Preface to the Second Edition

During the time that has elapsed between the first edition and the second edition of this book, there has been considerable improvement in the incorporation of flow cytometry immunophenotyping into hematology and pathology laboratories, including institutions where previous practice had relied heavily on traditional morphology and paraffin-based immunostaining. In addition, flow cytometry (FCM) immunophenotyping has also gained acceptance as a useful diagnostic tool for the identification of not only acute myeloid leukemias but also other myeloid disorders, both malignant and premalignant. During the same time, advances have been made in terms of instrumentation and commercially available reagents. For instance, the introduction of the T-cell receptor (TCR)-Vβ eight-tube kit has greatly facilitated the evaluation of some difficult to diagnose mature T-cell disorders, and the implementation of the DNA dye DRAQ5 has improved the grading of lymphoma subpopulations present in heterogeneous samples. The role of FCM analysis has also progressed beyond that of establishing a diagnosis to that of monitoring disease and providing prognostic information.

The second edition of this book reflects the recent advances in the FCM analysis of hematopoietic disorders. To this end, the chapters have been revised to incorporate additional text and figures. The focus of the book and its organization remain unchanged, however. The availability of new software tools has made it possible to add more case studies to the new companion CD-ROM, as well as to render the disk easier to use without the need to install a database engine. The listing of the case studies (and their diagnoses) is provided at the beginning of the book. The reader will do well not to omit the case studies from consideration as they supplement the information provided in the book.

It is hoped that this book will bring a better appreciation of the important role of FCM analysis in the diagnosis and management of hematopoietic disorders. When FCM is applied systematically, the potential exists to reduce the confusion that still exists in the current classification of certain malignant lymphomas and lymphoproliferative disorders.

Preface to the First Edition

Flow cytometry immunophenotyping of hematopoietic disorders is a complex and demanding exercise that requires a good understanding of cell lineages, developmental pathways, and physiological changes, as well as broad experience in hematopathology. The process includes several interrelated stages, from the initial medical decision regarding which hematologic condition is appropriate for FCM assay, to the final step of diagnosis whereby the FCM data is correlated with other relevant clinical and laboratory information. The actual FCM testing involves three major steps: preanalytical (specimen processing, antibody staining), analytical (acquiring data on the flow cytometer) and postanalytical (data analysis and interpretation). The literature, including the latest FCM textbooks, provides ample information on the technical principles of FCM such as instrumentation, reagents and laboratory methods, as well as quality control and quality assurance. Similarly, correlations of morphologic findings and phenotypic profiles have been well covered in many publications. In contrast, much less attention has been given to the other equally important aspects of FCM immunophenotyping, especially data analysis. The latter is a crucial step by which a phenotypic profile is established.

To bridge this gap in the literature, the focus of this book is more on FCM data analysis than laboratory methods and technical details. For the reader to become familiar with our data analysis strategy, an overview of our approach to the preanalytical and analytical steps is also presented, with an emphasis on the preanalytical aspects, which have been rarely touched upon in the literature.

The process of data analysis follows a practical and systematic approach, utilizing the visual patterns of the dual parameter displays rather than calculating a "percent positive" for each individual antibody. The FCM graphic displays presented throughout the book, together with the clinical case studies contained in the companion CD-ROM should facilitate the readers to gain an in-depth appreciation of this visual approach to data analysis. Via the case studies, the topics discussed in the textbook can be illustrated in greater detail, and the FCM diagnostic subtleties will become more apparent.

The book is designed for all laboratory professionals involved in the immunophenotyping of hematologic disorders, including pathologists, PhDs, and technologists working in FCM laboratories, residents and fellows in pathology and hematopathology training programs, as well as clinical hematologists with a special interest in this subspecialty. In terms of organization, this book breaks away from the traditional mold used in other textbooks. The chapters are not arranged by specific diagnosis (i.e., the end point of a diagnostic workup) but by how the data presents at the time of the diagnostic consultation. This organization reflects the real-life problem-solving methods applied daily in the laboratory, whereby the strategies employed differ depending on whether the cell population in the sample analyzed is heterogeneous or nearly homogeneous.

The few available books covering FCM phenotypes in hematologic malignancies have tended to focus more on leukemias than lymphomas. In this book, equal emphasis is given to both categories of disease, thereby providing considerably more information on lymphomas and chronic lymphoproliferative disorders. Furthermore, DNA cell cycle analysis is also

included in the FCM study of mature lymphoid malignancies, in which the DNA data have been proven to be of prognostic significance, thus permitting a more objective and reproducible grading of these tumors.

The approach to the classification of hematologic neoplasms employed in this book also departs from that used in the various existing classifications. The antigenic profiles of leukemias and lymphomas have been incorporated into the more recent classification schemes. However, the phenotypes of many disorders, in particular malignant lymphomas, have been derived from paraffin-based immunostaining instead of FCM studies, thus not taking into consideration the large amounts of valuable information provided by FCM immunophenotyping (e.g., a better appreciation of the pattern of antigenic density distribution and coexpression). The approach taken in this book is to simplify the classification (which should facilitate the comparison of results between different institutions) by utilizing the graphical patterns of phenotypic expression and the results of DNA cell cycle analysis where appropriate, together with other relevant clinical/laboratory data including the morphology of the submitted specimen. A more detailed discussion on the morphology of the bone marrow and peripheral blood manifestations of hematologic disorders can be found in our previous textbook (and its companion CD-ROM) entitled *Diagnostic Hematology: A Pattern Approach* (Arnold Publishers; distributed in the United States by Oxford University Press; ISBN 0-7506-4247-5).

For practical reasons, most of the FCM graphics in the book are presented as black-and-white illustrations. The dot plots in many of the case studies contained in the CD-ROM are, on the other hand, presented in color to facilitate the viewing of the cell cluster(s) of interest, especially for educational purposes. The use of color dot plots is popular in some laboratories. In our opinion, laboratory staff involved in FCM data analysis should be familiar with both black-and-white and color FCM displays however, rather than relying solely on the color format.

The list of suggested readings is not meant to be exhaustive. Many of the references were chosen mainly for the readers to obtain more depth on certain topics, for example, the maturation and differentiation of hematopoietic cells.