

ISHIK UNIVERSITY

FACULTY OF EDUCATION

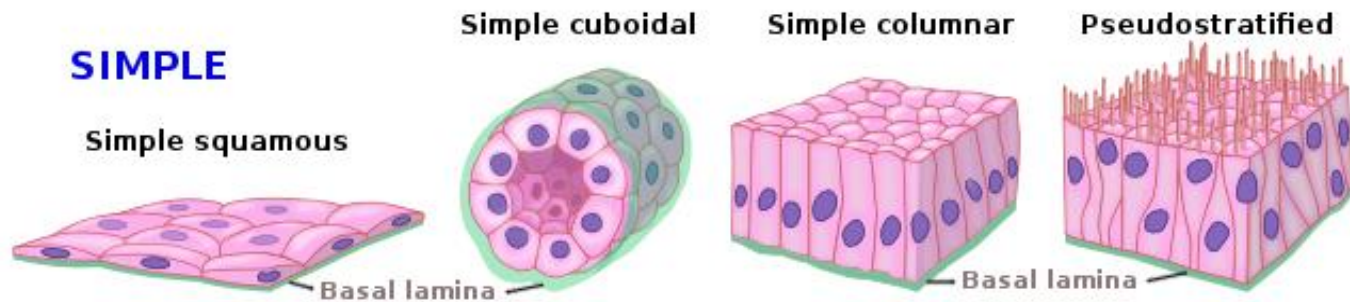
Department of BIOLOGY EDUCATION ISHIK UNIVERSITY

Lecture series in Histology for undergraduate students

PREPARED BY :

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analysis
 education organ cartilage
 exploration laboratory
 microscope disease scientific diagnostic
 cancer tissue biology cell sample
histology
 preparation section pattern micro
 research science
 medical art biopsy
 slide
 microscopy morphology animal
 shape



Dear students it is absolutely necessary to read these notes and do what it says if you would like to be a successful one while you are a student or in your future profession after graduation.

1- Students must arrive in the lecture hall **before few minutes of start**

2-Students **must read** the lecture before attending it .

3-Students must **try to answer** all the question marks inside the slides with other fellow students or alone.

4-Students must **participate** in the discussions during the lecture

Good Luck

Histology

Introduction:

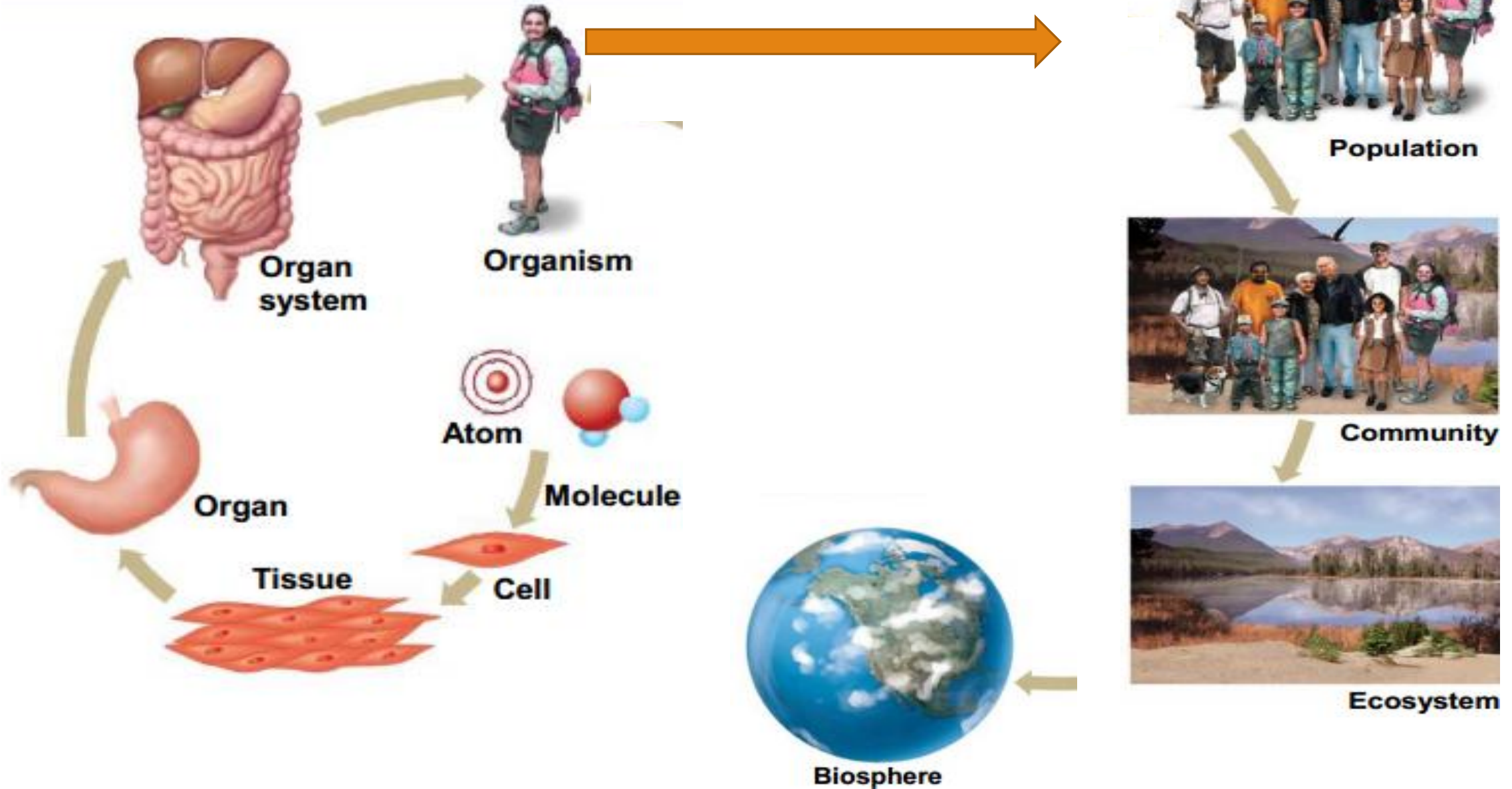
Histology is the science which deals with the study of cells and tissues of the body in relation to their **structure** and **function** using microscope.?

No cell can perform **all** functions necessary for life, therefore cells differentiate , group together into **tissues** performing **similar functions**.

Definition of tissues; they are groups of **similar cells and cell products** with a common function grouped together and form the body.

Despite its complexity, the human body is composed of only four basic types of tissues: **epithelial, connective , muscle. and nervous tissue.**

Levels of organization of living organisms (MAN)

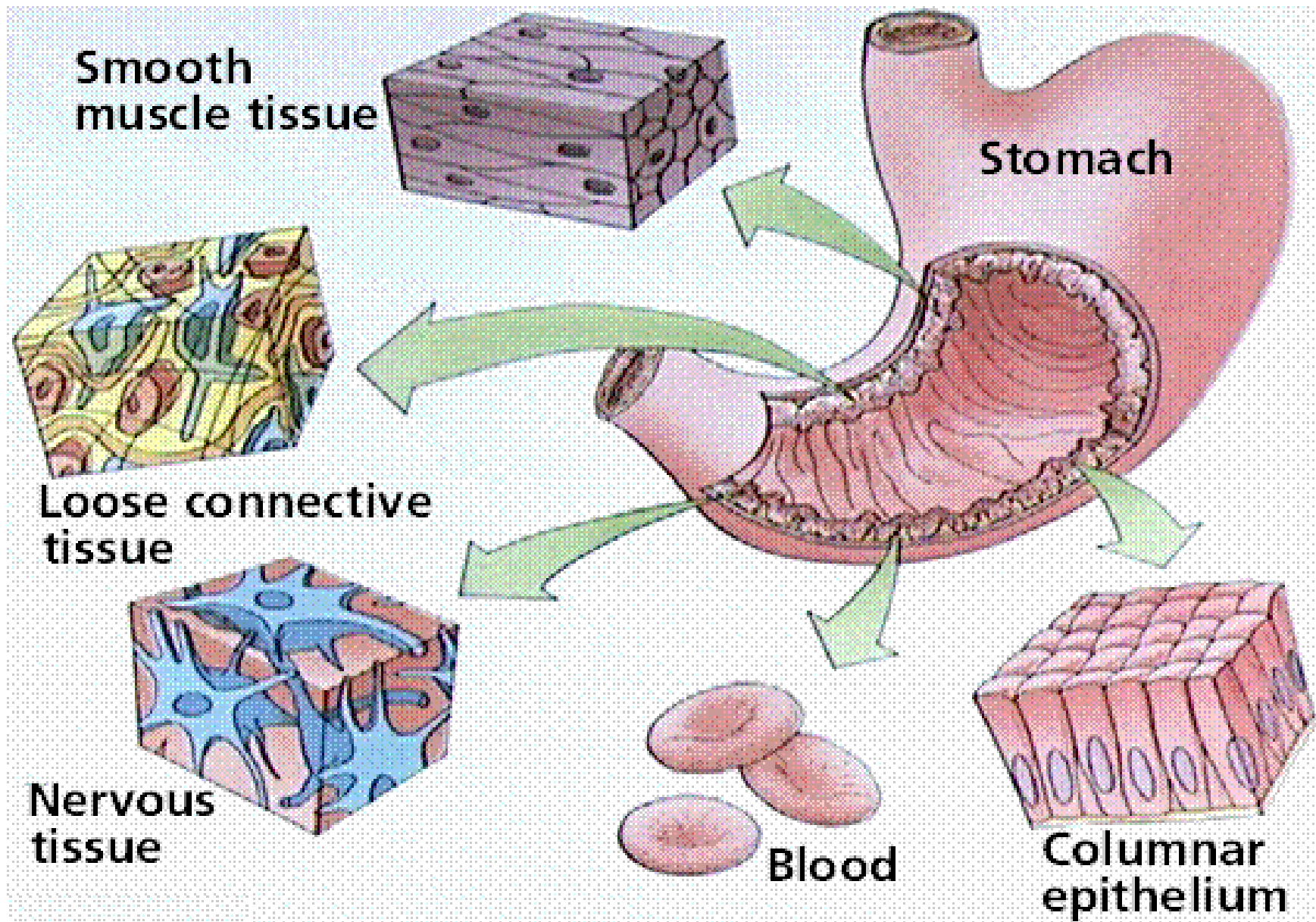


The importance of Histology:

1- Familiarity with histology helps students to know how cells and tissues develop complex organs and organ systems eventually the whole body.

2-Structure Reveals Function.

3-By examining tissues under microscope we can see any change in the tissues during disease (for better **diagnosis and treatment**).



Main Subjects included in this course :

- 1- The cell, its types ,functions and its structure and organelles .How cells form tissues ,organs , organ systems and organism (levels of organization).
- 2- **Histological techniques** : How tissues are prepared in the form of sections on glass slides for microscopic examination ?
- 3- **The four basic types of tissues** that make up the body (Epithelium. Connective, Muscle and Nervous Tissues),**their structure and location.**

Pt;1: Histological Techniques

Histology involves preparation of tissues for microscopical examination.

In order to study tissues with a microscope they must be preserved (fixed), embedded in paraffin wax , cut into very thin sections, mounted on glass slides and stained to be ready for microscopical examination, these steps are called **histological techniques**.

Summary of histological techniques: Embedding, cutting sections and mounting sections on slides



Fixation ➔ **Paraffin embedding**



Tissue blocks



Cutting sections using microtome



Mounting



Slide staining jars

Deparaffinized



Hot plate



Water bath -warm

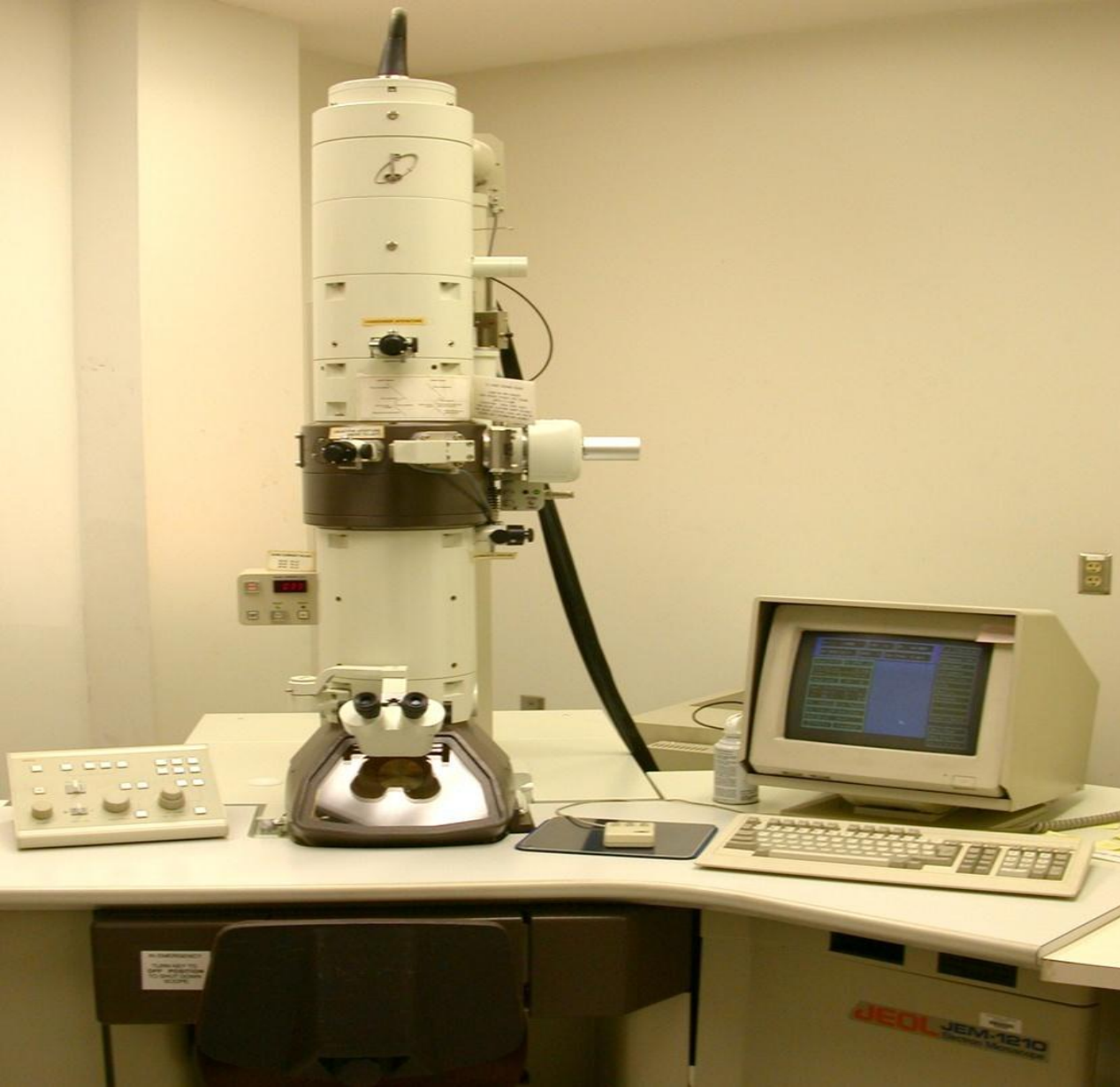
Histological Techniques:

Objectives:

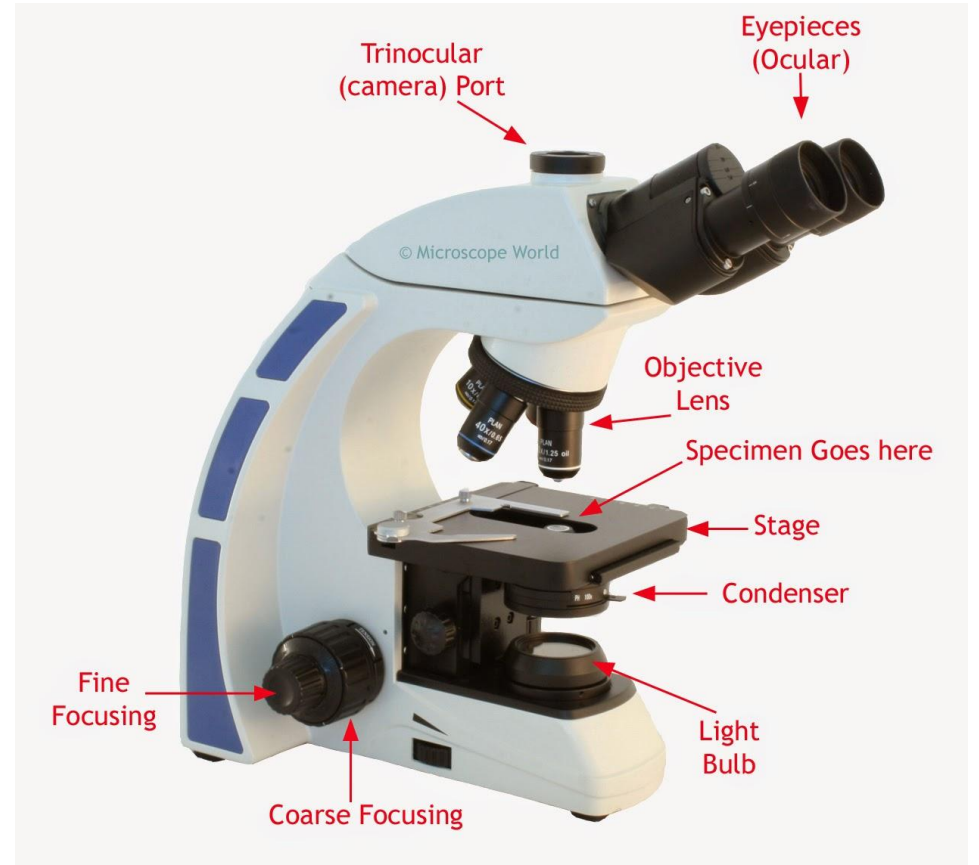
- 1- To be familiar with types of microscopes.
- 2- To be familiar as how to prepare tissues for microscopic examination including :Fixation, embedding, cutting thin sections, staining and mounting .

Types of Microscopes:

- 1-Light microscope
- 2- Electron microscope: Scanning / Transmission
- 3- Fluorescence microscope
- 4- Inverted microscope
- 5-Phase contrast microscope
- 6-Confocal microscope

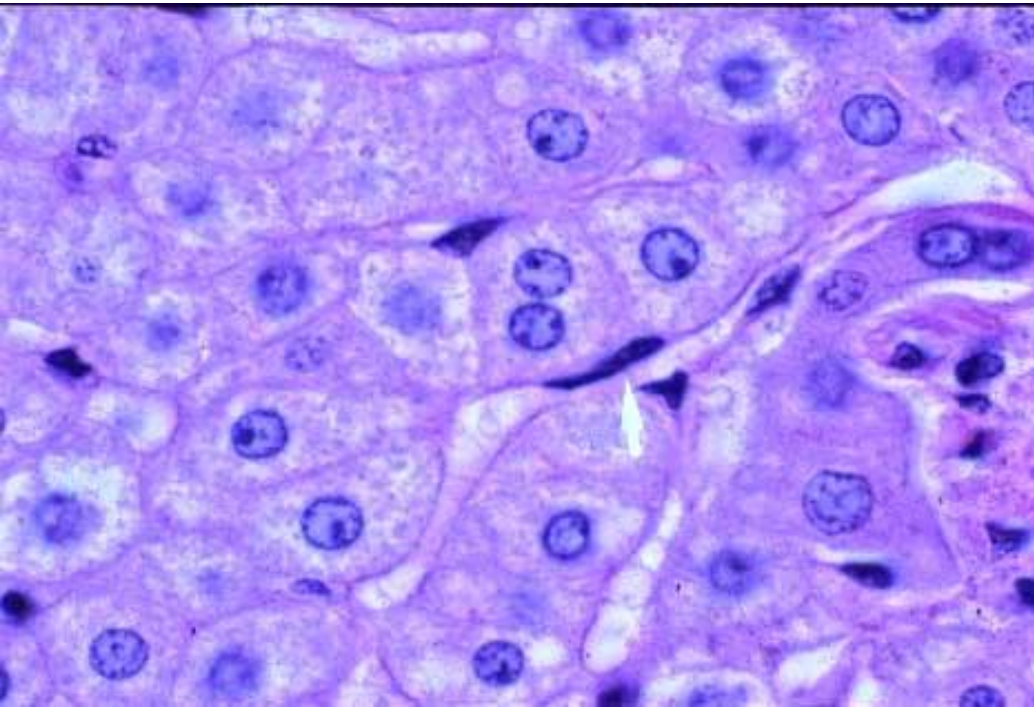
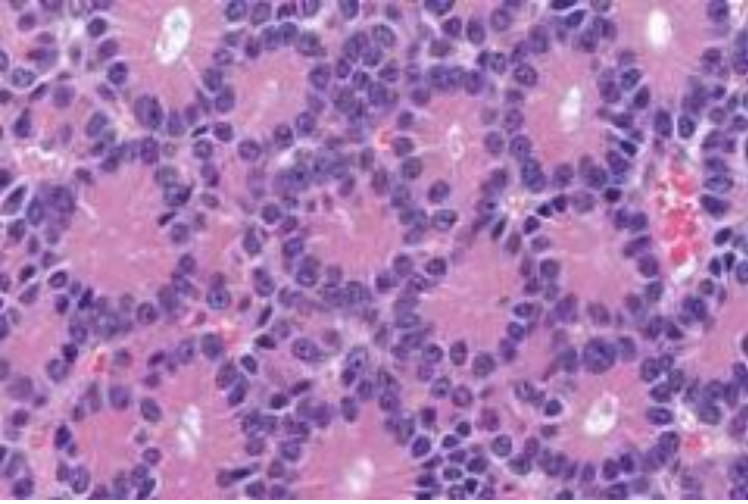


Electron microscope

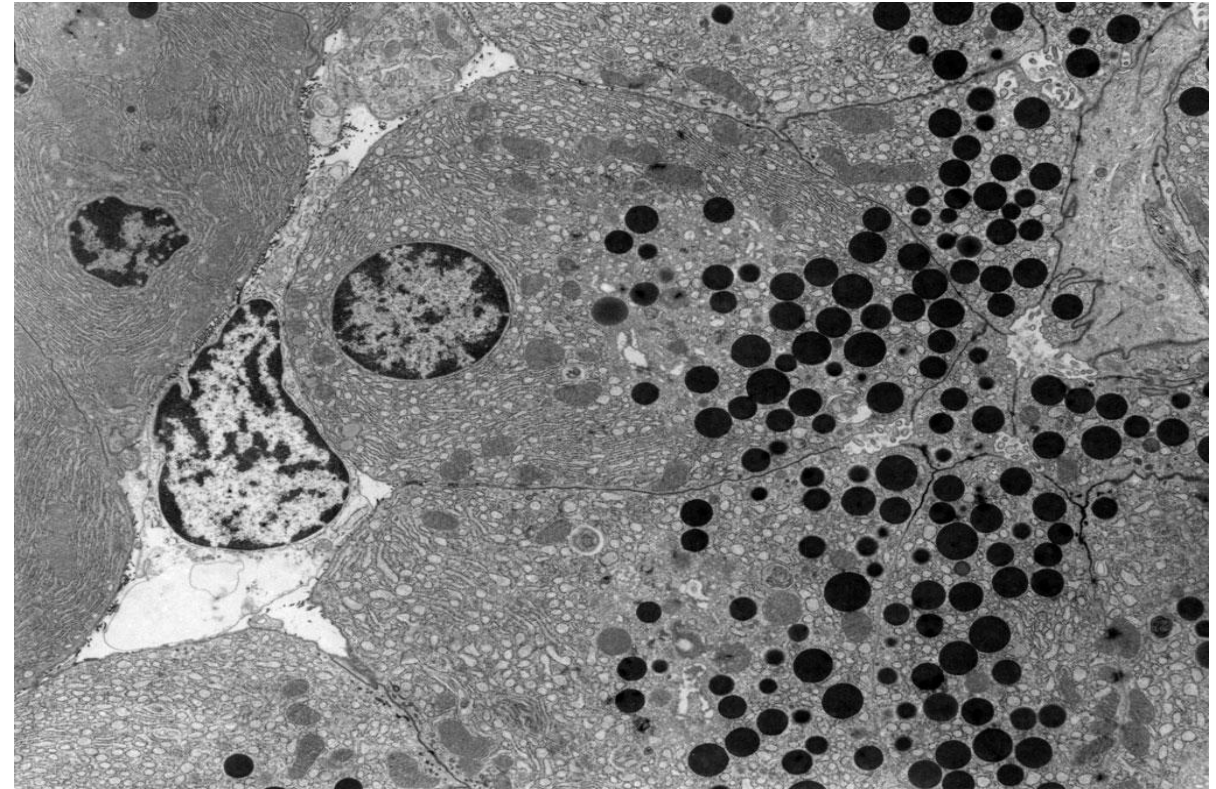


Light microscope

Sections from cuboidal epithelium



1-Light microscope image of simple cuboidal epithelium low & high powers



2- Electron microscope image of simple cuboidal epithelium

Tissue preparation for Microscope examination

1- Fixation

a-The purpose of fixation is to preserve tissues permanently in as life-like state as possible.

b-Fixation should be carried out as soon as possible after removal of the tissues to prevent autolysis.

c-There is no perfect fixative, though formaldehyde comes the closest.

Therefore, a variety of fixatives are available for use, depending on the type of tissue present and features to be demonstrated, most commonly used:(formol-saline & Bouin's).



Factors affecting fixation

- 1-The volume** of fixative is important. There should be a 10:1 ratio of fixative to tissue.
- 2-Increasing** the **temperature**, as with all chemical reactions, will increase the speed of fixation.
- 3-Concentration** of fixative should be adjusted down to the lowest level possible.
- 4-Time interval**: Also very important is time interval from removal of the tissues to the fixation.



2-Tissue Processing:

The technique of getting fixed tissue into paraffin then cutting sections followed by staining is called tissue processing. The main steps in this process are :

a-washing tissues from fixative then dehydration by series of alcohol

b-clearing.(using xylene to replace alcohol)

c- embedding in paraffin wax(making hard blocks)

d-cutting sections using microtome

e- mounting sections on glass slides

e- staining

g- coverslipping then examining by microscope



a. Dehydration: Gradual removal of water from the tissue using ascending grads of ethyl alcohol to prevent tissue shrinking.

b. Clearing: Replacement of alcohol in tissue by clearing fluid like **xylene**, benzene, or acetone.

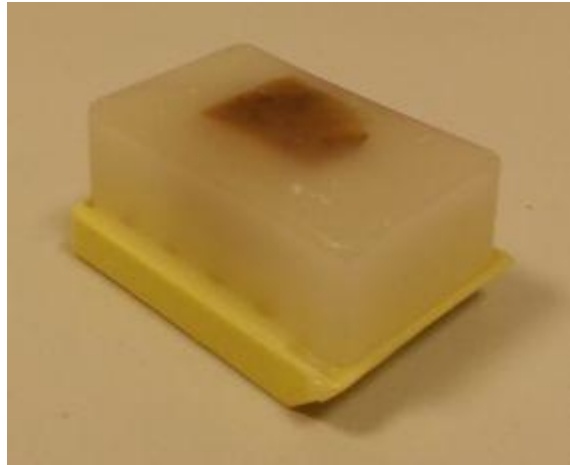
c. Embedding:

Tissues are impregnated in paraffin wax at what temperature ? .Make the blocks ready for sectioning.



d-Sectioning

- Once the tissues have been embedded, they must be cut into very thin sections (**4 to 6 microns**) that can be placed on a slide.
- This is done with **a microtome**. The important thing for proper sectioning is a **very sharp knife**.



e. Mounting:

Sections spread on the hot plate and mounted on glass slides



f- staining sections:

- The embedding process must be reversed in order to get the paraffin wax out of the tissue and allow water soluble dyes to penetrate the sections.
- Therefore, before any staining can be done, the slides are "**deparaffinized**" by running them through xylene then, to alcohols and lastly to water.
- **There are no stains that can be done on tissues containing paraffin.**



STAINING DISHES & RACKS



g-Coverslipping

The stained section on the slide must be covered with a thin piece glass to protect the tissue from being scratched, and to preserve the tissue section for years to come.



Frozen Sections

1. Frozen sections are performed with an instrument called **cryostat**.
2. The cryostat is just a **refrigerated** box containing a microtome.
3. The temperature inside the cryostat is about **-20 to -30 C**.
4. The tissue sections are cut and picked up on a glass slide.
5. The sections are dried and then stained.



Decalcification

EDTA can remove calcium safely, it works slowly, it penetrates tissue poorly, but it is expensive in large amounts.

Histological Techniques

Histology involves preparation of tissues for microscopical examination.

Basic methods of histological preparation of tissues include the following:

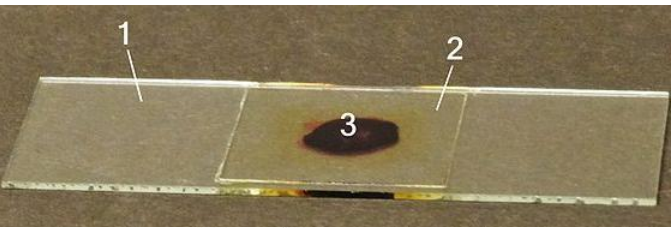
- a- Fix tissue example(using 4-10% formalin+ buffer).
- b- Dehydrate tissue using a series of alcohol
- c- Clear tissue from alcohol using xylene
- d-Embed tissue in hard medium such as wax.
- e- Section embedded tissue using a microtome
- f- Mount sections on glass slides
- g- Remove the embedding medium by immersing in xylene
- h- Stain tissue (e.g. hematoxylin – eosin)
- i- Examine tissue with microscope

Sectioning creates different “levels “ or planes of section (longitudinal, transverse or cross and oblique) according to the need..

Summary of histological techniques: Embedding, cutting sections and mounting sections on slides



Fixation → Paraffin embedding



Tissue blocks



Cutting sections using microtome



Mounting



Slide staining jars

Deparaffinized



Hot plate

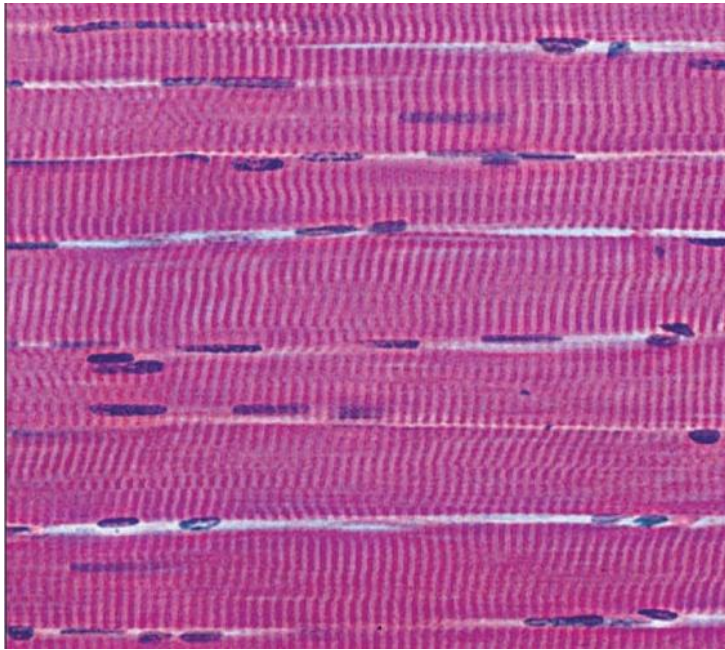


Water bath -warm

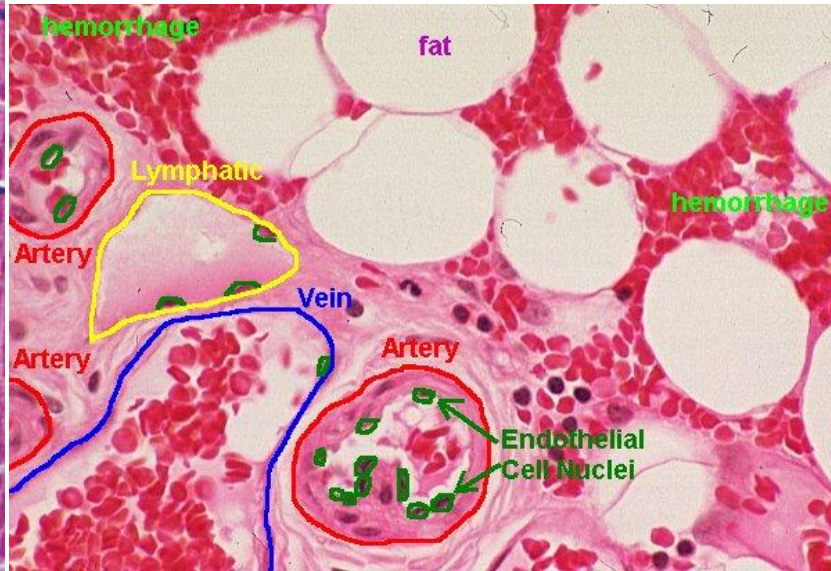
examples of some tissues



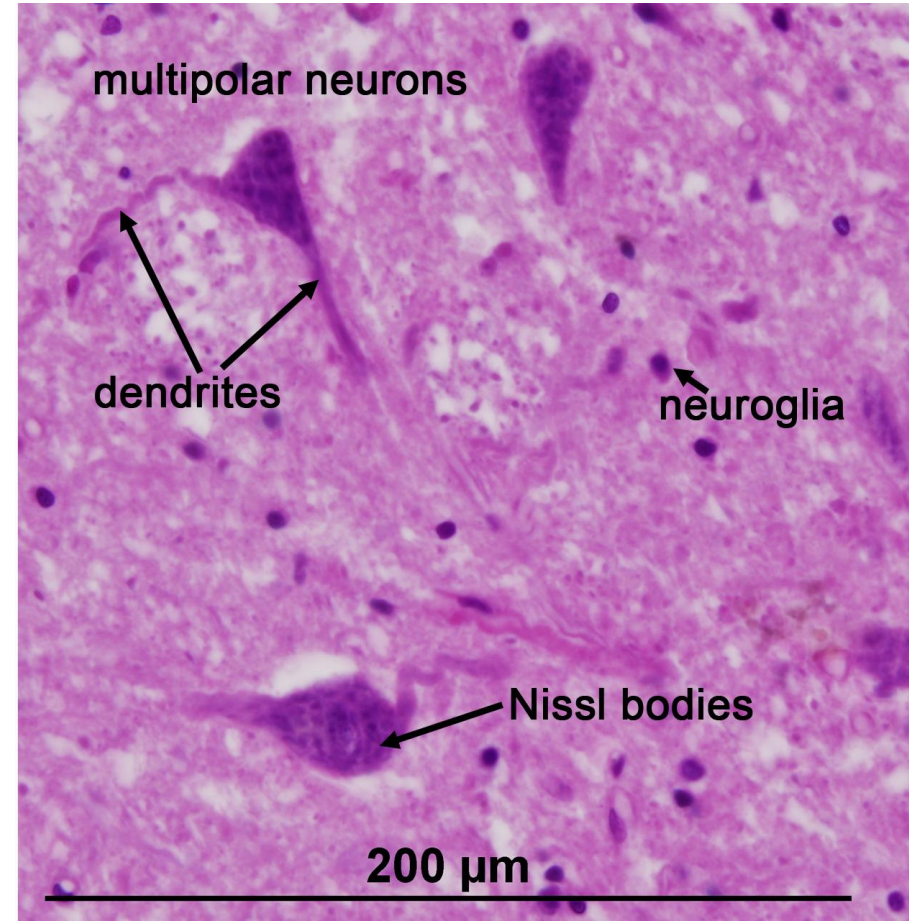
epithelium



skeletal muscle



connective tissue



nerve cells

THE END

References:

[Textbook of Histology](#) by Leslie Gartner pub: Elsevier 4th EDITION

[Wheater's Functional Histology](#) by Barbara Young Elsevier 6TH EDITION

Junqueira's Basic Histology: Text and Atlas, Thirteenth Edition 13th Edition LANG

[HISTOLOGY A TEXT AND ATLAS by Ross & Pawlina](#) / Lippincott Williams

and Wilkins

Fifth edition.

[Histology Books, Ebooks & Journals | US Elsevier Health](#)

[*www.us.elsevierhealth.com/medicine/histology*](http://www.us.elsevierhealth.com/medicine/histology)

[Normal histology, with special reference to the ... - Internet Archive](#)

[*https://archive.org/details/normalhistologyw00pier*](https://archive.org/details/normalhistologyw00pier). Internet Archive