Introduction to Histology
Histology

The term "Histology" is derived from the Greek word for a tissue "Histos", and "-logos" = “the study of”

Histology: Is the study of tissues and how they are arranged into organs.

Also called “Microscopic Anatomy” Or Microanatomy.
At the smallest level, your body is made up of atoms, which are tiny, non-living pieces of elements such as iron or oxygen. Atoms join together with other atoms to form molecules. Molecules are not alive, either. It takes trillions of molecules organized in a very specific way to make the smallest living thing – a cell.

At the largest level, your body is made up of systems. Each system does a particular job. The framework of bones that supports your body is called the skeletal system. The tubes that carry blood from your heart all around your body make up the circulatory system. All the systems work together to keep you going.
Levels of Organization

• **Cells** are the smallest units of life.

• **Cells** are arranged in organized groups called **tissues** to perform specific functions.

• **Tissues** are organized together to form **organs** to perform more complex functions.

• **Organs** work together in groups to form **systems** to perform higher order functions.

• **Systems** make up the individual **organism**.
Levels of Organization

- The Human Body
- Systems
- Organs
- Tissues
- Cells
- Molecules
- Chemicals
Levels of Organization

<table>
<thead>
<tr>
<th>Level of Organization</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Cell</td>
<td>Smallest unit of life.</td>
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<tr>
<td>Tissue</td>
<td>An association of cells with the same general structure and function.</td>
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<tr>
<td>Organ</td>
<td>An association of several tissue types that carry out a specific function.</td>
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<tr>
<td>Organ System</td>
<td>Two or more organs that work together to carry out a general function, such as digestion or movement.</td>
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Levels of Organization – Cells

Cell: Is the microscopic unit of structure and function. The human body contains 50 trillions of cells.

200 different types of cells are specialized for certain tasks: contraction, defense, secretion....

Example:
Red blood cells, nerve cells,....

Cells work together at certain task and form tissues that carry out a particular function.
Levels of Organization – Cells
Levels of Organization - Cells

cell membrane

cytoplasm

mitochondrion

nucleus

DNA

endoplasmatic reticulum

lysosome

ribosome

Golgi apparatus
Human development begins with a single cell, the fertilized egg.

Which divides to produce scores of identical, smaller cells.

The first tissues appear when these cells start to organize themselves into layers.

Three strata called Primary germ layers, which give rise to all of the body’s mature tissues.
The three layers are called:

1. **Ectoderm**: gives rise to the epidermis and nervous system.

2. **Mesoderm**: gives rise to muscle, bone and blood among other tissues.

3. **Endoderm**: gives rise to the mucous membranes of the digestive and respiratory tract and the digestive glands among other things.
Embryonic Tissues

16-day-old embryo (dorsal surface view)

Key:
- Blue = Ectoderm
- Red = Mesoderm
- Yellow = Endoderm

Muscle and connective tissue (mostly from mesoderm)
Nervous tissue (from ectoderm)
Epithelium
Yolk sac (lined with endoderm)

Amniotic cavity containing amniotic fluid

Amnion

Endometrium

Maternal blood pool

Chorion (derived from blastocyst cavity membrane)

Allantois

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**Endoderm layer becomes:**
1) Digestive system
2) Liver
3) Pancreas
4) Lungs (inner layers)

**Mesoderm layer becomes:**
1) Circulatory system
2) Lungs (epithelial layers)
3) Skeletal system
4) Muscular system

**Ectoderm layer becomes:**
1) Hair
2) Nails
3) Skin
4) Nervous system
Levels of Organization – Tissue

- **Tissue** is a group of similar cells and cell product that arise from the same region of the embryo and work together to perform a specific structural or physiological role in an organ.

- Tissue is composed of **cells** and **Matrix**.

- The matrix is composed of **fibrous proteins** and usually a clear gel variously known as **ground substance**, tissue fluid, extracellular fluid (ECF), interstitial fluid, or tissue gel.

- **The ground substance** contains water, gases, minerals, nutrients, wastes and other chemicals.
The primary Tissue classes

The four primary tissues are:
1. Epithelial Tissue.
2. Connective Tissue.

These tissues differ from each other in:
1. The types and functions of their cells.
2. The characteristics of the matrix (extracellular material) that surrounds the cells.
3. The relative amount of space occupied by cells versus matrix.
Epithelial tissue

Consists of a flat sheet of closely adhering cells, one or more cells thick, with the upper surface usually exposed to the environment or to an internal space in the body.

Epithelium covers the body surface, lines body cavities, forms the external and internal linings of many organs, and constitutes most gland tissue.
Connective tissue

Consists mostly of fibers and ground substances, with widely separated cells. It is the most abundant, widely distributed and histologically variable of the primary tissues.

It serves to connect organs to each other, support, protect organs, movement, storage and transport.

Include: fibrous tissue, cartilage, bone, blood and fat.
Muscular tissue

Consists of elongated cells that are specialized to respond to stimulation by contracting.

Its primary job is to exert physical force on other tissues and organs.

Not only do movement of the body but do such process as digestion, waste elimination, breathing, speech and blood circulation.
Consists of neurons or nerve cells and a much greater number of neuroglia or glial cells which protect and assist the neurons.

Neurons are specialized to detect stimuli, respond quickly and transmit coded information rapidly to other cells.
Levels of Organization – Organ

An organ is a structure with discrete boundaries that is composed of two or more of these tissue types (usually all four).
Levels of Organization – System

**System:** Is a group of two or more organs that work together to perform a specific function for the organism.

- Digestive system.
- Respiratory system.
- Circulatory system.
- Urinary system.
- Endocrine system.
Levels of Organization – System

1. Chemical Level
   - Atoms
   - Molecule (DNA)

2. Cellular Level
   - Smooth muscle cell

3. Tissue Level
   - Smooth muscle tissue
   - Serous membrane

4. Organ Level
   - Digestive system
   - Esophagus
   - Liver
   - Stomach
   - Pancreas
   - Gallbladder
   - Small intestine
   - Large intestine

5. System Level

6. Organismal Level
Preparation of tissues for study
Microtechnique

• Cells are too small to be seen by necked eye. They are studied with the help of microscopes.

• Microscopes are high resolution instruments that are used for observing fine details of very small objects.

• Microtechnique: is tissue preparation for microscopic examination.
Microscopy

- Scientists use microscopes to visualize cells that are too small with the naked eye.

- **Light microscopes (LM.s)**
  - Pass visible light through a specimen.
  - Magnify cellular structures with lenses.
  - Magnified images are typically from 10-1000X.

- **Electron microscopes (EM.s)**
  - Focus a beam of electrons through a specimen (TEM) or onto its surface (SEM).
  - Magnified images are typically from 1000X to 50,000X up to 2,000,000.
Light Microscope Parts
Electron Microscopy

• Transmission electron microscopy (TEM):
  • Provides for detailed study of the internal ultrastructure of cells.
  • A transmission electron microscope profiles a thin section of a specimen.

• The scanning electron microscope (SEM):
  • Provides for detailed study of the surface of a specimen.
  • Micrographs taken with a scanning electron microscope show a 3D image of the surface of a specimen.
Transmission Electron microscope (TEM)
Types of EM

- **Transmission electron microscopy** (TEM).
  - Views the ultrastructural details in shades of gray

- **Scanning electron microscopy**.
  - Views only the surface as a 3D image.
  - Usually the object is coated with a thin layer of heavy metal, such as gold deposited on the specimens surface. As the beam of electrons scans the surface → some are reflected while others are ejected → captured by electron detectors → displayed on a monitor as 3D image
Types of EM

Cilia

Transmission EM
Scanning EM
Use different methods for enhancing visualization of cellular structures

**TECHNIQUE**

**Bright field** (unstained specimen).
Passes light directly through specimen.
Unless cell is naturally pigmented or artificially stained, image has little contrast.

**RESULT**

![Image of bright field (unstained specimen)]

**Bright field** (stained specimen).
Staining with various dyes enhances contrast, but most staining procedures require that cells be fixed (preserved).

![Image of bright field (stained specimen)]
Use different methods for enhancing visualization of cellular structures

**TECHNIQUE**

**Phase-contrast:**
Enhances contrast in unstained cells by amplifying variations in density within specimen; especially useful for examining living, unpigmented cells.
Use different methods for enhancing visualization of cellular structures

**TECHNIQUE**

**Differential-interference-contrast (Nomarski):**
Like phase-contrast microscopy, it uses optical modifications to exaggerate differences in density, making the image appear almost 3D.

**Fluorescence:**
Shows the locations of specific molecules in the cell by tagging the molecules with fluorescent dyes or antibodies. These fluorescent substances absorb ultraviolet radiation and emit visible light.
Use different methods for enhancing visualization of cellular structures

**TECHNIQUE**

Confocal: Uses lasers and special optics for “optical sectioning” of fluorescently-stained specimens. Only a single plane of focus is illuminated; out-of-focus fluorescence above and below the plane is subtracted by a computer. A sharp image results, as seen in stained nervous tissue, where nerve cells are green, support cells are red, and regions of overlap are yellow.

**RESULT**
Preparation of tissue For Light Microscopy
( Tissue Processing)

• The aim of tissue processing is to embed the tissue in a solid medium firm enough to support the tissue and give it sufficient rigidity to enable thin sections to be cut, and yet soft enough not to damage the knife or tissue with preservation of the structure with the least possible alteration.
Tissue Processing

- Fixation
- Dehydration
- Clearing
- Embedding
- Sectioning
- Sliding
- Staining
- Mounting
Tissue Processing

(a) Fixation → Dehydration → Clearing → Infiltration → Embedding
Fixation

• Achieved by the influence of various chemical compounds called Fixative.
• Common methods - 10% formaldehyde.

• To preserve the microstructure of tissue as the living state and prevent the alteration of tissue.
• To prevent the autolysis.
• To prevent of bacterial digestion.
• To harden the tissue and making it to be cut easily.
Dehydration

• Is the removal of extractable water from the tissue.
• Graduated strength of ethyl alcohol is routinely employed at series consisting of 30, 50, 70, 95 and 100% alcohol produce good result.
Clearing

- The process of tissue to prepare it for embedding by using the chemical that is miscible with both the dehydrating agent and the embedding agent.

- **Xylene** and **chloroform** are the most commonly used.
  - Chloroform
  - Benzene / benzol
  - Xylene / xylol
  - Cedar wood oil
  - Benzyl benzoat
  - Methyl benzoat
Infiltration & Embedding

- **Impregnation (infiltration):** The tissue is kept in a wax bath containing molten paraffin wax (paraffin heated to 60 degree: melting point).

- **Embedding:** It is done by transferring the tissue which has been cleared of the alcohol and impregnated with wax to a mould filled with molten wax & is allowed to cool & solidify.

- After solidification, a wax block is obtained which is then sectioned to obtain ribbons.

- **Paraffin wax** is the most frequency used agent.
Sectioning

- Sectioning is carried out by the help of machine called **Microtome**.
- Usually 5-10 µm thick.
Sectioning
Sliding

- Clean microscopic glass slides are taken and the section which is floated in warm water is taken on the glass slide in such a way that no air bubble is trapped between them.
Staining

• Because the tissues of the body are colourless and are difficult to study their details. The staining technique enhance natural contrast and permits distinction to be made between them.

• The most commonly used dye is a combination of HEMATOXYLIN and EOSIN (H&E).

• By this method the nuclear structures are stained dark *purple* or *blue*.

• All cytoplasmic structures and intracellular substances are stained *pink* or *red*. 
Basophilia

- Basophilic structures (acidic components) are stained by basic dyes.
- Basic dyes are positive.
- Basophilic structures are negative (ex. DNA, RNA, ribosomes, RER).
- Basophilic = Blue.
- Nucleus.
Acidophilia

- Acidophilic structures (basic components) are stained by acid dyes.
- Acid dyes are negative.
- Acidophilic structures are positive (ex. Proteins, collagen, cytoplasm).

- Eosinophilic = Pink or red.
- Cytoplasm.
Mounting

• Applying a thin glass coverslips using mounting medium (DPX) to protect the section.
Microscopic Examination
Microscopic Examination