AXIALLY RESOLVED, VOLUMETRIC IMAGING BY LIHGT NEEDLE MICRSCOPY WITH SPATIALLY TRANSPOSED DETECTION

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In biological imaging, the rapid acquisition of three-dimensional (3D) volumetric images is essential for investigating the detailed and dynamical structure of specimens and its functionality. Although laser scanning microscopy utilizing confocal detection or multi-photon excitation is generally employed for this purpose, 3D images are constructed from a series of two-dimensional images acquired with a moving observation plane, which ultimately limits the acquisition speed. Here, we present a novel method that enables 3D image construction by only a single raster scan of an excitation light needle spot with an extended focal depth implemented in a two-photon excitation microscope [1]. In general, a light needle spot in laser microscopy merely provides a deep focus image with a loss of depth information. In our approach, however, the depth information is retrieved from the transposed lateral information on an array detector for emitted fluorescent signals converted into "Airy beam", which has non-diffracting and self-bending properties. Thus, 3D image is constructed from a single raster scan of a light needle without a moving observation plane, which can significantly reduce acquisition time.

In our experiment, we employed a 1040-nm femto-second pulsed laser as a two-photon excitation source and produced a light needle spot with a focal depth of ~15 μm at the focus of a water immersion objective lens with a numerical aperture of 1.15 by using a phase-only spatial light modulator (SLM). The emitted fluorescent signal was then converted into an Airy beam by applying a cubic phase modulation to another SLM placed on a detection path. The intensity distribution of the converted signals on an image plane was recorded by an electron-multiplying charge-coupled device (EMCCD) and used to construct the depth information at each scanning position. We acquired and constructed a 3D image of a biological sample and the depth information was correctly retrieved from a single raster scanning of a light needle [see Fig. 1]. The present result demonstrates the potential of our method to accelerate the acquisition speed for 3D volumetric imaging compared to conventional image stacking.

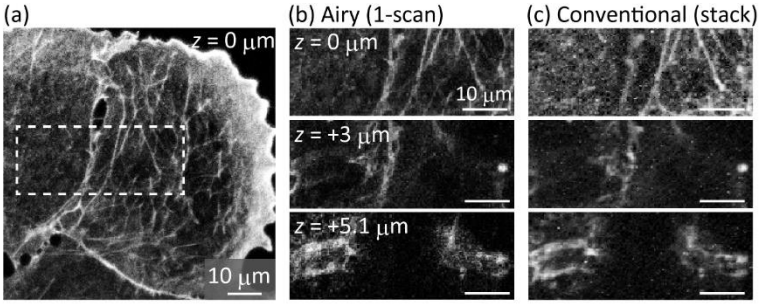

Figure 1: (a) Reconstructed image at z = 0 μm for F-actin in fixed COS-7 cells obtained from a single scanning of a light needle with Airy beam conversion. (b) Magnified views for the rectangular region shown in (a) for the different depth positions. (c) Corresponding images taken by the conventional image stacking.

[1] Y. Kozawa and S. Sato, "Light needle microscopy with spatially transposed detection for axially resolved volumetric imaging" Sci. Rep. **9**, 11687 (2019).