REVIEW ARTICLE

Flow Cytometer: The Need of Modern Hematology Laboratory

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ABSTRACT

Indeed, laboratory diagnosis is rapidly changing from what it was in the past to the present. Nowadays, flow cytometer (FCM) has become a novel introduction to the modern diagnostic technique, mainly in the field of hematology. In case of acute leukemia, peripheral blood, bone marrow examination, cytochemistry and immunohistochemistry for differentiation of myeloid or lymphoid lineage is required, which is feasible by flow cytometry. It has dramatically improved the diagnostic efficiency and reduced the duration of sampling along with better diagnostic outcomes as well as provided efficient therapeutic monitoring of any drug or drug regimen. It has also opened some more sensitive therapeutic plans, like monitoring "Minimal residual disease (MRD)", which is not possible without FCM. Detection of MRD has led to improved overall survival of patients. It has also opened up huge opportunities for research, which has become an important part of academic curriculum nowadays. Considering the importance and absolute necessity for better outcomes in hematology, the knowledge of basic principle of FCM becomes indispensable. Here, we try to elucidate the elementary components of this technique and also highlight its uses.

Keywords: Flow cytometer, hematology, immunohistochemistry

Flow cytometer (FCM) is a powerful technique for diagnosing multiple characteristics of a single cell. This technique is based on both qualitative and quantitative estimation. In the present era, FCM has made the transition from a research tool to a prerequisite in a laboratory dealing with hematolymphoid malignancy. It is useful not only in diagnosis for initiation of therapy, but also in therapeutic monitoring during follow-up. With the advent of prognostically useful antibodies, use of multicolor flow cytometry has become of utmost importance in the diagnosis and management of hematolymphoid diseases.[1] Looking at the importance of this equipment, we need to have an elementary knowledge of its principle.

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Literally, the word 'flow' means to pass, 'cyto' means cell and 'metry' means measurement. Thus, flow cytometry translates to the passage of cells in a single file (line or row) in front of a laser beam to be detected, counted and sorted. The cells are labeled with fluorochromes and when excited by laser beams of appropriate wavelength, they emit light (fluorescence), which is filtered and collected. Specialized software converts the result into a digitalized (numerical) value.[1]

Integral Components of FCM: The key components are being described below:

Fluidics: Cells of interest flow through a liquid stream called the sheath fluid. The speed of cells is higher than the speed of sheath fluid. This results in streamlining of cells in a single line (linear file). This mechanism is called hydrodynamic focusing. Up to 50,000 cells/sec can be measured, but the normal throughput is 1,000-10,000 cells/sec.[2]

Interrogation point: Inside a FCM, cells in suspension are drawn into a stream created by a surrounding sheath of isotonic fluid. This creates a laminar flow which enables the cells to pass individually through an interrogation point. At this point, fluorochrome tagged cells pass through a laser beam causing its light (and that of the fluorochromes present) to scatter in all directions. These are collected through optics that

direct the light to a series of filters and mirrors, which isolate particular wavelength bands.[3]

Scattering of light: Physical characteristics of a cell, such as size and internal complexity, like granularity, can help identify different cell populations like blasts, plasma cells, monocytes, etc. This diversity in different cell populations is identified using two parameters - forward and side scatter. Forward scatter (FSC) is based on two properties: size and refractive index. The FSC intensity is based on the particle's size and can also be used to distinguish between cellular debris and living cells. Side scatter (SSC) is based on the granularity or internal complexity. The more granular the cell, the more side scatter light is generated. Dead cells have lower FSC and higher SSC than living cells. The detector placed in the line of light beam measures forward scattering (FSC) [size] and that placed perpendicular to the light stream measures side scattering (SSC) [granularity, nuclear structure].[3-5]

Electronics: The light signals are detected by photomultiplier (PMT) tubes and undergo digitization for computer analysis. Figure 1 depicts a schematic diagram of the components of a FCM.

For all practical purposes, cells falling in the range of 3-20 μ diameter can be analyzed using this technique. Identification of cells at a frequency of as low as 0.0001% has been reported to be possible by flow cytometry. Fluorescent dyes may bind with different cellular components like DNA or RNA. Antibodies conjugated to fluorescent dye have the potential to bind specific proteins on cell membranes or inside the cells. When a fluorochrome labeled cell is passed through a light source, the fluorescent molecules get excited and achieve a higher energy state. As they return to their resting states, the fluorochromes emit light energy at different wavelengths.[3]

Several properties of a cell can be measured simultaneously by using multiple fluorochromes. Each fluorochrome with similar excitation wavelengths and different emission wavelengths (or colors) enables the measurement of several cell properties. Most commonly used dyes are propidium iodide, phycoerythrin, fluorescein, etc. Tandem dyes with internal fluorescence resonance energy transfer can create even longer wavelengths and more colors.[6]

Information about physical and chemical structure of cells gathered is used in diagnosis of diseases. Samples used are bone marrow aspirates, blood, body fluid and tissue. For tissue samples, dissociation to single cells is required. Equipment for tissue dissociation is available commercially.[7,8]

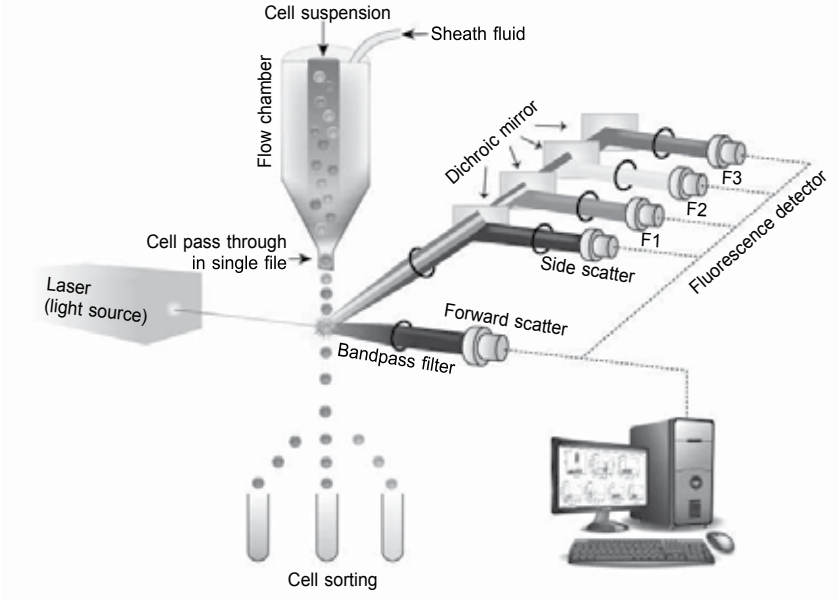


Figure 1. Schematic diagram of a flow cytometer.

Getting numerical values: Photons collected by detectors get converted into electrical energy (current) to give a digitized value through "Analog to Digital Converter".[9] Common softwares used are Caluja, CellQuest, Flowjo, FCS Express (FCS: Fluorescence-activated cell sorting), etc.

Gating: This refers to the isolation of subsets of cells on a plot. Gates can be visualized as barriers placed around cell populations having common characteristics like scatter or cluster of differentiation (CD) marker expression to isolate, quantify and study these subpopulations. Initially, cells are gated on the basis of FSC and SSC properties. After initial isolation and quantification of the population of interest, further division into subpopulations based on surface (or intracellular) markers is done. Back gating is a method for elimination of nonspecific staining and false positives. Here, the population identified by a particular gate is gated again on entirely different parameters for confirmation.[3,5,10]

In Figure 2, we can understand the different cell clusters produced after running in FCM. Each cluster is gated using specialized software (caluja) and it is analyzed for any abnormality. Cluster of blasts can be identified in the image and its percentage can be used for analyzing presence or absence of malignancy.

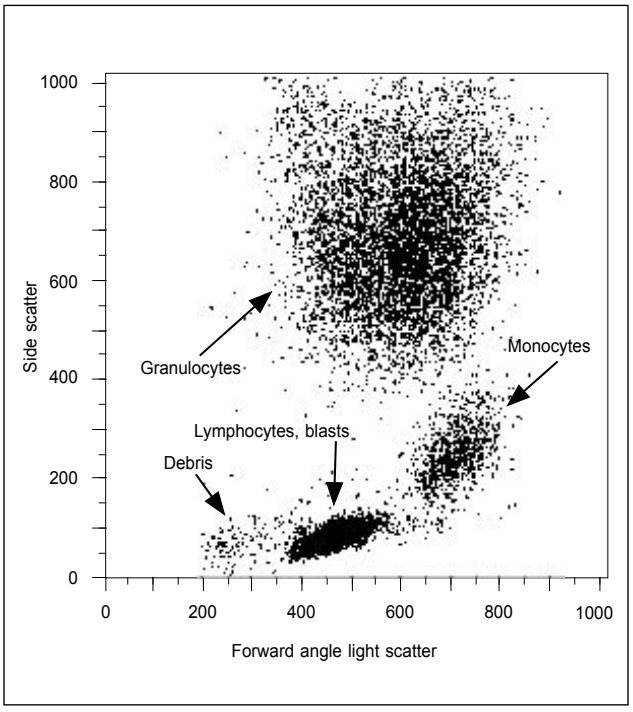


Figure 2. Dot plot of forward light scatter and side scatter.

Image source: Riley RS, Idowu M. Principles and applications of flow cytometry. Available at: http://www.flowlab-childrens-harvard.com/yahoo_site_admin/assets/docs/PRINCIPLESANDAPPLICATION.29464931.pdf

COMPARISON OF FLOW CYTOMETRY WITH IMMUNOHISTOCHEMISTRY

Immunohistochemistry, in the past decade, has been popularly called as the brown revolution, due to its surmount importance in diagnosis.

However, the emergence of flow cytometry has made it possible to overcome the shortcomings of immunohistochemistry (IHC). It is a time-consuming technique restricted by the use of limited number of CD markers on a particular tissue section. The quantification of cells and enumeration of the different cell subtypes are also not possible via this technique. On the other hand, using flow cytometry, multicolor immunophenotyping is possible whereby large number of CD markers can be used simultaneously. This makes it possible to analyze numerous cells at the same time. Numerous parameters can be examined at once. Sometimes, the presence of two co-existent pathologies may be detected. Dead cells may also be gated using the analysis.

There is no need of a tissue biopsy. Even small quantities of samples such as body fluids, peripheral blood or fine needle aspirate specimens can be processed and identification of cells at a frequency as low as 0.001% is possible. Studies show that it is a rapid process requiring less effort. The only drawback of FCM as compared to IHC is that unlike in IHC, the localization of antigen (nuclear, cytoplasmic or membranous) is not possible in FCM.[9,11]

USES OF FLOW CYTOMETRY

Flow cytometer is used to detect the size of cells and also to incorporate various hematological parameters, like to enumerate red blood cell (RBC), platelets size and white blood cell (WBC) based on light scatter. Moreover, bone marrow aspirate, cerebrospinal fluid and peripheral blood are all specimens that can be analyzed using flow cytometry (only viable cells can be analyzed). If the sample does not carry viable cells, flow cytometry analysis does not seem to be an option.[12,13] FCM is also used in predicting leukemic cell lineages in peripheral blood of dogs and cats.[14]

Measurement of DNA content was one of the earliest uses of flow cytometry. A 67% increase in DNA content was noted in malignant cells compared to nonmalignant cells. Tumor cells that are not diploid have an abnormal number of chromosomes that lead to an aneuploidy

Table 1. The Basic CD and Immune Cells Designation

Immune cell type	CD designation
B-cell	CD19, CD20, CD22
T-cell	CD1, CD3, CD4, CD5, CD7, CD8
Myeloid cells	CD13, CD33, CD117
Blasts	CD34, CD38, HLA DR
Monocyte	CD14, CD64
Macrophage	CD68, CD14, CD64, CD11b
Granulocytes	CD13, CD15, CD16, MPO
Megakaryocytes	CD41, CD42, CD62
RBC	CD36, CD235a

cell. Moreover, flow cytometric analysis of nuclear DNA content will demonstrate histogram peaks for nuclei of the sample that is in different phases of the cell cycle - G_0/G_1, S-phase and G_2/M. Thus, it is used to determine the DNA content and ploidy of tumors. Retrospective studies examined the relationship between DNA abnormalities and duration of survival in patients with oncologic disease in an attempt to predict prognosis.[3,15-17]

Phenotyping, the identification of particular observable characteristics, is one of the uses of flow cytometry in oncology. There are many phenotypic designations to differentiate healthy cells from tumor cells. Table 1 provides basic CD specification for common immune cell phenotypes. In addition, flow cytometry is used to identify the lineage of leukemic blood cells or to classify a lymphoma or leukemia as either T or B cells, which provides prognostic information of the disease. It also examines functions of natural killer cells and T-cell, which have shown some correlation with psychological distress of patients.[10,18-21]

Flow cytometry is used for assessment of the affected lymphoid tissue which is important for staging and classification of malignant lymphomas.

It is also used in laboratory diagnosis of immune-mediated cytopenias, like hemolytic anemia, thrombocytopenia and neutropenia, etc. Moreover, its sensitivity is more in comparison to conventional direct agglutination test (e.g., Coombs for immune-mediated hemolytic anemia [IMHA]). It is also used to monitor the progression of acquired immunodeficiency syndrome (AIDS) in humans with human immunodeficiency virus (HIV).[22,23]

CONCLUSION

To summarize, in the current scenario, flow cytometry forms an integral part of diagnosis, especially in hematolymphoid malignancies. It further aids in conducive disease management by guiding therapeutic protocols. Due to the accuracy and precision of this technique and its ability to use samples obtained from minimally invasive methods, like peripheral blood sampling, it is also widely used in assessing the response of the patient to treatment regimens. As of date, it can be said to be indispensable to the laboratory. In the times to come, FCMs can be expected to continue to decrease in energy consumption as well as size and increase in detection and precision measurements.

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