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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1. TEM image of the core NaYF₄:Yb³⁺,Tm³⁺ UCNPs

![TEM image of core NaYF₄:Yb³⁺,Tm³⁺ nanoparticles](image)

Figure S1. TEM image of core NaYF₄:Yb³⁺,Tm³⁺ 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 NaY₁₋ₓ₋ₓYb,Tm,F₄ (x and y denoting the molar concentrations in rare earth elements, respectively) and the shielding layer NaYF₄. The cores have an average diameter of \( D_1 = 32 \text{ nm} \), and the core-shell particles have an average diameter of \( D_2 = 42 \text{ nm} \). In the rate equation model, \( N_0 \) and \( N_{Yb0} \) denote the number densities of Tm³⁺ and Yb³⁺ 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 NaY₁₋ₓ₋ₓYb,Tm,F₄ is thus obtained

\[
M_{r1} = 22.99 + 88.91 \cdot (1 - x - y) + 173.04 \cdot x + 168.93 \cdot y + 19.00 \cdot 4 = 187.9 + 84.13 \cdot x + 80.02 \cdot y \text{ g/mol},
\]

while the molar weight of NaYF₄ is

\[
M_{r2} = 22.99 + 88.91 + 19.00 \times 4 = 187.9 \text{ g/mol}.
\]

The density of NaYF₄ crystal was determined to be \( \rho = 4.2 \text{ g/cm}^3 \) by X-ray energy dispersive spectroscopy (XEDS) analysis. The mass of the core portion in a single core-shell UCNP can be calculated by

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

while the mass of the shell portion can be calculated by

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

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

\[
Y:Yb:Tm = \frac{m_{\text{core}}}{M_{r1}} \cdot (1 - x - y) + \frac{m_{\text{shell}}}{M_{r2}} : \frac{m_{\text{core}}}{M_{r1}} \cdot x : \frac{m_{\text{core}}}{M_{r1}} \cdot y
\]
\[ = 94.858\%: 5.100\%: 0.042\% \]

\( x \) and \( y \) are thus determined to be

\[ x = 11.9\% , \]

and

\[ y = 0.098\% \]

The concentration of \( \text{Yb}^{3+} \) ions is obtained by

\[ N_{\text{Yb}0} = \frac{\rho \cdot 1}{M_{r1}} \cdot x \cdot N_A = 1.52 \times 10^{21} \text{cm}^{-3} \]

involving \( N_A \) the Avogadro constant. Similarly, the concentration of \( \text{Tm}^{3+} \) ions is obtained by

\[ N_0 = \frac{\rho \cdot 1}{M_{r1}} \cdot y \cdot N_A = 1.25 \times 10^{19} \text{cm}^{-3} \]

3. Estimated number of ions in a single nanoparticle

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

\[ n_{\text{Yb}} = N_{\text{Yb}0} \cdot V_{\text{core}} = 26080 \]

and

\[ n_{\text{Tm}} = N_0 \cdot V_{\text{core}} = 215 \]

for \( \text{Yb}^{3+} \) and \( \text{Tm}^{3+} \) 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

\[ C_0 = 1.6 \times 10^{-18} \text{cm}^3/\text{s}, \]

and

\[ C_1 = 6.2 \times 10^{-16} \text{cm}^3/\text{s}. \]

Since \( \text{NaYF}_4 \) is poorly characterized in bulk form, we compared such values with those reported in Ref. 28 for \( \text{LiYF}_4 \) crystal, which has similar phonon energies to \( \text{NaYF}_4 \). The selected value for \( C_0 \) here is several times smaller than that for sample no. 5 (\( C_0 = 9.1 \times 10^{-18} \text{cm}^3/\text{s} \)) 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 \( \text{Tm}^{3+} \) ions in this study, as \( C_0 \) decreases with the decrease of the molar concentration of \( \text{Tm}^{3+} \) 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 $^3\text{H}_5$ (Tm$^{3+}$) and $^3\text{H}_6$ (Tm$^{3+}$) (~8500 cm$^{-1}$) is similar to that between $^1\text{G}_4$ and $^3\text{H}_4$ ( (~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

![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.](Image)

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$^2$

![Simulated cumulative QY at the average power density of 1 W/cm$^2$.](Image)

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

![Graph showing the influence of ETU rates on the simulated signal gain.](image)

Figure S4. The influence of ETU rates (a) C₀, (b) C₁ and (c) C₂ on the simulated signal gain. The average power density was set to 1 W/cm², 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

\[ \text{MPE}_{\text{CW}} = 0.2C_A \text{ W} \cdot \text{cm}^{-2} \]  \hspace{1cm} (1)

where \( C_A \) is a wavelength dependent parameter,

\[ C_A = 10^{2(\lambda - 0.700)} \]  \hspace{1cm} (2)

with \( \lambda [\mu\text{m}] \) the wavelength. For the wavelength of 975 nm,

\[ C_A = 3.548 \]  \hspace{1cm} (3)

and

\[ \text{MPE}_{\text{CW}} = 709.6 \text{ mW} \cdot \text{cm}^{-2} \]  \hspace{1cm} (4)

b. For repetitive pulse excitation

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

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:

$$\text{MPE}_{sp} = 1.1C_A t^{0.25} \text{ J} \cdot \text{cm}^{-2}$$

$$= 1.1 \times 3.548 \times 0.02^{0.25} \text{ J} \cdot \text{cm}^{-2}$$

$$= 1.47 \text{ J} \cdot \text{cm}^{-2}$$

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

$$\text{MPE}: E = \text{MPE}_{sp} \times F = 2.9 \text{ W} \cdot \text{cm}^{-2}$$

The MPE found using Rule 2 is:

$$\text{MPE}: H_{\text{group}} = 1.1C_A t^{0.25} \text{ J} \cdot \text{cm}^{-2}$$

$$= 1.1 \times 3.548 \times 10^{0.25} \text{ J} \cdot \text{cm}^{-2}$$

$$= 6.94 \text{ J} \cdot \text{cm}^{-2}$$

In terms of average irradiance, the MPE is

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

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

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

**c. For single pulse excitation**

The MPE for the single pulse excitation can be calculated by

$$\text{MPE}_{sp} = \frac{1.1C_A t^{0.25}}{t} \text{ W} \cdot \text{cm}^{-2}$$

For a 50 ms single pulse,

$$\text{MPE}_{sp} = 36.9 \text{ W} \cdot \text{cm}^{-2}$$
Supporting references:
