Selective Two-Photon Imaging of a Biological Sample

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Abstract. The use of phase shaped 13 fs pulses to selectively enhance fluorophore excitation in biological samples is shown. Images of a mouse kidney sample show high contrast without the use of filters or tuning the laser.

1. Introduction

More than a decade ago, Denk and Webb [1] introduced two-photon microscopy. This method has since become very attractive for imaging biological samples due to its ability to provide high resolution images. Two-photon microscopy uses femtosecond laser pulses to increase the two-photon excitation efficiency. Although the use of shorter pulses would have advantages in efficiency and reproducibility, problems such as spectral dispersion have limited the use of pulses with duration less than 100 fs. Here, we demonstrate how compensating phase distortions introduced by high numerical aperture microscope objectives on 13 fs pulses allows us to take high-contrast, detailed, and reproducible images of biological samples. Through pulse shaping of our compensated pulses, we selectively excite different fluorophores within a mouse kidney sample, and show a further improvement in image detail.

Because the efficiency of two-photon excitation increases as pulse duration decreases, two-photon microscopy using the sub-10 fs pulses currently available would provide unprecedented image quality. However, shorter pulses are increasingly subject to spectral dispersion caused by phase distortions from lenses, mirrors, and especially from high NA microscope objectives, increasing pulse length and decreasing efficiency. Adjusting for the loss in signal by increasing pulse intensity causes an unacceptable amount of sample damage; therefore, these distortions must be eliminated so that a transform limited (TL) pulse is achieved. To this end, our group uses multiphoton intrapulse interference phase scan (MIIPS) [2]. The use of TL pulses results in images with a factor of 7 higher than those obtained with pulses corrected for linear chirp but not for high-order phase compensation, as illustrated in Fig. 1 [3]. An image taken with pulses without any phase compensation would be orders of magnitude less intense.

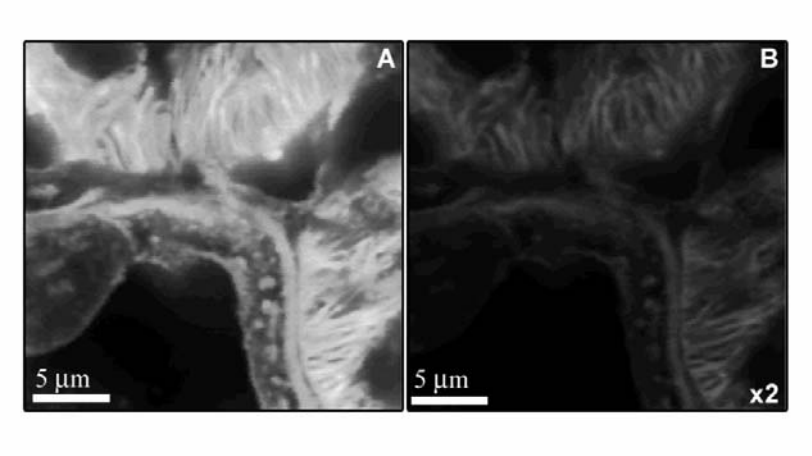

Fig. 1. a) Fluorescently labeled mouse kidney sample (FluoCells Prepared Slide #3), imaged with TL pulses. b) The same sample, imaged without compensation for high-order phase distortion. Intensity has been increased by a factor of 2 to show detail; actual signal intensity is decreased by a factor of about 7 as compared to image a). Both panels show a portion of the convoluted tubules, with actin-containing microvilli structures on the top and right sides of the panels.

The imaging experiments presented here were carried out with a titanium sapphire oscillator capable of producing sub-10 fs pulses (100 nm FWHM) centered around 800 nm, with a repetition rate of 97 MHz and average power of 250 mW. After passing through the MIIPS setup for compensation, the beam was brought into the rear port of a Nikon TE2000-U inverted microscope and focused onto the sample with a 60x/1.45 NA oil-immersion objective. The sample was raster scanned, and the data collected with a CCD camera.

In addition to gains in image intensity, the use of compensated ultrashort pulses for imaging allows pulse shaping, rather than tuning the laser's center frequency, to be used for selective two-photon excitation. Pulse shaping can tune the two-photon excitation wavelength to any region within the pulse's second harmonic spectrum without changing the laser's center frequency, producing selectivity while avoiding problems caused by the wavelength-dependent properties of the experimental optical components. This technique has been shown effective for imaging fluorescent beads and polymer-doped dyes [4], probing microscopic environments through biological tissue [5] and imaging biological specimens [6]. The large bandwidth of sub-10 fs pulses allows access to a wider range of excitation wavelengths than would be possible with longer pulses. The linear combination images in Figure 2 show selective imaging of a biological sample, taken under excitation with a 13 fs pulse shaped to optimize short-wavelength (a) and long-wavelength (b) excitation. A profile of the intensity of fluorescence emission along a vertical line drawn through the image (c) shows selectivity between the two excitation wavelengths.

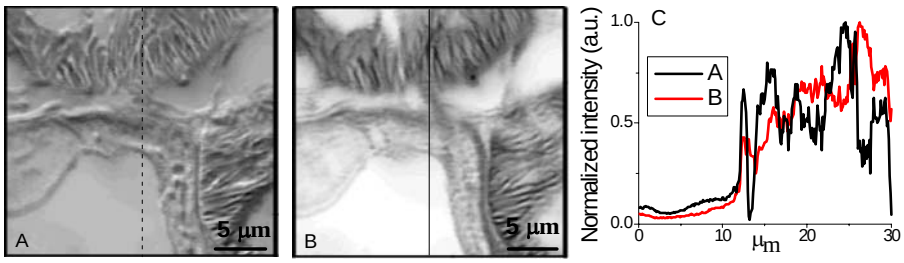

Figure 2. a) Fluorescently labeled mouse kidney sample (FluoCells Prepared Slide #3), imaged with pulses shaped to enhance two-photon excitation of shorter wavelengths. b) The same fluorescently labeled mouse kidney sample imaged with pulses shaped to enhance two-photon excitation of longer wavelengths. c) Plot of the intensity of one column from image A (dots) plotted against the intensity of the same column in image B (solid line).

Each image shown in Figs. 1 and 2 above was collected twice, in alternation, to check for reproducibility and photobleaching effects. Compensating phase distortions is particularly important for ensuring the reproducibility of the selectivity we achieved; because distortions vary from system to system and even from day to day, the phase shapes used for selectivity can only be meaningful if phase shaping is applied to compensated pulses. This is the reason why two-photon microscopy with uncompensated femtosecond pulses can have poor reproducibility.

In addition to the capabilities demonstrated here, pulse shaping of ultrashort pulses can be used to suppress harmful three-photon absorption [3] or to excite at multiple wavelengths simultaneously. Now that automated pulse shapers capable of measuring and correcting for phase distortions, as well as delivering phases for selective excitation, have become commercially available, these methods will open new contrast capabilities in the field of nonlinear optical imaging.

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References

1 W. Denk, J.H. Strickler, W.W. Webb, in *Science*, Vol. 248, 73-76, 1990.

2 B. Xu, J.M. Gunn, J.M. Dela Cruz, V.V. Lozovoy, and M. Dantus, in *J. Opt. Soc. Am. B*, in press, 2006.

3 J.M. Dela Cruz, V.V. Lozovoy, and M. Dantus, in *J. Photochem. Photobio.*, submitted, 2006.

4 I. Pastirk, J.M. Dela Cruz, K.A. Walowicz, V.V. Lozovoy, and M. Dantus, in *Optics Express*, Vol. 11, 1695, 2003.

5 J.M. Dela Cruz, I. Pastirk, M. Comstock, V.V. Lozovoy, and M. Dantus, in Proc. *Nat. Acad. Sci. USA*, Vol. 101, 16996-17001, 2004.

6 J.P. Ogilvie, D. Débarre, X. Solinas, J-L Martin, E. Beaurepaire, and M. Joffre, in *Optics Express*, Vol. 14, 759-766, 2006.