MEDICAL INSTRUMENTS-II

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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

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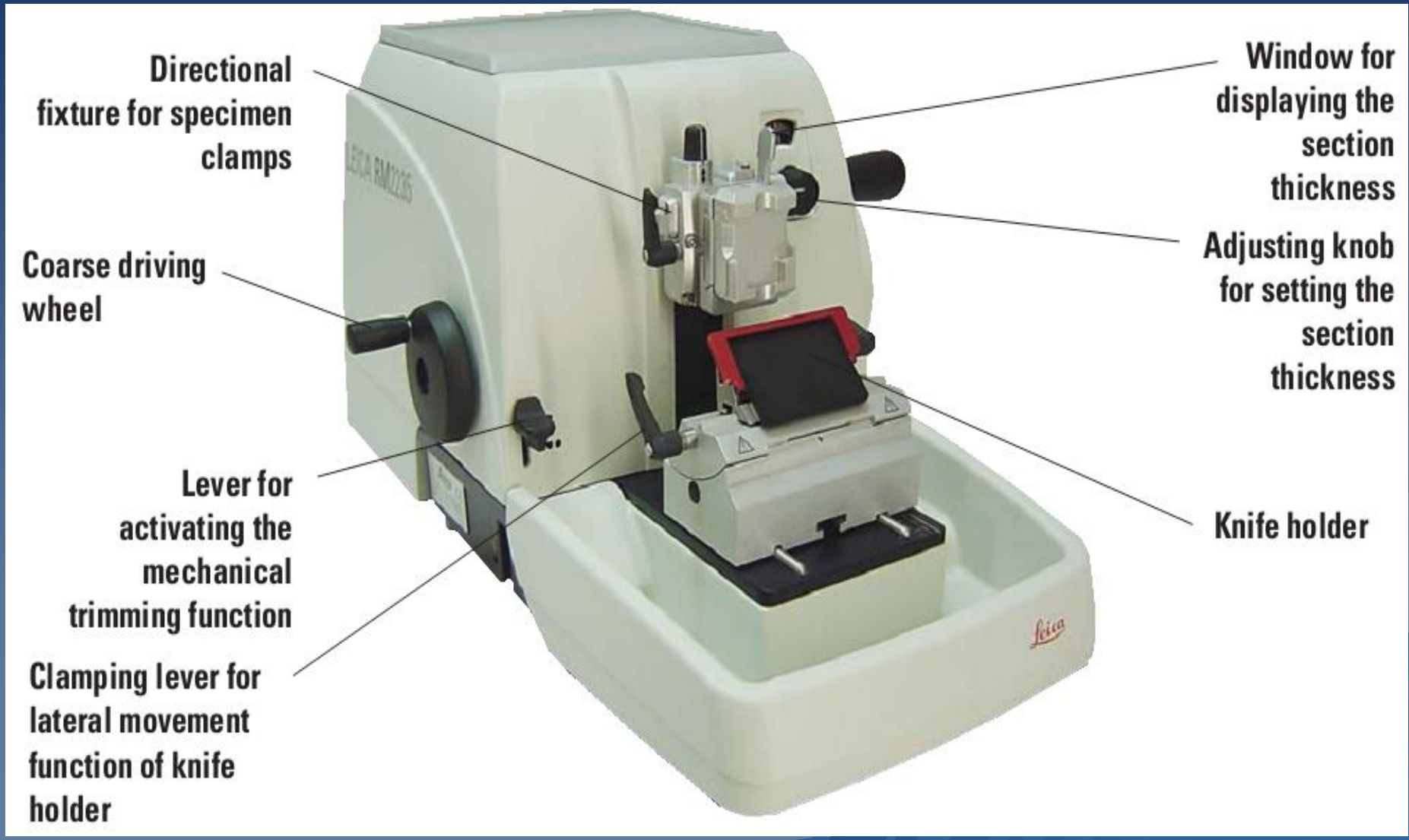


Rotary Microtome: Operation

- 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.

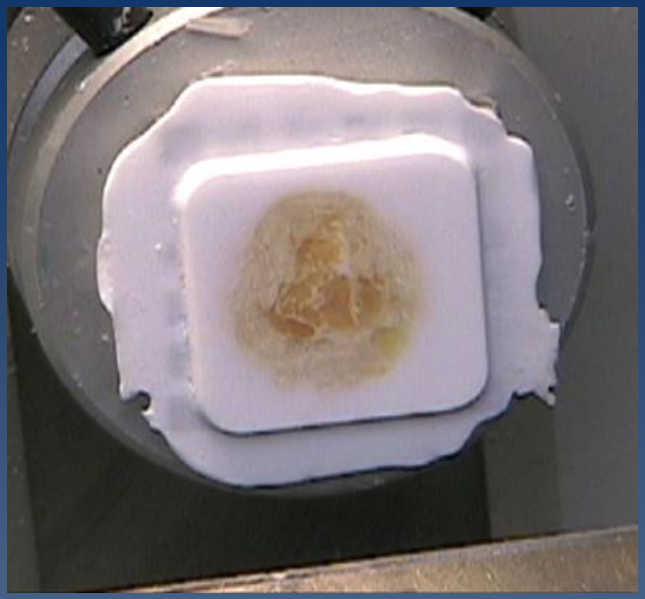
Rotary Microtome: Operation

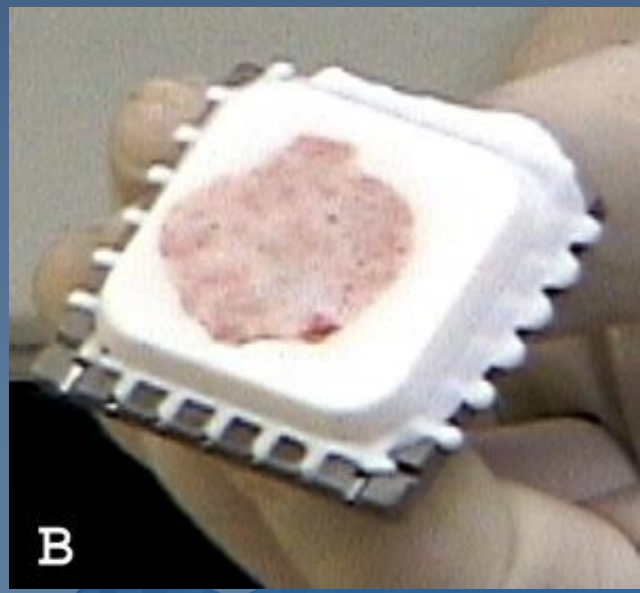
Trimming

- 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