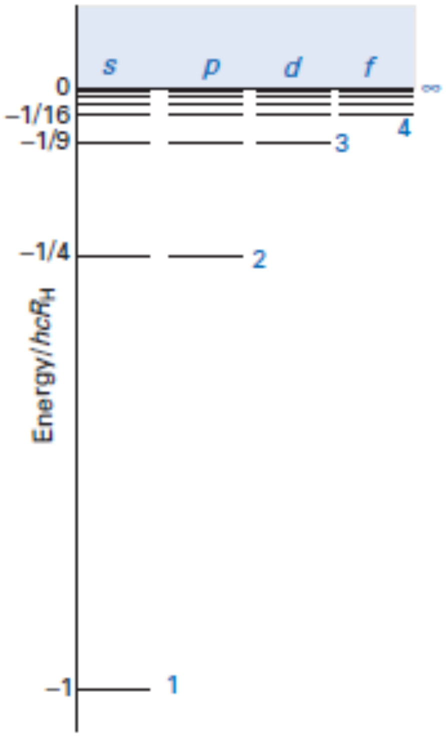
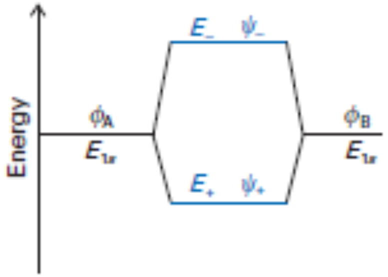
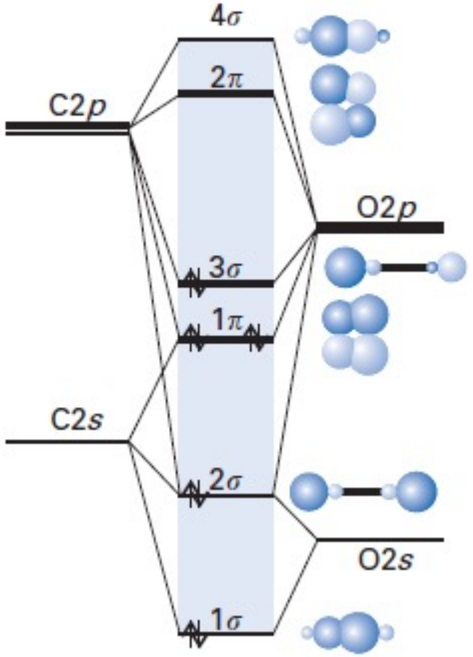
Introduction to Light microscopes

Atomic States

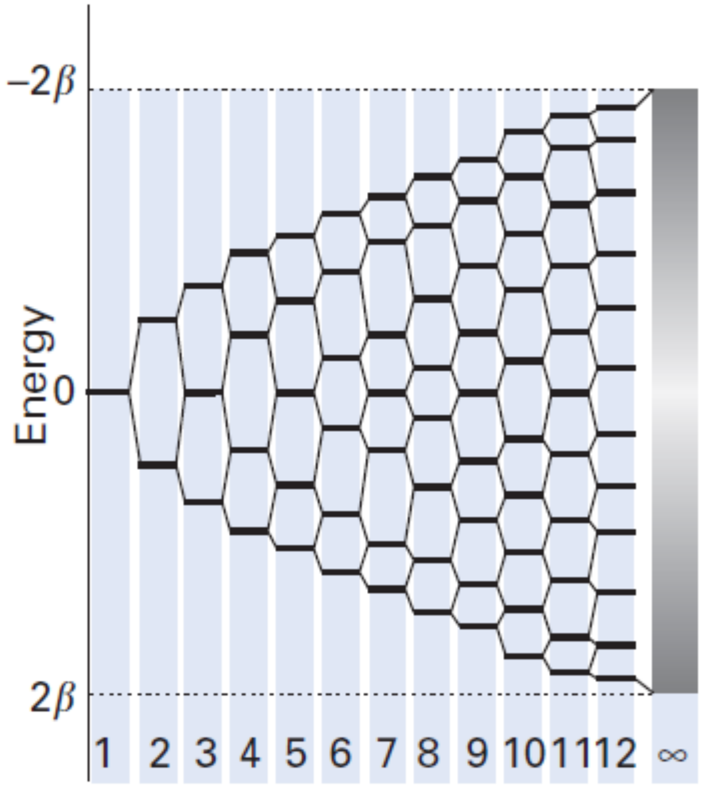


Molecular orbitals





String of N atoms – leading to solids



Lowest energy corresponds to completely bonded orbital with no nodes.
kth energy level is formed with k-1 nodes.

Remember, E, B are vectors $E = \begin{matrix} E_x \\ E_y \\ E_z \end{matrix}$

All of Maxwell's equation can be narrowed down to

$$\nabla^2 E = \epsilon_0 \mu_0 \frac{\partial \vec{E}}{\partial T}$$

$$\nabla^2 B = \epsilon_0 \mu_0 \frac{\partial \vec{B}}{\partial t}$$

Hence the Maxwell's equation will come to

$$\frac{\partial^2 E_x}{\partial^2 x^2} + \frac{\partial^2 E_x}{\partial^2 y^2} + \frac{\partial^2 E_x}{\partial^2 z^2} = \epsilon_0 \mu_0 \frac{\partial E_x}{\partial t}$$
$$\frac{\partial^2 E_y}{\partial^2 x^2} + \frac{\partial^2 E_y}{\partial^2 y^2} + \frac{\partial^2 E_y}{\partial^2 z^2} = \epsilon_0 \mu_0 \frac{\partial E_y}{\partial t}$$
$$\frac{\partial^2 E_z}{\partial^2 x^2} + \frac{\partial^2 E_z}{\partial^2 y^2} + \frac{\partial^2 E_z}{\partial^2 z^2} = \epsilon_0 \mu_0 \frac{\partial E_z}{\partial t}$$

The velocity of wave can be deciphered by comparing with standard wave equation

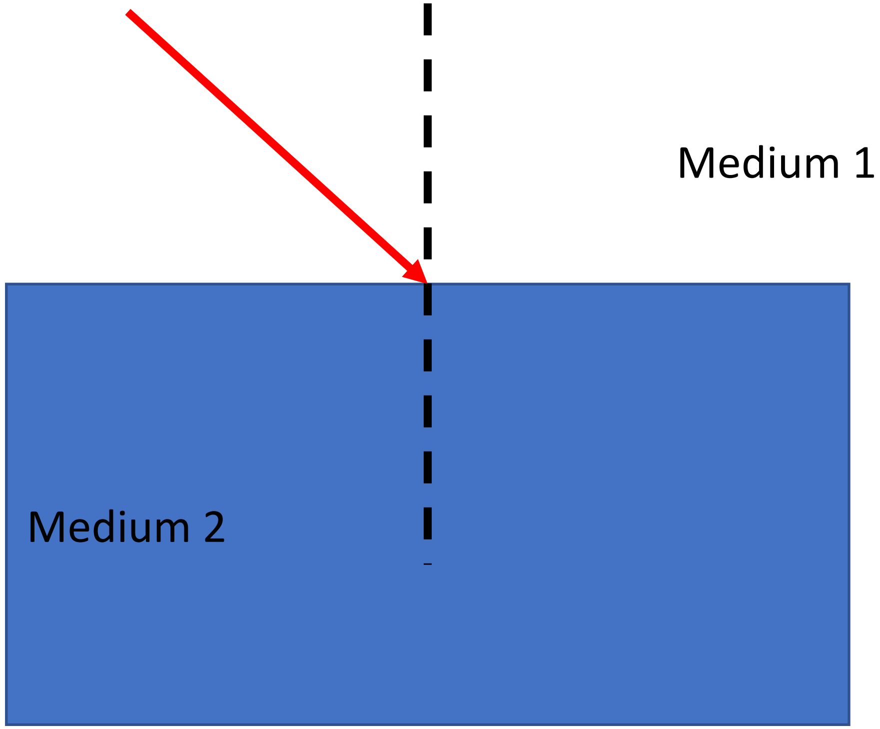
$$v = \frac{1}{\sqrt{\epsilon_m \mu_m}}$$

If the wave is moving in free space, $\mu_0 = 4\pi\ 10^{-7}\ m \frac{Kg}{C^2}, \epsilon_0 \mu_0 = 11.12\times10\ - 18\ s^2/m^2$

This gives the velocity of light in free space to be $C = 2.99792458\times10^8 \frac{m}{s}$

Light at the interface of materials

- We saw that the material determines the velocity, wavelength and such.
- So, what happens to the direction of propagation when light meets an interface between two materials ?
- What decides ?



The velocity of light is now different.

$$v = \frac{1}{\sqrt{\epsilon_m \mu_m}}$$

For each material, we can define a number called as refractive index –n

$$n = \frac{C}{v} = \sqrt{\frac{\epsilon_m \mu_m}{\epsilon_0 \mu_0}} = \sqrt{K_E K_M}$$

It is known that $K_M \cong 1$, for most materials and do not deviate from few parts in 10^{4}.

TABLE 3.2 Maxwell's Relation

Gases at 0°C and 1 atm		
Substance	$\sqrt{K_E}$	n
Air	1.000294	1.000293
Helium	1.000034	1.000036
Hydrogen	1.000131	1.000132
Carbon dioxide	1.00049	1.00045

Liquids at 20°C		
Substance	$\sqrt{K_E}$	n
Benzene	1.51	1.501
Water	8.96	1.333
Ethyl alcohol (ethanol)	5.08	1.361
Carbon tetrachloride	4.63	1.461
Carbon disulfide	5.04	1.628

Solids at room temperature		
Substance	$\sqrt{K_E}$	n
Diamond	4.06	2.419
Amber	1.6	1.55
Fused silica	1.94	1.458
Sodium chloride	2.37	1.50

But what happens when the light gets absorbed ?

We defined Absorbance

$$A(\lambda) = \epsilon l c = \log_{10} \frac{I_0}{I}$$

This means, the intensity of light passing through a material will decay exponentially $I = I_0 e^{-A}$

If we can take the length (l) outside, we rewrite the same equation as $I = I_0 e^{-\alpha t}$

α attenuation coefficient
t – thickness through which the light is propagating

The electric field in the light is taken to be $E = E_0 e^{i(kx - \omega t)}$

$$\tilde{n} = n - i\kappa$$

and $k = \dfrac{2\pi\tilde{n}}{\lambda}$

$$\kappa = \frac{\alpha\lambda}{4\pi}$$

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$$\tilde{n} = n - i\kappa$$

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$$\kappa = \frac{\alpha\lambda}{4\pi}$$

$\tilde{n}$ is the complex refractive index

Such that the wavelength in the medium $\lambda = \frac{\lambda_0}{n}$ where, n is the refractive index

κ , the complex part, is proportional to the attenuation coefficient.

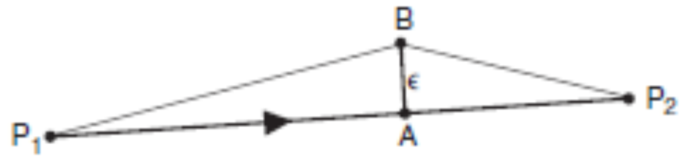
n and κ are related by Kramer-Kronig relation

$$n(\omega) = \frac{1}{\pi}\int_{-\infty}^{\infty} \frac{\kappa(\omega')}{\omega' - \omega} d\omega'$$

Light propagation:

- Rectilinear propagation

  Between two points light is assumed to travel in a straight line by Fermat's principle of least time

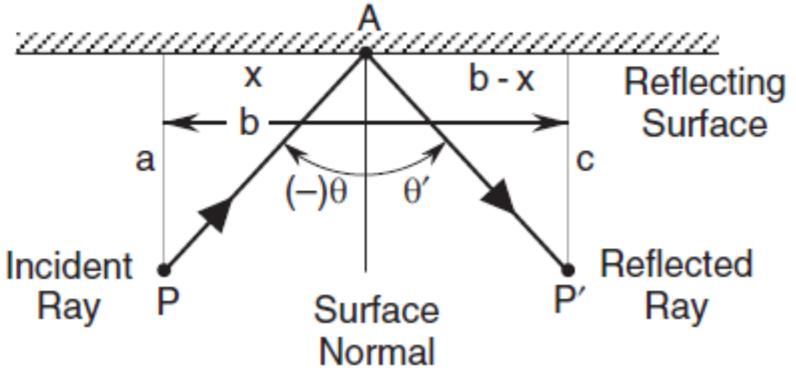


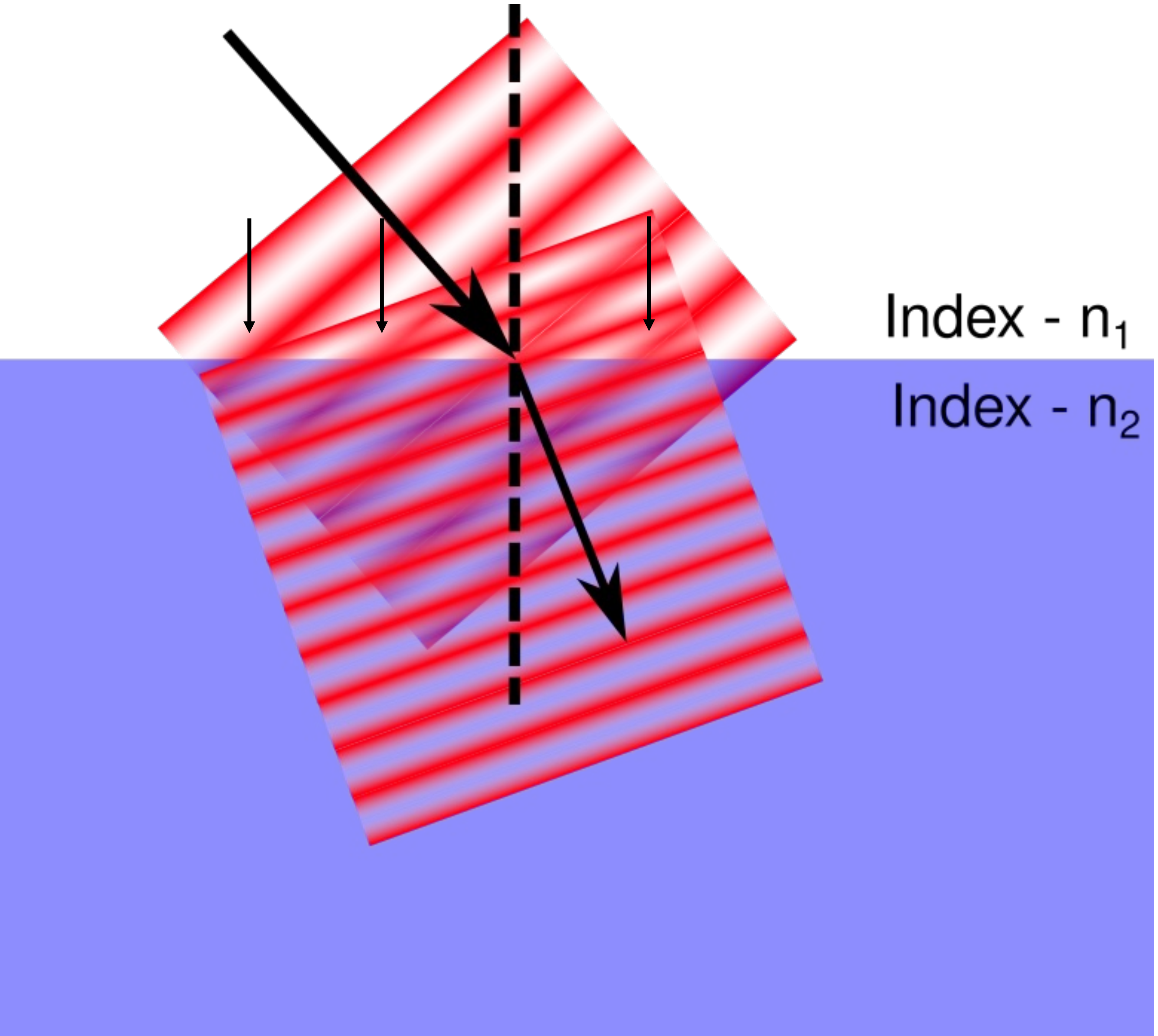
- Reflection:

At the interface between two media, the light gets reflected such that the incident ray and reflected ray are coplanar,

The angle of incidence = angle of reflection

$$\theta = -\theta'$$





Snell's law

Velocity of light $v_2 n_2 = v_2 n_2 = C * 1$

Where C – velocity of light in vacuum
n = 1 is the index of vacuum.

The process is elastic (No loss of energy)

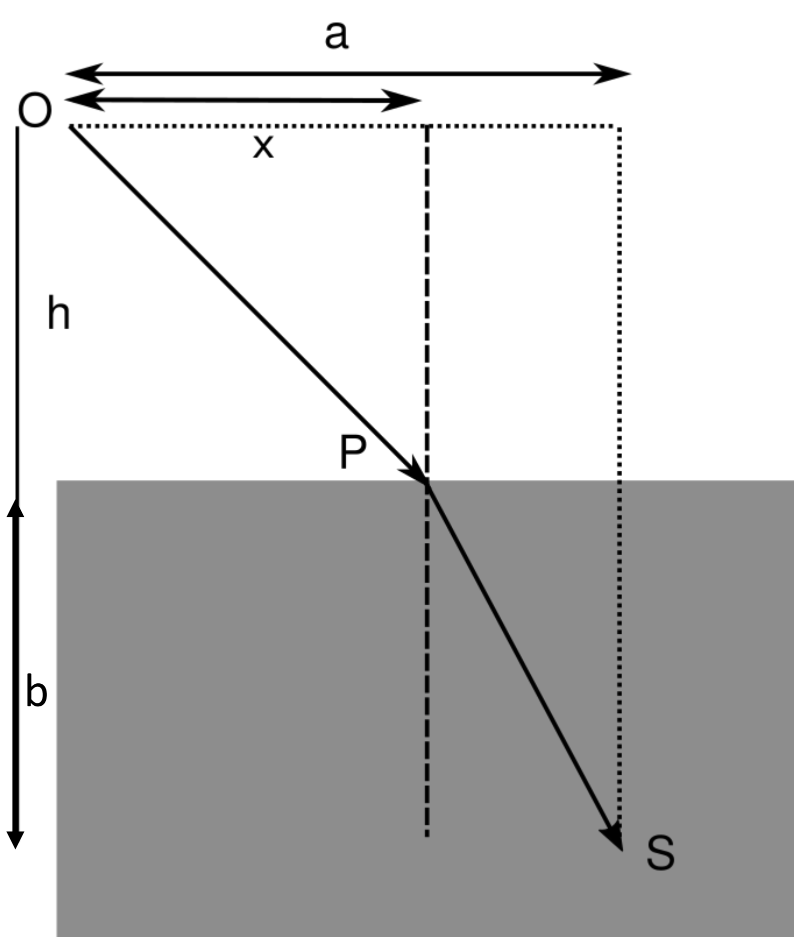
So $E_1 = h\nu_1 = E_2 = h\nu_2$

Therefore wavelength $\lambda_i = \frac{v_i}{\nu} = \frac{C}{n_i \nu}$

The light waves have to bend to accommodate for the change in the wavelength in phase velocity in different medium.

$$\frac{\sin\theta_1}{\sin\theta_2} = \frac{n_2}{n_1} = \frac{v_1}{v_2}$$

Derive Snell's law from Fermat's principle of least time



Fermat's principle:
Between 0 -> S, light travels the path that requires the least time

$$T = \overrightarrow{OP} + \overrightarrow{OS}$$

$$T = \frac{\sqrt{h^2 + x^2}}{v_1} + \frac{\sqrt{b^2 + (a\ - x)^2}}{v_2}$$

Minima can be obtained when $\frac{dT}{dx} = 0$ and we can arrive at the Snell's law.

$$\frac{dT}{dx} = \frac{x}{v_1\sqrt{h^2 + x^2}} - \frac{a\ - x}{v_2\sqrt{b^2 + (a - x^2)}} = 0$$

$$\frac{\sin\theta_i}{v_1} = \frac{\sin\theta_2}{v_2}$$

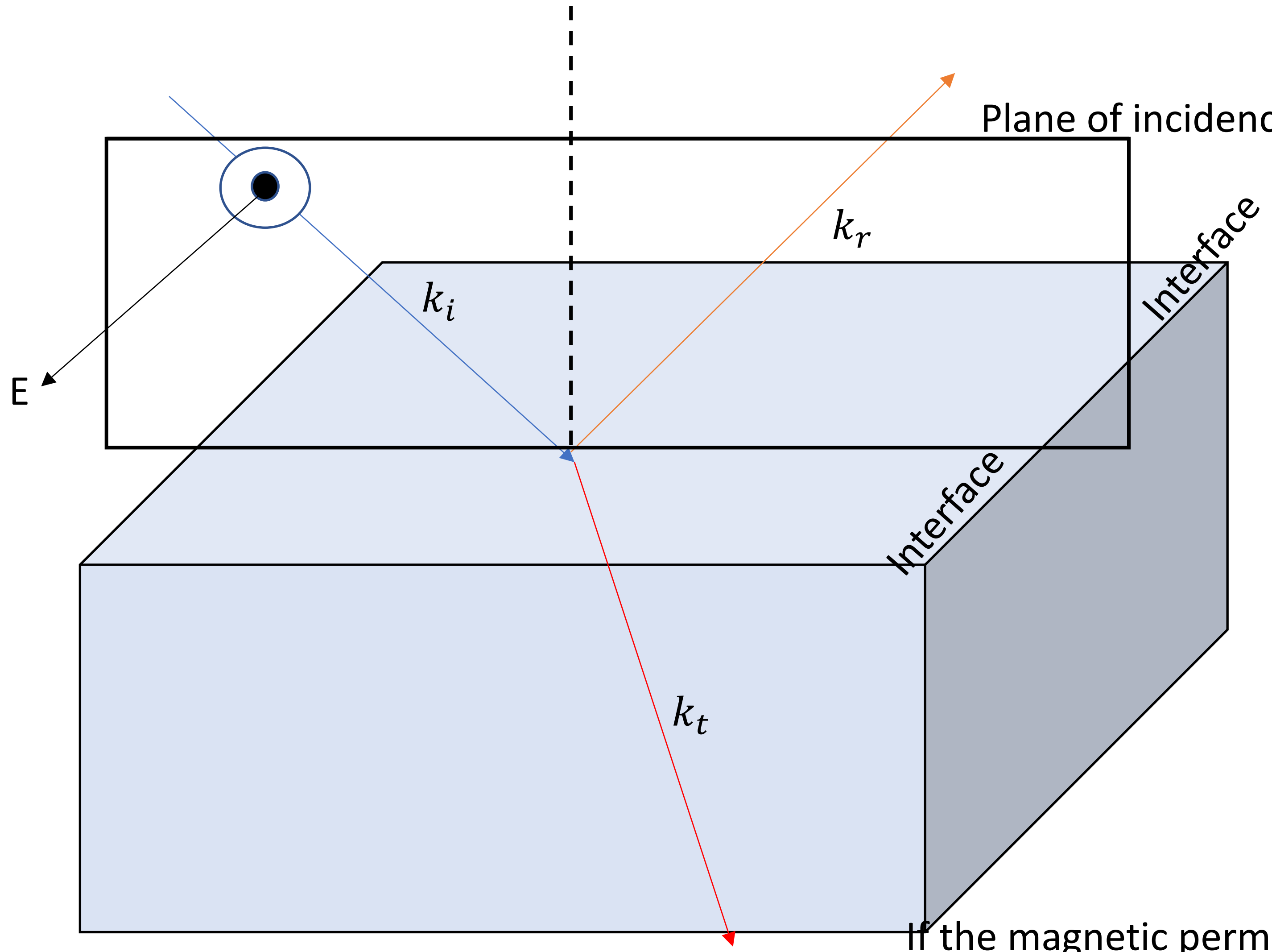
Intensities

Plane of incidence carries both the medium

Assume incident radiation is

$$E_i = E_{0i} \cos \overrightarrow{k_i} . \vec{r} \; - \omega_i t$$

Plane of incidence



Electric field normal to the plane of incidence

If the magnetic permeabilities does not change

$$r_\perp = \frac{E_{0r}}{E_{0i}} = \frac{\left(\frac{n_i}{\mu_i} \cos \theta_i \; - \frac{n_t}{\mu_t} \cos \theta_t\right)}{\frac{n_i}{\mu_i} \cos \theta_i + \frac{n_t}{\mu_t} \cos \theta_t}$$

$$r_\perp = \frac{n_i \cos \theta_i \; - n_t \cos \theta_t}{n_i \cos \theta_i + n_t \cos \theta_t}$$

When you have incidence with Electric field perpendicular to plane of incidence

$$r_\perp = \frac{E_{0r}}{E_{0i}} = \frac{\left(\frac{n_i}{\mu_i}\cos\theta_i \; - \frac{n_t}{\mu_t}\cos\theta_t\right)}{\frac{n_i}{\mu_i}\cos\theta_i + \frac{n_t}{\mu_t}\cos\theta_t}$$

$$T_\perp = \frac{E_{0r}}{E_{0i}} = \frac{\frac{2n_i}{\mu_i}\cos\theta_i}{\frac{n_i}{\mu_i}\cos\theta_i + \frac{n_t}{\mu_t}\cos\theta_t}$$

When you have incidence with Electric field parallel to plane of incidence

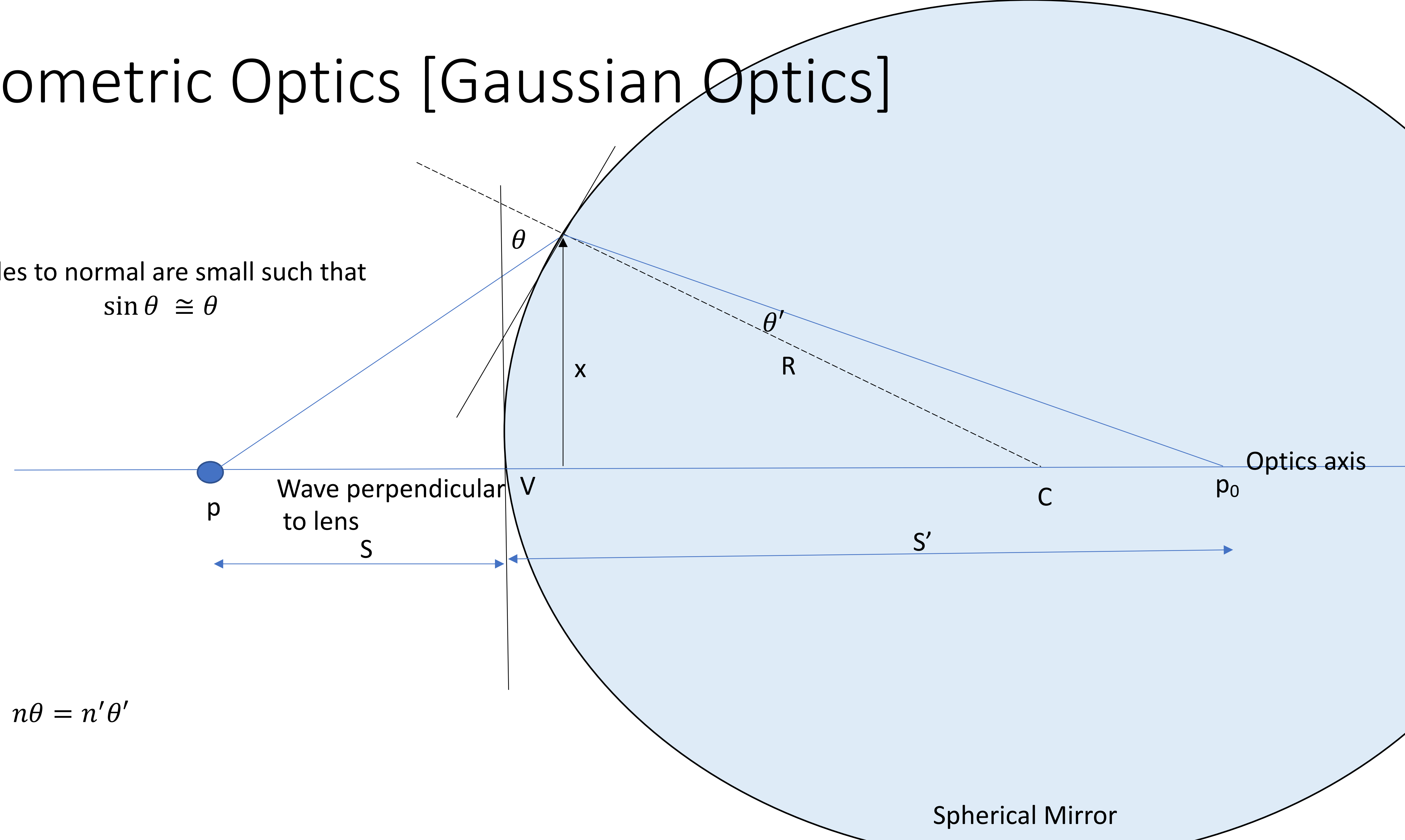
$$r_\parallel = \frac{E_{0r}}{E_{0i}} = \frac{\left(\frac{n_t}{\mu_t}\cos\theta_i \; - \frac{n_i}{\mu_i}\cos\theta_t\right)}{\frac{n_i}{\mu_i}\cos\theta_t + \frac{n_t}{\mu_t}\cos\theta_i}$$

$$r_\parallel = \frac{E_{0r}}{E_{0i}} = \frac{\left(\frac{2n_i}{\mu_i}\cos\theta_i\right)}{\frac{n_i}{\mu_i}\cos\theta_t + \frac{n_t}{\mu_t}\cos\theta_i}$$

Geometric Optics [Gaussian Optics]

Angles to normal are small such that

$\sin\theta \cong \theta$



$n\theta = n'\theta'$

Rules / Sign convention

- S positive to the left of V
- S’ positive to the right of V
- R positive if it is right of V

The equation is independent of x.

Ie in Gaussian optics,

All rays, independent of X, will meet at the Optics axis at point P_0

$$\frac{n'}{S'} + \frac{n}{S} = \frac{n' - n}{R}$$

Gaussian imaging equation

$$\frac{n'}{S'} + \frac{n}{S} = \frac{n' - n}{R}$$

First focal length/Object focal length:

object if imaged at ∞, $s' \rightarrow \infty$

The object then is at a point $S = \frac{n}{n'-n} R$ also called as f_o, object focal length/first focal length

If the object is at infinity, $S \rightarrow \infty$

Then, the image is formed at a distance $S' = \frac{n'}{n'-n} R$ otherwise called as image focal length f_i, second focal length

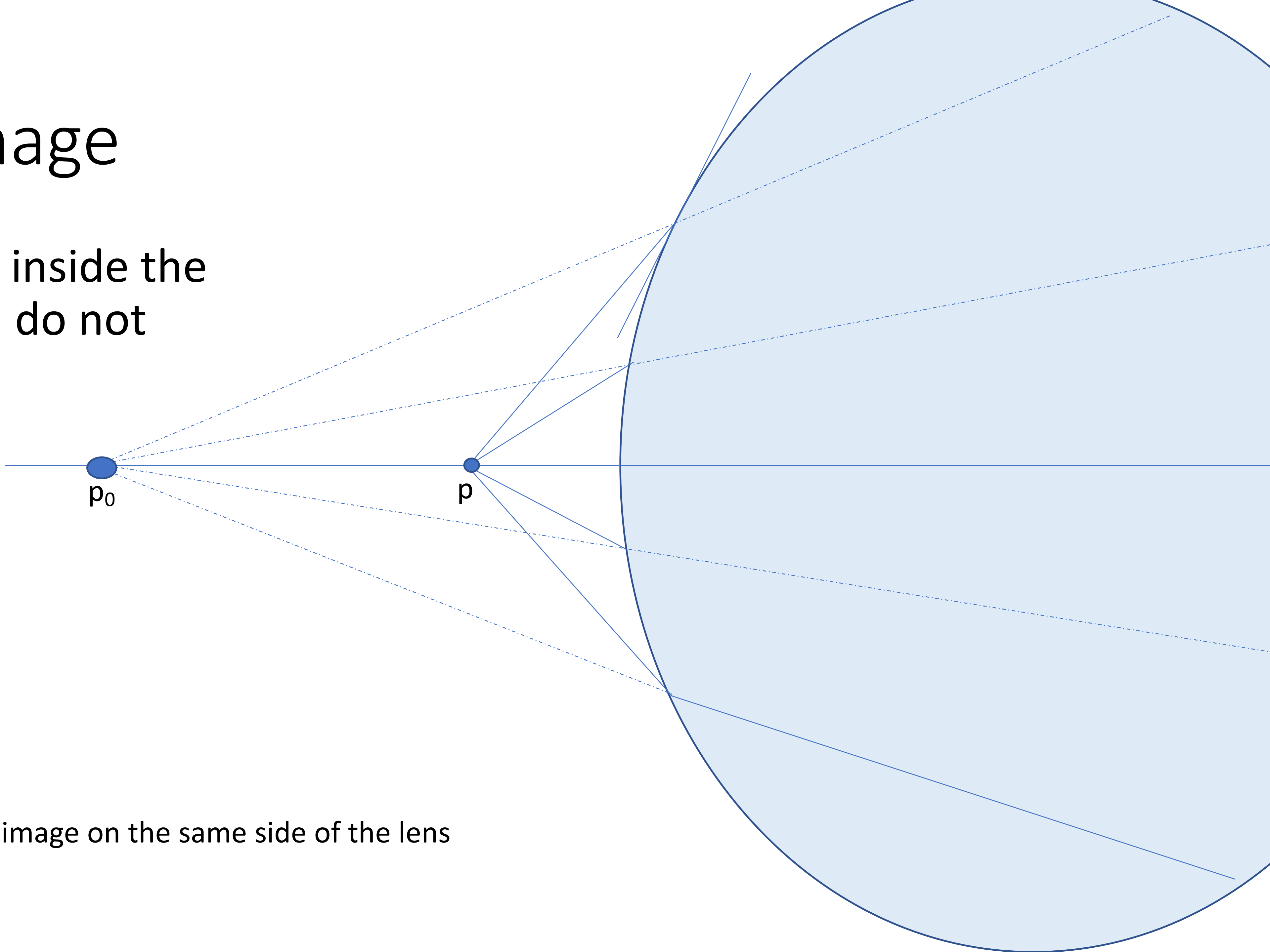
If the object $S < \frac{n}{n'-n} R$, then no positive solutions are available for the image

This means, the light diverges inside the second medium.

A virtual image can only be obtained. $S' < 0$

Virtual Image

- What if the rays inside the second medium do not converge ?

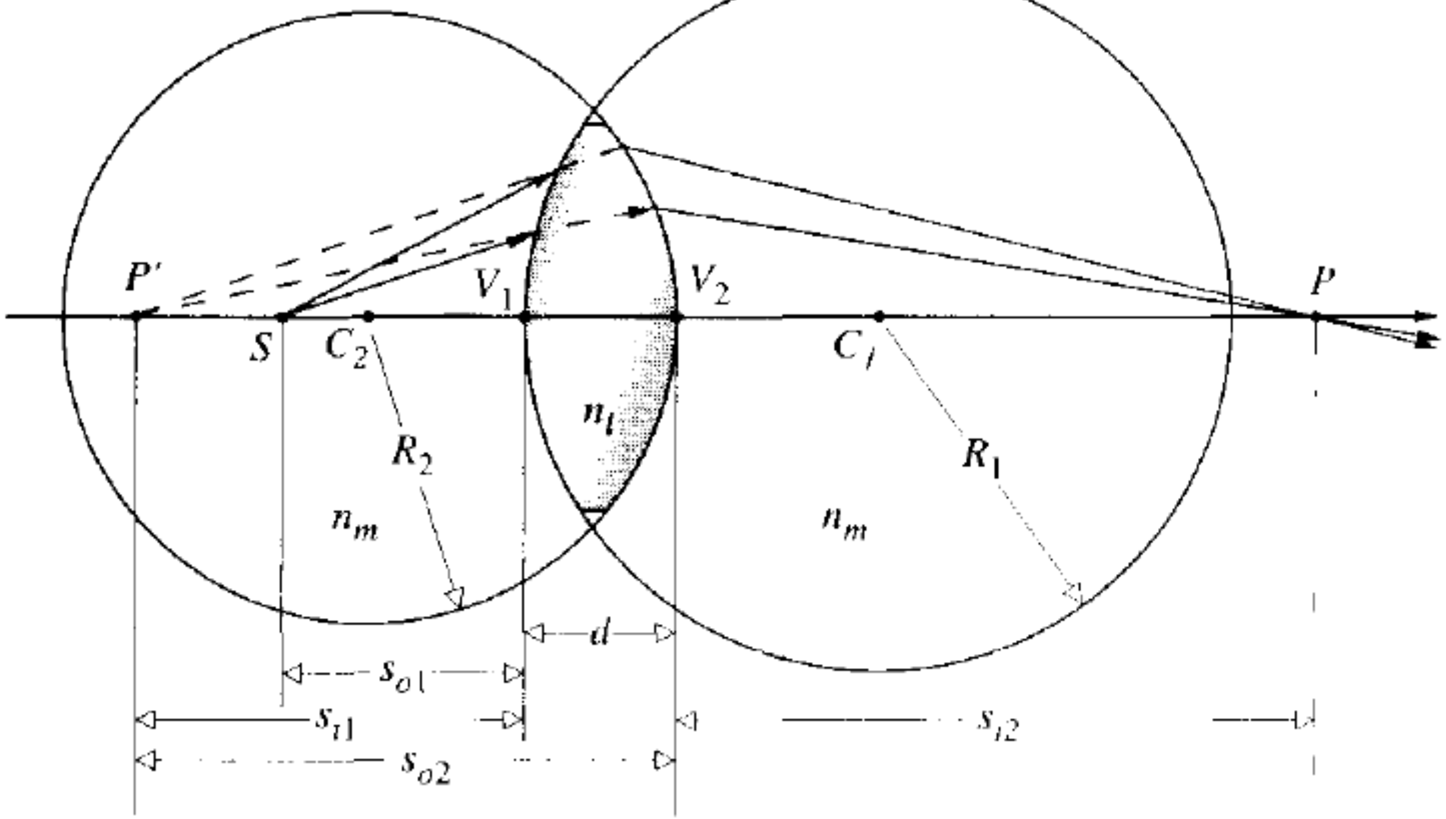


You have an virtual image on the same side of the lens

Case for a thin lens

$d \to 0$
$S_{01} \to S$
$S_{t2} \to S'$

(c)



Governing equation:

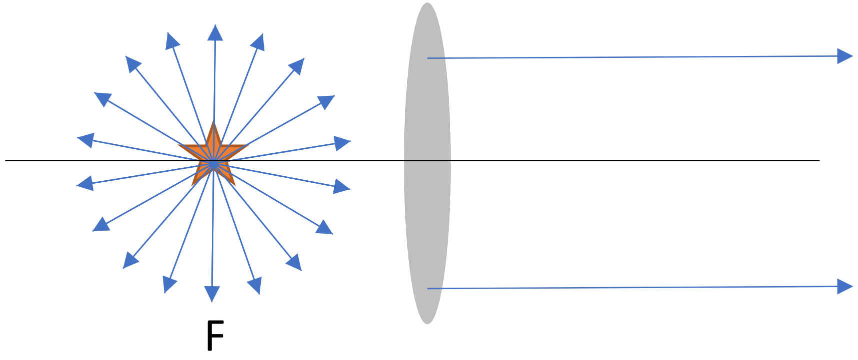
$$\frac{1}{S'} + \frac{1}{S} = (n_l - 1)\left[\frac{1}{R_1} - \frac{1}{R_2}\right]$$

We call $\frac{1}{f} = \left[\frac{1}{R_1} - \frac{1}{R_2}\right](n_l - 1)$

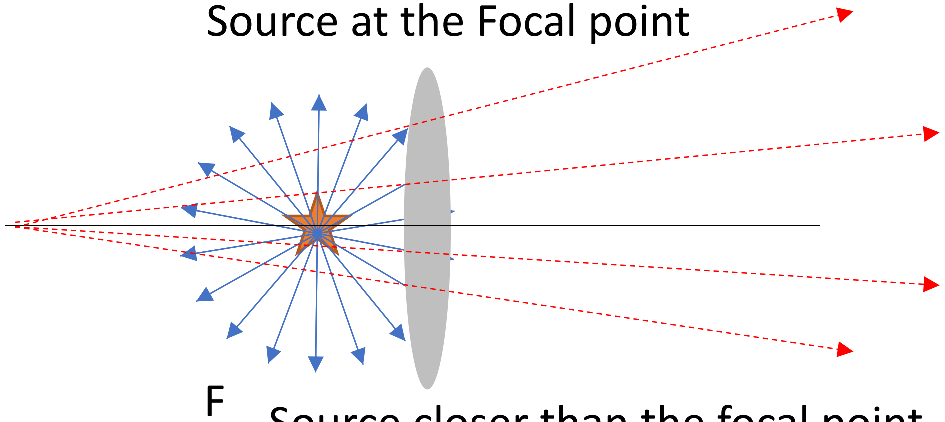
As focal length

Gives Thin-lens equation/ Lensmaker's equation $\frac{1}{S'} + \frac{1}{S} = \frac{1}{f}$

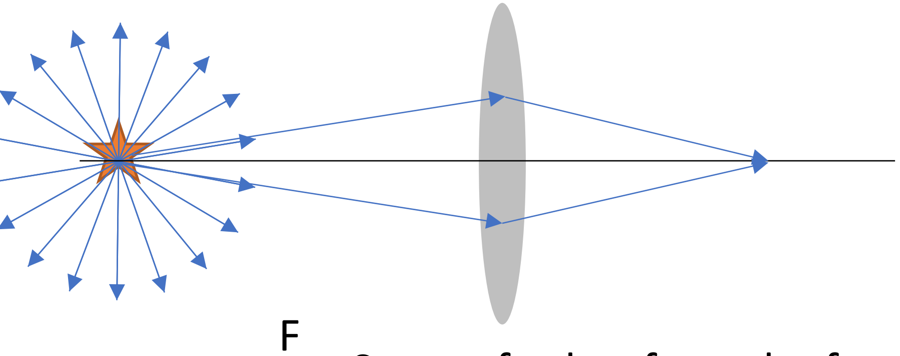
Lets us look objects away from optic axis



Source at the Focal point

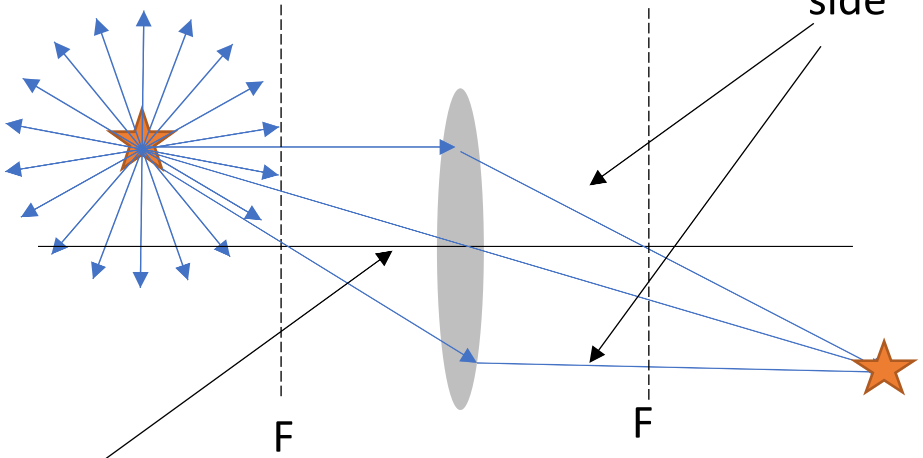


Source closer than the focal point



Source farther from the focal point

Ray passing through focal point should be parallel in the other side

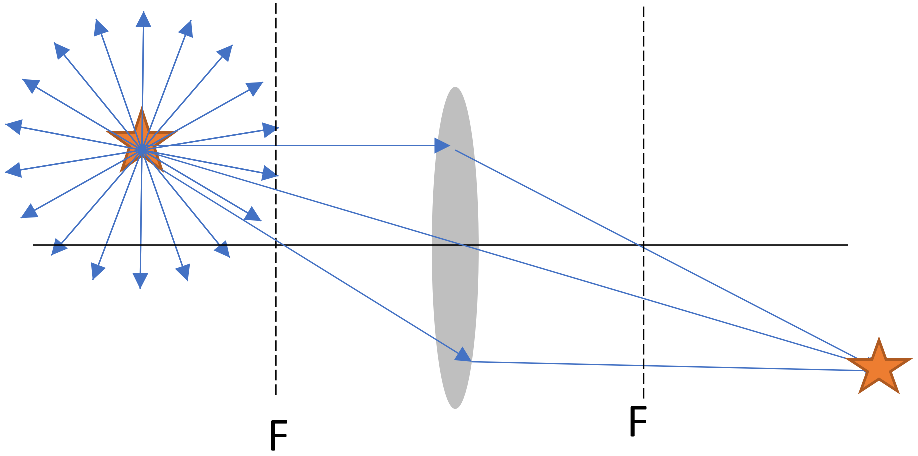


Source at the Focal point

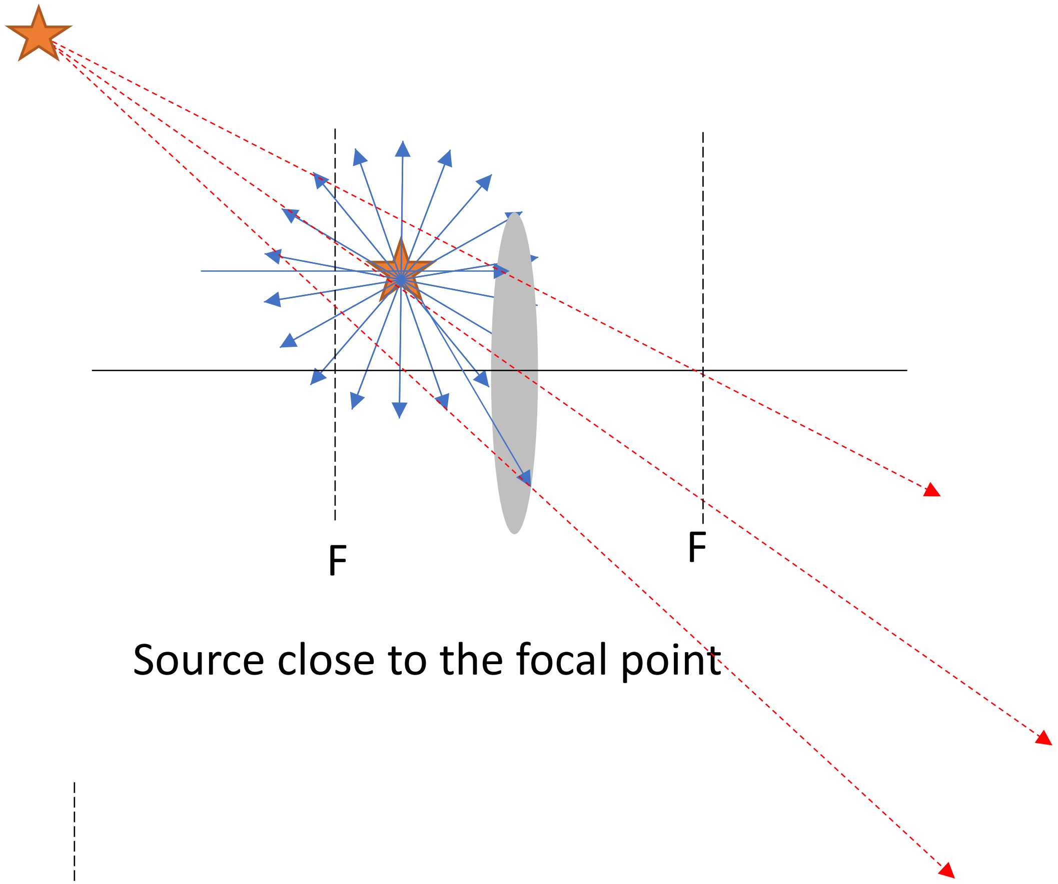
An inverted image is formed

Rays through the central point always goes undeflected

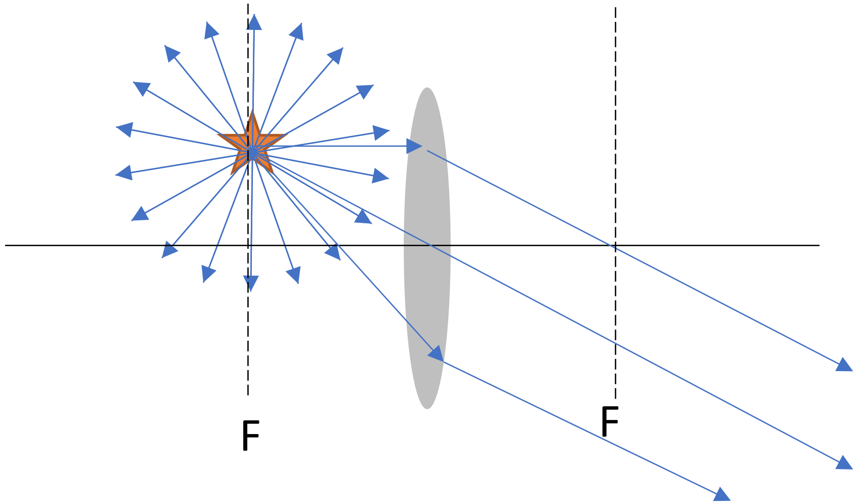
Remember, the point is called as image as at this point, the light rays again diverge seeming like a source



Source farther from the focal point



Source close to the focal point

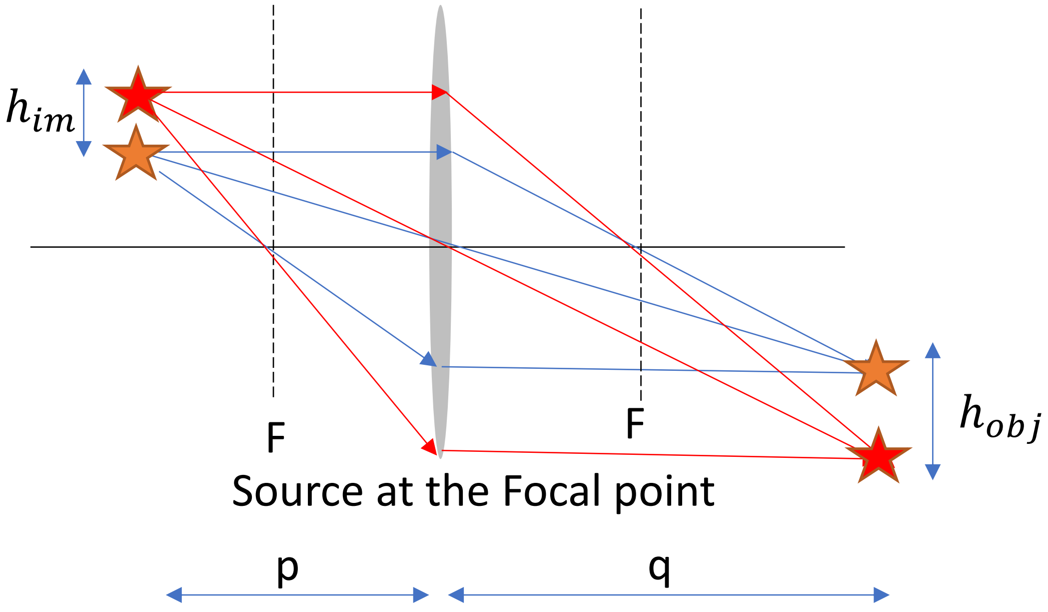


Source farther from the focal point

No image is formed.
Image at ∞

So what happens at the object at the focal point ?

Object, Image and Magnification



Magnification of this single lens is given

$$|m| = \frac{h_{img}}{h_{obj}}$$

Homework: Just using simple geometric arguments: prove that magnification:

$$m = \frac{q}{p}$$

- An object – Collection of point sources.
- A plane containing point sources normal to optic axis gives an image plane normal to optic axis!.
- Each point in the object will have a corresponding image point
- The image-point is obtained by co-incidence of atleast two rays.
- Ray 1 – which passes through the lens center – undeflected.
- Ray 2 – a line parallel to optic axis has to pass through the focal point on the otherside.

Summary

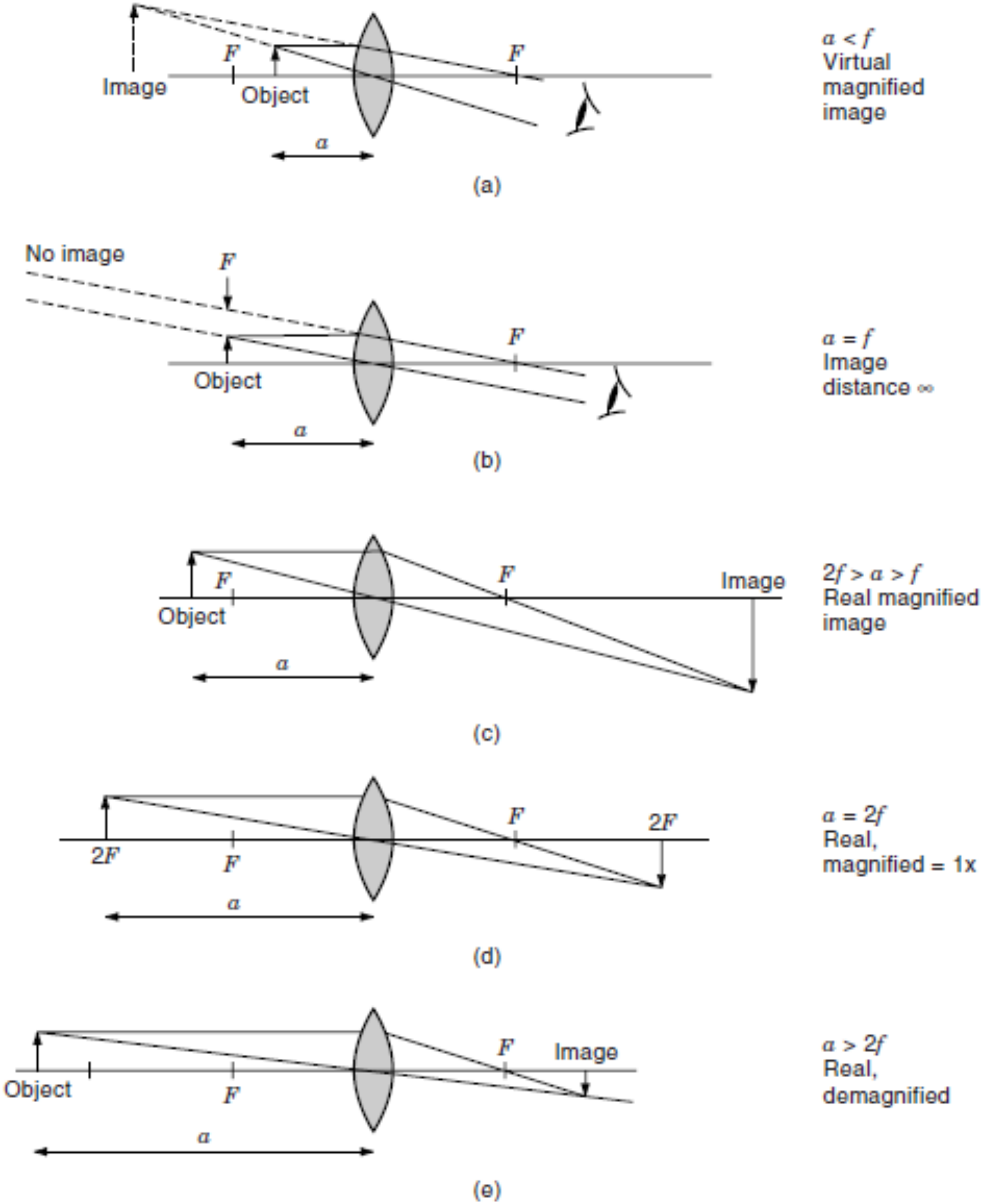


Image
F
Object
F
a
(a)
a < f
Virtual
magnified
image
No image
F
F
Object
a
(b)
a = f
Image
distance ∞
F
Object
F
Image
a
(c)
2f > a > f
Real magnified
image
2F
F
F
2F
a
(d)
a = 2f
Real,
magnified = 1x
Object
F
F
Image
a
(e)
a > 2f
Real,
demagnified

Table of reference:

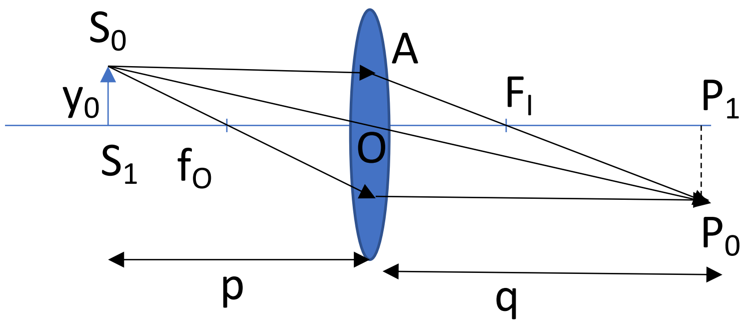
Object	Type	Location	Orientation	Relative size
∞ > p > 2f	Real	f < q <2f	Inverted	Minified
P = 2f	Real	Q = 2f	Inverted	Same size
f < p > 2f	Real	∞ > q >2f	Inverted	Magnified
p = f		±∞		
P < f	Virtual	q > p	Erect	Magnified
Anywhere	Virtual	q < f, p > q	Erect	Minified

For virtual image

Magnification $|M| = \frac{q}{p} = \left(\frac{q}{f} - 1\right)$

Magnification $|M| = \frac{q}{p} = \left(\frac{q}{f} + 1\right)$

y_i is negative by convention due to the downward direction of the image



Triangles AOF_i and $P_1P_0F_i$ are similar

Therefore: $\frac{y_0}{y_i} = \frac{f_i}{q - f_i}$

From the similarity of S_0S_1O and OP_1P_0

$$\frac{y_0}{y_1} = \frac{p}{q}$$

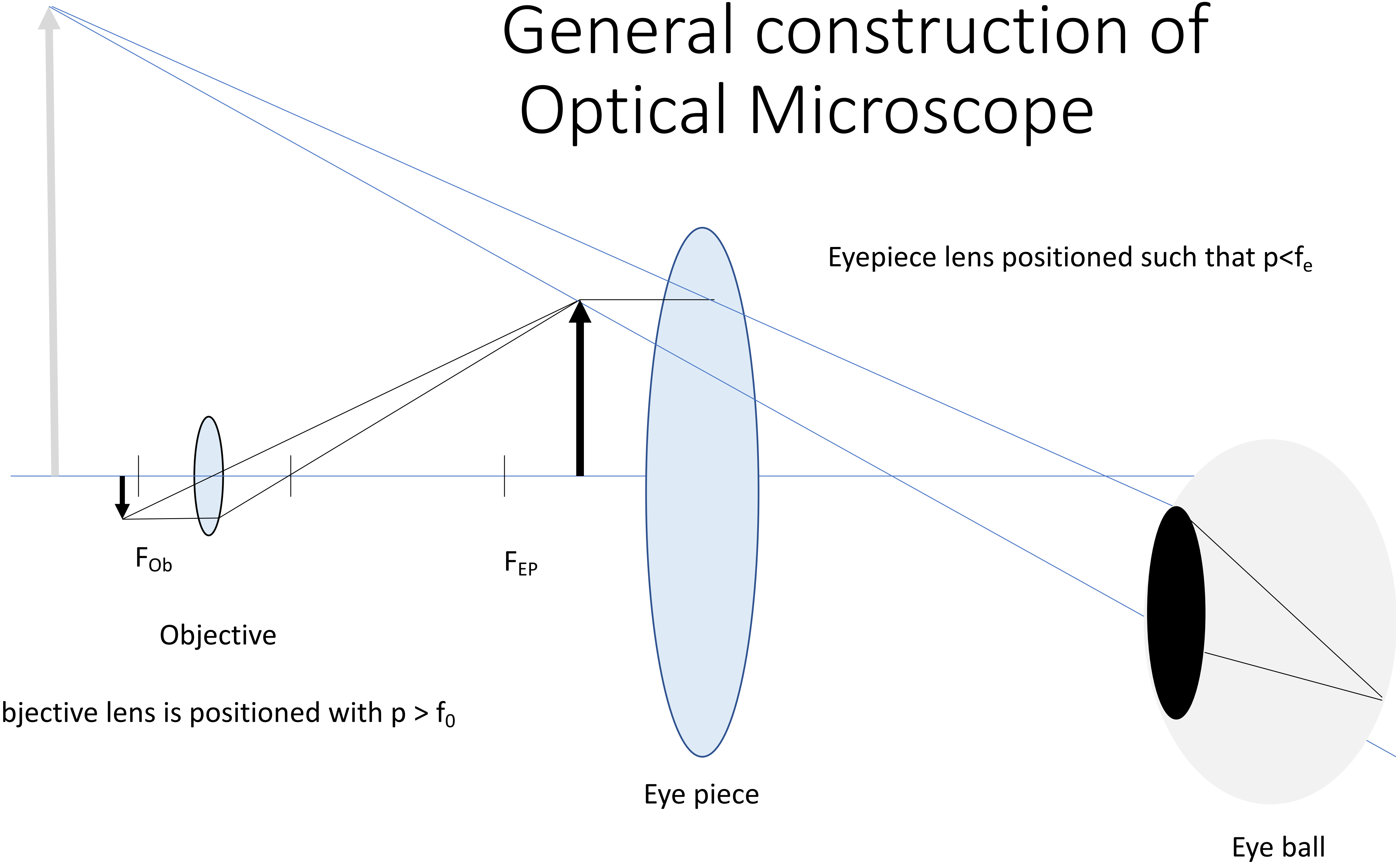
Also from the thin lens equation $\frac{1}{p} + \frac{1}{q} = \frac{1}{f}$

If we denote $x_0 = p - f$ and $x_i = q - f$

We can obtain the Newton's equations for magnification: $M = \frac{y_i}{y_o} = \left(-\frac{q}{p}\right) = -\frac{x_i}{f} = -\frac{f}{x_0}$

$$f^2 = x_0 x_i$$

General construction of Optical Microscope



Eyepiece lens positioned such that $p<f_e$

Objective lens is positioned with $p > f_0$

Magnification in a microscope

Magnification produced by the objective lens $M_o = \frac{q}{p} = \frac{q}{f_o} - 1 = \frac{q_0 - f_o}{f_o}$

Magnification of the eye-piece $M_e = \frac{q_e + f_e}{f_e}$

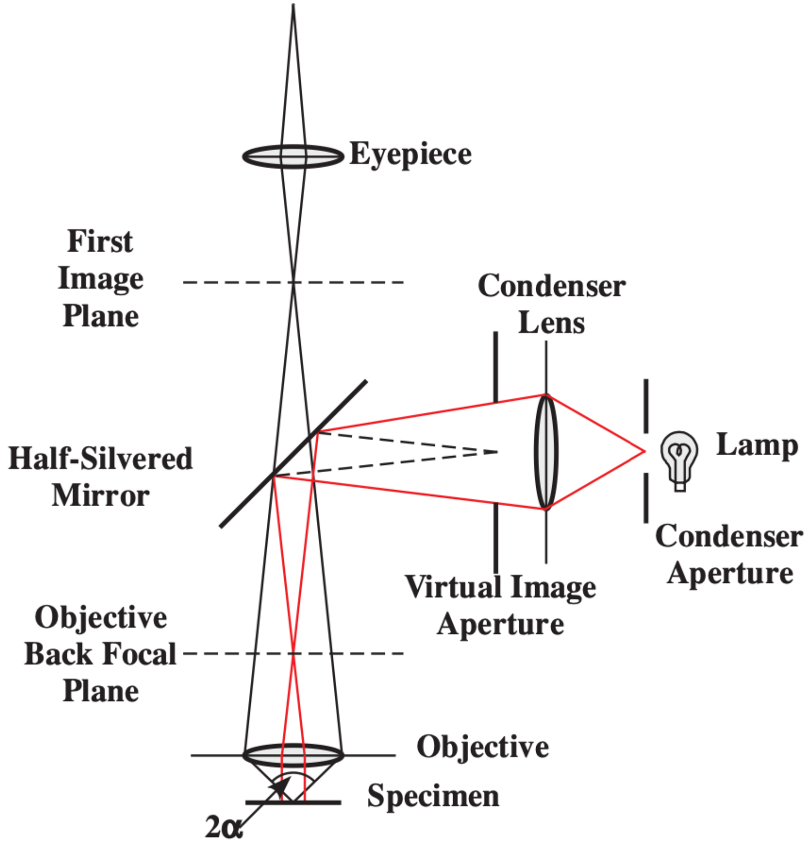
We call the term $q_0 - f_0 = t$ the tube length of the microscope.

Human eye has an number called the distance of distinct vision: D

If we place the eye at the exit of eye piece, $D = f_e + q_e$

And the eye piece magnification can be written as $M_e = \frac{D}{f_e}$

- The total magnification: $M = M_0\, M_e = \frac{tD}{f_o f_e}$

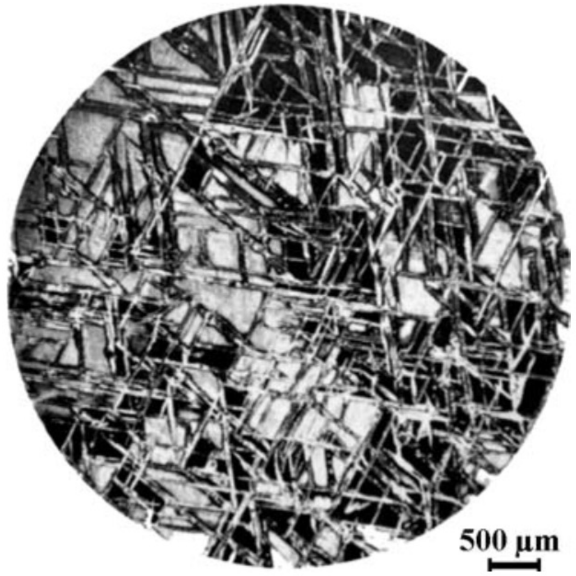


- Thus in-principle, one can achieve extra-ordinarily high magnification just with optical microscope!

- Why then are we seeing only 100X microscopes every where ? Why not higher ? Why do we need even sophisticated instruments ?

Optical microscope

- Primary tool for morphological characterization
  - In biological sciences thin slices of tissues are viewed in transmission mode
  - Contrast enhancements using fluorescent enhancement, dark field , differential interference contrast and phase contrast are employed
  - Metallurgical samples are typically investigated in reflection
  - Contrast are topological, differential absorption, reflection with optical interference



A “Widmanstatten” microstructure in steel. Specimen prepared by Henry Sorby (ca. 1864).

Examples of microscopic images

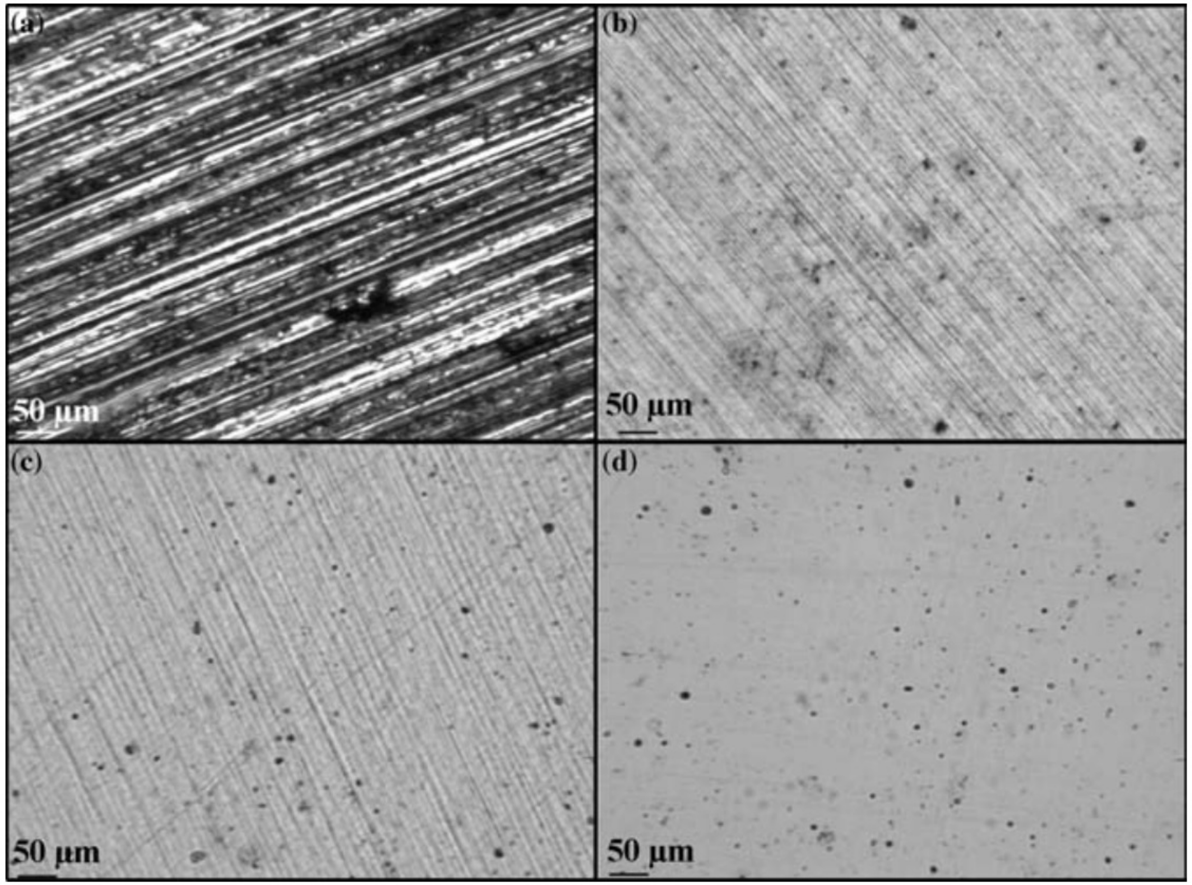


Figure 3.39 *Optical micrographs of 1040 steel after polishing with a sequence of diamond grits: (a) Rough-grinding to achieve a planar surface; (b) after polishing with 6 μm diamond grit; (c) after polishing with 1 μm diamond grit; (d) after polishing with 1/4 μm diamond grit.*

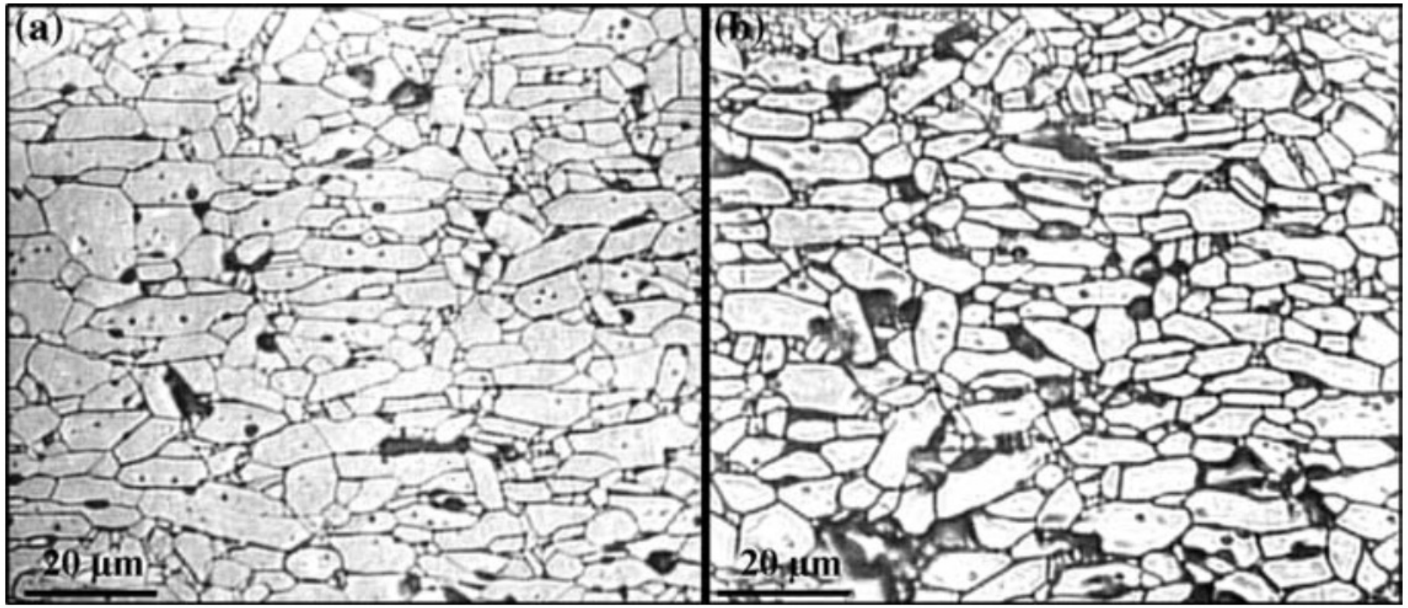
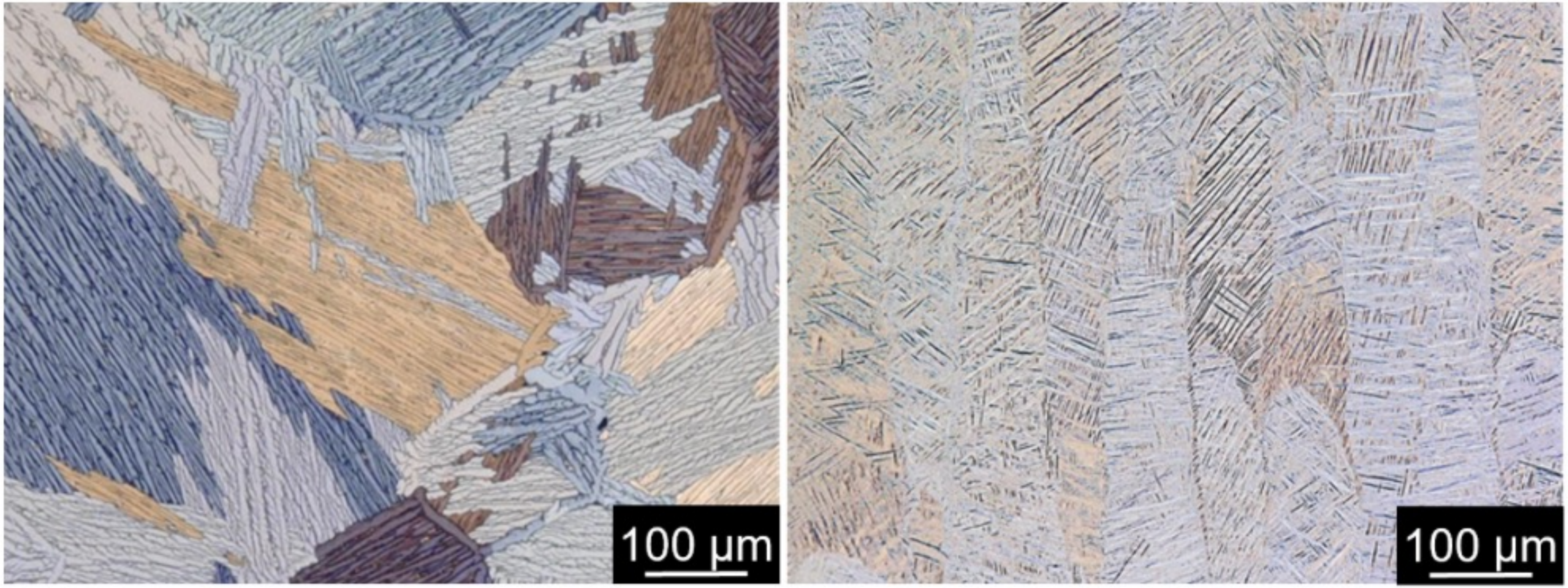


Figure 3.42 *Polished alumina thermally etched at 1200° C for 30 min shows poor contrast and fails to reveal all the boundaries (a). Thermal etching for 2 h at the same temperature clearly reveals all the grain boundaries but with some loss of resolution for the finest grains (b).*

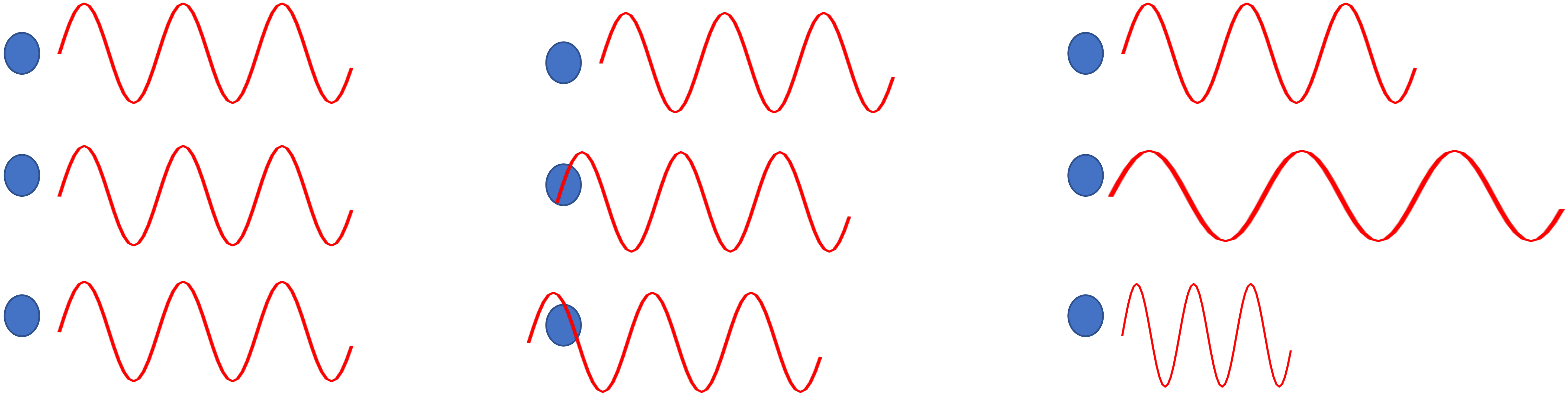


Light microscopic photograph of Ti-6AL4 prepared by KOH etch to show basket weave structure on the left and martensitic microstructure on the right.

What can the microscope can/cannot do ?

Wave properties of light:

- Coherence:
  - A property of the source of light
  - Consider an object: composed of multiple point light sources
  - These point sources can have the following property:

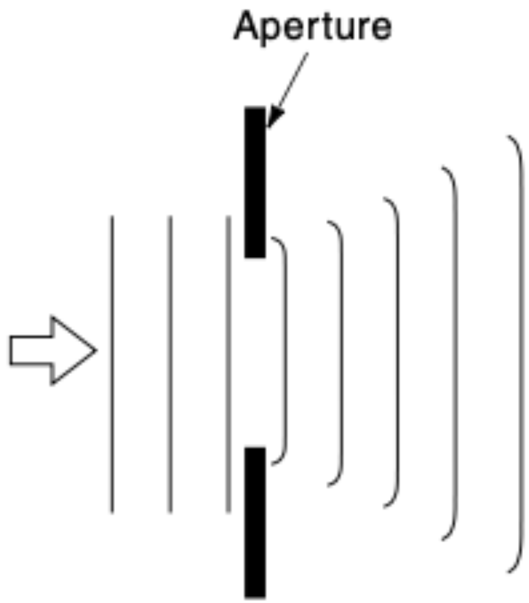


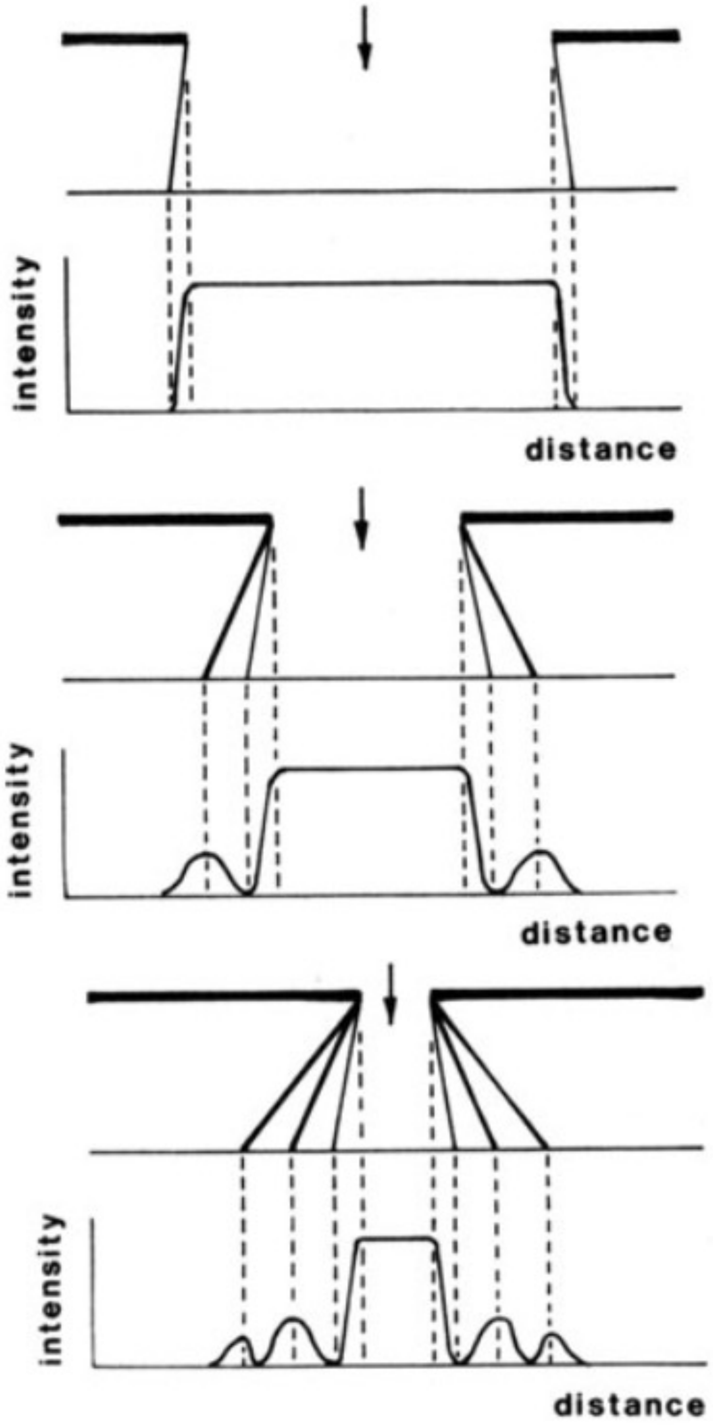
Monochromatic and coherent

Monochromatic and non-coherent

Non-monochromatic

Diffraction





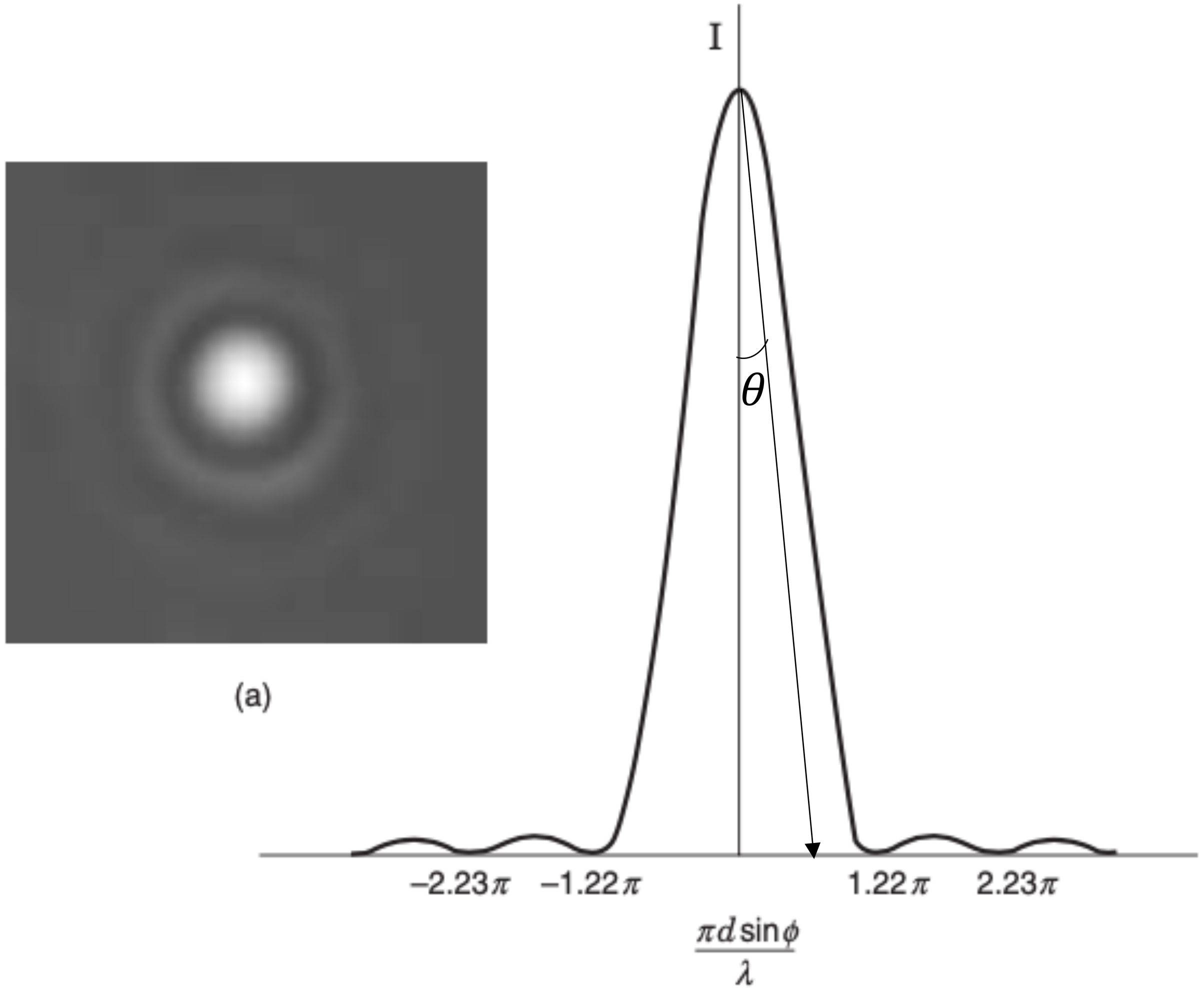
Light, being a wave, hugs an obstruction, much like a water wave.

As the width of the slit decreases, the extent of diffraction , wave bending also increases

This causes, interference between waves reaching to a point from different sources

Diffraction and interference are inevitable properties of waves

Diffraction from a circular slit



The intensity profile is an Airy disk

$$I(\theta) = I_o \left(\frac{2J_1\left(\frac{2\pi a}{\lambda} \sin\theta\right)}{\frac{2\pi a}{\lambda} \sin\theta} \right)^2$$

Where a is the radius of the aperture.

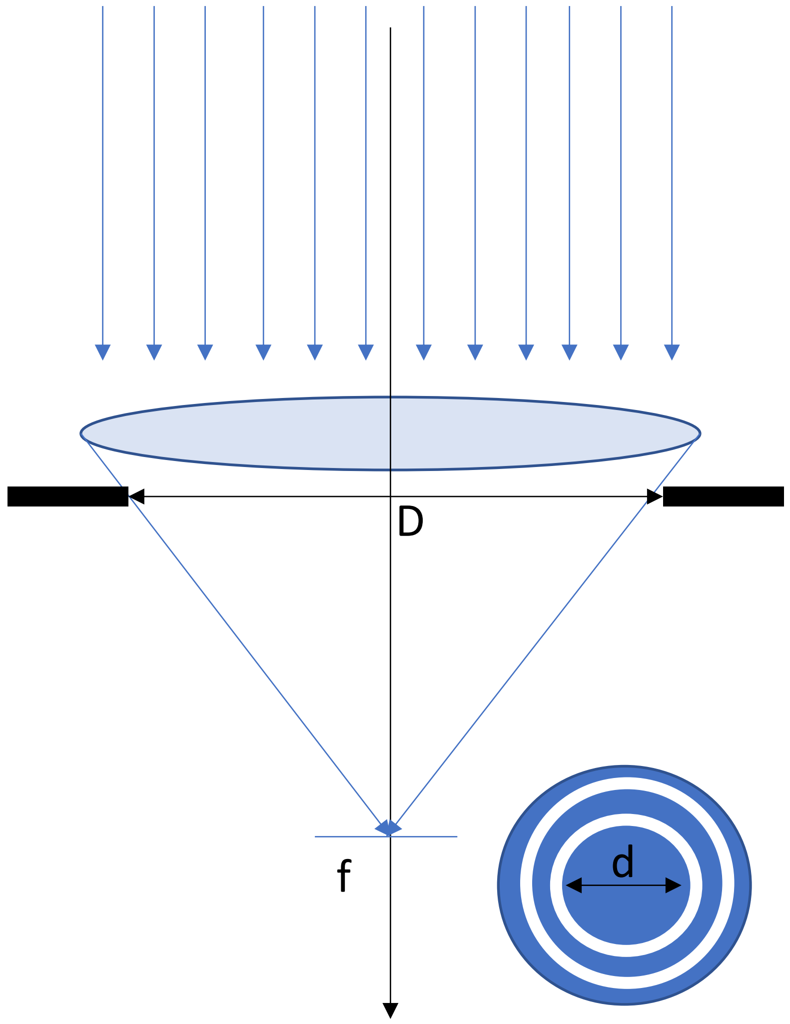
HW: plot the function for different disk openings ‘a’

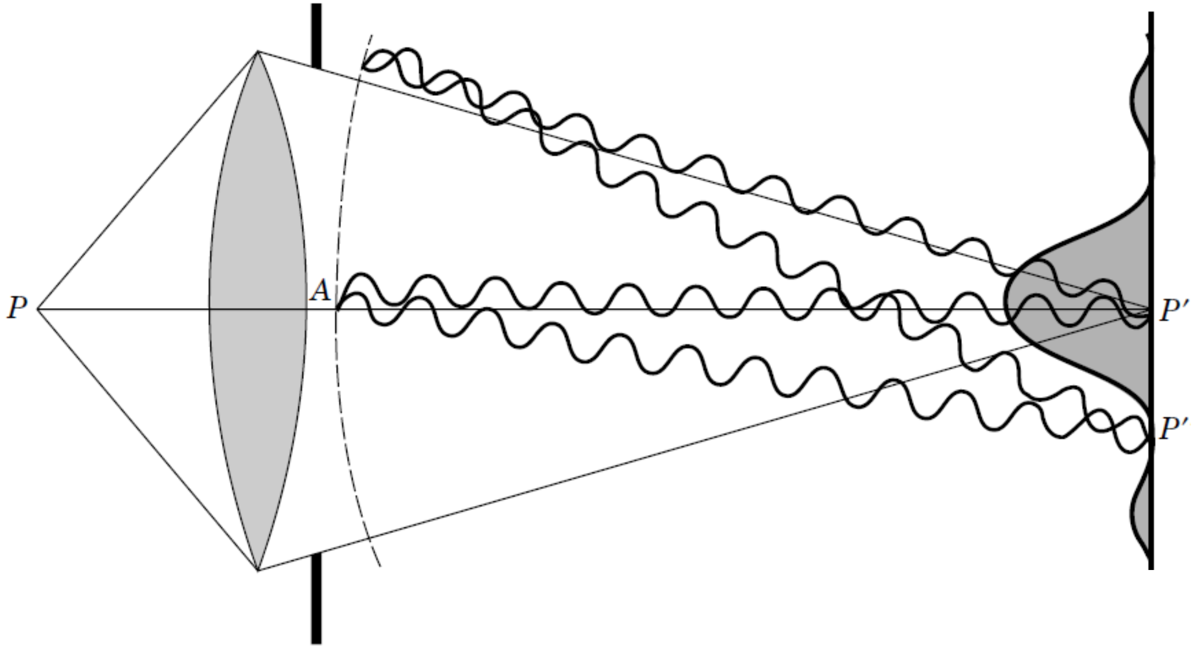
Diffraction in a lens

- Similar to the previous situation, when a collinear collection of rays are incident on a lens, it should focus the rays to the focal point as seen before!
- However, the point is not a point due to the diffraction effects just discussed. The point gets to be a disk! - Particularly an Airy disk of minimum diameter
- The size of the disk $d = 1.22\frac{\lambda f}{D}$

If $\sin\alpha \cong \frac{D}{2f}$

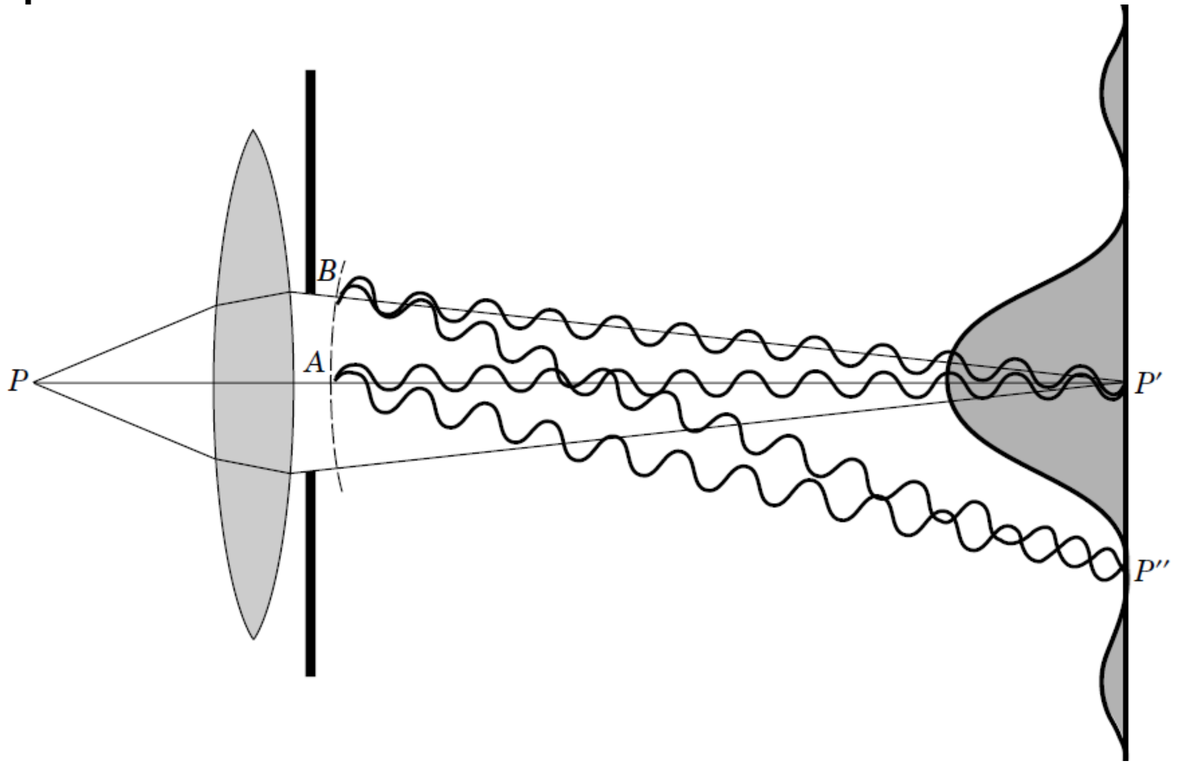
$$d = 0.61\frac{\lambda}{\sin\alpha}$$



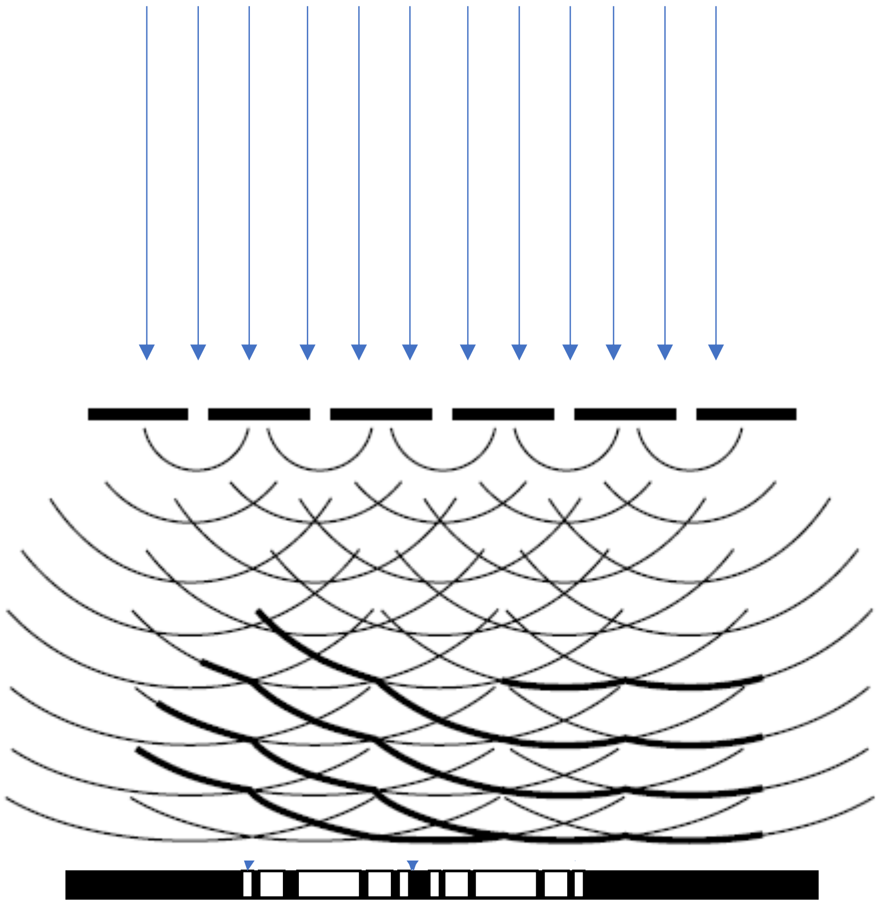


Effect of aperture opening

- As the width of aperture is reduced, number of light waves falling on a single point is reduced.
- The dots are less well defined
- It can also be looked at from the collection angle:
  - Larger the opening, larger the collection of incident radiation by the lens.

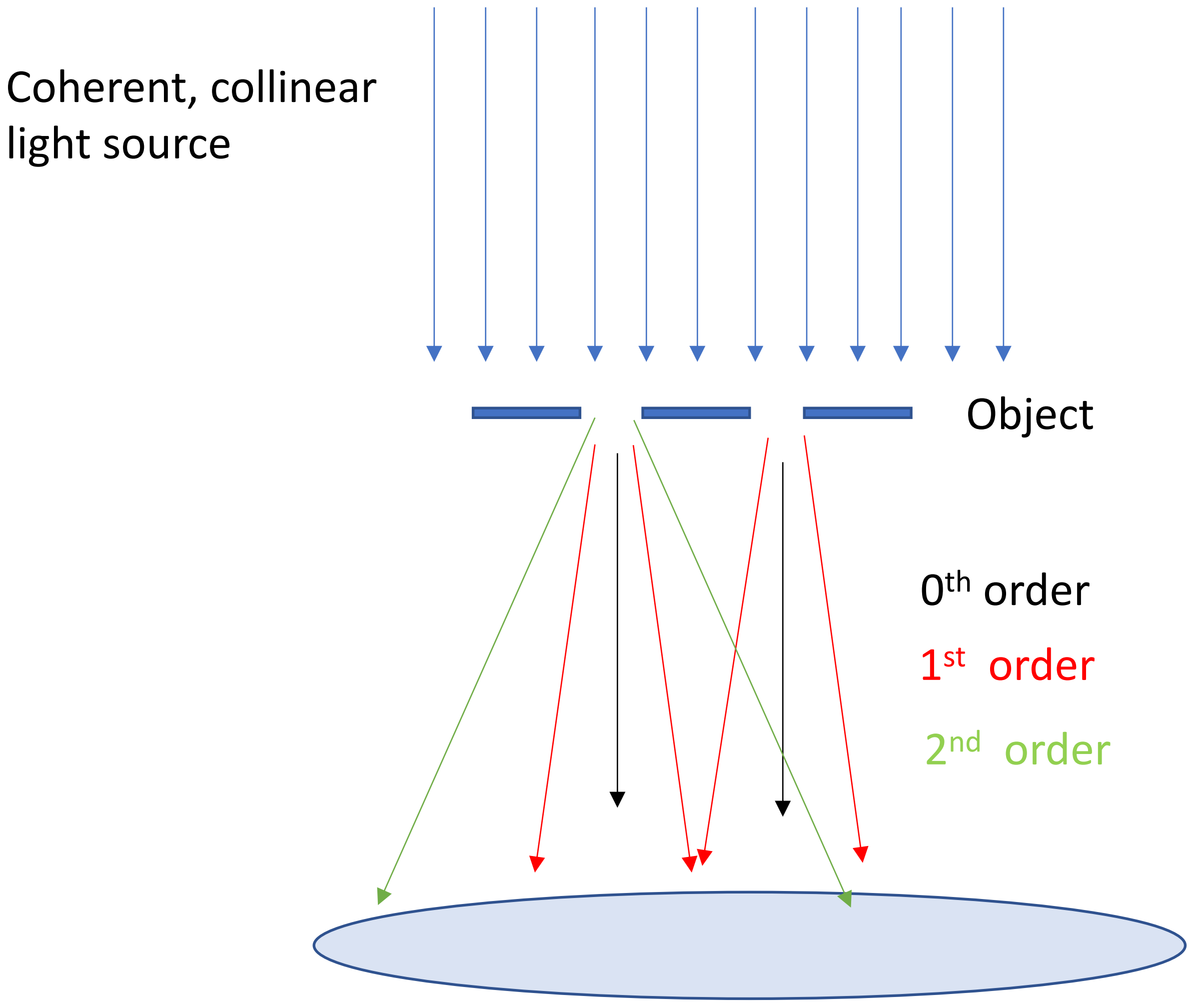


Diffraction from closely spaced particles



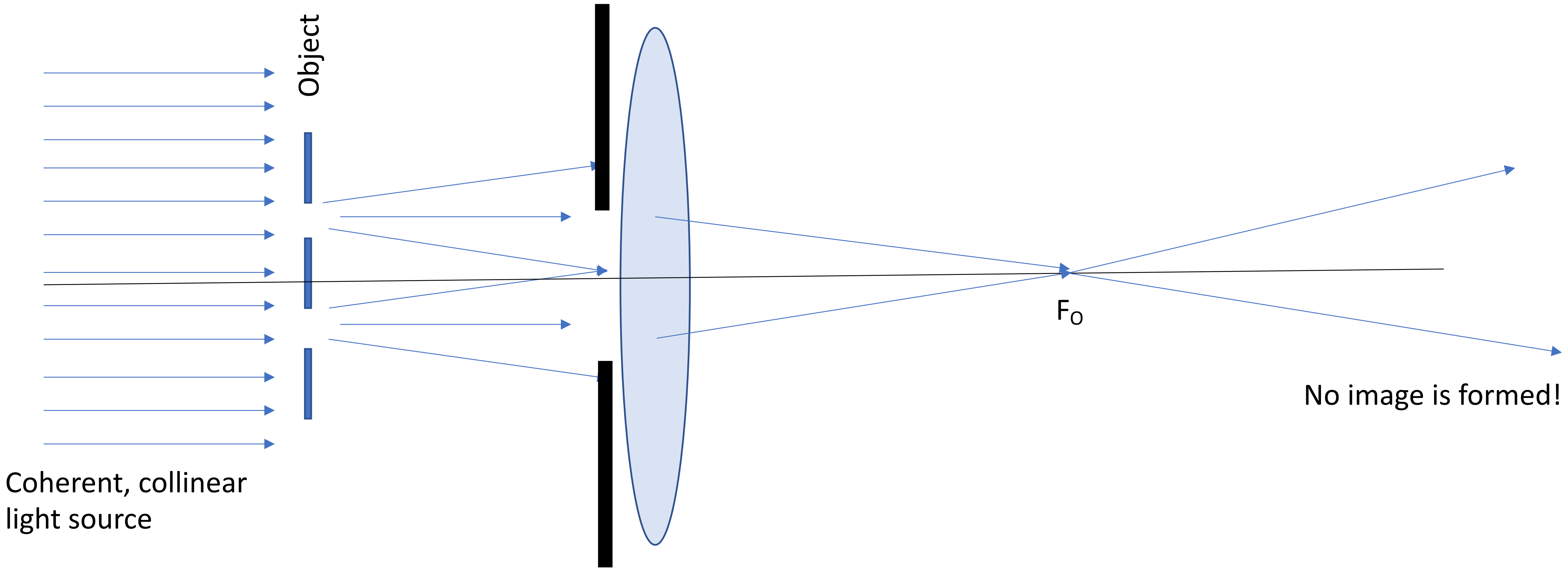
The famous double slit diffraction experiments

Photo screen



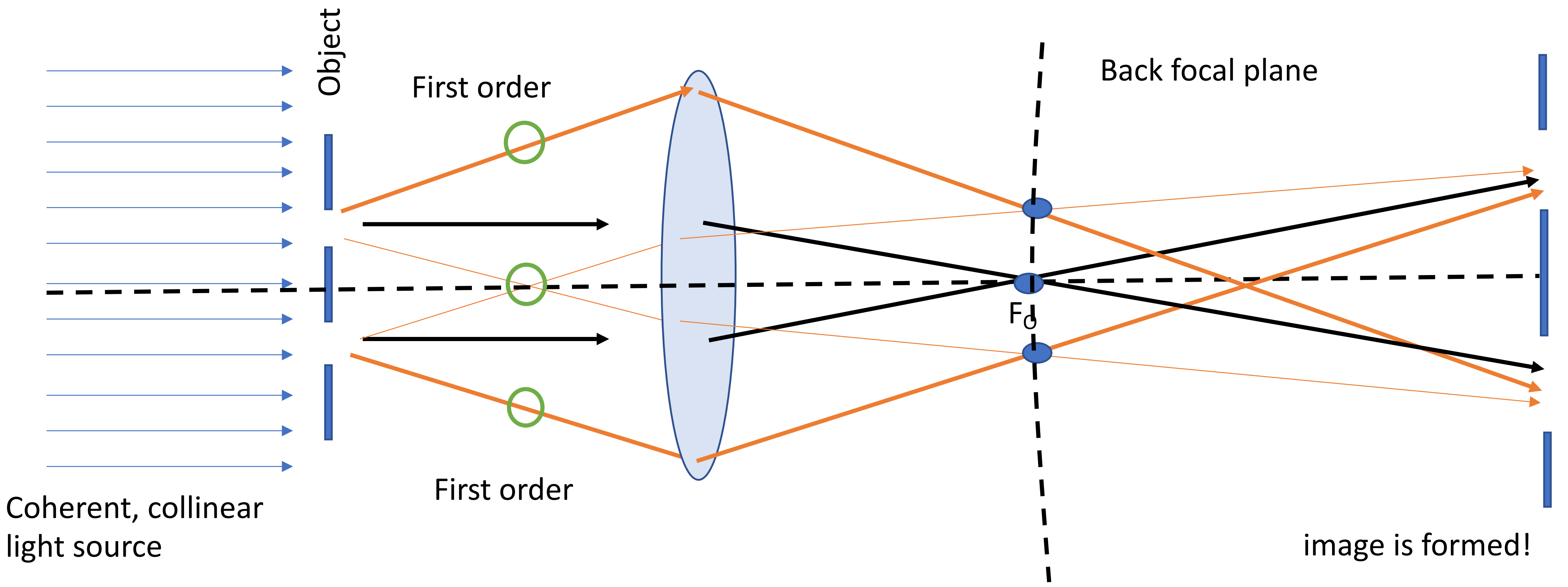
What happens when we converge such diffracted beams ?

Suppose your objective lens is small/ you have a small aperture



So, if we only collect the undiffracted (not deflected) light rays, No image is formed!

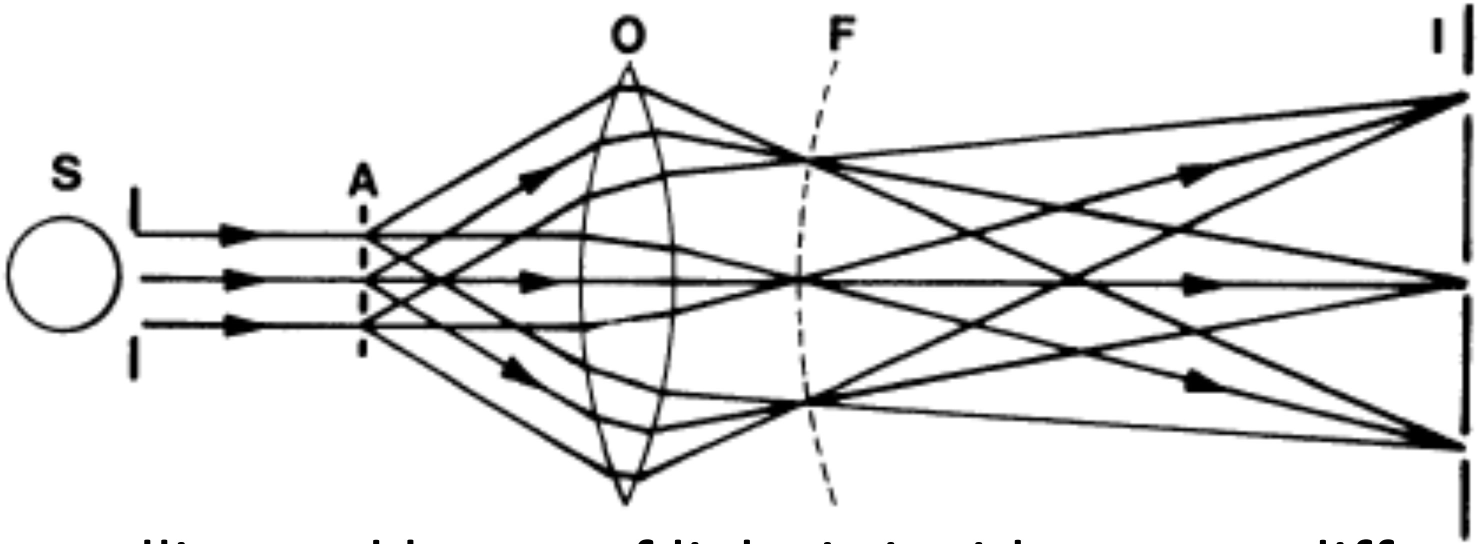
Suppose your objective lens is small/ you have a small aperture



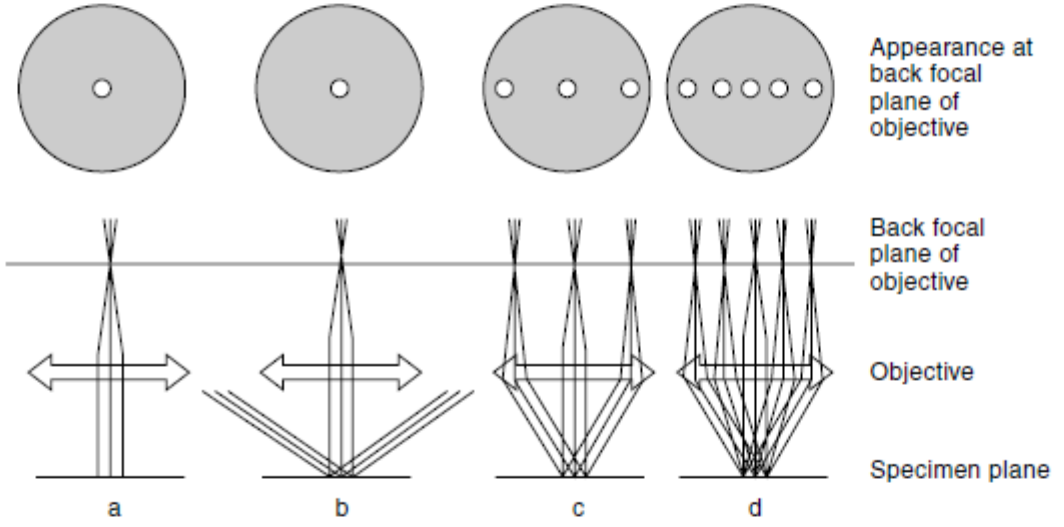
So, if we only collect the undiffracted (not deflected) light rays, No image is formed!

An image of the object is formed when atleast two diffracted beams are collected!

Properties of Image Formation



- When a collimated beam of light is incident on a diffracting object:
  - N-orders of diffracted collimated beams are formed.
  - 0^{th} order – non-diffracted light called as bright field. Collection of the 0^{th} order will form no image.
  - An 1^{st} order diffracted beams are all parallel
    - Parallel beams get focused to a point – focal plane
    - 1^{st} order beams differ by exactly 1 wave-length, hence are in phase
  - Angular separation between the diffracted beams decrease with the order
  - Angular separation between the beams increase as the object separation decreases



- The 1st order diffracted beams are all parallel
  - Parallel beams get focused to a point – focal plane
  - 1st order beams differ by exactly 1 wave-length, hence are in phase
  - They interact constructively giving corresponding bright spots in the focal plane
  - The dark areas behind the lens (focal plane) are regions of destructive interference.

If d is the gap spacing in the object, the spacing in the focal plane: $D \cong \frac{1}{d}$

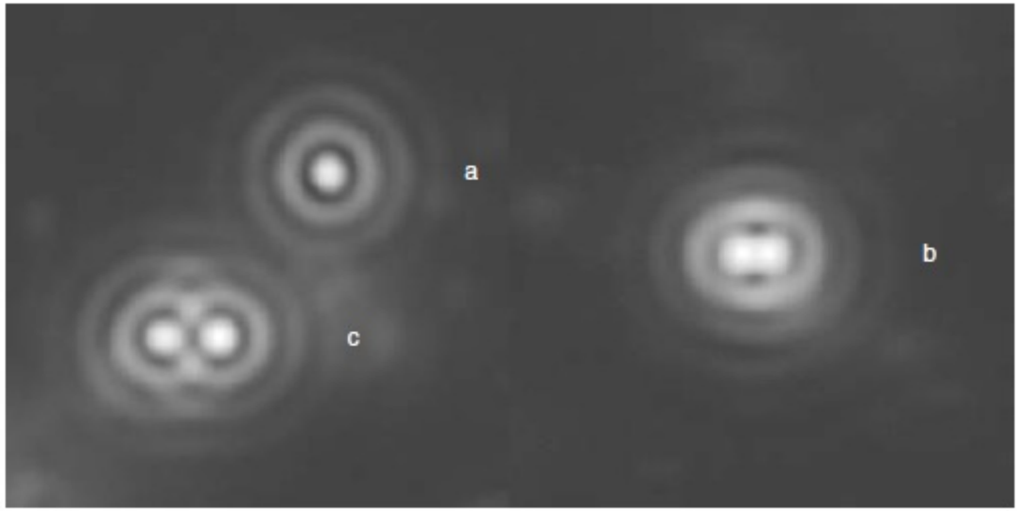
Abbe’s criteria

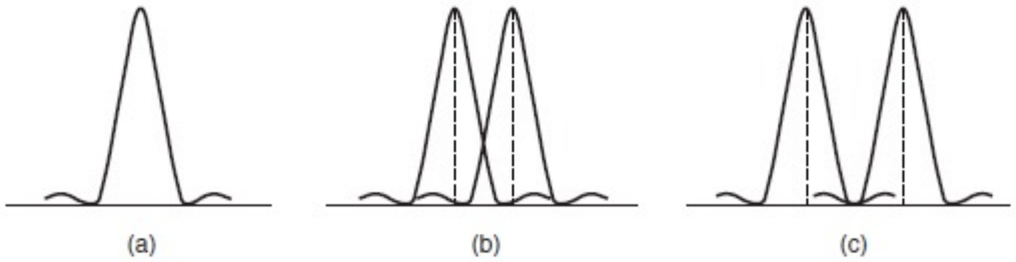
- Bright field (0^{th} order diffracted beam cannot form a distinct image)
- Collection of at least the first order forms a defined image
- Higher the number of diffracted beams captured, better is the resolution.
- Fortunately, the intensity of diffracted beams fall quickly with order, so first few orders are the most prominent
- However, smaller the particle size, larger the angle of diffraction
  - This means, even the first order diffracted line will be farther for smaller particles

If an object doesn’t diffract light/ the objective lens does not collect diffracted light, no image is formed.

Spatial Resolution of a microscope

- A point in the image plane (due to converging light beams of λ) will have a minimum diameter $0.61\frac{\lambda}{NA}$
- Two bright points in the image plane is considered to be resolved, when the peak of the second coincides with the first minima of the first source.





Remember, the limit is not about resolving the smallest particle!

It is about identifying two close lying particles are two separate particles.

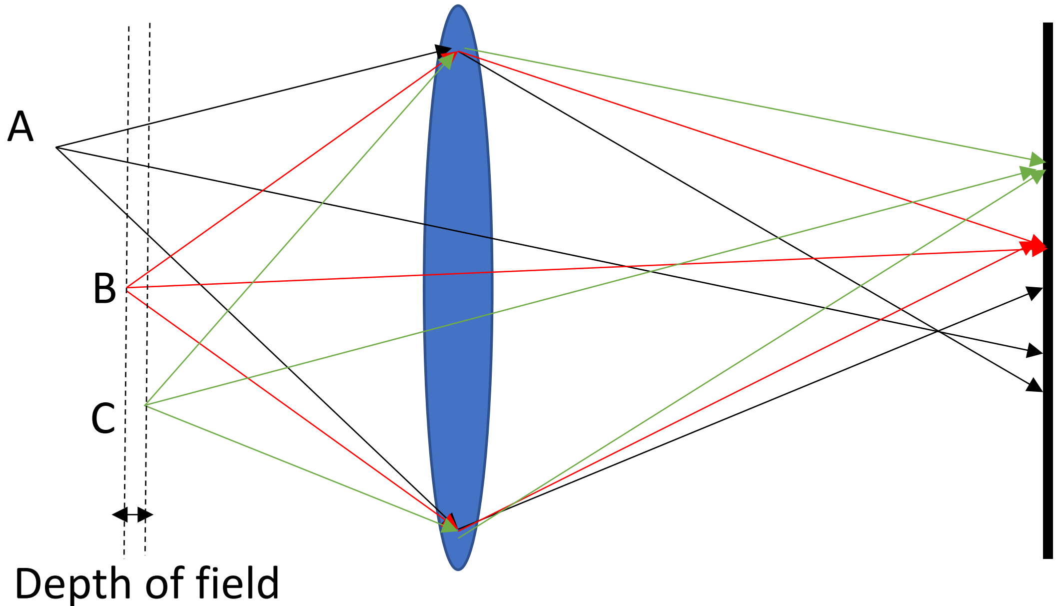
Controlling the spatial resolution.

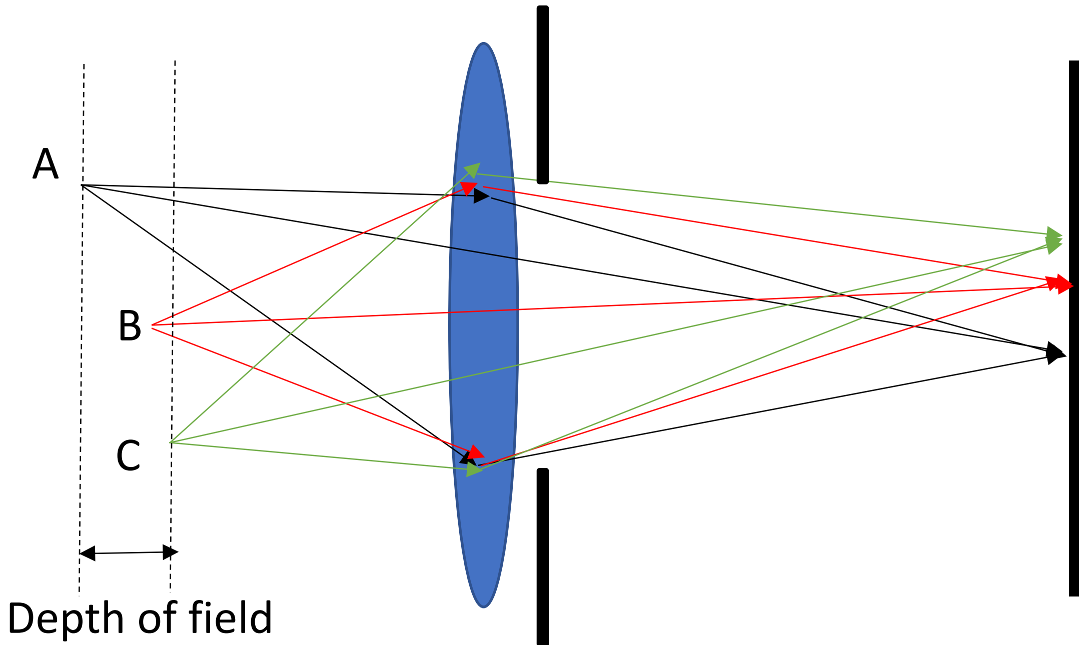
- Spatial resolution is limited by $d \cong 0.61 \frac{\lambda}{NA}$
  - Smaller resolution is obtained by increasing NA or reducing λ
  - Typically wavelength of illumination is not largely modified because
    - A blue and lower, the optical components start absorbing
    - UV light sources are typically more expensive
    - Human eye has very low sensitivity to blue (green is the most sensitive to eye)
  - NA can be increased by
    - Choosing a higher refractive index medium (Oil immersed lenses)
    - Increasing the acceptance angle (larger lenses)

Depth of Field

- This is the thickness of the optical section along the Z-axis within which the specimen are in focus

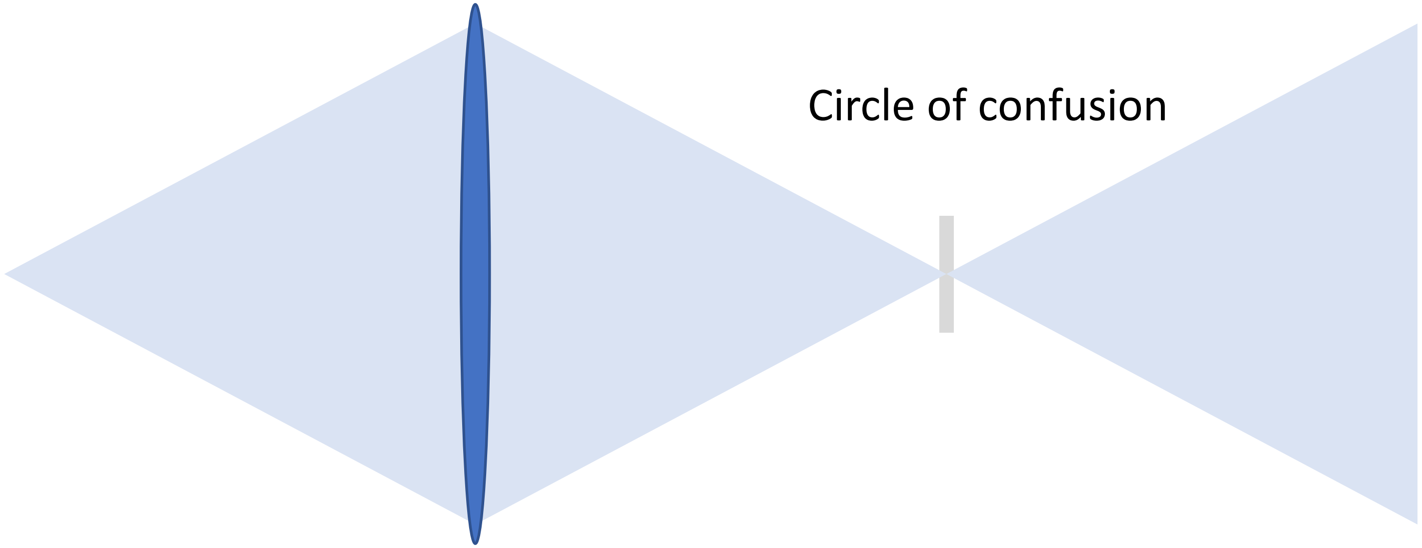
$$Z = \frac{n\lambda}{NA^2}$$



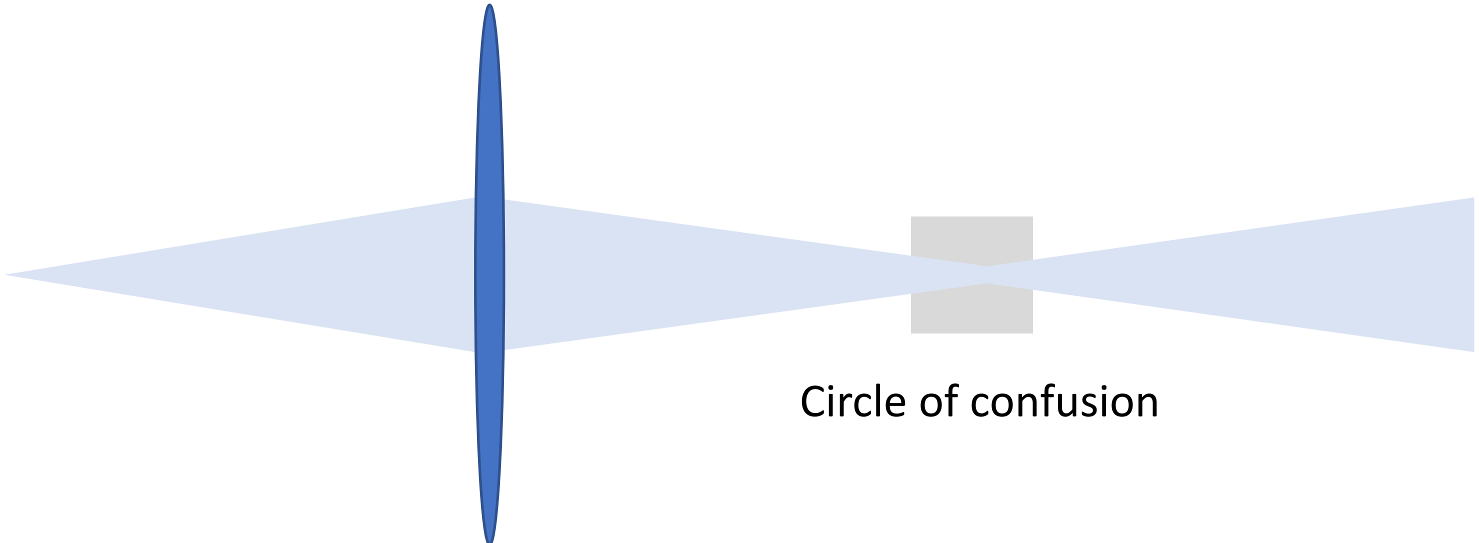


Depth of focus

Smaller apertures and stronger lens (smaller f) causes larger depth of focus



Larger aperture has steeper angles, the region in which the image will appear to be focused will be small



Smaller aperture openings leads to a larger circle of confusion, region in which the image will appear to be in focus

Resolution vs Depth of Field Trade-off

Maximum spatial resolution is obtained by having the largest NA (collection inside the lens).

This has the largest aperture opening.

However,

Largest NA, also degrades (1) depth of field. Only a very small region (thickness) is imaged.
(2) Has poorer contrast.

Lack of contrast in large NA objectives is due to two fold reasons:

1. Large NA also collects stray light
2. Large NA, has interference with light with large varying coherence. (Smaller NA, smaller ray bunches, better coherence)