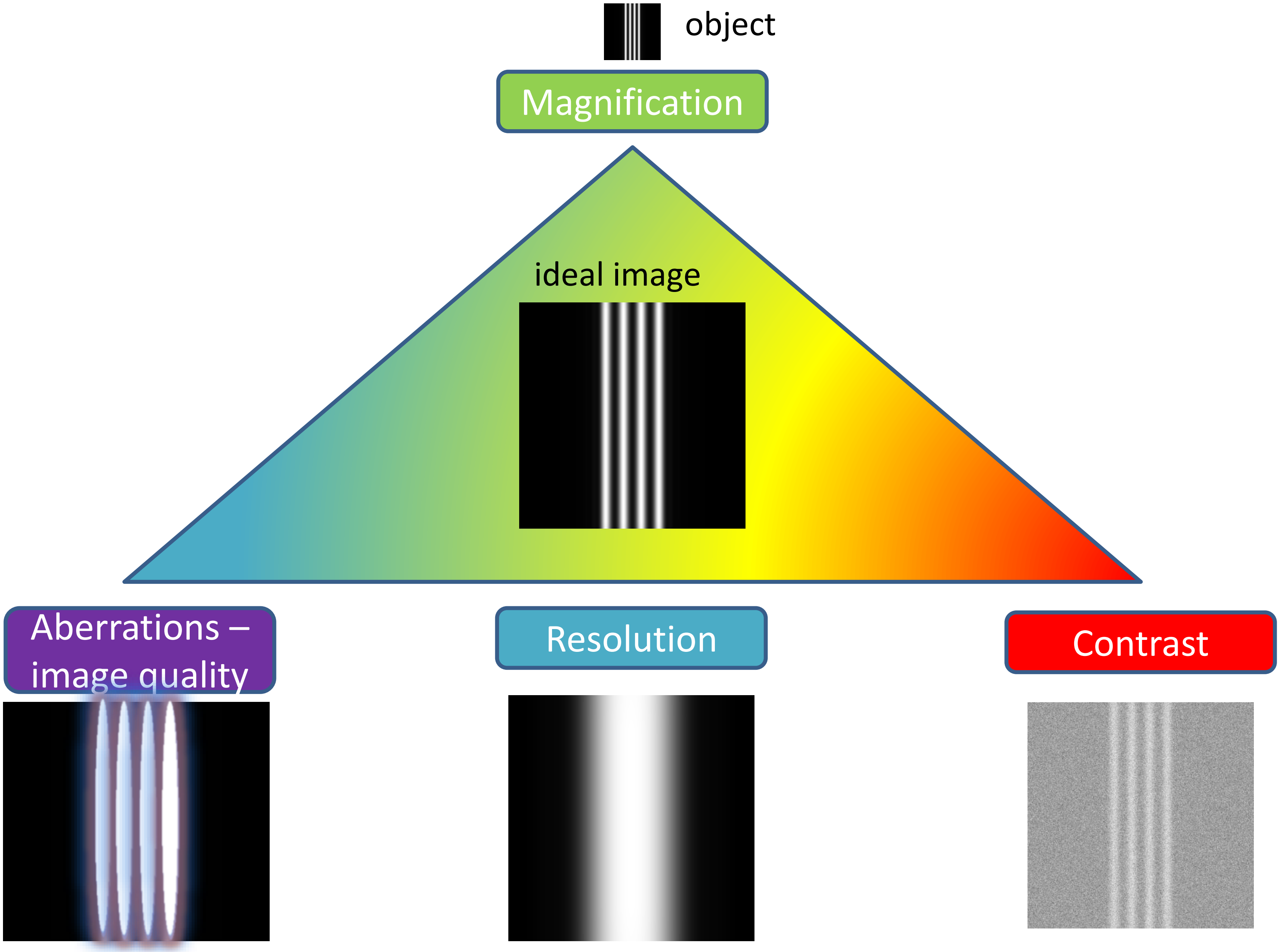
MICRO-561

Fundamentals of Biomicroscopy

Syllabus (tentative)

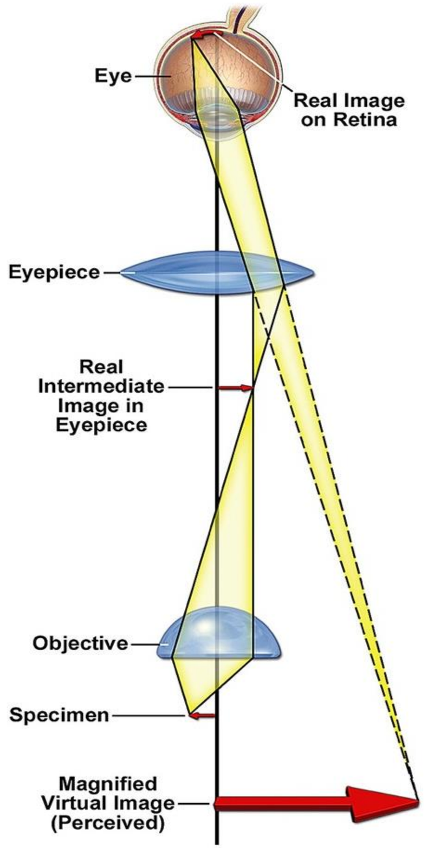
Lecture 1	Intro & Ray Optics-1
Lecture 2	Ray Optics-2 & Matrix Optics-1
Lecture 3	Matrix Optics-2
Lecture 4	Matrix Optics-3 & Microscopy Design-1
Lecture 5	Microscopy Design-2
Lecture 6	Microscopy Design-3
Lecture X	HOLIDAY
Lecture 7	Resolution-1
Lecture 8	Resolution-2
Lecture 9	Resolution-3
Lecture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

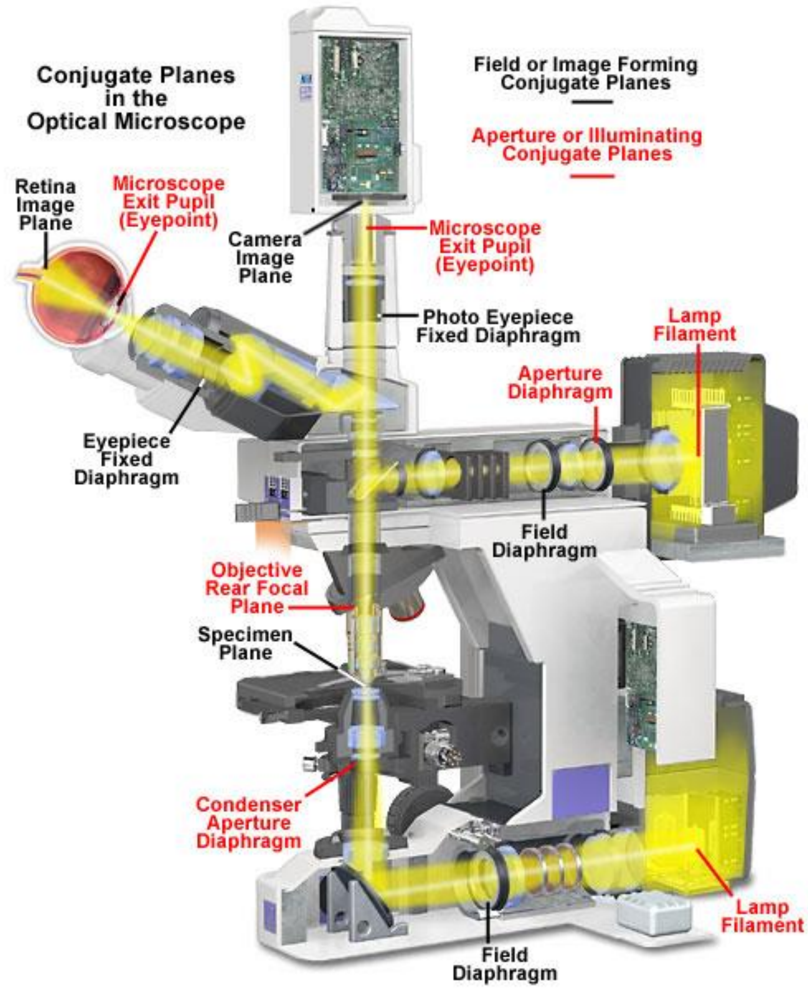
Important aspects for microscopy



Optical Microscope

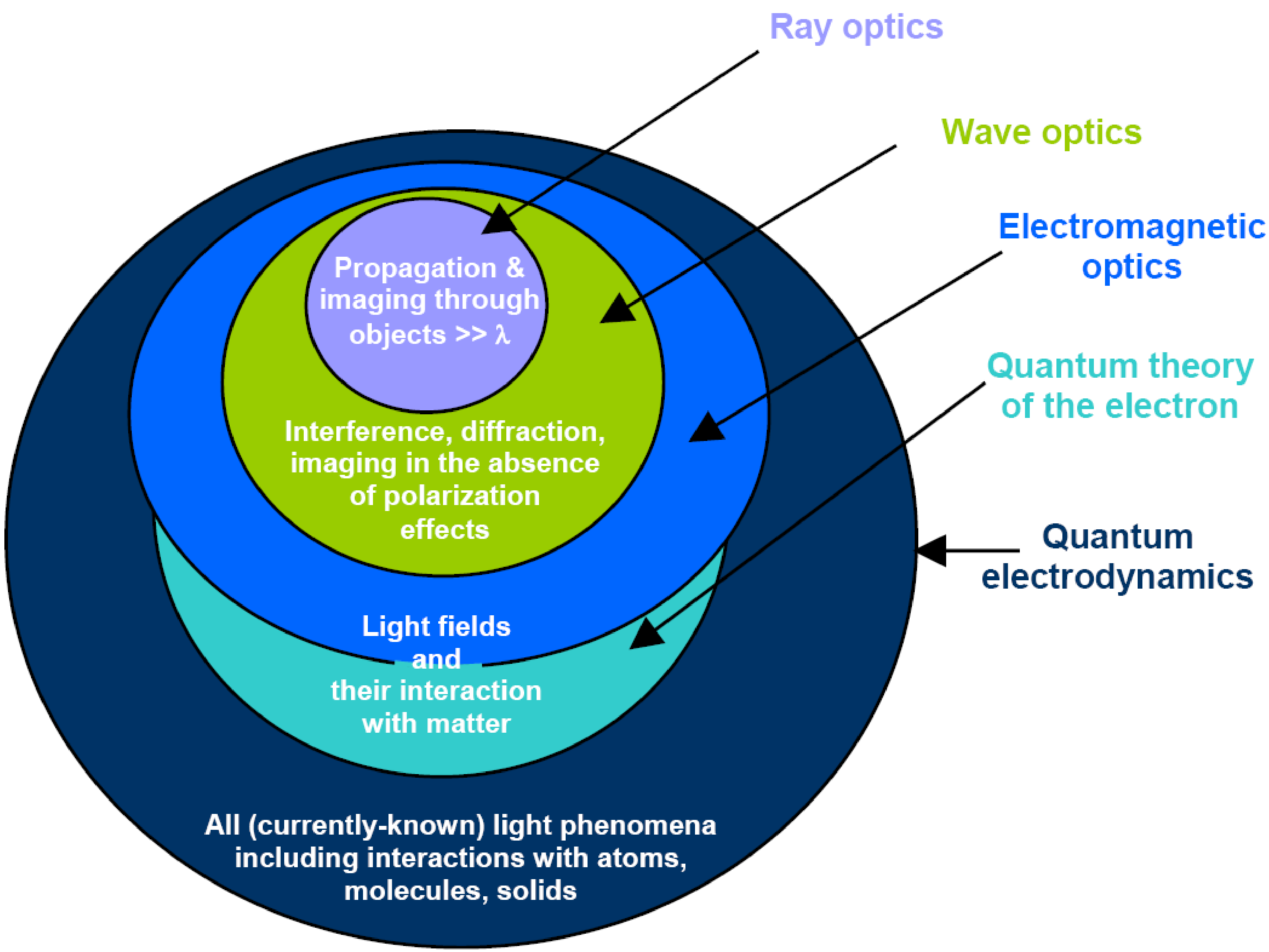
Optical train: **Consists of optical components such as lenses, diaphragms, filters..**



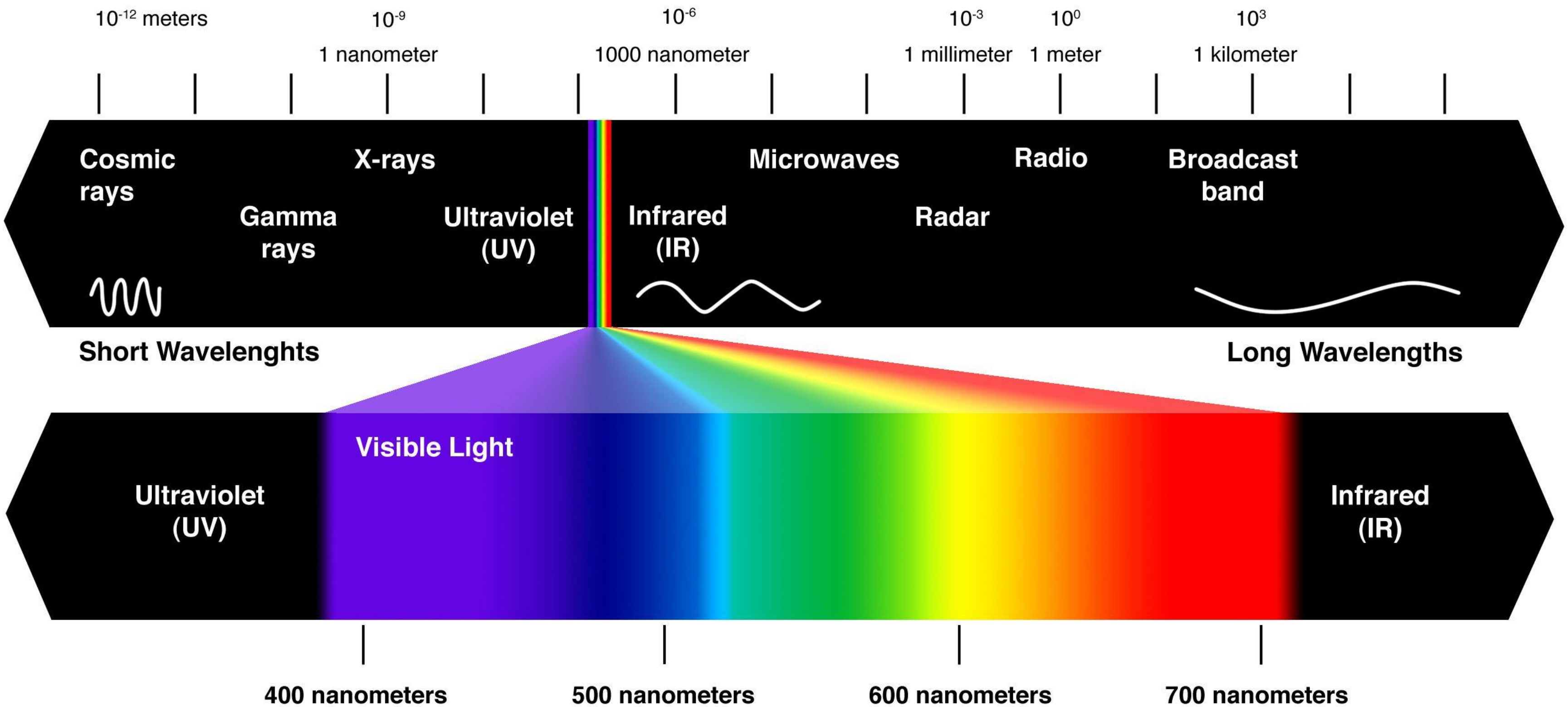


To understand and design an optical microscope, we need to describe light, its propagation and the interaction of light with object and various optical components.

Theories for Light (Optics)

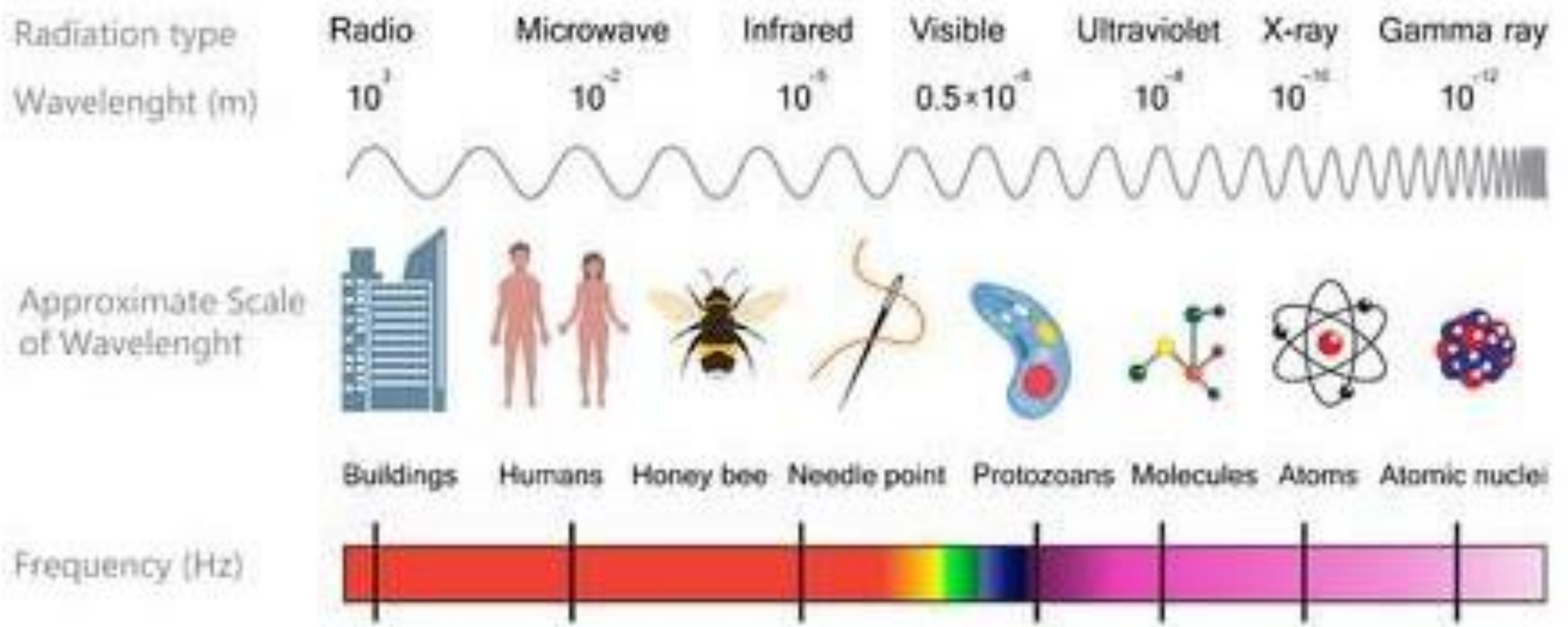


Electromagnetic spectrum



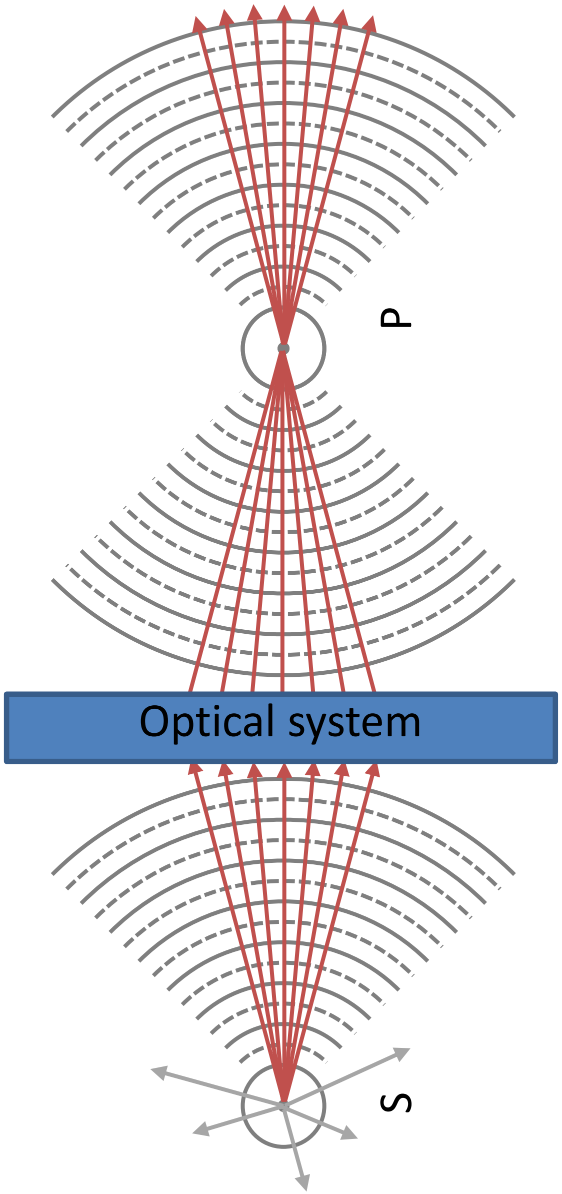
- λ for visible spectrum is 400 – 700 nm
- In order for ray optics to hold object size should be >> λ

Electromagnetic spectrum



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

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

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

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

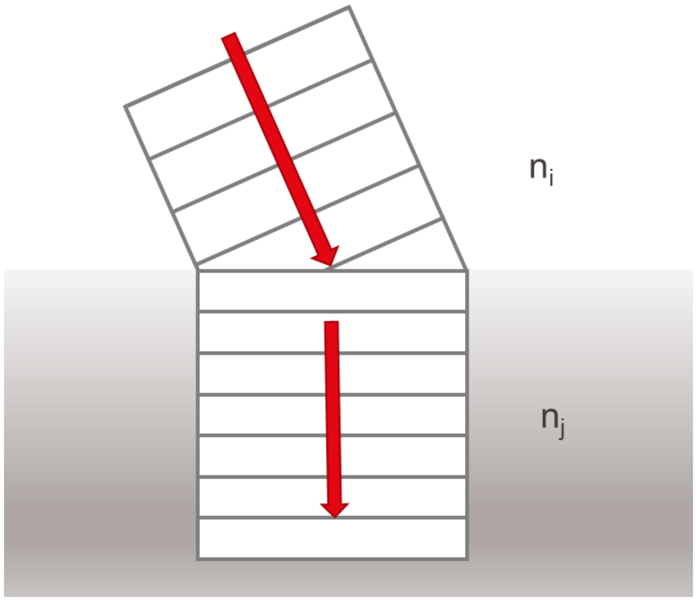
Optical medium & "**n**":

- The medium is called **"homogenous"** when its refractive index is constant, n=constant
- The medium is called **"inhomogenous"** when its refractive index is position dependent, n=n(r)

Light Propagation & Homogenous Medium

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

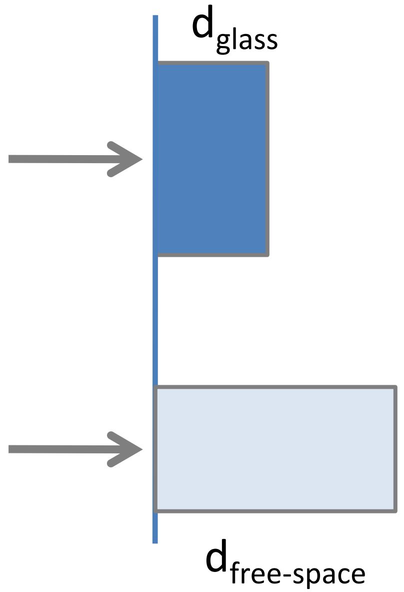
Medium	Refractive index n	Velocity in medium / km*s^{-1}
Air	1.0003	299203
Water	1.33	225032
Glycerol	1.47	203600
Immersion Oil	1.518	197162
Glass	1.56-1.46	191854-204995



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

- *Speed of light in glass is less than in air.*
- *For the same time duration, light will travel less distance in the glass compared to the propagation in free-space.*

Postulates of Ray Optics - 3

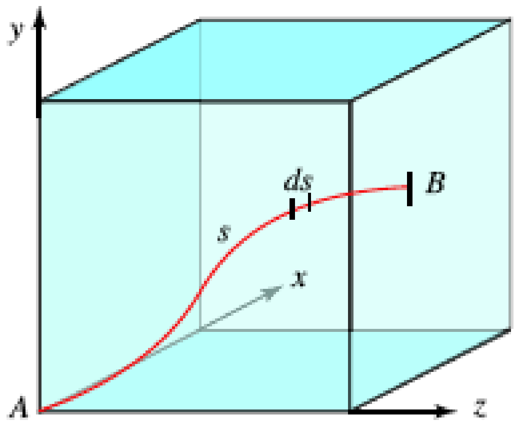
3. In an **inhomogeneous** medium the refractive index n(r) is a function of r (x,y,z).

Therefore, the optical path length is given as:

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

$$\Delta OPL(r) = n(r)\Delta S$$

$$\int_{\Delta S \to 0} \Delta OPL(r) = \int_{\Delta S \to 0} n(r)\Delta S$$



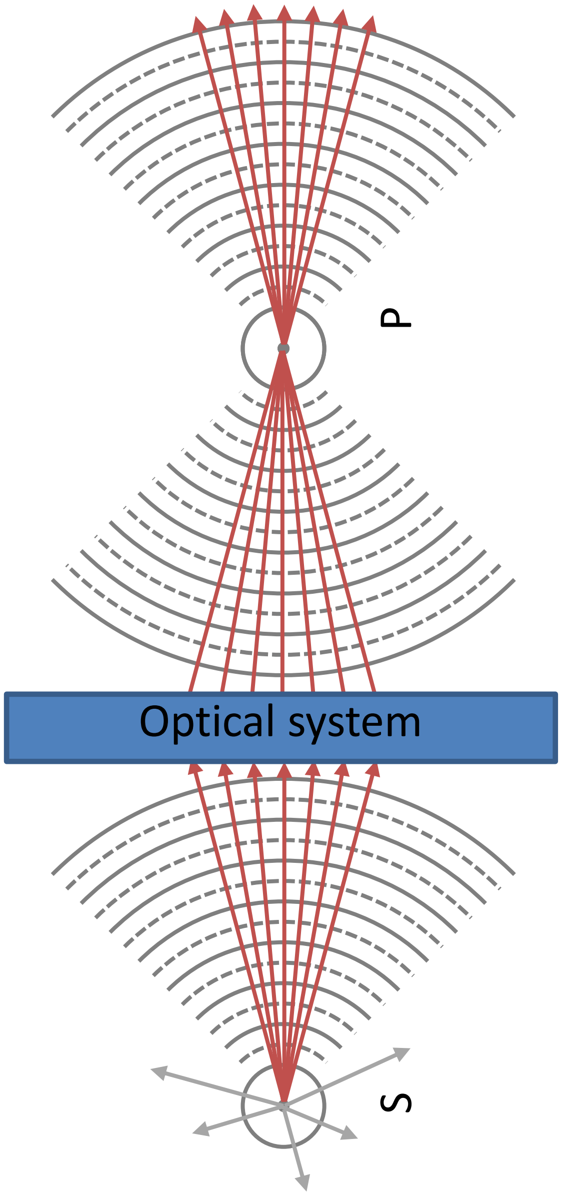
Fermat's Principle:

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:

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

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

Outline



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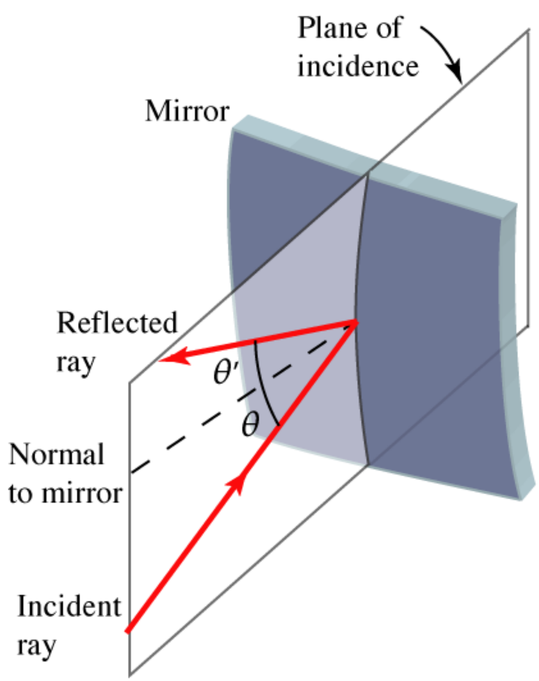
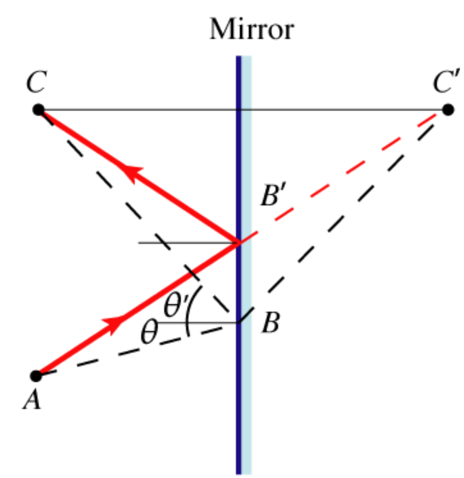
The Law of reflection

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 : $\theta' = \theta$



(a)



(b)

The Law of reflection

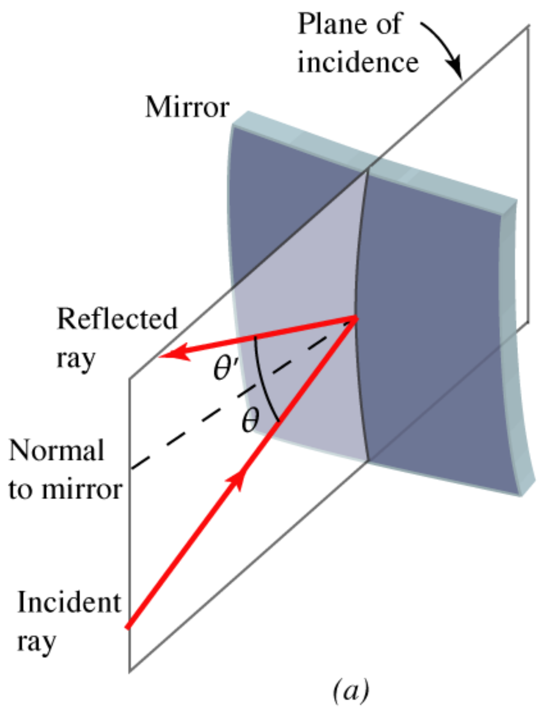
Proof:

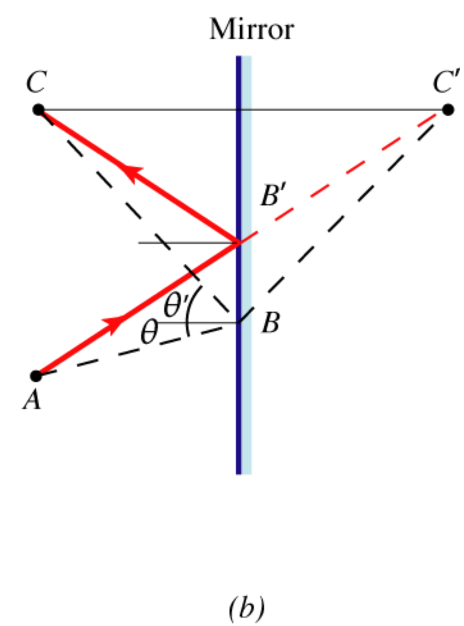
- Let's assume a ray travels from A to C by reflecting from B such that $\boldsymbol{\theta} \neq \boldsymbol{\theta}'$
- C' is the mirror image of C

- 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'

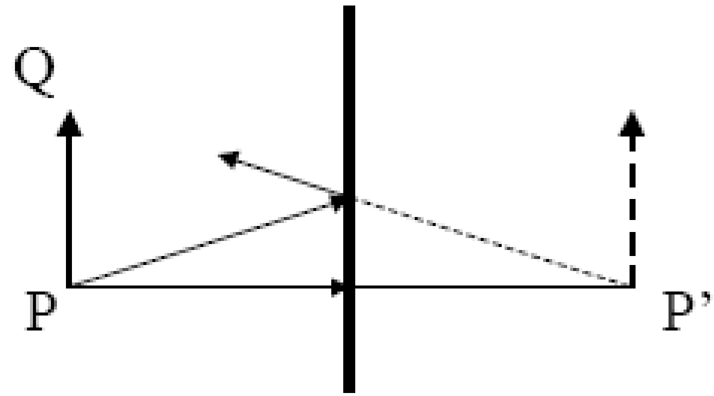
  Thus $\theta = \theta'$





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 a virtual image same as the object.

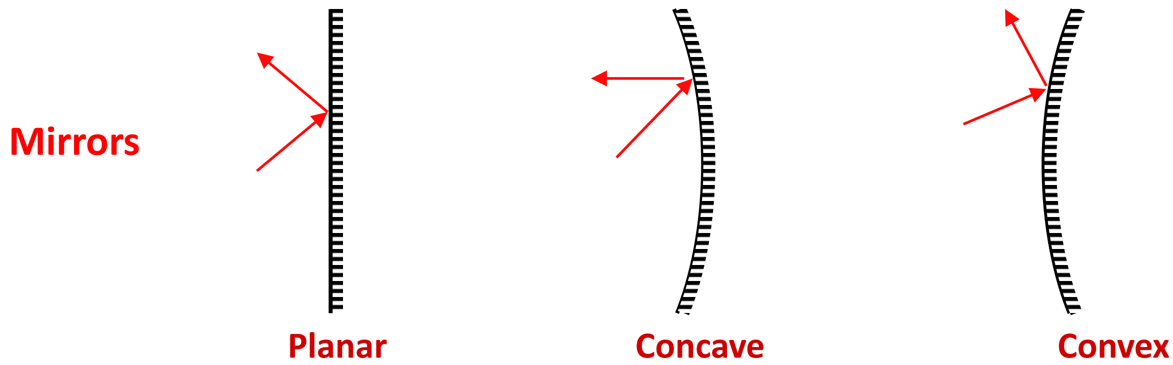
- Hence, the height h’ of the virtual image is same as h:

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

So, for a plane mirror m = +1

Components based on reflection: **Planar & Curved Mirrors**

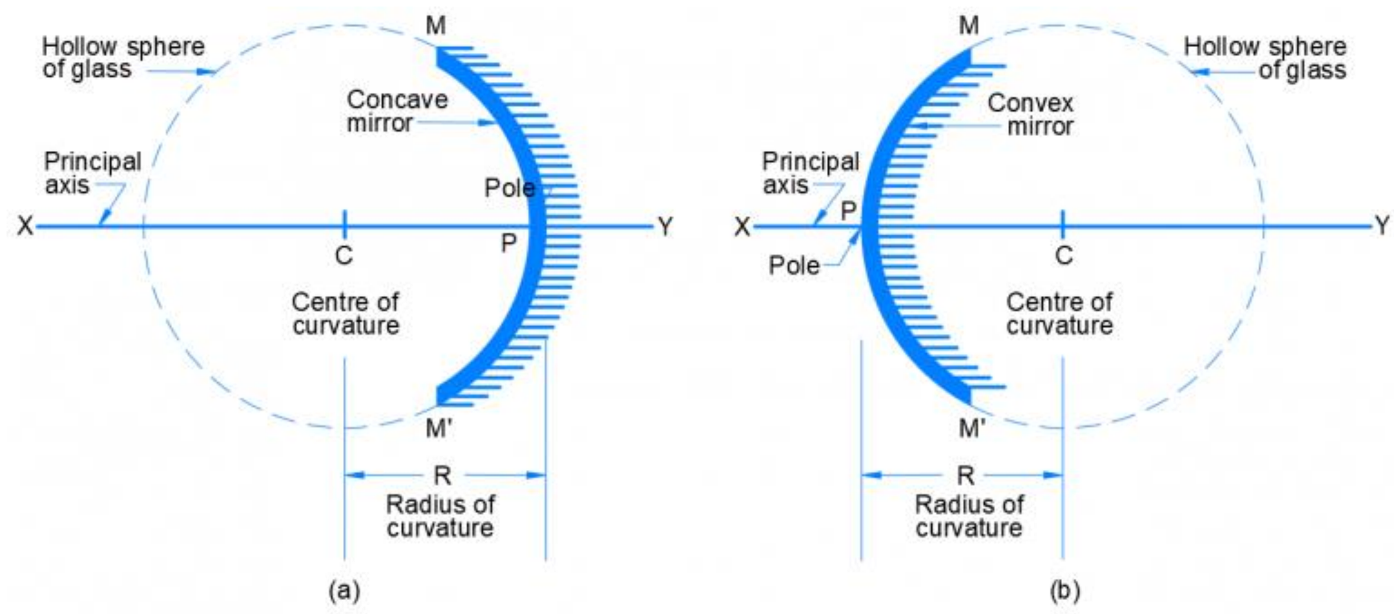
Reflective optical components (mirrors) a used to form an image, focusing and collimating light:



Imaging Concepts

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

Components based on reflection: **Curved Mirrors**



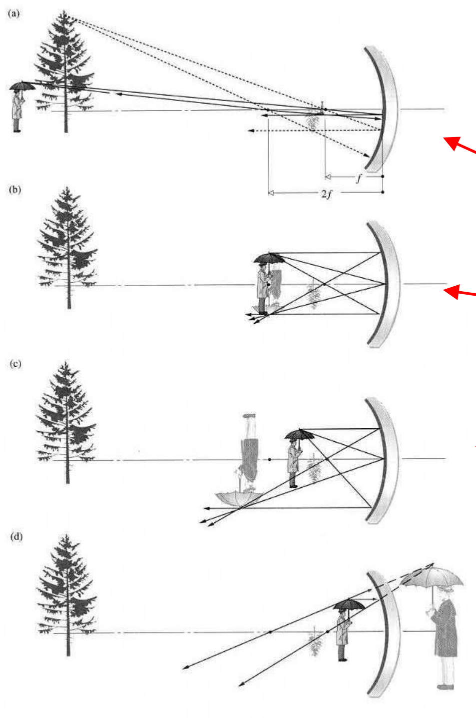
Key Terminologies:

- **Center of Curvature (C):** The hollow sphere's center, which represents a critical point for comprehending the mirror's behavior.
- **Pole (P):** The spherical mirror's central point. This is different from the center of curvature.
- **The radius of Curvature (R):** The distance between the center of curvature and the pole.
- **Principal Axis:** A straight line from the pole and the center of the mirror.
- **Focal Length (f):** Half of the radius of curvature, defining the focusing ability of the mirror.

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

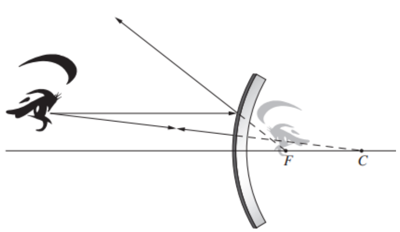
Curved Mirrors: Magnification

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



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	$\|s_i\| > s_0$	Inverted	Magnified



Convex mirrors

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

For the **image:**

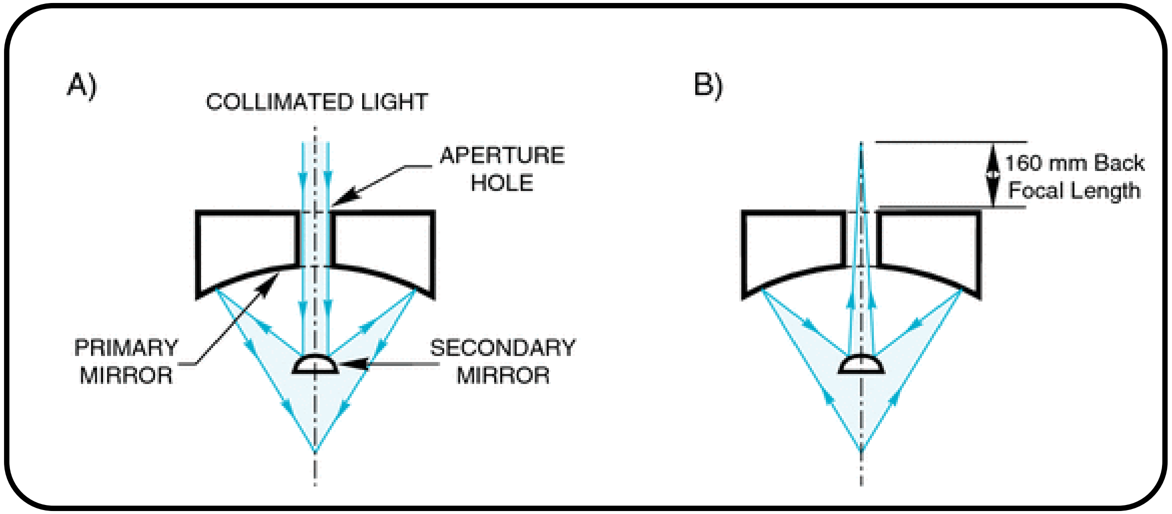
- If rays re-converge it corresponds to a **real image**
- If rays appear to emanate from then it is a **virtual image**

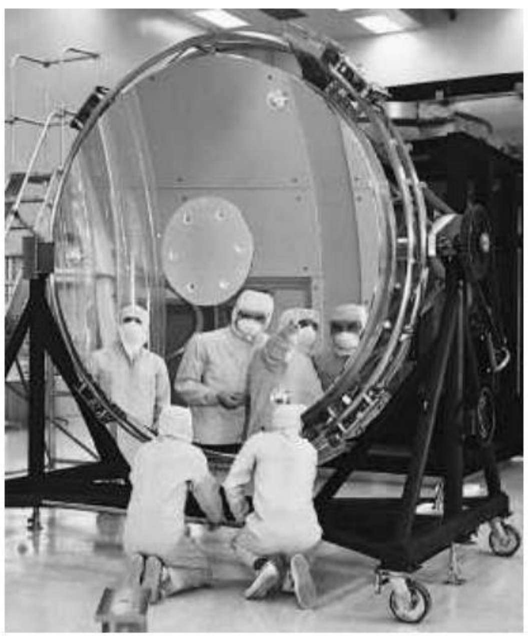
From E. Hecht, Optics Book

Curved Mirrors: Comparison Chart

BASIS FOR COMPARISON	CONVEX MIRROR	CONCAVE MIRROR
Meaning	Convex mirror implies the mirror whose reflecting surface is away from the center of curvature.	Concave mirror refers to the mirror whose reflecting surface is towards the center of curvature.
Shape		
Center of curvature	Lies behind the mirror	Lies in front of the mirror
Type	Diverging mirror	Converging mirror
Image	Virtual image is formed.	Real or virtual image is formed.
Used as	Rear view mirrors in cars and bikes.	Reflectors in projectors, searchlights etc.

Reflective microscope objective

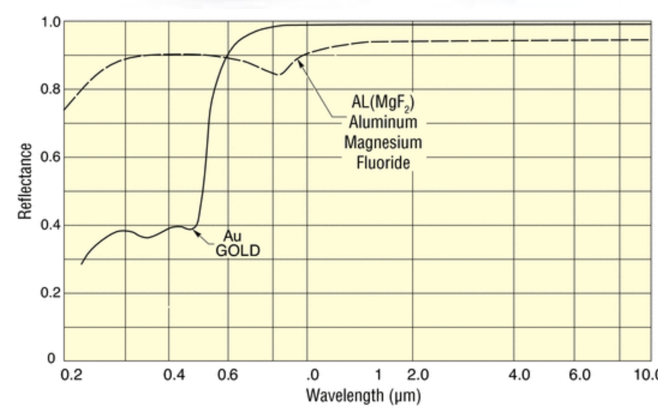




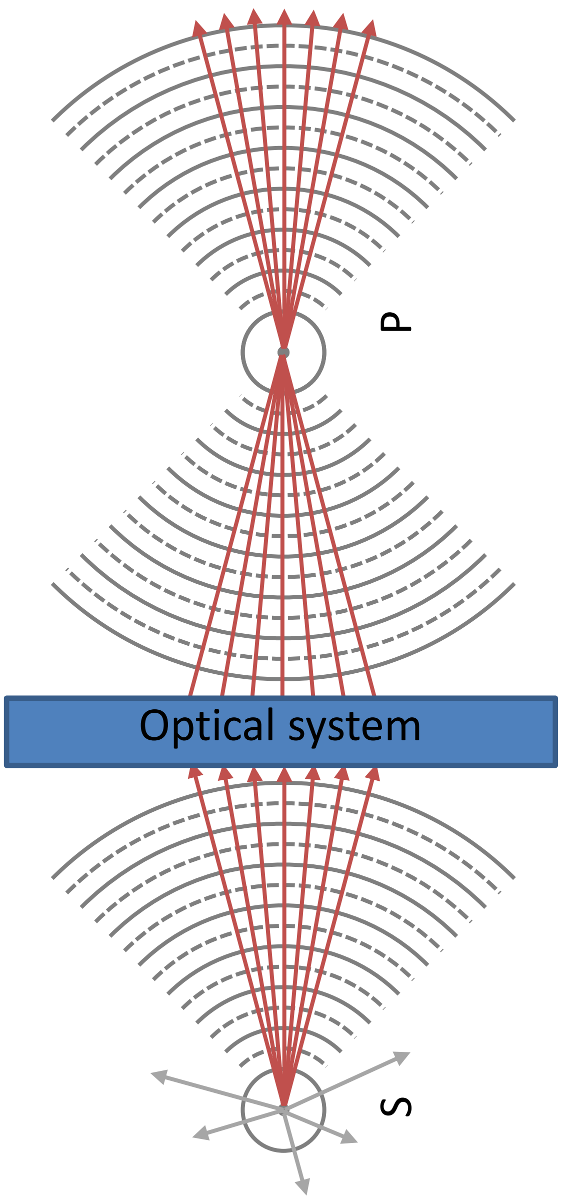
The 2.4-m-diameter hyperboloidal primary mirror of the Hubble Space Telescope. (NASA)

Application examples:

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



Outline



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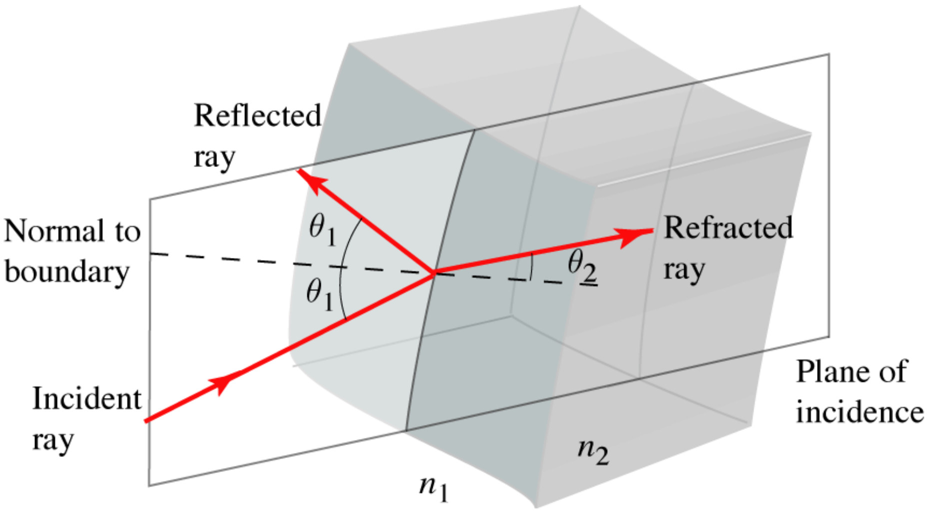
The Law of Refraction

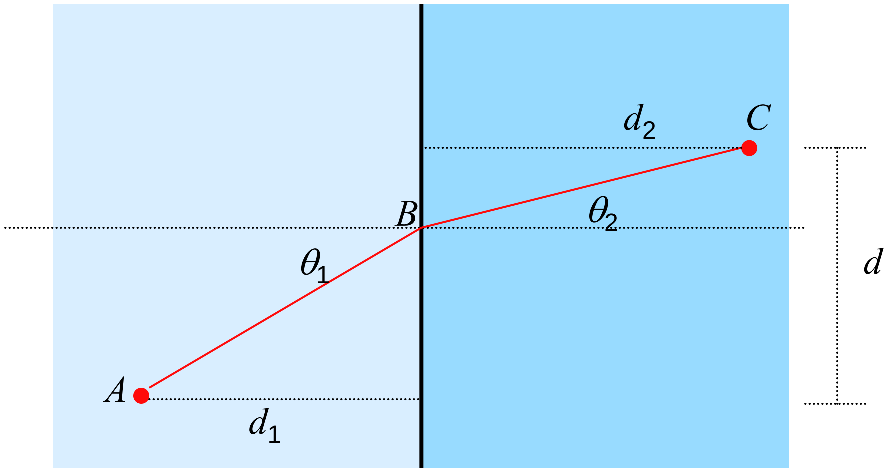
Refraction occurs at a boundary between two different media:

1- The refracted ray lies in the plane of incidence and follows the law of reflection ($\theta_{inc} = \theta_{reflect} = \theta_1$).

2- The angle of refraction ($\theta_{refract} = \theta_2$) is related to the angle of to the angle of incidence ($\theta_{inc} = \theta_1$) by the **Snell's Law**

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





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:

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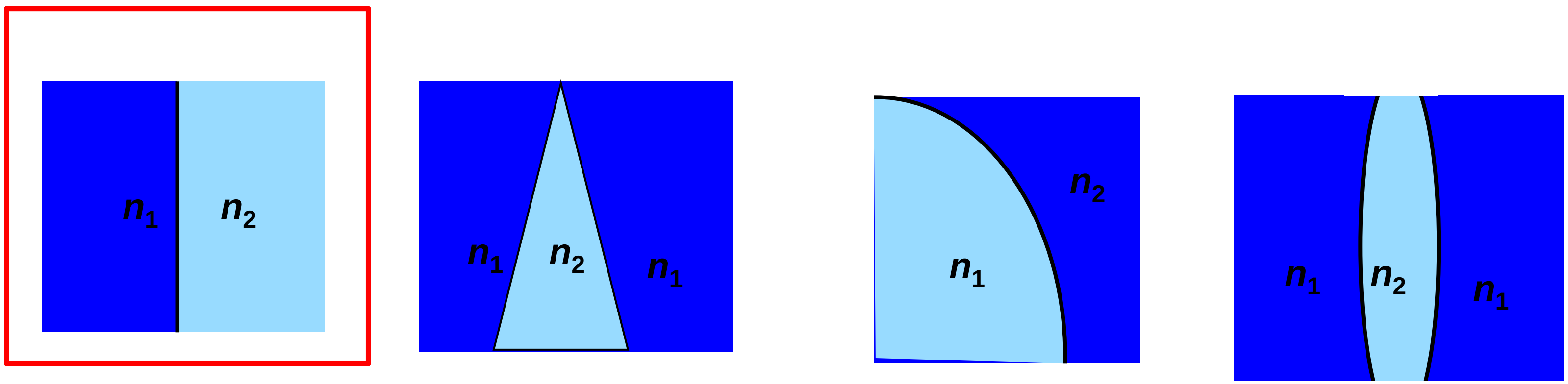
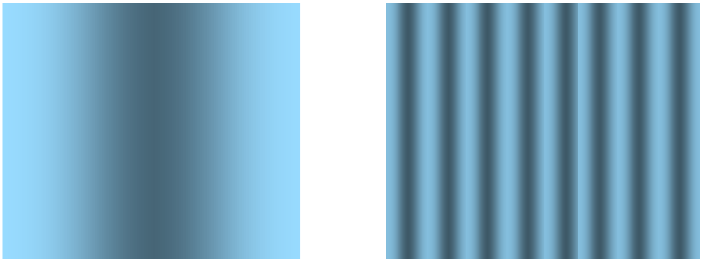
Boundaries Between Transparent Media

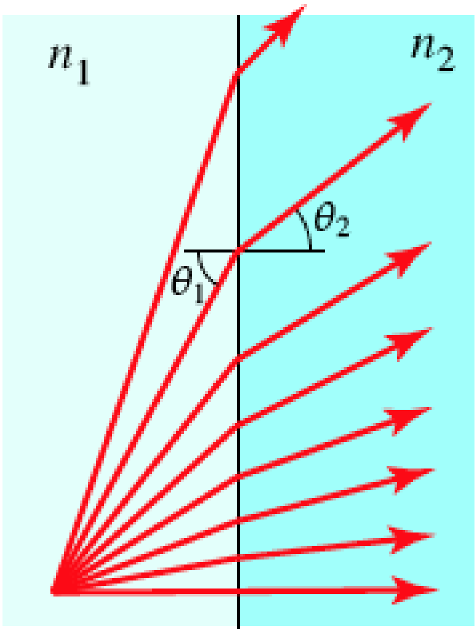


Graded-Index Media



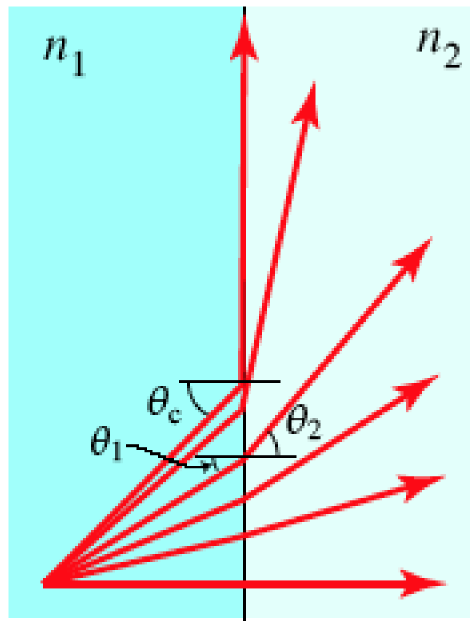
Planar Boundaries (application of Snell's Law)

$n_1 < n_2$

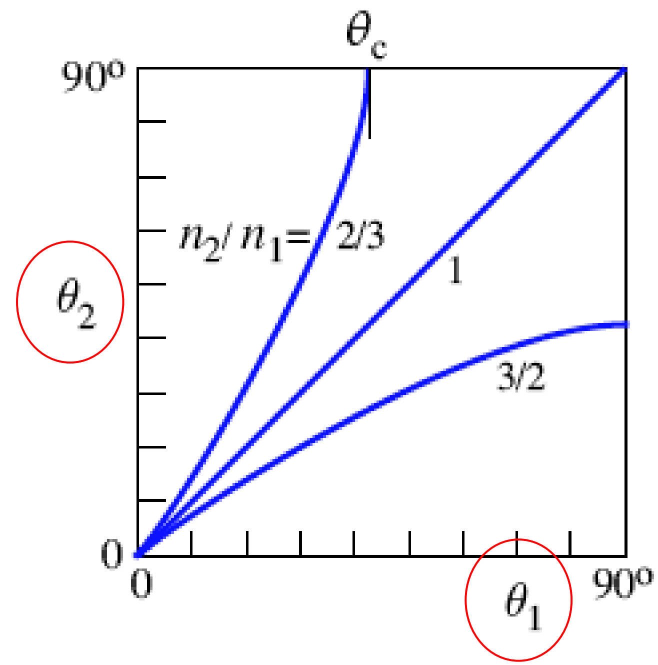


External refraction

$n_1 > n_2$



Internal refraction



Snell's Law:

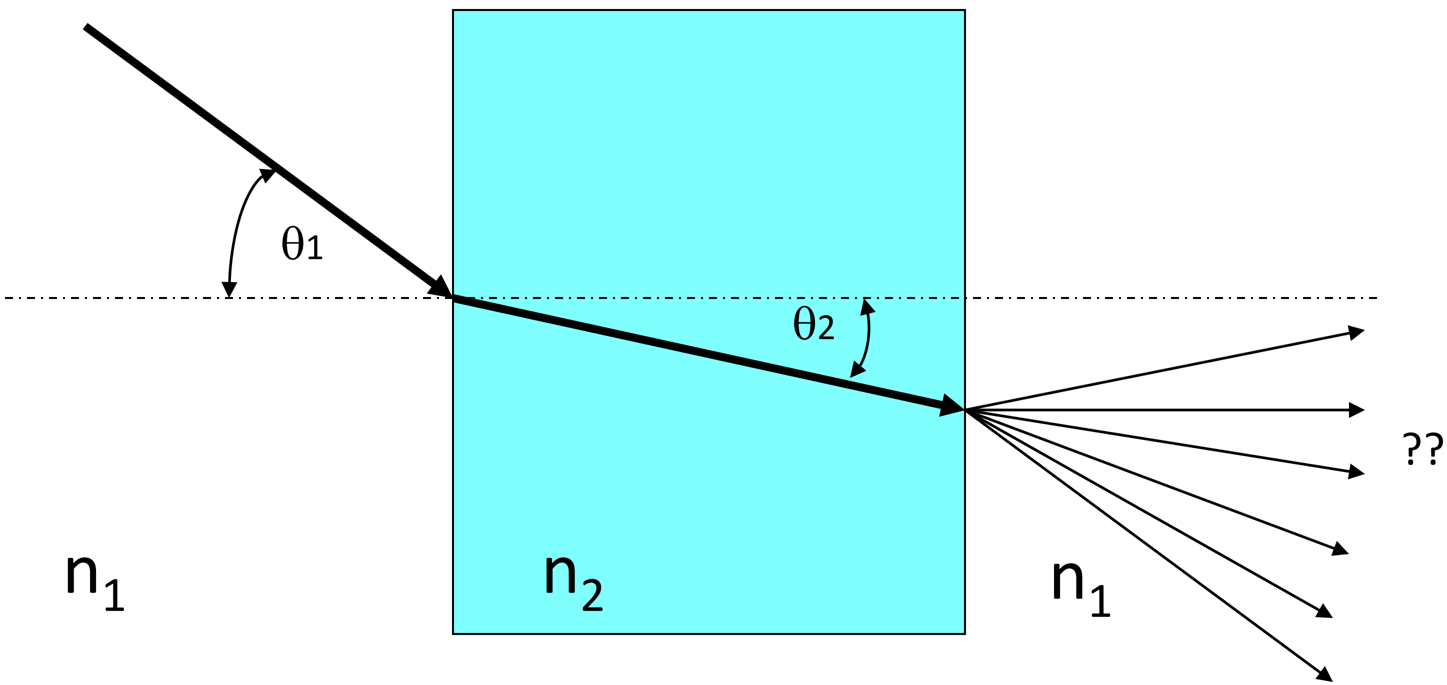
$$\sin\theta_1 = (n_2 / n_1)\sin\theta_2$$

Critical Incidence Angle:

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

Refraction:
light beam through a plane-parallel glass plate

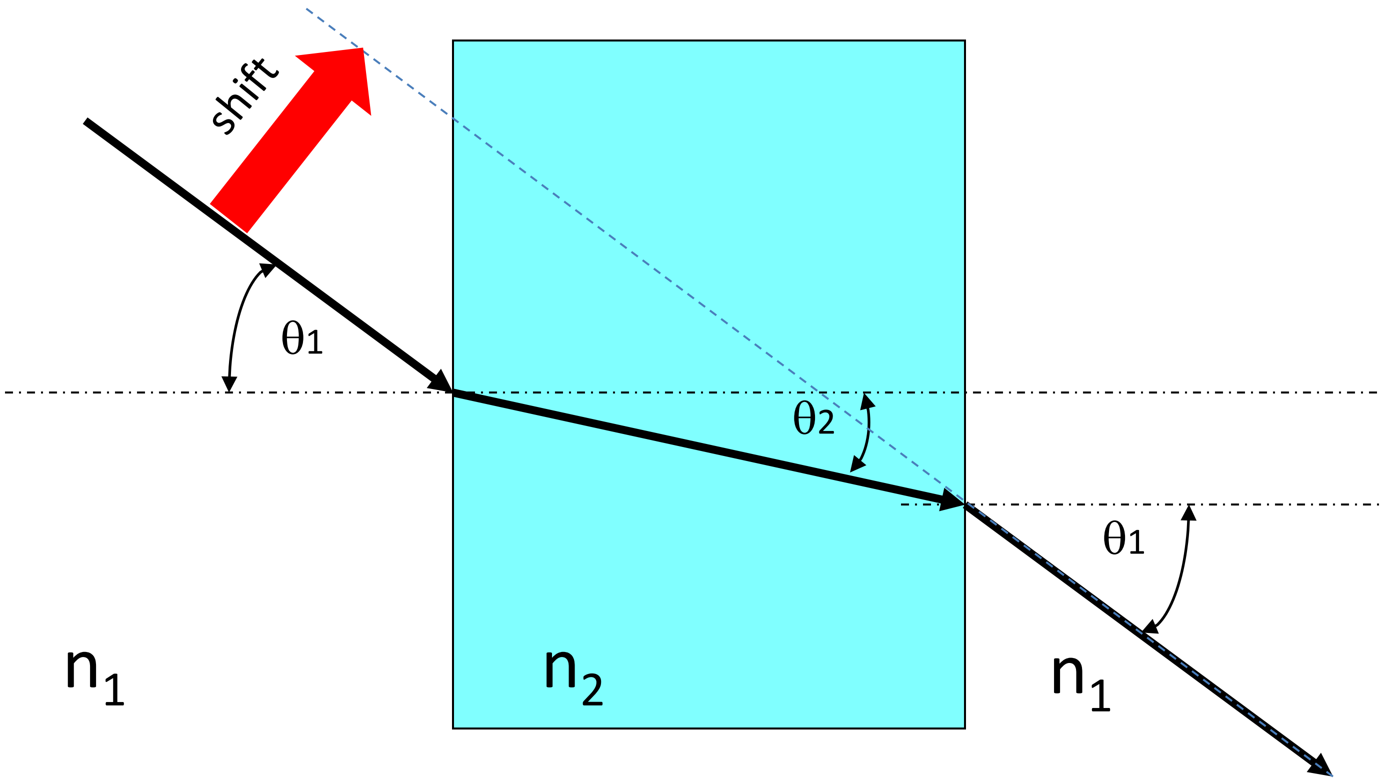
Snell's Law: $\eta 1 \sin \theta 1 = \eta 2 \sin \theta 2$



What is the angle of light at the exit of the glass plate?

Refraction:
light beam through a plane-parallel glass plate

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



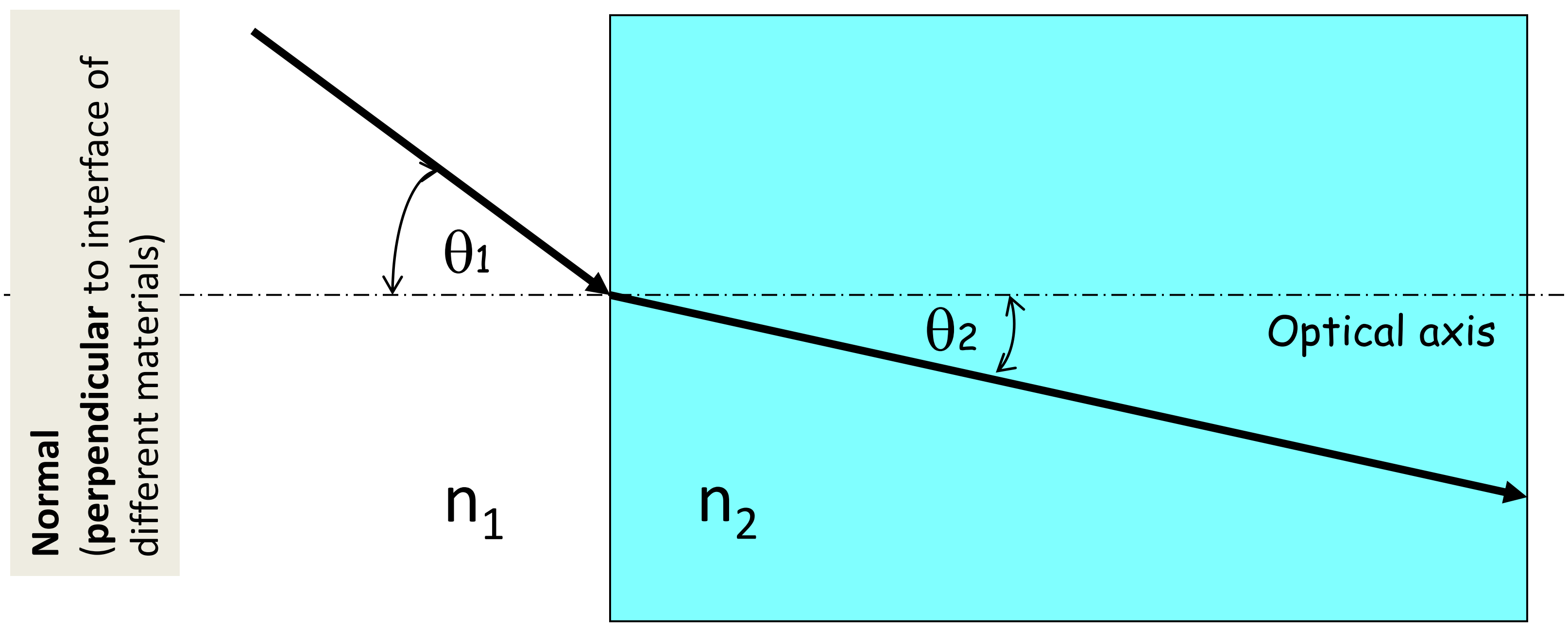
"Exit angle" is same as the incident angle ➔ the beam doesn't change deflection angle
➔ Thus, a parallel plate only results in a lateral shift in beam path.

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 **<u>paraxial approximation</u>** ➔ $n_1 \theta 1 \sim n_2 \theta 2$



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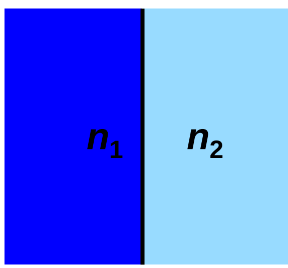

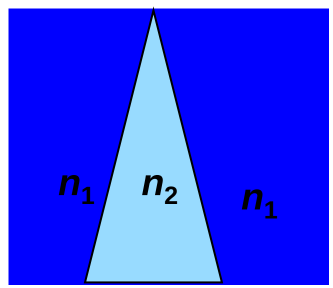

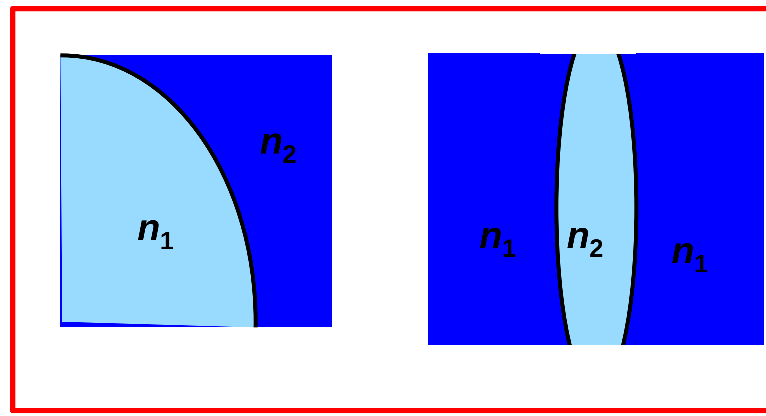

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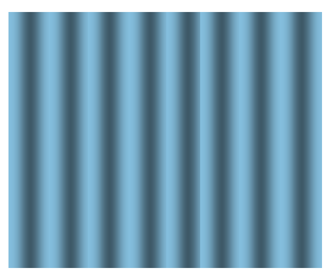
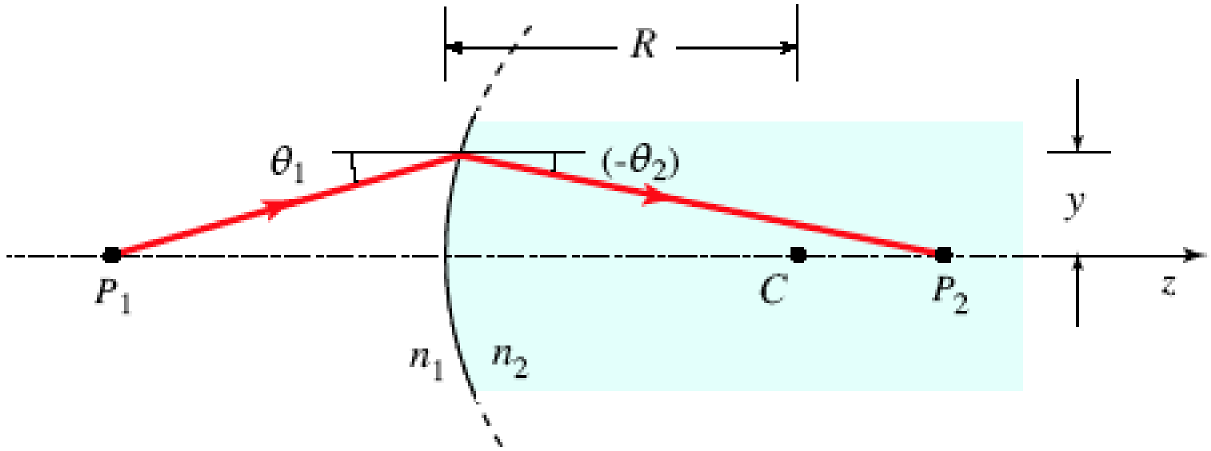


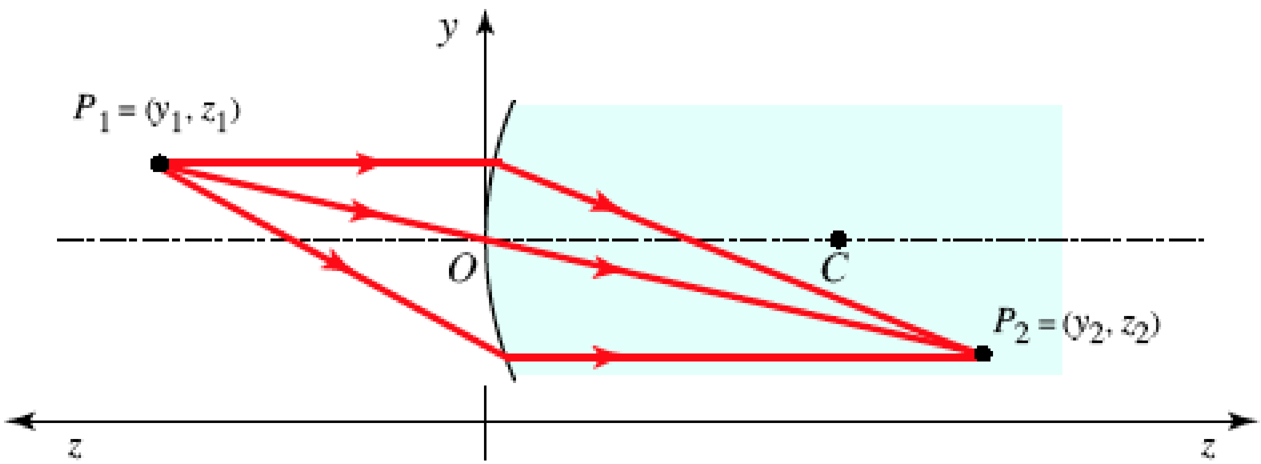
Spherical Boundary



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

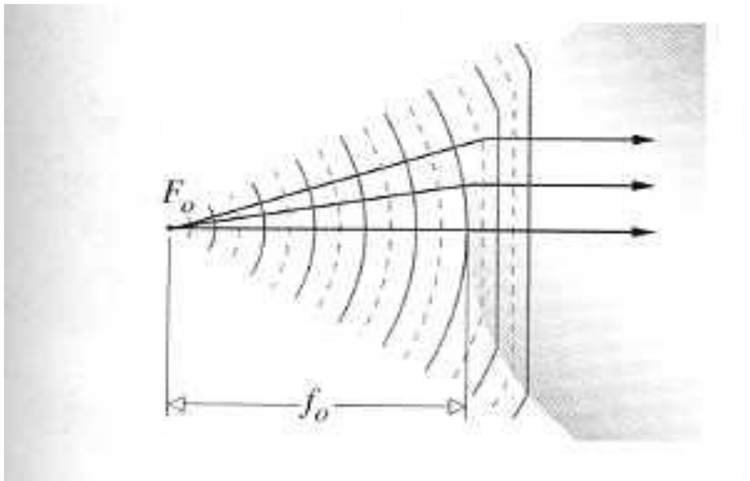
$$\frac{n_1}{z_1} + \frac{n_2}{z_2} \approx \frac{n_2 - n_1}{R}$$

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 is imaged at infinity:

$z_2 = \infty$

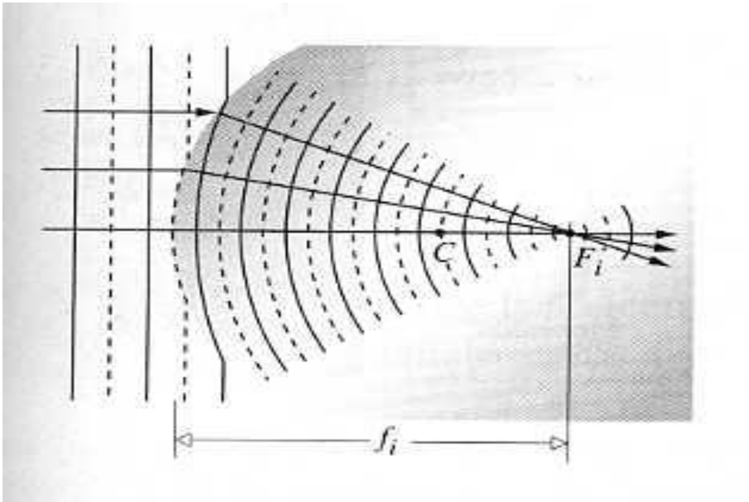
$z_1 = s_o = f$

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

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

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

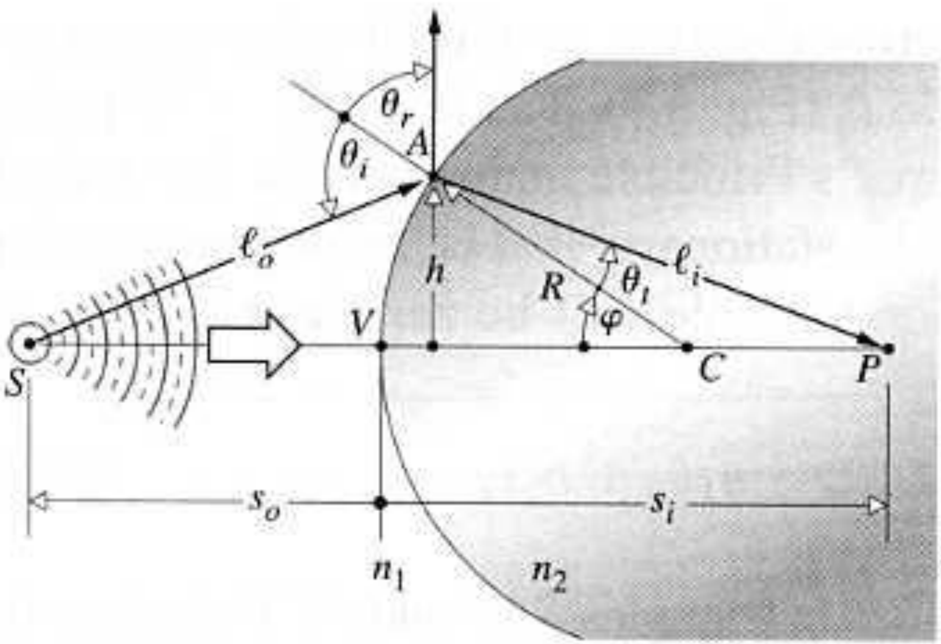
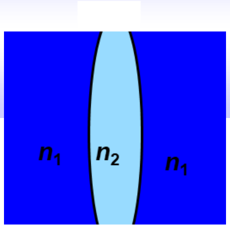


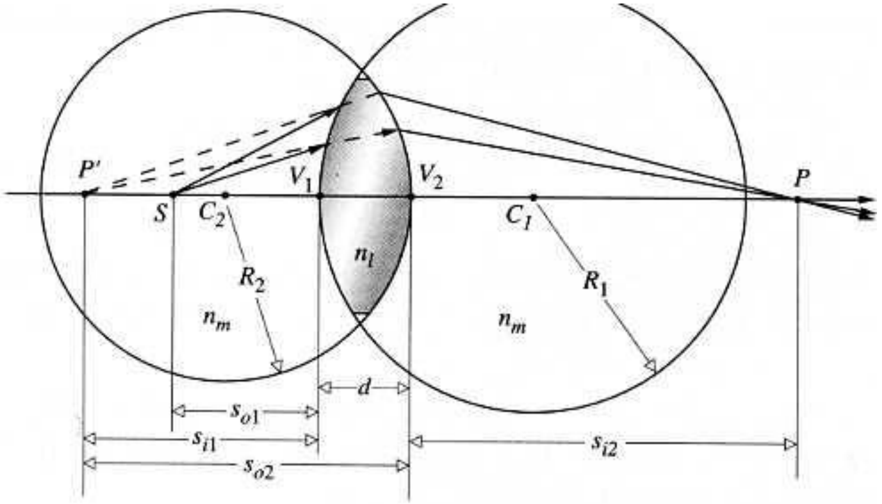
TABLE 5.1 Sign Convention for Spherical Refracting Surfaces and Thin Lenses* (Light Entering from the Left)

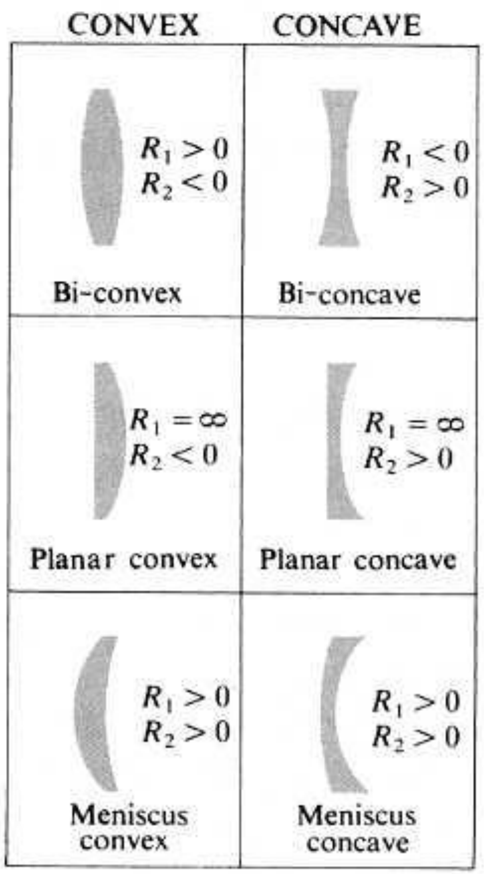
s_o, f_o	+ left of V
x_o	+ left of F_o
s_i, f_i	+ right of V
x_i	+ right of F_i
R	+ if C is right of V
y_o, y_i	+ above optical axis

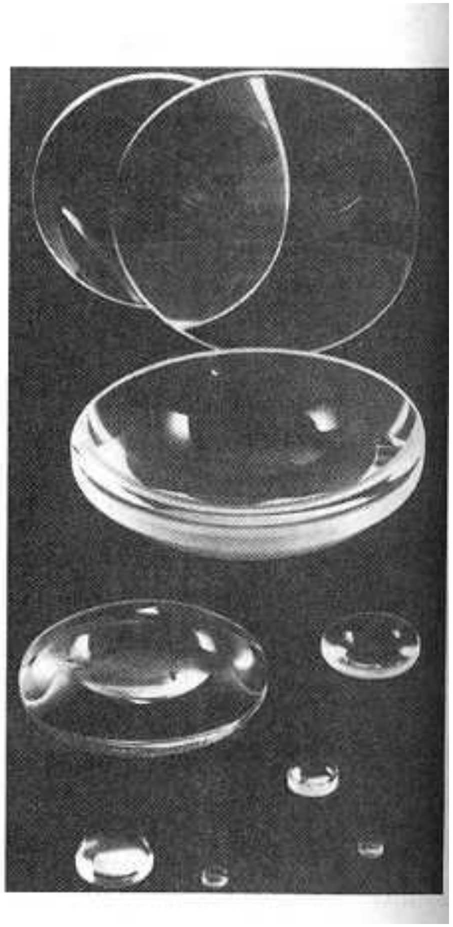
Spherical Lens



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







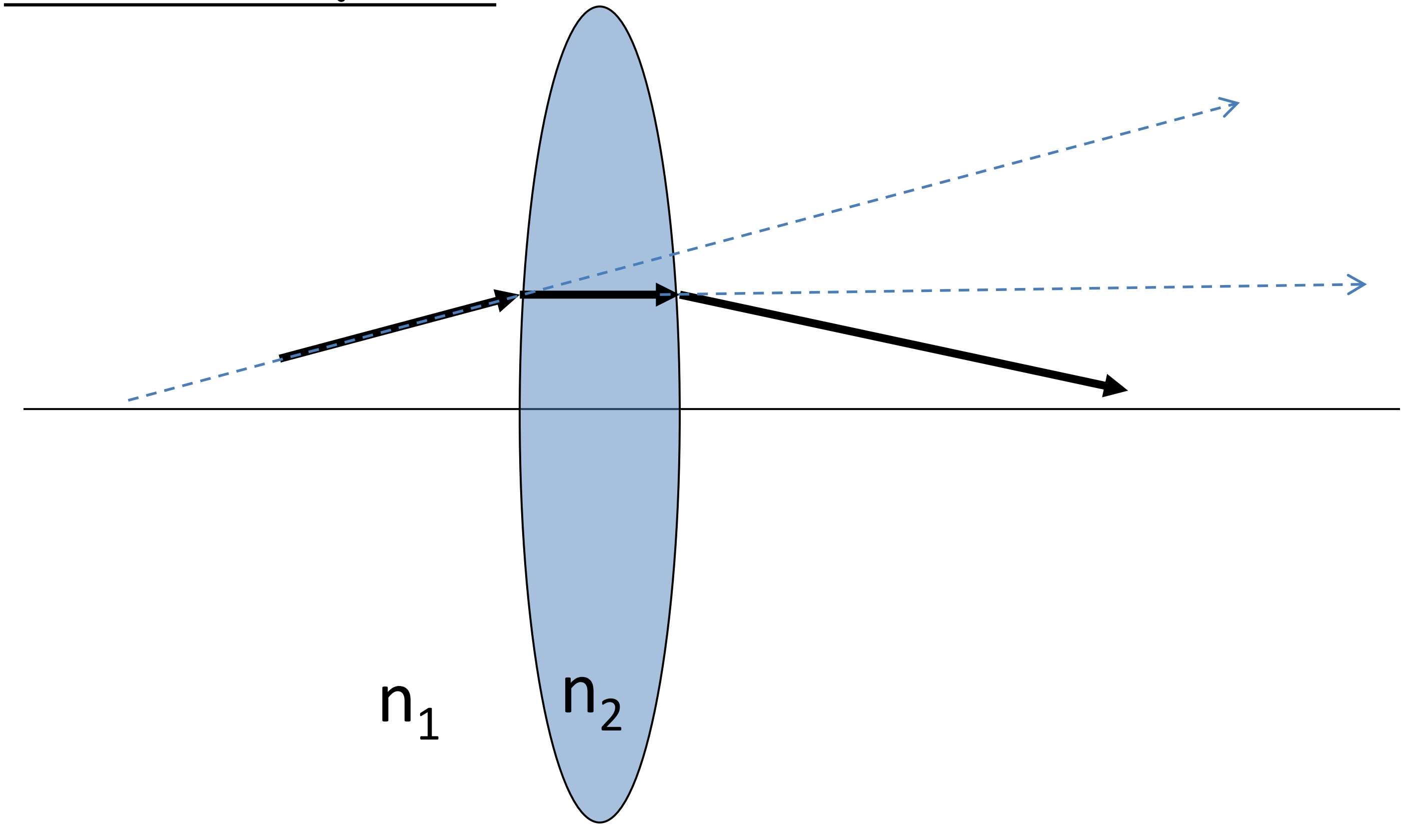
In order to find the total deflection angle, apply the following equation twice

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

Lenses

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

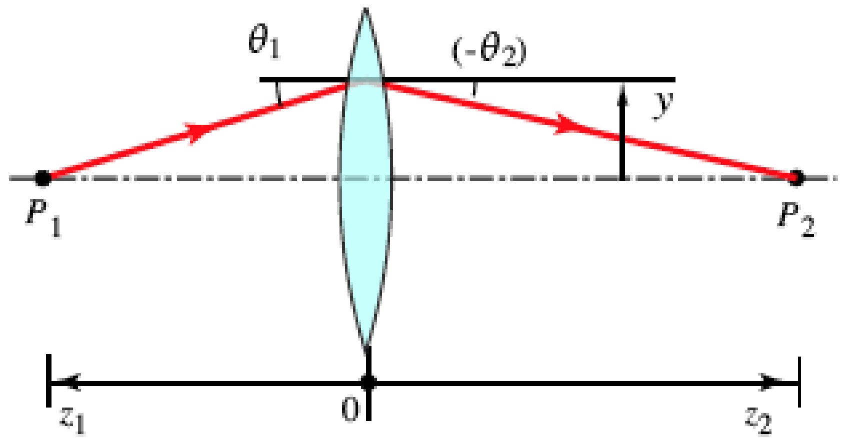
➔It has 2 **curved surfaces**



A lens changes the beam direction
→ Results in a beam deflection

Formulas for Thin Lenses

A thin lens has a thickness that is essentially negligible.



When this thin lens is in free space:

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

Lens maker's formula (thin lens equation)

Focal Length *f*

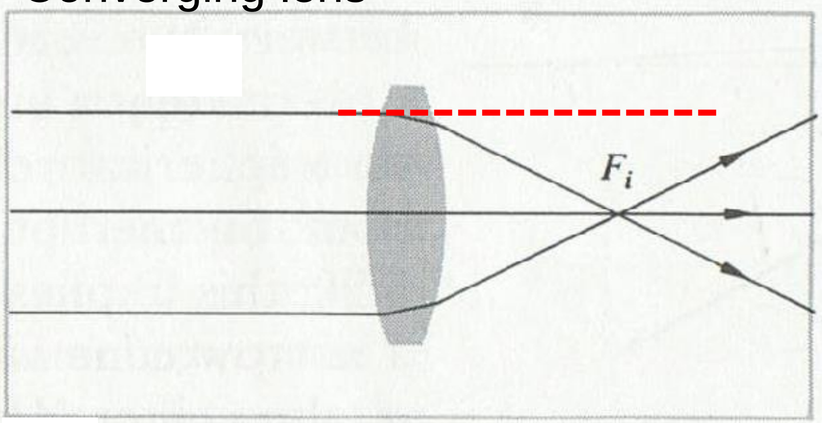
$$\frac{1}{f} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$$

Thin Lenses

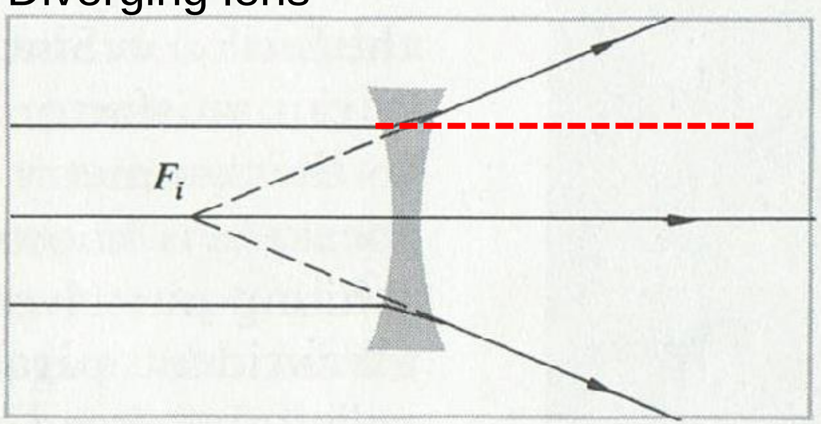
For a thin lens is in free space:

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

Converging lens

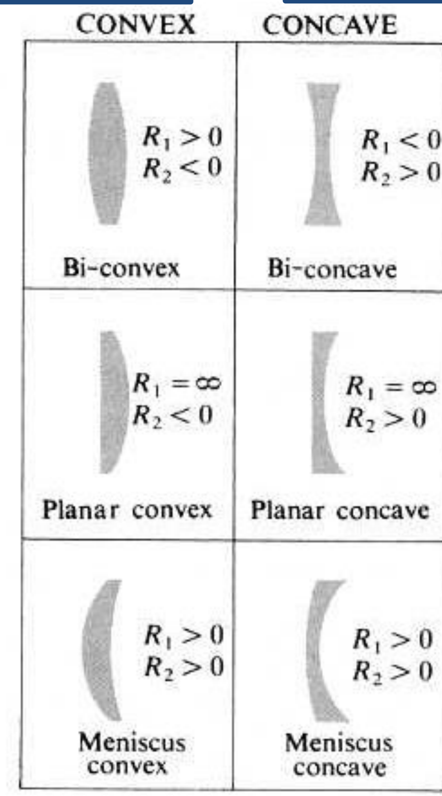


Diverging lens



f = positive
converging

f = negative
diverging



Determining the focal length of a thin lens

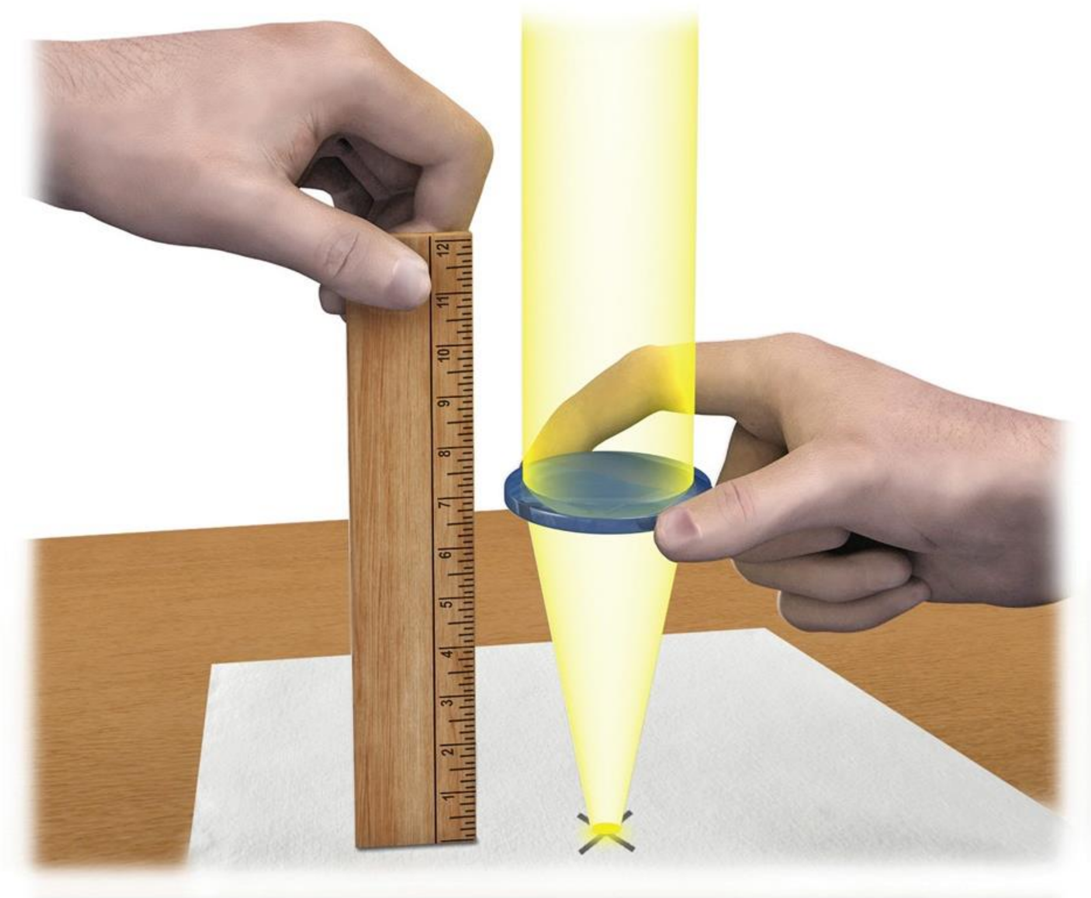


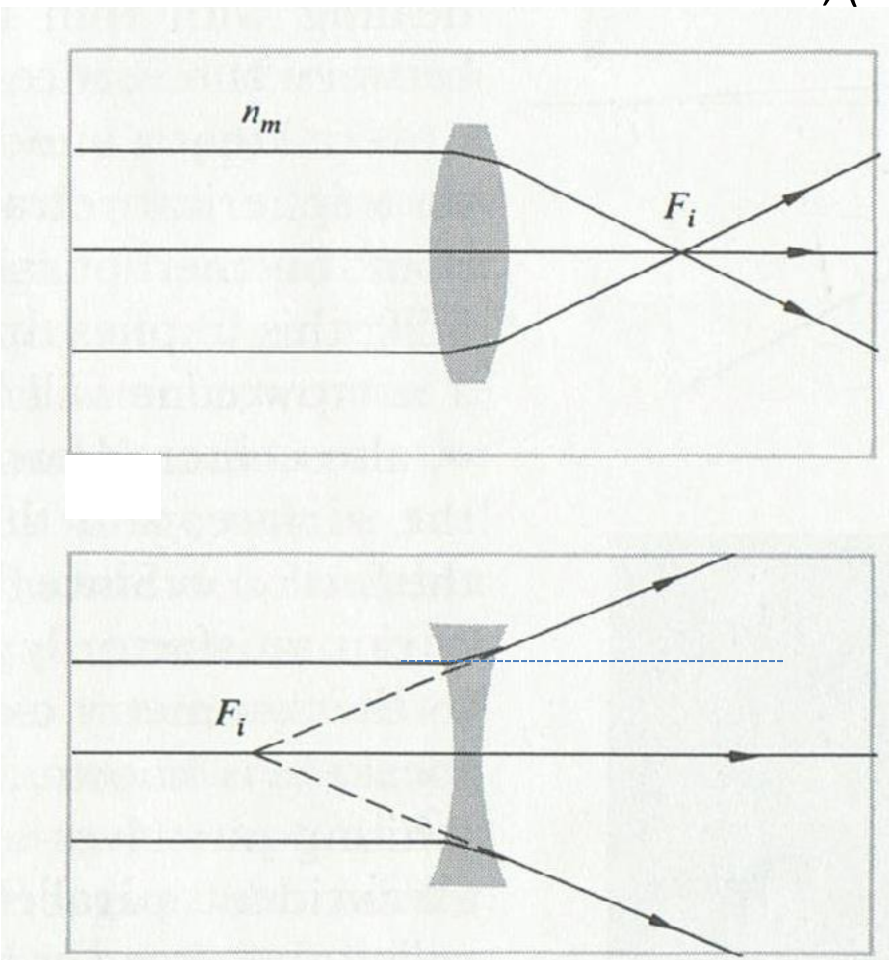
Figure 4.6

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

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

- Lens is made out of material with index $n_{lens,}$ which is bigger than 1
- Outside of lens is air with $n_{outside}$=1
- Thus, (n-1) > positive



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

Pay attention to medium dependence

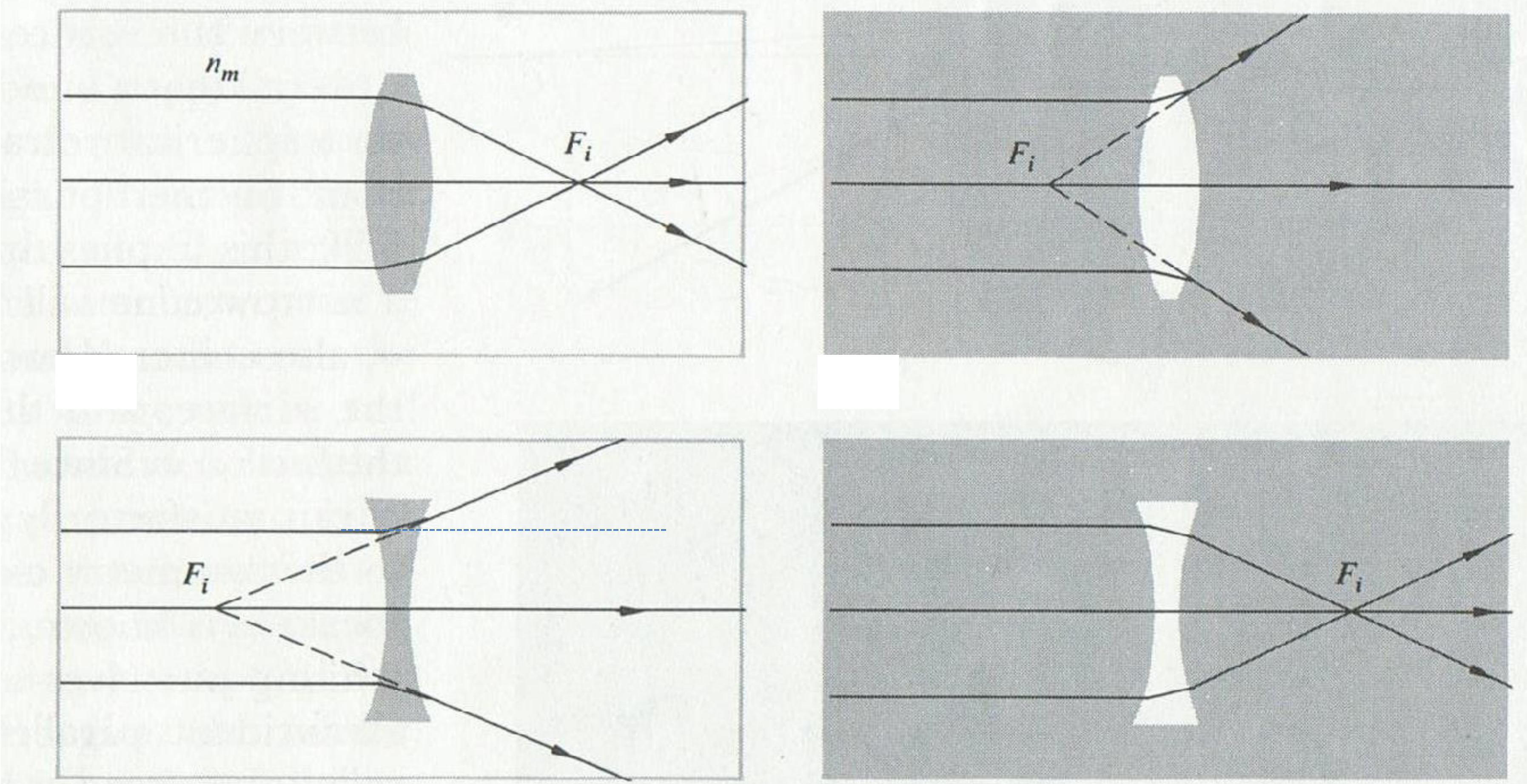
$$\frac{1}{f} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$$

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}



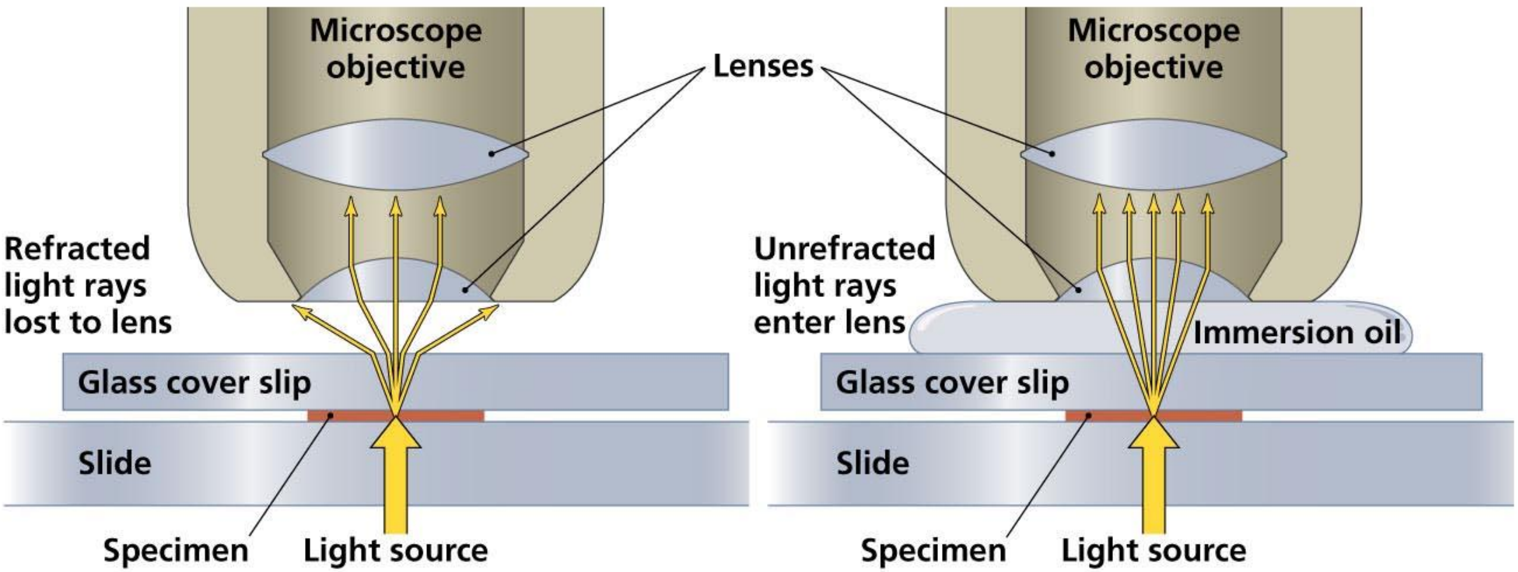
Pay attention to medium dependence

Do magnifying glasses work underwater?

Normal magnifying glasses do not work underwater because the refraction index of glass is almost equal to that of water.

DIVING MAGNIFYING GLASS

Medium dependence is important in microscopy



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