desired combination of light-scattering properties and fluorescent markers are tagged with an electrical charge, and can be detected into a separate tube for further analysis. Advantages of flow cytometry include; high sensitivity, rapid as many cells can be analysed in a short time frame, allows for the discrimmination of cell subtypes, and allows multiple parameters to be assessed simultaneously. A limitation of flow cytometry is the inability to determine the location of antigen. Cells passing through the flow cell are interrogated by the laser. Scattered and fluorescent light is directed through the series of mirror and filters to the appropriate photo-diode or PMT. there, the induced voltages are digitized and represented by the software in graphical form. Since each parameter of light scatter and fluorescence is recorded for each cell detected, results can be displayed that include any combination of parameters for the cell population being studied. Cells entering the flow cell are focused by the encircling sheath fluid and exposed to the laser beam one cell at a time. Scattered and fluorescent light beams are visualised leaving the flow chamber.

Flow cytometry applications

The flow cytometer can be used to detect and to classify leukaemias in order to inform treatment. The flow cytometer can be used to measure T-cell subpopulations, an important diagnostic indicator in AIDS. when the number of CD4+ T cells in the blood of a patient with AIDS falls below a certain level, the patient is at high risk for opportunistic infections.

Intracellular cytokine staining

Intracellular cytokine staining measures cytokine production in individual cells by flow cytometry. A transport inhibitor, such as brefeldin A, is added to block the secretion of the produced cytokines. The cells are incubated on a 96 well microplate, fixed, permeabilized, then stained.

Bromodeoxyuridine-based assays

Bromodeoxyuridine (BrdU) assays are used to assess cell division/proliferation. BrdU is an analogue for deoxythymidine and when introduced into cells, is rapidly phosphorylated to bromodeoxyuridine triphosphate, an analogue for deoxythymidine triphosphate, and is incorporated in its place into newly synthesised DNA. cells that divide following BrdU incorporation can then be identified using fluorescently labelled antibodies to BrdU. BrdU assays can be combined with cell-surface and intracellular markers for multiparameter staining. The greater the fluorescence intensity, the greater the proportion of anti-BrdU bound, the greater the concentration of BrdU, thus the greater the rate of proliferation.

Carboxyfloorescein succinimidyl ester assay

Carboxyfluorescein succinimidyl ester (CSFE) assays can also be used to assess cell division/proliferation. The uncharged acetyl groups of the CFSE molecule enable CFSE to enter a cell and are then cleaved by intracellular esterases, so that CFSE remains trapped in the cytoplasm. In the cytoplasm CFSE molecules are covalently attached to intracytoplasmic proteins. The amount of fluorescence emitted is cut in half each time a CFSE labelled cell divides. CFSE fluorescence can therefore be used to measure the number of times a cell has divided since addition of the CFSE.