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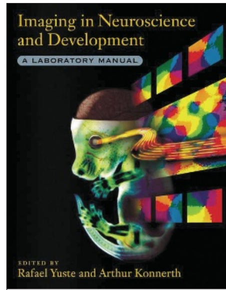
Illuminating live-cell imaging



Imaging in Neuroscience and Development: A Laboratory Manual

edited by Rafael Yuste and Arthur Konnerth

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Reviewed by Marla B Feller

Imaging techniques to visualize processes otherwise hidden from the naked eye have become an integral part of biomedical and neuroscience research. As we peruse the beautiful figures in the pages of major journals, we often ask ourselves, "How can I do that?" The answer to this question is likely to be provided in the second edition of *Imaging in Neuroscience and Development: A Laboratory Manual*, edited by Rafael Yuste and Arthur Konnerth.

The book originated at the Cold Spring Harbor summer course on imaging of neuronal structure and function of the nervous system. Course directors Yuste and Konnerth did a great service to the neuroscience community by compiling a manual that contains basic imaging principles as well as a long list of protocols. Though the concepts underlying imaging techniques are familiar to most people using a microscope, the devil is in the details. Why isn't my dye going into solution? How can I prevent my sample from bleaching faster than I can image? What camera do I need? It is in the answers to these sorts of questions where this manual succeeds by providing a practical guide for researchers and students who want to pursue imaging experiments.

A major emphasis of the second edition is live imaging of cellular processes. To this end, there is a chapter devoted to general methods for maintaining live specimens for imaging. There is also a greatly expanded section on imaging experiments used in the field of neurodevelopment, where imaging has proven to be particularly powerful. In development, there are many examples where simple observations give insights into mechanisms. For example, live imaging of morphological changes in developing neurons answers basic questions such as whether a local environment is attractive or repulsive to growth cones or how it affects axonal or dendritic arborizations. What is most striking about this section is how many of these processes—embryogenesis, synaptogenesis, dendritic development, axonal refinement—can be monitored live, either in cultured explants or in living animals.

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The manual begins (and ends) with basic principles of various forms of microscopy and detailed comparisons of light sources, cameras and optics systems to help investigators build an imaging system most appropriate for their application. There are several chapters devoted to two-photon microscopy, including detailed explanations on how to build one yourself! Topics range from the most practical questions, such as the difference between a video and CCD camera, to the more esoteric, such as strategies for obtaining images with a resolution that beats the diffraction limit.

The remaining sections are devoted to protocols from laboratories that have pioneered the techniques. Browsing through the index, I was struck by the amazing array of sophisticated strategies that are described. There is an excellent section describing the various methods of monitoring ion concentrations (primarily calcium, but also others, including sodium, chloride and pH) in different compartments of cells. There are protocols on measurement of cellular processes surrounding synaptic transmission, including exo- and endocytosis and receptor trafficking. An independent section on the design and use of genetically engineered probes shows how various indicators can be targeted to neurons of interest but can also be used to monitor intracellular processes such as movement of proteins and even gene expression in real time. A section is devoted to using light to stimulate neuronal responses, with a special emphasis on the technique of uncaging molecules either inside or outside cells.

There are several new protocols for measuring membrane potential, including the application of the nonlinear optical technique of second-harmonic generation, used for years by physicists and chemists to monitor structures and chemical reactions at surfaces. Second-harmonic generation requires similar equipment as two-photon fluorescence imaging (namely, a femtosecond laser and a photomultiplier tube) but has several advantages, including a unique specificity to changes in fluorescence of outer cell membranes. I provide this as an example of how even an experienced imager might be inspired to try new techniques after reading this manual.

Though the work presented in this book is impressive, future editions might be improved by a few changes. First, the organization could be streamlined. The editors are clear in stating that this edition should not function as a basic optics text. However, by having separate authors write the chapters that describe general principles, similar topics are seemingly disjoined by different styles and different levels of detail. Second, independent chapters could be devoted to the basic principles underlying, and strategies for dealing with, common problems encountered during imaging experiments, such as autofluorescence or bleaching. Third, protocols could be more generalized—in a few cases, the protocols are bulleted versions of methods sections from papers. For example, it would be helpful if the authors included unsuccessful approaches to prevent readers from going down the same misguided paths.

Despite these minor limitations, having all the information in one manual is enormously useful. The second edition of *Imaging in Neuroscience and Development: A Laboratory Manual* will almost certainly be a standard laboratory reference for many years to come. ■