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Transient absorption microscopy of gold nanorods as spectrally orthogonal labels in live cells†

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Gold nanorods (AuNRs) have shown great potential as bio-compatible imaging probes in various biological applications. Probing nanomaterials in live cells is essential to reveal the interaction between them. In this study, we used a transient absorption microscope to selectively image AuNRs in live cells. The transient absorption signals were monitored through lock-in amplification. This provides a new way of observing AuNRs with no interference from background autofluorescence.

Metal nanoparticles have shown many promising optical properties as well as other intrinsic characteristics that make them great candidates for biomedical researches. They have been demonstrated as molecular carriers, diagnostic probes, and therapeutic agents [1-4]. Among the variety of metal nanoparticles, gold nanorods (AuNRs) display particularly unique characteristics. They present a diverse range of optical processes, as their electron energy states can be finely regulated by size and aspect ratio of the rods [5]. Furthermore, AuNRs can generate surface plasmon and result in a high intensity local electro-magnetic field, through which the spontaneous Raman signal can be greatly enhanced [6]. AuNRs are strong light scatterers, which ensure their visualization by dark field microscopy [7-11]. AuNRs can also produce both single-photon and two-photon excitation luminescence (TPL) [12-14]. The TPL has shown higher intensity than two-photon excited cellular autofluorescence under the same irradiation power [15]. However, the TPL intensity of AuNRs is not as high as that of the common fluorescent dyes [15]. Moreover, when conjugating AuNRs with intrinsically fluorescent molecules, it can become difficult to distinguish TPL of AuNRs from exogenous fluorescence.

Herein we report a new imaging modality, transient absorption (TA), to selectively image AuNRs in live cells. TA is a mature method in spectroscopic measurement [16-19], and has been coupled with scanning microscopy as a contrast source to image single-walled carbon nanotubes and nanodiamonds, recently [20, 21]. TA, a multi-photon process, is the secondary absorption happening at excitation state after the system’s primary absorption, which can be characterized by absorption spectrum (Figure 1). This nonlinear optical response provides TA with intrinsic 3-dimensional sectioning capability. With transient absorption microscope (TAM), we can selectively highlight AuNRs from any fluorescent environment, providing a superior selectivity and background-free images.

We first studied the optical properties of AuNRs with TAM. The experimental setup has been reported previously [21]. Briefly, two spatially and temporally overlapped picosecond pulse lasers were introduced into a scanning microscope, serving as pump beam (1064 nm) and probe beam (tunable between 780-990 nm) in TAM. The TA signal strength is dependent on the incident power of laser beams. The power dependences on both pump and probe beams were verified by fixing the power of one beam, and altering power of the other beam. TA signal intensity was recorded against b

Fig. 1 Transient absorption of gold nanorods. (a) Primary absorption (pump) happened between the ground state (G) and the first excited state (E1). (b) Transient absorption (probe) is the transition between excited states after the primary absorption. (c) UV-Vis absorption spectrum of gold nanorods. The inset is TEM image of the same sample. The scale bar is 40 nm. (d) Schematic diagram of the transient absorption microscopy system. PD: Photodiode.

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the dependence of signal intensity against the delay time between two pulses (Figure 2 d). There was no signal when the probe pulse was ahead of the pump pulse. As the probe pulse was delayed with reference to the pump pulse, the TA signal firstly increased and then decreased. The asymmetric temporal behavior is a result of the transient nature in this process. The TA signal of AuNRs has much longer lifetime than the pulse width.

![Graph](image1)

**Fig. 2** Characterization of the transient absorption property of AuNRs. (a) The dependence of the TA intensity on the excitation power of the pump beam (1064 nm). (b) The dependence of the TA intensity on the excitation power of the probe beam (850 nm). (c) The dependence of the TA intensity on the probe wavelength. (d) The time-resolved TA intensity (red), with the stimulated Raman scattering signal of dodecane to present the system response (blue).

We then applied TAM to image the cell uptake of AuNRs. HeLa cells were incubated in the culture medium with AuNRs for 8 h, and then imaged with TAM using the wavelength combination of 1064 nm (pump) and 816.7 nm (probe). The imaging system is also capable of performing stimulated Raman scattering (SRS) microscopy [22]. SRS can directly probe Raman-active vibrations, without labeling, from specific chemical bonds or structures, for example, CH$_2$, which is abundant in cellular lipids. However, SRS can only be carried out when the two laser pulse trains are perfectly overlapped in time-domain. Through these wavelengths we used the SRS signal of CH$_2$ stretching (2845 cm$^{-1}$), when the time delay was adjusted to 0, to provide the lipid distribution inside live cells for comparison. When the SRS reveals the lipid components of live cells, we also observed the bright dots scattered in cells, mostly in cytoplasm (Figure 3 a)). The SRS signal of lipid was relatively weak and evenly distributed through the whole cell body except the nuclei, and clearly sketched the outline of cells.

![Graph](image2)

![Image](image3)

**Fig. 3** Imaging AuNRs in live cells. (a)-(c) Imaging AuNRs in live HeLa cells at different delay time between the pulse trains of pump and probe beams. Inset in (c) shows a zoomed region with AuNRs. (d) Two-photo luminescence (TPL) image of AuNRs-containing live HeLa cells stained with calcein AM. (e) TA image of AuNRs demodulated by a lock-in amplifier. (f) Merged image of (d) and (e) showing the distribution of AuNRs in a high fluorescent background. Inset in (f) shows a zoomed region, yellow indicates co-localized TPL and TA signal from AuNRs. Scale bar: 25 μm.

TA, like SRS, can probe the AuNRs in high spatial resolution at the diffraction limit. To verify whether the AuNRs form aggregates in aqueous solutions, we measured the hydrodynamic radius, $R_h$, of AuNRs that dispersed in the
cell culture medium by dynamic light scattering. The apparent R_h,app value determined at 30°C was 18 nm. The diameter was thus comparable to the length of AuNRs (40 nm) measured by TEM, suggesting that AuNRs stayed mainly as single particles in culture medium.

TA signal was acquired by a large-area photodiode that collected all the signals generated by laser excitation within the focal volume, including the fluorescence. However, the TA signal was encoded with the same modulation frequency as the pump beam while the fluorescence signal would not carry this frequency if it could not be excited by the pump laser. With lock-in amplifier which only demodulated the signal with the encoded pumping frequency, we should be able to easily decouple the weak TA signal from strong fluorescence background. We then tested the imaging capability of AuNRs using TAM with the existence of calcine AM, a fluorescent dye to indicate the cell viability [23, 24]. After the HeLa cells were cultured with AuNRs-containing culture medium for 8 h, calcine AM was introduced to stain the live cells. Soon all the live cells exhibited strong two-photon fluorescence under the excitation of probe laser (816.7 nm). This high-intensity green fluorescence spatially well overlapped with the AuNR’s two-photon luminescence emission and make the differentiation of them impossible through the wavelength selection under the two-photon imaging channel (Figure 3(d)). This disadvantage has been created various difficulties of applying AuNRs as labels in biological researches. However, the TA image (Figure 3(e) and (f)) shows only AuNRs with extremely clean background.

This spectral orthogonal imaging modality of AuNRs allows us to use them as a new category of labeling nanomaterials for bioimaging.

In summary, transient absorption can serve as a novel modality to image gold nanorods. The selective imaging capability can distinguish AuNRs from high backgrounds of Raman scattering or wavelength-overlapped fluorescence, avoiding the difficult identification of AuNRs with other optically active species. TAM, excited with near-infrared lasers, also provides the intrinsic 3D imaging capability with high spatial resolution, making it a suitable for live cell imaging using AuNRs as spectrally orthogonal labels. With the long-wavelength excitation provided by near-infrared lasers, TAM is a promising modality in tissue imaging, which would be hard for TPL or dark-field microscopy. By tuning the pump/probe wavelengths, hopefully, multiplex imaging can also be achieved. In addition, the absorption nature in TA process creates the possibility to quantitatively analyze single nanoparticles.

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