Book Review

Biophotonics

Gerard Marriott, Ian Parker, *Methods in Enzymology*, Volume 360, *Biophotonics*, Part A, 714 pages+xxxv, ISBN 0-12-182263-x, Academic Press, San Diego, California (2003), $149.95 hardcover.
Gerard Marriott, Ian Parker, *Methods in Enzymology*, Volume 361, *Biophotonics*, Part B, 590 pages+xxxv, ISBN 0-12-182264-8, Academic Press, San Diego, California (2003), $149.95 hardcover.

Reviewed by Barry R. Masters, Fellow of SPIE and OSA, consultant. E-mail: brmail2001@yahoo.com

Methods in Enzymology is a highly respected, widely available, and practical series of volumes that contains detailed protocols and descriptions of biochemical and biophysical techniques. While the early volumes elucidated techniques related to enzymology, hence the title of the series, later volumes are of deep interest to the readers of the *Journal of Biomedical Optics*. Typically, a volume is dedicated to a specific subject, for example, Volume 307, *Confocal Microscopy*.

In organizing their two-volume work *Biophotonics* (Parts A and B), the Editors, Gerard Marriott and Ian Parker, have deviated from the usual format. They have compiled fifty-three excellent chapters on the broad subject of biophotonics, which were written by internationally renowned scientists who work in twelve countries.

While there is unanimity among scientists that the field of biophotonics is a rapidly emerging field with origins in physics, biology, medicine, and engineering, there is less agreement to delineate what subjects constitute biophotonics. Biophotonics is a broad, interdisciplinary field of research and its scale ranges from single molecules to whole organisms. The editors of *Biophotonics* are to be congratulated for having made a judicious selection of both the topics to be covered and also the quality of the authors they have selected to present these topics.

I suggest that *Biophotonics* would be extremely useful to any researcher who is working in this expanding field. Undergraduate students beginning their research projects, graduate students, scientists, engineers, and clinicians involved with biophotonics would comprise the intended audience. Perusal of *Biophotonics* will demonstrate that the editors and the authors have produced two volumes which adhere to the highest standards of technical writing and illustration. I highly recommend *Biophotonics* as a very reliable and useful reference work. First I will point out general characteristics of these books which form the basis of my recommendation, and then I will discuss specific features of these books which substantiate my recommendation.

First, in keeping with its past as part of the *Methods in Enzymology* series, *Biophotonics* contains clear, detailed descriptions of methods, protocols, and techniques. These are presented in sufficient detail to be easily reproduced. Sources of chemicals and instrument components are given. In some cases, detailed parts lists as provided, together with the names and addresses of suppliers.

Second, the text is complemented and integrated with many excellent illustrations, tables, images, and graphs. The very high quality illustrations are well documented with legends and annotations. Many of the illustrations are in color which improves the visual understanding of complex images and graphics.

Third, each chapter contains a comprehensive set of references to the peer-reviewed literature. This makes it easy to obtain further details and to verify statements by the chapter authors.

Fourth, these are very practical books which are intended for the researcher engaged in experimental work. An important theme throughout the volumes is an emphasis on the limitations and validation of each experimental technique. These limitations are presented to the reader in the theoretical derivations of both the fundamental physics and the subsequent mathematical analysis of the data. Furthermore, the descriptions of the various instruments are replete with discussions of calibrations, as well as a comparison of alternative components or techniques.

The production quality of these volumes is very high. The reader is well served by two additional features: an author index which is useful to locate the authors of every cited reference, and a very thorough subject index.

Some specific features which substantiate my recommendation are selected from each volume of *Biophotonics*. They are an attempt to provide an evaluation of the volumes; while all of the chapters are excellent my selections were made to provide a diversity of topics. I begin with an evaluation of four chapters chosen from Part A. Ian Parker wrote ''Photonics for Biologists'' as a guide to optics, microscopy, photonic components, and the construction of complete instruments. This chapter represents a concise, but critical presentation of these topics. There are sections on cleaning optics, laser safety, and how to construct a total internal reflection microscope. A useful appendix provides photonics publications and web sites and a detailed listing of vendors. It is obvious to the reader that the author has a wide experience in the construction of optical instruments and that he clearly communicates the critical points. Michael J. Sanderson and Ian Parker contributed a chapter on ''Video-Rate Confocal Microscopy'' which is a ''Heathkit'' approach to constructing the complete instrument. Every aspect of the project is carefully described, starting with the acquisition of parts (a complete list of parts and vendors is included), the optical design, the mechanical design, and the electronic control systems. The authors' de-

scription is sufficiently complete for others to construct an identical microscope. I strongly concur with the authors' statement, ''the sense of accomplishment of building your own instrument should not be underestimated.'' In fact, as stated by the authors, the construction of biophotonic instruments has several advantages: a reduction in cost, the ability to modify and maintain the instrument, the instrument may be designed with characteristics that are not available in commercial instrument, and it is a great learning experience for graduate students.

Robert M. Clegg and coworkers wrote a chapter on ''Fluorescence Lifetime-Resolved Imaging: Measuring Lifetimes in an Image.'' The authors emphasize how the experimental methods are related to the fundamental mechanisms of fluorescence. They stress that an understanding of the photophysics of fluorescence helps to avoid pitfalls, problems, and false interpretations of the data. They begin with a rigorous analysis of the pathways and rates of deexcitation from the excited state. The authors include the key references to the field, including the 1951 German textbook by T. Förster and his papers of 1946 and 1948. In the second part of the chapter their emphasis is on the experimental realizations. Here the authors compare the various techniques and instrumentation and present a critical review of methods for data analysis and display. For example, they present a comparison of time domain and frequency domain measurements. Similarly, they compare scanning two-photon and full-field fluorescence lifetime-resolved imaging. The key theme is that by a rigorous understanding of the molecular processes researchers can optimize the use of fluorescence lifetime-resolved imaging to investigate their research problems. Fluorescence resonance energy transfer (FRET) is an expanding field and the inclusion of several chapters on this topic in *Biophotonics* attests to its importance.

Finally, Paul S. Maddox and coworkers contributed a chapter on ''Spinning Disk Confocal Microscope System for Rapid High Resolution, Multimode, Fluorescence Speckle Microscopy, and Green Fluorescent Protein in Living Cells.'' Their chapter is a guide to interfacing several commercial components to construct a unique instrument. Consistent with this aim the authors provide a list of instrument components and their sources. There is also a useful section on the rationale for component selection and a comparison of their instruments with alternatives. Their clear and detailed description should permit the reader to reproduce the confocal microscope. These summaries provide some insight into the style and contents of Part A of *Biophotonics*. There are many other comprehensive chapters which cover the topics of fluorescence, as well as the design, construction, and use of fluorescent probes, probes synthesized in the laboratory and those that are genetically encoded.

Part B of *Biophotonics* contains chapters on the development and applications of imaging technologies; again, the scale varies from single molecules to molecular motors, single ion channels, cells, and organisms. Daniel Axelrod, who is a long-time developer of total internal reflection fluorescence microscopy (TIRF), contributed a critical review on this technique which is also called ''evanescent wave microscopy.'' TIFR is a unique wide-field technique, that excites fluorescence of a specimen within <100 nm from the surface. The chapter is divided into theoretical considerations which include polarization effects, optical considerations including the description for construction of a prismless microscope (TIFR), and a detailed discussion of experimental considerations. A strong feature of TIFR is that it can readily be combined with many standard fluorescence spectroscopy microscopic techniques. This chapter provides the reader with sufficient details to construct and use a TIFR microscope.

Andrew C. Millard and coworkers contributed a chapter on second harmonic generation (SHG) imaging microscopy. This technique is part of the important field of nonlinear microscopy. Similar to two-photon excitation microscopy, second harmonic microscopy depends on the square of the incident light intensity. SGH microscopy offers intrinsic three-dimensional optical sectioning without the need for confocal apertures as well as greatly reduced out-of-plane photobleaching and photo-toxicity. It differs from other nonlinear microscopies in that SGH is restricted to specimens that lack a center of symmetry; for example, cellular membranes. The authors have contributed a clear description of the theory as well as a complete description of the instrumentation. The authors state it is critical to perform the appropriate controls in order to insure that the SHG signal is pure and not due to contributions from the fundamental or from autofluorescence. They illustrate the appropriate controls with examples from their experiments.

Another important technique is fluorescence correlation spectroscopy (FCS) which is described in a contribution by Joachim D. Müller, Yan Chen, and Enrico Gratton. FCS was first presented in a 1972 publication by D. Magde, E. Elson, and W. W. Webb. Over the years there have been advances in instrumentation; new stable light sources, improved light detectors, new microscopy techniques, and improved computer analysis techniques. Together these advances have resulted in new applications based on the enhanced sensitivity. FCS is a useful technique to determine the kinetic properties of a system at equilibrium. It differs from a wide variety of strong perturbation methods such as ''temperature-jump'' which may not be suitable for living cells. The basic idea is to extract the kinetic parameters from measurements of the fluorescence signal fluctuations. FCS measurements are now routinely performed on biological samples with single-molecule sensitivity. The authors explain how construct the FCS instrumentation, and how to analyze the data. The analysis of FCS is in some ways similar to the analysis of chemical kinetics. Experimental kinetic studies can only be shown to be consistent with a given kinetic mechanism—their uniqueness cannot be proven. Similarly, in FCS the data evaluation, which is the evaluation of the experimentally derived autocorrelation function, requires a model to be selected and then fit to the experimental data. A statistical criterion, such as the reduced chi-squared parameter, is typically used to evaluate the quality of the fit of the model to the data. What is unique in their chapter is the emphasis on the calibration of the instrument, their

careful and complete discussion of sample preparation and controls. The authors end their chapter with a discussion of a novel feature of FCS: measurements made in vivo. That novel capability is due to the fact that FCS does not require potentially damaging external perturbations, and the spatial resolution is excellent, being on the order of femtoliters.

Biophotonics includes several innovative chapters on the evolving subject of optical traps. Sarah E. Rice, Thomas J. Purcell, and James A. Spudich contributed a chapter on optical traps used to study molecular motors at the single molecule level. They include a detailed description on optical trap design and construction. Once the trap is constructed following their detailed description the next challenge is the recording of bead position on nanometer length scales and on millisecond time scales. The authors give complete instructions to perform this task based on the use of quadrant photodiode detectors, as well as alignment and calibration procedures. The detailed protocols for attaching cytoskeleton filaments to glass, attaching anti-GFP antibodies to microspheres, and the production of biotin-actin filaments is elucidated in their chapter. Their chapter is a guide to the construction, calibration, and use of optical traps.

A second chapter on ''optical tweezers'' by Steven B. Smith, Yujia Cui, and Carlos Bustamante has the title ''Optical-Trap Force Transducer that Operates by Direct Measurement of Light Momentum.'' As described in detail by the authors, many designs of optical traps suffer from various calibration problems that are difficult to circumvent. The authors describe a new, innovative, force-measurement technique, based on opposed-beam optical tweezers, that overcomes some of the outstanding limitations of previous designs. Their chapter covers the physical theory of the new technique, the details of instrument design and calibrations, and the acquisition of force-extension curves. The real advantage of this new technique is that the calibrations procedure is unaffected by bead size, shape, index of refraction, and location.

Single molecule studies is a rapidly emerging field and *Biophotonics* contains several chapters on this topic. An example is the chapter by Andrea M. Femino and coworkers on ''Visualization of Single Molecules of mRNA in Situ.'' They solved this difficult problem of using the technique of fluorescence in situ hybridization (FISH) together with a specially constructed epifluorescence digital imaging microscope, three-dimensional optical sectioning, constrained iterative deconvolution, and three-dimensional interactive analysis software. Each of these topics is carefully described and there is a complete step-by-step approach for the calibration, data acquisition, data analysis, and visualization. Experimental details are presented with sufficient detail to allow reproduction by other groups. The important emphasis is on the avoidance of artifacts and correct calibration techniques.

In summary, the two-volume set of *Biophotonics* constitutes an extensive set of protocols, techniques, instruments, and methods of analysis that constitute the state-of-the art. The books are extremely well organized, clearly written, profusely illustrated, and thoroughly documented. The editors and the diverse group of authors have succeeded in their aims and have produced a highly useful and recommended set of books.