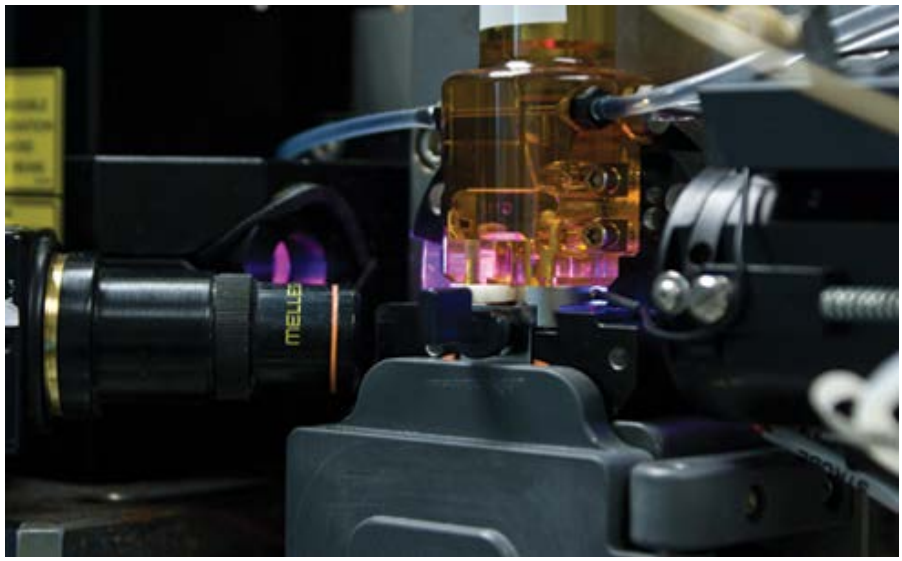
Flow cytometry and cell sorting

Written by Danielle Nicholson
Reviewed by Jack Dunne, Owl biomedical, Inc.

Of the techniques available to both the clinician and the researcher, few are as widely employed as flow cytometry. Cytometry or 'cell measurement' is a laser-based technology employed for counting, sorting, detecting biomarkers and protein engineering. By suspending cells in a fluid stream and passing them drop by drop, cell by cell through an electronic detection apparatus, a host of physical and chemical characteristics can be analysed using this advanced technology. With applications that range from common clinical laboratory tests, such as complete blood count and monitoring of the CD4 white blood cell count in HIV patients, to advanced, multi-colour flow cytometry used to identify subtypes of inflammatory cells active in diseases such as diabetes mellitus, flow cytometry has become rapid, flexible and sensitive.

Flow cytometry is performed on a variety of tissues, including peripheral blood, bone marrow aspirates, skin biopsies and tissue culture cell lines.



Did you know?

Subpopulations of cells thus identified can be isolated, or sorted, from the mixture at speeds of up to 40,000 cells per second and with a purity approaching 100%. This technique is called fluorescence-activated cell sorting, FACS.

MACS, magnetic-activated cell sorting (Miltenyi Biotech™) is another method used for separating mixed populations of cells. Here, the cells are incubated with magnetic nanoparticles coated with antibodies against a particular surface antigen. If the cells express this antigen, they attach to the magnetic nanoparticles. On a column placed in a strong magnetic field, cells attached to the nanoparticles (expressing the antigen) stay on the column (+) and the others pass through thus separating the mixture of cells (-).

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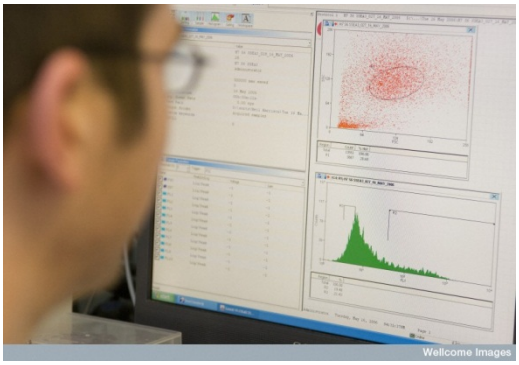
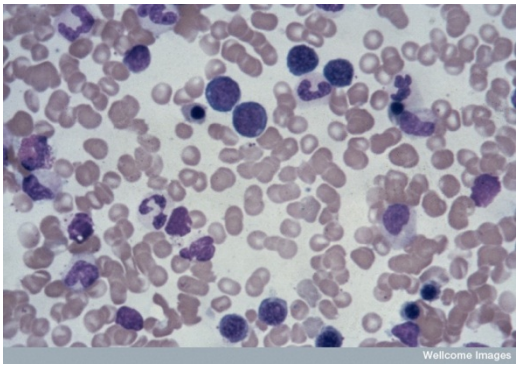
The Flow Cytometry Process

The process begins with the selection of fluorescent-labelled antibodies specific to cell-surface markers used to characterize the cell population of interest. These cell surface markers are typically glycoproteins called cluster of differentiation (CD) markers. They help separate and differentiate between cell subpopulations.

The sample is then processed, for example, with enzymatic degradation, centrifugation, and/or filtration to isolate the cells of interest, and the resulting cellular suspension is "stained" with the selected fluorescent antibodies. The single cell suspension is then introduced into the flow cytometer in a buffer solution called the sheath fluid, which flows toward a laser aimed at the solution's path. Because the flow of the liquid through the tubing is laminar, or sheet-like, and the diameter of the tubing narrows along its path, the cells are forced to line up single file as they approach the laser measuring single cells as they "flow" through the detector system. Flow cytometry allows for the identification and separation of subpopulations within a heterogeneous mixture of cells, such as bone marrow.





The fluorescent chemical bound to the antibody, called a fluorophore, is selected on the basis of the specific wavelength of laser present in the flow cytometer. If cells have the selected marker on the surface, the bound antibody– the fluorophore will absorb the laser energy and then release it in the form of a specific wavelength of light as the cells pass through the laser. The emitted light is detected by an optical system sensitive to various wavelengths, allowing for information on multiple surface markers to be read simultaneously. Subsequently the data is collected by an adjoining computer in the laboratory. Specially designed software graphically represents the distribution of the labelled cell populations in one-, two-, or three-dimensional formats for analysis.

Funded by the European Commission's FP7, REDDSTAR is a three year, 10 partner project that will comprehensively examine if stromal stem cells derived from bone marrow can safely control blood glucose levels while also alleviate damage caused by six diabetic complications. **www.REDDSTAR.eu**

SEVENTH FRAMEWORK PROGRAMME

REDDSTAR