

# BOOK REVIEW

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## Principles of Fluorescence Spectroscopy, Third Edition

Joseph R. Lakowicz, 954 pages +xxvi, illus., index, ISBN-13: 978-0387-31278-1, Springer, New York (2006), \$89.95, hardcover, with CD-ROM.

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Lakowicz has written a full-color, third edition of his classic textbook and reference, *Principles of Fluorescence Spectroscopy*, hereafter called *Principles*, that is easily understandable to students, scientists, engineers, and clinicians; specifically those individuals who have not studied physical chemistry (quantum chemistry, molecular spectroscopy, statistical mechanics, and kinetic theory) nor organic chemistry, and nevertheless are working with fluorescence techniques and optical microscopes, analytical instruments, diagnostic devices, or are developing new techniques based on these devices. I found the book to be eminently suitable for its intended audience. Lakowicz has extensive experience in fluorescence spectroscopy; he has taught the materials contained in the *Principles*, and he and his students and colleagues have made many significant contributions to the field. The well-tested and targeted audience-accepted approach is to teach the principles in a phenomenological manner that combines a concise, yet physically correct, qualitative exposition with carefully designed and executed multicolor diagrams, figures, and tables that contain experimental data. When necessary, the author provides the required equations that describe the phenomena. Furthermore, the author has designated advanced topics, which may be left out of the syllabus for an introductory course.

What is the scope of this book? The content of *Principles* covers the phenomena that one usually observes in the laboratory with a spectrofluorometer, a fluorescence microscope, and a frequency- or time-domain lifetime instrument. The author begins with a description of the components of these instruments: light sources, monochromators, filters, detectors, and polarizers. The next step is to introduce a variety of fluorophores and then to describe the techniques of characterizing their absorption and emission properties as well as environmental influences (temperature, solvent, quenching) on these properties. The amount of material allocated to each topic roughly correlates with the author's research interests and the impact of these disparate topics on the work of biological researchers. Therefore, the topics of fluorescence lifetime measurements, anisotropy decays, and resonance energy transfer from small molecules to macromolecules of biological interest predominate. The topics of fluorescence-lifetime microscopy, single-molecule detection, fluorescence correlation spectroscopy, metal-enhanced fluorescence, fluorescence

sensing, and multiphoton excitation microscopy are covered in less detail. The author is credited with describing such a panoply of topics, albeit with very different levels of coverage, within the almost one thousand pages of the volume.

In order to enhance the *Principles* as both a textbook and as a reference, the author provided a number of extremely useful features: glossaries of acronyms and mathematical terms (placed in the beginning of the book), problems and answers for each chapter, and appendices on corrected emission spectra, fluorescent lifetime standard, and additional readings. A CD-ROM that contains all of the figures is included. The detailed index facilitates access to the myriad topics. The publisher is to be commended for the high quality of book production, the extensive use of color, the selection of large type and leading (the space between lines) as well as a large format book with ample free space for writing notes. In addition to the copious use of color, the new edition is enriched with new chapters on significant emerging topics. The publisher included an erratum for one incorrect figure on page 434.

What potential readers will not find in the *Principles* is a rigorous physical explanation for the phenomena described that is based on quantum theory and quantum chemistry. For example, readers will not find the detailed physical theory for resonance energy transfer (references are given) nor will they find the physical basis for the interaction of light and matter that results in the spectroscopic properties of fluorescence molecules as described in the phenomenological descriptions and the copious experimental data that are provided in the *Principles*. Other physical questions that cannot be answered from reading the text include the following: how do heavy metals affect spin-orbital coupling, and why are specific molecules associated with an emission spectra that occurs with specific energies?

But these omissions are consistent with the author's pedagogical plan as previously described; without out prior knowledge of quantum theory, group theory, kinetic theory and statistical mechanics, the logical development of the above-mentioned topics cannot be developed from fundamental principles, and thus readers are dependent on the descriptive nature of the phenomena. Of course, a perusal of the advanced papers and books that are cited after each chapter would permit readers to gain a deeper understanding of the physical basis behind the phenomena, but only if they possessed the adequate background in physics and chemistry that is required to comprehend these materials. It is a tribute to the author that the *Principles* can teach fluorescence spectroscopy by describing the phenomena, illustrating the data, and presenting the basic concepts and principles that qualitatively explain them.

I now offer a more critical and detailed assessment of the individual chapters. I view the *Principles* as consisting of two

parts: the first part presents the principles of classical fluorescence spectroscopy in which the author presents the phenomena, the theories that explain the phenomena, and the instruments that measure the phenomena; in the second part, the author provides concise perspectives on rapidly emerging topics. The latter topics include the following: multiphoton excitation microscopy, fluorescence sensing, new and novel fluorophores, DNA technology, fluorescence-lifetime imaging microscopy (FLIM), single-molecule detection, fluorescence correlation spectroscopy (FCS), metal-enhanced fluorescence, and surface-plasmon-coupled emission. These latter topics (some may call them emerging advanced topics) are presented with significantly greater theoretical explanations than the material of the first part. These latter chapters, although concisely written, are augmented with carefully selected references that should be studied by the reader.

The introduction contains historical material on the independent observations made by Herschel, Jablonski, and Stokes. There is a reference to the two German papers published in 1916 by Smoluchowski, and a book that contains the English translations of these papers as well as biographic materials. Many of us use the equations that were first derived by Smoluchowski (on molecular diffusion and Brownian motion), but we know very little of his scientific accomplishments during his short life. His biography also includes side-by-side German and English versions of his key papers on diffusion (*Marian Smoluchowski, His Life and Scientific Work*, Polish Scientific Publishers, Warsaw, 1986). This portion of the book helps place the development of fluorescence and its role in science in the proper perspective. It is an interesting and informative section and I would have enjoyed reading even more about others who made major pioneering contributions to fluorescence spectroscopy.

The *Principles* addresses many of the difficulties that the

beginner spectroscopist confronts in the laboratory. Many a beginner is perplexed when the conversion between wavelength and wave number is required. The author's brief discussion of how to proceed is helpful. Anyone who has performed fluorescence measurements based on intensities is well aware of the numerous variables that confound the interpretation of their studies. Many of these problems are resolved when lifetime measurements are performed in either the time domain or the frequency domain. Of course, these techniques are associated with new complexities, especially when there are multiple lifetimes and/or multiple molecular configurations within the sample volume. Often the novice is confused by the terminology of fluorescence spectroscopy; for instance, when the terms fluorescence anisotropy and fluorescence polarization are taught. Perusal of the chapters on fluorescence anisotropy make for a clear understanding of each of these terms and the associated physical techniques and the assumptions that they are based on. Another insightful example is the section on multiphoton excitation fluorescence in which the author demonstrates that the anisotropy can reach a value of 0.5 and therefore exceed the magnitude of the one-photon limit of anisotropy that is 0.4.

The latter half of the book is especially exciting due to the exposition of emerging developments in fluorescence spectroscopy. The text appears to incorporate more of the physics that support the phenomena, the quality of the color illustrations are impressive, and the latter part of the book is written in a more engaging style.

Overall, *Principles* has an appropriate combination of clearly written text, well-designed schematic illustrations, and thoughtful examples of experimental data. All of these features make it a highly recommended and preferred textbook for teachers and beginning students in the field of fluorescence spectroscopy.