Preface

Each year in May, a new class of students joins the Analytical and Quantitative Light Microscopy course at the Marine Biological Laboratory in Woods Hole, Massachusetts. For three decades, this course, started by Shinya Inoue, has played a major role in the development and implementation of modern light microscopy. Much of the material in this book derives from lectures and laboratories in this course.

The first edition of this book appeared in 1998 as "Video Microscopy," and we find it remarkable that 15 years later we are putting the final touches on the fourth edition. By the time of the second edition, in 2003, it became clear that technology trends mandated that the name be changed to "Digital Microscopy." Like all technology, light microscopy is caught up in the whirlwind of Raymond Kurzweill's singularity. That is how we got from the early microscopes of Hooke and von Leuwenhoek to the modern microscope. It used to be that microscopes were limited by the fundament resolution limit of physical optics (a subject covered in Chapter 2). One of the great scientific advances of this past year was the discovery at European Organization for Nuclear Research (CERN) of the elusive Higgs boson. In a very real sense, the Large Hadron Collider at CERN is the world's largest microscope, and its resolution is limited by the same fundamental physics as the light microscope.

However, and increasingly, in biological science, this resolution limit has been overcome by super resolution techniques (such as structured illumination, PALM, STORM, and STED) (see Chapters 22 and 24) on the one hand and confocal microscope (Chapter 18) on another. Now we routinely speak about resolution 10 times finer than the classical limit. As shown in the chapter on Tip-Enhanced Raman Spectroscopy (Chapter 26) microscopy, two more orders of magnitude finer resolution can be achieved in some instances. It is not that the laws of physical optics no longer apply, but rather that they may be used in new ways by asking different questions. Understanding these subtle points is an important element of this book. It is the old question that scientists forever deal with—namely how to think "outside the box" in imaginative ways.

Whereas early microscopy largely focused on structural issues, biologists demanded, and technologists delivered, molecular identification as well. Now it is routine to look at a single-molecule level at the distribution and dynamics of individual molecular species on the timescales of the living cell. This revolution began with immunofluorescence, was expanded by the development of a host of molecular probes (see for instance Chapter 16), and finally by the development of the family of green fluorescent proteins, covered in Chapter 5, for which Osamu Shimomura, Martin Chalfie, and Roger Y. Tsien were awarded the Nobel Prize in Chemistry in 2008.

It is easy to sigh and ask where and when will it all end? The exciting point is that it will never end and we look forward to seeing the next advances and techniques in the years to come. This takes us to the principal goal that defines both this book and the Analytical and Quantitative Light Microscopy course. We hope to impart a real

understanding of the physics and chemistry involved in modern light microscopy. So when the next great question in biology arises, our readers and students will have the tools to respond with creative new solutions.

The preparation of a book of this sort cannot be done in isolation, and many people have contributed both directly and indirectly to this volume. First, we want to recognize Zoe Kruze, Acquisitions Editor at Serials and Elsevier Insights, for her infinite patience and professionalism in dealing with us and bringing this edition along to completion. Above all, we thank our contributors for taking the time to write articles covering the basics and practical aspects of digital microscopy. Although this may be less glamorous than describing the latest and greatest aspects of their work, their efforts will provide a valuable service to the microscopy community. We also thank the members of the commercial faculty of the Analytical and Quantitative Light Microscopy course for their years of patience, inspired teaching of both students and us. Through their interaction with students, these people, principals in the video and microscopy industries, have defined how the material covered in this book is best presented for use by researchers in biology. We are grateful that some of them have taken the time from their busy schedules to write chapters for this book. The job of editing a book like this is a dubious honor, and after a season of arm twisting, we can only hope that we have as many friends at the end of the process as we had at the beginning. Our gratitude also goes out to the many individuals who have and continue to administer the educational programs at the Marine Biological Laboratory. We pray that the Marine Biological Laboratory in its new association with the University of Chicago will never lose sight of its singular role in science education. Five generations of scientists are indebted to this remarkable institution.

In recognition of the contributions of the many past students of the Analytical and Quantitative Microscopy course, the commercial faculty, the instrument manufacturers, and the academic faculty, we respectfully dedicated this volume to them all.

Greenfield Sluder
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