

Preface

Negotiating the variety of biological applications available for a single-cell technology can be a daunting task in the twenty-first century. There was a time when flow cytometry offered an opportunity to analyze single cells by studying their phenotype or their cell cycle and provide an easy-to-achieve solution to well-known problems. But times have changed. The applications and opportunities for flow cytometry have never been more significant than they are today. New instrumentation, faster, more sensitive, more available, has moved the field. This is no more evident than in the opening chapter of this book, where a detailed review of cellular microenvironments promises to open a new era in understanding how therapeutic agents must be designed to accommodate the knowledge that can be obtained only using the techniques outlined in this book. This chapter is undoubtedly the most comprehensive review of microenvironment cytometry available today.

For three decades there have been proposals to move flow cytometry into the clinical microbiology laboratory, but complexity and cost have prevented any widespread adoption from a clinical perspective. This has changed with a chapter on rapid, low cost implementation using microtiter-plate analysis (now available on virtually every commercial instrument), whereby critical data such as antibiotic sensitivity can be achieved in a few hours on a routine basis. Similarly, technology advances in high throughput have made some approaches to drug screening almost routine, producing valuable data in a short period of time, both reducing the cost and decreasing the time to result. These processes demand more opportunities for analytical data reduction; a section of this book addresses this rapidly changing environment. Two other vital areas of single-cell analysis demand attention: standards/quality control and instrument sensitivity, and assay validation. These are addressed in two ways; a discussion of approaches to ensure that data are verifiable and quantifiable, as well as assay validation, and a discussion of the very sensors from which the signals we use are derived. In this unique chapter on photon detection, we bring the most up-to-date discussion of sensor technology available, with suggestions of how next-generation sensors may transform single-cell analysis, and in particular the analysis of very small particles where every photon counts. While the most modern technology available is presented, so too is a bird's eye

review of how the field of cytometry became a field to itself and how the very tools we have today evolved from the original discoveries.

In the clinical environment, for more than 30 years flow cytometry has been crucial to quantify circulating CD4+ T lymphocytes in patients with HIV infection, a parameter required first to start antiretroviral therapy, then to follow its efficacy. Over these decades, spectacular advancements in all the fields related to this technology have dramatically improved the capability to finely diagnose a large number of human diseases, and to identify the phenotype and function of new cell populations. So, several chapters in this volume focus on real-world applications in clinical environments. From the critical aspects of running multicenter studies to analysis of rare cells and the impact on patient diagnostics, important factors are identified. Further, the tremendous opportunities for evaluating the very detailed aspects of metabolic function of mixed populations of cells is one of the most challenging but highly rewarding features of single-cell analysis using flow cytometry. Indeed, this technology has allowed the opening of a completely new field that studies how metabolic changes play a role in determining the differentiation, maturation and functionality of immune cells.

This book covers a range of research and clinical applications and brings together a *state-of-the-art* focus on the uniqueness of cytometric tools.

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Single Cell Analysis

Contemporary Research and Clinical Applications

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