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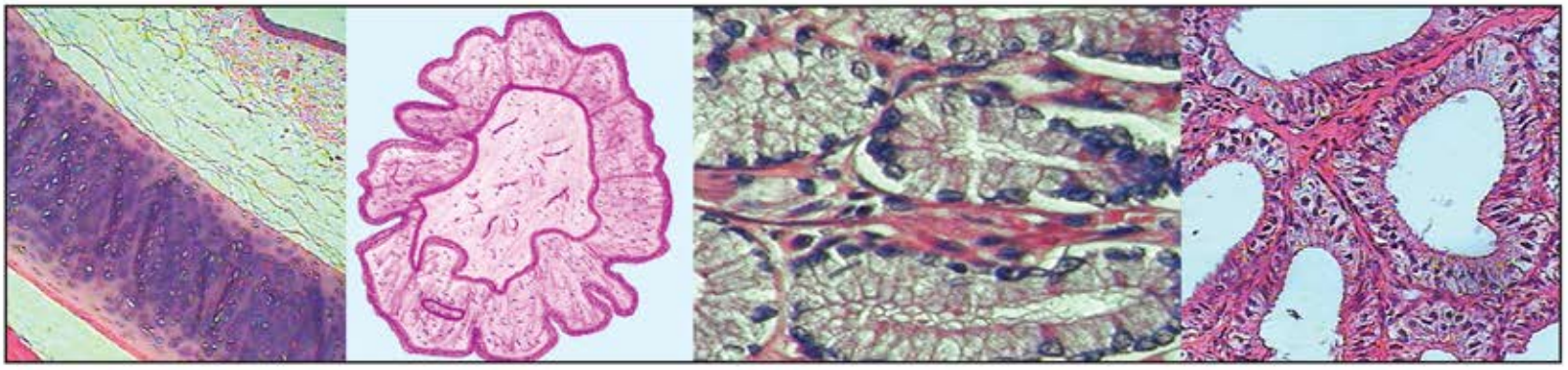
Basic Histology

A Color Atlas & Text



V Subhadra Devi

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2 Chapter

Microscopy

MICROSCOPE

- The principal instrument in histology is the compound microscope.
- A microscope is an instrument for viewing objects that are too small to be seen by the naked or unaided eye.
- The compound microscope is also called optical or light microscope as it uses lenses and light to enlarge the image.
- The light microscope allows us to magnify cells up to a thousand times and resolve details (distinguish two dots) as small as 0.2 micrometers.
- The compound microscope has two systems of lenses for greater magnification:
  - Ocular or eyepiece lens through which one looks.
  - Objective lens or the lenses (usually three) closest to the object.
- The magnifying power of lens in the eye piece is 10x and those in the objectives are 10x (low power), 40x or 45x (high power) and 100x (oil immersion).

PARTS OF A MICROSCOPE (FIGS 2.1A AND B)

The parts of light microscope can be broadly classified into three components:

1. Magnifying units—Object lenses and eye piece lens.
2. Illuminating units—condenser and mirror.
3. Mechanical units—Body tube with coarse and fine adjustments, inclination joint, mechanical stage, the focusing adjustment of condenser and supporting base.

Base

The bottom of the microscope, used for support and usually contains a light source or an illuminator.

Body Tube

- It separates the objective and the eyepiece.
- It keeps the alignment of optics.
- It sends light to the ocular lens.
- The optical tube length of the microscope is the distance between the back focal plane of the objective and the front focal plane of the eyepiece.

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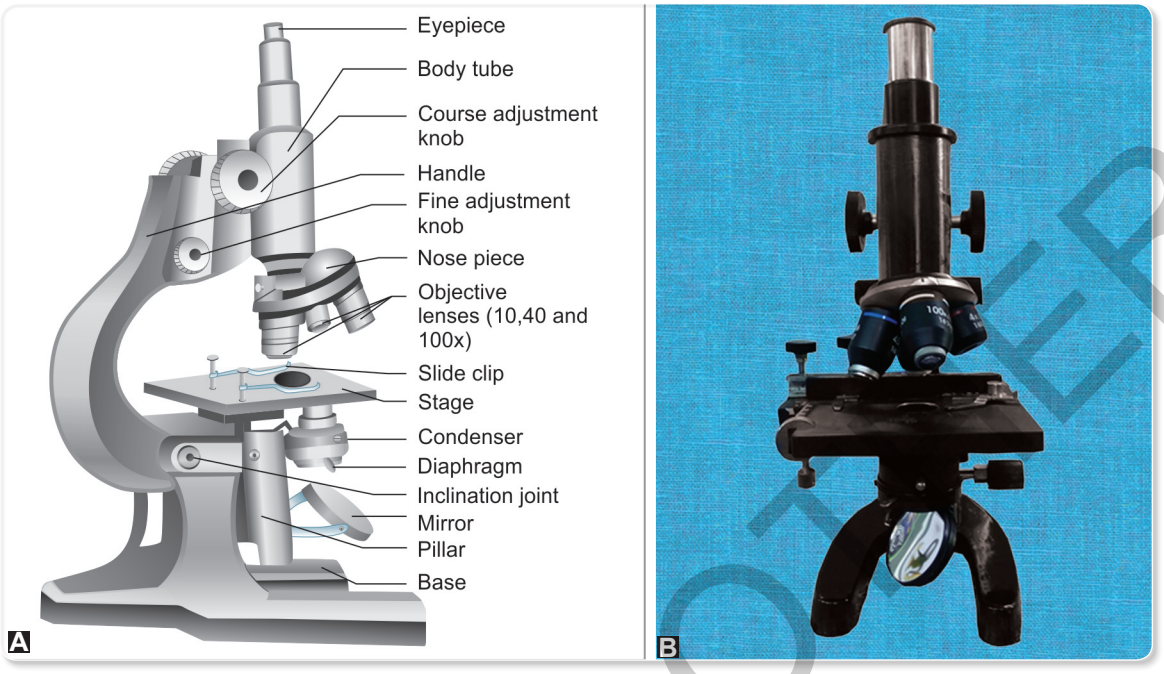

Figs 2.1A and B: Compound microscope

- It connects the eyepiece lens to the objective lenses.
- The front focal plane of the eye piece is arranged to coincide with the plane in which the image would be formed by the objective if there is no intervening field lens.
- The mechanical tube length is the distance between the upper edge of the ocular sleeve or draw tube and lower edge of the revolving nose piece. It is 160 mm and the objectives are adjusted to this distance.

Arm

Supports the tube (containing lens system) and connects it to the base.

Source of Illumination

- It can be mirror or an artificial Illuminator.
- If the microscope has a mirror, it is used to reflect light from an external light source (day light but not direct sun light) up through the bottom of the stage.
- The mirror is planoconvex and can be turned in any direction for reflecting light.
- A steady light source (opal electric bulb of 110 volts) is used in place of a mirror.
- Blue or green filters are used to reduce intensity.

Stage

- The flat platform to place the slide.
- Stage clips hold the slide in place.
- If the microscope has a mechanical stage it will be possible to move the slide by turning two knobs.
- One knob moves it left and right, the other moves it up and down.

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Ocular or Eyepiece Lens

1. The lens at the top of microscope through which we look.
2. It magnifies the image from the objective lens.
3. They are usually 5x, 8x, 10x or 15x power.
4. Eye pieces above 10x are not commonly used as they cause distortion and haziness of the image.
5. When looking through the microscope with one eye, the other eye should not be closed and it is advisable to use both eyes to avoid strain in one eye.

Revolving Nosepiece or Turret

This is the part that holds two or more objective lenses and can be rotated to easily change power.

Objective Lenses

- These magnify the image.
- The objectives are designated by their focal length and oculars by their factorial magnification.
- The focal length, magnification and numerical aperture of the objectives are generally inscribed on the objective mount.
- Usually, we find 3 or 4 objective lenses on a microscope. They almost always consist of 4x, 10x, 40x and 100x powers.

Condenser Lens

- The purpose of the condenser lens is to focus the light onto the specimen.
- Condenser lenses are most useful at the highest powers (400x and above). Microscopes with in stage condenser lenses render a sharper image than those with no lens (at 400x).
- If the microscope has a maximum power of 400x, we will get the maximum benefit by using a condenser lense rated at 0.65 NA or greater. For a magnification of 1000x, a focusable condenser lens with a NA of 1.25 or greater is required.
- The condenser lens can be moved up and down. It is set very close to the slide at 1000x and moved further away at the lower powers.

Diaphragm or Iris

- A rotating disk under the stage.
- It is used to stop the passage of light except for the light that passes through the aperture.
- This diaphragm has different sized holes and is used to vary the intensity and size of the cone of light that is projected upward into the slide.
- There is no set rule regarding which setting to use for a particular power. Rather, the setting is a function of the transparency of the specimen, the degree of contrast you desire and the particular objective lens in use.

Coarse and Fine Adjustment Knobs

- Coarse adjustment knob is used when focusing a specimen.
- Fine adjustment knob focuses a sharp image on the specimen.
- The larger coarse adjustment and smaller fine adjustment knobs are fixed to the arm.

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PRINCIPLES OF MICROSCOPY

- What one views through the microscope is not the specimen but the image of the specimen.
- In order to properly understand the images produced by microscope one has to understand how they are produced.
- The input requirements of optical system of human eye determines the output requirements of lens systems of the microscope.
- There are two important aspects in the principles of microscopy:
  - Resolution and Resolving power
  - Magnification

DEFINITIONS

Resolution

- Resolution is a measure of amount of clarity and sharpness of the object.
- It is the ability of microscope to distinguish between two points that are close together.
- The resolving power is defined as the minimum distance by which two points must be separated in order to perceive them as two separate points rather than as a single fused image.
- This should not be confused with magnification.
- Resolution determines the performance of a microscope.
- For calculating the resolving power of a microscope, the following physical parameters are required
  - Numerical aperture of objective (NA) and condenser lenses.
  - Wavelength of light.

Numerical Aperture

- Numerical aperture is a mathematical calculation of light gathering capability of a lens.
- The numerical aperture of a microscope objective is its ability to gather light and resolve fine details of specimen while working at a fixed distance from the specimen.
- It is represented by the symbol NA.
- The value of NA has relationship with both condenser lens and objective lens. For sharp images the NA of condenser should be equal or higher than the specific objective lens. The NA of each objective lens is inscribed on the metal tube and ranges from 0.25–1.4.
- Higher NA indicates better light gathering property of lens and better resolution. Higher NA means shorter working distance between lens and object. NA of more than 1.0 indicates use of immersion oil.
- The NA of most condensers are 1.25.
- The length of mechanical microscopic tube, thickness of the slide, thickness of cover slip and the media between objective lens and coverslip are also important variables to be considered for effective resolving of details.
- The standard length of the mechanical tube holding the eye piece lens and revolving nose piece containing objective lenses is 160 mm that is required for proper working of the lens.
- The ideal thickness of glass slide is 1.0 mm and that of coverslip is 0.17 mm. The commonly used medium between objective lens and coverslip is the oil. The focal length of the objective is 45 mm.
- An objective lens with a larger numerical aperture will visualize finer details than a lens with a smaller numerical aperture.
- NA of condenser lens is as important as that of objective lens. Closure of condenser diaphragm results in loss of resolution. A full aperture with oil immersion gives better resolution.

Numerical aperture of each objective

Objective	NA
10x	0.25
40x	0.65
100x	1.25

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Wavelength

- Resolution is also influenced by wavelength of light used to illuminate the specimen.
- If the space between two particles is smaller than the wavelength then their image cannot be resolved. Shorter wavelengths are capable of resolving details to a greater degree than larger wavelengths.

shorter wavelength ⇒ greater resolution

- Greatest resolving power in optical microscopy is with near-ultraviolet light that has shortest wavelength. This is followed by blue, green and red light in that order in their ability to resolve specimen details.
- Tungsten-halogen bulb is commonly used for illumination of specimen in light microscopy for generating white light.

Magnification

- Magnification increases the size of image.
- This is the ratio of image length to object length measured in planes perpendicular to optical axis.
- This is represented by the letter x.
- The magnification of a microscope is calculated by multiplying the eyepiece lens magnification by the objective lens magnification. For example,10x (eyepiece) × 100x (objective) = 1000x, i.e. the specimen is magnified 1,000 times to the normal eye sight.

Note: As magnification increases the resolution decreases and vice-versa.

Care of Microscope

How to focus your microscope:

- Lower the stage to the lower position using coarse focusing knob. Always start with 10x power objective.
- Place the slide on the stage and use slide clips on the top of the stage to hold the slide in place.
- While looking from the side, bring the objective lens down as close to the specimen as possible without touching it.
- Use the coarse adjustment knob to focus on the slide and use the fine adjustment knob until the object is in sharp focus.
- Adjust the light intensity by raising or lowering the condenser for best image.
- After focusing with 10x objective rotate 40x objective in position over the slide. Use only fine adjustment knob for sharp image.
- Rotate the objectives so that there is no lens directly over the slide. Place a drop of immersion oil on the slide and slowly rotate the 100x objective on the slide. Adjust light intensity if required.
- At the end of laboratory work lower the stage to the lowest position. Clean all lenses and keep the 10x objective in position.

DIFFERENT TYPES OF MICROSCOPES

Compound microscope/light microscope (see Figs 2.1A and B):

- It is also called optical or light microscope as it uses lenses and light to enlarge the image.
- The image seen is two dimensional.
- This is the most commonly used microscope.
- Both living and non-living individual cells can be observed.
- It has high magnification but low resolution.

Dissection microscope/stereo microscope (Fig. 2.2):

- It is light illuminated.
- The image that appears is three dimensional.
- It is used in dissection for better visualization of a larger specimen.
- Individual cells cannot be identified as it has low magnification.



Fig. 2.2: Dissection microscope

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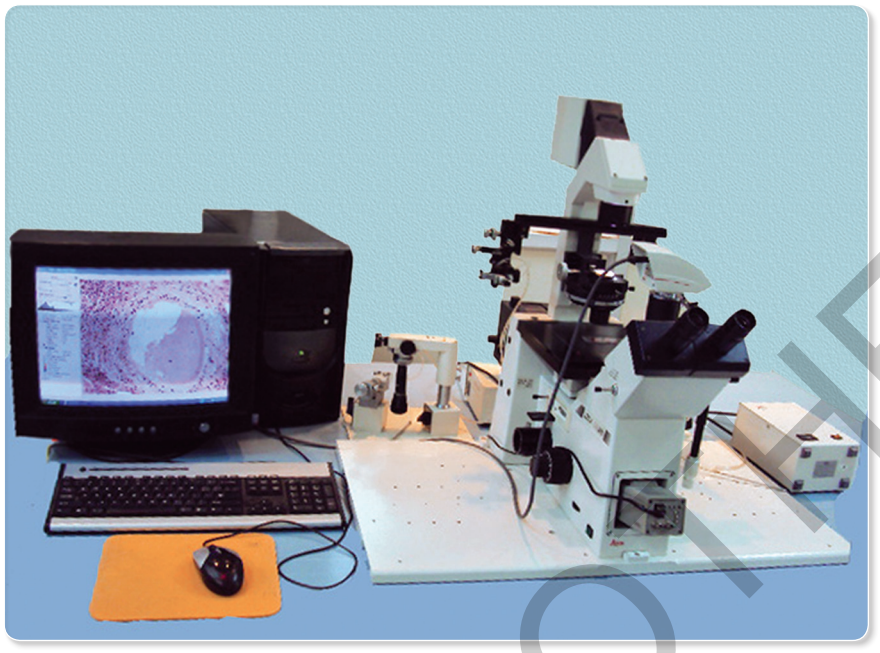

Fig. 2.3: Dark field, phase contrast, differential, interference contract and fluorescence microscopy as single unit

Dark field microscope (Fig. 2.3):
- Produces a bright image of the object against a dark background.
- Used to observe living, unstained preparations like uric acid and oxalate crystals in urine and *Treponema pallidum* the organism that causes syphilis.

Phase contrast microscope (Fig. 2.3):
- Enhances contrast between intracellular structures with differences in refractive index.
- Best method for observing living cells.

Differential interference contrast microscope (Fig. 2.3):
- Creates image by detecting differences in refractive indices and thickness of different parts of specimen.
- Excellent method to observe living cells.

Fluorescence microscope (Fig. 2.3):
- It exposes specimen to ultraviolet, violet, or blue light.
- Specimens are usually stained with fluorochromes.
- Shows a bright image of the object resulting from the fluorescent light emitted by the specimen.
- It is used for observing naturally fluorescent molecules like vitamin A and certain neurotransmitters, by injecting specific fluorescent molecules for detection of antigens or antibodies, for observing gap junctions between cells and in tracing neuronal pathways.

Stereo-zoom microscope (Fig. 2.4):
- It is used to study the surfaces of solid specimens or to carry out close work such as microdissection or microsurgery.
- These use lower power than compound microscopes.
- These are used to view the whole object.



Fig. 2.4: Stereo-zoom microscope

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Electron microscope:

- Uses a beam of high speed electrons instead of light.
- Electron particles bounce off from the specimen and form image on photographic plate or TV screen.
- It has better magnification and resolution than light microscope.
- It is used for observing small objects like viruses, DNA, parts of a cell.
- Types: It is of two types:
  - Scanning electron microscope (SEM) (Fig. 2.5)
    - » The image is seen in 3D.
    - » It has high magnification and high resolution.
    - » It uses electrons reflected from the surface of a specimen to create image.
    - » The specimen is coated in gold and the electrons bounce off to give exterior view of the specimen. The pictures are in black and white.
    - » Can magnify up to 100,000x.
  - Transmission electron microscope (TEM)
    - » This gives a 2D view.
    - » Thin slices of specimen are obtained.
    - » The electron beams scatter when they pass through thin sections of a specimen. Those that do not scatter (transmitted electrons) are used to produce image. Denser regions of specimen scatter more electrons and appear darker.
    - » It has high magnification and high resolution. Can magnify up to 250,000x.



Fig. 2.5: Electron microscope

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Salient Features

- *Basic Histology: A Color Atlas and Text* is brought out with an idea to facilitate basic understanding of histological structure of various tissues with a functional correlation.
- Covers three aspects relevant to histology:
  - Microscopic structure of a tissue or organ at various magnifications
  - Development of various tissues in brief was added at the end of each chapter which is a unique feature of this book
  - Clinical importance of tissues or their structural components in brief were presented for stimulating the reader to understand the importance of normal structure.
- Organization of microscopic pictures and figures:
  - An introduction figure for each system showing gross and microscopic components relevant to the system
  - Histological pictures at different magnifications for better understanding and a hand-drawn image for simplification of the subject
  - Flow charts and hand-drawn figures for certain ultrastructural details.
- Organization of content in each chapter:
  - With brief introduction or overview
  - Content distribution under various headings and sub-headings
  - Presentation of content in the form of points.
- Each chapter at the end highlights:
  - A table summarizing the microscopic structure of various organs discussed under that system, their function and location were presented in a table form for easy review before examination
  - Few MCQs of importance
  - Descriptive questions to facilitate the students in preparing for the examination.
- In the last chapter an overview of histology slides was presented that facilitates students for facing the practical examination with confidence.

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