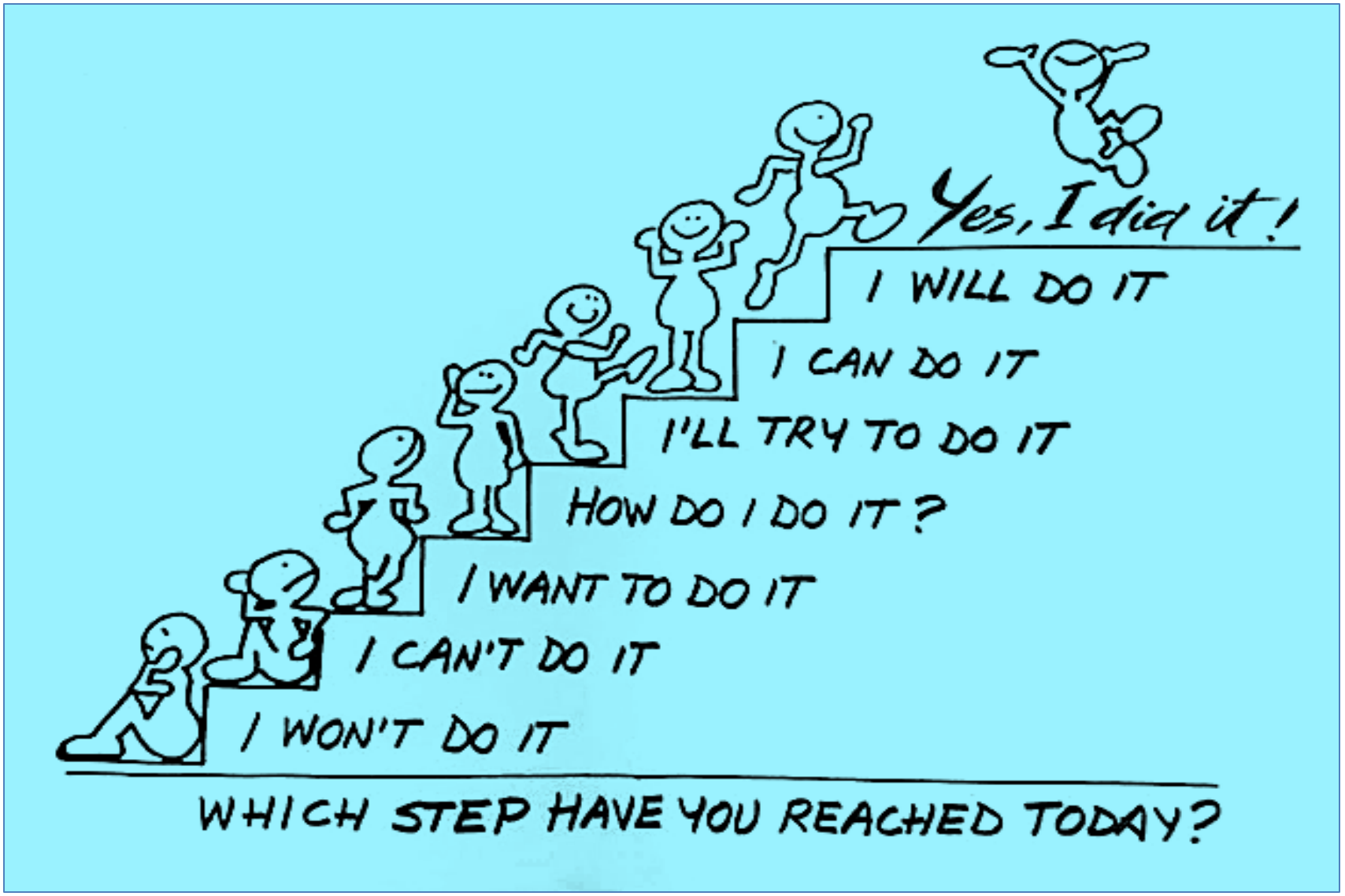
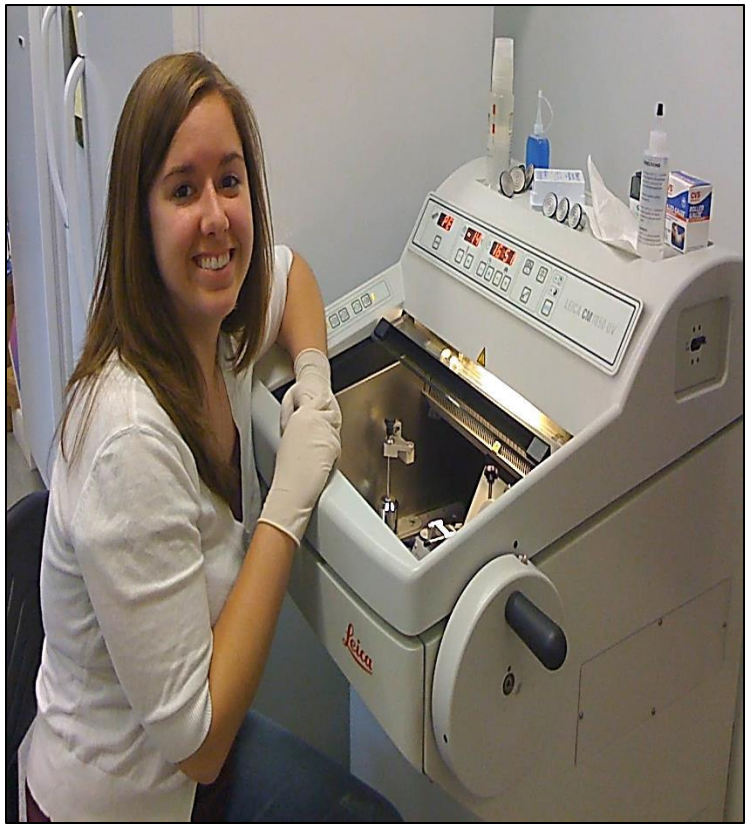
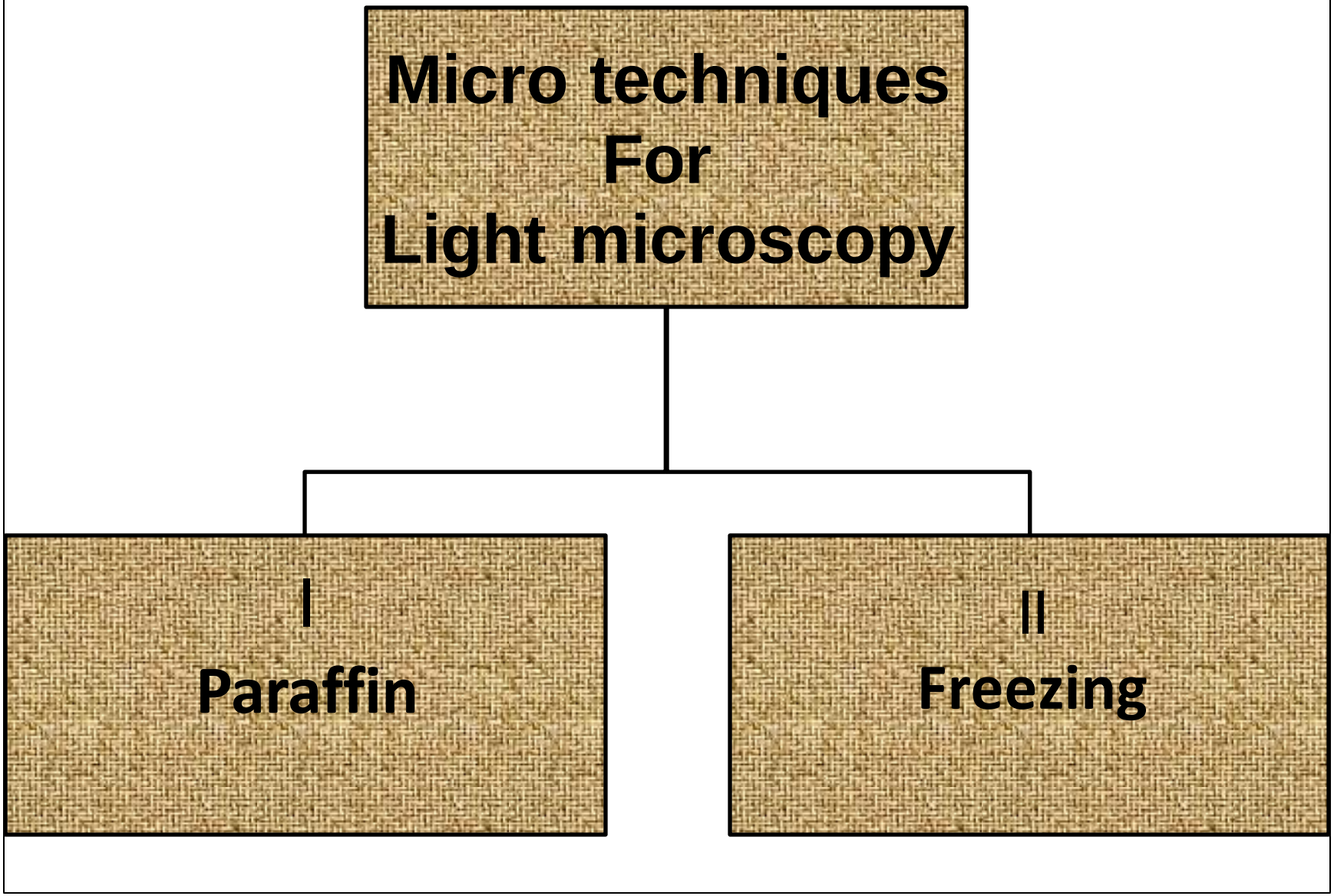
Think positive



Cell Bio-2
Micro-techniques



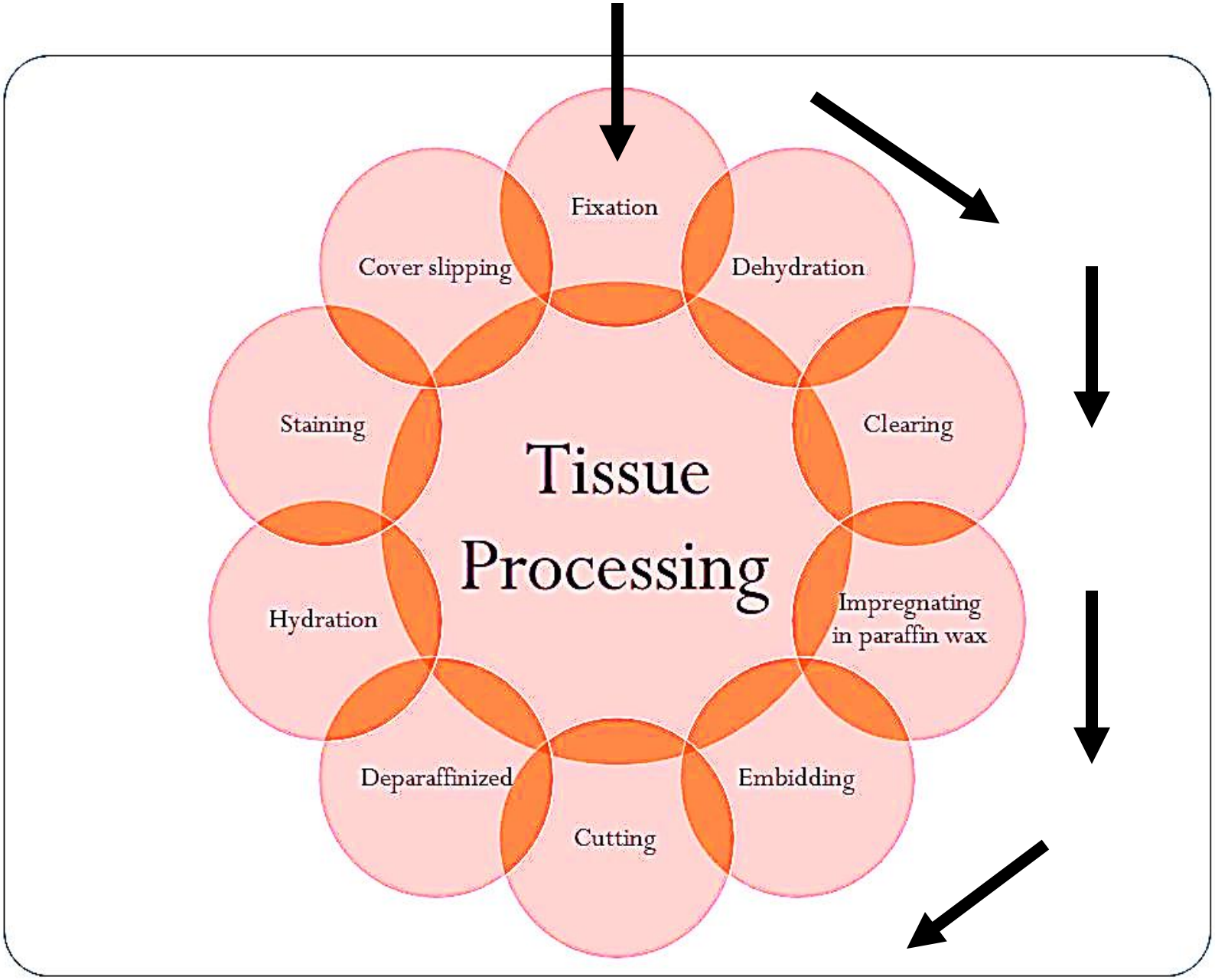

Micro techniques
For
Light microscopy
I
Paraffin
II
Freezing

I: Paraffin technique

- Technique used to prepare **the tissues** 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
4. **Sectioning :** by microtome
5. **Mounting :** on glass slides
6. **Staining of the sections**


Fixation
Dehydration
Clearing
Impregnating in paraffin wax
Embidding
Cutting
Deparaffinized
Hydration
Staining
Cover slipping
Tissue Processing

Fixation

- To maintain the tissue in as life-like state as possible,
- Sample for analysis is directly placed into a fixative solution upon removal from the body
- 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:

1. 10% formal saline: Most commonly used fixative

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

Advantage of fixation :

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

- Prevent putrefaction & stop autolytic changes by killing 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

Clearing : with this process the tissue become translucent the tissue is treated with xylol or benzol …to remove the alcohol

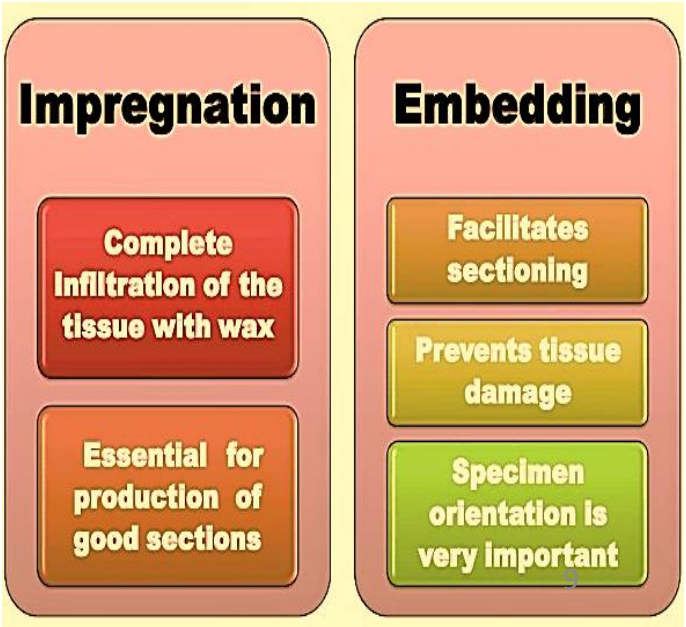
Impregnation & Embedding

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

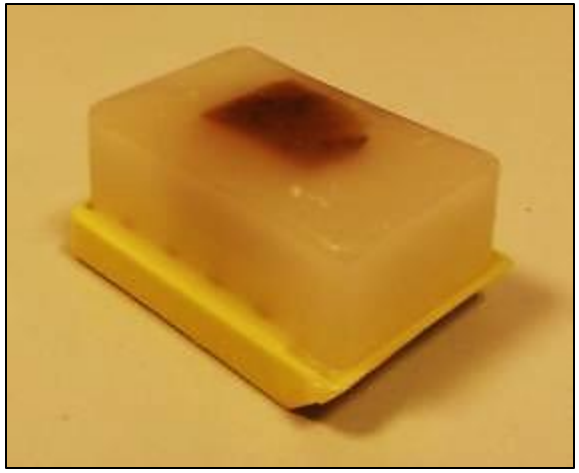


Automatic tissue processor

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

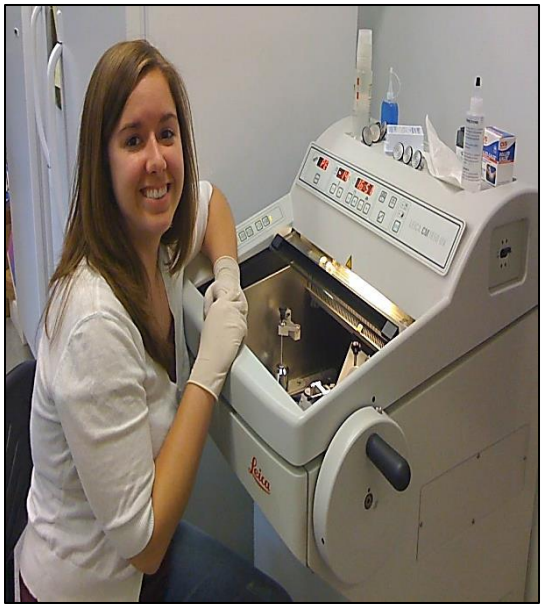
- A microtome is a mechanical device used to cut extremely thin slices of a fixed tissue block known as **sections.**
- It holds the block of hard paraffin with the tissue in its center against a sharp metal knife that used to cut the block into thin sections (3-10 microns) as it moves up and down.



mounting

Tissue sections are put on glass slides smeared with egg albumin , warmed on a hot plate to dry the. Sections are now ready to be stained

II. Freezing technique



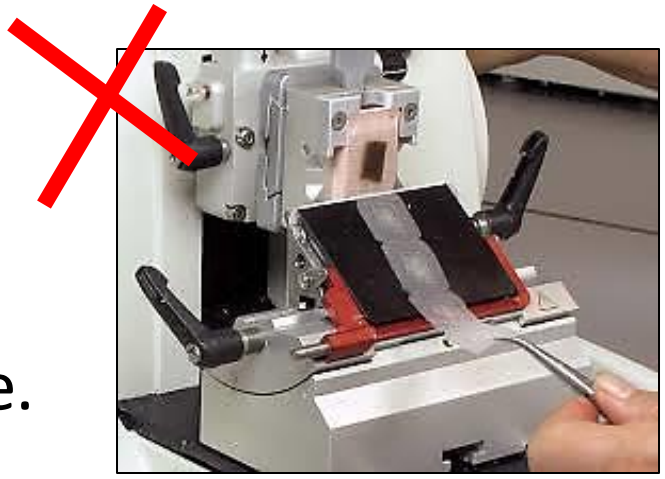
Fresh frozen tissues are cut using freezing microtome (cryostat). The sections then rapidly stained.

Advantages:

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

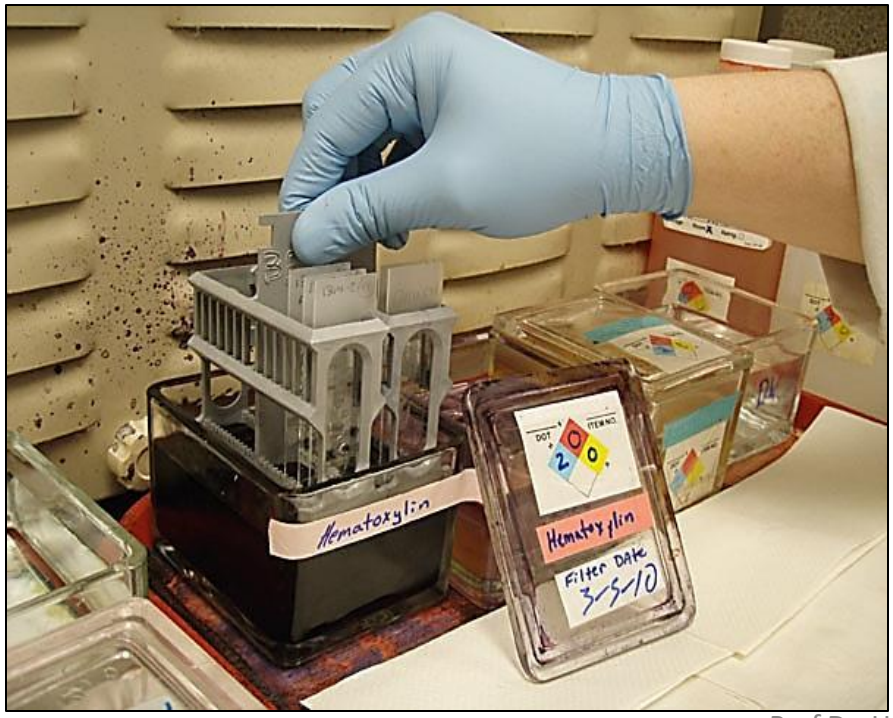
Disadvantages:

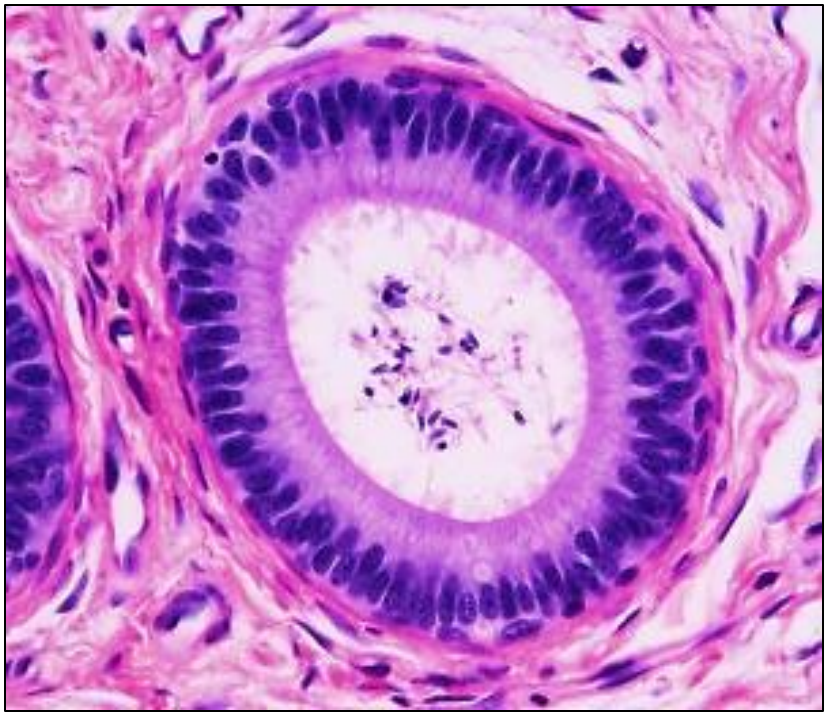
- Non serial & Fragmented sections .
- Cannot be preserved for a long time.



Staining

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





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, 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 (it has +ve charged proteins)

Acidophilic structure=red

Major Steps in the Routine H & E

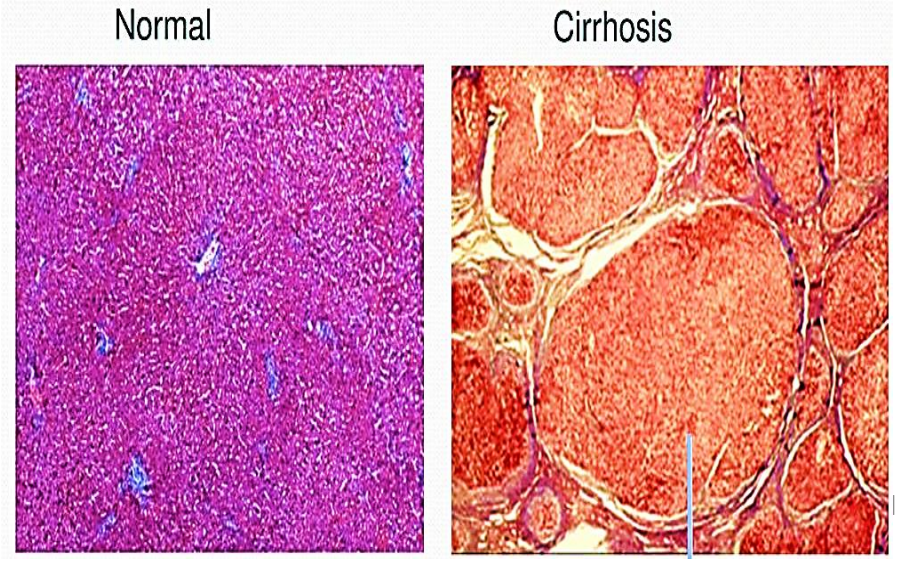
1. **De-paraffinization-**(removal of paraffin wax using xylene)
2. **Hydration**-(graded alcohols to water)
3. **Nuclear staining**-using Hematoxylin
4. **Differentiation**-Acid alcohol
5. **Bluing**-Ammonia Water
6. **Counterstaining**-using Eosin
7. **Dehydration**-(application of graded alcohol to 100% alcohol)
8. **Clearing**-Xylene (transition from alcohol to non-aqueous reagents)
9. *Note*: Water-rising steps are not shown

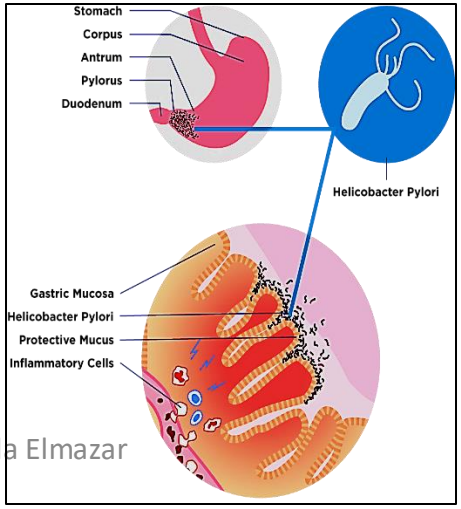
Steps of H& E staining

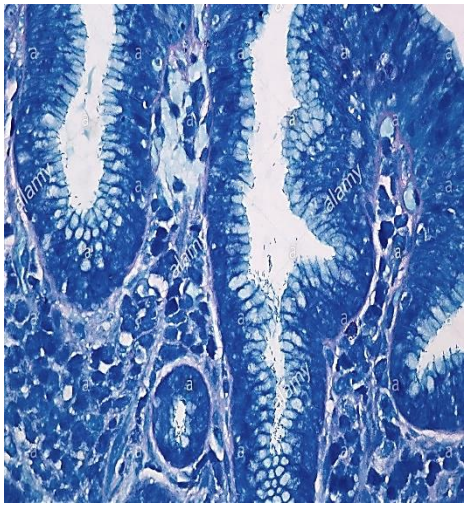
Automatic slides staining machine

The clinical values of special stains

- H& E is general stains shows what type of tissue is there
- Special stains answer specific questions like what type of cells & what type of tissues there
- Used in the diagnosis of medical diseases like Tichrome stain in case of Liver Cirrhosis
- Diagnosis of bacterial & fungal diseases e.g. H. Pylori







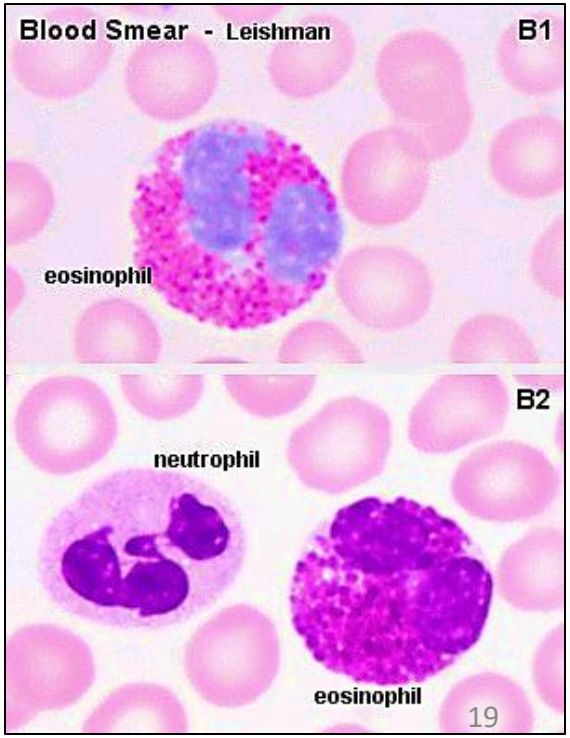
Vital stain:

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 with Trypan blue & Indian ink

Neutral stain:

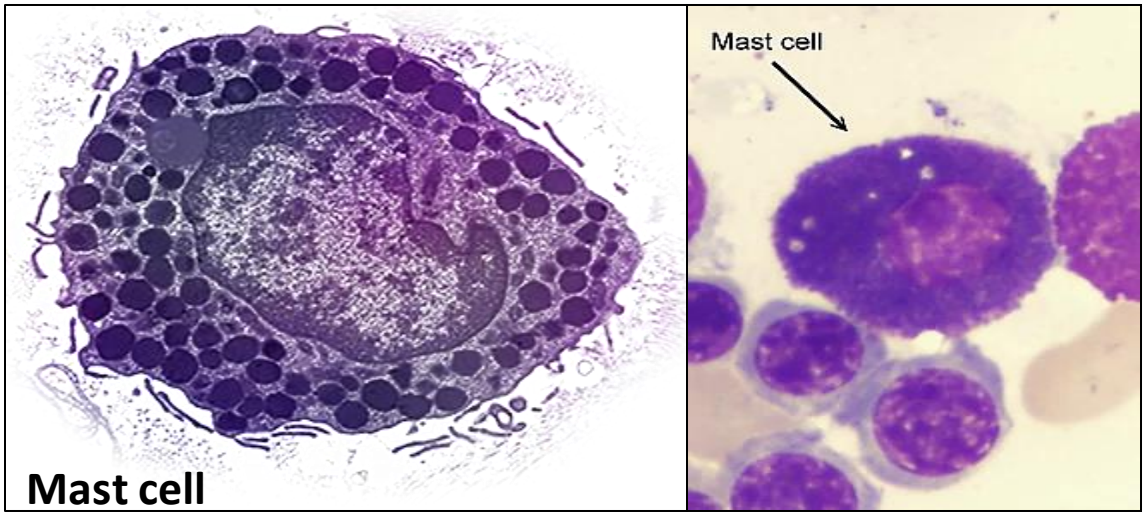
mixture of acidic & basis stains to stain nuclei & cytoplasm

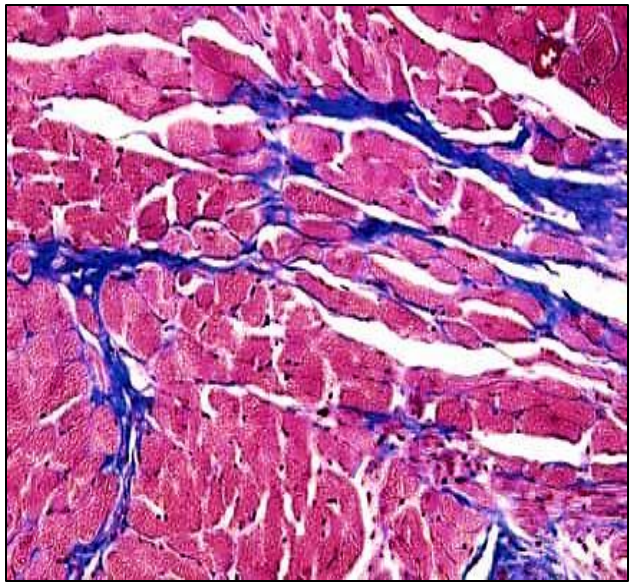
e.g. Leishman stain → stains blood films → demonstrate blood cells



Metachromatic stain:

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

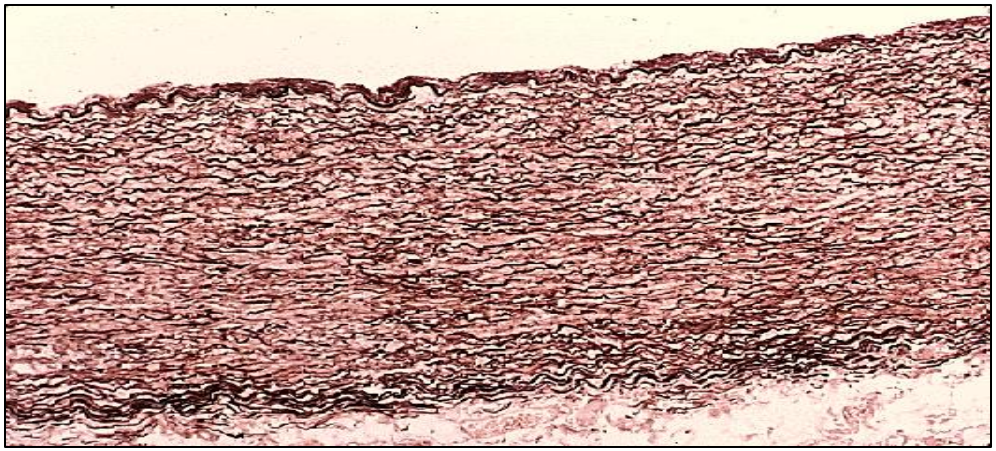




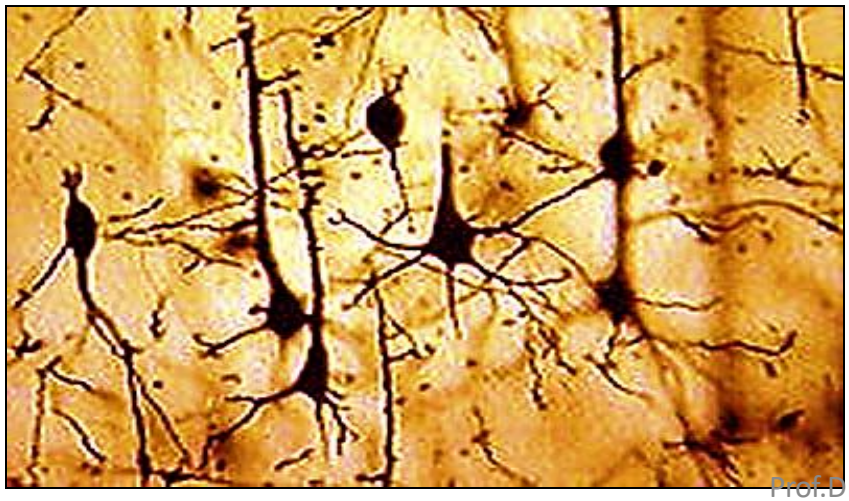
Trichrome stains: (Fibrous tissue)

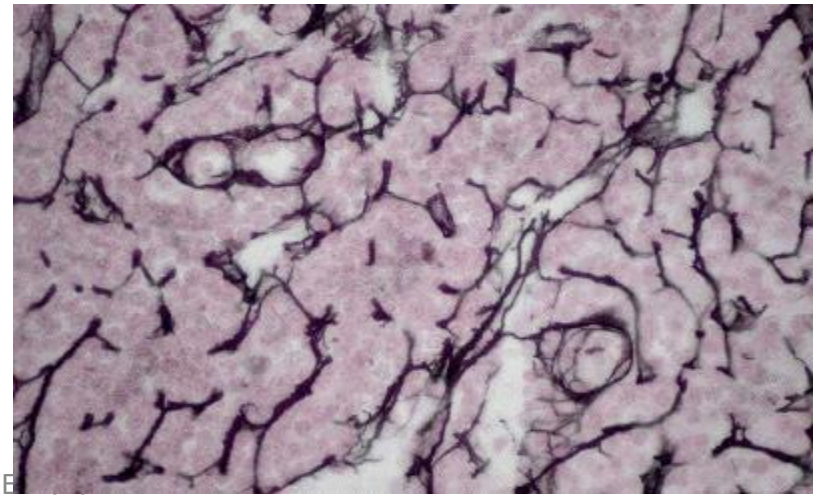
3 stains used in combination to give 3 colors to different tissue Components e.g. collagen fibers

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



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





- **Histochemical stains:**

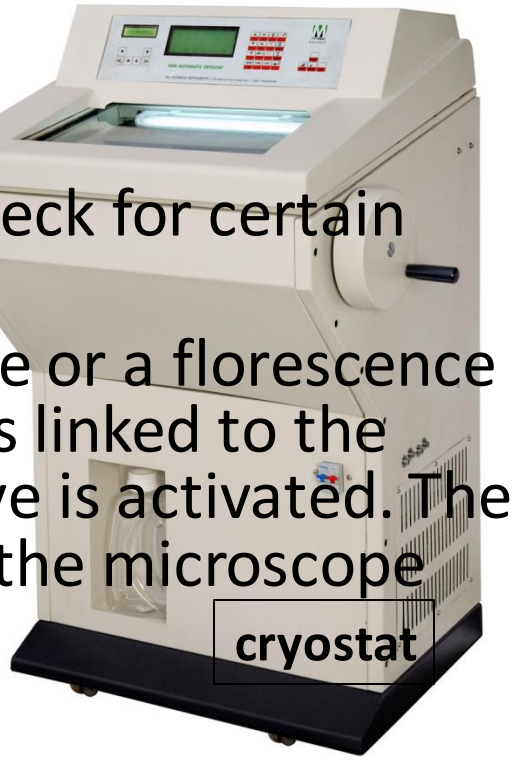
Stain used **selectively** to identify & demonstrate enzyme or chemical component of the cells (e.g. alkaline phosphatase enzyme) (Relate structure to function)

- **Immuno-histochemical (IHC) stains:**

Laboratory method that uses antibodies to check for certain antigens (markers) in a sample of tissue.

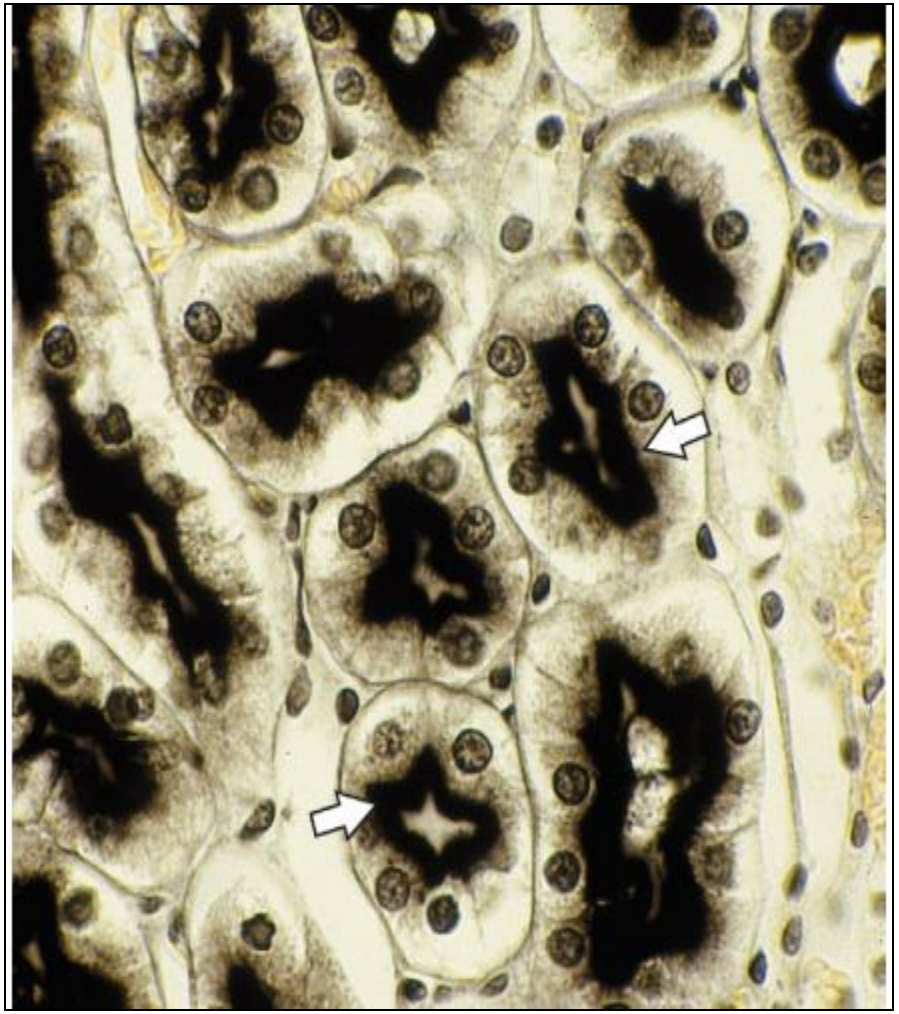
The antibodies are usually linked to an enzyme or a florescence dye (Labeled antibodies). After the antibodies linked to the antigen in the tissue sample the enzyme or dye is activated. The localization of the antigen can be seen under the microscope

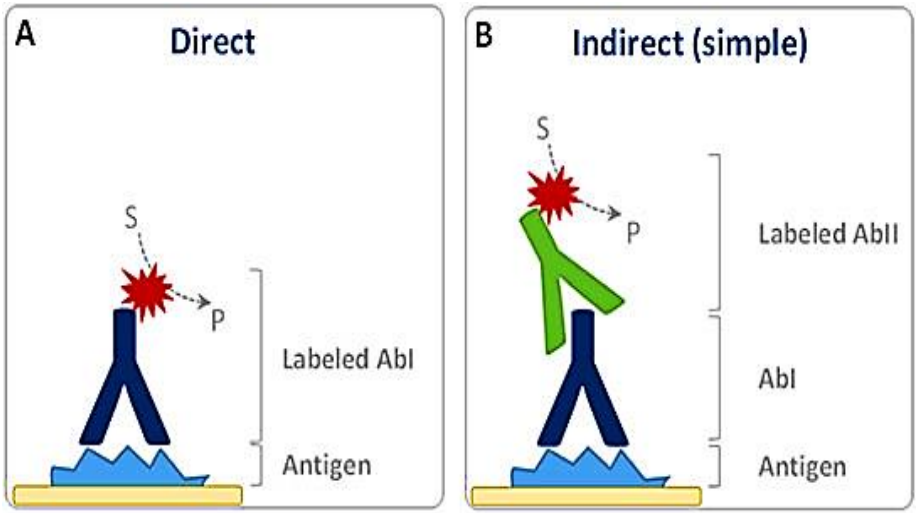
(labeling we use : Fluorescein , Rhodamine)

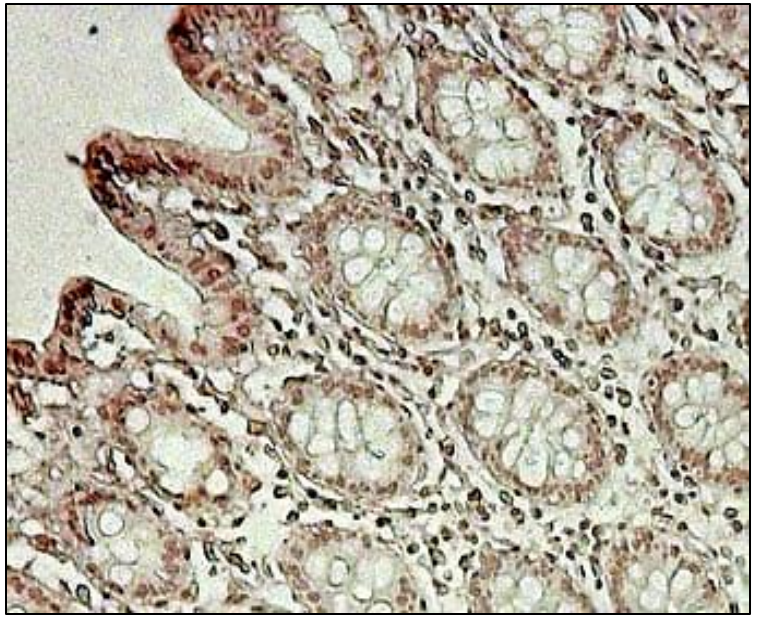

cryostat

This method use for:

Diagnosis of cancers (markers) and can tell the difference between different types of cancer . Specific tumor







Histochemistry
Alkaline phosphatase enzyme

immunohistochemistry

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)
- Detection of certain ions in the cell e.g. Ca, Fe….etc.

Protein electrophoresis: (BL serum protein electrophoresis)

Proteins carry a positive or a negative electrical charge, and they move in fluid when placed in an electrical field. Proteins will be separated according to their charge & molecular weight (e.g. M protein in multiple myeloma)

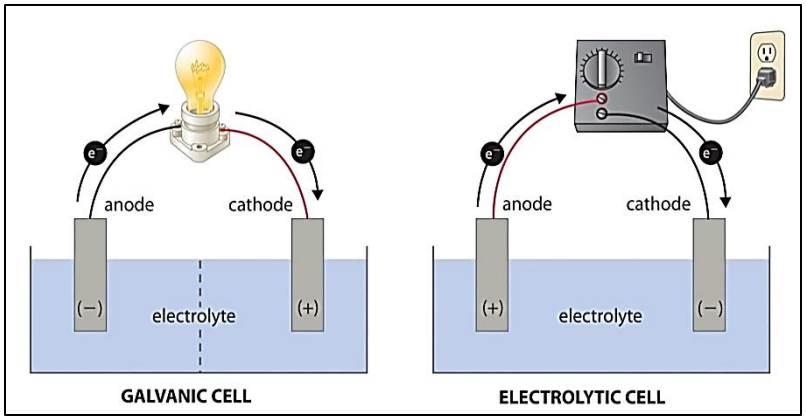
DNA electrophoresis: is technique used to identify & quantify DNA fragments (DNA fragments are -ve charged).

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

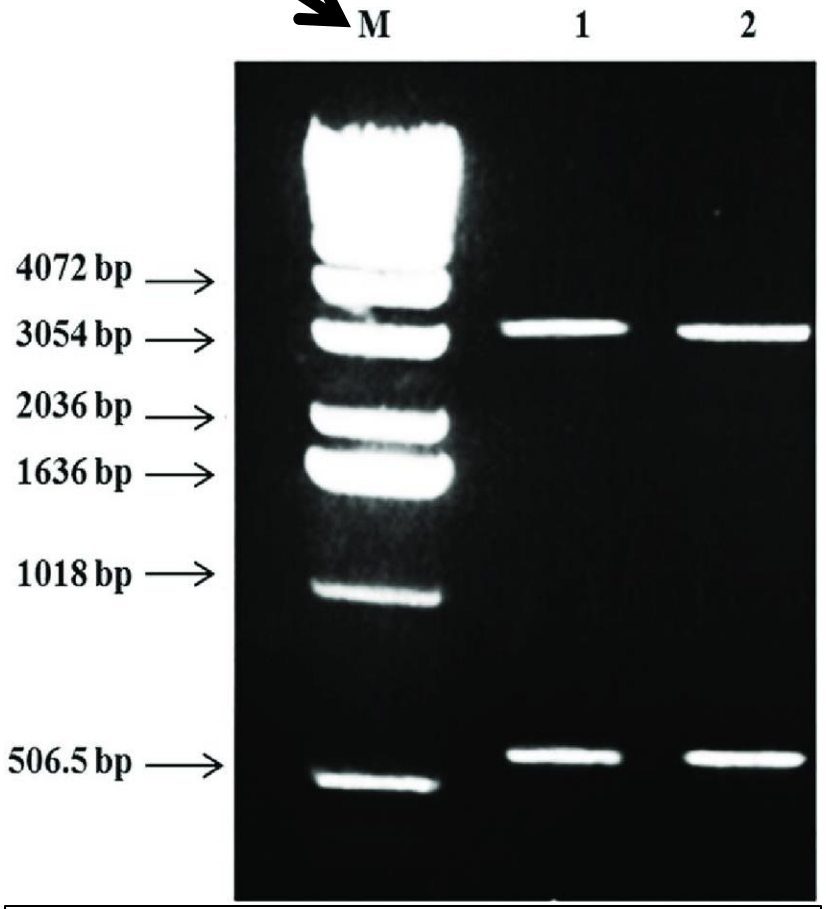
Samples are loaded into wells of an **agarose** or **acrylamide gel** and subjected to an electric field, causing the negatively charged nucleic acids to move toward the positive electrode.

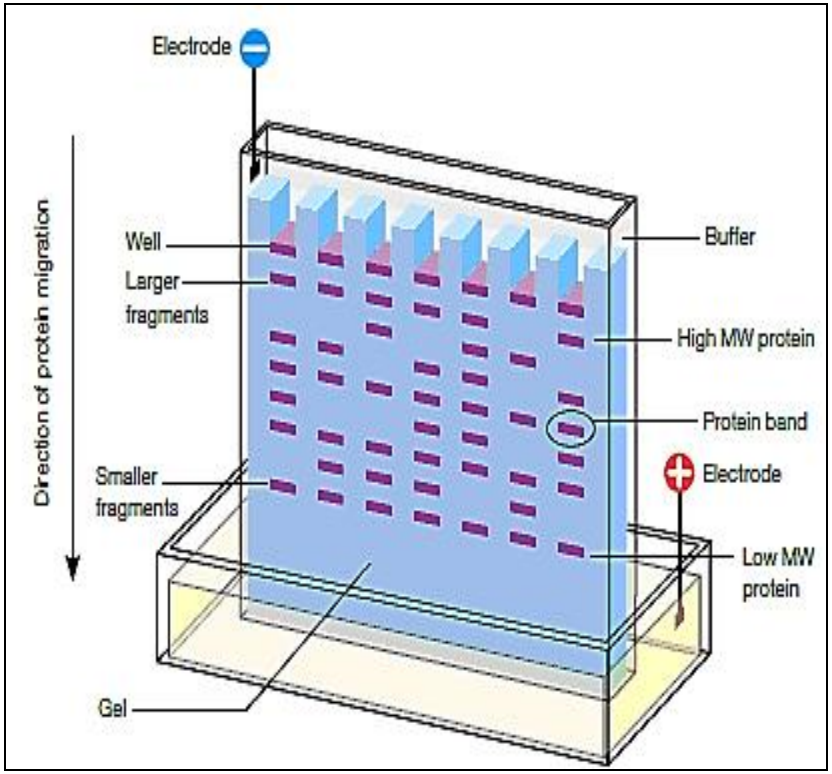
Small fragments will move faster than the large ones

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



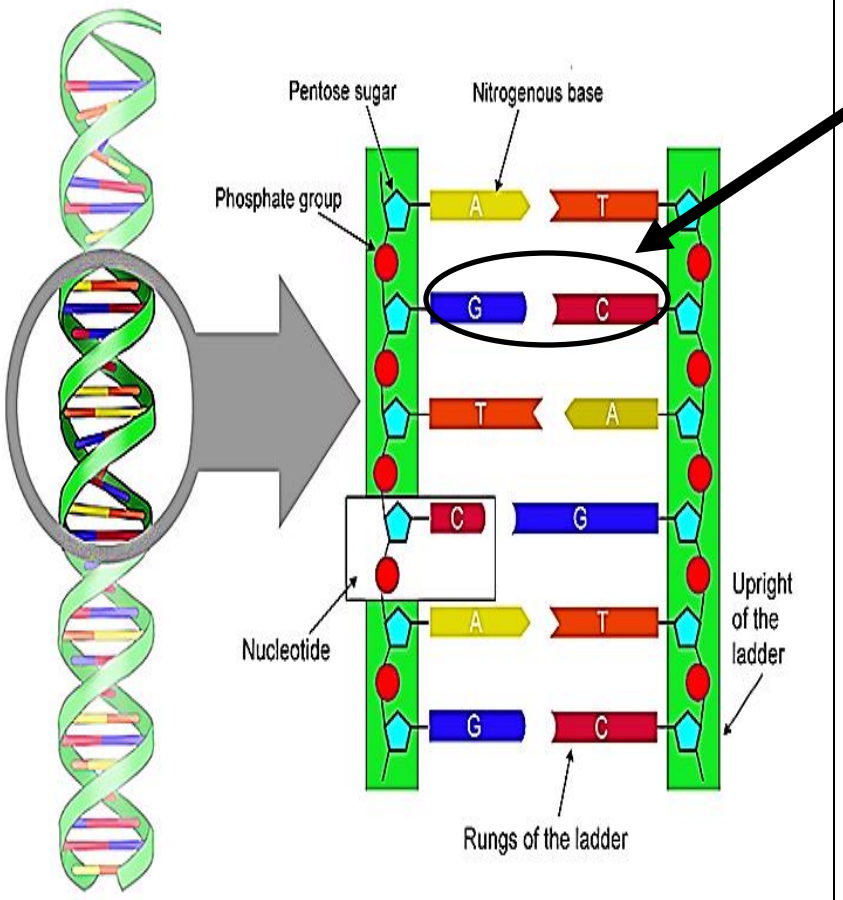
DNA Ladder: DNA fragments of known lengths used to estimate the size of unknown DNA molecule





Protein electrophoresis

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



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

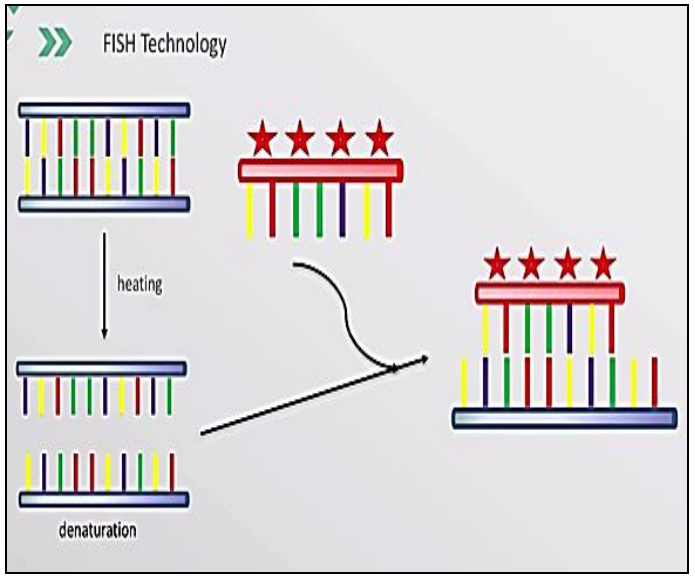
Fluorescent In situ hybridization (FISH technique):

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

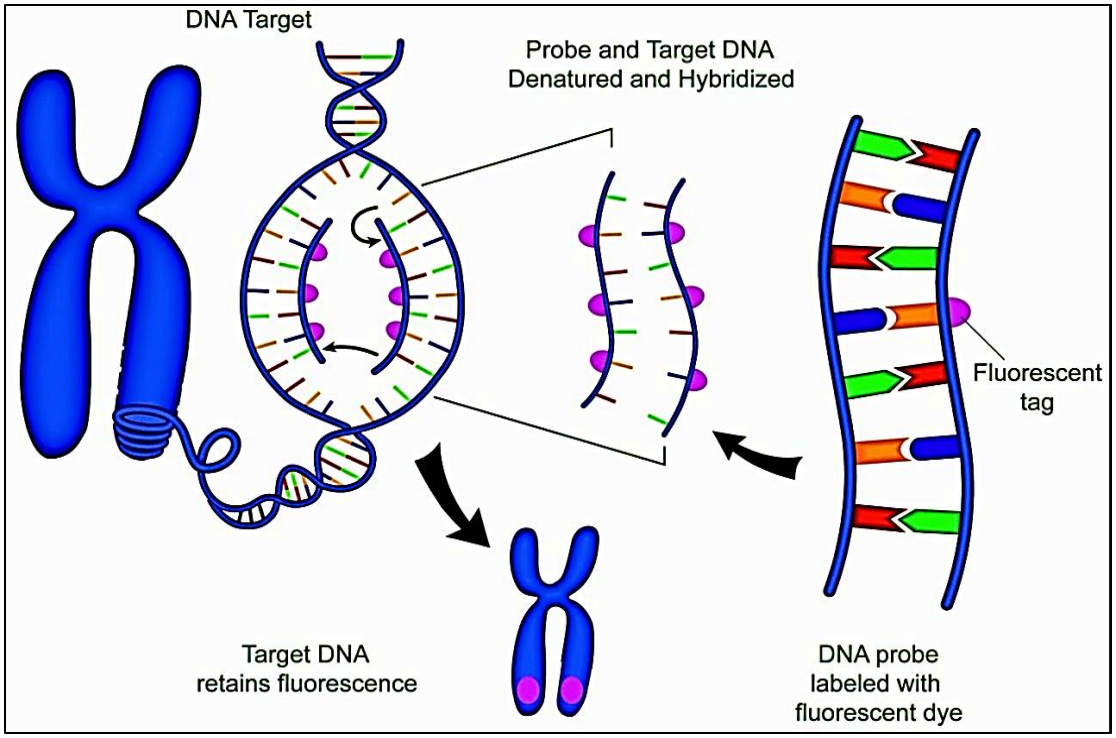
fluorescent probe:

fragment of DNA or RNA of variable length which can be radioactively labeled (probe)

It can be used in DNA or RNA samples to detect the presence or absence of nucleotide sequence that are complementary to the sequence on the probe .



Useful in detect chromosomal abnormalities


DNA Target
Probe and Target DNA
Denatured and Hybridized
Fluorescent
tag
Target DNA
retains fluorescence
DNA probe
labeled with
fluorescent dye


Fluorescence In Situ
Hybridization (FISH)
Imaging Systems
Market

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

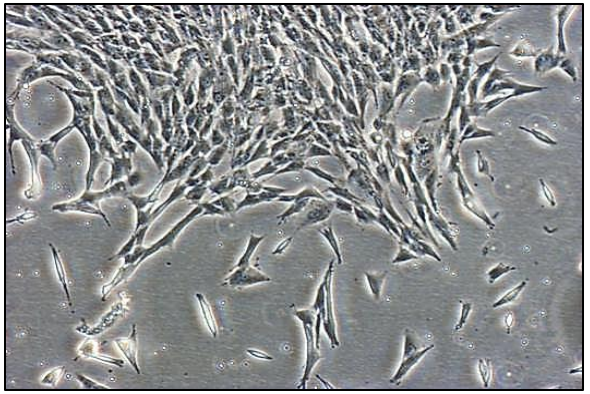
3- Used in cultivation of bacteria, viruses, in order to prepare different vaccination

4- Study the effects of new drugs

Cell culture

Cells can be isolated from the body for ***in vitro*** culture in several ways:

1- white blood cells (not RBCs) can be easily purified from blood and grown in culture.



2- Cells of tissues can be released from **tissues** by enzymatic digestion

Using enzymes such as collagenase and trypsin which break down the extracellular matrix.

Primary cultures:

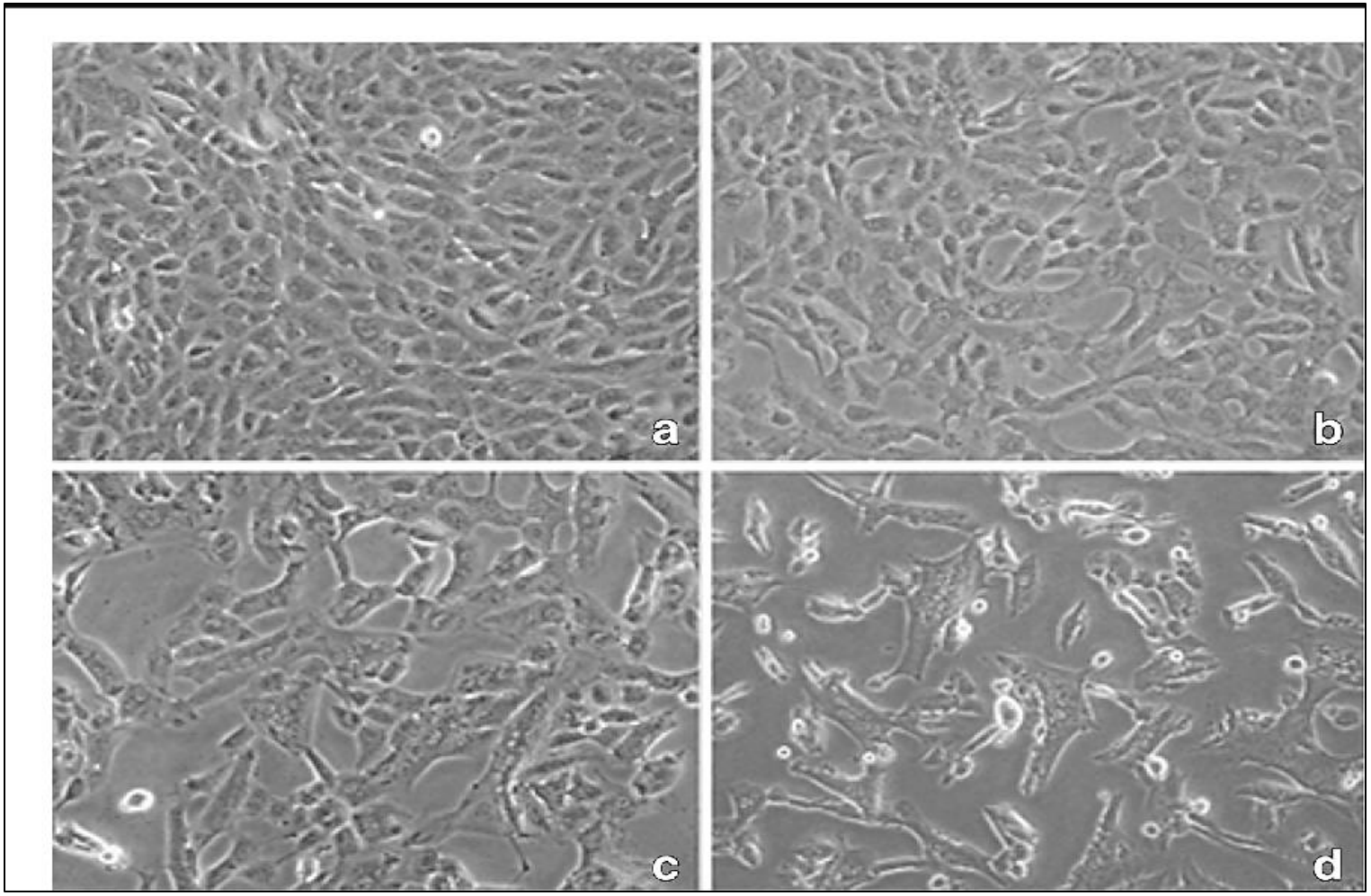
Refer to the cells that are cultured directly from a tissue (parent cells).

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:

- stage in which the cells (1ry or 2ry) become adherent to & covering most of the culture surface forming monolayer(e.g. 25%, 50%, 100%)



Different degree of confluency

cell line:

- a population of cells (clone)developed from a single cell and therefore consisting of cells with a uniform genetic make-up (phenotype & function)
- Cell lines have a limited life span, and as they are passaged

immortalized cell line

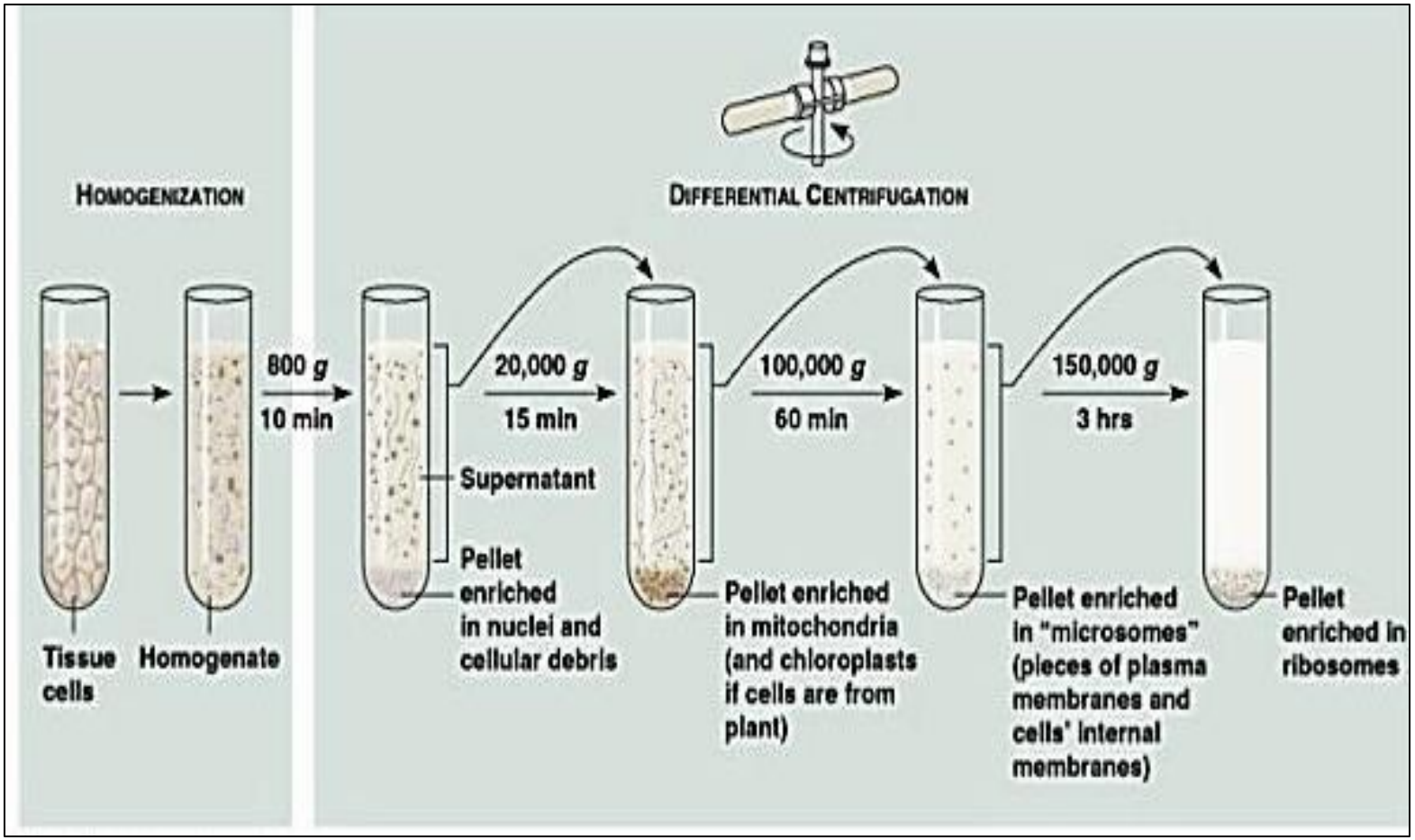
- Has acquired the ability to **proliferate indefinitely.**
- It is obtained from subcultures of the primary culture
- Normal immortalized cell line: stem cells
- Abnormal immortalized cell lines : cancer cells

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