



**Faculty of Applied
Science**

**Medical Analysis
Department**

Micritechnique (MA505)

Lecture 7

SPECIAL STAINS AND HISTOCHEMISTRY

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M.Sc. Biomedical Science Blood Science

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Learning outcome

- By the end of this lecture, students will be able to:
- Define special stains and explain why they are used in histopathology.
- Explain the basic principles of histochemical staining.
- Describe the principle and applications of PAS stain.
- Describe the principle and applications of Masson's Trichrome.
- Explain the use of Alcian Blue for acidic mucins.
- Explain the role of Reticulin staining in demonstrating reticular fibers.
- Identify appropriate stains for carbohydrates, connective tissue, mucin, and microorganisms.

Special Stains

- **Special stains** are staining techniques designed to demonstrate particular tissue components, microorganisms, or chemical substances that may not be clearly visible with routine H&E staining.
- **Examples**
- Carbohydrates → **PAS**
- Collagen → **Masson's Trichrome**
- Acidic mucin → **Alcian Blue**
- Reticular fibers → **Reticulin**
- Fungi → **GMS/PAS**
- Mycobacteria → **Ziehl-Neelsen**

What is Histochemistry?

- **Histochemistry** is the study and identification of chemical substances within cells and tissues by using specific chemical reactions that produce a visible microscopic result.

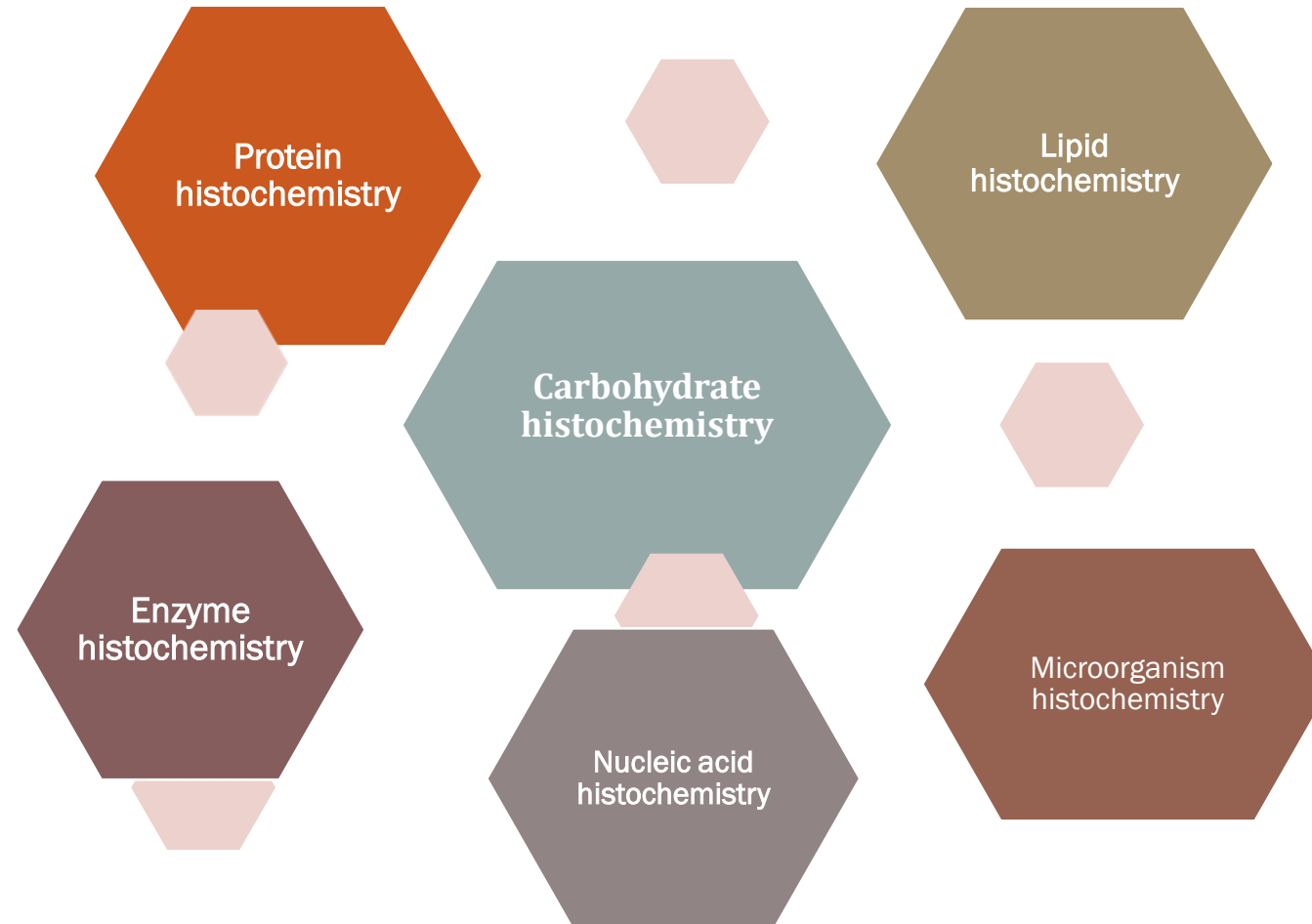
Histochemistry vs. Histology

Histology	Histochemistry
Studies tissue structure	Studies chemical composition
Focuses on morphology	Focuses on tissue substances
H&E commonly used	Special chemical reactions used
General tissue examination	Specific substance identification
Example: cell architecture	Example: glycogen detection

Principles of Histochemistry

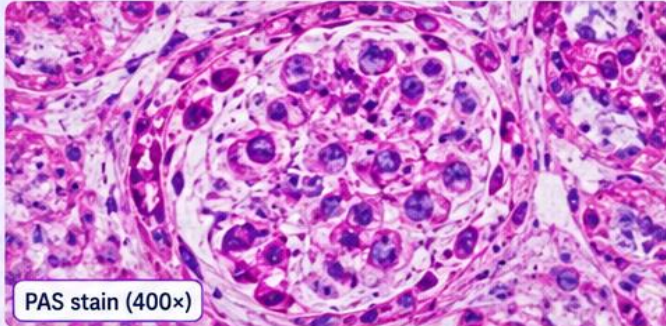
- **A. Specificity**
 - The reaction should identify a particular chemical substance as specifically as possible.
- **B. Localization**
 - The reaction should demonstrate **where the substance is located**.
 - For example:
 - **Glycogen → cytoplasm**
 - **Mucin → goblet cells**
 - **Basement membrane → extracellular location**
- **C. Chemical reaction**
 - The tissue substance reacts with a reagent to produce a visible product.
- **D. Visualization**
 - The final reaction product must be visible using:
 - Light microscopy
 - Fluorescence microscopy
 - Electron microscopy in some techniques

Types of Histochemical Techniques



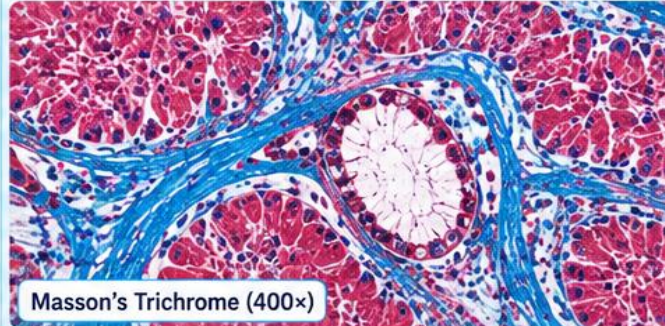
Types of Histochemistry

1. Carbohydrate Histochemistry (PAS)



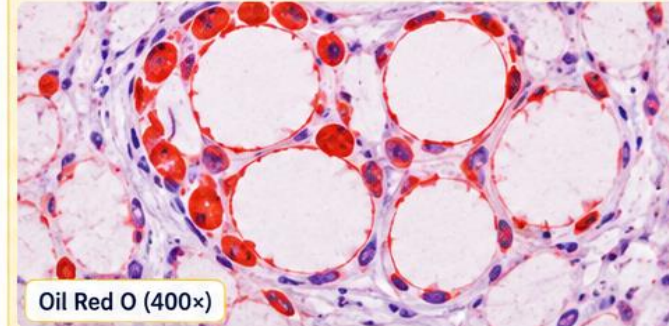
- Demonstrates carbohydrates (glycogen, mucins, glycoproteins).
- **Example:** PAS (magenta/pink color).

2. Protein Histochemistry (Masson's Trichrome)



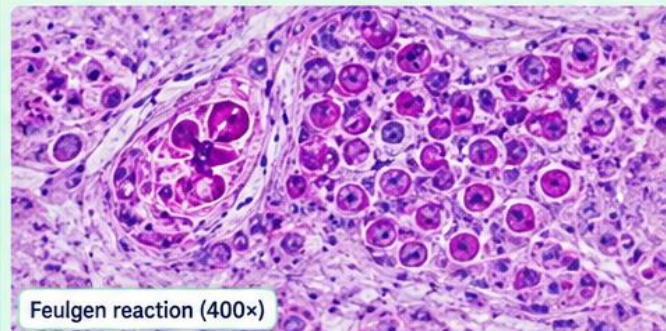
- Demonstrates specific proteins, especially collagen, reticular fibers and some amino acids.
- **Example:** Collagen (blue/green), muscle (red).

3. Lipid Histochemistry (Oil Red O)



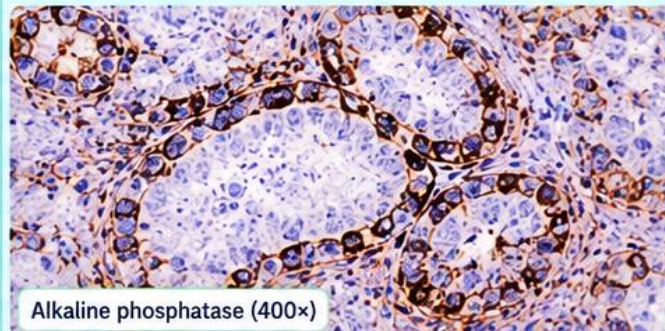
- Demonstrates lipids (neutral fats, phospholipids and other lipid substances).
- **Example:** Lipid droplets (red/orange color).

4. Nucleic Acid Histochemistry (Feulgen)



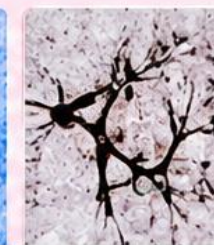
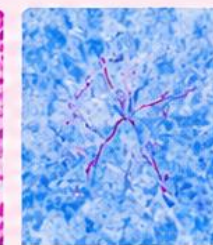
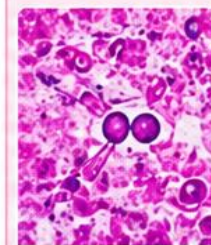
- Demonstrates DNA and RNA.
- **Example:** DNA (magenta/purple color).

5. Enzyme Histochemistry (Alkaline Phosphatase)



- Demonstrates the activity of specific enzymes within tissues.
- **Example:** Alkaline phosphatase (brown/dark color).

6. Microorganism Histochemistry



- Demonstrates microorganisms (bacteria, fungi, parasites).
- **Examples:** PAS / GMS (fungi), Ziehl-Neelsen (acid-fast bacilli), Gram (bacteria).

Carbohydrate histochemistry

- Used to demonstrate:
- Glycogen
- Mucins
- Glycoproteins
- **Example:** PAS

Protein histochemistry

- Used to demonstrate specific protein components.
- Examples include techniques for:
- Collagen
- Reticular fibers
- Certain amino acids

Lipid histochemistry

- Used to demonstrate:
- Neutral fats
- Phospholipids
- Other lipid substances
- Important point:
- **Frozen sections are often preferred for demonstrating lipids**, because routine paraffin processing may remove many lipids.
- Examples:
- Oil Red O
- Sudan dyes

Nucleic acid histochemistry

- Used to demonstrate:
- DNA
- RNA
- Examples:
- Feulgen reaction → DNA
- Methyl green-pyronin → DNA/RNA differentiation

Enzyme histochemistry

- Demonstrates the **activity of enzymes** within tissues.
- Examples:
 - Phosphatases
 - Esterases
 - Dehydrogenases
- Important concept:
 - Enzyme histochemistry demonstrates **enzyme activity**, not simply the presence of the enzyme protein.

Microorganism Histochemistry

- Specialized staining techniques to **demonstrate, identify, and localize microorganisms within tissue sections.**
- **Example**
- **Gram stain** → bacteria
- **Ziehl-Neelsen** → acid-fast bacilli
- **GMS/PAS** → fungi

Controls Histochemistry

- This is an important concept for Medical Analysis students.
- **Controls**
- **Positive control**
- A tissue known to contain the target substance.
- **Purpose:** Confirms that the staining procedure worked.
- **Negative control**
- A sample in which the target reaction should not occur.
- **Purpose:** Helps identify nonspecific staining.

Factors affecting histochemical results

- **Fixation**
- Fixative type
- Fixation time
- Tissue processing
- Reagent concentration
- pH
- Temperature
- Reaction time
- Section thickness
- Washing/differentiation

Diagnostic Applications

Target	Stain	Main Finding
Liver fibrosis	Masson's Trichrome	Collagen → Fibrosis
Fungal infection	PAS / GMS	Fungi
Mucin	Alcian Blue / PAS	Mucin
Mycobacterial infection	Ziehl–Neelsen	Acid-fast bacilli
Glycogen	PAS	Glycogen

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.
- Allen, D. C., & Cameron, I. R. (Eds.). (2012). *Histopathology specimens: Clinical, pathological and laboratory aspects* (2nd ed.). Springer.

Next lecture

- Artifacts, Quality Control and Diagnostic Applications