3D SUPER-RESOLUTION IMAGING IN TWO-PHOTON MICROSCOPY USING NON-LINEAR OPTIMIZATION

Simeng Chen, Jiun-Yann Yu, Jian Xing, Venkatalakshmi V. Narumanchi, James Folberth, Stephen R. Becker, and Carol J. Cogswell
University of Colorado at Boulder
Boulder, CO 80309, USA
Simeng.Chen@colorado.edu or cogswell@colorado.edu

KEY WORDS: Two-photon microscopy, 3D super-resolution, non-linear optimization.

We present a technique for achieving isotropic 3D super-resolution two-photon microscopy. Our previous work demonstrates that super-resolution for single photon microscopy can be achieved by constraining the excitation volume with a scanning focal spot, and numerically analyzing the acquired images with non-linear optimization [1, 2]. In this presentation, we show that the 3D resolution can be further improved by adopting two-photon fluorescence excitation. By performing Monte Carlo simulations on 3D synthetic phantoms, we demonstrate that the achievable super-resolution depends on the sparsity of the sample and signal-to-noise ratios. We confirm that 150nm isotropic super-resolution is achieved in 3D by imaging Thy1-YFP labeled dendritic spines in a mouse brain section (Fig.1). This is in contrast to the ~400nm XY resolution and ~900nm Z resolution of conventional two-photon microscopy, at a ~970nm excitation wavelength and a 1.4 objective numerical aperture.

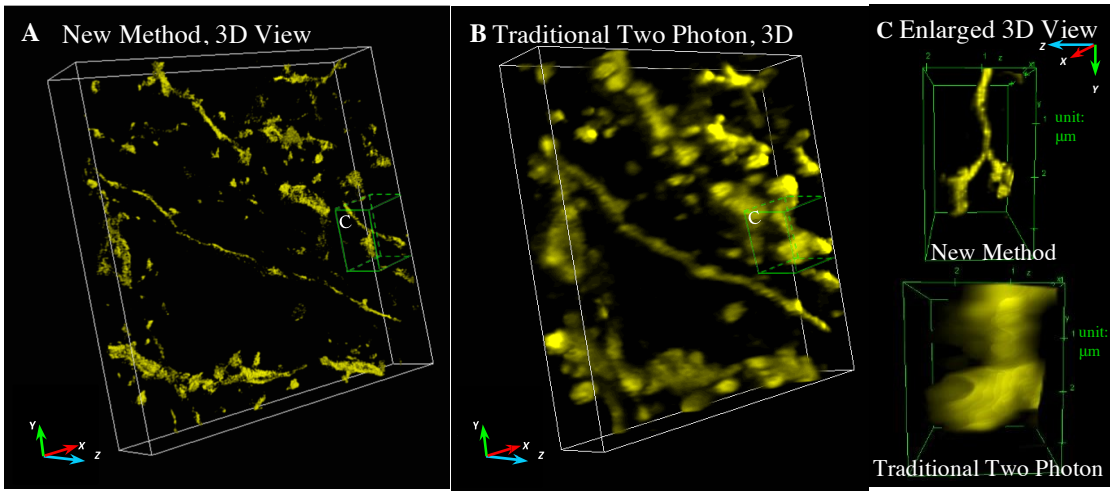

Figure 1. (A) 3D volume view (18 μm x 18 μm x 3.6 μm) of YFP-Thy1 labeled dendrites in a fixed 20 μm-thick mouse brain section imaged with our method, and (B) the corresponding view with traditional two-photon microscopy. (C) Higher-magnification views of the dendritic spines bounded by the cubes in (A) and (B) confirm isotropic 3D super-resolution using our new method.

[1] J.-Y. Yu et al., Achieving superresolution with focused spot illumination and non-negative inversions, *Opt. Express,* **26**, 8 (2018).
[2] C. J. Cogswell et al., Scanning EPIC Microscopy: Toward Simultaneous Super-Resolution of Continuous 3D Fluorescent Structures at Speed. Focus on Microscopy 2017, Bordeaux, France.