

Frozen & Related Sections

Frozen Sections

- Frozen sections are produced without the use of dehydration steps or overnight fixation.
- Needed: OCT (Optimal Cutting Temperature embedding material), mould(chuck) and means of rapidly freezing tissue.
- Frozen sections have important clinical and research applications.

e.g. in Mohs Micrographic Surgery, in animal research setting

Principle of Frozen Sections

- 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.

Theoretical Considerations

- The consistency of the frozen block may be altered by varying the temperature of the tissue.
 - Reducing the temperature will produce a harder block; raising the temperature makes the tissue softer.
- The majority of non-fatty unfixed tissues section well at -25°C . The sectioning of fixed tissue requires a block temperature of approximately -10°C or warmer.

Theoretical Considerations

- There is more water in fixed tissue; consequently, the tissue will have a harder consistency, requiring a higher temperature to obtain the ideal consistency for sectioning.

N.B for fatty unfixed tissue section well at a colder temperature or surround the tissue with OCT which helps keep the tissue together when cut.

Frozen Sections

Advantages	Disadvantages
Quick diagnostic procedure (≈ 10 mins)	Difficult to cut serial sections e.g. fatty tissue, bone and delicate structures i.e. ovarian epithelium
Every type of staining can be done	Sections cut are thicker.
Minimal shrinkage of tissues as compared to paraffin sections	Storage can be expensive (use of specialized equipment e.g. ultra-low temperature freezer)
Lipids and enzymes which are lost in routine paraffin sections can be demonstrated	Formation of ice-crystals can cause distortion in tissue morphology & difficult sectioning

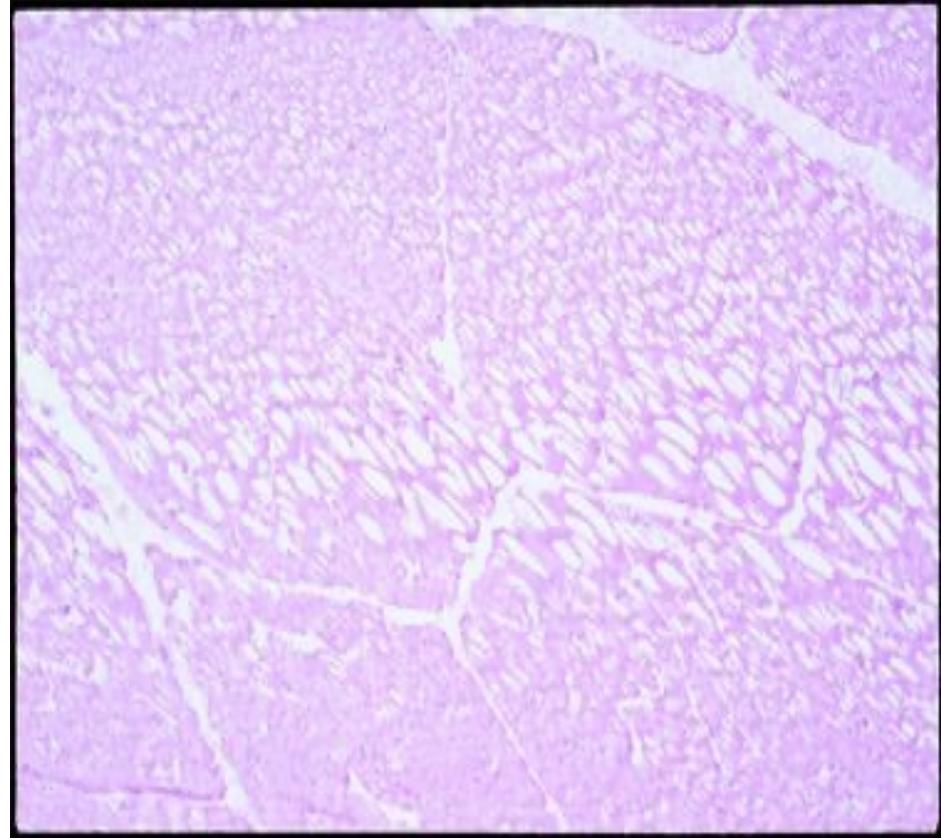
Uses of Frozen Sections

- 1) Rapid production of sections for intra-operative diagnosis.
- 2) Diagnostic and research enzyme histochemistry for labile enzymes.
- 3) Diagnostic and research non-enzyme histochemistry, e.g. lipids and some carbohydrates.
- 4) Immunofluorescent methodology.
- 5) Immunohistochemistry techniques when heat and fixation may inactivate or destroy the antigens.

Freezing of Fresh Unfixed Tissue

- The specimen should be frozen as rapidly as is possible without creating freeze artifacts.
- Examples of techniques for suitable freezing include:
 - i. Liquefied nitrogen (-190°C)
 - ii. Isopentane (2-methylbutane) cooled by liquid nitrogen (-150°C)
 - iii. Dry ice (-70°C)

- When freezing tissue for frozen sections, freeze artifact may occur.
- The water in the tissue freezes and forms ice crystals, and the crystal size and the quantity of crystals is proportional to the speed at which the tissue is frozen.
- The tissue is cut and placed on a microscope slide at room temperature; at this point the tissue is thawed. The thawing of the ice crystals produces freeze artifact that appears as holes or discontinuation of the tissue architecture when viewed microscopically.



Ice crystal artifact in a section of skeletal muscle

Fixed Tissue and the Cryostat

- The effect of freezing unfixed tissue is the diffusion of labile substances.
 - Diffusion of labile substances is enhanced when the section is cut in the cryostat, producing heat which causes slight thawing of the cut section.
 - This may not cause a problem for diagnosis, but it can affect the accurate localization of some abundant enzymes, such as acid and alkaline phosphatases.
- The tissue must be fresh and placed in formal calcium at 4°C for 18 hours.

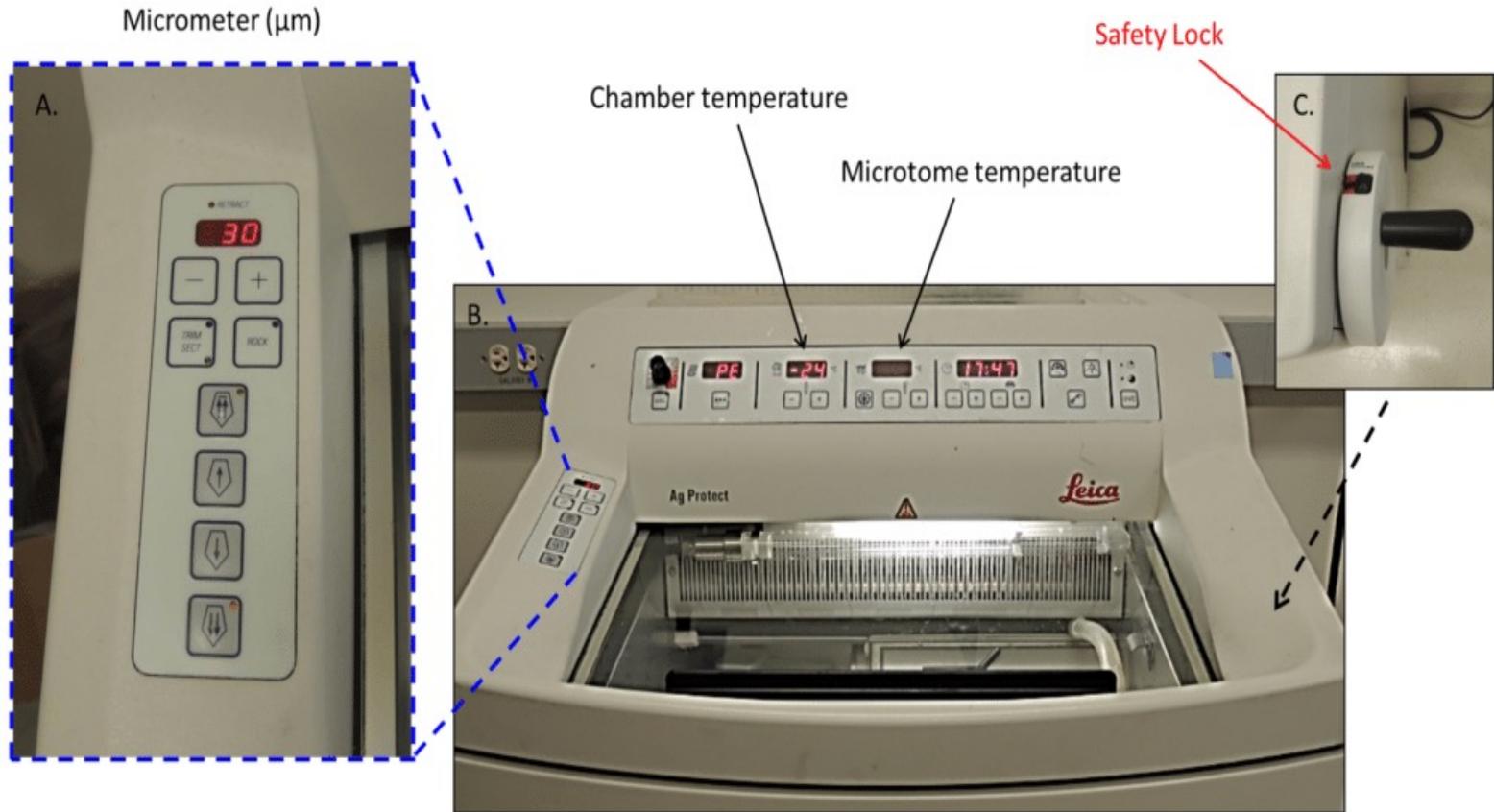
The Cryostat

- Is a refrigerated cabinet in which a specialty microtome is housed.



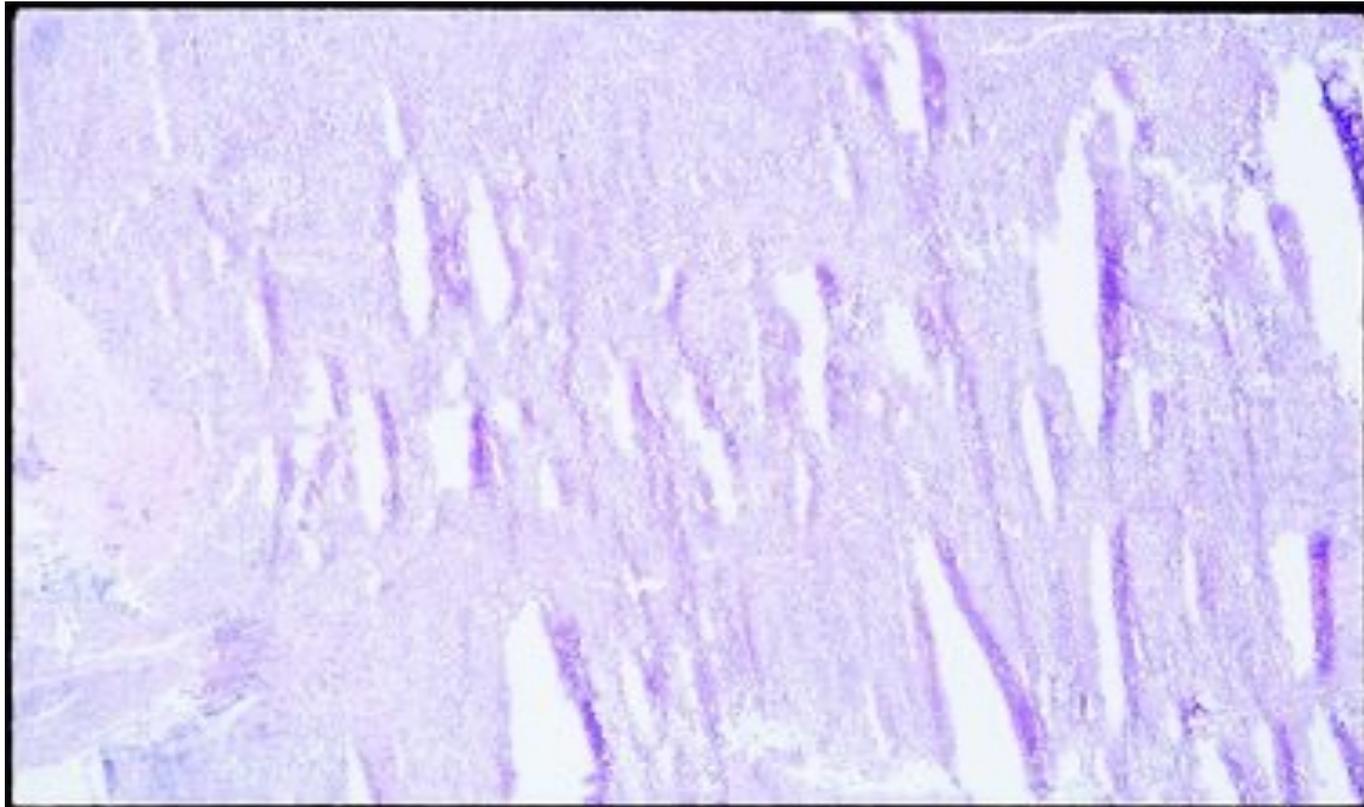
1. Smooth turning handwheel with 360° locking mechanism.
2. Double compressor system allows for fast freezing and robust lifespan of cryostat
3. Waste collection for liquid condensate is safe and easily accessible

The Cryostat



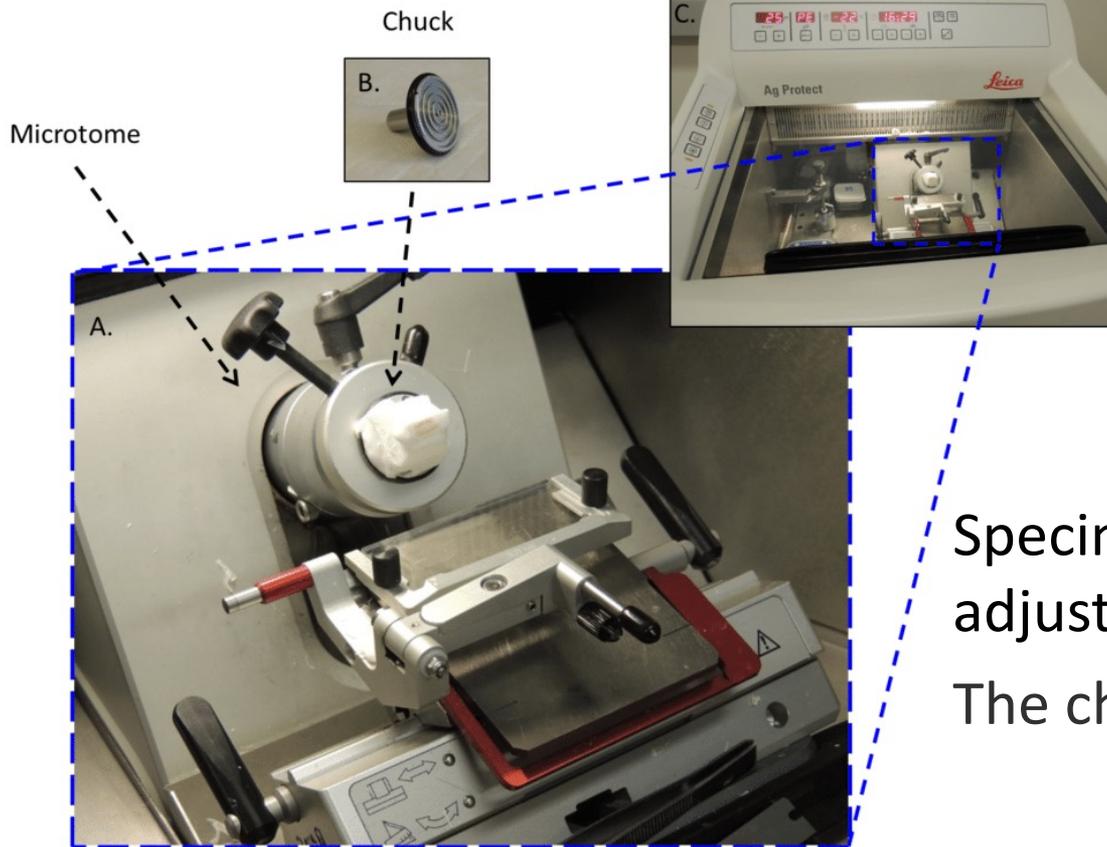
Cabinet temperature

- Most unfixed material will section well between -15 and -23°C .
- Tissues containing large amounts of water will section best at the warmer temperature, and harder tissues and those that contain fat require a colder temperature.
- Most fixed tissues will section best within the range of -7 to -12°C , depending on the hardness of the tissue.



Frozen section "shutters" caused by sectioning a block that was too cold

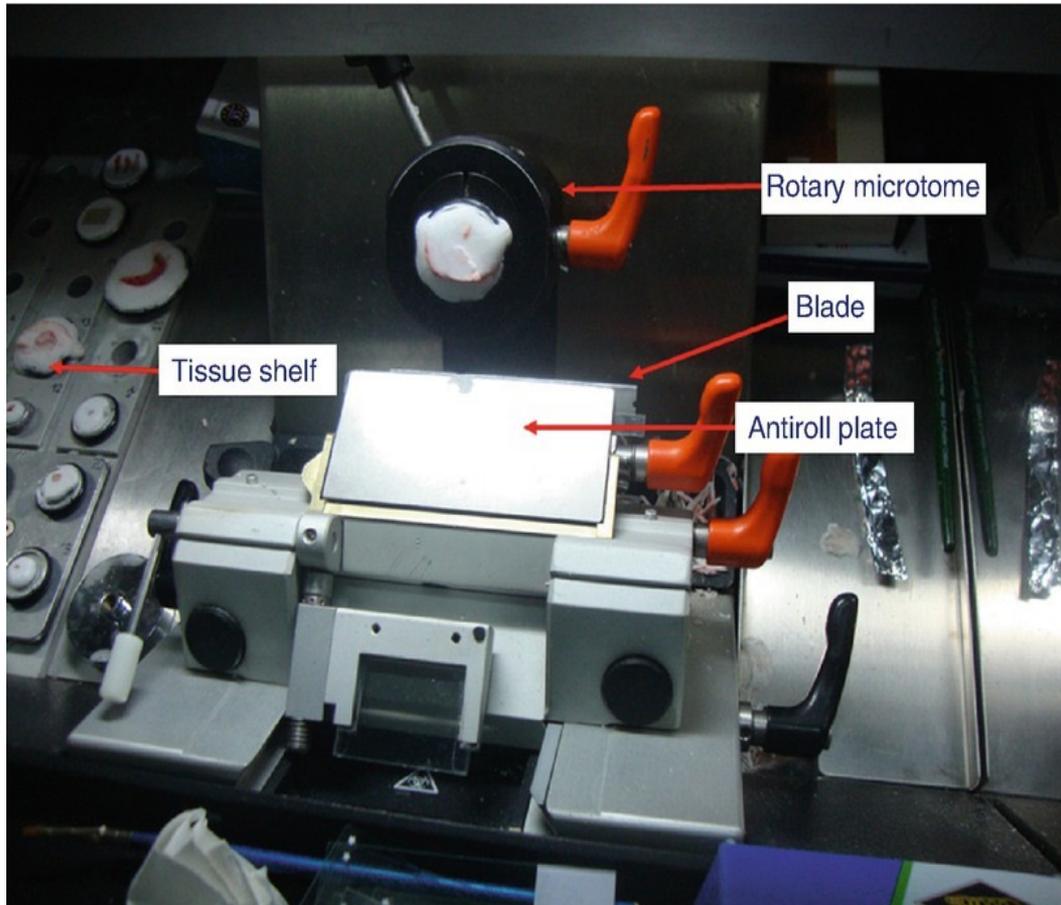
The Cryostat



Specimen holder is easily adjustable.

The chuck holds the tissue

The Cryostat



- Freezing shelf (tissue shelf) can hold 36 specimen discs (Chucks) at one time.
- Knife holder is fully adjustable with anti-rolling plate and blade guard.

Blade or knife

- Type of tissue and the procedures to be performed may dictate the use of a steel knife.
- A sharp edge is paramount in obtaining a quality frozen section. The microtome blade angle and block face angle should be closely monitored.
- Disposable blades have become routine in most clinical laboratories;
 - ✓ they produce a perfect edge and are instantly available.
 - ✓ rapidly cooled because of their size.

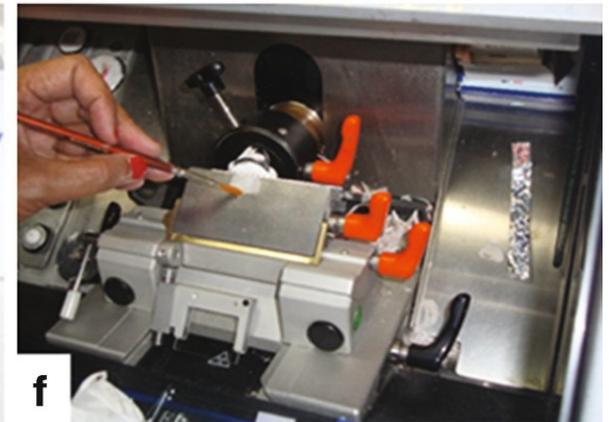
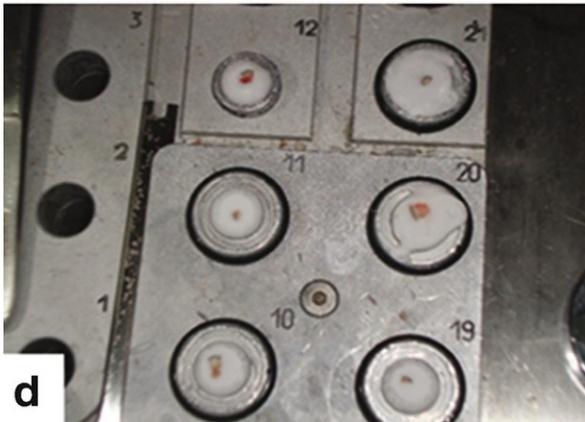
Anti-roll Plate

- Attached to the front of the microtome blade adaptor, is intended to stop the natural tendency of frozen sections to curl upwards on sectioning.
- Adjustments can be done to the anti-roll plate, it can be raised or lowered against the knife, to increase the angle between the knife and the anti-roll. It is the adjustments which determines the success of sectioning the tissue block.
- If the anti-roll plate is not working correctly, a sable hair brush can be used to manipulate the section.

Sectioning Technique

- Speed, tissue type, and temperature of the block and the cabinet play important roles.
- The cut section will rest on the surface of the blade holder after cutting; a room temperature slide is held above the section, where electrostatic attraction causes the tissue to adhere to the slide.

Cryostat Processing



(a) chuck is covered with OCT, (b) the tissue is now put on the chuck, (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

Cryostat Processing

Watch a video using this link:

<https://www.youtube.com/watch?v=tA7S75MFdNk>

Freeze Drying and Freeze Substitution

- Freeze drying and freeze substitution is too labor-intensive, time-consuming and capricious for clinical diagnostic use.
- These techniques are more commonly used in research.

Freeze Drying

- Freeze drying is the technique of rapid-freezing (quenching) of fresh tissue at -160°C , and the subsequent removal of water molecules (in the form of ice) by sublimation in a vacuum at a higher temperature (-40°C). The blocks are then raised to room temperature and fixed by vapor or embedded in a suitable medium.

There are four stages to freeze drying

1 - Quenching

- Instantly stops chemical reactions and diffusion in the tissue.
- It converts the tissue into a solid state in which unbound water in the tissue is changed into small ice crystals, which are subsequently removed in the drying phase.

2 - Drying

- Most time-consuming.
- Divided into three distinct steps:
 - i. Introduction of heat to the tissue to cause sublimation of ice.
 - ii. Transfer of water vapor from the ice crystals through the dry portion of the tissue.
 - iii. Removal of water vapor from the surface of the specimen.
 - i.e. a chemical trap containing a dehydrant chemical such as phosphorus pentoxide.

3 - Fixation and embedding

- Tissue is allowed to come to room temperature.
- The dried piece of tissue is extremely friable, and any undue pressure on it will cause the tissue to disintegrate into a fine powder.
- The delicate tissue is ready for embedding and sectioning or for fixing in a suitable vapor.

Vapor fixation

- A number of fixatives can be used in their vapor form, including formaldehyde, glutaraldehyde, and osmium tetroxide.
- Following fixation, the tissue is embedded in paraffin.

Applications of Freeze Dried Material

- Demonstrating fine structural details.
- Immunohistochemistry methods
- Demonstration of hydrolytic enzymes
- Autoradiography
- Mucosubstances
- Scanning electron microscopy
- Formaldehyde-induced fluorescence
- Proteins

Frozen Section Substitution

- Involves the rapid freezing of the tissue to -160°C in isopentane supercooled by liquid nitrogen.
- The sections are transferred to water-free acetone and cooled to -70°C for 12 hours.
- The sections are floated onto slides and allowed to dry.

Frozen Section Substitution...

- The histochemical method is applied. For most diagnostic purposes, cryostat sections preserve most tissue components

References

- Carson FL. **Histotechnology: a self-instructional text.** 2nd ed. Chicago: American Society of Clinical Pathologists Press; 1996.
- Suvarna, S.K, Christopher L, Bancroft J.D, **Bancroft`s Theory & Practice of Histological Techniques, 7th edition,** Churchill Livingstone, NY, U.S.A.