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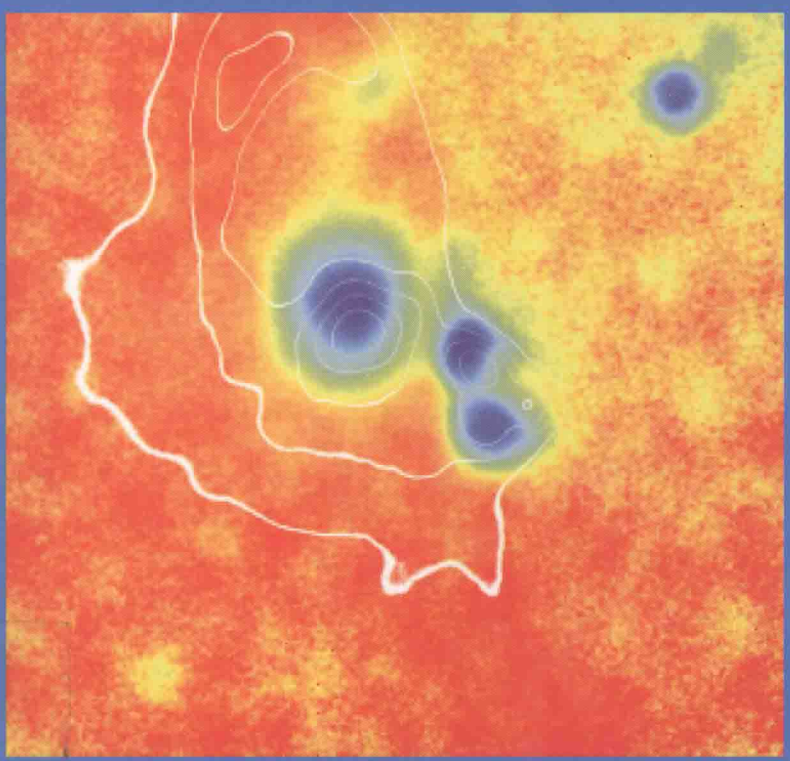
Bernard Valeur

Molecular Fluorescence

Principles and Applications



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Preface

This book is intended for students and researchers wishing to gain a deeper understanding of molecular fluorescence, with particular reference to applications in physical, chemical, material, biological and medical sciences.

Fluorescence was first used as an analytical tool to determine the concentrations of various species, either neutral or ionic. When the analyte is fluorescent, direct determination is possible; otherwise, a variety of indirect methods using derivatization, formation of a fluorescent complex or fluorescence quenching have been developed. Fluorescence sensing is the method of choice for the detection of analytes with a very high sensitivity, and often has an outstanding selectivity thanks to specially designed fluorescent molecular sensors. For example, clinical diagnosis based on fluorescence has been the object of extensive development, especially with regard to the design of optodes, i.e. chemical sensors and biosensors based on optical fibers coupled with fluorescent probes (e.g. for measurement of pH, pO_2, pCO_2, potassium, etc. in blood).

Fluorescence is also a powerful tool for investigating the structure and dynamics of matter or living systems at a molecular or supramolecular level. Polymers, solutions of surfactants, solid surfaces, biological membranes, proteins, nucleic acids and living cells are well-known examples of systems in which estimates of local parameters such as polarity, fluidity, order, molecular mobility and electrical potential is possible by means of fluorescent molecules playing the role of probes. The latter can be intrinsic or introduced on purpose. The high sensitivity of fluorimetric methods in conjunction with the specificity of the response of probes to their microenvironment contribute towards the success of this approach. Another factor is the ability of probes to provide information on dynamics of fast phenomena and/or the structural parameters of the system under study.

Progress in instrumentation has considerably improved the sensitivity of fluorescence detection. Advanced fluorescence microscopy techniques allow detection at single molecule level, which opens up new opportunities for the development of fluorescence-based methods or assays in material sciences, biotechnology and in the pharmaceutical industry.

The aim of this book is to give readers an overview of molecular fluorescence, allowing them to understand the fundamental phenomena and the basic techniques, which is a prerequisite for its practical use. The parameters that may affect the

characteristics of fluorescence emission are numerous. This is a source of richness but also of complexity. The literature is teeming with examples of erroneous interpretations, due to a lack of knowledge of the basic principles. The reader's attention will be drawn to the many possible pitfalls.

Chapter 1 is an introduction to the field of molecular fluorescence, starting with a short history of fluorescence. In Chapter 2, the various aspects of light absorption (electronic transitions, UV–visible spectrophotometry) are reviewed.

Chapter 3 is devoted to the characteristics of fluorescence emission. Special attention is paid to the different ways of de-excitation of an excited molecule, with emphasis on the time-scales relevant to the photophysical processes – but without considering, at this stage, the possible interactions with other molecules in the excited state. Then, the characteristics of fluorescence (fluorescence quantum yield, lifetime, emission and excitation spectra, Stokes shift) are defined.

The effects of photophysical intermolecular processes on fluorescence emission are described in Chapter 4, which starts with an overview of the de-excitation processes leading to fluorescence quenching of excited molecules. The main excited-state processes are then presented: electron transfer, excimer formation or exciplex formation, proton transfer and energy transfer.

Fluorescence polarization is the subject of Chapter 5. Factors affecting the polarization of fluorescence are described and it is shown how the measurement of emission anisotropy can provide information on fluidity and order parameters.

Chapter 6 deals with fluorescence techniques, with the aim of helping the reader to understand the operating principles of the instrumental set-up he or she utilizes, now or in the future. The section devoted to the sophisticated time-resolved techniques will allow readers to know what they can expect from these techniques, even if they do not yet utilize them. Dialogue with experts in the field, in the course of a collaboration for instance, will be made easier.

The effect of solvent polarity on the emission of fluorescence is examined in Chapter 7, together with the use of fluorescent probes to estimate the polarity of a microenvironment.

Chapter 8 shows how parameters like fluidity, order parameters and molecular mobility can be locally evaluated by means of fluorescent probes.

Chapter 9 is devoted to resonance energy transfer and its applications in the cases of donor–acceptor pairs, assemblies of donor and acceptor, and assemblies of like fluorophores. In particular, the use of resonance energy transfer as a 'spectroscopic ruler', i.e. for the estimation of distances and distance distributions, is presented.

In Chapter 10, fluorescent pH indicators and fluorescent molecular sensors for cations, anions and neutral molecules are described, with an emphasis on design principles in regard to selectivity.

Finally, in Chapter 11 some advanced techniques are briefly described: fluorescence up-conversion, fluorescence microscopy (confocal excitation, two-photon excitation, near-field optics, fluorescence lifetime imaging), fluorescence correlation spectroscopy, and single-molecule fluorescence spectroscopy.

This book is by no means intended to be exhaustive and it should rather be

considered as a textbook. Consequently, the bibliography at the end of each chapter has been restricted to a few leading papers, reviews and books in which the readers will find specific references relevant to their subjects of interest.

Fluorescence is presented in this book from the point of view of a physical chemist, with emphasis on the understanding of physical and chemical concepts. Efforts have been made to make this book easily readable by researchers and students from any scientific community. For this purpose, the mathematical developments have been limited to what is strictly necessary for understanding the basic phenomena. Further developments can be found in accompanying boxes for aspects of major conceptual interest. The main equations are framed so that, in a first reading, the intermediate steps can be skipped. The aim of the boxes is also to show illustrations chosen from a variety of fields. Thanks to such a presentation, it is hoped that this book will favor the relationship between various scientific communities, in particular those that are relevant to physicochemical sciences and life sciences.

I am extremely grateful to Professors Elisabeth Bardez and Mario Nuno Berberan-Santos for their very helpful suggestions and constant encouragement. Their critical reading of most chapters of the manuscript was invaluable. The list of colleagues and friends who should be gratefully acknowledged for their advice and encouragement would be too long, and I am afraid I would forget some of them. Special thanks are due to my son, Eric Valeur, for his help in the preparation of the figures and for enjoyable discussions. I wish also to thank Professor Philip Stephens for his help in the translation of French quotations.

Finally, I will never forget that my first steps in fluorescence spectroscopy were guided by Professor Lucien Monnerie; our friendly collaboration for many years was very fruitful. I also learned much from Professor Gregorio Weber during a one-year stay in his laboratory as a postdoctoral fellow; during this wonderful experience, I met outstanding scientists and friends like Dave Jameson, Bill Mantulin, Enrico Gratton and many others. It is a privilege for me to belong to Weber's 'family'.

Paris, May 2001 Bernard Valeur

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Prologue

La lumière joue dans notre vie un rôle essentiel: elle intervient dans la plupart de nos activités. Les Grecs de l'Antiquité le savaient bien déjà, eux qui pour dire "mourir" disaient "perdre la lumière".

Louis de Broglie, 1941

[*Light plays an essential role in our lives: it is an integral part of the majority of our activities. The ancient Greeks, who for "to die" said "to lose the light", were already well aware of this.*]