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Programme: M.Sc., Botany

Course Title : CELL BIOLOGY AND BIOINSTRUMENTATION
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Unit – IV
BIOINSTRUMENTATION
Topic: Centrifugation

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Centrifugation

- Centrifugation is a process that involves the use of the centrifugal force for the separation of mixtures. (Sedimentation)
- Depends on density of the particle, the size of the particle and the viscosity of the medium, apart from the centrifugal force.

- Heavier particles sediment quickly due to greater gravitational force on them.
- Lighter particles do not sediment easily – higher gravitational force.
- Centrifuges are used to increase the gravitational force on particles.

- A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed.
- is an equipment, generally driven by an electric motor (some older models were spun by hand), that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis.

- The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances to separate out along the radial direction (the bottom of the tube) - lighter objects will tend to move to the top (of the tube; in the rotating picture, move to the centre).

- **Principle:**
- If a particle (mass = m kg) spins in a centrifuge (radius r) at a velocity (v, m s-1) then the centrifugal force (F) acting on the particle equals m v2/r. The same particle experiences gravitational force (G, Newton) = m g (where g = acceleration due to gravity) F = m v2/r


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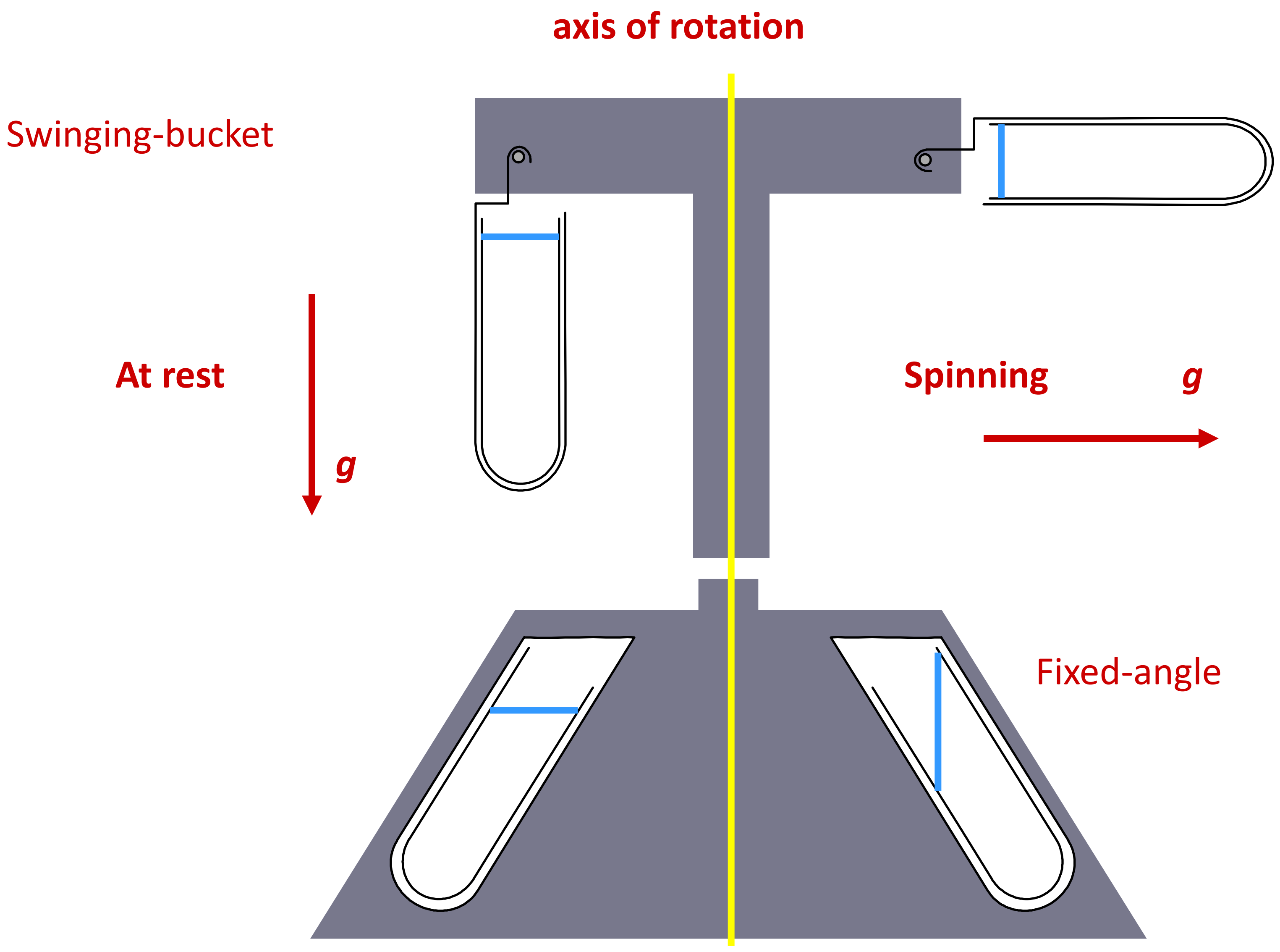
- Centrifugal force: (Isaac Newton) is the tendency of any rotating object to move away from its center of rotation.
- Therefore the centrifugal force on an object mainly depends on the mass of the object and velocity or speed of rotation.
- Centrifugal force is expressed as Relative Centrifugal force (RCF) in ‘g’ units.

Types of Rotors

- types of rotors:
- 1. Fixed angle
- 2. Swinging bucket

- Based on types by rotor design:
- Fixed-angle centrifuges are designed to hold the sample containers at a constant angle relative to the central axis.
- Centrifuge tubes containing the sample are kept at an angle 30° to the horizontal and the particles are subjected to a lateral force. When the particle strike the side of the tube, they are driven downwards and sediments.

Centrifuge rotors



- Sedimenting particles have only short distance to travel before pelleting
- Short run time
- Most widely used.

- Swinging head (or swinging bucket)
- The tubes spin horizontally and hence the particle to a constant force.
- Path length for the particle to travel is increased which is equal to the depth of the suspension.
- Therefore sedimentation time is increased with type of rotors. Longer distance of travel – better separation

Types of centrifuges

- Based on Speed:
- Low speed centrifuge (maximum 5000 rpm)
- High speed centrifuge (Max. 20,000 rpm)
- Ultracentrifuge (Max. 1,00,000rpm)

- Basically centrifuges have a rotor chamber (where the rotor is rotated around a fixed central axis with the help of an electric motor) and a control units to control the speed and temperature.
- Start by applying power and stopped by shutting off the power.

- High speed centrifuges include a timer, a dynamic brake and a calibrated speed control.
- At high speed the friction of the rotor with the air present in the rotor chamber produces considerable heat.
- Therefore rotor chamber is refrigerated.

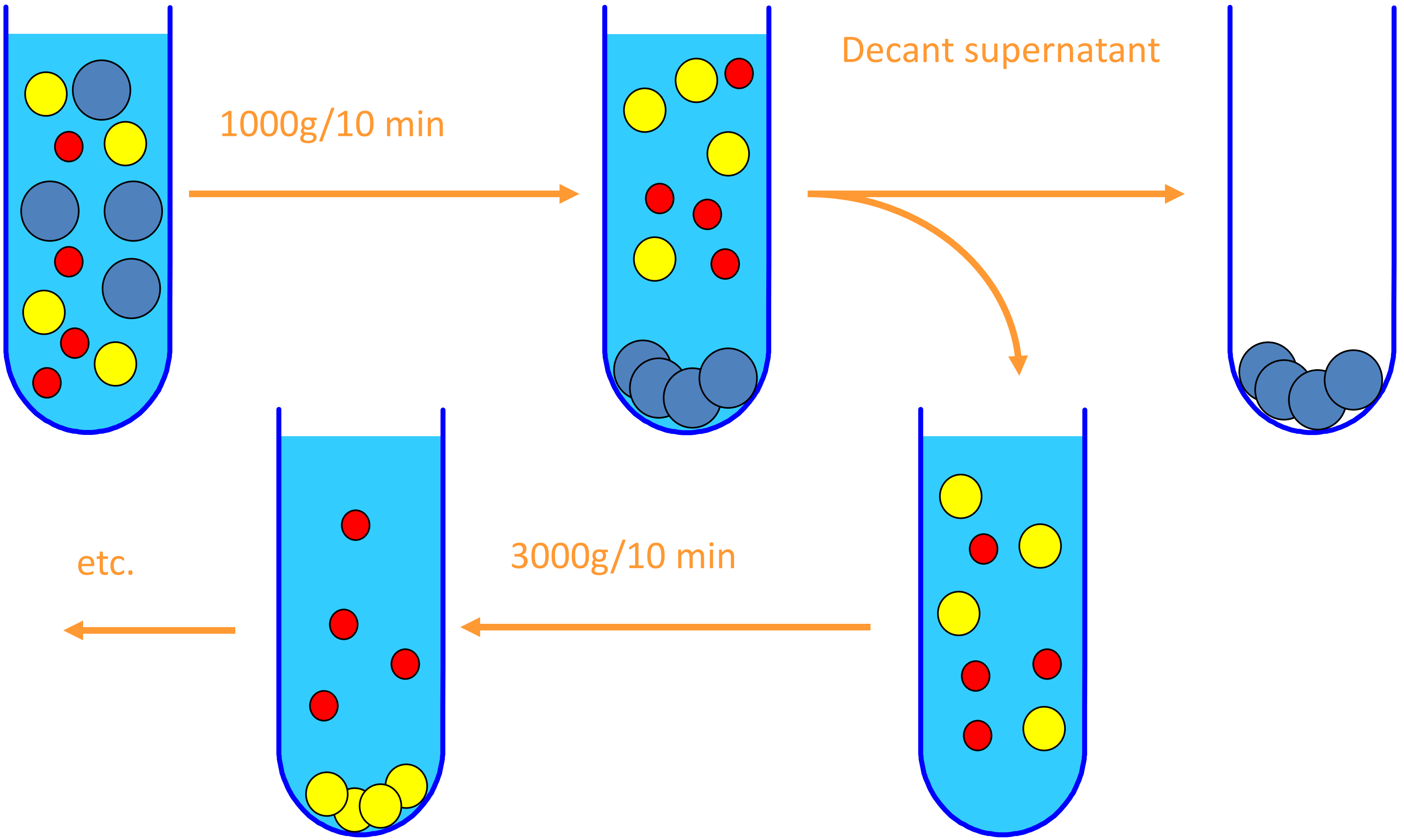
- Ultracentrifuges are more elaborate and are operated not only under refrigeration but also under vacuum to reduce friction.
- Two types: Analytical, Preparative
- Analytical: Properties of sedimenting particles such as their apparent molecular weight.
- Preparative: isolate and purify specific particles such as subcellular organelles.

- Types based on intended Use:
- Differential centrifugation: makes use of different speeds to effect sedimentation of different particles, step by step) and
- Density gradient centrifugation: makes use of different densities of the particles at same speed (two types)

Differential Centrifugation

- Centrifuge tube filled with a homogenous solution and centrifuged at required rpm for required time and temperature.
- Results in two fractions, a pellet at the bottom of the tube containing the sedimented material at that speed and a supernatant solution containing unsedimented material.
- **Rate of particle sedimentation depends mainly on its size and the applied *g*-force**

Differential centrifugation of a tissue homogenate (I)



- Two fractions are recovered by decanting the supernatant solution into a fresh tube and leaving the pellet in the tube.
- Supernatant can be re-centrifuged at a higher speed to effect further sedimentation of lighter particles with formation of new pellet
- And so on.

Size of major cell organelles

• Nucleus	4-12 μm
• Plasma membrane sheets	3-20 μm
• Golgi tubules	1-2 μm
• Mitochondria	0.4-2.5 μm
• Lysosomes/peroxisomes	0.4-0.8 μm
• Microsomal vesicles	0.05-0.3μm

Differential centrifugation of a tissue homogenate (II)

1. Homogenate – 1000g for 10 min
2. Supernatant from 1 – 3000g for 10 min
3. Supernatant from 2 – 15,000g for 15 min
4. Supernatant from 3 – 100,000g for 45 min

- Pellet 1 – nuclear
- Pellet 2 – “heavy” mitochondrial
- Pellet 3 – “light” mitochondrial
- Pellet 4 – microsomal

Differential centrifugation (IV)

- Poor resolution and recovery because of:
- Particle size heterogeneity
- Particles starting out at r_{min} have furthest to travel but initially experience lowest *RCF*
- Smaller particles close to r_{max} have only a short distance to travel and experience the highest *RCF*

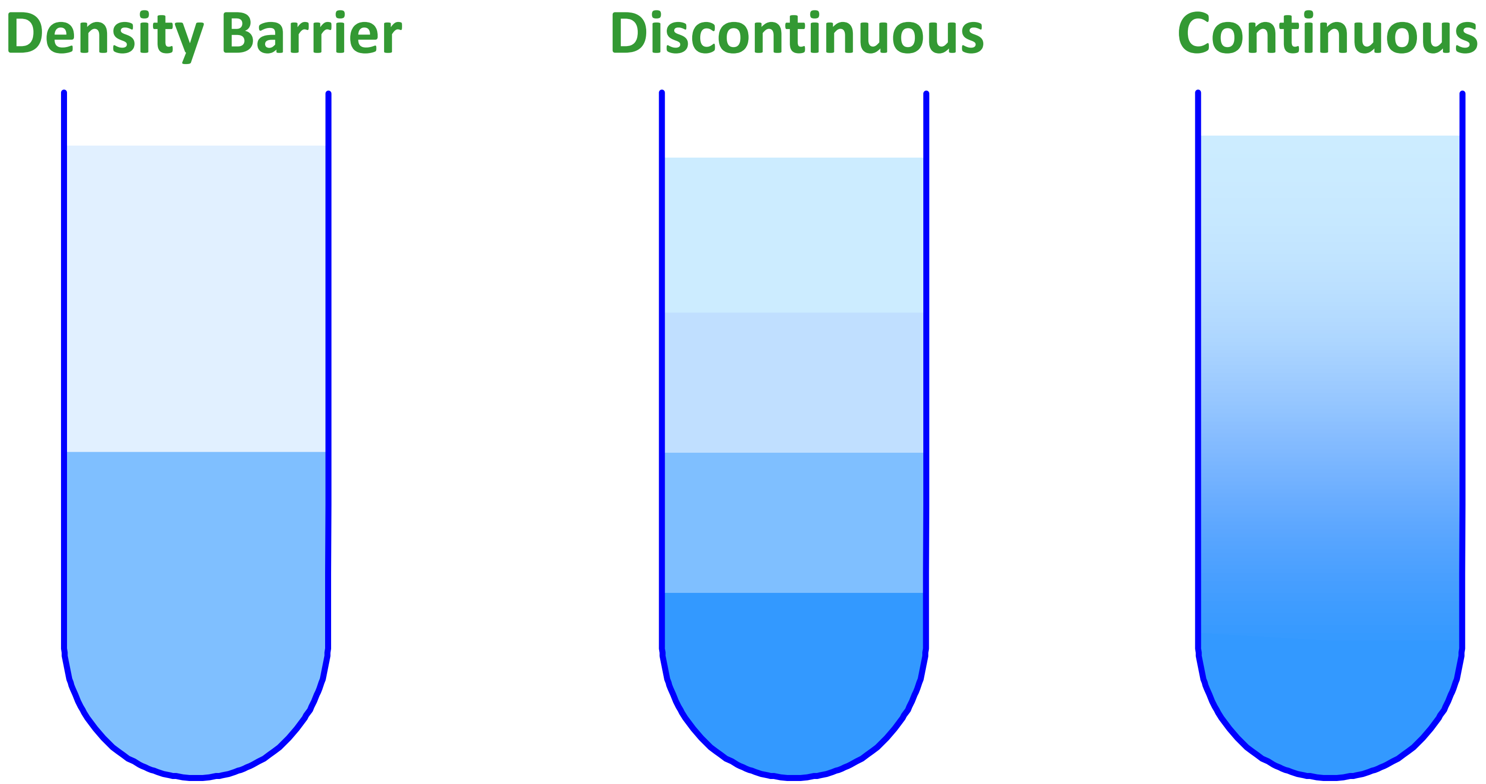
Density gradient centrifugation

- Two types: Zonal and Isopycnic
- 1. Zonal centrifugation using preformed gradient of different densities (linear of step gradients using glycerol or sucrose solutions.
- Mixture to be separated is layered on top of the gradient (Increasing concentration down the tube)
- Move down tubes at different rates.

Zonal centrifugation

- Sedimentation of the particles mainly depends on the particle’s sedimentation coefficient (density, size and shape).
- gradients can be formed in two ways:
- Step gradient
- Continuous (linear) gradient

Density gradient centrifugation



- 2. Isopycnic (same density) centrifugation self forming gradient during the centrifugation (e.g., Cesium chloride gradient).
- 10 – 100%
- 10, 20, 30, 40 …. 100%: step gradient
- Molecules separated on equilibrium position.
- Each molecule float or sinks to a position where density.

- Isopycnic centrifugation:gradient forming salt solution and sample solution throughly mixed – high speed -forms gradient of densities from top to bottom.
- Two solutions: 50% and 5% - forms gradient

- A particle will sediment through a solution if particle density > solution density
- If particle density < solution density, particle will float through solution

- Conversion of rpm to ‘g’:
- for any given rpm of the rotor, the g value can be calculated by the follwing equation:
- g= 11.17 x r (rpm/1000)2
- r= radius of rotor
- Eg. If the sample is spun at 10,000 rpm, g will be :
- g= 11.17 x 10.8 (10,000/1000)2 = 12,063.

- Thank You