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Yb^{3+}/Tm^{3+} co-doped $NaNbO_3$ nanocrystals as three-photon-excited luminescent nanothermometers

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ABSTRACT

In this paper we report on Thulium/Ytterbium codoped sodium niobate ($NaNbO_3$) colloidal nanocrystals as high resolution luminescent nanothermometers operating under three-photon excitation. The spectral shape of the Thulium ($^1G_4 \rightarrow {}^3H_6$) luminescence band at 480 nm after 980 nm (Ytterbium) excitation has been analyzed in detail in the biophysical temperature range. It has been found that the population redistribution among the thermally coupled stark levels could be successfully used for ratiometric thermal sensing. The thermal sensitivity of the three photon excited luminescence has been successfully used to evaluate, in a single-beam experiment, the light induced thermal loading taking place in 940 nm excited $NaNbO_3$:Tm^{3+}/Yb^{3+} nanocrystals.

1. Introduction

Temperature is a fundamental parameter whose knowledge is essential in a great variety of areas including industry, electronics, energy, engineering and so on. In the particular case of biomedicine accurate temperature sensing becomes essential since temperature is one of the most critical parameters affecting the dynamics of living specimens [1]. For instance, when the body temperature falls out of the biophysical range, 34–45 °C, irreversible effects such as damage and alterations of basic living functions can appear [2,3]. This is due to the strong temperature dependence of cellular dynamics, in which abnormal temperatures (different from 37 °C) could induce irreversible effects such as changes in membrane polarity and permeability, denaturalization of cellular components (enzymes, lipids, nucleic acids, etc.), modification of cell division rate and thermal activation/de-activation of intracellular reactions [4,5]. In addition, many diseases or health dysfunctions (such as tumor development, inflammation and circulatory problems) induce relevant local temperature variations in their early stages so the detection of intra-body small temperature anomalies could be used as an effective early detection procedure [6]. Thermal sensing does not only find application in diagnosis but also in therapy procedures. In particular, the application of hyperthermia treatments [7–9], where malignant cells are driven up to the cytotoxic level (above 45 °C), require continuous temperature reading so that dynamical adjustment of irradiation conditions would be achieved.

High resolution (sub-micrometric, sub-degree) thermal imaging in living systems, either at the single cell or at the small animal scales, is not an easy task at all. Indeed, it constitutes a very active and challenging research area. Among the different approaches proposed in the past for high resolution thermal imaging of biosystems, the use of Luminescent nanothermometers results of special interest [10]. A luminescent nanothermometer (LNThM) is referred as a luminescent nanoparticle (NP) with a strongly temperature dependent luminescence, so that a proper analysis of its luminescence could lead to an accurate thermal reading [11]. In recent years, a great number of LNThM has been introduced to the scientific community by numerous research groups including metallic NPs, semiconductor nanocrystals (Quantum Dots) and rare-earth doped nanocrystals [10–17]. Summarizing reviews of the different systems employed up to now as LNThM can be found elsewhere [10,11].

Among the different LNThM already demonstrated, those based on rare earth luminescent ions present clear advantages such as superior chemical and physical stability, low toxicity, narrow luminescence lines, and absence of aging effects among others. In most of the rare earth doped LNThMs, thermal reading becomes possible thanks to the existence of luminescence lines simultaneously generated from two electronic levels with very similar energy

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(separated by a reduced energy gap). When this energy separation between emitting electronic states or stark levels is comparable to or smaller than $k_B \times T_R = 208\,cm^{-1}$ (being k_B and T_R the Boltzman's constant and the room temperature, respectively), it is said that these electronic states are thermally coupled levels (TCLs). In this case, small temperature changes could produce remarkable changes in the population re-distribution and, consequently, on the intensity ratio between the different emission. "Ratiometric" thermal sensing [18–20] (temperature determination from the analysis of the intensity ratio between two luminescence lines) is possible. This possibility has been successfully demonstrated in a great variety of rare earth doped systems. Probably Er/Yb doped systems are the most popular ones. In these systems the thermosensitive Erbium emission is produced after energy transfer from Ytterbium ions, this being a two-photon (2P) process [21–23]. The fact that the luminescence providing thermal reading is excited by multi-photon processes becomes specially interesting since it opens the possibility of fluorescence thermal imaging with superior spatial resolution when compared to that achievable using one photon excited LNThMs (such as Neodymium doped NPs) [24,25]. 2P ratiometric thermal sensing was also demonstrated in infrared emitting Yb/Tm co-doped NPs with also excellent results [17,26,27].

As mentioned above, the main advantage of 2P over one-photon excitation is the substantial improvement achieved in the spatial resolution due to the second order nature of excitation process and the use of shorter emission wavelength [24]. Further resolution improvement would, therefore, be obtained if the thermo-sensitive luminescence would be excited under a three-photon (3P) excitation mechanism. Indeed, it has been reported how high resolution/high penetration images of living neurons can be achieved by using 3P excited luminescent nanoprobes [12]. Furthermore, S. Maiti et al. demonstrated that 3P microscopy could be used for high resolution imaging of serotonin distribution inside living cells [15] and Caillat et al. provided experimental evidence about the potential use of multiphoton upconversion rare earth doped nanocrystals for sub-diffractive microscopy [24]. In particular, Caillat and co-workers demonstrated that a 3P excited process gives a superior spatial resolution since it leads to a significant reduction in the effective laser spot size. There are in the literature recent works in which 3P excited emissions of rare earth ions have been tentatively explored for thermal sensing. This is the case of Yb/Tm and Yb/Ho co-doped luminescent systems [28–33]. In some cases, high resolution ratiometric thermal sensing has been demonstrated, but only using bulk materials and not in aqueous colloidal solutions, limiting their potential applications. At this point, the work published by X. Wang et al., [17] deserves special mention. Authors demonstrated the ability of the 3P exited emission of Tm ions from $NaYbF_4$ nanocrystals with remarkable sensitivity at low and high temperatures (100 and 700 K) but with almost vanishing sensitivity in the surroundings of room temperature [17]. This fact limits the application of the system proposed by X. Wang et al. for monitoring thermal events occurring at room temperature, such as biological related ones. To the best of our knowledge there is up to now no reports on 3P excited LNThMs with appreciable thermal sensitivity at room temperature, despite their intrinsic interest for high resolution thermal imaging of biosystems.

In this work we report on a 3P-excited LNThM constituted by water dispersible sodium niobate ($NaNbO_3$) NPs codoped with Thulium and Ytterbium ions that actuate as activator and sensitized centers, respectively. The $NaNbO_3$ host is a wide band gap (3.08 eV) pervoskite oxide with unique physical and chemical properties such as low density, high sound velocity, photo-refractive and photo-catalysis effects [34]. Alkali niobates have been studied for their promising non-linear optical properties, such as second harmonic generation [35] and recently cytotoxicity and degradation behaviors of potassium sodium niobate piezoelectric ceramics were explored for bio-medical applications [36]. Moreover, Er^{3+}/Yb^{3+} or Tm^{3+}/Yb^{3+}doped $NaNbO_3$ powders show significant upconversion emission in the visible region upon 980 nm excitation [13,37] so they can be used as multi-photon luminescent probes. In this work we report as the temperature sensitivity of the 3P excited Thulium emission, at around 480 nm, suffers in the surroundings of room temperature. The potential use of these spectral changes for thermal sensing is demonstrated and the obtained thermal sensitivities are compared to those previously reported for other LNThMs.

2. Experimental

2.1. Synthesis of nanoparticles

Thulium and Ytterbium co-doped single-phase $NaNbO_3$ nanocrystals ($Na_{0.94}Tm_{0.01}Yb_{0.05}NbO_3$, hereafter Tm/Yb:$NaNbO_3$ NPs) were synthesized by the Pechini sol–gel method, as described elsewhere [13]. 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 $Ln(NO_3)_3{\cdot}H_2O$ (Ln = Tm and Yb) (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. The rare earth concentrations used here were chosen to optimize the pure crystal phase structure (pure orthorhombic) and upconversion luminescence as we have previously investigated [37,38].

2.2. Three photon excited fluorescence thermometry experiments

Fluorescence experiments were performed by dispersing the Tm/Yb:$NaNbO_3$ NPs in distilled water at a concentration of 5% in mass and also in powder. The sample was optically excited with a temperature stabilized continuous wave (cw) diode laser (THORLABS with LD and EC controllers), at a central wavelength of 976 nm. Excitation power was controlled by controlling laser diode current. The generated fluorescence was collected by an optical fiber connected to a high resolution spectrometer (64 cm single-grated monochromator with 0.1 nm resolution - McPHERSON, model 207), that allows for accurate spectral analysis in the 300–1100 nm spectral range using an amplifier lock-in (Stanford Research System, model SR530) and an Hamamatsu S-20 photomultiplier tube. Temperature induced modifications in the visible luminescence of Tm/Yb:$NaNbO_3$ NPs were investigated by placing the colloidal solution or the powder inside a homemade temperature controller operating between room temperature up to 80 °C with an estimated temperature stability better than 1 °C. For laser induced heating, and in order to avoid excessive heating due to strong absorption at 976 nm, the Tm/Yb:$NaNbO_3$ NPs were optically excited with a 940 nm fs Ti:Sapphire laser (Coherent, model Chameleon Ultra II) with repetition rate of 80 MHz, i.e., practically in regime cw. The Ti:Sapphire laser was used in pump induced heating experiments instead of the laser diode because it could lead to larger pump intensities.