Experiment No: 1

Study of Compound Microscope

Objective:	To study the various parts and draw the compound microscope
Requirement:	Compound microscope

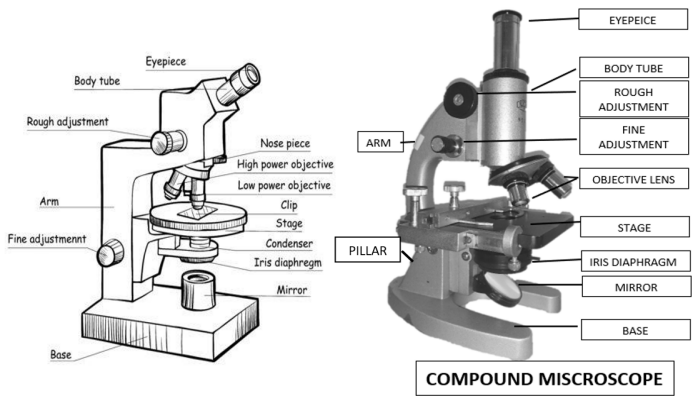
Principle

The compound microscope has a combination of lenses that enhances both magnifying power as well as the resolving power. The specimen or object, to be examined is usually mounted on a transparent glass slide and positioned on the specimen stage between the condenser lens and objective lens.

A beam of visible light from the base is focused by a condenser lens onto the specimen. The objective lens picks up the light transmitted by the specimen and create a magnified image of the specimen called primary image inside the body tube. This image is again magnified by the ocular lens or eye piece.

The compound microscope is called so because, in contrast to a single magnifying convex lens, it has two such lenses-the objective and the eyepiece. It magnifies the image of an object that is not visible to the naked eye to an extent where it can be seen clearly.

Procedure



COMPOUND MISCROSCOPE

Study the following parts of compound microscope as follows:

A. The support system

B. The focusing system

C. The optical system (magnification system)

D. The illumination system

A. The Support system

1. **Base:** It is a heavy-metallic, U-or horseshoe-shaped base or foot, which supports the microscope on the working table to provide maximum stability.
2. **Pillars**: Two upright pillars project up from the base and are attached to the C-shaped handle. The hinge joint allows the microscope to be tilted at a suitable angle for comfortable viewing.
3. **Handle (arm or limb):** The curved handle, which projects up from the hinge joint supports the focusing and magnifying system.
4. **Body tube:** fitted at the upper end of the handle, either vertically or at an angle, the body tube is the part through which light passes to the eyepiece. It can be raised or lowered by the focusing system.
5. **The stage:** It is a rectangular/square flat platform with an aperture in its center, and fitted to the arm below the objective lenses. The slide is placed on it and centered over the aperture for viewing. The light emerging from the condenser passes through the slide and objective into the body tube.

B. The Focusing system

It consists of two coarse and two fine adjustment screw-heads: It is employed for raising or lowering the optical system with reference to the slide till it comes to the focus. Thus, the adjustments place an objective lens at its optimal working distance. The coarse adjustment moves the optical system up down rapidly through a large distance. The fine adjustment is employed for precise focusing.

C. The Optical (Magnifying) system

1. **The body tube:** It is a hollow tubular part present between the upper ends of the objectives and eyepiece. The body tube can be moved up and down with the help of adjustment screws. The distance between the upper focal point of the eyepiece and the lower focal point of the objective is called the optical tube length, which is about 25cm.

2. **Eyepiece:** It fits into the top of the body tube and through it an image is seen. Most microscopes are provided with replaceable 5x, 8x, and 10x eyepieces, though 6x and 15x are also available. Each eyepiece has two lenses- one mounted at the top, **the eye lens** and the other, the **field lens**, is fitted at the bottom. The field lens collects the divergent rays of the primary image and passes these to the eye lens, which further magnifies the image.
3. **The nosepiece:** It is a circular metallic structure fitted at the lower end of the body tube and has fixed and revolving nosepiece. The revolving nosepiece carries interchangeable objective lenses. Its correct position being indicated by a click sound.
4. **Objective lenses:** The magnifying power of each lens and its numerical aperture (NA) provided rather than its focal length are written on each lens.
   a) *Low-power objective (10x: NA= 0.25)*: This lens magnifies the image 10 times. It is used for initial focusing and viewing a large area of the specimen slide.
   b) *High-power objective (45x: NA=0.65)*: This lens magnifies the image 45 times. Because of higher magnification, it is used for more detailed study of the material.
   c) *Oil-immersion objective (100x: NA=1.30)*: This lens magnifies the image 100 times. Since the lens almost touches the slide it has to be immersed in a special medium (cedar wood oil), a drop of which is first placed on the slide. The oil is used to increase the NA and thus the resolving power of the objective. As this lens gives a total magnification of 1000 times, it is employed for detailed study of blood cells and tissues.

D. The illumination system

1. **Source of light**: The source of light may be the diffuse, natural day light (sunlight) reflected and scattered by the atmosphere and its dust particles and reflected from the buildings. On bright, sunny days, the day light is the ideal for routine student work.

   If day light is not available, or is not sufficient, an artificial source of light a fluorescent tube fitted on the working table can provide enough light.
2. **The mirror**: A double sided mirror, in fact two mirrors, one flat or plane and the other concave, fitted back to back in a metal frame is located below the condenser. It can be rotated in any direction. The

plane mirror is used with a distant natural source of light. The concave mirror is used when the light source is near the microscope.

3. **The condenser**: It is a system of lenses fitted in a short cylinder that is mounted below the stage. It can be raised or lowered by a rack and pinion, and focusing the light rays into a solid core of light on to the material under study. It also helps in resolving the image.

a) The lens system: It is composed of two lenses. Since the condenser is a lens system, it has a fixed NA, which should be equal or less than that of the objective being used. With axes of the two being the same, all the light passing through the condenser is collected by the objective, thus allowing maximum clarity.

b) The iris diaphragm: It is fitted within the condenser to adjust the size of the aperture of the diaphragm and regulate the intensity of the light falling on the material under study.

Observation: Observe and draw a neat labelled diagram of compound microscope and measure the length of body tube, number of objectives, magnification of eyepiece, and type of stage.

Precautions

1. Always keep compound microscope in an upright position.
2. Avoid touching lenses with your hands.
3. Keep the microscope covered or in box, when not in use.

Result: Compound microscope studied in detail and its diagram has been drawn. A table of observation is made and values are recorded.

Experiment No: 2

General Techniques of Blood Collection

Objective:	To study the general techniques for the collection of blood
Requirement:	Syringe, needle, glass slides, cotton

PRINCIPLE

BLOOD SAMPLING

There are several methods of collection of blood. Following are the two most common methods:

Venipuncture

Advantages:

(i) Recommended for collecting large quantity of venous blood

(ii) The composition of blood is not significantly different from that of the capillary blood

(iii) Blood is obtained by a single puncture and repeated investigations can be carried out

Disadvantages:

The procedure requires a technical person with

(i) Skill and confidence

(ii) May also require assistance

(iii) Individuals to be cooperative

(iv) Complete aseptic precautions and

(v) Anticoagulant containers (vacutainer)

Prick Method*:* The method is used when only couple of drops of blood is sufficient/required.

Site for Pricking: A vascular site under good physical control is chosen. Pad of the thumb or great toe or the heel in infants and the ball of a finger

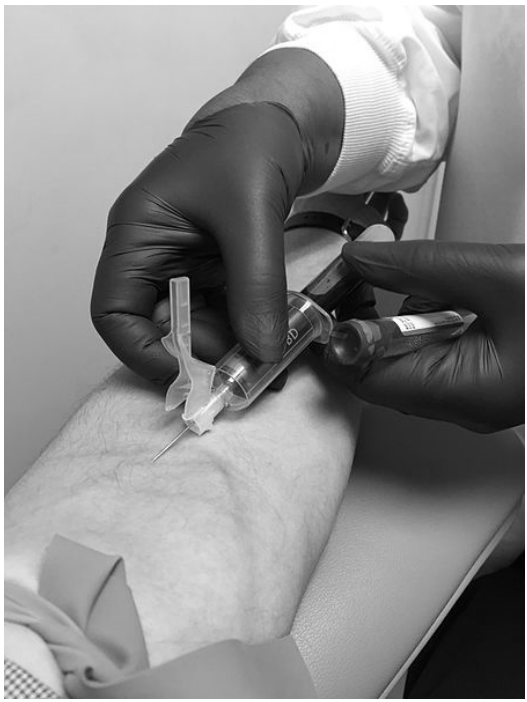
or the lobe of either ear in adults are the common sites. The skin of the part selected should be healthy and not be edematous, congested, bloodless, or cold. The pricking should not be undertaken if it is not possible to observe the aseptic precautions.

PROCEDURE

Venipuncture:

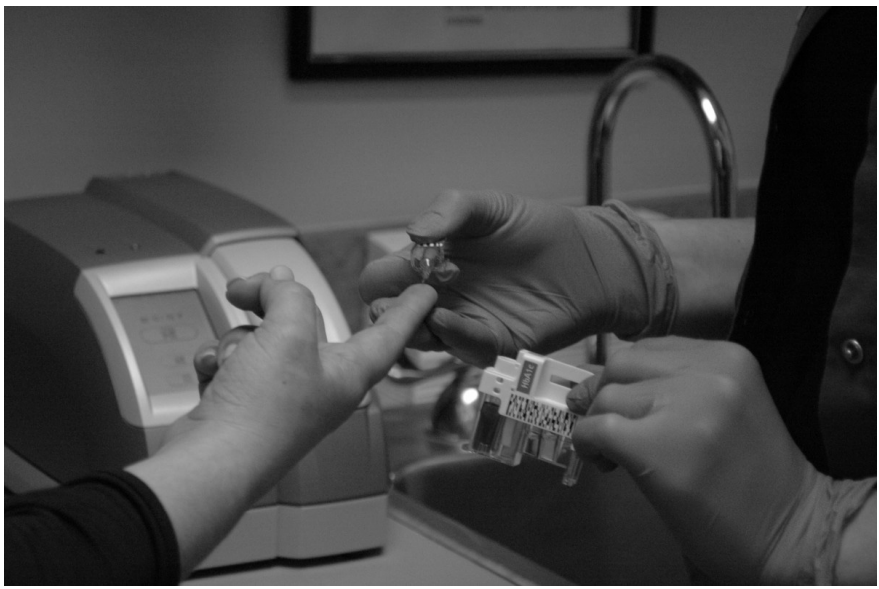
1. Let the subject lie down on the bed or sit on a chair to relax.
2. Reassure the patient/ individual and put him/her to ease.
3. Examine the cubital fossa of his left arm for a “suitable vein".
4. Obstruct the venous return either with the armlet of a blood pressure apparatus raising the pressure to 40 mm Hg level or with a strap of broad elastic or by compression of the arm above the vein.
5. Rest the fully extended arm comfortably on padding.
6. Clean the skin over the selected vein with spirit and allow it to dry.
7. Fix the selected vein by traction on the skin over it with the thumb of your left hand
8. Hold the syringe along the vein and the level of the needle.
9. Place your first finger nearest to the butt of the needle and the body of the syringe in the palm of your hand.
10. Locate a spot on the vein about half a cm below its maximal turgor pressure.
11. Puncture at the spot by pushing in the needle firmly and steadily forming an angle of 30° to 50° with the lower arm.
12. When you have punctured the skin, the resistance encountered is suddenly reduced.
13. Put a slight drag on the piston with your little or ring finger to induce a little negative pressure in the syringe.
14. Push the needle along the line of the vein and if necessary at a changed angle to puncture the vein.
15. When the vein is punctured blood enters the syringe and the resistance encountered for pulling the piston is suddenly reduced.
16. Fix the syringe with left hand and slowly withdraw piston with the right hand as the blood enters the syringe.

17. When sufficient amount of blood is withdrawn hold the syringe in the palm the little and ring fingers supporting the piston and the index finger on the butt of the needle.
18. Place a piece of sterile cotton wool soaked in spirit on the punctured site with the left hand and press lightly.
19. Withdraw the needle with the syringe and press the swab firmly on the punctured site.
20. Instruct the subject to hold it pressed.
21. Hold the syringe vertical with the piston supported.
22. Hold the collection tube with the left hand and slowly transfer the blood in it.
23. Discard the syringe and needle safely as per the guidelines.
24. Hold the collection tube in the palms of your hands and rotate it forwards & backward till the anticoagulant thoroughly mixes.
25. Label the container and keep aside for testing.



Obtaining Sample by the Prick Method

1. Clean and massage the finger with a dry clean sterile gauze swab soaked in methylated spirit.
2. Dip the left-hand ring finger in warm water at 40^{o} C for 3 to 5 minutes or rub the finger to facilitate the flow of blood.
3. Allow the finger to get dry.
4. Hold the finger to be pricked in your left hand.
5. Press the ball of the finger to raise the skin into a small ridge in the longitudinal axis.
6. Hold the disposable sterile lancet with the right-hand fingers.
7. Hold the finger so that the puncture faces you.
8. Mop the oozed plasma and first part of blood.
9. Wipe the lancet, clean and then dispose it.
10. Allow a drop of about 3 mm diameter of blood formed on the punctured site.
11. Collect it as desired.
12. When blood is collected successfully, apply a sterile gauze swab soaked in spirit and keep the site pressed for 1 minute or till the bleeding ceases.



Precautions

1. Wash your hands, clean with plenty of soap and water and dry them with a sterilized cloth piece or tissue paper.
2. Confirm that the packet of the irradiated sterilized syringe and the needle are sealed.
3. Do not touch the cutting portion of the sterile lancet or syringe needle or the site of the finger to be pricked or punctured.
4. Do not force blood collected in syringe into container through the needle as it causes hemolysis.
5. The back and forth movement of container for mixing with anticoagulant should not be too vigorous as it causes hemolysis.
6. Label the container with care to avoid error in labeling patient/ individual ID.

Observation: The students will observe both methods of blood collection and write their observation with respect to ease of collection, advantages, disadvantages, and challenges.

Result: Blood collection techniques have been performed and learned.