Ministry of Higher Education
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# Lab. 2
# Microtechniques
## "Histotechniques"

**Sum of steps used for preparation of a microscopic slide ready to examination**

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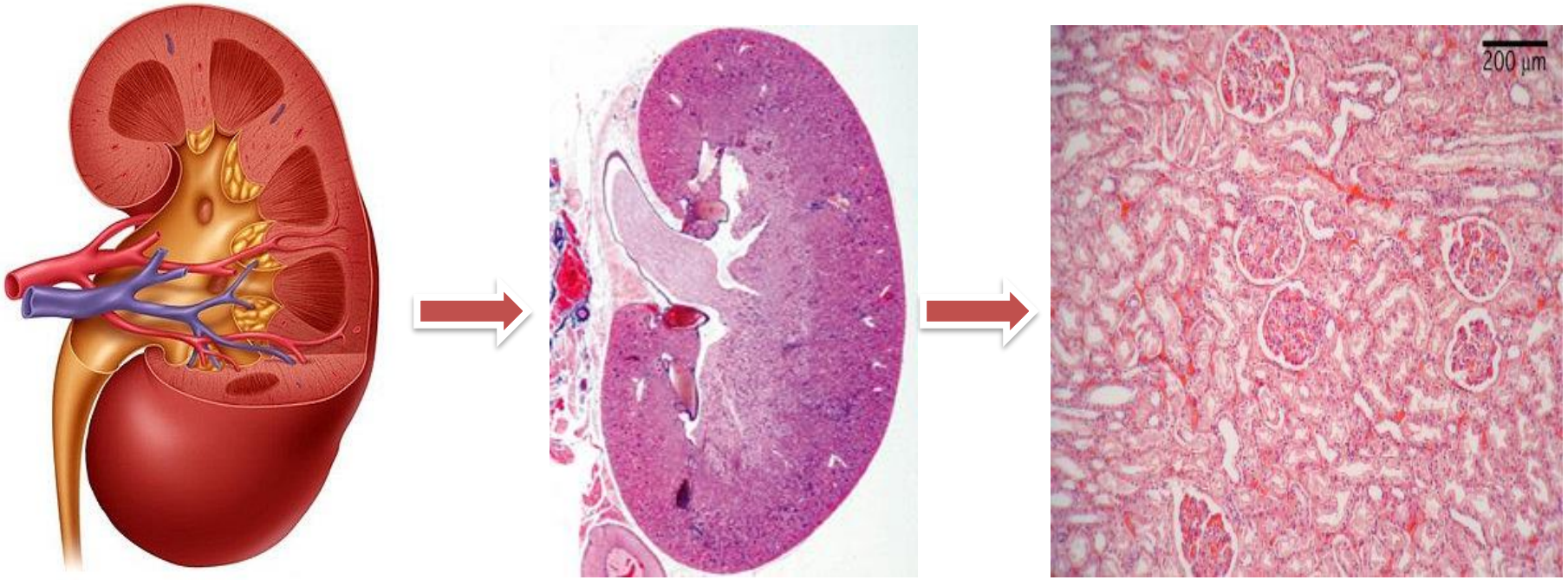
# ➢What is histology?

**Histology** is the study of the microscopic anatomy (microanatomy) of cells and tissues of animals and plants.



# Types of specimen

1. **Autopsy**

   post-mortem specimens

2. **Biopsy**

   specimens took from the living animals or humans…

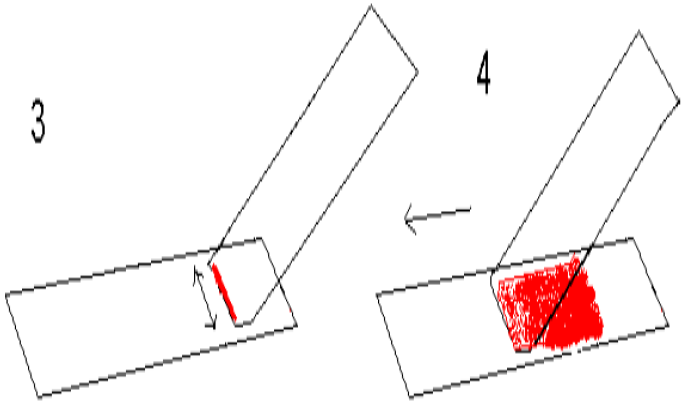
# Methods of preparing microscopic slides:

## 1. Sectioning method

Done by **Microtome**

## 2. Non-Sectioning method

Smear, squash, spreading



# Processing for slide preparation

To perform histological examination, must first prepare tissues:

1. Fixation
2. Dehydration
3. Embedding
4. Sectioning/mounting
5. Staining
6. Microscopic examination

# 1- Fixation

- **Fixation** is a complex series of **chemical events** that comes by reaction between the **fixative and protein** which form a gel like subs.
- **The aim of fixation:**

1- To prevent autolysis and bacterial attack.
2- To prevent changing of the tissue's volume and shape during processing.
3- prepare tissue for **clear staining** of sections.
4- To get tissue as **close** as their **living state.**

# Types of fixatives

- Formalin 10%  (very common)
- Bouin fixative (expensive)
- Alcohols (70%)
- Gluteraldehyde ( E.M)

# Washing

- The process to get rid of the excessive fixative solution.
- Depend on the type of the fixative used the different washer are used.

# 2- Dehydration

- **Remove water from fixed tissue**
  - graded alcohol series followed by "clearing agent" (xylenes, histoclear, toluene) to remove alcohol (making tissue somewhat transparent).
    - **miscible** = liquids will mix to form a homogenous solution

# Dehydration

50% EtOH / 50% Phosphate-buffered Saline (PBS)
75% EtOH / 25% PBS
90% EtOH / 10% PBS
95% EtOH / 5% PBS
100% EtOH
clearing agent
paraffin wax (if doing paraffin embedding/sectioning)

# 3- Clearing

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

- **miscible** = liquids will mix to form a homogenous solution

# Some clearing agents:

- Xylene
- Toluene
- Chloroform
- Benzene

## Choice of a clearing agent depends on:

1. Type of tissues to be processed
2. Safety factors.
3. Cost and convenience.
4. Speedy removal of dehydrating agent .
5. Minimal tissue damage .

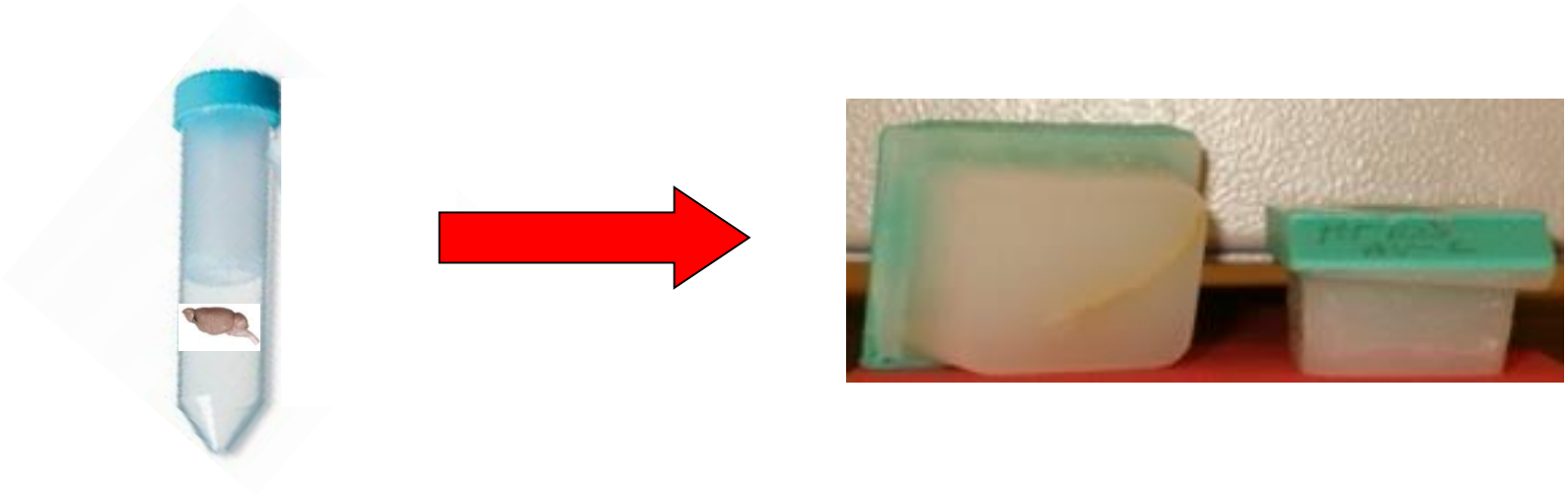
# 4- Embedding

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

- **Why so ?**
  - Structural support to tissue during sectioning
  - Makes tissue easier to cut
  - Tissue needs to withstand sectioning process
  - Components must stay in natural positions

# Embedding

Tissue embedded in "hard" Paraffin wax medium



# 5- Sectioning

- Using microtome to cut transparent thin slices from embedded tissue specimen & mount on microscope slides

- Why slices should be very thin?
  - Allows you to see internal structure of tissue.
  - Allows stains or specific markers such as antibodies to more easily infiltrate tissue.
  - Allows light to pass through tissue making structure visible.

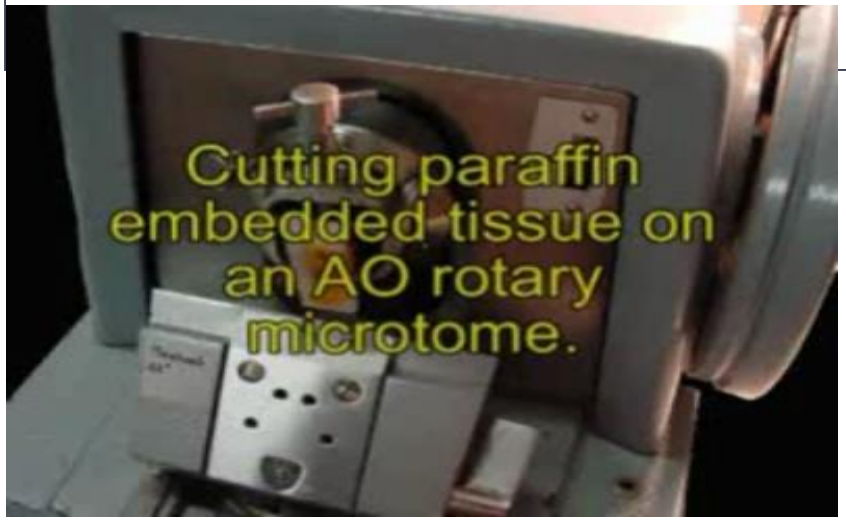
# Sectioning by microtome

- A **microtome** is a mechanical instrument used to cut biological specimens into very thin segments for microscopic examination.

**Types:**

- Rotary microtome
- Freezing microtome
- Sliding microtome
- Ultra microtome(E.M)





# Sectioning by microtome

- We use microtome to cut transparent thin slices from embedded tissue specimens
  - generally **5-15** mm sections
  - Thickness depends upon tissue, subsequent procedures treatment.

# Mounting sections

A. 40° C water bath

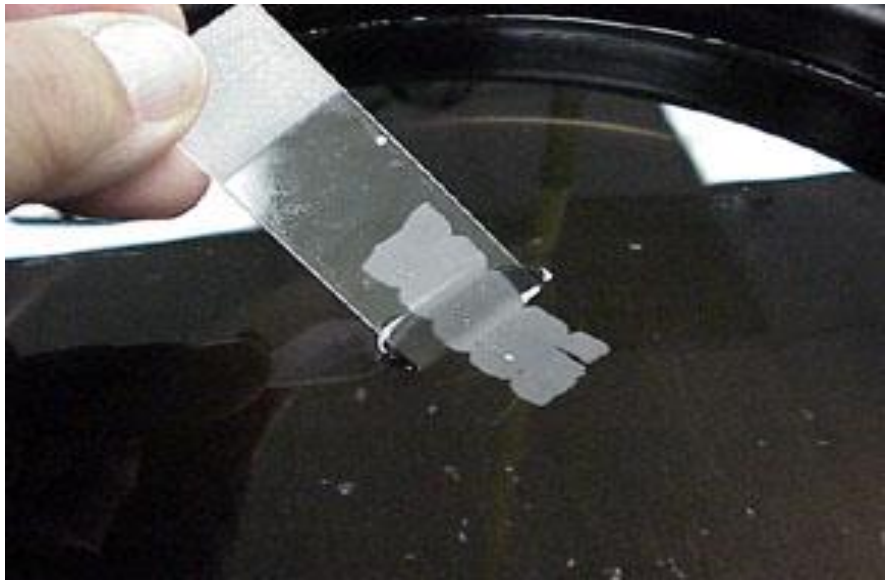
1. Flattens paraffin section

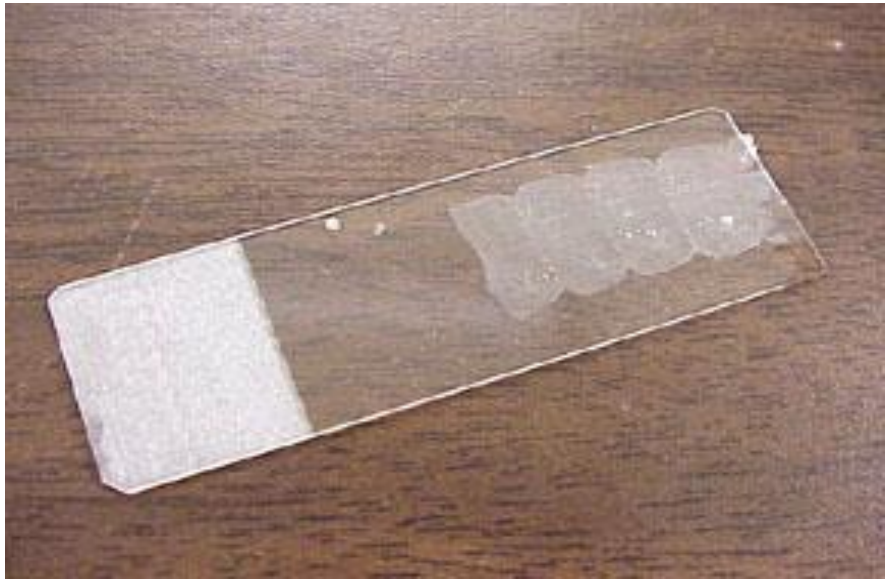
2. Permits mounting on slide

B. Gelatin & albumin

C. Glass slides

D. Oven / air dry





# 6- Staining

- **Use many techniques to stain tissue sections w/ dyes**
  - first must remove embedding medium, rehydrate(series alcohol) tissues (dyes generally in aqueous solution)
  - common stains - hemotoxylin & eosin (H&E), trichrome
- **Why staining is important?**
  - Creates higher contrast that allows observation of structures that are not visible in unstained tissues.
  - May reveal differences in chemical nature of certain regions of tissue.

# Coverslipping / Mounting

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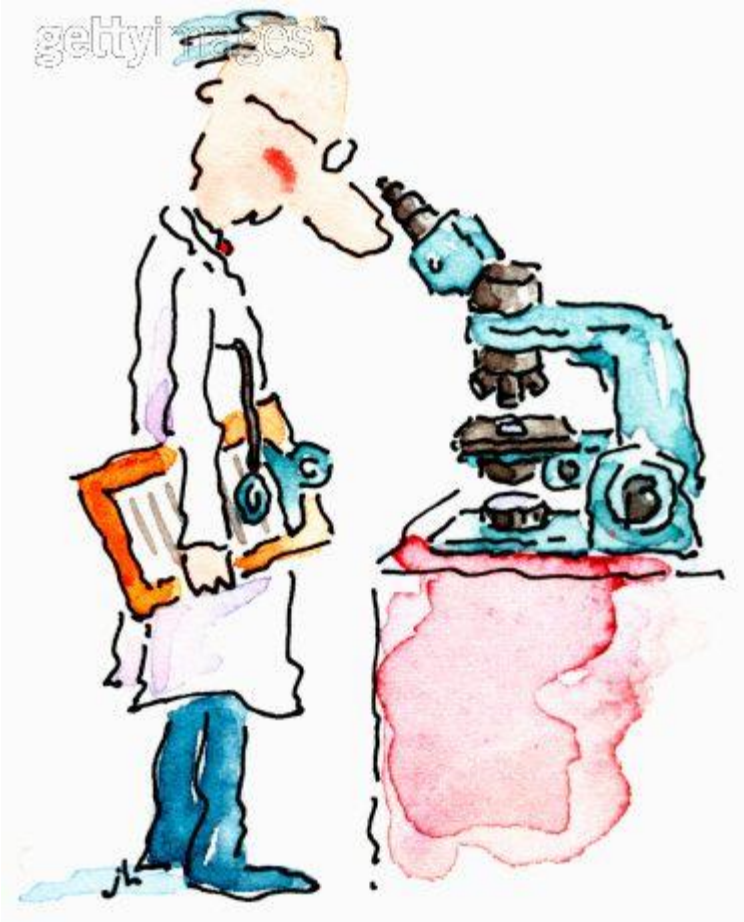
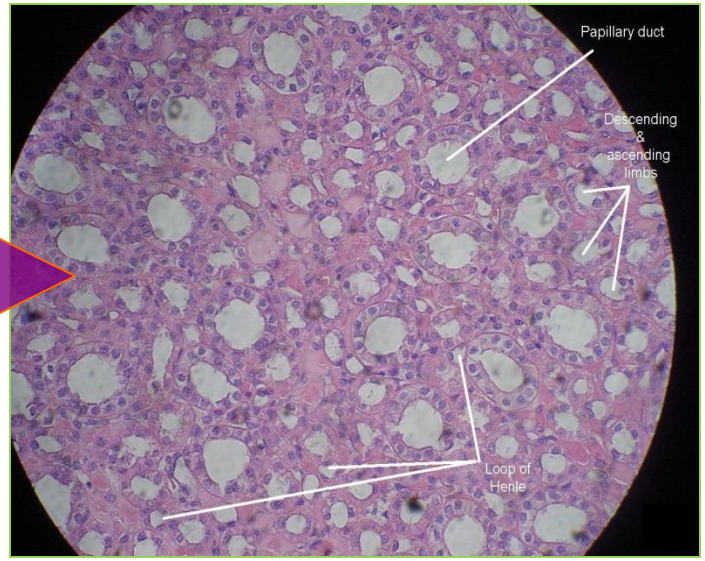
*A process in which we use a medium for adhesion of coverslip to the slide to protect the tissue from the dust, MO. & dirt.

**Processs:**

1. Remove the slides from the staining agent and dry it .
2. Coverslip using appropriate sized cover glass.
3. Label slides if necessary and arrange accordingly.

# The slides are ready to be examined Microscopically






# Thank you