MEDICAL INSTRUMENTS-II

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Course Content

General study of following instruments:

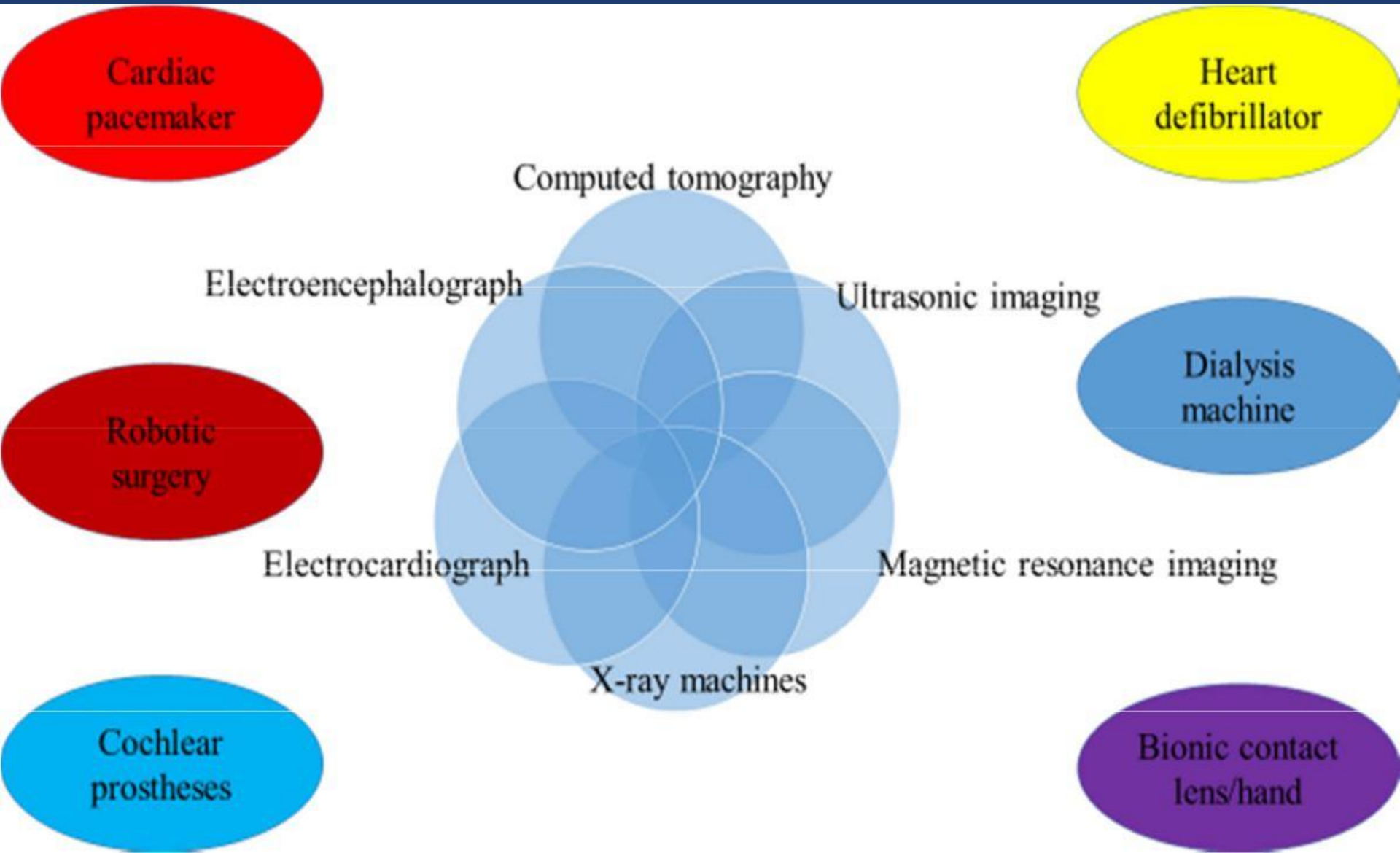
- Microtomes; parts, working, limitations & uses.
- ECG Machine.
- EEG Machine.
- Echo-Cardiography.
- Endoscopy Machine.
- X-Ray, MRI & Mammography Machines.
- Ultra Sound Machine.
- Anesthesia Machine.
- Dental Chair.
- Other specialised Machines used in fields of:
  - Surgery, Medicine, Gynae & Obstetrics, Eye, ENT, Paediatrics, Cardiology, Radiology, Orthopaedics, Neurosurgery, Urology and Dermatology etc.

PAPER PATTERNS & MARKS DISTRIBUTION OF UNIVERSITY EXAMINATIONS

TOTAL MARKS = 100 (having Theory Section only)

THEORY (100 marks)	
Question	**Marks for each Questions**
Question 01: MCQs (20 stems with 04 possible options only 01 correct)	01x20 = 20
Question 02: SEQs (08 stems Requiring short answer of all)	08x05 = 40
Question 03-05: LEQs (Requiring detailed answer of any 02 Qs)	02x15 = 30
Total Marks	**90**
INTERNAL ASSESSMENT (10 MARKS)	
Internal assessment Theory part	10
Total Marks	10
Grand Total Marks	**100**

Contributions of biomedical science, engineering and technology to equipment's and devices for clinical diagnosis, treatment, and rehabilitation practice



MEDICAL INSTRUMENTATION-II

MICROTOMY

Microtomy:

- Is the means by which tissue can be sectioned (cut) and attached to a surface (usually glass slide) for further microscopic examination.

Microtome:

- Basic instrument used in microtomy.
- Microtome is a mechanical device used for cutting biological specimens (biopsy) in to very thin uniform slices for microscopic study.
- First microtome invented by Hiss.

Microtome

- Machines that cut extremely thin sections from a sample for applications in histology or pathology
- Use special metal, glass or diamond blades, depending on the type of specimen and the desired thickness.
- Consists of a blade holding unit with a blade holder and clamps, an advancing mechanism, and a mechanism for adjusting section thickness.

Classes of microtomes

- There are 5 basic types of microtomes named according to the mechanism-
  - Rocking microtome
  - Rotary microtome
  - Base sledge microtome
  - Sliding microtome
  - Microtomes for frozen sections:
    - Freezing microtome.
    - Cryostat
  - Ultramicrotome

Rocking microtome

Rocking microtome:

- The Rocking microtome was invented in 1881 by Caldwell and Threlfall and improved on by Charles Darwin in 1985.
- Now it is manufactured by Cambridge and Baker.
- This microtome derives its name from the rocking action of the cross arm.
- Oldest in design, cheap , simple to use.
- Extremely reliable.
- Very minimum maintenance.

Mechanism of action:

- Knife is fixed, the block of the tissue moves through an arc to strike the knife.
- Between strokes the block is moved towards the knife for the required thickness of sections by means of a ratchet operated micrometer thread.
- Steady of the handle gives ribbons of good sections.

Rocking microtome:

Components

1. Knife clamps
2. Block holder
3. Tension adjustment
4. Microtome adjustment screw
5. Operating handle
6. Feed mechanism

Advantages:

1. Excellent for serial sectioning (60 to 90 sections ribbon)
2. Very small blocks can be cut
3. The instrument is cheap, reliable and easy to maintain

Disadvantages:

- Size of the block that can be cut is limited.
- Does not give flat sections because of the rocking movement sections are cut in a curved plane.
- Light weight, therefore, cutting hard tissues may give vibrations, thus it is advisable to fit it with screwed to the bench , to avoid movement during cutting.

Rotary Microtomes

Rotary microtome

- Most widely used, also called Minot microtome, after its inventor Professor.
- Very popular design all over the world.

Mechanism:

- The hand wheel rotates through 360 degree moving the specimen vertically pass the cutting surface (i.e knife edge) and returning it to the starting position.
- Block holder is mounted on a steel carriage which moves up and down in grooves and is advanced by a micrometer screw- cutting perfectly flat sections.

Rotary Micotome : Types

- Manually operated
- Semi-automated
- Fully automated

Rotary Micotome : Types

- **Manual:** Completely manipulated by the operator.
- **Semi-automated:** This auto cut microtome has a built-in motor derive with foot and hand control ; with suitable accessories, the machine can cut thin sections of 0.5 to 2.0 um thickness. (One motor to advance either the fine or coarse hand –wheel)
- **Fully automated:** Two motors that drive both the fine and the coarse advance hand-wheel. An example is automated cryostate.

Mechanism of block advancement:

- Retracting or
- non retracting.
- Retracting action moves the tissue block away from the knife on upstroke, producing a flat face to the tissue block.

Rotary Microtome parts

- The main components of a rotary microtome are:
  a. Base
  b. Cassette or Block holder
  c. Block adjustment screw
  d. Knife holder
  e. Blade clamps
  f. Angle of tilt adjustment
  g. Thickness gauge
  h. Operating handle
  i. Face plate
  j. Waste tray

Rotary Microtome parts

- **Microtome base plate or stage:** A platform which has rails that secure the knife holder base.
- **Knife holder base:** A part that anchors the knife holder to the microtome stage. The knife holder base can be moved toward or away from the block, but **MUST** be stationary and locked during microtomy.
- **Knife holder:** comprised of the:
  - **blade clamp** that holds the blade
  - **knife tilt** for adjusting the knife angle
  - **face plate** that guides the ribbons away from the blade and towards the operator.

Rotary Microtome parts

- **Cassette clamp or block holder:** Holds the paraffin block in place
  - moves up and down with each revolution while the blade is stationary
  - may have knobs that allow the user to manipulate the block face in various directions to bring the tissue in alignment with the blade.
- **Coarse hand wheel:** Moves the block holder either toward the knife or away from the knife.

Rotary Microtome parts

- **Advancement hand wheel:** Turns in one direction and advances the block toward the knife at the specified microns
  - Most hand wheels are equipped with a safety lock to prevent the wheel from releasing and having the block holder come down towards the blade while a block is inserted or removed. The safety lock should be used anytime the microtomist is not actively sectioning paraffin blocks.

Rotary Microtome parts

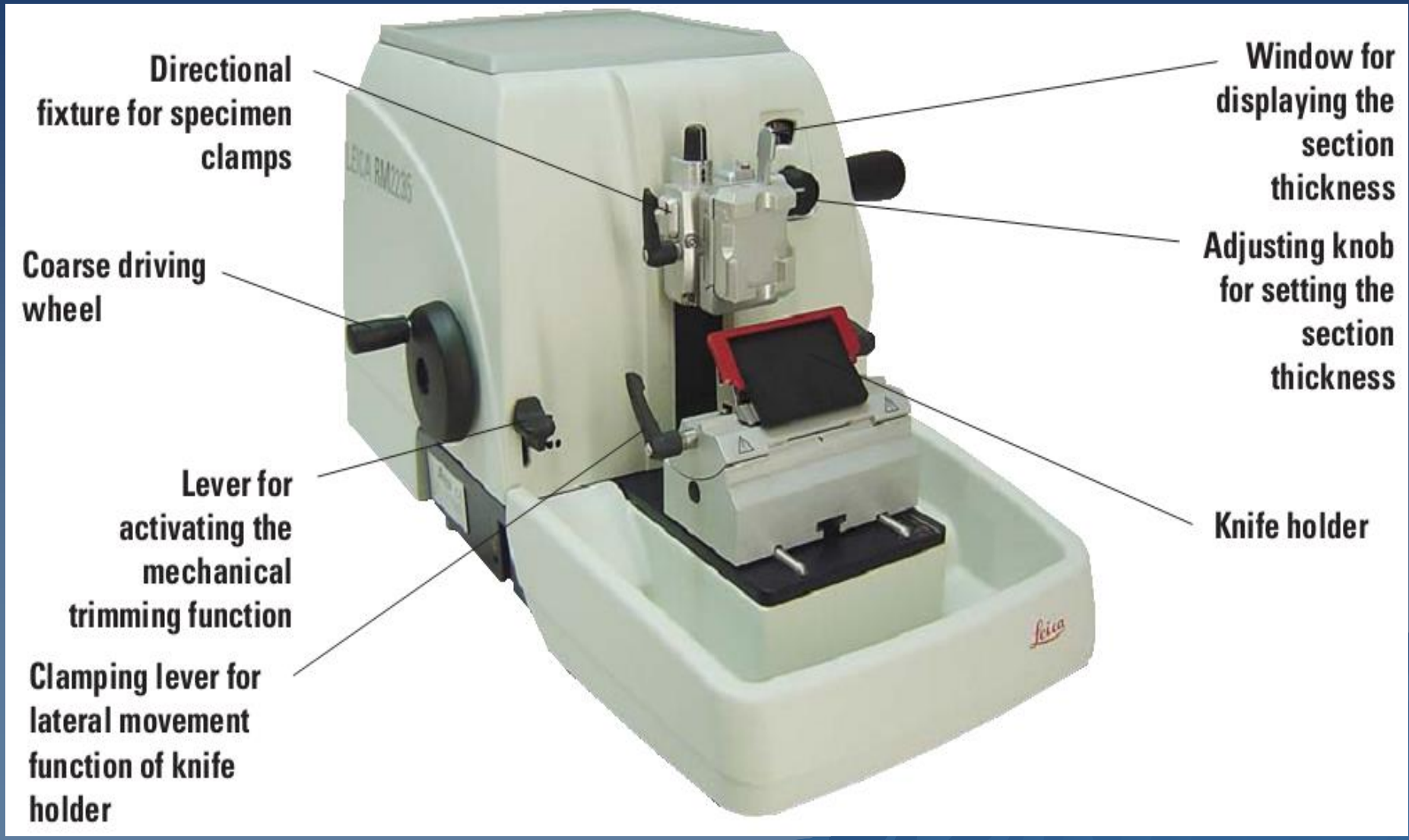
- **Micron adjustment:** Micron settings for section thickness can range from 1 to 60 microns on most microtomes.
- **Thickness gauge:**

  Micron settings for section thickness.

- **Base** on which whole microtome rests.

An Overview-Microtome Components

AN Overview-Microtome Components



Rotary Microtome: Operation

- 1. Fill the water bath with water and heat to 56˚C or just below the melting point of paraffin wax.
- To avoid microorganisms growth, the water bath should be carefully cleaned every day and the water in flotation bath should be discarded.
- 2. Put the paraffin blocks on a cold surface (e.g., refrigerated cold plate or ice cubes) to harden the cut surface. Avoid prolonged cooling and very cold surfaces as they may lead to cracking in the block surface.

Rotary Microtome: Operation

- **Fitting and Adjusting the Knife:**

3. Install a disposable blade in the microtome.
4. Set angle between the blade edge bevel and the block to 2–5 degrees (clearance angle). A correct angle should be set to avoid compression in cut sections and to reduce friction as the knife passes through the block. Angles in the above mentioned range are recommended for paraffin sections, but the exact angle is generally found by trial and error.
5. Lock the blade in place.
6. Lock the microtome hand-wheel.

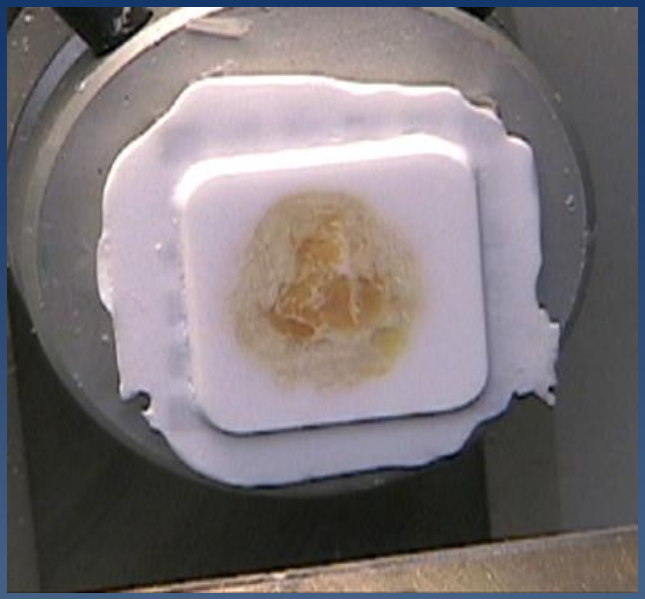
Rotary Microtome: Operation

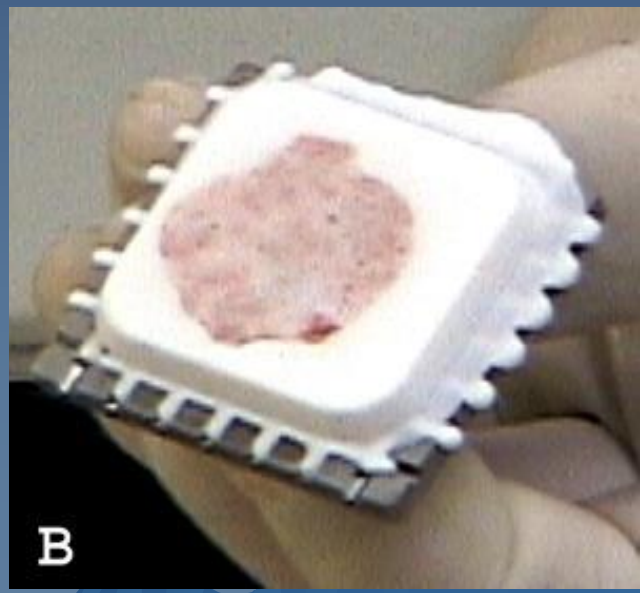
Trimming

- 7. Trim the edges of one block with a sharp razor blade so that the upper and lower edges of the block are parallel to the edges of the knife. Otherwise a ribbon cannot be cut. Keep 2–3 mm of paraffin wax around the tissue.
- 8. Fit the cassette paraffin block onto the cassette holder of the microtome. Orientate the block so that its greater axis is perpendicular to the edge of the knife, and also that the edge offers the least resistance (e.g., the smallest edge will be cut first).
- 9. Unlock the hand-wheel.

Rotary Microtome: Operation

- 10. Advance the block until it is in contact with the edge of the knife. Paraffin block edges must be parallel to the knife. If not, adjust the block orientation.
- 11. Set the section thickness around 15 microns.
- 12. Coarse cut the block at 15microns until the whole surface of the embedded tissue can be cut.
- 13. Lock the microtome hand-wheel.





Rotary Microtome: Operation

Sectioning

- 14. Return the trimmed block to cold plate for 1–2 min.
- 15. Set the section thickness to 4–5 um
- 16. Remove wax debris from the knife with alcohol. Avoid use of xylene to clean the paraffin debris as it often leaves an oily remnant on the knife and following sections will stick.
- 17. Move to an unused area on the blade or install a new disposable blade.

Rotary Microtome: Operation

- 18. Install the cassette paraffin block onto the cassette holder again.
- 19. Cut a series of paraffin sections. If sectioning is doing well, you will obtain a ribbon of serial sections.
- 20. Gently breath upon the sections to eliminate static electricity, to flatten the sections, and to facilitate the removal of the ribbon from the blade.
- 21. Separate the ribbon (including four to five sections) from the knife edge with a paint brush or forcep.

Floatation

- 22. Transfer the cut sections to the surface of the water bath.
- 23. Gently separate the floating sections on the water bath with pressure from the tips of forceps to remove fine wrinkles.
- 24. Collect sections on a clean glass slides. Hold the slide vertically beneath the section and lift carefully the slide up to enable tissue adherence.
- 25. Label slides with a histo-pen or pencil. Avoid pens with nonalcohol-resistant ink (ballpoint or felt-tipped pens).
- 26. Allow the slides to dry horizontally on a warm plate for 10 min to ensure that the section firmly adheres to the glass slide. Alternatively slides can be dried vertically in an oven for 20 min at 56°C.
- 28. Store the slides in dry boxes at room temperature. For immunohistochemistry, slides should be stored at 4°C to minimize antigen loss.

Advantages:

- Section thickness range: 1.0 - 60.0 µm
- Stable and less of vibration (Heavier and more stable)
- Excellent for routine and research work.
- Ability to cope with harder tissue.
- Easy adaptation to all types of tissues (hard, fragile, or fatty)
- Ideal for cutting serial sections
- Cutting large blocks
- Cutting angle of knife is adjustable.
- Large and heavier knife used-less vibration when cutting hard tissue.
- Technological advances in the automation improved the section quality, increased productivity and occupational safety.

Disadvantages:

- Complex design
- Initial cost is relatively higher
- Knife is placed in the blade holder up position and can be dangerous to the operator (to avoid this, knife guard/protector can be used)
- Not suitable for cutting large blocks

CARE OF THE ROTARY MICROTOME

- After cutting, brush away with soft brush: all accumulated paraffin and tissue
- Wipe clean all metal parts with xylol
- Avoid continuous application of xylol to the rest of the machine (can remove the paint)
- Dry the machine carefully especially the knife holder
- keep the machine well oiled to prevent rust formation
- keep the moving parts of microtome lubricated.
- Cover the microtome when not in use (prevent accumulation of dust and dirt which may interfere the sectioning)

Types of Microtomes

Type	Sample	Thickness	Application
Saw microtome	Hard and brittle material	> 30 microns.	hard materials (e.g. bone, teeth)
Sledge microtome	Embedded hard samples	1 – 60 microns	Bony Samples sliced , histo-enzymes study
Rotary microtome	Embedded samples	0.5 - 60 microns	Thin samples. Manual control
Vibrating microtome	Difficult/soft, Fresh/fixed samples	Fixed >10 microns Fresh > 30	Less pressure and sample disruption
Laser microtome	All samples	10-100 microns	No sample contact and no sample preparation
Cryo-microtomes	Frozen samples	upto 20 micron	Quick results
Ultra-microtomes		TEM 40-100nm SBFSEM 30-50nm	Extremely thin cuts for specialty microscopes

MEDICAL INSTRUMENTS-II

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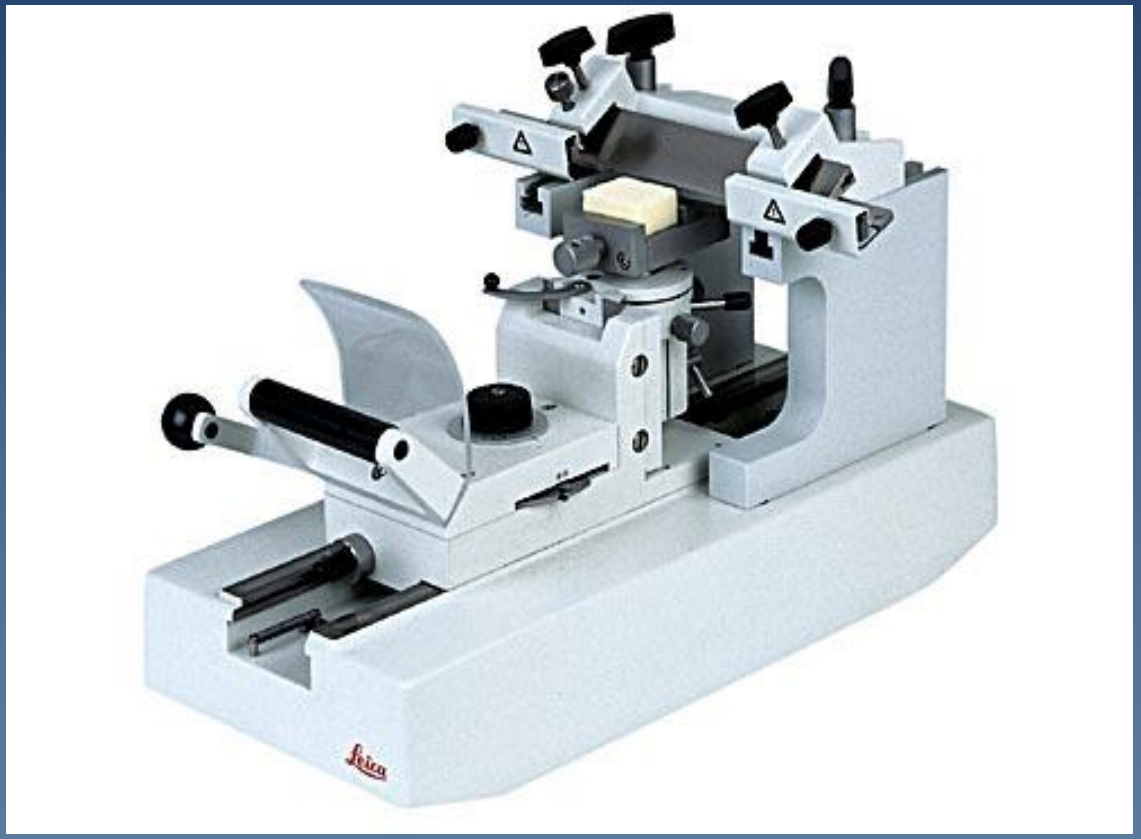
Problems in section cutting

Cause	remedy
A- When ribbon and consecutive sections are curved 1-leading and trailing edges of block not parallel 2- blade blunt in one area 3-Tissue varying in consistency	 1-trim with sharp scalpel until parallel 2-replace the blade 3-cool block with ice
B-Alternate sections thick and thin 1-Wax too soft for tissue 2- block or blade loose 3- insufficient clearance angle	 1-cool block with ice or re-embed in higher melting point wax 2-Tighten block and blade 3-Slightly increase clearance angle
C-Thick and thin zones parallel to knife edge(chatters) 1- Knife or block loose un holder 2- Excessive steep knife angle 3-tissue or wax too hard for sectioning 4-Calcified areas in tissue	 1- Tighten 2-reduce angle to minimum 3-use sharp heavy duty knife, use softening fluid on tissue 4-Rehydrate and decalcify, or surface decalcification

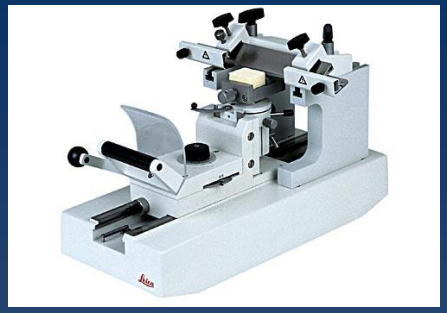
D- Splitting of sections at right angles to knife edge **1-Nick in blade edge** **2- Hard particles in tissue** **3-hard particles in wax**	**1-replace the blade** **2-if calcium decalcify if other remove with care.** **3- Re-embed in fresh filtered wax.**
E- sections will not join to form a ribbon 1- Wax too hard for sectioning conditions 2- Debris on blade edge 3- knife angle too steep or too shallow	1-warm or re-embed in lower melting point wax 2-clean with xylene moistened cloth. 3- Adjust to optimum angle.
F- sections become attached to block on return stroke 1-Insufficient clearance angle. 2- Wax debris on blade edge. 3-debris on block edge. 4- Static electrical charge on ribbon	1-increase clearance angle. 2- clean with xylene- moistened cloth. 3-trim edge with sharp scalpel. 4- humidify air with moist bath near the knife. Place bunsen burner near knife .

G. Areas of tissue in block not present in sections **1- incomplete impregnation of tissue.** **2- Wax block becoming detached.**	**1- return tissue to vacuum impregnating bath for a few hours or reprocess if fault I excessive.** **2- re-attach with hot spatula.**
H- Excessive compression of sections 1- Blunt blade 2- wax too soft for tissue or sectioning conditions	1- use different part or replace 2- cool block with ice or use higher melting-point wax.
I- Sections expand and disintegrate on water surface 1- Poor impregnation of tissue. 2- water temperature too high	1- return tissue to vacuum impregnating bath for a few hours 2- Cool
J- Sections roll into a tight coil instead of remaining flat on knife. 1- Blade blunt 2- section thickness too great for wax	1- Use different part or replace. 2-reduce section thickness or use slightly higher melting-point wax. Breathe on block as sections are being cut.

Sledge microtome

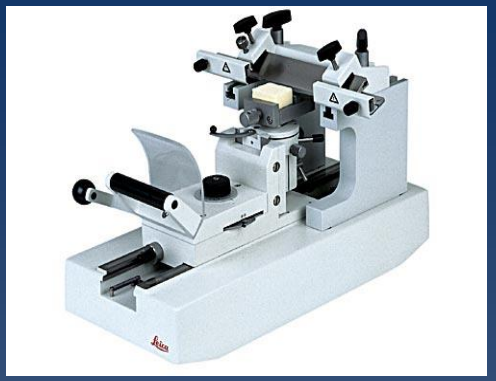


Sledge microtome



- Originally designed for cutting sections of very large blocks of tissue (eg. whole brains)
- Used primarily for large blocks, hard tissues, whole mounts.
- Especially useful in neuropathology and ophthalmic pathology.
- It is similar to a sliding microtome
- Usually a wedge-shaped knife is used.

Mechanism of action:



- The block holder is mounted on a steel carriage which slides backwards and forwards on guides against a fixed horizontal knife.

Components

1. Angle of tilt adjustment
2. Knife clamps
3. Block holder
4. Course feed adjustment
5. Operating handle
6. Thickness gauge
7. Adjustment locking nut
8. Block adjustment screw
9. Split nut clasp

Base Sledge microtome parts

- **Operating handle**
  - **handle** advances – the sledge, will move along the runners and will also advance the specimen, the selected amount (thickness).
- **Thickness control**
  - A knob, calibrated in microns, is used to select the thickness of sections to be cut from 1µm – 40µm, in 1µm steps. Always set the thickness by turning the knob anticlockwise. If the thickness has to be reduced then turn clockwise past the required thickness, then anticlockwise.

Base Sledge microtome parts

- **Coarse advance/trim control**
  - Quickly raises or lowers the specimen 450µm per turn.
- **Specimen holder**
  - Specimens are clamped in the holder fitted
  - can be raised and lowered or removed after releasing the clamp lever

- **Knife clamps**
  - Two screws, turned by a lever , are used to fix the knife in the knife clamp and the knife clamp in the knife block
  - The cutting angle of the knife is adjustable from 0-40º. Slew angles of the knife can be obtained by loosening the knife block clamp levers and the knife clamp levers and moving knife to slew angle required.
- **Knife guards**
  - Each knife guard can be moved to the central position to cover the working area of the knife, or to the outer position while section cutting.
  - The black knobs secure the guards in the chosen position.
- **Side pillars**
  - In normal use, these pillars slope inwards slightly. For large area sections they can be unbolted from the base, swapped left to right and refitted, so that they slop outwards, so providing more space.

OPERATION

- **Trimming the Specimen**
- Fit the specimen block securely into the appropriate block holder on the microtome to trim excess material from the surface of the specimen block:

a. First move the sledge towards the knife and adjust the specimen height using the coarse advance so that the specimen is just below the knife.

b. Set the thickness control to cut thick sections, eg: 20µm, and move the sledge back and forth repeatedly to trim the specimen.

c. Or set the thickness control to zero and alternately raise the specimen slightly using the coarse advance and move the sledge forward then backwards. This method is quicker but should only be used if the operator is experienced in the use of base sledge microtomes.

OPERATION

- **Fitting the Object Holder**
- Pull the sledge away from the knife holders. Secure the required object holder by placing it in the holder in the sledge slide. Set to correct height and lock by tightening clamp lever
- Note that the clamp lever has a ratchet with its movement limited to prevent the lever being left in positions where it could impact on other parts during sectioning.
- **Fitting and Adjusting the Knife**

a. Slacken the knife clamp levers just enough to let the knife slide into the knife clamps from the side.

OPERATION

b. Insert the knife, taking care to avoid touching the edge against the metal surfaces.

c. Check that the knife lies flat on the pad pieces in the knife clamps and that the heel of the knife is not caught up on the slot in the knife clamps.

d. Screw the knife clamp levers down just sufficiently to steady the knife in position.

e. Slacken the knife block clamp levers . Set the knife holders to the desired slew angle, ensure the knife clamps still cover the ends of the knife. Set the knife to the desired cutting angle and tighten the knife clamping levers.

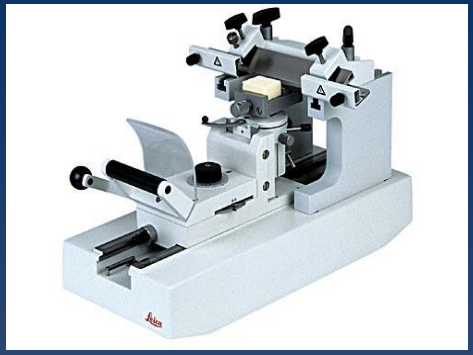
f. Check that the knife is still at the desired slew angles and tighten the knife block clamp levers.

Advantages:

- Heavy , very stable, not subject to vibration.
- Knife large(24 cm in length) and usually wedge shaped –less vibration .
- Adjustable knife holding clamps allow tilt and angle of the knife to the block to be easily set

  – used for cutting celloidin sections by setting the knife obliquely

  - paraffin wax embedded sections are more easily cut .



Disadvantages

- Slower in use than rocker or rotary microtome
- With practice, sections from routine paraffin blocks can be cut as quickly as on any other type of microtome.

Sliding microtome

- Designed for cutting celloidin-embedded tissue blocks.
- The knife or blade is stationary, specimen slides under it during sectioning.
- Also used for paraffin –wax embedded sections.

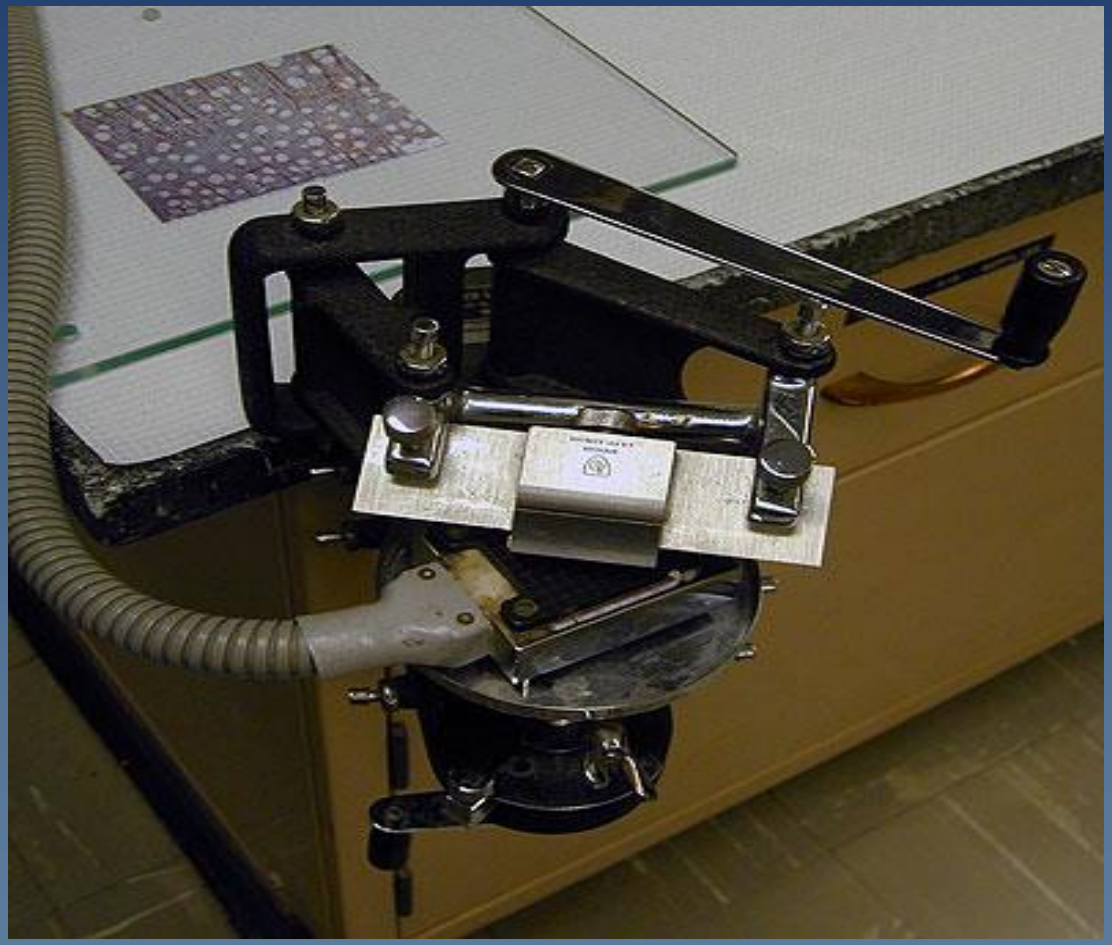
Freezing microtome

- Simple type of freezing microtome is clamped to the edge of a bench and is connected to a cylinder of carbon dioxide by means of a specially strengthened flexible metal tube.
- It consist of a radial arm attached to a central pivot. On this arm, two clamps hold a wedge profile microtome knife mounted with a simple block holder, with a cutting edge inclined in a horizontal plane.

Working principle

- The object is mounted on a block holder (chuck) known as freezing stage, with a centrally advancing screw. The block holder is perforated and attached to a feed pipe carrying carbon dioxide gas, which is sprayed on to the tissue for freezing. The knife moves over the block around a horizontal axis when once the tissue hardens.
- Thermoelectric cooling device units may be used in place of carbon dioxide gas to freeze the tissue and cool the knife. The cooling produced by thermoelectric units depends upon the flow of direct current, which may be regulated by means of power packs. In this stage, temperature can be reduced from ambient to -36C in 60 seconds, but the optimum cutting temperature for the tissue is usually about -20C.

Freezing microtome



Advantages:

- Freezing microtome is used in the demonstration of fat
- Ideal for brain sectioning.
- Simple design with no complex moving parts.
- Can be of diagnostic use when affordability of cryostat is not possible.
- Better demonstration of soluble and diffusible substances.
- Easy to operate and maintain.

Disadvantages:

- The sliding knife tends to jump on striking hard tissue.
- Difficult to sharpen the long knife.
- No serial sectioning possible.
- Sections less than 8 u cannot be cut under the best of conditions.

CRYOSTAT

- The first cryostat was introduced in 1959.
- (‘cryo’ meaning cold and ‘state’ meaning stationary)
- Cryostat is a refrigerated cabinet in which a microtome is housed. The microtome used is usually a rotary type, but may of sliding type or even rocking type and is rust proof. The microtome is mounted in a stainless steel cabinet at an angle of 45 degree. It has an antifogging air circulating system, a drain for defrosting and a shelf for four to six metal block holders. Cabinet temperature is -5 to -30 ^C. All microtome control operation are outside the cabinet.

  Harris International Microtome is most commonly used.

  This unit operates on the ‘open top-cold box’ principle.

Working principle

- To create a cold atmosphere around tissue block holder and microtome by means of a special refrigerator type compressor capable of taking temperature below -30 to -50 C. The reason for freezing the tissue is to provide a hardened matrix for sectioning the tissue and at the same time, preserving biochemical or immunological properties of a cell or tissue. The coolant used is usually Freon 22.

- **Advantages**
- Used extensively for rapid diagnosis, fat stains and enzyme histo-chemistry in neurological applications as well as in fluorescence microscopy.
- Both the knife and tissue are maintained at same low temperature.
- Capable of slicing sections as thin as 1 um
- Serial sectioning is possible.
- Automatic defrosting and sterilization
- Antifogging air circulatory system.

- **Disadvantages**
- Constant supervision and maintenance of temperature is required.
- The whole instrument should be kept in an air-conditioned room to prevent excessive cryostat compressor functioning.
- Lubricants of special type with a low congealing point have to be used. This prevents the lubricants solidifying at a cooler temperature within the chamber.
- Freeze artifacts seen as holes in the tissues.
- If the temperature is too low, the tissue become hard and crumbles, and becomes difficult to cut.
- Difficulty in sectioning fixed tissue.
- High cost of the instrument.

Difference between a Microtome and a Cryostat

- A Microtome is used to cut very thin sections at room temperature, on the other hand a Cryostat is used to cut frozen sections at sub zero temperatures (generally -30 deg C).

- A cryostat is used in situations where rapid analysis of tissues is required. The water rich tissue is frozen on a quick freezing shelf inside the cryostat, this makes it very hard and it is then ready to be cut into thin sections in a microtome, also placed inside the cryostat chamber.

- On the other hand to be cut in a simple microtome, the tissue needs to be first dehydrated and fixed in paraffin before it can be sectioned. It is a long procedure compared to quick sectioning in a cryostat.

- The quality of sections cut in a cryostat is inferior compared to those cut in a microtome because dehydrated and paraffin embedded tissues give better sections but when it comes to quick sectioning, Cryostat is the choice.

FIOW CHART


Tissue
Tissue Processing
Tissue embedded
Slice cut
Slice cut
Tissue
fiotation and dry
Cryostat section
HE Staining
Microscope
analyze

MEDICAL INSTRUMENTS-II

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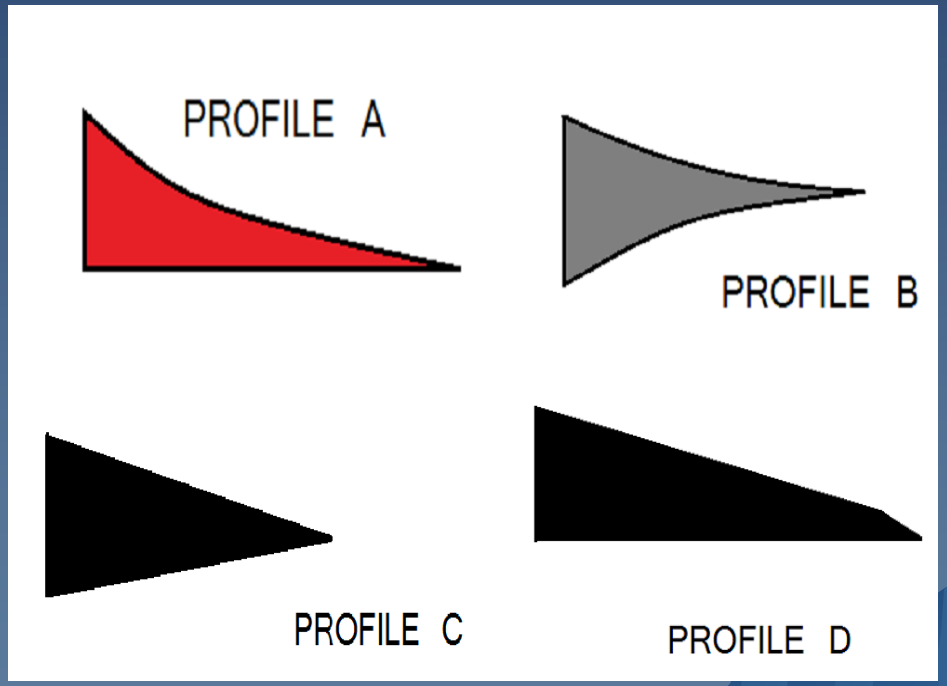
Microtome knives

- Developed to fit in specific types of microtomes and cope with different degrees of hardness of tissues and embedding media.
- Paraffin-wax embedded tissues knives are made of steel.
- Resin-embedded tissue is normally cut using glass knives.

Microtome knives: Classification

Knives are classified according to their shape when viewed in profile as:

- Plano-concave or A profile
- Biconcave or B profile
- Wedge or C profile
- Tool edge or D profile.



Wedge profile knife:

- Profile of this knife is wedge like, conical in shape and both surfaces are plane. Originally designed for cutting frozen sections in Freezing microtome and Cryostat but they can be used in Rotary microtome.

Advantages:

1. Gives great rigidity to the knife
2. Used for cutting all types of section on any microtome including paraffin section, celloidin section and Frozen section.
3. It can cut tough material.
4. Serial sections can be easily obtained.

Disadvantages:

1. A knife back is required when sharpening is done
2. Can not be used in rocking microtome because it give curly sections.

Plano-concave:

- It is plane on one side and concave on other side. Primarily used for cutting nitrocellulose –embedded tissues.
- Available with varying degrees of concavity.

Advantages:

1. Suitable for paraffin sections and celloidin sections.

Disadvantages:

1. Its edge is not ridge as wedge profile knife
2. Frozen section can not be cut by this type of knife.
3. For sharpening concave side of knife, a knife back is not required
4. For sharpening facets on plane surface knife back is required.

Biconcave knives:

- Concave on both sides
- Classical knife shape introduced by Heiffor.

Advantages:

1. Used with the rocking microtome.
2. Relatively easy to sharpen.
3. Section easily and smoothly run on its surface

Disadvantages:

1. Less rigid , prone to more vibrations and becomes blunt more easily.
2. Only paraffin section can be cut
3. With gradual adoption of more substantial microtomes , this knife design has lost popularity.

Tool edge(D-profile):

Called ‘chisel edge’, similar to a woodworker’s chisel. This knife is plane on both sides and has a steep cutting edge used primarily to section exceptionally hard tissue.

- Decalcified dense cortical bone.
- Un-decalcified bone.
- Stouter than conventional knives to give added rigidity.
- Edge may be coated with tungsten-carbide for increased life.

Disposable blades

- Used for routine microtomy and cryotomy.
- Provide a sharp cutting edge, produce flawless 2-4 mm sections.
- Disposable blade holders incorporated into the microtome.

Disposable blades

- Blade is coated with PTFE (polytetrafluoroethylene) allowing ribbons to be sectioned with ease.
- Over-tightening the disposable blade in the clamping device may cause cutting artifact such as thick and thin sections.

Glass and diamond knives

- Used in electron microscopy and with plastic resin-embedded blocks.

Knife angles

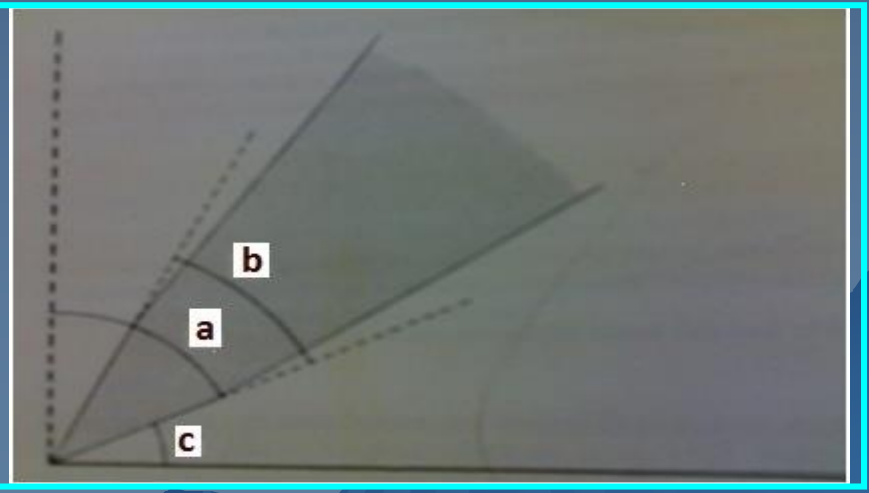
- Clearance angle: angle formed by a line drawn along the block surface and the lower bevel of the knife. To get good sections this angle should be proper not more than 20 to 50 degrees. This angle should neither too steep nor shallow.
- Rake angle: angle between the upper bevel of the knife and a line at 90 degrees to the block surface. For good sections this angle should be larger.

Angles associated with the knife edge.

a:rake angle

b:bevel

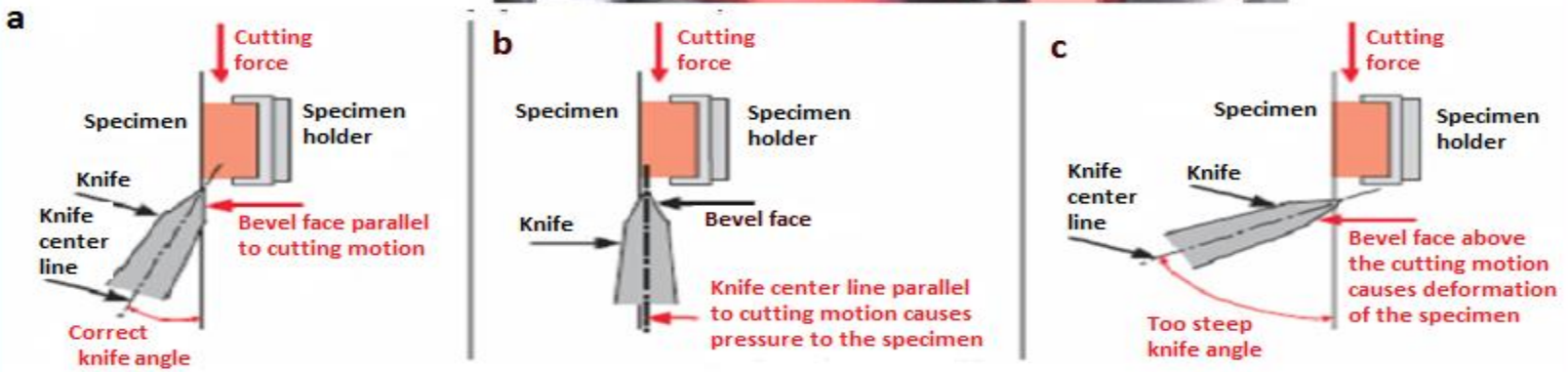
c:clearance angle.



Knife angles

- Bevel angle: angle of facets which meet to form the edge. It is usually from 27 to 32 degrees.
- Vary between 15 and 35 degrees.
- Longer facets and smaller bevel would give rise to a keener edge.
- Sharpness or acuity: reflection of light by the knife edge when viewed under the microscope.

Knife Angles in Microtomy



a: the correct knife angle

b: too shallow

c: too steep

Microtome knife sharpening

- Manual procedure or automatic procedure.

- 1) Abrasive grinding of the facets (HONING)
- 2) Polishing (STROPPING)

Abrasive grinding of the facets [HONING]

- Naturally occuring slabs of stone with varying abrasive properties:
- Stones : belgian black and arkansas, Aloxite and carborundum-composites.
- Lubricated with soapy water or light oil during use.

Abrasive grinding of the facets [HONING]

Glass plates:

- hand sharpening
- Readily available ,cheap
- Surface roughened to enable particles of abrasive to adhere to the glass .
- Easily cleaned after use.

Copper and bronze plates: automatic knife sharpening machines.

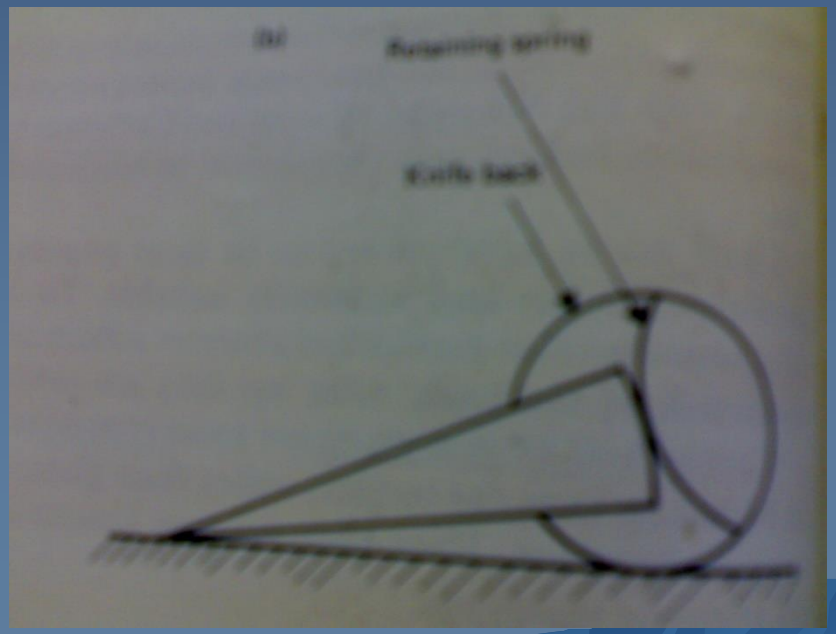
- Expensive , superior properties

Abrasives

- Aluminium oxide(alumina)
- Iron oxide(Jeweller’s rouge)
- Silicon carbide
- Diamond

Manual method

- Knife with a ‘back’ effectively raises the non cutting edge up off the hone.
- Back of the knife is ground simultaneously with the edge , hence reserved for use only with that particular knife.



Manual method

- Hone is placed on the bench on a non-skid surface (damp cloth) to prevent moving during honing.
- Small quantity of light oil or soapy water applied to the hone and smeared over the surface.
- Abrasive is applied to the glass or metal plate.
- Knife with handle and backing sheath is laid on the hone with cutting edge facing away from the operator , heel roughly in the centre of the nearest end of the hone.

Manual method

- Handle of the knife is held between the thumb and the forefinger .
- Thumb and forefinger of other hand rest on the other end of the knife to ensure even pressure along the whole edge of the knife.
- Knife is pushed forward diagonally from heel to toe ,turned over on its back and moved across the hone until the heel is in the centre with the cutting edge leading and then brought back diagonally.
- It is then turned over on its back and moved across the hone to its original position completing figure of eight movement.

Stropping

- Process of polishing an already fairly sharp edge.
- Types of strop: best strops made from hide from the rump of the horse marked ‘shell horse’.
- 2 types: flexible(hanging) and rigid.
- **Flexible type**:
- Back of the strop is made of canvas and is intended to support the leather during stropping.
- Strops should be kept soft by applying a small quantity of vegetable oil into the back of the leather

- Stropers should be kept free from grit and dust.
- **Rigid type**:
- Single leather strop stretched over a wooden frame to give a standard tension or a block of wood about 12x2x2 inches in size having a handle at one end with four grades of leather or even a soft stone cemented on each side.
- The sides of these strops are numbered and the knife is stropped on No1, then No2 and so on finishing on the finest leather.

Automatic knife sharpners

Automatic knife sharpners

- Two basic designs available.

1) knife is held vertically with revolving sharpening wheels grinding the cutting edge.

2) knife is held horizontally against the surface of a slowly rotating flat plate.

Automatic knife sharpners

- Plates – glass , copper or bronze charged with an abrasive.
- Glass plates need to be roughened before use to allow the abrasive particles to be held more easily in place.
- Copper and bronze plates used in conjunction with diamond paste, 6micrometer particle size being most appropriate for rough sharpening, and 1 micrometer for fine polishing.

Stropping Technique:

- Knife is laid on the near end of the strop with the cutting edge towards the operator (opposite direction to that used in honing.)
- Knife held with forefinger and thumb to facilitate easy rotation at end of each stroke.
- Action is exact opposite to that used in honing,using full length of the strop and stropping evenly the whole of the blade.


Thank You!

Microtomy- paraffin wax

- Factors involved in producing good paraffin-wax sections :

Temperature:

- Tissues are more easily sectioned at a lower temperature than that of the atmosphere.
- Lowering temperature brings tissues of differing composition to a more uniform consistency,degree of hardness-ensures a uniform cutting process.
- Blocks are cooled by keeping , face down on ice-tray (2-3min).

Knife angle

- Greater the rake angle(flatter the knife)more likely is a smooth plastic flow type cutting action.
- Higher rake angles are more suitable to softer tissues
- Lower rake angles for harder tissues.

Speed of cutting

- Soft tissues are cut more easily at a slow speed.
- Hard tissues are cut easily at a little fast rate.
- If sections are cut at too fast speed, compression will become more marked.
- If cut too slowly, difficult to maintain the rhythmic action required.

Slant

- Commonly used to refer to the relationship of the knife edge to the block when cutting nitrocellulose-embedded tissue on a sliding microtome.
- Advantages: larger area of the edge is employed.
- Resistance to cutting force is applied more gently.

Paraffin section cutting

- Equipment required:
- Microtome.
- Flotation(water bath)
- Slide drying oven or hot plate
- Fine pointed or curved forceps.
- Sable or camel haired brush.
- Scalpel.
- Slide rack.
- Clean slides.
- Teasing needle.
- Ice tray.
- Chemical resistant pencil or pen.

Cutting technique

- Insert apprropriate knife in the knife-holder of the microtome and screw it tightly in position.
- Correctly set the adjustable knife angles.
- Fix the block in the block holder of the microtome
- Move the block holder forward or upward until the paraffin wax is almost touching the knife edge.
- Ensure that the whole surface of the block will move parallel to the edge of the knife,

Cutting technique

- Trim the excess wax from the block surface and expose the tissue, advance the block by setting the thickness to about 15 micrometer.
- Care should be taken not to trim too coarsely as
  A. Small biopsies may be lost.
  B. Tissue in the block may be torn giving rise to considerable artifact.
  C. Unsuspected small foci of calcification may cause tears in the tissue and nicks in the knife.

- Once the surface of tissue has been revealed proceed to trim the next block.
- Replace the trimming edge by a sharp one and check it is tightly secured.
- Reset the thickness gauge to 4-5 micrometer.
- Insert the block to be cut and tighten securely.
- Bring the block face up until it nearly touches the knife edge.

- Paraffin-wax embedded tissue , sections are normally cut at a thickness of 4-5 micrometer.
- Thicker sections(10-20 micrometer): demonstrate certain features of the central
- nervous system.
- Thin sections(1-2 micrometer): for examining highly cellular tissue such as lymph nodes.
- The amount of advance is operator determined most commonly in graduated 1 micrometer stages.

- Paraffin wax embedded tissue: the properties of the wax causes each section to adhere by its edge to the previous forming a ribbon of sections Ribbons should be held gently with a fine moistened brush or with a pair of fine forceps.
- Holding the ribbons with the finger is to be discouraged : section and water bath may become contaminated with the operator’s exfoliated squames.
- Before being attached to the slides the creases must be removed and the sections flattened.
- This is achieved by floating them on warm water.

Section Cutting and Collection

Rotary microtome

Flotation (water bath)

Flotation(water bath)

- Thermostatically controlled water baths for floating out tissue ribbons after sectioning.
- To remove the creases and flatten the sections.
- Temperature of water in the bath should be 10 degree celcius below the melting point of paraffin employed.
- Distilled water may be used to prevent water bubbles from being trapped under the sections.
- Alcohol or a small drop of detergent may be added to the water to reduce the surface tension-to flatten out the sections with ease.

Flotation(water bath)

- Sections which are curled will flatten on warm water, creases removed.
- To remove air bubble, thick sections of wax which curl into a roll during trimming are used. Hold one roll in the end of a pair of forceps and bring the end of the wax roll up under the section to touch the air bubble.The bubble will adhere to the wax roll and come away with it when removed.

Mounting the section on a slide:

- A clean slide is half submerged in water and brought into contact with the edge of the section.
- Section approached from the side, straight approach will push the section away.
- Section oriented on wet slide using the edge of the forceps or dissecting needle.
- Section should be centrally positioned on the slide.
- Slide should be identified by inscribing the appropriate no.on the slide with a diamond pencil.

Drying oven or Hot plate

Drying oven or hot plate

- Drying oven :
- Mounted section placed in an oven at 50degree celcius for 1 hour to dry.
- Hot plates:
- Slide complete with section may be transferred directly to the surface of the hot plate maintained at a temperature of 55-60 degree celcius.and left for 15 min.
- Section left face up until water evaporates then turned over to prevent dust settling.
- Small creases disappear as the section warms up.

Brush and forceps

- Forceps, brushes or teasing needles for removal of folds, creases and bubbles that may form during the floating out of the section on water bath.
- Manipulating the section as it passes acrosss the edge of the blade.

Slides

- For normal routine work, 76x25 mm slides universally used.
- Thickness :1-1.2mm,do not break as easily.
- Larger slides for tissues such as eyes or brain.
- Chemical resistant pens and pencils routinely used to label the slide.

Section adhesives

Sections may detach from the slides:

- Exposure to strong alkali solutions during staining.
- Cryostat sections for immunofluorescense, immunohistochemistry ,or intraoperative diagnosis.
- Central nervous system tissues.
- Decalcified tissues.
- Tissues containing blood and mucus.
- Sections submitted to extreme temperatures.

- For sections from ester or polyester –wax embedded tissue , adhesives are mandatory.

Albumen

- Equal parts of glycerin,distilled water and egg white are mixed filtered through coarse filter paper.
- A crystal of thymol is added to inhibit the growth of moulds, solution kept in refrigerator.
- Small quantity of the solution is smeared over the surface of the slide immediately before mounting sections from the water bath.

Gelatin :

- May be used as a 0.5% solution in distilled water.
- Liable to be contaminated with moulds ,needs to be melted with gentle heat before use.

Araldite:

- Clean slides are coated with 1 in 10 dilution of the resin in acetone immediately before use.
- As section dries ,resin polymerizes forming a rigid bond between tissue and slide.

Starch:

- Successful adhesive .
- Lost popularity due to staining reactivity with many dyes.

Poly-L-lysine :

- As 0.1% solution, diluted further for use 1 in 10 with distilled water.
- Effectiveness diminishes in few days.

3-aminopropyltriethoxysilane(APES):

- Slides dipped in 2% solution of APES in acetone,drained,dipped in acetone, drained again.
- The process is complete when the slides are dipped in distilled water.
- Useful for cytology,for specimens that may be bloody or contain proteinaceous material.

Charged or plus slides: slides manufactured with a permanent positive charge.

- Coating slide with basic polymer in which a chemical reaction occurs leaving the amino groups linked by covalent bonds to silicon atoms of the glass.
- Superior in their resistance to cell and tissue loss during staining.

Tissues cut reasonably well if

- Properly fixed
- Decalcified if necessary.
- Completely dehydrated.
- Cleared and impregnated with paraffin wax.
- Sharp knife is rigidly held in a properly adjusted microtome.

Cutting dificult tissue

- Alternate sections being thick and thin.
- Only part of the tissue being cut.
- Sections extremely compressed.
- Divided into two groups:
- 1) tissue exceptionally hard and tough.
- 2) fragmentation of tissue occurs as it is cut.

Cutting dificult tissue

Hard tissues

- Decreasing the rake angle.
- Resharpening the knife.
- Softening agents:
  solution of 4 % phenol,
- Mollifex(British drug houses Ltd)soak the block for 30-60 minutes

- Fragmentation of tissue:
- Blood clots and lymphoid tissues :
  increasing the rake angle.
  coating the block with celloidin by a camel hair brush in between the sections.

Serial sections

Necessary to cut and preserve every section from a piece of tissue or from a specific area .

Required:

- To identify small ulcer
- Presence of malignant cells tracking along a lymphatic or neural sheath.
- Scarce organisms such as acid fast bacilli.
- In embryology.

Problems and solutions for paraffin section.

Problem:
Ribbon/consecutive sections curved.

1) Block edges not parallel
2) Dull blade edge.
3) Excessive paraffin.
4) Tissue varying in consistency

Solutions:

1) Trim block until parallel.
2) Replace balde.
3) Trim away excess paraffin.
4) Re-orient block

Problem: Thick and thin sections

1) Paraffin too soft for tissue
2) Insufficient clearance angle
3) Faulty microtome mechanisms
4) Blade or block loose in holder.

Solutions

1) Remove excess paraffin
2) Increase clearance angle.
3) Check for faults in microtome.
4) Tighten block and blade

Problem:

Sections will not form ribbons

1) Paraffin too hard for sectioning.
2) Debris on knife edge.
3) Clearance angle incorrect.

Solutions:

1) Re-embed in lower melting point paraffin.
2) Warm surface of block
3) Clean blade and back of blade holder
4) Adjust to optimal angle.

Problem:

sections attach to block on return stroke

1) Insufficient clearance angle.
2) Debris on blade edge.
3) Debris on block edge.
4) Static electricity on ribbon.

Solutions

1) Increase clearance angle.
2) Clean blade edge.
3) Trim edges of block
4) Humidify the air around the microtome.
5) Place static guard or dryer sheets near microtome.

Problem:
incomplete section

1) Incomplete impregnation of tissue with paraffin.
2) Tissue incorrectly embedded.
3) Sections superficially cut.

Solutions

1) Re-process tissue block.
2) Re-embed tissue;make sure orientation is correct and tissue is flat in mould.
3) Re-face block,cut deeper into the tissue.

Problem:
chatter-thick and thin zones parallel to blade edge

1) Knife or block loose in holder
2) Excessive knife tilt
3) Paraffin too hard for sectioning.
4) Calcified areas in tissue.
5) Overhydration of tissue.
6) Dull blade.

Solutions

1) Clean blade edge to remove excess paraffin.
2) Replace or use new area of blade.
3) Tighten the blade levers.
4) Reduce angle.
5) Rehydrate .
6) Re-embed in fresh paraffin.

Problem:

Splitting of sections at right angles to knife edge

1) Nicks in blade.
2) Hard particles in tissue.
3) Hard particles in paraffin.

Solutions

1) Use different part of blade or replace.
2) Calcium deposit-surface decal.
3) Mineral or other particle-remove with fine sharp pointed forceps

Trouble shooting

Problem	Possible cause	Corrective action
Possible faults		
1. Thick/thin sections The sections alternate between being thick and thin. In extreme cases, there are no sections whatsoever.	• The blade is not clamped properly.	• Reclamp the blade.
	• The blade is dull.	• Laterally displace the knife holder or insert a new blade.
	• The pressure plate is damaged or incorrectly adjusted.	• Insert a new pressure plate or use a new knife holder. • Readjust the pressure plate.
	• Clearance angle of the knife/blade too small.	• Methodically experiment with larger clearance angle settings until you have found the optimum angle.
2. Section compression The sections are very compressed, show folds or are squeezed together.	• The blade is dull.	• Use another area of the blade or a new blade.
	• The specimen is too warm.	• Cool the specimen before sectioning.
	• The sectioning speed is too fast.	• Reduce the sectioning speed.
3. "Stripes" in sections For knife holder	• There is an accumulation of paraffin on the rear pressure plate of the knife holder.	• Remove paraffin from this area on a regular basis.

Trouble shooting

4. Noises during sectioning The knife "sings" when sectioning hard specimens. The sections show scratches or chatter marks.	• The sectioning speed is too fast.	• Turn the handwheel at a slower speed.
	• The clearance angle is too wide.	• Methodically reduce the clearance angle setting until you have found the optimum angle.
	• Insufficient clamping of object and/ or knife holder.	• Check all screw and clamp connections on the object holder system and the knife holder. If necessary, tighten the levers and screws.
Instrument malfunctions		
1 There is no further feed motion and thus no sectioning.	• The front end position has been reached.	• Run the specimen back by turning the coarse driving wheel.
2. High blade consumption	• Too great of a sectioning force was applied.	• Adjust the sectioning speed and/or section thickness when trimming. Select a smaller section thickness, rotate the handwheel more slowly.

MAINTENANCE

- **Cleaning**
  - Wipe off sectioning debris etc with a paper towel. Alcohols (industrial methylated spirits, ethanol or methanol) may be used. Xylene may be used sparingly to remove built-up wax, but ensure surplus Xylene is wiped off.
  - Avoid harsh solvents such as acetone
- **Lubrication**
  - After cleaning, oil both the sliding surfaces of the sledge base with Microtome Sledge Oil. Now move the sledge several times over the full length of travel to distribute oil

MAINTENANCE

- **Decontamination**
  - It is the responsibility of the user to ensure that a decontamination procedure is employed which is appropriate to the nature of the work carried out.

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THANK YOU