VOLUMETRIC IMAGING UTILIZING LINEAR-SHIFT POINT-SPREAD FUNCTION BASED ON MULTIPLEXED COMPUTER-GENERATED HOLOGRAM

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Rapid acquisition of three-dimensional (3D) volumetric images of biological samples is crucial for capturing its dynamical behavior and functionality *in vivo*. However, increasing the acquisition speed in these techniques is challenging for usual light microscopy including laser scanning microscopy because, in principle, 3D images are constructed from multiple two-dimensional images by changing the observation plane. We have demonstrated 3D image acquisition based on two-photon excitation laser microscopy with a light needle spot with an extended focal depth, where axial information is retrieved by Airy beam conversion for fluorescent signals [1]. In this approach, the self-bending and parabolic propagation of an Airy beam plays a key role to construct 3D images. However, such propagation behavior poses the non-linear lateral shifting to the resultant point spread function (PSFs), leading to the depth-dependent variation of the axial resolution in reconstructed images.

Here, we propose a novel approach to extract axial information in light needle microscopy by utilizing a linear-shift PSF realized by multiplexed computer-generated hologram (CGH). We design a 16-multiplexed CGH, which converts the axial information to the lateral information [2] as shown in Fig. 1(a). The designed CGH results in the linear shift behavior for the PSF on the lateral direction (*H*) with respect to its axial position (*z*) as shown in Fig. 1(b). Applying this behavior to a two-photon excitation, light needle microscope equipped with an array detector enables video-rate acquisition of 3D volumetric images with a depth-independent axial resolution in an extended depth range.

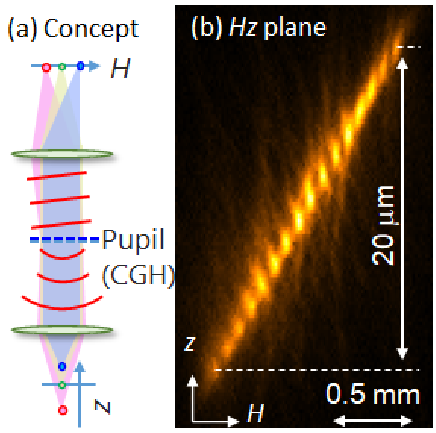

Figure 1: (a) Multiplexed CGH concept. (b) Lateral shift behavior for 16-multiplexed CGH with the depth range of 20 µm, observed for a 200-nm fluorescent beads under two-photon excitation with a 1040-nm laser source. Numerical aperture of an objective lens is 1.15.

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