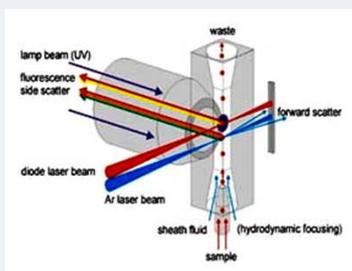


## Introduction

Flow cytometry (FCM) is a technique for simultaneously measuring certain physical and chemical features of individual particles in suspension as they pass through a fixed laser beam, on the basis of angular reflection of an incident laser light. The light reflected by such particles at a low angle ( $< 2^\circ$ ) is detected in the forward direction along the axis of the incident light and this "forward scatter" (FSC) is considered to be proportional to the relative size of the particle. Light reflected at  $> 2^\circ$  is detected at  $90^\circ$  or more to the axis of the incident light and is referred to as orthogonal or "side scatter" (SSC), which is considered to be proportional to the surface and/or internal complexity of the particle. Additional information about each of the particles in the suspension can readily be obtained if they are 'tagged' with a fluorescent dye delineating a particular physical or chemical characteristic. Under these conditions, when an incident (eg, argon-ion) laser light is applied, the energy absorbed by the fluorochrome is absorbed and subsequently emitted at a higher wavelength which is specific for the fluorochrome. The intensity of the emitted fluorescent signal is proportional to the level of fluorochrome associated with the particle. Moreover, multiple parameters can be measured simultaneously using a panel of different fluorescent markers, each with a unique emission signal. A flow cytometer (eg FACScan; Becton Dickinson Ltd) is optimized for the acquisition of the data, and appropriate software is then used to analyze similarities and differences between the particles in the population.

## Schematic illustration of a flow cytometer



The flow cytometer uses the principle of hydrodynamic focusing for presenting particles in a fluid suspension to a laser (or other light excitation source). The sample is injected into the center of a sheath flow and as each single particle passes through the laser beam, the emitted optical signals generated by each single particle are captured and displayed for subsequent analysis.

## Applications of FCM

Because cells are essentially 'particles', the FCM technique has had major application in the life sciences and is now widely used in plant biotechnology, microbiology, haematology, immunology, oncology and virtually all other areas of cell and molecular biology. This has been due, in part, to the development and availability of fluorescent-labelled reagents which are specific for nucleic acids and, in particular, antibodies which identify specific cell-associated proteins. The use of monoclonal antibodies (mAb) to detect and measure many thousands of such 'antigens' has now become fundamental in assessing cell proliferation, differentiation and function, and is especially important in the analysis of suspensions containing different types of cell, including abnormal diseased cells and also putative stem cells for clinical therapy. In addition, research in the Biomaterials and Tissue Engineering Division at the Eastman has pioneered the application of the FCM technique for analysis of the precise cellular effects of conventional, modified and newly-developed materials for orthopaedic, dental and soft tissue implant surgery and for determining the potential efficacy of procedures for tissue repair and regeneration. During the past 10 years these studies, generating more than 40 publications, have had a significant impact on the criteria for evaluating cellular responses to biomaterial formulations and have now become the gold standard for determination of 'biocompatibility'.

Frequency histograms (Figure left below) display relative fluorescence or scattered light signals plotted against the number of events. The simplicity of this type of display is the main attraction. To see the relative levels of other parameters which were collected at the same time, one needs to use one of the forms of bivariate displays namely dot, density or contour plots. In these type of displays, one parameter is plotted against another in an X versus Y axis display.

The bivariate display (Figure right below) plots one dot or point on the display related to the amount of parameter x and y for each cell which passed through the instrument. Dotplots are good for detecting small numbers of events which are well separated from the main populations of the cells present but give little or no indication of the relative density of numbers of events in populations. This is particularly true for large data files. This is one reason for using a density plot.

