# THE MICROSCOPE

## 1-Introduction :

The microscope is a valuable instrument. There are many small objects or details of objects which cannot be seen by the unaided human eye. The microscope magnifies the image of such objects thus making them visible to the human eye. Microscopes are used to observe the shape of bacteria, fungi, parasites and host cells in various stained and unstained preparations.

## 2-Types of Microscopy

Microscopes used in clinical practice are light microscopes. They are called **light microscopes** because they use a beam of light to view specimens.
**A compound light microscope** is the most common microscope used in microbiology. It consists of two lens systems (combination of lenses) to magnify the image. Each lens has a different magnifying power. A compound light microscope with a single eye-piece is called **monocular**; one with two eye-pieces is said to be **binocular**.
Microscopes that use a beam of electrons (instead of a beam of light) and electromagnets (instead of glass lenses) for focusing are called **electron microscopes**. These microscopes provide a higher magnification and are used for observing extremely small microorganisms such as viruses.

### Light microscopy

### Brightfield microscopy

This is the commonly used type of microscope. In brightfield microscopy the field of view is brightly lit so that organisms and other structures are visible against it because of their different densities. It is mainly used with stained preparations. Differential staining may be used depending on the properties of different structures and organisms.

### Darkfield microscopy

In darkfield microscopy the field of view is dark and the organisms are illuminated. A special condenser is used which causes light to reflect from the specimen at an angle. It is used for observing bacteria such as treponemes (which cause syphilis) and leptospires (which cause leptospirosis).

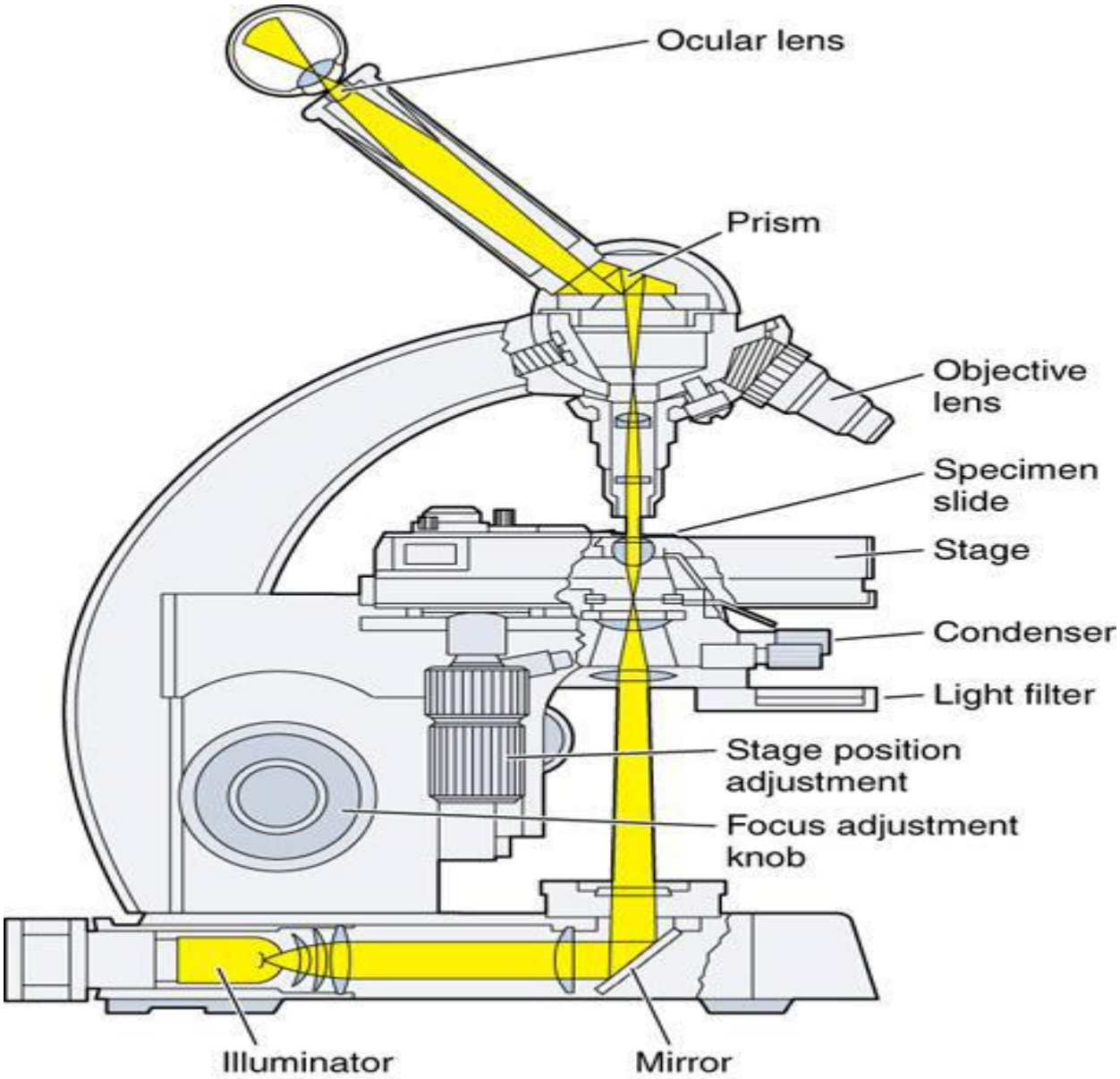
### Phase-contrast microscopy

Phase-contrast microscopy allows the examination of live unstained organisms. For phase-contrast microscopy, special condensers and objectives are used. These alter the phase relationships of the light passing through the object and that passing around it.

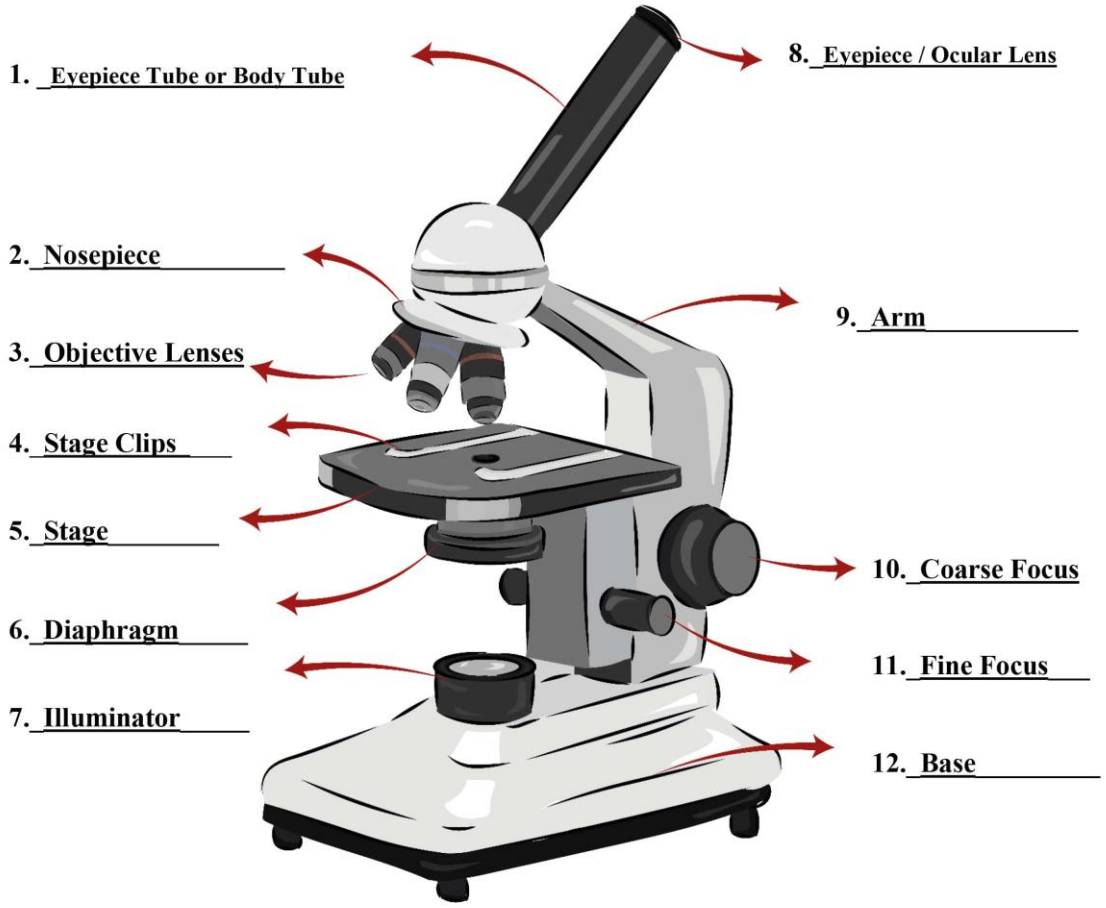
**Fluorescence microscopy**

In fluorescence microscopy specimens are stained with fluorochromes/ fluorochrome complexes. Light of high energy or short wavelengths (from halogen lamps or mercury vapour lamps) is then used to excite molecules within the specimen or dye molecules attached to it. These excited molecules emit light of different wavelengths, often of brilliant colours. Auramine differential staining for acid-fast bacilli is one application of the technique; rapid diagnostic kits have been developed using fluorescent antibodies for identifying many pathogens.

## 3- Parts of the Microscope



# Parts of a Microscope Worksheet




## Eye-pieces

- The specimen is viewed through the eye-piece (Fig. 3.2). It has a lens which magnifies the image formed by the objective. The magnifying power of the eye-piece is in the range 5x.20x. A movable pointer may be attached to the inside of theeye-piece.
- In binocular microscopes, the two eye-pieces can be moved closer or farther apart to adjust for the distance between the eyes by pulling. pushing motion or by moving a knurled ring.

## Microscope tube

The microscope tube is attached on top of the arm. It can be of the monocular or binocular type. It supports the eye-piece on the upper end.

## Mechanical tube length

- Mechanical tube length is the distance between the place where the objective is inserted and the top of the draw-tube into which the eyepieces fit.

- In modern microscopes it is not tubular; it contains prisms that bend the light coming up, thus providing a comfortable viewing angle (Fig. 3.3). In a binocular tube, the light is also split and sent to both eye-pieces.

## Nose-piece

The nose-piece is attached under the arm of the microscope tube. The nose-piece (Fig. 3.4) houses the objectives and rotates them. The objectives are arranged in sequential order of their magnifying power, from lower to higher. This helps to prevent the immersion oil from getting onto the intermediate objectives.

## Objectives

The numerical aperture (NA) is the measure of light-gathering power of a lens. The NA corresponding to the various magnifying powers of the objective is:

| Magnification | Numerical aperture |
|---|---|
| **10X** | **0.25** |
| **40X** | **0.65** |
| **100X** | **1.25** |

A high NA indicates a high resolving power and thus useful magnification (see page 10).

To provide the best image at high magnification, immersion oil is placed between the slide and the oil immersion objective (100x). Unlike air, immersion oil has the same refractive index as glass. Therefore, it improves the quality of the image. If immersion oil is not used, the image appears blurred or hazy.

## Mechanical stage

- The mechanical stage holds the slide and allows it to be moved to the left, right, forward or backward by rotating the knobs.
- It is fitted with fine vernier graduations as on a ruler. This helps in relocating a specific field of examination.

## Condenser

- The condenser (Fig. 3.6) illuminates the specimen and controls the amount of light and contrast. There are different types of condensers. Some condensers have a rack-and pinion mechanism for up-and-down adjustment.
- The NA of a condenser should be equal to or greater than that of the objective with maximum NA.

- An iris diaphragm is provided below the condenser. This adjusts the NA of the condenser when using objectives having low magnifying power.
- A swing-out type filter holder may be fitted above or under the condenser. In some microscopes the filter holder may not be swing-out type. The filter holder holds detachable filters when required.
- Condenser centring screws, when present, are used to align the condenser with the objective.
- A condenser raising knob may be present (if centring screws are notthere), or the distance may be fixed.

## Two-sided mirror

A mirror (Fig. 3.7) is the simplest illuminator. The two-sided mirror provides necessary illumination through reflection of natural or artificial light. It has two surfaces, one plain for artificial light and other concave for natural light. It is supported on two sides by a fork fixed on a mount in a way that permits free rotation.

## Built-in light sources

An illuminator is built into the base of the microscope. A halogen bulb provides the best illumination. On top of the illuminator is an in-built filter holder to fit the filter of desired quality.

## Filters

- Blue filters are used to change the light from ordinary electric bulbs into a more natural white light.
- Neutral density filters are used to reduce brightness without changing the colour of the background.
- Green filters may be useful in some situations.

The object of AFB (Ziehl.Neelsen) microscopy is to find AFB, which are stained red by carbol fuchsin. The intensity of the red colour decreases when blue/green filters are used. Blue/green filters are, therefore, not recommended for Ziehl.Neelsen microscopy.

Cedar wood oil should not be used as it leaves a sticky residue on the objective. If cedar wood oil is used, particular care then needs to be taken to ensure that the objective is thoroughly and promptly cleaned with xylene after each session of use. Petrol can be used in place of xylene for cleaning if xylene is not available.

Liquid paraffin should not be used as it has a low refractive index which produces an inferior image. It is also unsuitable for scanning specimens for long periods, as is required for accurate microscopy.

## Coarse and fine focusing knobs

The coarse and fine focusing knobs are used to change the distance between the specimen slide and the objective. The coarse focusing knob alters this distance rapidly and is used to bring the specimen into the field of view using an objective having low magnification power. The fine focusing knob changes the distance very slowly and permits better viewing of the object. One revolution of the fine focusing knob should generally move the mechanical stage by 100 mm. The movement should be smooth and free from jerks.

## Functioning of the microscope

There are three main optical pieces in the compound light microscope. All three are essential for a sharp and clear image. These are:

- Condenser
- Objectives
- Eye-pieces.

The condenser illuminates the object by converging a parallel beam of light on it from a built-in or natural source. The objective forms a magnified inverted (upside down) image of the object. The eye-piece magnifies the image formed by the objective. This image is formed below the plane of the slide. The total magnification of the microscope is the product of the magnifying powers of the objective and the eye-piece. For example, if the magnifying power of the eye-piece is 10x and that of the objective is 100x, then the total magnification of the compound light microscope is: 10x **X** 100x = 1000-fold magnification.

# 4 Routine Operation of the Microscope

- Ensure that the voltage supply in the laboratory corresponds to that permitted for the microscope; use a voltage protection device, if necessary.
- Turn on the light source of the microscope .
- With the light intensity knob, decrease the light while using the low magnification objective.
- Place a specimen slide on the stage. Make sure the slide is not placed upside down. Secure the slide to the slide holder of the mechanical stage
- Rotate the nose-piece to the 10x objective, and raise the stage to its maximum.
- Move the stage with the adjustment knobs to bring the desired section of the slide into the field of view.
- Focus the specimen under 10x objective using the coarse focusing knob and lowering the stage
- Make sure the condenser is almost at its top position. Centre the condenser using condenser centring screws if these are provided in the microscope. For this take out one eye-piece and while looking down the tube, close the iris diaphragm till only a pin-hole remains. Check if this is located in the centre of the tube.

- Open the condenser iris diaphragm to 70%.80% to adjust the contrast so that the field is evenly lighted
- Adjust the interpupillary distance till the right and left images become one .
- Focus the image with the right eye by looking into the right eye-piece and turning the focusing knob
- Focus the image with the left eye by looking into the left eye-piece by turning the diopter ring
- Put one drop of immersion oil on the specimen .
- Change to 100x objective Routine Operation of the Microscope
- Increase the light by turning the intensity knob until a bright but comfortable illumination is achieved.
- Focus the specimen by turning the fine focusing knob.
- When the reading/observation has been recorded, rotate the objective away from the slide.
- Release the tension of the slide holder, and remove the slide.
- If immersion oil was used, wipe it from the objective with lens paper or muslin cloth at the end of each session of use. In general, avoid wiping the objective except when it seems to be dirty.
- Turn off the light.
- Cover the microscope when not in use and take necessary precautions against fungus.

**Eye strain should not develop if the microscope is used properly.**

**Never adjust the stage upward while looking through the eye-piece. It will cause the objective to push against the slide and may damage it.**

**Only the 100x objective can be used for viewing under immersion oil. All other lenses are to be used without immersion oil; keep them dry and avoid applying oil or any liquid to these lenses.**

# 5 Maintenance of the Microscope

## Installation and storage

- Install the microscope on a sturdy, level table. Equipment and instruments which generate vibrations, such as centrifuges and refrigerators, should not be placed on or near this table.
- The height of the table should be convenient for the user. As an alternative or in addition, an adjustable stool should be made available to make microscopy comfortable.
- The table should be away from water, sinks, and racks containing chemicals, to prevent damage to the microscope from splashes or spills.
- If the microscope does not have a built-in light source then the table should be placed near a window away from direct sunlight and arrangements made for the provision of a lamp.
- In so far as is possible, the microscopy room should be free from dust and should not be damp.
- If the microscope is to be used every day, do not remove it from the site of installation, provided security is assured.
- When the microscope is not in use, keep it covered with a polythene or plastic cover and take necessary precautions against fungus.
- In humid areas, store the microscope every night in a cabinet fitted with an electric bulb (5 W or 40 W). This is switched on at night to reduce humidity.
- If the microscope is used intermittently and requires storage for prolonged periods, keep it in an air-tight plastic bag with about 100g of drying agent. Remember to regenerate/replace drying agents (silica gel or dry rice) fortnightly or as needed.
- If only a wooden box is available, keep the microscope in it with some dry silica gel or dry rice

## Maintenance of lenses

Avoid collection of dust and immersion oil on the objectives and eye-pieces by keeping the microscope covered. Do not allow immersion oil to touch any of the objectives other than the oil immersion objective. Always keep the eye-pieces in place to protect the inner surface of the objective. Close the holes of missing objectives in the nose-piece by using special caps that are provided, or by sealing with adhesive tape.

## Removal of dust from lenses

Check for dust or dirt on the lenses (eye-pieces, objective, condenser and illuminator lenses) if the image appears hazy or with black dots.

- If the black dot moves when the eye-piece is rotated, this means that the dust is on the eye-piece.
- If the black dot moves when the slide moves then the dust is present on the slide.

- If these two are ruled out, presume that the dust is on the objective. Dust on objectives shows as dots if it is inside. If the dust is outside the objective it shows as a hazy image.

**Do not remove the dust from the lenses by wiping these with a cloth as this can scratch the lens and damage it permanently. Use an airbrush or a camel-hair/artist.s brush.**

Dust can be removed with a camel-hair/artist.s brush or by blowing air over the lens with an airbrush. Dust on the inner surface of the objective can be removed by using a soft camel-hair brush (artist.s brush).

## Removal of oil from lenses

The presence of oil on the lenses produces a hazy image. The localization of oil can be done by the same method as has been described above for localization of dust.

**Oil should be removed with the help of lens paper using lens cleaning fluid as recommended by the manufacturer. This can be applied gently with lens paper. Do not use force to remove oil as this might result in scratches on the lens.**

If the field of view is not clear despite cleaning, and the microscope works well with another lens, then the lens has been permanently damaged and must be repaired or replaced.

If the field of view is not clear even after changing the lenses (objective and eye-piece) there is probably dirt or fungus on the tube prisms. These can be checked by removing the eye-pieces, and examining the upper part of the microscope tube with the light fully open. Fungus is seen as threads, dots or a woolly layer.

## Inspection of the objective

- Carefully unscrew the objective from the nose-piece.
- Gently remove one eye-piece to use as a magnifier (or use a magnifying glass).
- Grasp the objective in one hand with the front lens face up.
- Hold the eye-piece in the other hand with the top lens facing down.
- Bring the eye-piece very close to your eye and focus on the objective. Change the angle of the objective so that light can reflect off its surface. The two lens surfaces will be about 2.5 cm apart. Try to avoid letting them touch each other.
- Inspect the objective for scratches, nicks, cracks, deterioration of seal around the lens, or oil seepage into the lens.

# 6 Care of the Microscope

## After daily use

- Bring the variable voltage regulator setting to the minimum before turning off the lamp. Turn off the light source of the microscope.
- Gently wipe the immersion oil off the objective, condenser and mechanical stage with lens paper or muslin cloth.
- Replace the cover of the microscope and take necessary precautions against fungus.

## Each month

- Use an air brush to blow away dust. Clean the objectives, eye-pieces, and condenser with lens cleaning fluid. Do not put fluid directly on the lenses; instead, apply it to the lens paper and then clean.
- Remove the slide holder from the mechanical stage and clean.
- With a tissue moistened with water, wipe the dust off the body of the microscope and the window of the illuminator in the base of the unit.

## Every six months

Thoroughly inspect, clean, and lubricate the microscope after consulting the manufacturer's manual. This should preferably be done by professional service personnel.