**Dr.Iman**

**Microscopy**

The light microscope is the most important machine in medical laboratory .It uses avisible light source with asystem of condenser lenses to send the light through the object to be examined . The light microscope is used to enlarge small objects and to reveal their fine details .

The light microscope is composed of:

1-Frame and mechanical parts

2- Optical (magnification )system

3- Illumination system

**The Electron Microscope (EM):**

The Electron Microscope (EM) is better suited to study the details of cell than LM . The detailed morphology revealed by EM may be called fine or submicroscopic structures or ultrastructure .There are two type of the electron microscopes ,the transmission and scanning

**A. Transmission Electron Microscope (TEM):**

In this microscope , the ordinary light source is replaced by a beam of electrons, which are emitted by heated tungsten gun or filament (cathode) in a vacuum controlled system .There is a voltage difference between the cathode and the anode which accelerates the electron beam and attracts the electrons to the anode where they pass through its central hole .While passing in the microscope tube , the electron beam is subjected to electric coils with magnetic field , which deflect the electrons and change their path , thus called electromagnetic lenses . The image is viewed directly on a fluorescent screen because the human eye is not sensitive to electrons.

The tissue is treated with a double fixation method to preserve the ultrastructural details .Buffered gluteraldehyde is used as a first fixative , and then a special second fixative such as osmium tetra- oxide is used to stain lipids and proteins. The tissue then , embedded in resin or plastic in vacuum oven .The resulting blocks are very hard ; they are cut into very thin sections (40-90 nm) by the use of ultra –tome and a diamond or glass knife .

The thin sections are mounted are special 100-400 mesh copper grids, and stained with heavy metal such as lead and uranyl acetate – the image is seen on a screen as a block and white and recorded on photograph

\_ The electron microscope requires a vacuum-enclosed system , high voltage (60-120KV) ,and mechanical stability .

The high resolving power of EM made possible to study the details of interior of cell with a final magnification of 400000 times.

The use of high voltage (500000 – 1000000 V) allowed the use of relatively thicker sections to be examined to get 3-D images.

**B- Scanning Electron Microscope (SEM):**

This microscope provides a three dimensional image of the surface of fixed and dehydrated tissues; it has less resolving power than TEM. The sample is coated with gold , which emits secondary , electrons after being hit with a primary electron beam .The electrons do not pass through the specimen ,but scan sequentially the different surface points of the specimen and the resulting image will be in black and white color.

**Comparison between light (LM) and electron microscopes(EM)**

|  |  |  |
| --- | --- | --- |
| Electron Microscope | Light Microscope |  |
| Presented on a screen in shades of green . In photographs , image appears in grey scale or in black and white | Presented directly to the eye , Image keeps the color given staining | Image |
| High up tox400,000  (2,000,000)? | Up to X 1500 (times ) , wider field of sample view ; good orientation . | Magnification |
| High , 0.1nm | 0.1- 0.2 µm | Resolution |
| Tissue prcessing take one day at least . Frozen tissue cryofructure and histochemistry | By frozen section Sample can be prepared in 20 min. | Time Processing |
| Fewer | Many | Artefacts |
| Very thin 40-90nm | 1-10 µm | Section thickness |
| Obtained by thicker section of high high–voltage EM ,freeze –  fructured techniques and SEM | Can be constructed by serial section | 3-D image |
| Heavy metal | Routine H&E | Stain |
| Very small sample | Can be large and alive | Specimen size |
| Beam of electron | Visible light (electric) | Light source |
| Electromagnetic | Glass | Lenses |

**3- The Phase- Contrast Microscope**

This type is used to study the living and unstained tissues .Its idea is based upon the fact that light passing through the media of different refractive indices changes direction and speed, thus creating contrast .It is equipped with a lens system that enables it to convert the phase variation into intensity variations, which are perceived as differences in brightness, and the unstained object becomes visible.

**4-The Interference Microscope**

The microscope uses the same principle of the phase contrast microscope , but with two beams of lights, which interfere with one another in a way to provide precise information on the density of cellular region even in the living state.

**5- Fluorescence Microscope**

This microscope depend on the fact that certain substance present in nature emit light of longer visible wavelength on exposure to ultraviolet or lase light .This character is known as fluorescence and the molecules having this character will appear colored or bright in a dark field .Same of these substances are used to stain the cell components such as acridine orange which stain specifically nucleic acids . With DNA ,the acridine orange emit yellowish green light and RNA , acridine orange emits reddish orange light. This microscope is used to localize nucleic acids in the cells, to localize antigen-antibody complexes and to trace the pathway of nerve fibers.

**6-The polarizing Microscope**

This type uses two filters, one of them is located below the condenser , called the polarizer ; the other filter is localized between the objective lens and the eye piece and called analyzer . When the axes of the two polarizers become perpendicular on each other , no light passes resulting in a dark field scene .This type is used to examine a crystalline substance or well-ordered fibrous molecule , such as collagen , microtubules and microfilaments which appear light against the dark field .These substances alter the plane of entering polarized light and rotate the axis of the emerging light (birefringence).

**7-The Confocal Microscope**

This microscope uses a very small beam of laser light for illumination , and a highly computerized system . Its main principle is based on the fact that a very small laser beam originating from one thin plane of the section passes through a pinhole of plate , while the rest of beams coming from other planes are blocked by that plate . The small beam scans other planes of the section , and thus able to collect serial optical sections from thick specimens .Using filters to eliminate the unfocused images and the photomultiplier detectors within a highly computerized system , made obtaining of quality sharp three-dimensional image is feasible .It is used to optically dissect the specimen and study the structure of biologic material after fluorescent labeling of area of interest , thus con not be used as a routine procedure.

**8- X-ray Microscope**

Uses electromagnetic radiation in the soft X-Ray band to produce magnified images of objects. Since X-ray penetrate most objects, there is no specially prepare them for X-rayMicroscopy observations.

-Unlike visible light, X-ray do not reflect easily, and they are invisible to the human eye. Therefore, an X-ray Microscope exposes film or uses a charge- coupled device ( CCD) detector to detected X-ray that pass through the specimen.

- Its contrast imaging technology using the difference in absorption of soft X-rays in the water window region (wave lengths :2.34 - 4.4nm, energies: 280-530 ev) by carbon atom( main element composing the living cell) and the oxygen atom ( main element for water).

-Micro focus X- ray also achieves high magnification by projection.

- A micro focus X – ray tube produce X-rays from an extremely small focal spot(5µm down to 0.1 µm).

-The X – rays are in the more conventional X – ray range (20 to 300Kv) and they are not re- focused.

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**A microtome**

It is a sectioning instrument that allows for a cutting of extremely thin slices of material , known as sections. Microtomes are an important device in microscopy preparation , allowing for the preparation of samples for observation under light or electron microscopes.

**Microtomes blades:\*\***

**1-Steel blades** are used to prepare sections of animal or plant tissues for light microscopy histology .

**2-Glass Knives** are used to slices sections for light microscopy and slice very thin sections for electron microscopy.

**3-Industrial grade diamond knives** are used to slice hard materials such as bone , teeth and plant matter for both light microscopy and for electron microscopy.

**4-Gem quality diamond** knives are used for slicing thin sections for electron microscopy.

**Microtome type :\*\***

**1-Sledge microtome :**A sledge microtome is device where the sample is placed into fixed holder (shuttle),which then moves backwards and forewords across a knife.

**2-Rotary microtome :**This instrument is a common microtome design .This device operates with a staged rotary action such that the actual cutting is part of rotary motion. In a rotary microtome , the knife is typically fixed in a horizontal position.

**3- Cryomicrotome :**For the cutting of frozen samples, many rotary microtome can be adapted to cut in liquid nitrogen chamber , in a so called cryomicrotome setup.

**4-Ultramicrotome** :An ultra-microtome is main tool of ultramicrotomy.

**5-Vibrating microtome :**The vibrating microtome operates by cutting using a vibrating blade, allowing the resultant cut to be made with less pressure than would be required for stationary blade .The vibrating microtome is usually used for difficult biological samples. The cut thickness is usually around 30-50 µm for live tissue and 10-500 µm for fixed tissue .

**6-Saw microtome:** The saw microtome is especially for hard materials such as teeth or bones .The microtome of this type has a recessed rotating saw, which slices through the sample. The minimal cut thickness is approximately 30 µm , and can be made for comparatively large samples .

**Microtome Knives (Knife design and cut types ):\*\***

Generally, knives are characterized by the profile of the knife blade, which falls under the categories of **planar concave ,wedge shaped or chisel designs.**

**Applications of microtomes :**

The most common applications of microtomes are :

**\*Traditional Histology Technique**: Tissues are hardened by replacing water with paraffin. The tissue is thin cut in the microtome at thicknesses varying from 2 to 50 µm (micrometers) thick. From these the tissue can be mounted on a microscope slide , stained with appropriate aqueous dye (s) after prior removal of the paraffin, and examined using a light microscope.

**\*Cryosectioning Technique** : water –rich tissues are hardened by freezing and cut in the frozen state with a freezing microtome or microtome –cryostat; sections are stained and examined with light microscope. This technique is much faster than traditional histology (5 minute vs 16 hour ) and is used conjunction with medical procedures to achieve a quick **diagnosis**. Cryosections can also be used in **immunohistochemistry** as freezing tissue a stops degradation of tissue faster than using a fixative and does not alter or mask its chemical composition (antigenicity ) as much.

**\*Electron Microscopy technique**: After embedding tissues in epoxy resin, a microtome equipped with a **glass or gem grade** diamond knife is used to cut very thin sections(typically 60 to 100 nanometer ). Sections are stained with aqueous solution of an appropriate heavy metal salt and examined with transmission electron microscope **(TEM).** This instrument is often called **ultramicrotome**. The ultramicrotome is also used with its glass knife or an industrial grade diamond knife to cut survey sections prior to thin sectioning .These survey sections are generally 0.5 to 1 µm thick and are mounted on a glass slide and stained to locate areas of interest under a light microscope prior thin sectioning for the TEM. Thin sectioning for the TEM is often done with a gem quality diamond knife . Complementing traditional TEM techniques ultramicrotomes are increasingly found mounted inside an SEM chamber so the surface of the block face can be imaged at and then removed with the microtome to uncover the next surface which is ready for imaging .This technique is called Serial Block-Face Scanning Electron Microscope (SBFSEM).

**\*Botanical Microtomy Technique**: Hard materials like wood , bone and leather require a sledge microtome. These microtomes have heavier blade and cannot cut as thin as a regular microtome.

**\*Spectroscopy (especially FTIR or Infrared Spectroscopy )Technique** : Thin polymer sections are needed in order that the infra-red beam will penetrate the sample under examination. It is normal to cut samples to between 20 and 100 µm in thickness.