

MIIPS[®] Application Notes

High-order Dispersion Compensation for TPEF and SHG Imaging

High-order dispersion of ultrashort laser pulses (with ~100 nm bandwidth) accounts for significant reduction of two-photon excitation fluorescence (TPEF) and second harmonic generation (SHG) signal produced at the focal plane of a laser-scanning two-photon microscope. The second- and third-order corrections recover 20-40% of the signal intensity expected for a transform-limited (TL) laser pulse, while the rest depends on the proper compensation of higher-order terms. It can be accomplished through the use of a pulse shaper by measuring and correcting spectral phase distortions via multiphoton intrapulse interference phase scan (MIIPS[®]) method; see Figs. 1 and 2.

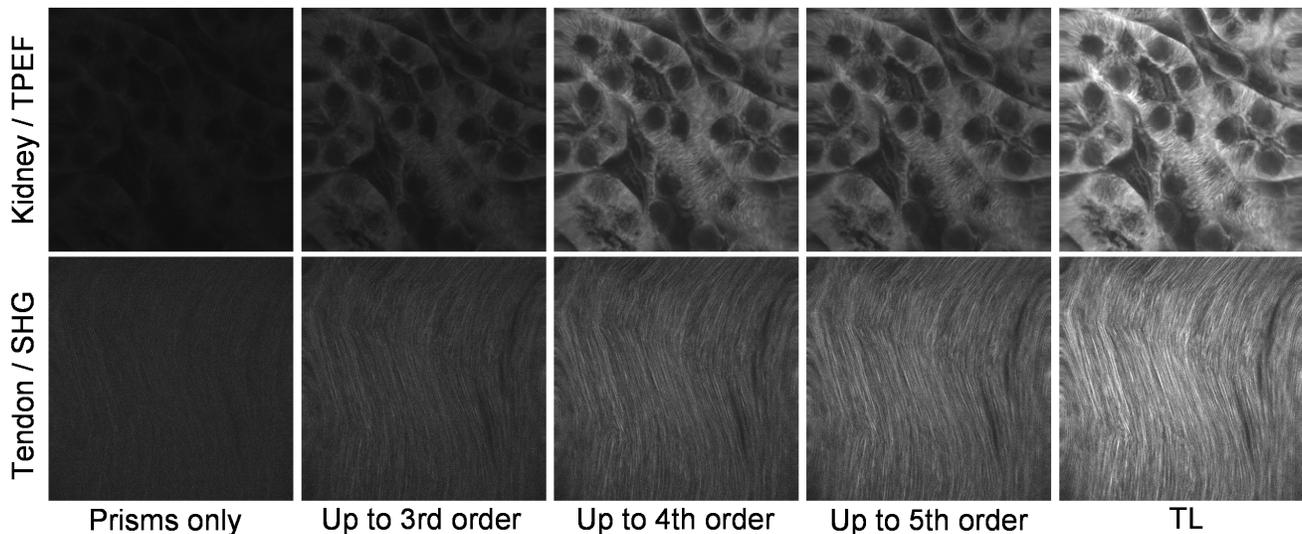


Fig. 1. SHG and TPEF imaging in the presence of high-order phase distortions. TPEF images of a fluorescently stained mouse kidney slide and SHG images of frozen rat tendon obtained after: compensation for second-order dispersion via a prism-pair compressor (First column); additional compensation for the third-order dispersion by a 4f pulse shaper. The residual phase distortion, found from a MIIPS[®] scan, is fitted with a third-order polynomial (Second column); compensation for up to the fourth-order dispersion (Third column); compensation for up to the fifth-order dispersion (Fourth column); Full phase compensation using MIIPS[®] (Fifth column). The 512 × 512-pixel images are acquired for the same average laser power of ~1 mW and averaged over 30 scans. The image size is 150 μ m × 150 μ m. (Image Courtesy of Dantus Research Group).

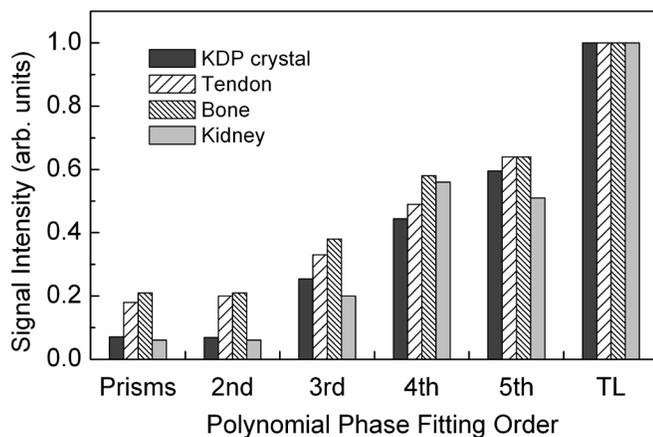


Fig. 2. Compensation of high-order phase distortions. Polynomials of increasing power, from two to five, are used to fit the measured correction phase. SHG or TPEF signal from various samples is recorded for every found fitting function, programmed onto the spatial light modulator. SHG imaging of frozen rat tendon and TPEF imaging of stained mouse kidney are done under conditions described in Fig. 1. Mouse footpad bone data is acquired with the laser power of 10 mW at the objective focus, averaging over 20 scans. Two-photon excitation in the bone sample produces both SHG and autofluorescence. (Courtesy of Dantus Research Group)

Reference

Y. Andegeko, D. Pestov, V.V. Lozovoy, M. Dantus, "Ultrafast multiphoton microscopy with high-order spectral phase distortion compensation," Invited paper in Proc. SPIE 7183, 71830W (2009).