

Journal of Biomedical Optics

BiomedicalOptics.SPIEDigitalLibrary.org

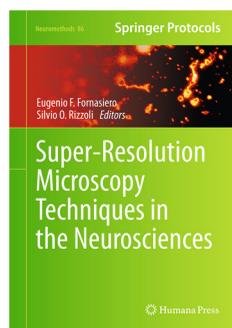
Super-Resolution Microscopy Techniques in the Neurosciences

Barry R. Masters

Super-Resolution Microscopy Techniques in the Neurosciences

Eugenio F. Fornasiero and Silvio O. Rizzoli, eds., 391 pages + xvi, ISBN: 978-1-62703-982-6, Humana Press/Springer, New York (2015), \$139.00, hardcover.

Reviewed by Barry R. Masters, Fellow of AAAS, OSA, and SPIE



The 2014 Nobel Prize in Chemistry was awarded jointly to Eric Betzig, Stefan W. Hell, and William E. Moerner “for the development of super-resolved fluorescence microscopy.” A clear introduction to this important and emerging field that includes video interviews with the Nobel Laureates can be found at: http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2014/popular-chemistryprize2014.pdf. Their paths to creative insight

and discovery are still an open question.

What is super-resolution? The term has many contextually different meanings, but with respect to optical microscopy with visible light it typically refers to Abbe’s 1873 publication on the physical limits of resolution in an optical microscope that operates under specific conditions. These conditions include the constraints of linear absorption and emission processes. Under these specified conditions the physical process of light diffraction limits the resolution of an optical microscope. For an illumination system with visible light the lateral resolution (x - y plane) is approximately 200 nm and the axial resolution is approximately 500 nm (z -direction). The term super-resolution optical microscopy refers to those techniques that can achieve resolution beyond the diffraction limit. The actual performance of an optical microscope is not only dependent on the optical resolution; it is also a function of the mechanisms that generate contrast and noise in the image. Optical aberrations also degrade the optical performance of the microscope.

Eugenio F. Fornasiero and Silvio O. Rizzoli of the Department of Neuro- and Sensory Physiology, University of Göttingen Medical Center, Göttingen, Germany, have edited a multicontributor highly recommended volume, *Super-Resolution Microscopy Techniques in the Neurosciences*, that will prove to be an exemplar for future books in this field. Many of the applications of super-resolution microscopy as described in this book involve investigation on synaptic processes, neurotransmitter release and uptake, the development and the plasticity of dendritic spines, and the *in vivo* images of the cortex of living animals. While this book has the word neurosciences in its title, and the volume is part of the series *Neuromethods*, I claim that this book will be extremely useful to scientists and engineers who work in a variety of disciplines and employ interdisciplinary approaches to problem solving.

Super-Resolution Microscopy Techniques in the Neurosciences presents the new techniques that achieve resolution beyond the diffraction limit by using nonlinear optical effects and new conditions outside of the conditions that Abbe stipulated in 1973.

The theoretical basis of each technique, its implementation in instrumentation and mathematical algorithms, its limiting assumptions, as well as a variety of key applications are all comprehensively described and critically discussed. In particular the problems and the limitations of each technique are carefully evaluated to a degree that was previously unknown in the literature. The key problems of probe development, specimen preparation, light damage to both the probe and the specimen, photon detector design and construction, mathematical algorithms used in the calculations, instrument control systems, specimen mechanical positional stability during the duration of the imaging process, and the control and mitigation of optical aberrations are faithfully discussed. Once the final composite or computed images are obtained, there are the confounding problems of image interpretation and analysis. These problems of image interpretation and the role of artifacts, both specimen and instrumentation derived, have always plagued optical microscopy and they still continue to do so.

The super-resolution techniques described, compared, and critically evaluated in this book have a common achievement: they provide investigators with a new set of techniques to study biological structure and processes at the nanometer scale. These optical imaging techniques bridge the scale between traditional optical microscopes with micron resolution and electron microscopes and near-field microscopes with atomic resolution. The development of microscopes that cover the entire range of scale provides the tools for further inquiry and potential discovery that will increase our knowledge and understanding of living processes.

This book describes a variety of techniques to achieve super-resolution imaging. These techniques include the following: methods that use stimulated emission to deplete excited states in a prescribed annular pattern (STED), methods that determine the positions on single fluorophores which can be used to form the composite fluorescent image of the specimen such as photoactivated light microscopy (PALM) and stochastic optical reconstruction microscopy (STORM), and methods that employ a structured pattern of the illumination such as structured illumination microscopy (SIM), as well as their many variants.

The book wisely includes comprehensive chapters on alternative methods to achieve super-resolution limited to one dimension, such as near-field optical microscopy, atomic force microscopy, x-ray microscopy, Raman microscopy—in particular nonlinear optical approaches towards subdiffraction resolution in CARS (coherent anti-Stokes Raman scattering) imaging—and electron microscopy photo-oxidation techniques.

The book is filled with critical comparisons, cautions, warnings, and limitations of the various techniques and the necessary specimen preparations. For example, a chart is provided that compares critical parameters for a variety of super-resolution techniques. The authors remind the reader, “As we reach higher resolution, the sizes of the fluorescent probes start to matter as much as their optical properties.” This limit points to the utility of probeless techniques of optical microscopy. Each chapter

provides a carefully selected and up-to-date list of key references. The lack of a useful listing of the patents that cover each of the super-resolution techniques is regrettable, as it would be beneficial for the correct attribution of simultaneous discovery of many of these methods. The full color images and the graphics in the book are all of a high standard both in design and in the production of the book. A useful index is provided.

In a book composed of many excellent chapters it is a difficult task to denote chapters that stand out for their comprehensive character, their integration of physical theory, mathematical analysis, instrument design, and a superb integration of clear text and graphics. Kai Wicker's chapter on "Super-resolution fluorescence microscopy using structured illumination," and Daniel Smeets, Jürgen Neumann, and Lothar Schermelleh's chapter on "Applications of three-dimensional structured illumination microscopy in cell biology: pitfalls and practical considerations," and Felipe Opazo's chapter on "Probing biological samples in high-resolution microscopy: making sense of spots," are singled out. They point out the potential utility of structured light microscopy—a wide-field technique based on the moiré effect—as it is evolving, and the enormous potential that it offers to researchers in the neurosciences and other interdisciplinary investigations, as well as the problems of the interpretation images composed of "spots" and their subsequent understanding in terms of biological function and structure. Their discussion of the theory in terms of Fourier analysis and reciprocal space is excellent.

The last three chapters in this book further express the recurring themes of limitations, problems, pitfalls, and cautions in the use of commercial microscopes, as well as investigator constructed microscopes that offer super-resolution. The ultimate goal of all microscopic images is a precise and accurate mapping of the specimen. Many of these techniques employ fluorescent probes, and their design parameters are crucial to successful implementation in super-resolution techniques. The labeling techniques and other details of specimen preparation are no less important. Two critical factors are label density and label distribution, and a proper understanding of these factors is important for optimal and accurate imaging that is a valid reflection of the structure of the specimen. The experimental operation of some super-resolution microscopic techniques require high light intensities to increase the numbers of photons that can be detected; but the high light intensities that improve the resolution in some techniques also increase the artifacts and photo-damage that is induced in the specimen. This is especially important for live cell imaging. The use of traditional methods that enhance contrast in traditional wide-field optical microscopy need to be altered to meet the requirements of super-resolution techniques.

Super-Resolution Microscopy Techniques in the Neurosciences is highly recommended as a critical guide to the variety of amazing techniques that can, when used with appropriate precautions and controls, further open up the nanoscale world of cell biology and neuroscience to investigators.