

Optical Microscopy: Emerging Methods and Applications by Brian Herman and John J. Lemasters, Editors

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The experimental arsenal of cell biologists and biophysicists has been strengthened by the merging of new optical microscopic techniques with digital image acquisition, processing, and analysis methods. These rapidly developing methodologies have spawned many books over the past 10 years, but new compendia are needed to keep up with current developments. This volume is a somewhat eclectic collection of chapters on subjects ranging from the tracking of single molecules along cell surfaces to the implementation of image cytometry for the diagnosis of cervical cancer. Several of the chapters are superb and provide sufficient detail for the novice to begin to design experiments around the methods described. However, the book ignores or gives only a superficial treatment to some very important topics in the field.

Fluorescent probes of cellular physiology are discussed in several chapters with varying degrees of overlap. Ratiometric imaging is covered from different points of view in the chapters by Bright, Poenie and Chen, Chew and Ljungström, Morris, Foskett, and Niggli et al. Poenie and Chen introduce the chemistry of fluorescent probes in Chapter 1. They provide a brief overview of indicators available for proteins, enzyme activity, membrane dynamics, membrane potential, and ion concentrations. Shortcomings and potential artifacts in the applications of these probes are highlighted; in particular, they describe efforts to design improved probes for calcium with reduced compartmentalization. The other chapters in this group touch on probe chemistry as well, while describing specific variations on the theme of ratiometric imaging—primarily for calcium distributions; the authors discuss applications from their own laboratories and present innovative approaches toward multi-parameter imaging.

Several chapters deal with instrumentation for modern quantitative optical microscopy, including some sophisticated systems for fluorescence in the chapters listed above. Among these, the chapter by Morris on rapid kinetic imaging of pH and calcium shows how to push available video technology to its limit by incorporating up to four cameras to simultaneously acquire images at four wavelength combinations. Several chapters include discussions of cameras and other imaging detectors. The chapter by Bookman and Horrigan is notable for its very thorough treatment of video-rate ccd cameras; it includes a careful comparison of spatial and temporal resolutions achievable with several commercially available devices. A surprising omission from this volume is a discussion of slow readout cooled ccd cameras,

which are becoming increasingly popular for low light level imaging applications requiring high photometric and spatial fidelity.

Descriptions of specialized instrumentation are also featured in several chapters dealing with sophisticated microscope techniques for quantitating molecular dynamics in cells. Single particle tracking is the subject of one of these; this technique combines high resolution microscopy with image processing and analysis to track the trajectories of gold particles conjugated to single molecules on the surface of cells with nanometer resolution. Total internal reflection fluorescence microscopy, the subject of a thorough chapter by Tamm, is used to study the dynamics of fluorescent molecules close to the glass coverslip; it is especially useful for investigations of cell-substratum interactions. A somewhat dense but quite complete treatment of Time-Resolved Fluorescence Lifetime Imaging is presented with valuable descriptions of technologies for ultra-fast image detection. Confocal microscopy is described in an inappropriately short chapter for such an important new technique.

Among the best of the contributions is the chapter by Kao and Adams on Photosensitive Caged Compounds. They provide a thorough description of the theory, chemistry, and applications of these important tools that permit the localized release of biologically active second messengers and neurotransmitters with a flash of uv light. The topic is covered concisely but in sufficient detail to give readers a good appreciation of both the potential and the pitfalls inherent in this technique. I am unaware of any other recent review with as broad and thorough a coverage of this important method.

Although this volume casts a wide net, it cannot be considered a comprehensive coverage of the subject. As mentioned above, the treatment of confocal microscopy is short and restricted to imaging fluorescent physiological indicators in living cells. The volume does not describe 3D imaging or morphometric applications of confocal microscopy. Perhaps this can be forgiven because of the availability of recent books devoted completely to confocal microscopy. Other important topics that are absent include digital deconvolution of 3D datasets from widefield microscopes, photobleaching recovery and photoactivation methods for studying molecular dynamics, and a thorough description of cooled slow readout ccd imagers. Overall, however, the thorough treatment of several important methodologies, especially those surrounding ratio imaging, should make this book a valuable resource for cell biologists interested in applying quantitative microscopy to their research problems.