Introduction to Laboratory

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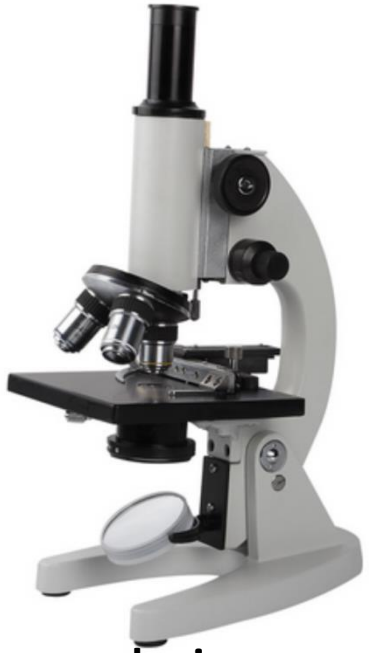
Necessary Instruments

1. A pair of forceps,
2. Two fine, long handle, dissecting needles,
3. Glass droppers,
4. Good and sharp razor,
5. Safety blade,
6. A fine brush,
7. A pair of sharpened pencils,
8. Pencil eraser,
9. A clean and soft handkerchief and
10. Practical record with cover file and spare pages, etc.

Microscope

1. 'The primary instrument of the biologists' .
2. It helps to increase the resolving power (property to distinguish objects lying very close as separate bodies) of human eye which fails to recognise objects lying closer between 0.01 to 0.25 mm.
3. Some common types of microscopes are listed below-
    1. **Dissecting microscope**,
    2. **Compound microscope**,
    3. Binocular microscope,
    4. Phase contrast microscope and
    5. Electron microscope.

Dissecting microscope



Compound microscope

Dissecting microscope

1. It consists of basal foot,
2. A vertical limb, stage and a lens.
3. The basal foot is a stand.
4. The limb has an attached stage made of glass plate.
5. A folded arm which can be moved vertically holds the lens.
6. A mirror is attached at the base of the limb.

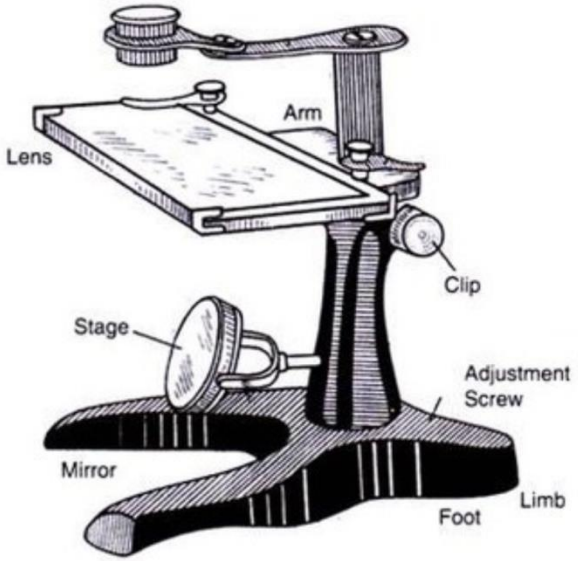
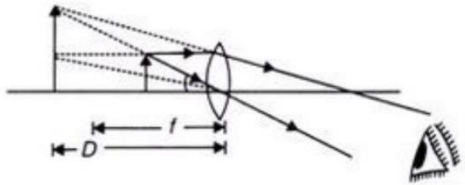
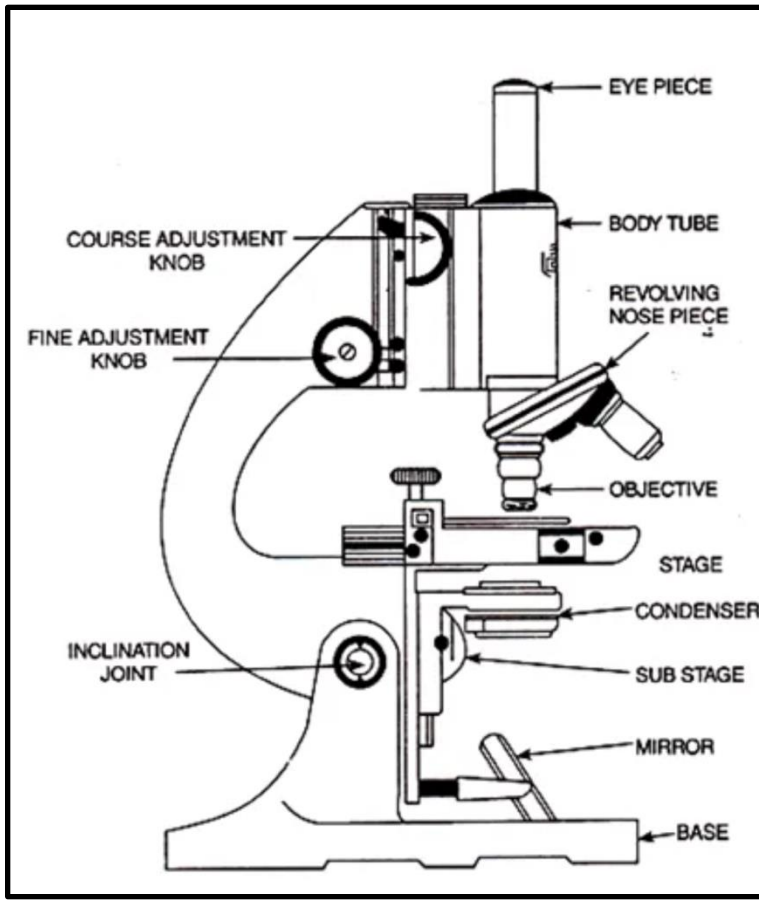


Fig. 6.1 Simple/dissecting microscope



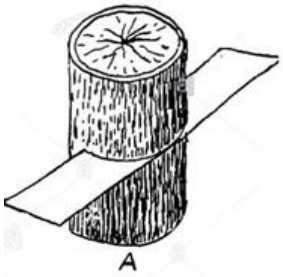
Compound microscope

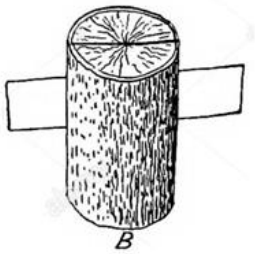
- It is one of the most commonly used and the most suitable microscope in the Botany Laboratory.
- At one time, it employs
  - one ocular (eye piece) and
  - one objective, in working position.
- It is also known as monocular-mono-objective microscope.



Laboratory Techniques

1. Section cutting: Sections of preserved material are cut in suitable planes for histological and ecological studies.
2. Planes. The following are a few commonly needed planes-
3. In case of cylindrical organs : (e.g., stems, roots, etc.).
    1. Transverse: The section is cut by passing razor's edge at right angles to the longitudinal axis.
    2. Longitudinal: The section is cut by passing razor's edge at right angles to the transverse axis.





Stains and staining

1. The selected sections need to be stained.
2. The stains help to distinguish different tissues, cells or inclusions from one another by developing specific colours.
3. Some of the commonly used stains.
   a) Acetocarmine:
   b) Aniline blue:
   c) Crystal violet:
   d) Erythrosine:
   e) Hematoxylins:
   f) Fast green:
   g) Light green:
   h) Safranin:

Specificity

Most of the stains are specific in reaction and are purposely used so that definite structures or substances are stained.

- Achromatic figure
  - Aniline blue
  - Erythrosine
  - Fast green
  - Light green
- Cellulose cell wall
  - Aniline blue
  - Delafield hematoxylin
  - Fast green
  - Light green
- Lignified cell wall
  - Crystal violet
  - Safranin
- Suberized cell wall
  - Safranin
- Cutinized cell wall
  - Crystal violet
  - Erythrosine
  - Safranin
- Nucleus
  - Crystal violet
  - Hematoxylin
  - Safranin

Stains

1. Bryophyta:
   a) Members are stained usually with safranin and mounted in 10% glycerine.
2. Pteridophyta and Gymnosperms:
   a) Representatives of these groups are stained by a double staining method.

Method of single staining

1. The material is kept in a watch glass.
2. A few drops of stain are added so that the material is immersed in the stain.
3. The material is allowed to remain so for a few minutes and allowed to take stain.
4. The time required varies with materials.
5. After the stain is taken up, the excess of stain is washed off.
6. The stained material is ready for mounting.
7. Demo:
    1. https://www.youtube.com/watch?v=YU0DYrU_0FM
    2. https://www.youtube.com/watch?v=GlCOQijkIKc

Collection

1. While on a collection trip, local or out-station, following things are to be carried along.
    1. Containers. For packing the collected material, preferably carry plastic unbreakable containers or polyethylene bags.
    2. Preservatives.
        1. Formalin-Acetic-Alcohol (FAA) or
        2. Alcohol 70% or
        3. Alcohol 90%, and/or
        4. Formalin 6%-10%.
    3. Other requirements:
        1. Scalpel,
        2. Knife,
        3. Blade,
        4. Forceps,
        5. Pencil,
        6. Paper,
        7. A hand lens,
        8. A bag or
        9. Vasculum for keeping plants or plant press with many newspapers or blotting papers.

Safranin Staining

1. Introduction:
   a) Safranin is a cationic dye
2. Properties
   a) Chemical name: 3,7-Diamino-2,8-dimethyl-5-phenylphenazinium chloride
   b) Molecular weight: 350.84
   c) Molecular formula: $C_{20}H_{19}C_lN_4$
   d) Solubility: Soluble in ethanol
   e) Color: Brownish red
   f) Odor: No specific odor
   g) Physical structure: Powder

Staining procedures

1. To prepare the staining solution;
   a) Add 20mg safranin powder to a 100ml beaker.
   b) Pour 20ml distilled water in the beaker and make 0.1% safranin staining solution by constant stirring.
   c) Filter the staining solution to avoid particles.

Double staining

1. Safranin:
   a) Add 20mg safranin powder to a 100ml beaker.
   b) Pour 20ml distilled water in the beaker and make 0.1% safranin staining solution by constant stirring.
2. Light Green:
   a) Transfer 20mg of fast green dye in another 100ml beaker.
   b) Make it 0.1% staining solution by adding 20ml distilled water in it.
3. Filter both the staining solutions to avoid particles.

Double Staining:

1. Safranin-Fast Green Method:

Select a thin section

↓

Stain with safranin (for 3 to 5 minutes)

↓

Wash with water

↓

Destain with acid alcohol (if need be)

↓

Wash thoroughly with water to remove the traces of acid

↓

Stain with fast green (for a few seconds to one minute)

↓

Wash with glycerine

↓

Mount in glycerine

2. Double staining Demo; https://www.youtube.com/watch?v=RO-SdUmxu5s