Preface to the special issue on advances in localization microscopy

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Single molecule localization microscopy (SMLM) has seen rapid innovation, driven by new methodologies that push the boundaries of resolution, precision, and applicability across biological and material sciences. This special issue of Optics Communications contains a selection of recent advancements that address critical challenges and broaden the scope of localization microscopy. Authored by leading experts in the field, these papers illustrate the power of interdisciplinary efforts in advancing the field.

One prominent theme in this issue is the **simultaneous measurement of molecule positions and orientations**, known as "Single Molecule Orientation and Localization Microscopy" (SMOLM). In this category, Jouchet, Roy, and Moerner present a deep learning approach that combines neural networks with PSF engineering to achieve simultaneous 3D localization and orientation measurements of fluorescent molecules at high precision. This method exemplifies how computational advances open up new possibilities for high-dimensional optimization problems. Munger et al. present an analytical study on the impact of excitation polarization on joint position and orientation measurements and provide a detailed analysis of potential biases, which are helpful to improve the reliability of SMOLM. Finally, Schneider et al. present a comprehensive simulation tool for visualizing PSF shapes, where the impact of several parameters such as molecule rotation can be evaluated and which assists researchers in designing SMLM setups and interpreting data.

A group of papers focuses on **engineering of point spread functions (PSF)** to enhance axial resolution and depth-of-focus. Fan et al. introduce temporal TIRF angle modulation combined with PSF engineering, which improves axial localization precision two-fold over classical TIRF-based SMLM. Gresil et al. contribute to this theme with a binary annular phase mask that regulates spherical aberration and extends the depth-of-focus for single-particle tracking in heterogeneous environments, enabling long-term tracking.

In terms of **TIRF microscopy design**, McCluskey and Dekker provide practical insights into the construction and optimization of a micromirror-based TIRF system for multicolor imaging. Their contribution aims to improve signal quality in complex biological systems where conventional dichroic methods limit detection efficiency. This guide offers invaluable advice for researchers looking to adopt or optimize similar setups in their labs.

The development of **calibration standards** also features prominently in this issue. Olevsko et al. introduce a color-coded nanometric ruler designed for axial calibration in super-resolution microscopy, providing a critical tool for validating 3D measurements at the nanoscale.

Lastly, Mitra et al. introduce an innovative **excitation control technique for long-duration imaging**. Their small-window illumination microscopy (SWIM) technique enables high-quality single-molecule imaging in *C. elegans* neurons by reducing autofluorescence and photobleaching, making it a promising approach for long-term studies in living organisms.

The papers included in this special issue collectively address a range of technical innovations, including deep learning-enhanced imaging, advanced PSF engineering, polarization analysis and calibration tools. Each of these contributions addresses a distinct challenge in localization microscopy. We are pleased to present these papers to our readership and hope that they will stimulate further developments in nanoscale imaging, thereby enabling researchers to explore complex molecular dynamics with unparalleled precision.

Sophie Brasselet is an optical physicist. She has obtained her PhD in 1997 at the University of Paris Sud and CNRS Bagneux with J. Zyss, on the study of multipolar molecules applied to nonlinear optics in polymers. She then spent two years (1998–2000) as a postdoctoral fellow at UCSD and Stanford University (USA) with W.E. Moerner on the development of single molecule localization imaging methods in cells. After six years at ENS Cachan (France) as an assistant professor, she was recruited as a CNRS researcher at Fresnel Institute (Marseille, France). She is now a CNRS research director and the director of this institute. She has developed non-linear optical microscopy and super-resolved fluorescence imaging based on the control of light polarization for the last fifteen years, with pioneering contributions to provide new imaging methods capable of accessing structural information in biological samples at the nanoscale. She is currently extending these approaches to 3D polarized imaging at high spatial resolution extending single molecule localization microscopy, or to fast, label free organization imaging in biological tissues applicable to biomedical optics. She has published more than 150 peer reviewed articles and given more than 100 invited conferences. She was awarded the CNRS silver medal (2016) as well as the Leon Brillouin Grand Prix of the Optical French Society (2022), and is an OPTICA fellow since 2020.

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Yoav Shechtman is an Associate Professor of Biomedical Engineering at the Technion - Israel Institute of Technology, specializing in optical nanoscopy and computational imaging. He holds dual B.Sc. degrees in Electrical Engineering and Physics (2007) and earned his Ph.D. at the Technion (2013). Following his Ph.D., Dr. Shechtman completed a postdoctoral fellowship at Stanford University, working with W.E. Moerner on point spread function (PSF) engineering for 3D and multicolor localization microscopy. His recent contributions include the application of deep learning to localization microscopy and to optical design, e.g. methods such as Deep-STORM, DeepSTORM3D, and derivatives that enable dense, multicolor, volumetric, and super-spatiotemporal-resolution microscopy. Specific applications include subcellular tracking and optical genome mapping, as well as efficient 3D-printing based fabrication of diffractive optics. Dr. Shechtman currently serves as associate editor for Biophysical Reports and has co-organized international conferences in computational and biomedical optics. Selected awards and recognitions include the 2018 Early Career Award of the International Association for Medical and Biological Engineering (IAMBE), the 2018 ERC starting grant, the 2020 International Union for Pure and Applied Biophysics (IUPAB) Young Investigator Prize and Medal, the 2021 Krill Prize for Excellence in Scientific Research, and 2023 Harrington fellowship at UT Austin, and the 2024 Hershel Rich Technion Innovation Award.

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