Microscopy Lab

Aim

Acquaintance with the use of microscopes for observing specimen

Background:

Scientists use devices called microscopes to examine the small sized objects, organization of tissues, details of cell structure etc. There are several types of microscopes, each of which has its uniqueness and utility.**For example:** Simple microscope and compound light microscope for observing whole mounts or details of tissue sections, different types of microbes etc.

Simple Microscope: A magnifying glass (A few mm)

Compound microscope: It uses a combination of lenses to bend light rays to visualize an enlarged image of minute objects. (1-100um sized objects)

Electron microscope: To visualize very small structures range (0.1 nm). Mainly two types:

- ❖ Scanning electron Microscope (SEM): For visualizing outer structures on cells/specimen
- ❖ Transmission electron Microscope (TEM): For visualizing internal structures of a cell.

The specimen has to be fixed and hence dead

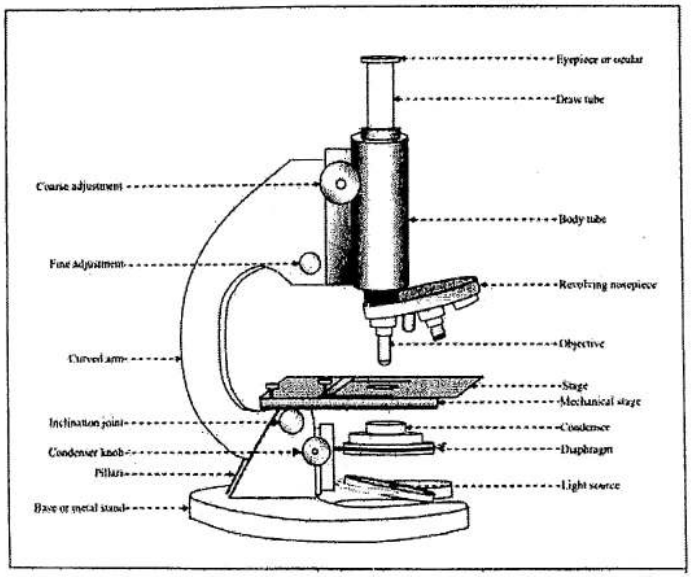
Confocal microscope: Also used for live imaging and at molecular level. E.g. to study protein-protein interaction

Terms to familiarize with

Magnification: The total **magnification** depends upon the power of the ocular times the power of the objective lens.

Resolution = lambda/2 (NA) The wavelength of light produced by most microscopes is 5000 Angstrom units (Å).

The *numerical aperture*, a measurement of the light-gathering ability of the objective, is printed next to or below the magnification on the barrel of the objective. To calculate the resolution in Angstroms for an objective, divide 5000 by the numerical aperture for the lens, then divide the result by 2. To convert the resolution to micrometers, divide the resolution in Angstroms by 10,000.



Points to consider before starting experiment with microscopes:

Cleaning of mechanical parts, lenses etc.

Carrying microscope: Grasp by its arm only if highly required. Put a hand at the bottom for support

Parts of a compound light Microscope:

Lenses (Eye piece or ocular, Nose piece, Objectives (4x, 10x, 40x, 100x also provided with a respective number called numerical aperture or NA). **10x/1.25,**

Mechanical stage, condenser, condenser focusing knob, Iris diaphragm, Course and fine adjustment,

Observation with scanner, low-power, and high-dry objectives:

1. As we will be using **binocular microscopes**, you have to adjust two eyepiecesas per your level of comfort in such a way that the two fields of views should converge to give you one field of view.
2. Place the slide on the stage. Use the knobs of the mechanical stage to move the slide, centering the object in the hole in the center of the stage.
3. Select the scanner (4X) objective. You should begin all microscopic observations on low power. This is the only way to properly focus the microscope. Grasp the nosepiece (not the objectives) and rotate it until the low power objective clicks into place. (If your microscope does not have a scanner lens, then begin with the low-power lens.)
4. Focus the microscope on the scanner power. With the coarse focus knob, raise the stage until it stops. With the fine focus, bring the object into sharp focus. You should be able to focus with only a slight movement of the fine focus.
5. Observe the slide under the scanner power. Use the mechanical stage knobs to find the object and center it in the field of view.
6. Set the proper illumination. Adjust the iris diaphragm until you can see the maximum amount of detail. Use the minimum amount of light necessary to get the best contrast and resolution.

7. Switch from scanner to low-power (10X) objective. After you have focused the object on the scanner, rotate the low-power lens into place. Do not change the focus knobs before moving the nosepiece. Microscopes used in microbiology are **parfocal**, which means that they keep their focus from one objective lens to the next. Once you have moved the low-power lens into place, bring the object into sharp focus with the fine focus knob. Use the iris control to set the best illumination.
8. Switch from low-power to high-dry. After you have focused the object on the low- power, rotate the high-dry lens into place. Do not change the focus knobs before moving the nosepiece. Once you have moved the high-dry lens into place, bring the object into sharp focus with the fine focus knob. Use the iris control to set the best illumination.

Using the oil immersion lens: (This section you will need to know for your next class to visualize slides of stained bacteria)

1. First, you must focus the microscope using the scanner, low power, and high-dry objectives.
2. With the microscope sharply focused with the high-dry lens, rotate the nosepiece halfway to the oil immersion objective, and place a small drop of immersion oil directly on the specimen. Continue to rotate the nosepiece until the oil immersion objective clicks into place. The oil should touch the bottom of the lens and fill the gap between the lens and the object. Focus with the fine focus only until the object comes into view.
3. Never use immersion oil with any lens other than the oil immersion lens. The low-power and high-dry lenses do not have the proper gaskets, so oil can seep into the objective and cause it to become permanently blurred.
4. Clean the oil immersion lens after each use, using the technique demonstrated by the instructor. You will use the oil immersion lens, the objective with the highest magnification, for most of your work. It is 100X on most microscopes, and it works properly only when used with immersion oil. The oil acts to reduce the amount of light lost by scattering, or refraction. This greatly improves the resolution at the highest magnification.

Preparing specimen slides for observations

Each student will be preparing two slides

1. **A plant cell (Onion peel) (<u>this practical</u>)**
2. **Bacterial Cell by Gram staining (<u>next practical</u>)**

Procedure for onion peel slide:

1. You will be given a small piece of onion.
2. Separate a thin, transparent peel from the inner surface of the scale leaf with the help of forceps.
3. Keep this peel in a watch glass containing water.
4. Add **two drops** of safranin stain in the watch glass to stain the peel.
5. Take a clean slide and put **a small drop** of glycerine in the centre of the slide.
6. With the help of a brush and needle transfer the peel on the slide. Glycerine prevents the peel from drying up.
7. Carefully cover it with a coverslip and avoid any air bubble from entering interring the coverslip.
8. Remove any excessive glycerine with a filter paper.
9. Observe the prepared mount of the peel under the low and high magnification of a compound microscope. Record your observations and draw the structure.