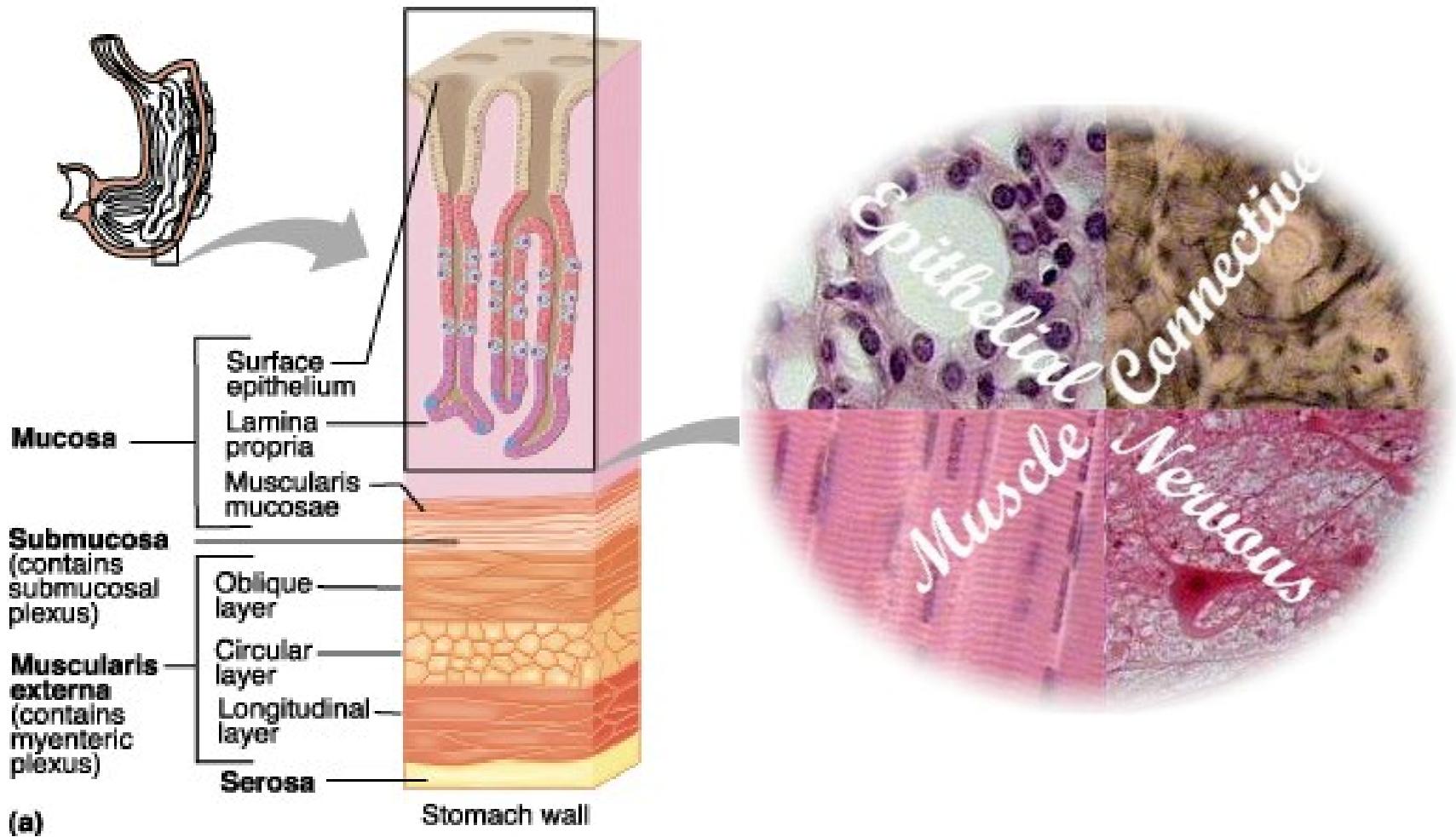


# General features of histology & its methods



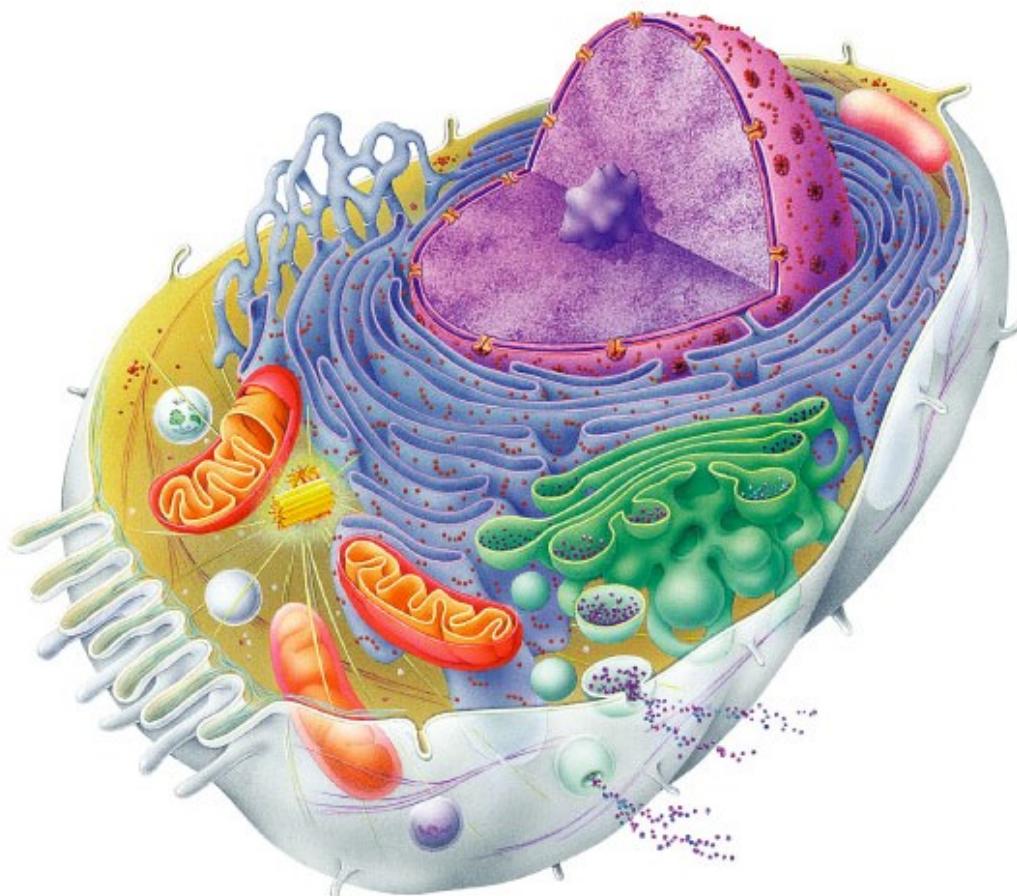
Lecture 1

# Histology - The study of tissues and microanatomy of organs

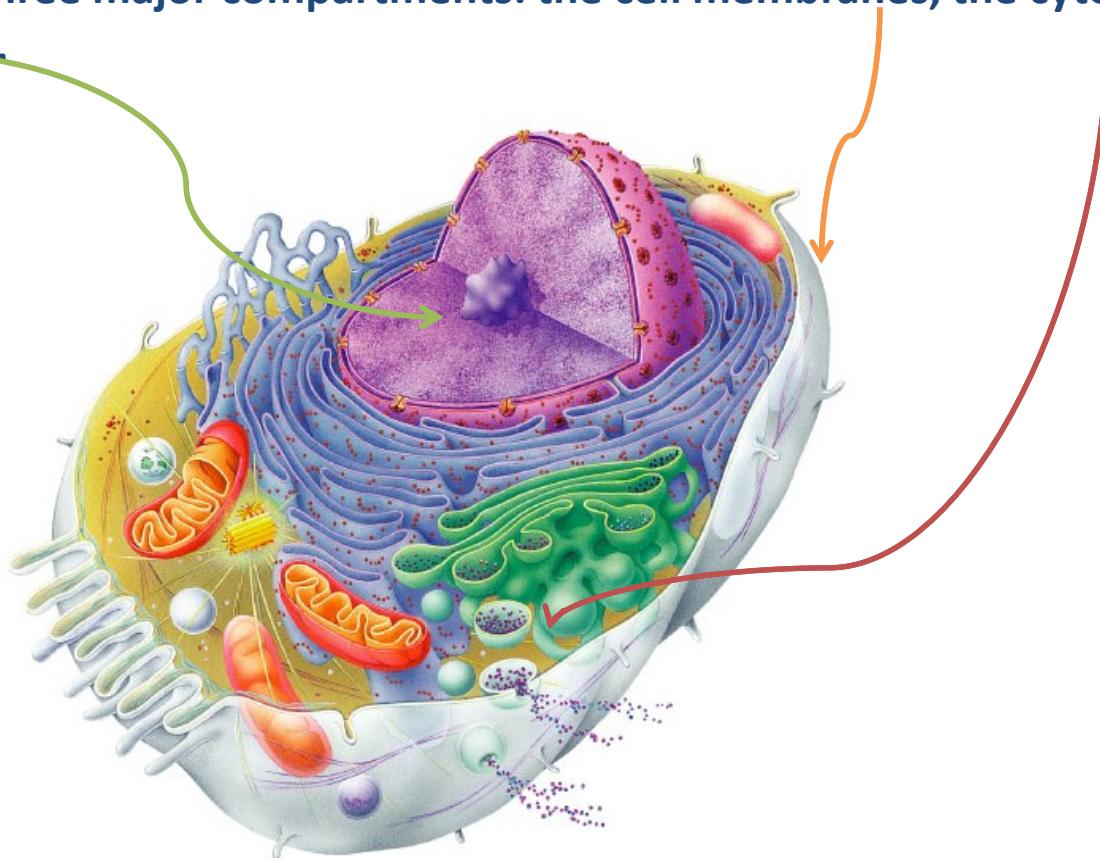


# Cytology - The Study of Cells

Cells are the smallest living structure.  
Cell - functional unit of the body



**Cells have three major compartments: the cell membranes, the cytoplasm and the nucleus.**

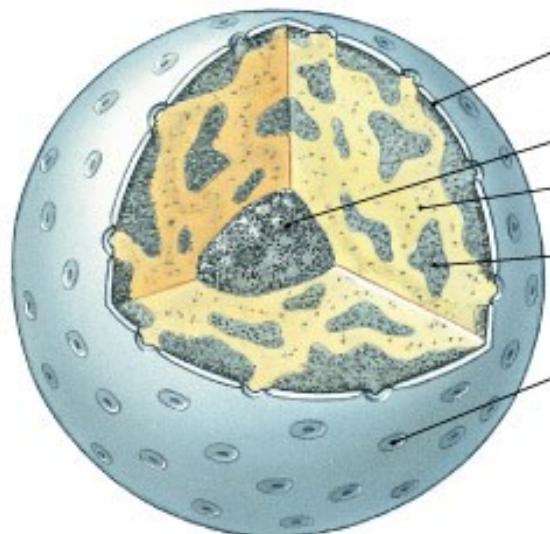
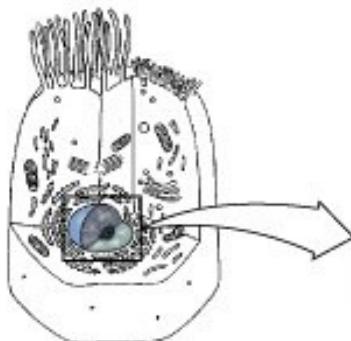


*Often synonymous with cytoplasm (protoplasm)*

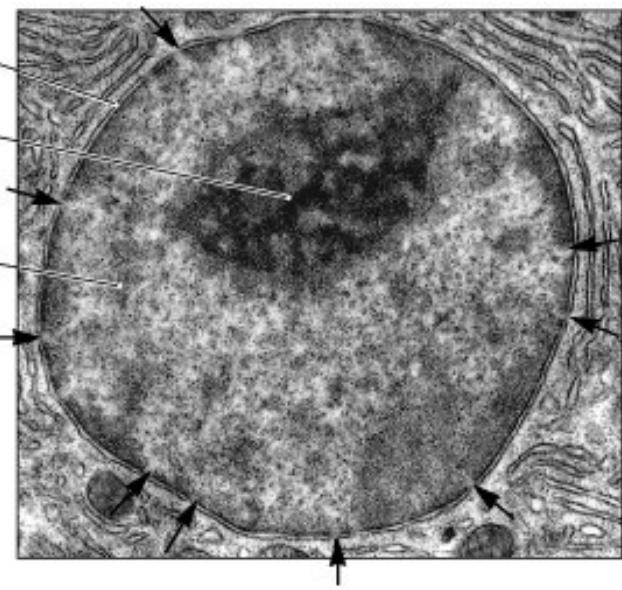
The cytoplasm contains **organelles** (“little organs”) and **inclusions** in an aqueous gel called the **cytosol**.

# Nucleus (= center)

- Visible with LM
- Membrane bound
- Many pores
- DNA
- 23 Pairs of Chromosomes » Except gametes
- Nucleolus



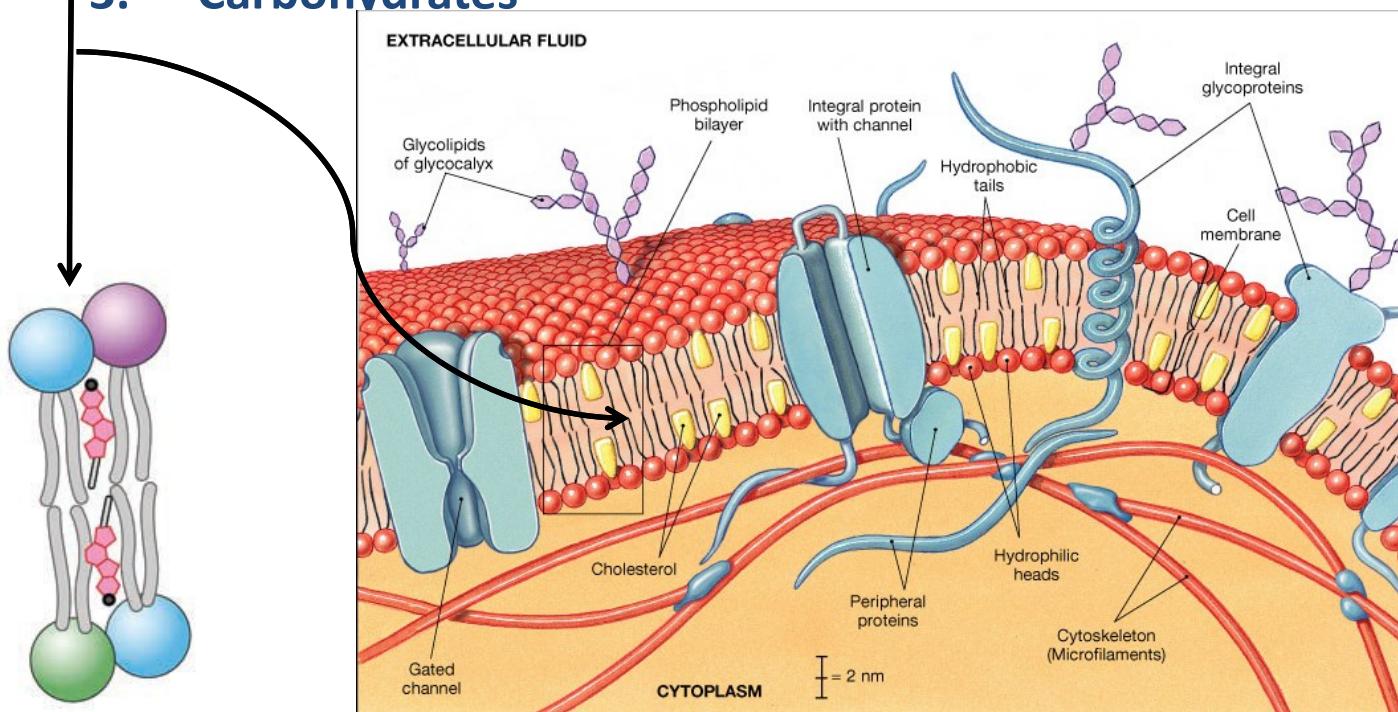
(a)



The **cell membranes** separate a cell from its environment. The outer cell membrane is called the **plasma membrane** or **plasmalemma**.

### Biochemical components:

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



The **plasma (cell) membrane**, a lipid bilayer that forms the cell boundary as well as the boundaries of many organelles within the cell;

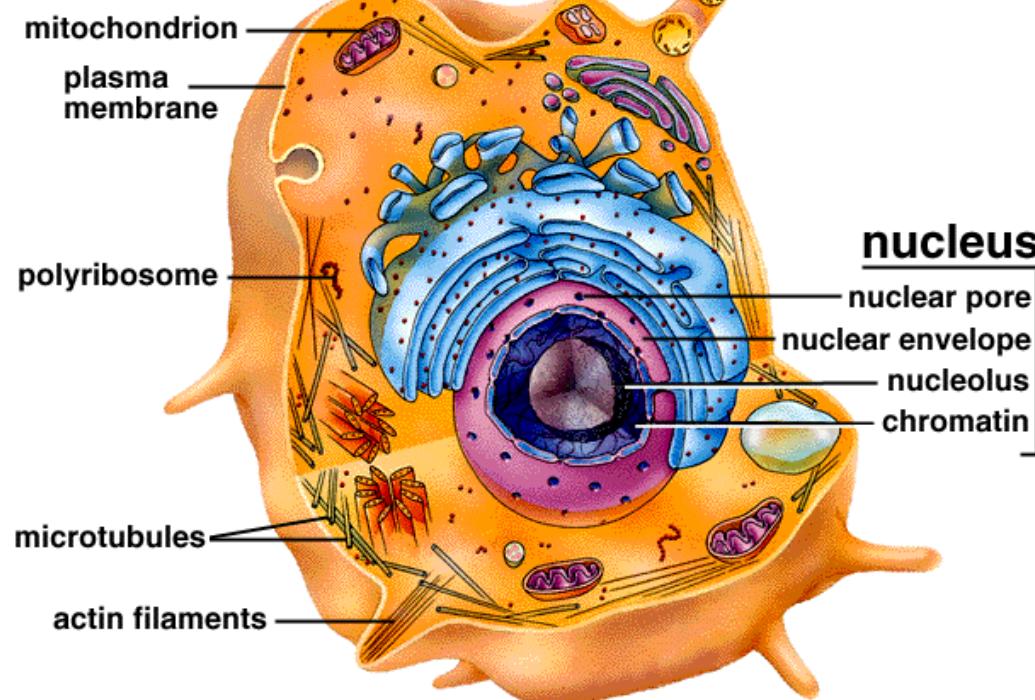
Organelles are described as membranous (membrane limited) or nonmembranous.

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

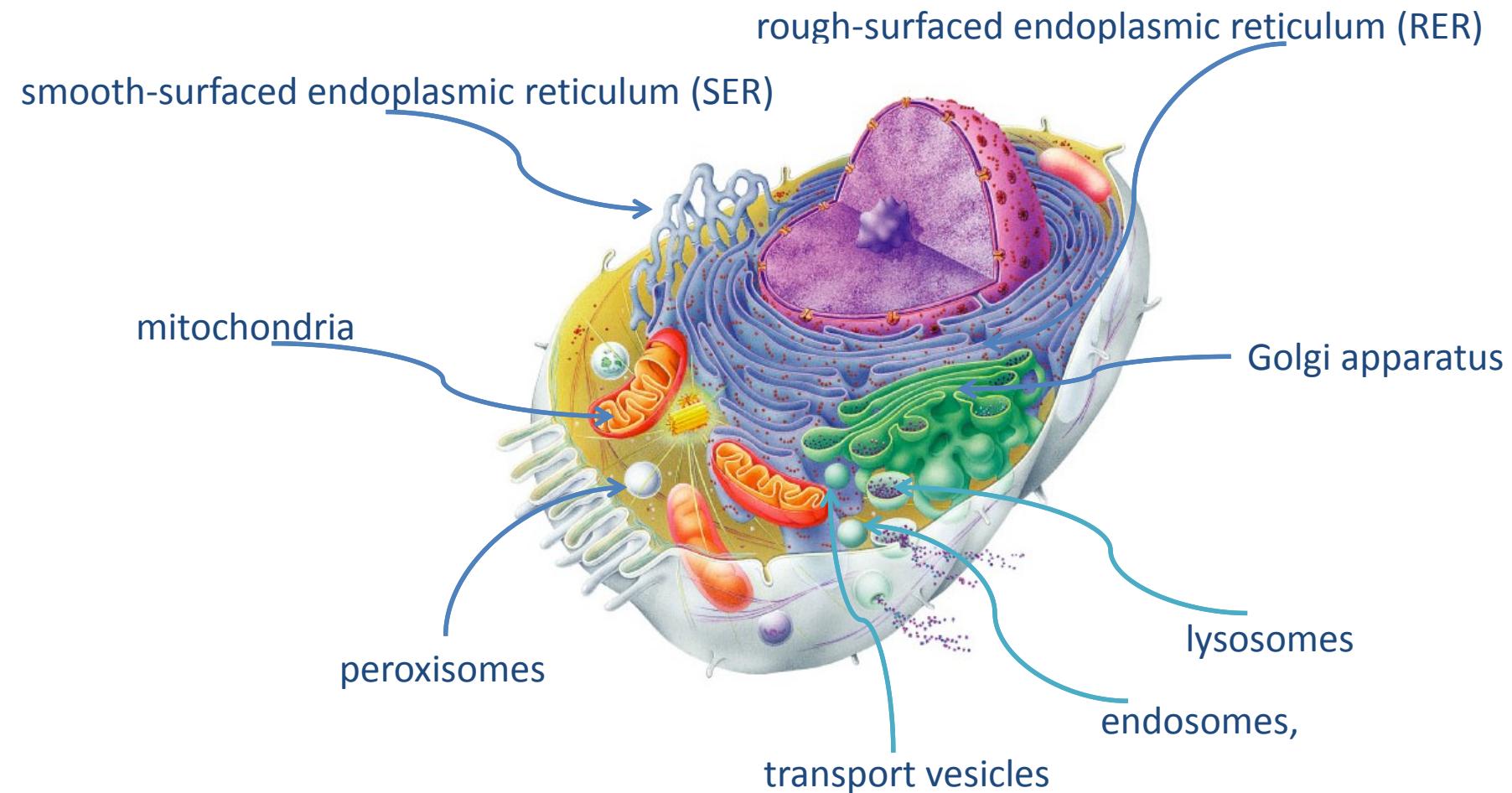
nonmembranous organelles without plasma membranes.

Sylvia S. Mader, Inquiry into Life, 8th edition. Copyright © 1997 The McGraw-Hill Companies, Inc. All rights reserved.

## Animal Cell Organelles (1)

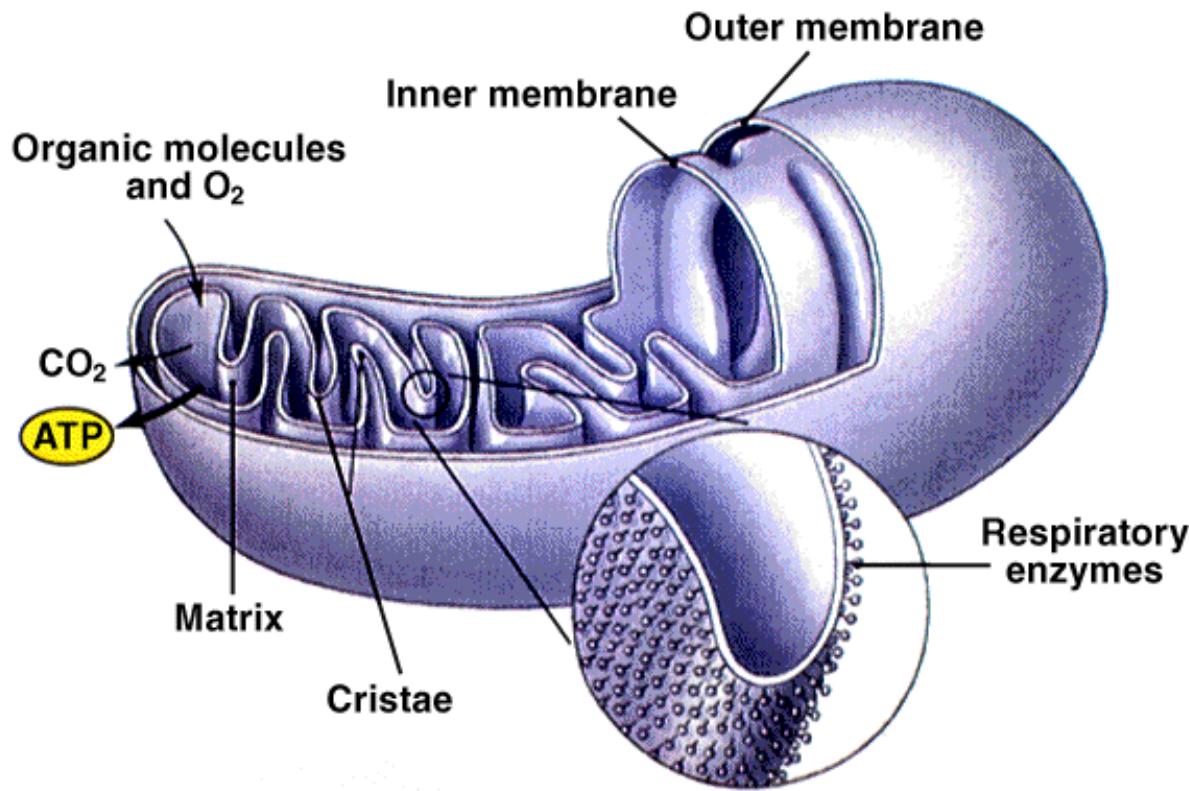


# The membranous organelles include:

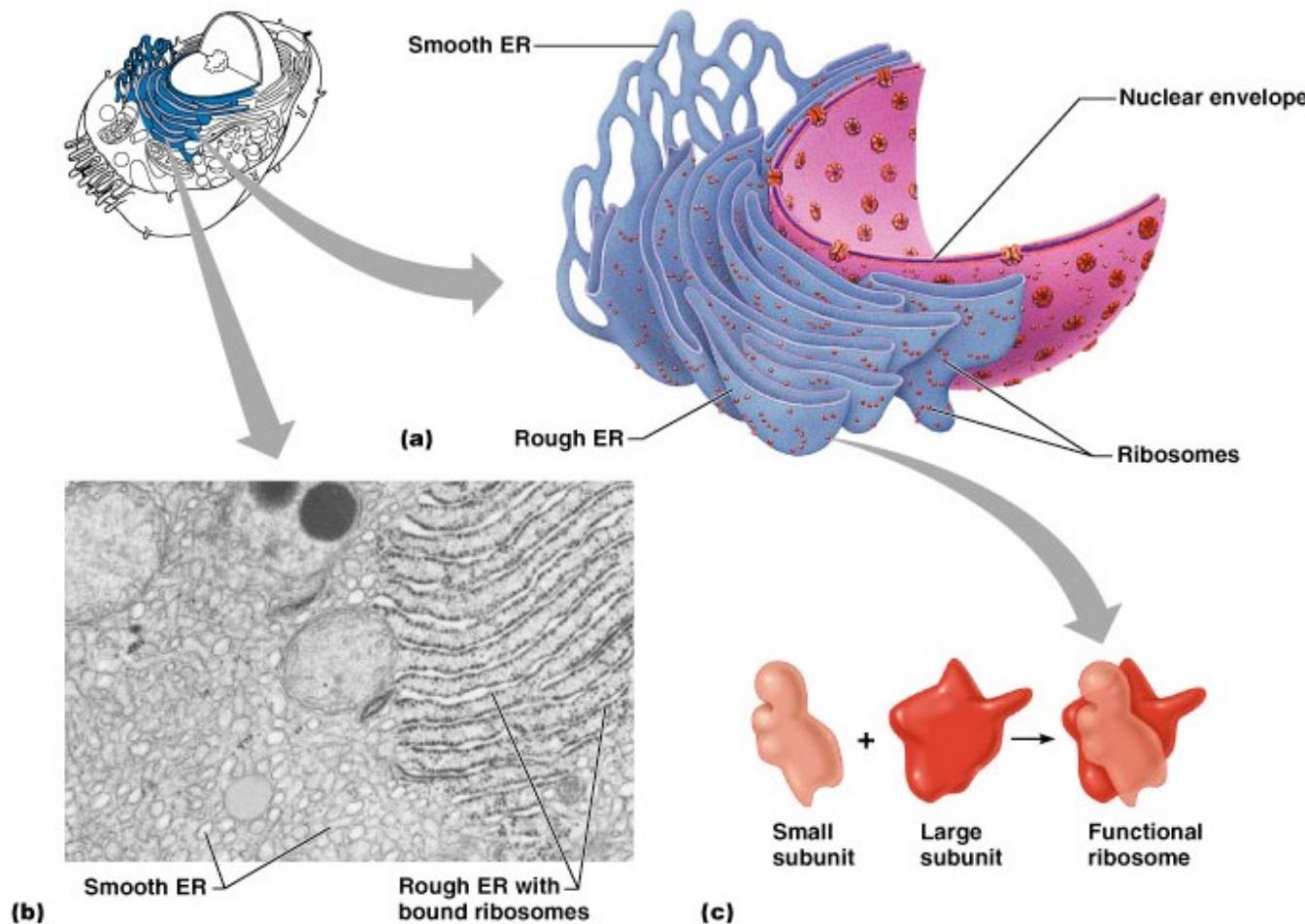


**Mitochondria**, organelles that provide most of the energy to the cell by producing adenosine triphosphate (ATP) in the process of oxidative phosphorylation

- Own genome
- Self-replicating



- **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 but not associated with ribosomes;



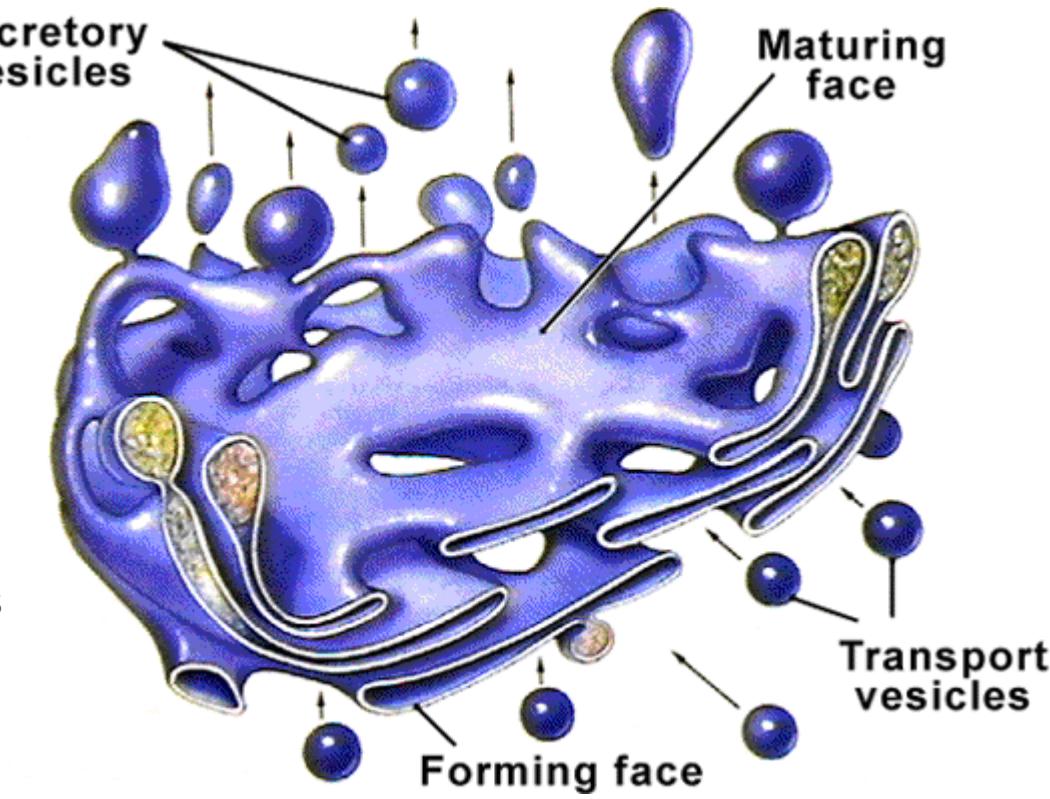
- **Golgi apparatus**, a membranous organelle composed of multiple flattened cisternae responsible for modifying, sorting, and packaging proteins and lipids for intracellular or extracellular transport;

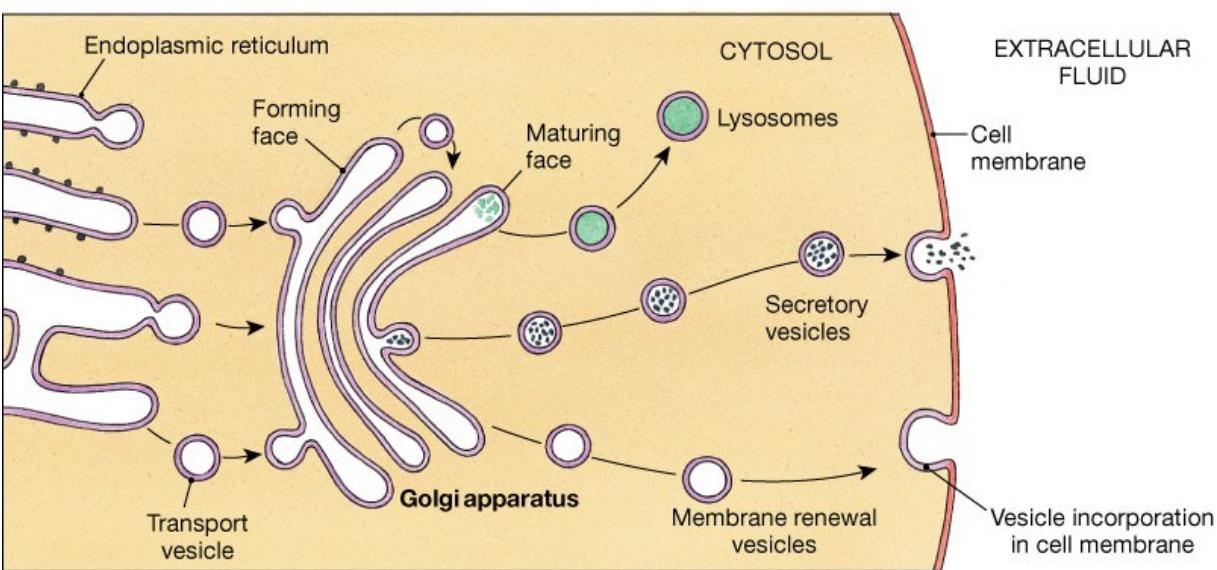
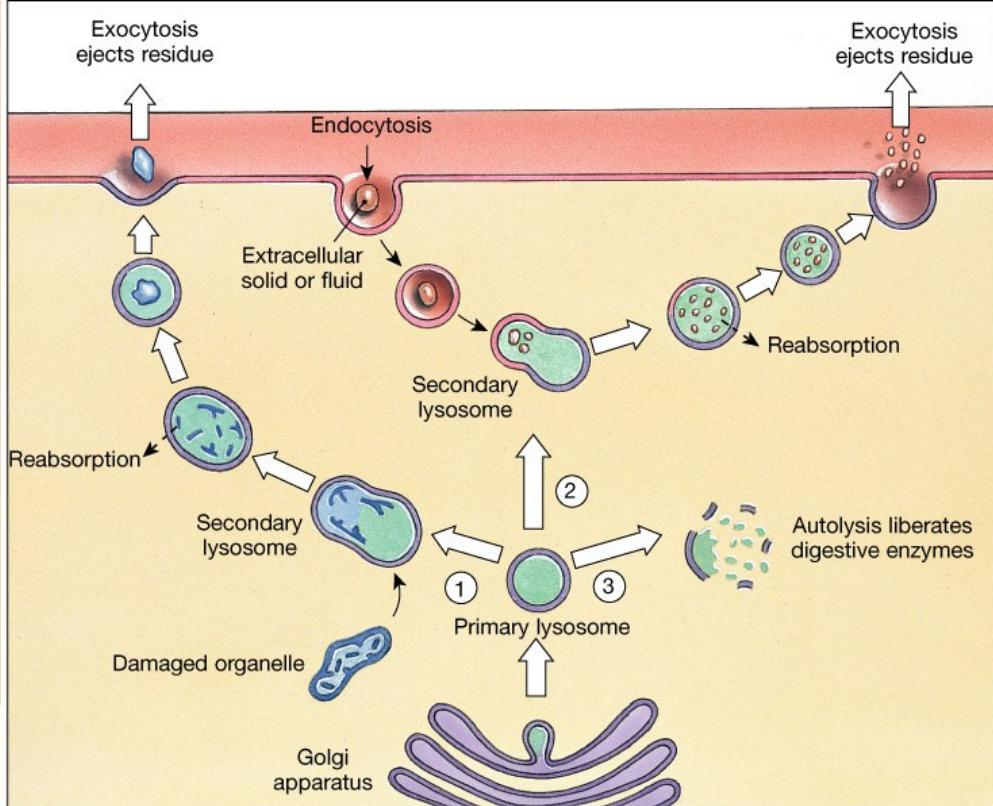
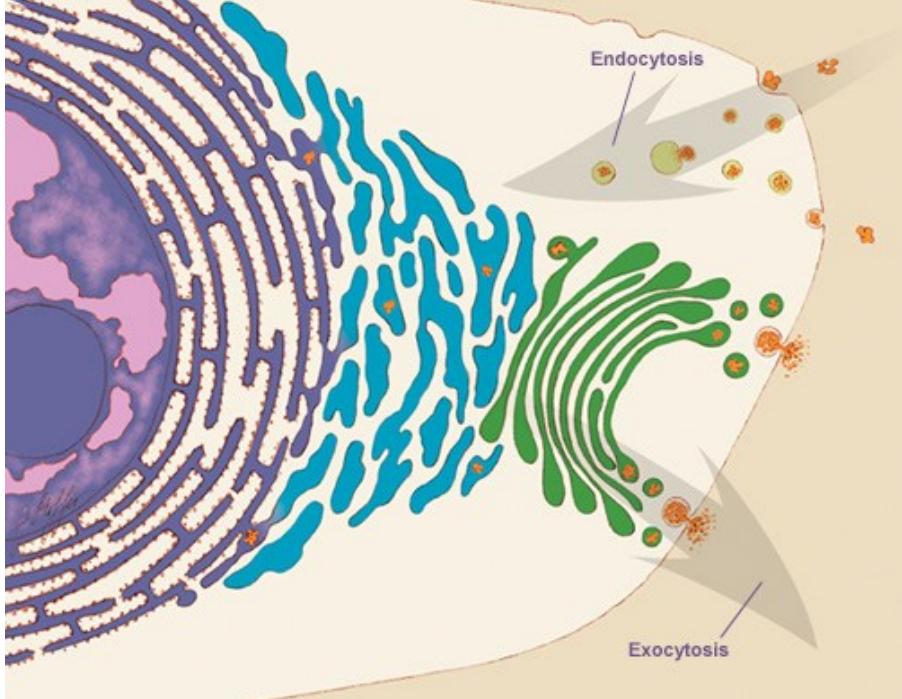
**Endosomes**, membrane-bounded compartments interposed within endocytic pathways that have the major function of sorting proteins delivered to them via endocytotic vesicles and redirecting them to different cellular compartments for their final destination;

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

**Transport vesicles**—including **Pinocytotic vesicles**, **Endocytotic vesicles**, and **Coated vesicles**—that are involved in both endocytosis and exocytosis and vary in shape and the material that they transport;

**Peroxisomes**, small organelles involved in the production and degradation of H<sub>2</sub>O<sub>2</sub> and degradation of fatty acids.



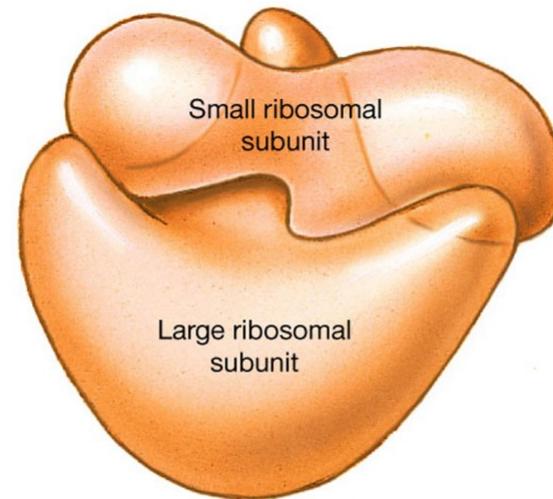
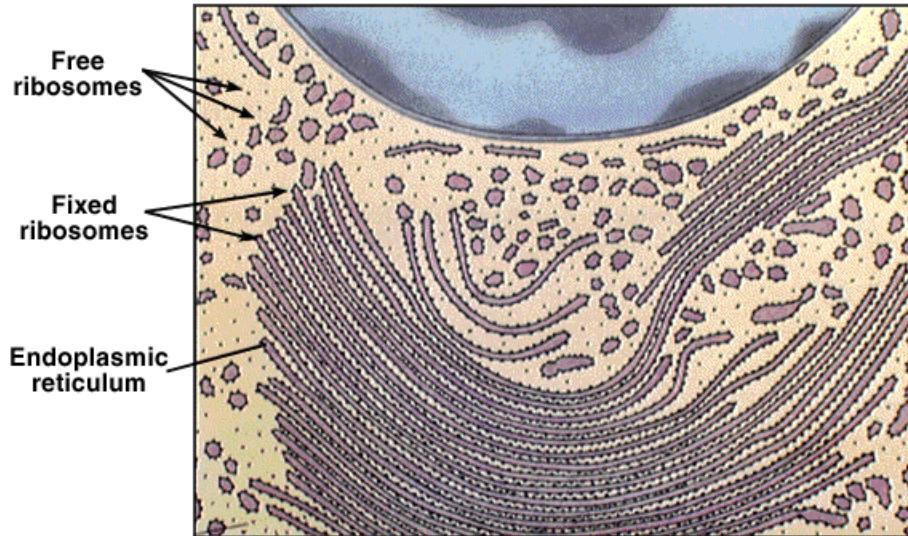


# The nonmembranous organelles include:

- **Microtubules**, which together with actin and intermediate filaments form elements of the **cytoskeleton** and continuously elongate (by adding tubulin dimers) and shorten (by removing tubulin dimers), a property referred to as **dynamic instability**;
- **Filaments**, which are also part of the cytoskeleton and can be classified into two groups—**actin filaments**, which are flexible chains of actin molecules, and **intermediate filaments**, which are ropelike fibers formed from a variety of proteins—both groups providing tensile strength to withstand tension and confer resistance to shearing forces;
- **Centrioles**, or short, paired cylindrical structures found in the center of the **microtubule-organizing center (MTOC)** or **centrosome** and whose derivatives give rise to basal bodies of cilia;
- **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).

# Ribosomes

60% RNA + 40% protein  
Protein Factories  
Fixed vs. free ribosomes



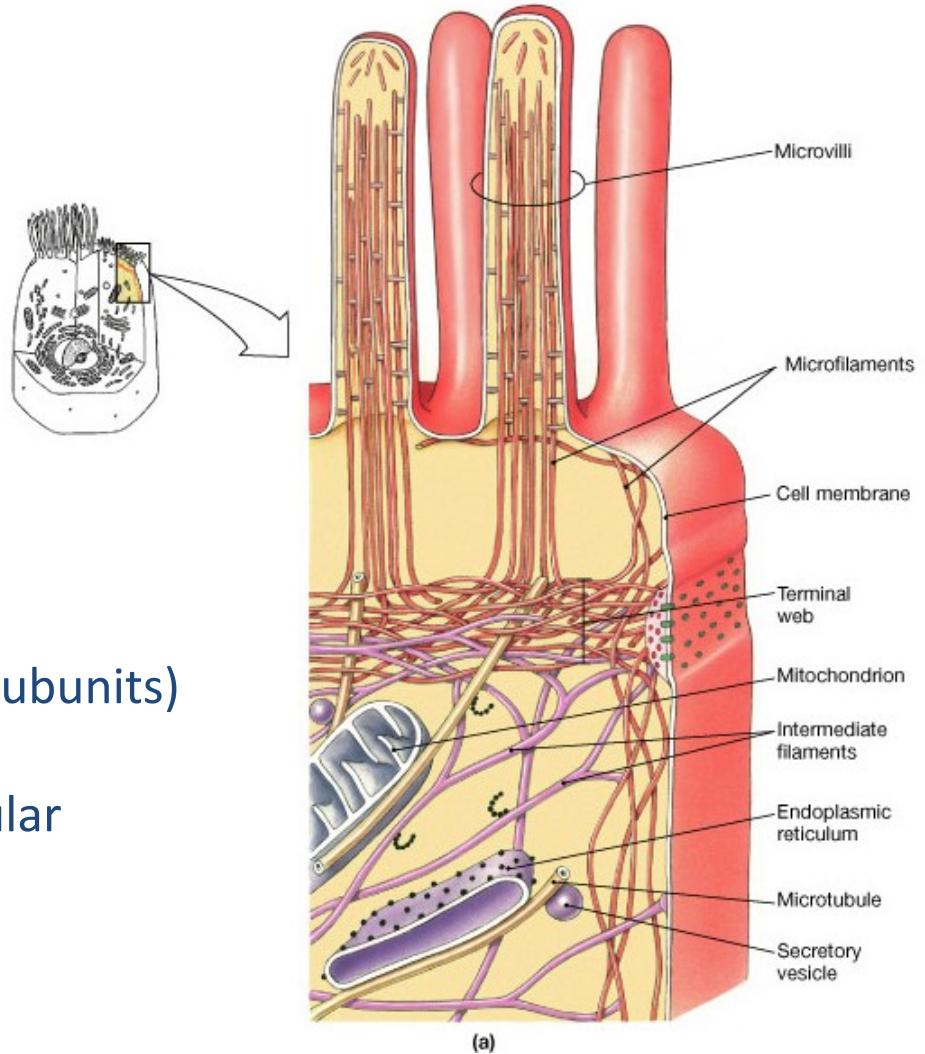
(b) Ribosome

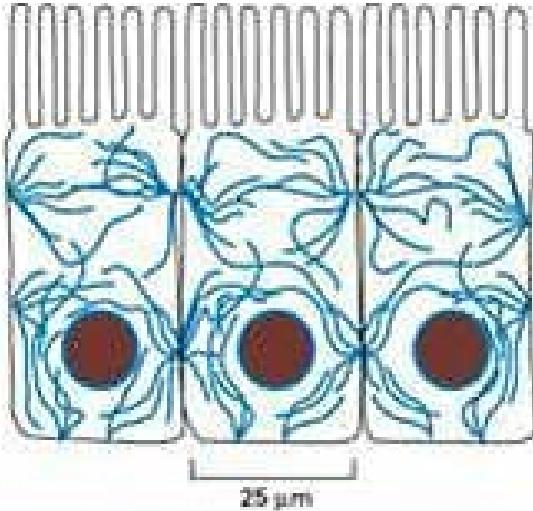
# Cytoskeleton

## 3 major components:

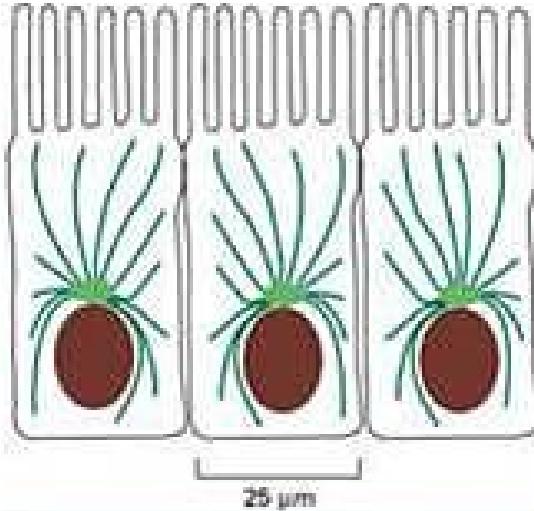
1. Microfilaments (mostly actin)
2. Intermediate filaments
3. Microtubules (composed of tubulin subunits)

**Function:** support & movement of cellular structures & materials

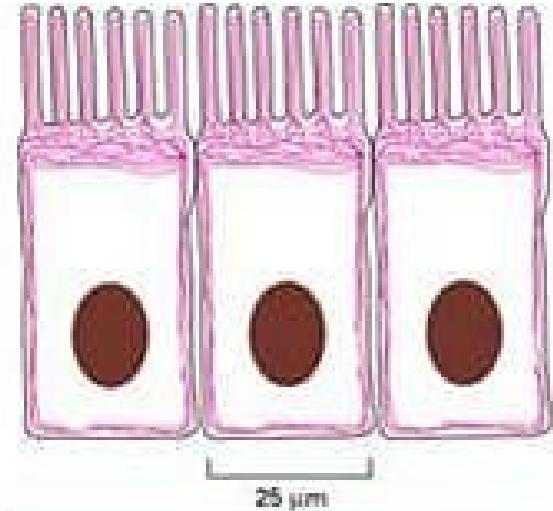




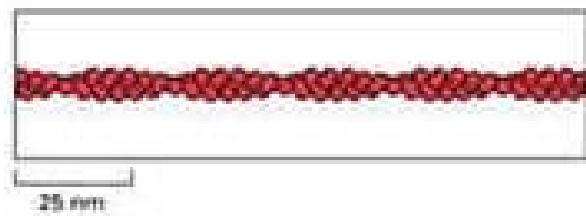
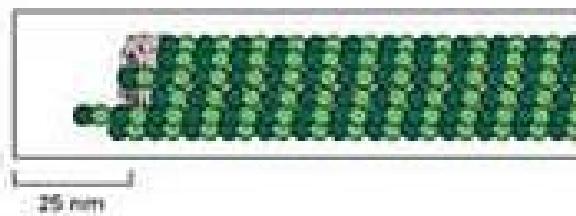
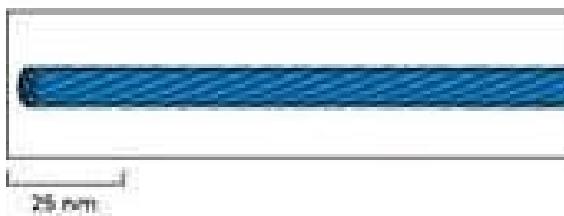
INTERMEDIATE FILAMENTS



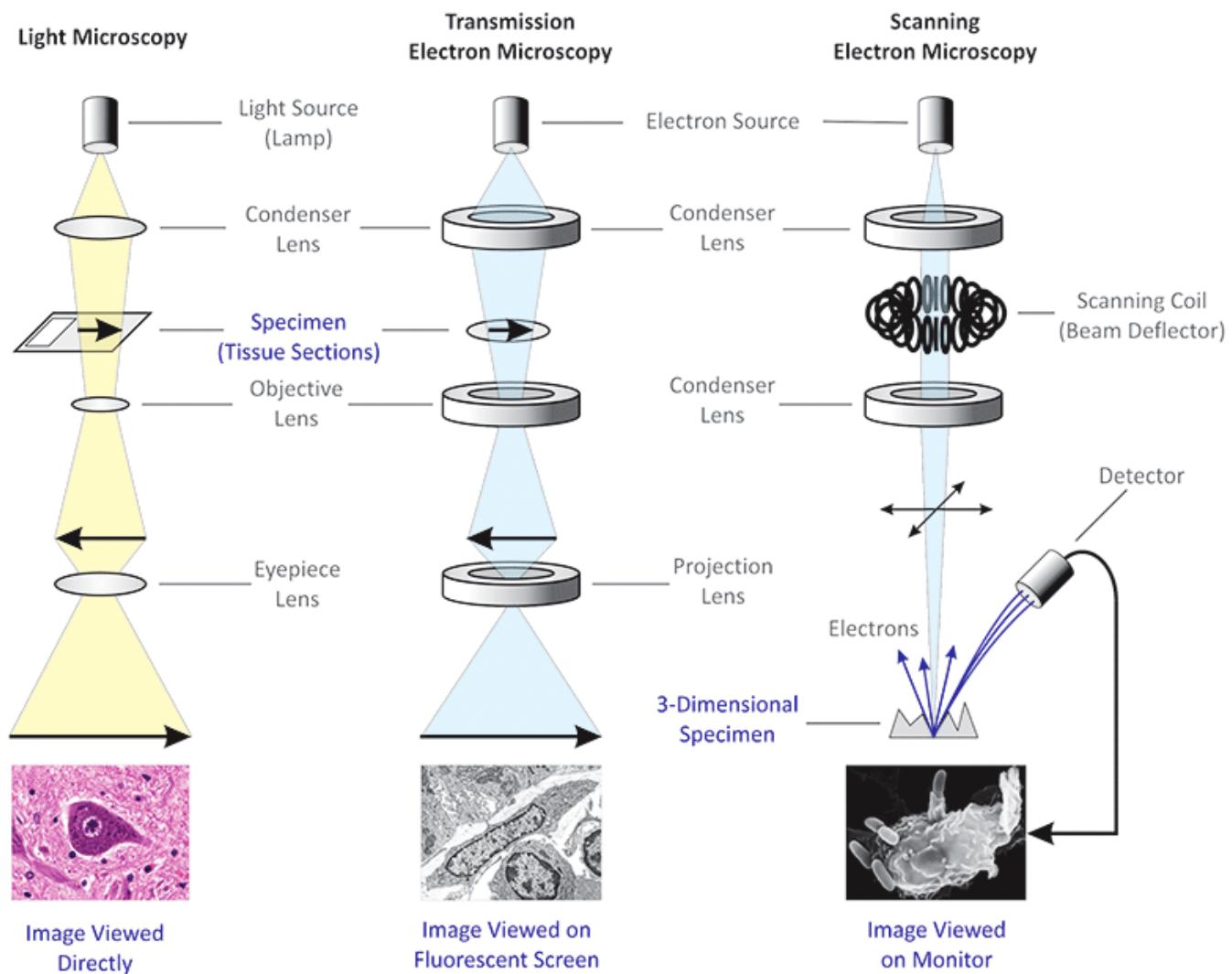
MICROTUBULES

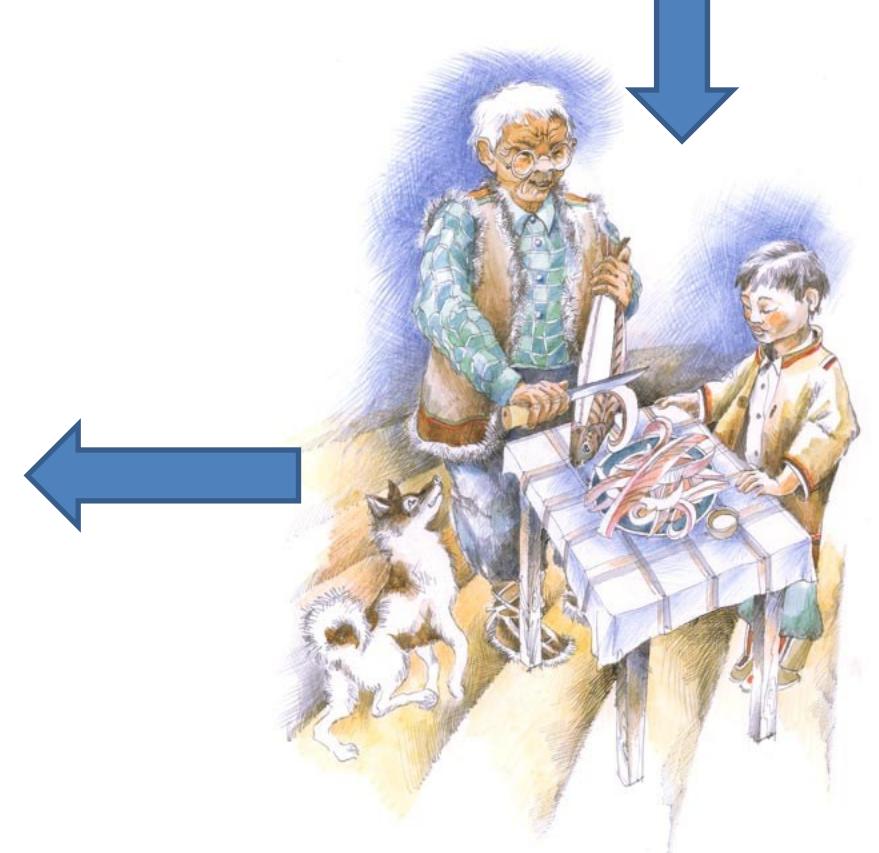
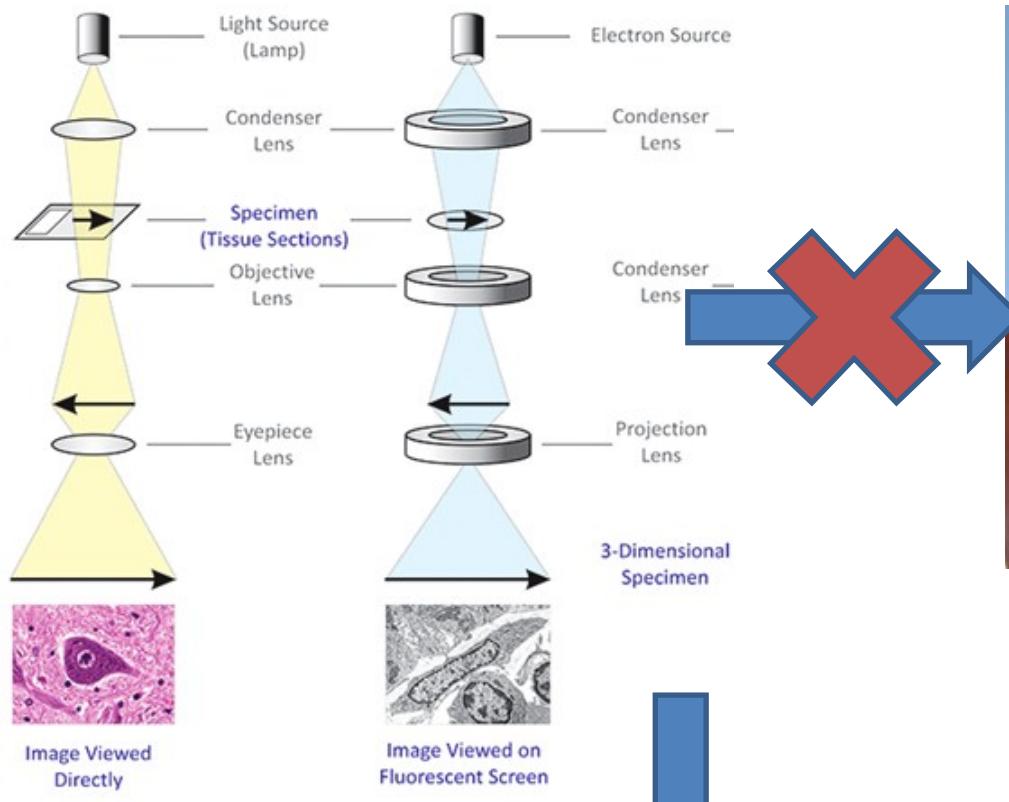


ACTIN FILAMENTS



# The methods used by histologists:



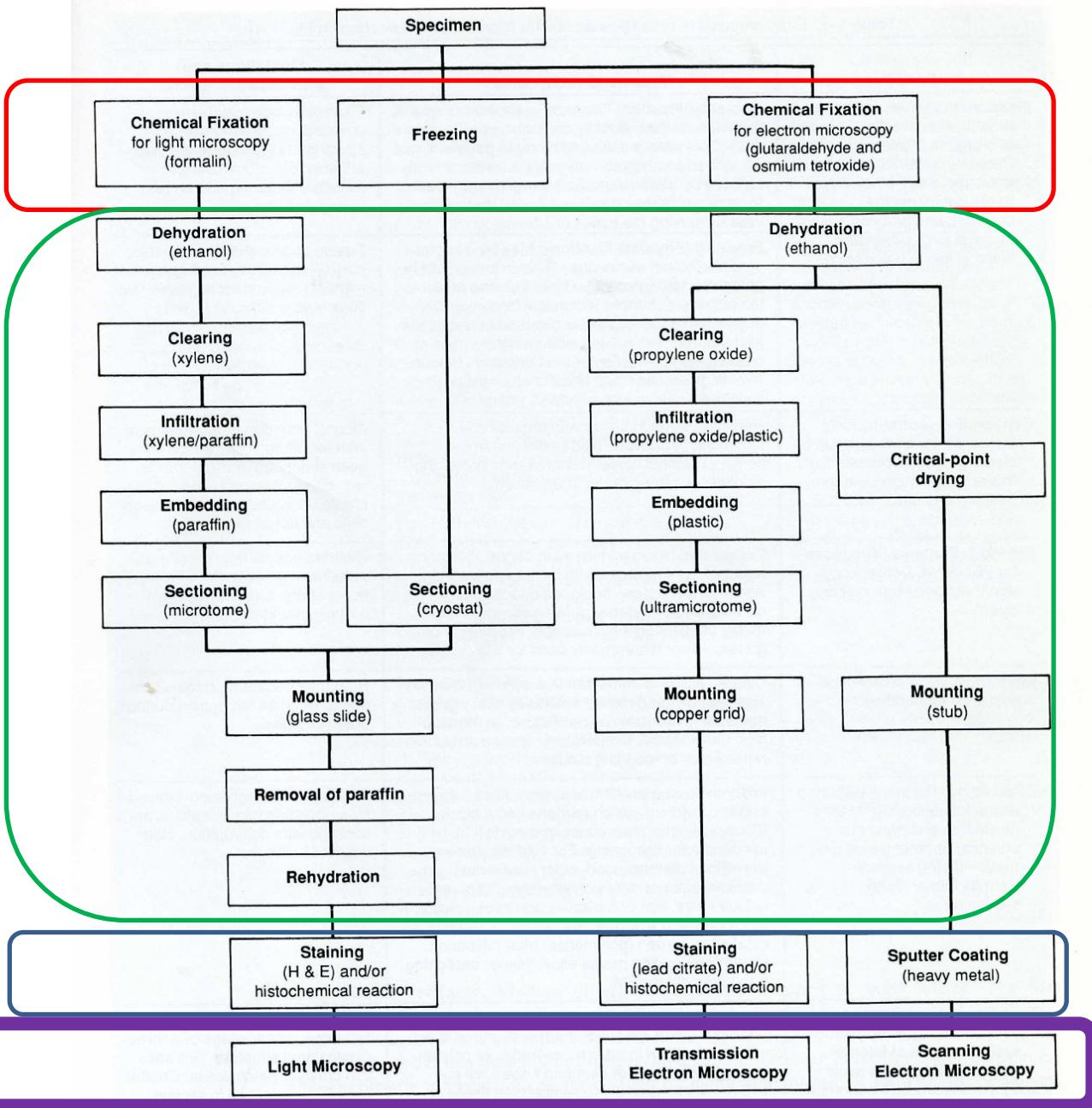


# TISSUE PREPARATION

Tissues are aggregates or groups of cells organized to perform one or more specific functions.

1. The first step in preparation of a tissue or organ sample is **fixation** to preserve structure.
2. In the second step, the specimen is prepared for **embedding** to permit sectioning.
3. In the third step, the specimen is **stained** to permit examination.

## The first step



# The first step

**Fixation**, usually by a chemical or mixture of chemicals, permanently preserves the tissue structure for subsequent treatments. Specimens should be immersed in fixative immediately after they are removed from the body.

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.

<p><b>Fixation:</b> Preserves structural organization of cells, tissues, and organs of interest. Prevents bacterial and enzymatic digestion, insolubilizes tissue components to prevent diffusion, and protects against damage from subsequent steps in tissue processing.</p>	<p><b>Chemical Fixation:</b> Common approach. Chemical fixatives used individually or in mixtures. Best results achieved by rapid penetration of living tissue with fixative. Small tissue pieces may be fixed by <b>immersion</b>. Entire organs may be fixed by <b>perfusion</b> (fixative pumped through vessels serving the tissue of interest).</p>	<p>Fixative-induced changes in chemical composition and fine structure may produce staining artifacts. Structural changes include denaturing and cross-linking proteins.</p>
	<p><b>Freezing (Physical Fixation):</b> May be used for light or electron microscopy. Tissue embedded in cryoprotectant (glycerin). Rapid freezing at low temperatures reduces ice crystal formation and associated artifacts. Allows tissue to be sectioned (or fractured) without dehydration or clearing. Faster than chemical fixation. Avoids dissolving lipids and denaturing fixative-sensitive proteins (eg, enzymes, antigens).</p>	<p>Frozen specimens do not last as long as chemically fixed specimens. Obtaining serial frozen sections is very difficult.</p>

## Properties of chemical fixatives and fixative mixtures.

Type	Actions	Examples
Aldehydes	React with amine groups, form cross-links among proteins, and cause coagulation (but not coarse precipitation) of tissue proteins. May interfere with periodic acid-Schiff (PAS) and Feulgen staining specificity.	<b>Formalin</b> (formaldehyde gas in water) commonly used for light microscopy. <b>Glutaraldehyde</b> commonly used for EM.
Oxidizing agents	Cross-link proteins and precipitate unsaturated lipids.	<b>Osmium tetroxide</b> . Often used with glutaraldehyde for EM (see Double Fixation below). Also, <b>potassium permanganate</b> and <b>potassium dichromate</b> .
Protein-denaturing agents	Normal protein shape maintained largely by ionic interactions with water molecules. Denature protein by removing associated water, changing the protein's shape. In absence of cross-linking agents, rehydrating tissue may restore protein conformation.	<b>Acetic acid, methanol, ethanol, acetone.</b>
Others	Unclear	<b>Mercuric chloride, picric acid.</b>
Mixtures	Exploit advantages and minimize disadvantages of various fixatives.	<i>Light microscopy:</i> <b>Bouin's fluid</b> (picric acid, formalin, acetic acid). <i>EM:</i> <b>Karnovsky's fixative</b> (paraformaldehyde and glutaraldehyde in buffered saline).
Double fixation	Used for EM. Specimen fixed in buffered glutaraldehyde, washed in phosphate buffer, and postfixed in buffered osmium tetroxide. Osmium reacts with lipids to form black precipitate that stains cell membranes.	

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# The second step

<b>Dehydration (substitution):</b> Eases penetration of tissue by clearing agent. Prepares fixed tissue for infiltration with embedding medium.	Replaces water in tissue with organic solvent; commonly, <b>ethanol</b> . Fixed tissue immersed in series of alcohol–water mixtures with increasing alcohol concentration, to 100% alcohol.	Alcohol may denature proteins of interest. Water loss causes uneven shrinkage of components with different water content. May create unnatural spaces between cells and tissue layers.
<b>Clearing:</b> Prepares fixed tissue for infiltration. Dehydrating agent replaced with clearing agent.	Dehydrated tissue immersed in series of clearing agent-alcohol mixtures with increasing clearing agent concentration, or placed directly into clearing agent. <b>Xylene</b> (paraffin solvent) commonly used for light microscopy. <b>Propylene oxide</b> (plastic solvent) commonly used for EM.	Clearing agents may denature proteins of interest. Some components shrink unevenly as their proteins denature.
<b>Infiltration:</b> Prepares cleared tissue for embedding.	Cleared tissue is immersed in a series of clearing agent-embedding medium mixtures with increasing embedding medium concentrations, at medium-high temperature. Evaporating clearing agent is replaced by embedding medium.	Heat may denature proteins of interest. Bubbles left behind during poor infiltration.
<b>Embedding:</b> Prepares infiltrated tissue for sectioning. Makes tissue firm and prevents crushing or other tissue disruption during sectioning. Permits thin, uniform sectioning.	Infiltrated tissue positioned in mold filled with embedding medium, which hardens into a block. Block is attached to a chuck that holds it in microtome for sectioning. <i>For light microscopy</i> , <b>paraffin</b> commonly used; other media are celloidin, plastics, and polyethylene glycol (water soluble) wax. <i>For EM</i> , plastics and epoxy resins (eg, <b>Epon</b> and <b>Araldite</b> ) are common. Require a catalyst to harden (polymerize) after infiltration. Harder embedding media allow thinner sectioning, a requirement for EM.	The improved sectioning allowed by embedding has limitations associated with dehydration, clearing, and infiltration.
<b>Sectioning:</b> Most tissues too thick and opaque for microscopic analysis of internal structure. Thin slices allow light or electrons to penetrate specimen and form image.	<i>For light microscopy</i> , standard rotary <b>microtome</b> with steel blade will cut 3–8- $\mu\text{m}$ sections of specimens embedded in paraffin, celloidin, or polyethylene glycol. Glass or diamond knives will cut 1–5- $\mu\text{m}$ sections of plastic-embedded tissue. Frozen sections, 5–25 $\mu\text{m}$ , are cut with a freezing microtome or in a <b>cryostat</b> (standard microtome in a refrigerated chamber). <i>For EM</i> , an <b>ultramicrotome</b> with a glass or diamond knife will cut very thin sections (0.08–0.1 $\mu\text{m}$ or up to 0.5 $\mu\text{m}$ for high-voltage EM). Ultramicrotomes include stereomicroscope to observe cutting.	Sections typically provide only a two-dimensional image of a three-dimensional structure. Dull knife can crush or pinch tissue. Chatter (wavelike variations in section thickness) results from knife vibration during sectioning. Burr on knife can tear tissue.

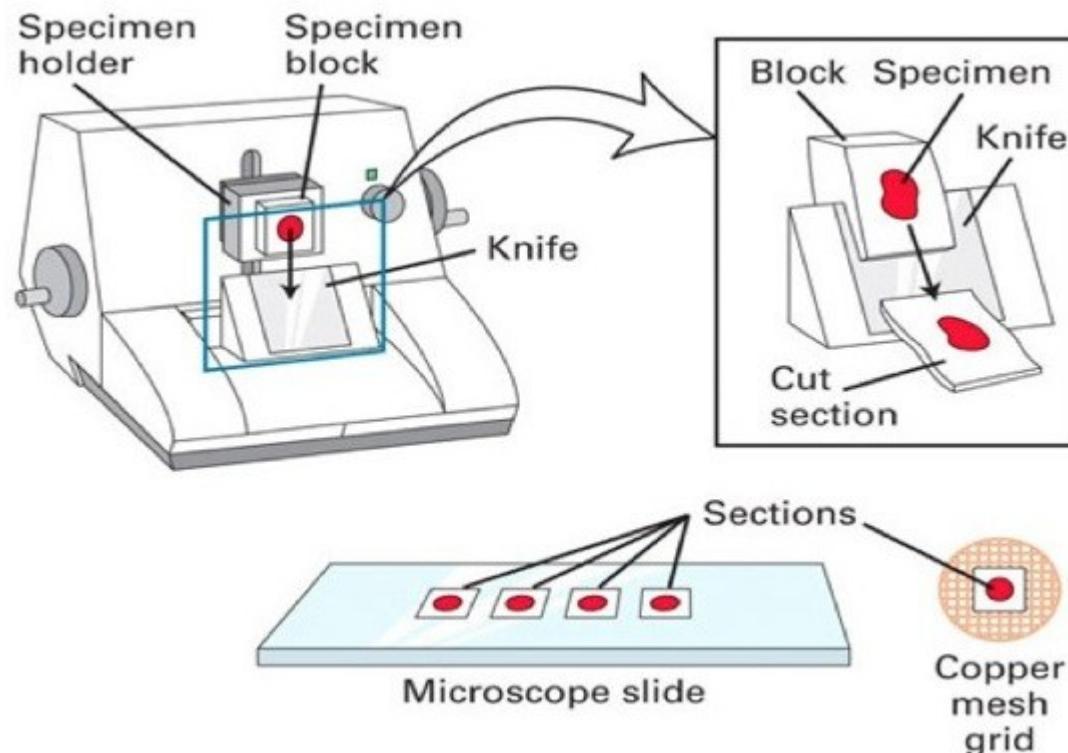


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## SECTIONING

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

- a) 7 micron sections for LM - placed on glass slides
- b) 70 millimicrons - placed on metal grids for TEM





# The third step

<p><b>Mounting:</b> Eases handling and decreases damage to specimen during examination.</p>	<p>For light microscopy, sections placed on <b>glass slides</b>, often precoated with thin layer of albumin, gelatin, or polylysine to improve attachment. After staining, sections covered with glass coverslips to preserve them for repeated examination. For EM, specimens mounted on <b>copper grids</b>. Electron beam cannot penetrate glass.</p>	<p>Tissue sections may develop folds, making some regions appear to have more cells and stain darker. For grid-mounted specimens, only portions lying between crossbars will be visible.</p>
<p><b>Staining:</b> Most tissue substructure is indistinguishable even at high magnification. Stains, ligands with specific binding affinities and optical properties, and radiolabels are used to localize and distinguish cell and tissue components. Knowledge of specificities of such substances (Table 1–3) provides additional information about structure and composition.</p>	<p><b>For light microscopy:</b> Once sections are on slide, paraffin is dissolved. Tissue may be rehydrated before staining. Plastic sections stained without removing plastic. Most stain affinities based on reciprocal acid–base characteristics of stain and tissue components. Acidic stains (eg, eosin) bind basic (ie, <b>acidophilic</b>) structures and compounds (eg, cytoplasmic proteins). Basic stains (eg, hematoxylin) bind acidic (ie, <b>basophilic</b>) tissue components (eg, nucleic acids in ribosomes). Stain mixtures reveal multiple cell components. Hematoxylin and eosin (H&amp;E), most common stain mixture for light microscopy, distinguishes nucleus from cytoplasm.</p>	<p>Acid–base boundaries may not correspond to boundaries between structures. Multiple staining procedures may be needed to characterize a particular cell or tissue component. Because colors are artifacts of staining, it is best to focus more on tissue component structure than color.</p>
	<p><b>For TEM:</b> Most stains (contrasting agents) for TEM chosen for electron-absorbing or -scattering ability and affinity for particular cell components. <b>Heavy metal salts</b>, such as lead citrate and uranyl acetate, are common. The fixative osmium tetroxide interacts with lipids to form electron-dense precipitate and doubles as a stain for cell membranes.</p>	<p>TEM stains stop electrons from penetrating. TEM images are shadows of heavy metal deposits. Actual tissue structures not seen.</p>
	<p><b>For SEM:</b> SEM specimens not stained per se. First subjected to <b>critical point drying</b>, to prevent artifacts related to surface tension. After dehydration, specimens soaked in liquid miscible with CO<sub>2</sub> or Freon and put in critical point chamber. Chamber is heated to a critical temperature (31 °C), raising pressure to a critical 73 atm, at which the gas and liquid phases exist without surface tension and liquid escapes specimen without altering structure. Specimen is then mounted on a stub and sputter-coated (sprayed) with fine mist of heavy metal particles (eg, gold) before viewing (Fig. 1–1).</p>	<p>SEM reveals surface architecture in exquisite detail, but heavy metal coating prevents electrons from penetrating to reveal internal structure.</p>

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# STAINING

Basic reaction of stains = attraction of opposites:

- a) Structures that stain with a basic stain = **BASOPHILIC** (stain acid component - Nuclei or RER in secretory cells)
- b) Structures that stain with an acidic stain = **ACIDOPHILIC** (stain basic component - "Normal" cytoplasm)

Special techniques of **Histochemistry** (localization of enzyme activity) or **Immunostaining** (antigen detection with antibodies)



Examples of common stains and their affinities.

<b>Application</b>	<b>Types</b>	<b>Stains</b>	<b>Affinity</b>
Light microscopy	Basic dyes	Hematoxylin, toluidine blue, methylene blue, alcian blue	Basophilic tissue components, eg, DNA, RNA, and polyanions such as sulfated glycosaminoglycans.
	Acidic dyes	Eosin, orange G, acid fuchsin	Acidophilic tissue components, eg, basic proteins in cytoplasm.
	Lipid-soluble dyes	Oil red O, Sudan black	Long-chain hydrocarbons (fats, oils, waxes).
	Multicomponent histochemical reaction	Periodic acid-Schiff (PAS) reaction	Complex carbohydrates (glycogen, glycosaminoglycans).
		Feulgen's reaction	Nuclear chromatin (DNA and associated proteins).
Transmission electron microscopy	Heavy metal (electron dense)	Uranyl acetate, lead citrate	Nonspecific; adsorb to surfaces and enhance contrast.
		Osmium tetroxide	Actually a fixative, but binds to phosphate groups of membrane phospholipids, enhancing contrast.
		Ruthenium red	Polyanions; complex carbohydrates, eg, oligosaccharides of the glycocalyx and glycosaminoglycans of the extracellular matrix.

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

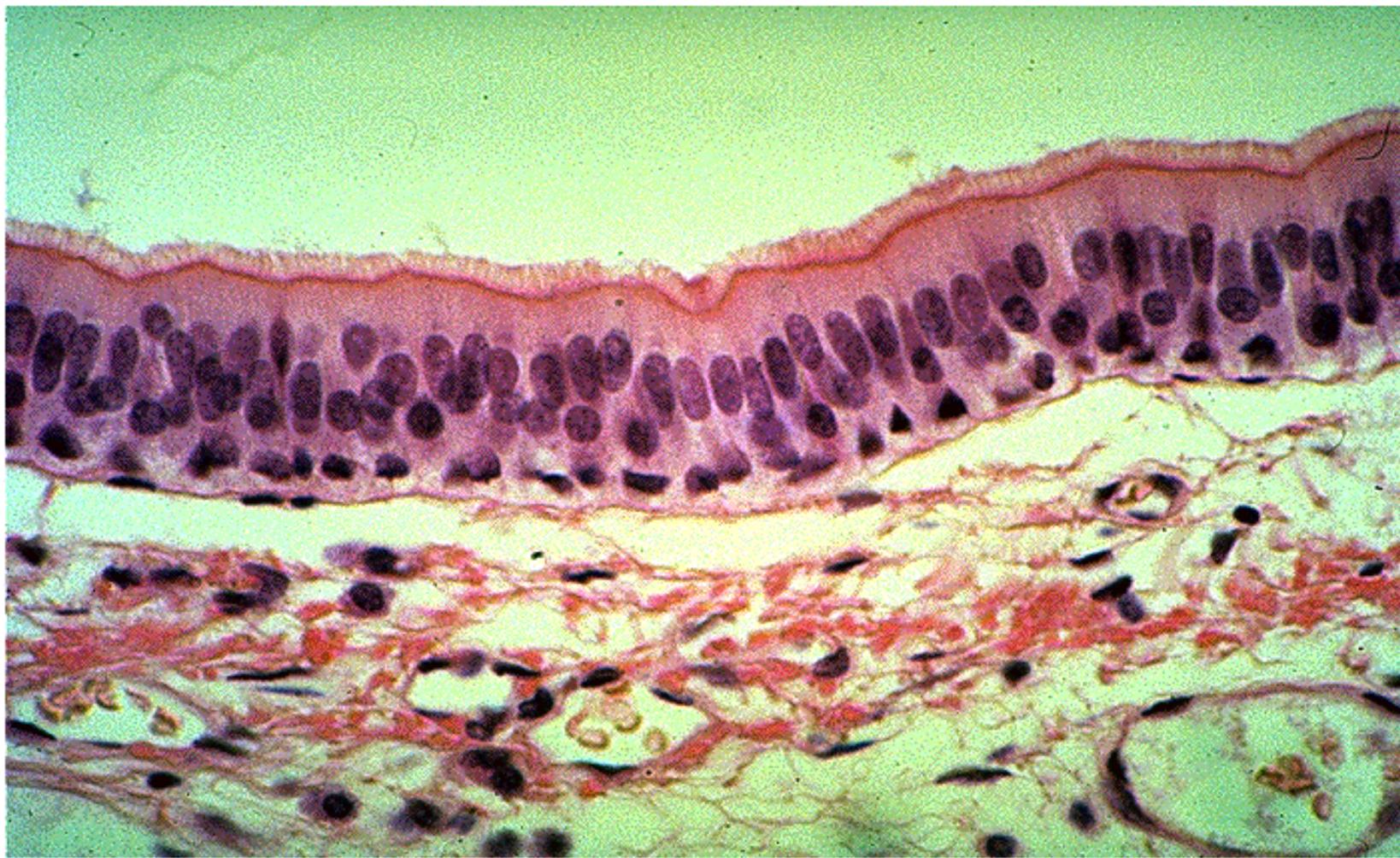
- **heterochromatin** and **nucleoli** of the nucleus (chiefly because of ionized phosphate groups in nucleic acids of both),
- **cytoplasmic components** such as RER (also because of ionized phosphate groups in ribosomal RNA),
- **extracellular materials** such as the complex carbohydrates of the matrix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

## Hematoxylin and Eosin (H&E)

Nuclei (DNA) and Rough Endoplasmic Reticulum (ribosomes-RNA) are acids and basophilic and stain blue with hematoxylin. Cytoplasmic proteins (acidophilic at neutral pH) stain pink with eosin.



**1) Hematoxylin & Eosin (H & E)** - most common stain - good for general structure

- a) nuclei = blue (basophilic) → Hematoxylin
- b) cytoplasm = pink (acidophilic) → Eosin

**2) Connective tissue stains** - both employ a nuclear, cytoplasmic, and a third stain specific for matrix

- a) **Masson's trichrome**
- b) **Mallory's triple C.T. stain**

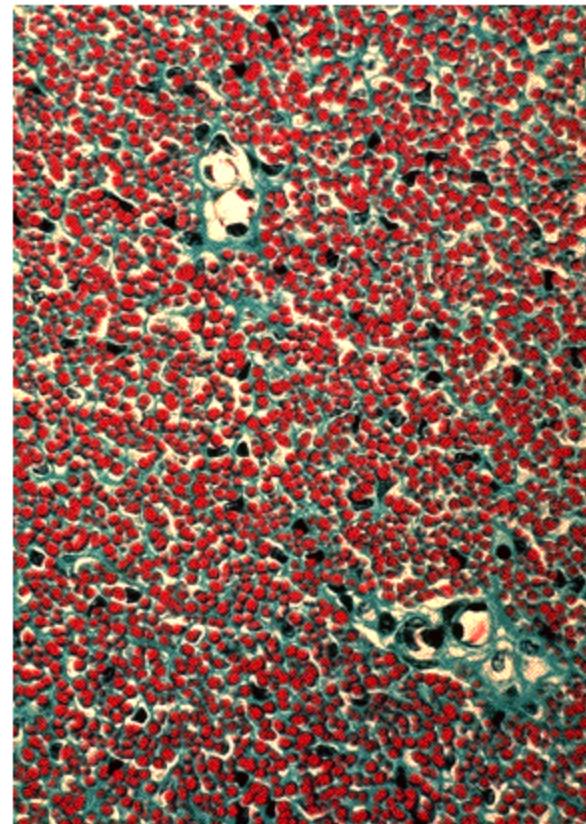
## Mallory's and Masson's Trichrome

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



### **3) Silver Impregnation**

- a) specificity provided by what silver is complexed to and pH of staining solution
- b) used to trace nerves, stain golgi, reticular fibers

### **4) PAS (Periodic Acid Schiff's)**

- a) Schiff reagent a stain called Basic Fuchsin = specific stain for carbohydrates = PAS stain

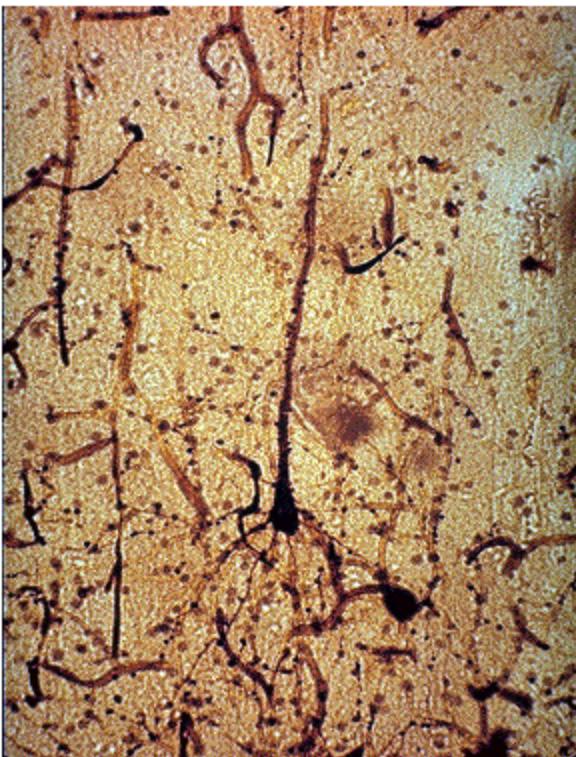
### **5) Wright Stain for blood smears**

- a) uses azure blue stains to stain WBC granules basophilic or neutrophilic
- b) used with eosin for RBS and eosinophilic

## Golgi's Silver Stain

Selective for nerve cells-neurons blacken when silver is reduced. Only a few cells stain which is useful for determining relations between nerve cell processes.

Pyramidal Cell in Cerebral Cortex

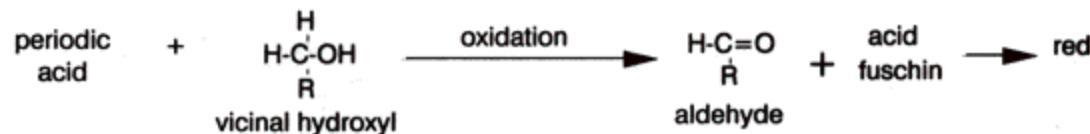


Synaptic Boutons on Purkinje Cell



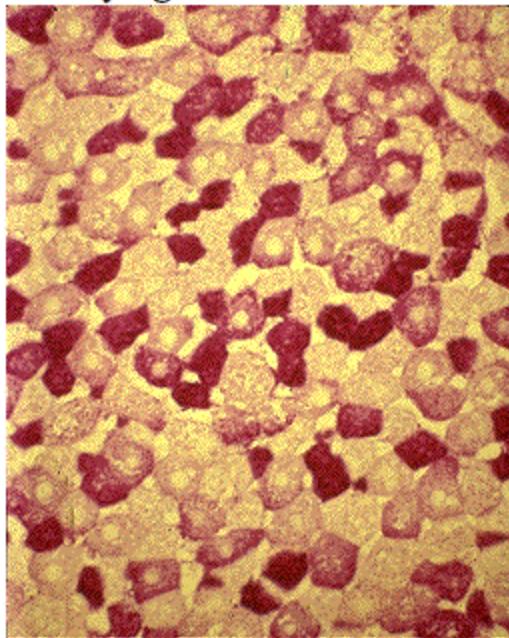
## Histochemical Stains

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

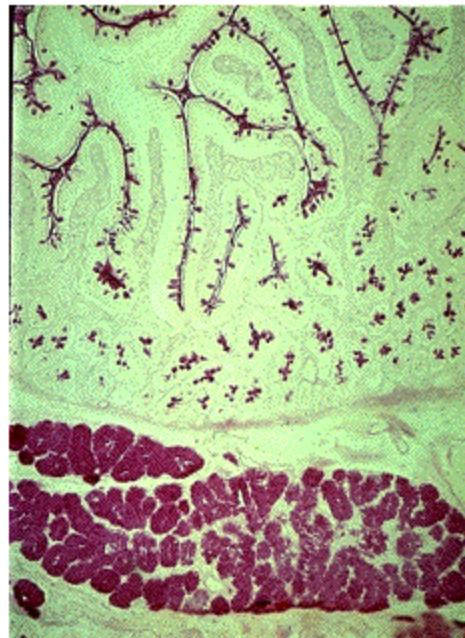


Stains mucopolysaccharides (intestinal mucosa & liver glycogen) and glycoproteins

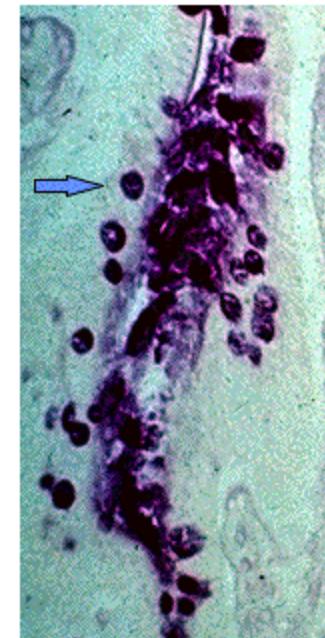
Glycogen in Liver Cells



Intestinal Mucosa



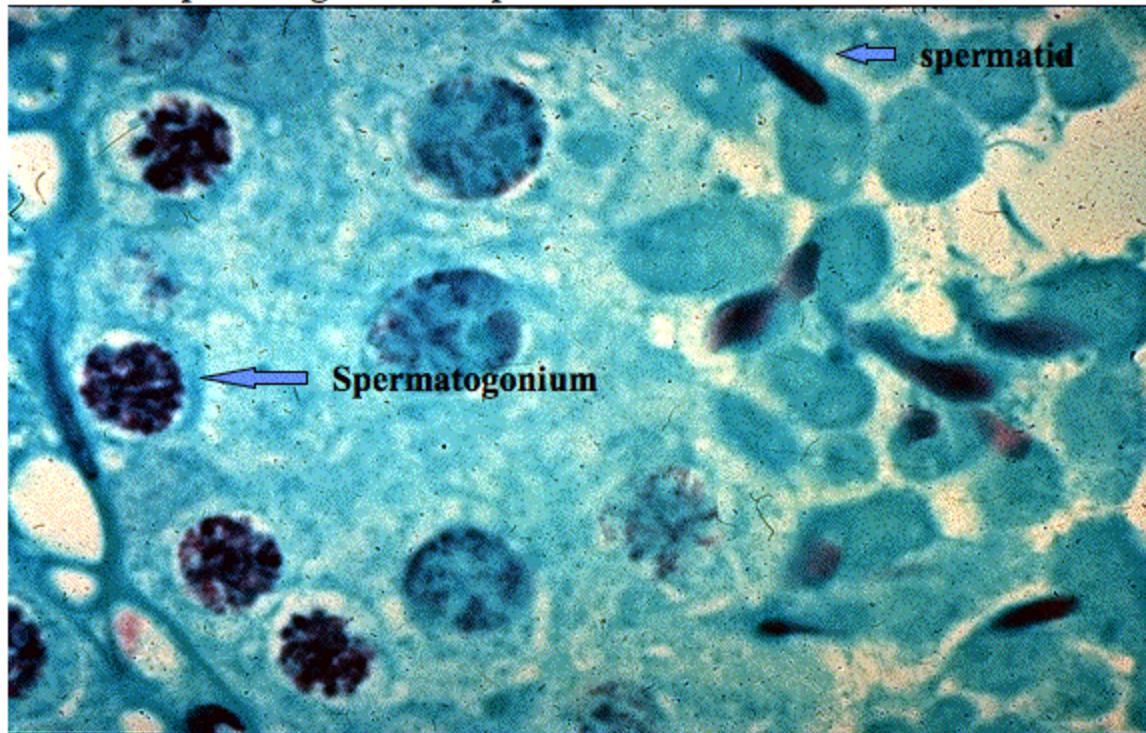
Goblet Cells



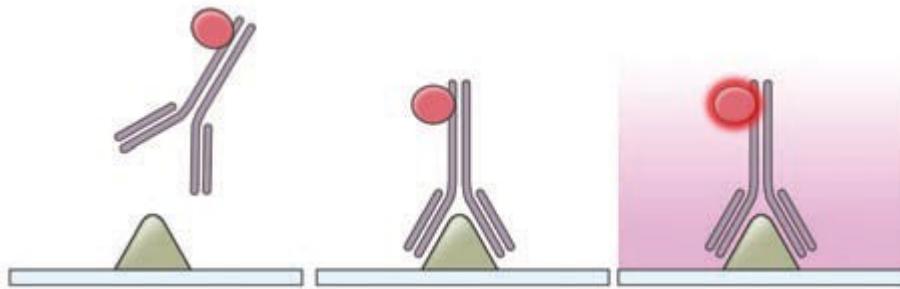
## Histochemical Stains

**Feulgen Reaction-** Mild acid hydrolysis removes RNA but not DNA and unmasks aldehyde groups of deoxyribose. Free aldehyde groups react with Schiff's reagent. Nuclei and chromatin are Feulgen positive and cytoplasm is Feulgen negative.

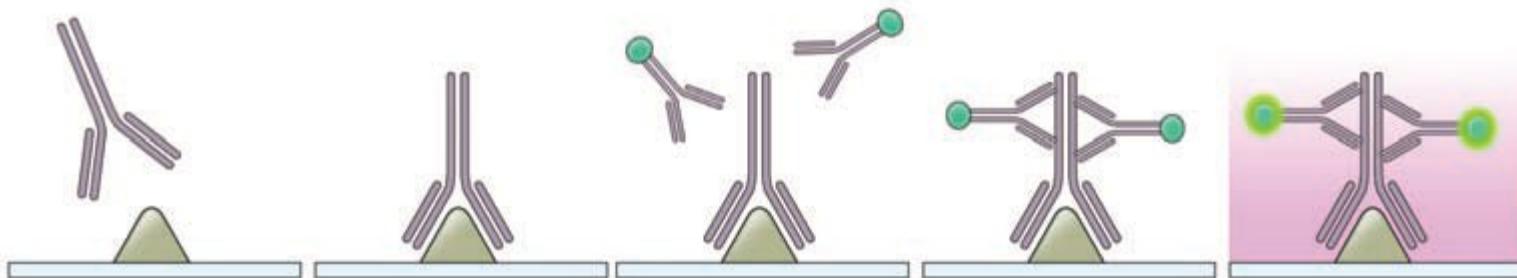
Spermatogonia and Spermatids in Seminiferous Tubule



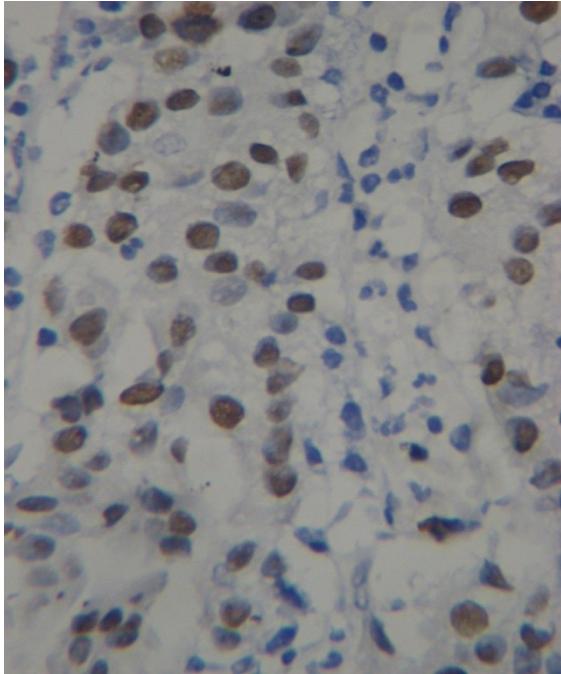
## DIRECT IMMUNOSTAINING



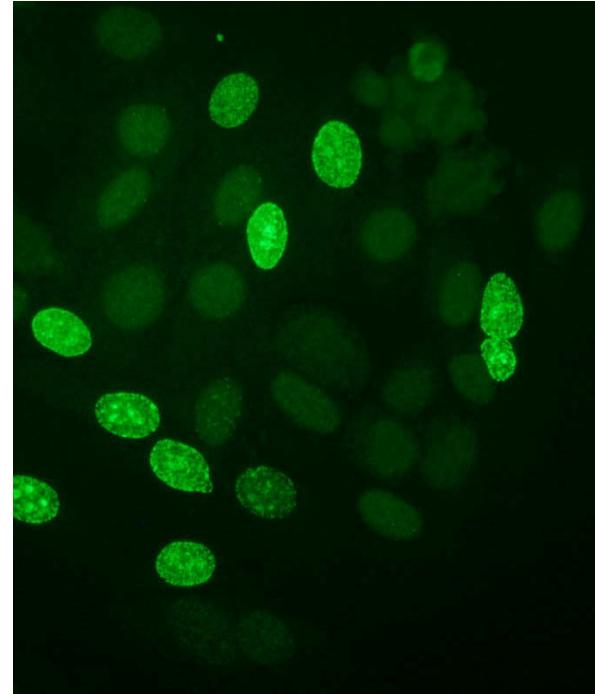
## INDIRECT IMMUNOSTAINING



# Immunostaining (antigen detection with antibodies)



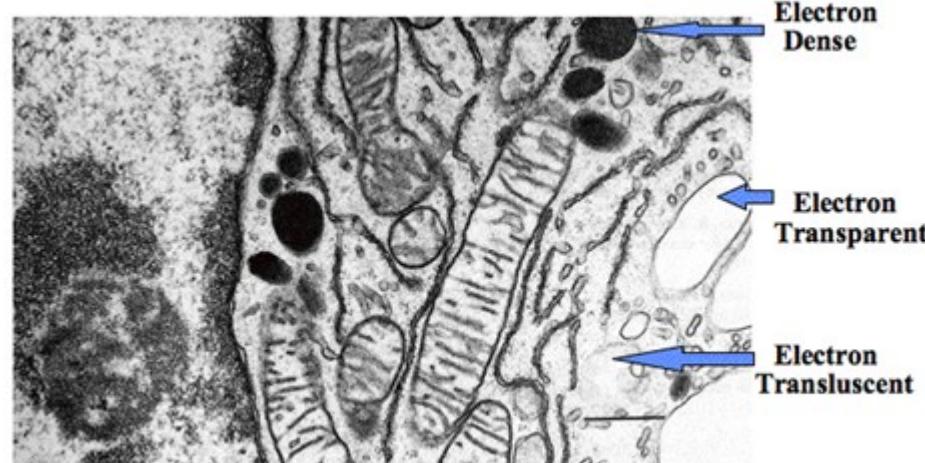
immunohistochemistry



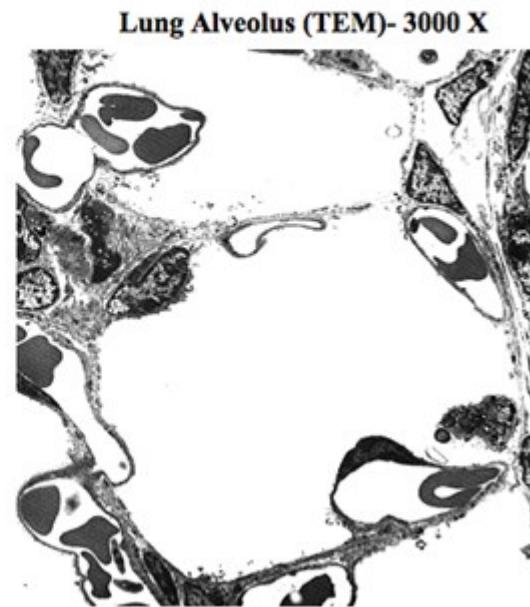
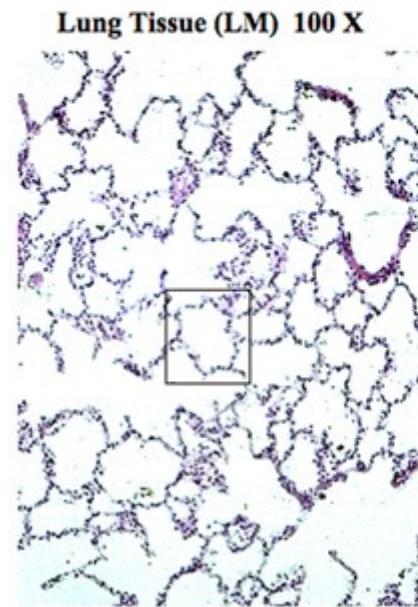
Immunofluorescent stain

For TEM - staining done with heavy metals = provide electron density to section - most commonly:

- 1) Uranyl Acetate
- 2) Lead citrate



#### Magnification with the Transmission Electron Microscope





# Thank you for attention

