

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

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

