Section 1: Synthesis of upconversion nanoparticles (UCNPs)

Reagents:

$YCl_3 \cdot 6H_2O$ (99.99%), $GdCl_3 \cdot 6H_2O$ (99.99%), $ErCl_3 \cdot 6H_2O$ (99.99%), $LuCl_3 \cdot 6H_2O$ (99.99%), NH_4F (99.99%), NaOH (99.9%), KOH (99.9%), oleic acid (OA, 90%), and 1-octadecene (ODE, 90%) were purchased from Sigma-Aldrich and used as received without further purification.

Method:

1. Synthesis of $NaYF_4$:Yb,Er core nanocrystals

In a typical experiment, 1 mmol $RECl_3 \cdot 6H_2O$ (RE=Y, Yb, Er) with the molar ratio of 78:20:2 were added to a flask containing 6 mL OA and 15 mL ODE. The mixture was heated to 160 °C under argon flow for 30 min to obtain a clear solution and then cooled down to about 50 °C, followed by the addition of 5 mL methanol solution of NH_4F (4 mmol) and NaOH (2.5 mmol). After stirring for 30 min, the solution was heated to 80 °C under argon flow for 20 min to expel methanol, and then the solution was further heated to 310 °C for another 90 min. Finally, the reaction solution was cooled down to room temperature, and products were precipitated by ethanol and washed with cyclohexane, ethanol and methanol for 3 times to get the $NaYF_4$:Yb,Er nanoparticles.

2. Synthesis of $NaYF_4$:Yb,Er nanorods

The longitudinal growth of $NaYF_4$:Yb,Er onto the $NaYF_4$:Yb,Er core nanocrystals was conducted via a successive layer-by-layer hot-injection protocol. Firstly, shell precursors were prepared: 1 mmol $RECl_3 \cdot 6H_2O$ (RE=Y, Yb, Er) with the molar ratio of 78:20:2 were added to a 50 mL flask containing 6 mL OA and 15 mL ODE. The mixture was heated to 160 °C under argon flow for 30 min to obtain a clear solution and then cooled down to about 50 °C, followed by addition of 5 mL methanol solution of NH_4F (4.0 mmol) and NaOH (2.5 mmol) and 3 mL methanol solution of KOH (2.0 mmol). After stirring for 30 min, the solution was heated to 80 °C under argon for 20 min to remove methanol, and then the solution was further heated to 150 °C for another 30 min. Finally, the reaction solution was cooled down to room temperature and labelled as $NaYF_4$:Yb,Er shell precursors.

For the longitudinal growth, 0.2 mmol core particles were added to a 50 mL flask containing 3 mL OA, 7 mL ODE, 69 mg NaOH and 77 mg KOH. The mixture was heated to 160 °C under argon flow for 30 min, and then the solution was further heated to 310 °C. After that, 0.2 mL of the shell precursors were immediately injected into the reaction mixture and ripened at 310 °C for 3 min followed by the same injection and ripening cycles for 50 times to get the nanorods with length of 60 nm (average). The injection and ripening cycles were adjusted to obtain nanorods of various lengths. Finally, the reaction solution was cooled down to room temperature and the formed nanorods were purified according to the procedures used for the purification of $NaYF_4$:Yb,Er core particles.

3. Synthesis of $NaYF_4$:Yb,Er@$NaGdF_4$ nanodumbbell

$GdCl_3 \cdot 6H_2O$ of 0.2 mmol were added to a 50 mL flask with 6 mL OA and 6 mL ODE. The mixture was heated to 160 °C under argon flow for 30 min to obtain a clear solution and cooled down to about 50 °C, followed by the addition of 4 mL methanol solution of NH_4F (0.8 mmol) and NaOH (0.5 mmol). After stirring for 30 min, the formed $NaYF_4$:Yb,Er cores (0.2 mmol in cyclohexane) were added and the solution was heated to 80 °C under argon flow for 20 min to expel methanol and cyclohexane, and then the solution was further heated to 310 °C for another 60 min. Finally, the reaction solution was cooled down to room temperature and the formed nanodumbbell were purified according to the procedures used for the purification of $NaYF_4$:Yb, Er core particles.

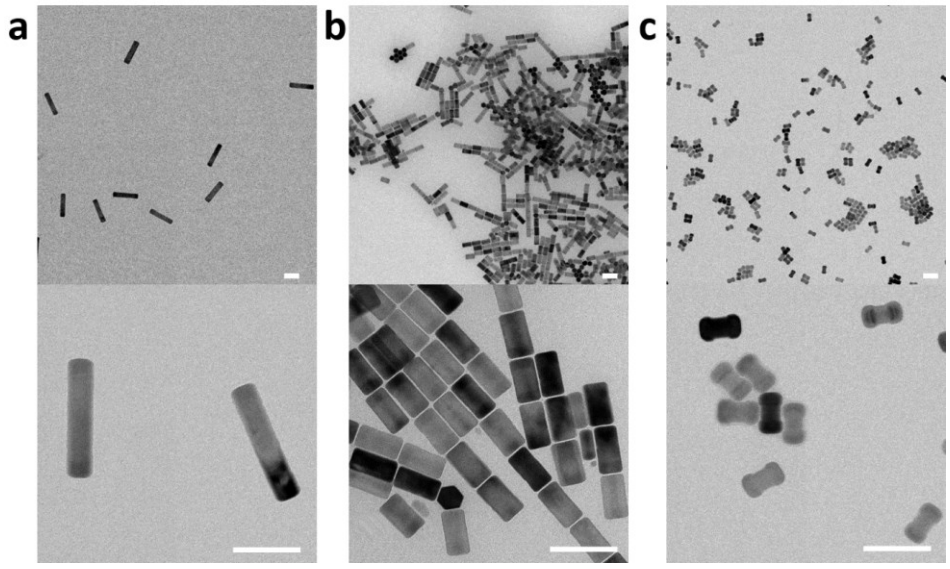

Figure S1. As-synthesized UCNPs for the silica coating. Scale bar: 100 nm.

Section 2: DNA modification onto UCNPs

Reagents:

Chloroform (99.8%) was purchased from RCI Labscan Limited and used directly without further purification.

The following DNA sequences were synthesized by Integrated DNA Technologies:

5'-AGTCTAGGATTCGGCGTGGGTTAATTTTT-3'

5'-AGTCTAGGATTCGGCGTGGGTTAATTTTT-phosphate-3'

5'-GAGCATGGTTTTAATTAAGCTCGCCATCAAATAGCTTT-3'

5'-phosphate-GAGCATGGTTTTAATTAAGCTCGCCATCAAATAGCTTT-3'

5'-AAAAA-FAM-3'

5'-AAAAAAAAAAAAAAAAAAAA-FAM-3'

Method:

UCNPs chloroform suspension of 400 μL and 300 μL of DNA (non-phosphorylated, phosphorylated and dye-labelled) water solution was mixed together in a small glass vial. Because chloroform is immiscible with water and of a higher density, there would form two layers in the vial. The vial was placed on a vortex machine and a gentle shake of 600 rpm was provide and lasted for two hours. After that, the UCNPs are pulled up to upper aqueous phase. The UCNPs were collected by centrifuge and purified by using ethanol and water separately to remove the excessive organic solvent and un-reacted DNA molecules. The final products were applied to do silica coating in the next step.

Section 3: Silica coating method

Reagents:

Cyclohexane (99.9%), Tetraethyl orthosilicate (TEOS, ≥99.0%), IGEPAL® CO-520 (average Mn 441), ethyl alcohol (≥99.5%) and ammonium hydroxide solution (30%) were purchased from Sigma-Aldrich and directly used without further purification.

Instruments:

TEM characterization of nanoparticles is done by FEI Tecnai T20 Transmission electron microscope. Fluorescence intensity of nanoparticles is measured by our home-made confocal microscope.

Method:

UCNPs (as-synthesized, non-phosphorylated DNA modified, or phosphorylated DNA modified) of 1 mg were purified by ethanol and water and again by ethanol. 40 μL of CO-520 and 300 μL of cyclohexane were mixed and used to disperse the UCNPs samples. It should be noticed here proper that ultrasonication is required. After that, 20 μL of 30% ammonium hydroxide solution together with 80 μL CO-520 were drop into the system. The liquid was sonicated until the it gets transparent. 0.5 μL of TOES was diluted by 1 mL cyclohexane, and the mixed solution was added into the micro-emulsion system dropwise under 800 rpm stir. After 10 hours, 500 μL of ethanol was dropped into the system to stop the silica coating reaction. After centrifuge, the products were purified by ethanol and water respectively for 3 times. Finally, the products were stored in water and directly dropped onto copper grids for TEM characterization.

To verify that the concentration of TEOS would not influence the anisotropic silica coating result, 2.5 μL of TEOS was diluted by 1 mL of cyclohexane and dropped into the system.

Silica coating experiment by Stöber method was quite similar to micro-emulsion method: 1 mg UCNPs (as-synthesized, non-phosphorylated modified or phosphorylated DNA modified) were suspended in 300 μL of ethanol together with 20 μL of 30% ammonium hydroxide solution. After that, 0.5 μL of TOES was diluted by 1 mL ethanol, and the mixed solution was added into system dropwise under 800 rpm stir. After 10 hours, 500 μL of ethanol was dropped into the system to stop the silica coating reaction. The same protocol was conducted to collect and purify the final products.

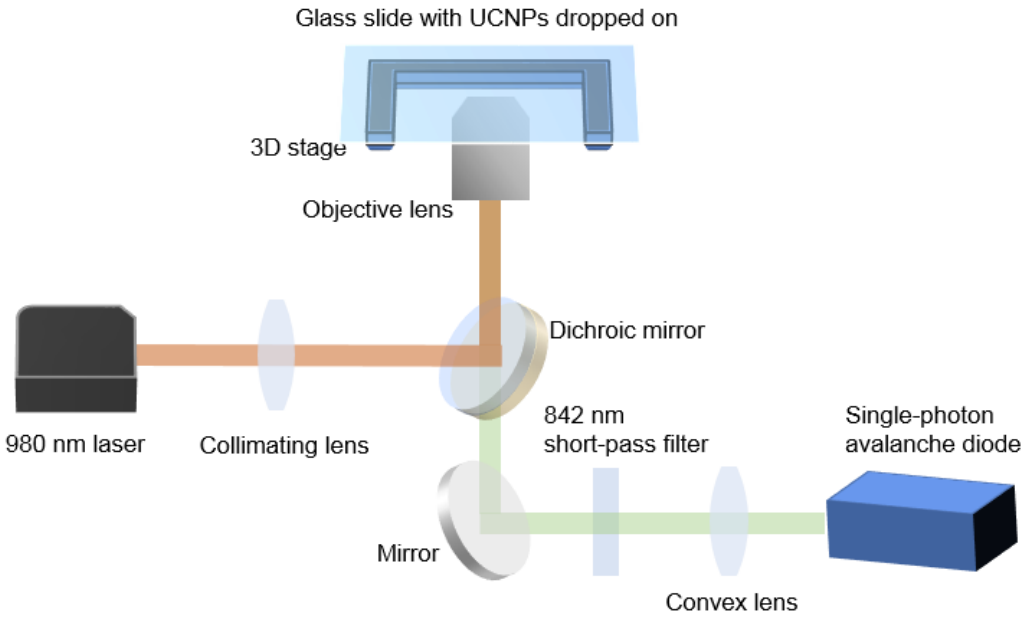

Figure S2. Optical layout for single particle measurement.

Section 4. Cell culture and particle treatment

Cell culture:

MDA-MB- 468 cells derive from human breast were used to study the cell uptake efficiency. The cells were cultured in incubator under the condition of 37℃ and 5% CO_2. Leibovitz's L-15 (Gibco) supplemented of 10% foetal bovine serum (FBS) medium was used to culture the cells. Subsequent subculture routine was conducted when 80% confluent has been reached. Cells were detached by Trypsin-EDTA (0.25%) and seeded 24 hours before the particle treatment. Specifically, 1x10,000 cells were cultured in 1 mL L-15 supplemented by 2% FBS, followed by overnight incubation (37℃, 5% CO_2) in a petri dish (23mm glass-bottom diameter, FluoroDish).

Particle treatment:

First, the original cell medium is removed from the petri dish. Instead, the cells were cultured in 1 mL cell medium containing UCNPs (0.03 μmol) for 3 hours (37 °C, 5% CO_2). Afterward, cell culture was removed, and the cells were purified by phosphate-buffered saline buffer (PBS, Gibco). After that, 200 μL of 4% paraformaldehyde (PFA) was added to fixed cytoskeleton for 10 min and then discarded. Then PBS was used again to remove all the traces of PFA, serum, and excess UCNPs by rising three times of fixed cells. Finally, the cells were covered by 500 μL PBS buffer in microscopy dish and ready for imaging experiment.

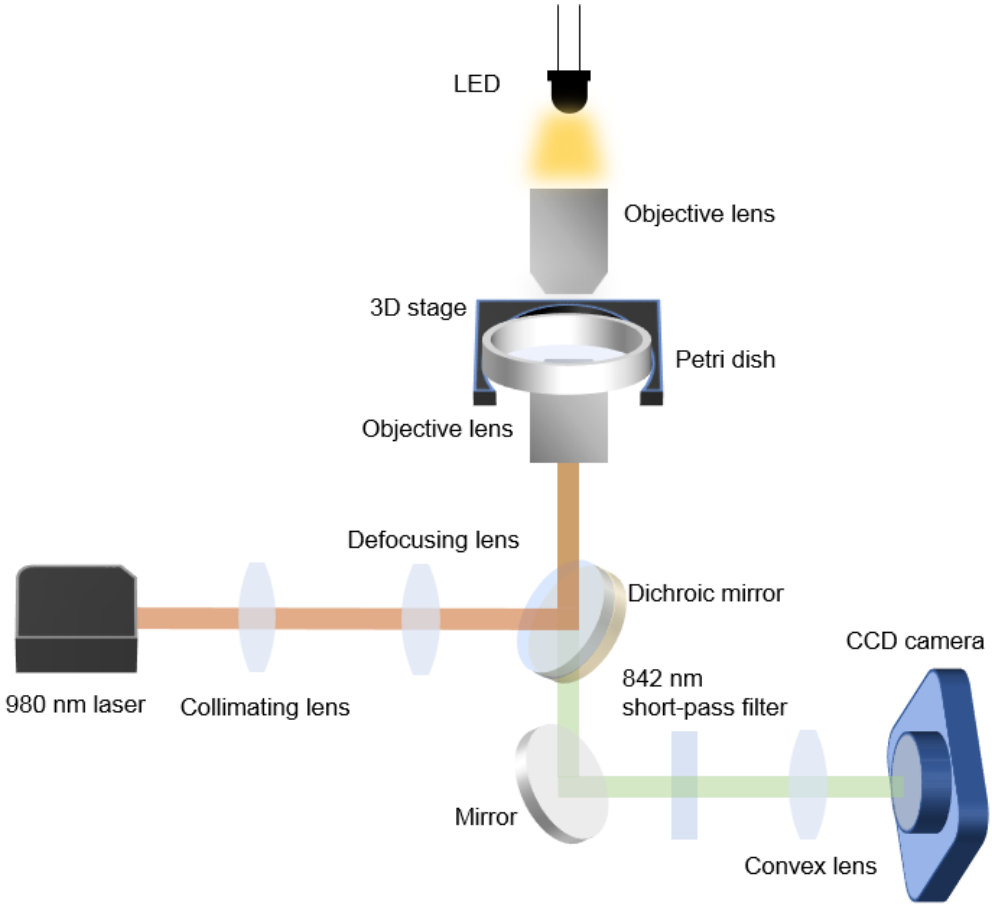

Figure S3. Optical layout for 3D localization of UCNPs inside the cell.

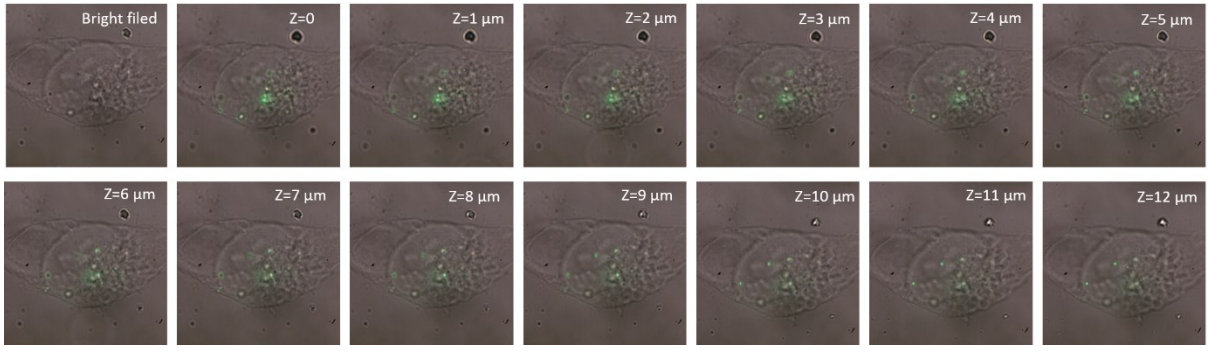

Figure S4. Images for 3D scanning of cell with anisotropic silica coated UCNPs inside.

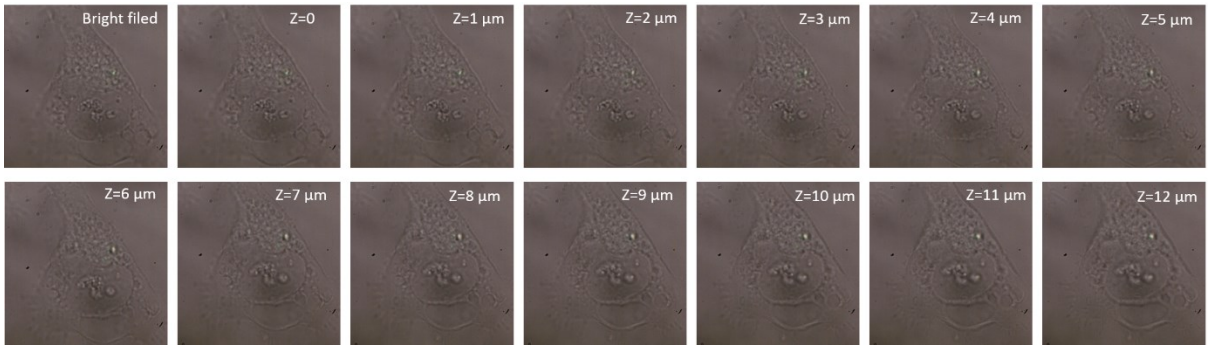

Figure S5. Images for 3D scanning of cell with fully silica coated UCNPs inside.

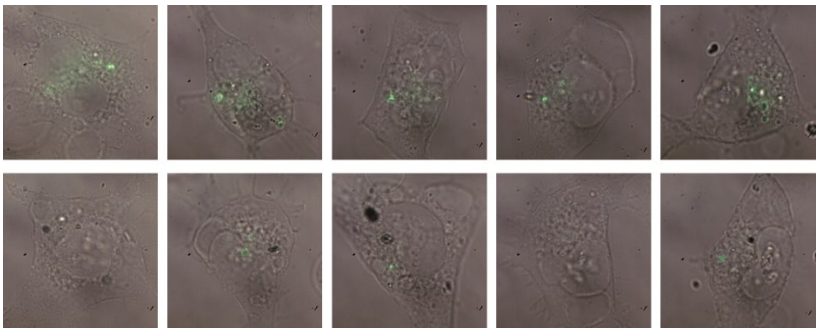

Figure S6. Images to show the difference of anisotropic silica coated UCNPs (upper) UCNPs with complete silica shell (below) in cell uptake efficiency.