

METHODS IN MOLECULAR BIOLOGY

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Light Microscopy

Methods and Protocols

Edited by

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Cover image: Photoactivated localization microscopy (PALM) detecting a bacterial membrane protein. Shown is a *Bacillus subtilis* cell expressing FloA-mNeonGreen. FloA is a bacterial flotillin-like protein, involved in membrane compartmentalization. PALM images were acquired in TIRF. Detected signals were filtered for PSF width (100–200 nm) and photon count (200–1000 photons). The average localization precision of detected FloA-mNeonGreen molecules is 25 nm.

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Preface

Recent achievements, such as the development of a new generation of nanoscopes surpassing the Abbe's diffraction limit or high-resolution approaches for deep imaging, such as light-sheet or two-photon excitation microscopy, have revolutionized light microscopy. In addition to the progress made in optical systems, novel genetically encoded fluorescent reporters and labeling methods allow investigation of biological processes as never previously achieved. Equally important, the information collected from imaging experiments has been dramatically augmented by the development and optimization of a plethora of image analysis tools and computational solutions that provide unbiased and systematic quantitative imaging. Today, therefore, light microscopy encompasses an extraordinary range of applications that can meet the needs of any biological system under investigation.

In this regard, we aimed at creating a book, which functions as a roundup user manual, addressing up-to-date light microscopy approaches and toolsets offered for live or fixed cell observations. Imaging strategies outlined in this book include confocal laser scanning and spinning disk confocal microscopy, FRET (fluorescence resonance energy transfer), FRAP (fluorescence recovery after photobleaching) and laser microsurgery experiments, light-sheet and two-photon excitation microscopy, PALM (photoactivated localization microscopy), STED (stimulated emission depletion), TIRF (total internal reflection fluorescence), and optical coherence microscopy. Here we describe the use of these imaging methodologies to study properties of a multitude of biomolecular targets in a broad range of model systems, ranging from bacteria over tissue to whole animal imaging.

These advanced fluorescence light microscopy methods are exploited to pinpoint and track single molecules, visualize and follow individual cells in living animals or plants, monitor biomolecular spatiotemporal dynamics, or obtain super-resolved images at nanometer resolution. Focus is placed on system instrumentation parameters providing step-by-step guidelines for microscope and experimental setup, as well as sample preparation protocols. Moreover, sophisticated labeling and detection methods are introduced, including tissues clearing, genetically encoded voltage indicators, reciprocal probes, or biosensors. Finally, detailed workflows on data analysis and data quantification are presented dependent on the imaging setup, target, or biological process of interest, including automated and high-content analyses.

This book can offer to the inexperienced user the possibility of a straightforward strategy to address biological questions by selecting the appropriate imaging system, preparation protocol, and data evaluation method based on the experimental model available. In parallel we are wishing to reinforce the experienced user with a variety of additional cutting-edge applications that can be complementary to routine practices and can increase the array of acquired observations and datasets. Finally, we anticipate that the book will additionally prove to be a robust teaching guide for light microscopy practical courses.

Editing this book has been a lengthy but most enjoyable quest. Firstly, we would like to thank our authors who accepted our invitation and generously introduced their expertise and protocols to the scientific community, while patiently went over revisions. We have been overwhelmed with the information and detailed methodologies, as well as image quality

included in the manuscripts, which have indeed exceeded our original expectations. We are familiar with the pains and joys of image acquisition and analysis and we are grateful for their efforts and dedication in bringing this work forward. Further, we would like to thank our series editor Prof. John M. Walker for his critical advice and help on the book preparation, as well as the staff at Humana Press for inviting us and greatly assisting us to edit this book and for giving us the opportunity to produce what we feel is today's Light Microscopy. Happy imaging!

Los Angeles, CA, USA
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