➢ Techniques used in Cell Biology

Cell Biology: The branch of biology which deals with the study of cell structure and function is called cell biology or cytology.

Microscopy: Micro-small and scope to look at.

It is a technique used to study small sized particles which can't be seen with naked eye. It is done with the help of instrument called microscope.

Types of microscopes

1. **Simple Microscope:** It is similar to magnifying glass which has only one convex lens.
2. **Compound light Microscope:** It is a type of microscope which uses a beam of light to illuminate objects. It has two lenses i.e. Eye piece and objective lens.
3. **Electron Microscope:** A type of microscope which uses a beam of electrons.

Types of Electron Microscope

1. **Transmission Electron Microscope (TEM):** It is used to study internal parts of Cell/object. A very small object is placed in vacuum section of TEM. Only dead cell can be studied as there is vacuum.
2. **Scanning Electron Microscope (SEM):** It is used to study 3-dimensional structure of an object. With SEM, we can view and take photograph of an object.

Resolution: The ability to distinguish between two closely placed objects/points is called resolution.

Magnification: The power of microscope to enlarge the actual size of an object is called magnification.

The compound light microscope uses two magnifying convex lenses.

1. Objective lens 2. Eye piece (lens)

Objective lens magnifies the object according to its power then this magnified image is further magnified by eye piece. Therefore total magnification of microscope is equal to product of powers of objective lens and eye piece.

i.e. Magnification power = Eye piece × Objective lens

40X × 20X = 800X

Microscope	Magnification	Resolution
Compound microscope	10000X	250nm
TEM	1000000X	0.14nm
SEM	300000X	2.3nm

Resolution power of compound microscope is 500X that of human eye. Resolution power of human eye is 1mm.

➢ Staining

Stain means dye or colour. The process of treating tissues with stain is called staining. Most of the biological substances are transparent in structure. For better visibility and understanding different stains are used to colour the tissues.

Vital Stains: Those stains which are used in low concentration are non-toxic are called vital stains e.g. methylene blue and neutral red (safranin). When two or more stains are used, the second one is known as counter stain.

Types of staining on basis of number of stains used:

1. **Simple Stain:** When just one stain is used during staining it is known as simple stain.
2. **Differential stain/counter stain:** When two stains are used simultaneously in the same process the second stain is called counter stain.
3. **Multiple stains:** When three or more stains are used simultaneously is called multiple stains.

Basic types

1. **Temporary stain:** A stain which can be removed by treating tissues with organic solvent is known as temporary stains.
2. **Permanent stain:** Stain which cannot be removed from tissues are called permanent stains.

Profile/list of Stains

Permanent stains		
Stains	**Final colour**	**Suitable for**
Aniline blue	Blue	Fungal hyphae and spores
Borax carmine	Pink	Nuclei, Obelia colony
Eosin	Pink/Red	Cytoplasm/Cellulose
Feulgen's stain	Red/Purple	Chromosomes
Leishman's stain	Red/Pink	Blood cells
Methylene blue	Red/Purple	Nuclei/lignin and plant tissues
Temporary stains		
Aniline sulphate	Yellow	Lignin
Iodine solution	Blue-black	Starch
Schulz's solution(chlorine-zinc-iodine)	Yellow/blue or violet	Lignin/cutin/proteins/starch/cellulose

➢ Cell Fractionation: Fraction means part/component.

It is a modern technique used to separate various components of cell.
Steps involved in cell fractionation:

1. Grinding of tissues
2. Homogenate formation
3. Centrifugation

1. **Grinding of tissues:** First the tissues are added liquid nitrogen (-196^{0}C), then grinded with the help of glass rod.
2. **Homogenate formation:** Formation of similar size particles is called homogenization. It is done with a physical method i.e. osmotic shock or liquid with proper osmotic pressure.
3. **Centrifugation:** The process through which components of cell are separated in centrifuge is called centrifugation. In this process the homogeneous mixture is kept in centrifuge and spun with different speed.

Speed I: 800g for 10 minutes

At these low speed large size particles e.g. nucleus, cell wall and unbroken cell separate in the form of pellet. Pellet: The material which precipitates down at the bottom is called pellet. Supernatant: The material still remains in solution is called supernatant.

Speed II: 20,000g for 15 minutes

When the supernatant is spun with the above medium speed, the pellet contains medium sized particles e.g. mitochondria and chloroplast.

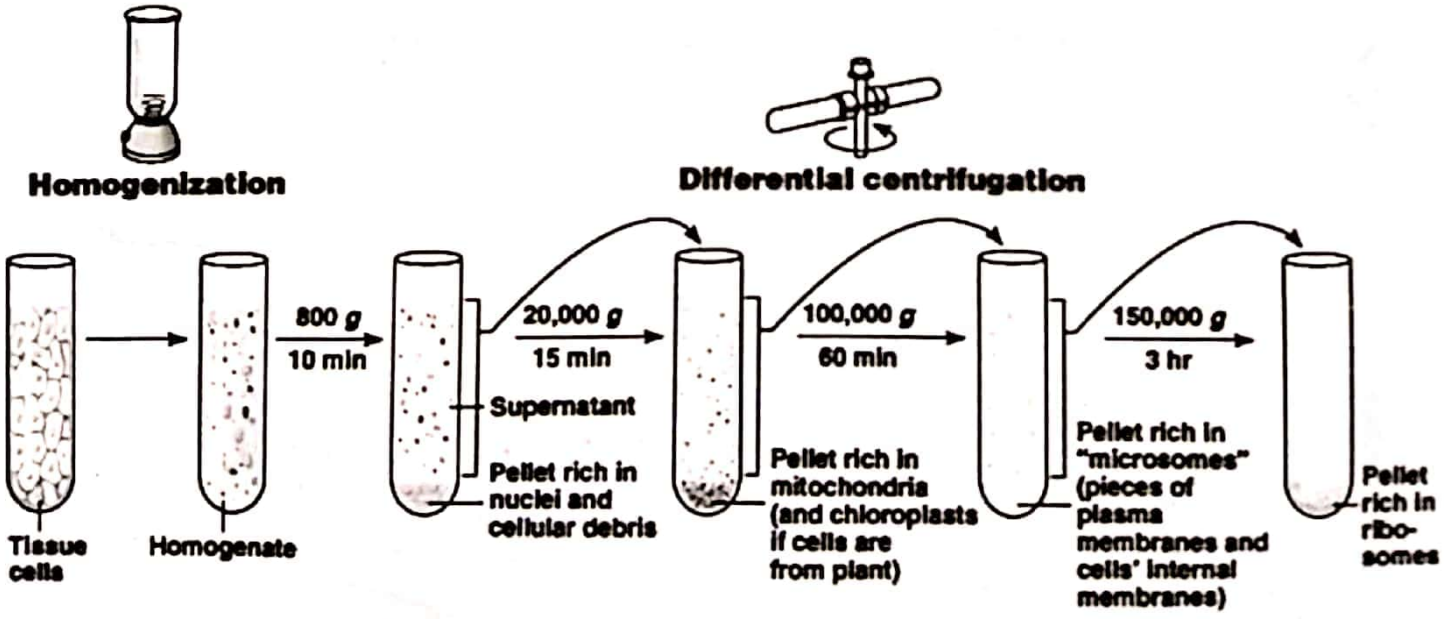
Speed III: 100,000g for 60 minutes

Next the supernatant is spun at the above high speed, the pellet is rich in small size particles e.g. microsomes, pieces of plasma membrane and cell internal membranes.

Speed IV: 150,000g for 3 hrs

Next the supernatant is spun at the above highest speed, the pellet is rich in smallest size particles e.g. ribosomes.

What is g? g stands for gravity.



Isolation of different cell organelles by differential centrifugation

Tissue Culture

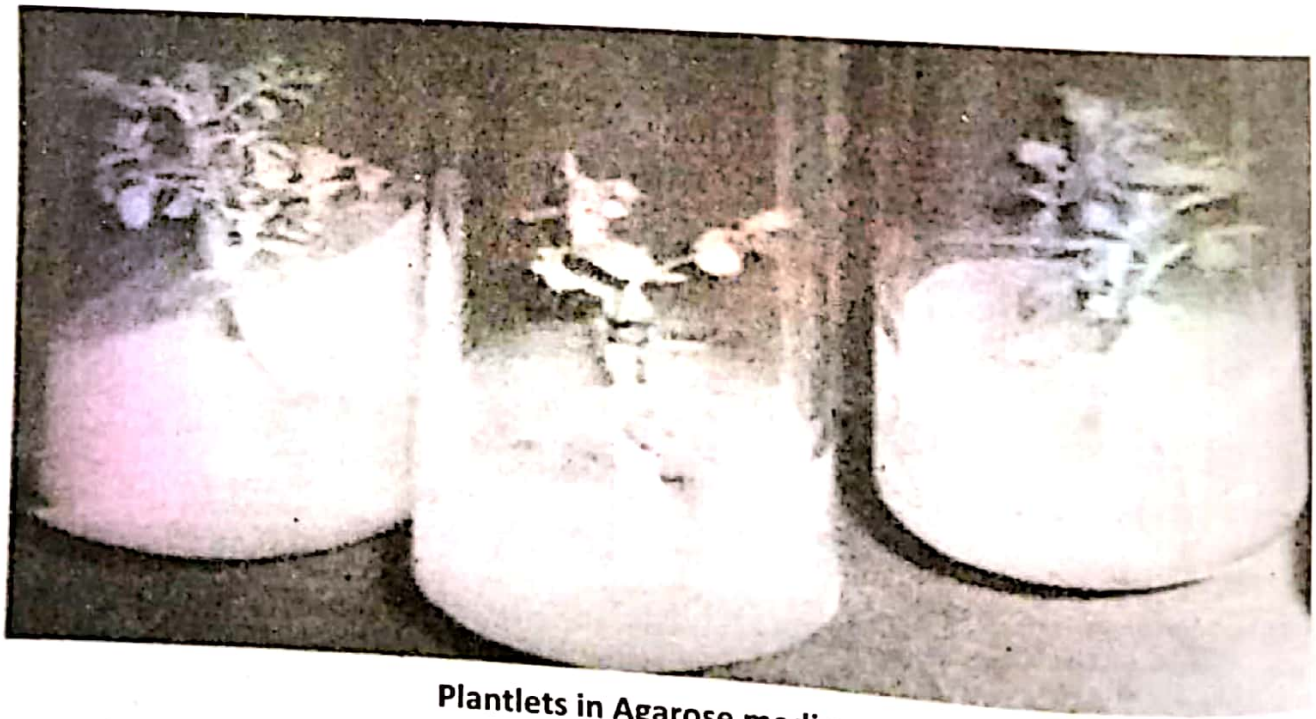
Definition: It is a technique which refers to the in-vitro growth of cells, tissues and organs on nutrient medium in aseptic condition.

Tissue culture is carried out on nutrient medium which may be liquid (broth) or semi-solid (agar). Liquid medium contains nutrients for growth of tissue in liquid form which is called broth. Semi-solid: Liquid medium is made viscous by the addition of agar. Agar is gelatinous substance obtained from red algae.

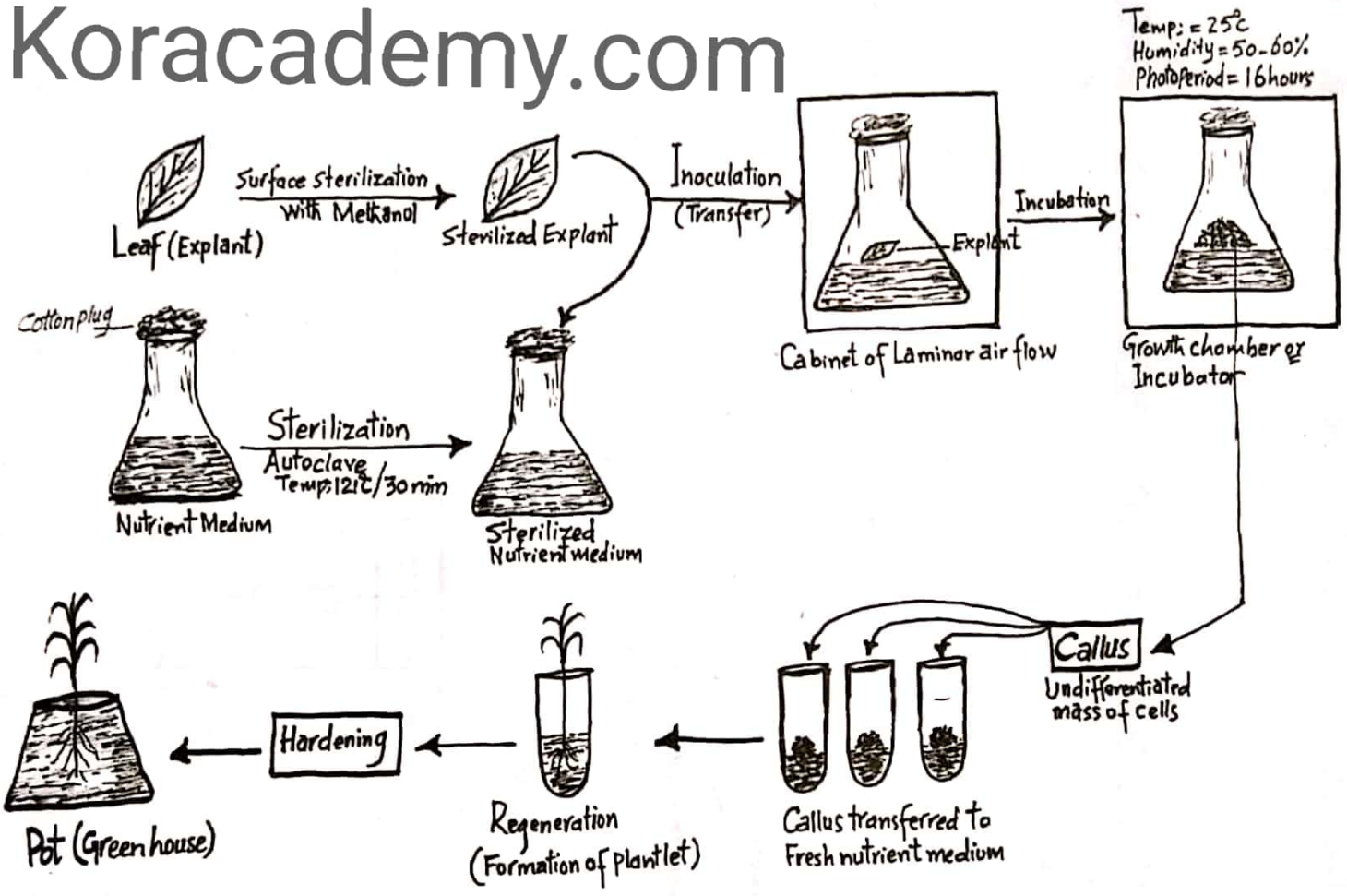
Procedure

The steps involved in the regeneration of a complete plant from an explant are given below:

1. **Preparation of suitable nutrient medium:** Suitable nutrient medium is prepared according to the need of tissue culture.
2. **Sterilization of nutrient medium:** Nutrient medium is sterilized at 120^0C for 30 minutes in autoclave (sterilizer).
3. **Selection of explant:** Any excised plant part to be used in tissue culture is called explant. (Explant may be shoot tip, root tip, embryo, endosperm or anther etc).
4. **Sterilization of explant:** Explant is surface sterilized with disinfectants e.g. methanol or ethanol.
5. **Inoculation (Transfer):** Explant is then transferred on the surface of nutrient medium in biosafety cabinet.
6. **Incubation:** The flask containing explant is transferred to incubator /growth chamber at 25°Cwith 60% humidity for photoperiod of 16 hrs. The mass of cells is developed after a definite period called Callus.
7. **Formation of plantlets:** Now callus is transferred to another nutrient medium which contain proper amount of hormones i.e. auxin and cytokinin. High auxin and low cytokinin concentration lead to roots induction. High cytokinin and low auxin concentration lead to shoot induction.
8. **Hardening:** The process in which plant is gradually exposed to environmental conditions for adjustment is called hardening.
9. **Plantlets/Seedling transfer:** When plantlets are hardened, they are transferred to suitable area like green house or field or pot.



Plantlets in Agarose medium



Sketch of the process of Tissue culture

➢ Advantage of tissue culture

1. This technique is used to produce organisms of valuable characteristics free from fungal or bacterial infections.
2. It acts as potential system for storage of templates.
3. It produces identical diploid clones of plant species unless affected by mutation during culture.
4. It helps to continue multiplication of desired plants species throughout the year irrespective of season.

➢ Chromatography (Chrome = colour, graphein = to write)

Chromatography is a technique used to separate various components of a mixture between two phases.

i. Stationary phase
ii. Mobile phase

i. Stationary Phase: The phase which does not move during the process is called stationary phase. Cellulose or silica contains water molecules act as stationary phase and cellulose and silica are just supporting material. Stationary phase may be liquid or solid.

ii. Mobile Phase:It is usually move during the process. It may be gaseous or liquid. Movement of substances depends on mass or density and charge / polarity.

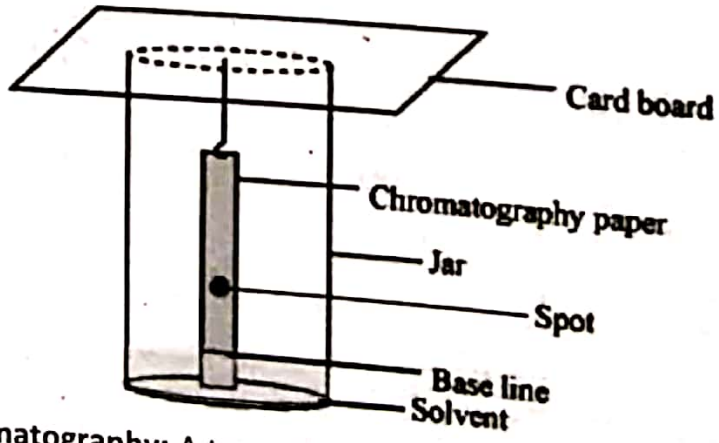
There are two types of chromatography.

1. Paper Chromatography
2. Column chromatography.

1. Paper chromatography

A type of chromatography in which filter paper is used. The mixture is spotted near one end of the filter paper, then dipped in a beaker containing organic solvent e.g. acetone etc. acetone move upward and separate the molecules through capillary action.

For example: Photosynthetic pigments, sugars, and amino acids are dissolved in suitable solvent (acetone) which run though filter paper (S.phase) with different speed through capillary action.



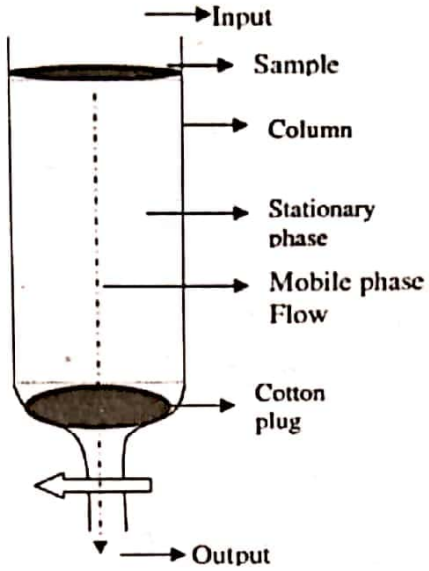
2. Column chromatography: A type of chromatography is which column / tube is used is called column chromatography.

Procedures: This tube is filled with silica which acts as stationary phase. Now solution is transferred to the tube, this solution cannot move through solid or semi-solid silica (S. Phase). So gas or liquid is used to increase the pressure and push the solution through silica.

Gas Chromatograph: When gas is used to increase the pressure and push the solution through silica it is called gas chromatography.

High Performance Liquid Chromatography (HPLC): When liquid is used to compel the solution to pass through silica it is called HPLC.

Column chromatography



Applications:

i. To determine the presence of cocaine in urine.

ii. To determine the presence of alcohol in blood.

iii. To determine the presence of lead in water.

➢ Electrophoresis: (Electro means charge and phoresis means being carried)

The technique used to separate charged molecules under the influence of an electric field at definite pH is called electrophoresis. Under the influence of electric field +vely charged particles will move towards cathode and –vely charged particles will move towards anode.

Separation of molecules depends on the following factors:

i. Amount of charge: Greater the amount of charge on particle, faster will be the moment of particle towards electrode and vice verse.

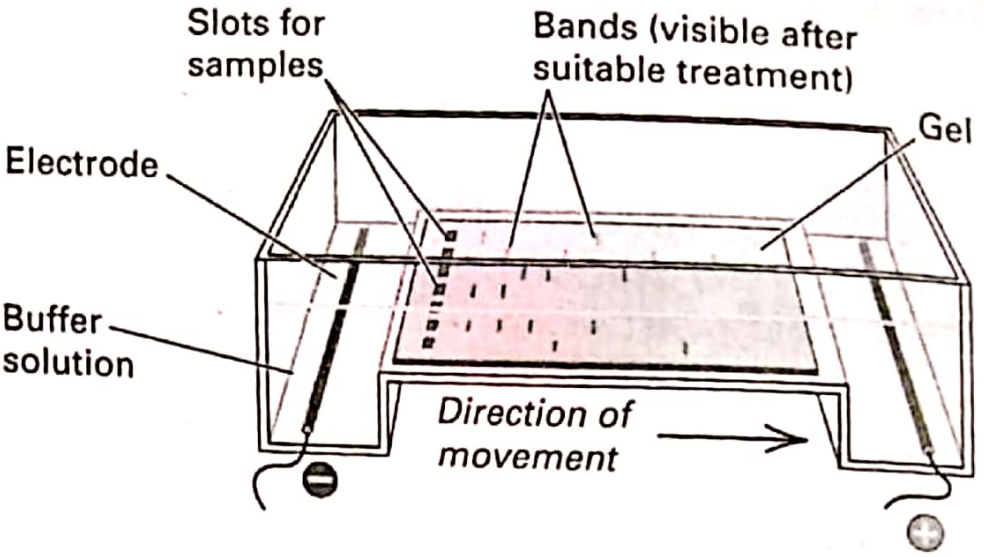
ii. Mass / Size: Smaller the molecules, faster will be the movement and vice versa.

iii. pH of the medium: Separation of molecules depends on suitable pH of the medium.

iv. Strength of electric field: Greater the strength of electric field faster will be the movement and vice versa.

Gel electrophoresis is a type of electrophoresis. Further Gel electrophoresis is of two types:

1. Agarose gel electrophoresis: It is used for the separation of DNA and RNA.
2. SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis): It is used for the separation of proteins.

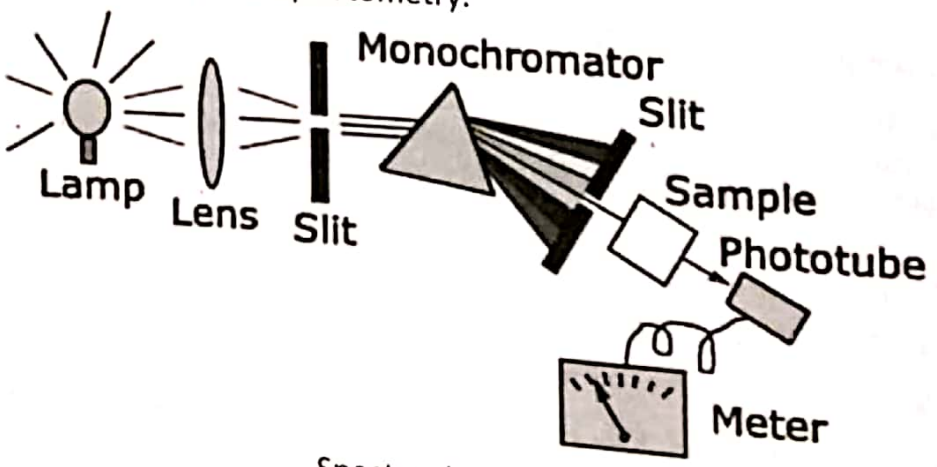


Agarose gel electrophoresis of DNA

Spectrophotometry

The technique used to measure different components of a mixture by the absorption of different wavelength of lights OR measurement of turbidity (cloudiness) of suspension is called spectrophotometry.

Explanation: More the amount of cells in suspension, the greater will be the turbidity. Cell mass of suspension is directly proportional to its turbidity/optical density. Turbidity (Cellmass) is directly proportional to absorption and inversely proportional to transmission. It is measured on the basis of optical density. The ability to absorb, transmit and reflect light is called optical density. Sugars, amino acids and pigments can be measured by spectrophotometry.



Spectrophotometer

Microdissection

A technique which involves to cut/dissect cells or its organelles under microscope is called micro dissection. The following techniques are used in microdissection:

i. **Chromosome microdissetion:** It involves the use of fine glass needle to remove a portion from chromosome under microscope.

ii. **Laser microdissetion:** It involves the use of laser light to cut the cell under microscope.

iii. **Laser capture microdissetion:** It involves the use of laser under microscope to cut the cells from tissue and adhere/attach with a photographic film.

➢ Micrometry

Micrometry is the measurement of actual size of an object using micrometer under microscope is called micrometry.

Types of micrometer: 1. Stage micrometer 2. Ocular micrometer

1. Stage micrometer: It is placed on stage of microscope. Its scale length is 1mm, divided into 100 divisions. Each division is equal to 0.01 mm or 10µm. Its each division appears small under low power of microscope while it appears large under high power of microscope.

2. Ocular micro meter: It is also called eye piece micrometer which is placed in ocular lens. It has scale of many divisions. Its division distance can be calculated as follow:

1. Place ocular micrometer in eye piece.
2. Mount/place stage micrometer on stage and bring its scale under the microscopic field.
3. Adjust the both scales in such a way that the lines of Ocular micrometer superimpose on the lines of stage. Adjust in such a way that there lines coincide one another.
4. The number of divisions on O.M equal to number of divisions on S.M are to be counted.

   The distance of 1 division of S.M is already known and distance of 1 division of O.M can be calculated as follow:

   ➢ **For low power of objective:**

   Let 50 divisions of O.M are equal to 25 division of S.M.

   50 divisions of O.M = 25 divisions of S.M.

   As 1 division of S.M = 10µm

   So 25 divisions on S.M. = 25 x 10µm = 250µm

   Now 1 divisions on O.M = $\frac{250}{50}$ µm

   = 5 µm

   Thus each division on O.M is 5µm for low power objective.

   ➢ **For medium power objective**

   Let 50 divisions on O.M = 10 divisions on S.M

   As 1 divisions on S.M = 10µm

   So 10 divisions on S.M = 10 x 10µm = 100 µm

   Now 50 divisions on O.M = 100µm

   So 1 division on O.M = $\frac{100}{50}$ = 2µm

   So 1 division on O.M = 2µm

   ➢ **For high power objective:**

   100 divisions on O.M = 10 Divisions on S.M

   10 divisions on S.M = 10x10µm = 100 µm

   100 divisions on O.M = 100 µm

   1 Divisions on O.M = $\frac{100\mu m}{100}$ = 1 µm

5. Now remove the S.M and place object on stage, the size of which is to be measured.

Suppose if nucleus of cell covers 2 divisions on high power objective then it size is 2x1 µm = 2 µm.