

# Supplementary Materials for

## **Multiplexed structured illumination super-resolution imaging with lifetime-engineered upconversion nanoparticles**

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This file includes:

Materials and Methods

Supplementary Figures. S1 to S5

## **Materials and Methods**

### **1. Nanoparticle synthesis**

#### **1.1 Synthesis of NaYF<sub>4</sub>: Yb, Er core nanoparticles**

We synthesized the NaYF<sub>4</sub>:20%Yb, 2%Er core nanoparticles by coprecipitation method. 1 mmol RECl<sub>3</sub> (YCl<sub>3</sub>·6H<sub>2</sub>O (0.78 mmol), YbCl<sub>3</sub>·6H<sub>2</sub>O (0.2 mmol) and ErCl<sub>3</sub>·6H<sub>2</sub>O (0.02 mmol) ) methanol solution together with 6 mL oleic acid (OA) and 15 mL 1-octadecene (ODE) were added to a 50 ml three-neck round-bottom flask under vigorous stirring. The resulting mixture was heated at 150 °C for 40 mins. Then the solution was cooled down to room temperature. A 6 mL methanol solution with 2.5 mmol NaOH and 4 mmol NH<sub>4</sub>F was added and stirred for 40 mins, and then the mixture was slowly heated to 150 °C and kept for 40 mins under argon flow to remove methanol and residual water. The solution was quickly heated at 300 °C under an argon flow for 90 mins. The final NaYF<sub>4</sub>:Yb,Er nanocrystals were dispersed in cyclohexane after washing with cyclohexane/ethanol/methanol several times.

Another three kinds of core nanoparticles were synthesized with different doping concentrations (NaYF<sub>4</sub>:20%Yb, 1.5%Er, NaYF<sub>4</sub>:30%Yb, 2%Er, NaYF<sub>4</sub>:30%Yb, 8%Er) using the same above method.

#### **1.2 Synthesis of NaYF<sub>4</sub>: 5%Yb, NaYF<sub>4</sub>: x%Yb, 20%Nd (x= 5, 15, 30) and NaYF<sub>4</sub> pure precursors**

The precursors were prepared similar as above process and stopped when the reaction solution was heated to 150 °C after adding NaOH/NH<sub>4</sub>F solution and kept for 40 min. The solution was cooled down to room temperature to yield the shell precursors.

#### **1.3 Synthesis of core@NaYF<sub>4</sub>:5%Yb core-shell nanoparticles**

We prepared the core@ NaYbF<sub>4</sub>:5%Yb core-shell nanoparticles by epitaxial growth method. The pre-synthesized NaYF<sub>4</sub>: Yb, Er core nanoparticles were used as seeds for shell modification. The core nanocrystals were added to a 50 ml flask with 3 ml OA and 8 ml ODE. The mixture was heated to 160 °C under argon for 30 min, and then further heated to 300 °C. The as-prepared shell precursors were injected into the reaction mixture about 0.02ml/2 min to get around 3 nm thickness shell. After the reaction, the solution was cooled down to room temperature and washed dispersed in

cyclohexane for next step epitaxial growth.

#### **1.4 Synthesis of core@NaYF<sub>4</sub>:5%Yb@NaYF<sub>4</sub>:x%Yb, 20%Nd (x=5, 15, 30) core-shell-shell nanoparticles and core @NaYF<sub>4</sub>:5%Yb @NaYF<sub>4</sub>: x%Yb, 20%Nd @NaYF<sub>4</sub> (x=5, 15, 30) core-shell-shell-shell nanoparticles**

The core-shell-shell (core-shell-shell-shell) nanoparticles were also prepared by epitaxial growth method described above and the core-shell samples (core-shell-shell) were used as the seeds.

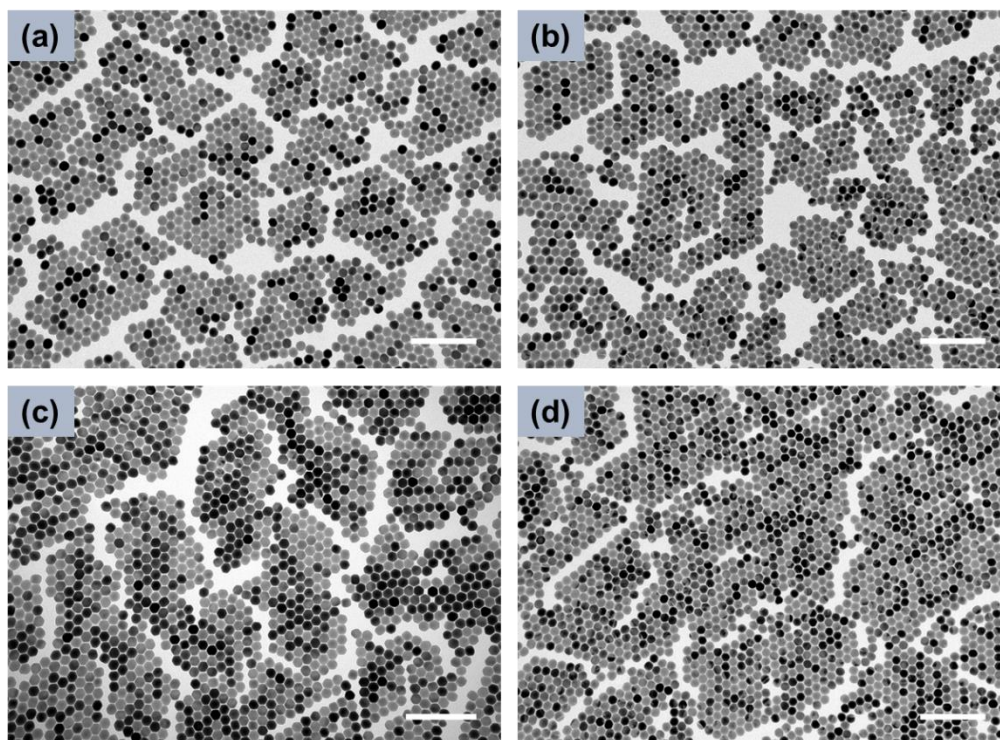
### **2. General materials characterization techniques.**

The shape of the nanoparticles were performed by transmission electron microscope (TEM), JEOL TEM-1400 at an acceleration voltage of 120 kV. The samples were prepared by dropping onto the carbon-coated copper grids.

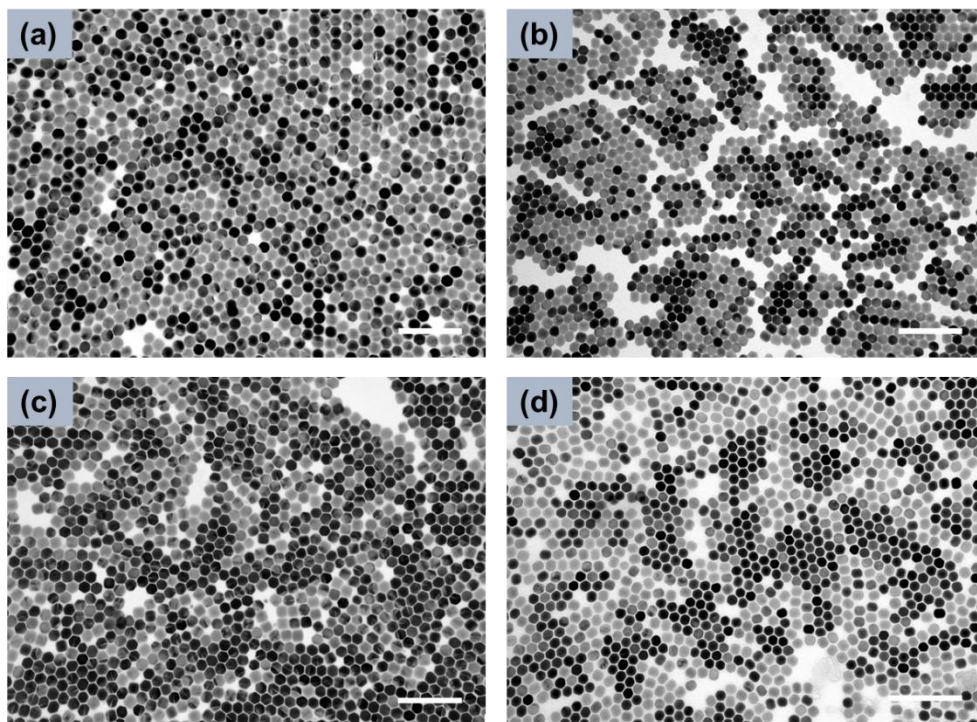
### **3. Preparation of sample slides for single nanoparticle measurements**

A coverslip was washed with ethanol by ultrasonication and dried. 10  $\mu$ l of the  $\tau^2$ -dots (diluted to 0.01 mg/ml in cyclohexane) was dropped onto the surface of cover slip and let it dry naturally. The coverslip was put onto a glass slide and squeezed out air bubbles.

## Supplementary Figures

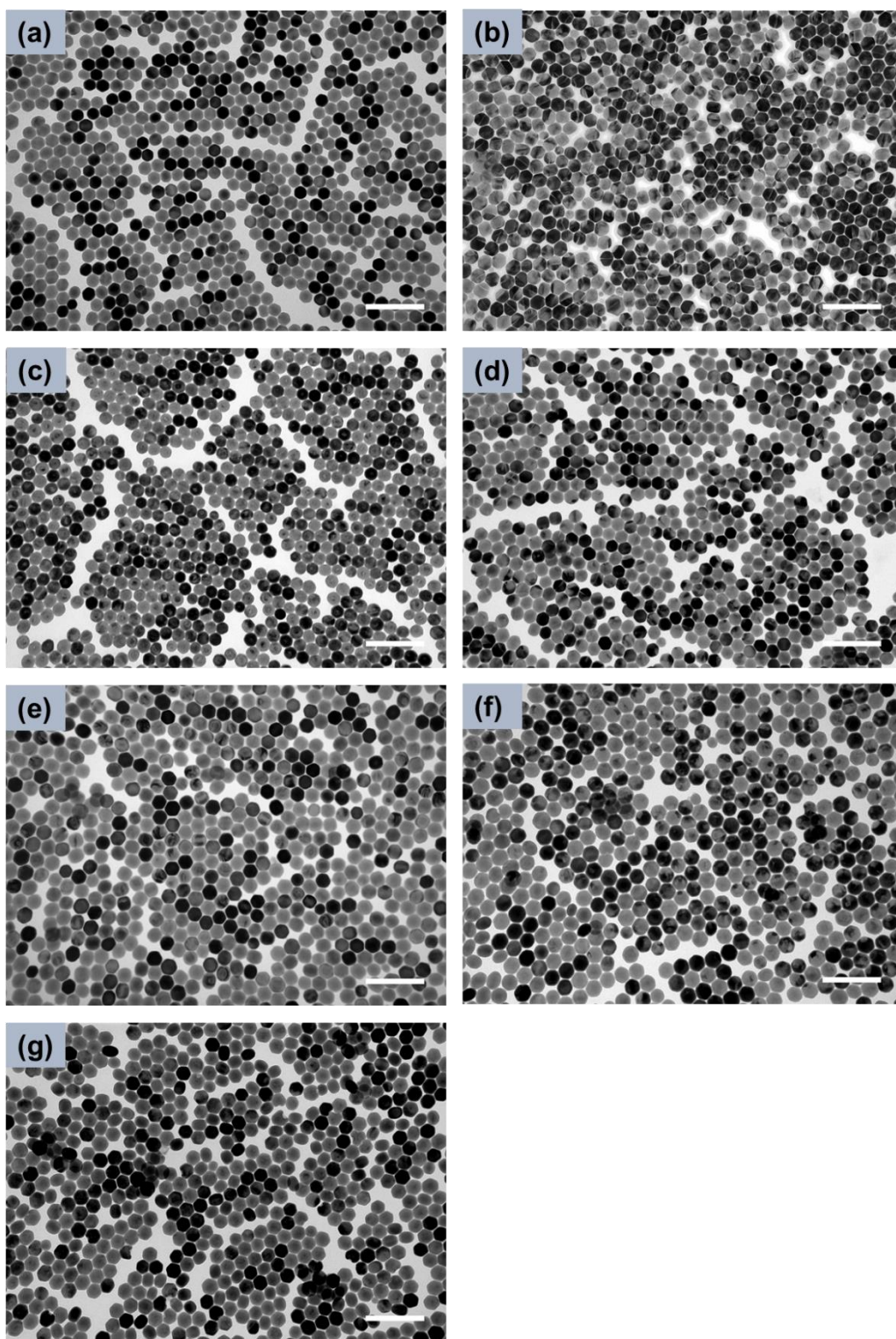


**Figure S1. TEM images of core samples.** (a)  $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$ , 2%  $\text{Er}^{3+}$  core nanoparticles. (b)  $\text{NaYF}_4\text{:}30\%\text{Yb}^{3+}$ , 8%  $\text{Er}^{3+}$  core nanoparticles. (c)  $\text{NaYF}_4\text{:}30\%\text{Yb}^{3+}$ , 2%  $\text{Er}^{3+}$  core nanoparticles. (d)  $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$ , 1.5%  $\text{Er}^{3+}$  core nanoparticles. Scale bar: 200 nm.



**Figure S2. TEM images of core-shell nanoparticles.** (a)  $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$ ,  $2\%\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$  nanoparticles. (b)  $\text{NaYF}_4\text{:}30\%\text{Yb}^{3+}$ ,  $8\%\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$  nanoparticles. (c)  $\text{NaYF}_4\text{:}30\%\text{Yb}^{3+}$ ,  $2\%\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$  nanoparticles. (d)  $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$ ,  $1.5\%\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$  nanoparticles. Scale bar: 200 nm.





**Figure S3. TEM images of core-shell-shell nanoparticles.**

(a)  $\text{NaYF}_4\text{:}30\%\text{Yb}^{3+}$ ,  $8\%\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$  @  $\text{NaYF}_4\text{:}15\%\text{Yb}^{3+}$ ,  $20\%\text{Nd}^{3+}$  nanoparticles.

(b)  $\text{NaYF}_4\text{:}30\%\text{Yb}^{3+}$ ,  $2\%\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$  @  $\text{NaYF}_4\text{:}15\%\text{Yb}^{3+}$ ,  $20\%\text{Nd}^{3+}$  nanoparticles.

(c)  $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$ ,  $1.5\%\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$  @  $\text{NaYF}_4\text{:}15\%\text{Yb}^{3+}$ ,  $20\%\text{Nd}^{3+}$

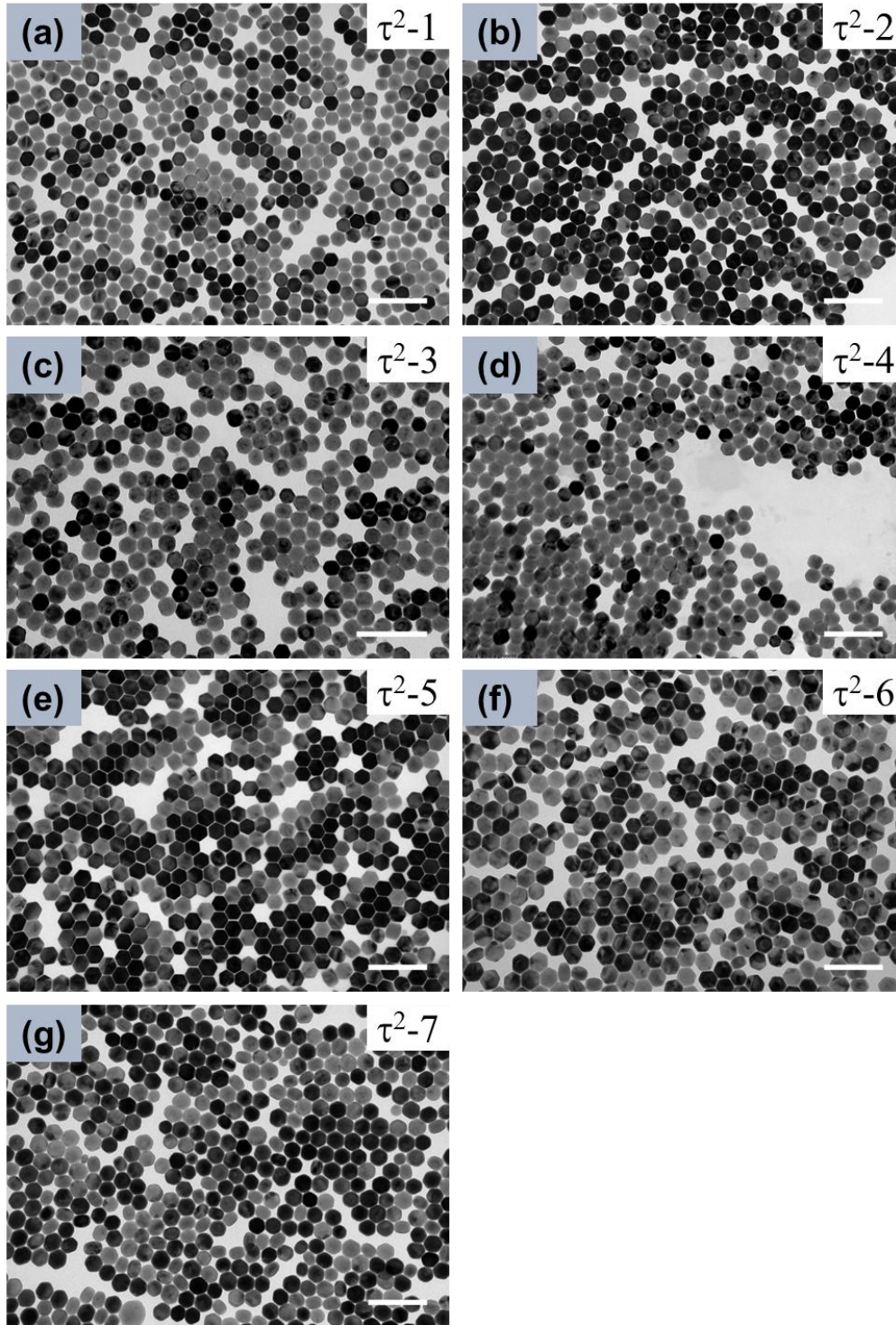
nanoparticles.

(d)  $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$ , 2%  $\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$ @  $\text{NaYF}_4\text{:}15\%\text{Yb}^{3+}$ , 20%  $\text{Nd}^{3+}$  nanoparticles.

(e)  $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$ , 2%  $\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$ @  $\text{NaYF}_4\text{:}15\%\text{Yb}^{3+}$ , 20%  $\text{Nd}^{3+}$  nanoparticles.

(f)  $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$ , 2%  $\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$ @  $\text{NaYF}_4\text{:}30\%\text{Yb}^{3+}$ , 20%  $\text{Nd}^{3+}$  nanoparticles.

(g)  $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$ , 1%  $\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$ @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$ , 20%  $\text{Nd}^{3+}$  nanoparticles. Scale bar: 200 nm.



**Figure S4. TEM images of core-shell-shell-shell nanoparticles.**

(a)  $\text{NaYF}_4\text{:}30\%\text{Yb}^{3+}$ ,  $8\%\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$  @  $\text{NaYF}_4\text{:}15\%\text{Yb}^{3+}$ ,  $20\%\text{Nd}^{3+}$  @  $\text{NaYF}_4$  nanoparticles ( $\tau^2$ -1).

(b)  $\text{NaYF}_4\text{:}30\%\text{Yb}^{3+}$ ,  $2\%\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$  @  $\text{NaYF}_4\text{:}15\%\text{Yb}^{3+}$ ,  $20\%\text{Nd}^{3+}$  @  $\text{NaYF}_4$  nanoparticles ( $\tau^2$ -2).

(c)  $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$ ,  $1.5\%\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$  @  $\text{NaYF}_4\text{:}15\%\text{Yb}^{3+}$ ,  $20\%\text{Nd}^{3+}$  @  $\text{NaYF}_4$  nanoparticles ( $\tau^2$ -3).

(d)  $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$ ,  $2\%\text{Er}^{3+}$  @  $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$  @  $\text{NaYF}_4\text{:}15\%\text{Yb}^{3+}$ ,  $20\%\text{Nd}^{3+}$  @

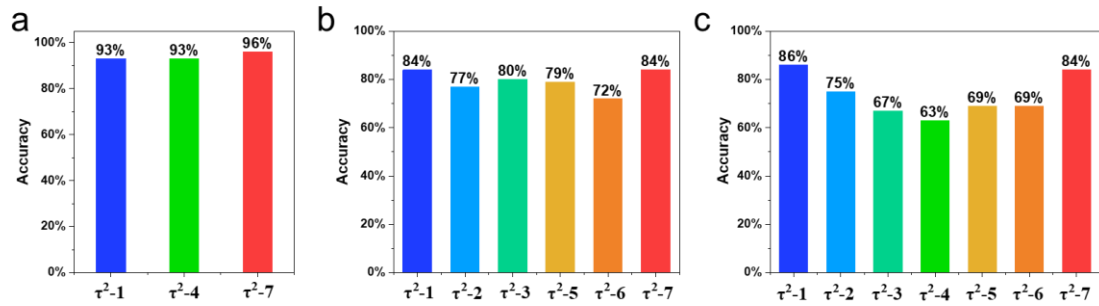


NaYF<sub>4</sub> nanoparticles ( $\tau^2$ -4).

(e) NaYF<sub>4</sub>:20%Yb<sup>3+</sup>, 2% Er<sup>3+</sup> @ NaYF<sub>4</sub>:5%Yb<sup>3+</sup>@ NaYF<sub>4</sub>: 15%Yb<sup>3+</sup>, 20% Nd<sup>3+</sup> @ NaYF<sub>4</sub> nanoparticles ( $\tau^2$ -5).

(f) NaYF<sub>4</sub>:20%Yb<sup>3+</sup>, 2% Er<sup>3+</sup> @ NaYF<sub>4</sub>:5%Yb<sup>3+</sup>@ NaYF<sub>4</sub>: 30%Yb<sup>3+</sup>, 20% Nd<sup>3+</sup> @ NaYF<sub>4</sub> nanoparticles ( $\tau^2$ -6).

(g) NaYF<sub>4</sub>:20%Yb<sup>3+</sup>, 1% Er<sup>3+</sup> @ NaYF<sub>4</sub>:5%Yb<sup>3+</sup>@ NaYF<sub>4</sub>: 5%Yb<sup>3+</sup>, 20% Nd<sup>3+</sup> @ NaYF<sub>4</sub> nanoparticles ( $\tau^2$ -7). Scale bar: 200 nm.



**Figure S5.** Mean classification accuracies of 3 and 7 types of  $\tau^2$ -dots with deep learning algorithms, based on 50 times random validation test for each type.