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Microtome- A Review

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ABSTRACT

Microtomy is a fundamental technique in histological and pathological practices, enabling the preparation of uniformly thin tissue sections for microscopic examination. This review provides a comprehensive analysis of microtomes and microtome knives, emphasizing their structural components, classification, operational principles, and applications in various domains of biomedical science. Microtomes are broadly categorized into manual and automated types, each suited for specific sectioning requirements such as routine histology, cryosectioning, electron microscopy, and hard tissue analysis. The distinctions among rocking, rotary, sledge, sliding, vibrating, and cryostat microtomes, as well as innovations like laser and computerized systems, are discussed in detail.

Equally critical to the microtomy process is the selection and maintenance of appropriate cutting tools. Microtome knives, constructed from materials such as high-carbon steel, tungsten carbide, glass, diamond, and sapphire, vary in sharpness, durability, and application. Their edge profiles ranging from plano-concave to wedge-shaped are tailored for different embedding media and tissue consistencies. Proper sharpening techniques, including honing and stropping, along with careful handling and storage, are essential to ensure section quality and prolong knife lifespan.

This review highlights the interplay between equipment design, material science, and technical precision in microtomy, underscoring its indispensable role in achieving reliable histopathological and research outcomes.

KEYWORDS: Microtomy, Microtome, Histological Techniques, Tissue Sectioning, Microtome Knives, Cryostat, Rotary Microtome

INTRODUCTION

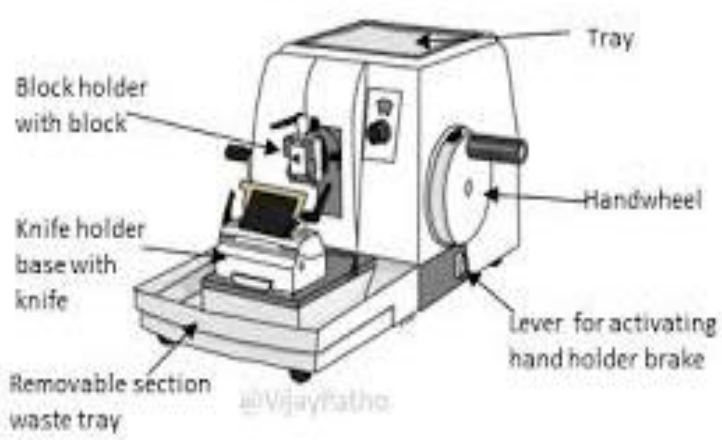
Microtomy- procedure in which the tissue is sectioned and attached to the surface of a glass slide for microscopic examination. Microtome- Greek8 mikros, “small”, and tome, “to cut”, tool used to cut extremely thin slices of sections.Chevalier,1839- introduced the term “microtome” Rudolf Thoma along with Rudolf Jung designed first microtome called “Thoma microtome”, beginning of a new era in histology. Most commonly used- rotary microtome.

APPLICATIONS OF MICROTOMES

- Traditional Histology Technique: tissues are cut at thicknesses varying from 2 to 50 µm.
- Cryosectioning Technique: faster than traditional histology (15 minutes vs 16 hours); for quick diagnosis.
- Electron Microscopy Technique: glass or gem grade diamond knife; thin sections (60 to 100 nanometer); examined with transmission electron microscope (TEM) and ultramicrotome used.
- Botanical Microtomy Technique: Hard materials- wood, bone and leather; sledge microtome used; heavier blades and cannot cut as thin as a regular microtome.

PARTS OF A MICROTOME

- Base (microtome body)
- Knife attachment and knife
- Material or tissue holder



CLASSIFICATION OF MICROTOMES

Manual microtome	Automatic microtome
Rocking microtome	Laser microtome
Rotary microtome	Computer microtome
Sledge microtome	Ultra-thin computer microtome
Freezing microtome	
Vibrating microtome	
Ultra microtome	
Cryostat	
Sliding microtome	
Saw microtome	

ROCKING MICROTOME

Oldest in design, cheap for sectioning paraffin blocks. Rocking action of cross arm and simple to use. Three moving parts, minimum maintenance

The tissue moves through an arc as it advances towards the knife held rigidly causing the sections to be cut in a curved plane

Disadvantage- thin sections difficult to obtain. Replaced by rotary microtome but nowadays used in portable cryostats

ROTARY MICROTOME

Used for cutting semi-thin to thin sections for light microscopy. Rotary action of hand wheel activating the advancement of a block toward knife.

The block moves up and down in a vertical plane in relation to the knife and cuts flat sections.

Advantage- more stable than rocking type, and ideal for cutting serial sections. Larger blocks of tissue is cut and cutting angle of the knife (tilt of knife) is adjustable. Heavier and larger knife- vibration can occur.

Sections of paraffin wax embedded tissues- 3 to 5µm; resin sections- 0.5 to 1µm

SLEDGE MICROTOME

Very large blocks of tissue (e.g. whole brain). The block-holder mounted on a steel carriage which slides backwards and forwards on guides against a fixed horizontal knife. Heavy and very stable and not subjected to vibration. Large knife(24 cm in length), wedge-shaped reduces possibility of vibration and requires less honing. The knife-holding clamps are adjustable

Disadvantage- very slow

SLIDING MICROTOME

Knife moved horizontally against a fixed block which progresses against it in an inclined plane. Used for paraffin-wax embedded and celloidin embedded sections

FREEZING MICROTOME

Frozen section. The machine clamped to edge of a bench and connected to cylinder of CO_2 by specially strengthened flexible metal tube. Knife is moved and tissue block remains static. The block moves by a pre-set amount, in microns, at the end of each cut. High quality, thin sections difficult to obtain. Tissue frozen without solid carbon dioxide or liquid nitrogen. Popular before cryostat. Two dissimilar metals are placed in apposition with one another and when a direct electric current passes through them, heat is generated on one surface and lost from the other- ‘Peltier’ effect

VIBRATING MICROTOME

Produces high quality sections of fresh, unfixed material from animal or botanical sources. Used to cut tissue which has not been fixed, processed or frozen. Enzyme histochemistry and ultrastructural histochemistry.
High speed vibration produced by razor blade. The amplitude of vibration adjusted by altering electrical voltage applied to the 'knife'. To prevent tearing, soft material is cut immersed in fluid and aids in dissipating heat.

ULTRA MICROTOME

Ultra-thin sections for light and electron microscopy. Tissue embedded in hard resin before cutting. Sections cut as thin as 10 nanometers.
Two mechanism: Thermal- heat induced expansion in a bifurcated metal strip. Mechanical- microprocessor coupled to a precise stepping motor controls the advance mechanism. Cutting stroke- motor driven provides regular, smooth motion for sections of even thickness and constant reproducibility. Knives- glass, diamond or sapphire. The block is brought to knife edge under microscopical control.

SAW MICROTOME

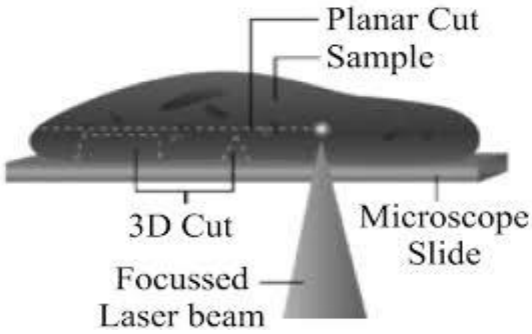
Cut sections from very hard material- undecalcified bone, glass or ceramics. Embedded in resins, moved extremely slowly against diamond coated saw rotating at approximately 600 rpm. Sections- 20 µm or greater
Does not produce very thin sections

CRYOSTAT

Microtome of any type but rustproof, enclosed and operated within a deep freeze cabinet. Cabinet fitted with a double glass window and door
Fluorescent light and fan for circulation of cool air. Temperature -10ºC to -40ºC. Rapid sectioning by incorporating an internal Freon quick freeze stage. This stage holds four block holders and after tissue is frozen transferred to microtome.Sections cut from 2 to 16µm

LASER MICROTOME

Precise, non-contact sectioning, designed to slice samples with high precision. Femtosecond laser technology. Slice thicknesses 5 to 100 µm.
Advantages- fewer artifacts and less time consumption

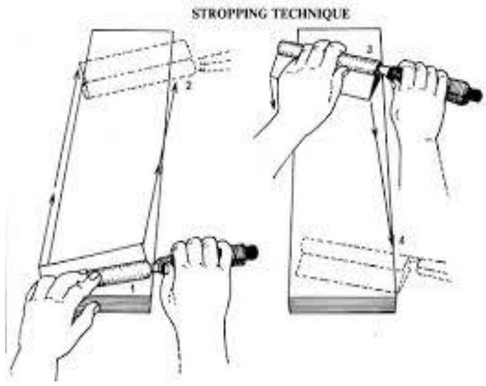



COMPUTERIZED MICROTOME

Equipped with advanced rapid thermostatic switch, semiconductor freezing, cryo scalped and cryoplate. Rapid freezing section or routine paraffin section (dual-purpose). Slice thickness1 to 25µm with least slice- 1µm and maximal slice- 32x32mm. Temperature of cryo scalpel and cryoplate- 0ºC ~-18ºC and -10ºC ~ -40ºC respectively

MICROTOME KNIVES

Central to production of good sections. First attempt- sharpened razor blade; but becomes blunt very quickly. In 1950 Latta and Hartmann discovered- freshly cut glass.For routine- wedge (C type) knife; plain on both sides. Size- 100 mm to 350 mm in length. Good quality of high carbon or steel; tempered at tip. Hardness of knife is essential to obtain good tissue sections. Sharpening- To achieve good sections; sharpened manually or by automatic machine. Honing- to remove nicks and irregularity from knife edge. Coarse and fine honing done using different abrasives. Stropping- to remove the “burr” formed during honing and to polish cutting edge.

CLASSIFICATION

Based on material of knife	Based on shape of the knife edge(profile)
Steel knives	Profile A: Strongly plano concave/ biconcave
Non-corrosive knives for cryostat	Profile B: Plano concave
Disposable blades	Profile C: Wedge shaped
Tungsten carbide knives	Profile D: Plane shaped
Glass knives	
Diamond knives	
Sapphire knives	

STEEL KNIVES

High quality carbon or tool grade steel. Heat treated to harden the edge
Steel- rust resistant, free from impurities and contain anti-corrosives.
Best knives- fully hardened. Only surface hardened lose the cutting edge very quickly

NON-CORROSIVE KNIVES FOR CRYOSTATS

Manufactured from hardened, heat treated stainless steel, free from impurities. 12 to 15% chromium

DISPOSABLE BLADES

Refined, thickened razor blades. Held in specially adapted knife holder- high quality sections. Replaced conventional microtome knives.
High quality stainless steel.Edge coated with platinum or chromium- strength and prolong cutting life

Teflon coated blades- cryostats due to reduced cutting resistance and minimal friction. Smaller, thinner blade reaches cryostat chamber temperature more rapidly. Held rigid in special holder to prevent vibration during cutting stroke

TUNGSTEN CARBIDE

Nonmagnetic and 100 times harder than hardened tool steel. Resistance to wear but brittle due to extreme hardness and handled carefully. 30,000 serial sections of undecalcified bone embedded in methacrylate can be obtained per sharpening

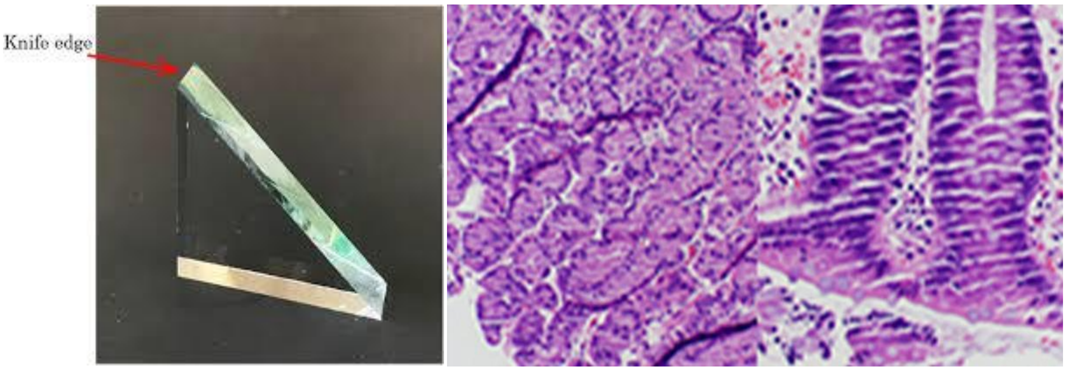
GLASS KNIVES

Ralph knives with edges of 25 or 38 mm- conventional sectioning- parallel to one surface of the glass. Ultramicrotomy- against / across thickness of glass. Glass knife holders available for use with rotary microtome. Hard but brittle; require care when handling

Deteriorate with storage due to changes in ‘flow’ or ‘strain’ of glass after fracture and from oxidation impurities in hardened glass after manufacture. Prepared immediately before us:

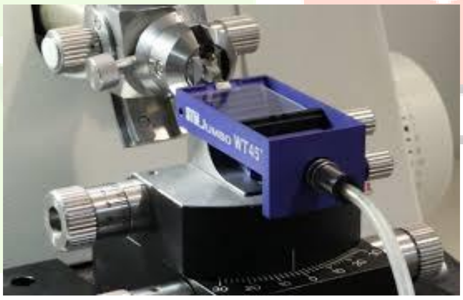
Ralph knives classified according to profile of cutting edge:
High profile- 1.0 to 1.5 cm cutting edge. Suitable for cutting sections from soft embedding medium- waxes.
Medium profile- 0.5 to 1.0 cm high cutting edge. Suitable for cutting sections from soft and hard plastics.

Low profile- less than 0.5 cm high cutting edge. Suitable for cutting sections from hard plastics.
Front profile- sloping edge artifact, unsuitable for use- due to inadequately securing the glass strip at each end



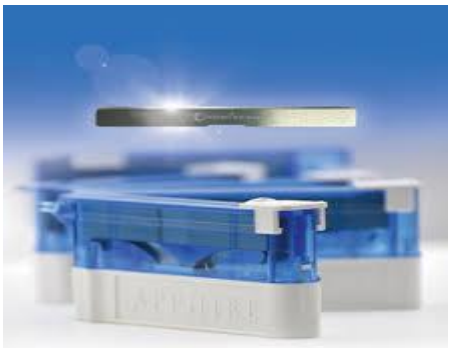
DIAMOND KNIVES

Gem quality diamonds without flaws. Very expensive, extremely durable due to its hardness. Very thin sections



SAPPHIRE KNIVES

Solid sapphire artificially produced from an alumina monocrystal under computer controlled thermal conditions. Harder than tungsten carbide or glass. High durability. Knife edge limited to 11 mm therefore special knife required.



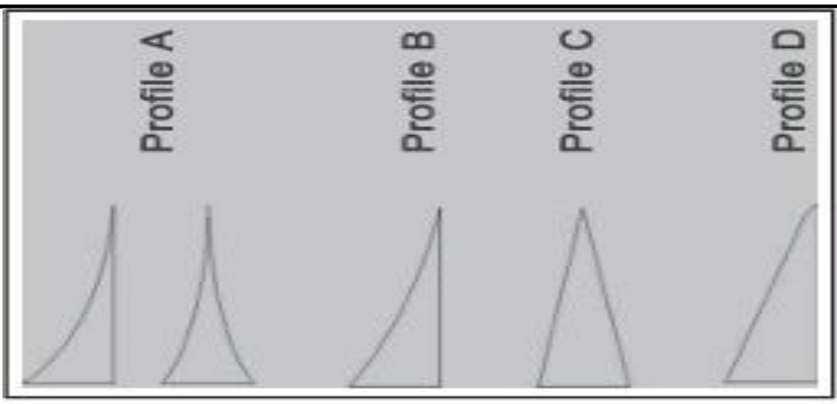
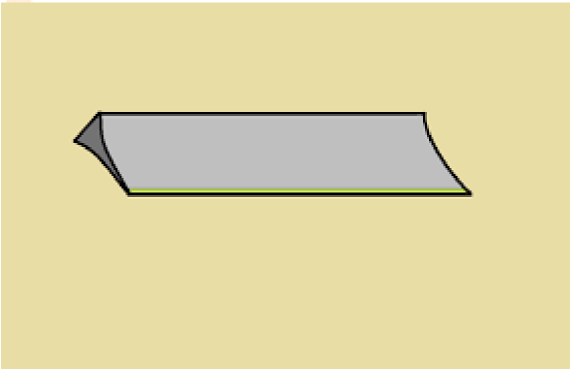


Figure 17. Profiles of the Knives.

PROFILE – A: STRONGLY PLANO CONCAVE/BICONCAVE

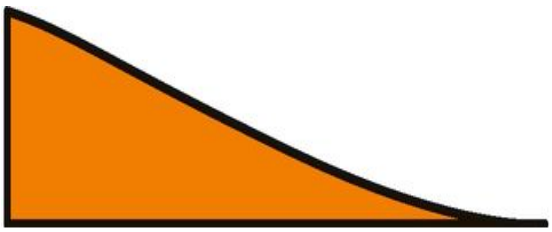
Plano concave- one surface straight other is hollow ground
Bi concave- two hollow ground surfaces.
Both knives extremely sharp



Used for- soft, celloidin embedded material or foam compounds.
Not suitable- hard materials, vibrate and produce chattering.
Best result- knife always oblique to the object when cutting sections

PROFILE – B: PLANO CONCAVE

Similar to profile – A knife but thicker back. Used for- too hard materials; softer materials embedded in paraffin wax. Positioned obliquely to material being sectioned. Varying degrees of concavity



PROFILE – C: WEDGE SHAPED

More rigidity. Used for- harder materials, paraffin wax embedded material, frozen, cryostat and for small, synthetic resin embedded material. Cutting plane transverse to object

PROFILE – D: PLANE SHAPED/TOOL EDGE SHAPED

Cut hard and tough material- greater stability. One bevel provides cutting edge, least sharp. Used for-synthetic resin blocks, hard materials embedded in paraffin wax and large wax blocks. Cutting edge produced by grinding bevel on each side of the knife for profiles – A, B and C and for profile- D onto the angled surface. Bevel faces- sharper angle than main surfaces

CARE OF MICROTOME KNIFE

Store knife in its box, when not in use. Cleaned with xylene before and after use. When stored for long time, smeared with grease or good grade of light oil. Knife edge should not be touched. Knife edge should never become badly nicked. Advisable to use separate knife for hard tissue like bone.

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