Introduction to imaging and modern microscope theory

EE231 Optics

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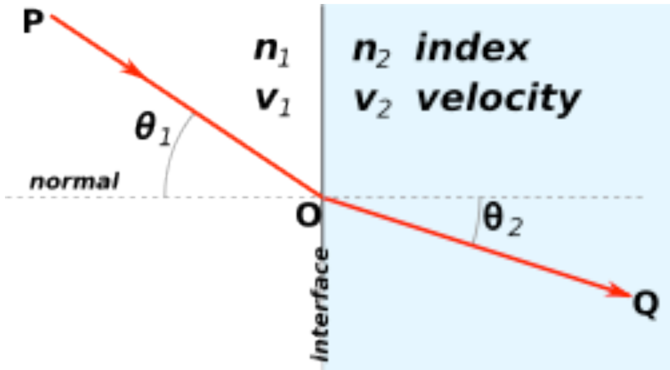
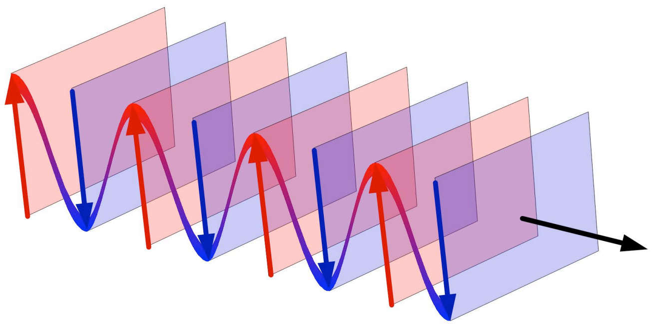
Geometric optics—process of light rays based on Fermat principle: *the path taken between two points by a ray of light is the path that can be traversed in the least time* (Fermat, 1650).

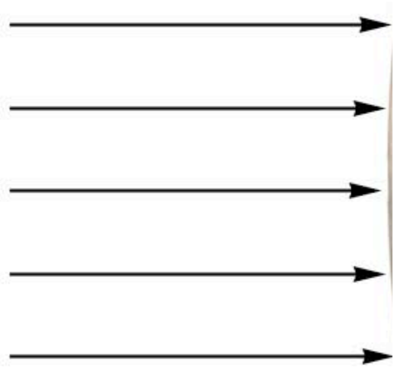


A plane wave: wave description



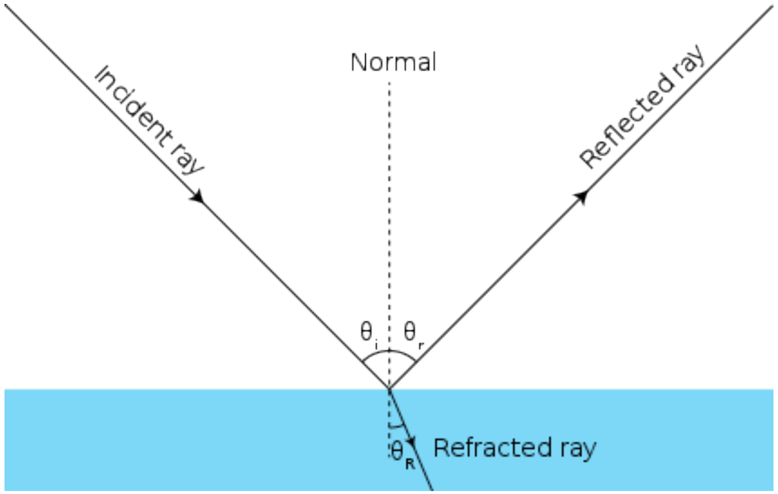
$$e^{i(\omega t-\mathbf{k}\cdot\mathbf{r})}$$

A plane wave: ray description



Incoming parallel rays

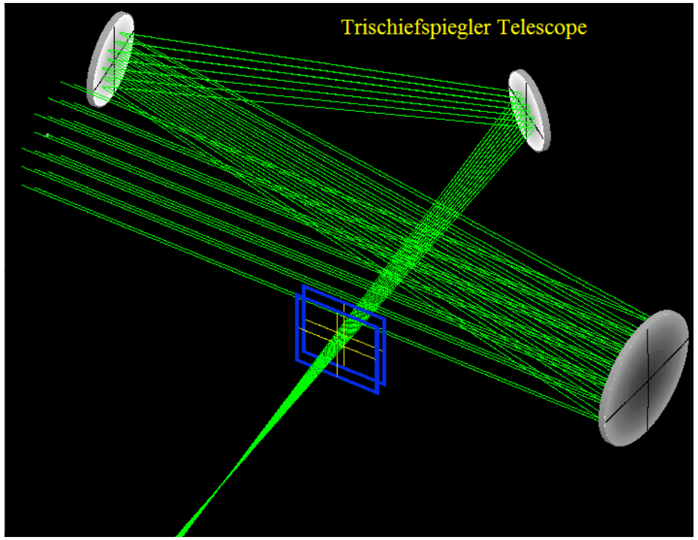
In geometrical optics, light matter interaction is described in terms of the laws of reflection and refraction.



Snell law:

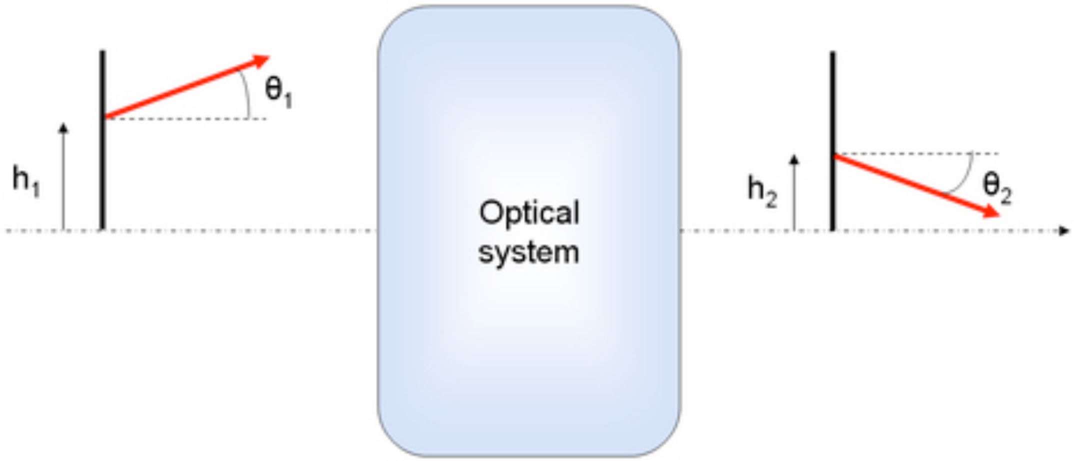
$$\theta_i = \theta_r$$

$$n_i \sin\theta_i = n_r \sin\theta_r$$

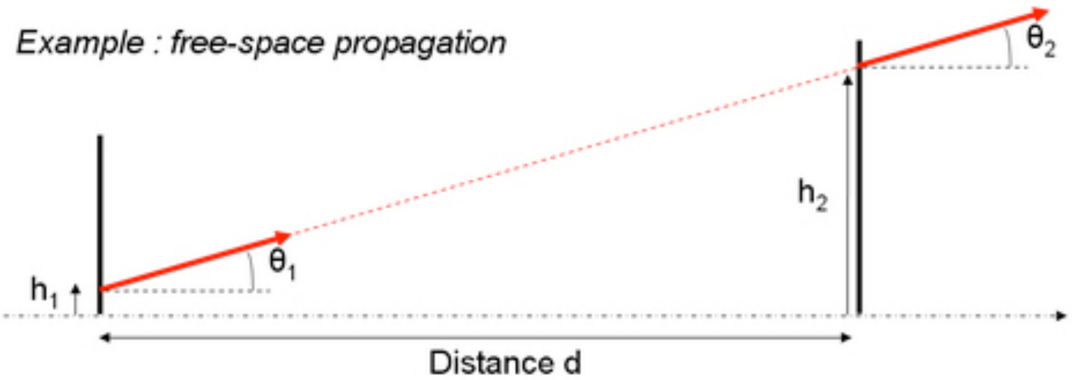


An example of ray propagation -> ray-tracing

ABCD Matrix



$$\begin{cases} h_i \equiv r_i \equiv y_i \\ \theta_i \equiv r_i^{'} \equiv \alpha_i \end{cases}$$



ABCD matrix: It can be utilized to describe a ray propagating through an optical element

$$\begin{bmatrix} r_{out} \\ r'_{out} \end{bmatrix} = \begin{bmatrix} A & B \\ C & D \end{bmatrix} \begin{bmatrix} r_{in} \\ r'_{in} \end{bmatrix}$$

For cascade of n optical elements, $\begin{bmatrix} A_t & B_t \\ C_t & D_t \end{bmatrix} = \begin{bmatrix} A_n & B_n \\ C_n & D_n \end{bmatrix} \cdots \begin{bmatrix} A_2 & B_2 \\ C_2 & D_2 \end{bmatrix} \begin{bmatrix} A_1 & B_1 \\ C_1 & D_1 \end{bmatrix}$

The translational matrix

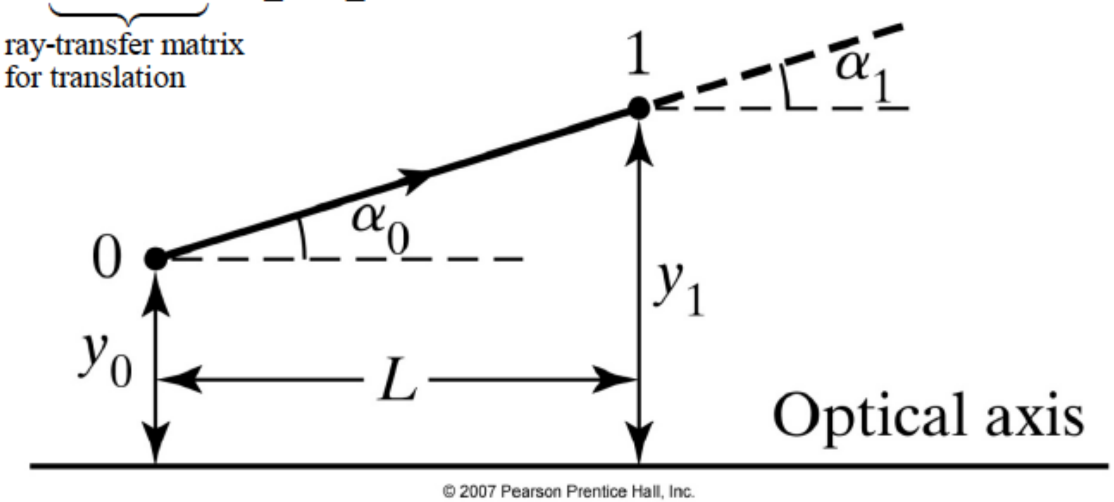
Consider simple tanslationof a ray in a homogeneous medium.

Translation from point 0 to 1 with paraxial approximation:

$\alpha_1 = \alpha_0$ and $y_1 = y_0 + L\tan\alpha_0 = y_0 + L\alpha_0$

We rewrite the equations:

$$\left.\begin{aligned} y_1 &= (1)\,y_0 + (L)\,\alpha_0 \\ \alpha_1 &= (0)\,y_0 + (1)\,\alpha_0 \end{aligned}\right\} \rightarrow \begin{bmatrix} y_1 \\ \alpha_1 \end{bmatrix} = \underbrace{\begin{bmatrix} 1 & L \\ 0 & 1 \end{bmatrix}}_{\substack{\text{ray-transfer matrix} \\ \text{for translation}}} \begin{bmatrix} y_0 \\ \alpha_0 \end{bmatrix}$$



F. Pedrotti, L. M. Pedrotti and L. S. Pedrotti, "Introduction to Optics" (Prentice Hall, 2007)

Refraction matrix

Consider refraction of a ray at a spherical interface (paraxial approximation):

Ray coordinates before refraction (y,α) and ray coordinates after refraction (y',α')

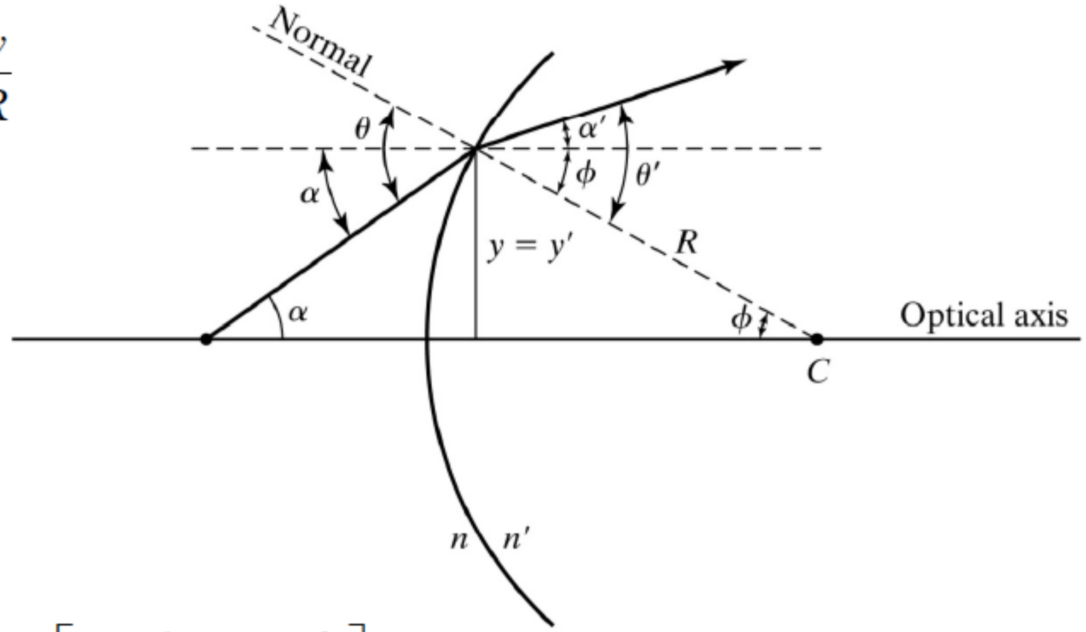
$$\alpha' = \theta' - \phi = \theta' - \frac{y}{R} \text{ and } \alpha = \theta - \phi = \theta - \frac{y}{R}$$

Paraxial form of Snell's law: $n\theta = n'\theta'$

$$\alpha' = \left(\frac{n}{n'}\right)\theta - \frac{y}{R} = \left(\frac{n}{n'}\right)\left(\alpha + \frac{y}{R}\right) - \frac{y}{R}$$

$$\alpha' = \left(\frac{1}{R}\right)\left(\frac{n}{n'} - 1\right)y + \left(\frac{n}{n'}\right)\alpha$$

The approximate linear equations:



$$\left.\begin{aligned} y' &= (1)y + (0)\alpha \\ \alpha' &= \left[\left(\frac{1}{R}\right)\left(\frac{n}{n'} - 1\right)\right]y + \left(\frac{n}{n'}\right)\alpha \end{aligned}\right\} \rightarrow \begin{bmatrix} y' \\ \alpha' \end{bmatrix} = \underbrace{\begin{bmatrix} 1 & 0 \\ \frac{1}{R}\left(\frac{n}{n'} - 1\right) & \frac{n}{n'} \end{bmatrix}}_{\text{Ray-transfer matrix for refraction}} \begin{bmatrix} y \\ \alpha \end{bmatrix}$$

If $R \rightarrow \infty$ we have transfer matrix for refration by plane interface: $\begin{bmatrix} y' \\ \alpha' \end{bmatrix} = \underbrace{\begin{bmatrix} 1 & 0 \\ 0 & \frac{n}{n'} \end{bmatrix}}_{\text{Refraction by a plane}} \begin{bmatrix} y \\ \alpha \end{bmatrix}$

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The reflection matrix

Consider refraction of a ray at a spherical interface (paraxial approximation):

Ray coordinates before refraction (y, α) and ray coordinates after refraction (y', α')

$$\alpha' = \theta' - \phi = \theta' - \frac{y}{R} \text{ and } \alpha = \theta - \phi = \theta - \frac{y}{R}$$

Goal: connect (y', α') to (y, α) by a ray transfer matrix for reflection by a concave mirror

Sign convention for the angles: $(+)$ pointing upward and $(-)$ pointing downward

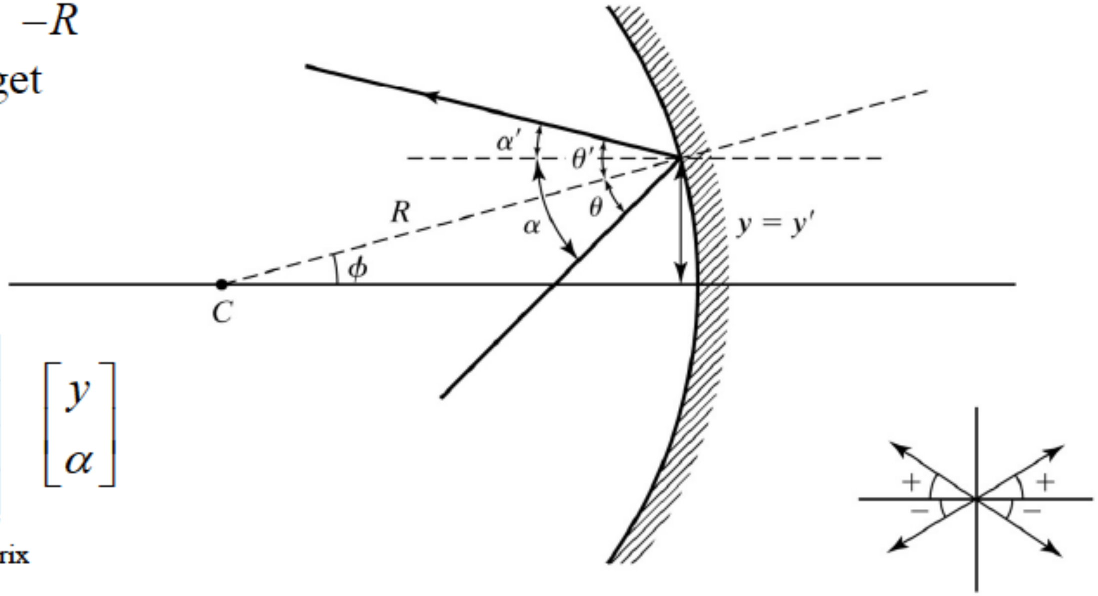
$$\alpha = \theta + \phi = \theta + \frac{y}{-R} \text{ and } \alpha' = \theta' - \phi = \theta' - \frac{y}{-R}$$

To eliminate θ and θ' we use $\theta = \theta'$ we get

$$\alpha' = \theta + \frac{y}{R} = \alpha + \frac{2y}{R}$$

The desired equations become:

$$\left.\begin{aligned} \mathrm{y}' &= (1)\, y + (0)\, \alpha \\ \alpha' &= \left(\frac{2}{R}\right) y + (1)\, \alpha \end{aligned}\right\} \rightarrow \begin{bmatrix} y' \\ \alpha' \end{bmatrix} = \underbrace{\begin{bmatrix} 1 & 0 \\ \dfrac{2}{R} & 1 \end{bmatrix}}_{\substack{\text{Ray-transfer matrix} \\ \text{for reflection}}} \begin{bmatrix} y \\ \alpha \end{bmatrix}$$



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Significance of system matrix elements

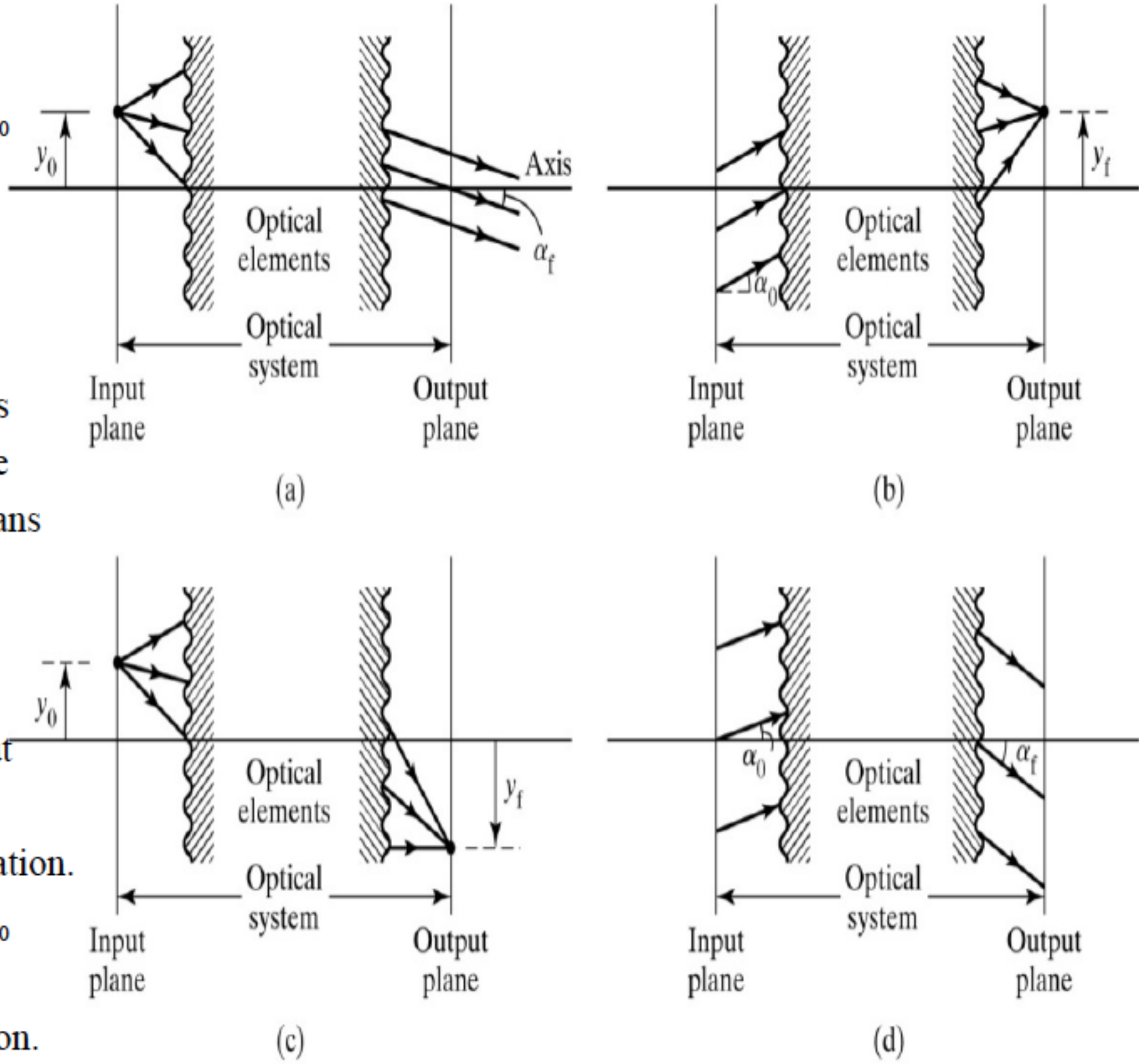
$$\begin{bmatrix} y_f \\ \alpha_f \end{bmatrix} = \begin{bmatrix} A & B \\ C & D \end{bmatrix} \begin{bmatrix} y_0 \\ \alpha_0 \end{bmatrix}$$

a) If $D = 0 \rightarrow \alpha_f = C y_0$ independent of α_0
All the rays leavoing the input plane will have the same angle at the output plane. Input plane is on the first focal plane.

b) If $A = 0 \rightarrow y_f = B\alpha_0$
means y_f is independent of y_0 that means all the rays departing input plane have the same height at the output plane. This means output plane is the second focal plane.

c) If $B = 0 \rightarrow y_f = A y_0$ All the points leaving the input plane at hight y_0 will arrive the output plane at height y_f output plane is image of the input plane.
$A = y_f / y_0$ corresponds to linear magnification.

d) If $C = 0 \rightarrow a_f = D\alpha_0$ independent of y_0
Input rays of all in one direction will produce output rays all in another direction. This is called (thelescopic system).



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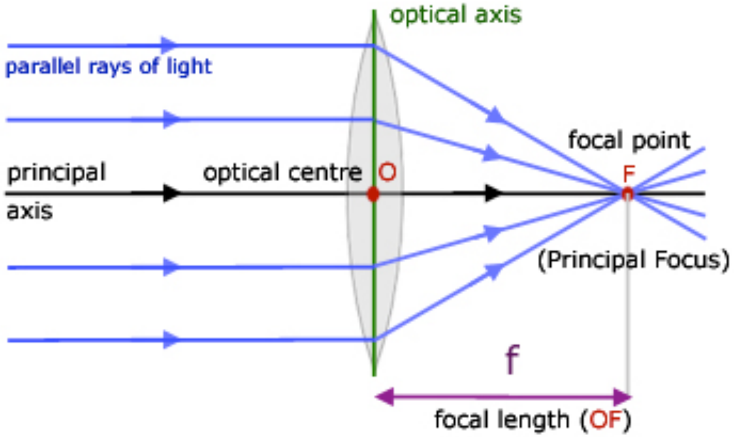
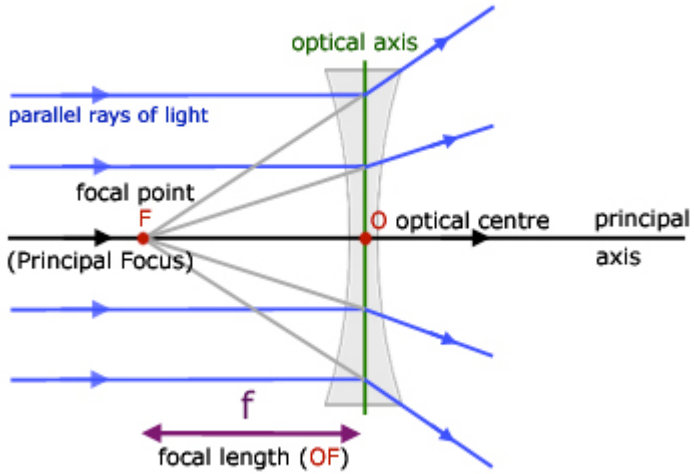
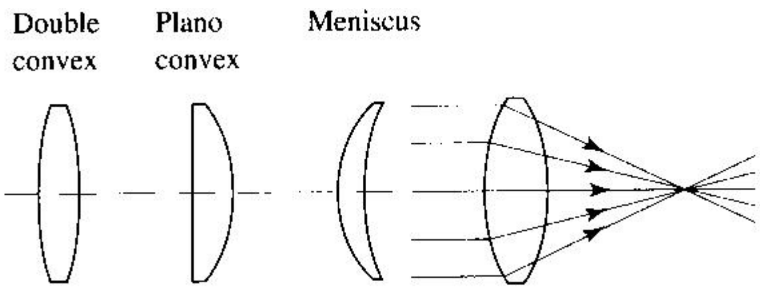
Lenses

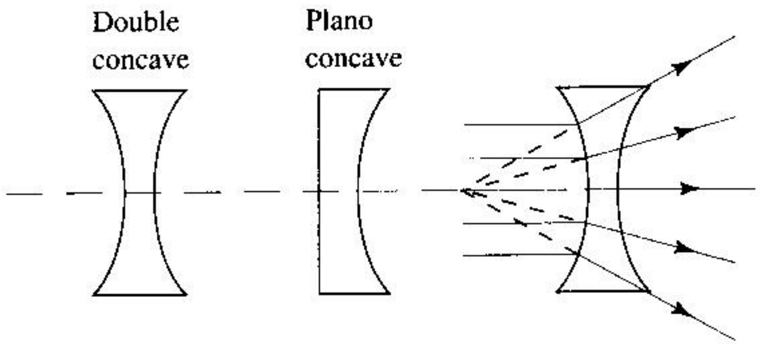
positive



negative







The thick lens and thin lens matrices

Goal: Applying the results for a thick lens

Let R represent a reflaction matrix and T represent tsranslation

$M=R_2TR_1$ the ray-transfer matrix for a thick lens can be written as:

$$M=\begin{bmatrix}1 & 0\\ \frac{n_L-n'}{n'R_2} & \frac{n_L}{n'}\end{bmatrix}\begin{bmatrix}1 & t\\ 0 & 1\end{bmatrix}\begin{bmatrix}1 & 0\\ \frac{n-n_L}{n_LR_1} & \frac{n}{n_L}\end{bmatrix}$$

For a thin lens $t\rightarrow 0$ in one environment $(n=n')$ the ray-transfer matrix becomes

$$M=\begin{bmatrix}1 & 0\\ \frac{n_L-n}{nR_2} & \frac{n_L}{n}\end{bmatrix}\begin{bmatrix}1 & 0\\ 0 & 1\end{bmatrix}\begin{bmatrix}1 & 0\\ \frac{n-n_L}{n_LR_1} & \frac{n}{n_L}\end{bmatrix}=\begin{bmatrix}1 & 0\\ \frac{n_L-n}{n}\left(\frac{1}{R_2}-\frac{1}{R_1}\right) & 1\end{bmatrix}$$

We exprss the lower left hand element in terms of the focal length:

$$\frac{1}{f}=\frac{n_L-n}{n}\left(\frac{1}{R_2}-\frac{1}{R_1}\right)$$ the lansmaker's formula

$$M=\begin{bmatrix}1 & 0\\ -\frac{1}{f} & 1\end{bmatrix}=\begin{bmatrix}A & B\\ C & D\end{bmatrix}$$

the ray-transfer matrix for a thin lens also known as the ABCD matrix.

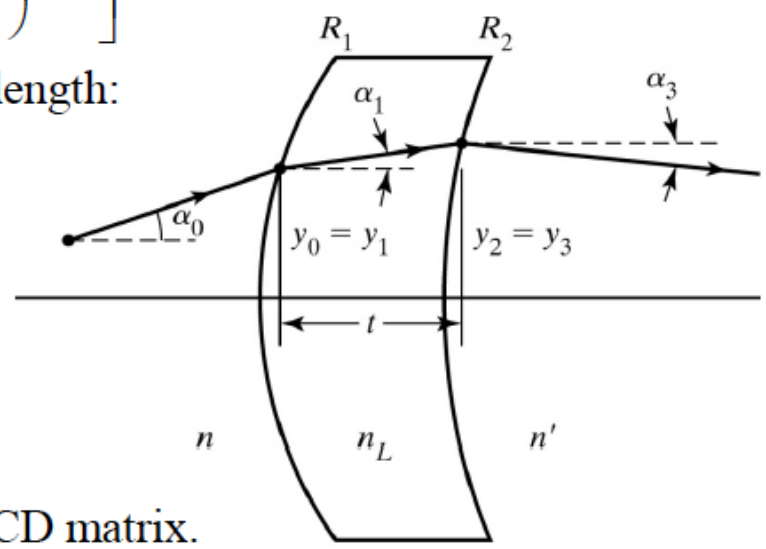


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Image Formation by thin Lenses

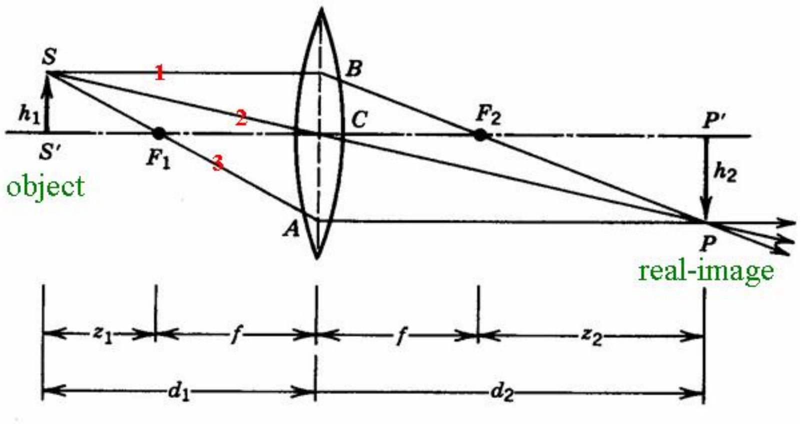
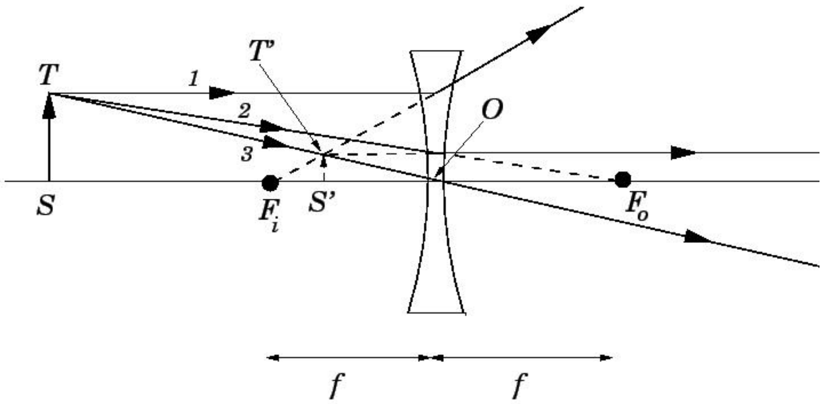


Image formation by a thin lens.



Lens equation: $\frac{1}{d_1} + \frac{1}{d_2} = \frac{1}{f}$ (Gaussian form)

$f^2 = z_1 z_2$ (Newtonian form)

Magnification

$$|M| \equiv \frac{h_2}{h_1} = \frac{d_2}{d_1}$$

Aberrations of Lenses

- **Primary Aberration** — image deviate from the original picture/the first-order approximation

  Monochromatic aberrations

  — Spherical Aberration

  — Coma

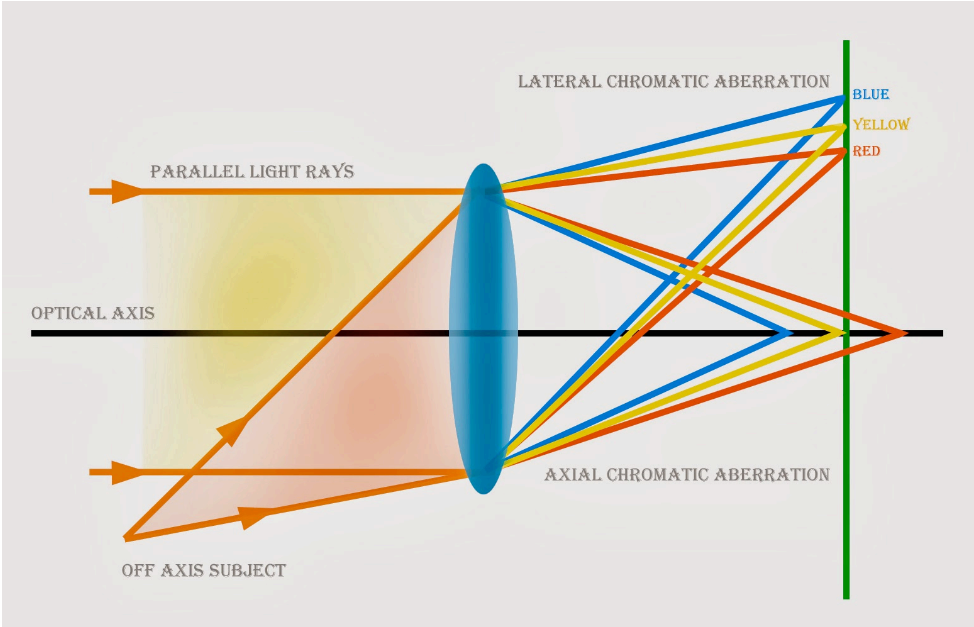
  — Astigmatism

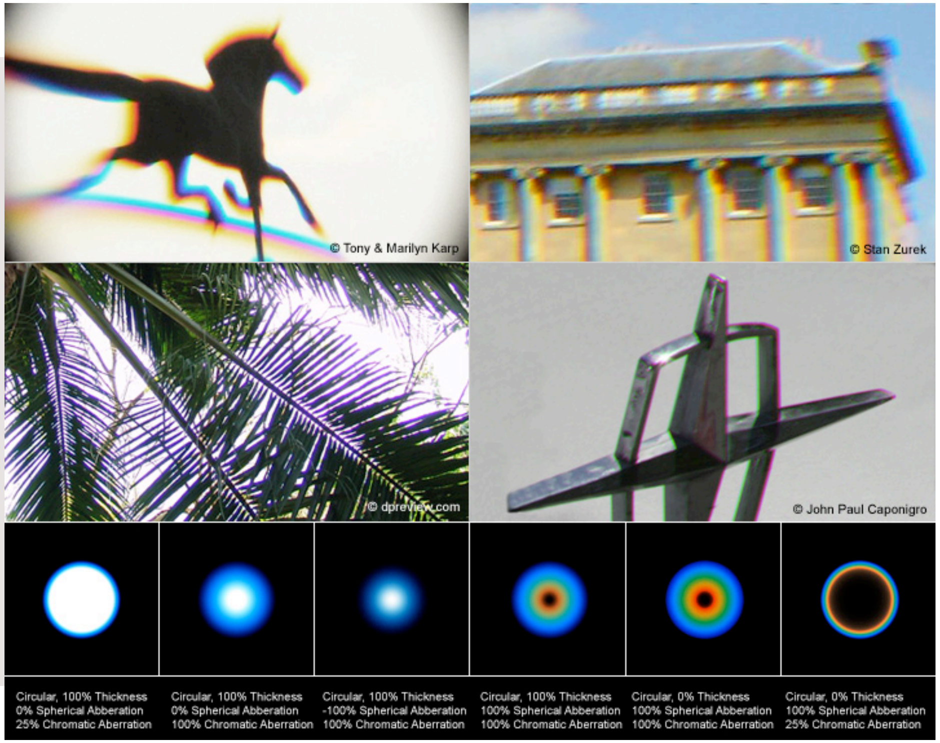
  — Curvature of field

  — Distortion

  Chromatic aberration

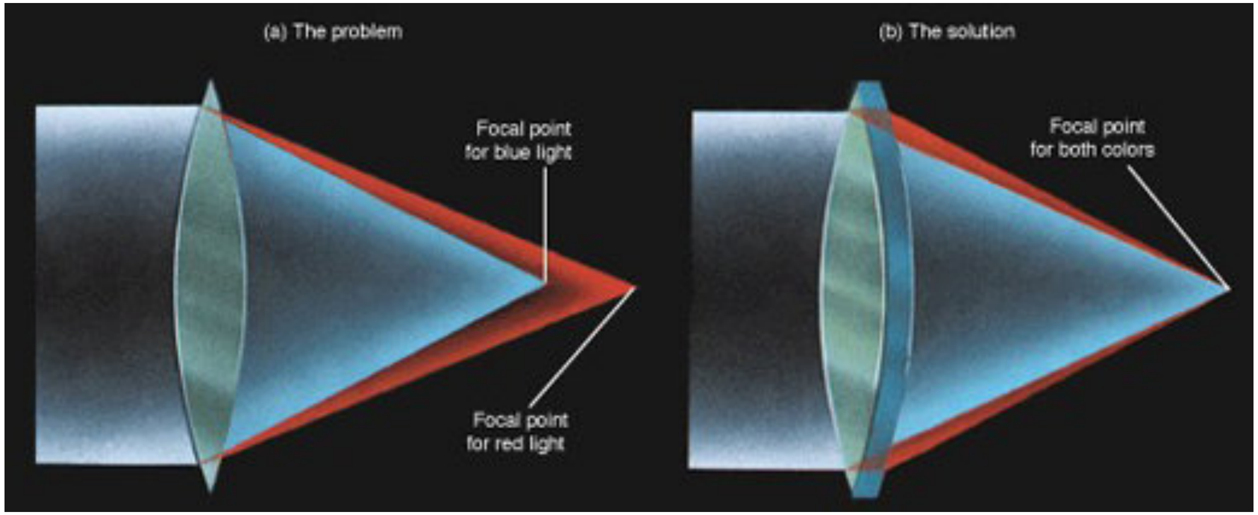
Chromatic Aberration



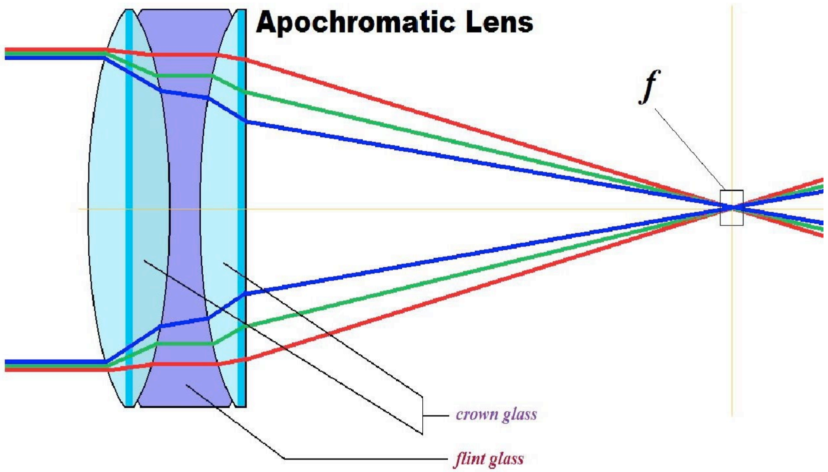


Due to the dispersive nature of the lens material, the focal lengths of lights with distinct wavelengths are different -> white spots are imaged as polychromatic spots

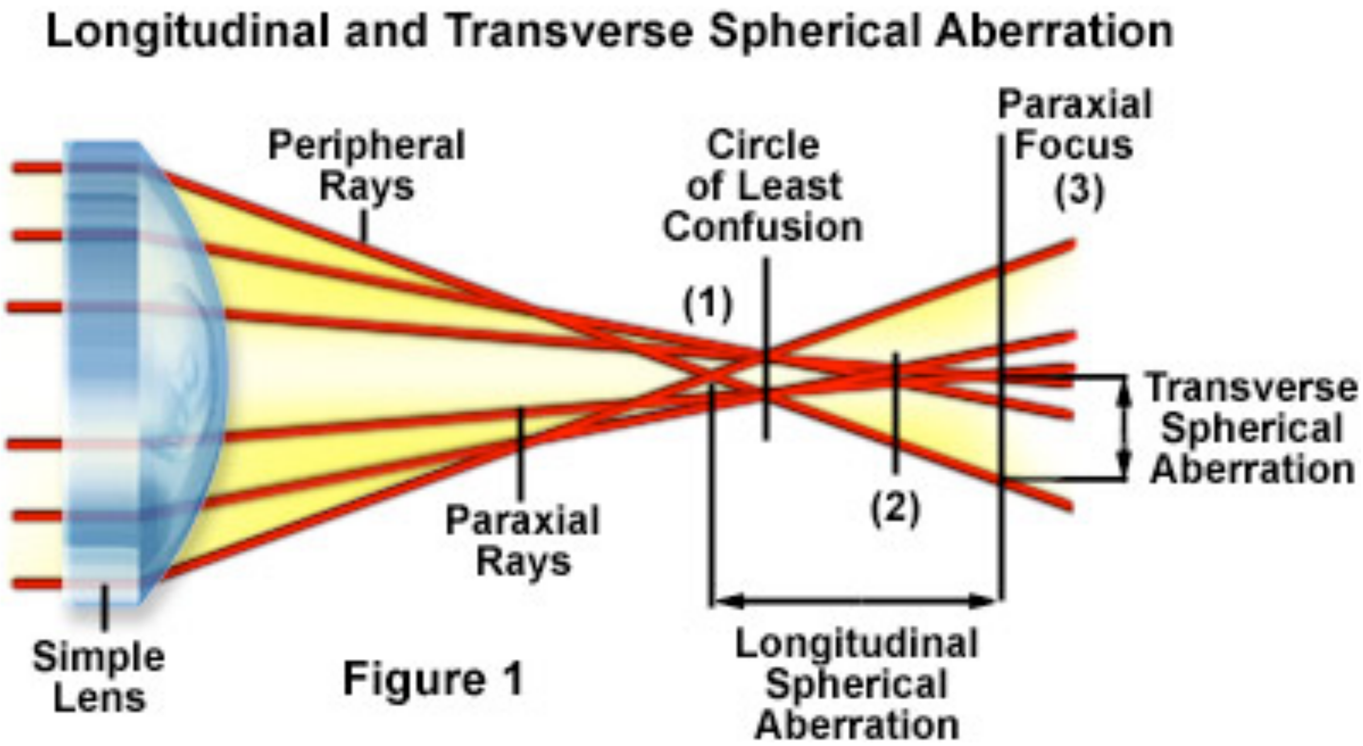
Solution of Chromatic Aberration-Using Doublet or triplet Lens



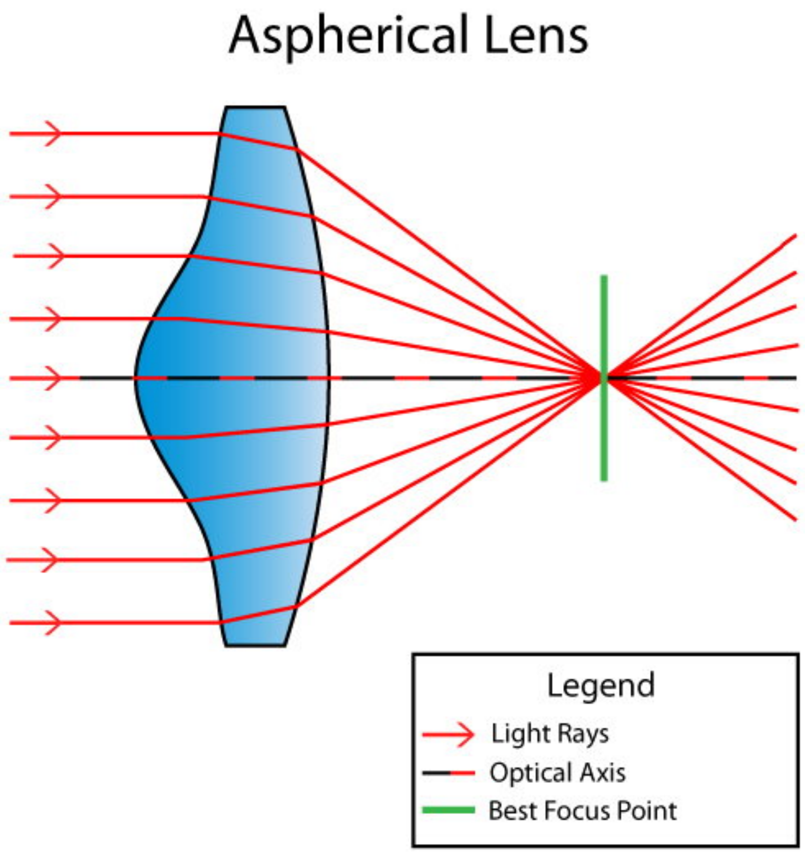
Achromatic doublet

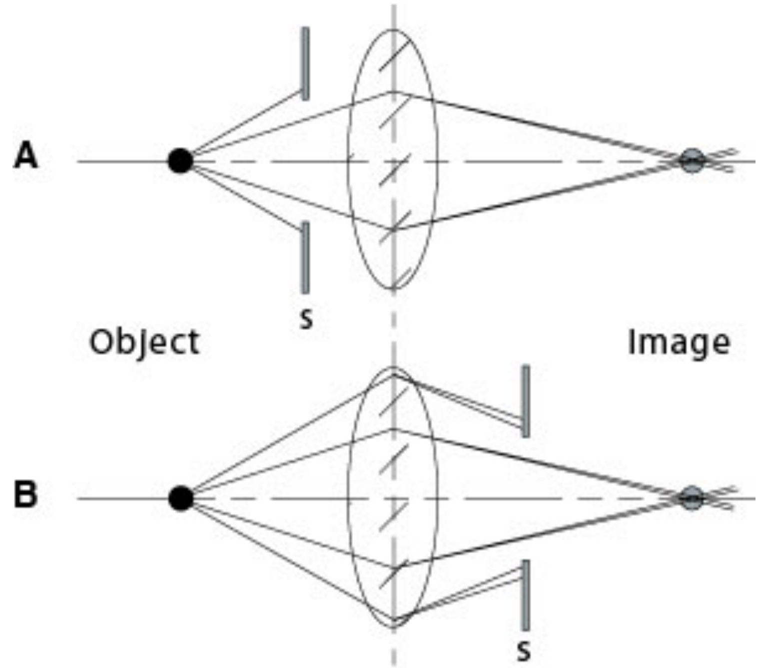


Spherical Aberration (SA)



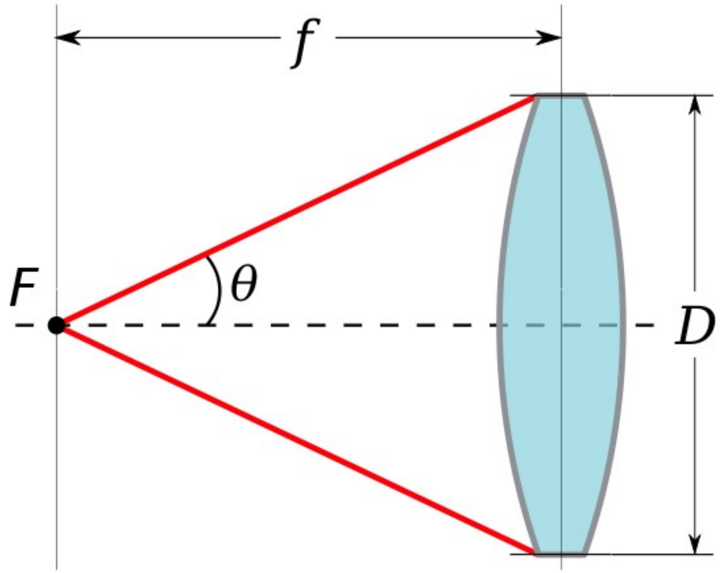
Solutions of Spherical Aberration- Using Aspherical Lens or Stop





Numerical Aperture (NA)

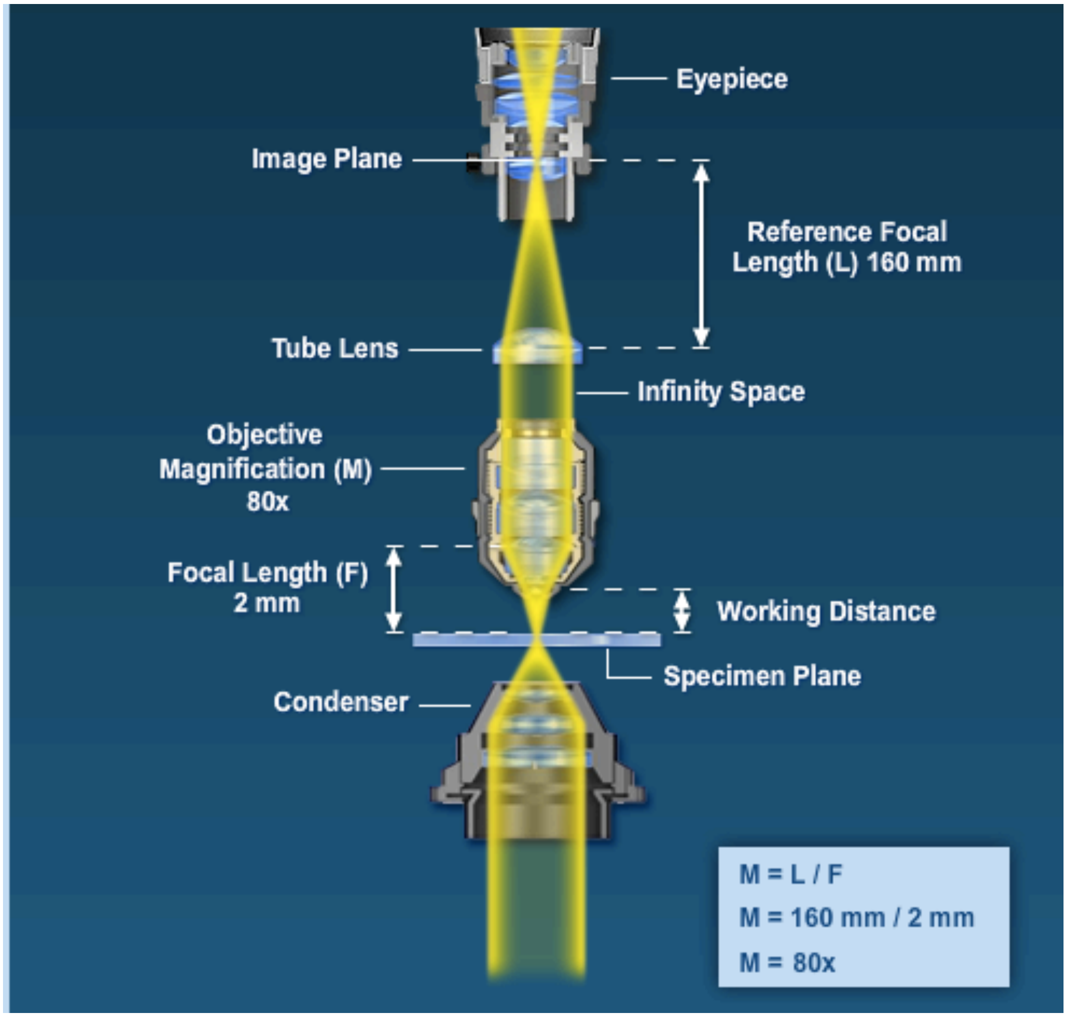
- The numerical aperture of an optical system is a dimensionless number that characterizes the range of angles over which the system can accept or emit light.



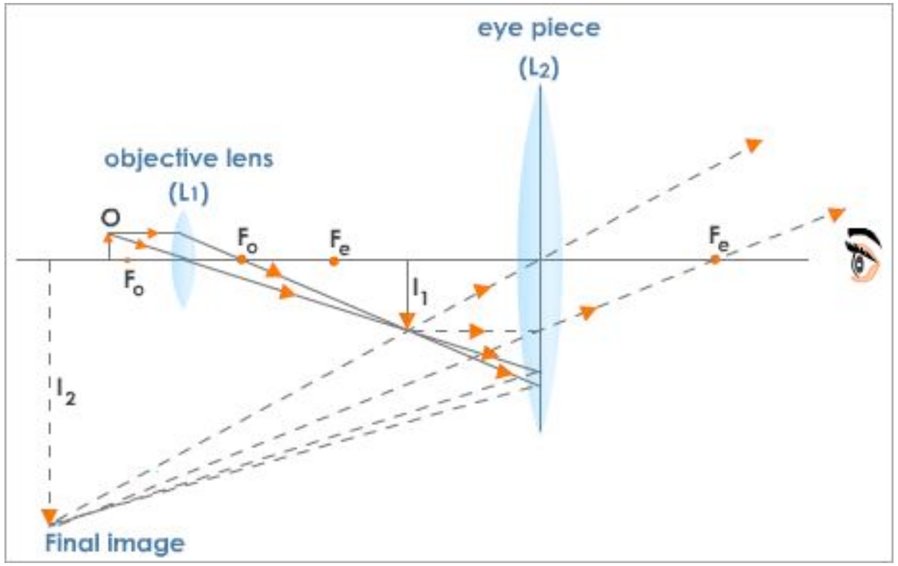
Medium of index n

$$NA = n \sin \theta$$

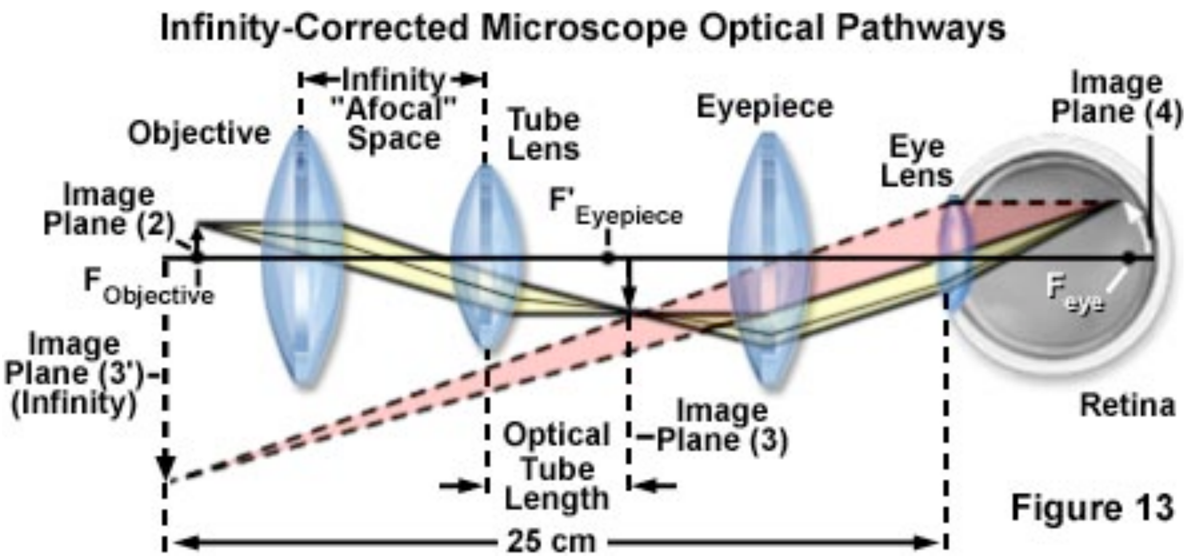
Optical Microscope



Microscope Theory

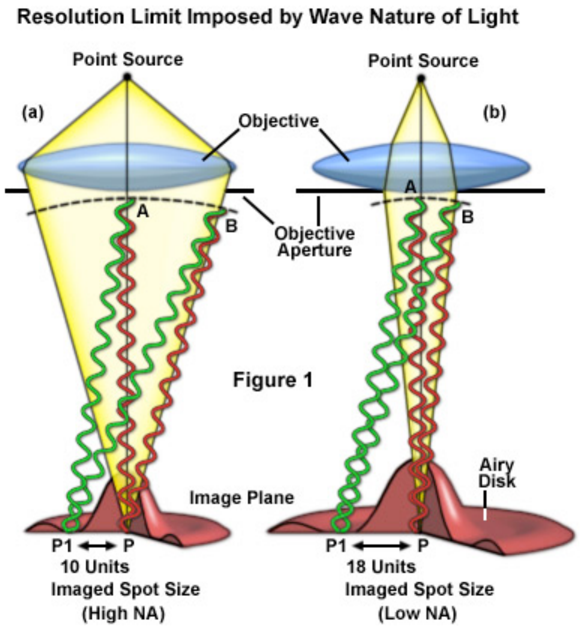


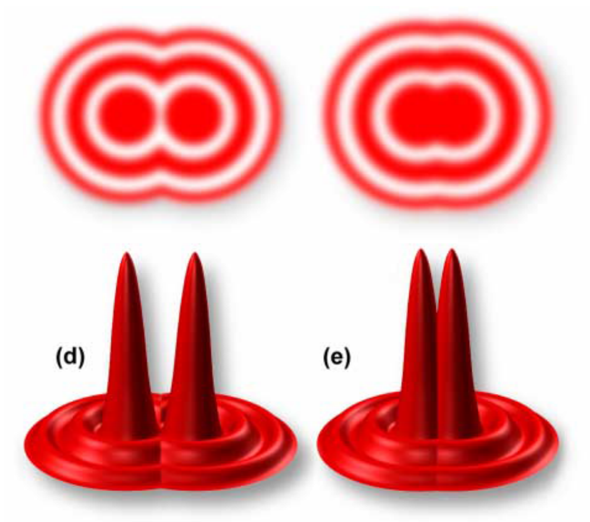
Traditional microscope:
An objective forms the image that is further magnified by the eyepiece lens.



Modern microscope:
The image is formed by an infinity corrected objective, which produces parallel rays, and a tube lens, which focus the rays into the intermediate image plane. The image is further magnified by the eyepiece lens.

Microscope Resolution



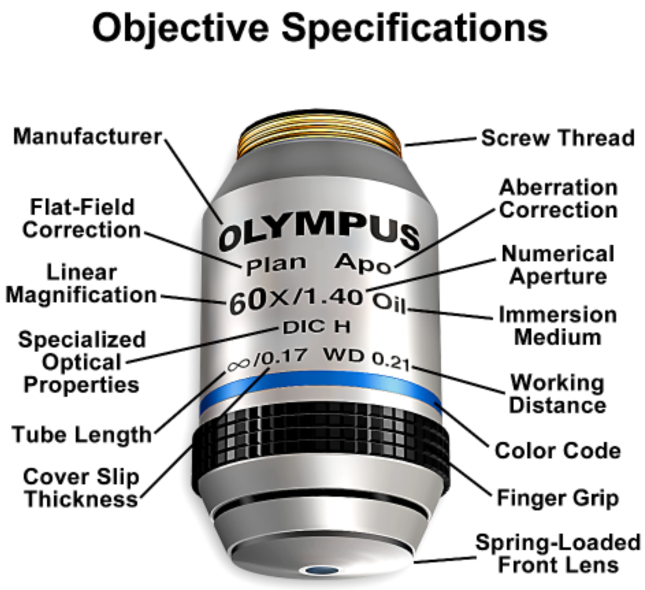


Due to the diffraction of light, a point source is imaged into a complex diffraction pattern composed by a bright spot and multiple interference fringes -> airy disk.
The diffraction limited (DL) resolution of the microscope is (Rayleigh limit):

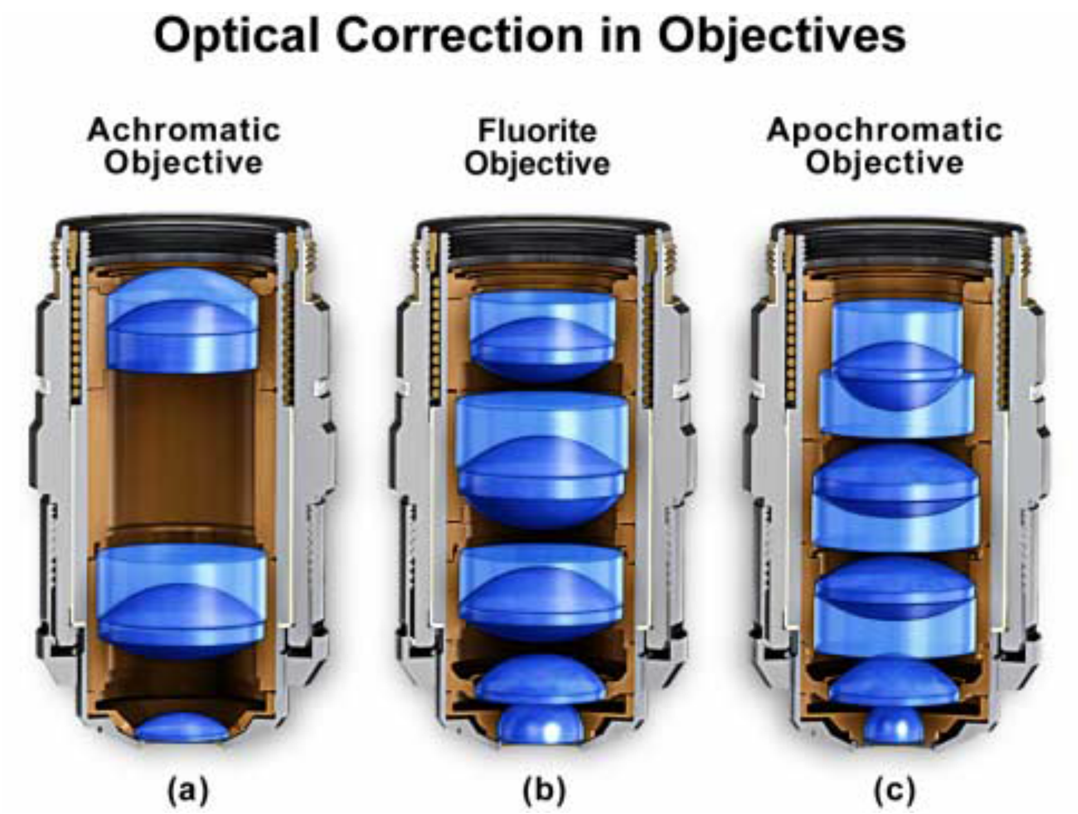
$$DL = 1.22 \frac{\lambda}{2 \cdot NA}$$

According to the Rayleigh criterion, two point sources observed in the microscope are regarded as being resolved when the principal diffraction maximum (the central spot of the Airy disk) from one of the point sources overlaps with the first minimum (dark region surrounding the central spot) of the Airy disk from the other point source.

Microscope Objectives

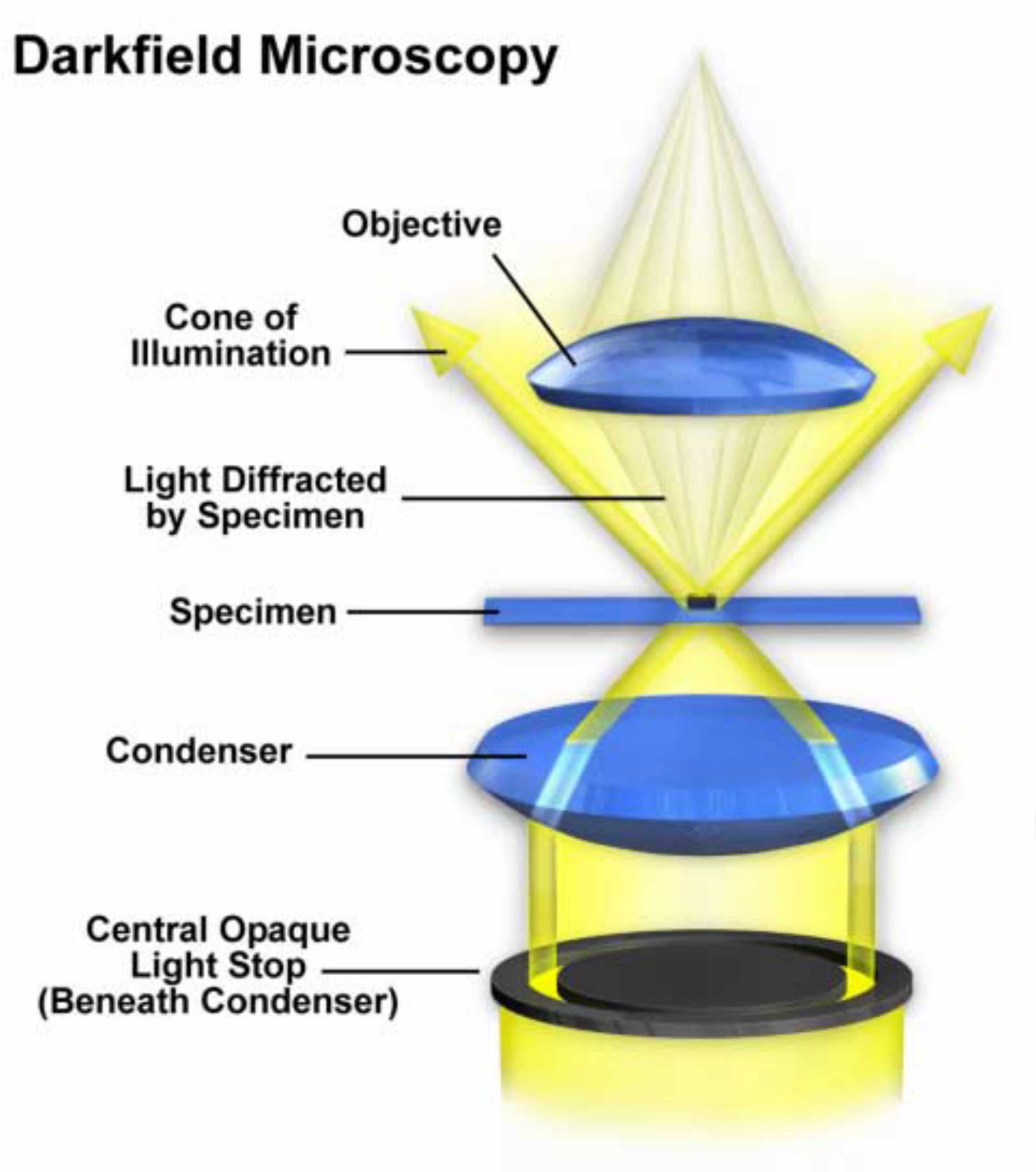


Microscope objectives can be quite complex optical systems, composed by many lens. All the information regarding the objective are engraved in the objective barrel.



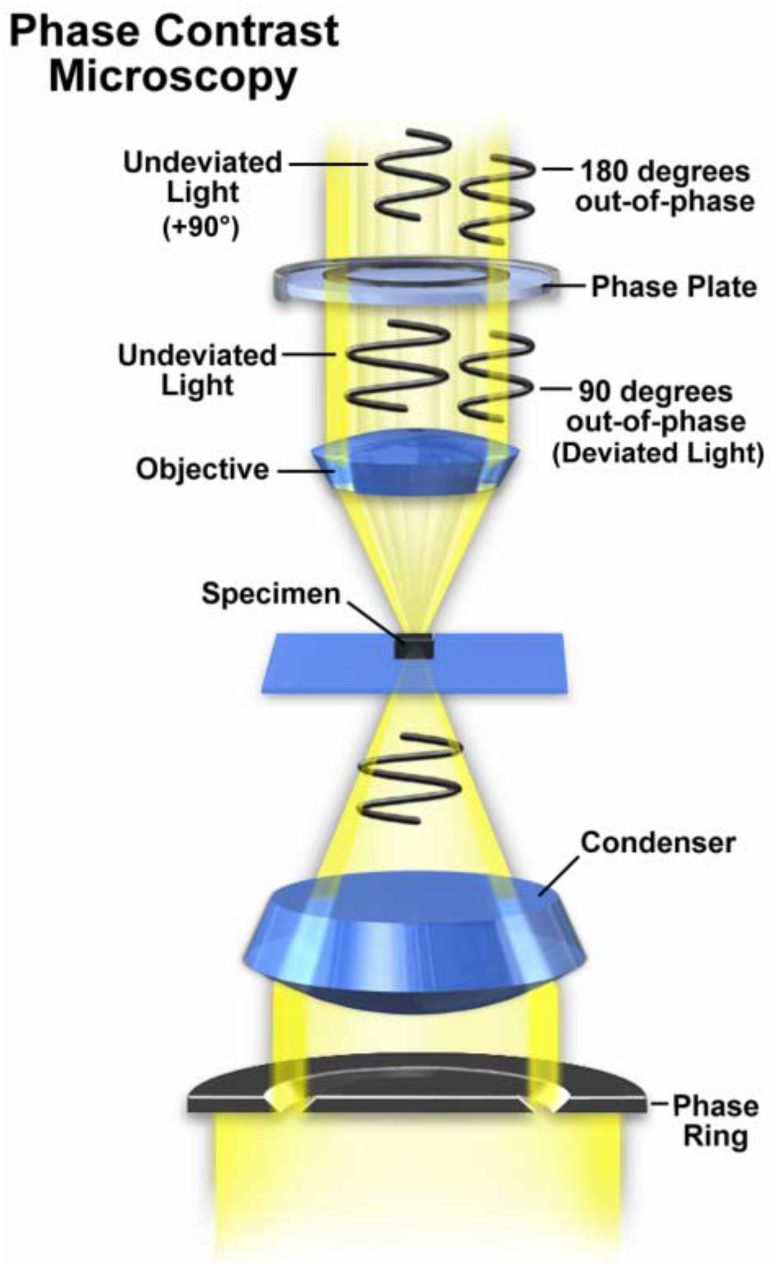
Aberrations are corrected at different levels, from achromatic objectives (the simplest and most economic) to apochromatic (most expensive)

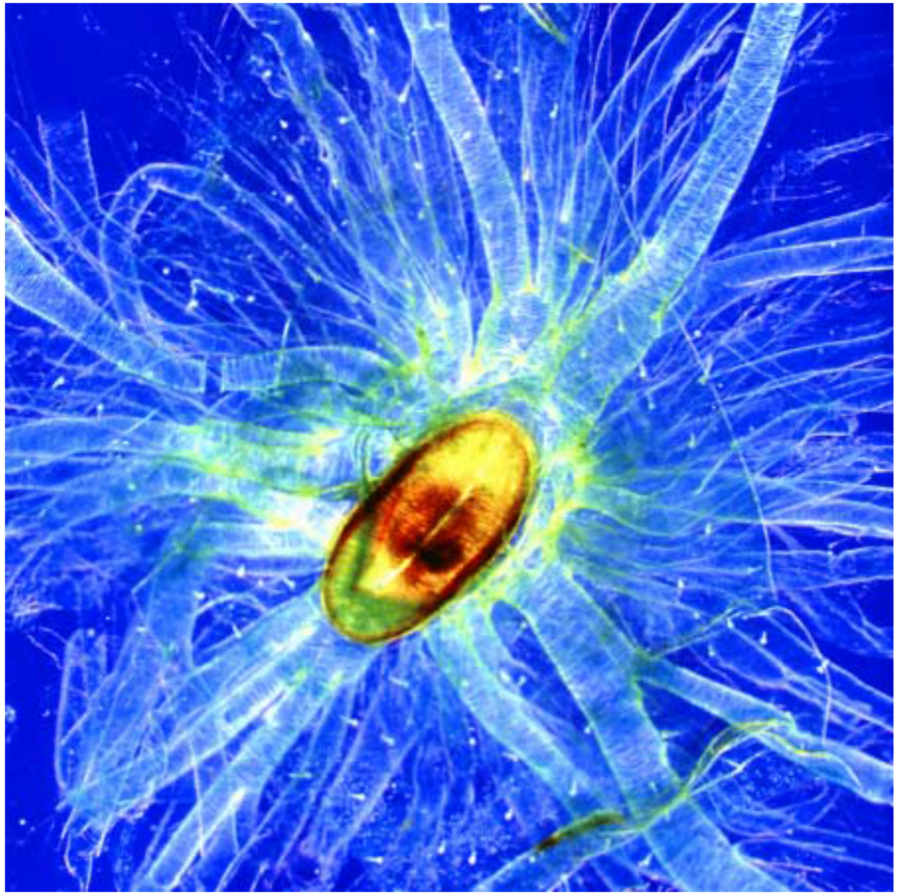
Microscopy Techniques



mysis zooplankton

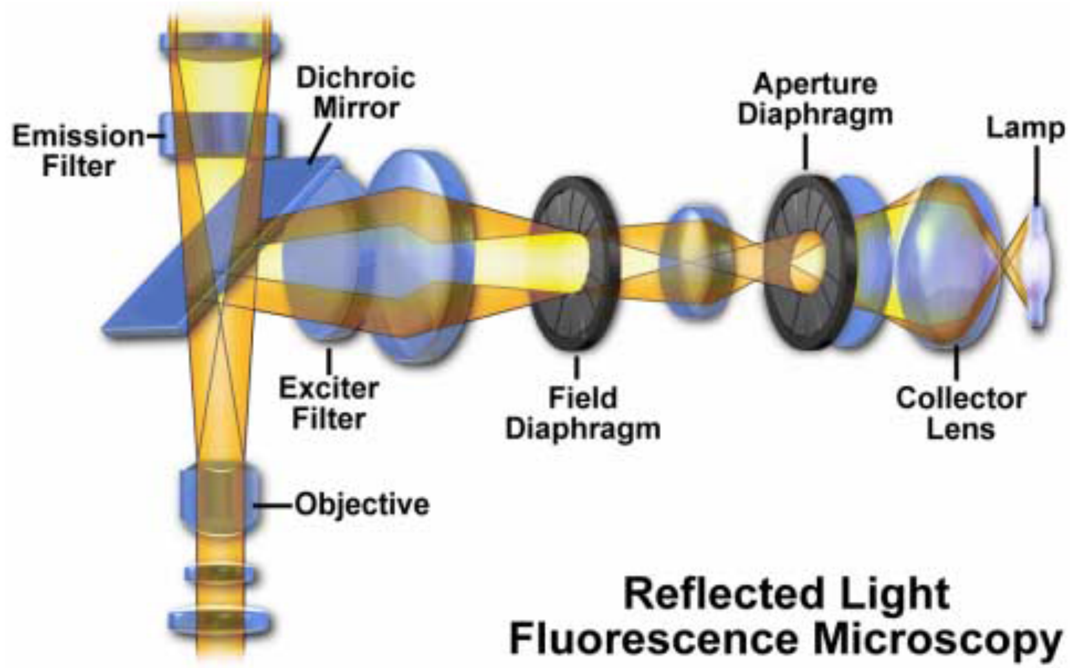
Microscopy Techniques

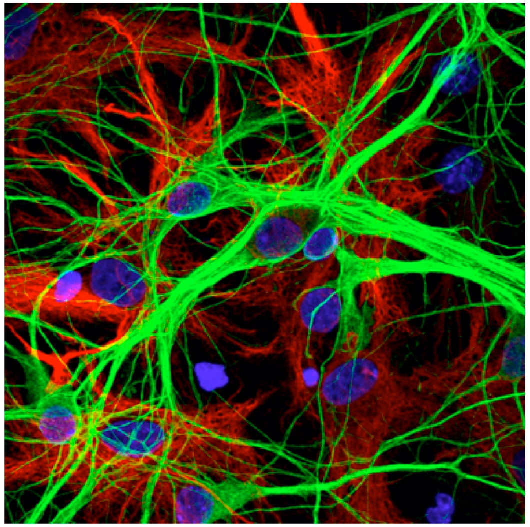


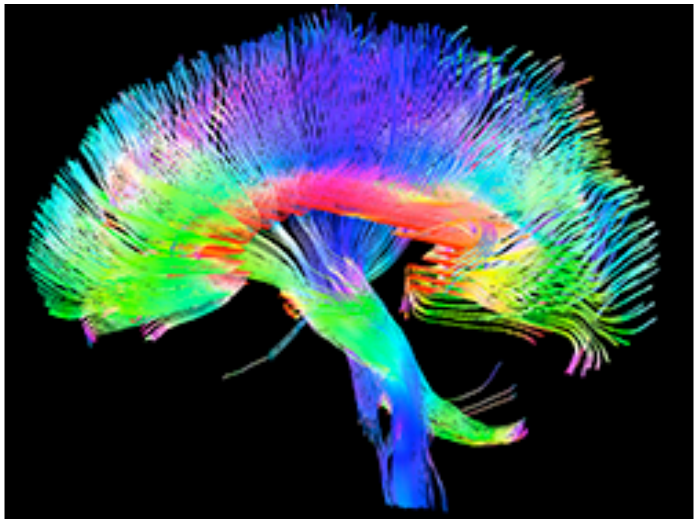


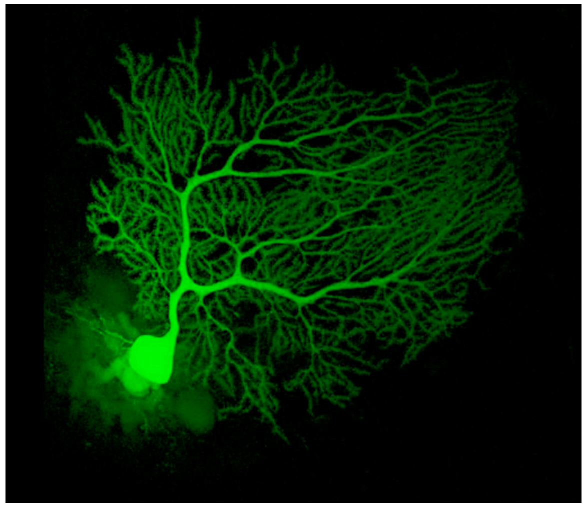
Silkworm

Microscopy Techniques




Nervous system


Brain neurons


Cerebellum