15/MHS06/005

MEDICAL LABORATORY SCIENCE

MLS 410 (BIOMEDICAL ENGINEERING)

QUESTION.

1. Discuss the physics of the light microscope, diagrams and illustrations needed.
2. Write notes on the ff biomedical equipment. Add notes on principle, brand, care and maintenance and cost (A) centrifuge (B) automatic tissues processor (C) microtome.

ANSWERS.

1. ***PHYSICS OF LIGHT MICROSCOPE.***

The microscope is an instrument used to view objects that are not visible to the naked eye.

The microscope was discovered in the early 1600s in the Netherlands and Denmark. The simplest compound microscope is constructed from two convex lenses, the first lens is the objective sense which has magnification values from 5X to 100X , in the standard microscopes the objectives are mounted in such a way that

The purpose of the microscope is to magnify small objects, and both lenses contribute to the final magnification.

Terms and principle commonly used in microscopy

Reflection :_ when a ray of light strikes a surface at an angle and it bounces back at an angle of equal size, it is said to be selected. Stray reflections inside the microscope interfere with the path of light rays and degrade that sharpness of the imagine.

Refraction : this is simply the bending of a light ray from the normal when it passes into the different optical medium. A normal line is the line perpendicular to a flat surface. This is caused by changes in the speed of light while passing from one medium into another of different optical density . When light enters a more dense medium it bends towards the normal line when entering a less dense field, light bends away from the normal line.

Refractive index : it is the measure of refraction and is measure as

$$\text{Refractive index } (n) = \frac{\text{velocity in vacuum (km/sec)}}{\text{Velocity in medium (km/sec)}}$$

It is proportional to the density of the medium. Refractive index can slo be defined as the relationship between the sine of the incident angle to the sine of the refracted angle.

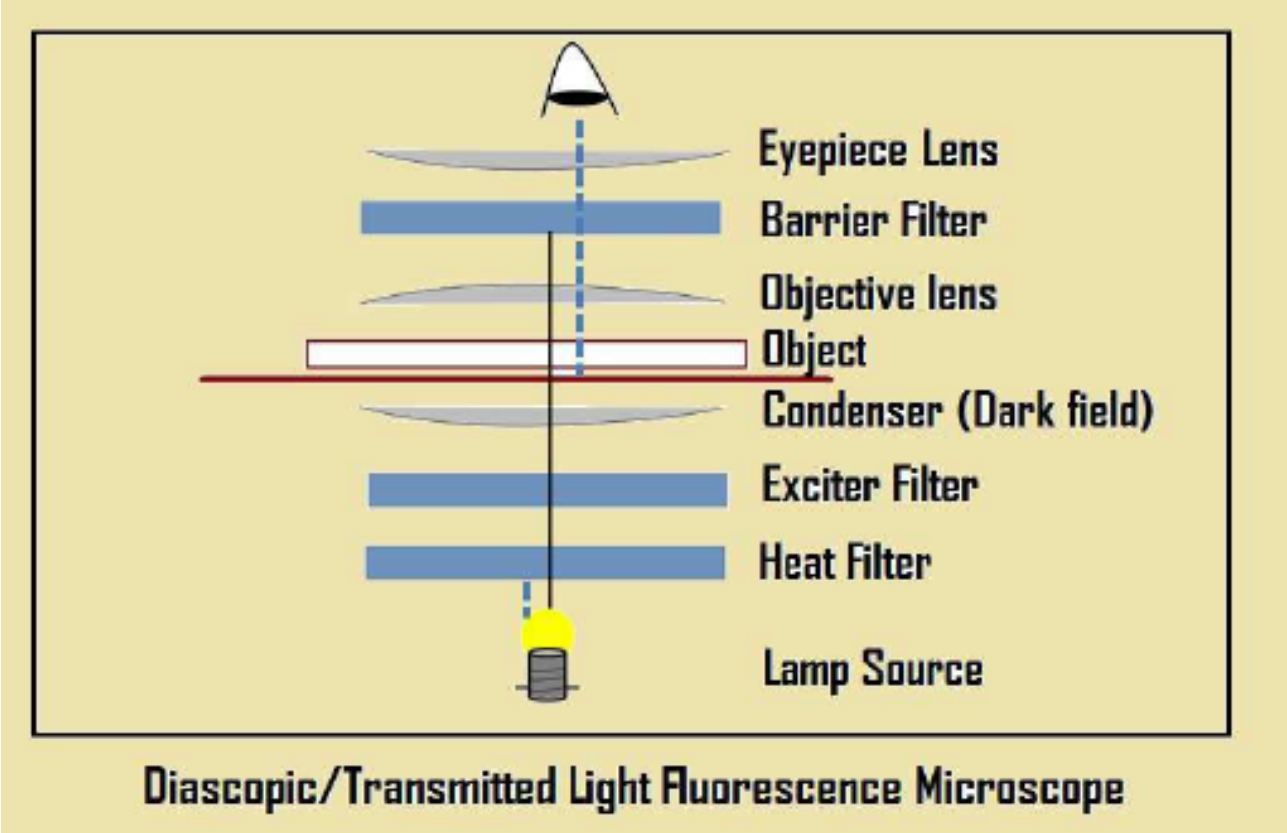
Lenses: in an optical system, the lens collects light rays from an object and redirects them to form a sharp, magnified image of the object in the imagine plane. There are two basic types of lenses used in microscopy - covering or positive lenses and diverging or negative lenses.

Principle focus and optical centre: the centre of lens surface on either side of a biconvex lens in called a centre of curvature. A straight line joining these two centres is the principal axis. A ray of light entering the lens along the principal axis does not refract and travels along the same line. Rays of light entering a converging lens parallel to the principal axis , however are refracted towards this axis. The point at which they meet is called principal

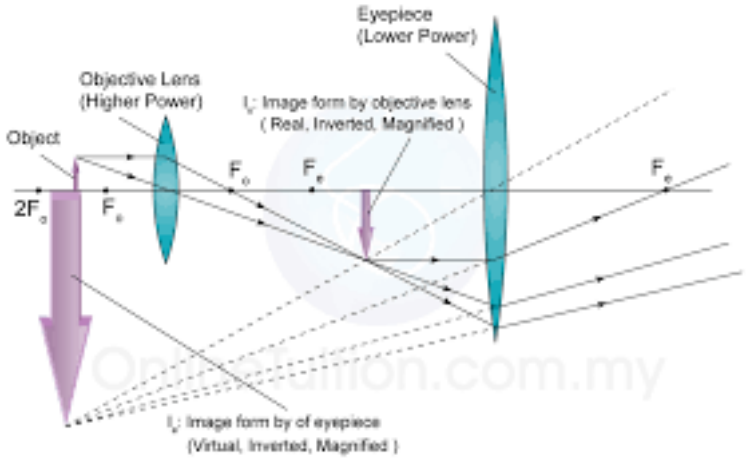
focus . A biconic lens has a principle focus on each side of the lens at an angle emerges parallel to the entering ray and will pass through the centre of the lens. Another ray entering similarly from the other surface of the lens also passes through the centre.

Magnification:

The magnification produced by a lens is defined as the ration of the distance between the lens and the imagine plane and the distance between the lens and the object . In simple words magnification is obtained by dividing the size of the image by the size of the object. In case of a convex lens the magnification is maximum when the object is placed just outside the principal focus of the Lens .



Diascopic/Transmitted Light Fluorescence Microscope



The object is slightly farther away from the objective lens than its focal length f_o, producing a case 1 image that is larger than the object. This first image is the object for the second lens, or eyepiece. The eyepiece is intentionally located so it can further magnify the image. The eyepiece is placed so that the first image is closer to it than its focal length f_e. Thus the eyepiece acts as a magnifying glass, and the final image is made even larger. The final image remains inverted, but it is farther from the observer, making it easy to view (the eye is most relaxed when viewing distant objects and normally cannot focus closer than 25 cm). Since each lens produces a magnification that multiplies the height of the image, it is apparent that the overall magnification m is the product of the individual magnifications: $m = m_o m_e$, where m_o is the magnification of the objective and m_e is the magnification of the eyepiece. This equation can be generalised for any combination of thin lenses and mirrors that obey the thin lens equations.

Normal optical microscopes can magnify up to 1500× with a theoretical resolution of −0.2 μm. The lenses can be quite complicated and are composed of multiple elements to reduce aberrations. Microscope objective lenses are particularly important as they primarily gather light from the specimen. Three parameters describe microscope objectives: the *numerical aperture* (*NA*), the magnification (*m*), and the working distance. The *NA* is related to the light gathering ability of a lens and is obtained using the angle of acceptance θ formed by the maximum cone of rays focusing on the specimen (see Figure 3a) and is given by $NA = n \sin \alpha$, where n is the

refractive index of the medium between the lens and the specimen and

THE PRINCIPLE OF LIGHT MICROSCOPE

The light microscope is an instrument for visualising fine detail of an object, it does this by creating a magnified image through the use of a series of glass lenses which first focus a beam of light onto or through an object and convex objective lenses to enlarge the image formed.

2. BIOMEDICAL EQUIPMENT

A. CENTRIFUGE.

A centrifuge is a piece of equipment generally driven by an electric motor that puts an object in rotation around a fixed axis , applying a force perpendicular to the axis to separate substances of different densities.

Principle of centrifuge.

The centrifuge works by using the sedimentation principle where by the centripetal acceleration causes denser substances and particles to move outward in the radical direction and at the same time objects which have less dense are displaced and moved to the centre.

Principle of operation.

Tubes in the centrifuge are tilted so centrifugal force can pull denser substances towards the bottom of the tube. The tube is placed in the centrifuge (the tube should be in the approbate size in order to prevent tube damage and a counter balance must always be used which means that the tubes opposite each other must be of equal mass), if the centrifuge has variable speed then enter the RPM, close the lid and turn on the timer and press start the its done remove the tube carefully after the centrifuge has completely stopped spinning to prevent remixing.

Brand of centrifuge

Care of centrifuge.

- the centrifuge should aways be placed on a flat surface
- It should be unplugged before cleaning
- Disposable gloves should be worn before handling the centrifuge
- May sure to follow the facility safety procedures when cleaning and disinfecting the centrifuge
- Dust accumulation must be prevented by putting a cover when not In use
- Do not use detergents or products that contains chlorine ions

- **As for cleaning the centrifuge**
- it should be clean at least weekly
- Remove the rotor and any sample holder
- Use a sponge , warm water an dish washing soap when cleaning
- Clean both the interior and exterior

Brands of centrifuge (types)

- clinical centrifuges: moderate speed devices used for clinical applications like blood collection tubes .

- Isopycnic centrifugation: often used to isolate nucleic acids such as DNA.
- sucrose gradient centrifugation : often used to purify enveloped viruses and ribosomes and also to separate cell organelles from crude cellular extracts.

- Microcentrifuges : this devices are for small tubes (micro tubes from 0.2ml to 2.0ml), up to 96 well plates .
- Multipurpose high speed centrifuges : devices for a broad range of tube sizes , high variability , big footprint
- Ultracentrifuge : because of the heat generated by air friction and the frequent necessity of maintaining samples at a given temperature many types of laboratory centrifuges are refrigerated and temperature regulated .
- Differential centrifugation : this is often used to separate certain organelles from whole cells for further analysis of specific parts of cells.

B. AUTOMATIC TISSUE PROCESSOR.

This is a device that processes tissue samples for sectioning and microscopic examination in the diagnostic laboratory, microscopic analysis of cells and tissues requires the preparation of very thin, high quality sections mounted on glass slides and appropriately stained to demonstrate and abnormal structures. This machine plays a big role in the preparation of the tissue by passing them through various chemicals.

PRINCIPLE OF AUTOMATIC TISSUE PROCESSOR .

The aim of tissue processing is concerned with the diffusion of various substances into and out of porous tissues.diffusion results from the tendency of processing reagents to equalise concentrations both inside and outside blocks of tissue.

SAFETY:

- caution when handling reagents
- Use disposable gloves when handling carcinogens or toxic materials

- Do not smoke or eat or drink close to the reagents or specimens

Types of automatic tissue processor.

There are two major types :

Tissue transfer processors

Fluid transfer processors

Tissue transfer processors

These processors are characterised by the transfer of tissues, contained within a basket, through a series of stationary reagents arranged in-line or in a circular carousel plan. The rotary or carousel is the most common model of automatic tissue processor, and is provided with 9-10 reagent and 2-3 wax positions, with a capacity of 30-110 cassettes depending upon the model. Fluid agitation is achieved by vertical oscillation or rotary motion of the tissue basket. Processing schedules are card-notched, pin or touch pad programmed. These processors allow maximum flexibility in the choice of reagents and schedules that can be run on them. These machines have a rapid turn-around time for day or night processing. In more recent models the tissue basket is enclosed within an integrated fume hood during agitation and transfer cycles thus overcoming the disadvantages of earlier styles.

Fluid transfer processors

In fluid-transfer units, processing fluids are pumped to and from a retort in which the tissues remain stationary. There are 10-12 reagent stations with temperatures adjustable between 30-45°C, 3-4 paraffin wax stations with variable temperature settings between 48-68°C, and vacuum-pressure options for each station. Depending upon the model these machines can process 100-300 cassettes at any one time. Agitation is achieved by tidal action. Schedules are microprocessor programmed and controlled and can

be viewed on a screen (see image below). Vacuum-pressure cycles coupled with heated reagents allow effective reductions in processing times and improved infiltration of dense tissues. Fluid-transfer processors overcome the main drawbacks of the tissue-transfer machines. Tissues are unable to dry out within the sealed retort and reagent vapours are vented through filters or retained in a closed-loop system. Processors are provided with alert systems and diagnostic programmes for troubleshooting and maintenance.

Care and maintenance of automatic tissue processor.

- accumulation of wax on any surface should be removed quickly .
- the melting point for the paraffin wax should be at 30 degrees
- Overflow or spillage should be cleaned up
- Timing should be checked when placing the cassettes in the processor
- clean outside of the instrument with xylene dampened cloth

Brands of Automatic tissue processor

- RSMT-101D 'CE' MARKED : brand name is radical
- KEDEE-TS6A automatic tissue processor

C. MICROTOME

This is a tool that is used to cut extremely thin slices of material known as sections, microtomes are used in microscopy which allow the preparation of samples which are to. Be observed under transmitted light or electron radiation.

Principles of microtome .

Microtome is a sectioning instrument that allows the cutting of extremely thin slices of a material known as sections. Microtome are used in transmitted or electrons radiation, it is a method for the preparation of thin section for materials such as bones , minerals and teeth.

Caring for the microtome knife

Before using the microtome knife

- the microtome knife must be coated with an oil mixture to prevent rust and corrosion when not in use
- Before using the knife, take a lint free facial tissue saturated in either xylene , benzene or acetone to remove the protective oil coating on the knife.
- Use a dry lint free facial tissue to wipe the knife clean and do NOT use gauze or any other coarse material because this will destroy the knife .
- The knife has already been stropped and its now ready for immediate use .

Care of microtome

- keep the edge of the knife clean at all times
- Spray or brush any household oil on the knife in order to prevent it from rusting (when not in use)
- Store the knife in its case to prevent oxidation from occurring
- If the use of a lab sharpener, send the knife out to be professional reconditioned
- Dust accumulation must be prevented by putting a cover when not In use
- Never adjust the screws too tightly that may cause binding.

Types of microtome

- rotary microtome
- Sliding or base siege microtome
- Cambridge rocking microtome
- Freezing microtomes

ROTARY MICROTOME: The Rotary microtome is so-called because of a Rotary action of the handwheel responsible for the cutting moment. The block holder is mounted on a steel carriage, which makes up and down in groves this type of instrument is the most ideal for routine and research work it is excellent for cutting serial sections.

SLIDING OR BASE SLEDGE MICROTOME :This is a large heavy instrument with a fixed knife beneath which the object moves mounted on a heavy sliding base containing the feed mechanism used primarily for cutting the sections of cellulose nitrate embedded tissues with an obliquely set knife.

CAMBRIDGE ROCKING MICROTOME: The instrument is so named because the arm has to move in a rocking motion while cutting the sections. It is a simple machine in which the knife is held by means of microtome thread. The rocking microtome was designed primarily for cutting paraffin wax sections but in an emergency use frozen section by inserting a wooden block in which the tissue is frozen.

FREEZING MICROTOME: This type has been designed for the production or preparation of frozen sections of fluid and non-fluid tissues usually without preliminary embedding. The object stage is connected to the cylinder of compressed carbon dioxide for the rapid cooling of the tissues and provisions are also made for the cooling of the knife.