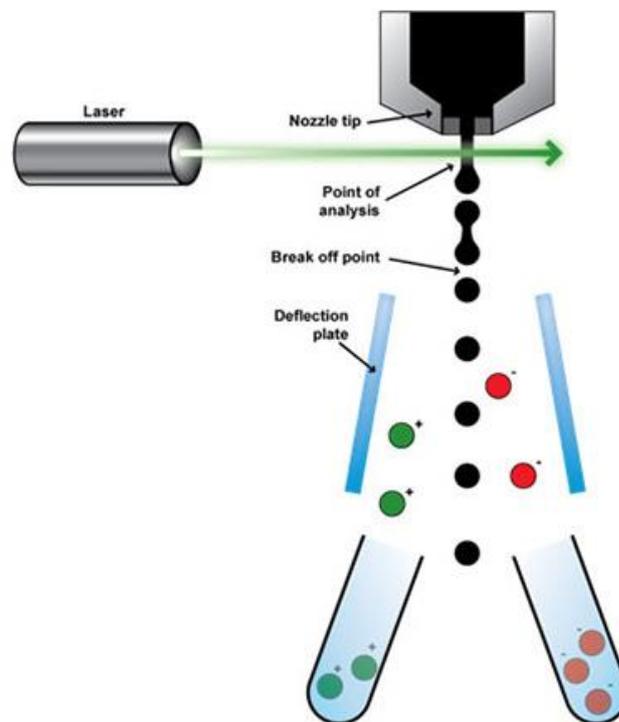


Fluorescence activated cell sorting of live cells

A description of fluorescence activated cell sorting of live cell populations.

Fluorescence activated cell sorting (FACS) of live cells separates a population of cells into subpopulations based on fluorescent labeling. Sorting involves more complex mechanisms in the flow cytometer than a non-sorting analysis. Cells stained using fluorophore-conjugated antibodies can be separated from one another depending on which fluorophore they have been stained with. For example, a cell expressing one cell marker may be detected using an FITC-conjugated antibody that recognizes the marker, and another cell type expressing a different marker could be detected using a PE-conjugated antibody specific for that marker. This is the basic task of flow cytometry.

Live cell sorting goes one step further:



1. Individual cells are “interrogated” by the laser as in a normal flow cytometer.
2. The machine is set up so that each individual cell then enters a single droplet as it leaves the nozzle tip. This drop is given an electronic charge, depending on the fluorescence of the cell inside the drop.
3. Deflection plates attract or repel the cells accordingly into collection tubes. For example:

A single FITC stained cell in a single droplet would be given a positive charge and be attracted to the right. Collection tubes to the right would collect all the positively charged FITC stained cell droplets.

A single PE stained cell in a single droplet would be given a negative charge and be attracted to the left. Collection tubes to the left would collect all the negatively charged PE stained cell droplets.

4. Sorted cell populations are then analyzed to ensure successful cell sorting.
5. Sorted cells can then be cultured.

The cells need to remain viable and without contamination for subsequent culture. See the tips below.

- Include serum in buffers.
- Avoid sodium azide in the buffers during staining as this can be toxic to cells and compromise the viability.
- The experiment should be undertaken in aseptic sterile conditions to ensure the cells do not become contaminated.
- It is not usually possible to do intracellular staining before sorting of live cells, as the permeabilization requires damage to the cell membrane which would compromise the cell viability.