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✡ Microscopy

* Light Microscope - Light through the specimen is transmitted from this instrument, therefore it is called transmission light microscope.

* Principle of Light Microscope:

→ Transmission,
→ Absorption
→ Diffraction
→ Reflection of Light wave

→ Light microscope consists of single lens or magnifying glass.

→ It is made up of more than one glass lens in combination therefore termed as compound microscope.

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→ Compound microscope

→ Condenser lens - focuses the light from the light source to the specimen.

→ Objective lens - facing towards object.

→ Eyepiece lens - close to eye <10X>

⇒ Objective

→ Has a small aperture and small focal length than that of eyepiece <referred as ocular>.

→ Responsible for producing a magnified image.

→ Available in different varieties <4X, 10X, 20X, 40X, 60X, 100X>.

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⇒ Eyepiece → Monocular - with single eyepiece
→ Binocular - with two eyepiece

→ Usually magnified by 10X.

→ If eyepiece of higher magnification is used, it enlarges the images without improving the resolution.

→ Light Microscope can view → Living / → Dead } Specimen

→ Visibility of magnified specimen depends on → Contrast / → Resolution

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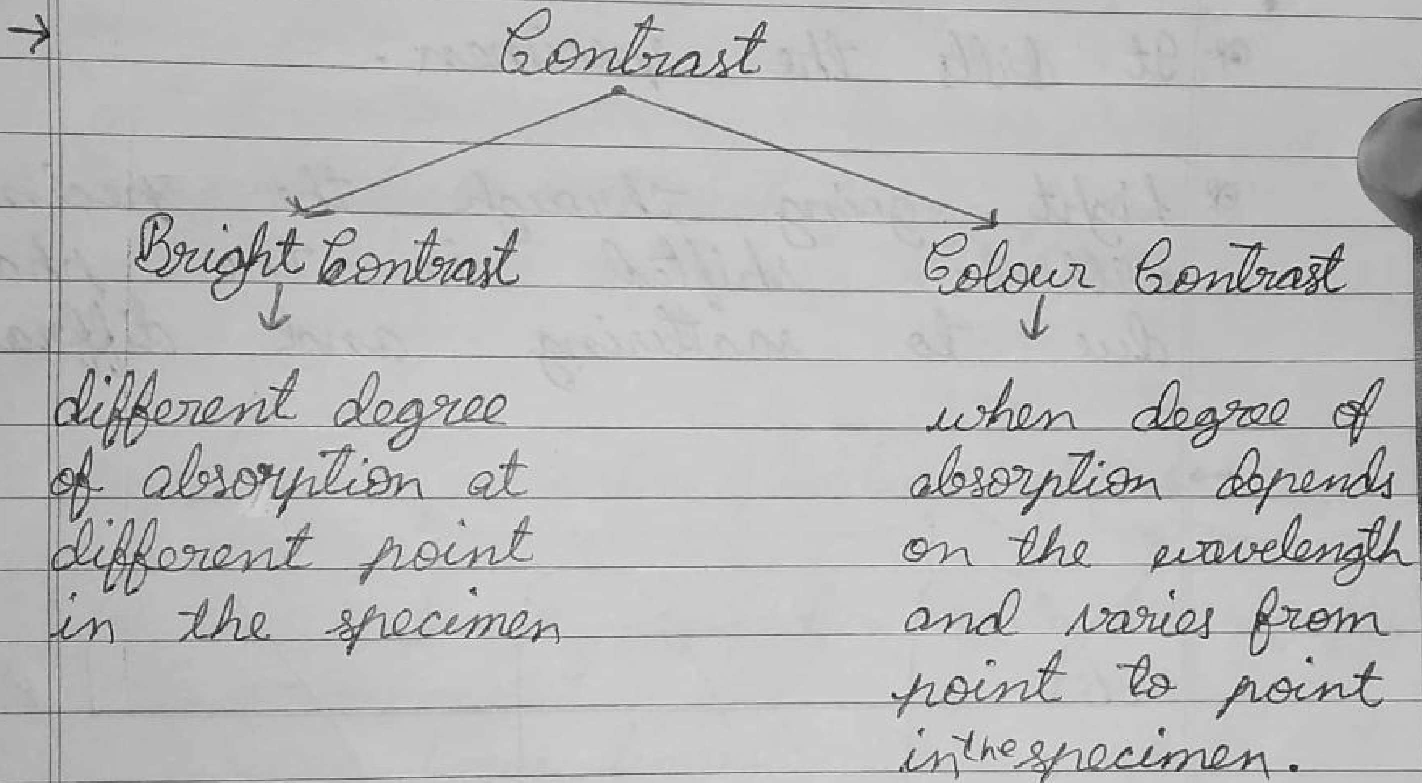
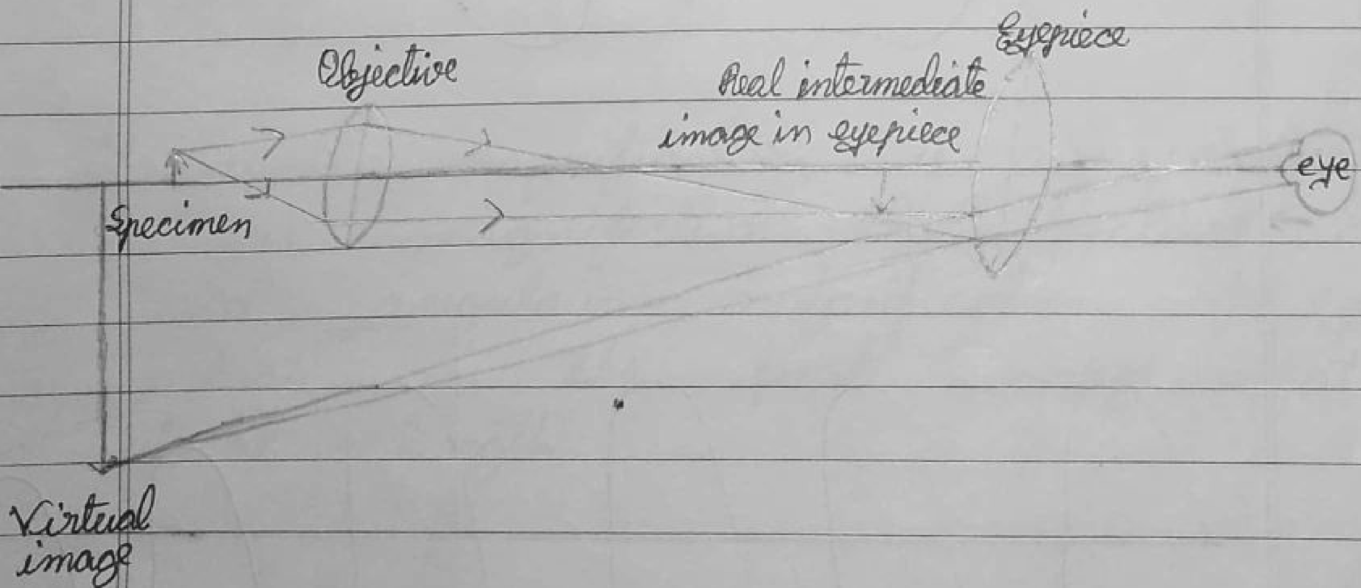
→ Contrast → different part of specimen absorb light to different degree

→





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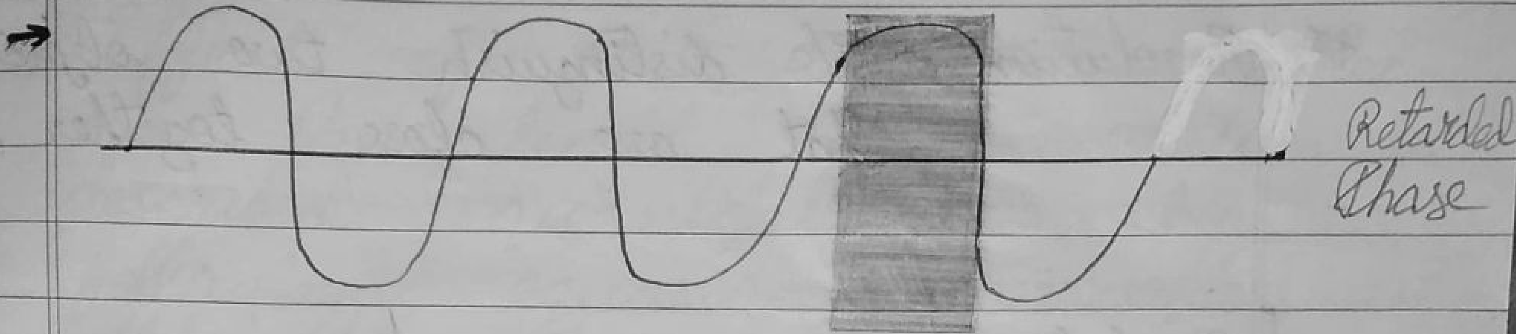

✡ Magnification → Product of magnification of eyepiece and objective.

Magnification = magnification of Objective + magnification of Eyepiece

magnification of Objective ↓ linear magnification ↓ linear dimension

magnification of Eyepiece ↓ Angular magnification

⇒ Overall magnification is a product of linear magnification of objective lens and angular magnification of eyepiece which is the first image at focal length.

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* They are rare recombinant which lack part of the normal phage genome and contain part of the bacterial chromosome located adjacent to the prophage attachment site.

* Formation of lambda gal <λ gal> and lambda bio <λ bio> transducing particles causes loss of some λ gene.

* λ gal particles lack the tail genes and sometimes head genes.

* Both of which are located at the right-end of the prophage.

* The λ bio particles lack genes from the left-end of prophage
<Int for integrase and Xis for excisenase>

* The number of missing phage genes depends on the position of the cut that generated the particles.

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* Resolution → To distinguish two objects that are close together.

Resolution (Resolving Power) $\propto \frac{1}{\text{limit of resolution}}$

Limit of Resolution - minimum distance between two points that allow for their discrimination & distinguish as two separate points.

→ Higher the resolving power, smaller will be the limit of resolution.

→ Limit of resolution depends on three factors:

i) wavelength (λ)

ii) refractive index (n)

iii) angular aperture (α)

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→ Effect of these three variables on the limit of resolution is described quantitatively by following equation known as Abbe Equation.

$$\text{Limit of Resolution} = \frac{0.61\lambda}{n \times \sin\alpha}$$

→ $(n \times \sin\alpha)$ is called as numerical aperture (NA) of objective lens.

→ NA is a measure of the ability of the lens to collect light from the specimen.

→ Lens with low NA (numerical aperture) collect less light than those of high NA (numerical aperture).

$$\text{Limit of Resolution} = \frac{0.61\lambda}{NA}$$

(NA = NUMERICAL APERTURE)

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→ From equation -

- numerator of equation should be as small as possible and denominator should be as large as possible.

Most Important → Resolution can be improved by shortening the wavelength of illuminating light, increasing the index of refraction on the objective lens and increasing the sin α.

- Angular α can be increased either by shortening the distance between the lens and object or by increasing the diameter of the lens.

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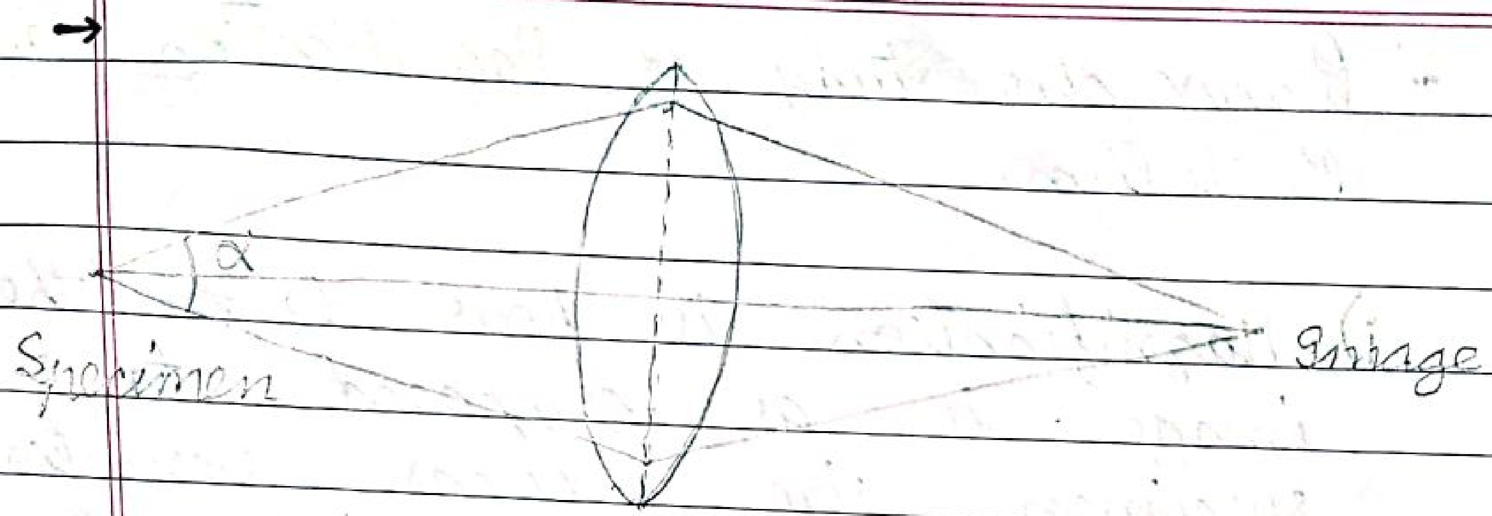
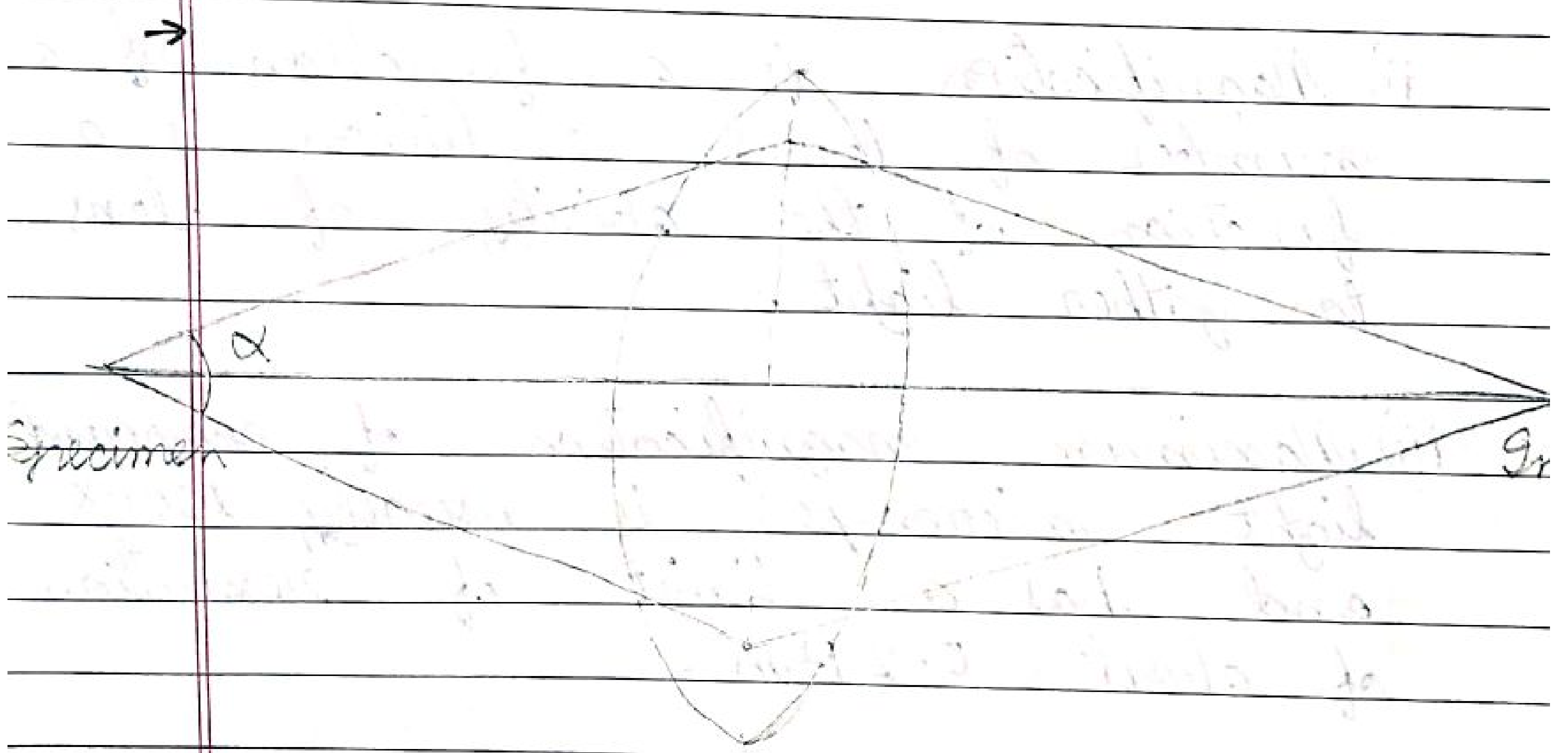


Objective lens with Low Aperture



Objective Lens with High Aperture

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* Comparable Study of Magnification and Resolution:

i) Magnification is how large the image is as compared to real specimen size whereas resolution is the amount of information that can be seen in the image, defined as the smallest distance below which two distinct objects will be seen as one.

ii) Magnification is a function of number of lenses, resolution is a function of the ability of lens to gather light.

iii) Maximum magnification of compound light microscope is usually 1500X and has a limit of resolution of about 0.2 μm.

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→ Some important points:-

* For best resolution specimen is illuminated with blue light of 450 nm.

* Angular aperture for best objective lens is about 70°.

* Maximum value of sin α is about 0.94.

* Refractive index is about 1.0.

* Lens designed for use is air the maximum numerical aperture is about 0.94.

* To increase numerical aperture, some microscope lenses are designed to be used with a layer of imersion oil between lens and specimen.

* Limit of resolution for a glass lens in air is roughly 300 nm.

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✱ Imersion Oil

→ Used to increase the resolving power to 0.61λ.

→ Has a higher refractive index than air and therefore allow the lens to recieve more of the light transmitted through the specimen.

→ Since the refractive index of imersion oil is about 1.5, the maximum numerical aperture for an oil imersion lens is about 1.5 × 0.94 = 1.4.

→ Thus the limit of resolution for a microscope that uses visible light is roughly 300 nm in air and 200 nm with an oil imersion lens.

→ The limit of resolution of an unaided <naked> human eye is 100 nm

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⇒ How it work < Mechanism >

i) In this system air is replaced by transparent oil < termed as imersion oil > of high refractive index < very similar to refractive index of glass >.

ii) Imersion oil such as paraffin oil, ceterwood oil has been placed at the interface between the objective lens and cover slip protecting the specimen < also between condenser lens and underside of the specimen slide >.

iii) If the air is present between the the cover slip and the objective lens, light is refracted, scattered and effectively lost.

iv) This happens because the refractive index of air is very different from that of glass if light passing through a glass-air interface

is refracted (bent to a large deg

v) By reducing the amount of refraction at this point, more of light can be directed to a narrow diameter lens of high power objective.

vi) The more the light, the clearer will be the image.

vii) Placing a material with a refractive index equal to that of glass in the air space between cover slip and objective, more light can be directed through the objective which improve resolution.

Imersion oil improve resolution by performing same function.

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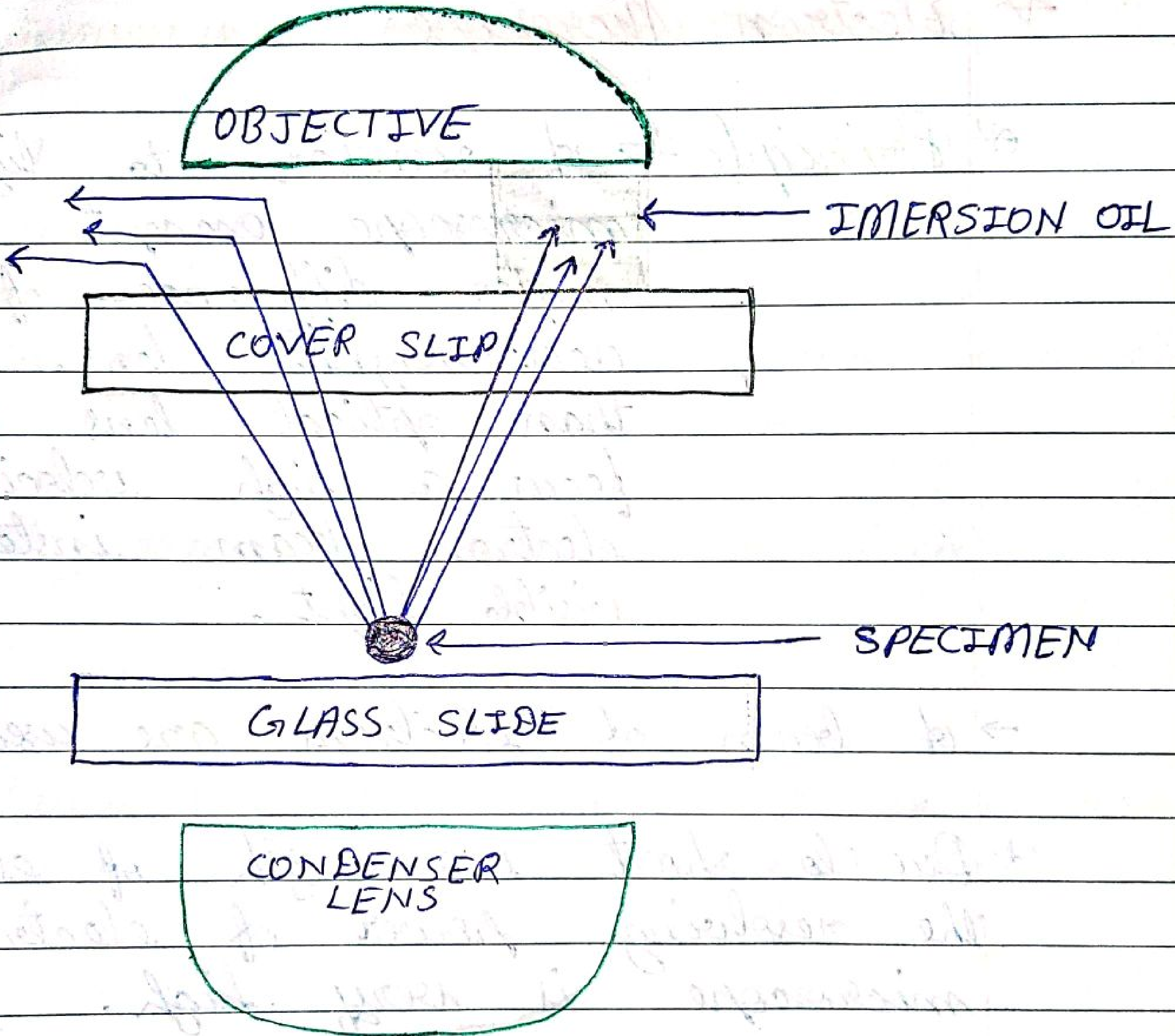

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✶ Electron Microscope

* Principle → A similar to light microscope except one major difference of using electromagnetic lens, rather than optical lens to focus a high velocity electron beam instead of visible light.

→ A beam of electrons are used.

* Due to short wavelength of electron the resolving power of electron microscope is very high.

* Not used to study live cells. It is used to study dead cells.

* It is of two types:

- Transmission electron microscope (TEM)
- Scanning electron microscope (SEM)

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