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

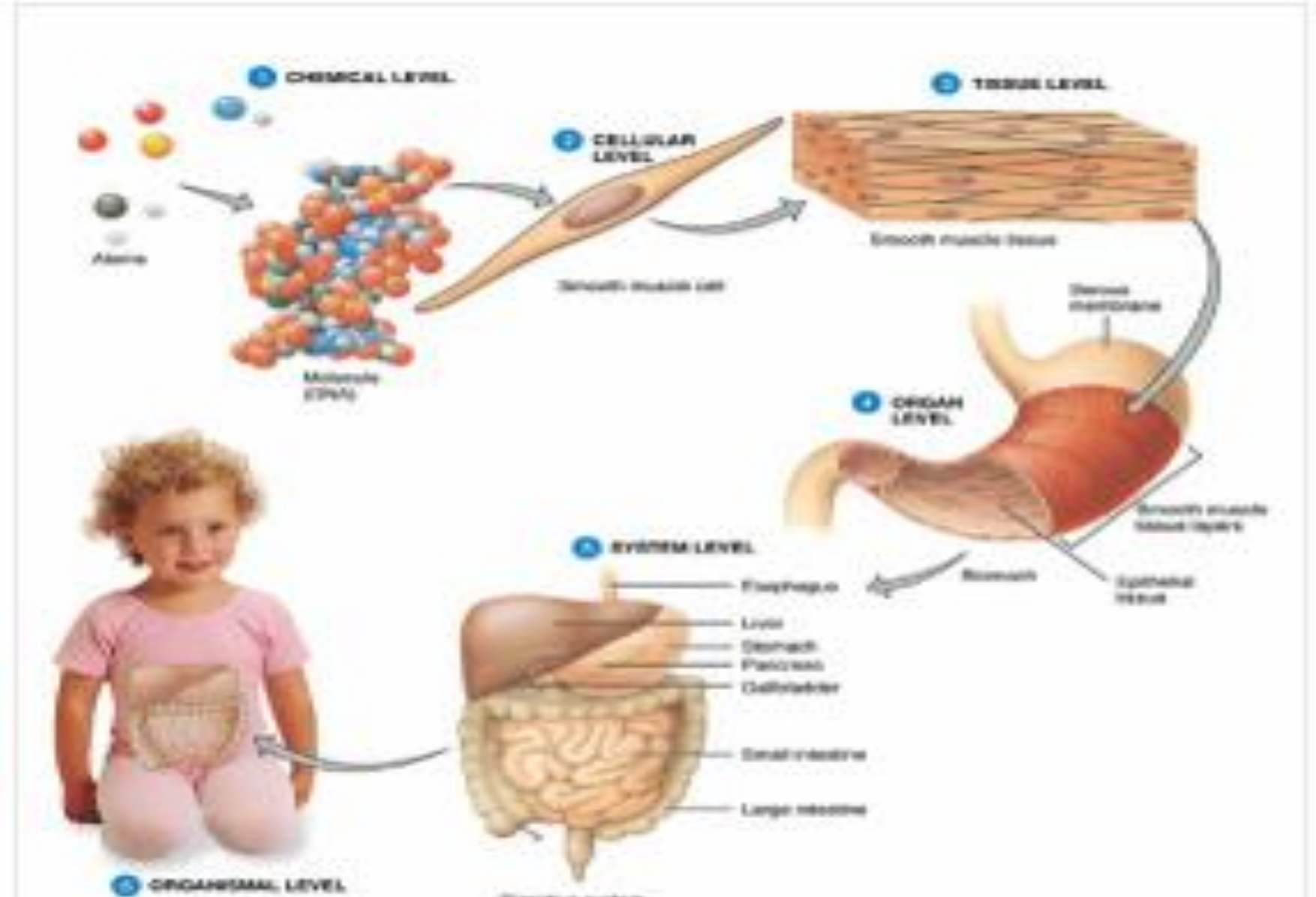
HISTOLOGY

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

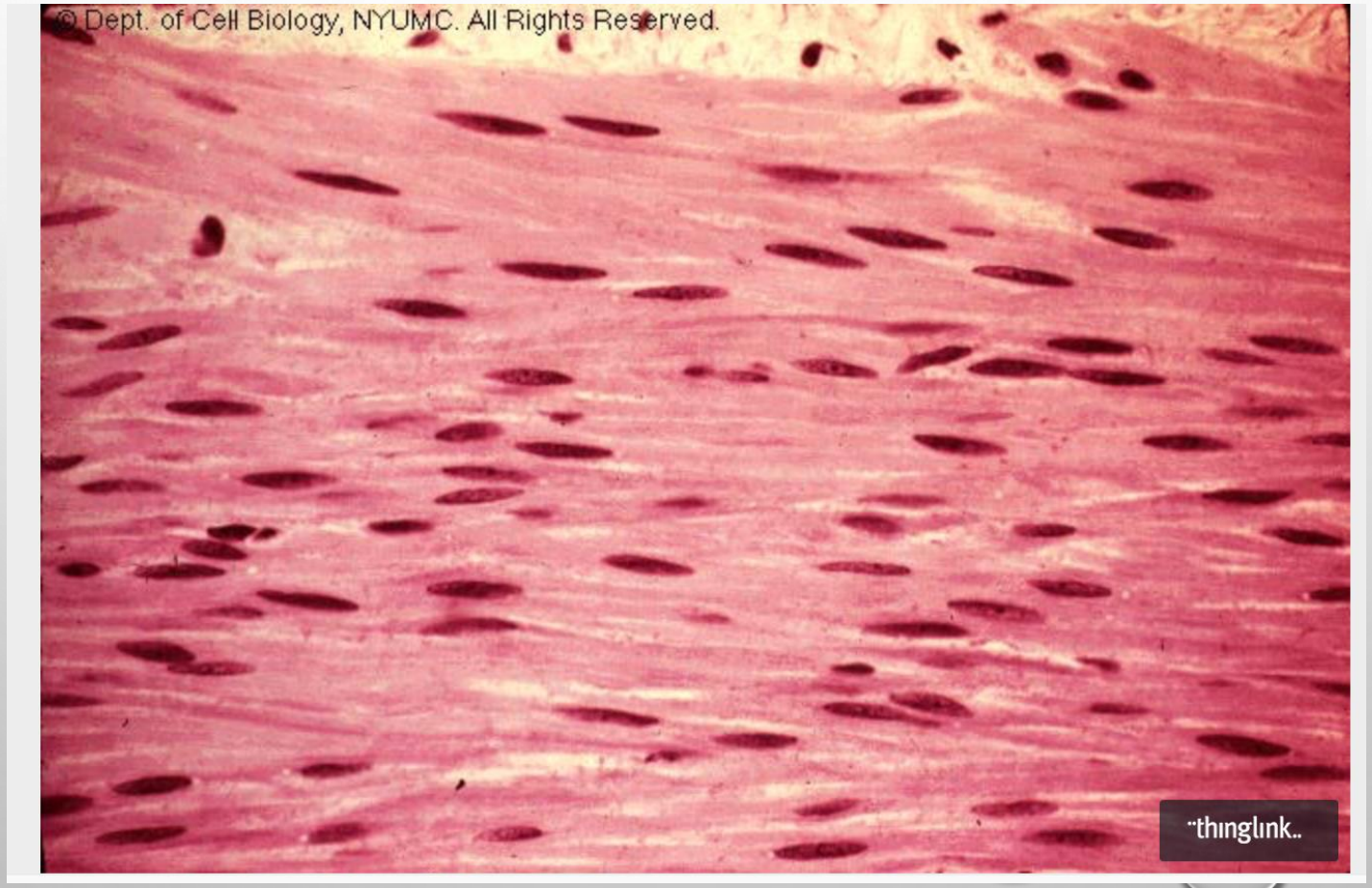
Histology

- 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



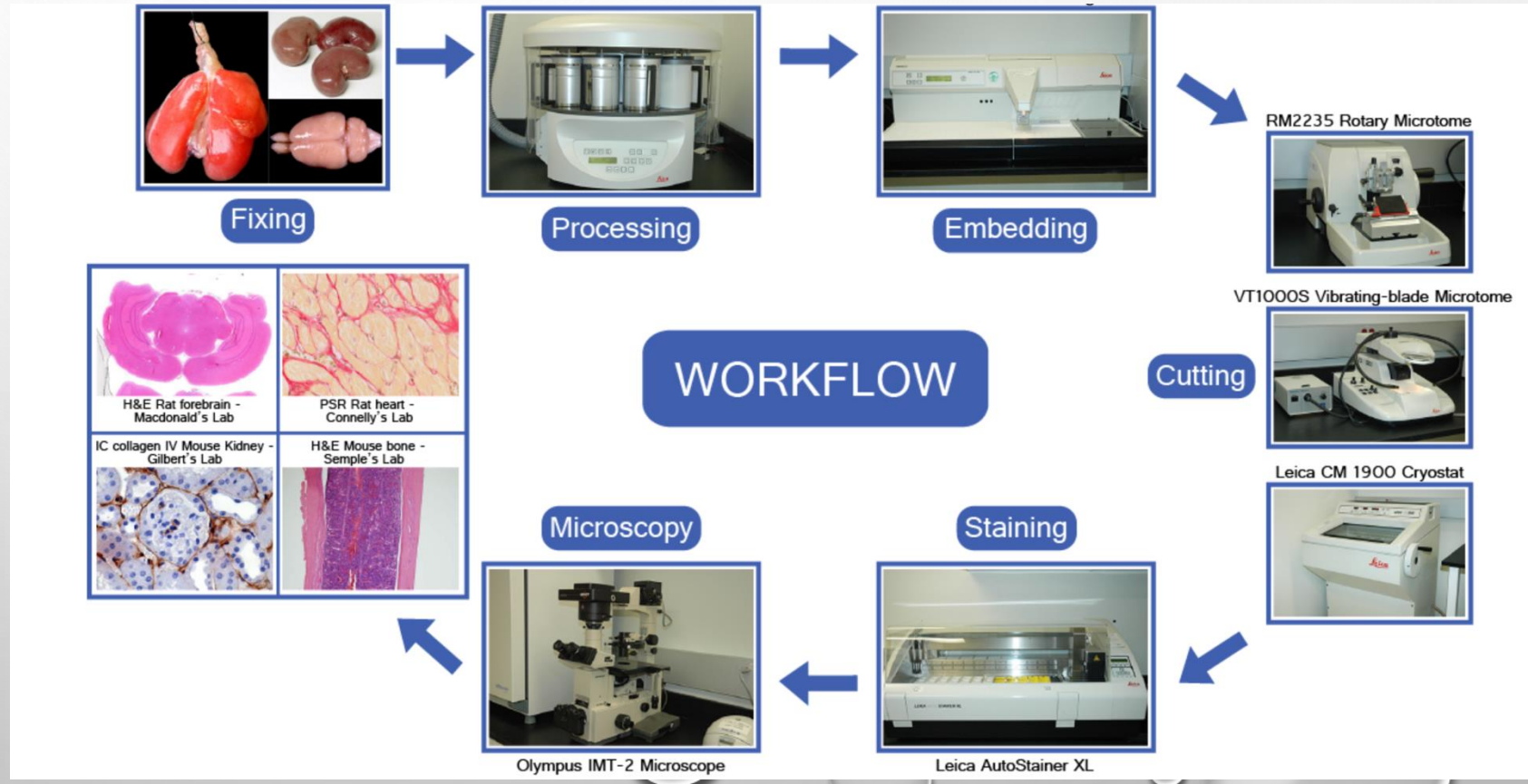
How did we get this image?



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

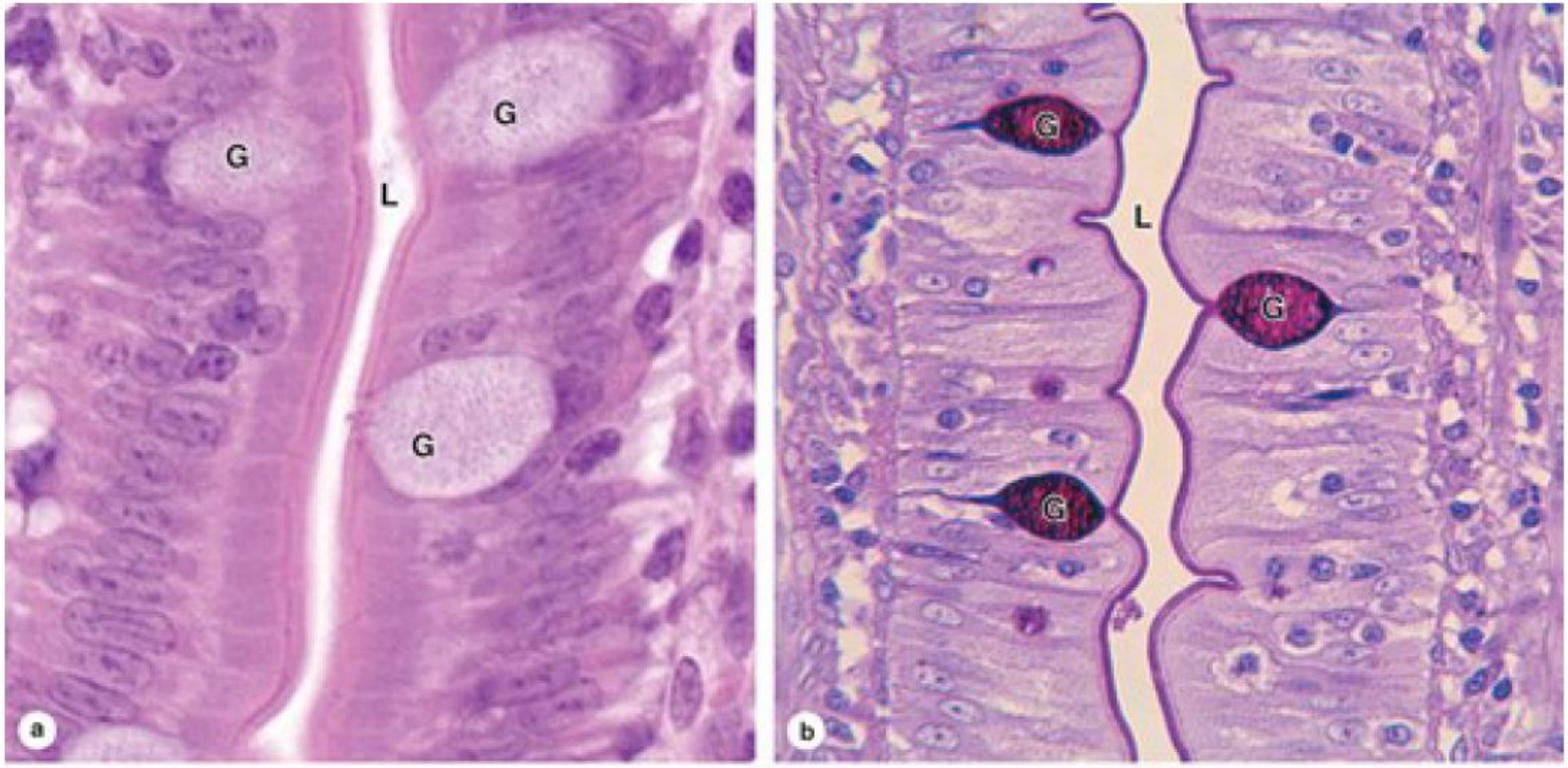
Staining And Stains

- 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.
- 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.

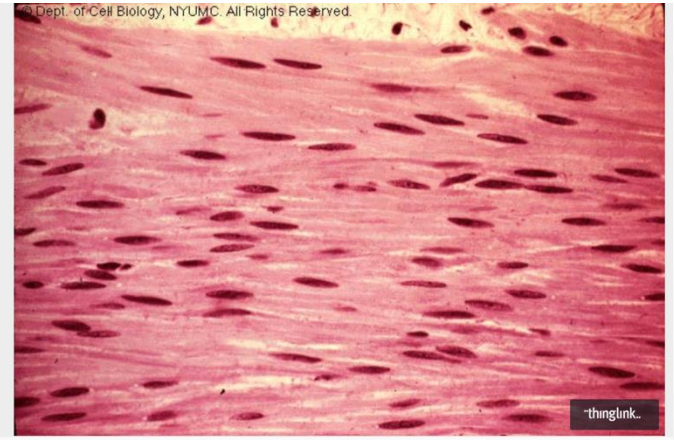
Staining And Stains-special stains

- **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.

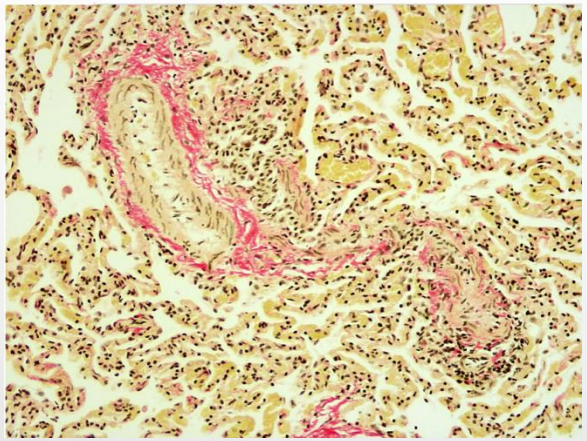
H&E PAS STAINING!



Examples Of Commonly Used Histological Stains

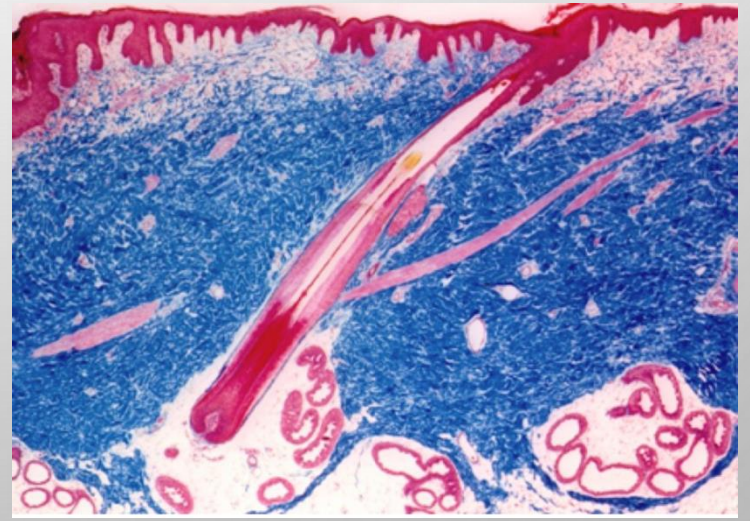


- Van Gieson method: collagen/pink, muscle/yellow.



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

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



MICROSCOPES

Types of microscope

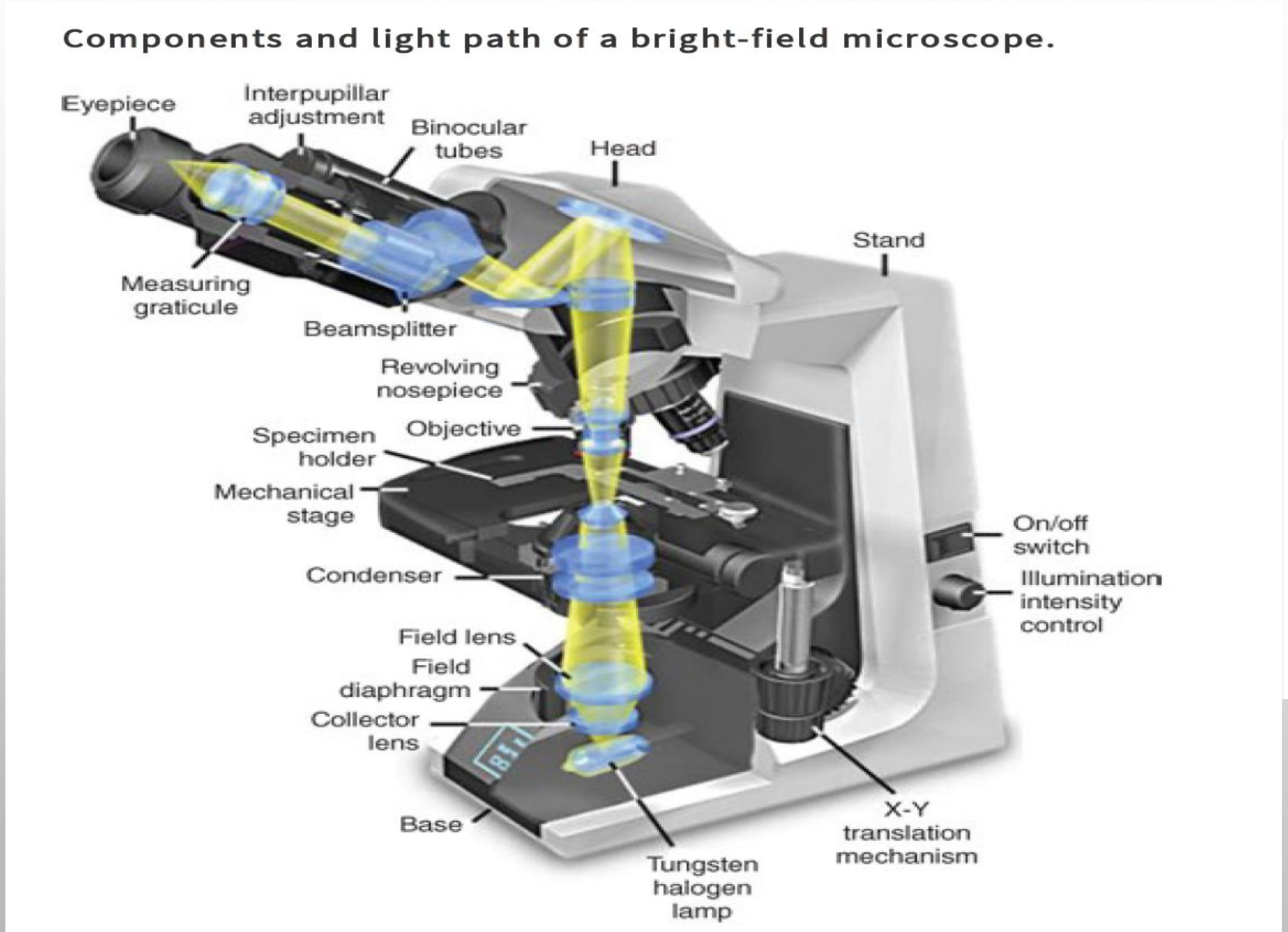
- **Light microscope.**

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

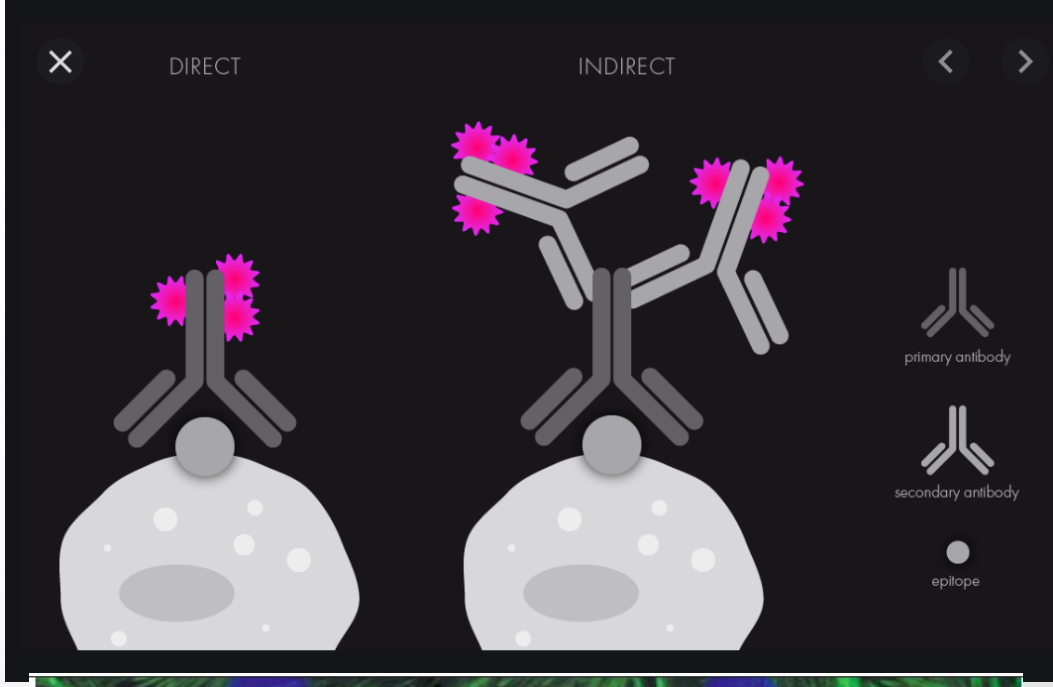
Light Microscope (Bright-field)



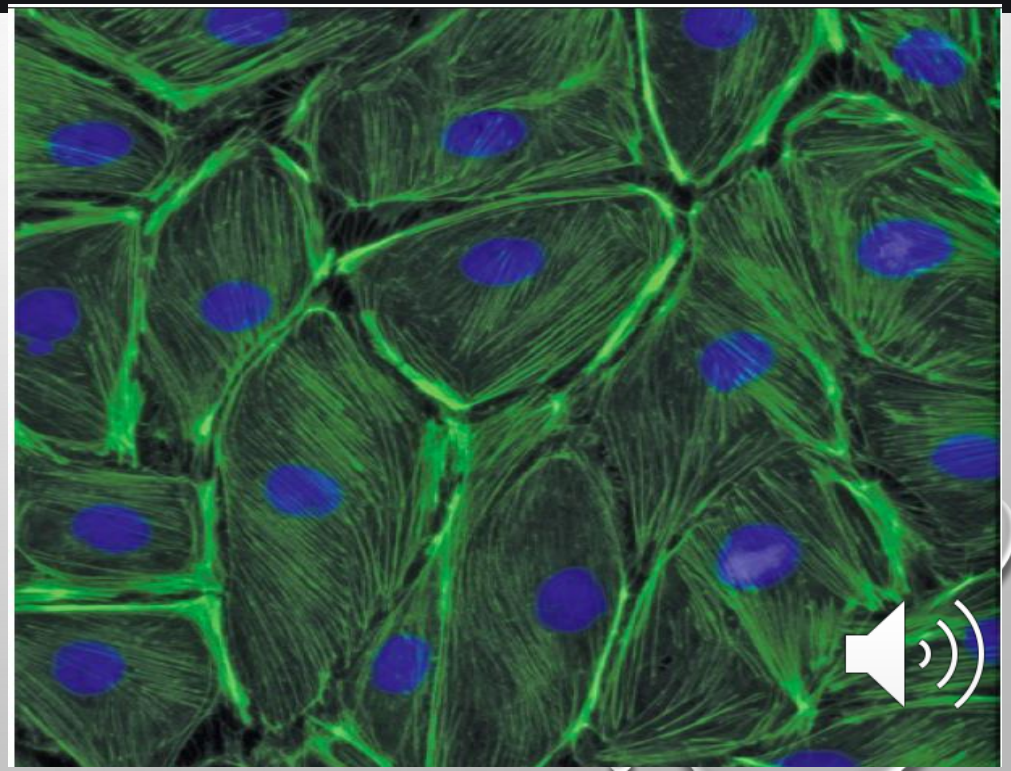
Bright-field Light Microscope

- 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.

Fluorescence Microscopy

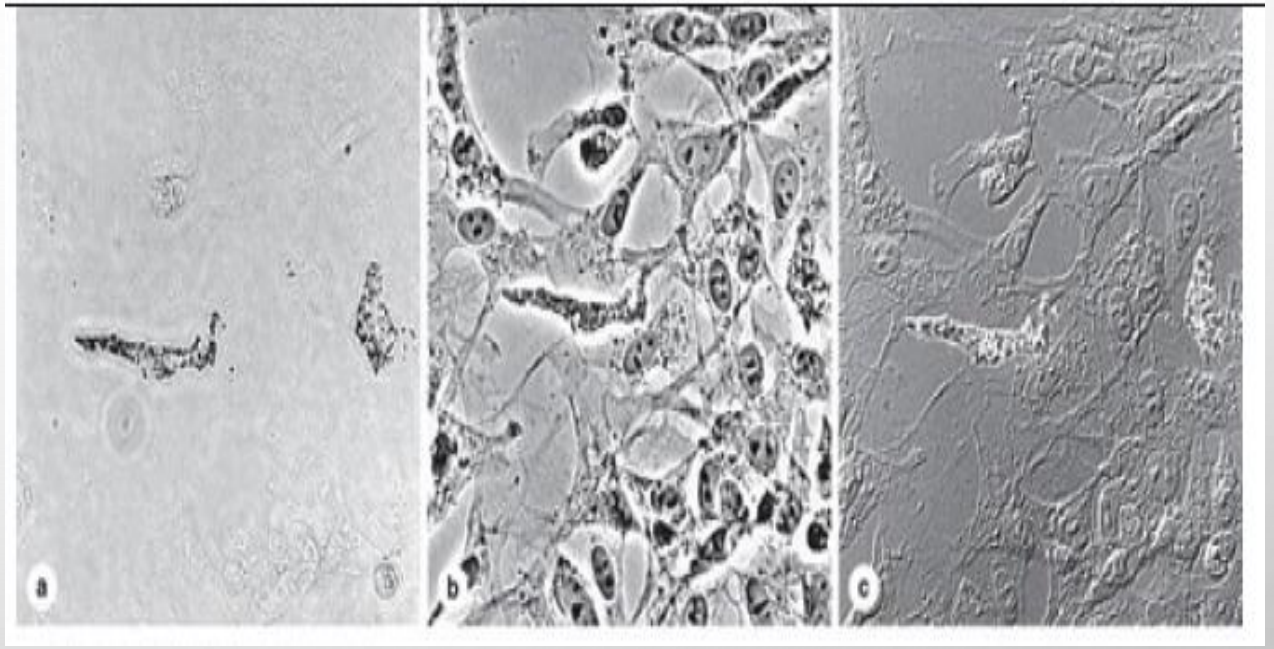


- **Fluorescence**: when certain cellular substances are irradiated by light of a proper wavelength, they emit light with a longer wavelength.
- In fluorescence microscopy, tissue sections are irradiated with
- Ultraviolet (UV) light and the emission is in the visible portion of the spectrum.
- The fluorescent 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.

Elecetron Microscope

- Interaction of tissue with a beam of electrons.

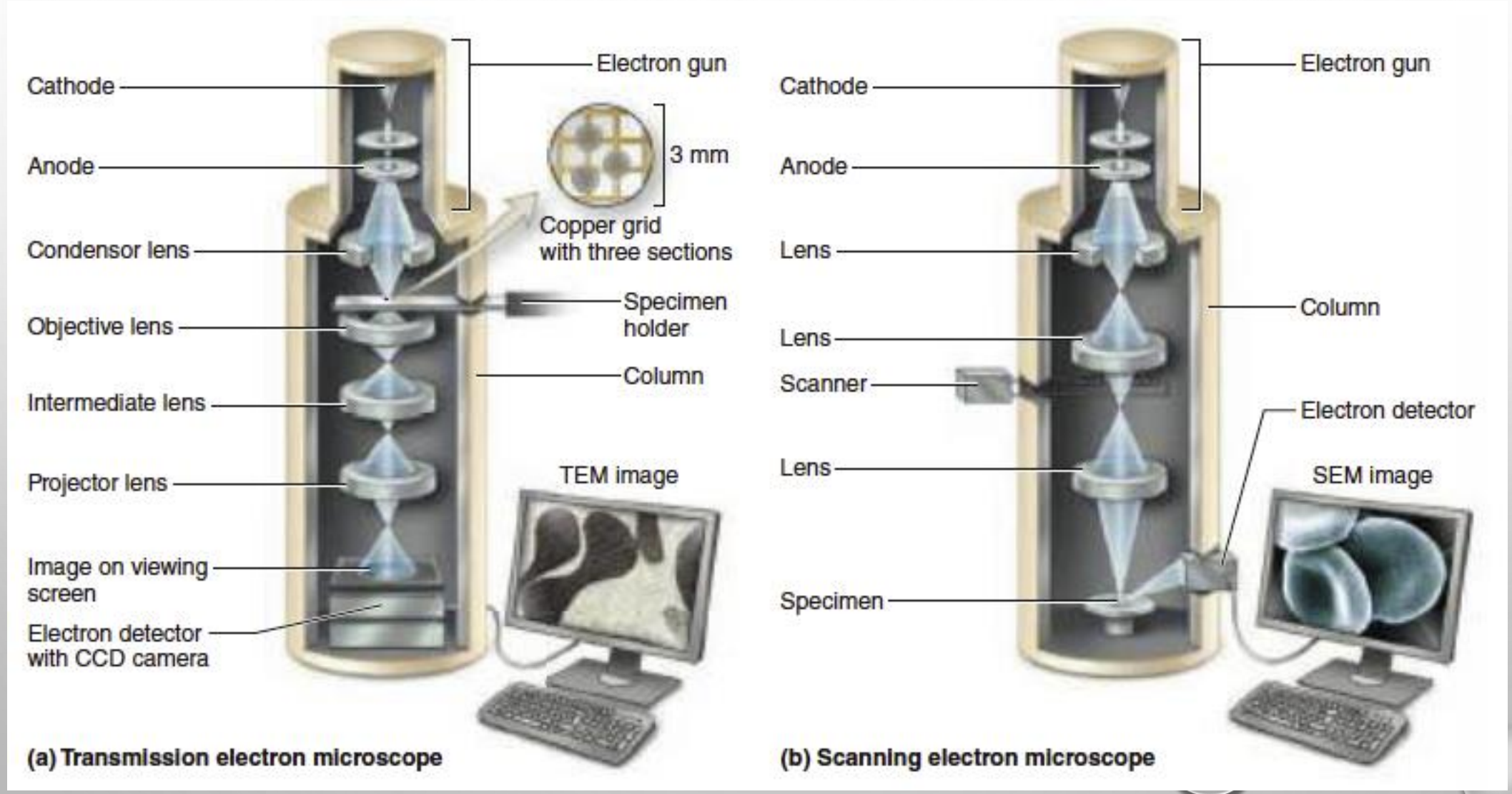
TEM

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

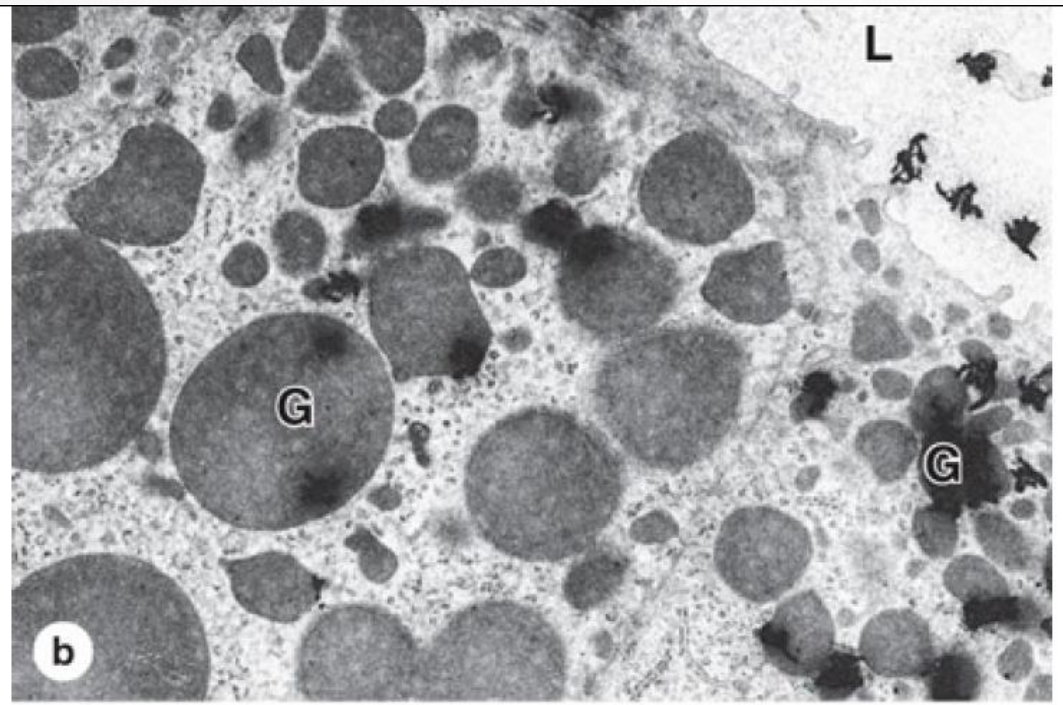
SEM.

- 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

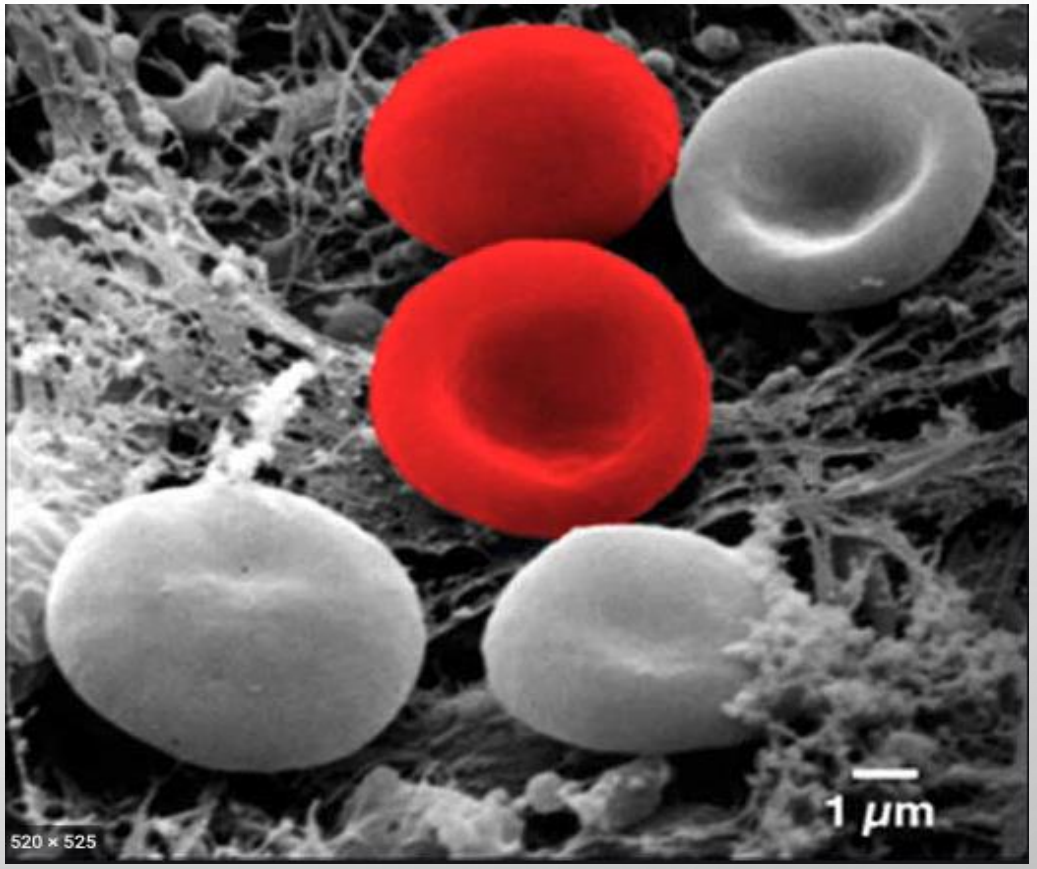


TEM



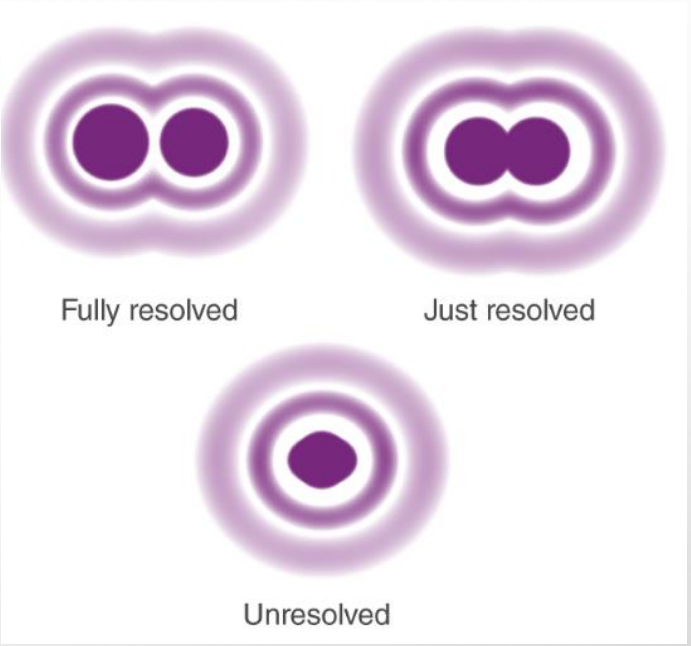
Source: Anthony L. Mescher: Junqueira's Basic Histology: Text and Atlas, 15th Edition.

SEM



Resolution

- **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.
- 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).