# Electronic Supplementary Information

## High-throughput In-focus Differential Interference Contrast Imaging of Three-dimensional Orientations of Single Gold Nanorods Coated with Mesoporous Silica Shell

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## Supplementary Movie

**Movie S1:** A movie showing DIC imaging of the embedded AuNRs (25 nm × 73 nm) measured by scanning in the z-direction with a vertical step size of 40 nm. The AuNRs have different focal planes in 2% agarose gel matrix.

# Experimental Section

### Preparation of Gold Nanorods

The AuNRs (10 nm × 30 nm) were synthesized based on a previously reported method.[1] The stock solution as seed was synthesized by mixing 250 μL of 10 mM $HAuCl_4$ with 7.5 mL of 100 mM CTAB solution. Then, 10 mM of $NaBH_4$ solution was prepared as reductant with fresh ice-cold water. A 600 μL of reducing solution was injected into the above gold, CTAB mixture under vigorous stirring for 2 min. After that, seed solution was aged for 1 hour under room temperature, undisturbed condition. For further growth of seed, we used aromatic additives, 2,6-dihydroxybenzoic acid. The growth solution was prepared by mixing 24 mL of 100 mM CTAB solution with 2 mL of 67 mM 2,6-dihydroxybenzoic acid solution. After complete dissolution of 2,6-dihydroxybenzoic acid solution, 170 μL of 10 mM $AgNO_3$ solution and 1 mL of 10 mM $HAuCl_4$ solution were added into the growth solution, respectively and stirred for 10 min. Subsequently, 140 μL of 100 mM L-Ascorbic acid solution were added. Finally, 600 μL of above seed solution was added into the growth solution. The final mixture was stirred for 30 s and left undisturbed at 27°C for 12 hours. The synthesized AuNRs were washed to remove excessive surfactant and unreacted chemicals using centrifuge.

### Preparation of Gold Nanorods Encapsulated with Mesoporous Silica

Mesoporous silica coating on AuNRs was performed according to the previously reported method with slight modification.[2-3] The proper concentration for the encapsulation, concentration of AuNRs and CTAB was 1.2 nM and 1.2 mM respectively. The mesoporous silica shell coating was conducted under basic condition to induce sol-gel reaction. The 30 μL of 100 mM of

NaOH solution was added into 10 mL of above gold nanorod solution. After 15 min, 20 μL of 20 v/v% TEOS diluted with methanol was added 10 times with 1 h intervals under magnetic stirring. Subsequently, another 30 μL of 100 mM NaOH solution was added and 20 μL of 20 v/v% TEOS diluted with methanol was added 10 times more. The mixture was aged for 24 h and washed three times with ethanol using centrifuge. The concentration of AuNRs was measured as previously reported.[4]

**Rotational Study under DIC Microscopy**

The sample glass slide was placed on a 360° rotating mirror holder affixed onto the microscope stge. By rotating the mirror holder 10° per step, the AuNRs@m$SiO_2$ were positioned in different orientations. DIC images at 750 nm were taken with the Andor EMCCD camera. The corresponding bandpass filter (750/±5 nm) were inserted in the beam path. The collected images were analyzed with MATLAB and NIH ImageJ.

**Measurement of AuNRs Embedded in Gel Matrix under DIC Microscopy**

In this study, the AuNRs without shell (25 nm × 73 nm) embedded in gel matrix were measured under DIC microscopy. All gels were prepared by dissolving the desired quantity of gel powder (w/w, 2%) in distilled water by boiling for 5 min on a hotplate. We then added the solution containing AuNRs into gels. A sample was prepared by spin casting the solution on the well-cleaned glass slide. Then, a 22 mm × 22 mm No. 1.5 coverslip (Corning, NY) was covered on the glass slide for measurements under optical microscopy. The DIC 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. The DIC images were recorded with Andor iXonEM+ CCD camera (iXon Ultra 897). The collected images were analyzed with MATLAB and NIH ImageJ.

# References


1. Yoon, S.; Lee, B.; Yun, J.; Han, J. G.; Lee, J.-S.; Lee, J. H., Systematic study of interdependent relationship on gold nanorod synthesis assisted by electron microscopy image analysis. *Nanoscale* **2017,** *9* (21), 7114-7123.
2. Yoon, S.; Lee, B.; Kim, C.; Lee, J. H., Controlled Heterogeneous Nucleation for Synthesis of Uniform Mesoporous Silica-Coated Gold Nanorods with Tailorable Rotational Diffusion and 1 nm-Scale Size Tunability. *Crystal Growth & Design* **2018,** *18* (8), 4731-4736.
3. Gorelikov, I.; Matsuura, N., Single-Step Coating of Mesoporous Silica on Cetyltrimethyl Ammonium Bromide-Capped Nanoparticles. *Nano Letters* **2008,** *8* (1), 369-373.
4. Orendorff, C. J.; Murphy, C. J., Quantitation of Metal Content in the Silver-Assisted Growth of Gold Nanorods. *The Journal of Physical Chemistry B* **2006,** *110* (9), 3990-3994.

# Supplementary Figures

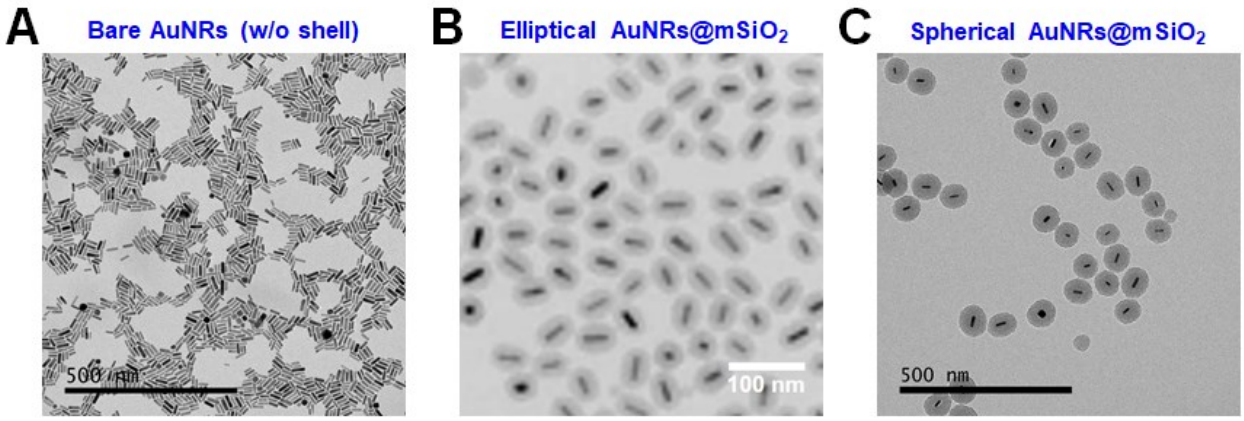


**Fig. S1** TEM images of **(A)** AuNRs without shell, **(B)** AuNRs coated with elliptical silica shell, and **(C)** AuNRs coated with mesoporous silica shell (AuNRs@$mSiO_2$).

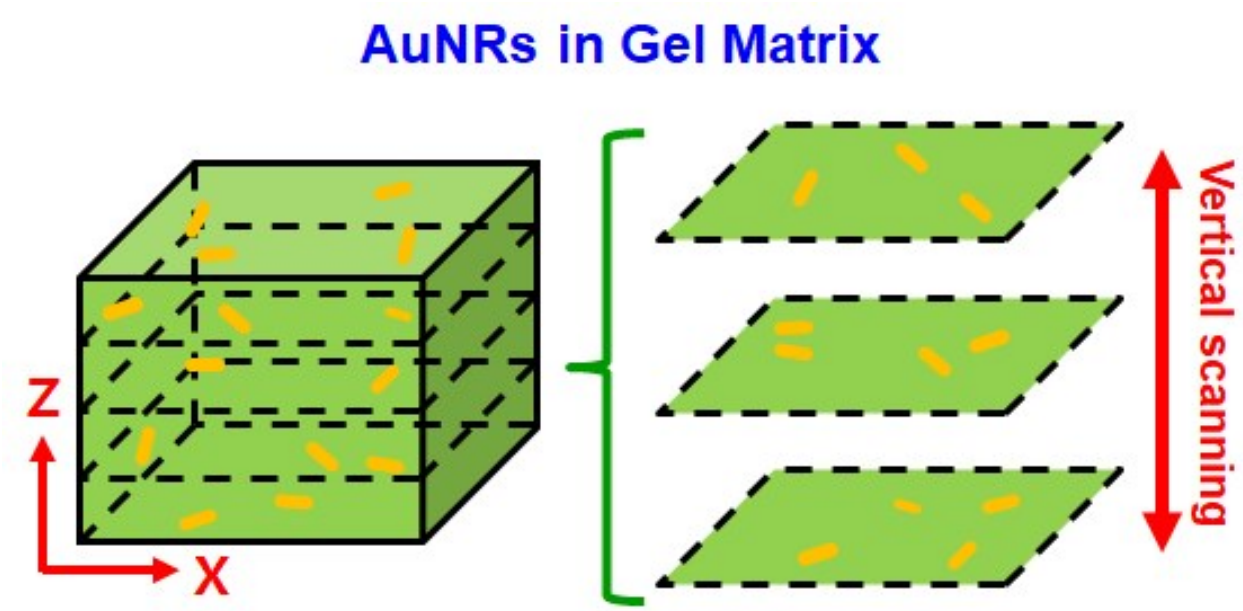


**Fig. S2** Schematic to show AuNRs embedded in a gel matrix at the different focal planes. The embedded AuNRs are measured by vertical scanning in the z-axis under DIC microscopy.

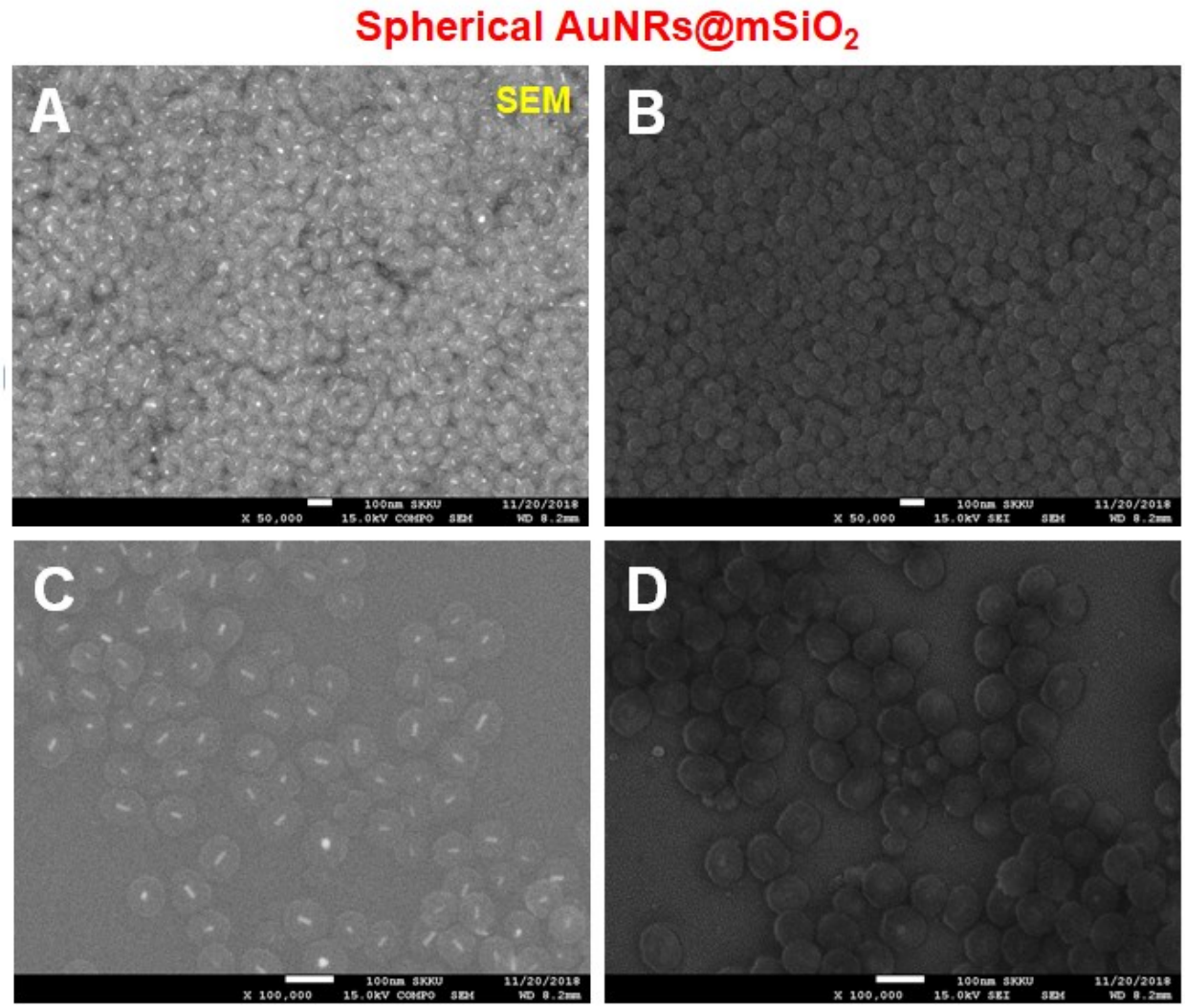


**Fig. S3 (A-D)** SEM images showing single AuNRs with random 3D orientations inside the spherical silica shell. Enlarged SEM images show the random orientations of AuNR cores in the shell (**C, D**).

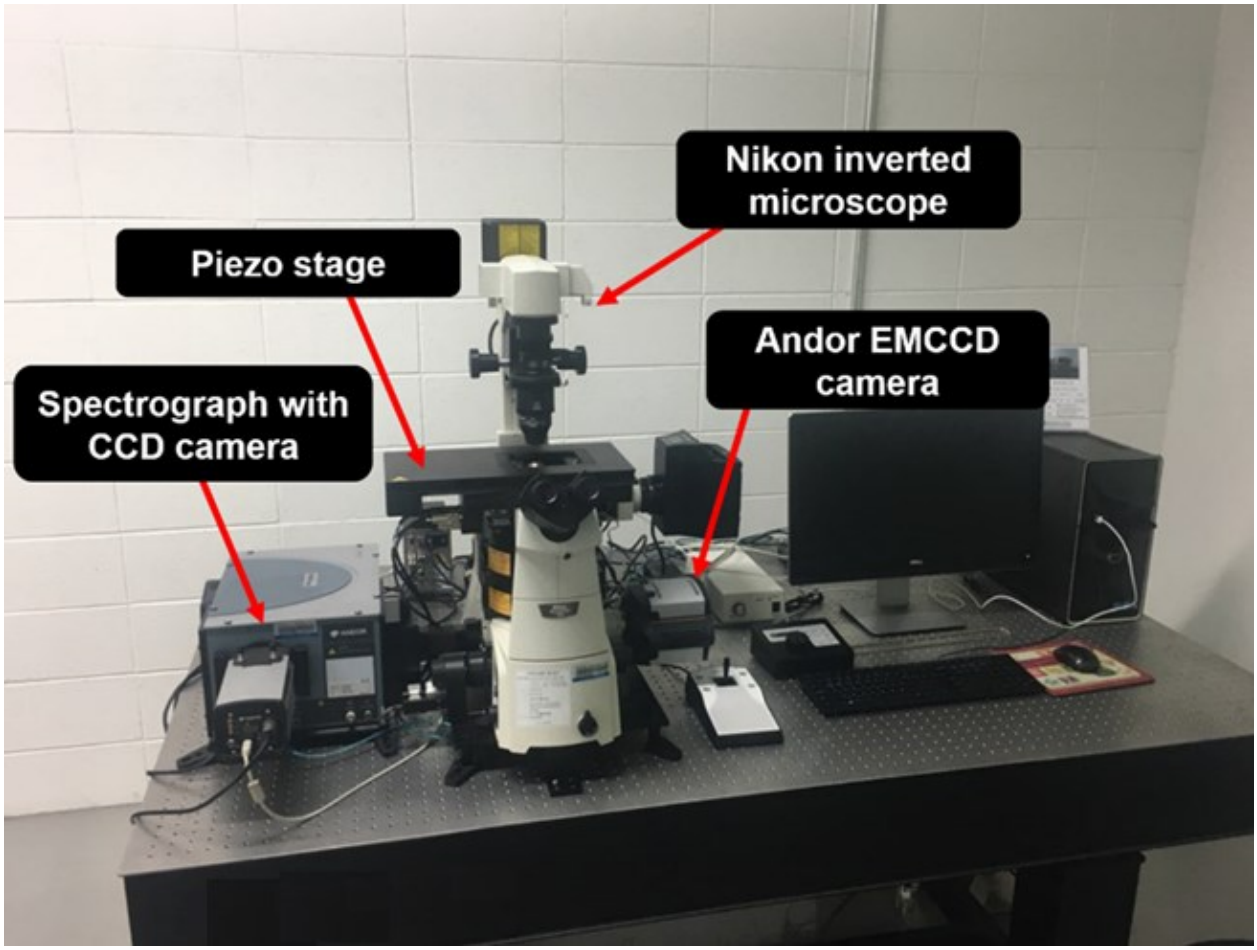


**Fig. S4** Photograph to show the experimental setup for single particle microscopy and spectroscopy.

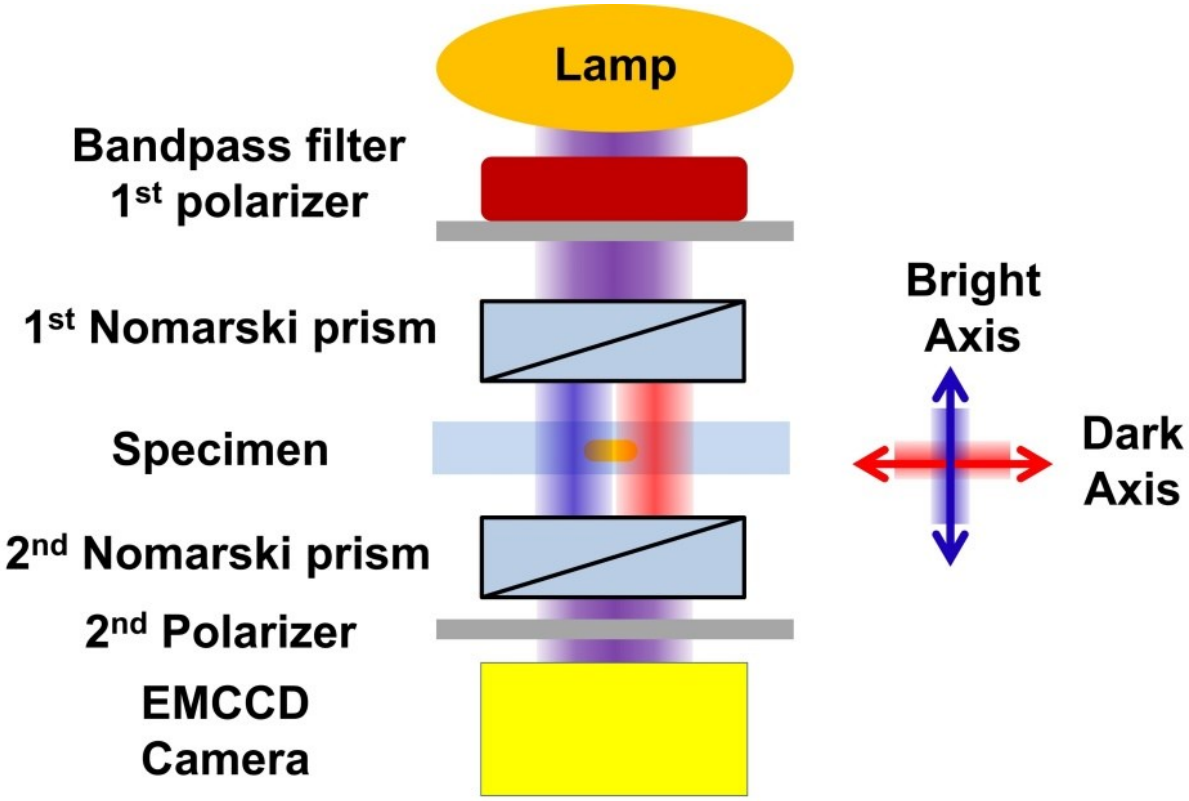


**Fig. S5** Schematic to depict the working principle of Nomarski-based DIC microscopy.

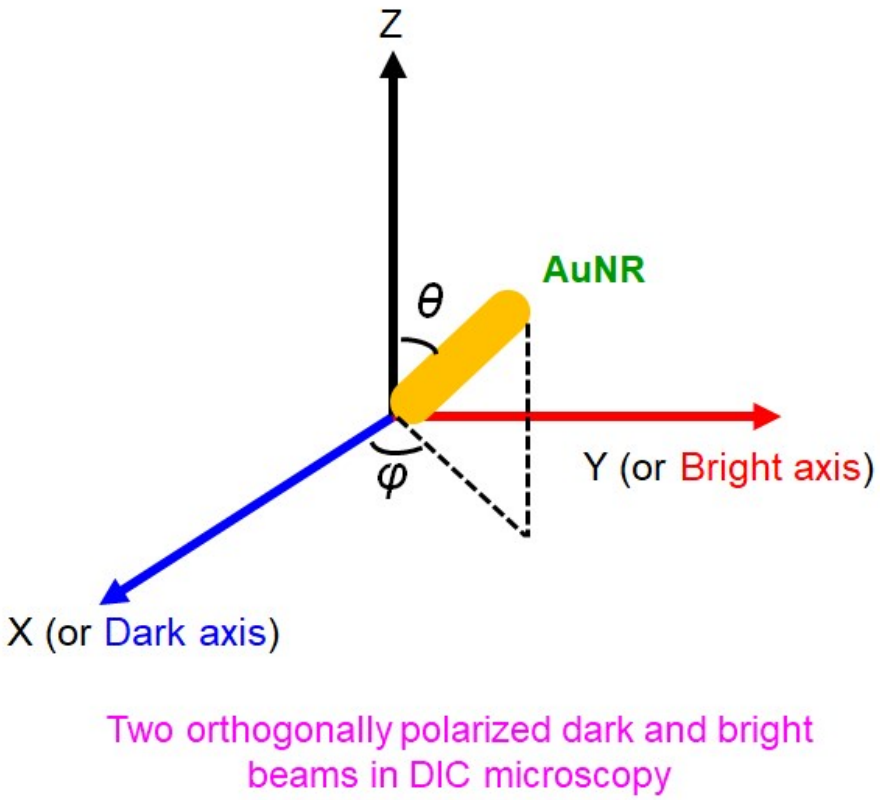


**Fig. S6** Definitions of the polar angle $\theta$ and the azimuthal angle $\varphi$ of a AuNR in 3D space under DIC microscopy with two orthogonally polarized dark and bright beams.

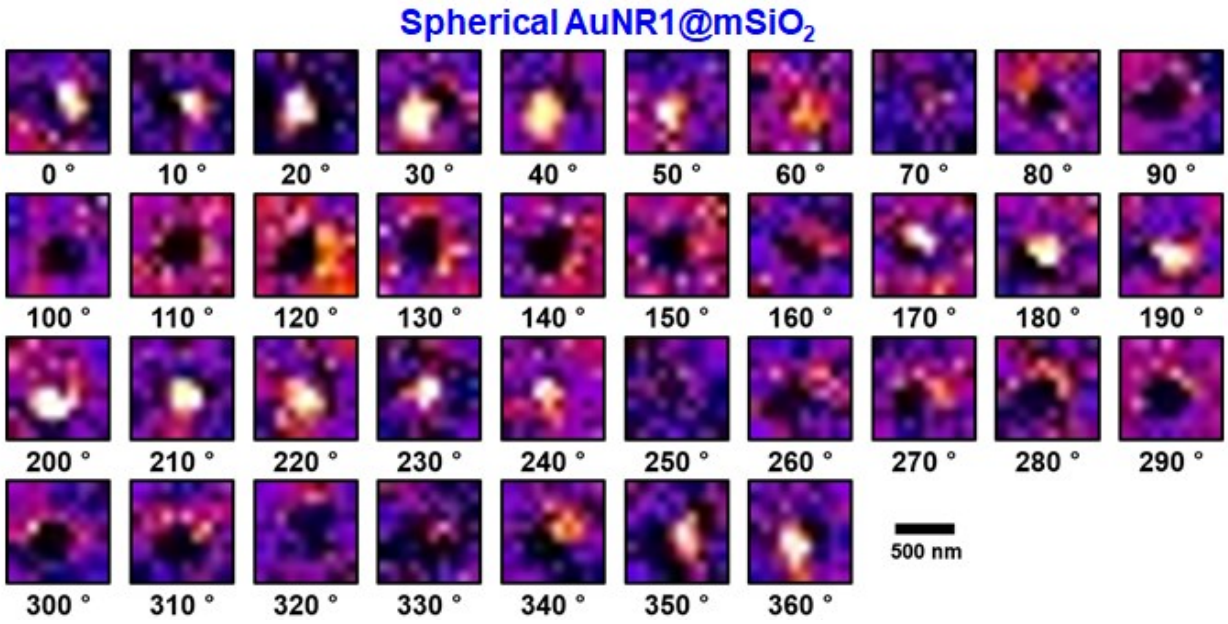


**Fig. S7** Complete set of DIC images of spherical AuNR1@$mSiO_2$ as a function of rotational angle with 10° increment from 0° to 360°.

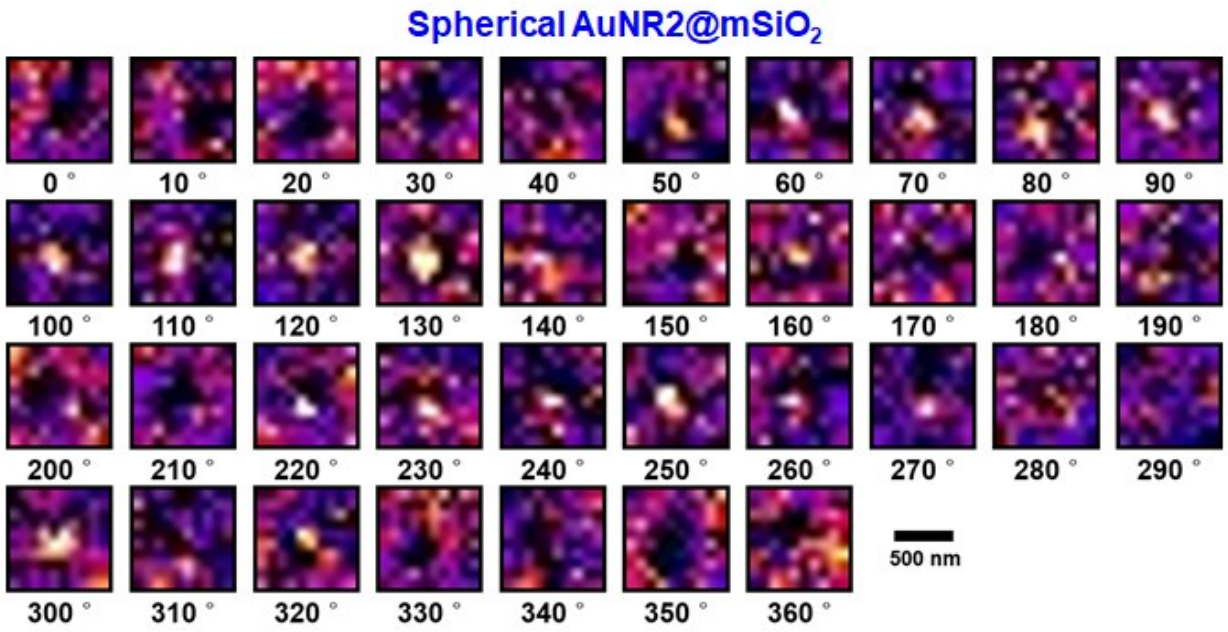


**Fig. S8** Complete set of DIC images of spherical AuNR2@mSiO$_2$ as a function of rotational angle with 10° increment from 0° to 360°.

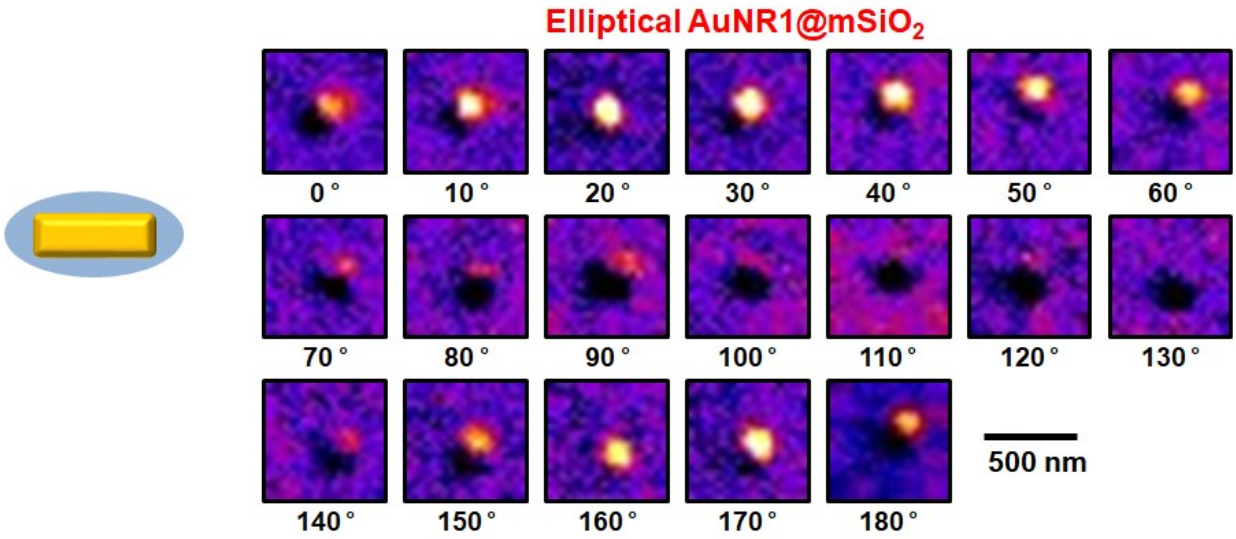


**Fig. S9** DIC images of elliptical AuNR1@$mSiO_2$ as a function of rotational angle with 10° increment from 0° to 180°.

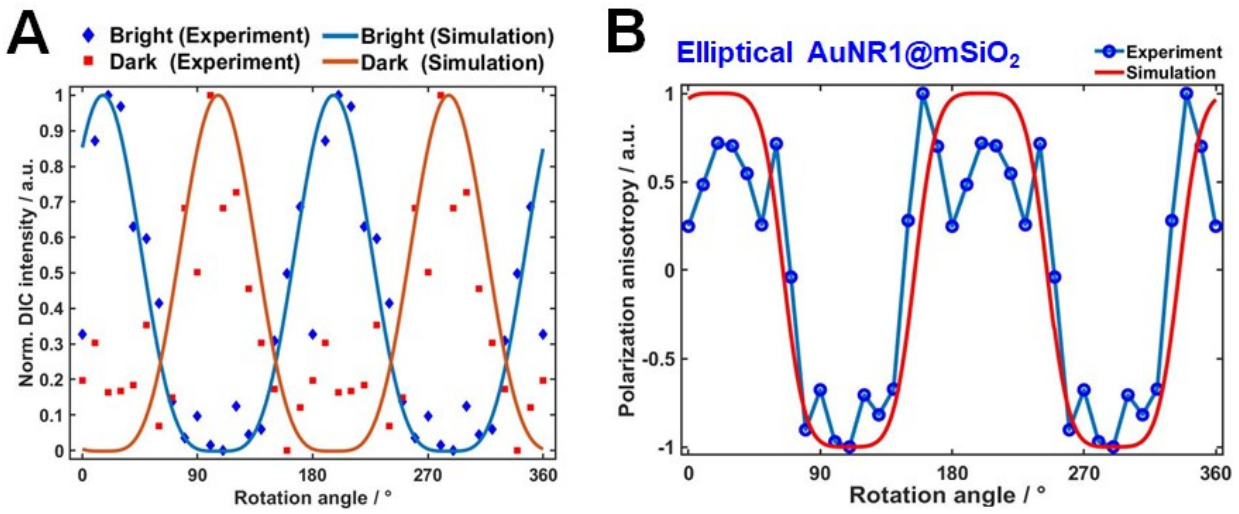


**Fig. S10 (A)** Normalized DIC intensities of elliptical AuNR1@mSiO$_2$ in Fig. S9 as a function of rotational angle. **(B)** Change in the DIC polarization anisotropy calculated from two dark and bright intensities in (A) for the elliptical AuNR1@mSiO$_2$.

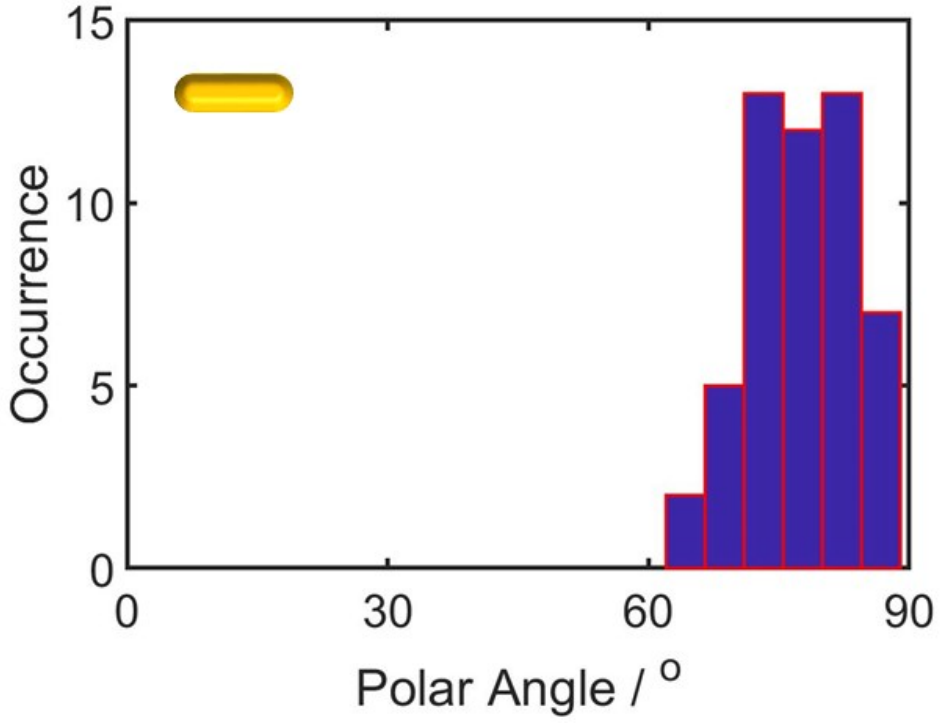


**Fig. S11** Histogram showing the polar angle distribution of bare AuNRs without the mesoporous silica shell, determined from the DIC measurement.

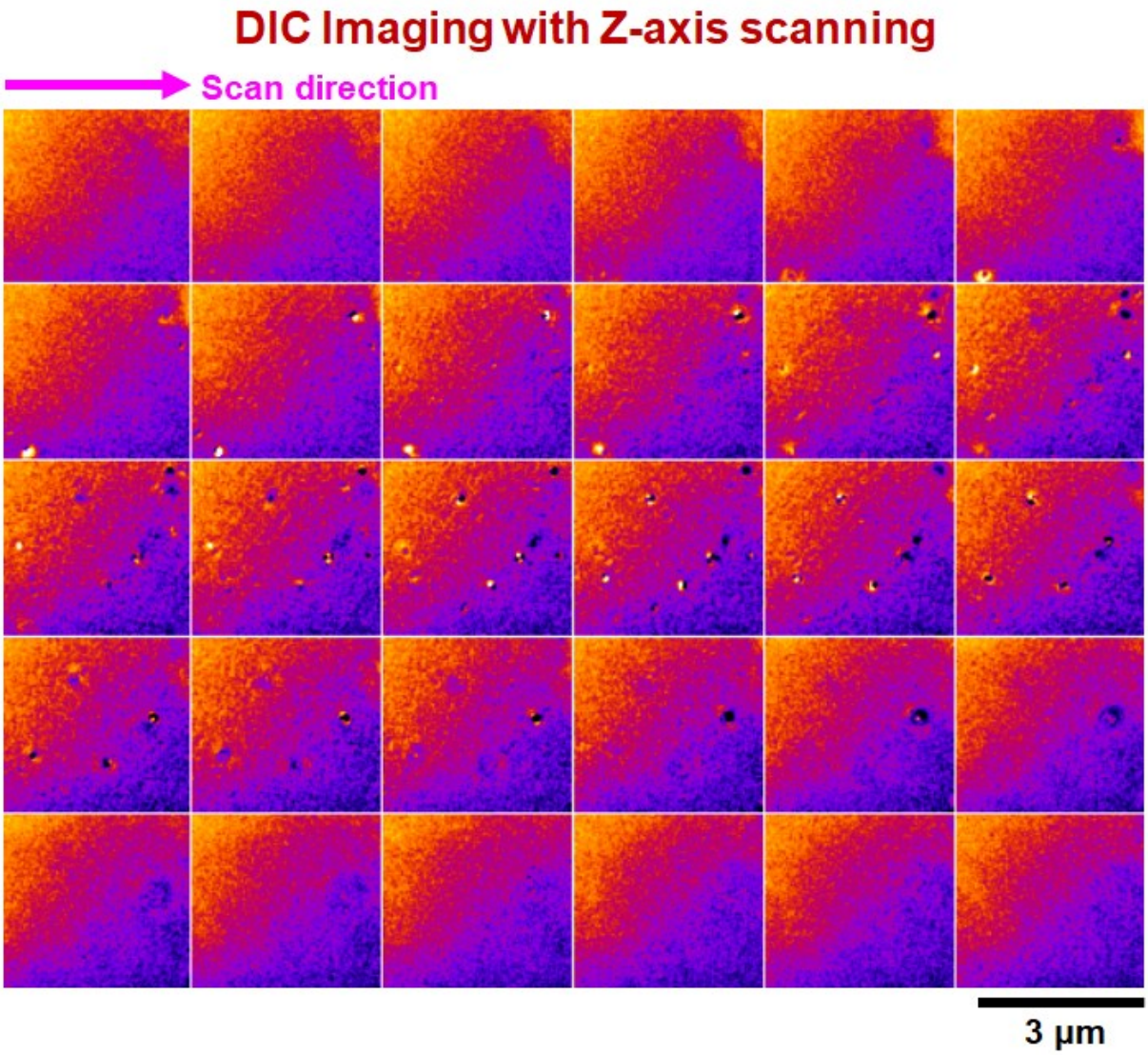


**Fig. S12** Consecutive DIC images of the embedded AuNRs (25 nm × 73 nm) measured by z-axis scanning with a vertical step size of 40 nm. The AuNRs in gel matrix have random 3D orienations at the different focal planes.