



LABROTORY INSTRUMENTATION AND TECHNIQUES

BY

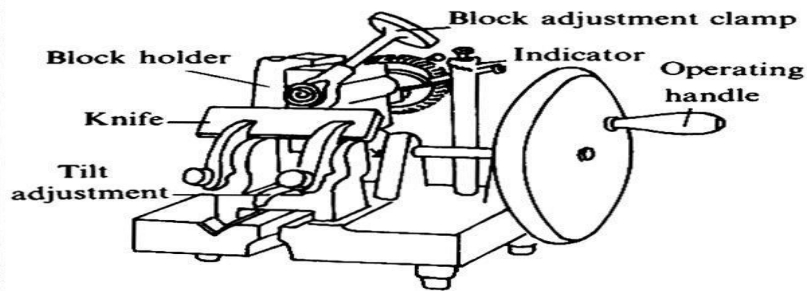
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LECTURE TEN

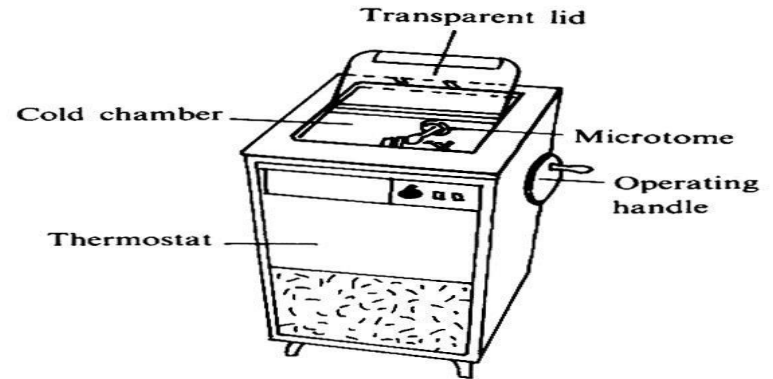
MICROTOMES

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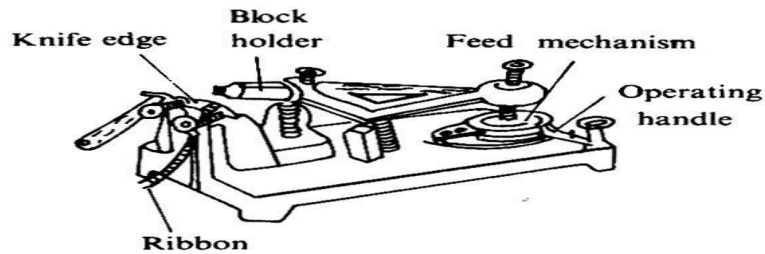
(1) Rotary microtome



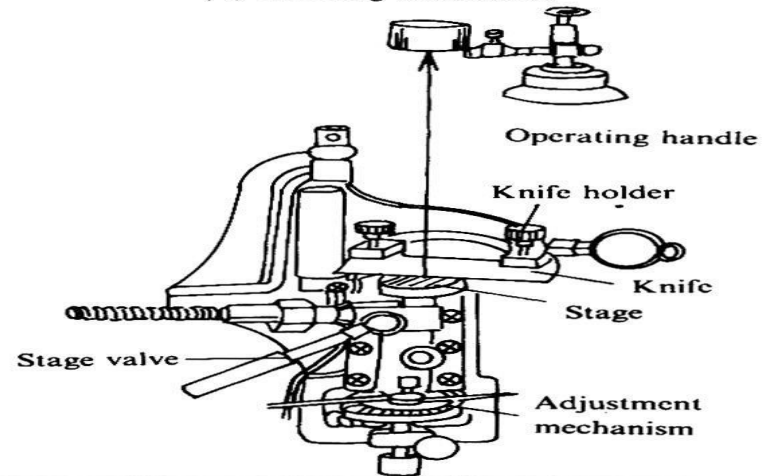
(2) Cryostat



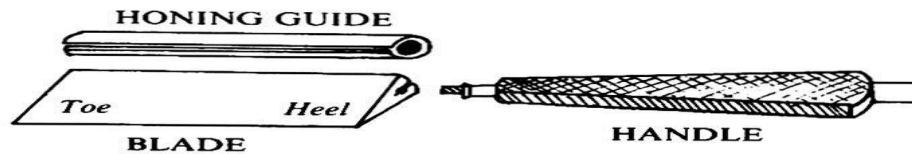
(3) Cambridge rocking microtome



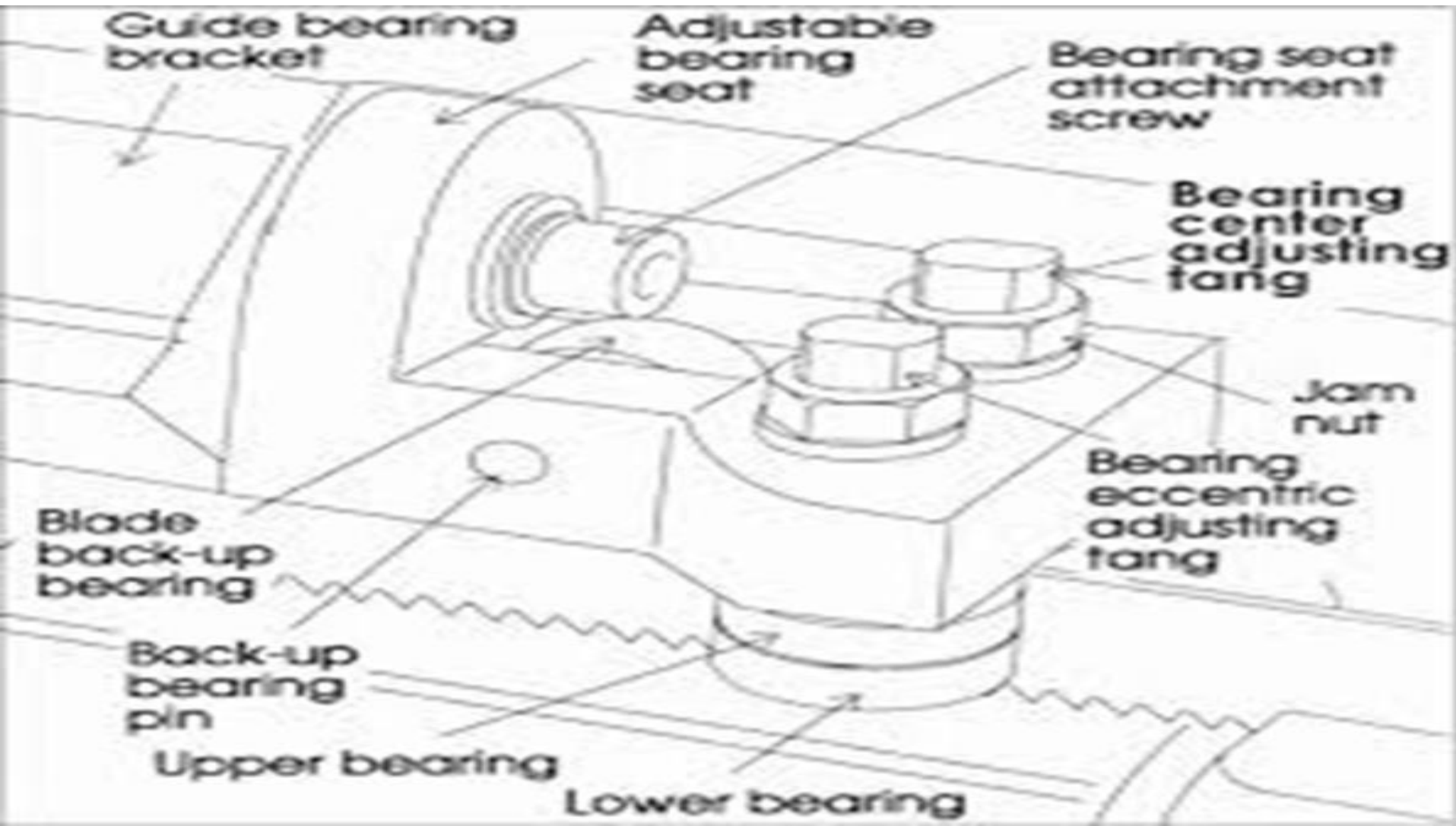
(4) Freezing microtome



MICROTOME KNIFE



A microtome is a tool used to cut extremely thin slices of material, known as sections. Various types of microtomes are available. Most commonly used microtome for routine histopathology is rotary microtome.



The most common applications of microtomes are:

Traditional Histology Technique: Tissues are hardened by replacing water with paraffin. The tissue is then cut in the microtome at thicknesses varying from 2 to 50 μm . From there the tissue can be mounted on a microscope slide, stained with appropriate aqueous dye(s) after prior removal of the paraffin, and examined using a light microscope.

Cryosectioning Technique: Water-rich tissues are hardened by freezing and cut in the frozen state with a freezing microtome or microtome-cryostat; sections are stained and examined with a light microscope. This technique is much faster than traditional histology (15 minutes vs 16 hours) and is used in conjunction with medical procedures to achieve a quick diagnosis. Cryosections can also be used in immuno histochemistry as freezing tissue stops degradation of tissue faster than using a fixative and does not alter or mask its chemical composition as much.

Electron Microscopy Technique: After embedding tissues in epoxy resin, a microtome equipped with a glass or gem grade diamond knife is used to cut very thin sections (typically 60 to 100 nanometer).

Sections are stained with an aqueous solution of an appropriate heavy metal salt and examined with a transmission electron microscope (TEM). This instrument is often called an ultramicrotome. The ultramicrotome is also used with its glass knife or an industrial grade diamond knife to cut survey sections prior to thin sectioning. These sections are of 0.5 to 1 μm thickness and are mounted on a glass slide and stained to locate areas of interest under a light microscope prior to thin sectioning for the TEM. Thin sectioning for the TEM is often done with a gem quality diamond knife.

Botanical Microtomy Technique: Hard materials like wood, bone and leather require a sledge microtome. These microtomes have heavier blades and cannot cut as thin as a regular microtome.

Rotary Microtome - It is most commonly used microtome. This device operates with a staged rotary action such that the actual cutting is part of the rotary motion. In a rotary microtome, the knife is typically fixed in a horizontal position. A rotary action of the hand wheel actuate the cutting movement. Here the advantage over the rocking type is that it is heavier and there by more stable. Hard tissues can be cut without vibration. Serial sections or ribbons of sections can easily be obtained. The block holder or block (depends upon the type of cassette) is mounted on the steel carriage that moves up and down and is advanced by a micrometer screw.

Auto-cut microtome has built in motor drive with foot and hand control. With suitable accessories the machine can cut thin sections of paraffin wax blocks and 0.5 to 2.0 micrometer thin resin sections.

Advantages

1. The machine is heavy, so it is stable and does not vibrate during cutting.
2. Serial sections can be obtained.
3. Cutting angle and knife angle can be adjusted.
4. It may also be used for cutting celloidin embedded sections with the help of special holder to set the knife.

Sledge Microtome is a device where the sample is placed into a fixed holder (shuttle), the sledge placed upon a linear bearing, a design that allows for the microtome to readily cut many coarse sections. Applications for this design of microtome are of the preparation of large samples, such as those embedded in paraffin for biological preparations. Typical cut thickness achievable on a sledge microtome is between 10 and 60 micron.

Cryomicrotome

For the cutting of frozen samples, many rotary microtomes can be adapted to cut in a liquid nitrogen chamber, in a so-called cryomicrotome setup. The reduced temperature allows for the hardness of the sample to be increased, such as by undergoing a glass transition, which allows for the preparation of semi- thin samples. However the sample temperature and the knife temperature must be controlled in order to optimise the resultant sample thickness

Ultramicrotome

A ribbon of ultrathin sections prepared by room temperature ultramicrotomy, floating on water in the boat of a diamond knife used to cut the sections. The knife blade is the edge at the upper end of the trough of water.

An ultramicrotome is a main tool of ultramicrotomy. It can allow for the preparation of extremely thin sections, with the device functioning in the same manner as a rotational microtome, but with very tight tolerances on the mechanical construction. As a result of the careful mechanical construction, the linear thermal expansion of the mounting is used to provide very fine control of the thickness

These extremely thin cuts are important for use with transmission electron microscope (TEM) and Serial Block-Face Scanning Electron Microscopy (SBFSEM), and are sometimes also important for light-optical microscopy. The typical thickness of these cuts is between 40 and 100 nm for transmission electron microscopy and often between 30 and 50 nm for SBFSEM. Thicker sections up to 500 nm thick are also taken for specialized TEM applications or for light microscopy survey sections to select an area for the final thin sections. Diamond knives (preferably) and glass knives are used with ultramicrotomes. To collect the sections they are floated on top of a liquid as they are cut and are carefully picked up onto grids suitable for TEM specimen viewing. The thickness of the section can be estimated by the thin-film interference colors of reflected light that are seen as a result of the extremely low sample thickness.

Vibrating microtome

The vibrating microtome operates by cutting using a vibrating blade, allowing the resultant cut to be made with less pressure than would be required for a stationary blade. The vibrating microtome is usually used for difficult biological samples. The cut thickness is usually around 30-500 μm for live tissue and 10- 500 μm for fixed tissue.

Saw microtome

The saw microtome is especially for hard materials such as teeth or bones. The microtome of this type has a recessed rotating saw, which slices through the sample. The minimal cut thickness is approximately 30 μm , and can be made for comparatively large samples.

Laser microtome

A conceptual diagram of laser microtome operation. The laser microtome is an instrument for contact free slicing. Prior preparation of the sample through embedding, freezing or chemical fixation is not required, thereby minimizing the artifacts from preparation methods. Alternately this design of microtome can also be used for very hard materials, such as bones or teeth as well as some ceramics. Dependent upon the properties of the sample material, the thickness achievable is between 10 and 100 μm . The device operates using a cutting action of an infra-red laser. As the laser emits a radiation in the near infra-red, in this wavelength regime the laser can interact with biological materials. Through sharp focusing of the probe within the sample, a focal point of very high intensity, up to TW/cm^2 , can be achieved. Through the non-linear interaction of the optical penetration in the focal region a material separation in a process known as photo-disruption is introduced. By limiting the laser pulse durations to the femtoseconds range, the energy expended at the target region is precisely controlled, thereby limiting the interaction zone of the cut to under a micrometre. External to this zone the ultra- short beam application time introduces minimal to no thermal damage to the remainder of the sample.

The laser radiation is directed onto a fast scanning mirror based optical system which allows for three dimensional positioning of the beam crossover, whilst allowing for beam traversal to the desired region of interest. The combination of high power with a high raster rate allows the scanner to cut large areas of sample in a short time. In the laser microtome the laser-microdissection of internal areas in tissues, cellular structures, and other types of small features is also possible.

MICROTOME KNIFE

It is the important instrument used to cut uniform thin serial sections of the tissue. Various types of knives are used with different microtomes. For routine purpose wedge (C type) knife is used. It is plain on both sides. The size varies from 100 mm to 350 mm in length. Microtome knives are made of good quality of high carbon or steel which is tempered at the tip.

Hardness of knife is essential to obtain good tissue sections.

Sharpening of microtome knife - To achieve good sections knife should be very sharp. The knife is put in the knife back to sharpen. Knife can be sharpened manually or by the use of automatic machine.

Honing - This is done to remove nicks and irregularity from the knife edge. Coarse and fine honing is done using different abrasives.

Stropping - The purpose of stropping is to remove the “burr” formed during honing and to polish cutting edge.

Other types of knives are diamond and glass knives. These knives are very expensive and used for ultramicrotomy.

Disposable knife – Nowadays these microtome blades are used. Two types of disposable blades are available.

1. Low profile blade - Usually used for cutting small and soft biopsies like kidney and liver biopsies.

2. High profile blade-Used for any tissue like myometrium, breast tumor or skin.

Advantages

1. Time is not spent in sharpening, honing or stropping the knife.

2. Resistant to both corrosion and heat.

3. Hardness of blade can be compared with the steel knife.

Disadvantages

1. Relatively expensive

2. Disposable blades are not as rigid as steel knife:

Care of the Microtome Knife

Care of the Microtome Knife

- 1- Store the knife in its box, when not in use.
- 2- The knife should be cleaned with xylene before and after use.
- 3- When knife is being stored for a long time, it should be smeared with grease or good grade of light oil.
- 4- Knife edge should not be touched.
- 5- Knife edge should never be come badly nicked. It is advisable to use separate knife for cutting hard issue like bone.
- 6-The above points are important if re usable knife is being used.

Points to remember

- 1 For routine histopathology rotary microtome is used.
- 2 Ultramicrotome is used to cut semi-thin sections or ultrathin sections.
- 3 Traditional type of knife requires honing and stropping to smoothen the cutting edge.
- 4 Disposable knives are expensive but do not need honing or stropping.
- 5 Knife edge is spoiled if properly decalcified tissue is not used.