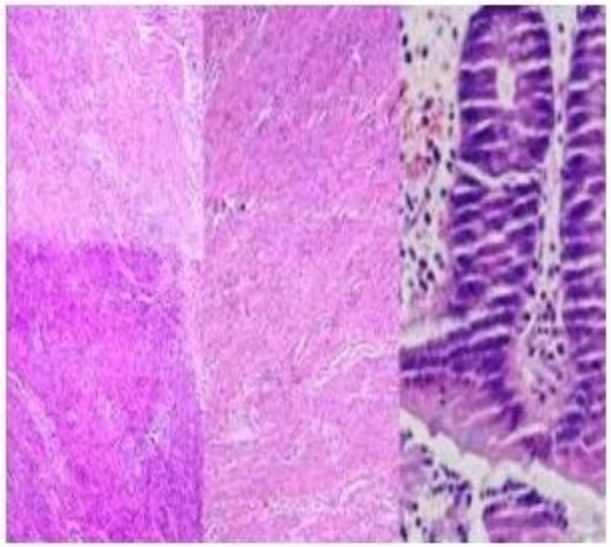
Histological techniques



By

Dr. Heba Sharaf Eldin

Assistant Professor of Histology & Cell Biology

ILos

1. Recognize the basic methods of studying histology.
2. Describe micro techniques used in LM (preparation & staining).

3. Describe microtechnique used in EM.

4. Compare between micro techniques used in LM & EM.

What *is* Histological techniques

Histological techniques

They are the methods by which histological sections are prepared for microscopic examination through series of processes

Preparation of tissues for study

- The *most common* procedure used in histologic research is the preparation of tissue sections or slices that can be studied with the light microscope.

- Under the light microscope, tissues are examined visually in a beam of transmitted light.

- Because most tissues and organs are too thick for light to pass through them, they must be sliced to obtain thin, translucent sections that are attached to glass slides for microscopic examination

What *is* the **ideal** tissue preparation method for microscopic examination

- That preserved the normal structure of the tissue on the slide

(has the same structure and molecular composition as it had in the body).

- However, artifacts, distortions, and *loss* of components due to the preparation process are often present

Basic histological methods

1. **Routine microscopic examination (LM & E/M)**
2. **Histochemistry & Cytochemistry**
3. **Immunocytochemistry**
4. Autoradiography
5. **Cell & Tissue Culture**
6. Hybridization techniques
7. Exfoliative Cytology
8. Cytogenetics
9. Bone Marrow smear **/ Blood film**
10. Fine needle aspiration / biopsy

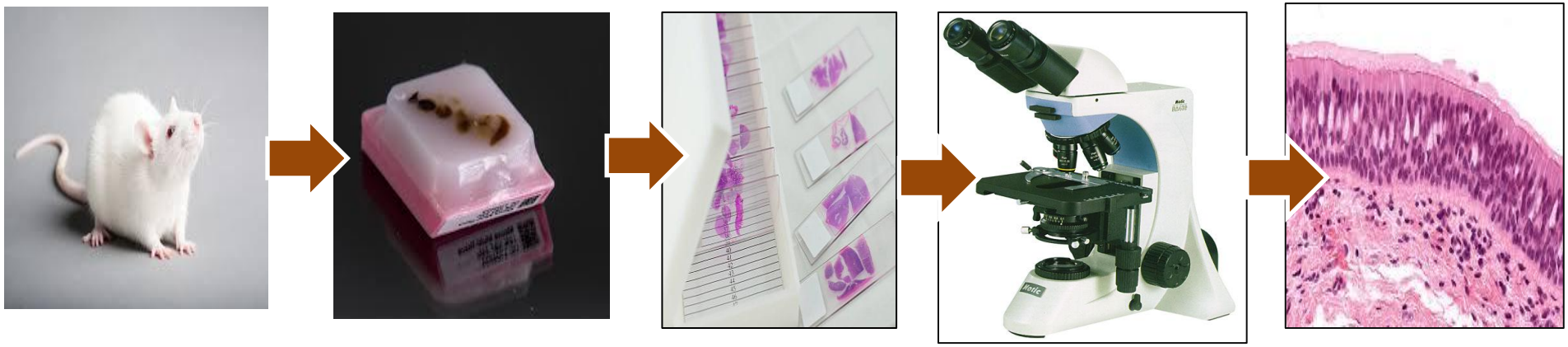
Steps of Preparation & Examination of Histological Sections

1- Sample collection

2- Tissue processing and sectioning

3- Staining

4- Microscopic examination.



Sample collection

Obtaining the tissue:

- Fresh piece of tissue from experimental animal (**rat**, mice, gunia pig, rabbit).
- Very small sample 0.5 cm (less than 1 cm) *to* allow entrance of fluids during procedure.

- **types:**

-Autoposy (Taken immediately after death).

-Surgical procedures

- **methods**

-Under anaesthesia

-Decapitation

Tissue processing and sectioning

Tissue processing

Replacement of all tissue water by medium to provide rigidity to tissue to enable adequate sectioning

Sectioning

Cutting the tissue into small slices

Tissue processing

- **Aim** : Replacement of all tissue water by medium to provide rigidity to tissue to enable adequate sectioning.
- **The used medium**:

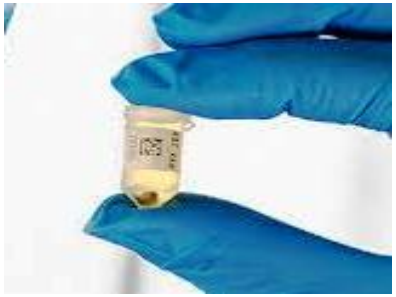
Differ according to the method of preparation &the microscope used for examination

For L.M examination:

- **Paraffin technique *(most common) :* Paraffin wax**
- **Celloidin technique : Celloidin**
- **Freezing technique**

For E.M examination:

Resin (Epon **)**

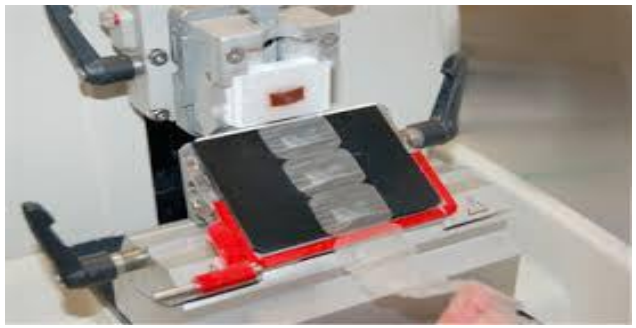


Sectioning

Cutting the tissue into thin sections by:

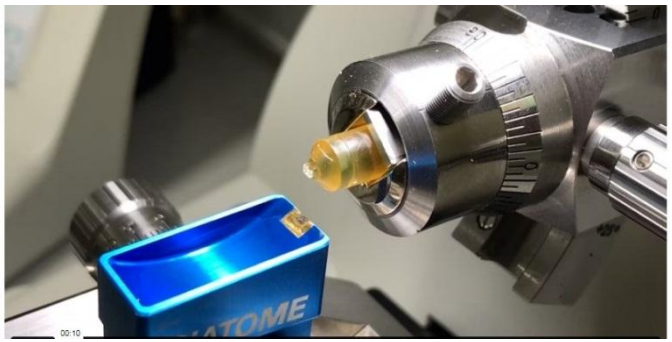
For L.M examination:

Rotatory microtome (paraffin sections)



For E.M examination:

Ultramicrotome (Resin sections)



Tissue processing for paraffin method

The most common procedure.

Can be divided into the following steps:

1-Tissue sampling

2- Fixation

3- Dehydration & clearing

4-Impergnation & embedding in paraffin

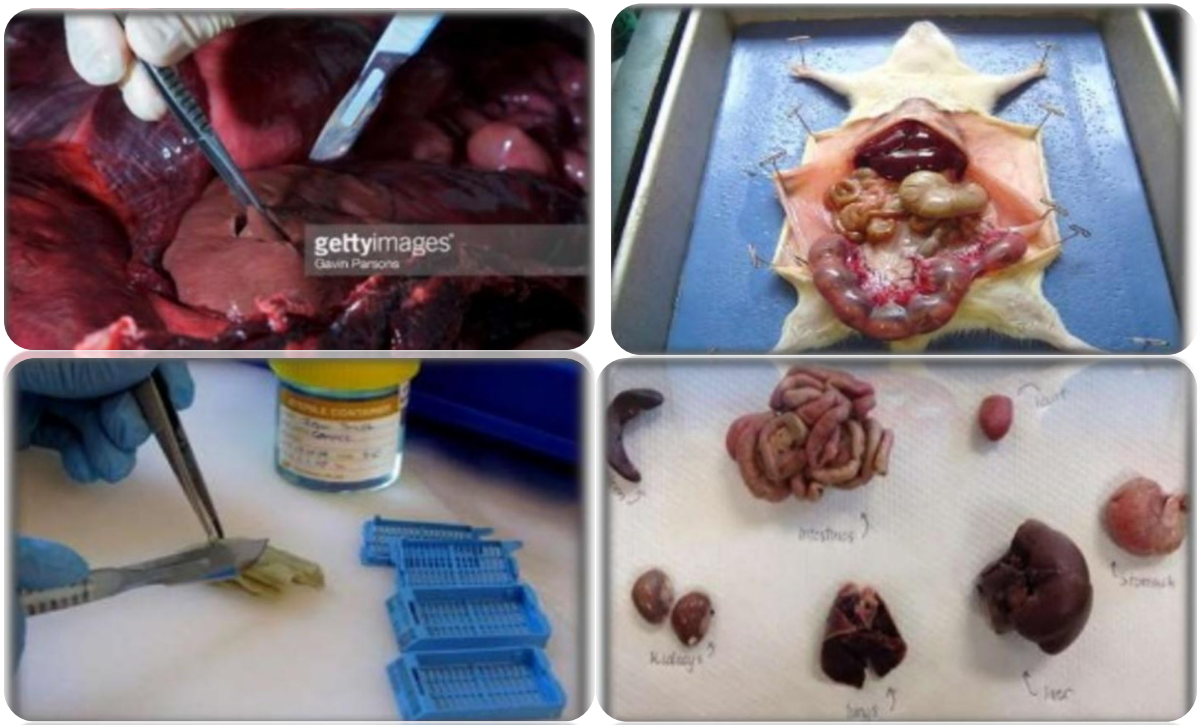
5-Sectioning with a microtome

6- Mounting on microscope slides

7-Staining

Tissue processing for paraffin method

1-Obtaining the tissue: very small and fresh.



Tissue processing for paraffin method

2-Fixation:

treatment of tissues by **putting them in a fixative**(chemical or mixture of chemicals).

Aims:

- Harden the tissue to help in section cutting.
- Coagulates tissue proteins so preserve the cellular structure of the tissue close to its natural state & preserve the relations of tissue components.
- Prevent enzymatic digestions of cells by autolysis
- Increase affinity of tissue for stains.

Types of fixative:

- Simple fixatives: e.g. **formol saline** (formalin 10%) ***(most common).***
- Combination of Simple fixatives: e.g. Bouins.

Duration: 24 hours

Tissue processing for paraffin method

3-Dehydration

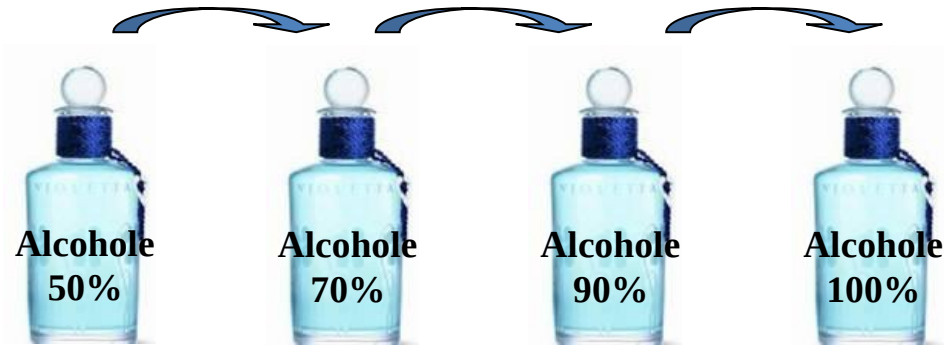
-Aim:

to remove excess water (why ????)

-Who?:

Putting the tissue pieces in **ascending grades of alcohol**(50%- 7o%- 90%) each for 30 m then **absolute alcohol** for 1 h. **Why???**

***Gradual dehydration*:** To prevent tissue shrinkage



Tissue processing for paraffin method

4- Clearing

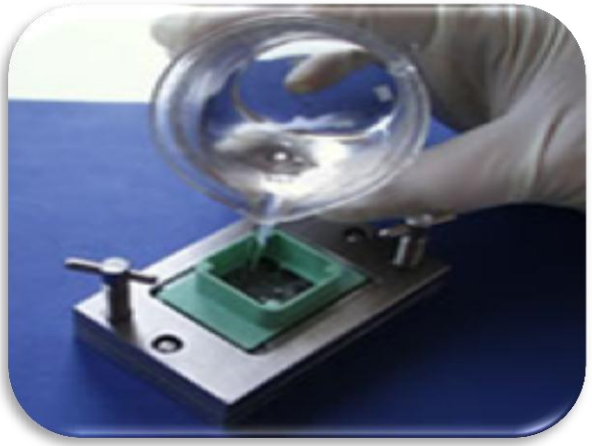
Aim: to replace alcohol

By: **Xylol**

For: 2 h (until tissue is translucent)

Tissue processing for paraffin method

5- Impregnation



By: melted soft paraffin wax

How: several change in oven

For : 2 h for each path

Aim:

-to replace xylol

- permeates the tissue and harden it from *inside*

Tissue processing for paraffin method

6- Embedding

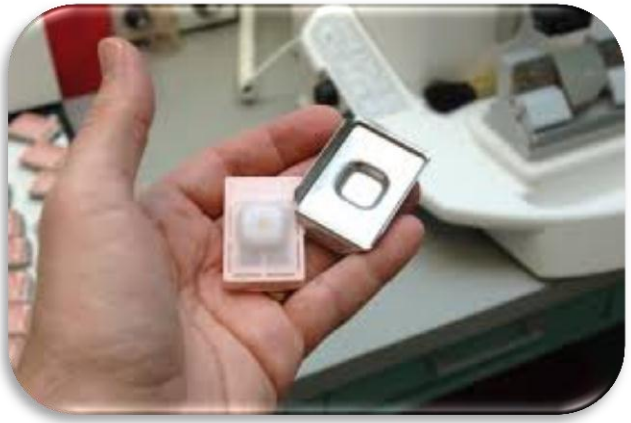
By: melted hard paraffin wax

Aim:

-to harden the tissue *from outside* forming paraffin **block**

- make thin section easy to cut

-preserve tissue for years

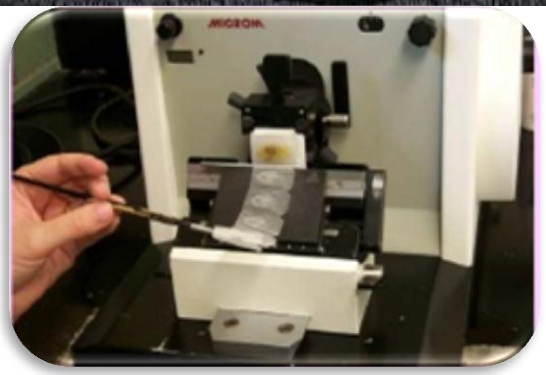


Tissue processing for paraffin method

7- section cutting

By: Rotatory microtome

Blocks are cut into thin sections (3-10micron).



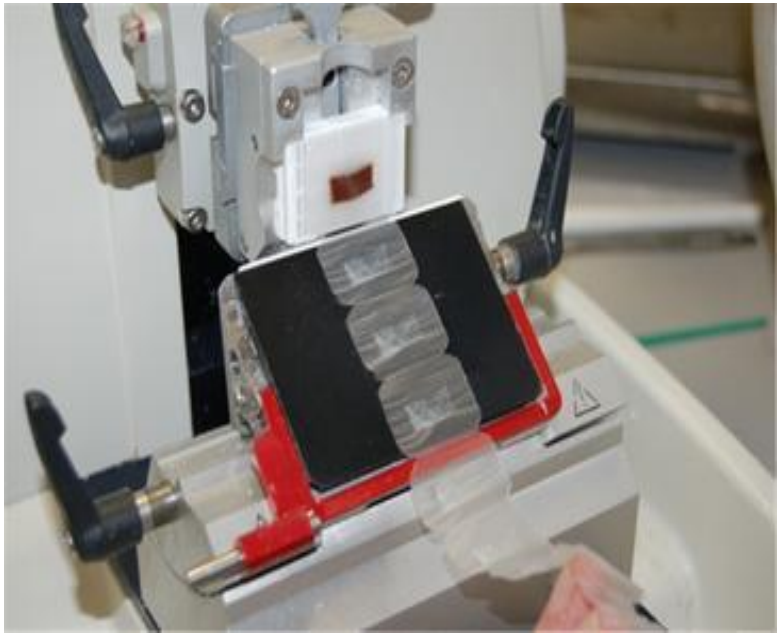
Tissue processing for paraffin method

8- Mounting sections

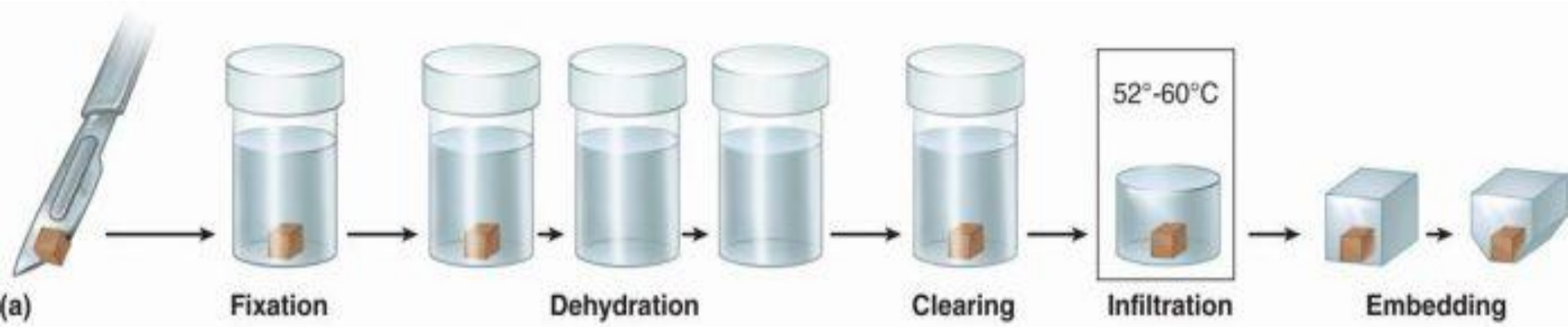
How???

-the sections are permanently attached to individual glass microscope slides.

-Slides are warmed on a hot plate, then dried in an incubator to be ready for staining .



Tissue processing for paraffin method



MEDICAL LABORATORY SCIENTIST
MLS

Tissue processing for celloidin method

- **Embedded :**in celloidin instead of paraffin
- **Cutting:** using sliding microtome.
- **When used:** Very large pieces…used for **brittle material**

Freezing technique

- **When used?**

- An urgent diagnosis is needed (during surgery)

-Enzyme & Lipids staining (histochemical study of very sensitive enzymes or small molecules)

- **Tissue sample:** Biopsy
- **Processing:** The biopsy is rapidly frozen in liquid nitrogen
- **Sectioning :**-microtome called a cryostat (subfreezing temperature is used)

-frozen sections are placed on slides for rapid staining and microscopic examination by a pathologist.

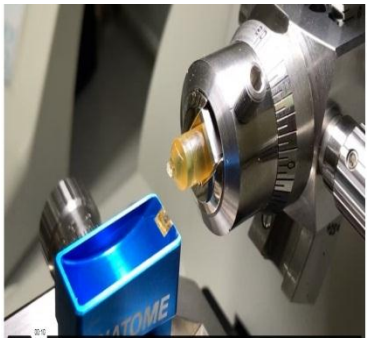
Preparation of sections for transmission electron microscope (TEM)

The *same* steps as in light microscope with some modifications

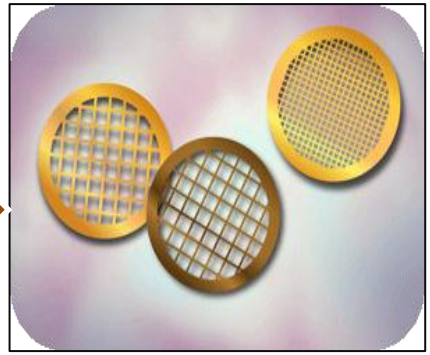
Tissue sampling	The piece of tissue must be very small (1 mm 3)
Fixation	**Glutaraldehyde**, then postfixed in **osmic acid**
Dehydration	in ascending grades of alcohol or acetone
Clearing	propylene oxide
Embedding	In epoxy resin
Sectioning and cutting	ultramicrotome with glass or diamond knives sections (50 - 100 nm)
Staining	salt of heavy metals
Mounting and examination	on copper grids

Preparation of sections for transmission electron microscope (TEM)

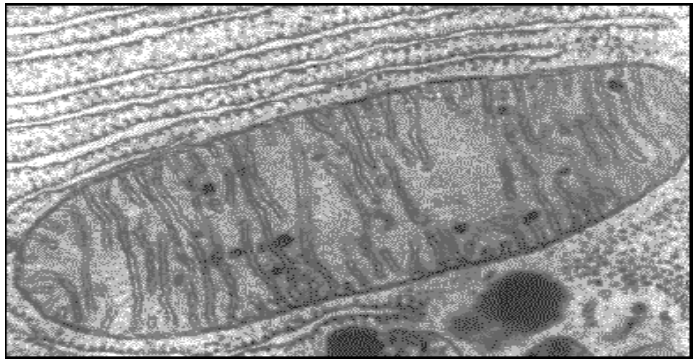
Epoxy resin



ultramicrotome



copper grids

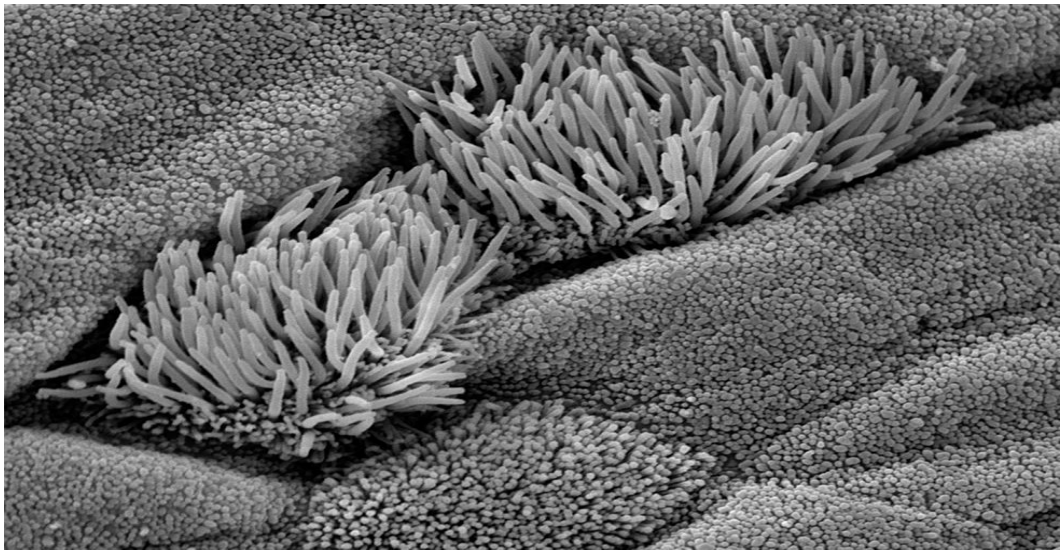


Technique :	L/ M	E / M
Fixation	Formalin 10%	Glutaraldhyde 4% & osmic acid
Clearing	Xylol	Propylene oxide
Embedding	Paraffin	Epoxy
Cutting	By metal knife	By glass knife or diamond knife
Thickness	Up to 10 microns	(50 - 100 nm)
Staining	Depends on colour	Depends on contrast
Spreading	Upon glass slide	Upon cupper grids

Scanning electron microscope (SEM)

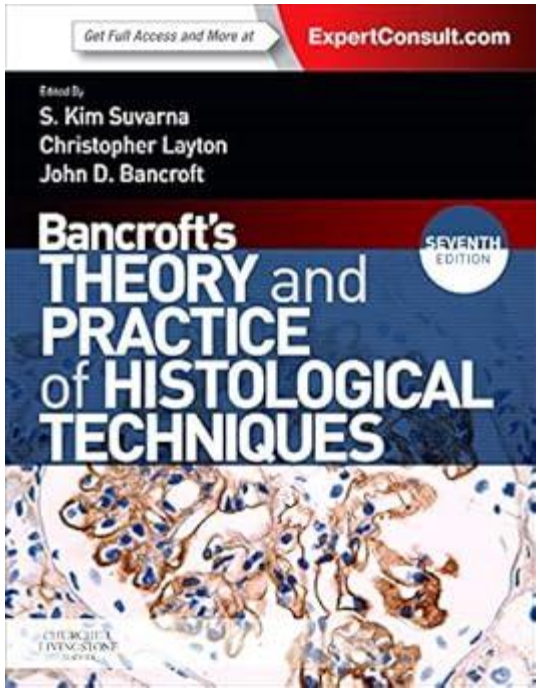
Specimen: *not be small* as in TEM

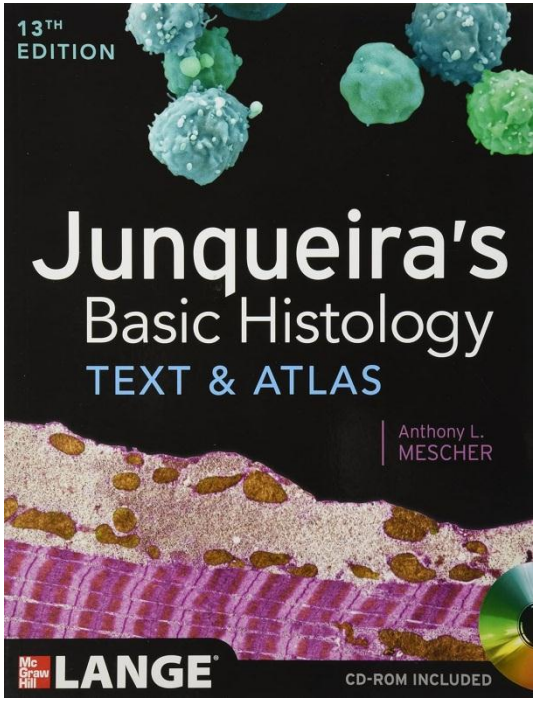
- More simple preparation
- The sample is *gold coated (3D)*
- It give data about the external surface (e.g. cilia)

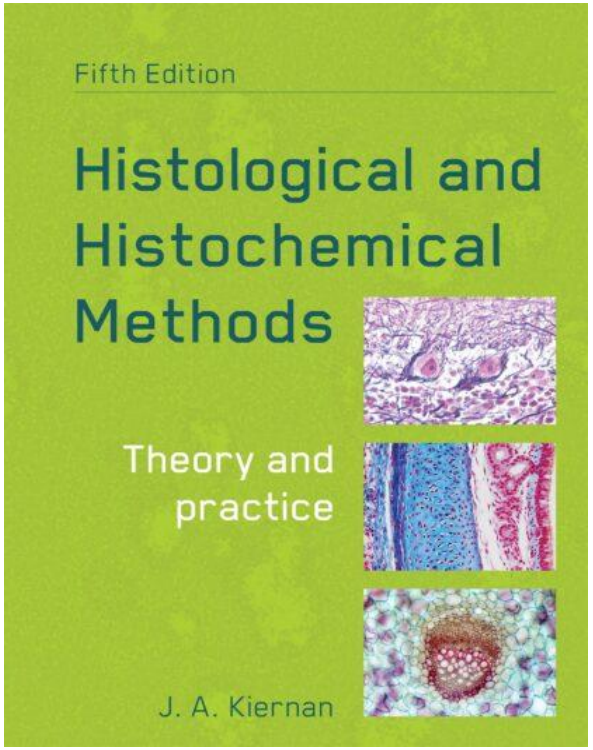


References

Text books







Web sites

www.histology-world.com

https://www.bbc.co.uk/bitesize/articles/zrp3ydm#znkd96f

TEST YOURSELF!

Which of the following is used for dehydration step in paraffin sections preparation?

1) Descending grades of alcohol
2) Ascending grades of alcohol
3) Xylol
4) Immersion in water

Which of the following techniques is used in operating room?

1) Paraffin technique
2) Celloidin technique
3) Freezing technique

Thank you