“Cell imaging” implies that the content of the volume might comprehensively cover the current major bio-imaging techniques, but as with any edited volume of contributed chapters, there must be a selection of topics. This volume is focused essentially on fluorescence microscopy techniques, and although there are sections on other light microscopy (LM) modes for completeness, there is scant reference to the wider world of cell imaging such as electron microscopy, atomic force microscopy or other complementary microscopy modes that do not depend on photons.

Chapter 1. The introductory chapter provides an overview indicating the importance of correlative approaches and the advantages of complementary methods. In addition to introducing subsequent chapters the author emphasizes the need for training in a specialist’s laboratory to gain a full appreciation of the practice of these sometimes sophisticated techniques.

Chapter 2. Mark Jepson gives a good account of the pros and cons of wide-field microscopy (WFM) vs. confocal laser scanning microscopy (CLSM) for particular fluorescence imaging applications. The now robust deconvolution algorithms for WFM enables it to compete with or supplant CLSM for acquiring high precision fluorescence information rapidly, especially in live cell studies and with relatively thin specimens. The criteria for successful deconvolved WFM imaging are discussed at length, as is the case for CSLM, emphasising the trade-offs among sensitivity, acquisition speed and spatial resolution. Because this discussion requires 17 pages, a summary table might have been useful, but the coverage is certainly informative.

Chapter 3. Koita Miura and Jens Riedorf consider image quantification and analysis including the basic use of densitometry and intensity measurements, and movement tracking using cross-correlation techniques. References to several Image J protocols allow the reader to experiment with this freely downloadable software for some of the protocols given, as well as indicating the commercial packages available. Procedures for basic microscopy are detailed, such as shading correction, pixel size determination, and tracking cell movement.

Chapter 4. Jon Lane and Howard Stebbing’s introduction to transmitted light imaging is possibly a fundamental chapter to be read first, because it relates to the importance of choosing an appropriate mode of microscopy. This overview, with emphasis on cell dynamics, provides a good grounding in the workings and typical applications of the main contrast modes used in transmitted LM. Phase contrast, differential interference contrast/Nomarski (DIC), and video-enhanced DIC microscopy are compared and illustrated with examples. Polariising and dark field imaging are also included, with brief reference to the bright field autoradiography approach. Finally, several alternative LM contrast modes are covered, their respective advantages discussed, and protocols for the most popular methods are provided.

Chapter 5. Returning to fluorescence, this chapter by Timo Zimmerman discusses spectral imaging. Here, problems with multi-channel fluorescence and overlap of signals are considered. The limitations of sequentially imaging multiple fluorophores to avoid cross-talk between them and the selection of filters are explained. Advantages of the alternative approach of spectral unmixing are presented, especially in relation to discrimination between signals from fluorescent protein variants. The principles behind the methods are compared, and applications in time-lapse and FRET are followed by an optimization and troubleshooting section. Image J plug-ins are again referred to and practical advice regarding the parameters of successful spectral unmixing are presented.
Chapter 6. Moving on to another biophysical experimental technique, John Presley discusses methods used to measure protein movements. Photobleaching, i.e. the loss of fluorescence due to continued exposure to excitation light, is often considered a negative outcome in fluorescence experiments. Turning this to advantage, FRAP (fluorescence recovery after photobleaching) and FLIP (fluorescence loss in photobleaching) have emerged as techniques for probing the movements of protein in the cell, its compartments, and within its membranes. A clear explanation is given of the FRAP experimental approaches used to measure the interactions between large and small proteins in the cytoplasm, organelles or membrane microenvironments by comparison of their diffusion coefficients. FLIP is considered in contrast, along with photo-activation, and an example is given of FRAP analysis of protein exchange between an organelle and the cytoplasm. Extensive protocols are included to guide the novice equipped with a confocal microscope who may wish to attempt FRAP analysis.

Chapter 7. For cell physiologists, Hadesmir et al. describe the background to, and recent advances in, calcium imaging from the early pre-confocal dyes through to the caged-calcium indicators and Ca-sensitive chameleon proteins. Their chapter is subdivided to address calcium measurement in mitochondria, ER, Golgi and the extracellular microenvironment using the required probes. Fluorescence changes reported by calcium-binding proteins are also covered, as is the usefulness of GAP-tagging in these proteins. Recommended protocols are described comprehensively for all these calcium imaging approaches.

Chapter 8. After a brief explanation of how total internal reflection fluorescence microscopy (TIRFM) works in principle, David Zenisek conveys how this high resolution mode of fluorescence imaging is undertaken in practice. Basically, TIRFM addresses cellular phenomena and related activities observable within the 100 nm range of the evanescent field generated when total internal reflection conditions are achieved, such as in the immediate vicinity of the surface membrane of a cell attached to a glass substrate. A concise table of applications directs the reader to key references, while the remainder of the chapter describes the different ways of performing TIRFM made possible by adding suitable hardware modules to existing wide field inverted microscopes.

Chapter 9. Jose-Angel Conchello describes how computational deconvolution of wide field fluorescence images represents the alternative approach to confocal or multi-photon instrumentation for recovering image fidelity that is degraded when light traverses the specimen and optical components. This chapter deals with mathematical solutions to the removal of out-of-focus light, and concepts of point spread function and frequency components in image restoration, together with several algorithms, are discussed. For those with a more physical background, there is much offered here, although as a mainstream cell biologist, I found its content rather challenging.

Chapter 10. The chapter by Miguel Branco et al. deals with correlative microscopy and the use of cryosections to increase visible detail in LM. Importantly, the use of cryosections for transmission electron microscopy (TEM) to allow observations at still higher resolution with immunogold labeling, is discussed also. Advantages of cryosection, such as penetration of antibodies into the section matrix and their use to localize RNA accurately in FISH analyses, are noted. Protocols are described for LM and TEM on the same specimen, and the authors discuss the options and technical difficulties encountered. Optimizing LM imaging can compromise the TEM approach and vice versa, so some strategy must be devised to suit both, particularly when fluorochromes and particulate gold reagents are used together. Although the authors’ experience is with studies of cell nuclei, and their examples are drawn from this, they demonstrate clearly the value of extending investigations to the EM level to obtain more detail.

Chapter 11. Returning to a purely fluorescence topic, Klaus Suhling’s chapter explains the basis and role of fluorescence lifetime imaging (FLIM) for cell biology. The biophysical environment within the cell can be monitored by measuring the decay of a fluorescent probe after excitation by FLIM techniques. Various parameters including oxygen, ion concentrations and pH levels can be determined; protein-protein interactions can also be monitored by fluorescence resonance energy transfer (FRET). Most of this chapter outlines the biophysics and instrumentation involved in the measurement of fluorescence decay, and offers an insight into the various implementations of FLIM. The authors point out that analysis of data derived from FLIM experiments involves specialist interpretation, hence a background in photonics might be desirable if attempting what can be complex experiments.

Chapter 12. FRET is discussed again in this chapter as a tool for measuring molecular proximities. Here Varma and Mayor discuss their methods for homo-FRET, in which interactions of
similar donor and acceptor fluorophores are investigated.

Chapter 13. This final chapter, by Elizabeth Roquemore, shows how scale-up of fluorescence microscopy approaches can be implemented in high content, high throughput screening for pharmaceutical assays. Automated preclinical drug screening by wide field microscopy permits subcellular localization for hundreds of cells at a time, a major advantage over other cell-based screening assays for drug discovery. The term “high content assay” is used to describe the number of cell parameters included in the information collected, including morphology and localized fluorescence intensity at the signal site. The choice of CLSM vs. spinning disc instruments is discussed with regard to versatility, and an overview table lists the respective capabilities of currently available systems. A similar compilation covers the diverse applications of this technology. Data acquisition and analysis from hundreds of cells in parallel requires a specific set-up optimized to yield statistically relevant data, especially for long-term imaging. Important criteria including speed of analysis and cell handling factors are discussed, and suitable methods are indicated in the protocols provided.

Appendices. The list of suppliers at the end of the book and the inclusion of a section on abbreviations at the start are to be commended. It is not clear, however, why the colour plates are disconnected from the appropriate text. There is usually an economic reason for this, but for a volume on fluorescence imaging where gray level images are fairly meaningless, repeated reference to the color section is obligatory. Consequently, when reading through the book, flipping to the color plate section became increasingly annoying.

Overall, however, the book brings together a multitude of fluorescence techniques and permits the reader to estimate their suitability and degree of difficulty for particular experimental needs. The book will appeal, therefore to both beginners and advanced microscopists wishing to explore new imaging directions or to become familiar with the main techniques in this rapidly advancing field. I recommend this volume as a standard source text for any laboratory using biological fluorescence imaging.

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