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 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.