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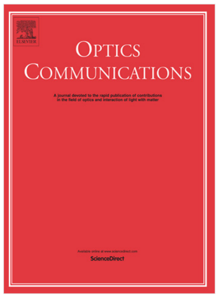

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Resistance to Optical Distortions in Three-dimensional Interferometric Temporal Focusing Microscopy

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Abstract

We analyze how optical distortions in deep imaging or dense scattering media influence three-dimensional interferometric temporal focusing (3D-ITF) imaging, and derive the distorted point spread function (PSF) and optical transfer function (OTF). Upon this, we find that 3D-ITF microscopy is less affected by optical distortions in terms of spatial resolution, artifacts and sectioning ability. Temporal focusing (TF) illumination supports a robust spatial modulation in 3D-ITF microscopy with distorted optics. As the modulation spatial frequencies are unique, they offer a redundancy in 3D-ITF microscopy. The redundancy in the spatial frequency of 3D-ITF images compensates for the loss of spatial frequency caused by the optical distortion in the emission OTF. The redundancy recovers the high spatial frequencies to prevent the degradation of spatial resolution. Moreover, the redundancy recovers the distortion in the emission OTF spectrum, which causes distorted shapes in the PSF such as side lobes and double peaks. Thus, there is a relatively lossless OTF spectrum in 3D-ITF compared to TF, and this leads to better trade-off between remaining noise artifacts and resolution after the Wiener filter correction.

1. Introduction

Temporal focusing (TF) technique utilizes a spatiotemporal modulation to gradually stretch the illumination laser pulse width out-of-focus. Therefore, TF offers an optical sectioning for wide-field multi-photon excitation fluorescence imaging [1-7]. On the other hand, structured illumination microscopy (SIM) [8-14] utilizes a spatially modulated illumination to down-convert the spatial frequencies beyond diffraction limitation in a raw image and then gives phase shifts in the modulation to form multiple raw images. SIM post-processes the raw images in order to separate the sub-images with different modulation frequencies and separately up-convert them. By combining the up-converted sub-images, SIM offers a spatial resolution beyond diffraction limitation and/or supports optical sectioning and/or removes background. It is convenient to combine TF and SIM via a digital micromirror device (DMD), which could be regarded as a blazed grating printed with programmable pattern. This combination [15,16], which we call interferometric temporal focusing (ITF) technique, is a win-win solution for both TF and SIM. It removes the scattered and out-of-focus emission in TF imaging through post-processing, while also reducing both the out-of-focus excitation in SIM raw images and the corresponding shot noise in the SIM reconstructed image by multi-photon TF excitation. Thus, ITF makes optical sectioned super-resolution images practically available in deep imaging or dense scattering media. Three-dimensional ITF (3D-ITF) microscopy [17] as shown in Fig. 1 adds axial spatial modulation in illumination [14] to further improve the sectioning ability and the axial resolution compared to two-dimensional ITF (2D-ITF), which has only lateral spatial modulation, at the cost of more raw-image acquisition but with the same setup complexity. There are methods like ITF, such as combining HiLo [18] with TF. These methods require fewer raw images to remove scattered and out-of-focus emission but lose the spatial resolution improvement [19-21].