VOLUMETRIC MULTICOLOR MULTIPHOTON MICROSCOPY FOR NEURON CONNECTIVITY AND CELL LINEAGE ANALYSIS

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KEY WORDS: Brainbow labeling, multiphoton microscopy, wavelength mixing, connectomics, cell lineage.

Reconstructing neural connectivity and cell lineage raises the challenge of imaging cubic millimeter volumes of tissue with suitable contrast, specificity and resolution. Our work aims at combining multicolor labeling and large-volume microscopy to reach this goal. The Brainbow labeling strategy [1] offers a large palette of fluorescent colors for cell tagging by random combinatorial expression of 3 fluorescent proteins (mCerulean, YFP and dTomato) in individual cells. It is however generally not straightforward to combine multiphoton imaging with multicolor labeling. We use the synchronization of two femtosecond laser pulses at 1100nm and 850nm to obtain simultaneous and optimal trichromatic two-photon excitation of Brainbow-labeled tissue, resulting in multicolor imaging with sub-cellular resolution over depths of a few hundreds of microns. [2,3]. Further extending the imaging depth in thick tissue samples is hampered by refractive index inhomogeneities that cause scattering and aberrations, thus preventing imaging with appropriate resolution and signal-to-noise ratio at large depths. We present our current advances towards combining multiphoton microscopy with recent approaches for large volume imaging such as clearing and serial tomography [4,5], and perspectives for analyzing brain tissue development.

References:

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