Supporting Information

On-Demand Intracellular Delivery of Single Particles in Single Cells by 3D Hollow Nanoelectrodes

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Figure S1. SEM images of the Raman-tagged gold nanorods with sizes of 25 × 90 nm (left) and 10 × 40 nm (right).
Figure S2. Raman spectrum of the Raman tag, Nile Blue A, adsorbed on the gold nanorods in which the 593 cm\(^{-1}\) and 633 cm\(^{-1}\) bands are selected for evaluation of the nanorod aggregation (Inset). Time traces of signal-to-baseline intensity of different Raman peaks and baseline (700 cm\(^{-1}\)) of 0.1 nanorod on average diffusing in a detection volume (Φ 1.2 × 5 µm) of the 60 × water immersion objective with N.A. = 1.0. (a). The corresponding correlation of the Raman peaks between 593 cm\(^{-1}\) and 663 cm\(^{-1}\) (b) or between 593 cm\(^{-1}\) and the baseline at 700 cm\(^{-1}\) (c) in which the R\(^2\) is the coefficient of correlation and \(<N>\) is the average number of nanoparticle in the detection volume. The high correlations between the Raman peaks or baseline indicate that no nanorod aggregation exists and the spectra of single nanorod in flow are stable. \(^1,^2\)
Figure S3. (a) Time traces of the nanorods (25 × 90 nm) translocating through the nanoelectrodes without cells under different electrophoresis bias voltage. (b) Raman time traces for a 0V bias applied. (c) Autocorrelation function (black dots) associated to the Raman time trace in (b), as well as its fit to a 2D diffusion model (black curve). (d) Raman time traces for a -2V bias applied. (e) Autocorrelation function (green dots) associated to the Raman time trace in (d), as well as its fit to a 2D diffusion model (green curve).
Table S1: Summary of fitting results extracted from the autocorrelation function of Raman data of translocated nanorods from 3 nanoelectrodes.

| Voltage (V) | $\tau_D$ (ms) | N     |
|-------------|---------------|-------|
| 0           | 170 ± 90      | 1.1 ± 0.2 |
| -0.5        | 100 ± 100     | 0.4 ± 0.2 |
| -1          | 90 ± 30       | 0.3 ± 0.1 |
| -1.5        | 70 ± 20       | 0.28 ± 0.02 |
| -2          | 69 ± 9        | 0.4 ± 0.1 |
Figure S4. Simulated electromagnetic field intensities distributions at 785 nm wavelength for (a) parallel nanorod at the center, (b) parallel nanorod near the edge, (c) perpendicular nanorod at the center, (d) perpendicular nanorod near the edge, of the nanoelectrode tip. Color bars indicate the field intensity enhancement, $|E/E_0|^2$. The field polarizations are all the same as in (a). The fields have higher intensities near the edge than at the center due to plasmonic coupling between the nanorod and the nanoelectrode.
Figure S5. Cell viability test before and after the electroporation and electrophoresis at -2V DC for 2 min; the white squares indicate the positions of the nanoelectrode arrays. Cells were stained with 1µl Calcein-AM (1 mg/ml, Life technologies) and 1µl ethidium homodimer-1 (2mM, Life technologies) in 1ml 1X PBS for 10min. The permeable non-fluorescent calcein-AM dye becomes green-fluorescent by esterase activity inside the cell. Therefore, only living cells will exert the green fluorescent color. Ethidium homodimer exerts red fluorescence when it intercalates with nucleic acid. As it is impermeable, this will only happen when the cell membrane is broken, indicating a dying/dead cell.
Figure S6. (a) A time trace of electrophoretic intracellular delivery of the nanorods at -2 V bias by nanoelectrode 5. (b) Bright-field optical images and Raman maps on the delivered cell and their overlaid images about the distribution of the nanorods. Raman maps took 5 to 10 min to finish, depending on the mapped area. Z indicates the position of the imaging and mapping focal plane. The images were taken at 3 different focal planes of the cells. The white dotted circles indicate the nanoelectrode positions and mark them with number (1 – 9). The white arrows show the color dots that correspond to the delivered nanorods in the cell at the center. The color bar represents normalized Raman intensity of the color dots. Nanoelectrode 2 and 4 without nanorod accumulations at the Z =1 µm Raman map showed nanorod accumulations after 10 min at the Z =2 µm map. The fact that the clogging were seen in the Raman mappings several minutes after the deliveries suggested that the intracellular molecule adsorption on the nanorods and the resultant clogging took minutes to happen.
Figure S7. A time trace of a clogged nanoelectrode with fluctuating baseline. However, such fluctuation was not observed in the time traces during the delivery that had almost zero baseline in Figure 5a and Supplementary Figure S6a. Positively biased electrophoresis could remove clogged gold nanorods, as the baseline decreased to near zero with increasing bias voltages.

References

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