
Preface

In the last edition, we noted that we were riding the crest of remarkable advances in innovative technologies. We did not anticipate that 6 years later we would still be in the midst of groundbreaking innovations that push the field of cytometry to new heights. Established as well as emerging methodologies are taking advantage of the availability of a plethora of cytometers, reagents, and analysis software. The development of new instrumentation has been greatly facilitated by compact and affordable solid-state lasers providing excitation wavelengths spanning the ultraviolet, violet, and near-infrared spectra. Accessibility to new fluorochromes covering the aforementioned spectra allows flexibility in panel design. It is now possible to perform measurements of increasing complexity in an attempt to answer questions proposed to unravel intricate communication networks in multicellular organisms. The ability of cytometry to simultaneously measure multiple aspects of cellular mechanisms down to the single cell/nucleus level holds promise for a profound understanding of homeostasis versus perturbation, the latter often leading to human diseases.

In the introductory chapter, Howard Shapiro, a pioneer in the field as well as a historian with an encyclopedic mind, recounts the landmark discoveries leading to the birth of flow cytometry. His insightful and philosophical account of the journey, both historical and personal, reveals how this enabling technology has made it possible to answer questions that no one even knew how to ask a few decades ago. In the concluding chapter, Howard reiterates the view that he expounded in the last edition on “The Cytometric Future: It Ain’t Necessarily Flow.” In it, he discusses alternative technologies that can also be used in the quest to improve human health.

The current edition aims to present established as well as emerging methodologies in cytometry. Each chapter explains the principles behind the methodology, presents step-by-step protocols, and highlights tips for successful execution. Quantitative fluorescence measurement is a well-established but underutilized technique that can benefit from a standardized procedure independent of instrument platforms and reagent differences. Hence, one chapter provides a detailed procedure for quantifying surface and intracellular protein biomarkers by calibrating the output of a multicolor flow cytometer in units of antibodies bound per cell. Other well-established methodologies include intracellular cytokine staining, apoptosis analysis, cell cycle analysis, tracking cell proliferation by dye dilution, and monitoring protein–protein interactions using Förster (fluorescence) resonance energy transfer (FRET). The utility of flow cytometry in basic science and clinical diagnosis is illustrated in the study of normal developmental stages of hematopoiesis and the detection of immune abnormality in peripheral blood cells of primary immunodeficiency patients, respectively. Additionally, two chapters provide the groundwork for designing and performing conventional flow cytometry. One of these chapters reviews current lasers available for flow cytometry, and provides guidance in matching laser wavelengths and characteristics to specific assays. The other chapter discusses strategies in panel design and optimization for simultaneous detection of more than 20 markers.

Some emerging methodologies, such as mass cytometry, vesicle flow cytometry, time-resolved flow cytometry, and real-time label-free deformability cytometry evolve around new or modified instrumentation. Mass cytometry, unlike conventional flow cytometry which uses a flow cytometer to analyze cells labeled with antibodies attached to fluorescent

tags, employs a mass spectrometer to analyze cells labeled with antibodies attached to heavy-metal isotopes. This novel technology enables simultaneous detection of more than 40 markers without significant signal spillover between detector channels. Vesicle flow cytometry, a challenge for conventional flow cytometers due to the small size of extracellular vesicles, can now be performed on flow cytometers equipped with high quantum efficiency avalanche photodiode array-based detectors. Extracellular vesicles are objects of interest as disease biomarkers and therapeutic targets. Fluorescence-triggered vesicle flow cytometry provides a general approach to the quantitative measurement of extracellular vesicle number, size, and surface marker expression. Time-resolved flow cytometry, an old concept dating back to 1992, is feasible with modifications to conventional flow cytometers. Time-resolved flow cytometry is broadly defined as the ability to measure the timing of fluorescence decay from excited fluorophores that pass through the flow cytometer. Because fluorescence lifetime is proportional to the quantum yield and independent of expression level, it measures the intrinsic brightness of a fluorophore. Fluorescence lifetime can also be used as a quantitative metric for FRET which is a nonradiative energy transfer event between a fluorescent donor and fluorescent acceptor molecule. Lifetime-based FRET measurements obviate the need to quantitate the acceptor and provide absolute FRET efficiencies based on the decrease of donor lifetime when it is quenched by energy transfer to the acceptor. Real-time label-free deformability cytometry leverages microfluidics to measure cell elasticity in engineered channel geometries of micrometer size. Appealing aspects of this technique include the possibility to characterize cells, and sensitively detect physiological and pathological changes in cell function without any external markers.

Other emerging methodologies involve new assaying techniques such as high-throughput cell surface profiling, single nuclei isolation and detection, and mRNA measurement. High-throughput cell surface profiling represents a rapid, simple, and cost-effective method to characterize the cell surfaceome. This monoclonal antibody-based screen is composed of 368 fluorochrome-conjugated cell surface protein-targeted antibodies arrayed into 96-well plates. Used in combination with a plate-based sample loading device for flow cytometers, it enables high-throughput analysis of an unprecedentedly large number of cell surface proteins in a single assay. The well-established method of direct isolation of nuclei from tissue and organ homogenates of plants has been extended to nuclei within homogenates produced from animal tissues and organs. The value of nuclear sorting for characterization of nuclear state has been recently enhanced by the development of molecular methods of RNA manipulation and amplification, allowing whole genome transcriptional analyses from single nuclei. Measurement of mRNA at the single cell level has been achieved recently with the branched DNA platform that is compatible with the detection of surface and intracellular antigens using monoclonal antibodies conjugated to fluorochromes, thus permitting simultaneous detection of mRNAs and proteins.

Data analysis is an integral and critical aspect of cytometry. High-dimensional data are generated as a result of combining a large number of measurements. Analysis of high-dimensional data incorporates dimensionality reduction algorithms and modeling. Unlike gating approaches, modeling lends itself to automation and accounts for measurement overlap among cellular populations. Designing models is greatly enhanced by a new technique called high-definition t-SNE mapping that can be used to visualize high-dimensional data as simple dot-plot displays. The chapter on data analysis introduces the concept behind building robust models and uses an example to illustrate how to build complex models that involve more than 35 correlated measurements.

We would like to thank John Walker for his invitation to participate once again in this exciting endeavor, his patience, and his expert editorial guidance. We are indebted 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.

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