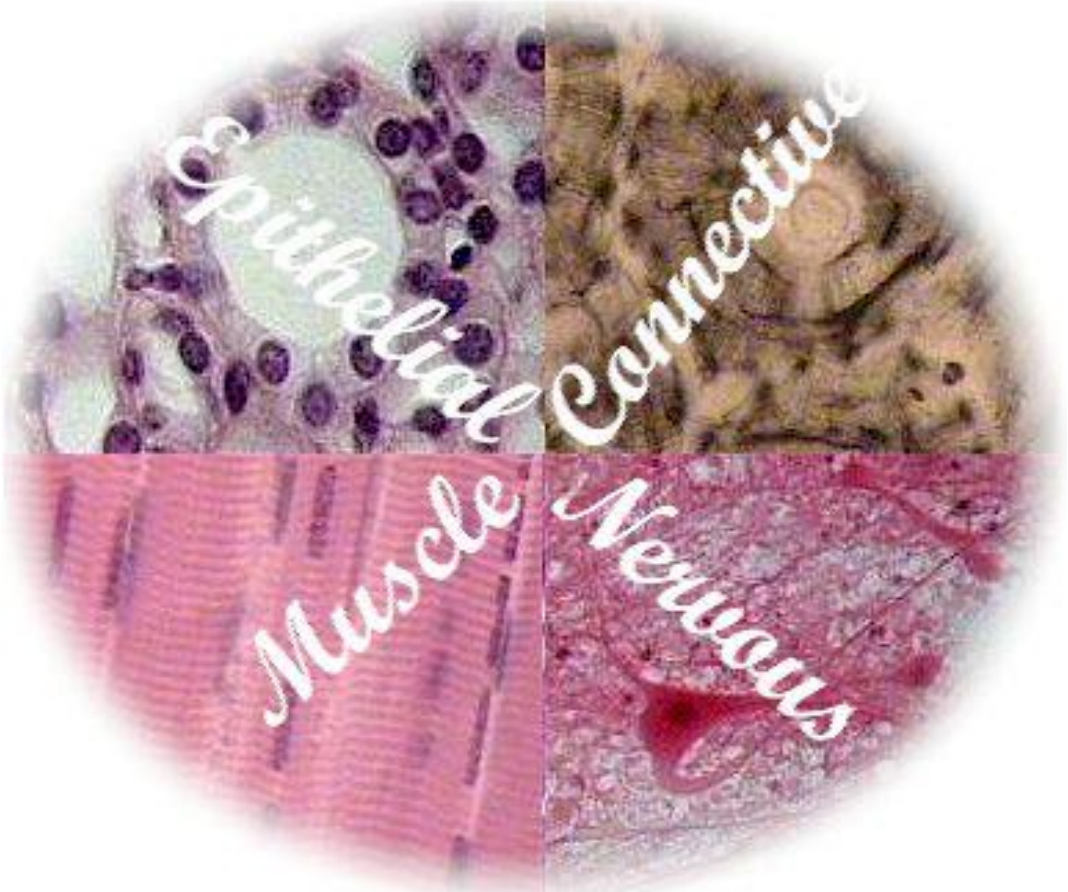
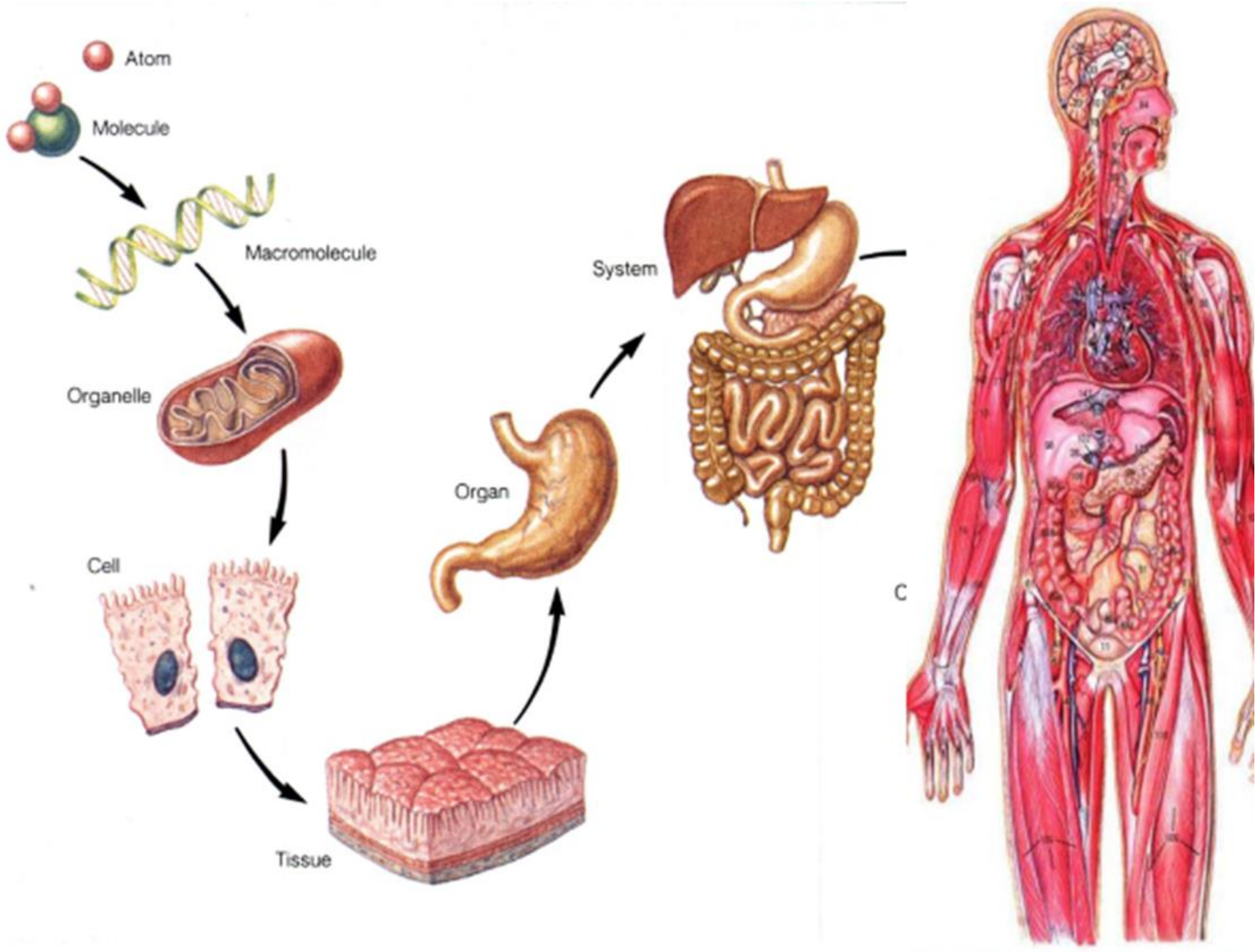
Subject and goals of Histology

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



Body organization



Histology

The fundamental aim of **histology** is to determine how tissues are organized at all structural levels, from cells and intercellular substances to organs.

Knowledge of microscopic structure and function of the basic tissues and organs is necessary to practice a wide variety of medical, pathologic, and surgical specialties.

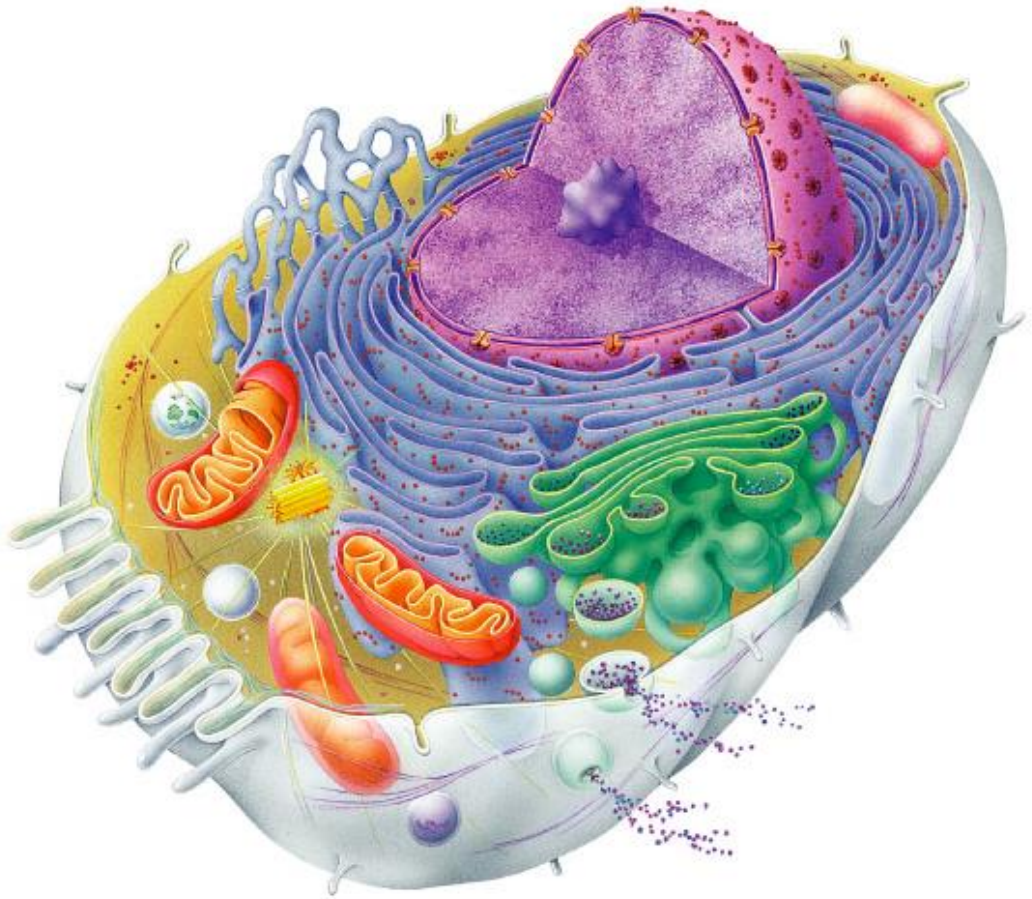
Cytology- the study of cells

Cell - functional unit of the body

- approximately 200 different cell types in the body
- cells vary in size and shape according to location and function

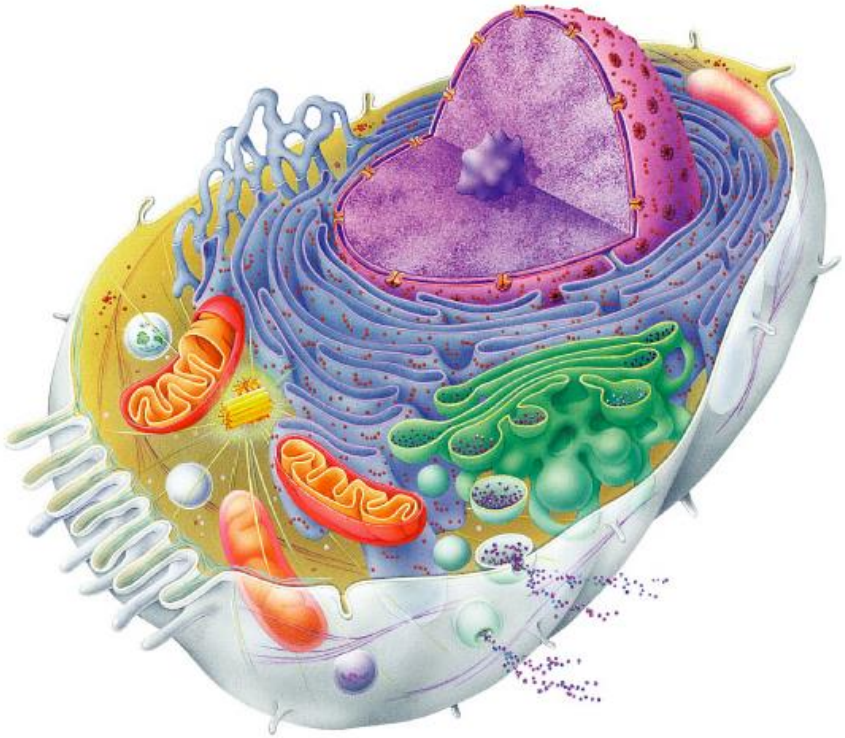
the largest - the mature human ovum (120 microns)

the smallest - the red blood cell (7-8 microns)



Cells have three major compartments:

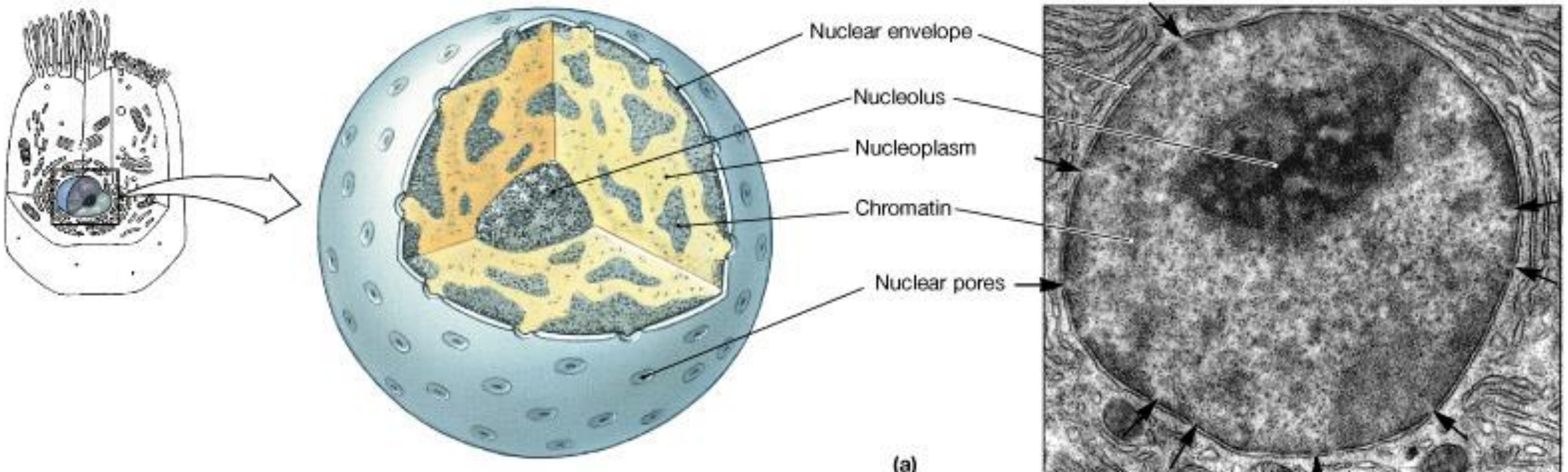
- **the cell membranes,**
- **the cytoplasm**
- **the nucleus.**



The cytoplasm contains ***organelles*** *(*“little organs”) and ***inclusions*** in an aqueous gel called the ***cytosol***.

Nucleus (= center)

- Houses the DNA
- produces ribosomes and messenger RNA
- Visible with LM
- Membrane bound
- Many pores
- 23 Pairs of Chromosomes (Except gametes)
- Nucleolus

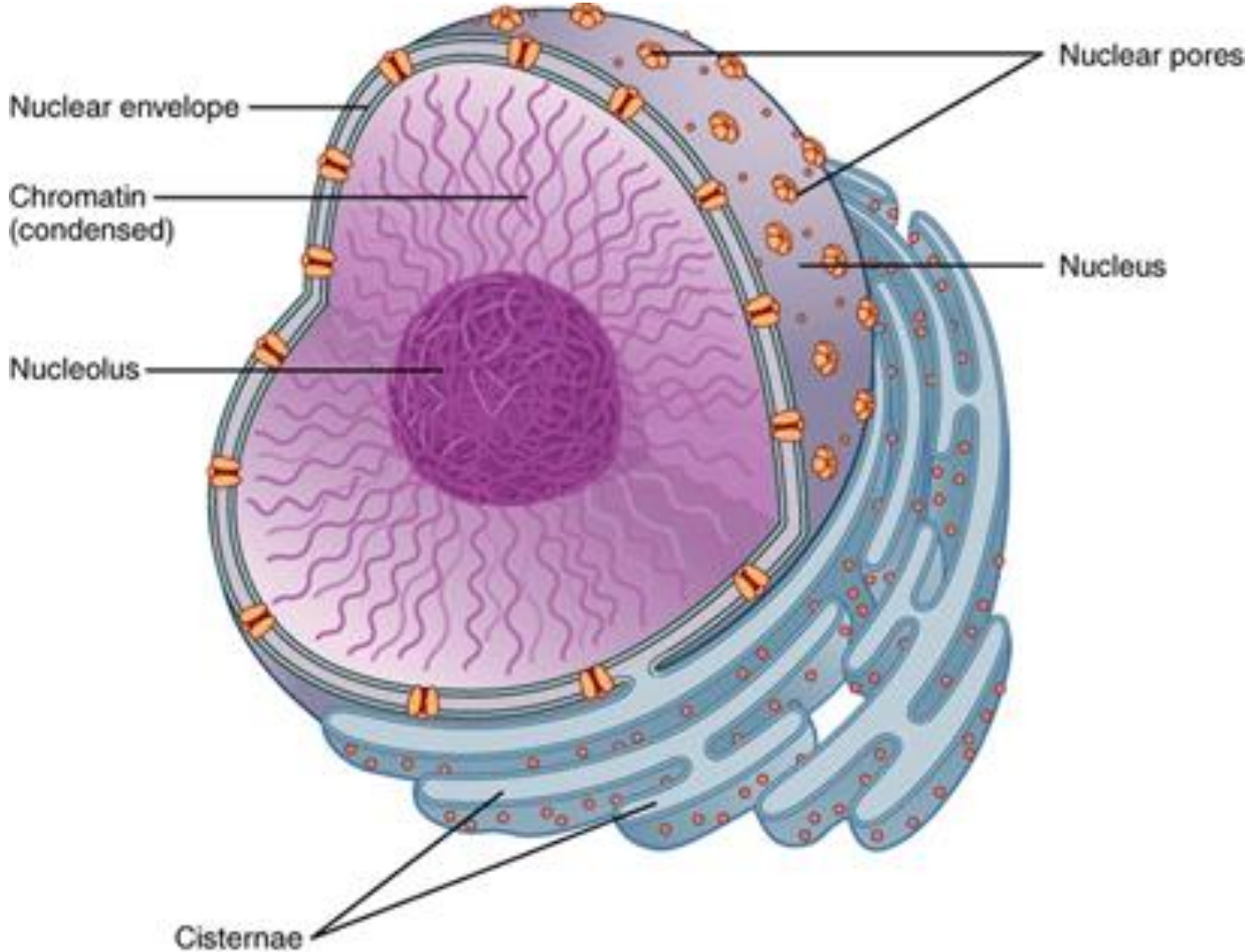


Chromatin is a complex of DNA and protein (known as histone)

Chromatin is the packaging of DNA

Chromatin condensation:

- **Euchromatin** is less condensed and can be transcribed (for cells with high transcriptional activity)
- **Heterochromatin** is highly condensed and cannot typically be transcribed. (for cell with low transcriptional activity)

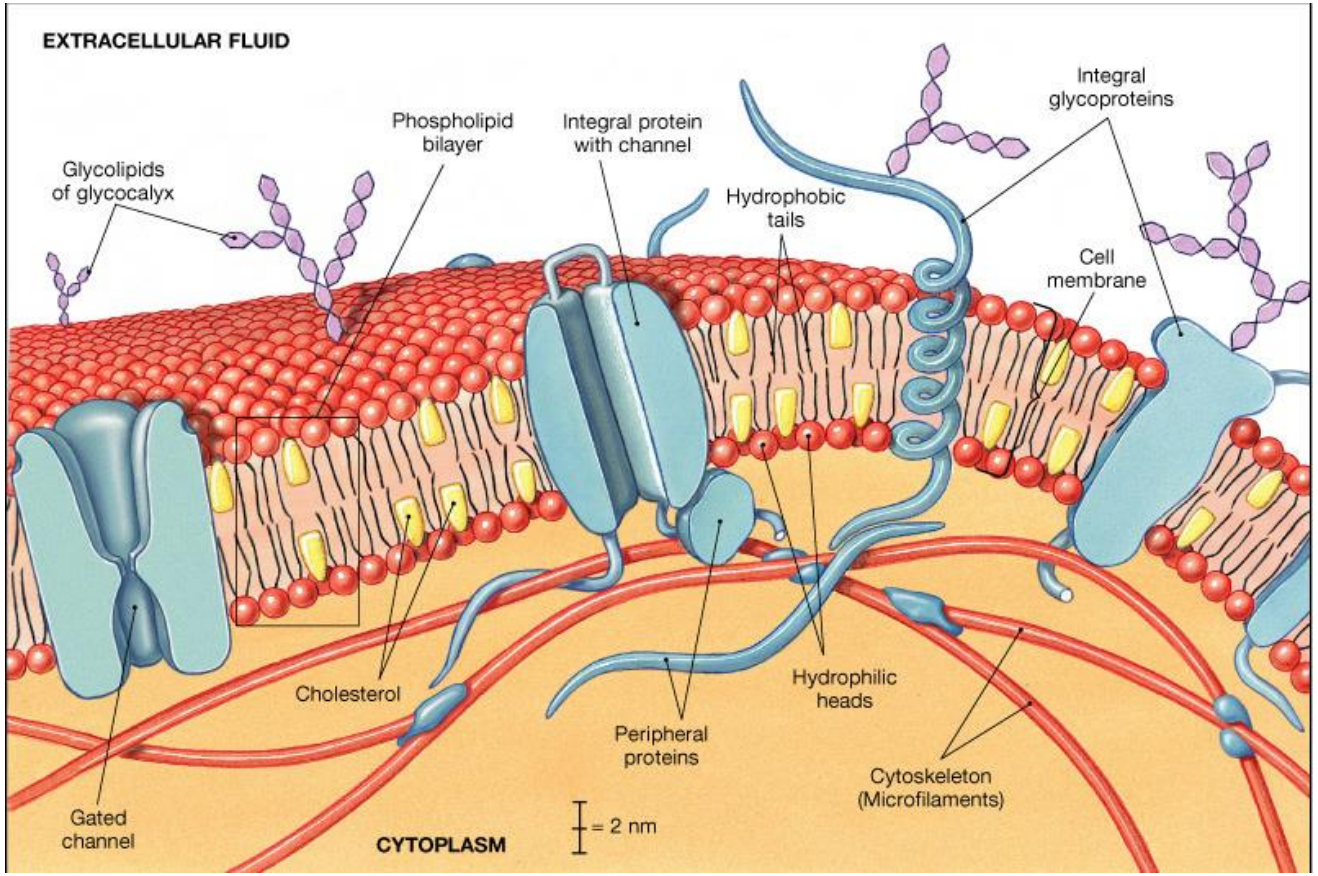


The plasma (cell) membrane

a lipid bilayer that forms the cell boundary as well as the boundaries of many organelles within the cell

Biochemical components:

1. **Lipids** (phospholipids, sphingolipids, cholesterol)
2. **Proteins** (integral membrane proteins (*transmembrane proteins*), peripheral membrane proteins)
3. **Carbohydrates**



The plasma (cell) membrane functions:

- **Membrane transport**
- **Cell adhesion** *(Proteins provide cell-to-cell attachment and cell-to-extracellular matrix anchorage)*
- **Intercellular communication** *(Transmembrane proteins assemble to form pores (gap junctions) between cells)*
- **Signal transduction** *(Following interaction with extracellular signals, e.g., hormones and growth factors, receptor proteins initiate intracellular signaling pathways)*

Membrane transport

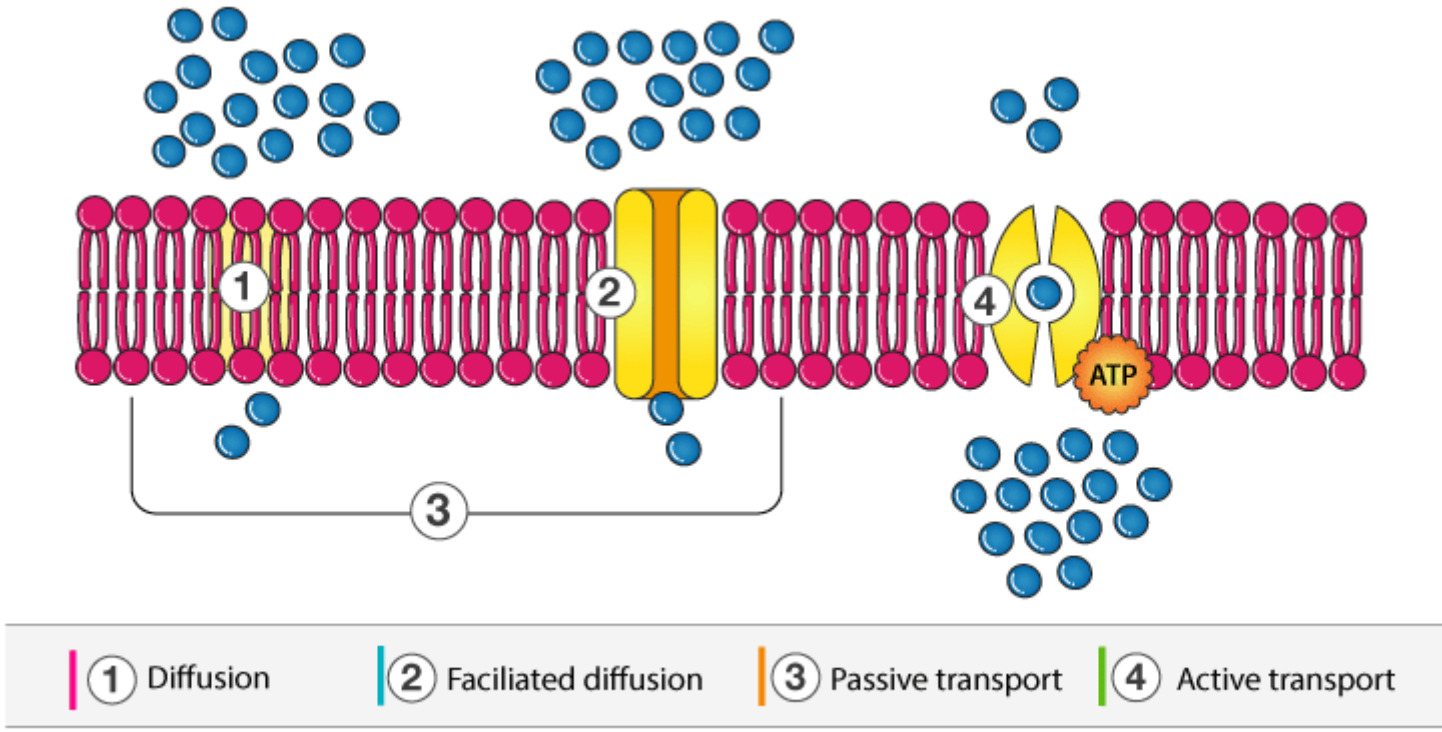
1. Diffusion

a. Passive diffusion

b. Facilitated diffusion. Utilizes transmembrane proteins to increase the permeability of the membrane to certain materials.

2. Active transport

Energy-requiring process of moving materials across the membrane.



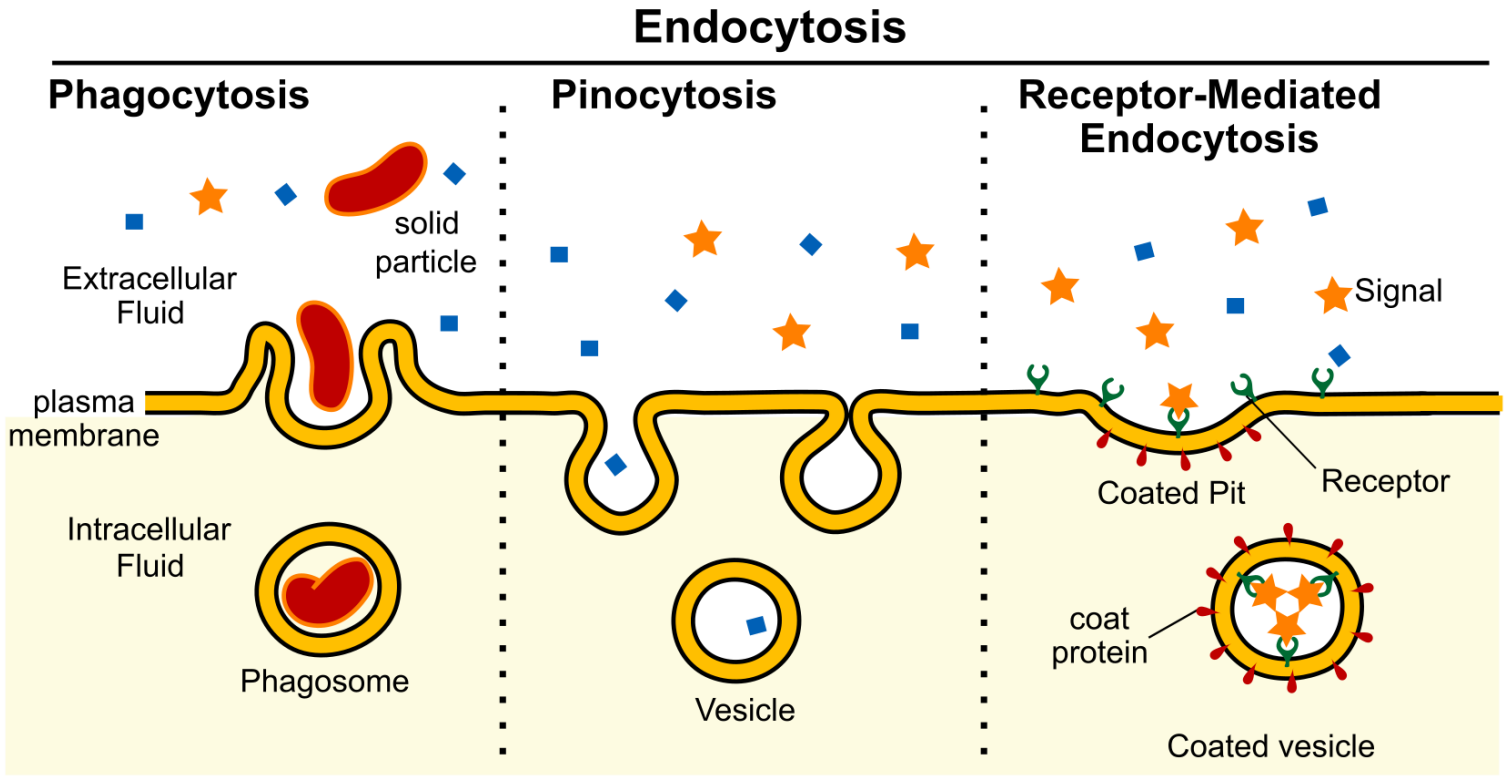
Membrane transport

3. Vesicular transport

a. Endocytosis. Internalization of small membrane vesicles formed from the plasma membrane

- Pinocytosis (cell drinking). Uptake of fluid into the cell by a continuous process
- Receptor-mediated endocytosis. Requires receptor-ligand binding for vesicle formation and internalization

b. Phagocytosis (cell eating). Ingestion of large particles (e.g. bacteria) into the cell; prominent in some macrophages and white blood cells.



Membrane transport

3. Vesicular transport

c. Exocytosis. Fusion of cytoplasmic vesicles with the plasma membrane and release of the vesicle contents to the outside of the cell

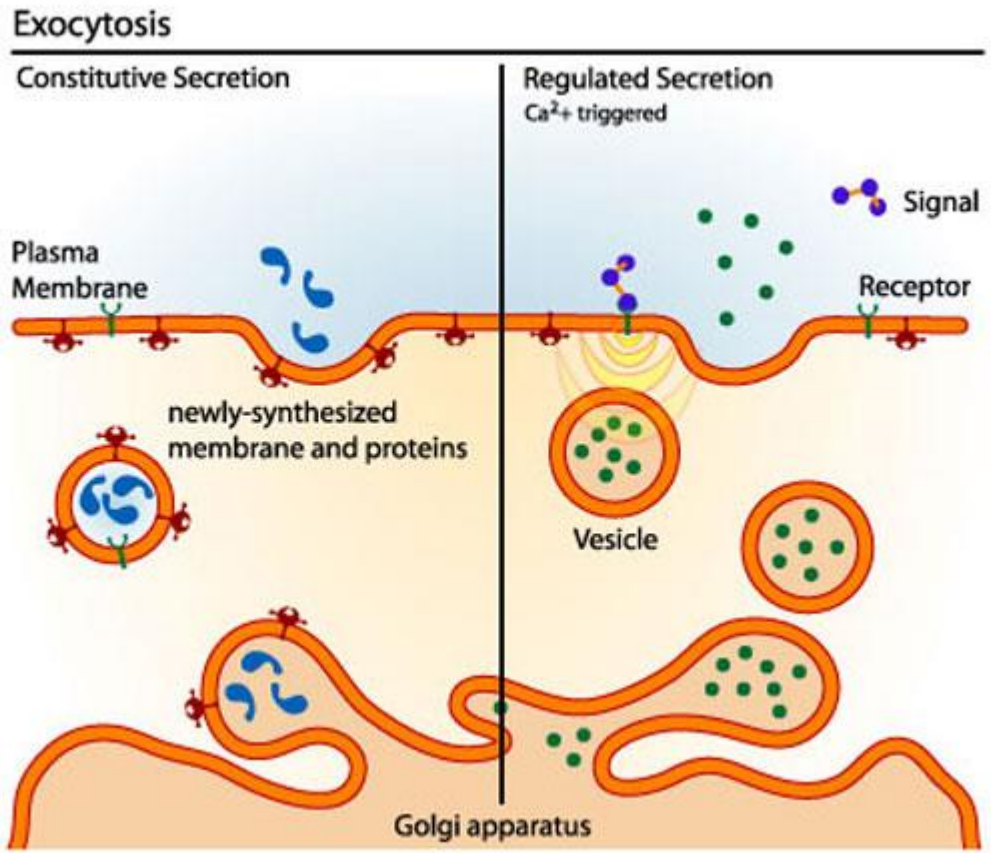
- Constitutive exocytosis. Continuous process that renews the

plasma membrane.

- Regulated exocytosis. Requires an extracellular signal for vesicle

fusion and release (e.g., hormone secretion)

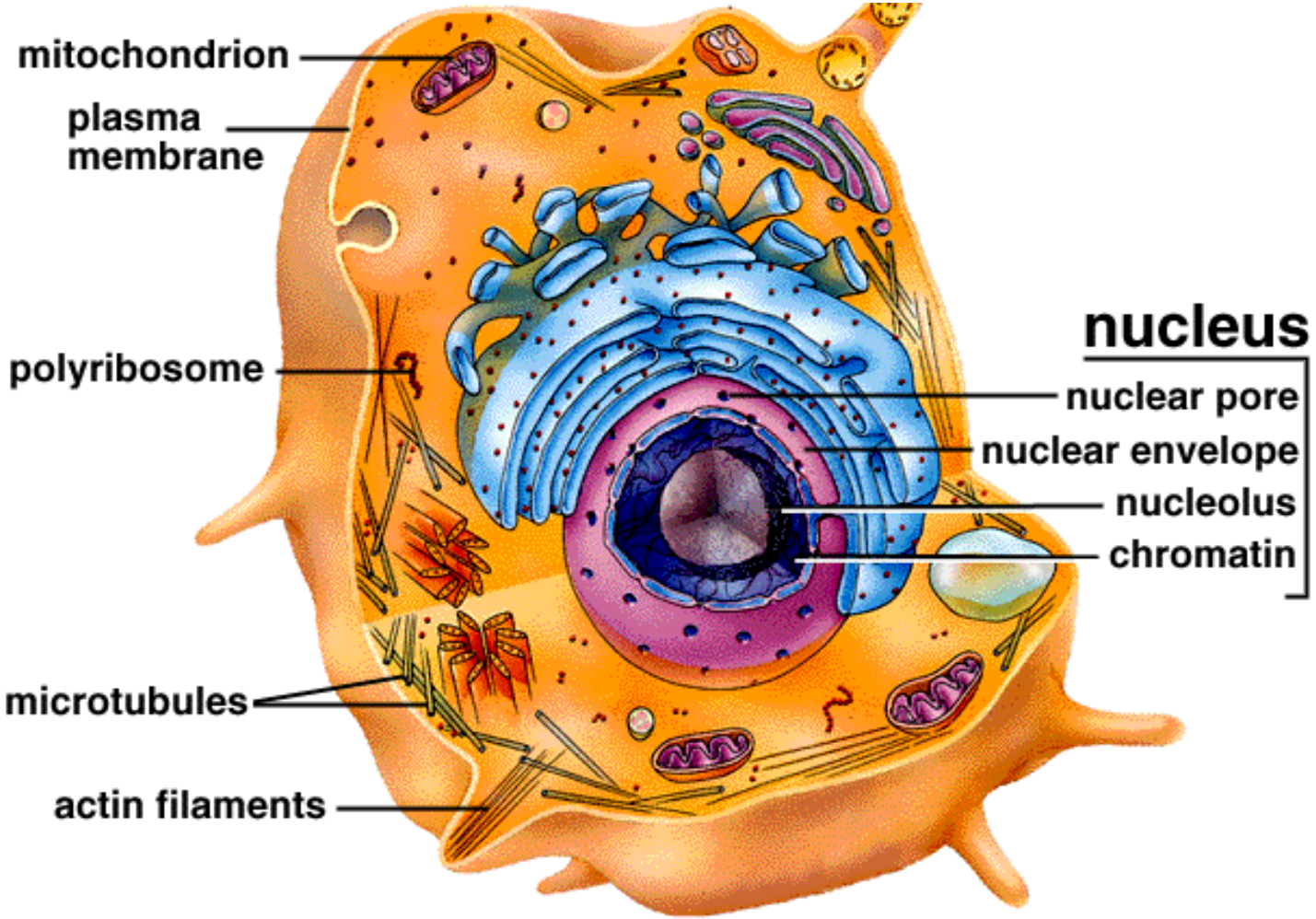
d. Transcytosis. Uptake of material on one side of a cell followed by transport and release from the opposite surface



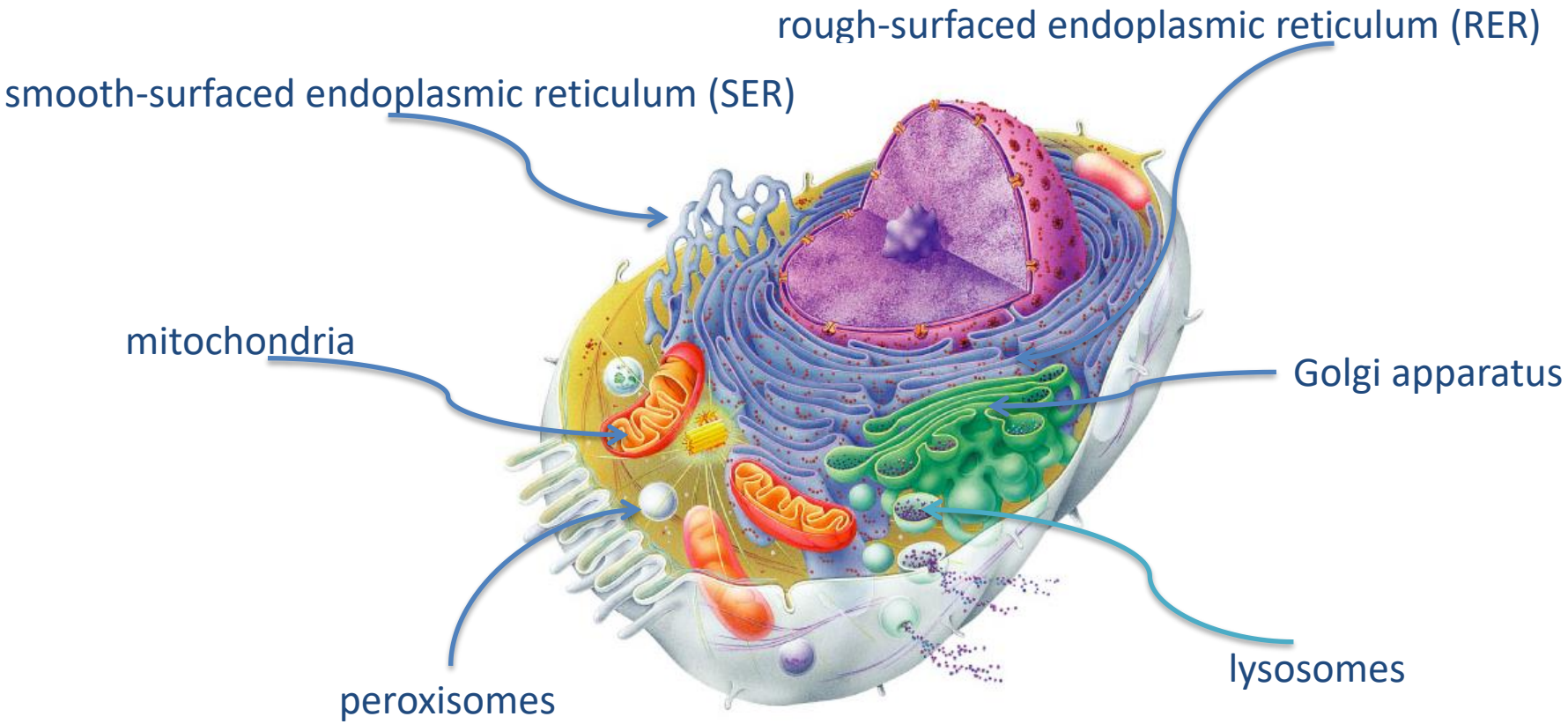
Organelles

membranous organelles
with plasma membranes that separate the internal environment of the organelle from the cytoplasm

nonmembranous organelles
without plasma membranes

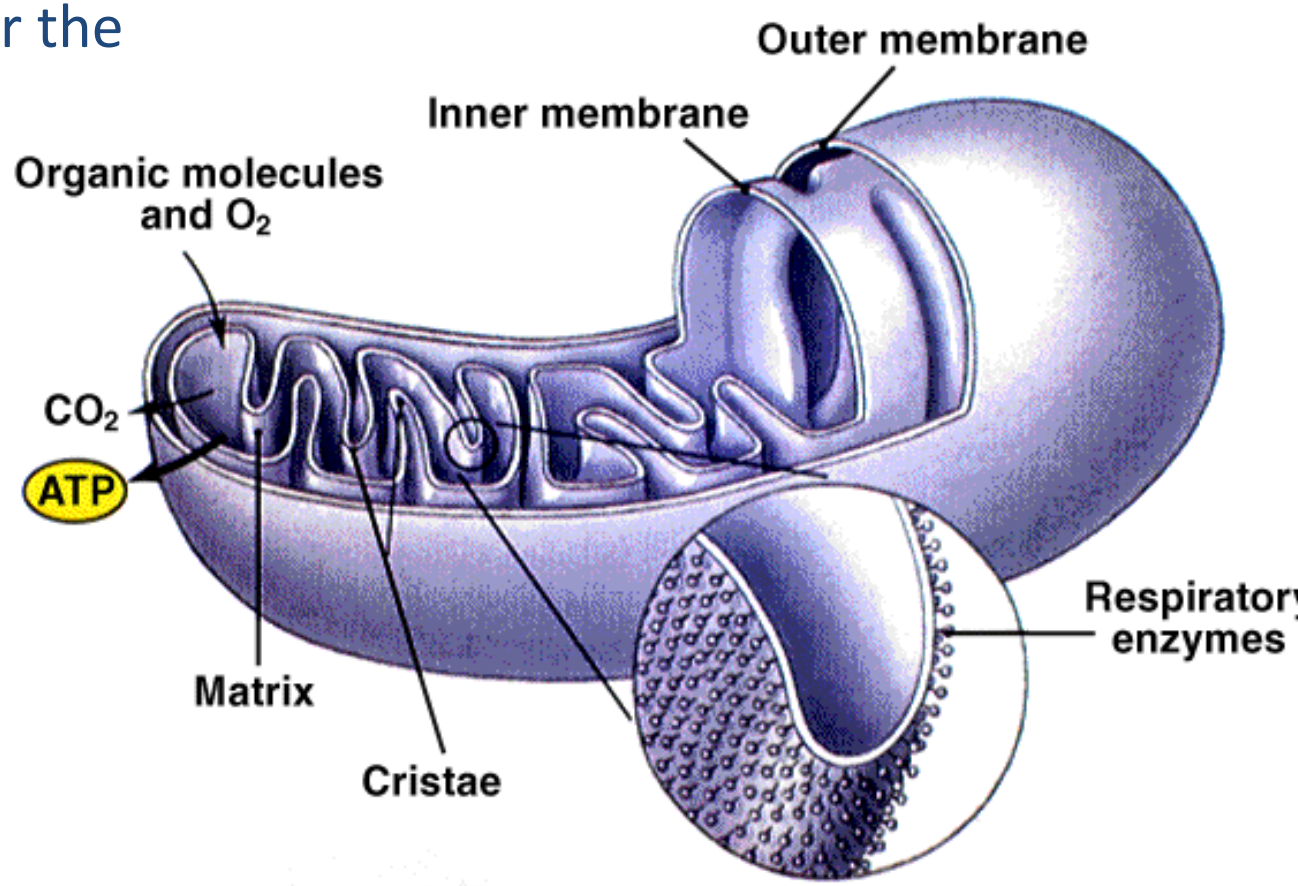


The membranous organelles:



Mitochondria

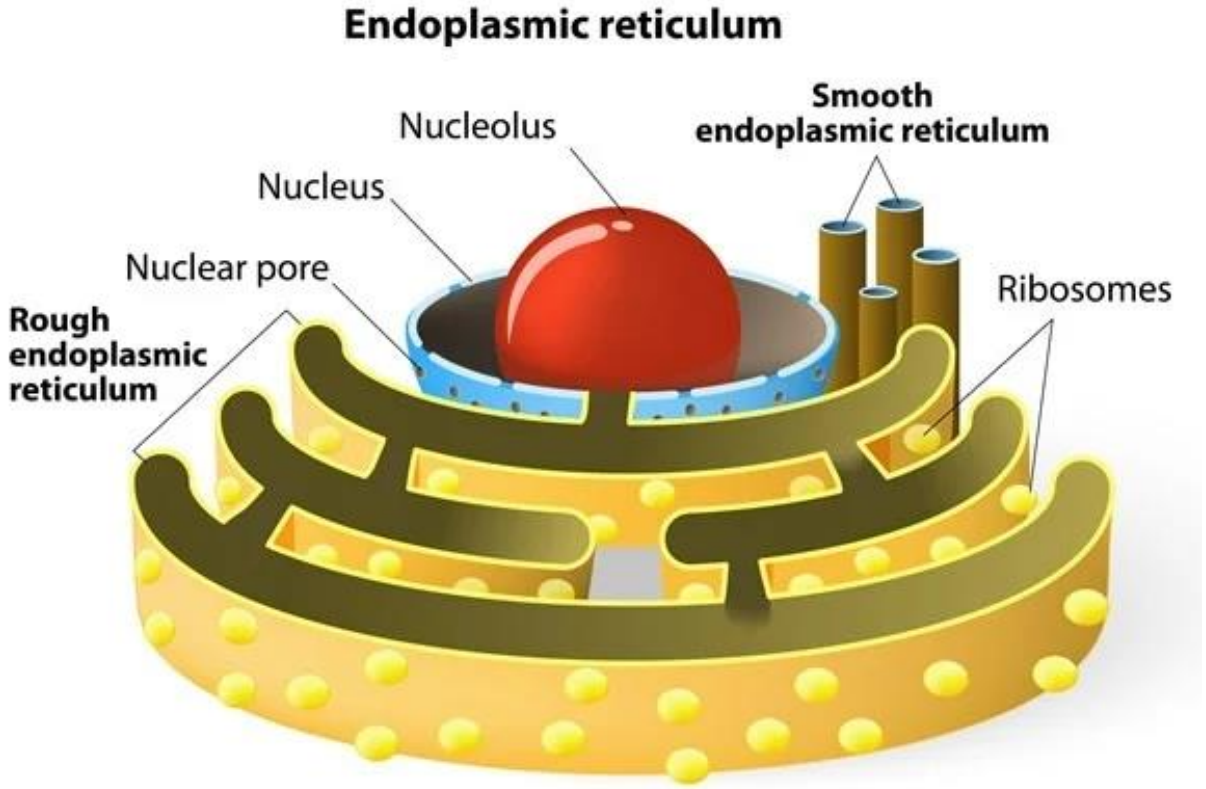
- “The powerhouses” of the cell
- They produce energy-rich molecules for the cell
- An oval-shaped
- A double membrane-bound organelle
- Has own genome
- Self-replicating
- The mitochondrial genome is inherited maternally



Endoplasmic reticulum

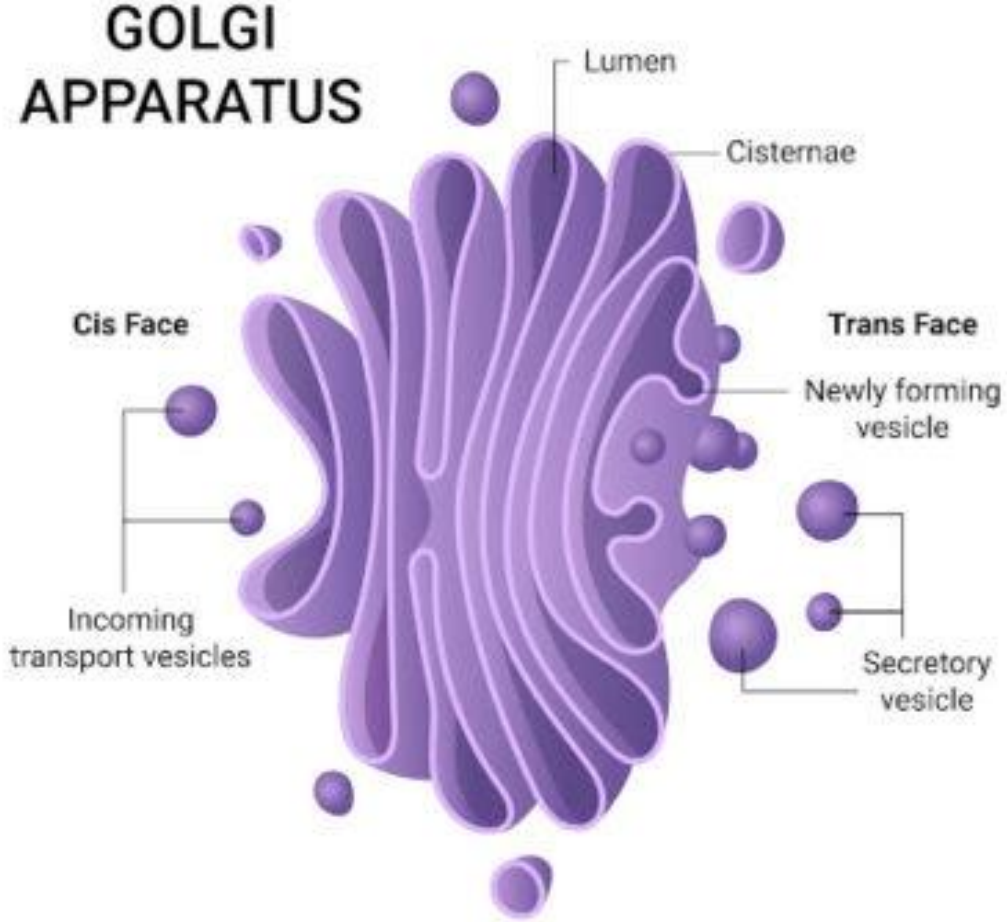
A network of membranous tubules, present within the cytoplasm of a cell

- **Rough-surfaced endoplasmic reticulum (RER)**, a region of endoplasmic reticulum associated with ribosomes and the site of protein synthesis and modification of newly synthesized proteins;
- **Smooth-surfaced endoplasmic reticulum (SER)**, a region of endoplasmic reticulum involved in lipid and steroid synthesis, detoxification, stores and mobilizes calcium, but not associated with ribosomes.



Golgi apparatus (Golgi Complex)

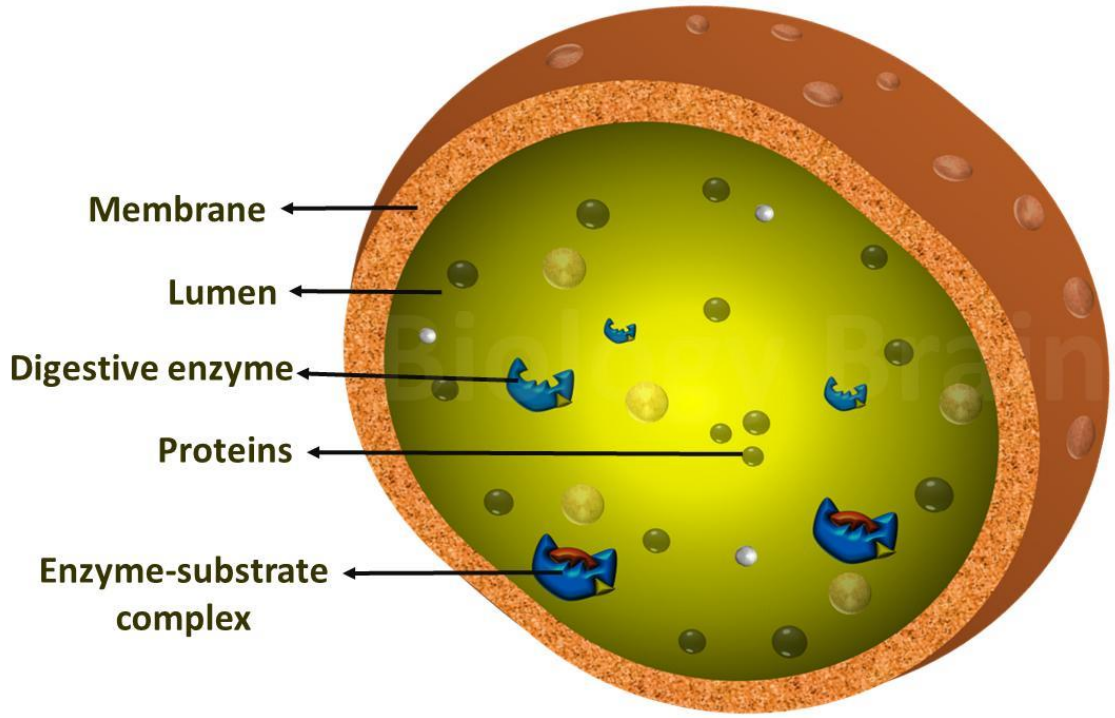
a membranous organelle composed of multiple flattened cisternae responsible for modifying, sorting, and packaging proteins and lipids for intracellular or extracellular transport



Lysosomes

small organelles containing digestive enzymes that are formed from endosomes by targeted delivery of unique lysosomal membrane proteins and lysosomal enzymes

- break down macromolecules into smaller molecules, which are then used to nourish the cell
- digest peptides, nucleic acids, carbohydrates, and lipids
- break down organelles that have reached the end of their life
- help fight off invading bacteria.



PRIMARY LYSOSOMES
VERSUS
SECONDARY LYSOSOMES

PRIMARY LYSOSOMES	SECONDARY LYSOSOMES
A membrane-bound sac, which buds from Golgi apparatus	A lysosome formed by the combination of a primary lysosome and an endosome
Smaller	Larger
Contain inactive digestive enzymes in the form of granules	Contain active digestive enzymes
Do not undergo digestion	Undergo digestion
Unable to eliminate their content to the outside	Can eliminate their content to the outside of the cell

Peroxisome

the peroxisome is a spherical organelle responsible for destroying its contents

Peroxisome is the site of fatty acid breakdown

It protects the cell from reactive oxygen species (ROS)* molecules which could seriously damage the cell.

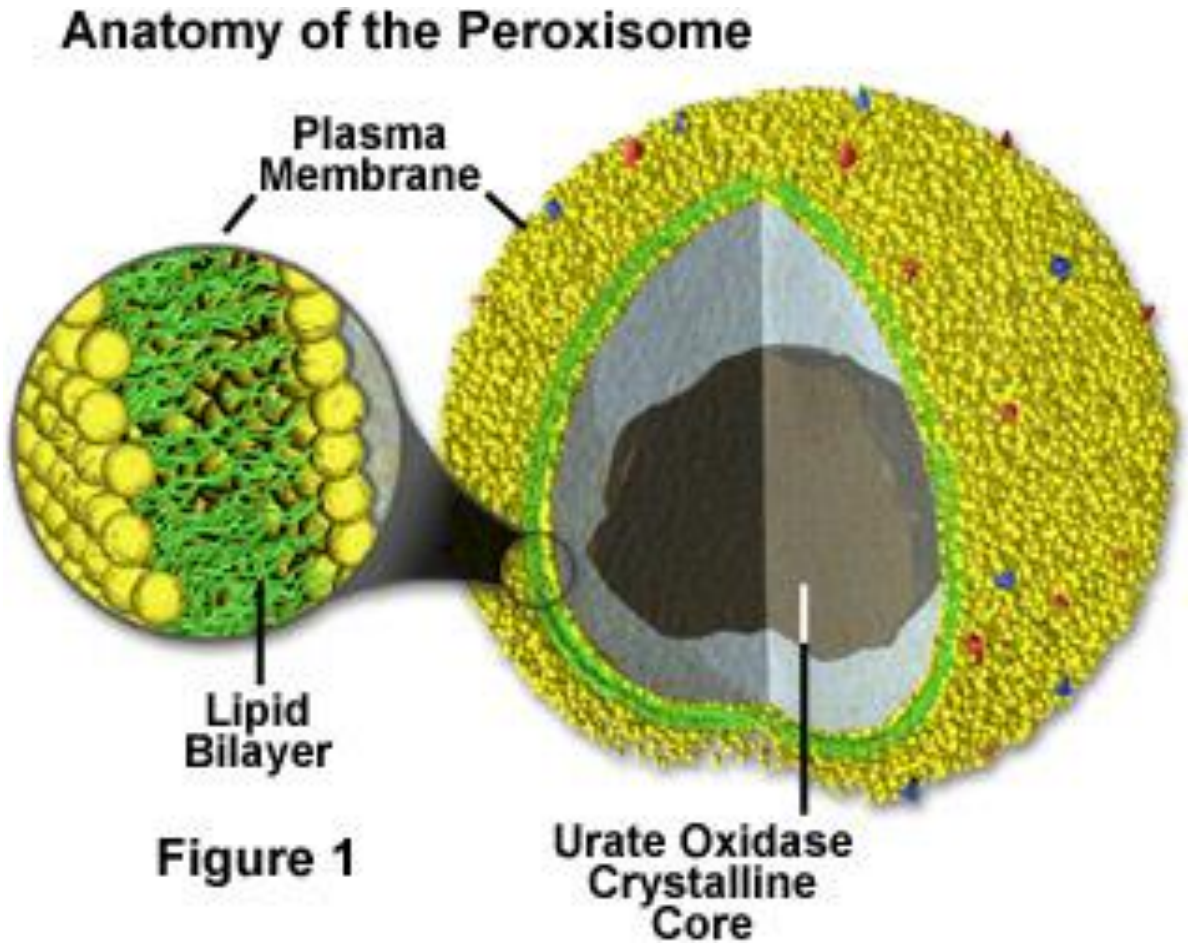
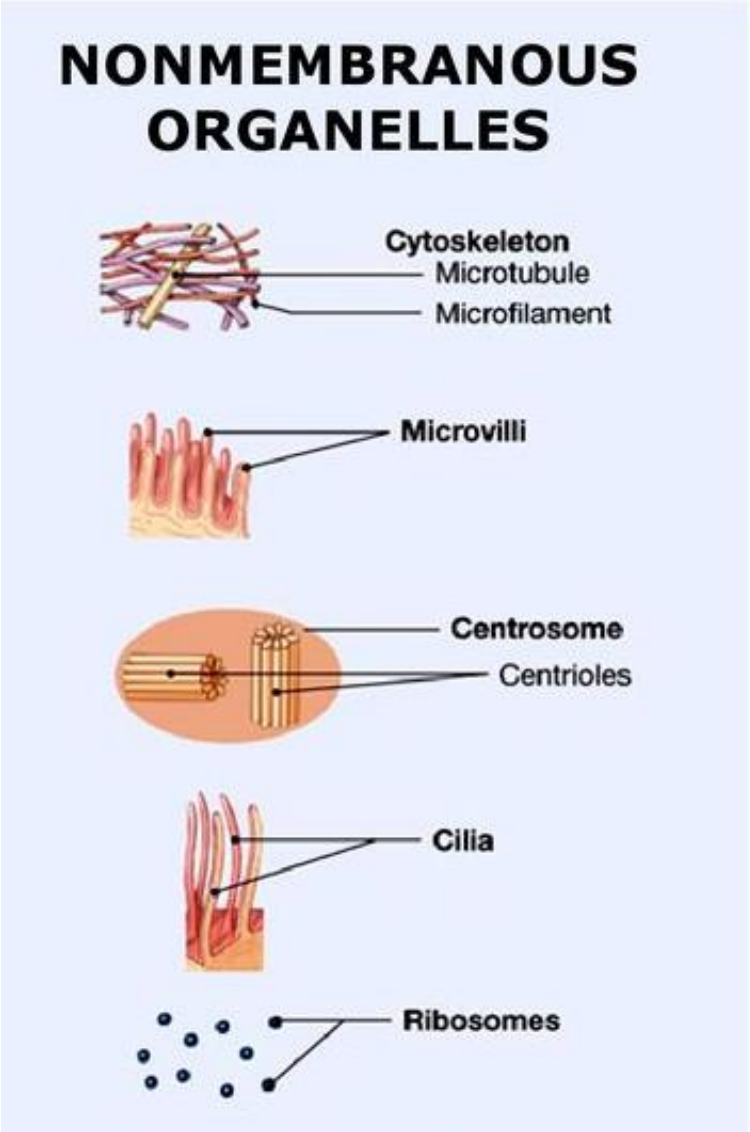


Figure 1

**ROSs are molecules like oxygen ions or peroxides that are created as a byproduct of normal cellular metabolism, but also by radiation, tobacco, and drugs. They cause what is known as oxidative stress in the cell by reacting with and damaging DNA and lipid-based molecules like cell membranes.*

The nonmembranous organelles

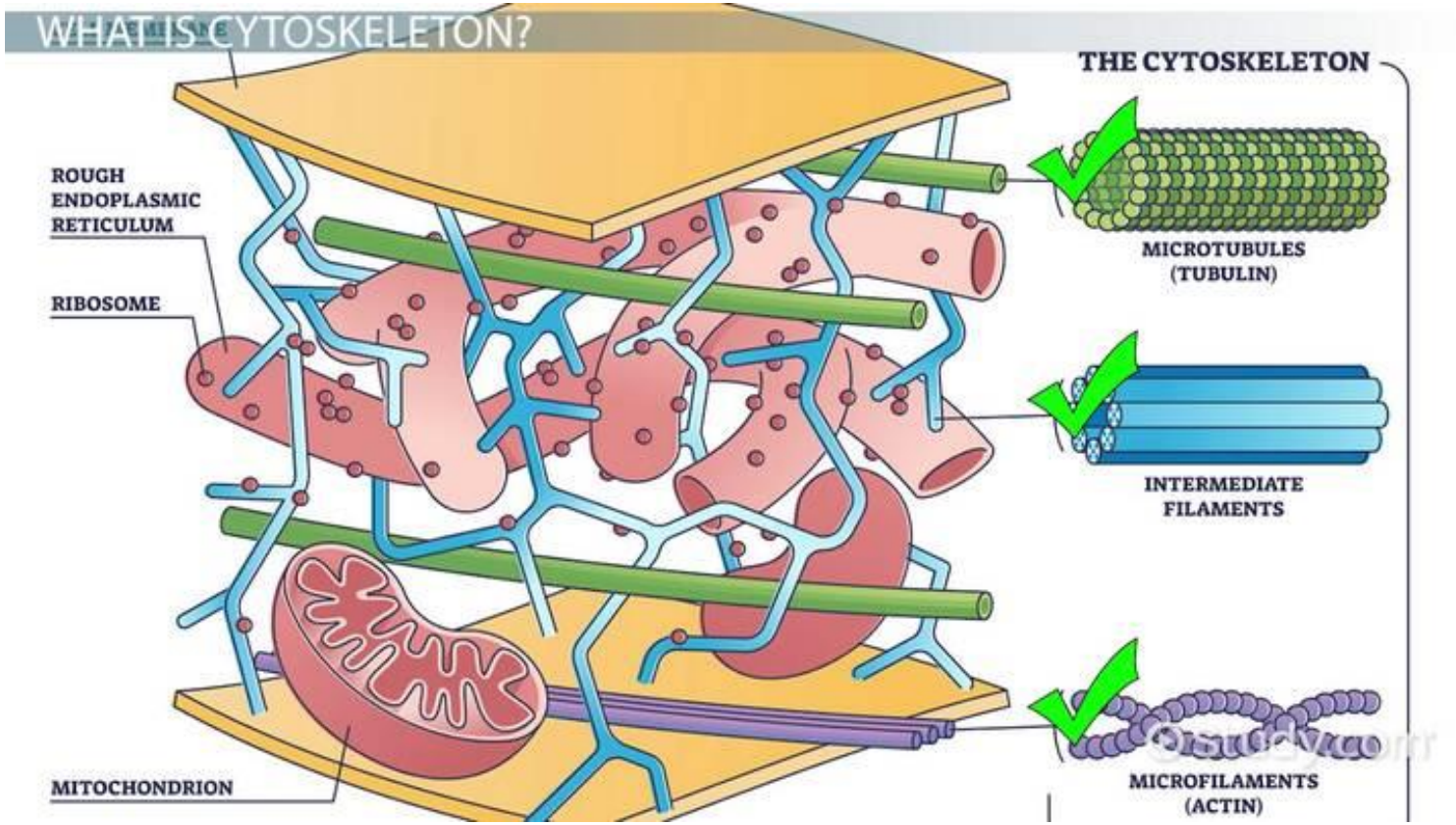


Cytoskeleton

3 major components:

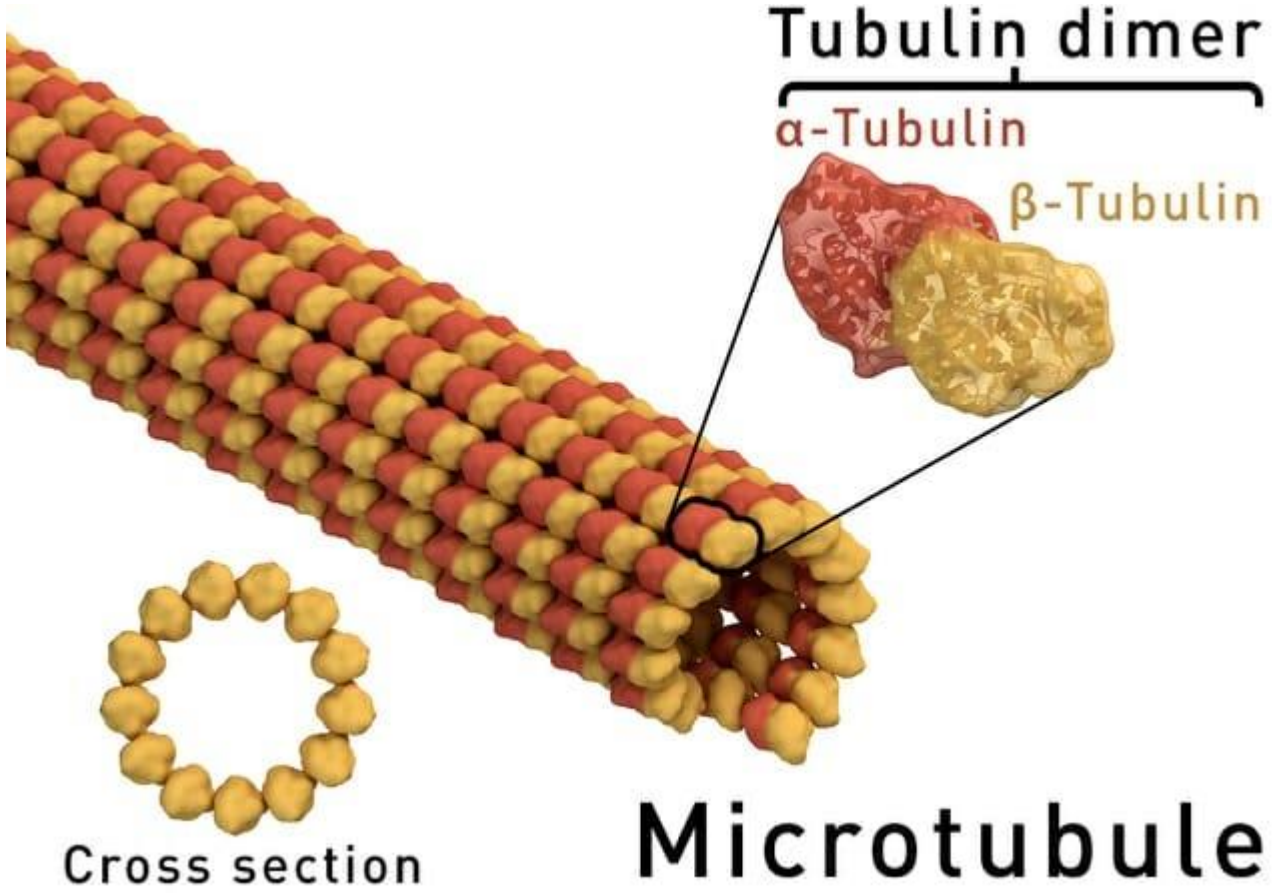
1. Microtubules (composed of tubulin subunits)
2. Intermediate filaments
3. Microfilaments (mostly actin)

Function: support & movement of cellular structures & materials



Microtubules

- Microtubules are small tubes made from the protein **tubulin.**
- Are found in **cilia and flagella**, structures involved in cell movement.
- Help provide pathways for secretory vesicles to move through the cell, and are even involved in cell division as they are a part of the **mitotic spindle**, which pulls homologous chromosomes apart.



Intermediate Filaments

- Smaller than the microtubules, but larger than the microfilaments.
- Are made of a variety of proteins*
- Very stable
- Help provide structure to the nuclear envelope and anchor organelles.

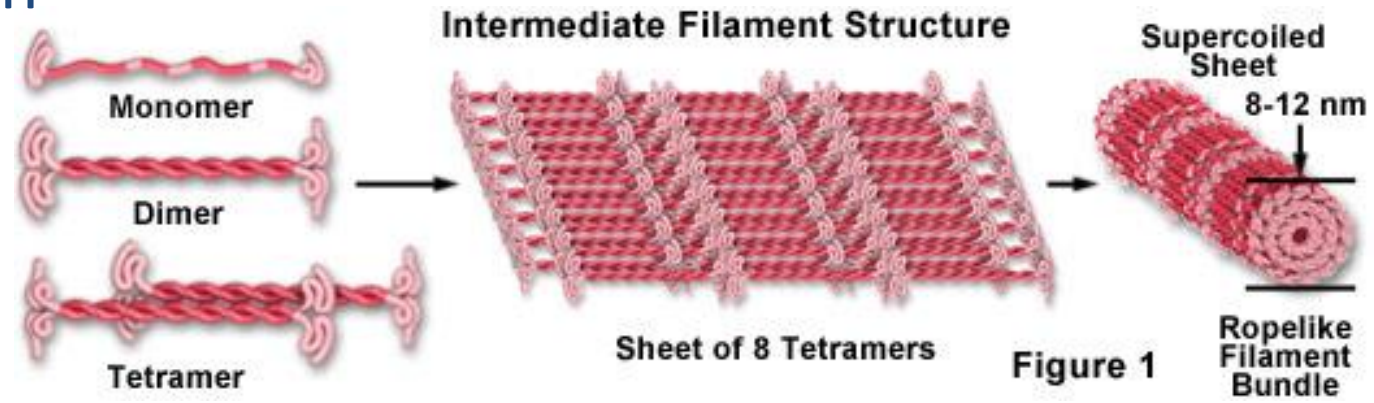
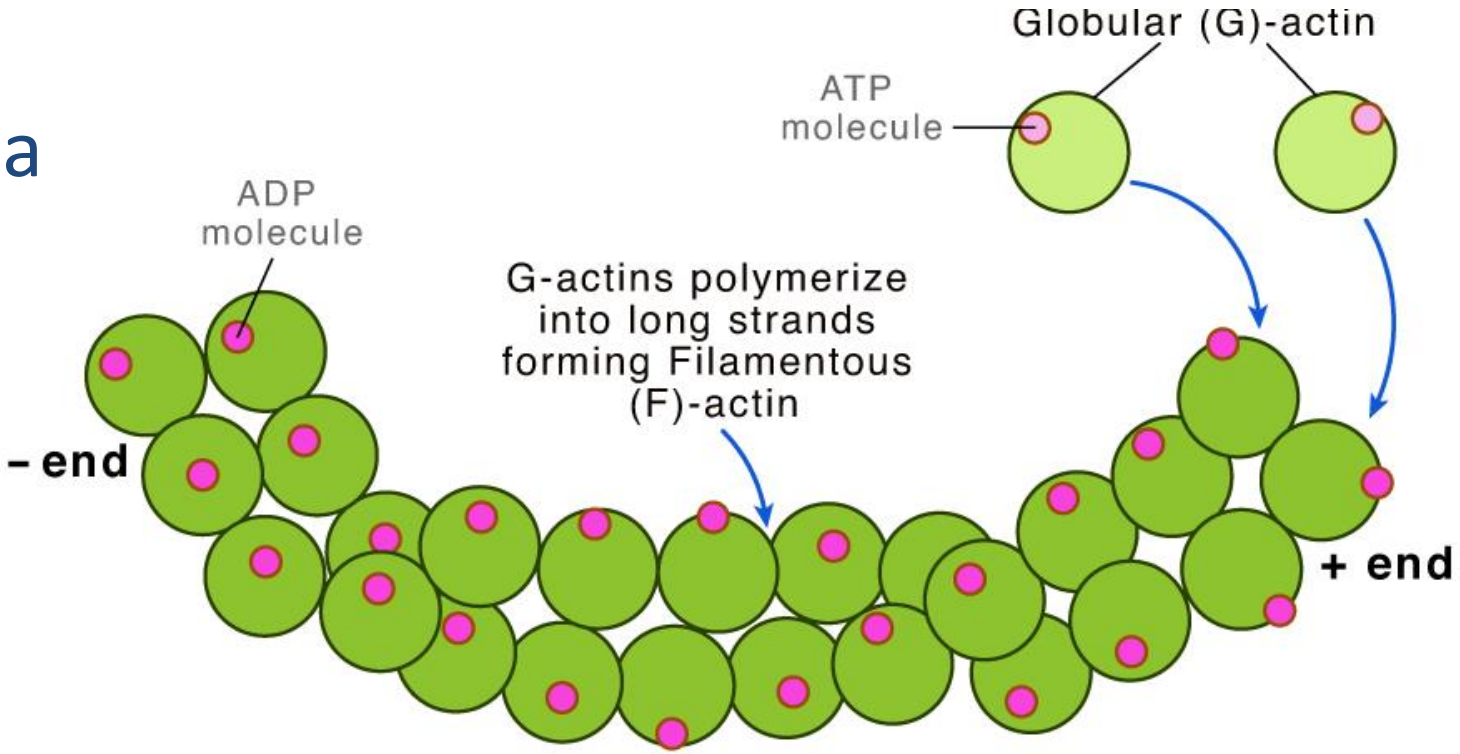


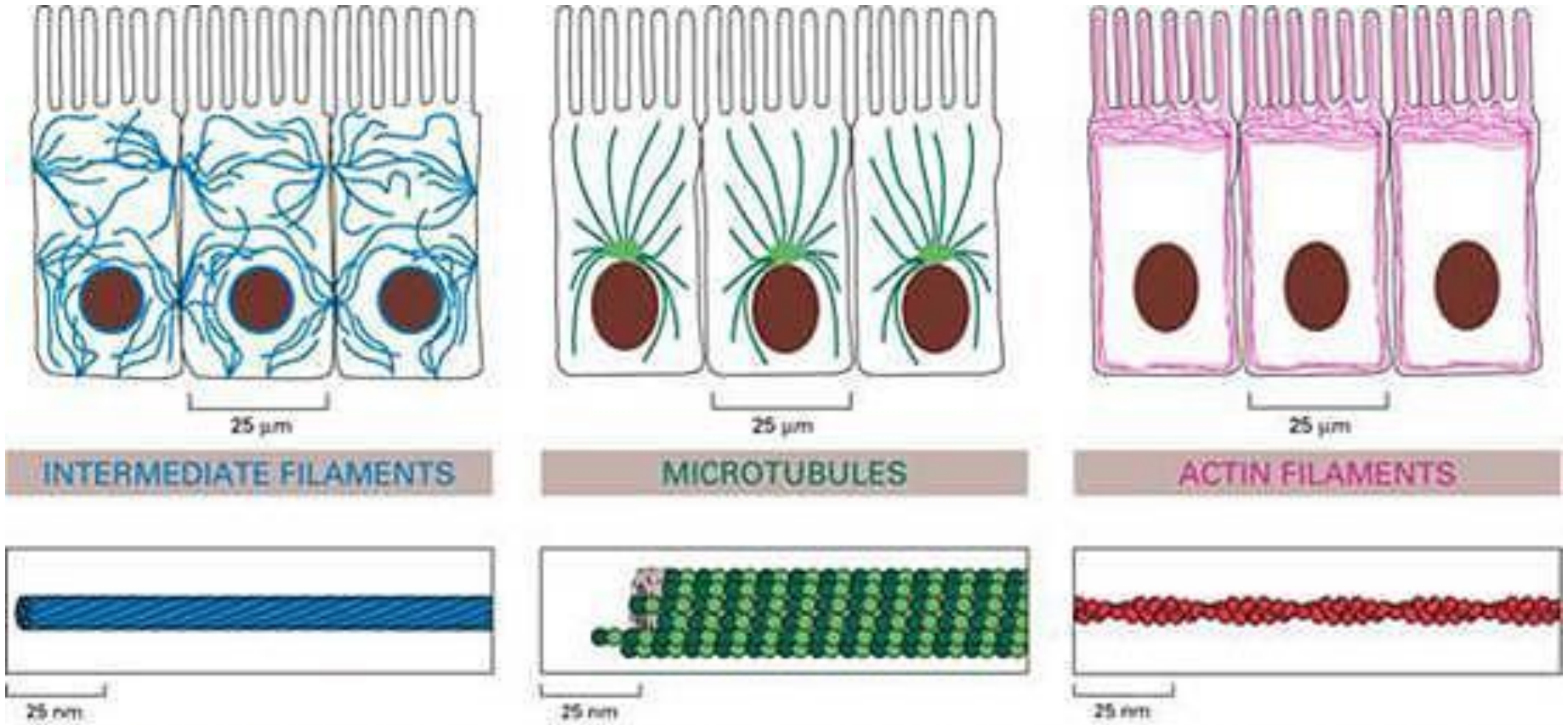
Figure 1

* *Cytokeratin for epithelial cells*
Desmin for muscle cells
Vimentin for fibroblasts and other CT cells
Glial fibrillary acidic protein for astrocytes and other glia

Microfilaments

- Are the thinnest part of the cytoskeleton
- Are made of actin
- Actin is both flexible and strong, making it a useful protein in cell movement.

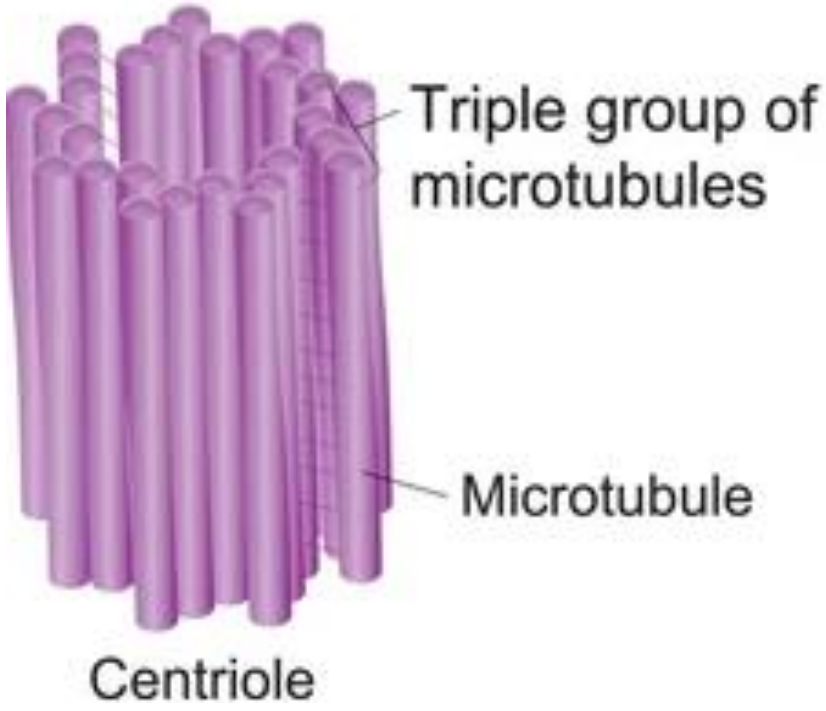



25 μm
INTERMEDIATE FILAMENTS
25 nm
25 μm
MICROTUBULES
25 nm
25 μm
ACTIN FILAMENTS
25 nm

Centrioles

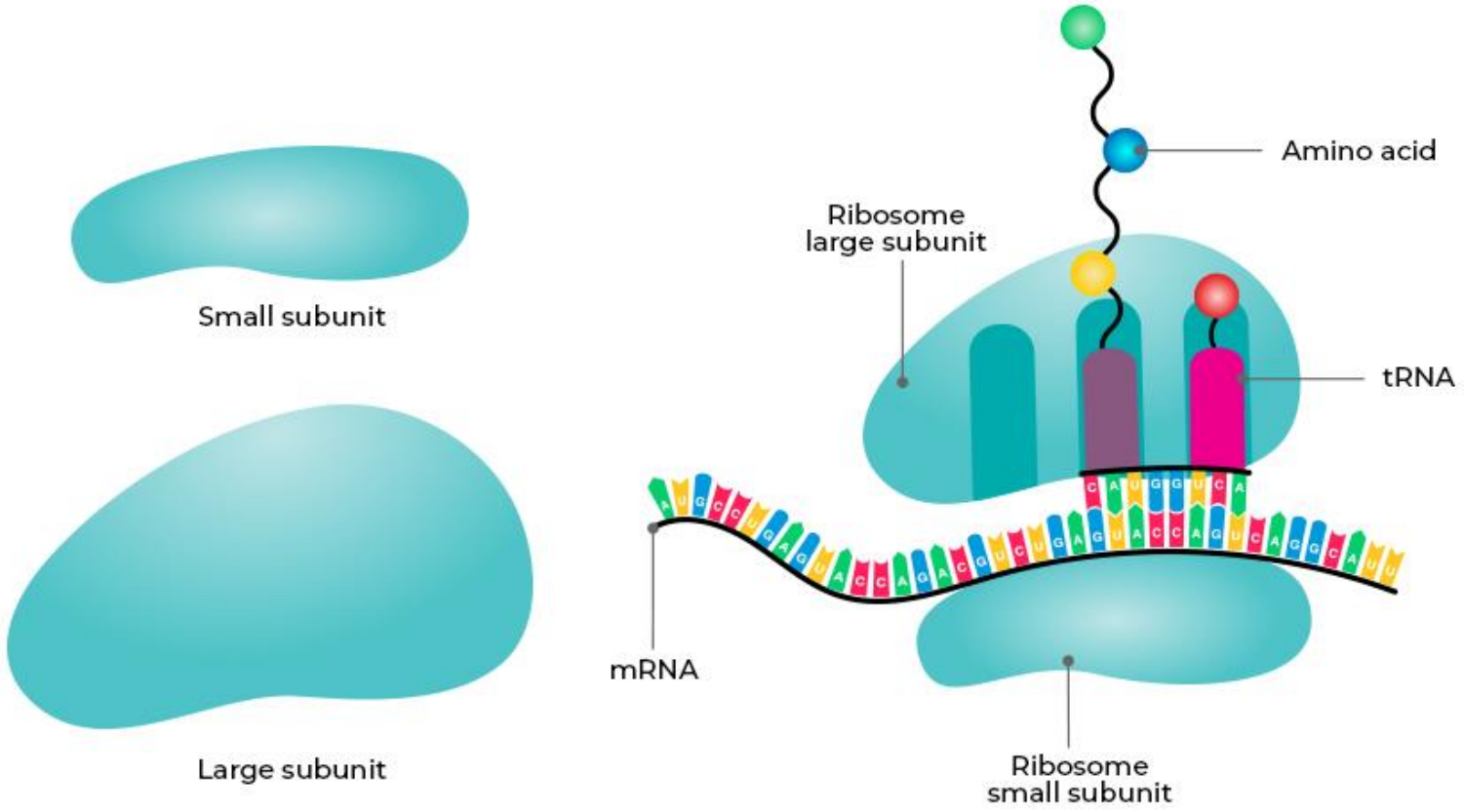
short, paired cylindrical structures found in the center of the **microtubule-organizing center** or **centrosome** and whose derivatives give rise to basal bodies of cilia and flagellum

- made up of 9 bundles of microtubules



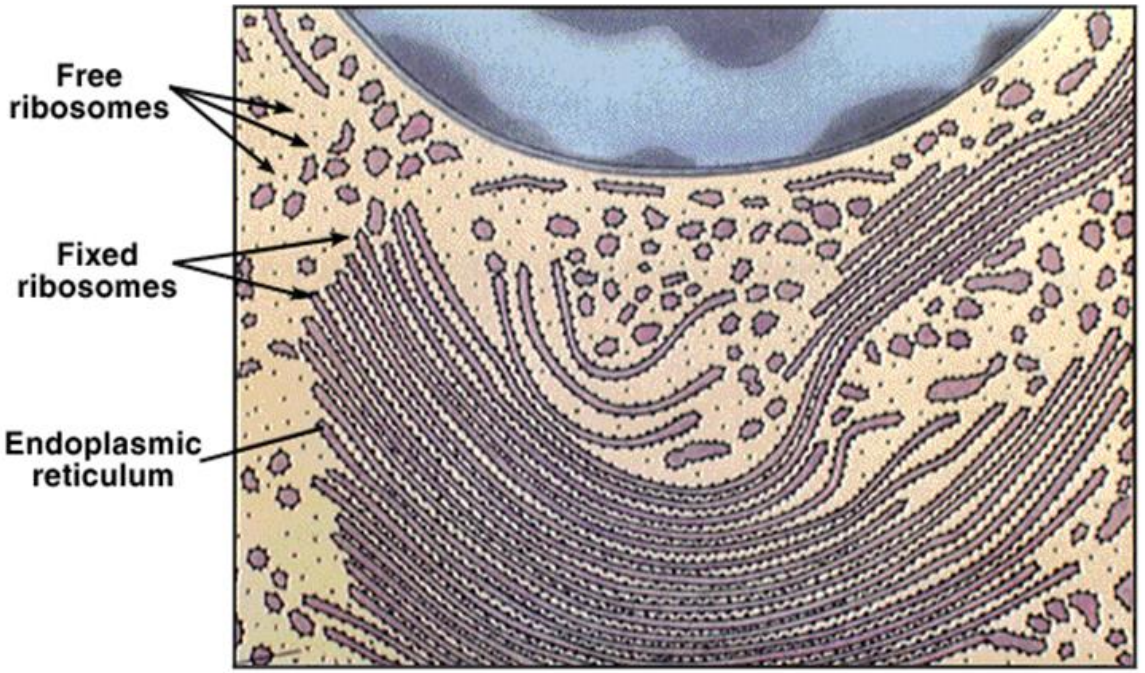
Ribosomes

structures essential for protein synthesis and composed of ribosomal RNA (rRNA) and ribosomal proteins (including proteins attached to membranes of the RER and proteins free in the cytoplasm).



Free ribosomes and bound ribosomes

- **Free ribosomes**
  - suspended in cytosol
  - synthesize proteins that function in cytosol
- **Fixed ribosomes**
  - attached to endoplasmic reticulum
  - synthesize proteins for export or for membranes



Inclusions

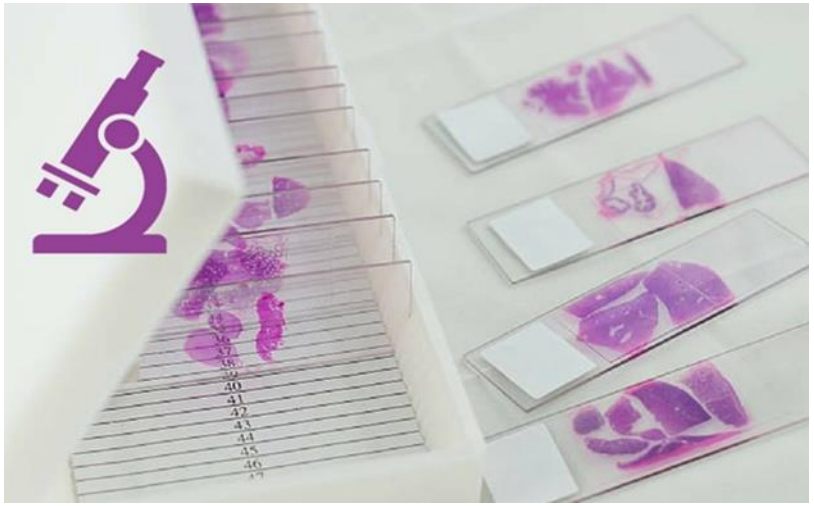
Cell inclusion are the non living temporary substance and can be the product of metabolism

- **Glycogen** – a storage form of glucose

Is abundant in cells of striated muscle and liver

- **Lipids** are storage form of triglycerides. Are stored in specialized cells (adipocytes) and as individual droplets (for ex. in hepatocytes)
- **Crystals** as crystalline forms of certain proteins (in cells of testis)
- **Pigments** (hemoglobin, melanin, membrane bound lipofuscin)

Methods of study



Histological slide

- preserve the vital state of structures
- be thin and transparent
- be contrast (studied structures should be clearly defined)
- must be preserved for a long time and used for re-examination

Preparation of histological slides for microscopy

1. Tissue fixation
2. Dehydration and Clearing
3. Infiltration and embedding
4. Sectioning
5. Staining

Fixation

Fixation stabilizes and preserves the tissue.

Fixation is used to:

- terminate cell metabolism,
- prevent enzymatic degradation of cells and tissues by autolysis (self-digestion),
- kill pathogenic microorganisms such as bacteria, fungi, and viruses, and
- harden the tissue as a result of either cross-linking or denaturing protein molecules.

Dehydration

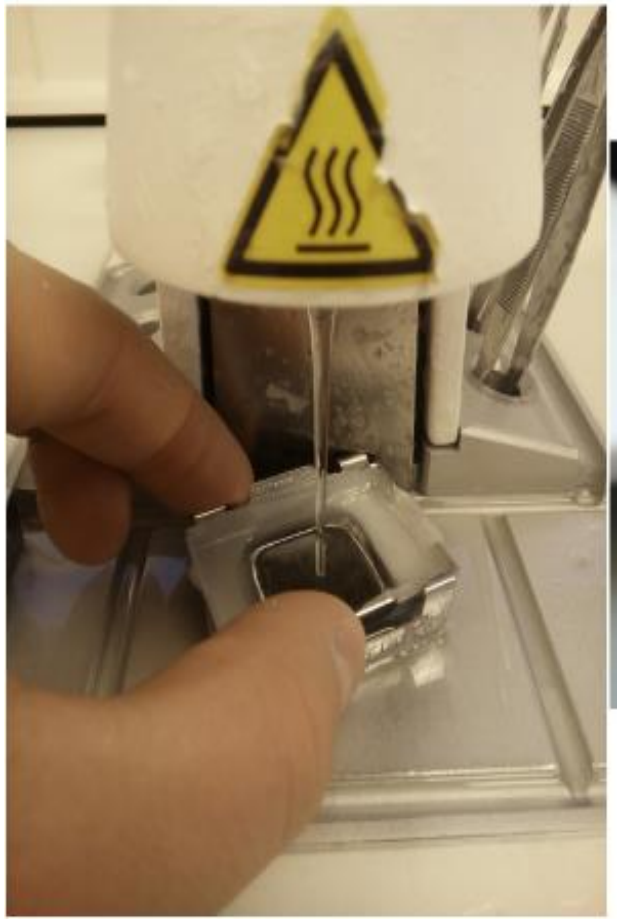
- To remove **fixative** and **water** from the tissue and **replace** them with dehydrating fluid.

Dehydrating (fluid) agent:

- Ethanol
- Methanol
- Isopropanol
- Acetone

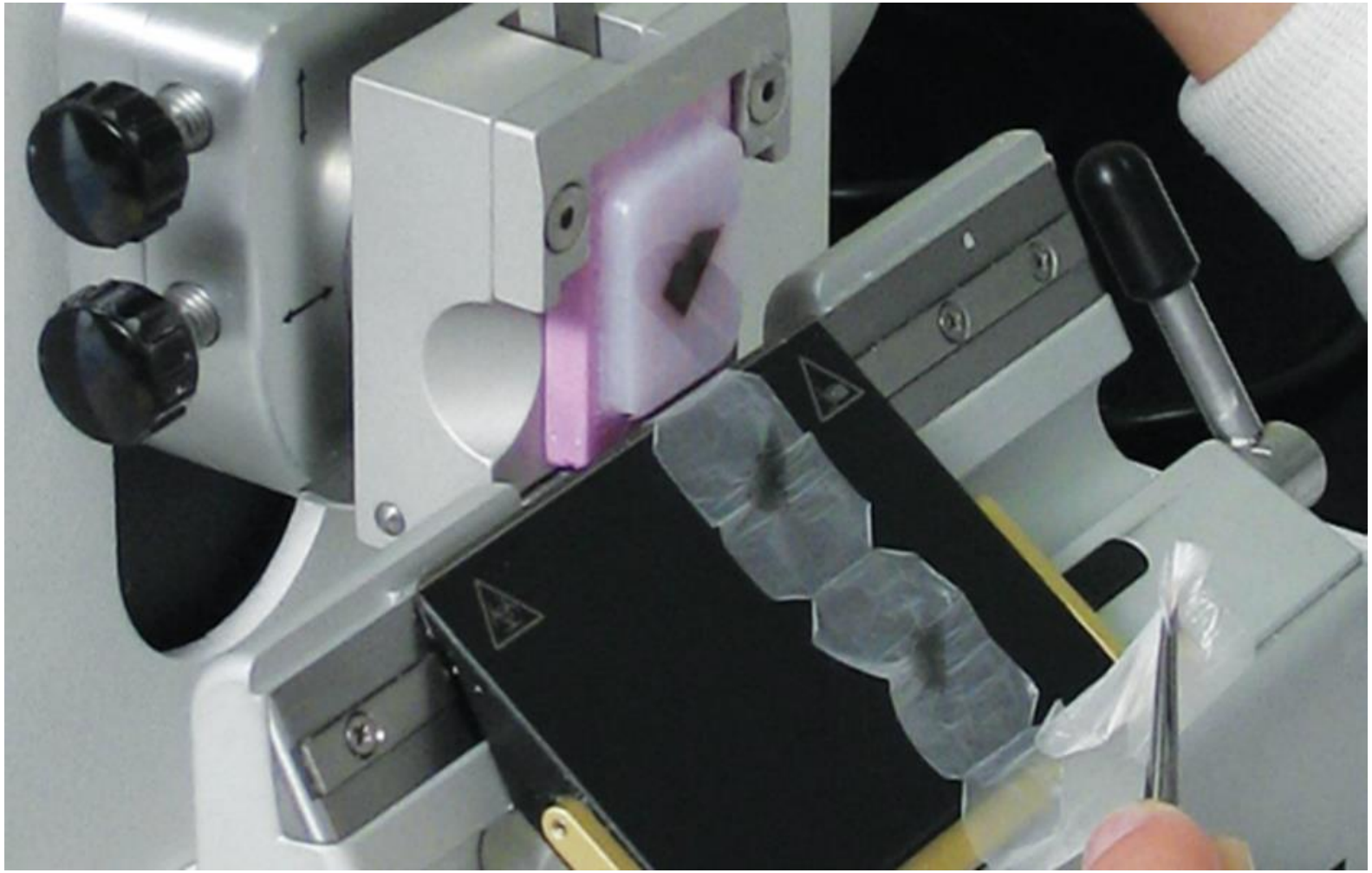
Embedding

Embedding converts the tissue into a solid form which can be sliced ("sectioned"). Usually using paraffin but also plastic.



Sectioning

Sectioning (slicing) provides the very thin specimens needed for microscopy



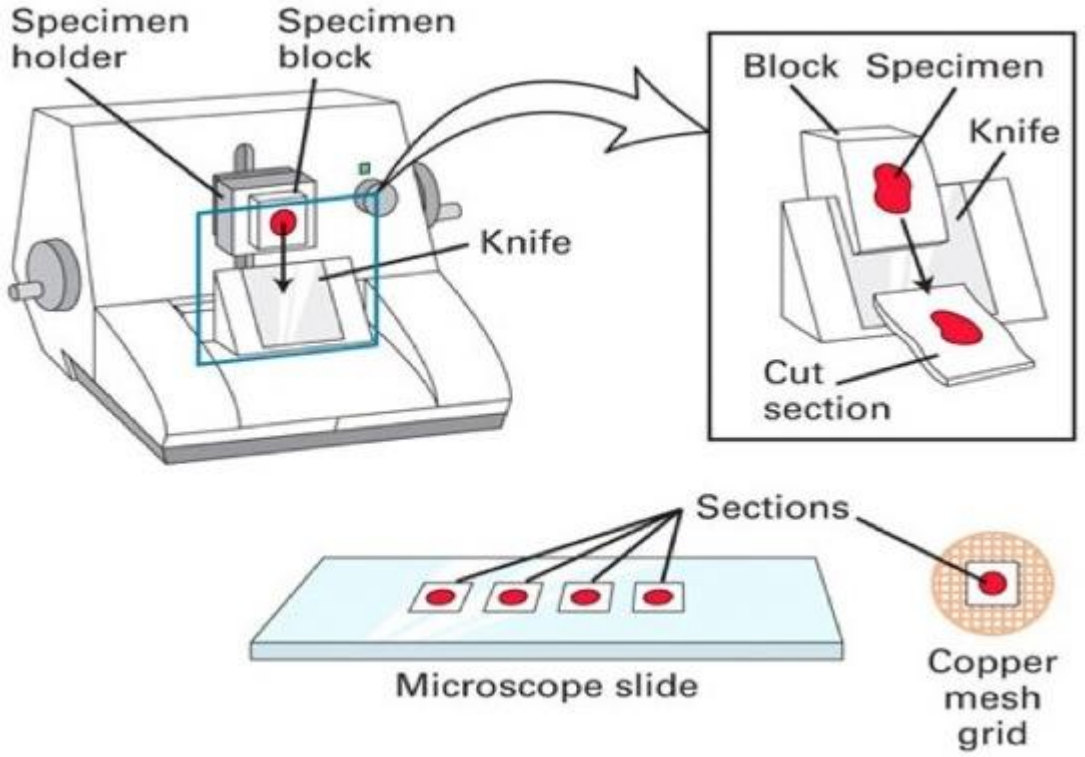
SECTIONING

To produce thin sections, embedded tissues are sectioned on a microtome

Section thickness:

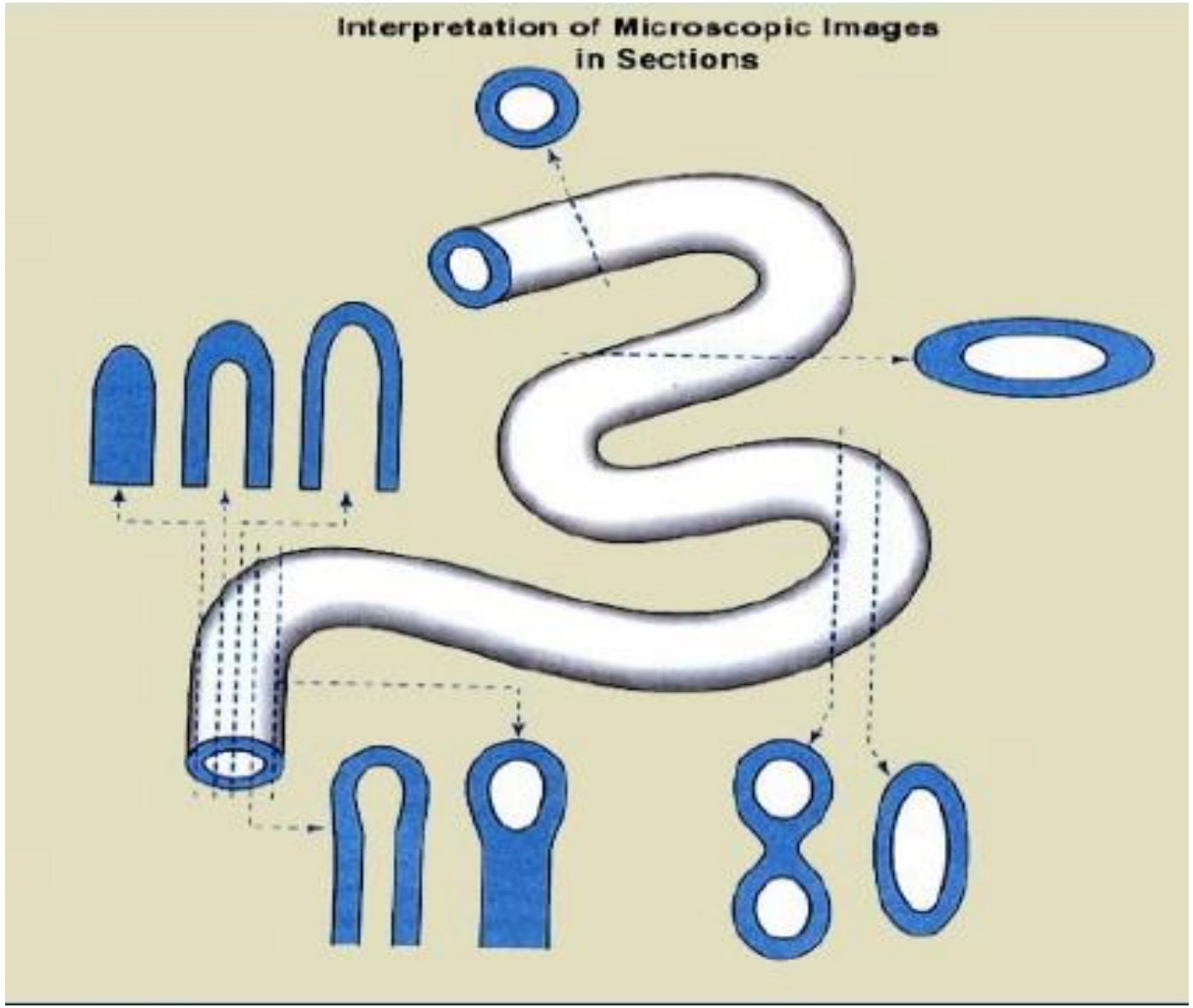
A. Light microscopy. 1-20 microns (placed on glass slides)

B. Electron microscopy. 60-100 nanometers (placed on metal grids for TEM)



Cross and longitudinal sections of one tissue

From 3D to 2D

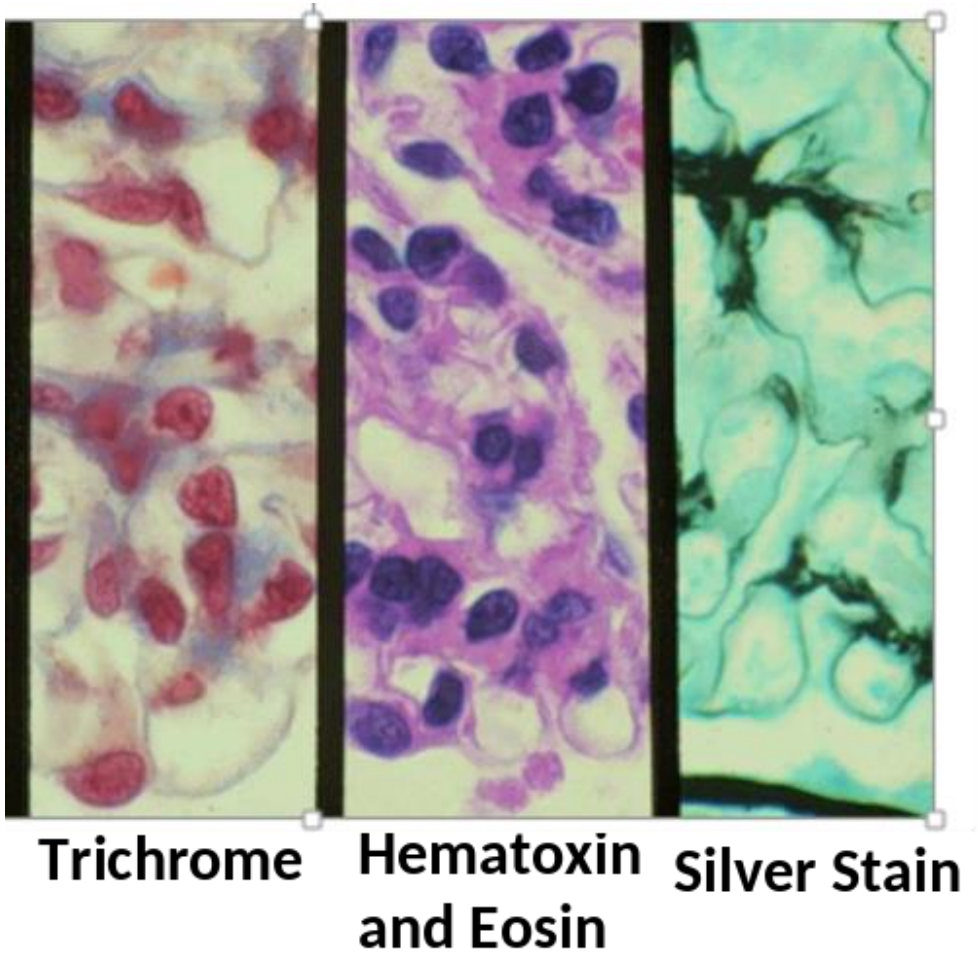


Staining

Most cells are transparent and appear almost colorless when unstained. Histochemical stains (typically hematoxylin and eosin) are used to provide contrast to tissue sections, making tissue structures more visible and easier to evaluate.

Staining

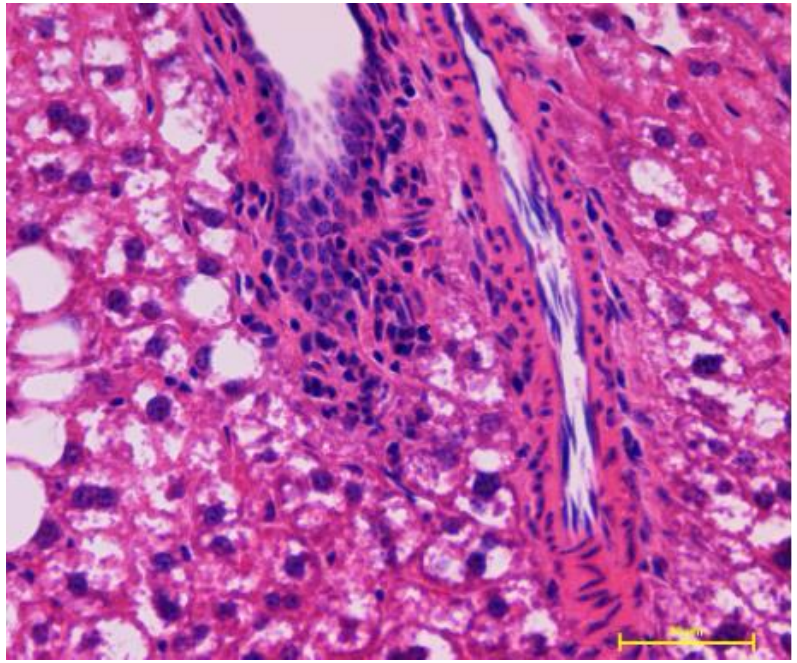
The staining process makes use of a variety of dyes that have been chosen for their ability (affinity) to stain various cellular components of tissue.



Tissue stained with three different staining methods

Hematoxylin & Eosin (H & E)

most common stain - good for general structure



•Dye	•Basic	•Acidic	•Structures stained	•Color
•Hematoxylin	•√	•	•Nuclei	•Blue to purple •
•Eosin	•	•√	•Proteins, basic regions of the cytoplasm, collagen fibers	•Pink

A limited number of substances within cells and the extracellular matrix display basophilia.

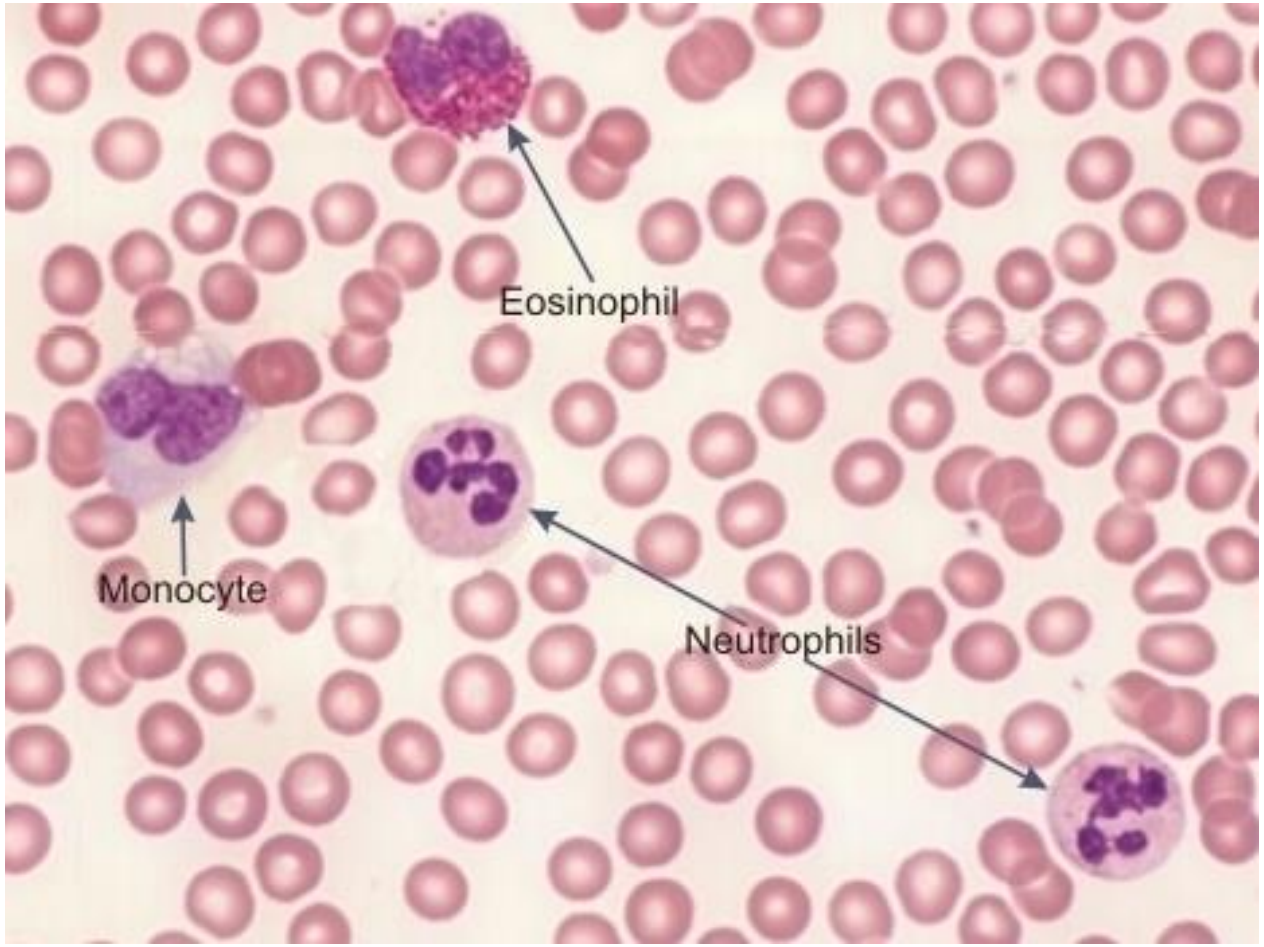
heterochromatin and **nucleoli** of the nucleus (chiefly because of ionized phosphate groups in nucleic acids of both),
cytoplasmic components such as RER (also because of ionized phosphate groups in ribosomal RNA),
extracellular materials such as the complex carbohydrates of the matrix of cartilage (because of ionized sulfate groups).

Staining with acidic dyes is less specific, but more substances within cells and the extracellular matrix exhibit acidophilia.

- most **cytoplasmic filaments**, especially those of muscle cells,
- most **intracellular membranous components** and much of the otherwise unspecialized cytoplasm,
- most **extracellular fibers** (primarily because of ionized amino groups).

Giemsa (May Grunwald Giemsa)

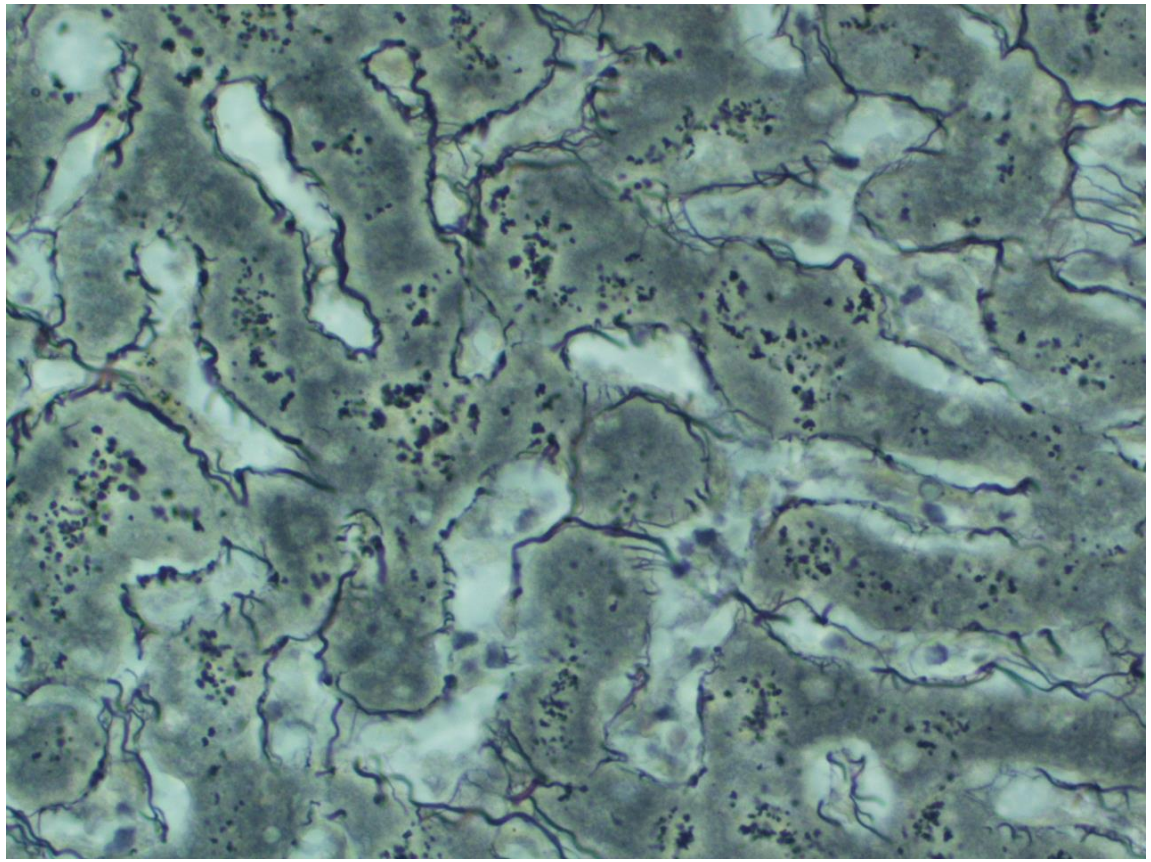
Stain of blood smears. This stain allows to visualize the different kind of blood cells.



Silver impregnation

Visualization of reticular connective tissue fibers.

Sugar residues in Reticular fibers have the capacity to bind silver salts, which reduced to metallic silver, give the typical black color.



Liver

Histochemistry

is used for the visualization of biological structures. As such, it is concerned with the identification and distribution of various chemical components of tissues.

It stains constituents of cells and biochemical tissues:

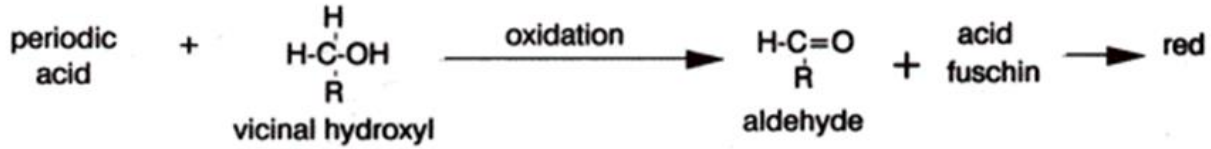
- mucins,
- lipids,
- nucleic acids,
- amyloid, and other proteins.

Periodic acid—Schiff stain (PAS)

This staining method is used for staining of **polysaccharides** such as glycogen, and **mucosubstances** such as glycoproteins, glycolipids and mucins in tissues

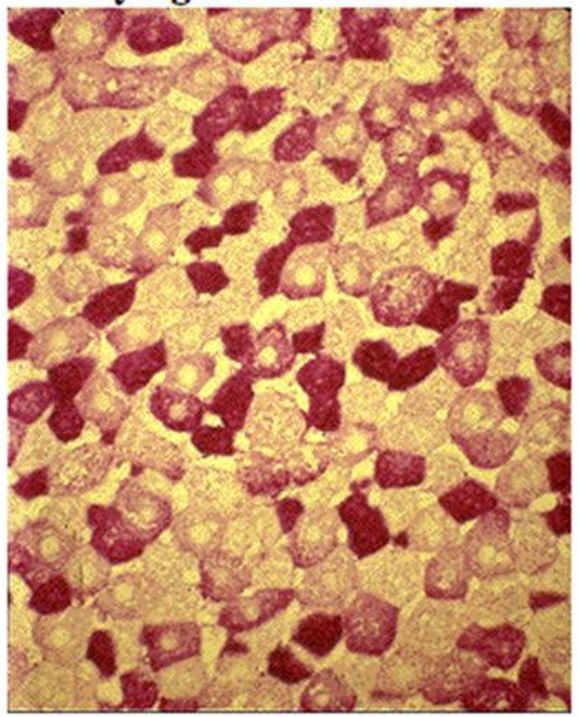
Histochemical Stains

Periodic Acid Schiff (PAS) (specific for hydroxyl groups of polysaccharides)

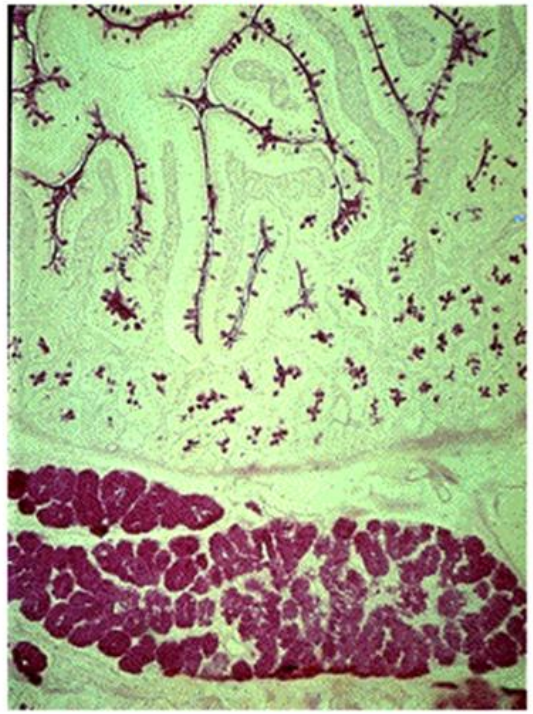


Stains mucopolysaccharides (intestinal mucosa & liver glycogen) and glycoproteins

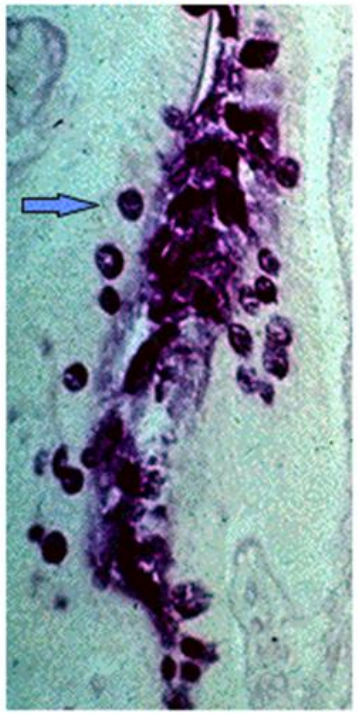
Glycogen in Liver Cells



Intestinal Mucosa



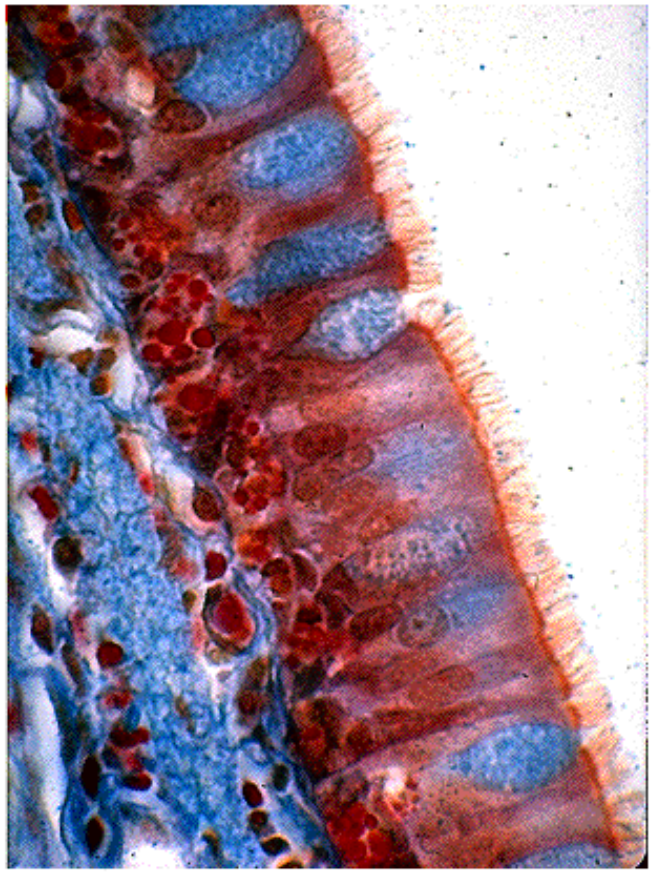
Goblet Cells



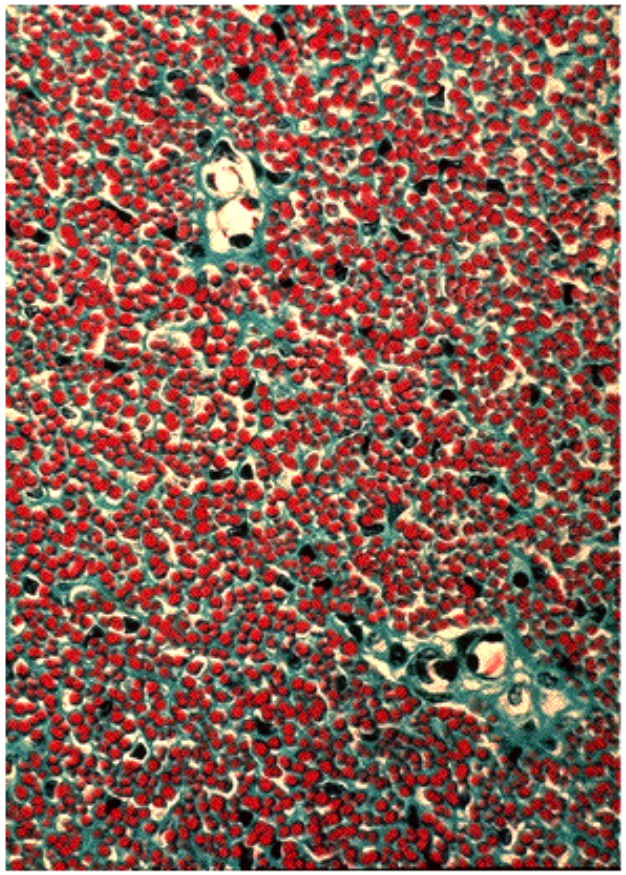
Mallory's and Masson's Trichrome

Combines several acidic dyes- collagen and reticular fibers stain blue, nuclei and cytoplasm stain red and elastic fibers stain yellow or pink

Tracheal Epithelium

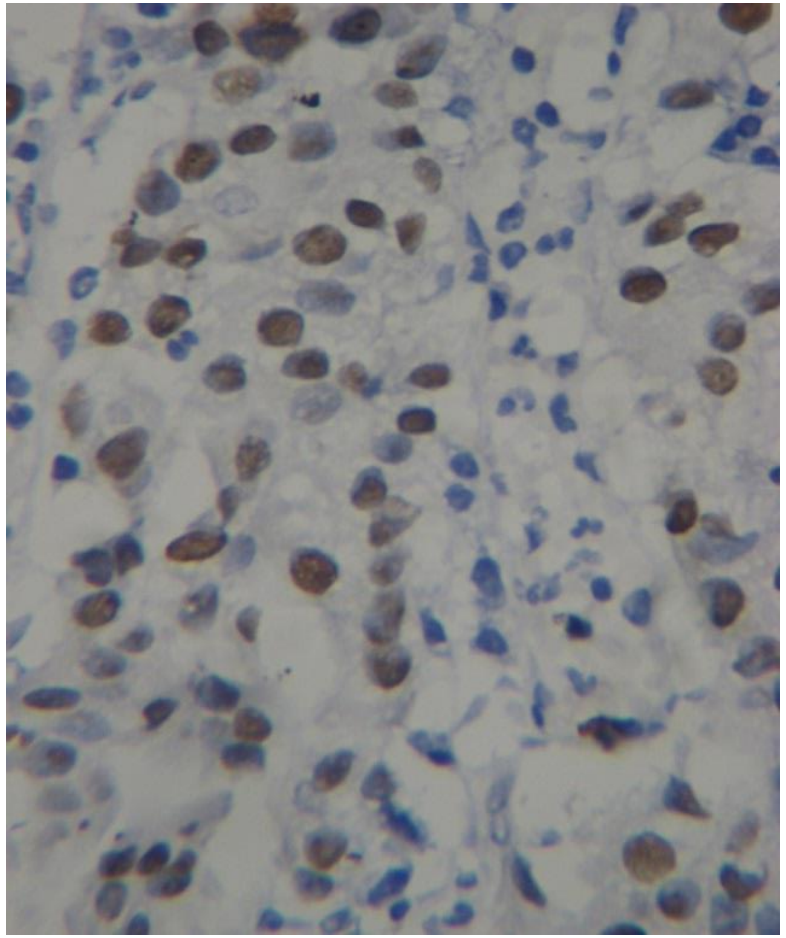


Dense, Regular, Elastic Connective Tissue

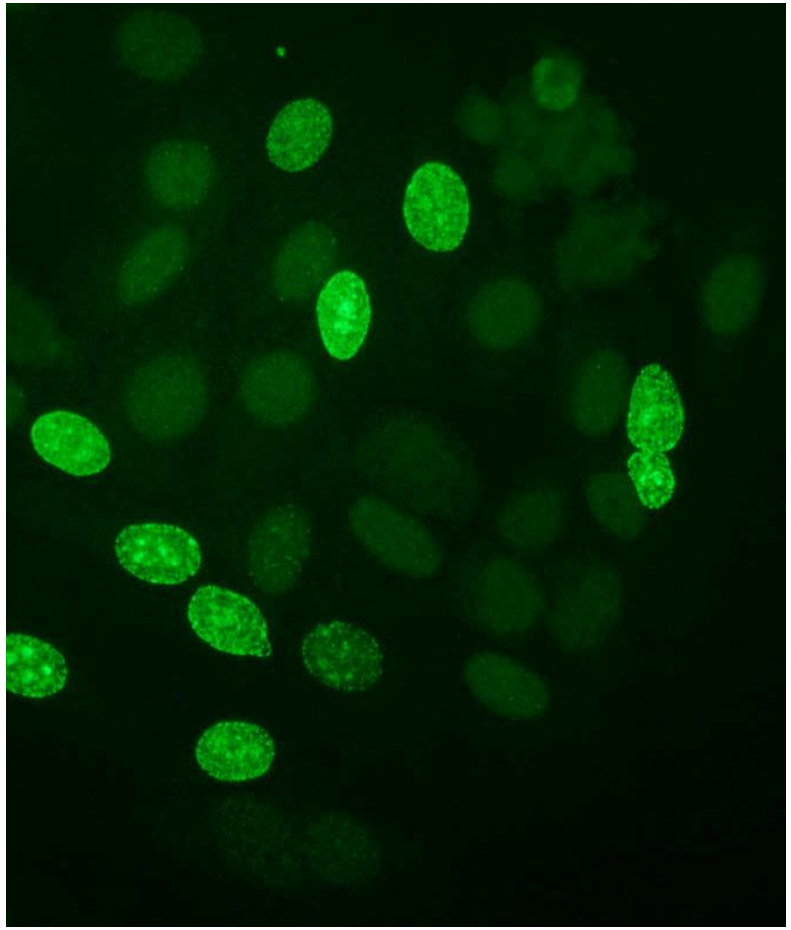


Stain type	Reagent	Color	Cell structure stained
Acid dyes	Eosin	Pink-red	Bind to and stain basic structures (or negatively charged structures), such as cationic amino groups on proteins. It stains them pink. **Cytoplasm**, muscle, connective tissue, colloid, red blood cells and decalcified bone matrix all stain pink.
Basic dyes	Hematoxilin	Blue	Binds to acidic structures, staining them blue to purple. It will bind and stain nucleic acids and some cytoplasmic substances. Therefore, **the nucleus stains blue.**
Metal stains	**Silver (Impregnation)** **Osmium (oxidation)**	**Brown/Black** **Black**	Golgi apparatus, reticular fibers through **sugar** residues Osmium is chemically bound to **fat**
Special stains: *Acid and basic dyes*	Giemsa Wright	Blue, purple pink-red, blue,	Used for blood and bone marrow smears (differential staining of blood cells)
	Goldner	Black, green, red, blue	Selective staining of connective tissue, muscle, fibrin, blood vessels.
Elastica	**Aldehyde Fuchsin** (Verhoeff)	Violet or purple	Visualization of elastic fibers

Immunohistochemical staining
(antigen detection with antibodies)

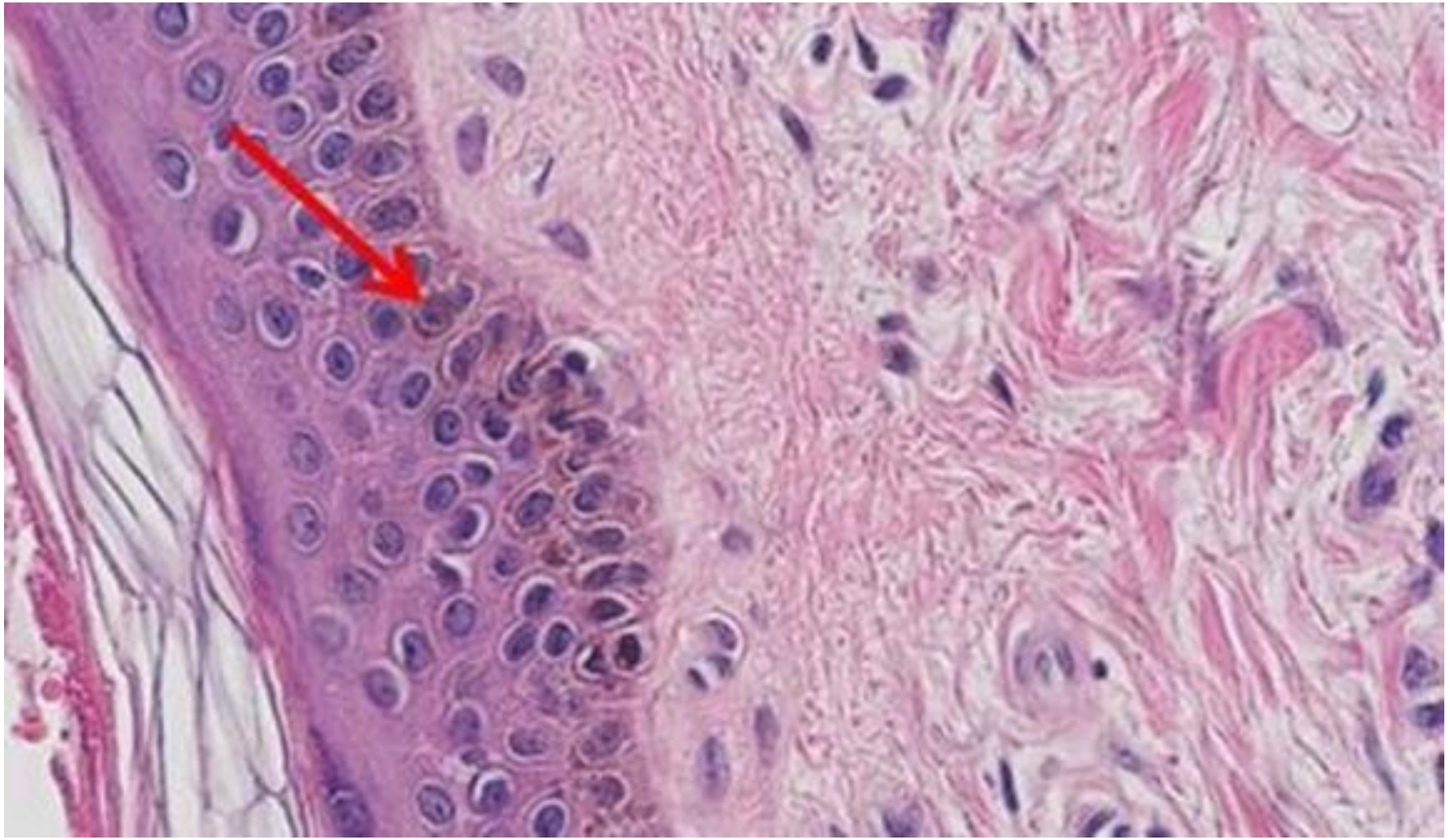


Immunohistochemistry



Immunofluorescent stain

Microscopy



Enjoy the histology labs!