MULTIPHOTON MICROSCOPY WITH PHASE COMPENSATED ULTRASHORT LASER PULSES

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ABSTRACT: The potential to greatly improve clinical biomedical imaging was evident since MPM was invented. With the high-peak power and low average power, nonlinear two-photon excitation has a number of advantages over confocal single-photon excitation [1]. Although shorter pulses can induce much higher two-photon excitation fluorescence (TPEF) and second-harmonic generation (SHG) signal, most research groups and instrument manufactures still use the same pulse duration (≥ 100 femtosecond) used in 1990. Shorter pulse duration can induce higher power density and thus greater nonlinear optical excitation, however, they are more greatly affected by high-order phase distortions primarily from the high numerical aperture microscope objective. It is therefore of great importance to accurately characterize the femtosecond pulse and correct the phase distortions at the sample [2].

In this work, multi-photon intrapulse interference phase scan (MIIPS) method was used to accurately measure and compensate the chromatic dispersion induced by the high numeric aperture objective with sub-12-fs ultrashort pulses. Autocorrelation was performed to confirm the delivery of the short pulse onto the focal spot. The two-photon imaging with transform-limited and group-delay dispersion corrected pulse of the same energy showed that 6-11 fold improvement in TPEF signal, and ~19 fold improvement in SHG signal from various specimens such as fixed and living cells, fixed mouse tissue and fresh rat tendon.

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