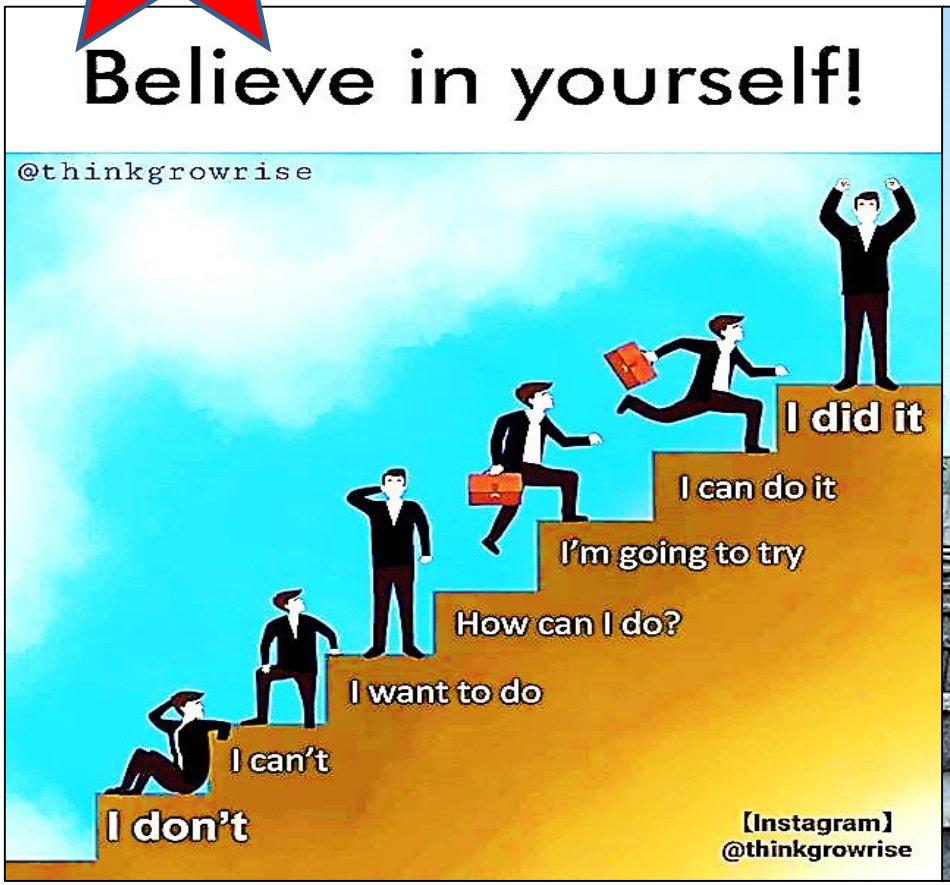
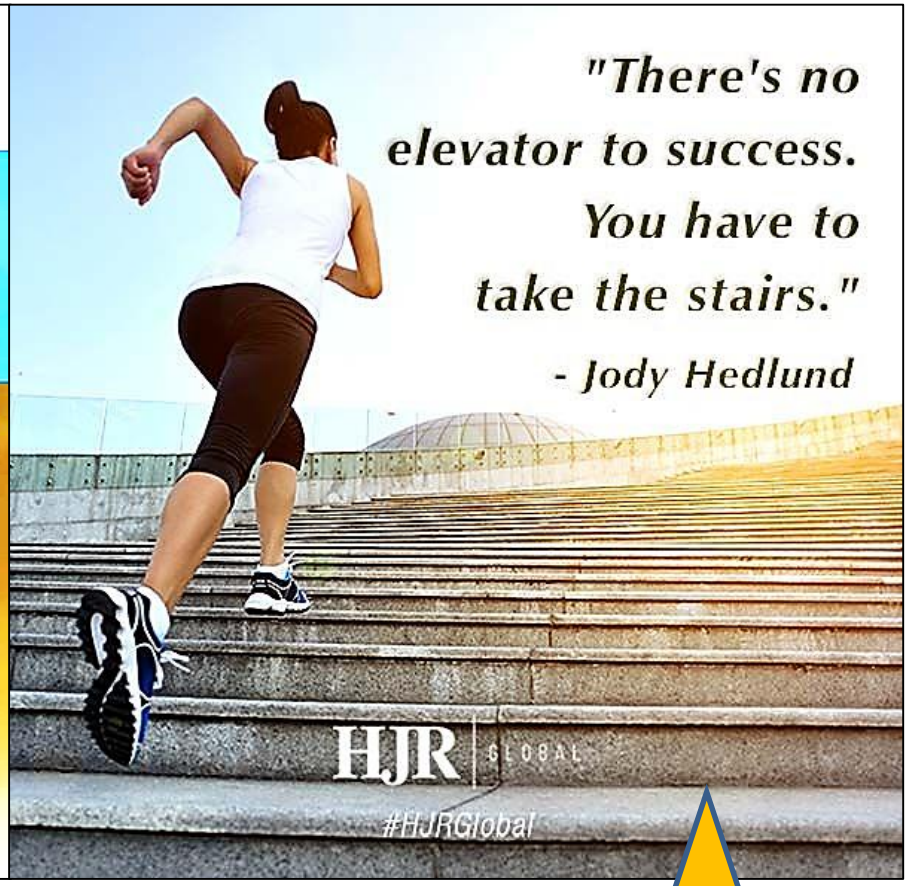
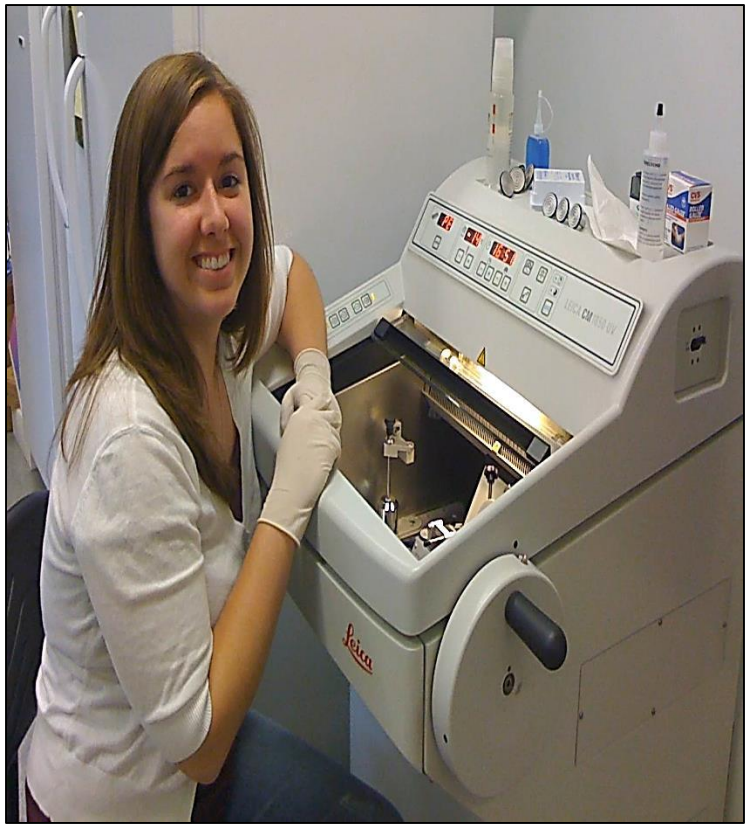
Think positive





Cell Bio-2
Microtechniques



Micro techniques For Light microscopy

To preserve the structure and chemical composition of the specimen

- I Paraffin
- II Freezing

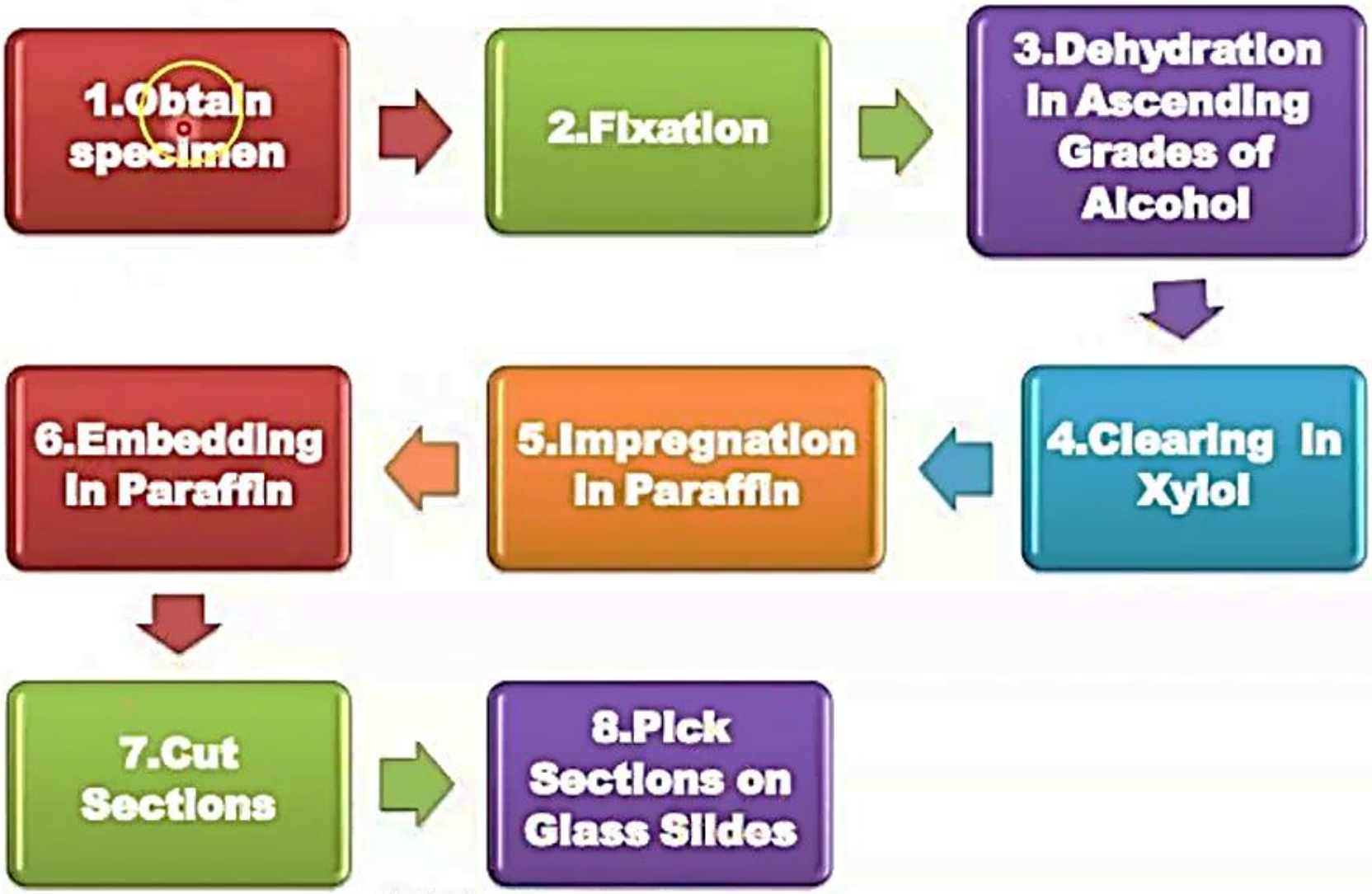
I: Paraffin technique

- Technique is histological method used to prepare **the tissue samples** for light microscopy

- it includes the following steps:

1. **Fixation :** in appropriate solution (formol saline)
2. **Dehydration and clearing :** in alcohol then xylol
3. **impregnation & Embedding :** in paraffin wax (Soft & hard)
4. **Sectioning :** by microtome
5. **Mounting :** on glass slides
6. **Staining of the sections**

Paraffin Technique



Fixation

- To preserve the structure of the tissue as in the life state
- upon removal from the body the sample Immediately placed in a fixative solution
- Fixation is done as soon as possible to prevent autolysis and to preserve the morphology.

For LM:

- Formol saline

For EM: a mixture of

- Glutaraldehyde
- Osmium tetroxide

ROUTINE FORMALIN FIXATIVES:

10% formal saline: Most commonly used fixative

Water (distilled)	900ml
Sodium chloride	8.5gm
Formalin	100ml

Advantage of the fixation step :

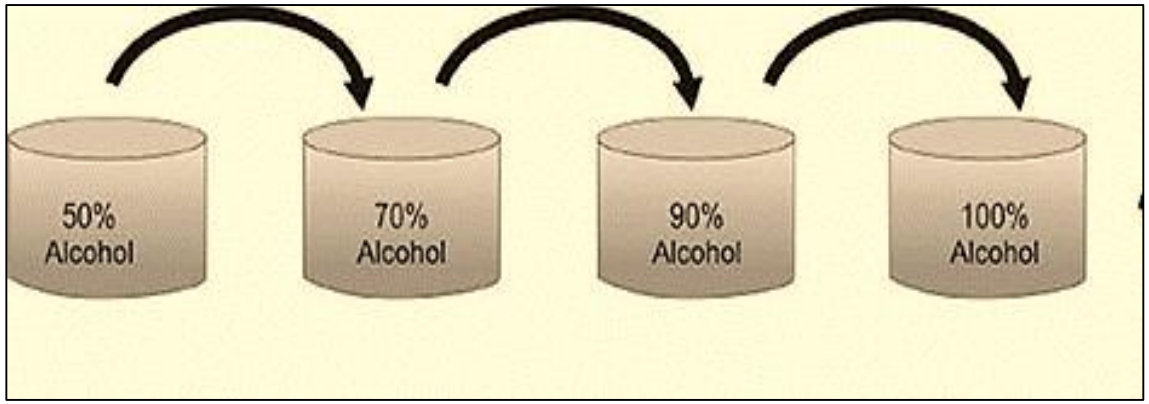
- Hardens the tissue by coagulating its protein → Facilitate the process of cutting & staining & examination

- Prevent putrefaction & stop autolytic changes by killing any bacteria

- Preserves the molecular & morphological structure of the tissue

Dehydration & Clearing

Dehydration : Is done by treating the specimen with ascending concentration of alcohol (50% → 70%→ 100%) ….. Gradual removal of water from the specimen (H_2O represent 70% of the body)



Clearing : with this process the tissue become translucent (to allow the light to penetrate the specimen)

the tissue is treated with xylol or benzol …to remove the alcohol

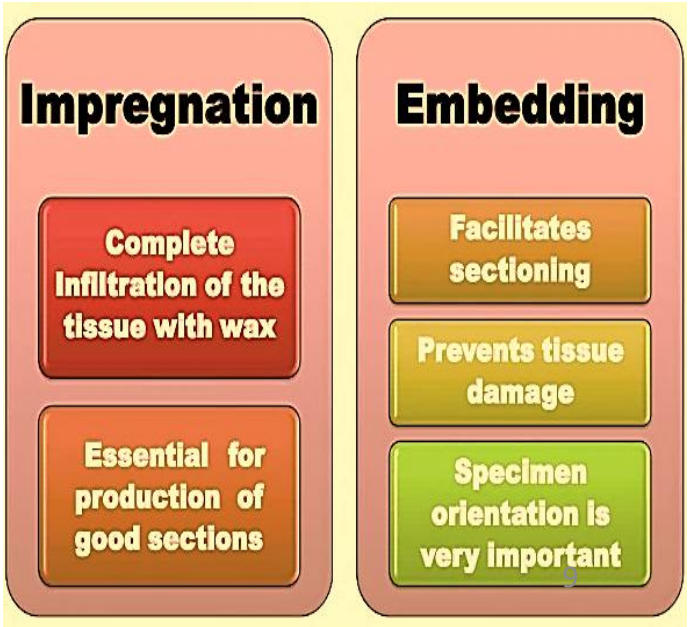
Impregnation & Embedding

Impregnation :

- Tissues are placed in molten soft paraffin wax
- The wax infiltrates the tissue & occupies all the spaces that were originally occupied with water

Embedding:

- Tissue are placed in molten hard paraffin wax
- The tissue is placed in the center of the paraffin, which hardens as it cools → paraffin block



Automatic tissue processor

Advantage of paraffin technique:

1- Disease diagnosis

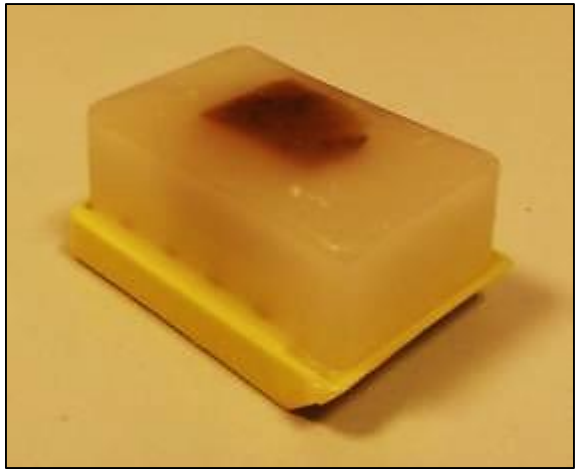
2- Research

3- Teaching

The steps required to take animal or human tissue from fixation to the state where it is completely infiltrated with soft paraffin wax then to be embedded in hard wax for section cutting on the microtome.

Sectioning by Microtome

- A microtome is a mechanical device used to cut extremely thin slices of a fixed tissue block known as **sections.**
- It holds the block of hard paraffin with the tissue in its center against a sharp metal knife that used to cut the block into thin sections (3-10 microns) as it moves up and down.

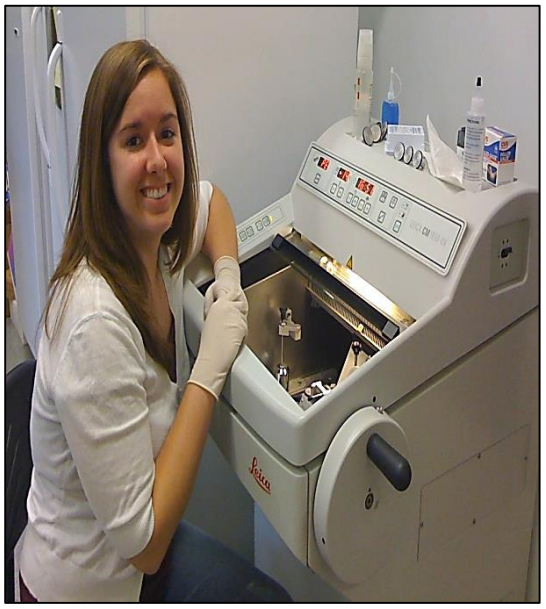


mounting

Tissue sections are placed on glass slides smeared with egg albumin , then warmed on a hot plate to dry.

The sections are now ready to **be stained**

II. Freezing technique



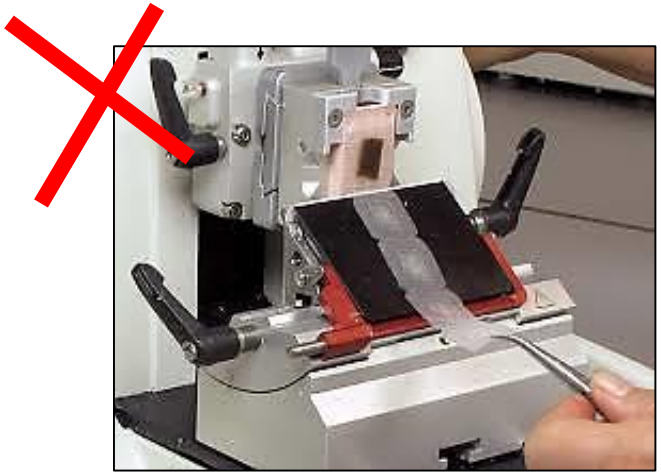
Fresh frozen tissues are cut using **cryostat** (Freezing microtome). The specimens placed in cold fluid called Isopentane/liquid nitrogen (-50 C) for fixation then rapidly stained.

Advantages:

- Rapid technique for diagnosis in operation rooms (tumors).
- No fixation , No dehydration & No chemicals are used, so useful for histochemical (enzyme staining) studies.

Disadvantages:

- Non serial & Fragmented sections .
- Cannot be preserved for a long time.



Applications of the freezing technique:

1- intraoperative diagnosis: checking if a tumor is completely removed

2- immunohistochemistry : when antigen preservation is crucial

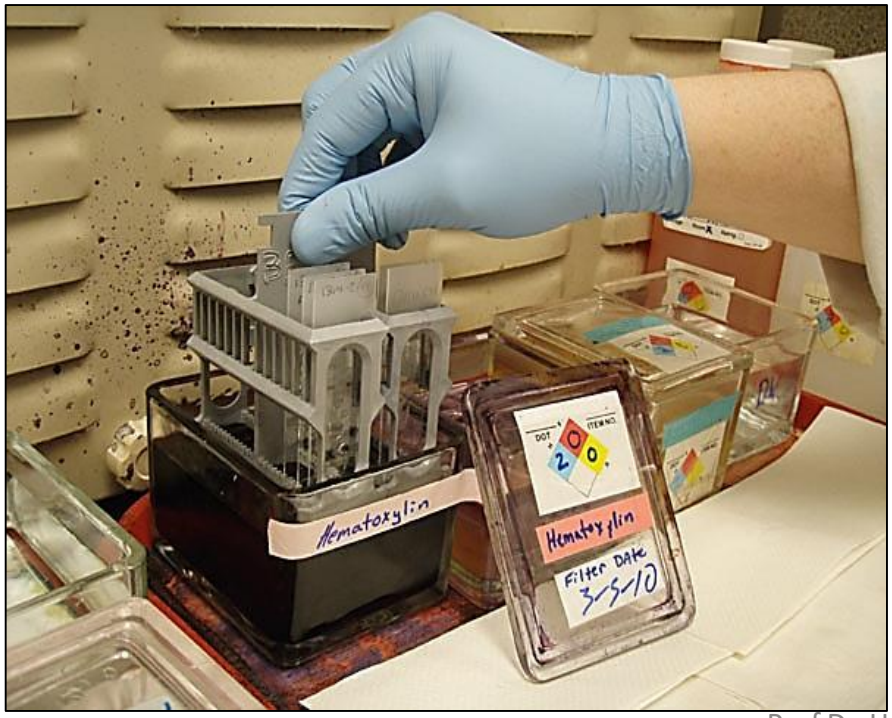
3- Enzyme histochemistry

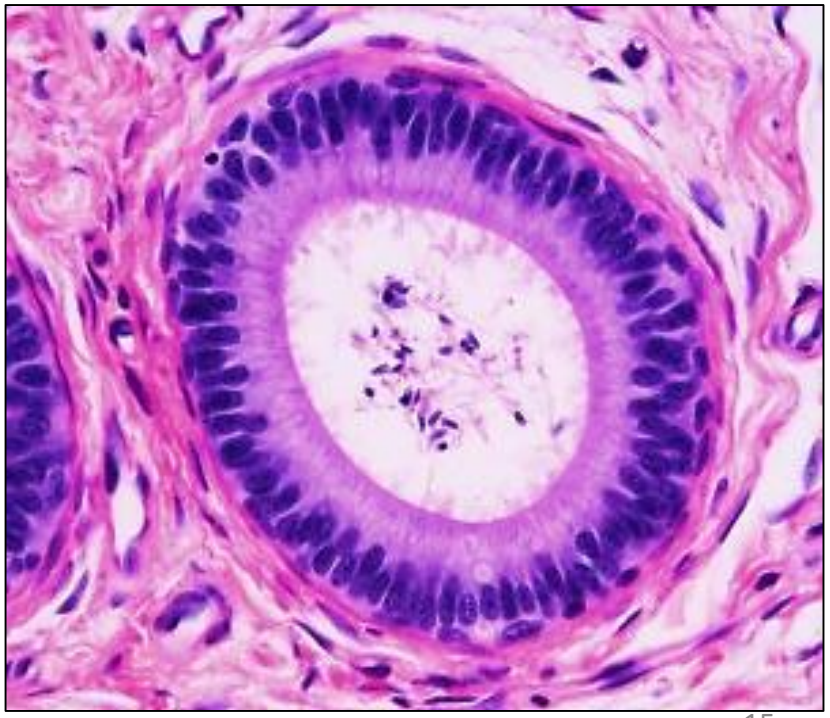
4- lipid demonstration

5- No need for dehydration or clearing

Staining

- The tissue sections that will be studied using the light microscope **must be stained** first since most tissues are colorless
- Dyes (stains) used are either **basic** or **acidic**





Staining

Used to visualize & distinguish the different parts of cells & tissues

Routine stains
H&E

Special stains
e.g. Ag & orcein, trichrome….etc

Common (Routine) histological stains H&E

- **Hematoxylin (H) :**

blue basic dye (+ve charged)

- Stains acidic (anionic –ve) components of the cell with a blue color e.g. nucleus, ribosomes (r-RNA)

Basophilic structure=blue

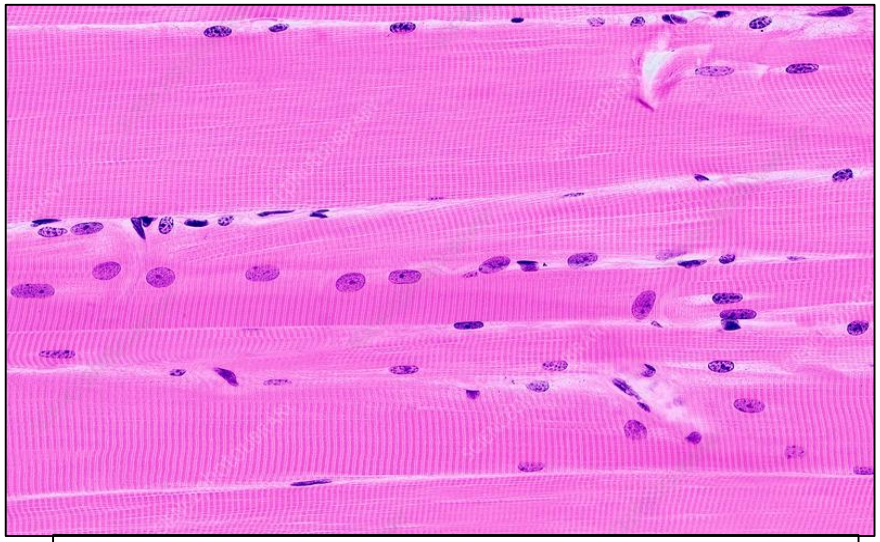
- **Eosin (E):**

red acidic dye (- ve charged)

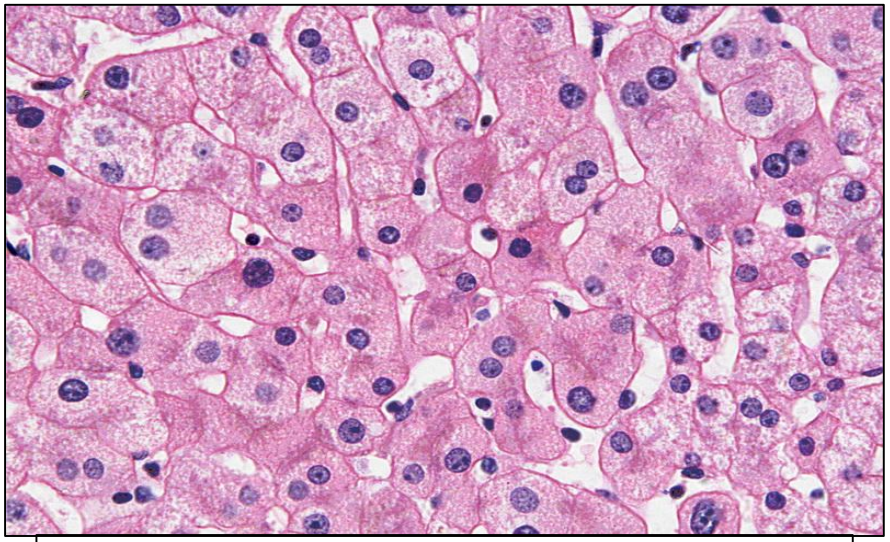
- Stains basic (cationic +ve) components of the cell with a red color e.g. cytoplasm, mitochondria, muscles !!

(it has +ve charged proteins)

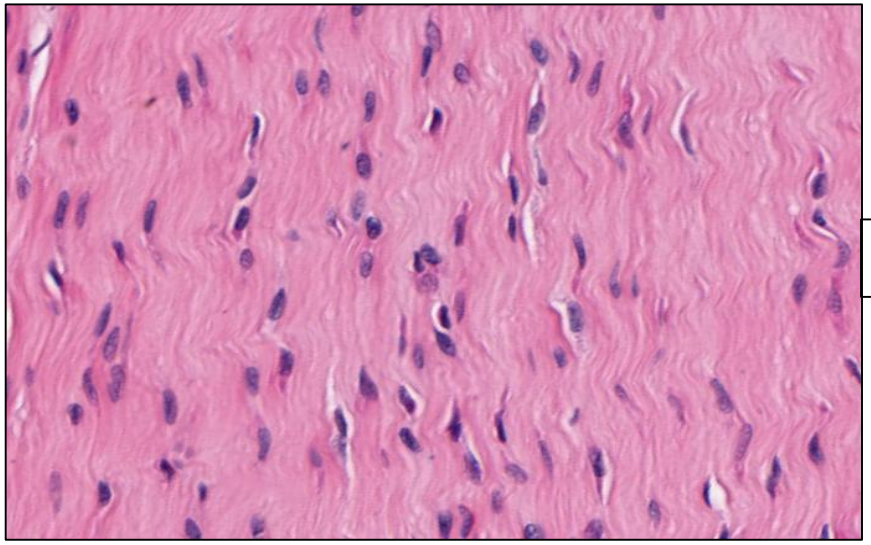
Acidophilic structure=red



L.S skeletal ms. fiber (contains actin & myosin) stained with H &E



Liver cells contains plenty of mitochondria stained with H &E

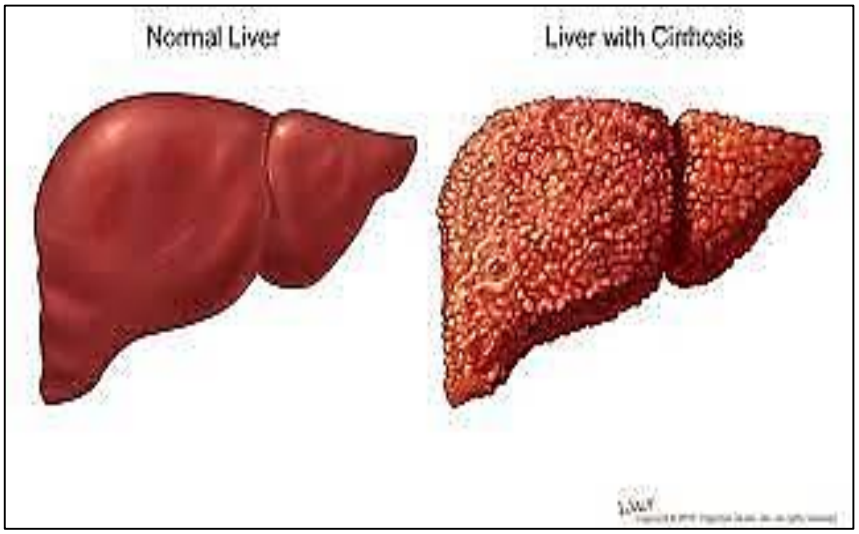


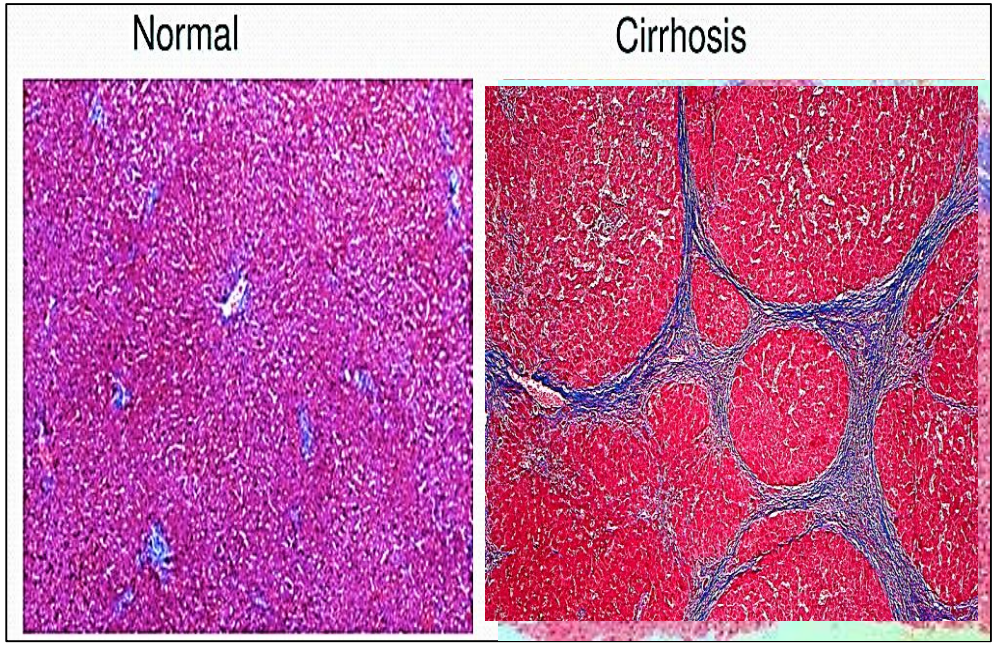
Collagen fibers stained with H & E

Automatic slides staining machine

The clinical values of special stains

- Special stains used to demonstrate tisuue components not easily visualized with H & E
- Also Used in the diagnosis of medical diseases like **Tichrome stain** in case of Liver Cirrhosis





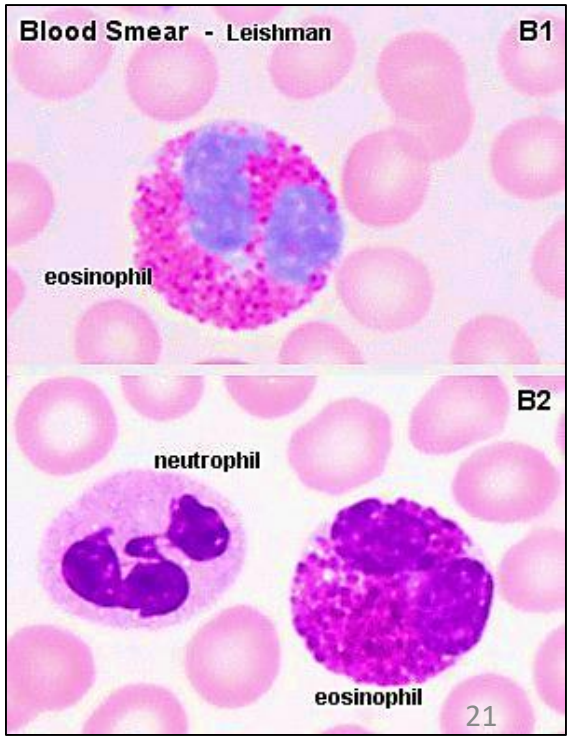
Vital stain: (test the viability of the cell)

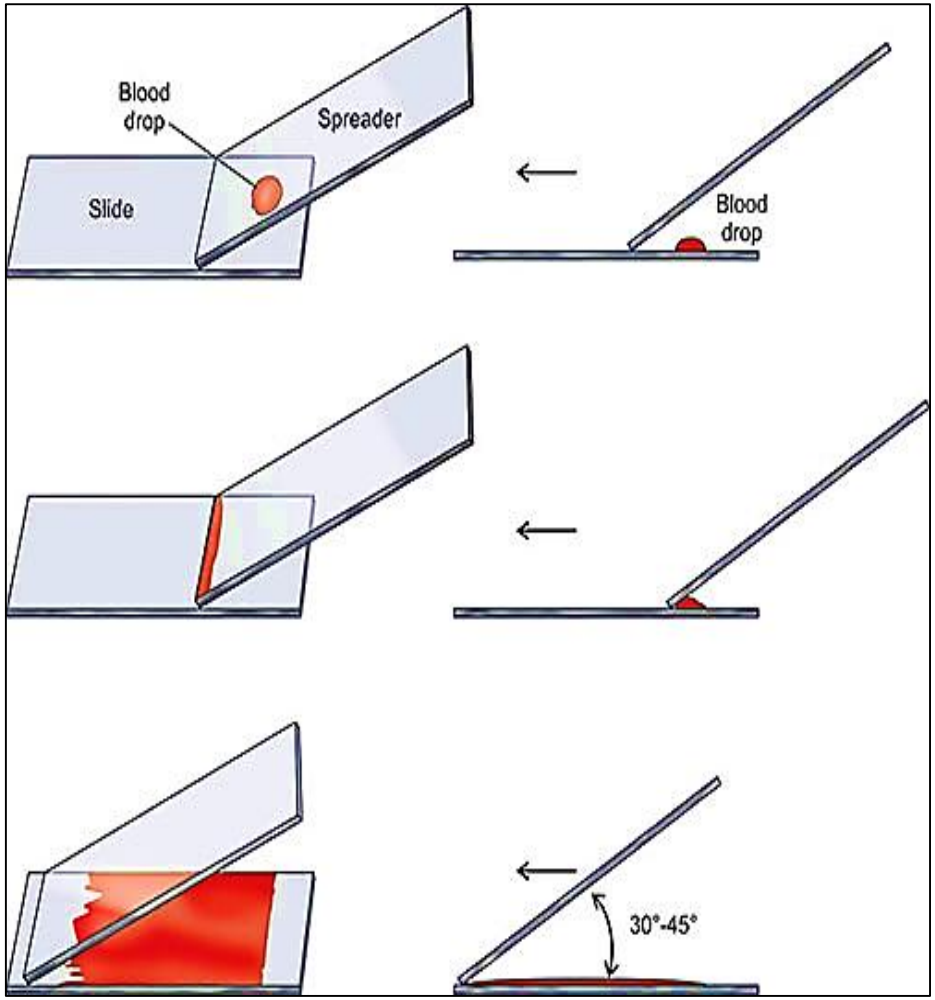
Stain the living cells inside the living animal. Done by injecting the dye into living animal prior to examine the tissue .e.g. staining phagocytic cells (macrophages) with Trypan blue & Indian ink

Leishman stain:

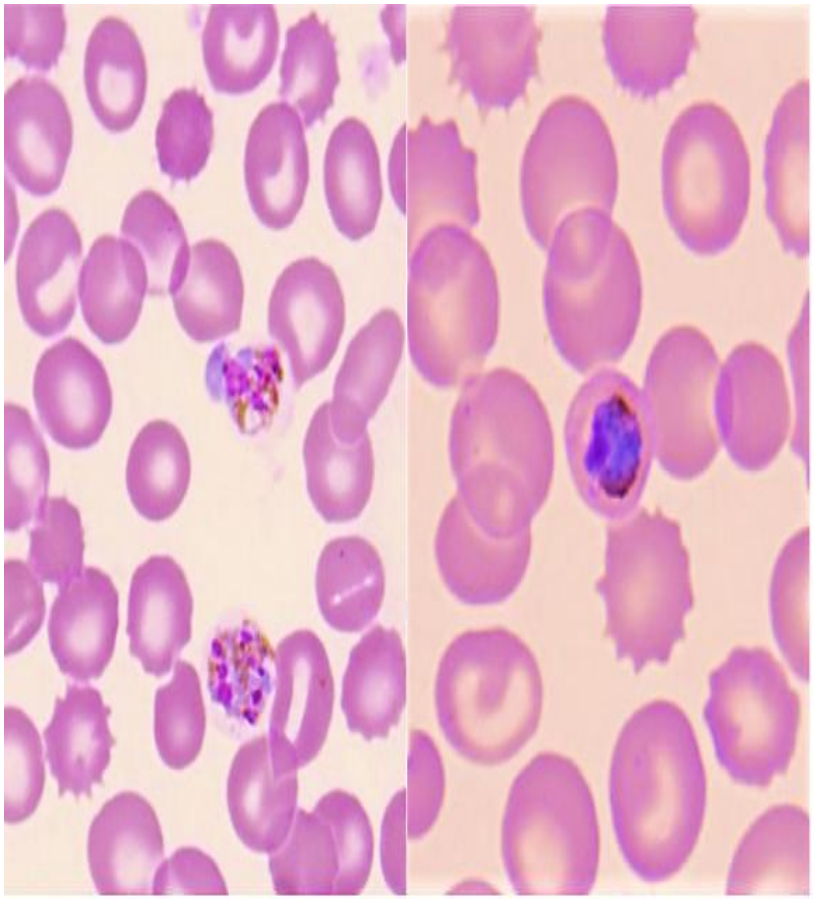
mixture of acidic & basis stains to stain nuclei & cytoplasm

(methylene blue & Eosin) It used to stain blood films to demonstrate white & red blood cells, as in e.g malaria parasite





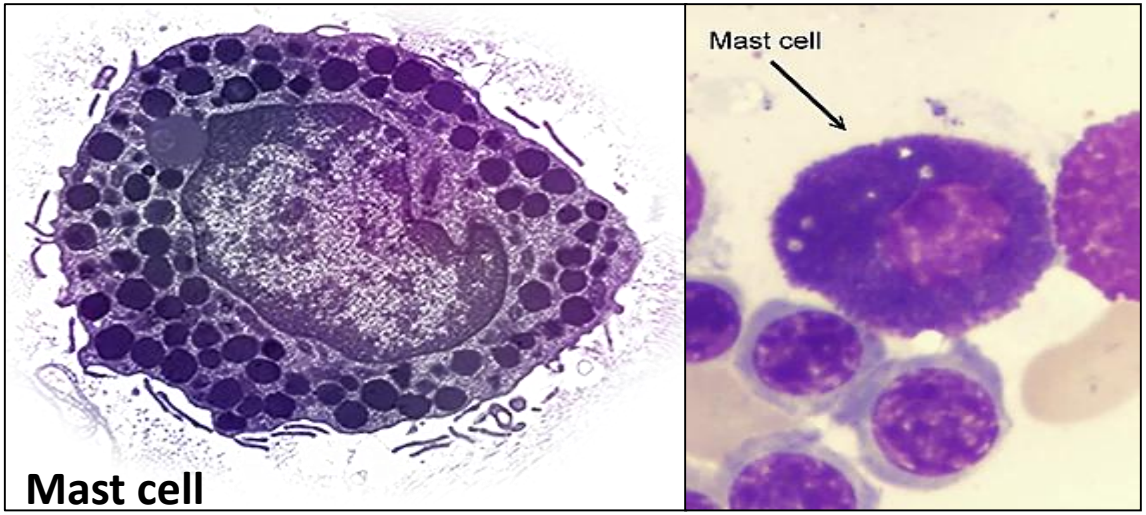
Technique of blood film

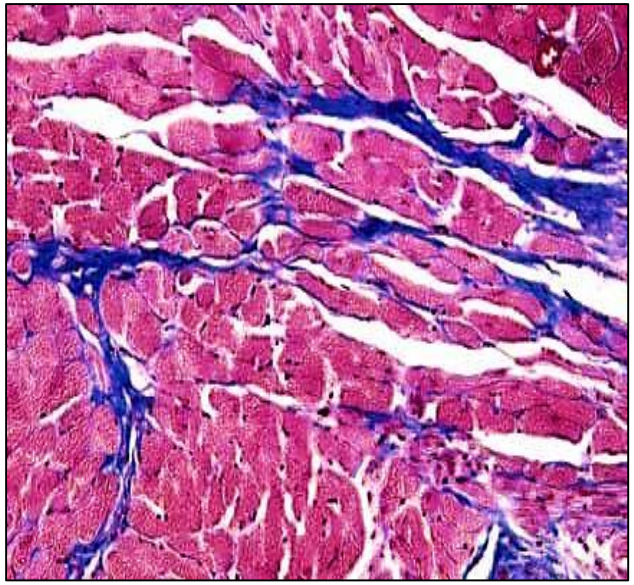


Red blood cells infected with malaria parasite

Metachromatic stain:

Stain gives the tissue new color different from that of the original stain e.g. Toluidine blue when stains Mast cells gives purple color (different from the blue color of the stain). Phenomenon called **metachromasia.**



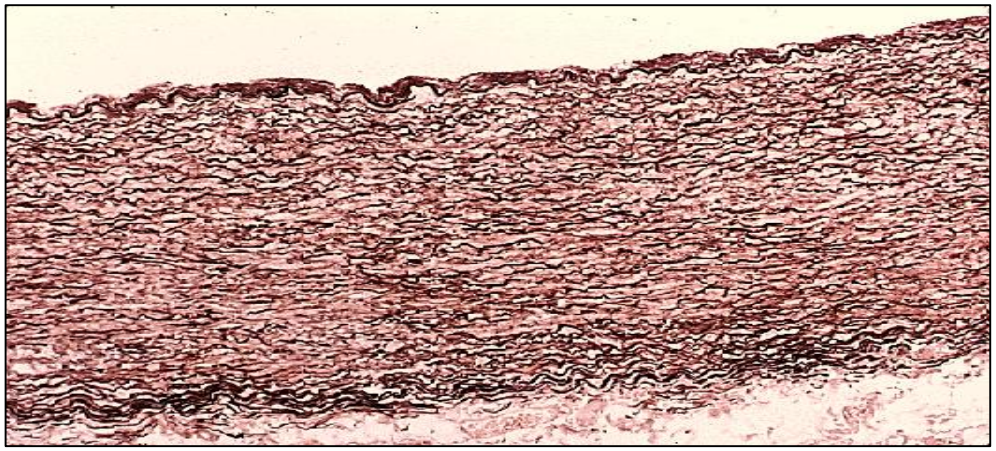


Trichrome stains: (connective tissue)

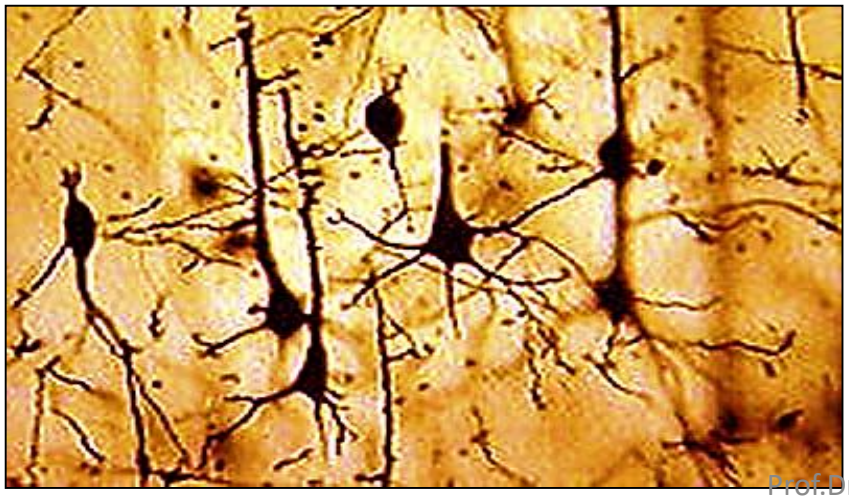
3 stains used in combination to stain different tissues

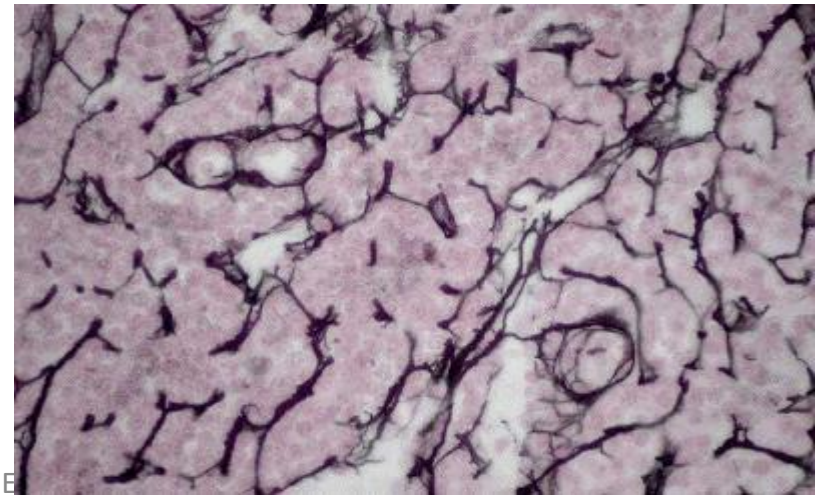
Components e.g. **collagen fibers** stained blue

Orcein stain : stains elastic fibers brown (wall of aorta)



Silver (Ag) stain: nerve cell brown & reticular fibers black





- **Histochemical stains:** (Relate structure to function)

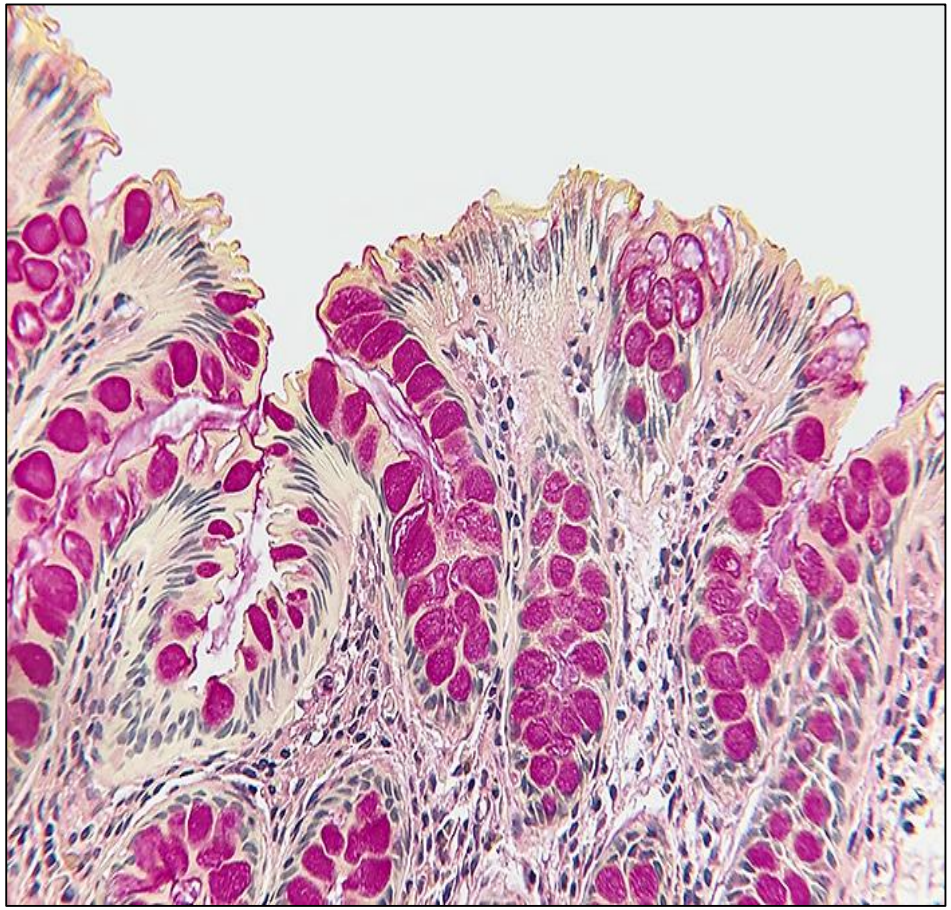
technique used **selectively identify & demonstrate the distribution of chemical substances or enzymes** within & between the cells e.g. mucine or alkaline phosphatase enzyme

Concept: enzyme in a cell or tissue converts into substrate → **colored product** that can be visualized at the site of its activity

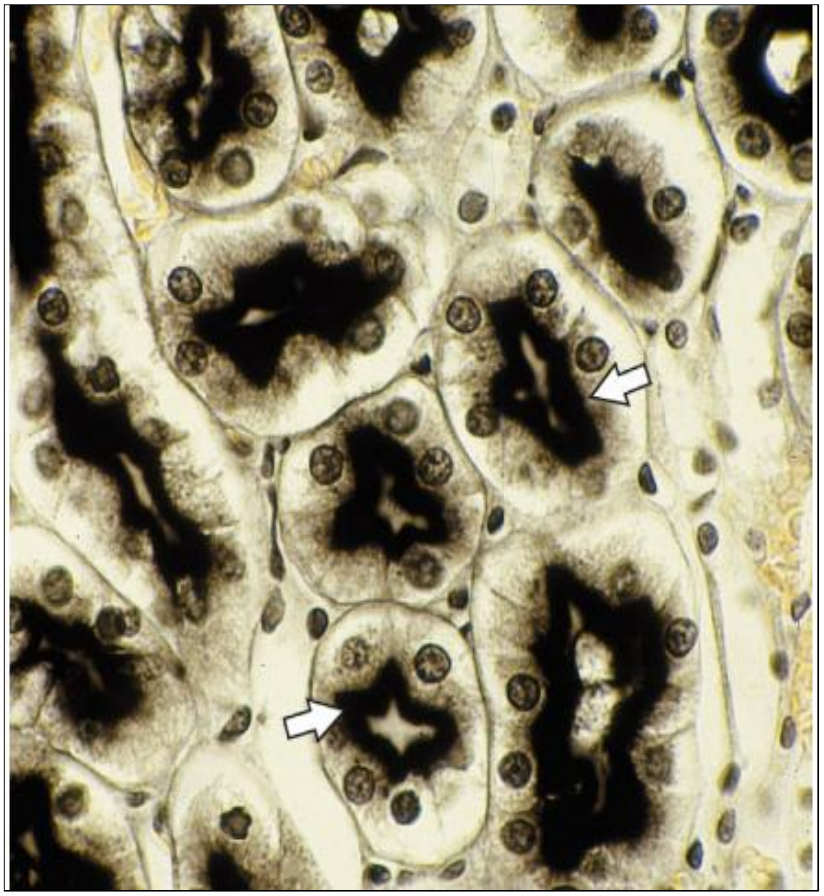
Purpose of histochemical staining

- Detect glycogen , lipids
- Detect enzyme activity
- Detect abnormal accumulation
- Assist in disease diagnosis (storage disorders , liver diseases)

Histochemical stains



for detection of mucin (e.g. glycogen) in mucous cells



(Alkaline phosphatase enzyme)
(this enzyme removes phosphate group from protein)

- **Immuno-histochemical (IHC) stains:**
- Laboratory method that **selectively identify antigens**
- using specific **antibodies** to check for these Antigens (proteins) in tissue samples.
- The antibodies are usually linked to an enzyme or a florescence dye (markers) (Labeled antibodies).

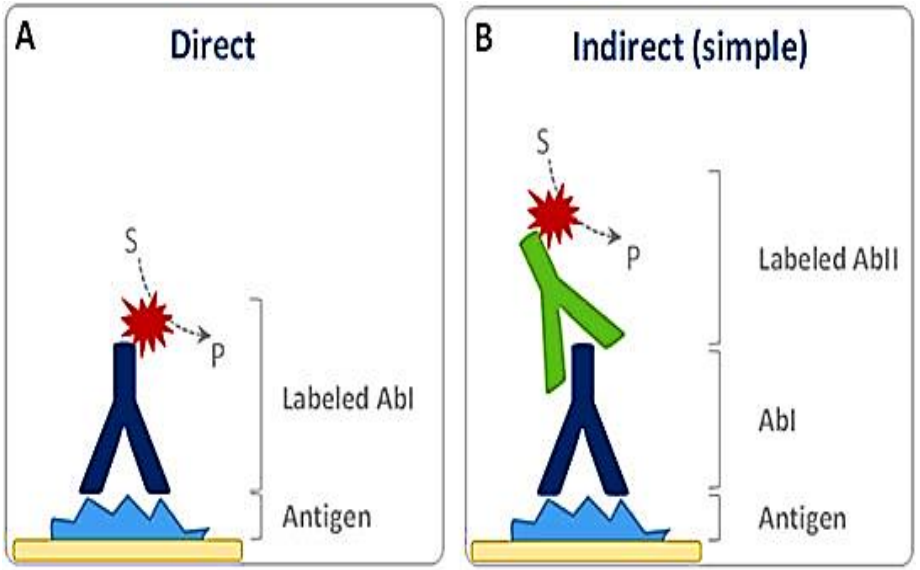
- After the antibodies are linked to the antigen in the tissue sample, the enzyme or dye is activated. The localization of the antigen can be seen under the microscope

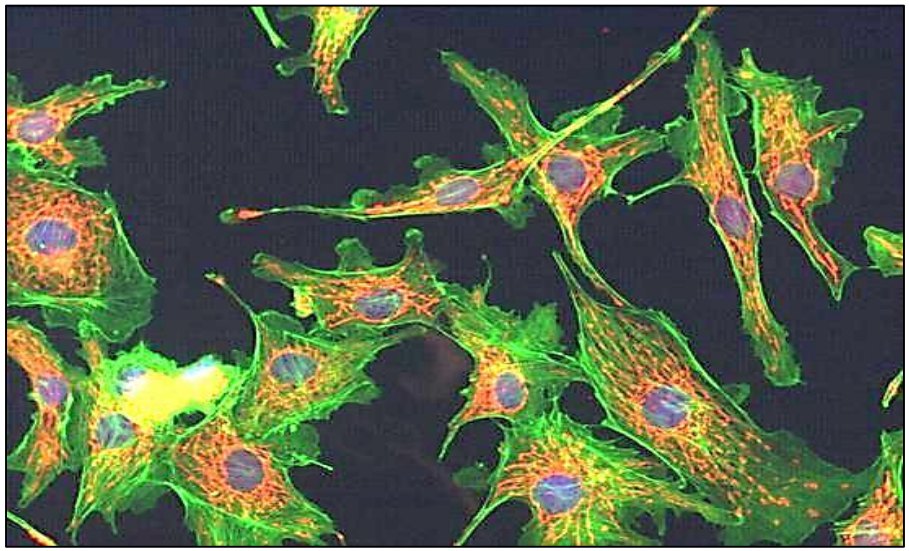
- Used in diagnosis of cancers (markers) and in the differentiation between different types of cancers

- Direct vs Indirect IHC

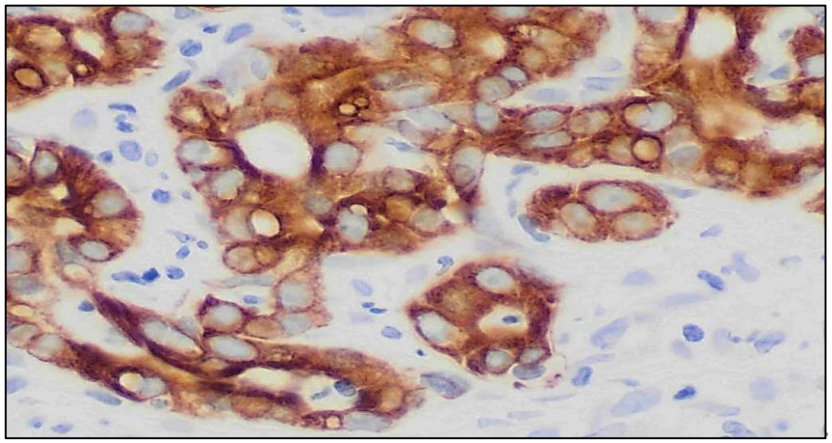
Direct uses labeled 1ry antibody. Less sensitive

Indirect uses unlabeled 1ry + labeled 2ry antibody. More sensitive , widely used in pathology labs





Immunohistochemistry (IHC)



Molecular analysis

It means **biochemical analysis** of certain components of the cell. It is usually quantitative in nature.

Examples are:

- Protein-electrophoresis
- DNA – electrophoresis
- Fluorescent In situ hybridization (FISH technique)
- Cell fractionation
- Detection of certain ions in the cell e.g. Ca, Fe….etc.

Protein electrophoresis: (serum , urine)

Proteins carry either positive/negative electrical charge, and can migrate in an electrical field. Proteins will be separated according to their charge & molecular weight (albumin. Globulin)

(Use to detect abnormal serum proteins :↑ increased gamma globulin protein in urin (M protein) = multiple myloma)

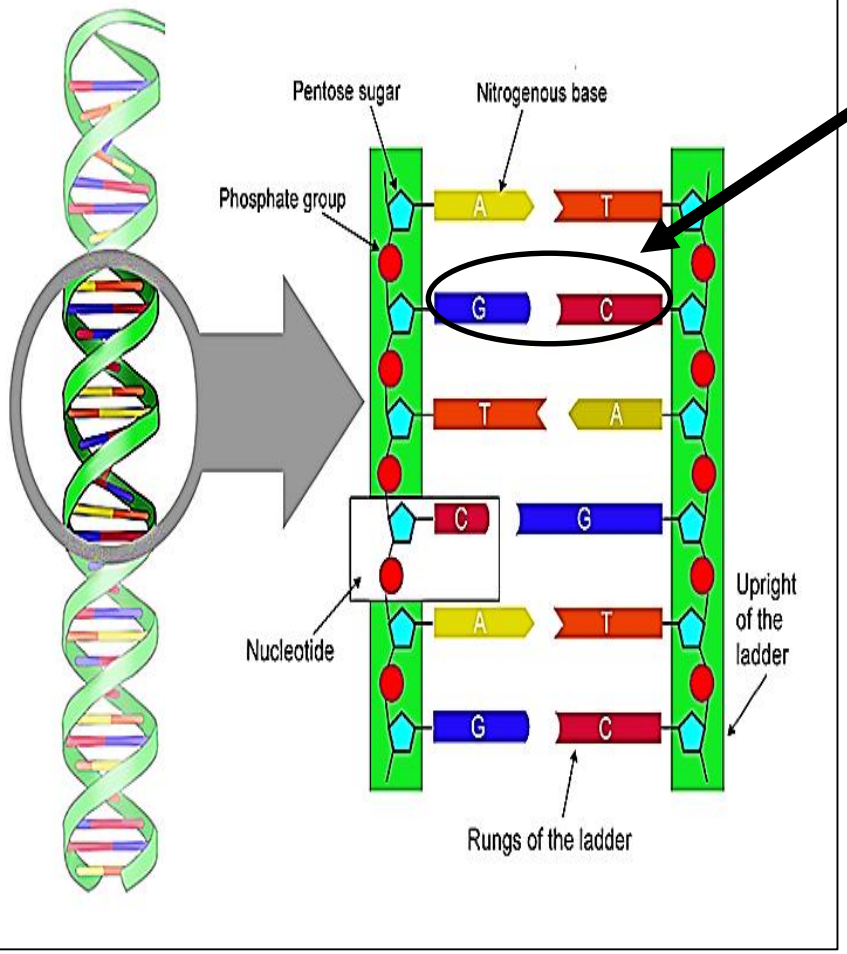
DNA electrophoresis: Technique used to identify & quantify (size) of DNA fragments (DNA fragments are -ve charged).

(in this case separation is based on length of the base pair)

Samples are loaded into wells of an **agarose** or **acrylamide gel** then subjected to an electric field, causing the negatively charged nucleic acids to move toward the positive electrode.

Small fragments will move faster than the large ones

(**DNA fingerprint , gene isolation, disputed paternity**)



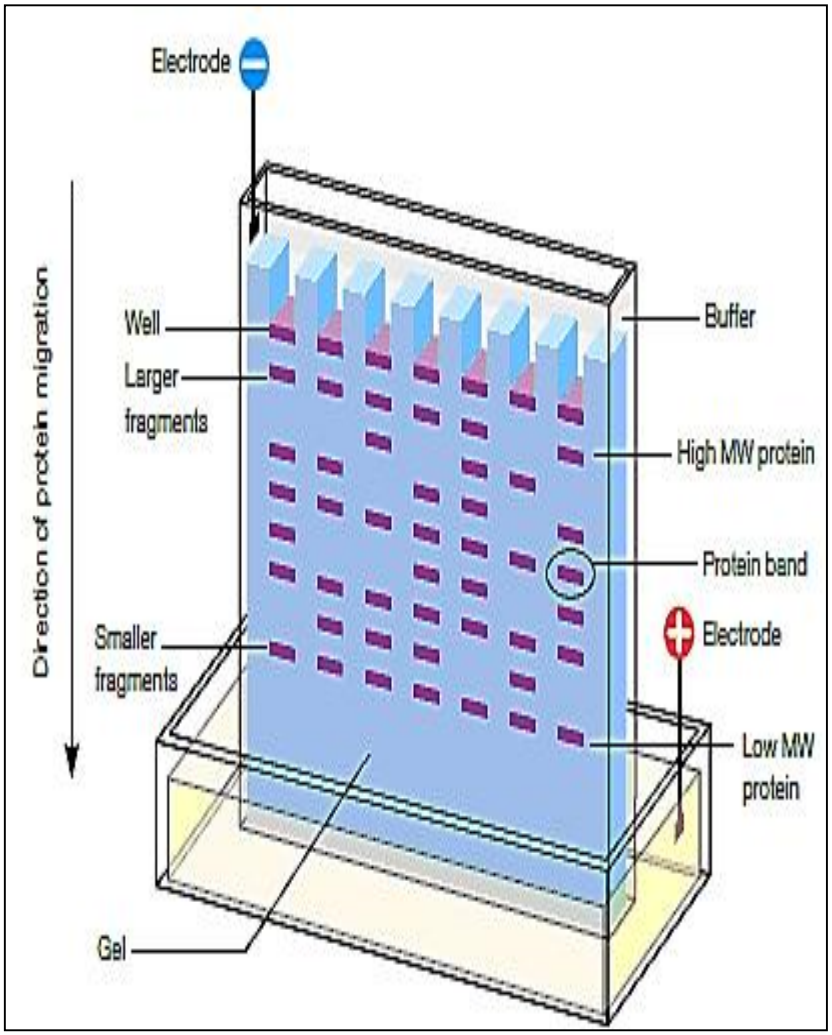
A base pair: is a unit consisting of two nucleobases bound to each other by hydrogen bonds.

They form the building blocks of the DNA double helix.

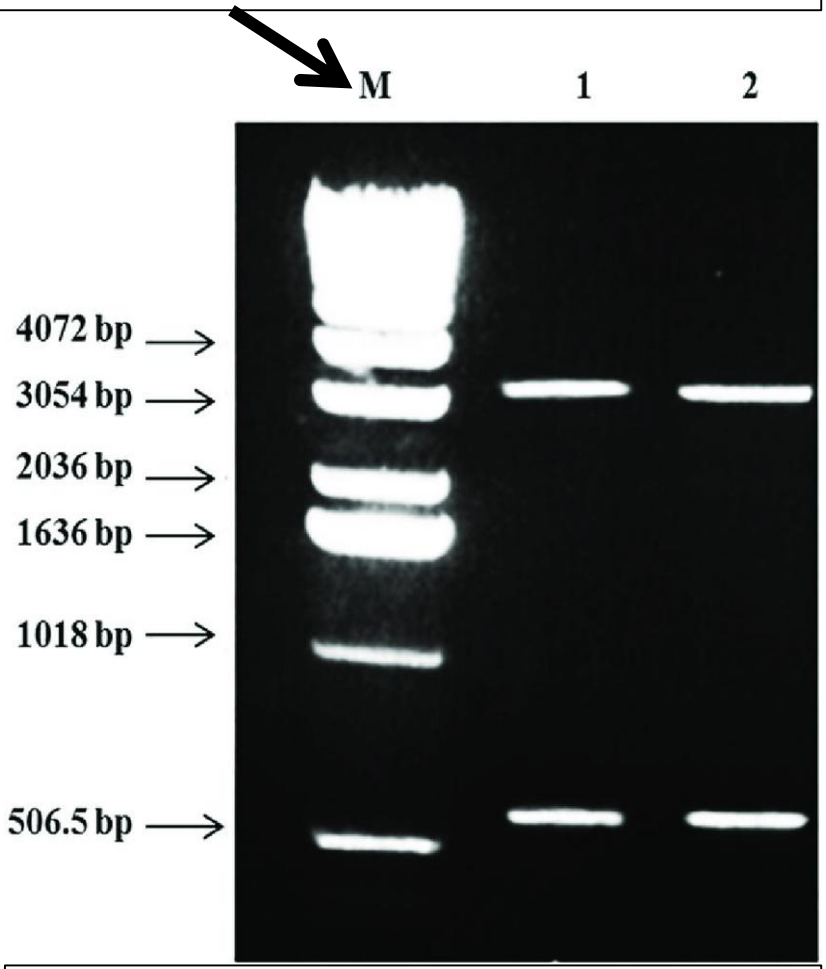
Sequence of bases on DNA determine genetic code for a trait

The human genome contains approximately 3 billion of these **base pairs**, which reside in the 23 **pairs** of chromosomes within the nucleus of all our cells

DNA Ladder: DNA fragments of known lengths used to estimate the size of unknown DNA molecule



Protein electrophoresis
Gel is used which is then stained to visualize the proteins



Fragment size usually referred to as base pair (bp). The shorter fragments travel faster

Fluorescent In situ hybridization (FISH technique):

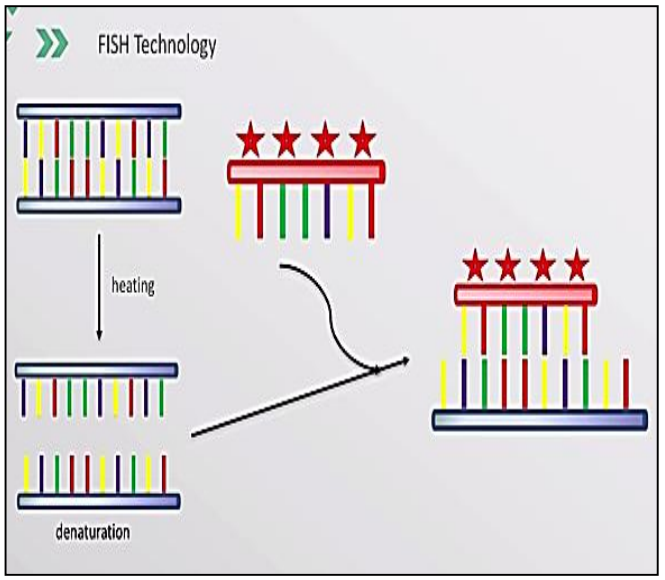
Molecular cytogenetic technique used to visualize and map the genetic material. Use to localize the site of specific genes / DNA sequence on chromosomes using a fluorescent probe

Fluorescent probe:

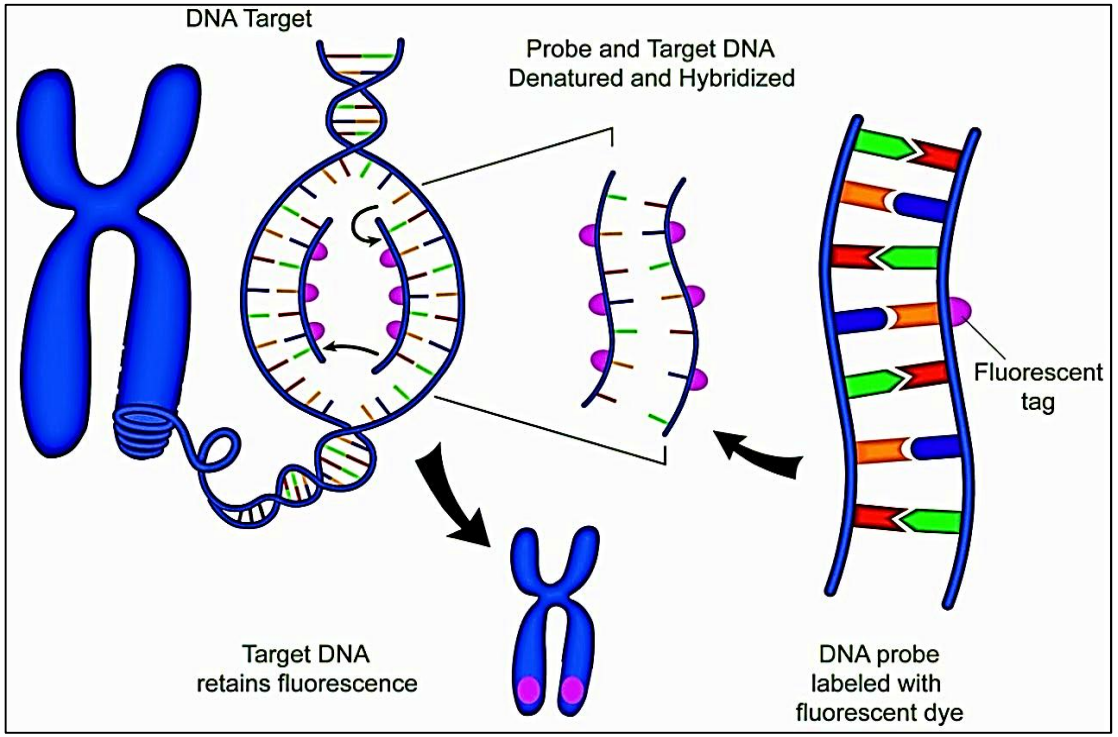
Fragment of DNA or RNA of designed to be complementary to a specific DNA sequence of interest & radioactively labeled

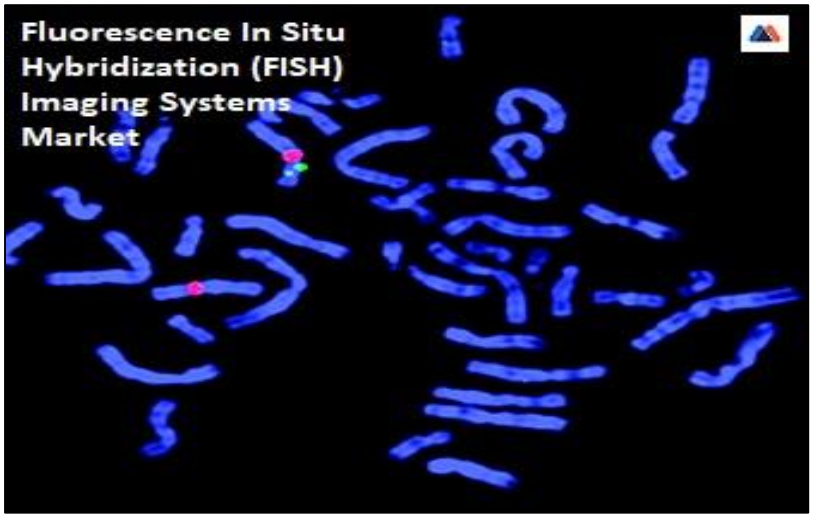
It can be used for DNA or RNA samples to detect the presence or absence of a nucleotide sequence that is complementary to the sequence of the probe .

Useful in detect chromosomal abnormalities



- **The probe hybridizes (binds) to the target DNA In situ (within the cell / nucleus)**
- **FISH helps "light up" specific genes or chromosomal regions**
- **The bound probes are visualized using fluorescence microscope**





Methods for study tissues

- **1-In vivo studies: within the living body .** Study of tissues after doing any experiment inside the living body (animal model based testing)

- **2-In vitro studies: outside the body** Study of tissues outside their normal biological context (cell based testing)

Cell and Tissue Culture

- **In vitro** cultivation of tissues & cells at defined temperature(37C) using an incubator & supplemented with a medium containing cell nutrients & growth factors(like animal serum) is collectively known **as tissue culture.**

- Different types of cells can grow in cultures as: white blood cells, fibroblasts, skeletal and cardiac muscle, epithelial tissue (liver, breast, skin, kidney) and many different types of tumor cells.

Medical uses of tissue culture:

1- used in studying chromosomal patterns of individuals …. Karyotyping, gene therapy

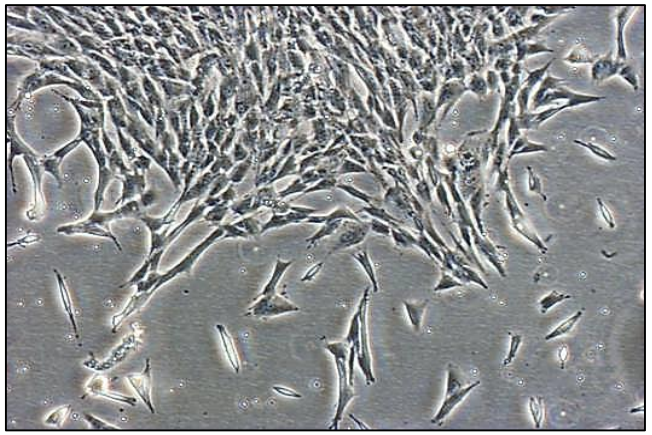
2- Used in researches of cancer

3- Used in cultivation of bacteria, viruses, in order to prepare different vaccination

4- Study the effects of new drugs

Cell culture

Cells can be isolated from the body for ***in vitro* cultures. Cells** can be released from **tissues** by enzymatic digestion Using enzymes such as collagenase and trypsin which break down the extracellular matrix.



Primary cultures:

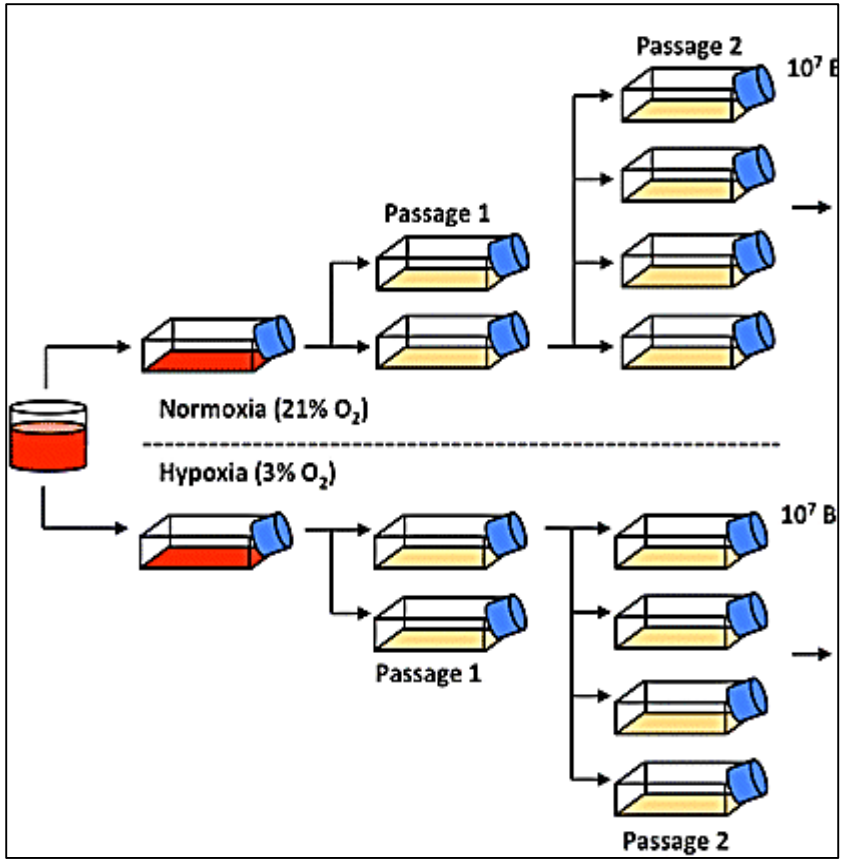
Refer to the cells that are cultured directly from a tissue (parent cells).

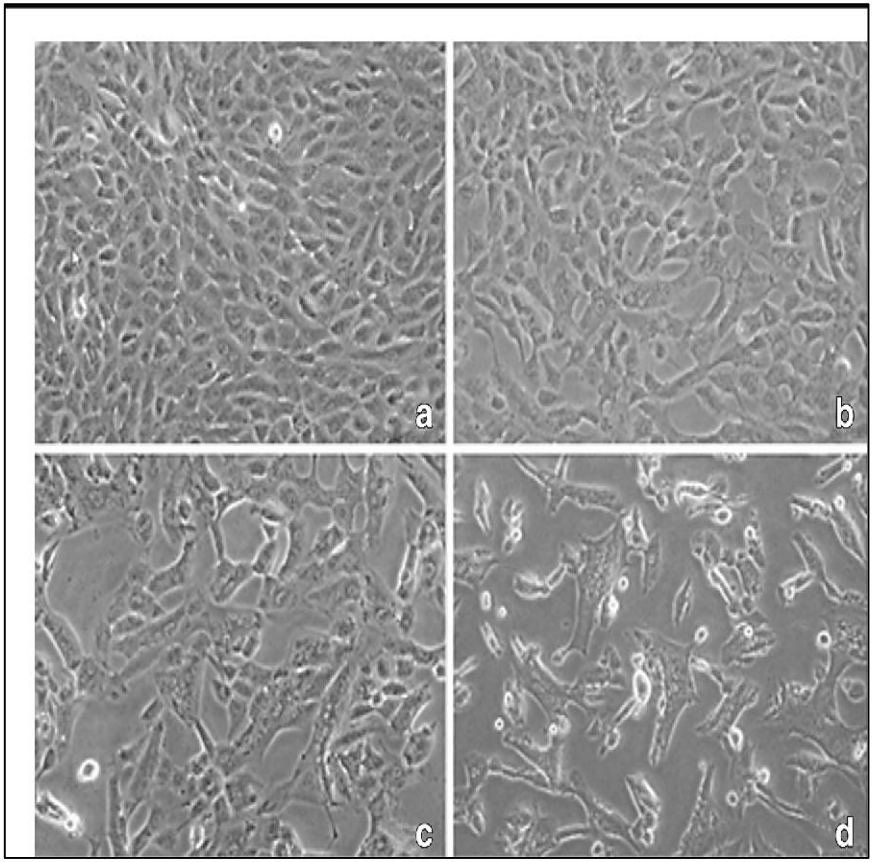
Secondary cultures:

- Once the parent cells reach confluence they have to be **sub-cultured** (i.e. passaged) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth

Confluence:

- Percentage of surface area in a culture vessel that covered by adherent cells (1ry or 2ry cultures) forming monolayer(e.g. 25%, 50%, 100%)





Cell passage = subculture

Different degrees of confluency

Cell line:

- a population of cells (clone) derived from a single cell that has been cultured in vitro. These cells have a **uniform** genetic make-up (phenotype & function)
- Cell lines have a limited life span, as they are passaged
- Origin : tissue , tumor or 1ry cells (Hela :cancer research)

immortalized cell line

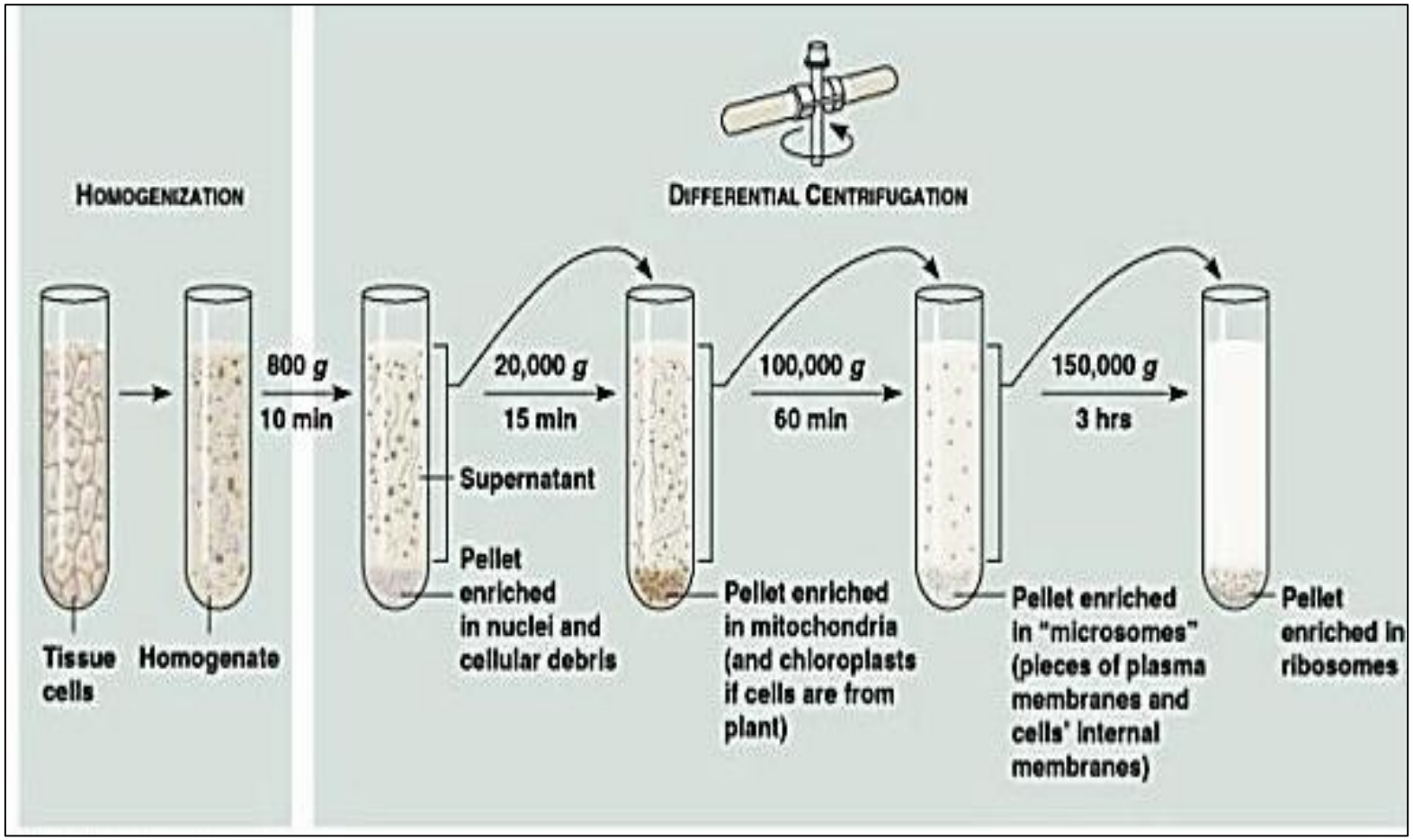
- Modified to **proliferate indefinitely.**
- It is obtained from subcultures of the primary culture
- Stem cells: share with IM cells; long – lived & self renew cells
- Abnormal immortalized cell lines : cancer cells

Cell fractionation

- It means isolation of the cell components (nucleus & organelles) while preserving its individual function to study the features of each.

- This is done by the use of **centrifugation** at different speeds and periods of time. The factor that determine whether a specific cell component ends up in the supernatant or the pellet is size and weight of component

- Nuclei are the first to be separated followed by different cell organelles



The sediment at the bottom of the tube is called **pellet**, the less dense component at the top is called **supernatant**

Thank you