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# Preface

Flow cytometry has evolved since the 1940s into a multidisciplinary field incorporating aspects of laser technology, fluid dynamics, electronics, optics, computer science, physics, chemistry, biology, and mathematics. Innovations in instrumentation, development of small lasers, discovery of new fluorochromes/fluorescent proteins, and implementation of novel methodologies have all contributed to the recent rapid expansion of flow cytometry applications. In this thoroughly revised and updated second edition of *Flow Cytometry Protocols*, time-proven as well as cutting-edge methods are clearly and comprehensively presented by leading experimentalists. In addition to being a valuable reference manual for experienced flow cytometrists, the editors expect this authoritative up-to-date collection to prove useful to investigators in all areas of the biological and biomedical sciences who are new to the subject.

The introductory chapter provides an eloquent synopsis of the principles and diverse uses of flow cytometry, beginning with a historical perspective and ending with a view to the future. Chapters 2–22 contain step-by-step protocols of highly practical and state-of-the-art techniques. Detailed instructions and helpful tips on experimental design, as well as selection of reagents and data analysis tools, will allow researchers to readily carry out flow cytometric investigations ranging from traditional phenotypic characterizations to emerging genomics and proteomics applications. Complementing these instructive protocols is a chapter that provides a preview of the next generation of solid-state lasers, and one that describes a rapid means to validate containment of infectious aerosols generated during high-speed sorting (Chapters 23–24).

An elegant new color-based “cell gating” strategy that promises to simplify the analysis and visualization of complex multiparameter data is introduced in this volume. The sophistication of multiparameter flow cytometry is best demonstrated in function-based studies where molecular processes can be examined at the single cell level. This is beautifully illustrated by a novel technique that involves measurement of intracellular kinase signaling cascades using highly specific antibodies that differentiate between the phosphorylated and nonphosphorylated states of proteins. Combined with immunophenotyping, discrete biochemical signaling events in individual cells within

heterogeneous populations can be deciphered. Other protocols are concerned with the characterization of T-cell subsets: intracellular cytokine staining can be used to quantify rare populations of antigen-specific T cells; cell tracking dyes can be combined with tetramer and cytokine staining to determine the frequency of antigen-specific - cell precursors destined to proliferate; and the cytolytic function of antigen-specific cytotoxic T lymphocytes can be assessed by a nonradioactive assay that monitors caspase activation in target cells, including primary cells of different lineages. Also described is a multidimensional approach to analyzing apoptosis that involves simultaneous analysis of caspase activation, annexin V binding to “flipped” phosphatidylserine residues, and membrane permeability to DNA binding dyes.

Investigators planning to enter the stem cell arena will be introduced to several techniques for the detection of candidate stem cells. Two methods exploit the ability of hematopoietic stem cells to efflux the vital dyes Hoechst 33342 and rhodamine 123. Because these methods obviate the necessity to identify cell surface antigens, they are widely applicable to other types of stem cells that may lack well-defined phenotypes. A third method can be used to investigate the role of the CD34 surface antigen in the biology of human hematopoietic stem cells. The ability to readily introduce genes into primary cells, such as hematopoietic stem cells, has facilitated studies of cell fate. The green fluorescent protein (GFP) from jellyfish and GFP-like proteins from corals have become commonly used markers for this purpose. Unlike other bioluminescent reporters, fluorescence of GFP and GFP-related proteins does not require exogenous substrates or cofactors. Several schemes for simultaneous detection of multiple fluorescent proteins are presented.

Cell isolation using the fluorescence-activated cell sorter (FACS) is an immensely powerful separation technique. The integration of FACS with microarray profiling will accelerate analysis of global gene expression within different cell types in complex tissues and organs. This recent development is discussed in one chapter in which practical details on microarray fabrication and hybridization are described. Also included are some innovative procedures for monitoring the interaction of biomolecules in living cells. Fluorescence resonance energy transfer (FRET) utilizing two spectral variants of GFP can be used to determine molecular association of proteins. Fusion of the proteins of interest to the GFP variants expands the horizon of FRET applications, which were previously limited by the availability of appropriate antibody pairs that would permit FRET to occur. Two new methods for discovering novel protein interactions are provided that take advantage of the high-throughput capability of FACS. The mammalian protein–protein interaction trap (MAPPIT) is a two-hybrid assay used to screen complex cDNA libraries for new protein interaction

partners. The second method allows rapid isolation of antigen-specific clones by flow cytometric screening of yeast surface display libraries. By displaying a library of single chain fragment variable antibodies (scFv) on the surface of yeast cells, the affinity of a clone can be characterized without the need for subcloning, expression, or purification of the scFv, and isolation of the highest affinity clones can be readily achieved by sorting.

In other chapters, both basic and clinical scientists will find useful protocols for cell cycle analysis of asynchronous populations, and for the determination of DNA ploidy, RNA content, and the proliferative status of tumor cells. Also presented are a FRET-based assay to monitor HIV-1 virion fusion in primary T lymphocytes, which can be adapted to study fusion mediated by envelope proteins from other viruses, and an *in situ* flow cytometric assay (flow-FISH) for assessment of changes in telomere length during aging and malignant transformation, which is an alternative to the more laborious terminal-restriction-fragment Southern blotting methodology.

Mounting interest in studying microorganisms parallels improved detection sensitivity of flow cytometers and development of microbe-specific methodologies. The chapter on multiparameter flow cytometry of bacteria provides reliable methods for assessment of the physiologic states of Gram-negative or Gram-positive organisms. Burgeoning application of multiparameter flow cytometry to microbial analyses is anticipated.

We would like to thank John Walker for inviting us to participate in this exciting endeavor and for his expert editorial assistance. We are especially grateful to all of the contributors for their enthusiasm. Their willingness to share their latest findings exemplifies the spirit of cooperation that is pervasive in the flow cytometry community.

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