Electronic Supplementary Information (ESI) for:

Deep tissue optical imaging of upconverting nanoparticles enabled by exploiting higher intrinsic quantum yield through using millisecond single pulse excitation with high peak power

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Content:

1. TEM image of the core $NaYF_4$:Yb^{3+},Tm^{3+} UCNPs

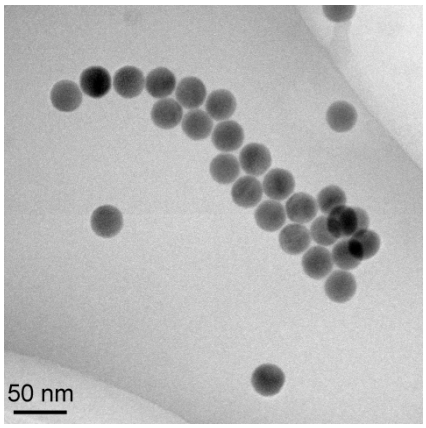

Figure S1. TEM image of core $NaYF_4$:Yb^{3+},Tm^{3+} nanoparticles. The mean diameter was determined to be approximately 32 nm.

2. The calculation of ion concentrations

The core-shell UCNPs used in this study are composed of two portions, *i.e.*, the optical active core $NaY_{1-x-y}Yb_xTm_yF_4$ (x and y denoting the molar concentrations in rare earth elements, respectively) and the shielding layer $NaYF_4$. The cores have an average diameter of D_1=32 nm, and the core-shell particles have an average diameter of D_2 = 42 nm. In the rate equation model, N_0 and N_{Yb0} denote the number densities of Tm^{3+} and Yb^{3+} ions in the core portion, respectively.

The overall molar ratio between the three rare earth elements in the core-shell UCNPs was determined to be Y:Yb:Tm = 94.858%:5.100%:0.042% by ICP-OES analysis on a PerkinElmer Optima 8300. The molar weight of $NaY_{1-x-y}Yb_xTm_yF_4$ is thus obtained

$$Mr_1 = 22.99 + 88.91 \cdot (1 - x - y) + 173.04 \cdot x + 168.93 \cdot y + 19.00 \times 4$$

$$= 187.9 + 84.13 \cdot x + 80.02 \cdot y \ \mathrm{g/mol},$$

while the molar weight of $NaYF_4$ is

$$Mr_2 = 22.99 + 88.91 + 19.00 \times 4 = 187.9 \ \mathrm{g/mol}\,.$$

The density of $NaYF_4$ crystal was determined to be $\rho = 4.2\ \mathrm{g/cm^3}$ by X-ray energy dispersive spectroscopy (XEDS) analysis. The mass of the core portion in a single core-shell UCNP can be calculated by

$$m_{\mathrm{core}} = V_{\mathrm{core}} \cdot \rho = \frac{4}{3}\pi\left(\frac{D_1}{2}\right)^3 \cdot \rho = 2.3 \times 10^{-17}\mathrm{g},$$

while the mass of the shell portion can be calculated by

$$m_{\mathrm{shell}} = V_{\mathrm{shell}} \cdot \rho = \left[\frac{4}{3}\pi\left(\frac{D_2}{2}\right)^3 - \frac{4}{3}\pi\left(\frac{D_1}{2}\right)^3\right] \cdot \rho = 2.9 \times 10^{-17}\mathrm{g}.$$

The overall molar ratio between the rare earth elements can be calculated by

$$\mathrm{Y:Yb:Tm} = \left[\frac{m_{\mathrm{core}}}{Mr_1} \cdot (1 - x - y) + \frac{m_{\mathrm{shell}}}{Mr_2}\right] : \frac{m_{\mathrm{core}}}{Mr_1} \cdot x : \frac{m_{\mathrm{core}}}{Mr_1} \cdot y$$

$$= 94.858\%: 5.100\%: 0.042\%$$

x and *y* are thus determined to be

$$x = 11.9\%,$$

and

$$y = 0.098\%$$

The concentration of Yb^{3+} ions is obtained by

$$N_{\mathrm{Yb0}} = \frac{\rho \cdot 1}{Mr_1} \cdot x \cdot N_{\mathrm{A}} = 1.52 \times 10^{21}\mathrm{cm}^{-3}$$

involving N_{A} the Avogadro constant. Similarly, the concentration of Tm^{3+} ions is obtained by

$$N_0 = \frac{\rho \cdot 1}{Mr_1} \cdot y \cdot N_{\mathrm{A}} = 1.25 \times 10^{19}\mathrm{cm}^{-3}$$

3. Estimated number of ions in a single nanoparticle

In a single nanoparticle, the numbers of ions can be obtained by

$$n_{\mathrm{Yb}} = N_{\mathrm{Yb0}} \cdot V_{\mathrm{core}} = 26080$$

and

$$n_{\mathrm{Tm}} = N_0 \cdot V_{\mathrm{core}} = 215$$

for Yb^{3+} and Tm^{3+} ions, respectively.

4. The selection of the ETU rates

a. The selection of C_0 and C_1

The power density dependent steady-state quantum yield of the used core-shell UCNPs have been measured and reported recently in our previous work [Liu *et al.*, Nanoscale, 2013, **5**, 4770-4775]. In this study, the ETU rates C_0 and C_1 were selected on the principle of giving the best fitting between the simulated and experimental results, which ends up with

$$C_0 = 1.6\times10^{-18}\ \mathrm{cm}^3/\mathrm{s},$$

and

$$C_1 = 6.2\times10^{-16}\ \mathrm{cm}^3/\mathrm{s}.$$

Since $NaYF_4$ is poorly characterized in bulk form, we compared such values with those reported in Ref. 28 for $LiYF_4$ crystal, which has similar phonon energies to $NaYF_4$. The selected value for C_0 here is several times smaller than that for sample no. 5 ($C_0=9.1\times10^{-18}$ cm^3/s) in Ref. 28 which has the most similar molar concentrations with the UCNPs investigated. This is reasonable considering the ~10 times less doping level of Tm^{3+} ions in this study, as C_0 decreases with the decrease of the molar concentration of Tm^{3+} ions, referring to the energy-transfer parameters for samples no. 2 and no. 4 in TABLE III reported in Ref. 28. The value for C_1 is on the same order of that reported in Ref. 28 for sample no. 5, exhibiting its reasonability.

b. The selection of C_2

The energy gap between states $^3H_5$ (Tm^{3+}) and $^3H_6$ (Tm^{3+}) (~8500 cm^{-1}) is similar to that between $^1G_4$ and $^3H_4$ ((~8620 cm^{-1})[27]. Thus, the energy mismatches of ETU0 and ETU1 are very similar. Using the energy-gap law formula [Diening *et al.*, J. Appl. Phys. 1998, **84**, 5900-5904], C_2 was estimated to be similar to C_0. In this study, we take the same value for C_2 with C_0.

5. Decay profiles of NIR UC emission from Tm^{3+} ions and the emission at 1035 nm from Yb^{3+} ions under 975 nm excitation

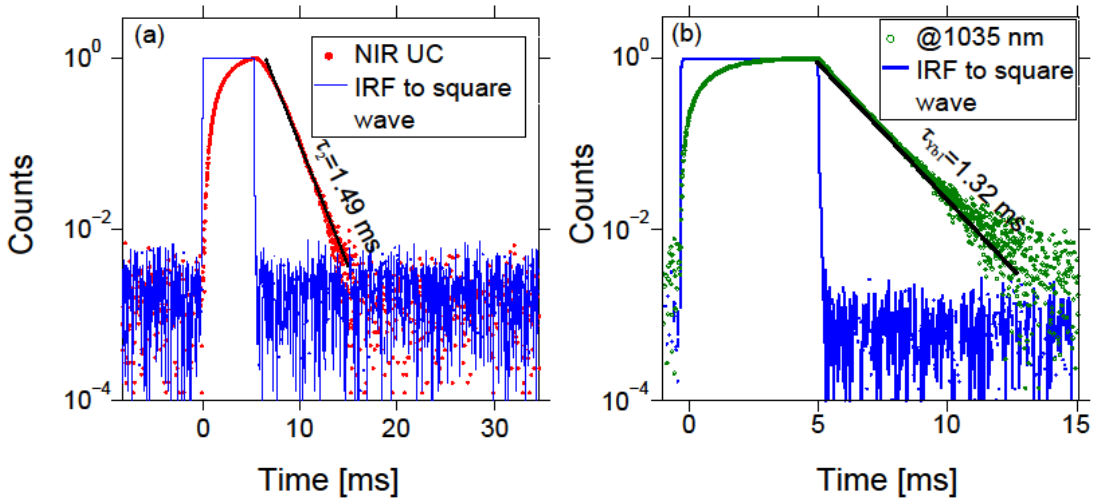

Figure S2. Decay profile of (a) the NIR UC emission at 800 nm from Tm^{3+} ions and (b) the emission at 1035 nm from Yb^{3+} ions under a square-wave excitation at 975 nm.

6. Simulated cumulative QY at the average power density of 1 W/cm^2

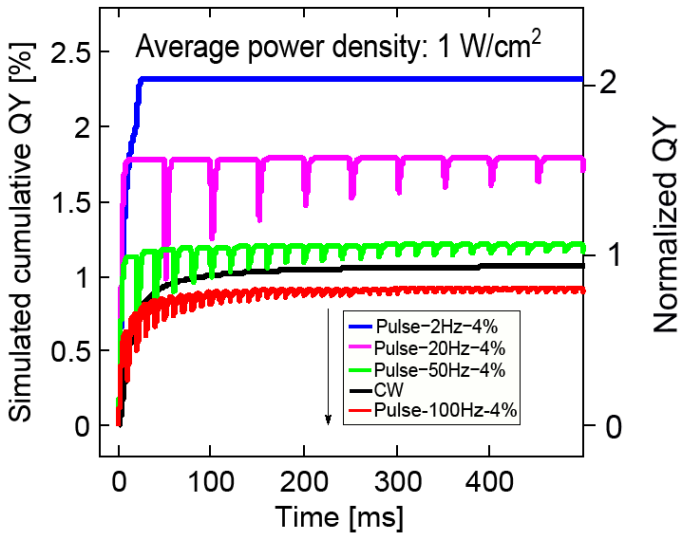

Figure S3. The temporally cumulative QY of NIR UC emission under CW excitation and under pulsed excitation with a fixed duty cycle of 4% and with various repetition rates. All the excitation approaches have the same average power density of 1 W/cm^2

7. The influence of ETU rates on the simulated signal gain

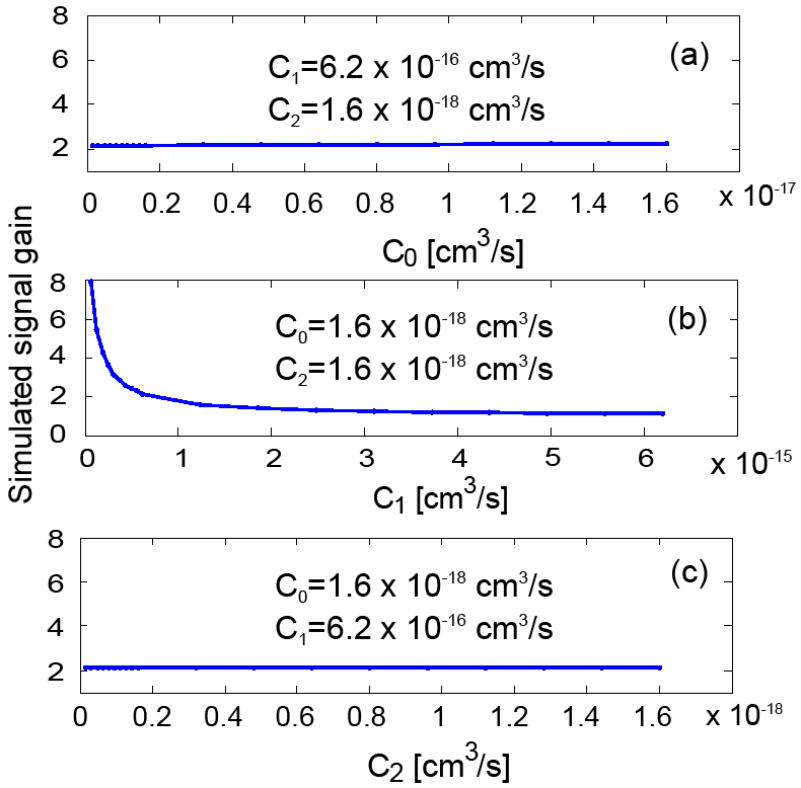

Figure S4. The influence of ETU rates (a) C_0, (b) C_1 and (c) C_2 on the simulated signal gain. The average power density was set to 1 W/cm^2, and the pulsed excitation has a 2 Hz repetition rate and 4% duty cycle in all simulations. Other parameter values were kept unchanged as in Table 1.

8. The calculation of maximum permissible power-density for exposure to human skin

a. For CW excitation

The Maximum permissible exposure (MPE) for skin exposure to a CW laser beam is given by

$$\mathrm{MPE_{CW}} = 0.2 C_\mathrm{A}\ \mathrm{W \cdot cm^{-2}} \tag{1}$$

where C_A is a wavelength dependent parameter,

$$C_\mathrm{A} = 10^{2(\lambda - 0.700)} \tag{2}$$

with λ [μm] the wavelength. For the wavelength of 975 nm,

$$C_\mathrm{A} = 3.548 \tag{3}$$

and

$$\mathrm{MPE_{CW}} = 709.6\ \mathrm{mW \cdot cm^{-2}} \tag{4}$$

b. For repetitive pulse excitation

For repetitive-pulse lasers, two rules apply to determine the MPEs for skin exposure.

Rule 1. Single-pulse limit.

Exposure of the skin shall not exceed the MPE based upon a single-pulse exposure.

Rule 2. Average-power limit

The average irradiance of the pulse train shall not exceed the MPE applicable for the total pulse train, duration *T*.

The MPE found using Rule 1 for a 975 nm laser where $T = 10$ s, pulse width $t = 20$ ms and $F = 2$ Hz is:

$$\begin{aligned} \mathrm{MPE_{sp}} &= 1.1 C_\mathrm{A} t^{0.25}\ \mathrm{J \cdot cm^{-2}} \\ &= 1.1 \times 3.548 \times 0.02^{0.25}\ \mathrm{J \cdot cm^{-2}} \\ &= 1.47\ \mathrm{J \cdot cm^{-2}} \end{aligned} \tag{5}$$

This MPE in terms of average power for Rule 1 is:

$$\mathrm{MPE}: E = \mathrm{MPE_{sp}} \times F = 2.9\ \mathrm{W \cdot cm^{-2}} \tag{6}$$

The MPE found using Rule 2 is:

$$\begin{aligned} \mathrm{MPE}: H_\mathrm{group} &= 1.1 C_\mathrm{A} t^{0.25}\ \mathrm{J \cdot cm^{-2}} \\ &= 1.1 \times 3.548 \times 10^{0.25}\ \mathrm{J \cdot cm^{-2}} \\ &= 6.94\ \mathrm{J \cdot cm^{-2}} \end{aligned} \tag{7}$$

In terms of average irradiance, the MPE is

$$\mathrm{MPE}: E = \frac{\mathrm{MPE}: H_\mathrm{group}}{10\ \mathrm{s}} = 694\ \mathrm{mW \cdot cm^{-2}} \tag{8}$$

Resultant MPE:

The MPE found using Rule 2 is the correct MPE to apply, since it is the smallest. The corresponding peak power-density in the pulse duration is

$$\rho = 694\ \mathrm{mW \cdot cm^{-2}} \times \frac{\frac{1}{2\ \mathrm{Hz}}}{20\ \mathrm{ms}} = 17.4\ \mathrm{W \cdot cm^{-2}} \tag{9}$$

c. For single pulse excitation

The MPE for the single pulse excitation can be calculated by

$$\mathrm{MPE_{sp}} = \frac{1.1 C_\mathrm{A} t^{0.25}}{t}\ \mathrm{W \cdot cm^{-2}} \tag{10}$$

For a 50 ms single pulse,

$$\mathrm{MPE_{sp}} = 36.9\ \mathrm{W \cdot cm^{-2}} \tag{11}$$

Supporting references:

1. H. Liu, C. T. Xu, D. Lindgren, H. Xie, D. Thomas, C. Gundlach and S. Andersson-Engels, *Nanoscale*, 2013, **5**, 4770-4775.

2. A. Diening, P. E.-A. Möbert and G. Huber, *J. Appl. Phys.*, 1998, **84**, 5900-5904.