MAJOR ASSIGNMENT.

MICROSCOPY.

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MICROSCOPY.

Synopsis :-

* Introduction.
* Magnification.
* Resolving Power.
* Types of Microscopes.
* Light Microscopes
    * Simple Microscope
    * Compound Microscope
    * Fluorescence Microscope.
* Electron Microscopes.
    * Transmission Electron Microscope. (TEM)
    * Scanning electron Microscope (SEM).

Introduction:-

* A microscope is an instrument that can be used to observe small objects, even cells. The image of an object is magnified through at least one lens in the microscope. This lens bends light toward the eye and makes an object appear larger than actually it is. We will see about more information of microscope in this Essay.

Magnification :-

* Magnification = $\frac{\text{Size of retinal image seen with microscope}}{\text{Size of retinal image seen with unaided eye}}$

Resolving Power :-

* The resolving power is the capacity of an optical system to show distinct images of points lying very close together. The resolving power of unaided human eye is 0.1 mm.
* The resolving power depends on the wave length of light and the numerical aperture of the objective lens.

Types of MicroScopes :-

* The microscopes are classified into three types based on the source of illumination. They are the following:
  * Light Microscopes.
  * Electron Microscopes.
  * x-Ray Microscopes.
* They are the types of Microscopes.

Light Microscopes :-

* There are so many types in microscope we will discussed about 3 types of microscope They are,
    * Simple microscope
    * Compound microscope.
    * Fluorescence microscope.

Simple Microscope :-

Definition :-

* A simple microscope is one that uses a single lens for magnification, such as a magnify a glass.
* It wes a lens to enlarge an object through angular magnification alone, giving the viewer an erect enlarged virtual image.

Principle :-

* It works on the principle that when a tiny object is placed within it focus, a virtual, erect and magnified image of the object is formed at the least distance.

Magnification :-

* The Magnifying power of a simple microscope is given by,
    * $M = 1 + D/F$.

Parts :-

The parts of a simple microscope maybe:-

(i) Mechanical parts:

* Metal Stand
* Stage.

ii) Optical parts :

* Mirror
* lens.

Applications:

* It is usually wed for the Study of microscopic algae, fungi and Biological Specimens.
* It is commonly wed by watchmakers and jewelers. to magnified view of fine parts.

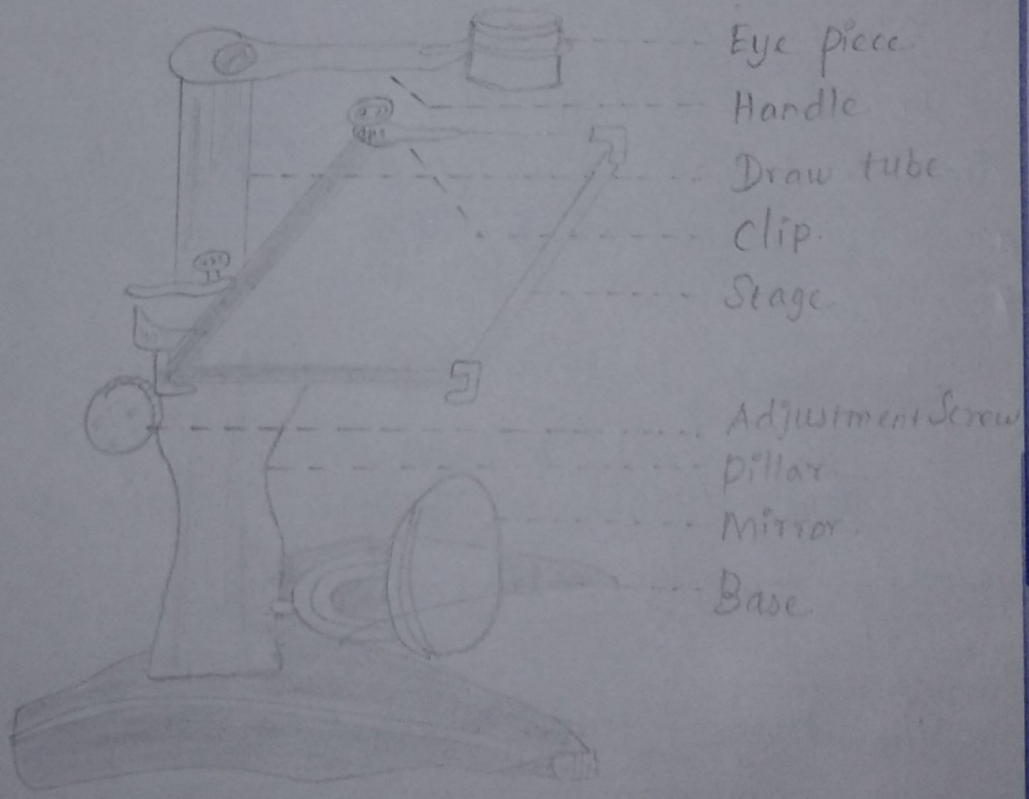

Fig.: Simple Microscope

Compound Microscope.

Definition:-

* The Compound microscope is an optical instrument to Magnify objects.
* It is formed by the Combination of two Simple microscopes
* It is a light microscope because light is the illuminating Source.

Principle:-

* It's work on the principle of optics. The lenses magnify objects. By Stacking lenses the magnification is increased.
* The Compound of microscope has a light Source, a diaphragm an object an objective lens and an eye piece.

Magnifying power:-

* The magnifying power of a Compound microscope is the ratio of the Size of the final image to the Size of the object

* $M = \frac{\text{Size of final image}}{\text{Size of the object.}}$

Resolving power:-

* The ability of the microscope to distinguish two very Small and closely Spaced objects as Separate entities is "Resolving power of the microscope".

* The Resolving power is improved by following factors.
  * High numerical aperture
  * Fully opened Condenser (diaphragm)
  * Shorter wavelength of light (blue light)
  * Immersion fluid like oil.

Numerical aperture: (NA).

* Numerical aperture is light gathering capacity Of the lens The NA of the lens is inscribed in the metal tube and it ranges from 0.25 to 1.4.

Structure :-

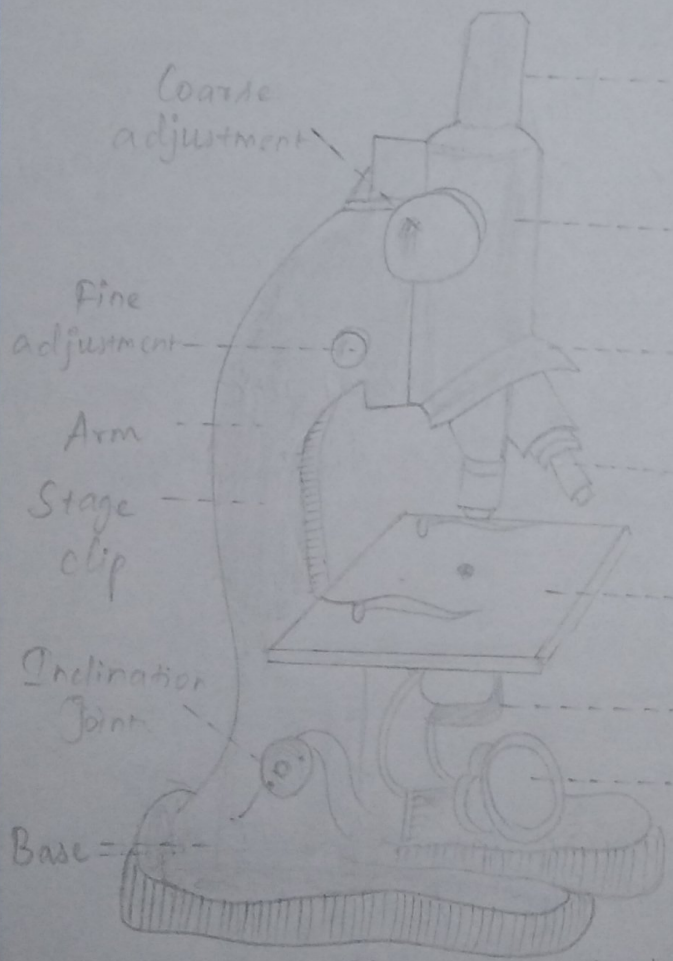

Fig: Compound Microscope

Parts of the Compound Microscope.

* The Compound microscope has the following Parts:

* **Reflecting mirror**: reflect the light
* Condenser: Focuses the reflect light to object
* Objective lens: Magnifies the Object
* Eye piece: Magnifies the object's image.
* Body tube: It is a tube with objective lens at the lower eye piece and upper end.
* Coarse adjustment: It moves the body to up, down
* Fine adjustment: It moves the body to up and down slowly.
* Stage: It is a platform with hole in centre.
* Stage clips: Hold the Slide.
* Nose-piece: Form of a rotating disc.
* Arm: Holds body tube and Coarse adjustment
* Inclination joint: permits tilting of the upper part
* Base or Foot: It keeps the body in position.

FLUORESCENCE MICROSCOPE.

* A Fluorescence Microscope is an optical microscope that uses Flouoresence instead of, or in addition to Scattering, reflection and attenuation or absorption to Study the properties of organic or inorganic Substances

* Fluorescence Microscope refers to any microscope that uses flourescence to generate an image, whether it is a more Simple set up like an epifluorescence microscopes or a more Complicated design Such as a Confocal microscope, which uses optical Sectioning to get better resolution of the fluorescence image.

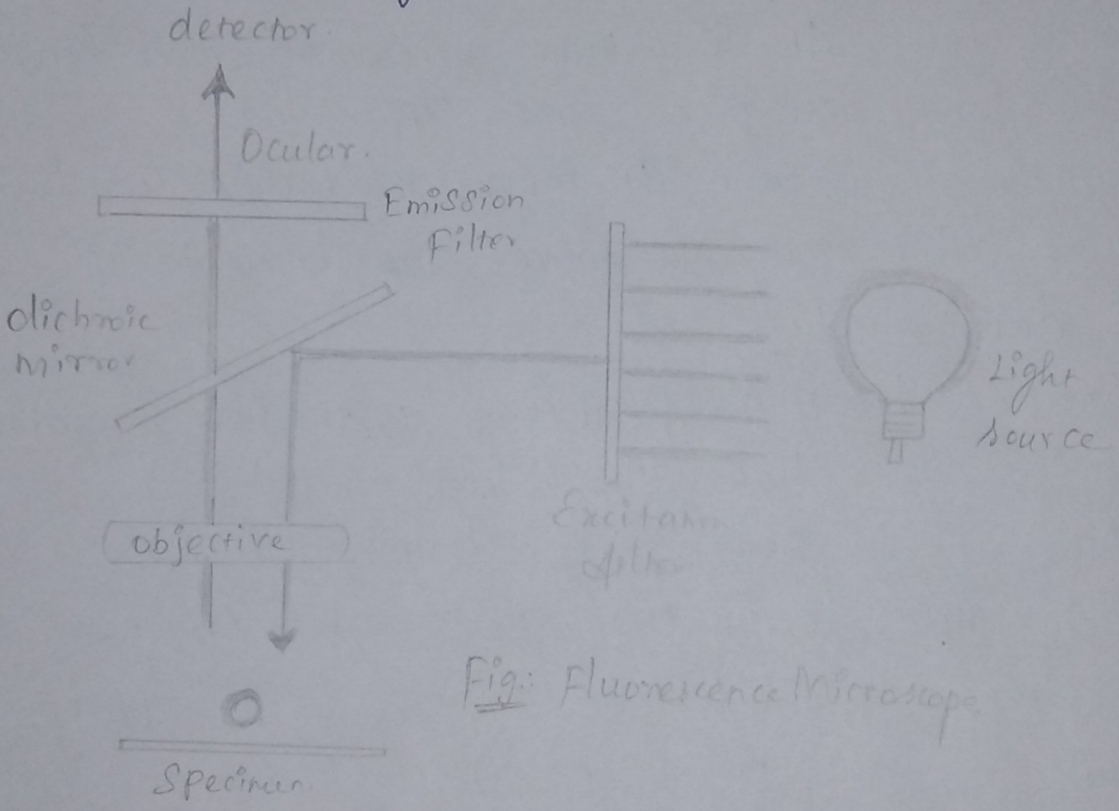

Fig: Fluorescence Microscope

Principle:-

* The Specimen is illuminated with light of a Specific wavelength, which is absorbed by the fluorophores, causing them to emit light of longer wavelengths.

The illumination light is Separated from the much weaker emitted fluorescence through the

the use of Spectral emission filter. Typical components of a fluorescence microscope are a light source

Electron Microscope.

* Electron microscope is a system of electro magnetic coils where electron beam is used as the source of illumination.
* Electron microscope gives a magnification of 2000 times than that of light microscope
* The first electron microscope designed by Knoll and Ruska in 1932
* There are two types of Electron Microscope
    * Transmission electron Microscope. (TEM)
    * Scanning electron Microscope. (SEM)

Transmission electron Microscope (TEM).

* Electron microscope in which electron beam is passed through the specimen to produce its image is called TEM.
* The First TEM was designed by Knoll and Ruska in 1931.

Principle:

* The basic principle of electron microscopes is similar to the optical principle of ordinary Compound Microscope
* Here, electron beam is substituted for light

beam and electromagnetic Coils are Substituted for optical lenses.

Instrumentation:

* The TEM Consists of an electrogun, Condenser, objective, amplifier, projector lenses and fluorescent Screen or photographic plate.
* Electron gun is the Source of electron beam used in this Microscope. It Consists of V-shaped filament
* There are two Condenser lenses just below the electron Gun. They are nothing but electromagnetic Coils.
* The objective lens is another electromagnetic Coil which is placed below the Specimen Stage
* The amplifier lens is yet other electromagnetic Coil Kept just below the objective lens.
* A projector lens Collects the magnified image and focuses it onto a fluorescent Screen photographic plate.

Application of TEM:

* TEM is an ideal tool for the Study of ultrastructure of Cells.
* It is used in the identification of plant and animal viruses based on their structural features.

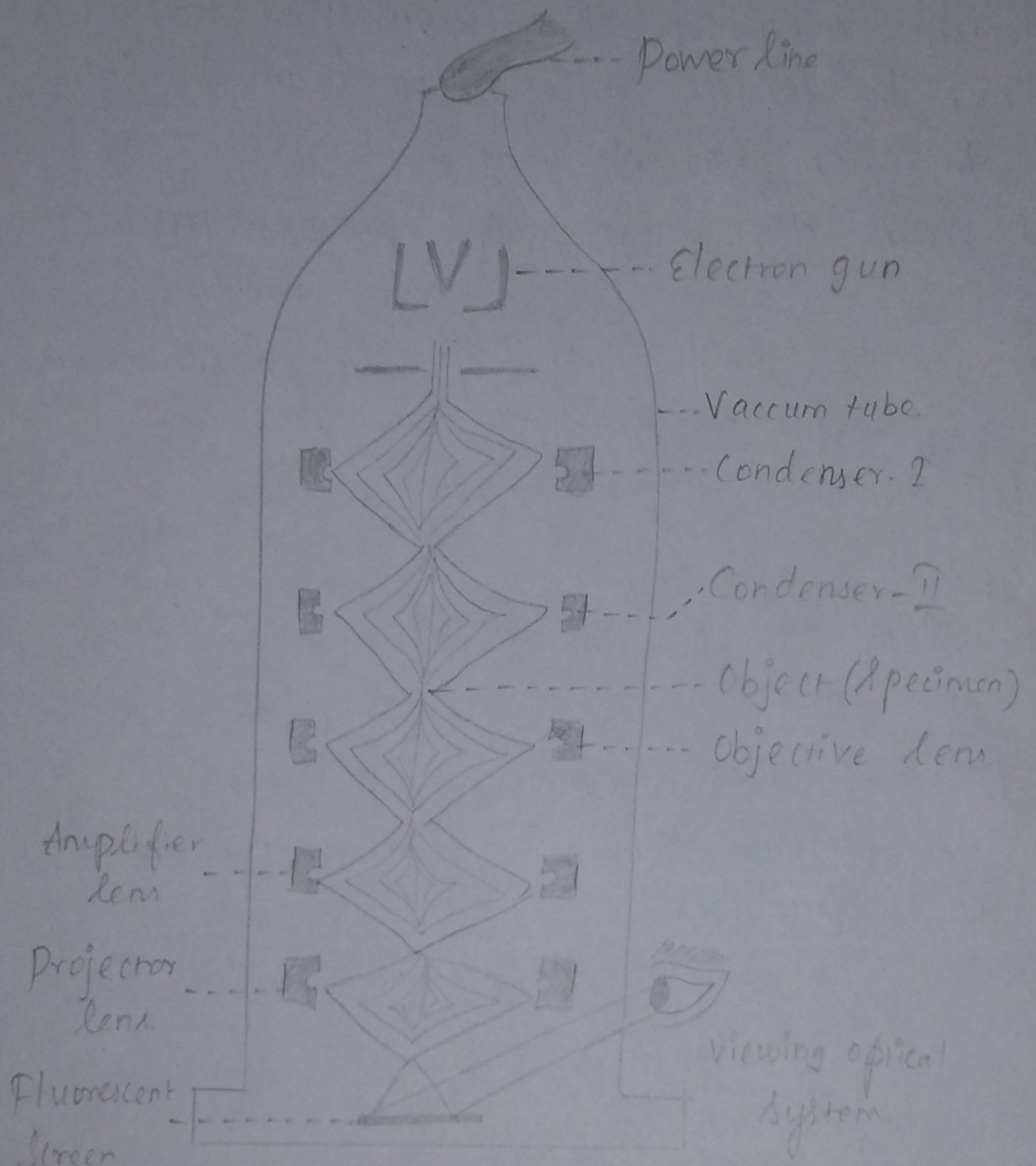

Fig.: Simplified diagram showing the electron path of TEM

* It is employed in the localization of nucleic acid, enzymes and protein in cells and cell organelles.
* It is used in Cancer research for the cytological observation of Cancer cells.

Scanning Electron MicroScope (SEM)

* Electron microscope that Scans the Surface of Specimen by passing an electron beam is called SEM. The SEM was designed by Max Knoll in 1935.
* It Scans the Surface of Specimen using electron beam more like a xerox machine Scanning the Surface of paper by laser beam It's very useful to Study the Surface architecture of Cell membrane, Organelles and others.

Principle:

* The SEM also uses an electron beam as illumination and electromagnetic Coils to direct the path of electron beam.
* When electron beam is focused on the Specimen, it produces Secondary electrons (SE), back Scattered electrons (BSE) and Characteristic X-rays.

Instrumentation:-

* The SEM Consists of an electron gun, two Condenser Coils, objective lens, Specimen Stage, grid, Scintillator, photomultiplier tube (PMT), Scanning Circuit and x-ray detector.
* Electron gun is the Source of electron beam used in this microscope
* There are two Condenser lenses just

below the electron gun. They are electromagnetic coils.

* The Specimen Srage is placed just below the deflection Coil. It is in a Slanting position (4.5°) in the electron path.

* Separate electron detectors are attached to the vaccum tube of SEM. Each of these Electron detector is formed of a Collector, a Scintillator and PMT.

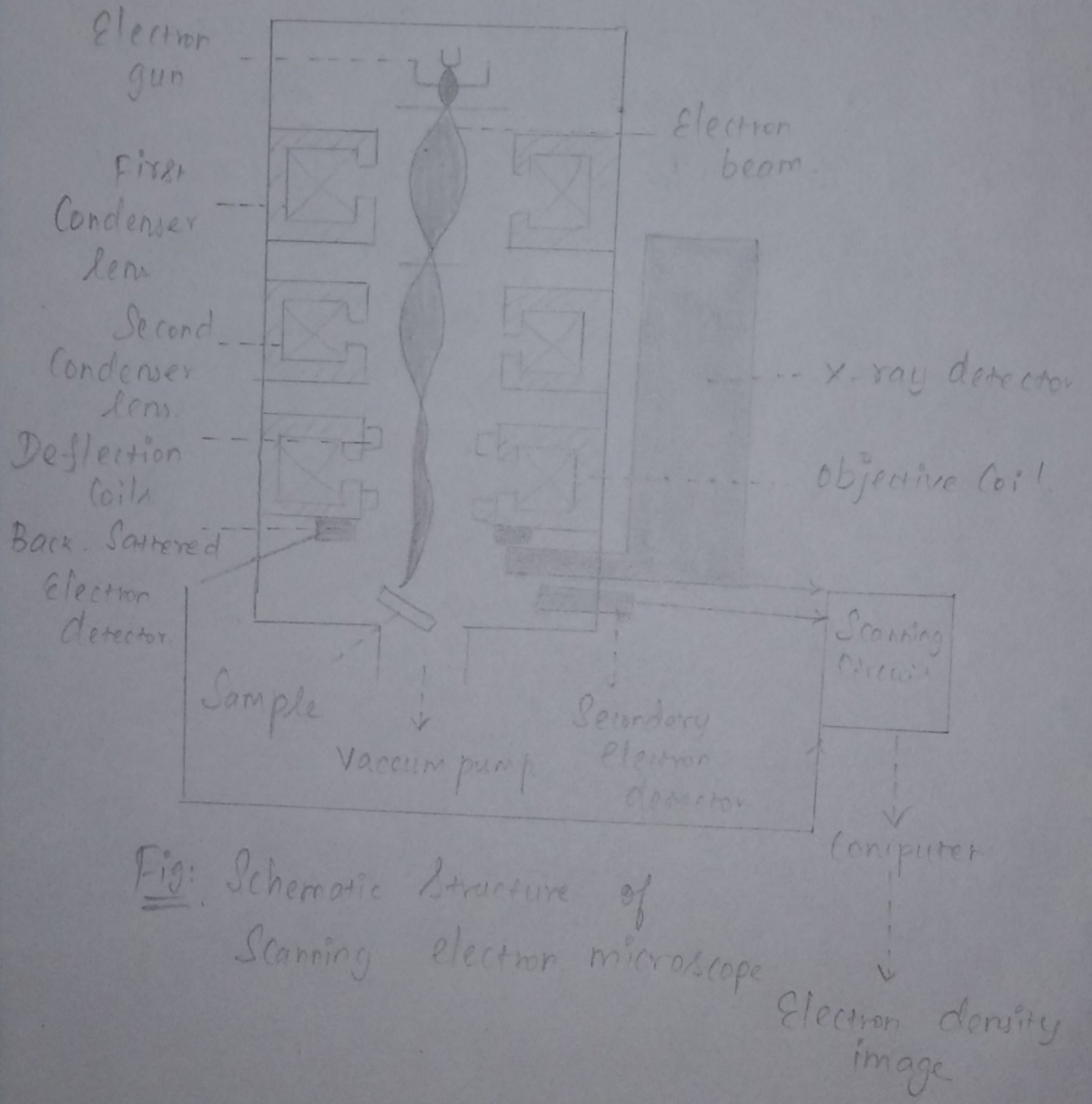

Fig: Schematic Structure of Scanning electron microscope

Application of SEM:

* SEM is very useful to view the surface architecture of microscopic creatures like bacteria, diatoms, pollen grains, nematodes and others.
* SEM gives the 3D structure of objects to reveal the structure of organism and organelles.
* SEM is employed in the analysis of structural features of compound eyes of insects.
* Hairs and scales on plant and animal surfaces are characterized with the SEM.
* SEM is used to study the surface of small archeological specimens and fossils.