

Flowcytometry: Principle, Interpretation of result and its role in solid tumors

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Abstract

Principle: Flowcytometer is equipment which has become an integral part for the diagnosis of hematolymphoid malignancies. The word 'flow' means to pass, 'cyto' means cell & 'metry' means measurement. The flowcytometry is the passage of cells in single file (line or row) in front of a laser to be detected, counted and sorted. Cells labeled with fluorochrome are struck by laser to emit light (fluorescence) at varying wavelengths. Fluorescence (photon) is filtered and collected to convert the result into a digitalized (numerical) value. The reading is done by specialized software. Cells of interest flow through a liquid stream. The speed of flow of cell is higher than the speed of fluid carrying the cell (sheath fluid). This results into arrangement of cells in a single line (single file). This mechanism is called Hydrodynamic focusing. Up to 50000 cells per second can be measured, but the normal throughput is 1000 – 10000 cells/ sec (1). Detectors of photons are called photomultiplier tube. The detector placed in the line of light beam measures Forward Scattering (FSC) [size] and that of placed perpendicular to the light stream measures Side Scattering (SSC) [granularity, nuclear structure]. For all practical purposes cells falling in the range of 3-20 μ in diameter can be analyzed (2). Identification of rare cells at frequencies as low as 0.0001% has been reported (3). Information about physical and chemical structure of cells is gathered, which is used in diagnosis of diseases. Samples used are bone marrow, blood, body fluid and tissue. For tissue dissociation of cell are required to separate it in to single cell. Equipment for Tissue dissociation is available commercially. For example GgentleMACS™ OctoDissociator. Getting numerical values: Detectors collect photon, gets converted into electrical energy (current) to give a digitized value through "Analog to Digital Converter". Simply the detector is left to emit the photons. The time taken by the detector to emit all the photons gives a numerical value. This can be plotted on a graph. Common soft wares in used are Caluja, CellQuest, Flowjo, FCS Express (FCS: Fluorescence activated cell sorting), etc. Uses of Flowcytometer: Immunophenotyping to differentiate Acute leukemia into Lymphoid/ myeloid/biphenotypic/bilineage. In Lymphoid malignancies, whether it is T-cell or B-cell. It has numerous uses in research work like DNA cell cycle/tumor ploidy, Chromatin structure, Total protein, lipids, surface charge, Enzyme activity, DNA synthesis, DNA degradation, Gene expression etc.

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