Applications of Flow Cytometry

Currently Flow cytometry has been proved as a powerful technique which is used to analyze the physical and chemical characteristics of a population of cells or particles. It is composed of three components (i) fluidics, (ii) optics, and (iii) electronics. In this process, fluorophore tagged cells or particles suspended in a fluid (Fluidics) are injected into the flow cytometer instrument. The sample is allowed to flow one cell at a time through a laser beam (Optics), light is absorbed and then emitted in a band of wavelengths. This scattered light is characteristic to the cells and their components. The scattered light is captured by detectors and processed by a computer to generate data (Electronics).

Flow cytometry is routinely used in basic research, clinical practice, and clinical trials. Applications of flow cytometry include:

Immunophenotyping

The most common application performed on the cytometer is immunophenotyping. This technique identifies and quantifies populations of cells in a heterogeneous sample - usually blood, bone marrow or lymph. These cell subsets are measured by labeling population-specific proteins with a fluorescent tag on the cell surface. In clinical labs, immunophenotyping is useful in diagnosing hematological malignancies such as lymphomas and leukemia.

Erythrocyte analysis

The use of flow cytometry for the detection and quantification of fetal red cells in maternal blood has increased in recent years.

Leukocyte analysis

Immunologic monitoring of HIV-infected patients is a mainstay of the clinical flow cytometry laboratory.

Cell Cycle Analysis

Flow cytometry can analyze replication states using fluorescent dyes to measure the four distinct phases of the cell cycle. Along with determining cell cycle replication states, the assay can measure cell aneuploidy associated with chromosomal abnormalities.

Apoptosis

In response to external physical and chemical stimuli, cells die by two ways, apoptosis and necrosis. These two distinct types of cell death, can be distinguished by flow cytometry on the basis of differences in morphological, biochemical and molecular changes occurring in the dying cells.

Cell Proliferation Assays

The flow cytometer can measure proliferation by labeling resting cells with a cell membrane fluorescent dye, carboxyfluorescein succinimidyl ester (CFSE). When the cells are activated, they begin to proliferate and undergo mitosis. As the cells divide, half of the original dye is passed on to each daughter cell. By measuring the reduction of the fluorescence signal, researchers can calculate cellular activation and proliferation.

Quantification of Soluble Molecules

Soluble antigens or antibodies can be quantified by flow cytometry if standard cells or beads are used. For example, OKT3 is a mouse anti-human antibody useful in treating transplant rejection. Circulating concentrations of OKT3 can be quantified by incubating with normal CD3-positive lymphocytes, followed by a fluorescently labeled anti-mouse antibody.

Membrane potential

Bacterial membrane potential may be analyzed using DiOC2, which exhibits green fluorescence in all bacterial cells, but shifts to red fluorescence as the dye becomes more concentrated in cells with larger membrane potentials. Mitochondrial membrane potential may be analyzed in the same manner with JC-1.

Estimation of ROS

The cellular ROS of the cells can be measured by employing several specific dyes eg. DCFDA, DHE, MitoSOX, etc.

Discrimination of live/dead cells

Live cells have intact membranes and are impermeable to dyes such as propidium iodide, which only leaks into cells with compromised membranes. Thiazole orange enters all cells, live and dead, to varying degrees. Thus a combination of these two dyes provides a rapid and reliable method for discriminating live and dead cells.

Intracellular cytokine detection

The cultured cells are stained with fluorescence-conjugated antibody for a target molecule. Finally, the labeled cells were analyzed by flow cytometry.