Bioluminescence and Fluorescence for In Vivo Imaging
Tutorial Texts Series

- **Polarization of Light with Applications in Optical Fibers**, Arun Kumar, Ajoy Ghatak, Vol. TT90
- **Digital Fourier Optics: A MATLAB Tutorial**, David G. Voeltz, Vol. TT89
- **Optical Design of Microscopes**, George Seward, Vol. TT88
- **Nanotechnology: A Crash Course**, Raúl J. Martin-Palma and Akhlesh Lakhtakia, Vol. TT86
- **Direct Detection LADAR Systems**, Richard Richmond, Stephen Cain, Vol. TT85
- **Fundamentals of Photonics**, Chandra Roychoudhuri, Vol. TT79
- **Matrix Methods for Optical Layout**, Gerhard Kloos, Vol. TT77
- **Practical Applications of Infrared Thermal Sensing and Imaging Equipment, Third Edition**, Herbert Kaplan, Vol. TT75
- **Bioluminescence for Food and Environmental Microbiological Safety**, Lubov Brovko, Vol. TT74
- **Introduction to Confocal Fluorescence Microscopy**, Michiel Müller, Vol. TT69
- **Artificial Neural Networks: An Introduction**, Kevin L. Priddy and Paul E. Keller, Vol. TT68
- **Basics of Code Division Multiple Access (CDMA)**, Raghuvir Rao and Sohail Dianat, Vol. TT67
- **Metrics for High-Quality Specular Surfaces**, Lionel R. Baker, Vol. TT65
- **Field Mathematics for Electromagnetics, Photonics, and Materials Science**, Bernard Maxum, Vol. TT64
- **High-Fidelity Medical Imaging Displays**, Aldo Badano, Michael J. Flynn, and Jerzy Kanicki, Vol. TT63
- **Thin-Film Design: Modulated Thickness and Other Stopband Design Methods**, Bruce Perilloux, Vol. TT57
- **Optische Grundlagen für Infrarotsysteme**, Max J. Riedl, Vol. TT56
- **An Engineering Introduction to Biotechnology**, J. Patrick Fitch, Vol. TT55
- **Image Performance in CRT Displays**, Kenneth Compton, Vol. TT54
- **Modulation Transfer Function in Optical and Electro-Optical Systems**, Glenn D. Boreman, Vol. TT52
- **Fundamentals of Antennas**, Christos G. Christodoulou and Parveen Wahid, Vol. TT50

(For a complete list of Tutorial Texts, see http://spie.org/x651.xml.)
Bioluminescence and Fluorescence for *In Vivo* Imaging

Lubov Brovko

Tutorial Texts in Optical Engineering
Volume TT91

SPIE
PRESS
Bellingham, Washington  USA

Downloaded From: https://www.spiedigitallibrary.org/ebooks/ on 01 Jul 2019
Terms of Use: https://www.spiedigitallibrary.org/terms-of-use
Introduction to the Series

Since its inception in 1989, the Tutorial Texts (TT) series has grown to more than 85 titles covering many diverse fields of science and engineering. The initial idea for the series was to make material presented in SPIE short courses available to those who could not attend and to provide a reference text for those who could. Thus, many of the texts in this series are generated by augmenting course notes with descriptive text that further illuminates the subject. In this way, the TT becomes an excellent stand-alone reference that finds a much wider audience than only short course attendees.

Tutorial Texts have grown in popularity and in the scope of material covered since 1989. They no longer necessarily stem from short courses; rather, they are often generated independently by experts in the field. They are popular because they provide a ready reference to those wishing to learn about emerging technologies or the latest information within their field. The topics within the series have grown from the initial areas of geometrical optics, optical detectors, and image processing to include the emerging fields of nanotechnology, biomedical optics, fiber optics, and laser technologies. Authors contributing to the TT series are instructed to provide introductory material so that those new to the field may use the book as a starting point to get a basic grasp of the material. It is hoped that some readers may develop sufficient interest to take a short course by the author or pursue further research in more advanced books to delve deeper into the subject.

The books in this series are distinguished from other technical monographs and textbooks in the way in which the material is presented. In keeping with the tutorial nature of the series, there is an emphasis on the use of graphical and illustrative material to better elucidate basic and advanced concepts. There is also heavy use of tabular reference data and numerous examples to further explain the concepts presented. The publishing time for the books is kept to a minimum so that the books will be as timely and up-to-date as possible. Furthermore, these introductory books are competitively priced compared to more traditional books on the same subject.

When a proposal for a text is received, each proposal is evaluated to determine the relevance of the proposed topic. This initial reviewing process has been very helpful to authors in identifying, early in the writing process, the need for additional material or other changes in approach that would serve to strengthen the text. Once a manuscript is completed, it is peer reviewed to ensure that chapters communicate accurately the essential ingredients of the science and technologies under discussion.

It is my goal to maintain the style and quality of books in the series and to further expand the topic areas to include new emerging fields as they become of interest to our reading audience.

James A. Harrington
Rutgers University
Contents

Preface ........................................................................................................................................... xi

Chapter 1 Overview of Bioluminescence; Examples of Bioluminescent Reactions ................................................................................................................................. 1
  1.1 History of Bioluminescence .................................................................................. 1
  1.2 Bioluminescent System of Bacteria .................................................................... 4
  1.3 Bioluminescent System of Insects (Fireflies) .................................................. 7
  1.4 Bioluminescent System of Ostracods (Vargula and Cypridina) ................. 10
  1.5 Coelenterazine-based Bioluminescent Systems ........................................... 12
    1.5.1 Calcium-dependent photoproteins ......................................................... 12
    1.5.2 Coelenterazine-based enzymatic bioluminescent systems ..................... 15
  1.6 Dinoflagellate Bioluminescence ........................................................................ 17
References .................................................................................................................................... 19

Chapter 2 The Family of Fluorescent Proteins ...................................................... 23
  2.1 Green Fluorescent Protein (GFP) from Aequorea Victoria and Its Mutants: Structure and Spectral Characteristics ................................................................. 23
  2.2 Other Accessory Fluorescent Proteins ............................................................ 27
  2.3 Red Fluorescent Proteins from the Discosoma Genus (DsRed): Structure and Spectral Characteristics ................................................................. 28
  2.4 Various Photoactivatable Fluorescent Proteins and Chromoproteins .............. 34
References .................................................................................................................................... 39

Chapter 3 Bioluminescence- and Fluorescence-based Cell Viability and Proliferation Assays .................................................................................................................. 43
  3.1 Principles of Bioluminescent Cell Viability and Proliferation Tests ......................... 43
    3.1.1 Viability/Proliferation tests based on firefly luciferase ....................... 44
    3.1.2 Viability/Proliferation tests based on bacterial luciferase ................ 45
    3.1.3 Viability/Proliferation tests based on Renilla and Gaussia luciferases ...... 46
3.1.4 Fluorescent proteins for cell proliferation and viability assays ......................................................... 46
3.2 Bioluminescence Environmental Toxicity Tests ................................................................................. 47
3.3 Bioluminescence Viability Tests for Pathogenicity Research and Drug Development ......................................................... 49
  3.3.1 Investigation of bacteria–host interaction and the efficiency of antimicrobial drugs using in vivo bioluminescence ................................................................................................................. 49
  3.3.2 Bioluminescence and fluorescence viability tests for oncology research and drug development .......................................................... 52
3.4 Fluorescence and Bioluminescence Tomography .............................................................................. 57
References ............................................................................................................................................... 58

Chapter 4 Real-Time In Vivo Monitoring of Gene Expression by Bioluminescence and Fluorescence Imaging ................................................................. 71
  4.1 Luciferase-based Reporters of Gene Expression ............................................................................. 73
  4.1.1 Bioluminescent gene reporters for clinical research ................................................................. 73
  4.1.2 Bioluminescent gene expression reporters for physiology research ......................................................... 74
  4.1.3 Bioluminescent gene expression reporters for viral research and bacteriology ......................... 76
  4.1.4 Bioluminescent gene expression reporters for toxicity testing ................................................................. 78
  4.2 Fluorescent Protein-based Reporters of Gene Expression and Their Applications ......................... 82
References ............................................................................................................................................... 84

Chapter 5 Bioluminescence and Fluorescence Imaging for In Vivo Real-Time Monitoring of Key Metabolites and the Intracellular Environment ......................................................... 87
  5.1 In Vivo Imaging of the Intracellular ATP Dynamic ................................................................. 87
  5.1.1 Bioluminescence in vivo ATP imaging ..................................................................................... 87
  5.1.2 Fluorescence in vivo ATP imaging ........................................................................................... 89
  5.2 In Vivo Bioluminescence Imaging of the Calcium Dynamic ................................................................. 91
  5.2.1 Photoproteins for in vivo calcium imaging ................................................................................. 91
  5.2.2 Fluorescent proteins as intracellular calcium indicators ................................................................. 95
    5.2.2.1 Nonratiometric single-fluorescent-protein-based GECI ...................................................... 95
    5.2.2.2 Ratiometric GECI based on fluorescence resonance energy transfer (FRET) between two fluorescent proteins ................................................................. 98
  5.3 In Vivo Optical Imaging of Enzymatic Activities ......................................................................... 102
  5.4 In Vivo Fluorescence Imaging of Hydrogen Peroxide ................................................................. 103
Contents

5.5 Genetically Encoded Fluorescence pH Indicators ................................ 104
5.6 Resonance-Energy-Transfer-based Sensors for In Vivo Detection of Signal Molecules ................................................................. 106
  5.6.1 Genetically encoded FRET-based sensor for in vivo cAMP monitoring .......................................................... 106
  5.6.2 Genetically encoded BRET-based sensor for in vivo cAMP monitoring .......................................................... 107
References ....................................................................................................................... 108

Chapter 6 Bioluminescence and Fluorescence Imaging for In Vivo Monitoring of Protein–Protein Interactions ........................................... 113
  6.1 Two-Hybrid System for In Vivo Monitoring of Protein–Protein Interactions ...................................................................................... 113
  6.2 Complementation Assay of Protein–Protein Interactions (‘Split-Protein’ Assay) .............................................................. 115
  6.3 Nonradiative Energy Transfer (FRET/BRET) for Investigation of Protein–Protein Interactions .................................................. 117
References ....................................................................................................................... 125

Chapter 7 Fluorescence and Bioluminescence Tomography—Current Status and Perspectives .................................................. 129
  7.1 Fluorescence Molecular Tomography Based on Genetically Engineered Probes ........................................................... 129
  7.2 Bioluminescence Tomography .............................................................................................................................. 130
References ....................................................................................................................... 132

Chapter 8 Review of Available Instrumentation for In Vivo Bioluminescence and Fluorescence Imaging ........................................... 135
  8.1 Plate Readers for Fluorescence and Bioluminescence In Vivo Imaging ...................................................................................... 136
  8.2 Overview of Systems for Macroscopic/Whole Body Bioluminescence and Fluorescence In Vivo Imaging and Image Processing Software .............................................................. 137
  8.3 Systems for Microscopic Luminescence In Vivo Imaging—Recent Innovations .............................................................. 142
  8.4 Concluding remarks .............................................................................................................................. 142
References ....................................................................................................................... 143

Index ....................................................................................................................... 145
Bioluminescence methods are gaining more and more attention among scientists due to their sensitivity, selectivity, and simplicity, along with the fact that bioluminescence can be monitored both in vitro and in vivo. Since the discovery of bioluminescence in the 18th century, enzymes involved in the bioluminescence process have been isolated and cloned. The bioluminescent reactions in several different organisms have also been fully characterized and used as reporters in a wide variety of biochemical assays. Discovery of green fluorescent protein (GFP) in 1961 initiated extensive research in the area of the naturally fluorescent proteins. As a result, Martin Chalfie, Osamu Shimomura and Roger Y. Tsien share the 2008 Nobel Prize in Chemistry for the work on the discovery of GFP and its application as a tagging tool in bioscience.

From the 1990s it became clear that luminescence can be detected and quantified directly from inside a living cell and, in some cases, from a whole living small animal. This gave rise to numerous possibilities for the in vivo monitoring of intracellular processes non-invasively using bioluminescent molecules and fluorescent proteins as reporters. The number of publications related to bioluminescence and GFP-based in vivo imaging has increased exponentially during recent years, indicating the huge interest and great potential of this technique.

Basics of bioluminescence and fluorescence systems are introduced and explained in the book, together with the principles of their application for in vivo imaging of intracellular processes. This book describes recent developments in optical (bioluminescence and fluorescence) imaging in the area of cell biology. Newly developed imaging methods allow transcriptional/translational regulation, signal transduction, protein–protein interaction, oncogenic transformation, cell and protein trafficking, as well as target drug action that can be monitored in vivo in real time with high temporal and spatial resolution, all providing researchers with priceless information on cellular functions in a timely and cost-effective fashion. Advantages and limitations of these novel luminescent methods are discussed, and possible future developments identified.

This book is intended for scientists and students involved in basic cell physiology research, for industry professionals, engineers, and managers involved in drug discovery and preclinical drug development. It will discuss the practical aspects of luminescence in vivo imaging for monitoring of intracellular processes. While some basic knowledge of biochemistry and biophysics is preferable, the book will include a brief review of fundamental principles that will allow people not familiar with these disciplines to grasp basic concepts.

Lubov Brovko
November 2010