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Control of selective two-photon fluorescence suppression by one-color pulse-pair excitation

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Dedicated to Professor Bishnu P Pal for his enormous contributions to the advancement of research and education in science and technology through his unique vision and outstanding dedication

For studying the selective excitation of a particular fluorophore in fluorescence microscopy, the effect of the laser pulse chosen is immense. To study the ramifications of this selection, we present a thorough study of one-color stimulated emission depletion of the two-photon fluorescence of selective fluorophores by changing the wavelength and delay between two identical laser pulses.

Keywords: Two-photon fluorescence, Stimulated emission, Pulse-pair excitation, One-color scheme, Multiphoton microscopy

1 Introduction

Due to its fleeting existence, femtosecond laser pulse has a correspondingly gigantic peak power, making it a good choice for use in multiphoton processes like two-photon fluorescence (TPF). TPF can itself be treated as a combination of two-photon absorption (TPA) processes followed by fluorescence emission. Ultrafast laser pulses can simultaneously excite a wide range of fluorophores encompassing a broad spectral range, even with a low two-photon absorption cross-section. Thus, the selective enhancement or suppression of fluorescence is of crucial importance as an experimental parameter in TPF microscopy [1]. Quantum control processes [2] using ultrafast laser pulse shaping [3] have been used in fluorescence microscopy [4-7] to differentiate between fluorochromes that have comparable properties [8,9]. Fluorescence process modulation is made possible by controlling the excited state population through the manipulation of net quantum interference by the accurate handling of pulse delay and phases. Such modulation is often used in pulse-pair [10,11] and pulse-train [12] excitation techniques. We have also shown such control using fluorescence microscopy in the solution phase. Coherent excitations aside, the excited state population can also be controlled by changing the interpulse delay between the pulses in the pulse-pair [13,14] and pulse-train [15] excitation incoherently. Here, the first pulse launches the wave packet from the ground state to the excited state, and the time-delayed pulse induces stimulated depletion through single-photon and two-photon absorption. We will describe mechanistically one-color stimulated emission suppression using the pulse-pair excitation process.

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2 Experimental Techniques

A mode-locked Ti:Sapphire oscillator (MIRA 900-F pumped by Verdi5, Coherent Inc., Santa Clara, California) tunable from 720 nm to 980 nm at a 76MHz repetition rate has been used as the laser system. The central wavelength of the laser was tuned at 730 nm and 750 nm with ~ 300fs pulse width. No TPF was observed from the samples below 730 nm central wavelength as the laser power was too low to generate TPF. The laser light was split and recombined using a Mach-Zehnder Interferometer to generate the pulse-pair before sending it to our multiphoton ready confocal microscope system (FV300 scan-head coupled with IX71 inverted microscope, Olympus Inc., Japan). The precise delay between the two pulses was controlled by introducing a retroreflecting mirror system mounted on a motorized linear stage (UE1724SR driven by ESP300, Newport Inc., Irvine, California) interfaced with a personal computer through a GPIB card (National Instruments, Austin, Texas) in one arm of the interferometer. In contrast, the other arm had the retroflector fixed on it. Scanning of the diffraction-limited focal spot across the focal plane by the raster scanning method using a pair of galvo-scanning mirrors has been done for image collection. Laser light was focused onto microscopic slides containing multi-labeled bovine pulmonary artery endothelial (BPAE) cells (F36924 and F14781, Molecular Probes) for imaging using an oil-immersion objective (UPlanApon 40x 1.4NA, Olympus). The image acquisition and analysis were performed using the FLUOVIEW (Olympus, Japan) software. The emission spectra of MitoTracker Red dye were taken using a fluorescence spectrometer (LS 55, PerkinElmer) from a 10^{-5} M solution of MitoTracker Red CMXRos (M-7512, Molecular Probes) dissolved in water. MitoTracker Red's fluorescence spectrum was matched with its reported value of 560nm to 700nm to obtain its quantitative spectra extending up to 750nm. The emission spectra of DAPI and Texas Red were obtained from their reported values. The laser light spectrum was collected through a multi-mode optical fiber connected to a spectrometer (HR2000, Ocean Optics). The field auto-correlation trace was collected at the sample stage by replacing the microscopic slide with an amplified Si photodiode (PDA100A-EC, Thorlabs, Newton, New Jersey) connected to a digital oscilloscope (waveRunner 6100A, LeCroy), which is connected to a personal computer.

3 Results and Discussions

Excitation with ultrafast laser pulses of high spectral bandwidth [16] involves more than one vibrational sub-state. The first pulse launches the population from the ground vibrational state of the ground electronic state to many folded vibrational energy states of an excited electronic state. Eventually, the population reaches the ground vibrational state of that excited electronic state. A time-delayed pulse at the red edge of the fluorescence spectra is used [17] for stimulating fluorescence depletion. In general, in two-photon fluorescence microscopy, the central wavelength of the laser is nearly two times the excitation wavelength maxima. So, if the fluorescence has a long tail, even a small part of that same laser light can be used for the single-photon stimulated emission process. Depending upon the excitation and emission spectra of chromophores, selective one-color stimulated emission is thus easily achieved in two-photon fluorescence microscopy.

Figure 1(a) shows the excitation and emission spectra of the DAPI, MitoTracker Red, and Texas Red [18] dyes, along with the spectra of laser pulses centered at ~730 and ~750 nm. Fluorescence of the DAPI dye dies long before the laser wavelength, whereas the fluorescence of MitoTracker Red and Texas Red extend slightly up to 750nm. The 725 nm to 750 nm region of Fig 1(a) has been zoomed in on and is shown in Fig 1(b). For stimulated emission, the spectrum of the laser light and fluorescence must overlap, and in order to achieve this within our experimental setup, the laser wavelength center was tuned to ~ 750 nm and ~ 730 nm. As the laser FWHM at these two wavelengths are ~ 3 nm, this indicates that the transformed limited pulse-width of the laser is ~ 300 fs, and this is also confirmed from their auto-correlation traces (Fig 2) measured at the sample stage. It is also evident from Fig 2 that the two-pulse interference extends to the ~900 fs region.

In order to eliminate the effect of field interactions on fluorescence dynamics, the minimum delay between the two pulses was kept at 900 fs. As the excited state lifetimes for the dyes are ≤ 1 ps [19], we varied the delay between the two pulses from 900 fs to 1 ps with a step size of 5 fs. In Fig 3, the upper two images depict BPAE cells with nuclei stained by DAPI and mitochondria stained by MitoTracker Red at 730 nm. In the same figure, the lower images depict BPAE cells with nuclei stained by DAPI and the F-actin, anti-bovine α-tubulin mouse monoclonal 236-10501 stained by Texas Red excited at 750 nm.

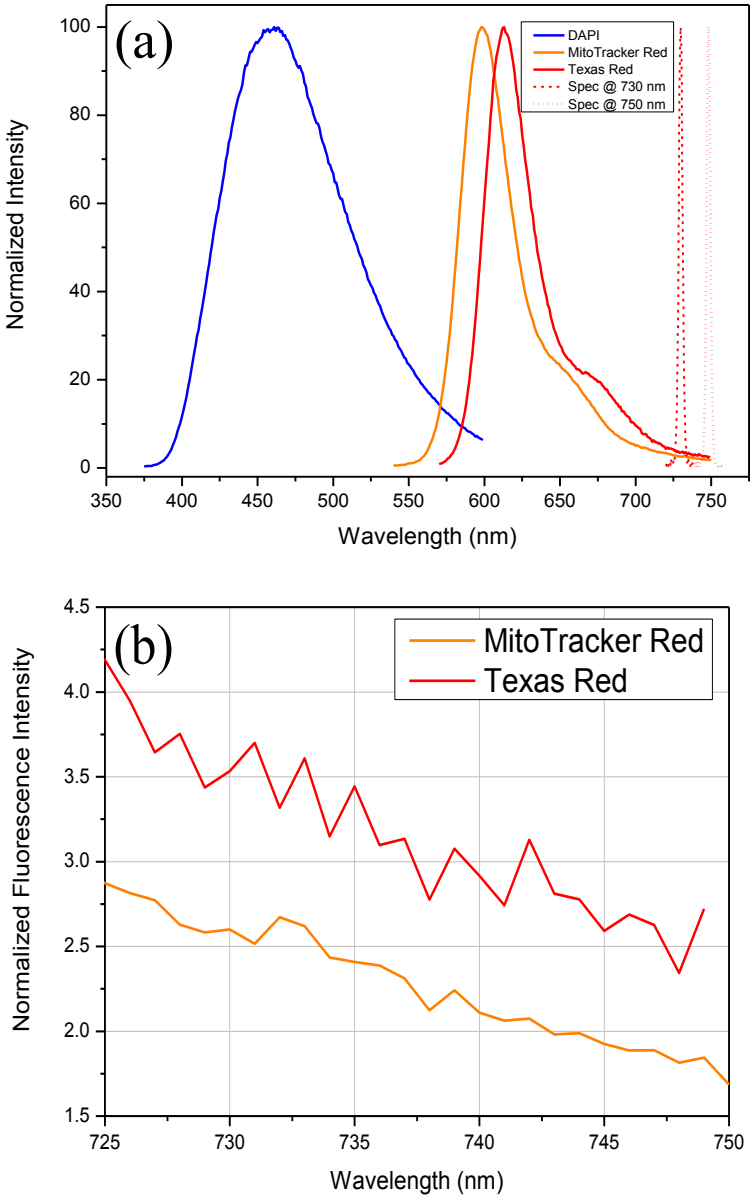

Fig 1. (a) Fluorescence spectra of DAPI, MitoTracker Red, and Texas Red dyes. (b) Zoomed portion of the spectra of MitoTracker Red and Texas Red around the laser excitation wavelengths.

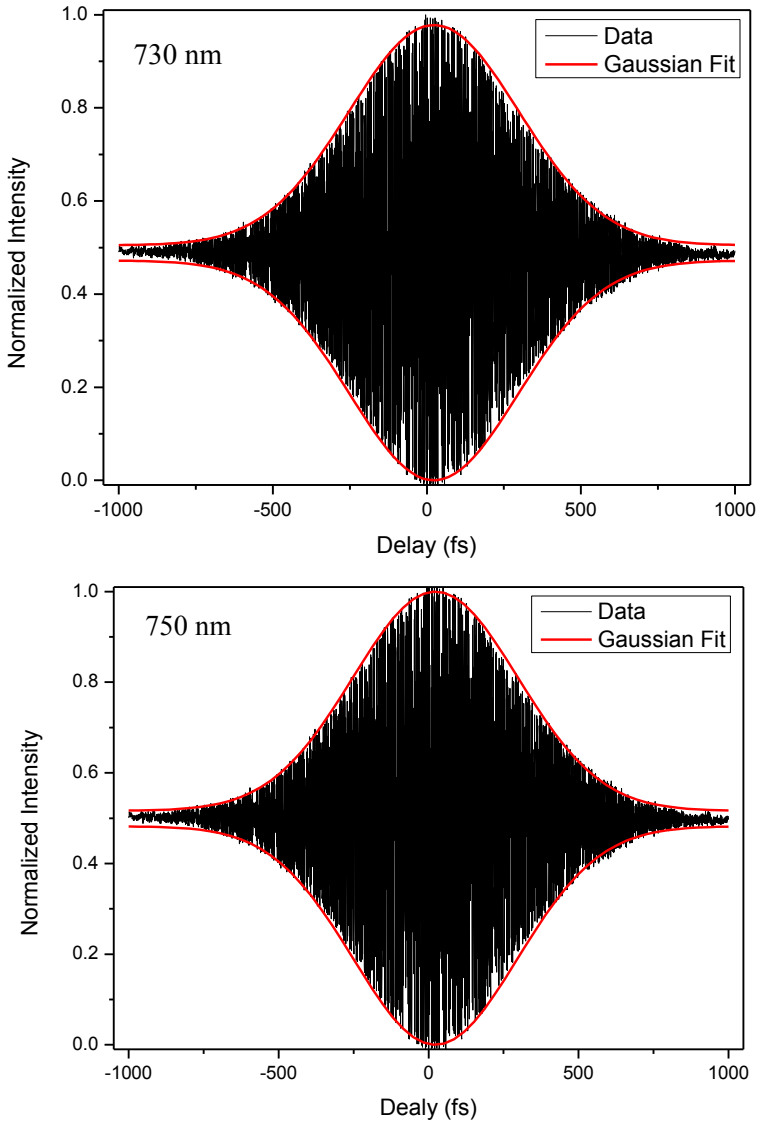

Fig 2. Field autocorrelation trace at 730 and 750 nm laser pulses.

At 750 nm wavelength and 1 ps delay, Texas Red fluorescence falls to ~ 86% of its value at 900 fs delay. At one ps delay, the fluorescence from MitoTracker Red decreases to ~ 79% of its value at 900fs delay at 730 nm wavelength (Fig 4a). Previously, we have shown that for Texas Red, at 730 nm, the decrease of fluorescence intensity on moving from 900 fs to 1 ps delay is ~ 65% [14]. But in all cases, the fluorescence of DAPI shows almost similar behavior with a drop of ~ 91%. It is to be noted that DAPI has a broad excitation profile at ~ 360 nm, whereas both MitoTracker Red and Texas Red have sharp excitation profiles at ~580 nm and ~600 nm, respectively. These bands can be attributed to the $S_1 \leftarrow S_0$ transition. MitoTracker Red and Texas Red chromophores also have another weak excitation band at ~370 nm, corresponding to $S_{n>1} \leftarrow S_0$. Using 730 nm and 750 nm lasers, we get the $S_1 \leftarrow S_0$ transition for DAPI, while for MitoTracker Red and Texas Red, the $S_{n>1} \leftarrow S_0$ transition occurs. As the DAPI fluorescence dies out long before the exciting laser

light wavelength, both the pulses take part only in TPA processes. But, for the MitoTracker Red and Texas Red chromophores, the first pulse induces only TPA while the second pulse induces both the TPA as well as the single-photon stimulated emission process.

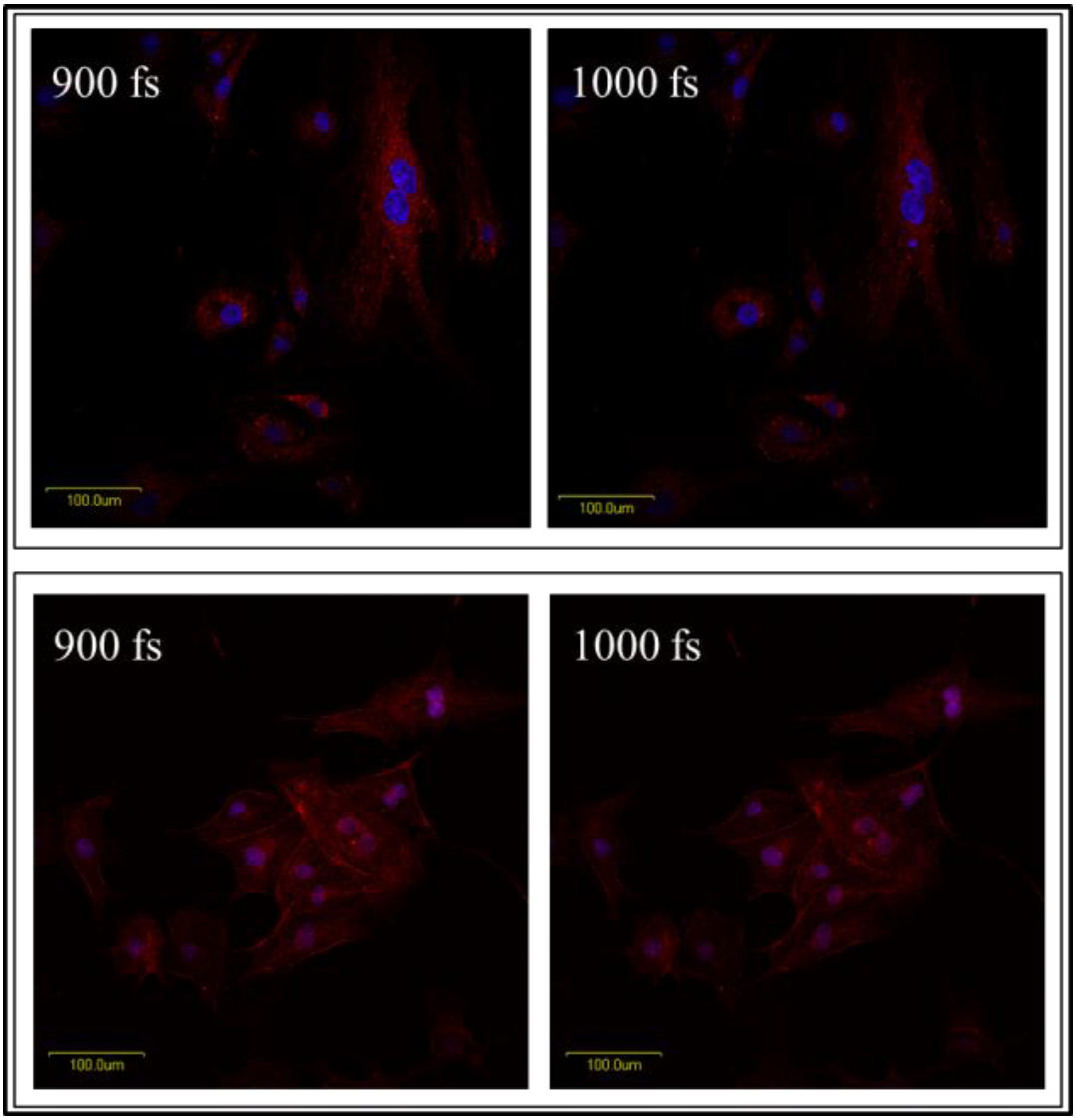

Fig 3. The two images in the top are of BPAE cells at 730 nm excitation [14]. Here, the nuclei are stained by DAPI, and the mitochondria are stained by MitoTracker Red. The two images at the bottom are of BPAE cells at 750 nm excitation. Here, the nuclei are stained by DAPI while the F-actin, and α-tubulin Mouse anti-Bovine monolonal: 236-10501 (Invitrogen™) are stained by Texas Red.

From Fig 1, the fluorescence intensity of Texas Red is 2.34401 at 748 nm and 3.53266 at 730 nm, while for MitoTracker Red, the fluorescence intensity is 2.6002 at 730 nm. It is clear that with an increase in fluorescence intensity, i.e., the overlap region between the laser spectrum and the fluorescence spectrum, selective two-photon fluorescence suppression becomes higher (Fig 4b). This result, in turn, confirms

our proposed mechanism for the two-photon fluorescence suppression by one-color stimulated emission [14,15]. The pulse-train excitation is a better choice compared to the pulse-pair excitation [15] for slower timescales (20 ps – 50 ps) due to the gradual decrease in the pulse energy of successive pulses in the pulse-train excitation scheme. However, it cannot be used beyond a certain delay time due to physical constraints. This limitation can be overcome using a pulse-pair excitation scheme where the excited state population of selective fluorophores can be greatly modulated within the fast excited state vibrational relaxation time (≤ 1 ps).

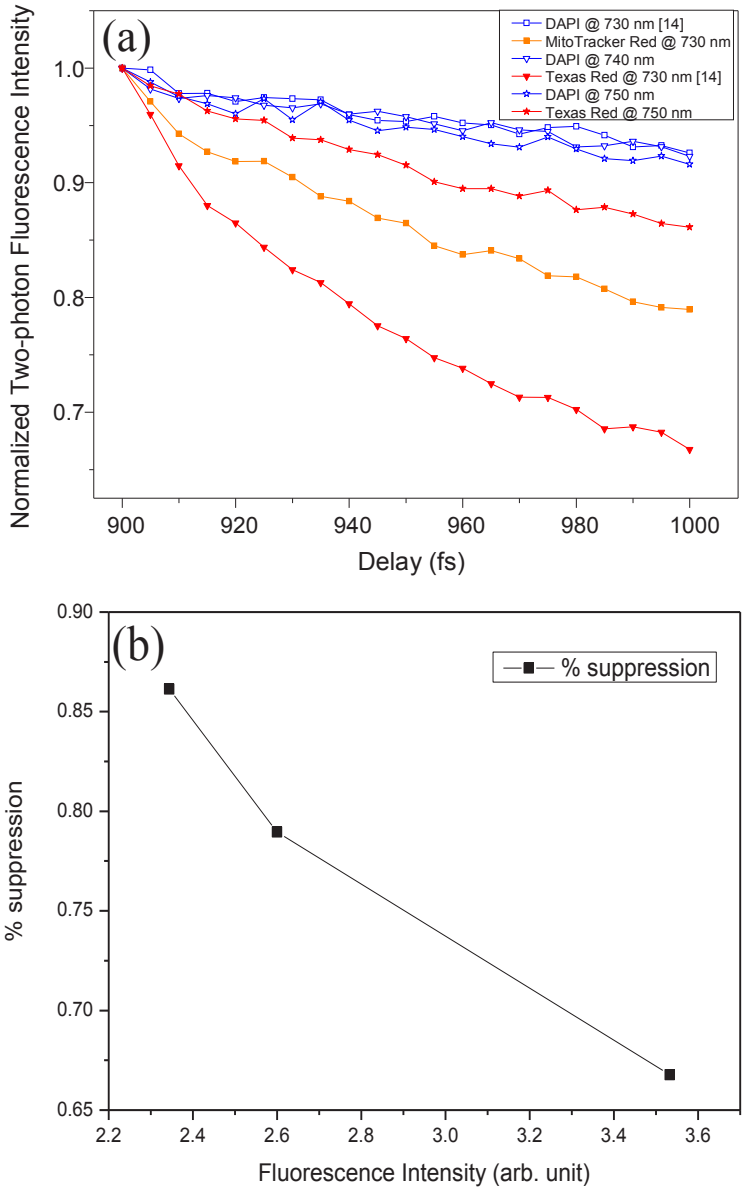

Fig 4. (a) Selective two-photon fluorescence suppression of MitoTracker Red and Texas Red compared to DAPI under pulse-pair excitation. (b) Variation of Variation of TPF suppression with increasing fluorescence intensity.

4 Conclusions

We have shown that different chromophores can be excited simultaneously due to broad TPA absorption spectra using ultrafast laser pulses. Furthermore, we have shown that tuning the laser spectral characteristics can yield better control on the selective suppression of the chromophore fluorescence with almost similar fluorescence properties.

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References

1. Wessendorf M W, Brelje T C, Multicolor Fluorescence Microscopy Using the Laser-Scanning Confocal Microscope, *Neuroprotocols*, 2(1993)121–140.
2. Brixner T, Damrauer N H, Niklaus P, Gerber G, Photoselective adaptive femtosecond quantum control in the liquid phase, *Nature*, 414(2001)57–60.
3. Goswami D, Optical pulse shaping approaches to coherent control, *Phys Rep*, 374(2003)385–481.
4. Pastirk I, Dela Cruz J M, Walowicz K A, Lozovoy V V, Dantus M, Selective two-photon microscopy with shaped femtosecond pulses, *Opt Express*, 11(2003)1695–1701.
5. Dela Cruz J M, Pastirk I, Comstock M, Lozovoy V V, Dantus M, Use of coherent control methods through scattering biological tissue to achieve functional imaging, *Proc Natl Acad Sci* (*USA*), 101(2004)16996–17001.
6. Ogilvie J P, Débarre D, Solinas X, Martin J-L, Beaurepaire E, Joffre M, Use of coherent control for selective two-photon fluorescence microscopy in live organisms, *Opt Express*, 14(2006)759–766.
7. Isobe K, Suda A, Tanaka M, Kannari F, Kawano H, Mizuno H, Miyawaki A, Midorikawa K, Multifarious control of two-photon excitation of multiple fluorophores achieved by phase modulation of ultra-broadband laser pulses, *Opt Express*, 17(2009)13737–13746.
8. Roth M, Guyon L, Roslund J, Boutou V, Courvoisier F, Wolf J-P, Rabitz H, Quantum Control of Tightly Competitive Product Channels, *Phys Rev Lett*, 102(2009)253001; doi.org/10.1103/PhysRevLett.102.253001.
9. Petersen J, Mitrić R, Bonačić-Koutecký V, Wolf J-P, Roslund J, Rabitz H, How Shaped Light Discriminates Nearly Identical Biochromophores, *Phys Rev Lett*, 105(2010)073003; doi.org/10.1103/PhysRevLett.105.073003.
10. Scherer N F, Ruggiero A J, Du M, Fleming G R, Time resolved dynamics of isolated molecular systems studied with phase-locked femtosecond pulse pairs, *J Chem Phys*, 93(1990)856; doi.org/10.1063/1.459456.
11. Scherer N F, Carlson R J, Matro A, Du M, Ruggiero A J, Romero-Rochin V, Cina, J A, Fleming G R, Rice S A, Fluorescence-detected wave packet interferometry: Time resolved molecular spectroscopy with sequences of femtosecond phase-locked pulses, *J Chem Phys*, 95(1991)1487; doi.org/10.1063/1.461064.
12. Rebane A, Sigel C, Drobizhev M, Interference between femtosecond pulses observed via time-resolved spontaneous fluorescence, *Chem Phys Lett,* 322(2000)287–292.
13. De A K, Goswami D, Ultrafast pulse-pair control in multiphoton fluorescence laser-scanning microscopy, *J Biomed Opt,* 14(2009)064018; /doi.org/10.1117/1.3268440.
14. De A K, Roy D, Goswami D, Selective two-photon fluorescence suppression by ultrafast pulse-pair excitation: control by selective one-color stimulated emission, *J Biomed Opt,* 6(2011)100505; doi.org/10.1117/1.3645082.
15. De A K, Roy D, Goswami D, Selective suppression of two-photon fluorescence in laser scanning microscopy by ultrafast pulse-train excitation, *J Biomed Opt*, 15(2010)060502; doi.org/10.1117/1.3509383.
16. Dyba M, Klar T A, Jakobs S, Hell S W, Ultrafast dynamics microscopy, *Appl Phys Lett*, 77(2000)597–599.
17. Rankin B R, Moneron G, Wurm C A, Nelson J C, Walter A, Schwarzer D, Schroeder J, Colón-Ramos D A, Hell S W, Nanoscopy in a Living Multicellular Organism Expressing GFP, *Biophys J*, 100(2011)L63–L65.
18. https://www.lifetechnologies.com/in/en/home/brands/molecular-probes.html

19. Min W, Lu S, Chong S, Roy R, Holtom G R, Xie X S, Imaging chromophores with undetectable fluorescence by stimulated emission microscopy, *Nature*, 461(2009)1105–1109.

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