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Series Editor: Javid Atai

Malini Olivo
U. S. Dinish *Editors*

Frontiers in Biophotonics for Translational Medicine

In the Celebration of Year of Light (2015)

Springer

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Javid Atai

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Foreword I

The UN proclaimed 2015 as the International Year of Light and Light-based Technologies, emphasizing achievements in the optical sciences and their importance to humankind. Light in the ultraviolet, visible, and infrared spectral region is a fundamental tool of human inquiry. This miniscule region of the vast electromagnetic spectrum is the only one that provides molecular information directly. Because molecules are the building blocks of life, the importance of light in the life sciences and medicine cannot be overemphasized.

Man-made high-resolution optical imaging dates to 350 years ago, when planar optical microscopy enabled visualization of subcellular structures, the basis of histology. However, light scattering in biological tissue presents a multitude of challenges to optical penetration. Wavefront aberration limits planar microscopy to tens of microns of penetration. Three centuries later, the advent of the laser and other new light sources enabled a host of new microscopic technologies. Tomographic optical microscopy—such as confocal microscopy, two-photon microscopy, and optical coherence tomography—beats the wavefront aberration limit by suppressing multi-scattered signals, but is still limited by diffusion to ~ 1 mm of penetration. Three-dimensional photoacoustic microscopy and photoacoustic computed tomography have conquered the diffusion limit by combining diffuse-light excitation and unscattered ultrasonic wave detection and advanced the penetration limit by nearly two orders of magnitude. Currently, penetration is confined only by dissipation. Wavefront engineering with internal guide stars, a nascent innovation, promises to shatter the dissipation limit and approach the absorption limit for whole-body penetration in human tissue.

This timely book highlights selected advances in biomedical optical technologies made toward translational biomedicine. The chapters are contributed by thought leaders responsible for cutting-edge developments in their areas of research. The technologies that have been commercialized for clinical or preclinical applications include optical coherence tomography, fluorescence lifetime microscopy, photoacoustic microscopy, and photoacoustic computed tomography.

Upcoming technologies with excellent translation potential include Raman spectroscopy, plasmonic sensing, nanoparticle-enhanced endoscopy, in situ gas spectroscopy, and diffuse optics. Also important is the chapter from a leading medical instrumentation company, highlighting their view of optical imaging in clinical laparoscopic surgery.

While the twentieth century was revolutionized by physical sciences, this century will belong to engineering and life sciences. As an interface between these disciplines, biomedical optics is particularly exciting. Our field will undoubtedly see even greater fruition by leveraging the previous century's invention—such as large-scale semiconductors, computers, lasers, nanotechnology, and ultrafast detectors. Let us join hands and use light to eradicate the most challenging diseases and make the world a healthier place to live.

Prof. Lihong V. Wang
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Foreword II

Optical imaging is not an emerging technology anymore in the world of molecular imaging. This book is a perfect testament of the advent of a new era in optical bioimaging and biosensing development which has already shown its impact in preclinical research, cancer detection, drug development, prognosis and diagnosis, image-guided therapies at the bedside, and many other areas of biomedicine. The potential of optical imaging remains considerable. Modern personalized medicine calls for noninvasive and nonionizing high performance imaging methods, to preserve cell integrity and allow harmless repeated explorations over time. Optical imaging meets increasingly those requirements with the rapid advances in biophotonics which provide cost-effective methods allowing deeper penetration into tissues and organs and ever-increasing specificity and resolution. These methods offer other significant competitive advantages: they are amenable to combination with other imaging modalities such as MRI and they can be used label-free or in conjunction with versatile intrinsic and extrinsic metabolic/chemical probes, dyes, conjugates, and contrast media to document in a targeted manner, cell physiology and function, molecular mechanisms, and gene expression.

In this context, this book is particularly timely, especially when its publication occurs in 2015, the International Year of Light and Light-based Technologies. It has been assembled by two world leading specialists in biophotonics, Professor Olivo and Dr. Dinish in Singapore, who have been successful in gathering contributions from the best international experts in the field of advanced optical imaging. The 11 chapters cover selected hot topics illustrating the high translational potential of biophotonics together with several actual transfers from bench to bedside. No doubt this opus will be of utmost interest for scientists and clinicians, both specialists and newcomers in this exciting field of modern optical bioimaging methods and their applications to medicine.

Prof. Patrick J. Cozzone
Executive Director of the Singapore Bioimaging Consortium
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About the Editors

Prof. Malini Olivo is currently the head of Bio-Optical Imaging Group, Singapore Bioimaging Consortium, A*STAR, Singapore, e-mail: malini_olivo@sbic.a-star.edu.sg; malini.olivo@nuigalway.ie. She holds a Stokes Professorship at the National University of Ireland and Royal College of Surgeons, Dublin, Ireland. She obtained a Ph.D. in Bio-Medical Physics in 1990 and did her postdoctoral training between 1991 and 1995 in University College London, UK, McMaster University and University of Toronto, Canada.

Since 1995 she has been very active in pioneering biophotonics research in Singapore. In 2015 she was awarded the OSA Fellowship for her pioneering contribution to photomedicine in the area of clinical biophotonics for diagnostics and therapeutics of cancer. The International Society for Photonics and Optics conferred an award for her contribution as a woman in photonics. She has won numerous awards in recognition of her contribution to biophotonics in Singapore, Ireland, and United States. She has published over 300 peer reviewed scientific papers, 12 book chapters, one book, and 23 patents. She has secured >US$20 million in research grants over the years and serves in the editorial board of photonics journals. Malini Olivo is well recognized internationally in her field and serves in numerous scientific advisory boards in the area of Photonics in Medicine.

Dr. U. S. Dinish is a biophysicist working as Research Scientist at Bio-Optical Imaging Group, Singapore Bioimaging Consortium, under Agency for Science Technology and Research (A*STAR), Singapore, e-mail: dinish@sbic.a-star.edu.sg. He obtained his Ph.D. in bio-optics and imaging in 2005 from Nanyang Technological University (NTU), Singapore. His current research interests include Nano-biophotonics and its applications in translational medicine and biomedical optical instrumentation and spectroscopy. He holds nine patents/patent applications and has published over 80 international journal papers and conference proceedings/presentations. He also authored four book chapters and won young investigator and best paper awards in international conferences in 2006 and 2012.

Dinish organized and chaired special sessions on bio-optical imaging and sensing in international photonics conferences. He is serving as reviewer for 14 leading journals in the field of optics, bio-optics, nanotechnology, and nanomaterials and won the certificate of appreciation for excellent review service (2012, 2013, and 2014) from the chief editor of Journal of Biomedical Optics (JBO), SPIE, USA. Currently, he is serving as the editorial board member of the journal 'Scientific Reports' (Nature Publishing Group) in biological physics category since 2014. Dinish was also nominated as consulting editor for the 'International Journal of Nanomedicine' (Dove press) since 2015.

Chapter 1
Fluorescence Lifetime Spectroscopy and Imaging Techniques in Medical Applications

Dimitris Gorpas and Laura Marcu

Abstract This chapter reviews the fluorescence lifetime techniques currently applied in biomedical diagnostics. Specifically the chapter focuses on time-resolved fluorescence spectroscopy (TRFS) and fluorescence lifetime imaging (FLIM) technologies for in vivo tissue characterization, with special emphasis on the translational potential of these techniques and the prospects of autofluorescence to provide intrinsic contrast for the assessment and diagnosis of human diseases. The use of these techniques in a number of medical applications, including cancer (gastrointestinal tract, lung, head and neck, brain and breast), skin and eye diseases, and atherosclerotic cardiovascular disease, are discussed and their recent developments towards translational medicine are highlighted.

1 Introduction

Certain molecules in biological tissues have the ability to emit light for a short duration (typically $<10^{-8}$ s), following the absorption of photons. This process is called fluorescence. The measurement of fluorescence emission is an established method for resolving the molecular composition of biological samples, using a variety of instruments, i.e. spectrophotometers, microarrays, microscopes, and endoscopes. Processing of fluorescence signal can reveal not only information on the specific molecular constituents but also on the local environment surrounding a specific fluorescence molecule or fluorophore. Fluorescence contrast and dynamics are often characterized by studying the excitation and emission spectra, quantum efficiency, polarization and fluorescence lifetime [1–6]. Among the most common

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tissue fluorophores are the aromatic amino acids (tyrosine, tryptophane, and phenylalanine), structural proteins (elastin, collagens, and collagen cross-links), enzyme metabolic co-factors [nicotin-amide adenine (phosphate) dinucleotide (NAD(P)H and flavin adenine dinucleotide (FAD)], lipid components and porphyrins. The optical properties of these fluorophores have been extensively studied, reviewed, and reported in literature [2–5, 7–9]. Detection of fluorescence emitted from intrinsic tissue fluorophores strongly depends on the surrounding micro-environment, making their in vivo characterization a challenging procedure [4].

The fluorescence emission of exogenous molecular probes has been also used for tissue diagnosis. With well-defined optical properties, exogenous probes are primarily used in near-infrared applications [10–14], where the background signal (autofluorescence) is minimal and excitation light penetrates deeper in tissue. However, among the numerous molecular probes under investigation only a few have been approved for human use by the U.S. Food and Drug Administration (FDA). These include fluorescein and indocyanine green (ICG) [7, 14], as well as porphyrin-based photosensitizers used in photodynamic therapy [15–21].

This chapter focuses on applications assessing the sources of endogenous fluorescence lifetime contrast and their prospects for in vivo tissue characterization and diagnosis. An in depth discussion of such applications is provided in the following sections.

2 Fluorescence Contrast for Tissue Characterization

Fluorescence measurements are commonly used for quantification of the optical properties of biological cells and tissues. Their interpretation can provide biochemical, functional and structural information of the sources of fluorescence contrast within these biological entities. Changes in tissue fluorescence properties can result from pathological transformations, therapeutic interventions, or during disease development, and thus intrinsic fluorescence has been frequently exploited as a diagnostic tool. Applications are diverse, ranging from cancer and intravascular diseases diagnostics to assessment of bioengineered tissues [2–5, 7–9, 15, 17, 20, 22–32]. Improvement in light delivery and collection systems via fiber optics is one of the key factors that lead to increased scientific and clinical interest towards tissue fluorescence measurements. Fiberoptic based systems have allowed for the development of non- or minimally invasive modules even for remote tissue fluorescence acquisition by using endoscopes or catheters [33–35]. Numerous published studies have clearly demonstrated the applicability of fluorescence spectroscopy and imaging for the assessment of various tissue pathologies.

Both steady-state (intensity and/or spectrum) and time-resolved (time- or frequency-domain) fluorescence measurements (Fig. 1) have been investigated for quantitative and qualitative compositional and pathophysiological analysis of biological tissues. Due to their rather simple and low-cost hardware implementation, the steady-state methods have been mostly exploited for human tissue diagnostics

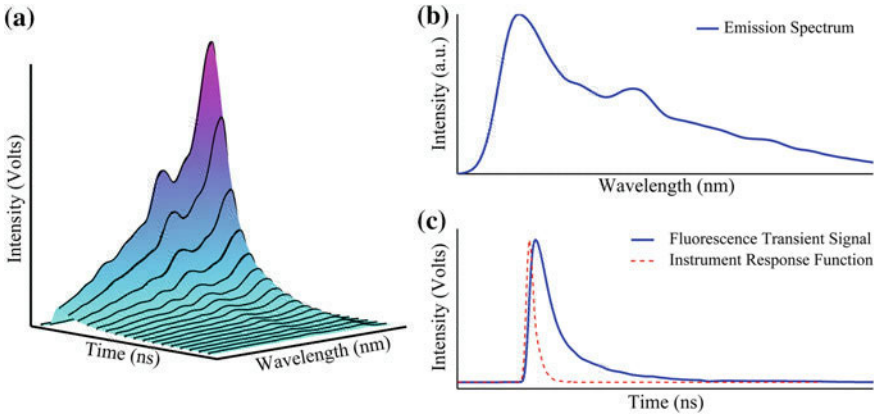

Fig. 1 **a** Spectro-temporal profile of fluorescence emission induced by sequential excitation light stimuli; **b** Fluorescence emission spectrum depicting integrated intensity values per wavelength; **c** Fluorescence transient signal as a result of a single excitation light stimulus

in vivo. Over the past decade commercial systems have emerged. One of the first fluorescence systems commercially available was the Light-Induced Fluorescence Endoscopy (LIFE) device from Xillix Technologies Corp. More recently Novadaq Technologies Inc. released the updated version of LIFE system, known as PINPOINT Endoscopic Fluorescence Imaging System, previously known as Onco-LIFE Autofluorescence Endoscopic Technology [36]. Karl Storz has also released an endoscopic system known as Video Bronchoscope for Autofluorescence Diagnostics, which was previously known as D-Light® System [37]. Both these systems have been developed to analyze fluorescence signals and visualize, through contrasting color assignment, bronchi and gastrointestinal (GI) tract diagnostic outcomes. The fluorescence excitation light for both of them is in the blue region of spectrum (400–450 nm for PINTPOINT and 380–440 nm for Video Bronchoscope for Autofluorescence Diagnostics), while the fluorescence detection is performed in the green region (470–560 nm) for the first system and in the green and red regions (475–800 nm) for the second. Other endoscopic fluorescence systems available nowadays are the SAFE-3000 system (408 nm excitation/430–700 nm detection) from Pentax [38], the AFI-Lucera® system (395–445 nm excitation/490–700 nm detection) from Olympus [39], DAFE (390–470 nm excitation/475–650 detection) from Richard Wolf [40], and Cellvisio (488/600 nm excitation/505–700/680–900 nm detection) from Mauna Kea Technologies [41] that can provide real-time microscopic images from endogenous or exogenous fluorophores.

Commercial systems based on combined fluorescence and reflectance spectroscopy were also developed for the diagnosis of cervix cancer. Namely two such systems are Luma Cervical Imaging System (337 nm excitation/360–720 nm detection) from MediSpectra Inc. (now acquired from SprectraScience Inc. [42]) and LuViva® Advanced Cervical Scan (multiple excitation and detection wavelength combinations through filter wheel) from Guided Therapeutics [43].