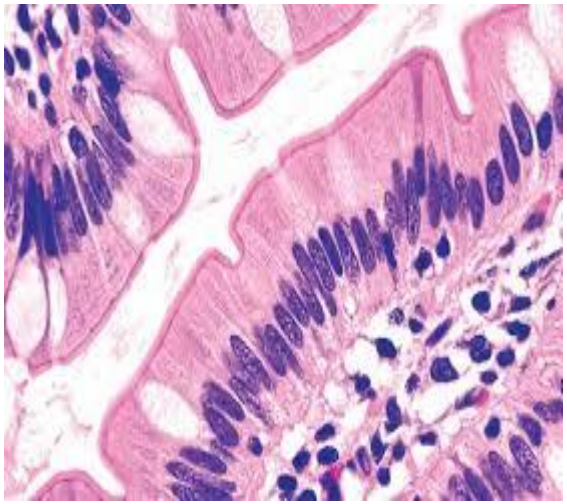
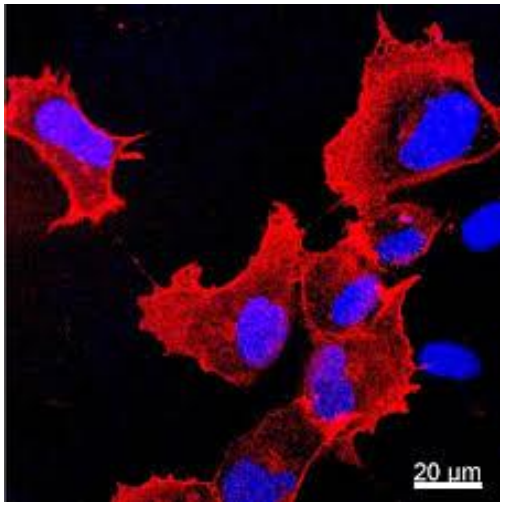
Histological techniques-3





By

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LEARNING OUTCOMES

- Give an account on the basic methods of studying histology especially **histochemistry** and **immunohistochemistry**

- Illustrate different **types of microscope.**

Basic histological methods

1. Routine microscopic examination (LM & E/M)
2. Histochemistry & Cytochemistry
3. Immunocytochemistry
4. Cell & Tissue Culture
5. Exfoliative Cytology
6. Bone Marrow smear / Blood film
7. Fine needle aspiration / biopsy

Histochemistry

- **Aim of the technique :**

identify and localizing specific **cellular structures** using a **specific enzymatic activity** present in those structures.

- **Steps of Enzyme histochemistry :**

(1) Tissue sections are immersed in a solution containing the **substrate of the enzyme** to be localized.

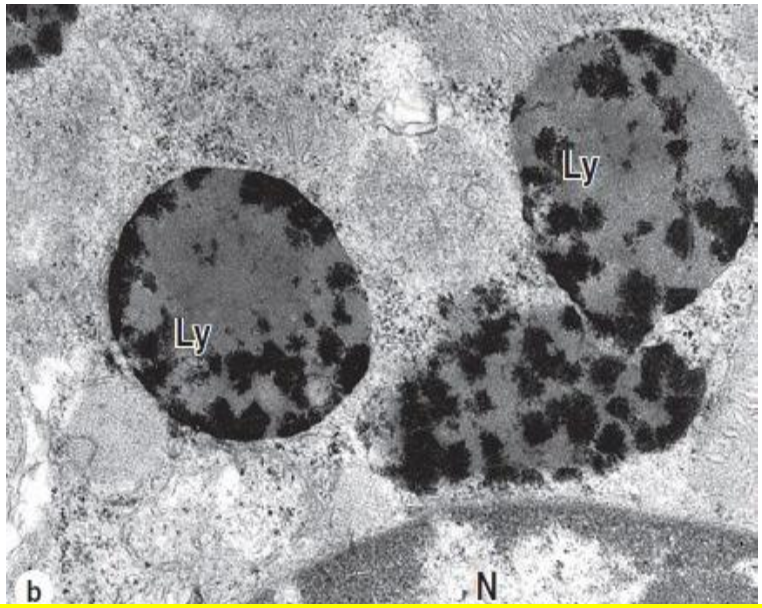
(2) The enzyme is allowed to act on its substrate.

(3) the section is put in contact with **a marker compound** that reacts with the **product** of the enzymatic action on the substrate.

4) The **final product**, precipitates over the site of the enzymes, allowing the region to be localized microscopically. As it will be **insoluble** and can be visible by *light or electron microscopy* by having color or electron density.

- **Examples :**
- **Alkaline phosphatase enzyme in renal tubules.**

L.M: strong activity of this enzyme at the apical surfaces of the cells (white arrows)



E.M: electron dense particles

Immuno-histochemistry

Aim of the technique

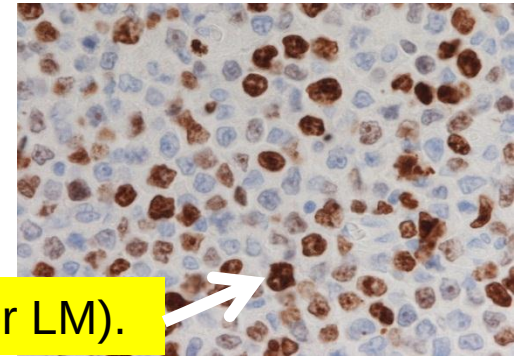
-Localization of specific proteins. *Any macromolecule* (Cytoplasmic-Nuclear-Lipids-Proteins).

Principle:

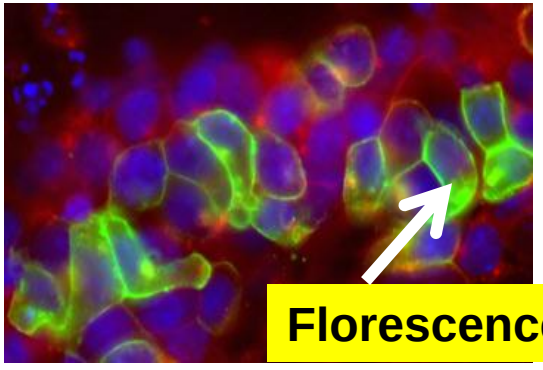
-using specific antibodies to attach to their antigens in a sample of tissue (antigen-antibody reaction). then visualized and localize the reaction under microscope.

The antibodies are usually linked to a marker (labeled) such as:

- An **enzyme** (for LM).
- **Florescence dye** (for Florescent Microscopy).
- **Gold particles** (for EM).
- The reaction is visualized by identification of the marker.

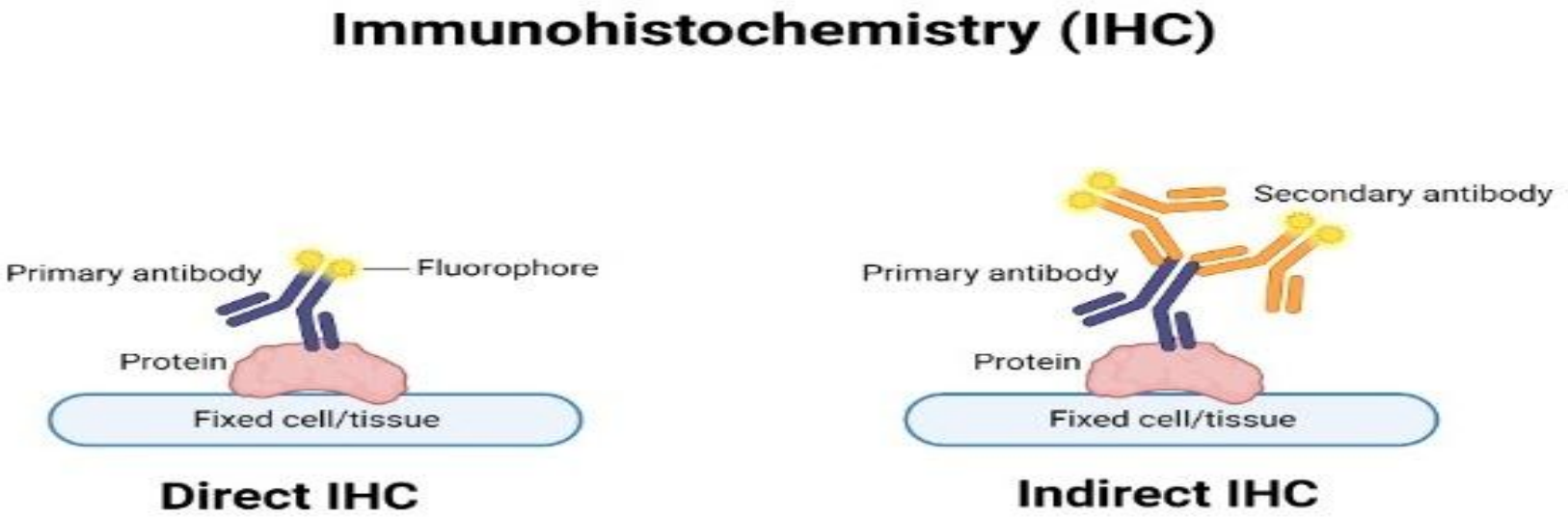


An **enzyme** (for LM).



Florescence dye

Types of Immuno-histochemistry



Direct method	indirect method
• Fast to get results • Labeling intensity is low	• Getting results takes longer • More sensitive • Labeling intensity is **high (the reaction is good visualized)** as many 2ry antibodies are attached to the 1ry antibody.

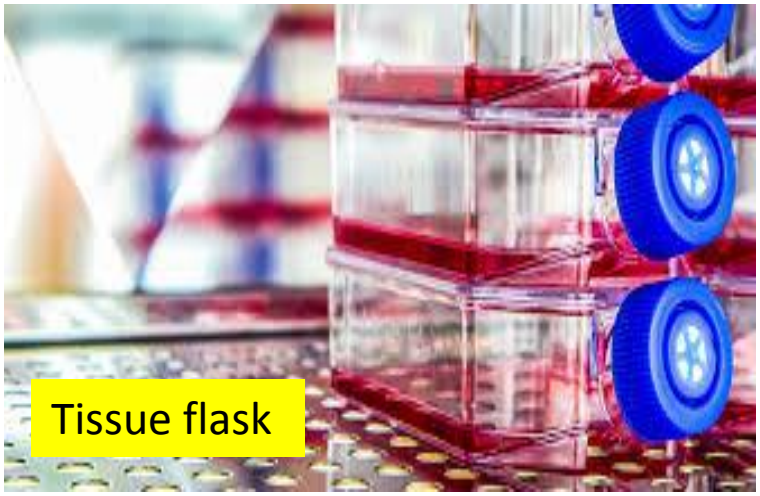
Cell & Tissue Culture

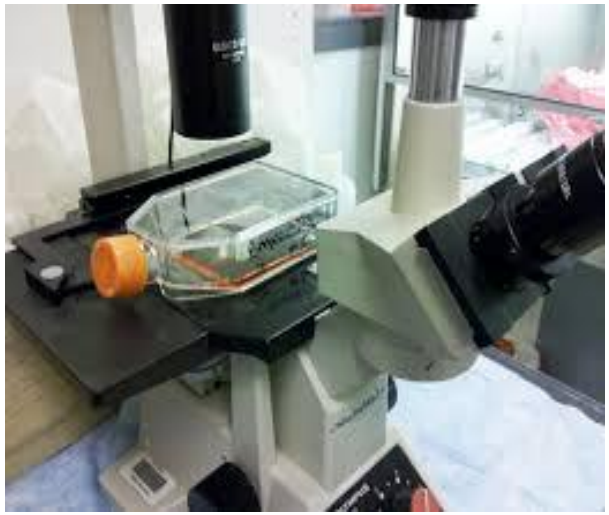
Aim:

Isolating the cells and maintain their growth outside the body (***in-vitro***) in an artificial growth media.

Examination:

by Phase-contrast light microscopy.





Exfoliative Cytology

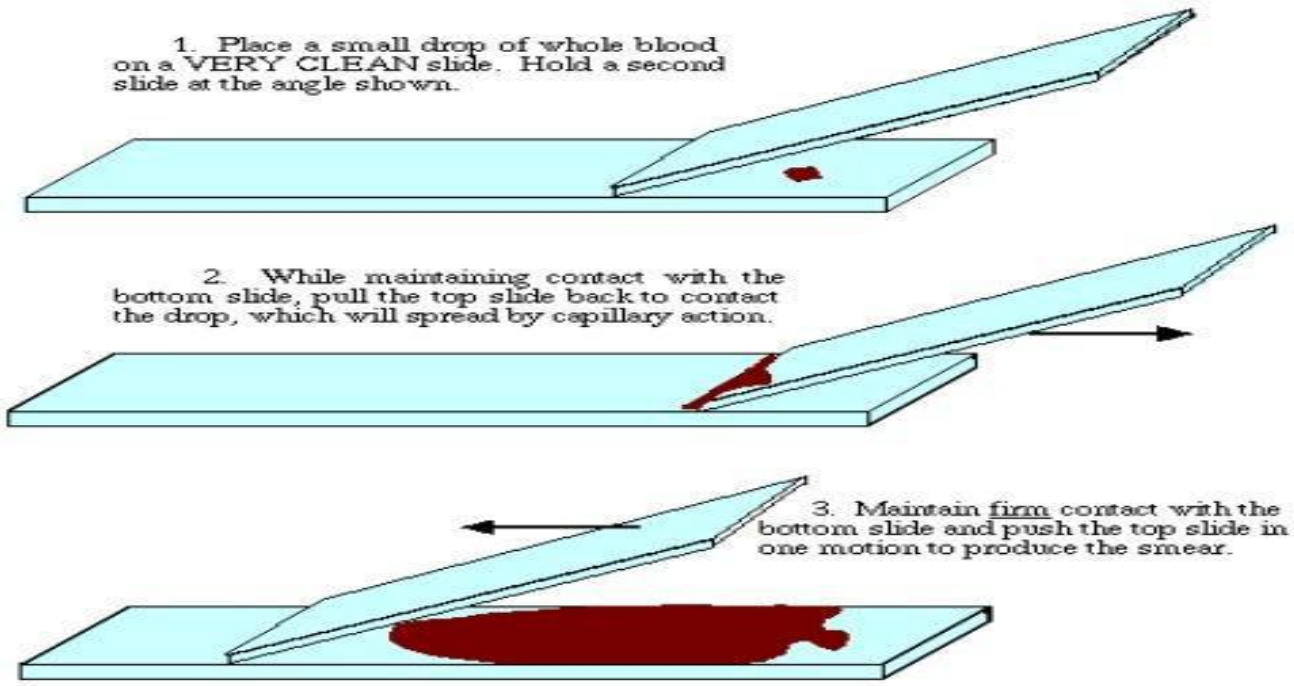
Microscopic examination of cells that normally desquamated from epithelial surfaces of the body or smears &swabs of fresh soft tissues.

e.g:

- Buccal swabs for PCR to diagnose corona virus.

Bone Marrow smear / Blood film

Thin layer of fresh body fluids spread on a slid, stained and examined



Fine needle aspiration / biopsy

- Is a procedure to obtain a **sample of cells from the body** for laboratory testing.

- Needle biopsy may be used to take tissue or fluid samples from muscles, breast, liver

Microscope

- The machine by which we can see very small things.

- *Micro* (means small) and *scope* (means something for looking with).

- The importance of a microscope is its ability to magnify small objects and to reveal their fine details

Common Types of Microscopy

1. Light microscope (LM)
2. Electron microscope (TEM & SEM)
3. Phase contrast microscope
4. Fluorescence microscope

1-Light Microscope (LM)

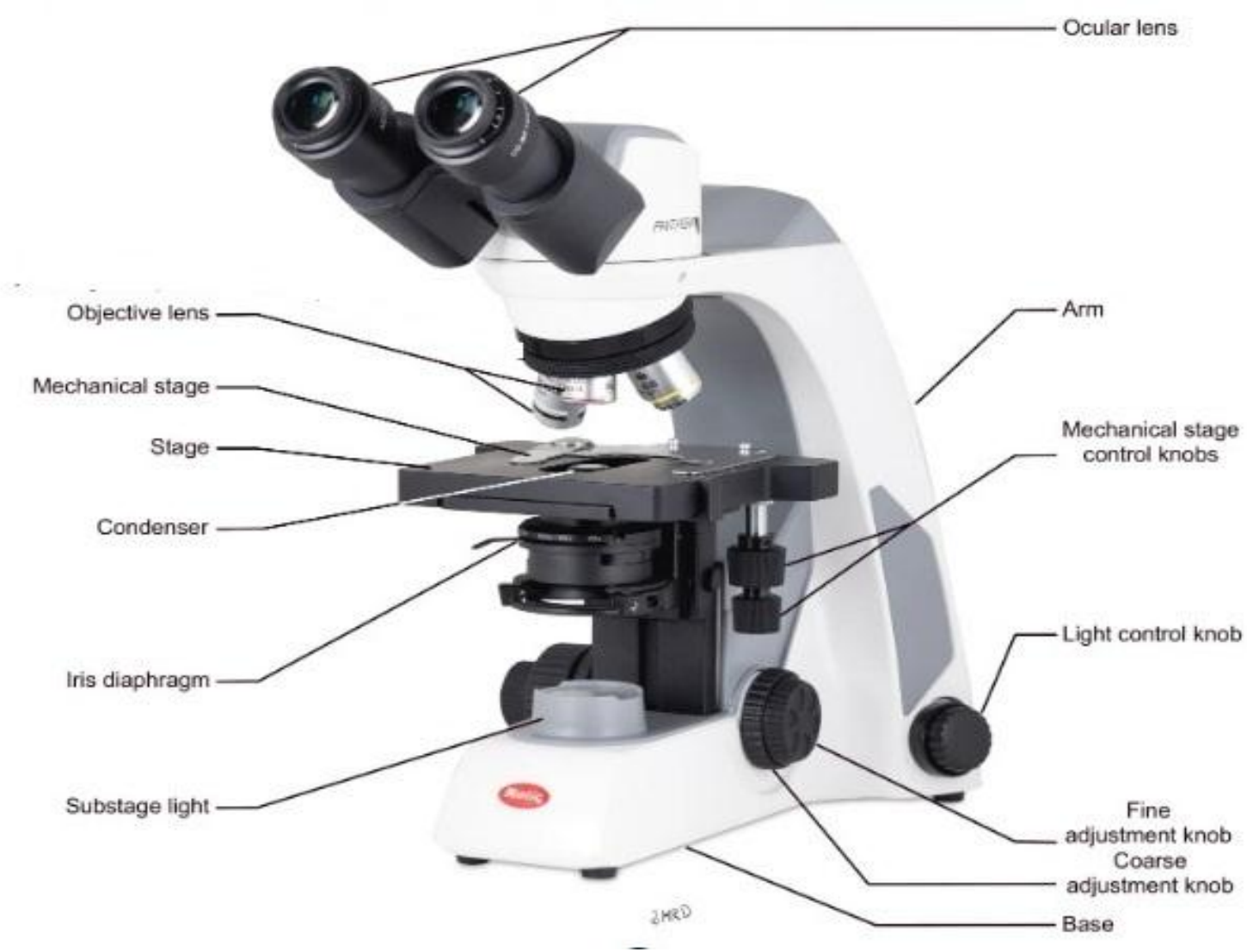
- Is the standard optical instrument for examination of histological sections and generating magnified images.
- Light microscope uses visible light source + condenser lens (to send light through the object).

The image of this object is magnified by two sets of lenses:

1. Ocular lens (Mag.=10)
2. Objective lenses (Mag.= 5 ,10 , 40,100)

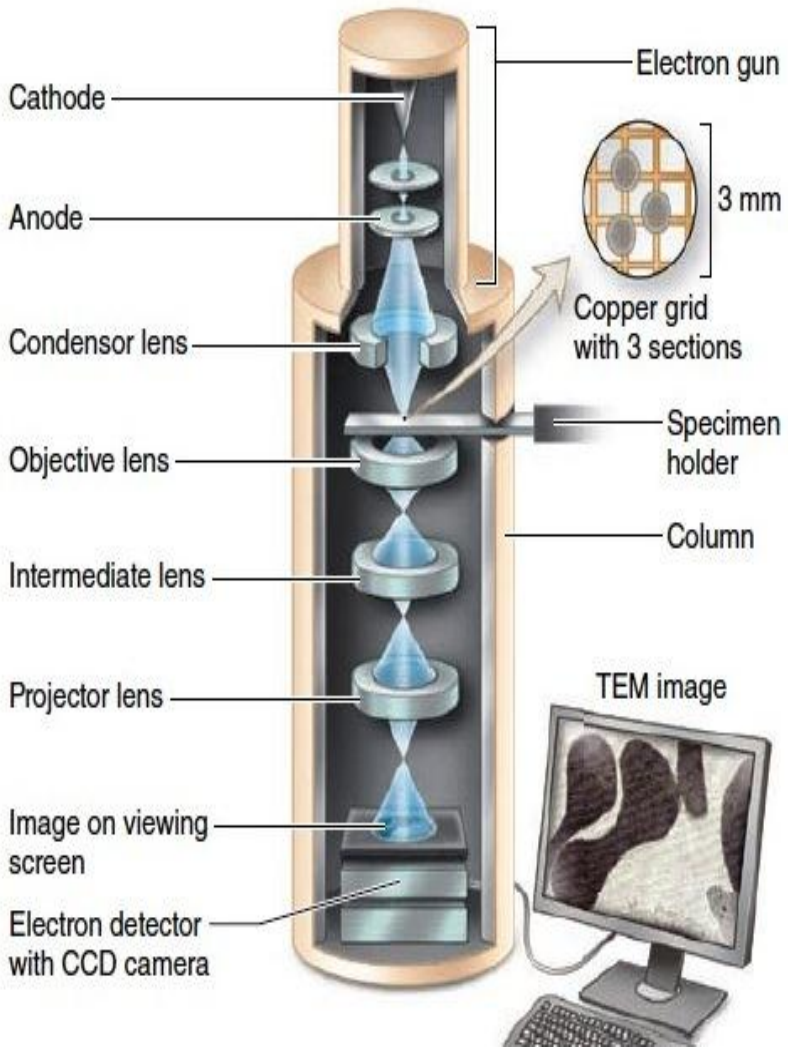
- **Total magnification power** = 1 x 2
  e.g.10 X40 =400

Parts of Light Microscope (LM)



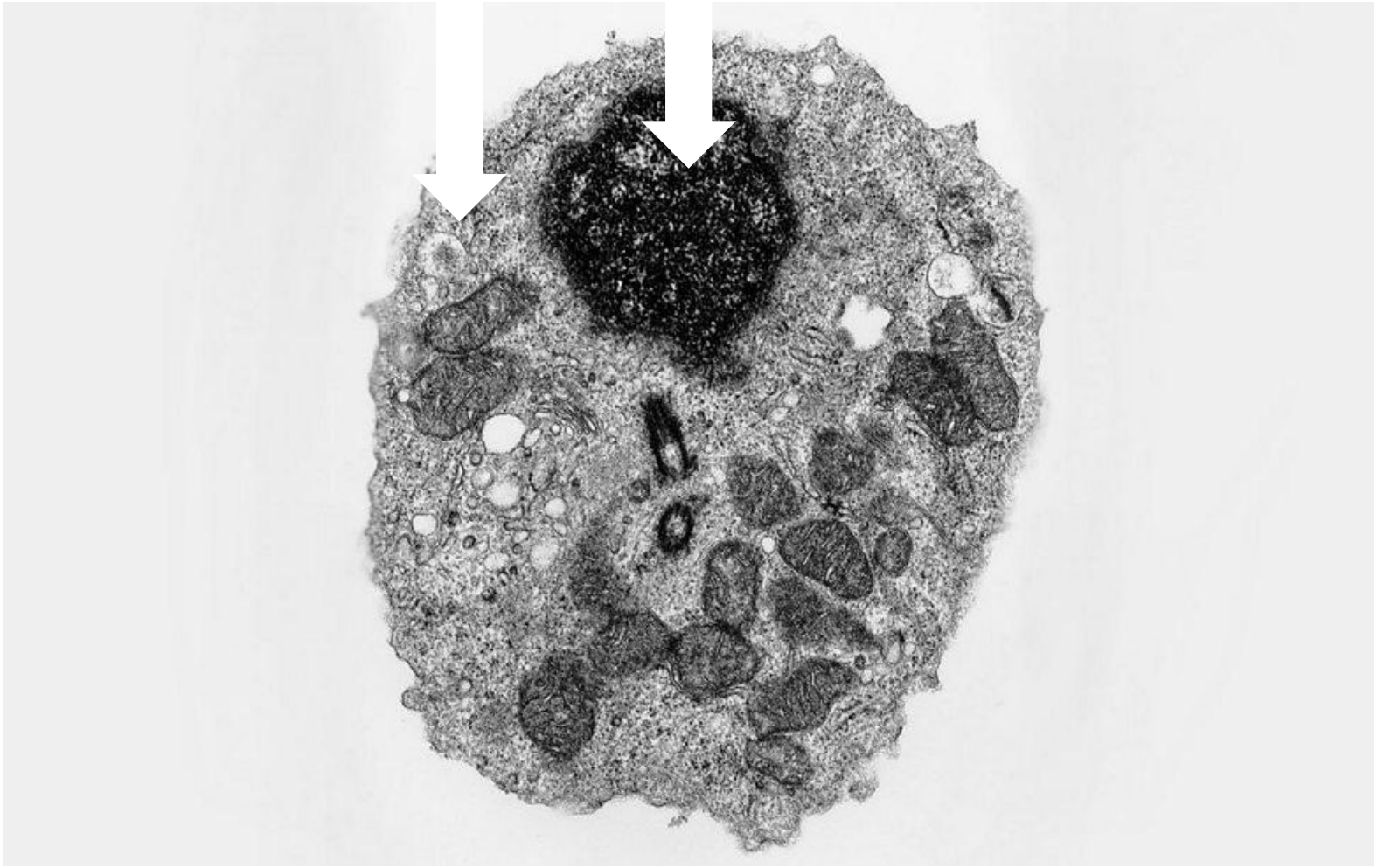
2- The Electron Microscope (EM)

- **Used to achieve greater magnification &resolution of images.**
- **Beam of electrons** is used as *source of light.*
- Beam passes through a vacuum tube.
- The lenses are **electromagnetic coils.**
- The first lens is a condenser focusing the beam of electrons on a section of the specimen.
- Electrons transmitted through the specimen reach the objective
- lens, which forms a focused, magnified image that is then magnified further through other lenses and captured on a viewing screen.

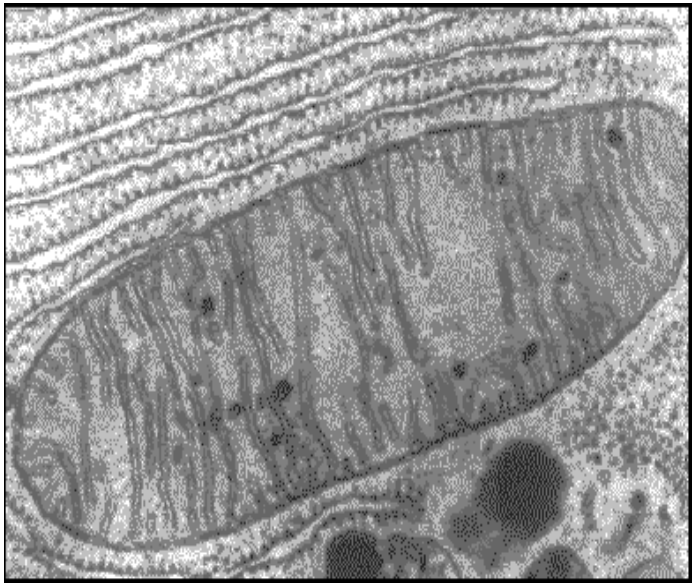
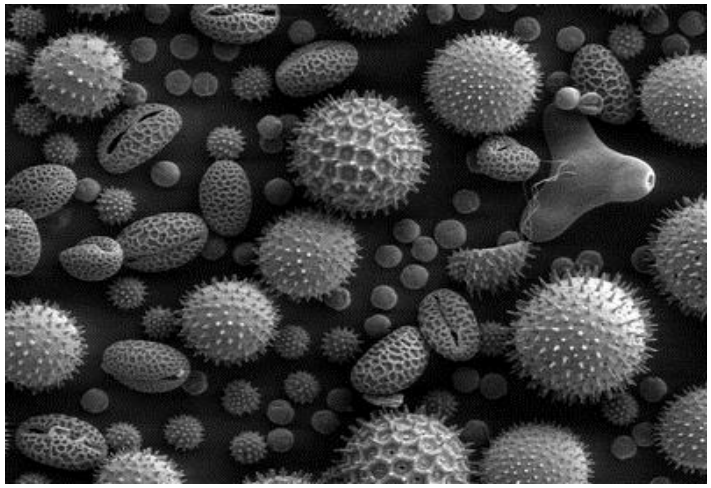


- The image appears on screen
- Images can be detected as:

Light areas (electron lucent) & dark areas (electron dense)

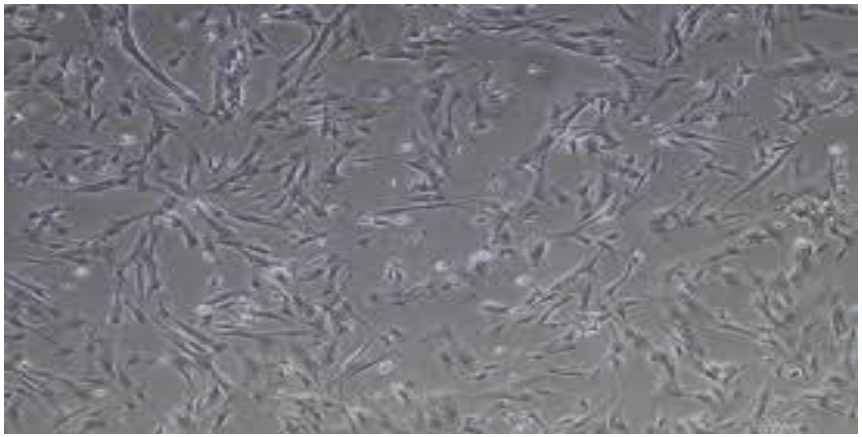


Types of EM

Transmission EM (TEM)	Scanning EM (SEM)
electron beams pass through the specimen. It shows the details of internal structures of cells 	a special type of EM where electron beams are reflected from the surface of coated specimen. This gives a three dimensional (3 D) image of a specimen. 

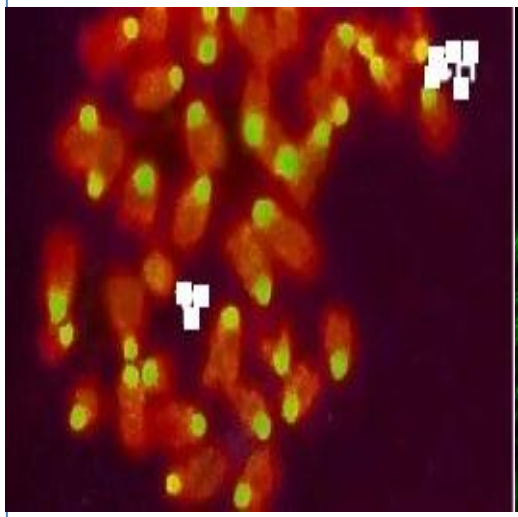
3-Phase contrast microscope

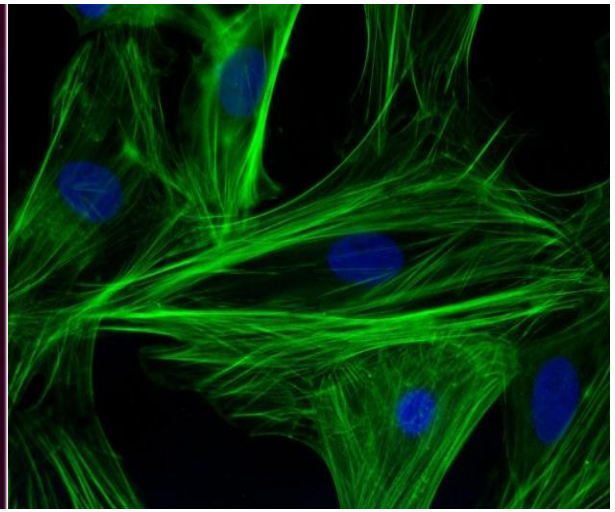
- Used to study **transparent unstained objects.**
- **Principle:** The speed of light changes when passes through cellular and extracellular structures & with different refractive indices.
- Objects appear lighter or darker to each others.
- It is useful in tissue culture to examining living cells & tissues.



4- Fluorescence Microscopy

- The light source is **ultraviolet rays** which pass through the tissue.
- Fluorescent compounds with affinity for specific cell macromolecules such as DNA and RNA may be used as fluorescent stains.
- Under fluorescence microscope, these nucleic acids emit fluorescence, allowing them to be localized separately in cells.





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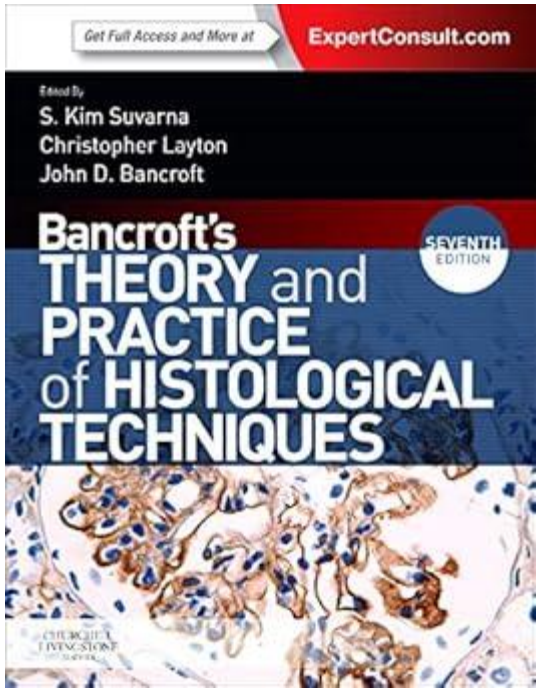
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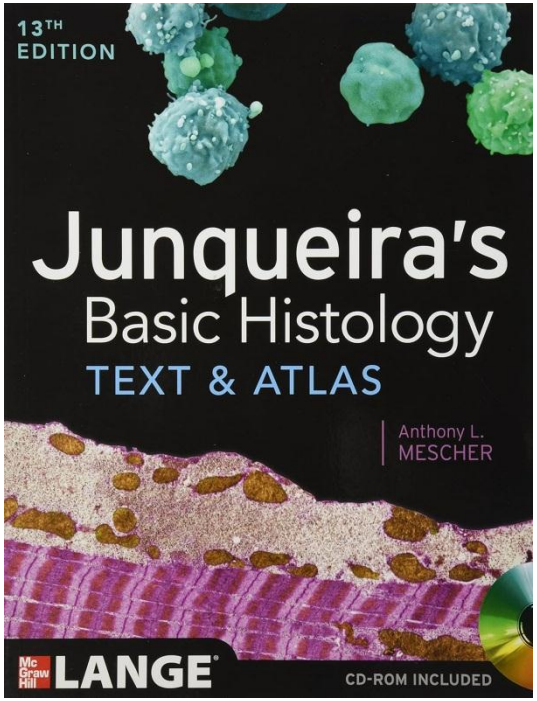
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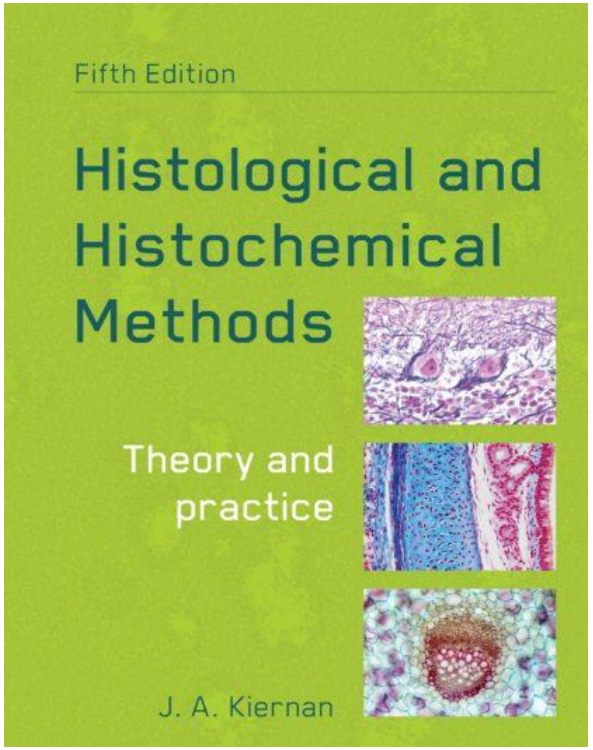
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