Histology

premed- JU 2018

◉ Sheet ○ Slides

Number

2

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In the previous lecture we talked about **Histology** which is the branch of science that deals with the microscopic study of normal tissue.

- Another important concept we must know which is **Histopathology**

What is Histopathology?

- Histopathology is the branch of science which deals with the microscopic study of the affected by disease.

Histopathology is the diagnosis of disease through Histology –through microscopy-

- **The tissues that we use for study can be obtained from**:
- **Biopsies**: Living tissue
- **Autopsies**: Dead tissue

→ In the case of Histopathology, we use **Biopsies**.

- We prepare our samples -Biopsies- using **Microtechniques**.

★ **Microtechniques:** is tissue preparation for microscopic examination.

Microtechniques usually involves hardening of the tissue followed by sectioning -cutting-.

- We have two types of techniques that we use:

1)Paraffin technique: This technique ends in sample embedded in paraffin wax.

→ So, when you hear the word **Paraffinize tissue** this means that we prepare it using the Paraffin technique.

→ During the Paraffin techniques tissues are hardened by replacing water with paraffin wax.

2)Freezing technique (Cryosections): Water-rich tissues are hardened by freezing and then when they are frozen, we cut them.

- Freezing techniques are Faster than traditional histology (20 min. VS 16 hrs.)
- We use Freezing techniques in biopsy during surgeries Rather than Paraffin techniques to make quick decisions.

Why we use Freezing techniques rather than paraffin techniques?

- Because the paraffin techniques take a long time and they aren't useful for making quick decisions, whereas in freezing techniques we snap freeze - immediately freeze- our specimen by throwing it into liquid Nitrogen.
  - We can also use Freezing techniques in **Immunohistochemistry** (immunofluorescence).

Why do we use freezing techniques in Immunohistochemistry??

- Because freezing tissue doesn't alter or mask it's chemical composition.

What do we mean by immune technique??

- It is a technique we use when we have some proteins that have antigens on them and we want to visualize those antigens, so to see the antigen an antibody must attach to the antigen, then if the antibody is attached to the antigen and gave you a signal, you'll notice it there.
- But if we use the normal fixation techniques (Paraffin techniques) the antigen will be crosslinked with another antigen (because it's a protein), so the antibody will not be able to bind to that antigen, and we will not be able to visualize this antigen.
- So, to avoid crosslinking with other antigens we use freezing techniques rather than paraffin techniques.

- **So, in general we use Freezing techniques:**

1- If we need quick decisions.
2- If we need to do immunohistochemistry.

Steps used for preparing tissues in Histotechniques (Paraffin techniques):

1- Identification and labeling of the specimen.
2- Fixation
3- Dehydration.
4- Clearing.
5- Impregnation (infiltration).
6- Embedding.
7- Section cutting.
8- Staining.
9- Mounting.

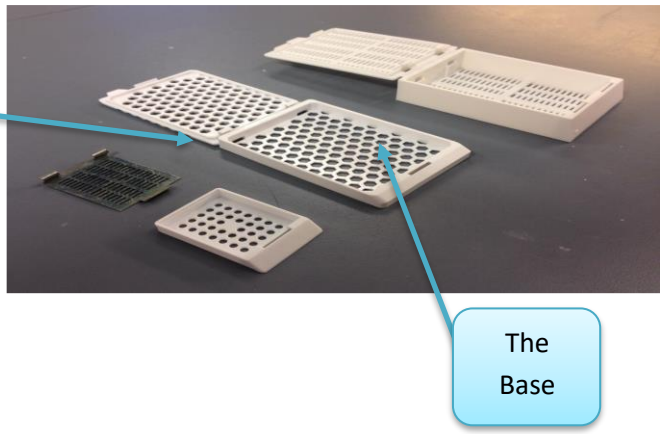
❖ Notice that the first step that we should do before any step is **Identification and Labeling**, if we don't identify our sample, we will lose our work.

- In labeling we must write three parts of the patient's name, in addition to the hospital number.
- This is a very important step because in Histopathology we deal with hundreds of samples at the same time, and if you don't identify your samples you will lose them.

➢ **Cassette**:

- We do all the sample preparation processes in a tool called a Cassette.
- This Cassette has many pores so that the materials can enter to the sample.
- The Cassette is made up of 2 parts:

1- **The Base**
2- **The cover**



So, you but your specimen in the Base of the cassette and then close it by the cover then you start the **Fixation**

❖ **Fixation**:

- It's a process by which the constituents of cells and tissues are fixed in physical and chemical state so that they will withstand subsequent treatment with various reagents with minimum loss of architecture.
- Fixation is achieved by exposing the tissue to chemical compounds called: **Fixatives**

Fixatives: Chemical compounds that prevent autolysis and bacterial decomposition and preserves tissue in their natural state and fix all components.

- We have Different types of tissue Fixatives:

✓ For **Light microscope** → We use **Paraformaldehyde** (Formalin).
✓ For **Electron microscope** → We use **Glutaraldehyde**
✓ For **Electron microscope** in addition to Glutaraldehyde we use → **Osmium tetroxide**

Osmium tetroxide has 2 Functions

- ✓ **Fixation**
- ✓ **Staining** (Osmium is a metal, so if you add it to the material it will stain for electron microscopy)

➡ Because Staining is additional step of fixation, we call it **Post-Fixation,** and we call Osmium **Post-Fixative**.

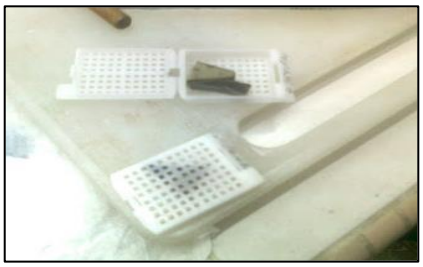
➡ **So, in general for electron microscopy we have 2 steps:**

- ➢ The first is with Glutaraldehyde.
- ➢ And the second is with Osmium tetroxide.

➡ Notice that no Fixative will penetrate a sample thicker than **1 Cm**

- ➢ We Call the process of cutting → **Grossing**
- ➢ In Grossing we section our sample to smaller parts so that the fixative can penetrate them

Specimen is placed in cassette



Cassettes are collected in Fixatives

- **10% Formalin**= 10ml paraformaldehyde + 90ml of water.

❖ **Tissue Processing**:

- In order to cut thin sections of the tissues, it should have suitable hardness and consistency when presented to the knife edge.
- These properties can be imparted by infiltration and surrounding the tissue with:

✓ Paraffin wax
✓ Various types of resins
✓ Freezing

Tissue processing can be subdivided into:

1-Dehydration **2- Clearing** **3- Impregnation (Infiltration)**

❖ **Dehydration**:

- It's the process in which the water content in the tissue to be processed is completely removed by passing the tissue through increasing concentration of dehydration agents.
- Tissues are dehydrated by using increasing strength of alcohol.
- Water is replaced by **Diffusion.**
- During Dehydration water in tissue has been replaced by alcohol.
- Then in the next step alcohol should be replaced by paraffin wax

⇒ Because paraffin wax is not alcohol soluble, we replace alcohol with a substance in which wax is soluble, this step is called: **Clearing**

❖ **Clearing**:

- Clearing is replacing the dehydrating fluid with a fluid that is totally miscible with both the **dehydrating fluid** (alcohol) and the **embedding medium** (wax)

➢ **Some of the clearing agents:**

1- Xylene
2- Toluene
3- Chloroform
4- Benzene

❖ **Impregnation**: (Infiltration)

- In this process the tissue is kept in a wax bath containing molten paraffin wax.

⇒ The duration of the procedure can be noted down as

➢ **Fixation:**

1- 10% Formalin saline (I) → 1.5 hrs.
2- 10% Formalin saline (II) → 1.5 hrs.

➢ **Dehydration:**

1- 80% alcohol → 1 hour
2- 95% alcohol (I) → 1 hour
3- 95% alcohol (II) → 1 hour
4- Absolute alcohol 100% (I) → 1 hour
5- Absolute alcohol 100% (II) → 1 hour
6- Absolute alcohol 100% (III) → 1 hour

➢ **Clearing:**

1- Xylene (I) ⟶ 1.5 hrs.

2- Xylene (II) ⟶ 1.5 hrs.

➢ **Infiltration:**

1- Paraffin Wax (I) ⟶ 1.5 hrs.

2- Paraffin Wax (II) ⟶ 1.5 hrs.

⟹ **We can do Tissue processing by 2 methods:**

➢ **Manual Tissue processing**: Using my hand, I remove the cassette from one material into the other.

❖ We start with Formalin, then we go to the alcohol (70% Ethanol), then to the second alcohol (80% Ethanol), then to the third alcohol (90% Ethanol), then to the fourth alcohol (100% Ethanol), then to the clearing material (Xylene), and then the Wax.

➢ **Mechanical Tissue processing**: In this method we just put the cassette in the first jar then a mechanical arm (mechanical device) move it from one jar to the other.

- Temperature is maintained around 60^{O} C

Tissue Baskets

Tissues Processor

❖ **Embedding**:

- It's a process by which impregnated tissues are surrounded by a medium such as agar, gelatin, or Wax which when solidified will provide sufficient external support during sectioning.

⟹ The main difference between infiltration and Embedding is that Embedding is External (Happening outside the tissue), whereas Infiltration is Internal (Happening inside the tissue).

So, when we have wax **inside the tissue** → **Infiltration**

Whereas, when we have wax **outside the tissue** (in a mold) → **Embedding**

- Embedding is done by transferring the tissue to a mold filled with molten wax which can cool and solidify
- After solidification a wax block is obtained which is then sectioned to obtain ribbons of sections.

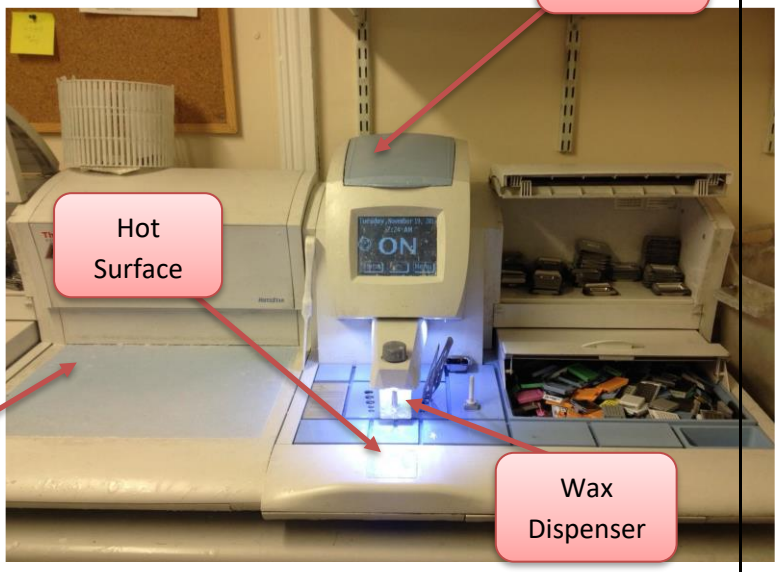
For Embedding we need three main things:

1- The Paraffin Wax (it's solid at first and we need melt it).
2- The molds.
3- The Embedding center (Embedding Station).

Paraffin Wax

The molds

The Embedding Center



The parts of the Embedding Center:

1- Wax Reservoir: We take the solid wax and put it in this reservoir, then this container is heated to 60° C allowing the wax to melt.
2- Hot surface: we bring the mold and put it on this surface down the wax dispenser.
3- Wax dispenser: This dispenser dispense wax into the mold, then we orient the sample in the proper way
4- Cold plates: Then we put the mold on the cold plates allowing the wax to solidify.

➢ **The important functions of Embedding:**

1- **Easier Handling** : I can easily hold the block and take it for sectioning.

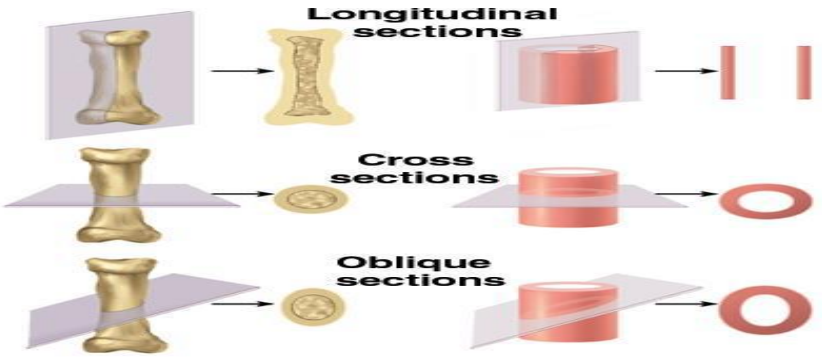
2- **Orientation** : I want to orient my sample in the most efficient way for my particular need.

➢ **Types of tissue sections:**

1- **Longitudinal section**: Tissue cut along the longest direction of an organ.

2- **Cross section**: Tissue cut perpendicular to the length of an organ.

3- **Oblique section**: Tissue cut at an angle between a cross and longitudinal section.



➢ Orientation of Tissues in the block :

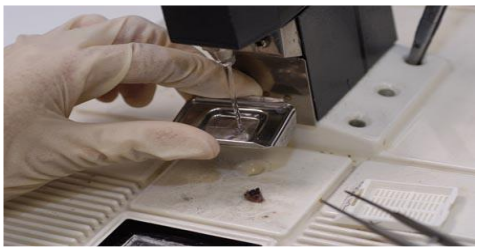
- Correct orientation of tissue in a mold is the most important step in embedding.

The orientation of tissue can be in either **Cross section** or **Longitudinal section**

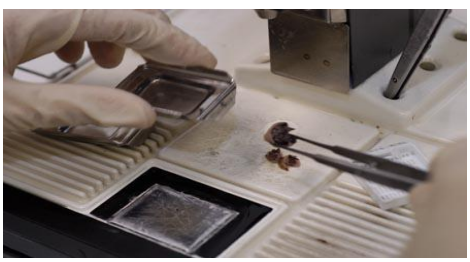
- Incorrect placement of tissues may result in diagnostically important tissue elements being missed or damaged during microtomy.

➡ **General Embedding Procedure:**

1- Fill the mold with paraffin wax.



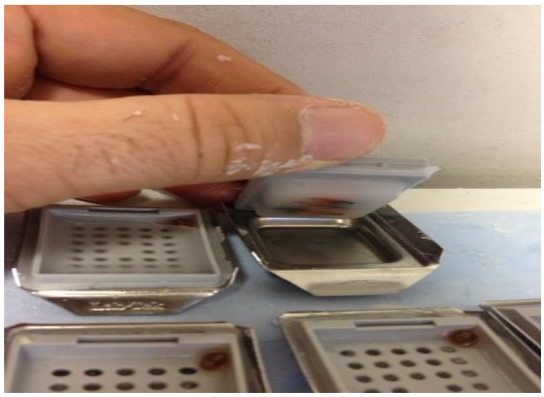
2- Using the warm forceps select the tissue, take Care that it does not cool in the air



3- Orienting the tissue in the mold.

4- Cool the block on the cold plate.

5- Remove the block from the mold.



6- Blocks of embedded tissue are usually trimmed to remove the excess wax on the surface.

❖ **Trimming**: Gresley cutting your sample so that you remove the excess wax and you reach your sample of interest.

❖ **Sectioning**:

- It's the procedure in which the blocks which have been prepared are cut or sectioned and thin strips of uniform thickness are prepared.

➢ For sectioning we use an instrument called ⟶ **Microtome**.

➢ **We have different types of microtomes:**

1- **Rotary microtome.**
2- **Freezing microtome.**
3- **Ultra-microtome.**

★ Rotary microtome:

- It's an instrument used for **light microscopy** preparation to get **thin sections**.

How does the Rotary microtome work?

- The rotary microtome has a handle, when we rote the handle, the sample will move up and down and a bit forward to reach the knife so sectioning can begin.

Sectioning (Ribbon of sections)

★ **Freezing microtome**: (Cryostat)

- We use this microtome for **Cryo sections** (Frozen sections).

★ **Ultra-microtome**:

- It's an instrument used for **Electron microscopy** preparation to get **Ultra-thin sections**.
- The typical thickness of tissue cut is between 20-100 nm for Transmission Electron Microscope (TEM)

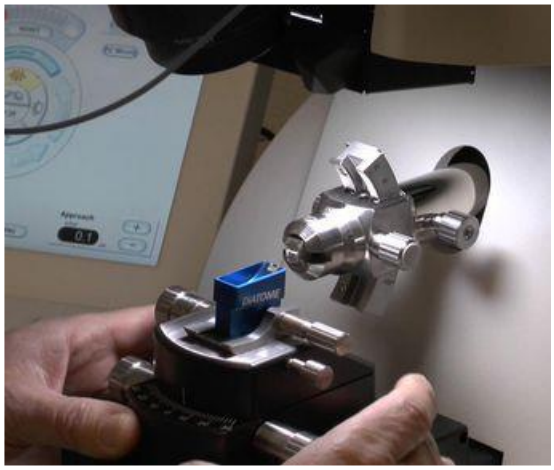
For Ultra-microtome we have different types of knifes:

1- **Glass knife** : Cheaper.
2- **Diamond knife** : More expensive.

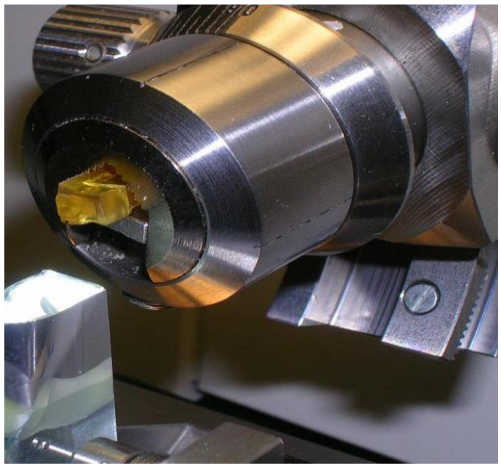
➢ When we need a very thin sections and we need to magnify to a large degree we must use the **Diamond knife**, Why??

Because the glass may leave some marks on our sample (Because the glass isn't very sharp).

Ultra-microtome



Diamond Knife

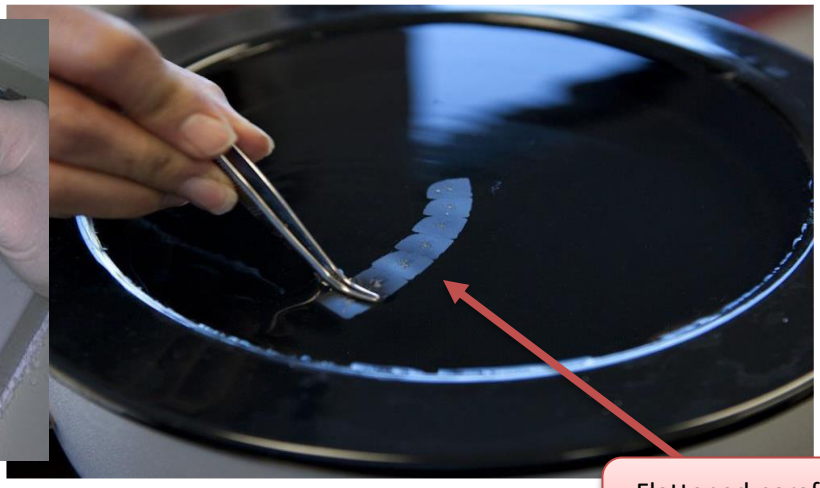


Glass Knife

➡ In the next step we move the ribbon into a **thermostatically controlled water path.**

- This water path must be maintained at a temperature 5-6 degrees below the melting point of the paraffin wax (it must be at 50-54° C), so that when you put your sample on the slide it will be flat not wrinkled (it's like ironing your section)

Taking the **Floating** sections onto the slide



Flattened paraffin sections

- Then we take the section on the slide, notice that the section is flat , no air no stretch or breaks.

➢ One of the adhesives used for fixing the sections on the slides → **Albumin solution.**

❖ **Staining**:

- Staining is a process by which we give color to a section.

Why do we do staining??

✓ To bring out the particular details in the tissue under study.

- We have many types of stains that can be used, but the most common stains that are used for Light microscopy → **Hematoxylin & Eosin**.

➡ Stains can be classified into 2 classes:

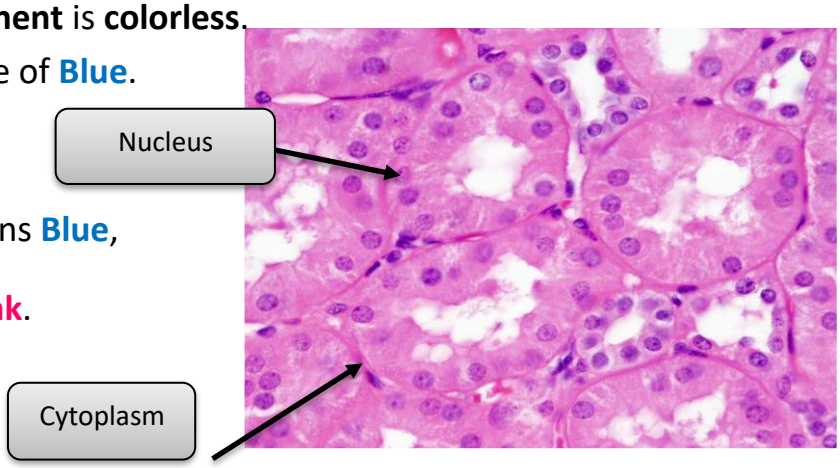
1- **Acid Stains** : (Ex: Eosin)
2- **Basic Stains :** (Ex: Hematoxylin)

★ **Acid Dyes:**

- We use Acid Dyes (Acid Stains) to stain **basic components** of the sample, Ex: Eosin stains Cytoplasm.
- When we add **Acid Dyes** to the sample → **The Basic Component** is **colored** whereas the **Acid Component** is **colorless.**
- The color imparted is shade of **Red**/**Pink.**

★ **Basic Dyes:**

- We use Basic Dyes (Basic Stains) to stain **acidic components** of the sample, Ex: Hematoxylin stains nucleus.
- When we add **Basic Dyes** to the sample → **The Acid Component** is **colored** whereas **The Basic Component** is **colorless**.
- The color imparted is shade of **Blue**.



➡ Notice that the **nucleus** stains **Blue**, whereas the **cytoplasm** stains **Pink**.

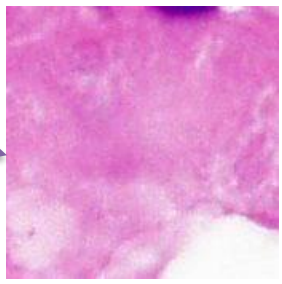
- **We have 2 types of staining:**

1- **Manual Staining**.

2- **Automatic Staining**.

➡ So, we start staining by putting the sample in the Hematoxylin so that the nucleus will be stained, then we remove it and wash it to remove excess Hematoxylin, then we put it in the Eosin so that the Cytoplasm will be stained, then we remove it and wash it to remove excess Eosin.

Look at this picture, why we cannot see any nucleus in this cell??



- Because the level of the section was either above or beyond the level of the nucleus.

Manual Staining

Automatic Staining

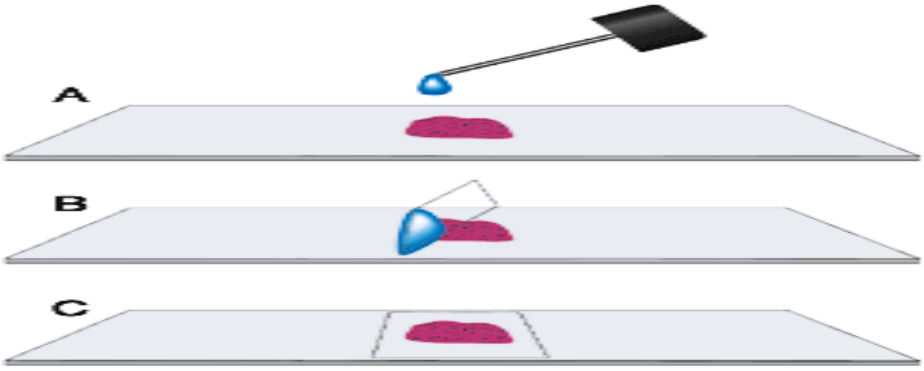
❖ **Mounting**:

- It's a process in which the stained section on the microscope slide is mounted using mounting medium dissolved in Xylene.
- The most common **Mountant** (mounting media) that we use is **DPX** (Distrene Dibutyl phthalate Xylene).

Why do we specifically use DPX??

- Because the diffraction index of this material is close to the diffraction index of the glass

➡ During Mounting we first put **a drop of the mountant** on the slide then we cover the slide using a **Coverslip** to protect the sample.



All the previous steps were to the light microscopy, What about Electron microscopy?

1- In Electron microscopy we use **Electron beam** instead of **light**.
2- We use **Glutaraldehyde** instead of **Paraformaldehyde** as a Fixative.
3- We use **Ultra-microtome** instead of **Microtome**
4- We use **Propylene Oxide** instead of **Xylene**.
5- We use **Resin** instead of **Paraffin wax**.
6- We Produce **Ultra-thin sections** (0.02-0.1 µm) instead of **Thin sections**.
7- We use **Metal grid** (Metal Mesh) instead of **Glass Slides**.