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DEPARTMENT: MEDICAL LABORATORY SCIENCE

COURSE CODE: MLS 410

COURSE TITLE: BIOMEDICAL ENGINEERING

  **THE PHYSICS OF A LIGHT MICROSCOPE** A light microscope is a biology laboratory instrument or tool, that uses visible light to detect and magnify very small objects, and enlarging them.

* 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.
* 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
* What is seen in the microscope as an enlarged clear image of the specimen is known as the virtual image. To calculate the magnification, multiply the objective and eyepiece objective magnification together. The magnification is standard, i.e not too high nor too low, and therefore depending on the magnification power of the lenses, it will range between 40X and 100oX.
* Calculation of magnification = Magnification of objective lens/magnification of the eyepiece lens.



**THE CENTRIFUGE**

* Centrifugation is a technique of separating substances which involves the application of centrifugal force.
* The particles are separated from a solution according to their size, shape, density, the viscosity of the medium and rotor speed.
* **A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis of spin that can be very strong**

 **PRINCIPLE OF A CENTRIFUGE**

* In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it floats to the top.
* The greater the difference in density, the faster they move. If there is no difference in density, the particles stay steady.
* To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful “centrifugal force” provided by a centrifuge.
* A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward).
* The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction.
* At the same time, objects that are less dense are displaced and move to the center.
* In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low- density substances rise to the top.

  **BRANDS OF CENTRIFUGE**

* **LW SCIENTIFIC**
* **THERMO SCIENTIFIC**
* **UNICO**
* **BECKMAN**
* **SORVALL**
* **CLAYADAMS. ETC.**

 **CARE AND MAINTENANCE**

**Avoiding Rotor Failures**

The centrifugal field which accelerates the separation process also exerts large forces on the rotor material. If a rotor fails, the centrifuge is severely damaged as well. For this reason, some simple precautions should be observed

Rotors are designed to be run up to their maximum speed with a load of a specific weight. One should never attempt to run a rotor at a speed higher than the one designated by its manufacturer. Also, if high density solutions (greater than 1.2 g/mL, for instance) are used, the run speed must be reduced to prevent undue stress on the rotor. Consult your instruction manual for exact directions.

**Tube Breakage**

Glass tubes can break during centrifugation, due either to improper loading or inherent defects. Any glass fragments must be removed from the buckets, adapters, rubber liners, and rotor chamber before the next run is made. If you find gray dust, which results from sandblasting of the rotor chamber by glass particles, it must be cleaned up too. You should make several dry runs without samples, and clean the chamber between each run to be sure this dust is eliminated from the centrifuge.

**Chemical Resistance**

If you plan to centrifuge any uncommon solvents or solutions, consult your manual to be sure they are compatible with the various plastics and metals comprising the centrifuge, the rotor, the tubes, and other accessories. These same precautions must be observed with any solvents used for sterilization purposes. A table of 19 chemical resistances for common centrifuge materials is available from Beckman Coulter.

**Aerosol Generation**

If any liquid is spilled on a rotor, it will be dispersed as a particulate mist when the centrifuge is run. Part of this mist will be fine enough to form a relatively stable aerosol which will tend to be dispersed throughout the laboratory. Such spills should be thoroughly cleaned up before running the centrifuge.

**Handling Human Samples**

Human blood or blood components can transmit an infectious disease or virus if the patient or donor carries these. Blood should be handled with respect for this possibility during all laboratory manipulations, including centrifugation.

**When in doubt, refer to your instruction manual**

From time to time, you’ll have questions about the actual operation and maintenance of your centrifuge. The instruction manual provided with each instrument is designed to answer these questions. It should be read before making your first run, and kept handy for future reference.

 **COST OF A CENTRIFUGE**

Approximately 45$ i.e 16,000 naira

 **AUTOMATIC TISSUE PROCESSOR**

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 ATP machine plays a big role in the preparation of the tissue by passing them through various chemicals; a major process called **TISSUE PROCESSING which involves:**
(i) **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**

(ii) **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

(iii) **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.

(iv) **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.

(v) **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**

 **PRINCIPLE**

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.

 **BRANDS**

* ASP6025 S.
* HistoCore PELORIS 3 Premium **Tissue Processing** System.
* HistoCore PELORIS 3.
* ASP6025.
* Leica ASP300S.
* RemoteCare.
* Leica TP1020.
* Archive.

 **CARE AND MAINTENANCE**

* + Any spillage or overflow should be cleaned immediately
	+ Accumulation of wax on any surface should be removed
	+ Timing should be checked when placing the cassette processor
	+ The Temperature of the paraffin wax should be set above 3°c above the melting point of wax

 **COST**

  Automatic Tissue Processor WT-TS3A Price. US $3600-$3800

 **MICROTOME**

**microtome** is a sectioning instrument that allows the cutting of extremely thin slices of a material known as section . **microtome** are used in microscopy , allowing for the preparation of sample for observation under transmitted light or electrons radiation.

**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.**

**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 cits a semi thin section with a thickness of as low as .5 micron meter.**

 **Care and maintenance**

* + - When leaving the microtome, even for a short time, ensure that the blade guard is in place.
		- Before using your knife, take a lint-free facial tissue saturated in either zylene, benzene or acetone to remove the protective oil coating on the knife. C. Use a dry, lint-free, facial tissue to wipe your knife clean. DO NOT USE GAUZE or any other coarse material; it will destroy the edge of your knife

 **BRANDS**

* AGD Biomedicals
* Alltion (Wuzhou)
* Amos scientific
* ANA-MED
* Auxilab S.L.
* Boeckeler Instruments, Inc.
* Breukhoven
* Bright Instruments

 **COST**