Preface

Live-cell investigations to a great extent rely on far-field optical microscopy techniques such as wide-field, or confocal microscopy, because fluorescence detection of tagged molecules such as proteins offers large specificity and sensitivity and lens-based far-field optics allows the observation of the cellular interior with minimal invasion. Over the centuries, major efforts have been exerted to improve the resolution of far-field light microscopy, which for long meant perfecting lenses and other optical elements. The limits of this strategy became evident with the discovery of the diffraction barrier in the nineteenth century. From then onwards, it was clear that light cannot be focused to a spot smaller than about half the wavelength of light, i.e., to about 200–300 nm. This meant that molecules, such as proteins, or any other features that are closer together than this range, cannot be discerned. For example, protein distributions cannot be visualized at smaller scales in a cell. The diffraction barrier clearly impeded the applicability of far-field optical microscopy in the life sciences. Therefore, over the decades, efforts have been undertaken to overcome this major limitation, but it was not until at the end of the twentieth century that this barrier was overcome. Since then, a multitude of super-resolution far-field fluorescence microscopy or nanoscopy techniques have been developed, such as STED, GSD, RESOLFT, (f)PALM, (d)STORM, GSDIM, etc., and it has become clear that lens-based fluorescence microscopes can resolve features at the nanometer scale.

At their very basis, all current fluorescence nanoscopy techniques share the same operational principle: nearby sample molecules are no longer discerned just by the phenomenon of focusing light but by prompting them to briefly assume (at least) two different states. Usually, the (two) states that are selected for separation are a fluorescence on- and an off-state. In other words, turning molecules on and off is employed so that the signal does not stem from all molecules simultaneously. Thus, the use of two different states for molecular distinction renders nearby objects distinguishable when illuminated by the same diffraction pattern, yielding images with nanoscale resolution.

Unfortunately, even up to date, there is a lot of confusion about the basics, similarities, and differences of the various nanoscopy approaches, as well as about

their potential. Parts of these concerns are based on contingent issues such as an increased setup complexity, phototoxicity, and demands on labeling. Yet, recent years have seen major improvements, allowing the widespread onset of turn-key optical nanoscopes into open facilities of biological institutes. Here, a major drive has been the developments in lasers and optics technology but specifically also in labeling techniques.

As a matter of fact, in optical nanoscopy, fluorescent labels must fulfill two roles. First, they have to highlight the structures of interest, giving bright signal that may be distinguishable from other molecular tags. Secondly, the labels have to provide the pair of different states, say the on- and off-states required for discerning nearby molecules. Therefore, it is the symbiosis of molecular tags and optical design that makes the microscopes of today sharp and bright.

This book tackles all of the above issues. In 11 chapters distinguished scientists and leaders in the respective fields elaborate on the basics of the different nanoscopy approaches and their recent advancements, on improved labeling technology, as well as give an overview of representative applications.

The first part of the book introduces different optical nanoscopy approaches. Chapter “STED Fluorescence Nanoscopy” (by C. Eggeling and S. W. Hell) introduces STED microscopy, the earliest far-field fluorescence nanoscopy approach. The chapter describes the advances that have overcome initial system complexities, resulting in multiple applications of this technique. Chapter “Super-Resolution Imaging Through Stochastic Switching and Localization of Single Molecules: An Overview” by X. Zhuang and co-workers introduces the STORM approach, guiding the reader through the basics and selected applications of this technique and of the related approaches (f)PALM, GSDIM, etc. In chapter “A Practical Guide to dSTORM: Super-Resolution Imaging with Standard Fluorescent Probes”, the approach called dSTORM by M. Sauer is presented. Similar to GSDIM or blink microscopy, the great achievement here was the investigation of how to implement any organic dye or fluorescent protein into the concept of STORM/(f)PALM, which were initially restricted to a certain class of photoswitchable dyes. Specifically, in dSTORM dedicated buffer conditions for mounting of samples have been developed.

The second part of the book focuses on the demands that optical nanoscopy has put on the fluorescence labels. Chapter “Single-Molecule Photocontrol and Nanoscopy” by W. E. Moerner and co-workers depicts the development of eYFP and novel photoactivatable organic dyes for their use in (d)STORM/(f)PALM type of nanoscopy. Chapter “Probes for Nanoscopy: Fluorescent Proteins” by U. Nienhaus and co-workers provides an overview of fluorescent proteins, with a special focus on their usage in optical nanoscopy. In chapter “Tailoring Fluorescent Labels for Far-Field Nanoscopy”, D. A. Yushchenko and M. P. Bruchez highlight the advances in fluorophore development for a range of existing techniques. Emphasis is placed on the requirements these distinct methods demand from the fluorophores in order to maximize resolution, particularly in live-cell imaging. P. F. Aramendia and M. L. Bossi (chapter “Probes for Nanoscopy: Photoswitchable Fluorophores”) discuss on how photochromic organic dyes can be designed to fulfill the

requirements of a suitable probe for different fluorescence nanoscopy strategies. In chapter “Far-Field Nanoscopy with Conventional Fluorophores: Photostability, Photophysics, and Transient Binding”, P. Tinnefeld and his team give an overview of their work on using conventional organic fluorophores for (d)STORM/(f)PALM type nanoscopy, with a special focus on the importance and principles of photostability and blinking characteristics of these probes.

The third part gives specific developments and selected applications. In chapter “NASCA Microscopy: Super-Resolution Mapping of Chemical Reaction Centers” J. Hofkens, B.J. Roeffaers and co-workers introduce their concept of NASCA nanoscopy to visualize single chemical reaction centers with sub-diffraction resolution using special fluorogenic probe molecules. M. H. Ulbrich (chapter “Counting Molecules: Toward Quantitative Imaging”) shows how single-molecule fluorescence can be used to analyze protein–protein interactions by enabling the direct visualization of protein complexes and the number and species of their constituent subunits. Finally, in chapter “In Vivo Tracking of Single Biomolecules: What Trajectories Tell Us About the Acting Forces” M. Brameshuber and G. J. Schütz introduce the reader to the concept of the tracking of single biomolecules in living cells, and how their trajectories can report on acting forces, e.g., by the underlying cytoskeleton.

Emerging nanoscopy techniques are flourishing and have started revealing exciting new biology, such as insights of the cytoskeleton, protein complexes, or virus maturation. Meanwhile, fluorescence nanoscopes are commercially available and new probes are developed at fast pace. The collection of chapters in this book can present only a glimpse on these exciting developments. The work presented, however, gives a good overview into different approaches of this interdisciplinary and rapidly developing field. Fluorescence nanoscopy involves a new physics and new chemistry to reveal new biology! We are grateful to the authors for their outstanding contributions and believe that the book will be stimulating for the community of microscope developer, microscopy users, and all interested readers.

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