AXIAL RESOLUTION IMPROVEMENT IN SCANNING BESSEL BEAM LIGHT-SHEET MICROSCOPY WITH COMPLEMENTARY BEAM

Xianghua Yu, Hao Jia, Yanlong Yang, Xing Zhou, Shaohui Yan, Junwei Min, Tong Peng, Ming Lei, and Baoli Yao*
State Key Laboratory of Transient Optics and Photonics, Xi'an Institute of Optics and Precision Mechanics, Chinese Academy of Sciences
No.17 Xinxi Road, Xi'an 710119, China
E-mail: yaobl@opt.ac.cn

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Light-sheet microscopy (LSM) is widely used for in-vivo imaging of cells or micro-organs volumetrically with high temporal-spatial resolution and low photobleaching [1]. A scanned Gaussian beam can form a light-sheet, which has to be made a trade-off between the field-of-view (FOV) determined by the Rayleigh range of Gaussian beam and the light-sheet thickness related to the Gaussian beam waist. The employment of propagation-invariant beams such as Bessel beam into the scanning light-sheet microscopy tremendously expands the FOV, especially in scattering media [2]. However, the side lobes of Bessel beam will create significant out-of-focus background when scanned in light-sheet microscopy, limiting the axial resolution of the imaging system. Here, we propose an approach to overcoming this issue by scanning the sample twice with zeroth-order Bessel beam and another type of propagation-invariant beam, complementary to the zeroth-order Bessel beam, which greatly reduces the out-of-focus background created in the first scan. The axial resolution can be enhanced by subtraction of the two scanned images, and is comparable to the lateral resolution across a whole field-of-view of 200μm×200μm×100μm. We showed the optimization procedure to form the complementary beam with a spatial light modulator, and verified the superior axial imaging performance experimentally with fluorescent beads.

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