Introduction

Flow cytometry is a technique which allows us to obtain information about the physical and chemical characteristics of cells or particles in suspension by passing them through a beam of laser light. There is a wide range of applications, from clinical diagnostics (mainly in Haematological Oncology or Immunology) to complex research projects in biomedicine or cellular biology in general.

After the first unsuccessful attempts to automatically count cells during the first decades of the 20th century, the field gained ground during the Cold War in the 50s with the search for ways to study bacteria due to the dangers of biological weapons. The development of the flow chamber in the following decade was followed by the first flow experiments and the commercial production of units started in the 70s. At the same time, using the same principles cell separation technology advanced using electrostatic deflection to separate cells in a hybrid between flow cytometry and an ink injection printer. From this technology came the name FACS (fluorescence activated cell sorting).

The development of monoclonal antibodies in the 80s, along with improvements in flow cytometers allowed them to be used in clinical diagnostics. From the study of subpopulations of CDF+ lymphocytes to track the development of HIV, through diagnostics for haematological disorders and studies of the cell cyle (ploidy) it is routinely used in laboratories and clinics. The possibilities of using the technology for cell sorting is usually reserved for research applications.

Description

A flow cytometer has three basic principles:

Hydraulic System
Pneumatic and fluid controls are necessary to establish a laminar flow that enables cells in suspension to pass the flow chamber individually and in a stable manner.

Optical System
This involves lasers, filtres and lenses. The light source is normally produces by one or more lasers. In most cytometers a gas laser (usually argon) is used with air refrigeration to produce a monochromatic light of 488 nm as the principal laser. The light excites the fluorochromes which produces scattering and this is used to provide information about the characteristics of the cell. The latest technologies use solid state lasers, which are replacing gas lasers as they use less power and are more durable. The presence of several lasers along with the primary source increase the range of fluorochromes that can be read from violet to far red. The scattered light and

fluorescence are collected via a system of filtres and mirrors that direct the signal to the sensors (photodiodes/photomulipliers).

Electronic-Informatics System
The photomultipliers convert disperse light (photons) to electric signals (voltage). These analogic signals are amplified and converted into digital signals for visualization via the computer. There are now new systems of conversion to digital values which allow the modification of compensation values of fluorescence signals and the subsequent application of lin-log scales.

The perfect integration of these systems allows for rapid accurate analysis of a large number of cells in a short time.

Process

The cell suspension is prepared previously with monoclonal antibodies conjugated with different fluorochromes (FITC, PE, PerCP, PECy5, APC etc) or fluorescent probes (PI, DHR, DiOC etc) and is introduced into the hydraulic system so that the cells pass the flow chamber individually, where they are intercepted by the light. Contact of the laser beam with the cell produces two different types of scatter; forward and side (90º). Forward scatter yields information on cell size, while side scatter reveals its complexity. The fluorochromes of the monoclonal antibodies or probes present in the membrane or the interior of the cell are excited by the laser light and generate fluorescence, which is collected by the optical system and subsequently processed and digitalized.

Applications

- Detection of subpopulations of cells (immuophenotyping)
- Apoptosis
- Cell Cycle
- Viability
- Funcational assays (metabolism, oxidation, proliferation, intracellular calcium, pH etc)
- Cell separation
- Multiplex assays (beads, arrays)