Nonlinear Optical Properties of Gold Nanorods (GNRs) under FS Laser Excitation near the Third Optical Tissue Window and Application for Multichannel Cellular Imaging

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Abstract— Gold nanorod (GNR) was a promising bioimaging probe in the third optical tissue window (1600–1800 nm). In this paper, hydrophilic GNRs with proper dimensions were synthesized according to the classical seed mediated method. Their nanostructures were taken with a transmission electron microscope and their extinction spectra were recorded on a UV-vis scanning spectrophotometer. Their nonlinear optical features were recorded by a home built measurement system, under the femtosecond (fs) laser excitation of 1580 nm, which was selected as the demonstration of the third optical tissue window. A sharp third harmonic generation (THG) signal and a mild three photon luminescence (3PL) signal could be clearly distinguished. These signals could be utilized for multichannel nonlinear bioimaging in the third optical tissue window.

Negatively charged polystyrene sulfonate (PSS) and positively charged polyallylamine hydrochloride (PAH) were separately coated onto the positively charged GNRs through electrostatic adherence. The PAH-PSS coated GNRs were added in the culture medium of HeLa cells. Multichannel nonlinear optical imaging of cancer cells stained with GNRs was performed with a 1580 nm fs laser coupled scanning microscope. The signals were filtered by a 665 nm dichroic mirror, and then splitted by another 560 nm dichroic mirror into the green and red imaging channels. HeLa cells can be clearly visualized in both green and red channels with good contrast. For HeLa cells without GNRs treatment, there were no such signals detected in these channels.

This was a pioneer work of the multichannel nonlinear bioimaging in the third optical tissue window with GNRs and would be helpful for our further studies on in vivo bioimaging in this window with deeper penetration.

1. INTRODUCTION

Gold nanorod (GNR) is a type of metal nanoparticle of tens of nanometers in cylinder cap shape, with a transversal surface plasma resonance peak at around 520 nm and a tunable longitudinal surface plasma resonance (LSPR) peak. It had wide applications in bioimaging and biosensors, and some related studies have been performed in our group [1, 2]. Besides, GNR is appropriate for nonlinear (e.g., two-photon luminescence) bioimaging [3, 4].

Three-photon luminescence (3PL) is a process in which one single molecule absorbs three photons and emits one new photon with higher frequency. As the 3PL signal had a cubic power dependence on the input laser excitation, there would be a rather small focus on the sample, and photobleaching out of focus can be excluded in bioimaging [5]. Together with near infrared (NIR) excitation, the autofluorescence and photodamage towards biosamples were very small, as the energy of photon was small when its wavelength was long. As the excitation wavelength was far from the absorption of the nanoparticles, the photobleach was greatly reduced. In third harmonic generation (THG), three incident photons combine to generate a photon with tripled frequency/energy. In addition to the advantages of 3PL, THG produces no photodamage or photobleach in bioimaging as it is a thermal-free parametric process [6].

In this paper, we studied various nonlinear optical effects (3PL, SHG, THG) of GNRs, with the fs laser excitation from 1500 nm to 1600 nm, which was near the third optical tissue window (1600–1800 nm). In this window, scattering loss of light is very small and the absorption loss of light is not so distinct. Thus, nonlinear optical bioimaging can achieve very deep imaging depth, if fs-excitation with its wavelength in this optical window is adopted [7]. We further performed multichannel (3PL and THG) nonlinear optical imaging of cancer cells stained with GNRs, with a 1580 nm-fs laser coupled scanning microscope. The results would be very helpful to our further studies on applying GNRs in deeper nonlinear optical (THG and 3PL) *in vivo* bioimaging.

2. EXPERIMENTS

The gold nanorods used in our experiments were synthesized according to the seed mediated method suggested by Nikoobakht et al. with minor modifications [8, 9]. The extinction spectra of GNRs were recorded on a UV-vis scanning spectrophotometer (UV-2550, Shimadzu), and the nanostructures were taken with a transmission electron microscope (TEM, JEM-1200EX, Jeol).

The nonlinear optical features of GNRs were recorded by a home built measurement system (As shown in Fig. 1(a)). An optical parametrical oscillator (OPO) pumped by a mode-locked Ti: sapphire fs laser (200 fs, 76 MHz) was used as the excitation source, with output wavelength ranging from 1200 nm to 1600 nm. The linearly polarized near infrared (NIR) laser light was filtered by a 1000 nm long pass filter (Thorlabs), and was converted to be a circularly polarized light by a quarter-wave plate (Union Optic) to suppress the THG signals from the glass slide surface [6]. The laser beam was then directed into a microscope structure and focused onto the sample by a $10\times/0.25$ NA lens (Nikon). The signal was collected along the forward direction through a $40\times/0.75$ NA object lens (Nikon) and coupled into a fiber, which was connected to a spectrometer (PG2000, Ideaoptics Instruments). The as synthesized GNRs were uniformly dropped onto a glass slide and dried for the measurement.

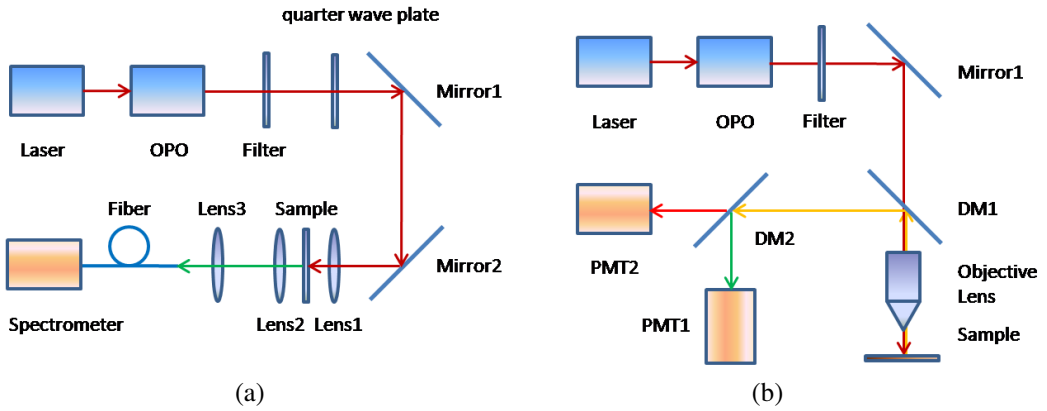

Figure 1: Schematic illustration of (a) the setup for nonlinear optical spectra measurements and (b) the fs laser coupled scanning microscope.

The multichannel nonlinear optical imaging of cells was taken by a scanning microscope (MPM 200, Thorlabs) coupled with the aforementioned fs OPO (as shown in Fig. 1(b)). In the microscope, the fs laser beam (1580 nm) was reflected), focused onto the sample by a $25\times/1.05$ NA objective lens (Olympus). The signal from the sample was epi-collected with the same objective lens, filtered by the 665 nm dichroic mirror, and was splitted by another 560 nm dichroic mirror (DM2, 560–850 nm long-pass, FF560-Di03-25 × 36, Semrock). The optical signals in different imaging channels (green imaging channel: <560 nm; red imaging channel: $560\sim665$ nm) were collected by two separate photomultiplier tubes (PMTs, H7422-40, Hamamatsu). For the bright field image, the sample was illuminated by a halogen lamp and the photos were taken with a charge coupled device (CCD) equipped on the microscope.

Positively charged GNRs were encapsulated with polystyrene sulfonate (PSS, negative charge) and polyallylamine hydrochloride (PAH, positive charge) separately through electrostatic adherence [10]. HeLa cells were cultivated in Dulbecco minimum essential media (DMEM) with 10% fetal bovine serum (FBS), 1% penicillin, and 1% amphotericin B, at 37°C and 5% CO_2. The PAH-PSS-coated GNRs were added in the culture medium. After incubation, the cells were gently washed thrice by phosphate buffered saline (PBS, pH = 7.4, 10 mM) and imaged by the aforementioned scanning microscope.

3. RESULTS AND DISCUSSION

The extinction spectrum and TEM image of as-synthesized GNRs were shown in Fig. 2. They were monodispersed and had a cylinder cap shape, with a dimension of 65×25 nm. The LSPR peak of the GNRs was at 614.5 nm.

Due to the wavelength limitation of the OPO output ($1200\sim1600$ nm), 1580 nm was selected as the demonstration wavelength of the third optical tissue window. The nonlinear emission spectrum

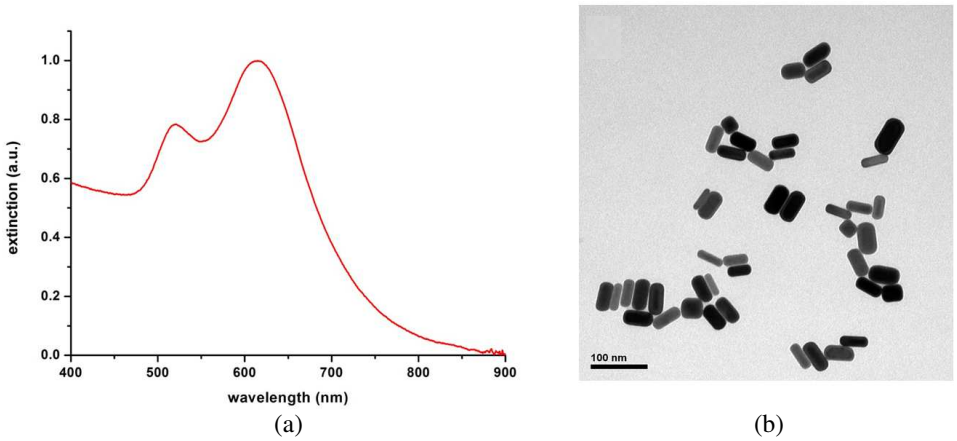

Figure 2: (a) The extinction spectrum and (b) TEM image of GNRs.

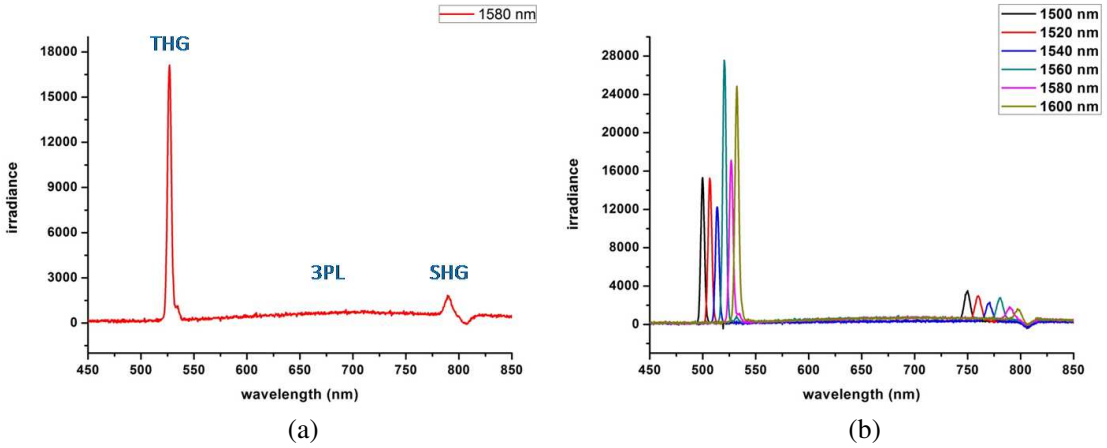

Figure 3: The nonlinear emission spectra of GNRs excited at (a) 1580 nm and (b) 1500–1600 nm.

of GNRs excited at 1580 nm was shown in Fig. 3(a). A sharp THG (at 527 nm) and a relatively weaker SHG (at 790 nm) can be easily discriminated from the spectra. 3PL was recognized by the slight apophysis between THG and SHG signals, and the lower intensity was partially due to the insufficient collection of the objective in the forward direction. The nonlinear optical spectra of GNRs under the fs excitation with various wavelengths were shown in Fig. 3(b). Tunable THG (from 500 nm to 533 nm) and SHG (from 750 nm to 800 nm) signals could be clearly observed, while the envelops of 3PL spectra were almost the same.

Nonlinear optical images of HeLa cells (stained with GNRs) were performed with the 1580 nm-fs laser coupled scanning microscope. HeLa cells can be clearly visualized with good contrast in both green (Fig. 4(a)) and red channels (Fig. 4(b)), and they matched perfectly in the merged image (Fig. 4(c)). There were no optical signals detected in these channels for HeLa cells without GNRs treatment. As SHG at 790 nm was out of the detected wavelength range of the red channel (560 $\sim$ 665 nm), it did not contribute any signals to Fig. 4(b). Thus, the contrast in Fig. 4(b) should be completely from the 3PL of GNRs, according to the spectrum in Fig. 3(a). THG and very small part of 3PL of GNRs contributed to the contrast in the green channel (Fig. 4(a)). As the signal in the green channel was stronger than that in the red channel, it indicated THG signal was stronger than 3PL and it was the main contribution for the green channel. Fig. 4(d) was the bright field image of the rectangle in Fig. 4(c). As we can see, they coincided very well. In our further study, we will apply GNRs in deeper nonlinear optical *in vivo* bioimaging, with the fs laser excitation in the third optical tissue window (1600–1800 nm), where light experiences less attenuation in biological tissues.

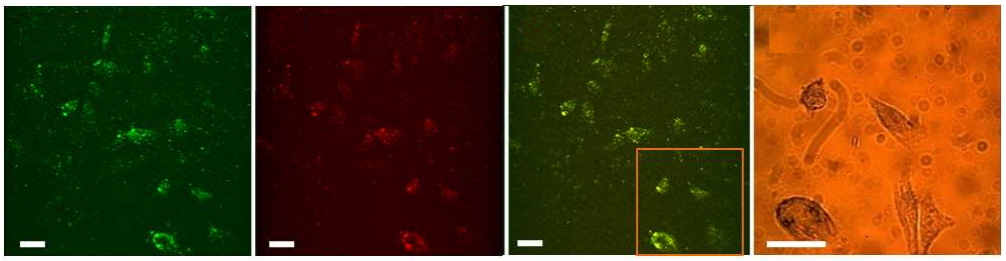

Figure 4: (a)–(c) The nonlinear optical images and (d) bright field of HeLa cells. The scale bar is 20 μm.

4. CONCLUSION

The nonlinear optical responses of GNRs near the third optical tissue window were studied, and THG and 3PL were found to be available. Multichannel (THG and 3PL) nonlinear optical imaging of cancer cells stained by GNRs was firstly demonstrated. The results would be helpful in our further studies on *in vivo* bioimaging in the third optical tissue window.

ACKNOWLEDGMENT

This work was partially supported by the National Basic Research Program (973) of China (2011CB 503700), the Ministry of Science and Technology of China (973 Program; 2013CB834704), the National Science Foundation of China (61275190).

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