

BOOK REVIEW

Molecular Imaging, FRET Microscopy and Spectroscopy

Eds., Ammasi Periasamy, Richard N. Day, 312 pages +xv, ISBN 0-19-517720-7, illus., index. Oxford University Press, New York (2005), \$95.00 hardcover.

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Förster resonance energy transfer (FRET), one mechanism of energy transfer between molecules, is a rapidly evolving group of powerful spectroscopic techniques with the capability to measure interactions on the molecular scale. A properly designed, executed, analyzed, and validated FRET measurement can provide the researcher with a proximity indicator of molecular interactions in the interior and on the surface of cells.

The experimental evidence in support of the transfer of excitation energy between atoms in the gas phase dates from the Göttingen studies of Cario and Franck in 1922. Later in Paris, J. Perrin and F. Perrin (father and son) performed luminescence polarization quenching experiments on dye molecules in solution that supported the idea of long-range energy transfer.

It was the seminal work of Th. Förster working in Göttingen who provided a brilliant phenomenological theory, initially based on a classical treatment of intramolecular resonance, and subsequently based on a quantum mechanical theory. Förster based his theory on the previous Perrin theory that incorporated Coulombic interaction and the dipole-dipole approximation; however, he accounted for the observation that broad bands are involved in molecular vibronic transitions. Förster's theory is based on Fermi's golden rule (actually derived by Dirac) and the second-order perturbation theory, which treats the weak coupling between donor and acceptor molecules.

Förster made his theory accessible to a wide range of scientists by grouping numerous physical terms such as the Franck-Condon integrals into experimentally accessible parameters such as the overlap integral, the lifetime of the donor molecule, the index of refraction of the medium, and the orientation parameter that is related to the relative orientation of the transition dipole moments for the donor-acceptor molecular pair.

While FRET spectroscopic techniques were first developed for dye molecules in solution, they rapidly developed into microscopic techniques that are applicable to live cells, and that is the origin of its great utility by cell biologists.

Without careful validation, the Förster theory with its R^{-6} dependence on intramolecular distance between the donor and acceptor pairs would not be useful. The correct priority for this validation is a 1965 paper from the Harvard laboratory

of Blout. Using rigid steroids with attached donor and acceptor molecules, Latt, Cheung, and Blout demonstrated that the R^{-6} dependence on intramolecular distance is valid. In a very clever set of studies, Stryer and Haugland in 1967 demonstrated the distance dependence of Förster's theory over a range of donor-acceptor molecular pairs that were synthesized with a variety of molecular spacers. The measured efficiency of energy transfer showed the R^{-6} dependence predicted by Förster.

The experimental implementation of FRET is ultimately limited by the selectivity and the sensitivity of the measurement as well as models used in the analysis. The intensity-based methods are limited by spectral bleed-through due to the inadequacy of the filters and dichroics used in the microscopes. There is the possibility of distributions of donor and the acceptor separation distances, as well as distributions of the relative orientations of the electronic transition moments. Other confounding variables include the theoretical value of the orientation parameter κ , which is typically, although not always, experimentally verified, assumed to correspond to an average for the case of free-rotation of the donor and the acceptor molecules, and to a lesser extent the assumed value for the refractive index of the media.

Molecular Imaging, FRET Microscopy and Spectroscopy provides the reader with a single source containing chapters on a variety of modern FRET techniques with applications to cell biology. It aims to introduce FRET techniques to cell biologists, and to address the multitude of confounding variables in the instrumentation and data analysis. In their preface the editors state that this book is intended for graduate students, postdoctoral fellows, and scientists who are new to state-of-the-art FRET microscopy imaging systems.

The editors are well versed in FRET; each year at the University of Virginia they teach and organize a short course on FRET. The content of the course follows the interests of the editors, and this is also seen in the selection of chapter topics and authors. For example, the preponderance of chapters is concerned with intensity-based techniques.

Readers with a strong physics background would be better served by a book with more physical rigor in the description of fluorescence and FRET. The presentation of the theory of FRET is very weak. Clegg wrote the foreword that briefly mentions Coulombic coupling and dipole-dipole interactions and resonance; however, nowhere in the book are these terms explained either classically, nor quantum mechanically. The chapter on the basics of fluorescence and FRET qualitatively presents some of the theory in a manner that is very similar to the material in Lakowicz's *Principles of Fluorescence Spectroscopy*. While Clegg's brilliant, comprehensive chapters on fluorescence resonance energy transfer are cited, they did not serve as exemplars for the theory chapter in the book.

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does not provide financial disclosure of all authors when they discuss commercial products; however, the book lists six commercial companies that supported the publication of the book. This arrangement resulted in two authors who are listed as employees of companies that supported the book, and their chapters do not have substantially new material.

Overall, the production quality of the text is variable. The equations are clear and distinct. The figures show variable quality from very good to poor. Some figures composed of multiple single figures are reproduced at a size in which the axes of the graphs and the numbering of the axes become illegible. Finally, several typos, errors, and omissions appear that should be corrected in a published erratum.

The strength of this volume is its description of a variety of experimental techniques for the use of FRET in cell biology.

Although FRET is an important and widely-used approach, there are many technical difficulties that confound the experimental results. *Molecular Imaging, FRET Microscopy and Spectroscopy* provides the reader with a practical and realistic guide that will help the reader optimize the use of FRET for solving biological problems. While the lack of balance between intensity-based and lifetime techniques follows the interests of the editors, this is an incomplete view that may serve to prejudice the reader who is new to the field. The lack of depth in the explanation of FRET theory suggests that the target audience is primarily the biology community. However, this book can also be a valuable resource to engineering/physics students since it contains excellent examples of biological applications that may inspire new technical approaches.