Simultaneous Multi-Color 4Pi-SMS Imaging Using Salvaged Fluorescence

Yongdeng Zhang, Lena K. Schroeder, Mark D. Lessard, Phylicia Kidd, Jeeyun Chung, Yuanbin Song, Lorena Benedetti, Yiming Li, Jonas Ries, Pietro De Camilli, James E. Rothman, David Baddeley, Joerg Bewersdorf
Department of Cell Biology, Yale School of Medicine, New Haven, CT, USA
Email: yongdeng.zhang@yale.edu

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Combining the molecular specificity of fluorescent probes to label multiple sub-cellular targets with whole-cell three-dimensional (3D) imaging at nanoscale resolution, is extremely valuable for investigating the spatial organization of intricate organelles or protein complexes which often feature important details at the tens of nanometer scale. As the optical gold standard in 3D super-resolution microscopy, 4Pi-SMS [1] has recently revealed a diverse range of nanostructures in cells and microgels. While single-color nanoscopy can successfully address questions concerning sub-diffraction sized structures, many biological applications require multi-color imaging to investigate targets of interest in context with other molecules or organelles. However, high quality two-color 4Pi-SMS imaging has remained challenging preventing its realization in whole cells. Additionally, three-color 4Pi-SMS imaging has been completely unexplored due to numerous difficulties. Here we present a super-resolution light microscopy method that allows simultaneous two- and three-color imaging of whole cells at ~20 nm 3D resolution with minimal cross-talk and negligible chromatic aberrations. We demonstrate its potential for cell biological research by imaging the highly convoluted Golgi apparatus and endoplasmic reticulum-plasma membrane (ER-PM) contact sites in cultured mammalian cells and synaptonemal complexes in mouse spermatocytes - structures that have traditionally been the imaging realm of electron microscopy (EM). The presented concept of utilizing salvaged fluorescence in ratiometric imaging is generally applicable in the majority of single-molecule based super-resolution microscopy.

[1] Huang, F. et al. Ultra-High Resolution 3D Imaging of Whole Cells. *Cell* 166, 1028-1040 (2016).