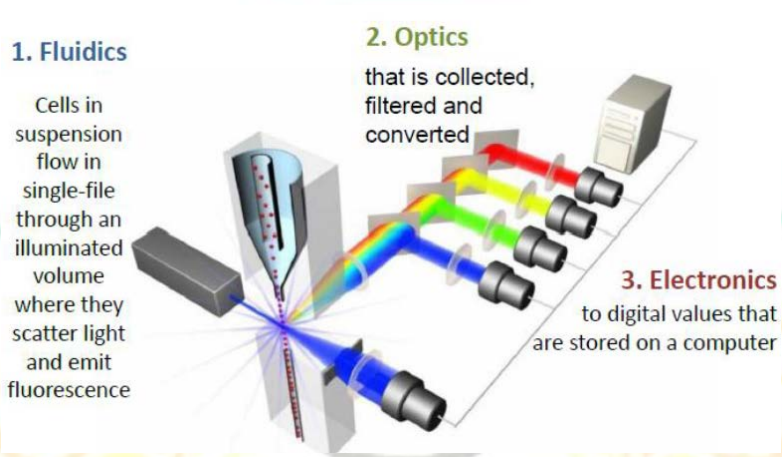
Flow Cytometer

Introduction: A cytometer is used for counting and measuring the physical and chemical characteristics of cells and other biological particles. A flow cytometer is different from other cytometers in that a single-cell suspension is passed through it in a fluid stream. A flow cytometer is a complex combination of optics, fluidics, and electronics. A labelled cell is forced through the system, causing the cell to scatter light and emit fluorescence. This output is sensed by photodetectors and then amplified and converted to digital signals for storage in computers. This stored data can be displayed or used for further analysis.



The flow cytometer is widely used in research as well as in clinical immunology and hematology to perform rapid immunophenotyping, cell sorting, and DNA analysis.

Principle: Prepared single cell or particle suspensions are necessary for flow cytometric analysis. Various immunoflurescent dyes or antibodies can be attached to the antigen or protein of interest. The suspension of cells or particles is aspirated into a flow cell where, surrounded by a narrow fluid stream, they pass one at a time through a focused laser beam. The light is either scattered or absorbed when it strikes a cell. Absorbed light of the appropriate wavelength may be re-emitted as fluorescence if the cell contains a naturally fluorescent substance or one or more fluorochrome-labeled antibodies are attached to surface or internal cell structures. Light scatter is dependent on the internal structure of the cell and its size and shape.

Fluorescent substances absorb light of an appropriate wavelength and reemit light of a different wavelength. Fluorescein isothiocyanate (FITC), Texas red, and phycoerythrin (PE) are the most common fluorescent dyes used in the biomedical sciences. Light and/or fluorescence scatter signals are detected by a series of photodiodes and amplified. Optical filters are essential to block unwanted light and permit light of the desired wavelength to reach the photodetector. The resulting electrical pulses are digitized, and the data is stored, analyzed, and displayed through a computer system. The end result is quantitative information about every cell analyzed (Fig. 1). Since large numbers of cells are analysed in a short period of time (>1,000/sec), statistically valid information about cell populations is quickly obtained.

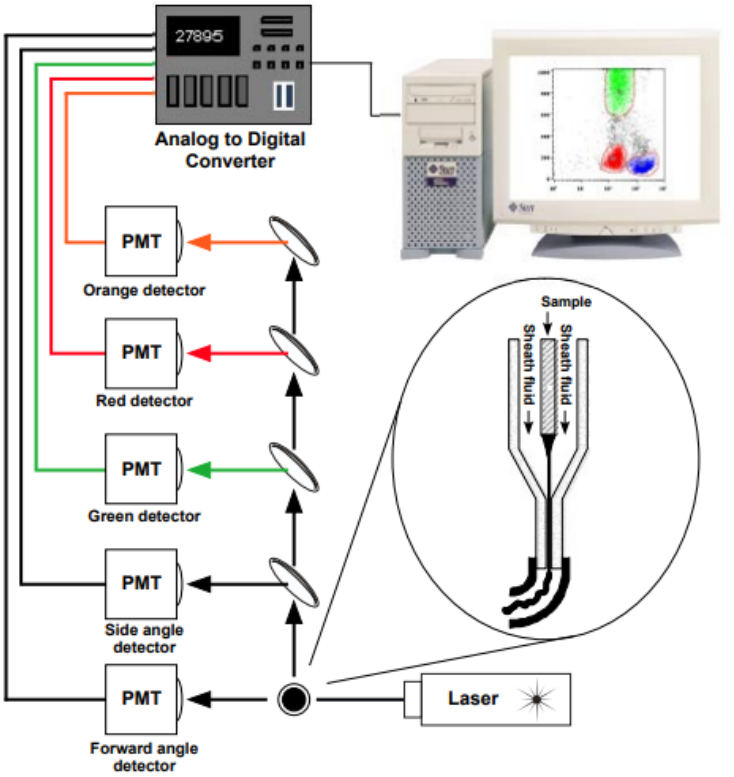
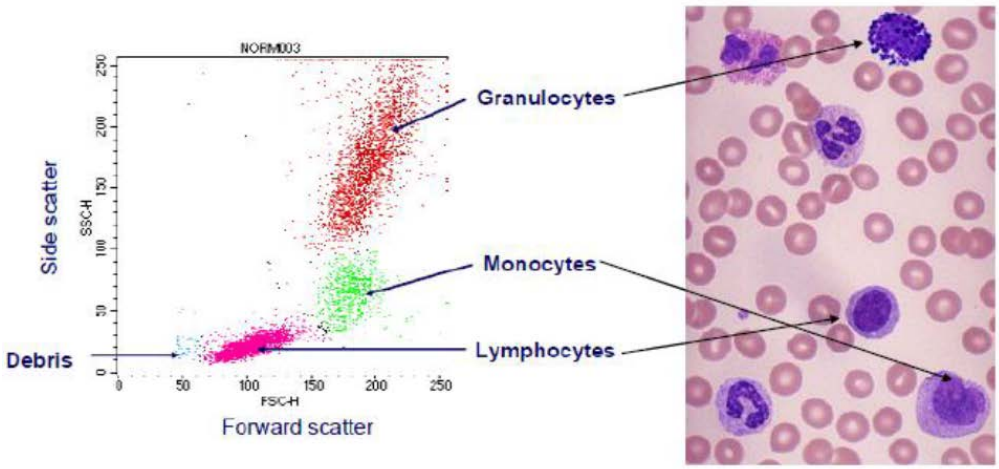


Fig. 1 - Working of Flow Cytometer (see Principle for details)

Applications of Flow Cytometry

1. **Phenotypic characterization of blood cells**

   Immunophenotyping or phenotypic characterization of cells is the identification and quantification of a specific cell group in the mixed population using flow cytometry e.g. immune cells of the blood. Immunophenotyping of the cells can be accomplished by staining of a single cell simultaneously with two or more antibodies.



2. **Measurement of apoptosis markers**

Many different flow cytometric methods for the assessment of apoptosis in cells have been described including

a. cell viability,
b. measuring plasma membranes changes,
c. detection of active caspase-3 ,
d. DNA fragmentation,
e. detection of mitochondrial proteins.

3. **Intracellular cytokine detection**

To detect intracellular cytokines, cultured cells were treated with Golgi stop (1:1500) for 4 h at 37°C. At the end of incubation, cells were first washed and labeled with cell surface markers for T cells. Following washing, cells were fixed and permeabilized for intracellular staining of cytokines. After fixation and permeabilization, fluorescence-conjugated antibody for a target molecule was added to the cells. Finally, the labeled cells were analyzed by flow cytometry.