Nguyen D, Diamond LW, Braylan RC. Flow Cytometry in Hematopathology: a Visual Approach to Data Analysis and Interpretation


Field of medicine: Hematology, immunology.


Audience: Clinicians and researchers in the field of hematology, pediatricians, immunologists, hematopathologists, and specialists in laboratory medicine requiring novel insights in the immunophenotyping of hematopoietic malignancies.

Purpose: Flow cytometry (FCM) immunophenotyping has been incorporated into hematology laboratories as a necessary diagnostic and prognostic tool. In the recent years, advances have been made in both instrumentation and commercially available reagents. This progress was accompanied with the development of new FCM clinical applications in a wide spectrum of hematologic disorders. This book provides a systematic clinically oriented guide to FCM data analysis and interpretation, crucial for obtaining reliable and reproducible data, and could certainly serve as a valuable source of information in FCM testing. As authors stressed in the preface, the book is aimed to “simplify the classification by utilizing the graphical patterns of phenotypic expression and the results of DNA cell cycle analysis,” together with other “relevant clinical/laboratory data.”

Content: The book includes 5 well illustrated and clearly written chapters: 1) Approach to FCM: General Considerations; 2) FCM Immunophenotyping and DNA Analysis: Practical Aspects That Can Affect Data Analysis and Interpretation; 3) FCM Data Analysis on Nearly Homogeneous Samples; 4) FCM Data Analysis on Heterogeneous Specimens; and 5) FCM Interpretation and Reporting. The text is easy to follow and includes clearly presented information and practical instructions for FCM testing, with numerous, mostly black and white dot-plots and histograms. In addition, the book provides a list of 100 case studies with an accompanying CD-ROM. Starting from the third chapter, case studies are given as examples of the practical application of certain pattern analysis, such as different subtypes of myeloid or T- or B-cell leukemia, several forms of lymphoma, some viral infections that affect hematopoietic cells, and other hematopoietic disorders. At the end, there is a list of selected reading in the field of FCM, Appendix with instructions how to use the case study CD-ROM, and Index of basic terms, which enables quick orientation.

The first chapter, entitled Approach to FCM: General Considerations, gives a short introduction to general aspects and basic principles in FCM testing, potential applications and pitfalls and, finally, an overview of the immunologic markers currently in use in the FCM laboratory.

The following chapter, entitled FCM Immunophenotyping and DNA Analysis: Practical Aspects That Can Affect Data Analysis and Interpretation, includes practical guidelines for the critical steps in FCM testing, starting with the sample selection, preparation, and staining. The chapter continues with detailed instruction on how to perform data acquisition, antibody panel design, FCM immunophenotyping data presentation, and how to approach DNA data analysis. Each subsection describes specific points in FCM
testing, such as instrument calibration, color compensation, exclusion of nonviable cells, antibody selection, analysis panels, etc.

The next two chapters, entitled FCM Data Analysis on Nearly Homogeneous Samples and FCM Data Analysis on Heterogeneous Specimens, focus on the practical approaches for applying FCM testing on different types of hematopoietic cell specimens in a variety of hematologic diseases. This part of the book is probably the most important, especially for the experienced users who already acquired basic concepts of FCM method and need more advanced instructions. The material is divided into two parts: the analysis of a nearly homogeneous sample (third chapter) and the analysis of heterogeneous samples (fourth chapter), pointing out the important differences between those sample types and quite distinct requirements of performing FCM testing in respect to sample heterogeneity.

The chapter describes the procedures important for FCM data acquisition and analysis, including adjustment of FCM parameters, dot plot design, definition of lineage antigenic features, identification of physiological vs pathological samples, etc. Numerous dot plot and histogram figures, together with the accompanying CD-ROM, make understanding and application even easier. In addition, the third chapter contains highly illustrative color figures (plates; total 57) showing cell smears and dot plots in different disorders.

The last chapter, entitled FCM Interpretation and Reporting, summarizes FCM data interpretation and result reporting, which is best carried out using “pattern approach.” The chapter is divided into two parts according to the maturity status of the affected cell lineage. These parts are subdivided into illustrated paragraphs describing specific hematopathological condition, in respect to specific phenotypic features and recommended FCM pattern design.

**Highlights:** The authors were successful in reducing the confusion that still exists in FCM testing in the field by providing systematical instruction for FCM data analysis and interpretation. The accompanying CD-ROM with 100 case studies can serve as a highly illustrative appendix for easier understanding of FCM data analysis in a wide spectrum of hematologic disorders. The book helps in the integration of FCM results and other relevant laboratory and clinical information, in order to improve and facilitate diagnosis and monitoring in hematological diseases.

**Related reading:** The list of suggested reading is provided at the end of the book to enable readers to obtain “more in-depth knowledge on certain topics,” such as consensus recommendations for different disease classification, phenotypic characterization of certain cell lineage, hematopoietic cell differentiation, optimization of multiparameter FCM analysis, etc. The authors recommend their previous textbook (Nguyen D, Diamond L. Diagnostic hematology: a practical approach. Arnold publishers; 2000), which complements the presented book with details on “the morphology of the bone marrow and peripheral blood manifestations of hematologic disorders.”