**MATRIC NUMBER**: 17/MHS06/068

**COURSE CODE**: MLS 410

**COURSE TITLE**: BIOMEDICAL ENGINEERING

**ASSIGNMENT**

**Question**
1. Discuss the physics of the light microscope diagrams and illustrations needed

2. Write notes on the following biomedical equipment. Add notes on principle, brand, care and maintenance and cost A. Centrifuge B. Automatic Tissues processor C. Microtome

**Answer**

**Question 1**

**Introduction**

A light microscope is a biology laboratory instrument or tool, that uses visible light to detect and magnify very small objects, and enlarging them. It is an important device that enables us to visualize minute objects (animate and inanimate) that cannot be seen with our naked eyes.

They use lenses to focus light on the specimen, magnifying it thus producing an image. The specimen is normally placed close to the microscopic lens.

Microscopic magnification varies greatly depending on the types and number of lenses that make up the microscope. Depending on the number of lenses, there are two types of microscopes i. e Simple light microscope (it has low magnification because it uses a single lens) and the Compound light microscope (it has a higher magnification compared to the simple microscope because it uses at least two sets of lenses, an objective lens, and an eyepiece). The lenses are aligned in that, they can be able to bend light for efficient magnification of the image.

The functioning of the light microscope is based on its ability to focus a beam of light through a specimen, which is very small and transparent, to produce an image. The image is then passed through one or two lenses for magnification for viewing. The transparency of the specimen allows easy and quick penetration of light. Specimens can vary from bacterial to cells and other microbial particles.

As mentioned earlier, light microscopes visualize an image by using a glass lens and magnification is determined by, the lens’s ability to bend light and focus it on the specimen, which forms an image. When a ray of light passes through one medium into another, the ray bends at the interface causing refraction. The bending of light is determined by the refractive index, which is a measure of how great a substance slows the speed of light. The direction and magnitude of the bending of the light are determined by the refractive indexes of the two mediums that form the interface, a medium with a lower refractive index such as glass to air, it normally speeds up the light penetration and making light bend away from the normal and when light is passed through a medium with a greater refractive index such as air to glass, it normally slows down and bends towards the normal, perpendicularly to the surface.

**Major Parts of Microscope**

**Frame work of the microscope**

This includes;

* **An arm (Stand):**- The basic frame of the microscope to which the base, body and stage are attached.
* **A stage** :- The table of the microscope where the slide or specimen is placed
* **A foot or base** :- is the rectangular part up on which the whole instrument rest

**Focusing system**

This encompasses;

* **Coarse adjustment** :- The coarse focusing adjustment is controlled by a pair of large knobs positioned one each each side of the body
* Rotations of these knobs move the tube with its lenses, or in some microscope the stage, up or down fairly rapidly
* **Fine adjustment**: - While low power objectives can be focused by the course adjustment, high power objectives require a fine adjustment.
* **Condenser adjustment**: - The condenser adjustment is focused is focused usually by rotating a knob to one side of it. The condenser aperture is adjusted by the iris diaphragm, which is found just below the condenser is to condense the light required for visualization

**Magnification system**

This comprises;

**Objectives**:- Objectives are components that magnify the image of the specimen to form the primary image. For most routine laboratory work, 10x, 40x and 100x (oil immersion) objectives are adequate.

**Eyepiece**: - The eyepiece is the upper optical component that further magnifies the primary image and brings the light rays to a focus at the eye point. It consists of two lenses mounted at the correct distance. It is available in a range of magnifications usually of 4x, 6x, 7x, 10x, 15x and sometimes as high as 20x.

**N.B**: The number of eyepiece on microscopes can be classified as Monocular and binocular microscopes.

**Illumination system**

**Condenser**:- This is a large lens with an iris diaphragm. The condenser lens receives a beam from the light source and passes it to the objective

**The Iris**:- This is a mechanical device mounted underneath the condenser and controls the amount of light entering the condenser.

 Source of illumination-Day light or Electric light

**WORKING PRINCIPLE OF A MICROSCOPE**

If an object is put between these two mediums i.e between water and air, in this case, a prism, the prism will bend the light at an angle. This is how the microscopic lenses work, they bend the light at an angle. The lens (convex) on receiving the light rays, it focuses the rays at a specific point known as the focal point (F-point). The measure of distance from the center of the lens and the focal point is known as the focal length.

A microscope uses lenses whose strength is predetermined, in that, the strength of a lens is directly related to the focal length i.e short focal length magnifies objects more than lenses with a long focal length.

Microscopy works strictly with a factor of resolution whereby resolution being the ability of a lens to be able to differentiate small objects that are closely packed together. The resolution of a light microscope is determined by a numerical aperture of its lens system and by the wavelength of the light it employs; a numerical aperture a definition of the light wavelengths produced when the specimen is illuminated.

A minimum distance (d) between two objects that distinguishes then to be two separate entities, determined by the wavelengths of the light can be calculated by an Abbe equation using the wavelength of the light that illuminated the specimen (Lambda, λ) and the numerical aperture (NA, n sin Ɵ) i.e. d=0.5 λ/n sin Ɵ

In microscopy we take advantage of waveform properties of light. These waves when produced at a particular source vibrate at right angles to the line of propagation. Each wave has a peak and trough. The distance traveled forward by the light ray is one wavelength (lambda). Wavelength varies with the color and intensity of the source.



**How the image is formed**

The structures the light microscope is called upon to resolve exert only a small influence on the light they transmit. What is changed is the phase of momentary vibration. Conventional bright field illumination will lack contrast and the details of the sample remain invisible. When the emerging waves have acquired a larger phase difference due to changes in refractive index, greater contrast is produced. This manifests itself by an edge effect (diffraction, refraction, and reflection). Sample details may be resolved in a number of ways. When a light passes through stained structures intensity is reduced selectively depending on the color and density of the sample as the light is absorbed. Selective absorption of wavelengths of white light produces colored light. Refraction changes the direction of a light ray as it passes from one medium to another. The shorter the wavelength, the greater the refractive angle. Diffraction is the bending of light rays around objects with sharp edges. A new wave front is created at this edge. Diffraction can be useful, but can also reduce resolution. When light is dispersed it is separated into its constituent wavelengths as a result of refraction on entering a transparent medium. Contrast can be defined as a steep slope between bright and dark image points. Adequate contrast MUST be achieved before the specimen can be resolved.

Two terms that are often confused, but are central to microscopy are magnification and resolution. Magnification is the degree by which dimensions in an image are, or appear to be, enlarged with respect to the corresponding dimensions in the object. Resolution is the act or result of displaying fine detail in an image. Magnification without resolution would be meaningless.

The theoretical resolution of the light microscope was first defined by Abbe in the following equation.

Abbe's equation for theoretical resolution of the light microscope:

$$d={1.22×λo}/{n sin µ}$$

d = distance of resolution

𝛌 = wavelength of incident light

µ = ½ angle of incoming light





The basic principle of the light microscope is shown in the image above. An image of the object (specimen) is formed by the objective lens, which typically provides a magnification in the range 10x to 100x. This magnified image is then viewed through the eyepiece (ocular), whose magnification is usually 10x. The microscope consists of two lens systems, the objective and the eyepiece. The objective forms a real image of the specimen, and this image is viewed through the eyepiece forming a virtual image at infinity. The total magnification of a microscope is obtained by multiplying the objective and eyepiece magnifications. Typically total magnifications are in the range 100x to 1000x, but rather as a virtual image viewed through the eyepiece.

This is the reason why so-called immersion objectives are often used. The space between specimen and objective is then filled with a fluid (often oil) with a high refractive index to increase the numerical aparture.

**Magnifying power of objectives**

**Low power objectives**: This can be odentified by such markings as 10x, or 16mm or 2/3 inch. This objective is generally used for rapid scanning of the microscopic field.

**High dry objective**: This objective is marked 40x, or 4m, or 1/6 inch. The colour code is a blue ring. This objective gives highest magnification in dry objectives.

**Oil Immersion objective**: This is the objective with the highest magnification in the ordinary light microscope. It is marked 100x,or 2mm, or ½ inch. It is denoted by a white ring. This objective is always used in conjunction with immersion oil. Oil, such as cedar wood oil, having the optical density as glass, is placed between the object and the lens to eliminate the refraction of light. The oil used for this purpose is called immersion oil and it enables the light to pass in a straight line from glass through the oil and back to the lens glass as through it were passing through the glass all the way

**Resolving Power**

The limit of useful magnification is set by the resolving power of the microscope. Resolving power of the microscope objective is its ability ro reveal closely adjacent points as separate and distinct. Quantitatively, it is a capacity to distinguish two neighboring points as separate entities. It depends largely upon the angle of light entering the objective and the refractive index of the medium between the object and the objective. The presence of oil between the object and the objective conserves many of the light rays which, would otherwise have been lost by defraction.

 Resolving Power (r) = $\frac{0.61×λ}{NA}$

Where 𝛌 is the wavelength of light used and NA is the numerical aperture. For green light, where lambda is approximately 540nm and the numerical aperture of the lens used is 1.4, the resolving power is about 200nm. Thus means that the minimum distance between the two points, to be seen separate and distinct, should be 200nm. If it is less than this, they will appear as a single object. When further magnification of the two points fails to show them separate and distinct, it is called empty magnification.

**Numerical Aperture**

The numerical aperture (NA) is defined as the product of the refractive index of the medium outside the lens (n) and the sine of half of the angle of the cone of the light absorbed by the front lens of the objective (u).This is expressed mathematically as



NA = n sin u = n $\frac{AD}{AC}$

Some objectives have the numerical aperture engraved on them. The numerical aperture of the commonly used objective.

The numerical aperture is in many ways more important than the magnification. This is because an increase in NA results in an increase in resolution.

**Focal Length and Working Principle**

The focal length of a lens is the distance between the optical centre and the point at which the parallel rays of light passing through it come to a critical focus. Since it is not possible to determine the exact optical centre of the objective, the term equivalent focal length is used. The focal length, however, is of less practical value than the working distance which is distance between the front lens of the objective and the object in focus. The working distance is much less than the focal length and it is of practical importance, especially with high power objective where the use of the wrong working distance can result in the damage to the front lens of the objective. To reduce such damage to the a minimum, spring loaded objectives are in use.

In modern microscope, objectives are par-fo-cas. This means that when an objective is used to focus on an object the objective will still be in focus with a small touch of the fine adjustment when another objective is swung into position. This prevents the possibility of damaging the object while changing from a low power to a high power objective during focusing. It also prevents the length of the objective from been scratched.

**Question 2**

Write notes on the following biomedical equipment. Add notes on principle, brand, care and maintenance and cost A. Centrifuge B. Automatic Tissues processor C. Microtome

**Answers**

1. **CENTRIFUGE**

A centrifuge is a laboratory device that is used for the separation of fluids, gas or liquid, based on density. Separation is achieved by spinning a vessel containing material at high speed; the centrifugal force pushes heavier materials to the outside of the vessel

**PRINCIPLES OF CENTRIFUGATION**

A centrifuge is used to separate particles suspended in a liquid according to particle size and density, viscosity of the medium, and rotor speed.

Within a solution, gravitational force will cause particles of higher density than the solvent to sink, and those less dense than the solvent to float to the top. Centrifugation takes advantage of even minute differences in density to separate particles within a solution.

As the rotor spins around a central axis, it generates a centrifugal force acting to move particles away from the axis of rotation. If the centrifugal force exceeds the buoyant forces of liquid media and the frictional force created by the particle, the particles will sediment.

**Centrifuge Rotor Types**

There are two very common rotor designs: fixed angle, and swinging bucket. The fixed angle rotor is designed to hold tubes in a fixed position at a fixed angle relative to the vertical axis of rotation (up to about 45°). Centrifugation will cause particles to sediment along the side and bottom of the tube. The swinging bucket design allows the tubes to swing out from a vertical resting position to become parallel to the horizontal during centrifugation. As a result, sediment will form along the bottom of the tube.

Fixed angle rotors are ideal for pelleting applications either to remove particles from a suspension and discard the debris or to recover the pellet, whereas swinging bucket rotors are best for separating large volume samples at low speeds and resolving samples in rate-zonal (density) gradients.Centrifuges may be classified based on maximum speeds, measured as revolutions per minute (RPM). Speeds range from 0-7,500 RPM for low-speed centrifuges, all the way to 20,000 RPM or higher.

Centrifuge rotor speed is often expressed as RCF in units of gravity (x g) for various procedures. However, many centrifuges display speed as revolutions per minute (RPM), necessitating conversion to ensure the correct experimental conditions. The following formula is used to convert RPM to RCF, where R is the rotor radius (cm) and S is the speed (RPM):

g = (1.118 x 10-5) R S2

**CENTRIFUGE CARE AND MAINTENANCE**

A few simple steps can keep a centrifuge functioning properly and reduce the risk of damage or injury.

* Keep the centrifuge properly lubricated. O-rings are the main source of protection against sample leakage, and must be lubricated prior to installation of a new rotor or following cleaning. Any threaded components should also be cleaned regularly and lubricated with an approved grease to ensure proper operation and to prevent cross-threading and corrosion.
* Ensure all users are aware of how to properly operate the centrifuge, including ensuring buckets are properly seated in their pins, balancing tubes in the rotor, operating rotors within stated guidelines for speed and maximum compartment mass, and avoiding scratching the rotor.
* Inspect critical components, and look for signs of wear including scratches, or effects of chemical exposure on the rotor.
* Pay close attention to noise, vibration, shaking, or grinding and stop the unit immediately if this occurs.

**Centrifuge Cleaning**

* Regularly clean the centrifuge with neutral cleaning solutions (alcohol or alcohol-based disinfectant) applied with a soft cloth to rotors and accessories. Daily cleaning should include the interior portion of the centrifuge, the rotor chamber, and surfaces with electronic components, such as touchscreens and keypads.
* It is important to be aware of the different types of samples used with the centrifuge and any specific products or protocols necessary for cleaning spills.
* Ensure a sturdy, level worksurface
* Always ensure the centrifuge is on an appropriate surface prior to operation.
* Balance the centrifuge
* Running an unbalanced centrifuge may cause significant damage, and injure the operator and other laboratory personnel. The total mass of each tube should be as close as possible- this becomes increasingly important at very high rotor speeds. Balancing masses to the nearest 0.1 gram is advisable, and it is important to balance tubes by mass, not volume. For example, do not balance a sample consisting of liquid with a higher or lower density than water with an equal volume of water.
* Do not open the lid while the rotor is moving
* Many centrifuges have a “safety shutoff”. However, this will only stop power to the rotor, which will still spin due to its own inertia for some time until it is slowed to a stop by friction.
* If the centrifuge is wobbling or shaking, pull the plug
* A little vibration is normal, but excessive amounts can mean danger. First, double check that the tubes are correctly balanced. If this does not resolve the issue, do not operate the centrifuge until it has been serviced by the manufacturer or dealer.

**BRANDS OF CENTRIFUGE**

* Eppendorf Tubes
* ELMI CM-7S Clinical
* Scilogex
* Ample Scientific Champion F-33D
* Unico C806 Power Spin Model FX Centrifuge
* Premiere XC-2415 Bench-Top Centrifuge

**COST OF CENTRIFUGE**

The cost of centrifuge in Nigeria ranges between 50,000 to about 500,000 naira depending on the type needed and brand

1. **AUTOMATIC TISSUE PROCESSING**

A tissue processor is a device that prepares 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 (slices) mounted on glass slides and appropriately stained to demonstrate normal and abnormal structures.The Automatic Tissue Processor machine plays a big role in the preparation of the tissue by passing them through various chemicals; a major process called TISSUE PROCESSING

Tissues to be processed are cut into small pieces to ensure the tissue fits into the tissue cassettes.

Smaller tissues (2-4 um) will be processed faster than the whole tissue or organ.
These tissue cassettes are packed into the oscillating tissue basket to tissue prior to fixation.

It undergoes different processes such as:

**FIXATION** – this is the process of preserving or fixing tissues by passing them through chemicals called fixatives. The fixatives will help protect the tissue from decay and autolysis. Routine fixative of use is 10% formalin

**DEHYDRATION** – this is the process of removing water molecules from the tissue by passing the tissue through ascending grades of alcohol. E.g methanol, acetone, 70-100% alcohol

**CLEARING** – this is the process of removing alcohol from the tissue by passing it through chemicals that will remove the alcohol molecules. These agents are called clearing agents. Xylene is mostly used for clearing.

**INFILTRATION** – this is the process of filling intracellular spaces left in the tissue by paraffin wax. This will help confer a bit of rigidity to the processed tissue.

**EMBEDDING**- this last step is manually done. This has to do with immersing the processed tissue into a mould containing liquid paraffin wax. This is for external support so that the tissue won’t crumble during microtomy

**WORKING PRINCIPLE OF AUTOMATIC TISSUE PROCESSOR MACHINE**

Most Automatic tissue processor machines are easy-to-program interface. The processor model has ten 1.8L (60.9oz.) reagent beakers and two 1.8L (60.9oz.) wax baths.
The tissue basket oscillates up and down in each station at three-second intervals to ensure thorough and even mixing of the reagents and optimum tissue infiltration. Infiltration time is separately programmable for each station. Up to nine programs may be run with immediate or delayed starting times.
When it’s time for tissue to be transferred to the next beaker or jar, the cover of the machine is raised up, and the lifting mechanism carefully removes the tissue basket and gently transfers it to the next beaker. When the infiltration time for any particular station is exceeded, a warning message will display, indicating the station number and excess time. Controls are arranged by functionality with an LCD to indicate operational parameters. Reagent container lids have seals to minimize operator exposure to hazardous fumes.
Tissue basket immediately immerses in a station in the event of power loss to protect samples from drying out. When power is restored, program will resume. In the event of long-term power failure, wax is liquified. Capacity of tissue basket is 80 cassettes.Vacuum configurations hasten infiltration, allowing pressure to be applied to any station in either manual or automatic operation. Fume control configurations extract fumes with a fan and pass them through an internal carbon filter.
For added efficiency, these models feature a two-part containment shield surrounding the reagent container platform.

**Automatic Tissue Processing Machine Processing Time Schedule**

Processing schedule varies and it depends on the following:
(i) Nature and size of tissue
(ii) Urgency

Beaker I – fixative (formalin) 1-2 hours
Beaker II – fixative 1 hour
Beaker III – fixative. 30- 45 minutes
Beaker IV – 70% alcohol. 30 minutes
Beaker V – 90% alcohol. 30 minutes
Beaker VI – Absolute alcohol. 1 hour
Beaker VII – Absolute alcohol. 1 hour
Beaker VIII – Methanol 30 minutes
Beaker IX – Xylene. 1-2 hours
Beaker X – Xylene 45 minutes – 1 hour
Wax bath I (done at 45°c) 2 hours
Wax bath II. 2 hours

Remember that the nature of tissue, size and urgency determines the processing time schedule.

**CARE AND MAINTAINANCE OF AUTOMATIC TISSUE PROCESSOR**

 1. Any spillage or overflow should be cleaned immediately.

2. Accumulation of wax on any surface should be removed.

3. The temperature of the paraffin wax bath should be set to 3oC above the melting point of wax.

4. Timings should be checked when placing the cassettes in the processor

**BRANDS OF TISSUE PROCESSOR COMPANIES**

KEDEE-TS6A, Automatic Tissue

Leica tissue processor

DIDAC tissue processor

Radical- RSMT-101D tissue processor

Yorco Tissue processor

Microm STP 12O

**COST OF TISSUE PROCESSOR**

The cost of tissue processor in Nigeria ranges between 1.4 million to 5 million naira depending of the brand and properties.

1. **MICROTOME**

A microtome is a specialized precision cutting instrument, which accurately and repeatedly slices sections from a block of embedded tissue. Different kinds of microtomes are used to section paraffin and plastic embedded tissues as well as the specialized microtomes used to section frozen tissues. In any microtome a sharp knife and the tissue block are held in a fixed relation to each other. With each pass of the tissue past the knife it advances the tissue block a preset amount—the section thickness. For frozen sections the section thickness typically ranges from 8 to 15 µm, for wax sections 4–10 µm, and for plastic histological sections 0.5–3 µm.

Microtome use steel, glass and diamond blades depending upon the the specimen being sliced and the desired thickness of the section being cut . steel blades are used to prepare sections of animals or plant tissues for light microscopy histology. Glass knives are used to slice sections of light microscopy and to slice very thin sections for electron microscopy. Industrial grade diamond knives are used to slice hard materials such as bones, teeth and plant matter for both light microscopy and for electron microscopy gem quality diamond knives are used for slicing thin sections of electron microscopy.

**PRINCIPLE:**

Microtome is a sectioning instrument that allows the cutting of extremely thin slices of a material known as section. Microtomes are used in microscopy, allowing for the preparation of sample for observation under transmitted light or electrons radiation. It is a method for the preparation of thin section for materials such as bones, minerals, and teeth.

**WORKING PRINCIPLE:**

 Microtome is a common instrument. This device operates with a staged rotary action such that the cutting is part of the rotary motion. In a rotary microtome, blade is fixed in horizontal position. Through the motion of the sample holder, the sample is cut by the knife position, at which point the fresh section remains on the knifes, at the highest point of the rotary motion, the sample holders is advanced by the same thickness as the section that is to be made, allowing for the next section to be made by three movement against the fixed blade which is the;

* Downward movement
* Forward movement
* And Backward movement

The flywheel is many microtomes can be operated by hands. This has the advantages that clean cut be made, as the relatively large mass of the fly wheel prevents the sample from being stopped during the sample cut. It cuts thickness between 1 and 60 micron meter. For hard material, its cuts a semi thin section with a thickness of as low as .5 micron meter.

**CARE AND MAINTAINACE OF MICROTOME**

* Daily Care Routine daily care consists of removing sectioning debris from the working area, brushing debris from the knife and cleaning as appropriate.
* first remove the microtome knife/blade holder and clean it
* remove the debris trays and clean
* wipe covers and remove excess debris as required
* Ensure that liquids do not enter the interior of the instrument during operation and maintenance
	+ Before uninstalling the knife/blade holder from the microtome always make sure to detach the knife/ blade wearing cut-resistant gloves and store the knife/blade in a safe place.
	+ Always store knife/blade in an appropriate place when not using them, for example, store it in the dedicated knife case.
	+ Never place a knife anywhere with the cutting edge facing upwards and never try to catch a falling knife.
	+ If the knife/blade holder is not fixed, for example, during installation or cleaning, pay special attention that the knife/blade holder is not falling off.
	+ Whenever possible, fix the knife/blade holder to avoid falling off.
* Do not use any solvents containing acetone or xylene for cleaning.
* When using cleaners, please comply with the safety instructions of the manufacturer and the laboratory safety regulations
* Never use a sharp or hard tool to scrape the instrument surface.
* Never soak the accessories in any cleaning solvent or water.
* Clean steel knives using an alcohol-based solution or acetone.
* For cleaning and removal of paraffin, do not use xylene or cleaning fluids containing alcohol (for example glass cleaner).
* Before each cleaning, carry out the following preparatory steps:
	+ Raise the specimen clamp to the upper end position and activate the handwheel lock.
	+ Remove the blade from the blade holder and insert it in the receptacle at the bottom of the dispenser, or remove the knife from the knife holder and put it back in the knife case.
	+ Remove knife holder base and knife holder for cleaning.
	+ Remove the specimen from the specimen clamp.
	+ Remove section waste with a dry brush
	+ Remove specimen clamp and clean separately.

**Microtome Knives and Blades**

* Great care must be exercised when handling knives and or disposable blades.
* Knives/blades must be stored in their boxes when not in use
* Knives /blades fitted to the microtome must be properly guarded
* Particular care must be taken during cleaning and knife sharpening

**BRANDS OF MICROTOME COMPANIES**

ThermoFisher scientific

Sigma

Leica

BIOBASE

Myr

**COST OF MICROTOME**

The cost of centrifuge in Nigeria ranges between 400,000 to about 3.5 million naira depending on the type needed and brand