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1.319 μm excited thulium doped nanoparticles for subtissue thermal sensing with deep penetration and high contrast imaging

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ABSTRACT

We report on the potential application of NIR–NIR two-photon excited fluorescence of Tm^{3+} doped sodium niobate ($NaNbO_3$:Tm) nanoparticles with both emission (800 nm) and excitation (1.319 μm) operating respectively within first and second biological windows for high resolution and deeper penetration bio-images. *Ex vivo* tissue depth using 1.319 μm was achieved as high as twice in comparison with the already conventional 800 nm excitation. The spectral profile of Tm^{3+} upconversion luminescence was analyzed in detail in the biophysical temperature range. A twofold enhancement of the solely detected 800 nm upconversion emission was obtained in the 22–90 °C temperature interval. The population redistribution of the thermally coupled sub-levels was demonstrated useful for ratiometric nanothermometry. The outstanding penetration depth together with the fluorescence thermal sensitivity make the $NaNbO_3$:Tm nanocrystal an excellent candidate as multifuncitional nanoprobe for high contrast and highly penetrating fluorescence imaging.

1. Introduction

Advances in luminescent nanoparticles (LNPs) based nanotechnologies have been the subject of intense study in the last decade owing to mainly their great potential of applicability in modern biophotonics [1–3]. Amongst many applications it can stand out in luminescent nanothermometer (LNTh) [4–8], high resolution bio-imaging, and hyperthermia for diagnosis and therapy of diseases [2,9–13]. The LNTh exploits the luminescence properties of LNPs whose emission is strongly affected by small temperature variations. A proper spectral analysis of the luminescence may be used to determine the actual nanoparticleís temperature as well as the local temperature in which they are incorporated. The LNTh has been mainly used in biomedicine towards the knowledge of the local temperature of many systems, being of particular interest in biological ones (such as living cells) [7,14,15], as well as in real-time temperature control in the treatment of cancerous tumors in small animal through hyperthermia [8,16–18].

Considering the different LNThs already demonstrated elsewhere [4,5], one can highlight those based upon emission signals from lanthanide-doped upconversion nanoparticles (UCNPs) [19–22] and also downconversion nanoparticles (DCNPs) [8,10,11,14,23–25]. Typically, UCNPs based LNThs use successive absorption and/or energy transfer (ET) of near-infrared (NIR) photons to promote population to higher excited-states which eventually lead to emission of higher-energy photons with wavelengths residing in the ultraviolet, visible, or NIR spectral regions [19,21,26–29]. The multiphoton absorption based excitation process, in principle, leads to higher spectral and spatial resolutions [2,9,30–32]. However, the majority of LNThs based NPs using upconversion (UC) emission, operate in the visible region of the electromagnetic spectrum and in this case conventional multiphoton and epifluorescence microscope could be easily adequate for both imaging and in some cases even sensing [7,33]. Nevertheless, despite the good results obtained so far, the practical application of LNThs with visible emitting LNPs is severely limited in biological systems because the light emitted in this wavelength range exhibits reduced penetration depth into human body mainly due to the combination of scattering (owing to tissue inhomogeneity) and absorption of light (caused by different compounds present in real tissues such as blood) by human tissues [34].

In order to overcome those technical obstacles, a great deal of effort has been paid to the development of LNPs operating within the so-called “biological windows” (BWs) of human tissues [9,16,34]. BWs are spectral regions where tissues are partially transparent, thus the light with these particular wavelengths can achieve large penetration depths into tissues [10,14,35]. Traditionally, two BWs are well defined: the first BW (I-BW, which

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extends from 650 up to ~940 nm) and the second BW (II-BW, 1000–1400 nm). These two BWs are separated by the water absorption band peaked at 980 nm (peak of absorption of the main ion used as sensitizer, Yb^{3+}) that limits the penetration length of light into tissues. This large penetration depth into tissues is caused in the I-BW by a negligible absorption of typically components present in tissues, such as water and hemoglobin. However, in the II-BW it is caused by reduced scattering coefficient associated with these longer wavelengths [34,36]. Accordingly, the light penetration depth is limited and the imaging quality is reduced. Therefore, an ideal photo-luminescent probe applied *in vitro* and *in vivo* imaging should have high photoluminescence quantum efficiency; appropriate spectral range of 700–940 nm or 1000–1400 nm for emission/excitation wavelengths to minimize background emission and provide deep light penetration in the tissues; and a large spectral shift between the emission and the excitation light to minimize excitation-light-induced background [11,37].

Most recently, many research groups have exploited the Nd^{3+} ion as a sensitizer due to its main absorption band around 800 nm that lies within the I-BW. This idea is very interesting as to DCNPs operating at the I-BW [11,14,38] and at the II-BW [8,10,31,39], as well as to UCNPs actuating in the visible region [7,13,40,41]. On one hand, NIR-to-NIR UCNPs for *in vivo* bioimaging has already been demonstrated of whole–body animal, but only using Yb^{3+} as sensitizer [21,42,43], limiting the imaging resolution due to water absorption and therefore the tissue penetration depth. In these cited works, the used activator was the Tm^{3+} ion, which used the NIR emission ($^3H_4 \rightarrow {}^3H_6$) within "optical transmission window". On the other hand, NIR emission from Tm^{3+} ions is usually accompanied of competitive blue and ultraviolet UC luminescences and this limitation in emission selectivity prevents the progress of multiplexed applications. As a matter of fact, emission selectivity is still a challenge so far. To address this problem, a series of strategies have been developed, including controlling the lanthanide concentration [44], using pump power as a controller [45,46], incorporating extrinsic energy level from other lanthanide or transition metal ions and manipulating the energy transfer by introducing acceptors within the NPs in specific positions [44,47]. However, diverse effects could occur when using the aforementioned approaches. For example, ions concentration in excess could lead to concentration quenching and as such reducing the UC emissions; although the very interesting multi-shell manipulation to favor or disfavor specific emission, it is a tedious fabrication process that would demand a lot of tests to an effective result; power controller would not be effective for several specific application, mainly in biosystems; etc. Therefore, the efficient modulation of UC selectivity still requires great effort of the scientific community. However, in the present work we report on a two-photon (TP) excited single Tm^{3+} doped sodium niobate ($NaNbO_3$) UCNP addressing many of the pointed out open issues such as: NIR-to-NIR UCNPs with emission and excitation within I-BW (800 nm) and II-BW (1.319 μm), respectively; the 800 nm emission is very efficient and the sole emission detected, then presenting more of 500 nm of separation to the excitation; and thermal sensitivity was obtained at this emission band in the surrounding of bio-physiological temperature range. The $NaNbO_3$ host is a wide band gap (3.08 eV) perovskite oxide with unique physical and chemical properties such as low density, high sound velocity, photo-refractive and photo-catalysis effects [48]. The non-linear optical properties of alkali niobate have been studied, such as second harmonic generation [49]. Recently the degradation behavior and the properties of cytotoxicity of potassium sodium niobate piezoelectric ceramics were used for bio-medical applications [50]. Moreover, two and three-photon excited LNTh based on UC emission in the visible region of Er^{3+}/Yb^{3+} and Tm^{3+}/Yb^{3+} co-doped $NaNbO_3$ powders, respectively, under 980 nm excitation have been demonstrated [19,20].

2. Experimental section

2.1. Synthesis

Thulium doped single-phase $NaNbO_3$ nanocrystals ($Na_{(1-0.01\,x)}Tm_{(0.01\,x)}NbO_3$ where x = 1, 3 and 5, hereafter $NaNbO_3$:xTm NPs) were synthesized by the Pechini sol–gel method, as described elsewhere [51]. Briefly, about 19.2 g of citric acid (Aldrich, 99.5%) was added to 10 ml of water under stirring and heating at 75 °C. After dissolution, 0.1836 g of ammonium niobium oxalate was dissolved and then stoichiometric quantities of Na_2CO_3 (Aldrich, 99.9%), and $Tm(NO_3)_3{\cdot}H_2O$ (Aldrich, 99.99%) were added to the above transparent solution. Finally, 5.6 ml of ethylene glycol (Aldrich, >99%) was added to the solution under regular stirring for 1 h at 75 °C and then the reaction mixture was cooled to room temperature. A gel was obtained after a heat treatment at 90 °C for 3 days. The gel was first heated at 400 °C for 2 h, where the carbaneous precursor was formed and then a white fluffy powder was obtained at 800 °C, which was the heat treatment temperature for 3 h.

2.2. Experimental apparatus

Fluorescence experiments were carried out in powder and also by dispersing the $NaNbO_3$:xTm NPs in distilled water generally at a concentration of 3–5% in mass. The excitation source was a continuous-wave krypton-arc lamp-pumped Nd:YAG laser (Quantronix 416-2L) operated at 1.319 μm. The laser delivered a maximum power of 1.5 W and which could be controlled using a polarizer. The pump beam was focused down onto the samples by a 5 cm focal length lens and the beam waist at the samples location was ~40 μm. Thus, excitation density into the samples of up to 60 KW/cm^2 was achieved for the laser maximum output power of 1.5 W. The fluorescence signal was collected directly from the sample by means of a 200 μm diameter fiber-bundle in a direction perpendicular to the pump beam. Neither a collecting lens nor a signal amplifying mechanism was used in all the measurements herein reported. No signal light by the pump laser itself was detected in the spectral range of the emission signals herein reported, even knowing the potential of the investigated sample to second harmonic generation [49]. Temperature induced modifications in the NIR luminescence of $NaNbO_3$:xTm NPs were investigated by placing the powder or colloidal solution inside a homemade temperature controller operating between room temperature up to 200 °C with an estimated temperature stability better than 1 °C. The particle size and morphology were evaluated using transmission electron microscope (TEM, TECNAI20, resolution of 0.2 nm) with an accelerating voltage of 200 kV. For TEM investigations, powders were suspended in water solution, and a drop of this suspension was put on a holey carbon-coated film supported on a 300 mesh copper grid.

3. Results and discussion

Fig. 1(a) and (b) exhibits TEM images of the $NaNbO_3$:Tm NPs used along this work. The synthesized sodium niobate nanocrystals have cube geometry with average edge dimensions of 70 ± 10 nm, similarly as recently obtained and published [19,52]. All the as-synthesized NPs are dispersible in distilled water with an outstanding colloidal property and without any evidence of precipitation during weeks, tested for NPs concentration of 3% in mass (NPs in mass per distilled water in mass). Fig. 1(c) shows typical room-temperature UC luminescence spectra of radiation emanating from the NPs doped with 1.0 mol% of Tm^{3+} ions under excitation at 1.319 μm, for three different pump powers. It can be seen that the