Laboratory Instruments

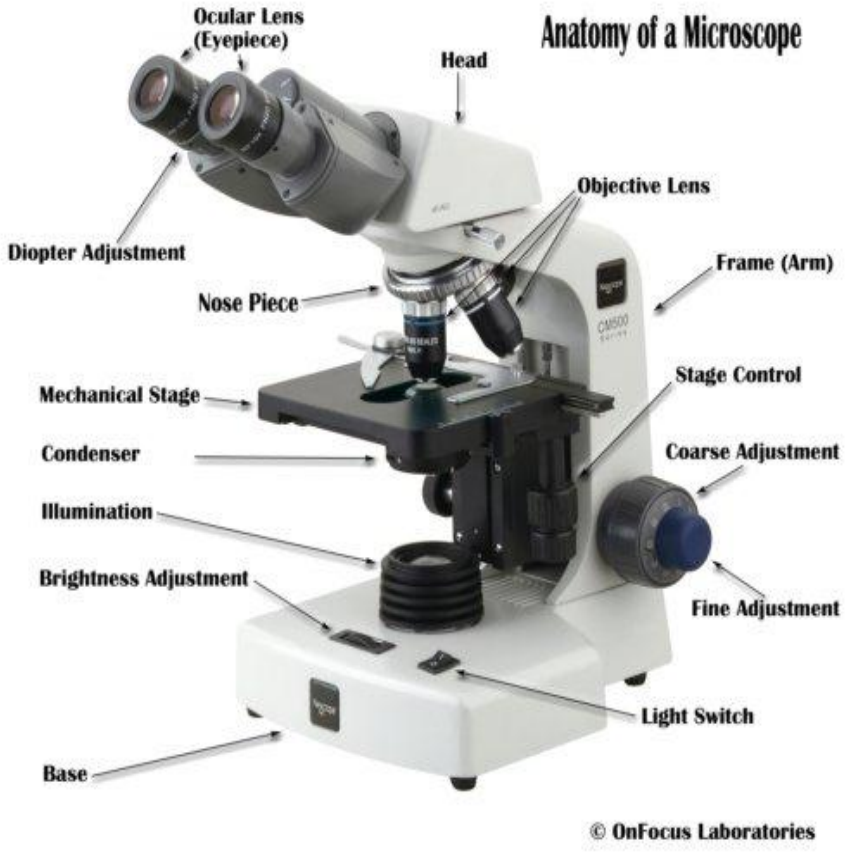
Microscope

It magnifies the image of the object to be visualized through it. Normally, the laboratory microscopes provide a magnification of x 40 (scanner), x 100 (low power), x 400 (high power) and x 1000 (oil immersion). The total magnification is obtained by multiplying the magnification of the objective with that of the eyepiece.

Parts of the Microscope

It has three sets of parts. They are the:

1- Stand
2- Mechanical adjustments
3- Optics or the lenses



Special Applications of Microscope

*Phase contrast illumination

This is needed to visualize transparent microorganisms suspended in a fluid. Ray of light travels in a wave from in a straight line. Two such rays travelling together are said to be in phase, and they produce a brighter illumination. If, however, these rays are out of step with each

other, they are said to be out of phase. They interfere and produce less bright illumination. Phase contrast microscopy makes use of this property of rays to help or hinder each other and thereby resulting increased contrast in the microscopic image.

The desired effect is brought about by placing an annulus in the condenser and a phase plate in the objective. A circle is engraved in the phase plate which matches the ring of beam coming through the condenser and annulus. This circle makes the wave take a longer or a shorter step, so becoming out of phase with those waves which pass through the rest of the plate.

Supposing that the specimen is suspension-free fluid, the only light that reaches the eye is that which goes from the annulus through the phase plate. Whereas presence of organisms would diffract and scatter the light. The light passing through the fluid gets out of phase with the light that has the organisms stand out in contrast to their background.

Equipment Needed

An annulus, a phase plate and a telescope that is needed for adjusting the rings of both annulus and the phase plate.

Method

1- Focus the specimen with the right objective after illuminating the microscope.
2- Place the matching annulus at its position.
3- Remove the eyepiece and put the telescope in its place, adjust it till the two rings, one bright and one dark are in focus.
4- Adjust condenser screws till the bright annulus rings fits exactly into the darker ring of the phase plate.
5- Remove the telescope, replace the eye piece, focus and examine the specimen.

Importance

This method is made use of for examining live organisms, e.g.

a- *Vibrio cholera*
b- Amoebae
c- Trypanosomes
d- *Trichomonas*
e- Other flagellates

It can also be used for platelet counting and for examining routine urine specimens.

Demerits

a- A halo is seen around each particle, it gives a false appearance of its structure.
b- In addition, some resolution power is lost but this is more than compensated for by the increased contrast that is produced.

*Dark Ground illumination

This method too, is used for visualizing organisms can be seen. In this method the light that is seen comes only from the microorganisms themselves and not from the light source. Hence, the organisms are brightly illuminated against a dark background.

Equipment Needed

1- An oil immersion dark ground condenser with the centering screws.
2- A funnel stop for insertion in 100 x objective to reduce its the numerical aperture(NA) and exclude light coming directly from the source.
3- A brightly illuminated microscope lamp.
4- Scratch less slides note more than 1 mm thick.

Method

1- Fit the dark ground condenser, raise it to stage level.
2- Place the cover slipped specimen on the thin polished glass slide. Both, the coverslip and the slide should be absolutely clean.
3- Place a drop of immersion oil between the condenser and the slide.
4- Adjust light source and the mirror properly.
5- Focus 10 x objective and observe.
6- Focus condenser up or low so that the ring ultimately becomes just a spot of light. Focus this spot right in the center.
7- Use 40 x objective, if needed, use the 100 x oil immersion by inserting the funnel stop into it.

Demerits

1- Focusing and/or centering of condenser is difficult as is the alignment of the lamp.
2- Difficulties may arise under the following circumstances:

- Smear traces on the slide or coverslip.
- If the specimen is dense.
- A bubble is present in the immersion oil.
- Insufficient oil contact below or above the slide.

Importance

1- This method is of particular importance for the examination of *Treponema* group of organisms.
2- It can also be of use for *Microfilariae*, for the sheath of the pathogenic forms can be clearly seen which otherwise needs to be stained.
3- For examining the rapid movement of *Vibrio cholera*.
4- In addition, this method can be used for:
   - *Leptospira*
   - *Borrelia*
   - *Spirillum* species.

(The ideal objective for dark ground illumination is the 50 x fluorite as this lens gives a clear, sharp and a well-illuminated image).

*Fluorescence Microscopy

This method entails the illumination of particles/ microorganisms (previously stained with a fluorescent dye) with ultraviolet (UV) light into visible light (yellow or orange), by lengthening their wavelength. The procedure is made use of for visualizing, besides other things, mycobacteria glowing against a black background.

All other wavelengths emitted by the lamp except the ultraviolet (UV) are to be filtered off (by using appropriate optical filters) and no harmful rays of UV light should reach the observes eye (by using an immersion dark ground condenser as described for previous method). Again, another filter is used to remove all unwanted fluorescent light by placing a secondary or a barrier filter above the eyepiece.

Equipment Needed

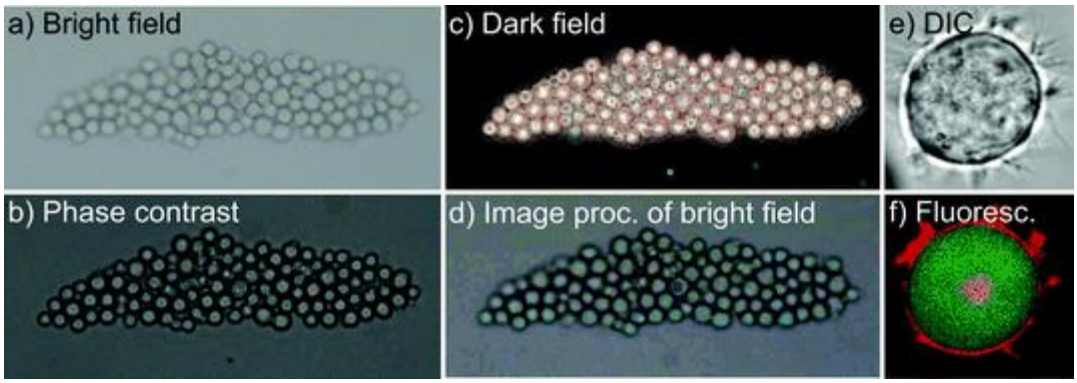
1- A fluorescent lamp (mercury vapour or quartz iodine, the latter is better, being cheaper, lighter and easier to use).
2- A blue (primary or exciting) filter, generally a BG12.
3- A yellow (secondary or barrier)filter.
4- An immersion dark ground condenser.
5- A nonfluorescent immersion oil, e.g. liquid paraffin.

Importance

1- For identifying mycobacteria.
2- It is used extensively in fluorescent antibody techniques used in parasitology and bacteriology.
3- It is also used widely in histology of kidney, skin, etc., where immune/autoimmune basis of disease is expected. In fact, anything can be confirmed with high degree of sensitivity and specificity, if

antibodies against it (later tagged with a fluorescent dye) can be produced.

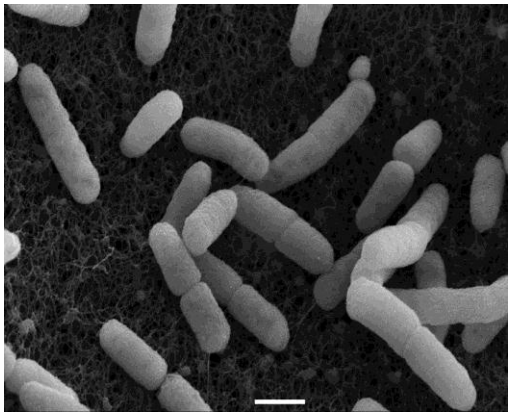
4- Used widely in cytogenetic.



*Electron Microscope

Basic Principle

The resolution of the light microscope has been shown to be limited by the numerical aperture(NA) and the wavelength of light employed. As the degree of correction in glass lenses is very high, the main limitation is imposed by the light (e.g., half wavelength of light), giving a normal resolution of approximately 250 nm; and when UV light is used, a resolution of about 100 nm. By the substitution of an electron beam for light rays, a much greater degree of resolution can be obtain; since at an acceleration of 50000 volts, electrons have a wavelength of only 0.001 nm; therefor, a theoretical resolving power of 0.0005 nm could be attained, which would enable molecules to be seen. Unfortunately, the degree of correction that is currently feasible with transmission electron microscope(TEM) lenses will permit a resolution of only 0.25 nm, but this is still a thousand times greater than the possible with the light microscope. A further difficulty with the TEM is that, since electrons have poor penetrating power, the sections to be examined must be very thin, less than 50 nm thick. This necessitates the use of special hard embedding media (plastics) and special ultra-microtomes to cut such thin sections. Steel knives cannot be used to cut these sections; either glass or diamond knives are used.



Weighing Scales or Analytical Balance

Weighing Scales: For weighing large quantities.

Analytical Balance: For accurate weighing of smaller quantities.

Use and Care

1- The weighing equipment must be placed on a firm bench, away from vibration, draughts, direct sunlight and just.
2- It should be kept perfectly horizontal by altering the screws on which the equipment stands.
3- Chemicals, etc. should never be placed directly on the pans. Weigh them in a container.
4- Never tough the weights with hands, handle them with forceps.
5- The balance should be at rest before adding or removing the weights or chemicals.
6- Before taking the reading, the glass window of the instrument should be closed.

Electronic analytical balance are also available.

Centrifuge

Centrifuge is used to sediment or deposit rapidly particles such as cells which may be suspended in a fluid. The speed is expressed as **rpm**, i.e. revolution per minute.

Relative Centrifugal Force(RCF)

More important than rpm is relative centrifugal force(**RCF**). RCF is expressed as the acceleration due to gravity or G (dynes per cm). the formula is:

$G = 0.00001118 \times (r) \times (n)^2$

Where *r* = radius in centimeters

and *n* = revolutions per minute

The time of centrifugation is equally important. The tubes should be spun for a definite period to obtain the desired effect.

Types of Centrifuge

Hand Centrifuge: Fixed to the bench, the handle is rotated manually. It gives low speeds only.

Motor-driven Centrifuge: Operated through mains electricity supply. The tubes may be kept in a fixed angle head or in a swing out head.

Microhematocrit Centrifuge: Also motor driven for finding out packed cell volume(PCV) of red blood cells(RBCs). In this, blood-filled capillary tubes are spun and later the percentage of RBC-filled column is estimated.

Use and Care

1- Use centrifuge tubes made of strong glass and they should not be too long.
2- The opposite tube should be balanced properly.

3- The centrifuge speed should be increased gradually.
4- The instrument should be kept clean. If something spills over inside, it should be cleaned and the instrument disinfected if necessary.

Other Necessary Equipment

Serological Water Bath: It is electrically heated and has a thermostatic temperature regulator. It can provide temperature ranging from room temperature to 100̊ C. various sizes to suit various workloads are available.

Incubator: Works on electricity and regulates temperature thermostatically. Necessary for various investigations where body temperature 37˚C (or otherwise) incubation is required.

Hot Air Oven: This is used for drying and sterilizing glassware. This too is thermostatically controlled and electrically heated. Its looks like an incubator.

**Glassware : Explain glassware which are necessary to work in the laboratory????