معامل التأثير العربي 2.23 لسنة 2025	مجـلـة الـتــربــوي Journal of Educational	
العدد 28 ــ يناير 2026		

مجلة التربوي

مجلة علمية محكمة تصدر عن كلية التربية الخمس

جامعة المرقب

العدد الثامن والعشرون (28)

يناير 2026م

- المجلة ترحب بما يرد عليها من أبحاث وعلى استعداد لنشرها بعد التحكيم .
- المجلة تحترم كل الاحترام آراء المحكمين وتعمل بمقتضاها .
- كافة الآراء والأفكار المنشورة تعبر عن آراء أصحابها ولا تتحمل المجلة تبعاتها .
- يتحمل الباحث مسؤولية الأمانة العلمية وهو المسؤول عما ينشر له .
- البحوث المقدمة للنشر لا ترد لأصحابها نشرت أو لم تنشر .

مجلة التربوي
Journal of Educational
معامل التأثير العربي 2.23 لسنة 2025
العدد 28 – يناير 2026

ضوابط النشر:

يشترط في البحوث العلمية المقدمة للنشر أن يراعى فيها ما يأتي :

- أصول البحث العلمي وقواعده .
- ألا تكون المادة العلمية قد سبق نشرها أو كانت جزءا من رسالة علمية .
- يرفق بالبحث تزكية لغوية وفق أنموذج معد .
- تعدل البحوث المقبولة وتصحح وفق ما يراه المحكمون .
- التزام الباحث بالضوابط التي وضعتها المجلة من عدد الصفحات ، ونوع الخط ورقمه ، والفترات الزمنية الممنوحة للتعديل ، وما يستجد من ضوابط تضعها المجلة مستقبلا .

تنبيهات :

- للمجلة الحق في تعديل البحث أو طلب تعديله أو رفضه .
- يخضع البحث في النشر لأولويات المجلة وسياستها .
- البحوث المنشورة تعبر عن وجهة نظر أصحابها ، ولا تعبر عن وجهة نظر المجلة .

Publication Guidelines:

Research papers submitted for publication must adhere to the following:

- The principles and rules of scientific research.
- The material must not have been previously published or be part of an academic thesis.
- The research must be accompanied by a linguistic endorsement according to a prepared template.
- Accepted research will be edited and corrected according to the reviewers' opinions.
- The researcher must comply with the journal's guidelines regarding the number of pages, font type and size, time periods granted for modifications, and any future guidelines established by the journal.

Notices:

- The journal reserves the right to edit the research, request modifications, or reject it.
- The publication of research is subject to the journal's priorities and policies.
- Published research reflects the views of the authors and does not represent the views of the journal.

مجلة التربوي
Journal of Educational
معامل التأثير العربي 2.23 لسنة 2025
العدد 28 ــ يناير 2026

Short Review of Flow Cytometry (FCM)

Nada Mukhtar Rifai[1], Wedad Mohamed Omran Alkut[2], Mrim Omran Ashkorfo[3], Njia Mild A. Rajab Aser[4]

Department of Biology - Faculty of Sciences - Elmergib University [1,2,3,4]

nmrifai@elemergib.edu.ly [1] wmalkut@elmergib.edu.ly [2]

moashkorfo@elmergib.edu.ly [3] nmrajab@elmergib.edu.ly[4]

Abstract

Flow cytometry is a powerful lab technique, that quickly analyses physical and chemical features (such as size, shape, and internal complexity) of single cells as they flux in a fluid flood through lasers. Individual cells or particles suspended in a buffer are passed through one or more lasers. Two types of tests are performed on each cell/particle: visible light scattering and one or more fluorescence parameters. Both tests are run independently. Flow cytometry is a fundamental tool with many applications and has become essential in fields as diverse as immunology, cell biology, molecular biology, cancer research, and infectious disease surveillance (1).

Furthermore, it allows sorting out different populations of cells from a variety of tissues at a same given time for further analysis. The significant development in the flow cytometry technique, since its initiation in the 1960s, has been a crucial component of modern clinical diagnostics and biomedical research.

Key words: Flow cytometry, fluorescence, cells, lasers, and fluid

Introduction

Flow cytometry is a vital tool for use in biomedical study and clinical research laboratory. Flow cytometers count a diversity of cell belongings and supply a means of distinguishing individual cells within a population. The term ccytometry refers to a set of means for quantitatively investigating and analyzing a cell's population. It is used flaming labelling systems, such as immunohistochemistry, to obtain data about a large amount of cells (1). Essentially, a FCM functions like a fluorescence microscope by analysing elements that are in motion within a suspension. Fluorescence microscopy employs a wide variety of chromophore probes to study cellular components and is particularly favored when examining a minor quantity of cells is enough or as only qualitative data are needed. FCM aids in characterizing various cell categories such as: organelles, nucleic acids, enzymes, and other

substances present in cell populations. Similar to microscopy, flaming analyses are utilized to attach to the objects being studied. (2). By means of the name proposes, Flow FCM is a technique uses laser beam to detect a single cells' flow in liquid to identify a number of different parameters (3). The term "flow cytometry" refers to the process of measuring individual cells (cyto) as they pass through a number of detectors. Over the past few decades, flow cytometry has been used more often in research and clinical settings (4). Numerous disciplines, including pathology, immunology, biology, genetics, parasitology, immunology, microbiology, biological oceanography, and medical research, use flow cytometry (5). Fluorescence-tagged antibodies are employed in molecular biology. These particular antibodies attach to antigens on the target cells and aid in providing details about particular traits of the cells under study in the FCM (6). It is widely used in the medicine field, particularly in haematology, tumour immunology, transplantation, genetics and chemotherapy (7).

Flow Cytometry Principle

The fundamental principles of physics, such as fluidics, optics, and electronics, are essential to all types of cytometry (8). A fast method for detecting cells as they proceed through a liquid fluid under a laser light beam is called FCM. The visual and fluorescence properties of single cells or any other material, including nuclei, chromosomal arrangements, and microbes, are calculated by FCM. Physical characteristics can be settled particular cell populations such as size, which is represented by forward angle laser scatter, and internal density, which is represented by right-angle scatter (9). Different immunofluorescent stains can attach with special cellular elements and antibodies, which could be linked to the protein or antigen of interest (10).

Ssuspended cells are drawn into a flow cell where, bounded by a slight stream of liquid, they flow individually through a directed laser beam of a particular wavelength aimed toward a hydrodynamic liquid flow (11) .Then, the current distributes across the light beam, numerous detectors are aimed, one of them is Forward Scatter (FSC), which is in the same direction with the light beam and several would be vertical to it (Side Scatter) (SSC) and one or more fluorescent sensors (12). Every floating unit from 0.2 to 150 micrometres that passes within the beam distributers, and the fluorescent particles are stimulated to a greater energy condition (13). At the rest state, fluorochromes release light energy at longer wavelengths. By using many fluorochromes, with same excitation wavelengths and

various release wavelengths, different cellular features can be determined at the same time.

The released light is emitted in all orientations and the light gathered by optics that direct it to filters and dichroic mirrors, which in turn separate certain bands of wavelengths (figure 1). Photomultiplier tubes record light signals and digitized for computer interpretation (4) .

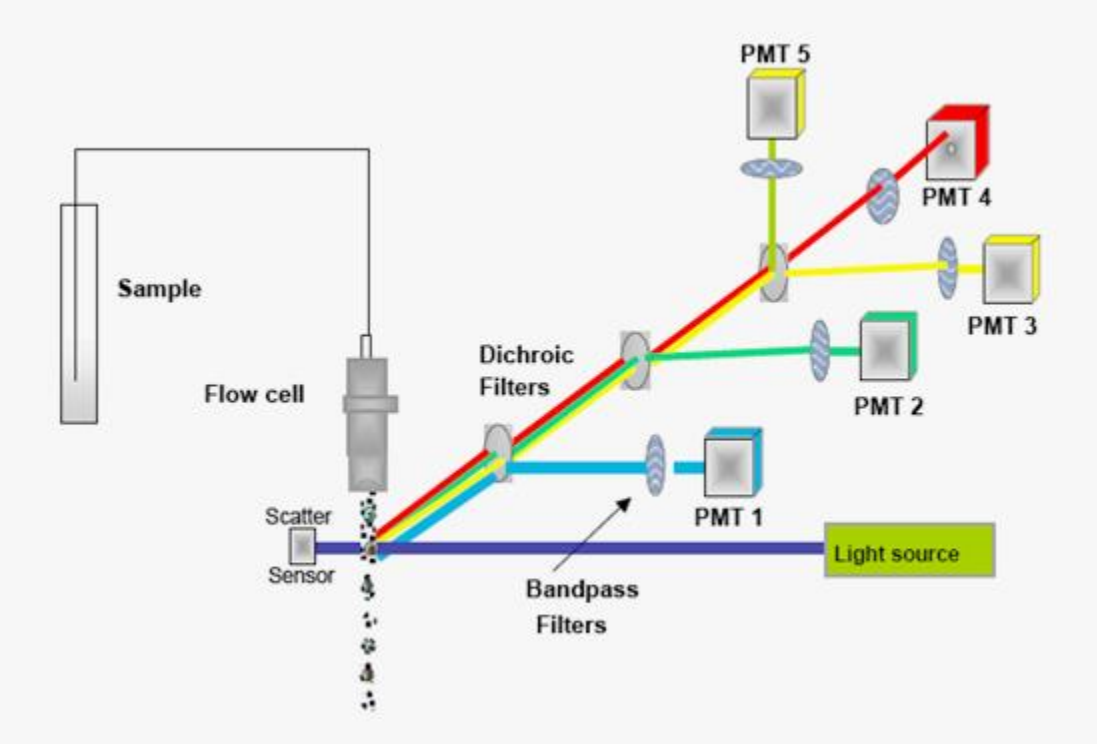

Figure1: diagram of flow cytometer. (From Murphy RF (2006)).

Flow Cytometry Clinical Applications

FCM is a versatile method for assessing a wide range of cellular properties and activity levels. Flow cytometry can quantify cell size, surface area, cell contents (such as granularity in immune cells), and cell cycle stage. The number of signalling chemicals within a cell, the phosphorylation state of target proteins, and calcium flux can all be measured to determine cellular activity. FCM is commonly utilized to calculate cells, with different cells identified by the expression of unique markers. This is especially useful for immunological cells, whose proportions can vary drastically between disease states . The variety of uses of this technique is very broad

to be reviewed in detail here, only a few linked topics are covered. Premier usages of FCM were the analysis of the cell cycle by measuring cellular DNA composition, this technique is one of the most significant clinical applications (15). Studying the DNA content provides a lot of information regarding the cell cycle. Many studies are conducted by applying specific DNA stain. In every analysis, the fluorescence emitted is relative to the DNA content display in the cells, taking into account the staining conditions (16). Thus, the effects of the additional stimuli on the cell cycle. For instance, medication therapies or transfected genes. Instead, we can see the DNA profile of a subset of cells specified by our antigen (17), or by combining the measurement of DNA content with the quantification of an antigen to quantify its expression during the cell cycle (17).

Each cell have approximately the same amount of cellular content. For an instant, the DNA forms a peak. In a standard DNA histogram, one peak is the G1 phase and the other with twice the channel value is the G2/M phase of the cell cycle (18). Consequently, all cells in the G1 phase absorb approximately the similar volume of stain and all flame in a specific channel. Propidium iodide (PI) is a fluorescent dye that preferentially binds and detected cellular DNA (19). Additionally, Cells are fixed in ethanol for example Hoeschst 33342, DAPI, mithramicin, propidium iodide, 7 aminoactonomycin D, DRAQ5 and TO-PRO-3. Moreover, these dyes used to computed stained DNA and split live and dead cells in unfixed samples (20). When DNA binds, fluorescence increases dramatically. It needs permeabilization of the plasma membrane (21). The usefulness of DNA scattering in cytokinetics occurs from the fact that it is visible. Gl phase cells have a stable (1x) DNA content, G2+M phase cells have a constant (2x) DNA content. S phase cells have a middle DNA content and form a range between the peaks of the two phases (22). Furthermore, there are a lots of techniques can be used to investigate cell scattering, for example; custom software and mathematical algorithms. Histograms are then operated to assess cellular distribution across a series phases of cell cycle (23). the proportion of cells in the G1, S, G2/M phases displays by the histogram. Though, various implications can be made about the progress of cells cycle, the evidence obtained is primarily static (8).

Moreover, the most popular flow cytometric tests of the cell cycle is built on bivariate analysis of DNA content with respect to 5-bromo-2-deoxyuridine (BrDU) which is combined into DNA in position of thymidine through cell progression in S phase (24). In both vitro and vivo, living cells have to be exposed to BrDU. The ingested sign can then be exposed immunocytochemically (16).

Revealing of BrdUrd in DNA provides information on cell cycle kinetics when samples are taken at different time intervals (25). The bivariate investigation of the DNA in relation to the incorporated BrDU thus shows cells in S phase; that is due to the differences in DNA content and for the cells have incorporated the analog (24). Cells with the same DNA content but in separate metabolic chambers cannot be distinguished by monoparametric analyses. Nonetheless, multiparametric analyses are typically employed to enhance cycle phase classification (16). Therefore, multiparametric analysis of DNA and RNA (26) or DNA and proteins (27), can be used to better evaluate the link between DNA division and cell growth. For instance, in certain biological systems, RNA content can be used as well.

Furthermore, phase scrutiny using FCM is intriguing for both basic and medicinal research. It enables in vitro testing of novel medications, such as antitumoural factors (22), in pharmacology to create novel treatments. Pathological cells can be identified in oncology by examining the DNA content and their distribution throughout different phases (28).

Furthermore, a lot of research has been done on immunolabelling, which often has two peaks. Unlabelled cells are shown in one peak (negative), and labelled cells are shown in another peak (positive). To determine the number of cells in a peak, these peaks can be evaluated using either flow cytometry or numerical fits (29). However, in a 2-parameter scatterplot with cell property magnitudes as onward and side scattering amount, the channels are distributed on the Yand X axes, while every cell has a specific intensification and is characterized by a point on a scatterplot.

As a result, subpopulations of cells with identical characteristics are displayed as clusters that might be replaced by sketching lines surrounding them (gate) to establish the number of cells in each cluster, as several cells with the same features, share the same point position (29). However, this method has had an important effect in several scopes. There is a small number of technologies capable of evaluating several parameters on miniature samples in a limeted period of time. There are some limitations to flow cytometry; This breaks down into fundamental physical constraints such as particle speed (laminar flow is preserved as soon as the Reynolds quantity is lower than 2300 (30). Therefore, the highest speed that keeps laminar stream is inversely relative to the pipe diameter (30). Fluorescence efficiency is because the fluorescence of a fluorochrome cannot be improved by increasing the excitation power.

One of the main limitations is the limited fluorescence lifetime (30) .The practical limits of fluorescence measurements include the need for suitable linearity to achieve quantitative results. Logarithmic amplifiers must have a known response for accurate data use, particularly at high signal levels, where measurement accuracy is affected by the sample flow alignment with the excitation light. Additionally, fluorescence sensitivity is critical for distinguishing weakly fluorescent populations from background noise at low signal levels (30).

Future Directions

1. Mass cytometry: Cell analysis using mass spectrometry (31-32). A novel method called mass cytometry analyzes cells using mass spectrometry, enabling the detection of up to 100 parameters .

2. Single-cell analysis: A thorough examination of a single cell (33). Flow cytometry and other methods are used in the developing field of single-cell analysis to thoroughly examine individual cells .

3. Image cytometry: Integrating image methods with flow cytometry (34-35). Image cytometry is a new technique that combines flow cytometry with imaging techniques, allowing for the analysis of cell morphology and fluorescence (36-37).

Reference

1- Heller, D. (2004) A Review and Applications of Flow Cytometry. University of Illinois at Urbana-Champaign.1-13.
2- Cunninqham, R, E (1998) Flow Cytometry. AFIP, 653-667 .
3- Givan, A. (2001) Flow Cytometry: First Principles. New York: Wiley-Liss. p273.
4- Brown, M, and Wittwer, C, (2000) Flow Cytometry: Principles and Clinical Applications in Hematology. Clinical Chemistry 46:8(B). 1221–1229.
5- Darzynkiewicz, Z., & Juan, G. (2001). Cell cycle analysis by flow cytometry. Methods in Cell Biology, 63, 261-280.
6- Nirav, K. Gheewala, Gautam, B. Sonara, Tejal, N. Gheewala, Renuka, R. Parmar, Gautam H. Viradiya, Lodhiya D.J. (2009) Flow cytometry: A cytometric technique for living cells. Pharmacy. 3:114-119
7- Freedman, J, and Lazarus, A, H. (1995) Applications of flow cytometry in transfusion medicine. 9: 87-109.

8- ormerod, M,G. (1994) Analysis of DNA: general methods. In flow Cytometry: A Practical Approach, 2nd ed. (Ormerod, M.G.,ed.) IRL Press, Oxford, UK,pp. 118-135.
9- Robinson, J. P., & Roederer, M. (2015). Flow Cytometry: The Basics. Springer.
10- Mandy, FF., Bergeron, M., Minkus, T. (1995) Principles of flow cytometry. Transfuse Sci. 16:303–14.
11- WWW. biology. Berkeley.edu/crl/ flow Cytometry basic.
12- Steinkamp, J, A., (2002) Flow Cytometry. LIFE SCIENCES. P 1-5
13- Givan, A, L,. (2001) Principles of flow cytometry: an overview. Methods Cell Biol. 63:19-50.
14- Murphy RF (2006) Basic flow cytometry theory. Flow cytometry talks. Purdue University Cytometry Laboratories .
15- Nunez, R., (2001) DNA Measurement and Cell Cycle Analysis by Flow Cytometry. Curr. Issues Mol. Biol . 3(3): 67-70 .
16- Jayat, C, Ratinaud, M, (1993) Cell cycle analysis by flow cytometry: Principles and applications. Biol Cell. 78, 15-25.
17- Landberg, G, Ten, EM, Roos, G, (1990) Flow cytometric multiparameter analysis of proliferating cell nuclear antigen/cyclin and Ki-67 antigen: a new view of the cell cycle. PubMed med. 187(1):111-8.
18- Chapman G, .V (2000) Instrumentation for flow cytometry. J Immunol Methods. 243:3-12.
19- Belloc, F., Dumain, P., Boisseau, M., R, Jalloustre,C, Reiffers , J Bernard , B Lacombe F (1994) A flow cytometric method using Hoechst 33342 and propidium iodide for simultaneous cell cycle analysis and apoptosis determination in unfixed cells. Cytometry, 1;17(1):59-65 .
20 - http:/ science. Cancerreseachuk.org/sci/facs major apps/cell cycle analysis
21 -Melamed, R, Lindmo, T, Mendelsohn, M.L. and et al (1990) Flow Cytometry and Sorting, 2nd ed. Wiley-Liss, New York. 22- 32.
22- Gray, J, W., Dolbear, F., Pallavicini, G, Beisker, W. and Aldman , F. (1986) Cell cycle analysis using flow Cytometry. 49, 2, 237-255.
23- Pierrez , J, and Ronot , X (1992) Flow cytometric analysis of the cell cycle: mathematical modeling and biological interpretation. Acta Biotheoretica. 40: 131-137 .
24 -Rabinovitch, P, S. Kubbies, M. Chen, Y. C, and Hoehnh (1988) BrdU-Hoechst Flow Cytometry: A Unique Tool for Quantitative Cell Cycle Analysis. Acad mic. 174. 309-318.

25- Ormerod, M, G, (2000) Flow Cytometry: A Practical Approach. Oxford University Press, Oxford.
26- Darzynkiewicz, Z, Sharpless T, Staiano-Coico L, Melamed MR. (1980) Subcompartments of the G1 phase of cell cycle detected by flow cytometry. Proc Natl Acad Sci USA 77:6696-6700.
27- Darzynkiewicz, Z., Crissman H.A. & Robinson, J.P.(1994) Flow cytometry In Methods in Cell Biology. 2nd edn. 2, B.
28- Bowen, W.P. & Wylie, P.G. (2006).Application of laser-scanning fluorescence microplate cytometry in high content screening. Assay Drug Dev. Technol. 4, 209–221.
29- Ochatt, S, J. (2006) Flow cytometry (ploidy determination, cell cycle analysis, DNA content per nucleus). Medicago. 1-13.
30- Hoffman, R, A.(2008) Flow Cytometry: Instrumentation, Applications, FutureTrends and Limitations. Springer Ser Fluoresc. 308-339.
31- Bandura, D. R., & Baranov, V. I. (2009). Mass cytometry: a new approach to single-cell analysis. Analytical Chemistry, 81(16), 6813-6822.
32- Spitzer, M. H. & Nolan, G. P. (2016).Mass Cytometry: Single Cells, Many Features. Cell 165, 780–791.
33 -Bendall, S. C., & Nolan, G. P. (2012). From single cells to deep phenotyping: the evolution of mass cytometry. Nature Methods, 9(7), 651-658.
34- George, T. C., & Basiji, D. A. (2006). Distinguishing modes of cell death using the ImageStream multispectral imaging flow cytometer. Cytometry Part A, 69A(10), 1053-1063.
35 - McKinnon K. M. (2018). Flow Cytometry: An Overview. Current protocols in immunology, 120, 5.1.1–5.1.11.
36 - Rees, P., Summers, H. D., Filby, A., Carpenter, A. E. & Doan, M. (2022). Imaging flow cytometry. Nat. Rev. Methods Primer 2, 1–13.
37- Psarra, K. & Fleva, A. (2023). History of Flow Cytometry. in Intraoperative Flow Cytometry, 3–7 .