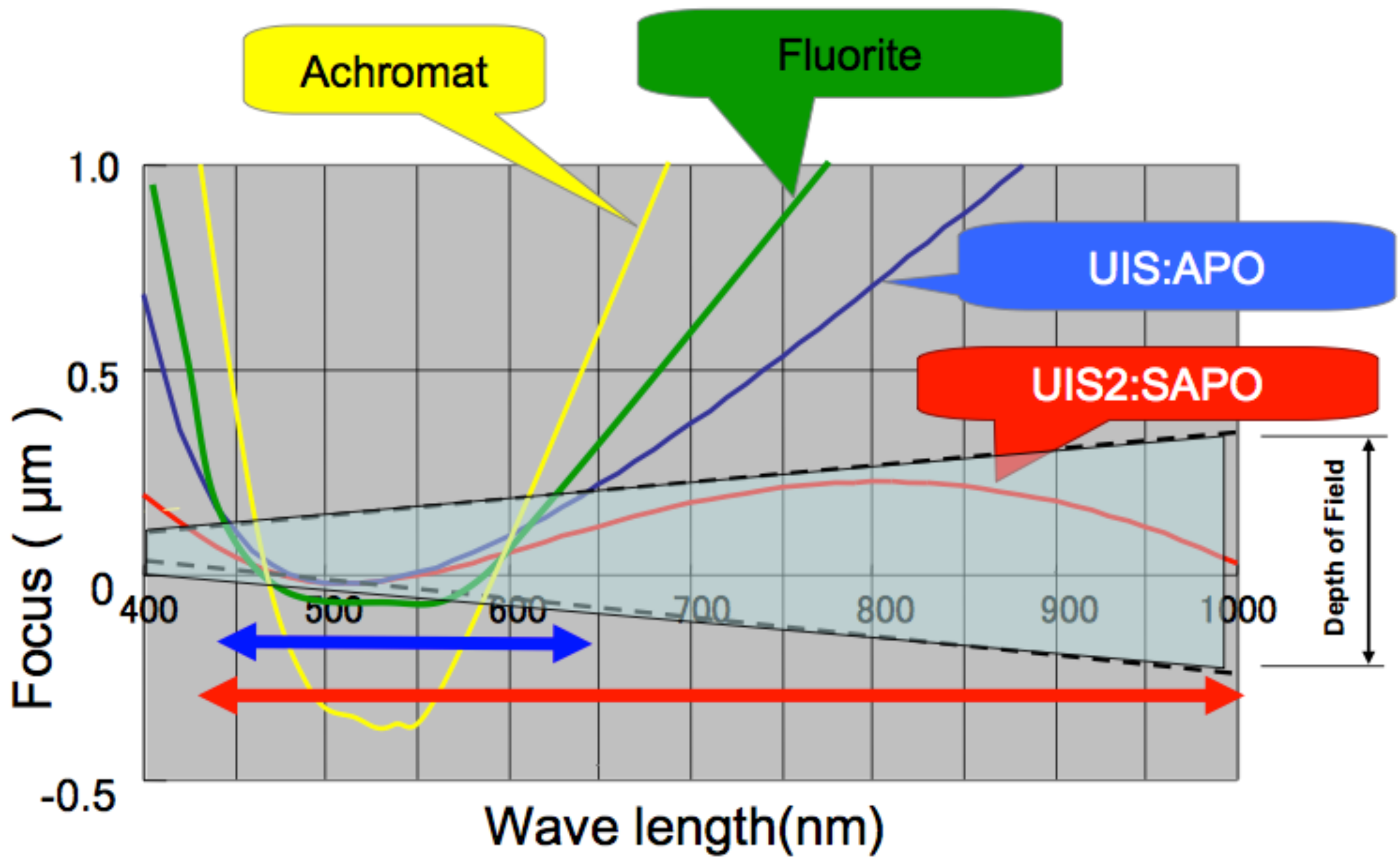
More $$$ = Better Aberration Correction

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, WI, 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.)

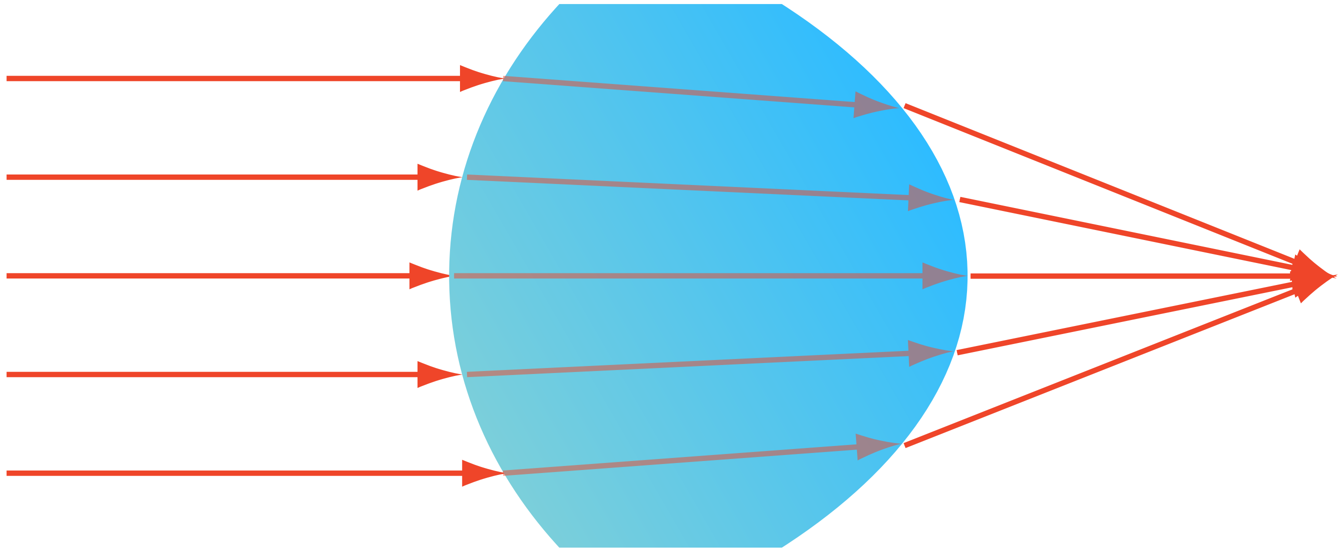
Approximations

Brutalizing optics into 3 limiting regimes

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

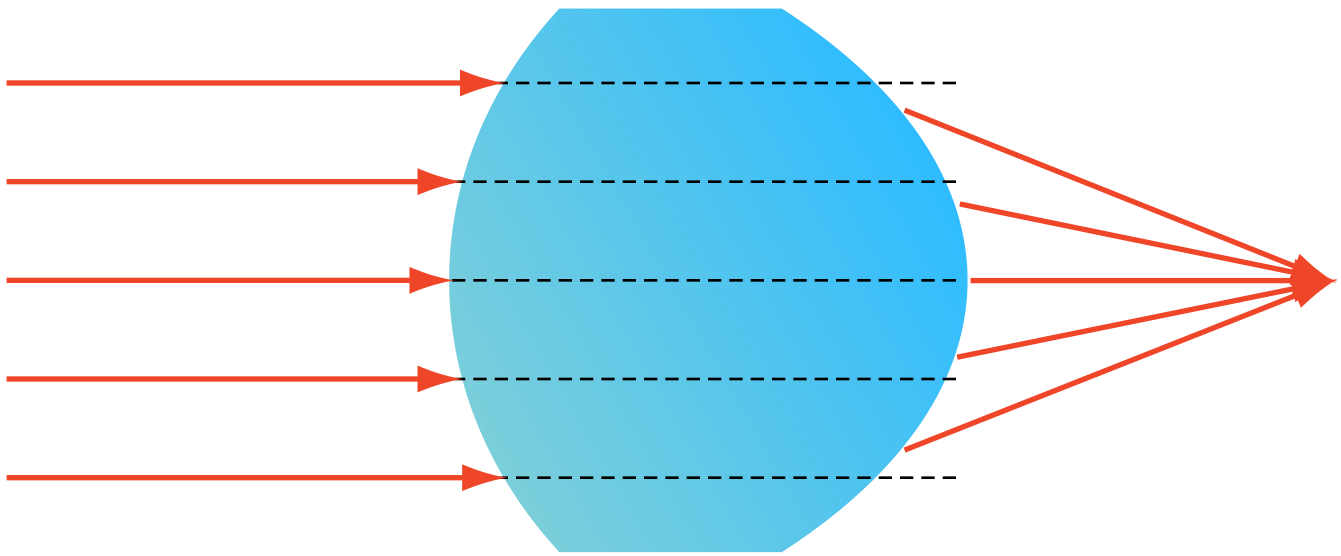
Thick Lenses

Focal length is measured from principal planes



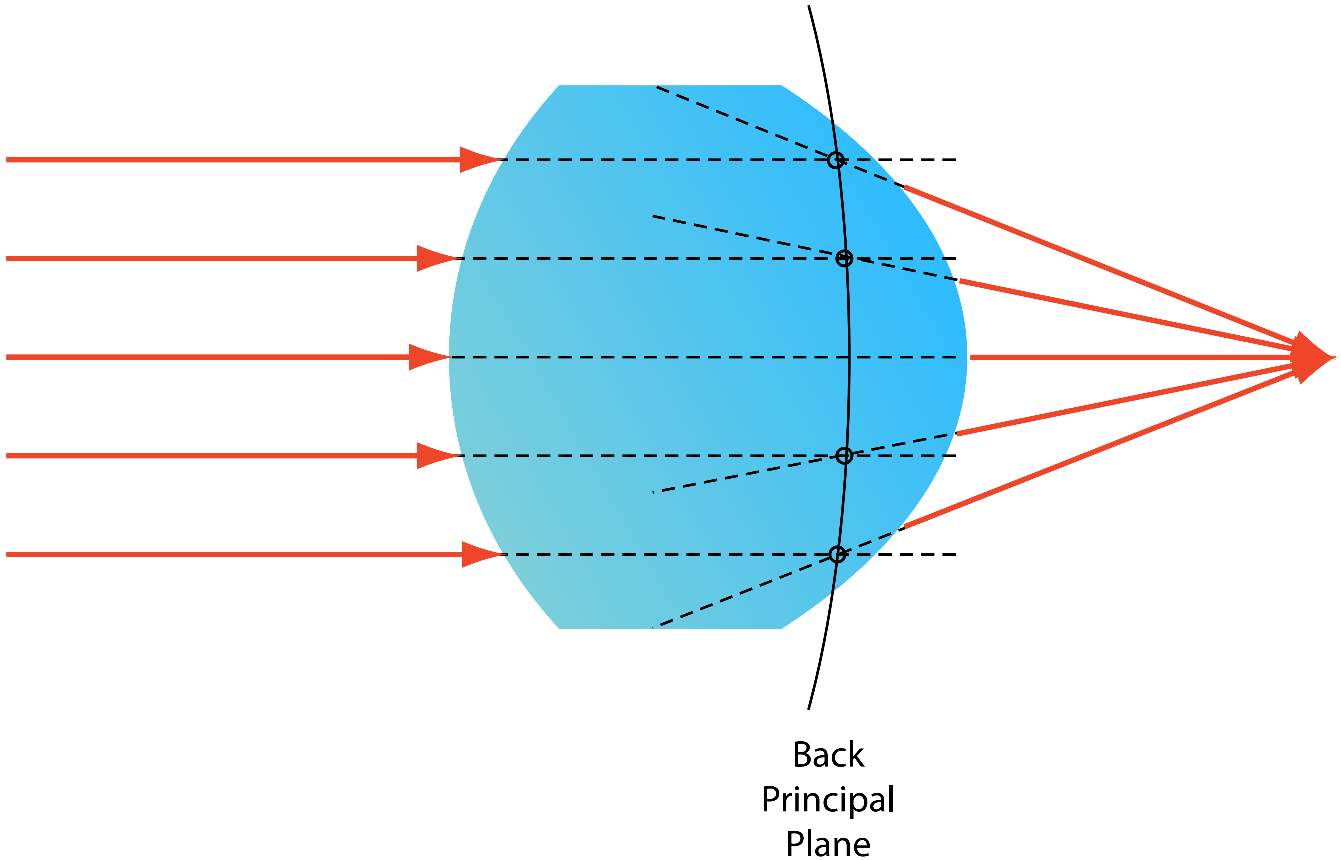
Thick Lenses

Focal length is measured from principal planes



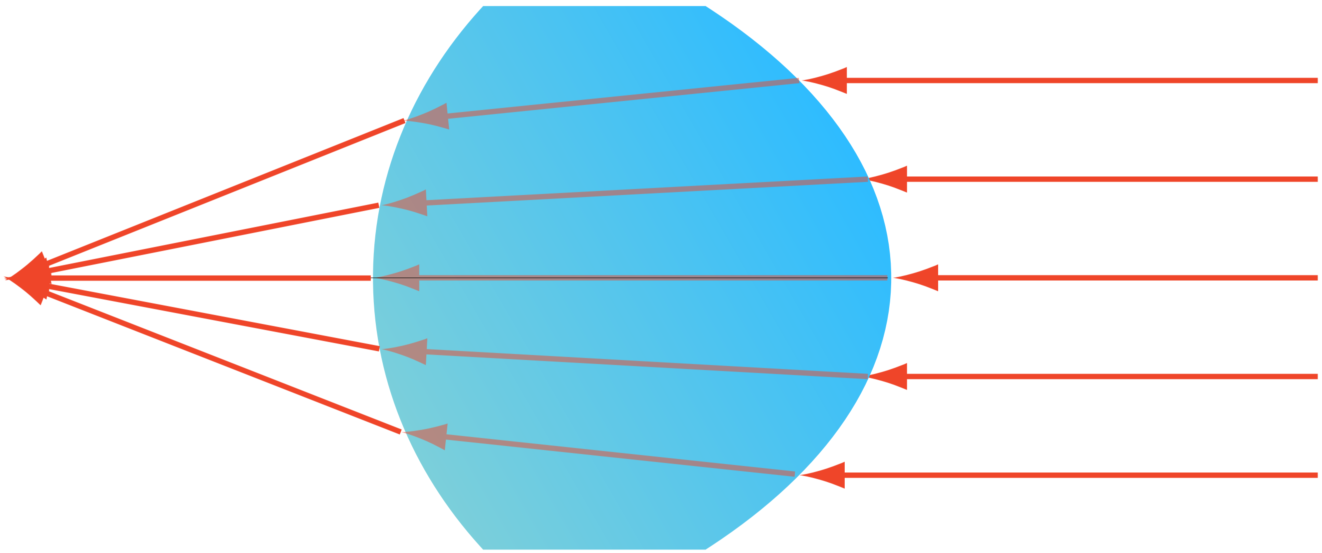
Thick Lenses

Focal length is measured from principal planes



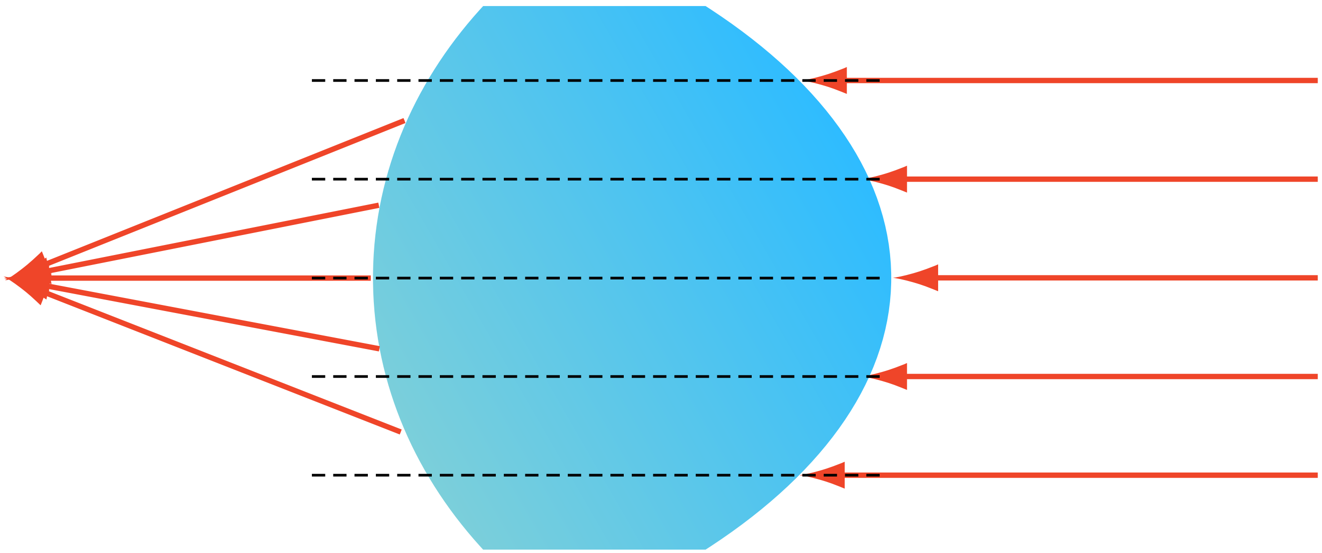
Thick Lenses

Focal length is measured from principal planes



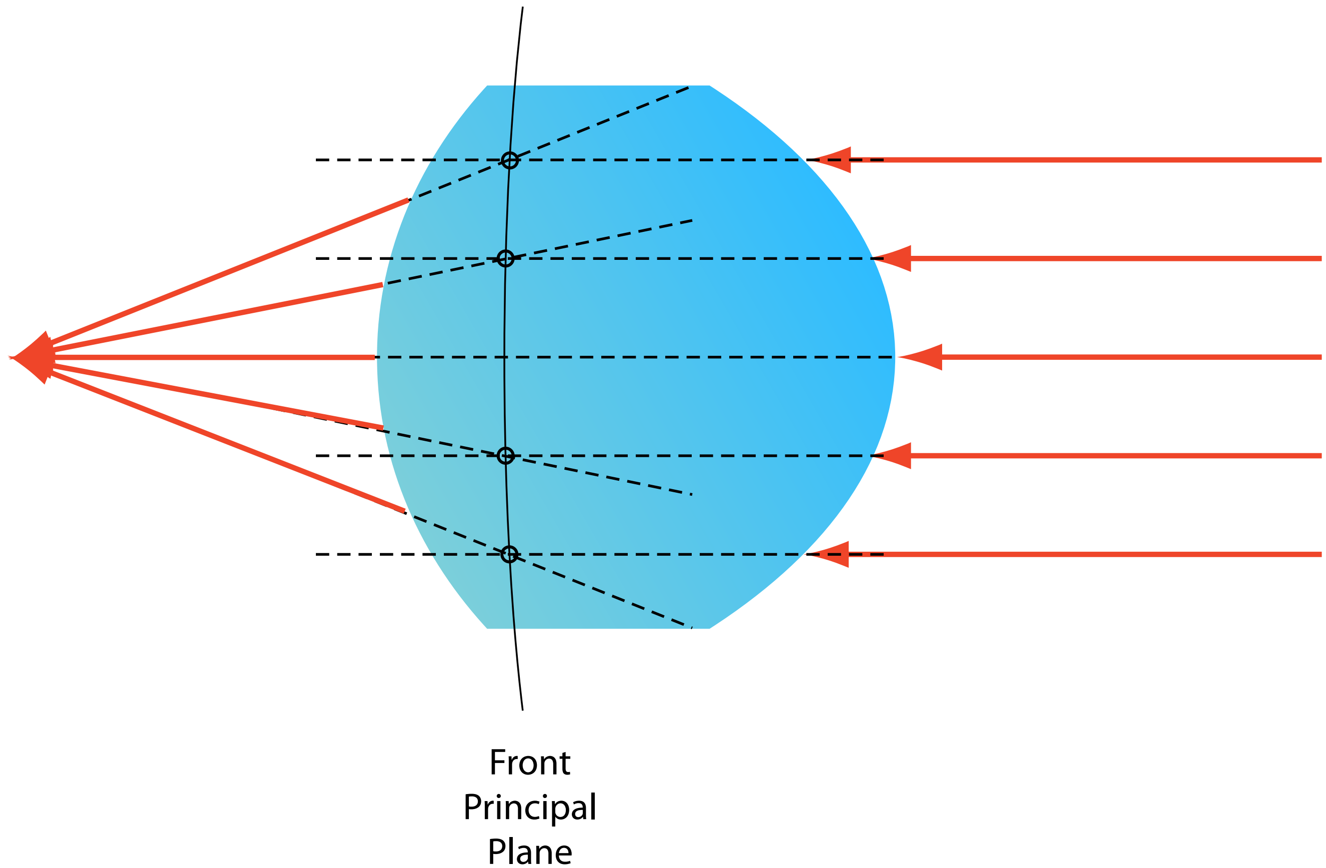
Thick Lenses

Focal length is measured from principal planes



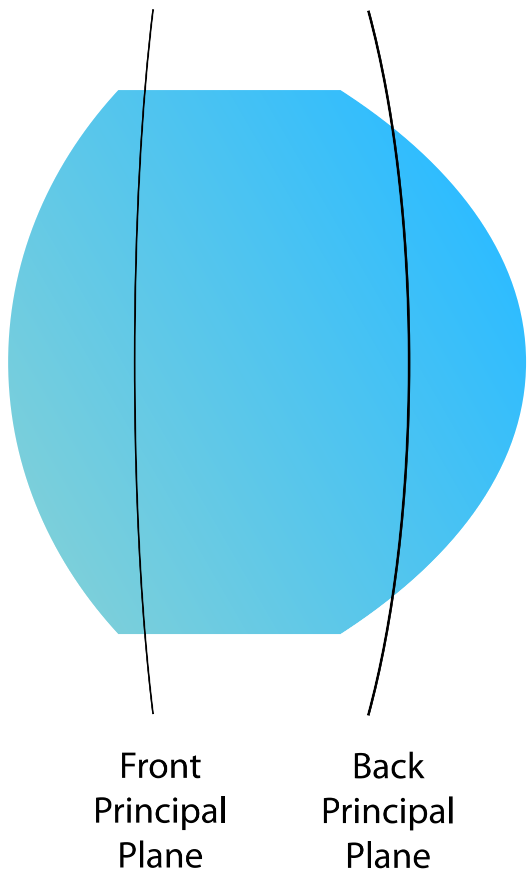
Thick Lenses

Focal length is measured from principal planes



Thick Lenses

Focal length is measured from principal planes



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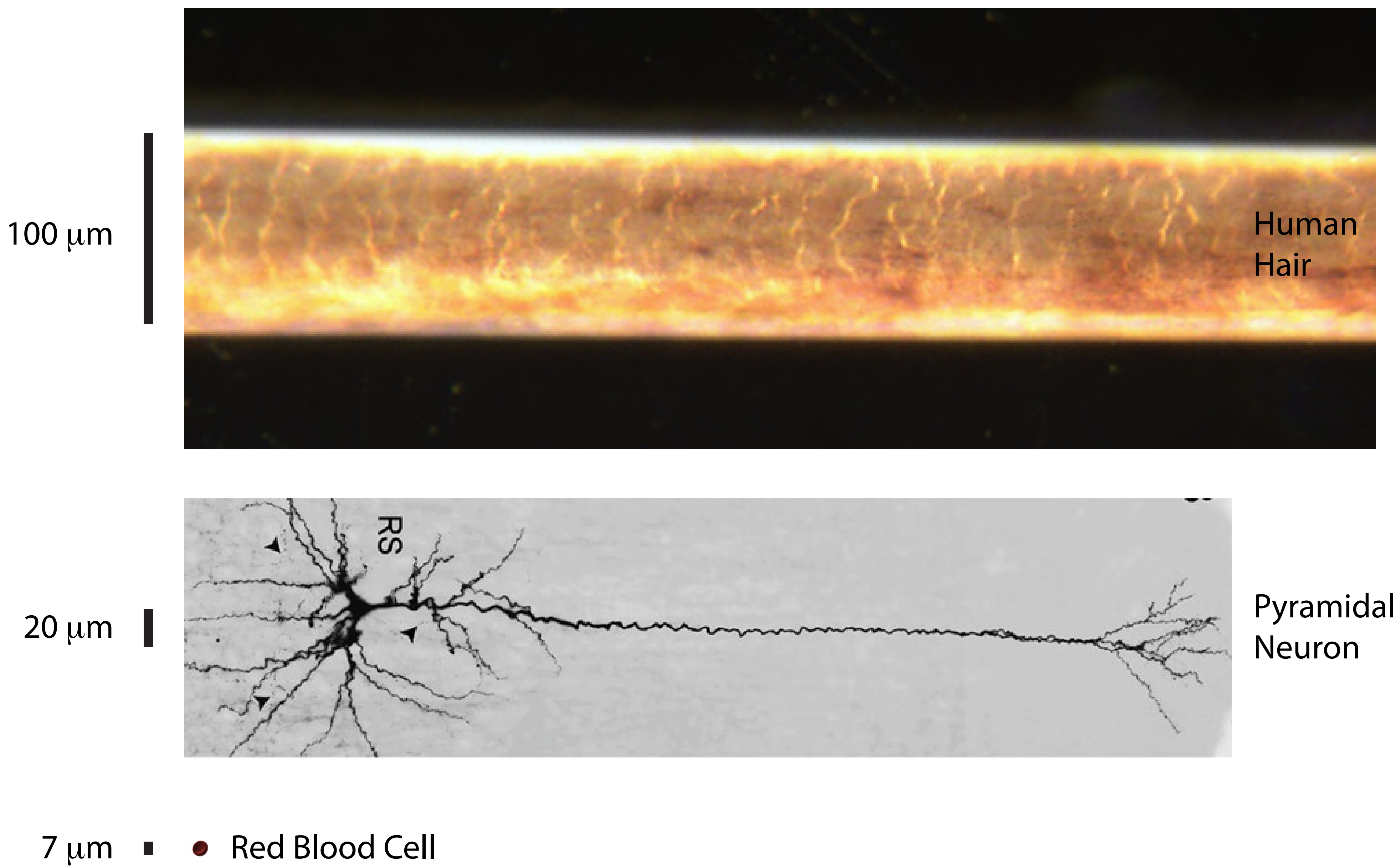
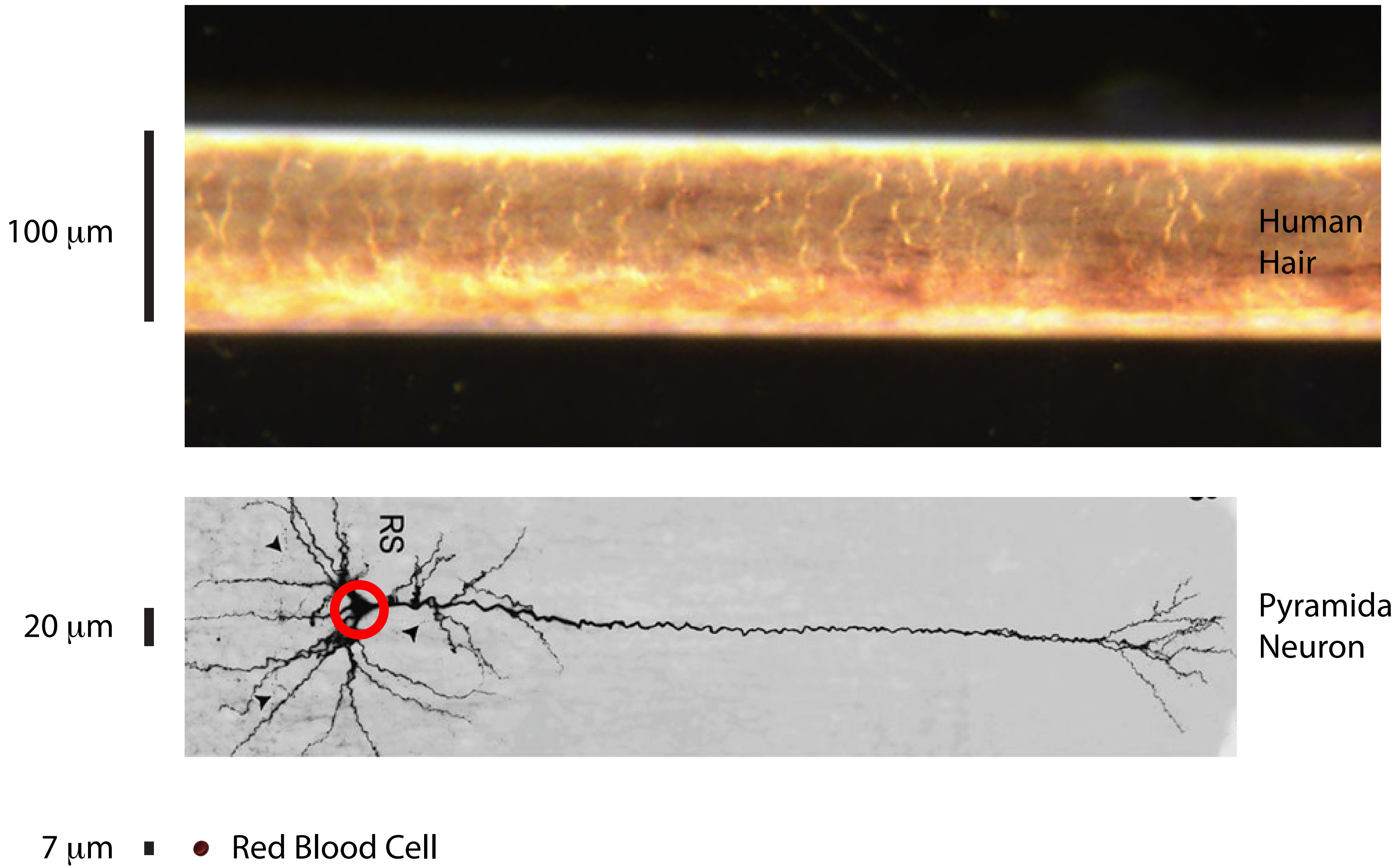
Sense of Scale



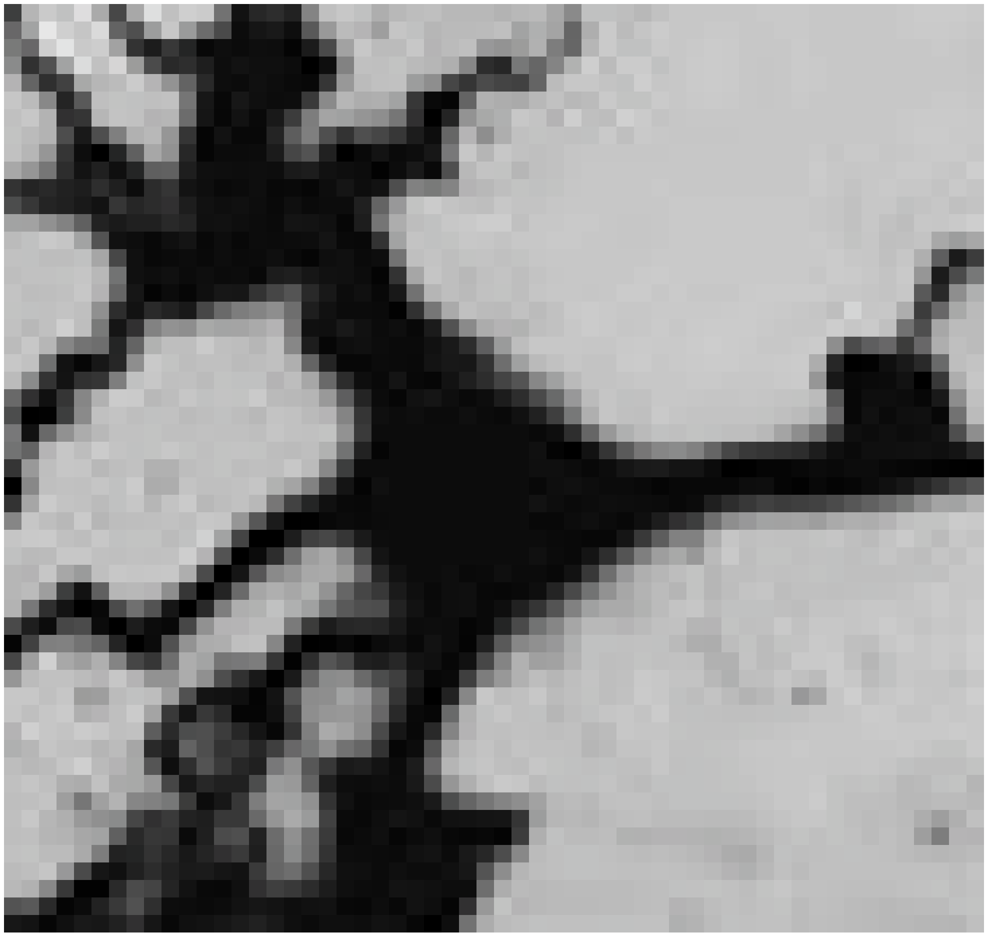
Sense of Scale



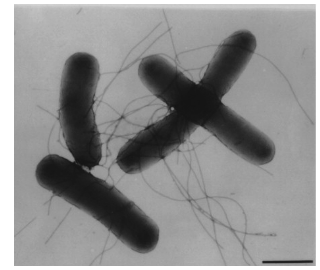
Sense of Scale

Pyramidal Neuron Cell Body (~10 μm)

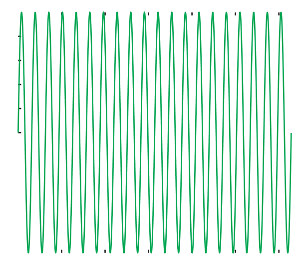
20 μm

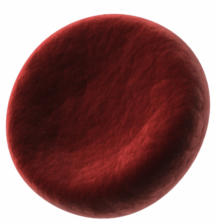


Bacterium (1 x 5 μm)



20 cycles of green light
($\lambda = 0.5$ μm)



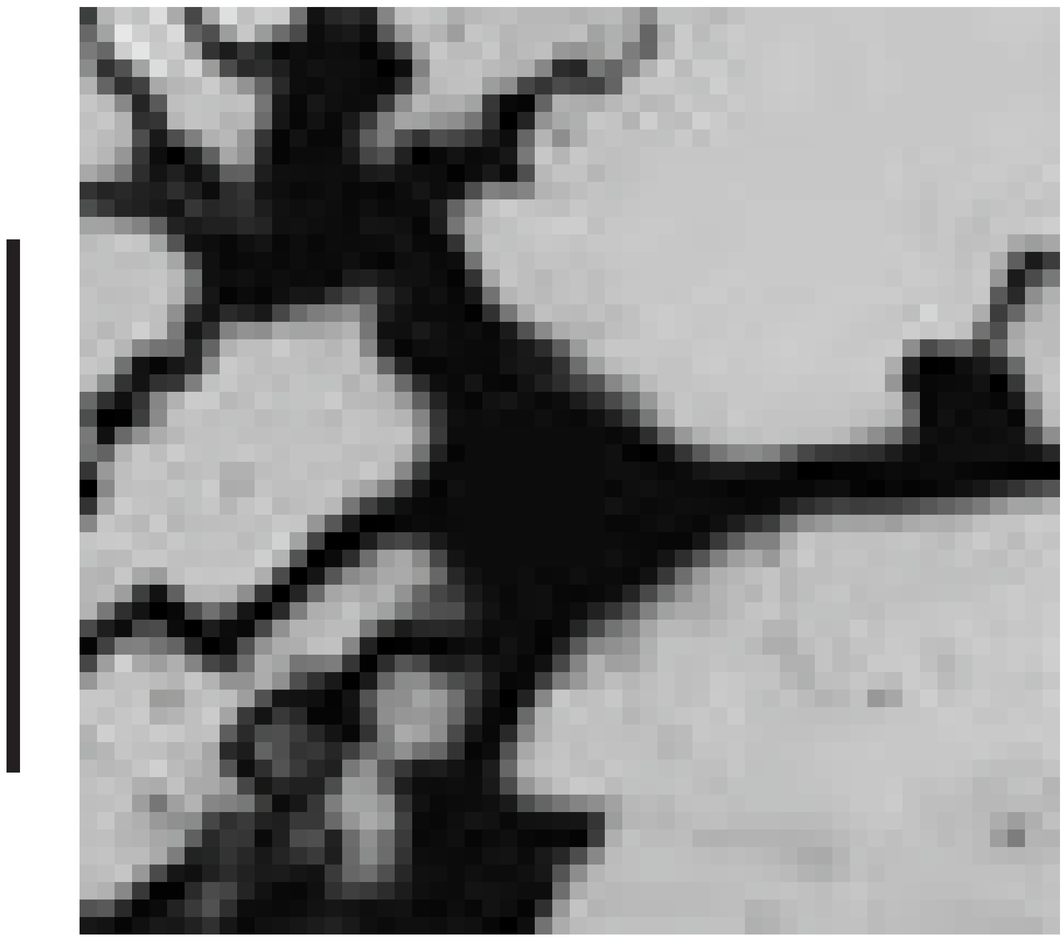


Red Blood Cell (7 μm)

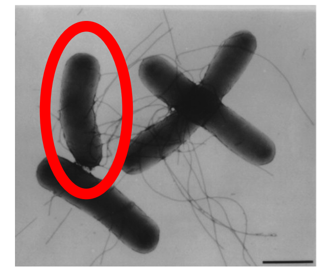
Sense of Scale

Pyramidal Neuron Cell Body (~10 μm)

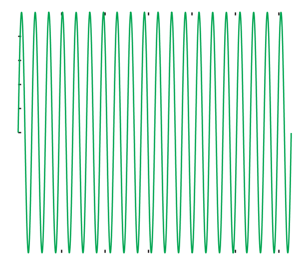
20 μm

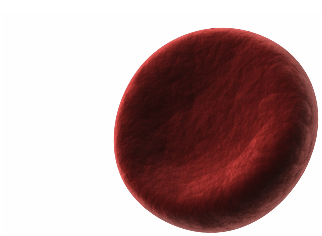


Bacterium (1 x 5 μm)



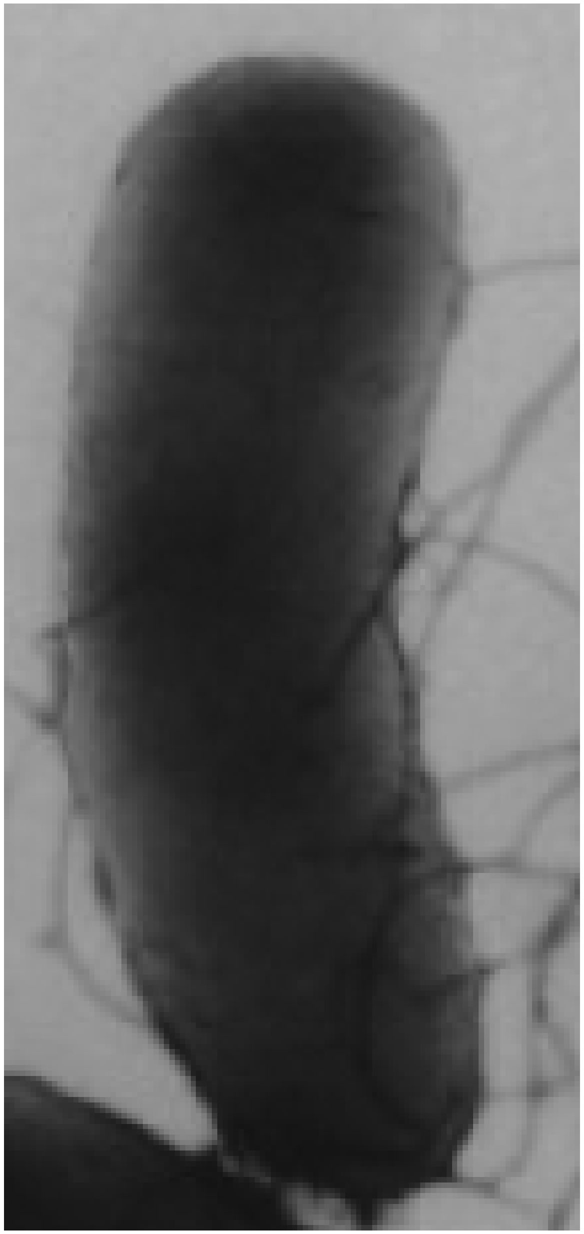
20 cycles of green light
(λ = 0.5 μm)

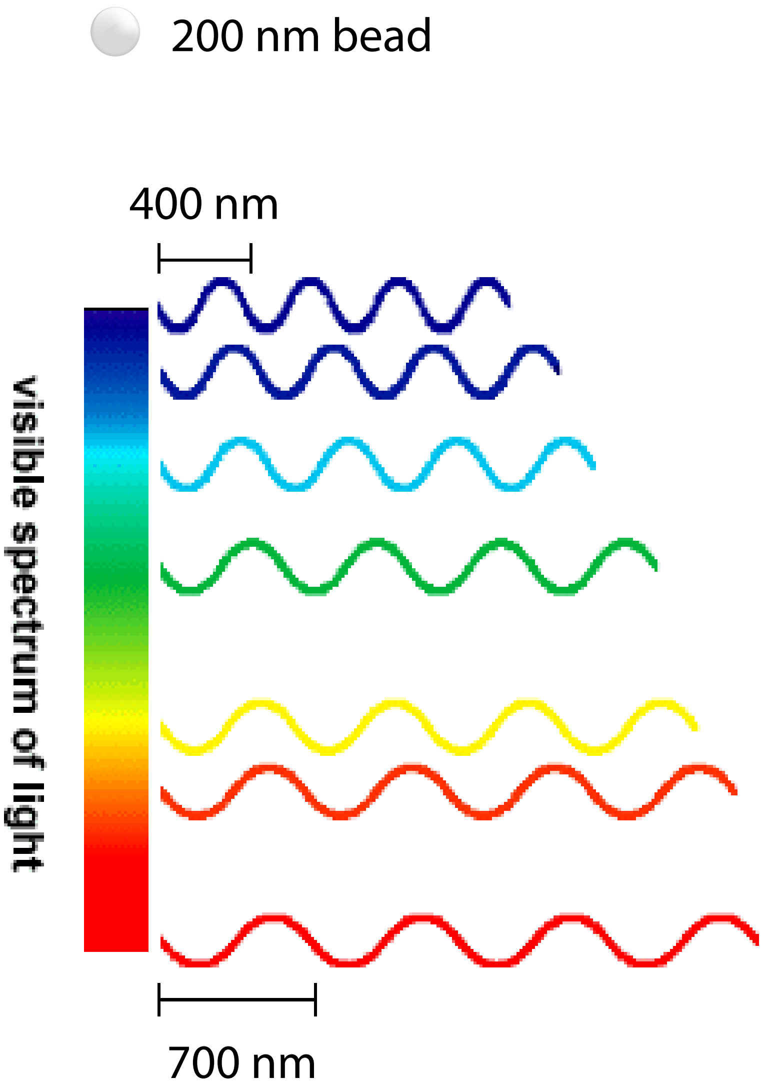




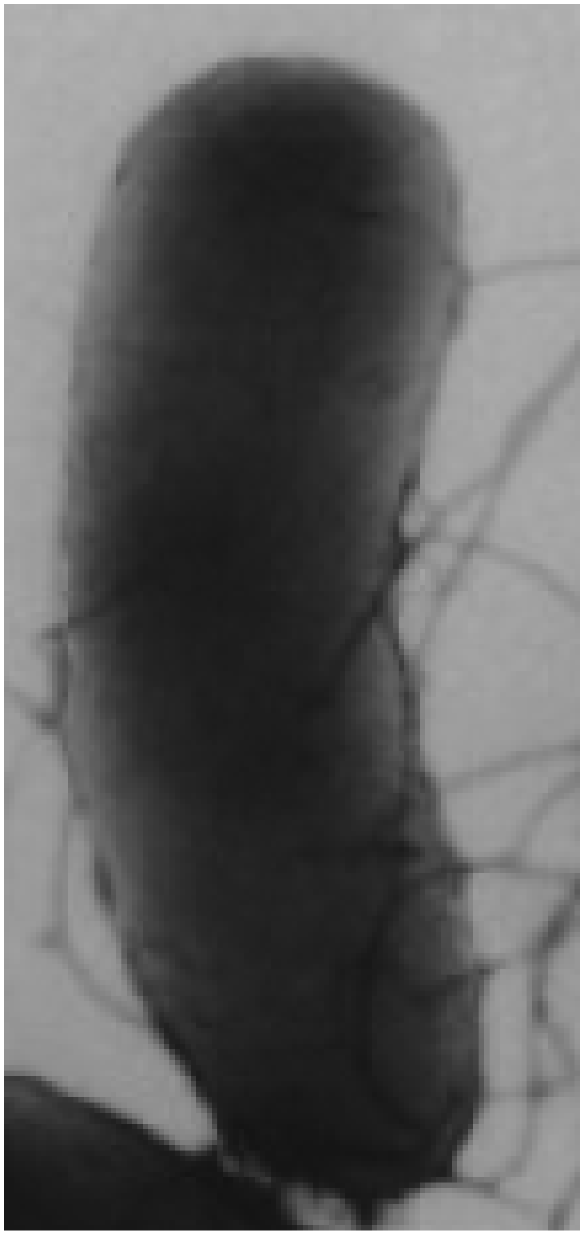
Red Blood Cell (7 μm)

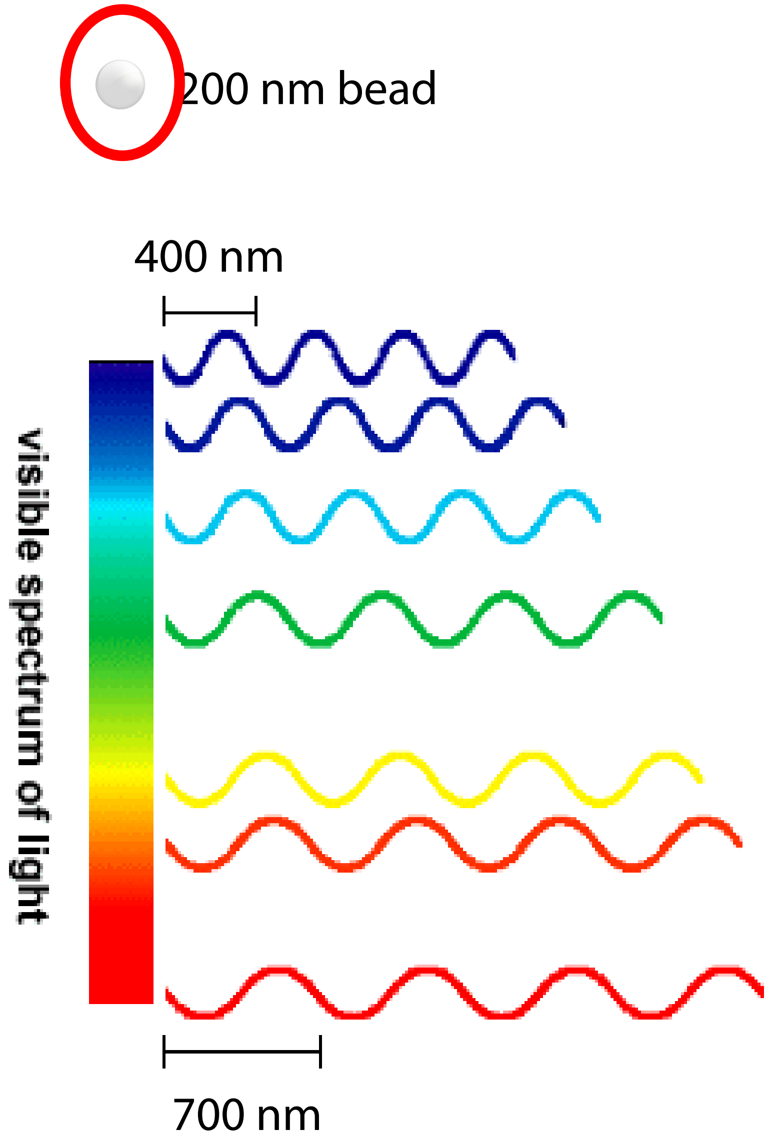
Sense of Scale





Sense of Scale





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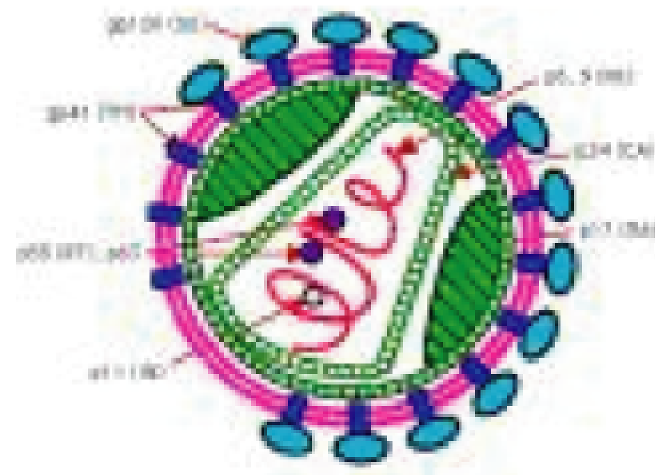
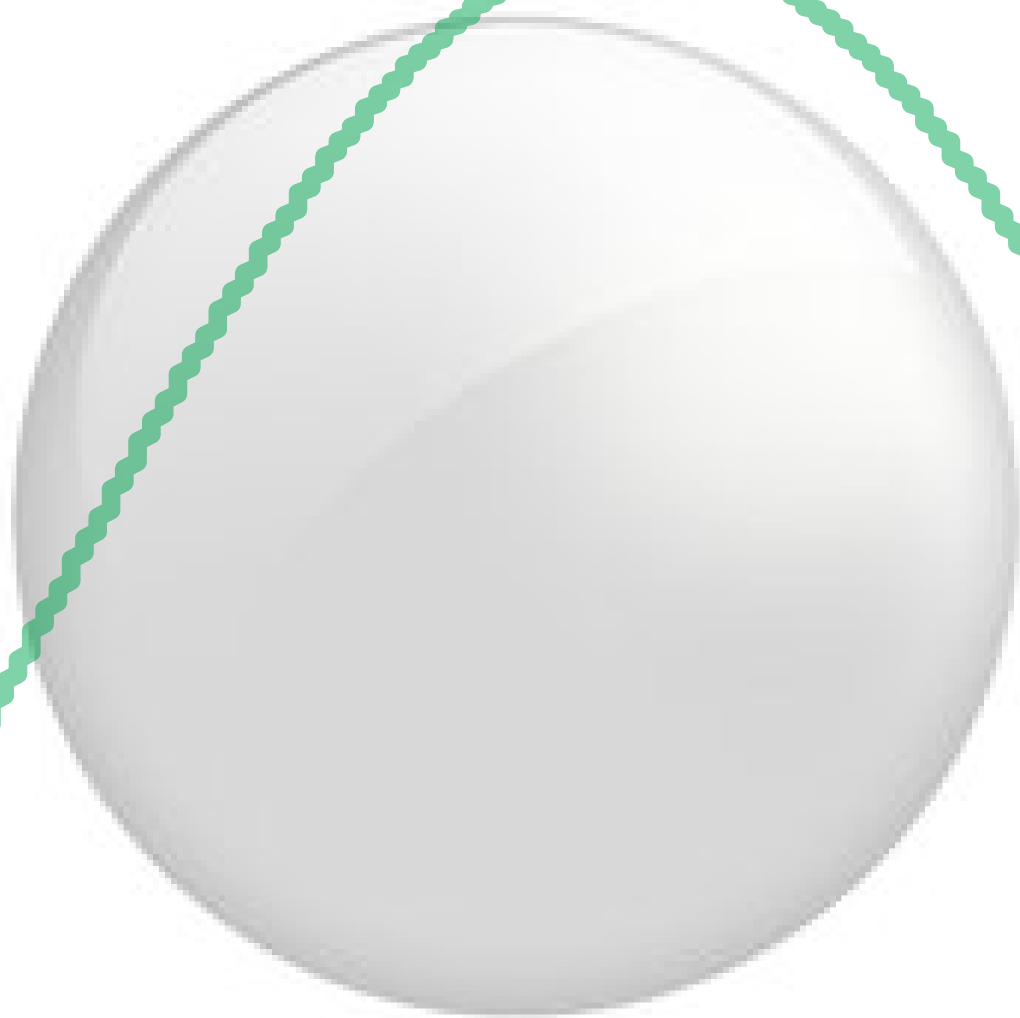
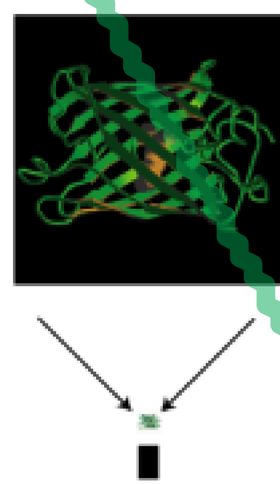


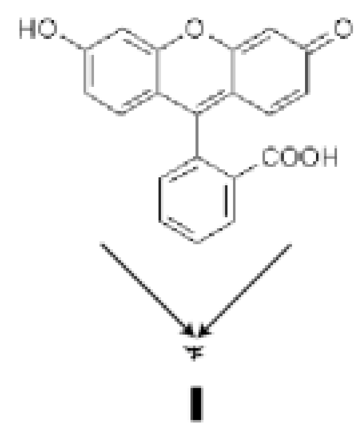
75 nm Virus Particle



200 nm bead



4 nm
Green Fluorescent
Protein



2 nm
Fluorescein
Molecule