How many microscopists does it take to decorate a living room?

It depends on how thinly you slice them…

Microtomy :

- Is the means by which tissue can be sectioned and attached to a surface for further microscopic examination.

Microtome:

- Basic instrument used in microtomy.
- Mechanical device for cutting thin uniform slices of tissue – sections.

Types of microtomes

- There are 5 basic types of microtomes named according to the mechanism-

- Rocking microtome
- Rotary microtome
- Base sledge microtome
- Sliding microtome
- Freezing mictotome.

Rocking microtome:

- Name derived from the rocking action of the cross arm.
- Oldest in degisn, 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 backward and forward movement of the handle gives ribbons of good sections.

Disadvantage:

- Size of the block that can be cut is limited.
- Sections are cut in a curved plane:
  (Microtomes designed to cut perfectly flat sections; the block moving through an arc at right angles to the knife edge are available.)
- Light instrument : advisable to fit it into a tray which is screwed to the bench , or to place it on a damp cloth to avoid movement during cutting.

Rotary microtome

- First machine designed by Professor Minot,hence often referred to as the "Minot Rotary".

Mechanism:

- The hand wheel rotates through 360 degree moving the specimen vertically past the cutting surface 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.

Advantages:

- Ability to cut thin 2-3 μm sections.
- Easy adaptation to all types of tissues (hard, fragile, or fatty) sectioning.
- Ideal for cutting serial sections: large number of sections from each block.
- Cutting large blocks
- Cutting angle of knife is adjustable.
- Large and heavier knife used-less vibration when cutting hard tissue.
- Heavier and more stable .

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. Also good for wood!

Mechanism of action:

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

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- true only when change from one instrument to another is made .
- 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

- Gives best results for cutting frozen sections.
- Machine is clamped to the edge of a bench and connected to a cylinder of CO2 by means of a specially strengthened flexible metal tube.

Freezing microtome

- Knife freezing attachment is supplied with most machines.
- Separately controlled flow of CO2 on the edge of the knife - to delay the thawing of sections on the knife and make it possible to transfer them directly from knife to slides.
- Sections thickness gauge is graduated in units of 5 micrometer instead of 1micrometer.

Vibrating microtome

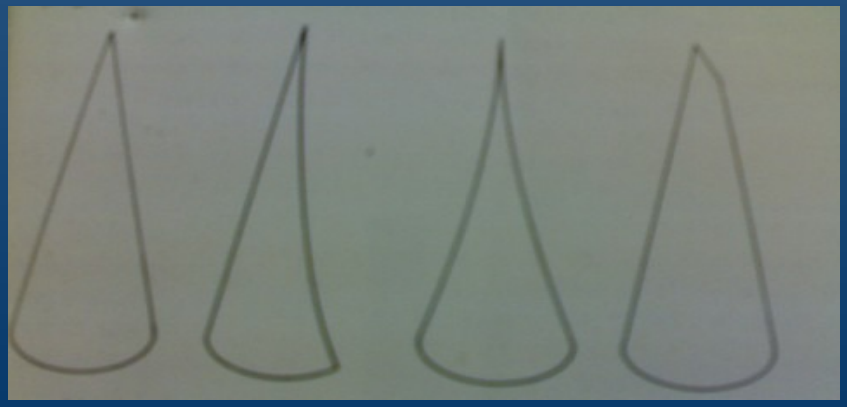
- Designed to cut tissue which has not been fixed, processed or frozen.
- Used in enzyme histochemistry ,ultrastructural histochemistry.
- During sectioning, the tissue is immersed in either water ,saline or fixative.
- It is cut by a vibrating razor blade , at thickness generally greater than used for paraffin wax.
- Tissues are cut at a very slow speed to avoid disintegration.

Microtome knives

- Developed to fit 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.

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

- Wedge.
- Planoconcave.
- Biconcave.
- Tool edge or D profile.



Wedge :

- Originally designed for cutting frozen sections
- Gives great rigidity to the knife
- Used for cutting all types of section on any microtome.

Plano-concave:

- Used primarily for cutting nitrocellulose – embedded tissues.
- Available with varying degrees of concavity.

Biconcave :

- Classical knife shape introduced by Heiffor.
- Used with the rocking microtome.
- Relatively easy to sharpen.
- Less rigid , prone to more vibrations.
- 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.

Used primarily to section exceptionally hard tissue.

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

Disposable blades

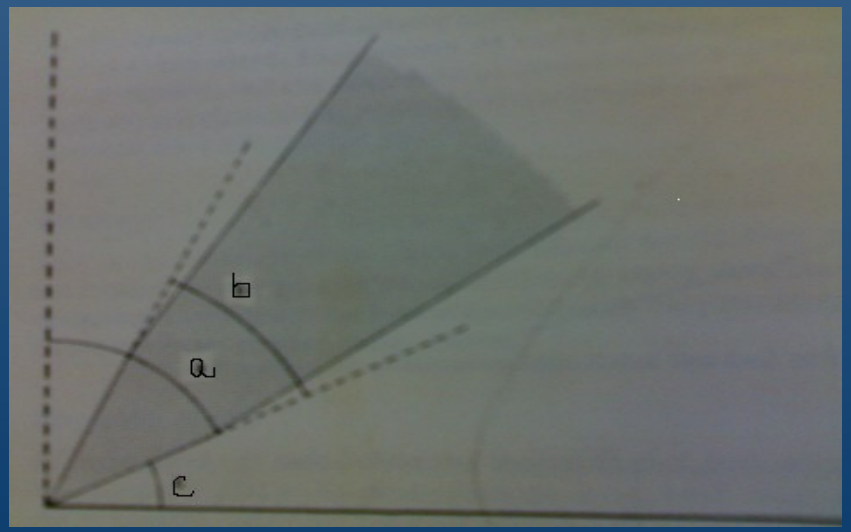
- Used for routine microtomy and cryotomy.
- Provide a sharp cutting edge, produce flawless 2-4 μm sections.
- Disposable blade holders incorporated into the microtome or an adapter.

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.

Knife angles

- Clearance angle: angle formed by a line drawn along the block surface and the lower bevel of the knife.
- Rake angle: angle between the upper bevel of the knife and a line at 90 degrees to the block surface.



Angles asociated with the knife edge.
A:rake angle; b:bevel ; c:clearance angle.

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 artefact.

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.

Flotation (water bath)

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- 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.

THANK YOU