Nanoscale

COMMUNICATION

A facile strategy is proposed to simultaneously enhance the intensity (10 times) and accelerate the transients (one fifth) of the 455-nm emission of Yb^{3+}/Tm^{3+} co-doped $NaYF_4$ nanocrystals via highly-doped sensitizers and a sandwich structure. This study enables one to implement UCNPs-based super-resolution nanoscopy at a scanning speed of 10 μs/pixel, which allows UCNP-based STED imaging of biological activities with higher time resolution.

Keywords: Upconversion nanoparticles, STED, super-resolution, lifetime, fast imaging

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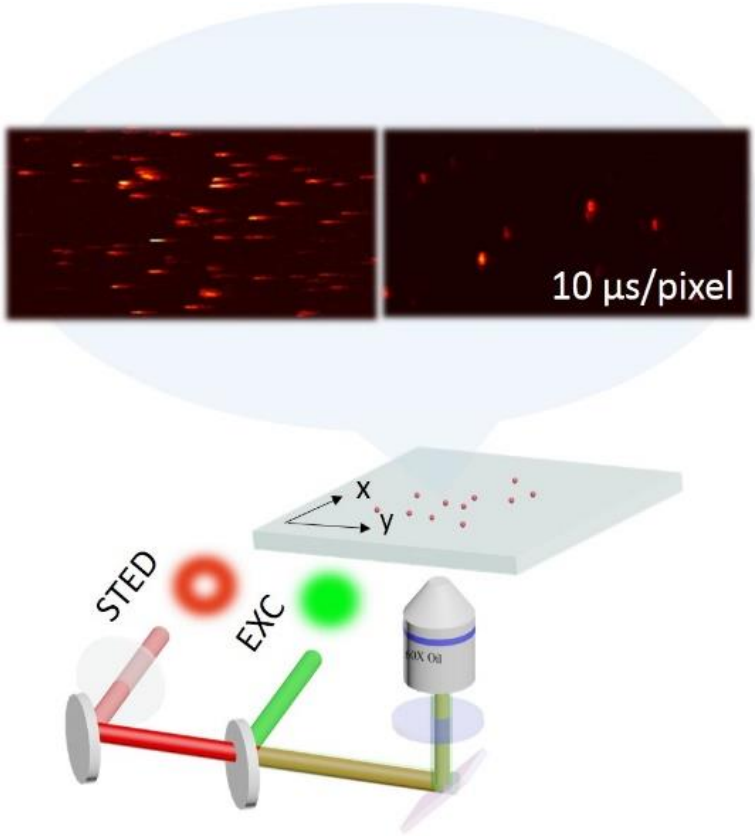
Fast upconversion super-resolution microscopy with 10-μs/pixel dwell times

TOC figure



Supporting Information

Fast upconversion super-resolution microscopy with 10-µs/pixel dwell times

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Supplementary methods

Materials and Reagents

Yttrium (III) acetate hydrate (99.9%), ytterbium (III) acetate hydrate (99.9%), thulium (III) acetate hydrate (99.9%) were purchased from Sigma-Aldrich. Sodium sulfate (98.0 %), (Sodium hydroxide (NaOH, >98%), ammonium fluoride (NH_4F, >99.99%), 1-octadecene (90%) and oleic acid (90%) were purchased from Aladdin®, China. Methanol (reagent grade), ethanol (reagent grade), cyclohexane (reagent grade) were purchased from Sinopharm Chemical Reagent Co., China. All the reagents were used as received without further purification.

Synthesis of $NaYF_4$ @ $NaYbF_4$:10%Tm^{3+} UCNPs

Synthesis of 12-nm core $NaYF_4$ nanoparticles

The designed nanoparticles were synthesized following the reported protocols with some modifications.[1-3] In a typical synthesis, 5 mL $Y(CH_3COO_2)_3$ stock solution, 10 mL oleic acid and 10 mL 1-octadecene were added into a 100 mL flask, the mixture was heated to 150℃ under stirring for about 40 mins, and then cooled down to room temperature. Then, the weighted 0.34 g NH_4F and 2.03 g sodium sulfate were quickly added and then mixed with lanthanide-oleate precursor solution uniformly. Next the reaction system was heated to 120℃ and kept at this temperature for 30 mins under an argon atmosphere, followed by incubated at 300℃ for 30 mins. Subsequently, the mixture was cooled down to room temperature. After precipitation, the core $NaYF_4$ were washed for several times using ethanol and cyclohexane. And finally, the sample was re-dispersed in 20 mL fresh cyclohexane for use.

Synthesis of 29-nm $NaYF_4$@ $NaYbF_4$:10%Tm^{3+} nanoparticles

A typical procedure is as follows: 1.8 mL of $Yb(CH_3COO_2)_3$ and 0.2 mL of $Tm(CH_3COO_2)_3$ stock solution were added into a 50 mL flask, together with 3 mL of oleic acid and 8 mL of 1-octadecene. The mixture was heated to 150℃ under stirring for about 30 mins, and then cooled down to room temperature. Then 2 mL of as- prepared core nanoparticle suspension was injected into the reaction flask as a growth template, followed by 4 mL of NH_4F-methanol stock solution (0.4 M) and 1 mL of NaOH-methanol stock solution

(1 M). Subsequently, the reaction system was kept at 50℃ for 30 min, and then was heated to 100℃ to remove methanol and residual water under vacuum for about 30 mins, Afterwards, the mixture was heated to 300℃ and incubated at this temperature for 1 hour under argon atmosphere, then the mixture was cooled down to room temperature naturally. After precipitation, the $NaYF_4$@$NaYbF_4$:10%Tm^{3+} UCNPs were washed for several times using ethanol and cyclohexane. And finally, the sample was re-dispersed in 8 mL fresh cyclohexane.

Characterization methods

Transmission electron microscopy (TEM)

TEM images were obtained on an electron microscope (JEM-2100HR, JEOL). The size and shape of the nanoparticles were determined from TEM images, the powder X-ray diffraction (XRD) patterns were recorded using X-ray diffraction (PANalytical X'Pert PRO diffract meter) with the Cu-Kα radiation at room temperature.

Luminescence transient measurement

Emission transient measurements of UCNPs were performed by modulating the 975-nm excitation laser with a chopper (model SR540, Stanford) and recording the time-resolved luminescence intensity. We use a pair of 50 mm lens combinations to place the chopper on the focal plane where the beams converge. The chopper modulation frequencies was set to 1000 Hz. The emission photons, passing through a 448-nm band-pass filter (F4, ET448/×19, Chroma), were detected by a photo multiple tube (PMT), and the trigger signal from the chopper was synchronized with a time-correlated single-photon counter (NanoHarp, Picoquant).

Optical measurement setup

We built a home-made optical system to perform the spectrum collection and imaging experiments (Figure. S1). The 810-nm CW laser was generated from an Ti: sapphire laser as the depletion light. The doughnut light was generated from a phase plate (RPC photonics VPP1a). At the same time, the 975-nm CW light beam was obtained from diode Laser. A 715-nm long pass filter (F2, FF01-715/lp-25, Semrock) was used to purify the laser spectra. These two lasers were spatially overlapped by using a polarizing beam splitter (PBS) and then directed into a multiphoton laser scanning microscope system. The spatially overlapped lasers were reflected by a 694-nm dichroic mirror (DM Chroma), and focused on the sample using an objective (60x NA=1.35, Olympus). The emission luminescence was collected by the same objective, and captured by a spectrometer or a photo multiple tube (PMT).

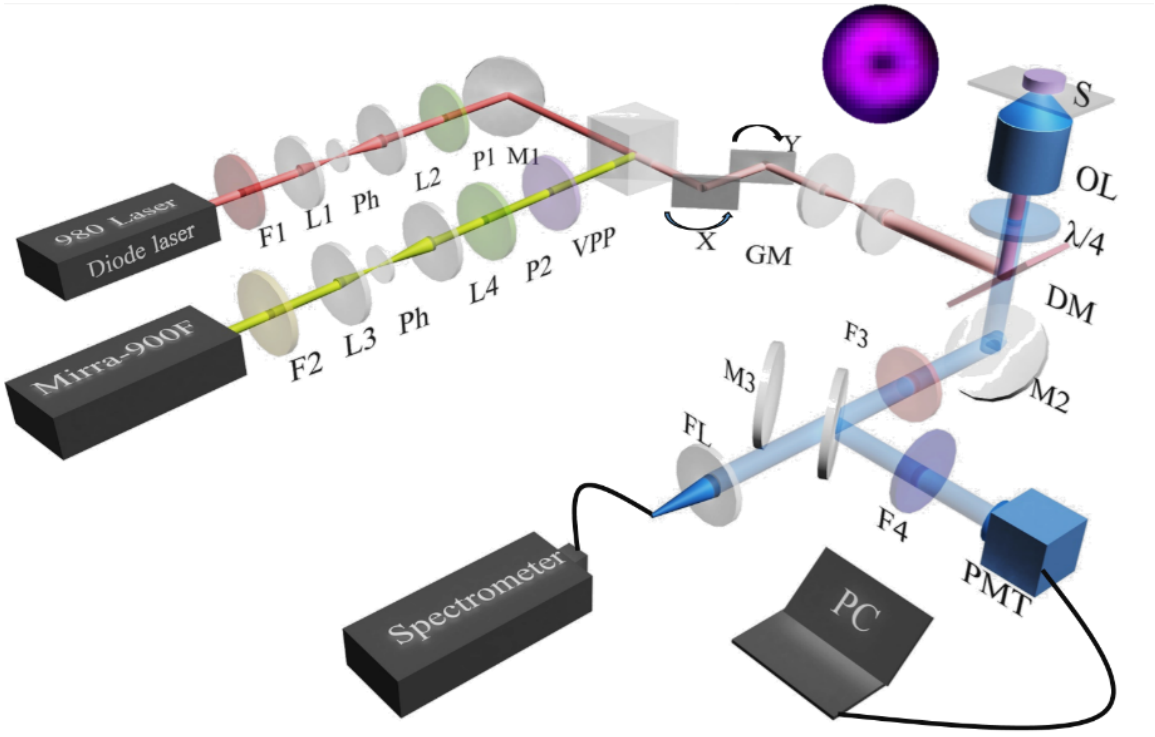

Figure S1. The scheme of the home-built STED setup. F1: 975-nm longpass Filter, F2: 715-nm longpass Filter, F3: 665-shortpass Filter, F4: 435-bandpass Filter, L1, L3, L4: Lens focus 50mm, L2: focus lens 100mm, Ph: Pinhole, P1, P2: half wavelength plate, VPP: phase plate, GM: lens combination. DM: 694-nm dichroic mirror. OL: objective lens; FL: focus lens; PMT: photomultiplier tubes. M1, M2, M3: sliver reflection mirrors.

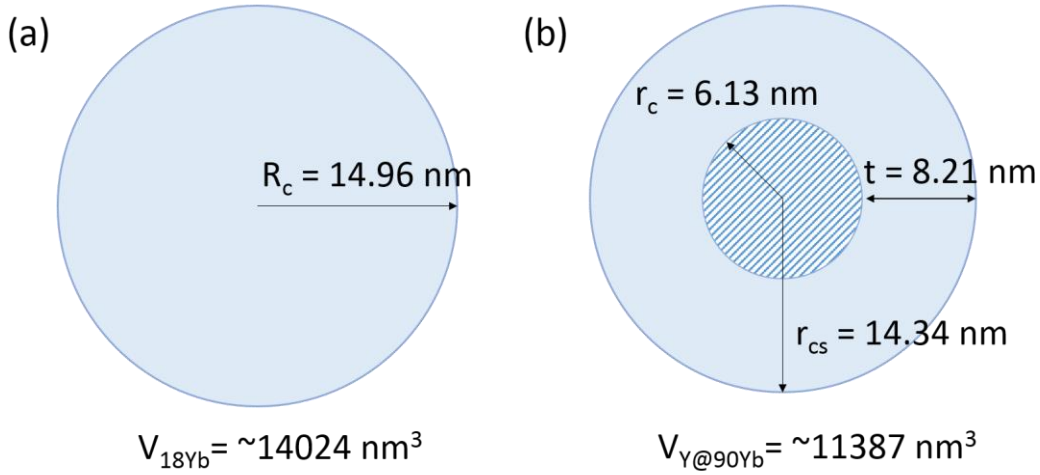

Figure S2. Schematic figure of single $NaYF_4$:18%Yb^{3+},10%Tm^{3+} (a) and $NaYF_4$@$NaYbF_4$:10%Tm^{3+} nanoparticle (b). The emitting volumes for 18Yb and Y@90Yb nanoparticles have been provided. The calculation process, as following.

One single UCNP was treated as a sphere and its volume (V) was calculated using the following equation:

$$V = \frac{4}{3}\pi r^3 \quad (1)$$

Where r is the radius of the nanoparticle, estimated from TEM images. The calculations of the emitting volumes for the 18Yb and Y@90Yb nanoparticles are depicted in Fig. S2 (a) and Fig. S2 (b), respectively. The emitting volume was calculated to be ~14024 nm^3 for 18Yb nanoparticle and ~11387 nm^3 for Y@90Yb nanoparticle.

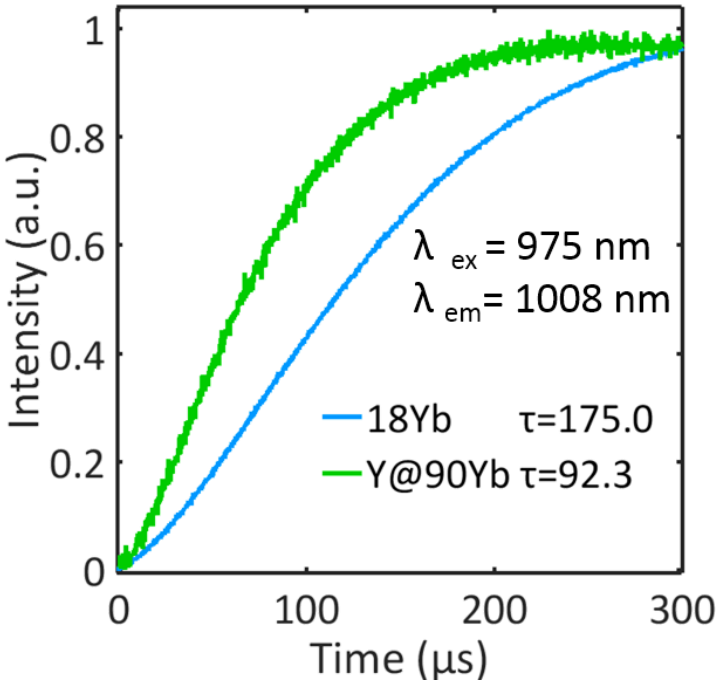

Figure S3. Rise kinetics of the Yb^{3+} 1008 nm were observed in $NaYF_4$@$NaYbF_4$:10%Tm^{3+} UCNPs than those in $NaYF_4$:18%Yb^{3+},10%Tm^{3+} UCNPs.

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