

RECONSTRUCTION APPROACHES FOR MULTIFOCUS-SIM DATA

Aurélie Jost^{1,2}, Sara Abrahamsson^{3,4} & Rainer Heintzmann^{1,2}

¹ Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller-University Jena, Germany

² Leibniz Institute of Photonic Technology, Jena, Germany

³ The Rockefeller University, New York, NY, USA

⁴ SciLifeLab, Stockholm, Sweden

E-mail : aurelie.jost@leibniz-ipht.de

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Recent developments in fluorescent microscopy aim at fast high-resolution imaging. 3D Structured illumination microscopy (SIM) typically acquires 15 raw images per imaged slice and mechanically repositions the stage for a focal series. After processing, the SIM image features super-resolved details at a two-fold improved resolution compared to the diffraction limit.

Multifocus microscopy (MFM) is a detection method that enables instantaneous acquisition of typically nine focal planes. Merging SIM excitation and a MF detection scheme (MF-SIM) enables to reach fast 3D super-resolution imaging.

Each such recorded MF-SIM exposure contains 9 zones, each corresponding to a different focal position. Thus, we need to acquire only 15 exposures to obtain the equivalent of a SIM acquisition series. Here, we explore possibilities and pitfalls of reconstructing such data where the sample is not moving with respect to the illumination pattern. One approach is to process each slice individually. Both methods – the classical Fourier-based approach and thick slice blind-SIM [2] – can be used to achieve high resolution and optical sectioning.

References;

[1] Abrahamsson, S. *et al.* Fast multicolor 3D imaging using aberration-corrected multifocus microscopy (MFM). *Nat. Methods* 10, 60-63 (2013).

[2] Jost, A. *et al.* Optical sectioning and high resolution in single-slice Structured Illumination Microscopy by *thick slice* blind-SIM reconstruction. *PLoS ONE* 10(7), e0132174 (2015).