Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Transient absorption microscopy of gold nanorods as spectrally orthogonal labels in live cells

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1. Methods and Materials

Synthesis of AuNRs

$HAuCl_4 \cdot 3H_2O$, $AgNO_3$, ascorbic acid, sodium borohydride and other chemicals were purchased from major suppliers such as Sigma-Aldrich and Alfa Aesar, and were used as received. The UV-Vis spectra were recorded with a Genesys 10s UV-Vis spectrophotometer.

AuNRs were synthesized using the seed-mediated growth method developed by Murphy et al [1]. Briefly, a seed solution of gold nanoparticles with an average diameter of around 4 nm was produced by the reduction of $HAuCl_4 \cdot 3H_2O$ (5 mL, 5 mM) with $NaBH_4$ (0.6 mL, 10 mM) in the presence of a cationic surfactant cetyltrimethylammonium bromide (CTAB, 5 mL, 200 mM). The resulting seed nanoparticles were used in the synthesis of AuNRs. In a typical procedure, to 8 mL of 0.1 M ascorbic acid solution was added 100 mL of 0.10 M CTAB, 2 mL of 25 mM $HAuCl_4$, 1.25 mL of 10 mM $AgNO_3$, and 2 mL of 0.5 M H_2SO_4 under gentle stirring at ambient temperature. Finally, 240 μL of the seed solution was added to the mixture, and the resulting solution was stirred for 1 min and then left undisturbed overnight at room temperature. According to TEM images, the average length, diameter, and aspect ratio of AuNRs were calculated to be 67 ± 8 nm, 16 ± 3 nm, and 4.2 ± 0.5, respectively. The concentration of AuNRs can be calculated by means of their UV-Vis spectra and the extinction coefficient at the longitudinal plasmon peak.

Transient absorption microscopy (TAM)

The TAM was constructed on a modified two-photon scanning microscope system. Light source for TAM is a pump source integrated optical parametric oscillator, also known as one-box OPO (picoEmerald, APE, Berlin, Germany). This one-box OPO can simultaneously provide two spatially and temporally overlapped pulse trains, with a repetition rate of 80 MHz. The wavelength of one beam is 1064 nm (the pump beam in TAM), and the other is tunable between 780-990 nm (the probe beam in TAM). Pulse duration is 8 ps for the pump beam and 6 ps for the probe beam. The two beams are sent into an inverted multiphoton microscope (IX81/FV1000, Olympus, Japan). Intensity of the pump beam is modulated with an electro-optic modulator (EO-AM-NR-C2, Thorlabs, USA) at 9.825 MHz. The two beams are collinearly aligned and focused by a 60X water immersion objective (UPLSAPO 60XW, Olympus, Japan), where they scan over the sample through galvano mirrors in the scanning unit. After transmitting through the sample, the probe beam is collected by a water immersion condenser (N.A. 0.9, Olympus, Japan), then focused to a large area photodiode (FDS1010, Thorlabs, USA). The photo-electric signal was sent into a home-made lock-in amplifier. After demodulation, the amplified signal was sent into an A/D converter, and then used to reconstruct images. Stimulated Raman scattering signal of paraffin's CH_2 stretching mode (2845 cm^{-1}) was acquired to characterize the system response and to evaluate the correlation between the two pulse trains.

Cell culture

The Hela cells were maintained in Dulbecco's Modified Eagle Medium(DMEM) complete media containing 10% Fetal Bovine Serum(FBS), and 1% penicillin-streptomycin mix. Cells were seeded into 35mm petri-dish at a concentration of $10^5/cm^2$. Cells were cultured in a humidified incubator at 37 °C and 5% CO_2 atmosphere.

Cell uptake of AuNRs

Hela cells were passaged into petri-dish with glass bottom. Cultured as mentioned above. Before cells grew to confluence, changed medium with AuNRs. Stocked AuNRs were added to culture medium with ratio of 1:1000. Then, changed the AuNRs-contained medium. Cultures the cells in incubator for 8h before imaging.

3-Dimensional time-lapse imaging

Hela cells were prepared as described above. AuNRs were added to Hela cells 2 hours before imaging. Wash the cells with PBS to remove remaining AuNRs in culture medium. Then an automatic 3-D time-lapse imaging was performed for about 20 mins.

Particle size measurement

AuNRs were suspended in culture medium 1X(1μg/mL) and 10X concentrations. Suspensions are then used to measure particle size distribution with light scattering analyzer (ZetaPALS, Brookhaven Instruments Co., NY, USA).

2. Supplementary Figures

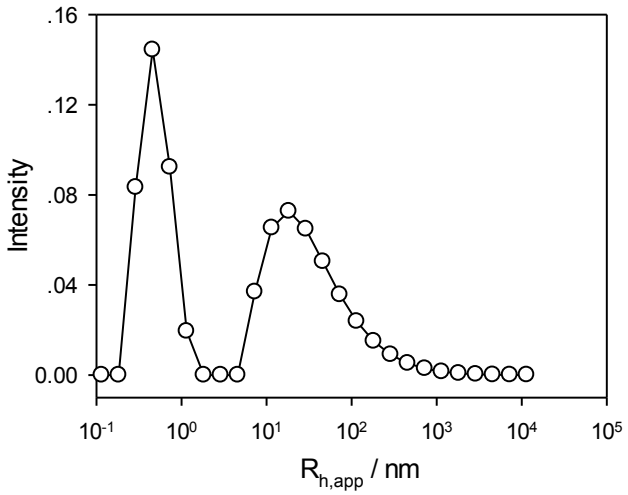

Fig S1. The appearance hydrodynamic radius ($R_{h,app}$) of AuNRs in cell-culture medium measured by dynamic laser light scattering at 30°. The distribution with the average $R_{h,app}$ about 0.5 nm is attributed to the diffusion caused by the culture medium, while the distribution with the average $R_{h,app}$ about 18 nm is caused by the diffusion of AuNRs.

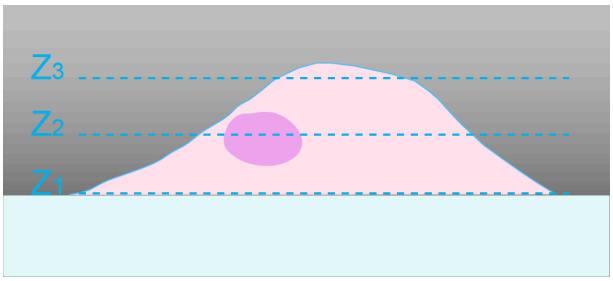

Fig S2. Illustration of focal plane selected in 3-D time-lapse. z_1, z_2, and z_3 corresponds to movies S1 - S3.

3. Supplementary Movies

Movie S1-S3: Motion of AuNRs in live HeLa cells lasting for ~ 20 min at different focal planes, as shown in Fig S2.

Movie S4: 3D distribution of AuNRs in live Hela cells. Faint blue showed the cell profiles. Bright green/white showed the AuNRs. Color code remains the same in the movies.

Reference

1, T. K. Sau, C. J. Murphy, *Langmuir*, 2004, **20**, 6414.