

Lab Practical One

Week 1: Lab Methods and Organelles

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Week 1: Lab Methods and Organelles

Background

- **Describe the steps in tissue preparation for microscopy:**
 - **Fixation:** typically the first step in the preparation of histological sections in where tissues samples are treated with fixatives as a means to preserve and protect from biological decay.
 - **Fixatives:** solutions, compounds, or others means meant to either disable degradative enzymes, induce cross-linking (stabilizing proteins), or protect from extrinsic damage.
 - **Embedding:** the process of placing tissues in a harder medium (e.g., paraffin and plastic resins) as a means to allow for thin slicing of tissue.
 - Embedding occurs later in the process of preparation; once a tissue is fixed it must first undergo a series of steps:
 - **Dehydration:** the removal of water using ethanol.
 - **Clearing:** replacement of an organic solvent miscible with both alcohol and the embedding medium, giving a translucent appearance.
 - **Infiltration:** evaporation of the clearing solvent via exposure to heat (50–60 °C) promoting the final embedding of tissue into the medium.
 - **Staining:** used as a means to increase contrast in tissue or specific features of tissue that are of interest as most biology tissue has very little inherent contrast.
 - **Basophilic:** dyes that have an affinity for **anionic** (net negative charge) cells parts.
 - E.g., hematoxylin, toluidine blue, alcian blue, and methylene blue.
 - **Acidophilic:** dyes that have an affinity for **cationic** (net positive charge) cell parts.
 - E.g., eosin, orange G, and acid fuchsin
- **What does H & E Stain?**
 - Hematoxylin (H) and eosin (E) stains are among the most commonly used stains.
 - As mentioned above, hematoxylin acts as a **basophilic dye**, turning negatively charged organelles like the cell nucleus, RNA-rich regions of cytoplasm, cartilage, anywhere from blue → purple.
 - Eosin acts as an **acidophilic dye**, typically turning cationic structures pink; sometimes it is considered to be a **counterstain**, i.e., typically a secondary dye that is meant to distinguish features.
- **What does PAS Stain?**
 - Periodic acid-Schiff (PAS) utilizes hexose rings of polysaccharides and other carbohydrate rich structures to stain macromolecules purple → magenta.

- **Describe Enzyme Histochemistry.**

- Enzyme histochemistry is a method for localizing cellular structures using specific enzymatic activity in such structures.
- Preservation of enzymes often requires non-fixed or mildly fixed tissue and generally adhere to the following steps:
 1. Tissues sections are immersed in solution containing the substrate of the enzyme to be localized.
 2. The enzyme is exposed to and allowed to act on the substrate.
 3. A marker compound is introduced and reacted with the product from step 2.
 4. Location is determined via precipitation of the insoluble product, which must be visible a light or electron microscopy, over the site of the enzyme.
- Phosphatase, dehydrogenase, and peroxidase are common examples of enzymes detected with histochemistry.

- **How does Immunohistochemistry work?**

- Immunohistochemistry (IHC): the use of labeled antibodies and antigens to identify and localize many proteins and macromolecules that lack specific enzymatic activity.
- Visualization of such interactions are commonly accomplished with either:
 - Chromogenic immunohistochemistry (CIH): use of antibodies conjugated to an enzyme that catalyzes a color-producing reaction.
 - Immunofluorescence: tagging of a fluorophore (fluorescein, rhodamine) to an antibody.
- Common used in diagnosis of abnormal cells such as those in cancerous tumors.

Microscopic Techniques

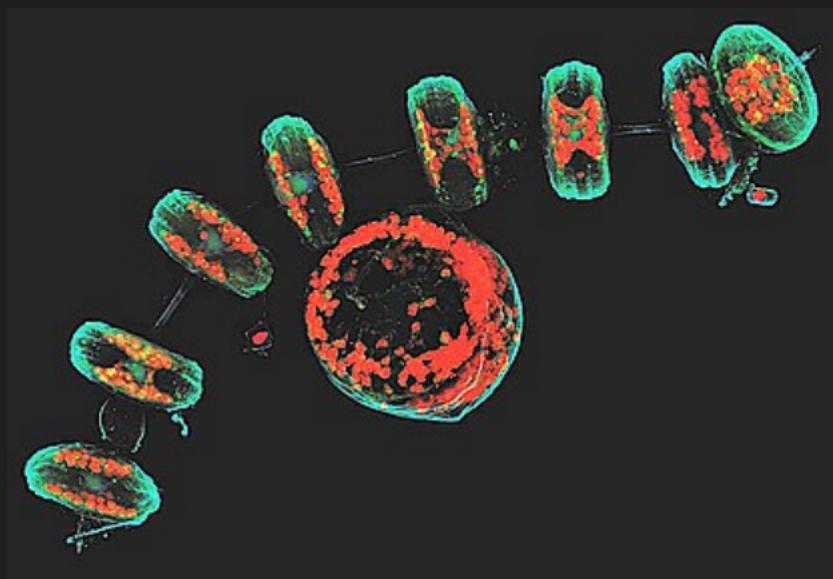
- **Bright field:** a very common method that uses ordinary light and stained structures to discern differences and cell structures.



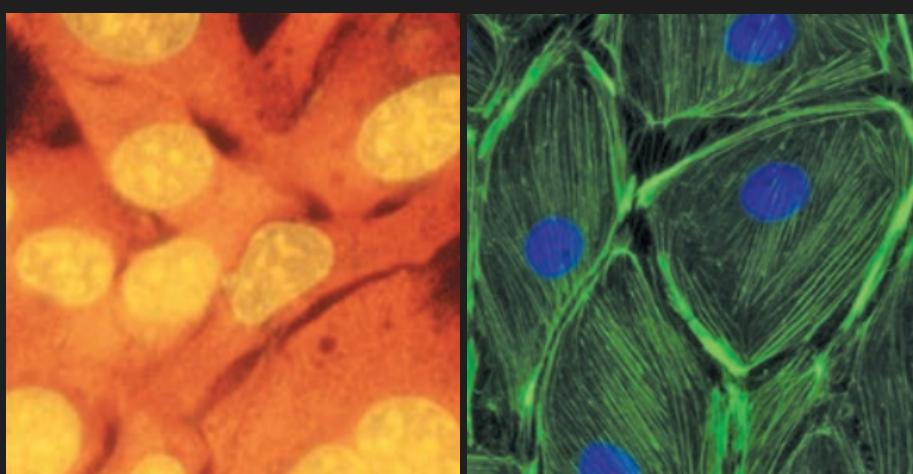
- **Phase contrast:** uses differences in refractive index of natural cell and tissue components; allows for observation of living cells.



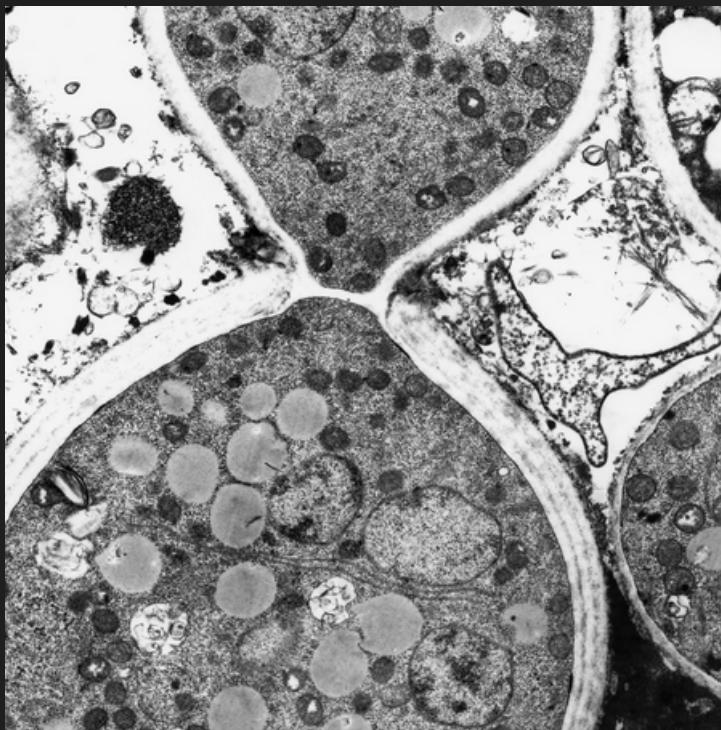
- **Confocal:** use of scans at successive focal planes with a more focused light beam, usually from a laser, to construct a 3D image.



- **Fluorescent:** use of longer wavelengths emitted via specific perturbations of cellular substances using a specific wavelength (usually UV). Useful for identification of cells and components that have affinity for specific fluorescent compounds.



- **Transmission Electron Microscopy (TEM)**: a high resolution (3 nm) that allows for particles to be magnified many times via transmission of electrons through a specimen; very thin tissue sections are used and a flat image is made from the intersection of the electron beam and the sample.

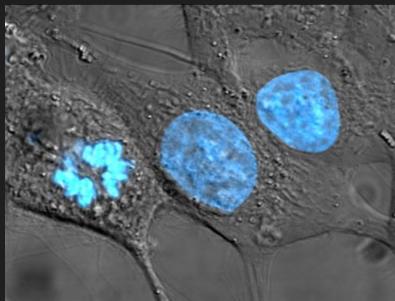


- **Scanning Electron Microscopy (SEM)**: used to provide a high resolution view of the surface of cells, tissues, and organs. Unlike TEM, SEM does not intersect with the specimen, instead the electrons are reflected by a coating and then collected, analyzed, and used to generate a 3D view.

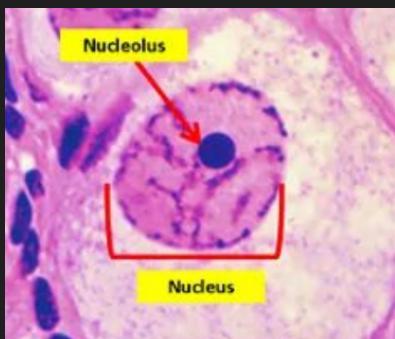


Organelles and Cytoplasmic Inclusions

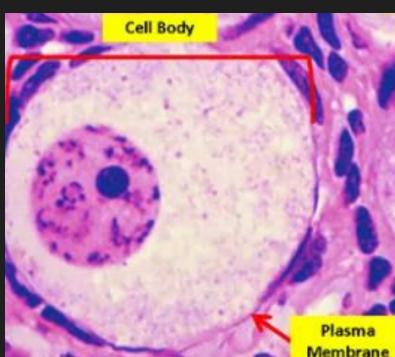
- **Nucleus:** a large membrane bound organelle that contains chromatin, the nucleolus, and nucleoplasm.
Size: **5–20 μm** , the largest organelle.



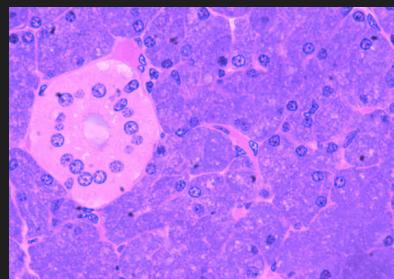
- **Nucleolus:** large, dense structure within the nucleus that functions in the synthesis of ribosomes.
Size: **0.5–5 μm** , smaller than nucleus.



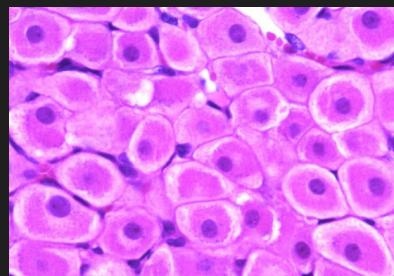
- **Plasma Membrane:** phospholipid bilayer containing various elements, acts as a physical barrier to regulate internal environment; maintains charge and functions in cell communication.
Size: **5–10 nm**, very thin—3 orders of magnitude less than width of nucleus.



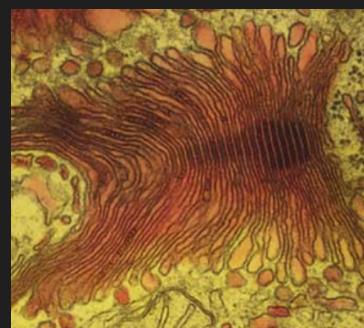
- **Rough ER:** interconnect membrane that modifies, transports, and stores proteins produced by attached ribosomes.
Size: **5–8 nm thick, 20–30 nm wide**, membrane of rER thin, while the lumen is wide and surrounds nucleus (often).



- **Smooth ER:** Like rER, but lacking ribosomes; synthesizes, transports, and stores lipids; metabolizes carbohydrates, toxins (of various types); and forms vesicles and peroxisomes.
Size: lumen is **30–60 nm** thick, larger than rER; overall fraction of cell varies.

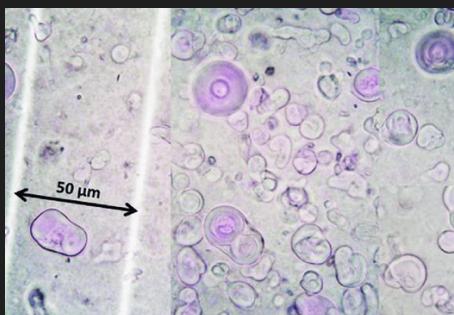


- **Golgi Apparatus:** modifies, packages, and sorts materials that arrive from the ER; forms secretory vesicles/lysosomes.
Size: **2–5 mm**, stacks of flat membranes, can occupy large portion of cell.



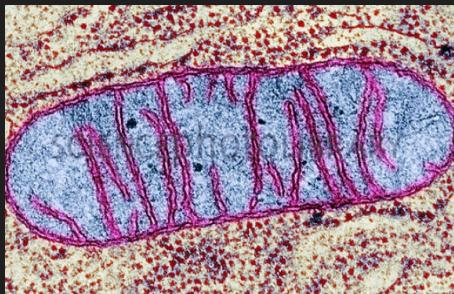
- **Vesicles:**

Size: 30–100 nm, large range varies among different types of vesicles, still relatively small.



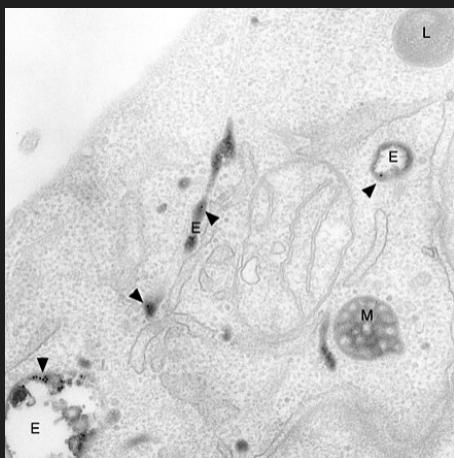
- **Mitochondria:**

Size: 0.5–1 μm, decently sized, but still smaller than other organelles in the cell.

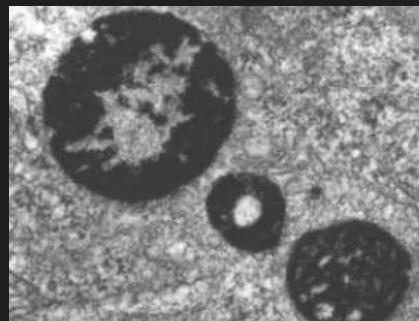


- **Endosomes:** collection of intracellular sorting organelles, originating from trans Golgi network that move molecules and ligands to lysosomes (L), or recycled back to cell membrane.

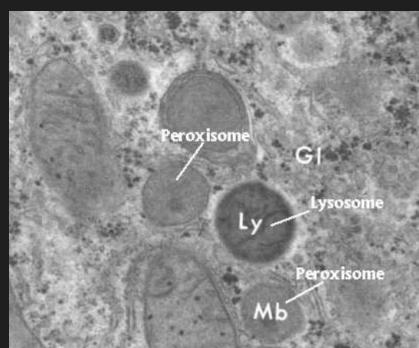
Size: 1–50 μm, depends on stage; (E)rly forms dynamic tubular network, can be long. (M)vbs (late) lack tubes. TEM, not light microscope below*



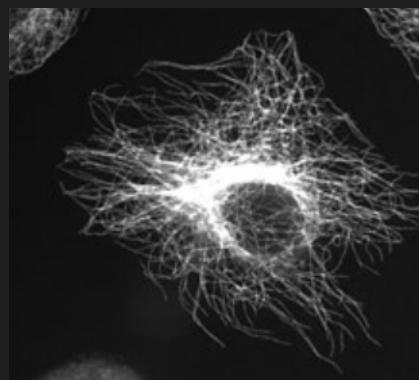
- **Lysosomes:** spherical-shaped from Golgi, contains digestive enzymes to break down microbes and materials. Size: 0.5–1 μm, rather small, TEM used below instead of light microscope.



- **Peroxisomes:** formed via ER or fission; contains enzymes to break down specific harmful substances, also used for beta oxidation of fatty acids. Size: 0.1–1 μm, typically smaller than lysosomes, can be similar sized.

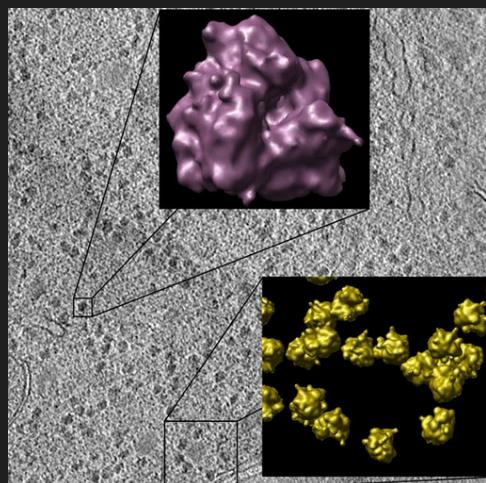


- **Cytoskeleton:** organized network of {actin, micro-, intermediate} filaments, and microtubules and other proteins. Size: 7 nm, varies, depending on structure and filaments used.



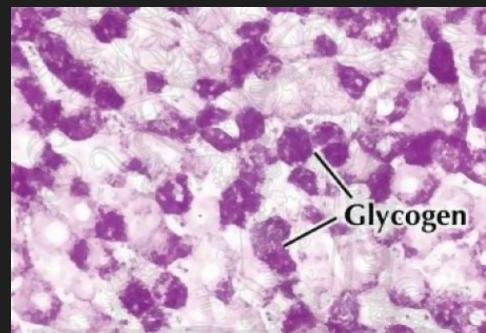
- Ribosomes:** composed of protein and rRNA and engage in protein synthesis; can be on rER, in plasma membrane, in lysosomes, and free in cell.

Size: 20–30 nm, varies, organized into both large and small subunits.



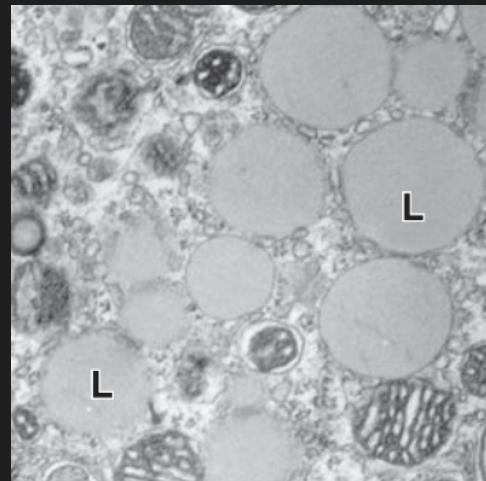
- Glycogen granules:** aggregates of carbohydrate polymer in which glucose is stored, notably in liver cells

Size: 20–30 μm, many can cluster.

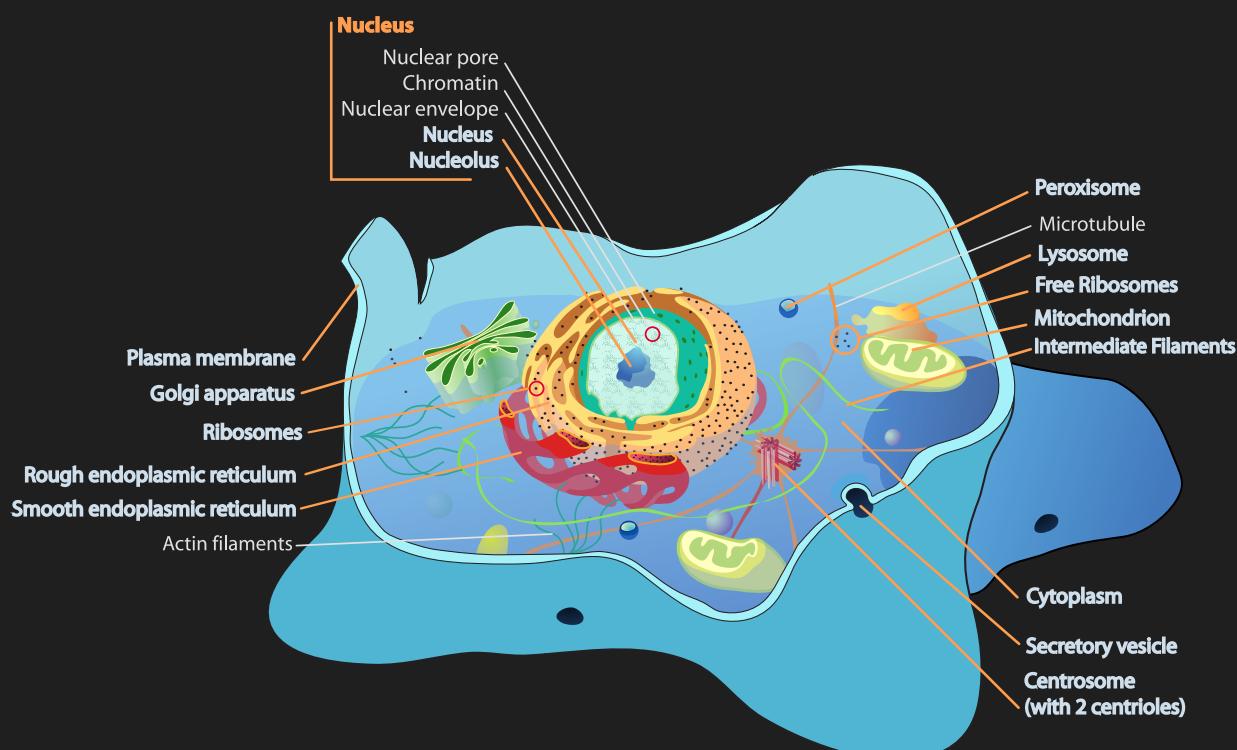


- Liquid droplets:** accumulations of lipid-filling adipocytes.

Size: 20–30 μm, can be removed.



Labeled Diagram of a Eukaryotic Cell



Mitotic Phases



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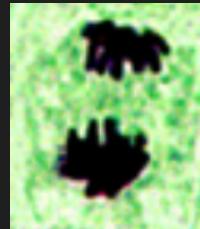
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Apoptosis Events

- **DNA fragmentation:**
- **Decrease of cell volume:**
- **Membrane Blebbing:**
- **Formation of apoptotic bodies:**

Features and Functions

- **Stratified squamous epithelium:**

- Features:

- Functions:

- **Simple cuboidal epithelium:**

- Features:

- Functions:

- **Skeletal muscle:**

- Features:

- Functions:

- **Cardiac muscle:**

- Features:

- Functions:

- **Smooth muscle:**

- Features:

- Functions: