Practical Cell Analysis

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Dept of Chemistry & Biochemistry, Texas Tech University, USA

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For Mimi, Anya, Natalya, and Micah.

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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.

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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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

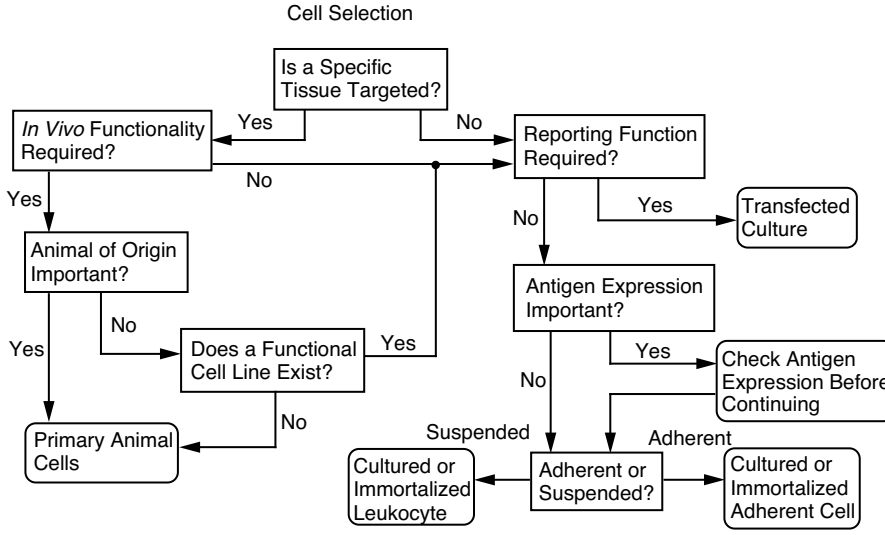

Figure 1.1 Selection of cells based on experimental need and type of analysis. Where *in vivo* functionality is required, primary cells are typically needed, unless a cultured cell line retains the desired phenotype. For cultured cells, antigen expression, morphology, and so on must be considered

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

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[a]	Moderate to high	Low
Cost to Obtain[c]	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[b]	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

[a] 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.
[b] Before death, senescence, or differentiation from their original form.
[c] Maintenance costs, after obtaining the cells, are assumed to be equivalent.

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

Table 1.2 Example cell lines used in bioanalysis

Cell line	Organism	Tissue	Growth type	Morphology	Disease state	Notes
Chinese Hamster Ovary CHO	Chinese Hamster	Ovary	Adherent	Epithelial		
HeLa	Human	Cervix	Adherent	Epithelial	Adenocarcinoma	Aggressive growth properties, high cross-contamination probability
Jurkat	Human	Blood	Suspended	Lymphoblast	T-cell lymphoma	Used in apoptosis studies as a positive control
U-937	Human	Blood	Suspended	Monocyte	Lymphoma	
NIH-3T3	Mouse	Embryo	Adherent	Fibroblast	None	
RBL-1	Rat	Blood	Suspended	Lymphoblast	Leukemia	
HT-29	Human	Colon	Adherent	Epithelial	Colorectal adenocarcinoma	Variable size and morphology (relative to normal epithelial cells)
HL-60	Human	Blood	Suspended	Myeloblastic	Leukemia	
Molt-3/Molt-4	Human	Blood	Suspended	Lymphoblast	Leukemia	Both cell lines come from same patient and express CD4
HuT 78	Human	Cutaneous T cell	Suspended	Lymphoblast	Sezary syndrome	Expresses CD4, variable morphology, clumping behavior
Raji	Human	Blood	Suspended	Lymphoblast	Lymphoma	Expresses CD19, good B cell model
PC-12	Rat	Adrenal Gland	Adherent/ Clustered	Polygonal/ Aggregates	Pheochromo-cytoma	Can differentiate into neuron-like cells
CCEM-CRF	Human	Blood	Suspended	Lymphoblast	Leukemia	Expresses CD3 and CD4
HL-1 (10)	Mouse	Heart	Adherent	Cardiomyocyte	None	Immortal and exhibits contractile behavior. Excellent heart model
C166-GFP	Mouse	Yolk Sac	Adherent	Endothelial	None	Clone of C166 line, expresses GFP
CCD-1064Sk	Human	Skin	Adherent	Fibroblast	None	Senescent after 54 passages, 46 chromosomes, not immortalized
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. Well-characterized 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 T lymphocytes 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.

NIH-3T3 Cells. The NIH-3T3 cell line is an embryonic mouse fibroblast. It is used extensively in microfluidic testing as a model system for adherent cells [3], due in part to its genetic and morphological stability. The cell line is used as a feeder layer for other cultures such as keratinocytes.

Rat Basophilic Leukemia (RBL-1) Cells. The RBL-1 line is a lymphoblast and is used as a model for suspended-cell analysis [3,4]. It is a stable cell line that can be used for a variety of cytometry and separation applications. Amaxa (www.amaxa.com) has detailed protocols for green fluorescent protein (GFP) transfection of RBL-1 cells using their products.

HT-29 Human Colon Adenocarcinoma Cells. The colorectal adenocarcinomaHT-29 line can be used for a variety of cancer-cell studies, [5] as well as experiments using model adherent epithelial cells. This cell line is highly aneuploid (chromosome count rages from 68–72). The cells grow with a large variation in size and morphology, an indicator of their virulent nature. HT-29 cells are tumorigenic and should be handled with care to avoid cross-contamination.

HL-60 Cell Line. The HL-60 line is a suspended myeloblast that is used because it is a robust cell for microfluidic applications [6]. It produces tumor necrosis factor-α (TNF-α) upon stimulation [7].

Molt-3 and Molt-4 Cells. Molt-3 cells are leukemia T lymphoblasts that express CD4 (among other antigens), making them excellent models for CD4 + T lymphocyte separation or sorting models (Chapter 5). They can be used in development of CD4 + T cell isolation by microfluidic FACS or MACS, or by affinity separations [8] before blood samples are evaluated. The same leukemia patient produced the Molt-4 T lymphoblast cell line, which expresses CD4 as well as CD3 (among others). Molt-3 and Molt-4 cell lines have different numbers of chromosomes, and both can be used for a variety of separation, flow cytometry, and FACS applications (see Chapter 5).

Human T Cutaneous Lymphocyte (HuT 78) Cells. The HuT 78 cell line is another CD4 + cell line that can be used for cytometry and cell separation applications [9]. This cell line has a more variable morphology than other lymphoblast lines discussed so far. HuT 78 cells are a robust line, but exhibit clumping behavior in culture, requiring mechanical disruption