

Cell Fractionation

Principle, Homogenization, Centrifugation Techniques — Study Notes

1. Introduction

Cell fractionation is a technique used to separate and isolate different cellular components (organelles, membranes, and macromolecules) based on their size, density, and other physical properties, while preserving their structural and functional integrity. It is a foundational tool for studying the biochemistry and function of individual organelles in isolation.

2. Principle

Cells are first broken open (homogenized) to release their internal contents without destroying the organelles themselves. The resulting homogenate is then subjected to centrifugal force, which separates components according to their size, shape, and density. Larger, denser structures sediment (pellet) at lower centrifugal speeds, while smaller, less dense structures require higher speeds and longer durations to sediment.

3. Homogenization

The tissue or cell sample is disrupted in a chilled, buffered, isotonic medium (commonly containing sucrose) to release organelles while minimizing damage. Common homogenization methods include:

- Mechanical disruption using a Potter–Elvehjem homogenizer (Teflon pestle in a glass tube)
- Mortar and pestle grinding (often with sand or liquid nitrogen for tough tissues)
- Sonication (ultrasound-induced disruption)
- Osmotic lysis (swelling and bursting cells in hypotonic medium)
- High-pressure homogenization or use of a blender for bulk tissue

Key precautions: the homogenization medium should be cold (to reduce enzymatic degradation), isotonic or appropriately buffered (to prevent organelles from swelling or shrinking), and of correct pH, so that organelles remain intact and functional after isolation.

4. Differential (Sequential) Centrifugation

This is the most common first step in cell fractionation. The homogenate is centrifuged repeatedly at progressively increasing speeds and durations. After each run, the pellet (containing the heaviest remaining component) is collected, and the supernatant is transferred to a fresh tube and centrifuged at a higher speed to sediment the next-lightest component.

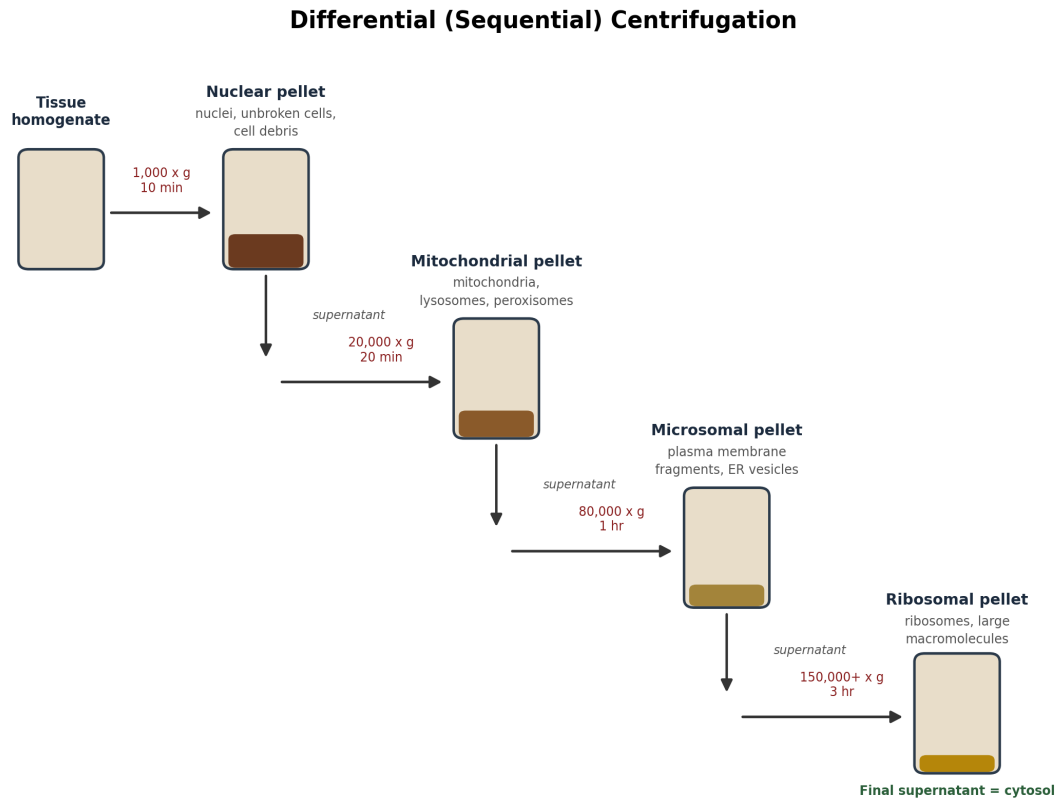


Fig. 1 — Differential centrifugation: increasing centrifugal force and time at each step pellets progressively smaller/lighter cellular components.

Typical sedimentation order (heaviest to lightest):

Speed (approx.)	Time	Pellet obtained
1,000 x g	~10 min	Nuclei, unbroken cells, cytoskeletal debris
20,000 x g	~20 min	Mitochondria, lysosomes, peroxisomes
80,000 x g	~1 hour	Plasma membrane fragments, microsomes (ER vesicles)
150,000+ x g	2–3 hours	Ribosomes, large macromolecular complexes

The final supernatant remaining after the last spin is the **cytosol** (soluble cytoplasmic fraction), free of organelles.

5. Density Gradient Centrifugation

Differential centrifugation alone often gives impure fractions, since organelles of similar sedimentation rate may co-pellet. Density gradient centrifugation improves purity by separating organelles according to their precise density, using a pre-formed gradient (commonly of sucrose or Percoll) inside the centrifuge tube.

The crude organelle fraction (or homogenate) is layered on top of the gradient and centrifuged at high speed for an extended period. Each organelle migrates through the gradient until it reaches the point where the surrounding medium density equals its own — its **isopycnic (equal-density) point** — where it forms a discrete band. This is also called isopycnic or equilibrium density gradient centrifugation.

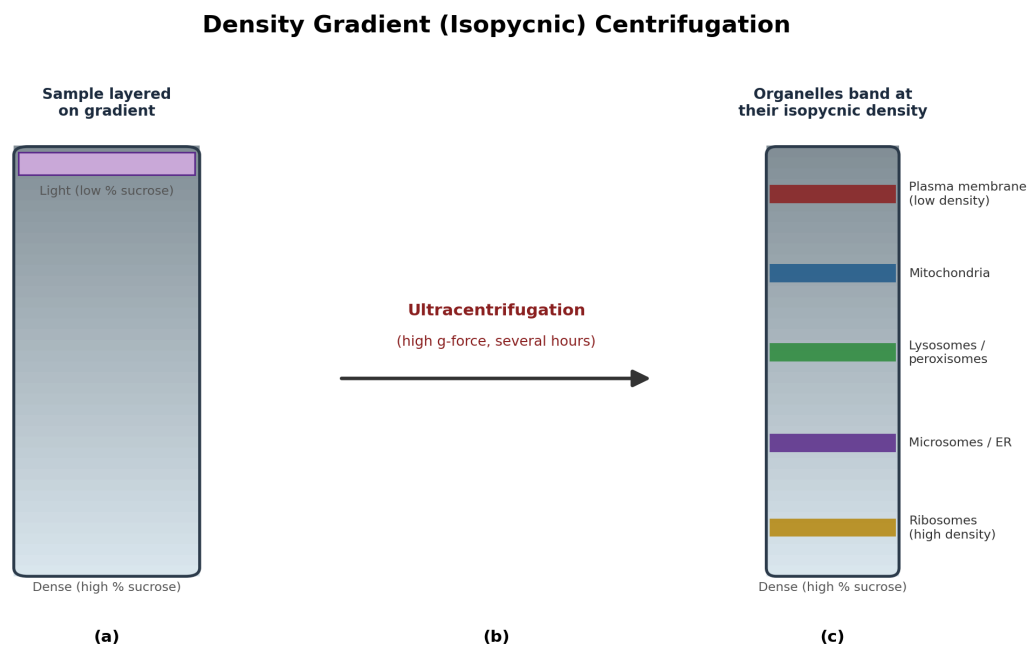


Fig. 2 — Density gradient (isopycnic) centrifugation: the sample is layered onto a sucrose gradient (a) and, after ultracentrifugation (b), organelles band at their characteristic density (c).

6. Differential vs. Density Gradient Centrifugation

Feature	Differential centrifugation	Density gradient centrifugation
Basis of separation	Size and sedimentation rate	Buoyant (isopycnic) density
Medium	Uniform (e.g. plain buffer)	Pre-formed density gradient (e.g. sucrose)
Purity of fractions	Moderate — fractions often mixed	High — sharp, discrete bands
Typical use	Initial, crude separation	Further purification of a crude fraction

7. Applications

- Isolating specific organelles (mitochondria, nuclei, ribosomes, ER) for biochemical study
- Studying organelle-specific enzyme activities and metabolic pathways
- Purifying membranes for protein and lipid composition analysis
- Isolating viruses and macromolecular complexes
- Preparing cell-free systems (e.g. for in vitro protein synthesis or DNA replication studies)

Note: Marker enzymes are often used to confirm fraction identity and purity — e.g. succinate dehydrogenase for mitochondria, acid phosphatase for lysosomes, and catalase for peroxisomes.