Preparation of tissues for study

❖ HISTOLOGY :

Is the branch of science which deals with microscopic study of **normal tissue** .

❖ HISTOPATHOLOGY :

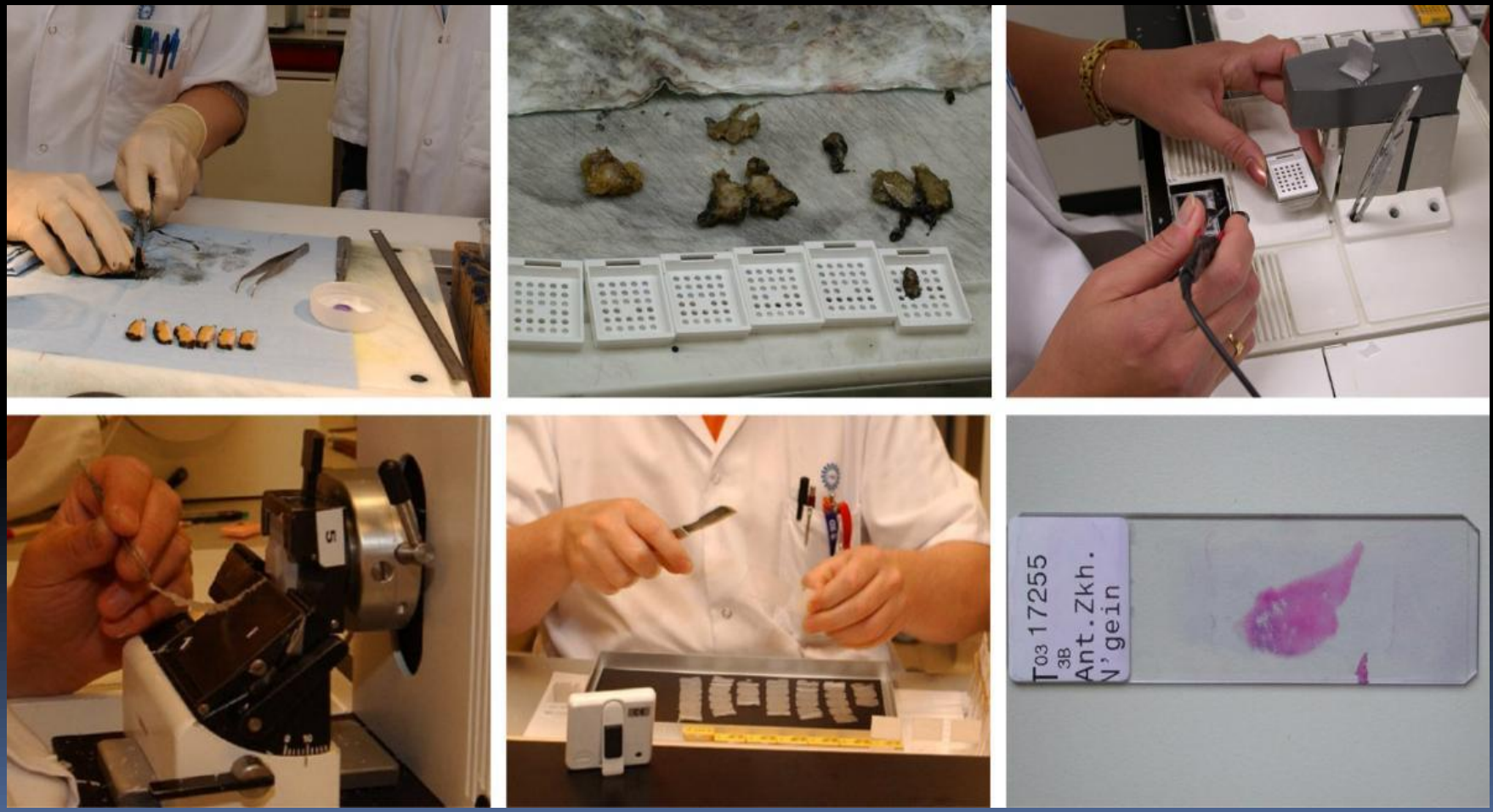
Is the branch of science which deals with microscopic study of **tissue affected by disease.**

Tissue can be obtained from:

- Biopsies
- Autopsies

Microtechnique

- Microtechnique : is tissue preparation for microscopic examination.



Microtechnique

- Most tissues are thick , so they must be sliced to obtain **thin , translucent sections.**

- It usually involves hardening of the tissue followed by sectioning (cutting).

- There are different methods used, however the basic principles are similar.

- **Paraffin technique**
- **Freezing technique**

Histological Techniques:

1. Paraffin technique:

- Tissues are hardened by replacing water with **paraffin**.
- The tissue is then cut in the microtome at thicknesses varying from 2 to 25 μm thick.
- The tissue can be mounted on a microscope slide, stained and examined using a light microscope.

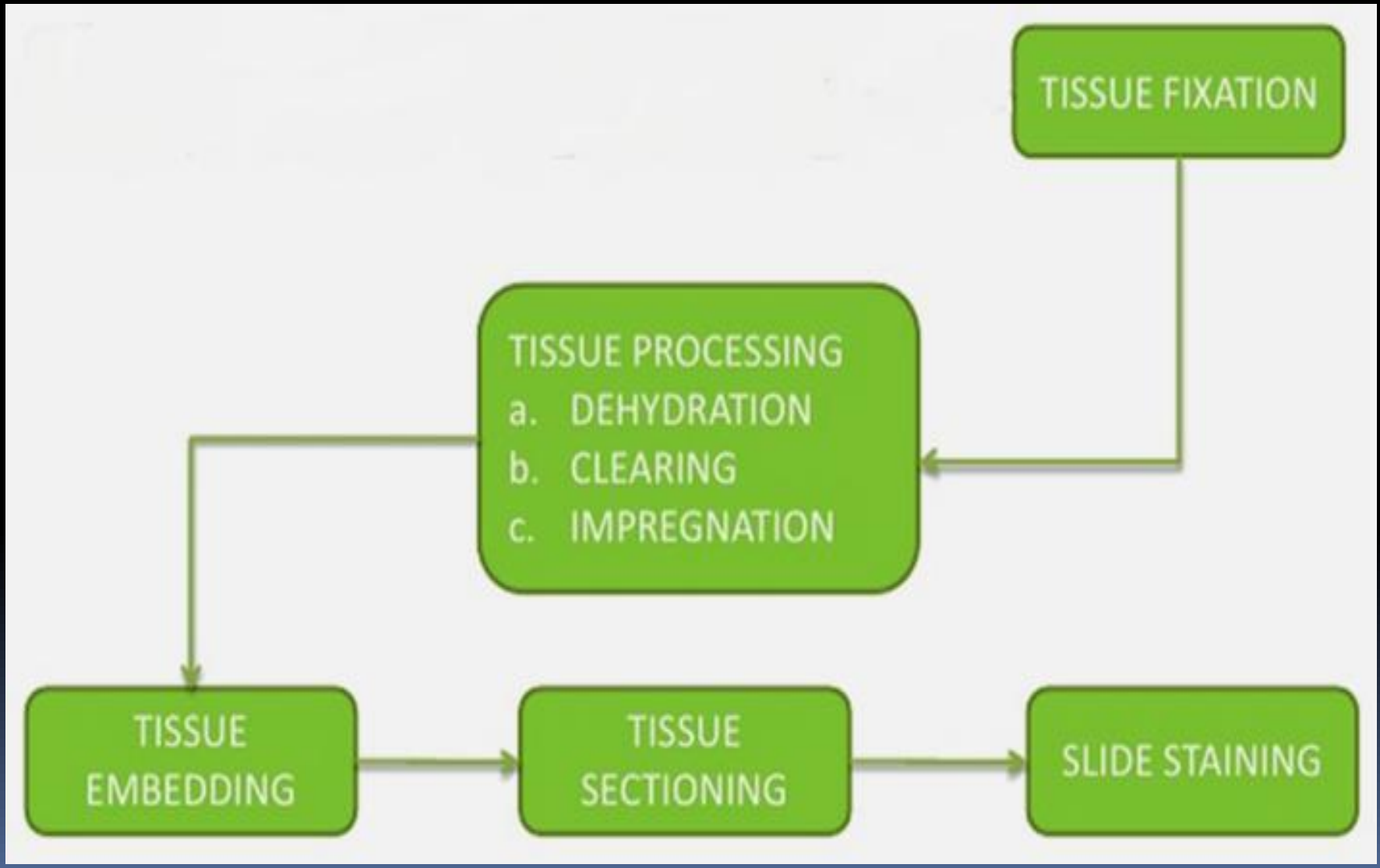
2. Freezing technique:

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

- This technique is **much faster** than **paraffin** technique (5 -20 minutes vs. 16 hours).
- Are used in **operations** to achieve a quick diagnosis.
- Cryosections can also be used in **immunohistochemistry** as freezing tissue does not alter or mask its chemical composition as much as preserving it with a **fixative**.


fixation
HISTOLOGICAL TECHNIQUE
clearing
wax impregnation
dehydration
HISTOLOGY

Histological Technique



Protocols followed in Histotechniques:

1. Receipt & Identification.
2. Labeling of the specimen with numbering.
3. Fixation.
4. Dehydration.
5. Clearing.
6. Impregnation (infiltration).
7. Embedding.
8. Section / cutting.
9. Staining.
10. Mounting.

Specimen Dissection - the stage

Dissecting and Specimen Sampling

Taking a representative sample of the tissue

Specimen


10% Neutral Buffered Formalin
pH 6.8 - 7.2 at 25°C
Name: Mickey Mouse
Spec: Rt nasal polyp
DOB: 18/11/28
Date: 26/11/13
Dr. Makewell

Fixation

- Is the process by which the constituents of cells and tissue are fixed in **a physical and chemical state** so that they will withstand subsequent treatment with various reagents with minimum loss of architecture .

- This is achieved by exposing the tissue to chemical compounds, call **fixatives**.

Properties of an Ideal Fixative

- Prevents autolysis and bacterial decomposition.
- Preserves tissue in their natural state and fix all components.
- Make the cellular components insoluble to reagent used in tissue processing.
- Preserves tissue volume.
- Avoid excessive hardness of tissue.

- **Important Factors in fixation:**

• Fresh tissue.

• Proper penetration of tissue by fixatives.

• Correct choice of fixatives.

- This should be approximately 10-20 times the volume of the specimen.
- Fixative should surround the specimen on all sides.

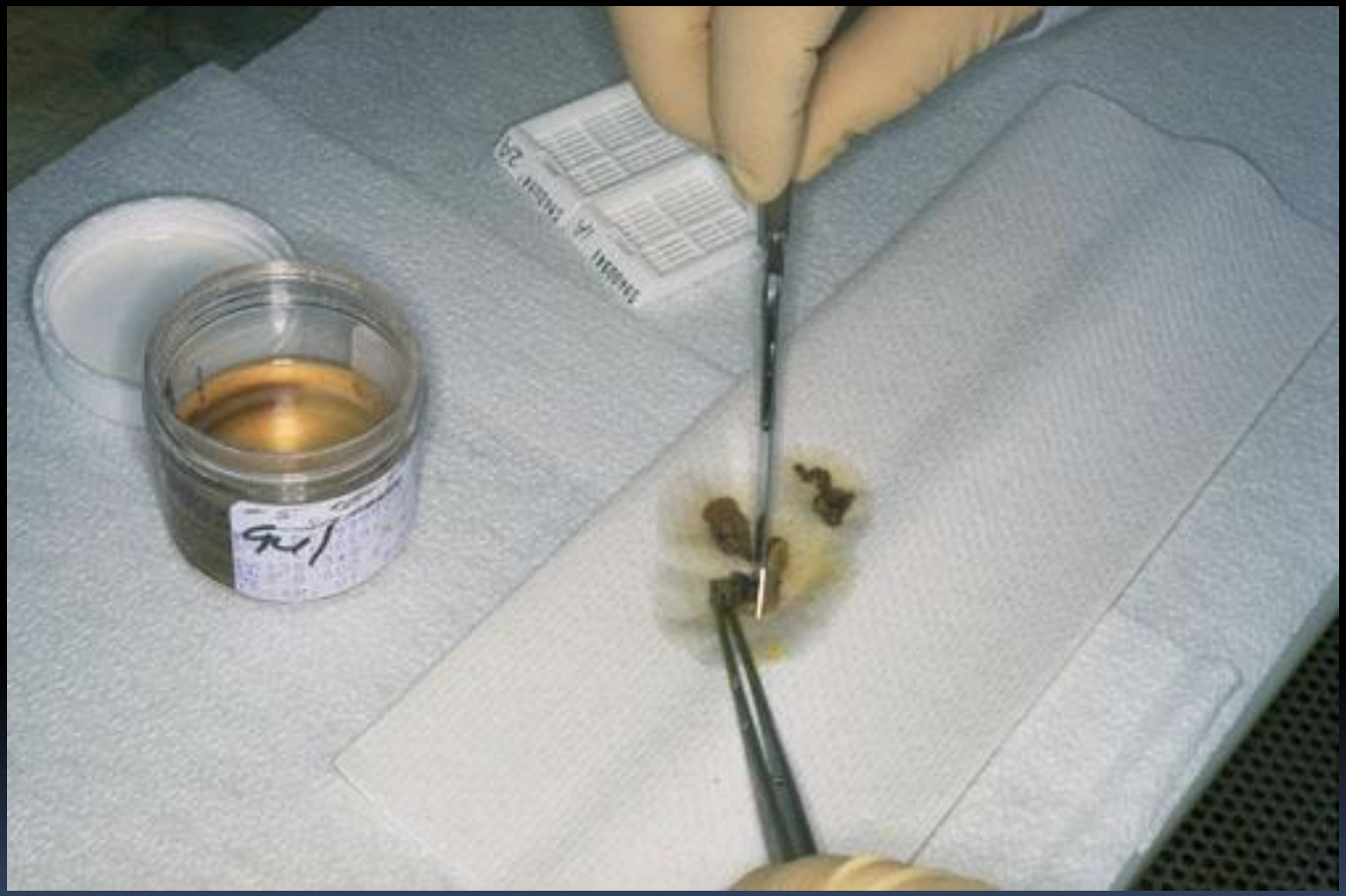
Tissue fixatives

- **There are many tissue fixatives i.e**

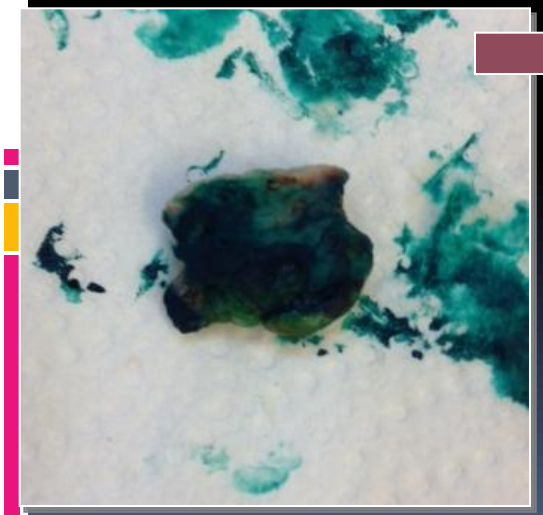
- Buffered formalin (light microscope preparation)
- Buffered gluteraldehyde (electron microscope preparation).
- Osmium tetraoxide (electron microscope preparation, preserve and stain).
- Zenker’s formal saline
- Bowen’s fluid

- No fixative will penetrate a piece of tissue thicker than 1 cm.
- For dealing with specimen thicker than this, cut slices not thicker than 5 mm are recommended.

Cassette



Specimen bits are placed in porous cassettes

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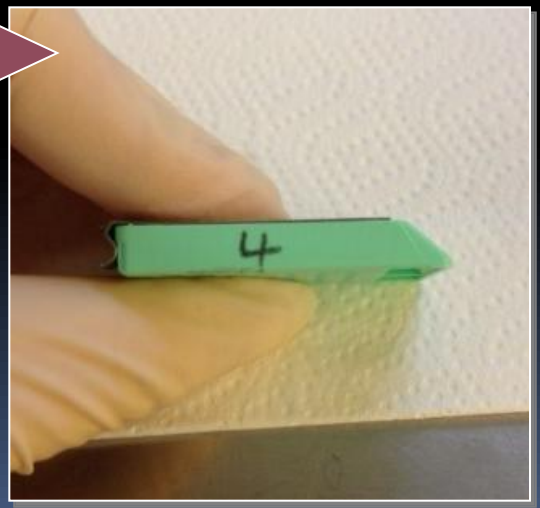
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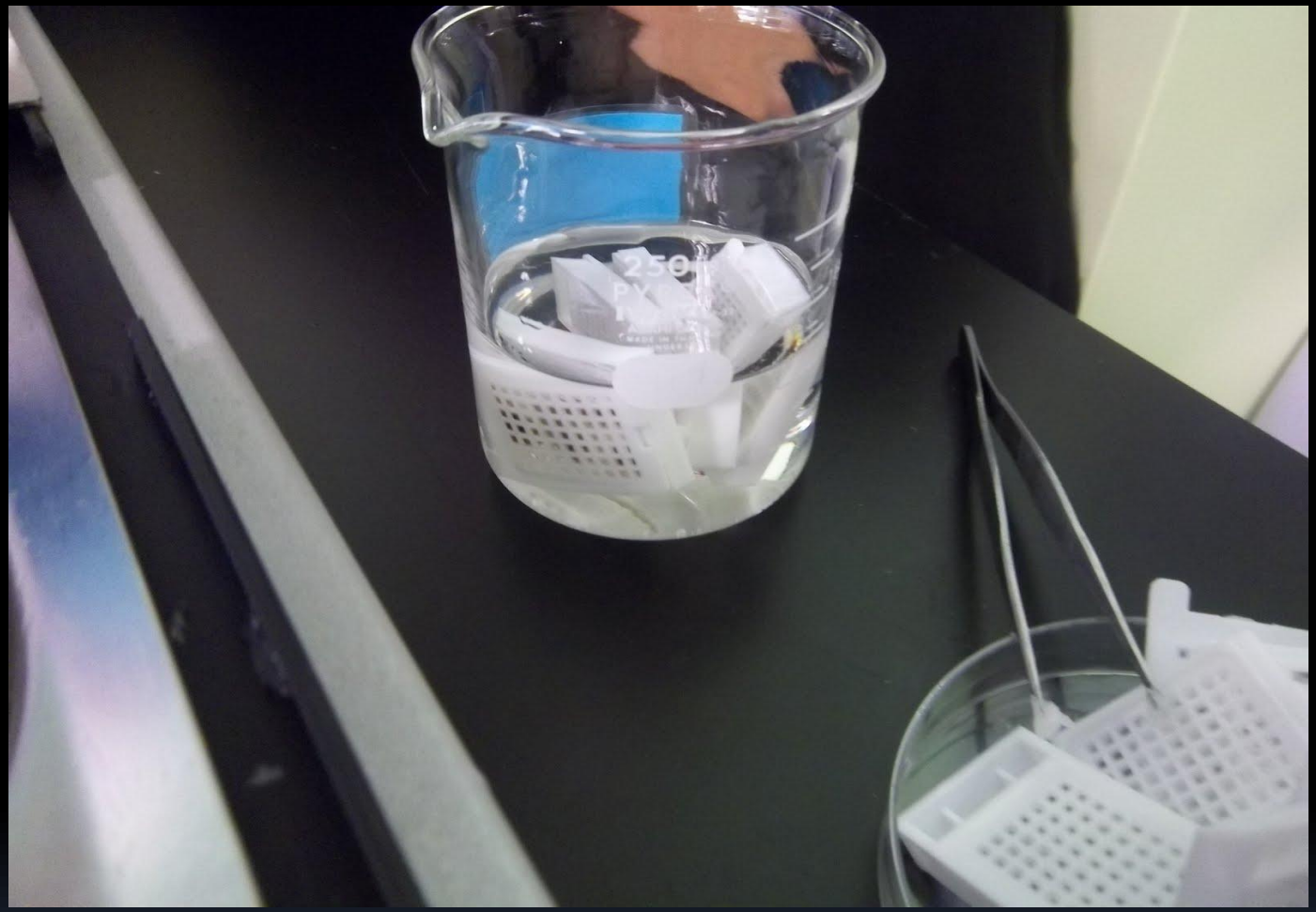


Cassettes are collected in fixatives 10% formalin

1mm/hour fixation ~ 6 hour

Tissue Processing

It can be subdivided into:

a) Dehydration
b) Clearing
c) Impregnation (infiltration)

Dehydration (removal of water)

It is the process in which the water content in the tissue is completely removed by passing the tissue through increasing concentrations of dehydrating agents.

- Tissues are dehydrated by using increasing strength of alcohol; e.g.

  80%, 95% and 100%.

- Replace water by diffusion.

- During dehydration water in tissue has been replaced by alcohol.
- The next step alcohol should be replaced by paraffin wax.
- As paraffin wax is not alcohol soluble, we replace alcohol with a substance in which wax is soluble. This step is called **clearing**.

Clearing

- Replacing the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid and the embedding medium.

- **Some clearing agents:**

- Xylene.
- Toluene.
- Chloroform.
- Benzene.

Impregnation (infiltration):

- In this the tissue is kept in a wax bath containing molten paraffin wax.
- The wax is infiltrated in the tissue which increases the optical differentiation & hardens the tissue & helps in easy sectioning of the tissue.

Types of tissue processing

There are two types :

1. Manual Tissue Processing
2. Mechanical Tissue Processing

Manual Tissue Processing

- In this process the tissue is changed from one container of reagent to another by hand.

Note:

The processing, whether manually or mechanically, involves the same steps and reagents in same sequence.

Mechanical Tissue Processing

- In this the tissue is moved from one jar to another by mechanical device.
- Timings are controlled by a timer which can be adjusted in respect to hours and minutes.
- Temperature is maintained around 60 ºC (infiltration).
- Automatic tissue processor:

➢ Overnight
➢ 12 Baths.
➢ 16 hours .

Tissue basket

Tissues processor

Mechanical Tissue Processing

Fixation

1. 10 % Formalin saline (I) for 1.5 hours
2. 10 % Formalin saline (II) for 1.5 hours

Dehydration

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

Clearing:

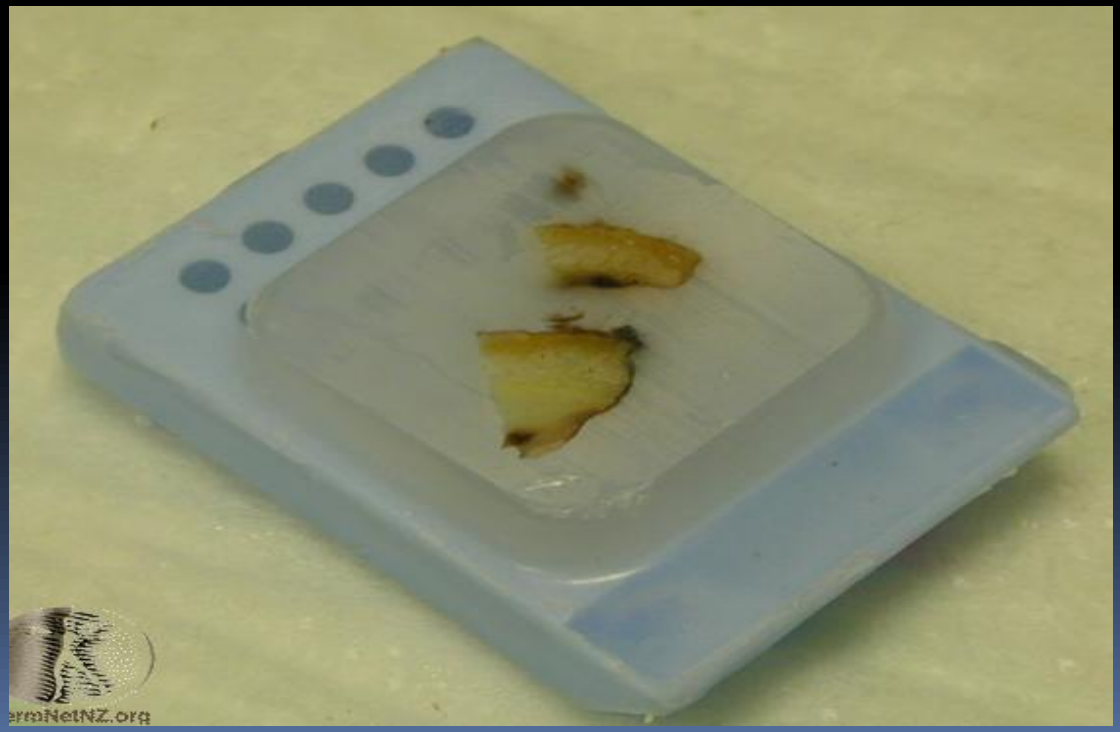
1. Xylene (I) for 1.5 hours
2. Xylene (II) for 1.5 hours

Infiltration:

1. Paraffin wax (I) for 1.5 hours
2. Paraffin wax (II) for 1.5 hours

Embedding

Is the process by which iimpregnated tissues are surrounded by a medium such as agar, gelatin, or wax which when solidified will provide sufficient external support during sectioning.



Embedding:

- It is done by transferring the tissue which has been impregnated with wax to a mould filled with molten wax & is allowed to cool & solidify.

- After solidification, a wax block is obtained which is then sectioned to obtain ribbons.

Embedding tools

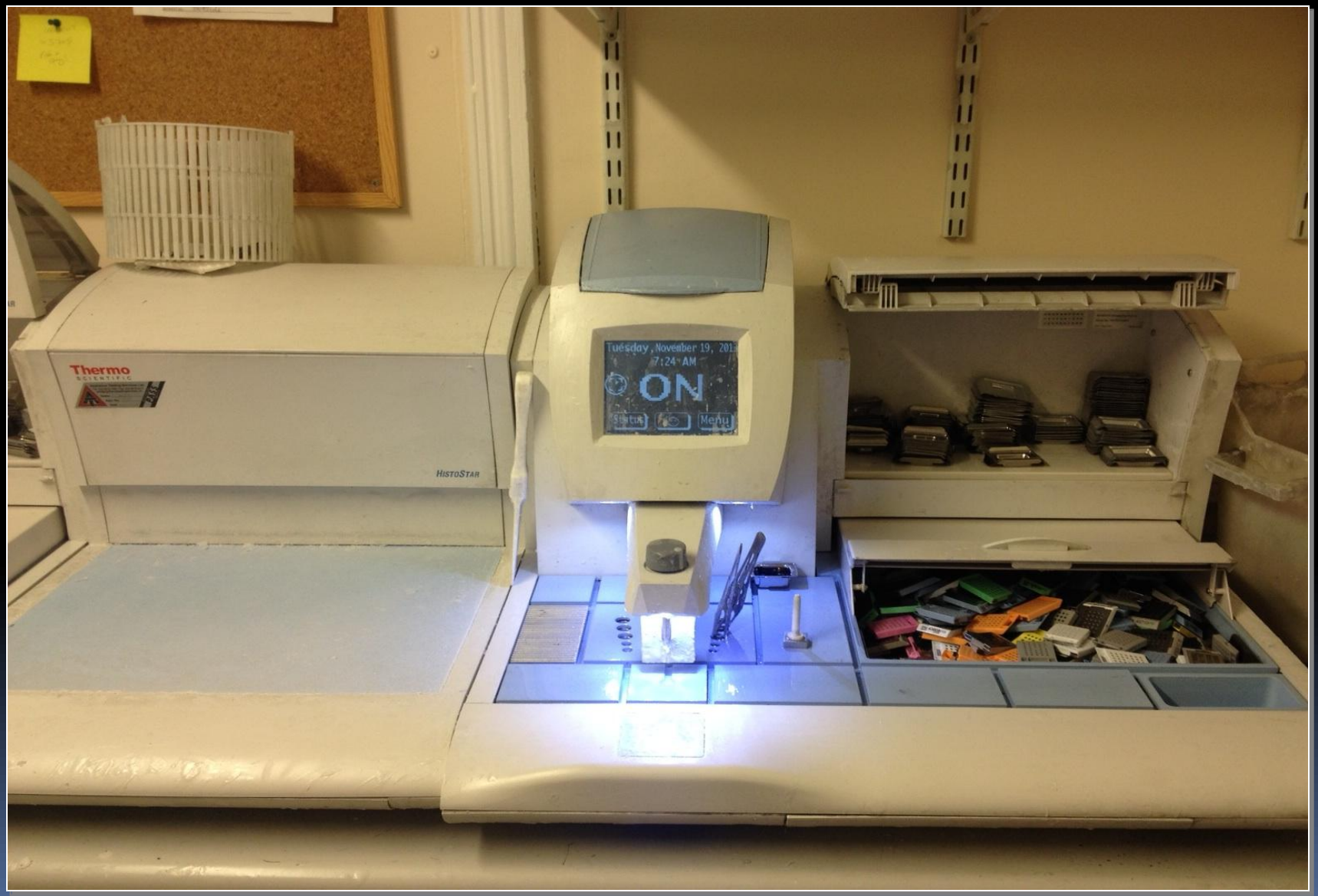
Moulds

Paraffin wax

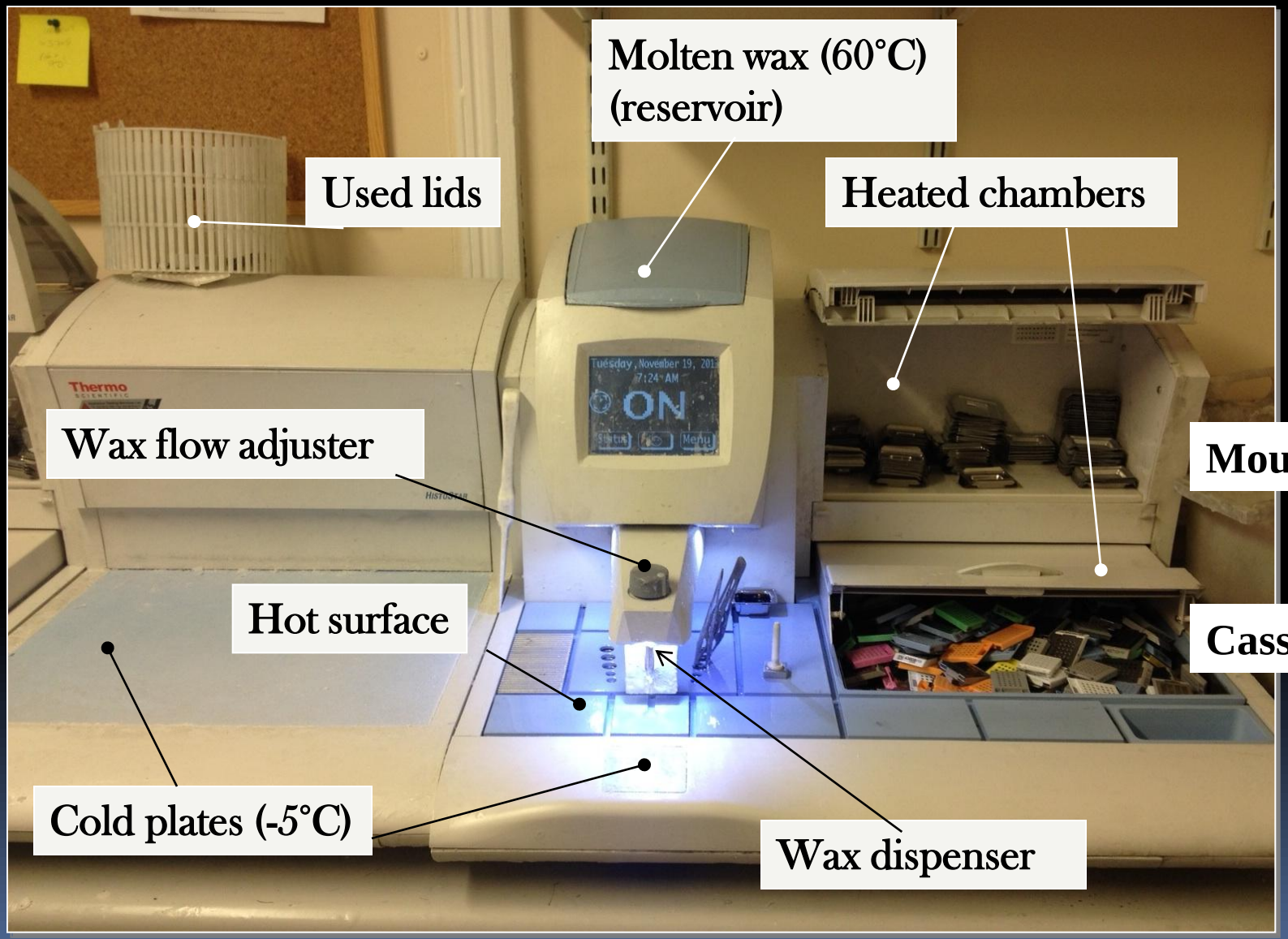
Paraffin wax:

Is a polycrystalline mixture of solid hydrocarbons. Paraffin wax is traditionally marketed by its melting points which range from 39°C to 68°C.

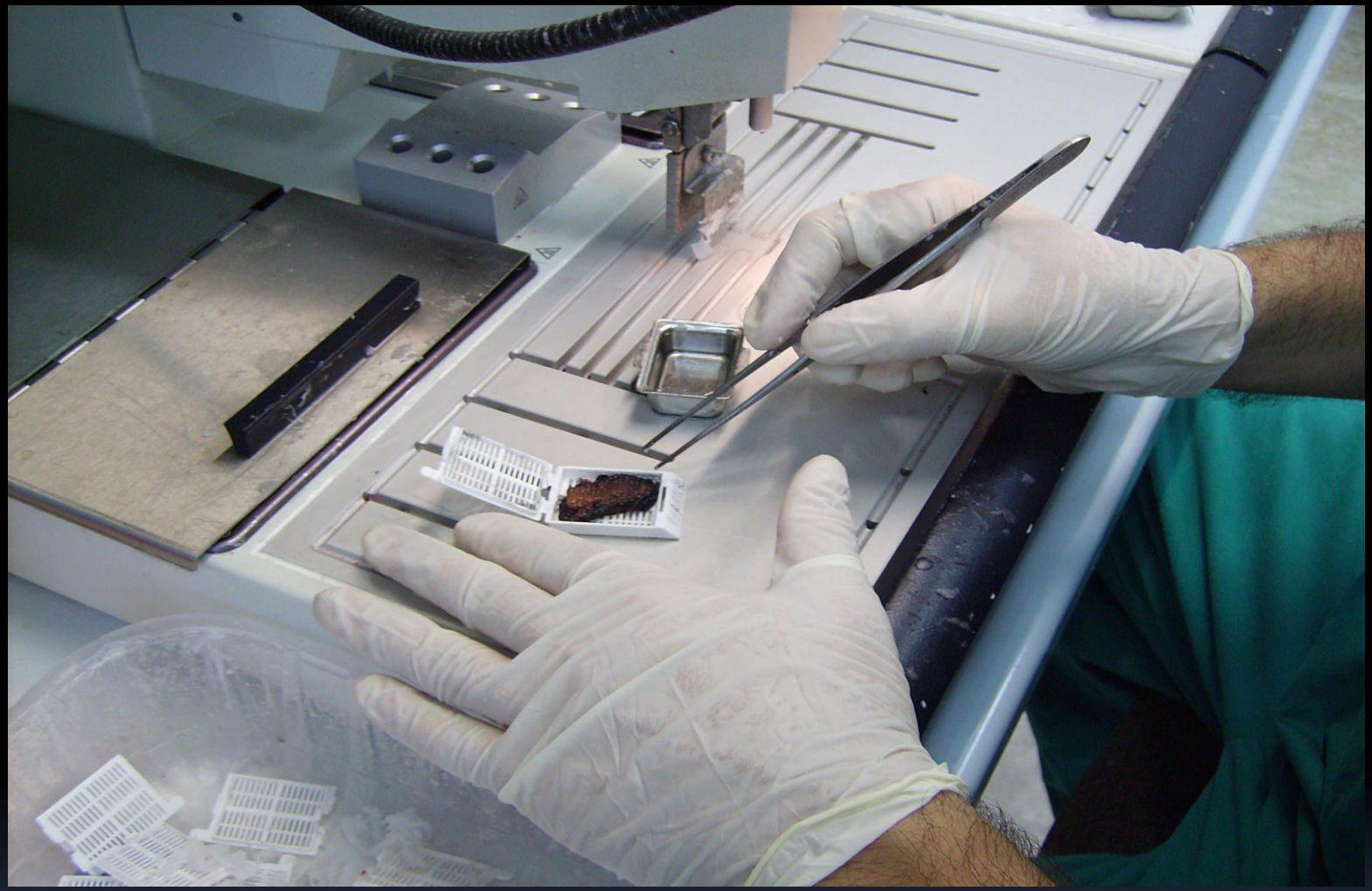
Embedding Centre



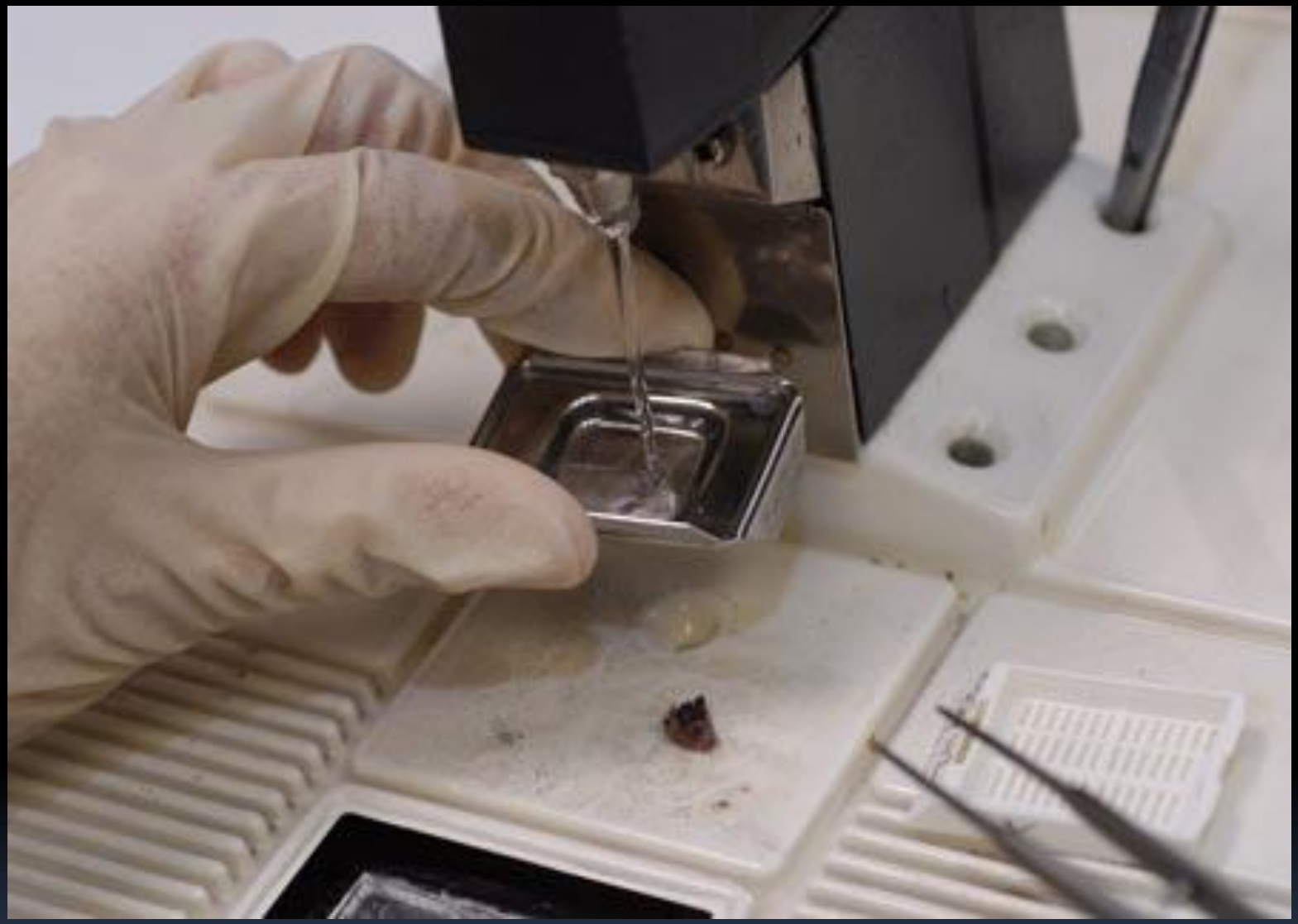
Embedding Centre



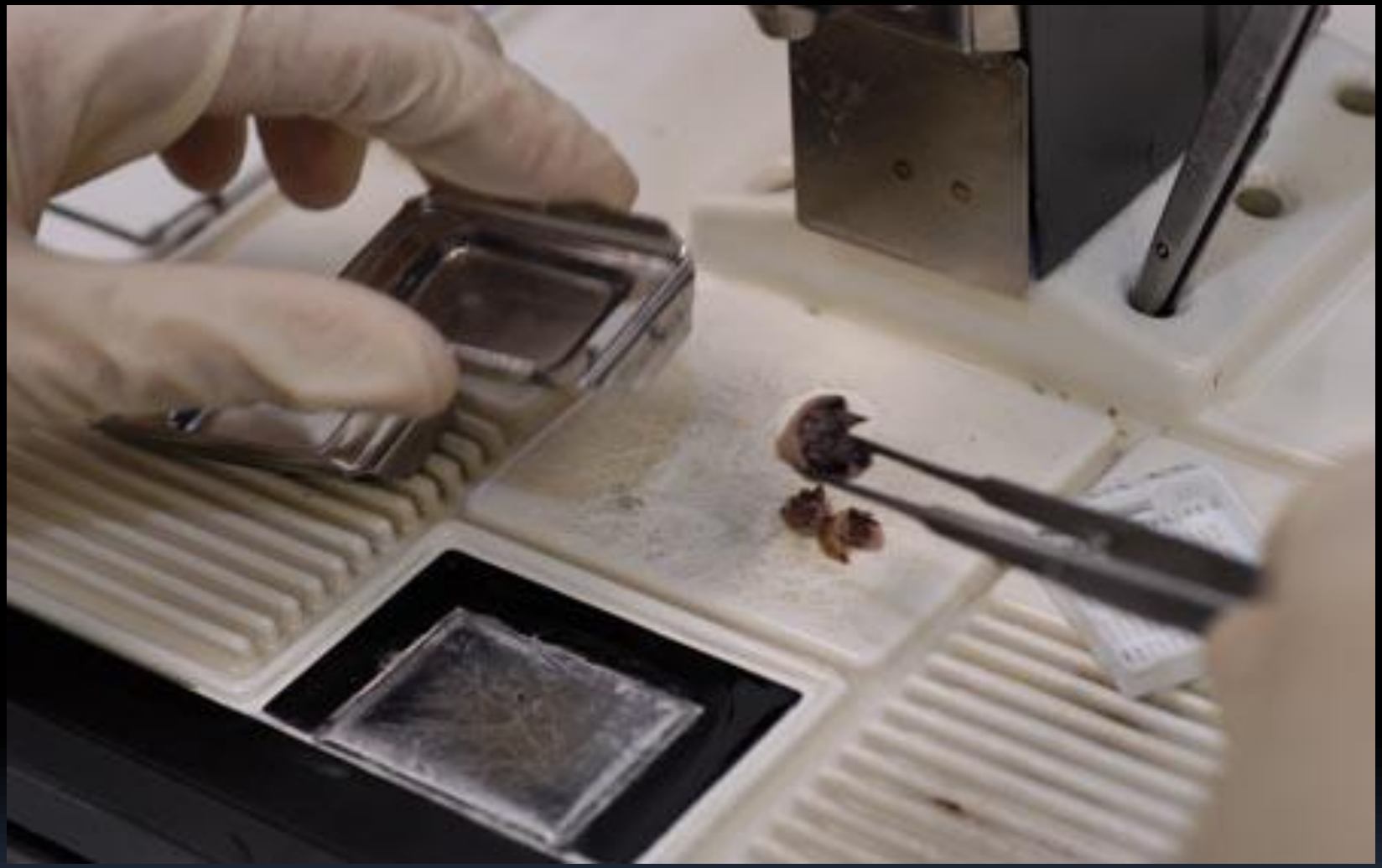
General Embedding Procedure



Open the tissue cassette.



Fill the mould with paraffin wax.



Using warm forceps select the tissue, taking care that it does not cool in the air.

Orienting the tissue in the mould.

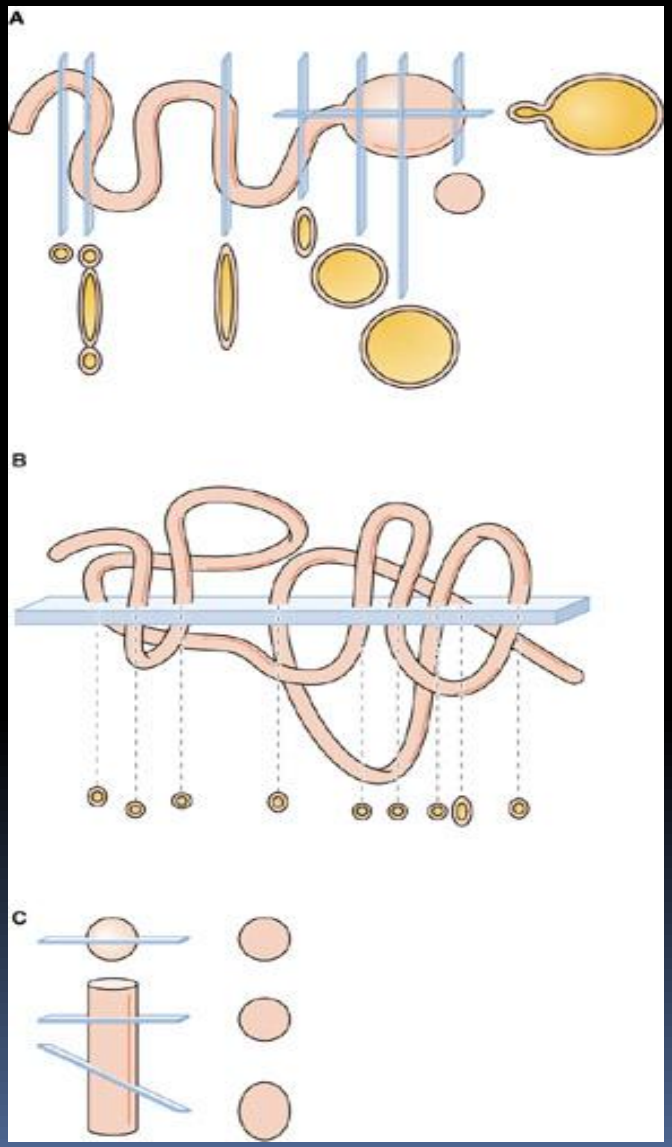
Orientation Of Tissue In The Block

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

1. cross section

2. longitudinal section

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

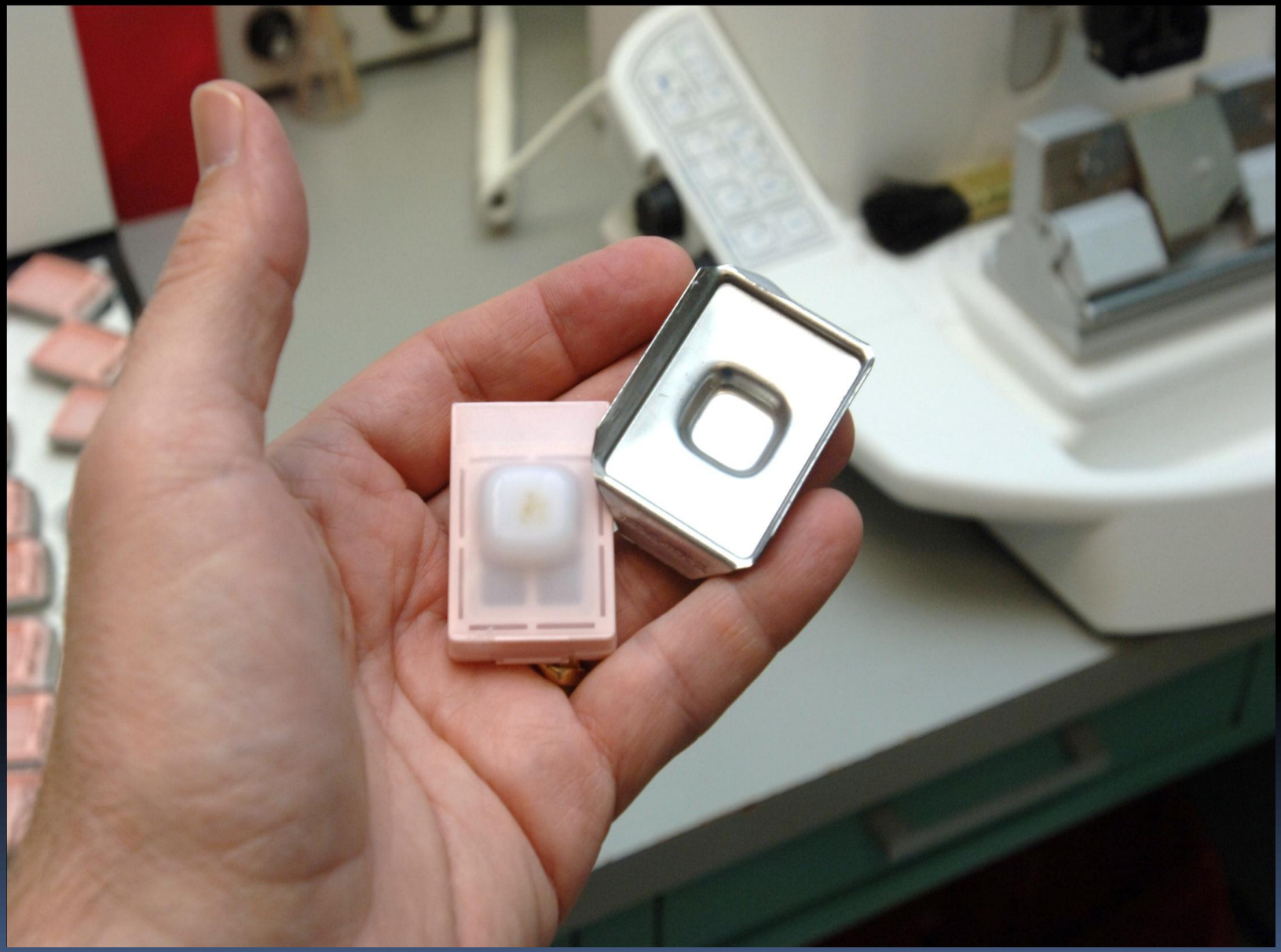


Chill the mould on the cold plate and firming the tissue into the wax with warmed forceps.

Cool the block on the cold plate.

Blocks on the cold plate.

Remove the block from the mould.



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

Sectioning

Sectioning (Section Cutting) :

- **I**t is the procedure in which the blocks which have been prepared are cut or sectioned and thin strips of uniform thickness are prepared.

- The instrument by which this is done is called as a **Microtome.**

TYPES OF MICROTOMES:

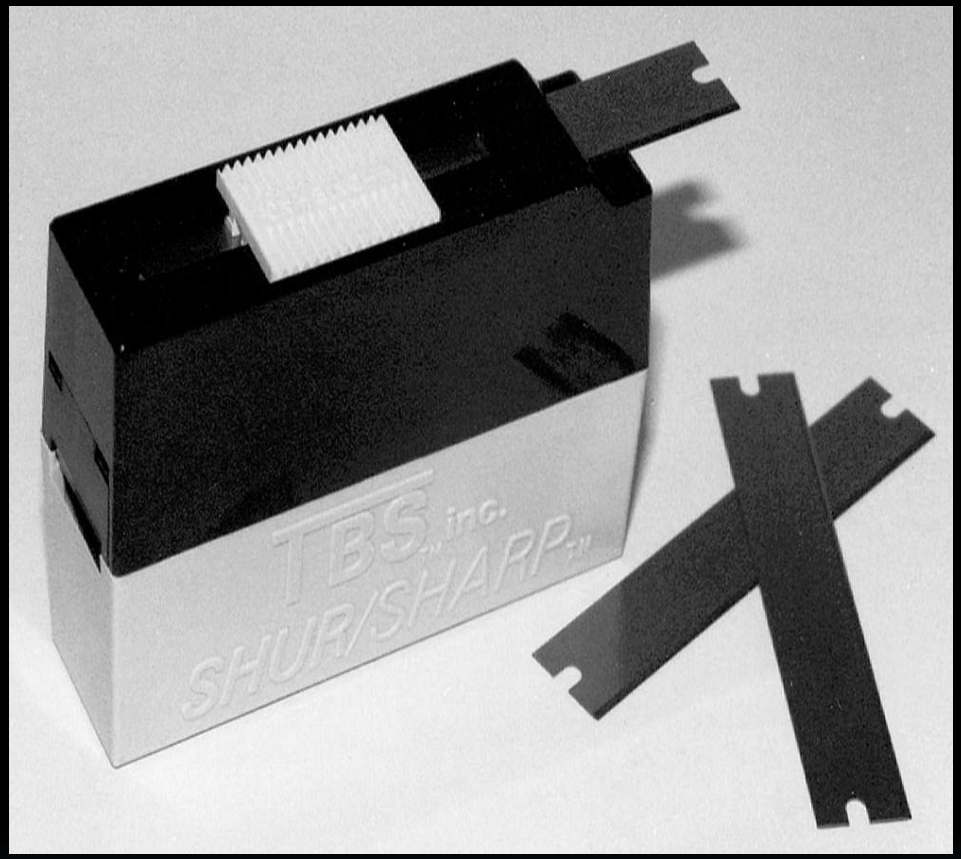
- Sliding microtome.
- Rotary microtome.
- Rocking microtome.
- Freezing (cryostat) microtome.
- Base sledge microtome.
- Ultramicrotome.

Main parts of all microtomes :

1. Base (microtome body).
2. Knife and its holder.
   - Steel Knives / Disposable Blades.
   - **Non-corrosive Knife**
   - Glass Knives.
   - Diamond Knives.
3. Block holder.
4. **Micron adjustment** (section thickness).

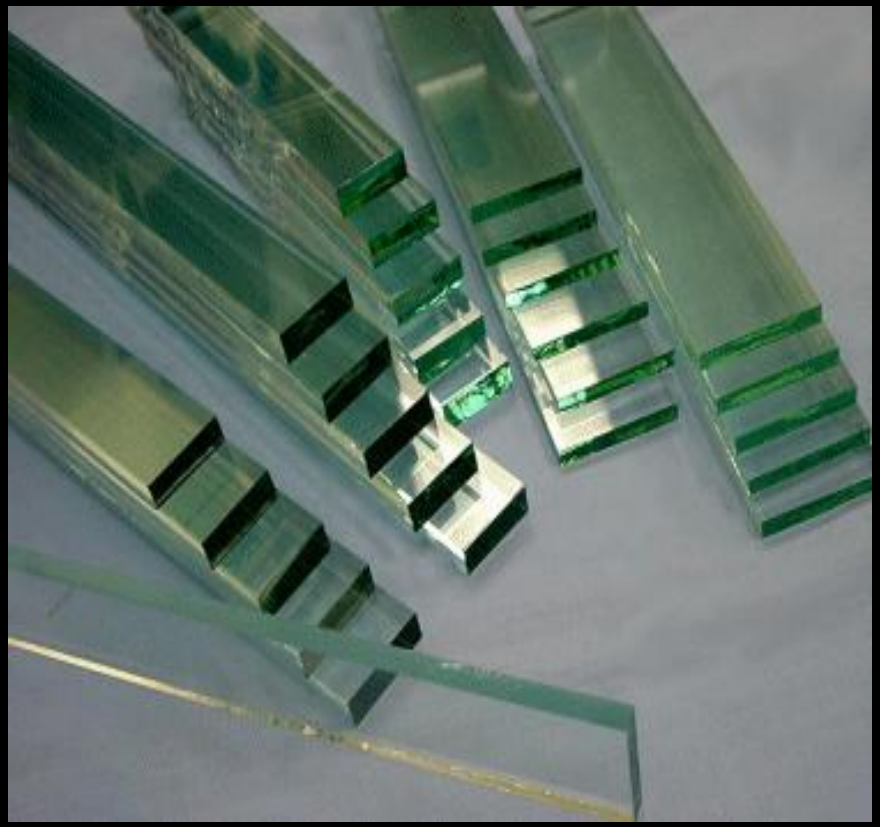
Steel Knives

(for rotary microtome)



Disposable Blades

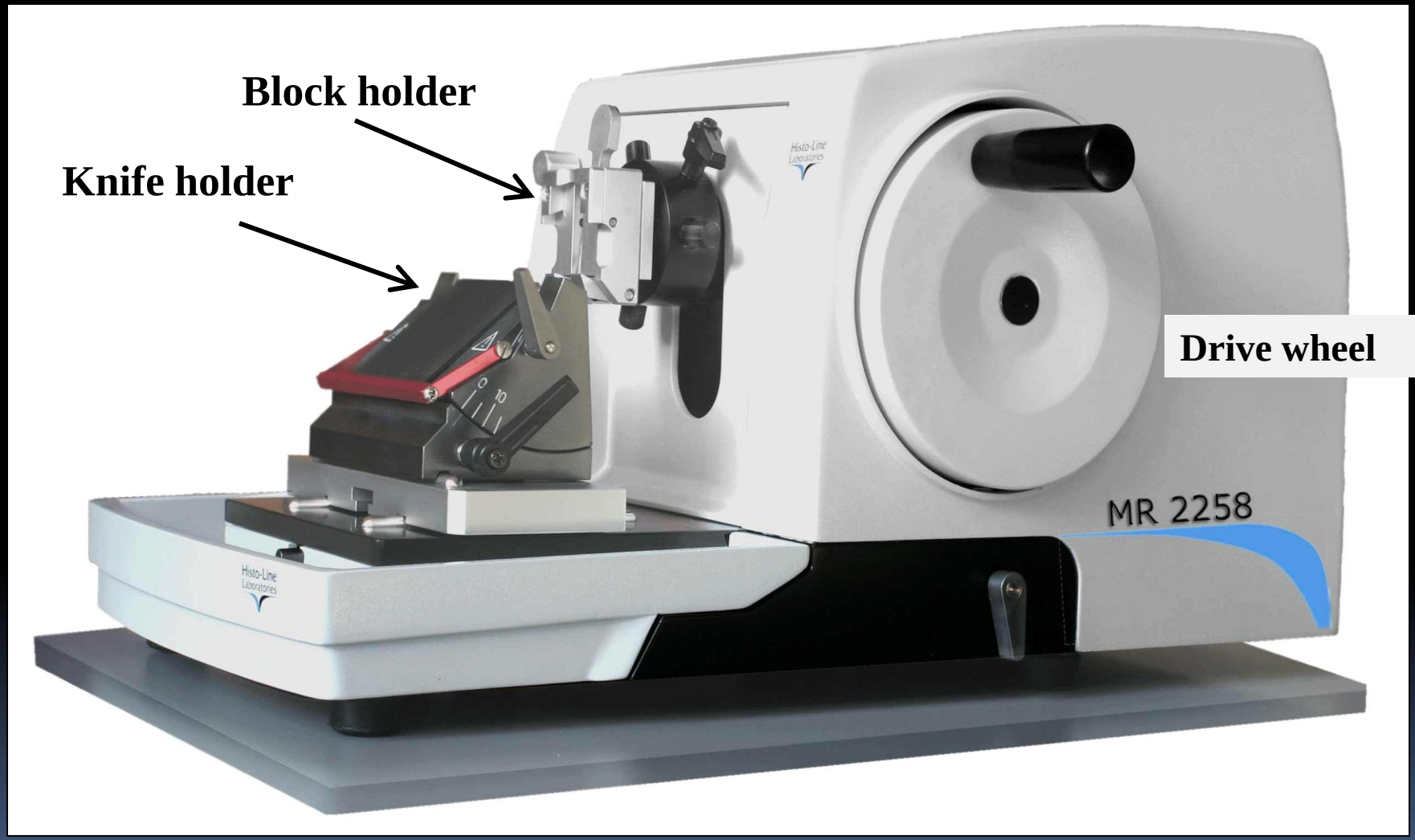
(for rotary microtome)



Glass Knives

(for ultramicrotome)

Rotary Microtome




KD-2268
KEDEE
Micron adjustment
(section thickness)
1-60µ
Paraffin Block
Steel knife
KEDEE

Rotary Microtome

The knife is stationary (fixed) and the block holder is moved up and down in a vertical plane by the rotary action of the hand wheel.

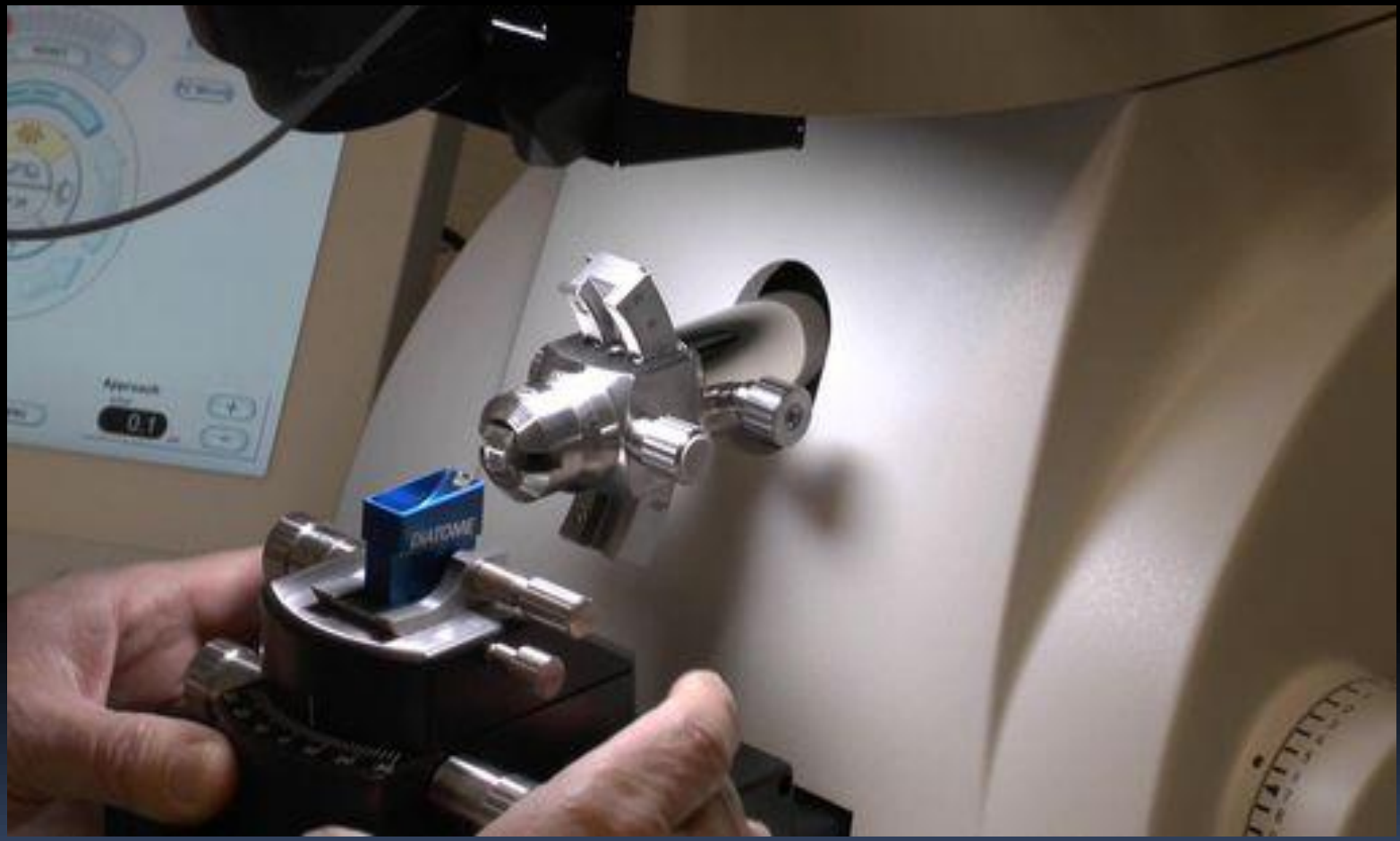
- It is the most commonly used.
- Suitable for paraffin embedded sections.
- It is suitable for cutting of small tissues .
- Ideal for cutting serial sections.

Ultra Microtome

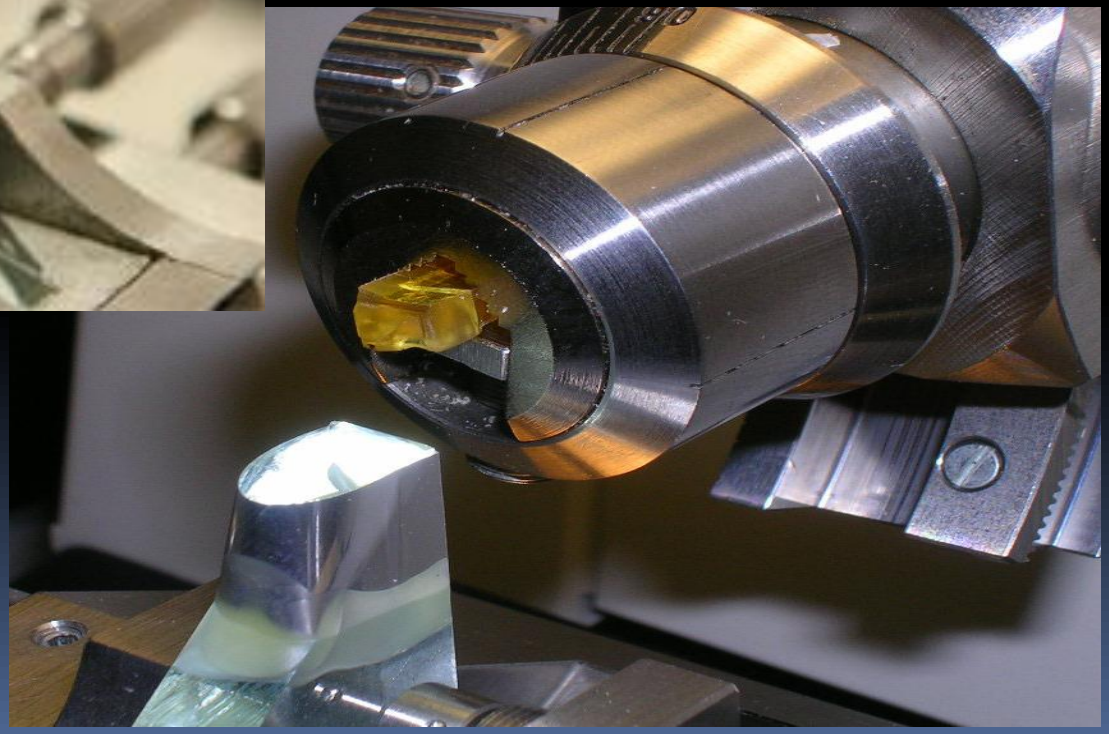
Ultra Microtome

- ✓ Used for very thin section (up to 1 µm).
- ✓ The typical thickness of tissue cut is between 40 -100 nm for TEM.
- ✓ Examine by Transmission electron microscpy (TEM).
- ✓ Knife: Diamond or Glass.

Ultra Microtome



Ultra Microtome



Freezing (Cryostat) microtome

- Frozen tissue embedded in a freezing medium is cut on a microtome in a cooled machine called a **cryostat.**

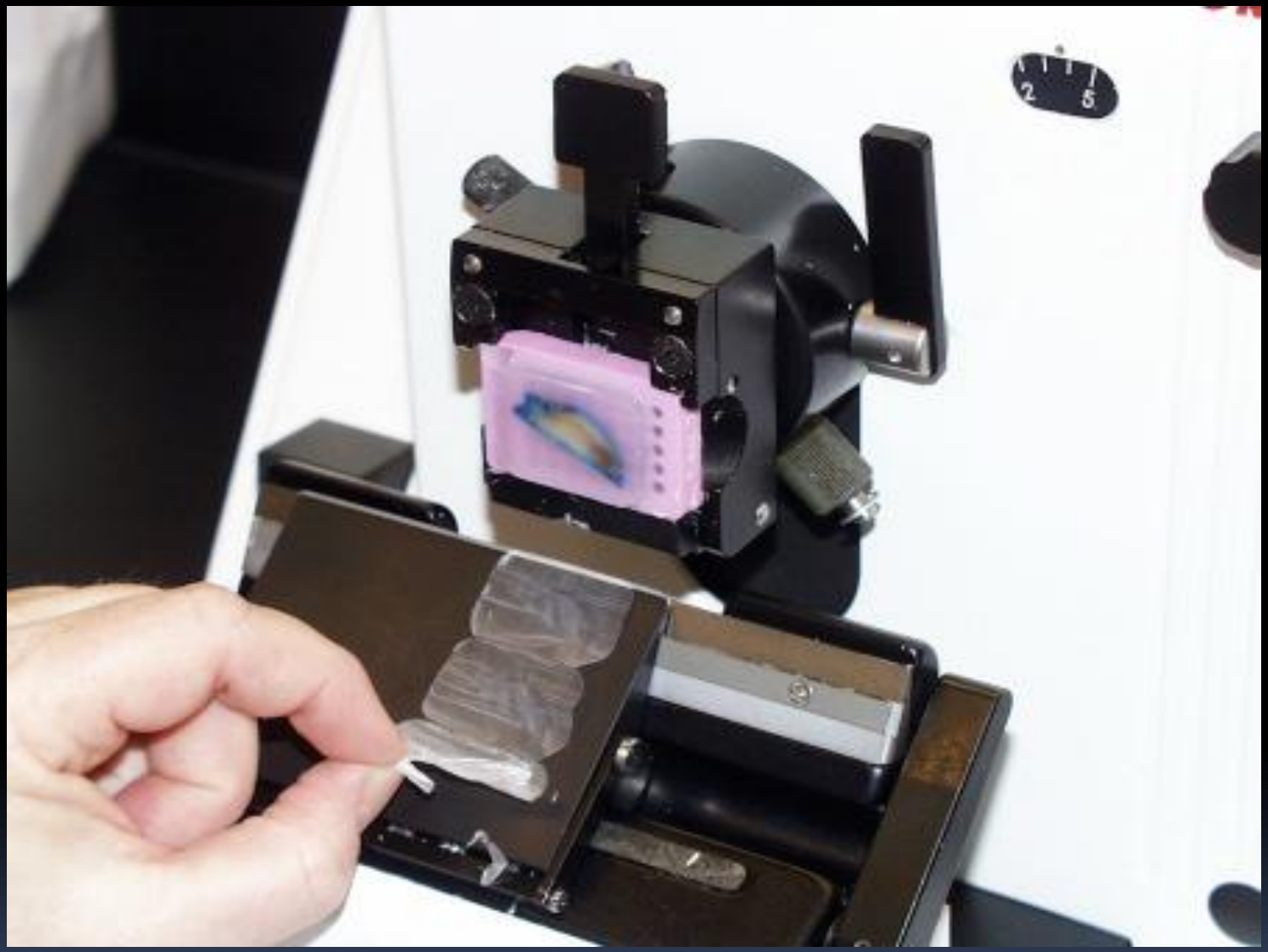
Freezing (Cryostat) microtome

Freezing (Cryostat) microtome

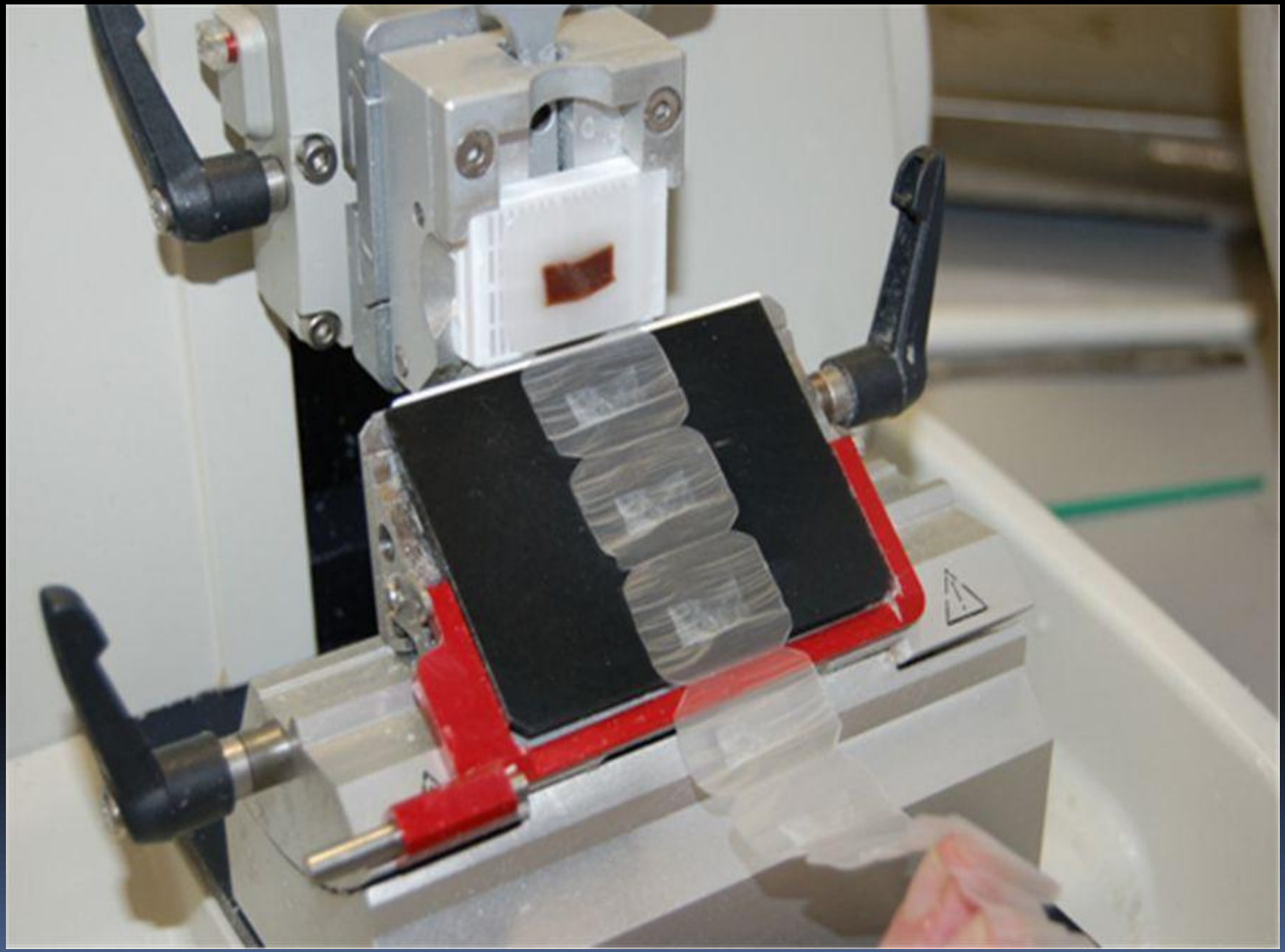
Sectioning procedure


Leica


FINESSE
NEXT
CLEAR
RETRACT
RUN
STOP
MODE
MEMORY
AUTO
TRIM SECTION
ROCK
RUN
STOP

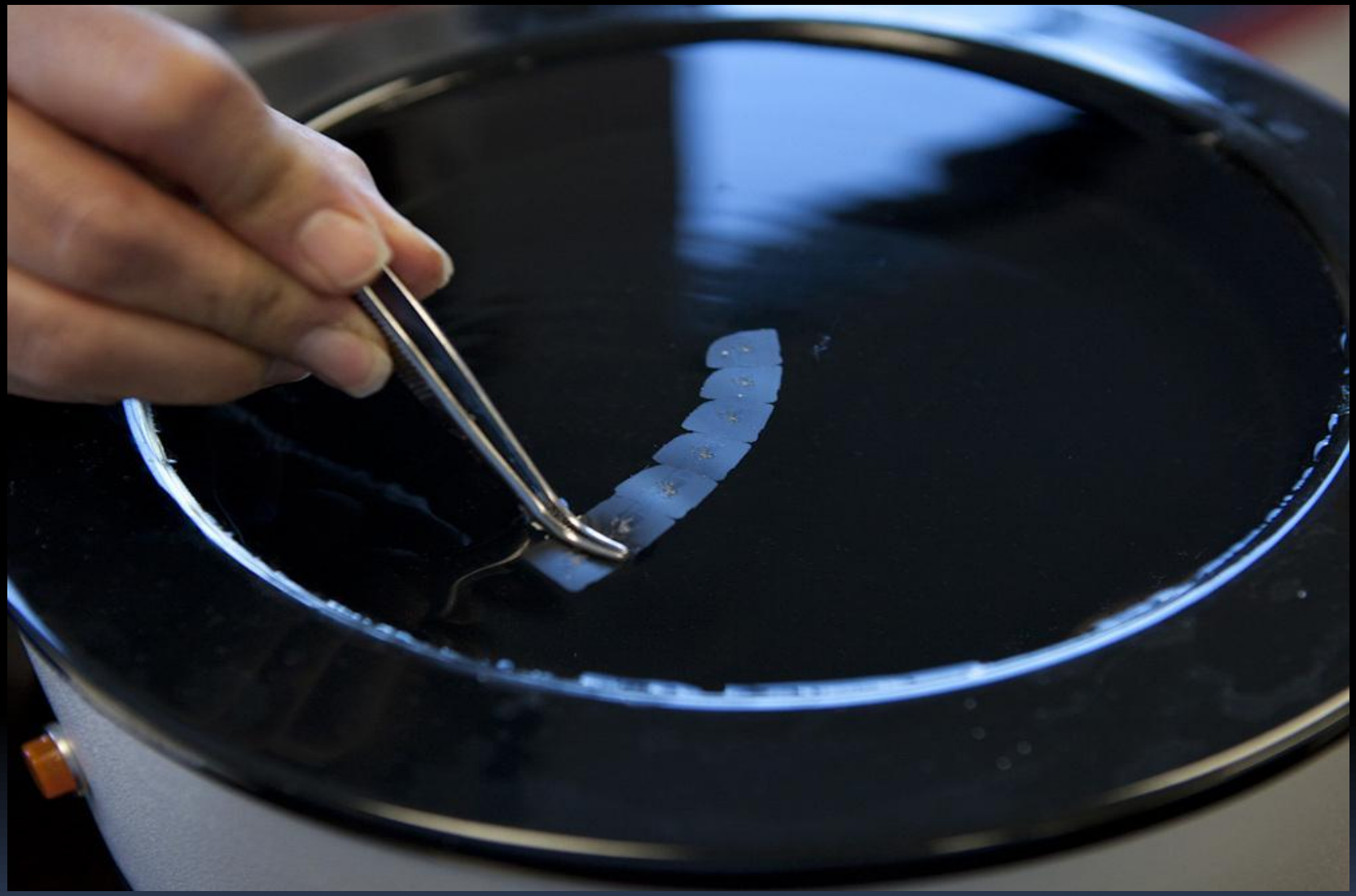


Ribbon of sections



Tissue floatation bath

- It is a thermostatically controlled water bath.
- It is maintained at a temperature maintained 5 – 6 degree below the melting point of paraffin wax.



Flattens paraffin sections

Ribbon sections are taken on hot water bath.

Taking the floating sections onto slide.

Adhesives used for fixing the sections on the slides

Albumin solution (Mayor’s egg albumin)

Taking the section onto slide.

Flat, no air bubbles, no stretch or breaks.

Staining

Staining

- Staining is a process by which we give **color** to a section which bring out the particular details in the tissue.

- The most commonly used stain in routine practice is Haematoxylin & eosin stain.

- **Staining steps**:

  a. rehydration

  b. stain

  c. dehydration

- **Classification of Stains:**

- Acid stains
- Basic stains
- Neutral stains

Acid Dyes

In an acid dye:

- The **basic component** is colored and the acid component is colorless.
- Acid dyes stain basic components e.g. **eosin** stains cytoplasm.
- The color imparted is shade of **red**.

Basic Dyes

In a basic dye

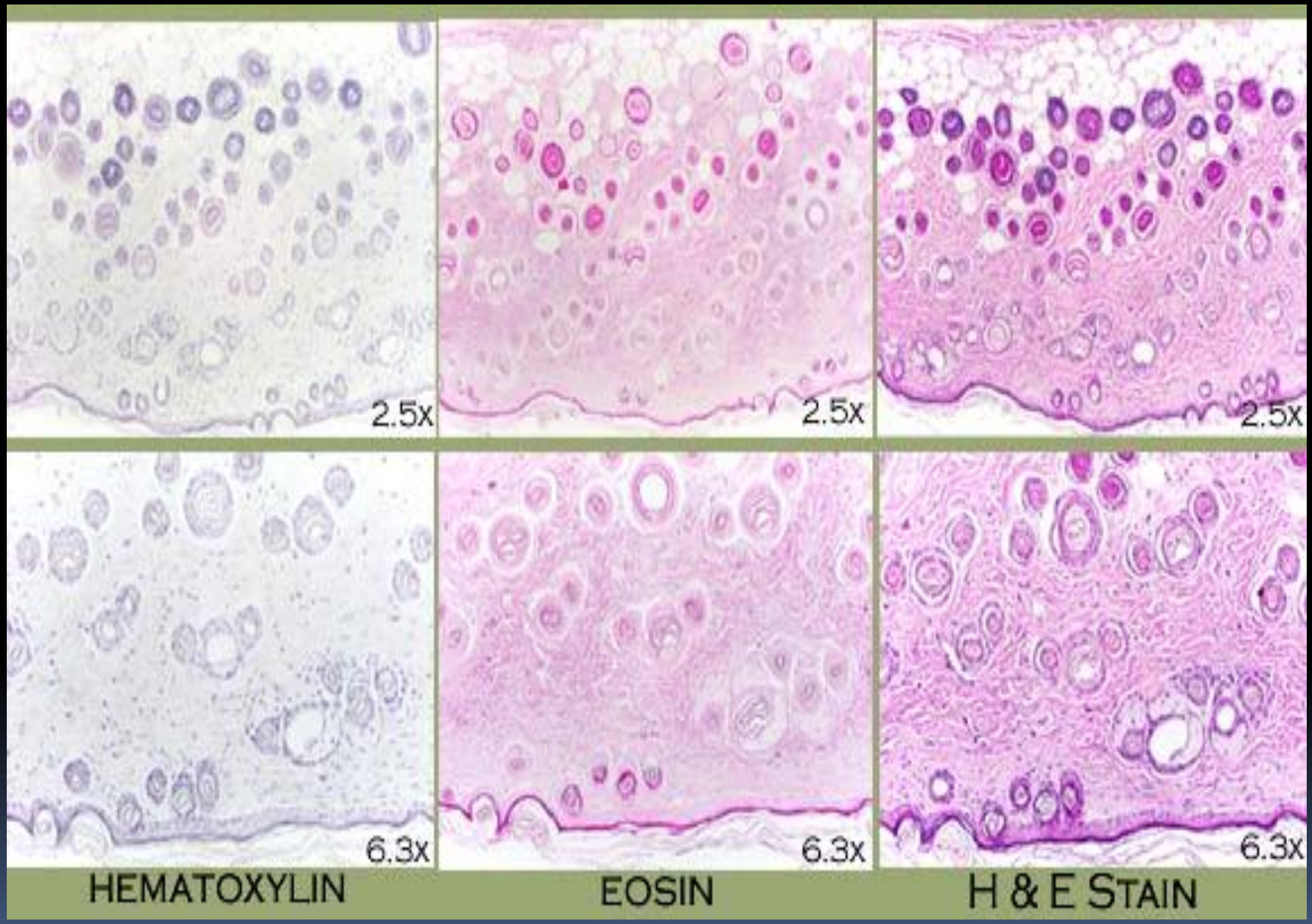
- **The acid component** is colored and the basic component is colorless.
- Basic dyes stain acidic components e.g. **Hematoxylin** stains nucleus.
- The color imparted is shade of **blue.**

Hematoxylin and Eosin (H & E)

Hematoxylin: stains acidic molecules shades of blue.

Eosin: stains basic materials shades of red, pink and orange.

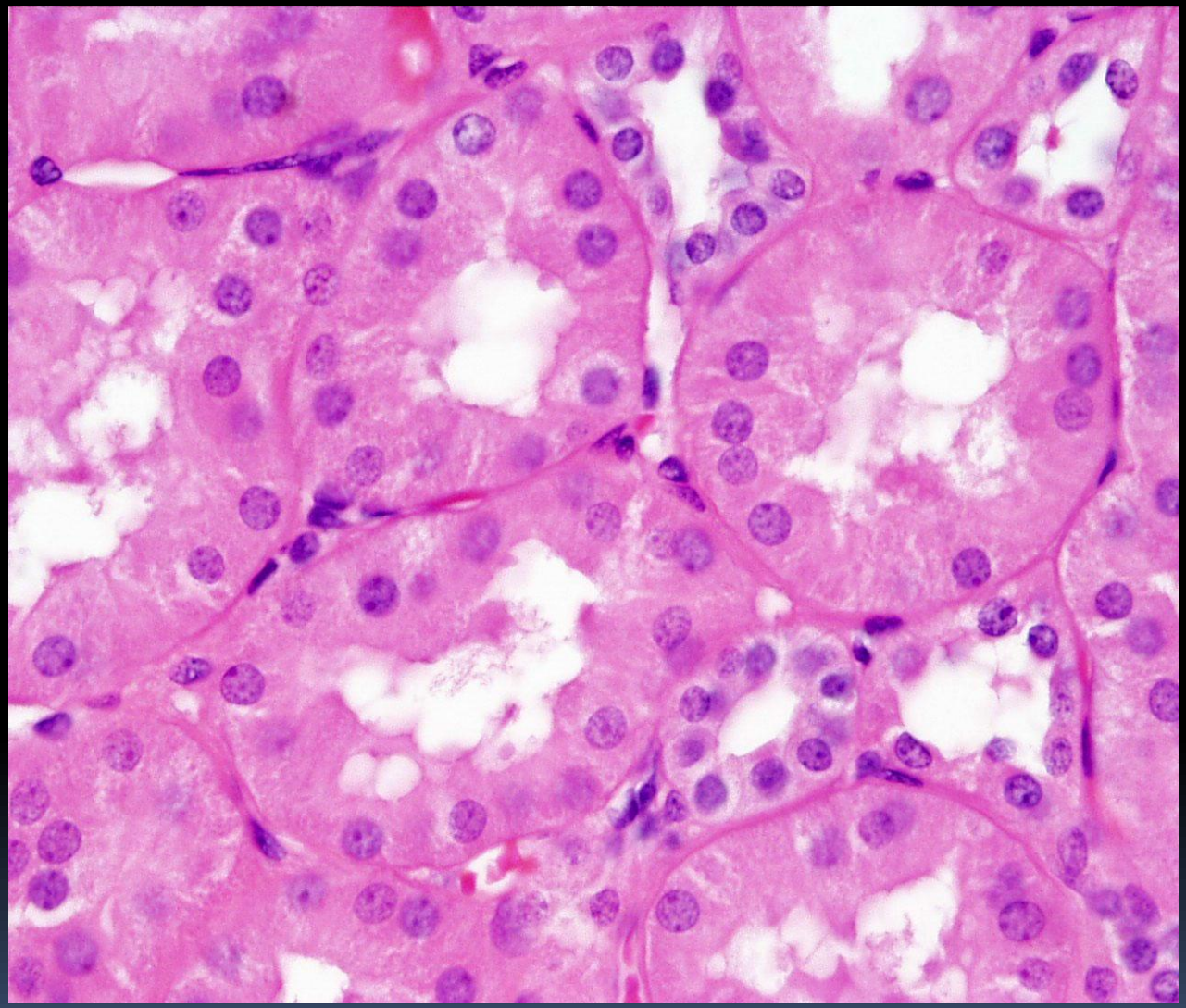
H & E stains are universally used for routine histological examination of tissue sections.


2.5x
2.5x
2.5x
6.3x
6.3x
6.3x
HEMATOXYLIN
EOSIN
H & E STAIN

Result :

The nucleus stains **Blue** .

The cytoplasm stains **pink.**



Neutral Dyes

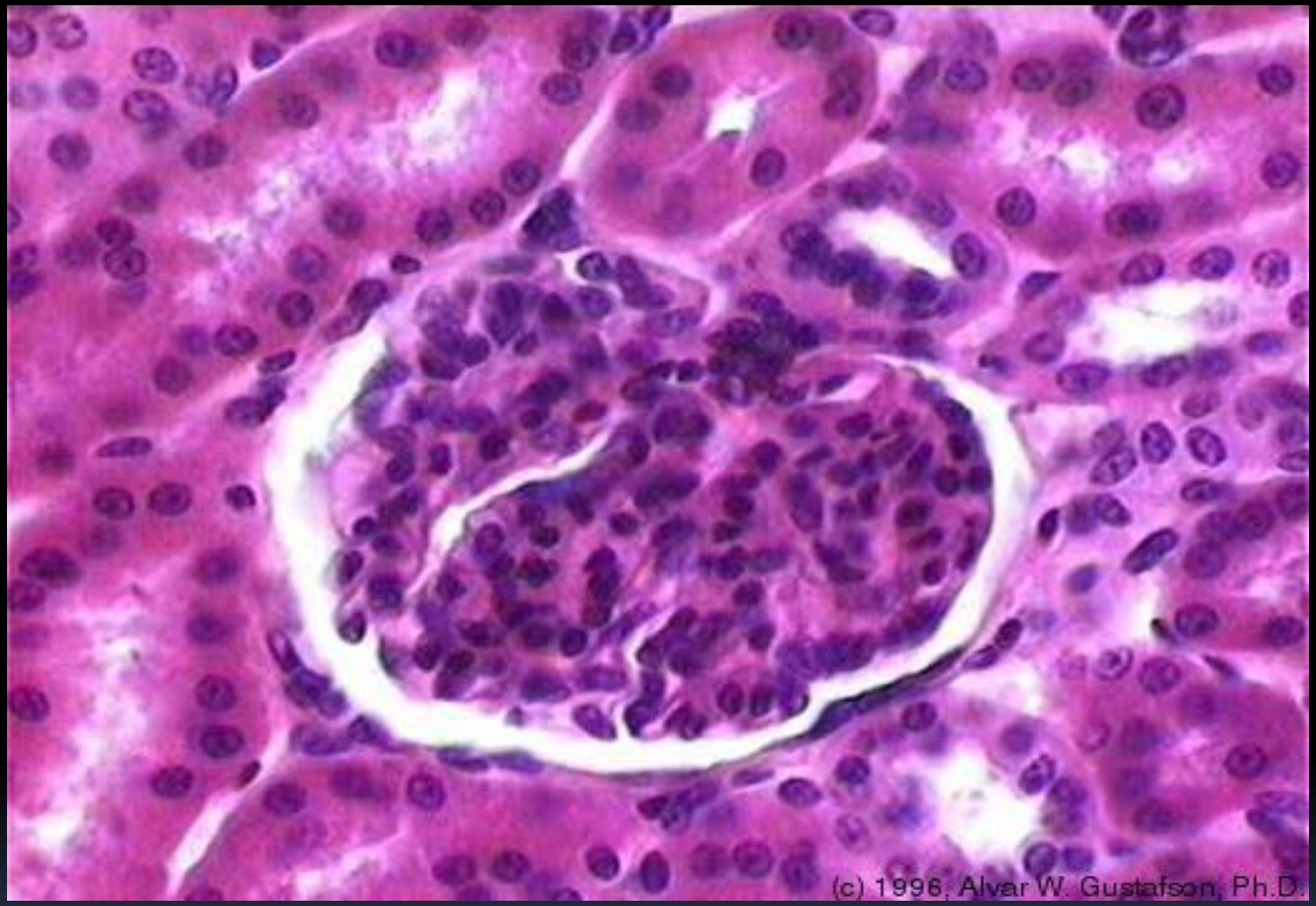
- When an acid dye is combined with a basic dye a neutral dye is formed.
- As it contains both colored radicals, it gives different colors to cytoplasm and nucleus simultaneously.

Special stains

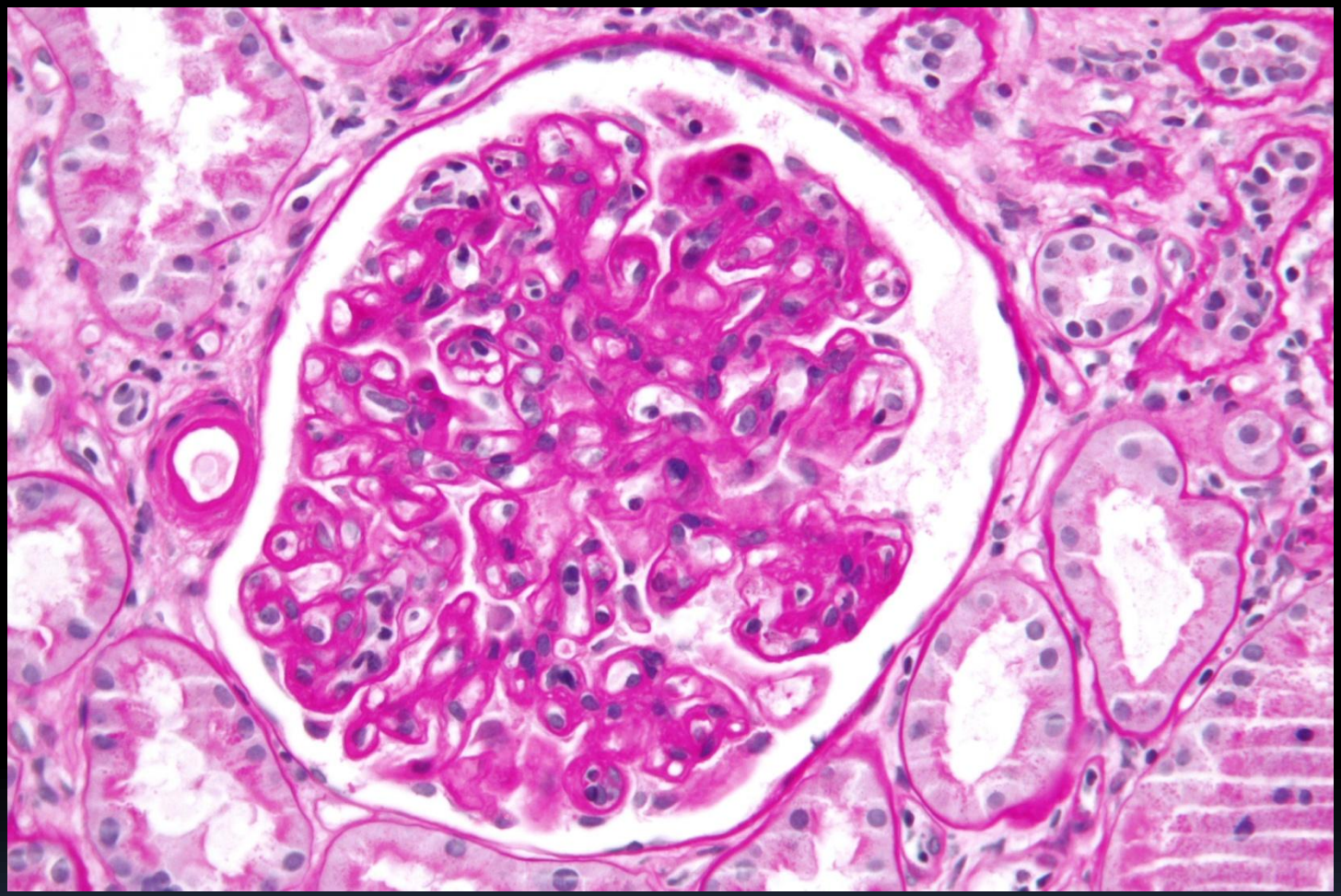
- When a specific components of tissue e.g. fibrous tissue, elastic tissue, nuclear material is to be stained, certain special stains are used which specifically stain that component tissue.

Examples :

1. PAS.
2. Maisson’s trichrome.
3. Silver.

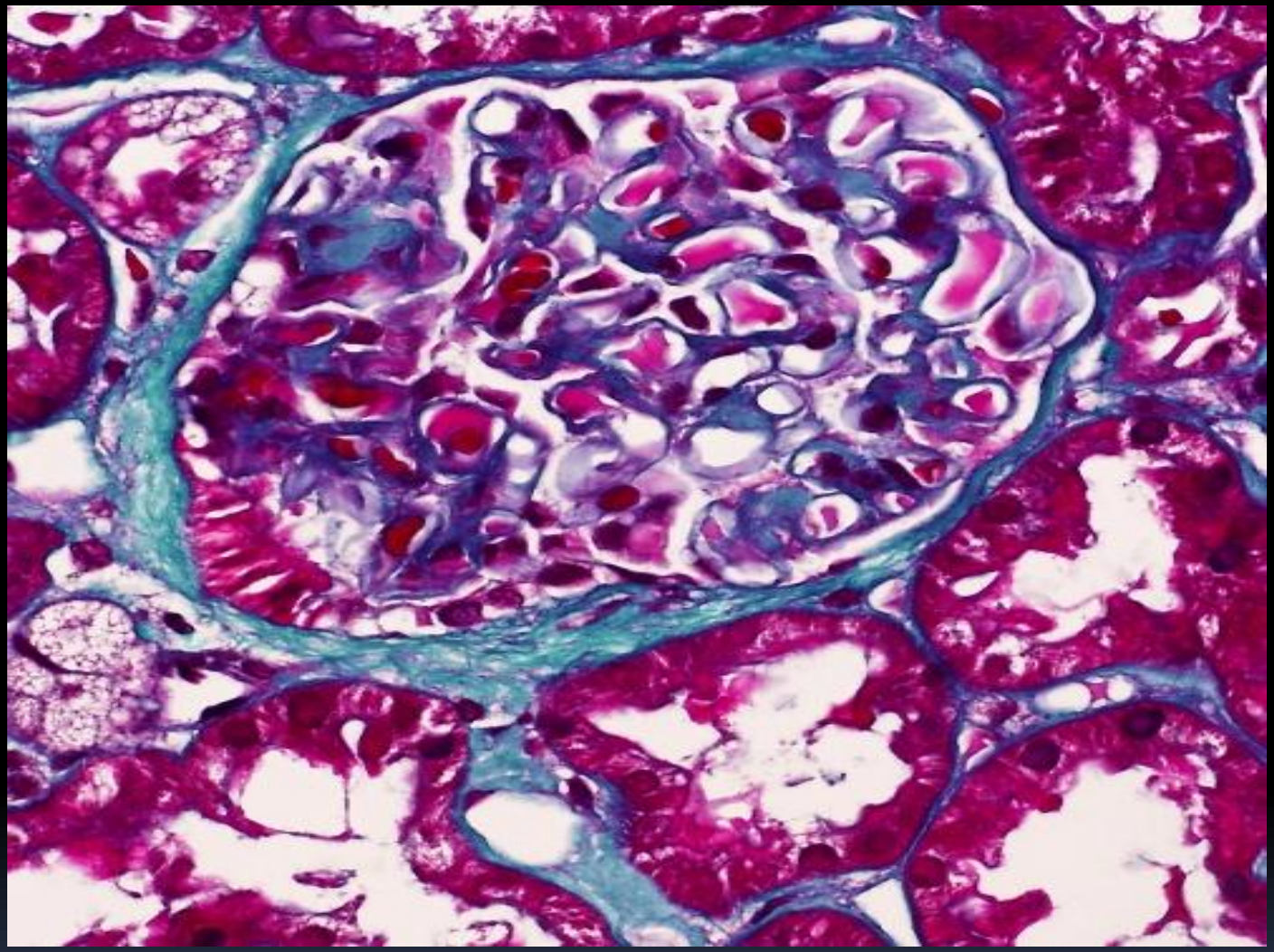


Normal glomerulus of kidney is stained with H&E

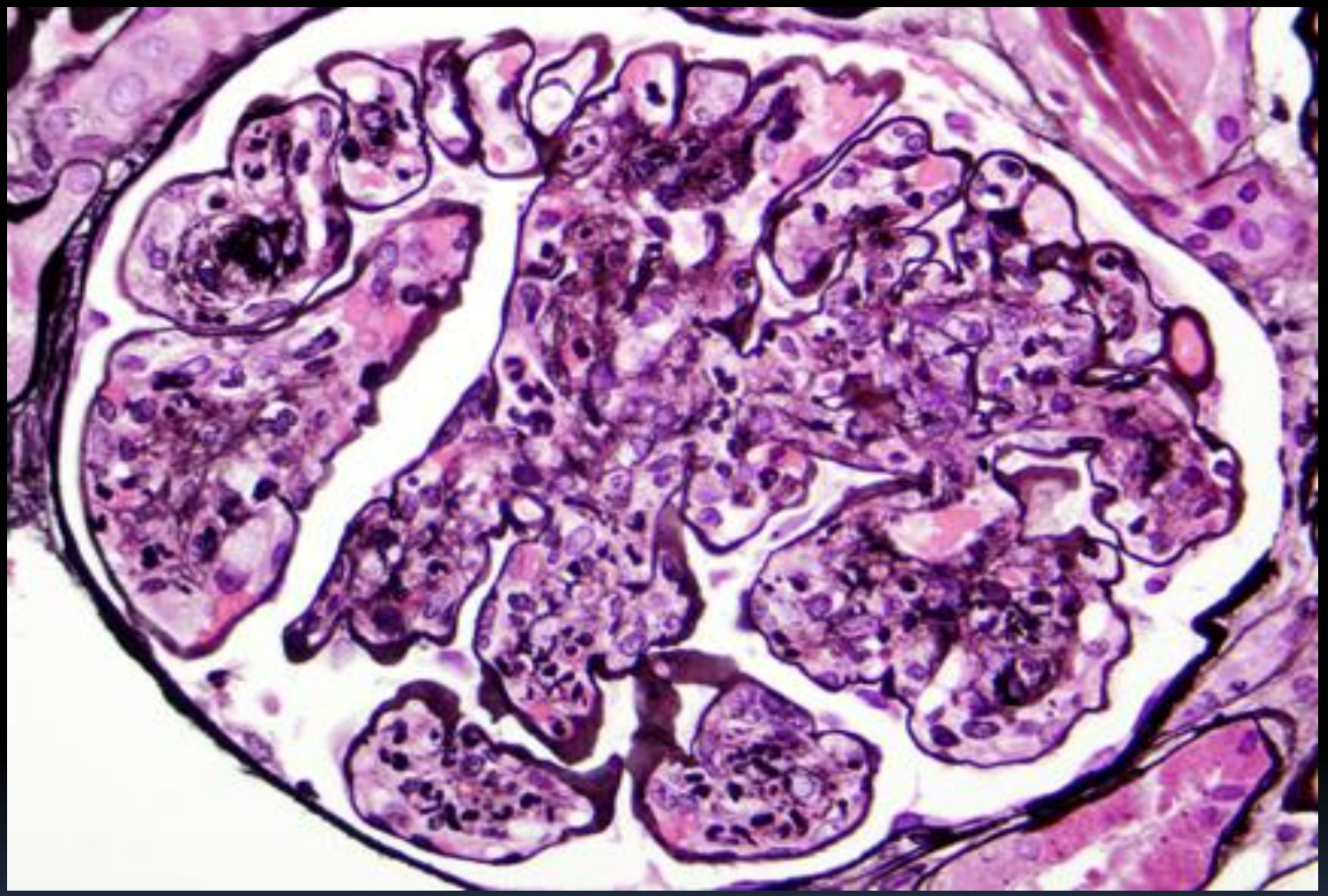


Normal glomerulus of kidney is stained with PAS

detects glycogen, glycoproteins, glycolipids and mucins in tissues

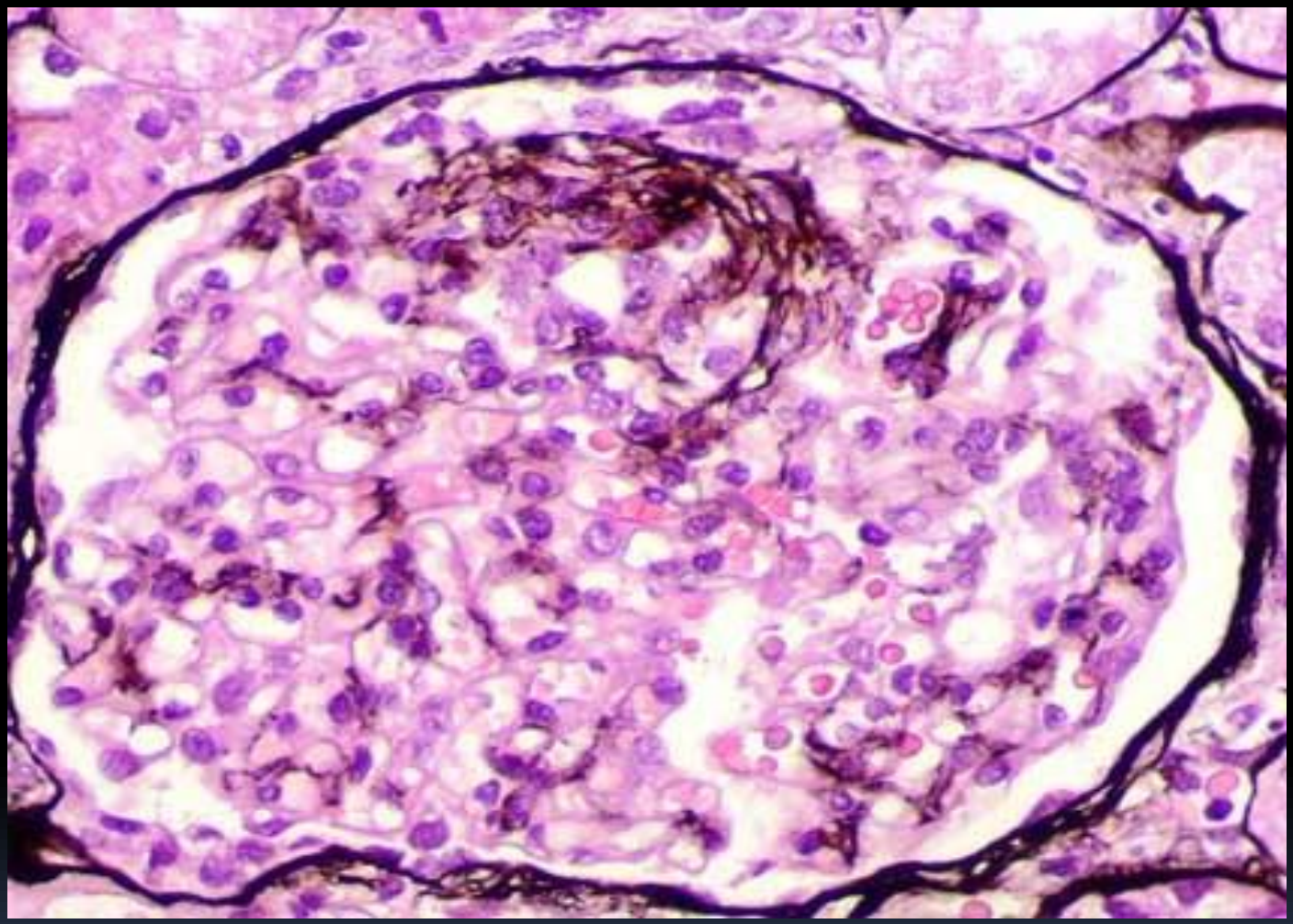


Normal glomerulus of kidney is stained with Masson_trichrome



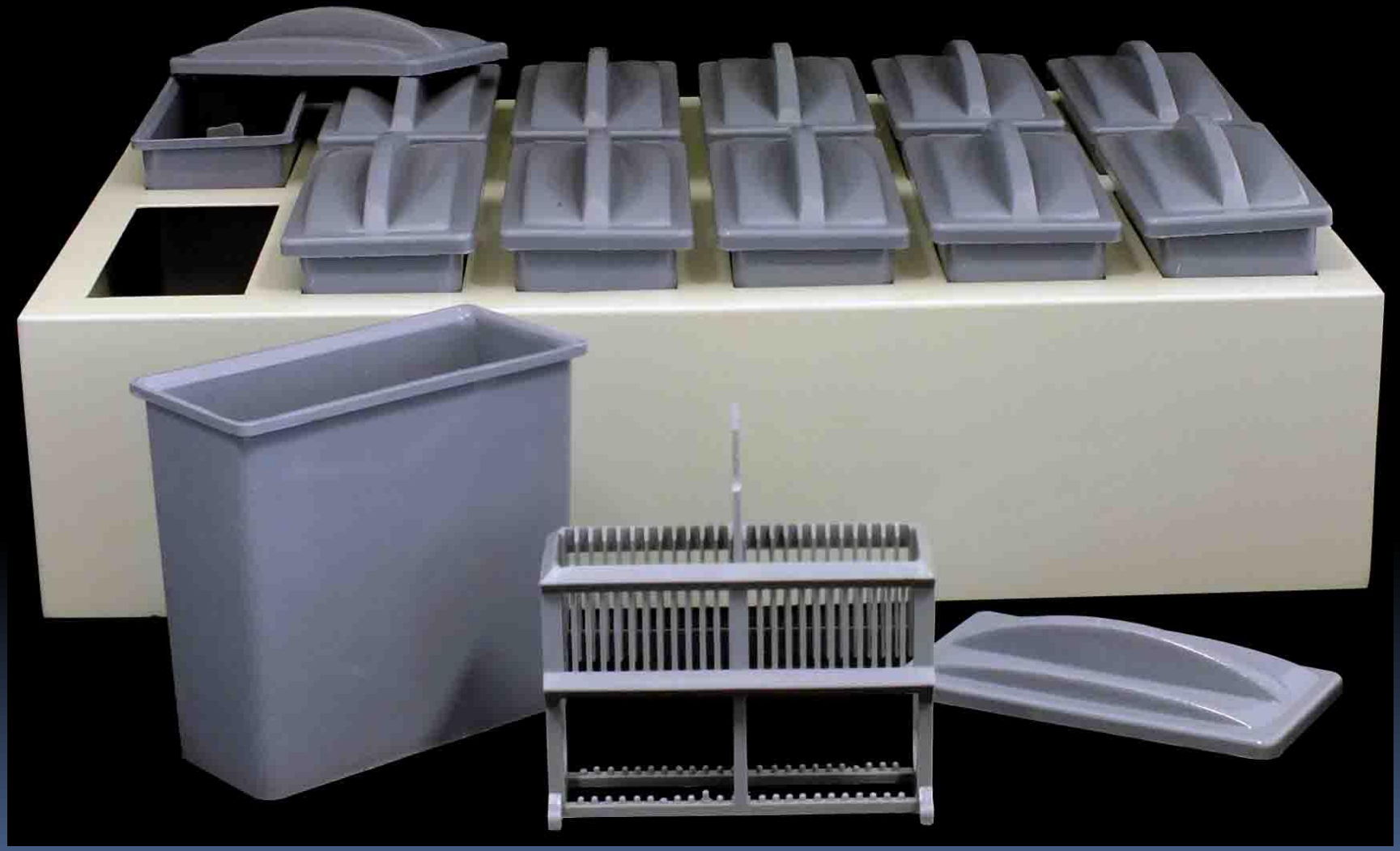
Normal glomerulus of kidney is stained with methenamine silver.

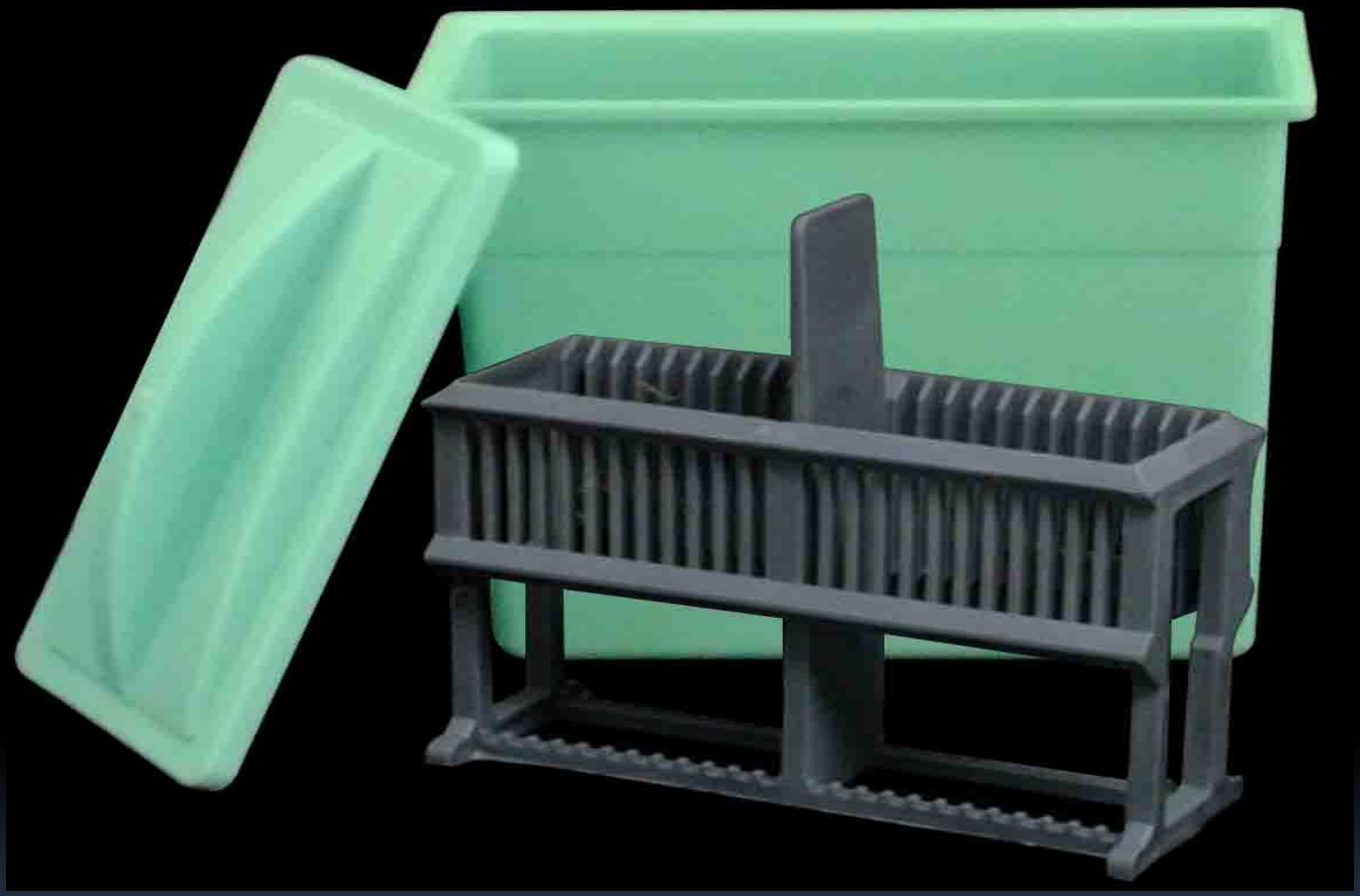
Silver stains reticular fibres (type III collagen).



Normal glomerulus of kidney is stained with methenamine silver

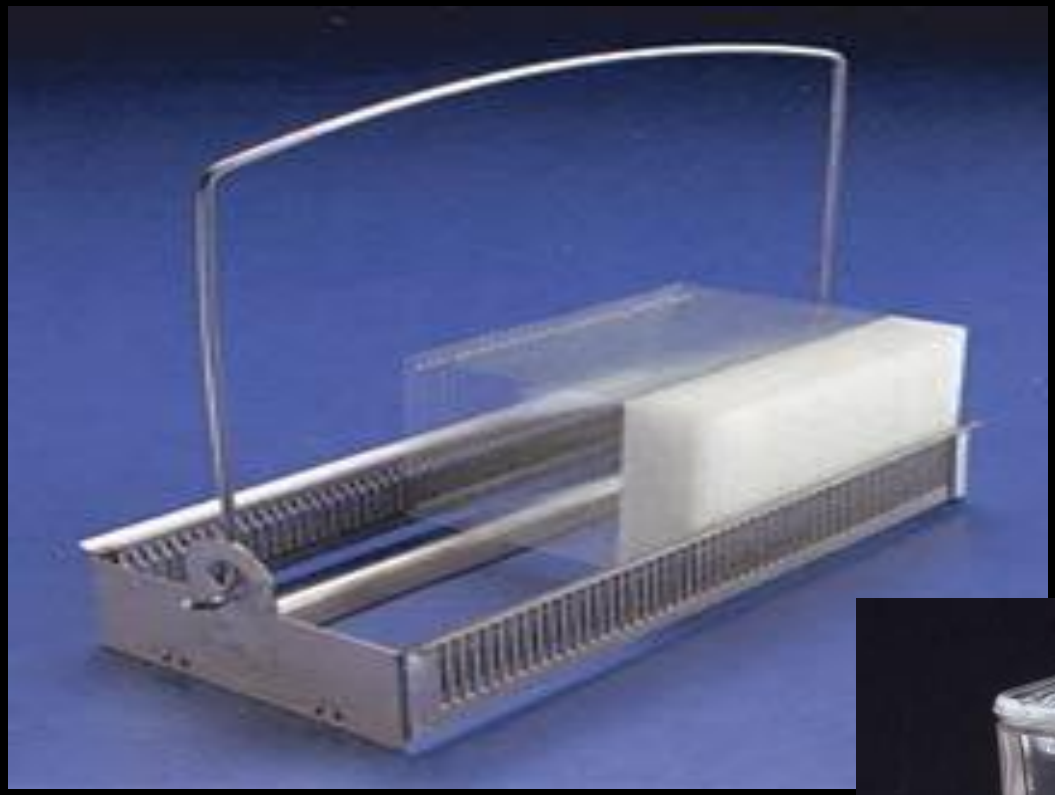
Staining Tools





Slide rack


Liver Spleen
Brain Rat
Lung Mouse
Lung Mouse
Mouse Uterus
Kidney Mouse
Lion Testis
Lion Testis
Pup
12 June 13



Slide rack

Solutions

Procedure of staining:

- There are two types of staining:

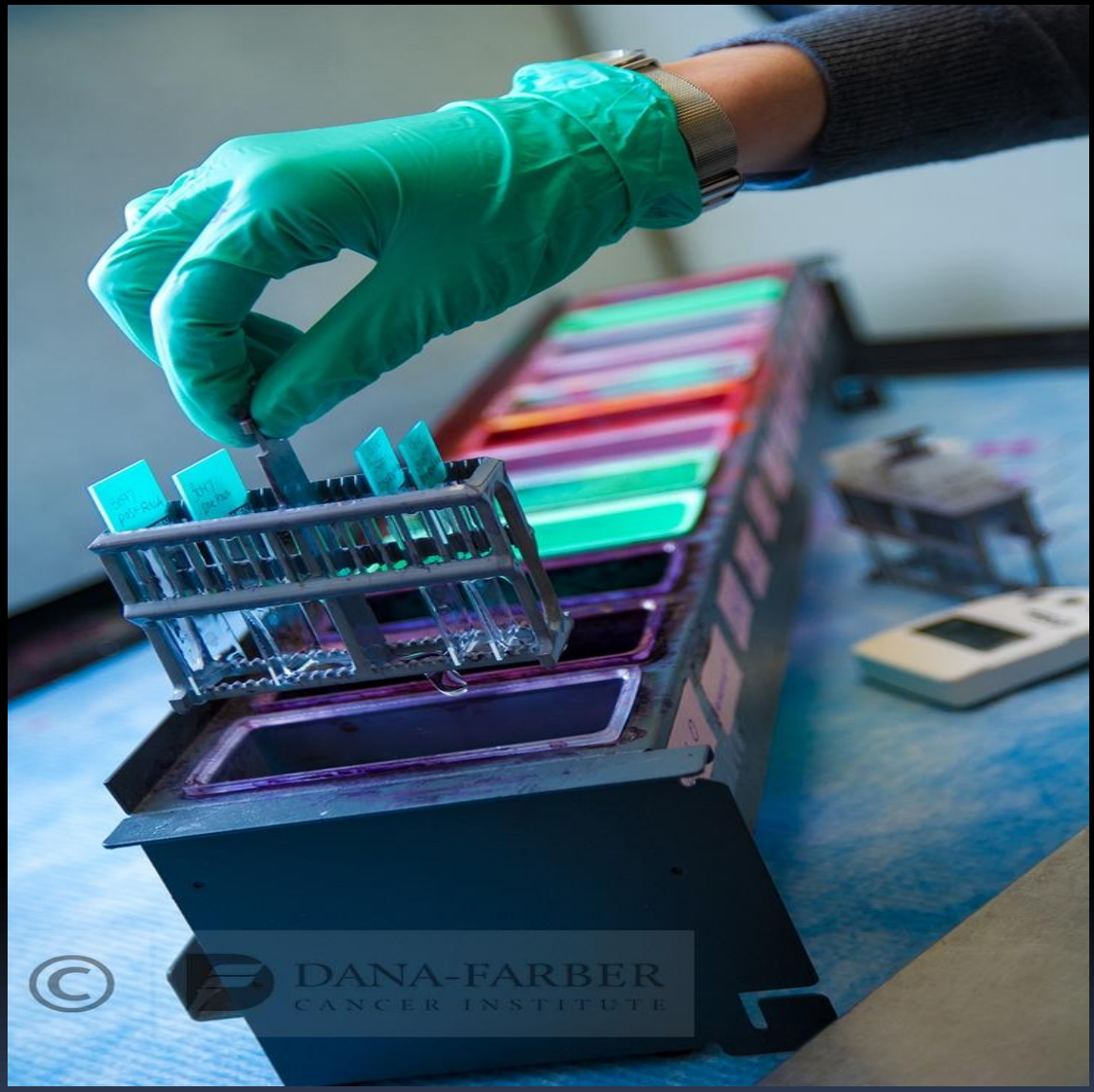
- Manual Staining
- Automatic staining

- Slides stained either manually or by automatic stainer, pass through same sequences of reagents.

Manual Staining

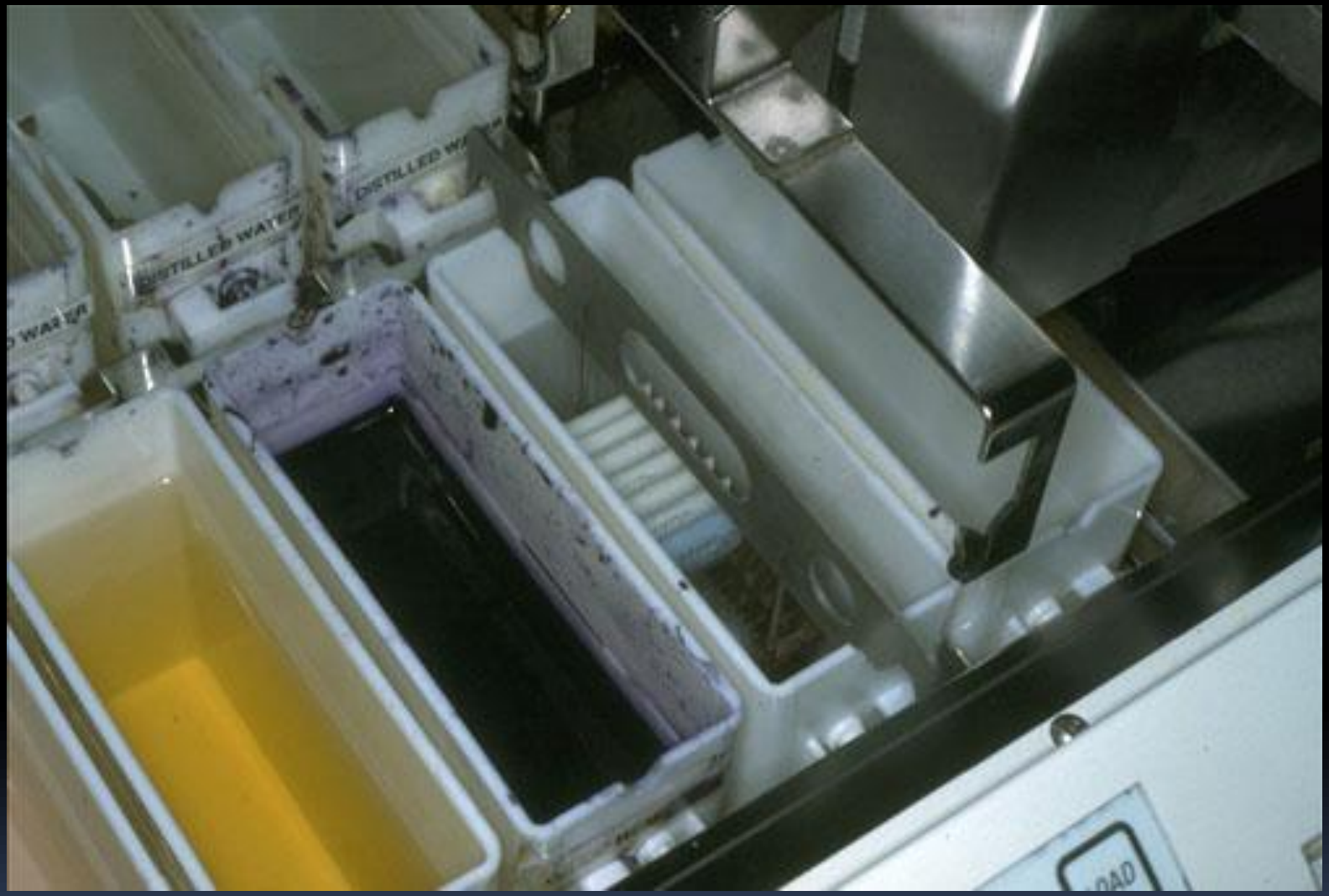
- Different reagent containers are placed in a special sequence and the slides are removed from one container to another manually.

Manual Staining



Manual Staining

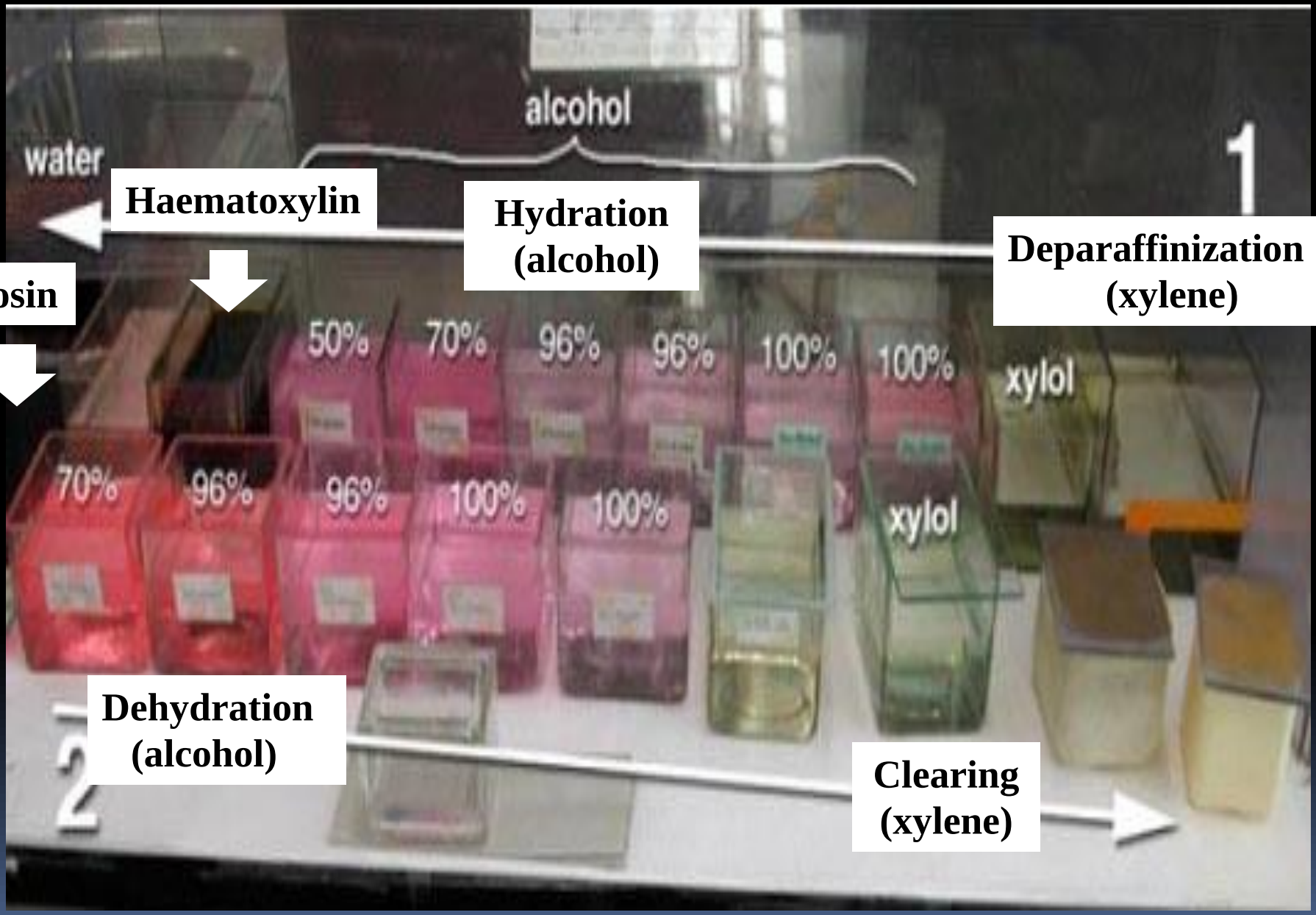
Automatic stainer



Automatic stainer

Procedure :

1. Deparaffinization with xylene
2. Hydration (hydrate to water 100%, 95%, 70% ethanol)
3. Wash under water
4. Stain with Haematoxylin for 10-15 min
5. Wash with water
6. Differentiate with 1 % acid alcohol (lithium carbonate)
7. Wash with water
8. Stain with 1% Eosin for 4-5 min
9. Dehydration (70%, 95% and absolute 100% alcohols)
10. Clearing with xylene
11. Dry
12. Mount


alcohol
water
1
Haematoxylin
Hydration
(alcohol)
Deparaffinization
(xylene)
Eosin
50%
70%
96%
96%
100%
100%
xylol
70%
96%
96%
100%
100%
xylol
Dehydration
(alcohol)
2
Clearing
(xylene)

Mountining

Mountining:

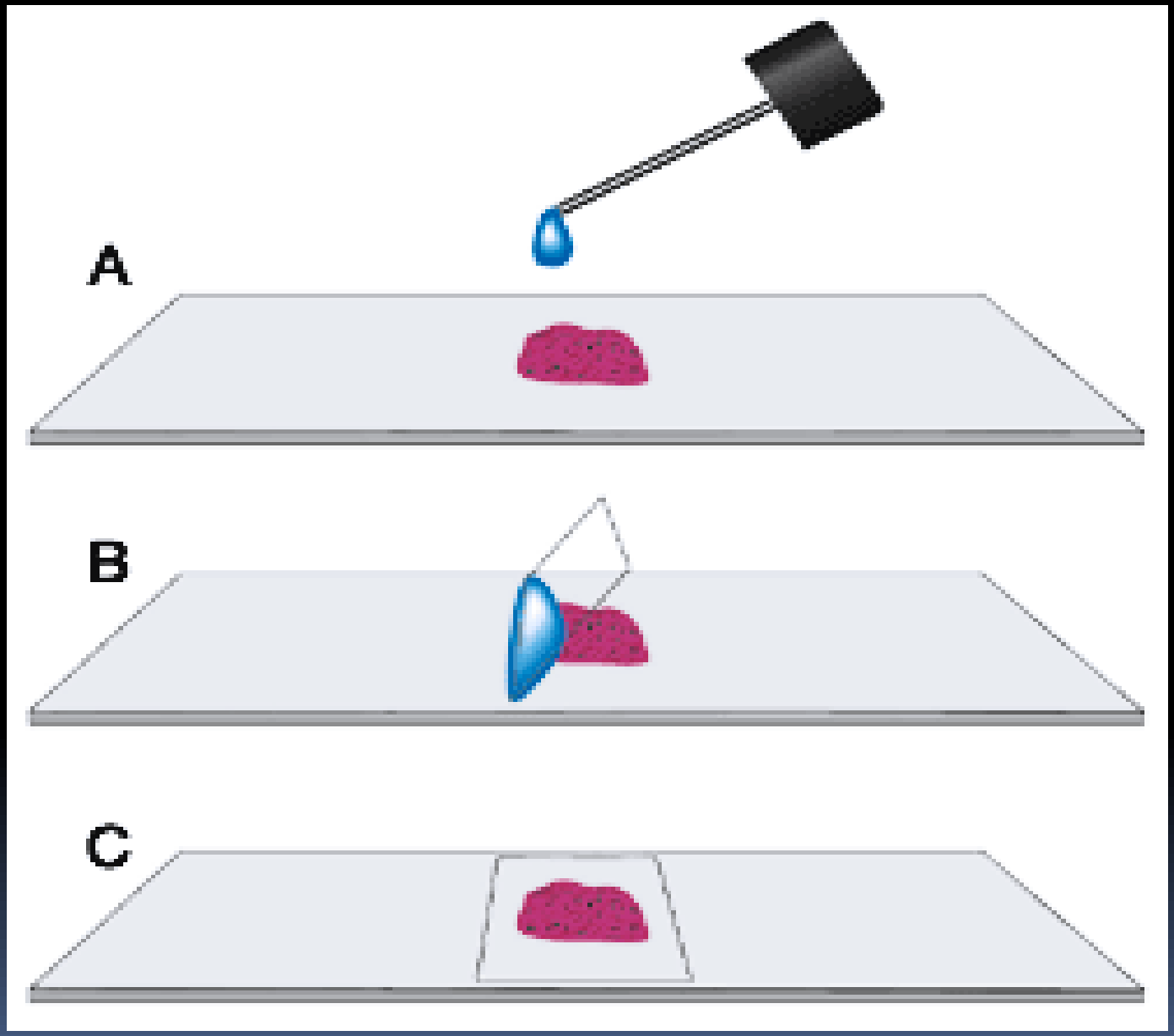
Stained section on microscope slide then is mounted using mounting medium dissolved in xylene.

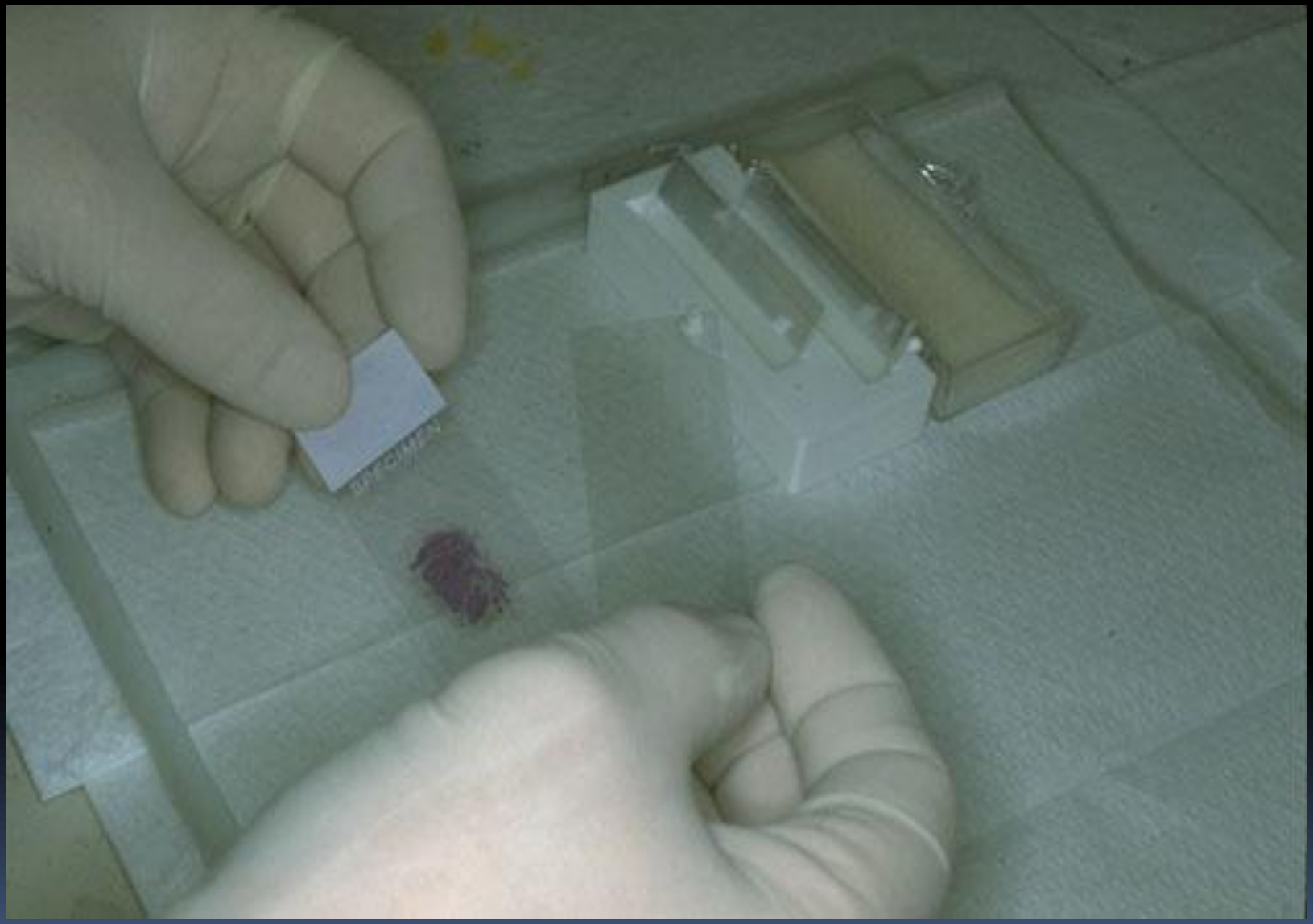
Examples of Mountants :

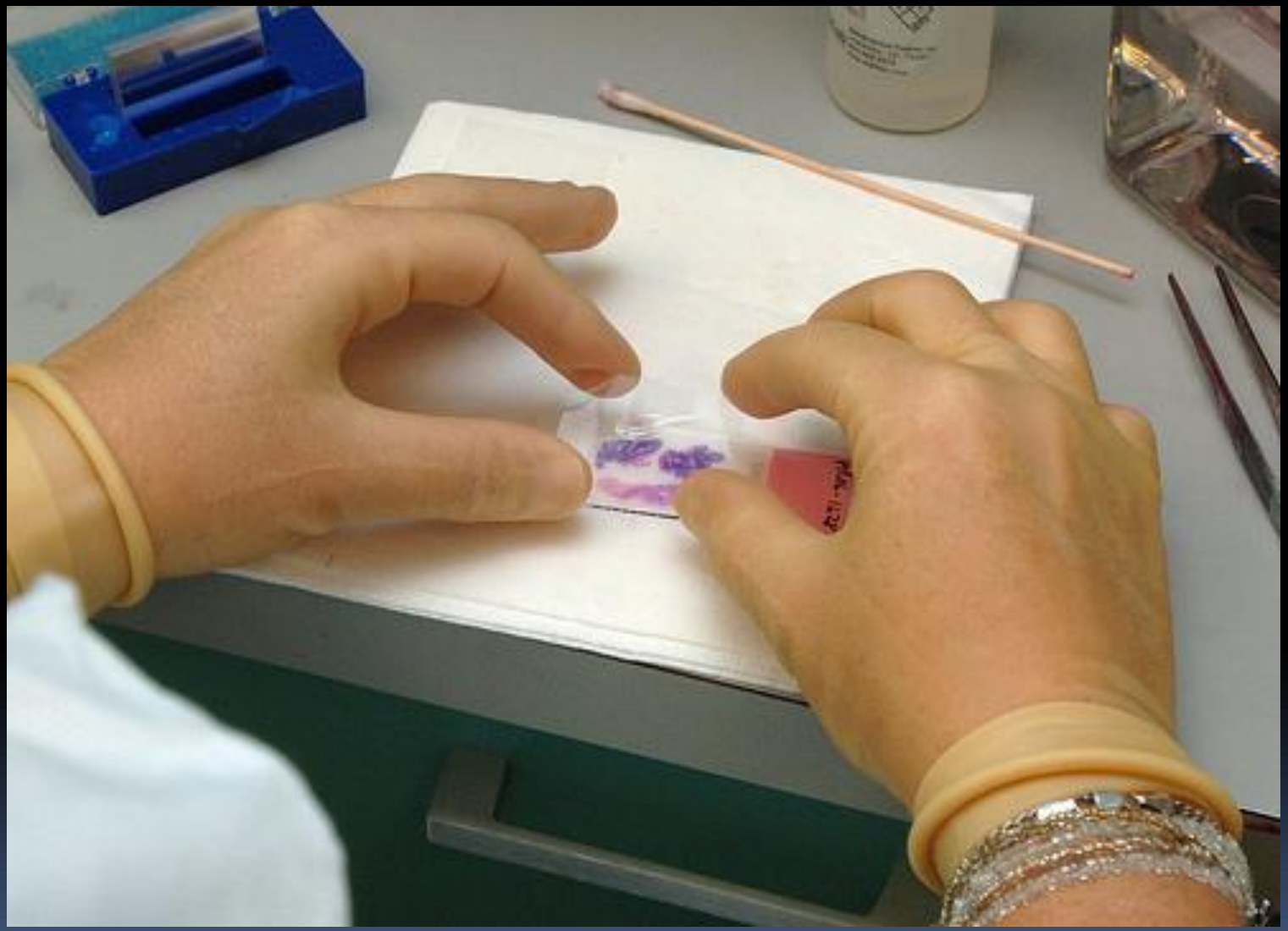
- ✓ DPX (Distrene Dibutyl phthalate Xylene).
- ✓ Canada Balsam
- ✓ Colophonium resin
- Terpene resin

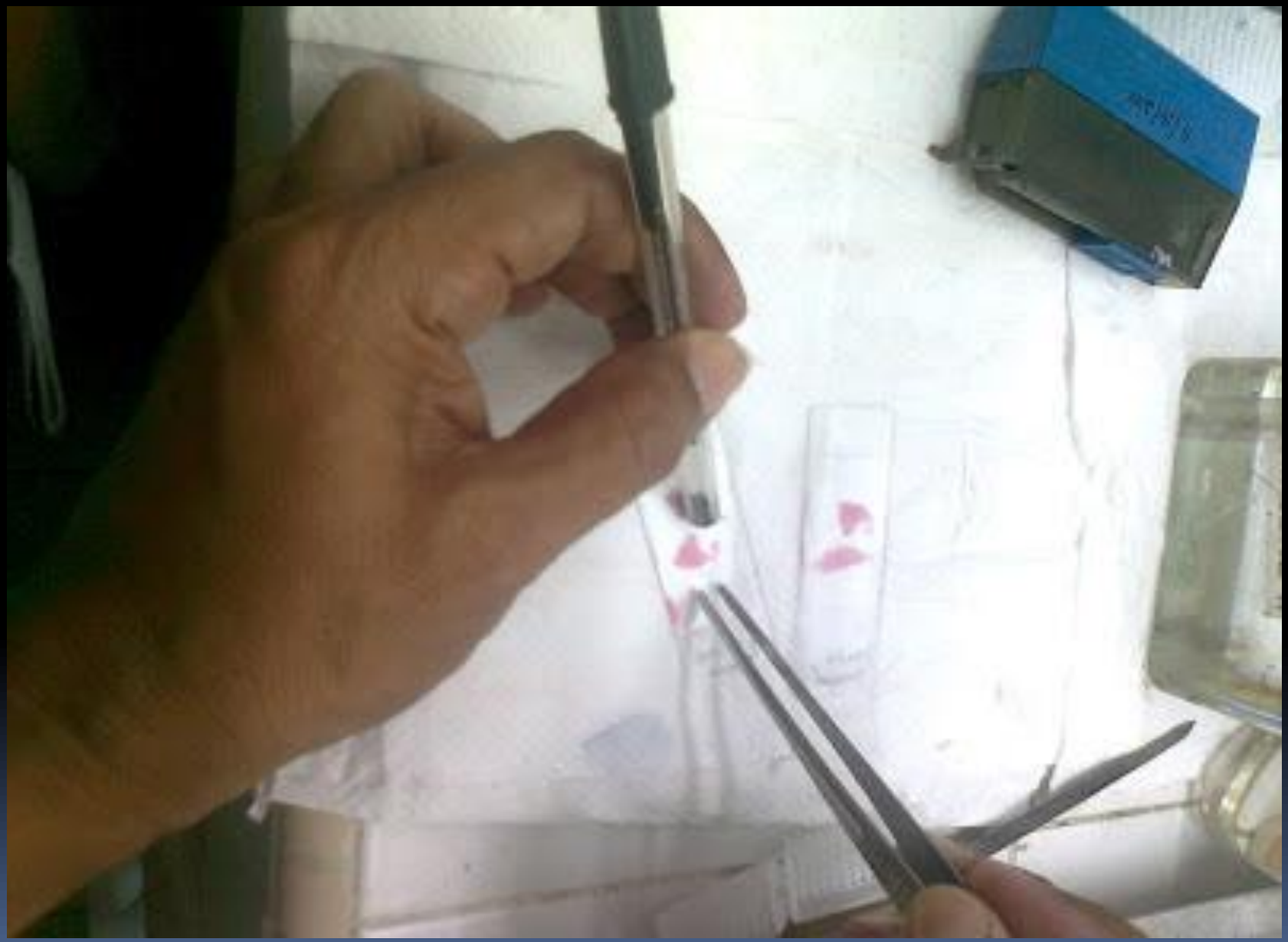
- A coverslip is placed on top, to protect the sample.

- Evaporation of xylene around the edges of the coverslip, dries the mounting medium and bonds the coverlips firmly to the slide.

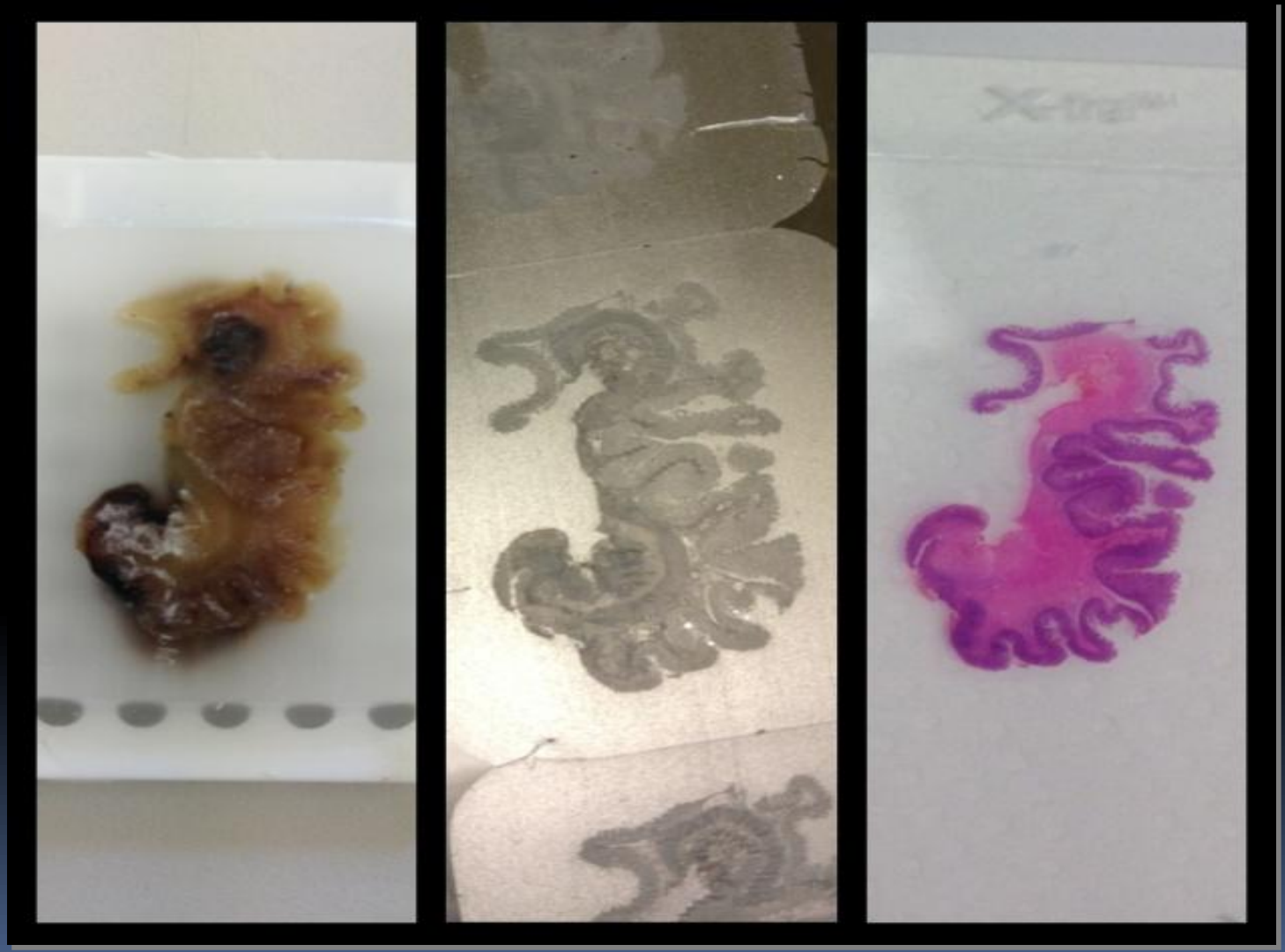

A
B
C



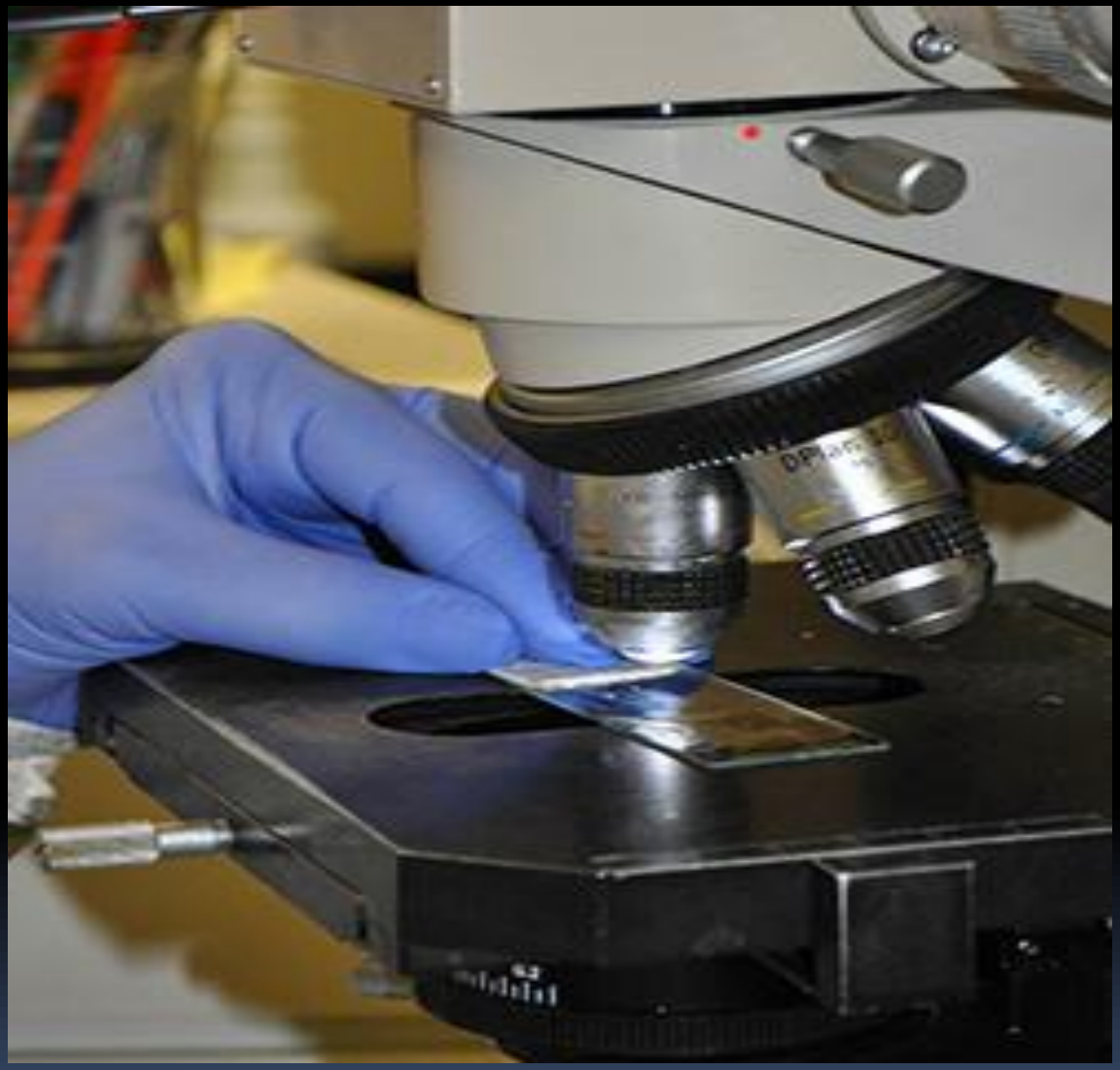




Imagination



Light Microscopic Examination



Microscopic Examination

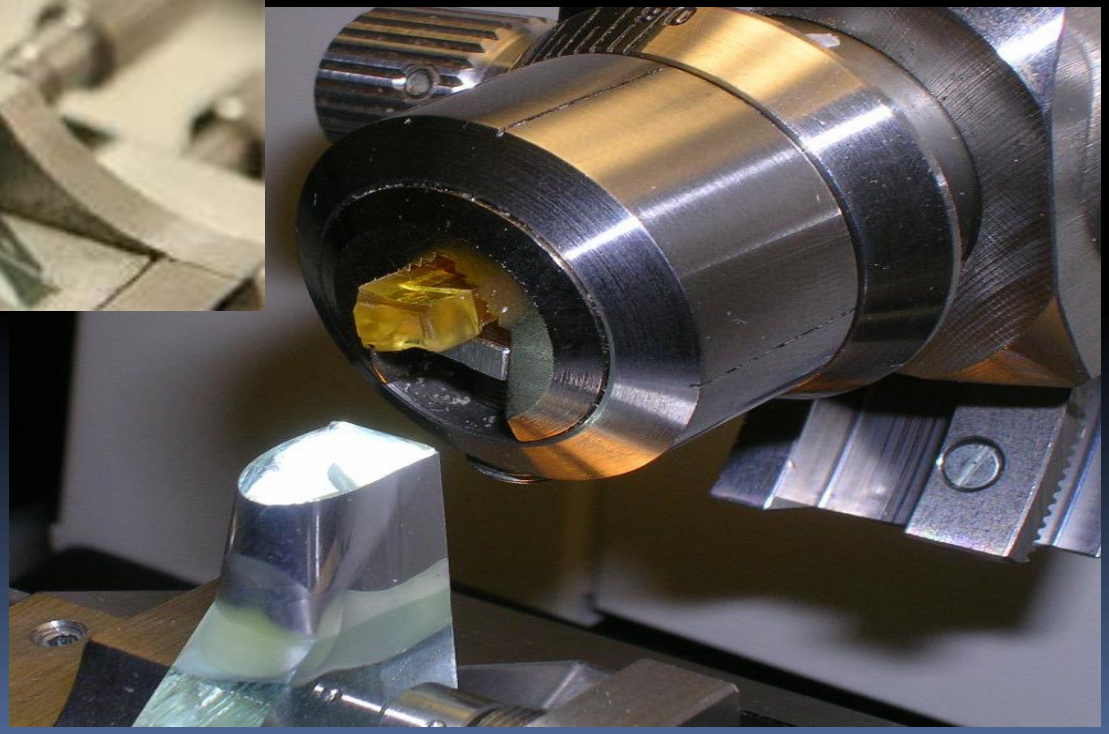
Concerning Light Microscopy

- Use beam of transmission light.
- Magnified images are typically from 10-1000x.

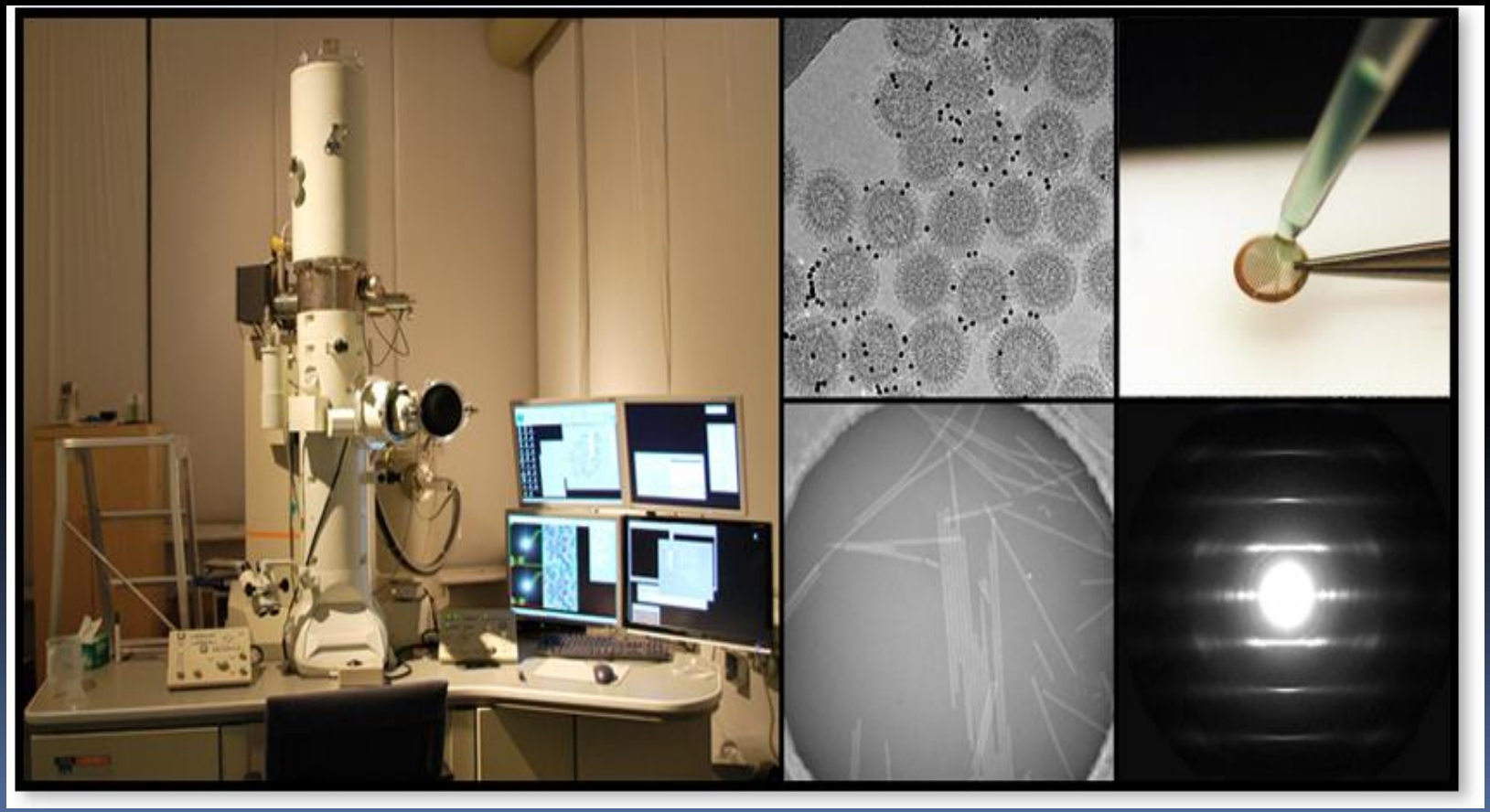
Concerning Electron Microscopy

- ✓ Electron beam instead of light.
- ✓ Magnified images are typically from 1000X to 50,000X.
- ✓ Gluteraldehyde & Osmium tetraoxide fixative.
- ✓ Embedding is in hard epoxy plastic.
- ✓ Ultramicrotome.
- ✓ Diamond or Glass knives.
- ✓ Thin section (0.02 -0.1 μm).
- ✓ Specimen is mounted on a metal grid.

Ultra Microtome



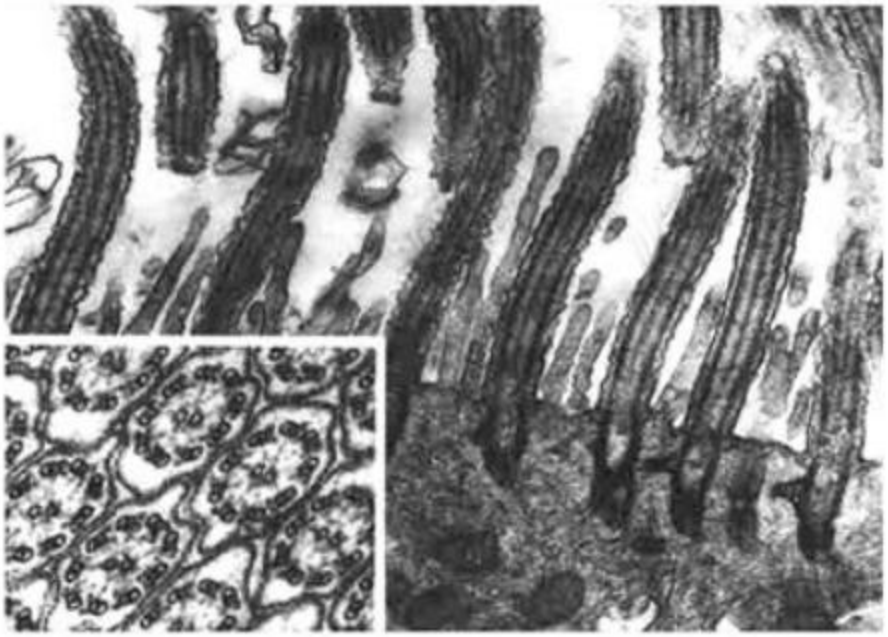
FOR **transmission electron microscopy**, a diamond knife (or glass) mounted in an ultramicrotome is used to cut 0.02- 0.1 μ thick tissue sections which are mounted on a 3-mm-diameter copper grid. Then the mounted sections are treated with the appropriate **stain.**



Transmission Electron microscope (TEM)

Types of EM

Cilia



Transmission EM

Scanning EM