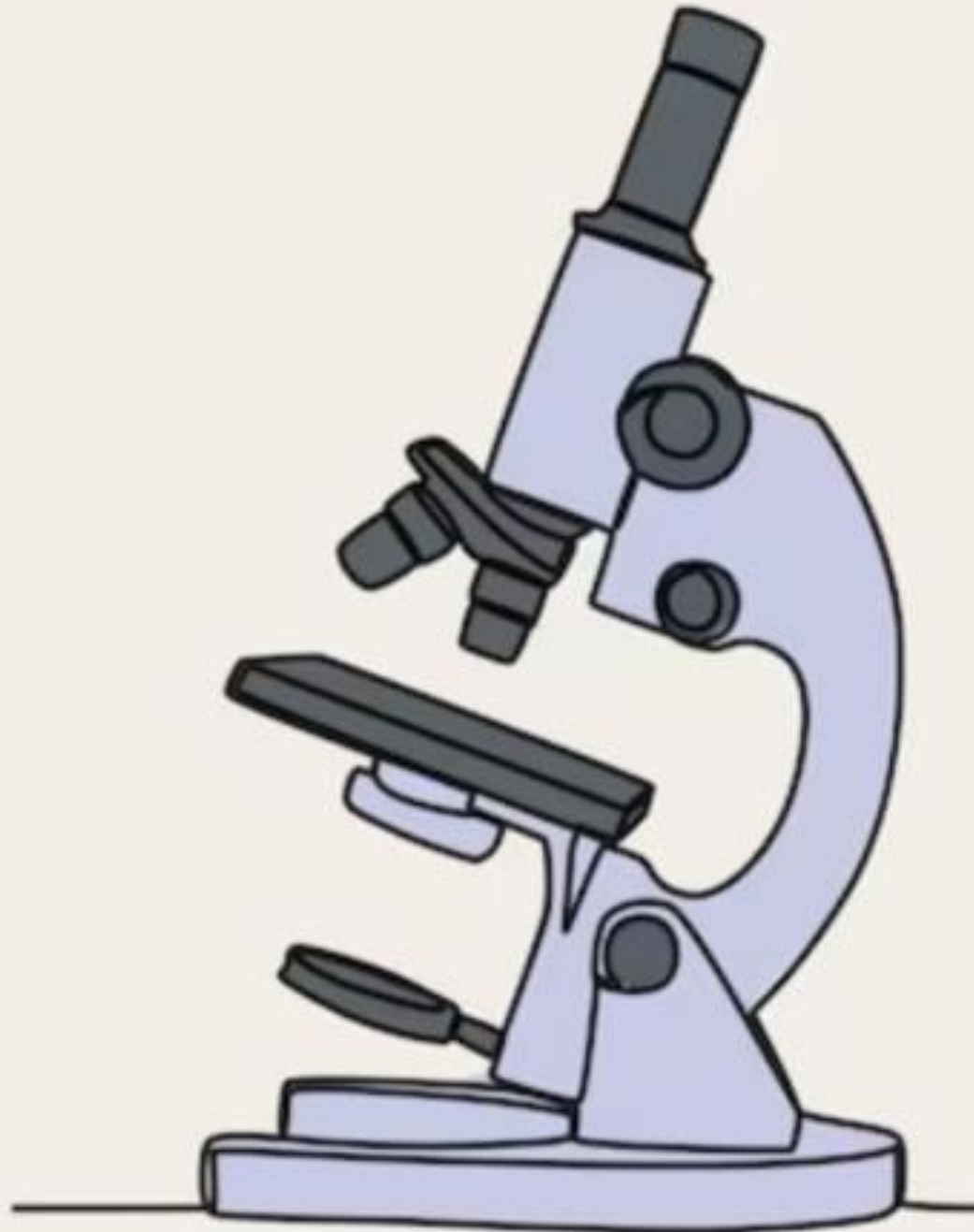


Histology

Modified n. 1

Writer: Raya Abu limoon
& Retaj

Corrector: Hind Suhwail



Human Histology

REFERENCE: JUNQUEIRA'S BASIC HISTOLOGY,
TEXT AND ATLAS, 15TH EDITION, BY ANTHONY L.
MESCHER, CHAPTER 1.

TOPICS TO BE COVERED

1. OVERVIEW

2. EPITHELIUM

3. CONNECTIVE TISSUE الأنسجة الضامة

4. CARTILAGE

5. BONE

6. MUSCULAR TISSUE الأنسجة العضلية

7. NERVOUS TISSUE الأنسجة العصبية

HISTOLOGY

- MICROSCOPIC ANATOMY!
- **HISTO= WEB OR TISSUE**
- LOGOS= STUDY
- THE STUDY OF CELLS AND THE **EXTRACELLULAR** MATRIX

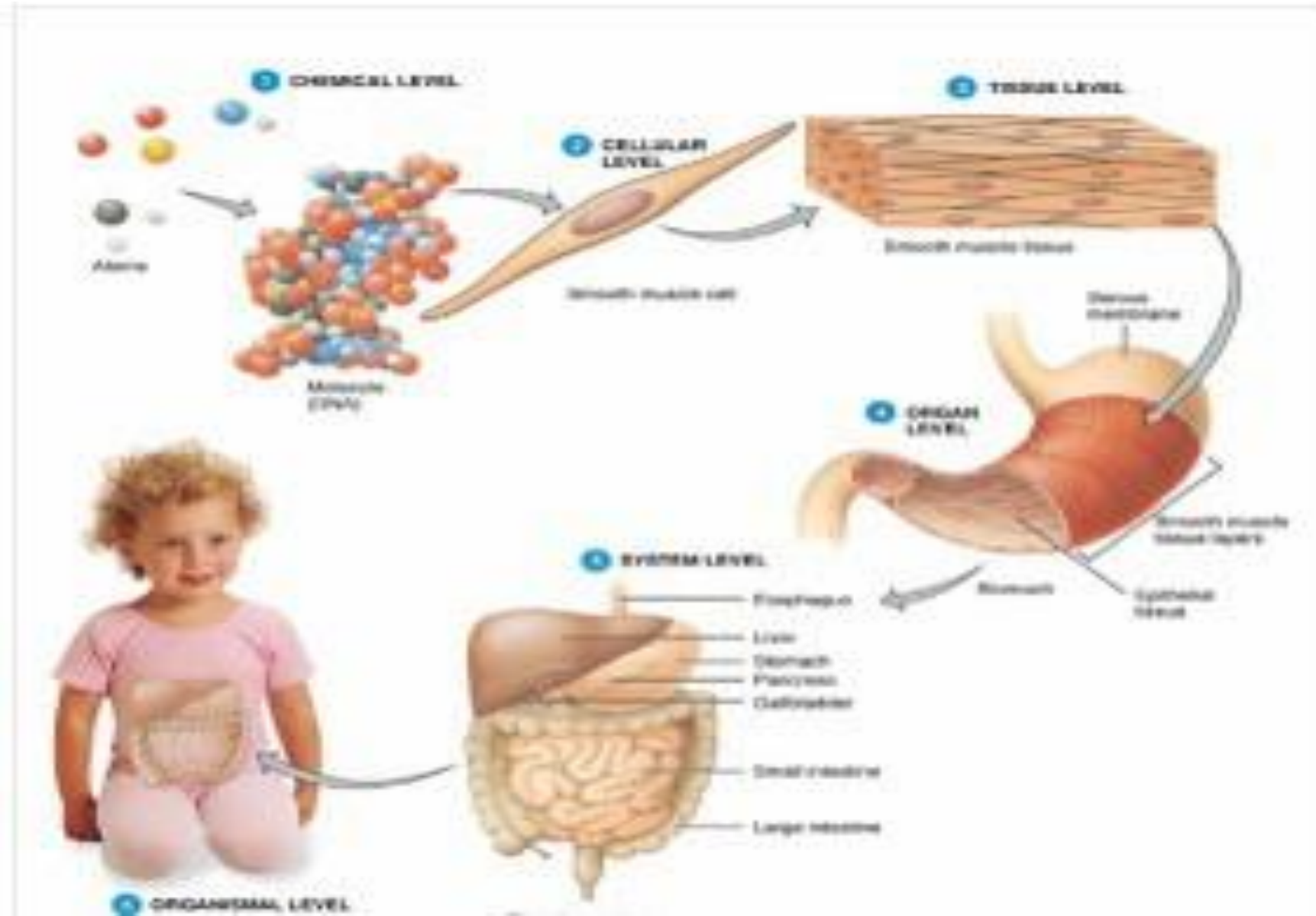
Can contain material :fibers , electrolytes .fluids, proteins....

HISTOLOGY

Histology is the study of the tissues of the body and how these tissues are arranged to constitute organs.

- Tissue is composed of cells and ecm (extracellular matrix)

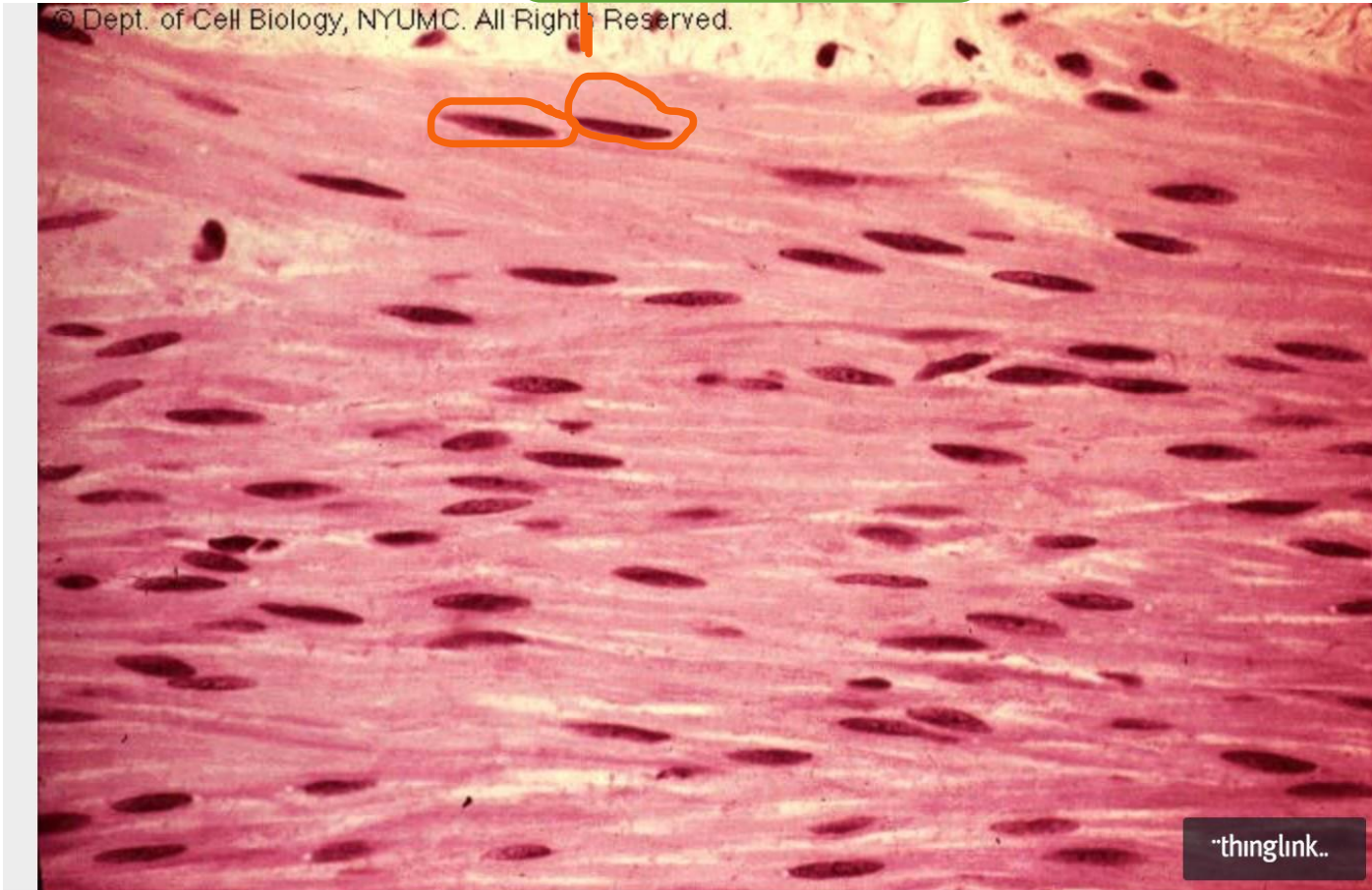
LEVEL OF ORGANIZATION



Just
Take
General
idea

HOW DO WE GET THIS IMAGE?

Nucleus



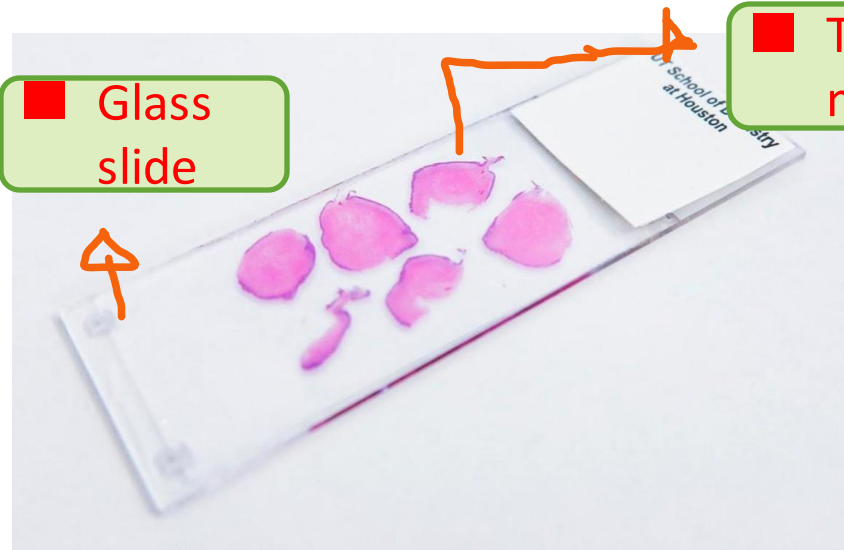
*this is a colored tissue
(histological section)

*you cannot see clear borders for the
cells in light microscope, but you can
know their number based on the
number of nuclei

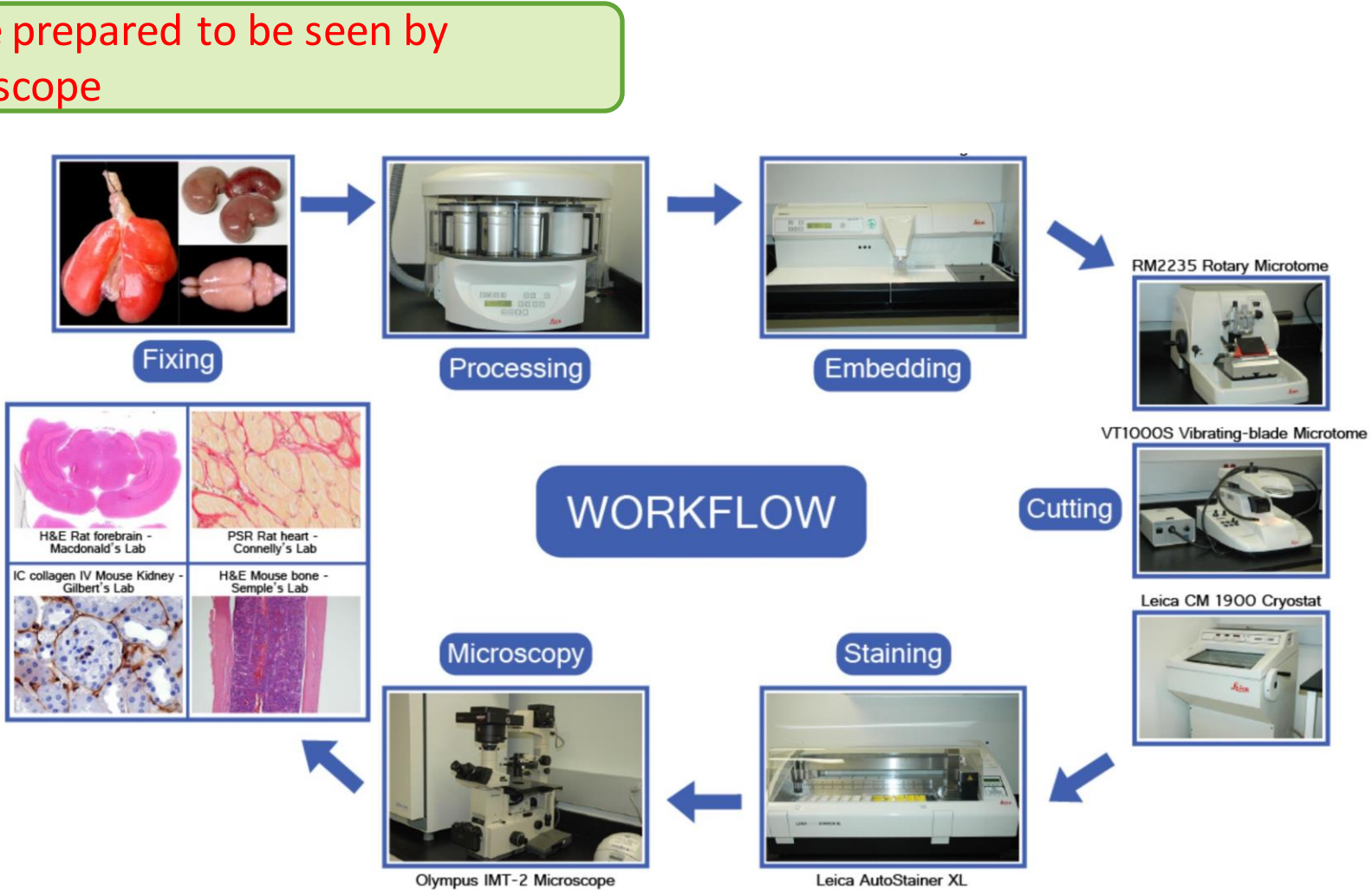
*this is a smooth muscle tissue (you
will know how to distinguish by
experience)

*to visualize something in
particular we need to add special
stains

Tissue Processing For Histology



<https://www.youtube.com/watch?v=4DJm4NLECQs>



Tissue Preparation For Light Microscopy

- **Fixation:** small pieces of tissue are placed in solutions of chemicals that cross-link proteins and inactivate degradative enzymes, which preserves cell and tissue structure.(

■ because tissue will degrade after several hours without fixation in room temperature)
(e.g. formaldehyde)

- **Dehydration:** the tissue is transferred through a series of increasingly concentrated alcohol solutions, ending in 100%, which removes all water.
- **Clearing:** alcohol is removed in organic solvents in which both alcohol and paraffin are miscible.

THIS SLIDE WAS MISSED

Tissue Preparation For Light Microscopy

Infiltration: the tissue is then placed in melted paraffin until it becomes completely infiltrated with this substance. •

Embedding: the paraffin-infiltrated tissue is placed in a small mold with melted paraffin and allowed to harden.

Trimming: the resulting paraffin block is trimmed to expose the tissue for sectioning (slicing) on a microtome.

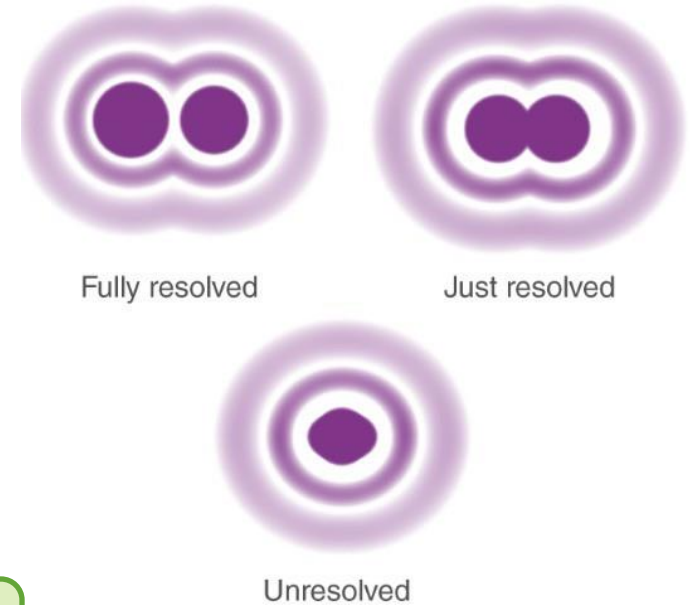
MICROSCOPES

- LIGHT MICROSCOPE >> *its types* (OTHERS: CONFOCAL, FLUORESCENCE, PHASE-CONTRAST) INTERACTION OF LIGHT WITH TISSUE. RESOLUTION AROUND 3 μ M.

■ It uses light. The bigger the lense ..the more details , the result images can be coloured

- ELECTRON MICROSCOPE. *it uses electrons* . INTERACTION OF TISSUE COMPONENTS WITH BEAMS OF ELECTRONS. RESOLUTION AROUND 3

NM ■ the result images are all in black, gray and white.



TYPES OF MICROSCOPE

- **Light microscope.**

1. Bright-field microscopy
2. Fluorescence microscopy
3. Phase-contrast microscopy
4. Confocal microscopy
5. Polarizing microscopy

- **Electron microscope**

- 1 . Transmission electron microscopy

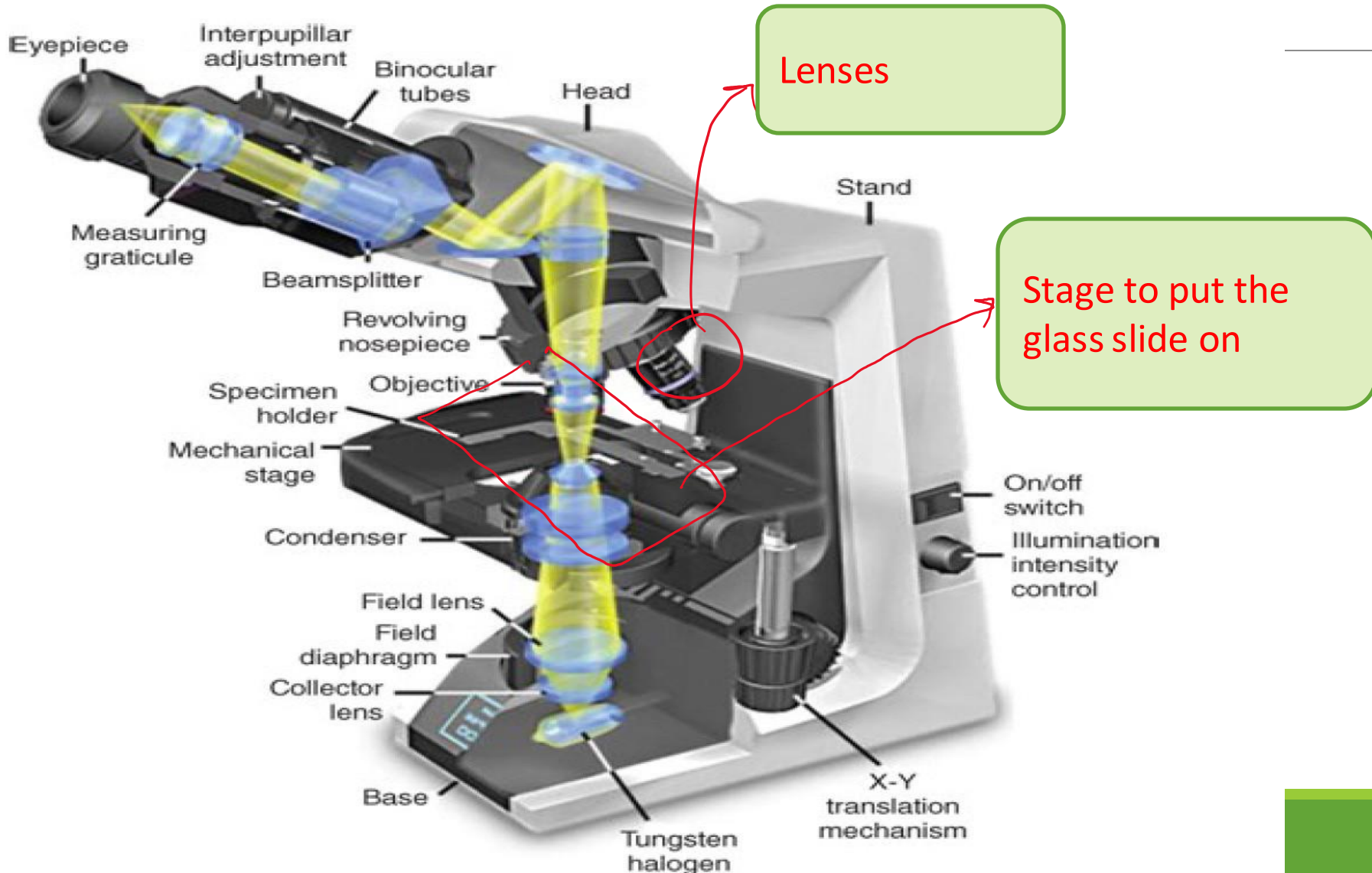
■ (gives 2D images) transmission means that the electron interacts with the tissue, some of them (electrons) are reflected and the others are absorbed

- 2 . Scanning electron microscopy

■ (gives 3D images)

LIGHT MICROSCOPE (BRIGHT-FIELD)

Components and light path of a bright-field microscope.



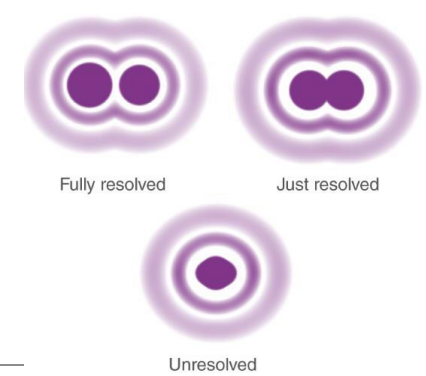
BRIGHT-FIELD LIGHT MICROSCOPE

- Stained tissue is examined with ordinary light passing through the preparation.
- Includes an optical system and mechanisms to move and focus the specimen.
- The **condenser** collects and focuses a cone of light that illuminates the tissue slide on the stage.
- **Objective** lenses enlarge and project the illuminated image of the object toward the eyepiece, x4, x10
- The two **eyepieces** or oculars magnify this image another x10 and project it to the viewer, yielding a total magnification of x40, x100, or x400.

After multiplying them

RESOLUTION

The resolution gives the scientists new way to study the tissue



- **Resolving power:** the smallest distance between two structures at which they can be seen as separate objects.

The smaller the distance between the two things, the higher the Lense you need

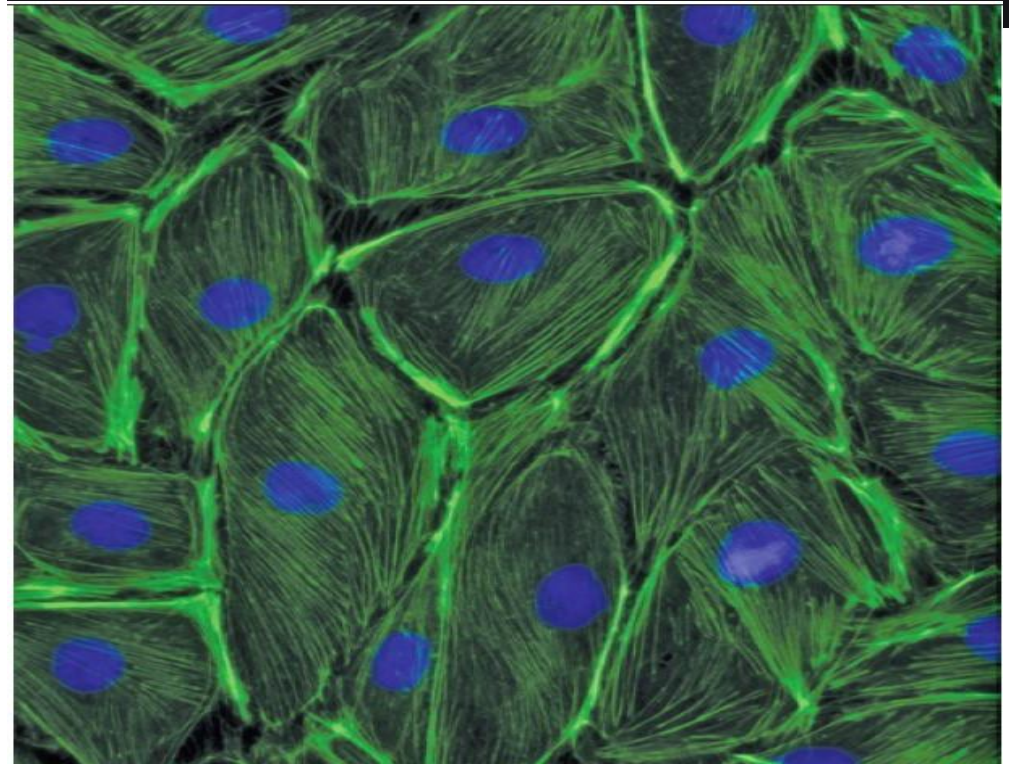
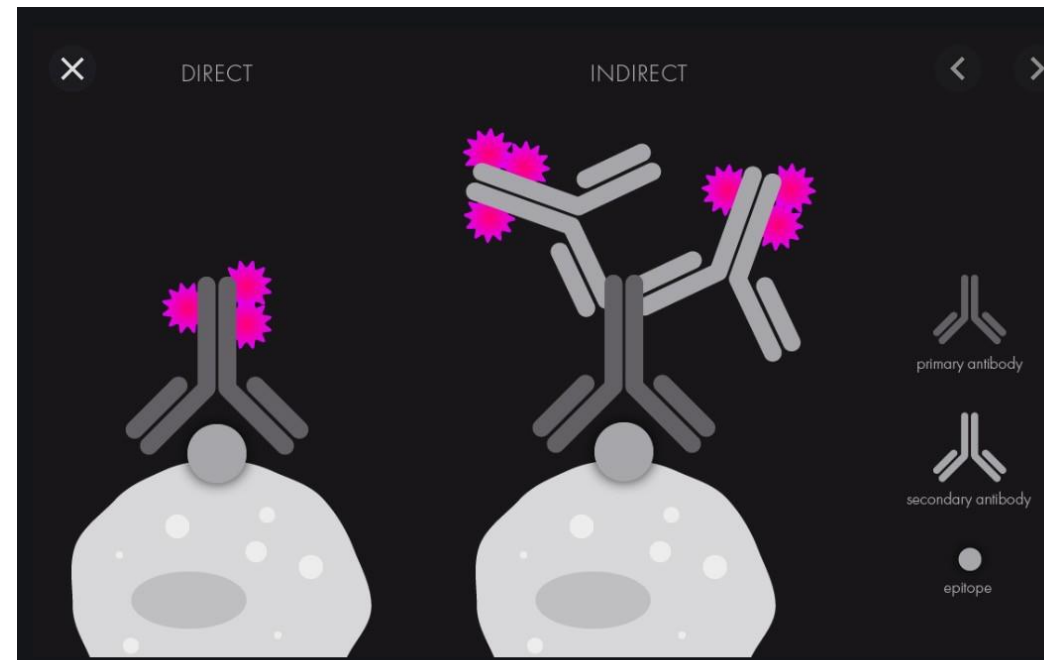
- The maximal resolving power of the light microscope is approximately $0.2 \mu\text{m}$ --- can permit clear images magnified 1000-1500 times. **Not the best**

- Objects smaller or thinner than $0.2 \mu\text{m}$ (such as a single ribosome or cytoplasmic microfilament) cannot be distinguished.
- The microscope's resolving power determines the quality of the image, its clarity and richness of detail, and depends mainly on the quality of its objective lens.
- **Magnification** is of value only when accompanied by high resolution

FLUORESCENCE MICROSCOPY

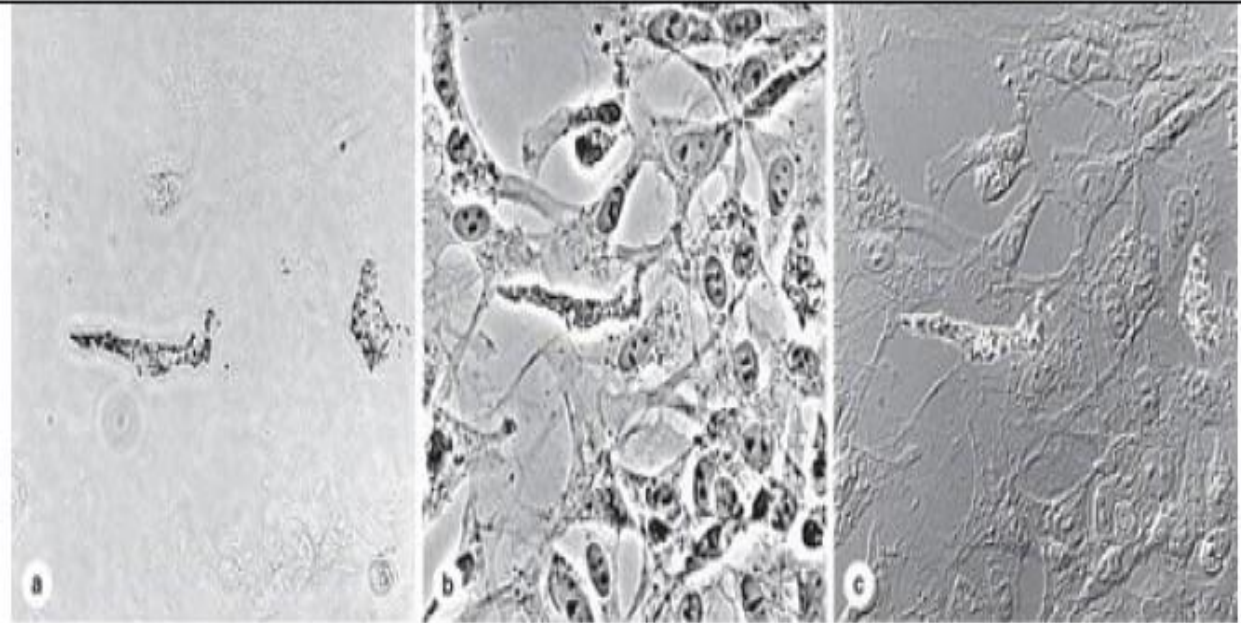
Uses specific stain to recognize things

- **Fluorescence**: when certain cellular substances are irradiated by light of a proper wavelength, they emit light with a longer wavelength.
- In fluorescence microscopy, tissue sections are 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. For fluorescent microscopy the instrument
- Has a source of UV or other light and filters that select rays of different wavelengths emitted by the substances to be visualized.



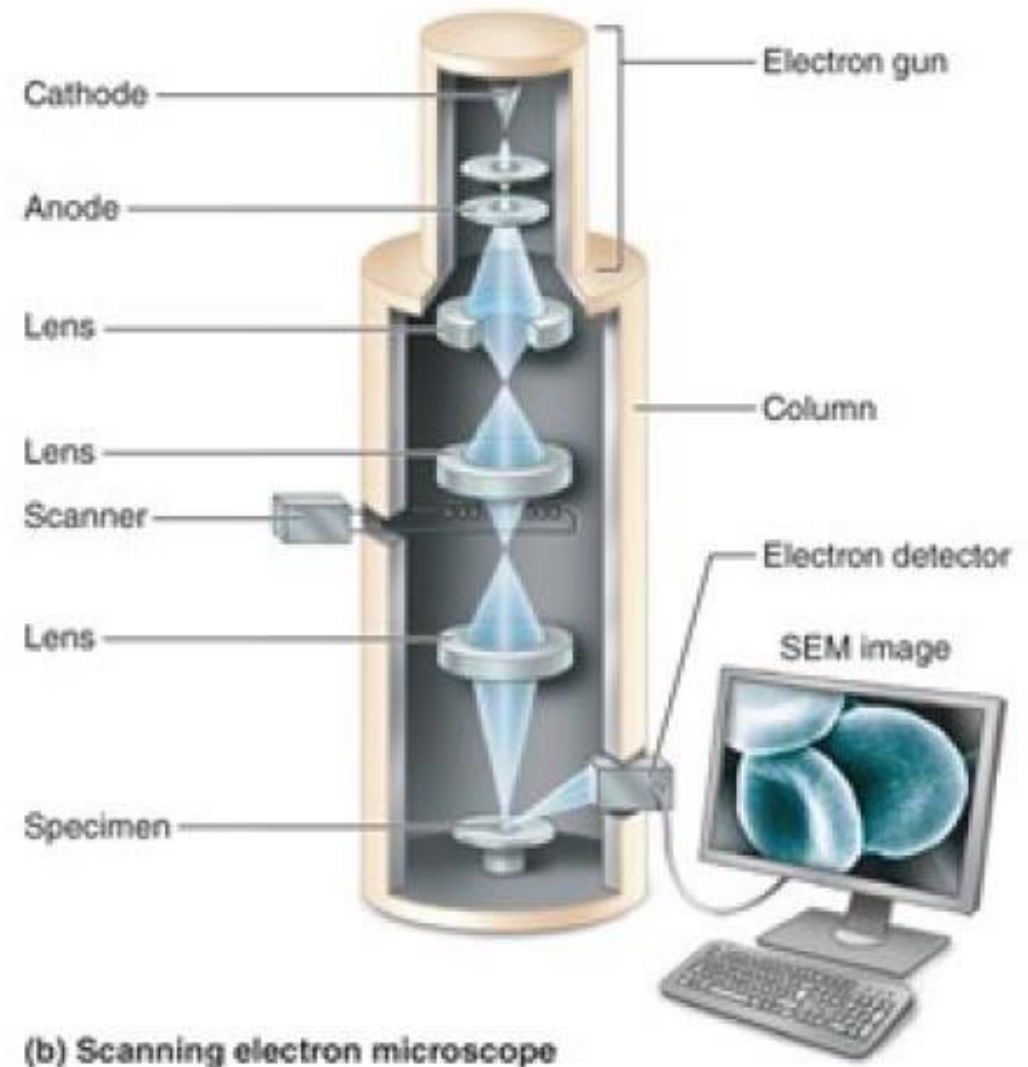
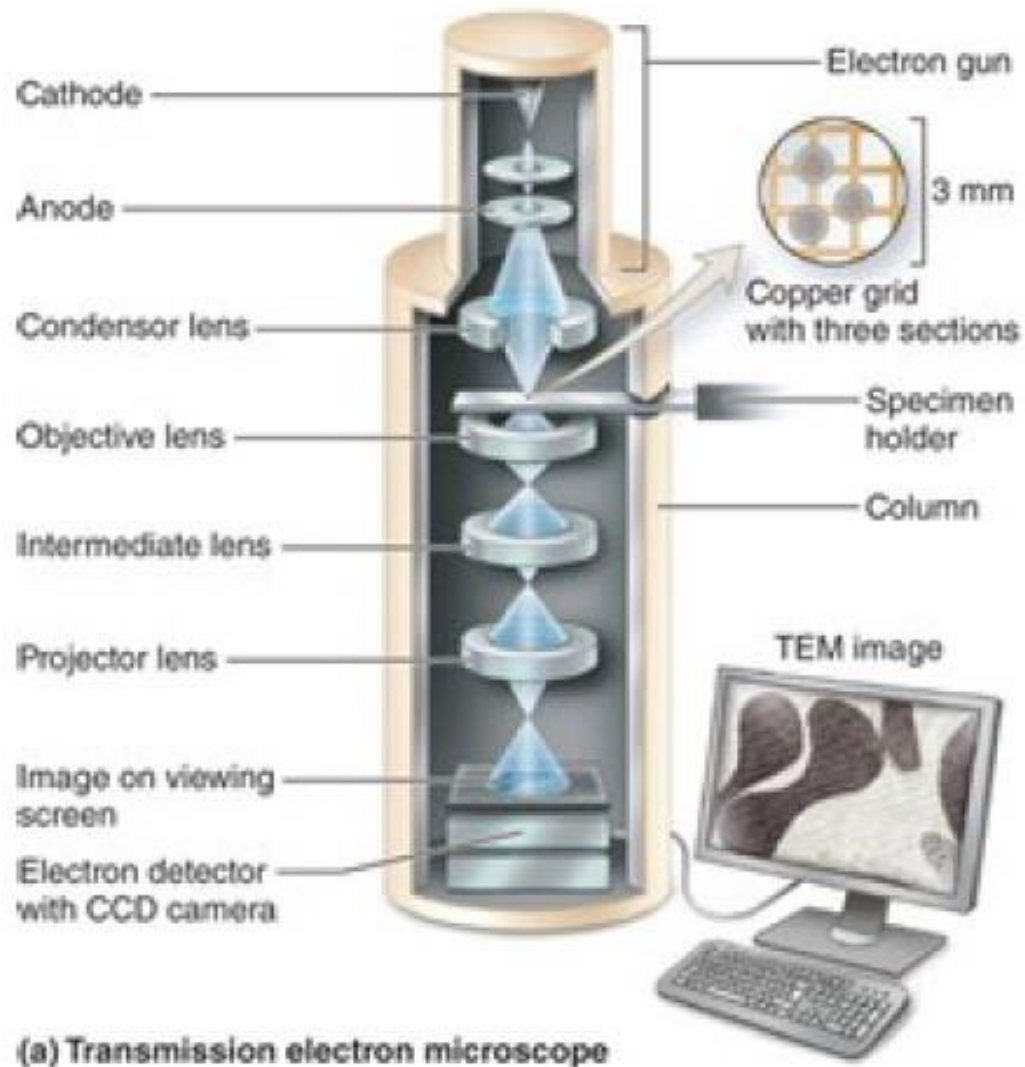
Phase-contrast Microscopy

- Study unstained cells and tissue sections (colorless; similar optical densities).
- Uses a lens system that produces visible images from transparent objects and can be used with living, cultured cells.



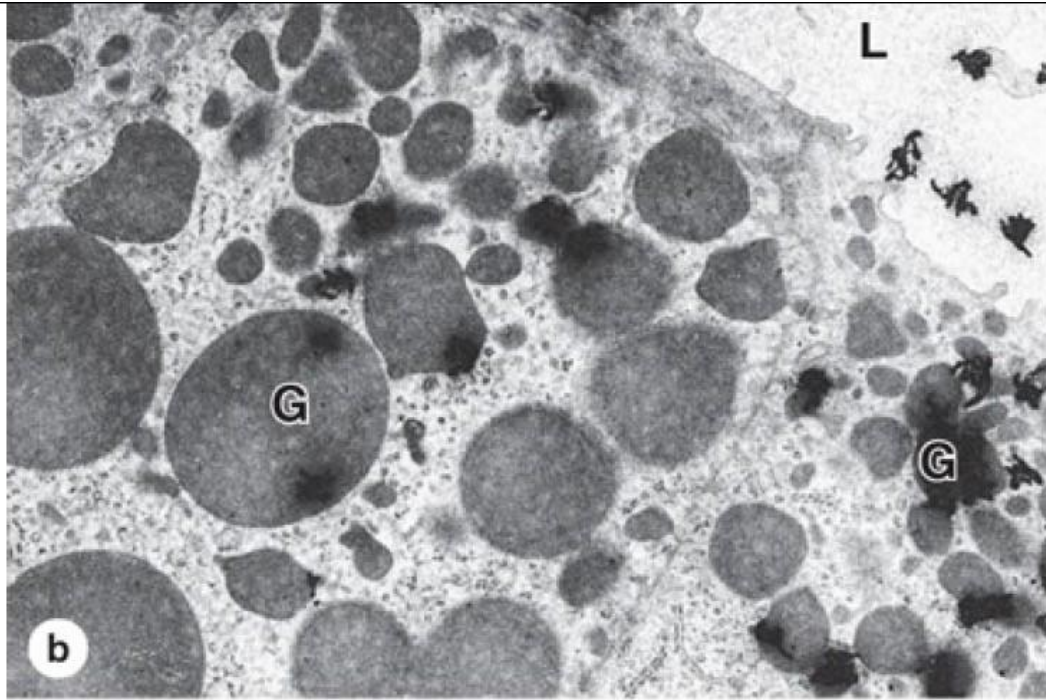
- Is based on the principle that light changes its speed when passing through cellular and extracellular structures with different refractive indices--- appear lighter or darker in relation to each other.

Electron Microscope



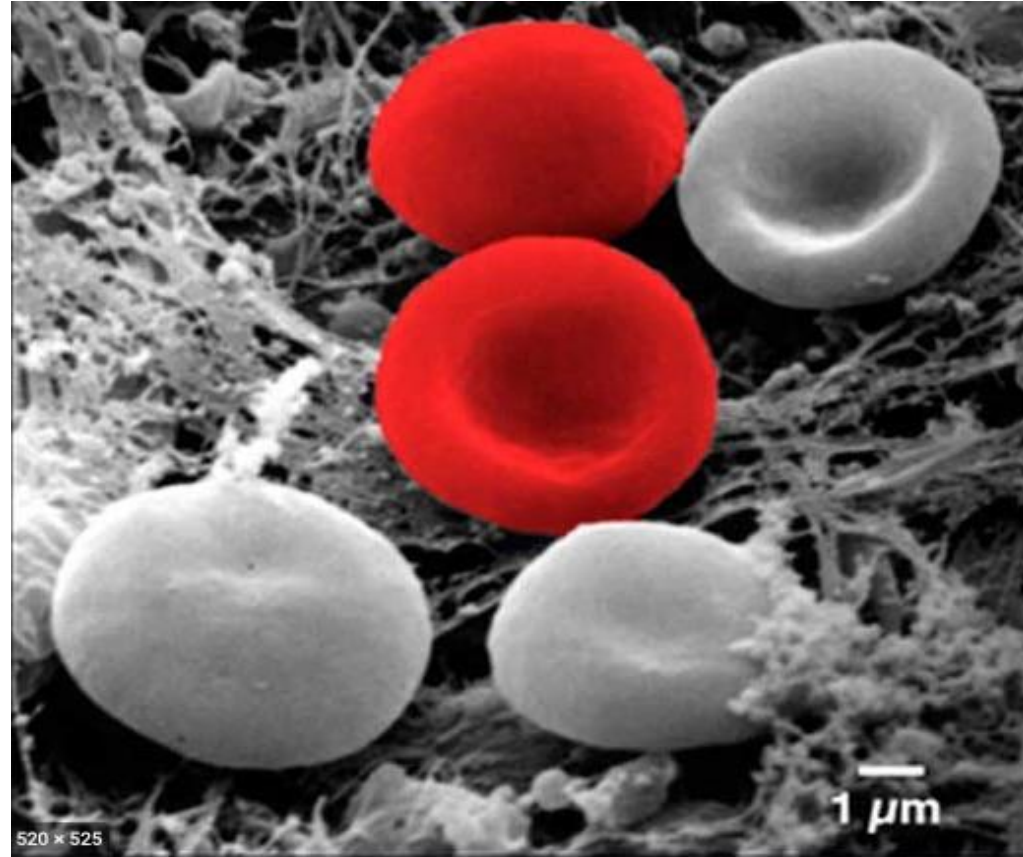
- Confocal microscopy achieves high resolution and sharp Focus by using
- (1) a small point of high-intensity light, often From a laser, and
- (2) a plate with a pinhole aperture in front of The image detector. The point light source, the focal point of
- The lens, and the detector's pinpoint aperture are all optically
- Conjugated or aligned to each other in the focal plane (confocal),
- And unfocused light does not pass through the pinhole.
- This greatly improves resolution of the object in focus and
- Allows the localization of specimen components with much

TEM



Source: Anthony L. Mescher: Junqueira's Basic Histology: Text and Atlas, 15th Edition. Copyright © McGraw-Hill Education. All rights reserved.

SEM



Staining And Stains

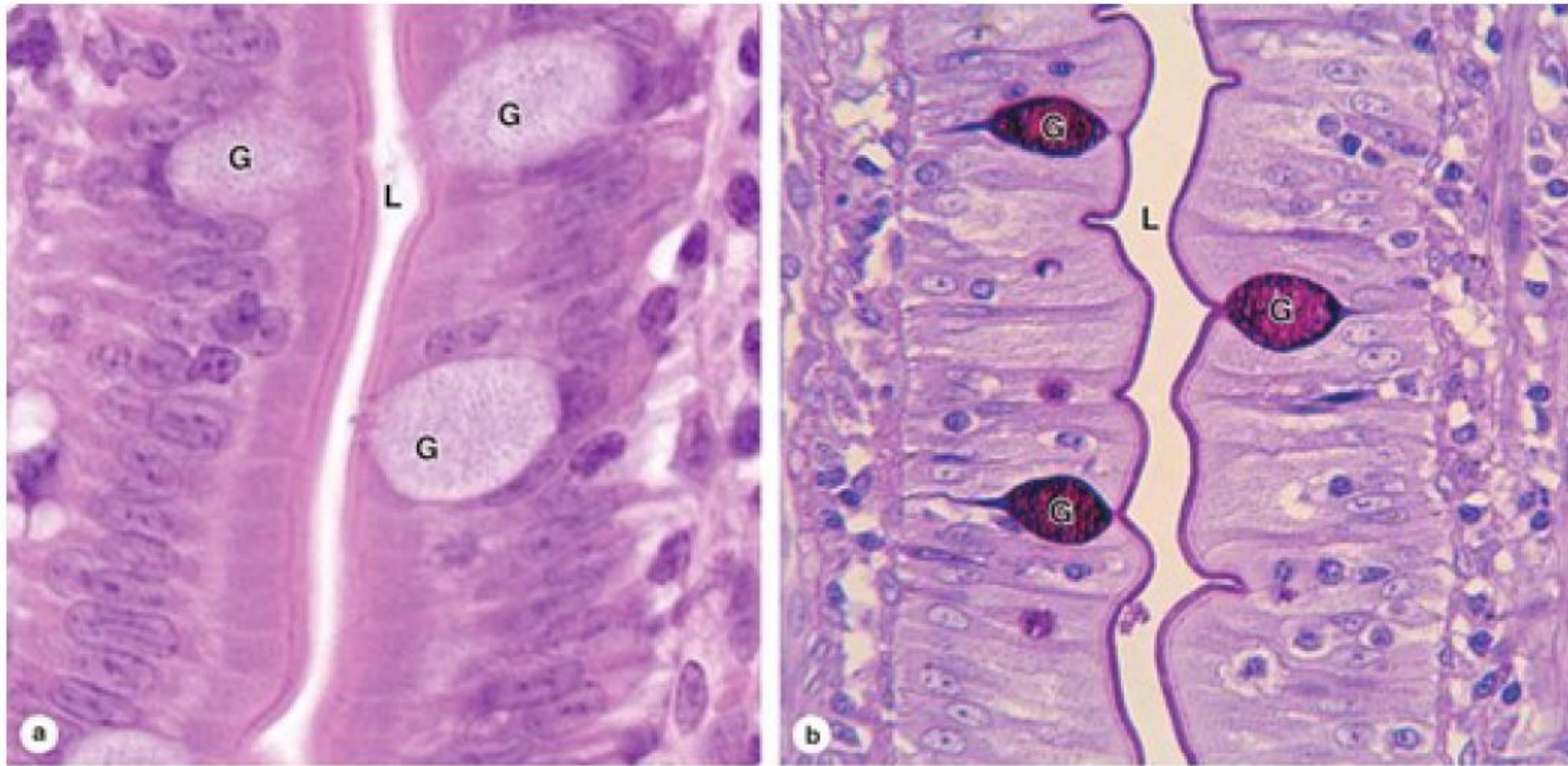
- Most cells and extracellular material are completely colorless!
- Dyes forming electrostatic (salt) linkages with ionizable radicals of macromolecules in tissues.
- Cell components such as nucleic acids with a net negative charge (anionic) have an affinity for basic dyes and are termed **basophilic**;
- Cationic components, such as proteins with many ionized amino groups, stain more readily with acidic dyes and are termed **acidophilic**.
- Basic dyes include toluidine blue, alcian blue, and methylene blue.
- **Hematoxylin** behaves like a basic dye, staining basophilic tissue components. The main tissue components
- DNA, RNA, and glycosaminoglycans: ionize and react with basic dyes do so because of acids in their composition
- Acid dyes: eosin, orange g, and acid fuchsin stains mitochondria, secretory granules, and collagen are acidic.

Staining And Stains

- **Trichrome** stains allow greater distinctions among various extracellular tissue components, eg, masson trichrome.
- **The periodic acid-schiff (PAS)** reaction utilizes the hexose rings of polysaccharides and other carbohydrate-rich tissue structures and stains such macromolecules distinctly purple or magenta.
- **Sudan black:** lipid-soluble dyes --stains lipids; avoiding the processing steps that remove lipids, such as treatment with heat and organic solvents which can be useful in diagnosis
- Can employ
- **Metal impregnation:** less common methods. Using solutions of silver salts to visual certain ECM fibers and specific cellular elements in nervous tissue.

H&E

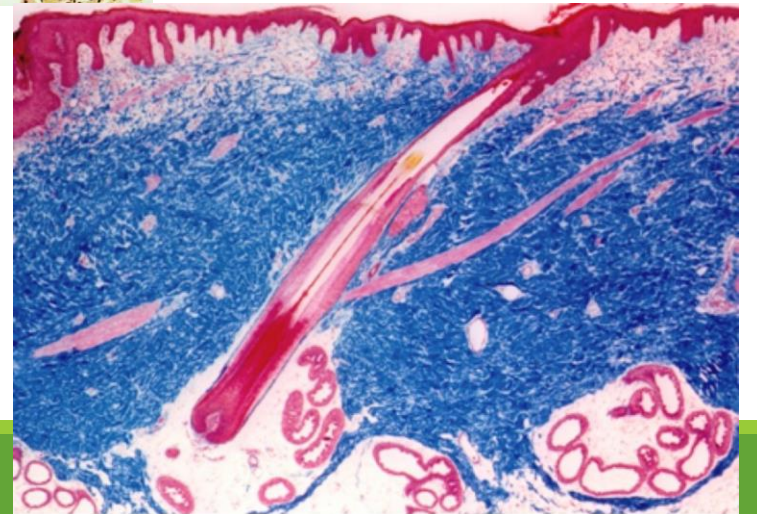
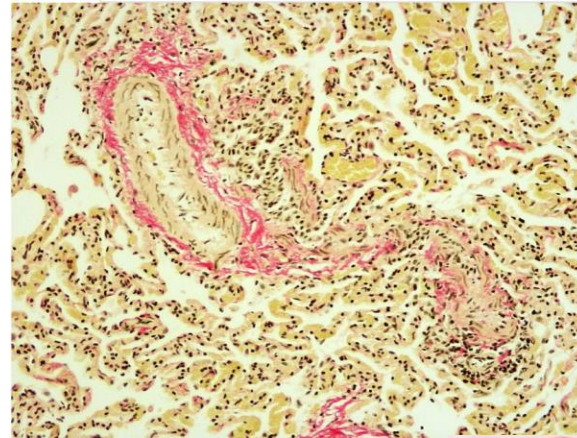
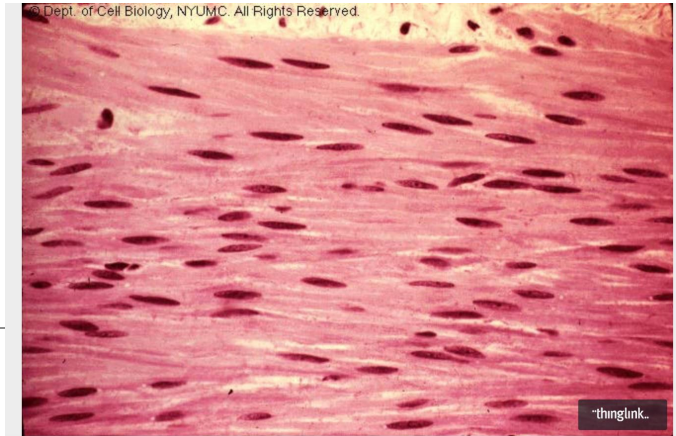
PAS STAINING!



Source: Anthony L. Mescher: Junqueira's Basic Histology: Text and Atlas, 15th Edition. Copyright © McGraw-Hill Education. All rights reserved.

Examples Of Commonly Used Histological Stains

- Hematoxylin and eosin (H&E): nucleus/blue, cytoplasm/pink
- Van gieson method: collagen/pink, muscle/yellow.
- Trichrome method: three color system to emphasize support fibers: connective tissue/blue, cytoplasm/pink, nuclei/dark brown.



TISSUE TYPES

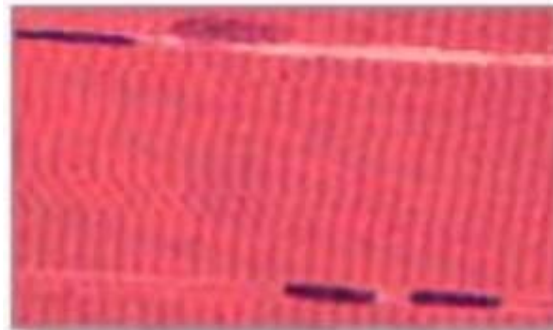
- EPITHELIUM
- CONNECTIVE TISSUE
- MUSCULAR TISSUE
- NERVOUS TISSUE



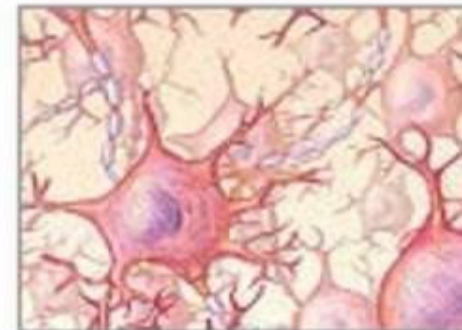
Connective tissue



Epithelial tissue



Muscle tissue



Nervous tissue



Test bank

1- is the main purpose of the Fixation process?

- A. Eliminating bacteria
- B. Enhancing color
- C. Preserving cell and tissue structure
- D. Nourishing tissues
- E. Repairing cellular damage

2- What is the final step in the tissue processing procedure before sectioning?

A. Dehydration

B. Fixation

C. Clearing

D. Trimming

E. Infiltration

3- Which type of microscope relies on the interaction of light with tissue?

- A. Light microscope
- B. Confocal microscope
- C. Fluorescence microscope
- D. Phase-contrast microscope
- E. Electron microscope

4- What is the typical resolution of an electron microscope?

A. 3 millimeters

B. 3 micrometers

C. 3 nanometers

D. 3 centimeters

E. 3 kilometers

5- What is the primary method of illumination in a bright-field light microscope?

A. Fluorescent light

B. Laser light

C. Ordinary light passing through the preparation

D. Polarized light

E. Ultraviolet light

- 6- What type of specimens can be studied using phase-contrast microscopy?
- A. Stained cells
 - B. Unstained cells
 - C. Tissue sections with different refractive indices
 - D. Tissue sections with similar optical densities
 - E. Non of the above

- 7- Which staining technique employs a three-color system to emphasize support fibers, staining connective tissue blue, cytoplasm pink, and nuclei dark brown?
- A. Hematoxylin and eosin (H&E) stain
 - B. Van Gieson method
 - C. Trichrome method
 - D. Periodic acid-schiff (PAS) reaction
 - E. Sudan black stain

- 8- What type of light is used to irradiate tissue sections in fluorescence microscopy?
- A. Infrared light
 - B. Ultraviolet (UV) light
 - C. Visible light
 - D. X-rays
 - E. Gamma rays

9- What is the function of the objective lenses in a bright-field light microscope?

A. Collect and focus a cone of light

B. Enlarge and project the image toward the eyepiece

C. Illuminate the tissue slide on the stage

D. Magnify the illuminated image of the object

E. Adjust the focus of the specimen

10- What is meant by "resolving power" in microscopy?

A. The maximum magnification achievable

B. The ability to distinguish between two separate structures

C. The amount of light passing through the preparation

D. The thickness of the tissue sample

E. The distance between the condenser and the objective lens

11-What is the affinity of cell components with a net negative charge for in staining?

A. Basic dyes

B. Acidic dyes

C. Neutral dyes

D. Fluorescent dyes

E. Inert dyes

12- Which dye stains acidic cell components like mitochondria, secretory granules, and collagen?

A. Toluidine blue

B. Alcian blue

C. Methylene blue

D. Hematoxylin

E. Eosin

13- What is the staining method that utilizes the hexose rings of polysaccharides and carbohydrate-rich tissue structures, staining such macromolecules distinctly purple or magenta?

- A. H&E stain
- B. Sudan black stain
- C. Periodic acid-schiff (PAS) reaction
- D. Trichrome stain
- E. Metal

14- What is the primary determinant of a microscope's resolving power?

A. The quality of its objective lens

B. The magnification achieved

C. The type of light source used

D. The thickness of the specimen

E. The distance between the eyepiece and the specimen

15- What happens when certain cellular substances are irradiated by light of a proper wavelength in fluorescence microscopy?

- A. They absorb light with a shorter wavelength
- B. They emit light with a longer wavelength
- C. They reflect light with the same wavelength
- D. They become transparent
- E. They change color

16- What staining method is commonly used to stain the nucleus blue and the cytoplasm pink?

A. Hematoxylin and eosin (H&E) stain

B. Van Gieson method

C. Trichrome method

D. Periodic acid-schiff (PAS) reaction

E. Metal impregnation

- 17- What principle is phase-contrast microscopy based on?
- A. Absorption of light by cellular structures
 - B. Refraction of light through transparent objects
 - C. Reflection of light off of stained cells
 - D. Diffraction of light through tissue sections
 - E. Changes in the speed of light passing through

18- Which staining technique involves the use of lipid-soluble dyes to stain lipids, thereby avoiding the processing steps that remove lipids, such as treatment with heat and organic solvents?

- A. H&E stain
- B. Sudan black stain
- C. Periodic acid-schiff (PAS) reaction
- D. Trichrome stain
- E. Metal impregnation

- 19- What is the primary advantage of phase-contrast microscopy?
- A. It allows for the study of stained cells and tissue sections
 - B. It produces visible images from transparent objects
 - C. It utilizes a lens system that enhances color contrast
 - D. It is specifically designed for use with fixed cells only
 - E. It relies on the absorption of light by cellular structures

1-C
2-D
3-A
4-C
5-C

6-B
7- C
8-B
9-A
10-B

11-A
12-E
13-C
14-A
15-B

16-A
17-E
18-B
19-B

كله النا والهستو النا

By : Mahmoud Hasan

V2. check SLIDE 10

Also Q7 in the testbank