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COURSE TITLE: BIOMEDICAL ENGINEERING

ASSIGNMENT

1. Discuss the physics of the light microscope, diagrams and illustrations needed
2. Write notes on the ff biomedical equipment. Add notes on the principle, brand, care and cost of; A. Centrifuge, B. Automatic tissues processor, C. Microtome.

ANSWERS

1. The **optical microscope**, also referred to as a **light microscope**, is a type of microscope that commonly uses visible light and a system of lenses to generate magnified images of small objects. Optical microscopes are the oldest design of microscope and were possibly invented in their present compound form in the 17th century. Basic optical microscopes can be very simple, although many complex designs aim to improve resolution and sample contrast. The microscope is one of the most expensive and delicate instrument used in the clinical laboratory. Because it can be really misused, it is important for medical laboratory personnel to conversant with the working principle of the commonly used LIGHT MICROSCOPE and should know the how to use and maintain it correctly.

WORKING PRINCIPLE

The microscope magnifies the image of the object being viewed through it. An ordinary magnifying glass is referred to a compound microscope or a light microscope. The magnification of the object is produced by the combined action of two lenses, the objective lens near the viewer's eye. The specimen to be viewed with the light microscope has to be sufficiently thin so that light can pass through it. Some light is absorbed while passing through the specimen, and a contrast may be produced due to differences in light absorption by different parts of the specimen. However, the optical system of the bright-light microscope does not reveal much contrast in the unstrained preparation. Therefore, the contrast needs to be enhanced with staining.

- **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 reflected. Reflection not only occurs when light passes through air and strikes an object, but also when its strikes an interface between air and glass. Stray reflections inside the microscope interfere with the path of light rays and degrade the sharpness of the image.

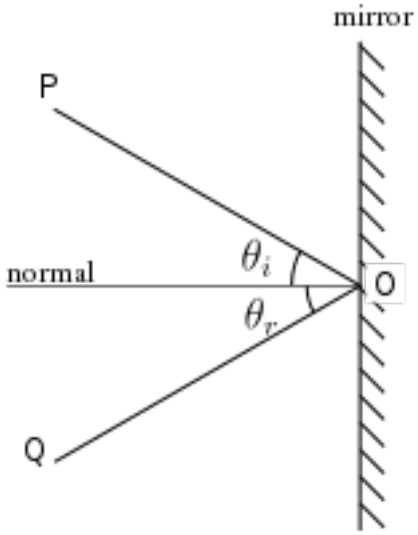
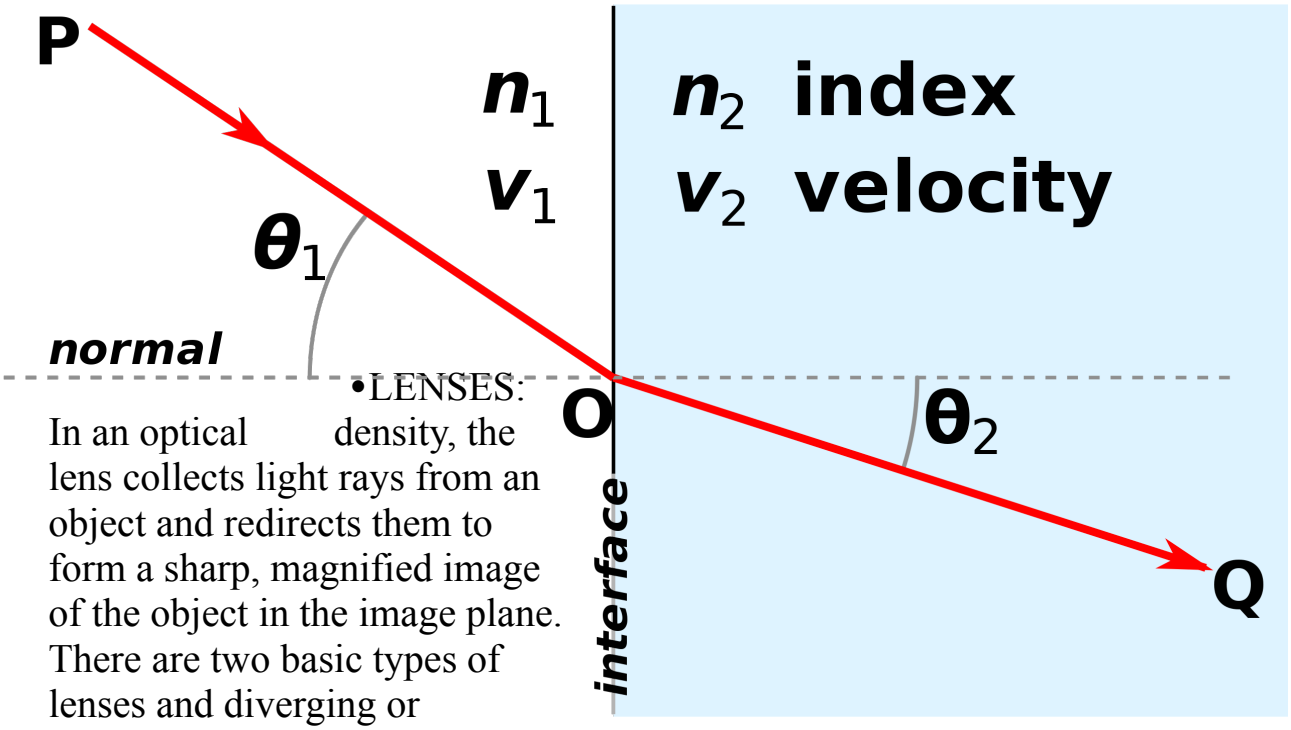


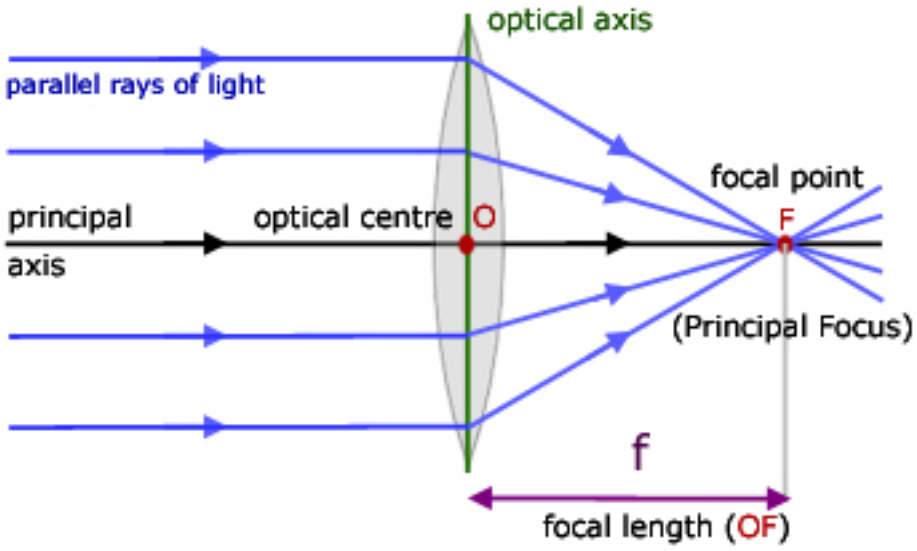
DIAGRAM OF A SPECULAR REFLECTION

- **REFRACTION**: Refraction is simply the bending of a light ray from the normal when it passes into a different optical medium. A normal line is the line perpendicular to a flat surface. Refraction id caused by changes in the speed of light while passing from one medium into another 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. Optical media include glass such as (filters, slides, lenses, coverslips) , air, immersion oil.

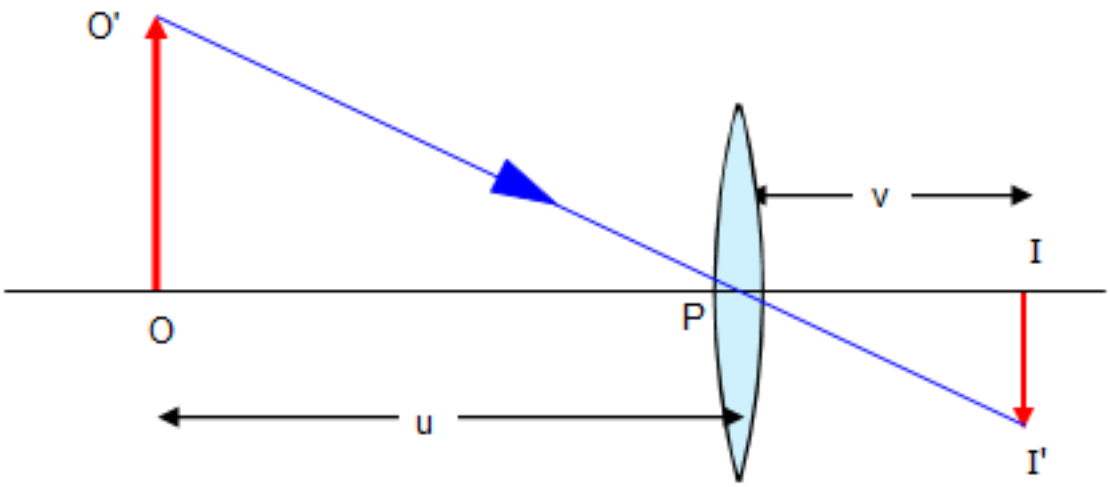


•LENSES: In an optical density, the lens collects light rays from an object and redirects them to form a sharp, magnified image of the object in the image plane. There are two basic types of lenses and diverging or negative lenses. The converging lens is convex and directs light to point. The diverging lens is concave and it bends light outward. Several combinations of these two basic types are possible. However, double convex lens is the most common used in microscopy.

- **PRINCIPLE FOCUS AND OPTICAL CENTRE**: The centre of the lens surface on either side of a biconvex lens is called a centre of curvature. A straight line joining these two centres is the **principle axis.** A ray of light entering the lens along the principlal 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 of at which they meet is called the **principal focus.** A biconvex lens has a principle focus on each side of the lens. A ray of light entering a converging 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. The pont at which these two rays cross is called the **optical centre (O)** of the lens. The distance between the optical centre and the principle focus is the **focal length** of the lens.

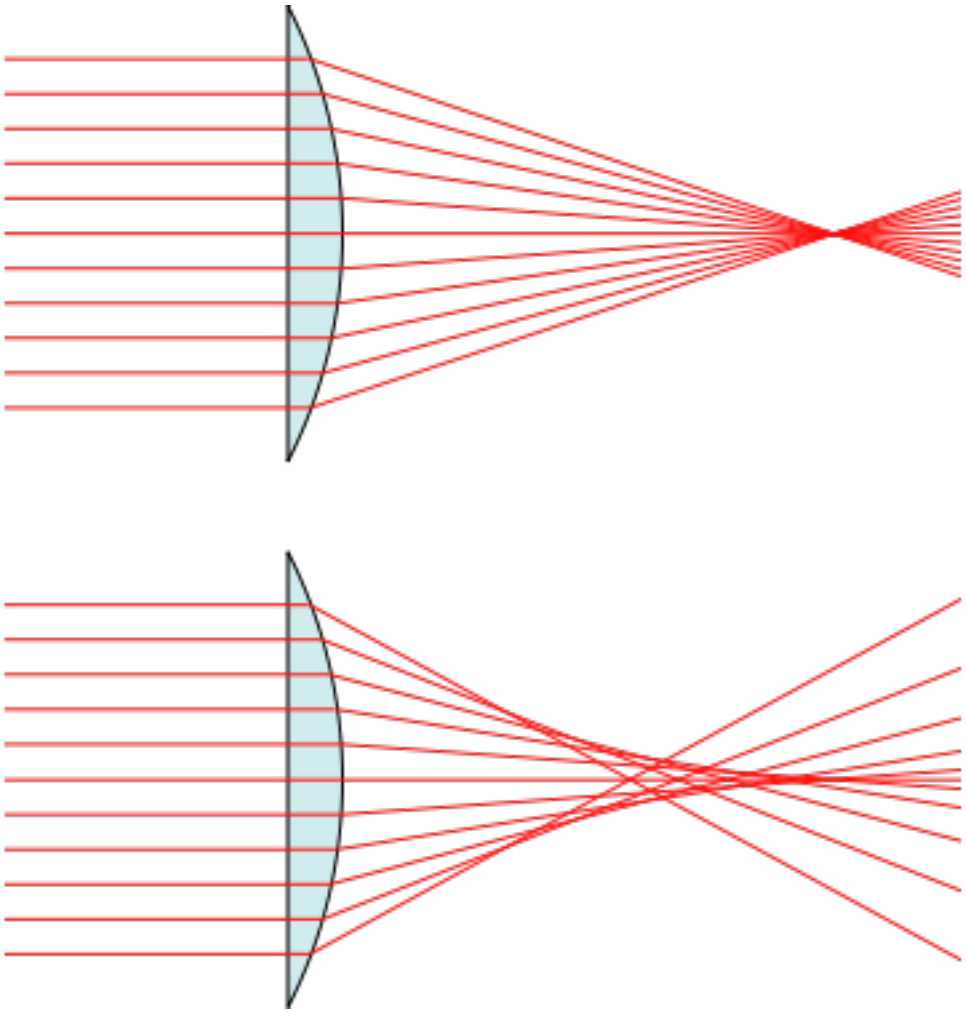


- **MAGNIFICATION**: The magnification produced by a lens defined as the ratio of the distance between the lens and the image plane, and the distance between the lens and the object. In simple words, magnification is obtained by dividing the size of the image by the size of the object. In case of a convex lens, the magnification is maximum when the object is placed just outside the principal focus of the lens.



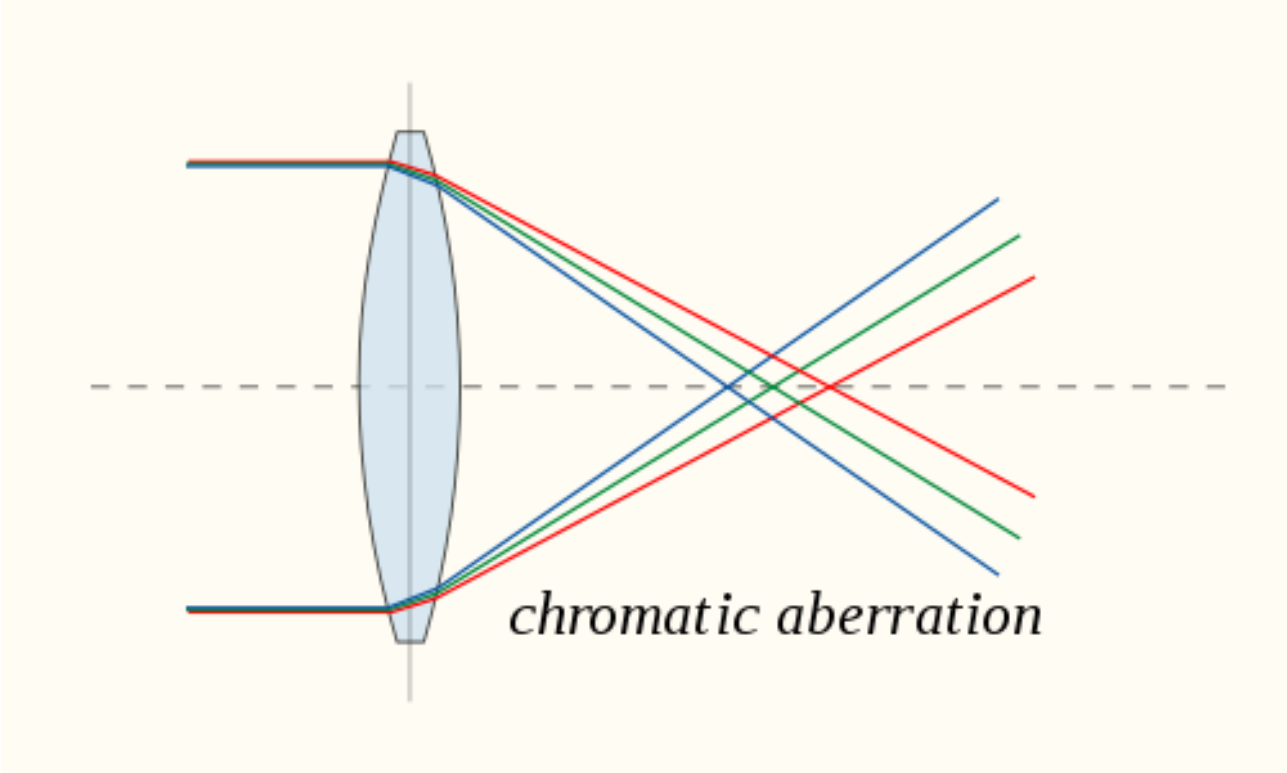
- **DEFECTS IN LENS SYSTEMS**: While lenses provide the desirable aspect of magnification, they also have limitations caused by behaviour of light. In microscopy, these limitations arise from two causes— the shape of the lens, and the presence of different wavelengths in the white light used. These limitations give rise to two defects, namely spherical aberration and chromatic aberration. These two aberrations are corrected by compounding lenses of varying refractive indices and dispersing abilities to one compound lens.

A. Spherical Aberration: Is the indistinct or fuzzy appearance of images due to nonconvergance of rays of light to a common focus. The spherical aberration occurs when the edge of the lens gives a slightly higher magnification than its centre. This results in the loss of contrast, resolution, clarity and overall focus. Spherical aberration is the a property of those lenses that have less than perfect spherical shape and it increases with increase in the thickness of the biconvex lens. It can be corrected by compounding it with a biconcave lens that brings the image to a sharp focus.



B. Chromatic Aberration: Is defined as the fuzzy appearance of the image due to to non-convergence of rays of white light to a common focus. This is the condition in which the image is surrounded by a multi-coloured ring, with a blue light being slightly more magnified than the red. It is caused by splitting of white light into its component colours while passing through a biconvex lens, which acts as a prism. When light passes through a prism, the light of shorter wavelength, like blue, is refracted more strongly than that of a longer wavelength such as red. Thus, the light of one colour is projected at a greater

magnification than another resulting in the appearance of coloured fringes in the image of the object.
Chromatic aberration in modern microscopes is controlled by proper combination of lenses. Achromatic lenses are corrected for one colour while the apochromatic are for three colours.



Chromatic aberration of a single lens causes different wavelengths of light to have differing focal lengths.

QUESTION 2

CENTRIFUGE: It is used to rapidly sediment particles as cells, which maybe suspended in a fluid. A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a force perpendicular to the axis of spin (outward) that can be very strong.

PRINCIPLE: The centrifuge works using the sedimentation principle, where the centrifugal 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. The

greater the CF, the faster and more effective is the sedimentation. This centrifugal force, which is the outward pull due to rotation, is relative to the speed of centrifuging in revolutions per minute (rpm). The actual sedimentation achieved, however, depends on the radius of the centrifuge. The radius of the centrifuge is the distance between the centre of the centrifuge shaft and the tip of the centrifuge tube.

HISTORY: English military engineer Benjamin Robins (1707–1751) invented a whirling arm apparatus to determine drag. In 1864, Antonin Prandtl proposed the idea of a dairy centrifuge to separate cream from milk. The idea was subsequently put into practice by his brother, Alexander Prandtl, who made improvements to his brother's design, and exhibited a working butterfat extraction machine in 1875.

CARE:

- Always place the centrifuge on a flat surface first.
- Always unplug the power cord before cleaning.
- Emergency phone numbers and procedures should be posted and kept up to date.
- Wear disposable gloves.
- Follow your facility's safety procedures when cleaning and disinfecting the centrifuge.
- Before moving the centrifuge to a new location, the exterior and interior surfaces should be cleaned and disinfected.
- Plug in centrifuge only when completely dry.
- Use a non corrosive disinfectant to clean the centrifuge from time to time.
- Do not try to stop the centrifuge by hand while still rotating.

COST: Ranging from prices of N25,000 - N107,000 depending on the brand and type.

AUTOMATIC TISSUE PROCESSOR: 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. Tissue processing involves four stages namely; FIXATION, DEHYDRATION, CLEARING AND IMPREGNATION.

HISTORY: Tissue processing has been in existence as far back as in the late 18th century. Major breakthroughs about the basic components of tissue were made possible because of tissue processor. Tissue processing was done manually in the 18th and 19th century. A cruel long process that took days and sleepless nights to achieve this feat. This discomfort forced scientists into looking for a better and a more efficient way to process tissues.
The first automatic tissue processors were introduced during the first half of the 20 th 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.

PRINCIPLE: The first automatic tissue processors were introduced during the first half of the 20 th 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. 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.

CARE:

- Any spillage or overflow should be cleaned immediately.
- Accumulation of wax on any surface should be removed.
- The temperature of the paraffin was bath should be set to 30C above the melting point of wax.
- Timings should be checked when placing the cassettes in the processor.

COST: Ranging from prices $6,550.00 - $14,800 depending on the brand and type.

MICROTOME: A microtome (from the Greek *mikros*, meaning "small", and *temnein*, meaning "to cut") is a tool used to cut extremely thin slices of material, known as sections. Important in science, microtomes are used in microscopy, allowing for the preparation of samples for observation under transmitted light or electron radiation.

Microtomes use steel, glass, or diamond blades depending upon the specimen being sliced and the desired thickness of the sections being cut. Steel blades are used to prepare sections of animal or plant tissues for light microscopy histology. Glass knives are used to slice sections for light microscopy and to slice very thin sections for electron microscopy. 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. Gem quality diamond knives are used for slicing thin sections for electron microscopy.

Microtomy is a method for the preparation of thin sections for materials such as bones, minerals and teeth, and an alternative to electropolishing and ion milling. Microtome sections can be made thin enough to section a human hair across its breadth, with section thickness between 50 nm and 100 μm.

MICROTOME

HISTORY: In the beginnings of light microscope development, sections from plants and animals were manually prepared using razor blades. It was found that to observe the structure of the specimen under observation it was important to make clean reproducible cuts on the order of 100 μm, through which light can be transmitted. This allowed for the observation of samples using light microscopes in a transmission mode.

One of the first devices for the preparation of such cuts was invented in 1770 by George Adams, Jr. (1750–1795) and further developed by Alexander Cummings. The device was hand operated, and the sample held in a cylinder and sections created from the top of the sample using a hand crank.

In 1835, Andrew Prichard developed a table based model which allowed for the vibration to be isolated by affixing the device to the table, separating the operator from the knife.

Other sources further attribute the development to a Czech physiologist. Several sources describe the Purkyne model as the first in practical use.

The obscurities in the origins of the microtome are due to the fact that the first microtomes were simply cutting apparatuses, and the developmental phase of early devices is widely undocumented.

At the end of the 1800s, the development of very thin and consistently thin samples by microtomy, together with the selective staining of important cell components or molecules allowed for the visualisation of microscope details.

Today, the majority of microtomes are a knife-block design with a changeable knife, a specimen holder and an advancement mechanism. In most devices the cutting of the sample begins by moving the sample over the knife, where the advancement mechanism automatically moves forward such that the next cut for a chosen thickness can be made. The section thickness is controlled by an adjustment mechanism, allowing for precise control.

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

CARE:

- Keep the edge of your knife clean at all times.
- Spray or brush any household oil on your knife to prevent rust when not in use.
- Store your knife in its case to prevent oxidation from occurring.
- If using a lab sharpener, periodically send your knife out to be professionally reconditioned.
- Dust accumulation must be prevented by putting a cover when not in use.
- Wipe the moving parts regularly with good neutral oil to lubricate and avoid rust.
- Never adjust screw too tightly that they may cause binding.

COST: Ranging from prices $1500 - $10,300 depending on the brand and type.