COHERENT STRUCTURED ILLUMINATION PROVIDES A LATERAL RESOLUTION OF $\lambda/4n$ IN LIGHT SHEET-BASED FLUORESCENCE MICROSCOPY

Bo-Jui Chang, Victor Didier Perez Meza, and Ernst Hans-Karl Stelzer
Physical Biology, Buchmann Institute for Molecular Life Sciences (BMLS)
Goethe University Frankfurt am Main
Max-von-Laue-Str. 15, D-60438 Frankfurt am Main, Germany
E-mail: bo-juan.chang@physikalischebiologie.de

KEY WORDS: light sheet, structured illumination, super resolution, 3D, SIM, LSFM, SPIM, DSLM, mitochondria

We combine two techniques, super resolution structured illumination microscopy (SIM)[1][2] and light sheet-based fluorescence microscopy (LSFM)[3][4], to provide coherent structured illumination light sheet-based fluorescence microscopy (csiLSFM). SIM has always been an attractive option for super resolution imaging with a moderate excitation intensity as well as a higher acquisition speed[1] compared to e.g. STED, GSD, d/STORM and PALM. LSFM features low photo-toxicity and low photo-bleaching, as well as the inherent true optical sectioning because of its illumination scheme. Furthermore, LSFM is particularly well-suited for thick specimens. Therefore, we aim to improve the lateral resolution in LSFM without losing any of its advance.

Previously, we introduced the concept of csiLSFM, showed its resolution of $126\pm7$ nm, and demonstrated its application with GFP-tagged mitochondria in yeast. Due to the improvement of the csiLSFM system and our recent development of SIM reconstruction algorithm (see Abstract by Perez Meza), we now achieve true counter-propagating light sheets illumination and generate the interference pattern close to $\lambda/2n$. Consequently, an ultimate resolution of $\lambda/4n$ in linear structured illumination is now possible. We achieve a resolution of $88\pm10$ nm with 40 nm fluorescent beads, detected by a water-immersion, NA1.0, 63x objective lens at an emission wavelength of 515 nm (Figure 1).

Figure 1. The resolution-improved images (maximum intensity projection) of 40 nm fluorescent beads embedding in 1% Phytagel. Lucy-Richardson deconvolution was implemented in both the wide-field and SIM images. The image stacks comprise 50 planes spaced by 100 nm.