

Supplementary Materials for

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

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Materials and Methods

1. Nanoparticle synthesis

1.1 Synthesis of NaYF₄: Yb, Er core nanoparticles

We synthesized the NaYF₄:20%Yb, 2%Er core nanoparticles by coprecipitation method. 1 mmol RECl₃ (YCl₃·6H₂O (0.78 mmol), YbCl₃·6H₂O (0.2 mmol) and ErCl₃·6H₂O (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 NH₄F 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 NaYF₄: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 (NaYF₄:20%Yb, 1.5%Er, NaYF₄:30%Yb, 2%Er, NaYF₄:30%Yb, 8%Er) using the same above method.

1.2 Synthesis of NaYF₄: 5%Yb, NaYF₄: x%Yb, 20%Nd (x= 5, 15, 30) and NaYF₄ pure precursors

The precursors were prepared similar as above process and stopped when the reaction solution was heated to 150 °C after adding NaOH/NH₄F solution and kept for 40 min. The solution was cooled down to room temperature to yield the shell precursors.

1.3 Synthesis of core@NaYF₄:5%Yb core-shell nanoparticles

We prepared the core@ NaYbF₄:5%Yb core-shell nanoparticles by epitaxial growth method. The pre-synthesized NaYF₄: 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@NaYF₄:5%Yb@NaYF₄:x%Yb, 20%Nd (x=5, 15, 30) core-shell-shell nanoparticles and core @NaYF₄:5%Yb @NaYF₄: x%Yb, 20%Nd @NaYF₄ (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 μ l of the τ^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

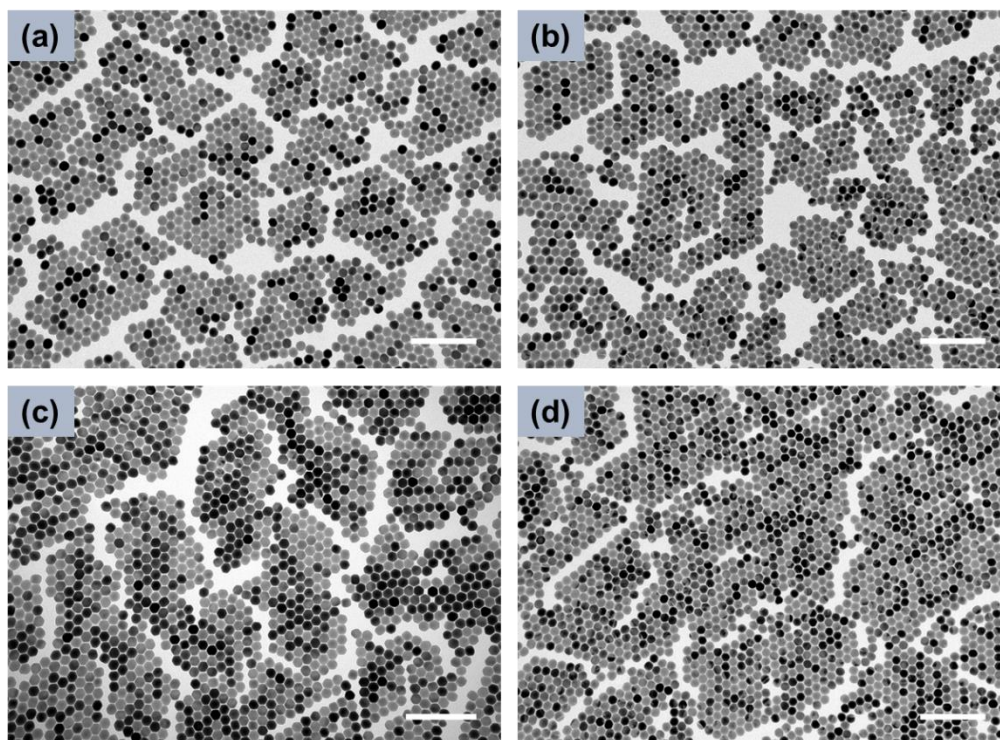


Figure S1. TEM images of core samples. (a) $\text{NaYF}_4:20\%\text{Yb}^{3+}$, 2% Er^{3+} core nanoparticles. (b) $\text{NaYF}_4:30\%\text{Yb}^{3+}$, 8% Er^{3+} core nanoparticles. (c) $\text{NaYF}_4:30\%\text{Yb}^{3+}$, 2% Er^{3+} core nanoparticles. (d) $\text{NaYF}_4:20\%\text{Yb}^{3+}$, 1.5% Er^{3+}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

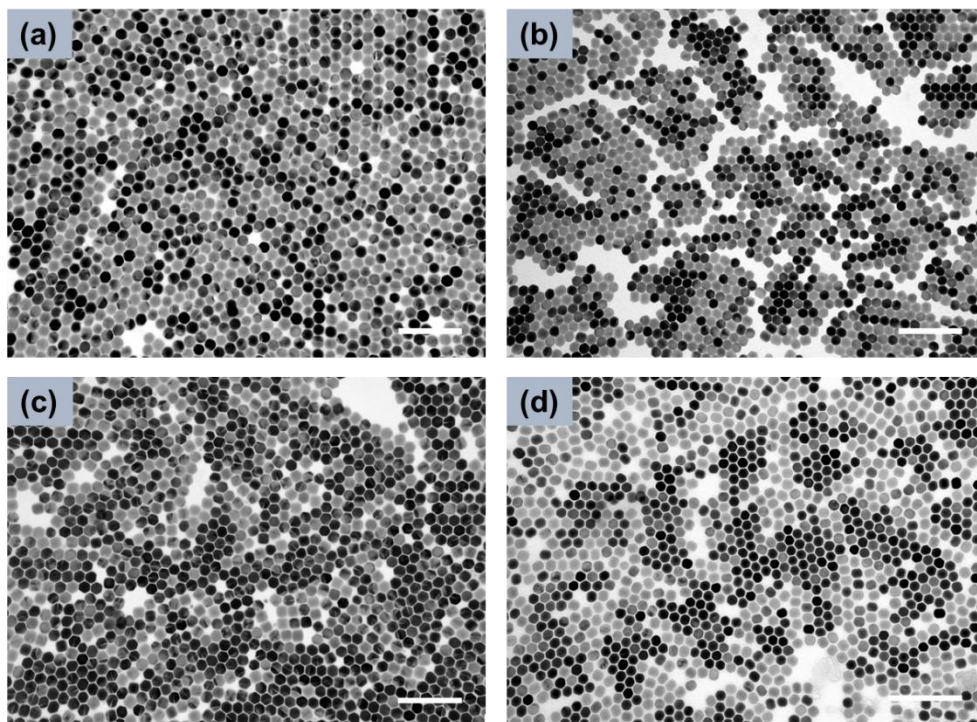


Figure S2. TEM images of core-shell nanoparticles. (a) $\text{NaYF}_4\text{:}20\%\text{Yb}^{3+}$, $2\%\text{Er}^{3+}$ @ $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$ nanoparticles. (b) $\text{NaYF}_4\text{:}30\%\text{Yb}^{3+}$, $8\%\text{Er}^{3+}$ @ $\text{NaYF}_4\text{:}5\%\text{Yb}^{3+}$ nanoparticles.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

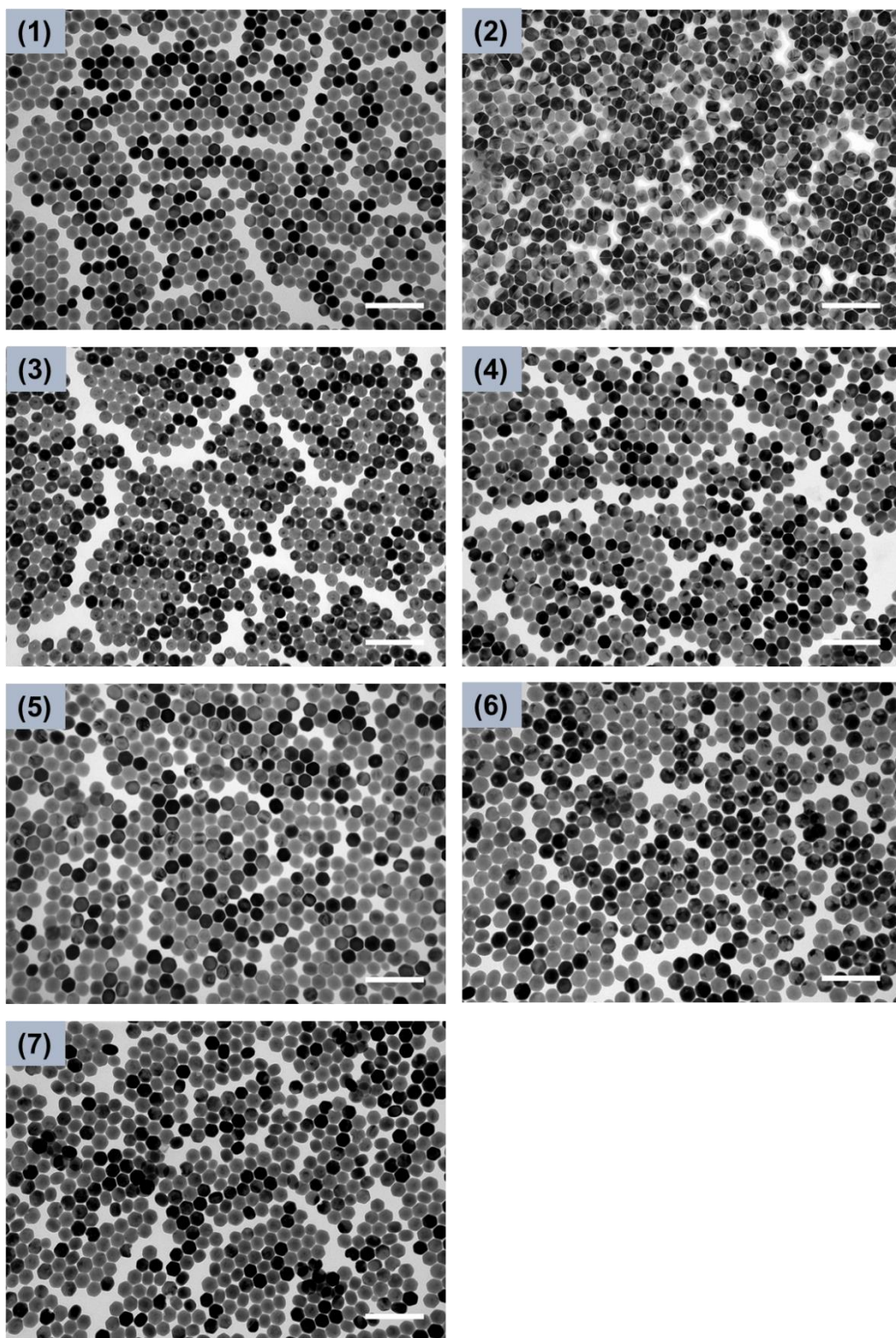


Figure S3. TEM images of core-shell-shell nanoparticles.

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

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

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

nanoparticles.

(4) NaYF₄:20%Yb³⁺, 2% Er³⁺ @ NaYF₄:5%Yb³⁺@ NaYF₄: 15%Yb³⁺, 20% Nd³⁺ nanoparticles.

(5) NaYF₄:20%Yb³⁺, 2% Er³⁺ @ NaYF₄:5%Yb³⁺@ NaYF₄: 15%Yb³⁺, 20% Nd³⁺ nanoparticles.

(6) NaYF₄:20%Yb³⁺, 2% Er³⁺ @ NaYF₄:5%Yb³⁺@ NaYF₄: 30%Yb³⁺, 20% Nd³⁺ nanoparticles.

(7) NaYF₄:20%Yb³⁺, 1% Er³⁺ @ NaYF₄:5%Yb³⁺@ NaYF₄: 5%Yb³⁺, 20% Nd³⁺ nanoparticles. Scale bar: 200 nm.

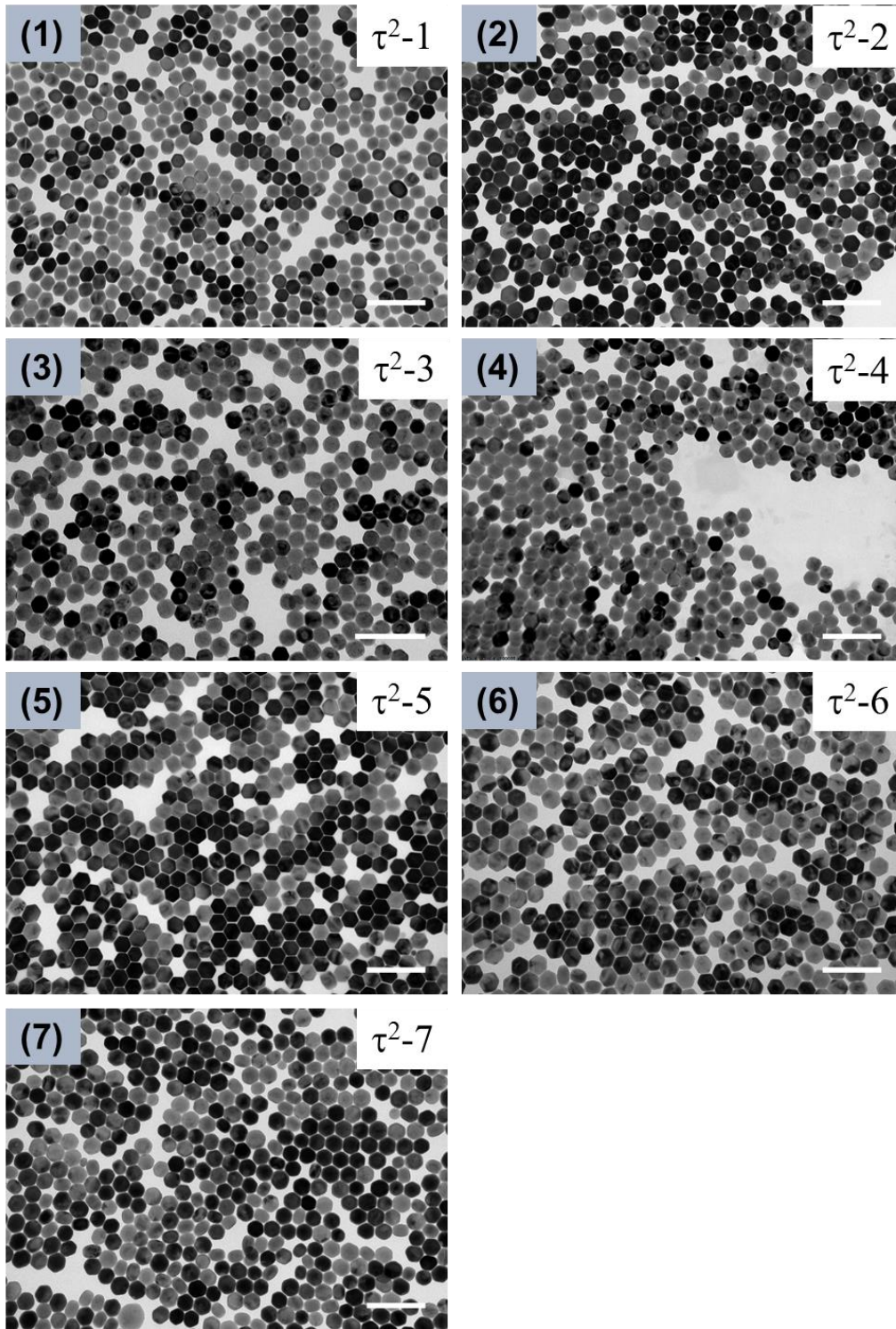


Figure S4. TEM images of core-shell-shell-shell nanoparticles.

(1) $\text{NaYF}_4:30\%\text{Yb}^{3+}$, $8\%\text{Er}^{3+}$ @ $\text{NaYF}_4:5\%\text{Yb}^{3+}$ @ $\text{NaYF}_4:15\%\text{Yb}^{3+}$, $20\%\text{Nd}^{3+}$ @ NaYF_4 nanoparticles (τ^2 -1).

(2) $\text{NaYF}_4:30\%\text{Yb}^{3+}$, $2\%\text{Er}^{3+}$ @ $\text{NaYF}_4:5\%\text{Yb}^{3+}$ @ $\text{NaYF}_4:15\%\text{Yb}^{3+}$, $20\%\text{Nd}^{3+}$ @ NaYF_4 nanoparticles (τ^2 -2).

(3) $\text{NaYF}_4:20\%\text{Yb}^{3+}$, $1.5\%\text{Er}^{3+}$ @ $\text{NaYF}_4:5\%\text{Yb}^{3+}$ @ $\text{NaYF}_4:15\%\text{Yb}^{3+}$, $20\%\text{Nd}^{3+}$ @ NaYF_4 nanoparticles (τ^2 -3).

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

NaYF₄ nanoparticles (τ^2 -4).

(5) NaYF₄:20%Yb³⁺, 2% Er³⁺ @ NaYF₄:5%Yb³⁺@ NaYF₄: 15%Yb³⁺, 20% Nd³⁺ @ NaYF₄ nanoparticles (τ^2 -5).

(6) NaYF₄:20%Yb³⁺, 2% Er³⁺ @ NaYF₄:5%Yb³⁺@ NaYF₄: 30%Yb³⁺, 20% Nd³⁺ @ NaYF₄ nanoparticles (τ^2 -6).

(7) NaYF₄:20%Yb³⁺, 1% Er³⁺ @ NaYF₄:5%Yb³⁺@ NaYF₄: 5%Yb³⁺, 20% Nd³⁺ @ NaYF₄ nanoparticles (τ^2 -7). Scale bar: 200 nm.

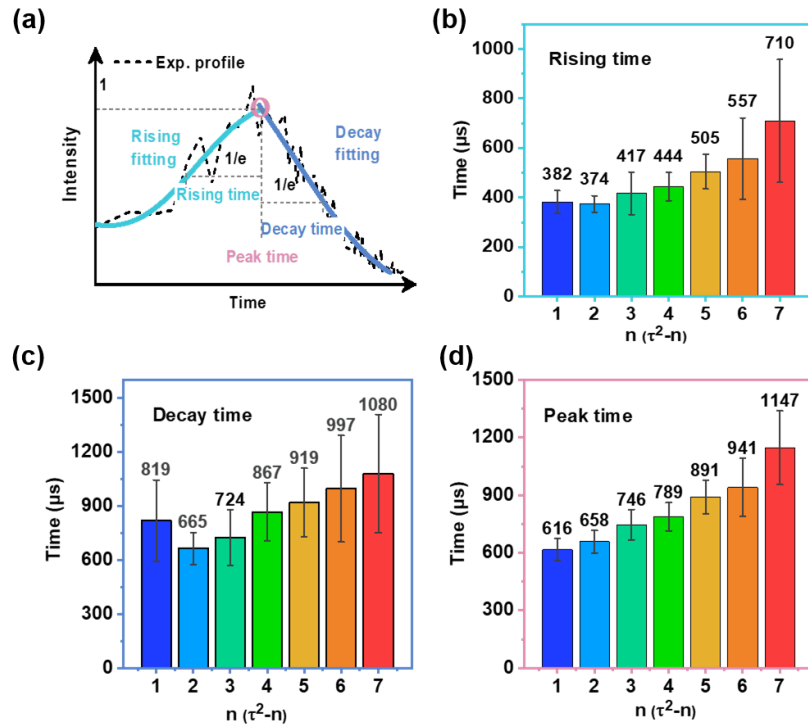


Figure S5. Characteristic parameters of lifetime curves of τ^2 dots. (a) Illustration of the rising time, decreasing time and peak time of τ^2 -1 - τ^2 -7 dots and corresponding results (b-d).

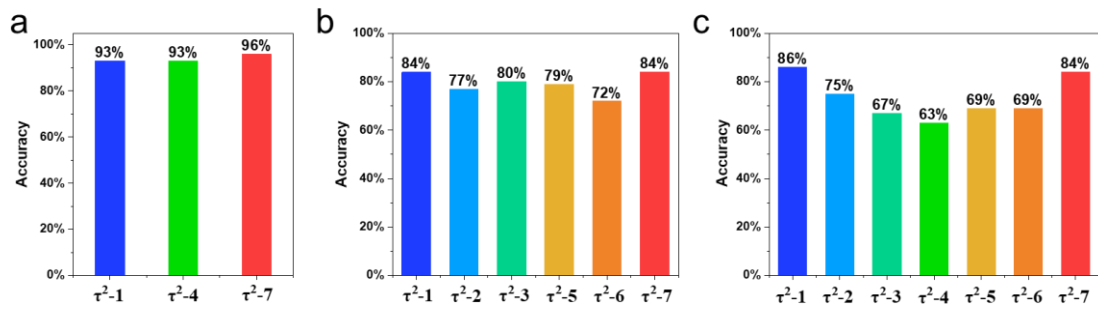


Figure S6. Mean classification accuracies of 3 and 7 types of τ^2 -dots with deep learning algorithms, based on 50 times random validation test for each type.