Supplementary Information

Fast and background-free three-dimensional (3D) live-cell imaging with lanthanide-doped upconverting nanoparticles

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Chemicals. Yttrium(III) chloride hexahydrate ($YCl_3 \cdot 6H_2O$), ytterbium(III) chloride hexahydrate ($YbCl_3 \cdot 6H_2O$), erbium(III) chloride hexahydrate ($ErCl_3 \cdot 6H_2O$), oleic acid, 1-octadecene, ammonium fluoride (NH_4F), sodium hydroxide (NaOH), methanol (CH_3OH), and ethanol (CH_3CH_2OH) were purchased from Sigma-Aldrich Chemical Co. 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (mPEG) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG-amine) were obtained from Avanti Polar Lipids, Inc. All other chemicals and organic solvents used were of reagent grade or better.

Synthesis of $NaYF_4$:Yb^{3+},Er^{3+} UCNPs. Synthesis of $NaYF_4$:Yb^{3+},Er^{3+} upconverting nanoparticles (UCNPs) were conducted according to previously reported procedure.[1,2] Briefly, $YCl_3 \cdot 6H_2O$ (0.78 mmol), $YbCl_3 \cdot 6H_2O$ (0.20 mmol), $ErCl_3 \cdot 6H_2O$ (0.02 mmol) were mixed with oleic acid (8 mL) and 1-octadecene (15 mL) in a 50 mL flask under an atmosphere of nitrogen and heated to 160°C form a clear solution. The solution was left stirring for 30 minutes at 160°C under vacuum. The reaction mixture was then cooled down to room temperature and 10 mL methanol containing NaOH (2.5 mmol) and NH_4F (4 mmol) were added into the flask and stirred for 30 minutes. In order to remove methanol, the solution was heated to 100°C and stirred for 10 minutes. Subsequently, the solution was heated to 300°C and maintained for 1h. After cooling to room temperature, the UCNPs were precipitated and washed by addition of ethanol for three times via centrifugation. The pellet can easily be re-dispersed in hexane.

Passivation of $NaYF_4$:Yb^{3+},Er^{3+} UCNPs. Passivation of UCNP surface was conducted according to previously reported procedure.[2] UCNPs in chloroform (2 mg/mL, 5 mL) were added to the mixture of mPEG (15 mg) and DSPE-PEG-amine (5 mg) in chloroform (5 mL).

After evaporating chloroform, the residue was incubated at 65°C for 1 h. Water (10 mL) was added to the residue. After filtration with a 0.2 μm cellulose acetate syringe filter, the solution was washed with water twice via centrifugation and then stored in water (2 mL).

UCNPs characterization. The shape, size, and uniformity of synthesized UCNPs were measured with a transmission electron microscope (JEM-2100F, JEOL). The hydrodynamic sizes of UCNPs dispersed in water were determined using a particle size analyzer (ELSZ-1000, Otsuka Electronics Co. Ltd). X-ray diffraction (XRD) patterns were obtained with a benchtop XRD instrument (MiniFlex II, Rigaku) to determine the crystal structure of the UCNPs. The elemental composition and content of UCNPs were measured with an inductively coupled plasma mass spectrometer (ICP-MS, ELAN 6100, PerkinElmer).

Measurement of the emission spectra of UCNPs-PEG-amine. The emission spectra of UCNPs-PEG-amine (dispersed in water), whose concentration was adjusted to 5 mg/mL, were recorded by excitation at 980-nm CW diode laser (P161-600-980A, EM4 Inc.). The emission was collected by an optical fiber and detected by a spectrometer (HR2000+, Ocean optics).

Preparation of HeLa cells sample. HeLa cells (ATCC) were cultured in the Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), 1% penicillin and streptomycin (Gibco) at 37°C in a humidified 5% CO_2 incubator. At 70% confluence, cells were washed with phosphate buffered saline (PBS, Gibco), detached with trypsin (Trypsin-EDTA solution, WEL GENE), and suspended in DMEM medium. 1 mL of the cell suspension at a cell density of 5×10^4 cells/mL were seeded on 24 well plate (Corning), and incubated at 37°C, 5% CO_2 overnight. For RFP labeling of nuclei, CellLight® Nucleus-RFP, BacMam 2.0 reagent (Life Technologies) was treated according to

the manufacturers' instructions. After culture for 16 h, the medium was changed to 800 μL of UCNP-containing medium (1.25 mg/mL). After additional incubation for 2 h, 1 mL of UCNPs internalized HeLa cells (2 x 10^4 cells/mL) were seeded on the cover glass-bottomed dish (SPL), and incubated for 12 h. The cell samples were then washed and used for live-cell imaging. For fixed-cell imaging, they were treated with 4% paraformaldehyde (Biosesang).

Wide-field epi-fluorescence microscope with a motorized stage. In this study, wide-field epi-fluorescence microscope setup (Fig. S1) for UCNPs imaging in the HeLa cells was composed of inverted microscope (IX73, Olympus), a 980-nm NIR diode laser (P161-600-980A, EM4 Inc.), an electron multiplying charge coupled device (EMCCD) camera (iXON3, Andor Technology), motorized microscope stage (Mac6000, Ludl Electronic Products Ltd.) and live-cell incubation chamber (TC-L-10, Live Cell Instrument) on the stage. The incubation chamber kept the cell sample under 37^{o}C and 5% CO_2 during imaging. The luminescence signal from UCNPs was collected by the microscope objective lens (Apo N, NA 1.49, 60X, oil immersion, Olympus). 980-nm laser beam was reflected by dichroic beam splitter (T950spxr, Chroma Technology) and directed to the microscope objective lens. The laser beam was focused on the back focal plane of objective by a plano-convex lens (focal length = 300 mm). The illumination area on the sample surface was circular with the diameter of ~66 μm and typical power density of illumination was 731 W/cm^2. After exciting sample, the emission beam (centered at 525 nm, 540 nm, 655 nm) from the UCNPs sample was passed through the same objective, the dichroic beam splitter and a band pass emission filter (ET700sp-2p, Chroma Technology). Outside the microscope, emission beam was magnified further by a set of convex lens and finally directed to the EMCCD camera. A sample of UCNPs in HeLa cell was placed on the microscope stage and was translated by motorized microscope xyz stage. Z-sectioning was conducted by moving microscope objective, which

was controlled by the home-made LabVIEW (National Instruments) codes.

The accuracy of UCNP localization (3D). In order to calculate errors in coordinates of localized UCNPs (Δx), the spots were analyzed by the equation derived by Thompson et al.,[3]

$$\Delta x \approx \sqrt{\frac{s^2 + a^2/12}{N}} \qquad (1)$$

where N is the number of photons detected, s the standard deviation of the Gaussian fit, and a the pixel size. N was estimated by the equation provided by the manufacturer (Andor Technology).

$$N \approx \frac{(I - O) \times P}{EM \times QE} \qquad (2)$$

(I: signal intensity within ROI, O: bias offset, P: preamp, EM: EM gain, QE: quantum efficiency)

Typically, N = 3000, s = ~200 nm, and a was ~128 nm. Therefore, the value of accuracy in terms of error of localization (Δx) was *ca.* 4 nm. Likewise, Δy turned out to be also *ca.* 4 nm through the same procedure. In equation (1), the size of the pixel was replaced by the gap between the stack (~500 nm) for estimation of Δz, and s was 847 nm, yielding the error (Δz) of *ca.* 15 nm.

Image processing. Image processing was carried out with LabVIEW program and ImageJ FIJI software (http://fiji.sc/Fiji).

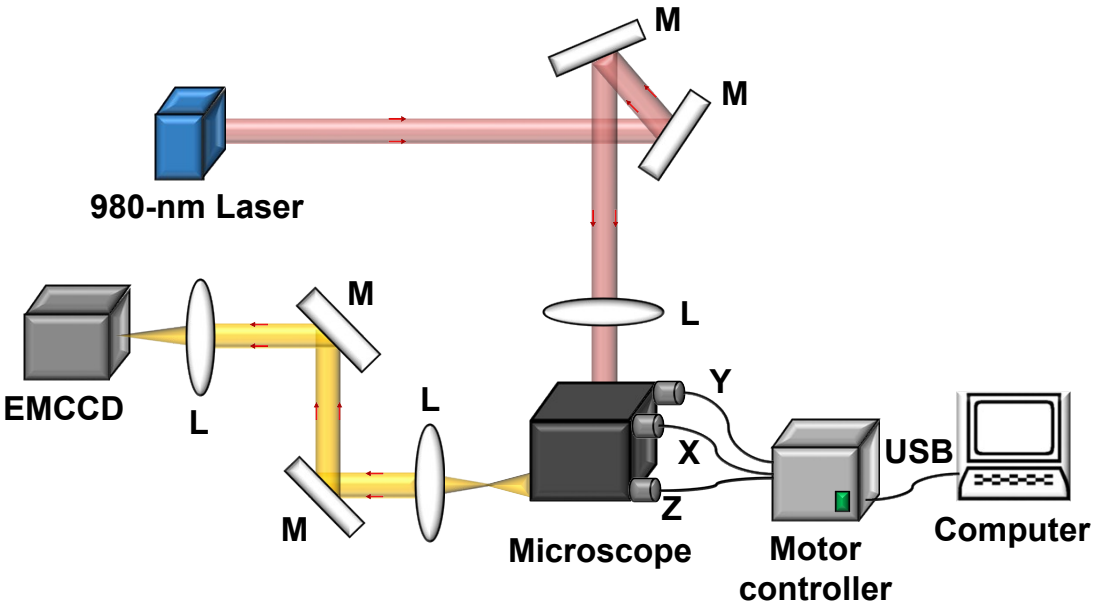

Fig. S1 The schematic of wide-field epi-fluorescence microscope setup with z-sectioning capability, which is composed of a 980-nm diode laser, an inverted microscope, a motorized xyz stage connected to a PC through a controller, and EMCCD (L: lens, M: mirror).

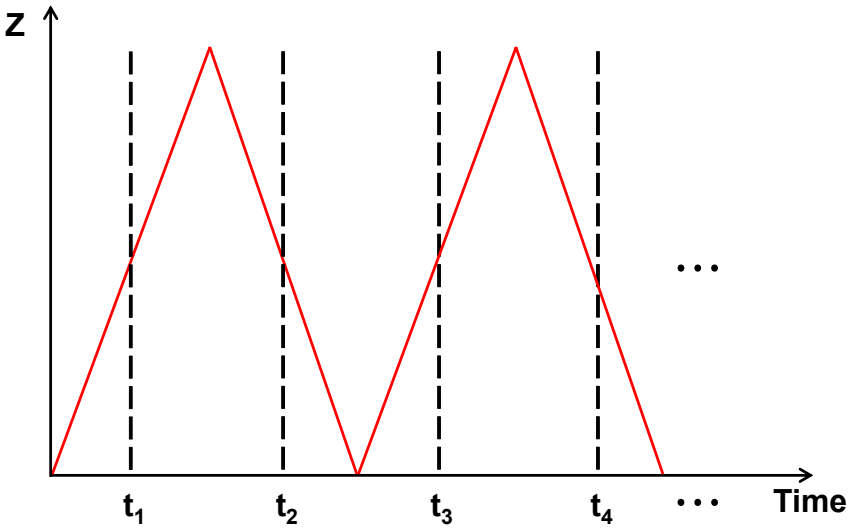

Fig. S2 Z-scanning profile (red) *vs.* time. The stack images were acquired continuously during upward and downward movement of the objective (z-scanning), which was controlled by LabVIEW codes. The time points were defined as the mid-point of each scan. The time intervals (t_i-t_{i-1}) were 1 second typically. The set of images acquired during the downward scanning were inverted such that the z-coordinates are consistent to those during the upward scanning.

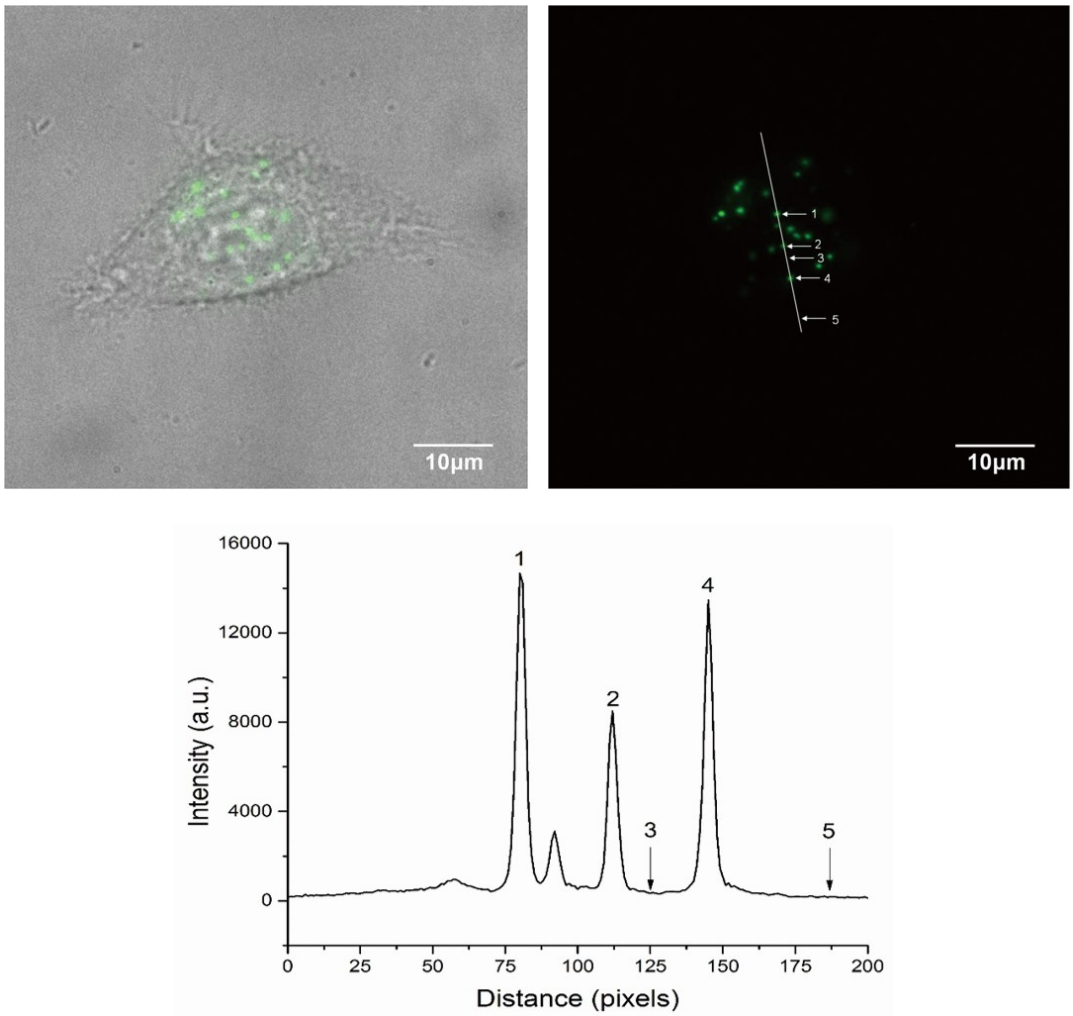

Fig. S3 The absence of autofluorescence. The merged image (top left) indicates that the UCNPs (green) are internalized into a HeLa cell. The intensity of upconversion luminescence from individual UCNPs was analyzed along the white line (top right), and the result is plotted (bottom). The numbers 1, 2, and 4 designate single UCNPs, 3 intracellular space, and 5 extracellular space. Note that the intensity of the regions 3 is nearly zero indicating that the autofluorescence background is totally absent.

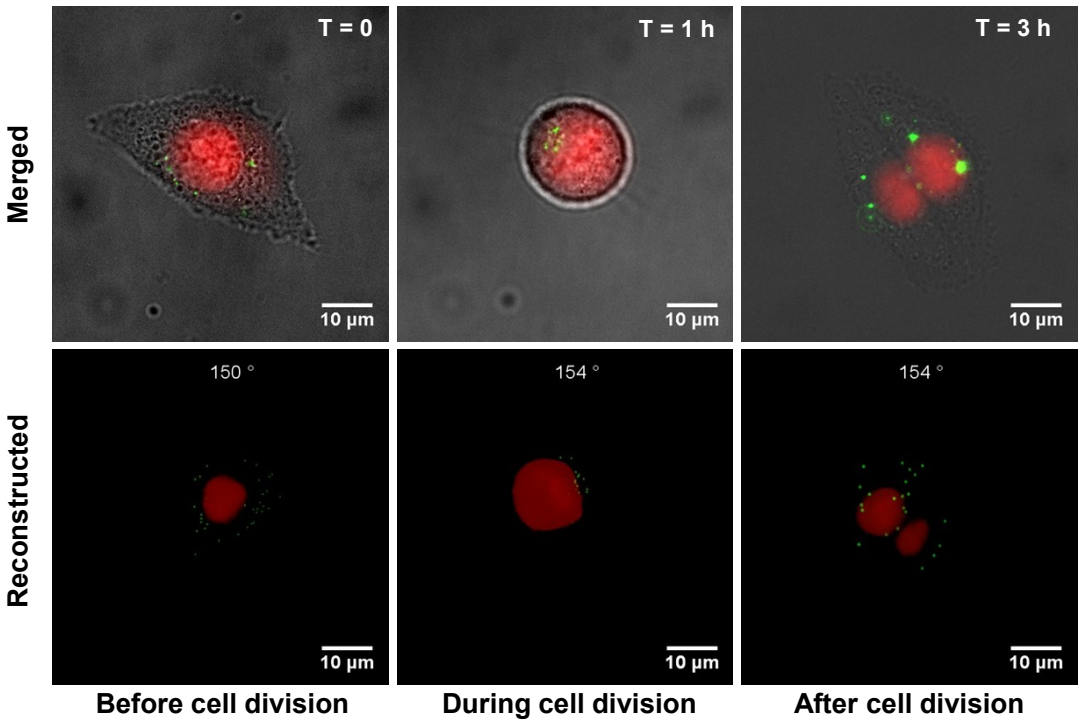

Fig. S4 Merged and reconstructed luminescence images taken during the division of a HeLa cell. (Top) 2D wide-field epi-fluorescence images show blurred luminescence of UCNPs. On the other hand, the 3D images (bottom), reconstructed by centroid determination and Gaussian fitting, clearly display the distribution of UCNPs in the cytosol. The overall 3D images indicate, even during the cell division, the endocytosed UCNPs never enter the nuclei.

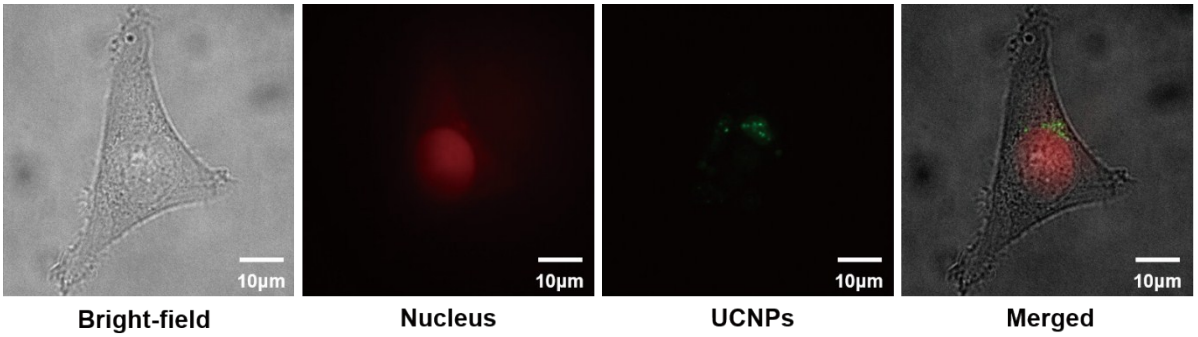

Fig. S5 2D Wide-field bright-field image, luminescence images, and merged image of a HeLa cell with internalized UCNPs (green). The nucleus was labeled with nucleus-labeling RFP (red).

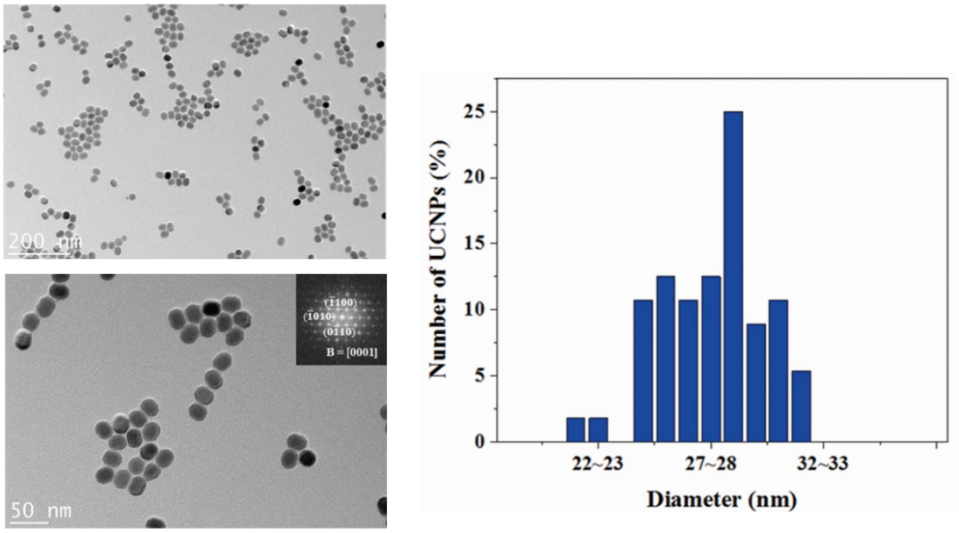

Fig. S6 TEM images and size distribution analysis of bare NaYF4:Yb^{3+}, Er^{3+} UCNPs before PEG passivation. The UCNPs were well-dispersed and uniform in size. The selected area electron diffraction (SAED) pattern confirmed the identity of a perfect hexagonal close-packed (HCP) structure of the synthesized UCNPs (bottom left, inset). The 50 UCNPs were randomly selected and the size distribution was analyzed (right). The average diameter of the UCNPs was 27.6 ± 2.3 nm.

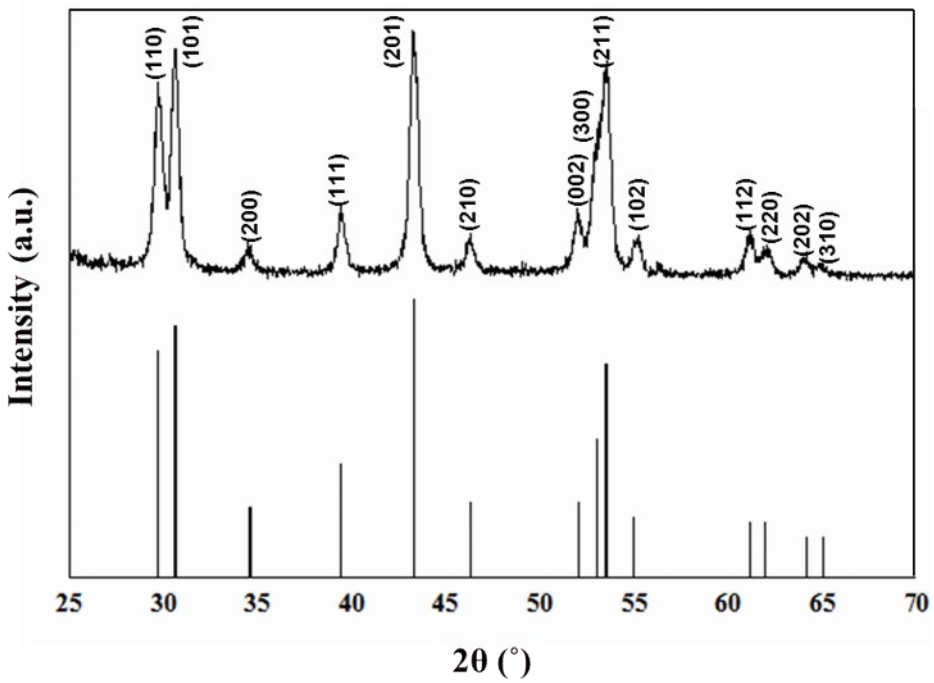

Fig. S7 The experimental powder XRD pattern of UCNPs (upper) is well indexed to the hexagonal phase β-$NaYF_4$ (lower, JCPDS No. 16-0334).

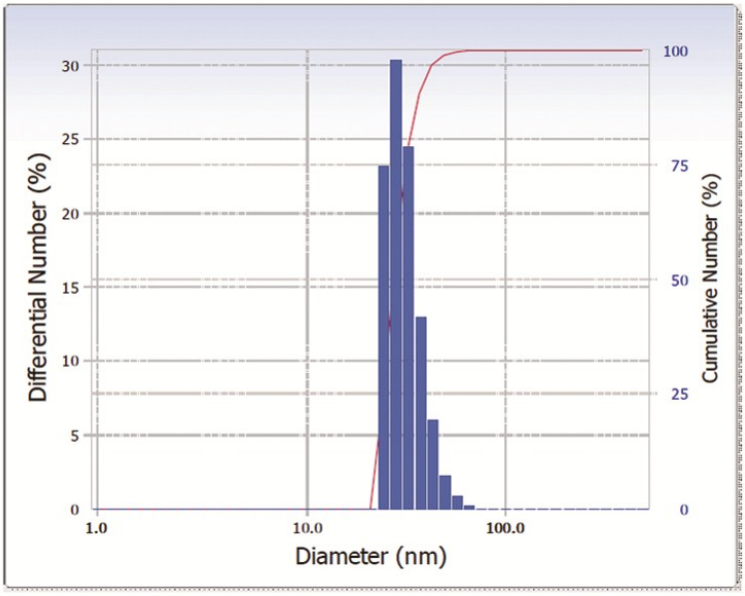

Fig. S8 Dynamic light scattering (DLS) size distribution histogram of PEG-phospholipids-coated UCNPs (UCNP-PEG-amine). The hydrodynamic diameter is 32.6 ± 6.9 nm.

Table S1. Elemental compositions of UCNPs analyzed with ICP-MS.

	Y (mol%)	Yb (mol%)	Er (mol%)
Molar Ratio (%)	77.3	20.1	2.6

Movie S1. A raw imaging data in real time showing repetitive z-sectioning for 3D imaging or 3D particle tracking in a live cell.

Movie S2. A rotating view of 3D images with localized UCNPs. The nucleus was labeled with RFP and excited by 532 nm laser separately and its image was merged with UCNP image.

References

1 Z. Li, Y. Zhang and S. Jiang, *Adv. Mater.*, 2008, **20**, 4765.

2 Y. I. Park, H. M. Kim, J. H. Kim, K. C. Moon, B. Yoo, K. T. Lee, N. Lee, Y. Choi, W. Park, D. Ling, K. Na, W. K. Moon, S. H. Choi, H. S. Park, S. Y. Yoon, Y. D. Suh, S. H. Lee and T. Hyeon, *Adv. Mater.*, 2012, **24**, 5755.

3 R. E. Thompson, D. R. Larson and W. W. Webb, *Biophys. J.*, 2002, **82**, 2775.