Flow Cytometry

Aim:

Demonstration of Flow Cytometry through experimental sample.

Introduction:

Flow cytometry is a standard laser-based technology that is used in the detection and measurement of physical and chemical characteristics of cells or particles in a heterogeneous fluid mixture. The properties that can be measured by this process include a particle's size, granularity or internal complexity, and fluorescence intensity. These characteristics are determined using an optical-to-electronic coupling system that detects the cells based on laser scattered by the cells. Cell components are fluorescently labelled and then excited by the laser to emit light at varying wavelengths. The fluorescence can then be measured to determine the amount and type of cells present in a sample. Up to thousands of particles per second can be analysed as they pass through the liquid stream. A beam of laser light is directed at a hydrodynamically-focused stream of fluid that carries the cells. Several detectors are carefully placed around the stream, at the point where the fluid passes through the light beam. One of these detectors is in line with the light beam and is used to measure Forward Scatter or FSC. Another detector is placed perpendicular to the stream and is used to measure Side Scatter (SSC). Since fluorescent labels are used to detect the different cells or components, fluorescent detectors are also in place. The suspended particles or cells, which may range in size from 0.2 to 150µm, pass through the beam of light and scatter the light beams. The fluorescently labelled cell components are excited by the laser and emit light at a longer wavelength than the light source. This is then detected by the detectors. The detectors therefore pick up a combination of scattered and fluorescent light. This data is then analyzed by a computer that is attached to the flow cytometer using special software. The brightness of each detector (one for each fluorescent emission peak) is adjusted for this detection. Using the light measurements, different information can be gathered about the physical and chemical structure of the cells. Generally, FSC can detect the cell volume whereas the SSC reflects the inner complexity of the particle such as its cytoplasmic granule content or nuclear structure.

Principle:

The basic principle of flow cytometry is based on the measurement of light scattered by particles, and the fluorescence observed when these particles are passed in a stream through a laser beam.

Fluidics

- The purpose of the fluidics system is to transport particles in a fluid stream to the laser beam. To accomplish this, the sample is injected into a stream of sheath fluid (usually a buffered saline solution) within the flow chamber.
- The design of the flow chamber allows the sample core to be focused in the center of the sheath fluid where the laser beam then interacts with the particles.
- Focusing is achieved by injecting the sample suspension into the center of a sheath liquid stream. The flow of the sheath fluid moves the particles and restricts them to the center of the sample core.

Optics System

- The optical system of the cytometer consists of excitation optics and collection optics.
- The excitation optics consists of the laser and lenses that are used to shape and focus the laser beam to the flow of the sample.
- The collection optics consist of a collection lens to collect light emitted after the particle interacts with the laser beam and a system of optical mirrors that divert the specified wavelengths of the collected light to designated optical detectors.
- fter a cell or particle passes through the laser light, the rays emitted on the side and the fluorescence signals are directed to the photomultiplier tubes (PMTs), and a photodiode collects the signals.
- To achieve the specificity of a detector for a particular fluorescent dye, a filter is placed in front of the tubes, which allows only a narrow range of wavelengths to reach the detector.

Electronics system

- The electronic system converts the signals from the detectors into digital signals that can be read by a computer.
- Once the light signals strike one side of the PMT or the photodiode, they are converted into a relative number of electrons that are multiplied to create a more significant electrical current.
- The electrical current moves to the amplifier and is converted to a voltage pulse.
- The highest point of the pulse is achieved when the particle strikes the center of the beam, in which case the maximum amount of scatter or fluorescence is achieved.
- The Analog-to-Digital Converter (ADC) then converts the pulse to a digital number.

Requirements:

1. Flow cytometer
2. Sheath fluid
3. Filtered decontamination solution
4. Filtered distilled water
5. Facs tubes
6. Trypsin
7. PBS
8. DCFH-DA stain

Procedure:

- Harvest the cells using trypsin.
- Centrifuge the cell suspension to pellet down the cells.
- After centrifugation, discard the supernatent and wash the pellet once with 1X PBS.
- After washing, stain the cells with 10 uM concentration of DCFH-DA and incubate at 37 degree for 30 minutes in dark condition.
- After incubation is over, acquire the data through flow cytometry in the FL-1 or FITC channel.
- Analysze the data.

Important Instructions:

- Before starting the experiment, flow cytometer should be washed throughly using distilled water and decontamination solution.
- Before running in the flow cytometers, the cells under analysis must be in a single-cell suspension.
- Clumped cultured cells or cells present in solid organs should first be converted into a single cell suspension before the analysis by using enzymatic digestion or mechanical dissociation of the tissue, respectively.
- It is then followed by mechanical filtration should be done to avoid unwanted instrument clogs and obtain higher quality flow data.