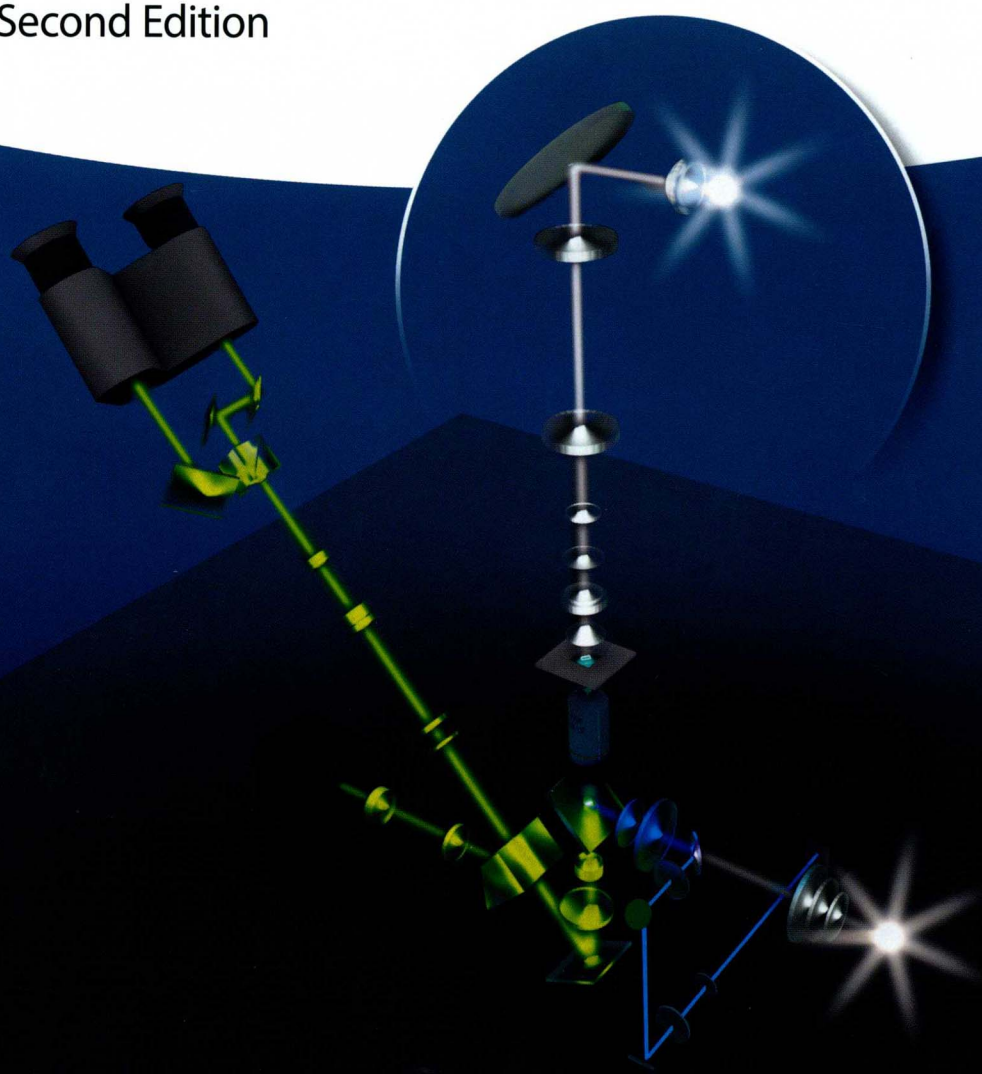


Edited by Ulrich Kubitscheck

Fluorescence Microscopy

From Principles to Biological Applications

Second Edition



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WILEY-VCH

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Preface to the Second Edition

What is This Book?

This book is both a high-level textbook and a reference work for researchers applying high-performance microscopy. It provides a comprehensive yet compact account of the theoretical foundations of light microscopy, the large variety of specialized microscopy techniques, and the quantitative utilization of light microscopy data. It will enable the user of modern microscopy equipment to fully exploit the complex instrumental features with knowledge and skill. These diverse goals were approached by recruiting a collective of leading scientists as authors. We applied a stringent internal reviewing process to achieve homogeneity, readability, and optimal coverage of the field. Finally, we took care to reduce redundancy as far as possible.

Why This Book?

Meanwhile, there are numerous books on light microscopy on the market. At a closer look, however, many available books are written at an introductory level with regard to the physics behind the often sophisticated techniques. Or, they represent rather a collection of review articles on advanced topics. Books *introducing* a wide range of techniques such as light sheet microscopy, fluorescence resonance energy transfer, stimulated emission depletion, or structured illumination microscopy, together with the required basics and theory, are rare. Even the basic optical theory such as the Fourier theory of optical imaging or topics such as the sine condition are seldom introduced from scratch. With this book, we fill this gap.

Is This Book for You?

The book is aimed at advanced undergraduate and graduate students of the biosciences and researchers entering the field of quantitative microscopy. Since they are usually recruited mostly from natural sciences, that is, physics, biology, chemistry, and biomedicine, we addressed the book to this readership. Readers

would definitely profit from a sound knowledge of physics and mathematics. This allows diving much deeper into the presented material than without such knowledge. However, all authors are experienced in teaching university and summer courses on light microscopy and for many years explored the best ways to present the required knowledge. Hopefully, you will find that they have come up with good solutions. In case you see room for improvement, please let me know.

How Should You Read the Book?

Students who require an in-depth knowledge should begin at their level of knowledge, either Chapter 1 (Introduction to Optics) or Chapter 2 (Principles of Light Microscopy). Beginners should initially omit advanced topics, for example, the section on Differential Interference Contrast (Section 2.6.4). Principally, the book is readable without the “text boxes,” which present either historical summaries or theoretical derivations. They are meant to provide a good understanding of theory and scientific reasoning. Then, they should proceed through Chapters 3 (Fluorescence Microscopy), 4 (Labeling Techniques), and 5 (Confocal Microscopy). Chapters 6 (Two-photon Microscopy) and 7 (Light Sheet Microscopy) are on alternative approaches to achieving optical sectioning, and more. Chapters 8–10 cover the most important super-resolution techniques, and Chapters 11–13 cover advanced topics and special techniques. They should be studied according to the reader’s interest and requirement.

What Updates Done to the First Edition?

Most importantly, we added three new chapters and therefore cover now all major currently used approaches to fluorescence microscopic imaging. These chapters focus on two-photon microscopy, localization-based super-resolution microscopy, and light sheet microscopy. These topics were really missing in the first edition. I am happy that very distinguished authors have now contributed these materials. Of course, numerous typos and mistakes were corrected. All chapters were carefully revised to improve the accessibility, and updated; many figures were redrawn or exchanged. Some chapters were almost completely rewritten. Also, we shortened the lists of references as much as possible to reduce the volume of the book. The chapters were rearranged to follow the logic described above: (i) foundations, (ii) general fluorescence microscopy, (iii) optical sectioning approaches, (iv) high-resolution imaging, and (v) advanced quantitative techniques. Finally, I added a second appendix, which gives a very short introduction into the computational treatment of images. It is meant to serve as a bridge to the extensive existing literature on this topic.

Website of the Book

There is a website containing material extending the contents of this book www.wiley-vch.de/home/fluorescence_microscopy2. There you will find all figures as JPGs for use in courses and additional illustrative material such as movies.

Personal Remarks on the History of This Book

I saw one of the very first commercial laser scanning microscopes in the lab of Tom Jovin at the Max-Planck Institute of Biophysical Chemistry, Göttingen, at the end of the 1980s, and was immediately fascinated by the images produced by that instrument. In the beginning of the 1990s, confocal laser scanning microscopes began to spread over biological and medical labs. At that time, they usually required really dedicated scientists for proper operation, filled a small laboratory room, and were governed by computers as big as today's refrigerators. The required image processing demanded substantial investments. At that time, Reiner Peters and I noticed that biologists and medical scientists needed an introduction to the physical background of optics, spectroscopy, and image analysis for coping with the new techniques. Hence, we offered a lecture series entitled "Microscopes, Lasers and Computers" at the Institute of Medical Physics and Biophysics, University of Münster, Germany, which was very well received. We began to write a book on microscopy containing the material we had presented, which we thought should not be as comprehensive as Jim Pawley's "Handbook" but should offer an accessible path to modern quantitative microscopy. We invested almost 1 year into this enterprise, but then gave up ... in view of the numerous challenging research topics that kept us busy, the insight into the dimension of this task, and the reality of career requirements. We realized we could not do it alone.

In 2009, Reiner Peters, now at The Rockefeller University, New York, organized a workshop on "Watching the Cellular Nanomachinery at Work," and gathered some of the current leaders in microscopy to report on their latest technical and methodological advances. On that occasion, he noted that the book that had been in our minds 15 years ago was still missing ... and contacted the speakers of his meeting. Like many years before, I was excited by the idea to create this book, and together we directly addressed the lecturers of the meeting and other experts in the field and asked for introductory book chapters in the areas of their methodological expertise. Most of them responded positively, and thus began the struggle for an introductory text. Unfortunately, Reiner could not keep his position as an editor of the book due to further obligations, so I had to finish our joint project. Here is the result, and I very much hope that the authors have succeeded in transmitting their ongoing fascination for advanced light microscopy. To me, microscopy appears as a century-old tree that began another phase of growth about 40 years ago, and since then has shown almost every year a new branch with a surprising and remarkable technique offering exciting and fresh scientific fruits.

Acknowledgments

Finally, I would like to thank some people who have contributed directly and indirectly to this book. First of all, I would like to name Prof. Dr Reiner Peters. As mentioned, he invited me to the first steps to teach microscopy and to the first attempt to write this book. Finally, he launched the initiative to create this book as an edited work. Furthermore, I would like to thank all authors who invested their expertise, time, and energy in writing, correcting, and finalizing their respective chapters. They are all very respected colleagues, and some of them became my friends during this project. Also, I thank some people who were earlier collaborators or colleagues and helped me to learn more and more about microscopy: Prof. Dr Reinhard Schweitzer-Stenner, Dr Donna Arndt-Jovin, Prof. Dr Tom Jovin, Dr Thorsten Kues, and Prof. Dr David Grünwald. Likewise, I acknowledge the project editors responsible at Wiley-VCH for this project, Dr Andreas Sendtko, Dr Gregor Cicchetti, and Anne du Guerny, who wholeheartedly supported this project and showed very professional patience when yet another delay occurred, but also pushed when required. Last but not least, I would like to thank my collaborator Dr Jan Peter Siebrasse and my wife Martina, who patiently listened to my concerns when yet another problem occurred.

Bonn, Germany
January 20th, 2017

Prof. Dr. Ulrich Kubitscheck

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