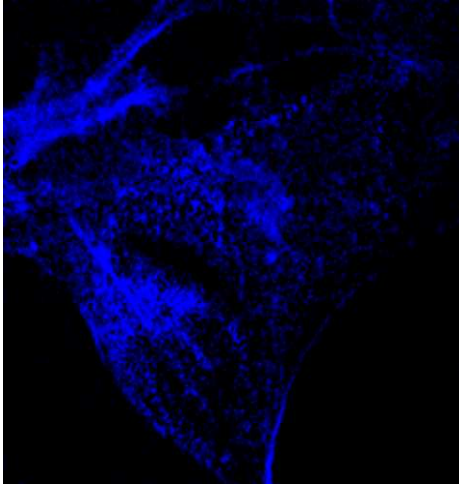


MIIPS™ application notes

What can you do with MIIPS™?

Selective Two-Photon Excitation for Biomedical Imaging

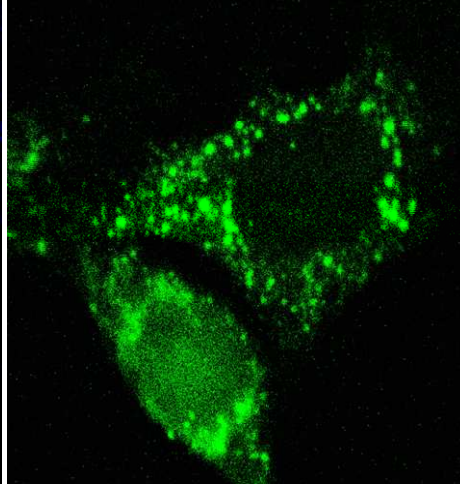
Blue Phase



Actin

Phalloidin - 350

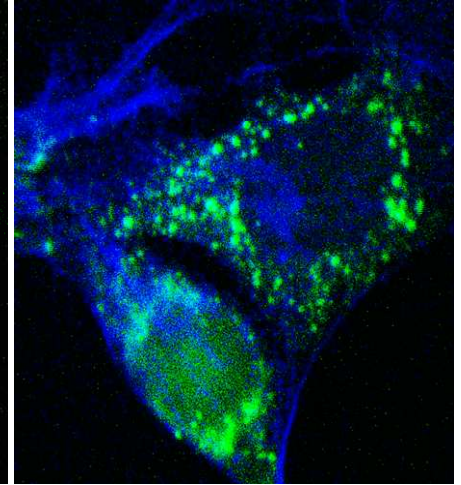
Red Phase



Mitochondria

MitoTracker-488

Merged



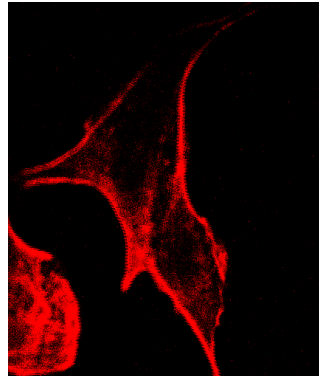
Selective two-photon excitation using MIIPS.
Figures courtesy of the Dantus Research Group.

Two-photon microscopy has several advantages over conventional imaging techniques including improved resolution, higher penetration through thick samples, decreased background scattering and less potential for photo-induced damage. Ultrashort pulses (~12fs pulses implemented here), when used for two-photon imaging, can bring about two significant advantages. First, two-photon excitation depends on the peak intensity squared. Second, a broader range of frequencies can be accessed. The application of these advantages depends on being able to correct for the phase distortions introduced by the high numerical aperture microscope objective, and for this we use multiphoton intrapulse interference phase scan (MIIPS™).

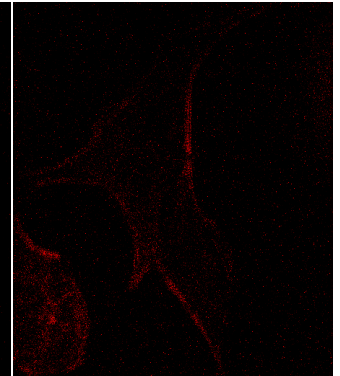
Once the transform limited (TL) pulse is achieved using MIIPS™, other pulse shapes can be introduced by the pulse shaper. When introducing different phase functions, the fundamental spectrum does not change and therefore any wavelength-dependent effects in the beam optics are eliminated. This is important to ensure accurate registration between the images obtained for the different shaped pulses. Pulse shaping can be used to enhance two-photon excitation at shorter or at longer wavelengths while suppressing two-photon excitation at the opposite end of the spectrum. Due to this selective excitation, the different pulse shapes can be used to create images that distinguish between sub-cellular structures with different two-photon excitation spectra. These images can then be compiled to form an image that shows much more detail and thus, increases the ability to distinguish among different sub-cellular structures.

It is evident, from our preliminary experimental data, that pulse shaping of broad bandwidth pulses to gain contrast has a significant potential for biomedical imaging. The use of the MIIPS technique to create perfect TL pulses followed by pulse shaping to selectively excite different regions in a sample, results in very detailed images. The method used here can be extended to take advantage of differences in the nonlinear optical susceptibility of the individual components. Similarly, it can be used in coherent anti-stokes Raman scattering.

GVD, TOD and higher compensated pulses using MIIPS



GVD-only compensated pulses



Benefits of using MIIPS for phase distortion elimination (image on left) vs. simple GVD compression are evident in this image of Actin: Phalloidin – 568 (Courtesy of Dantus Research Group).

References:

- B.W. Xu, J.M. Gunn, J.M. Dela Cruz, V.V. Lozovoy, M. Dantus, *J Opt Soc Am B Opt phys*, 23, 750-9 (2006).
L.T. Schelhas, J.C. Shane, M. Dantus, *Nanomedicine: Nanotechnology, Biology, and Medicine* 2, 177– 181 (2006).

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