JHARKHAND Rai University
RANCHI

Bachelor of Science in Medical Laboratory Technology (BMLT)

HISTOPATHOLOGY & HISTOTECHNIQUES

LAB. – I

SEMESTER: FOUR (IVth)

PRACTICAL LABORATORY MANUAL

INDEX

Experiment No. 01

AIM: - Basic Principles in Histology -Tissue Processing.

Introduction

Histology is the study of microscopic structure of tissues. Tissue processing is a sequence of procedures by which fresh biological tissues are preserved, hardened, and prepared for sectioning and staining so that they can be examined under a microscope. Proper tissue processing is essential to maintain cellular architecture and prevent autolysis and putrefaction.

Principle

Tissue processing involves the removal of water from tissues and its replacement with a medium (paraffin wax) that provides sufficient rigidity to allow thin sectioning. This is achieved through a series of steps including fixation, dehydration, clearing, infiltration, embedding, sectioning, and staining.

Requirements / Materials

- Fresh tissue specimen
- Fixative (10% formalin)
- Graded alcohol series (70%, 80%, 90%, absolute alcohol)
- Clearing agent (xylene)
- Paraffin wax
- Embedding moulds
- Microtome
- Glass slides and cover slips
- Water bath
- Stains (e.g., Hematoxylin and Eosin)
- Mounting medium

Procedure

1. Fixation

- Fresh tissue is immediately placed in 10% formalin.
- Fixation preserves tissue structure and prevents decomposition.

2. Dehydration

- Fixed tissue is passed through increasing concentrations of alcohol (70% → 80% → 90% → absolute alcohol).
- This step removes water from the tissue.

3. Clearing

- Alcohol is replaced by a clearing agent such as xylene.
- Tissue becomes transparent and ready for wax infiltration.

4. Infiltration (Impregnation)

- Tissue is placed in molten paraffin wax.
- Wax infiltrates the tissue spaces and provides support.

5. Embedding

- Tissue is oriented properly in a mould filled with molten paraffin wax.

- Wax is allowed to solidify, forming a tissue block.

6. Sectioning

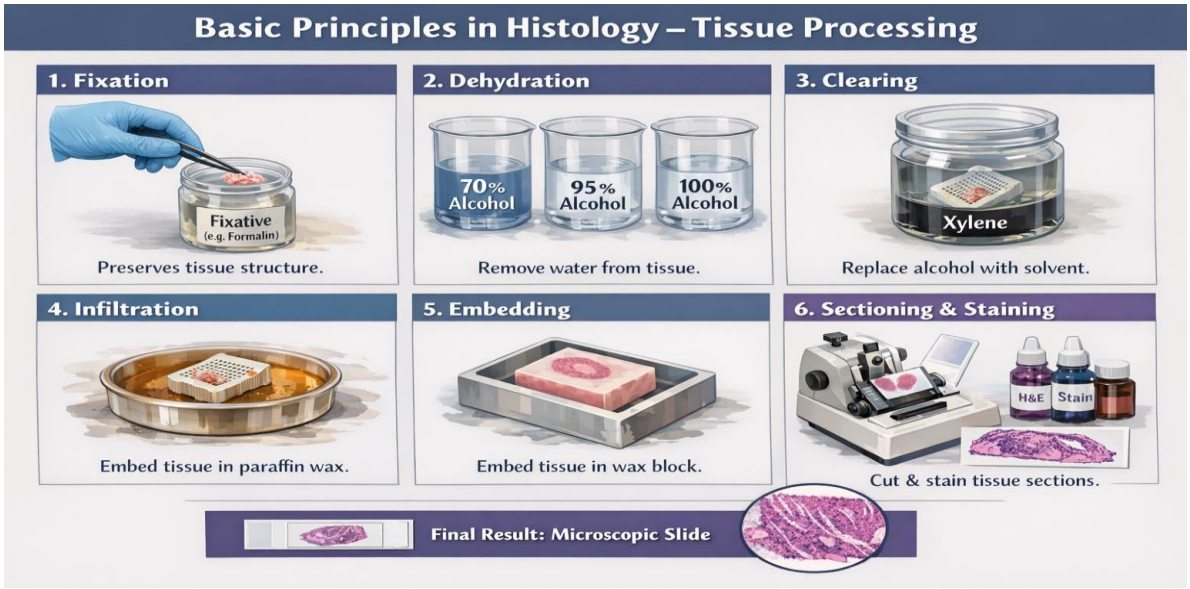
- Thin sections (3–5 µm) are cut using a microtome.
- Sections are floated on a warm water bath and mounted on glass slides.

7. Staining

- Sections are stained using Haematoxylin and Eosin (H&E) or other special stains.
- Staining provides contrast and highlights tissue structures.

8. Mounting

- Stained sections are mounted with a mounting medium and covered with a cover slip.



Observation

- Well-preserved tissue architecture is observed under the microscope.
- Cells, nuclei, and extracellular components are clearly visible.

Result

The tissue is successfully processed and permanent histological sections are obtained for microscopic examination.

Precautions

- Tissue should be fixed immediately after removal.
- Proper timing must be followed in each processing step.
- Avoid overheating paraffin wax.
- Handle chemicals like formalin and xylene with care.

Experiment No. 02

AIM: - To study the principle, construction, working, and applications of different types of microscopes including Light Microscope and Electron Microscope.

Introduction

A microscope is a scientific instrument used to observe objects that are too small to be seen with the naked eye. Microscopes play a vital role in biological, medical, and material science studies. Based on the source of illumination used, microscopes are mainly classified into Light Microscopes and Electron Microscopes.

Classification of Microscopes

1. Light Microscopes
- Simple microscope
- Compound microscope
- Phase contrast microscope
- Fluorescence microscope

2. Electron Microscopes
- Transmission Electron Microscope (TEM)
- Scanning Electron Microscope (SEM)

Light Microscope

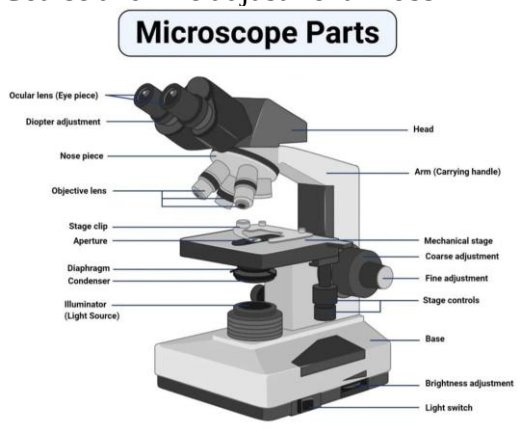
A light microscope uses visible light and a system of glass lenses to magnify the image of a specimen. It is widely used in routine laboratory and educational studies.

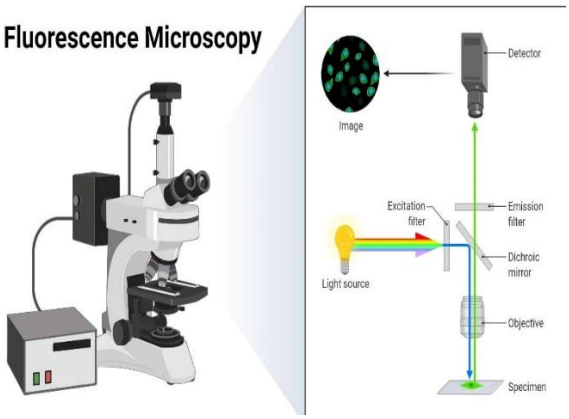
Principle of Light Microscope

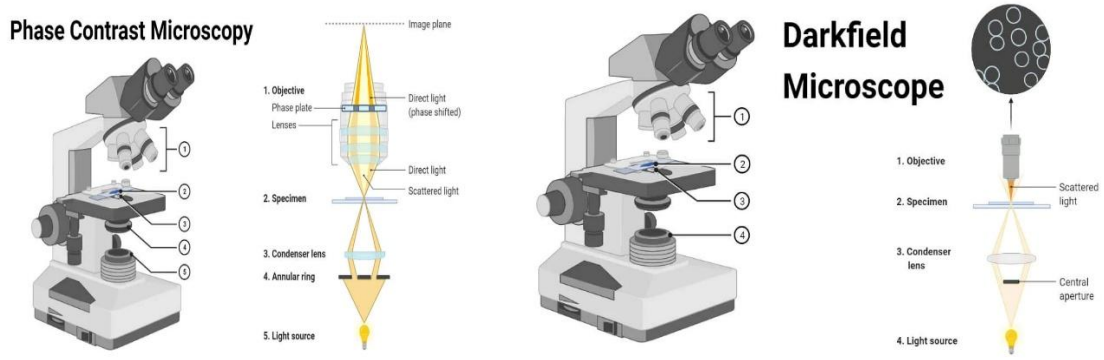
The principle of a light microscope is based on the refraction of light rays through glass lenses. When light passes through a specimen and lenses, a magnified virtual image is produced.

Parts of Light Microscope

Eyepiece (Ocular lens)
Objective lenses
Revolving nosepiece
Stage and stage clips
Condenser
Iris diaphragm
Light source or mirror
Coarse and fine adjustment knobs







Working of Light Microscope

The specimen is placed on the stage and illuminated using a light source. Light passes through the condenser and specimen, then enters the objective lens to form a magnified image. This image is further enlarged by the eyepiece for observation.

Applications of Light Microscope

Study of cells and tissues
Observation of microorganisms
Histology and pathology laboratories
Routine clinical investigations

Limitations of Light Microscope

Limited resolving power
Cannot visualize ultrastructural details
Maximum magnification up to 1000–1500×

Electron Microscope

An electron microscope uses a beam of electrons instead of light to obtain very high magnification and resolution. Electromagnetic lenses are used to focus the electron beam.

Principle of Electron Microscope

The principle is based on the wave nature of electrons. Electrons have a much shorter wavelength than visible light, allowing greater resolving power.

Transmission Electron Microscope (TEM)

In TEM, a beam of electrons passes through an ultra-thin specimen. The internal structures of the specimen scatter electrons differently, forming a detailed image.

Scanning Electron Microscope (SEM)

In SEM, electrons scan the surface of the specimen. The reflected electrons produce a three-dimensional image of the surface topography.

Applications of Electron Microscope

Ultrastructural study of cells and organelles
Study of viruses and bacteria
Material science and nanotechnology
Advanced medical and biological research

Advantages of Electron Microscope

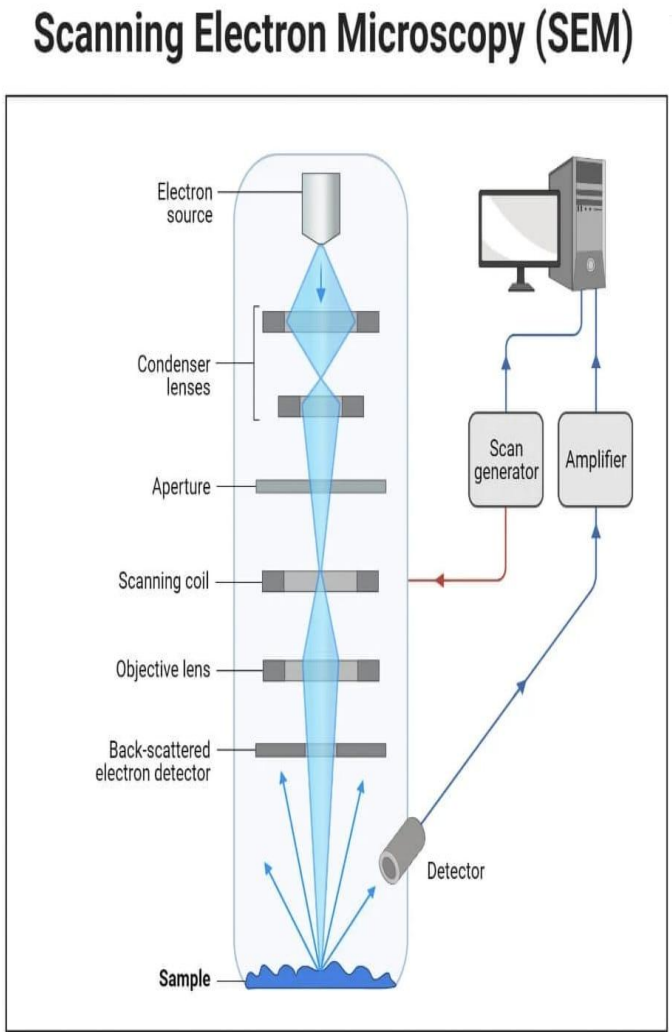
Very high magnification (up to 1,000,000×)
High resolving power
Detailed structural visualization

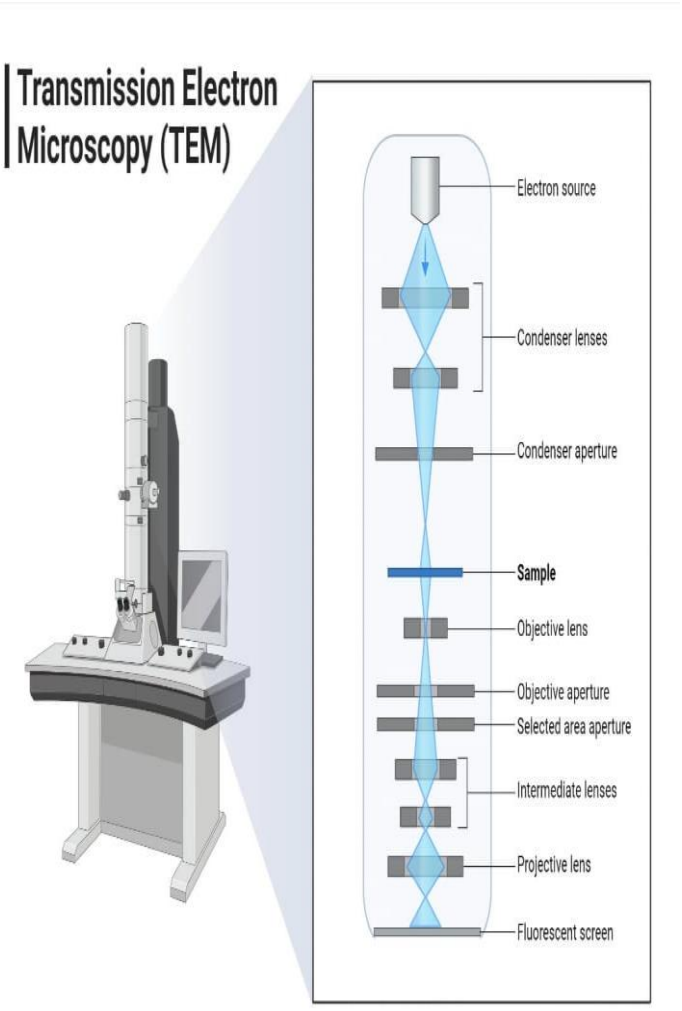
Limitations of Electron Microscope

Expensive equipment
Complex sample preparation
Specimens must be observed in vacuum

Conclusion

Both light and electron microscopes are essential tools in scientific research. While light microscopes are suitable for routine laboratory work; electron microscopes are indispensable for advanced ultrastructural studies.





Experiment No. 03

Aim: To Perform Haematoxylin & Eosin (H&E;) Staining

Principle:

Haematoxylin and Eosin (H&E;) staining is a routine histological staining method. Haematoxylin is a basic dye that stains acidic components (basophilic structures) such as nuclei blue or purple. Eosin is an acidic dye that stains basic components (acidophilic structures) such as cytoplasm and extracellular matrix pink.

Requirements:

- Tissue sections mounted on glass slides
- Xylene
- Graded alcohol (100%, 95%, 70%)
- Distilled water
- Haematoxylin stain
- Eosin stain
- Acid alcohol (1% HCl in alcohol)
- Ammonia water / Bluing agent
- DPX mountant and coverslip
- Staining jars and forceps

Procedure:

- Deparaffinization: Place slides in xylene for 5–10 minutes (2 changes).
- Hydration: Pass slides through descending grades of alcohol (100%, 95%, 70%) and rinse in water.
- Stain in Haematoxylin for 5–10 minutes.
- Wash in running tap water.
- Differentiate in 1% acid alcohol for a few seconds.
- Wash in water and blue in ammonia water for 1–2 minutes.
- Rinse in water.
- Counterstain with Eosin for 1–2 minutes.
- Dehydrate through ascending grades of alcohol (70%, 95%, 100%).
- Clear in xylene (2 changes).
- Mount with DPX and place coverslip.

Observation:

Nuclei appear blue or purple. Cytoplasm appears pink. Muscle fibers are pink, collagen is light pink, and red blood cells appear bright red or orange.

Result:

The tissue section was successfully stained using H&E; stain showing clear differentiation between nucleus and cytoplasm suitable for microscopic examination.

Precautions:

- Ensure complete deparaffinization before staining.
- Do not over-differentiate in acid alcohol.
- Avoid over-staining with eosin.
- Handle slides carefully to prevent tissue damage.
- Use fresh reagents for best results.

Experiment No. -04

Aim- To study and identify the morphological characteristics of different cells and tissues from prepared microscopic slides.

Principle

Morphology is the study of form and structure. Each cell and tissue in the body has a distinct shape, size, arrangement, nucleus, and cytoplasmic features. By observing these features under a microscope, cells and tissues can be identified and classified.

Requirements

- Compound microscope
- Permanent microscopic slides
- Immersion oil (if required)
- Lens paper
- Practical record book and pencil

Specimens to be Examined and Their Morphology

1. Squamous Epithelial Cells

Morphology:

- Cells are flat, thin, and polygonal
- Central, flattened nucleus
- Cytoplasm is scanty
- Cells are closely packed with no intercellular space

Location: Buccal cavity, alveoli of lungs
Function: Protection, diffusion

2. Cuboidal Epithelial Cells

Morphology:

- Cells are cube-shaped
- Round, centrally placed nucleus
- Arranged in a single layer
- Clear cell boundaries

Location: Kidney tubules, glands
Function: Secretion and absorption

3. Columnar Epithelial Cells

Morphology:

- Cells are tall and column-shaped
- Elongated nucleus near the base
- May show goblet cells
- Free surface may have microvilli

Location: Intestine, stomach
Function: Absorption and secretion

4. Blood Cells

(a) Red Blood Cells (RBCs)

Morphology:

- Biconcave disc shape
- No nucleus
- Uniform in size

Function: Transport of oxygen

(b) White Blood Cells (WBCs)

Morphology:

- Larger than RBCs
- Prominent nucleus
- Irregular shape

Function: Défense and immunity

(c) Platelets

Morphology:

- Small, irregular cell fragments
- No nucleus

Function: Blood clotting

5. Muscle Tissue

(a) Skeletal (Striated) Muscle

Morphology:

- Long, cylindrical fibres
- Cross striations present
- Multiple peripheral nuclei

Function: Voluntary movement

(b) Smooth Muscle

Morphology:

- Spindle-shaped cells
- Single central nucleus
- No striations

Function: Involuntary movements

(c) Cardiac Muscle

Morphology:

- Branched fibres
- Single central nucleus
- Intercalated discs present

Function: Pumping of blood

6. Nervous Tissue

Morphology:

- Neurons consist of:
  - Cell body
  - Axon
  - Dendrites
- Neuroglial cells present

Function: Transmission of nerve impulses

7. Connective Tissue

(a) Areolar Tissue

Morphology:

- Loose arrangement of fibres
- Fibroblast's present
- Intercellular matrix abundant

(b) Cartilage

Morphology:

- Chondrocytes in lacunae
- Homogeneous matrix

(c) Bone

Morphology:

- Osteocytes in lacunae
- Haversian system present

Function: Support, protection, binding

Procedure

(Morphological Study of Cells and Tissues)

1. Ensure that the compound microscope is clean and properly adjusted.
2. Take the permanent slide of the given specimen and place it carefully on the microscope stage**.**
3. Fix the slide using the stage clips to prevent movement.
4. Adjust the mirror or light source to obtain proper illumination.
5. Focus the specimen first under low power objective (10×) to locate the tissue and get a general view.
6. Once the specimen is clearly visible, rotate the nosepiece to high power objective (40×) for detailed observation.
7. Observe the shape of cells, size, nucleus, cytoplasm, arrangement of cells, and any special features such as striations, branching, or lacunae.
8. Identify the specimen by comparing observed features with standard morphological characteristics.
9. Draw a neat, labelled diagram of the specimen in the practical record.
10. After observation, remove the slide carefully and clean the microscope lenses.

Observation

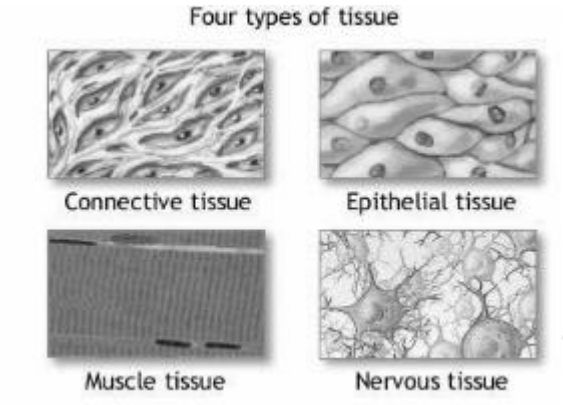
The given specimen was observed under low and high-power magnification.
The following morphological features were noted:

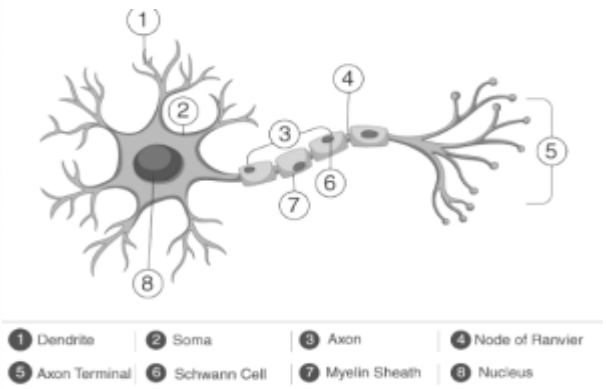
- **Cell shape and size:** Cells showed characteristic shapes such as flat, cuboidal, columnar, elongated, or branched depending on the tissue.
- **Nucleus:** The nucleus was observed to be centrally placed, peripherally placed, elongated, or absent in some cells (e.g., RBCs).
- **Cytoplasm:** Cytoplasm was clear and distinct in most cells; striations were seen in striated muscle tissue.
- **Cell arrangement:** Cells were arranged in layers (epithelial tissue), scattered in matrix (connective tissue), or in long fibres (muscle tissue).
- **Special features:**
    - Striations in skeletal muscle
    - Intercalated discs in cardiac muscle
    - Axon and dendrites in nervous tissue
    - Lacunae in cartilage and bone

Based on these observations, the specimen was identified.

Result

The given slide was examined successfully and the cells and tissues were identified based on their morphological characteristics such as cell shape, nucleus, cytoplasm, and arrangement under the microscope.





Experiment No. 05

Aim- To study and interpret different histological slides and identify tissues and organs based on their microscopic structural features.

Principle

Histology is the study of microscopic structure of tissues. Each tissue and organ show characteristic cellular arrangement, staining pattern, and structural organization when stained (commonly with Haematoxylin and Eosin). By carefully observing these features under a microscope, the slide can be correctly interpreted and identified.

Requirements

- Compound microscope
- Permanent histological slides
- Light source
- Lens paper
- Practical record book

Procedure (Detailed Interpretation Steps)

1. Switch on the microscope and adjust proper illumination.
2. Place the given histological slide on the microscope stage and secure it with clips.
3. Focus the slide under low power objective (10×) to obtain a general view of the tissue.
4. Observe the overall architecture**:**
    - Arrangement of cells
    - Presence of layers
    - Tissue organization
5. Switch to high power objective (40×) for detailed study.
6. Observe and note:
    - Shape and size of cells
    - Nature and position of nucleus
    - Cytoplasm appearance
    - Presence of connective tissue, fibres, or spaces
7. Identify special structures such as:
    - Glands
    - Blood vessels
    - Lacunae
    - Striations
    - Nerve fibres
8. Correlate the observed features with standard histological characteristics.
9. Identify the slide and draw a neat, labelled diagram in the record book.
10. Clean the microscope and remove the slide carefully after observation.

Observation (General Format)

The histological slide shows the following microscopic features:

- Cells are distinct and well stained**.**
- Nuclei appear darkly stained (blue/purple) due to haematoxylin.
- Cytoplasm appears pink due to eosin staining.
- Cells are arranged in a specific pattern characteristic of the tissue.
- Supporting connective tissue and blood vessels are visible where applicable.

Interpretation of Common Histological Slides

1. Epithelial Tissue

Interpretation:

- Cells arranged in continuous layers
- Minimal intercellular space
- Basement membrane present
- Cells may be squamous, cuboidal, or columnar

Identification: Epithelial tissue

2. Muscle Tissue

(a) Skeletal Muscle

- Long cylindrical fibres
- Cross striations present
- Multiple peripheral nuclei

(b) Smooth Muscle

- Spindle-shaped cells
- Single central nucleus
- No striations

(c) Cardiac Muscle

- Branched fibres
- Central nucleus
- Intercalated discs present

3. Connective Tissue

(a) Areolar Tissue

- Loose arrangement of fibres
- Fibroblast's present
- Abundant ground substance

(b) Cartilage

- Chondrocytes in lacunae
- Homogeneous matrix

(c) Bone

- Osteocytes in lacunae
- Haversian system visible

4. Nervous Tissue

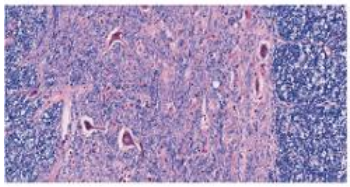
- Neurons with large cell body
- Axon and dendrites visible
- Supporting neuroglial cells present

Result

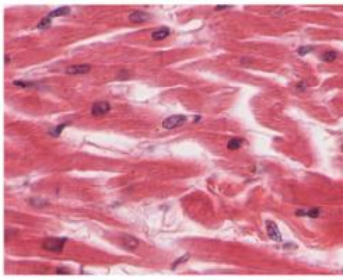
The given histological slide was observed and correctly interpreted based on its microscopic structural features, and the tissue/organ was identified successfully.

Precautions

- Always focus under low power first
- Handle slides carefully
- Do not allow lens to touch the slide
- Clean lenses after use

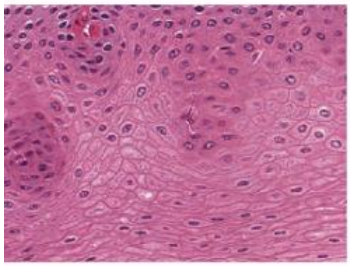


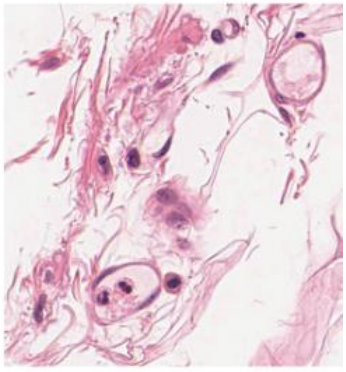
Nervous tissue
- Brain
- Spinal cord
- Nerves



Muscle tissue
- Cardiac muscle
- Smooth muscle
- Skeletal muscle

Epithelial tissue
- Lining of GI tract organs and other hollow organs
- Skin surface (epidermis)





Connective tissue
- Fat and other soft padding tissue
- Bone
- Tendon