

Ex. No: 2 Microscopy: various types of microscopes. Observing sections of specimen using Electron microscope; Preparing specimen for observation-Fixative preparation and fixing specimen for light microscopy studies in cereals

The term microscopy refers to the study of microscopes. Microscope is a Greek word meaning (MikronG – small, SkopeinG – to see) to see small things. It is an instrument used to produce an enlarged image of a small object, which otherwise would be invisible to naked eyes. This is achieved by a combination of several lenses and a light source to illuminate the object. According to the source of light employed for illumination, microscopes can be classified as 1. Light microscope 2. X ray microscope and 3. Electron microscope.

1. Light microscopes

These microscopes use ordinary light as source of illumination. These can be of several types.

i) Simple microscope

These are similar to magnifying glasses and consist of one or several lenses.

These microscopes produce erect virtual image on the retina. Eg. Hand lenses, Spectacles and dissecting microscopes.

ii) **Compound microscope**

A compound microscope consists of three types of lens systems viz., Condenser, lens system, Objective lens system and Eyepiece lens system. The condenser lens system occurs beneath the specimen and its function is the collection and focusing of the light rays on the object or specimen, which is placed on the stage of the microscope. The objective lens system remains near and above the specimen. It produces and magnifies the image of the specimen. The eyepiece or ocular lens system remains near the eyes of the observer and it magnifies and forms the secondary image of the primary image previously produced by the objective.

Apart from these lens systems, the compound microscope consists of the following components

- a) The body of the microscope is called stout having a stout base, which is horseshoe shaped and a slightly curved limb. By adjusting the hinge joint of the limb, the stout can be tilted backwards slightly
- b) A mirror with a plain side and a concave side is attached to the base of the stout. It is provided with a cymbal adjustment and can be manipulated in any way depending on the light

source so as to reflect the source light on to the object through the condenser lens. c) The platform on which objects are placed is called stage of the microscope. It consists of a central aperture and clips. The stage is attached to the stout. d) The body tube, which is vertical or slanting, is attached to the limb. The body tube can be lowered or lifted up. e) Fixed to the lower end of the body tube is the revolving nosepiece which carries the objectives.

iii) Ultra Violet microscope

In UV microscopes, instead of ordinary light, invisible UV rays of short wave lengths are employed for the illumination of objects. The glass lenses are replaced with lenses of fused quartz. These microscopes are efficient in qualitative and quantitative determinations of different cellular components.

iv) Phase contrast microscope

These microscopes are employed to study materials showing little or no contrast in their properties like colour, density etc. This is achieved by passing the light rays through glass of two thicknesses so that the rays passing through thickest glass travel slowly. These microscopes are used mainly for scanning of unstained cells for division.

v) Fluorescence microscope

Fluorescent microscopes substitutes' fluorescence in UV light for the colour of the specimen. Mercury lamps are used as UV source. These microscopes are used in microbiological and histological studies and to some extent in chromosome banding technique.

2. X-Ray microscope

This microscope is used for analysis of the three dimensional structures of micro molecules and macromolecules of the cell. The molecules to be investigated must be present in crystalline form. In X ray microscopes, electromagnetic lenses or reflecting curved mirrors focus the x-ray beams and image are formed on a film.

3. Electron microscope

Things that are too small to be seen with a light microscope can be seen with an electron microscope. Instead of using lens to bend beams of light, an electron microscope uses a magnetic field to bend beams of electrons. The electrons are released from the cathode in an electron gun. These beams travel in straight line in vacuum. They can be concentrated and can be deflected in an electrostatic or magnetic field. This microscope can magnify objects 3,00,000 times. The magnified images are projected on a fluorescent screen where they are visible and are photographed. This microscope is classified into two types. i) Transmission Electron Microscope (TEM): In this type of microscopes, the electrons emitted by the cathode ray tube

passes through the object and are focused on to the fluorescent screen by an electromagnetic objective lens. This is used to study parts inside the cell. ii) Scanning electron Microscope (SEM): This microscope is used to scan the surface of the objects. It produces three-dimensional images of the surface architecture.

Method of Measurement of cells

The cells are the fundamental units of life forms and are of microscopic size. The cells and their components are usually measured in micrometer (μm) or microns (μ) and Angstroms (AO). In light microscope the cells are measured by an ocular micrometer disc and a stage micrometer. The ocular micrometer disc is usually a glass plate, which is placed on the ocular diaphragm. The micrometer disc must be calibrated before measurements are made. A calibration is made for each ocular lens, length of body tube and objective to be used. The stage micrometer is usually a glass slide on which a scale of known intervals has been marked. When the ocular micrometer disc and the stage micrometer are graduated in the same units, the calibration of the ocular micrometer disc can be done easily. This is done by focusing on the stage micrometer, setting the zero line on it coincident with the zero line on the ocular micrometer disc, and reading across the scale to the next two lines that are coincident with each other. The magnification factor is obtained by dividing the known value on the stage micrometer into the ocular value. Then the size of any object viewed in the light microscope will be the dimension shown on the ocular disc scale divided by the magnification factor.

Exercise

i) Draw the diagram of a compound microscope and label its parts. ii) Draw the optical path ways of light and electron microscope.