Book Review

Imaging: A Laboratory Manual

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Imaging: A Laboratory Manual is a useful book for students and researchers interested in the rapidly advancing broad field of biological optical imaging. I recommend Imaging as a general and introductory textbook and laboratory manual for undergraduate and graduate laboratory courses in biological optical imaging. Imaging provides a balance of introductory technical materials with clear and precise experimental protocols, technical limitations, cautions, and detailed technical insights. It is modern in its coverage and includes chapters on modern microscopies, including super-resolution imaging, and linear and nonlinear optical microscopy.

I stress the words “optical imaging” because this large and comprehensive volume does not adequately discuss nonoptical techniques, such as micromagnetic resonance imaging, microcomputed tomography, and cyroelectron microscopy. While Imaging contains a chapter on atomic force microscopy, other books are required to provide the theory and experimental protocols for the wide range of scanning-probe techniques. Similarly, the active field of laser trapping, with its seminal applications to the biomedical sciences, requires other books. Clearly, the scope of Imaging is molecular imaging and cellular indicators (more accurately molecular location, conformation, molecular interactions, and motility and colocalization), which the reader will agree is well described in this practical book. The topics not covered in Imaging are predominately nonoptical techniques and provide information on molecular and supermolecular structures (proteins, cellular organelles such as ribosomes, nuclear pores, membrane channels, viruses, and changes in these structures at the atomic level).

Another broad area that Imaging does not address is the field of medical optical imaging. This includes endoscopes, colposcopes, dermatoscopes, and ophthalmoscopes. These important devices are based on optical imaging techniques, such as linear and nonlinear spectroscopy and optical low-coherence tomography. As the editor, Rafael Yuste, states in the preface, Imaging is the first volume in a series, and other specialized areas of imaging will appear in forthcoming volumes of the series. Hopefully, they will address functional magnetic resonance imaging, positron emission tomography, and other techniques of medical imaging.

The subtitle, A Laboratory Manual is what differentiates the books in this series from the many textbook and reference books that introduce and provide comprehensive summaries of the field of imaging. The practical benefit of a laboratory manual is that the reader can have the manual opened and flat on the laboratory bench and the book will guide (in many cases with the help and assistance of a mentor who is experienced in the particular instrumentation and associated laboratory techniques as well as the safety aspects required to work with lasers, high voltages, and the chemical and biological hazards) the students in constructing the instrumentation, calibrating the instruments, preparing the specimens, and evaluating the resulting images and numerical data. An extremely useful antecedent is the series Methods in Enzymology. In that series, the protocols were extremely detailed and tested prior to their publication. I would hope that the protocols in Imaging would also have been independently tested and verified before they appear in the book, but that point is not clear. It would seem probable that the chapters and the protocols in Imaging derives from the course notes that were used to teach the courses on imaging and microscopy that were offered at the Cold Spring Harbor Laboratory, at Cold Spring Harbor, New York. The Woods Hole Marine Biology Laboratory also provides courses on optical microscopy and analytical and quantitative light microscopy, and their course notes provide similar materials as those in Imaging. I found the protocols in Imaging to be variable in detail. Most of the protocols consisted of substantial details that covered instrumentation, specimen preparation, image and data acquisition, image and data analysis, and interpretation and limitations of the techniques. Other protocols were too brief to provide an adequate guide for the reader.

At the end of the book are several useful appendices and an index. One of the appendices consists of a useful glossary. Because two-dimensional images preclude the reader from appreciating the dynamics of cellular and tissue imaging, the publisher provides a link to the book’s Web site, which contains several movies that are freely available to the reader. The inclusion of appendices that prepare the reader to protect both themselves and the equipment may have their origins in good legal advice; nevertheless, these chapters are mandatory reading and in my opinion, a pointer to these appendices printed in large red letters should appear in the front of the book. These critical appendices include the following: “Safe Operation of a Fluorescence Microscope,” Care and Cleaning of Optical Equipment, and “Cautions,” which describes the hazardous materials that are covered in the book.

Imaging is divided into three sections: Instrumentation, Labeling and Indicators, and Advanced Microscopy. The latter section is further subdivided into the following topics:
molecular imaging, cellular imaging, tissue imaging, fast imaging, and uncaging. The chapter “Temporal Focusing Microscopy,” by Dan Oron and Yaron Silberberg of the Weizmann Institute of Science, Rehovot, Israel, is a seminal example of how the invention of new technologies is rapidly incorporated into novel imaging instruments.

Optical microscopy is advancing at a rapid pace, and the impact on our understanding of the biomedical sciences is monumental. These developments can be divided into two closely related advances: (i) new instrumentation for optical imaging, new light sources, and new light detectors, and (ii) new genetic and extrinsic fluorescent probes and indicators. The probes and indicators provide increased sensitivity and specificity. Nevertheless, there are limitations of current technology; optical microscopy is subject to optical aberrations from the instrumentation and the specimen, photobleaching and photodamage of the probes and the live specimens, a small field of view, unintentional effects of the overexpressed genetically expressed fluorescent probes, the signal-to-noise ratio of the detector and associated electronics, sensitivity and selectivity of the signal, background, Poisson statistics, and data-acquisition time. That is why the field is so active. These are serious problems that researchers (many who contributed to Imaging) are working diligently and creatively to overcome. Although many of the researchers depend on fluorescent probes to provide molecular specificity, there are alternatives in the form of probeless coherent imaging. This is exemplified in the contributed chapter “Coherent Raman Tissue Imaging in the Brain,” from researchers in the laboratory of X. Sunney Xie.

I now provide some general comments on the utility and pedagogical style of Imaging. One outstanding feature incorporated in the book is the discussion of technical details that are necessary for a given technique to work. As the editor points out in his preface, these technical details are often not explicitly stated in the methods sections of scientific publications. In this aspect, Imaging performs a great service to the aspiring researcher who is new to a particular imaging technique and its associated specimen preparation. The “Protocols” sections list the reagents, the equipment, and the experimental method. I was particularly pleased to see the protocol sections with the title “Troubleshooting.” Here the reader is given a list of potential problems associated with a given technique and specimen preparation, and then the reader is exposed to suggested solutions. The protocols are augmented with links to Web sites and include clear and well-designed color illustrations and many examples of biological optical images in full pseudocolor. The reader will also find the worked quantitative examples of calculations very helpful.

Next, I review a representative chapter of Imaging and highlight its critical features. Imaging presents several techniques to achieve super-resolution fluorescent imaging. Chapter 35 presents one approach, stochastic optical reconstruction microscopy (STORM), which was developed in the laboratory of Xiaowei Zhuang and her students and collaborators at Harvard University. There are several optical techniques that overcome the Abbe-defined diffraction limit of resolution. Each of these techniques has inherent benefits as well as limitations. As the authors of the chapter point out, “nanoscale precision in single-molecule localizations do not, however, translate directly into image resolution.” STORM is based on molecules that can be optically switched from a nonfluorescent to a fluorescent state, thus providing the required sparse sets of fluorescent molecules in a small region of the sample. Variants of this technique were independently developed by a number of groups. A substantial part of the chapter is devoted to a comprehensive overview of the characteristics of photoswitchable fluorophores. Next, the reader learns how to construct a system for STORM by modifying a wide-field microscope. The authors provide a critical summary of STORM data analysis: fluorescent molecule peak identification and fitting, postprocessing, drift correction of the sample and stage, and finally, image rendering. The chapter also presents a technique to localize each fluorophore in three-dimensional (3-D) space and an example of whole-cell 3-D STORM imaging of mitochondria. There are protocols for the transfection of genetically encoded photoswitchable probes and one for the preparation of photoswitchable-labeled antibodies.

A deep understanding of the theory is important in order to optimize the experimental instrumentation and design, as well as the capability to correctly analyze and interpret the acquired data. To this end, the readers can augment their knowledge and understanding on specific topics of theory and instrument design and construction by careful reading of the references associated with each chapter in Imaging. The reader will gain an appreciation of the diversity of optical methods that are available and under continuous improvement and development. The selection of a specific optical imaging technique should be highly dependent on the biological question that the researcher is investigating. In some cases, the use of multiple optical imaging techniques will yield knowledge that would be unobtainable by a single imaging technique.

Imaging is a highly recommended laboratory manual that is optimally suited for a practical course in general microscopy of biological specimens. There is no substitute for a “hands-on” practical laboratory course on optical microscopy, but an experienced mentor is invaluable to guide the novice in the conundrums and intricacies of the imaging methodologies.

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