Preface

MANY OF THE REMARKABLE IMAGES AND ANALYSES of the dynamic properties of molecules in living cells described in this book stem from technical advances of the early 1970s. This was the period when commercially available epifluorescence microscopes equipped with efficient dichroic filter systems were introduced. These instruments permitted cell biologists to take full advantage of indirect immunofluorescence techniques and, for the first time, to photograph cells with 15–60-second exposure times, albeit on 35 mm film. Epifluorescence also made it possible to use the highest numerical aperture oil immersion objectives as both condenser and objective lenses. Accompanying these developments in microscopy, advances in the production and use of antibodies permitted the widespread use of double or even triple label indirect immunofluorescence, thereby providing investigators with the opportunity to carefully examine the associations between different components within the same subcellular compartment. Subsequently, developments in fluorescence in situ hybridization (FISH) permitted the localization of individual genes on chromosomes and specific mRNAs. The more recent introduction of confocal, deconvolution, and multiphoton microscopes, along with continuous advances in image-capturing devices, have made it possible to improve resolution, map in significant detail the three-dimensional organization of structures within cells, and capture images with unprecedented speeds, frequently in the range of milliseconds.

The images produced using high-resolution immunofluorescence techniques have revealed the remarkable complexity of cellular architecture and provided important insights into major cellular structures, including the different cytoskeletal systems, the nucleus and its various compartments, and membranous organelles. However, images of fixed and stained cells are static, providing only a brief snapshot of the organization and properties of these cellular structures. This major drawback stimulated cell biologists and microscopists to develop methods for studying specific types of molecules or multicomponent complexes in living cells, using the sensitivity of fluorescence-based imaging. At first, this involved the tracking of the incorporation of proteins (e.g., in the case of the cytoskeletal systems, tubulin, vimentin, and actin) directly conjugated with a fluorochrome such as X-rhodamine. Such derivatized proteins are usually microinjected into cells, and over time, they are incorporated into the structures containing their endogenous counterparts. This permits the investigator to carry out time-lapse observations and undertake fluorescence recovery after photobleaching (FRAP) experiments to monitor the dynamic properties of specific cellular proteins in live cells. However, the fluorescence signal is usually weak under these conditions,

and the overall photobleaching of specimens in the microscope field undermines the number of time-resolved images that can be captured.

The amazing discovery in the early 1990s that genetically encoded fluorescent proteins could be expressed in cells and organisms through the use of green fluorescent protein (GFP) represented a revolution in the field of cell biology. The use of GFP-tagged proteins has many advantages as described throughout this book, but the greatest of these is the ability to capture more time-resolved images, providing researchers with the opportunities to observe the dynamic properties of tagged molecules for extended periods of time and extract quantitative information from living cells.

The specific methods used for live cell imaging described in the 35 chapters in this book have been written by world-renowned authorities. Each chapter is accompanied by remarkable images and, in many cases, movies on the accompanying DVD that clearly demonstrate the enormous benefits derived from the coordinated use of the technical advances in microscopic imaging. These advances have made it possible for researchers to delve into the mysteries of subcellular architecture and function in unprecedented ways. As a result, cell biologists are now undertaking experiments and analyses of living cells that, until a few years ago, they never dreamed would be possible.

The book is divided into two major sections. In the first, "Detection and Approaches to Live Cell Imaging," the technical aspects of live cell imaging are presented. Here, the reader learns about GFP variants, how best to express a GFP fusion protein in cells, and the different imaging modalities that can be used in conjunction with GFP fusion proteins. In the second section of the book, "Imaging of Live Cells and Organisms," specific examples are presented. These examples utilize GFP and various live cell imaging approaches to study the dynamics of subcellular components or processes from single molecules to single cells in culture, tissues, and animals. Each chapter is written in a fashion that is extremely useful for both those who have never used live cell imaging in their research programs, as well as for the more expert microscopist. In addition, this book provides an excellent reference for students interested in learning about the way in which live cell imaging can be used to extract important functional information from cells or organisms that cannot be obtained by other more traditional biochemical, molecular, or genetic methodologies.

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