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Tissue Imaging and Multidimensional Spectroscopy Using Shaped Femtosecond Laser Pulses

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We use rapidly updatable, femtosecond pulse shaping and multidimensional spectroscopy to make new targets accessible by nonlinear optical imaging. For example, we observe two-photon absorption (TPA), sum frequency absorption (SFA) and self phase modulation (SPM). Detection of TPA and related effects, such as the local quantum yield (fluorescence/absorption) permits direct observation of important endogenous molecular markers which are invisible in multiphoton fluorescence microscopy; it also permits excitation in the long-wavelength water windows which have significantly reduced scattering, but little endogenous two-photon fluorescence. The fundamental problem is that at the powers one might reasonably apply to tissue (e.g. 5 mW from a modelocked laser) typically 10^{-6} of the light is removed by TPA, with the rest lost to scattering and linear absorption; and SPM does not broaden the spectrum in the dramatic way associated with (for example) continuum generation. A variety of solutions to these problems using femtosecond pulse shaping will be presented. The simplest solution, which uses amplitude modulation of a fs pulse train, has led to high quality microscopic images of the melanin distribution in melanotic lesions, and has led to discrimination between the different types of melanin in melanosomes. Shaping individual pulses instead of the envelope permits high sensitivity detection of both SPM and TPA via spectral hole refilling combined with heterodyne detection. We manufacture laser pulses with a narrow (ca. 3 nm) spectral hole, which can only be refilled by nonlinear processes; TPA causes refilling 180 degrees out of phase with the wings of the pulse, SPM is 90 degrees out of phase. By inserting a phase-coherent pedestal in the hole, then repeating the experiment with a different phase on a timescale rapid compared to any physiological processes, we can extract the phase of the refilling, hence the relative contributions of SPM and TPA. This method can extract excellent signatures from hemoglobin as well as melanin. We have also used it to image neurons firing in tissue, and to characterize off-diagonal peaks of contrast agents in two-dimensional spectra.