DUAL COLOR 3-PHOTON MICROSCOPY OF NERVOUS TISSUE WITH A MULTIBAND MEGAHERTZ OPA

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KEY WORDS: multiphoton microscopy, three-photon, tissue imaging, scattering, OPCPA.

Two-photon (2P) microscopy has become the gold standard for high-resolution fluorescence imaging of biological tissues. However, 2P fluorescence imaging in scattering tissues such as the brain of mammalian models remains limited to depths of 500-600 µm when using 900 nm excitation wavelength. This is principally caused by scattering, which degrades the excitation confinement together with the signal-to-background ratio at large depths. One recently demonstrated and promising approach to reach greater depths is to use a three-photon (3P) excitation mechanism and to shift the excitation towards longer wavelengths, in order to both reduce scattering and improve excitation confinement [1]. Water absorption should be minimized when shifting the excitation towards the short-wavelength infrared) range, so that 1.3 µm and 1.7 µm appear as optimal wavelengths for deep-tissue microscopy [1, 2].
We developed an original dual-color SWIR source that simultaneously emits ultrashort pulses at 1.3 and 1.7 µm with characteristics optimized for 3P microscopy: sub-70 fs duration, 1.25 MHz repetition rate, and µJ-range energy [3]. In turn, we achieve simultaneous 3P excitation of green and red fluorescent proteins in nervous tissue *ex vivo* and *in vivo* [3].

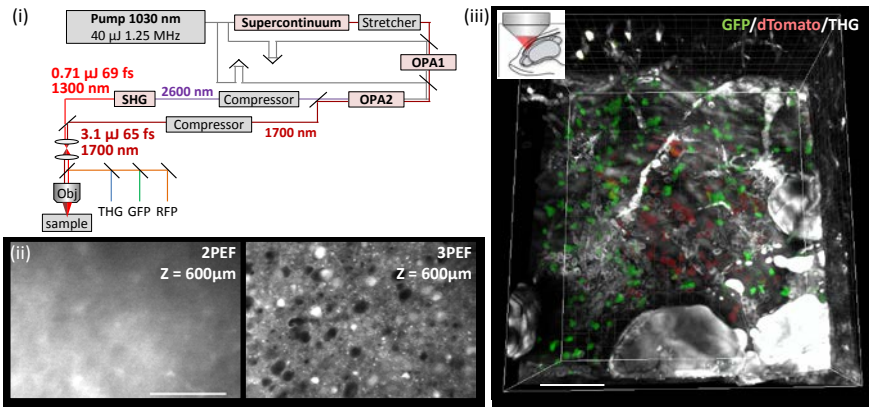

Figure 1: (i) OPA source providing simultaneous excitation at 1.3 and 1.7 µm. (ii) Comparison of 2P and 3P imaging of tdTomato-labeled fixed mouse brain tissue at a depth of 600µm. (iii) Dual-color 3P imaging in a live adult fish brain. Scale bars, 100 µm. Adapted from [3].

References:

[1] N.G. Horton et al, "In vivo three-photon microscopy of subcortical structures within an intact mouse brain," *Nature Photonics* (2013).
[2] D.G. Ouzounov et al, "In vivo three-photon imaging of activity of GCaMP6-labeled neurons deep in intact mouse brain," *Nature Methods* (2017).
[3] K. Guesmi, L. Abdeladim, et al, "Dual-color deep-tissue three-photon microscopy with a multiband infrared laser," *Light: Science and Applications* (2018).