Electronic Supplementary Information

Single-Particle Spectroscopy and Defocused Imaging of Anisotropic Gold Nanorods by Total Internal Reflection Scattering Microscopy

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Experimental Methods

Simulation of Scattering Patterns of AuNRs

We used the simulation program developed by Enderlein and Böhmer[1]. The program is designed to calculate the characteristic intensity distribution from and emitter with three perpendicular emission dipoles of different emission strength. It has been widely used to determine the spatial orientation of single dye molecules[1, 2]. The simulation program is a special Matlab based utility with a graphics user interface (GUI) for easy calculation. This program allows us to calculate exactly the defocused (or focused) images of single molecules. For using the GUI, one should download the files from the website (https://www.uni-goettingen.de/de/imaging+of+single+molecules/512319.html).

The parmeters that can be input are : the numerical aperture of the objective lens, magnification of imaging, extent of defocusing (or defocusing distance in micrometers), κ and R. For defining the emission strength ratios of the three independent dipoles (Fig. S9), we input the parameter κ and R into the program. The ratio κ defines the ratio of the emission strength of the *b*- to *c*- dipole (transverse dipoles, Fig. S9) as shown below.

$$I_b/I_c = (1-\kappa)/(1+\kappa) \qquad \text{(Eq. 1)}$$

In this study the emission strength of the *b*- dipole is assumed to be same as that of the *c*-dipole. In addition, the ratio R defines the emission strength of the *a*- dipole (or longitudinal dipole) to the combined *b*- and *c*- dipoles (or transverse dipoles) as shown below.

$$R \times I_a + (1-R) \times (I_b + I_c) \qquad \text{(Eq. 2)}$$

When *R* is 1, we only have the contribution from a- dipole (longitudinal dipole) to the image patterns. However, the other two transverse dipole (b- and c-) start to contribute to the image patterns with decreasing the ratio *R*. That is, lower *R* values indicate more contributions from the two transverse dipoles. Therefore, we were able to calculate the scattering patterns of a AuNR by adjusting the important parameters.

Calculation of the Signal-to-Noise Ratios of Single AuNRs

The signal-to-noise ratios in the cross-sectional scattering intensity profiles (focused and defocused image patterns) of AuNRs were calculated as shown in Eq. 3 and Fig. S7. The signal was obtained by subtracting the average background intensity (I_b) from the maximum signal intensity (I_{max}). A noise was computed by taking the standard deviation of the background intensity (I_b). Therefore, the signal-to-noise ratio was calculated by Eq. 3.

$$\mathrm{S/N} = (I_{\mathrm{max}} - I_{\mathrm{b,ave}}) / I_{\mathrm{b,std}} \qquad \text{(Eq. 3)}$$

Calculation of Optical Extinction Spectra of AuNRs

We used Gans theory[3] with the known dielectric function for gold[2] to simulate the optical absorption spectra of randomly oriented AuNR colloids by varying the refractive index of surrounding medium (*n*). According to Gans theory, the extinction coefficient γ of randomly oriented particles in the dipole approximation is given by

$$\gamma = \frac{2\pi N V \varepsilon_m^{3/2}}{3\lambda} \sum_j \frac{(1/P_j^2)\varepsilon_2}{(\varepsilon_1 + \frac{1-P_j}{P_j}\varepsilon_m)^2 + \varepsilon_2^2} \qquad \text{(Eq. 4)}$$

N: the number of particles per unit volume

V: the volume of each particle

ε_{m}: the dielectric constant of the surrounding medium, ε_m is equal to n^2

λ: the wavelength of the interacting light

ε_1 and ε_2: the real and complex part of the material dielectric function

P_j: the depolarization factors for the three axes A, B, C of the rod with $A>B=C$

$$P_A = \frac{1-e^2}{e^2}\left[\frac{1}{2e}\ln\left(\frac{1+e}{1-e}\right)-1\right] \qquad \text{(Eq. 5)}$$

$$P_B = P_C = \frac{1-P_A}{2} \qquad \text{(Eq. 6)}$$

$$e = \sqrt{1-\left(\frac{B}{A}\right)^2} \qquad \text{(Eq. 7)}$$

Eq. 4 for γ was plotted as a function of n while the aspect ratio (A/B) of AuNRs was fixed to the values of 1.9 and 2.4 the average aspect ratios of two differently sized AuNRs in this study.

References

1. M. Böhmer and J. Enderlein, *Journal of the Optical Society of America B*, 2003, **20**, 554-559.
2. M. A. Lieb, J. M. Zavislan and L. Novotny, *Journal of the Optical Society of America B*, 2004, **21**, 1210-1215.
3. S. W. Prescott and P. Mulvaney, *Journal of Applied Physics*, 2006, **99**, 123504.

Supplementary Figures

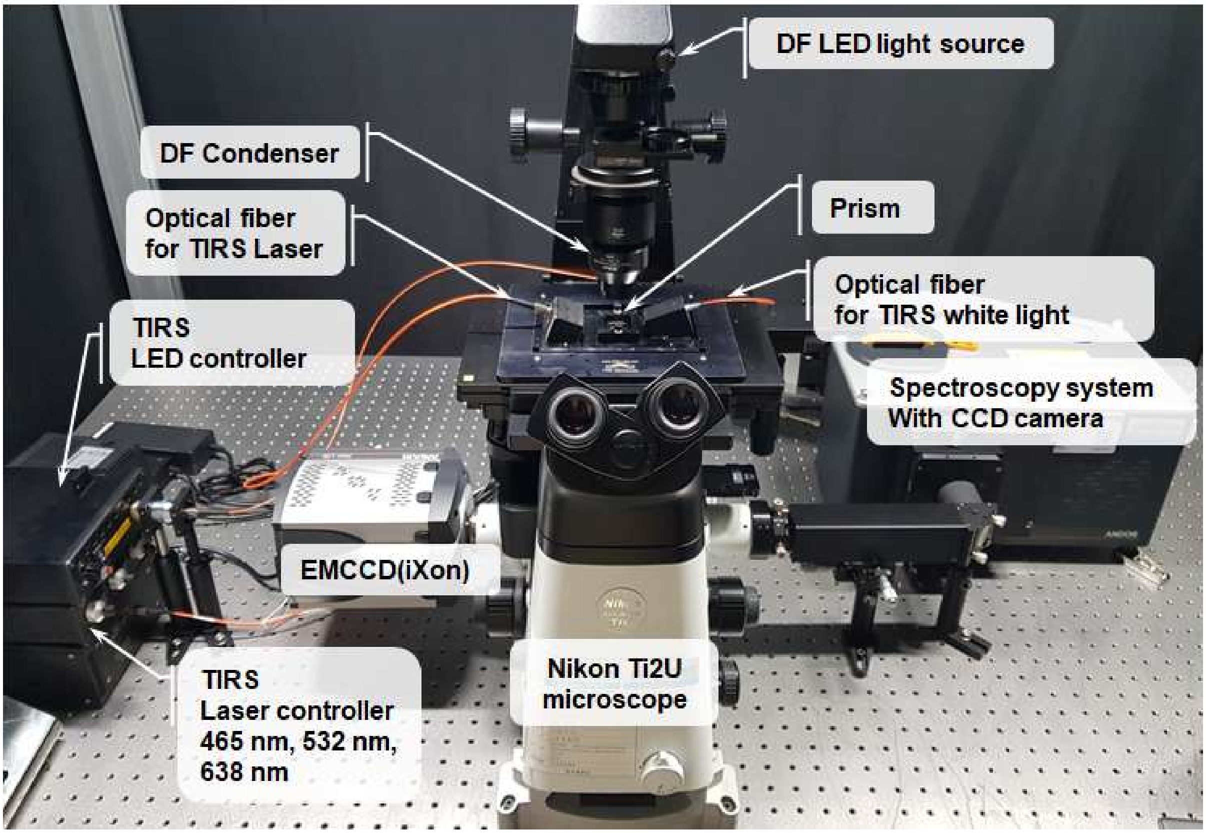

Fig. S1 A photograph to show inverted Nikon microscope for DF and TIRS microscopy.

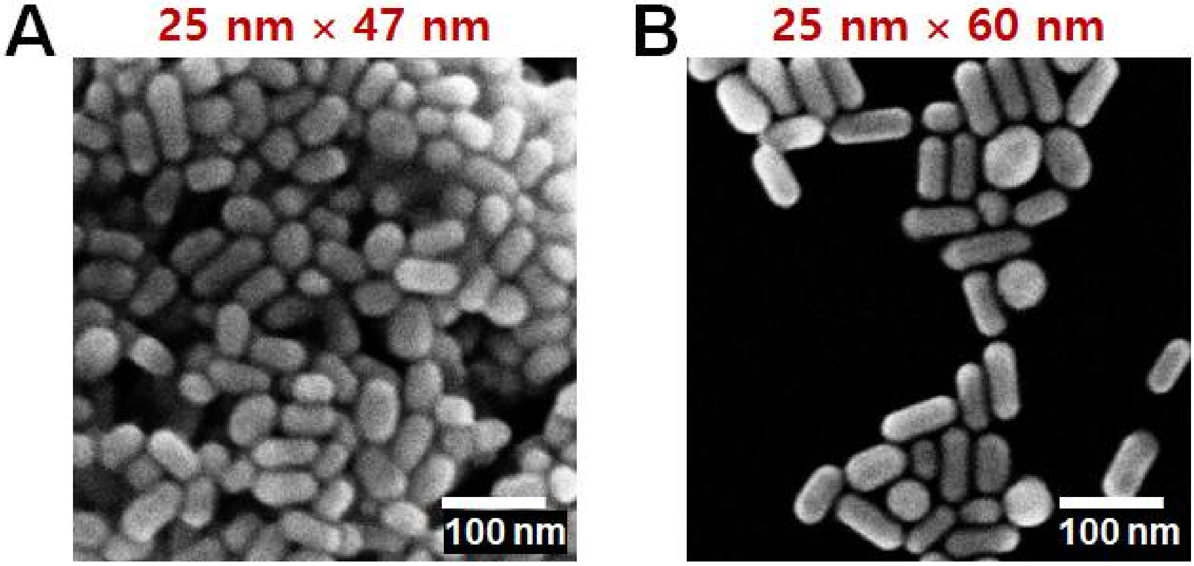

Fig. S2 (A, B) SEM images of AuNRs with two different sizes (25 nm × 47 nm, 25 nm × 60 nm) at the low magnification.

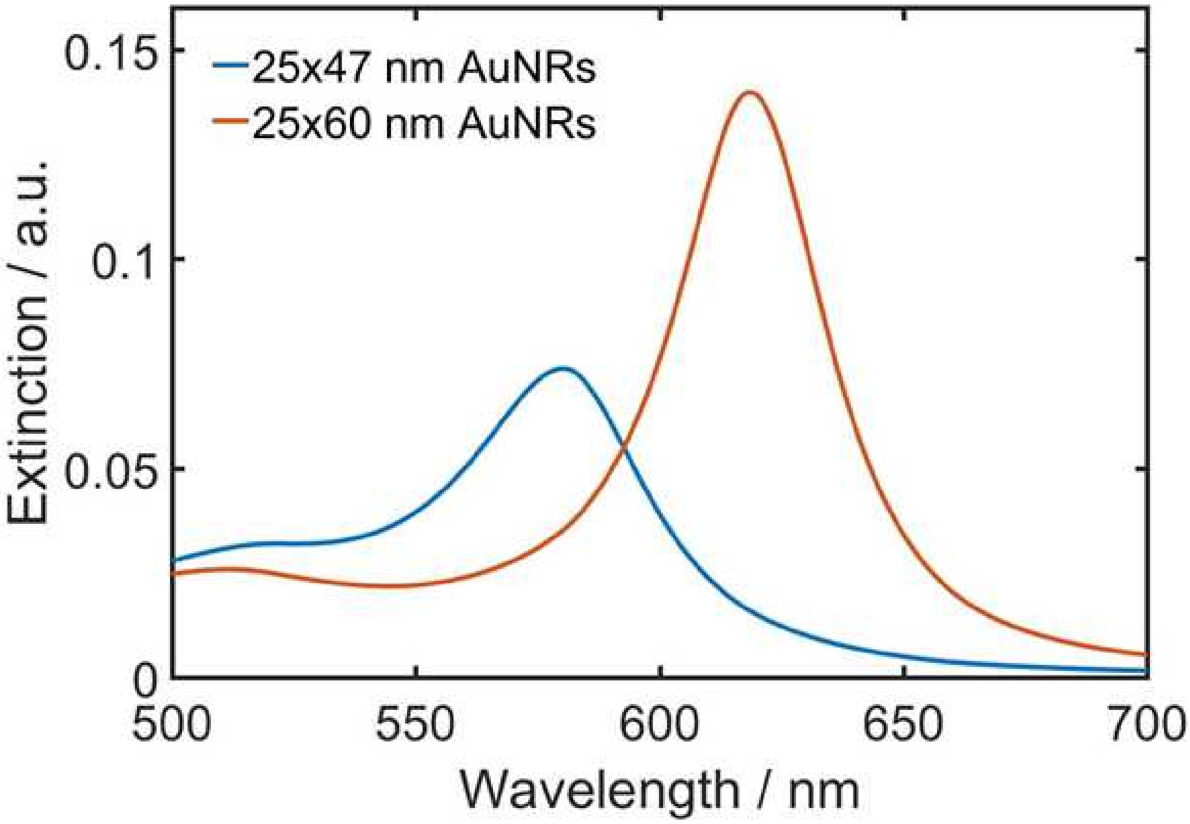

Fig. S3 Calculated extinction spectra of AuNRs with two different ARs (1.9, 2.4) in water.

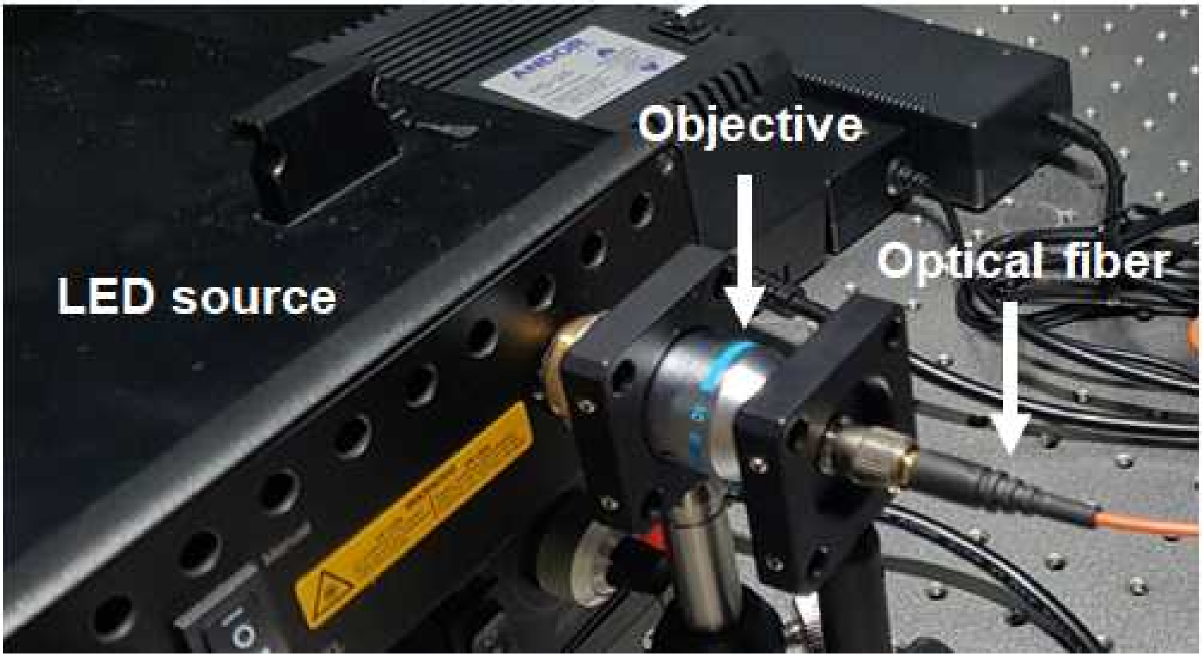

Fig. S4 A photograph showing the illumination of LED (white-light) in TIR microscopy. After the light emitted from the LED source passed through the 40x objective, it was focused on one end of the optical fiber.

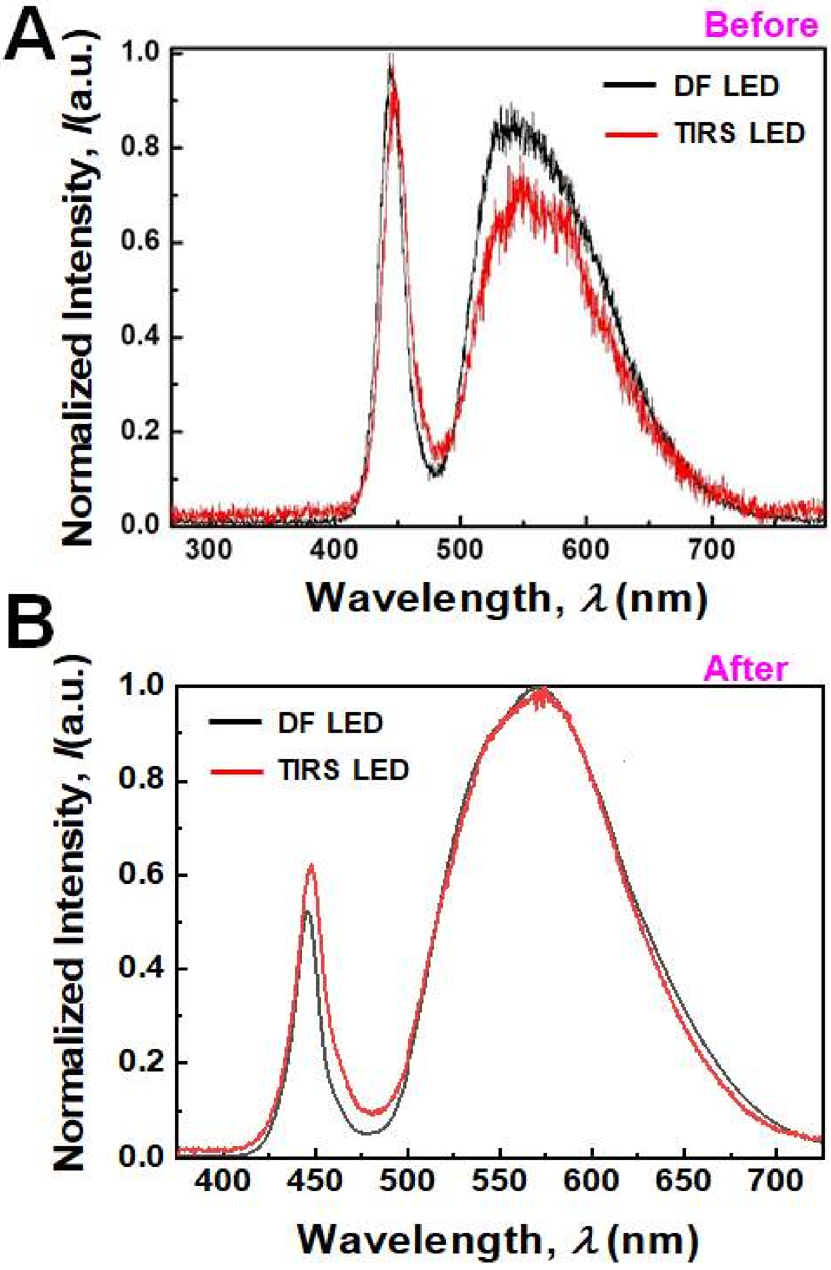

Fig. S5 Overlaid lamp spectra of two LED light sources used for DF and TIRS microscopy before **(A)** and after **(B)** the spectral calibration.

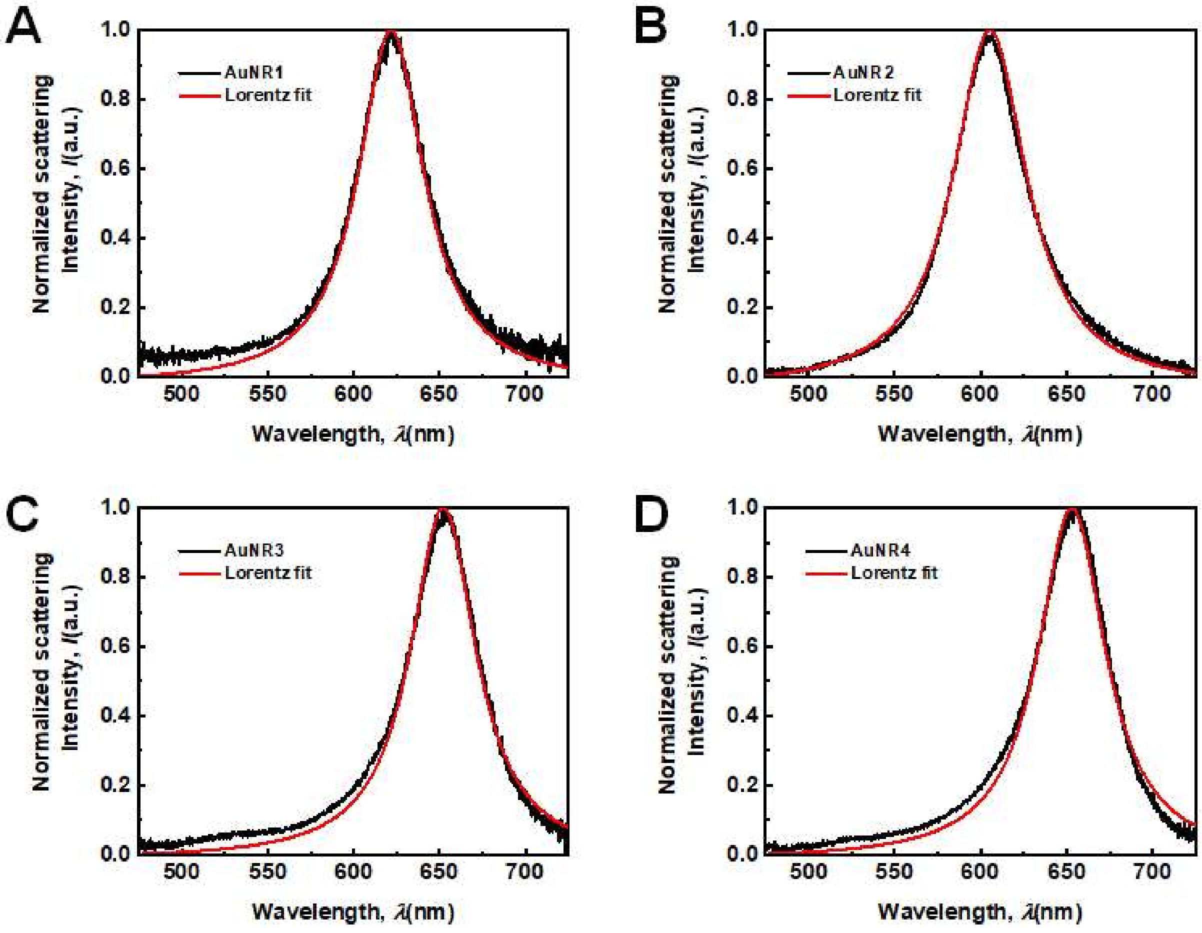

Fig. S6 (A-D) Single particle TIRS spectra of the four AuNRs in Figs. 4 and 5 fitted with the Lorentzian function. The experimental spectra (black curve) were fitted well by the Lorentzian function (red curve).

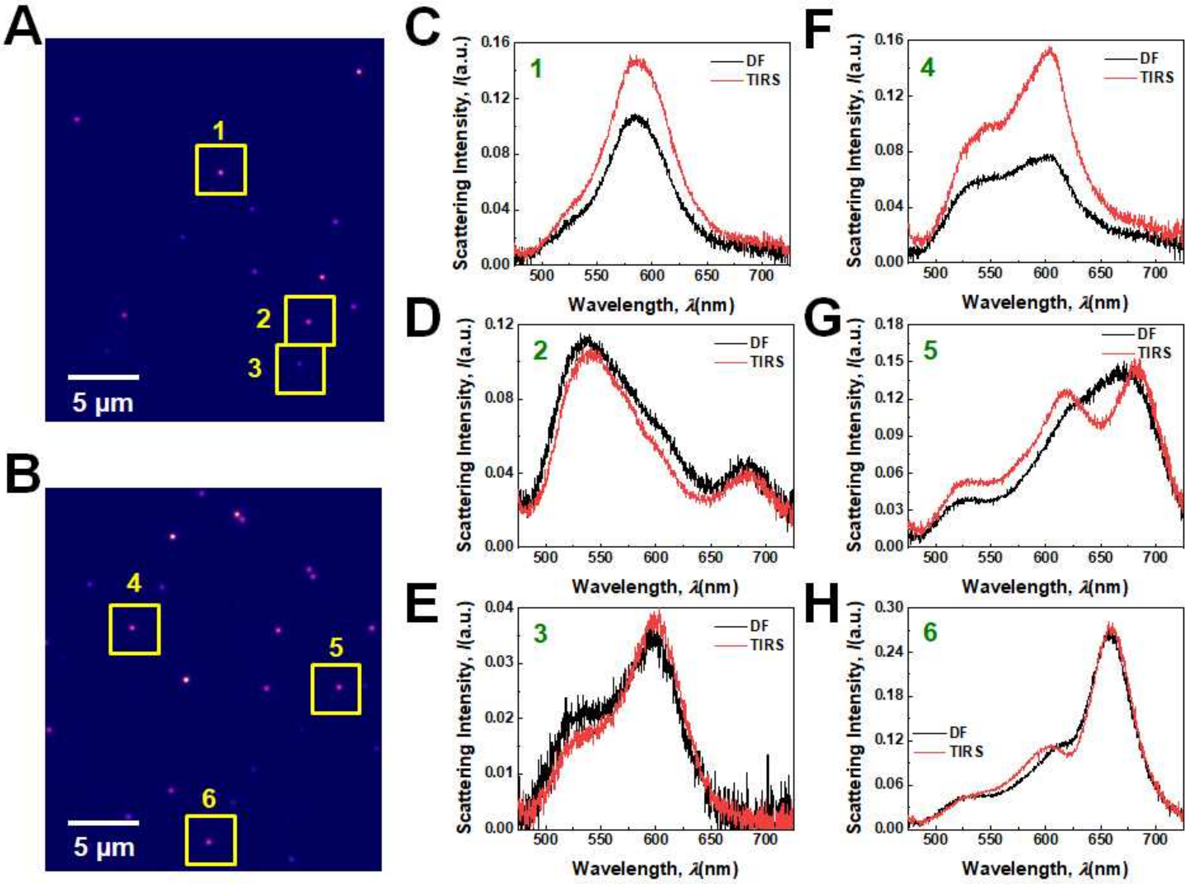

Fig. S7 (A) DF Scattering image of AuNRs with an average size of 25 nm × 47 nm. **(B)** DF Scattering image of AuNRs with an average size of 25 nm × 60 nm. **(C-H)** Scattering spectra of AuNRs squared in A and B. The scattering spectra having multiple broad peaks differ from the typical single particle scattering spectra showing a single LSPR peak for AuNRs.

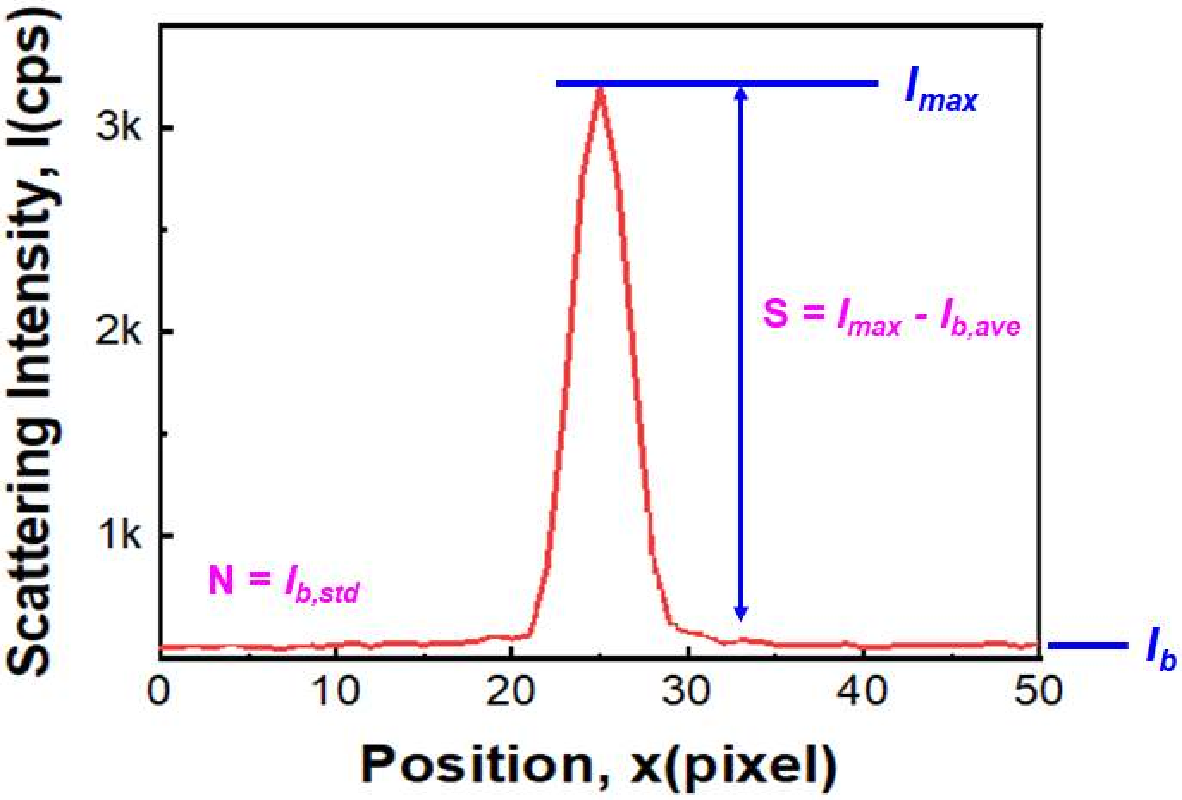

Fig. S8 Calculation of the signal-to-noise ratio (SNR) in the cross-sectional intensity profile of a AuNR.

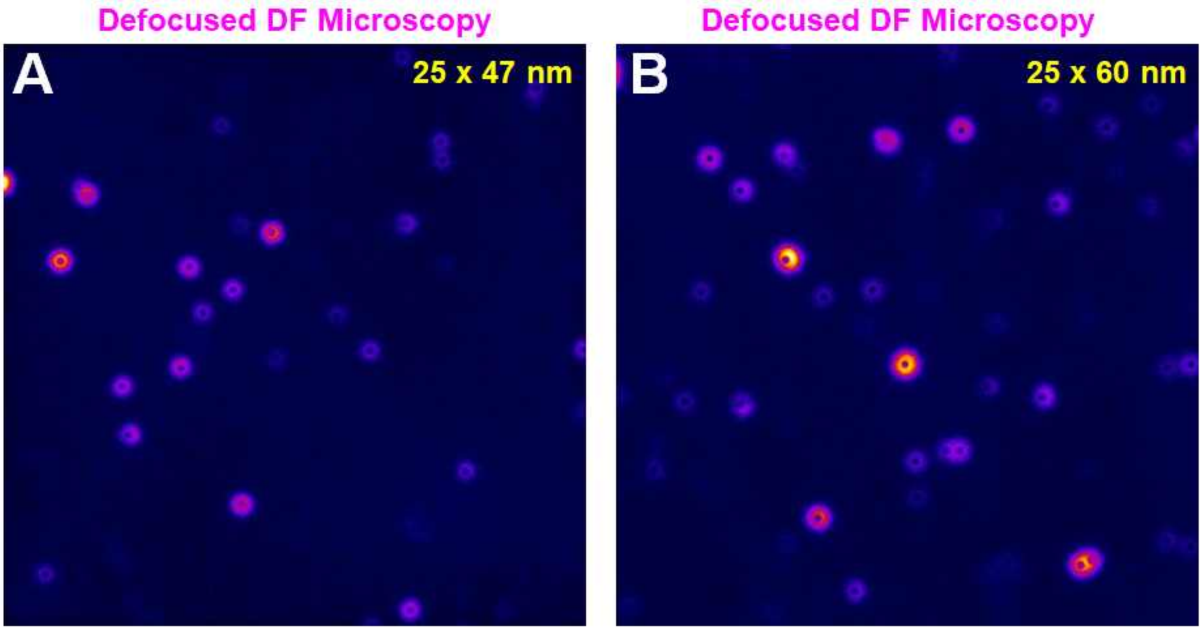

Fig. S9 (A) Defocused DF scattering image showing many AuNRs with an average size of 25 nm × 47 nm. **(B)** Defocused DF scattering image showing many AuNRs with an average size of 25 nm × 60 nm. The opening in the doughnut-shaped scattering pattern was observable for some AuNRs on the glass slide. CTAB and salts that may be present in the AuNR preparation can affect the attachment of AuNR to the glass surface and in-situ spatial heterogeneity.

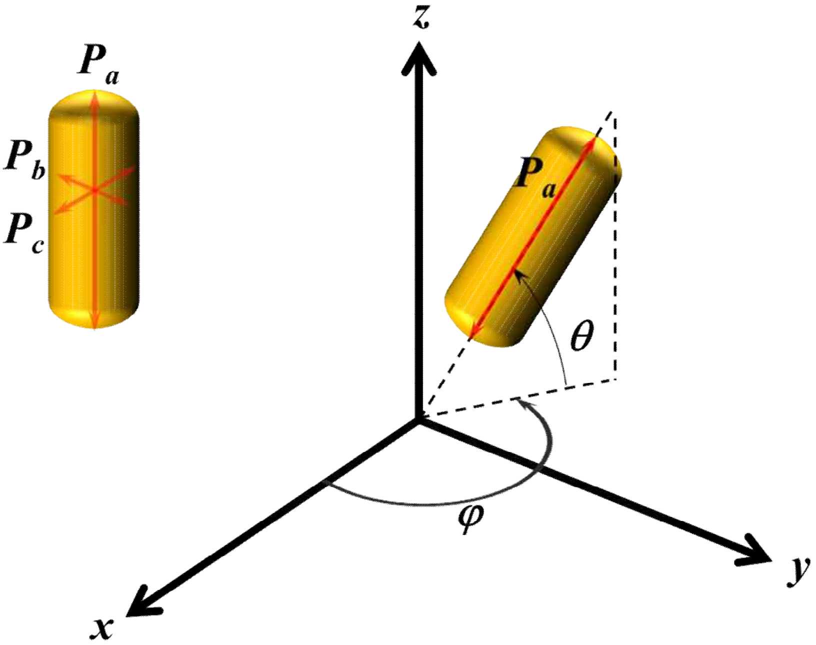

Fig. S10 Definition of azimuthal angle (φ) and polar angle (θ) of a AuNR in 3D space.