# **Practical Cell Analysis**

Dimitri Pappas Dept of Chemistry & Biochemistry, Texas Tech University, USA



## **Practical Cell Analysis**

# **Practical Cell Analysis**

Dimitri Pappas Dept of Chemistry & Biochemistry, Texas Tech University, USA



This edition first published 2010 © 2010 John Wiley & Sons, Ltd

Registered office

John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com.

The right of the author to be identified as the author of this work has been asserted in accordance with the Copyright, Designs and Patents Act 1988.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by the UK Copyright, Designs and Patents Act 1988, without the prior permission of the publisher.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The publisher is not associated with any product or vendor mentioned in this book. This publication is designed to provide accurate and authoritative information in regard to the subject matter covered. It is sold on the understanding that the publisher is not engaged in rendering professional services. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

The publisher and the author make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties, including without limitation any implied warranties of fitness for a particular purpose. This work is sold with the understanding that the publisher is not engaged in rendering professional services. The advice and strategies contained herein may not be suitable for every situation. In view of ongoing research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of experimental reagents, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each chemical, piece of equipment, reagent, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. The fact that an organization or Website is referred to in this work as a citation and/or a potential source of further information does not mean that the author or the publisher endorses the information the organization or Website may provide or recommendations it may make. Further, readers should be aware that Internet Websites listed in this work may have changed or disappeared between when this work was written and when it is read. No warranty may be created or extended by any promotional statements for this work. Neither the publisher nor the author shall be liable for any damages arising herefrom.

Library of Congress Cataloging-in-Publication Data

Pappas, Dimitri.

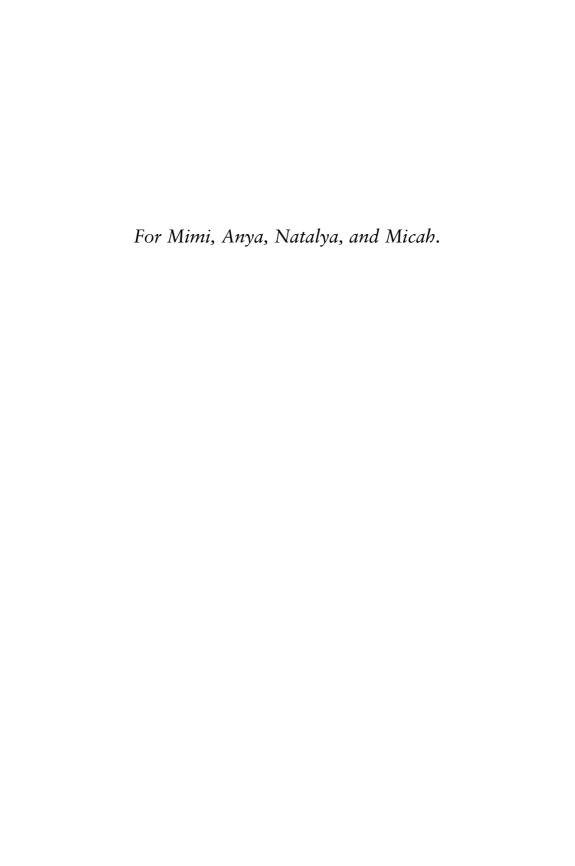
Practical cell analysis / Dimitri Pappas. p. cm. Includes bibliographical references and index. ISBN 978-0-470-74155-9 (cloth) 1. Cytology–Technique. I. Title. QH585.P357 2010 571.6028–dc22

2009049247

A catalogue record for this book is available from the British Library.

ISBN 978-0470-74155-9

Set in 10.5/13pt, Sabon by Thomson Digital, Noida, India. Printed and bound in Great Britain by TJ International, Padstow, Cornwall.



## Contents

Pγ	eface		xiii
Αc	knowl	edgments	xix
1	Getti	ng Started (and Getting the Cells)	1
	1.1	Introduction	1
	1.2	The Driving Need	2
	1.3	Primary and Cultured Cells	3
	1.4	Choosing a Cultured Cell	6
	1.5	Choosing Primary Cells	11
	1.6	Easily Obtainable Primary Cells	14
	1.7	Primary Cells from Tissues	16
	1.8	Purifying Primary Cells	21
	1.9	How Long do Primary Cells Remain Primary?	24
	1.10	Obtaining Primary Cells from a Commercial Source	25
	1.11	Bacteria and Yeast	26
	1.12	Practical Aspects of Cell Culture	27
	1.13	Safety Aspects of Primary and Transformed	
		Cell Lines	29
	1.14	Transfection of Primary and Transformed	
		Cell Lines	30
	1.15	Conclusion	32
	Refer	ences	32
2	The C	Cell-Culture Laboratory (Tools of the Trade)	35
	2.1	Introduction	35
	2.2	Issues Concerning a Cell Laboratory	36
	2.3	Setting up a Cell Culture Laboratory	44
	2.4	Cell Line Storage	48

#### viii CONTENTS

	2.5	Personal Protective Equipment	51
	2.6	Cell and Sample Handling	52
	2.7	Common Analytical Instrumentation for Cell Culture	53
	2.8	Considerations when Setting up a Cell-Culture	
		Laboratory	57
	2.9	Establishing and Regulating a Culture Facility	61
	2.10	Conclusion	63
	Refer	ences	63
3	Main	taining Cultures	65
	3.1	Introduction	65
	3.2	Medium	66
	3.3	The Use of Medium in Analysis, and Alternatives	71
	3.4	Culturing Cells	73
		Protocol 3.1: Sub-Culture of Adherent Cells	75
	3.5	Growing Cells in Three Dimensions	77
	3.6	Sterility and Contamination of Culture	79
	3.7	Storage of Cell Samples and Cell Lines	80
		Protocol 3.2: Cryopreservation of Mammalian Cells	83
		Protocol 3.3: Retrieval of Cells from Liquid-Nitrogen	
		Storage	84
	3.8	Conclusion	86
	Refe	erences	86
4	Micro	oscopy of Cells	89
	4.1	Introduction	89
	4.2	Microscope Types	90
	4.3	Culturing Cells for Microscopy	95
	4.4	Signals, Background, and Artifacts in Optical	
		Microscopy	101
	4.5	Staining Cells for Fluorescence Microscopy	104
		Protocol 4.1 Fixation of Cells for Immunochemical	
		Staining	111
	4.6	Multiple Labels	113
	4.7	,	116
	4.8	Spatial Resolution in Optical Microscopy	117
	4.9	Image Saturation and Intensity	119
	4.10	Atomic Force and Environmental Scanning	
		Electron Microscopy	120
	4.11	Conclusion	121
	Refer	ences	122

CONTENTS	ix
----------	----

5	Separ	rating Cells	125
	5.1	Introduction	125
	5.2	The Cell Sample	126
	5.3	Label-Free (Intrinsic) Separations	131
	5.4	Immunomagnetic Sorting	134
	5.5	Cell-Affinity Chromatography	137
	5.6	Affinity Chemistry Considerations in CAC and	
		MACS Separations	142
		Protocol 5.1: Screening of Antibody Clones	145
	5.7	Elution in Cell-Affinity Chromatography	147
	5.8	Nonspecific Binding in Cell Separations	148
	5.9	Separation of Rare Cells	151
	5.10	Fluorescence-Activated Cell Sorting	152
	5.11	Sorting Parameters	156
	5.12	Other Separation Techniques and Considerations	156
	5.13	Conclusion	160
	Refer	ences	161
6	Flow	Cytometry: Cell Analysis in the Fast Lane	165
	6.1	Introduction	165
	6.2	The Cell Sample	167
	6.3	Flow Cytometer Function	169
	6.4	Obtaining or Finding a Flow Cytometer	177
	6.5	Using Flow Cytometers	178
	6.6	Setting up a Flow Cytometer for Multi-Color Staining	181
	6.7	Analyzing Flow Cytometry Data	185
	6.8	Example Flow-Cytometry Assays	189
	6.9	No-Flow Cytometry	191
	6.10	Conclusion	192
	Refer	ences	192
7	Analy	zing Cells with Microfluidic Devices	195
	7.1	Introduction	195
	7.2	Advantages of Microfluidics	196
	7.3	Considerations of Microfluidics and Cells	198
	7.4	Obtaining Microfluidic Cell Devices	202
	7.5	Microfluidic Flow Cytometry	209
	7.6	Cell Separations	213
	7.7	Analysis of Cell Products	215
	7.8	Cell Culture	219
		Protocol 7.1: Low-Shear Cell-Culture Chip	222

#### x CONTENTS

	7.9	Conclusion	225
	Refer	ences	225
8	Statis	tical Considerations	229
U	8.1	Introduction	229
	8.2	Types of Error	230
	8.3	Figures of Merit in Statistical Analysis of Cells	236
	8.4	Limits of Detection and Quantitation (of Cell)	240
	8.5	Methods to Improve Cell Statistics	242
	8.6	Comparing Analytical Values	243
	8.7	Rejecting Data: Proceed With Caution	245
	8.8	Conclusion	245
	Refe	rences	246
9	Proto	cols, Probes, and Standards	247
	9.1	Introduction	247
	9.2	Cell Transfection and Immortalization (Chapter 1)	247
		Protocol 9.1: Transfecting Cells with Polyamine	
		Reagents	248
		Protocol 9.2: Stable Transfection using Polyamine	
		Delivery	249
		Protocol 9.3: Transfection Using Electroporation	251
		Protocol 9.4: Cell Immortalization Using	
		hTERT Transfection	255
	9.3	Calculating Relative Centrifugal Force (RCF) and	
		Centrifuge Rotor Speed (Chapter 2)	256
	9.4	Fluorescence Methods (Chapters 4 and 6)	256
		Protocol 9.4: Apoptosis Detection Using	
		Fluorophore-Conjugated Annexin-V and a	
		Viability Dye	257
		Protocol 9.5: Apoptosis Detection Using	
		Fluorogenic Caspase Probes	263
	9.5	Surface Modifications for Cell Analysis	
		(Chapters 5 and 7)	266
		Protocol 9.6: Covalent Linkage of Proteins	
		(Nonantibody) to Glass by Microcontact Imprinting	266
		Protocol 9.7: Covalent Linkage of Antibodies to Glass	269
		Protocol 9.8: Noncovalent Attachment of Antibodies	
		to Glass #1	271
		Protocol 9.9: Noncovalent Attachment of Antibodies	
		to Glass or PDMS #2	272
		Protocol 9.10: Blocking Endogenous Biotin	273

9.6	Flow Cytometry and Cell Separations	
	(Chapters 5 and 6)	274
	Protocol 9.11: Cell Cycle Measurements by Flow	
	Cytometry	274
	Protocol 9.12: Antigen Density Measurements	
	in Flow Cytometry	275
	Protocol 9.13: Antigen Density Measurements Using	
	Fluorescence Correlation Spectroscopy	279
	Protocol 9.14: Cell Proliferation Using Anti-CD71	
	Staining (Chapters 4 and 6)	281
9.7	Fluorescent Labels and Fluorogenic Probes	
	(Chapters 4–7)	283
Re	ferences	284
Index		287

### **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 multiparameter 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

#### xviii PREFACE

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).

#### xx ACKNOWLEDGMENTS

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.

# 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

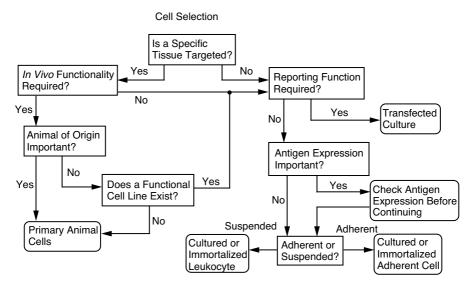
cells. No matter what the driving factor, the choice of cell type, and the origins of that cell sample are as critical as any aspect of the instrument design or sample-processing protocol. An excellent technique with the wrong cell type (e.g., an antibody–antigen mismatch) or a cell type that fails to attract interest, can fail as assuredly as a technique with poor figures of merit.

This chapter covers the types of cells one may consider for a cell-analysis technique, primary or cultured, animal type, prokaryotic/eukaryotic, and so on. Some example protocols for primary cells are given, as well as sources for cultured cells. More importantly, the little-shared pitfalls of choosing and obtaining cell cultures are also discussed, as well as methods to avoid them. Later chapters in this book cover the analytical techniques; for now, the discussion will be restricted to the cell as a commodity in the laboratory.

#### 1.2 THE DRIVING NEED

Are the cells specific to a biochemical or medical problem? If so, then much of decision work is eliminated, although obtaining the cells may not be much easier. If not, are the cells going to serve as validation of a method? In this case, does the species matter, or the antigen expression? Is cell morphology important? Figure 1.1 depicts some of the questions one may consider while choosing the cell type if a specific species/tissue type are not already defined. For example, if a group has received funding to study smooth muscle cells, then the species may not matter, but the cell type does without question. In this case, the decision of whether to use primary or secondary cells must be made (Section 1.3). Figure 1.1 will guide users who need a cell type to demonstrate a method. For example, if a new cell culture device is developed specifically to accommodate suspended cells (see Chapter 7), then almost any suspended cell will suffice, and therefore one may opt for a well-characterized, immortalized cell line of a particular species, one that can be perpetuated indefinitely in the lab for long-term quality control. In other cases, it may be prudent to use an established cell line that is closely associated with the analytical technique in question.

Certain cell-analysis techniques require some type of reporting capability or response to a stimulus. Cells transfected to express green or other fluorescent proteins are useful in imaging cell growth in culture devices, for detection in cell separations, and so on. Cells transfected for a specific study, such as using cyan and yellow fluorescent proteins attached to two different target proteins, can be developed in one's own laboratory, and



**Figure 1.1** Selection of cells based on experimental need and type of analysis. Where *in vivo* functionality is required, primary cells are typically needed, unless a cultured cell line retains the desired phenotype. For cultured cells, antigen expression, morphology, and so on must be considered

become an invaluable resource. Methods for transfection, as well as methods for labeling cells with large and/or impermeant materials are discussed at the end of this chapter.

#### 1.3 PRIMARY AND CULTURED CELLS

When first developing a technique, it is tempting – and often advisable – to begin with a cultured cell line that is well characterized and easy to obtain. However, cultured cells have many drawbacks, which preclude their use in all applications. For example, if a particular organ, tissue, or animal is to be studied, it is more straightforward to obtain cells from that animal and tissue type rather than hunt for a cultured cell line of the equivalent type. Of course, if human cells are of interest, then primary tissue and cells are difficult to obtain, and a cultured line may be the only choice. There are several disadvantages and advantages to primary and cultured cells, which are summarized in Table 1.1.

This discussion will begin with cultured cells. If cultured cells are the chosen route to cell analysis, then the benefits of relatively stable cell behavior, morphology, and growth are apparent immediately. A short

#### 4 GETTING STARTED (AND GETTING THE CELLS)

Table 1.1 Comparison of primary and cultured cell lines

	Primary cells	Cultured cells
In Vivo Cell Function Retained	Yes, until differentiation	Most cell lines do not retain in vivo functionality
In Vivo Morphology Retained	Yes, until differentiation	Many cell lines (e.g., leukocytes) retain in vivo morphology
Change in Protein Expression	No, until differentiation	Yes
Ethical Challenges <sup>a</sup>	Moderate to high	Low
Cost to Obtain	Moderate	Low
Human Cells Readily Available	No, samples are difficult to obtain	Yes, many cell lines are derived from humans
Animal Cells Readily Available	Yes	Yes
Cell Longevity <sup>b</sup>	Cells reach senescence or differentiate after a number of passages	Most cell lines replicate indefinitely (some become senescent after a number of passages)
Immortalization	Primary cell lines are not immortal	Most cultured lines are immortal
Aneuploid	No, unless diseased cells are targeted	Many cell lines are aneuploid, or become so after multiple passages

<sup>&</sup>lt;sup>a</sup> Cultured cells pose few ethical restrictions, as the initial harvesting has already been conducted. Primary cells will require IACUC approval for animals or IRB approval for human subjects. <sup>b</sup> Before death, senescence, or differentiation from their original form.

survey of recent published literature will reveal that while a vast number of cultured (immortalized) cells are used, there are a handful that are used with a high degree of popularity in cell analysis (Table 1.2). However, one should carefully research which cell types would be best for a given analysis, rather than following the lead of others, or using the same cell lines as another laboratory just to ease the decision-making process.

When selecting a cultured cell, one must decide on what is most important; cell morphology, growth, expression of a reporter, and so on. The first and easiest decision is to narrow down what cell type is to be analyzed. If neuronal activity is of interest, then the cell line PC12 may be a logical choice. PC12 cells differentiate in the presence of nerve growth factor [1], but otherwise remain immortalized, allowing for a permanent culture to be established, but for differentiated cells to be generated when needed. Regardless of cell type, one must also address the differences in functionality between cultured and primary cells. For example, while some cells can be differentiated in culture to take on

<sup>&</sup>lt;sup>c</sup> Maintenance costs, after obtaining the cells, are assumed to be equivalent.

 Table 1.2
 Example cell lines used in bioanalysis

Chinese         Chinese         Ovary         Adherent         Epithelial         Adenocarcinoma         Aggressis           Ovary CHO         Human         Cervix         Adherent         Epithelial         Adenocarcinoma         Aggressis           Jurkat         Human         Blood         Suspended         Lymphoblast         T-cell lymphoma         Used in a positiv           VU-937         Human         Blood         Suspended         Lymphoblast         Lymphoma         Used in a positiv           NIH-3T3         Mouse         Embryo         Adherent         Fibroblast         Lymphoma         Used in a positiv           NIH-3T3         Rat         Blood         Suspended         Lymphoblast         Leukemia         Polorectal           HT-29         Human         Blood         Suspended         Lymphoblast         Leukemia         Both cell           HuT-80         Human         Blood         Suspended         Lymphoblast         Leukemia         Both cell           HuT 78         Human         Blood         Suspended         Lymphoblast         Leukemia         Clumpical           Raji         Human         Blood         Suspended         Lymphoblast         Lymphoma         Expresses	Cell line	Organism	Tissue	Growth type	Morphology	Disease state	Notes
Human Blood Suspended Lymphoblast T-cell lymphoma Blood Suspended Lymphoblast T-cell lymphoma Colon Adherent Epithelial Colorectal Adherent Colon Suspended Lymphoblast Colorectal Adherent Blood Suspended Lymphoblast Leukemia Adherent Blood Suspended Lymphoblast Leukemia Adherent T cell Human Blood Suspended Lymphoblast Leukemia Colorectal Adherent Adherent Blood Suspended Lymphoblast Leukemia Colorectal Adherent Adherent Colorectal Lymphoblast Colorectal Adherent Adherent Cardiomyocyte None Suspended Lymphoblast Lymphoma Gland Clustered Aggregates Cardiomyocyte None Skin Adherent Endothelial None Sin Adherent Fibroblast Down Syndrome	Chinese Hamster Ovary CHO	Chinese Hamster	Ovary	Adherent	Epithelial		
Human Blood Suspended Monocyte Lymphomas Dlood Suspended Embryo Adherent Fibroblast Colonectal Blood Suspended Lymphoblast Leukemia Adherent Blood Suspended Lymphoblastic Leukemia Adherent Blood Suspended Lymphoblastic Leukemia Adrenal Blood Suspended Lymphoblast Colorectal adenocarcinoma Blood Suspended Lymphoblast Cukemia T cell T cell Cutameous Suspended Lymphoblast Cukemia Adrenal Adherent/ Polygonal/ Pheochromo-cytoma Gland Clustered Aggregates Cardiomyocyte None Cardiomyocyte Mouse Heart Adherent Endothelial None Skin Adherent Fibroblast None Sin Adherent Fibroblast Down Syndrome	HeLa	Human	Cervix	Adherent	Epithelial	Adenocarcinoma	Aggressive growth properties, high
Human Blood Suspended Monocyte Lymphoma  Lolon Adherent Eibroblast None  Lymphoblast Colorectal  Blood Suspended Lymphoblast Leukemia  Adherent Eibroblast Colorectal  Adherent Eibroblast Colorectal  Adherent Blood Suspended Lymphoblast Leukemia  Lymphoblast Cleukemia  Lymphoblast Colorectal  Adherent Adherent Polygonal/ Polygonal/ Polygonal/  Adherent Adherent Adherent Adherent Adherent Cland Clustered Agergates  Mouse Heart Adherent Cardiomyocyte None  Gland Suspended Lymphoblast Leukemia  Adherent Adherent Adherent Cardiomyocyte None  John Syndrome  Human Skin Adherent Eibroblast None  Sith Adherent Eibroblast None  Endorthelial None  Fibroblast Down Syndrome	Jurkat	Human	Blood	Suspended	Lymphoblast	T-cell lymphoma	Used in apoptosis studies as a
Human Blood Suspended Lymphoblast Leukemia adenocarcinoma Blood Suspended Lymphoblastic Leukemia adenocarcinoma Blood Suspended Lymphoblast Leukemia T cell Human Blood Suspended Lymphoblast Lymphoblast Carary syndrome Adrenal Adherent/ Polygonal/ Pheochromo-cytoma Gland Clustered Aggregates Cardiomyocyte None Heart Adherent Cardiomyocyte None Skin Adherent Eibroblast None Skin Adherent Eibroblast None Sin Adherent Eibroblast Down Syndrome	U-937 NIH-3T3	Human Mouse	Blood Embryo	Suspended Adherent	Monocyte Fibroblast	Lymphoma None	
Human Colon Adherent Epithelial Colorectal adenocarcinoma Suspended Myeloblastic Leukemia Suspended Lymphoblast Leukemia T cell Human Blood Suspended Lymphoblast Lymphoma Dave Adrenal Adherent Polygonal/ Pheochromo-cytoma Cland Clustered Aggregates Gland Suspended Lymphoblast Leukemia Adherent Adherent Cardiomyocyte None Suspended Lymphoblast Leukemia Adherent Adherent Cardiomyocyte None Gland Suspended Lymphoblast Leukemia Adherent Adherent Endothelial None Sin Adherent Fibroblast Down Syndrome	RBL-1	Rat	Blooď	Suspended	Lymphoblast	Leukemia	
Human Blood Suspended Lymphoblast Leukemia  Myeloblastic Leukemia  Lymphoblast Leukemia  Lymphoblast Leukemia  Lymphoblast Lymphoma  Lymphoblast Loukemia  Lymphoblast None  Lymphoblast Loukemia  Lym	HT-29	Human	Colon	Adherent	Epithelial	Colorectal adenocarcinoma	Variable size and morphology (relative to normal enithelial cells)
78 Human Gutaneous Suspended Lymphoblast Leukemia 78 Human Cutaneous Suspended Lymphoblast Sezary syndrome 7 T cell 7 Human Blood Suspended Lymphoblast Lymphoma 7 Gland Clustered Aggregates 7 Mouse Heart Adherent Cardiomyocyte None 7 GFP Mouse Yolk Sac Adherent Endothelial None 7 Adherent Fibroblast Down Syndrome 8 Juman Skin Adherent Fibroblast Down Syndrome	HL-60	Human	Blood	Suspended	Myeloblastic	Leukemia	
Human Cutaneous Suspended Lymphoblast Sezary syndrome T cell Human Blood Suspended Lymphoblast Lymphoma Adrenal Adherent/ Polygonal/ Pheochromo-cytoma Gland Clustered Aggregates Gland Suspended Lymphoblast Leukemia L(10) Mouse Heart Adherent Cardiomyocyte None1064Sk Human Skin Adherent Fibroblast None oit 532 Human Skin Adherent Fibroblast Down Syndrome	Molt-3/Molt-4	Human	Blood	Suspended	Lymphoblast	Leukemia	Both cell lines come from same patient and express CD4
Human Blood Suspended Lymphoblast Lymphoma Adrenal Adherent/ Polygonal/ Pheochromo-cytoma Gland Clustered Aggregates Blood Suspended Lymphoblast Leukemia Heart Adherent Cardiomyocyte None 7-1064Sk Human Skin Adherent Fibroblast None oit 532 Human Skin Adherent Fibroblast Down Syndrome	HuT 78	Human	Cutaneous T cell	Suspended	Lymphoblast	Sezary syndrome	Expresses CD4, variable morphology, clumping behavior
Human Skin Adherent Fibroblast Down Syndrome  Adherent Fibroblast Leukemia  Mouse Yolk Sac Adherent Endothelial None  Human Skin Adherent Fibroblast Down Syndrome	Raji DC 12	Human D 3+	Blood	Suspended	Lymphoblast	Lymphoma	Expresses CD19, good B cell model
Human Blood Suspended Lymphoblast Leukemia Mouse Heart Adherent Cardiomyocyte None Mouse Yolk Sac Adherent Endothelial None ik Human Skin Adherent Fibroblast None Human Skin Adherent Fibroblast Down Syndrome	rC-12	Kat	Adrenal Gland	Adnerent/ Clustered	rolygonal/ Aggregates	глеоспгото-сутота	Can differentiate into neuron-like cells
Mouse Yolk Sac Adherent Endothelial None Ik Human Skin Adherent Fibroblast None Human Skin Adherent Fibroblast Down Syndrome	CCEM-CRF	Human	Blood	Suspended	Lymphoblast	Leukemia	Expresses CD3 and CD4
Mouse Yolk Sac Adherent Endothelial None k Human Skin Adherent Fibroblast None Human Skin Adherent Fibroblast Down Syndrome	NL-1 (10)	asnom	пеап	Adnerent	Cardionnyocyte	Nolle	nimorial and exhibits contractile behavior. Excellent heart model
ik Human Skin Adherent Fibroblast None Se Human Skin Adherent Fibroblast Down Syndrome Se	C166-GFP	Mouse	Yolk Sac	Adherent	Endothelial	None	Clone of C166 line, expresses GFP
Human Skin Adherent Fibroblast Down Syndrome	CCD-1064Sk	Human	Skin	Adherent	Fibroblast	None	Senescent after 54 passages, 46 chromosomes, not immortalized
chrom	Detroit 532	Human	Skin	Adherent	Fibroblast	Down Syndrome	Senescent after 30 passages, 47 chromosomes

phenotypes similar to primary cells, other cells cannot be made to resemble their primary counterparts. If muscle contraction is required, then there are several cell lines that retain contractile function, even if some of the morphological features are lost.

When considering cultured cells, one must consider the end experiment. If fluorescent protein reporting, or other mutations, are required, then transformation of a primary or cultured cell line is required (Section 1.14). Will adherent or suspended cells be necessary? If one is validating a culture device (Chapter 7), then adherent cells – regardless of type – will be necessary. However, for validation of flow cytometry, MACS, affinity separations, and FACS analyses of suspended cells will make sample preparation easier. Suspended cells, particularly blood cells, are readily available in both primary- and cultured-cell samples. The benefit of suspended cells is that culture (for proliferating cells) is simple, and the ethical issues of extracting primary cells in this manner are fewer than when tissue samples are required.

#### 1.4 CHOOSING A CULTURED CELL

Once the decision to obtain a culture from a transformed cell line has been made, Figure 1.1 can help decide on the specific cell type. First, if reporting functionality is required, a cell line already transformed - such as the mouse endothelial cell line C166-GFP from the American Type Culture Collection – can be used to demonstrate devices or develop analytical methodology before other cell lines are tested. It is also possible, as noted below, to transfect a cell line, provided the researcher has met all guidelines for transforming cells and handling recombinant DNA, and so on. The latter approach allows one to develop many different cell lines into reporter clones, but requires additional infrastructure and cost. If a fluorescent cell is needed, and there is no need to tie the fluorescent protein to the expression of a particular protein, then several cell lines are readily available commercially (See Table 1.2). It is also possible to use a long-term (1–5 days) fluorescent tracer, such as the CellTracker series of dyes from Invitrogen, to render a cell fluorescent for tracking or detection purposes.

Whether or not reporting functionality is needed, there are applications that require cells of a certain tissue or animal type. If this is the case, then one must exercise caution to avoid choosing a cell line that lacks a key feature. For example, not every cancer cell line displays tumorigenic activity if injected into animals or cultured with other tissues. If the cell

analysis in question in this example is to study tumorigenesis, then this critical aspect of the cell line must be investigated before a cell line is selected. Similarly, antigen expression is critical for many applications, such as flow cytometry and affinity or MACS cell separations. Many transformed cell lines are derived from cancerous cell lines that have deviated from the original tissue. Antigen expression should therefore be investigated before acquisition or at the least tested by flow cytometry before the cell line is used in additional analyses.

In some cases, morphology (beyond adherent or suspended), species, functionality, and antigen expression are unimportant. Examples of these cases include the development of a new analytical or culture technique, where the cells in question must simply survive long enough to prove the concept will work. In these circumstances, a well-characterized cell line that will grow under a wide range of conditions may be the ideal choice. These so-called "lab weeds" are robust cell lines that are used routinely around the world. Examples such as Chinese Hamster Ovary (CHO), Jurkat, HeLa, and other cell lines are nearly as ubiquitous as the analytical balance or pH meter in modern cell laboratories. These cell types are also available in various transfected clones for a wider range of options. Wellcharacterized standard cell types are not always the best choice, as they suffer from problems associated with immortalization (see below) and often offer the bottom-line choice. There are thousands of cultured cells from different species, tissues, and phenotypes available. Straying from the standard choices may allow for greater impact in one's research.

While several commercial sources of immortalized cell lines exist, two of the largest are the American Type Culture Collection (ATCC, www. atcc.org) and the Health Protection Agency Culture Collection (HPACC, also known as the European Collection of Cell Cultures, http://www. hpacultures.org.uk). These organizations house thousands of cell lines from a variety of origins, and are always a good starting point when acquiring cultured cell lines. Other cell types are available from biotechnology vendors, and some specialized cell lines are available only from particular institutes or individual investigators. Cells from ATCC and HPACC are typically classified by animal and tissue of origin, disease type (if applicable), morphology, growth properties, DNA profile, age/ethnicity of human source (if applicable), and so on. In most cases, the cell line is shipped as a cryopreserved aliquot (see Chapter 3 for detailed protocols on cryopreservation and thawing of cells), which must then be thawed and cultured. It is important to note that some cell cultures have stipulations for use. For example, use of the U-937 monocyte line [2] requires that the original paper is cited in all published work with that cell line. Listed below

are some common lines used in cellular analysis. These cell lines do not represent an exhaustive list, but rather offer a few examples from several different cell types. The cells lines listed below are listed in no particular order, and only the parent (i.e., no mutants) cell line is discussed.

Chinese Hamster Ovary Cells. This double-X chromosome female cell line was derived from the Chinese Hamster (Cricetulus griseus). This cell line is proline-dependent, and has been transformed into several other clones (more than 30 mutants at ATCC alone) with different gene expression or transfection. The CHO cell line is epithelial in phenotype, and is used in applications involving microfluidic design and validation, and is also a popular host for transfection and genetic studies.

HeLa Cells. These cervical adenocarcinoma cells were isolated from an African American woman named Henrietta Lacks. Mrs. Lacks's cancer was so virulent that her cells, still used around the world today, have infiltrated many cell lines (see Chapter 3). Despite the vigorous growth of HeLa cells, and their propensity to cross-contaminate other cultured lines (e.g., the CCL-13 liver culture), they are useful both in tests of proliferation, and in viral studies. Since the cells replicate so rapidly, they can be used to generate large amounts of viruses, and can also be used to test anticancer activity of new compounds. Researchers using HeLa cells should, however, take precautions to isolate HeLa cell lines from other cell cultures to avoid cross-contamination. Like CHO cells, the HeLa cell line has been transfected into multiple clone types.

Jurkat Cells. Jurkat cells were derived from a 14-year-old male with acute T-cell leukemia. It is a suspended lymphoblast cell that expresses the CD3 antigen on its surface. Jurkat cells are relatively uniform in size for a cancerous cell line. Jurkat cells are Tlymphocytes and, unlike some cancerous cell lines, readily undergo apoptosis using either the caspase-8 (mitochondria-mediated) or caspase-9 (receptor-mediated) pathways. Jurkat cells therefore are used as positive controls for apoptosis initiators and inhibitors. They are also useful for flow cytometry (either bench-top or microfluidic) as they are suspension cells.

*U-937 Cells.* U-937 monocytes are derived from a lymphoma patient. Originating from blood cells, they are suspended and therefore useful for microfluidic applications of cell separation, among other applications. Like Jurkat cells, they express the CD95 (Fas) antigen and are useful in apoptosis studies.