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**DEPARTMENT OF BIOTECHNOLOGY**

# CELL BIOLOGY AND GENETICS LAB MANUAL

**Prepared by**

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## CELL BIOLOGY AND GENETICS LAB

**Sub Code: BT36L** **Contact Hours: 3 /Week**

**Credits: 0:0:2** **Total Hours: 39**

**Prerequisites:** Students should know basic biology and theoretical concepts of cell Biology and genetics

**Course objective:** The objective of this course is:

1. To give practical experience in understanding different cell structure, its organelles and multiplication
2. To learn the laws of heredity with practical emphasis on inheritance

**Course outcomes**: After successful completion of this lab course, student:

Course Outcome 1(CO 1): would be able to identify and outline the structure of an eukaryotic cell at different magnification, measure the cell length and breadth using micrometry, differentiate stages of Mitosis and meiosis, isolate chloroplasts and estimate chlorophyll content in plant sample, record growth of single cells.

Course Outcome 2(CO2) would be able to isolate the DNA, identify and distinguish different blood cells, to solve simple genetic problems and analyze Human karyotype and pedigree.

**List of experiments**

1. Principle and utility of microscopy.
2. Observation of distinguishing features of different eukaryotic cells.
3. Measurement of stomatal cells
4. Preparation of blood smear and differential staining of blood cells.
5. Identification of Blood groups
6. Study of divisional stages in Mitosis.
7. Study of divisional stages in Meiosis.
8. Isolation of plant cellular DNA.
9. Observation of growth and differentiation in single cells.
10. Isolation of chloroplasts.
11. Simple genetic problems solving
12. Human Karyotype analysis
13. Simple Mendelian traits in humans and pedigree analysis.

**Cell Biology & Genetics Laboratory Manual**

Experiment No: 1

**Principle and utility of microscopy**

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The function of any microscope is to enhance **resolution**. The microscope is used to create an enlarged view of an object such that we can observe details not otherwise possible with the human eye. Because of the enlargement, resolution is often confused with **magnification**, which refers to the size of an image. In general, the greater the magnification, the greater the resolution, but this is not always true. There are several practical limitations of lens design which can result in increased magnification without increased resolution.

A compound microscope is composed of two elements; a primary magnifying lens and a secondary lens system, similar to a telescope. Light is caused to pass through an object and is then focused by the primary and secondary lens. If the beam of light is replaced by an electron beam, the microscope becomes a transmission electron microscope. If light is bounced off of the object instead of passing through, the light microscope becomes a dissecting scope. If electrons are bounced off of the object in a scanned pattern, the instrument becomes a scanning electron microscope.

### Bright Field, Dark Field, Phase Contrast

All microscopes actually allow visualization of objects through minute shifts in the **wavelength phase** as the light passes through the object. Further image forming can be had through the use of color, or through a complete negative image of the object. If the normal phase shift is increased (usually by 1/4 wavelength), then the microscope becomes a **phase contrast microscope**. Phase contrast microscopes can be designed to have medium phase or dark phase renditions, by altering the degree of additional shift to the wavelength from 1/4 to 1/2 wavelengths, respectively.

If the beam of light is shifted in phase by a variable amount, the system becomes a **differential interference contrast microscope**. If the light image is reversed, then the microscope becomes a **dark field microscope**. All standard bright field microscopes can be readily converted to dark field by inserting a round opaque disk beneath the condenser. Dark field microscopy was first utilized to examine trans-filterable infectious agents, later to be termed viruses, and to determine that they were particulate in nature. Small objects, even those below the limits of resolution, can be detected easily with dark field, as the object appears to emit light on a dark field.

Finally, if the normal light microscope is functionally turned upside down, the microscope becomes an **inverted microscope**. This is particularly useful in tissue culture since it allows observation of cells through the bottom of a culture vessel, without opening the container, and without the air interface normally present between the objective and the surface of the culture. By adding phase contrast optics to the inverted microscope, it is possible to monitor tissue cultures directly, without the aid of stains or other enhancements.

## The Bright Field Microscopy

**Materials**

- Binocular Microscope
- Microscope slide with sample

**Procedure**

1. Pick up a microscope from the cabinet by placing one hand under the base and the other on the arm of the microscope. Most microscope damage is due to careless transport. It is important that you carry the microscope securely, with two hands, and in an upright position.
2. Place the microscope in front of you, unwind the power cord and plug it in. You will notice that all parts of the microscope are now conveniently located for your use, with an uninterrupted view of the stage, and the focus controls are conveniently at arms-length.
3. Note the magnification power and the numerical aperture of the lenses which are on your microscope's nose-piece. These values are stamped or painted onto the barrels of the objectives.
4. Prepare microscope slide with the sample. Place the slide on the stage and ensure that it is locked in place with the slide holder.
5. Rotate the condenser focusing knob to move the condenser to its highest position of travel. Although there is an ideal location for the condenser, the correct position of the condenser will vary slightly for each objective. Unless directed otherwise, it will not be necessary to move the condenser during any of the intended uses in this course.
6. If, however, you wish to find the ideal location, focus the microscope on any portion of a slide, and then simply close down the condenser aperture and move the **condenser** until you have a sharply focused view of the condenser aperture (usually with a slight blue hazy edge). If you do this, you can then open the aperture until it just fills the field of view (different for each objective). This is the correct location and use of the condenser and aperture and the condenser should not be moved from this position. Never use the condenser aperture for control of light intensity. Control of light intensity is the purpose of the variable rheostat (dimmer switch, or voltage regulator) on the light source.
7. Looking down into the microscope, adjust the eyepieces to your interpupillary distance and diopter. The microscope is equipped with a knob between the eye tube extensions for this adjustment. Many microscopes simply require pushing the eye tubes together or apart directly. Move the eye tubes back or forth until you see one uniform field of view. The first time you use the microscope, adjust the eyepieces for your personal comfort. Note that modern microscopes have HK (high eye point) eyepieces and consequently you need not remove eyeglasses if you are wearing them. Quite the contrary, they should be worn to prevent eyestrain while you constantly shift from looking through the microscope.
8. Begin by focusing the microscope on any object within the field of view.
    - Find a suitably contrast location in the center of the field of view and close your left eye. Using the coarse and fine adjustments, focus until you obtain a sharp image with your right eye only!
    - Now close your right eye and adjust the focus of the left eyepiece by rotating the diopter adjusting ring located on the left eyepiece. Do not readjust the focus of the

left eye with the coarse or fine adjustments of the microscope - use the adjustment ring on the eye tube.

10. All subsequent uses of the same microscope will involve use of the coarse and fine focus adjustments. That is, step 9 need only be performed once at the beginning of your lab. It may, of course, be checked periodically if desired, and will need to be readjusted if someone else uses your microscope.

**Unless otherwise instructed, do not use the preset device!**

11. Always begin focusing the microscope with the 10X magnification. Even if you are going to use the 100X, it is more efficient to begin with the 10X and then move up to the power desired.

12. The objective lenses are parfocal, which means that if one is focused, each of the others is approximately in focus when revolved into position. Rotate the coarse focus control until the slide is as close to the 10X objective as possible. Move the stage manipulators until a portion of the slide is directly under the objective and focus carefully on the object in view.

13. After adjusting the focus at 10X, center the object to be viewed, and rotate the nosepiece to the next highest magnification. Use the fine focus control only for the 40X or 100X objectives.

14. Manipulate the fine focus to obtain the sharpest image. During use of the microscope, one hand should remain on the fine focus as constant readjustment will be called for. Use the other hand to manipulate stage movements.

15. Note that the microscope is typically designed so that one revolution of the fine focus knob raises or lowers the microscope stage 0.2 mm. This permits direct readings on the fine focus knob scale to 0.002 mm (2 microns) and can be used to determine the thickness of materials being examined.

16. Use the 40X objective, center the object you wish to view and rotate the objective turret to bring the 40X objective into position. Is there any change in the orientation of the image?

17. In your observation book, draw the images of the sample at 10X and 40X and report the changes observed.

Experiment No: 2

## Observation of distinguishing features of prokaryotic and eukaryotic cells

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**Aim:** To observe the characteristics of prokaryotic and eukaryotic cells.

**Materials required**: Slides, cover slips, stains, microscopes and sample.

**Procedure**:

Prokaryotic sample
Prepare a smear of the bacterial suspension on a sterilized clean slide and stain by Gram staining procedure and observe under different magnification.

Eukaryotic sample
Peel off the epidermis of onion fleshy leaves and place on a drop of saffranin on a clean slide and observe at different magnification.

Report the differences in cell morphology between them.

Experiment No: 3

## Measurement of Onion epidermal cell

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**Aim**: To measure the dimension of a microscopic an object using ocular and stage micrometer

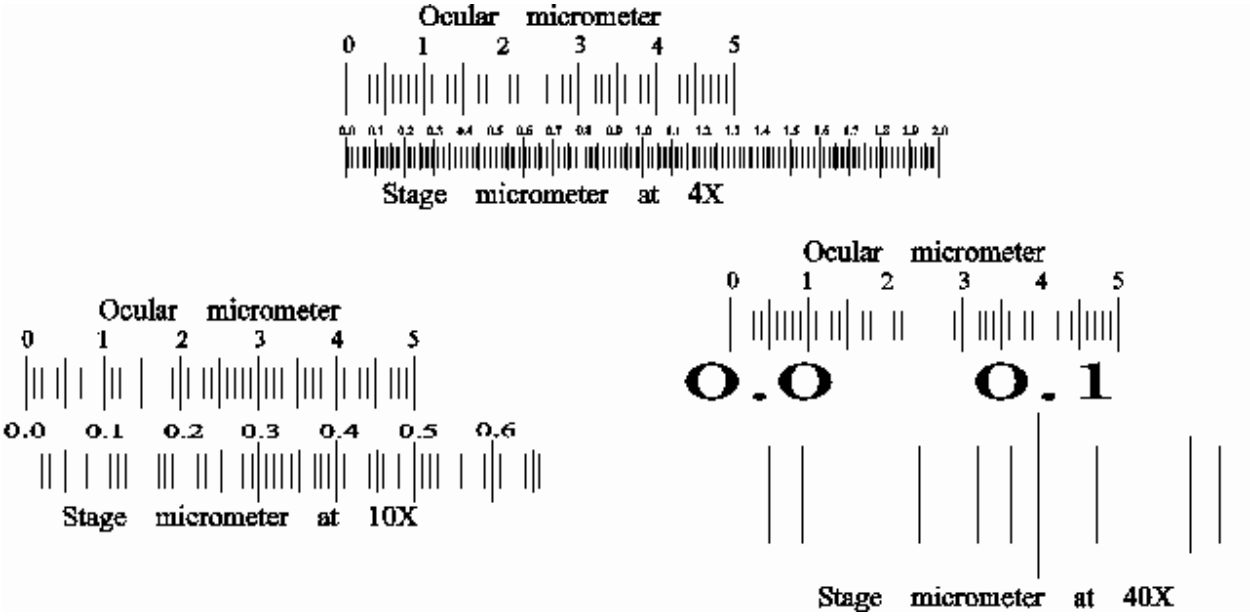
**Principle**: To measure an object seen in a microscope, an ocular micrometer serves as a scale or rule. This is simply a disc of glass upon which equally spaced divisions are etched. The rule may be divided into 50 subdivisions, or more rarely 100 subdivisions. To use the ocular micrometer, calibrate it against a fixed and known ruler, the stage micrometer. Stage micrometers also come in varying lengths, but most are 2 mm long and subdivided into 0.01 mm (10 micrometer) lengths. Each objective will need to be calibrated independently. To use, simply superimpose the ocular micrometer onto the stage micrometer and note the relationship of the length of the ocular to the stage micrometer (Refer to Figure 1). Note that at different magnifications, the stage micrometer changes, but the ocular micrometer is fixed in dimension. In reality, the stage micrometer is also fixed, and what is changing is the power of the magnification of the objective.

### Materials required

- Microscope
- Ocular micrometer
- Stage micrometer
- Millimeter ruler
- Prepared slide with sample

### Procedure

1. Place a stage micrometer on the microscope stage, and using the lowest magnification (4X), focus on the grid of the stage micrometer.
2. Rotate the ocular micrometer by turning the appropriate eyepiece. Move the stage until you superimpose the lines of the ocular micrometer upon those of the stage micrometer. With the lines of the two micrometers coinciding at one end of the field, count the spaces of each micrometer to a point at which the lines of the micrometers coincide again as seen in Figure 1.




3. Since each division of the stage micrometer measures 10 micrometers, and since you know how many ocular divisions are equivalent to one stage division, you can now calculate the number of micrometers in each space of the ocular scale.
4. Repeat for 10 X and 40 X and 100X. Record your calculations below.

| Microscope magnification | |
|---|---|
| Value for each ocular unit at 4X | |
| Value for each ocular unit at 10X | |
| Value for each ocular unit at 40X | |
| Value for each ocular unit at 100X | |

5. Using the stage micrometer, determine the smallest length (in microns) which can be resolved with each objective. This is the **measured** limit of resolution for each lens.
6. On a clean slide, place a small bit of onion epidermal peel and stain with saffranin. Place a coverslip and foucs the slide to view epidermal cell
7. Coincide one end of the cell with "0" position of the ocular micrometer and measure the length of the cell in terms of number of ocular- stage division.
8. Turn the eye piece around and measure the breadth of the cell.
9. Using the calculated values for your ocular micrometer, determine the dimensions of the onion epidermal cell found on your microscope slide, and add the dimensions to your drawing.

Experiment No: 4

## Preparation of blood smear and differential staining of blood cells.

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**Aim**: Preparation of blood smear and to observe different cell types in blood.

**Materials required:** Sterile needle, clean slide, blood sample, spirit lamp, Leishman's stain, blotting paper and cover slips.

**Procedure:**

1. Slightly warm the clean slides using spirit lamp and place a drop of blood.

2. Prepare a thin smear using another slide.

3. Dry the smear by waving the slide in air to prevent undue shrinking of blood cells.

4. Cover the air dried smear with 3-4 drops of Leishman's stain for one minute to effect fixation.

5. After one minute, flood the slide with drops of distilled water (double the number of stain drops) and leave for 15-20mins.

6. After 20 mins, rinse the slide with distilled water and dry by using blotting sheet and examine the smear under microscope.

7. Report the different types of cells observed in your sample.

Experiment No: 5

## Study of divisional stages in Mitosis.

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**Aim**: Preparation of onion root tip squash and observation of different stages of cell division.

**Materials required**: Onion root tips, glass slides, cover slips, watch glass, blade, brush, blotting paper, spirit lamp, 1N HCl, glacial acetic acid and carmine.

**Preparation of stain**: Acetocarmine is prepared by dissolving 1g of carmine in boiling glacial acetic acid. Then, it is filtered through a filter paper to obtain the desired intensity of stain.

**Procedure:**

1. Collect 1cm long two to three bits of onion root tips in a watch glass and was wash thoroughly using distilled water.

2. Drain off the distilled water and add 1 drop of 1N HCl plus nine drops of acetocarmine.

3. Warm the watch glass gently for 5mins keep it aside for cooling.

4. Take a bit of the tip on a clean slide, place enough drops of acetic acid to it to place a cover slip.

5. Cover the glass slide with blotting paper and apply pressure with the help of a thumb to get squash and proper spreading of cells.

6. Remove the blotting paper, clean the slide with tissue paper, observe under microscope and report the stages observed by you.

Experiment No: 6

## Study of divisional stages in Meiosis

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**Aim**: To observe the different stages of Meiosis using permanent slides of grasshopper's testes.

**Procedure:**

Observe permanent slides of different stages of meiotic division in Grasshopper's testes and report.

Experiment No: 7

# Isolation of plant cellular DNA

**Aim**: A simple and rapid method for isolation of cellular DNA

**Materials required:** coconut endosperm, Sodium chloride, Sodium Citrate, Mortar and pestle, centrifuge tubes and absolute alcohol.

**Procedure:**

(All steps to be carried out at $5^{o}C$)

1) Grind about 200mg of the tissue (coconut endosperm or spleen/heart/testis/kidney of any vertebrate) in about 5ml of Saline Citrate Solution (85ml of 0.9% sodium chloride solution + 15ml of 0.5% Sodium Citrate Solution – pH 7.4).
2) Transfer the homogenate into a centrifuge tube and make up the volume to 10ml with Saline Citrate Solution (SSC).
3) Centrifuge at 3000rpm for 8 minutes and discard the supernatant.
4) Rehomogenize the pellet with 5ml of SSC.
5) Adjust the volume to 10ml, centrifuge at 3000rpm for 8 minutes and discard the supernatant.
6) Then, suspend the pellet in 10ml of 12% sodium chloride solution and centrifuge at 10,000rpm for 15 minutes using a refrigerated centrifuge.
7) Transfer the supernatant into a 30ml test tube and add 2-3 volumes of absolute alcohol.
8) Gently mix it by inverting the tube. The white fibrous DNA precipitates.
9) Spool the fibrous white DNA by winding around a clean sterile bent glass rod.

**B) QUANTIFICATION OF DNA**

The presence of DNA in solution can be checked by the following methods.

a) 1) Transfer the spooled fibrous DNA into a 1.5ml eppendorf tube, add 1ml of 70% alcohol.

2) Centrifuge for 5 minutes at 10,000 rpm and discard the supernatent.

3) The pellet containing the DNA is dried, dissolved in 2ml distilled water and optical density is read in a spectrophometer at 260nm wavelength.

Experiment No: 8

## Observation of growth and differentiation in single cells

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**Aim**: To Observe growth and differentiation in single cells (pollen grains) by hanging drop culture method.

**Principle**: The hanging drop culture method involves suspending the pollen grains in a drop of nutrient medium on a cover glass hanging over a shallow depression. A thin film of Vaseline is applied around the rim of the cavity in a slide to raise the height of a cover glass when placed inverted and also to prevent the evaporation of the culture medium.

**Materials required:** Cavity slides, cover glasses, Petri dishes, Petroleum jelly or Vaseline, culture medium (Breubaker and Kwak medium, 1963).

Breubaker and Kwak medium: Sucrose – 100gm/lt, $H_3BO_3$- 100gm/lt, $CaNO_3$- 300mg/lt , $MgSO_4$- 200mg/lt and pH-7.3.

**Factors necessary for pollen germination:**

i) Relative humidity

ii) Sucrose: carbohydrate source, acts as a respiratory substrate, controls osmotic potential

iii) Boron: In the form of boric acid is necessary for pollen germination. In its presence pollen tubes are rigid and straight.

iv) Calcium: Supplemented in the form of calcium nitrate. Pollen wall is pecto-cellulosic in nature, calcium nitrate helps in the translocation of substances necessary for pollen germination.

v) Temperature: 18-30 ° C is supposed to be the optimum temperature.

**Procedure:**

1. Collect fully open flowers of Hibiscus, Impatiens and *Vinca rosea*. Select two or three flowers of each type that are mature and shedding pollen. The ripeness of the pollen seems to be critical to success, so sampling from more than one flower increases the chance of successful germination.
2. Carefully place a drop of Breubaker and Kwak's medium on a coverslip.
3. Carefully remove an anther containing mature pollen from a flower and gently touch it to the surface of medium. Or Transfer any pollen from the needle by tapping the needle against a pair of forceps placed next to the slide.You should see the pollen float onto the surface of the drops.
4. Clean the needle thoroughly and repeat step 3 so that you have two slides with pollen for each of the types of flower you are investigating.
5. Cavity slide, a special type of glass slide, which has a small concave portion at the middle, is used to practically "hang"or suspend the culture droplet on the coverslip, by raising the margin of the cavity using petroleum jelly. View the droplet as it is

suspended or "hanging" from the side. It is a "hanging drop" slide because the droplet remains untouched due to the concave shape of the slide and it just hangs from the cover glass.

6. Place a filter paper in each of the Petri dishes, moisten the paper with water and replace the lids and set aside. Note the time of adding pollen to the medium and place the slides in the Petri dishes. Handle the slides with great care so that the drops of pollen medium remain in the centre of the slides.
7. Remove the slide from the growth chamber, dry the bottom of it, and place it on the stage of the compound microscope.
8. Draw what you see at three different times, being certain to record the length of the pollen tube at each time and check after about 45 minutes for signs of germination.
9. Calculate the pollen germination percentage.

Germination % = number of germinated pollen per field/total number of pollen per field X100

Experiment No: 9

## Estimation of amount of chlorophyll present in the leaf tissue

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**Aim**: To estimate the amount of chlorophyll present in the given sample

**Principle**: Chlorophyll is extracted in 80% acetone and the absorption at 663nm, 645nm and 652nm are read through a spectrophotometer and using this absorption, amount of chlorophyll is estimated.

**Materials required**: Acetone, leaf tissue (Spinach), Mortar and pestle, centrifuge tubes

**Procedure:**

1) 1g of finely cut and well mixed representative sample of leaf is weighed into a clean mortar.
2) The tissue is ground into a fine pulp with the addition of 20ml 80% acetone.
3) The homogenate is then centrifuged at 5000 rpm for 5 minutes and the supernatant is transferred to a 100ml volumetric flask.
4) The residue is ground with 20ml of 80% acetone and centrifuged at 5000rpm for 5minutes.The supernatant is transferred to the volumetric flask.
5) The above step is repeated till the residue is colourless.
6) The mortar and pestle are washed thoroughly with 80% acetone and the clear washings are collected in the volumetric flask and the volume is made up to 100ml with 80% acetone.
7) The absorption at 663nm, 645nm and 652nm are read against a blank of 805 acetone solution.

**Calculations**

**mg chlorophyll a per gram tissue = $12.7(A_{663})-2.69(A_{645})$ x V/1000xW**

**mg chlorophyll b per gram tissue = $22.9(A_{645})-4.68(A_{663})$ x V/1000xW**

**mg chlorophyll a (total) per gram tissue = $20.2(A_{645})-8.02(A_{663})$ x V/1000xW**

Where 'V' final volume of chlorophyll extract in 80% acetone; 'W' is fresh weight of tissue collected

**Report:**

The total amount of chlorophyll present in the spinach leaf sample---------

Amount of Chlorophyll a ---------- and Chlorophyll b is ----------

Experiment No: 10.

**Genetic problem solving**

**Aim**: Solving Genetic problems which obey Mendelian laws.



Experiment No: 11.

## Human Karyotype analysis

**Aim**: Analyze the Human karyotype chart for different genetic disorders



Experiment No: 12.

Pedigree analysis

**Aim**: Analysis of human pedigree for the charts