Quantitative Biomedical Optics

This is the textbook and reference resource that instructors, students, and researchers in biomedical optics have been waiting for. Comprehensive and up-to-date, it covers a broad range of areas in biomedical optics, from light interactions at the single-photon and single-biomolecule levels, to the diffusion regime of light propagation in tissue.

Subjects covered include spectroscopic techniques (fluorescence, Raman, infrared, near-infrared, and elastic scattering), imaging techniques (diffuse optical tomography, photoacoustic imaging, several forms of modern microscopy, and optical coherence tomography), and laser-tissue interactions, including optical tweezers.

Topics are developed from the fundamental principles of physical science, with intuitive explanations, while rigorous mathematical formalisms of theoretical treatments are also provided.

For each technique, descriptions of relevant instrumentation and examples of biomedical applications are outlined, and each chapter benefits from references and suggested resources for further reading, and exercise problems with answers to selected problems.

Irving Bigio is Professor of Biomedical Engineering and Electrical Engineering at Boston University. His research activities address the interactions of light with cellular and tissue structures on the microscopic and mesoscopic scales. He pioneered methods of elastic scattering spectroscopy and has developed practical diagnostic and sensing applications that have been demonstrated in large clinical studies. He has co-authored over two hundred scientific publications and is an inventor on nine patents. Trained in optical physics, he gains satisfaction from explaining the fundamentals of complex phenomena in biomedical optics on an intuitive level. He believes that historical developments in physics theory and artistic expression have influenced each other, leading to parallels between the concepts of physical science and the movements in art. He is convinced that Vincent Van Gogh understood the scattering of starlight by interstellar dust (and was aware of spiral galaxies), as evidenced by Starry Night. He is also convinced that the medical field will finally “discover” the benefits of various clinical applications of biomedical optics.

Sergio Fantini is Professor of Biomedical Engineering and Electrical & Computer Engineering at Tufts University. His research interests in biomedical optics are in the area of diffuse spectroscopy and imaging of biological tissue. He contributed to the development of quantitative frequency-domain methods for absolute tissue oximetry, spectral imaging approaches to optical mammography, and the assessment of cerebral hemodynamics in the human brain. He has co-authored about two hundred scientific publications and is an inventor on 11 patents. He thinks that Falstaff and Otello, by Verdi and Boito with due credit to Shakespeare, and Beethoven’s Opus 131 are among the greatest expressions of the human mind. He is still waiting to witness Fiorentina win the title in the Italian Serie A. While waiting, he is performing translational research aimed at developing quantitative diffuse optical methods for clinical applications.

"Bigio and Fantini provide a long-needed introduction to the field of biomedical optics and biophotonics, adding spice to the presentation of the basics with historical and etymological gems. The conversational tone of the book is very welcome, and allows room for the clear explanation of subtleties not always clarified in other discussions. The book is a wonderful introduction to the field. It balances rigor with readability. Bravo!"

Steven L. Jacques, Oregon Health & Science University

"This book about biomedical optics provides a remarkably comprehensive introduction to the field. The text is carefully and affectionately developed with quantitative rigor, and it is written in a clear, easy-to-understand style that helps students develop intuition. The subject matter covers basics of linear and nonlinear optical spectroscopy, static and dynamic light scattering and more advanced topics such as light transport through highly scattering tissues, acousto-optics and opto-acoustics, and imaging from microscopy to tomography. The book should prove useful as a textbook for courses targeting both advanced undergraduates and graduate students in science, engineering, and medicine. It will also be a valuable reference for researchers working at the frontiers of knowledge."

Arjun G. Yodh, University of Pennsylvania

"Bigio and Fantini's comprehensive text on Biomedical Optics provides a wonderful blend of accessible theory and practical guidance relevant to the design and application of biomedical optical systems. It should be required reading for all graduate students working in this area."

Rebecca Richards-Kortum, Rice University

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Quantitative Biomedical Optics

Theory, Methods, and Applications

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To our families, mentors, teachers, and students

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Preface

A textbook for a new field based on old concepts

Biomedical optics is a field that is both new and ancient. From the vantage point of the natural sciences and engineering, this is a newly developing interdisciplinary field, dealing with the application of optical science and technology to biological and biomedical problems, including clinical applications. On the other hand, the field has been around for thousands of years in a less quantitative way. Physicians' eyes have served as optical spectrographs and sensors, with the brain serving as a database repository and providing the computational power (of a massively parallel computer) for pattern recognition. For example, physicians have known for a long time that a Caucasian patient with yellowing of the skin (or of the sclera of the eye) is likely to be suffering from liver disease. If the patient is flushed red, he/she might be running a fever, and if a local tissue area appears flushed and red, an inflammation is indicated; and the bluish appearance of a patient's lips and nail beds might be indicative of hypoxia. Now that the modern approach has become more quantitative and is developing new technologies, however, the field is growing and beginning to have a major impact on bioscience and healthcare. The emerging field combines the observational with the mathematical and computational, benefits from recent advances in optical technologies, and is coupled with a more rigorous physical-science approach that seeks to understand the basic underlying principles.

This textbook provides a broad survey of the field and covers the basics of a quantitative approach to the subtopics, taking advantage of the powerful tools offered by mathematics, physics and engineering. This quantitative approach and the didactic style, coupled with the description of representative applications and problem sets that accompany each chapter, are designed to serve the needs of students and professionals in engineering and the physical sciences. Students of the biological sciences will also find the text useful, especially if they have a good mathematical background. The basic material about general concepts and methods that are directly relevant to biomedical optics, including some topics of medical statistics, are described at an introductory level, whereas selected topics are covered in greater depth and treated at a more advanced level. Consequently, by proper selection of the material, this textbook can be used for upper-level undergraduate courses, as well as more advanced graduate-level courses on biomedical optics.

The coverage of this book is broader than that of other currently available texts in the field, teaching a broader range of topics under the umbrella of biomedical optics and explaining the interrelationships among them. It also emphasizes aspects in all three areas of theory, instrumentation, and biomedical applications to provide a comprehensive view of each biomedical optics technique that is presented. As a result of its broad coverage, the book can also serve as a reference resource for researchers in biomedical optics. Although the survey is broad, it is by no means exhaustive. Some topics have been bypassed, mainly because they pertain to areas that have not yet developed to the point that they are based on a mathematical formalism or a clear understanding of the physical principles. This book also leaves to others the general field of photonic biosensors, which is broad enough to merit a textbook of its own.

Is it biomedical optics or biophotonics?

One might ask the simplest of questions: what is the proper name for the field, *biomedical optics* or *biophotonics*? The question may be tackled from an etymological point of view by recognizing that the Greek word "οπτική" (optiki) is associated with *vision* while the Greek word "φωτόνιο" (fotonio) is associated with *light*. This seems to suggest that biophotonics may be a more appropriate term for a field in which light is used to interrogate biological systems and to interact with them. One should recognize, however, that the word *optics* has been traditionally used to describe the science of light, which only during the twentieth century was recognized to consist of quanta for which the word *photons* was coined. Therefore, biomedical optics conjures up the more classical elements of lenses, fiber optics, lasers (not so classical) and, perhaps, ophthalmic applications, whereas biophotonics might appear to speak to the quantum-mechanical or statistical nature of light and its interaction with biological tissue. If electronics refers to the generation, manipulation and detection of electrons, then, by analogy, photonics refers to the generation, control and detection of photons, the quantum units of light.

It is arguable that optics and photonics speak to the wave-like and particle-like nature of light, respectively. Although a debate of sorts had been ongoing since the days of Newton as to the true nature of light, modern physicists explain that the two are intertwined (if not entangled!) and the distinction is more philosophical. Historically, the mathematical and physics tools of both approaches have been shown to produce the same results, in most cases, when describing the same phenomena. An instructive view of the matter can be gained from examining how the language of physics itself varies with the frequency (or wavelength) when describing electromagnetic radiation. For low frequencies, such as radio waves, the wave nature completely dominates and enables explanation of all phenomena of interest, given that the photon energy is extremely low ($<10^{-4}$ eV), and a detected radio-wave field is composed of a large number of photons. At very high frequencies, as with "hard" X-rays or γ-radiation, the photon energy is much larger ($>10^4$ eV), and formalisms for interactions focus on the photonic nature of the field (with the exception of the methods for X-ray crystallography). The intersection of the two regimes happens in the range centered on visible light (characterized by photon energies of 1.6–3.1 eV) where both the photoelectric effect and the wave-nature of light are important.

In this textbook, we utilize whichever approach is simpler and more intuitive for understanding a specific concept. Thus, it is easier to think of the wave-nature of light when explaining, for example, the interference effects relevant to optical coherence tomography, whereas it is conceptually simpler to think of photons as particles when describing the random diffusion of photons (also referred to as *photon migration*) in densely scattering media, such as most biological tissues. In the end, we have chosen the word *Optics* in the title of this book, perhaps because one speaks more naturally about, say, "optical diagnostics" or "optical microscopy" or "optical fibers," rather than "photonic diagnostics" or "photonic microscopy" or "photonic fibers."

Why is the field of biomedical optics important?

The dramatic growth of the field in recent years is a consequence of the realization that optical methods offer the potential to have a significant impact on the broad field of health care, and also to provide novel tools for an increasingly quantitative approach to biology. When used for measurement and diagnostic purposes in living systems, light is, under most circumstances, essentially noninvasive. Thus, for biomedical applications there is a growing list of distinct advantages:

- Light (at visible and near-infrared wavelengths) is non-ionizing radiation, and at sub-thermal levels has no cumulative effect on tissue.
- Light can be used to reveal much about tissue that cannot be determined by other imaging or sensing modalities.
- Light can travel farther into tissue than one might think. Although scattering is strong, near-infrared light is only weakly absorbed in tissue, and can diffuse

across several centimeters of tissue, enabling, for example, imaging and sensing of structures and function in solid organs, such as skeletal muscles, breast or brain.

- Optical fibers can be used to deliver and collect light, permitting access to remote sites within the body, mediated by endoscopes, catheters or needles.
- Properties of tissue that are not commonly or readily monitored in real time can be measured with light, enabling new types of diagnostic measurements, e.g., blood oxygenation without drawing blood, cellular nucleus size, etc.
- Optical imaging typically features high temporal resolution, down to the millisecond range.
- The spatial resolution of optical imaging techniques scales with the penetration depth, from sub-micron in microscopy applications at depths up to $\sim$100 μm, to several millimeters for diffuse optical imaging at depths of centimeters in tissue. Importantly, throughout the range, optical methods offer functional information not available with other imaging modalities.
- New methods of therapy can be accomplished with light, enabling new ways to treat diseases or repair problems in tissue. The use of light enables interactions to be highly specific as a consequence of wavelength selectivity, spatial selectivity (with tight focusing), temporal selectivity (with ultrashort laser pulses), or cellular and molecular selectivity (with molecular targeting agents).

In short, biomedical optics is ideally suited to serve the trend of modern clinical medicine: the development of noninvasive or minimally invasive diagnostics and therapeutics. It also opens new avenues for biomedical research at the cellular and molecular levels.

Physical modeling in quantitative biomedical optics

In the field of physics, the expression "simplicity is elegance" has been passed down as gospel since Albert Einstein's time. The elegance of simple physical models, however, does not always go hand in hand with the complexity of biological systems. Figure 0.1(a) shows an elegantly simple physicist's view of a chicken, in the spirit of the old joke: "a physicist postulates a chicken as a sphere of uniform density." The chicken is represented in a less simple and more realistic form in Figure 0.1(b). Of course, the simple chicken model of Figure 0.1(a) is far from representative of the complexity of a real chicken, which, some might argue, is more closely represented by the more sophisticated and more complex model of Figure 0.1(b). The question that must often be tackled in quantitative biomedical optics is whether the added complexity of more sophisticated models and

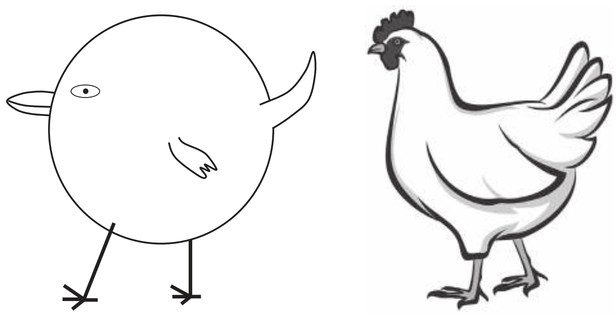

Figure 0.1

(a) An overly simplified view of a chicken; (b) a more sophisticated and complex representation of a chicken. Is it always better to use a more complex model for a biological system? This is a key question in quantitative biomedical optics. (Figure 0.1(b) courtesy of Cliparts.co, http://cliparts.co/clipart/186502.)

treatments is really required by the specific application in hand, and whether it truly teaches more about reality. For example, if one needs to discriminate a chicken from a giraffe, the simple representation of Figure 0.1(a) might be adequate (assuming a physicist would postulate a giraffe as a long cylinder of uniform density!), but it would not be adequate to model shape variations that would distinguish a hen from a rooster, for which the more sophisticated model of Figure 0.1(b) may be necessary. Given the complexity of biological systems, finding a good compromise between the simplicity of physical models and their appropriate representation of the biological parameters of interest is a critical objective to be achieved in quantitative biomedical optics. In writing this book, we have endeavored to strike the right balance, so that the basic physical principles can be understood, while useful quantitative results can be obtained from their application.

Organization of the book

We have strived to present the broad range of topics covered in this book in a consolidated way. We have cross-referenced material from different chapters every time ideas presented in one area had relevance or implications in other areas. We have also used consistent symbols and notations, and we have paid particular attention to the physical dimensions and associated units. For example, even though the scattering phase function (p) is dimensionless, by expressing it in units of sr^{-1} one immediately appreciates its meaning of a probability per unit solid angle. This also results in its discrimination from the scattering probability

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per unit scattering angle (indicated in this text as p_θ), when the latter is expressed in units of rad^{-1}. As another example, we have explicitly discussed the units of diffuse reflectance and their implications in understanding its physical meaning.

In our descriptions and derivations, our goal is to provide a clear presentation of the basic principles and their implications, and we have included references in the bibliographic sections of each chapter to direct the interested reader to more advanced material or in-depth treatments. For example, we have not presented the solutions to the diffusion equation in a variety of tissue geometries (slab, sphere, cylinder, etc.); rather, we have focused on the two ideal cases of infinite and semi-infinite media, to introduce and discuss the basic parameters in play, their interdependence, and the roles they play in optical measurements in the diffusion regime. Our aim is to strike a good balance between presenting the material in a comprehensive, rigorous, and quantitative fashion, while keeping a descriptive tone with intuitive explanations and illustrative examples.

The chapters of this book are arranged in the following four groupings:

I. **Chapters 1–4.** These provide the broad underpinnings for the field. Chapter 1 defines the ***nomenclature*** that we adopt for this book, including the symbols and units, along with the rationale for many of the choices. Chapter 2 provides a broad overview of the ***optical properties of biological tissues***, and introduces the constituents that contribute to those properties. The quantitative formalisms for representing those optical properties are also presented. Chapter 3 teaches the ***basics of biomedical statistics***, in the language of probability theory, especially as applied to diagnostic tests that may be the translational goal of many of the technologies described in this book. Chapter 4 is an overview of the ***basics of optical spectroscopy***, including a comparison of the merits of different types of spectroscopy as applied to tissue diagnostics; also included are overviews of the optical-science basics of instrumentation for tissue spectroscopy and of optical fibers.

II. **Chapter 5–8.** These chapters present in-depth developments of four classes of tissue spectroscopy, predominantly related to superficial measurements or measurements in the sub-diffuse regime. Chapter 5 covers ***fluorescence spectroscopy and imaging***, starting with the molecular physics of fluorescence, followed by listings of the important endogenous fluorophores and exogenous reporter fluorophores. Then, instrumentation and methods are described, including methods for fluorescence lifetime and polarization measurements. Chapter 6 covers spectroscopic methods for measurement of ***vibrational modes of biomolecules***, starting with the molecular physics concepts of vibrational transitions, followed by discussions of ***IR-absorption spectroscopy*** and ***Raman scattering spectroscopy***. Chapter 7 teaches the physics of ***scattering by single dielectric particles***, including Mie theory, and lays the groundwork for measurements of elastic scattering; also covered are dynamic light scattering from single particles and Doppler flowmetry. Chapter 8 extends the principles established in Chapter 7 to measurements of ***multiply scattered light in the sub-diffuse regime***, and the extraction of tissue optical properties from measurements at short source-detector distances.

III. **Chapters 9–15.** These chapters cover the theoretical formalisms and a range of measurement methods for light transport in the diffuse regime. Chapter 9 introduces the ***Boltzmann transport equation*** and derives its best-known approximations, including the ***diffusion equation*** in various forms and the constraints of boundary conditions. Chapter 10 teaches the basics and formalisms of ***continuous-wave tissue spectroscopy***, with special attention to applications in the diffusion regime. Chapter 11 presents formalism and methods of ***time-domain spectroscopy*** in the diffusion regime, with a special emphasis on the features of the photon time-of-flight distribution. Chapter 12 formulates the ***frequency-domain spectroscopy*** method in the diffusion regime, including a detailed development of the concept of photon-density waves. Chapter 13 presents a broad overview of the types of ***instrumentation and experimental methods for diffuse tissue spectroscopy***. Chapter 14 focuses on methods of ***optical imaging in the diffusion regime***, and covers a broad array of parameter sensitivities and methods for solution of the inverse imaging problem. Chapter 15 concludes this grouping by presenting several exemplary areas of ***applications of diffuse optical methods***, for both clinical and pre-clinical use.

IV. **Chapters 16–19.** These chapters cover methods of higher-resolution imaging in tissue and several advantageous areas of laser-tissue interactions. Chapter 16 introduces the basic concepts of ***acousto-optic and opto-acoustic methods*** for imaging at various length-scales based on the interaction or combination of ultrasound and light in tissue. Chapter 17 starts with the basics of a classical compound microscope, then teaches the basics of several classes of ***modern optical microscopy*** that are important in biomedical science, and concludes with recent developments of super-resolution microscopy. Chapter 18 introduces the fundamentals of ***optical coherence tomography***, implemented with both time-domain and frequency-domain methods, and includes some practical considerations and applications. Finally, Chapter 19 overviews the fundamentals of ***optical tweezers***, with related instrumentation and applications, and presents a survey of the more practical types of ***laser-tissue interactions*** from sub-thermal tissue treatments to microsurgical techniques.