lecture (1)

MICROSCOPY AND MICROTECHNIQUE

Histology: is the science of studying tissues and cells

MICROSCOPY

The use of any microscope is to serve two main functions; magnification and resolution.

Magnification: is the increase in linear dimensions of an object without optical defects.

- This is done through 2-stage magnifying system. The specimen is magnified first by many objective lenses system, then by a second lens, ocular lens or eye-piece lens.
- The total magnification of the instrument is the product of magnification resulting from the objective and the ocular lenses.
- Magnification should be hand in hand with clarity (resolution) otherwise the image is blurred.

Resolution: is the power of the microscope to distinguish fine details.

- The limit of resolution (L.r) is defined as the shortest distance between two details below which they appear as one or cannot be seen at all with the microscope.
- The resolution power of the microscope increases as the limit of resolution decreases.

The L.r. of the human eye is 200 um. While that of the ordinary light microscope is 0.24 um and it is 0.24 nm in electron microscopes.

1mm =1000 um 1um=1000 nm

Types of microscopes:

1-The ordinary light microscope: in which artificial lamp light is used for illumination. The optical components consist of 3 systems of lenses: condenser, objective and eye piece. The details of its components are shown in Fig. (1). It is used for examination of fixed cells and tissues

2-Phase contrast microscopy:

It permits direct observation of living cells.

It is based on the principle that light changes its speed when passing through cellular and extracellular structures with different refractive indices. These changes are used by the phase contrast system to cause the structures to appear lighter or darker relative to each other.

3. Fluorescent microscopy:

Some components are auto fluorescent such as vitamin A .Fluorescent stains that have affinity to certain macromolecules may be used e.g. acridine orange which can combine with DNA and RNA.

4. Electron microscopy:

There are three types of electron microscopes:

A- Transmission electron microscopes (TEM):

- It is used to examine the fine details of the cells and tissues.
- The illumination source is electron beam emitted by heating a cathode (tungsten filament).It is of very short wave length (λ).
- The beam follows a straight bath through vacuum.
- It is deflected and refracted when it passes through electro-magnetic lenses.
- The L.r is 0.24 nm.
- The magnification power is several thousand times.
- The resulting magnified image is projected on a fluorescent screen and could be photographed.

B. High voltage electron microscope (H.V.E.M.):

- It is supplied with high voltage electric current resulting in emitting electron beam of higher penetrating power.
- It is used to examine relatively thick sections (5 um.) and in studying living cells.

C- Scanning electron microscope (S.E.M.):

- It is used to study the details of surfaces and contours of the cells.

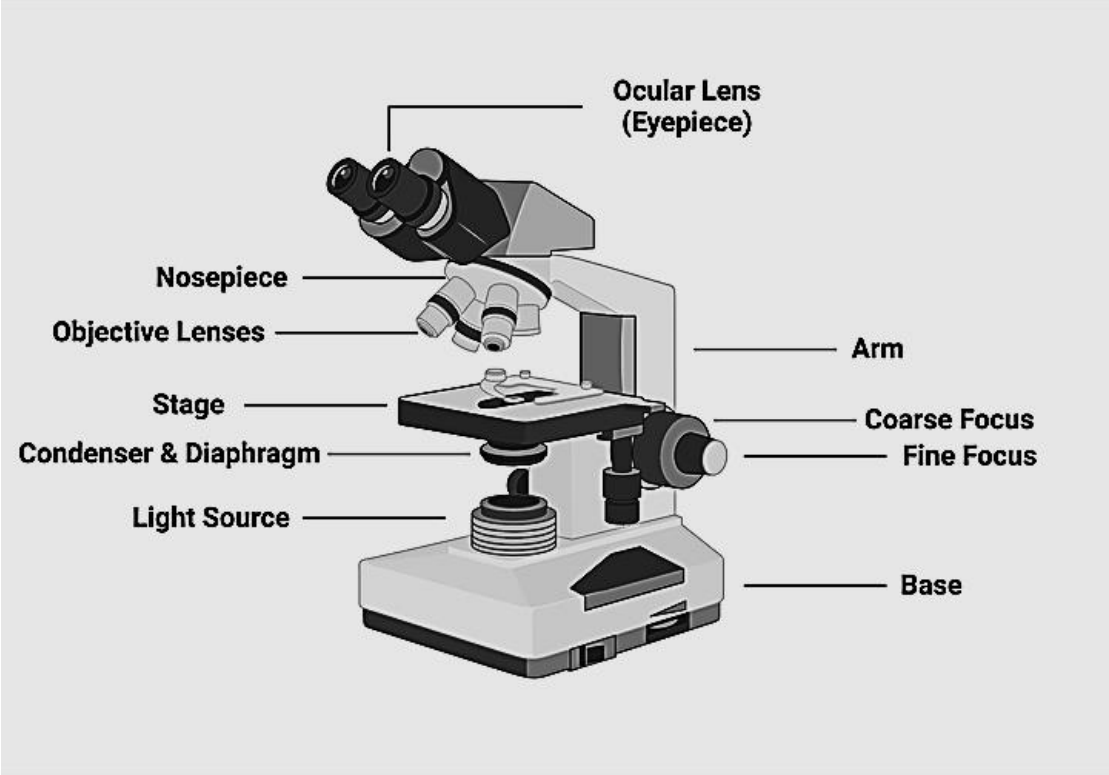

Fig. (1): Schematic drawing of the different parts of the ordinary light microscope (A) and the optical bath through it.

MICROTECHNIQUE

Methods of studying cells:

The microscopic study of cells and tissues includes 2 major techniques:

a- Living cells b- fixed cell

A: Examination of living cells: (tissue culture)

- It is known as in vitro study.
- The piece of tissue is treated with trypsin to dissociate its components and the cells are put in sterile dish with the appropriate culture medium.
- It may be **primary, secondary or established cell line.**
- **It is used to:**

✓ Study the effect of a toxic substance on certain cells.

✓ To make karyotypes for chromosomal study.

- ✓ To form clones of cells from one cell.
    - **In established cell line,** the cells are kept culture after culture

***B: Examination of fixed tissues and cells* by sectioning**

The piece of tissue (0.5 – 1 cm) is taken by biopsy, surgical excision or postmortem undergoes several steps to be prepared in the form of a histological slide. There are several methods used to prepare sections:

I- Paraffin techniques for examination by light microscope: will be sicussed in setails in practical sessions.

II. Frozen sections:

It is used to prepare fresh and fixed sections where histochemical reactions especially enzyme histochemistry are to be applied and intraoperative .

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III. Freeze drying technique:

The tissue is immediately frozen in liquid helium (-170c) and is kept under vacuum. Water evaporates and the tissue is directly embedded in paraffin.

IV. Preparation of tissues for electron microscopy (EM):will be discussed in practical sessions.

Ultrathin sections is needed for examination by EM (Figure 2)

Special tissue preparations for microscopy:

a. Filming: used in examination of tissue fluids e.g. blood film. Fig. 3

B. Smearing: used in examination of sticky secretions e.g. bone marrow smear.

c. Teasing: suitable for examination of fibers e.g. muscle and nerve fibers.(fig. 4)

d.Spreading: suitable for examination of thin tissue e.g. loose connective tissue.

e. Grinding: used to examine bone by ground preparation.

Fig. 2: Transmission electron microscope

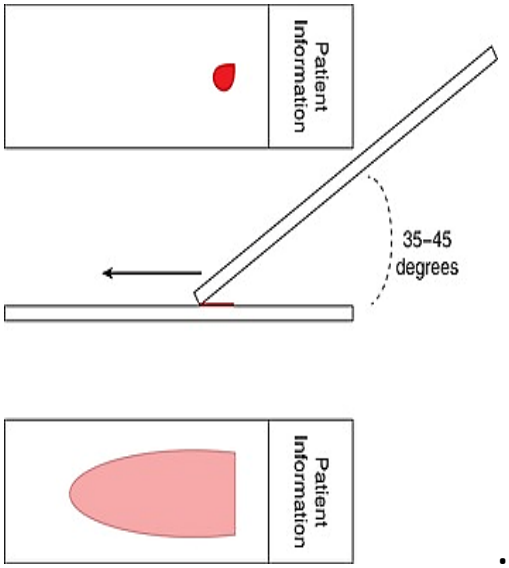

Fig. 3: Blood film

fig. 4 Teased muscles

Types of staining methods:

1- General histological methods as H&E.

2- Special histological methods: In these methods certain dyes are known to be attached to structures or a particular structure in cells and tissues respectively as silver stain.

3- Vital staining methods:

these include 2 different main techniques:

A-Vital staining technique: staining of tissues in the living state inside the body e.g. staining of macrophages using Indian ink.

B-Supra vital staining technique: staining of tissues in the living state outside the body e.g. staining of mitochondria in a fresh state using Janus green B.

4- Histochemical methods: a particular component such as an enzyme, glycogen or fat is localized inside the cells by using either physical or chemical procedures on histological sections.

The Stain:

The stain is a coloring agent which can penetrate and be retained by tissue elements.

For routine uses, hematoxylin and eosin (H&E) are used as general histological stain.

Hematoxylin gives deep purple satin while eosin gives pink to red color.

Substance is coloured when it has a specific group called a **"chromophore"** which shows absorption band in the region of visible light.

The stain can be retained by the tissue components when it has a specific group called **"auxochrome"** of the dye which may be acidic or basic group that can be retained by the stained material by reacting with it.

Basophilic and Acidophilic staining :

Hematoxylin stain is a basic dye that has the ability to stain acidic structures in the cell (e.g. nuclei) and the stained structure is known as basophilic structure.

Eosin is an acidic dye that has the ability to stain basic structures in the cell (e.g. the cytoplasm) and the stained structure is known as eosinophilic or acidophilic structure.