3D COMPUTATIONAL SUPER-RESOLUTION IMAGING OF BRAIN SAMPLES ACHIEVED IN SLIGHTLY-MODIFIED CONVENTIONAL AND TWO-PHOTON MICROSCOPES 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: 3D super-resolution, 3D brain imaging, computational microscopy

It is generally assumed that the only approach to breaking the 3D classical resolution barrier, with more than a factor of three resolution improvement, is through manipulating the fluorophores or their excitation processes. In this talk, we report a new approach to achieving 3D super-resolution imaging, without the requirements of photo-switchable fluorophores, that can provide more than 3 times resolution improvement in all three dimensions. 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]. Here, we support these findings with super-resolved 3D images of mouse retina and Drosophila brain sections. We also present results that shows that our new method can be used in a modified two-photon microscope to produce super-resolved images that are isotropic in x, y and z dimensions. Figure 1 confirms that ***150nm isotropic super-resolution*** is achieved in 3D by imaging Thy1-YFP labeled dendritic spines in a mouse brain section. 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.

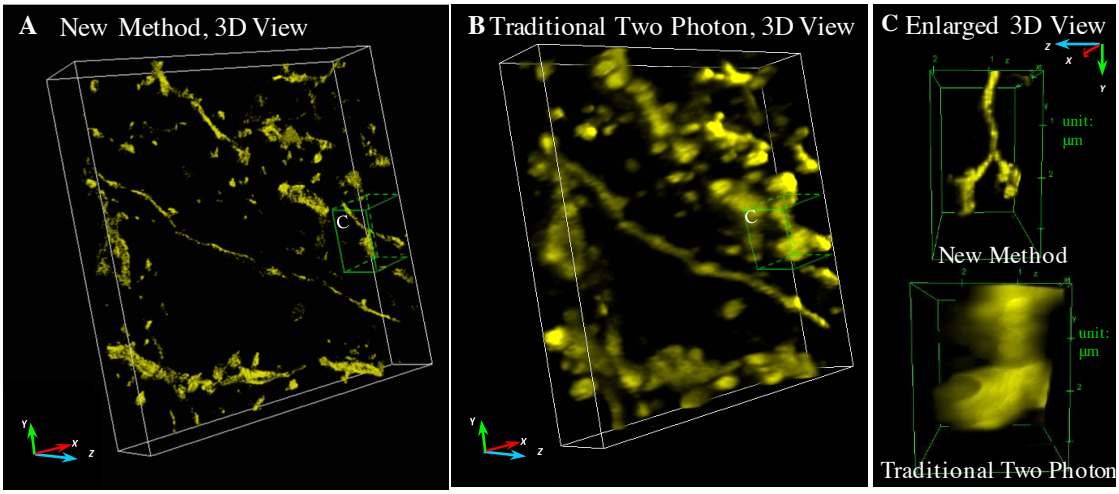

Figure 1. (A) 3D volume view (18 μm x 18 μm x 3.6 μm) of YFP-Thy1 labeled dendrites in a fixed 20 μm-thick mouse brain section imaged with our method, and (B) the corresponding view with traditional two-photon microscopy. (C) Higher-magnification views of the dendritic spines bounded by the cubes in (A) and (B) confirm isotropic 3D super-resolution using our new method. NA=1.4.

[1] Yu, J.-Y., Becker, S. R., Folberth, J., Wallin, B. F., Chen, S., and Cogswell, C. J., "Achieving superresolution with illumination-enhanced sparsity," *Opt. Express* **26**, 9850-9865 (2018).

[2] Xing, J., Chen, S., Becker, S. R., Yu, J.-Y., and Cogswell, C. J., "ℓ_1-regularized maximum likelihood estimation with focused-spot illumination quadruples the diffraction-limited resolution in fluorescence microscopy." *Opt. Express* **28**, 39413-39429 (2020).