Preface

Almost 400 years ago, Robert Hooke and Antonie van Leeuwenhoek first viewed individual cells through their early microscopes and with careful observation laid the foundation of modern cell biology. Since then, optical microscopy has been a major driving force of biological discovery. For the longest time, microscopy was limited by the inability to acquire reproducible images—not too long ago microscopic observation could only be documented by manual drawing, which requires a specialized skill set and is arguably highly subjective. Even micrography on photographic film has only limited quantitative value. Over the last two decades, however, ever-accelerating advances in optics, electronics, and digital camera technology in combination with the rise of fluorescent proteins have transformed fluorescence light microscopy from a descriptive, observational tool to a truly quantitative method.

Digital cameras are now more sensitive than the human eye, and fluorescence from individual molecules can routinely be detected. The dynamics of movement, intensity fluctuations, or distribution of fluorescently labeled structures in living cells or organisms can be measured providing important information about biological processes. This is the exciting new world of quantitative cell biology, and ideally scientific conclusions will continue to rely less and less on "representative images," but instead on quantitative analysis of digital data. Although microscopy is one of the most direct tools available to ask a scientific question, images can also be dangerously deceiving. In our modern world, the deceptive nature of images is all around us from advertising to the daily news, but in science, we must learn to objectively analyze the underlying data instead of blindly believing what we think we can see. Any digital photomicrograph is only a representation of reality that needs to be carefully scrutinized and interpreted in order to reach valid conclusions, and any analysis can only be as good as the original data.

The goals of this book are to provide the reader with a practical understanding of how digital image data are generated by modern fluorescence microscopy modalities, to outline technical and fundamental reasons that limit the accuracy and precision with which these data can be analyzed, and to provide guidance into cutting-edge technologies and image analysis that are expanding the abilities of traditional microscopy. Chapters 1–6 cover basic principles of quantitative fluorescence microscopy as well as technical aspects of objective lenses, cameras, microscope maintenance, modern live-cell imaging setups, and the properties of fluorescent proteins. Chapters 7–17 give an overview of different fluorescence microscopy techniques ranging from more established confocal imaging to state-of-the-art super-resolution strategies that circumvent the diffraction limit, and light-sheet microscopy allowing unparalleled isotropic observation of biological specimens in three dimensions. Finally, the remaining chapters describe more specific and advanced microscopy and image analysis methods. We were striving for a balanced mixture of basic information and more advanced topics, and hope that this collection will be a useful resource to anyone who wishes to venture into the brave new world of

quantitative fluorescence microscopy. We would like to thank the students of the Quantitative Imaging: From Cells to Molecules course at Cold Spring Harbor Laboratory for many inspiring discussions. Last but not least, we thank all the authors who have contributed their expertise to this volume.

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