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MIIPS® Application Notes

Nonlinear optical imaging with ultrashort laser pulses

Since the introduction of two-photon microscopy by Webb and Denk it has been known that the two-photon excitation fluorescence (TPEF) and second harmonic generation (SHG) signal intensity should be linearly proportional to the inverse of the pulse duration. To utilize ultrashort pulses, however, chromatic dispersion must be compensated. We use multiphoton intrapulse interference phase scan (MIIPS®) method [1] to measure and then cancel phase distortions, obtaining transform limited (TL) pulses at the focal plane.

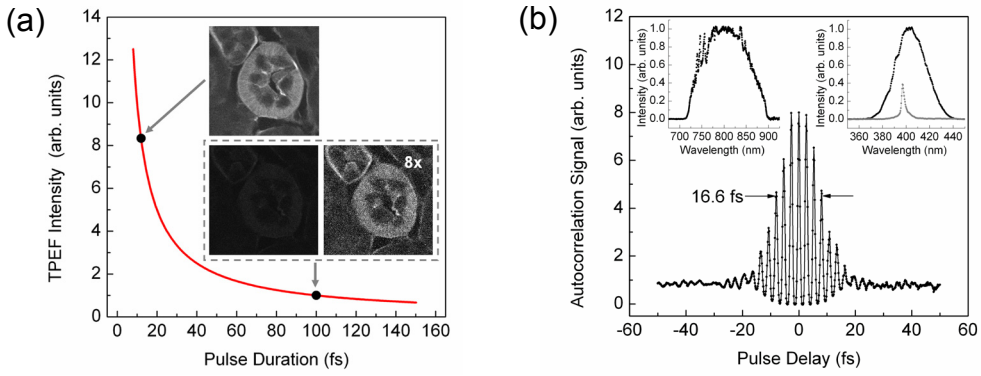

Fig. 1. (a) Dependence of TPEF intensity on laser pulse duration. TPEF imaging of a mouse kidney (Molecular Probes, F-24630), with 12 fs and 100 fs laser pulses. The average laser power on the sample and other acquisition parameters are the same. (b) Interferometric autocorrelation of the MIIPS-corrected pulse at the focus of the objective. Phase-amplitude shaping is used to split the laser pulse into two attenuated replicas with adjustable time delay. The integrated SHG signal from a 100 μm KDP crystal at the objective focus is recorded as a function of the pulse timing, controlled by the pulse shaper. The figure is adapted from ref. [2].

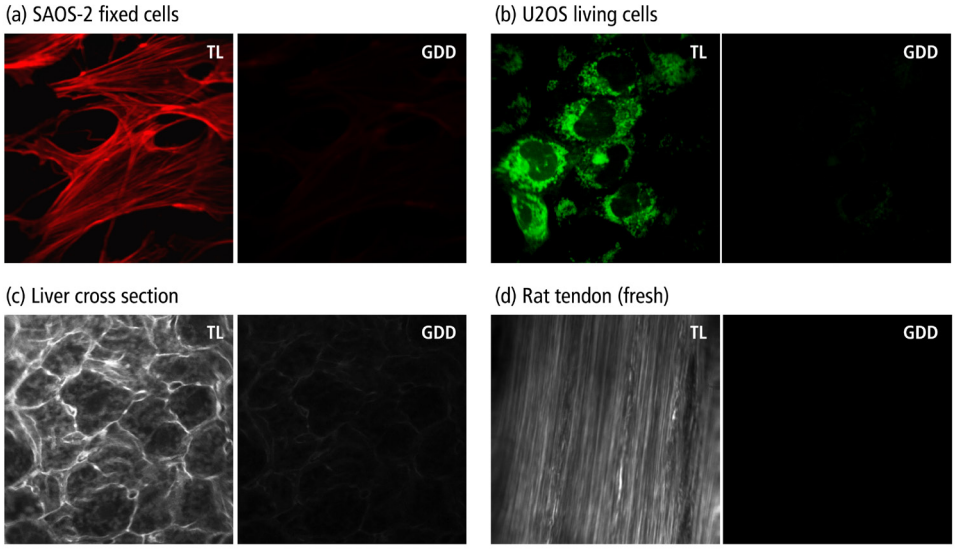

Fig. 2. TPEF/SHG imaging with TL pulses and laser pulses compensated for group delayed dispersion (GDD) on: (a) SAOS-2 fixed cells stained with phalloidin 568. TPEF signal obtained with TL pulses had an 11-fold greater intensity compared to the signal acquired when GDD-only compensation was used. (b) U2OS living cell stained with MitoTracker 488. The measured gain in TPEF was ~6. (c) Mouse liver tissue cross-section stained with MitoTracker 488 and phalloidin 568. The gain factor was ~7. (d) SHG image of a fresh rat tendon with the observed gain of ~19. The images were taken using a Zeiss LD C-APOCHROMAT 40x/1.1 NA objective. Image size is 150 μm. TL pulse duration for all images is 12-13 fs. The figure is adapted from ref. [2]

References

[1] Y. Coello *et al.*, J. Opt. Soc. Am. B **25**, A140-A150 (2008).
[2] P. Xi *et al.*, J. Biomed. Optics **14**, 014002 (2009).