Electronic Supplementary Information

One-scan fluorescence emission difference nanoscopy with excitation orthogonalized upconversion nanoparticles

Bingru Huang [a], Qiusheng Wu [a], Xingyun Peng [a], Liqin Yao [a], Dengfeng Peng[b*], Qiuqiang Zhan[a,b*]

[a]*Centre for Optical and Electromagnetic Research (COER), Guangdong Provincial Key Laboratory of Optical Information Materials and Technology, South China Academy of Advanced Optoelectronics, South China Normal University (SCNU), 510006 Guangzhou, China.*

[b]*Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education, College of Optoelectronic Engineering, Shenzhen University, 518060 Shenzhen, China.*

Corresponding authors:

**E-mail:* zhanqiuqiang@m.scnu.edu.cn *(Qiuqiang Zhan)*

**E-mail:* pengdengfeng@szu.edu.cn *(Dengfeng Peng)*

Experimental section

Materials.

Yttrium (III) acetate hydrate (99.9%), ytterbium (III) acetate hydrate (99.9%), thulium (III) acetate hydrate (99.9%) and Erbium (III) acetate hydrate (99.9%) were purchased from Sigma-Aldrich. 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.

Instrumentation.

Transmission electron microscopy (TEM) were obtained on a JEM-2100HR microscope. 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-K_α radiation at room temperature.

Synthesis of $NaYF_4$: Er^{3+} (2 mol%) @$NaYF_4$@$NaYF_4$:Yb^{3+}/Tm^{3+}(20/2 mol%) UCNPs.

The nanoparticles of $NaYF_4$:Er^{3+} (2 mol%) @ $NaYF_4$ @ $NaYF_4$:Yb^{3+} /Tm^{3+}(20/2 mol%) (Er@Y@Yb/Tm) were synthesized following the method reported in previous work. In the synthesis of core $NaYF_4$:Er^{3+} UCNPs, 5 mL aqueous solution of $Y(CH_3COO)_3$, $Er(CH_3COO)_3$ with a total lanthanide amount of 1 mmol was added into a 100 ml flask containing 7.5 mL oleic acid and 17.5 mL 1-octadecene. The mixture was heated to 150℃ under stirring for 40-50 mins to form the precursor. When it was cooled down to room temperature, 10 mL NH_4F-methanol stock solution (0.4 M) and 2.5 mL NaOH-methanol stock solution (1 M) were mixed and injected into the flask quickly. Subsequently, the mixture was stirred at 50 ℃ for 30 mins, and then was heated to 100 ℃ under vacuum to pumping out of methanol and residual water for ca. 30 mins.

With the protection of an Argon atmosphere, the whole system was heated to 300℃ and reacted for 1.5 hours. After cooled down to room temperature, 10 mL of ethanol was added for centrifugation treatment. The resulting core UCNPs were washed several times with ethanol and cyclohexane, and finally dispersed in 8 mL of cyclohexane. The core-shell $NaYF_4$:Er^{3+}@$NaYF_4$ UCNPs and core-shell-shell Er@Y@Yb/Tm UCNPs follow the same protocols except different proportions of each kind of lanthanide stock solution was applied in each of synthesis procedure.

Synthesis of $NaYF_4$: Yb^{3+}/Er^{3+} (18/2 mmol%) UCNPs.

In the synthesis of Yb^{3+}/Er^{3+} co-doped UCNPs, 5 mL aqueous solution of $Y(CH_3COO)_3$, $Yb(CH_3COO)_3$, $Er(CH_3COO)_3$ with a total lanthanide amount of 1 mmol was added into a 100 ml flask containing 7.5 mL oleic acid and 17.5 mL 1-octadecene. the following synthetic procedure was identical to $NaYF_4$: Er^{3+} (2 mmol%) UCNPs, and finally dispersed in 8 mL of cyclohexane.

Optical microscopy and spectroscopy.

A home-built optical system was built to perform the spectrum collection and imaging experiments (Scheme 2). An 808-nm CW laser was obtained from a single mode optical fiber coupled laser (Laser-1) and was used to excite the Er^{3+} singly doped core. A wavelength tunable Ti Sapphire laser (Laser-2, Mira 900, Coherent) was used to explore the optimal wavelength to excite the Yb^{3+}/Tm^{3+} doped shell of Er@Y@Yb/Tm UCNPs and generate 940-nm laser for exciting Yb^{3+}/Tm^{3+}. A 715-nm long-pass filter F1 (FF01-715/LP, Semrock) was used to purify the laser spectrum. Using a polarizing beam splitter (PBS), these two lasers were spatially overlapped and directed into a multiphoton laser scanning microscope system. Two rotatable halve wave plates (P1, P2) were used to adjust the laser power behind the PBS. A clockwise vortex phase plate (VPP-1a, RPC Photonics) was insert into the 808-nm laser path to convert it to hollow beam. Another half-wave plate (P3) combined with a quarter wave plate (QWP) were used to transfer the hollow beam into right-hand circular polarization. The spatially overlapped lasers were reflected by a 775-nm

short pass dichroic mirror (DM1, Chroma), and focused on the sample using an objective (NA=1.35, Olympus). The emission fluorescence was collected by the same objective, and then detected by two photomultiplier tubes (PMT1 and PMT2) after filtered by a 694-nm short pass filter (F2, Semrock), and separated by a 505-nm dichroic mirror (DM2, Olympus). A band-pass filter F3 (FF01-466/40, Semrock) set in front of PMT1 was used to purify the blue emission from the Yb^{3+}/Tm^{3+} doped shell, so that we get a solid spots image excited by 940-nm Gaussian laser beam in the blue channel. And the band-pass filter F4 (ET550/40m, Chroma) set in front of PMT2 was used to purify the green emission from the Er^{3+} doped core, hence the green channel recorded a hollow spots image excited by 808-nm hollow laser beam. The fluorescence emission difference imaging can be obtained by subtracting these two images directly with proper weight.

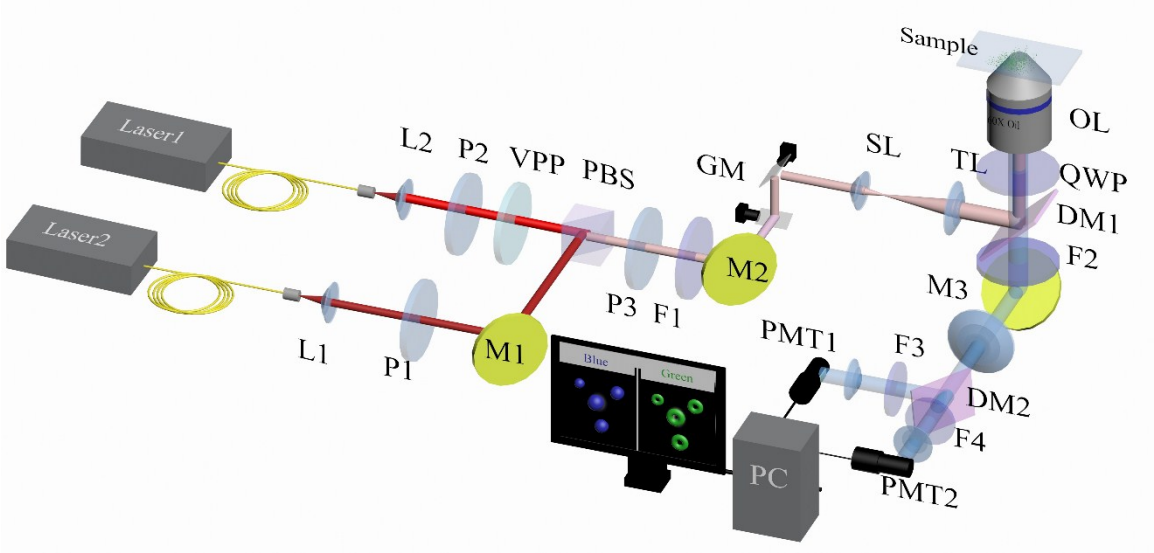

Figure S1 | Schematic diagram of the one-scan FED system. L1, L2: Collimate lens. P1, P2, P3: Half wave plates. VPP: Vortex phase plate. PBS: Polarized beam splitter. M1, M2, M3: Sliver reflection mirrors. F1: 715-nm long-pass filter, F2: 694-nm short-pass filter, F3: blue band-pass filter, F4: green band-pass filter. DM1: 775-nm short-pass dichroic mirror. DM2: 505-nm long-pass dichroic mirror. OL: Objective lens. PMT1, PMT2: Photomultiplier tubes.

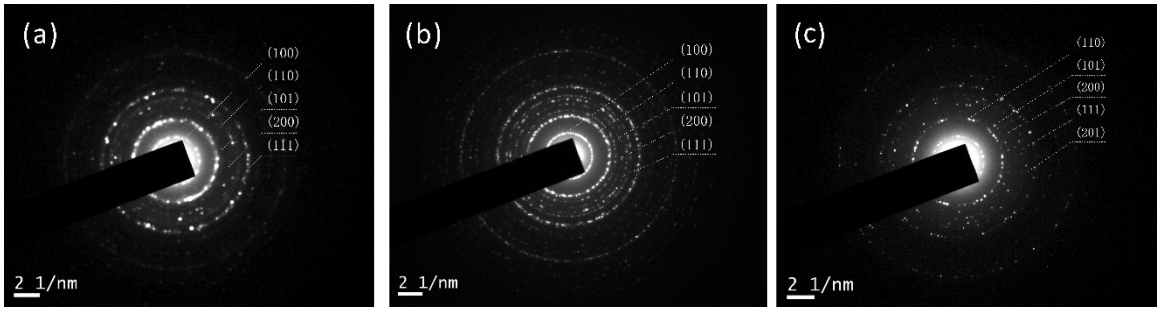

Figure S2 | The selected area electron diffraction (SAED) patterns of the as-synthesized (a) core, (b) core-shell, (c) core-shell-shell UCNPs.

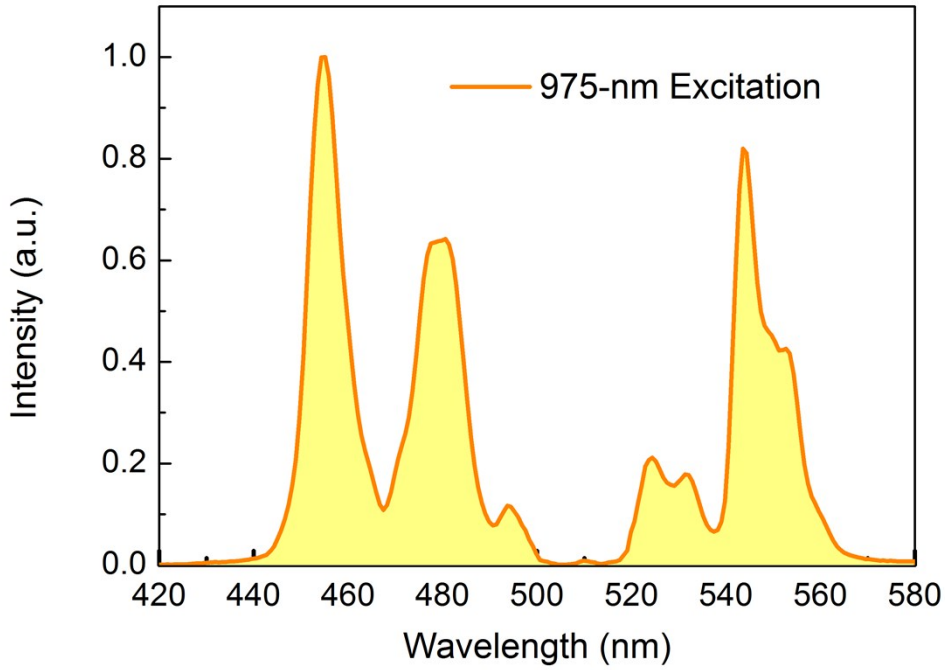

Figure S3 | The normalized upconversion luminescence spectrum of $NaYF_4$:2%Er^{3+}@$NaYF_4$@$NaYF_4$:20%Yb^{3+}/2%Tm^{3+} nanoparticles under 975-nm excitation, 1.25 MW/cm^2.

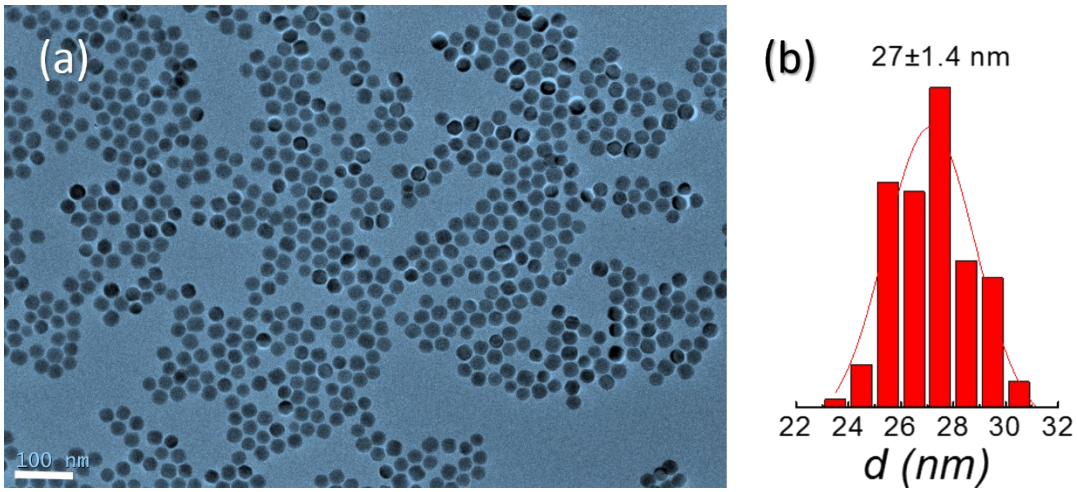

Figure S4 | TEM images of $NaYF_4$:18%Yb^{3+}/2%Er^{3+} nanoparticles with corresponding size distribution. Scale bar: 100 nm.