

# DYNAMIC SELECTIVE MICROSCOPY OF FLUORESCENT SPECIES *IN VIVO* WITH RAPIDLY PHASE-SHAPED BROADBAND PULSES

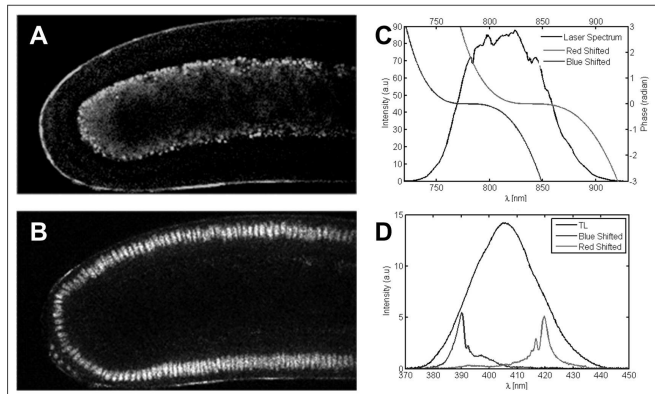
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**KEY WORDS:** coherent control, two-photon excited fluorescence, two-dimensional spatial light modulator, embryo imaging

We report dynamic selective imaging of two different fluorescent species in a biological specimen using spectral phase shaping of a single broadband laser beam, with short pixel dwell time (10 $\mu$ s) and high resolution (0.8 NA). The underlying principle of the experiment is coherent control of two-photon absorption: when the spectral phase of a broadband laser beam is anti-symmetric with respect to a frequency component  $f$ , constructive interference occurs in the second-harmonic spectrum at frequency  $2f$ , while destructive interference occurs at other frequencies. This allows selective two-photon excitation of a particular fluorophore.



**Figure 1** *In vivo* imaging of fluorescently labeled *Drosophila* embryos using (A) blue shifted pulse shaping for preferential endogenous fluorescence excitation and (B) red-shifted pulse-shaping for preferential GFP excitation. Laser spectrum (C) and applied spectral phases. Corresponding second harmonic spectra (D) measured at the focus.

Phase shaping was performed with a two-dimensional spatial light modulator (SLM) combined with a scanning mirror allowing switching between two different programmable pulse shapes at a high (kHz) rate. The pulse shaper was used for both characterizing and controlling the spectral phase of sub-15fs pulses at the focus of a high NA microscope objective. Rapid switching between two phase shapes (shown in Figure 1) was then used to quasi-simultaneously excite alternatively GFP and endogenous fluorescence at different stages of a developing *Drosophila* embryo. We obtained a two-order of magnitude faster imaging capability compared to previously reported acousto-optic filter-based phase shaping experiments ([1]). The demonstration of *in vivo* imaging with rapidly shaped pulses, short pixel acquisition times, and high NA opens up the possibility of multiplexed microscopy of biological samples using a single broadband laser source.

[1] Ogilvie et al, Opt. Express 14, 759 (2006).