

Tissue Microtomy: Principle and Procedure

5

5.1 Introduction

After embedding the tissue and preparing the block, the next step is microtomy. The word “microtomy” is originated from the Greek language. *Mikros* means small and *temnein* means to cut. So the word “microtomy” means to cut the tissue in thin sections. For successful microscopic examination, it is necessary to have thin sections of the tissue by microtomy.

5.2 Microtomes

It is the main instrument by which we cut the embedded tissue in the paraffin block as thin section. The different types of microtomes in the traditional histology laboratory are:

- Rotary microtome
- Rocking microtome
- Base sledge microtome
- Sliding microtome
- Cryomicrotome
- Ultramicrotome
- Laser microtome

(a) *Rotary microtome* (Fig. 5.1): This is the most commonly used microtome in routine laboratory. The cutting blade is kept in horizontal position, and the block containing tissue moves up and down with the help of rota-

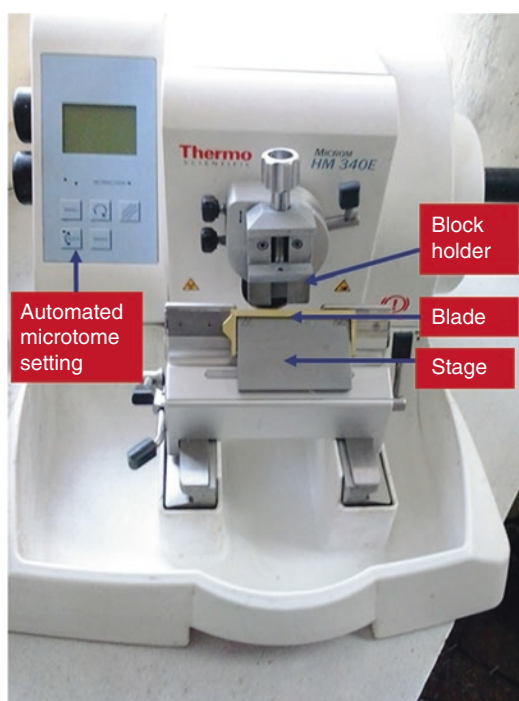


Fig. 5.1 Semiautomated rotary microtome

tory handle attached with the microtome. In each 360° rotation of the wheel handle, the block moves down followed by up, and the tissue is cut as thin ribbon. This microtome has the option to be semiautomated or automated with the adjustment and control of the movement of the block and angle of the knife.

Advantages:

1. Good-quality 2–3- μm -thin section is possible.
2. Heavy and stable automated rotary microtome reduces health hazard and gives the best-quality section.
3. Good tissue ribbon production.
4. Easy-to-cut various types of tissue: firm, fragile, small biopsy, etc.

Disadvantages:

1. Expensive.
 2. Unsuitable to cut large block.
 3. Knife faces up and so may be dangerous to the technical staff.
- (b) *Rocking microtome:* The rocking microtome is also known as Cambridge rocking microtome. The word “rocking” is used as there is a rocking action of the microtome like arm movement. In this type of microtome, the knife is static, and the block of tissue moves in a rocking motion (arc-like movement of the block). This is one of the oldest designs of the microtome. The microtome can cut thin section with ribbons and is ideal for serial section. The sections are slightly curved in this microtome.

Advantages:

1. Thin section
2. Easy to operate
3. Low-cost instrument and reliable

Disadvantages:

1. Tissue is curved and the microtome does not provide flat section.
 2. As the microtome is of light weight so vibration may occur.
- (c) *Base sledge microtome:* In sledge microtome the block is fixed in a static position within a steel carriage. The knife slides to and fro over the top of the block. This microtome is the best for large tissue sample or the hard tissue. The tissue sections are usually thick (more than 10 μm) in base sledge microtome.

Advantages:

1. Hard tissue can be cut.
2. Large tissue sample can be cut.
3. The best microtome for ophthalmology and large neuropathology section.

Disadvantages:

1. Difficult to get thin section.
 2. Large slides are required.
- (d) *Sliding microtome:* In this microtome the knife is static, and the block moves horizontally over the knife.

Advantages:

1. Large sections can be cut.
2. Mainly used for celloidin-embedded tissue.
3. Simpler design and easy maintenance.
4. Brain sections can be cut better by this type of microtome.

Disadvantages:

1. The knife may glide in case of hard tissue and may jump.
 2. Long knives are difficult to sharpen.
- (e) *Cryomicrotome:* This type of microtome is used for the cutting tissue for frozen sample (Fig. 5.2). The sample is made hard in liquid nitrogen and then cut by the microtome in the chamber that contains liquid nitrogen.

Advantages:

1. To get rapid section for rapid diagnosis
2. To study nerve biopsy
3. To study enzyme histochemistry

Disadvantages:

1. It needs continuous supervision to maintain the temperature.
2. Freezing artefact is often seen.
3. Very expensive instrument.
4. Fixed tissue is very difficult to cut.

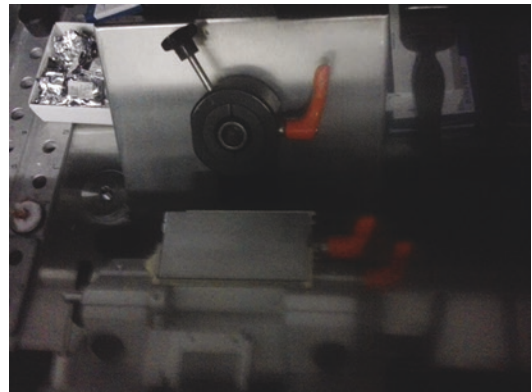


Fig. 5.2 Cryomicrotome used in frozen section

- (f) *Ultramicrotome*: Ultramicrotome is used to cut ultrathin sections for transmission electron microscopy. Sections are cut between 40 and 100 nanomicon thickness with the help of glass knife or diamond knife. The tissue is at first trimmed to make small block of 1×1 mm size, and then the section is cut by this ultramicrotome with the help of optical microscope. The cut section is allowed to float on the water hold by a boat and then finally picked up on a metal grid.
- (g) *Laser microtome*: In this ultramodern microtome, the laser beam is used to cut the biological section without any processing or embedding the material. Infrared laser beam with ultrashort pulse duration is applied, and therefore almost no heat is generated, and the tissue is cut without any thermal effect.

5.2.1 Microtome Knife

The microtome knife is important to cut uniform and thin section of tissue (Box 5.1). These are made of steel. Various types of knife profiles are available for different types of microtomes. The most commonly used knife profile is Profile C or wedge profile. The various types of microtome knife include (Fig. 5.3):

1. *Plano concave (Profile A)*: One side of the knife is plain and the other side is concave. Originally these knives were used for cutting celloidin-embedded tissue. This is a very sharp knife and is used for cutting soft tissues. However, presently these knives are less frequently used.
2. *Biconcave (Profile B)*: The knife is concave on both sides. The knife was used for rocking microtome. The concavity of the knife is often difficult to identify. This is a less rigid type of knife and often vibrates during cutting.
3. *Wedge (Profile C)*: This knife is plain on both sides. This is the most widely used knife for routine microtomy and it is compatible with the different type of microtome. This type of knife is also easy to sharpen.
4. *Tool edge (Profile D)*: The knife resembles chisel used in wood working. Both sides of the knife are plain; however the cutting edge is

Box 5.1 Microtome Knives

Types based on shape:

- Plano concave (Profile A):
 - Very sharp knife and is used for cutting soft tissues
- Biconcave (Profile B):
 - Used for rocking microtome
 - Vibrates during cutting
- Wedge (Profile C):
 - Most commonly used
- Tool edge (Profile D):
 - Used for hard tissue

Types based on disposability:

- Permanent
- Disposable
 - Low-profile blade: To cut small biopsy
 - High-profile blade: To cut firm to hard tissue

Materials used in knife:

Conventional knife: Steel

Diamond knife: Made of diamond and used to cut epoxy resin blocks

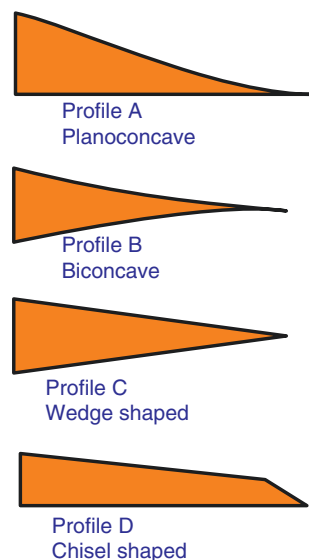


Fig. 5.3 Different types of microtomy knives based on shape. Profile C is the most commonly used knife

step. The tool edge knife is mainly used to cut the hard tissue such as decalcified bone. The knife is difficult to sharpen and is not recommended presently.

5.2.2 Disposable Knife

Nowadays disposable blade is used in many laboratories to save time to sharpen. Two types of disposable blades are available:

1. *Low-profile blade*: These blades are used to cut small biopsy or soft large tissue.
2. *High-profile blade*: These are used to cut firm to relatively hard tissue such as the uterus, heart, etc.

Advantages:

1. Easy to replace within seconds.
2. No need to sharpen.
3. The overall cost of disposable blade system is low as there is no need of any knife sharpener or abrasive powder to sharpen the knife.

Disadvantages:

1. They are not very rigid like ordinary knife, and therefore vibration effect may be seen.

5.2.3 Materials Used in Knife

Conventional knife: The conventional microtome knives are made of very high-quality carbon or steel material that are usually tempered from the edge to inside one third of the width. The desirable hardness of the knife is in between 400 and 900 Vickers hardness scale.

The diamond knife: This is a costly knife and is used to cut epoxy resin blocks in electron microscopy. Special care is needed to sharpen this knife.

Glass knife: This is used for ultramicrotomy to cut very hard tissue. The cutting edge of the knife is parallel to one surface of the glass.

5.2.4 Angles of Knife

The various angles formed by the knife include (Fig. 5.4):

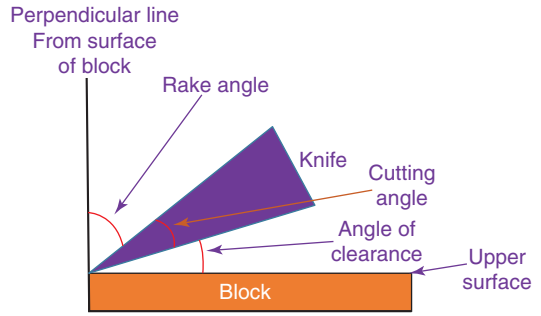


Fig. 5.4 Schematic diagram of angles of knife

Rake angle: It is the angle between the upper bevel of the knife and the perpendicular line drawn from the surface of the block. Increased rake angle makes the section cutting easier.

Angle of clearance: It is the angle between the lower bevel of the knife and the surface of the block. It is usually around 5° . The angle of clearance is related with the friction between the block and knife. Lower the angle of clearance, the less will be the compression on the block.

Bevel angle: This is the angle in between the two planes of the knife.

Cutting edge: This is the straight line formed by the intersecting of the two planes of the bevel.

5.3 Microtome Knife Sharpening

Sharpening of the microtome knife is needed to get good sections. The following methods help in sharpening of knife:

5.3.1 Manual Method

Honing Abrasive grindings are used to sharpen the knife. Stone can be used to sharpen the knife. Light oil can be used to lubricate the stone before use.

Iron oxide, aluminium oxide and silicon carbide are usually used as abrasive. The handle of the knife is hold by one hand, and the other hand holds the front end of the knife. The knife is pushed forward and diagonally over the slab

several times. Same procedure is followed in other surface of the knife also.

Stropping It helps to polish the cutting edge of the knife that is already sharpened by honing and also to remove any “burr” formed during honing. Strop is made of leather and it should be free from any dust or grit.

Automatic Knife Sharpener Presently automatic knife sharpener is available in the market. The knife is placed horizontally on the surface of the rotating plate made of glass or copper coated with abrasive agents.

5.3.2 Factors Involved in Cutting

1. *Temperature*: Lowering the temperature facilitates section cutting.
2. *Angle of rake*: Higher rake angle helps in smooth flow of ribbons. Lower rake angle is used for hard tissue.
3. *Consistency of tissue*: Soft tissue is cut at a slow rate than the hard tissue.

5.4 Sectioning the Paraffin Block

The following instruments are essential for section cutting:

1. Microtome with blade
2. Water bath
3. Paraffin block with embedded tissue to cut
4. Ice tray
5. A blunt forceps or camel brush
6. Slide rack with slides

Water Bath (Floatation Chamber) Water bath is used to float the tissue after cutting (Fig. 5.5). The temperature of the water bath is usually controlled automatically by a thermostat. The temperature of water in the water bath should be 10 °C below the melting point of the embedded paraffin wax and is usually kept in 40–50 °C. It is necessary to prevent formation of any air bubbles within the water bath. For adequate floating of

the tissue, one can add a few drops of alcohol or little amount of detergent. This reduces the surface tension of the water and tissue floats smoothly.

Blunt Forceps and Camel Hair Brush Blunt forceps helps to manipulate the floating tissue section (Fig. 5.6). Camel hair brush is used to clean the blade.

Slide Rack with Clean Glass Slides The clean slides are kept in the slide rack. The slides can be already labelled by diamond pencil or on the frosted side by lead pencil. Alternatively this can be marked after lifting the tissue section.

Adhesive In case of routine section and staining, no adhesive is required. However, in certain situations we use cell adhesive material such as:



Fig. 5.5 Water bath used in microtomy. The constant temperature (usually 40–50 °C) is maintained in the water bath. This is usually 5–10 °C lower than the melting point of the paraffin



Fig. 5.6 Blunt forceps and brush used in section cutting

1. Brain sections
2. Decalcified tissue
3. Using strong alkali at the time of staining

The most commonly used adhesives include:

- *Mayer's egg albumin and glycerol*: This is prepared by mixing
 - White part of egg: 100 ml.
 - Glycerol: 100 ml.
 - Homogenize the mixture thoroughly, and filter it by gauze piece. Add few crystals of thymol to prevent bacterial growth.
- *Poly-L-lysine*: This is a good adhesive and does not produce any background staining. The slides are coated with poly-L-lysine diluted by distilled water (1:10) before using.
- *3-Aminopropyltriethoxysilane (ACEP)*: The slides are dipped in the dilute ACEP solution in acetone (2%) and then dried before using.
- *Permanent positively charged slides*: Here the slide is tempered in such a way that the slide

surface is always positively charged. It is easier to lift the tissue section with these slides. The positively charged slides are also excellent for lifting frozen section tissues.

5.4.1 Steps of Tissue Sectioning (Fig. 5.7)

1. *Trimming the tissue*: Trimming of the tissue is needed to expose the tissue piece within the paraffin wax for cutting. The block is fixed in the chuck of the microtome, and the paraffin is cut till the tissue is fully exposed. Adequate caution should be taken not to overcut tissue as this may produce various artefactual changes.
2. *Cooling the block*: After the initial trimming, the blocks are kept for cooling for 15–20 min. This will help to maintain the same consistency of the paraffin and tissue. This helps in easy cutting.



Trimming of tissue



Cooling the block by ice



Cutting the tissue and making ribbons of tissue



Floating the tissue in Water bath



Picking up the tissue on slide and drying

Fig. 5.7 Different steps of section cutting are highlighted here. At first the tissue is trimmed. Then the block is cooled in ice. The block is placed in the microtome, and the angle of clearance is adjusted to 5 °C. The tissue is gently cut,

and the tissue ribbon is placed with the help of brush on the water bath. Then the tissue is picked up by a putting a glass slide perpendicularly in front of it, and then when the tissue is touched, the slide is withdrawn vertically

3. *Cutting proper:* The block is fixed in the chuck of the microtome. The cutting surface of the block should be parallel to the knife. The angle of clearance should be only $2-5^\circ$ to have good section (Fig. 5.8). The tissue in the block is cut by gentle, smooth and slow stroke. The ribbon-like tissue sections are produced. The tip of the ribbon is held by forceps, and the end part of the ribbon is removed from the knife edge by brush. In case of any difficulty to get the flat section, the cutting surface should be gently warmed by warm water.

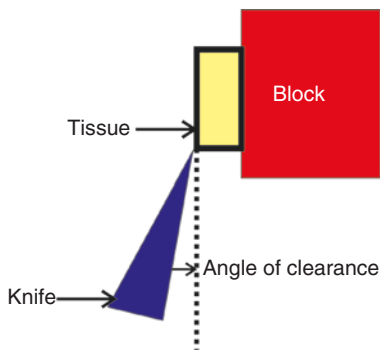


Fig. 5.8 The schematic figure of the tissue and the position of knife making the correct angle of clearance which is 5°

4. *Floating the ribbon:* The ribbon of the tissue is floated in the water bath, and this makes the tissue flat and removes any wrinkling of the tissue. With the help of the forceps, the individual sections are separated from each other. As mentioned before, the temperature of the water bath should be constantly maintained below the melting point of the paraffin wax. In case of temperature variation in the bath, the air bubbles may be formed that may rupture the tissue.
5. *Picking up the tissue:* The slide is placed vertically within the water bath in front of the tissue, and when the tissue is touched, the slide is withdrawn vertically from the water. The tissue pickup process must be gentle and smooth. To prevent any mix-up, the water bath should be cleaned immediately after cutting each block.
6. *Drying the section:* The slide containing the picked-up section is kept in slide rack. The slides are now kept in hot oven to get dry. The temperature of the oven should be slightly more than the melting point of the paraffin.

Various problems may occur in tissue sectioning (Figs. 5.9, 5.10, 5.11, 5.12 and 5.13) [1]. These are enumerated below in Table 5.1.

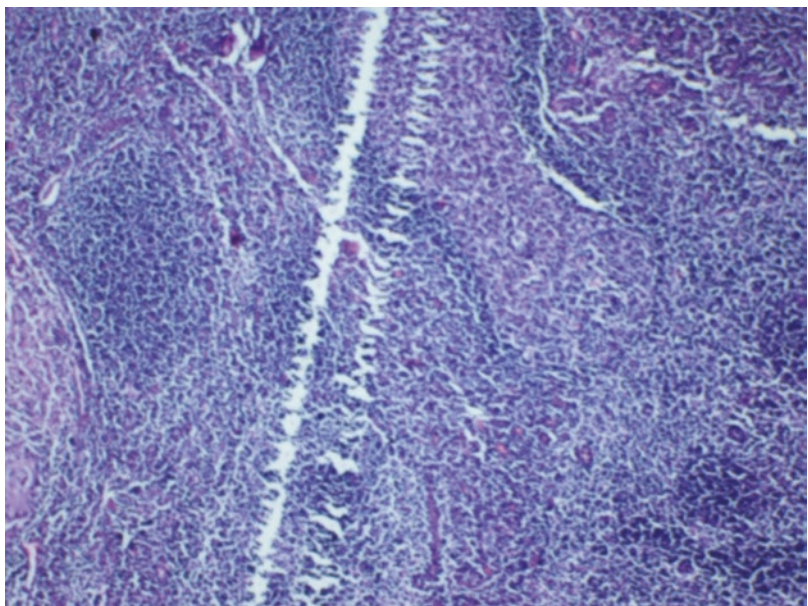


Fig. 5.9 Tear in the tissue due to uneven cutting edge of the knife

Fig. 5.10 Large hole in the tissue due to the bad processing

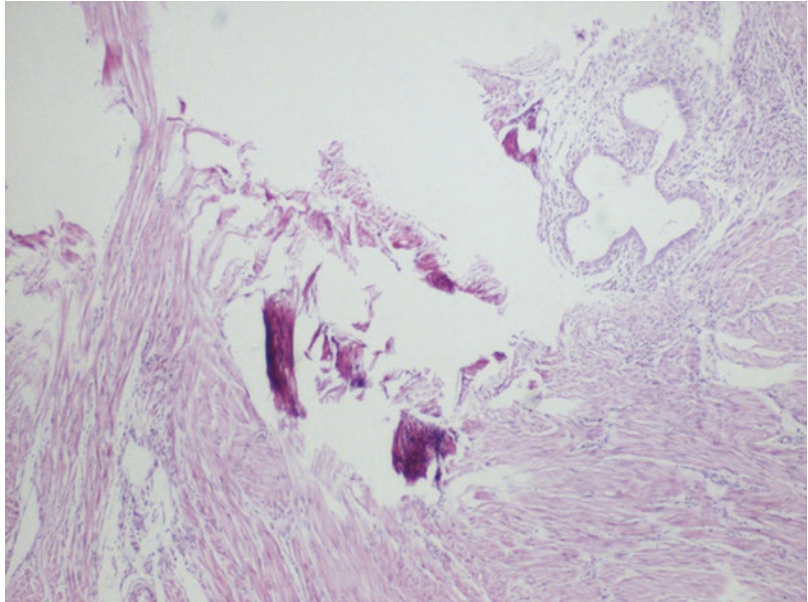


Fig. 5.11 Multiple air bubbles have ruptured the tissue. The air bubbles are produced due to uncontrolled temperature in the water bath. Note the characteristic multiple irregular tear of the tissue

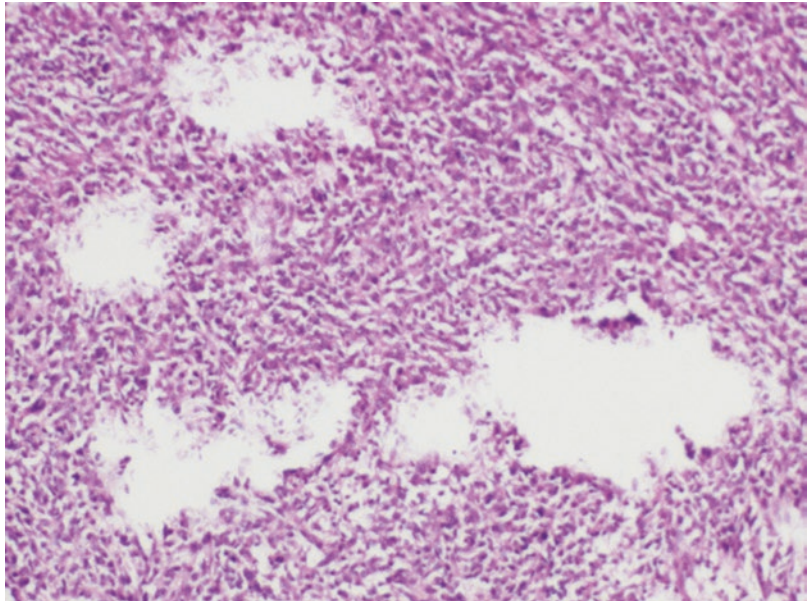


Fig. 5.12 Freezing artefact of the tissue due to immediate putting the tissue from the refrigerator to formalin. Note the regular spaces in between the tissues due to melting of ice crystals

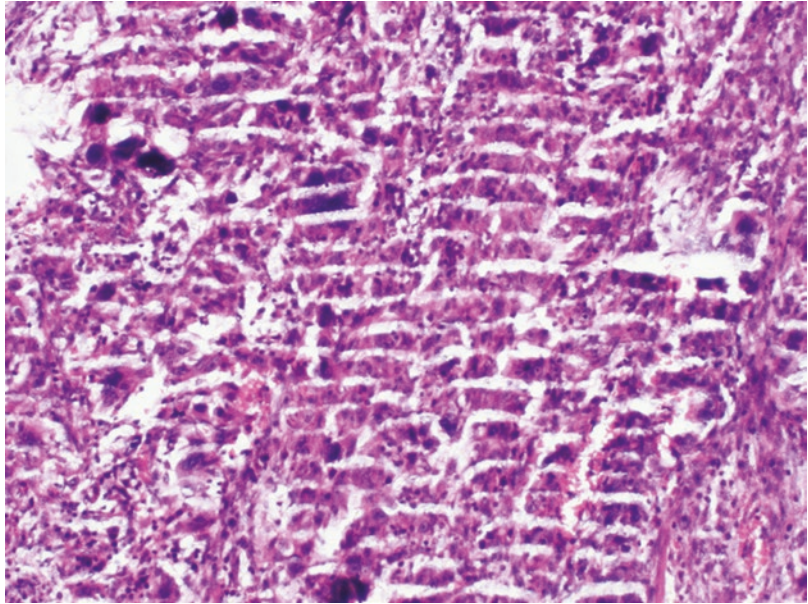


Fig. 5.13 Uneven staining pattern due to poor deparaffinization of the tissue

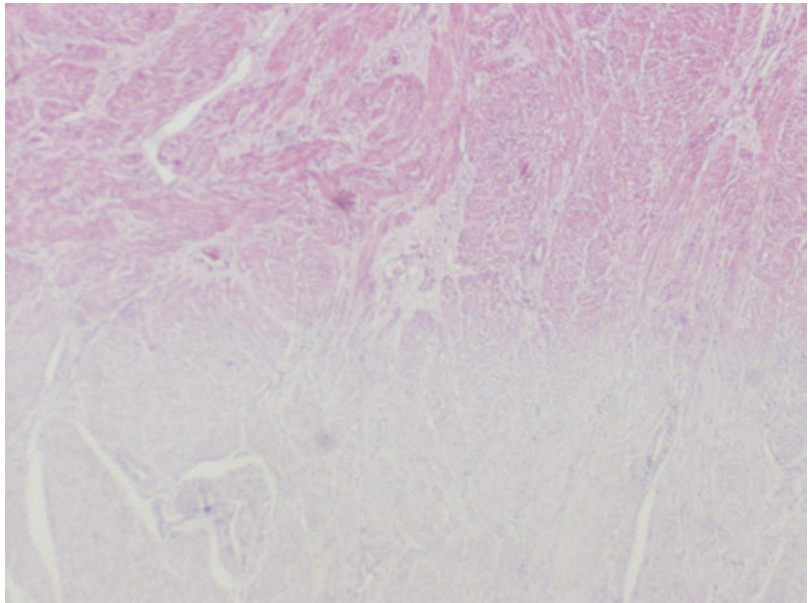


Table 5.1 Troubleshooting in tissue sectioning

Faults	Source of faults	Solution
<i>Ribbons not formed:</i> the tissue is twisted and curled or sticks to the knife	<ul style="list-style-type: none"> • Paraffin is hard • Clearance angle is incorrect: small • Dirty knife • Too cold or hot room temperature 	<ul style="list-style-type: none"> • Select low melting point paraffin • Correct the knife alignment • Clean the blade • Adjust the room temperature
<i>Ribbons are curved:</i> the ribbons are not straight and are coiled	<ul style="list-style-type: none"> • Edges of the block are not parallel: the sides of the block and the knife edge are not in right angle • Surface of the block is uneven • Blade is not sharp • The paraffin is too much 	<ul style="list-style-type: none"> • The block holder should be aligned properly • Trimming is needed • Sharpen the knife or replace the blade • Trim and remove the excessive paraffin
<i>Ribbons are excessively compressed and wrinkled</i>	<ul style="list-style-type: none"> • Block is warm • Dull blade/knife • Paraffin is soft and sticky • Knife clearance is not optimum and is too small • Rotation of the microtome is clumsy 	<ul style="list-style-type: none"> • Cool the block • Replace the blade or sharpen the knife • Cool the block and then try to cut; otherwise the paraffin should be replaced with higher melting point • Increase the knife clearance • Needs consistent and gentle rotation of the wheel
<i>Thick and thin section (chatter)</i>	<ul style="list-style-type: none"> • Knife or block is loose • Blunt knife • The clearance angle is very small • Paraffin is soft and sticks to the knife 	<ul style="list-style-type: none"> • Tighten the knife clamps or the chucks • Sharpen the knife or change the blade • Adjust the clearance angle • Clean the knife and try to remove the attached paraffin
<i>Section attaches to the block</i>	<ul style="list-style-type: none"> • Static electricity is generated in the knife or ribbon • The clearance angle is defective 	<ul style="list-style-type: none"> • Apply ionizer to remove the static charge, and/or clean the blades, holder, etc. with alcohol • Increase the clearance angle
<i>Tear or scratches in the section</i>	<ul style="list-style-type: none"> • Defect in the blade: a nick or jagged knife edge • Dirt in the knife • Sharp particle in the tissue • Sharp particle in the paraffin 	<ul style="list-style-type: none"> • Hone the knife/use other part of knife/change the blade • Clean the blade • Decalcify the tissue • Try to remove sharp particle by scalpel
<i>Tissue disintegrate in the water bath</i>	<ul style="list-style-type: none"> • Too hot water • Faulty and incomplete processing 	<ul style="list-style-type: none"> • Temperature should be lowered down • Reprocess the tissue
<i>Large holes in the tissue</i>	<ul style="list-style-type: none"> • The underprocessed tissue ruptures when comes in contact with warm water 	<ul style="list-style-type: none"> • Reprocess the tissue

Reference

1. Peachey LD. Thin sections. I. A study of section thickness and physical distortion produced during microtomy. *J Biophys Biochem Cytol.* 1958;4(3):233–42.

Frozen Section: Principle and Procedure

6

6.1 Introduction

The frozen section is the rapid tissue section by cooling the tissue with the help of cryostat to give immediate report of the tissue sample. This is especially needed in large hospital to diagnose the lesion or extent of the lesion at the time of operation. The cryostat is the instrument that has the arrangement to freeze the tissue and also to cut the frozen tissue for microscopic section.

6.2 Indications of Frozen Sections

The frozen section is used mainly for immediate diagnosis of the lesion for management and to know the extent of the lesion [1–3] (Box 6.1). It is also helpful to do enzyme immunocytochemistry and immunofluorescence study. At

Box 6.1 Indications of Frozen Section

- Rapid diagnosis of the lesion for intra-operative management
- To know the extent of the lesion
- To do enzyme immunocytochemistry
- To do immunofluorescence study
- To stain lipid and certain carbohydrate in the tissue

times, frozen section tissue is used for the demonstration of fat and carbohydrate in the tissue sample.

6.3 The Principle of Frozen Section

The rapid freezing of the tissue sample converts the water into ice. The firm ice within the tissue acts as embedding media to cut the tissue. Lowering the temperature makes the tissue more firm, whereas increasing temperature makes the tissue softer.

Cryostat Machine Proper (Fig. 6.1)

Temperature range in the machine: The cryostat machine has the usual temperature range from 0 °C to –35 °C. The most of the tissue is sectioned properly between –15 °C and –25 °C. The water-containing tissues can be sectioned in higher temperature, and fat-containing tissue needs much lower temperature to cut.

Rotary microtome (Fig. 6.2): Rotary microtome is placed inside the cabinet of the cryostat. Here the knife is fixed and the tissue is moved with the help of a rotary wheel.

Tissue shelf (Fig. 6.2): Just in one side of the microtome, there is a tissue shelf to keep the tissue. In this place the samples are kept for freezing. Usually the temperature of tissue shelf is lower than the overall cabinet temperature.

Fig. 6.1 Cryostat machine with its parts

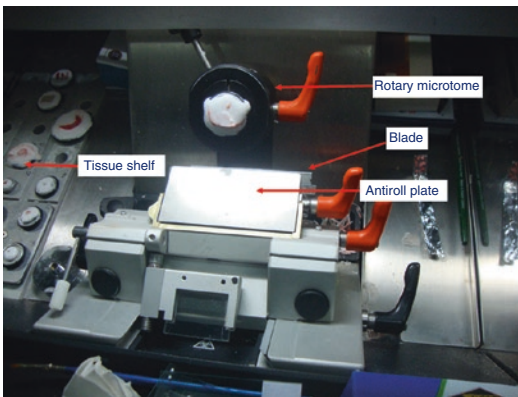
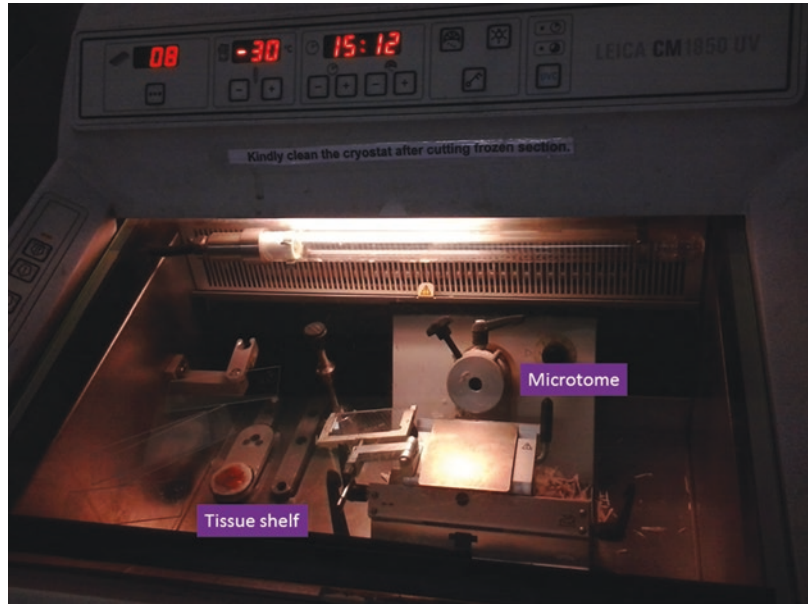


Fig. 6.2 Microtome, blade, antiroll plate and tissue shelves are shown

Place to keep the brush and knife holder: Just in front of the microtome machine, there remains a small place to keep the brush and knife holder.

Knife or blade: Nowadays, low- or high-profile disposable blades are used. The blade should be properly fixed to the holder to get an even pressure in the whole length. Alternatively Profile C steel blade is also used. The angle of the knife is kept in between 5° and 7° .

Antiroll plate (Fig. 6.2): Just in front of the knife, there is an antiroll plate that prevents the rolling of the cut tissue. It is usually a glass plate

within a metal frame. The undersurface of the plate has free space, and there is a gap between the knife and the plate.

Alternating to antiroll plate, a cool sable hair brush can be used to get unrolled tissue.

Specimen holder: The specimen holder or chuck is supplied by the manufacturers in different sizes and shapes. Usually these are round metal structures.

Embedding medium: This medium is used to hold the tissue over the chuck. Presently optimum cutting temperature (OCT) compound is used as embedding medium. The OCT is made of water-soluble glycols and resin.

6.4 Cryostat Sectioning

The process of the cryostat sectioning needs the following steps.

1. *Grossing and cutting the specimen* (Box 6.2): The cutting surface of the tissue should be smooth. The following steps in grossing of the tissue are mandatory for accurate reporting:
 - *Identify the tissue sample of the patient and the requisition form:* This is the first and foremost part of the frozen tissue grossing.

Box 6.2 Grossing for Frozen Section Tissue

- *Identify the tissue sample of the patient.*
- *Clinical information:* provides possible differential diagnosis.
- *Tissue appearance:* colour, texture, nodule, any suture.
- *Anatomy of the tissue:* identify the resection planes and margins.
- *Colour the resection planes and margins.*
- Section cutting:
 - Use sharp blade.
 - First cut the most important area.
 - Give gentle pressure and avoid too much pressure.
- Cytology preparation: if needed make
 - Imprint smear
 - Scrape smear
 - Crushed smear

- *Salient clinical information:* The essential clinical information is very helpful as it guides the pathologist to reach the possible differential diagnosis.
- *Tissue appearance:* The gross appearance of the tissue such as colour, texture, consistency and any suture to mark the anatomical position.
- *Resection margin:* It is very important to identify the resection margins of the tumour. The resection planes and margins should be inked thoroughly. The different colours of ink can be used for medial and lateral margin identification.

Cutting the tissue: The tissue should be fresh without any fixative. The tissue should be preferably dry, and it should not be wrapped in gauze piece. Any suture, staple or sharp hard structure should be removed from the tissue sample. Now the tissue is cut into small pieces as it facilitates freezing. Take multiple sections of the tissue to understand the main pathology and to minimize the error. Use a new sharp scalpel blade, and first cut the most important area that needs microscopic examination. It is preferable to use gentle

stroke of the scalpel rather than too much pressure.

Cytology of the tissue: At times the imprint of the tissue on the slide provides good morphological details such as lymphoma of the lymph node. Similarly crushing of tissue also provides excellent morphological details such as in case of tissue of the brain tumour.

1. *Tissue embedding in the mould* (Fig. 6.3): The small piece of the tissue is kept in the centre of the mould, and then the OCT is poured over it in excess. Then the tissue holder or chuck is firmly placed over the tissue with overflowed OCT.
2. *Tissue loading in the frozen section chamber:* The tissue is now placed in the frozen section chamber, and cold spray can be used to make the process faster.
3. *Loading the blade:* The cutting knife or blade is now loaded and the proper alignment is done.
4. *Trimming the tissue:* The loss of normal or natural colour to whitish colour indicates that the tissue is frozen. The frozen tissue in the tissue holder is now placed in the holder of the microtome. The block is trimmed to remove the excess OCT and to get the smooth tissue surface for sectioning.
5. *Sectioning* (Fig. 6.3): The tissue is now cut gently and is spread over the antiroll plate with the help of a brush. The brush should be cooled. The tip of the tissue is guided by the brush.
6. *Section lifting:* The glass slide of normal room temperature is pressed firmly over the tissue section, and normally the tissue sticks immediately.
7. *Fixation:* The tissue should be immediately fixed in methanol for 1 min or 95% ethanol for few seconds. Rapid fixation within few seconds is mandatory. In case of delayed fixation, the cells are swollen, and the cytoplasmic margin may be ruptured giving hazy appearance of the margin of the cells.

Troubleshooting in frozen section: Various problems may arise during the cutting of frozen section tissue. This has been highlighted in Table 6.1.

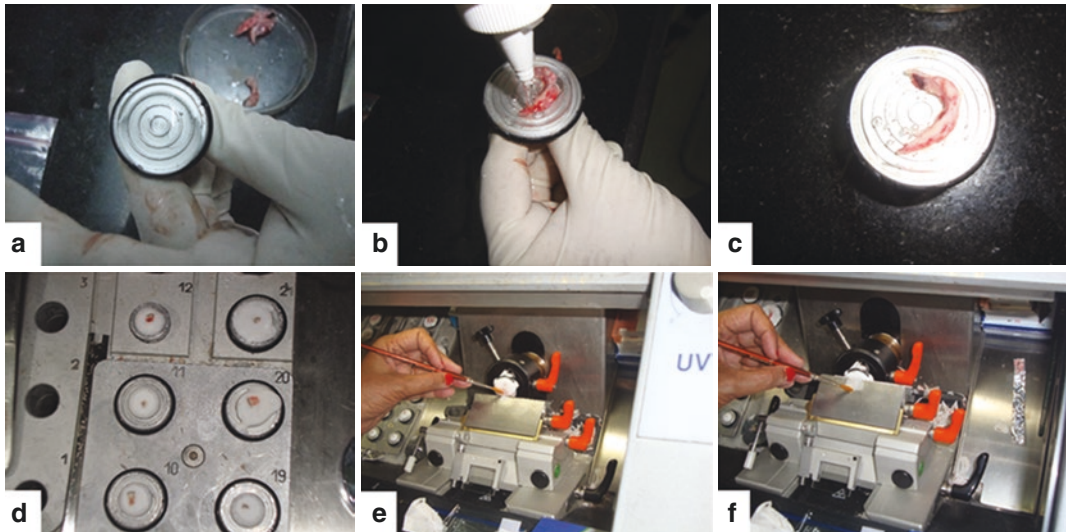


Fig. 6.3 Cryostat processing: (a) mould is covered with OCT, (b) the tissue is now put on the block, (c) OCT is flooded over the tissue, (d) the tissue now is put in the cooling chamber, (e) the brush guides the tip of the tissue, (f) the tissue section is gently spread over the antiroll plate and later picked up by touching a glass slide

Table 6.1 Troubleshooting in frozen section

Problems	Cause	Solution
Freezing artefact	Formation of ice crystal within the tissue. Water-containing tissue shows more such artefact	<ul style="list-style-type: none"> Freeze the tissue rapidly, i.e. snap freezing The tissue specimen should not be in saline solution before freezing
Uneven tissue embedding	The surface of the tissue is uneven, and the vital information may be lost	<ul style="list-style-type: none"> Make tissue even at the cutting surface before freezing
Block is loosen during chucking	The chuck may be too cold when the tissue is placed on it	<ul style="list-style-type: none"> Take the tissue out and reattach it on a clean chuck which is not too cold
Tissue crumpled	The tissue in the block is warm or too cold	<ul style="list-style-type: none"> Make the block of tissue in the optimum temperature: -15°C to -20°C
Chattering artefact	The temperature of the block is too cold, and the tissue becomes hard. The blade will cut the tissue thick and thin in regular interval	<ul style="list-style-type: none"> Bring the block in optimum temperature. Pressing the cut surface of the block by gloved finger may make the block warmer
Thin stripe in tissue	The perpendicular tear in the tissue is due to nicks on the blade	<ul style="list-style-type: none"> Replace the blade by a sharper one
Widely striped tissue and also tearing of the tissue	This may happen if the tissue is sticking with the blade	<ul style="list-style-type: none"> Clean the blade or replace with a new one

Fixation: Immediate dip in 95% ethyl alcohol for a few seconds fixes the tissue.

6.5 Staining

We commonly use haematoxylin and eosin (H&E) and toluidine blue stain.

6.5.1 H&E Staining

- Rinse the slide in tap water.
- Put in haematoxylin for 1 min.
- Rinse in tap water for 5 s.
- Rinse in Scott's tap water for 5 s for bluing.
- Dip in eosin for 20 s.
- Rapidly rinse in tap water.

- 95% ethanol for 10 s.
- 100% ethanol for 10 s.
- 100% ethanol for 10 s.
- Dip in xylene for 20 s.
- Mount by DPX.

6.5.2 Toluidine Blue Stain

This is a very simple stain and takes only a few seconds. The drops of toluidine blue stain are put on the section, and the coverslip is put on the section. The slide is now ready to see. The histopathologist feels more comfortable in H&E stain than this unfamiliar toluidine blue stain.

6.6 Factors Affecting the Good-Quality Section

The common factors responsible for the good-quality smear include:

- *Temperature*: when the temperature falls, water within the tissue becomes frozen and gives the tissue hard consistency. The optimum temperature of frozen tissue is in between -15°C and -25°C . Warm tissue remains soft and sections crumple. On the other hand, the overcooled tissue becomes very hard and brittle and produces again bad-quality crumpled section. Moreover the hard tissue may cause “chattering” artefact and also thick and thin sections. Different tissue contains variable amount of fat and water. The consistency of different tissue varies, and therefore the optimum temperature to cool the tissue varies considerably. Table 6.2 shows the optimum temperature of different organs to have good frozen section.
- *Tissue consistency*: other than the optimum cooling temperature, the consistency of tissue has significant effect on cutting such as:
 - (i) Fatty tissue: It is difficult to cut the fatty tissue in frozen section. Fat may smear

Table 6.2 Optimum temperature for frozen section

Tissue	Optimum temperature
Brain, liver, spleen	-7°C to -10°C
Rectum, uterus, adrenal, muscle, skin	-12°C to -15°C
Heart, lung, intestine, pancreas, ovary, cervix, prostate	-16°C to -20°C
Bone marrow, breast	-20°C to -25°C

on the knife and may make problem in cutting.

- (ii) Collagenous tissue: The firm collagenous tissue is difficult to cut.
 - (iii) Necrotic tissue: Soft necrotic tissue may create considerable problem as they may fall from the slide making hole in the section. It is preferable to take only viable tissue for frozen section.
 - (iv) Bony hard tissue: Hard tissue like bone or cartilage may damage the blade significantly. In this situation a new section can be processed, or new blade can be used.
- *Tissue size*: The size of the tissue sample should be small as the larger tissue takes much longer time to freeze.

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