Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supplementary Materials for

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

Baolei Liu[a,b], Jiayan Liao[a], Yiliao Song[c], Chaohao Chen[a], Lei Ding[a], Jie Lu[c], Jiajia Zhou[a]*, Fan Wang[a,b]*

[a]Institute for Biomedical Materials and Devices (IBMD), Faculty of Science, University of Technology Sydney, NSW 2007, Australia
[b]School of Electrical and Data Engineering, Faculty of Engineering and IT, University of Technology Sydney, NSW 2007, Australia
[c]Centre for Artificial Intelligence, Faculty of Engineering and IT, University of Technology Sydney, NSW 2007, Australia

*e-mail: jiajia.zhou@uts.edu.au; fan.wang@uts.edu.au

This file includes:

Materials and Methods

Supplementary Figures. S1 to S7

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

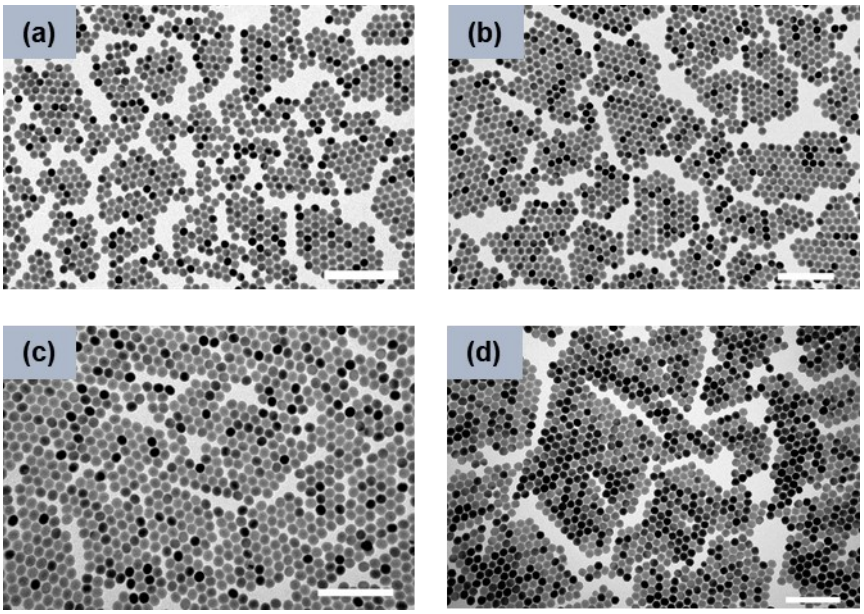

Figure S1. TEM images of core samples. (a) $NaYF_4$:20%Yb^{3+}, 2% Er^{3+} core nanoparticles. (b) $NaYF_4$:30%Yb^{3+}, 8% Er^{3+} core nanoparticles. (c) $NaYF_4$:30%Yb^{3+}, 2% Er^{3+} core nanoparticles. (d) $NaYF_4$:20%Yb^{3+}, 1.5% Er^{3+} core nanoparticles. Scale bar: 200 nm.

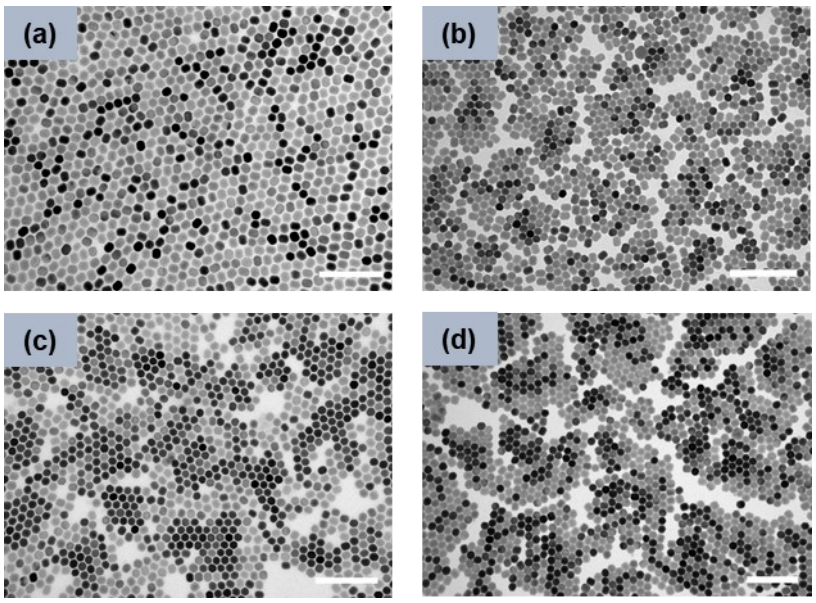

Figure S2. TEM images of core-shell nanoparticles. (a) $NaYF_4$:20%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+} nanoparticles. (b) $NaYF_4$:30%Yb^{3+}, 8% Er^{3+} @ $NaYF_4$:5%Yb^{3+}

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.

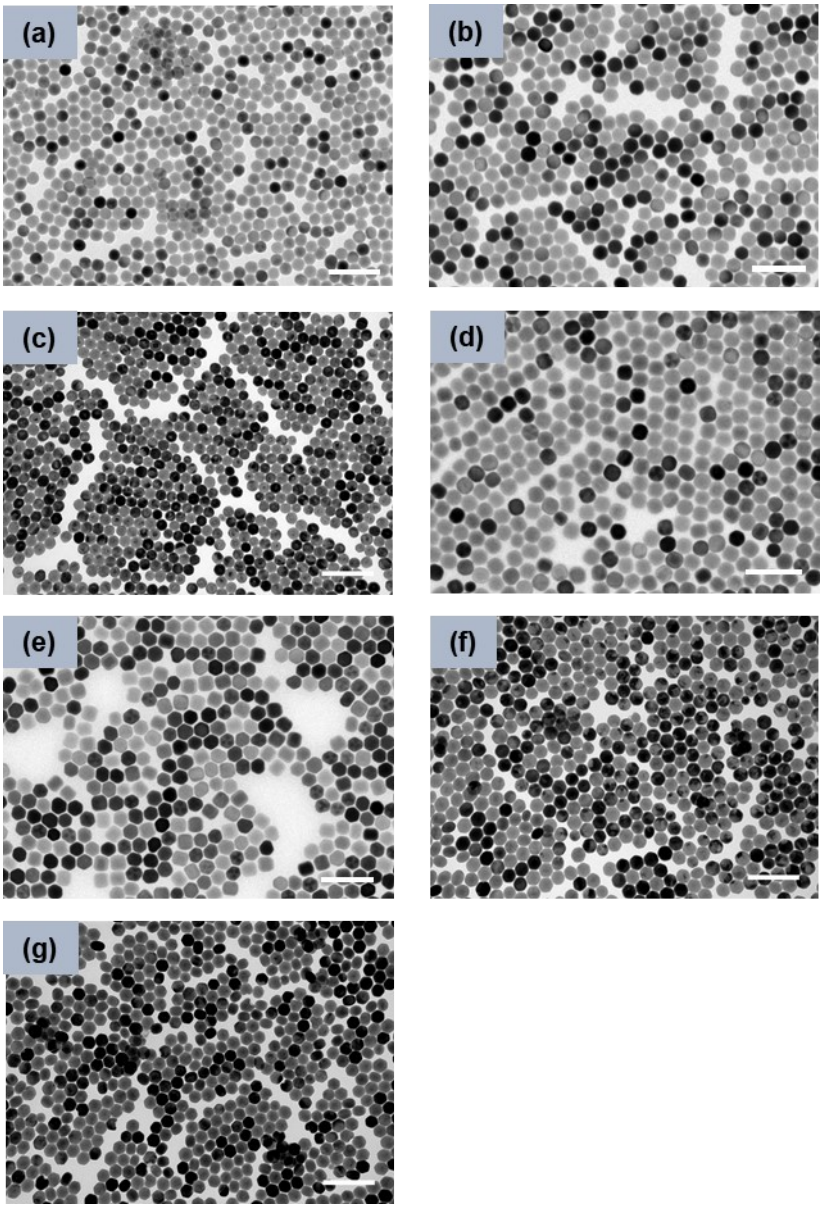

Figure S3. TEM images of core-shell-shell nanoparticles.
(a) $NaYF_4$:30%Yb^{3+}, 8% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 15%Yb^{3+}, 20% Nd^{3+} nanoparticles.
(b) $NaYF_4$:30%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 15%Yb^{3+}, 20% Nd^{3+} nanoparticles.
(c) $NaYF_4$:20%Yb^{3+}, 1.5% 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.

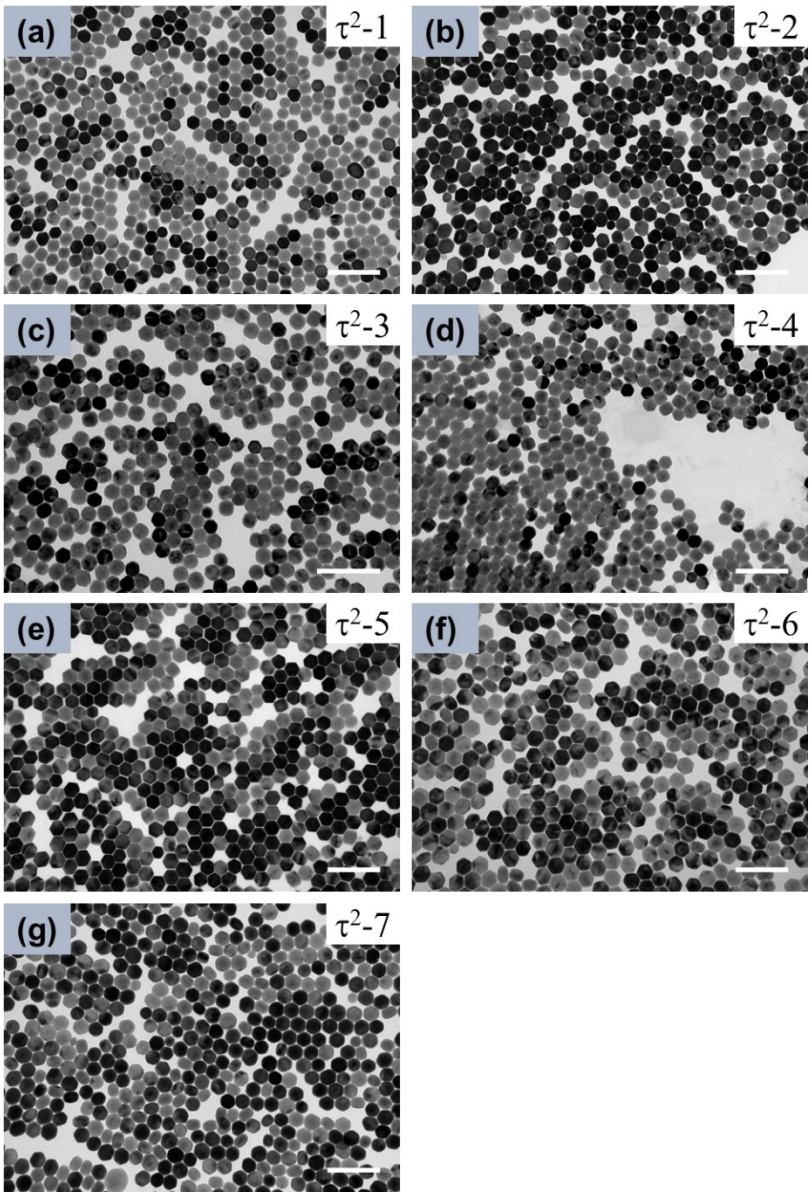

Figure S4. TEM images of core-shell-shell-shell nanoparticles.
(a) $NaYF_4$:30%Yb^{3+}, 8% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 15%Yb^{3+}, 20% Nd^{3+} @ $NaYF_4$ nanoparticles (τ^2-1).
(b) $NaYF_4$:30%Yb^{3+}, 2% Er^{3+} @ $NaYF_4$:5%Yb^{3+}@ $NaYF_4$: 15%Yb^{3+}, 20% Nd^{3+}@ $NaYF_4$ nanoparticles (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.

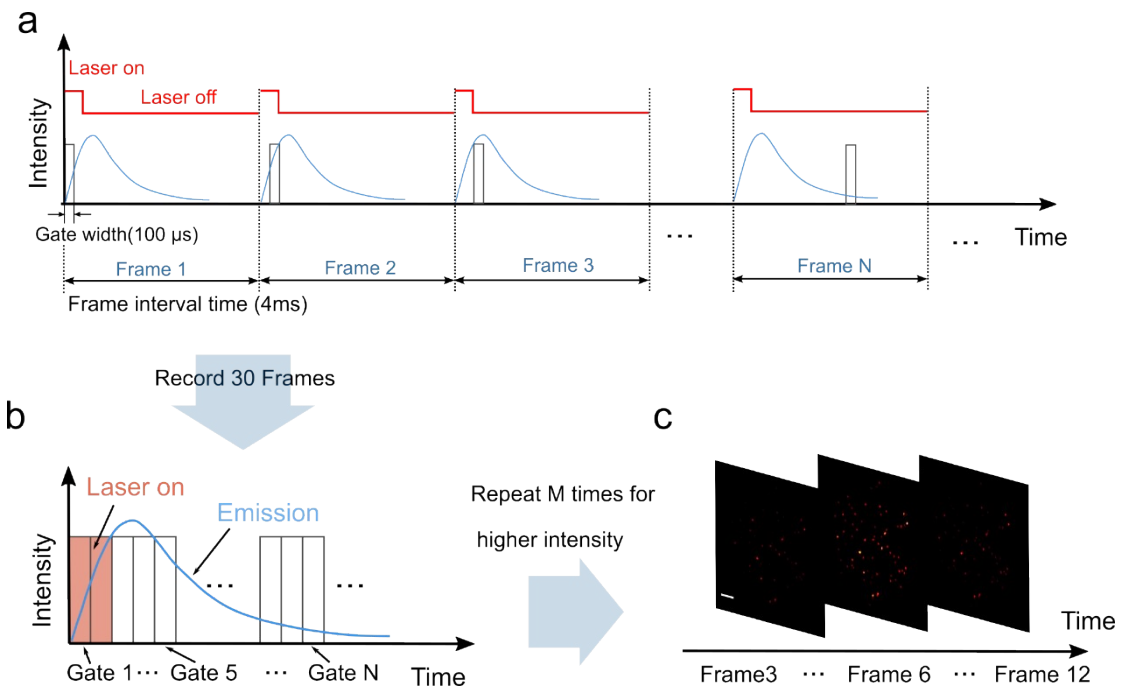

Figure S5. The principle for time-gated wide-field imaging. (a) The camera is gated with an exposure time of 100 μs, and in each frame, only a gated image can be detected. The imaging gate will be shifted to the next 100 μs. The lifetime curve/images (b) can be reconstructed by 30 frames. To obtain a 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).

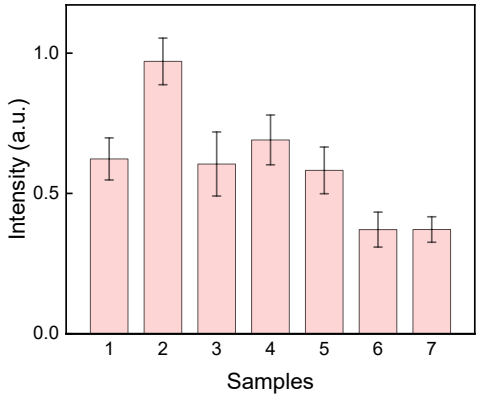

Figure S6. The statistics of the fluorescence intensities for different samples (τ^2-1 to τ^2-7). The intensities are measured from different single nanoparticles for each τ^2-dots.

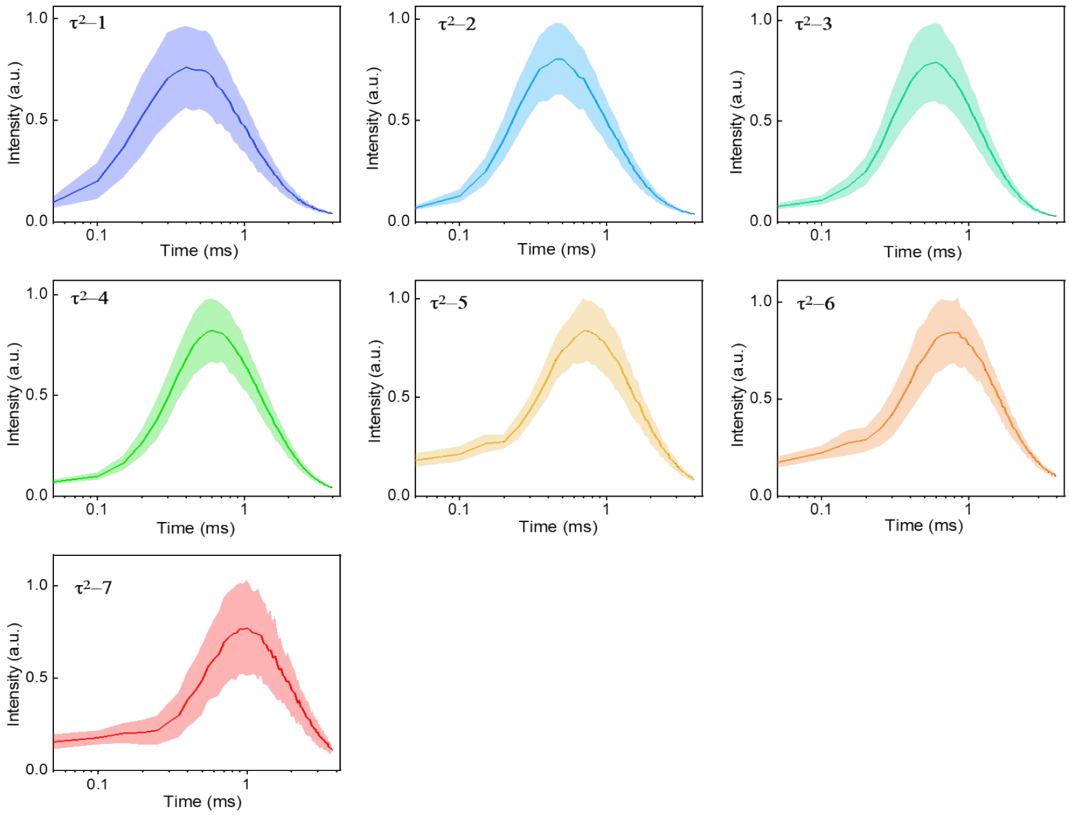

Figure S7. The statistics of normalized lifetime profiles for different samples (τ^2-1 to τ^2-7).