HANDBOOK OF

OPTICAL BIOMEDICAL DIAGNOSTICS

SECOND EDITION

Volume 2: Methods

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SPIE PRESS
Bellingham, Washington USA

Library of Congress Cataloging-in-Publication Data

Names: Tuchin, V. V. (Valerii Viktorovich), editor.
Title: Handbook of optical biomedical diagnostics / Valery V. Tuchin, editor.
Other titles: Optical biomedical diagnostics
Description: Second edition. | Bellingham, Washington : SPIE Press, [2016] | Includes bibliographical references and index.
Identifiers: LCCN 2015038341| ISBN 9781628419092 (v. 1 : alk. paper) | ISBN 9781628419139 (v. 2 : alk. paper)
Subjects: | MESH: Diagnostic Imaging–methods. | Microscopy, Confocal. | Spectrum Analysis.
Classification: LCC R857.O6 | NLM WN 180 | DDC 616.07/54–dc23
LC record available at http://lccn.loc.gov/2015038341

Published by

SPIE
P.O. Box 10
Bellingham, Washington 98227-0010 USA
Phone: +1 360.676.3290
Fax: +1 360.647.1445
Email: books@spie.org
Web: http://spie.org

Printed in the United States of America.
First printing.

Images on the cover were reprinted with permission from the following publications:
- A. Doronin, C. Macdonald, and I. Meglinski, "Propagation of coherent polarized light in highly scattering turbid media," *J. Biomed. Opt.* **19**(2), 025005 (2014).
- S. J. Kirkpatrick and D. D. Duncan, "Noncontact microstrain measurements in orthodontic wires," *J. Biomed. Mater. Res.* **29**, 1437–1442 (1995).

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Preface

This *Handbook* is the second edition of the monograph initially published in 2002. The first edition described some aspects of laser–cell and laser–tissue interactions that are basic for biomedical diagnostics and presented many optical and laser diagnostic technologies prospective for clinical applications. The main reason for publishing such a book was the achievements of the last millennium in light scattering and coherent light effects in tissues, and in the design of novel laser and photonics techniques for the examination of the human body. Since 2002, biomedical optics and biophotonics have had rapid and extensive development, leading to technical advances that increase the utility and market growth of optical technologies. Recent developments in the field of biophotonics are wide-ranging and include novel light sources, delivery and detection techniques that can extend the imaging range and spectroscopic probe quality, and the combination of optical techniques with other imaging modalities.

The innovative character of photonics and biophotonics is underlined by two Nobel prizes in 2014 awarded to Eric Betzig, Stefan W. Hell, and William E. Moerner "for the development of super-resolved fluorescence microscopy" and to Isamu Akasaki, Hiroshi Amano, and Shuji Nakamura "for the invention of efficient blue light-emitting diodes which has enabled bright and energy-saving white light sources." The authors of this *Handbook* have a strong input in the development of new solutions in biomedical optics and biophotonics and have conducted cutting-edge research and developments over the last 10–15 years, the results of which were used to modify and update early written chapters. Many new, world-recognized experts in the field have joined the team of authors who introduce fresh blood in the book and provide a new perspective on many aspects of optical biomedical diagnostics.

The optical medical diagnostic field covers many spectroscopic and laser technologies based on near-infrared (NIR) spectrophotometry, fluorescence and Raman spectroscopy, optical coherent tomography (OCT), confocal microscopy, optoacoustic (photoacoustic) tomography, photon-correlation spectroscopy and imaging, and Doppler and speckle monitoring of biological flows.[1–45] These topics—as well as the main trends of the modern laser diagnostic techniques, their fundamentals and corresponding basic research

on laser–tissue interactions, and the most interesting clinical applications—are discussed in the framework of this Handbook. The main unique features of the book are as follows:

1. Several chapters of basic research that discuss the updated results on light scattering, speckle formation, and other nondestructive interactions of laser light with tissue; they also provide a basis for the optical and laser medical diagnostic techniques presented in the other chapters.
2. A detailed discussion of blood optics, blood and lymph flow, and blood-aggregation measurement techniques, such as the well-recognized laser Doppler method, speckle technique, and OCT method.
3. A discussion of the most-recent prospective methods of laser (coherent) tomography and spectroscopy, including OCT, optoacoustic (photoacoustic) imaging, diffusive wave spectroscopy (DWS), and diffusion frequency-domain techniques.

The intended audience of this book consists of researchers, postgraduate and undergraduate students, biomedical engineers, and physicians who are interested in the design and applications of optical and laser methods and instruments for medical science and practice. Due to the large number of fundamental concepts and basic research on laser–tissue interactions presented here, it should prove useful for a much broader audience that includes students and physicians, as well. Investigators who are deeply involved in the field will find up-to-date results for the topics discussed. Each chapter is written by representatives of the leading research groups who have presented their classic and most recent results. Physicians and biomedical engineers may be interested in the clinical applications of designed techniques and instruments, which are described in a few chapters. Indeed, laser and photonics engineers may also be interested in the book because their acquaintance with a new field of laser and photonics applications can stimulate new ideas for lasers and photonic devices design. The two volumes of this *Handbook* contain 21 chapters, divided into four parts (two per volume):

- Part I describes the fundamentals and basic research of the extinction of light in dispersive media; the structure and models of tissues, cells, and cell ensembles; blood optics; coherence phenomena and statistical properties of scattered light; and the propagation of optical pulses and photon-density waves in turbid media. Tissue phantoms as tools for tissue study and calibration of measurements are also discussed.
- Part II presents time-resolved (pulse and frequency-domain) imaging and spectroscopy methods and techniques applied to tissues, including optoacoustic (photoacoustic) methods. The absolute quantification of the main absorbers in tissue by a NIR spectroscopy method is discussed. An example biomedical application—the possibility of monitoring brain activity with NIR spectroscopy—is analyzed.

- Part III presents various spectroscopic techniques of tissues based on elastic and Raman light scattering, Fourier transform infrared (FTIR), and fluorescence spectroscopies. In particular, the principles and applications of backscattering diagnostics of red blood cell (RBC) aggregation in whole blood samples and epithelial tissues are discussed. Other topics include combined back reflectance and fluorescence, FTIR and Raman spectroscopies of the human skin *in vivo*, and fluorescence technologies for biomedical diagnostics.
- The final section, Part IV, begins with a chapter on laser Doppler microscopy, one of the representative coherent-domain methods applied to monitoring blood in motion. Methods and techniques of real-time imaging of tissue ultrastructure and blood flows using OCT is also discussed. The section also describes various speckle techniques for monitoring and imaging tissue, in particular, for studying tissue mechanics and blood and lymph flow.

Financial support from a FiDiPro grant of TEKES, Finland (40111/11) and Academic D.I. Mendeleev Fund Program of Tomsk National Research State University have helped me complete this book project. I greatly appreciate the cooperation and contribution of all of the authors and co-editors, who have done a great work on preparation of this book. I would like to express my gratitude to Eric Pepper and Tim Lamkins for their suggestion to prepare the second edition of the *Handbook* and to Scott McNeill for assistance in editing the manuscript. I am very thankful to all of my colleagues from the Chair and Research Education Institute of Optics and Biophotonics at Saratov National Research State University and the Institute of Precision Mechanics and Control of RAS for their collaboration, fruitful discussions, and valuable comments. I am very grateful to my wife and entire family for their exceptional patience and understanding.

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April 2016

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Part III: Scattering, Fluorescence, Infrared, and Raman Spectroscopy of Tissues

This part of the *Handbook* describes the basic principles and diagnostic applications of optical techniques based on detecting and processing the scattering, fluorescence, FT IR, and Raman spectroscopic signals from various tissues, with an emphasis on blood, epithelial tissues, and human skin.

Chapter 1 covers the approaches to quantitative measurement of the spontaneous aggregation kinetics of red blood cells in whole blood samples and the biophysical and clinical importance of these measurements. It is shown that real-time measurement of the backscattered light intensity provides information on a number of important characteristics of blood related to the hemorhological status of the donor. Although there are a number of parameters influencing the aggregation and disaggregation measurements, statistically significant correlations with different diseases can be obtained that have high diagnostic value for clinicians. The relation of blood aggregation and sedimentation measurements is also discussed. It is shown that the new emerging modality of laser manipulation and trapping (laser tweezers) is very helpful when studying the individual features of interaction between cells, measuring the corresponding forces and the kinetics of cells aggregation and disaggregation.

Chapter 2 overviews the principles and applications of light scattering spectroscopy of epithelial tissues. It describes novel techniques capable of identifying and characterizing pathological changes in these tissues at the cellular and sub-cellular levels and providing structural and functional information about the tissue. The discussion is focused on studying epithelial morphology in living tissues without tissue removal aiming at noninvasive or minimally invasive detection of precancerous and early cancerous changes in a variety of organs such as esophagus, colon, uterine cervix, oral cavity, lungs,

and urinary bladder. The main goal of this chapter is to provide the readers with basic tools necessary to understand the potentials of biomedical light scattering spectroscopy, including sufficient medical and biological background and principles of light scattering by cells and sub-cellular structures. The relation of single and multiple scattering in tissue is particularly considered. Finally, the applications of various types of light scattering in detection of early cancer and precancerous conditions are reviewed. In addition, several recently developed clinical tools are described including the endoscopic polarized scanning spectroscopy (EPSS) instrument, which is compatible with existing endoscopes. It scans large areas of the esophagus chosen by the physician and has the software and algorithms necessary to obtain quantitative, objective data about tissue structure and composition, which can be translated into diagnostic information in real time. This process enables the physician to take confirming biopsies at suspicious sites and minimize the number of biopsies taken at nondysplastic sites. Another newly developed technique, called confocal light absorption and scattering spectroscopic (CLASS) microscopy, combines light-scattering spectroscopy (LSS) with confocal microscopy. In CLASS microscopy, light-scattering spectra are the source of the contrast. Another important aspect of LSS is its ability to detect and characterize particles well beyond the diffraction limit.

Chapter 3 discusses the applications of reflectance and fluorescence spectroscopies for the assessment of the optical properties of human skin in relation to different diseases, environmental factors, and the effectiveness of various treatments. Applied to the skin *in vivo*, these techniques provide information on the structure of epidermis and dermis, on the quantity and density of blood vessels, on the concentration and spatial distribution of chromophores and fluorophores in skin, and on the nature of skin metabolic processes. The authors discuss the potential advantages and possible applications of the combined use of reflectance and fluorescence spectroscopy of skin for the evaluation of erythema and pigmentation indices, the determination of hemoglobin oxygenation and concentration, and the investigation of the efficacy of topical sunscreens. Simple models are used to analyze changes in skin reflectance and fluorescence spectra as a result of morphological and functional alterations in skin, or as a result of treatment effects. Such changes can be monitored by imaging techniques, in particular, in polarized light and analyzing the color characteristics of the reflected light. Ways to improve the accuracy of skin diagnostics and the efficiency of skin therapy by analyzing and controlling the skin optical parameters are also discussed in this chapter. In particular, the authors demonstrate how to control the sensitivity of skin reflectance spectra by compression and stretching. A special emphasis is made on the potentialities of immersion optical clearing and corresponding decrease in the scattering coefficient in tissue studies. Ways to raise the efficiency of optical clearing, e.g., by

accelerating the penetration of the index-matching compounds by enhancing skin permeability through creating a lattice of microzones (islets) of limited thermal damage in the stratum corneum, are also discussed.

Chapter 4 discusses the basic principles and potentialities of *in vivo* diagnostics of human skin by vibrational spectroscopic techniques, namely, Fourier transform infrared spectroscopy and confocal Raman microspectroscopy. The detailed information on the molecular composition, structure, and organization of the skin and, in particular, the content of water and natural moisturizing factor in human skin epidermis that can be obtained with these techniques is highlighted. The results of the research, reviewed in this chapter, provide the means for various applications of these techniques in cosmetics, pharmacology, clinical diagnosis, treatment monitoring, and surgery. A large part of the chapter is devoted to the resonance Raman spectroscopy of cutaneous carotenoids. These substances form an antioxidant network of living skin and quick *in vivo* measurement of their amount in skin is very important when estimating the status of a human organism. Distribution of carotenoids in the human skin and the factors influencing their concentration are discussed.

Finally, Chapter 5 overviews different fluorescence technologies used in biomedical diagnostics. It provides information on the basic principles of fluorescence spectroscopy, microscopy, and imaging, including the continuous-wave, time-gated, and time-resolved variants. Theory and applications to cell biology of total internal reflection fluorescence spectroscopy and microscopy, energy transfer spectroscopy and wide-field 3D microscopy (including structured illumination and light sheet microscopies) are described in detail. This is followed by a discussion of the principles as well as current and possible future applications of laser scanning and multiphoton microscopy. In the last part of the chapter, the super-resolution and single-molecule detection possibilities are briefly discussed.

Overall, the chapters provide readers with knowledge of a very important and quickly developing field of optical biomedical diagnostics.

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