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
4. CARTILAGE
5. BONE
6. MUSCULAR TISSUE
7. NERVOUS TISSUE

HISTOLOGY

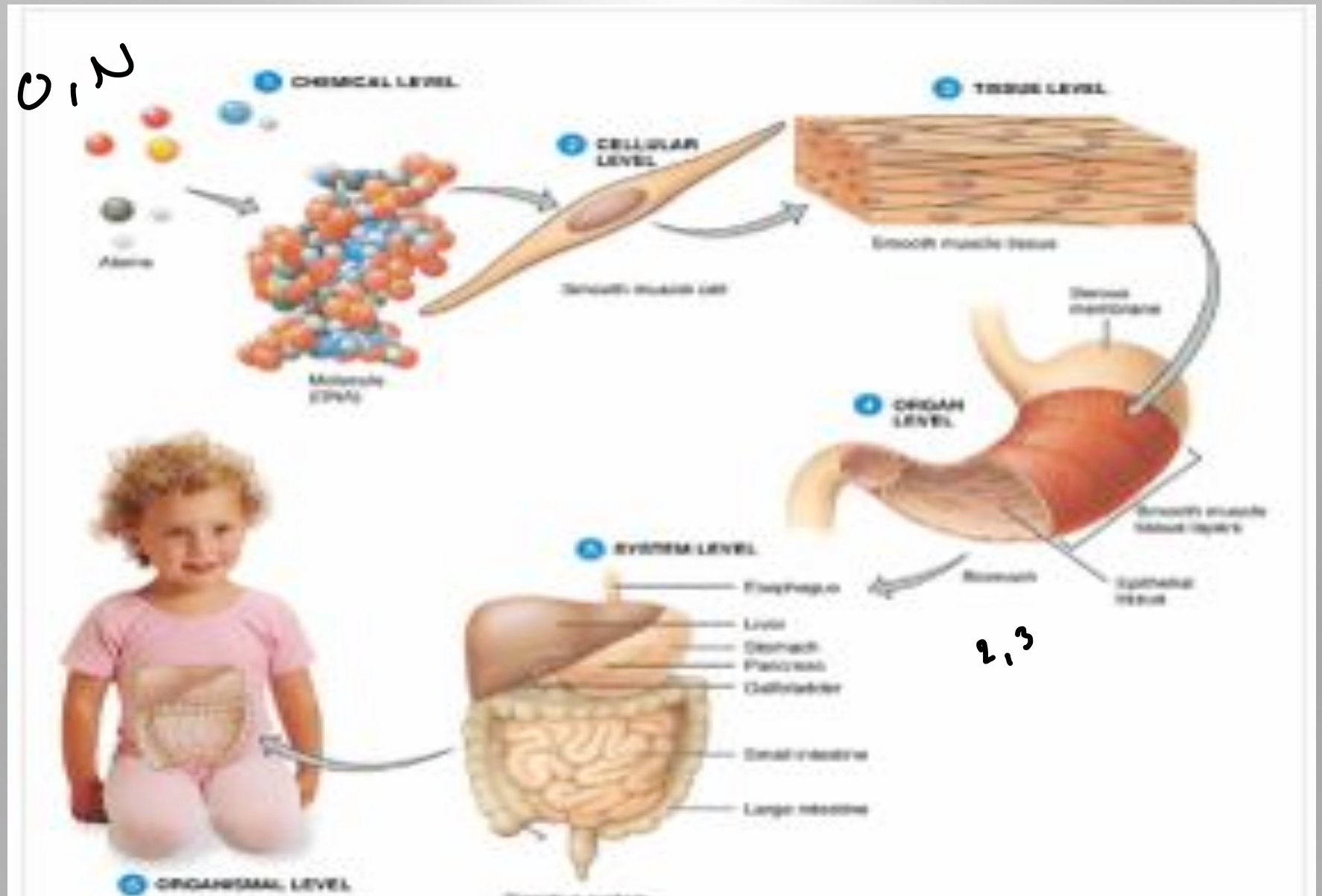
- MICROSCOPIC ANATOMY!
- *HISTO= WEB OR TISSUE*
- *LOGOS= STUDY*
- THE STUDY OF CELLS AND THE EXTRACELLULAR MATRIX

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

C, O, N

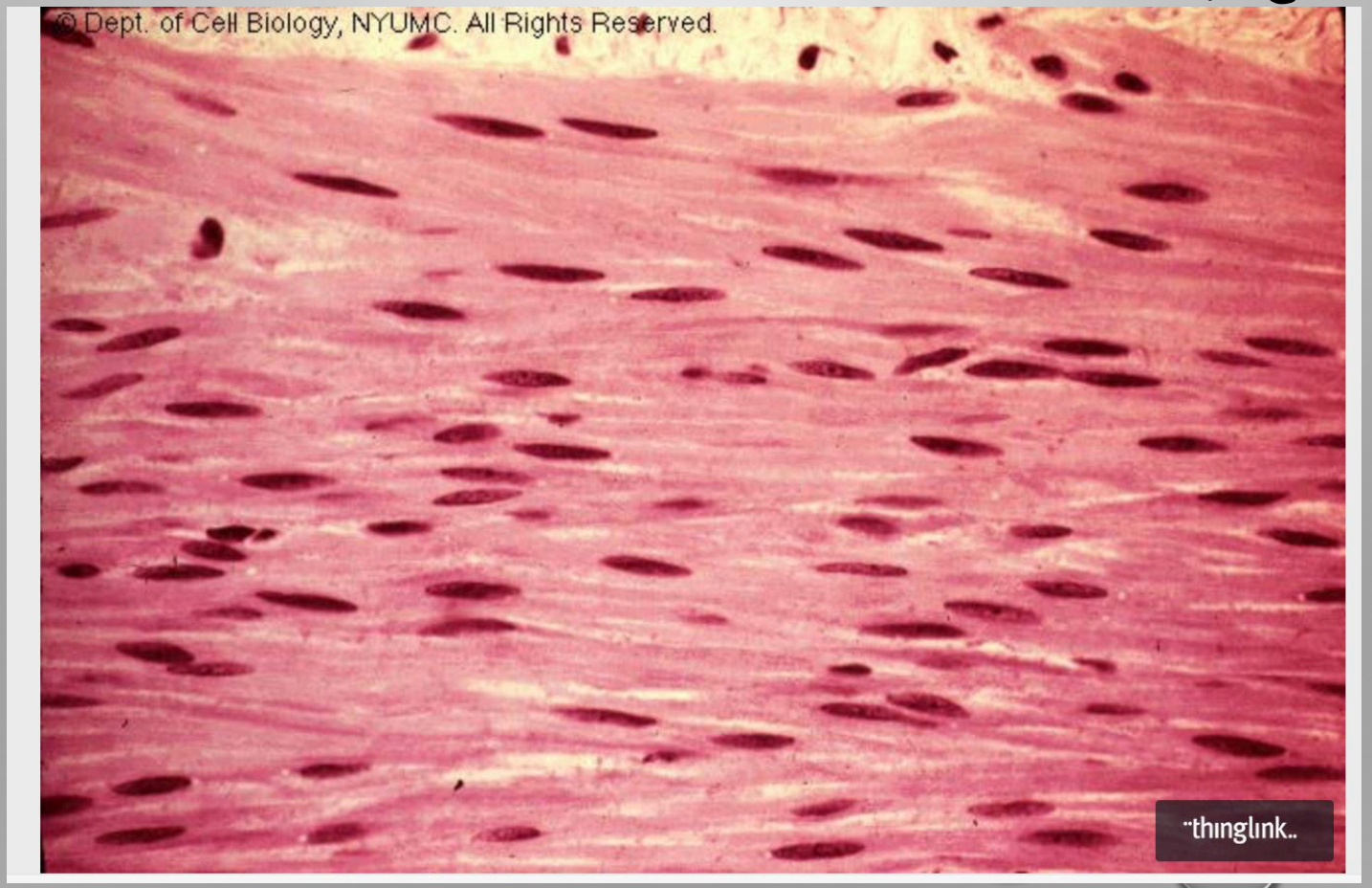


2, 3

connective more than one type of tissue

How did we get this image?

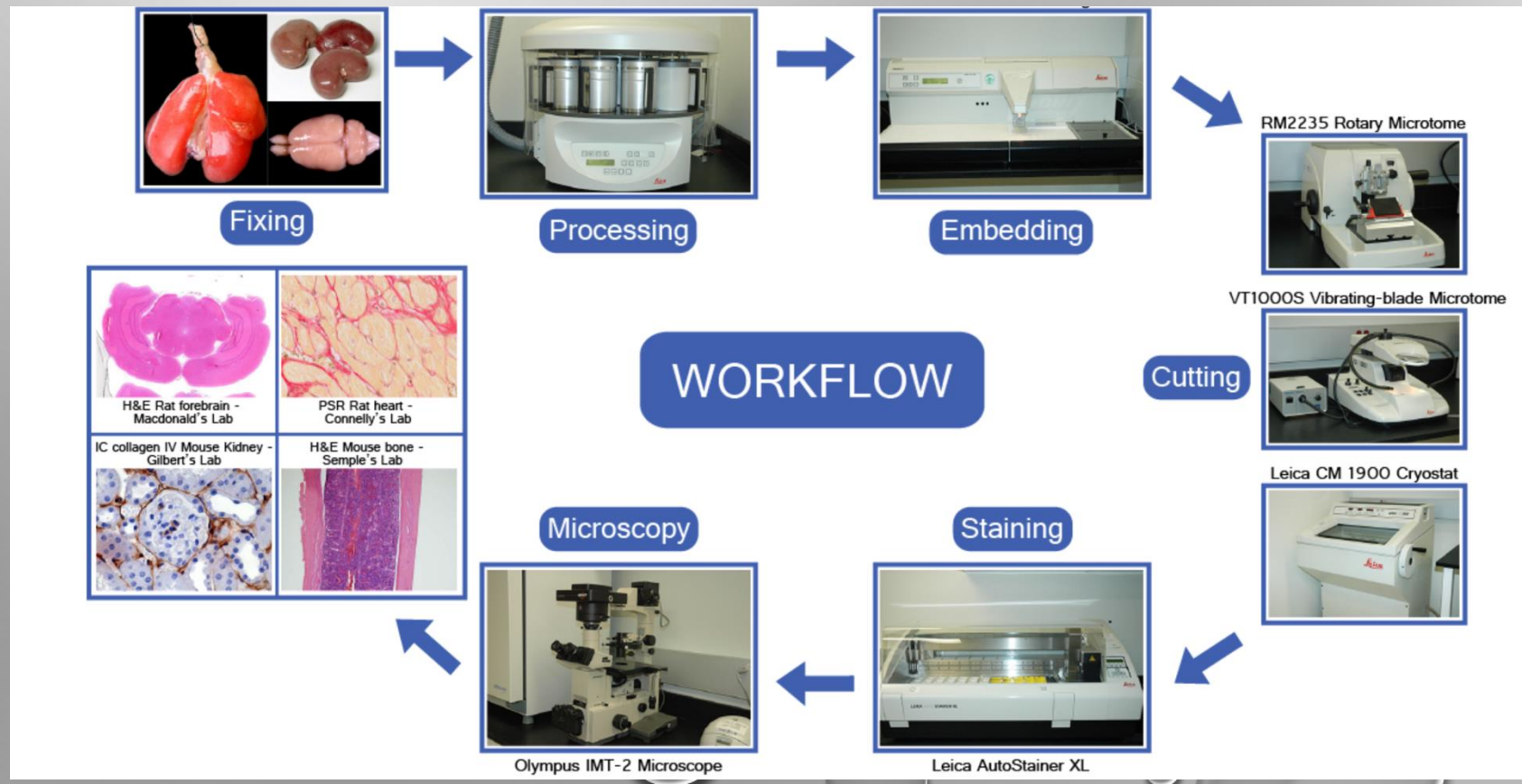
Pigment stain smooth Muscle



Tissue Processing For Histology

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

Paraffin block



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.
- **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.

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.

STAINING & MICROSCOPES

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. -charged bluish
- 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. + charged pinkish

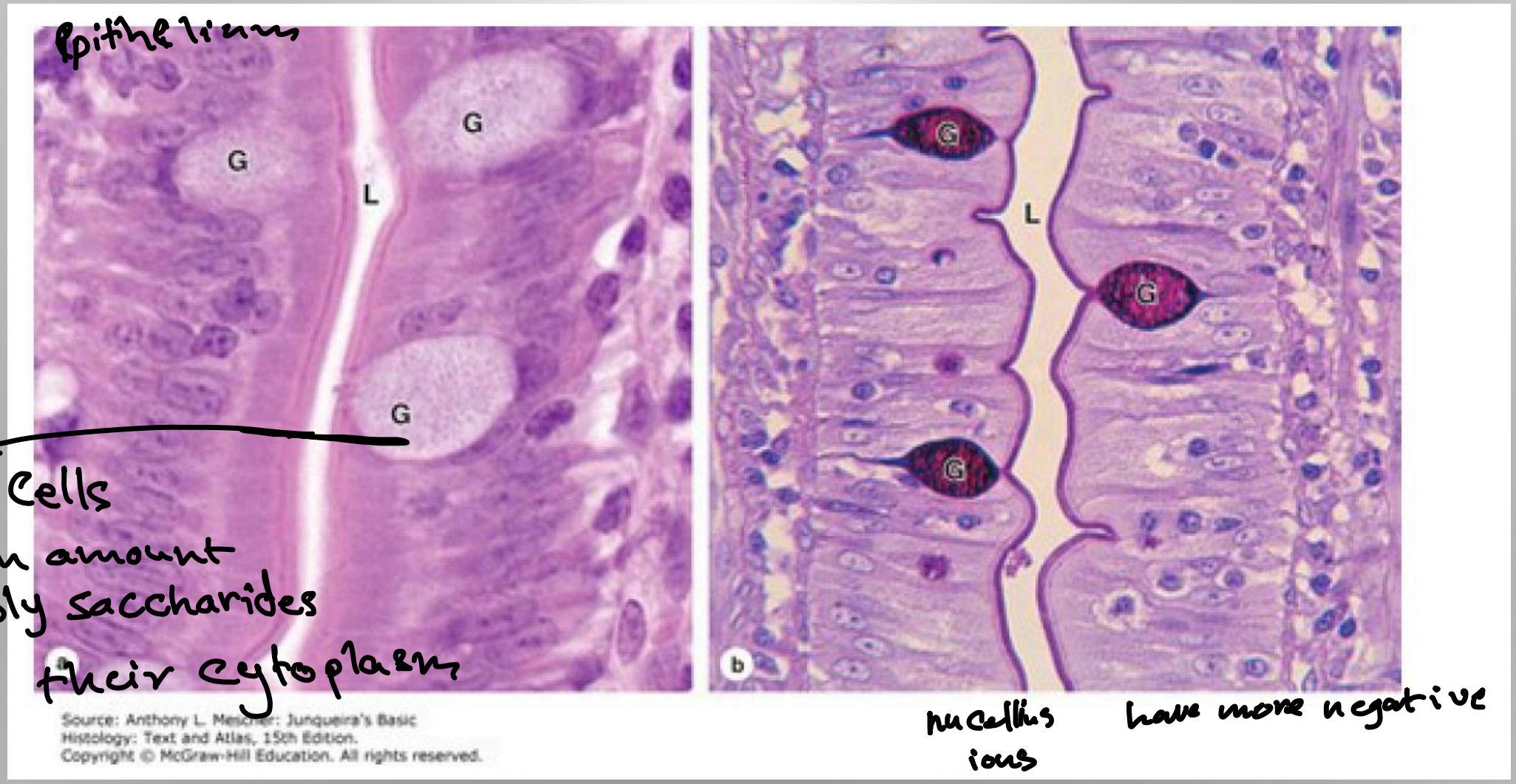
Staining And Stains-special stains

- **Trichrome** stains allow greater distinctions among various extracellular tissue components, e.g., 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 --satins lipids; avoiding the processing steps that remove lipids, such as treatment with heat and organic solvents which can be useful in diagnosis
- **Metal impregnation:** less common methods. Using solutions of silver salts to visual certain ECM fibers and specific cellular elements in nervous tissue.
- **Immunostaining**: immunofluorescence and immunohistochemistry.

nuclie will react with it and cytoplasm react with it

H&E

PAS STAINING!



goblet cells have an amount of poly saccharides inside their cytoplasm

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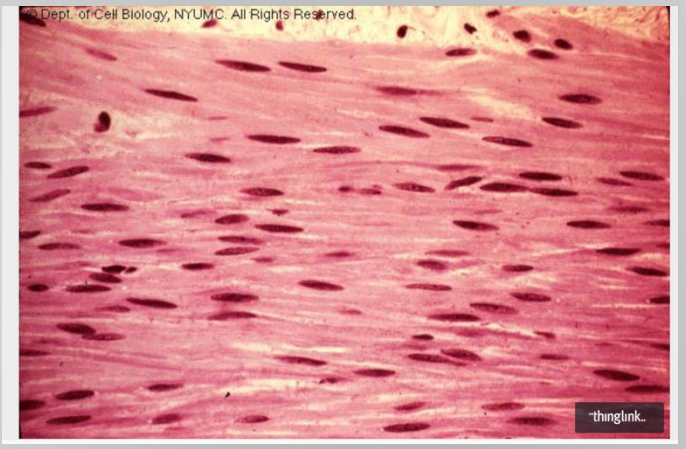
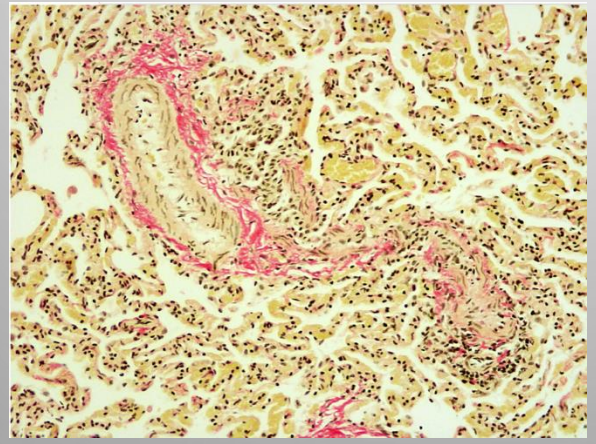
nucellus ions have more negative

Examples Of Commonly Used Histological Stains



for tracking collagen

- Van Gieson method: collagen/pink, muscle/yellow.

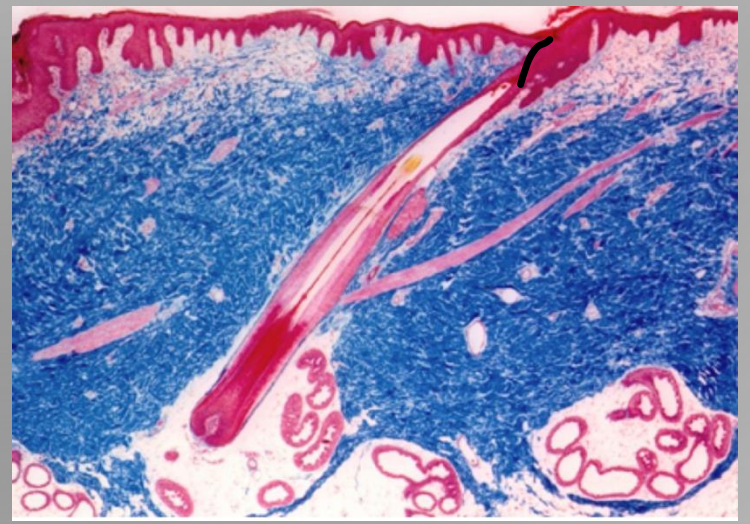


stains the muscles in a yellow color

- Hematoxylin and eosin (H&E): nucleus/blue, cytoplasm/pink

Smooth muscles

- Trichrome method: three color system to emphasize support fibers: connective tissue/blue, cytoplasm/pink, nuclei/dark brown.



skin

MICROSCOPES

Types of microscope

- **Light microscope.**

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

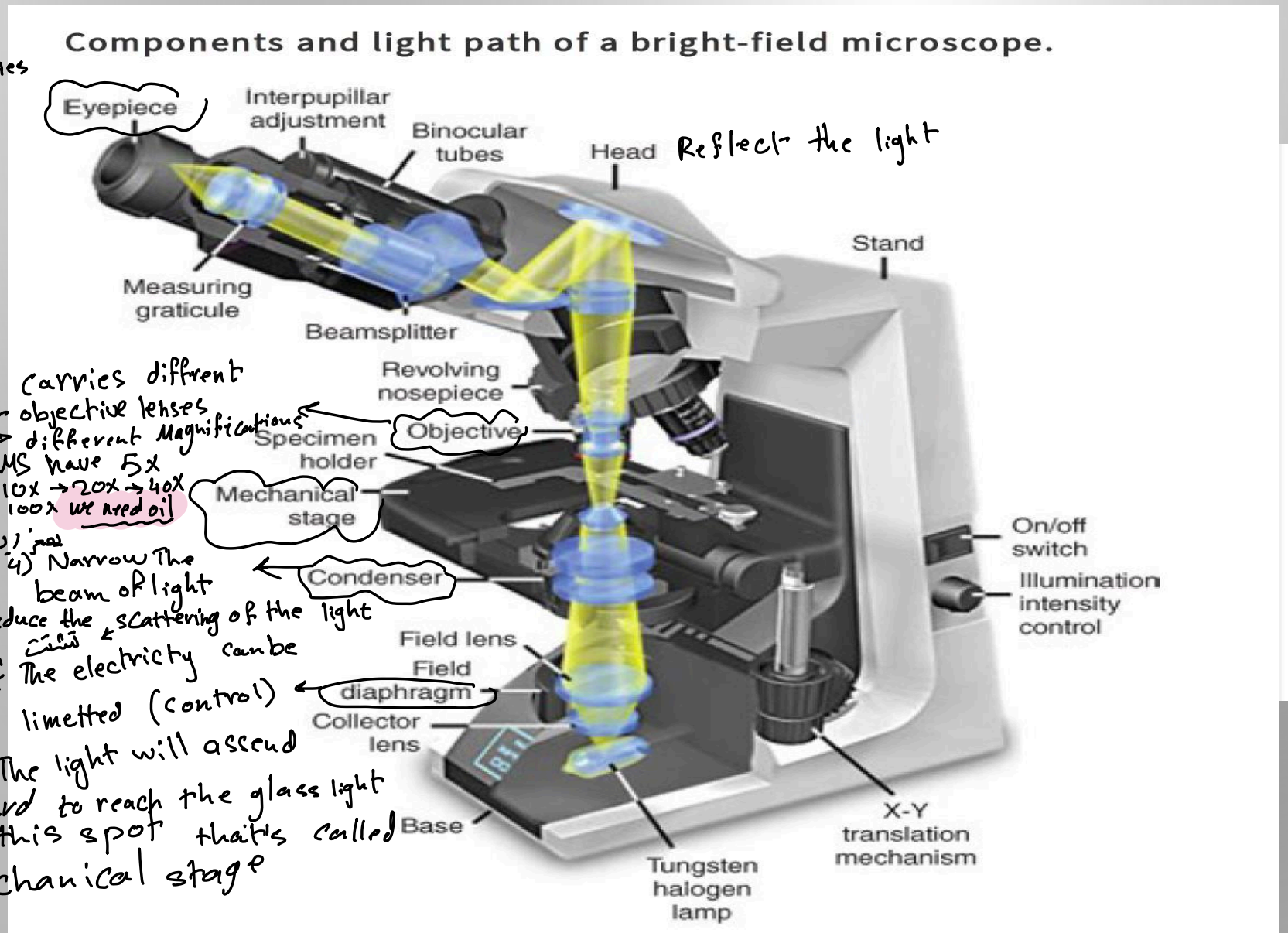
- **Electron microscope**

1. Transmission electron microscopy
2. Scanning electron microscopy

Light Microscope (Bright-field)

final magnification = M of objective x M eye piece

magnifies 10X



Reflect the light

carries different objective lenses different Magnifications

most MS have 5X then 10X → 20X → 40X some → 100X we need oil

4) Narrow The beam of light to reduce the scattering of the light

2) The electricty can be limetted (control)

3) The light will assend upward to reach the glass light on this spot that's called mechanical stage

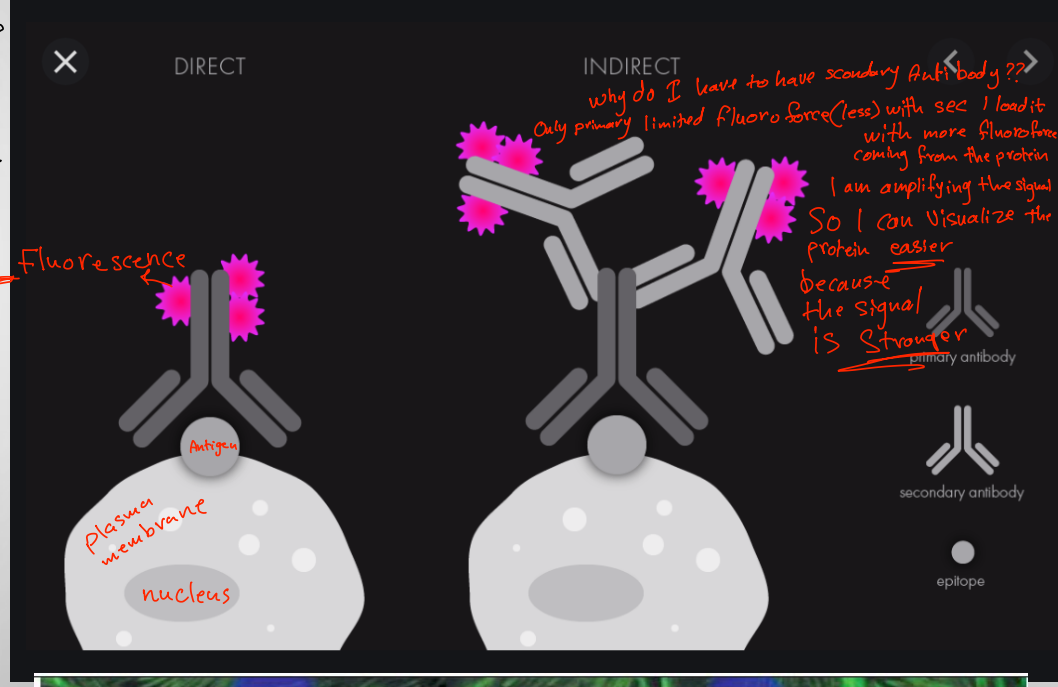
① the most commonly used and the Cheapest
they connect to electricity → Turn on the Tungsten halogen lamp

Bright-field Light Microscope

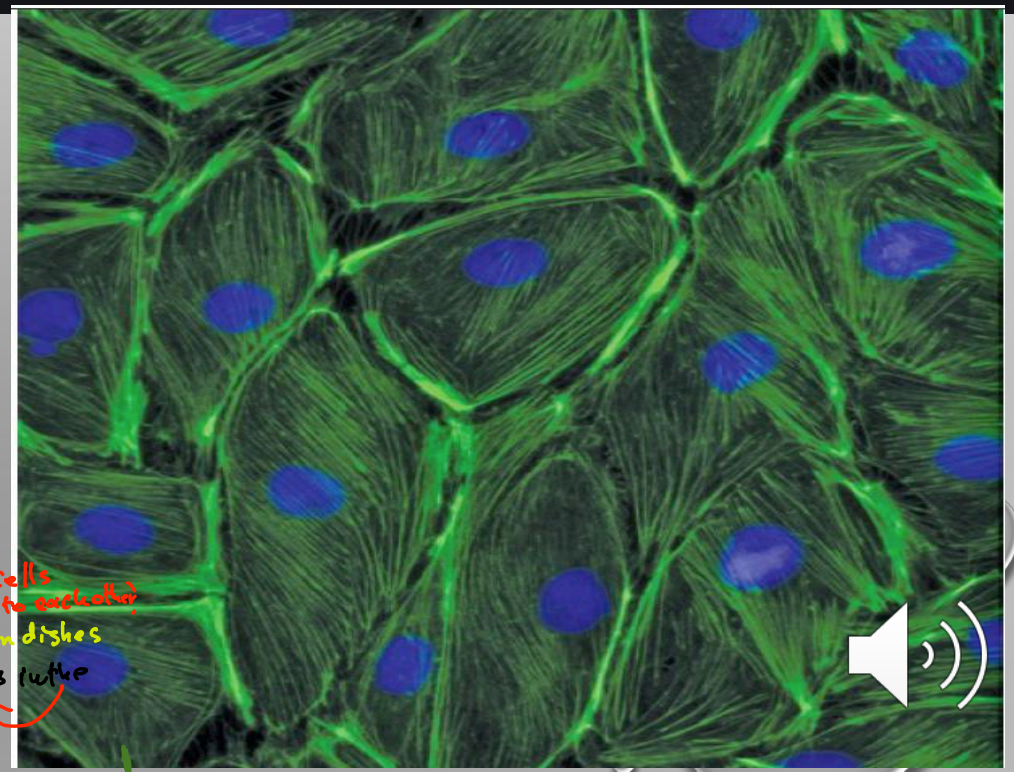
- Stained tissue is examined with ordinary light passing through the preparation.
- Includes an optical system and mechanisms to move and focus the specimen.
- The **condenser** collects and focuses a cone of light that illuminates the tissue slide on the stage.
- **Objective** lenses enlarge and project the illuminated image of the object toward the eyepiece.
- The two **eyepieces** or oculars magnify this image another 10X and project it to the viewer, yielding a total magnification of 40X, 100X, or 400X.

who we combine microscopes with immuno staining??
I'm targeting a specific protein so I purchase Antibody that will bind to the protein and we have fluorforce on their surface so I process my section and go to fluorescence Microscope...

Fluorescence Microscopy



- **Fluorescence**: when certain cellular substances are irradiated by light of a proper wavelength, they emit light with a longer wavelength. particular
- 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.



blue: nucleus
In this image we have targeted something specific How??

Cultured cells (cells next to each other)
Cells grown on dishes
we but cells in the incubator

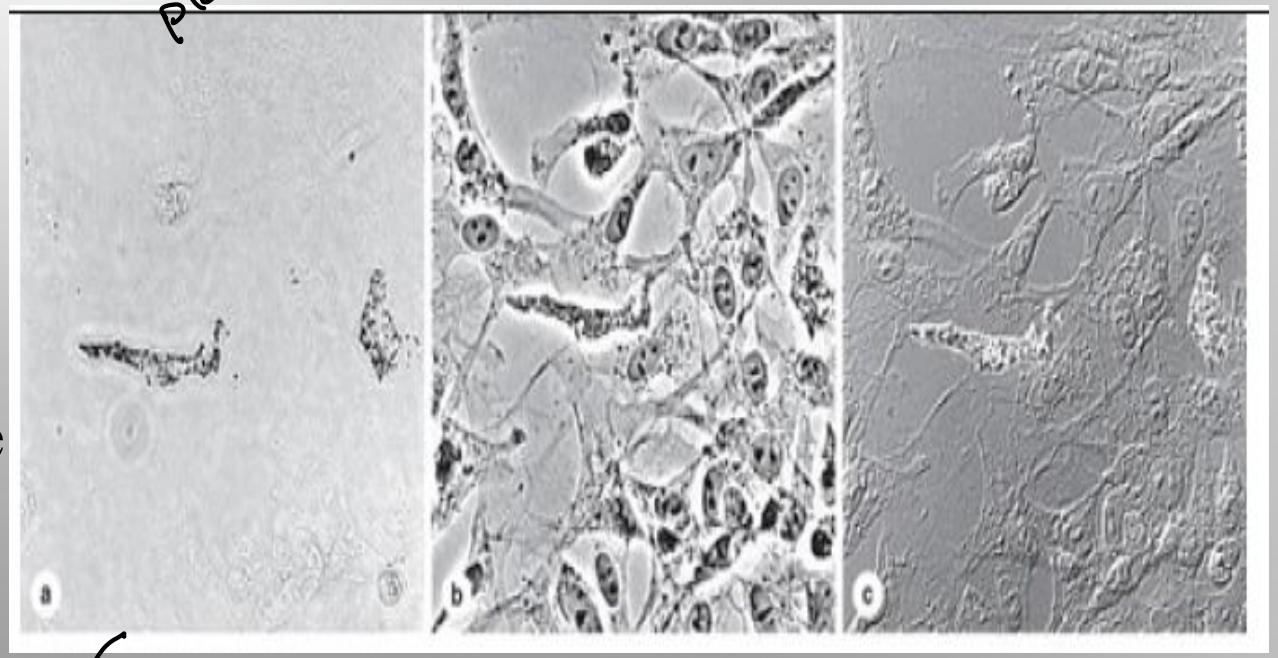
we use the florescence to visualize immuno stained sections in this image we have targeted filamentous (Protein) concentrated under the plasmalamina

Phase-contrast Microscopy

Can I visualize the cells without killing them (without staining)??

Culture plate

- 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.
- lenses detect different refractive indices مؤشرات انكسار
- 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.



The round structure are the nuclei
the processes that the cells send to eachother
cells in culture depends on the trend to be social
and they communicate thatis onemethod to keep the cells alive

Elecetron Microscope

- Interaction of tissue with a beam of electrons.

depends on how the beam of electrons react with the tissue

TEM

- The electron beam passes the tissue.

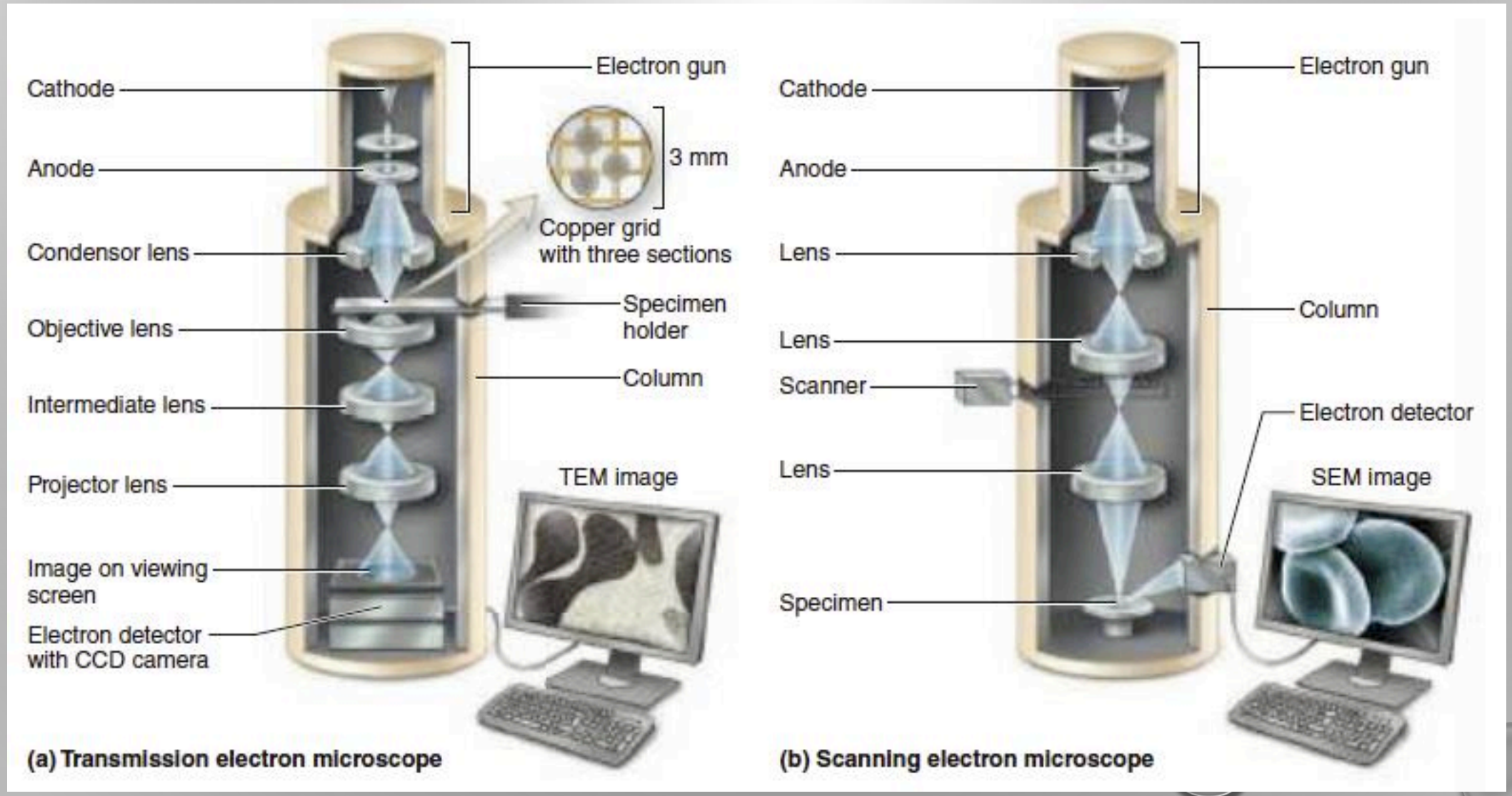
Reflected
absorbed
pass

- Very high magnification
- Very thin sections, 40-90 nm.
- Electron beam interact with tissue producing black, white and shades of gray images.

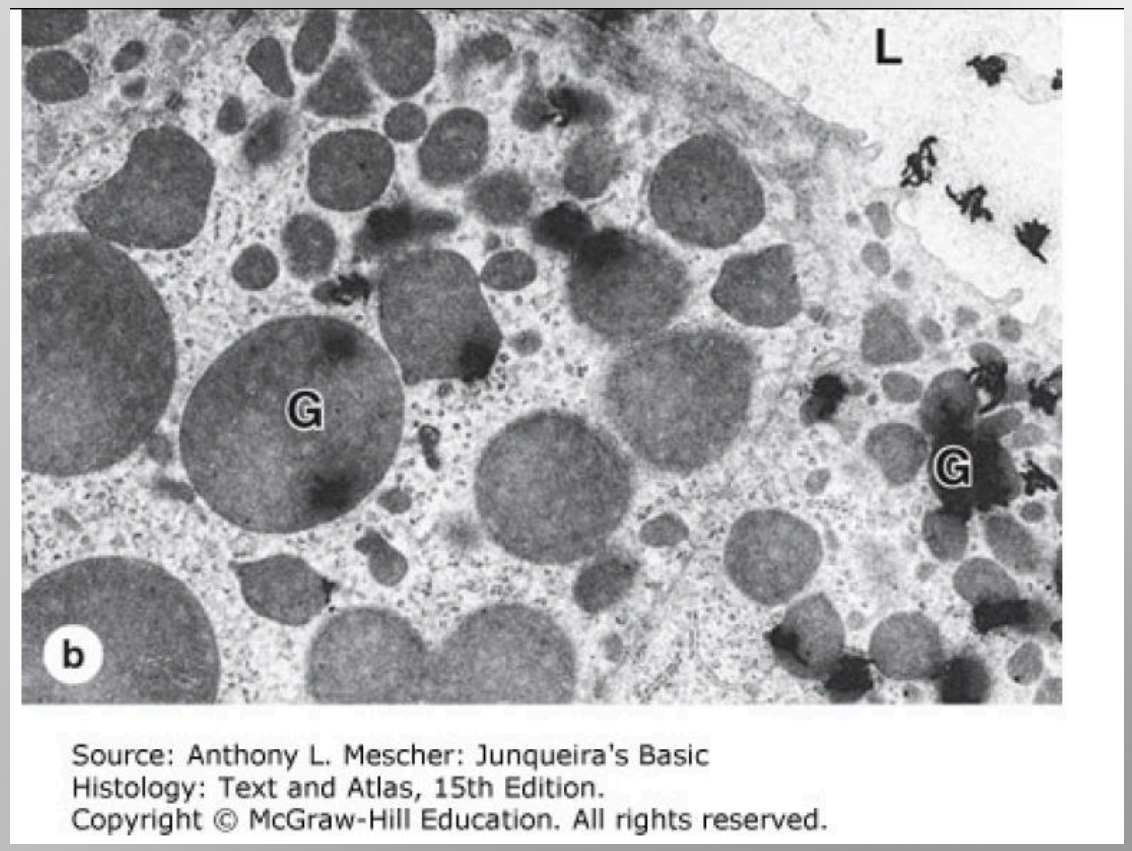
SEM.

- The electron beam does not passe the tissue.
- The surface of cells and tissue is coated with heavy metals (gold)---which reflect the electrons---producing 3D images which is a recording of the specimen topography.

Electron Microscope



TEM



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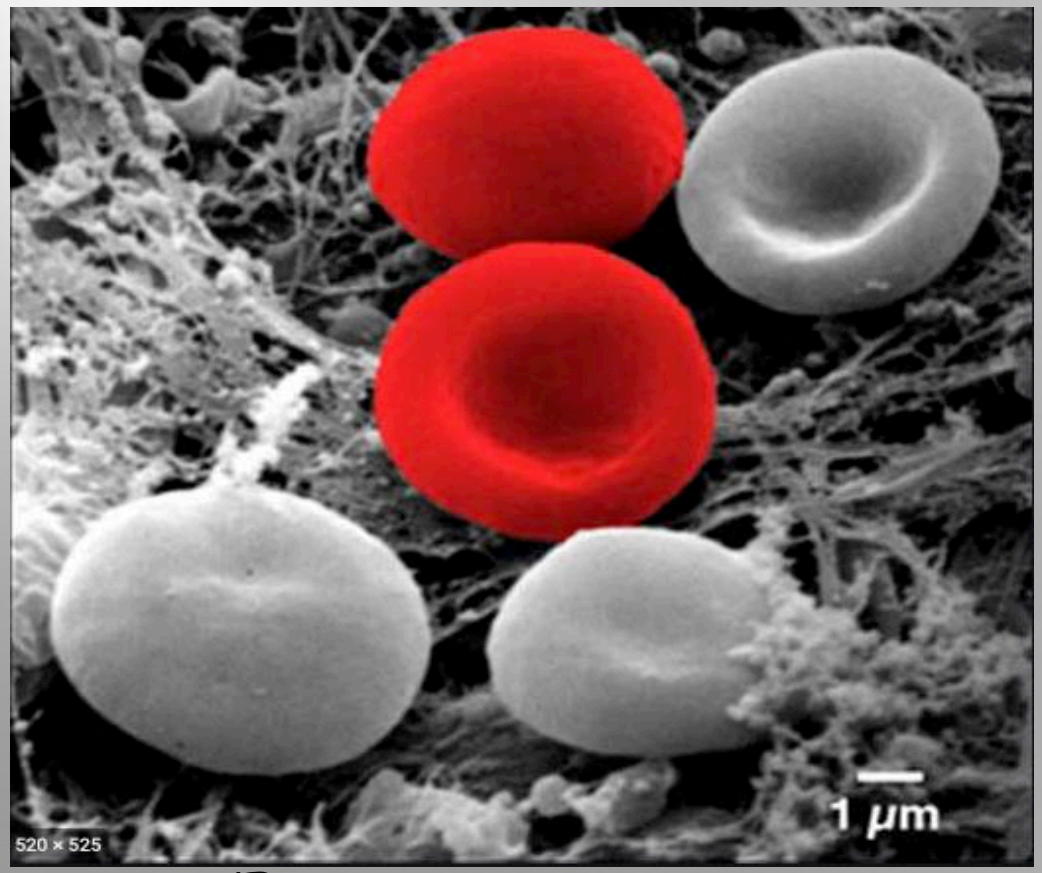
2D

SEM

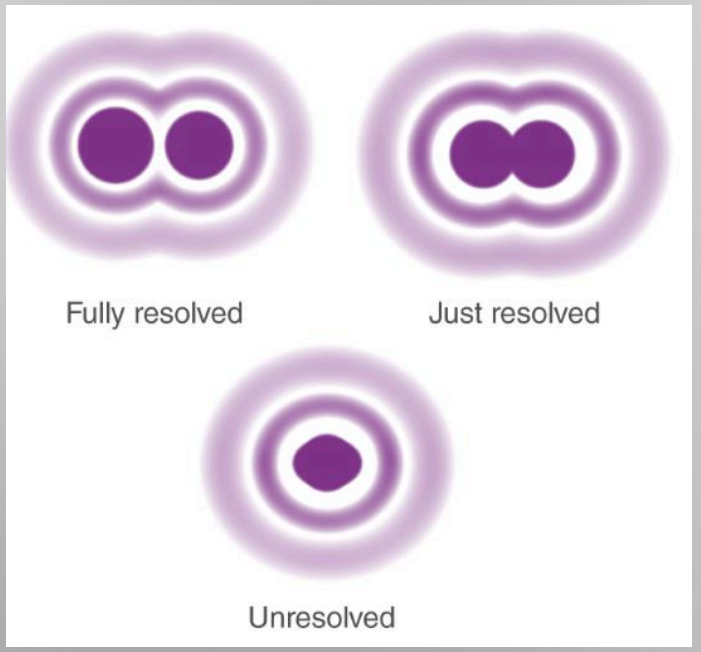


3D

Resolution

how finely detailed the images that we're looking at

- **Resolving power**: the smallest distance between two structures at which they can be seen as separate objects.
- The maximal resolving power of the light microscope is approximately 0.2 µm--- can permit clear images magnified 1000-1500 times.



- Objects smaller or thinner than 0.2 µm (such as a single ribosome or cytoplasmic microfilament) cannot be distinguished.

higher → 2 objects
less will appear touching (just result) → 1 object

- 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.
- Resolving of TEM is 3 nm (electron wavelength is shorter than that of light).