Multicolor Imaging based on Interferometric Information in 4Pi Single-Molecule Switching Nanoscopy

Kevin Hu[1,2], Yongdeng Zhang[2], and Joerg Bewersdorf[1,2]
[1]Department of Biomedical Engineering
[2]Department of Cell Biology
Yale University
333 Cedar Street, New Haven, CT, USA
Email: k.hu@yale.edu

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By utilizing a dual-objective setup in single-molecule switching nanoscopy (SMSN; e.g. PALM, STORM or FPALM), it is possible to obtain three-dimensional (3D) images at 10 to 20 nm resolution [1,2]. We have recently expanded this technology to the imaging of thick cells through the addition of deformable mirrors in each arm of the 4Pi-interferometric cavity and new image processing algorithms, significantly increasing the application range [3].

Here we present a novel scheme for simultaneous multicolor imaging in whole-cell 4Pi-SMSN (W-4PiSMSN). This filter-free approach extracts the color-information from the interference pattern of the single-molecule fluorescence by taking advantage of the wavelength-dependence in the interference intensities. We demonstrate that this approach can be used to separate two or more emission spectra in the phase space, allowing for probe identification without any modifications to the beam path. With the example of Alexa Fluor 647 and CF 680, both of which are compatible with SMSN, we show a quantitative analysis of the dependences between obtainable spatial resolution, cross-talk and coherence length.

Our new approach represents a novel path to extract color-information from SMS super-resolution data.

References

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