Super-Resolution Imaging and Rotational Tracking of Surface Enhanced Raman Scattering Nanoparticles in Living Cells

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INTRODUCTION

Conventional super-resolution imaging of SERS is challenging as Raman signal is generated from the virtual state which cannot be easily modulated as fluorescence either sequentially or stochastically. Here, we have successfully developed a novel super-resolution technique, which enables SERS as imaging contrast with a resolution of approximately 50 nm. By modulating the polarization angle of the excitation laser, the SERS nanorods display a dramatic anisotropy effect, allowing nanoscale orientation calculations by our newly developed Super-resolution Dipole Orientation Mapping (SDOM) algorithm[1]. With this new technique, the ultrastructure of SERS nanorods labelled vesicles in macrophage has been successfully revealed. We have further unlocked the intracellular behavior of SERS nanorods by nanoparticle tracking in live macrophages. The rotational dynamics as well as the position can be obtained simultaneously at super-resolution scale, which paves a new avenue for subcellular super-resolution imaging with SERS effect, shedding light on wider biological applications.

METHODS

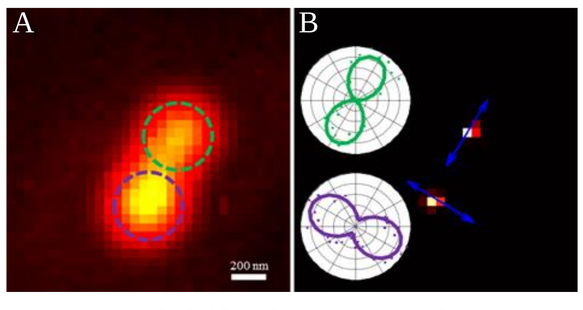

Fig.1 (A) Wide-field and (B) super-resolution orientation mapping of two randomly oriented SERS nanorods. The diagrams are polar plots of modulated SERS signal.

System design of SERS nanoscopy

Epi-illumination microscopy with a linearly polarized 647-nm excitation laser, modulated by a rotary half-wave plate (HWP), was undertaken to obtain images of SERS nanorods. The camera readout signal was synchronized with the rotary HWP so as to obtain the polarization angle of the linearly polarized light during the image collection.

Super-resolution Dipole Orientation Mapping (SDOM) algorithm[1]

Typically, a super-resolved image was reconstructed by the sparsity-enhanced deconvolution algorithm[2] from 10 polarization modulated SERS images with 18° polarization rotation per frame. The effective dipoles are described with their intensity g_0 and dipole orientation α . Under polarized excitation, the intensity is denoted by $g(r,\varphi)$:

$$g(r,\varphi) = g_0(r)\cos^2(\alpha-\varphi)$$

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:

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

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. The polarization-variant intensity could be expressed as:

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

The dipole orientation α was hence extracted using the least-squares estimation of the cosine-squared function.

RESULTS

The SERS tags in the experiments are silica-coated gold nanorods loaded with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) as the Raman reporter. By modulating the polarization angle of the excitation laser, the SERS nanorods display a dramatic anisotropy effect, allowing nanoscale orientation calculations by the SDOM algorithm. Single particle tracking of SERS nanorods provides not only the particle trajectory, but also the orientation at super-resolution. The position and orientation information give further insight into the dynamic process of labeled molecules, most possibly to unravel the working mechanism of molecule interaction such as protein diffusion and nanomotion.

Polarization effect of SERS nanorods

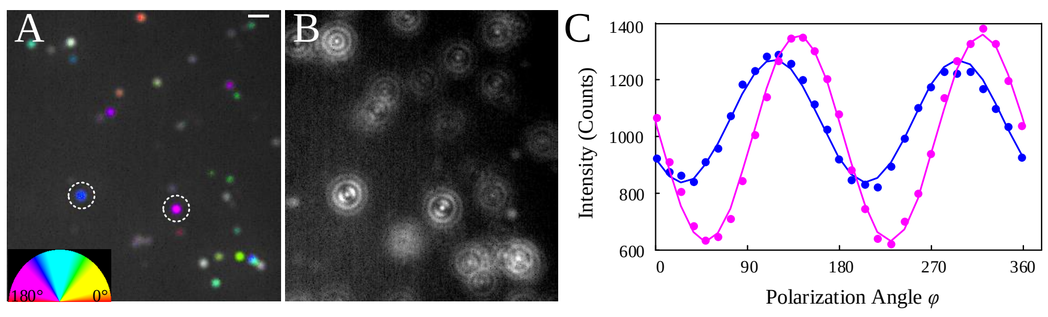

Fig.2 (A) A colour-coded map of randomly oriented SERS nanorods following excitation with linearly polarized 647-nm laser (scale bar, 1 μm). Note: The colour bar indicates the orientation from 0° to 180° . (B) A defocused pattern[3] of SERS nanorods at the defocused value of 1.5 μm with one-second integration time. (C) The averaged intensity plots of SERS nanorods (circled in A) as a function of the polarization angle. The solid curves correspond to the best fitting using a cosine-squared function.

Super-resolution imaging of SERS nanorods with orientational information

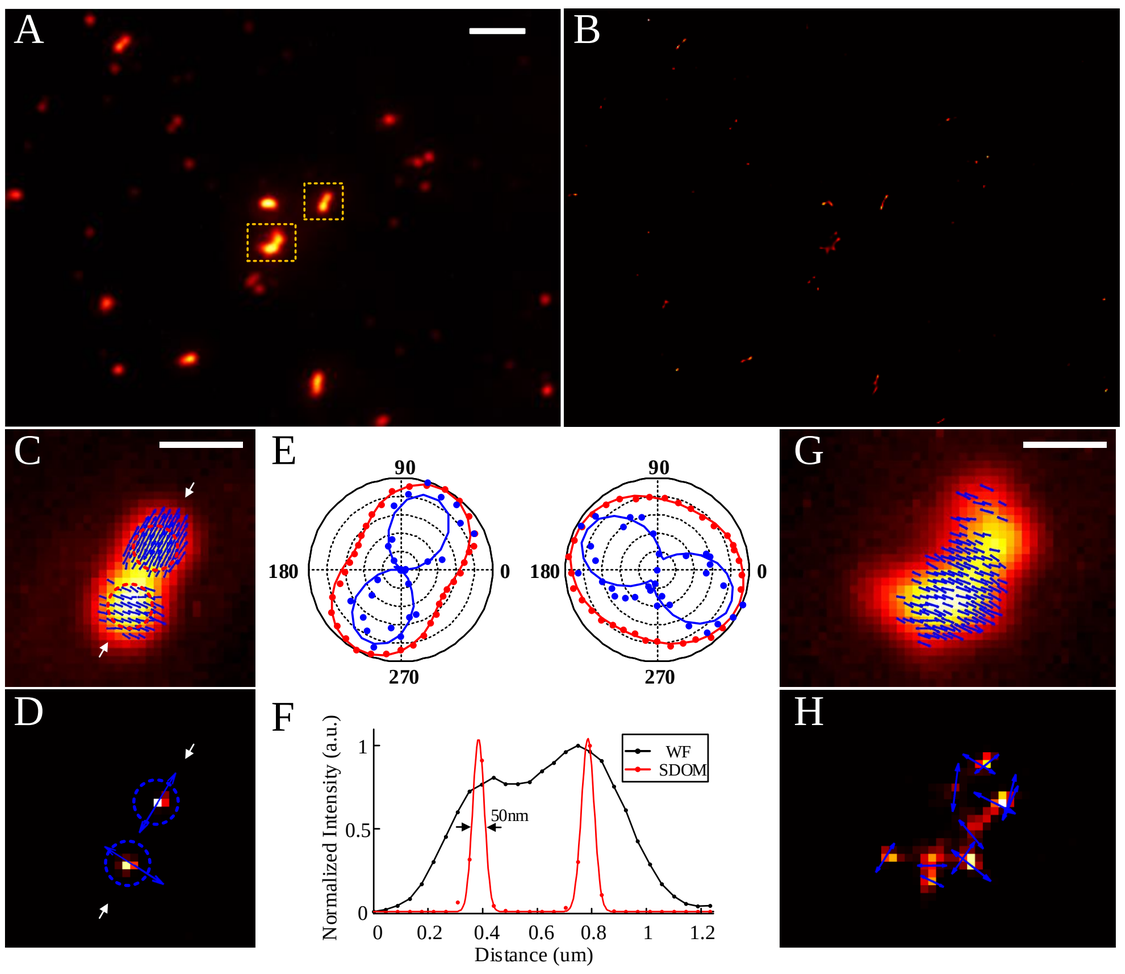

Fig.3 Super-resolution imaging and orientation mapping of SERS nanorods by polarization modulation. (A) Wide-field and (B) reconstructed polarization-based super-resolution image of randomly oriented SERS nanorods dispersed over a coverslip (scale bar, 2 μm); (C, G) Enlarged wide-field images; and (D, H) SDOM images of SERS emitters indicated by the yellow boxes in (A) (scale bars, 500 nm). Note: The direction of blue arrows indicate the dipole orientation; (E) Intensity polar plots of the upper and the lower SERS nanorods in wide-field (C) and SDOM (D) images. The dots correspond to the experimental data and the solid curves correspond to the best fitting using cosine-squared function; and (F) Intensity profiles at the cross-sections of (C) and (D). The FWHM of the fitted Gaussian curve is 50 nm.

Dynamic super-resolution SERS nanorods tracking inside macrophages

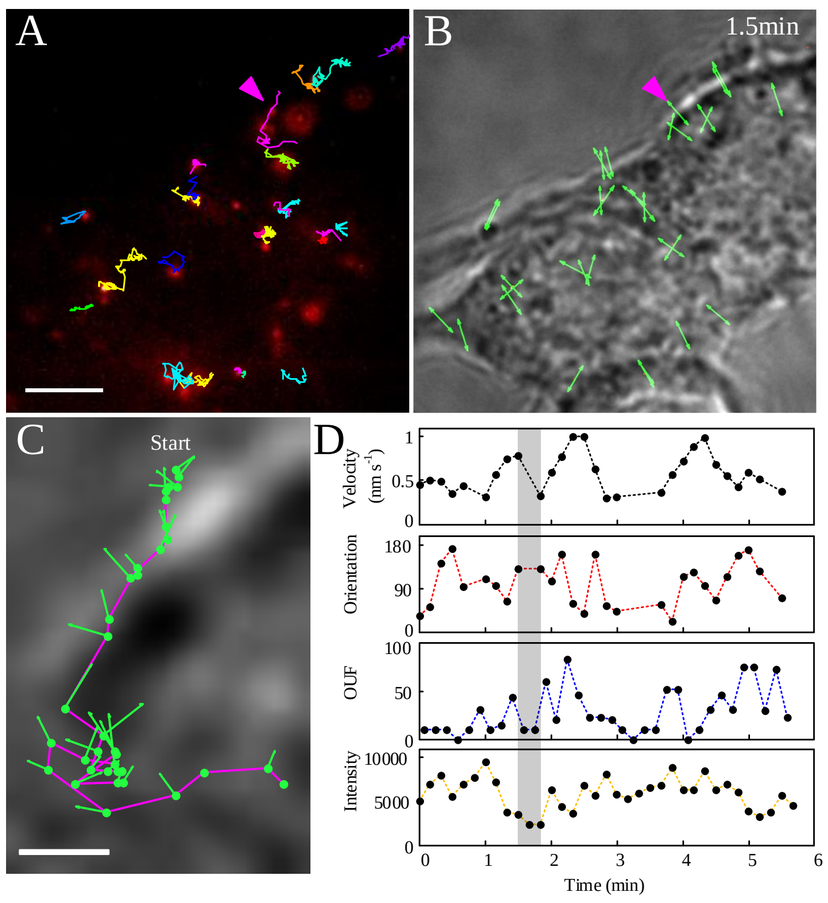

Fig.4 (A) Cellular trajectory of SERS nanorods in living mouse macrophages within 15 minutes. (B) Bright-field image overlaid with SDOM image of endocytosed SERS nanorods at time point of 1.5 min. Measured polarization orientation of detected particles are shown by the direction of green lines, respectively. The lines are placed with their center at the position of the particles. (C) The trajectory and rotational dynamics of the corresponding SERS nanorods marked by carmine triangle in (A) and (B). (D) Time-lapse analysis of the velocity, orientation, OUF and intensity of the representative SERS nanorod.

References:

(1) Zhanghao, K.; Chen, L.; Yang, X.; Wang, M.; Jing, Z.; Han, H.; Zhang, M. Q.; Jin, D.; Gao, J.; Xi, P., Super-resolution dipole orientation mapping via polarization demodulation. Light-Science & Applications 2016, 5 (10).

(2) Hafi, N.; Grunwald, M.; van den Heuvel, L. S.; Aspelmeier, T.; Chen, J.; Zagrebelsky, M.; Schütte, O. M.; Steinem, C.; Korte, M.; Munk, A., Fluorescence nanoscopy by polarization modulation and polarization angle narrowing. Nature methods 2014, 11 (5), 579-584.

(3) Böhmer, M.; Enderlein, J., Orientation imaging of single molecules by wide-field epifluorescence microscopy. JOSA B 2003, 20 (3), 554-559.

(4) Harmsen, S.; Wall, M. A.; Huang, R.; Kircher, M. F., Cancer imaging using surface-enhanced resonance Raman scattering nanoparticles. Nature Protocols 2017, 12 (7), 1400-1414.

CONCLUTION

In cellular tracking analysis of SERS nanorods in living mouse macrophages, the particle trajectory and the rotational dynamics were both recorded at super-resolution scale. The extreme photophysical stability of SERS nanoparticles enable stable observations over a long period. This opens up new windows for possible applications of SERS nanoparticles as a novel reagent in super-resolution microscopy and single particle analysis. This technique could also be highly valuable in screening nanotherapeutics for various diseases, as it could be used to study interactions between nanomaterials and various cells[4].

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