SECTION 1 Hematology

Experiment No. 1

Date: .....

AIM

To study a compound microscope.

Specific Learning Objectives (SLOs)

At the end of this practical, each student should be able to:

1. Identify all the parts of a compound microscope and describe their functions.
2. Describe the mechanism of formation of image with the help of a ray diagram, of a compound microscope.
3. Adjust the condenser and diaphragm of microscope according to objective lens used.
4. Calculate the total magnification of the microscope for the given objective lens.
5. Describe the role of oil in oil immersion lens.
6. Describe the precautions for care and handling the microscope.

Domain: ***Shows***
Level: ***Shows how***
Aligning teaching learning methods: ***Demonstration***
Aligning assessment methods: ***Practical/OSPE/Viva voce***
No. of procedures to be done independently for certification: ***0***

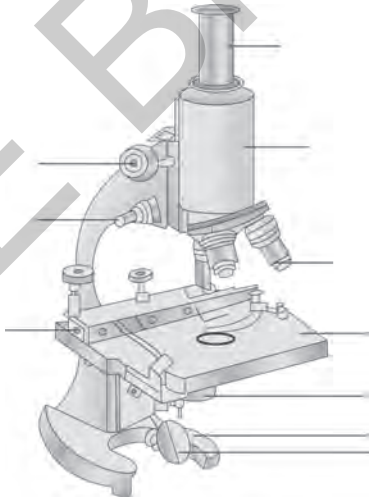


Fig. 1.1: Label the parts of a compound microscope.

Microscope

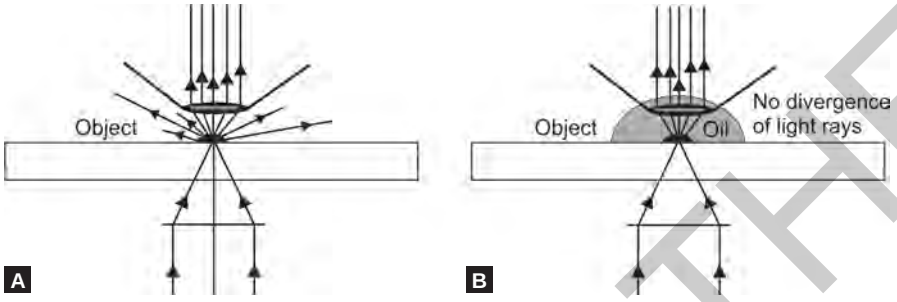
It is an instrument used to visualize tiny objects which cannot be seen with the naked eye.

Parts of Microscope

A compound microscope essentially consists of the following parts:

1. **The stand:** It comprises of a horse-shoe shaped heavy foot to provide stability; and a **limb** which bears the optical system. The limb is attached to the foot by a hinge joint so that microscope can be adjusted at a comfortable angle for the observer.
2. **The optical system:** It is mounted on the tube and consists of two parts:
   i. An **external tube** which bears a revolving nose piece at its lower end in which inter-changeable three objective lenses of various magnifications are fitted:

a. **Low power (10X):** Magnifies the image by 10 times
b. **High power (40X):** Magnifies the image by 40 times
c. **Oil immersion (100X):** Magnifies the image by 100 times. It is most frequently used in hematology because of its greater magnification and resolution. While using this lens, a drop of cedar wood oil is put on the slide for better resolution.
The cedar wood oil has a refractive index of 1.33, which is similar to the refractive index of glass. The oil prevents the divergence of light rays emerging through the object on the slide and provides a sharper focus.



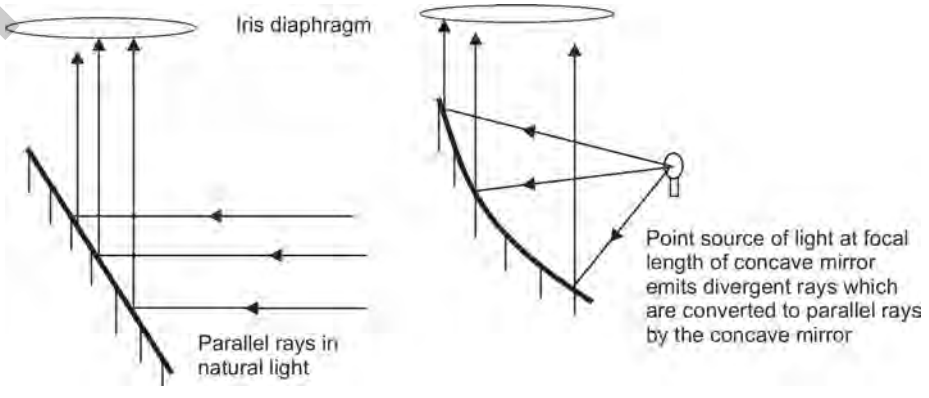
Figs. 1.2A and B: (A) Oil immersion lens without oil; (B) Oil immersion lens used with oil.

The objective lenses can be identified by magnification imprinted on them.
ii. An **inner draw tube** that carries the eye piece (magnification 10X) at its upper end. Length of optical tube is 250–260 mm.

3. **The body:** The body consists of two mechanisms: The coarse adjustment and fine adjustment. With the help of these adjustments the height of the tube can be adjusted in such a way that the objective lens can be positioned at its optimal working distance (i.e., its focal length) from the object to be examined. The fine adjustment corresponds to a movement of 0.002 mm of the tube. It is used for accurate focusing.
4. **The stage:** It is a platform that accommodates a glass slide on which the object to be examined is mounted and it has an aperture in the center to permit light to reach the object. A calibrated mechanical stage is present on the fixed stage which helps to move the object from side to side and before backward.
5. **The substage:** It lies beneath the stage and can be lowered or raised by means of screw. The stage is fitted with a condenser and iris diaphragm.
   i. The **condenser** consists of two lenses. It serves to condense the rays of light and focuses them on the object. It converges the parallel rays of light on the object, kept on the focus of condenser/objective lens.

   Note: The position of condenser varies with the objective lens used.

   ii. Immediately below the condenser is the **iris diaphragm** that can control the amount of light reaching the object. The light rays entering the iris diaphragm should be parallel.
6. **The mirror:** Below the condenser a double reflecting mirror is present, which is plane on one side and concave on the other.
   a. The plane side of the mirror is used when the source of light is diffuse such as daylight. So that the parallel incident rays remain parallel, when they enter the iris diaphragm.



b. Concave mirror is used when source of light is from point source such as bulb. The concave mirror converts the divergent rays into parallel rays before they enter the iris diaphragm.

Note: Higher the magnification, smaller is the area that can be examined at a time. Hence, it is always advisable to focus a slide under low power (10X) first.

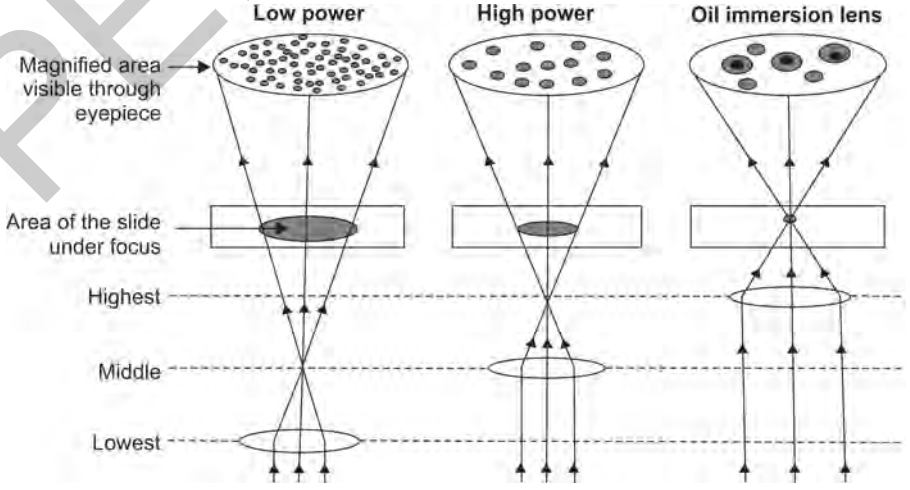
PRECAUTIONS

1. A clear image can never be seen through dirty lenses. Make sure that the eye piece as well as all the objective lenses are clean; do not rub them with dry cotton or rough cloth. Apply xylene to the lenses (to dissolve the grease). Use a piece of muslin cloth or softest material available. **Always clean the microscope lenses before and after use**.
2. Do not clean the objective with alcohol as it may dissolve the cement which unites the component lens.
3. Make minimal and careful use of ONLY the fine adjustment while using the high power and oil immersion lenses.
4. If the microscope has to be moved, it should be held upright by the limb keeping a hand below its foot.
5. When it is not in use keep it covered by dust cover.

Focusing of an Object according to the Lens

1. Place the slide on fixed stage.
2. Use light source.
3. Adjust the mirror *(Plane mirror for natural light and concave mirror for artificial light)*.
4. Adjust the microscope for low power, high power or oil immersion as per the table given below:

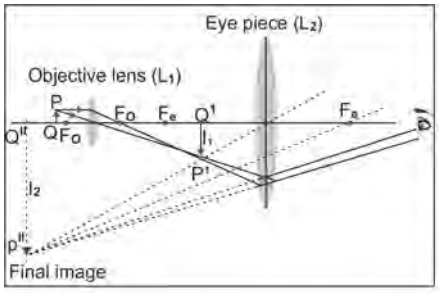
Lens	*Low power*	*High power*	*Oil immersion*
Position of condenser	Lowest (because the clarity of image will distort due to excessive brightness at higher position)	Mid way	Highest (highest position of the condenser)
Iris diaphragm	Partially open	Fully open	Fully open (to allow maximum light)
Lens used	10x	40x	100x
Focal length	16 mm	4 mm	2 mm
Use of oil	No	No	Cedar wood oil used
Color band on the objective lens	Brown	Green	Black



5. Using the coarse adjustments screw, focus the object and then use fine adjustment for accurate focusing. See the object.

Image Formation

Draw the diagram showing image formation in a compound microscope.



First image: Real and inverted
Final image: Virtual, inverted and highly magnified*.

LET'S THINK!!

Q1. Why is the optical tube of a standard length?

.....
.....
.....
.....
.....
.....
.....

Q2. Why cedar wood oil is used in the oil immersion lens?

.....
.....
.....
.....
.....
.....
.....

Q3. Mention the other substances used instead of cedar wood oil.

Q4. Define magnification. How will you calculate it?

Q5. Define resolution.

Q6. Why the oil immersion lens has a pin hole aperture?

Q7. What is refractive index of glass and cedar wood oil?

DEMONSTRATION NOTES

Experiment No. 2

Date: .....

INTRODUCTION

An in-depth knowledge of the microscope is very essential for the student as he is a beginner. He should also know the common objects which appear as artifacts that can misguide the student when performing hematological experiments. This experiment again provides a chance to the student to get familiar with the microscope regarding its proper use, handling, care and maintenance.

AIM

To study the common objects.

Specific Learning Objectives

At the end of this practical, each student should be able to:

1. Identify various common objects, which frequently act as artifacts in the microscopy.
2. Describe three identification features each of these common objects artifacts.

Domain: ***Shows***

Level: ***Shows how***

Aligning teaching learning methods: ***Demonstration***

Aligning assessment methods: ***Practical/OSPE/Viva voce***

No. of procedures to be done independently for certification: ***0***

APPARATUS

About 8–10 clean, grease-free glass slides and cover slips.

COMMON OBJECTS

1. **Cotton fibers:** Look like long, semi-transparent ribbon like filaments which are twisted at regular intervals.
2. **Dust particles:** Look different in size with varying shapes like angular, irregular polygonal, appear light or dark brown, black or yellow in color, e.g., mica, silicon, graphite and carbon.
3. **Human hair:** Look like long filaments and are cylindrical in shape. It has three layers, i.e., inner medulla, outer cortex surrounded by cuticle.
4. **Starch granules (Potato):** Stains with iodine dye. Look oval or pear shaped. Hilum is present at one end and is surrounded by concentric rings.
5. **Starch granules (Rice):** Stains with iodine dye. Look hexagonal in shape with hilum at the center.
6. **Air bubble:** Look round in shape and are of various sizes. They are surrounded by a dark ring with a clear center.

OBSERVATIONS

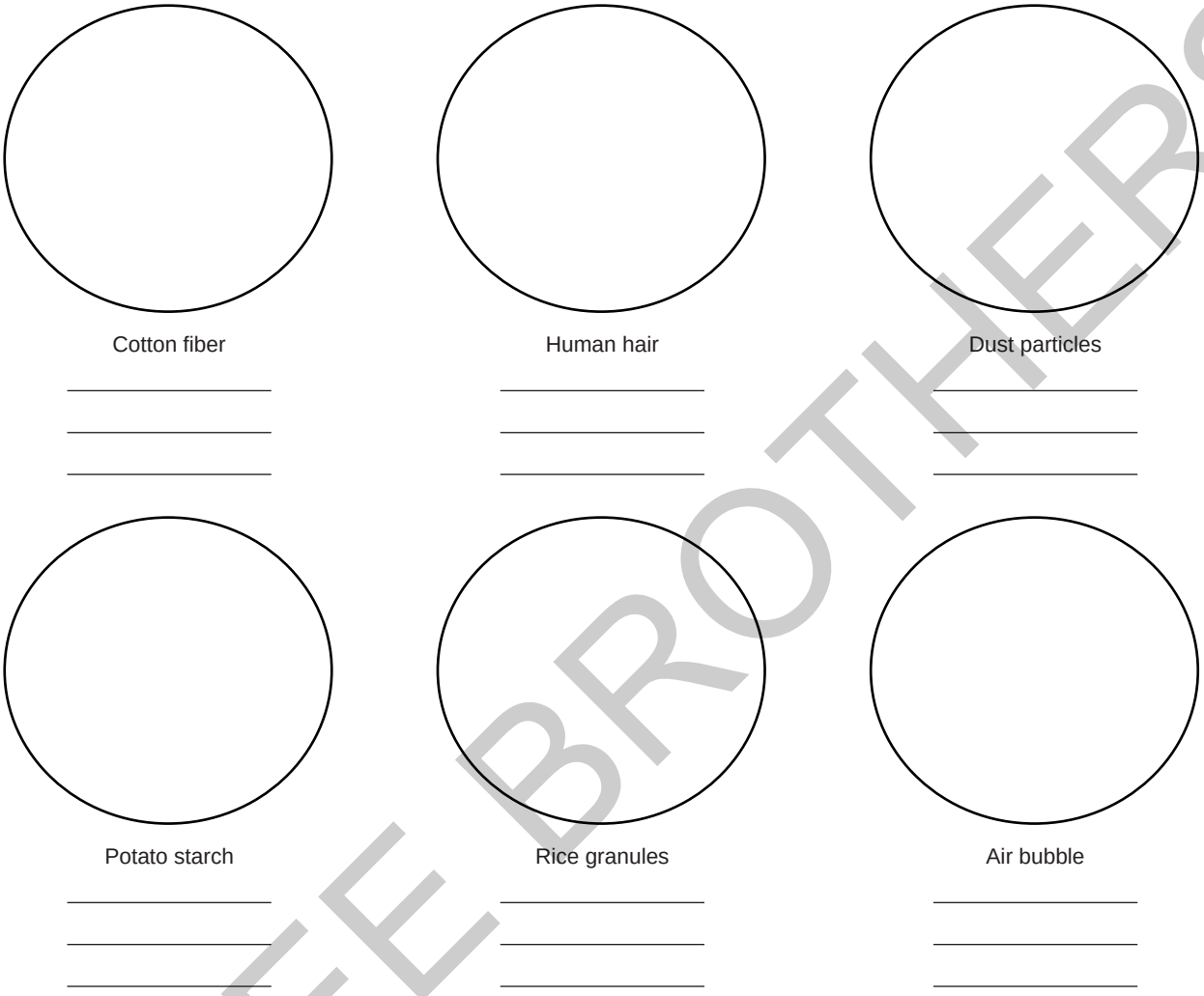


Fig. 2.1: Draw the light microscopic picture of common objects *(For color version, See Plate 1)*

DEMONSTRATION NOTES

Experiment No. 3

Date: .....

INTRODUCTION

Blood investigation makes an essential tool that helps the clinician to arrive at a conclusive diagnosis. Accuracy and precision in the method of collection of blood is thus very important as any technical error could mislead the diagnosis and create a potential life-threatening hazard for the patient.

AIM

Collection of a blood sample.

Specific Learning Objectives

At the end of this practical, each student should be able to:

1. Enumerate different possible routes of the blood sample collection avoiding the sites not recommended for this purpose.
2. Know the complete protocol for aseptic precautions to be taken before the sample procurement.
3. Identify the correct vial (with or without a specific anticoagulant) for transferring the sample before lab investigations.

Domain: ***Knows***
Level: ***Knows how***
Aligning teaching learning methods: ***Demonstration***
Aligning assessment methods: ***OSPE/Viva voce***
No. of procedures to be done independently for certification: ***0***

APPARATUS

Sterile needle/lancet, blood collection vial [plain, ethylene diamine tetra-acetic acid (EDTA)], sterile cotton swab, rubbing alcohol, tourniquet, adhesive dressings.

I. Capillary (Peripheral) Blood

It is obtained by puncturing the skin and drawing a small amount of blood.

Site:

- Adult—Finger
  Ear lobe (not used these days)
- Infant—deep puncture of the plantar surface of the heel.

PROCEDURE

1. ***Finger prick***:
   - Clean the finger (or the area) with a spirit swab.
   - Allow the spirit to dry.
   - Using a sterile disposable 24 gauze needle or a lancet to prick the finger on the lateral side bold enough to get a free flow of blood (approximately 5 mm deep).

LET'S THINK WHY?

Q1. Why should the little finger and thumb should not be pricked?

.....

.....

Q2. Why is spirit allowed to dry before pricking?

.....

.....

Precaution:

Do not squeeze the finger, as it would result in oozing of tissue fluid along with the blood.

2. ***Ear lobe prick*:**
   - Rub the ear gently in between the fingers till it becomes warm
   - Now prick with a sterile pricking object and collect the blood in a capillary tube.
3. ***Heel prick*:**
   - Preferred in infants
   - A deep prick is made on the medial or lateral parts of the plantar surface of heel.

LET'S THINK WHY?

The central and posterior aspect of heel should not be pricked?

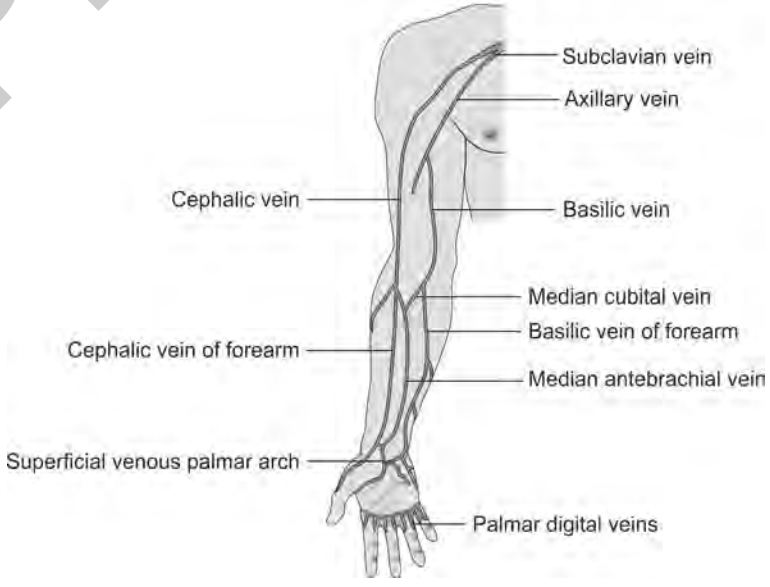
.....

.....

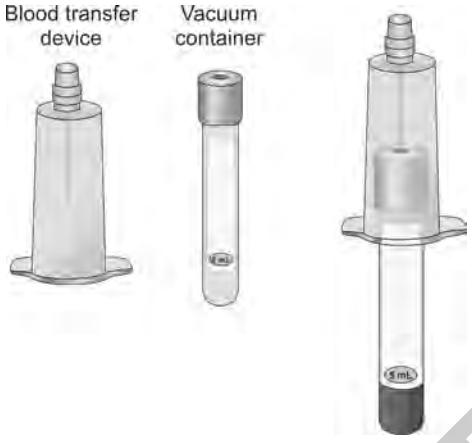
II. Venous Blood

It is a common practice nowadays to draw venous blood.

Site: Median cubital vein by means of a dry, disposable plastic syringe.



With all aseptic precautions blood is withdrawn and transferred to the appropriate desired vials.



	Plain vial	*EDTA vial*	*Citrate vial*	*Heparin vial*
Indications				
Contraindications				

ANTICOAGULANTS

Anticoagulants are chemical substances that prevent coagulation of blood.

Commonly used anticoagulants are EDTA, trisodium citrate, and heparin cofactor.

1. EDTA and trisodium citrate removes calcium which is an essential factor for coagulation.
   Calcium is either precipitated as insoluble crystals of oxalate or bound in a nonionized form. EDTA is used for complete blood counts, peripheral blood films and ESR and is avoided in coagulation studies. Citrate is used for coagulation studies, ESR and is avoided in complete blood counts.
2. Heparin binds to antithrombin and thus interferes with the coagulation cascade. It is used for finding out osmotic fragility and pH of blood and is avoided in blood counts.
3. In case we want to perform the laboratory tests in serum, no anticoagulant is used.

LET'S THINK!!

Q1. What is the difference between capillary blood and venous blood?

.....
.....
.....
.....
.....
.....
.....
.....
.....
.....
.....
.....
.....