MULTIPHOTON EXCITATION OF BIOLOGICAL SAMPLES WITH FOURIER-LIMITED, BROAD-SPECTRUM, ULTRASHORT LASER PULSES

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Broad spectrum ultrashort-pulse lasers with pulse durations below 15 fs provide peak powers equal or higher than standard 2p laser systems but with lower average power and the ability to simultaneously excite multiple fluorophores [1]. Such lasers emit with over 200 nm spectral bandwidth @-10 dB centred around 800 nm supporting a pulse duration of less than 10 fs. Unfortunately, the dispersion introduced by conventional laser-scanning and focusing optics limits the use of such lasers by stretching the initially short pulse width by several orders of magnitude. Furthermore, the ultra-broadband nature of few-cycle pulses requires precise dispersion compensation up to at least third-order [1], which is beyond the capabilities of standard pulse compressors such as those based on dispersive prisms.

We have solved these problems by carefully measuring the dispersion of a Zeiss LSM780 MP system and using a chirped-mirror-based custom variable pre-compensation unit to negate the dispersion introduced by the laser scanning optics. The pre-compensation unit design accounts for third order terms, in order to have a near flat phase in the sample plane of the microscope (shortest possible pulse duration). The full pre-compensation does not use pulse shapers, allowing 60% transmission of the laser system and reducing the average power needed for imaging. Laser pulse measurements performed in the sample plane of the microscope using the d-scan technique show a near Fourier limited pulse duration of 10 fs.

A unique setup allowed us to seamlessly switch back and forth between a Ti:Sa Laser (Coherent Chameleon) and the pre-compensated Thorlabs Octavius. This allowed us to fairly compare, for the first time, excitation efficiency and photo bleaching rates for both systems. In drosophila larvae, approximately 3 times the average power was required for Ti:Sa long pulses (140 fs) to achieve the same level of GFP fluorescence intensity compared to compressed broadband pulses. Photo-bleaching rates were similar for both lasers at the same sample intensity; however the higher average power needed for the long-pulse Ti:Sa laser rapidly resulted in sample destruction. Compressed broadband pulses were also more efficient for the excitation of fluorescence from confetti mice expressing CFP, GFP, and RFP in the same compartment. At the same average power level in the sample plane, the fluorescence intensity produced by compressed broadband pulses was 2 – 3 times brighter than for long-pulse Ti:Sa pulses. When normalised against GFP intensity, compressed broad-spectrum pulses generated 10% more signal in the blue and red channels compared to uncompressed Ti:Sa laser pulses at 810 nm. These data support the superior imaging capabilities of compressed few-cycle laser pulses in 2p microscopy of biological samples.

[1] A. Klinger et. al, “Intravital autofluorescence 2-photon microscopy of murine intestinal mucosa with ultra-broadband femtosecond laser pulse excitation: image quality, photodamage, and inflammation,” J Biomedical Optics, 20(11), 116001 (2015)