

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.”

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Quantitative Biomedical Optics

Theory, Methods, and Applications

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CAMBRIDGE
UNIVERSITY PRESS

Shaftesbury Road, Cambridge CB2 8EA, United Kingdom

One Liberty Plaza, 20th Floor, New York, NY 10006, USA

477 Williamstown Road, Port Melbourne, VIC 3207, Australia

314–321, 3rd Floor, Plot 3, Splendor Forum, Jasola District Centre, New Delhi – 110025, India

103 Penang Road, #05–06/07, Visioncrest Commercial, Singapore 238467

Cambridge University Press is part of Cambridge University Press & Assessment,
a department of the University of Cambridge.

We share the University's mission to contribute to society through the pursuit of
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www.cambridge.org

Information on this title: www.cambridge.org/9780521876568

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First published 2016

A catalogue record for this publication is available from the British Library

ISBN 978-0-521-87656-8 Hardback

Additional resources for this publication at www.cambridge.org/9780521876568

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Cambridge University Press & Assessment
978-0-521-87656-8 — Quantitative Biomedical Optics
Irving J. Bigio, Sergio Fantini
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To our families, mentors, teachers, and students

Contents

<i>Preface</i>	<i>page xxi</i>
1 Nomenclature	1
1.1 Describing the optical radiation field and its interactions with tissue	2
1.2 Quantities describing the optical radiation field	4
1.3 Quantities describing the optical properties of tissue and the interactions between the radiation field and tissue	10
2 Overview of tissue optical properties	19
2.1 Absorption and scattering coefficients	20
2.2 Survey of the primary chromophores in tissue	21
2.3 Non-chromophore absorbers	30
2.3.1 Lipids	30
2.3.2 Water, water, everywhere – and vibrational modes	31
2.4 The scattering properties of tissue	33
2.4.1 Sources of scattering in tissue	33
2.4.2 The scattering cross section and its relation to the scattering coefficient	34
2.4.3 The anisotropy factor in tissue	35
2.4.4 The reduced scattering coefficient in tissue	38
2.5 The Beer-Lambert law to describe absorption and weak scattering	39
Appendix 2.A: Tabulated near-infrared spectra of HbO, Hb, cytochrome <i>c</i> oxidase, water and lipids	41
3 Introduction to biomedical statistics for diagnostic applications	60
3.1 The importance of proper statistics	61
3.2 Assessing the efficacy of dichotomous diagnostic tests	62
3.2.1 Sensitivity, specificity, positive and negative predictive values	65
3.3 Test accuracy	68

3.4	Dichotomous decisions based on a continuous variable	69
3.4.1	The ROC curve	70
3.5	Required sample size and reliability of a test	72
3.5.1	Example 1: testing sensitivity and specificity with pre-diagnosed sets	74
3.5.2	Example 2: determining Se and Sp for a population sample with an expected prevalence	75
3.5.3	Example 3: measuring the prevalence of a disease in a population	76
3.6	Prospective vs. retrospective studies	78
4	General concepts of tissue spectroscopy and instrumentation	83
4.1	Atomic spectroscopy	84
4.2	Molecular spectroscopy: from diatomic molecules to biological molecules	87
4.3	Electronic transitions – the issue of electron spin	91
4.3.1	Fluorescence and phosphorescence	92
4.4	Vibrational transitions: IR absorption spectroscopy and Raman spectroscopy	95
4.5	Scattering by particles: elastic-scattering spectroscopy	97
4.6	Relative merits of different types of tissue spectroscopy	98
4.6.1	Fluorescence measurements and spectroscopy	98
4.6.2	Infrared-absorption spectroscopy	99
4.6.3	Raman spectroscopy	100
4.6.4	Elastic-scattering spectroscopy and diffuse reflectance spectroscopy	100
4.7	Introduction to general principles of spectroscopic measurements and instrumentation	101
4.7.1	What is light?	101
4.7.2	Anatomy of a spectrometer	104
4.7.2.1	Dispersive element: prisms	105
4.7.2.2	Dispersive element: diffraction gratings	106
4.7.2.3	Exemplary configuration of a grating spectrometer	107
4.8	Basics of optical fibers	108
4.8.1	Numerical aperture of a fiber	110
4.8.2	Basic fiber types	110
4.8.3	Single-mode vs. multimode fibers	111
4.8.4	Graded-index fibers	112
4.8.5	Imaging fiber bundles	113
4.8.6	Photonic-crystal fibers (PCF)	114

5	Autofluorescence spectroscopy and reporter fluorescence	118
5.1	Fluorescence emission relevant to measurements in biological systems	119
5.1.1	Quantum yield	122
5.1.2	Excitation-emission matrix	122
5.1.3	Fluorescence intensity vs. fluorophore concentration	124
5.2	Fluorescence lifetime	125
5.3	Endogenous fluorescent molecules	126
5.4	Exogenous “reporter” fluorophores	129
5.4.1	Exogenous fluorophores in common clinical use: fluorescein and indocyanine green	129
5.4.2	Drugs that are fluorescent	132
5.4.3	Fluorescent biomolecular probes	133
5.4.4	Nanoparticles as fluorescent biomarkers: quantum dots	133
5.4.5	Molecular beacon probes based on oligonucleotide hybridization	135
5.5	Instrumentation for fluorescence sensing and imaging	136
5.5.1	Instrumentation for point measurements	136
5.5.2	Instrumentation for fluorescence imaging	138
5.6	Fluorescence lifetime spectroscopy	140
5.6.1	Measurement of multiple fluorophores	141
5.6.2	Example applications of fluorescence lifetime measurements	143
5.7	Polarization and anisotropy of fluorescence emission	143
5.7.1	Combining polarization and time-dependent fluorescence measurements	145
5.8	Sensing molecular dynamics with fluorescent biomarkers: FRET	146
6	Raman and infrared spectroscopy of vibrational modes	153
6.1	Vibrational modes in biological molecules	153
6.1.1	Frequencies of some biomolecular vibrational modes	155
6.1.2	The complexity of biological molecules and feature assignment	157
6.2	Semi-classical derivation of Raman scattering	160
6.2.1	Wavelength dependence of the scattered field from molecules	163
6.2.2	A little bit less simplification	166
6.3	IR-absorption spectroscopy	167
6.4	Is it IR-active, Raman-active or both?	169
6.4.1	Homonuclear diatomic molecules	170
6.4.2	Heteronuclear diatomic molecules	170
6.4.3	Linear triatomic molecules	171
6.4.4	Nonlinear triatomic molecules	172

6.5	Isotopic shifts	173
6.6	Enhancements of Raman scattering	174
6.6.1	Resonance-Raman scattering (RRS)	174
6.6.2	Surface-enhanced Raman scattering (SERS)	175
6.6.3	Coherent anti-Stokes Raman spectroscopy (CARS)	175
6.7	Instrumentation for IR-absorption and Raman spectroscopy	177
6.7.1	Fourier-transform IR (FTIR) spectroscopy	177
6.7.2	Basic instrumentation for Raman spectroscopy of biological samples	178
6.7.3	Instrumentation invoking fiberoptic probes	179
6.7.4	Data treatment for Raman spectra of biological media	180
6.8	Examples of pre-clinical and clinical applications of Raman spectroscopy	181
7	Elastic and quasi-elastic scattering from cells and small structures	186
7.1	Sources of light scattering in biological systems	187
7.2	Scattering by a single particle: coordinates and formalism	191
7.2.1	Rayleigh scattering	194
7.2.1.1	Polarized Rayleigh scattering	197
7.2.2	Mie theory	197
7.3	Exact and numerical calculations of scattering by single particles of arbitrary shape	207
7.4	Single-scattering methods to study cell properties: instrumentation and applications	211
7.4.1	Measuring the scattering phase function: methods and devices	211
7.4.2	Flow cytometry	213
7.4.3	Measurements of single scattering events in tissue in a transmission geometry	214
7.4.4	Measurement of singly scattered photons in a backscattering geometry	215
7.4.4.1	Scattering from cell monolayers	215
7.4.4.2	Selection of singly backscattered light from bulk tissue	216
7.4.4.3	Angle-resolved low-coherence interferometry (aLCI)	218
7.5	Scattering from particles in motion	219
7.5.1	Doppler shifts and laser Doppler velocimetry	219
7.5.2	Dynamic light scattering	222

8 Diffuse reflectance spectroscopy at small source-detector separations	229
8.1 Practical optical geometries	230
8.1.1 Monte Carlo simulations: where have the collected photons been?	230
8.2 Representing tissue scattering properties in the partially diffuse regime	233
8.2.1 Importance of the phase function for short source-detector separations – phase functions for multiple scattering	235
8.2.2 Higher moments of the phase function	236
8.2.3 The Henyey-Greenstein phase function	238
8.2.4 The modified Henyey-Greenstein phase function	238
8.2.5 Mie-theory phase function for multiple scattering	239
8.3 Representing tissue absorption properties in the partially diffuse regime	241
8.3.1 Correction for the vessel-packing effect	242
8.4 Empirical models for the diffuse reflectance	243
8.4.1 A representative empirical model	246
8.4.2 Lookup tables	249
8.5 The inverse problem: extracting tissue properties	249
8.5.1 Varying the source-detector separation	250
8.5.2 Measurements at multiple wavelengths	251
8.6 Special distances for diffuse reflectance measurements: “isosbestic” points	252
8.6.1 Source-detector distances for which the average photon pathlength is insensitive to the tissue reduced scattering coefficient	253
8.6.2 Source-detector separations for which the reflectance is insensitive to the tissue reduced scattering coefficient	255
8.6.3 Source-detector separation for which the reflectance is insensitive to the phase function parameter γ	256
8.7 Typical instrumentation for diffuse reflectance spectroscopy	257
8.7.1 Common components for DRS	258
8.7.2 Calibration of system response	259
8.7.3 Tissue “phantoms” for validation	259
8.8 Examples of clinical and pre-clinical applications of DRS with incoherent light	261
8.8.1 Elastic-scattering spectroscopy for detection of dysplasia	261
8.8.2 Measurement of drug concentrations in tissue	263
8.9 Quasi-coherent variants of diffuse reflectance spectroscopy	265
8.9.1 Diffuse correlation spectroscopy in the short source-detector distance regime	265

8.9.2	Laser speckle contrast imaging	267
8.9.3	Low-coherence enhanced backscattering	269
9	Transport theory and the diffusion equation	277
9.1	The Boltzmann transport equation	278
9.2	Expansion of the Boltzmann transport equation into spherical harmonics	282
9.3	The P_N approximation	287
9.4	The P_1 approximation	288
9.5	The diffusion equation	293
9.6	Boundary conditions	295
9.6.1	Infinite medium	295
9.6.2	Boundary between a scattering medium and a non-scattering medium: mismatched refractive index conditions	297
9.6.3	Boundary between a scattering medium and a non-scattering medium: matched refractive index conditions	302
9.6.4	Boundary between two scattering media	303
9.7	The microscopic Beer-Lambert law	305
9.8	The fluorescence diffusion equation	306
9.9	The correlation diffusion equation	308
	Appendix 9.A: Low-order spherical harmonics	309
	Appendix 9.B: The dependence of the diffusion coefficient on absorption	310
10	Continuous-wave methods for tissue spectroscopy	317
10.1	The objective of tissue spectroscopy	318
10.2	CW tissue spectroscopy with diffusion theory	319
10.2.1	CW solution for an infinite, homogeneous medium	319
10.2.2	Determination of the tissue optical properties with CW diffusion theory	322
10.3	The modified Beer-Lambert law	324
10.4	Continuous-wave diffuse reflectance	327
10.4.1	Total diffuse reflectance vs. single-distance diffuse reflectance	327
10.4.2	Diffuse reflectance with transport theory	330
10.4.3	Two-flux Kubelka-Munk theory	332
10.4.4	Diffusion theory for single-distance reflectance and total diffuse reflectance	334
10.4.5	Spatially modulated spectroscopy	339
10.4.6	Monte Carlo simulations: limits of validity of continuous-wave diffusion theory	340
	Appendix 10.A: The Dirac delta	342

11 Time-domain methods for tissue spectroscopy in the diffusion regime	348
11.1 Diffusion theory: time-domain solution for an infinite, homogeneous medium	349
11.2 Moments of the photon time-of-flight distribution	354
11.3 Time-domain diffuse reflectance	357
11.4 Limits of validity of time-domain diffusion theory	361
12 Frequency-domain methods for tissue spectroscopy in the diffusion regime	365
12.1 Basic concepts of frequency-domain tissue spectroscopy	365
12.2 Diffusion theory: frequency-domain solution for an infinite, homogeneous medium	367
12.3 Absolute measurements of μ_a and μ'_s with frequency-domain spectroscopy	374
12.4 Photon-density waves	375
12.4.1 The wavelength of photon-density waves	377
12.4.2 The phase velocity of photon-density waves	378
12.4.3 The attenuation length of photon-density waves	379
12.5 The frequency domain as the Fourier transform of the time domain	380
12.6 Frequency-domain diffuse reflectance	381
12.7 Limits of validity of frequency-domain diffusion theory	385
Appendix 12.A: Fourier integrals of time-domain Green's functions	385
12.A.1 Frequency-domain fluence rate in an infinite medium	386
12.A.2 Frequency-domain diffuse reflectance from a semi-infinite medium	386
13 Instrumentation and experimental methods for diffuse tissue spectroscopy	391
13.1 Light sources	391
13.1.1 Relevant properties of light sources for diffuse optical spectroscopy	391
13.1.2 Spectral distribution of illumination	392
13.1.3 Pulsed and modulated sources	393
13.1.4 Maximum permissible levels of optical exposure of the skin	394
13.2 Methods for delivering and collecting light	396
13.2.1 Optical fibers	396
13.2.2 Direct illumination and light collection	397
13.3 Optical detectors	397
13.3.1 General description of optical detectors	397
13.3.2 Spectral sensitivity	401
13.3.3 Linearity and dynamic range	401

13.3.4	Temporal response	402
13.3.5	Responsivity and sensitivity	403
13.3.6	Shot noise	405
13.4	Experimental approaches for time-resolved spectroscopy	407
13.4.1	Time domain	407
13.4.1.1	Streak camera	408
13.4.1.2	Time-correlated single-photon counting	409
13.4.1.3	Time gating	410
13.4.2	Frequency domain	411
13.4.2.1	Homodyne detection	412
13.4.2.2	Heterodyne detection	414
14	Diffuse optical imaging and tomography	420
14.1	Collective photon paths in a scattering medium	421
14.1.1	Sensitivity function of a given optical signal to a specific optical property	421
14.1.2	The CW region of sensitivity	422
14.1.2.1	Infinite geometry	423
14.1.2.2	Semi-infinite geometry	426
14.2	Backprojection methods	429
14.3	Diffuse optical imaging with time-gated approaches	431
14.4	Spatial frequency-domain imaging	433
14.5	Diffuse optical tomography: the forward problem	433
14.5.1	Deterministic forward models: transport theory and diffusion theory	435
14.5.1.1	Analytical solutions	436
14.5.1.2	Finite-difference method (FDM)	437
14.5.1.3	Finite-element method (FEM)	437
14.5.2	Stochastic models and methods: Monte Carlo simulations and random walk theory	438
14.6	Diffuse optical tomography: the inverse problem	440
14.6.1	Linear methods based on perturbation theory	440
14.6.1.1	Linearization for absorption and diffusion perturbations	441
14.6.1.2	The CW sensitivity function for absorption perturbations in infinite and semi-infinite media	443
14.6.1.3	Discretization of the problem into a linear system of equations	448
14.6.2	Nonlinear, iterative methods based on calculation of the Jacobian	449
14.7	Regularization of the inverse imaging problem	452
14.8	Contrast and resolution in diffuse optical imaging	453

14.8.1	Optical properties contrast	453
14.8.2	Image contrast	455
14.8.3	Spatial resolution	456
Appendix 14.A:	Fluence rate Green's functions for a semi-infinite medium	457
15	In vivo applications of diffuse optical spectroscopy and imaging	467
15.1	Oximetry	468
15.1.1	The oxygen dissociation curve of hemoglobin	468
15.1.2	Pulse oximetry	471
15.1.3	Tissue oximetry	474
15.1.3.1	Oxygen saturation of hemoglobin in blood-perfused tissue	474
15.1.3.2	Redox state of cytochrome <i>c</i> oxidase	475
15.2	Skeletal muscle studies	478
15.2.1	The oxygen dissociation curve of myoglobin	478
15.2.2	Blood volume, blood flow, and oxygen consumption in skeletal muscle	479
15.2.3	NIRS measurements of muscle metabolism during exercise	482
15.2.4	Confounding factors in optical studies of skeletal muscle	484
15.3	Functional brain investigations	484
15.3.1	Effects of brain activation on hemoglobin and oxidized cytochrome concentrations	484
15.3.2	Modeling the hemoglobin concentration dependence on CBV, CBF, and CMRO ₂	487
15.3.3	Diffuse correlation spectroscopy (DCS) to measure cerebral blood flow	489
15.3.4	Optical imaging of intrinsic signals (OIS): high-resolution brain mapping	489
15.3.5	Functional near-infrared imaging (fNIRI): noninvasive brain mapping	492
15.4	Optical mammography	496
15.5	Small-animal imaging	501
15.6	Prospects of diffuse optics for biomedical applications	503
16	Combining light and ultrasound: acousto-optics and opto-acoustics	512
16.1	Basic concepts of ultrasound imaging	513
16.1.1	The nature of ultrasound	513
16.1.2	The source of contrast in ultrasound imaging	515
16.1.3	Ultrasound transducers	516
16.1.4	Spatial resolution in ultrasound imaging	517

16.2	Acousto-optic spectroscopy and imaging by ultrasonic tagging of light	518
16.2.1	Mechanisms of ultrasonic modulation of light intensity	518
16.2.2	Experimental methods to detect ultrasonically tagged light	522
16.2.3	Applications of ultrasonically tagged light	525
16.3	Photoacoustic imaging	526
16.3.1	Generation of ultrasound by pulsed illumination	526
16.3.2	Instrumentation for photoacoustic imaging	530
16.4	Photoacoustic tomography and microscopy	532
16.4.1	The range of penetration depths afforded by photoacoustic imaging	532
16.4.2	Photoacoustic tomography (PAT)	533
16.4.3	Photoacoustic microscopy (PAM)	534
16.5	Applications of photoacoustic imaging	536
16.5.1	Imaging of tissue vascularization and microvasculature	536
16.5.2	Hemoglobin saturation and blood flow	537
16.5.3	Other applications	538
17	Modern optical microscopy for biomedical applications	543
17.1	Basic elements and theory of a classical microscope	544
17.1.1	Magnification	544
17.1.2	Resolving power and the point spread function	546
17.1.2.1	The optical transfer function	550
17.1.3	Enhancement of resolution with immersion objective lens	551
17.2	Microscopic imaging based on phase contrast (PC) and differential interference contrast (DIC)	552
17.2.1	Phase contrast imaging with spatially coherent illumination	552
17.2.2	Phase contrast imaging with incoherent illumination	555
17.2.3	Applications of phase contrast and DIC microscopy	558
17.3	Optical sectioning: confocal microscopy	558
17.3.1	Basic design of a confocal microscope	560
17.3.2	Axial resolution of a confocal microscope	562
17.3.3	Application example of in vivo confocal microscopy	564
17.4	Nonlinear optical microscopy	566
17.4.1	Multi-photon excitation fluorescence microscopy	567
17.4.2	Harmonic generation microscopy	570
17.4.2.1	Phase shifts and radiation patterns	572
17.4.2.2	Symmetry of the sample and enabled applications	573
17.5	Super-resolution microscopy	574

17.5.1	Direct super-resolution: near-field scanning optical microscopy (NSOM)	575
17.5.2	Point-source localization techniques for super-resolution	576
17.5.3	Stimulated emission depletion (STED) microscopy	577
17.5.4	Stochastic methods of super-resolution	578
18	Optical coherence tomography	585
18.1	The coherence length of light	586
18.1.1	Coherence length of a short laser pulse	588
18.1.2	Coherence length of a long-pulse or continuous light source	590
18.2	Time-domain optical coherence tomography	592
18.2.1	Sources of contrast and basics of OCT	592
18.2.2	Detection of the TD-OCT signal	595
18.2.3	Scanning of TD-OCT to achieve an image	596
18.2.4	Maximum sectioning depth and transverse resolution	597
18.3	Frequency-domain optical coherence tomography	598
18.3.1	Spectral-domain OCT	598
18.3.2	Swept-source OCT	599
18.3.3	The SNR advantage of FD-OCT	599
18.4	Doppler OCT	600
18.5	Instrumentation for OCT	601
18.5.1	Optical components	601
18.5.2	Light sources and detectors	602
18.5.3	Transverse scanning	603
18.6	Speckle in OCT images	604
18.7	A sampling of OCT applications	605
19	Optical tweezers and laser-tissue interactions	612
19.1	Optical tweezers	613
19.1.1	Forces resulting from radiation pressure	613
19.1.2	Trapping force for particles larger than the wavelength	615
19.1.3	Trapping of particles much smaller than the wavelength	619
19.1.4	Trapping of particles similar in size to the wavelength	621
19.1.5	Generic instrumentation for optical tweezers	622
19.1.6	Calibration of trapping force	624
19.2	A sampling of applications of optical tweezers	625
19.3	Sub-thermal, thermal, and ablative applications of lasers	627
19.3.1	Sub-thermal irradiation	628
19.3.2	Non-ablative thermal effects	630
19.3.2.1	Laser tissue welding	631
19.3.2.2	Interstitial laser thermotherapy	632
19.3.3	Tissue ablation and microsurgery	633

xx Contents

19.3.3.1	Laser-assisted in vitro fertilization	634
19.3.3.2	Laser refractive surgery	635
19.3.3.3	Some cosmetic applications in dermatology	637
	<i>Answers to selected problems identified by *</i>	645
	<i>Table of symbols</i>	649
	<i>Table of acronyms</i>	654
	<i>Index</i>	659

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 $\sim 100\ \mu\text{m}$, 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

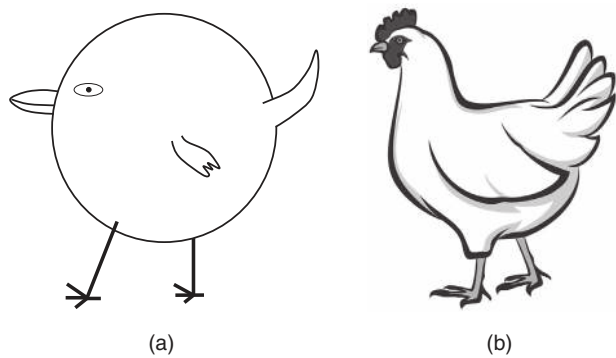


Figure 0.1

(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

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.