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 $NaYF_4$: Yb, Er core nanoparticles

We synthesized the $NaYF_4$:20%Yb, 2%Er core nanoparticles by coprecipitation method. 1 mmol $RECl_3$ ($YCl_3 \cdot 6H_2O$ (0.78 mmol), $YbCl_3 \cdot 6H_2O$ (0.2 mmol) and $ErCl_3 \cdot 6H_2O$ (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 ºC 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_4F 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 ºC under an argon flow for 90 mins. The final $NaYF_4$: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 ($NaYF_4$:20%Yb, 1.5%Er, $NaYF_4$:30%Yb, 2%Er, $NaYF_4$:30%Yb, 8%Er) using the same above method.

1.2 Synthesis of $NaYF_4$: 5%Yb, $NaYF_4$: x%Yb, 20%Nd (x= 5, 15, 30) and $NaYF_4$ 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_4F solution and kept for 40 min. The solution was cooled down to room temperature to yield the shell precursors.

1.3 Synthesis of core@$NaYF_4$:5%Yb core-shell nanoparticles

We prepared the core@ $NaYbF_4$:5%Yb core-shell nanoparticles by epitaxial growth method. The pre-synthesized $NaYF_4$: 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@$NaYF_4$:5%Yb@$NaYF_4$:x%Yb, 20%Nd (x=5, 15, 30) core-shell-shell nanoparticles and core @$NaYF_4$:5%Yb @$NaYF_4$: x%Yb, 20%Nd @$NaYF_4$ (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 μl 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

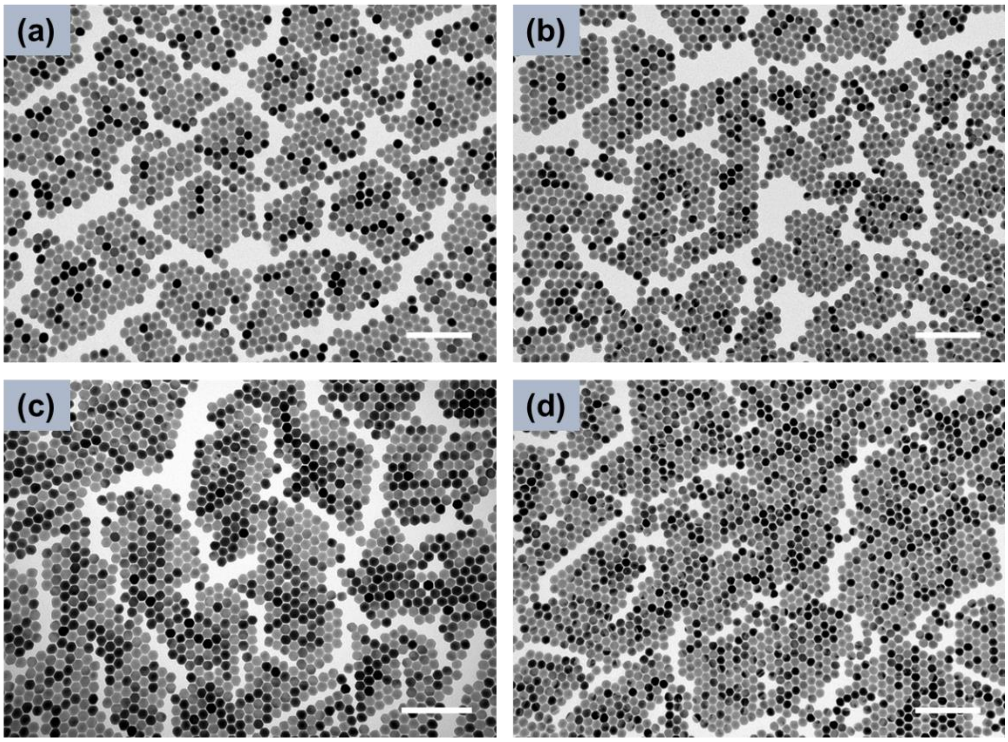

Figure S1. TEM images of core samples. (a) $NaYF_4$:20%Yb^{3+}, 2% Er^{3+} core nanoparticles. (b) $NaYF_4$:30%Yb^{3+}, 8% Er^{3+} core nanoparticles. (c) $NaYF_4$:30%Yb^{3+}, 2% Er^{3+} core nanoparticles. (d) $NaYF_4$:20%Yb^{3+}, 1.5% Er^{3+} core nanoparticles. Scale bar: 200 nm.

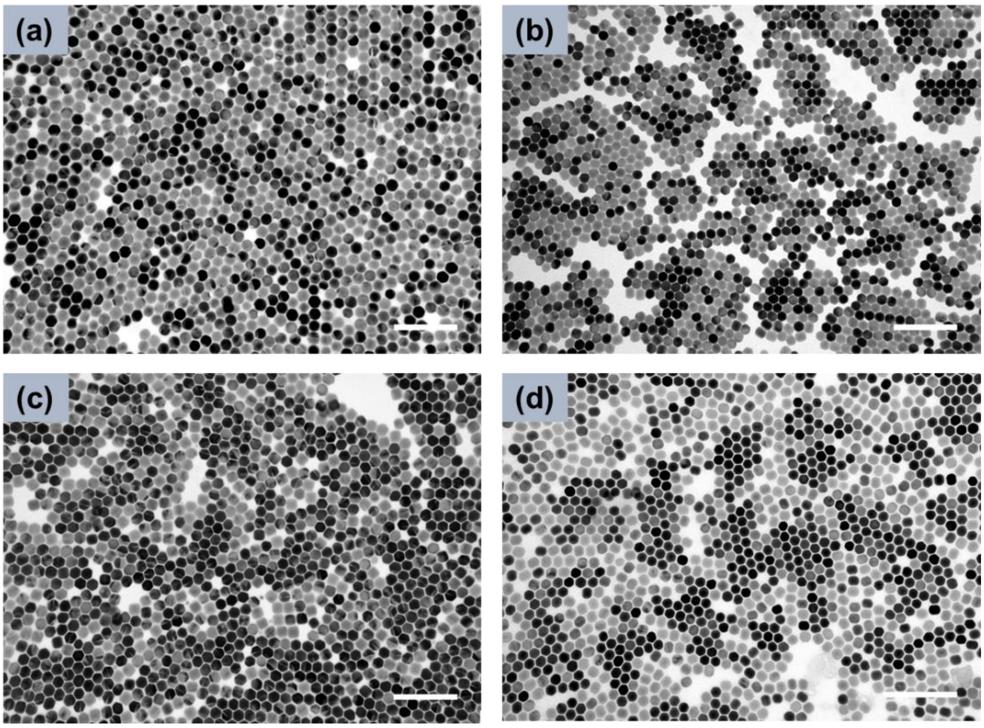

Figure S2. TEM images of core-shell nanoparticles. (a) $NaYF_4$:20%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+} nanoparticles. (b) $NaYF_4$:30%Yb^{3+}, 8% Er^{3+} @ $NaYF_4$:5%Yb^{3+} nanoparticles. (c) $NaYF_4$:30%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+} nanoparticles. (d) $NaYF_4$:20%Yb^{3+}, 1.5% Er^{3+} @ $NaYF_4$:5%Yb^{3+} nanoparticles. Scale bar: 200 nm.

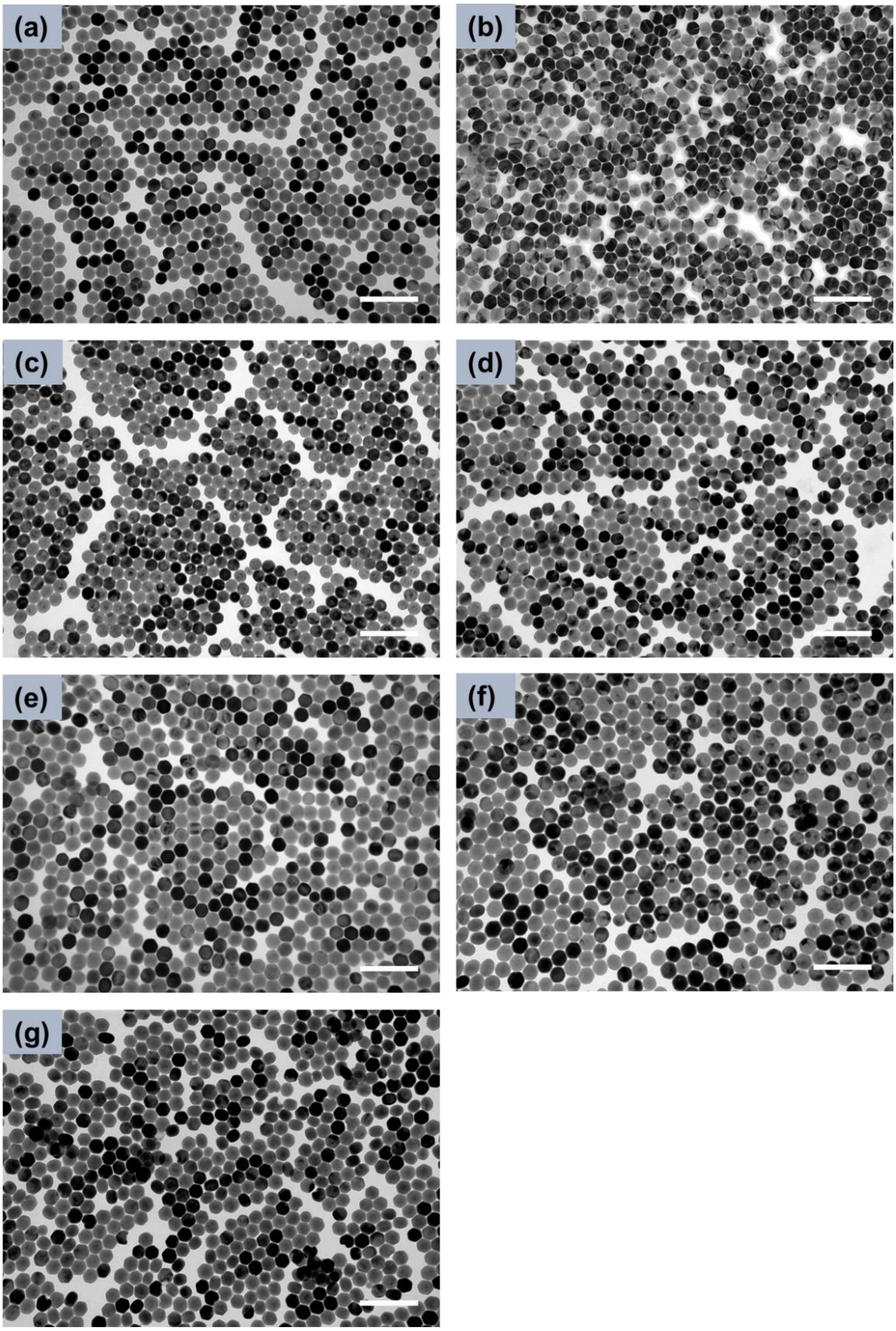

Figure S3. TEM images of core-shell-shell nanoparticles.

(a) $NaYF_4$:30%Yb^{3+}, 8% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 15%Yb^{3+}, 20% Nd^{3+} nanoparticles.

(b) $NaYF_4$:30%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 15%Yb^{3+}, 20% Nd^{3+} nanoparticles.

(c) $NaYF_4$:20%Yb^{3+}, 1.5% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 15%Yb^{3+}, 20% Nd^{3+}

nanoparticles.

(d) $NaYF_4$:20%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 15%Yb^{3+}, 20% Nd^{3+} nanoparticles.

(e) $NaYF_4$:20%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 15%Yb^{3+}, 20% Nd^{3+} nanoparticles.

(f) $NaYF_4$:20%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 30%Yb^{3+}, 20% Nd^{3+} nanoparticles.

(g) $NaYF_4$:20%Yb^{3+}, 1% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 5%Yb^{3+}, 20% Nd^{3+} nanoparticles. Scale bar: 200 nm.

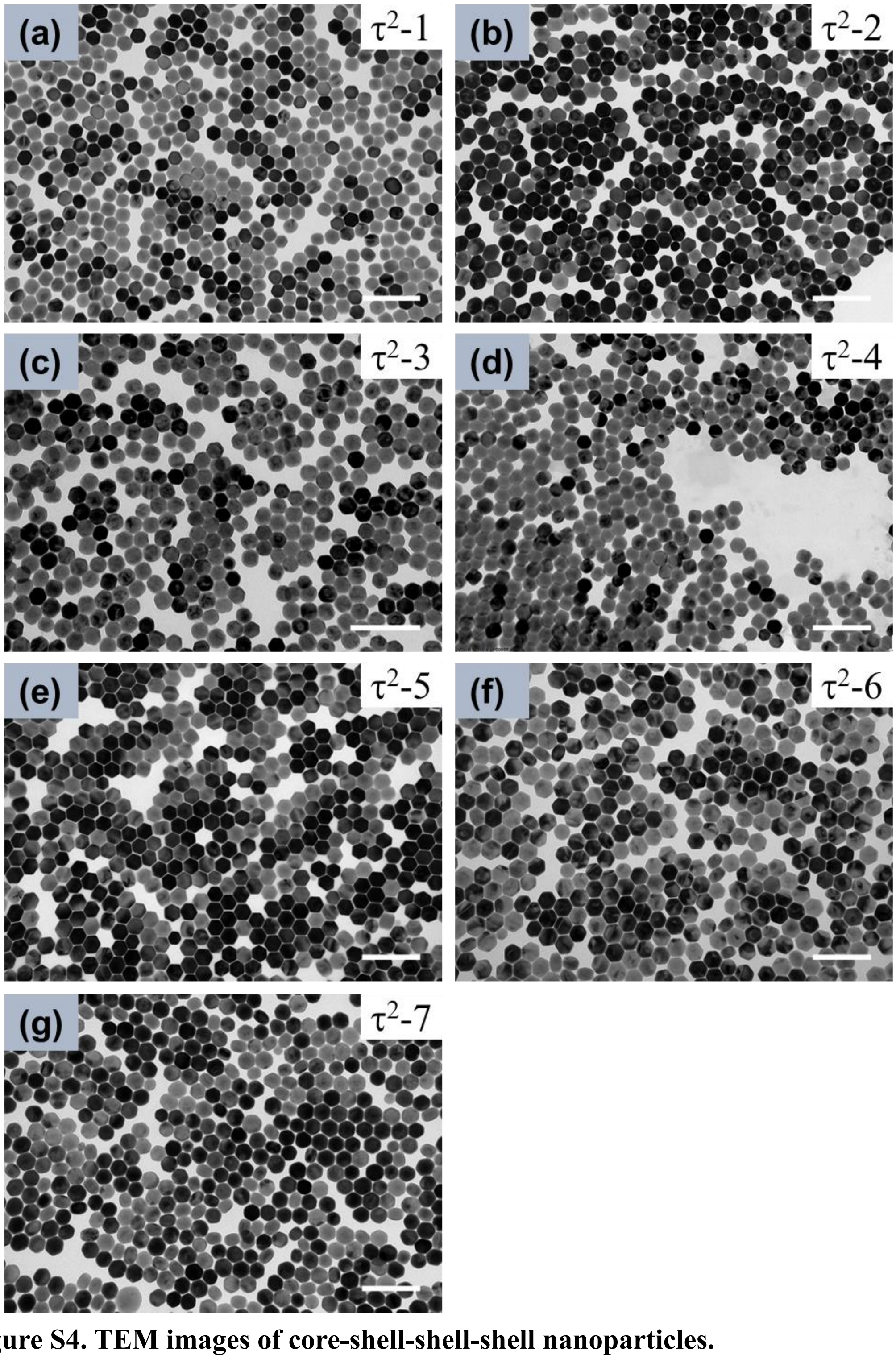

Figure S4. TEM images of core-shell-shell-shell nanoparticles.

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

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

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

(d) $NaYF_4$:20%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 15%Yb^{3+}, 20% Nd^{3+} @

$NaYF_4$ nanoparticles (τ^2-4).
(e) $NaYF_4$:20%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 15%Yb^{3+}, 20% Nd^{3+} @ $NaYF_4$ nanoparticles (τ^2-5).
(f) $NaYF_4$:20%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 30%Yb^{3+}, 20% Nd^{3+} @ $NaYF_4$ nanoparticles (τ^2-6).
(g) $NaYF_4$:20%Yb^{3+}, 1% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 5%Yb^{3+}, 20% Nd^{3+} @ $NaYF_4$ nanoparticles (τ^2-7). Scale bar: 200 nm.

Table S1. The average rising, peak and decay time of used lifetime-engineered upconversion nanoparticles (**τ^2** dots)

τ^2-x dots	Rising time (μs)	Peak time (μs)	Decay time (μs)
1	272.0	400.1	942.4
2	302.9	500.2	970.8
3	350.2	580.6	968.2
4	388.4	629.7	1117.0
5	462.9	724.9	1211.4
6	479.2	757.0	1221.0
7	589.3	982.1	1332.9

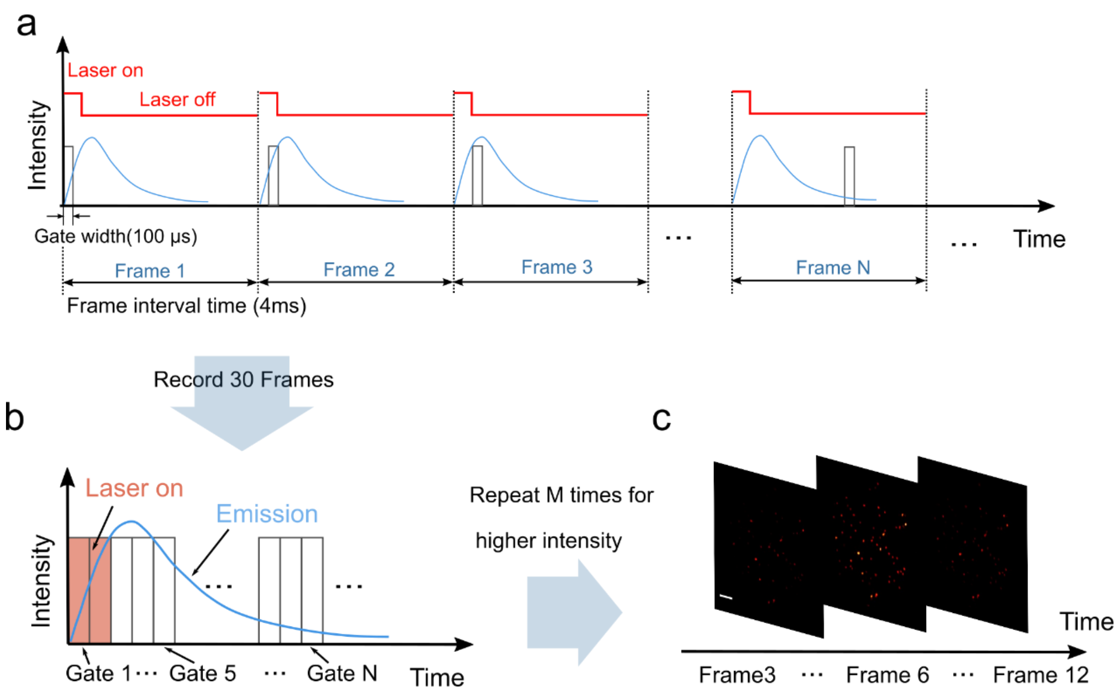

Figure. S5 The principle for time-gated wide-field imaging. (a) The camera is gated with an exposure time of 100 μs, and in each frame, only a gated image can be detected. The frame interval time is set as 4 ms in order to avoid the effect from the last lifetime circle. The imaging gate will be shifted to the next 100 μs. The lifetime curve/images (b) can be reconstructed by 30 frames. To obtain a better signal-to-noise ratio, we repeat the process for M times (e.g M=7500 for TR-SIM, and M=3000 for TR-WF) and integrate the signal to obtain a series of time-resolved images (c).

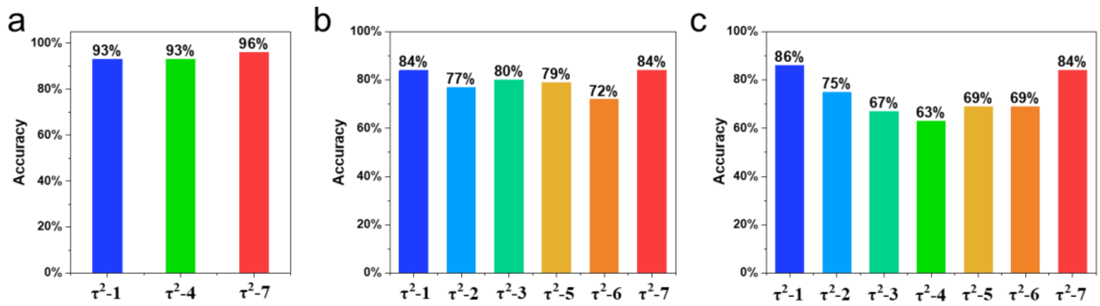

Figure S6. Mean classification accuracies of 3 and 7 types of τ^2-dots with deep learning algorithms, based on 50 times random validation test for each type.