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A LABORATORY MANUAL

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ACCOMPANYING MOVIES

Movies are freely available online at www.cshprotocols.org/imaging.

CHAPTER 2
Imaging Single Receptors with Quantum Dots

MOVIE 2.1. Diffusion and stabilization of single QD-GlyRs (green). Time-lapse recording (1200 images at 1 Hz; acquisition time, 75 msec). Synapses are labeled with FM4-64 (red).

CHAPTER 68
Imaging Microglia in Brain Slices and Slice Cultures

MOVIE 68.1. Migration of microglia (MG) cells in a rat hippocampal tissue slice following bath application of exogenous ADP (1 mM). The red fluorescence is BodipyTR-ADP.

MOVIE 68.2. Branch extension and subsequent migration of IB4 lectin-labeled MG in rat hippocampal tissue slices following application of exogenous ADP (1 mM).

CHAPTER 86
Two-Photon Imaging of Neuronal Structural Plasticity in Mice during and after Ischemia

MOVIE 86.1. Targeting individual brain arterioles for photoactivation of Rose Bengal. The movie has been recorded as epifluorescence with a charge-coupled device (CDD) camera on a microscope equipped with a 40×, 0.8-NA water-immersion lens over a time period of 120 sec and shows the blood vessel in sextuple time (movie plays in 20 sec) during targeting with 532-nm laser light. While the laser beam position is fixed (bright spot in the middle of the image), the specimen is being moved to target multiple sites within the blood vessel. Approximately 80 sec after start of photoactivation (≈14 sec in the movie), the blood flow appears to be blocked. See Figure 3 for single frames of this movie and further description. A small amount of background light was added to permit viewing of areas not subjected to photoactivation; this procedure was only used for creation of this movie and was never employed during an actual experiment. (Reprinted, with permission, from Sigler et al. 2008, ©Elsevier.)

CHAPTER 87
Two-Photon Imaging of Microglia in the Mouse Cortex In Vivo

MOVIE 87.1. Three-dimensional distribution of enhanced yellow fluorescent protein (eYFP)-expressing neurons (yellow) and enhanced green fluorescent protein (eGFP)-positive microglia (green) in the neocortex of a Thy1-eYFP x Cx3cr1-eGFP mouse implanted with an open skull window. The images are maximum-intensity side projections from a stack of fluorescence images. Individual focal planes were recorded in 2.5-µm steps starting from 800 µm depth below the pia mater to the cortical surface.

MOVIE 87.2. A time-lapse recording showing enhanced green fluorescent protein (eGFP)-expressing microglia (green) and enhanced yellow fluorescent protein (eYFP)-expressing Purkinje cells (red) in the cerebellar cortex of an anesthetized adult Thy1-eYFP x Cx3cr1-eGFP mouse. Images were recorded near a bend in the cerebellar folium, which allowed the molecular and Purkinje cell layers to be visualized within a single image. Microglial processes are highly motile. Neurons appear structurally stable during the same time interval. Elapsed time in minutes is shown in the *upper right* corner. Scale bar, 15 µm.

MOVIE 87.3. A video clip taken in the cerebellar cortex of an anesthetized mouse expressing enhanced green fluorescent protein (eGFP) in microglia. The video shows a microglial process at high magnification. Fine protrusions continually palpate the cellular environment. Elapsed time in minutes is indicated in the *upper right* corner. Scale bar, 5 µm.

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MOVIE 87.4. This exemplary video shows phagocytosis of cellular debris by microglia. Fluorescence data were recorded in the cerebellar cortex of an adult Cx3cr1-eGFP mouse expressing enhanced green fluorescent protein (eGFP) in microglia (*left*) following mechanical injury to the cortex and topical application of the red fluorescent dye sulforhodamine 101 (SR101) (*center*). The *right* panel shows an overlay of the eGFP and SR101 signals. The bright red cell is an injured Purkinje neuron that has accumulated SR101. Time after mechanical injury is indicated in the *upper right* corner. Scale bar, 10 µm.

CHAPTER 88

Two-Photon Imaging of Immune Cells in Neural Tissue

MOVIE 88.1.The dynamic interaction between immune cells expressing tdRFP (red) and neural processes expressing eGFP (green) in an experimental autoimmune encephalomyelitis (EAE)-affected mouse. The visualization was performed by dual near-infrared/infrared (NIR/IR) excitation two-photon microscopy in the brain stem of anesthetized mice.

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Preface to the Book Series

To train young people to grind lenses… . I cannot see there would be much use…because most students go there to make money out of science or to get a reputation in the learned world. But in lens-grinding and discovering things hidden from our sight, these count for nought.

—Antonie van Leeuwenhoek

Letter to Gottfried Leibniz on 28 September 1715 in response to Leibniz' request that he should open a school to train young people in microscopy

You can observe a lot just by watching.

—Yogi Berra

ONE OF THE CENTRAL THEMES OF BIOLOGY IS the constant change and transformation of most biological systems. In fact, this dynamic aspect of biology is one of its most fascinating characteristics, and it draws generation after generation of students absorbed in understanding how an organism develops, how a cell functions, or how the brain works. This series of manuals covers imaging techniques in the life sciences—techniques that try to capture these dynamics. The application of optical and other visualization techniques to study living organisms constitutes a direct methodology to follow the form and the function of cells and tissues by generating two- or three-dimensional images of them and to document their dynamic nature over time. Although it seems natural to use light to study cells or tissues, and microscopists have been doing this with fixed preparations since van Leeuwenhoek's time, the imaging of living preparations has only recently become standard practice. It is not an overstatement to say that imaging technologies have revolutionized research in many areas of biology and medicine. In addition to advances in microscopy, such as differential interference contrast or the early introduction of video technology and digital cameras, the development of methods to culture cells, to keep tissue slices alive, and to maintain living preparations, even awake and behaving, on microscopes has opened new territories to biologists. The synthesis of novel fluorescent tracers, indicator dyes, and nanocrystals and the explosive development of fluorescent protein engineering, optogenetical constructs, and other optical actuators like caged compounds have made possible studies characterizing and manipulating the form and function of cells, tissues, and circuits with unprecedented detail, from the single-molecule level to that of an entire organism. A similar revolution has occurred on the optical design of microscopes. Originally, confocal microscopy became the state-of-the-art imaging approach because of its superb spatial resolution and three-dimensional sectioning capabilities; later, the development of two-photon excitation enabled fluorescence imaging of small structures in the midst of highly scattered living media, such as whole-animal preparations, with increased optical penetration and reduced photodamage. Other

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nonlinear optical techniques, such as second-harmonic generation and coherent anti-Stokes Raman scattering (CARS), now follow and appear well suited for measurements of voltage and biochemical events at interfaces such as plasma membranes. Finally, an entire generation of novel "superresolution" techniques, such as stimulated emission depletion (STED), photoactivated localization microscopy (PALM), and stochastic optical reconstruction microscopy (STORM), has arisen. These techniques have broken the diffraction limit barrier and have enabled the direct visualization of the dynamics of submicroscopic particles and individual molecules. On the other side of the scale, light-sheet illumination techniques allow the investigator to capture the development of an entire organism, one cell at a time. Finally, in the field of medical imaging, magnetic resonance scanning techniques have provided detailed images of the structure of the living human body and the activity of the brain.

This series of manuals originated in the Cold Spring Harbor Laboratory course on Imaging Structure and Function of the Nervous System, taught continuously since 1991. Since its inception, the course quickly became a "watering hole" for the imaging community and especially for neuroscientists and cellular and developmental neurobiologists, who are traditionally always open to microscopy approaches. The original manual, published in 2000, sprang from the course and focused solely on neuroscience, and its good reception, together with rapid advances in imaging techniques, led to a second edition of the manual in 2005. At the same time, the increased blurring between neuroscience and developmental biology made it necessary to encompass both disciplines, so the original structure of the manual was revised, and many new chapters were added. But even this second edition felt quickly dated in this exploding field. More and more techniques have been developed, requiring another update of the manual, too unwieldy now for a single volume. This is the reasoning behind this new series of manuals, which feature new editors and a significant number of new methods. The material has been split into several volumes, thus allowing a greater depth of coverage. The first book, *Imaging: A Laboratory Manual*, is a background text focused on general microscopy techniques and with some basic theoretical principles, covering techniques that are widely applicable in many fields of biology and also some specialized techniques that have the potential to greatly expand the future horizon of this field. A second manual, *Imaging in Neuroscience: A Laboratory Manual*, keeps the original focus on nervous system imaging from the Cold Spring Harbor Imaging course. A third volume, *Imaging in Developmental Biology: A Laboratory Manual*, now solely deals with developmental biology, covering imaging modalities particularly suited to follow developmental events. There are plans to expand the series into ultrastructural techniques and medical-style imaging, such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET), so more volumes will hopefully follow these initial three, which cover mostly optical-based approaches.

Like its predecessors, these manuals are not microscopy textbooks. Although the basics are covered, I refer readers interested in a comprehensive treatment of light microscopy to many of the excellent texts published in the last decades. The targeted audience of this series includes students and researchers interested in imaging in neuroscience or developmental or cell biology. Like other CSHL manuals, the aim has been to publish manuals that investigators can have and consult at their setup or bench. Thus, the general philosophy has been to keep the theory to the fundamentals and concentrate instead on passing along the little tidbits of technical knowledge that make a particular technique or an experiment work and that are normally left out of the methods sections of scientific articles.

This series of manuals has only been possible because of the work and effort of many people. First, I thank Sue Hockfield, Terri Grodzicker, Bruce Stillman, and Jim Watson, who conceived and supported the Imaging course over the years and planted the seed blossoming now in these manuals and, more importantly, in the science that has spun out of this field. In addition, the staff at CSHL Press has been exceptional in all respects, with special gratitude to John Inglis, responsible for an excellent team with broad vision, and David Crotty, who generated the ideas and enthusiasm behind this new series. Also, Inez Sialiano, Mary Cozza, Michael Zierler, Kaaren Janssen, Catriona

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Simpson, Virginia Peschke, Judy Cuddihy, Martin Winer, Kevin Griffin, Kathleen Bubbeo, Lauren Heller, Susan Schaefer, Jan Argentine, and Denise Weiss worked very hard, providing fuel to the fire to keep these books moving, and edited them with speed, precision, and intelligence. More than anyone, they are the people responsible for their timely publication. Finally, I honor the authors of the chapters in these books, many of them themselves past instructors of the CSH Imaging course and of similar imaging courses at institutions throughout the world. Teaching these courses is a selfless effort that benefits the field as a whole, and these manuals, reflecting the volunteer efforts of hundreds of researchers, who not only have taken the time to write down their technical knowledge but have agreed to generously share it with the rest of the world, are a beautiful example of such community cooperation. As Leibniz foresaw, "lens grinding" is a profession that is indeed meaningful and needs the training of young people.

RAFAEL YUSTE

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Preface

DIRECTLY SEEING THE NERVOUS SYSTEM IN ACTION—be it a vesicle releasing transmitter, a neuron integrating synaptic input in its dendrites, or a neuronal population generating patterns of activity—is always a fascinating experience and provides us with a sense of immediate and credible understanding. In recent years, our ability to directly observe neural events at various spatial and temporal scales has enormously expanded because of the development of new imaging technologies as well as novel functional indicators. In this book we try to capture a snapshot of these developments, providing an overview of the currently applied imaging approaches for visualization of neural dynamics. As the field is continually and rapidly developing, our collection of chapters represents, however, an intermediate report at most. It is safe to predict that soon further innovations will permit even more detailed insights into the dynamic organization of the nervous system.

This manual is part of the series of laboratory manuals that has now emerged from the previous laboratory manual *Imaging in Neuroscience and Development*. In the spirit of the whole series the chapters provide short overviews of specific methods and applications as well as step-by-step experimental protocols with many practical tips. *Imaging in Neuroscience* is centered on the original neuroscience focus of the previous editions, and many of the core chapters were maintained and updated. Nonetheless, the volume has undergone substantial changes. Some of the old chapters were merged in revised form into the accompanying *Imaging: A Laboratory Manual* and *Imaging in Developmental Biology: A Laboratory Manual*, which provide details about microscopy techniques and applications in developmental biology, respectively. Most notably, more than 50 entirely new chapters have been included in *Imaging in Neuroscience* in addition to the revised chapters (making up more than half of the book). This large expansion clearly reflects the rapid progress of imaging applications in neuroscience during the past 5 years. For example, in complement to biosensors for reading out neural activity, new classes of "bio-actuators" (e.g., Channelrhodopsin-2) have entered the scene that now enable precise and specific control of neurons with light, thus opening a wide field of applications. Similar great advances have occurred in the area of in vivo imaging of neural activity. Dynamic properties of various cell types (including glia) are now studied in living animals with unprecedented detail, often taking advantage of genetic means for cell identification or labeling. In fascinating work, high-resolution imaging studies have been extended to awake animals, directly linking cellular and network events to behavior. Moreover, the use of animal models of brain diseases has expanded the application of these imaging techniques, thereby helping to identify key cellular processes that occur in brain pathologies.

We have divided the manual into eight sections that are roughly ordered according to the spatial level of neural organization: from molecules to synapses, cells, networks and brain areas. Section 1 starts by introducing molecular tools for labeling receptors and cells and for optical readout and control of neural activity. These tools are applied, for example, to study mechanisms of axonal propagation of excitation and synaptic transmission (Section 2). Section 3 moves to the cellular level, focusing in particular on the integration of synaptic inputs in neuronal dendrites. A further inte-

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grative level of neural organization is reached in Sections 4 and 5, where functional properties of neurons and neural circuits are investigated in brain slice preparations (in vitro) and in living animals (in vivo), respectively. Section 6 adds chapters on studies of the dynamic properties of various glial cells, a currently expanding field of investigation. Many new chapters are also found in Section 7, which highlights the emerging approaches of performing high-resolution imaging studies during behavior. Finally, Section 8 provides examples of imaging studies in various animal models of disease. The manual ends with a series of appendices, including a glossary of imaging terms, useful information on spectra, lenses, and filters, and instructions for handling imaging hardware safely.

We enjoy remembering the wonderful time we had discussing the books' concept and content with James Sharpe, Rachel Wong, and Rafael Yuste. Rafa's enthusiasm and drive in putting together this book series were just exceptional. Special thanks go to the entire editorial and production team at CSHL Press for a superb job on finalizing this manual, in particular to David Crotty, Kathleen Bubbeo, Mary Cozza, and Inez Sialiano. We are also thankful to Benjamin Grewe, Anja Gundlfinger, Henry Lütcke, David Margolis, José María Mateos, Morgane Roth, and Marcel van 't Hoff for help with proofreading. Last but not least, we would like to thank our families for their support, patience, and love.

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