

ULTRA CENTIFUGATION

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CENTRIFUGATION

A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed. In biology, the particles are usually cells, sub cellular organelles, viruses, large molecules such as proteins and nucleic acids.

BASIC PRINCIPLE OF CENTRIFUGATION

- A particle, whether it is precipitate, a macromolecule or cell organelle when rotated at high speed is subjected to a centrifugal force.
- Centrifugal force is defined as $F = m\omega r$
- Where, F = intensity of centrifugal force
 - m = effective mass of sedimenting particle
 - ω = angular velocity of rotation
 - r = distance of migrating particles from central axis of rotation

BASIC PRINCIPLE OF CENTRIFUGATION

- The magnitude of the induced gravitational field is measured in terms of the G value: a G value of 1000 refers to an induced field that is thousand time stronger than that due to gravity. The G value which is also referred to as the RCF (relative centrifugal force) value depends on the rotation speed as well as the manner in which the centrifuge tubes are held by the rotor.

- $G = r \omega^2 r / g$
- $\omega = 2\pi n$
- Substituting for ω
- $G = r (2\pi n)^2 / g$
- $G = r (2 * 3.14 * [n/60])^2 / 9.81$
- $G = 1.12 * 10^{-3} r n^2$
- $G = 1.12 * 10^{-3} r (\text{RPM})^2$
- Where:
- r = distance from the axis of rotation (m)
- ω = angular velocity (radians/s)
- g = acceleration due to gravity (m/s²)
- n = rotation speed, RPM

INTRODUCTION - ULTRA CENTRIFUGATION

- It is invented by Theodor Svedberg in 1923, for which he was awarded Noble prize in 1926.
- It is an important tool in biochemical research. It imposes high centrifugal force on suspended particles and separates them on the basis of difference in molecular weight.
- For example:-
 - RBC are separated from plasma or blood
 - nuclei from mitochondria
 - One protein from another complex mixture
 - Isolation of DNA, RNA and lipids

INTRODUCTION

- Its rotational speed is up to 80000 rpm.
- It create a centrifugal force up to 600000g

Types of Centrifuges & Its Uses & Characteristics

Types of Centrifuges & applications

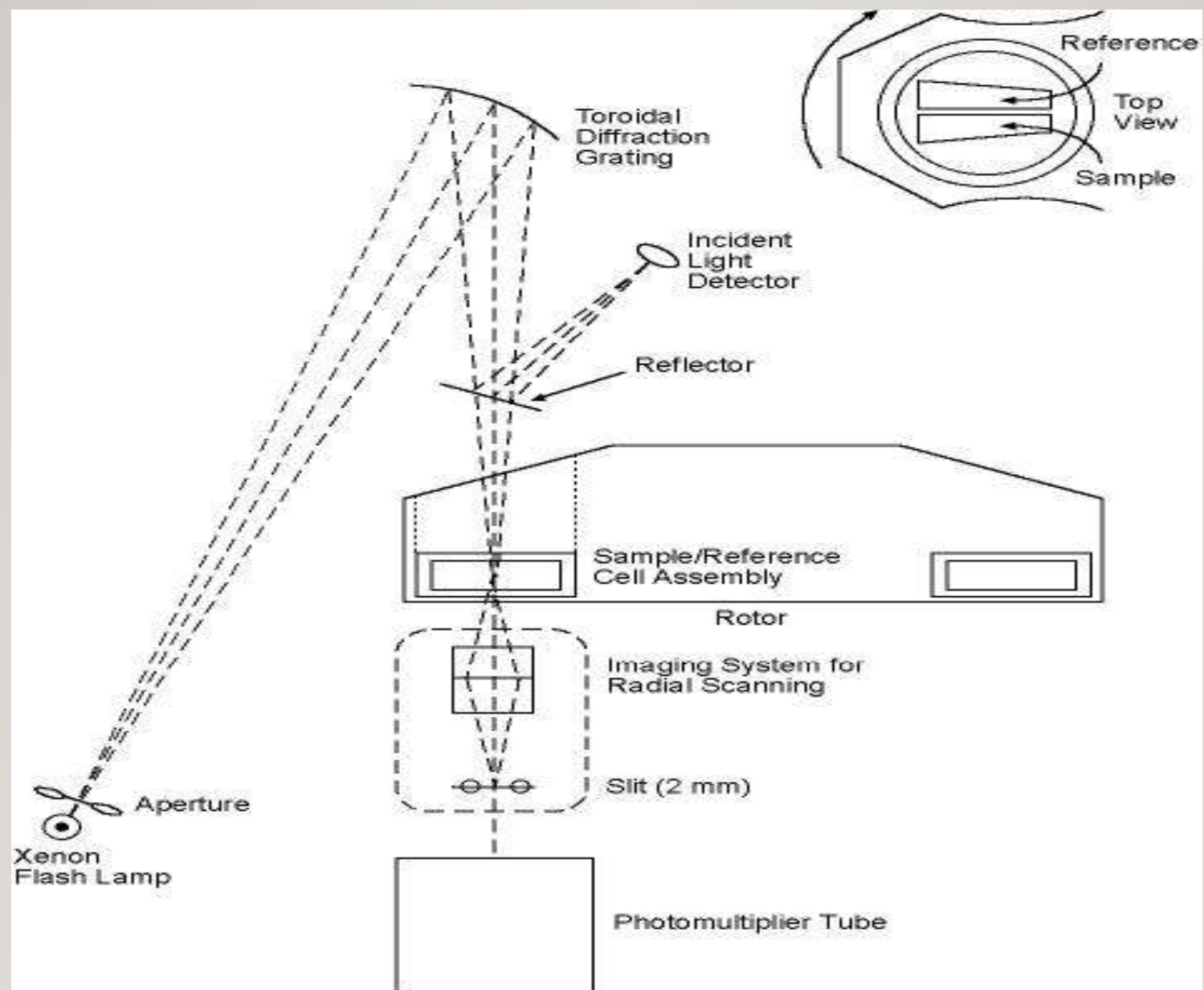
Characteristic	Types of centrifuge		
	Low Speed	High Speed	Ultracentrifuge
Range of Speed (rpm)	1-6000	1000-25,000	20-80,000
Maximum RCF (g)	6000	50,000	6,00,000
Refrigeration	some	Yes	Yes
Applications			
Pelleting of cells	Yes	Yes	Yes
Pelleting of nuclei	Yes	Yes	Yes
Pelleting of organelles	No	Yes	Yes
Pelleting of ribosomes	No	No	Yes
Pelleting of Macromolecules	No	No	Yes

TYPES OF ULTRA CENTRIFUGATION

- There are two types of ultra centrifugation:-
 - A. Analytical ultra centrifugation
 - B. Preparative ultra centrifugation

ANALYTICAL ULTRA CENTRIFUGATION

- The aim of analytical centrifuge is to study molecular interaction between macromolecules or to analyse the properties of sedimenting particles.
- The sample can be monitored in real time through a optical detection system using UV light absorption or through interference of optical refractive index sensitive system.



PREPARATIVE ULTRA CENTRIFUGATION

- The aim of preparative centrifuge is to isolate and purify particles such as sub-cellular organelles.
- These are available in wide variety of rotors.
- Most rotors are design to hold tubes that contains the samples. E.g. swinging bucket rotors and fixed angle rotors.

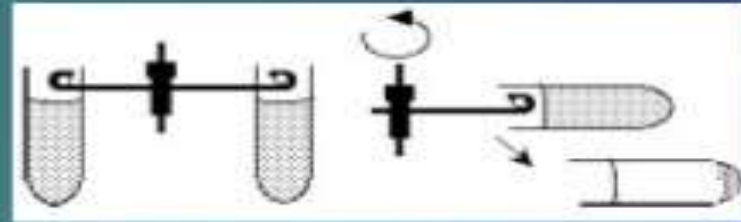
Centrifuge Rotors

- **Fixed Angle Rotor**



Sedimenting particles have only short distance to travel before pelleting. Shorter run time. The most widely used rotor type.

- **Swinging Bucket Rotor**



Longer distance of travel may allow better separation, such as in density gradient centrifugation. Easier to withdraw supernatant without disturbing pellet.

USES OF ANALYTICAL CENTRIFUGE

- There are two kind of experiments that can be performed using analytical centrifuge:-

Sedimentation velocity experiment:-

The objective of sedimentation velocity experiment to interperate the entire time-
course of sedimentation.

This determine the shape and mass of dissolved macromolecules and their size distribution.

USES OF ANALYTICAL CENTRIFUGE

Sedimentation equilibrium experiment:-

Sedimentation equilibrium experiments are design to determine the final steady state of the experiment.

USES OF PREPARATIVE CENTRIFUGE

Differential ultra Centrifugation:-

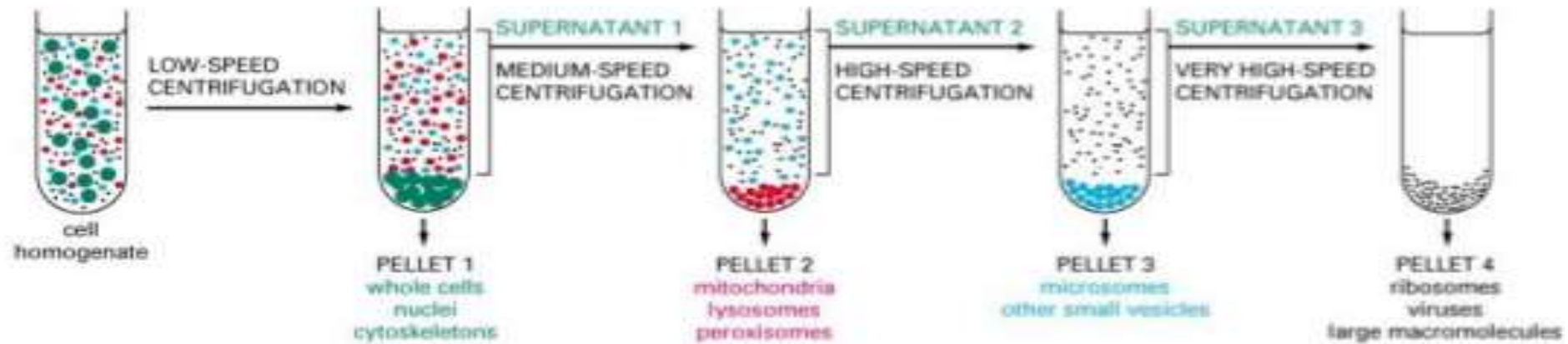
It is a common procedure in microbiology and cytology to separate organelles from whole cell for further analysis of those components.

In this process a tissue sample is first homogenized to break the cell membrane and mix-up the cell content. The homogenate is then subjected to repeated centrifugation each time removing the pellet and increasing the centrifugal force.

DIFFERENTIAL CENTRIFUGATION

Repeated centrifugation at progressively higher speeds will fractionate cell homogenates into their components.

Centrifugation separates cell components on the basis of size and density. The larger and denser components experience the greatest centrifugal force and move most rapidly. They sediment to form a pellet at the bottom of the tube, while smaller, less dense components remain in suspension above, called the supernatant.



USES OF PREPARATIVE CENTRIFUGE

Density gradient centrifugation:-

It allows separation of many or all components in a mixture and also allows for their measurement.

It divided in two categories –

1. Rate zonal
2. Isopycnic

USES OF PREPARATIVE CENTRIFUGE

Rate zonal:-

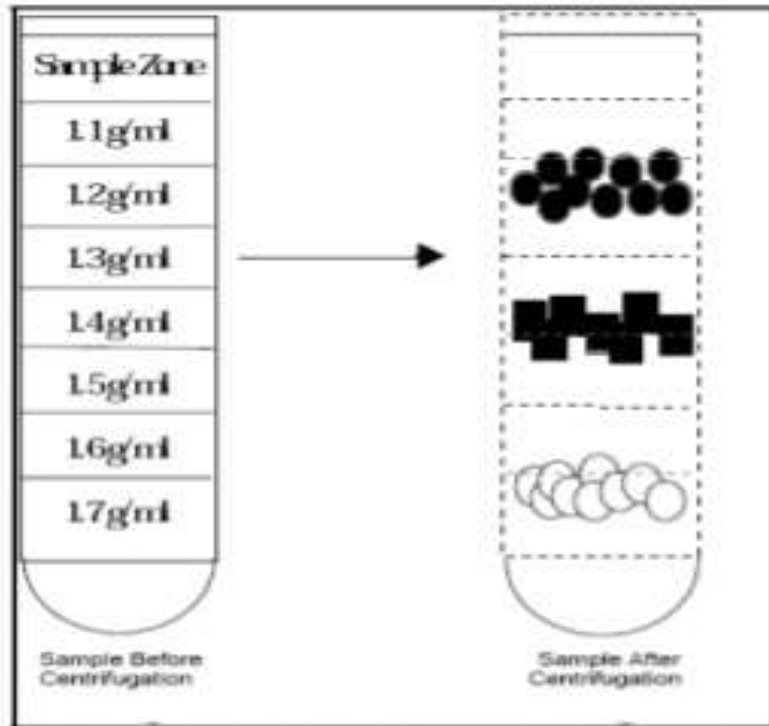
In the rate zonal the solution have density gradient and the sample has density which is greater than all the layer in solution.

The sample is applied in a thin zone at the top of the centrifuge tube.

Under centrifugal force the particles will begin to separate according to their zones (Density gradient).

The particles will sediment in separate zones according to their shape and size.

Rate zonal centrifugation



- The particles will begin sedimenting in separate zones according to their size shape and density.

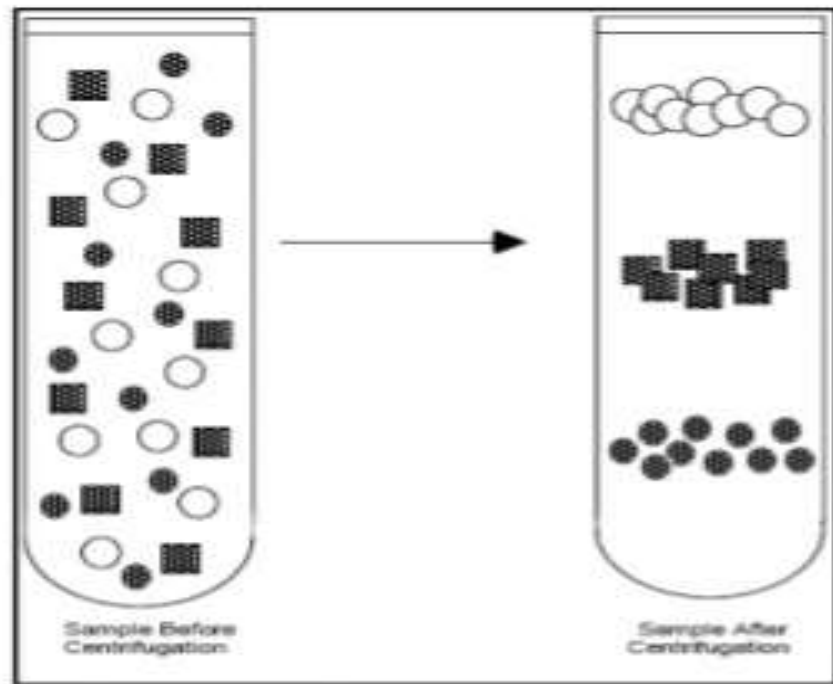
USES OF PREPARATIVE CENTRIFUGE

Isopycnic:-

Isopycnic means of the same density. In this technique molecules are separated on equilibrium position.

Each molecule floats or sink to its equivalent density position.

Isopycnic or sedimentation equilibrium centrifugation



- In Isopycnic centrifugation separation of particles occurs into zones on the basis of their density differences, independent of time.

FUNCTION OF ANALYTICAL CENTRIFUGE

It uses small sample size (less than 1mm).

In built optical system to analyse the progress of separated molecules.

It uses relatively pure samples.

It is used to determine sedimentation coefficient and molecular weight of the molecules.

FUNCTIONS OF PREPARATIVE CENTRIFUGE

Large sample size can be used.

No inbuilt optical system for read out.

Less pour sample can be used.

It can be used to determine sedimentation coefficient and molecular weight of molecules.

It is generally used to separate organelles and cell component.

Application in Water Treatment

Centrifugation

Separation of solid substances from highly concentrated suspensions

Separation of oily suspensions

Separation of oily concentrate d sludge

Separation of heavy particles and large-sized grains by cycloning

Commercial applications

- **Centrifuges with a batch weight of up to 2,200 kg per charge are used in the sugar industry to separate the sugar crystals from the mother liquor .**
- **Standalone centrifuges for drying (hand-washed) clothes – usually with a water outlet.**
- **Large industrial centrifuges are also used in the oil industry to remove solids from the drilling fluid.**



OTHER APPLICATIONS

- ◉ Separating chalk powder from water
- ◉ Removing fat from milk to produce skimmed milk
- ◉ Separating textiles
- ◉ Removing water from lettuce after washing it in a salad spinner
- ◉ Separating particles from an air-flow using cyclonic separation

REFERANCE

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THANKYOU