# Preface

Phenotypic cell-based assays provide a means to quantify specific biological processes, such as protein trafficking and cytokinesis. The assays, used for years in basic research, are becoming more sophisticated and are increasingly applied in drug discovery. Fluorescence microscopy is the primary mode of phenotypic analysis. For example, microscopy-based ''imaging'' of biological responses measured through the localization or expression of cellular proteins is most often achieved with immunofluorescent probes, genetically encoded fluorescent protein fusions, and fluorescent dyes.

Here we describe the use of automated fluoresence microscopy in the analysis of a wide cross-section of cellular biology, including: (chapter), the cell cycle (1, 16, 24), enzymes controlling epigenetic events (2, 29), translocation of ligand-dependent transcription factors exemplified with the steroid hormone nuclear receptors (3, 11, 14, 29), translocation of ligand-independent transcription factors, $\beta$-catenin in primary human preosteoblasts (9), NFAT measured through adenovirus delivery of an NFAT-nitroreductase reporter gene (14), NF$\kappa$B p65 in TNF-$\alpha$-induced HUVEC (15), NF$\kappa$B p65 and c-Jun in IL-1$\alpha$ stimulated HeLa cells (20) and FKHR in response to PI3 kinase signaling (27), cell motility (13), lipid modification of proteins by palmitic acid (10), GABA A receptor trafficking in cultured neurons visualized with quantum dots (12), focal adhesion complex remodeling via labeled paxillin (13), E-selectin and VCAM-1 expression in TNF-$\alpha$-induced HUVEC (15), apoptosis (16), embryonic stem cell cardiogenesis (17), intracellular calcium dynamics in drug-sensitive and -insensitive human breast cancer MCF-7 cell lines (18) and heterogeneous primary neuronal cultures (19), mitogen-activated protein kinase signaling (20, 21–23), cellular morphology (24), the p53:hdm2 protein-protein interaction (27), ATPases (30), and G protein-coupled receptor signaling (4–8, 25, 29), a major therapeutic target class. Additionally, chapter 13 describes the use of a cDNA expression library to facilitate the identification of structural or organelle-specific proteins based on localization of the expressed proteins as fusions to YFP.

Development of automated light microscopes capable of rapidly imaging cell populations in 96-, 384- and 1536-well plates enables the aforementioned breadth of biological assays for screening of libraries of small molecules (13, 23, 29) and nucleic (13–15, 27, 28) acids. An example of a chemical library screen is described in Chapter 23. Accompanied by Chapters 21 and 22, this segment outlines the development of a high throughput screening (HTS)

cellular assay to identify p38 MAPK inhibitors, deployment of the assay in a 32,000 compound screen, and the selectivity profiling of candidate actives identified from the screen. Defining a compound's specificity through classification based on cellular phenotype is becoming an increasingly important application for automated microscopy. In Chapter 24, a multivariate approach using a cell-line panel categorizes compounds having different mechanisms of action, illustrating the power of cellular phenotype to distinguish the potential ''off-target'' effects of new substances.

Recently available genome-scale cDNA and siRNA libraries (see Chapter 28, Table I) are complemented by current advances in automated high-throughput microscopy and quantitative image analysis. As with small molecule library handling, storage, and delivery to cell cultures, genomic libraries have their own unique requirements. Nucleic acid-based libraries are made to enter mammalian cells by transfection or viral delivery, and the advantages and limitations of each are discussed in several chapters of this volume (13–15, 27, 28). Methods describing the preparation, handling, and arraying in microtiter plate format of plasmid cDNA or shRNA libraries and high-throughput transfection protocols (28), the use of pooled RNA duplexes (13), and strategies for the assessment of transfection efficiency variation in the context of HTS (27, 28) are also explored.

The microscope has undergone a remarkable evolution since Hans and Zacharias Janssen made their first compound microscope in Holland *ca.* 1590. Likewise, developments in image acquisition and analysis have made equally great strides from the first sketches of ''cells'' within slices of cork published by Robert Hooke in 1665. The systems described throughout this volume represent the current state-of-the-art custom and commercial automated microscope-based imagers and software. Differences between platforms include the nature of sample illumination, light collection, field of view, autofocus mechanism (the subject of Chapter 32), throughput, and analysis software, to name a few; the merits of each depend on the application (25). For example, in the high-throughput screening of large chemical libraries, the need for sample throughput may outweigh the wide range of excitation and emission wavelength options required in the detailed kinetic investigation of multiple subcellular fluorescent markers for a more limited number of samples.

The instruments described in this volume include the INCell 1000 and 3000 (1, 4–6, 8, 17, 27), Discovery-1 (2, 3, 26), Cell Lab IC100 Image Cytometer (3, 10, 11), Opera (7), ArrayScan HCS (9, 20–23), Pathway BioImager (18, 19), several custom platforms based on the Olympus Model IX71 (12, 13, 32) and Model IX81 inverted microscopes (13), and the inverted Zeiss Axiovert 100M epifluorescent microscope (24). In addition, a cytometry-based cellular imaging instrument, the Explorer, containing no microscope optics and designed for cellular assays where moderate resolution of cellular features is acceptable, but

high sample throughput is required, is described in Chapter 29. Another detector, the Plate::Vision multimodal reader, illustrates how microscopy can be used to enhance the screening of non-cellular assays. Here an array of 96-minilenses creates a 'quasiconfocal detection zone' that samples a ~10 nL volume within the well of a microtiter plate and can enable very high screening throughput (30). The refinement of components for automated microscopy is the subject of the final chapters. A ''systems cell biology'' approach aims to correlate multiple cellular biomarkers from relevant cell types to develop predictive tools for drug discovery in Chapter 31. On the hardware side, a digital autofocus solution to accompany high numerical aperture oil immersion lenses is described in Chapter 32, and a review of the fluorescence lifetime imaging microscope, enabled by the streak camera (33), illustrate technologies that will enable the next generation automated microscopes.

Regardless of the specific microscopy platform, investigators operating with large sample populations will benefit from an integration of sample preparation, imaging, data collection, processing, and storage. Chapters 2, 5, 6, 7, 13, 26, and 28 describe in varying detail the approaches taken by several laboratories to prepare a scaleable screening infrastructure.

Currently the applications of automated light microscopy are bounded only by our imagination and the fundamental physical properties of light. The latter limitation is surmountable by techniques that allow microscopic inspection of individual molecules and atoms, such as atomic force and electron microscopy. Applying these technologies in a manner described here for light microscopy will be a topic for future volumes in this series.

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