

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 **eonsin (E)** stains are among the most commonly used stains.
 - As mentioned above, hematoxylin acts as a **basophilic dye**, turning negatively charge 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?**

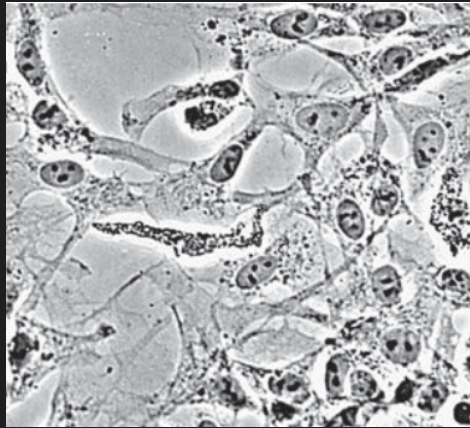
- Immunohistochemistry (IHC): the use of labeled antibodies and antigens to identify and localize many proteins and macromolecules that lack specific enzymatic activity.
- Visualization of such interactions are commonly accomplished with either:
 - Chromogenic immunohistochemistry (CIH): use of antibodies 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

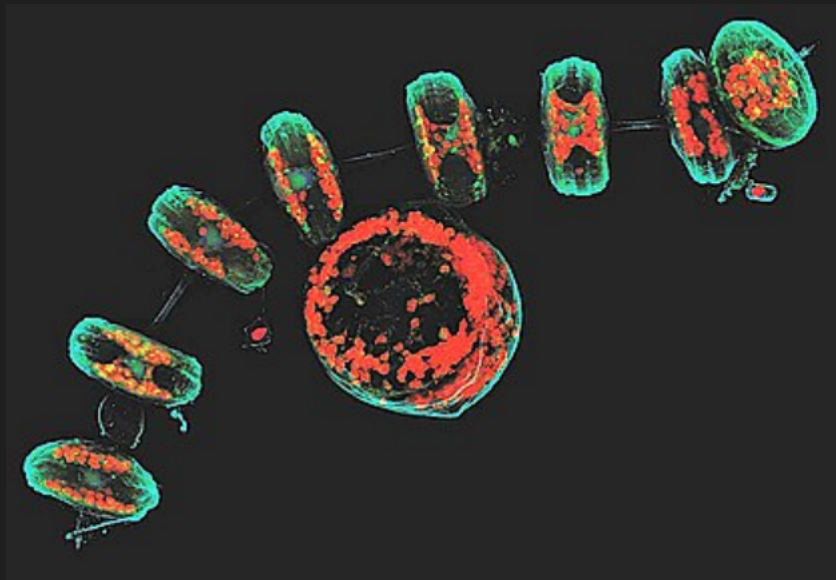
- **Bright field:** a very common method that uses ordinary light and stained structures to discern differences and cell structures.



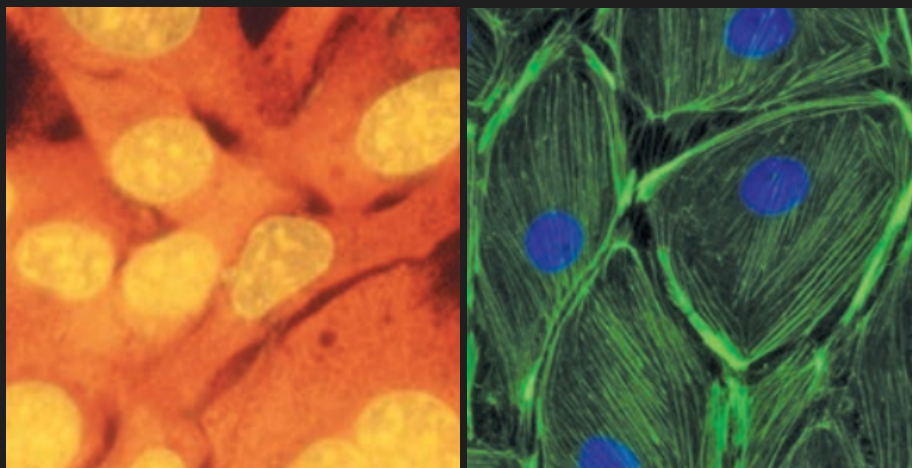
- **Phase contrast:** uses differences in refractive index of natural cell and tissue components; allows for observation of living cells.



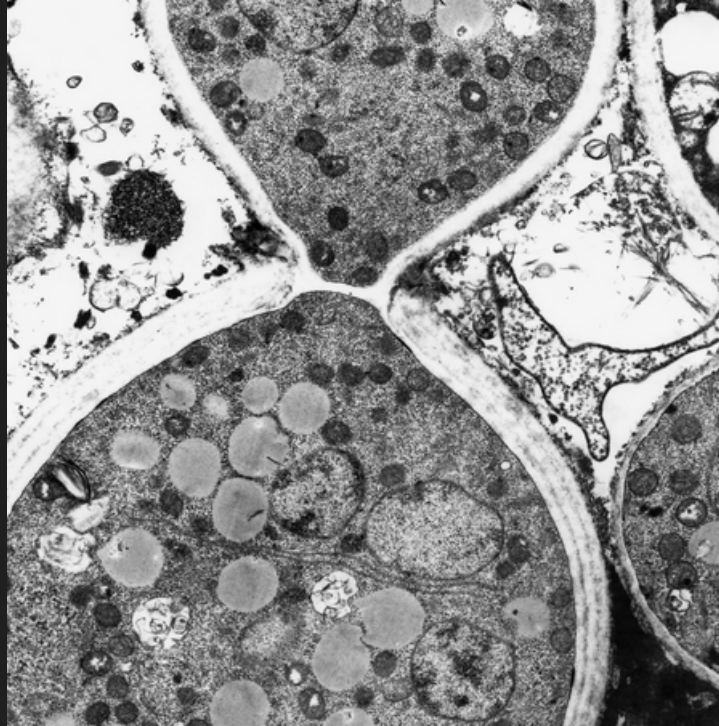
- **Confocal:** use of scans at successive focal planes with a more focused light beam, usually from a laser, to construct a 3D image.



- **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.



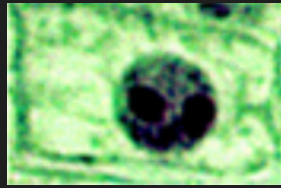
- **Scanning Electron Microscopy (SEM):** used to provide a high resolution view of the surface of cells, tissues, and organs. Unlike TEM, SEM does not intersect with the specimen, instead the electrons are reflected by a coating and then collected, analyzed, and used to generate a 3D view.



Organelles and Cytoplasmic Inclusions

Organelles and Cytoplasmic Inclusions			
Structure	Size	Light Microscopic Features	Function
Nucleus	5–20 µm; largest organelle		
Nucleolus			
P. Membrane			
Rough ER			
Smooth ER			
Golgi Body			
Vesicles			
Mitochondria			
Endosomes			
Lysosomes			
Peroxisomes			
Cytoskeleton			
Ribosomes			
Glycogen			
L. Droplets			

Mitotic Phases



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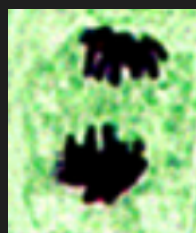
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Apoptosis Events

- 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: