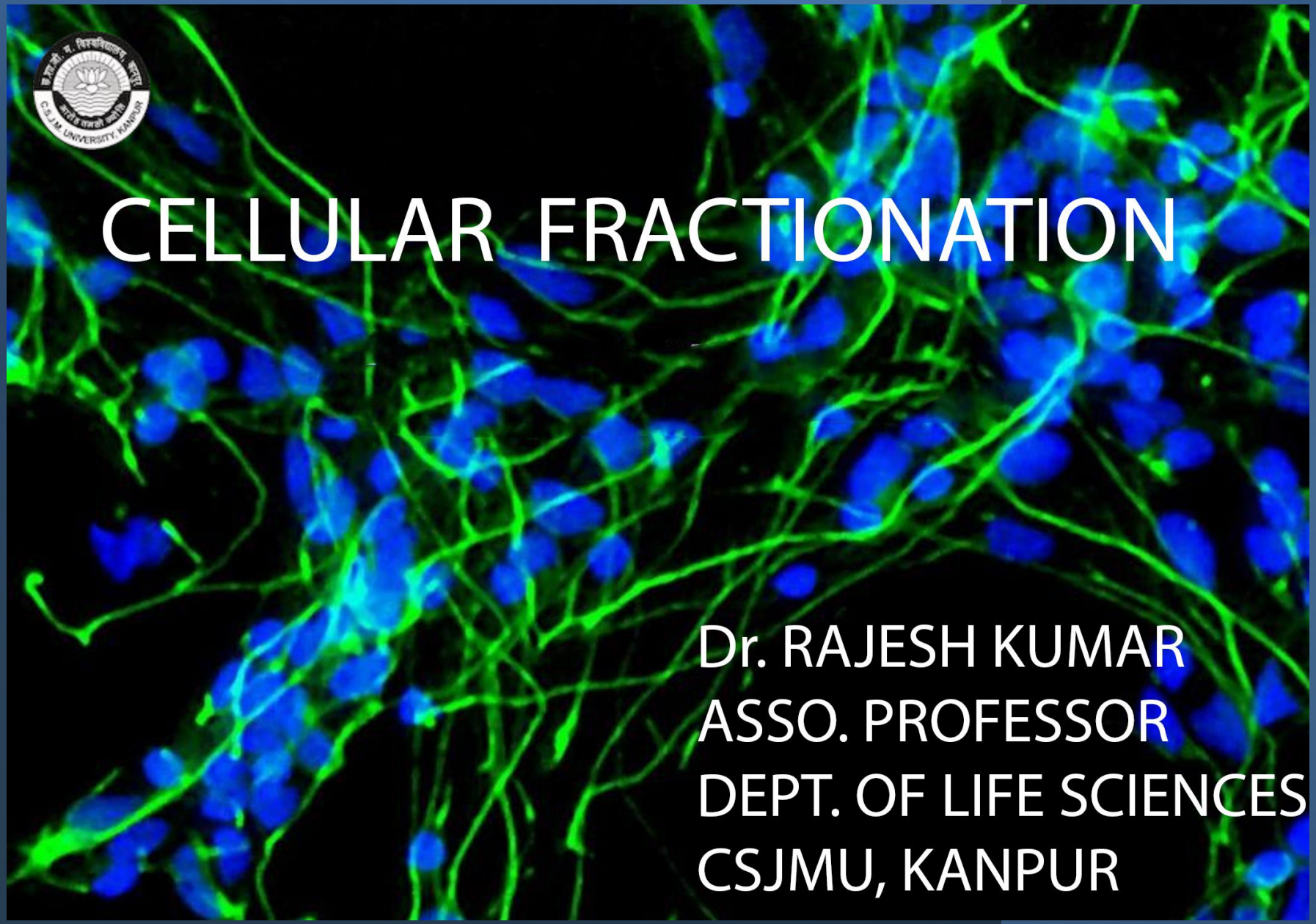

CELLULAR FRACTIONATION

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INTRODUCTION

Cellular Fractionation is a process used to separate the cellular components while preserving their individual functions of each component. It is an important step for studying a specific intracellular structure, organelle, proteins, or assess possible associations between these macromole cular structures.

Which provide various information like; buoyant density, surface change dens ity, size and shape. This is a method that was originally used to demonstrate the cellular location of various biochemical processes.

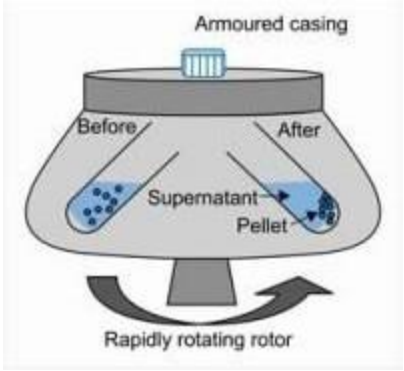
Other uses of Subcellular fractionation is to provide an enriched source of a protein for further purification, and facilitate the diagnosis of various disease states. It is a method that dissects cells into various organelle according to their shape, size, density, the viscosity of medium and rotor speed under the influence of an external applied centrifugal force.

PRINCIPLE OF CENTRIFUGATION

The principle of the centrifugation technique is to separate the particles suspended in liquid media under the influence of a centrifugal field.

Which is the process involve the principle of sedimentation. The sedimentation is a phenomenon where suspended material settles out of the fields by gravity.

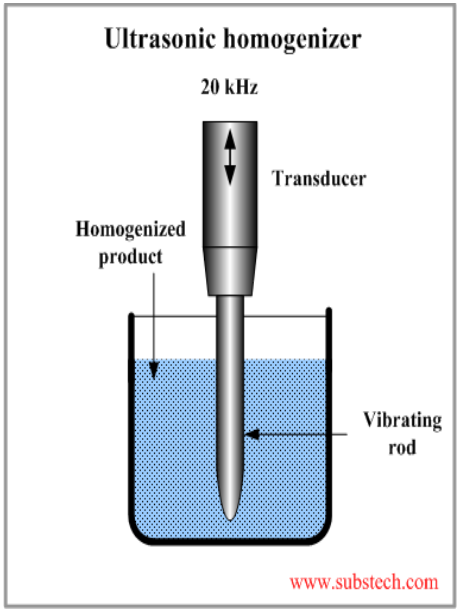
$S = v/w2.r$



Step One: Homogenization-

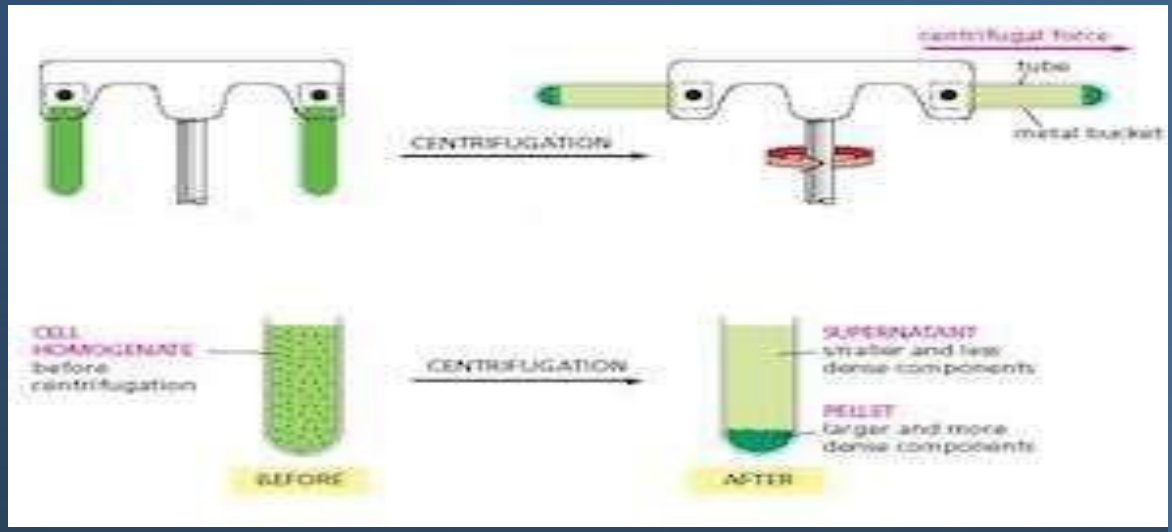
Tissue is typically homogenized in a buffer solution that is isotonic to stop osmotic damage. Mechanisms for homogenization include grinding, mincing,chopping, pressure changes, osmotic shock, freeze-thawing, and ultra-sound. The samples are then kept cold to prevent enzymatic damage.

It is the formation of homogenous mass of cells (cell homogenate or cell suspension). It involves grinding of cells in a suitable medium in the presence of certain enzymes with correct pH, ionic composition, and temperature. For example, pectinase which digests middle lamella among plant cells.



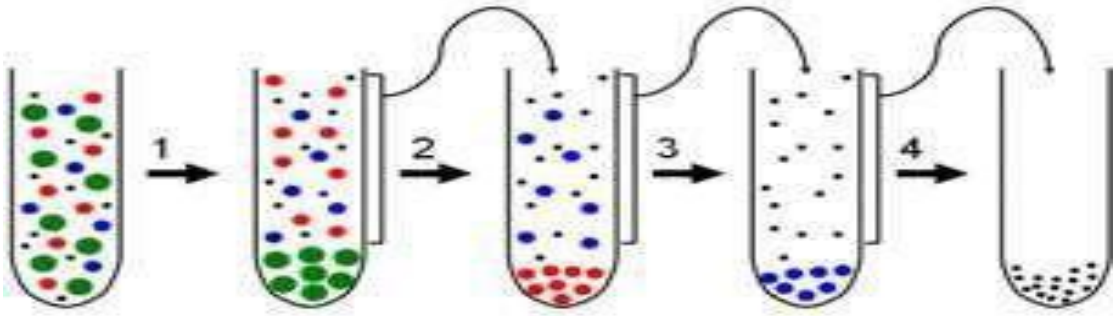
Step Two: Separation

Cell homogenates are separated into fractions by spinning them super-fast in a process called centrifugation. Centrifugation produces force that are thousands of time higher than gravity, and cellular components are pushed toward the bottom of the container. Centrifugation applies enough force to cell homogenates to allow different cellular fractions to separate based on properties such as mass, density, and shape, as shown here in the figure here. Centrifugation causes components that are too heavy to resist the force of gravity to move to the bottom of the tube.

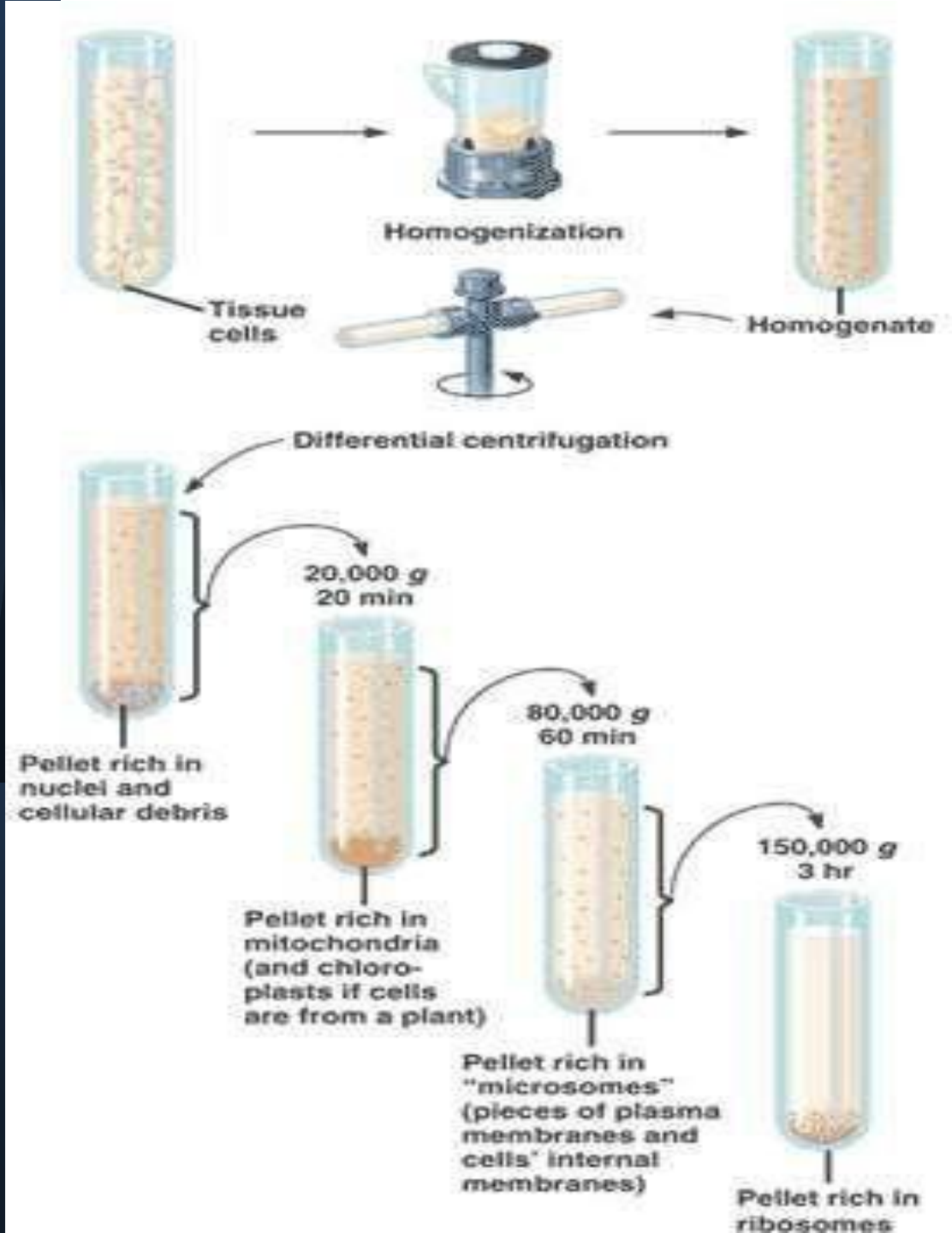

centrifugal force
tube
metal bucket
CENTRIFUGATION
CELL HOMOGENATE
before centrifugation
CENTRIFUGATION
SUPERNATANT
smaller and less dense components
PELLET
larger and more dense components
BEFORE
AFTER

Step Three: Purification

Purification is achieved by differential centrifugation– the sequential increase in gravitational force results in the sequential separation of organelles according to their density. Because the different fractions will be collected by centrifuging the sample several times.



SUMMARY OF CELL FRACTIONATION



- An enzyme that is known to be localized exclusively in the target organelle.
- Isolation of any organelle requires a reliable test for the presence of the organelle.
- Marker enzymes also provide information on the biochemical purity of the fractionated organelles. The presence of unwanted marker enzyme activity in the preparation indicates the level of contamination.

ENZYMES ARE USED AS MARKER DURING SUBCELLULAR FRACTIONATION

S No.	COMPONENT	MARKER	E.C NUMBER
1	Nuclei	DNA Polymerase RNA Polymerase	2.7.7.7 2.7.7.6
2	Plasma Membrane	5-Nucleotidase Leucine aminopeptidase	3.1.3.5 3.4.1.1
3	Endoplasmic Reticulum Cytosol	Gloucose-6-phosphodiesterase Aldolase	3.1.3.9 4.1.2.7
4	Mitochondria	Succinate dehydrogenase Cytochrome oxidase	1.3.99.1 1.9.3.1
5	Lysosome	Acid phosphatase Aryl Sulfatase-C	3.1.3.2 3.1.6.1
6	Peroxisomes	Catalase Peroxidase	1.11.1.6 1.11.1.7
7	Golgi Bodies	Sialyl transferase	2.4.99.1

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