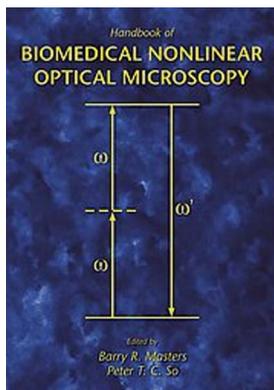


BOOK REVIEW

Handbook of Biomedical Nonlinear Optical Microscopy

Barry R. Masters and Peter T. C. So, Editors, 896 pages, ISBN13: 978-0195162608, Oxford University Press, New York (2008), \$150 hardcover.

Reviewed by William W. Mantulin, University of California-Irvine, Beckman Laser Institute and the Laboratory for Fluorescence Dynamics, Irvine, California.



On occasion the stars align and practicing scientists working in a burgeoning new field are presented with a guide for navigation through the uncharted heavens. Such a guide for the field of nonlinear optical spectroscopy and microscopy has emerged with the publication of a conceptually interesting, information-rich, well-written, comprehensive, current, and eminently practical handbook.

Co-edited by two renowned Massachusetts Institute of Technology scientists, Barry R. Masters and Peter T. C. So, the *Handbook of Biomedical Nonlinear Optical Microscopy* weighs in at 896 pages and 33 chapters. An international group of 56 scientific contributors provide the individual chapters, which have been woven into a coherent tapestry by fine editing. Stand-alone graphics complement the text and relevant literature citations guide the reader to pertinent source material. The foreword by Watt W. Webb, a pioneer in the field, touches on themes developed in the handbook: historical antecedents, theoretical development, technology, biomedical applications, and future directions. The editors' preface lays out their logic for organizing the handbook into four parts (history, spectroscopic basis, technology, and biomedical applications) including introductory overviews to each section. The overviews introduce the themes and content of each of the four parts and, as appropriate, comment on future perspectives. The handbook ends with a comprehensive and useful index.

The first section, Part I (introduced by Masters), is rather unique and delves deeply into the historical development of the field (Chap. 1), including a biography of the scientific life of Maria Göppert-Mayer (Chap. 2), the scientist acknowledged with laying out the theoretical framework for two-photon excitation processes. Parenthetically, it was surprising to learn that this outstanding scientist, who was awarded a Nobel Prize in physics in 1963 for her work on the nuclear shell model, spent the early decades of her scientific career without a faculty appointment. Of particular interest to this reader was the heretofore unpublicized link between the theoretical formulations of Göppert-Mayer and the earlier studies

of Paul Dirac on second-order time-dependent perturbation theory and virtual states. I had always linked perturbation theory with the development of quantum mechanics, but Chap. 3 describes how quantum mechanics incorporated earlier formulations of perturbation theory from astronomy and celestial mechanics. The fourth chapter provides a translation (German to English) of the two seminal Göppert-Mayer publications (1929 and 1931) on two-quantum processes, supplemental material from Dirac (radiation and perturbation theory) and Kramers and Heisenberg (radiation dispersion), and extensive commentary by Masters cementing the evolution and linkages of these seminal publications.

Part II, introduced by Robert Boyd and Barry Masters, provides three chapters detailing the physical processes of nonlinear optical spectroscopy: saturated fluorescence, harmonic generation, and stimulated light scattering. These chapters are particularly important because they provide the physical and mathematical basis for understanding nonlinear optical phenomena, which are developed from the perspective of instrumentation, signal analysis, microscopic imaging, and biomedical applications in Parts III and IV. Chapter 5 (by Masters and So) details the classical and quantum mechanical aspects of molecular spectroscopy with explanations of key physical and mathematical concepts (and equations) culminating with a comparison of single and multiphoton processes. In Chap. 6 on harmonic generation, Boyd starts with Franken's discovery (1961) of optical second harmonic generation and proceeds through the theory of nonlinear optical susceptibility, quantum mechanical treatment of nonlinear susceptibility, and descriptions of (n 'th) harmonic generation with mention of applications to surface nonlinear optical materials and optical microscopy. Chapter 7 by Eric Potma and Sunney Xie explores the theory of spontaneous and coherent Raman scattering in a progression from classical Raman to coherent anti-Stokes Raman spectroscopy (CARS). CARS-relevant issues of resonant and nonresonant contributions to spectral properties, suppression of nonresonant background, phase matching, field distribution under tight focusing, and forward (as well as *epi*- or backward) CARS are clearly presented mathematically and through illustrations.

The emergence of nonlinear microscopy in the 1990s clearly coincided with the availability of robust femtosecond lasers and low-noise photodetectors with single photon sensitivities. As the largest section in the handbook, Part III begins with an introduction by Peter So and Daekeun Kim and proceeds to 19 chapters detailing various aspects of nonlinear optical instruments for microscopic imaging and analysis, that is, the practical implementation of the theoretical discussion of Part II. These chapters offer the reader a plethora of critical and useful technical information about hardware, data analysis, lasers, detectors, multiphoton excitation, harmonic generation, multispectral imaging, CARS, polarization micros-

copy, fluctuation correlation spectroscopy (FCS), Förster resonance energy transfer (FRET), diffraction unlimited far-field fluorescence microscopy, high-speed imaging, photobleaching, and lifetime imaging, all within the context of nonlinear optical phenomena. For example, Chap. 8 (by John Girkin) discusses laser sources for nonlinear microscopy and delves into mode-locking and methods of mode-locking with practical information about Ti:sapphire lasers (including commentary on commercially available systems), pulse shaping, and dispersion. Girkin further introduces possible directions for future improvements to lasers for nonlinear optics in the form of saturated Bragg reflectors in passive mode-locking systems and vertical-external-cavity surface-emitting laser systems. The chapter concludes with several sections of practical advice for the use of femtosecond lasers touching on laboratory environment and diagnostic equipment in the form of autocorrelators (pulse length measurement), spectrometers, and photodiode detectors. This same theme of imparting practical and useful information is carried on in Chap. 12 (by Siavash Yazdanfar and Peter So) about signal detection and processing in nonlinear optical microscopes. This chapter examines technical aspects of detector types [photomultiplier tubes, photodiodes, avalanche photodiodes, and various cameras (CCD, streak, CMOS)], detector selection criteria, spectral/temporal noise, and signal conditioning circuitry. Other chapters deal with fluorescence-related topics, such as the presentation in Chap. 13 (by Chris Zu and Warren Zipfel) of multiphoton excitation of fluorescence probes and practical techniques for estimating the excitation cross section of dyes; Chap. 19 (by Sebastian Wachsmann-Hogiu and Daniel Farkas) of multispectral and fluorescence lifetime imaging including spectral and spatial multiplexing techniques; Chap. 21 (by Wolfgang Becker and Axel Bergmann) on fluorescence lifetime imaging by frequency domain and time domain (analog and photon counting) approaches to fluorescence microscopy; and two chapters on FRET phenomena and theoretical development (Chap. 22 by Masters and So) and FRET microscopy (Chap. 23 by Ye Chen, Horst Wallrabe, and Ammasi Periasamy). Nonlinear optical microscopy affords excellent spatiotemporal resolution as described in Chap. 25 (by Suzzette Pabit and Keith Berland) on two-photon fluorescence correlation spectroscopy (including photon counting histogram analysis) and Chap. 26 (by Edward Brown, Ania Majewska and Rakesh Jain) on fluorescence photobleaching recovery (including compartmentalization analysis). The examination of tissue by multiphoton polarization microscopy (Chap. 20 by Yen Sun et al.) reveals new insights into the architecture of skin, and in Chap. 14 Karsten König describes the potential for cellular photodamage during multiphoton microscopy due to inherently high transient light intensity. Breaking the diffraction barrier with stimulated emission approaches has facilitated the development of single molecule studies, and this approach is detailed in Chap. 24 (by Andreas Schönle et al.). They discuss a particularly interesting example of how this "superresolution" informs neurobiology by establishing resident protein distributions during recycling of synaptic vesicles in neuronal cells. Following the themes es-

tablished in Part II, two chapters examine second harmonic generation microscopy (Chap. 15 by Jerome Mertz) and its application to protein arrays (such as collagen) in tissue (Chap. 16 by Paul Campagnola). Chapter 17 (by Eric Potam and Sunney Xie) expands their theoretical discussion in Part II into an examination of CARS experiments including light sources, scanning microscopes, microspectroscopy, and suppression of nonresonant background signals. In the biological realm, CARS microscopy has proven to be adept at imaging lipids in adipose tissues and cells: however, it has also found applications in materials science with high-resolution mapping of lithographic line patterns in photoresists. Part III provides the reader with substantial experimental and technical information to illuminate the theoretical description of the physical processes involved in nonlinear optical phenomena.

The final section, Part IV, introduced by Bruce Tromberg's discussion of current and emerging applications of multiphoton microscopy (MPM), provides six chapters describing biomedical applications in immunology, neurophysiology, embryology, cellular metabolism, and dermatology. Chapter 28, by David Kleinfeld and coworkers, explores the imaging of electrical and hemodynamic networks within the brains of live animals by MPM, leading to insights about interactions between neuronal activity and blood flow. In Chap. 29, Rakesh Jain and coworkers use intravital MPM for studying tumor-associated blood and lymphatic vessels through various methodologies such as dorsal skinfold chambers and cranial windows. These studies are also conducive to examination (by second harmonic generation) of the influence of fibrillar collagen in the tumor extracellular matrix on tumor cell metastasis and drug transport. Applications to developmental biology described in Chap. 31 by Irina Larina and Mary Dickinson span (nondestructive) longitudinal imaging (overextended phases of development) of embryonic nervous, visual, and cardiovascular systems. These studies underscore the particular importance of genetically modified fluorescent protein (e.g., green fluorescent protein) expressing animals in imaging studies. For studies in tissue, the importance of coupling 3-D imaging capabilities with temporal resolution are highlighted in Chap. 30, by Ian Parker and Michael Cahalan, which discusses visualization of lymphocyte mobility, chemotaxis, and antigen recognition in lymphoid organs and *in vivo* lymph nodes in anesthetized mice. As described in Chap. 32 by Masters and So, MPM has enhanced fundamental understanding of skin architecture, biology, and pathology through noninvasive imaging to depths of hundreds of microns. The optical signals arise from intrinsic components [cellular NAD(P)H and flavoproteins fluorescence] or second harmonic generation from extracellular proteins (collagen and elastin). Extrinsic indicators (fluorophores) are powerful tools for quantifying transdermal transport and skin pathologies. The final chapter, Chap. 33 (by Masters), discusses how intrinsic spatiotemporally resolved signals from MPM in a variety of tissues such as neural, corneal, pancreatic islets, and skin are useful for studying (glycolytic and oxidative) cellular metabolism. The applications of nonlinear optical spectroscopy with an emphasis on biological and biomedical subjects described

Book Review

in Part IV provide the reader with a broad exposure to this rapidly developing and vastly important field.

In summary, the *Handbook of Biomedical Nonlinear Optical Microscopy* provides the reader, both neophyte and veteran, ample material for exploring theoretical foundations, historical development, practical implementation, and varied practical applications. In addition to comprehensive coverage of material relevant to nonlinear optical microscopy, the editors have added reader aids such as two levels of presentation complexity with advanced topics including mathematically and physically rigorous explanations of theory, spectroscopy, instrumentation, and applications. The qualitative descriptions of nonlinear optical phenomena are clear and augmented with historical insights, physical models, analogies to classical and quantum theories, and “discussions” illuminating certain key concepts and equations. The reader is afforded the choice as to how deeply to delve into the theoretical and practical aspects of the field by this presentation design. If required, the profound explanation is provided. In this manner, the handbook is useful to the novice researcher as well as the seasoned practitioner. Since the handbook covers not only fluorescence phenomena but also second harmonic generation and stimulated light scattering, it casts a broad net for all researchers concerned with nonlinear optical spectroscopy and microscopy. The chapters on instrumentation and practical issues are particularly useful. Part IV, which describes various biomedical applications of nonlinear optical spectroscopy, in particular regarding microscopy-based imaging, is an exciting group of chapters because the real-world benefits of this technology

are highlighted. Overall, the editors are to be congratulated for this outstanding effort. It is a handbook that deserves a place on the bookshelf of every researcher considering or conducting biomedical optical studies. On my bookshelf, it's front and center.



William W. Mantulin received his BS degree in chemistry from the University of Rochester in 1968. He continued graduate studies in physical chemistry at Northeastern University with a focus on optical spectroscopy and received his PhD in 1972. After graduation, he accepted a postdoctoral fellowship in the Department of Biochemistry at Texas Tech University to study DNA intercalation reactions. In 1974, he moved to the University of Illinois at Urbana-

Champaign to develop frequency domain methods for studying biological fluorescence applications. In 1976, he joined the staff at the Baylor College of Medicine. As an assistant professor he led a research program in lipoprotein dynamics. In 1986, he returned to the University of Illinois to establish—in collaboration with Enrico Gratton—the Laboratory for Fluorescence Dynamics (LFD), a National Institutes of Health supported technology development center. As director, he helped guide the LFD to become a world-recognized, state-of-the-art fluorescence laboratory, serving local, national, and international scientists. In addition, he pursued an active research program in biological fluorescence and optical tissue imaging as an adjunct professor of biochemistry and biophysics. In 2006, Dr. Mantulin, along with the LFD, transferred to the University of California-Irvine (UCI). Currently, he is leading a neurophotonics program at UCI's Beckman Laser Institute and continuing as a principal research scientist at the LFD.