

How Flow Cytometry Works

Flow cytometry operates by passing cells or particles suspended in a liquid through a laser beam. The cells scatter light and emit fluorescence when labeled with specific fluorochromes, often bound to antibodies. The instrument captures this emitted light to analyze parameters like:

- Cell size
- Granularity (internal complexity)
- Presence and abundance of surface and intracellular markers (using fluorescently tagged antibodies or probes)

The technology allows researchers to:

- Quantify cell populations
- Analyse cell-cycle status
- Investigate protein expression patterns (differentiation, activation)
- Assess intracellular activation pathways
- Measure different metabolic processes.
- Sort specific cells for further analysis
- Characterise and sort pluripotent stem cells

Technologies in Flow Cytometry: Conventional vs. Spectral

Conventional Flow Cytometry

In conventional flow cytometry, detectors are designed to capture light emitted at specific wavelengths corresponding to the fluorochromes used in the experiment. The system uses fixed optical filters to collect the emitted light and separate the signals based on predetermined wavelengths.

Advantages:

- Simple and well-established technology
- Reliable for basic, routine cell analysis with a limited number of colors (usually up to 8-10 colors)
- Lower cost and easier to set up for standard assays

Disadvantages:

- Limited parameter capacity (fewer channels)
- Fluorescence overlap between channels can complicate the analysis

Best for:

- Routine analysis of relatively simple experimental designs where only a few markers need to be analyzed.

Spectral Flow Cytometry

Spectral flow cytometry uses multiple detectors to capture light across a broad spectrum, rather than relying on fixed filters for specific wavelengths. The technique then applies sophisticated computational algorithms to "unmix" the overlapping signals, allowing for greater flexibility and the ability to analyze many more parameters simultaneously.

Advantages:

- Enables analysis of more than 30 parameters simultaneously, ideal for complex, high-dimensional data
- Reduced spectral overlap compared to conventional systems
- Greater flexibility in experimental design (e.g., using a wider range of fluorochromes)
- Autofluorescence handling. Some cells and tissues have high levels of autofluorescence. Spectral flow cytometry allows for autofluorescence correction, leading to better resolution of the data.

Disadvantages:

- Requires more advanced data analysis software and techniques
- More complex experimental setup and panel design

Best for:

- High-dimensional studies, such as in depth immunophenotyping in complex populations, and autofluorescent samples.

Fluorescence-activated Cell Sorting (FACS)

Cell sorting refers to isolating specific populations of cells from a mixed sample based on distinct characteristics. Flow cytometry-based sorting is an indispensable tool for obtaining pure cell populations for further analysis, culturing, or downstream applications. With advanced sorting capabilities, researchers can:

- Sort single cells for single-cell sequencing or cloning
- Isolate rare cell populations (e.g., tumor-infiltrating lymphocytes, stem cells)
- Purify live cells for functional assays or tissue culture