Electronic Supplementary Information

Characteristic Image Patterns of Single Anisotropic Plasmonic Nanoparticles Embedded in Gel Matrix

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This document contains experimental methods and additional supporting figures (Fig. S1 to S9).

Experimental Methods

1. Optical Microscopy

An upright Nikon Eclipse 80i microscope was used in this study. DIC microscopy consists of a set of two Normarski prisms, two polarizers, and a quarter-waveplate. The samples were illuminated through an oil immersion condenser (numerical aperture (NA) = 1.4), and, after passing through the sample, the signals were collected by a Plan Apo oil-immersion objective (100×, NA = 1.4). A bandpass filter with central wavelength of 700 nm (full width at half maximum: ±10 nm) was obtained from Thorlabs (Newton, NJ) and inserted into the microscope's beam path to illuminate the samples in our study. A Hamamatsu complementary metal-oxide-semiconductor (CMOS) camera was employed to record highly detailed DIC images of AuNRs. In DF mode, the microscope utilized a Nikon Plan Fluor 100× 0.5-1.3 oil iris objective and a Nikon DF condenser.

2. Sample Preparation

AuNRs with an average size of 25 nm × 73 nm were purchased from Nanopartz (Loveland, CO, USA). The AuNR colloid solution was first diluted with 18.2-MΩ pure water to a proper concentration. The diluted solution was then sonicated for 15 min at room temperature and mixed with a solution containing 1 μm microspheres. The microspheres were used as a position reference in this study and allowed us to find the same particles during the correlation study. In this study, the AuNRs are embedded in gel matrix. All gels were prepared by dissolving the desired quantity of gel powder (w/w, 0.5%) in distilled water by boiling for 5 min on a hotplate. We then added the mixed solution containing both AuNRs and micospheres into gels. A sample was prepared by spin casting the mixture on the well-cleaned glass slide, and the thickness of gel matrix was ~ nm. Then, a 22 mm × 22 mm No. 1.5 coverslip (Corning, NY) was covered on the glass slide for measurements under optical microscopy.

3. DF and DIC Imaging of AuNRs in Gel matrix

The sample glass slide was placed onto the microscope stge. The DF images of AuNRs under randomly-polarized white light illumination were obtained by using a motorized rotary stage from Sigma Koki (SGSP-60YAM) coupled to the fine-adjustment knob on the microscope. The motor was controlled by Intelligent Driver, CSG-602R (Sigma Koki). We scanned in the z-direction with a vertical step size of ~40 nm. We employed a Nikon Plan Fluor 100× 0.5-1.3 oil iris objective and a Nikon DF condenser for taking DF images for AuNRs. We then carried out DIC imaging for the same AuNRs at 700 nm by scanning in the z-direction that allows us to find the exact focal plane of AuNRs embedded in gel matrix. The DIC and DF images were recorded with the Hamamatsu CMOS camera. The collected images were analyzed with MATLAB and NIH ImageJ.

4. Simulation of Scattering Image Patterns of AuNRs in This Study

We used the simulation program developed by Enderlein and Böhmer.[1] The program is designed to calculate the characteristic intensity distribution from an 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 (http://www.joerg-enderlein.de/imagingOfSingleMolecules.html).

The parameters 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. 1A), we input the parameter κ and R into the program. The ratio κ defines the ratio of the emission strength of the b- to the c-dipole (transverse dipoles, Fig. 1A) as shown below.

$$I_b / I_c = (1-\kappa)/(1+\kappa)$$

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.

$$R \times I_a + (1-R) \times (I_b + I_c)$$

When R is 1, we only have the contribution from a-dipole (longitudinal dipole) to the image patterns. However, the other two transverse dipoles (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.

5. Preparation of Synthetic Lipid Bilayers on Glass Slides

The phospholipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC, Avanti Polar Lipids) solution in chloroform was first dried by a nitrogen steam and followed by at least 3 hr drying under vacuum at room temperature to remove the residual chloroform. The dried lipids were stored in a -20°C freezer.

Phosphate buffered saline (1× PBS, pH 7.4) was used to bring the final concentration to 0.5 mg/mL. The cloudy solution containing multilamellar vesicles was obtained after swelling in the PBS buffer solution for min with several times vortexing. The suspension solution was then forcing through a 100 nm pore size polycarbonate membrane at least 21 times to prepare the solution with large unilamellar vesicles using a mini-extruder (Avanti Polar Lipids, Alabaster, AL). The resulted solution was kept in a 4°C fridge.

The planar bilayer was formed by incubating the large unilamellar vesicles solution on a freshly cleaned glass slide in a chamber created by two double-sided tapes and a clean coverslip for 10 min. After that, PBS was used to remove the excess lipids. AuNR solution was then introduced into the chamber with the membrane for optical imaging.

References

1. M. Böhmer and J. Enderlein, *J. Opt. Soc. Am. B*, 2003, **20**, 554-559.
2. M. A. Lieb, J. M. Zavislan and L. Novotny, *J. Opt. Soc. Am. B*, 2004, **21**, 1210-1215.

Supplementary Figures

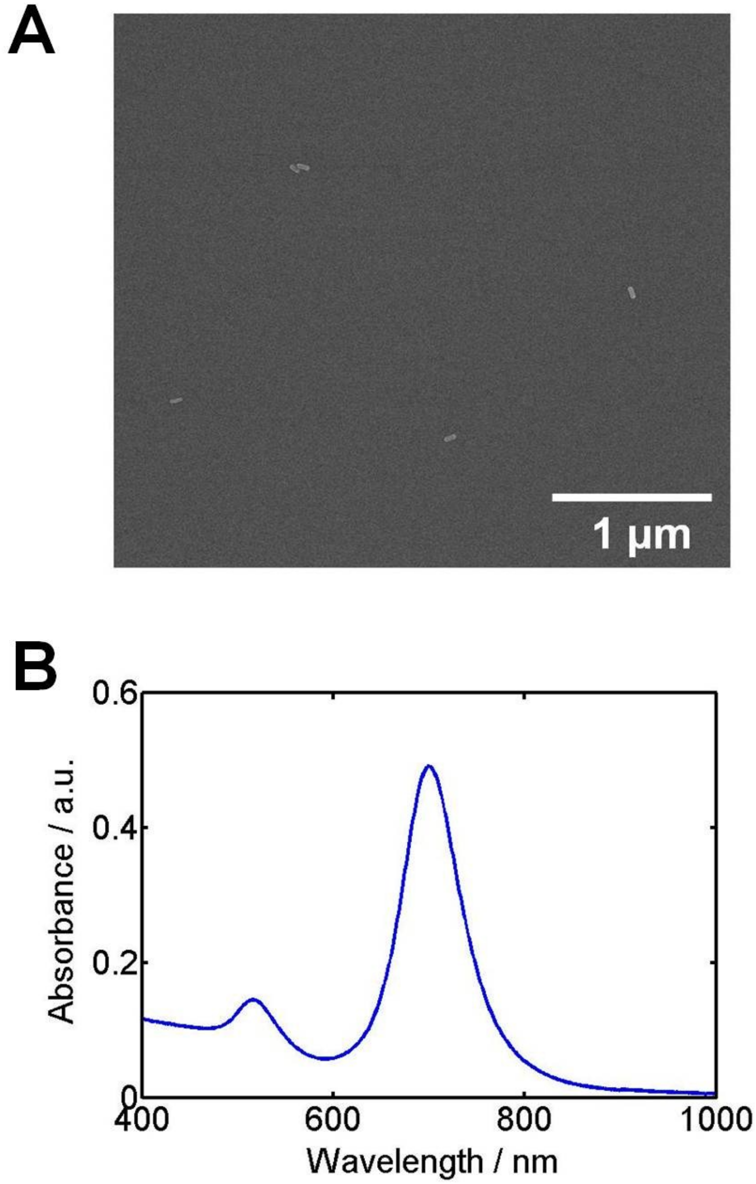

Fig. S1 SEM image (A) and a UV-Vis spectrum (B) of AuNRs used in this study. The UV-Vis spectrum of AuNRs were measured in water.

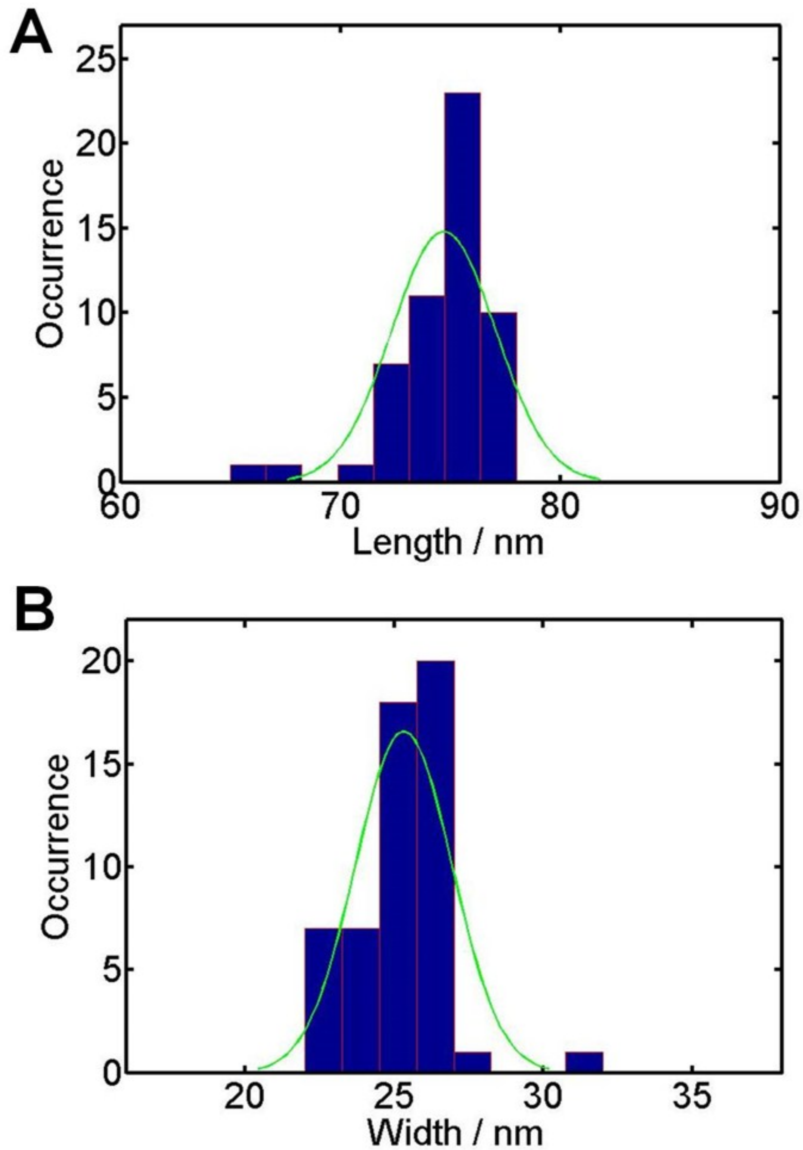

Fig. S2 Size distribution of AuNRs. The average length and width are 73.7 nm (±3.38 nm) and 25.4 nm (±1.65 nm), respectively.

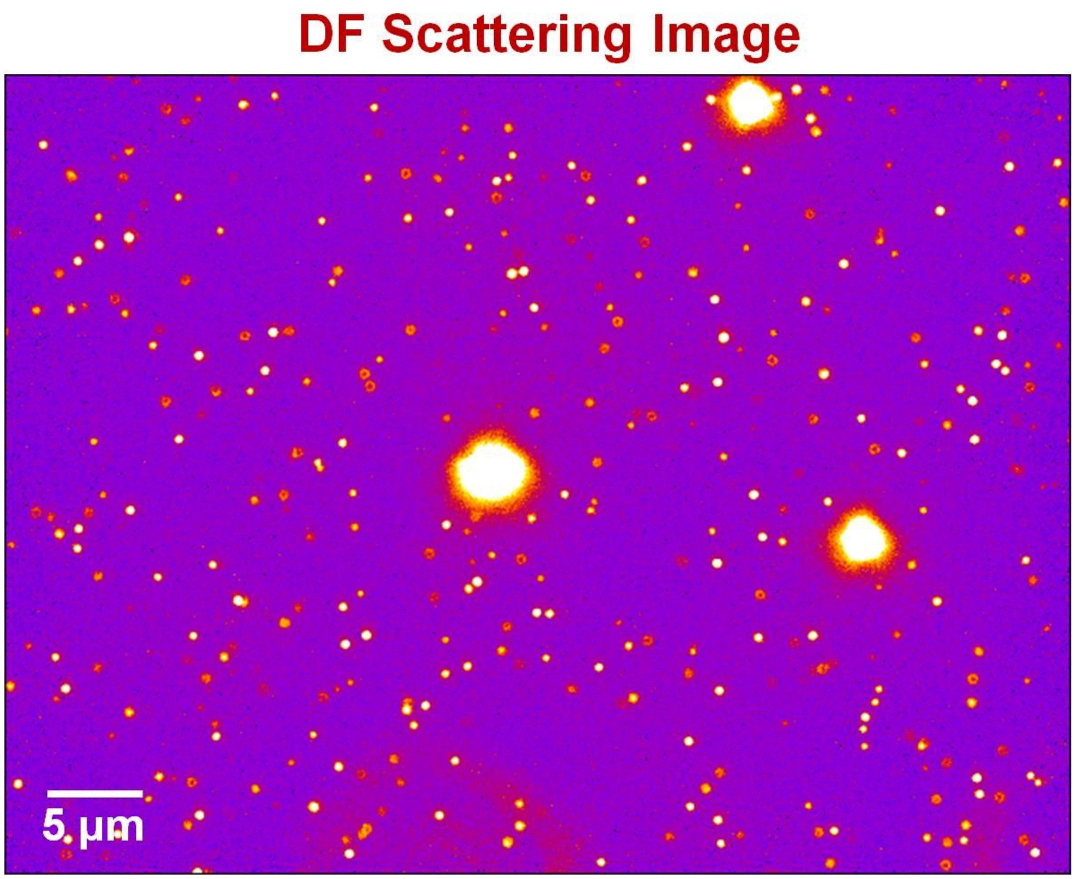

Fig. S3 DF scattering image of many AuNRs in gel matrix.

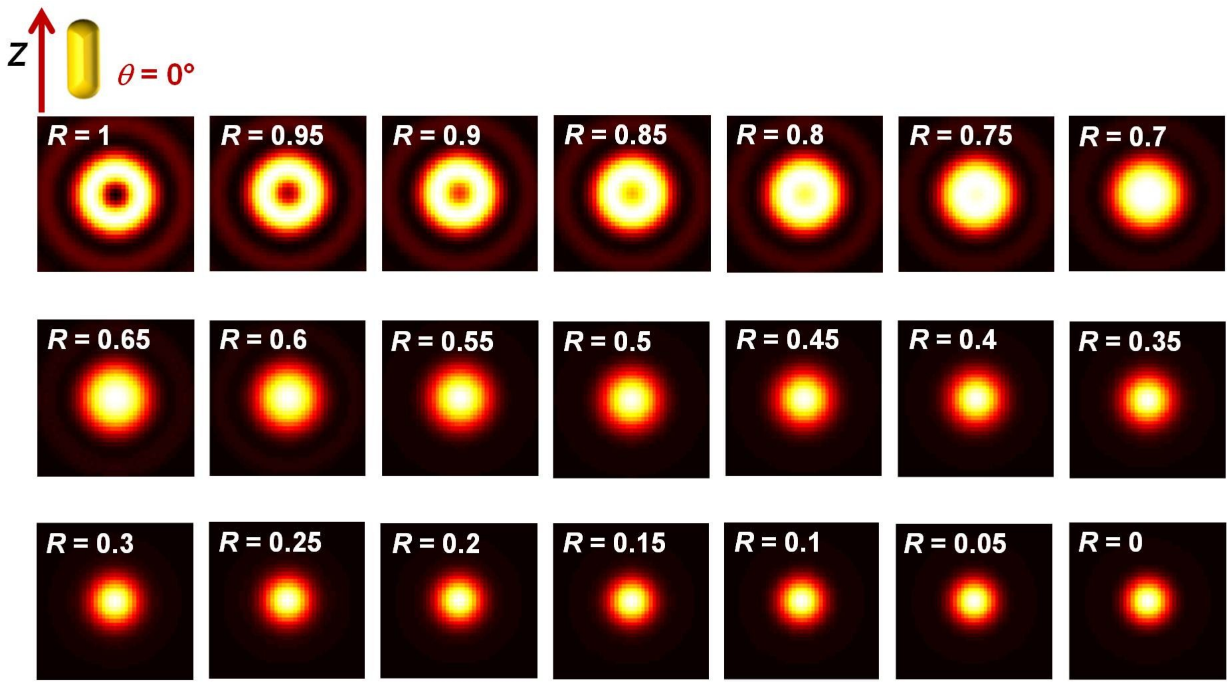

Fig. S4 The simulated scattering patterns of a AuNR by varying the parameter R from 1 to 0 in the focal plane. In this simulation, the polar angle θ of a AuNR was set to 0°. A doughnut-shaped scattering pattern is changed to a solid bright spot with decreasing the R value.

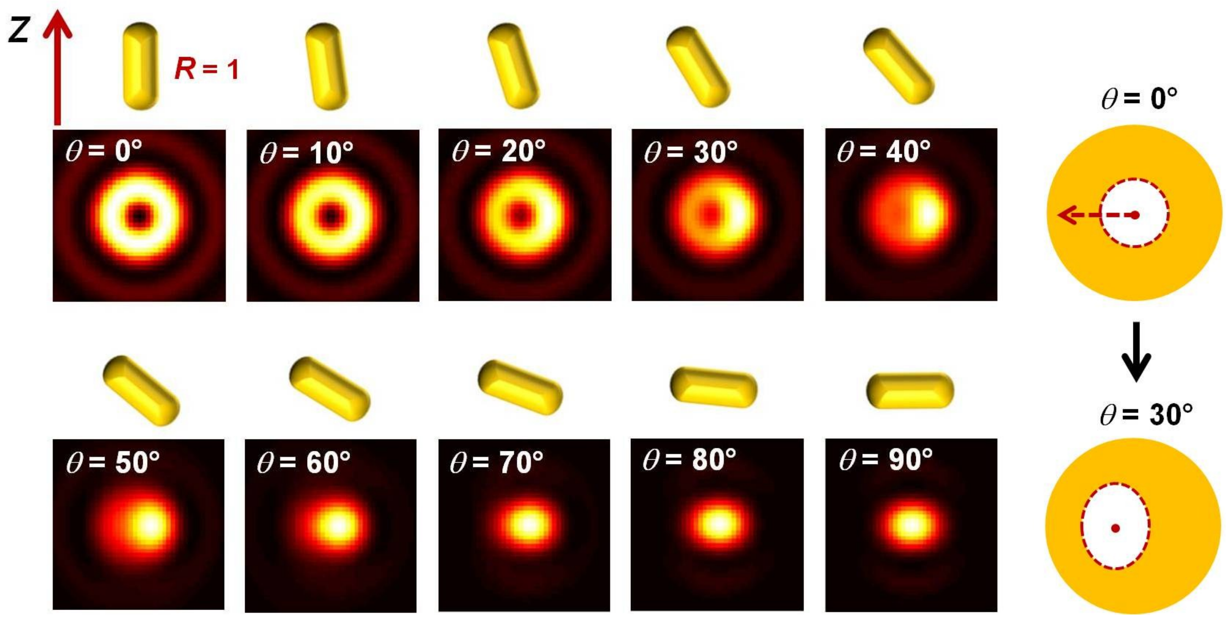

Fig. S5 The simulated scattering patterns of a AuNR by varying the polar angle *θ* of a AuNR from 0° to 90° at the fixed ratio *R* of 1 in the focal plane. It is found that the center moves toward the edge of a pattern with increasing the polar angle *θ* from 0° to °. In addition, a doughnut-shaped scattering pattern is changed to a solid bright spot with increasing the polar angle *θ*.

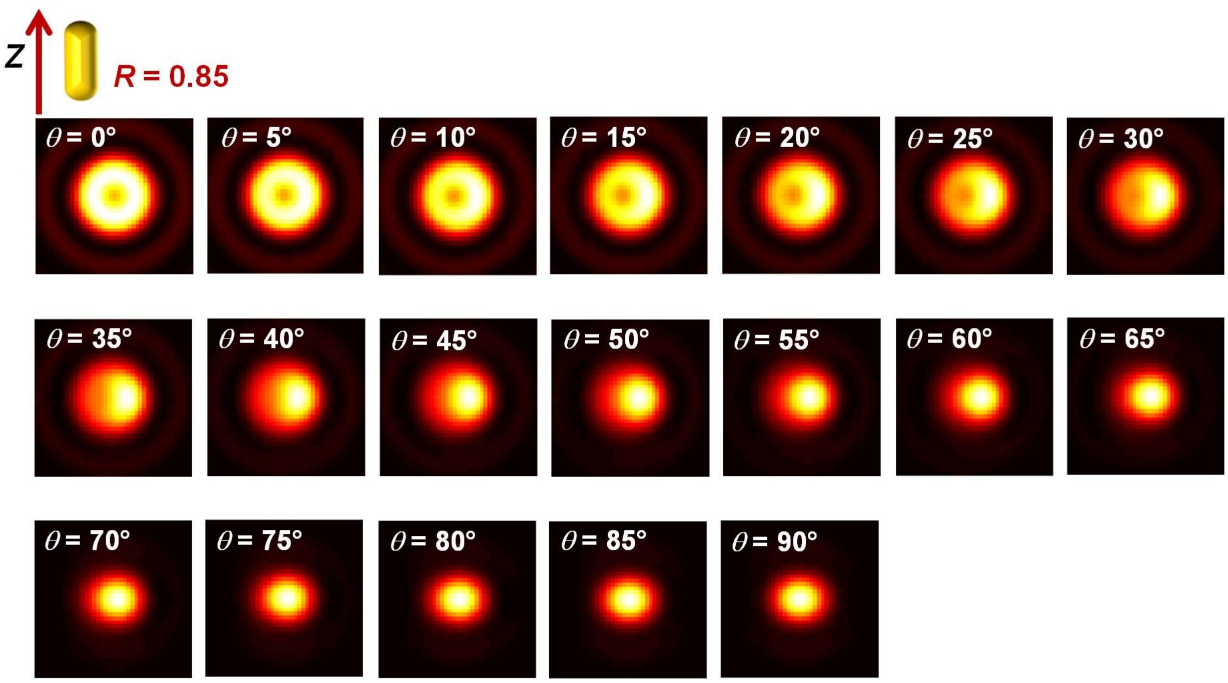

Fig. S6 The simulated scattering patterns of a AuNR by varying the polar angle θ of a AuNR from 0° to 90° at the fixed ratio *R* of 0.85 in the focal plane.

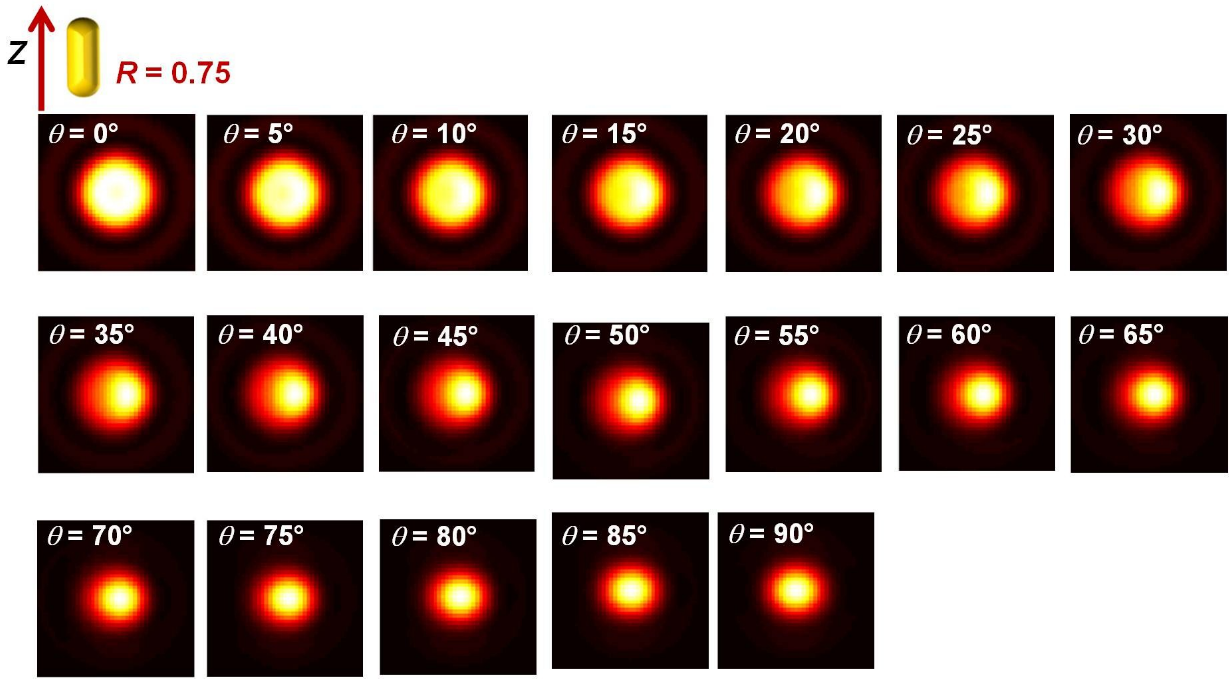

Fig. S7 The simulated scattering patterns of a AuNR by varying the polar angle θ of a AuNR from 0° to 90° at the fixed ratio R of 0.75 in the focal plane.

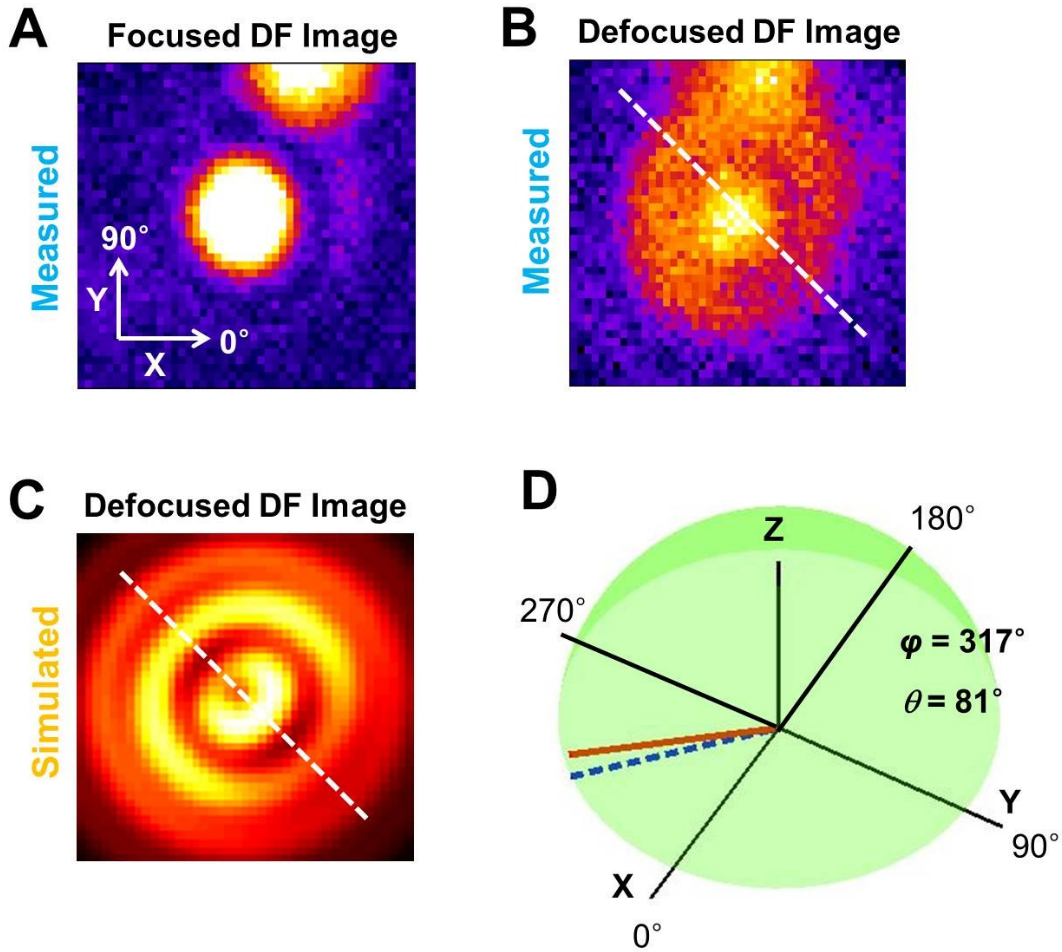

Fig. S8 (A) Focused DF image of single AuNR with a solid bright spot. (B) Defocused DF image at the defocusing distance of ~1 μm. (C) The corresponding best-fit simulated scattering pattern. The polar and azimuthal angles of the AuNR are estimated to be 81° and 317°, respectively. (D) The 3D illustration of the estimated orientation (φ=317° and θ=81°).

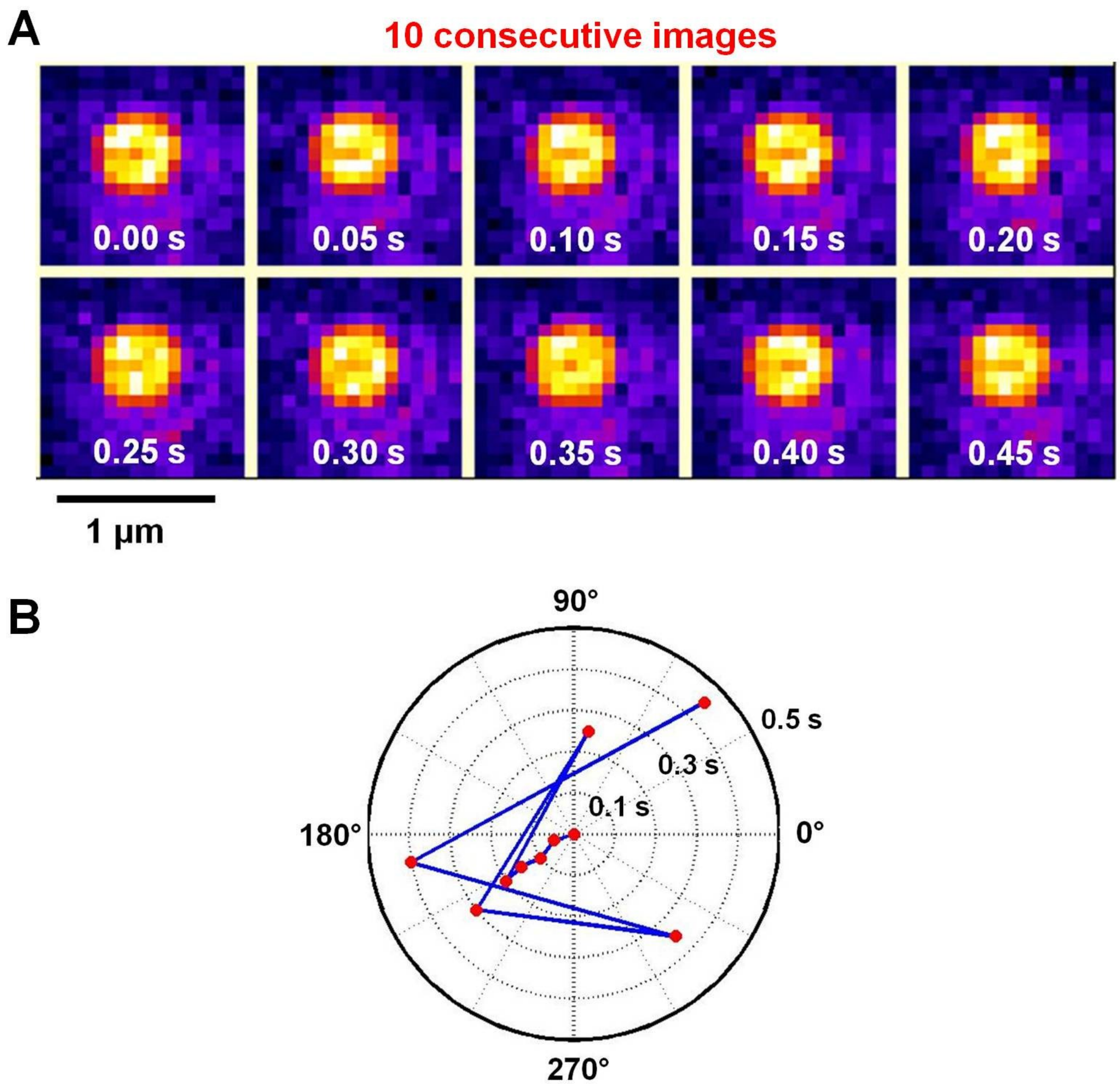

Fig. S9 Another example of direct observation of the orientation of a AuNR rotating on synthetic membrane. (A) 10-successive DF images of a AuNR are shown as a function of time. The temporal resolution is 50 ms. It is found that the doughnut-shaped patterns are changed as a function of time. (B) Polar graph to present the azimuthal angle of the AuNR as a function time for the image sequence of (A).