Section 1

Haematology Experiments

Experiment 1.1

To study the compound microscope and observe common interfering objects under low power and high power

OBJECTIVES

At the end of the practical class student should be able to—

1. Identify the various parts of microscope and the objective lenses with their magnification power.
2. Focus the objects under low power, high power and oil immersion lens of the microscope.
3. Tell the function of condenser and its position while using low power, high power and oil immersion objectives.
4. Tell various types of surfaces of the mirror (reflecting mirror) present in the microscope and in which situation they are used.
5. tell the types of images formed in the microscope.
6. Identify the interfering objects may be present in the visual field (in slide), e.g. sand particles, starch particles, cotton fibers, hair, fat globules and air bubbles.
7. Tell basic principle of use of plane and concave mirrors in compound microscope.

APPARATUS

A compound microscope, glass slide, coverslip, sand, milk, wool thread, cotton fibers, hair and starch granules (crushed potato).

Compound Microscope

Microscope was invented by Antony Leeuwen Hoeck. Various parts of the microscope (Fig. 1.1.1) are as follows:

1. **Base:** Horseshoe-shaped base which provides stability to the microscope.
2. **Limb:** It joins the base to the optical part of the microscope by hinge joint.
3. **Handle:** It is a curved part which joins body tube to the stage of microscope.
4. **Body tubes:** Outer vehicle tube is attached with the handle. It can be moved up and down by coarse and fine adjustment screws, present on the upper part of the handle. Upper end of the inner tube has eyepiece of ten times (10x) magnification lens. Lower end of outer tube has revolving nose piece which contains three objective lenses with low power (10x), high power (40 or 45x) and oil immersion lens (100x).

   Identification of objectives: Each objective marked with its magnifying power, that is 10x, 40x, 45x and oil.

Magnification by using various objectives:

Total magnification = Magnification by eyepiece × magnification by objective

i. Low power (10x): 10 × 10 = 100 times
ii. High power (45x): 10 × 45 = 450 times
iii. Oil immersion lens (100x): 10 × 100 = 1000 times

5. **Fixed stage:** It is a fixed square platform with a hole in the centre. Slide or counting chamber is placed on it and light rays fall on the slide through central hole.
6. **Mechanical stage:** Two clips present on the fixed stage, are used to shift the slide side to side and

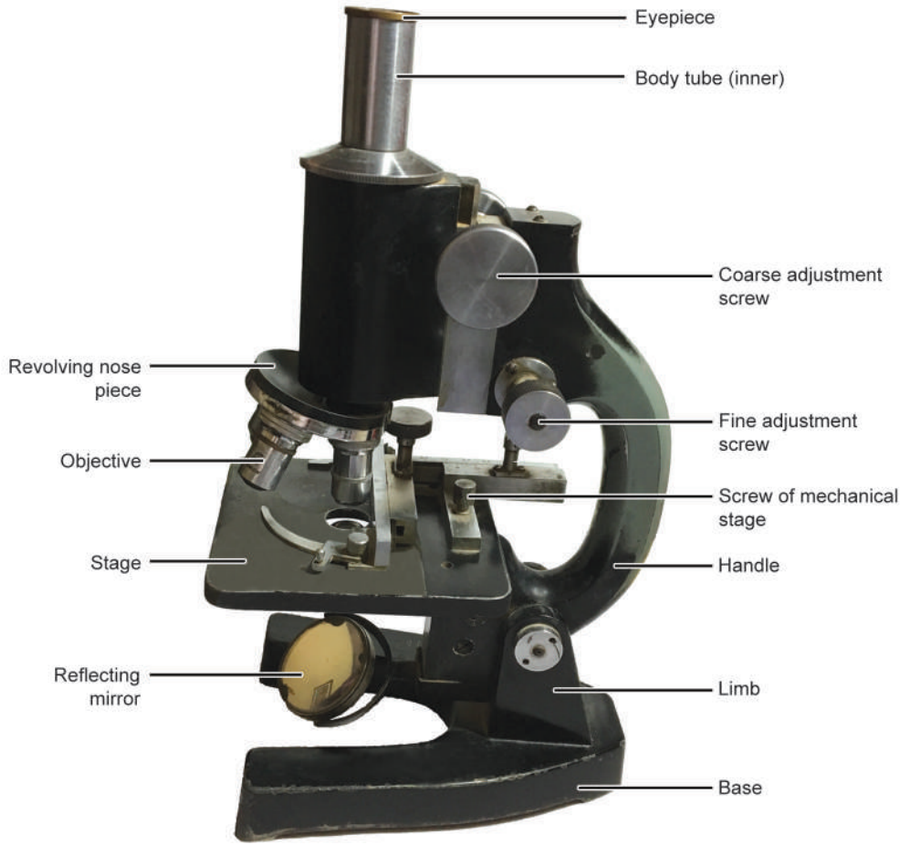


Fig. 1.1.1: Compound microscope (monocular).

backward and forward with the help of two screws present on the stage.

7. **Substage:** Under the fixed stage there is movable stage which has a diagram and a condenser. Aperture size of the diaphragm can be adjusted with the help of a knob present on its side.
   - **Condenser:** It is made up of two convex lenses. It condenses the light rays on the object. It can be moved up or down with the help of a screw present at the lowest part of the handle.
   - **Reflecting mirror:** There is a reflecting mirror having two reflecting surfaces, plane and concave below condenser. It reflects the light from light source to the object.

     Plane mirror is used when natural light is used and concave mirror when artificial light is used. The parallel rays come from plane mirror when light rays are coming from distant source (natural light).

     Concave mirror also gives parallel rays when rays are coming from near source of light (artificial light) but it does not give parallel rays when light rays are coming from distant source (Fig. 1.1.2).

Image Formation in a Compound Microscope

i. Objective forms real, inverted and enlarged image.
ii. Eyepiece forms virtual, erect and enlarged image (Fig. 1.1.3).

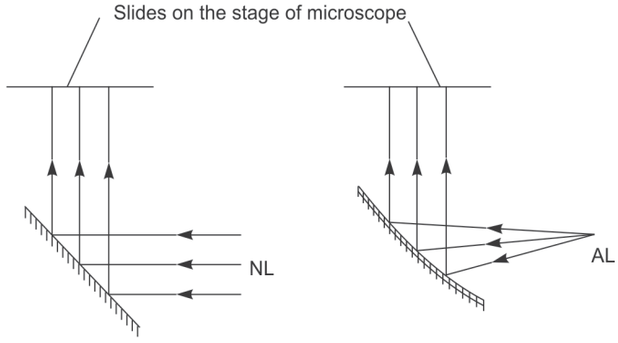


Fig. 1.1.2: Reflection of light rays from plane mirror and concave mirror of a compound microscope when the source of light is natural (NL) or artificial (AL).

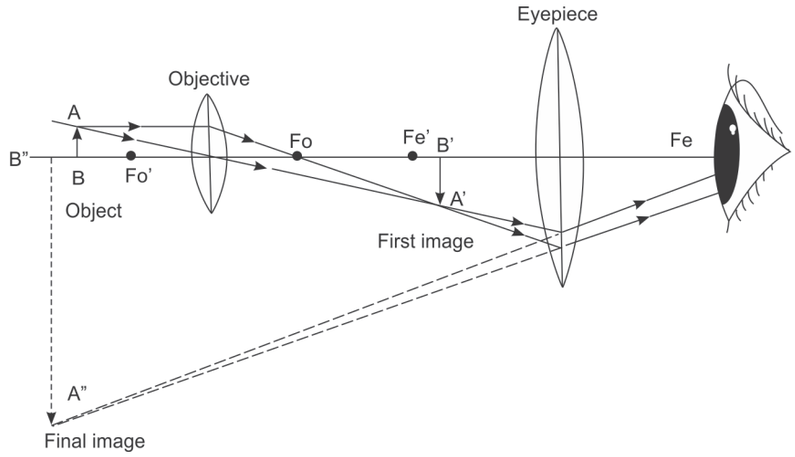


Fig. 1.1.3: Image formation in a compound microscope.

Adjustment of Intensity of Illumination

General Principle

When we use low power (10×) objective, a large area of field is visualized, so we need less illumination. When oil immersion lens (100×) is used, very small area of the field is visualized, requiring highest illumination so that sufficient light reaches up to the eyepiece (Fig. 1.1.4).

Means to increase the intensity of illumination:

1. Aperture size of diaphragm
   Small size—less illumination
   Big size—more illumination
2. Position of condenser
   Lowest position—minimum illumination
   Highest position—maximum illumination

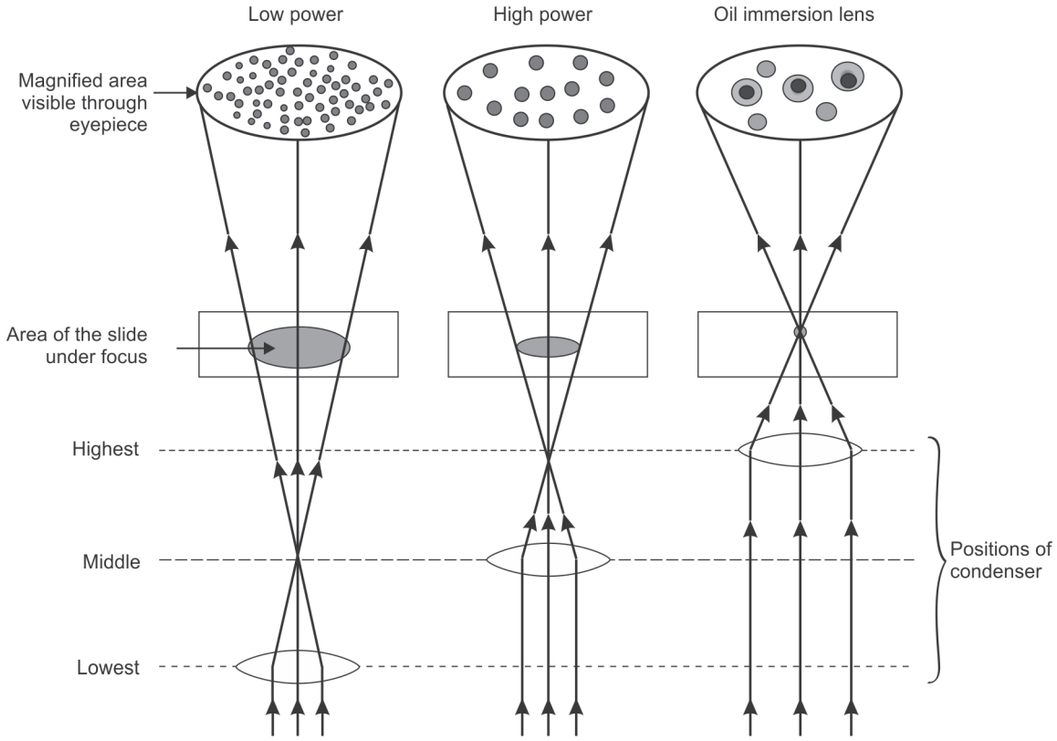


Fig. 1.1.4: Position of condensers in relation to examination of slide under various objectives of a compound microscope.

3. Type of reflecting mirror
   Plane mirror—less illumination
   Concave mirror—more illumination
4. In case of binocular microscope there is no reflecting mirrors and light source is electrical, so illumination can be adjusted with the help of light switch available in the base of microscope and condenser.

Use of Cedarwood oil or Liquid Paraffin in Oil Immersion Objective

Refractory index of cedarwood oil or liquid paraffin is equal to that of glass, so it prevents the scattering of light rays and the image will be more clear when we use it with oil immersion lens (Fig. 1.1.5).

Binocular compound microscope (Fig. 1.1.6)

- Two eyepieces are there in the microscope.
- No reflecting mirror
- Inbuilt light source which can be recharged.
- ***At the time of focusing, stage move up or down not the body tube holding eyepiece.***

Focusing of an Object under Microscope

a. Low Power

i. Place the slide on the stage of microscope and bring the area of the slide over the hole present in the stage with the help of screws attached with mechanical stage.
ii. Then bring low power objective over the slide and adjust its position a few mm above the slide. During this process constantly look from the side of the microscope so that it will not touch the slide.
iii. Adjust illumination by adjusting the aperture of diaphragm, position of condenser and selection of proper mirror.
iv. With the help of coarse adjustment screws move the objective up and simultaneously look into the microscope till the object is visualized.
v. Now with the help of fine adjustment screw further focus the object till it becomes clearly visible.

b. High Power

i. First focus the object under low power.
ii. Turn the nose piece and bring high power objective over the slide.
iii. Adjust the fine adjustment screw till view becomes clear.
iv. Adjust the illumination so that object becomes very clear.

c. Oil immersion Lens

i. Put a drop of cedarwood oil after placing the slide on the stage just over the area which has to be focused.
ii. Dip the oil immersion lens very carefully with the help of coarse adjustment screw by constantly looking from the side of microscope so that it will just dip in the oil without touching the slide.
iii. Do fine focusing with the help of fine adjustment screw by raising the objective till its view becomes very clear.
iv. Illumination is adjusted to the maximum by all the ways discussed (fully open diaphragm and highest position of condenser).

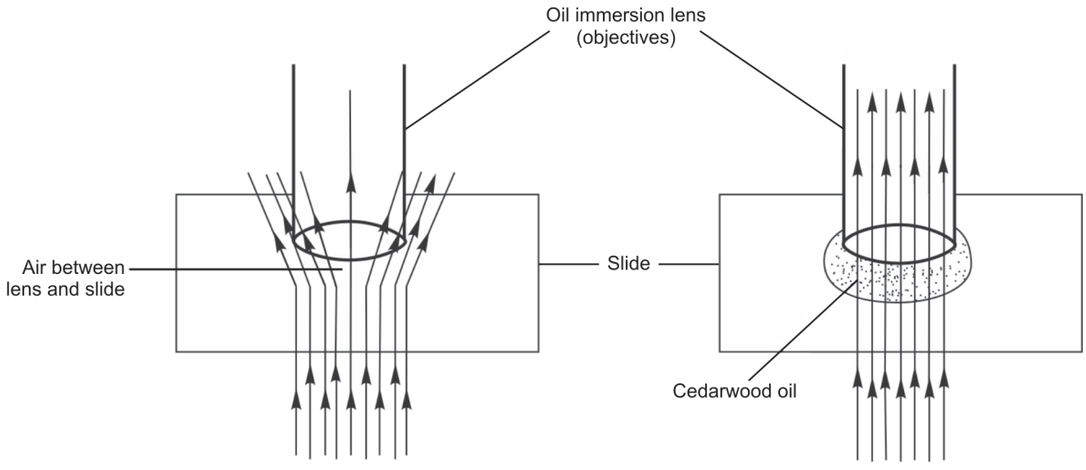


Fig. 1.1.5: Cedarwood oil prevents scattering of light rays.

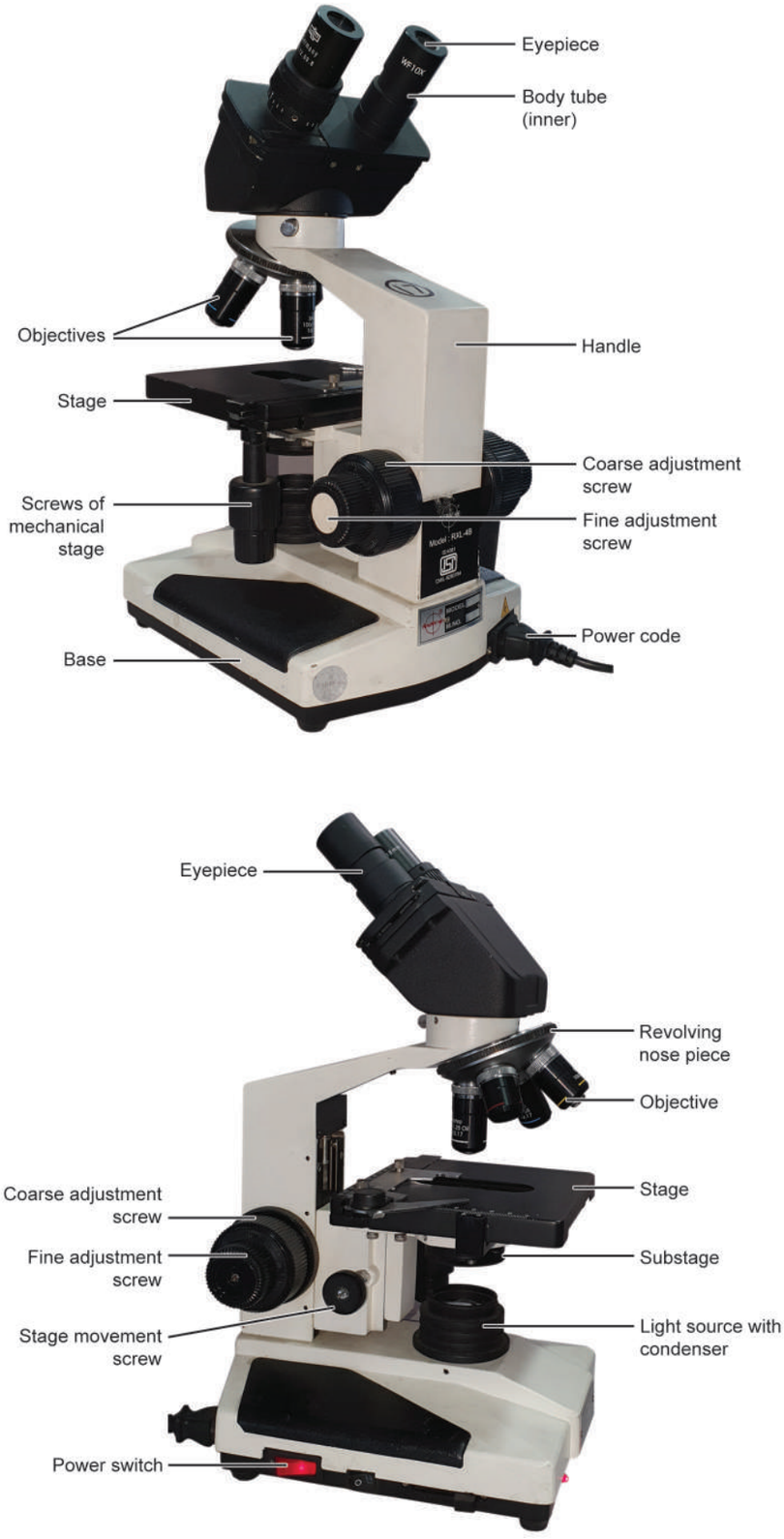


Fig. 1.1.6: Binocular compound microscope.

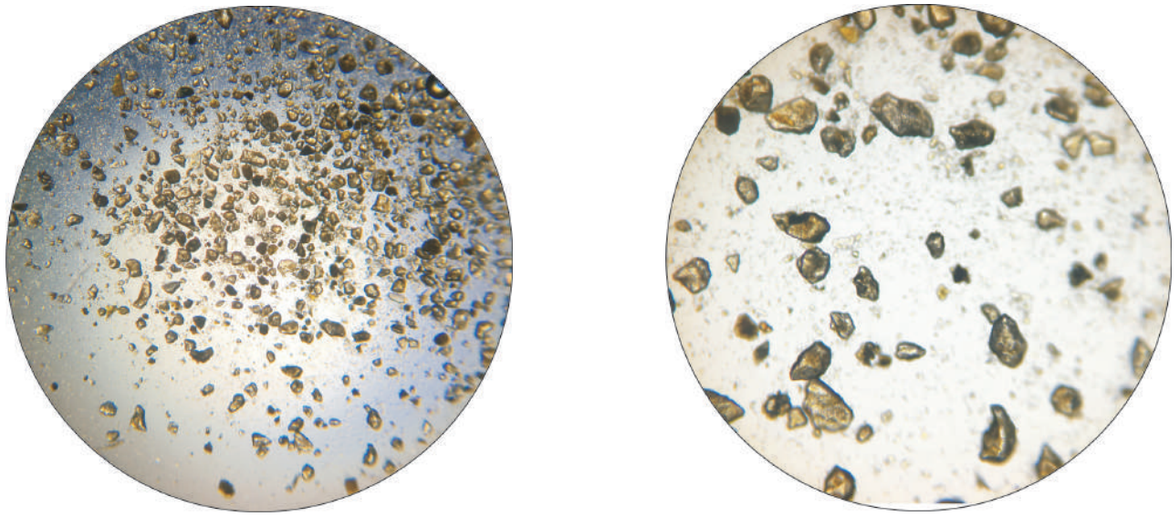
PRECAUTIONS

1. Eyepiece and objective of the microscope must be clean.
2. Do not use dry cotton to clean the lens. Xylene with soft cloth should be used for this purpose.
3. Do not use spirit to clean lenses since it may dissolve the fixing material of the lens.
4. Do not lower the objective grossly without looking from the side of the microscope.
5. Always keep the microscope vertical at the time of shifting it from one place to the other.

Focussing

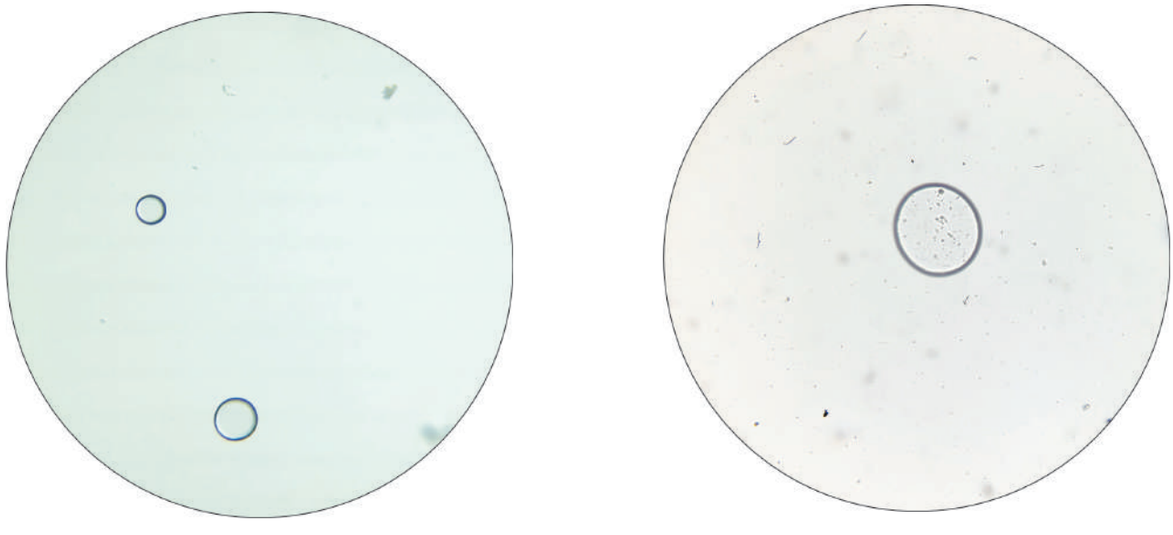
Focus all the objects first in low power and then under high power as follows:

1. **Sand (dust) particles:** Place a little quantity of sand mixed in water on the slide and put a coverslip on it and examine. Translucent and opaque particles of different shapes and sizes are seen. (Fig. 1.1.7)
2. **Air bubble:** Put a drop of distilled water on the slide and place a coverslip over it, in such a way that it will have air bubbles.



(a) (b)

Fig. 1.1.7: Sand (dust) particles under (a) low power (10×) and (b) high power (40×) of microscope.



(a) (b)

Fig. 1.1.8: Air bubble (a) low power (10×) and (b) high power (40×) of microscope.

Air bubbles are clear spaces with dark boundaries because of refraction from the boundaries of air bubble (Figs 1.1.8 and 1.1.9).

3. **Fat globule:** Take a drop of milk on the slide and put a coverslip. Fat globules are seen as circular objects having fine clear boundary (Fig. 1.1.10).
4. **Wool fibers:** Place wool fibers on a drop of water on the slide and put a coverslip over it and examine under microscope.

   These fibers are translucent having no twists.
5. **Cotton fibers:** Put some cotton fibers in a water drop on glass slide, cover it with coverslip and examine under microscope.

   These are translucent fibers having twists (oblique marking) at various sites (Fig. 1.1.11).
6. **Human hair:** Place some hair on a slide, cover it with coverslip and examine.

   These are dark coloured objects some time lighter in the centre and darker in periphery (cortex and medulla) (Fig. 1.1.12).
7. **Starch particles:**
   i. **Unstained:** Take a drop of mixture of crushed potato in water on a slide and put a coverslip on it. Examine under microscope.

      Starch particles are pear shaped objects with concentric rings and pointed hilus on one side (Fig. 1.1.13a).

   ii. **Stained:** Add a drop of iodine solution to starch particles and examine under microscope. Particles appear blue in colour having concentric rings and pointed hilus on one side (Fig. 1.1.13b).

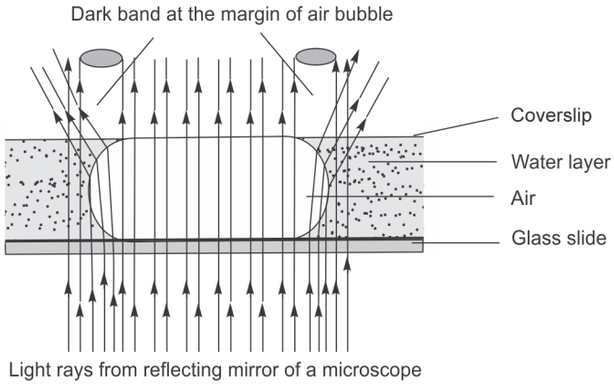


Fig. 1.1.9: Appearance of boundaries of air bubble under oil immersion lens.

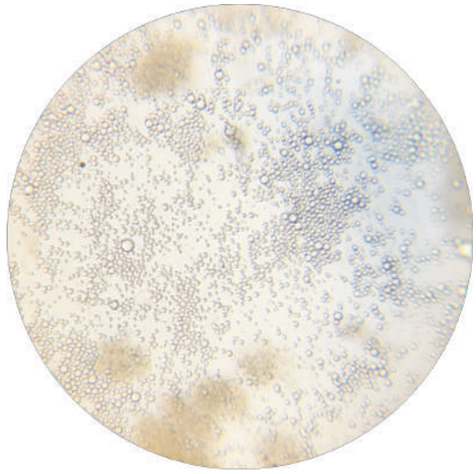
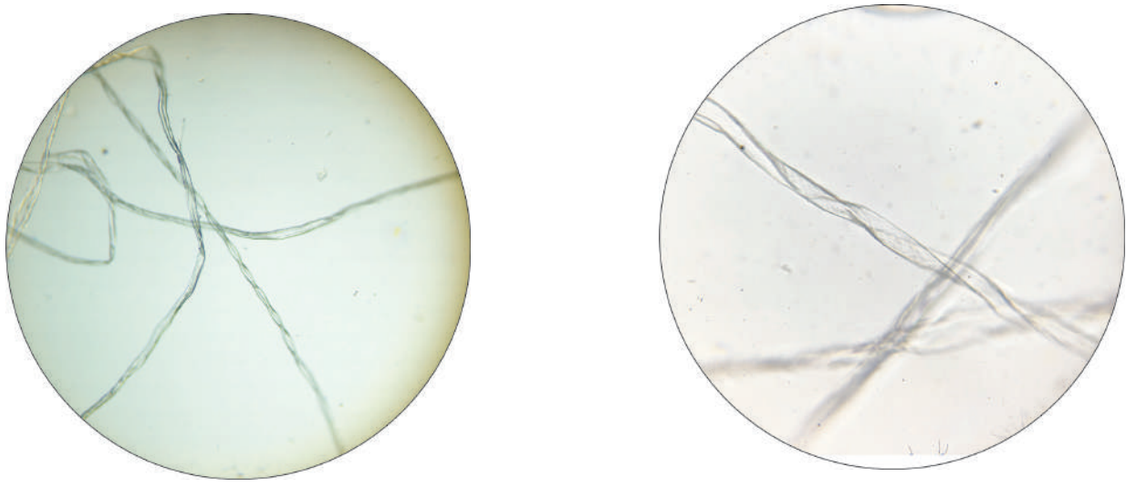


Fig. 1.1.10: Fat globule, high power (40×) of microscope.



(a) (b)

Fig. 1.1.11: Cotton fibers (a) low power (10×) and (b) high power (40×) of microscope.

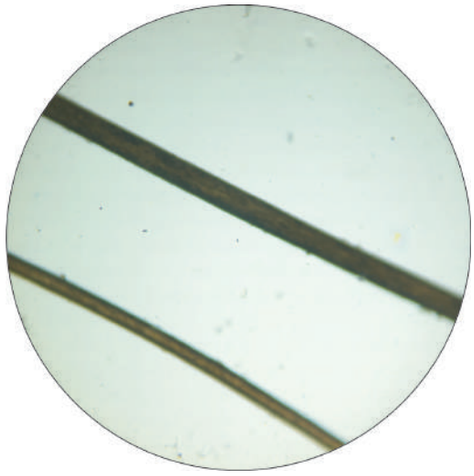


Fig. 1.1.12: Human hair, high power (40×) of microscope.

QUESTIONS AND ANSWERS

Q.1. How will you identity the various objectives?

Ans. *See* text

Q.2. What is the role of cedarwood oil/liquid paraffin in oil immersion lens?

Ans. Cedarwood oil has the same refractory index as that of glass so prevents the scattering of light rays coming from the slide and makes the view more clear.

Q.3. What is the function of condenser and what should be its position while using low power, high power and oil immersion objective?

Ans. *See* text

Q.4. What are various types of surfaces of the mirror present in the microscope and in which situation they are used?

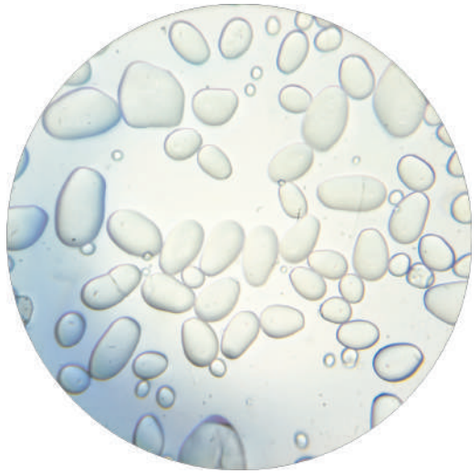
Ans. *See* text

Q.5. What type of image do you observe in the microscope?

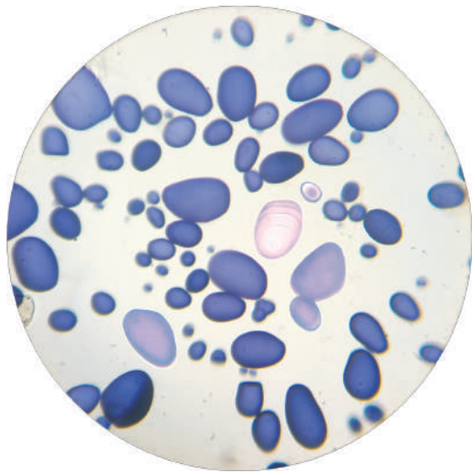
Ans. *See* text

Q.6. Why is the boundary of air bubble darker?

Ans. Light rays coming from the boundaries of air bubble divert their path and because of deficiency of light in this region they look dark.



(a)



(b)

Fig. 1.1.13: Starch particles under high power (40×) of microscope (a) unstained and (b) stained.

Observations and Diagrams

Observations and Diagrams *(Contd.)*

Student's Notes

Experiment 1.2
To study haemocytometer and to collect blood sample

A. HAEMOCYTOMETER

OBJECTIVES

At the end of the practical class student should be able to—

1. Identify RBC and WBC pipette.
2. Focus Neubauer's chamber under low power, high power and oil immersion lens.
3. Identify the various squares of Neubauer's chamber.
4. Tell the size and volume of big, medium and small squares.
5. Charge the Neubauer's chamber.

Haemocytometer is an apparatus used to do count of various blood cells (RBC, WBC, eosinophil and platelets). It consists of RBC and WBC pipette, and a thick slide (Neubauer's chamber).

1. RBC Pipette

i. This consists of a glass stem having capillary tube in it which opens in a bulb containing red bead and opposite to the bulb again there is a small stem. This small stem is connected to the red coloured mouthpiece with the help of a rubber tube (Fig. 1.2.1).

ii. The stem has three markings, 0.5, 1.0 and 101. From the tip of the pipette to the marking 1.0 there are 10 equal divisions/parts. These are simple divisions not any specific unit like—mm, ml, and cu. mm.

iii. The stem has the capacity of one part and bulb has 100 parts.

iv. Bead of the pipette serves two purposes, one mixing of the blood with diluting fluid and other act as an identification mark of RBC pipette.

2. WBC Pipette

This pipette is similar in shape except size of bulb is smaller, markings are 0.5, 1.0 and 11, and bulb contains white bead and colour of the mouthpiece is white (Fig. 1.2.2).

3. Neubauer's Chamber (Counting chamber)

i. This is a thick glass slide having central platform divided into two positions with the help of H shape groove or trench (Fig. 1.2.3).

ii. On both the sides of lateral groove there are raised ridges of a height of 0.1 mm (1/10 mm) from the central platform. When a coverslip is placed on the ridges, a space of 0.1 mm height is created below the coverslip on the central platform.

iii. Counting chamber (grid) is made up of ruled area of 3 mm × 3 mm size on each central platform (Fig. 1.2.4). Each central area is further divided by triple lines into 9 squares of equal size (1 square mm each).

iv. Four corner squares are further divided into 16 squares of equal size. These four corner squares are used to do total leucocytic count (TLC).

v. Volume of each big square is 0.1 cu mm (1 mm × 0.1 mm).

vi. Central big square is divided into 25 (medium size) squares each having arm 1/5 mm. Area of each medium size square is 1/25 mm^2 (1/5 × 1/5) and the volume of each square is 1/250 cu mm (1/25 × 1/10).

vii. Further these medium size squares are divided into 16 small squares of equal size. Area of each small square is 1/20 mm^2 (1/5 × 1/4). The area of each square is 1/400 mm^2 (1/20 × 1/20) and volume is 1/4000 mm^3 (1/400 × 1/10). Total

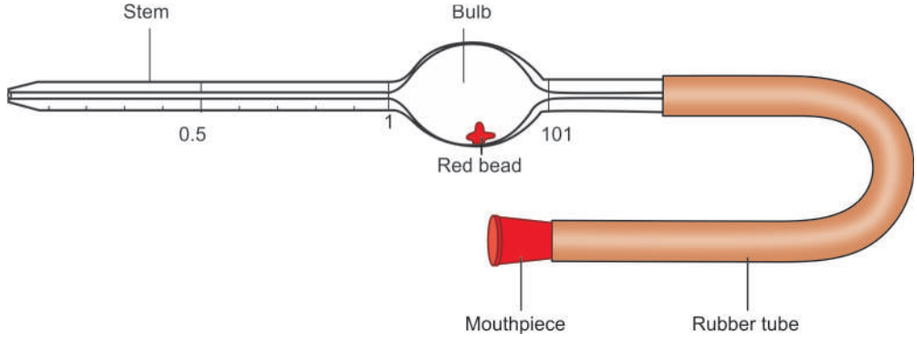


Fig. 1.2.1: RBC pipette.

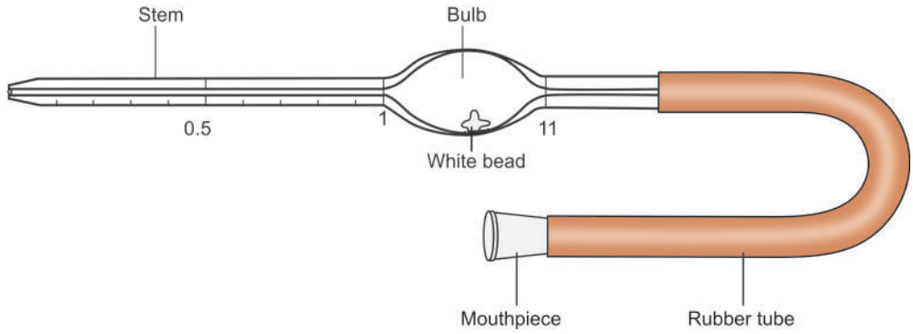


Fig. 1.2.2: WBC pipette.

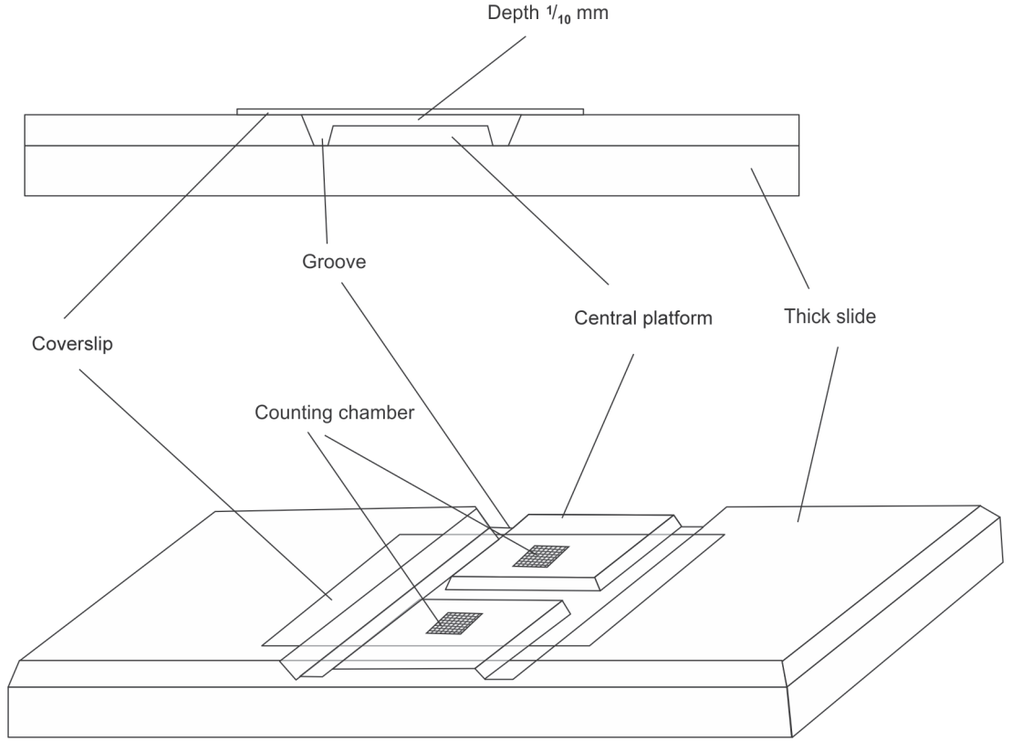


Fig. 1.2.3: Neubauer's chamber (improved), side view (upper) and surface view (lower).

number of smallest squares in big central square are 400 (25 × 16).

viii. RBCs are counted in 5 medium size squares (R1, R2, R3, R4, R5), four corners and one central.

Use of Counting Chamber

RBC chamber: It is used in RBC, platelet and reticulocyte count.

WBC chamber: It is used in WBC and eosinophils count.

PROCEDURE

i. Clean the Neubauer's chamber in soap solution and let it dry.

ii. Place it on the stage of microscope and focus counting chamber in low power and study all the squares carefully.

iii. Turn the nose piece of microscope and focus the chamber under high power. Study all the squares carefully.

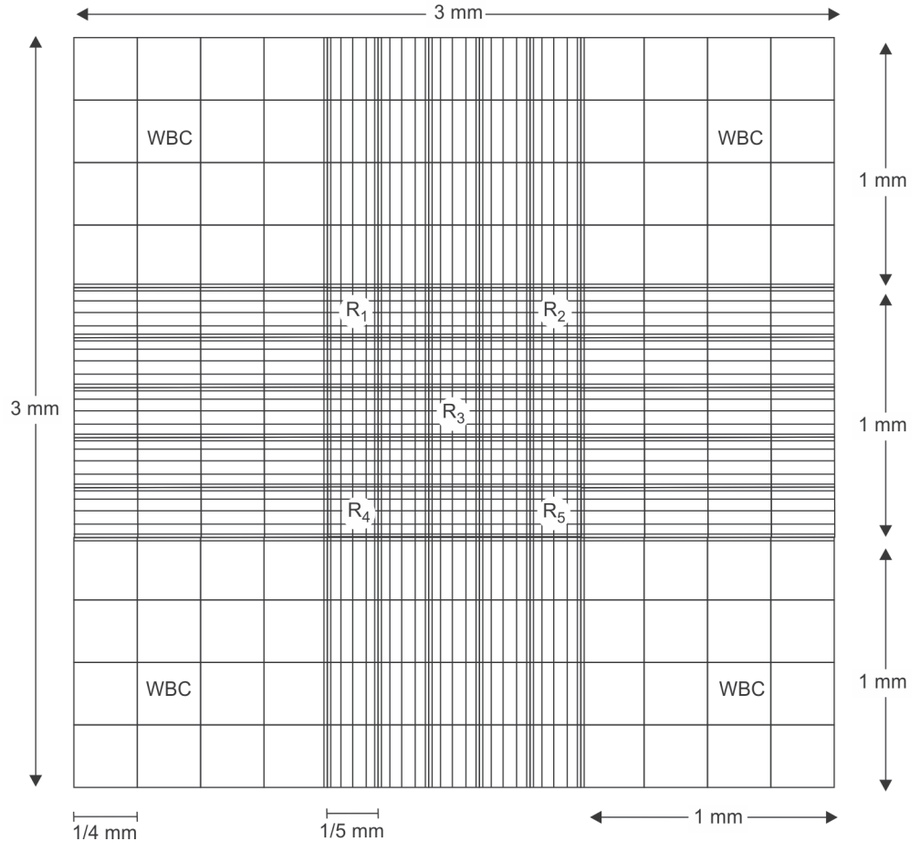


Fig. 1.2.4: Counting chamber (Neubauer's chamber) under low power of a compound microscope.

Charging of Chamber

This involves introducing diluted blood in Neubauer's chamber below coverslip.

Remove the chamber from the stage of the microscope and place a coverslip over the chamber. Now take coloured solution (eosin in water) in the RBC pipette and touch the tip of pipette near the edge of the coverslip on central platform at an angle of about 45 degree. As the fluid enters below the coverslip because of capillary action, immediately remove the pipette.

PRECAUTIONS

1. Chamber should be clean, dry and grease free.
2. There should not be overcharging (flow of fluid in side trenches) or undercharging (incomplete filling of the chamber).
3. Do not hold the coverslip from its flat side (it will leave fingerprints), hold it from the edges.
4. If overcharging is there, wash the chamber and recharge.

B. COLLECTION OF BLOOD SAMPLE

OBJECTIVES

At the end of the practical class student should be able to—

1. Collect blood sample of capillary blood and venous blood.
2. Tell the names of common anticoagulants used and their mechanism of action.
3. Tell the significance of mixed oxalate.
4. Tell the precautions to be taken in collecting and transferring blood.
5. Dispose of the needle, syringe and cotton swab after collection of blood sample.
6. Explain why ring finger of left hand is selected to collect the capillary blood sample.

Blood is collected for various haematological investigations.

Capillary Blood

i. It can be collected from fingertip and ear lobule in adult and heal in case of newborn or infant.
ii. Take 20–24 gauge sterilised disposable hypodermic needle for this purpose.
iii. Clean ring finger of left hand (preferably ring finger) with spirit swab and left it dry. Sterilisation is effective when spirit dries up and spirit causes haemolysis when it comes in contact with blood.
iv. Give 2–3 mm deep prick at centre of the tip of ring finger so that free flow of blood will be there.
v. After collection of blood sample put a spirit swab at pricking site and hold it there for 2–3 minutes (till bleeding stops).
vi. Do not squeeze the finger for collection of blood, it will cause dilution of blood because tissue fluid mixes with blood.

Venous Blood

i. Take 5 ml disposable syringe with 19–20 gauge needle.
ii. Support the arm of the subject on the edge of the table and locate the vein in antecubital fossa (area).
iii. Clean the area with spirit swab and let it dry. Do not touch the area again with finger once it is cleaned.
iv. Apply rubber or cloth tourniquet firmly around the arm to occlude venous return. Ask the subject to close and open the fist repeatedly so as vein gets engorged with blood.
v. Place the thumb of your left hand on the skin about 4–5 cm distal to the vein to be pricked so that vein will not slip.
vi. Introduce the needle into the skin and push the needle on the side of vein, when needle enters the vein resistance felt to cease.
vii. Draw the blood in syringe slowly to the required quantity (slowly means not faster than the filling of vein).
viii. Release the tourniquet and withdraw the needle after putting the fresh spirit swab at the site of the puncture of skin.
ix. Ask the subject to press the swab at the site of puncture for 2–3 minutes (till bleeding stops).
x. Eject the blood from the syringe after removing needle in the vial having anticoagulant. For serum no anticoagulant is required in the vial.

Anticoagulant Used

1. Ethylene diamine tetracetic acid (EDTA):
   - Calcium chelating agent
   - No effect on blood cells
   - 2.4 mg dry powder in a vial for 2 ml of blood. (1.2 mg/ml of blood)
   - Used for ESR and PCV measurement.
2. Sodium citrate:
   - Calcium chelating agent
   - 0.4 ml of 3.8% solution for 1.6 ml of blood
   - Used for ESR and collection of blood from donors
3. Double oxalate mixture:
   - Potassium oxalate and ammonium oxalate in the ratio of 2:3.
   - Single oxalate is not used mainly to measure PCV. Because potassium oxalate causes shrinkage of cells and ammonium oxalate causes increase in volume of the cells.
4. Heparin:
   - Solution or powder may be used according to need.
   - Does not affect cell volume.

PRECAUTIONS

1. Must not interchange the pricking needle with other students.
2. Preferably use a needle for pricking once.
3. If necessary to reuse the needle in the same subject, sterilise it on spirit lamp not with spirit (as spirit does not kill hepatitis virus).
4. Do not use single oxalate anticoagulant for measuring PCV.
5. Destroy the needle before disposing it off in needle destroyer.
6. During collecting blood from blood vessel for investigation must wear gloves.
7. If you get prick accidentally from the needle which has been used to collect the blood sample must inform to the senior doctor immediately for further necessary actions.

QUESTIONS AND ANSWERS

Q.1. Why ring finger of left hand is preferred for pricking?

Ans. Because palmar fascia does not extend up to ring figure. So there are no chances of spread of infection, if occurs during pricking. Other reason is the ring finger of left hand comes in contact least as compared to the other fingers of same hand or of the opposite during working, so the chances of pain and infection are less.

Q.2. Why should we not squeeze the finger for taking the blood?

Ans. *See* text

Q.3. How will you differentiate between RBC and WBC pipette?

Ans. *See* text

Q.4. What are the mechanisms of action of different anticoagulants?

Ans. *See* text

Q.5. What measures will you take to prevent the haemolysis at the time of collection of blood?

Ans. Haemolysis can be prevented by:

i. Using the clean vial to collect the sample.
ii. Taking the sample by wide gauze needle (No. 24 or less).
iii. Withdrawing the blood slowly from the vein.
iv. Delivering the blood gently after removing the needle from the syringe in the vial.

Student's Notes

Experiment 1.3 (PY 2.11b)
Determination of red blood cell (RBC) count

OBJECTIVES

At the end of the practical class student should be able to—

1. Do RBC count of his own blood.
2. Tell normal value of RBC count.
3. Name the contents of Hayem's fluid and their role in fluid.
4. Tell in which magnification RBCs are counted.
5. Tell rule of counting of cells.
6. Enumerate physiological and some pathological conditions which effect erythrocyte count.
7. Tell the precautions to be taken in this investigation.
8. Tell the possible sources of errors effecting the count.
9. Calculate the dilution factor.
10. Tell the precautions to be taken in this experiment.
11. Tell in spite of all the precautions what are the chances of error.

APPARATUS

Neubauer's chamber (thick slide), RBC pipette, diluting fluid, microscope, coverslip, pricking needle and spirit swab.

RBC Diluting Fluid (Hayem's Fluid)

1. Sodium chloride (NaCl): 0.5 gm, to maintain isotonicity of fluid
2. Sodium sulphate (Na_2SO_4): 2.5 gm, which prevents rouleaux formation.
3. Mercuric chloride ($HgCl_2$): 0.25 gm, acts as preservative (antibacterial and antifungal)
4. Distilled water (H_2O): 100 ml.

PRINCIPLE

Red blood cells are counted in diluted blood and actual count is calculated by multiplying by dilution factors.

PROCEDURE

1. Take about 3–5 ml Hayem's fluid in a watch glass.
2. Prick the ring finger after cleaning it with spirit swab.
3. Wipe off the first drop of blood. Suck the next drop in RBC pipette exactly up to 0.5 mark, taking care that there should be no air bubble. If excess blood has been drawn, remove it by touching the pipette on the cotton swab very carefully.
4. Wipe off the blood sticking around the tip of the pipette with cotton swab.
5. Now suck the Hayem's fluid in the pipette up to mark 101.
6. The pipette is then kept horizontally between palms and rolled gently for a minute to mix the blood with diluting fluid.
7. Focus Neubauer's chamber under low power (10×) objective of microscope.
8. Remove the chamber from microscope and place a coverslip on it.
9. Discard first 2–3 drops of fluid from the pipette which is unmixed fluid present in the stem of the pipette.
10. Charge the Neubauer's chamber:
    i. Small drop of fluid is allowed to form at the tip of the pipette.
    ii. Bring the tip of the pipette near the edge of the coverslip on central platform in such a way that it will make an angle of about 45° with central platform.
    iii. Fluid will be drawn in capillary space below the coverslip. Once the fluid enters below coverslip immediately, remove the pipette from central platform.
11. Wait for 3–5 minutes to settle the cells and put the charged Neubauer's chamber under low of microscope. With the help of fine focussing see that there should be the uniform distribution of RBCs, if it is not recharge the chamber again.
12. Focus the Neubauer's chamber under (40´) high power and count the cells in five medium size square shown in Fig. 1.3.1 (R1, R2, R3, R4 & R5). There are 16 small squares in each medium size square so the total small squares are 80.

Rules for Counting the Cells

i. Any cell which is lying half or more than half or complete is counted in the same square.
ii. Counting is started from left and upper border of square.
iii. Among the tripple line central line should be considered as a main line for counting of cells.
iv. Cells of lower and right border should also counted at the end of each row.

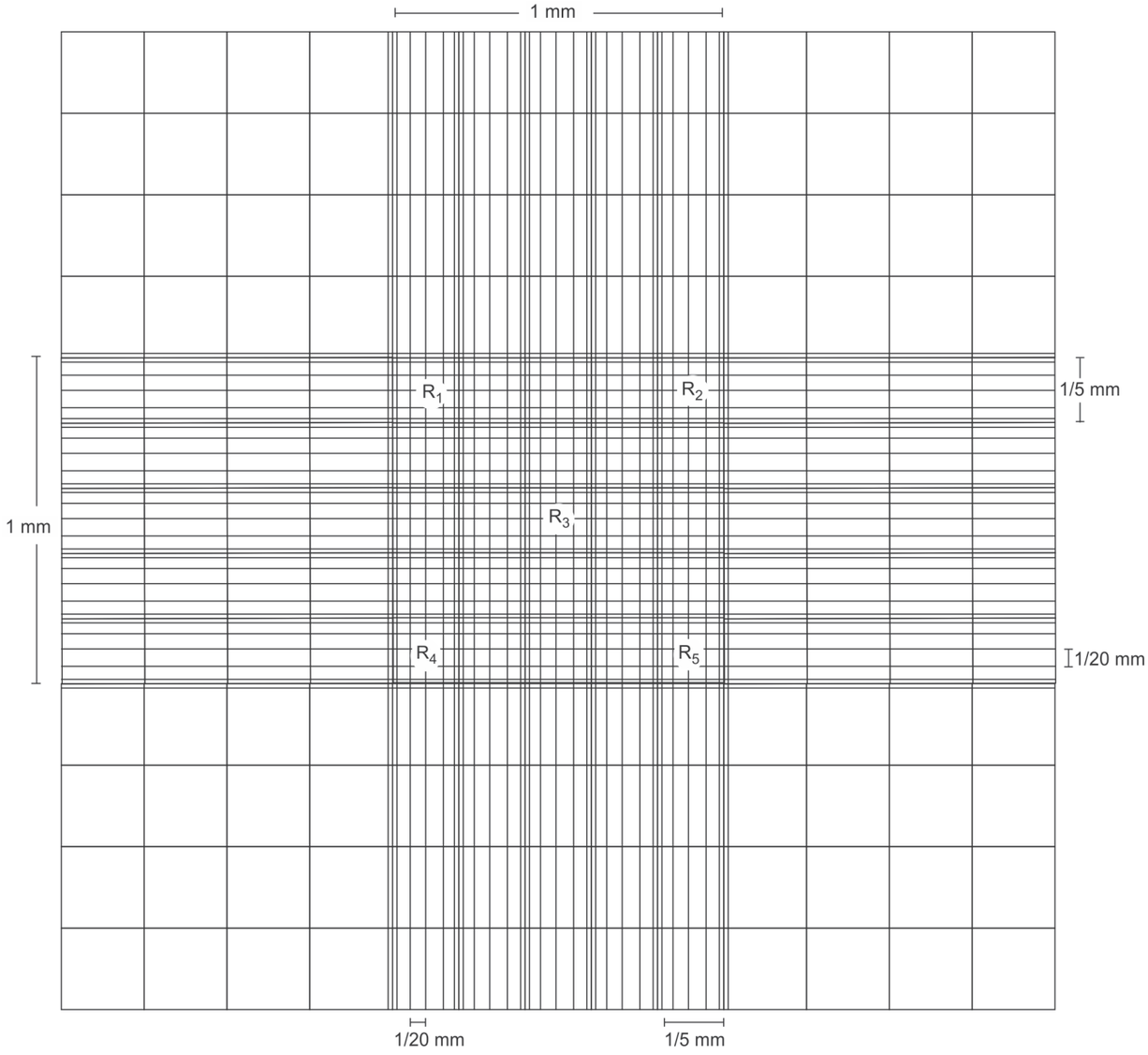


Fig. 1.3.1: Counting chamber (Neubauerís chamber) under low power of a compound microscope.

For example: In medium size square R1 (Fig. 1.3.2) the number of cells are as follows:

Square 1 – 2 cells
Square 2 – 1 cell
Square 3 – 2 cells
Square 7 – 1 cell
Square 9 – 2 cells
Square 11 – 1 cell
Square 12 – 1 cell
Total cells in R1 square = 10.

During counting enters the number of cells present in different small squares in the square drawn on a paper in a similar way as in Neubauer's chamber (Fig. 1.3.2).

CALCULATIONS

Dilution factor: 0.5 part of blood mixes in total 100 parts of mixture (99.5 parts diluting fluid). Fluid present in the stem (1.0 part) does not take part in mixing. This is why 2–3 drops of fluid (present in stem) is discarded before charging the chamber.

$$\text{Dilution factor} = \frac{\text{Total volume of bulb (100 parts)}}{\text{Volume of blood taken (0.5 part)}}$$

$$= \frac{100}{0.5} = 200$$

Area of medium size square (R)
$= 1/5 \times 1/5 = 1/25 \text{ mm}^2$

Depth of chamber $= 1/10$ mm

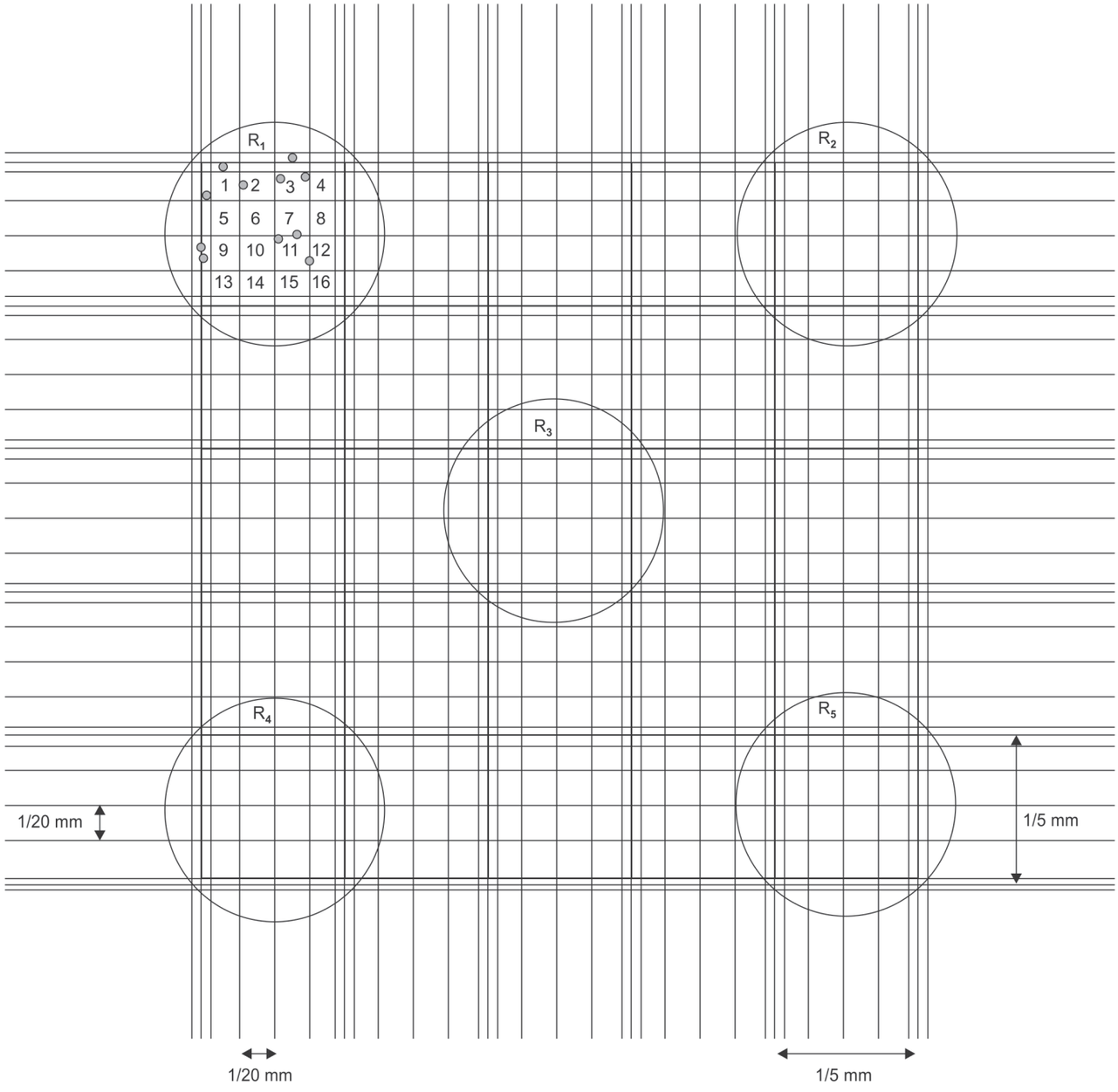


Fig. 1.3.2: Each circle denotes the field which is visualised at one time under high power of a compound microscope.

Volume of each medium size square (R)
= 1/25 × 1/10 = 1/250 cu mm

Total volume of 5 squares (R1+R2+R3+R4+R5)
= 1/250 × 5 = 1/50 cu mm

Suppose total number cell counted in five
(R1 + R2 + R3 + R4 + R5) squares = N

Number of cells counted in 1/50 mm^3 in
diluted blood = N

Number of cells counted in 1 mm^3 diluted blood
= N × 50

Number of cells in 1 mm^3 undiluted blood
= N × 50 × 200

RBC count = N × 10000/mm^3

Observations and Calculations

Normal RBC Count

In adult male: 5.5 million/cu. mm (5–6 million)
In adult female: 4.8 million cu. mm (4.5–5.5 million)

RESULT AND COMMENTS

Write down the result and comment accordingly

PRECAUTIONS

1. RBC pipette, Neubauer's chamber and coverslip should be clean and dry.
2. Hold the coverslip from its edges not from its flat surfaces.
3. Don't take more time in filling the pipette with blood (blood will clot in the pipette).
4. There should not be undercharging or overcharging.

Physiological Variations

1. Age: In newborn baby it is more.
2. Sex: More in male.
3. High attitude: Increase RBC count at high attitude.

Pathological Variations

1. Hypoxia
2. Polycythemia vera.

QUESTIONS AND ANSWERS

Q.1. What is the normal value of RBC count in adult male and female?

Ans. *See* text

Q.2. How will you differentiate RBC and WBC pipette?

Ans. *See* text

Q.3. What are the functions of bead in the pipette?

Ans. *See* text

Q.4. What are the units of markings on the pipette?

Ans. *See* text

Q.5. How much is the dilution in RBC pipette and why?

Ans. *See* text

Q.6. Why there is a need to dilute the blood for RBC count?

Ans. Because the RBC count is very high and in undiluted blood it is very difficult to count the cells under microscope.

Q.7. What are the other uses of RBC pipette?

Ans. RBC pipette can be used for platelets count and sperm count.

Q.8. Why is it important to discard 2–3 drops of fluid before charging the chamber?

Ans. *See* text

Q.9. How is the RBC count affected, when the chamber is under or overcharged?

Ans. *See* text

Q.10. How will you clean the pipette blocked by a blood clot?

Ans. It should be cleaned with N/10 HCl or hydrogen per oxide.

Q.11. What is the importance of rules of counting of RBC?

Ans. If we follow this rule during counting of RBCs, we will not miss any cell and will not count any cell twice.

Q.12. Will you count the WBCs during RBC count and how will it affect RBC count?

Ans. WBCs should be avoided during counting of RBCs but smaller white are difficult to exclude during counting. Even if these cells are included during counting it will not affect the RBC count because WBC count is very less as compared to RBC count.

Q.13. What is the approximate error in RBC count by this method?

Ans. It is about + 11%.

Q.14. Is there any other accurate method for determination of RBC count?

Ans. Yes, it is done by electronic cell counter which is accurate and more reliable method. By this method error is about 1%.

Q.15. What are the physiological factors responsible for polycythemia?

Ans. *See* text

Q.16. What is the composition of Hayem's fluid and what is the role of various ingredients in fluid?

Ans. *See* text

Student's Notes