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Coherent Control in Multiphoton Fluorescence Imaging

Arijit Kumar De* and Debabrata Goswami

Department of Chemistry, Indian Institute of Technology Kanpur, UP - 208016, India.

Abstract

In multiphoton fluorescence laser-scanning microscopy ultrafast laser pulses, *i.e.* light pulses having pulse-width 1 picosecond ($1 \text{ ps} = 10^{-12} \text{ s}$), are commonly used to circumvent the low multiphoton absorption cross-sections of common fluorophores. Starting with a discussion on how amplitude modulation of ultrashort pulse-train enhances the two-photon fluorescence providing deep insight into laser-induced photo-thermal damage, the effect of controlling time lag between phase-locked laser pulses on imaging is described. In addition, the prospects of laser pulse-shaping in signal enhancement (by temporal pulse-compression at the sample) and selective excitation of fluorophores (by manipulating the phase and/or amplitude of different frequency components within the pulse) are discussed with promising future applications lying ahead.

Keywords

Coherent control; time-domain control; ultrafast laser pulses; multiphoton imaging; amplitude modulation; pump-probe spectroscopy; ultrafast imaging; pulse shaping

1. INTRODUCTION

Coherent control or quantum control refers to controlling the light-matter interaction using the phase-coherence of laser light [1, 2]. Early attempts in frequency domain control by precise excitation of a single vibrational mode using near-monochromatic continuous wave (CW) lasers were largely doomed by the fact that rapid energy dissipation among other vibrational modes (known as intra-molecular vibrational energy redistribution or IVR) results in loss of coherence. However, owing to the fleeting existence, ultrashort laser pulses can launch coherent vibrational wave-packets since the vibrational time period is much longer than the temporal width of the pulse. Ultrafast laser pulses are generated by constructive interference among different longitudinal optical modes sustained by the laser cavity which is known as 'mode-locking' [3-5]. This results in the generation of train of phase-locked laser pulses with repetition rate equal to the inverse of the round-trip time of a pulse within the laser cavity. Femtosecond ($1 \text{ fs} = 10^{-15} \text{ s}$) laser pulses have been shown to probe the vibrational 'dephasing' (*i.e.* intra-molecular loss of coherence) in real-time by precise tuning of time delay between two pair of pulses; this is known as pump-probe spectroscopy and has been pioneered by the research groups of Hochstrasser, Fleming and

Zewail [6]. Control over the time delay between pump and probe pulses imparts control over the time-evolution of the wave-packet and this is known as the pump-pump (or pump-dump) scheme of quantum control originally proposed by Tannor, Kosloff and Rice [7]. A somewhat different but equivalent control scheme, proposed by Shapiro and Brumer, exploits the idea of controlling the phase of different optical frequency components (or laser modes) within a pulse and thereby achieving the control by quantum interference between multiple light-matter interaction paths [8]. This is achieved by 'laser pulse-shaping' which makes use of a grating-spherical lens (or grating-concave mirror to eliminate chromatic aberration) combination for maximum spatial separation of the spectral components on a plane (known as the 'Fourier plane') and subsequent recombination by using another grating-spherical lens combination; a spatial light modulator (SLM) kept at the Fourier plane can modulate the phases (and/or amplitudes) of various frequency components and thereby giving a pulse shaped in time owing to the inverse Fourier relationship between the time and frequency components of a mode-locked pulse [9]. By using a programmable SLM one can generate pulses of various shapes and get the desired pulse shape obtained by iteratively solving the time-dependent Schrodinger equation as described by the 'optimal control theory' [10] or iteratively search for the optimal pulse shape by using learning algorithms where a series of experiments are carried out in a feedback loop until the optimal condition is reached [11].

Since mode-locked ultrafast lasers are used in multiphoton fluorescence microscopy [12] to make use of the gigantic instantaneous peak power for circumventing the low multiphoton absorption cross-sections of common fluorophores, all the above mentioned methods of time-domain coherent control have exciting applications in imaging with laser pulses in numerous ways. In this proceeding, we discuss few recent studies from our group showing control by ultrafast pulse-pair excitation with applications in multi-photon imaging.

2. METHODOLOGIES

The schematic of the experimental set-up is shown in figure 1a. In our experiment, the laser system was a mode-locked Ti:saph laser (Mira900-F pumped by Verdi5, Coherent) producing femtosecond laser pulse trains at 76 MHz repetition rate having tunability in the range of 720-980 nm. We used ~200 fs pulsed excitation centered on 780 nm. The laser beam was separated in two parts by using a beam-splitter and recombined using another beam splitter after passing the two split beams over almost equal distance. One of the beams was passed through a retro-reflecting mirror mounted on a mechanical stage (UE1724SR driven by ESP300, Newport). The collinearly propagating beams were sent to a home-made autocorrelator containing a second harmonic generation (SHG) crystal (type-1 beta-barium borate or BBO, Casteck). The BBO output was detected by a photo-multiplier connected to a digital oscilloscope (TDS 224, Tektronix). The delay stage and the oscilloscope were interfaced with a personal computer using a GPIB card (National Instruments) and the data were collected using LabVIEW software.

The combined beam was sent to a multi-photon-ready confocal microscope system (FV300 scan-head coupled with IX71 inverted microscope, Olympus). For imaging purposes, all slides were purchased from Molecular Probes and all images were taken using an oil-

immersion objective (UPlanApoN 60X 1.42NA, Olympus). The principle of laser scanning is shown in figure 1b. In laser-scanning microscopy (LSM) the image acquisition is achieved by point-by-point illumination resulting in pixilated-image construction and the high scanning speed ensures laser dwell time on each pixel over very small time window ($\sim 10 \mu\text{s}$); therefore each pixel is illuminated by $\sim 1,000$ low energy ($\sim 1 \text{ nJ}$) laser pulses. As shown in figure 1b, a pair of scanning mirrors quickly switches the focused laser beam among different locations of the sample.

3. RESULTS AND DISCUSSIONS

The interferometric autocorrelation signal is shown in figure 2. The autocorrelation width was found to be $\sim 320 \text{ fs}$ corresponding to a pulse width $\sim 225 \text{ fs}$ (using Gaussian envelope approximation).

Several research groups have shown the applications of control schemes in various non-linear imaging *e.g.* multiphoton fluorescence microscopy [13] and coherent anti-Stokes Raman scattering (CARS) microscopy [14]. However, all of them used amplified laser pulses with slow repetition rate ($\sim 1 \text{ kHz}$) which is much slower compared to fast scanning speed of LSM. High repetition rate (HRR) laser pulses are not used to study ultrafast molecular dynamics since molecular de-excitation processes are not over within the time lapse between two successive pulse ($\sim 10 \text{ ns}$). However, we have recently shown that photo-thermal effects are manifested by finite temperature rise resulting from the pile-up effect of myriads of laser pulses over a finite time window much longer than temporal separation of pulses and demonstrated that amplitude modulation of a train of pulses at $\sim 1 \text{ kHz}$ frequency results in ‘complete’ removal of such deleterious effects leading to significant fluorescence enhancement [15]. The results showed that laser-induced photo-thermal damage is largely governed by ‘transparent’ solvent and the time scale for pile-up effect depends on the heat transfer parameters of the solvent. These findings are crucial for live cell imaging where photo-damage can largely affect the viability of the live specimen. This is precisely the reason why measurement of non-linear absorption coefficients by z-scan method using amplitude-modulated highly repetitive pulse train yields similar results using amplified low repetition pulses [16].

Despite having no pile-up effect as imparted by HRR lasers, amplified lasers suffer from poor signal-to-noise ratio and fluctuations of carrier-envelope phase from pulse to pulse. Also the very high pulse energy ($\sim 1 \mu\text{J}$) results in pulse-saturation effects while tightly focusing with a high numerical aperture objective. An alternative method is to use HRR laser excitation with the sample solution kept in a flowing condition. Another possibility is to use an amplitude-modulated excitation. Figure 2 shows a comparison among different methods. The last two methods result directly from the fact that pile-up effect is a long time effect. Exactly similar condition is achieved during laser scanning since each pixel is illuminated for a time period that is not sufficient for building up the pile-up effect. Thus HRR lasers cause minimal photo-damage in LSM.

With this logic of using HRR laser in LSM, we split each pulse of the pulse train into two pulses and delayed one pulse with respect to the other just like in pump-probe spectroscopy.

However, in pump-probe experiments the probe pulse, made sufficiently weak compared to the pump pulse, is allowed to excite a sample volume less than that excited by the pump and finally separated from the pump pulse either by non-collinear overlapping or using other techniques while maintaining the collinear geometry (*e.g.* using polarizing beam-splitters when polarization of pump and probe pulses are different or filtering out the probe as in ‘two-color’ or ‘non-degenerate’ pump-probe studies where pump and probe frequencies are different). In contrast, our ‘pump-probe’-like experiment involved ‘degenerate’ (*i.e.* having same spectral content), iso-energetic and co-propagating pump and probe pulses illuminating the same sample volume but at different times. The delay between the pump and probe pulses were varied at an interval of 250 fs from zero delay (*i.e.* when pump and probe pulses are temporally overlapped) up to 5 ps which is much shorter than the time lapse (~ 10 ns) between the successive laser pulses as shown in figure 1a. This means that each pixel in an image now results from excitation by $\sim 1,000$ pairs of pump-probe pulses. For every pixel, both the pump and probe pulse triggered the same two-photon induced fluorescence and we collected the total fluorescence signal due to both of them under different polarization of the pump and probe pulses. We collected series of images (each of 512×512 pixels) for different time delay between the pump and probe pulse. Putting all these images together resulted in a movie that visually revealed ultrafast dynamics within a cell (see snapshots under multimedia figures below). To our best knowledge, this is the first demonstration of simultaneous spatial and temporal resolution.

We are presently building up a concave grating based pulse shaper for further applications of coherent control in imaging. The beauty of using concave gratings over planar ones is that it can easily induce linear frequency sweep across the pulse (*i.e.* ‘chirp’) which can be immediately characterized by field (or interferometric) autocorrelation technique. Due to different velocities of different frequency components within a pulse, a pulse is always chirped when it passes through a material medium; this is known as group velocity dispersion (GVD). The two-photon absorption rate have been found to vary inversely with pulse width [17]. We are presently investigating pre-chirp compensation using the concave grating where negative GVD imparted by the pulse shaper counter-balances the positive GVD imposed by the optical elements within the microscope (*e.g.* objective) to achieve transform-limited pulse at the sample yielding efficient two-photon absorption [19]. This will lead to further applications of pulse shaping in imaging, *e.g.* selective excitation of fluorophores [20].

4. CONCLUSIONS

Thus we show how ideas of time domain coherent control can be directly implemented in multiphoton fluorescence LSM. Further research in the field of coherent control by laser pulse shaping is currently being pursued in the authors’ laboratory.

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REFERENCES

- [1]. Rice, SA.; Zhao, M. Optimal Control of Molecular Dynamics. Wiley; New York: 2000.
- [2]. Shapiro, M.; Brumer, P. Principles of the Quantum Control of Molecular Processes. Wiley-Interscience; Hoboken, NJ: 2003.
- [3]. Milonni, PW.; Eberly, JH. Lasers. John Wiley & Sons, Inc.; 1988.
- [4]. Rulliere, C., editor. Femtosecond Laser Pulses: Principles and Experiments. Second Ed. Springer; New York: 2005.
- [5]. Diels, J.; Rudolph, W. Ultrafast Laser Pulse Phenomena: Fundamentals, Techniques and Applications on a Femtosecond Time Scale. Academic Press; 2006.
- [6]. Zewail AH. Laser Femtochemistry. Science. 1988; 242:1645. [PubMed: 17730575]
- [7]. Tannor DJ, Kosloff R, Rice S. Coherent pulse sequence induced control of selectivity of reactions: Exact quantum mechanical calculations. J. Chem. Phys. 1986; 85:5805.
- [8]. Brumer P, Shapiro M. Laser control of molecular processes. Annu. Rev. Phys. Chem. 1992; 43:257. [PubMed: 18397166]
- [9]. Weiner AM, Leaird DE, Patel JS, Wullert JR. Programmable Shaping of Femtosecond Optical Pulses by Use of 128-Element Liquid Crystal Phase Modulator. IEEE J. quant. Electron. 1992; 28:908.
- [10]. Shi S, Woody A, Rabitz H. Optimal Control of Selective Vibrational Excitation in Harmonic Linear Chain Molecules. J. Chem. Phys. 1988; 88:6870.
- [11]. Judson RS, Rabitz H. Teaching Lasers to Control Molecules. Phys. Rev. Lett. 1992; 68:1500. [PubMed: 10045147]
- [12]. Denk W, Stickler JH, Webb WW. Two-photon laser scanning fluorescence microscopy. Science. 1990; 248:73. [PubMed: 2321027]
- [13]. Dela Cruz JM, Pastirk I, Comstock M, Lozovoy VV, dantus M. Use of coherent control methods through scattering biological tissue to achieve functional imaging. PNAS USA. 2004; 10:16996. [PubMed: 15569924]
- [14]. Dudovich N, Oron D, Silberberg Y. Single-pulse Coherently Controlled Nonlinear Raman Spectroscopy and Microscopy. Nature. 2002; 418:512. [PubMed: 12152073]
- [15]. De AK, Goswami D. Exploring the Nature of Photo-Damage in Two-Photon Excitation by Fluorescence Intensity Modulation. J. Fluorescence. DOI: 10.1007/s10895-008-0405-3.
- [16]. Jana A, De AK, Nag A, Goswami D, Bharadwaj PK. Diaza-18-crown-6 Based Chromophores for Modulation of Two-Photon Absorption Cross-Section by Metal Ions. J. Organometallic Chem. 2008; 693:1186.
- [17]. Xu C, Webb WW. Measurement of two-photon excitation cross sections of molecular fluorophores with data from 690 to 1050 nm. J. Opt. Soc. Am. B. 1996; 13:481.
- [18]. Tang S, Krasieva TB, Chen Z, Tempea G, Tromberg BJ. Effect of pulse duration on two-photon excited fluorescence and second harmonic generation in nonlinear optical microscopy. JBO Lett. 2006; 11 020501-1.
- [19]. Fu L, Jain A, Cranfield C, Xie H, Gu M. Three-dimensional nonlinear optical endoscopy. JBO Lett. 2007; 12 040501-1.
- [20]. Brixner T, Damrauer NH, Niklaus P, Gerber G. Photosensitive adaptive femtosecond quantum control in the liquid phase. Nature. 2001; 414:57. [PubMed: 11689940]

Fig. 1a.

Schematic of the experimental set-up (the autocorrelator and microscope are not shown).

Abbreviations: BS: beam splitter, M: mirror.

Fig. 2b.

Principle of laser-scanning, light paths due to two different positions of the scanning mirror are shown as solid and dashed lines. Abbreviations: L; lens, O: objective, S: sample, SM: scanning mirror.

Fig. 2.
Interferometric autocorrelation trace.

Fig. 3.
Different experimental schemes to remove the pile-up effect of pulse train.

Video 3.

Snapshot (delay ~ 1 ps) of bovine pulmonary-artery endothelial (BPAE) cells under horizontally polarized pump and vertically polarized probe excitation (a) and horizontally polarized pump and circularly polarized probe excitation. <http://dx.doi.org/10.1117/12.807687.1> and <http://dx.doi.org/10.1117/12.807687.2>