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**COURSE TITLE: BIOMEDICAL ENGINEERING**

**COURSE CODE: MLS 410**

**ASSIGNMENT TITLE: BIOMEDICAL EQUIPMENTS**

**QUESTIONS**

1. Discuss the physics of light microscope diagrams and illustrations needed.
2. Write notes on the following biomedical equipment. Add notes on principle, brand, care and maintenance and cost.
3. Centrifuge
4. Automatic Tissues Processor
5. Microtome.

**ANSWERS**

**1.**

A light microscope is an instrument that uses visible light and magnifying lenses to examine small objects not visible to the naked eye, or in finer detail than the naked eye allows. The usefulness of any microscope is that it produces better resolution than the eye. Resolution is the ability to distinguish two objects as separate entities, rather than seeing them blurred together as a single smudge. Light microscopes play an important role in many research laboratories, including electron microscopy facilities. Samples for light microscopy are prepared in an ever-increasing number of techniques, and can range from sliced biological organisms and tissue cultures to materials science and geological samples. Light and electron microscopes have many similarities in their optical principles.

**PRINCIPLES OF LIGHT MICROSCOPY**

 The light microscope is an instrument for visualizing 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.

**HOW THE IMAGE IS FORMED**

Conventional brightfield 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.

**THE THEORETICAL RESOLUTION OF LIGHT MICROSCOPY**

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:

The light microscope has various components. The first and the most important element are the lenses.



The six simple lenses. A, B, & C are converging or positive lenses while D, E, & F are concave or negative lenses.



 Converging and diverging lenses. Parallel rays enter a convex lens converge and meet at the principle focus. The distance between the center of the lens and the principal focus is the focal length. Parallel rays entering a concave lens diverge and never meet.

The Objective Lens is the first part of the imaging system; the objective lens forms a primary, enlarged image of the object. Very fine details are distinguished with the objective lens. The eyepiece sometimes called the ocular lens, is the second lens, which forms a secondary, further enlarged image. By multiplying the magnifying power of the objective lens and the magnifying power of the ocular the final magnification is found. A Substage Condenser lens is the third optical component. It is placed on a platform beneath the object. Light is directed through the substage condenser and converges to a point at the position of the specimen. The light rays diverge as they pass through the specimen and form an inverted cone, whose base is just large enough to fill the aperture of the objective. The size of the light beam is controlled by a diaphragm beneath the condenser called the aperture diaphragm. The light source should contain both a lens to project an image of the lamp filament called a field condenser and a diaphragm to control the size of the illuminated field called a field diaphragm.

**Typical lamp for light microscope**

Kohler Illumination is the most common method of illumination. In Kohler illumination the image of the source is projected by the field condenser onto the substage condenser, to the top of the plane of the object. This method assures even illumination. Modern light microscopes use several different modes of operation depending on the needs of the investigator. The most common of these being brightfield microscopy in which direct light passes through the objective aperture and illuminates the background against which the image is seen. Since the structural elements being resolved have little variance in refractive index, the image will lack contrast and the details remain invisible. Small structure detail can be revealed by changing the absorption of the object by means of staining.

 Many microscopes have special lenses that allow an investigator to image using phase-contrast. This method reveals details in specimens possessing very slight differences in optical path or refractive index in the surrounding media. Phase contrast requires no staining and can be used with living tissue. Contrast is produced in this method by phase differences in the light leaving the object to amplitude differences in the image. A phase plate alters the optical path length traveled by diffracted light from that traveled by direct light.

Light waves in brightfield and phase contrast after passing through an object. Pathway A represents the light wave before encountering the object. Wave B represents the wave phase after passage in brightfield (unstained mode). C compares the wave phase of an object viewed with phase contrast.

Differential interference contrast (DIC) differs from phase contrast in that the image has a strong relief and three-dimensional appearance. It must be remembered that the impression of surface details are the results of the optics and not the specimen for most biological samples. The optics for DIC consist of a polarizer at the light source and Wollaston prisms in the condenser and above the objectives. The beam passes through the polarizer, enters the first prism where it is split in two. One beam vibration is parallel to the prism and one is perpendicular. Both beams pass through the specimen in parallel in close proximity and are recombined in the second prism.



Differential Interference Contrast Schematic.

Darkfield microscopy is a mode in which direct light is prevented from passing through the objective aperture, but a hollow cone of light forms an apex in the plane of the specimen. The image is formed by light scattering from features of the object. Details appear bright white against a dark background. Darkfield can also be used for living bacteria, algae and plankton.

Abbe in order to ease in identification of lens quality devised an equation for numerical aperture. Numerical aperture numbers can assist in comparing angles of dry, water immersion, and oil immersion objectives. Note the similarity to Abbe’s equation for theoretical resolution. This number is found on all objective lenses.

N.A. = n sin u

n = refractive index of medium

u = 1/2 the angle of light rays taken in when focused on the object.

When choosing an objective another consideration is depth of field. Depth of field is the distance from the nearest part of the subject in acceptable focus to the farthest part of the subject in acceptable focus. The efficiency (resolution) of a lens is inversely proportional to the depth of field

N.A. .25 .30 .50 .65 .85 .95

Depth (in microns) 8.0 5.5 2.0 1.0 .25 .10

Variation in Depth of Field with Change in N.A.

Two aberrations within lenses detract from Abbe's equation of theoretical resolution. These aberrations are called spherical aberration and chromatic aberration. Spherical Aberration occurs when outer rays entering a lens are diffracted differently from those entering near the center. A solution for reducing spherical aberration is introducing a diaphragm or aperture.

Spherical aberration of a simple lens. A. Under correction. B. Over correction.

The thickness of the cover glass should be chosen according to specifications of a particular objective. Deviation from the required thickness results in over correction or under correction of spherical aberration. Chromatic Aberration occurs as white light entering a lens is broken into a spectrum from red to violet. Violet rays (more energetic) are refracted more than the red rays (less energetic). Consequently, an uncorrected lens will be surrounded by color fringes. The more expensive lenses have a higher degree of correction.

**2(a)** **CENTRIFUGE**

A centrifuge is a motor-driven device used for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed. 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 (isopycnic conditions), the particles stay steady. Laboratory centrifuges are widely used in hospitals or other industries where they are used for extracting suspended material from a variety of medium. 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.

**PRINCIPLE**

The centrifuge involves principle of centrifugation, where the acceleration at centripetal force causes denser substances to separate out along the radial direction at the bottom of the tube. 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.

**-**

* + - * Speed of sedimentation (ultra-centrifuge or high-speed centrifuge).
			* Presence/absence of vacuum (ultra-centrifuge or small bench top)
			* Temperature control refrigeration.
			* Volume of sample and capacity of centrifugation tubes.

**BRANDS OF CENTRIFUGE**

* Excelsa® I 2206 (FANEM®)

Blood Bank Centrifuge/ For biology/ Laboratory. Speed: 300rpm – 5,000rpm.

* Rotofix 32 A (HETTICH)

Laboratory centrifuge/ Medical/ Benchtop/ High-performance. Speed: 0rpm- 6,000rpm.

* Eba 270 (HETTICH)

Laboratory centrifuge/ medical/ compact/ swing-out. Speed: 0rpm-4,000rpm.

* Robotic 380, Rotina 380 RC Robotic (HETTICH)

Robotic centrifuge/ Laboratory/ Clinical/ Benchtop. Speed: 0rpm-5,100rpm.

* Cmone (AESTHETIC GROUP)

Manual centrifuge/ Clinical/ Multifunction/ Benchtop. Speed: 100rpm-3,000rpm.

* C 2201 (LISTON)

Laboratory centrifuge/ medical/ benchtop/ low- speed. Speed: 100rpm-3,600rpm.

* CN-45 (ORMA)

Laboratory centrifuge/ clinical/ for teaching/ vertinary. Speed: 300rpm- 4,500rpm.

* KR41 (THERMO SCIENTIFIC)

Laboratory centrifuge/ clinical/ Multifunction/Compact. Speed:0rpm-4,250rpm.

* Z206-A (BENCHMARK SCIENTIFIC)

Laboratory centrifuge/ clinical/ For biology/ benchtop. Speed: 200rpm-6,000rpm.

* CD-0412 (PHOENIX INSTRUMENT)

Clinical centrifuge/ benchtop. Speed: 300rpm-4,500rpm.

**MAINTENANCE AND CARE OF A CENTRIFUGE**

* 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.
* Scheduling regular preventive maintenance with a trained technician for your centrifuge is vital because it increases the durability and functionality of the centrifuge.
* 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.

**(b) AUTOMATIC TISSUE PROCESSOR**

The automatic tissue processor is a machine that prepares tissue samples for sectioning and microscopic examination in the diagnostic laboratory. It combines proven technology and modern design, for automated animal and human tissue processing. 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.** The first automatic tissue processors were introduced during the first half of the 20th century. In the USA, they were produced under the name of **Auto-Technicon** and in the UK under the name of **Histokine**, and later by other companies. These devices have slowly evolved to be safer to use, handle larger specimen numbers, process more quickly and to produce better quality outcomes.

The automatic tissue processor processes specimen overnight for about 16-18hours. It is operated in a closed system which assist with keeping exposure to toxic vapours to a minimum when they are vented to the outside air or through a filter. Several reagents are placed in the processor which include: 10% neutral buffered formalin, 70% alcohol, 80% alcohol, 90% alcohol, 95% alcohol, absolute alcohol, xylene and paraffin wax. Automatic tissue processors utilize heat at approximately 45oc along with vacuum and pressure to aid in the processing of the tissue. There are twelve cylinders in this device, nine glass cylinders and three wax cylinders which are twelve processing stages and they are:

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

**PRINCIPLE**

 Time required for tissue processing may be considerably reduced when tissue is suspended in fluid, continuously agitated. Moved from one reagent to another whenever desired, not restricted by working hours. Processors are configured with present interval for different schedules of suspension, agitation and automatic changeover.

**BRANDS OF AUTOMATIC TISSUE PROCESSOR**

1. **KD-TS6A, Automatic Tissue Processor**

This processor is used to process animal and human tissues automatically. It is accurate and easy to use and maintain. It is an excellent choice for histology and pathology labs of hospitals and research institutions.

**Main Technical Data**

* Steps of processing tissue: 12 steps.
* Glass containers: 9 with capacity of 1.2L each.
* Wax cups: 3 with capacity of 1L each. Temperature range: 45~850C±3
* Process: Each cup working hours between 0-59hr59min. Delay process: 0-59hrs59min.
* Vertically reciprocal motion of tissue basket: 3 times/min.
* Tissue basket specification: Barrel tissue basket-￠95x80mm. Layer nacelle-￠95x80mm, Five layers.
* Weight: ＜60kg.
* External dimension: (diameter) 660 x (high)550 x 680(Tip top) mm.
* Rated voltage: 220v, 50Hz or 110V, 60Hz
1. **KD-TS6B, Automatic Vacuum Tissue Processor**

This processor uses Vacuum function to accelerate the speed of tissue processing for animal and human tissues automatically. It is accurate and easy to use and maintain. It is an excellent choice for histology and pathology labs of hospitals and research institutions.

**Main Technical Data**

* Steps of processing tissue: 12 steps.
* Glass containers: 9 with capacity of 1.2L each.
* Wax cups: 3 with capacity of 1L each. Temperature range: 45~85C±3
* Process: Each cup working hours between 0-59hr59min. Delay process: 0-59hrs59min.
* Vacuum: ≤ 0.053Mpa.
* Vertically reciprocal motion of tissue basket: 3 times/min.
* Tissue basket specification: Barrel tissue basket-￠95x80mm. Layer nacelle-￠95x80mm, Five layers.
* Weight: ＜60kg.
* External dimension: (diameter) 660 x (high)550 x 680(Tip top) mm.
* Rated voltage: 220V, 50Hz or 110V, 60Hz
1. **KD-TS3D1, Automatic Tissue Processor**

**Technical specification:**

* Numbers of stations: 12 (no.10, 11 and 12 are wax dispensing cylinder)
* Volume of each station: 2 litres
* Range of temperature: ambient-80 C
* Temperature tolerance: +/-1 C
Processing time of each station: anytime (No.1 station) 0-24 hrs (other stations)
* Time of exchange station, delay, leachate:10-60s (adjustable), and leachate, basket shaking work above cylinder
* Tissue protecting stations: No 1 - No 7 (adjustable)
* Voltage: AC220V/50Hz, AC110V/60Hz
* Power: 1000VA
* Dimension: 1135x480x490 mm
* Weight: 88kg
1. **KD-TS3D, Automatic Tissue Processor**

**Technical specification:**

* Numbers of cylinder: 12 (no.10, 11 and 12 are wax dispensing cylinder)
* Volume of each cylinder: 1.8 litre
* Processing time of each cylinder: anytime (No.1 cylinder) 0-24 hrs (other cylinders)
* Time of exchange cylinder, delay, leachate:10-60s (adjustable), and leachate, basket shaking  work above cylinder
* Voltage: AC220V 50Hz, AC110V 60Hz
* Power: 1000VA
* Dimension: 1135x480x490 mm

Weight: 88kg

1. **KD-TS3S, Automatic Tissue Processor (Double tissue basket)**

**Technical specification:**

* Numbers of cylinder: 14 (10 reagent cylinders, No.11, 12, 13, 14 are wax dispensing cylinders)
* Volume of each cylinder: 1.5 Liter
* Range of temperature: ambient-80 C
* Precision tolerance: +/-1%
* Processing time of each cylinder: 0-99 hrs (No.1 and No.2 cylinders), 0-24 hrs (No.3-No.14 cylinders)
* Time of exchange cylinder, delay, leachate: 10-60s (adjustable), and leachate, basket shaking work above cylinder
* Times of basket shaking: 0-6 times /min(adjustable)
* Protecting station of tissue: No.7 for A program, No.5 for B program
* Voltage:AC220V/50Hz、AC110V/60Hz
* Power:550VA
* Dimension:1250x430x490 mm

weight:85kg

**MAINTENANCE AND CARE OF AN 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 cassette in the processor.
5. Clean outside the instrument with xylene dampened cloth.
6. Close retort chamber, press CLEAN for the clean cycle to start automatically.
7. A quality control chart is recommended to ensure that reagents are monitored and changed frequently as per manufacturer’s instruction.

**(c) 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 present amount—the section thickness. These sections are stained using suitable staining techniques followed by observing them under the microscope.

**TYPES OF MICROTOMES**

1. Rotary microtome.
2. Sliding or Base Sledge Microtome.
3. Cambridge rocking microtome.
4. Freezing microtome.
5. Cryostat microtome
6. Ultramicrotome
7. Rocking microtome

**PRINCIPLE**

 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. It I a method for the preparation of thin section for materials such as bones, tissues, minerals and teeth.

**BRANDS OF MICROTOME**

1. [Leica RM2125 RTS](https://www.leicabiosystems.com/histology-equipment/microtomes/leica-rm2125-rts/) ([The Essential Microtome](https://www.leicabiosystems.com/histology-equipment/microtomes/leica-rm2125-rts/))
2. [HistoCore BIOCUT](https://www.leicabiosystems.com/histology-equipment/microtomes/histocore-biocut/) ([Manual Rotary Microtome](https://www.leicabiosystems.com/histology-equipment/microtomes/histocore-biocut/))
3. [HistoCore MULTICUT](https://www.leicabiosystems.com/histology-equipment/microtomes/histocore-multicut/) ([Semi-Automated Rotary Microtome](https://www.leicabiosystems.com/histology-equipment/microtomes/histocore-multicut/))
4. [HistoCore AUTOCUT](https://www.leicabiosystems.com/histology-equipment/microtomes/histocore-autocut/) ([Automated Rotary Microtome](https://www.leicabiosystems.com/histology-equipment/microtomes/histocore-autocut/))
5. [Leica RM Cool Clamp](https://www.leicabiosystems.com/histology-equipment/microtomes/leica-rm-coolclamp/) ([Improved workflow and uniform paraffin sections](https://www.leicabiosystems.com/histology-equipment/microtomes/leica-rm-coolclamp/))

**MAINTENANCE AND CARE OF MICROTOME**

1. Routine daily care consists of removing sectioning debris from the working area, brushing debris from the knife and cleaning as appropriate.
2. Knives/blades must be stored in their boxes when not in use.
3. Knives /blades fitted to the microtome must be properly guarded.
4. Take particular care to clean the contact surfaces of disposable blade holders. A build-up of debris can prevent the blade from seating properly and causes instability during section cutting.
5. In the event of a breakdown, a qualified person should be called.

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