

Introduction to Light microscopes

Characterization Technique 1

All of Maxwell's equation can be narrowed down to

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

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

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

Hence the Maxwell's equation will come to

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

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

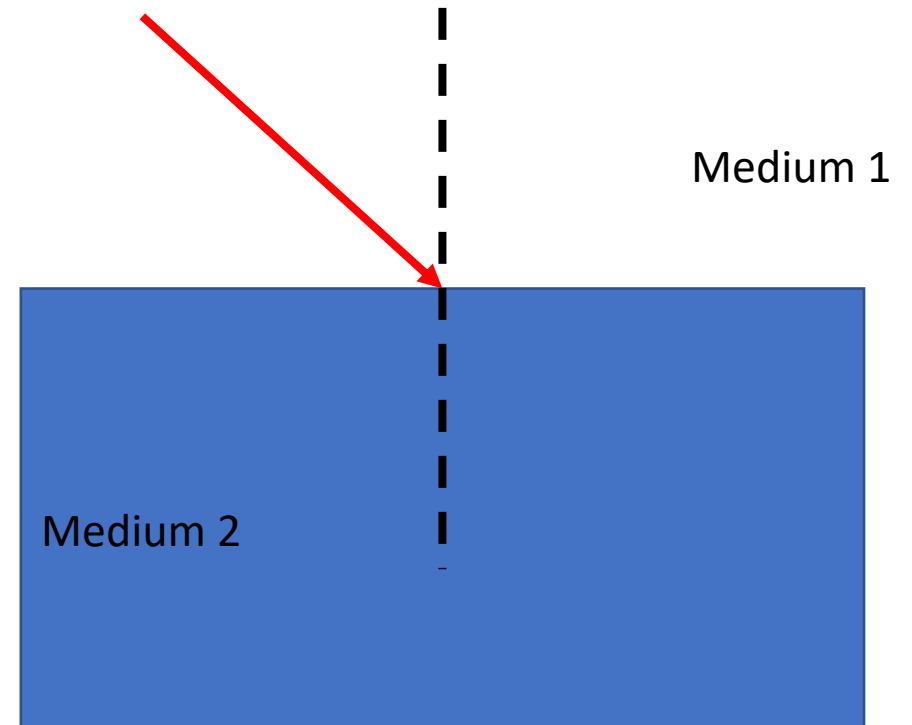
$$v = \frac{1}{\sqrt{\epsilon_m \mu_m}}$$

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

This gives the velocity of light in free space to be $C = 2.99792458 \times 10^8 \frac{\text{m}}{\text{s}}$

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 10⁴.

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

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

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 $E = E_0 e^{i(kx - \omega t)}$

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

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

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

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

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

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

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

$\tilde{n}$ 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.

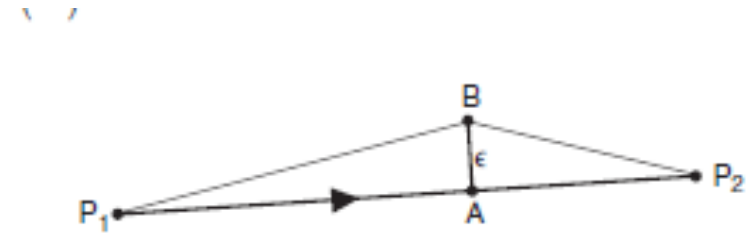
n and κ are related by Kramer-Kronig relation

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

Light propagation and Reflection

- Rectilinear propagation

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

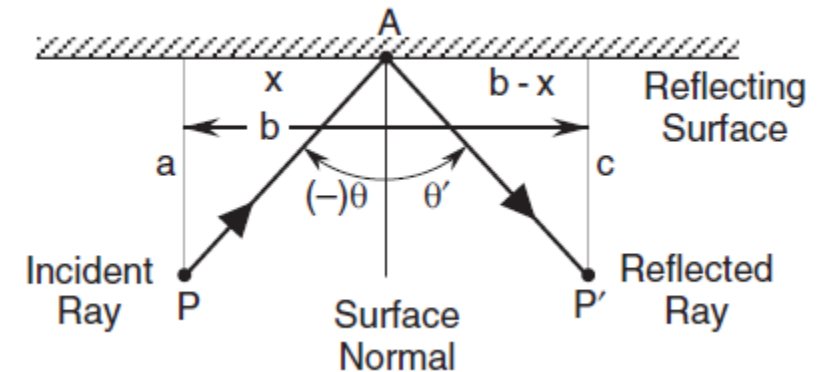


- Reflection:

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 $v_2 n_2 = v_1 n_1 = C$

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

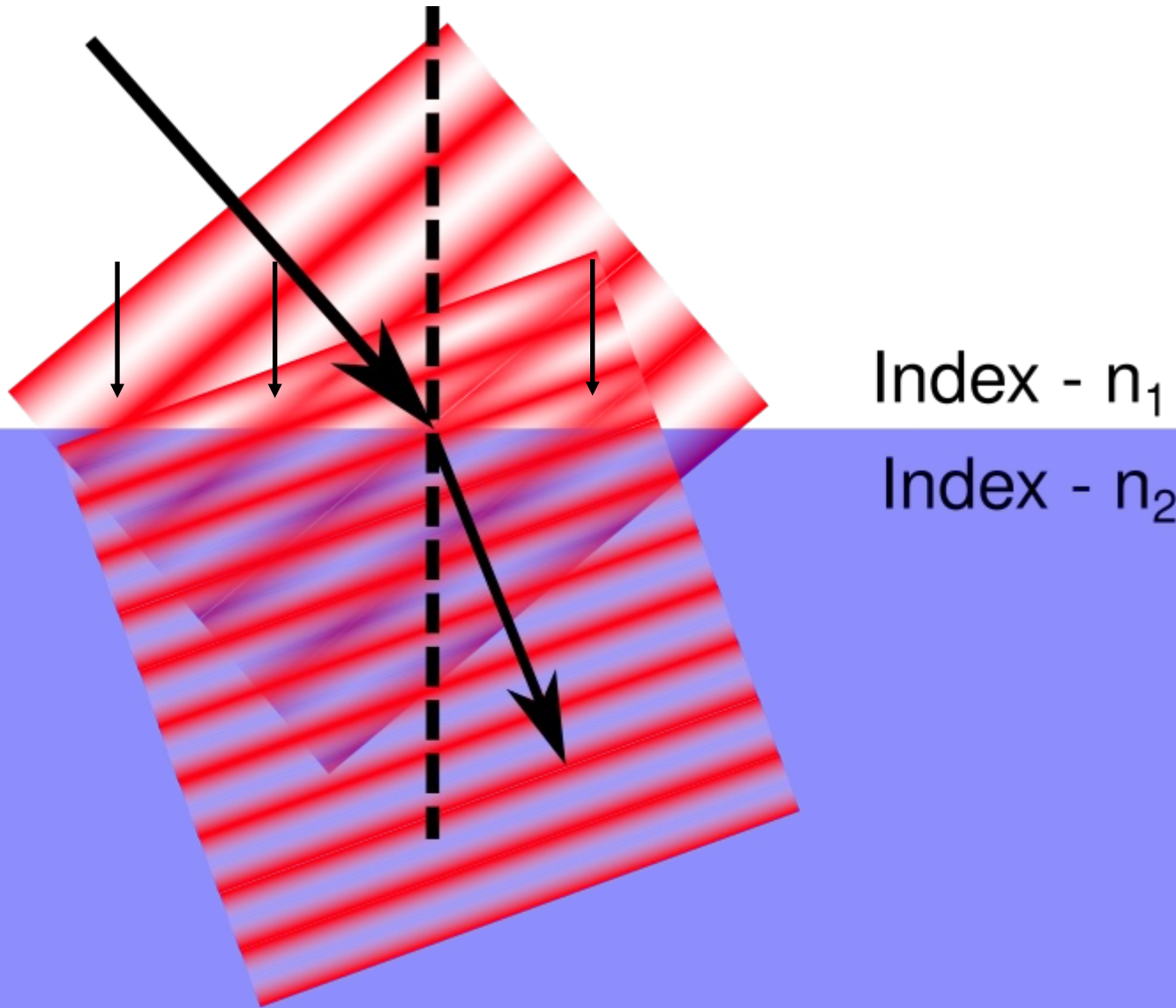
The process is elastic (No loss of energy)

$$\text{So } E_1 = h\nu_1 = E_2 = h\nu_2$$

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

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

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

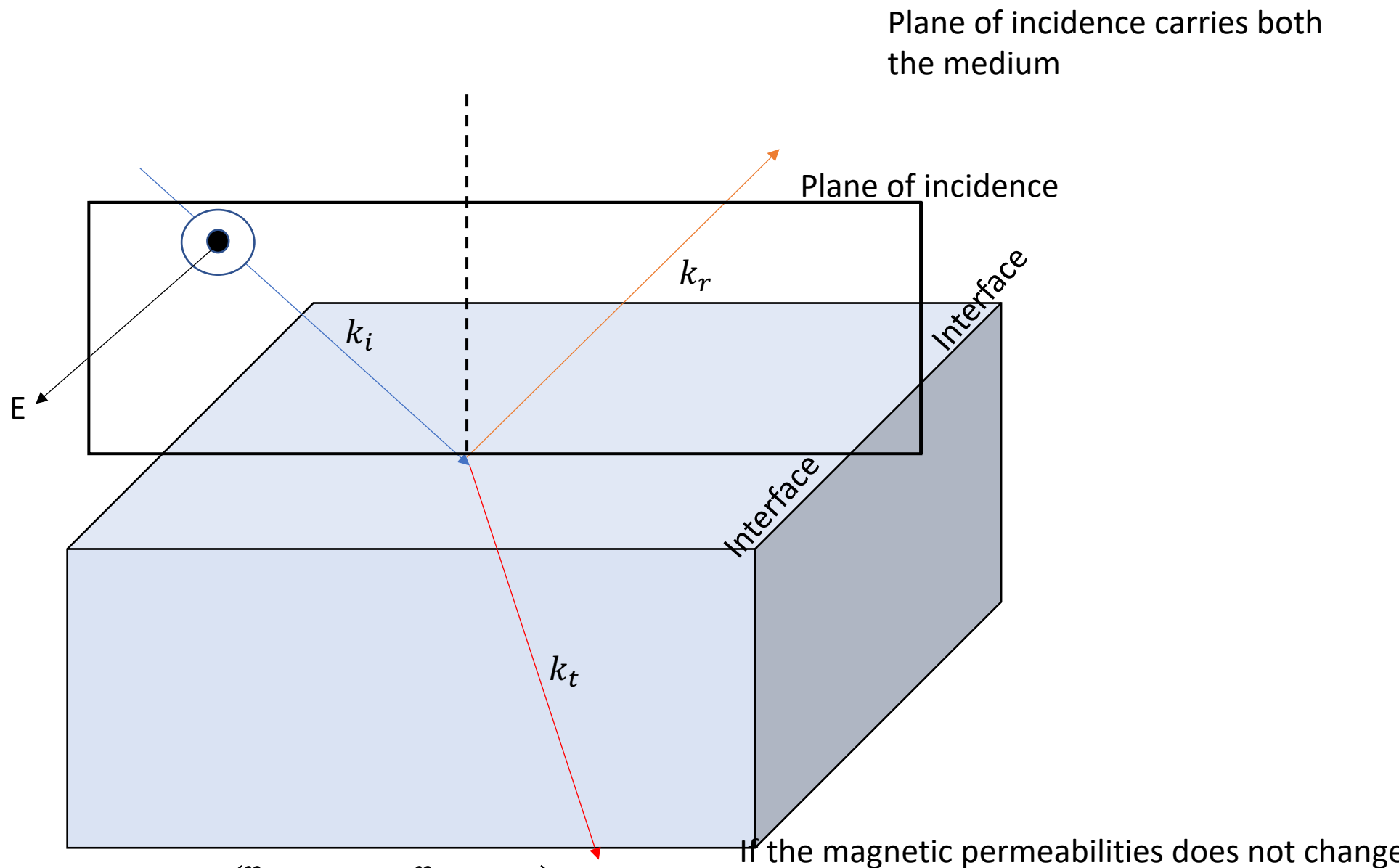


Intensities

Assume incident radiation is

$$E_i = E_{0i} \cos \vec{k}_i \cdot \vec{r} - \omega_i t$$

Electric field normal to the plane of incidence



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

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

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

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

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

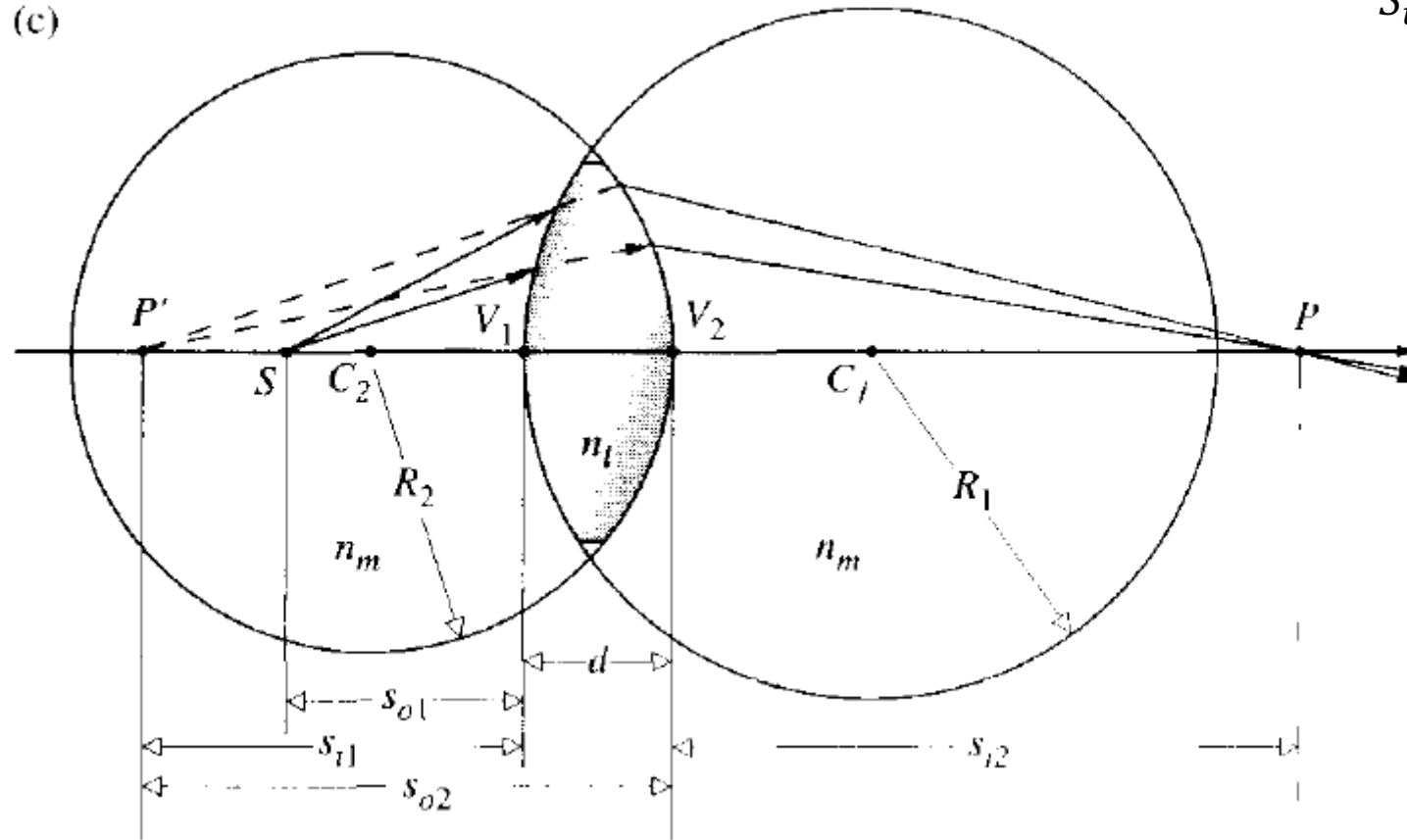
When you have incidence with Electric field parallel to plane of incidence

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

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

Case for a thin lens

$$\begin{aligned} d &\rightarrow 0 \\ S_{o1} &\rightarrow S \\ S_{t2} &\rightarrow S' \end{aligned}$$



Governing equation:

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

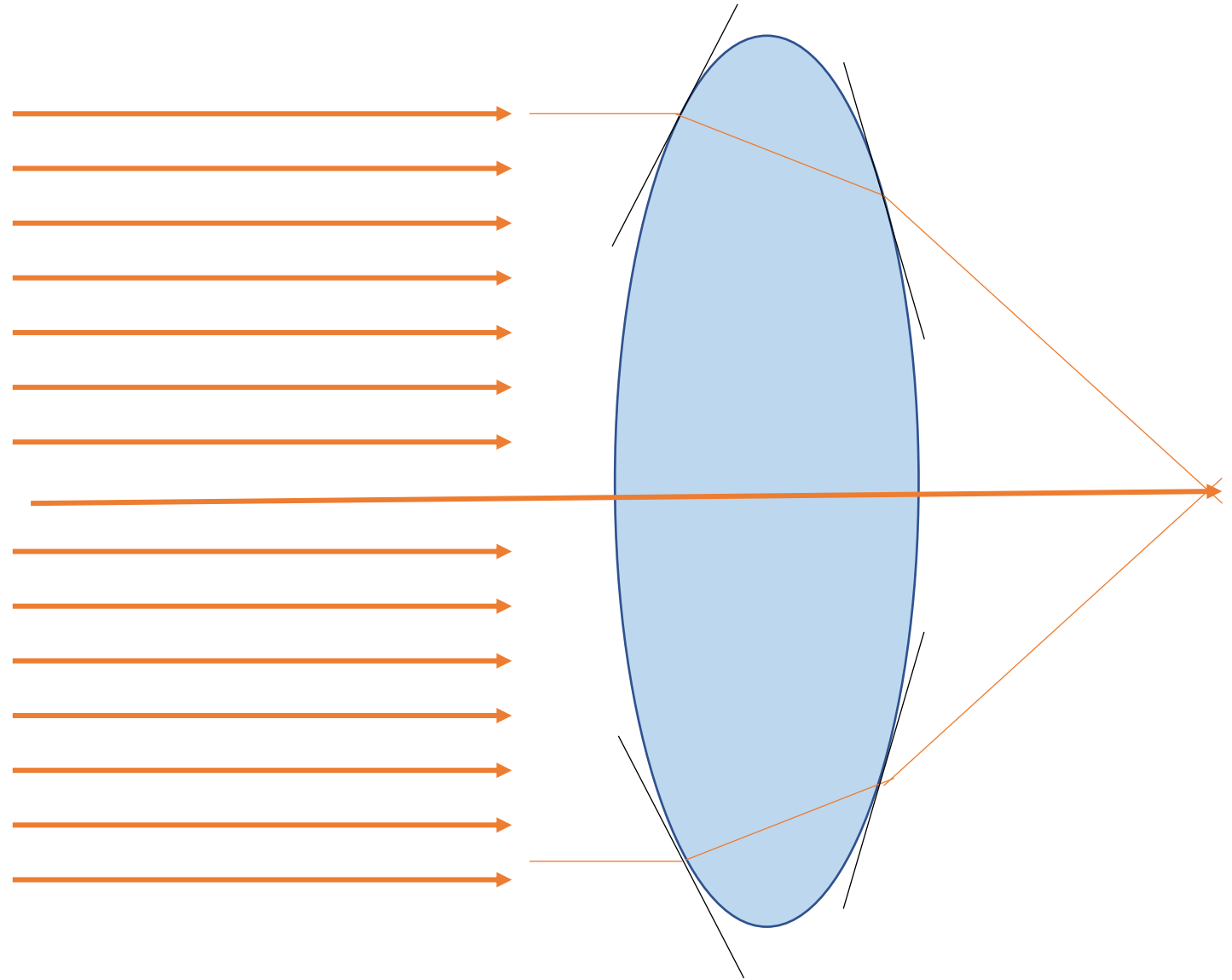
We call $\frac{1}{f} = \left[\frac{1}{R_1} - \frac{1}{R_2} \right] (n_l - 1)$

As focal length

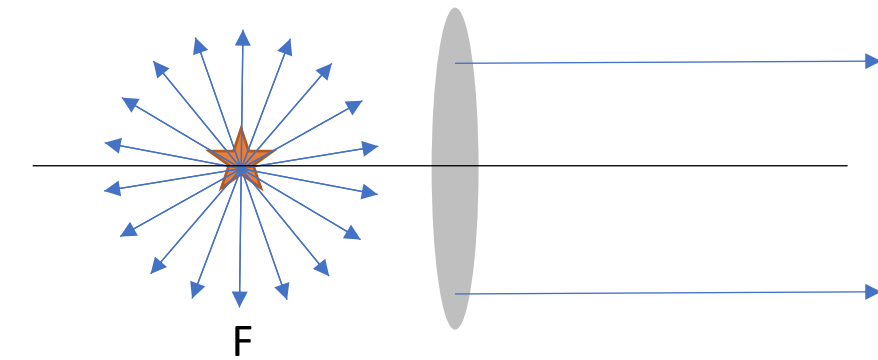
Gives Thin-lens equation/ Lensmaker's equation $\frac{1}{S'} + \frac{1}{S} = \frac{1}{f}$

Light in our grasp

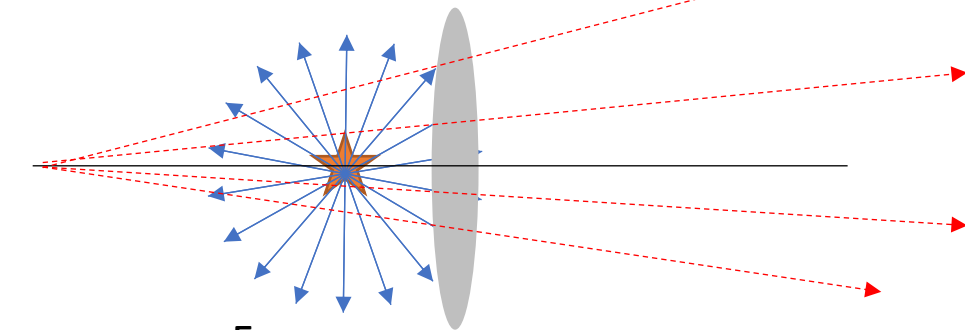
- Assume a parallel beam of light incident on a convex lens
- At each interface, the light bends following Snell's Law
- A point of intersection of all the beams can be expected.
- The point is characteristic of the lens and is called the focal point of the lens



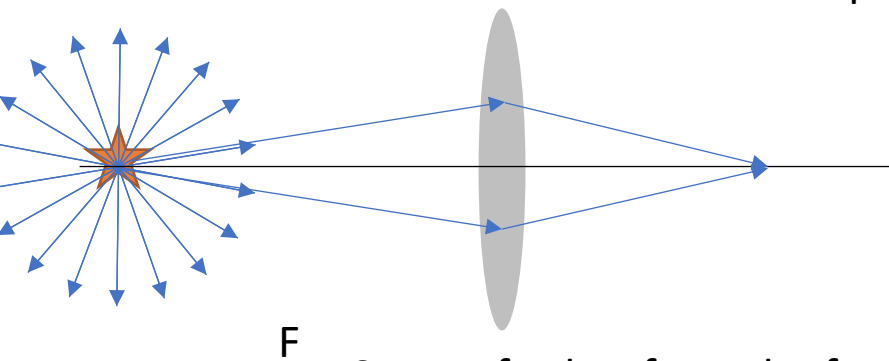
Lets us look objects away from optic axis



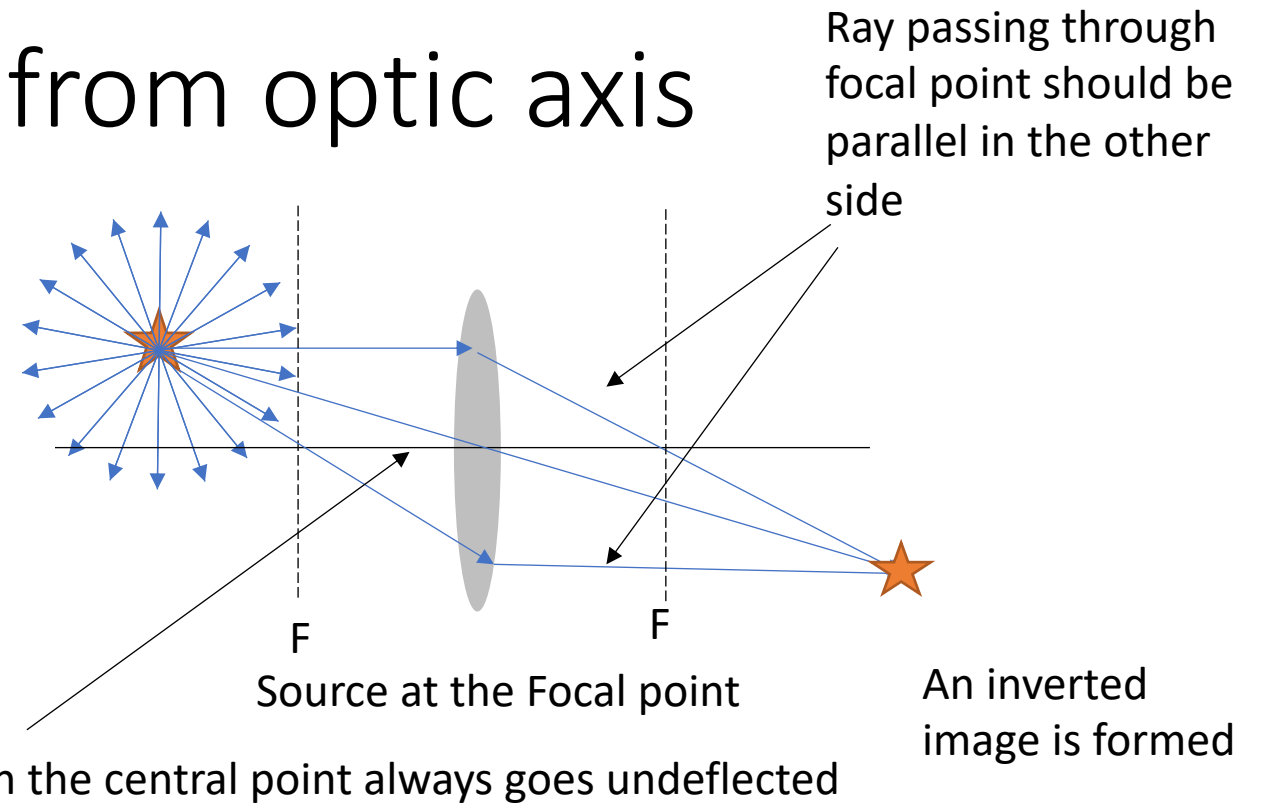
Source at the Focal point



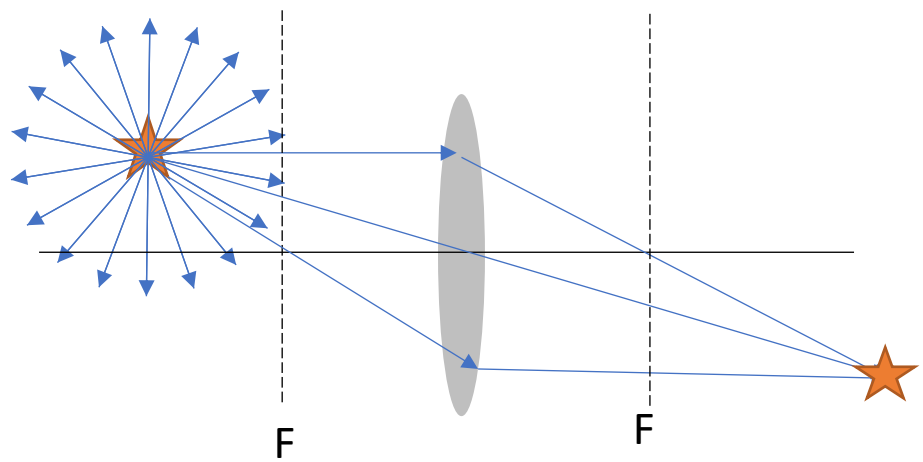
Source closer than the focal point



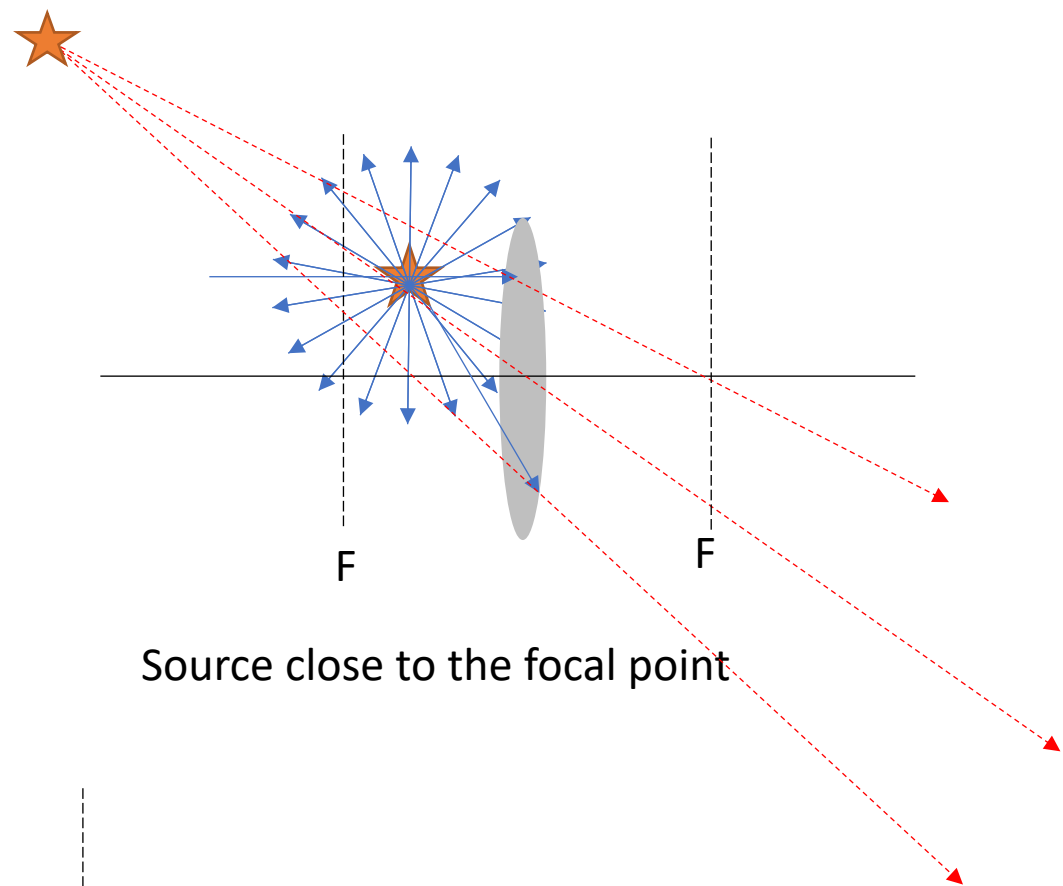
Source farther from the focal point



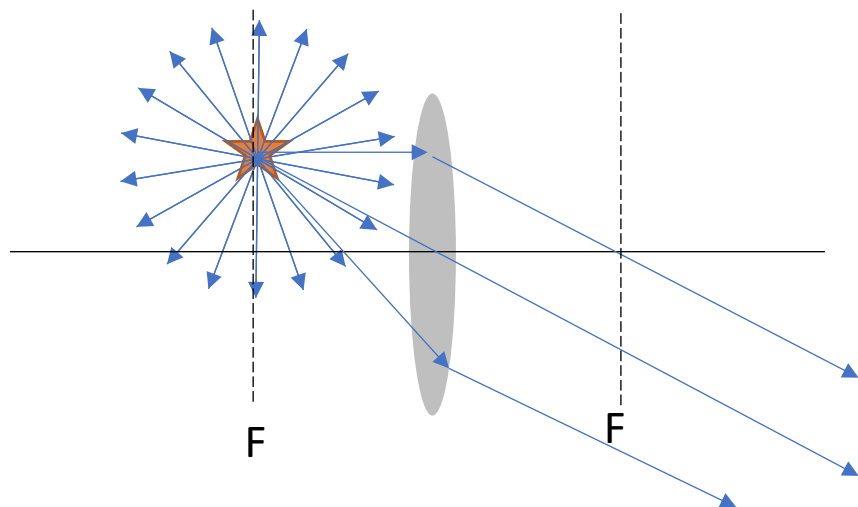
Remember, the point is called as image as at this point, the light rays again diverge seeming like a source



Source farther from the focal point



Source close to the focal point

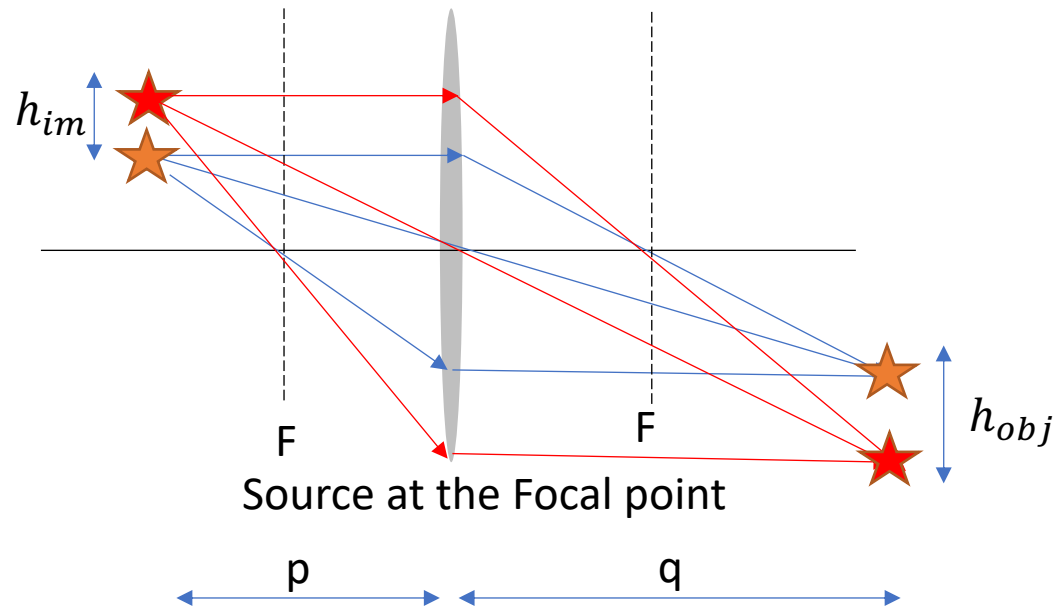


Source farther from the focal point

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

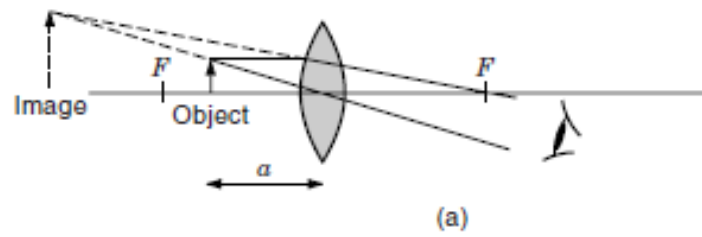
$$|m| = \frac{h_{img}}{h_{obj}}$$

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

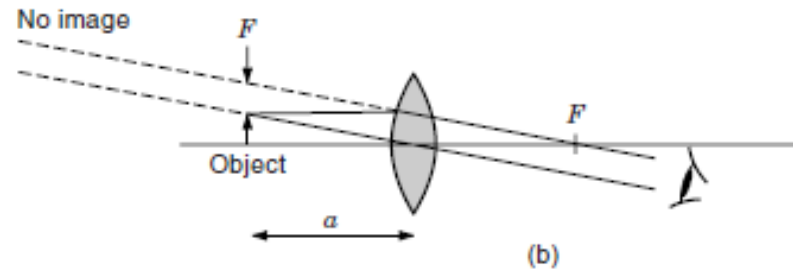
$$m = \frac{q}{p}$$

- 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 coincidence of at least 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.

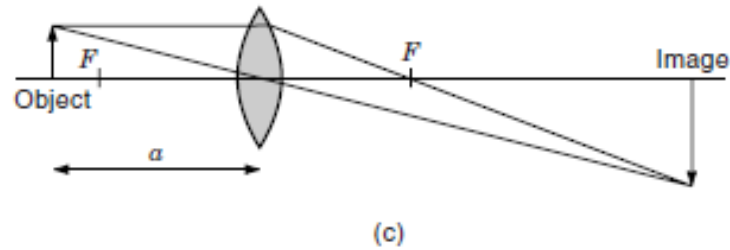
Summary



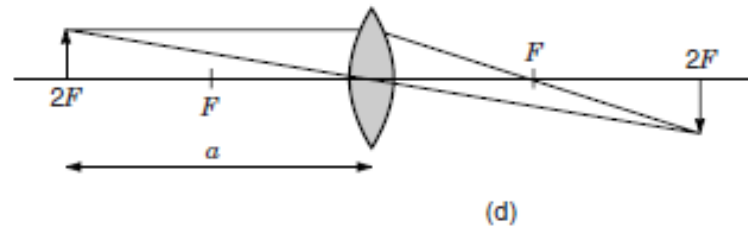
$a < f$
Virtual
magnified
image



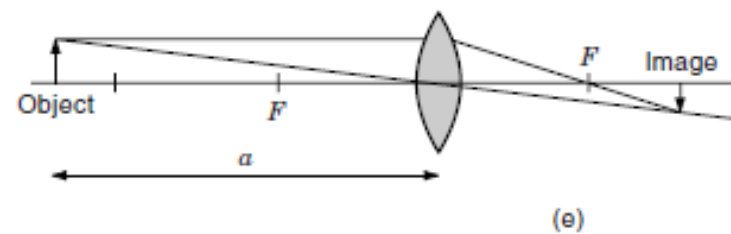
$a = f$
Image
distance ∞



$2f > a > f$
Real magnified
image



$a = 2f$
Real,
magnified = 1x



$a > 2f$
Real,
demagnified

Table of reference:

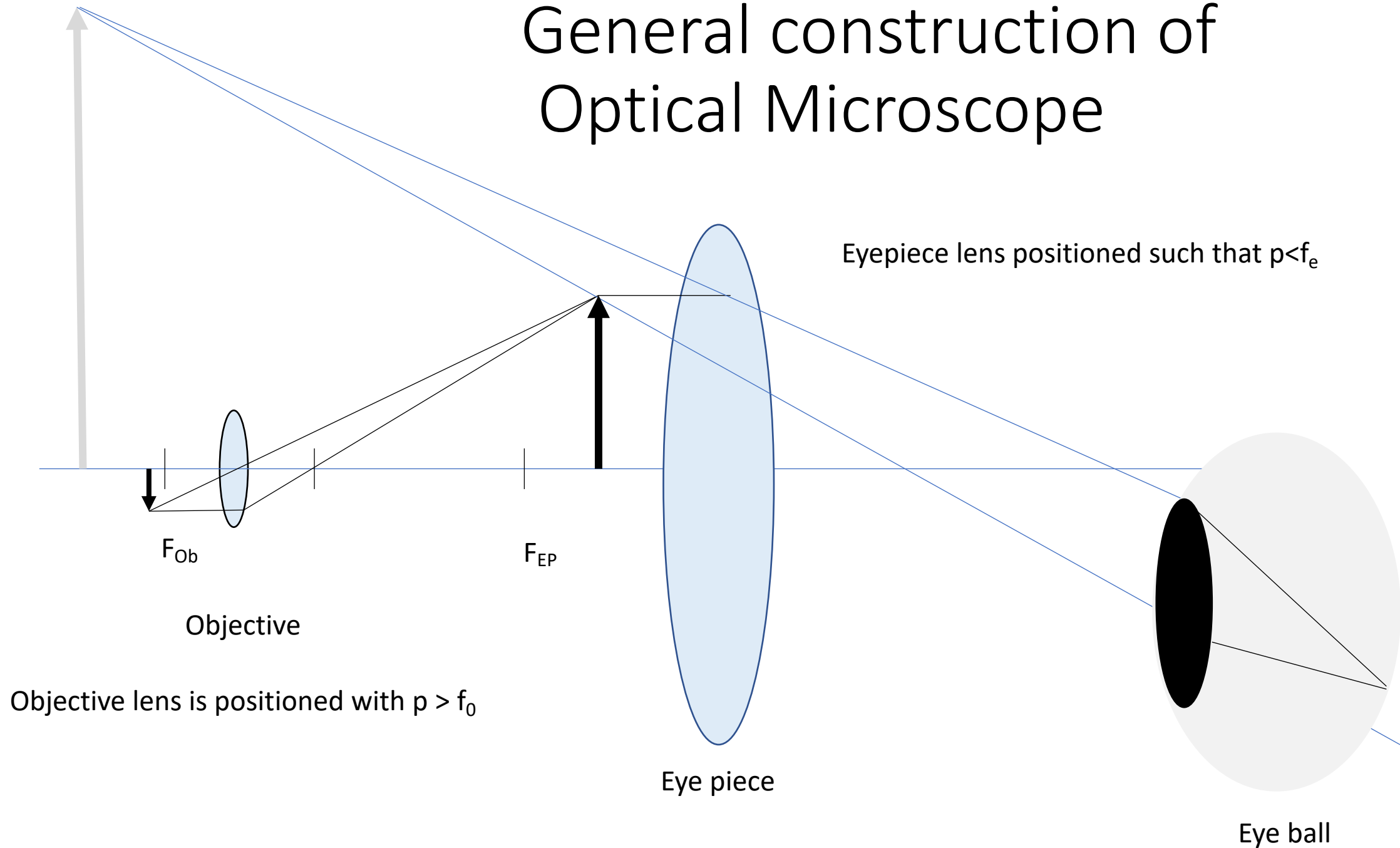
Object	Type	Location	Orientation	Relative size
$\infty > p > 2f$	Real	$f < q < 2f$	Inverted	Minified
$P = 2f$	Real	$Q = 2f$	Inverted	Same size
$f < p > 2f$	Real	$\infty > q > 2f$	Inverted	Magnified
$p = f$		$\pm\infty$		
$P < f$	Virtual	$q > p$	Erect	Magnified
Anywhere	Virtual	$q < f, p > q$	Erect	Minified

For virtual image

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

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

General construction of Optical Microscope



Magnification in a microscope

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

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

We call the term $q_o - f_o = t$ the tube length of the microscope.

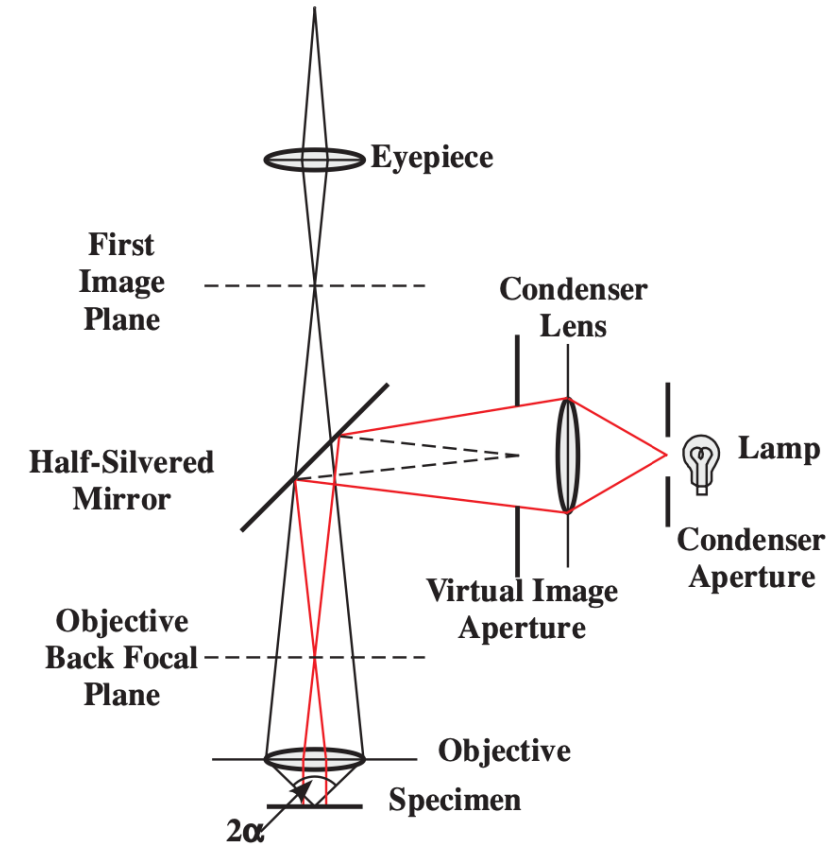
Human eye has an number called the distance of distinct vision: D

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: $M = M_o M_e = \frac{tD}{f_o f_e}$

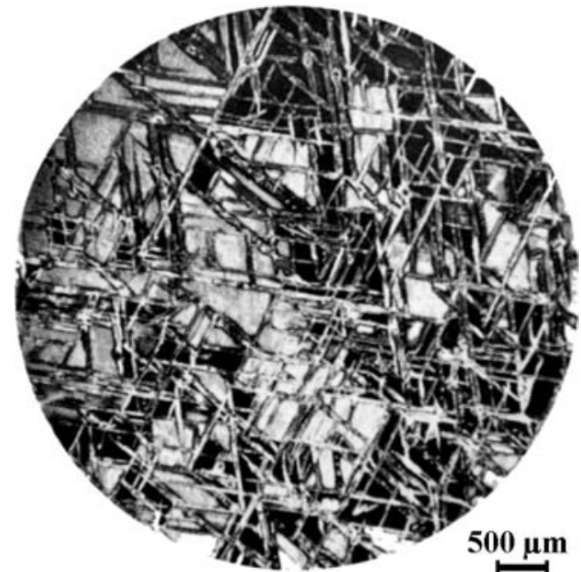
- Thus in-principle, one can achieve extra-ordinarily high magnification just with optical microscope!
- Why then are we seeing only 100X microscopes every where ? Why not higher ? Why do we need even sophisticated instruments ?



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

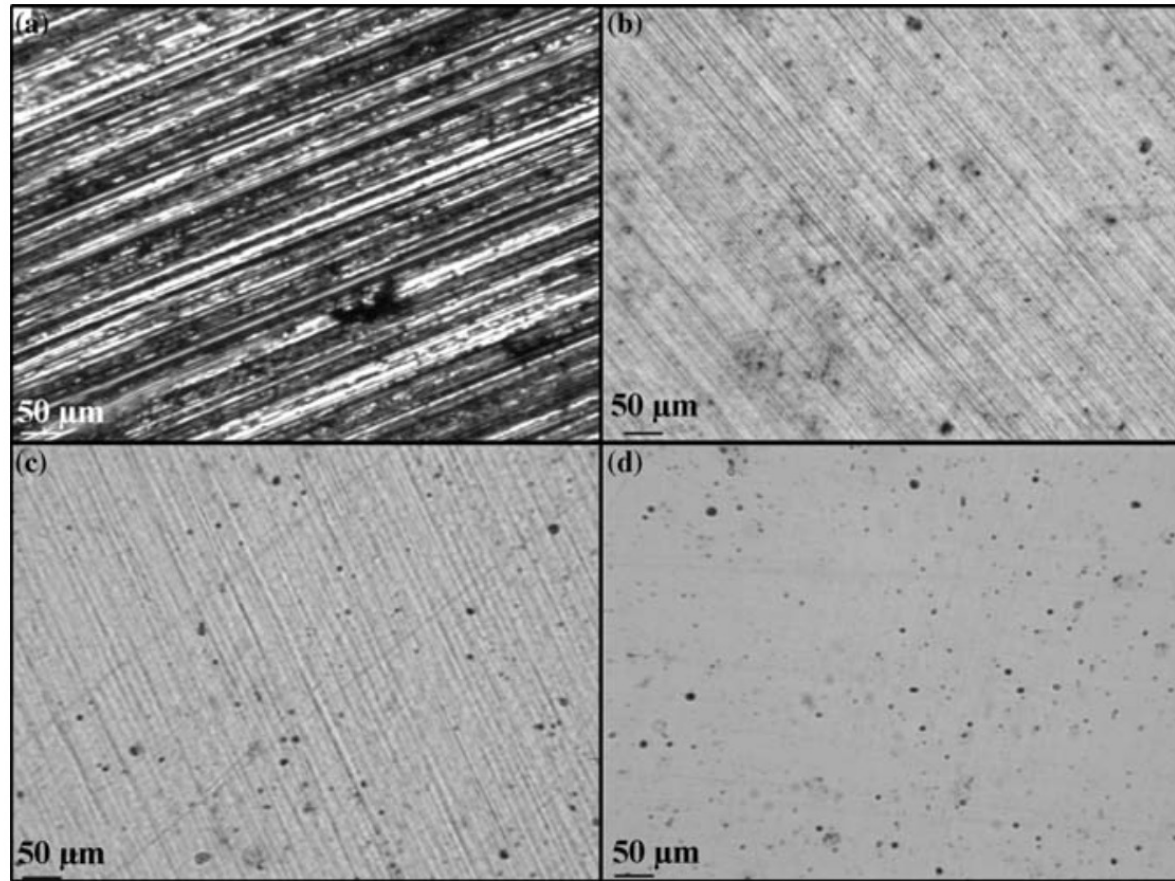


Figure 3.39 Optical micrographs of 1040 steel after polishing with a sequence of diamond grits: (a) Rough-grinding to achieve a planar surface; (b) after polishing with $6\text{ }\mu\text{m}$ diamond grit; (c) after polishing with $1\text{ }\mu\text{m}$ diamond grit; (d) after polishing with $1/4\text{ }\mu\text{m}$ diamond grit.

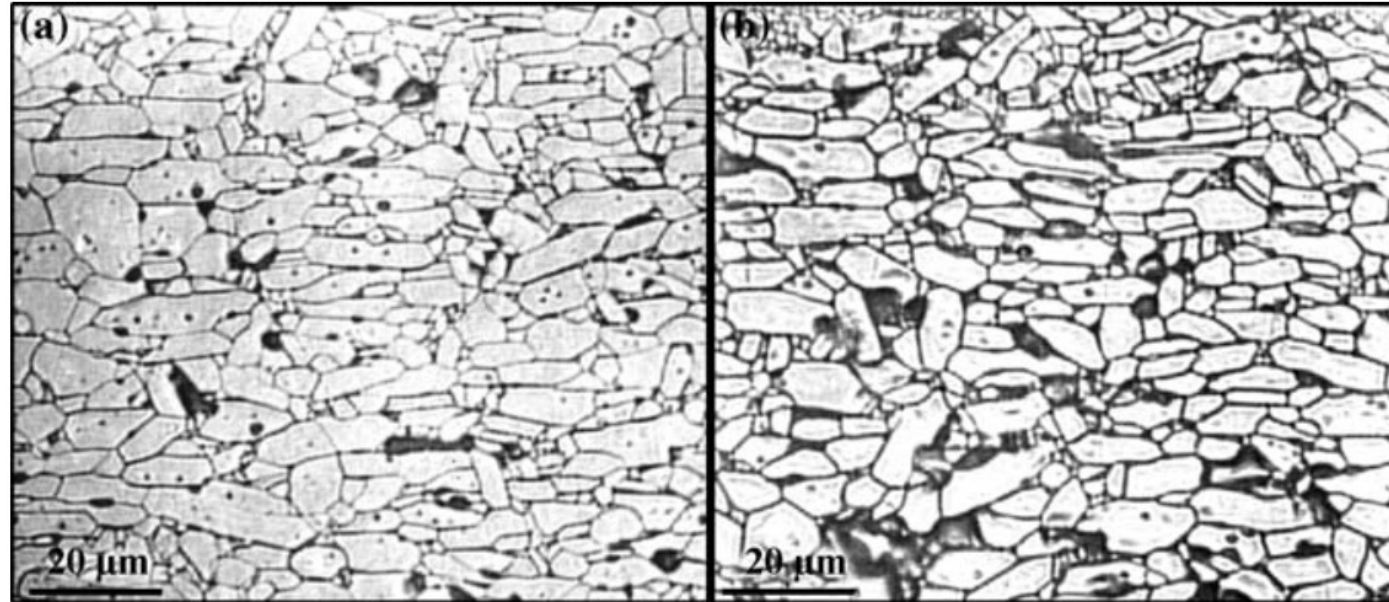
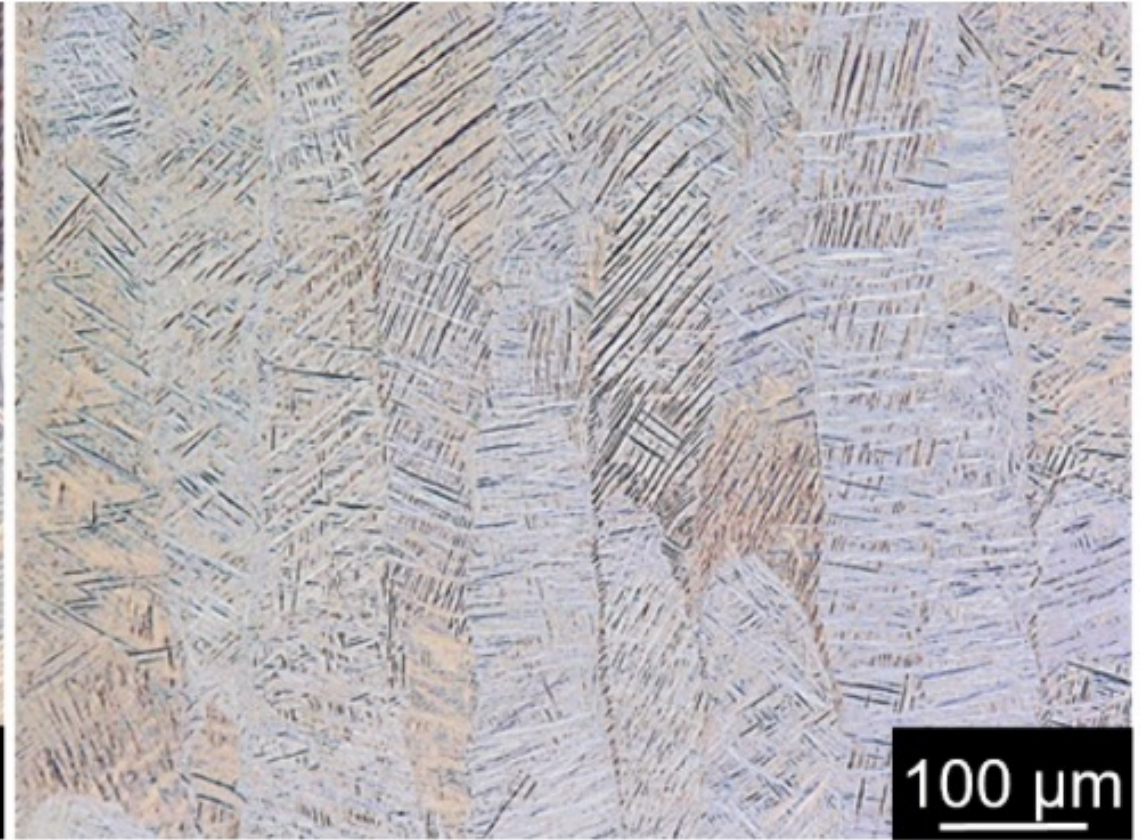
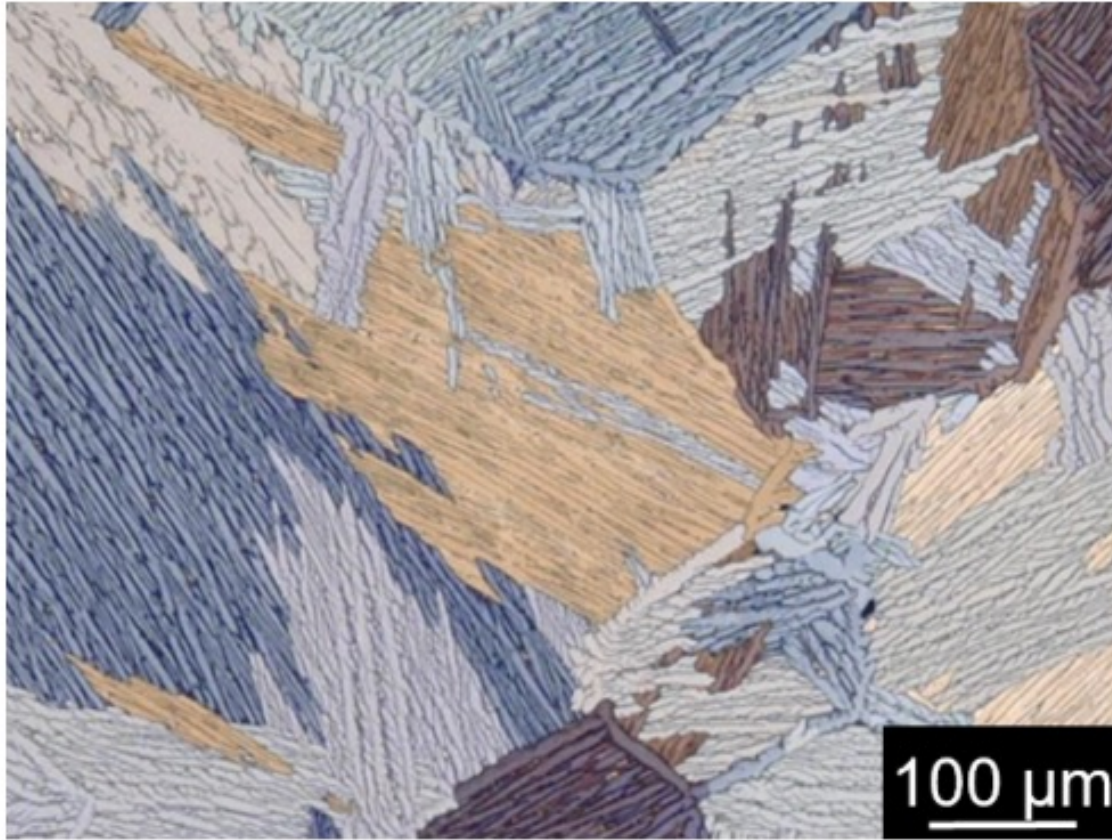


Figure 3.42 Polished alumina thermally etched at 1200°C for 30 min shows poor contrast and fails to reveal all the boundaries (a). Thermal etching for 2 h at the same temperature clearly reveals all the grain boundaries but with some loss of resolution for the finest grains (b).



Light microscopic photograph of Ti-6Al4V prepared by KOH etch to show basket weave structure on the left and martensitic microstructure on the right.

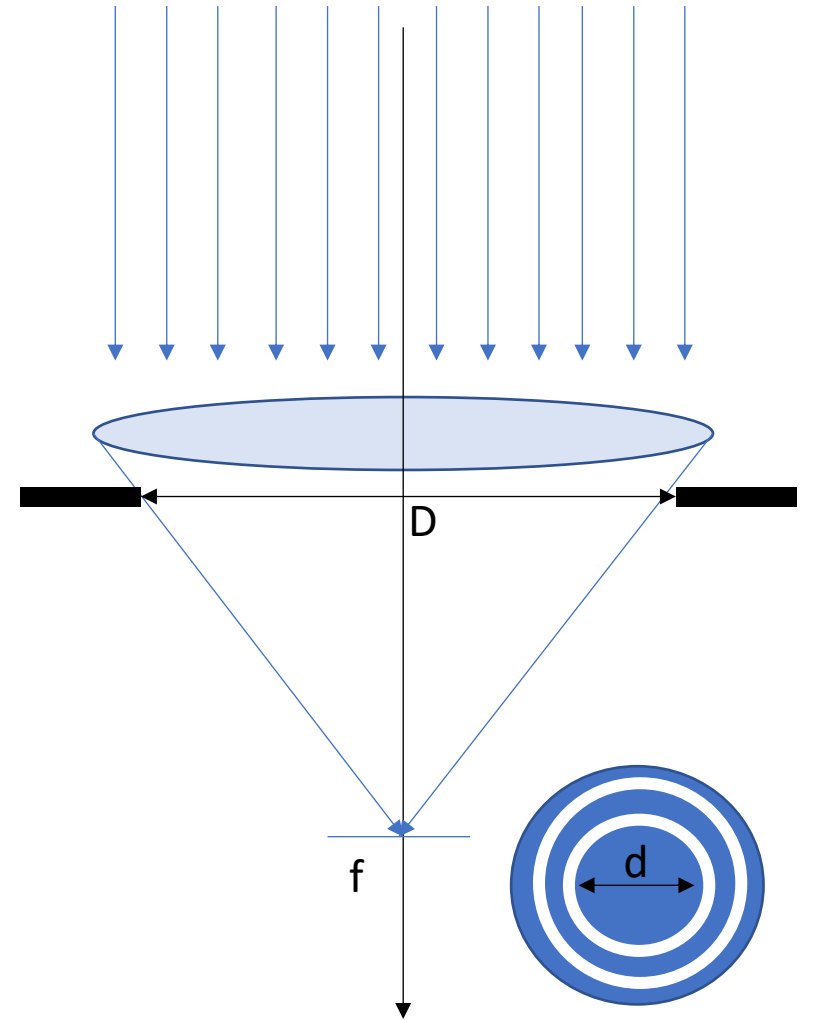
What can the microscope
can/cannot do ?

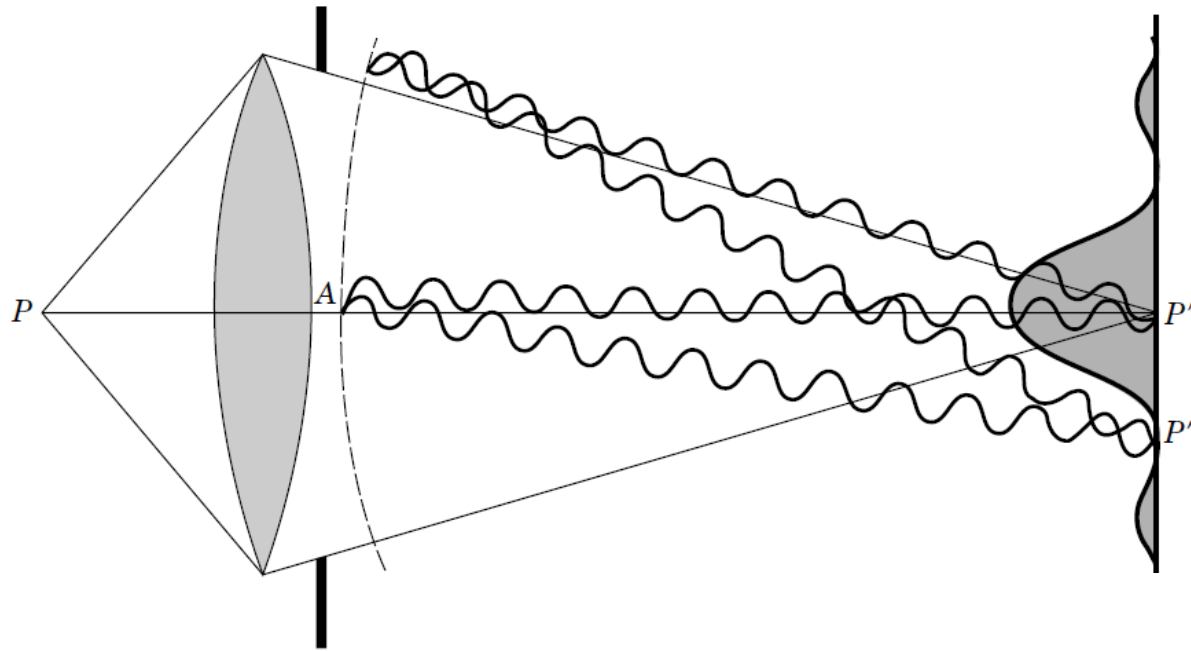
Diffraction in a lens

- 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 $d = 1.22 \frac{\lambda f}{D}$

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

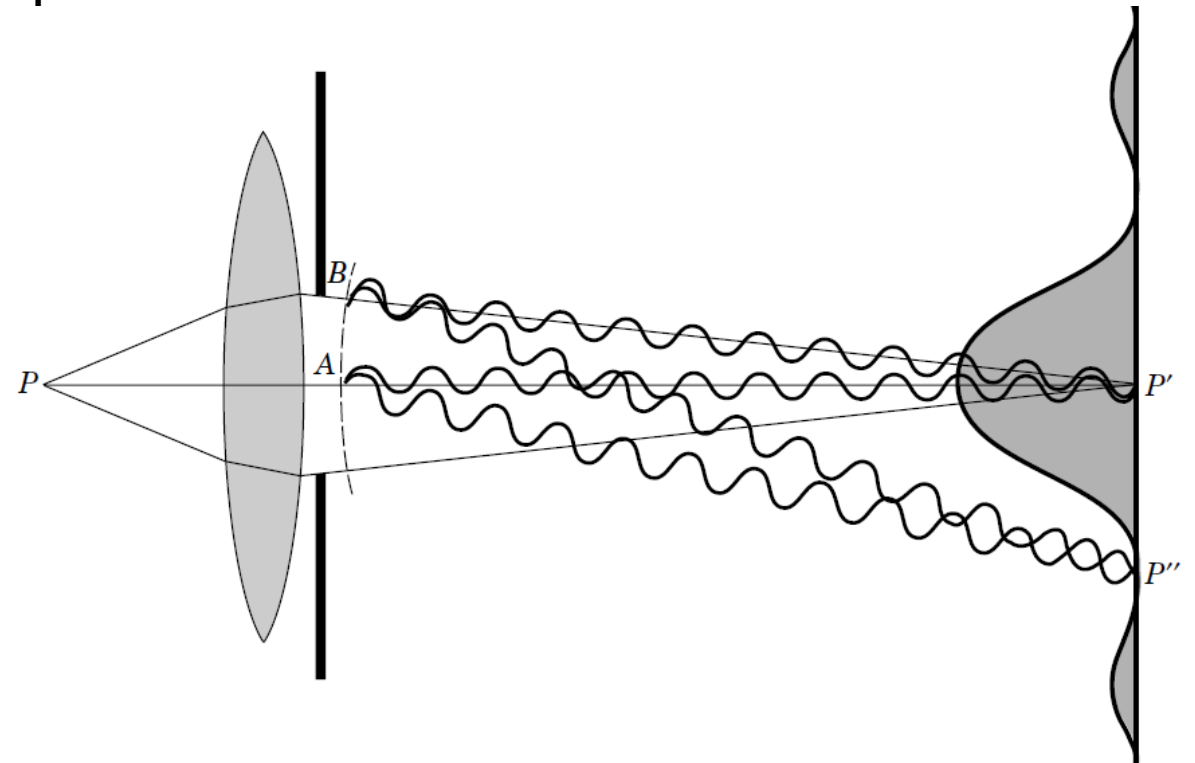
$$d = 0.61 \frac{\lambda}{\sin \alpha}$$



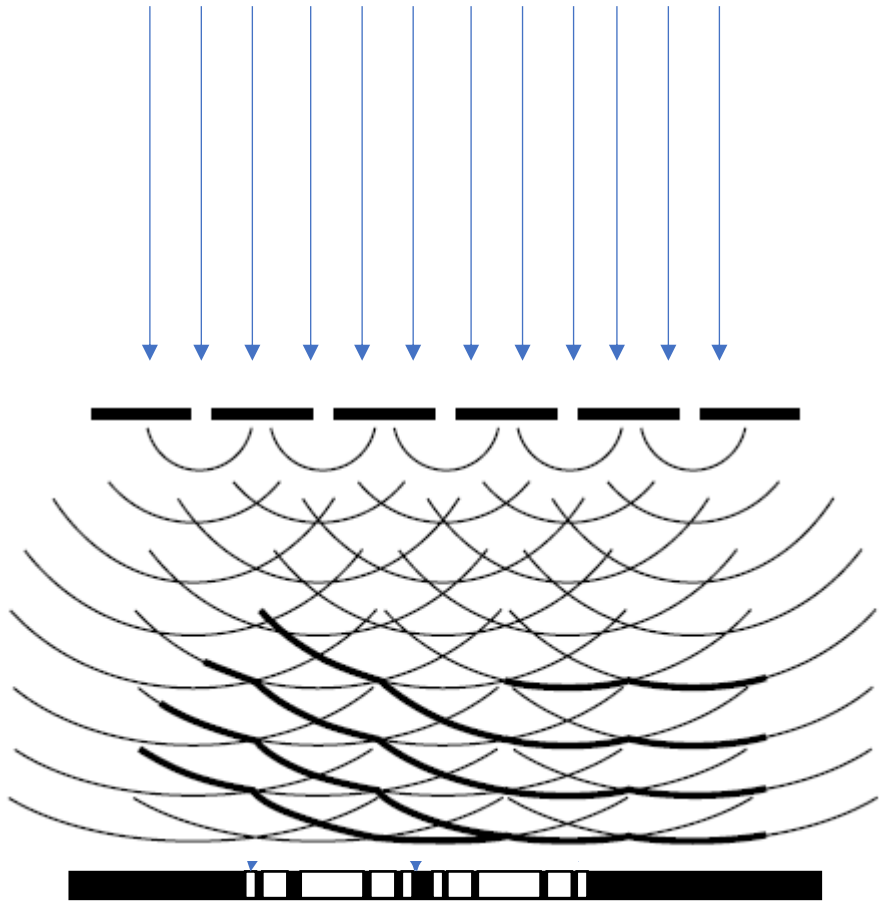


Effect of aperture opening

- 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.

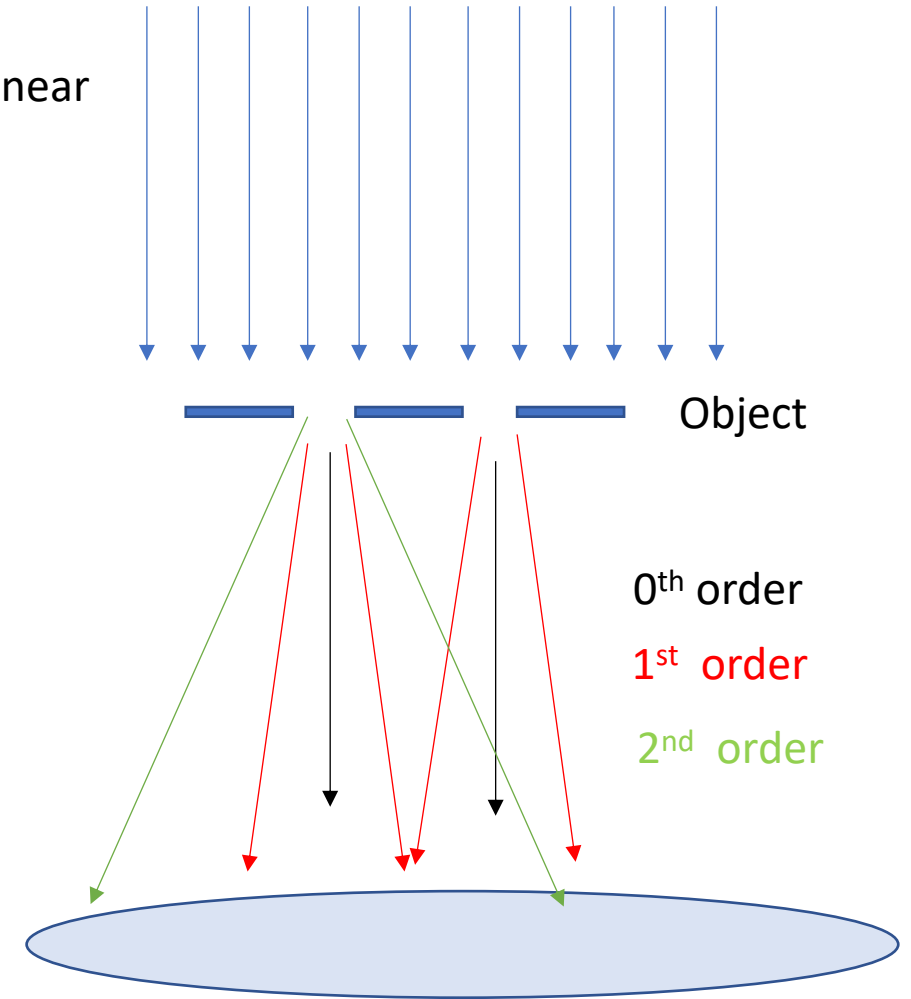


Diffraction from closely spaced particles



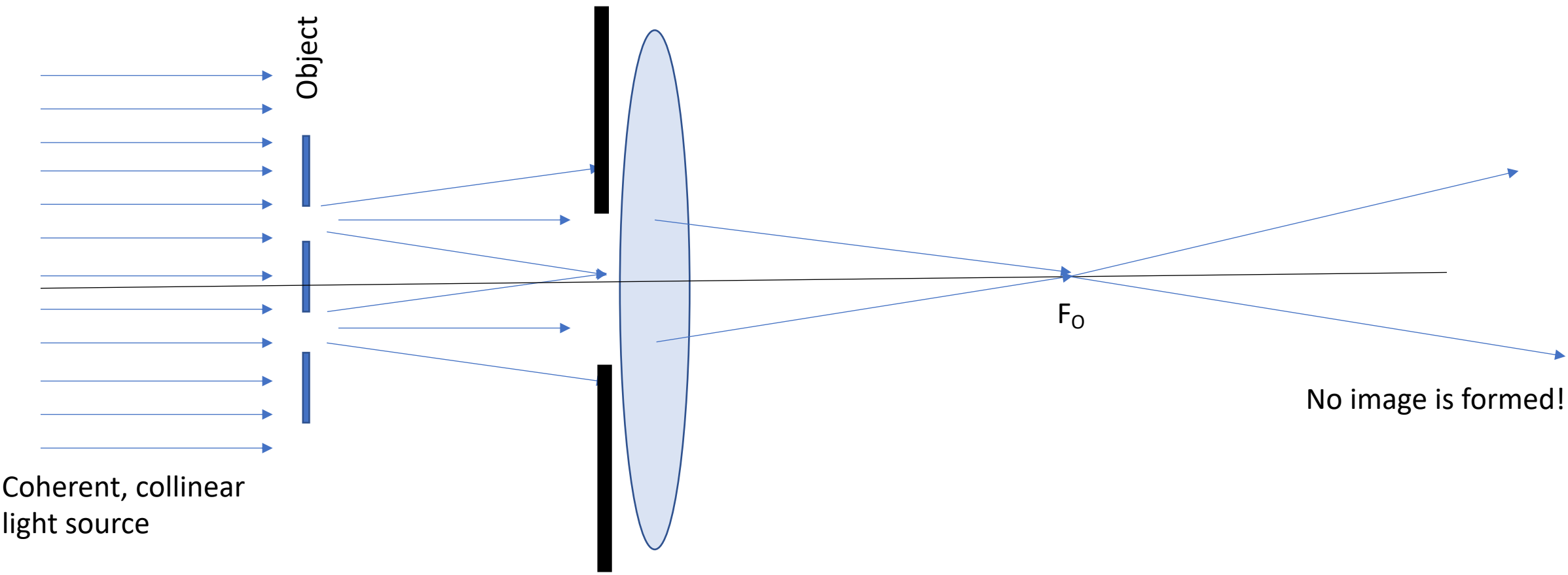
The famous double slit diffraction experiments
Photo screen

Coherent, collinear
light source



What happens when we converge such diffracted beams ?

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

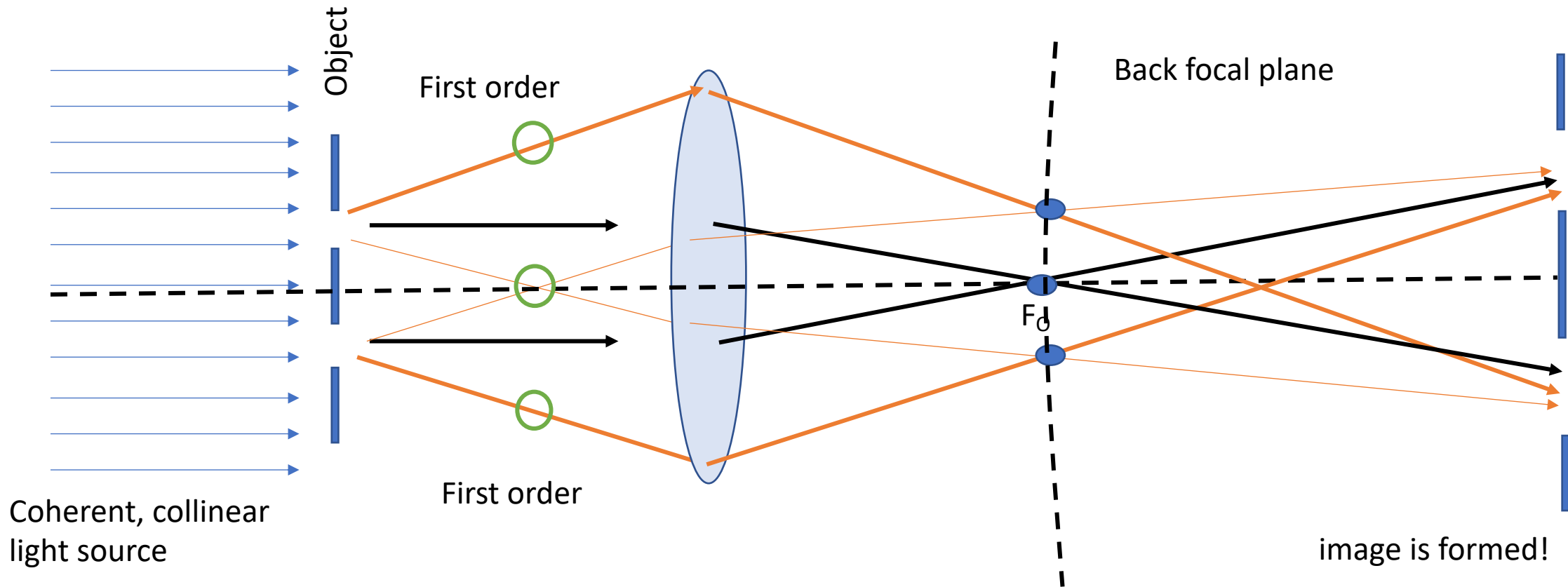


Coherent, collinear
light source

No image is formed!

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!

Abbe's criteria

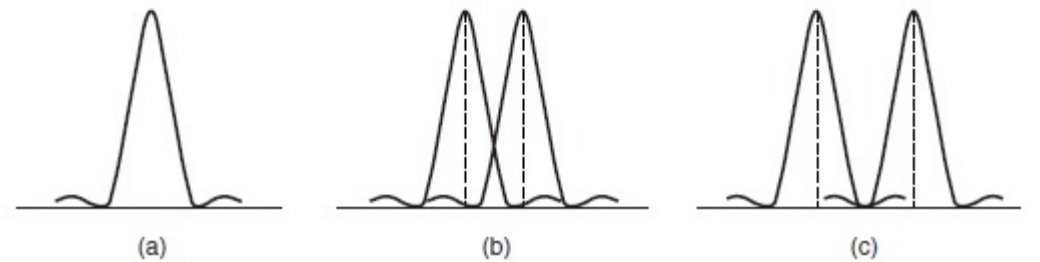
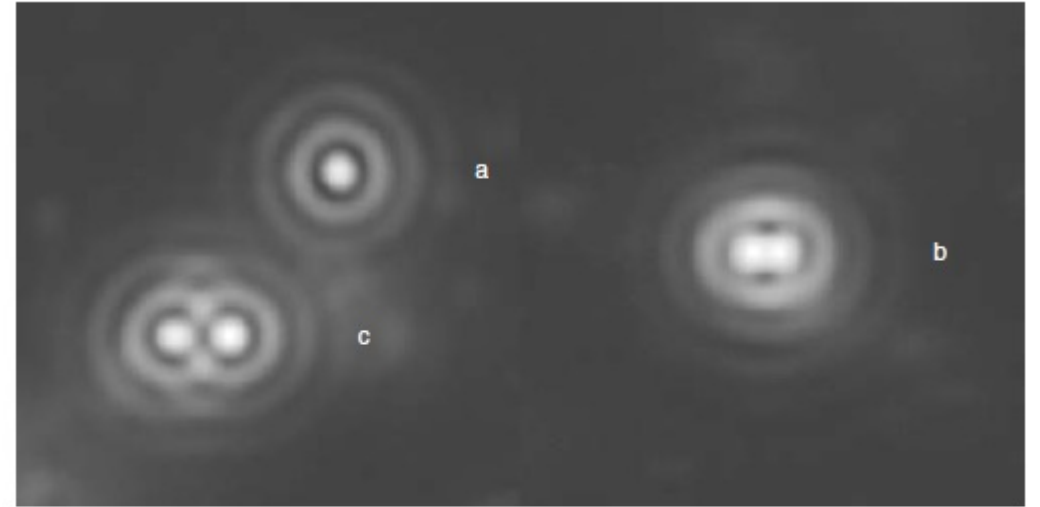


- Bright field (0^{th} order diffracted beam cannot form a distinct image)
- Collection of at least the first order forms a defined image
- Higher the number of diffracted beams captured, better is the resolution.
- Fortunately, the intensity of diffracted beams fall quickly with order, so first few 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.

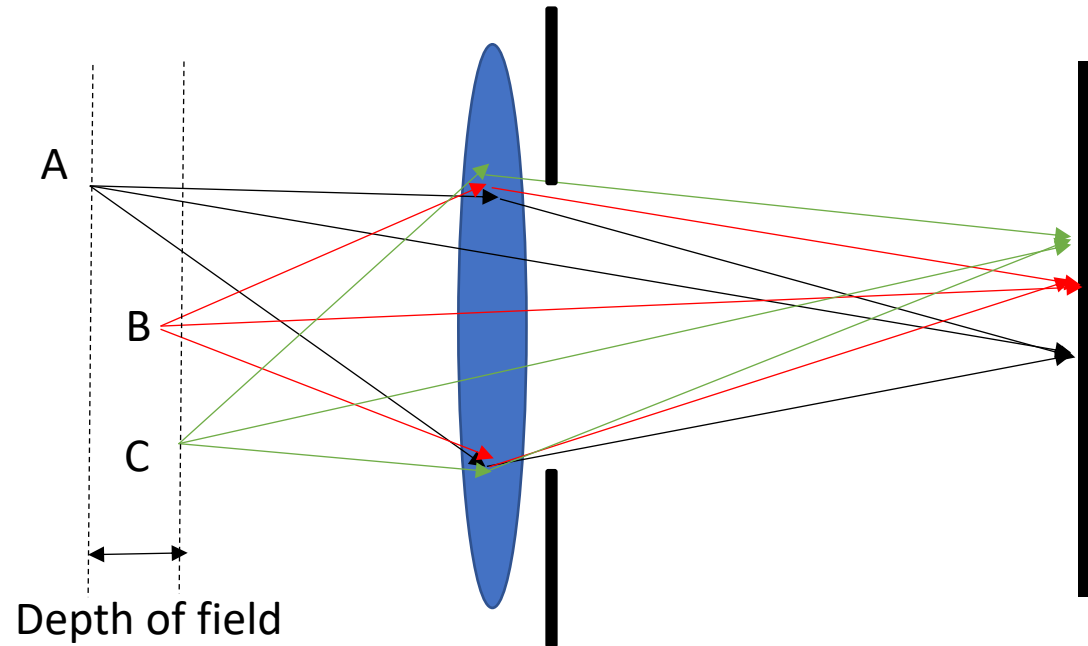
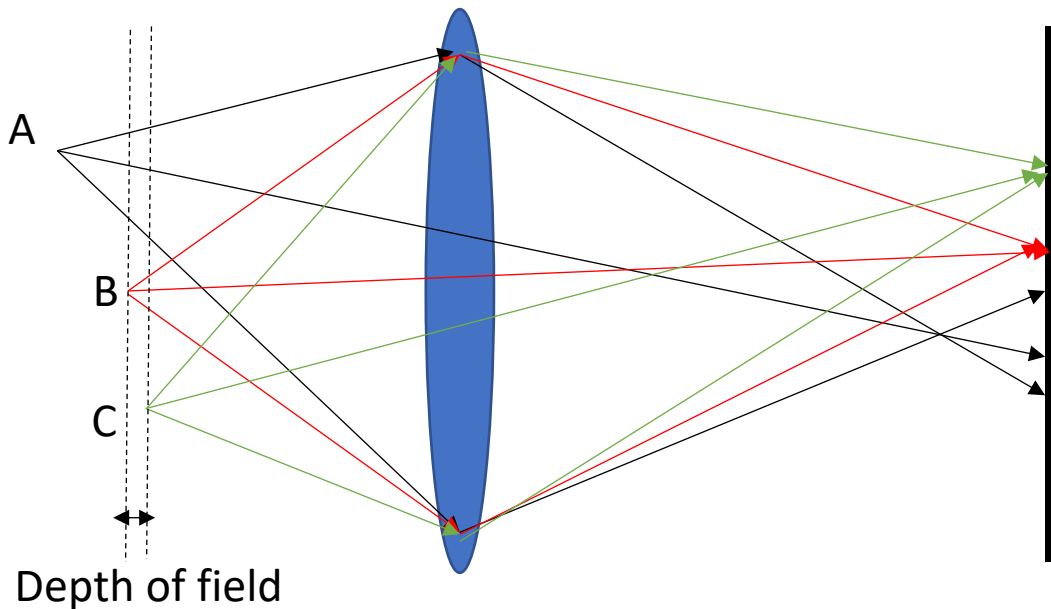
Controlling the spatial resolution.

- 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

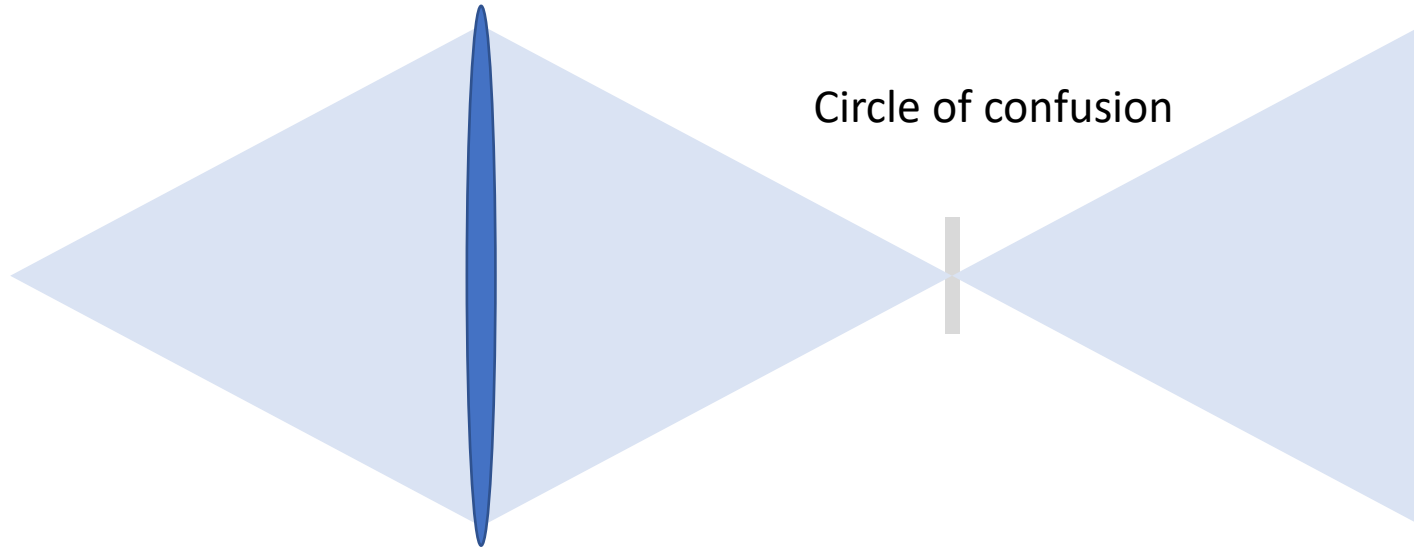
- This is the thickness of the optical section along the Z-axis within which the specimen are in focus

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

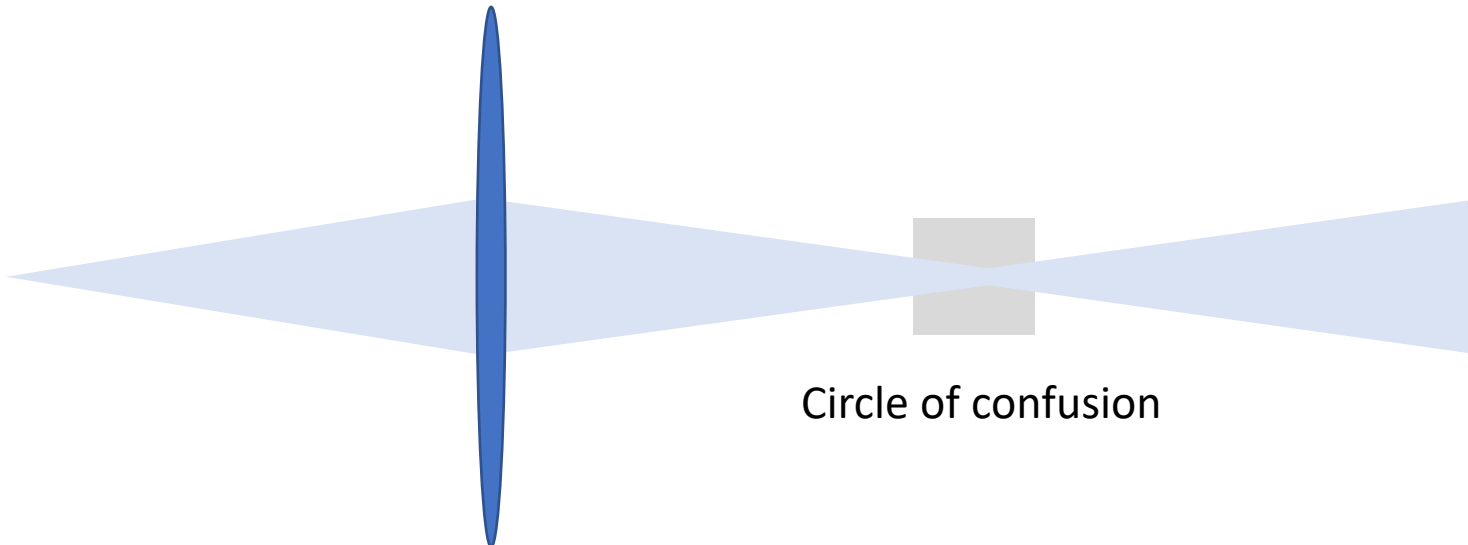


Depth of focus

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)