

Ex.No.1

HANDLING OF MICROSCOPE

AIM

To understand the proper care and maintenance of microscope.

PRINCIPLE

A simple microscope works on the principle that when a tiny object is placed within its focus, a virtual, erect and magnified image of the object is formed at the least distance of distinct vision from the eye held close to the lens.

The magnifying power of a microscope is given by:

$$M = 1 + D/F$$

D = least distance of distinct vision

F = focal length of the convex lens

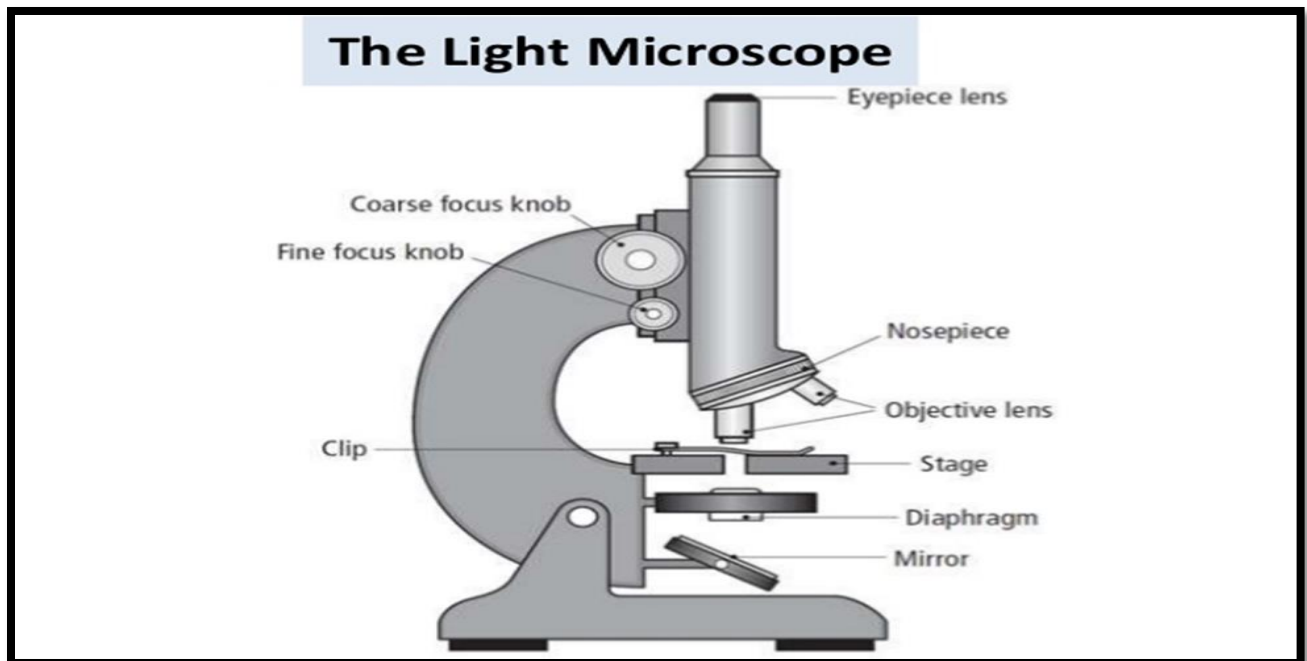
The focal length of the convex lens should be small because smaller the focal length of the lens, greater will be its magnifying power. The maximum magnification of a microscope is about 10, which means that the object will appear 10 times larger by using the simple microscope

PROCEDURE

A microscope is a high quality instrument and should last 25-30 years if treated properly and with care.

- When moving the microscope, always carry it with both hands. Grasp the arm with one hand and place the other hand under the base for support.
- Turn the revolving nose piece so that the lowest power objective lens is "clicked" into position (This is also the shortest objective lens).
- The microscope slide should be prepared by placing a coverslip or cover glass over the specimen. This will help to protect the objective lens if they touch the slide.
- Place the microscope slide on the stage and fasten it with the stage clips. You can push down on the back end of the stage clip to open it.

- Look at the objective lens and the stage from the side and turn the coarse focus knob so that the objective lens moves downward. Move it as far as it will go without touching the slide
- Now, look through the eyepiece and adjust the illuminator (or mirror) and diaphragm for the greatest amount of light.
- Slowly turn the coarse adjustment so that the objective lens goes up (away from the slide). Continue until the image comes into focus.
- Use the fine adjustment, if available, for fine focusing. If you have a microscope with a moving stage, then turn the coarse knob so the stage moves downward or away from the objective lens.
- Move the microscope slide around so that the image is in the center of the field of view and readjust the mirror, illuminator or diaphragm for the clearest image.
- Now, you should be able to change to the next objective lens with only minimal use of the focusing adjustment.
- Use the fine adjustment, if you cannot focus on your specimen, repeat steps with the higher power objective lens in place. When finished, lower the stage, click the low power lens into position and remove the slide.



Ex.No.2

CYTOCHEMICAL STAINING OF PROTEINS BY COOMASSIE BRILLIANT BLUE STAIN

AIM:

To identify the presence of proteins in plant or animal cell by cytochemical staining technique.

PRINCIPLE:

The Coomassie Brilliant Blue G-250 dye has three forms: anionic (blue), neutral (green), and cationic (red). In an acidic environment, the red dye is converted into its blue form after binding to the protein of interest. If no protein binds to the dye, then the solution will remain brown. The CBB stain forms a strong, noncovalent complex with the carboxyl group of the protein by van der Waals force and the amino group through electrostatic interactions. During the complex formation, the red form of the Coomassie dye first donates its free electron to the ionizable groups on the protein causing a disruption of the protein's native state and exposing its hydrophobic pockets. These pockets non-covalently bind to the non-polar components of the dye via van der Waals forces. The ionic interaction further strengthens the bond. This complex formation enables the detection of the proteins separated by the gel.

REAGENTS REQUIRED:

1. 2.5% glutaraldehyde
2. Paraffin
3. Coomassie brilliant blue R dye
4. Clarke's solution
5. Ethanol

PROCEDURE

1. Fresh plant or animal tissue is first fixed in 2.5% glutaraldehyde and embedded in paraffin.
2. Section the material, deparaffinise (if necessary) and bring down to water.
3. Stain in 0.02% Coomassie brilliant blue R 250 in Clarke's solution (pH 2.0).
4. Rinse in Clarke's solution.
5. Destain in fresh Clarke's solution for 20 minutes.
6. Dehydrate in 98% and absolute ethanol for 5 minutes and mount.

RESULT:

Proteins stains in blue colour.

Ex.No.3

CYTOCHEMICAL STAINING OF RNA BY METHYL GREEN-PYRONIN

AIM:

To identify the presence of RNA in plant or animal cell by cytochemical staining technique.

PRINCIPLE:

Methyl green is an impure dye containing methyl violet. When methyl violet has been removed by washing with chloroform, the pure methyl green appears and is specific for DNA. Both dyes are cationic, when used in combination methyl green binds preferentially and specifically to DNA, and pyronin binds RNA.

REAGENT REQUIRED:

Fixation:

Carnoy is preferred, but formalin is acceptable.

Staining Solution:

Methyl green pyronin

Mix 9ml of 2% methyl green dye, 4ml of 2% pyronin Y, 23ml of Acetate buffer pH(4.8) and 14ml of Glycerol before use.

PROCEDURE

1. Take sections down to water.
2. Rinse in acetate buffer pH 4.8.
3. Place in methyl green-pyronin Y solution for 25 min.
4. Rinse in buffer.
5. Blot dry.
6. Rinse in 93% ethanol, then in absolute ethanol.
7. Rinse in xylene and mount.

RESULTS

DNA Stains in Greenish blue colour.

RNA stains in red colour.

Ex.No.4

CYTOCHEMICAL STAINING OF POLYSACCHARIDE BY TOLUIDINE BLUE

AIM:

To identify the presence of polysaccharides in plant and animal cells by Cytochemical staining technique.

PRINCIPLE:

Mast cells are found in the connective tissue and their cytoplasm contains granules (metachromatic) composed of heparin and histamine. Toluidine blue should stain mast cells red-purple (metachromatic staining) and the background blue (orthochromatic staining). Metachromasia, tissue elements staining a different color from the dye solution, is due to the pH, dye concentration and temperature of the basic dye. Blue or violet dyes will show a red color shift, and red dyes will show a yellow color shift with metachromatic tissue elements.

REAGENT REQUIREMENTS:

Fixation: 10% Formalin.

Sections: paraffin sections at 5 μ m.

1. Toluidine Blue Stock Solution:

Mix 1g of Toluidine blue O (Sigma) in 100ml of 70% alcohol

2. Sodium Chloride (1%):

Mix 0.5g of Sodium chloride in 50ml of Distilled water (make this solution fresh each time).
Adjust pH to 2.0~2.5 using glacial acetic acid or HCl.

3. Toluidine Blue Working Solution (pH 2.0~2.5):

Mix 5ml of Toluidine blue stock solution in 45ml of 1% Sodium chloride (pH 2.3). The pH should be around 2.3 and less than 2.5 .

Make this solution fresh and discard after use. pH higher than 2.5 will make staining less contrast.

PROCEDURE:

1. First select fresh, frozen or chemically fixed and paraffin embedded tissue.
2. Deparaffinize and hydrate sections to distilled water.

3. Stain sections in toluidine blue working solution for 2-3 minutes.
4. Wash in distilled water, until most of the excess stain has washed out.
5. Dehydrate quickly through 95% or 100% alcohol (10 dips each since stain fades quickly in alcohol).
6. Clear in xylene or xylene substitute for 3 minutes.
7. Coverslip with resinous mounting medium.

RESULTS:

Sulphated and carboxylated polysaccharides stains in pink to reddish pink colour.

Background stains in blue colour.

EX.No.5

STUDY OF MITOSIS IN ONION ROOT TIP

AIM

To study the different stages of mitosis in onion root tip from temporary slides.

REQUIRMENTS

1. Onion root tips
2. Slides
3. Coverslips
4. Filter papers
5. Acetocarmine stain
6. Dissecting needles
7. Forceps
8. Watch glass
9. Microscope
10. Spirit lamp
11. Incubator
12. 1N HCl
13. Acetic acid
14. Methanol
15. Formalin

PROCEDURE

To make slide of onion root tip

- Remove the old roots from onion and put in bottle full of water. New roots appear in 4-5 days.
- Cut the root tips when the roots are about 2-3cm in length.
- Root tips are then transferred to FFA fixative for 24 hours. (FFA fixative = 40% formalin, 90% alcohol and glacial acetic acid in the ratio of 1:9:5)
- Transfer the root tips to 1:1 solution of conc.HCl : alcohol for 15-20 minutes and the these can be preserved in 70% alcohol for long.
- To make the slide, transfer the root tips to 1N HCl/0.4N HCl in a watch glass and incubate it for 5minutes at 60 C.
- Decant 1N HCl/0.4N HCl and add few drops of acetocarmine in watch glass and warm it till vapours are seen (Do not allow the solution to boil).
- Place the root tips on a slide, cut the tips and discard the rest of the root.
- When the tip is sufficiently stained, place the coverslip over it.

- Squash the root tip with the help of a needle or forcep after keeping it in folds of blotting paper. Even gentle pressure can be applied with the thumb for uniform spreading of the cells.
- Observe the slide first under low power and then under high power locating a specific area.
- Examine different stages of mitosis.

RESULT AND OBSERVATION

Mitosis is defined as “A process in which chromosome of a nucleus split longitudinally into two halves and their split halves migrate to the two poles constituting two daughter nuclei which are identical to each other as well as to the parent nucleus both qualitatively and quantitatively.”

The process of mitosis is completed under the following stages;

1. Interphase

- Stages in between two successive cell divisions and hence a non-dividing phase.
- Large and distinct nucleus with nuclear membrane.
- Nucleolus clearly visible.
- Nucleus contain chromatin network.

2. Prophase

- Enlarged nucleus and occupies most of cell volume.
- Chromatin network gets condensed and appear as long thread-like structure called chromosomes.
- Each chromosome consists of two chromatids held together by centromere.
- Nucleolus gradually disappears by the end of prophase.
- Nuclear membrane starts disappearing.

3. Metaphase

- Nuclear membrane completely disappear.
- Chromosomes become shorter and thicker and hence become distinct and clearly visible under the compound microscope.
- Chromosomes orient themselves towards the equator with their centrosomes arranged on a equatorial line forming metaphase plate. Out of two chromatids, one faces one pole and the other chromatid faces the opposite pole.
- Series of spindle fibres attach the centrosomes to the opposite poles.

4. Anaphase

- Centrosome of each chromosome divides into two so that each chromatid gets its own centromere.
- Spindle fibres gradually shorten so that each chromatid with its centromere is pulled towards its respective pole with the centromere proceeding towards pole and chromosome arms trailing behind.

- Each chromatid now behaves as a independent chromosome and is thus known as daughter chromosome.

5. Telophase

- At each pole, the daughter chromosomes start uncoiling, elongate and become thin and invisible.
- Nuclear membrane reappears.
- Nucleolus gets reconstituted.
- Two daughter nuclei are formed at the two poles of a cell.
- The two daughter nuclei are similar to the parent nucleus both qualitatively and quantitatively.

AIM

To study the various stages of meiosis in onion flower buds through temporary slides.

REQUIREMENTS

1. Onion flower buds
2. Slides
3. Coverslips
4. Filter papers
5. Acetocarmine stain
6. Dissecting needles
7. Forceps
8. Watch glass
9. Microscope
10. Spirit lamp
11. Acetic acid
12. Methanol

PROCEDURE

1. Onion root buds are placed in fixative which contains acetic acid and methanol in the ratio 3:1.
2. Open up floral bud and extract the anther.
3. Tease these anthers on a slide containing few drops of acetocarmine.
4. Warm the slide over spirit lamp for a minute.
5. Put the coverslip and squash it by pressing it with thumb or back of a dissecting needle after keeping slide between folds of filter paper.
6. Observe the slide under microscope.

RESULT AND OBSERVATION

Meiosis consists of two divisions – Meiosis I and Meiosis II. Meiosis I is the reductional part of the division as the chromosome number is reduced to half. Meiosis II is the equational division similar to mitosis.

Stages of Meiosis I

Meiosis I is divided into the following stages;

1. **Prophase I**

- It is divided into five sub stages.

Leptotene

- Chromosomes appear as long thread like structures.
- Chromosomes show beaded appearance due to chromomere.
- Nuclear membrane is clearly visible.

Zygotene

- Chromosomes are short and thick.
- Homologous chromosomes start pairing and form a bivalent. Pairing of homologous chromosomes is known as Synapsis.

Pachytene

- Pairing of homologous chromosomes is completed and they form bivalent or tetrads.
- Crossing over takes place between non-sister chromatids of homologous chromosomes.

Diplotene

- Paired chromosomes start separating.
- Chromosomes are held together at chiasmata i.e., the point of crossovers.

Diakinesis

- Chromosomes are short and condensed.
- Terminalization of chiasma takes place.
- Separation of paired chromosomes is completed.
- Nucleolus and nuclear membrane start disappearing.

2. Metaphase I

- Nuclear membrane and nucleolus completely disappeared.
- Chromosomes arrange in the center of cell. i.e., called Equatorial plate or metaphase plate.
- Spindle fibres are attached to centromeres of homologous chromosomes.

3. Anaphase I

- Homologous chromosomes move towards opposite poles.
- No division of centromere takes place.
- Number of chromosomes at each pole is reduced to half.

4. Telophase I

- Chromosomes reach the poles.
- Chromosomes start uncoiling and become thin.
- Nucleolus and nuclear membrane start reappearing.

Stages of Meiosis II

- It is similar to mitosis but there is no DNA duplication before it.
- Telophase I may or may not be followed by Interphase II but without DNA duplication.
- Meiosis II is divided into following stages;
 1. **Prophase II**
 - Chromosomes again reappear. Each chromosome has two chromatids.
 - Spindle fibres start appearing.
 - Nucleolus and nuclear membrane start disappearing.
 2. **Metaphase II**
 - Nucleolus and nuclear membrane are completely disappeared.
 - Chromosomes orient themselves at the metaphase plate.
 - Centromere divides separating the two chromatids, each chromatid having its own centromere.
 3. **Anaphase II**
 - Each chromatid has its own centromere.
 - Centromeres are attached to spindle fibres of opposite poles.
 - Chromatids start moving to opposite poles by shortening of spindle fibres.
 4. **Telophase II**
 - Chromosomes again uncoil to form chromatin network.
 - 4 Haploid nuclei are seen in a cell after appearance of nuclear membrane and nucleolus.

Cytokinesis

Cytoplasm is distributed to 4 nuclei in case of plant cell.

AIM:

To identify the presence of mitochondria in cabbage.

PRINCIPLE:

Mitochondria are the cytoplasmic organelle found in variable numbers in all plant animal cells. Large number of mitochondria in the cells can change the appearance of cells. Mitochondria are considered the ‘power houses’ of the cell as many of the energy producing biochemical reactions like oxidative phosphorylation and Krebs cycle activity takes place in mitochondria. Mitochondria can be demonstrated by electron microscopy, enzyme histochemistry and histological methods however electron microscopy is the most satisfactory method. Histopathological methods such as Altman’s technique for mitochondria is simple and useful for demonstration of mitochondria.

REAGENT REQUIRED:**Fixation**

Champy’s fluid is usually recommended, Helly’s fluid works equally as well.

Aniline-acid fuchsin – saturated solution of acid fuchsin in 5% aniline in distilled water.

Differentiator 1

Saturated alcoholic picric acid 10 ml

30% alcohol 40 ml

Differentiator 2

Saturated alcoholic picric acid 5 ml

30% alcohol 40 ml

PROCEDURE

1. Take sections down to water.
2. Flood sections with aniline-acid fuchsin solution.
3. Gently heat the slide until steam rises and leave for 5 min.
4. Rinse in tap water.
5. Differentiate in solution 1 until the excess red stain is removed.
6. Completely differentiate in solution 2, controlling microscopically.
7. Dehydrate rapidly in two changes of absolute alcohol.
8. Clear in xylene and mount in DPX.

Results

Mitochondria stains in red colour.

Background tissues stains in yellow colour.

Ex.No.8 SEPARATION OF PLANT PIGMENTS BY PAPER CHROMATOGRAPHY

AIM

To separate and determine the Rf value of plant pigments by paper chromatography.

PRINCIPLE

Paper chromatography is a useful technique in the separation and identification of different plant pigments. In this technique, the mixture containing the pigments is first applied as a spot to the paper about 1.5 cm from the bottom edge of the paper. The paper is then placed in a container with the tip of the paper touching the solvent. Solvent is absorbed by the paper and moves up the paper by capillary action.

The solvent carries the dissolved pigments as it moves up the paper. The pigments are carried along at different rates. Therefore, the less soluble pigments will move slower up the paper than the more soluble pigments. This is known as developing a chromatogram.

REAGENTS REQUIRED

1. Fresh spinach leaves of fresh green leaves.
2. Whatman filter paper No.1,
3. Chromatography chamber,
4. Chromatography solvent (9:1 petroleum ether & acetone),
5. Plastic pipettes
6. Pencil
7. capillary tube

PROCEDURE

Freshly green leaves were taken and washed with distilled water. 5ml of acetone was added to leaves and crushed thoroughly with the help of mortar and pestle. The green color solution was extracted. The plant extract are now used for paper chromatography.

A strip of chromatography paper (18 cm long) was taken and drawn a line 2 cm from the pointed end of the paper by using a pencil lightly. The plant extract was applied in the center of the chromatography paper by using capillary tube.

5ml of solvent was poured into the chromatographic chamber and the paper is dipped in it. The solvent carries the dissolved pigments as it moves up the paper. The different pigments were differently absorbed and hence are carried through different distances on the paper. When the solvent travelled about $\frac{3}{4}$ of the paper, the paper was removed carefully and air dried.

Chlorophyll b - Olive green

Chlorophyll a - Blue green

Xanthophyll - Yellow-brown

Carotene – Yellow

Rf equation = $\frac{\text{Distance travelled by the pigments}}{\text{Distance travelled by the solvent}}$

Distance travelled by the solvent

RESULT

