
Preface

Flow cytometry is riding the crest of unprecedented advances in innovative technologies. Improvement in instrumentation, lasers, fluorophores, and data analysis software have facilitated the development of new applications as well as the optimization of existing applications. This thoroughly revised up-to-date edition highlights the expanding contribution of flow cytometry to basic biological research and diagnostic medicine.

The introductory chapter presents a historical perspective documenting valuable contributions of pioneers in the field. An eloquent synopsis of the principles of flow cytometry provides a solid foundation for the understanding of basic applications. Modern flow cytometry has been evolving toward high-dimensional complexity. A novel concept, which underlies an accurate and efficient strategy for analyzing complex multiparametric data, is introduced. Great strides have been made toward quantitative fluorescence measurement, bead-based multiplexed analysis, semiautomated high-throughput flow cytometry, and fluorescence resonance energy transfer the analysis of protein interactions. Other applications range from polychromatic phenotypic characterizations to genomic and proteomic analyses. Technologies utilized encompass conventional flow cytometry and imaging cytometry. The prevalence of aerosol-generating cell/particle sorting warrants a detailed description of standard safety measures. The last chapter poignantly asserts that while flow and imaging cytometry evolve on a platform of costly sophisticated technologies, minimalist imaging cytometry holds promise for field-research applications in resource-challenged environments.

The utility of multiparametric flow cytometry is best demonstrated in function-based studies. Assessment of cytotoxic effector activity and regulatory T cell functions can be determined using cell tracking dyes, phenotypic markers, and viability probes. Intracellular cytokine staining is routinely used to visualize antigen-specific T cells. Combined with functional and phenotypic markers, specific cellular subsets can be further dissected. Phospho flow deciphers intracellular kinase signaling cascades by using highly specific antibodies which differentiate between the phosphorylated and nonphosphorylated states of proteins. Combined with immunophenotyping, discrete biochemical signaling events in individual cells within heterogeneous populations can be carefully examined. The complex progression of apoptotic death can be evaluated by monitoring multiple apoptotic characteristics simultaneously. Employing multiple antibodies to detect epitopes on cell cycle-regulated proteins provides more information than the measurement of DNA content alone.

Single-cell resolution and high-throughput capability make flow cytometry amenable to the identification of rare cells within a population. Technical aspects of rare event detection are discussed in the context of practical examples. Direct investigation of distinct cellular subsets in normal hematopoietic development versus hematologic diseases is critical to the understanding of disease initiation and progression. High-resolution polychromatic fractionation of hematopoietic precursors dissects developmental stages and identifies cellular subsets with defined lineage potentials. Carefully devised protocols in the study of human hematologic disorders enable the diagnosis and monitoring of patients with leukemia and lymphoma, or primary immunodeficiency diseases. Cell-derived microparticles, which are implicated in pathogenesis, can be analyzed by conventional as well as imaging flow cytometry.

The impact of fluorescent proteins (FPs) on bioscience is underscored by the award of the 2008 Nobel Prize in Chemistry to three scientists involved in the discovery and subsequent optimization of the green fluorescent protein (GFP). Unlike other bioluminescent reporters, fluorescence of GFP and GFP-related proteins does not require exogenous substrates or cofactors. GFP from jellyfish and GFP-like proteins from other marine organisms, as well as recently engineered nontoxic red-shifted variants, have played vital roles in the noninvasive detection of genes transferred into living cells. Several schemes for the simultaneous detection of multiple FPs in living cells were presented in the second edition. The applications described in this edition further illustrate the broad utility of FPs in flow cytometry. The study of plant biology has been accelerated by the use of FP expression constructs. Other downstream applications involve genome-wide expression profiling and mass spectrometry, two analysis platforms that readily interface with flow cytometry. Such integration bodes well for the study of systems biology.

We would like to thank John Walker for inviting us to participate in this exciting endeavor again and for his expert editorial guidance. We are especially grateful to all of the contributors for their enthusiasm and generosity. Their willingness to impart their knowledge exemplifies the spirit of cooperation that is pervasive in the cytometry community. As the “Notes” section is a hallmark of this series, we are proud to present chapters that contain up to 59 notes!

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