

Microscope

→ An optical instrument which is used to produce a magnified image of a small object which cannot be viewed by naked eyes.

→ Microscopy : "Micro" - means small
"scopy" - means to trace

— It is the technical field associated with the use of microscopes.

Principles of a Microscope

• Resolution : The power of a lens to capture in-depth details of an object.

→ High resolution 100 - 200 nm
It focuses on shorter wavelengths

• Magnification : The ratio of the size of the image to the size of the object.

• Numerical aperture : It is a number which determines the ~~resolving~~ ability of a lens to resolve fine details in an object being observed.

• Chromatic Aberrations : The effect produced due to refraction ~~of light~~ of different light-wavelengths at different angles, thus resulting in a failure to focus.

Simple Microscope

- It is an age old microscope ~~was~~ developed for viewing micro-objects.
- It is a modern version / modified design of light microscope.
- It can resolve upto $1 \mu\text{m}$ & its magnification ranges from 0 to $10\times$.

Working Principle : When an object is placed within its focus, then a virtual, erect & magnified image is formed at the least distance of distinct vision.

Parts Mechanical → (a) Base : A heavy metal base along with a vertical rod is present.

It supports the whole microscopic system. Stability is provided to all other parts.

(b) Stage : A rectangular metal plate is fitted with the vertical rod. It has a small hole at its centre, which let pass the light rays. When specimen is to be examined, it is placed in the stage and over that hole.

Optical → (a) Mirror : A plano-convex mirror is present below the stage attached with the vertical rod. It focuses light from the surrounding to the specimen.

(b) lens : A single biconvex lens is present above the stage. It magnifies the image and makes it visible to the viewer through the eye piece.

Standard theory
delectos

Compound Microscope

- It was introduced for more detailing of specimens.
- A compound microscope has essentially two or more lenses (double convex) fixed at the two extremities of a hollow tube.
- The lower lens is called the objective lens - used for viewing of the specimen.
- The upper lens is called the ocular or eye-piece.

Parts

Mechanical : a) Head/Body → It houses the optical parts in the upper part of the microscope.

(b) Base : It is a heavy metallic structure which provides support & stability to the other parts & houses the illuminator.

(c) Arms : It connects the base and supports the head.

Optical : (a) Ocular/Eyepiece → It is present at the top of the microscope which lets us view the image.

(b) Objective lens → The lens which is used to examine the sample. It is present near the sample. They range from 4X - 100X and include 3, 4, or 5 lenses on a microscope.

(c) Nosepiece → The rotating turret which houses the objective lenses, so that different objective can be conveniently selected.

(d) Coarse & Fine Adjustments : Coarse screw is used for moving the stage to bring it in focus. Fine screw is used for sharp focussing even under low light.

(e) Illuminator : light source in a microscope which allows viewing of the ~~object~~ specimen. It is either natural light or artificial light.

(f) Aperture : It is a tiny hole at the centre of the stage which allows light to reach the specimen.

(g) Condenser : It is used to collect & focus the light from the illuminator to the specimen.

Comparison Microscope

→ It ~~comp~~ is a device which is used to analyse side by side specimens.

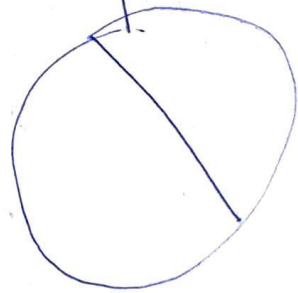
→ Two microscopes are connected by an optical bridge thus resulting in a split view window for viewing the separate objects simultaneously.

→ Calvin Goddard in 1920.

→ The optical bridge consists of a series of lenses and a mirror which brings back the image at a single eye-piece.

→ The viewer views through the eye-piece as in a conventional microscope, except a line separates the circular view field.

→ Not to rely on old memory.



Stereoscope

→ It is basically used for 3-D view of the specimen.

→ Some features of the sample cannot be clearly analyzed under a conventional microscope. So, stereoscopes are used for 3-D viewing of the sample.

→ In this type → two optical paths are present; providing slightly diff. view angles at the left & right eye.

→ It works on the light reflection principle rather than transmission.

Phase Contrast

A sample can be examined by light radiations and difference in contrast b/w the cell & the phase plate.

→ Features are same as compound, only exceptions
- presence of an opaque disc with transparent thin ring, phase plate and condenser.

→ The phase plate acts as an optical disc.




→ When light passes through the sample, then radiations are bent due to varying density of the specimen.

→ This light is converted & displayed as image.

Polarizing Microscope

→ Polarized light is mainly used for enhancing the contrast of an image by the use of birefringent materials.

→ Polarized microscopy involves polarized light to investigate the optical properties of various elements.

→ A polarizer  is used to filter the selected light-waves or radiations to pass through  and  collects it in a single plane.

→ In analyzer a second polarizer is used to control the quantity & direction of the light that illuminates a sample.

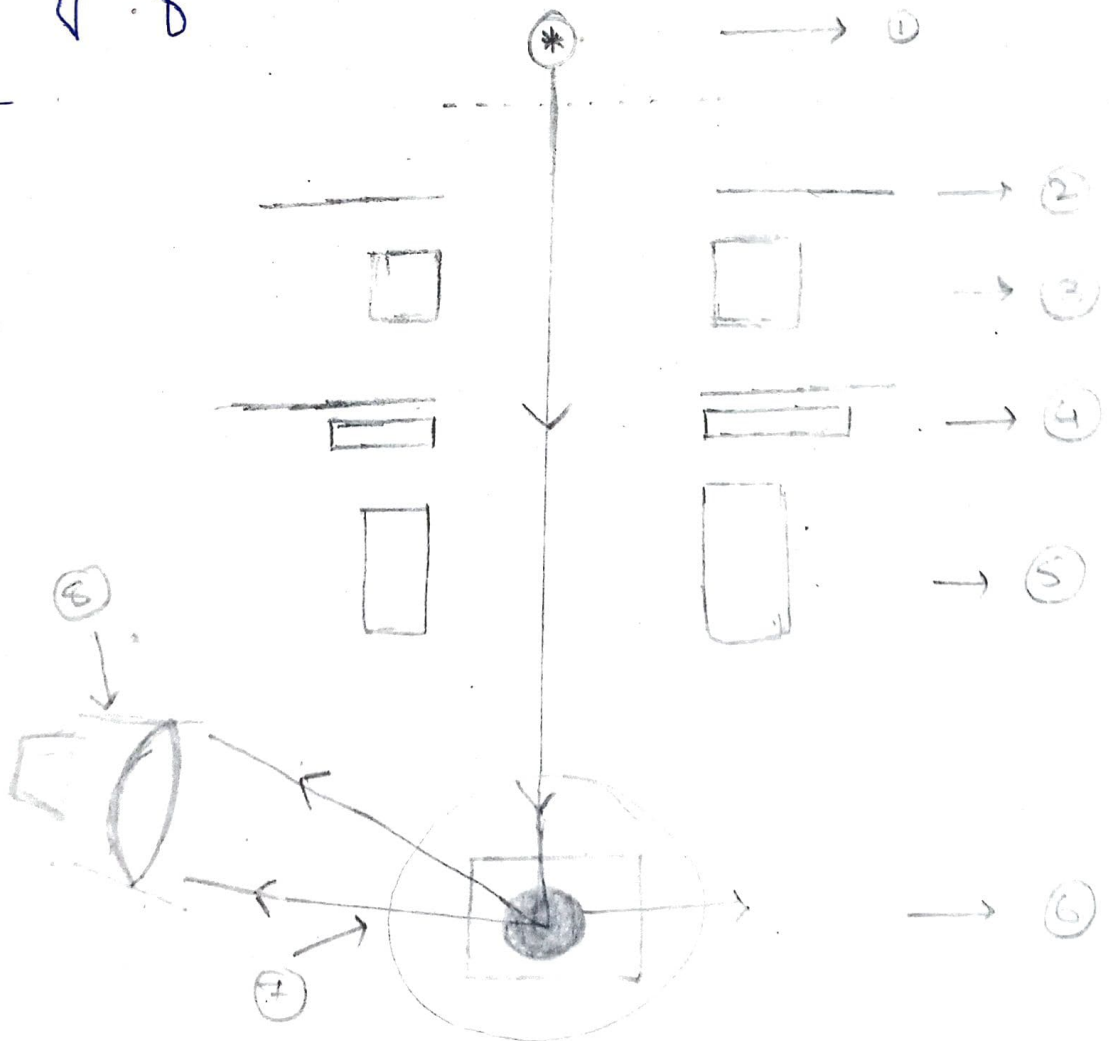
Due to the use of these filters the light waves vibrate in a single direction.

Fluorescence Microscopy

A fluorescence microscope is an optical instrument which uses fluorescence & phosphorescence for analyzing the samples.

→ In this case UV light is allowed to pass through the specimen. The photons get absorbed by the specimen & thus becomes excited. Upon relaxation, they emit photons. The particles that absorb light are called fluorophores. These fluorophores emit radiation at a much longer wavelength thus giving fluorescence.

SEM



Isopycnic Centrifugation

- This process depends on density only.
- There is not much difference in density gradient of the mediums.
- The denser medium is placed always at the bottom and lighter medium at the top.
- Here, it is not necessary for the sample to be layered over at the top of ~~every~~ the medium. But it can be mixed with the medium or can even be poured at the bottom of the tube and then the medium.

Density will be nearly \uparrow towards the bottom of the tube.

Neurosis : The person gets depressed or mentally unstable but never loses his touch with reality.

Its ability to gather and resolve details at a fixed object distance.

Chromatic Aberrations

Define microscope - It is an optical instrument that helps us to view an object which is not possible to see in unaided eye.

Micro - small
"scopy" means - to look

Principles [Numerical Aperture (NA): It measures the ability of lens to collect light from the specimen]

Magnification → It is the ratio of size of image to the size of object.

Resolution → It is the power of the lens to capture the in-depth details of an object. High Resolution → 100-200 nm

Types of Microscopes :- It focuses on shorter wavelengths.
Earlier days magnifying :-

1) Optical

2) Electronic

3) Scanning Probe Microscope

Optical

- 1) Simple
- 2) Compound
- 3) Comparison
- 4) Stereo
- 5) Phase Contrast
- 6) Fluorescence
- 7) IR microscope
- 8) X-ray Microscope
- 9) Polarizing Microscope
- 10) Ultrasonic Microscope

Electronic

- Scanning Electron Microscope (SEM)
- Transmission Electron Microscope (TEM)

Scanning Probe

- Scanning Tunneling Microscope
- Atomic Force Microscope

* Simple Microscope

- It's the simplest form of microscope.
- It helps us to see micro-objects.
- Mag. — 0 to 10 X
- It's originally ~~designed~~ made and a modified design of light microscope.

— Mechanical System

- ~~Base~~ Base
- Arms
- Adjustment Screws

- Movable arm
- Single lens
- Stage clips
- Stage

• Convex lens

Optical System

- Reflection pathway

— helps us to magnify the image using a reflection pathway and also makes the object visible through

→ It focuses the light on the object and uses the magnified image that includes the reflection path and an eyepiece.

Uses

- Normally to view hair sampling, chemicals (powdered or debris); biologically

COMPOUND MICROSCOPE

→ It has two converging lenses.

Compound microscope consists essentially of two or more double convex lens fixed in the two extremities of the hollow cylinder. The lower lens is called the objective & upper lens is called the eyepiece or ocular lens.

→ Compound microscope helps in viewing a smaller object or a specimen such as cell structures, which cannot be seen at lower levels of magnification.

2 - consists of structural & optical ~~components~~ parts.

- Structural Compounds

1. ~~Head~~ Head - for viewing
2. Base -
3. Arm -

- Optical Components

1. Illuminator
2. Condenser - intensity of light (controls)
3. Eye piece
4. Objective lens [$4\times \times 100\times$ - Magnification]

- Aperture
- Stage

Uses

- In the examination of soil, paints, fibers and toolmarks etc.
- It is used in ballistic purpose for the examination of ammunition, cartridge case and bullets.
- Fingerprint & toolmark examination.
- Serology (blood & semen) - just for viewing the physical appearances.
- Toxicology; traces of foreign materials
- Explosives

Simple (Naked)

✓ There is no rigorous requirement of research.

ii) It has a single lens

iii) Magnifying power upto $10\times$

(iv) Resolve below $1\mu\text{m}$

(v) level of magnification is 1.

(vi) Natural light source used.

(vii) Magnification is adjustable.

Compound

i) Used for magnifying micro-organisms like algae, fungi & bacteria etc.

ii) It has a double lens ($3-5$ objective lens are used)

iii) Magnification is $4\times$ to $100\times$.

(iv) Resolve below $0.2\mu\text{m}$

(v) level of magnification is higher upto $10\times$.

(vi) Illuminator is used.

(vii) Not adjustable magnification.

COMPARISON

It is a device which is used to analyse side by side specimens. It consists of 2 microscopes connected by an optical bridge which results in split view window to separate objects viewed simultaneously. This avoids the observer to rely on a memory when comparing two objects under a conventional microscope, the modern instrument has an optical, mechanical & electronic refinements.

Uses

- Examination of tool marks, bite marks, fired bullets & cartridge cases

* Side by side view

* Superimposition technique.

- It also help us to examine & analyze the object by superimposition technique.

Stereoscope

* After viewing in every other, specimen is viewed for 3-D.

* Some characteristics of physical evidences are not possible to study in compound because it gives 2-D structural details.

* But sometimes the 3-D image of several physical evidences is needed for forensic examination.

* For 3-D analysis of evidence, stereoscope is used.

* It is having prism, placed in the light-path & radiates the light which enters into the sample.


* The magnification ranges b/w 10X & 25X.

• Counterfield notes

• determine secrets of handwriting strokes.

Phase Contrast Microscope

A sample can be examined by light-radiations and by ~~the~~ difference ~~in~~ in the contrast- b/w the cell & the phase plate.

The features  are same as a compound microscope except, it contains condenser and an opaque disc ^{under} ~~the~~ phase plate. This phase plate works ~~as~~ as an optical disc i.e., when light-passes through the sample the light radiations bent- due to the variation in density i.e., the specimen. The light-passing through the sample is converted & displayed in the form of image.

Uses

- Soil analysis
- examination of paint, fibers, (same as other microscopes)
- u u decomposed body
- u u insects
- u u forensic entomology

Fluorescence Microscope

It is a type of microscope in which UV radiation is allowed to pass through the sample, is absorbed by the molecule of the specimen & this emits a light. A radiation at different wavelengths by which respected samples can be analysed.

Uses


- Examination of ink
- Currency notes and passports
- It helps us in case of drownings
- Analysis of blood samples

Polarizing Microscope

— Involves the use of polarized light to investigate the optical properties of various elements.

→ It is a contrast-enhancing technique which allows to evaluate the composition and 3-D structure of a specimen.

→ It uses polarizing filters to make use of polarized light by configuring the movement of light waves & forcing their wave vibrations in simple directions.

→ Polarized light — When a ray of light passes through a Nicol's prism, then there is no diversion and the light rays are aligned in the path of light; ~~then the ray passes~~ then the ray is  aligned in a single direction either upwards or downwards.

Uses

- to highlight the feature of crystals, fibers, minerals, polymers & ceramics.
- It is used in geology, chemistry, botany & metallurgy.

Electron Microscope

A type of microscope which uses a beam of electrons to illuminate a specimen

to create a high magnified image.

Specimens smaller than 0.2 nm can be examined & analyzed.

The image produced by EM are always in 2-D structural pictures, that indicates black and white. But may be coloured artificially.

Electron Microscope

- drying declaratio
- sections

↳ Scanning Electron Microscope (SEM)

It is a type of electron microscope that produces a image of a sample by scanning it with the focus beam of electrons.

These electrons interacts with the surface producing various signals that can be detected and contains an information about the sample surface of topography and composition.

The most common SEM is used for the detection of secondary electrons emitted by the atoms ~~excited~~ excited by the electron beam.

By scanning the sample & collecting the secondary electron with the detector, an image is displayed by displaying the topography of the surface.

• Magnification^m : power of SEM $\rightarrow 1000X - 10,000X$

Uses : It produces 3-D structural imaging.

- GSR
- Fire arm identification
- Examination of Paint
- Handwriting Examination
- Surface Imaging
- Analyze the gold materials.

2) Transmission Electron Microscope (TEM)

It is a technique in which a beam of electron is transmitted through the specimen and as it passes, through the image is formed from the ~~attraction~~ interaction of electrons through the specimen & the image is magnified & focused onto the photographic films. It is capable of imaging at a higher resolution than other microscopes.

- Magnification - $10,000X - 1,00,000X$
- It produces a 2-D structural imaging.

Q Diff b/w SEM & TEM

SEM

TEM

- | | |
|--|--|
| i) Based on scattered electrons | i) Based on transmitted electrons. |
| ii) It focuses on the surface of a specimen. | ii) It focuses a detailed structure & its composition of a specimen. |
| iii) No such modifications. | iii) Sampling in TEM has to be thinner stain. |
| iv) As compared to TEM resolution is low. | iv) High resolution power. |

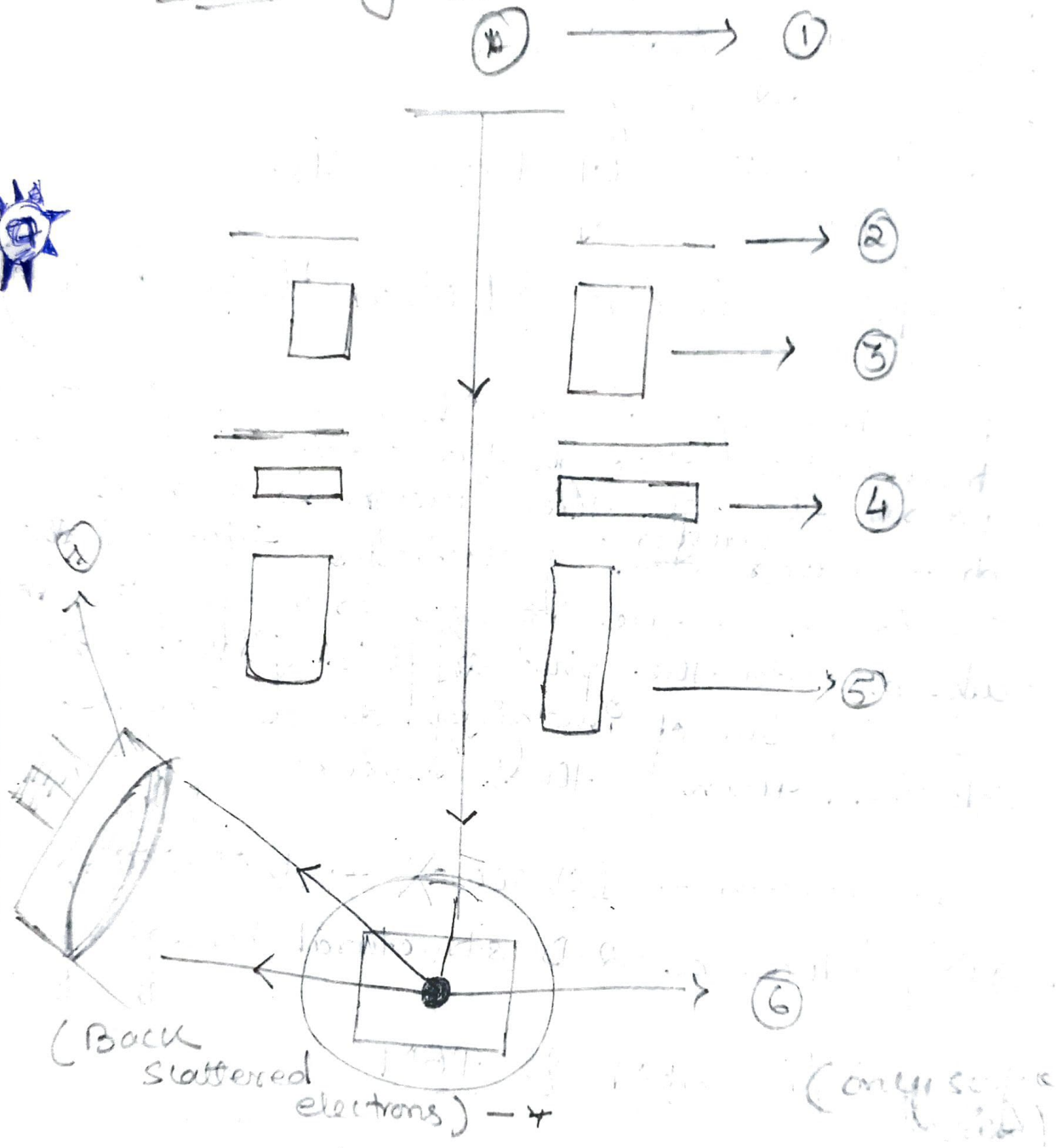
v) Shows 2-D Image

vi) We can add up magnifications $1000\times - 10,000\times$.

v) Shows 2-D Image.

vii) Magnification ranges $10,000\times - 1,00,000\times$

Block diagram of SEM



① - Source (Acts as an electron beam)

② - ~~Illuminating~~ ^{directing} of Aperture

③ - Condensor lens

④ - Magnetic lenses

⑤ - Scanning coils (Objective Lens)

⑥ - Specimen

⑦ - Detector

- ↓ The rays which are reflected from the specimen.
- ↓ Detector - Everhard-Thornley detector

TEM

Overall view, inner composition in depth,
Qualitative & quantitative.