

The synthesis and upconversion properties of new promising biomarkers: NaYF₄:Yb³⁺,Tm³⁺ nanocrystals

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I . Introduction

Recently, fluorescent labelling has attracted a great deal of interest due to its diverse applications in the fields of single virus tracing^[1], ion channel detection^[2], gene delivery^[3] and DNA sequencing^[4], etc. Traditionally, organic dyes and fluorescent proteins are utilized in imaging of cells and tissues. These fluorophores emit Stokes shifted light upon ultraviolet (UV) and visible excitation, which make them suffering the intrinsic drawbacks of severe photobleaching and autofluorescence^[5, 6].

Recent developments of quantum dots (QDs) have brought a new dimension to bioimaging^[7, 8]. QDs can emit large Stokes shifted and narrow emission spectra under the excitation of UV light, the wavelengths of which can be adjusted by changing their size and aspect ratios. QDs are more stable against photobleaching and have high quantum efficiency. Their main defects are their toxicity and low penetration of the excitation light^[9, 10, 11].

Upconversion (UC) nanoparticles have revealed to be an excellent kind of biomarkers in bioimaging due to their unique optical property to emit large anti-Stokes shifted emission upon near-infrared (NIR) excitation^[12, 13]. The “optical transmission window” of the biological tissues in NIR range allows for deeper light penetration and results in lower autofluorescence and reduced light scattering, thus producing increased image contrast^[14, 15]. In this summary, we report the synthesis and UC properties of new promising biomarkers, NaYF₄:Yb³⁺, Tm³⁺ nanocrystals, emitting at 800 nm.

II. Experimental

Chemicals

NaF, NaOH, and oleic acid were purchased from the Beijing Chemical Reagent Company. Y(NO₃)₃•6H₂O, Yb(NO₃)₃•6H₂O, and Tm(NO₃)₃•6H₂O were supplied by the Shanghai Chemical Reagent Company. All chemicals were of analytical grade and used without any further purification.

Solvothermal Synthesis of NaYF₄ Nanocrystals.

The NaYF₄:Yb³⁺, Tm³⁺ upconverting nanocrystals were synthesized through a solvothermal method. In a typical synthesis, 20 ml oleic acid, 1.2 g sodium hydroxide, 7 ml ethanol, 9 ml water, 0.6 ml aqueous solution of thulium ions, 3.4 ml aqueous solution of sodium fluoride were added to a 50 ml autoclave tube under agitation. And then the reaction was controlled at 180°C for 8 hours. After the reaction was cooled to room temperature, the nanocrystals were collected at the bottom of the vessel. These as-prepared nanocrystals are nearly monodispersed in cyclohexane transparently with oleic acid coating^[16, 17].

Surface silica-coating

In order to make as-prepared nanocrystals water-soluble, silica-coating was performed through a reverse microemulsion method^[18].

Transmission Electron Microscopy (TEM)

The size and morphology of the raw and silica-coated NaYF₄:Yb³⁺, Tm³⁺ nanocrystals were characterized using a JEM-2010HR transmission electron microscope (TEM). Samples were prepared by placing a drop of dilute cyclohexane or water dispersion of corresponding nanocrystals on the surface of a copper grid.

Fluorescence Spectroscopy

The upconversion (UC) emission spectra were measured under a power-tunable 980 nm laser diode

excitation and detected with a lens-coupled monochromator attached with a photomultiplier.

III. Results and Discussion

Figure 1 shows a TEM micrograph of the NaYF₄:Yb³⁺, Tm³⁺ nanocrystals. As evident in the figure, these nanocrystals are nearly monodispersed in cyclohexane, which were uniform in size with a narrow size distribution. The average diameter of the nanocrystals is about 20 nm. This is of significance at the starting point for developing UC biomarkers, since it provides the possibility to perform surface coating and functionalization, in order to make them biocompatible.

Figure 2 shows the TEM image of the SiO₂-coated NaYF₄:Yb³⁺, Tm³⁺ nanocrystals. The nanoparticles represent good dispersity and relatively homogeneous size distribution, which are prerequisites of being served as biomarkers.

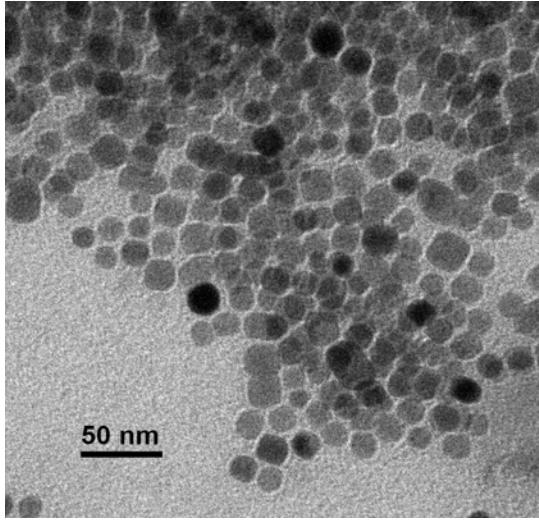


Figure 1 The TEM micrograph of the NaYF₄:Yb³⁺, Tm³⁺ nanocrystals

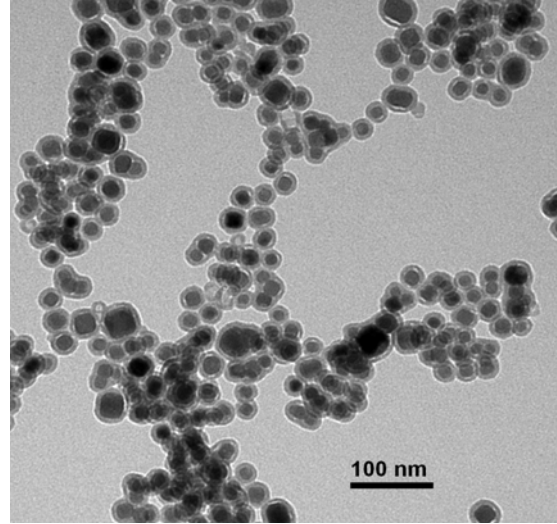


Figure 2 The TEM image of the SiO₂-coated NaYF₄:Yb³⁺, Tm³⁺ nanocrystals

Figure 3 shows the UC emission spectrum of the NaYF₄:Yb³⁺, Tm³⁺ nanocrystals. The observed UC bands at 477 nm and 800 nm can be assigned to intra-4f electronic transitions ¹G₄→³H₆ and ³H₄→³H₆ of Tm³⁺ ions, respectively^[17]. The blue emission center at 477 nm is only visible for higher pump intensities. The pump power dependence of the 800 nm line was investigated. It is known that the number of photons that are required to populate the upper emission state can be obtained by the

following relation: $I_f = P^n$ (1),

where I_f is the fluorescent intensity, P is the pump laser power, and n is the number of the laser photons required. As shown in the inset of **Figure 3**, the n value for the emission center at 800 nm is 2.0, which means that a two-photon process is involved to populate state ³H₄ (Tm³⁺). Because of the quadratic dependence, there is an inherent 3-D localization of 800 nm UC emission, which can be utilized in the development of 3-D imaging systems^[15].

Figure 4 shows the energy levels of Tm³⁺ and Yb³⁺ ions, as well as the proposed mechanism to produce the 477 nm and 800 nm fluorescences. Here Yb³⁺ ions act as sensitizers, which absorb the energy of the pump laser and transfer to nearby Tm³⁺ ions. First, an Yb³⁺ ion in the ²F_{7/2} ground state absorbs a 980 nm photon and transit to excited state ²F_{5/2}. Then it transfers the energy to a neighbouring Tm³⁺ ion in the ground state ³H₆, and drops back to the ground state ²F_{7/2}. At the same time, the Tm³⁺ ions was excited to a ³H₅ excited state, and then decayed to a lower excited state ³F₄. The Tm³⁺ ion in the ³F₄ state was excited to ³F_{2,3} excited states after absorbing another pump photon energy from Yb³⁺ through

a nonradiative energy-transfer process, and then decayed to $^3\text{H}_4$ excited state. The 800 nm UC emission was generated when the Tm^{3+} ions in the $^3\text{H}_4$ excited state transitioned to the ground state $^3\text{H}_6$ ^[17].

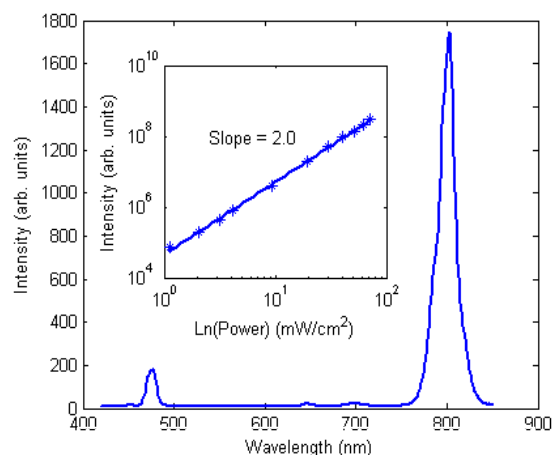


Figure 3 UC emission spectrum of the $\text{NaYF}_4:\text{Yb}^{3+}, \text{Tm}^{3+}$ nanocrystals

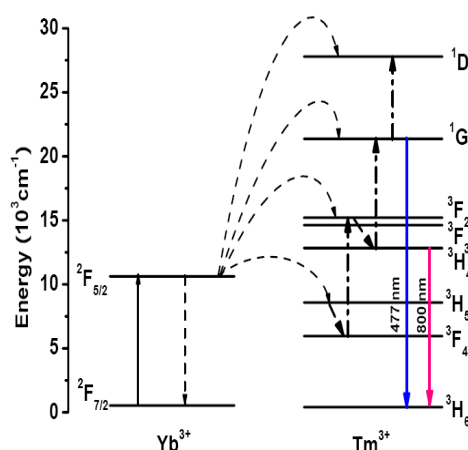


Figure 4 The energy levels of Tm^{3+} and Yb^{3+} ions and the proposed mechanism of upconversion emission

IV. Conclusion

In summary, nearly monodispersed $\text{NaYF}_4:\text{Yb}^{3+}, \text{Tm}^{3+}$ nanocrystals with uniform size were prepared through a solvothermal method. The surface coating with silica was performed via a reverse microemulsion. The UC 800 nm emission of the as-prepared nanocrystals resulted from a two-photon process.

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