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Polarization-based super-resolution imaging of surface-enhanced Raman scattering nanoparticles with orientational information

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1. Super-resolution Dipole Orientation Mapping (SDOM) algorithm

In SDOM[1], the effective dipoles are described with their intensity g_0 and dipole orientation α. Under polarized excitation, the intensity is denoted by $g(r,\varphi)$:

$$g(r,\varphi) = g_0(r)\cos^2(\alpha - \varphi) \tag{1}$$

where r is the position of the pixel and φ is the polarization direction of the excitation. The super-resolution reconstruction process is a convolution with the system PSF $U(r)$ with Poisson noise model. Hence, the acquired image would be:

$$I(r,\varphi) \sim Possion\left\{I_0(\varphi)\left[\int U(r-r')g(r',\varphi)dr' + b(r)\right]\right\}. \tag{2}$$

Here, to extract the dipole orientation $g(r,\varphi)$ from the detected image series $I(r,\varphi)$, maximum a posteriori (MAP) is applied, followed by extraction of the intensity g_0 and dipole orientation α via least-squares estimation of the cosine-squared function. The fitting algorithm set the intensity threshold to avoid invalid angle fitting in some pixels. The polarization-variant intensity could be expressed as:

$$g(r_i,\varphi) = \sum_{j=1}^{n}\frac{M_j}{2}\cos(2\alpha_j - \varphi) + \sum_{j=1}^{n}\frac{M_j}{2}. \tag{3}$$

We can separate the AC and DC components of Eq. (3) as following:

$$A=\sqrt{(\sum_{j=1}^{n}\frac{M_j}{2}\cos 2\alpha_j)^2+(\sum_{j=1}^{n}\frac{M_j}{2}\sin 2\alpha_j)^2}\ ,\ B=\sum_{j=1}^{n}\frac{M_j}{2}, \tag{4}$$

where A is referred as orientation amplitude which contains the dipole orientation signal, and B as super-resolution translation which contains the super-resolution signal. We define OUF as $OUF=A/B$ to describe the orientation uniformity of dipoles. The value of OUF indicates the faithfulness of the dipole orientation, which is denoted by the length of the dipole in the subsequent imaging results. The orientation mapping algorithm sets the OUF and the goodness of fit as threshold to mark the valid nanoparticles and orientation. Some pixels may loss orientation information due to poor polarization modulation, or low intensity. All the invalid orientations is set to '0'.

2. Sample preparation

Synthesis of AuNRs

Gold nanorods were synthesized by a silver ion-assisted seed-mediated method.[2] Typically, the seed solution was prepared by the addition of $HAuCl_4$ (10 mM, 0.25 mL) into cetyltrimethylammonium bromide (CTAB, 100 mM, 10 mL) in a 50 ml bottle with gentle mixing for 2 min. A freshly prepared, ice-cold $NaBH_4$ solution (10 mM, 0.6 mL) was then injected rapidly into the mixture solution, followed by vigorously stirring for 2 min. The seed solution was kept at 25℃ for 2 h before use. The growth of Au nanorods occurred in a growing solution containing $HAuCl_4$ (10 mM, 4.5 mL), $AgNO_3$ (10 mM, 0.36 mL) and CTAB (100 mM, 90 mL) in a 50-mL plastic tube. Subsequently, ascorbic acid (100 mM, 0.72 mL) was added. When the growing solution turned colorless, the seed solution (0.12 mL) was injected. The solution was gently mixed for 120 s and left undisturbed at 28℃ for 6 h. The growth process was stopped by removing the excess CTAB using centrifugation at a speed of 12000 rpm for 15 min and repeated for 3 times. The AuNRs were stored at 4℃ before use.

DTNB loading and silica coating of AuNRs

A self-assembly monolayer (SAM), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), served as Raman reporter, was adsorbed onto the surface of AuNRs substrate.[3] Briefly, 100 μl DTNB (10 mM) ethanol solution was added dropwise into AuNRs in 6 portions over 3 h. The resulting solution was allowed to react overnight, followed by centrifugation to remove free DTNB. A protective silica shell can provide chemical and mechanic stability, minimizing other unwanted signals. Therefore the as-prepared DTNB-AuNRs were further coated with SiO_2 shell by a Stöber's method.[4] Typically, 40 μl NaOH (250 mM) was added to the DTNB-AuNRs solution to achieve alkaline environment for the hydrolysis of tetraethyl orthosilicate (TEOS). 12 ul×3 TEOS (10% v/v) was dropwise added to the resulting solution for 4 hours. TEOS was allowed to form SiO_2 onto the surface of the DTNB-AuNRs for 4 hours. DTNB-AuNRs@SiO_2 nanoparticles were collected by washing 3 times with DI water.

All chemicals mentioned about were purchased from Sigma-Aldrich (St. Louis, MO, USA), and used without further purification except when mentioned specifically.

3. Instrumentation

The particle extinction spectra were measured using UV-visible spectrophotometer (Thermo Scientific Evolution 220, US). The SERS spectra of the AuNR-DTNB@SiO_2 were obtained by exciting with lasers with wavelength of 532 nm, 633 nm and 785 nm, respectively (LabRAM Aramis, Horiba Jobin Yvon, France). The morphology of the obtained AuNR-DTNB@SiO_2 was characterized using both transmission electron microscopy (FEI Tecnai G2 T20, USA) and scanning electron microscopy (Hitachi S-4800, Japan).

4. Additional experimental results

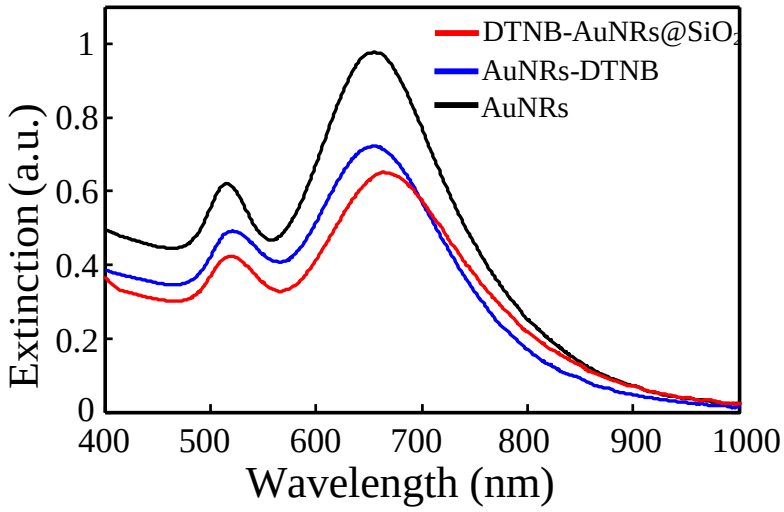

Figure S1 Extinction spectra of the as-prepared gold nanorods (AuNRs), DTNB modified AuNRs and AuNR-DTNB@SiO_2. All Nanorods exhibited a weak transverse plasmon band at 520 nm and a strong longitudinal SPR peak at 650 nm. The red-shift of longitudinal plasmon peaks between AuNR-DTNB@SiO_2 and AuNRs indicates the successful formation of silica shell on the DTNB-labeled AuNRs.

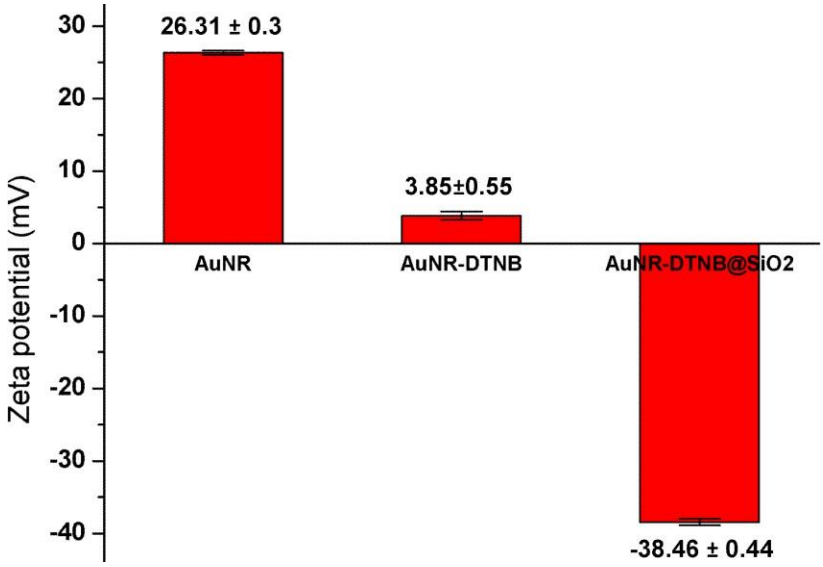

Figure S2 Zeta potential of the obtained AuNRs, DTNB modified AuNRs and AuNR- DTNB@SiO_2.

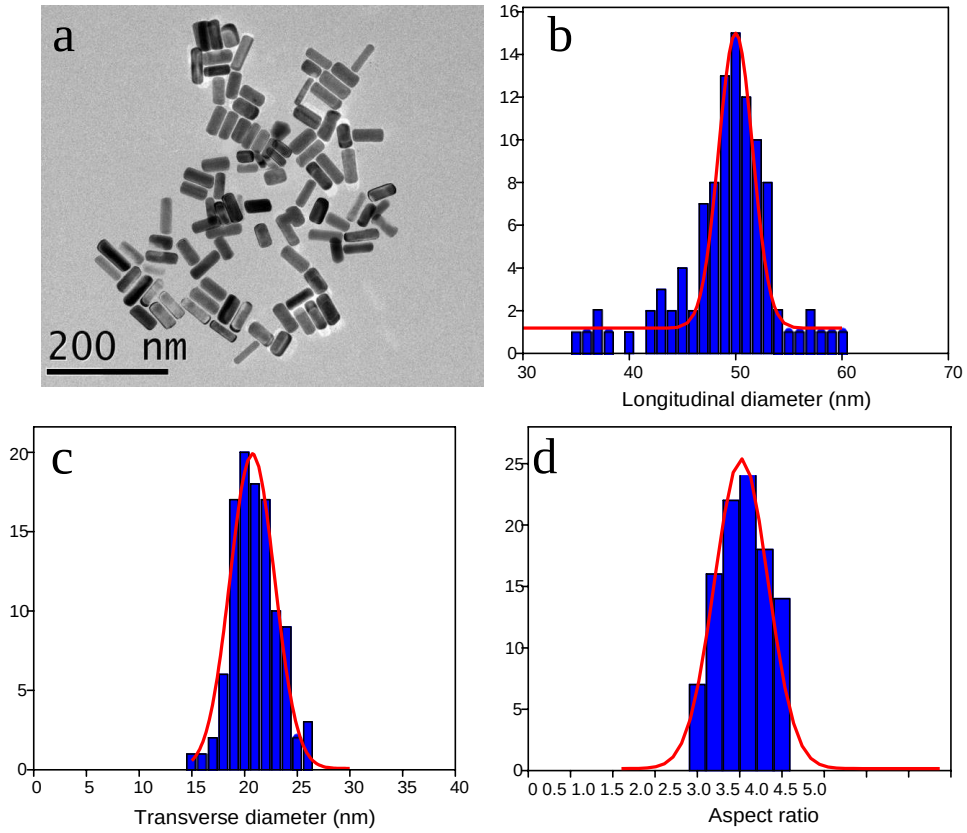

Figure S3 (a) TEM image of SERS nanorods. (b) The longitudinal and (b) transverse diameter distribution of 100 SERS nanorods obtained from the TEM images. (d) Distribution of the aspect ratio.

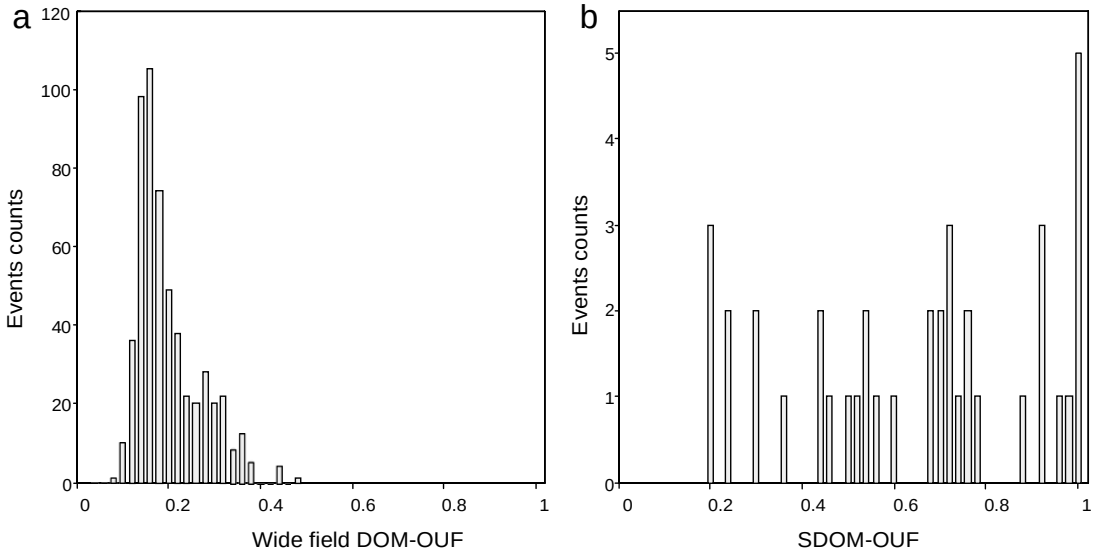

Figure S4 OUF histogram of (a) wide-field and (b) SDOM images of SERS nanorods.

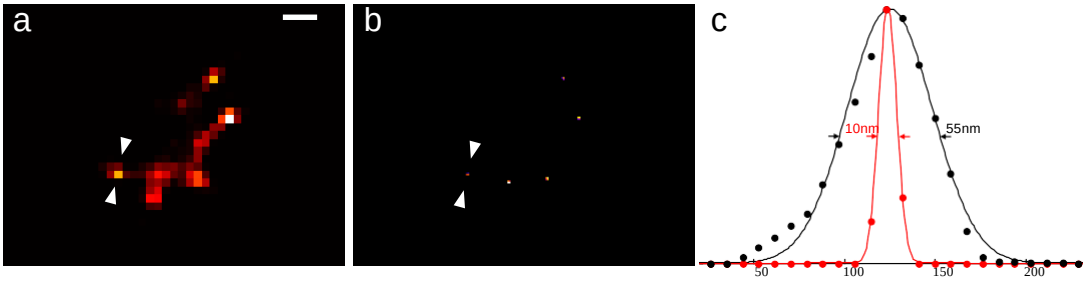

Figure S5 Super localization based on SERS-SDOM. (a) SERS nanoparticles resolved by SDOM. Scale bar, 200 nm. (b) Super localization of SERS nanorods by Gaussian Fitting to (a). (c) Intensity profiles at the cross-section in (a) and (b).

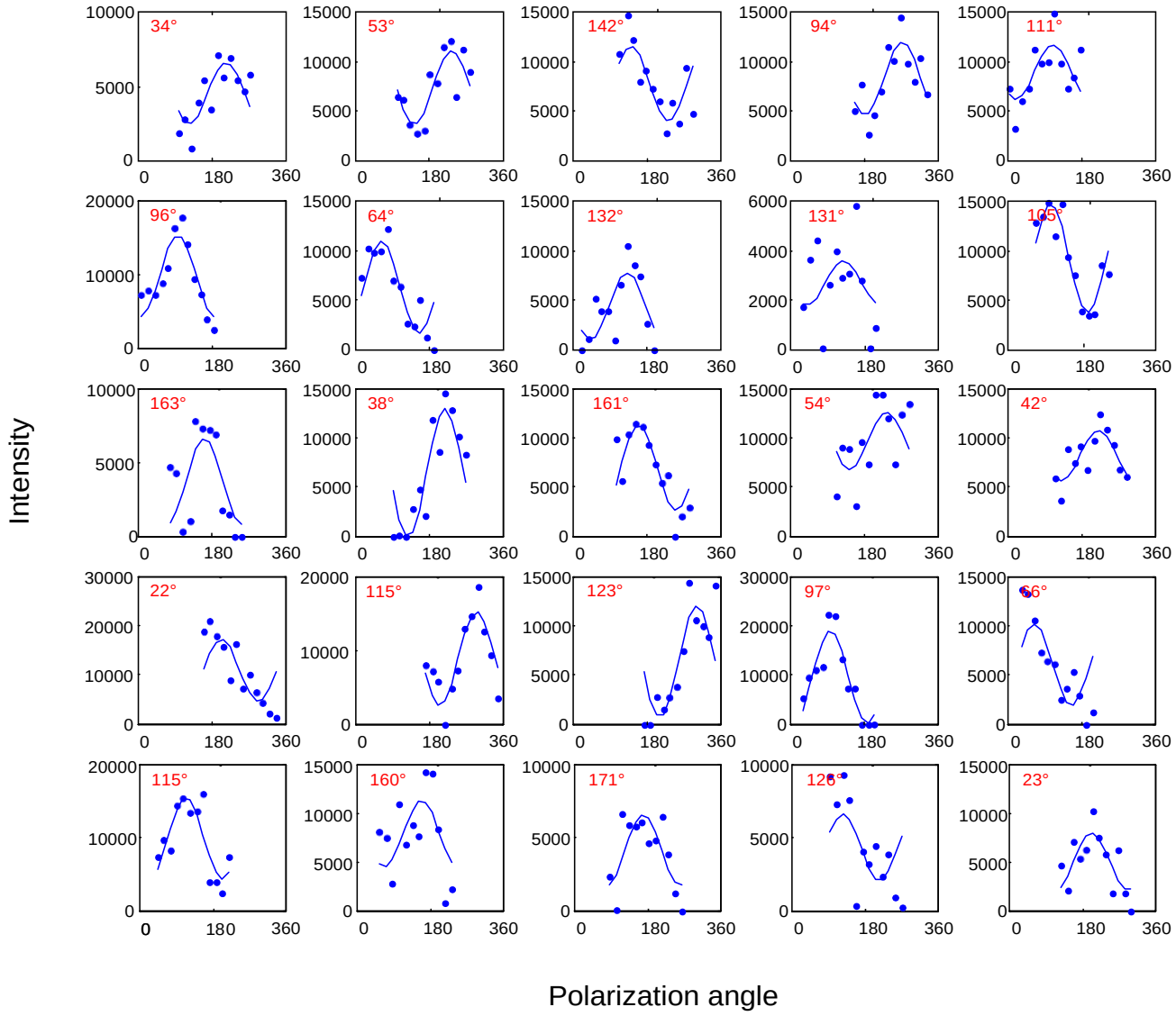

Figure S6 Polarization modulation of SERS signals, obtained from each tracking point.

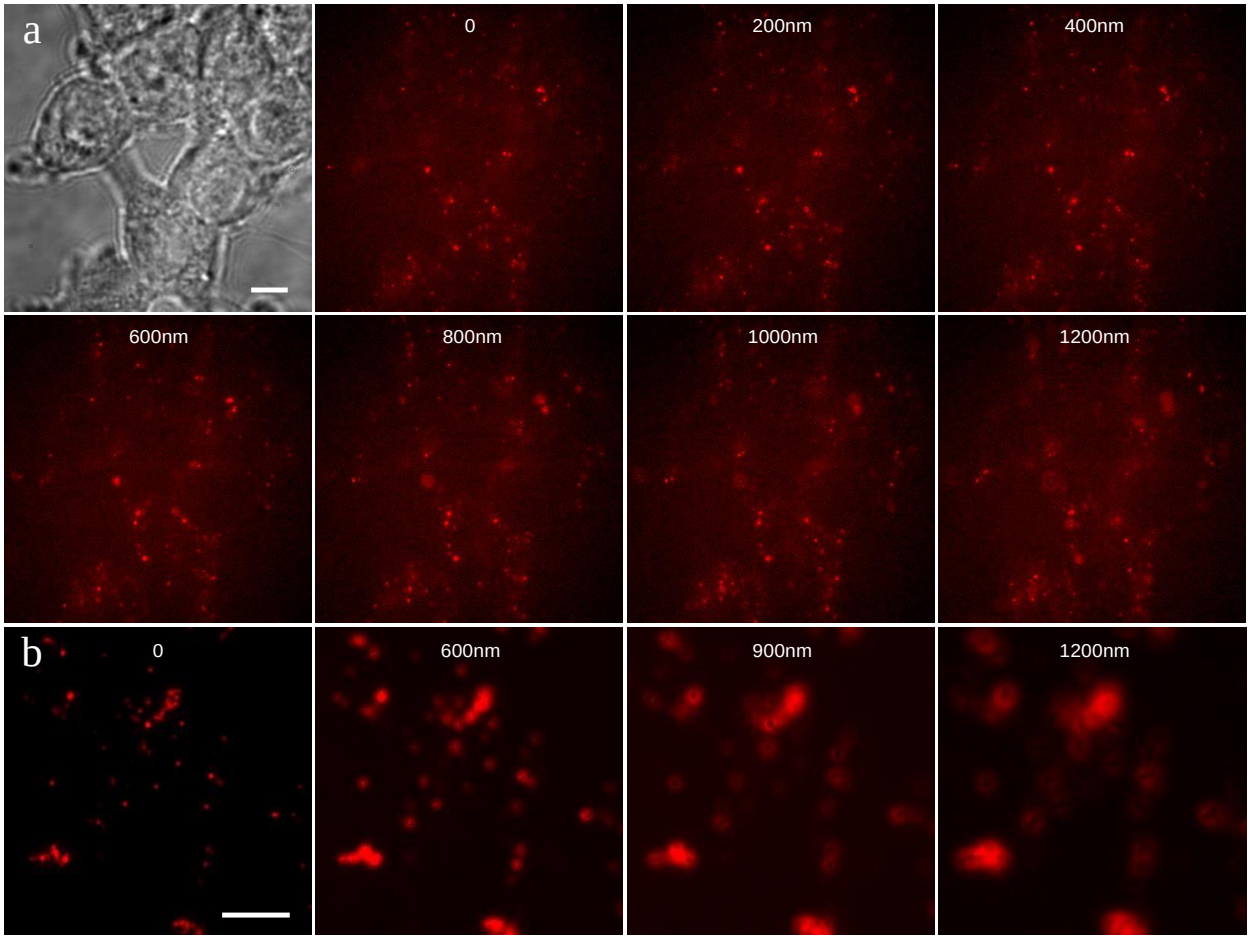

Figure S7 (a) Bright field image of mouse macrophages and defocused wide-field image of SERS nanorods internalized by endocytosis with defocusing distance varying from 0 to 1200 nm. (b) Defocused wide-field image of SERS nanorods deposited on the glass slides with defocusing distance varying from 0 to 1200 nm. The exposure time was 300 ms.

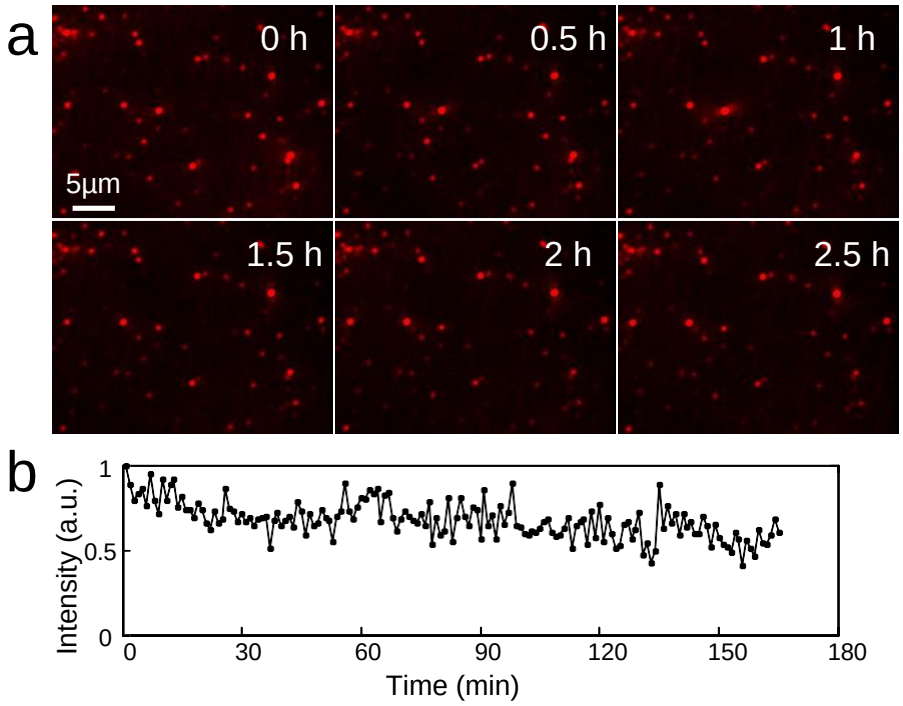

Figure S8 Photobleaching resistance of SERS nanorods. (a)Wide-field images of SERS nanorods at different time points under successive 647 nm illumination with strong laser power of 100W/cm^2. The camera exposure time was 100 ms. (b) Normalized average SERS intensity curve record every minute in (a) with continuously irradiation for more than two hours before the SERS signal subsided to half of the original intensity.

5. Cell culture and labeling with SERS nanorods

2X10^5 mouse macrophages (RAW 264.7) per well were seeded on 35-mm dish in phenol red free Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 g/L glucose with GlutaMAX™ (Invitrogen, Carlsbad, CA) and supplemented with 10% Fetal Bovine Serum (FBS, Hyclone), and 1% penicillin–streptomycin at 37 °C (5% CO2 in 100% humidity) overnight. 1-mL DMEM containing 10 nM/mL SERS nanorods was pipetted into the dish after warming to 37°C. The dish was then placed in 5% CO_2 at 37 °C for 1 hour to allow cellular uptake of SERS nanorods. Next,

the cells were fixed with 4% paraformaldehyde (PFA) in PBS for 10 min and stored in PBS for optical imaging.

6. Supplementary videos

Video S1: Polarization modulation of randomly orientated SERS nanorods excited with different polarization angle

Video S2: Real-time nanoparticle tracking by wide-field and SERS-SDOM

References

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4. M. Schütz, B. Küstner, M. Bauer, C. Schmuck and S. Schlücker, *Small*, 2010, **6**, 733-737.