Cost-effective Structured Illumination Microscopy with a Digital Mirror Device and coherent excitation light

Alice Sandmeyer[1], Mario Lachetta[1], Hauke Sandmeyer[2], Wolfgang Hübner[1], Thomas Huser[1], Marcel Müller[1,3]

[1] Biomolecular Photonics, Faculty of Physics, University of Bielefeld, Germany
[2] Numerical Simulations and Field Theory on the Lattice, Faculty of Physics, University of Bielefeld, Germany
[3] Laboratory for NanoBiology, Department of Chemistry, KU Leuven, Belgium
E-Mail: asandmeyer@physik.uni-bielefeld.de

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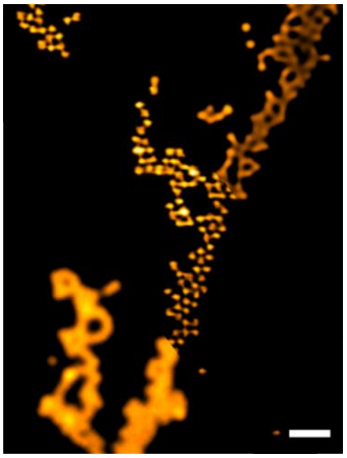
Structured illumination microscopy (SIM) has become an essential and successful super-resolution imaging tool with application in medical and biological research. Its low phototoxicity and high imaging speeds make it especially well-suited for live-cell imaging tasks. Here, we present a robust and cost-effective approach for a SIM excitation system and microscope.

We are adopting the Gustafsson approach for creating structured illumination, i.e. by interfering two coherent laser beams in the sample [1]. The resulting sinusoidal intensity pattern has to be shifted by three phases and three angles to allow for a near-isotropic 2x resolution enhancement in two dimensions. Thus, nine raw frames are recorded and result in one computationally reconstructed super-resolved image. To obtain the maximum modulation depth, and therefore the best spatial reconstruction, the phase, intensity and polarization of the two interfering beams must be precisely controlled and transmitted through the optical system.

We utilize a Digital Mirror Device (DMD) to create the required two beams. Compared to other spatial light modulator approaches [2], these devices exhibit a "blazed grating" effect which has to considered. To model this effect, computational simulations were performed to compare the results of the theoretical blazed grating effect with the experimental results at the setup. To reduce the overall system costs, we employ an industry-grade laser source (typically used in light-show applications) for excitation and an industrial CMOS camera for detection [3]. Combined with the cost-effective DMD, we created a cost-efficient SIM microscope, which is nonetheless capable of fast super-resolution imaging (see figure). Furthermore, we implemented real-time fairSIM image reconstruction, so that live reconstruction can be performed [4]. This is convenient because it provides immediate feedback about the usability of the data and the image position and enables real-time maneuvering of samples with super-resolution.



Comparison of a SIM (middle), wide-field (lower left) and filtered wide-field (upper right) fluorescence image of 200 nm TetraSpeck beads. Exposure time per raw frame is 20 ms. Scale bar, 1 µm.

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