Superior optical nonlinearity of an exceptional fluorescent stilbene dye

He, Tingchao; Sreejith, Sivaramapanicker; Gao, Yang; Grimsdale, Andrew C.; Zhao, Yanli; Lin, Xiaodong; Sun, Handong

2015

Superior optical nonlinearity of an exceptional fluorescent stilbene dye

Tingchao He, Sivaramapanicker Sreejith, Yang Gao, Andrew C. Grimsdale, Yanli Zhao, Xiaodong Lin, and Handong Sun

---

Superior optical nonlinearity of an exceptional fluorescent stilbene dye

Tingchao He,[1,2] Sivaramapanicker Sreejith,[3] Yang Gao,[4] Andrew C. Grimsdale,[4] Yanli Zhao,[3] Xiaodong Lin,[1,a)] and Handong Sun[2,a)]

[1]College of Physics Science and Technology, Shenzhen University, Shenzhen 518060, China
[2]Division of Physics and Applied Physics, Centre for Disruptive Photonic Technologies (CDPT), School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371
[3]Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371
[4]School of Materials Science and Engineering, Nanyang Technological University, Singapore, Singapore 639798

Strong multiphoton absorption and harmonic generation in organic fluorescent chromophores are, respectively, significant in many fields of research. However, most of fluorescent chromophores fall short of the full potential due to the absence of the combination of such different nonlinear upconversion behaviors. Here, we demonstrate that an exceptional fluorescent stilbene dye could exhibit efficient two- and three-photon absorption under the excitation of femtosecond pulses in solution phase. Benefiting from its biocompatibility and strong excited state absorption behavior, *in vitro* two-photon bioimaging and superior optical limiting have been exploited, respectively. Simultaneously, the chromophore could generate efficient three-photon excited fluorescence and third-harmonic generation (THG) when dispersed into PMMA film, circumventing the limitations of classical fluorescent chromophores. Such chromophore may find application in the production of coherent light sources of higher photon energy. Moreover, the combination of three-photon excited fluorescence and THG can be used in tandem to provide complementary information in biomedical studies. © 2015 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4915311]

Nonlinear upconversion of visible and infrared light to higher frequencies has been studied extensively and used for various applications such as bioimaging,[1] optical limiting,[2] higher energy coherent light sources,[3] and upconverted lasing.[4] Designing materials exhibiting nonlinear upconversion behavior of light has been a major challenge in the field of materials science for the last 25 years.[5] Such materials are of great scientific and technological interest not only for important application but also for the fundamental studies of how soft matters interact with light.

Multiphoton absorption (MPA) and multiple order harmonic generation (MOHG) are two of the most important techniques that can be used to achieve nonlinear upconversion of light.[6] There have been a large body of prior literatures which discussed in detail the MPA or MOHG of various fluorescent dyes individually, but reports of combination of different nonlinear upconversion behaviors are scarce.[7–9] From the viewpoint of applications, combining different nonlinear optical properties together should be extremely important. Unfortunately, most known organic molecules have relatively small MPA or/and MOHG, especially in the case of solid phases. The absence of such combination of MPA and MOHG in solid phases should be due to poor fluorescent emission and unsuitable stacking behavior of chromophores, which hinders the full exploitation of such kinds of nonlinear optical materials. Therefore, there is an urgent need to develop nonlinear upconversion materials with the proper combination of MPA and MOHG properties.

Herein, we report our studies on a stilbene dye which possesses strong fluorescence quantum yields (QYs) and excellent solubility in organic solvents, as well as moderate fluorescence QY in solid phases. In such case, this stilbene dye could avoid the fluorescence quenching in different phases to achieve various applications. In solution, the chromophore exhibits large two- and three-photon absorption (abbreviated as 2PA and 3PA) under the excitation of femtosecond pulses (100 fs, 1000 Hz), as well as the excited state absorption (ESA) under the excitation of nanosecond pulses (6 ns, 10 Hz) that can further boost its optical nonlinearity, which allows for applications in both bioimaging and optical limiting. Interestingly, both 3PA and third-harmonic generation (THG) have been observed simultaneously in the chromophore/PMMA films, which would be especially useful for the applications of non-invasive and spatially localized *in vivo* bioimaging, as well as higher energy coherent light sources.

In this letter, the chromophore was dissolved in solution and PMMA film for comparison purpose. First, stock solutions were prepared by dissolving chromophore into organic solvents, with a concentration of 1 mg/ml. And they were further diluted for optical studies at desired concentrations. For the solid state film, the chromophore was doped into a PMMA polymer host, with a doping level was 5 wt. %.

The chemical structure of stilbene chromophore is shown in Fig. 1(a). Figure 1(b) depicts the normalized UV-vis absorption and one-photon excited fluorescence spectra of stilbene chromophore in chloroform ($CHCl_3$) and PMMA film. The absolute QYs for chromophore in $CHCl_3$ and PMMA films were 94.65% and 1.69%, respectively. Although the stilbene

a)Authors to whom correspondence should be addressed. Electronic addresses: linxd@szu.edu.cn and hdsun@ntu.edu.sg
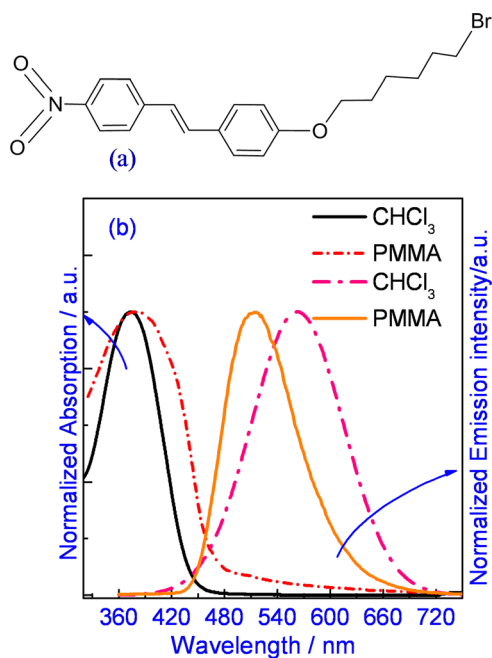
FIG. 1. (a) Molecular structure of the stilbene chromophore studied. (b) Normalized UV-Vis absorption and fluorescence spectra of stilbene chromophore in CHCl$_3$ and PMMA film.

FIG. 2. 2PA (a) and 3PA (b) spectra of stilbene chromophore in different solvents. The solid lines are provided as a guide.

chromophore exhibited small change in the absorption spectra (corresponding to ground state absorption) in different media, their emission maximum wavelengths underwent dramatic changes. In comparison to the fluorescence spectrum in CHCl$_3$ solution ($\lambda = 565$ nm), the chromophore in PMMA showed blue-shifted fluorescence spectrum ($\lambda = 515$ nm). Such dramatically different fluorescence emission properties for the stilbene chromophore can be explained in terms of the strong intramolecular charge-transfer character in different organic solvents and the molecular packing induced crystallographic modification in solid phase, rendering it to reach large optical nonlinearity.[10]

From the linear absorption spectra of stilbene chromophore in different media, it was expected that the excitation of both solution and PMMA film of the stilbene chromophore in the range of 700–900 nm and 1100–1500 nm, requires 2PA and 3PA behaviors. Upon excitation at above wavelength range, the chromophore in CHCl$_3$, with a concentration of 1×10^{-4} M, indeed exhibited bright fluorescence emission even excited with low power intensity. For the measurement of molecular MPA cross-sections, we used two- and three-photon excited fluorescence spectra methods.[11,12] 2PA and 3PA spectra were measured by comparison to 10 μM Rhodamine 6G with a charge-coupled device (CCD) camera after passing through a monochromator. No matter what medium (organic solvents or PMMA film) was used, the two- and three-photon excited fluorescence spectra for the chromophore showed little difference from those excited with one-photon, revealing that the same transition processes were involved in the situations of one- and multiphoton excitation. From the 2PA and 3PA spectra presented in Figs. 2(a) and 2(b), it can be seen that the chromophore presented good 2PA in the range of 700–900 nm and 3PA in the range of 1100–1500 nm due to the large intramolecular charge transfer property.[13] For example, the maximum 2PA
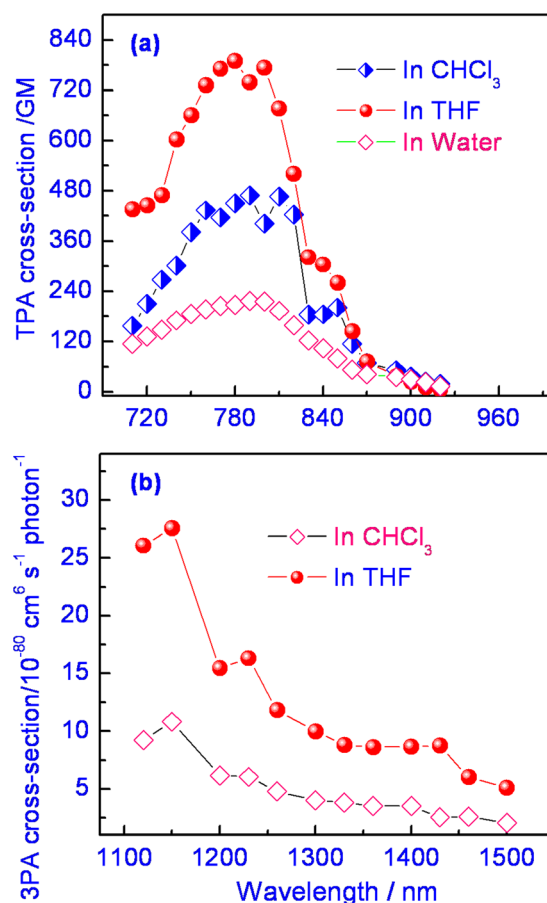
and 3PA cross-sections of stilbene chromophore in CHCl$_3$ were 470 GM and 11×10^{-80} cm^4 photon^{-1} s^{-1}, respectively. In order to assess the effect of the molecular environment and solvent polarity on the MPA properties, we also investigated the effect of the solvent polarity on MPA properties. It was found that with the addition of a solvent with moderate polarity, such as tetrahydrofuran (THF), the molecular MPA cross-sections were enhanced greatly. The corresponding molecular maximum 2PA and 3PA cross-sections in THF were 791 GM and 28×10^{-80} cm^4 photon^{-1} s^{-1}, respectively. Interestingly, the molecular nanoaggregates prepared by using a solvent-exchange process, in which the proportion of THF in water was 2% (v/v) and the final dye concentration was 1×10^{-4} M, also possessed moderate TPA cross-sections (Fig. 2(a)), strongly suggesting the potential applications of our organic nanoparticles in biological imaging.

As the compounds displayed very good solubility (up to 30 mg/ml) and large TPA in organic solvents, it allowed us to investigate their optical limiting properties by measuring the relationship between the output intensity and the input intensity, which is one of main applications for two-photon absorbing dyes.[14] In this work, both femtosecond pulses (1000 Hz, 100 fs, 800 nm) and nanosecond pulses (10 Hz, 6 ns, 800 nm) were used as light sources for comparison purpose. The measured transmitted intensity through a 1 cm path length quartz cuvette filled with sample with a concentration of 10 mg/ml in

CHCl$_3$ as a function of the incident intensity using nonlinear transmission method are shown in Fig. 3. The transmittance loss from the pure solvent and cuvette was subtracted. No matter what excitation pulses were used, the sample presented strong optical limiting behaviors.

It was worth noting that there were prominent differences between the experimental results achieved under the excitation of femtosecond and nanosecond pulses. For the irradiation of femtosecond pulses, the threshold value of optical limiting was about 30.1 J cm^{-2}. The main optical limiting mechanism under fs pulsed laser should be intrinsic 2PA of the stilbene chromophore.[15] In the case of nanosecond pulses excitation, the threshold value (12.2 J cm^{-2}) was much lower than that using femtosecond pulses excitation. Obviously, in this case, some other processes which coexisted with TPA additionally decreased input optical intensity present, making even more contribution to the optical limiting capability of the sample than a 2PA process.[15,16]

In view of large width of nanosecond pulses used, it was expected that 2PA induced ESA (2PA-ESA) process should become the chief reason for more superior optical limiting behavior.[15,16] Regarding the detailed 2PA-ESA process (due to the absorption of a singlet excited state or a triplet excited state), femtosecond pumped transient absorption and nanosecond flash photolysis experiments remained desirable.[17,18] Even so, the good optical limiting behaviors under the excitation of femtosecond and nanosecond pulses suggested the great potentials in many applications, such as reshaping and optical stabilization.[19]

To elucidate the potential application of stilbene chromophore as a biomarker, two-photon confocal microscopic studies of stilbene chromophore in HeLa cells were performed. 3-(4,5-Dimethylthiazol-2-yl)–2,5-diphenyltetrazolium bromide (MTT) assay revealed that the cellular viability of HeLa cells was greater than 80% after incubation with 200 μM probe for 24 h, indicating low cytotoxicity of the probe (Fig. 4(a)). Inspired by this observation we further carried out the two-photon fluorescent measurements in live cells using the molecular probe. To study the two-photon imaging capability, we incubated stilbene chromophore with
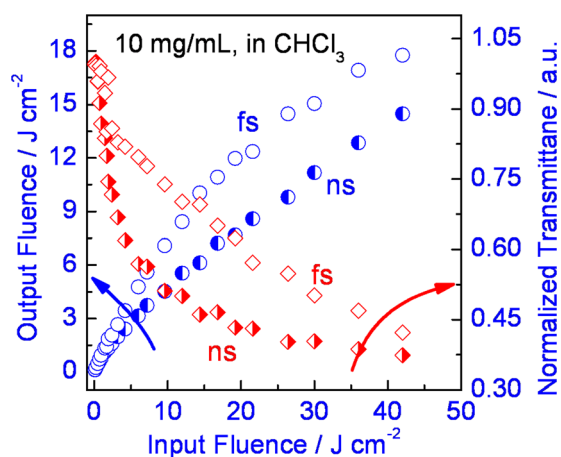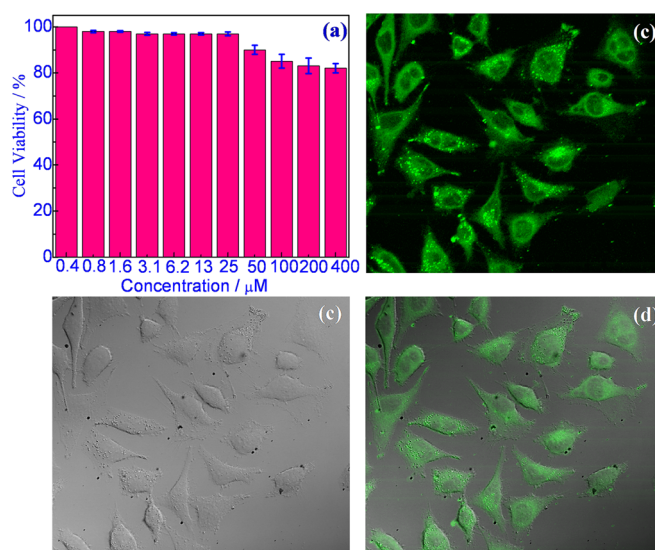

FIG. 4. Two-photon microscopy images of HeLa cells under excitation at 800 nm. (a) Relative viabilities of HeLa cells treated with stilbene chromophore at different concentration. The error bar represents experimental mean standard deviation. (b) The bright field image of live HeLa cells stained with 1×10^{-5} M probe for 4 h. (c) The corresponding dark field image of HeLa cells, and the emission was collected at 500–600 nm. (d) The overlay image of (b) and (c). Their high overlap indicates that stilbene chromophore is internalized in cells and can be used for imaging applications.

HeLa cells, which were imaged using two-photon fluorescence microscope under 800 nm excitation. Figure 4(b) depicts the bright field of HeLa cells endocytosed with 10 μM stilbene chromophore. Upon two-photon excitation at 800 nm, the mixed solution showed strong green fluorescence (Fig. 4(c)). This clearly indicated that the stilbene chromophore could be used as a two-photon fluorescent marker to image the cells for future drug studies. The overlay image (Figs. 4(b) and 4(c)) further confirmed the localization of stilbene chromophore in the cytoplasm. Thus, the *in vitro* two-photon imaging demonstrated promising application potential of the stilbene chromophore for practical two-photon bioimaging.

Although multiphoton excited fluorescence (MPEF) is usually the primary signal source in multiphoton microscopy (MPM), MOHG can also be used for the application of bioimaging. In fact, MOHG imaging was one of the earliest forms of biological nonlinear microscopy.[20] In addition, MOHG represents the most important frequency conversion technique to achieve the high energy coherent light source. Contrary to SHG, THG process is a more general effect because the symmetry induced selection rules are more relaxed for the most of the known media. The effect is driven by the third-order susceptibility tensor $\chi^{(3)}$.[21]

The combination of MPA excited fluorescence with MOHG generated by organic molecules is vital for the multi-color nonlinear microscopy of living tissue.[22–24] However, such kinds of bifunctional molecules are scare. Interestingly, for PMMA films of stilbene chromophores, when it was excited in the second near-Infrared window (1 kHz, 100 fs, 1200–1500 nm), MPEF/MOHG coregistration can easily be achieved by using a simple backscattering geometry.

Figure 5(a) shows emission spectra as functions of the excitation intensity, excited at 1200 nm. The emission spectra

FIG. 3. Measured output fluence and nonlinear transmission versus input fluence through chromophore CHCl$_3$ solution (10 mg/ml) under the excitation of femtosecond and nanosecond pulses at the wavelength of 800 nm.
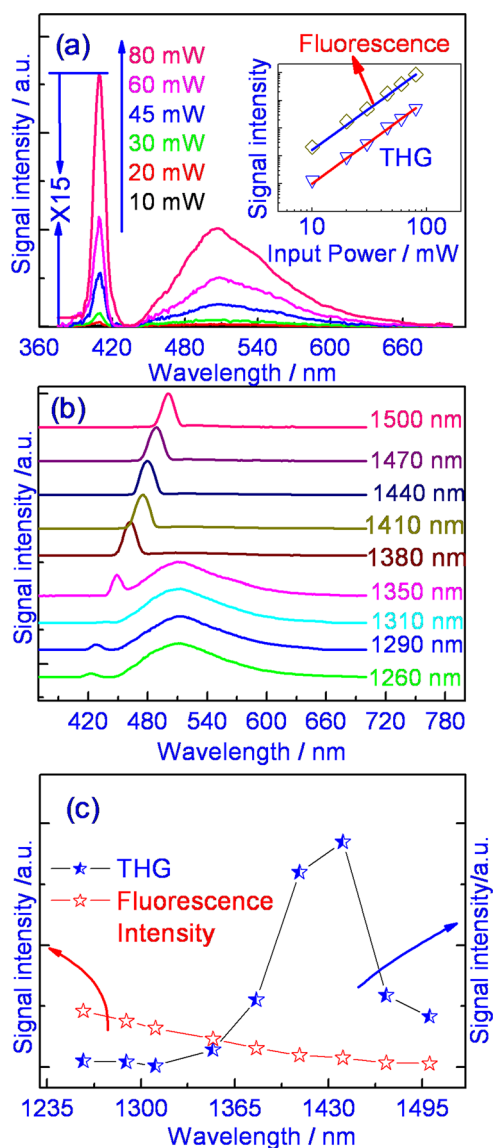
FIG. 5. (a) The signal intensity of THG and 3PA as a function of the input intensity excited at 1200 nm measured from the stilbene chromophore/PMMA solid film. Inset: Their logarithmic plots of the intensities as a function of the excitation intensity. (b) Wavelength dependent emission spectra consisting of THG and 3PA. (c) Comparison of signal intensity between THG and 3PA at different excitation wavelength.

consisted of a narrow peak and a broad feature from 430 to 660 nm, with a maximum at 515 nm, which corresponded to fluorescence emission of stilbene chromophore. As for the narrow peak, it was manifested at one-third the excitation wavelength and a bandwidth in accordance with the excitation laser spectral width. For the emission spectra achieved under different excitation intensity, both the broad band emission and single narrow line could be characterized with a cubic increase of the peak intensity with respect to the excitation intensity. Such excitation dependence clearly suggesting the signature of 3PA and THG.[21] It should be pointed out that in the range of our excitation wavelength and excitation intensity the THG signal from the optical glass substrate is very weak and barely noticeable. Therefore, the observed signals should solely result from the PMMA film of stilbene chromophores.

Inspection of the emission spectra depicted in Fig. 5(b) reveals the excitation wavelength dependence of spectra of

three-photon excited fluorescence and MOHG, while Fig. 5(c) compares the signal intensity between THG and 3PA at different excitation wavelength. For the wavelength-dependent experiments, the input intensity of pulses at different wavelengths was set to be the same. All the measurements were carried out at room temperature. Distinct differences in the emission profiles at different excitation wavelength were observed. The emission spectrum achieved under the excitation of 1260 nm was dominated by three-photon excited fluorescence, accompanied by a weak THG. With increasing excitation wavelength, the peaks of fluorescence emission band remained unchanged while the wavelength of THG shifted towards longer energy and always located at the tripled frequency of fundamental light. Meanwhile, the relative contribution of the peak intensity derived from THG steadily increased to dominate at the excitation wavelength of 1380 nm. Such trend was determined by the different wavelength dependent spectral response and the reabsorption behavior of MPA and MOHG in stilbene chromophore in PMMA film. Although there was competition between MPA and MOHG, it would not weaken the importance of this molecule. The combination of MPA and MOHG in the PMMA film of stilbene chromophores could be easily achieved through the suitable choice of excitation wavelength. The combination of strong 3PA and MOHG of stilbene chromophore should be due to their π-stacking building in solid film, like the structures of other strongly dipolar chromophore.[8,25] It can be further confirmed by the absence of THG in organic solvents. The coregistration of MPA and MOHG was also reported in collagen across a broad excitation wavelength range in reflection geometry.[26]

In summary, superior MPA and MOHG properties have been observed from an exceptional fluorescent stilbene chromophore, which has never been reported in previous stilbene derivatives. The chromophore solution exhibits good 2PA and 3PA because of its intrinsic strong intramolecular charge transfer, suggesting its potential in bioimaging. In addition, the chromophore is found to show superior optical limiting under the excitation of nanosecond pulses due to the contribution of 2PA-ESA. Remarkably, the chromophore/PMMA film simultaneously possesses strong MPA and MOHG under the excitation of femtosecond pulses in the second biological window. The stilbene chromophore with such excellent nonlinear optical properties will prove to be extremely useful biological fluorescent labels and to be vital for the application of higher energy coherent light sources.

[1]H. A. Collins, M. Khurana, E. H. Moriyama, A. Mariampillai, E. Dahlstedt, M. Balaz, M. Kuimova, M. Drobizhev, V. X. D. Yang, D.

Phillips, A. Rebane, B. V. Wilson, and H. L. Anderson, Nat. Photonics **2**, 420 (2008).

[2]T. C. He, W. Wei, L. Ma, R. Chen, S. Wu, H. Zhang, Y. Yang, J. Ma, L. Huang, and G. G. Gurzadyan, Small **8**, 2163 (2012).

[3]M. Cazzanelli, F. Bianco, E. Borga, G. Pucker, M. Ghulinyan, E. Degoli, E. Luppi, V. Véniard, S. Ossicini, D. Modotto, S. Wabnitz, R. Pierobon, and L. Pavesi, Nat. Mater. **11**, 148 (2012).

[4]Y. Wang, V. D. Ta, Y. Gao, T. C. He, R. Chen, E. Mutlugun, H. V. Demir, and H. D. Sun, Adv. Mater. **26**, 2954 (2014); Q. Zheng, H. Zhu, S. Chen, C. Tang, E. Ma, and X. Chen, Nat. Photonics **7**, 234 (2013).

[5]W. Jie, X. Chen, D. Li, L. Xie, Y. Y. Hui, S. P. Lau, X. Cui, and J. Hao, Angew. Chem., Int. Ed. **54**, 1185 (2015); M. Tsang, G. Bai, and J. Hao, Chem. Soc. Rev. **44**, 1585 (2015).

[6]G. S. He, L. Tan, Q. Zheng, and P. N. Prasad, Chem. Rev. **108**, 1245 (2008); M. Drobizhev, N. S. Makarov, S. E. Tillo, T. E. Hughes, and A. Rebane, Nat. Methods **8**, 393 (2011).

[7]T. C. He, Y. Gao, R. Chen, L. Ma, D. Rajwar, Y. Wang, A. C. Grimsdale, and H. Sun, Macromolecules **47**, 1316 (2014).

[8]Y. A. Getmanenko, J. M. Hales, M. Balu, J. Fu, E. Zojer, O. Kwon, J. Mendez, S. Thayumanavan, G. Walker, Q. Zhang, S. D. Bunge, J. Bredas, D. J. Hagan, E. W. Van Stryland, S. Barlow, and S. R. Marder, J. Mater. Chem. **22**, 4371 (2012).

[9]C. Yu, S. Tai, C. Kung, W. Lee, Y. Chan, H. Liu, J. Lyu, and C. Sun, Opt. Lett. **33**, 387 (2008).

[10]C. Chen, T. Hinoue, I. Hisaki, M. Miyata, and N. Tohnai, Tetrahedron Lett. **54**, 1649 (2013).

[11]L. Guo and M. S. Wong, Adv. Mater. **26**, 5400 (2014).

[12]H. H. Fan, L. Guo, K. F. Li, M. S. Wong, and K. W. Cheah, J. Am. Chem. Soc. **134**, 7297 (2012).

[13]X. Wang, Y. Zhou, W. Yu, C. Wang, Q. Fang, M. Jiang, H. Lei, and H. Wang, J. Mater. Chem. **10**, 2698 (2000).

[14]Q. Zheng, S. K. Gupta, G. S. He, L. Tan, and P. N. Prasad, Adv. Funct. Mater. **18**, 2770 (2008).

[15]L. Wang, Y. Zhao, J. Guan, and F. Wu, Appl. Phys. Lett. **102**, 251906 (2013).

[16]J. E. Ehrlich, X. L. Wu, I.-Y. S. Lee, Z.-Y. Hu, H. Röckel, S. R. Marder, and J. W. Perry, Opt. Lett. **22**, 1843 (1997).

[17]K. D. Belfield, M. V. Bondar, S. Yao, I. A. Mikhailov, V. S. Polikanov, and O. V. Przhonska, J. Phys. Chem. C **118**, 13790 (2014).

[18]A. H. Shelton, R. S. Price, L. Brokmann, B. Dettlaff, and K. S. Schanze, ACS Appl. Mater. Interfaces **5**, 7867 (2013).

[19]G. S. He, H. S. Oh, and P. N. Prasad, Opt. Lett. **36**, 4431 (2011).

[20]J. Lin, W. Zheng, Z. Wang, and Z. Huang, Appl. Phys. Lett. **105**, 103705 (2014).

[21]R. W. Boyd, *Nonlinear Optics* (Academic Press, USA, 2008).

[22]D. De'barre, W. Supatto, A. Pena, A. Fabre, T. Tordjmann, L. Combettes, M. Schanne-Klein, and E. Beaurepaire, Nat. Methods **3**, 47 (2006).

[23]W. R. Zipfel, R. M. Williams, R. Christie, A. Y. Nikitin, B. T. Hyman, and W. W. Webb, Proc. Natl. Acad. Sci. U.S.A. **100**, 7075 (2003).

[24]S. Zhuo, J. Yan, Y. Kang, S. Xu, Q. Peng, P. T. C. So, and H. Yu, Appl. Phys. Lett. **105**, 023701 (2014).

[25]G. Ramos-Ortiz, M. Cha, S. Thayumanavan, J. C. Mendez, S. R. Marder, and B. Kippelen, Appl. Phys. Lett. **85**, 179 (2004).

[26]A. Zoumi, A. Yeh, and B. J. Tromberg, Proc. Natl. Acad. Sci. U.S.A. **99**, 11014 (2002).