FLOW CYTOMETRY

Flow cytometry (FC) is a technique used to detect and measure physical and chemical characteristics of a population of cells or particles. In this process, a sample containing cells or particles is suspended in a fluid and injected into the flow cytometer instrument. The sample is focused to ideally flow one cell at a time through a laser beam, where the light scattered is characteristic to the cells and their components. Cells are often labeled with fluorescent markers so light is absorbed and then emitted in a band of wavelengths. Tens of thousands of cells can be quickly examined and the data gathered are processed by a computer. Flow cytometry is routinely used in basic research, clinical practice, and clinical trials. Uses for flow cytometry include:

- Cell counting
- Cell sorting
- Determining cell characteristics and function
- Detecting microorganisms
- Biomarker detection
- Protein engineering detection
- Diagnosis of health disorders such as blood cancers

A flow cytometry analyzer is an instrument that provides quantifiable data from a sample. Other instruments using flow cytometry include cell sorters which physically separate and thereby purify cells of interest based on their optical properties.

Modern flow cytometers are able to analyze many thousands of particles per second, in "real time" and, if configured as cell sorters, can actively separate and isolate particles with specified optical properties at similar rates. A flow cytometer is similar to a microscope, except that, instead of producing an image of the cell, flow cytometry offers high-throughput, automated quantification of specified optical parameters on a cell-by-cell basis. To analyze solid tissues, a single-cell suspension must first be prepared.

Components of flow cytometer:

A flow cytometer has five main components: a flow cell, a measuring system, a detector, an amplification system, and a computer for analysis of the signals. The flow cell has a liquid stream (sheath fluid), which carries and aligns the cells so that they pass single file through the light beam for sensing. The measuring system commonly uses measurement of impedance (or conductivity) and optical systems - lamps (mercury, xenon); high-power water-cooled lasers (argon, krypton, dye laser); low-power air-cooled lasers (argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet) resulting in light signals. The detector and analog-to-digital conversion (ADC) system converts analog measurements of forward-scattered light (FSC) and side-scattered light (SSC) as well as dye-specific fluorescence signals into digital signals that can be processed by a computer. The amplification system can be linear or logarithmic.

The process of collecting data from samples using the flow cytometer is termed "acquisition". Acquisition is mediated by a computer physically connected to the flow cytometer, and the software which handles the digital interface with the cytometer. The software is capable of adjusting parameters (e.g., voltage, compensation) for the sample being tested, and also assists in displaying initial sample information while acquiring sample data to ensure that parameters are set correctly. Early flow cytometers were, in general, experimental devices, but technological advances have enabled widespread applications for use in a variety of both clinical and research purposes. Due to these developments, a considerable market for instrumentation, analysis software, as well as the reagents used in acquisition such as fluorescently labeled antibodies have been developed.

Modern instruments usually have multiple lasers and fluorescence detectors. The current record for a commercial instrument is ten lasers and 30 fluorescence detectors. Increasing the number of lasers and detectors allows for multiple antibody labeling, and can more precisely identify a target population by their phenotypic markers. Certain instruments can even take digital images of individual cells, allowing for the analysis of fluorescent signal location within or on the surface of cells.

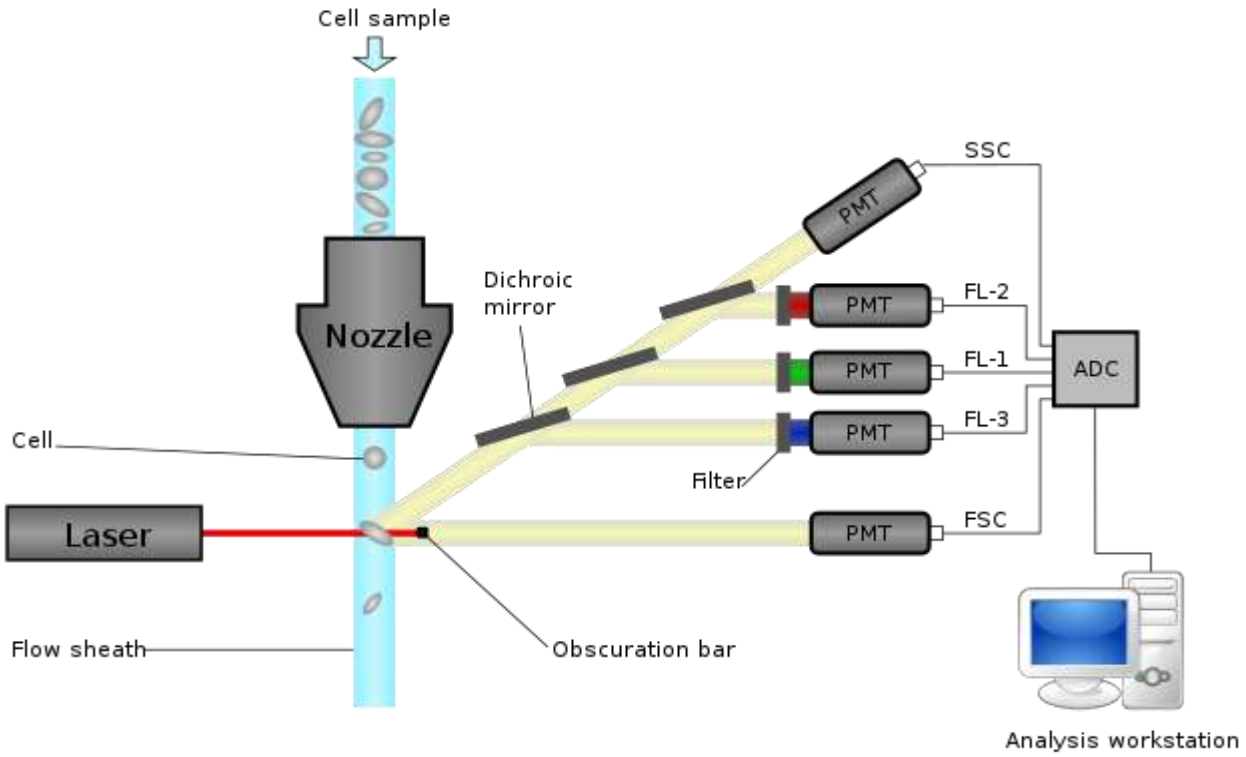

Fig. Components of a flow cytometer

Fluidics system of a flow cytometer

Cells must pass uniformly through the center of focused laser beams to accurately measure optical properties of cells in any flow cytometer. The purpose of the fluidic system is to move the cells one by one through the lasers beam and throughout the instrument. Fluidics in a flow cytometer with cell sorting capabilities also use the stream to carry sorted cells into collection tubes or wells.

Hydrodynamic focusing

For precise positioning of cells in a liquid jet, hydrodynamic focusing is used in most cytometers. The cells in suspension enter into the instrument enclosed by an outer sheath fluid. The sample core is maintained in the center of the sheath fluid. The sample input rate or how fast the cells flow through to the laser interrogation can be controlled by the pressure of the sheath fluid on the sample core. Under optimal conditions, the central fluid stream and sheath fluid do not mix.

Optical filters

Light emitted from fluorophores are in a spectrum of wavelengths, so combining multiple fluorophores may cause overlap. To add specificity, optical filters and dichroic mirrors are used to filter and move light to the detectors such as photomultiplier tubes (PMTs) or avalanche photodiodes (APD).[12] Optical filters are designed as band pass (BP), long pass (LP), or short pass (SP) filters. Most flow cytometers uses dichroic mirrors and band pass filters to select specific bands of the optical spectrum.