



**Faculty of Applied
Science
Medical Analysis
Department
Micritechnique (MA505)**

Lecture 3

TISSUE PROCESSING

Kovan Faidhalla

M.Sc. Biomedical Science Blood Science

(Summer school)

August 20, 2026

Outlines



- What Is Tissue Processing?
- What are the purpose of tissue processing?
- Principles of Tissue Processing
- What is Dehydration?
- Why is dehydration necessary?
- Common Dehydrating Agents
- What is Clearing?
- Infiltration
- Factors Affecting Paraffin Infiltration
- Class activity
- References
- Next lecture

Learning outcome

- By the end of this lecture, students will be able to:
- Define tissue processing and explain its purpose in histopathology.
- Describe the principles of **dehydration, clearing, and paraffin infiltration**.
- Discuss factors affecting the quality and speed of tissue processing.
- Identify common processing problems and explain their causes.
- Select appropriate processing conditions for different tissue types.
- Explain how improper processing affects subsequent sectioning and staining.

What Is Tissue Processing?

- **Tissue processing** is the series of chemical steps used to prepare a fixed tissue specimen for embedding in a suitable medium, usually paraffin wax, so that thin sections can be produced for microscopic examination.

What are the purpose of tissue processing?



Remove water from the tissue.

Replace water with a solvent compatible with paraffin.

Replace the solvent with molten paraffin.

Provide sufficient tissue support for sectioning.

Preserve tissue morphology as much as possible.

Principles of Tissue Processing

- Routine paraffin processing consists mainly of three major stages:

- **1. Dehydration**

- Removal of water from tissue



- **2. Clearing**

- Replacement of alcohol with a substance miscible with paraffin



- **3. Infiltration**

- Replacement of clearing agent with molten paraffin

What is Dehydration?

- Dehydration is the process of **removing water from fixed tissue**, usually by using increasing concentrations of alcohol.
- **Why is dehydration necessary?**
- Paraffin wax is not miscible with water.

Common Dehydrating Agents

- **Ethanol**
 - The most commonly used dehydrating agent.
 - Typical sequence:
 - **70% → 80% → 95% → 100% ethanol**
 - The gradual increase minimizes tissue distortion.
- **Other agents**
 - Isopropanol
 - Methanol
 - Acetone

Why Use Graded Alcohol?

- A sudden transfer from tissue containing a large amount of water directly into absolute alcohol can cause:
- Excessive shrinkage
- Hardening
- Tissue distortion
- Poor staining
- Therefore, tissues are generally transferred through **increasing concentrations of alcohol.**
- **Example**
- **70% → 80% → 95% → 100% → 100%**

Factors Affecting Dehydration

- **1. Tissue size**
 - Small tissue pieces dehydrate faster than large specimens.
- **2. Tissue thickness**
 - Thicker specimens require longer processing.
- **3. Alcohol concentration**
 - Higher concentrations remove water more rapidly.

4. Temperature

Increasing temperature generally accelerates processing but may increase tissue damage.

5. Agitation

Movement of the reagent around tissue can improve penetration.

6. Duration

Insufficient time results in incomplete dehydration.

Excessive dehydration may cause:

Tissue hardness

Shrinkage

Brittleness

What is Clearing?

- Clearing is the process in which the dehydrating agent, usually alcohol, is replaced by a solvent that is **miscible with both alcohol and paraffin**.
- Many clearing agents make tissue appear more transparent or "clear."

Common Clearing Agents

- **Xylene**
- One of the most commonly used clearing agents in routine histopathology.
- Advantages:
 - Rapid
 - Effective
 - Compatible with paraffin
- **Other clearing agents**
 - Toluene
 - Chloroform
 - Cedarwood oil

How Do We Know Clearing Is Complete?

- Properly cleared tissue should generally:
- Become relatively translucent.
- Allow the clearing agent to penetrate the tissue.
- Be ready for paraffin infiltration.

- **Incomplete clearing**
- If alcohol remains in the tissue:

Infiltration

- Infiltration is the process of replacing the clearing agent inside the tissue with a supporting medium, usually **molten paraffin wax**.
- **Main purpose**
- To provide sufficient structural support for cutting thin sections.

Paraffin Wax

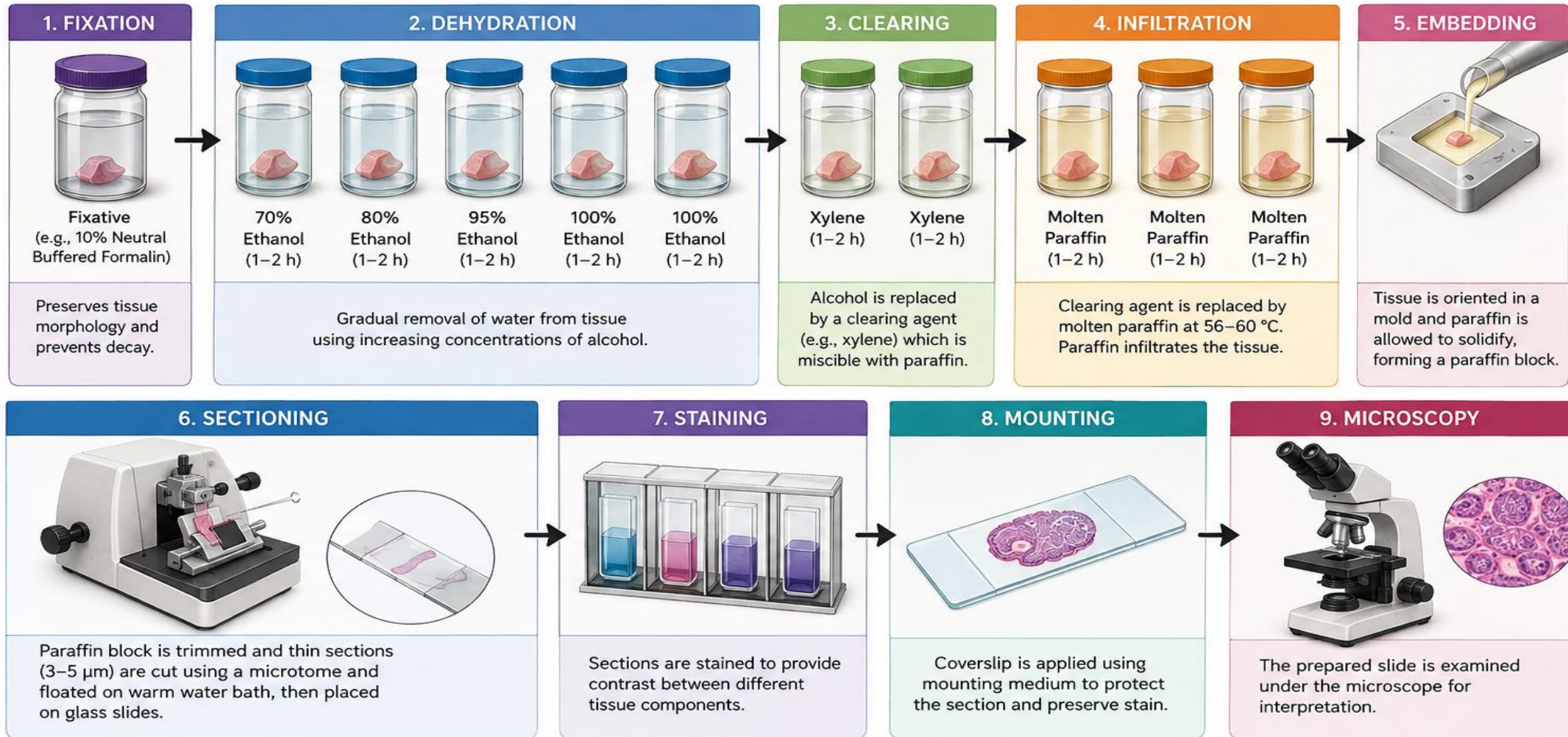
- Paraffin is commonly used because it:
- Provides good tissue support.
- Allows thin sections to be produced.
- Is relatively inexpensive.
- Is suitable for routine histology.
- Can be removed during staining.

Factors Affecting Paraffin Infiltration

- **Tissue size**
- Large tissues require longer infiltration.
- **Tissue density**
- Dense tissues may require additional time.
- **Temperature**
- Temperature should be sufficiently high to keep paraffin molten but not unnecessarily high.
- **Paraffin quality**
- Contaminated or poorly maintained paraffin can negatively affect tissue quality.
- **Vacuum**
- Vacuum processing can improve penetration of paraffin into some tissues.
- **Duration**
- Insufficient infiltration produces poorly supported tissue.

STEPS OF TISSUE PROCESSING

From fixed tissue to paraffin block ready for sectioning



SUMMARY FLOW

Fixation → Dehydration → Clearing → Infiltration → Embedding → Sectioning → Staining → Mounting → Microscopy

 **Key Point:** Proper tissue processing is essential to preserve morphology, ensure good sectioning, and obtain high-quality microscopic results.

Under-Processing vs Over-Processing

Under-processing	Over-processing
Incomplete dehydration	Excessive dehydration
Incomplete clearing	Excessive clearing
Poor paraffin infiltration	Tissue becomes hard/brittle
Soft tissue	Excessive shrinkage
Poor sectioning	Difficult sectioning
Poor tissue support	Tissue fragmentation

Common Processing Problems

- Tissue remains soft
- Tissue is excessively hard
- Tissue Shrinkage

Tissue remains soft

- Possible causes:
 - Incomplete dehydration
 - Incomplete clearing
 - Inadequate paraffin infiltration
 - Tissue too thick
- **Solution**
 - Review the processing schedule and reagent quality.

Tissue is excessively hard

- Possible causes:
 - Excessive dehydration
 - Prolonged exposure to alcohol
 - Excessive processing temperature
 - Prolonged exposure to certain clearing agents
- **Result**
 - Sectioning becomes difficult and tissue may crack or fragment.

Tissue Shrinkage

- **Possible causes**
- Excessive dehydration
- Prolonged processing
- High temperatures
- Strong chemical exposure

Why is this important?

Shrinkage can alter:

Tissue architecture

Cell relationships

Morphometric measurements

Diagnostic interpretation

Class Activity 1 and 2

- **Class activity**
- **5 MQCs questions you should select the correct answer.**
- **Time : 2 min**
- **Marks: 5 marks**
- **Class activity 2**
- **5 matching question you must match the correct answer.**
- **Time : 2 min**
- **Marks: 5 marks**

What is the main purpose of tissue dehydration?

- A. To remove paraffin from tissue
- B. To remove water from tissue
- C. To stain tissue components
- D. To harden paraffin

Which of the following is commonly used as a clearing agent in routine histopathology?

- A. Ethanol
- B. Formalin
- C. Xylene
- D. Paraffin

What is the correct sequence of the main stages of tissue processing?

- A. Clearing → Dehydration → Infiltration
- B. Infiltration → Dehydration → Clearing
- C. Dehydration → Clearing → Infiltration
- D. Dehydration → Infiltration → Clearing

What is the main purpose of paraffin infiltration?

- A. To remove water from tissue
- B. To preserve tissue by fixation
- C. To provide structural support for sectioning
- D. To stain the tissue

Which of the following is most likely to occur when tissue is excessively dehydrated?

- A. Tissue becomes excessively soft
- B. Tissue becomes hard and brittle
- C. Tissue becomes completely transparent
- D. Tissue becomes stained

Class activity 2

Column A	Column B
1. Dehydration	A. Provides support for sectioning
2. Clearing	B. Removes water from tissue
3. Paraffin infiltration	C. Produces thin tissue sections
4. Xylene	D. Replaces alcohol and prepares tissue for paraffin
5. Microtomy	E. Commonly used clearing agent

References

- Bancroft, J. D., & Gamble, M. (Eds.). (2019). Theory and practice of histological techniques (8th ed.). Elsevier.
- Suvarna, S. K., Layton, C., & Bancroft, J. D. (Eds.). (2019). Bancroft's theory and practice of histological techniques (8th ed.). Elsevier.

Next lecture

- Tissue Embedding