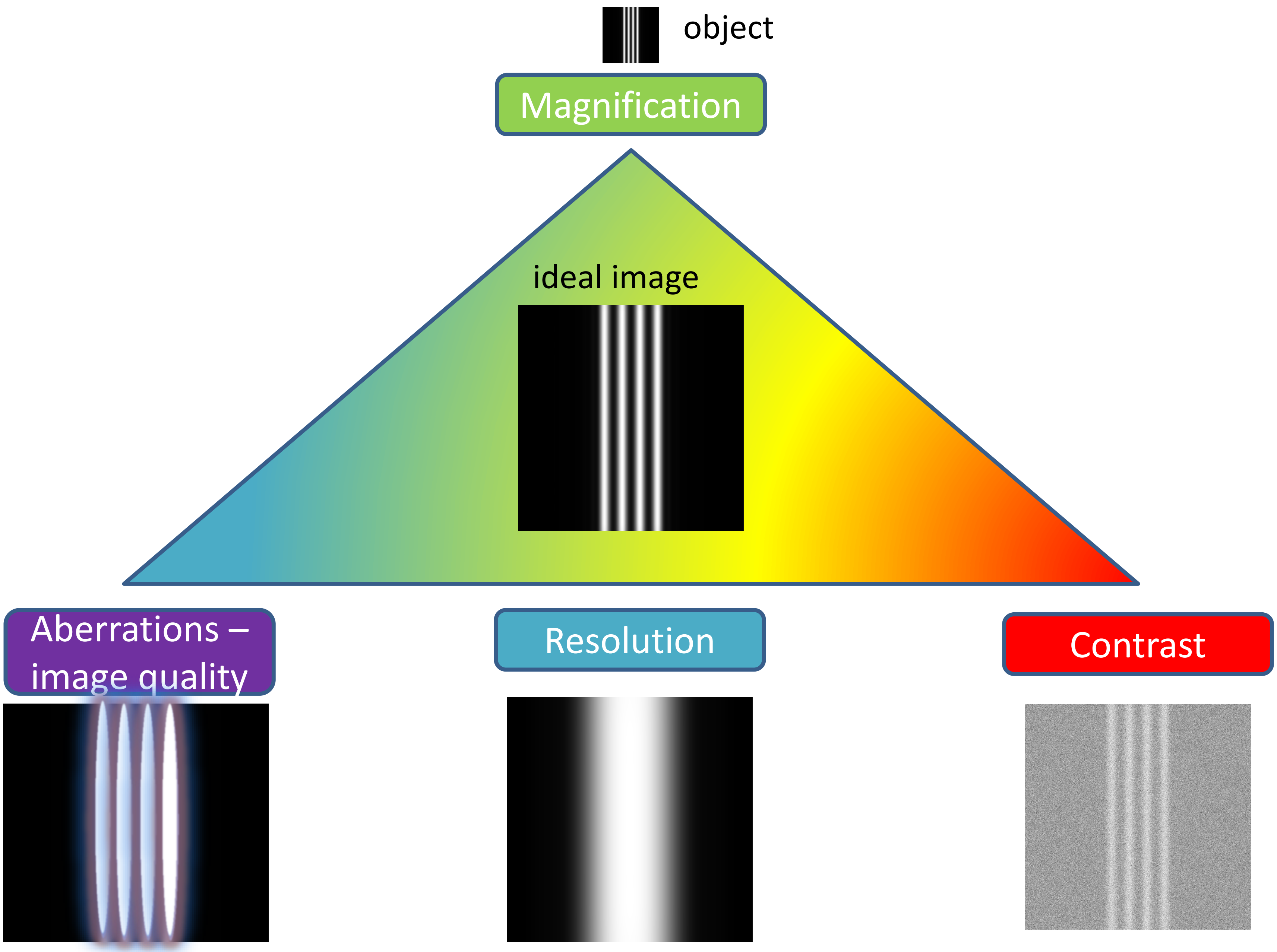
MICRO-561

Biomicroscopy I

Syllabus (tentative)

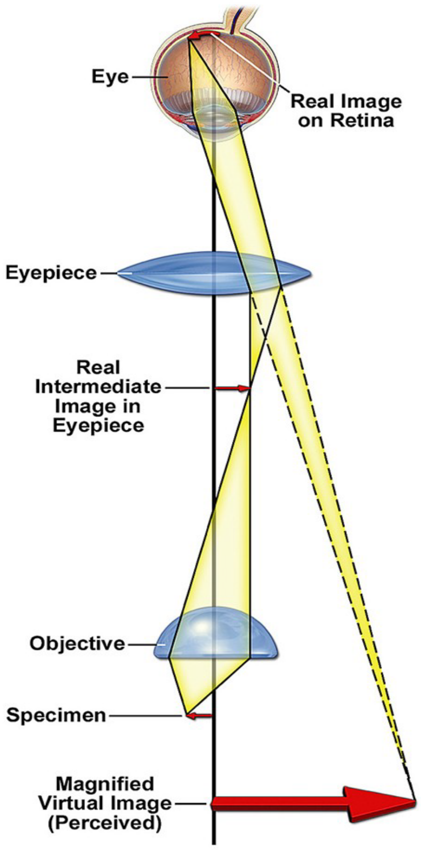
Lecture 1	Ray Optics-1
Lecture 2	Ray Optics-2 & Matrix Optics-1
Lecture 3	Matrix Optics-2
Lecture 4	Matrix Optics-3 & Microscopy Design-1
Lecture 5	Microscopy Design-2
Lecture 6	Microscopy Design-3
Lecture X	HOLIDAY
Lecture 7	Resolution-1
Lecture 8	Resolution-2
Lecture 9	Resolution-3
Lecture 10	Contrast & Fluorescence-1
Lecture 11	Fluorescence-2
Lecture 12	Sources & Filters
Lecture 13	Detectors
Lecture 14	Bio-application Examples

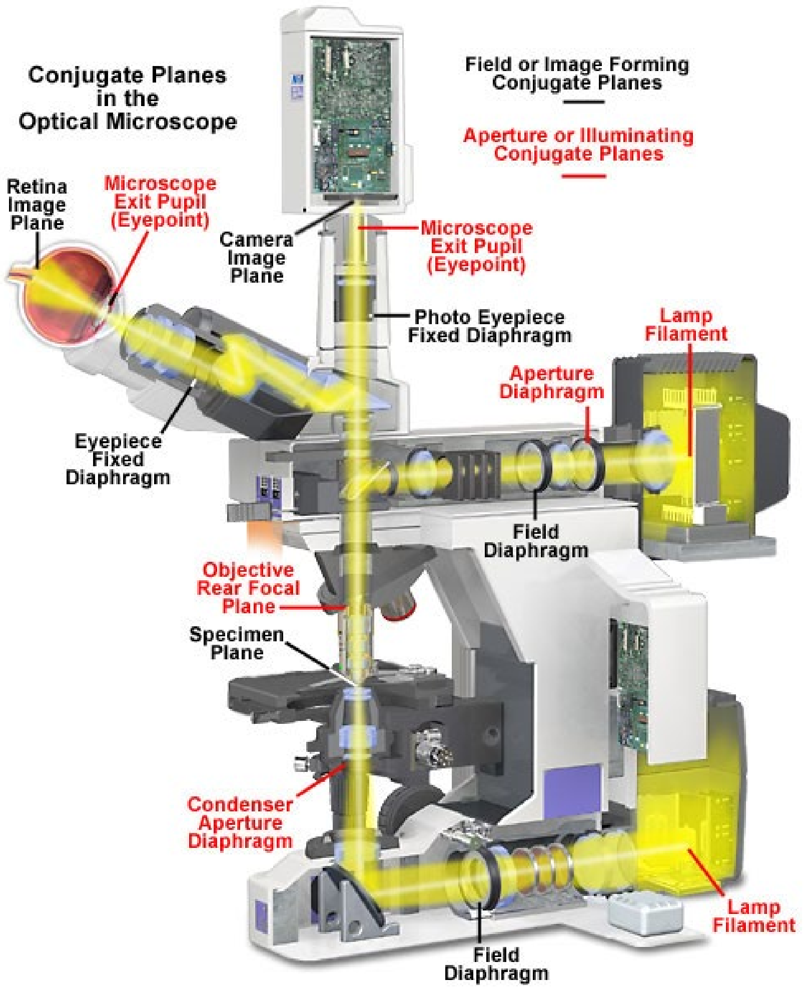
Important aspects for microscopy



Optical Microscope

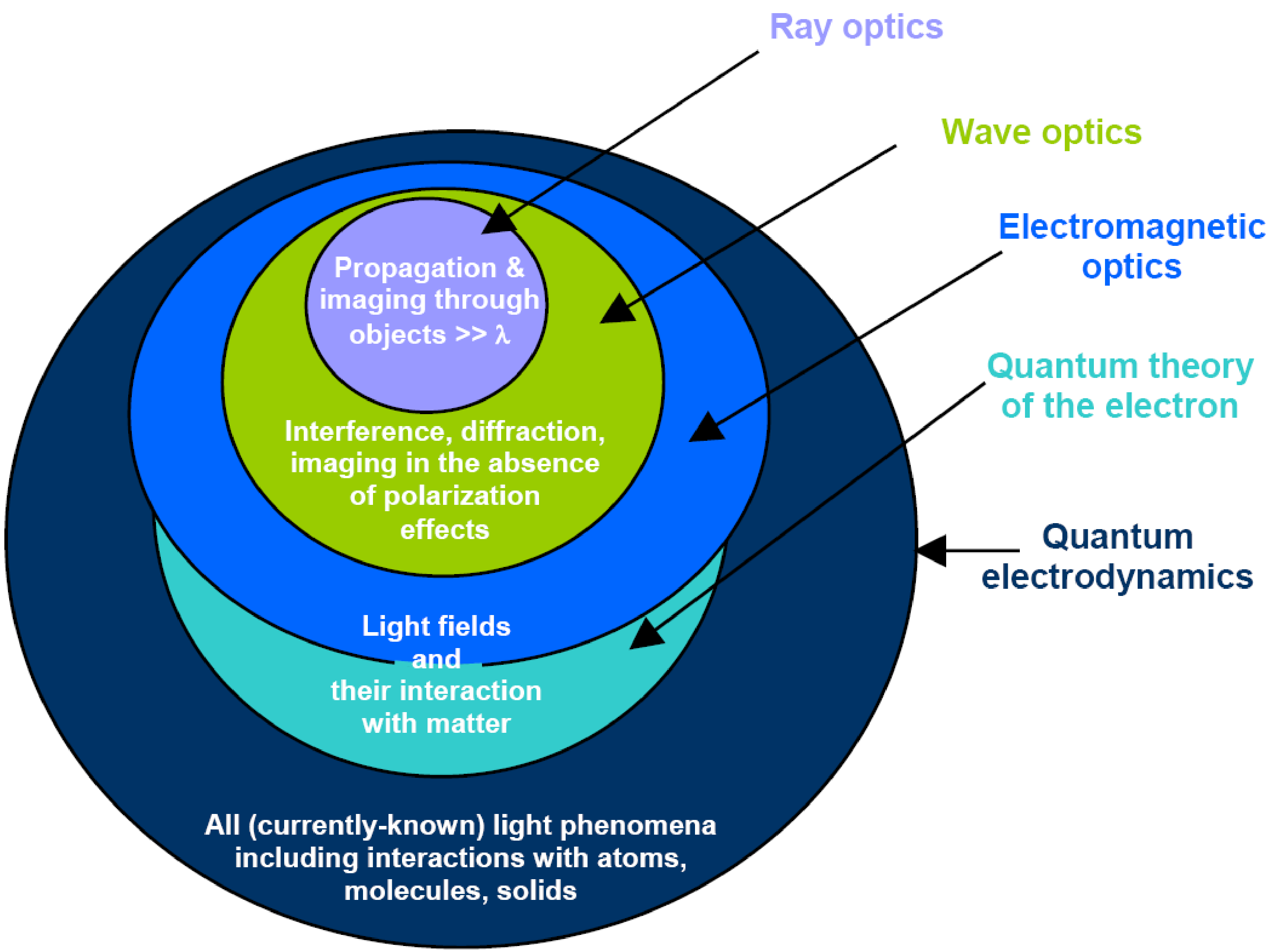
Optical train: Consists of optical components such as lenses, diaphragms, filters..



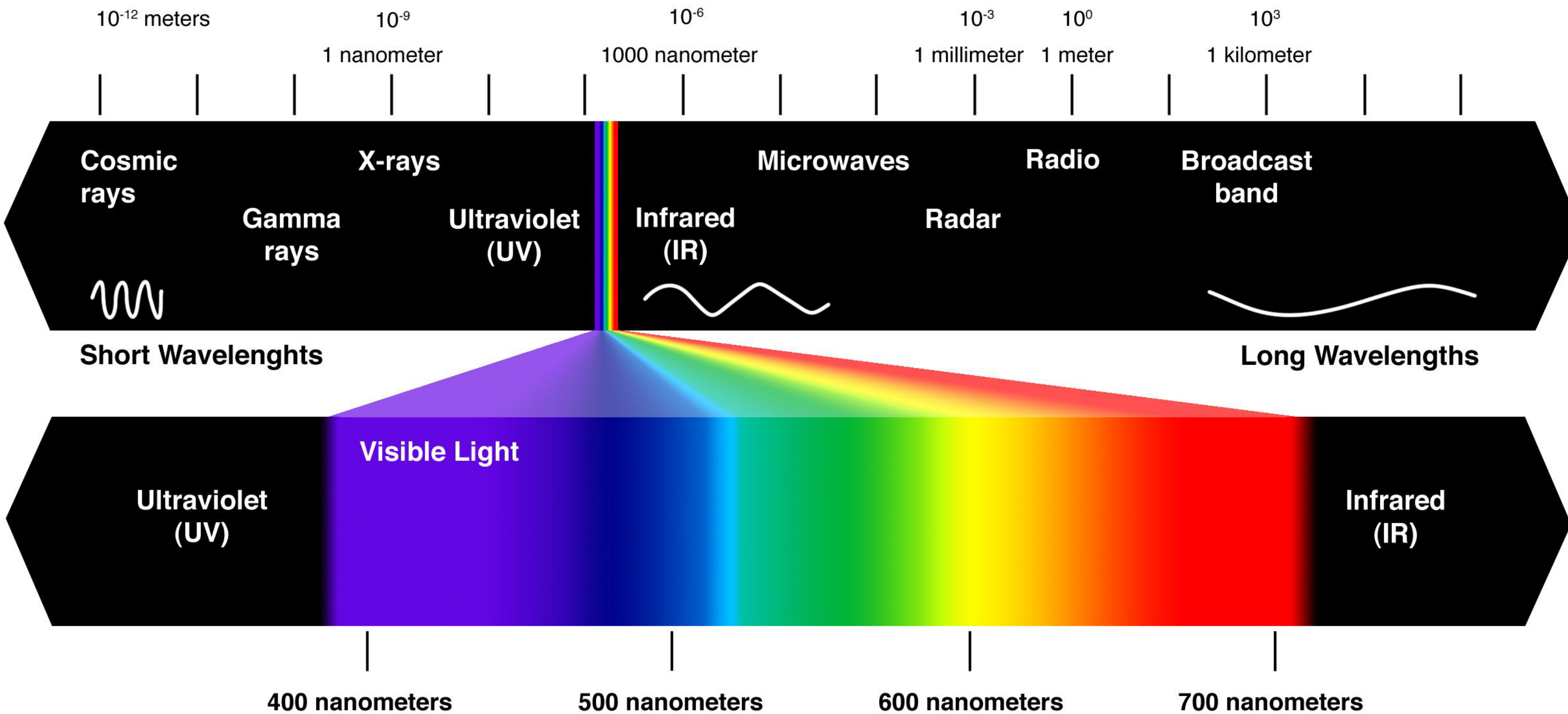


To understand and design an optical microscope, we need to describe light, its propagation and the interaction of light with object and various optical components.

Theories for Light (Optics)

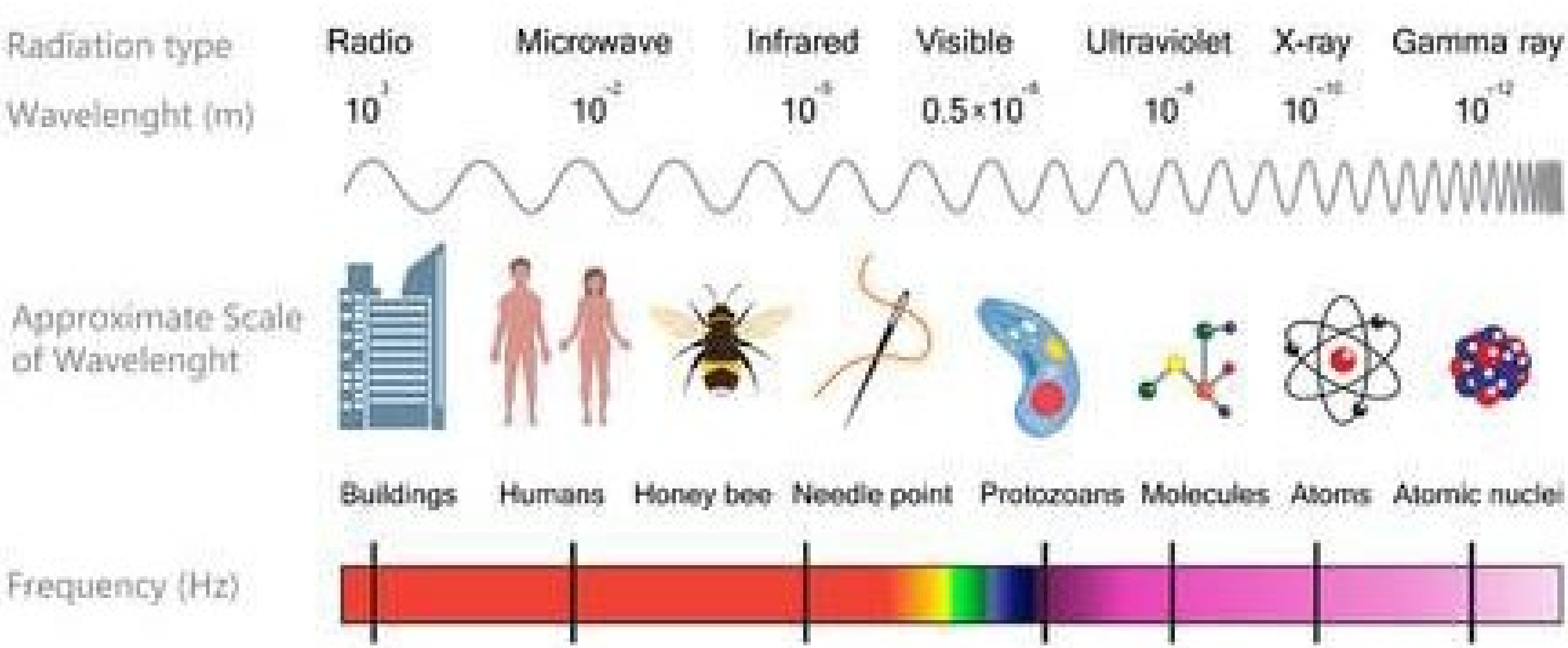


Electromagnetic spectrum



- λ for visible spectrum is 400 – 700 nm
- In order for ray optics to hold object size should be >> λ

Electromagnetic spectrum

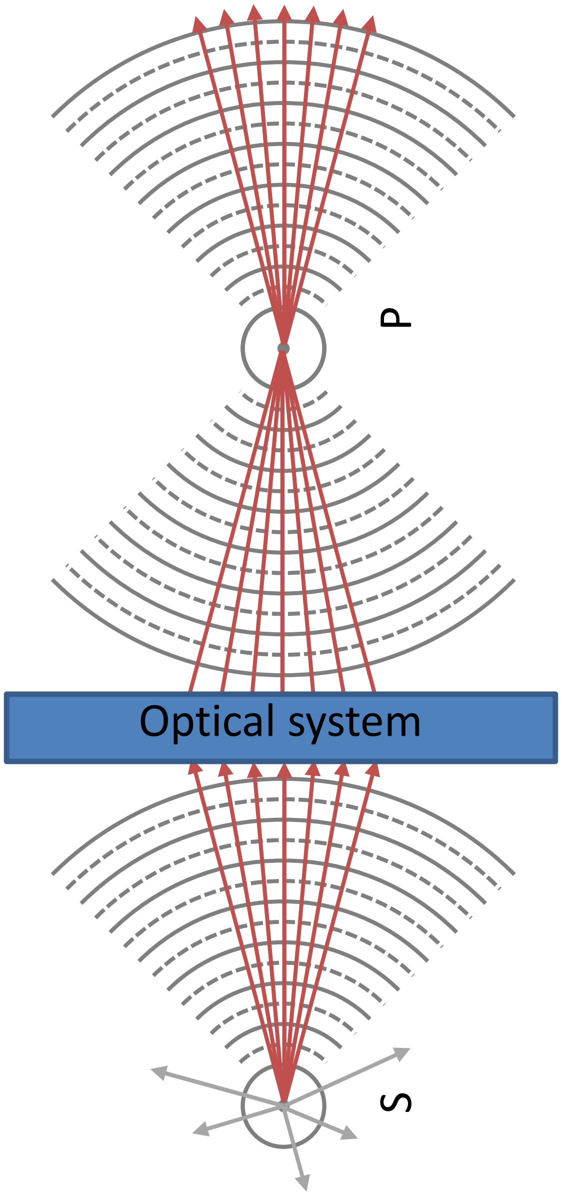


- λ for visible spectrum is 400 – 700 nm
- In order for ray optics to hold object size should be >> λ

Ray Optics

- Ray Optics – Simplest theory to describe light
- Light is described by rays that obey certain geometrical rules
  - Linear propagation in homogeneous media
  - $\lambda_0 \rightarrow 0$
  - No diffraction and interference
- Useful in description of optical instruments
- Ray Optics is also called Geometrical Optics

Outline



- Introduction to ray optics
  (a.k.a. geometrical optics)
  - Postulates of ray optics
  - Law of reflection
  - Law of refraction
- Basic optical components
  - Mirrors
  - Lenses
- Principle Rays
  - Ray tracing with a positive thin lens
  - Ray tracing with a negative thin lens

Postulates of Ray Optics -1

1) Light travels in the form of **rays** emitted by a light source and observed by an optical detector.

2) An optical medium is characterized by its **refractive index *n*** and the speed of light in medium is $c = c_o/n$

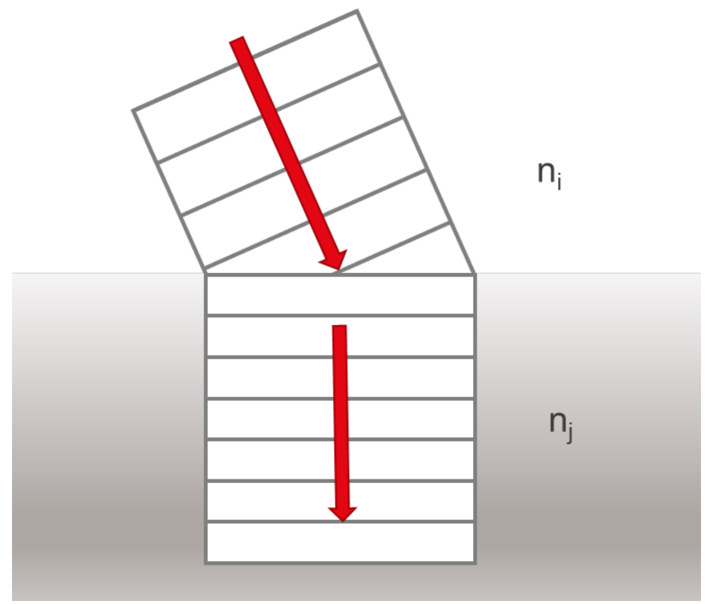
c_o = speed of light in free space (n=1 in free space)

c = speed of light in the medium = c_o/n

Light Propagation & Medium

$$n_{medium} = \frac{velocity_{vacuum}}{velocity_{medium}} = \frac{2.992926 \bullet 10^{8} \frac{m}{s}}{velocity_{medium}}$$

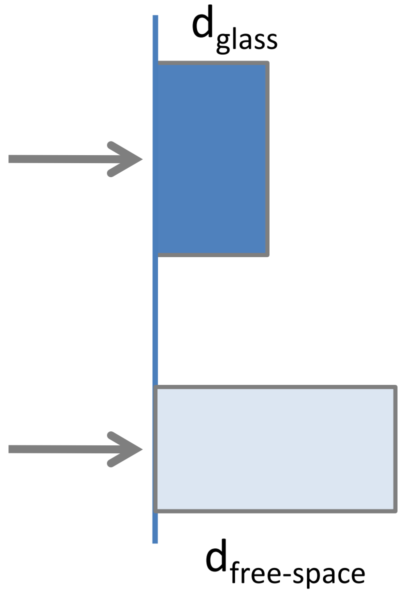
Medium	Refractive index n	Velocity in medium / km*s^{-1}
Air	1.0003	299203
Water	1.33	225032
Glycerol	1.47	203600
Immersion Oil	1.518	197162
Glass	1.56-1.46	191854-204995



Speed of light in a material is less than its speed in vacuum

Optical Path Length - OPL

$$\text{Optical Path Length (OPL)} = n_{medium} \times d_{medium}$$



Example:
For $n_{glass} = 1.5$,
if $d_{glass} = 1.0$ mm , then OPL = 1.5 mm

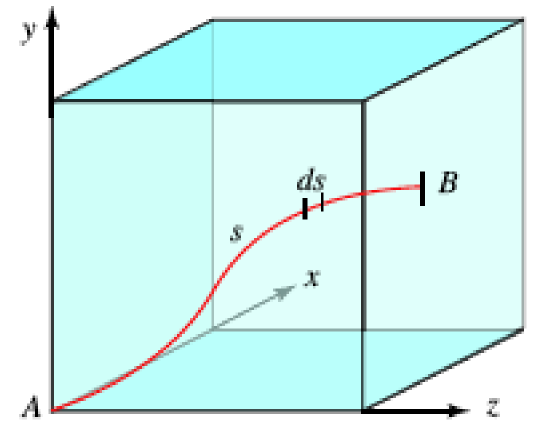
Postulates of Ray Optics - 3

3. In an **inhomogeneous** medium the refractive index n(r) is a function of r (x,y,z).

Therefore, the optical path length is given as:

$$\boxed{\int_A^B n(\mathbf{r})\, ds}$$

$$\boxed{\begin{gathered}\Delta OPL(r) = n(r)\Delta S \\ \int_{\Delta S \to 0} \Delta OPL(r) = \int_{\Delta S \to 0} n(r)\Delta S\end{gathered}}$$



Note:

- **Inhomogenous** medium means refractive index is position dependent, n=n(r)
- **Homogenous** medium means refractive index is constant, n=constant

Fermat's Principle:

Rays traveling between two points follow a path such that the time of travel (or optical path length) is an extremum relative to neighboring paths:

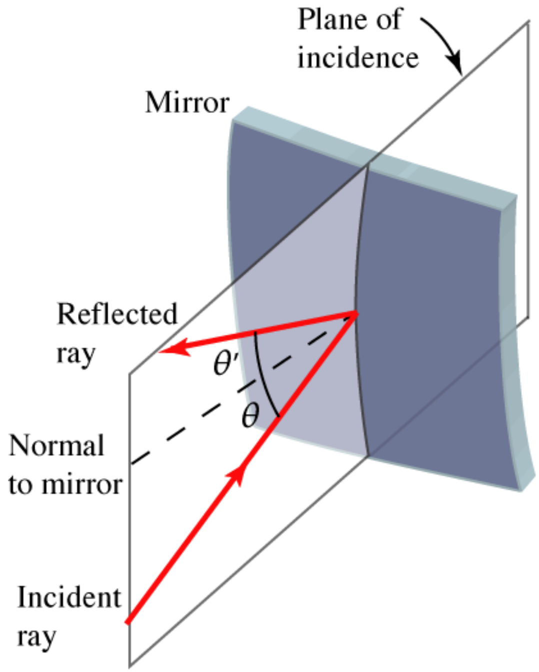
$$\boxed{\delta \int_A^B n(\mathbf{r})\, ds = 0}$$

- The extremum is usually a minimum: rays travel along the path of least time.
- If minimum time is shared by more than one path, all paths are followed simultaneously by the rays.

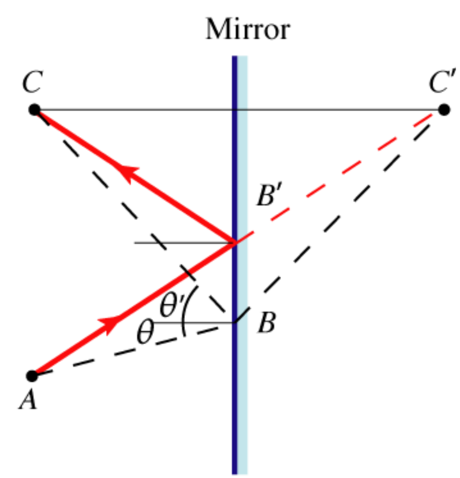
The Law of reflection

Reflection from a mirror or a boundary between two different media:

1- The reflected ray lies in the plane of incidence (this is linked to the conservation of momentum)
2- The angle of reflection is equal to the angle of to the angle of incidence : $\boxed{\theta' = \theta}$



(a)



(b)

The Law of reflection

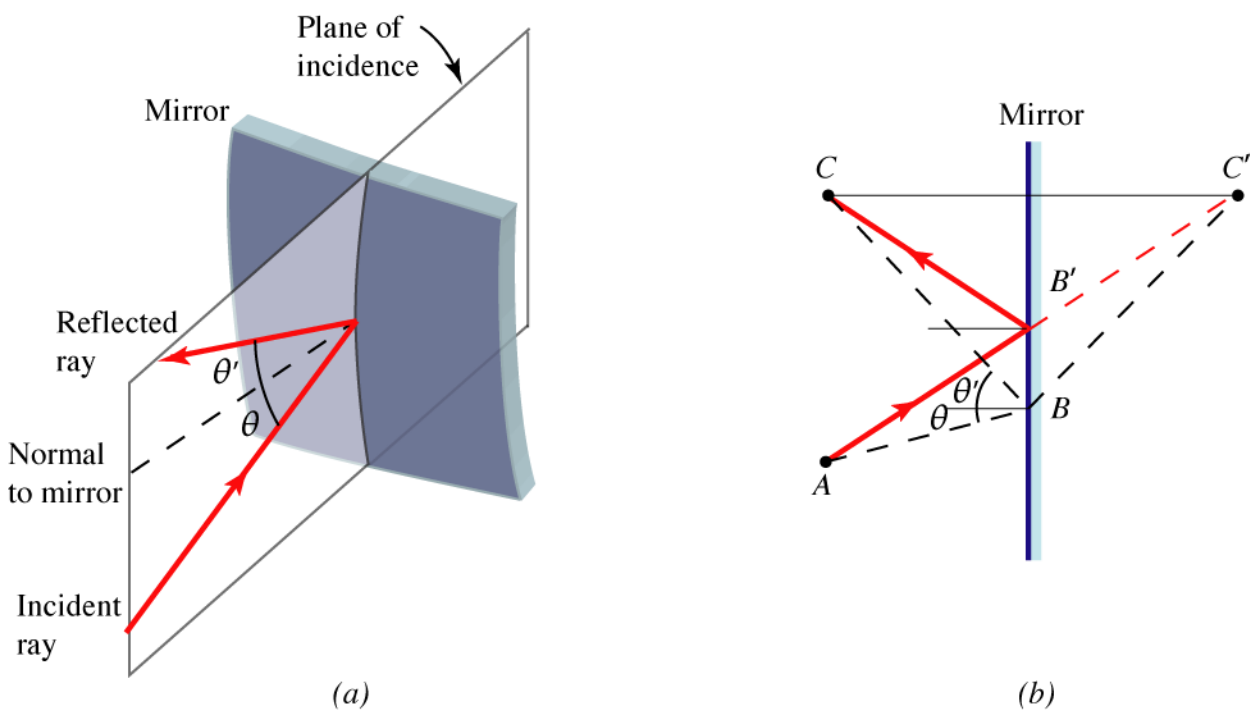
Proof:

- Let's assume a ray travels from A to C by reflecting from B such that $\boldsymbol{\theta} \neq \boldsymbol{\theta}'$
- C' is the mirror image of C

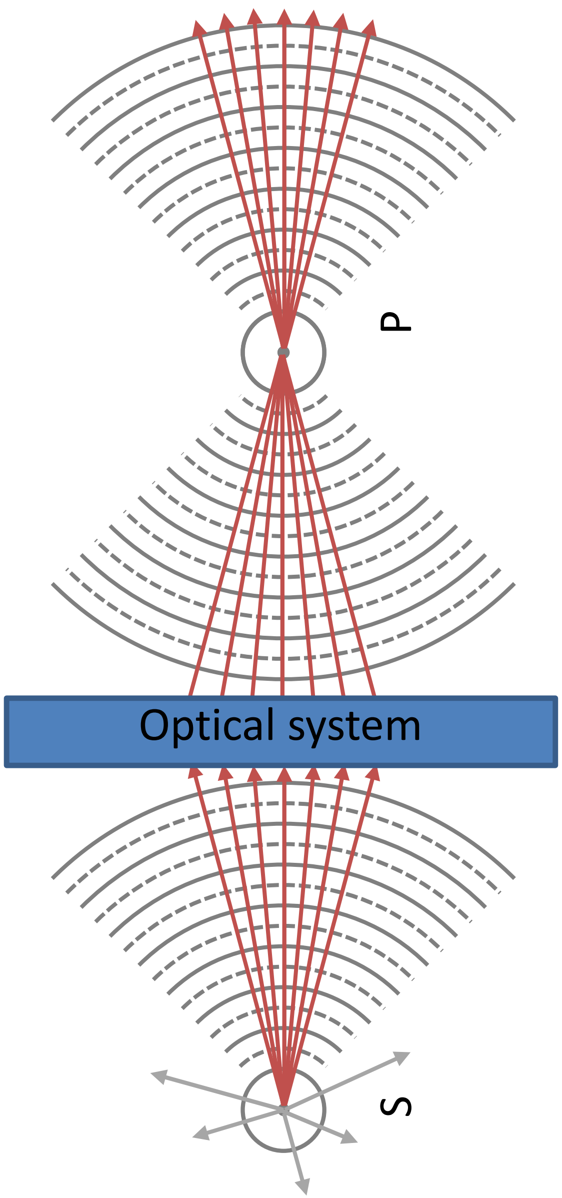
- AB+BC will be min when AB+BC' is minimum
- AB+BC' can be minimum if it is a straight line

  Thus, this requires that B=B'

  Thus $\theta = \theta'$



Outline



- Introduction to ray optics (a.k.a. geometrical optics)
  - Postulates of ray optics
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  - Law of refraction
- **Basic optical components**
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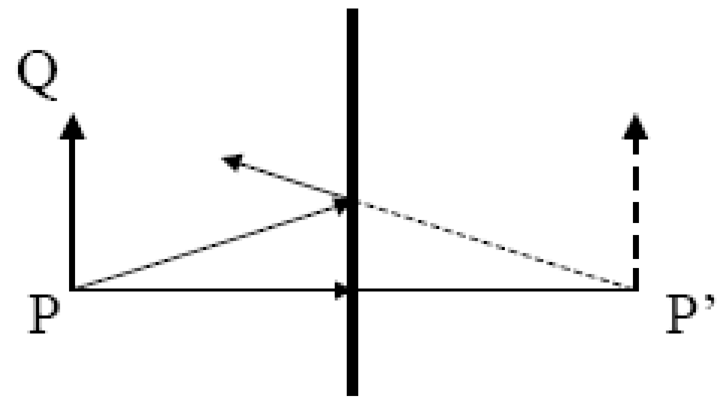
Example: Law of reflection for a planar mirror

For an extended object (e.g. the arrow PQ) of height, h:



- All points comprising the object (which is an arrow in this case) will produce virtual images similar to P'.

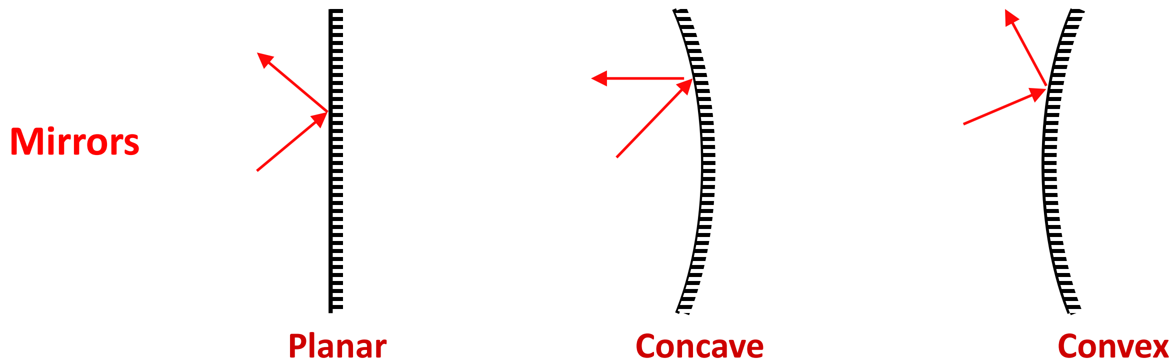
- Hence, a virtual image of height h' = h:

We define the magnification m as, $m = \dfrac{h'}{h}$

So m = +1 for a plane mirror.

Components based on reflection: <u>**Planar & Curved Mirrors**</u>

Components are used to form an image, focusing and collimating light:

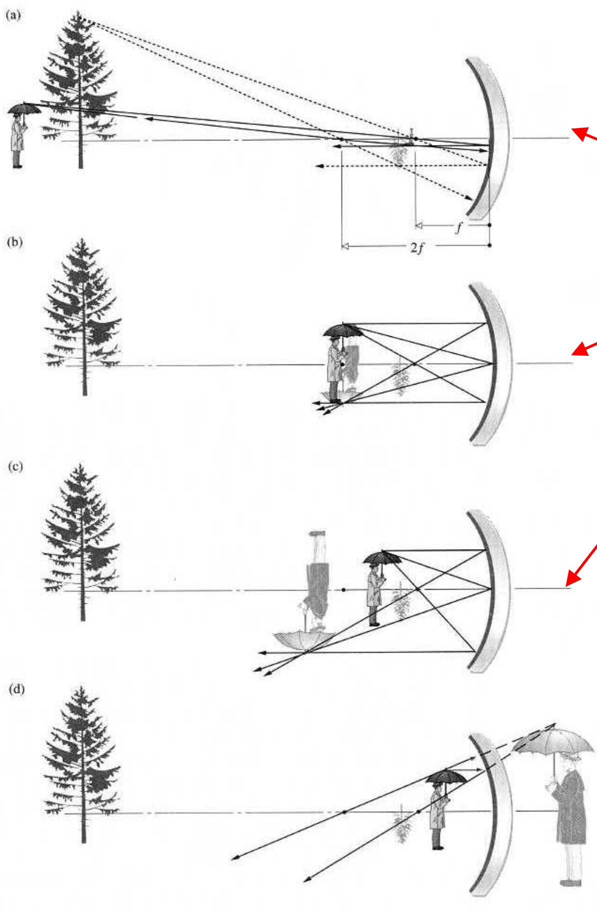


Imaging Concepts

- Object
- Image
  - Real
  - Virtual
  - Upright
  - Inverted
- Magnification
- Focus/Collimation

Magnification

For a spherical mirror, we can define radius of curvature (R) and focus (f = R/2)



E. Hecht, Optic, 7th edition, p.376

Concave mirrors

Object	Image			
Location	Type	Location	Orientation	Relative Size
$\infty > s_0 > 2f$	Real	$s < s_i < 2f$	Inverted	Minified
$s_0 = 2f$	Real	$s_i = 2f$	Inverted	Same size
$f < s_0 < 2f$	Real	$\infty > s_i > 2f$	Inverted	Magnified
$s_0 > f$	Virtual	$\lvert s_i \rvert > s_0$	Inverted	Magnified

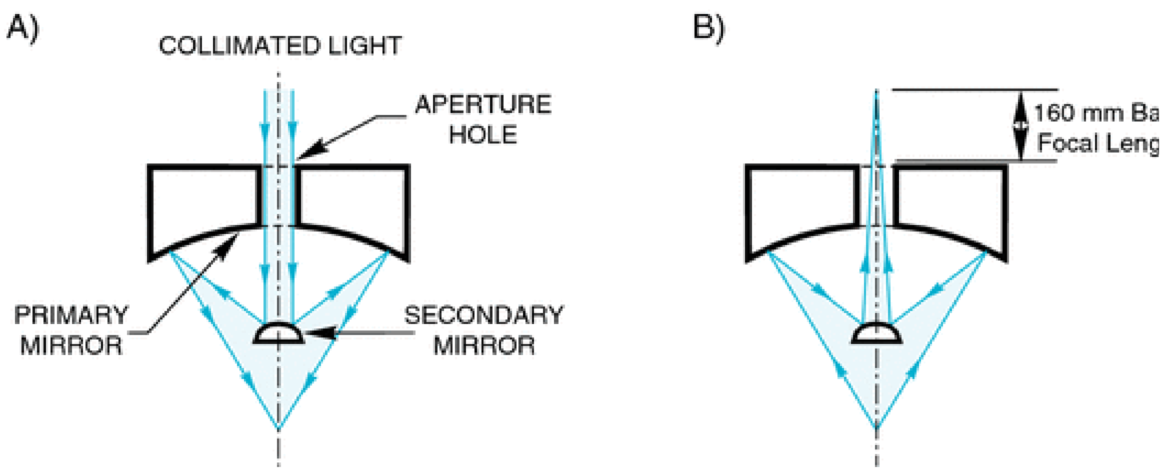
Convex mirrors

Object	Image			
Location	Type	Location	Orientation	Relative Size
Anywhere	Virtual	$\lvert s_i \rvert > \lvert f \rvert$ $s_0 > \lvert s_i \rvert$	Erect	Minified

For the **image:**

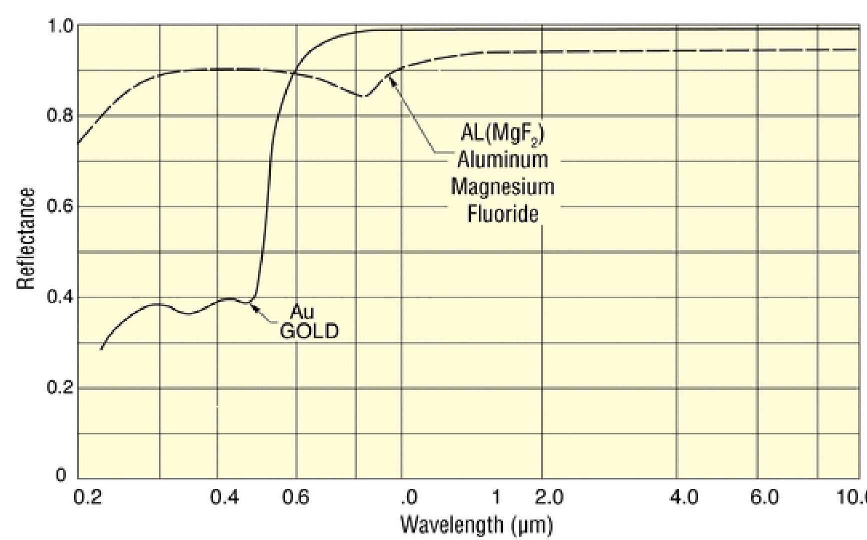
- If rays re-converge it corresponds to a **real image**
- If rays appear to emanate from then it is a **virtual image**

Reflective microscope objective

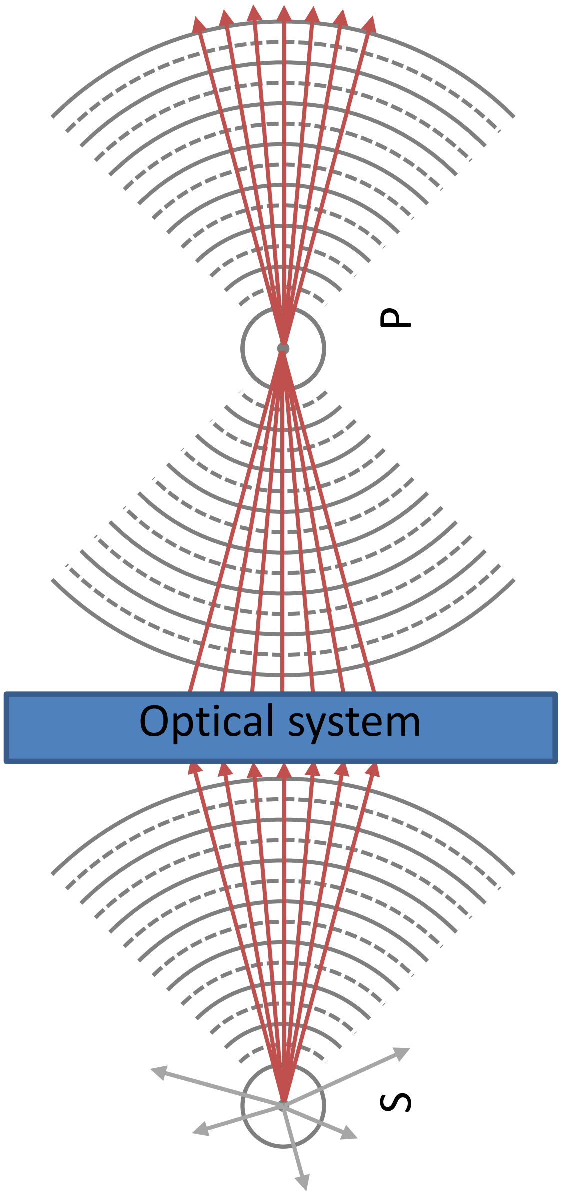


Application examples:

- Microscopy and spectroscopy in infrared is mainly based on reflective optics
- FT-IR Spectroscopy



Outline



- Introduction to ray optics (a.k.a. geometrical optics)
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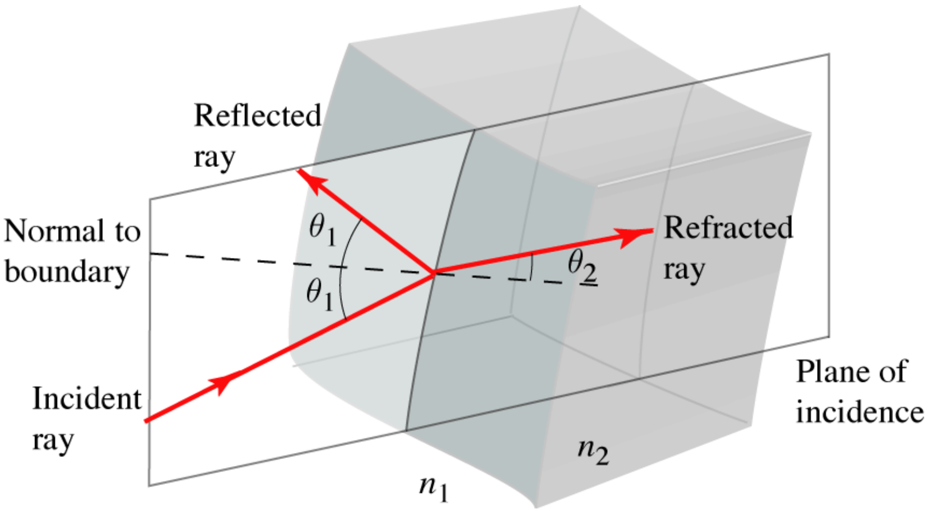
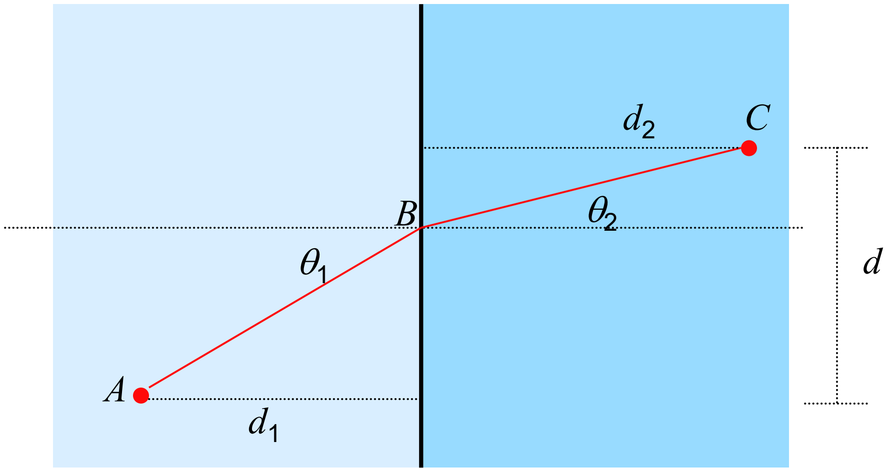
The Law of Refraction

Refraction occurs at a boundary between two different media:
1- The refracted ray lies in the plane of incidence (conservation of momentum ..)
2- The angle of refraction is related to the angle of to the angle of incidence by Snell's Law

Snell's Law: $\boxed{n_1 \sin\theta_1 = n_2 \sin\theta_2}$





Proof:

- Minimize **optical path length** OPT=$\boldsymbol{n_1} d_1 \sec\theta_1 + \boldsymbol{n_2} d_2 \sec\theta_2$
- Under the constraint that $d_1 \tan\theta_1 + d_2 \tan\theta_2 = d$

Application of Law of Refraction:

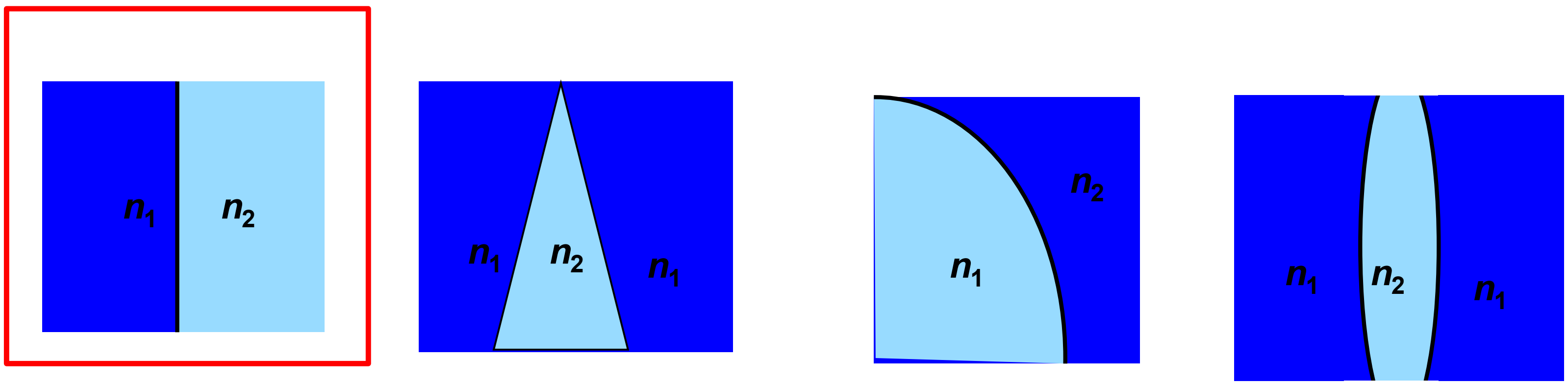
Refraction from a boundary between two different media:

1- The refracted ray lies in the plane of incidence (conservation of momentum ..)

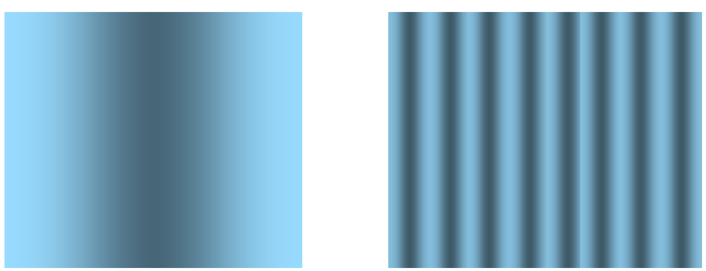
2- The angle of refraction is related to the angle of to the angle of incidence by Snell's Law

$$\boxed{n_1 \sin\theta_1 = n_2 \sin\theta_2}$$

Boundaries Between Transparent Media

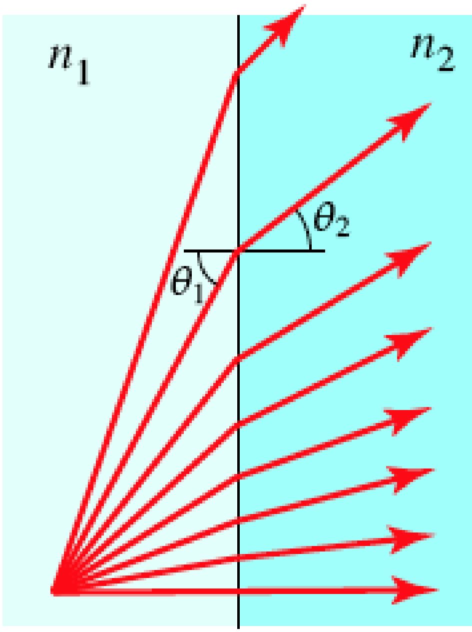


Graded-Index Media



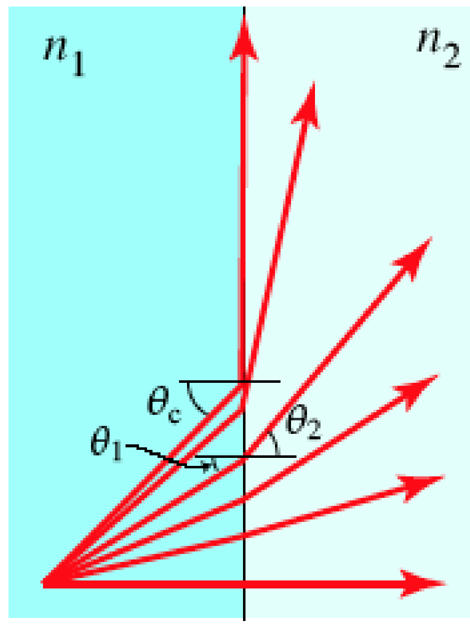
Planar Boundaries (application of Snell's Law)

$n_1 < n_2$

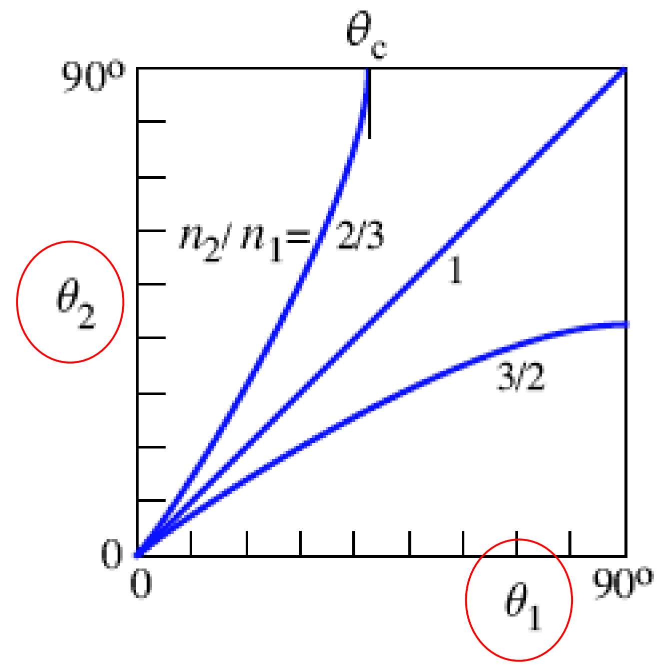


External refraction

$n_1 > n_2$



Internal refraction



Snell's Law:

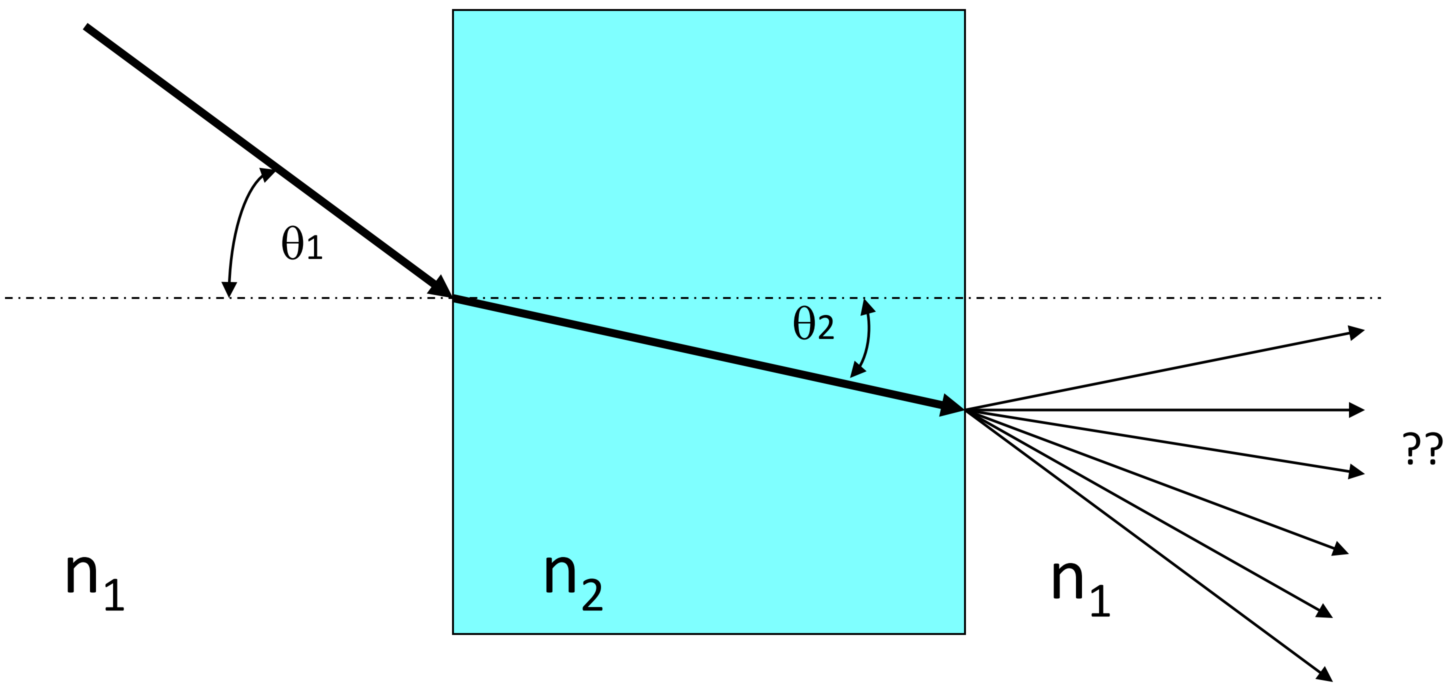
$$\boxed{\sin\theta_1 = (n_2/n_1)\sin\theta_2}$$

Critical Incidence Angle:

$$\boxed{\theta_c = \sin^{-1}\frac{n_2}{n_1}} \quad \theta_2 = \pi/2$$

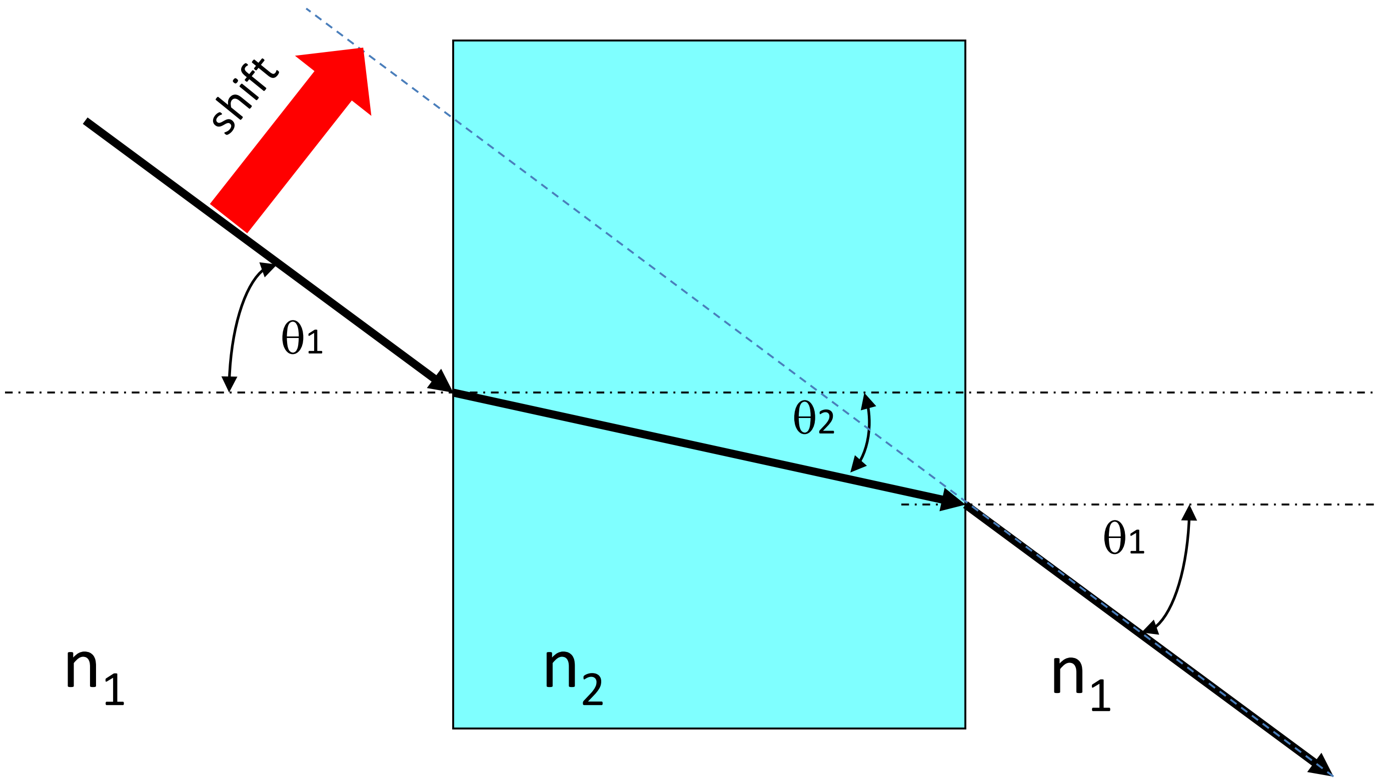
Refraction:
light beam through a plane-parallel glass plate

Snell's Law: $\eta 1 \sin \theta 1 = \eta 2 \sin \theta 2$



Refraction:
light beam through a plane-parallel glass plate

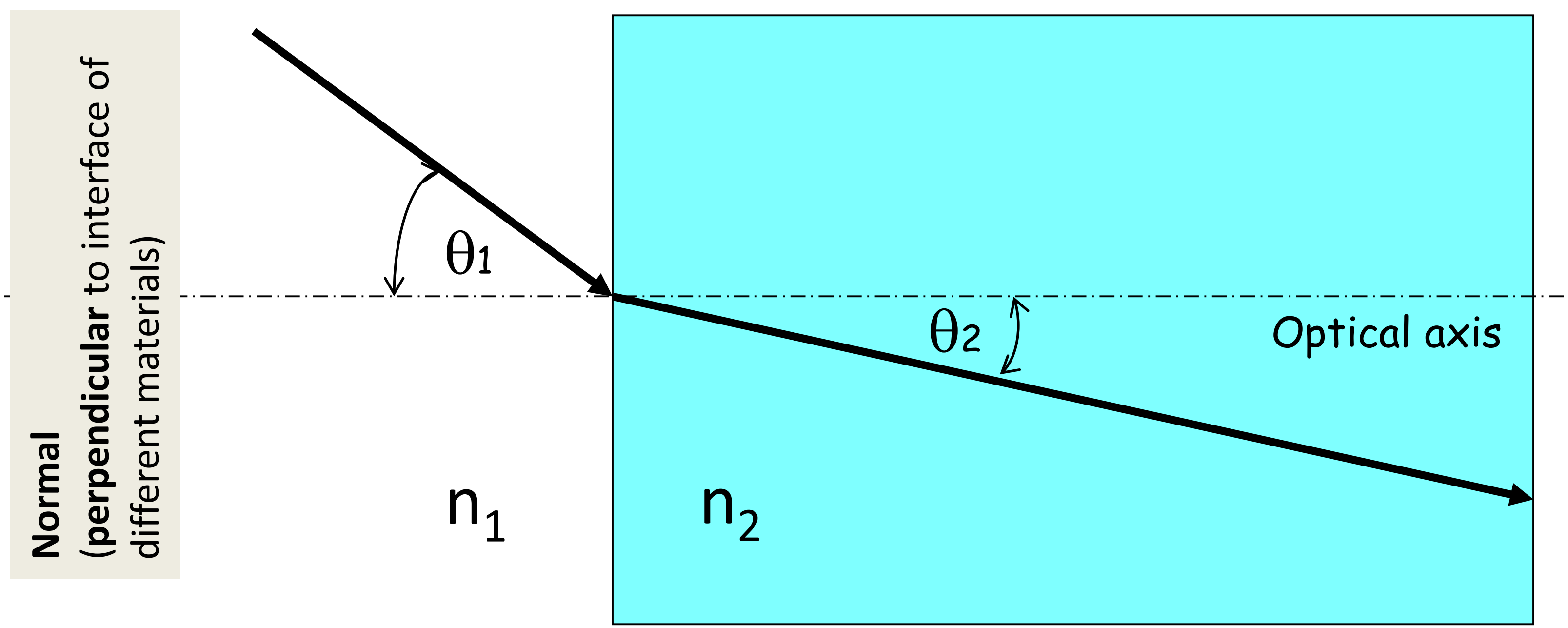
Apply Snell's Law twice ➔ $\eta 1 \sin \theta 1 = \eta 2 \sin \theta 2$



Exit angle is same as the incident angle ➔ the beam doesn't change deflection angle
➔ Thus a parallel plate only results in a lateral shift in beam path

Refraction under paraxial approximation:

- Snell's Law ➔ $n_1 \sin \theta 1 = n_2 \sin \theta 2$
- Under paraxial approximation ray propagates close to the optical axis so that θ1 and θ2 are very small.
- Snell's Law under **paraxial approximation** ➔ $n_1\ \theta 1 \sim n_2\ \theta 2$



Application of Law of Refraction:

Refraction from a boundary between two different media:

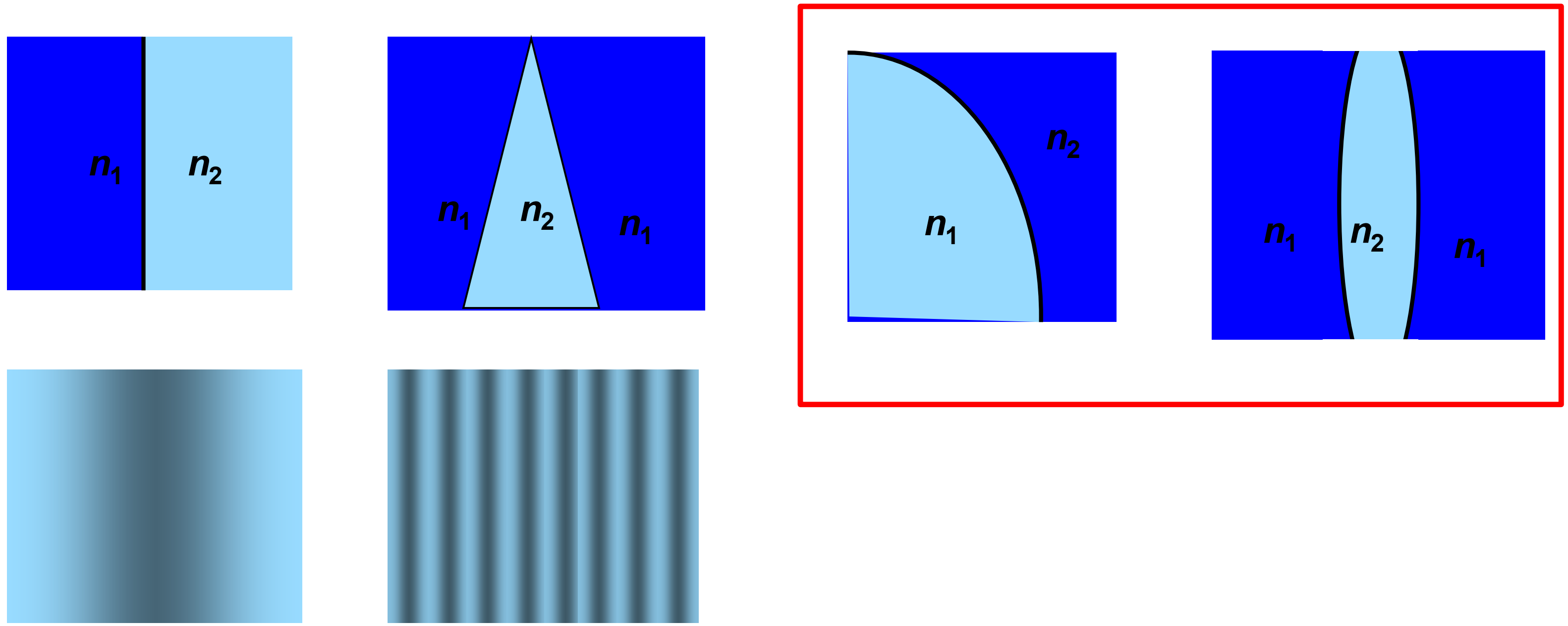
1- The refracted ray lies in the plane of incidence (conservation of momentum ..)

2- The angle of refraction is related to the angle of to the angle of incidence by Snell's Law

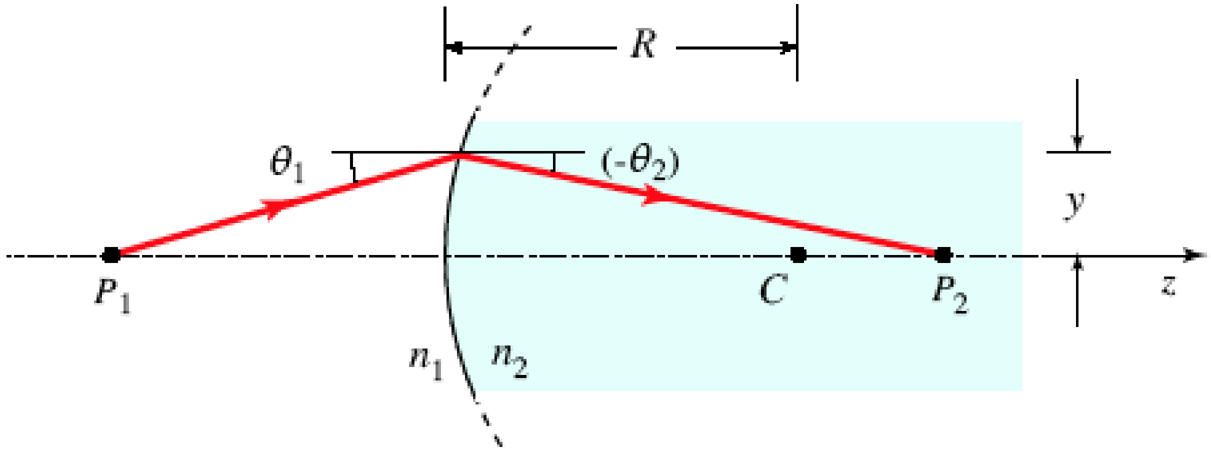
$$\boxed{n_1 \sin\theta_1 = n_2 \sin\theta_2}$$

Boundaries Between Transparent Media

Graded-Index Media



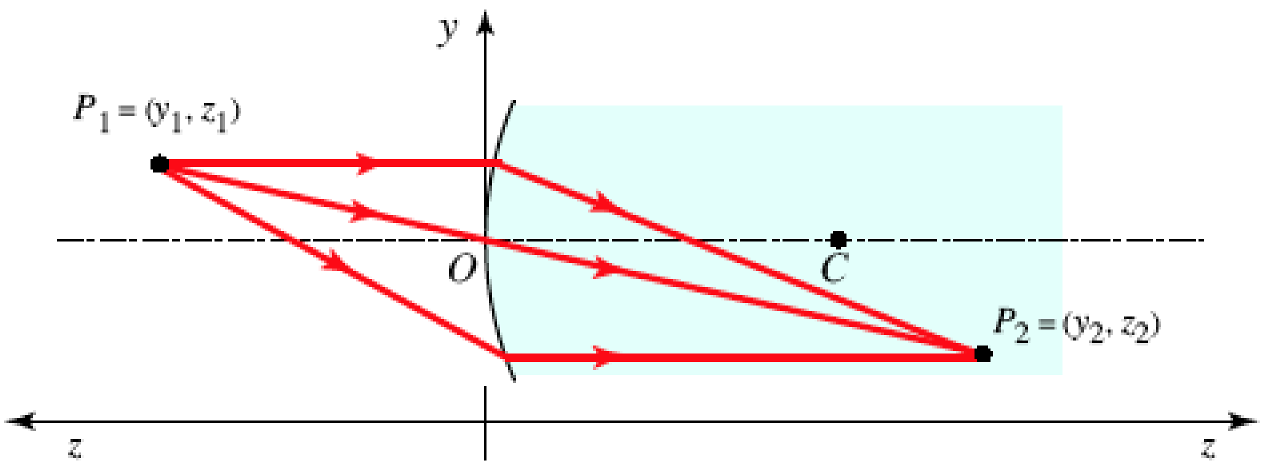
Spherical Boundary



Ray Deflection

Paraxial Ray Approximation

$$\theta_2 \approx \frac{n_1}{n_2}\theta_1 - \frac{n_2 - n_1}{n_2}\frac{y}{R}$$



Imaging Condition

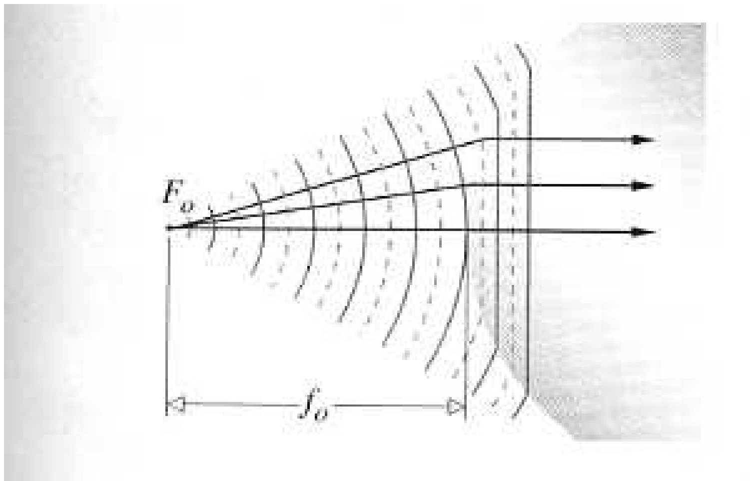
$$\frac{n_1}{z_1} + \frac{n_2}{z_2} \approx \frac{n_2 - n_1}{R}$$

Magnification

$$y_2 = -\frac{n_1}{n_2}\frac{z_2}{z_1}y_1$$

Object focus / Image focus

$$\frac{n_1}{z_1} + \frac{n_2}{z_2} \approx \frac{n_2 - n_1}{R}$$



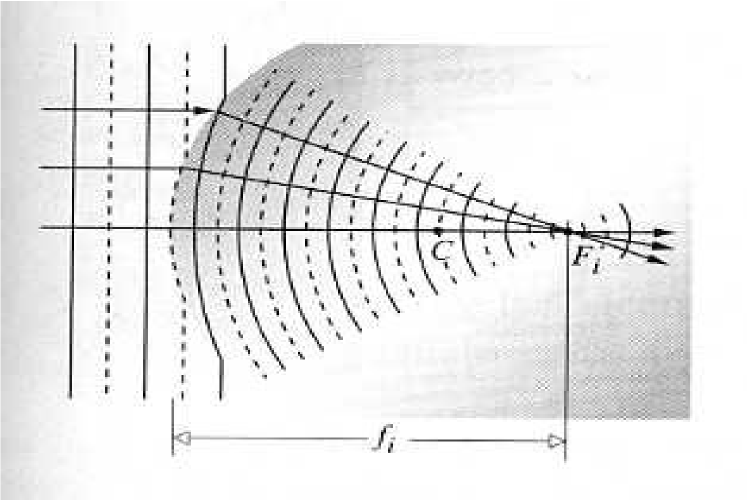
If the point F_0 imaged at infinity:

$z_2 = \infty$
$z_1 = s_o = f$

$$\frac{n_1}{s_0} + \frac{n_2}{\infty} = \frac{n_2 - n_1}{R}$$

$$s_0 \equiv f_0 = \frac{n_1}{n_2 - n_1} R$$

Object focal length
(first focus)



The point where the image is formed when $s_0 = \infty$

$$\frac{n_1}{\infty} + \frac{n_2}{s_i} = \frac{n_2 - n_1}{R}$$

$$s_i \equiv f_i = \frac{n_2}{n_2 - n_1} R$$

Image focal length
(second focus)

Sign convention for refractive surfaces

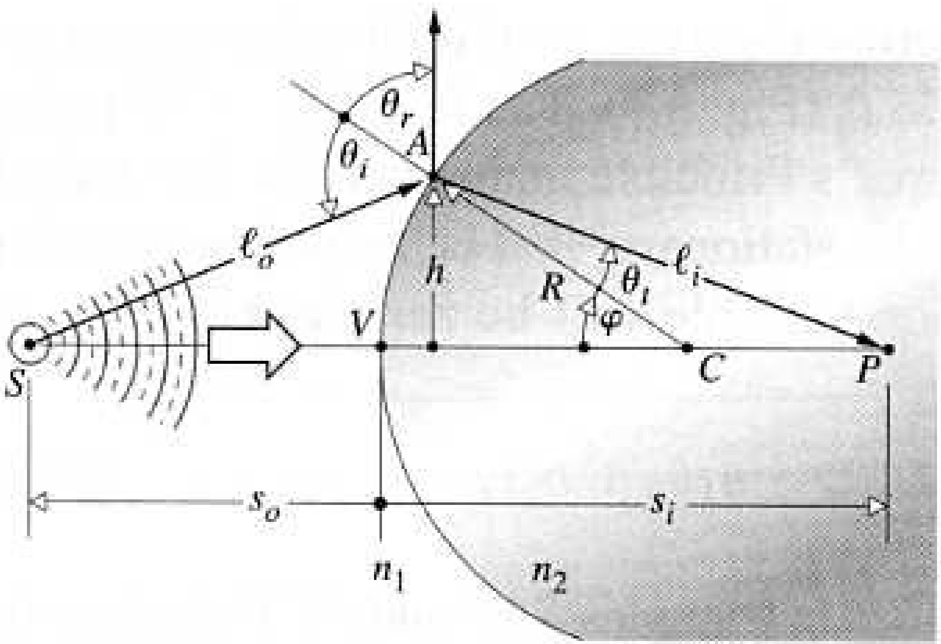
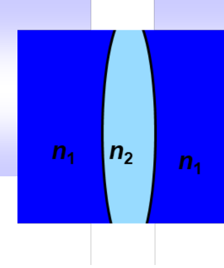


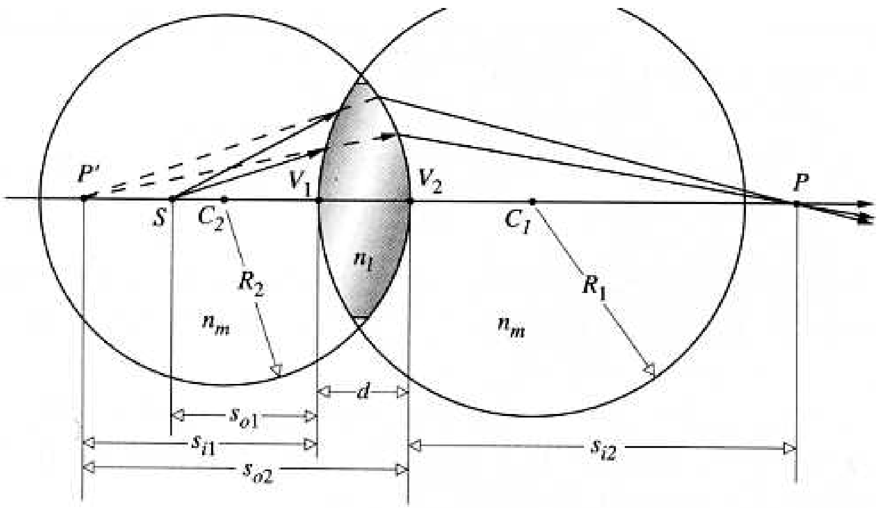
TABLE 5.1 Sign Convention for Spherical Refracting Surfaces and Thin Lenses* (Light Entering from the Left)

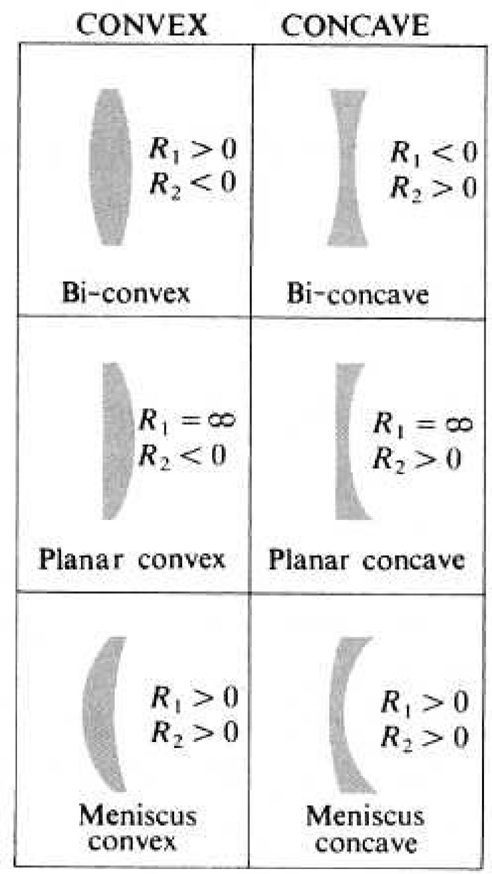
s_o, f_o	+ left of V
x_o	+ left of F_o
s_i, f_i	+ right of V
x_i	+ right of F_i
R	+ if C is right of V
y_o, y_i	+ above optical axis

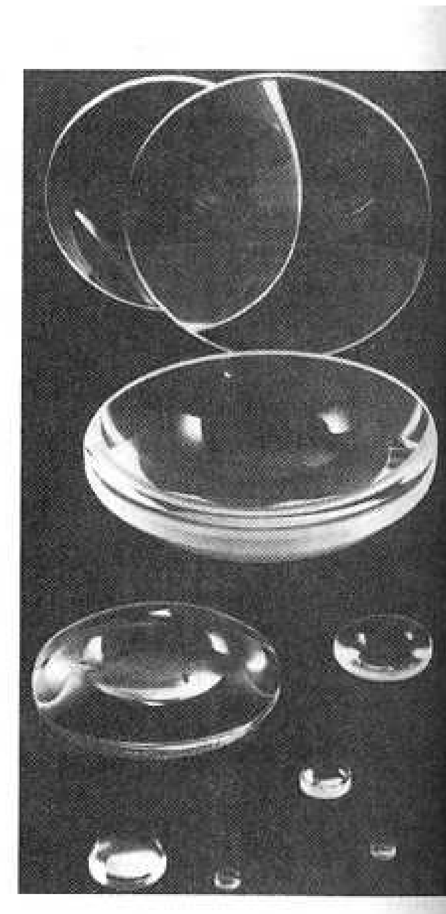
Spherical Lens



A spherical lens has **two boundaries**.
Example: Biconvex spherical lens







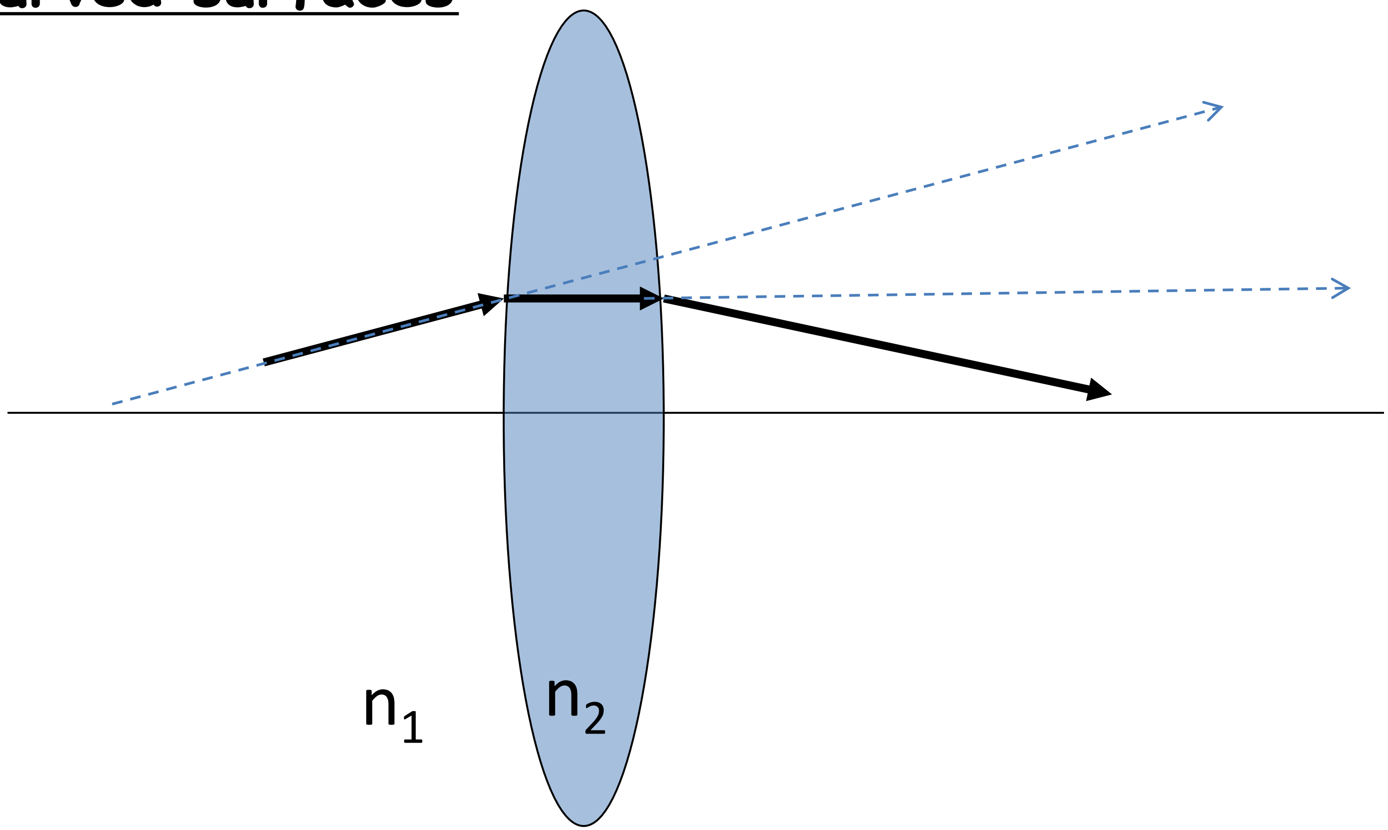
In order to find total deflection angle, apply following equation twice

$$\theta_2 \approx \frac{n_1}{n_2}\theta_1 - \frac{n_2 - n_1}{n_2}\frac{y}{R}$$

Lenses

Apply Snell's Law to something as complex as a lens:

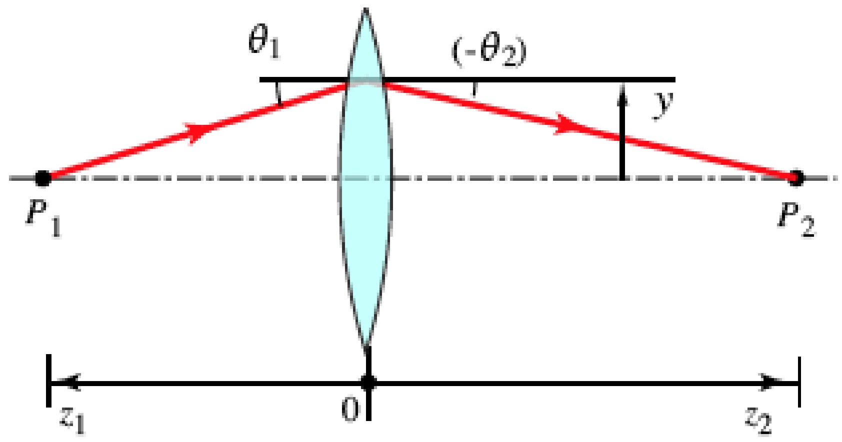
➔It is a **curved surfaces**



Changes beam direction
→ Results in a beam deflection

Formulas for Thin Lenses

A thin lens has a thickness that is essentially negligible.



<u>When this lens is in free space:</u>

Ray Deflection $\theta_2 = \theta_1 - \frac{y}{f},$

Lens maker's formula (thin lens equation)

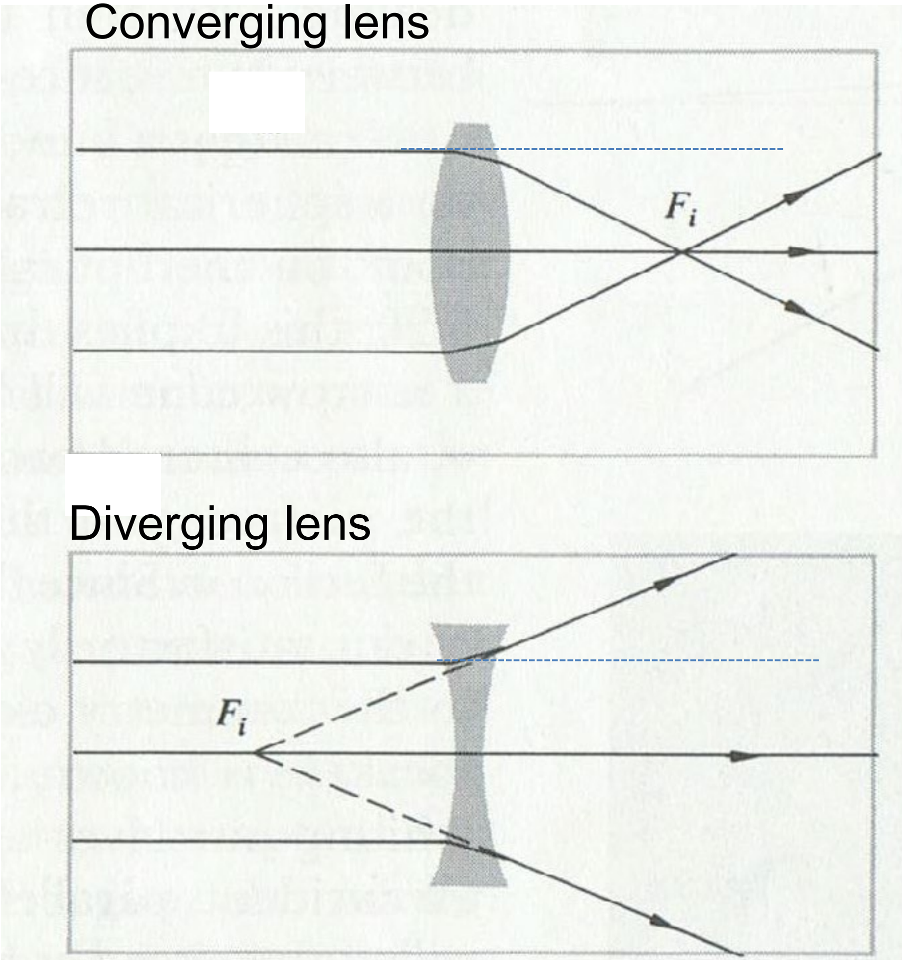
Focal Length *f* $$\boxed{\frac{1}{f} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)}$$

Thin Lenses

<u>For a thin lens is in free space:</u>

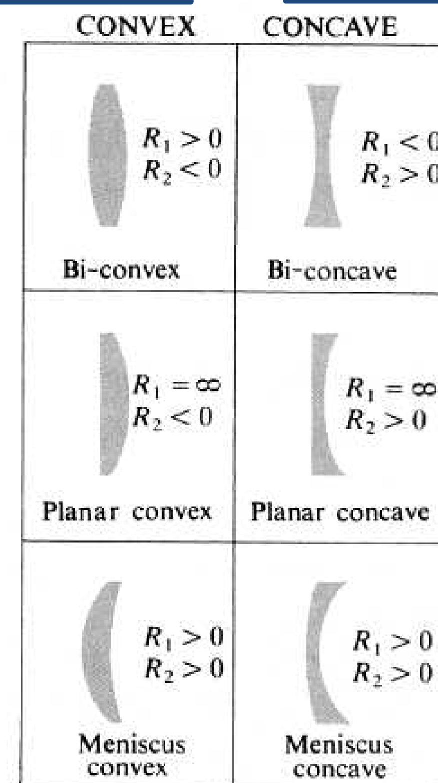
$$\frac{1}{f} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$$

Converging lens



f = positive
converging

f = negative
diverging



Determining the focal length of a thin lens

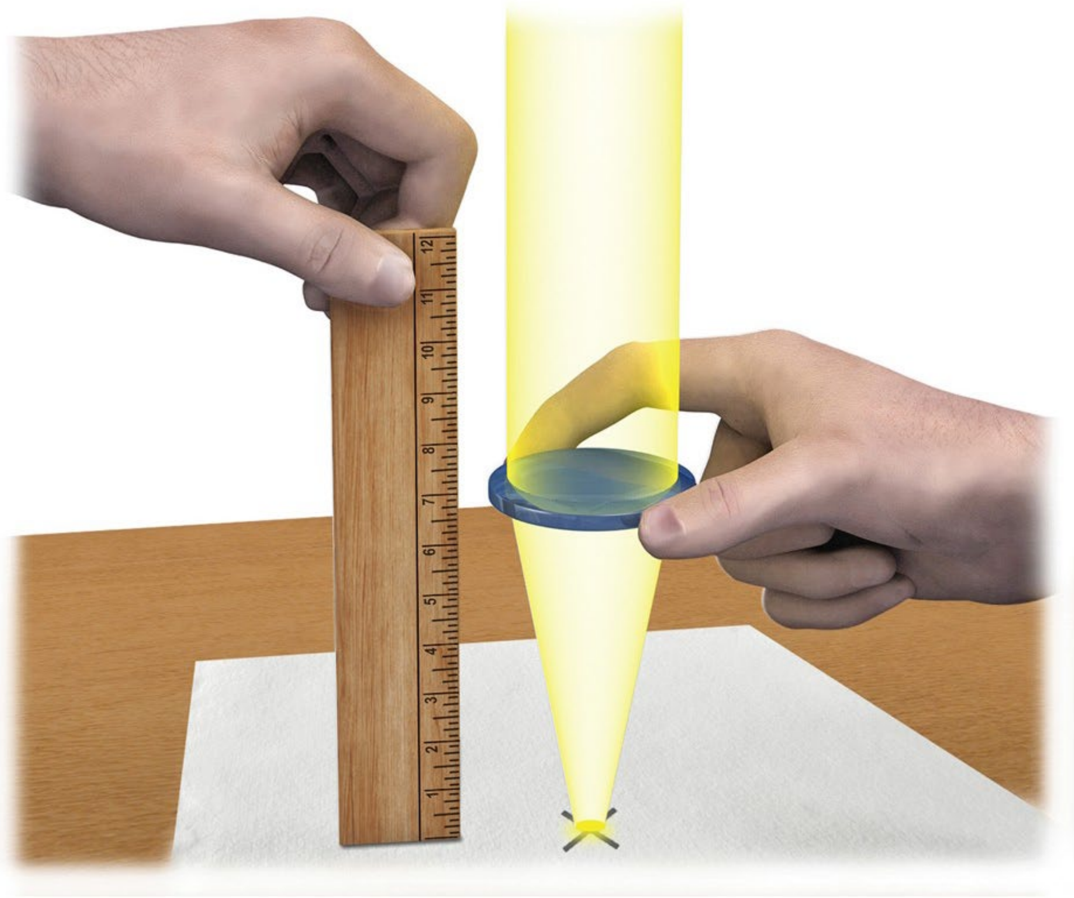


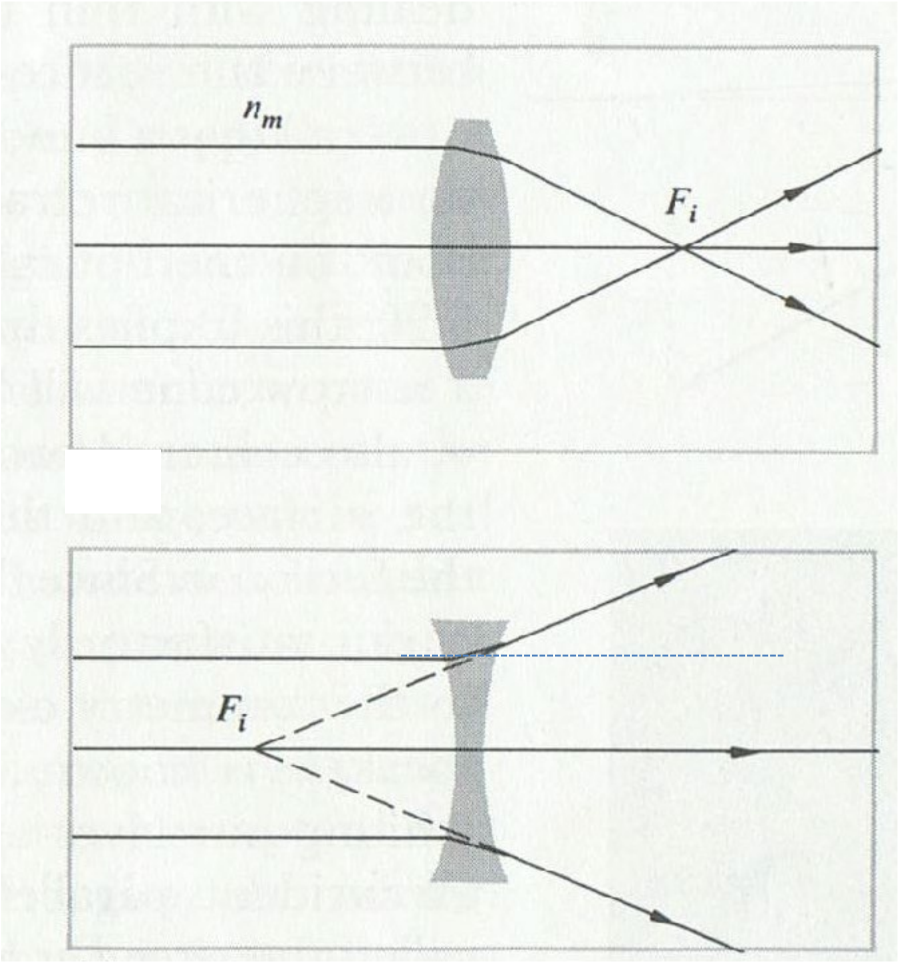
Figure 4.6

Determining the focal length of a simple lens. The image of a distant source is projected by the lens on a viewing surface; the focal length is the distance between the focal plane, and the lens as measured with a ruler.

Pay attention to medium dependence

$$\boxed{\frac{1}{f} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)}$$

- Lens is made out of material with index n (n>1)
- Outside of lens is air with n=1



These pictures will be valid as long as $n_{outside}$ is smaller than n_{lens}

Pay attention to medium dependence

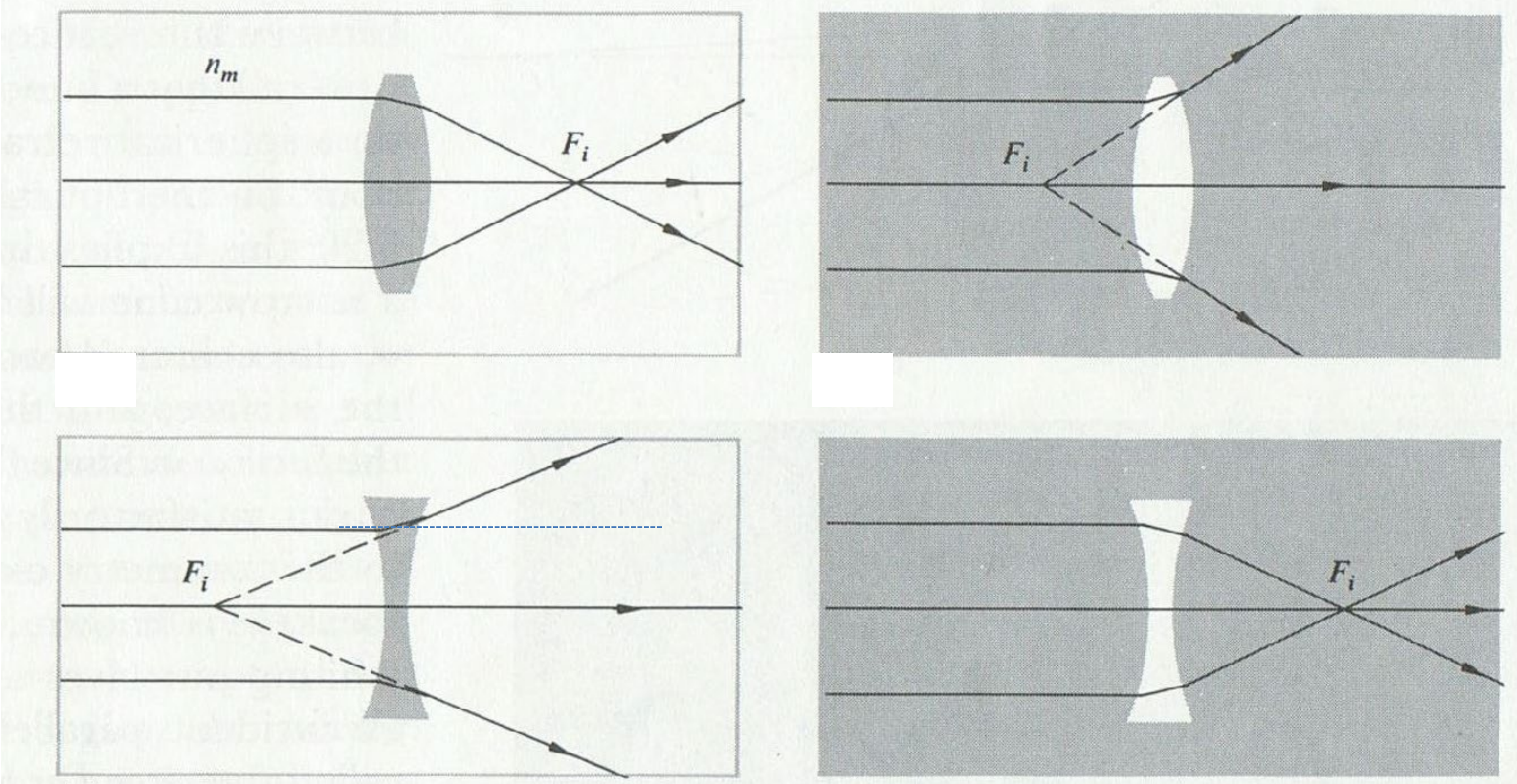
$$\boxed{\frac{1}{f} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)}$$

Generalized formula

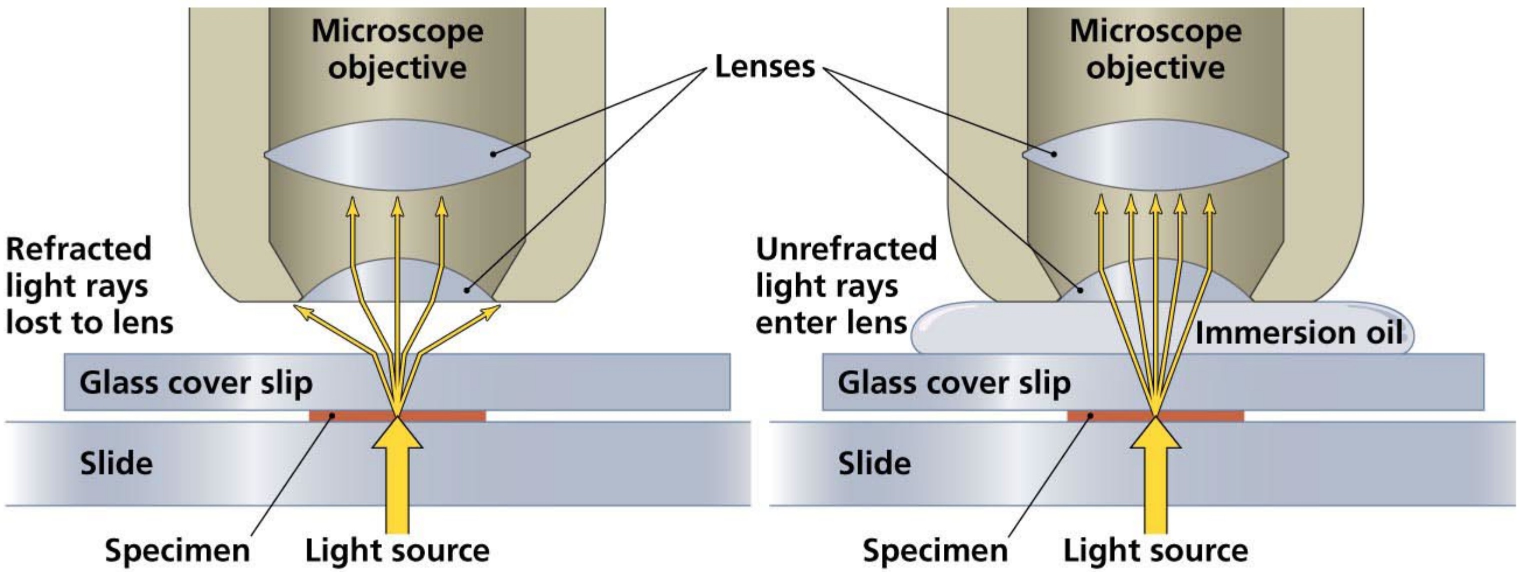
$$\frac{1}{f} = \frac{n_{lens} - n_0}{n_0}\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$$

$n_{outside}$ is smaller than n_{lens}

$n_{outside}$ is bigger than n_{lens}



Medium dependence is important in microscopy



(a) Without immersion oil (b) With immersion oil