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A Book Review:

Imaging Neurons, A Laboratory Manual

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The resurgence of microscopy in recent years is certainly not news to readers of this journal. But, for many of us, this resurgence has placed additional strains on our already busy lives to keep current on new technologies and techniques. Fortunately, books such as "Imaging Neurons. A Laboratory Manual" (Edited by Rafael Yuste, Frederick Lanni and Arthur Konnerth) provide comprehensive and timely reviews to help us keep abreast of the field.

The book evolved from a summer course taught by the editors and many of the contributors at the Cold Spring Harbor Laboratory, and although it obviously targets neurobiologists, many of the techniques are applicable to other biological systems. An impressive feature of the manual is the breadth and detail of topics covered. More than 100 experts in the field contributed 60 chapters on modern techniques for studying the structure and function of cells by optical methods.

The editors state in their preface that this manual is not a textbook on microscopy, although several chapters provide excellent overviews on optics as well as citations to more detailed references on the subject. Rather, the editors elected to emphasize experimental protocols with particular attention to practical techniques. Toward that end, the manual is divided into seven major sections that are described in greater detail below. Additional general information is found in the extensive appendix, which includes a glossary of microscopic terms, tables of excitation and emission spectra for the more common fluorochromes with recommended filter sets for each fluorochrome, a color rendition of the electromagnetic spectrum, instructions on the care and cleaning of lenses, an extensive list of addresses of manufacturers and suppliers (with web addresses), and more.

The first section gives an overview of optical systems, arc lamps, liquid-crystal and acousto-optic tunable filters, video (including infrared) microscopy and grating image systems for optical sectioning. A number of figures are included to highlight the components and optical pathways for each of the different imaging modalities. Practical instructions such as aligning a phase contrast microscope in 9 easy steps are sprinkled throughout. The chapters on video microscopy are particularly timely considering the recent advances in digital imaging and computer technologies. There is a brief introduction to the analysis of large data-sets generated by dynamic imaging as exemplified by experimental methods to determine changes in fluorescence of a voltage-sensitive dye as a function of time in the rat somatosensory cortex. This section concludes with methods for maintaining cells and tissue slices on the microscope, which sets the stage for many of the subsequent chapters applying imaging techniques to living cells. Practical considerations such as temperature, media and photodynamic damage are covered.

The second section highlights laser scanning confocal microscopy. The first two chapters in this section provide brief, but well written summaries of basic principles of confocal microscopy. The remaining contributions give examples of applications including studies of neuronal development and calcium dynamics in various neuron compartments (more on this later in the manual). The practical nature of the chapters includes a brief description for pre-

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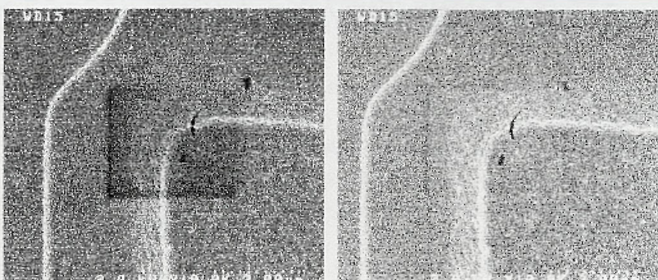

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Multiphoton microscopy is a topic covered by seven chapters in Section 3. The four applications described in this section will give the reader a good appreciation of the complexity and microanalytical powers of the technique. One that caught my attention was the chapter on two-photon imaging of living neurons (S.M. Potter) because it included his website with representative movies and images. It is worth the visit (http://www.caltech.edu/~pinelab/movies.html) although don't try downloading through a modem.

Section 4 addresses techniques relying on photoactivation of molecules protected by chemical groups which can be removed by any light source with sufficient energy in the near-UV range. Several chapters provide examples of how these techniques allow investigations of transient kinetic events associated with receptors and other cellular constituents. For example, "caged" neurotransmitters (*e.g.*, glutamate) can be applied to single cells and the "release" of the neurotransmitter experimentally regulated by photoactivation with light spots that can be controlled in size and duration. Adequate references are included in each chapter for those who wish to obtain additional information on the techniques.

The largest number of chapters (15) are devoted to the field of calcium imaging covered in Section 7. This large number by itself reflects the importance of the study of calcium in cell biology. Much of the success of these techniques relates to the availability and quality of various fluorescent calcium indicators, which are reviewed in the introductory chapters. Contributions that follow discuss techniques for loading calcium into the cell using either acetoxymethyl-ester derivatives that mask the negatively charged indicators or electrophysiological patch-clamp methods where the indicators are introduced into the cell through glass pipets. Other chapters describe specific methods for measuring calcium fluxes in dendrites of neurons in brain slices as well as the retina, presynaptic calcium transients, intramitochonrial calcium concentrations, and calcium oscillations in non-excitable cells such as lymphocytes and astrocytes.

Section 6 includes techniques that utilize optical imaging based on intrinsic signals including voltage-sensitive dyes. The final two chapters in this section demonstrate how these imaging techniques can be used to visualize dynamic subcellular events such as synaptic vesicle recycling and exocytosis of single secretory granules in chromaffin cells.

The last section (Section 7) covers recent applications of luminescence imaging and green-fluorescent protein (GFP). The chapter on cellular imaging of bioluminescence describes the use of luciferase to study transciptional events in single cells. Further refinements of these techniques to include mutants of GFP that alter their spectral characteristics depending on pH are used in one chapter to study proteins associated with secretory vesicles.

I sought in this brief review to describe the manual in such a way that readers can gain an appreciation of its scope and to summarize its content for those who might be interested in specific applications covered in the book. I think it is safe to say there is something for everyone in this book, at least with respect to light microscopy of fluorescent molecules, and it provides an excellent overview of modern techniques to study structural/functional relationships in living cells. The blend of theoretical chapters with practical step-by-step techniques will ensure that it finds a place on the bookshelves of many cell biologists, especially those interested in excitable cells. The book is not for the novice. ■

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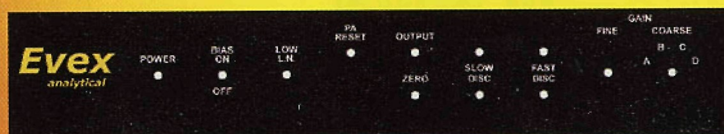

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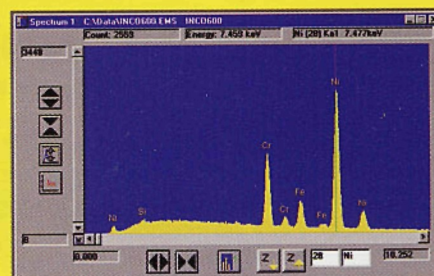

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