Two-photon luminescence and stimulated emission depletion with gold nanorods by a single wavelength

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Abstract Single-wavelength two photon STED technique simplifies common STED setup, and decreases the cost of the system. However, this technique limits the fluorescent dye to a few kinds. Because the dye should be excited and depleted by the same single wavelength laser. And the gold nanorods can be excited by near-infrared pulsed laser and produces two photon luminescence, which is brighter than general two-photon autofluorescence by three orders. We found that the gold nanorods can also be depleted by near-infrared pulsed laser. We studied the stimulated emission depletion of gold nanorods with a single wavelength laser in the near infrared. Two photon luminescence was excited with a femtosecond pulse, then depleted by stimulated emission with a stretched pulse. The depletion efficiency with different wavelength was measured to pick out the optimal wavelength. We showed that this method can be applied to super-resolved STED microscopy, which combines the high brightness of the nanorods and the simplicity of the single-wavelength STED system.

Keywords: nanorod, luminescence, STED, super-resolution

1. Introduction

Stimulated emission depletion (STED) microscopy has been developed by Hell and Wichmann two decades ago, providing spatial resolution well beyond the diffraction limit [1]. STED microscopy is generally based on the fluorescence switching because of stimulated emission depletion produced by a second red shifted beam, named STED beam. The STED beam is normally shaped to a doughnut-like distribution featuring zero intensity in the center. Earlier STED microscopy used two synchronized pulsed lasers: one for excitation of typically less than 100 ps duration and an another 250 ps pulse for depletion, which requires a relatively complex and expensive setup [1-3]. And later STED microscopy has been developed with continuous wave (CW) lasers, simplifying the implementation [4]. Moreover, researchers have coupled STED with two-photon microscopy to combine the advantages of two-photon microscopy with the super-resolution ability of STED microscopy [5-7]. All the above-mentioned STED microscopy, the excitation and depletion are implemented by two different wavelengths. And single-wavelength two-photon excitation stimulated emission depletion microscopy has been developed, simplifying the STED optical scheme by using a single wavelength for both two photon excitation and depletion [8-9]. However, this single-wavelength STED limits the fluorescent dye to a few kinds. Because the dye should be excited and depleted by the same single wavelength laser.

On the other hand, gold nanorods (NRs) has attracted much interest for their highly efficient absorption in the near-infrared (NIR) region. Gold nanorods exhibit highly efficient two-photon luminescence, significant enhanced by longitudinal surface plasmon resonance (LSPR) [10]. And Two-photon luminescence (TPL) has been regarded as a serial process, that involves sequential absorption of photons and emission from the recombination of electrons in the sp-band and holes in the d-band [11]. And several progresses have been made in understanding the optical properties of gold nanorods [11-12]. Their LSPR peak can be finely tuned from visible light to NIR region, as a function of aspect ratio [12]. Furthermore, the LSPR are sensitive to the polarization of the incident excitation [13]. And researchers have made efforts to explore the applications of gold nanorods as contrast agents for imaging [14]. And NRs were used for molecularly specific nonlinear imaging, and the results showed that the TPL intensity from NRs labeled cancer cells was three orders of magnitude brighter than the two-photon autofluorescence (TPAF) from the unlabeled cancer cells.
We found that the NRs could also be depleted by near-infrared pulsed laser. And we aimed to combine the high brightness of the nanorods and the simplicity of the single-wavelength STED system.

2. Materials and methods

2.1. Experimental setup

The scheme of our experimental setup is shown in Fig. 1. An ultrafast tunable (680-1080 nm) Ti:sapphire laser (Chameleon Ultra II; Coherent) was used as light source for both two-photon excitation and stimulated emission of the gold nanorods. The laser beam is split into two paths by a polarizing beam splitter (PBS), and the relative power of the two paths can be tuned by a half-wave plate in front of the PBS. The first beam is used to excite the gold nanorods and the second beam is used to deplete the excited state by stimulated emission. And the power of the second beam is further adjusted by another pair of PBS and half-wave plate. The STED beam is expanded with two lenses, and its pulses is stretched with 30-cm glass rod and 100-m polarization-maintaining optical fiber in order to avoid any linear or nonlinear excitation. On this beam path, after the fiber, an optical delay line, made with a pair of mirrors mounted on a linear stage, is used to temporally overlap the pulses of the two beams at the sample. And the final position of the delay line is chosen by optimizing the maximum depletion. And a 0 to 2π vortex phase plate is used to create doughnut-like pattern at the focus. The two-photon excitation beam path is expanded and recombined with the STED beam by a PBS, then their polarization is made circular by a quarter wave-plate. With a pair of mirrors, the recombined beams are imported into the infrared port of the confocal unit of the microscope (Leica TCS SP8; Leica Microsystems). The two beams are raster scanned into the back aperture of an water-immersion objective lens (N.A. = 1.20 , 63 ×, Leica) with a set of galvanometric scanning mirrors. Emitted light is passed through a laser filter (blocks >680 nm), detected with a photomultiplier tube. And the system collected emission light between 500 and 650 nm.

2.2. Sample preparation

Gold nanorods were synthesized in solutions by a seed-mediated method as reported previously [16, 17]. The gold nanorod samples for imaging were prepared by dispersing 5 µl dilution onto a glass slide and covered by a coverslip.

3. Results

Figure 2 shows a transmission electron microscopy (TEM) image of synthesized gold nanorods. We first examined the depletion of the two-photon luminescence of the gold nanorods with the single wavelength (770 nm). As shown in Figs. 3(a)-(c), we can observe obviously luminescence quenching at the presence of STED beam, and luminescence recovery without STED beam. The two-photon luminescence quenching is about 75%, when the STED pulse is on, which can be observed from Fig. 3(g).
Fig. 2 TEM image of gold nanorods.

In order to pick out the optimal wavelength for both excitation and depletion, we evaluated the two-photon luminescence with and without the STED beam, and the depletion efficiency as function of wavelength. Figure 4 shows the wavelength dependencies, remaining the two-photon and STED intensities constant. The black line shows the luminescence induced by the two-photon beam without the presence of the STED beam. Meanwhile we measured the luminescence while superimposing the two-photon and the STED beam (red line, Fig. 4). And then the depletion efficiency was calculated, as shown in Fig. 3 with the green line. As seen from Fig. 4, obvious depletion (depletion efficiency >0.7) can be observed over the measured wavelength from 730 nm to 820 nm. And the maximum excitation was produced at the wavelength of 770 nm, and the maximum depletion was obtained at the wavelength of 760 nm. Because the NRs are bright contrast agent, we chose the wavelength of 760 nm for latter experiment for the better depletion.

We analyzed the depletion efficiency by measuring the luminescence depending on the STED beam intensity, while keeping 2P excitation power constant at 6 mw. The results are reported in Fig. 5. The curve shows the luminescence reduction due to the STED effect. As predicted, luminescence quenching increases exponentially with the STED beam intensity.

Fig. 3. The depletion of the two-photon luminescence of the gold nanorods. (a)-(c) are the luminescence images, and (d)-(f) are the transmission image with the wavelength equal to 770 nm. (d) Quantification of the signal intensity from the nanorods in (a)-(c).

Fig. 4. Two-photon luminescence with (red dots) and without (black squares) the STED beam, and the depletion efficiency (green triangles) as function of wavelength, keeping the two-photon and STED intensities constant.

Fig. 5. Depletion of the gold nanorods luminescence, 2P excited and depleted at 760 nm, as a function of the STED beam power $P_{\text{STED}}$. The power of 2P beam is 6 mw.
We used the above optical setup to image gold nanorods to demonstrate the ability of single wavelength 2P STED to image with sub-diffraction limited resolution, with the STED beam on or off. And the results are shown in Fig. 6. Comparison of 2P imaging, single wavelength 2P STED reveals an improvement of lateral resolution when the STED laser is on (Fig. 6 (a) and (b)). And the resolution was quantitatively calculated. Under 2P imaging, images of displayed a full-width at half maximum (FWHM) of 265 nm, shown in Fig. 6(c). In contrast, single wavelength 2P STED images exhibited a FWHM of 190 nm, shown in Fig. 6(d), indicating about 1.4-fold improvement in resolution.

Fig. 6. Two-photon luminescence images of gold nanorods acquired with 2P (a) and single wavelength 2P STED (b). The images were collected by switching the STED laser on and off. (c) and (d) are the profile of intensity along the dashed lines in (a) and (b), respectively.

4. Discussion and conclusion

We showed that gold nanorods can be depleted by near-infrared pulsed laser, which induce two-photon luminescence of the nanorods. With the properties, gold nanorods can be used for single wavelength two-photon STED imaging, which simplifies the optical setup in terms of number of dichroic filters and laser sources of traditional STED microscopy. And The depletion efficiency with different wavelength was measured to pick out the optimal wavelength for single wavelength two-photon STED imaging of gold nanorods. The resolution of single wavelength 2P STED image indicated about 1.4-fold improvement in resolution than that of 2P image. And the possible reason of a little resolution improvement is that the two beam are not well overlapped in the 3D space. And we are making the effort to optimize the arrangement of the beams to improve the resolution. The proposed method combines the high brightness of the nanorods and the simplicity of the single-wavelength STED system, which seems a promising technique to imaging of biological samples at nanoscale resolution.

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References

[1] S. W. Hell and J. Wichmann, "Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy," Opt. Lett. 19(11), 780-782 (1994).
[2] T. A. Klar, S. Jakobs, M. Dyba, A. Egner, and S. W. Hell, "Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission," Proceedings of the National Academy of Sciences 97(15), 8206-8210 (2000).
[3] G. Donnert, J. Keller, R. Medda, M. A. Andrei, S. O. Rizzoli, R. Lahrman, R. Jahn, C. Eggeling, and S. W. Hell, "Macromolecular-scale resolution in biological fluorescence microscopy," Proceedings of the National Academy of Sciences 103(31), 11440-11445 (2006).
[4] K. I. Willig, B. Harke, R. Medda, and S. W. Hell, "STED microscopy with continuous wave beams," Nat. Methods 4(11), 915-918 (2007).
[5] G. Moneron and S. W. Hell, "Two-photon excitation STED microscopy," Opt. Express 17(17), 14567-14573 (2009).
[6] J. B. Ding, K. T. Takasaki, and B. L. Sabatini, "Supraresolution Imaging in Brain Slices using Stimulated-Emission Depletion Two-Photon Laser Scanning Microscopy," Neuron 63(4), 429-437 (2009).
[7] Q. Li, Y. Wang, D. Chen, and S. S. Wu, "2PE-STED microscopy with a single Ti:sapphire laser for reduced illumination," Plos One 9(2), e84846 (2014).
[8] T. Scheul, C. D’Amico, I. Wang, and J. C. Vial, "Two-photon excitation and stimulated emission
depletion by a single wavelength,” Opt. Express 19(19), 18036-18048 (2011).

[9] P. Bianchini, B. Harke, S. Galiani, G. Vicidomini, and A. Diaspro, "Single-wavelength two-photon excitation-stimulated emission depletion (SW2P-STED) superresolution imaging," Proceedings of the National Academy of Sciences 109(17), 6390-6393 (2012).

[10] M. B. Mohamed, V. Volkov, S. Link, and M. A. El-Sayed, "The 'lightning' gold nanorods: fluorescence enhancement of over a million compared to the gold metal," Chem. Phys. Lett. 317(6), 517-523 (2000).

[11] K. Imura, T. Nagahara, and H. Okamoto, "Near-Field Two-Photon-Induced Photoluminescence from Single Gold Nanorods and Imaging of Plasmon Modes," The Journal of Physical Chemistry B 109(27), 13214-13220 (2005).

[12] T. Wang, D. Halaney, D. Ho, M. D. Feldman, and T. E. Milner, "Two-photon luminescence properties of gold nanorods," Biomed. Opt. Express 4(4), 584-595 (2013).

[13] H. Wang, T. B. Huff, D. A. Zweifel, W. He, P. S. Low, A. Wei, and J. X. Cheng, "In vitro and in vivo two-photon luminescence imaging of single gold nanorods," Proceedings of the National Academy of Sciences 102(44), 15752-15756 (2005).

[14] X. Huang, I. H. El-Sayed, W. Qian, and M. A. El-Sayed, "Cancer Cell Imaging and Photothermal Therapy in the Near-Infrared Region by Using Gold Nanorods," J. Am. Chem. Soc. 128(6), 2115-2120 (2006).

[15] N. J. Durr, T. Larson, D. K. Smith, B. A. Korgel, K. Sokolov, and A. Ben-Yakar, "Two-Photon Luminescence Imaging of Cancer Cells Using Molecularly Targeted Gold Nanorods," Nano Lett. 7(4), 941-945 (2007).

[16] B. Nikoobakht and M. A. El-Sayed, "Preparation and Growth Mechanism of Gold Nanorods (NRs) Using Seed-Mediated Growth Method," Chem. Mater. 15(10), 1957-1962 (2003).

[17] T. K. Sau and C. J. Murphy, "Seeded High Yield Synthesis of Short Au Nanorods in Aqueous Solution," Langmuir 20(15), 6414-6420 (2004).