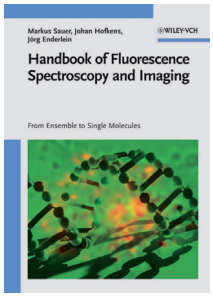
Handbook of Fluorescence Spectroscopy and Imaging

The terms "Fluorescence Spectroscopy" and "Fluorescence Imaging" comprise a diverse collection of experimental techniques used widely in the physical and life sciences to probe a variety of processes and phenomena. In the life sciences, fluorescence techniques have been instrumental in shaping our current knowledge of the structure and dynamics of biomolecules, and how (bio)molecular interactions determine their biological functions. In the last two decades the detection of individual molecules in vitro has revolutionized the field by providing a means by which the dynamic behavior of different subpopulations of biomolecules can be measured directly. In addition, in recent years, technical advances in fluorescence imaging and the development of genetically-encoded fluorescent probes have made it possible to visualize single molecules inside cells with unprecedented resolution.

The *Handbook of Fluorescence Spectroscopy and Imaging* aims, in the words of the authors, to become a ready reference for graduate students and researchers interested in using advanced fluorescence techniques and single-molecule fluorescence spectroscopy and imaging. Yet, those expecting a comprehensive treatment of these topics will be disappointed, as the book covers a rather small range of subjects that do not necessarily represent with justice the diverse variety of fluorescence techniques that are used in biology today. The analysis and interpretation of time-resolved fluorescence data represents, for instance, a notable omission. Time-resolved intensity and fluorescence polarization decays are crucial for the elucidation of solvent relaxation effects, quenching mechanisms and energy transfer processes, all of which are extensively used in the investigation of the structure and dynamics of biomolecules and the characterization of biomolecular interactions. In addition, the "imaging" part promised in the title is not covered as adequately as one might expect for a handbook. While the readers will find a chapter that reviews the newly-developed (and fascinating) field of super-resolution imaging, they will not be exposed to the less exotic but much more widely used imaging techniques such as fluorescence lifetime imaging (FLIM), multi-photon confocal microscopy, and FRET imaging. The book is organized in nine chapters that, although not signed by any individual author, have been obviously written independently without a common style or thread. Because the different chapters have been written at significantly different levels, most readers will find a section of the book that will fit their level and interest, but most will likely not find the book useful as a whole. The chapter on FRET, for instance, is written at a fairly low level, with just the basic equations needed to introduce the topic and a qualitative description of some applications, while the chapter on correlation techniques is exceptionally rigorous and mathematically intensive, but provides almost no examples of applications. Those readers that will appreciate the rigorous treatment of fluorescence correlation spectroscopy will likely find the rest of the book rather trivial, while those with no expertise in the field will miss the opportunity to appreciate the potential that the technique has to offer in the life sciences.

The first chapter in the book covers the basic principles of fluorescence spectroscopy. The chapter is written at a fairly low level (starting with the electromagnetic spectrum, the particle-in-the-box, and the Lambert–Beer law), and it is too brief for those who actually need this introduction to appreciate the rest of the book. Chapters 2 and 3 are devoted to fluorescent labels and fluorophore labeling, and stand out from the rest for they contain information that will appeal to amateurs and experts alike as is not readily available in review articles or introductory books. Chapter 2 introduces the reader to the different types of fluorescent labels used in single-molecule fluorescence research, focusing on the photophysical properties that are relevant for these types of experiments, while Chapter 3 offers an introduction to fluorescence labeling of biomolecules, and includes a section on genetically-encoded fluorescent proteins. Chapter 4, a very short section on fluorophore selection for single-molecule fluorescence spectroscopy, repeats some of the material included in Chapters 2 and 3 and could have been easily integrated into the preceding chapters. Chapter 5 covers fluorescence correlation techniques, and represents an abrupt change in level and style. This chapter contains a rigorous and math-intensive treatment of fluorescence correlation spectroscopy that will appeal to those who are extensively familiarized with the technique, but will likely overwhelm those who have had little or no exposure to the topic. The section on rotational diffusion is particularly noteworthy and will greatly interest those seeking a theoretical treatment of the topic. Despite this rigor, the authors have chosen to omit other fluctuation techniques such as those based on brightness analysis, those that involve more than one color, and image correlation techniques that have proven to be particularly powerful for research in vivo.

The transition into Chapter 6, devoted to FRET, is again abrupt. This chapter aims to



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introduce the basic concepts of FRET and provide a collection of discussed applications, but will likely disappoint beginners and experts equally. The chapter contains typos, inaccuracies, omissions, and mistakes that will confuse the non-experts and frustrate those who might consider using this book as an educational tool in their research labs or in the classroom. An equation that supposedly describes the use of anisotropy to determine FRET efficiencies, for instance, does not agree with any of the formal treatments of the topic described in the literature, and is not backed up by any reference. Whether this equation is valid in any particular situation is not clear, and I would be concerned by the possible misconceptions that such a simplified treatment of the topic can generate. Two out of the five values of Förster radius listed in table 6.1 do not match the values reported in the references provided by the authors. In particular, the value for the Cy3–Cy5 pair, perhaps the most commonly used FRET pair in the life sciences, has been misquoted by a whopping 18 Å, rendering it essentially nonsensical. A rather long list of other typos and inaccuracies puts this chapter below the bar. Readers interested in the fundamentals and applications of FRET will find a better value in Lakowicz's *Principles of Fluorescence Spectroscopy* or Valeur's *Molecular Fluorescence*, and those interested in single-molecule applications will be better off reading some of the many excellent reviews written on the topic.

The remainder of the book is devoted to photoinduced electron transfer (Chapter 7), super-resolution imaging (Chapter 8) and single-molecule enzymatics (Chapter 9). The level of these chapters is adequate for the non-experts, and their style and breadth is similar to what the reader will find in many of the review articles that these and other authors have written in scientific journals. The book ends with an index that is particularly disappointing for a handbook that aims to become a reference for students and researchers. There are no entries for the words "anisotropy", "quenching", "triplet", "lifetime", "quantum yield", "Jablonski", "polarization", and many other fundamental keywords and concepts in fluorescence. These topics are of course mentioned in different chapters, but readers will be left alone navigating the different chapters with the hope of finding the information they seek. In addition, the quality of some color illustrations is substandard, and some important figures that should have been printed in color appear in black and white. Readers will encounter very evident typos in some equations, misprints in chemical structures, the omission of proper references for much of the data presented in tables, and other glitches that one would not expect to see in a rather pricey book from a renowned publisher.

To summarize, the book contains some good chapters that will appeal to a diversity of readers, but will leave those hoping to find a comprehensive treatment of fluorescence techniques and imaging looking elsewhere.

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