RAPID VOLUMETRIC IMAGING WITH BESSEL-BEAM THREE-PHOTON MICROSCOPY

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Three-photon fluorescence microscopy is known for its ability for extending imaging depth in scattering tissues *in vivo*, which permits identification of neuronal structures and activities up to 1 mm of mouse brain [1]. However, it suffers from a slow scanning speed due to the low-repetition-rate laser sources used for three-photon excitation. For volumetric imaging, the imaging speed is even slower because of the serial focal scanning in axial dimension.

Here we present a rapid volumetric three-photon fluorescence microscopy based on the axially elongated Bessel focus. We used a refractive axicon to generate a Bessel beam, and by scanning the needle-like laser beam, a 300-μm × 300-μm × 300-μm volume with a single scan at 5 Hz could be obtained.

Using axially elongated Bessel focus to extend the depth of field has also confirmed to be an effective solution for two-photon microscopy [2]. Nonetheless, the intensity distribution of zero-order Bessel function in the transverse plane shows strong side lobes, which results in hazy backgrounds, especially when a high NA is used. Meanwhile, because of the power-cubed dependence, three-photon excited Bessel beam has much less side lobes compared to 2PM. We testified the results by theoretical calculation, and practical imaging test of fluorescent beads. Therefore, the volumetric three-photon fluorescence imaging could obtain better signal to background ratio, which was shown by the imaging results in fruit flies and zebrafish larvae *in vivo*.

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