Faculty of Applied Science

Medical Analysis Department

Microtechniques (MA505)

Lecture 1

INTRODUCTION TO MICROTECHNIQUES

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Learning outcome

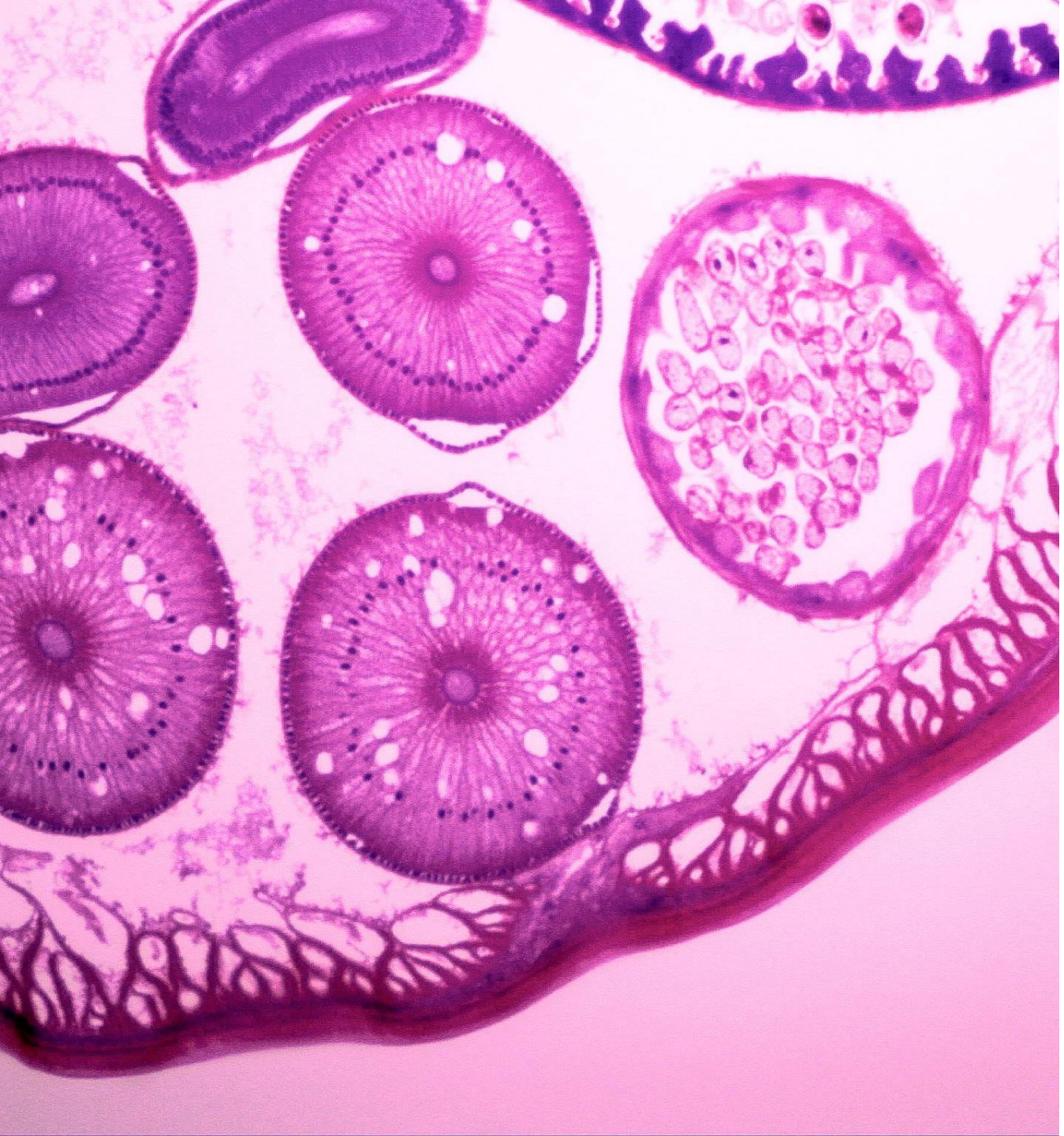
- By the end of this lecture, students will be able to:
- **Define** microtechnique and explain its role in histopathology.
- **Distinguish** histology, histopathology, cytology, biopsy and surgical specimens.
- **Describe** the complete pathway from tissue collection to microscopic examination.
- **Explain** the principles and purpose of fixation, dehydration, clearing, infiltration and embedding.
- **Describe** microtomy, section thickness, ribbon formation and common sectioning artifacts.
- **Explain** the basic principles of H&E staining and mounting.
- **Recognize** major safety hazards and quality-control points in a histology laboratory.

Outline

- Introduction and terminology
- Tissue specimens and workflow
- Fixation
- Dehydration, clearing and infiltration
- Paraffin embedding and tissue orientation
- Microtomy and sectioning
- H&E staining and other stains
- Mounting, microscopy, safety and quality control
- Question bank
- References

What Is Microtechnique?

- Microtechnique is the collection of methods used to prepare biological tissues for microscopic examination as a technical foundation of diagnostic histopathology.
- The aim is to preserve tissue architecture, produce thin sections and create sufficient optical contrast for microscopy.
- It connects specimen collection and processing with histological or pathological interpretation.



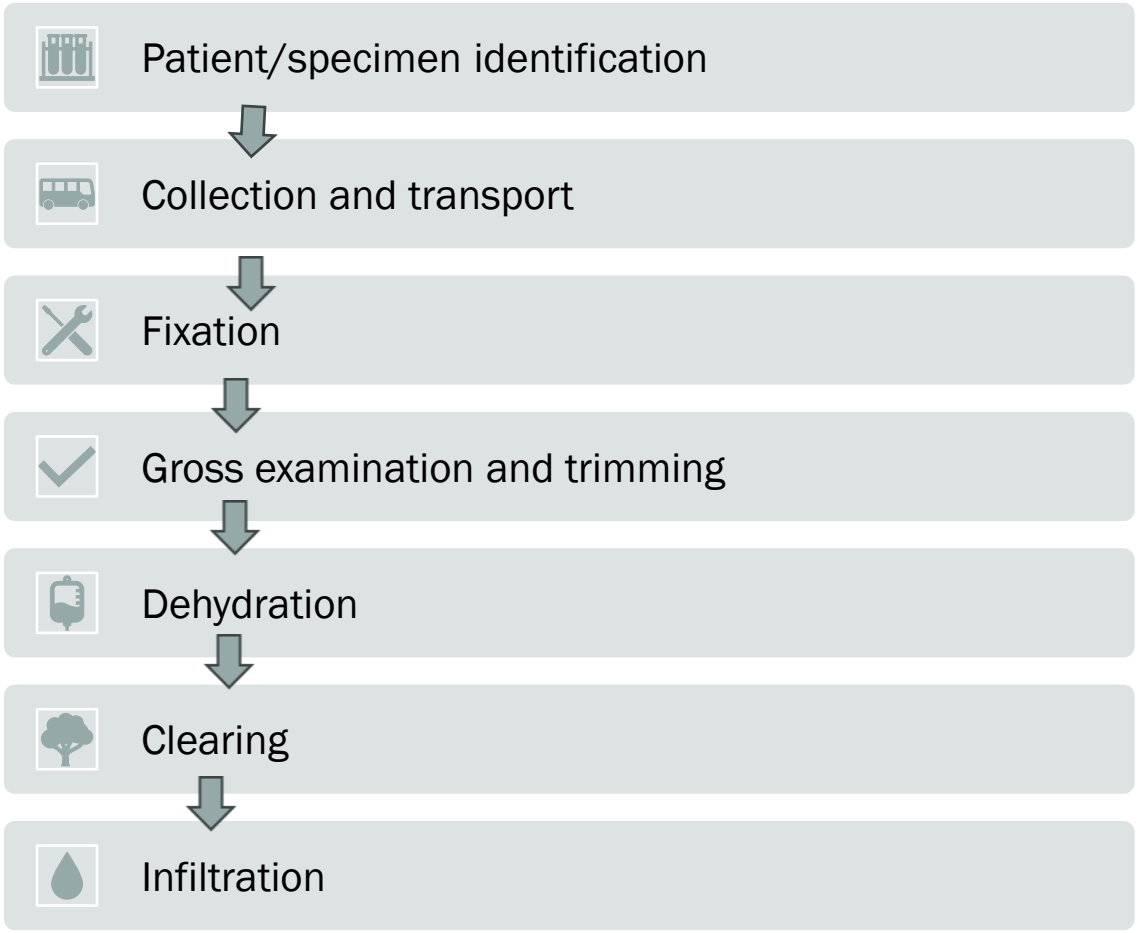
Why Is Microtechnique Important?

- **Preservation**: limits autolysis and putrefactive changes after tissue removal.
- **Processing**: converts preserved tissue into a form that can be supported and sectioned.
- **Sectioning**: produces thin, reproducible sections suitable for light microscopy.
- **Staining**: creates contrast so nuclei, cytoplasm and tissue structures can be distinguished.

Terminology related to Microtechniques

- **Histology** — study of the microscopic structure of normal tissues.
- **Histopathology** — study of microscopic structural changes associated with diseases.
- **Cytology** — study of cells or groups of cells, often without preserving full tissue architecture.
- **Biopsy** — tissue removed from a living patient for diagnostic examination.
- **Surgical specimen** — tissue or organ removed during a operation and submitted for examination.

Steps from Patient to Microscope



Embedding

Sectioning (microtomy)

Staining

Mounting/coverslipping

Microscopic examination and reporting

Specimen Collection and Handling

- Correct patient identification and specimen labeling are essential.
- Prompt fixation is important for routine formalin-fixed tissue.
- Tissue dimensions influence fixation and processing quality.
- Communication of clinical information can help guide specimen handling and downstream examination.

What is fixation

- **Fixation** is the process of preserving tissue morphology and stabilizing cellular components after removal from the body.
- It reduces **autolysis** and **putrefaction** and helps maintain tissue architecture.
- **Poor fixation** can lead to loss or distortion of important microscopic features.

Common Fixatives

- **10% neutral buffered formalin** is widely used for routine paraffin histopathology.
- Other fixatives are selected for specific applications, such as **alcohol-based fixatives** or specialized electron-microscopy fixatives.
- Fixative choice depends on specimen type, intended test and laboratory protocol.
- Do not treat all tissues identically: fixation requirements vary with size, composition and diagnostic purpose.

Factors Affecting Fixation

Tissue thickness: thinner pieces permit better penetration.

Fixative volume and tissue-to-fixative relationship.

Time: insufficient fixation can impair morphology; excessive fixation may affect some downstream tests.

Temperature and composition of the fixative.

Delay before fixation can increase autolytic change.

Consistent validated protocols are essential for diagnostic quality.

Gross Examination and Trimming

- Gross examination documents specimen type, dimensions and visible features.
- Tissue is selected and trimmed into pieces suitable for fixation and processing.
- Representative areas are chosen for diagnostic examination.
- Orientation and margin information may be important in surgical pathology.
- Proper trimming supports adequate reagent penetration and consistent processing.

Dehydration

- **Purpose**: remove water from fixed tissue.
- Routine paraffin processing commonly uses a graded series of alcohol concentrations.
- Gradual dehydration helps reduce abrupt changes in tissue composition.
- **Under-dehydration** can leave residual water and interfere with clearing and paraffin infiltration.
- **Over-dehydration** can make some tissues excessively hard or brittle.

Clearing

- **Purpose**: replace the dehydrating agent with a substance compatible with the embedding medium.
- **Xylene** is a traditional clearing agent in paraffin histology.
- Successful clearing allows subsequent paraffin infiltration.
- **Under-clearing** can interfere with paraffin penetration.
- **Over-processing** can contribute to hard or brittle tissue and staining problems.

Infiltration

- Infiltration is the replacement of the clearing agent within tissue spaces by the embedding medium.
- **Paraffin wax** is commonly used for routine light-microscopy tissue sections.
- Complete infiltration provides internal support during sectioning.
- Inadequate infiltration may produce poorly supported tissue and difficult sectioning.

Paraffin Embedding

- After infiltration, tissue is positioned in a mold containing molten paraffin.
- The block is cooled so the paraffin solidifies around and within the tissue.
- Embedding creates a stable support for microtomy.
- Correct tissue orientation is essential for obtaining the intended plane of section.
- Record specimen identification clearly on the block and associated documentation.

Tissue Orientation

- Orientation determines which anatomical plane will appear in the final section.
- **Correct orientation** can reveal epithelial surfaces, vessel walls, biopsy architecture or surgical margins appropriately.
- **Incorrect orientation** may produce an incomplete or misleading representation.
- Embedding should therefore be planned according to specimen anatomy and diagnostic requirements.

The Microtome

- A microtome is a precision instrument used to cut thin sections from an embedded tissue block.
- Common routine equipment: rotary microtome with a disposable or reusable blade.
- Important components include the **specimen holder**, **blade holder**, **thickness** setting and feed mechanism.
- Blade condition, block temperature and instrument settings influence section quality.

Sectioning and Section Thickness

- Routine paraffin sections are commonly a few micrometres thick; exact thickness depends on tissue and laboratory protocol.
- Sections should be continuous, even and free of avoidable distortion.
- After cutting, sections may form a **ribbon**.
- Sections are floated on a warm water bath to help flatten them before collection on slides.
- **Slides** are labeled and handled carefully to preserve specimen identity.

Ribbon Formation

- A **ribbon** is a sequence of consecutive paraffin sections that remain attached edge-to-edge during cutting.
- Good ribbon formation supports efficient collection of serial sections.
- Ribbon quality depends on wax properties, blade condition, block temperature, cutting angle and technique.
- Poor ribbons may appear fragmented, compressed, wrinkled or irregular.

Common Sectioning Artifacts

- **Compression —** tissue appears shortened or distorted in the direction of cutting.
- **Chatter —** repetitive bands or vibrations appear across the section.
- **Wrinkles/folds —** parts of the section overlap rather than lying flat.
- **Knife lines —** linear marks may arise from blade damage or debris.
- **Thick/thin sections —** inconsistent section thickness reduces microscopic quality.

Water Bath and Slide Pickup

- Sections are floated on a warm water bath to reduce wrinkles and facilitate flattening.
- Water temperature should follow the validated laboratory protocol and wax characteristics.
- The flattened section is picked up onto a clean, appropriately labeled glass slide.
- Slides are dried according to laboratory protocol before staining.
- Good slide handling reduces section loss and contamination.

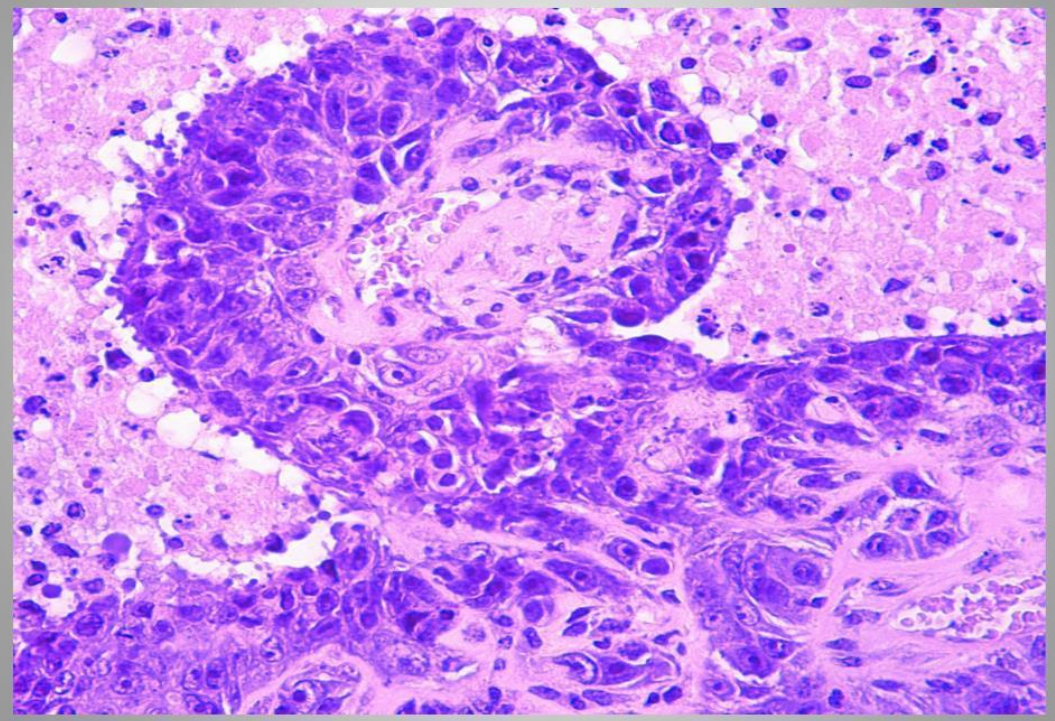
Why Do We Stain Tissue?

- Most unstained tissue has limited natural contrast under routine bright-field microscopy.
- Staining increases contrast and highlights different cellular and extracellular components.
- Routine H&E provides a broad overview of tissue morphology.
- Special stains and immunohistochemistry are selected when additional diagnostic information is required.

Hematoxylin and Eosin (H&E)

- **Hematoxylin** primarily highlights **nuclei** in blue-purple tones.
- **Eosin** is an acidic dye that produces pink to red staining of many **cytoplasmic** and **extracellular components.**
- H&E is the routine foundation for morphological assessment in histopathology.
- Staining quality depends on fixation, processing, section quality, reagent condition and protocol control.

Hematoxylin (nucleus) and Eosin (cytoplasm) stain



Special Stains: Why and Examples

- Special stains are used to demonstrate particular tissue components or microorganisms.
- **PAS** — commonly demonstrates glycogen and other carbohydrate-rich structures and can aid assessment of fungi and basement membranes.
- **Masson's trichrome** — useful for distinguishing connective tissue/collagen from other tissue components.
- **Ziehl–Neelsen** — used for acid-fast organisms in appropriate clinical contexts.
- **Congo red** — used to demonstrate amyloid with characteristic staining and birefringence.

Mounting and Cover slipping

- After staining, sections are prepared for permanent or appropriate mounting.
- Clearing improves optical compatibility with mounting media.
- A coverslip protects the stained tissue and provides a stable optical surface.
- Correct mounting minimizes bubbles, folds and contamination.
- Slides should be labeled clearly and stored appropriately.

Microscopic Examination

- The pathologist or trained professional evaluates tissue architecture and cellular morphology.
- Begin with low magnification to assess overall architecture, then use higher magnification for detail.
- Assess specimen adequacy, orientation, tissue preservation and staining quality.
- Interpretation should distinguish true biological findings from preparation artifacts.

Laboratory Safety

- Treat human tissue as potentially infectious and follow institutional biosafety procedures.
- Use appropriate PPE and safe specimen-handling practices.
- Formalin and organic solvents require appropriate ventilation, storage and chemical-safety controls.
- Microtome blades are sharps: use blade guards and safe handling procedures.
- Dispose of biological waste, sharps and chemical waste according to institutional requirements.
- Never improvise chemical protocols without laboratory authorization.

Quality Control in Microtechnique

- Correct specimen identification at every stage.
- Appropriate fixation and processing.
- Correct tissue orientation and complete paraffin infiltration.
- Consistent section thickness and minimal artifacts.
- Controlled staining reagents and validated staining procedures.
- Clean, intact, correctly labeled slides.
- Documentation and traceability of processing and staining steps.

Troubleshooting:

- Poor morphology ⟶ review fixation, tissue thickness and processing.
- Hard/brittle tissue ⟶ consider over-processing or excessive dehydration/clearing.
- Poor paraffin infiltration ⟶ consider incomplete processing or tissue composition.
- Wrinkled sections ⟶ review water-bath conditions and section handling.
- Knife lines ⟶ inspect blade and block for debris or damage.
- Uneven H&E staining ⟶ review deparaffinization, reagent condition, timing and section quality.

Questions bank

- What is the main purpose of fixation?
- Why is dehydration required before paraffin embedding?
- What is the role of a clearing agent?
- Why is tissue orientation important during embedding?
- What instrument is used to section paraffin blocks?
- What is a ribbon in microtomy?
- What are the primary roles of hematoxylin and eosin?
- Name two common sectioning artifacts.
- Why is specimen identification a critical quality-control issue?

References

- Suvarna, K. S., Layton, C., & Bancroft, J. D. (2019). *Bancroft's theory and practice of histological techniques* (8th ed.). Elsevier.
- Pawlina, W. (2023). *Histology: A text and atlas: With correlated cell and molecular biology* (9th ed.). Wolters Kluwer.

Next lecture

- Tissue fixation
- You can use this link to have overview about the topic
- https://www.youtube.com/watch?v=ItpO9tGZpc0