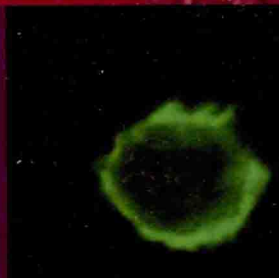
DIMITRI PAPPAS

Practical Cell Analysis



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Preface

This text came about for one good reason. As analytical chemistry and biology move closer together, biologists are performing increasingly sophisticated analytical techniques on cells. At the same time, chemists turn to cells as a relevant and important sample to study using newly developed methods. In both fields, there is a level of knowledge, usually passed down from researcher to researcher, which is not commonly found in the literature. Techniques, hints, and tips that can save time and effort – or avoid artifacts – that are "common knowledge" to one field are often hidden to another. For example, learning flow cytometry is often an art, as the number of adjustable parameters can turn a well-prepared sample into garbage once data acquisition begins. Similarly, developing a microfluidic culture device requires an understanding of the cell biology that dictates cell adhesion, growth, and response to shear stress. Setting up a culture lab, while trivial to a biologist, can be initially viewed as a daunting task by a chemist trained in classical procedures. Conversely, many analytical techniques require an intimate knowledge of how to properly acquire data. An understanding of the analytical principles, and the cell biology, can lead to successful research combining both.

WHY STUDY CELLS?

Research involving biological systems can occur on several levels. Each level of research, from molecule to organism, has distinct advantages and disadvantages, depending on the problem under investigation. The molecular level of bioanalytical research can elucidate interactions between the underlying machinery of a biological process. Molecular analysis, while highly detailed, lacks the *in vivo* mechanisms that often interact on a

higher level than the enzyme–substrate (or similar) case. *In vivo* work includes the full interaction of the living system. When looking at the entire organism, particularly a complex one like a mammal, it is difficult at times to separate the response of interest from all of the potential interfering signals and artifacts. Cellular analysis – whether with primary or immortal cells – lies in between the full-fledged organism and its molecular underpinnings. Molecular processes can be studied in living cells, and many observations of living cells can be used to predict the *in vivo* process. In addition, cell research often has fewer restrictions that *in vivo* work (especially if primary cells from one animal will be used for many experiments). In many cases, cells of interest contain most – or all – of the *in vivo* functionality, or can be used to extrapolate response from the entire organism. In the case of blood cells, the response of the organism can be readily determined from the cell sample in most instances. Pancreatic islets, while technically clusters of cells, can be isolated to study the production of insulin for diabetes research. Muscle cell contraction, on the other hand, can be studied on the cellular level, but lacks the anchoring to a physical frame that is found *in vivo*. Therefore, cell research must be conducted judiciously, so that experiments are warranted and can be used to understand organism response.

From the earliest days of cell analysis, it has been a marriage of the tools and methods that has allowed scientists to peer into the cell and unravel its mysteries. From the simplest light microscope to the newest microfluidic device, the ability to analyze the cell as an analyte, and as a container of analytes, has enabled a host of biomedical problems to be studied.

STUDYING CELLS

When faced with a biomedical problem to investigate or solve, the choice of both cell type (the sample) and analytical method are critical. Often, more than one technique will yield comparable information. In other cases, two or more techniques can be used to provide complimentary information. For example, fluorescence microscopy can yield high spatial and temporal resolution images of cell structure and morphology, but with low cell counts. Flow cytometry, in most cases, cannot yield any morphological information. However, the high cell counts and multi-parameter measurements can compliment data obtained by fluorescence microscopy. Cell culture on a microfluidic device can be coupled to fluorescence imaging, or cell separations. In many flow cytometry applications – particularly those involving rare cells – a cell-separation

step beforehand can enrich cell concentrations and provide better results.

This book discusses cell analysis from setup of a laboratory for cell work to using specific analytical methods. The goal of this book was to create a practical guide for working with cells in an analytical instrumentation setting. Therefore, Chapter 1 deals with acquiring cells, cell types, and how to choose a cell line or primary cell. Chapter 2 discusses the cell laboratory itself, from sterile handling equipment, incubators, and common items found in a cell lab. Floor plans of two laboratories serve as examples of the ergonomics to consider when working with cultures in a sterile manner. Chapter 3 discusses culture medium, additives, and the practical aspects of maintaining cells for analysis.

From an analytical standpoint, an understanding of the intricacies of cells can avoid many artifacts. For example, Chapter 4 discusses microscopy (e.g., light transmission, fluorescence, and atomic force) techniques for cells. In the case of fluorescence microscopy, the cell is a fixed object that is subject to photobleaching, toxicity, and loss of viability in long-term imaging. Understanding how to avoid photobleaching, and how to develop a chamber amenable to long-term cell imaging, can enable long-term experiments with high temporal resolution. Techniques to maintain cell viability in microscopy are also critical, especially for biological processes, which can take significantly longer than many chemical reactions (traditional chemists are not concerned with viability). Staining techniques, artifacts when making sensitive fluorescence measurements, and the sacrifice between strong statistics and spatial resolution are all discussed.

Chapter 5 deals primarily with cell separations, including fluorescence-activated cell sorting (FACS). Cell separation techniques are becoming both increasingly popular and diverse. Methods of producing a pure cell sample, based on differences in size, morphology, electrical properties, or antigen expression can be used individually or in tandem. Separations of living cells are both an analytical (i.e., cell isolation and counting) and a preparative method, an enabling technology for other analyses. Whether the separation method involves magnetic particles, droplet sorting, affinity chromatography, or other approaches, the fundamental aspects of cell isolation and reducing false positives are present in every separation strategy. Methods to reduce nonspecific capture, to enrich rare cells, and to combine techniques for greater separation power are all presented.

While FACS separations are discussed from a principle standpoint in Chapter 5, the mechanisms and detection are discussed alongside flow cytometry in Chapter 6. Flow cytometry is one of the earliest cell analysis

techniques. While it has matured and evolved over the decades, new methods and instrumentation continue to make this a vibrant field. Flow cytometry is often heralded as an objective technique (relative to microscopy, which can be highly subjective). However, given the number of parameters that must be set for an analysis, it is possible to skew data, or to produce artifacts. Compensation, the effect of detector sensitivity, and multiple occupancies are just some of the obstacles to obtaining suitable data from a flow cytometer. Once a good routine has been established with the instrument, a flow cytometer is then capable of producing a wealth of information from a cell sample.

Microscopy, cell separations, and flow cytometry are some of the most common cell analyses performed around the world. They are, largely, macrofluidic systems requiring large sample volumes and a greater degree of operator intervention. The continuing interest in "lab-on-a-chip" (microfluidic) devices has created a new form of cell analysis, where the fluid scales approach the scale of the cells themselves. Chapter 7 discusses microfluidic fabrication methods and ways to analyze cells by microfluidics. Many of the techniques discussed in preceding chapters can be applied to or integrated with microfluidic devices to increase information content or expand analytical capabilities.

HOW I GOT INTO THIS

My graduate and post-doctoral background are, I must admit, in no way related to cellular analysis. I studied laser excitation of a small cloud of cesium atoms. In fact, I don't recall making a single solution in the 5 years I spent in Jim Winefordner's and Nico Omenetto's laboratories at the University of Florida. What I did learn, aside from some fun and interesting spectroscopy, was the ability to apply analytical thinking to new problems. Therefore, when I left Gainesville, FL, for the equally humid shores of Houston, Texas, I was prepared for my new life as a bioanalytical chemist at NASAs Johnson Space Center. As a contractor with Wyle Life Sciences, I was thrust into a dynamic (and fun) group of people cramped into a lab roughly the size of a small recreational vehicle. I had never seen a cell incubator before, or even a cell since I was in high school biology class. Immersion is the best learning strategy, and within a week I was feeding my own, sterile culture of baby hamster kidney cells, the weed cell of our lab. It was during those few years at NASA that I realized two very important things. First, cell analysis – setting up a lab, maintaining cultures, handling cells – was not as difficult as first perceived. The second

thing I noticed – and this is no slight to my biologically inclined colleagues – is that biology and chemistry are often quite different things, despite our best efforts to integrate the two. Biologists have a wealth of unwritten knowledge for cell handling and culture, but still like to use gels – those antiquated slabs of acrylamide that are like cavemen's clubs compared to modern electrophoresis methods. There was at times real disconnection between the chemists – whose idea of a clean sample was one that was not turbid – and the biologists. Yet we shared common ground and common problems. This book, therefore, aims to bridge some of those problems and make connections between the two fields. For the analytical chemist, this book is aimed to orient him or her to the cell-culture laboratory, and the practices and considerations of measuring cells. For the biologist, newer – but readily available – technology is discussed to enable new biological analyses.

Rather than list new techniques that may never find commercial or academic fruition, this book is aimed at the practical, and at the readily implemented. Not every reader will have access to two- and multi-photon excitation microscopes, discussed in Chapter 4. However, everyone will be able to construct his or her own perfusion chamber for microscopy, for a minimal financial investment. This book contains numerous figures, flow charts, and tables aimed at deciding which techniques/samples to choose, and how to troubleshoot unforeseen problems as they arise. To keep the book as practical as possible, I have limited theoretical discussion when deemed excessive or unnecessary. It is my hope that this book rests on the laboratory bench (preferably away from the blood-borne pathogens), rather than on a shelf in the lab.

HOW THIS BOOK IS PUT TOGETHER

This book is meant to be a useful, practical guide. Much like a good manual or cookbook, the information should be easy to find. The main chapters (1–7) deal with the fundamentals and applied aspects of each technique. Chapter 8 discusses statistical considerations of analyzing cells. While some protocols are found in their respective chapters, many of the protocols (particularly those that can be applied to more than one technique) are placed in Chapter 9. Chapter 9 also contains several tables of useful probes and standards that can be used in many different cell analyses. Within each chapter, useful hints and tips are emphasized for easy reference. Like any new idea or technique, there is a bit of trial and error, of learning, in the cell-analysis process. This book aims to share

some of these lessons and point out pitfalls and obstacles along the way. Cell analysis is an exciting field that truly has limitless possibilities. As new problems arise that can be solved with cells, new analytical techniques are needed. The marriage of cell biology and analytical chemistry is a sensible one, and, with care, that union can help to understand some of the major health problems facing the world today.

Acknowledgments

This book is the product of a year of research and writing. During that time, and in the years leading up to it, several people influenced the material, or were responsible for some of the career turns that led me to start writing this book in the summer of 2009. I will, undoubtedly, have forgotten someone in this list of acknowledgments, but I will start with those who made this book a reality. Jenny Cossham of John Wiley & Sons worked with me from the book's conception to its final publishing. Jenny's initial email was what started this project, and her hard work and constant support were integral to its success. Gemma Valler and Zoe Mills, my production liaisons, were always quick with answers and enthusiasm. I am grateful for my current and former graduate students (Kelong Wang, Sean Burrows, Ke Liu, Randall Reif, Michelle Martinez, Yu Tian, Peng Li, and Yan Liu) and undergraduates (Charmaine Aguas, Ximena Solis-Wever, Brandon Cometti, and Molly Marshall, among others). Their dedication to our research efforts allowed me to focus on this and other projects. My current and former colleagues at Texas Tech made life easy for me while I wrote this book. I must also thank my former colleagues at Wyle Life Sciences and NASA Johnson Space Center, from whom I learned many of the tricks I've shared in this book. Ariel Macatangay, Grace Matthew, Dianne Hammond, and Sarah Wells were instrumental in my introduction to the world of cell analysis. Jim Winefordner, Nico Omenetto, Ben Smith, and my colleagues at the University of Florida taught me how to approach problems with an open mind. I would also like to thank Bob Kennedy of the University of Michigan and Edgar Arriaga of the University of Minnesota for their support of my research career; better advice would have been hard to find. This work was supported by a grant from the Robert A. Welch Foundation (Grant D-1667).

I would finally like to thank my wife, Mimi, for her unflagging support of both this book and my academic career. Her constant editorial guidance and patience made this work possible. Most of this book was written while my children slept at night, and so it is to those pleasant dreams that I dedicate much of this work.

Contents

1

Getting Started (and Getting the Cells)

1.1 INTRODUCTION

In any type of cellular analysis, one must consider both the analytical technique to use, as well as the cell type. Rather than start this text with a discussion of how to set up a cell-analysis lab (Chapter 2) or maintain cultures (Chapter 3), this chapter discusses the practical aspects of obtaining cells, regardless of what analysis is required. There are two possible scenarios in which an analytical scientist encounters cells; either the cells define the problem, or the scientist is in search of cells to validate a technique. In the case of the former, the application drives the cell type. When the pioneers of flow cytometry began their work decades ago, the samples dictated how the instrumentation would take form. The main cell types of interest at the time were blood cells – for both their tremendous health relevance and for their suspension qualities – as well as cells removed for gynecological screening, among others. The need for fast cell measurements (Chapter 6) drove the technology, but the cell samples were ready and waiting for their scientific counterparts.

In other cases, the scientist finds himself or herself with an exciting new technology that may one day change the landscape of cell research in a manner similar to microscopy and flow cytometry. The technique, perhaps first tested with beads or some other cell simulant, now requires "the real thing." Perhaps the scientist has already validated this method with cultured cell lines, and wants to move on to the truly "real thing," primary

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