

**Mustansiriyah University**

**College of Medicine**

**Department of Anatomy, Histology**

**& Embryology**

The background of the title section features a faint, light-colored illustration of a laboratory microscope and various pieces of glassware, including test tubes and beakers. A red laser line is shown passing through the microscope's eyepiece and objective lenses, focusing on a small object on the stage.

**MEDICAL BIOLOGY  
LABORATORY MANUAL  
FOR  
FIRST YEAR STUDENTS**

**BY**

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**Attention**

- ☒ **This manual must be used in combination with *diFiore's Atlas of Histology*, 11<sup>th</sup> edition. All the pages and figure references for the tissue slides pictures refer to this atlas.**
  
- ☒ **The subjects of Electron microscope, Histological techniques, and Cell division was adapted from Junqueira's Basic Histology, 14<sup>th</sup> edition.**
  
- ☒ **Most of the slides are stained by Hematoxylin& Eosin, except certain slides in which name of the stain is mentioned at the appropriate place in the manual.**

## **General Instructions**

1. **No materials may be taken out of the histology laboratory!**

2. **Microscopes:**

a) Two students are assigned to one microscope; therefore, **cooperation is necessary!** Please keep your scope clean and in proper working condition. At the end of each laboratory session, return your microscope to its appropriate place.

b) Carefully read the instructions in this manual on proper care and handling of the microscope. If you don't remember anything else remember to **CHECK TO SEE THAT THE CONDENSER IS ALL THE WAY UP AND THAT THE LIGHT IS OFF.**

3. **Slide Trays:**

a) You are sharing slide Trays with other students. Please cooperate by leaving them in good order!

b) Before each lab, check for damage and/or lost slides. Report any damage or loss immediately. The instructors will check the microscopes and slide trays periodically, and **you will be charged \$50.00 per damaged/lost slide.**

c) At the end of each lab, return your slide trays to the instructor.

4. Any demonstration slides and electron micrographs the instructors set out may be used for examination purposes. If any of this material is to be used, you will be notified either verbally or by notes written on the blackboard.

5. Each laboratory session is scheduled for TOW HOURS; it will be necessary for you to use this time efficiently. This will greatly decrease the time you will need for review.

6. Keep the lab tables clean.

7. The instructors are available any time during lab. Do not hesitate to ask for help when you need it. No question is "stupid" or "dumb".

8. **Students are strongly advised to bring both their ATLAS and their NOTEBOOK to the laboratory!**

## Lab. 1

# Microscope

### A. Conventional Light Microscope

This is the type of microscope used in this lab. The major parts of the instrument will be named and a method for the effective use of the microscope will be outlined.

The **light microscope** uses lenses and light to enlarge the image and is also called **bright field** or **compound** microscope.

The light microscope has two systems of lenses for greater magnification:

- 1) The ocular, or eyepiece lens that one looks into and
- 2) The objective lens, or the lens closest to the object



#### ☒ Parts of the microscope:

1. **Eyepiece (Ocular Lens):** the lens at the top that you look through. They are usually 10X or 15X power.
2. **Tube:** Connects the eyepiece to the objective lenses
3. **Arm:** Supports the tube and connects it to the base. It is used along with the base to carry the microscope
4. **Base:** The bottom of the microscope, used for support
5. **Illuminator:** A steady light source (110 volts) used in place of a mirror.
6. **Stage:** The flat platform where you place your slides.
7. **Stage clips:** hold the slides in place.
8. **Stage controls:** two knobs that move the stage in 4 directions (to examine different areas of the slide).
9. **Revolving Nosepiece or Turret:** This is the part that holds the objective lenses and can be rotated to easily change power.
10. **Objective Lenses:** Usually you will find 3 or 4 objective lenses on a microscope.

They almost always consist of: 4X (scanning objective), 10X (low power objective), 40X (high power objective) and 100X (oil immersion objective).

They are changed by rotating the turret, usually in a clockwise direction

When coupled with a 10X (most common) eyepiece lens, we get total magnifications of 40X (4X \* 10X), 100X, 400X and 1000X.

The shortest lens is the lowest power; the longest one is the lens with the greatest power.

Total magnification power = magnification of ocular lenses multiplied by magnification of objective lenses

**11. Condenser:** It is a rotating disk under the stage. A lever projects from the condenser that is used to vary the size of the opening of the condenser (**iris** or **diaphragm**) and thus vary the intensity and size of the cone of light that is projected upward into the slide. There is no set rule regarding which setting to use for a particular power. Rather, the setting is a function of the transparency of the specimen, the degree of contrast you desire and the particular objective lens in use.

**12. Coarse adjustment knob:** This is used to focus the microscope. It is always used first, and it is used only with the scanning (4X) and low power (10X) objectives.

**13. Fine adjustment knob:** This is used to focus the microscope. It is used with the high-power (40x) objective to bring the specimen into better focus.

☒ **Notes:**

The magnification power of the light microscope is limited to 1000-2000 X.

The resolving power (resolution) of the light microscope is 0.2 μm.

✱ *The resolving power (resolution) is defined as the smallest distance between two particles at which they can be seen as separated objects.*

The human eye has a resolution of **0.2 mm**.

### ☒ The Proper Use of the Microscope

In examining a slide with the light microscope, the following sequence of steps should be used:

1. Place the slide on the stage and hold it in place with the stage clips.
2. Select the very low (scanning) objective (4x) first and while looking from the side, raise the stage with the coarse adjustment knob to make the slide as close to the lens as possible without touching it.
3. Now, look through the eyepiece lens and focus upward (using coarse adjustment knob only) until the image is sharp and clear. If you can't get it in focus, repeat the process again.
4. Scan the entire section (*often tissue and organ identification can be made at this magnification*). Select an area or areas for study at higher magnification.
5. Rotate the revolving nosepiece to place the low-power objective (10x) in the optical axis. When turning the nosepiece, grasp the nosepiece itself or the part of the objective adjacent to the nosepiece to avoid excess stress on the objective.
6. Look again through the eyepiece and do minor adjustments with the fine adjustment knob until the image is sharp and clear.
7. Proceed to the next step in magnification, if necessary, which is high dry (40x).

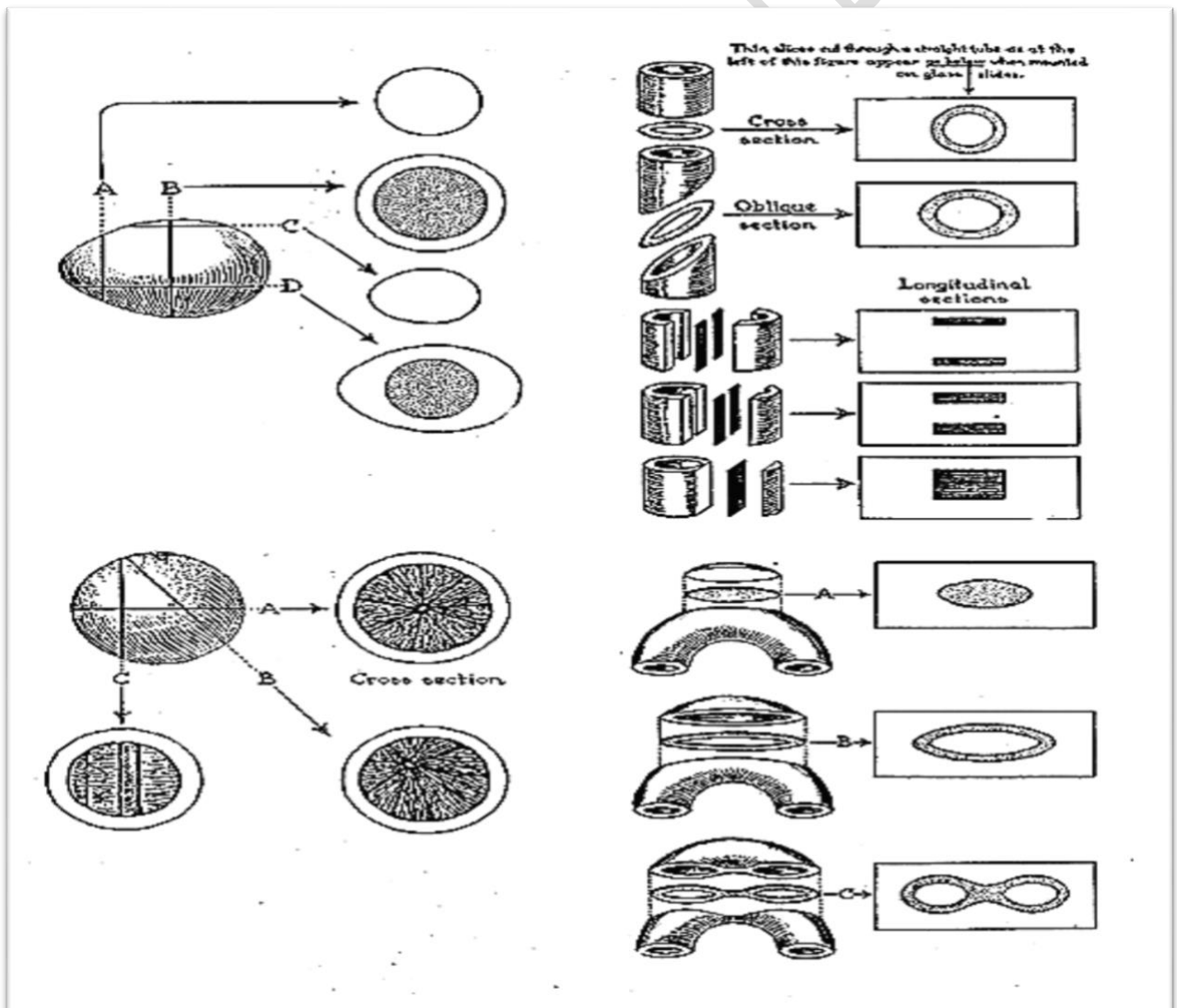
In this Lab, you will use only the 4X, 10X and 40X lenses.

☒ Notes:

- ☺ Both eyes should be open when viewing through the microscope.  
This prevents eye fatigue, which occurs when the non-viewing eye is kept closed. Keeping both eyes open does take some practice, but it is highly recommended.
- ☺ Also, you should never let your eye touch the ocular lens.  
If your eyelashes touch the lens you are to close.
- ☺ Always remove eyeglasses when viewing through a microscope.  
If your eyeglass lens touches the microscope it may get scratched.

## Tissue Sections

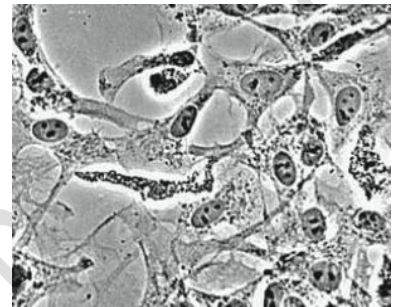
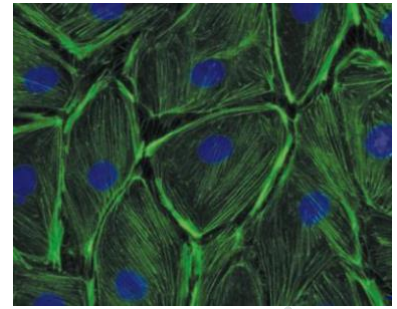
Most of the tissues you will be examining with the microscope are thin slices (called **sections**) of the tissues and organs being studied, such as **cross section**, **oblique section** and **longitudinal section**. The following image show you how correlate between the section and the plane of sectioning through the tissue.





## **B. Specialized Light Microscope**

1. **Fluorescence Microscope**: When certain cellular substances are irradiated by light of a proper wavelength, they emit light with a longer wavelength— a phenomenon called **fluorescence**. In **fluorescence microscopy**, tissue sections are usually irradiated with ultraviolet (UV) light and the emission is in the visible portion of the spectrum. The fluorescent substances appear bright on a dark background.
2. **Phase-Contrast Microscope**: uses a lens system that produces visible images from unstained, transparent objects and, importantly, can be used with living, cultured cells. Cellular structures appear lighter or darker in relation to each other because of light refraction.
3. **Confocal Microscope**: it produces higher resolution and sharper focus than bright field microscope. It uses computer assisted lens system to produce many serial images that can be digitally reconstructed into 3D image.



## **C. Electron Microscope:**

An electron microscope is a type of microscope that produces an electronically-magnified image of a specimen for detailed observation. The electron microscope is an imaging system that permits high resolution (0.1 nm). In practice, however, a resolution of 1 nm in tissue sections is considered satisfactory. The electron microscope (EM) uses a particle beam of electrons to illuminate the specimen and create a magnified image of it. The microscope has a greater resolving power than a light-powered optical microscope, because it uses electrons that have wavelengths shorter than visible light, and can achieve magnifications of up to 2,000,000X, whereas light microscopes are limited to 2000X magnification.

The electron microscope uses electrostatic and electromagnetic "lenses" to control the electron beam and focus it to form an image. These lenses are analogous to, but different from the glass lenses of an optical microscope that form a magnified image by focusing light on or through the specimen.

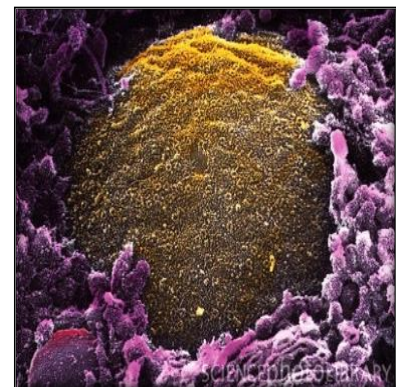
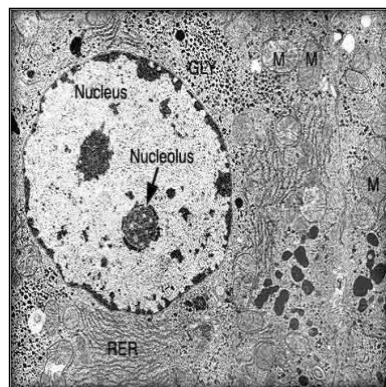
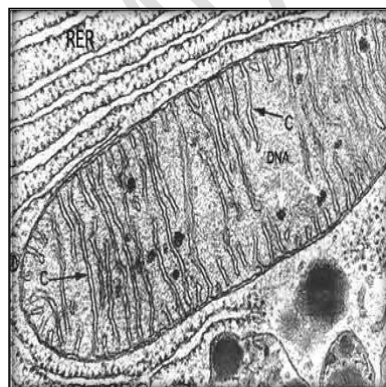
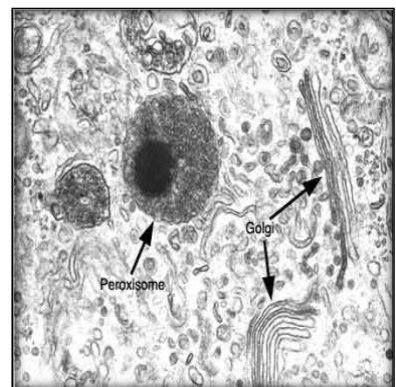
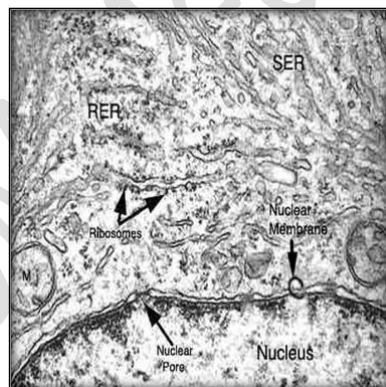
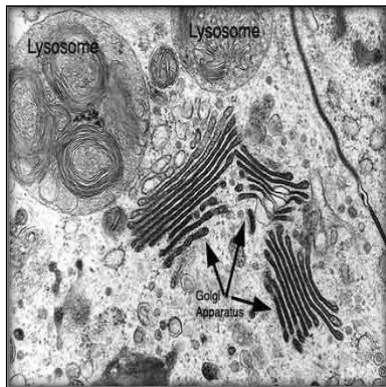
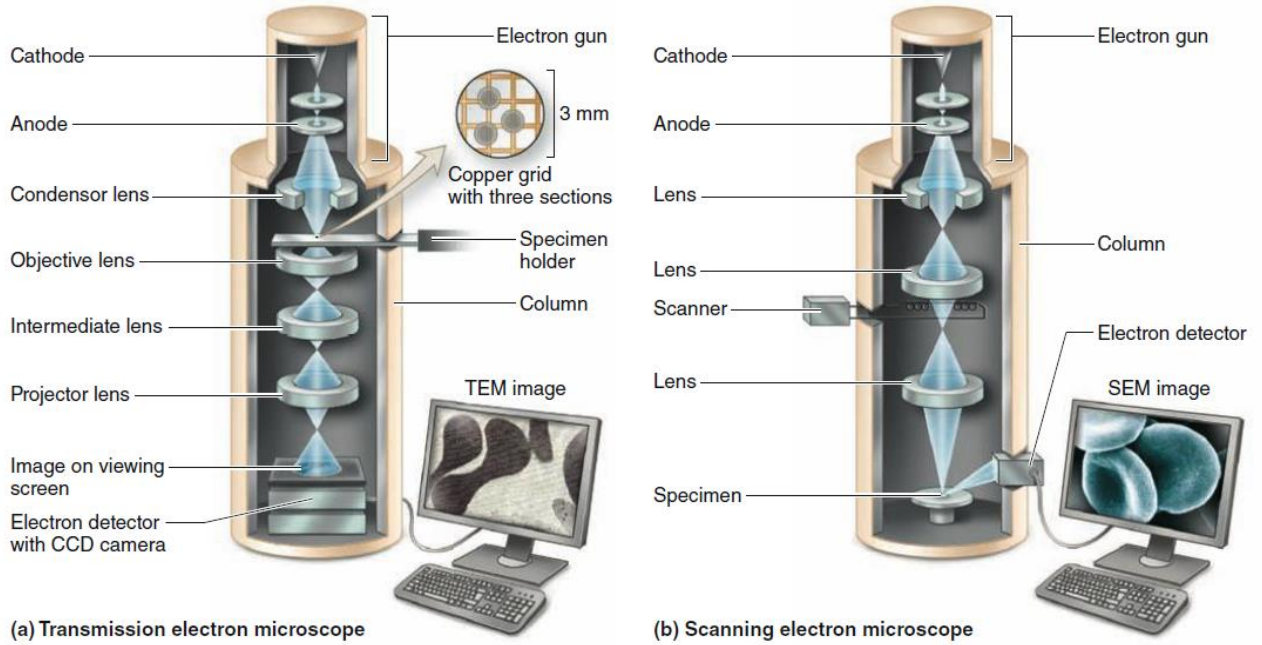
### **Types of Electron Microscope (EM):**

#### **1. Transmission Electron Microscope (TEM):**

The original form of electron microscope, the transmission electron microscope (TEM) uses a high voltage electron beam to create an image. The electron beam focused by electrostatic and electromagnetic lenses in a way roughly analogous to what occurs in the optical microscope (condenser, objective lens system), and transmitted through the specimen that is in part transparent to electrons and in part scatters them out of the beam. When it emerges from the specimen, the electron beam carries information about the structure of the specimen that is magnified by the objective lens system of the microscope. The spatial variation in this information (the "image") is viewed by projecting the magnified electron image onto a fluorescent viewing screen.

**2. Scanning Electron Microscope (SEM):**

Is used to provide a three-dimensional image of cells. In SEM the electron beam does not pass through the specimen. Instead, the surface of the cell is coated with a heavy metal, and a beam of electrons is used to scan across the specimen. Because the resolution of scanning electron microscopy is only about 10 nm, its use is generally restricted to studying whole cells rather than subcellular organelles or macromolecules.



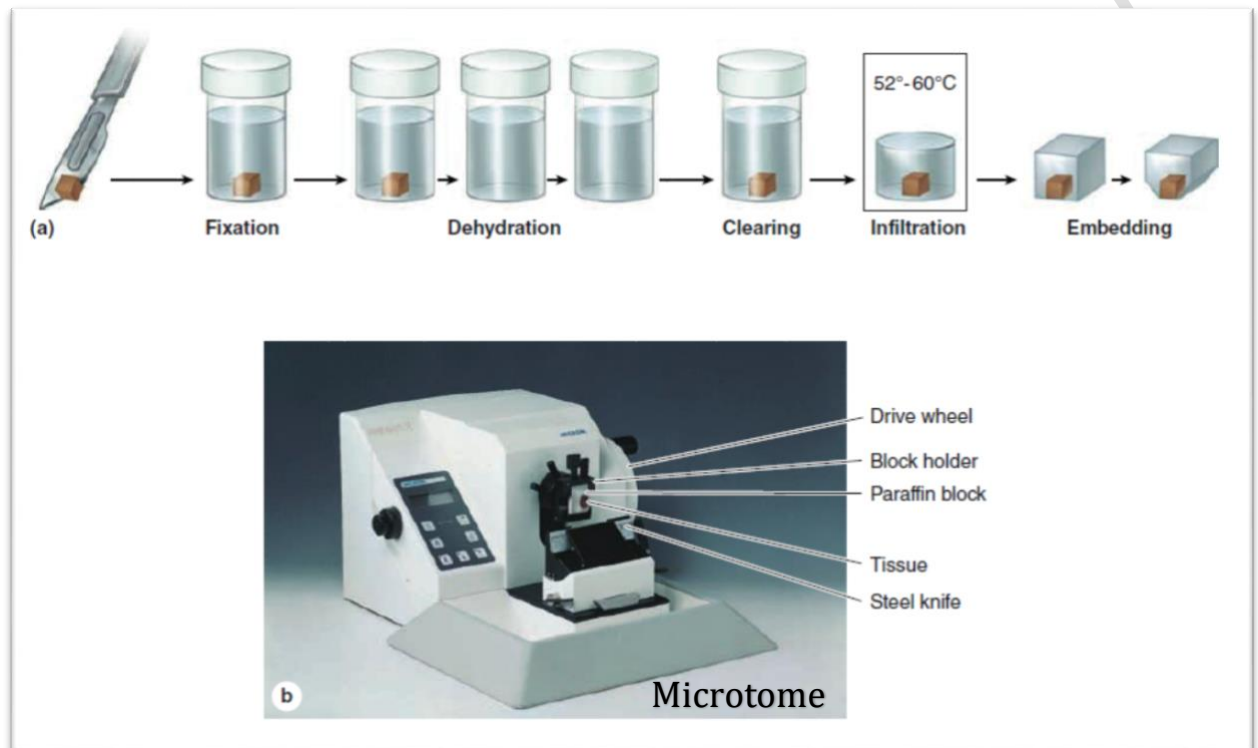
The 2D images are from TEM of cell organelles and nucleus. The 3d image (colored) is from SEM of nuclear membrane



## Lab. 2

### General Principles of Tissue Preparation and Staining

The study of tissue structure relies on the preparation of tissue samples in ways that allow their structural details to be viewed at light or electron microscopic levels. Because tissues and organs are usually too thick for light to pass through them, they must be sectioned to obtain thin, translucent sections and then attached to glass slides before they can be examined.



#### The basic steps used in tissue preparation for histology are:

##### ❖ Fixation

Fixation, usually done by a chemical or mixture of chemicals, permanently preserves the tissue structure for subsequent treatments. Fixation is used to:

1. Terminate cell metabolism.
2. Prevent enzymatic degradation of cells and tissues by autolysis (self-digestion).
3. Kill pathogenic microorganisms such as bacteria, fungi, and viruses.
4. Harden the tissue as a result of either cross-linking or denaturing protein molecules.
5. Transform the contents of the cell from a semifluid to a semisolid and prepare the cell contents for visualization with stains, dyes, or metallic salts.

##### ➤ **Fixatives:** commonly used fixatives are

1. **Formalin:** (mixture of formaldehyde and alcohol). It penetrates the tissue rapidly, leaves no residues, and requires little or no washing of the tissue to remove.
2. **Aldehydes:** such as glutaraldehyde and paraformaldehyde, are excellent fixatives for light microscopic applications and are also widely used in electron microscopy.
3. **Other fixatives:** Picric acid, Alcohols, Mercuric chloride, and Acetic acid

- **Methods of Fixation:** Fixation is usually accomplished in one of two ways.
1. **Immersion fixation.** The fixative is prepared and a small piece of tissue removed and immersed in the fixative. The advantage of this method is that the fixative can penetrate from all sides of the tissue block. This method is useful when the tissue sample is small.
  2. **Perfusion fixation.** In this method the fixative is perfused through the intact vascular system of the organism. After perfusion, the tissue samples are removed and placed in more of the same fixative used in the perfusion. This method provides superior fixation of large pieces of tissue.

**FREEZING** is also used as a method of fixation, especially in the clinical setting when a rapid diagnosis is needed during a medical procedure. A fresh tissue sample is retrieved from the patient or organism and immersed in liquid carbon dioxide or in a substance cooled extremely rapidly by dry ice. The best results are obtained with small tissue samples that are rapidly cooled to very low temperatures ( $-40^{\circ}$  to  $-60^{\circ}$  C).

### ❖ Processing of Fixed Tissue

Once the tissue sample is satisfactorily fixed, it must be taken through a series of steps that result in a thin slice of tissue mounted on a glass slide (for LM) or an even thinner slice mounted on a copper grid (for EM). In general, this process requires three basic steps:

1. **Dehydration:** The goal of dehydration is to remove the water from the tissue. The most common method is to start with **alcohol** in a concentration of 70% to 80%. Recall that alcohol will mix with water; therefore, it can be used to remove water from the tissue. The tissue sample is passed, stepwise, through progressively higher concentrations (from 70%–80% up to 100%) of alcohol. Through this process, the water is completely removed from the tissue and replaced with alcohol.
2. **Clearing:** The clearing reagent is a substance that will mix both with alcohol and with the embedding medium. The most commonly used are **xylene**, **toluene**, and **chloroform**. The tissue is passed from 100% alcohol through changes of the clearing reagent. This stepwise process progressively removes the alcohol from the tissue and replaces it with the clearing reagent.
3. **Impregnation and embedding:** Because the clearing reagent will mix with the embedding medium, the tissue sample is taken from the last step in this reagent and placed in melted embedding medium (**paraffin wax**). The sample is then progressively passed through several changes of the embedding material. This stepwise process progressively removes the clearing reagent and replaces it with the embedding medium that will harden when cooled.

**Embedding** is generally done in two steps.

- First, the tissue is removed from the last impregnation step and immediately placed in melted medium in a vacuum oven. The last traces of the clearing reagent and any minute bubbles are removed by vacuum.

- Second, the tissue sample is oriented in an **embedding mold**. The mold is then filled with melted medium and allowed to cool so the medium hardens. A similar set of steps are followed when a **polymer** is used as the embedding media for electron microscopy.

### ❖ Sectioning and Mounting

Sectioning of prepared tissues is done on **microtomes** that are designed to accommodate sections mounted in paraffin, frozen in ice, or embedded in plastic. The sections are cut using extremely sharp metal or glass knives, removed from the edge of the knife either as individual sections or as ribbons of sections, and floated in water (usually warmed).

For most applications in LM, the sections range in thickness from about **5 to 12  $\mu\text{m}$**  (for paraffin embedded tissue) and from about **0.5 to 2.0  $\mu\text{m}$**  (for plastic embedded tissue). For EM, glass or diamond knives are used to cut extremely thin sections ranges from 80 to 110 nm.

After the sections are cut, they are mounted on slides using a mounting substance such as **DPX**.

### ❖ Staining

The goal of staining tissue slices is to use substances to impart color to various components of the section, making these components available for study.

The simplest way by which stains function is to exploit the electrostatic interactions between the stain molecules and components of the cell; positive charges on cellular structures attract negatively charged stain molecules and vice versa.

- **Basic dyes** carry positive charges and are, consequently, known as cationic dyes; they are attracted to negative charges within the tissue. **Hematoxylin** and **toluidine blue** are commonly used basic (cationic) dyes. They stain nuclear DNA, cytoplasmic RNA, sulfonated polysaccharides such as chondroitin sulfate, and polycarboxylic acids such as hyaluronic acid.
- **Acidic dyes** carry negative charges and are, consequently, known as anionic dyes; they are attracted to positive charges within the tissue. **Eosin Y** is a commonly used acidic (anionic) dye. It stains many proteins (and, therefore, stains many structures within the cell), and acid dyes also stain extracellular structures such as collagen.

### ➤ Commonly used stains

**Hematoxylin and Eosin (H&E)** is, by far, the most commonly used combination stain. This combination of cationic and anionic dyes results in most constituents of the cell (RNA, DNA, polysaccharides, and others) being stained with various tones of either blue (hematoxylin) or pink (eosin). This method also stains extracellular collagen.

Other stains: **Mallory trichrome stain; Wright stain; Silver stains; periodic acid-Schiff (PAS) reaction; Iron hematoxylin; and Giemsa stain**

## ❖ Immunocytochemistry and Immunohistochemistry (ICC & IHC)

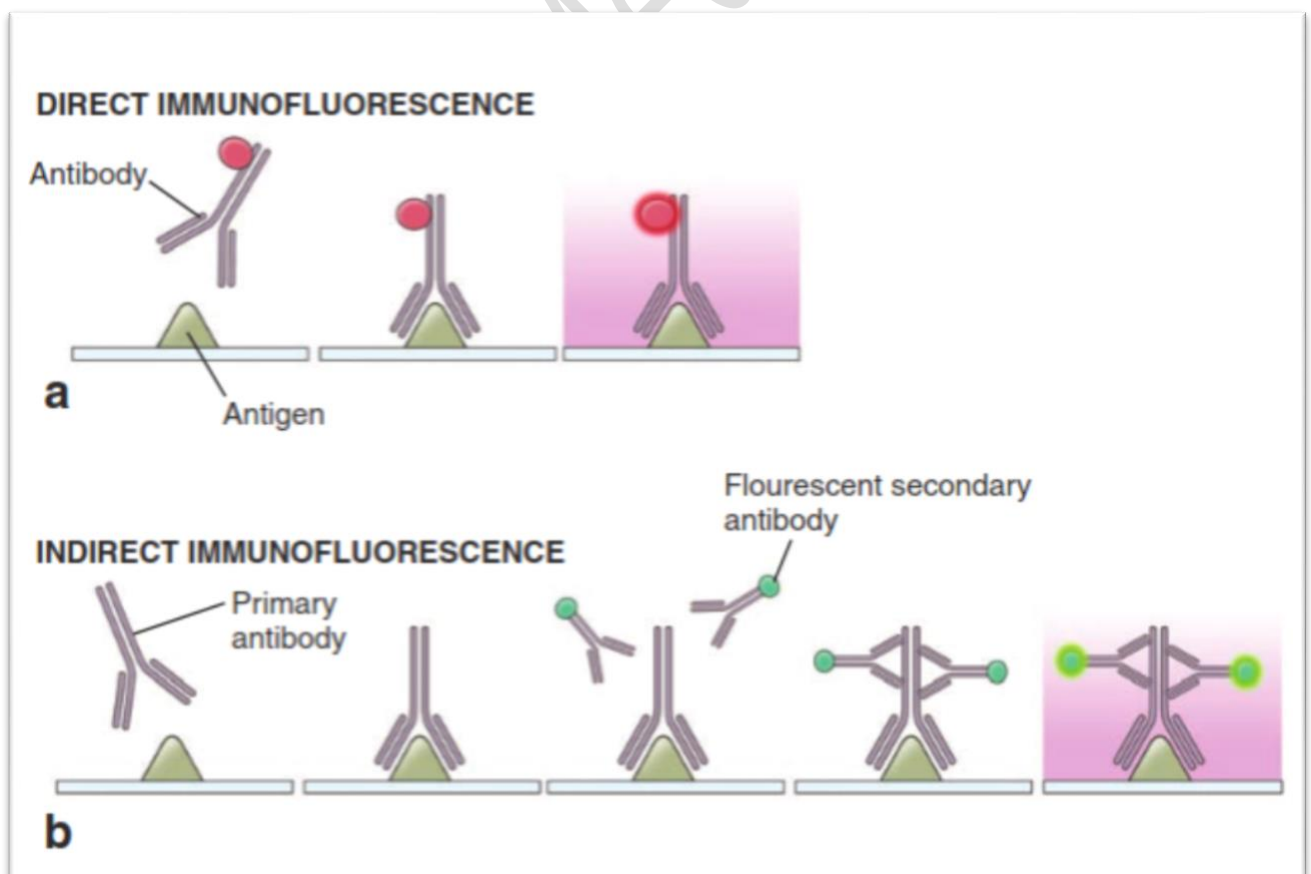
They are specialized methods that can be used to precisely localize enzymes or large molecules (macromolecules) within the cell or on its membrane. The immune system of the body is able to defend itself against foreign molecules (**antigens**) by producing specific types of proteins (**antibodies**). ICC & IHC methods use this feature of cells to visualize specific molecules.

For LM, an antibody is produced against a specified protein or molecule, and the antibody is coupled with a **fluorescent dye**, such as **fluorescein** or with an **enzyme** such as **peroxidase**. When this labeled antibody attaches to a specific antigen and is examined under microscope, it will show a color, thereby specifically identifying the location of that molecule.

This is the **direct method**: An antibody is produced, coupled to a dye, attached to an antigen, and, thus, becomes visible.

In the **indirect method**, unlabeled antibodies (**primary antibodies**) are produced in one animal (e.g. mice or rabbit) against a specific antigen and then applied to a tissue to which they attach. The unlabeled antibodies are visualized by exposing them to labeled antibodies (**secondary antibodies**) that are made in another species (e.g. goat) and that are directed against the immunoglobulins from the first species.

One way to visualize this is as follows: An antibody (primary antibody) is produced, not labeled, and attached to an antigen; a second antibody (secondary antibody) is produced, coupled with a dye, attached to the first unlabeled antibody, and, thus, becomes visible.





## Lab. 3

### Buccal Smear

The cells, which line the inside of your cheeks, form a mucous membrane and are classified as a stratified squamous epithelium tissue. These flat, scale-like buccal cells (pronounced, "buckle") resist friction and are shed constantly as the tissue is renewed. By gently scraping the inside of your cheek, these cells can be collected, and when smeared and stained, may be used to illustrate a number of important biological phenomena including cell and tissue structure.

#### **Slide Preparation:**

1. Clean a microscope slide well with soap & water, dry with a paper towel.
2. Cleanse very thoroughly under the nail of your index finger.
3. Place a *small* drop of distilled water in the center of the very clean slide.
4. *GENTLY* scrap the inside of your cheek with your finger nail to pick up some of the shed stratified squamous cells.
5. Express the material from under your nail by pressing with your thumb and press the material into the drop of water on the slide, mix and spread the material around to the size of a small circle.
6. Dry the slide by waving in air
7. Fix the smear by flooding with 90% alcohol for **5** minutes. Dry by waving in air.
8. Stain with hematoxylin for **5** minutes.
9. Rinse in tap water for **5-10** minutes.
10. Counterstain with eosin for **1/2 - 1** minute, then rinse with tap water.
11. Rinse in distilled water, and dry with a paper towel.
12. Examine under microscope, first with the **4x** objective, scanning the entire field to find a well-distributed region with individual cells (no big clumps). Then view with the **10x** and **40x** objectives. Note the nucleus (staining blue) and cytoplasm (staining pink).

## Lab. 4

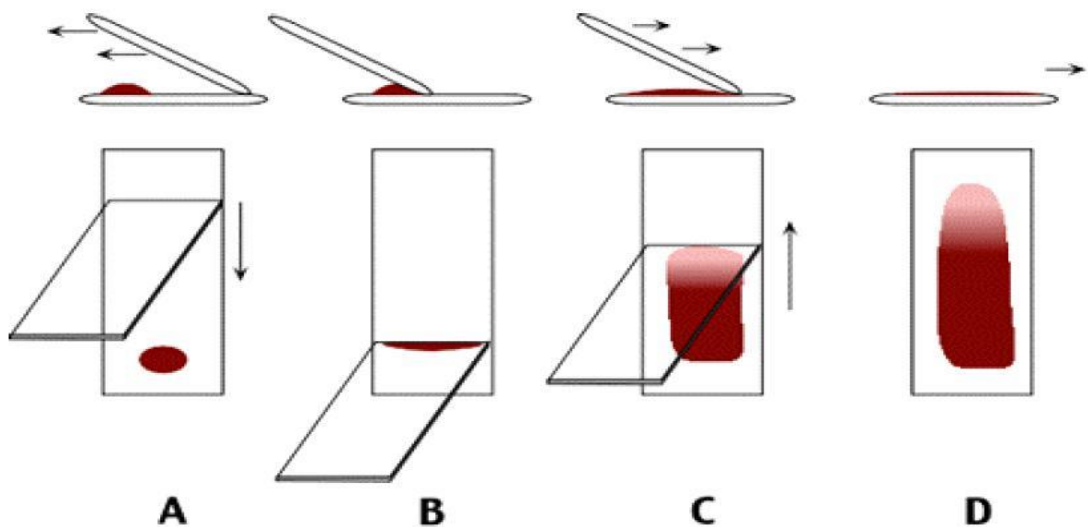
### Blood Smear

#### Slide Preparation

1. Take a clean slide.
2. Sterilize the tip of your finger by washing with water, then in 95% alcohol.
3. Prick the tip of your finger with a lancet, and then discard the first drop of blood.
4. Let the second drop of blood touch one end (distal 1/3) of your slide.
5. Place the slide on a flat surface, and hold the narrow side of the other edge of the slide between your left thumb and forefinger
6. With your right hand, place the smooth clean edge of a second (spreader) slide on the specimen slide, just in front of the blood drop.
7. Hold the spreader slide at a 30-45° angle and draw it back against the drop of blood.
8. Allow the blood to spread almost to the edges of the slide.
9. Push the spreader slide forward with one light, smooth, and fluid motion. A thin film of blood in the shape of a bullet with a feathered edge will remain on the slide.
10. Dry the smear slide by waving in air.

#### Lieshman's Stain

1. Add sufficient amount of Lieshman's stain with buffer to cover the blood smear, leave to fix for 1 minute.
2. Add distilled water; allow dilute stain to act for 10 minutes.
3. Tip of the staining solution and rinse the slide with distilled water until the smear appear pink.
4. Blot dry with a filter paper.
5. Examine under microscope with **4x**, **10x**, and then **40x** to identify blood cells (RBCs and WBCs).



## Lab. 5

### Mitosis

The period of cell division, or **mitosis** (Gr. *mitos*, a thread), is the only cell cycle phase that can be routinely distinguished with the light microscope. During this process, the parent cell divides, and each of the daughter cells receives a chromosomal set identical to that of the parent cell. The period between mitoses is called **interphase**, during which the DNA is replicated, centrosomes and centrioles are duplicated, and the nucleus appears as it is most commonly seen in histological preparations. The process of mitosis is subdivided into four phases.

1. **Prophase**: during which:-

- a. The nucleolus disappears, and the replicated chromatin condenses into discrete rod-shaped bodies, **the chromosomes**, each consisting of duplicate sister chromatids closely associated longitudinally.
- b. Outside the nucleus, the centrosomes with their centrioles separate and migrate to opposite poles of the cell and organize the microtubules of the mitotic spindle.
- c. Late in prophase, the nuclear envelope breaks down when proteins of the nuclear lamina and inner membrane are phosphorylated.

2. **Metaphase** : during which :-

- a. The condensed chromosomes attach to microtubules of the mitotic spindle at large electron-dense protein complexes called **kinetochores**, which are located at a constricted region of each chromatid called the **centromere**.
- b. The chromosomes are moved to the equatorial plane of the now more spherical cell. Kinetochore microtubules bound to sister chromatids are continuous with centrosomes at opposite poles of the mitotic spindle.

3. **Anaphase** : during which:-

- a. The sister chromatids separate from each other and are slowly pulled at their kinetochores toward opposite spindle poles by kinesin motors moving along the microtubules.
- b. Also, dynamic changes in the lengths of the microtubules occur as the spindle poles move farther apart.

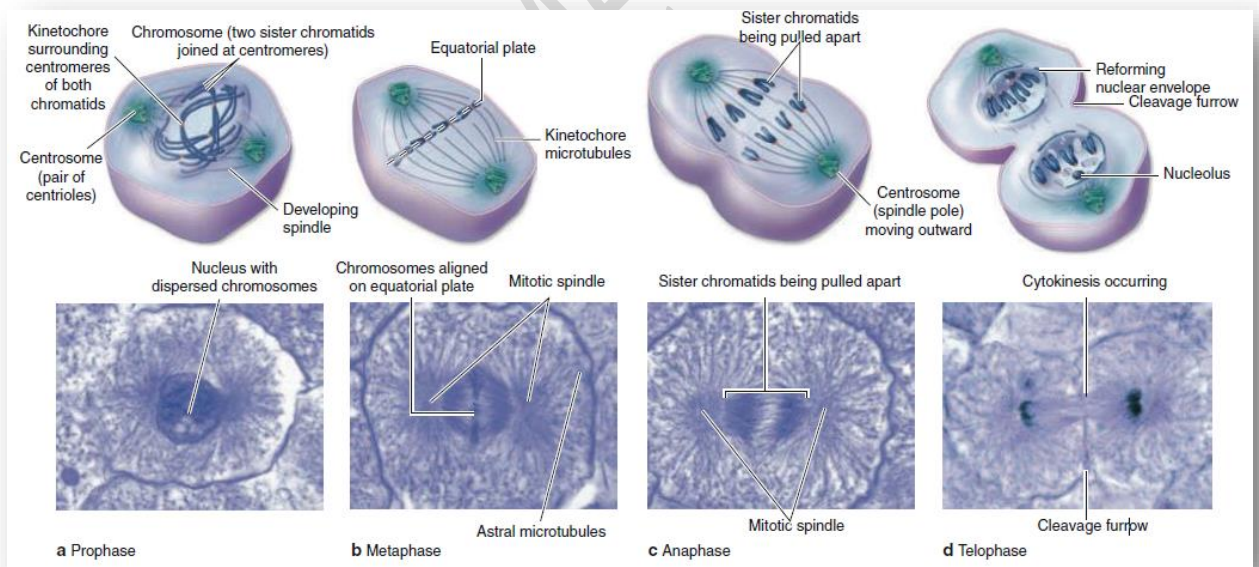
#### 4. **Telophase** : during which:-

- a. The two sets of chromosomes are at the spindle poles and begin reverting to their decondensed state (chromatin threads).
- b. Microtubules of the spindle depolymerize and disappear.
- c. The nuclear envelope begins to reassemble around each set of daughter chromosomes.
- d. Reappearance of the nucleolus.

#### Cytokinesis

A belt-like **contractile ring**, containing actin filaments associated with myosins, develops in the peripheral cytoplasm at the equator of the parent cell. Then constriction of this ring produces a **cleavage furrow** and progresses until the cytoplasm and its organelles are divided in two daughter cells, each with one nucleus.

Mitotic cells are often difficult to identify conclusively in sectioned adult organs but can be recognized in rapidly growing tissues by their condensed chromatin (dark nuclei).





## Lab. 6

### Epithelial tissue – Part 1

#### Simple Epithelium

##### 1. Simple Squamous Epithelium

Slide: artery x.s

Atlas: p.175 Fig. 8.1

- 🔍 At 4x & 10x identify the inner layer at the lumen of the artery.
- 🔍 At 40x see layer of squamous epithelial cells with dark elongated nuclei (black dots).

##### 2. Simple Cuboidal Epithelium

Slide: kidney tubules

Atlas: p.179 Fig.8.4

- 🔍 At 4x & 10x see the inner layer of the renal tubule.
- 🔍 At 40x see cells with rounded nuclei and no clear cell boundaries.

##### 3. E.g. 1: Simple Columnar Epithelium (stomach)

Slide: columnar epithelium

Atlas: p.33 Fig.2.4

- 🔍 At 4x & 10x identify and locate the edge at one side of the tissue.
- 🔍 At 40x see elongated cells with elongated oval nuclei located at base of the cells, with pink cytoplasm.

##### e.g. 2: Simple columnar epithelium with striated border

Slide: small intestine

Atlas: p.35 Fig.2.5

- 🔍 At 4x & 10x identify and locate irregular projections (villi) at one side of the tissue.
- 🔍 At 40x see elongated cells with elongated oval nuclei located at base of the cells, with pink cytoplasm and identify brush border (dark pink layer at the surface of the cells).

#### Pseudostratified Epithelium

##### 1. Ciliated pseudostratified columnar epithelium

Slide: trachea

Atlas: p.37 Fig.2.6

- 🔍 At 4x & 10x locate the pink side of the tissue (epithelium).
- 🔍 At 40x see epithelial cells with oval nuclei located at different levels, and with ciliated cells surface.

##### 2. Non-ciliated pseudostratified columnar epithelium

## Lab. 7

### Epithelial Tissue – part 2

#### Stratified epithelium

##### 1. A. stratified squamous keratinized epithelium

Slide: skin

Atlas: p.41 Fig. 2.10

- 🔍 At 4x & 10x look at the outer dark border of the tissue (epidermis)
- 🔍 At 40x see epithelium with several layers of cells and different shapes nuclei, at superficial layer you can see squamous cells with elongated flat nuclei, and on the epithelium there is a layer of keratin (dark wavy lines).

##### B. stratified squamous non-keratinized epithelium (esophagus)

Slide: stratified squamous epithelium

Atlas: p.39 Fig. 2.9

- 🔍 At 4x & 10x look at the outer dark border of the tissue.
- 🔍 At 40x see epithelium with several layers of cells and different shapes nuclei, at superficial layer you can see squamous cells with elongated flat nuclei.

##### 2. Stratified cuboidal epithelium

Slide: skin

Atlas: p.41 Fig. 2.11

- 🔍 At 4x locate small rounded structures (sweat glands) inside the skin, the dark pink ones are the ducts of the gland.
- 🔍 At 10x look at the wall of the duct.
- 🔍 At 40x see several layers of cells, and the superficial layer have cuboidal cells with rounded nuclei.

##### 3. Stratified columnar epithelium

##### 4. Transitional epithelium (relaxed urinary bladder)

Slide: transitional epithelium

Atlas: p.37 Fig. 2.7

- 🔍 At 4x look at pink side of tissue.
- 🔍 At 10x & 40x see several layers of cells with similar shapes (rounded) nuclei, the superficial layer have large dome-shaped cells with large rounded nuclei.

## Lab. 8


### Epithelium – part 3


#### Glandular epithelium

Classified according to the number of cells:-

##### 1. Unicellular gland

Slide: small intestine (goblet cells) Atlas: p.295 Fig.13.3 ; p.297 Fig.13.4

 At 4x & 10x locate the irregular projections(villi).


 At 40x see rounded empty cells with basal nuclei scattered between columnar epithelial cells.

##### 2. Multicellular gland

A. Classified according to morphology

###### 1. Simple straight tubular gland

Slide: small intestine Atlas: p.45 Fig. 2.12


 At 4x locate intestinal villi.

 At 10x see simple tubular glands lined with goblet cells and columnar cells with striated borders.

###### 2. Simple branched tubular gland

Slide: pyloric stomach Atlas: p.45 Fig. 2.13


 At 4x locate lining area of the stomach.

 At 10x see simple branched tubular gastric glands lined with columnar cells.

###### 3. Simple coiled tubular gland


Slide: skin (sweat gland)

 At 4X locate the area of the sweat glands.

 At 10x see light pink structures(secretory portion) and dark pink structures (duct portion).

###### 4. Simple branched alveolar(acinar) gland

Slide: human skin hair follicle Atlas: p.219 Fig.10.2 ; p.223 Fig.10.4


 At 4x look at the end of the hair follicle.

 At 10x & 40x see sebaceous gland(pale pink) filled with secretory cells and form number of alveoli near hair follicle.

###### 5. Compound tubular gland

Slide: duodenum

Atlas: p.293 Fig.13.1 p.295 Fig. 13.2


 At 4x look at the submucosa of duodenum

 At 10x & 40x see duodenal glands (Brunner glands) light staining mucous secreting tubule like secretory portion glands.

###### 6. Compound alveolar gland

Slide: mammary gland

Atlas: p.483 Fig. 19.24

 At 10x see the irregular dark pink structures (acini) at the center of the tissue.


## 7. Compound tubuloacinar gland


e.g : salivary glands.

### B. Classified according to the type of secretion

#### 1. Serous gland

Slide: parotid gland Atlas: p.253 Fig.11.13; p.259 Fig.11.16

 At 4x & 10x see the slide filled with several dark acini (serous).

 At 40x see serous acini composed of pyramidal cells arranged around a small lumen, & these cells have small, rounded nucleus located in basal zone of deeply basophilic cytoplasm.

#### 2. Mucous gland

Slide: small intestine


The e.g is goblet cells


#### 3. Mixed (seromucous) gland

Slide: submandibular gland

Atlas: p.255 Fig. 11.14; p.259 Fig.

11.17

 At 4x & 10x see the slide is mostly light stained.

 At 40x see mucous acini composed of columnar mucous cells arranged around a large lumen, and these cells oval or flattened nuclei at the base of the cell with pale, almost colorless staining cytoplasm.

See **serous demilune** which is a mucous alveoli surrounded by one or more groups of serous cells (in the form of crescent).

### C. Classified according to method of secretion


#### 1. Exocrine gland

e.g. : all the above glands

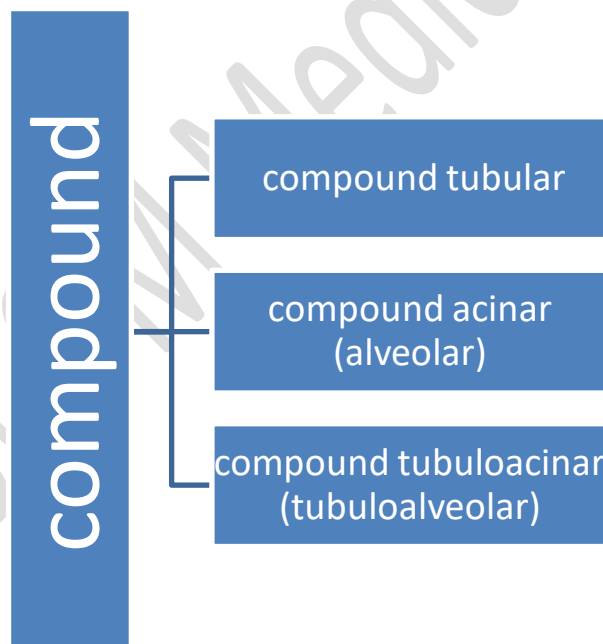
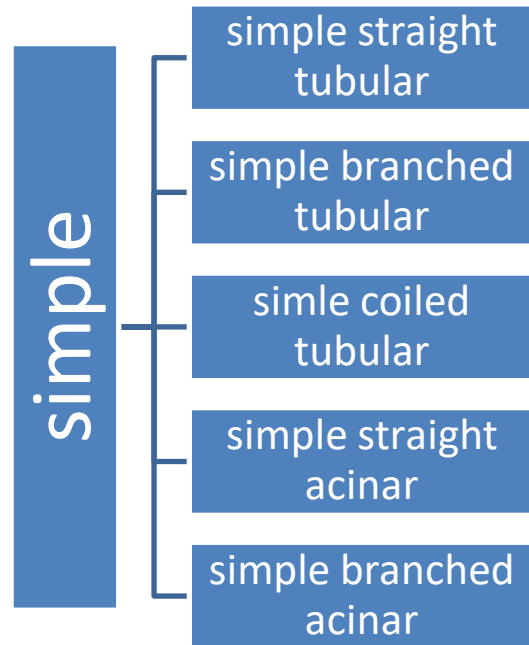
#### 2. Endocrine gland

Slide: thyroid gland

Atlas: p.397 Fig. 17.6

 At 10x see several rounded structures (follicles) filled with orange material (colloid).





**A diagram representing the classification of Glandular epithelial tissue. Note that Compound glands only classified according to the shape of the secretory part**

## Lab. 9

### Connective Tissue

#### Connective tissue cells

##### 1. Fibroblast & Fibrocyte

Slide: white fibrous tissue

Atlas: p.57 Fig.3.1

- 🔍 At 40x see the Fibroblast as elongated cell with some cytoplasmic projections, the nucleus is oval with sparse chromatin(light), and has one or two nucleoli. While Fibrocyte is smaller, without cytoplasmic projections, and have smaller, oval, dark nucleus.

##### 2. Macrophage

Slide: lymph node

Atlas: p.199 Fig. 9.4

- 🔍 At 10x look at the periphery of lymphatic nodules.
- 🔍 At 40x see the macrophage as rounded cells with irregular outline, have large nucleus with light acidophilic cytoplasm, and contain phagocytic inclusions.

##### 3. Mast cell

Slide: areolar tissue

Atlas: p.57 Fig. 3.1

- 🔍 At 40x see mast cell as oval cell with small, pale, centrally placed nucleus and cytoplasm filled with large, dark staining, closely packed granules.

##### 4. Plasma cell

Slide: small intestine

Atlas: p.59 Fig. 3.2

- 🔍 At 10x locate the submucosa (area beneath the villi).
- 🔍 At 40x see plasma cell as oval or rounded cell with small eccentrically placed nucleus that have clock face appearance (chromatin form peripheral clumps at the margin of the nucleus).

##### 5. Adipocyte

Slide: adipose tissue

Atlas: p.67 Fig. 3.11

- 🔍 At 40x see adipocyte as clear cell with narrow rim of cytoplasm and a small eccentrically placed nucleus. The large fat globule of the living cell has been dissolved during tissue preparation.

#### Connective tissue fibers

##### 1. Collagen fibers (tendon)

Slide: white fibrous tissue

Atlas: p.65 Fig. 3.8 & 3.9


- 🔍 At 4x & 10x see pink bundles of collagen fibers.
- 🔍 At 40x see collagen fibers as large, wavy fibers that stain pink and form thick bundles with elongated nuclei of fibroblasts.


## 2. Elastic fibers

Slide: aorta (human)

Atlas: p.179 Fig. 8.4

Stain: Orcein stain

 At 10x look inside wall of the aorta to locate irregular zigzag lines.

 At 40x see elastic fibers as thin, single, wavy fibers.

## 3. Reticular fibers


### Connective tissue types

#### A. Connective tissue proper

##### 1. Loose (areolar) connective tissue

Slide: areolar tissue


Atlas: p. 57 Fig. 3.1

 At 10x & 40x see areolar tissue with all types of connective tissue cells and fibers (arranged in irregular sparse way), with predominance of fibroblast and macrophages.

##### 2. Dense regular connective tissue

Slide: dense C.T (tendon)

Atlas: p.65 Fig. 3.9


 At 10x see collagen fibers arranged in compact, parallel bundles, and between them rows of fibroblasts.

##### 3. Dense irregular connective tissue

Slide: skin

Atlas: p.63 Fig. 3.6

 At 10x locate the tissue at the lower part of the dermis of skin.

 At 40x see collagen fibers as large thick bundles sectioned in different planes since they course in various directions. There are fibroblasts compressed among the fibers.


#### B. Specialized connective tissue

##### 1. Embryonic C.T.

###### a. Muroid C.T.


Slide: muroid tissue

Atlas: p.61 Fig.3.3

 At 4x & 10x see numerous fibroblasts with fine collagen fibrils course everywhere between them.

###### b. Mesenchymal C.T.


Slide: embryonic connective tissue

 At 4x & 10x see numerous undifferentiated cells, **mesenchymal cells**, characterized by oval nuclei with prominent nucleoli and fine chromatin. They possess many thin cytoplasmic processes and are immersed in an abundant and viscous extracellular substance containing few fibers.

##### 2. Adipose C.T.

Slide: adipose tissue

Atlas: p.67 Fig. 3.11

 At 4x & 10x see large collection of adipose cells (fat cells), which are close together, separated by small amount of C.T.

## Lab. 10

### Cartilage

#### 1. Hyaline cartilage

Slide: hyaline cartilage

Atlas: p.73 Fig.4.2; p.75 Fig.4.3, 4.4



At 4x & 10x see vertical bar of cartilage.



At 40x see numerous ovoid spaces termed lacunae which contain one chondrocyte or isogenous group of chondrocytes. The matrix usually has basophilic stain. See the perichondrium surrounding the cartilage.

#### 2. Elastic cartilage

Slide: elastic cartilage

Atlas: p.77 Fig. 4.5, 4.6

Stain: Orcein stain



At 4x & 10x see elastic fibers in the matrix (deep purple fibers). See large chondrocytes in lacunae, and the presence of perichondrium.

#### 3. Fibro-cartilage

Slide: fibro-cartilage

Atlas: p.77 Fig. 4.7



At 4x & 10x see the matrix is permeated with many small, parallel collagen fibers. See small chondrocytes in lacunae arranged in rows (not random arrangement or in isogenous groups). A perichondrium is absent.



## Lab. 11

### Bone

#### Bone Types

##### 1. Compact bone

Slide: Ground bone Atlas: p.93 Fig.4.17; p.95 Fig.4.19

- 🔍 At 4x & 10x see bone lamellae containing osteocytes in almond-shaped lacunae from which radiate minute canals, the canaliculi. See the external circumferential lamellae, internal circumferential lamellae, interstitial lamellae, and osteons (haversian system).
- 🔍 At 40x see haversian system consists of number of concentric lamellae surrounding a central canal (which in living state contains reticular C.T., blood vessels, and nerves).

##### 2. Spongy(cancellous) bone

Slide: developing bone Atlas: p.91 Fig.4.15, 4.16

- 🔍 At 4x & 10x see bony trabeculae, marrow cavities, and periosteum.
- 🔍 At 40x see lacunae with osteocytes inside the trabeculae, osteoblast as group of cells in linear arrangement at the margin of the trabeculae, osteoclast as large, individual, multinucleated cells at the margin of trabeculae.

##### 3. Decalcified bone

Slide: decalcified bone

- 🔍 At 10x & 40x see haversian canal, Volkmann canal, periosteum (adjacent to skeletal muscles), endosteum (adjacent to bone marrow).

#### Bone Development

##### 1. Intramembranous bone formation

Slide: membrane bone (developing) Atlas: p.89 Fig.4.13, 4.19

- 🔍 At 4x & 10x see periosteum, bone trabeculae, primitive marrow cavities.
- 🔍 At 40x see osteocytes, osteoblasts, and osteoclasts.

##### 2. Intracartilaginous (endochondral) bone formation

Slide: cartilage bone (developing) Atlas: p.81 Fig.4.8; p.83 Fig.4.9, 4.10

- 🔍 At 4x see cartilaginous part at upper part of tissue and developing bone in the middle and lower part of the tissue.
- 🔍 At 10x see in cartilaginous part the 5 zones of differentiation (zone of reserve cartilage, zone of proliferating cartilage, zone of hypertrophic cells and lacunae, zone of calcifying cartilage, zone of erosion and ossification). In the developing bone part see bone collar (periosteal bone) at the margin, bone spicules in the center, and periosteum (outer and inner layer).

## Lab. 12


### Bone Marrow


#### 1. Red bone marrow smear

Slide: red bone marrow smear, human

Stain: Giemsa stain

Atlas: p.111 Fig.5.12; p.113 Fig.5.13


 At 10x identify the slide.


 At 40x see :-

- a. **Proerythroblast**: large size; large round or oval nucleus occupies most of the cell with prominent nucleoli; thin rim of intense basophilic cytoplasm.
- b. **Normoblast**: small size; acidophilic cytoplasm; small round nucleus.
- c. **Reticulocyte**: small size, acidophilic cytoplasm with small round basophilic dot (remnant polyribosome).
- d. **Myeloblast**: large size; deeply basophilic cytoplasm (sometimes contain granules); large nucleus with 2-3 pale nucleoli.
- e. **Metamyelocyte (mature one called *band cell*)**: nucleus oval or band-shaped, cytoplasm with specific granules.
- f. **Megakaryoblast**: very large size; basophilic cytoplasm; very large, unevenly rounded nucleus.

#### 2. Bone marrow section


Slide: red bone marrow section Atlas: p.109 Fig. 5.11

 At 10x see connective tissue stroma, sinusoids, hemopoietic cells, and megakaryocytes.

 At 40x see features of **Megakaryocyte** as huge size cell with slightly acidophilic cytoplasm filled with fine granules; large, lobulated nucleus.

#### 3. Yellow bone marrow

Slide: white bone marrow

 At 10x see abundance of adipose cells with few hemopoietic cells.


## Lab. 13


### Blood

Slide: human blood smear

Stain: Giemsa stain

Atlas: p.101 Fig.5.1, 5.2 ; p.103 Fig.5.3, 5.4 ; p.105 Fig.5.5, 5.6, 5.7 ; p.107 Fig.5.8, 5.9 ; p.109 Fig.5.10

 At 10x identify the slide as blood smear.

 At 40x see :-

1. **Red blood cells** (RBC count in adult female is about 3.9-5.5 million/microliter, while in adult male it is about 4.1-6 million/microliter) as orange-pink, enucleated cells which are uniform in size.
2. **Leukocytes** (6000-10000/mm<sup>3</sup>):
  - A. Granulated leukocytes:
    1. **Neutrophils** (60-70%): have nucleus with 3-5 lobes, and cytoplasm with fine violet or pinkish granules.
    2. **Eosinophils** (2-4%): have bilobed nucleus, and cytoplasm filled with large, bright pink granules.
    3. **Basophils** (0.5-1%): have S-shaped nucleus, and cytoplasm with dark blue or brown granules that vary in size and obscure the nucleus.
  - B. Agranulated leukocytes:
    1. **Lymphocytes** (20-30%): are variable in size (small, medium, or large) with large, rounded, dark-staining nucleus that occupies a large portion of the cell. There is a narrow rim of basophilic cytoplasm.
    2. **Monocytes** (3-8%): are the largest of the leukocytes, the nucleus is light-staining, oval or horseshoe-shaped, and cytoplasm is lightly basophilic.
3. **Platelets** (150-400 ×10<sup>3</sup>/mm<sup>3</sup>): are small irregular masses of basophilic cytoplasm, and they tend to form clumps in smears.

## Lab. 14

### Muscular Tissue

#### 1. Skeletal muscles

Slide: striated muscle x.s, l.s

Atlas: p.119 Fig. 6.1

Stain: iron hematoxylin

At 4x & 10x see in t.s. muscle fascicles, perimysium, endomysium, muscle fibers, myofibrils, and nuclei of muscle fibers (located peripherally). While in l.s. see muscle fibers with cross striations.

At 40x see in l.s. cross striations, elongated or oval nuclei of muscle fibers at the periphery.

#### 2. Smooth muscles

Slide: smooth muscle l.s.

Atlas: p.131 Fig. 6.11, 6.12

At 10x & 40x see spindle shaped, individual muscle fibers. The nucleus is placed in the widest part of each fiber. The fibers are arranged so that the thick parts of fibers are adjacent to the thinner ends of others. No striations.

Slide: smooth muscle c.s.

Atlas: p.131 Fig. 6.11, 6.12

At 10x & 40x see sections of different diameters, the largest sections contain the nucleus.

#### 3. Cardiac muscles

Slide: cardiac muscle l.s.

Atlas: p.127 Fig. 6.8; p.129 Fig. 6.10

Stain: iron hematoxylin

At 10x & 40x see branching muscle fibers, cross striations (at regular intervals), **intercalated discs** (broader, occur at irregular intervals), Nucleus (centrally placed within the fibers).

Slide: cardiac muscle c.s.

Atlas: p.127 Fig. 6.8

Stain: iron hematoxylin

At 10x & 40x see sections of similar diameters, each section has centrally placed nucleus, a clear zone of perinuclear sarcoplasm (free of myofibrils).

## Lab. 15

### Nervous Tissue


#### A. Cells of nervous system

##### 1. Neuron (nerve cell)

Slide: spinal cord 4 levels human

Atlas: p.139 Fig. 7.1, 7.2; p.145 Fig. 7.5

 At 4x look at anterior horn of spinal cord.

 At 10x & 40x see neuron with large central nucleus (light staining) with a prominent nucleolus (dark staining), several cell processes (dendrites), and a single axon which arise from a clear area, the axon hillock. Neuroplasm contains basophilic substances (Nissl's bodies).

##### 2. Glial cells (supporting cells)

Slide: spinal cord 4 levels human

Atlas: p.139 Fig. 7.1, 7.2; p.145 Fig. 7.5


 At 10x look at anterior horn of spinal cord.


 At 40x see nuclei of neuroglial cells (oval or rounded, dark staining, small in size), cytoplasm is not stained.

#### B. Peripheral Nerve


Slide: nerve x.s. and l.s. Atlas: p.163 Fig.7.18; p.159 Fig.7.15

In L.S. :-

 At 10x see epineurium, perineurium, endoneurium, fasciculi of nerve fibers, Schwann cell nuclei.

 At 40x see axons (slender threads), neurolemma (Schwann's sheath), neurolemma nucleus (Schwann cell nucleus), node of Ranvier (gap between adjacent Schwann cells), and endoneurium.

In X.S. :-

 At 10x & 40x see bundles (fasciculi) of nerve fibers, perineurium, endoneurium, Schwann cell nucleus, neurokeratin network, and axons.