

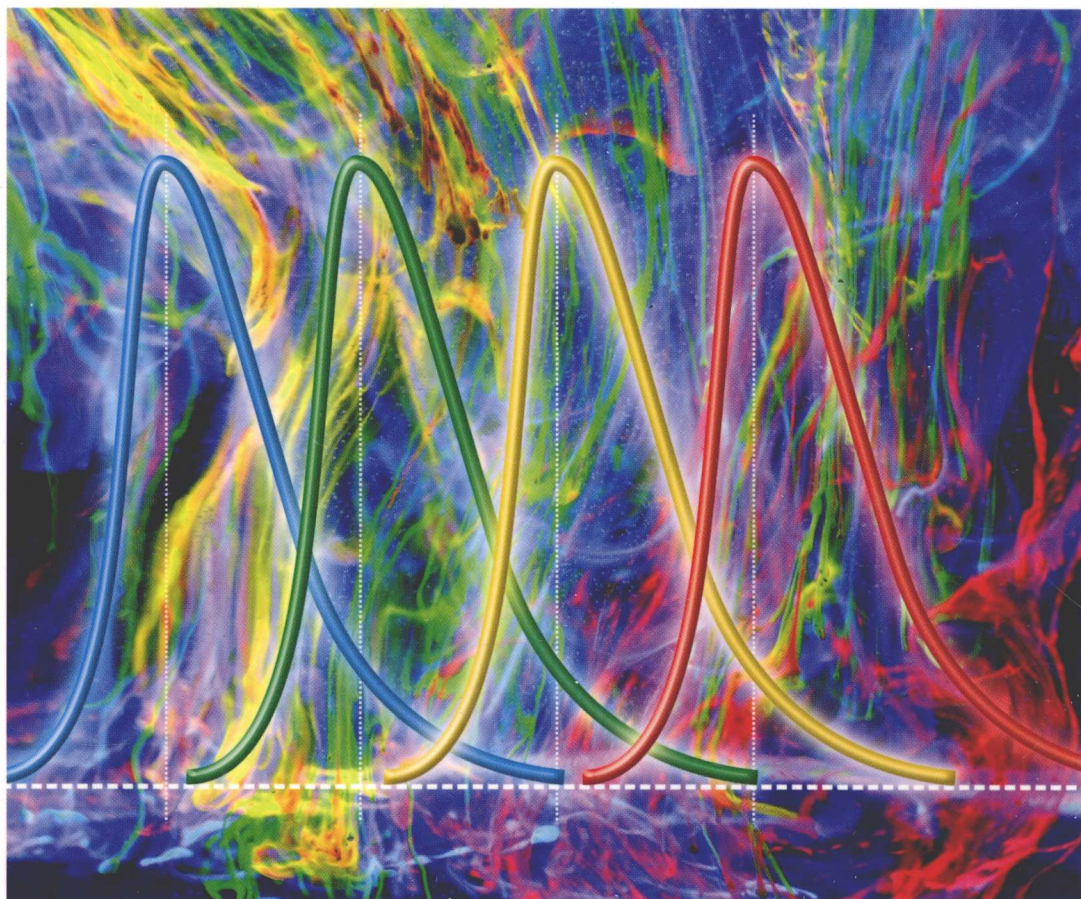
Bernard Valeur and  
Mário N. Berberan-Santos

 WILEY-VCH

# Molecular Fluorescence

Principles and Applications

Second Edition



*Bernard Valeur and Mário Nuno Berberan-Santos*

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Principles and Applications

Second Edition



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## Preface to the First Edition

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 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, that is, 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 are 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 toward 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.

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, 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 also wish 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*

## Preface to the Second Edition

The present second edition comes out 10 years after the first one. In the interval, numerous developments of fluorescence in various fields have appeared.

Fluorescence appears to be more than ever an outstanding tool for investigating not only living cells and biological tissues but also colloids, polymers, liquid crystals, and so forth. In life sciences, the use of fluorescent proteins (Nobel prize 2008) and semiconductors nanocrystals as tracers are two major advances that are discussed in this new edition. Fluorescence has also become extensively used as a tool for sensing chemical species in biology, medicine, pharmaceuticals, environment, and food science. In addition, fluorescence determination of physical parameters (pressure, temperature, viscosity) merits discussion.

The present edition is divided into three parts: principles, techniques, and applications. An appendix providing the absorption and emission characteristics of the most common fluorescent compounds has been added.

No major changes have been made in the chapters relevant to the principles, as the fundamentals of fluorescence remain the same. However, the historical section of Chapter 1 has been extended, and significant additions have been made to Chapter 4 dealing with structural effects on fluorescence.

The techniques are collected in the second part. Those that were previously considered as advanced techniques in the first edition are now currently used and are thus described in line with the more conventional techniques. Special attention has been paid to the recent developments in fluorescence microscopy, fluorescence correlation spectroscopy, and single molecule fluorescence spectroscopy.

In the third part, applications of fluorescence are presented with emphasis on fluorescence sensing of physical parameters and chemical species. A new chapter is devoted to autofluorescence and fluorescence labeling in biology and medicine. In the last chapter, which is also new, further applications are described: whitening agents, nondestructive testing, food science, forensics, counterfeit detection, and art. All these applications show the great versatility of fluorescence and its ability to reveal what is invisible to the eye thanks to its outstanding sensitivity.



## Acknowledgments

The authors wish to thank all their colleagues who participated in fruitful discussions on the various aspects of fluorescence described in this book. The list is too long to be given here.

B.V. acknowledges the Conservatoire national des arts et métiers, the Ecole normale supérieure de Cachan and the Centre national de la recherche scientifique for constant support and for providing facilities. He is very grateful to Prof. Mário N. Berberan-Santos for accepting to contribute to this second edition, and for helpful discussions.

M.N.B.S. acknowledges the Instituto Superior Técnico and Fundação para a Ciência e a Tecnologia for the facilities and financial support, and is very grateful to Prof. Bernard Valeur for his invitation, and for many years of advice and fruitful collaboration.

## 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.]*

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