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Fluorescence Lifetime Spectroscopy and Imaging: Principles and Applications in Biomedical Diagnostics

Laura Marcu, Paul M. W. French, and Daniel S. Elson, eds., 554 pages + xvi, ISBN: 978-1-4398-6167-7, CRC Press, Boca Raton, Florida (2015), $179.95, hardcover.

Reviewed by Barry R. Masters, Fellow of AAAS, OSA, and SPIE

The combination of spectroscopy and microscopy has provided the researcher and the clinician with a variety of optical devices that are both useful and prevalent in both the laboratory and the clinic. Typically, spectroscopic studies were based on intensity measurements. The absorption spectrum, emission spectrum, anisotropy, and the fluorescence lifetime were measured. Biophysicists and later cell biologists began to use fluorescent molecules to investigate the structure and the function of biological molecules such as antibodies and proteins. Later, more complex molecular assemblies such as cell membranes or the Golgi apparatus were investigated using fluorescence microscopy. Then these techniques were applied to tissues and organisms, and finally to *in vivo* human studies, such as the skin, the eye, and the heart.

These fluorescent microscopic techniques were based on intensity measurements of live cells, tissues, and organs in which there is a significant degree of tissue heterogeneity, light scattering, and a multitude of physical factors and conditions that affect the intensity measurements. The implementation of fluorescent lifetime measurements and imaging in the field of biomedicine is an attempt to overcome the inherent difficulties of intensity measurements in absolute units. This alternative methodology is based on the assumption that within the duration of the fluorescence decay, the fluorescence lifetime measurements are independent of the fluorophore concentration, the instrumentation for the excitation, and the detection instrumentation of the fluorescence decay signal. The modern advances are due to several factors: ultrafast lasers and light sources, advances in analog and digital signal processing, advances in detector design, and new analytical methods to analyze the decay data, and the ability to form images that are based on the decay data. The new instrumentation and analytical approaches are still dependent of the numbers of detected photons and the signal-to-noise ratios, but they are useful for live cell studies and human clinical studies when they are validated with appropriate control measurements. The new advances in both instrumentation and data analysis techniques that are comprehensively and clearly elucidated in this book permit the application of these methods to investigations of live cells and to *in vivo* studies.

The editors (physicists and engineers) and the contributors to *Fluorescence Lifetime Spectroscopy and Imaging* have produced a recommended book that provides a good starting place for the researcher or the clinician who is considering the implementation of these techniques in their research or instrument development. The anticipated audience for the book includes graduate students and established researchers and biomedical engineers. The book is divided into several parts: fluorescence measurements, instrumentation to measure fluorescence lifetimes, the analysis of lifetime data, applications in the field of oncology and ophthalmology, and the use of exogenous probes.

The photophysics is clearly described. The text and its associated figures are well integrated and work in a synergistic fashion to aid the understanding of the reader. The rigorous development of the mathematics is what separates this book from others. The extensive selection of the references is appropriate to each chapter. I found the parts of the book that critically discuss the theory, instrumentation, and methods of data analysis to be comprehensive, up-to-date, critical, and rigorous. The sections of the book that discuss lifetime fluorescence instrumentation and those on the analysis of fluorescence lifetime data are outstanding and extremely useful. This book is an excellent single source for investigators who are contemplating the use of these techniques into their research problems. The text is augmented by excellent tables and figures. The inclusion of a very comprehensive chapter on fluorescence lifetime imaging in turbid media is required reading for all those who plan to use these techniques in live cells and with *in vivo* applications. The chapter on light transport in turbid media introduces the radiative transfer equation and then introduces several approximations with a clear exposition of the inherent assumptions. A discussion of the inverse problem in diffusion imaging is required, and the book provides a good discussion.

On the other hand, I found the chapters on medical applications to be less rigorous, and perhaps over simplified in the treatment of the interpretation of the fluorescence from the intrinsic metabolic probe NAD(P)H. In the Preface the editors state: "However, autofluorescence from naturally occurring (endogenous) fluorophores in biological tissues can provide label-free contrast between different [metabolic] states of tissue for medical research and clinical applications." Furthermore, "This book particularly concerns the application of time-resolved (lifetime) measurement to improve the quantitative molecular assessment of biological tissues using fluorescence, which can be usefully applied to both endogenous and exogenous fluorophores in biological tissue."

In vivo human tissue and organs such as the skin, the heart, and the eye are complex highly scattering structures composed of various cell types and sometimes with layered structures composed of distinct cell types. In the case of spectroscopic measurements that are made over the component layers of different cell types, the data is confounded due to averaging over the distinct cell types within the microscopic observation field of the specimen. For example, in the cornea, each of the cell layers within the epithelial layer are composed of different

cell types with different metabolic pathways. The same confounding conditions hold for the anterior epithelial layers and the single posterior endothelial cell layer. Similar complexities hold for the structure of human skin, which varies in structure over the body. The human retina presents a similar situation with various layers containing many types of cells and fluorophores.

In the past, the interpretation of spectroscopic measurement on live tissues such as those listed above was based on the emission, absorption, and lifetime measurements made on pure isolated compounds such as NADH in a buffer solution, or in isolated liver mitochondria. It is dangerous to take spectroscopic data from one tissue or organelle and generalize to other tissues. Furthermore, many of the so-called functional spectroscopic measurements that are made on *in vivo* human tissues and organs make the assumption that the emission and the absorption measurement can be used to determine the source of the fluorescence. Typically, that assumption is not validated. The absorption and the emission spectra from complex *in vivo* human tissues are confounded by the presence of multiple fluorophores with overlapping spectra. The mathematical methods used to separate the putative sources of the combined spectra do not yield a unique solution as the problem is ill-posed. The best statistical analysis of the data may not yield the correct spectra from the complex mixture. While this sounds daunting, there are techniques to help validate the assertion that the emission spectrum is from NAD(P)H. In NAD(P)H cellular metabolic redox imaging of the *in situ* cornea, the addition of cyanide—a known inhibitor of the mitochondrial respiratory chain—slowly increases the fluorescence intensity of the NAD(P)H. The usual assumption that fluorescence can monitor cellular metabolic states must be validated on a tissue-by-tissue basis.

Fluorescence Lifetime Spectroscopy and Imaging is a valuable modern compendium of this exciting field of research that extends innovative fluorescence techniques to clinical diagnosis and treatment. It provides the reader with an excellent source for a detailed discussion of the techniques, their implementation in innovative instrumentation, the techniques of analysis and their limitations, and a number of examples of applications to the field of biomedicine.