SUPPORTING INFORMATION

Enhanced upconversion Luminescence through core/shell structures and its application for detecting organic dyes in opaque fishes

Pan Hu,[a] Xiaofeng Wu,*[,a] Shigang Hu,[a] Zenghui Chen,[b] Huanyuan Yan,[c] Zaifang Xi,[a] Yi Yu,[a] Gangtao Dai,[b] and Yunxin Liu*[,b,d]

[a]School of Information and Electrical Engineering, [b]Department of Physics and Electrical Science, [c]College of Mechanical and Electrical Engineering, Hunan University of Science and Technology, Xiangtan 411201, china.
[d]INPAC-Institute for Nanoscale Physics and Chemsitry, KU Leuven, Celestijnenlaan 200D B-3001, Belgium.

Experimental

Reagents and Chemicals. Reagents of Oleic Acid (OA) (90%), Octadecene (ODE) (90%), NaOH (98%), NH_4F (98%), Methanol (99%), Polyethylene Glycol (PEG) (90%), Sodium Fluorescein, Rhodamine B, $RECl_3 \cdot 6H_2O$ (RE=Lu, Y, Yb, Gd and Er/Tm) (99.99%), C_2H_5OH (90%), Cyclohexane (90%), were purchased from Sigma-Aldrich. Deionized water was used to deploy solution. All other reagents were of A.R and all chemicals can be used directly without further purification.

Synthesis. Upconversion nanoparticles were synthesized by our previous route [28]. Typically, aqueous solution of $RECl_3 \cdot 6H_2O$ (0.4mmol, RE=Lu, Yb and Er/Tm) is added to a flask of 50 ml with OA (4ml) and ODE (4ml). The mixed solution was heated to 160 ^{0}C for 30 min to remove residual water. Then, the mixture was cooled down to room temperature, and 5ml methanol solution with 0.05g of NaOH and 0.56g of NH_4F was added and stirred for 30 min at the temperature of 50 ^{0}C to evaporate methanol. The mixed solution was heated to 310 ^{0}C and reacted at the temperature of 310 ^{0}C for 60 min with argon atmosphere flowing through the reaction solution. Finally, the reaction was cooled down to room temperature naturally, and the nanoparticles were collected with ethanol by centrifugation at 10000 R/min for 5 min. The obtained precipitate was washed with ethanol and cyclohexane three times by centrifugation. In a subsequent shell growth procedure, the nanoparticles were added to a 50ml flask and the mixed solution (the solution comprise as front) was heated to 310 ^{0}C under an argon atmosphere for 25 min, The nanoparticles were collected with

ethanol by centrifugation at 10000 R/min for 5 min. The obtained precipitate was washed with ethanol and cyclohexane three times by centrifugation .The obtained white powder was the as-prepared nanoparticles.

Surface Modification of OA-UCNPs with PEG. The surface modification with PEG was conducted according to previous protocols with slight modification. In brief, 1ml cyclohexane solution of $NaLuF_4$ nanoparticles with oleic acid was added with ethanol and acetic acid aqueous solution stocked for 30 min, and subsequently precipitated by centrifugation at 3500 R/min for 5 min with the supernatant discarded. The collected nanoparticles were dispersed in aqueous solution with a drop (~0.6ml) of PEG, then the precipitates were separated by centrifugation after vigorous stirring for 1 hour at 20 °C, rinsed with ethanol three times to remove the unreacted PEG, and readily dissolved in water. The prepared solution was filtered through a 0.22-um syringe filter to remove lager aggregates. PEG-UCNPs were thus formed.

In vivo animal experiments. These opaque fishes were obtained from the pond. The opaque fishes were cultured in ordinary different concentrations of organic dyes. PEG-UCNPs in 0.9% NaCl saline solution were subcutaneously injected into gastro cavity of opaque fishes. The fluorescence imaging was collected by a Sony multiple CCD camera under the excitation of a 980 nm laser diode. The laser power density was safe for opaque fishes located at ~0.2W/mm^2 according to previous report.

In vitro tissues experiments. The opaque fishes were cultured in ordinary different concentrations of organic dyes. The opaque fish slices were washed two times with phosphate buffered saline (PBS), incubated with PEG-UCNPs conjugates at 20 °C for 2 h, and washed with PBS to remove any superfluous reagents before cell imaging. Imaging of the PEG-UCNPs uptook by opaque fish tissues was carried out using Olympus BX43 fluorescence microscopy under the excitation of a NIR 980 nm laser. The multicolor fluorescence was collected by a Tucsen H-694CICE digital camera. All studies were carried out at room temperature.

Characterization. The morphology and particles size of the synthesized nanoparticles were characterized by a H-7605c transmission electron microscope (TEM), using an accelerating voltage of 80 kV and a JEM 3010 high-resolution transmission electron microscopy operating at 200 KV (HRTEM). The photoluminescence spectra were measured by a Hitachi F-2700 fluorescence spectrophotometer equipped with a 980 nm laser as the excitation source. The photos of upcanversion luminescence were taken on Olympus BX43 inverted fluorescence microscope with a sony multiple CCD camera and a Nikon camera D3200 under the excitation of a NIR 980 nm laser. The low noise infrared diode laser is purchased from Changchun New Industries Optoelectronice Tech, which has the power density of 0.2W/mm^2 and the line width of 20 nm (970~990 nm). We used a 900 nm shortpass filter in the fluorescent imaging by Olympus BX43 inverted fluorescence microscope and taking photos by a Nikon camera D3200, to avoid the excitation light of 980 nm. Yes, it is sure that there is ~1% leakage of 980 nm signal from our filter [900 nm shortpass filter (Fig.S1)]. In order to confirm whether the 980 nm leakage light induced the imaging, we purchased a 980nm notch filter from an American company “Thorlabs” to remove completely the 980 nm excitation light when

collecting the emission light. The transmission spectra of the 980nm notch filter was listed Fig.S2. It is clear that no leakage of 980 nm light occurs after adding this 980nm notch filter.

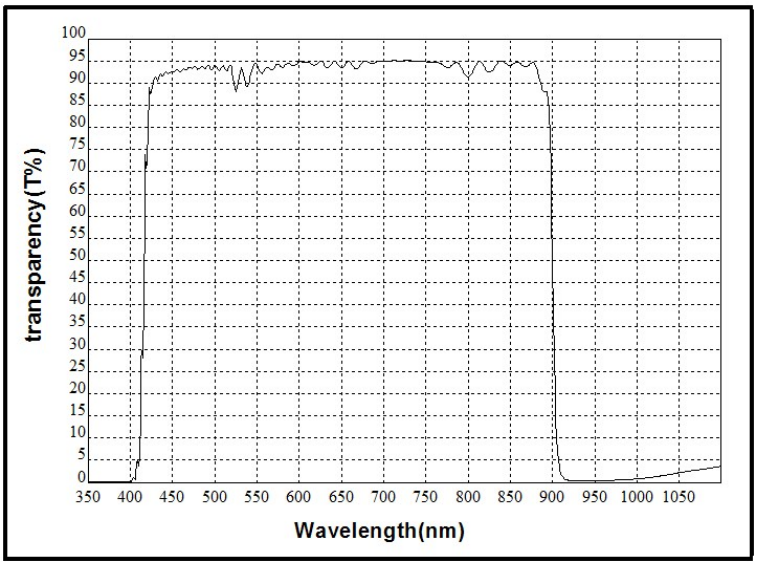


Fig. S1. The parameters of the 900 nm shortpass filter

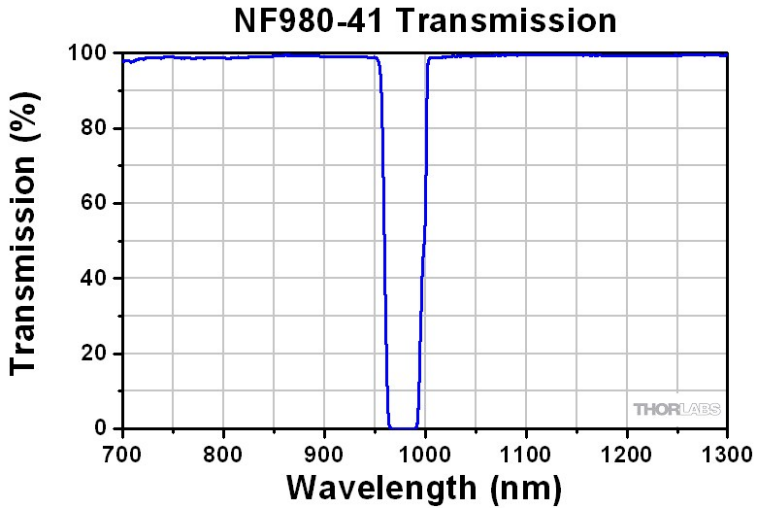
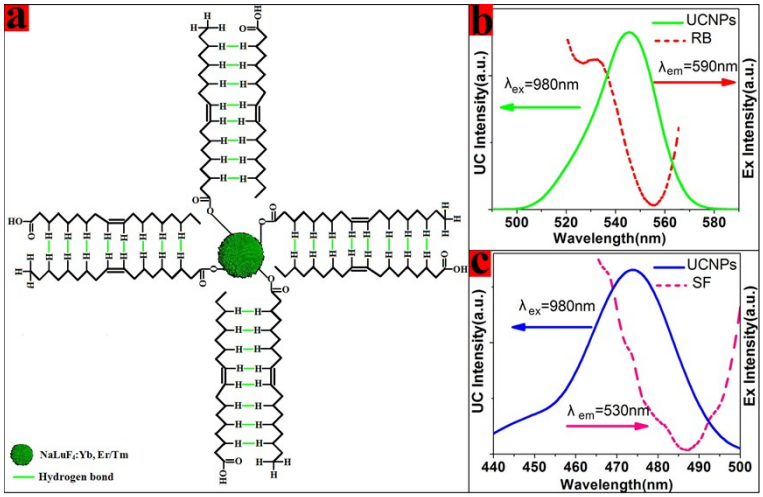


Fig.S2. The parameters of the 980 nm notch filter



Scheme S1 (a) Schematic illustration of free carboxyl groups on the surface of UCNPs. (b) The UC fluorescence spectrum of $NaLuF_4$:18%Yb^{3+},2%Er^{3+} nanocrystals (λ_{ex}=980 nm) and excitation spectrum of Rhodamine B (λ_{em}=590 nm). (c) The UC fluorescence spectrum of $NaLuF_4$:18%Yb^{3+},0.5%Tm^{3+} nanocrystals (λ_{ex}=980 nm) and excitation spectrum of sodium fluorescein (λ_{em}=530 nm).

Results and Discussion

The composition and phase purity of $NaLuF_4$ (core-only) and $NaLuF_4$/$NaGdF_4$ (core-shell) UCNPs are investigated by X-ray powder diffraction (XRD). As shown in Fig. S3, the core nanocrystals are actually composed of the cubic and the hexagonal phases, while the cubic one dominates the phase structures. The core−shell nanocrystals can also be indexed to the mixture of hexagonal and cubic phases, but the hexagonal one dominates the phase structures.

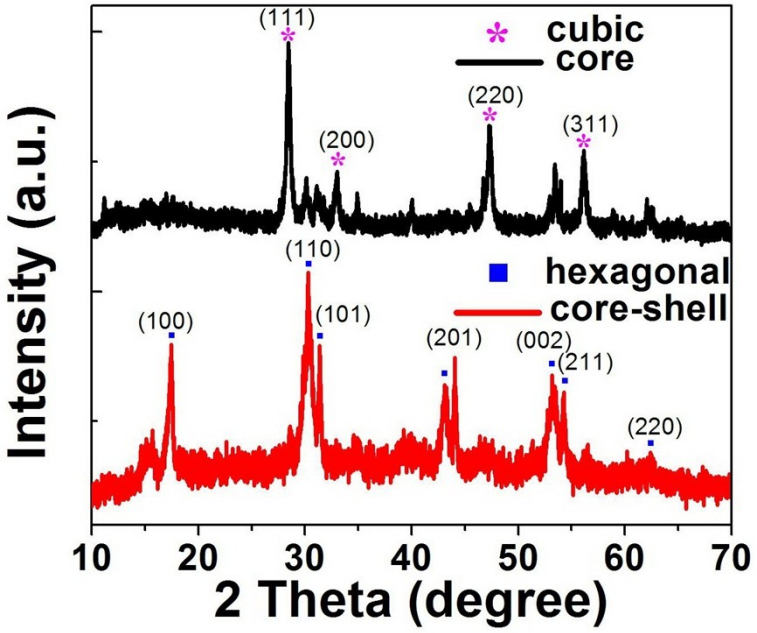


Fig.S3. XRD patterns of $NaLuF_4$:18%Yb^{3+},2%Er^{3+} (core) and $NaLuF_4$:18%Yb^{3+}/2%Er^{3+}/$NaGdF_4$ (core-shell) UCNPs.

The absorption spectra in Fig.S4 show that the nanoparticles had no absorption peak in visible and the main absorption spectra in UV.

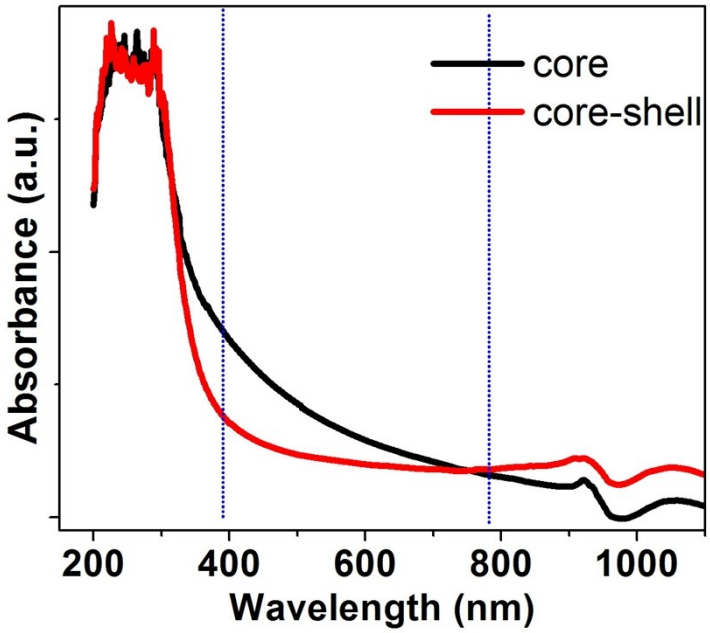


Fig.S4. The UV-visible absorption spectra of $NaLuF_4$ (core) and $NaLuF_4/NaGdF_4$ (core/shell).

The quantum efficiency of the sample. Based on the following Equation (1), the upconversion quantum yields (QY) for blue and green emission are calculated and listed in Table 1.

Table.1. The quantum efficiency of the samples.

Sample	QY (blue emission)	QY (green emission)	QY (red emission)
$NaLuF_4:Yb^{3+},Er^{3+}$		0.143%	0.030%
$NaLuF_4:Yb^{3+},Er^{3+}@NaYF_4$		0.470%	0.088%
$NaLuF_4:Yb^{3+},Er^{3+}@NaLuF_4$		0.262%	0.051%
$NaLuF_4:Yb^{3+},Er^{3+}@NaGdF_4$		0.201%	0.039%
$NaLuF_4:Yb^{3+},Tm^{3+}$	0.041%		0.003%
$NaLuF_4:Yb^{3+},Tm^{3+}@NaYF_4$	0.129%		0.011%
$NaLuF_4:Yb^{3+},Tm^{3+}@NaLuF_4$	0.093%		0.008%
$NaLuF_4:Yb^{3+},Tm^{3+}@NaGdF_4$	0.069%		0.005%

The QY is defined as:

$$QY = \frac{PE}{PA} = \frac{\frac{I_{EM}}{E_{EM}}}{\frac{I_{Ab}}{E_{Ab}}} = \frac{I_{EM}}{I_{Ab}} * \frac{E_{Ab}}{E_{EM}} = \frac{\eta_{UE} \ * \ E_{Ab}}{\sum E_{\lambda} \ * \ V_{IIRST}} \quad (1)$$

where QY is the quantum yield, the energetic upconverison efficiency η_{UE} of $NaLuF_4:Yb^{3+},Er^{3+}/NaLuF_4$ core/shell nanoparticles is approximately calculated to be 0.544% according to QY of 0.3% from Frank C. J. M. van Veggel's reporter in [Nanoscale, 2010, **2**, 1417–1419] due to a fact that the upconversion efficiency of $NaLuF_4:Yb^{3+},Er^{3+}/NaLuF_4$ core/shell nanoparticles is close to that of $NaYF_4:Yb^{3+},Er^{3+}$, E_{λ} is the energy of defined wavelength, V_{IIRST} is the Intensity Ratio of Single emission wavelength to Total emissions. PE and PA are the photons emitted and the photons absorbed, respectively. I_{EM} and I_{Ab} are the intensity of emission and absorption lights, respectively. E_{EM} and E_{Ab} are the energy of an emitting photon and an absorption photon, respectively. The sum of $E_{\lambda} \times V_{IIRST}$ represents the normalization of emission photons with different energies which can be determined by the emission spectra.

Detection of basic organic dyes. The UC fluorescent spectra of UCNPs@RB system with various concentration of Rhodamine-B were also investigated and shown in Fig.S5. It is observed that the green emission band centered at 545 nm and yellow emission band centered at 590 nm dominate the UC fluorescence, which are ascribed to the 4f-shell electronic $^4S_{3/2} \rightarrow {}^4I_{15/2}$ transition of Er^{3+} and exciton recombination radiation in Rhodamine-B achieved by LRET from $NaLuF_4$ nanoparticles (Donors) to Rhodamine-B (Acceptors) under 980 nm laser excitation. More importantly, the detection sensitivity of these LRET based nanoprobes is higher by several orders of magnitude than that of conventional approach where the green pump power is directly applied for detecting Rhodamine-B. This way can be extended to detect the concentration of SF. The upconversion fluorescent nanoprobes with an acidic ligand can quickly capture the basic sodium fluorescein in solution and forms a close UCNPs@SF system. The UC luminescence spectra of UCNPs@SF system with various concentration of sodium fluorescein solution were also investigated and shown in Fig.S6. There are two main peaks located at 476 nm and 521 nm on upconversion fluorescent spectra, which are ascribed to the $^1G_4 \rightarrow {}^3H_6$ transition of Tm^{3+} and exciton recombination radiation of sodium fluorescein accompanied by LRET from $NaLuF_4$ nanoparticles (Donors) to sodium fluorescein (Acceptors).

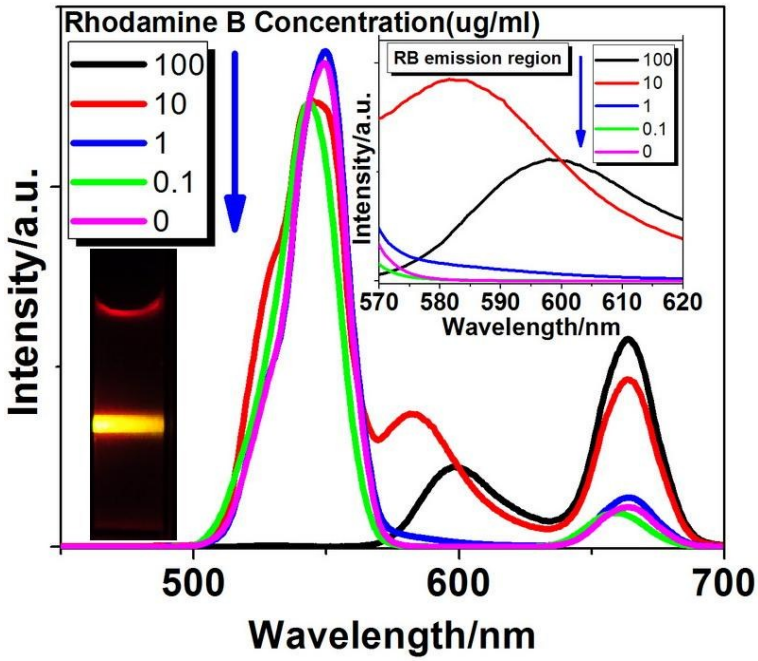


Fig. S5. Evolution of the fluorescence of UCNPs@RB with different concentration of RB (from 100 to 0μg/ml) under the excitation of 980 nm laser. Photo: the fluorescence imaging was collected by a Nikon camera D3200 under the excitation of a 980 nm laser diode. Inset: the corresponding magnification of the UCL spectra of UCNPs@RB between 570 nm and 620 nm.

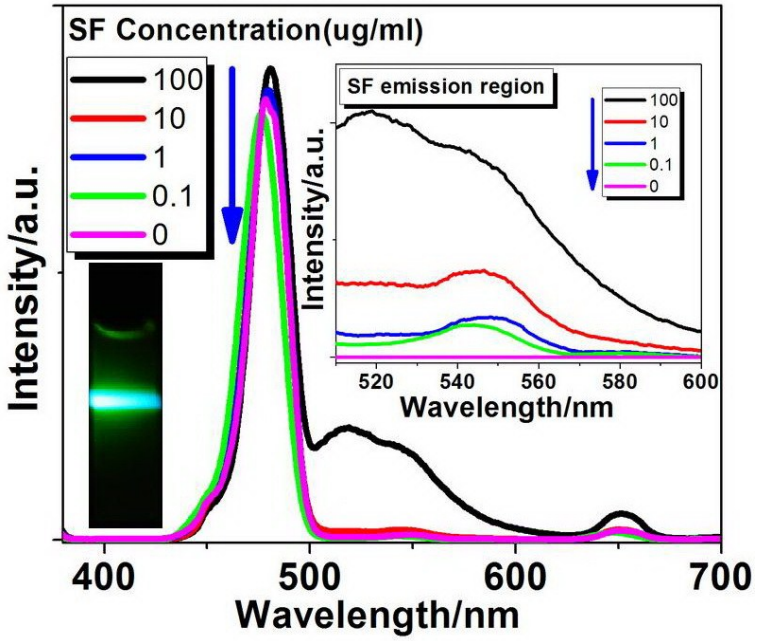


Fig. S6. Evolution of the fluorescence of UCNPs@SF with different concentration of SF (from 100 to 0μg/ml) under the excitation of 980 nm laser. Photo: the fluorescence imaging was collected by a Nikon camera D3200 under the excitation of a 980 nm laser diode. Inset: the corresponding magnification of the UCL spectra of UCNPs@SF between 510 nm and 600 nm.

In vivo Imaging of opaque fishes. Conventional bright field imaging is restricted to the ultrathin slices, which is gradually substituted by fluorescent imaging in vivo or

in vitro. The most popular fluorescent imaging is based on the visible emission of fluorescent proteins under the excitation of ultraviolet light. Regretfully, the background autofluorescence of the biological tissues limited its application for high resolution imaging. To overcome this drawback, upconversion fluorescent nanoprobes are rapidly developed in the last decade owing to their merits including absence of background autofluorescence, high detection sensitivity, well photostability, and deep penetration into tissues when excited by NIR 980 nm diode laser. Here, we present the application of upconversion nanoprobes for imaging of opaque fish in vivo. Metabolism is an important factor for maintaining basic vital movement, which is also a significant characteristic for heredity and tissue variation. For investigating the influence of organic dyes on the metabolism, the living opaque fishes cultured with different concentration of basic dyes (e.g., Rhodamine B, sodium fluorescein) were incubated in normal water at about 20 °C for various time, which were imaged afterward using a Nikon camera D3200 in bright field. We can noticeable that the opaque fishes were reared in high concentrations of organic dyes die rapidly by observing. This illustrates that organic dyes have a big impact on metabolism of opaque fishes. Meanwhile, the combination of UCNPs and fluorescent dyes can avoid this problem and show background-free images. The fluorescent imaging with UCNPs and fluorescent dyes are actually based on an upconversion luminescence resonant energy transfer from UCNPs to fluorescent dyes. The opaque fishes are first incubated in aqueous solution of dye and then in the aqueous solution of hydrophilic green UCNPs. The green upconversion fluorescent nanoprobes with an acidic ligand can quickly capture the basic Rhodamine-B in vivo and form a close system of UCNPs@RB. The UCNPs@RB system can emit yellow light due to luminescence resonant energy transfer from UCNPs to RB under the excitation of 980 nm infrared light, which is actually composed of the green emission of $NaLuF_4$:18%Yb^{3+},2%Er^{3+} nanoprobes and the red emission of Rhodamine-B, as shown in Fig.S7. This UCNPs@dyes system can be extended to other basic dyes. E.g., the upconversion nanoprobes with an acidic ligand can alos quickly capture the basic sodium fluorescein in opaque fishes and form a close UCNPs@SF system. The UCNPs@SF system can emit cyan light due to luminescence resonant energy transfer from UCNPs to SF under the excitation of 980 nm infrared light, which is actually composed of the blue emission of $NaLuF_4$:18%Yb^{3+},0.5%Tm^{3+} nanoprobes and the green emission of sodium fluorescein , as shown in Fig.S8. This combination of UCNPs and fluorescent dyes supplies researchers with another way for differential imaging with high intensity ratio of signal to noise.

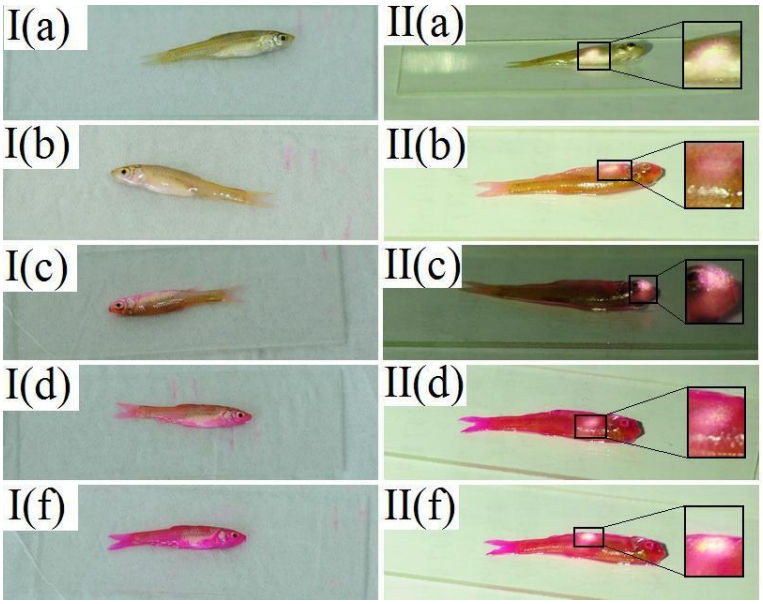


Fig. S7. Left column: conventional optical bioimaging in bright-field of the opaque fishes. **Right column:** In vivo upconversion luminescence imaging of the opaque fishes after cultured with different concentrations of Rhodamine B for one day using $NaLuF_4$:18%Yb^{3+},2%Er^{3+} nanocrystals as probes. Concentration of RB equals to II (a) 0μg/ml, II (b) 10μg/ml, II (c) 100μg/ml, II (d) 500μg/ml and II (f) 700μg/ml under the excitation of a 980nm laser diode.

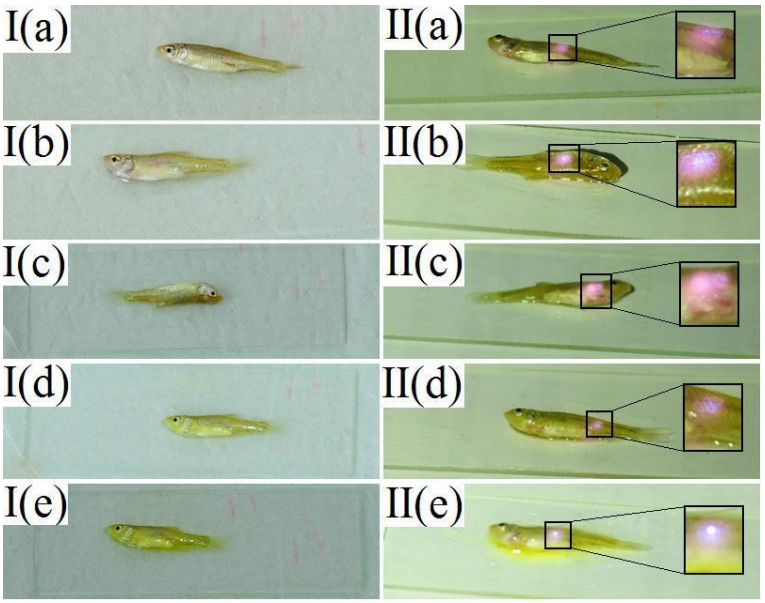


Fig. S8. Left column: conventional optical bioimaging in bright-field of the opaque fishes. **Right column:** In vivo upconversion luminescence imaging of the opaque fishes after cultured with different concentrations of sodium fluorescein for one day using $NaLuF_4$:18%Yb^{3+},0.5%Tm^{3+} nanocrystals as probes. Concentration of SF equals to II (a) 0μg/ml, II (b) 10μg/ml, II (c) 100μg/ml, II (d) 500μg/ml and II (f) 700μg/ml under the excitation of a 980nm laser diode.

In Vitro imaging of opaque fish tissue. The imaging of the biological tissue and cell units by the fluorescent labeling has wide application. In order to further explore

the effect of organic dyes with different concentrations on the opaque fish body tissue. The opaque fishes slices were immersed with aqueous solution of $NaLuF_4$:18%Yb^{3+},2%Er^{3+}@RB, and $NaLuF_4$:18%Yb^{3+},0.5%Tm^{3+}@SF at 20 °C for 1 h and washed with fresh PBS to restrain the crystallization of sodium salts in opaque fish tissue and remove excess upconversion nanoparticles, which were subsequently imaged by confocal fluorescent microscopy under excitation of 980 nm diode laser equipped with a Tucsen H-694CICE CCD camera. It can be seen clearly in Fig.S9 that opaque fish tissues were successfully labeled by $NaLuF_4$:18%Yb^{3+},2%Er^{3+} upconversion nanoparticles. It can be seen clearly in Fig.S9A that RB solution can easily enter the opaque fish tissue. It is noted that upconversion nanoparticles located only around in the tissue edge and no distribution in other tissue region (Fig.S9B) and the color of fluorescent imaging of the opaque fish tissue is green not yellow (Fig.S9C). This is due to a fact that the RB solution and UCNPs combined at the level of molecules. This indicates that RB has remarkable influence on the tissue volume and metabolism. The similar phenomenon is also observed by comparing the fluorescent imaging of the opaque fish tissue incubated with $NaLuF_4$:18%Yb^{3+},0.5%Tm^{3+} (Fig.S10). It also can be seen clearly in Fig.S10 that opaque fish tissues were successfully labeled by $NaLuF_4$:18%Yb^{3+},0.5%Tm^{3+} upconversion nanoparticles. It is noted that upconversion nanoparticles located only around in the tissue edge and no distribution in other tissue region (Fig.S10B). But SF solution does not enter the opaque fish tissue obviously and combined with UCNPs at the level of molecules as RB solution (Fig.S10C). This indicates that SF has remarkable influence on the tissue volume and metabolism.

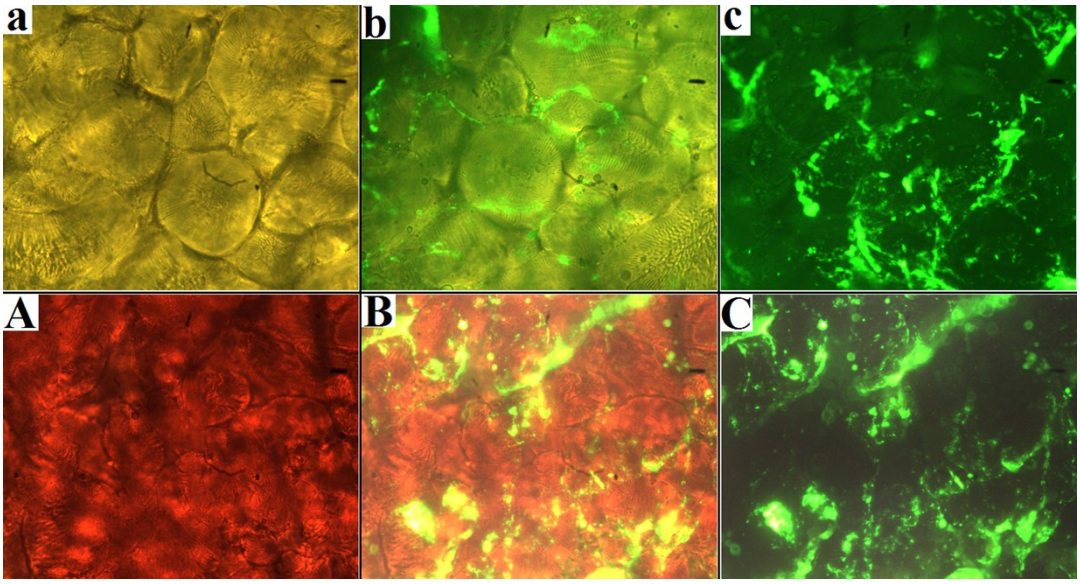


Fig. S9. Right column: In vitro fluorescence microscope tissue imaging of the opaque fishes slices after cultured in aqueous solution with different concentrations of Rhodamine B for one day using $NaLuF_4$:18%Yb^{3+},2%Er^{3+} nanocrystals as probes. **Left column:** conventional slice transmission imaging of the opaque fishes. **Middle column:** the overlay of the left and right columns. Concentration of RB equals to 500μg/ml under the excitation of a 980nm laser diode.

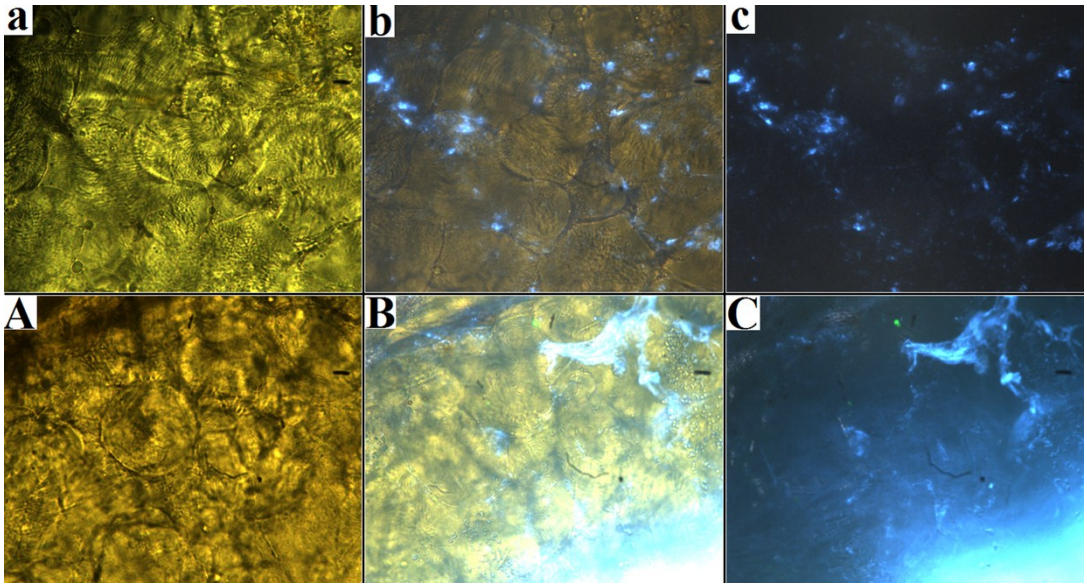


Fig. S10. Right column: In vitro fluorescence microscope tissue imaging of the opaque fishes slices after cultured in aqueous solution with different concentrations of sodium fluorescein for one day using $NaLuF_4$:18%Yb^{3+},0.5%Tm^{3+} nanocrystals as probes. **Left column:** conventional slice transmission imaging of the opaque fishes. **Middle column:** the overlay of the left and right columns. Concentration of SF equals to 500μg/ml under the excitation of a 980nm laser diode.

In order to confirm whether the 980 nm leakage light induced the imaging, we purchased a 980nm notch filter from an American company "Thorlabs" to remove completely the 980 nm excitation light when collecting the emission light.

From Fig.S11, it is determined that the "980 nm notch filter" can absorb the green emission centered at ~540 nm of upconversion nanoparticles to emit red light centered at ~678 nm. As a result, it is observed that the in vivo upconversion luminescence imaging of the opaque fishes has red background (due to 678 nm emission) (Fig. S12 and Fig.S13).

It is noted that no visible emission was observed when directly radiating the "980 nm notch filter" with 980 nm laser power.

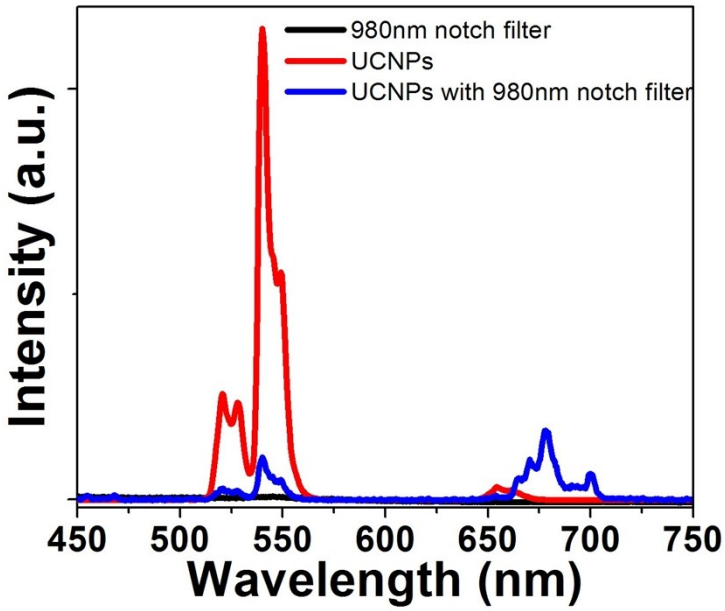


Fig.S11. The fluorescence emission spectra of 980nm notch filter, UCNPs and UCNPs with 980nm notch filter under the excitation of 980 nm laser.

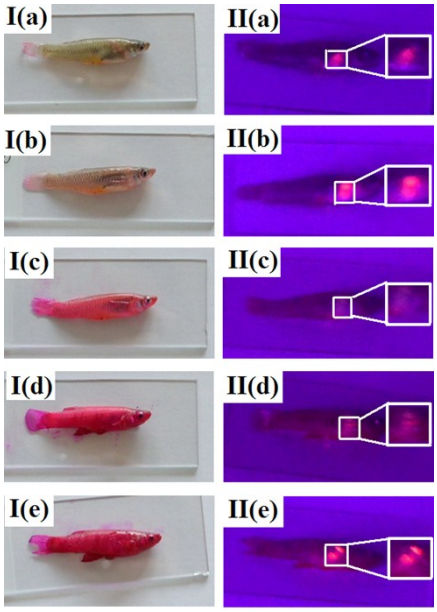


Fig.S12. Left column: conventional optical bioimaging in bright-field of the opaque fishes. **Right column:** In vivo upconversion luminescence imaging of the opaque fishes after cultured with different concentrations of Rhodamine B for one day using $NaLuF_4$:18%Yb^{3+},2%Er^{3+} nanocrystals as probes. Concentration of RB equals to II (a) 0μg/ml, II (b) 10μg/ml, II (c) 100μg/ml, II (d) 500μg/ml and II (f) 700μg/ml use a 980nm notch filter under the excitation of a 980nm laser diode.

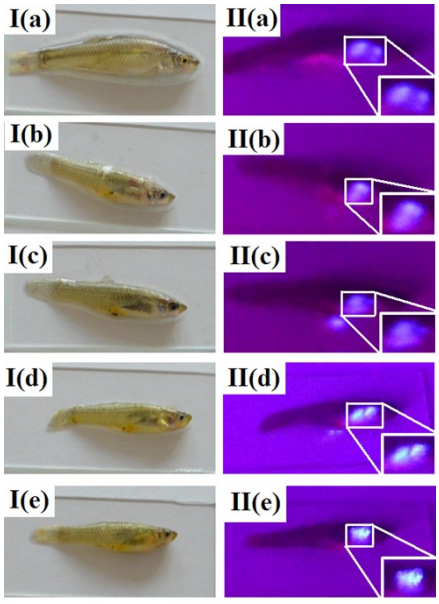


Fig.S13. Left column: conventional optical bioimaging in bright-field of the opaque fishes. **Right column:** In vivo upconversion luminescence imaging of the opaque fishes after cultured with different concentrations of sodium fluorescein for one day using $NaLuF_4$:18%Yb^{3+},0.5%Tm^{3+} nanocrystals as probes. Concentration of SF equals to II (a) 0μg/ml, II (b) 10μg/ml, II (c) 100μg/ml, II (d) 500μg/ml and II (f) 700μg/ml use a 980nm notch filter under the excitation of a 980nm laser diode.