**Centrifuges**

Centrifuges are devices used in a variety of scientific and technical applications which spin carrier vessels (centrifuge tubes) at high rotation speeds and very high centrifugal force. The centrifugal force (expressed as # *gravities* or, # x*g*) generated is proportional to the rotation rate of the rotor (in rpm) and the distance between the rotor center and the centrifuge tube. Therefore, a given centrifuge may use multiple rotor sizes to give flexibilty in choosing centrifugation conditions. Each centrifuge has a special graph, a nomograph, or a table which relates rotation rate (rpm) to centrifugal force (xg) for each size of rotor it accepts.

Typically, the material to be "spun" is placed in a centrifuge tube which is then placed in a rotor. The rotor is generally a dense metal which dissipates heat quickly, and is of sufficient mass that it generates momentum, i.e., once its spinning it requires little energy to keep it going. Centrifuges generally work under vacuum and are refrigerated to reduce heating caused by frictional forces as the rotor spins. Rotors are usually stored in refrigeration units to keep them at or near the operating temperature.

Centrifuges come in all shapes and sizes, and the rotors vary, therefore, the universal and transferable unit of centrifugation is centrifugal force in gravities (xg). Different makes of centrifuges use different rotors and each model comes with a table or a graph that relates centrifugal force to rotational speed (rpm) for each rotor (or swing buckets) it can use. In lab write-ups you should ALWAYS report the centrifugal force used (#gravities) and duration of time at that force because centrifugal force is the only transferable unit between different centrifuges.

**Differential Centrifugation:**

A commonly used technique for cell fractionation, called *differential centrifugation*, is used to separate particles from a liquid medium or to separate particles of different masses into separate fractions of the supernatant. We will use this technique in a several ways in this course.

1. In the Bio 242 Amylase lab we will use centrifugation to pellet the cellular debris and excess starch during the enzyme extract preparation. The enzyme, which is soluble, will remain in the supernatant. During the actual experiment, we will use centrifugation to separate the enzyme (soluble) from its substrate (insoluble amylose-azure) to stop the reaction.

2. In the molecular labs we will use centrifugation to promote a chemical reaction by forcing small quantities of reactants together in the bottom of micro-centrifuge tubes. We will also use centrifugation to prepare bacterial cells for transformation by alternately pelleting them and then re-suspending them with different chemical solutions.

3. In the Hill Reaction lab we will use a multi-step differential centrifugation (Fig. 9-3) to isolate cell organelles (chloroplasts) from crude cellular homogenate. Because the organelles have much less mass than the cell wall components, the first pellet that forms at low centrifugal force is primarily cellular debris. The organelle fraction is then pelleted at higher centrifugal force.

**Centrifuge Cautions:**

These cautions presume you have had proper instruction in the use of the centrifuge AND have read the instructions for using the instrument thoroughly.

1. Make sure the correct rotor is being used and that it is installed properly on the spindle. Make sure the rotor is secured before starting a run. On the prep centrifuges the rotor cap screws onto the spindle.

2. ***Balance the load in the roto***r - every tube **must** have a balance tube in the opposite slot with the same volume of fluid. Imbalanced rotors can damage or destroy the machine, and, in some instances kill people.

3. Make sure you are using the appropriate centrifuge tube for the job - they can rupture at too high a speed. You may need special, high density tubes for high force centrifugation.

4. Pre-cool the centrifuge and the rotor before use. Rotors should be stored in a refrigerator when possible.

5. DO NOT attempt to override any safety features of the centrifuge.

6. NEVER leave the centrifuge unattended until it reaches maximum speed and is going smoothly.

**centrifuge** is a piece of [laboratory equipment](https://en.wikipedia.org/wiki/Laboratory_equipment), driven by a motor, which spins liquid samples at high speed. There are various types of centrifuges, depending on the size and the sample capacity.

There are various types of centrifugation:

* [Differential centrifugation](https://en.wikipedia.org/wiki/Differential_centrifugation), often used to separate certain organelles from whole cells for further analysis of specific parts of cells
* [Isopycnic centrifugation](https://en.wikipedia.org/wiki/Isopycnic_centrifugation), often used to isolate nucleic acids such as [DNA](https://en.wikipedia.org/wiki/DNA)
* [Sucrose gradient centrifugation](https://en.wikipedia.org/wiki/Sucrose_gradient_centrifugation), often used to purify enveloped viruses and ribosomes, and also to separate cell organelles from crude cellular extracts

There are different types of laboratory centrifuges:

* **Microcentrifuges**

(devices for small tubes from 0.2 ml to 2.0 ml (micro tubes), up to 96 well-plates, compact design, small footprint; up to 30,000 g)

* **Clinical centrifuges**

(moderate-speed devices used for clinical applications like blood collection tubes)

* **Multipurpose high-speed centrifuges**

(devices for a broad range of tube sizes, high variability, big footprint)

* [**Ultracentrifuges**](https://en.wikipedia.org/wiki/Ultracentrifuges)

(analytical and preparative models)

Because of the heat generated by air friction (even in ultracentrifuges, where the rotor operates in a good vacuum), and the frequent necessity of maintaining samples at a given temperature, many types of laboratory centrifuges are refrigerated and temperature regulated.

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**Centrifuge tubes** are precision-made, high-strength tubes of [glass](https://en.wikipedia.org/wiki/Glass) or [plastic](https://en.wikipedia.org/wiki/Plastic) made to fit exactly in rotor cavities. They may vary in capacity from 50 mL down to much smaller capacities used in micro-centrifuges used extensively in molecular biology laboratories. Micro-centrifuges typically accommodate disposable plastic micro-centrifuge tubes with capacities from 250 [μL](https://en.wikipedia.org/wiki/Microlitre" \o "Microlitre) to 2.0 [mL](https://en.wikipedia.org/wiki/Millilitre" \o "Millilitre).

Glass centrifuge tubes can be used with most solvents, but tend to be more expensive. They can be cleaned like other [laboratory glassware](https://en.wikipedia.org/wiki/Laboratory_glassware), and can be [sterilized](https://en.wikipedia.org/wiki/Sterilization_(microbiology)) by [autoclaving](https://en.wikipedia.org/wiki/Autoclaving). They must be handled with care, since small scratches can cause failure under the strong forces imposed during a run. Glass tubes are inserted into soft rubber sleeves to cushion them during runs. Plastic centrifuge tubes, especially tend to be less expensive and, with care, can be just as durable as glass. Water is preferred when plastic centrifuge tubes are used. They are more difficult to clean thoroughly, and are usually inexpensive enough to be considered disposable.

Disposable plastic "Eppendorf tubes" of 0.5ml to 2ml are commonly used in micro-centrifuges. They are molded from a flexible transparent plastic similar to [polythene](https://en.wikipedia.org/wiki/Polythene), are semi-conical in shape, with integral, hinged sealing caps.

Larger samples are spun using centrifuge bottles, which range in capacity from 250 to 1000 milli litres. Although some are made of heavy glass, centrifuge bottles are usually made of shatter-proof plastics such as polypropylene or polycarbonate. Sealing closures may be used for added leak-proof assurance.

Types by rotor design:

* Fixed-angle centrifuges are designed to hold the sample containers at a constant angle relative to the central axis.
* Swinging head (or swinging bucket) centrifuges, in contrast to fixed-angle centrifuges, have a hinge where the sample containers are attached to the central rotor. This allows all of the samples to swing outwards as the centrifuge is spun.

**Microcentrifuges** [[edit](https://en.wikipedia.org/w/index.php?title=Centrifugation&action=edit&section=2)]

Micro-centrifuges are used to process small volumes of biological molecules, [cells](https://en.wikipedia.org/wiki/Cell_(biology)), or [nuclei](https://en.wikipedia.org/wiki/Cell_nucleus). Micro-centrifuge tubes generally hold 0.5 - 2.0 mL of liquid, and are spun at maximum angular speeds of 12,000–13,000 rpm. Micro-centrifuges are small enough to fit on a table-top and have rotors that can quickly change speeds. They may or may not have a[re frigeration](https://en.wikipedia.org/wiki/Refrigeration) function.

**High-speed centrifuges** [[edit](https://en.wikipedia.org/w/index.php?title=Centrifugation&action=edit&section=3)]

High-speed or super speed centrifuges can handle larger sample volumes, from a few tens of milli litres to several litres. Additionally, larger centrifuges can also reach higher angular velocities (around 30,000 rpm). The rotors may come with different adapters to hold various sizes of [test tubes](https://en.wikipedia.org/wiki/Test_tubes), bottles, or [micro titer plates](https://en.wikipedia.org/wiki/Microtiter_plate).

**Fractionation process** [[edit](https://en.wikipedia.org/w/index.php?title=Centrifugation&action=edit&section=4)]

General method of fractionation: Cell sample is stored in a suspension which is:

1. Buffered - neutral pH, preventing damage to the structure of proteins including enzymes (which could affect ionic bonds)
2. Isotonic (of equal water potential) - this prevents water gain or loss by the organelles
3. Cool - reducing the overall activity of enzyme released later in the procedure

* Cells are homogenised in a blender and filtered to remove debris
* The homogenised sample is placed in an ultracentrifuge and spun in low speed - nuclei settle out, forming a pellet
* The supernatant (suspension containing remaining organelles) is spun at a higher speed - chloroplasts settle out
* The supernatant is spun at a higher speed still - mitochondria and lysosomes settle out
* The supernatant is spun at an even higher speed - ribosomes, membranes settle out

The ribosomes, membranes and Golgi complexes can be separated by another technique called density gradient centrifugation.

**Ultra centrifigations**[[edit](https://en.wikipedia.org/w/index.php?title=Centrifugation&action=edit&section=5)]

*Main articles:*[*Differential centrifugation*](https://en.wikipedia.org/wiki/Differential_centrifugation)*, [Isopycnic centrifugation](https://en.wikipedia.org/wiki/Isopycnic_centrifugation" \o "Isopycnic centrifugation) and*[*ultracentrifugation*](https://en.wikipedia.org/wiki/Ultracentrifugation)

[Ultracentrifugation](https://en.wikipedia.org/wiki/Ultracentrifugation) makes use of high centrifugal force for studying properties of biological particles. Compared to micro centrifuges or high-speed centrifuges, ultracentrifuges can isolate much smaller particles, including ribosomes, proteins, and viruses. Ultracentrifuges can also be used in the study of membrane fractionation. This occurs because ultracentrifuges can reach maximum angular velocities in excess of 70,000 rpm. Additionally, while micro centrifuges and super centrifuges separate particles in batches (limited volumes of samples must be handled manually in test tubes or bottles), ultracentrifuges can separate molecules in batch or continuous flow systems.

In addition to purification, analytical ultracentrifugation (AUC) can be used for determination of the properties of macromolecules such as shape, mass, composition, and conformation. Samples are centrifuged with a high-density solution such as [sucrose](https://en.wikipedia.org/wiki/Sucrose), [caesium chloride](https://en.wikipedia.org/wiki/Caesium_chloride" \o "Caesium chloride), or [iodixanol](https://en.wikipedia.org/wiki/Iodixanol" \o "Iodixanol). The high-density solution may be at a uniform concentration throughout the test tube ("cushion") or a varying concentration ("[gradient](https://en.wikipedia.org/wiki/Gradient)"). Molecular properties can be modeled through [sedimentation](https://en.wikipedia.org/wiki/Sedimentation) velocity analysis or sedimentation equilibrium analysis. During the run, the particle or molecules will migrate through the test tube at different speeds depending on their physical properties and the properties of the solution, and eventually form a pellet at the bottom of the tube, or bands at various heights.