Supplemental Document

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Uptake quantification of gold nanoparticles inside of cancer cells using high order image correlation spectroscopy: Supplemental Document

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Methods

Simulation of HICS

The HICS validity was tested on simulated DFM images of varying concentrations. We have followed the methodology used by Sergeev et al. Firstly DFM images of randomly distributed AuNP scatterers (monomers and dimers only for a bimodal distribution) are simulated by convolving 2D gaussian spots fixed with beam waist representing the numerical aperture of an objective lens, on the pre-randomized positions of particles. The gaussian spot intensity reflects integrated visible range (400 – 700 nm) scattering spectrum of monomer and dimers of AuNPs. Typical image size was 256 x 256 pixels, while the beam waist was 5 pixels. Dimer scattering intensity can vary anywhere between 2 and 4 times of that of a monomer (i.e., $2 < \alpha_2 < 4$), depending on the gap distance between nanoparticles. In the simulation we have put α_2 value at 4 (gap distance of 2 nm). Gaussian noise with varying mean was added on pixel to with signal-to-noise ratio (SNR) to be varied between 2 ~ 100 to mimic the experimental condition. The range was correctly subtracted without affecting the HICS analysis. Mathematica 12 (Wolfram Research) was used as the software for image simulation and HICS analysis. The PC used had Intel Xeon Processor (3.6GHz) with 32GB RAM and Windows 10 as its OS. Typical run time was less than 0.1 second for generating a 256 x 256 image and analysing HICS.

Sample preparation for AuNPs on coverslip glass and experimental validation of HICS

The accuracy of HICS analysis was validated against dark-field scattering images of randomly distributed low-concentration AuNPs on a coverslip glass for a non-cellular environment similar to the simulated images. We purchased AuNPs of 80 nm diameter coated cetyltrimethylammonium bromide (CTAB) from NanoSeedz Ltd (NS-80-50, Hong Kong). AuNPs were diluted to 1:10 with distilled water then particles were sonicated for 10 min to minimize aggregation. A drop cast of AuNPs on the coverslip glass was dried at room temperature. We used commercial dark-field microscopy (Nikon Eclipse Ti-S and Nikon DS-Filc colour CCD camera) for imaging and a coupled spectrometer (SpectraPro 300i, Acton Research) for scattering spectroscopy. 100W halogen lamp was used as a white light source, which passed through a dry condenser with numerical aperture 0.8 - 0.95 NA (ring-illumination). Then an objective lens with 0.5 NA (40 x magnification) collected dark-field scattering images and spectrum of particles.

The following steps were then taken for the HICS analysis

(1) Once the DFM images were taken, we selected a square region in the acquired image where only monomers and dimers are present for the HICS analysis. The measured high-order autocorrelation function $g_{n,m}^{meas}(0,0)$ was calculated on these images using Eq. (3) in the main text.

(2) In order to extract the noise-corrected high-order autocorrelation function $g_{n,m}^{sig}(0,0)$ the background noise was subtracted and filtered from the image for the HICS processing using the method provided by Petersen et al [ref. 55 in the main manuscript]. The zero-lag, noise-corrected high-order autocorrelation function $g_{n,m}^{sig}(0,0)$ is expressed as

$$g_{n,m}^{sig}(0,0)=\frac{g_{n,m}^{meas}(0,0)\langle i_{meas}\rangle^{n+m}-g_{n,m}^{bn}(0,0)\langle i_{bn}\rangle^{n+m}}{\left(\langle i_{meas}\rangle-\langle i_{bn}\rangle\right)^{n+m}}, \qquad \text{(S1)}$$

where $\langle i_{meas}\rangle$ is the measured pixel intensity average of the image, $\langle i_{bn}\rangle$ is the measured pixel intensity average of the background noise image, and $g_{n,m}^{bn}(0,0)$ is the background noise correlation function. This equation is also used to noise-correct the HICS simulations in Section 2

(3) From the noise-corrected autocorrelation function $g_{n,m}^{sig}(0,0)$ together with Eq. (4)-(6) in the main text simultaneous equations are built-up and solved to extract the required parameters, $\langle N_1\rangle$, $\langle N_2\rangle$ and α_2.

(4) Monomer and dimer spot numbers in the images could be counted manually (spectrum counting method). Monomers are easily identified in the image due to their monomodal scattering intensity and distinct green colour (SPR peak at 550 nm). Monomer number dominated the image (~ 80% of the spots), and the rest of the spots were identified as aggregates. Dimers have their total intensity between two to four times that of monomers (i.e., $2 < \alpha_2 < 4$) with yellow to red colour (SPR peak within 550 - 700 nm)

Sample preparation for HeLa cells uptake with AuNPs

HICS analysis is extended to study cellular uptake of AuNPs in HeLa cells. HeLa cells (human cervical carcinoma) were cultured in microslide chamber (Abidi, AU) by Dulbecco's Modified Eagle Medium (DMEM, 10% v/v), fetal bovine serum (FBS, 1% v/v), glutamine 1%, 5% penicillin/streptomycin and amphotericin B. All the supplements were purchased from Sigma Aldrich. Cells were then incubated in a humidified atmosphere (95% air, 5% carbon dioxide v/v) at 37 ℃ for 24 hours to attach in to the chamber. A solution of 80 nm spherical AuNPs coated with CTAB (NS-80-50, NanoSeedz Ltd) was diluted and sonicated for 10 min to prevent aggregation. CTAB is cationic (ζ-potential ~ +40 mV), therefore are favorably taken up by negatively charged HeLa cell membrane [1]. 100µl of solution was added to the cultured HeLa cells and incubated for desired amount of time (0.5, 2, 4, 6, 8, 10, 24, and 48 hours). After the incubation, cells were fixed by 3.7% formalin for 15 minutes and then were washed six rounds by PBS for 30 minutes. To make chamber ready for microscopy imaging, a coverslip is attached to the top of the chamber with mounting medium (Aquatex, Merck Millipore, AU). DFM, described in section 3 was used to image the cells with AuNPs.

Fig. S1. Confocal laser scattering microscopy of AuNPs inside cells.

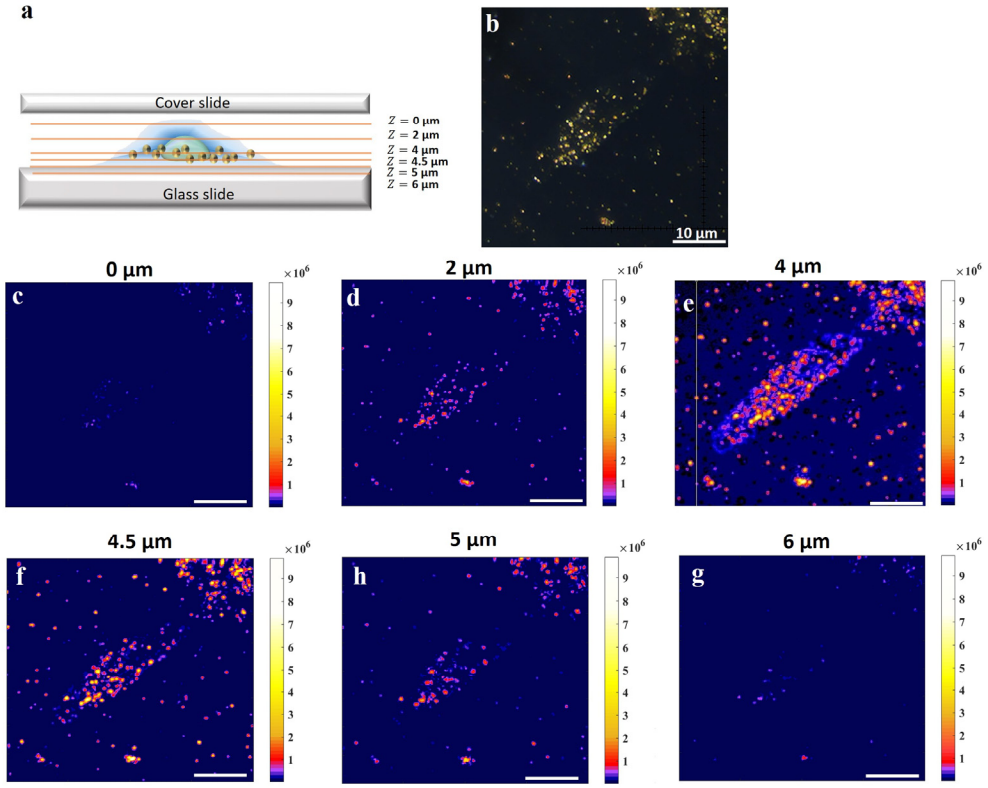

Fig. S1. A series of confocal laser scattering microscopy images of a cell with AuNPs internalised at different *Z* positions, from 0 to 6 µm depth. (a) Schematics of the sample structure and confocal z-sectioning depth. (b) DFM image of the same region. (c) z = 0 µm, (d) z = 2 µm, (e) z = 4 µm, (e) z = 4.5 µm, (f) z = 5 µm, (g) z = 6 µm. The cells are incubated with AuNPs for 10 hours. Laser wavelength was 530 nm continuous wave, and objective lens used was 0.7 NA. Point detector used was an avalanche photodiode (SPCM-AQR-14, Perkin-Elmer, USA). The images show that at z = 4 µm the scattering signal from the embdded particles reaches maximum and the cell outline (blue) is also visible. The highest particle scattering intensity and their concentration occur at the largest cell area, indicating that the particles imaged are internalized inside the cell.

References

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