



**Faculty of Applied
Science**

**Medical Analysis
Department**

Micritechnique (MA505)

Lecture 6

ROUTINE HISTOLOGICAL STAINING

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M.Sc. Biomedical Science Blood Science

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Outline

- What Is Histological Staining?
- Why Do We Stain Tissue?
- Acidic and Basic Dyes
- What is Haematoxylin?
- Why Do We Need Special Stains?
- Class activity
- References
- Next lecture

Learning outcome

- By the end of this lecture, students will be able to:
- Define histological staining.
- Explain why tissue sections require staining.
- Describe the basic principles of staining.
- Differentiate between acidic and basic dyes.
- Explain the H&E staining principle.
- Describe common special stains and their applications.
- Identify common staining problems.

What Is Histological Staining?

- **Histological staining** is the process of applying dyes or chemical reagents to tissue sections to increase contrast and make cellular and tissue structures visible under the microscope.
- **Why is staining necessary?**
- Unstained tissue is usually:
- Almost transparent
- Low in contrast
- Difficult to distinguish under bright-field microscopy

Why Do We Stain Tissue?

- **Visualization**
- Makes cells and tissue structures visible.
- **Differentiation**
- Distinguishes nuclei from cytoplasm.
- **Diagnosis**
- Helps identify pathological changes.
- **Identification**
- Demonstrates specific substances such as:
 - Glycogen
 - Mucin
 - Collagen
 - Elastic fibers
 - Microorganisms

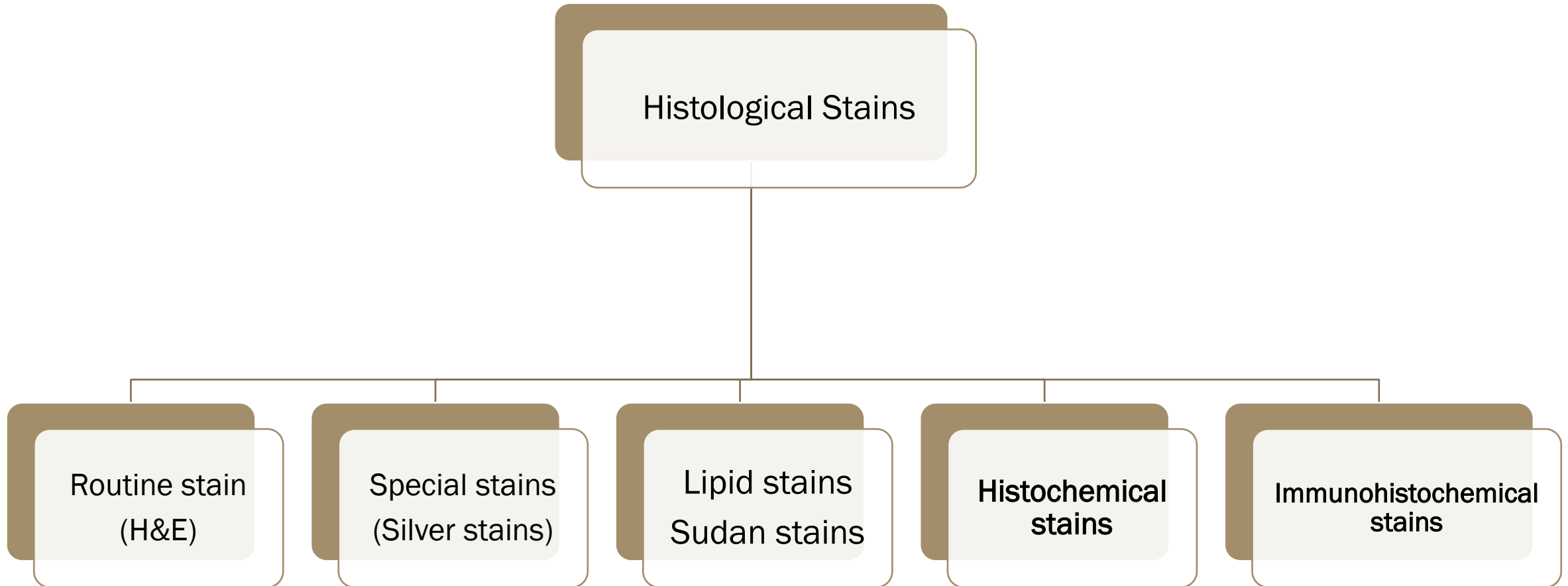
Basic Principle of Staining

- The interaction between a stain and tissue may depend on:
- Electrical charges
- Chemical groups
- Solubility
- Binding affinity
- Oxidation/reduction reactions

Acidic and Basic Dyes

- **Basic dyes**
- Basic/cationic dyes are attracted to **negatively charged tissue components**.
- Examples: DNA, RNA, and Some acidic mucopolysaccharides.
- Basophilic
- They appear blue/purple with hematoxylin.
- **Acidic dyes**
- Acidic/anionic dyes bind preferentially to **positively charged proteins**.
- **Acidophilic / Eosinophilic**
- They commonly appear pink/red with eosin

Major Types of Histological Stains



The Most Important Routine Stain Hematoxylin & Eosin — H&E

- **Why is H&E important?**
- H&E is:
 - The most commonly used routine histological stain.
 - Relatively simple.
 - Suitable for examining general tissue morphology.
 - Widely used in histopathology.

What is Haematoxylin?

- **Hematoxylin:** is a natural dark dye extracted from the **heartwood** of the **logwood tree** (*Haematoxylum campechianum*) and used widely in histology to color cell nuclei blue or purple.

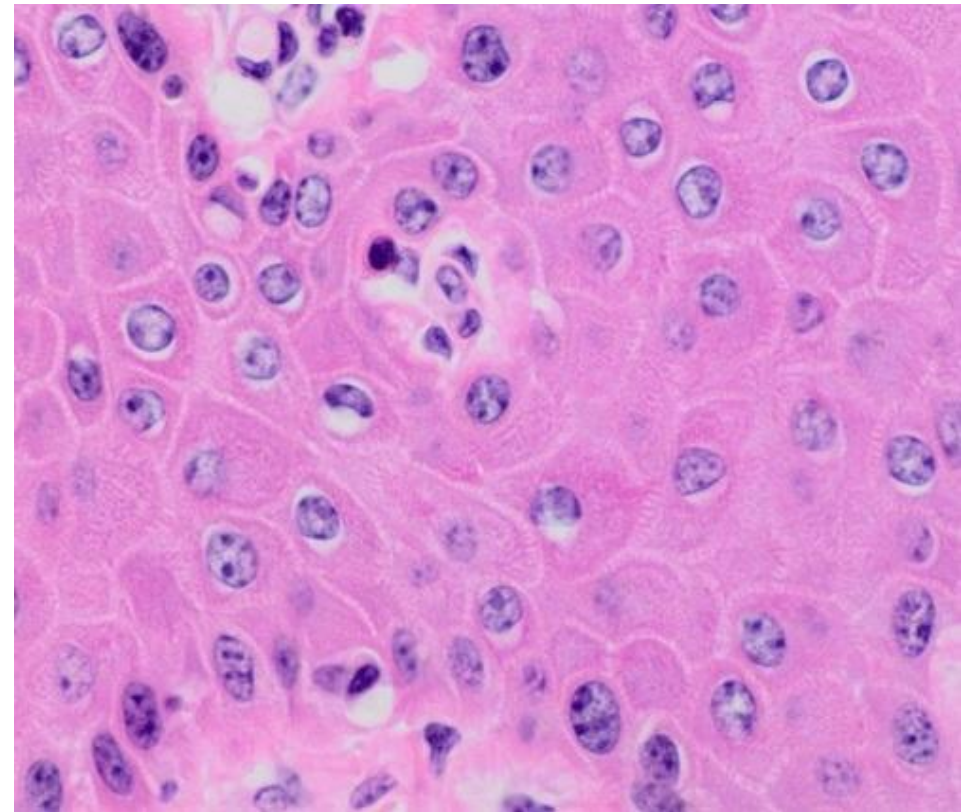
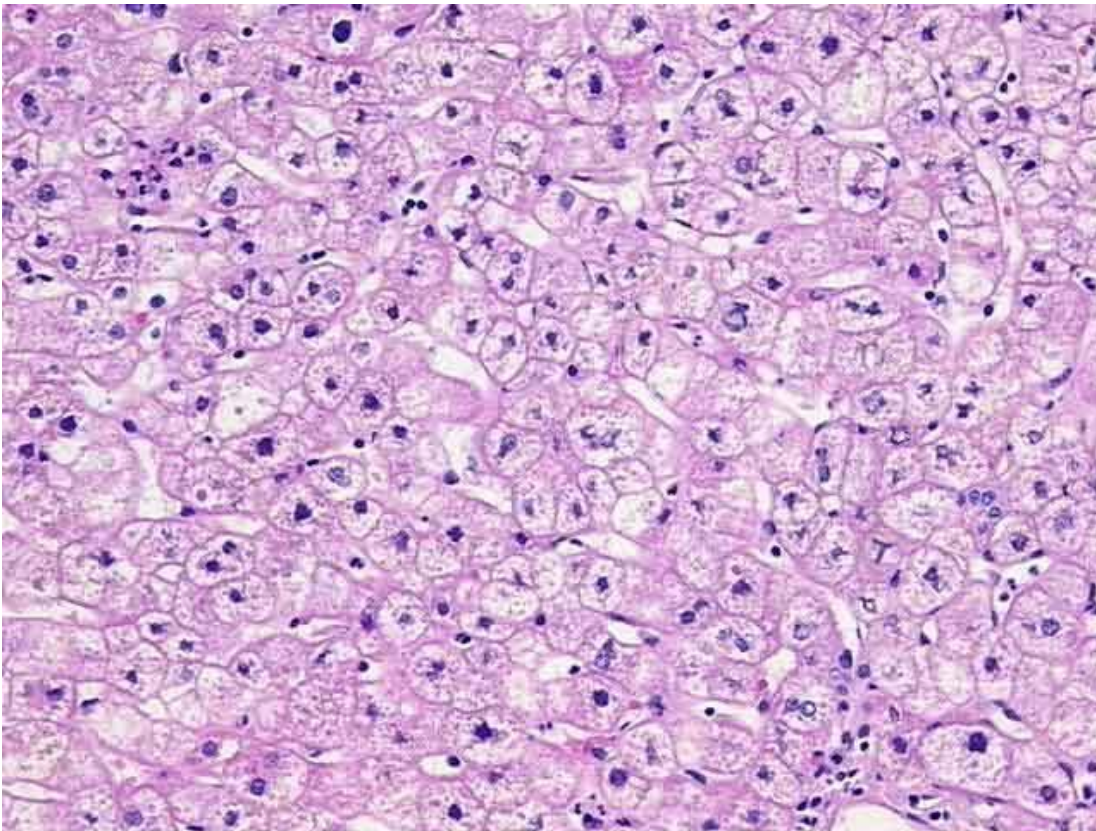


What is Eosin?

- Eosin: an acidic, pink-red fluorescent dye used in histology and pathology to stain cell cytoplasm, collagen, and extracellular proteins.



What structures can you identify?



H&E Color Interpretation

- **Nucleus** → Hematoxylin
- **Cytoplasm** → Eosin
- **Collagen** → Eosin
- **Red blood cells** → Strong eosinophilia
- **Hematoxylin = nuclear detail**
- **Eosin = cytoplasmic/extracellular protein detail**

Deparaffinization

- Deparaffinization is the essential laboratory process of removing paraffin wax from formalin-fixed, paraffin-embedded (FFPE) tissue sections before staining or molecular analysis.
- Stains are aqueous or require access to the tissue.

Hydration

- After removing paraffin:
- **Tissue is gradually brought back to water.**
- Example:
- **Absolute alcohol**
- ↓
- **95% alcohol**
- ↓
- **70% alcohol**
- ↓
- **Water**
- **Purpose**
- Prepare the tissue for aqueous staining solutions.

Differentiation and Bluing

- **Differentiation**
- Removes excess hematoxylin.
- **Bluing**
- Changes the hematoxylin color from reddish-purple to a more stable:
- **Blue-purple**
- **Why is this important?**
- It improves nuclear contrast.

Dehydration, Clearing & Mounting

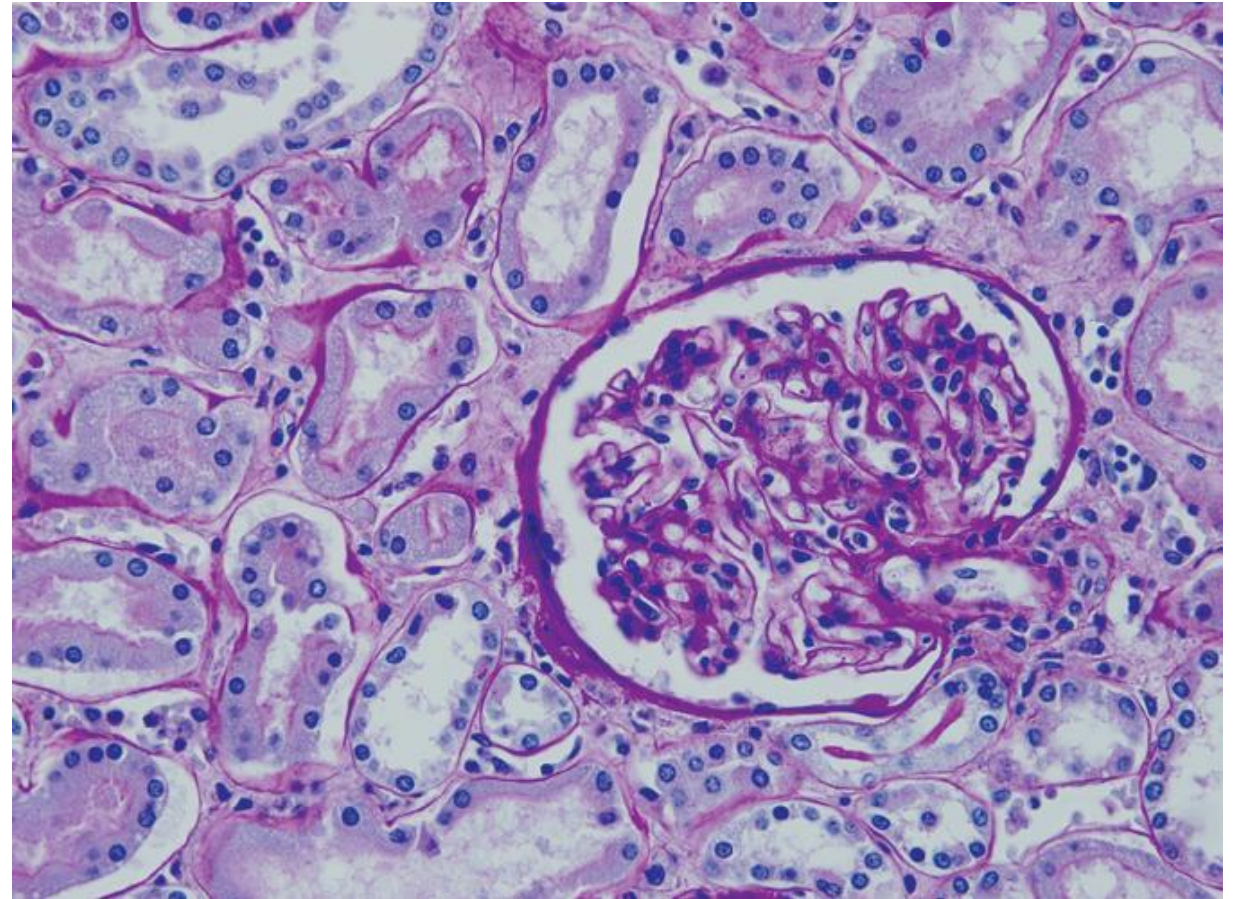
- After staining:
- **1. Dehydration**
- Remove water using alcohol.
- **2. Clearing**
- Replace alcohol with a clearing agent.
- **3. Mounting**
- Apply mounting medium and coverslip.
- **Final goal:**
- **Permanent, clear, microscope-ready slide**

Why Do We Need Special Stains?

- H&E provides excellent general morphology.
- But sometimes we need to answer a specific question:
- **Examples**
- **Is glycogen present?**
- → PAS
- **Is there increased collagen?**
- → Masson's trichrome
- **Is acidic mucin present?**
- → Alcian blue
- **Are acid-fast organisms present?**
- → Ziehl-Neelsen
- **Are reticular fibers present?**
- → Silver stain

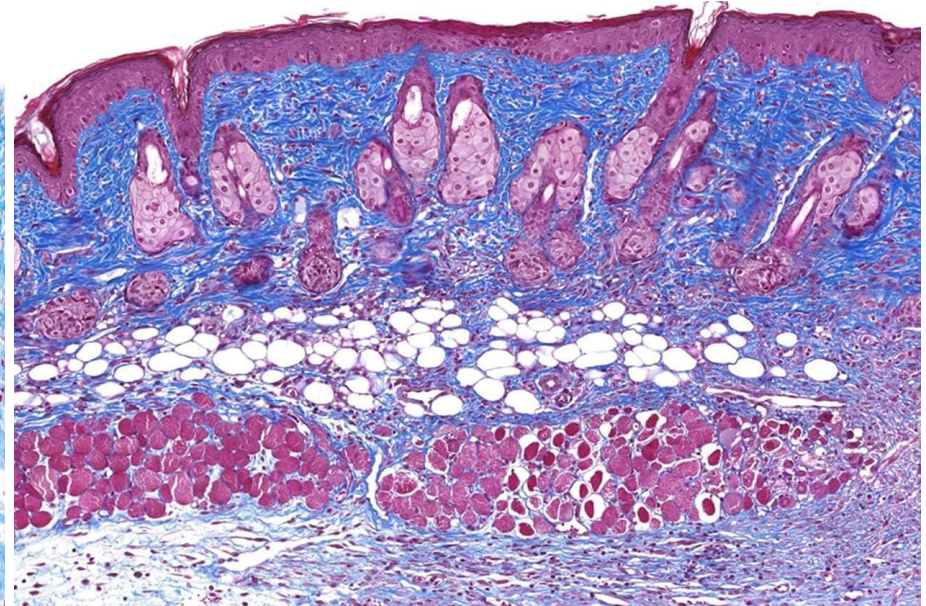
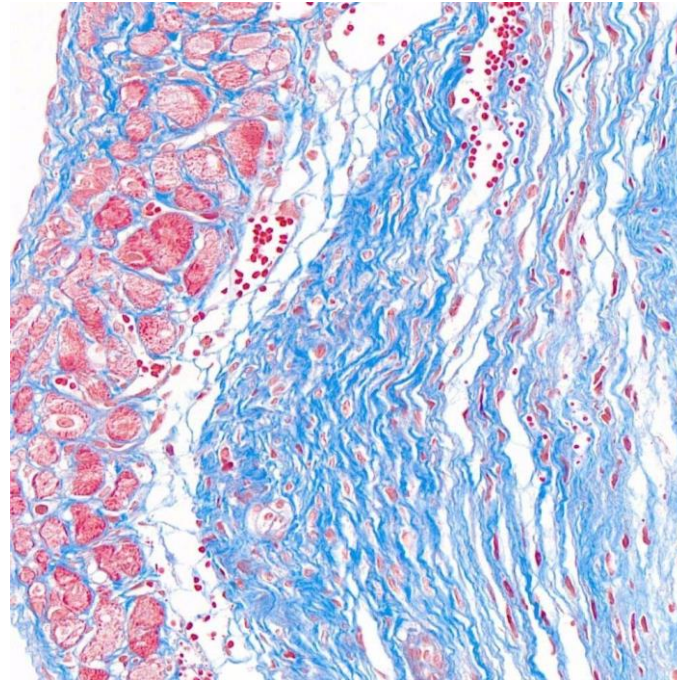
Periodic Acid–Schiff (PAS)

- **Main targets**
- Glycogen
- Neutral mucins/glycoproteins
- Basement membranes
- Some fungal structures
- **Typical positive color:**
- **Magenta / purple-red**
- **Common applications**
- Kidney
- Liver
- Intestine
- Fungal organisms
- Basement membranes



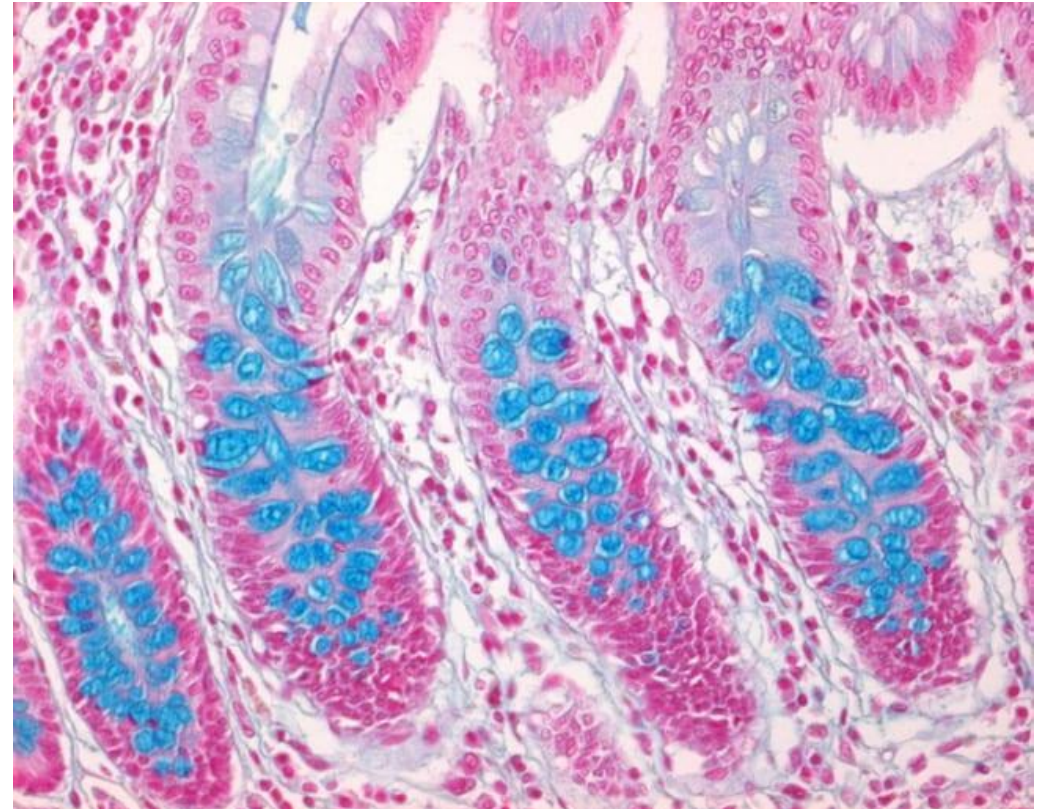
Masson's Trichrome

- **Masson's Trichrome**
- **Main purpose**
- To distinguish:
- **Collagen**
- Muscle
- Cytoplasm
- Nuclei
- **Typical colors**
- **Collagen → blue/green**
- **Cytoplasm/muscle → red**
- **Nuclei → dark blue and black**



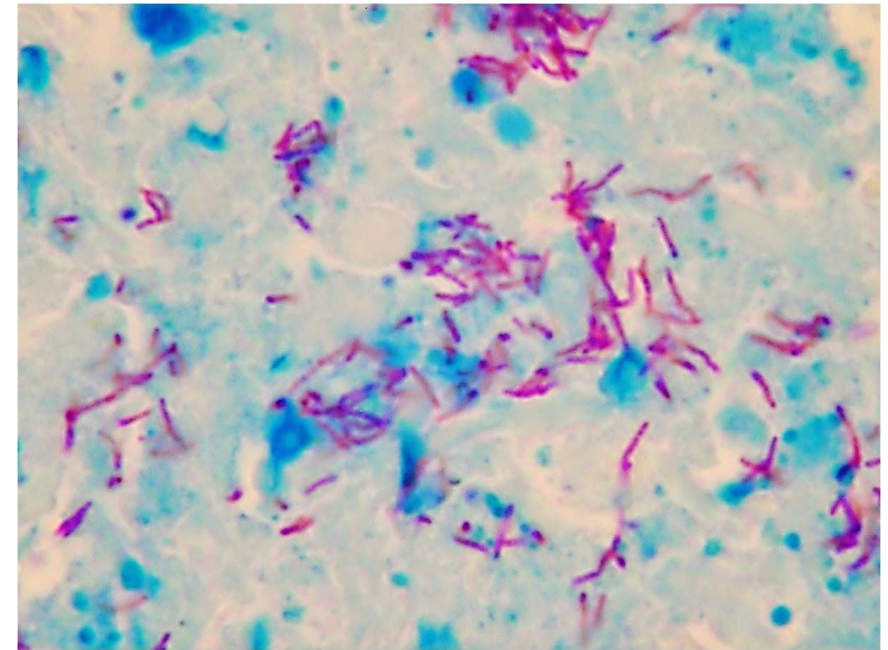
Alcian Blue Stain

- **Main target**
- **Acidic mucins**
- **Common locations**
- Goblet cells
- Intestinal mucosa
- Respiratory epithelium
- **Typical color**
- **Blue**



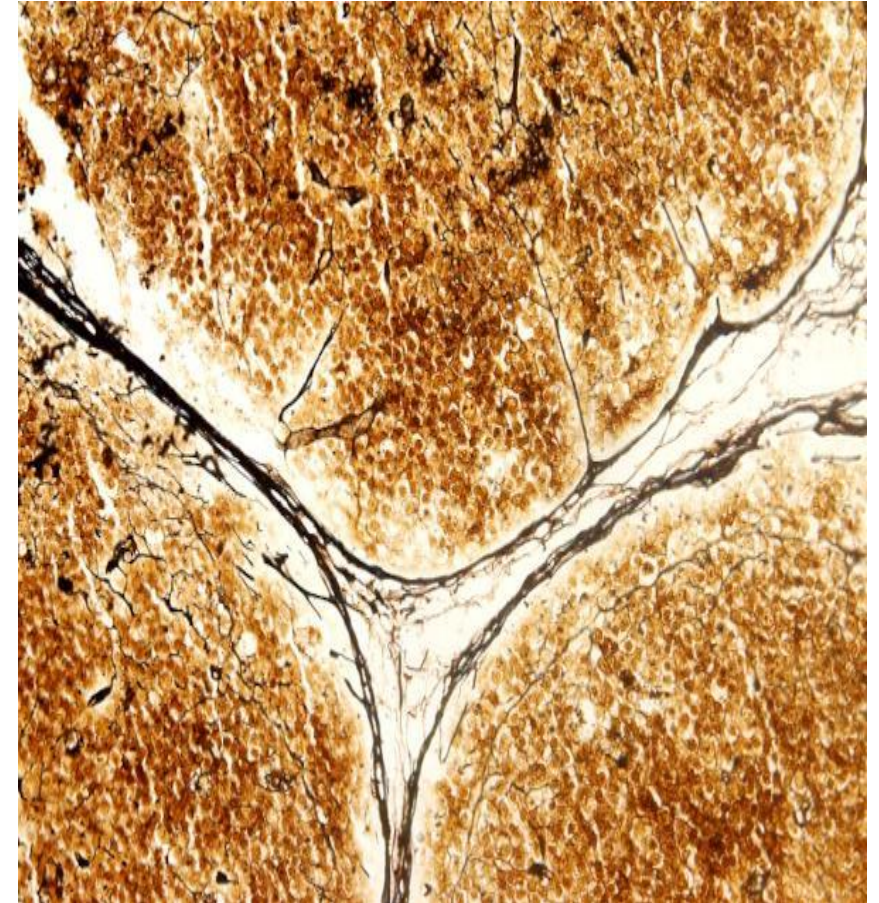
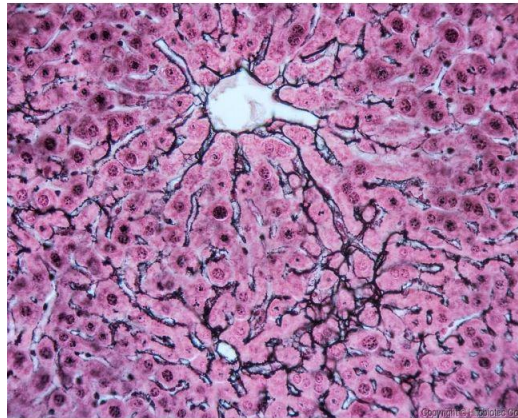
Ziehl-Neelsen Stain

- **Acid-Fast Stain**
- **Main target**
- **Acid-fast bacilli**
- Especially:
- **Mycobacterium**
- **Typical result**
- Acid-fast organisms → red/pink
- Background/counterstained material → blue



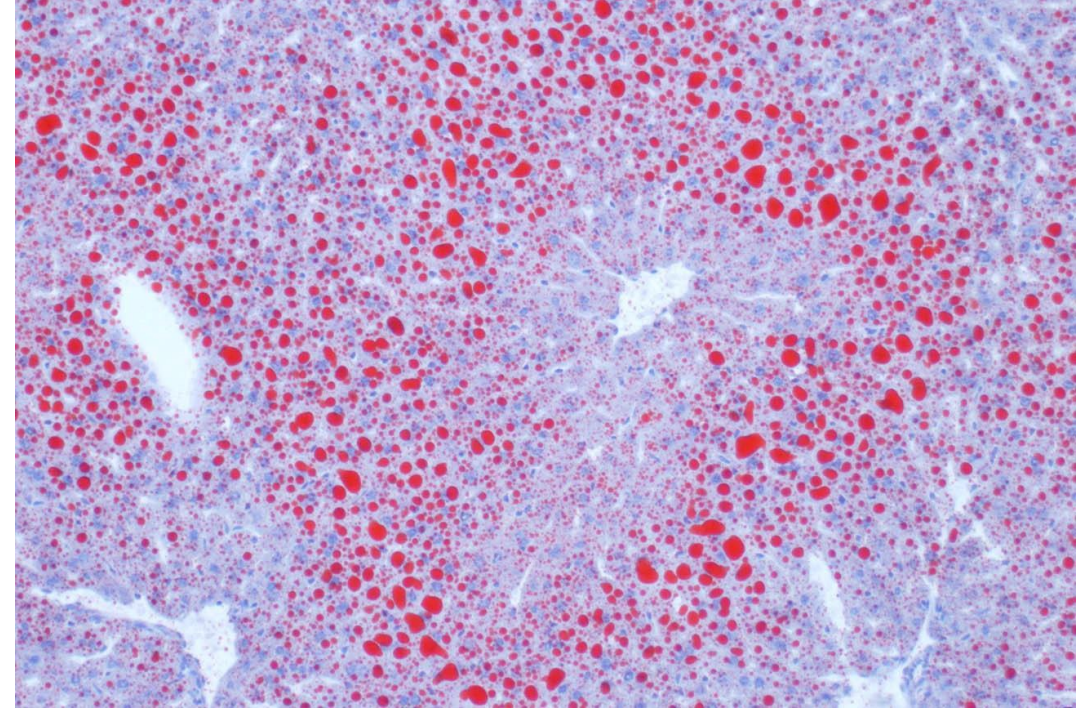
Silver Stains

- **Main target**
- **Reticular fibers**
- Reticular fibers are primarily associated with:
- **Type III collagen**
- **Typical appearance**
- **Black**
- **Common tissues**
- Lymph node
- Bone marrow
- Liver
- Spleen



Lipid Stains

- **Examples**
- Sudan III
- Sudan IV
- Oil Red O
- **Main target**
- **Lipids**
- **Important technical point**
- Many routine paraffin-processing steps remove lipids.



Comparison of Major Stains

Stain	Main target	Typical appearance
H&E	General morphology	Purple + pink
PAS	Glycogen, basement membranes, some mucins/fungi	Magenta
Masson's trichrome	Collagen	Blue/green
Alcian blue	Acidic mucins	Blue
Ziehl-Neelsen	Acid-fast organisms	Red/pink
Silver stain	Reticular fibers	Black
Verhoeff	Elastic fibers	Black/dark
Oil Red O	Lipids	Red

Class activity 1

Complete the missing word in this table with suitable word

Stain	Main target	Typical appearance
	General morphology	
PAS		Magenta
Masson's trichrome	Collagen	
Alcian blue		Blue
	Acid-fast organisms	
Silver stain	Reticular fibers	
	Elastic fibers	Black/dark
	Lipids	Red

Class activity 2

- **Write the summery about todays topic?**

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

- Special Stains and Histochemistry