Multi-photon evanescent wave (MPEW) excited lanthanide-doped upconverting nanoparticles (UCNPs) for fast single particles tracking and live cell membrane imaging

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Abstract: We propose a novel multi-photon evanescent wave (MPEW) excitation modality for upconverting nanoparticles (UCNPs) based microscopy. Experimental results show this new imaging method for UCNPs is nonscanning, ultrahigh contrast and high spatiotemporal resolution.
OCIS codes: (190.4180) Multiphoton processes; (240.6690) Surface waves; (180.4315) Nonlinear microscopy

1. Introduction

Upconverting nanoparticles (UCNPs) constitute a novel type of contrast agent with interesting and unique properties for luminescence bioimaging. Superior to organic dyes and quantum dots (QDs), UCNPs can emit anti-Stokes shifted light after being excited by 900-1000 nm CW laser light. Today, the research topic of *UCNPs* for biomedical applications is extremely popular, in particular related to optical bioimaging. UCNPs exhibit practically no autofluorescence, large anti-Stokes shifts, sharp emission band, nonphotobleaching, nonblinking, deep detection ability and a high spatial resolution[1]. In recent years UCNPs have therefore attracted remarkable attention in the biophotonics area. UCNPs have widely been employed in microscopy, small animal diffusion imaging, diffusion optical tomography, multimodal animal imaging, biosensing and photodynamic therapy (PDT) [2, 3].

Due to the multiphoton excitation process involved, UCNP-based microscopy can yield high resolution only with CW excitation [4]. Apart from their anti-Stokes emission characteristics, UCNPs are highly photostable and display non-blinking emission in contrast to QDs. Compared with red and blue organic dyes, green UCNPs exhibit exceptional photostability [5]. The absence of photobleaching and photoblinking enables precise tracking of single UCNPs. Thus, individual UCNPs possess such ideal properties suitable for single nanoparticles imaging, which enable one to use single UCNPs for probing single proteins, such as mapping single proteins moving through a cell, neurons cell interaction, and the process in brain cells connecting together to form a synapse [6].

However, almost all the UCNPs based microscopy studies were performed in confocal modality, where long scanning time is required [7]. It is because that the long lifetime in emissive organic dyes and nanoparticles would result in relatively low photoluminescence intensity under the unsaturated power density level. Thus, it is not possible to fleetly track single particles in order to record very quick bioprocesses. Wide field microscopy could be an alternative resolution for time-consuming laser scanning upconversion microscopy. However, the excitation light has to go through cell samples and growth media. This will induce heating effect (to damage the cell activity) and unwished scattering of both excitation and emission (to blur the image and to decrease resolution) [1, 8]. We propose a novel multi-photon evanescent wave excitation modality for single lanthanide-doped UCNPs imaging and tracking as well as *in vitro* cancer cell membrane imaging. Experimental results show this new imaging method for UCNPs is nonscanning, ultrahigh contrast and high spatiotemporal resolution.

2. Principles and configuration of the system setup

In total internal reflection microscopy (TIRM), a small portion of the reflected light penetrates through the interface and propagates parallel to the surface in the plane of incidence creating an electromagnetic field in the liquid adjacent to the interface. This field is termed the evanescent wave (EW), and is capable of exciting emissive samples (or being scattered by nanopatilces) residing in the immediate region near the interface. The EW intensity I decays exponentially with the increasing distance z from the interface according to the following equation:

$$I(z) = I_0 e^{-z/d} \tag{1}$$

Here, I_0 is the intensity at the interface. The characteristic penetration depth (d) at λ, the wavelength of incident light in a vacuum, is given by:

$$d = \lambda \Big/ (4\pi \sqrt{n_1^2 \sin^2\theta - n_2^2}) \quad (2)$$

where n_1 is the refractive index of the cover glass; n_2 is the refractive index of the water solution and θ is the incident angle on the glass/water interface. The penetration depth d is independent of the incident light polarization direction, and decreases as the reflection angle gets larger. This value is also dependent upon the refractive indices of the media present at the interface and the illumination wavelength. In general, the value of d is on the order of the incident wavelength, or perhaps somewhat smaller. When the incident angle equals the critical value, d goes to infinity, and the wavefronts of refracted light are normal to the surface.

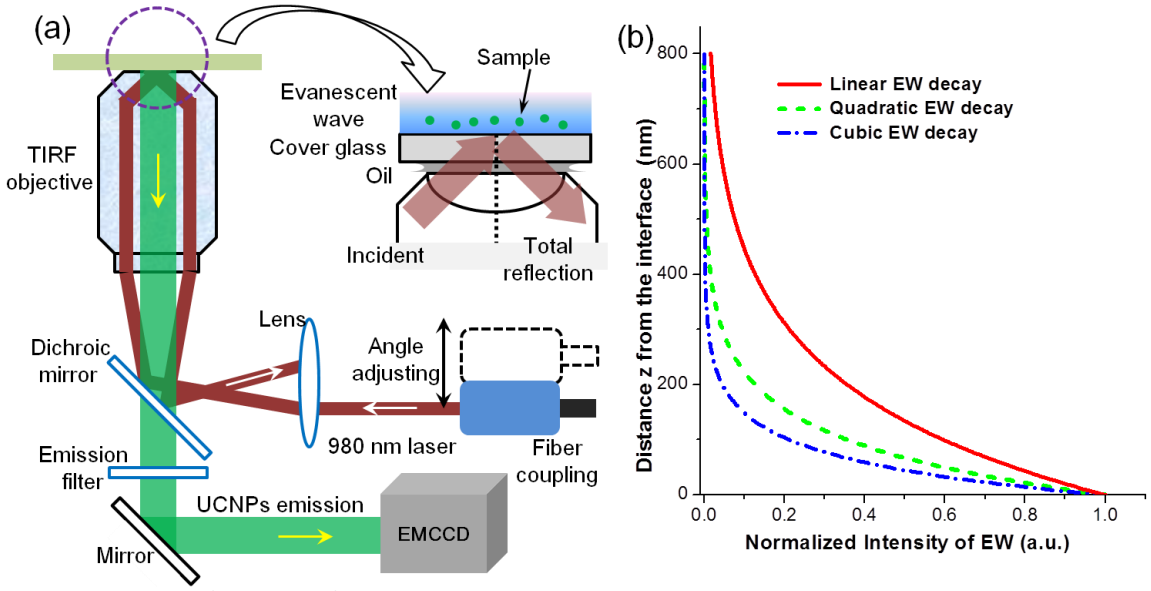

Fig. 1 (a) Schematic diagram for the setup of the proposed objective-based TPEW imaging system. Detailed light-path is shown with red standing for excitation laser beam (980 nm) and green light standing for luminescence signal. TIRF objective: 100 ×, NA=1.45. (b) Calculated intensity decay curves of EW excited luminescence for three cases: linear excitation process, two-photon excitation process (green and red emission of UCNPs) and three-photon excitation process (blue emission of UCNPs).

In this paper the TPEW was performed based on an Olympus TIRM illuminator accessory for the inverted IX-71 microscope, as shown in fig. 1(a). A 980-nm single mode laser is coupled to the microscope via an external port. The alignment of the input fibre connector with the microscope optical path is adjustable in order to optimize the incident light angle through high numerical aperture objectives. Appropriate Dichroic mirror and filter were selected for excitation and emission. The upconversion luminescence from UCNPs went into an EMCCD (Andor 885). Different from linear fluorescence, multi-photon process of UCNPs will shorten the detection depth. According to Eq. (2), the value of d for 980-nm laser EW is 193.6 nm (n_1(cover glass)=1.518, n_2(water)=1.33, incident angle 66^o). The corresponding detection depths for two-photon excitation (green and red emission of UCNPs) and three-photon (blue emission) excitation are 96.8 nm and 64.5 nm, respectively. The three decay curves were shown in Fig. 1(b).

3. Results and discussion

The used $Er^{3+}/Ho^{3+}/Tm^{3+}$-doped $NaYF_4$ UCNPs in this work were synthesized and surface functionalized according to our previously reported work[1]. These prepared UCNPs sample were employed to perform fast single nanoparticles tracking and live cell membrane imaging. TIRM imaging mode can only detect the UCNPs located within the depth of 96.8 nm (two-photon $NaYF_4$:Yb^{3+}/Ho^{3+}) and thus eliminate the background of z direction. Compared to Fig. 2 (a), Fig. 2 (b) have no scattering background signal as well as higher spatial resolution. Then highly diluted UCNPs aqueous suspension of different emissions (only with result of $NaYF_4$:Yb^{3+}/Er^{3+} shown in Fig. 2(c)) were used to perform single particle tracking. MPEW excitation doesn't need scanning and facilitates fleet real-time monitoring the moving of single nanopartiles. One particle was analyzed with 100 ms record time interval and the results were shown in picture No. 1 to 25. The detailed moving trace of this UCNP can be record during the time of 2.5 s, which probably is not enough for finishing one scanning in UCNPs based laser imaging system. Single particle imaging and fast tracking were well demonstrated, which is significantly advantageous over other UCNPs imaging modality. TIRM modality is a powerful tool for cell membrane study. In this work well functionalized UCNPs were also employed to label HeLa cancer cells[9]. The cancer cells experiments were performed similar to our previous work[1]. As shown in Fig. 3, the positions of UCNPs luminescence agree well with the position of cancer cells, which demonstrate the successful live cell membrane imaging of MPEW excited UCNPs.

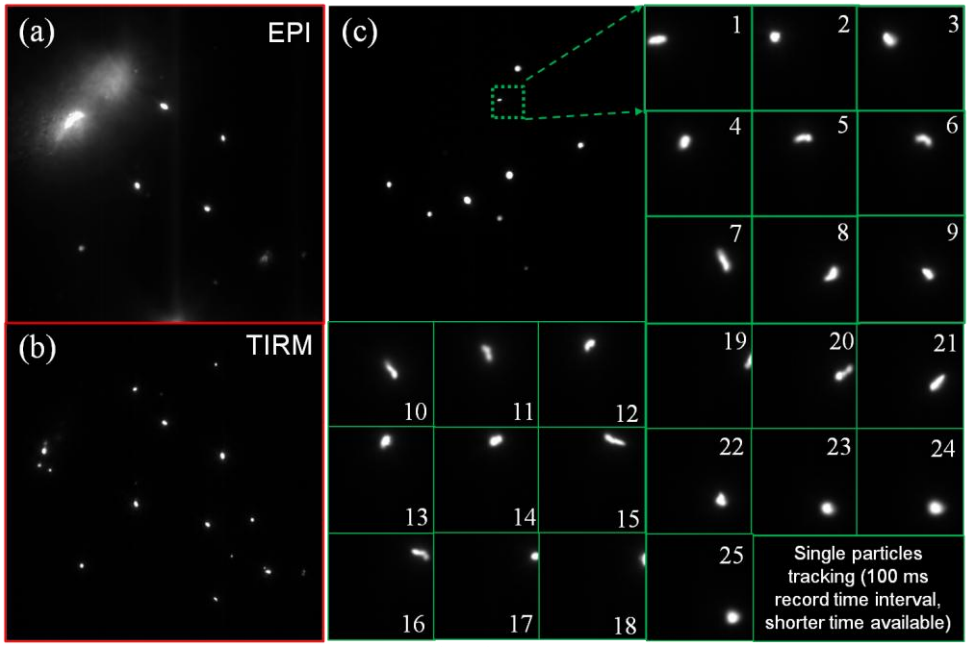

Fig. 2 Comparison of luminescent microscopy from $NaYF_4$:Yb^{3+}/Ho^{3+} in epifluorescence (a) and TIRM imaging (b) modes at water-glass interfaces; fast single particle tracking in water solution (c): picture NO.1 to 25 shows the fast moving with a record time interval of 100 ms.

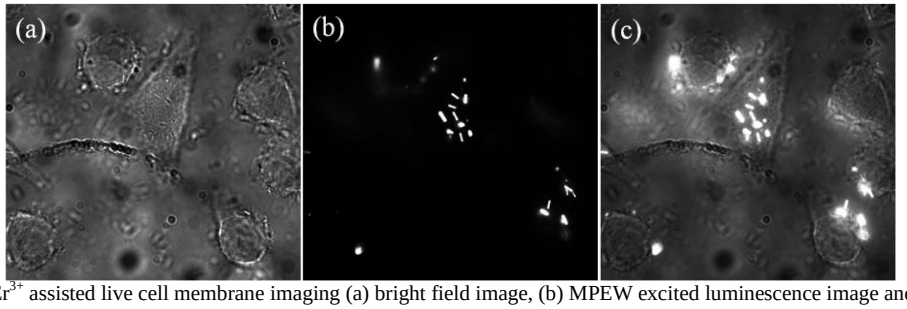

Fig. 3 $NaYF_4$:Yb^{3+}/Er^{3+} assisted live cell membrane imaging (a) bright field image, (b) MPEW excited luminescence image and (c) overlay image.

Conclusion

For the first time a novel MPEW was proposed to excite lanthanide-doped UCNPs for microscopy. The analyzed experimental results reveal that this new imaging method for UCNPs is nonscanning, ultrahigh contrast and high spatiotemporal resolution. This novel technique is very promising for many applications, such as single particles imaging, single protein tracking and single events monitoring happed in the live cell membrane.

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