

Histopathological Diagnosis

PREPARATION OF HISTOLOGICAL SPECIMENS



CUTTING

- Using the **microtome**.
- A microtome is a mechanical instrument used to cut biological specimens into very **thin segments** for microscopic examination.
- Most microtomes use a steel blade and are used to prepare sections of animal or plant tissues for histology.
- The most common applications of microtomes are :
 - 1- Traditional histological technique:**
 - 2- Cryosection:**
 - 3- Electron microscopy:**
 - 4- Botanical microtomy:**




1- Traditional histological technique:

tissues are hardened by replacing water with paraffin. The tissue is then cut in the microtome at thicknesses varying from 2 to 25 micrometers thick.

2- Cryosection:

water-rich tissues are hardened by freezing and cut frozen; sections are stained and examined with a light microscope.

This technique is much faster than traditional histology (5 minutes vs. 16 hours) and are used in operations to achieve a quick diagnosis.



3- Electron microscopy:

after embedding tissues in epoxy resin, a microtome equipped with a glass or diamond knife is used to cut very thin sections (typically 60 to 100 nanometers).

Sections are stained and examined with a transmission electron microscope. This instrument is often called an ultramicrotome.

4- Botanical microtomy:

hard materials like wood, bone and leather require a sledge microtome. These microtomes have heavier blades and cannot cut as thin a regular microtomy.



These paraffin sections are then placed in a 60°C oven in order to melt the wax which helps to adhere the sections to the slide in readiness for the next stage of staining.



Parameters used in histological diagnosis of neoplasm

- Gross appearance
- Microscopic appearance
 - *histological pattern*
 - *tumor cell cytology*
- Immunohistochemistry
- Histochemistry
- Cytogenetics /molecular pathology
- Electron microscopy

The Frozen Technique

In this method, the fresh tissues are frozen hardened and cut with a freezing microtome in the cryostat apparatus **within few minutes.**

- It is a **quick and simple method** which is commonly used **during operations** for rapid diagnosis of tumors e.g. carcinoma.
- The chemistry of tissues is preserved because we use no heat and no chemical solvents.



The Frozen Technique

Can be used in Histochemistry or IHC to demonstrate enzymes and chemical components of tissues.

Disadvantage: It gives not-serial thick sections which may fragment into small pieces, so they are very difficult to be stained and to be stored.



Paraffin-embedded tissue vs Frozen section

	Paraffin-embedded tissue section	Frozen section
Specimens	Fixed tissues	Fresh tissues
Making time	24-48 hours	10-20 minutes
Saving time	Permanent	Months
Morphology under microscopy	Clarity	Opacity
Application	Pathological Diagnosis	Intraoperative consultation

IMPORTANT

- Reporting time: 20 minutes
- Specimen should be sent fresh; without formalin.
- In some case the pathologist may make touch preparation of the received specimen.

