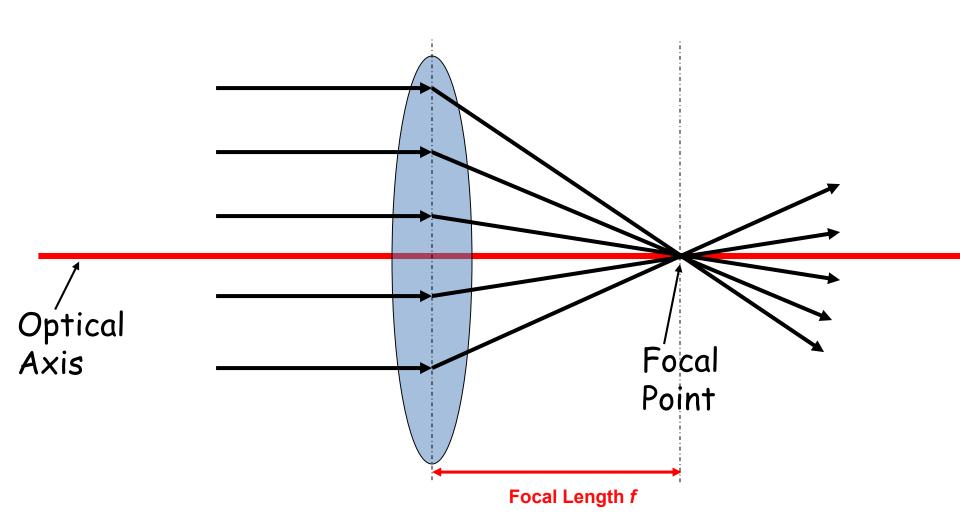
MICRO-561

Biomicroscopy I

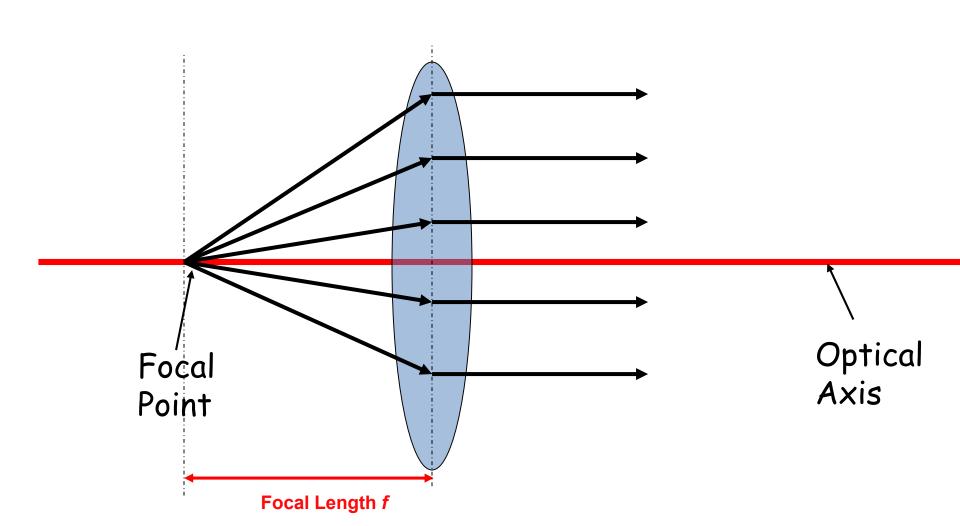
Syllabus (tentative)

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 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

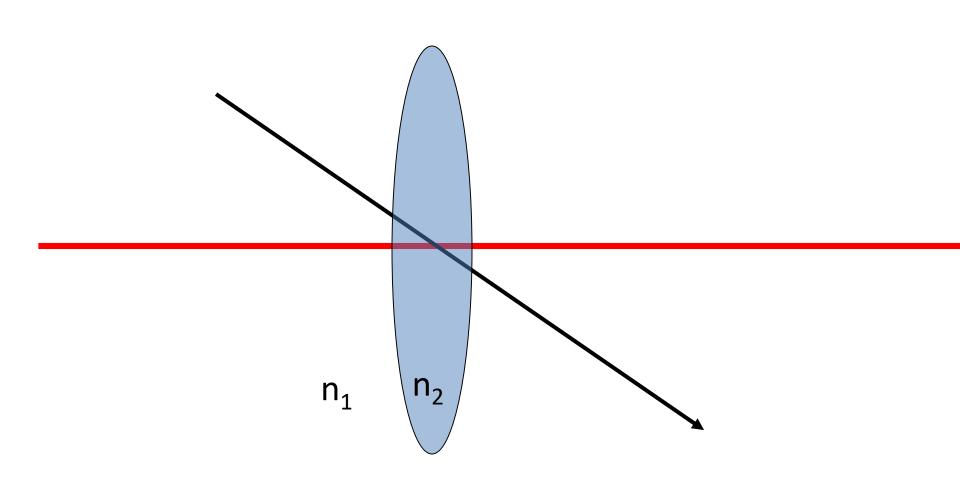
1. The Parallel Ray: Light rays that enter the lens parallel to the optical axis leave the lens by passing through Focal Point



2. The Focal Ray: Light rays that enter the lens from the focal point exit the lens parallel to the optical axis.

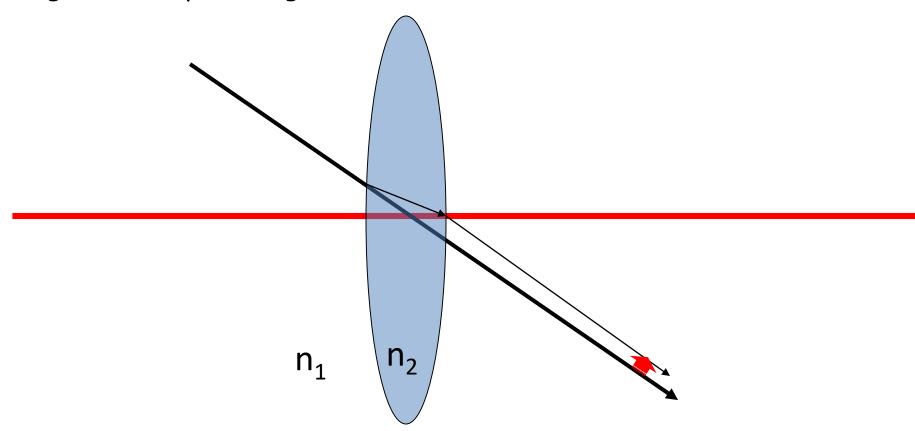


3. The Chief Ray: A ray that enters through the center of lens goes straight, without deviation

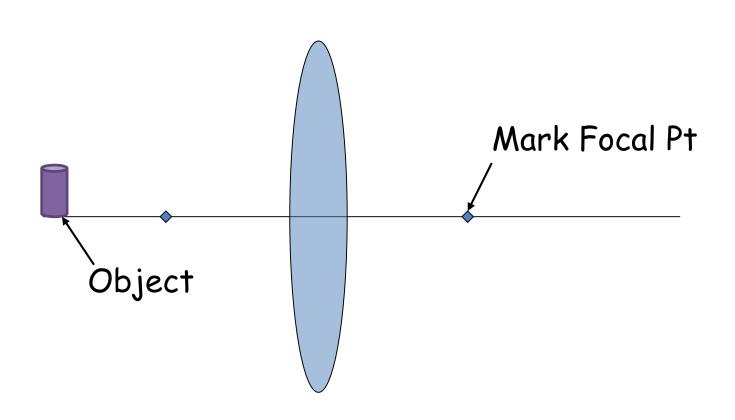


3. 3. The Chief Ray: A ray that enters through the center of lens goes straight, without deviation

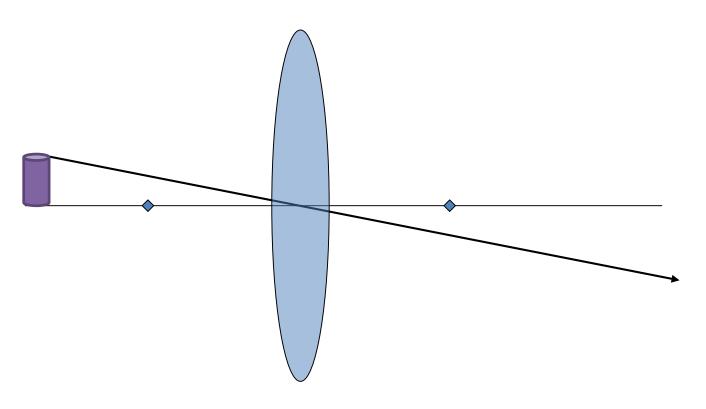
Correction: Note that normally there is a shift, but this shift can be neglected only if the glass is thin.



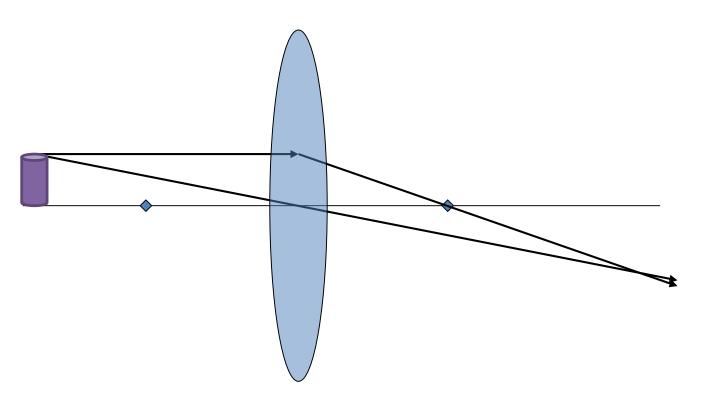
Let's applying ray tracing using **three principle rays** to an extended object, and find its image



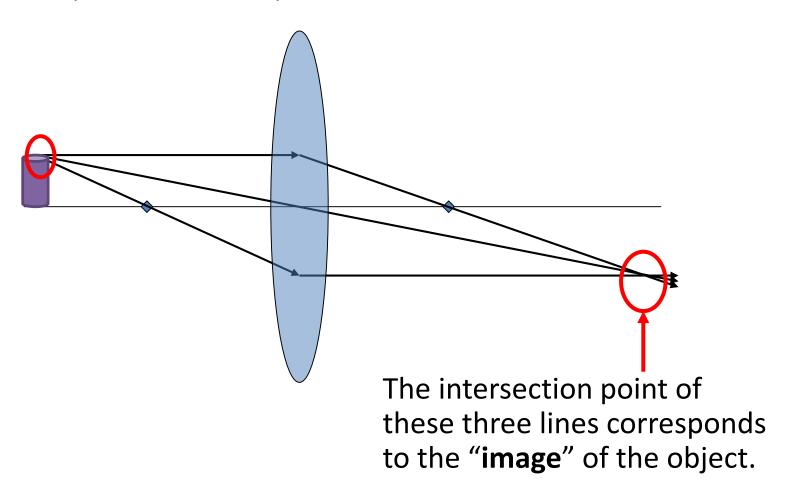
1. The Chief Ray: A ray enters through the center of the lens goes straight



2. The Parallel Ray: Light rays that enter the lens parallel to the optical axis leaves through Focal Point

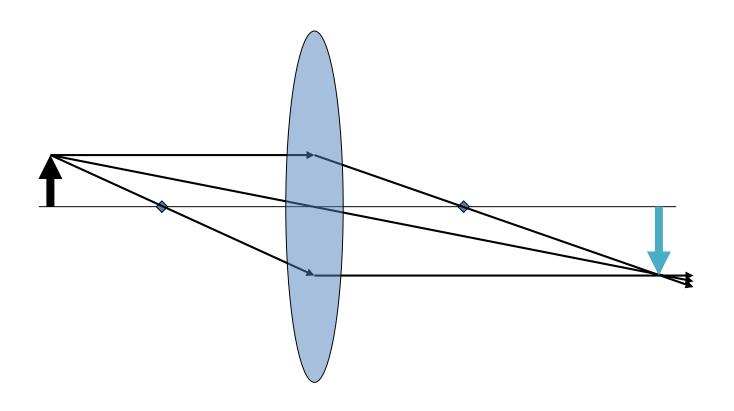


3. The Focal Ray: Light rays that enter the lens from the focal point, exit parallel to the optical axis.

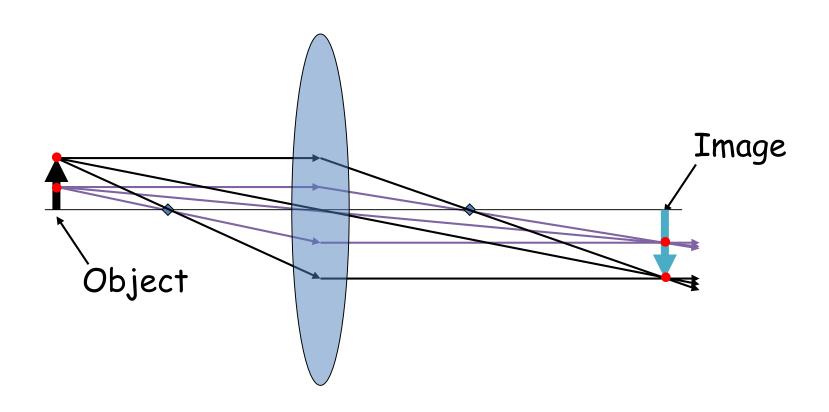




Ray tracing convention generally uses arrows to represent the object.

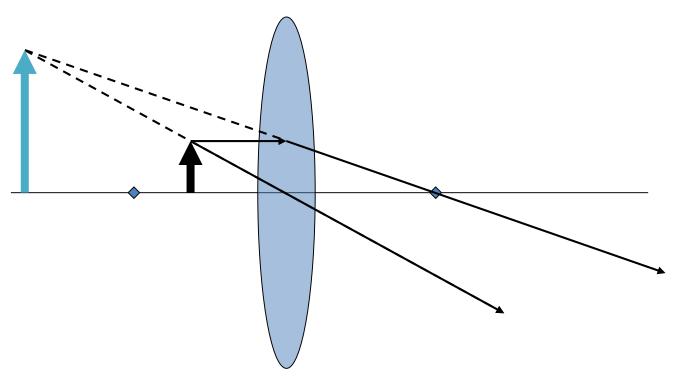


Same three rays can be applied for each point along the object.

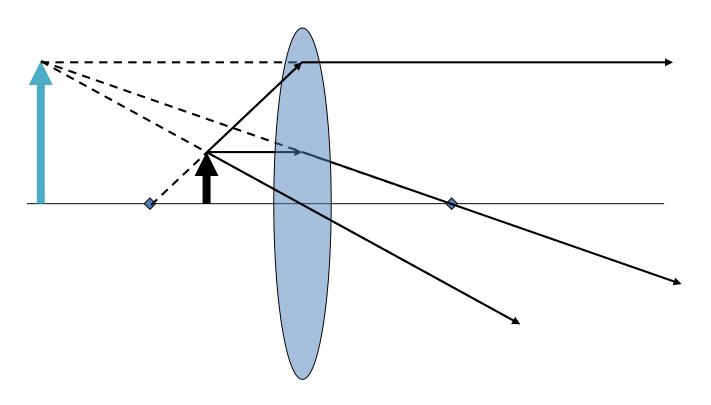


For object within the focal point:

- intersection point occurs for the extension of the rays (dashed lines), and it is at the back side of the object
- This corresponds to a "virtual image"

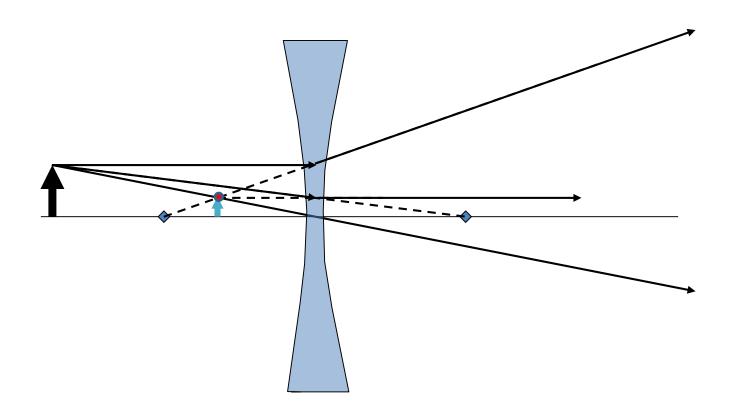


- Only need two rays are sufficient to locate the image.
- Of course one can use all the rules to trace three (or more) rays, and the intersection point will remain same

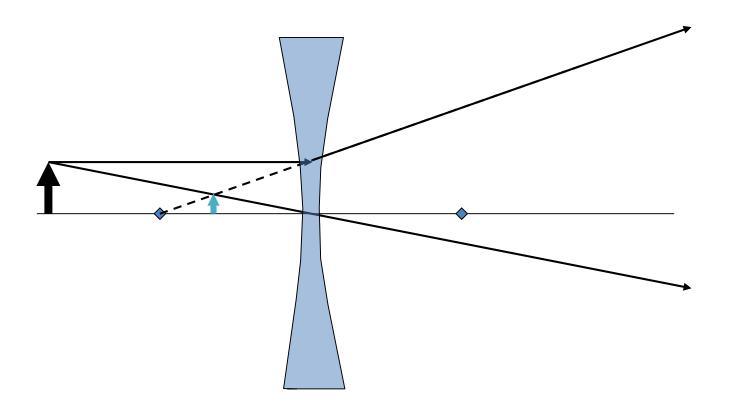




Same three rules can be applied to a concave (diverging) lens.



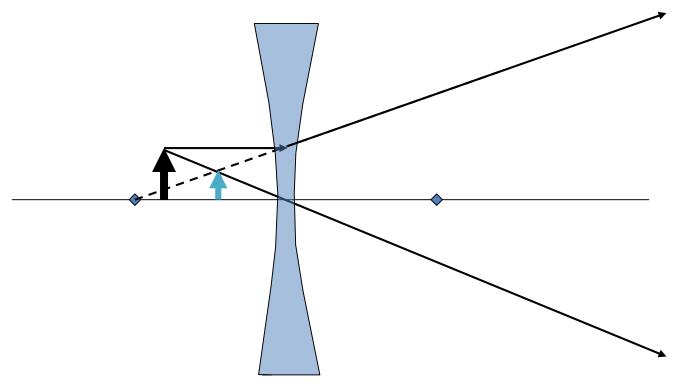
Again, two rays are enough to locate virtual image.



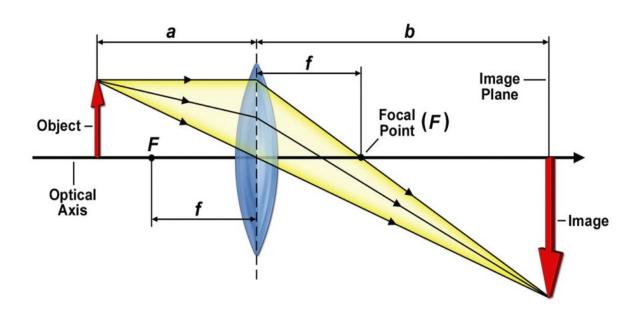


Concave lens makes a virtual image that is smaller than the object

....no matter where the object is located.



Formulas for thin lens: object-image relationship



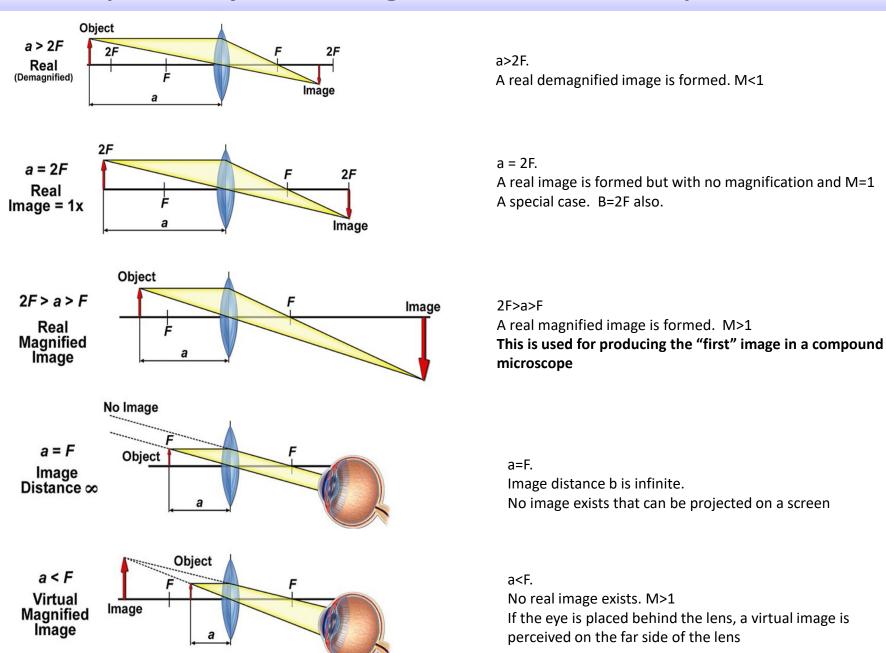
$$\frac{1}{f} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$$

Focal length (lens maker's formula)

$$\frac{1}{f} = \frac{1}{a} + \frac{1}{b}$$
 Imaging condition

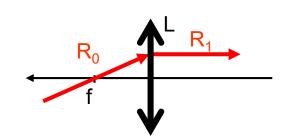
$$M = \frac{b}{a}$$
 Magnification

Example: object-image relation for a positive lens



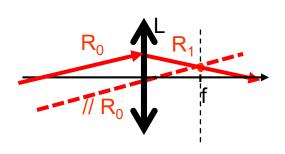
Ray Tracing - General

- 1) The chief ray: rays that pass through optical center
 - → not deviated
- 2) The parellel rays: Rays parallel to optical axis
 - → through focal point
- 3) The focal rays: Rays pass through focal point
 - → parallel to optical axis



4) The general ray: Ray with any given angle

- Draw a ray that is parallel to R₀ that passes through the center of the lens, dashed red line.
- This is called helper ray, //R₀ ray
- Draw a line vertical to the optical axis in the focal plane, black dashed line
- Find the intersection point of these two dashed lines
- The incoming ray R₀ refracts by the lens and passes through this intersaction point

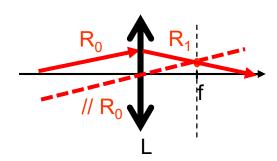


Ray Tracing - General

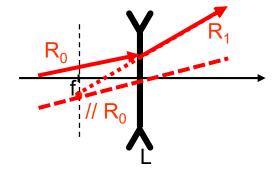
4) General ray: Ray with any given angle

- Draw a ray that is parallel to R₀ that passes through the center of the lens, dashed red line.
- This is called helper ray, //R₀ ray
- Draw a line vertical to the optical axis in the focal plane, black dashed line
- Find the intersection point of these two dashed lines
- The original ray refracts by the lens and passes through this intersaction point

Convergent lens:

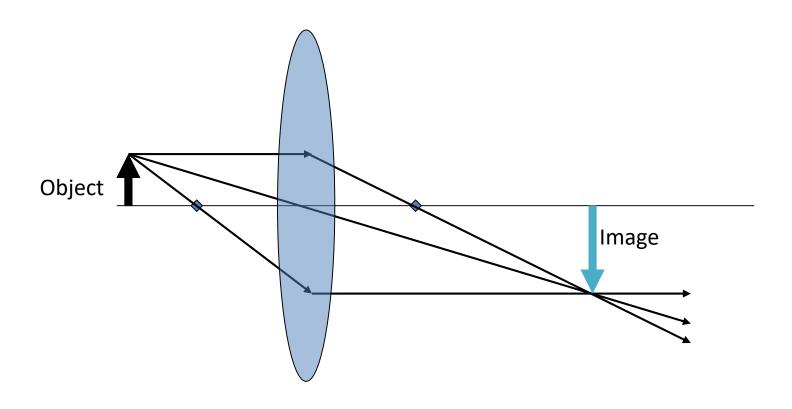


Divergent lens:



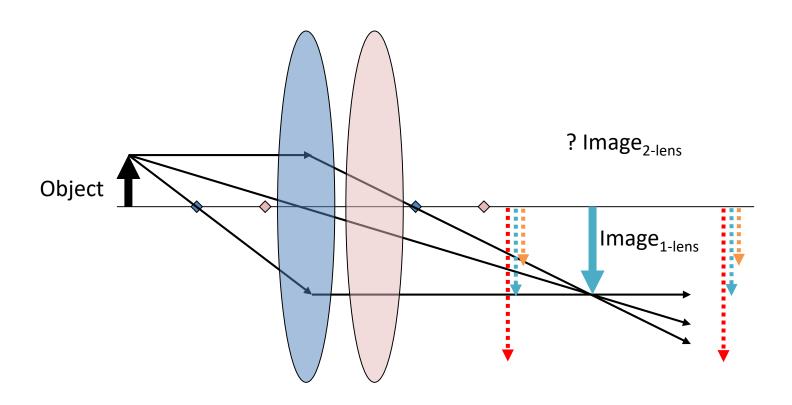
Ray Tracing – Example

Example-1: Let's begin with one convex lens.



Ray Tracing - Example

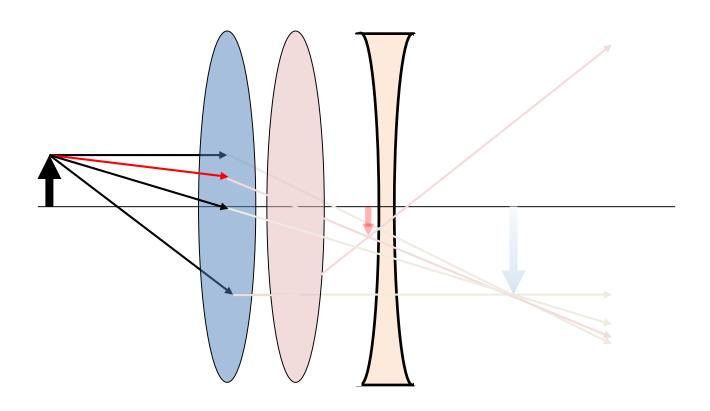
Example-2: Add a second convex lens.



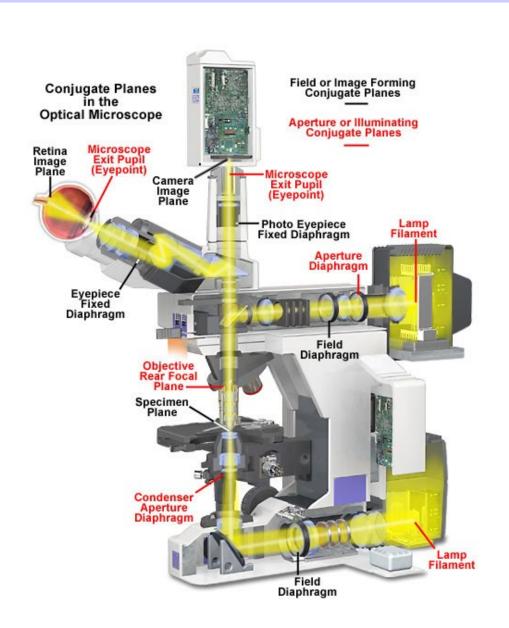


Ray Tracing - Example

.. it will get complicated (and not practical) for multiple lens system:



Microscope – An Optical System



Microscopes (optical imaging systems) contain multiple lenses.

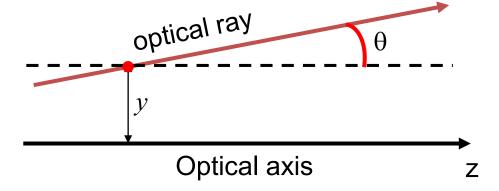
Q: How could we handle ray bending after many refracting/reflecting components?

A: Matrix approach is an alternative option.

The Ray Vector

A light ray can be defined by two co-ordinates:

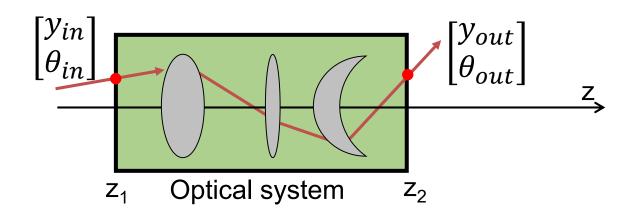
- its position, *y*
- its slope, θ



These two parameters define a **ray vector**, which will change as the ray propagates or passes through the optical elements.

$$\begin{bmatrix} y \\ \theta \end{bmatrix}$$

Matrix Optics



- An optical system is a set of optical components placed between two transverse planes at z_1 and z_2 , which are referred as the input plane and the output plane.
- The system is characterized by its effect on an incoming ray of arbitrary position & direction (y_{in}, θ_{in})
- The system steers the ray so that it has a new position and direction at the output plane, (y_{out}, θ_{out})
- In the paraxial approximation, when all angles are sufficiently small (i.e. $\sin(\theta) \approx \theta$) the relationship between (y_{in}, θ_{in}) and (y_{out}, θ_{out}) coordinates is linear and given by:

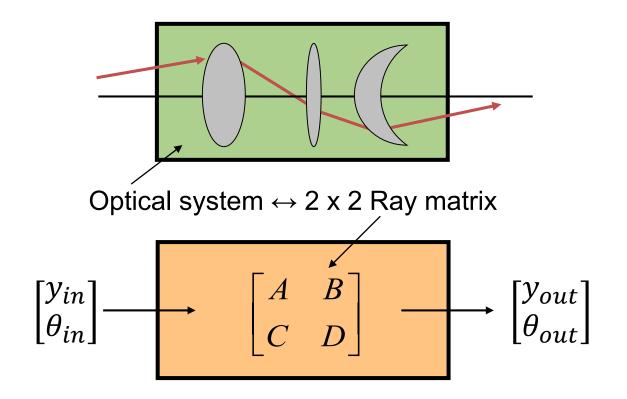
$$y_{out} = Ay_{in} + B\theta_{in}$$

$$\theta_{out} = Cy_{in} + D\theta_{in}$$

A, B, C and D are real numbers

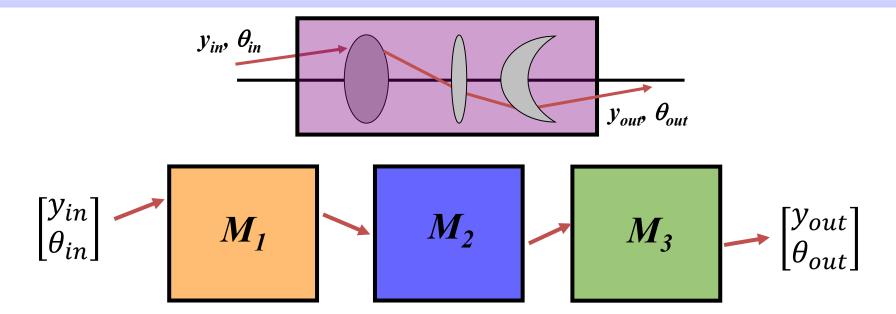
Ray Matrices

For optical systems with multiple components, we can define a 2 x 2 ray matrices.



These matrices are often called ABCD Matrices.

Cascaded Elements



we simply multiply ray matrices.

$$\begin{bmatrix} y_{out} \\ \theta_{out} \end{bmatrix} = M_3 \left\{ M_2 \left(M_1 \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix} \right) \right\} = M_3 M_2 M_1 \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix}$$

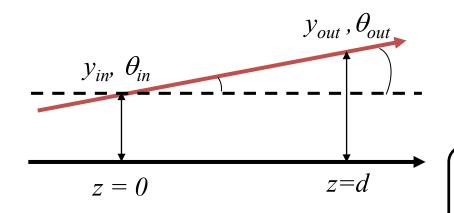
Notice the order in the multiplication: 3-2-1

Looks like the order is reversebut it makes sense when you think about it.

PS: Convention follows "Fundamentals of Photonics" by Saleh & Teich. Relevant pages are on Moodle

Ray matrix for propagation in free space or a medium

If y_{in} and θ_{in} are the position and slope upon entering, let y_{out} and θ_{out} be the position and slope after propagating from z=0 to z.



$$y_{out} = y_{in} + d\theta_{in}$$
$$\theta_{out} = \theta_{in}$$



$$y_{out} = 1.y_{in} + d.\theta_{in}$$

$$\theta_{out} = 0.y_{in} + 1.\theta_{in}$$



$$M_{prop} = \begin{bmatrix} 1 & d \\ 0 & 1 \end{bmatrix}$$

$$\begin{bmatrix} y_{out} \\ \theta_{out} \end{bmatrix} = \begin{bmatrix} 1 & d \\ 0 & 1 \end{bmatrix} \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix}$$

Obtain the terms by comparing the format:

$$y_{out} = Ay_{in} + B\theta_{in}$$

 $\theta_{out} = Cy_{in} + D\theta_{in}$

$$A = 1$$
; $B = d$; $C = 0$; $D = 1$

Ray Matrix for Planar Interface

1) At the interface:

$$y_{out} = y_{in}$$

2) Now calculate θ_{out} .

Snell's Law says:
$$n_1 \sin(\theta_{in}) = n_2 \sin(\theta_{out})$$

which becomes for small angles: $n_1 \theta_{in} = n_2 \theta_{out}$



Rewrite these for matrix notation:

$$y_{out} = 1.y_{in} + 0.\theta_{in}$$

$$y_{out} = 1. y_{in} + 0. \theta_{in}$$

$$\theta_{out} = 0. y_{in} + (\frac{n1}{n2}). \theta_{in}$$



Obtain the terms by comparing the format:

 n_2

$$y_{out} = Ay_{in} + B\theta_{in}$$

 n_1

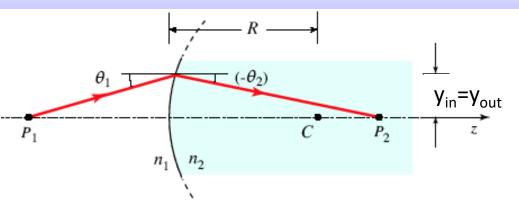
$$\theta_{out} = Cy_{in} + D\theta_{in}$$

$$A = 1$$
; $B = 0$; $C = 0$; $D = \frac{n1}{n2}$

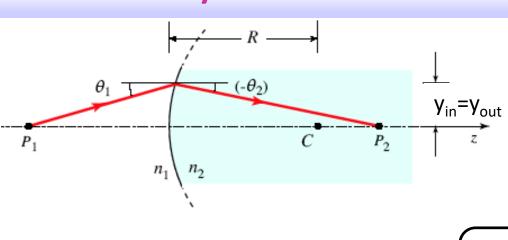


$$\begin{bmatrix} y_{out} \\ \theta_{out} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & n_1/n_2 \end{bmatrix} \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix} & \& & M_{planar-interface} = \begin{bmatrix} 1 & 0 \\ 0 & n_1/n_2 \end{bmatrix}$$

Ray Matrix for Curved Interface

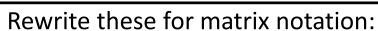


Ray Matrix for Curved Interface



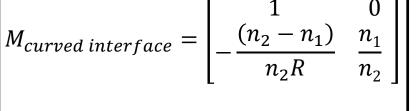
- 1) At the interface: $y_{out} = y_{in}$
- 2) For θ_{out} , use Snell law :

$$\theta_{out} = {n_1/n_2 \theta_{in} + {n_1/n_2 - 1 y_{in}/R}}$$



$$y_{out} = 1.y_{in} + 0.\theta_{in}$$

$$\theta_{out} = [\binom{n_1}{n_2} - 1)/R]. y_{in} + \binom{n_1}{n_2}.\theta_{in}$$



Obtain the terms by comparing the format: $y_{out} = Ay_{in} + B\theta_{in}$

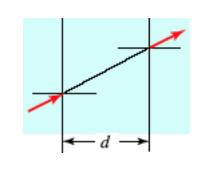
$$\theta_{out} = Cv_{in} + D\theta_{in}$$

$$\theta_{out} = Cy_{in} + D\theta_{in}$$

$$A = 1; \quad B = 0; \quad C = -\frac{(n_2 - n_1)}{n_2 R} \quad D = \frac{n_1}{n_2}$$

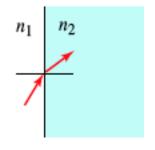
$$\begin{bmatrix} y_{out} \\ \theta_{out} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ -\frac{(n_2 - n_1)}{n_2 R} & \frac{n_1}{n_2} \end{bmatrix} \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix}$$

Matrix Optics – Basic Functions



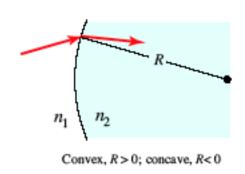
$$M_{prop} = \begin{bmatrix} 1 & d \\ 0 & 1 \end{bmatrix}$$

Planar boundary



$$M_{planar-interface} = \begin{bmatrix} 1 & 0 \\ 0 & n_1/n_2 \end{bmatrix}$$

Spherical boundary



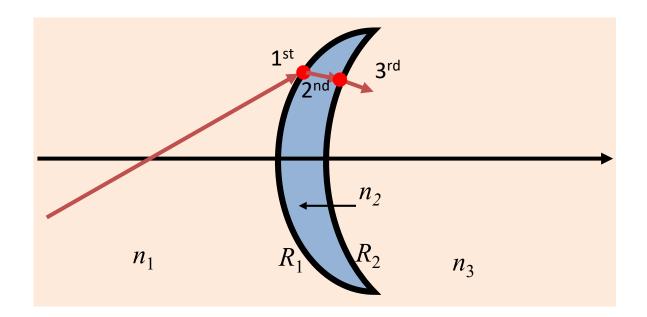
$$M_{curved\ interface} = \begin{bmatrix} 1 & 0 \\ -\frac{(n_2 - n_1)}{n_2 R} & \frac{n_1}{n_2} \end{bmatrix}$$

Note that when $R \rightarrow \infty$, curved surface becomes planar Thus, $C \rightarrow 0$ for M_{curved}

Example: Ray matrix of a lens

When a light ray interacts with a lens, it experiences:

- 1) The 1st curved interface having a curvature of R₁
- 2) Then, *propagates* in the lens
- 3) Then, the 2nd curved interface having a curvature of R₂



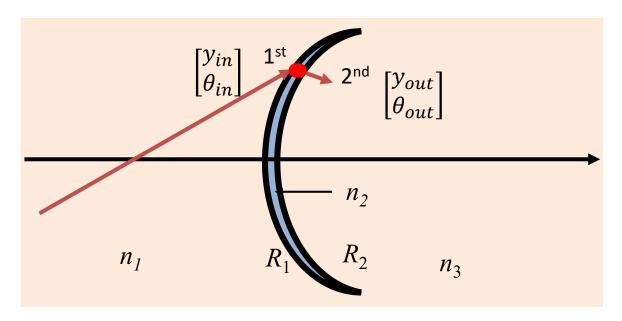
For a thin lens:

- we can neglect propagation inside the lens.
 Thus, in thin lens, light experiences only two curved interfaces.

Example: Ray matrix of a thin lens

With a thin lens, light experiences:

- 1) First, the curved interface having a curvature of R₁
- 2) Next, the second curved interface having a curvature of R₂

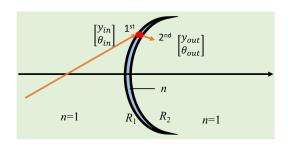


$$\begin{bmatrix} y_{out'} \\ \theta_{out'} \end{bmatrix} = M_{1^{st}curved\ interface} \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix} \qquad \qquad \begin{bmatrix} y_{out} \\ \theta_{out} \end{bmatrix} = M_{2^{nd}curved\ interface} \begin{bmatrix} y_{out'} \\ \theta_{out'} \end{bmatrix}$$

$$\begin{bmatrix} y_{out} \\ \theta_{out} \end{bmatrix} = M_{2^{nd}curved\ interface} \times M_{1^{st}curved\ interface} \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix}$$
 Remember reverse order!
$$\begin{bmatrix} y_{out} \\ \theta_{out} \end{bmatrix} = M_{N} \dots M_{3} M_{2} M_{1} \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix}$$

$$\begin{bmatrix} y_{out} \\ \theta_{out} \end{bmatrix} = M_N \dots M_3 M_2 M_1 \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix}$$

Example: Ray matrix of a thin lens



$$\begin{bmatrix} y_{out} \\ \theta_{out} \end{bmatrix} = \begin{bmatrix} M_{2^{nd} \ curved \ interface} \times M_{1^{st} \ curved \ interface} \end{bmatrix} \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix}$$
$$\begin{bmatrix} y_{out} \\ \theta_{out} \end{bmatrix} = M_{thin \ lens} \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix}$$

$$\mathbf{M} = \left[\begin{array}{cc} 1 & 0 \\ -\frac{(n_2-n_1)}{n_2R} & \frac{n_1}{n_2} \end{array}\right]$$
 Remember matrix for curved interface
$$n_1 \qquad n_2$$

$$n_2$$
 Convex, $R > 0$; concave, $R < 0$

$$\mathsf{M} = \left[\begin{array}{cc} 1 & 0 \\ -\frac{(n_2 - n_1)}{n_2 R} & \frac{n_1}{n_2} \end{array} \right] \qquad M_{1^{st}curved\ interface} = \left[\begin{array}{cc} 1 & 0 \\ -(n-1) & \frac{1}{n} \end{array} \right] \qquad n_1 = 1 \\ n_2 = n \qquad n_2 = n$$

$$M_{2^{nd}curved\ interface} = egin{bmatrix} 1 & 0 \ -(1-n) & n \ R_2 & n \end{bmatrix} & n_1 = n \ n_2 = 1$$

$$M_{thin \ lens} = M_{2^{nd} curved \ interface} \times M_{1^{st} curved \ interface} = \begin{vmatrix} 1 & 0 \\ -(1-n) \\ \hline R_2 & n \end{vmatrix} \begin{vmatrix} 1 & 0 \\ -(n-1) & 1 \\ \hline nR_1 & n \end{vmatrix}$$

$$M_{thin\ lens} = \begin{bmatrix} 1 & 0 \\ -(n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right) & 1 \end{bmatrix}$$
 \rightarrow This can be written as: $M_{thin\ lens} = \begin{bmatrix} 1 & 0 \\ -\frac{1}{f} & 1 \end{bmatrix}$

Remember Lens-Maker's Formula From Ray Optics

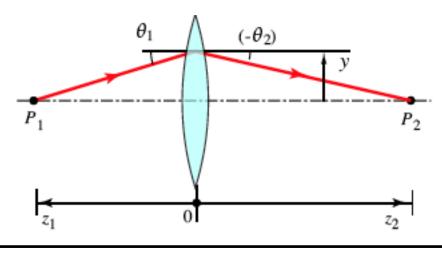
• where,
$$\frac{1}{f} = (n-1) \left(\frac{1}{R_1} - \frac{1}{R_2} \right)$$



Link between ray tracing & matrix representation

THIN LENS:

- Position is ~nearly same immediately after the ray exits a thin lens
- Thin lens only deflects the ray (i.e., change the outgoing angle) according to the law of refraction



From Ray Tracing

Ray Deflection:

$$heta_2 = heta_1 - rac{y}{f},$$

Focal length:

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

Imaging Condition:

$$\frac{1}{f} = \frac{1}{z_{obi}} + \frac{1}{z_{im}}$$

Magnification:

$$\mathsf{mag} = \frac{z_{im}}{z_{obj}}$$

From Matrix Representation

$$\mathbf{M} = \begin{bmatrix} 1 & 0 \\ -\frac{1}{f} & 1 \end{bmatrix}$$

$$\mathbf{M} = \begin{bmatrix} 1 & 0 \\ -\frac{1}{f} & 1 \end{bmatrix} \longrightarrow \begin{bmatrix} y_{out} \\ \theta_{out} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ -1/f & 1 \end{bmatrix} \begin{bmatrix} y_{in} \\ \theta_{in} \end{bmatrix}$$

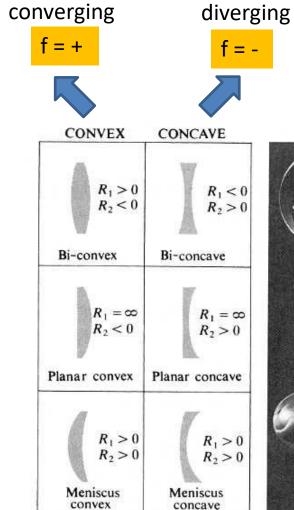
Ray Deflection :
$$\theta_{out} = \theta_{in} - \frac{y_{in}}{f}$$



Matrix Representation of a Thin Lens

Lens maker's formula

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

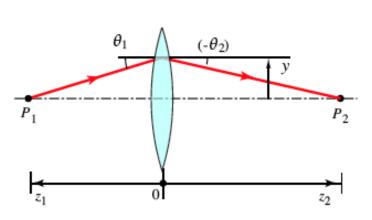


$$\mathbf{M} = \left[\begin{array}{cc} 1 & 0 \\ -\frac{1}{f} & 1 \end{array} \right]$$

- Positive radii indicate convex surfaces
- Negative radii indicate concave surfaces
- Negative angles point downward from the z-axis in the direction of ray propagation

Matrix Representation of Lenses & Curved Mirrors

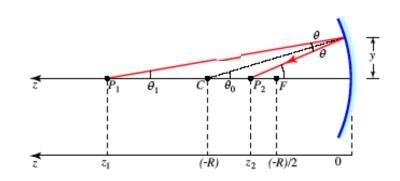
Under Paraxial Approximation $\rightarrow \sin \theta \approx \tan \theta \approx \theta$



- 1) Position is ~nearly same immediately after the ray exits a thin lens
- 2) Thin lens only deflects the ray (i.e., changes the outgoing angle) according to the law of refraction

Ray Deflection :
$$heta_2 = heta_1 - rac{y}{f},$$

$$\mathbf{M} = \begin{bmatrix} 1 & 0 \\ -\frac{1}{f} & 1 \end{bmatrix}$$



- 1) Position is ~same immediately after the ray reflects from a curved mirror
- 2) Mirror only reflects the ray (i.e., changes the outgoing angle) according to the law of reflection

Ray Deflection :
$$(-\theta_2) + \theta_1 pprox rac{2y}{-R}$$

(from ray optics)

$$\mathbf{M} = \begin{bmatrix} 1 & 0 \\ \frac{2}{D} & 1 \end{bmatrix} \qquad R = 2f$$



Lenses & Mirrors

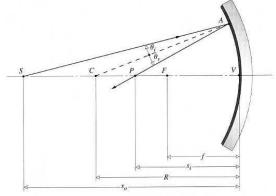
Lens maker's formula

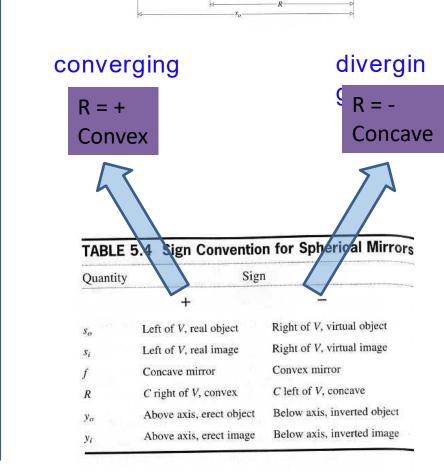
$$\frac{1}{f} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$$

converging diverging CONVEX CONCAVE $R_1 > 0$ $R_2 < 0$ $R_1 < 0$ $R_2 > 0$ Bi-convex Bi-concave Planar convex Planar concave $R_1 > 0$ $R_2 > 0$ Meniscus Meniscus

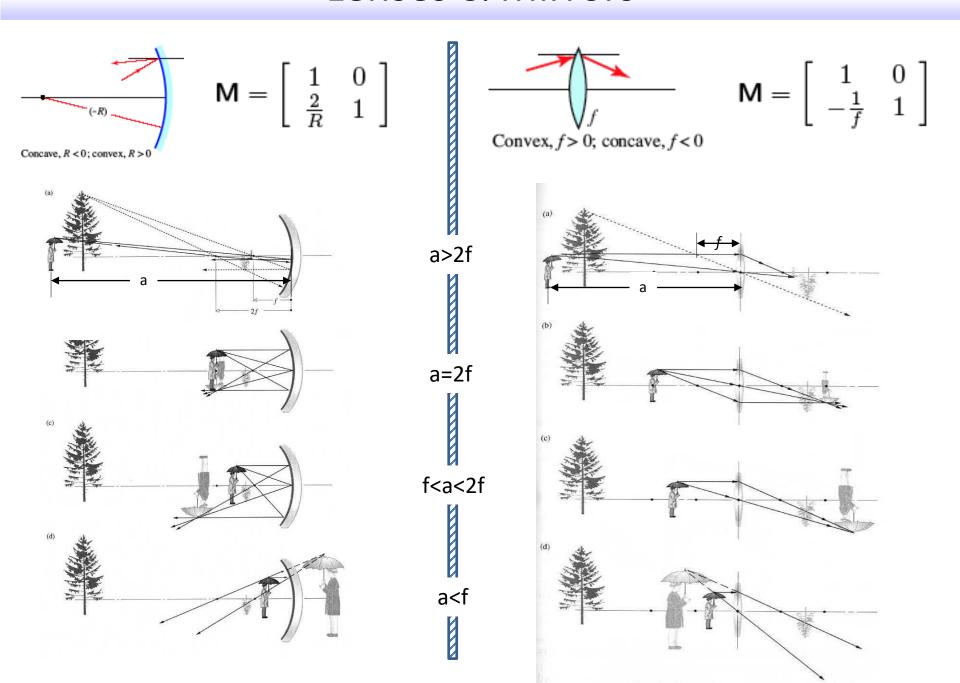
convex

concave



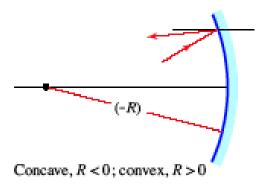


Lenses & Mirrors



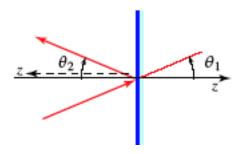
Matrix Representation for Mirrors

Spherical mirror



$$\mathbf{M} = \left[\begin{array}{cc} 1 & 0 \\ \frac{2}{R} & 1 \end{array} \right]$$

Planar mirror



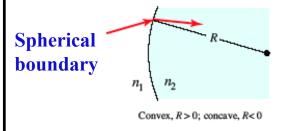
$$\mathbf{M} = \left[\begin{array}{cc} 1 & 0 \\ 0 & 1 \end{array} \right]$$

Corresponds to the limit case that $R \rightarrow \infty$ Thus, in ABCD matrix C element becomes 0

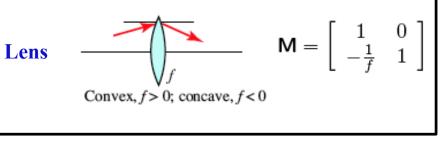
Matrix optics for basic functions & components - Summary

Propagation
$$\mathbf{M} = \begin{bmatrix} 1 & d \\ 0 & 1 \end{bmatrix}$$

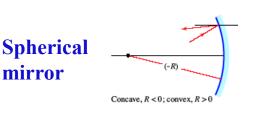
Planar boundary
$$M = \begin{bmatrix} 1 & 0 \\ 0 & \frac{n_1}{n_2} \end{bmatrix}$$



$$\mathbf{M} = \begin{bmatrix} 1 & 0 \\ -\frac{(n_2 - n_1)}{n_2 R} & \frac{n_1}{n_2} \end{bmatrix}$$



$$\mathbf{M} = \left[\begin{array}{cc} 1 & 0 \\ 0 & 1 \end{array} \right]$$



$$\mathbf{M} = \left[\begin{array}{cc} 1 & 0 \\ \frac{2}{R} & 1 \end{array} \right]$$

Example: Consecutive Lenses

Suppose we have an "optical system" consisting of two thin lenses right next to each other with no space in between.

When light enters this system, it experiences:

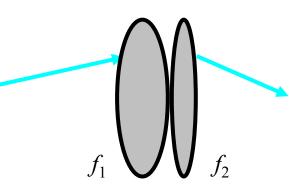
- Initially, 1st lens with focal length of f₁
- Then, 2nd lens with focal length of f₂

$$M_1 = M_{thin \ lens \ with \ f_1}$$

$$M_1 = \begin{bmatrix} 1 & 0 \\ -\frac{1}{f_1} & 1 \end{bmatrix}$$

$$M_2 = M_{thin lens with f_2}$$

$$M_2 = \begin{bmatrix} 1 & 0 \\ -\frac{1}{f_2} & 1 \end{bmatrix}$$



$$M_{total} = M_2 \times M_1$$

$$M_{total} = \begin{bmatrix} 1 & 0 \\ -\frac{1}{f_2} & 1 \end{bmatrix} \times \begin{bmatrix} 1 & 0 \\ -\frac{1}{f_1} & 1 \end{bmatrix}$$

$$M_{total} = \begin{bmatrix} 1 & 0 \\ -\frac{1}{f_1} - \frac{1}{f_2} & 1 \end{bmatrix}$$

$$\frac{1}{f_{total}} = \frac{1}{f_1} + \frac{1}{f_2}$$

$$\frac{1}{f_{total}} = \frac{1}{f_1} + \frac{1}{f_2}$$
 $M_{total} = \begin{bmatrix} 1 & 0 \\ -\frac{1}{f_{total}} & 1 \end{bmatrix}$