JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

AN OVERVIEW OF FLOW CYTOMETRY-ITS WORKING, FLUOROCHROMES, DOT PLOT AND APPLICATIONS.

Dr.Rajeev Ramachandra Kolgi[1*]
1. Department of Chemistry and Biochemistry, Government Science College Bengaluru-01. Karnataka, India.

Abstract

An instrumental method for physical characterization of cells such as size, granularity based on their light scattering and fluorescence properties, is a popular technique used for both clinical and research purposes. This article covers working and brief applications of flow cytometry in research, cell cycle and ploidy analysis, cancer treatment, organ transplantation, platelet disorders and immunotyping.

Key words: flow cytometry, cell size, granularity, light scattering.

Introduction

Flow cytometry is a powerful tool that enables accurate measurement of cellular characteristics**.** Different cellular parameters can be analysed by the flow cytometry such as size, shape, density, DNA, RNA and protein contents. Analysis of apoptosis, membrane structure, ploidy analysis, immunophenotyping, cell cycle flow cross matching, stem cell enumeration, and diseases of RBC and platelets can be done using flow cytometry.

Working

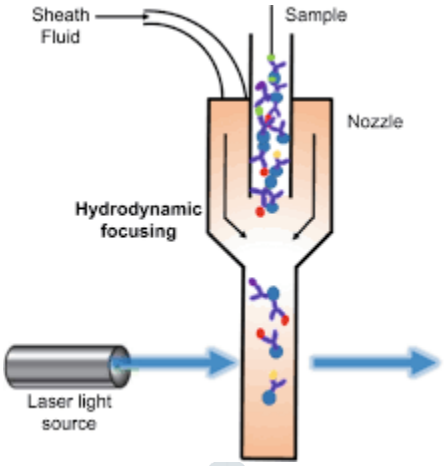


Fig 1: Flow cytometry

Laser emits light of one single wavelength hits the cell and as a result two events are possible forward and side scattering. Forward scattering is directly proportional to the size of the cell. Smaller cells will scatter less, whereas side scattering measures the cell complexicity.

Flow cytometry consists of three components the fluidics system, optical and electronics system. The fluidics system involves single file flow suspension of cells responsible for transporting the sample from the sample tube to the flow cell. The machine has flow cell in which there is a sheath fluid the sample runs from the middle of this fluid, a force is generated due to which the cells of the sample run one by one in the downward direction. The cells realeased from the flow cell come in contact with the laser light one by one in downward direction The optical system includes excitation light sources, lenses and filters used to collect and move light around the instrument and detector system. The electronics system are the brains of flow cytometry converts fluorescent signals into digital values, through computers the analysis of signals is done. The data can be displayed in the form of a two dimensional dot plot.

The more the complexity of the cell the more the light it scatters to the sides. The cell having less number of granules will scatter in the direction of less light. Generally small peaks are observed for the cells which are less granular.

Signal from a cell is detected in the form of Voltage pulse, is defined by its area, height and width. Height indicates the intensity of the signal and width is time taken for a cell to pass through the laser beam.

FLUOROCHROMES

Fluorochromes are chemical compounds that emit light upon light excitation, typically contain aromatic groups, planar or cyclic molecules

Fluorochromes used in flow cytometry may be linked to antibody molecules that will bind specifically to cellular components that may attach directly to specific cellular components in a non covalent way (Barnett et al 2013).The fluorophores used for labeling proteins, lipids, and other organic molecules bind covalently. Cyanines, succinimide esters belongs to this category. Fluorochromes used as markers of DNA, RNA bind non covalently. Acridine orange, olivomycins, chloromycin are few of the examples. Fluorescein isothiocyanate (FITC) and Phycoerythrin (PE) are the most frequently used fluorochrome pair for antibody labeling.

BRIEF APPLICATIONS OF FLOW CYTOMETRY

DIFFERNCIATION OF APOPTOSIS AND NECROSIS

Characterization of morphological and biochemical changes play a key role in distinguishing apoptosis from necrosis. Phosphotidyl serine is located on the inner leaflet of the cell membrane in normal viable cells, however it is translocated to the outer leaflet of plasma membrane in apoptic cells. This principle of irregular distribution of lipids is exploited for the detection of apoptosis cell by flow cytometry. The exposed phosphotidyl serine is detected by Ca^{+2} dependent Annexin V, and propidium iodide stains the necrotic cells, thus the apoptotic and necrotic cells can be differentiated.

Cell cycle analysis

Amount of DNA changes in cells as they progress through cell cycle can be analysed. The amount of DNA in a cell can be measured using flow cytometry and fluorescent dye that binds DNA.The greater the fluorescence the greater is the amount of DNA.Cells are disrupted and treated with protease later fixation of cells is done before staining, the cells are treated with RNase to separate it from DNA. Propidium iodide binds to DNA and amount of DNA present can be estimated.

The amount of DNA in a cell changes in G_1, G2 and S phase. Twice the DNA is present in G_2 phase compared to G_1 phase. Thus correlation of cellular DNA content with cell progression can be studied by flow cytometry.

Cancer treatment

Some therapeutic antibody drugs have been developed to target cells that have particular CD markers. Rituximab a monoclonal antibody by expressing the cell surface molecule CD-20 induces the killing of malignant B cells which

are destroyed through antibody dependent phagocytosis.The CD-20 antigens expressed on the surface of malignant B lymphocytes.

Flow cytometry utilizes maturation and development patterns during hematopoiesis as foundation for diagnosis of various disorders. Learning about CD markers is key in understanding the flow cytometry. The commonly used CD markers for particular type of cell are listed below.

Type of cell	CD markers
B lymphocyte	CD 20, CD22, CD 19
T helper cells	CD3, CD4.
Cytotoxic T cell	CD3, CD8
Natural killer cells	CD16, CD56
Stem cells	CD 34, CD38, CD 117
Granulocyte	CD 13, CD33, CD 15
Monocyte	CD 64, CD4, CD14, CD16

PLOIDY ANALYSIS

Ploidy determination is important in basic research and characterizing tumors. Due to replication errors cancer cells have irregular number of chromosomes (Darzynkiewicz et al 2010).

A large number of haploids usually need to be tested for breeding purposes. Ploidy analysis widely used to determine genome size and ploidy levels. This technique when coupled with Fluorescence activated cell sorting complete genome amplification can be done. By using several molecular markers haploid nuclei genotyping can be done by gene amplification (Pabla Aleza et al 2019).

ORGAN TRANSPLANTATION

Immunological rejection remains a major barrier to successful organ transplantation. Flow cytometry has become useful tool for monitoring immunological responses in transplant recipients. The flow cytometry determines the adequacy or inadequacy of immunosuppressive therapy through T cell receptor analysis (T.Shanahan 1997). Flow cytometry play a significant role for the immune monitoring by measuring the number of T, B and NK cells in patients who are maintained on immunosuppressive therapy after receiving organ transplant.

PLATELET DISORDERS

Normal platelets have two receptors GP IIB and IIIA. Whereas in case of Glanzmann thrombasthenia there is defect in platelet aggregation by blocking glycoprotein IIB and IIIA receptors on their platelets plasma membrane, hence it

gives negative results for CD 41 and CD 61.In acute lymphoblastic leukemia lymphoblasts are CD45 low or negative with low SSC. Monocytic leukemia typically express CD14 and CD64.

ASSESSMENT OF AUTOPHAGIC PROCESS

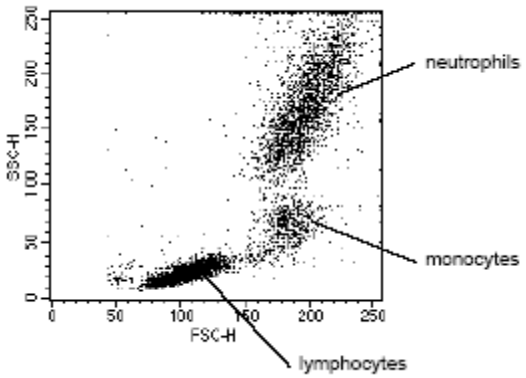
The use of flow cytometry to study the autophagic process has recently led to the development of numerous assays measuring various aspects of the autophagic process. This include the detection of autophagic markers, the microtubule associated protein LC3B.

Quantifying autophagy measuring LC3B puncta and autolysosome formation in cells using multispectral imaging flow cytometry (Pugsley H R 2017). The use of multispectral imaging flow cytometry has been gaining popularity due to its ability to aquire images of every cell and assesses autophagy in quantitative manner.

IMMUNOPHENOTYPING

Immunophenotyping analysis of heterogeneous population of cells on the markers present on the cell surface, cytoplasm, nucleus. Common technique for identification of acute leukemia's. Flow cytometry plays a role in the diagnosis of number of immunodeficiencies.

By using monoclonal antibodies conjugated to a fluorescent molecule and specific to different structure on lymphocytes the various subsets can be identified. To stain a sample antibodies are mixed with white blood cells and allowed to bind. RBC are removed by lysis.The resulting cells are allowed to run through flow cytometry, which counts and identifies the cells by passing them in a laser beam. Photomultifier tubes detect light scatter at various angles and the antigens on the cells can be identified. The data are electronically stored for analysis and can be displayed in the form of dot plot. By analyzing dot plot as shown in figure below it can be concluded that eosinophils, basophils, and neutrophils are having complex granularity compared to monocyte. Lymphocytes are found to small in size with fewer granularities.



Conclusion

Flow cytometry is powerful tool that enables rapid, accurate measurements of cellular characteristics and provides unparalleled insights into heterogeneity of cellular populations. Due to its great potential flow cytometry has been expanded to diverse fields of biological sciences and is routinely used in clinical, diagnostic and applied research.

References

1. Weir EG, Borowitz MJ (2001) Flow cytometry in the diagnosis of acute leukemia. Seminars in hematology 38: 124–138 6 Huh YD, Ibrahm S (2000) Immunophenotyping

2. Nagugen D, Diamond LW, Braylan RC (2003) Flwocytometery in Hematopathology: a visual approach to data analysis and interpretation. Humana Press New Jersey.

3. Z. Yang, D.J. Klionsky, Nat. Cell Biol. 12 (2010) 814–822. 475 [6] J.N. Ge, D. Huang, T. Xiao, Z. Wang, X.L. Li, H. Xiao, D.D. Tao, J.P. Gon, Chin. J. 476 Cancer 27 (2008) 102–108. 477

4. M. Komatsu, Y. Ichimura, Genes Cells 15 (2010) (2009) 923–933. 478

5. Wood BLaB, MJ (2010) the flow cytometric evaluation of hematopoietic neoplasia. In: Clinical diagnosis and management by laboratory methods (Henry). W.B. Saunders

6. Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, van't Veer MB (1995) Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia 9(10):1783–1786

7.Darzynkiewicz Z, Juan G, Li X, Gorczyca W, Murakami T, Traganos F. Cytometry in cell necrobiology: analysis of apoptosis and accidental cell death (necrosis) *Cytometry.* 1997;27:1–20.

8. Darzynkiewicz Z, Li X, Bedner E. Use of flow and laser-scanning cytometry in analysis of cell death. *Methods Cell Biol.* 2001; 66:69–109.

9.Webb E.S, Liu P, Baleeiro R, Lemoine N.R, Yuan M, and Wang Y. Immune checkpoint inhibitors in cancer therapy. *J Biomed Res*. 2018; 32(5):317–3.

10. Danova M, Torchio M, Comolli G, Sbrana A, Antonuzzo A, and Mazzini G. The role of automated cytometry in the new era of cancer immunotherapy. *Mol Clin Oncol*. 2018 ;9(4):355-3