Supporting Information for

Inconspicuous reactions identified by improved precision of plasmonic scattering dark field microscopy imaging using silver shell-isolated nanoparticles as internal references

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Apparatus. UV-3010 spectrophotometer (Hitachi, Tokyo, Japan) were used to measure the UV−vis absorption of AgNPs and AgSHINs. The morphology and size of AgNPs were imaged by SEM (S-4800, Hitachi, Tokyo, Japan). DP80 single chip true color CCD camera (Olympus, Japan) was used to take DFM images. The scattering light of AgNPs and AgSHINs was acquired by Image-Pro Plus 6.0 (IPP) software (Media Cybernetics, USA).

Reagents and Materials. Silver nitrate, sodium citrate, (3-aminopropyl) trimethoxysilane and sodium silicate were purchased from Sigma-Aldrich，hydrogen peroxide was purchased from Taixin Chemical Company(Chongqing, China). Positive slide glass was purchased from Dingguo Biotechnology Co. Ltd (Beijing, China).

Results and discussion

Characterization information of AgNPs.

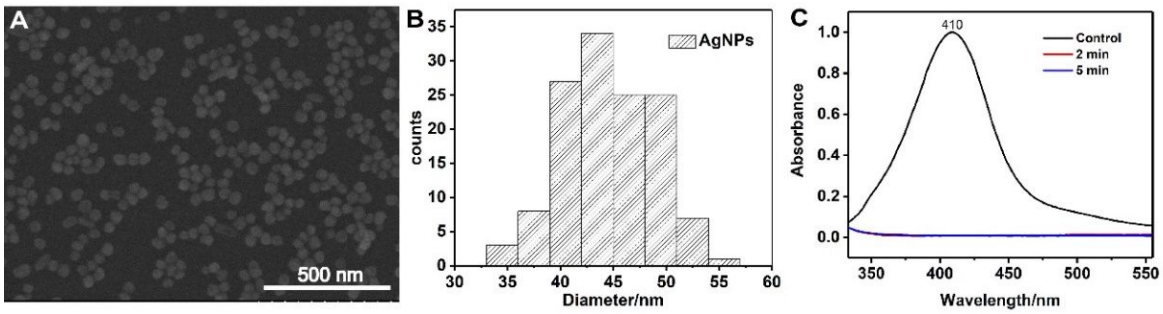

Figure S1. Scanning electron microscopy (SEM) image (A) and the size distribution (B) of AgNPs, (C) Time-dependent absorption spectra of AgNPs in a 6 wt % H_2O_2 solution. All of the absorptions are normalized to the original intensity before H_2O_2 was added (control).

Characterization information of AgSHINs.

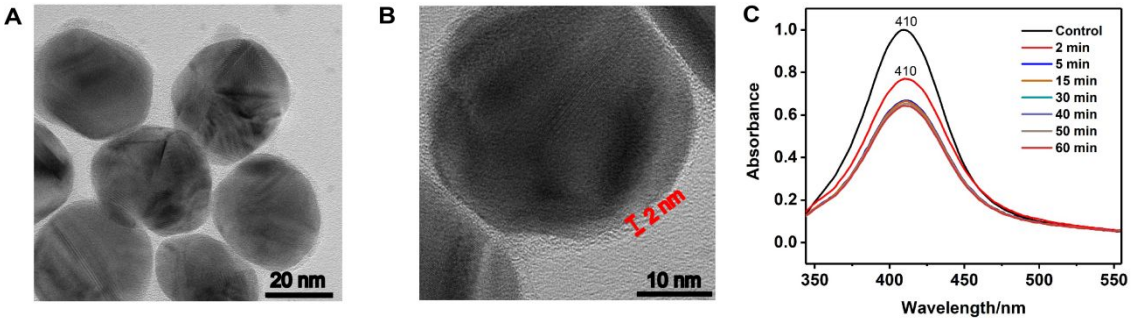

Figure S2. Transmission electron microscopy (TEM) image (A) of AgSHINs and the 2 nm SiO_2 shell (B) of AgSHINs. (C) Time-dependent absorption spectra of SiO_2-shell coated AgNPs in a solution containing 6 wt % H_2O_2, all of the absorptions are normalized to the original intensity before H_2O_2 was added (control).

Scattering intensity and RGB values of a typical SiO_2-shell coated AgNPs.

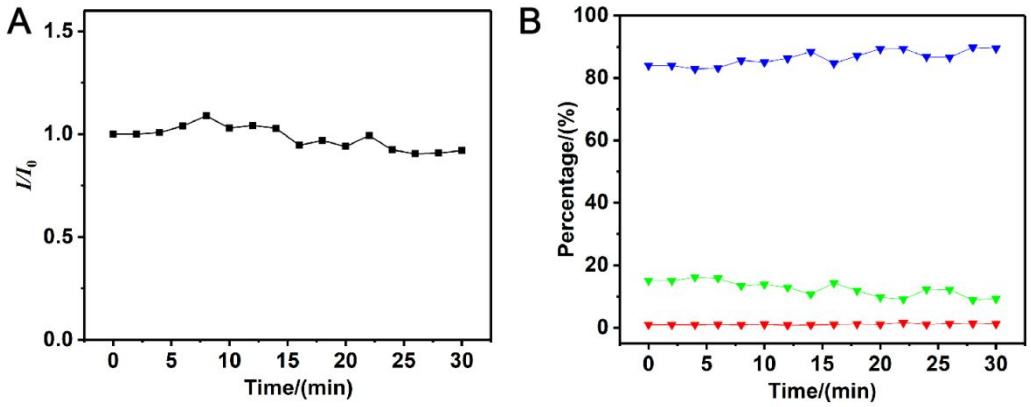

Figure S3. The real-time analysis of the plasmonic scattering intensity (A) and RGB values (B) of a typical SiO_2-shell coated AgNPs (the blue circled one in Figure 1D) against time scale.

The DFM images of AgSHIN and AgNPs.

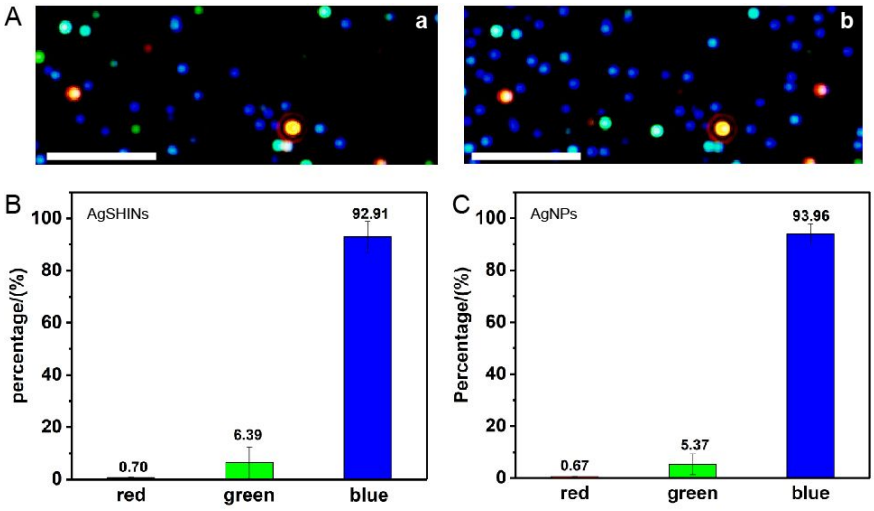

Figure S4. (A) The DFM images of AgSHINs IR (a) and AgNPs deposited later (b), a few spots of DFM images represent assemble or non-spherical nanoparticles. (B) The RGB values distribution of AgSHINs IR (B) and AgNPs (C), ten AgSHINs IR and ten AgNPs from A were analyzed. The scale bar is 20 μm for all DFM images.

The DFM images of the amalgamation of AgSHINs and AgNPs.

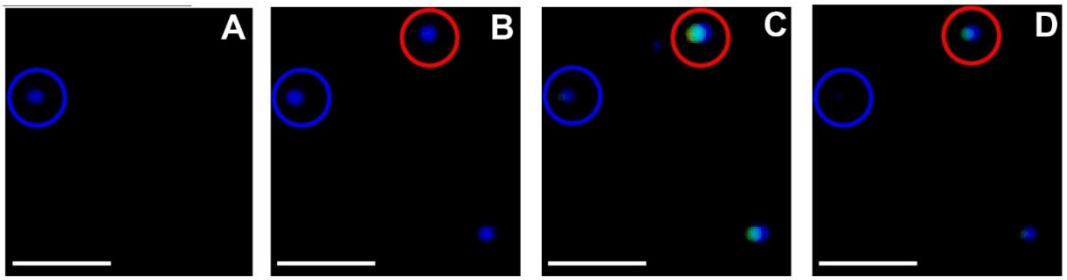

Figure S5. The DFM images of AgSHIN and AgNPs in different exposure time at amalgamation process (AgSHIN is represented by blue circle and AgNP is represented by red circle). (A) The local DFM images of the AgSHIN IR. (B) The same region of DFM image while AgNPs are deposited later. (C) The DFM image of AgSHIN IR and AgNPs bathed with 1.0×10^{-6} M mercury solution for 5 seconds. (D) The same DFM image just decreased the exposure time from 455 ms to 191 ms. The scale bar is 8 μm for all DFM images.

Effect of operation on scattering spectrums

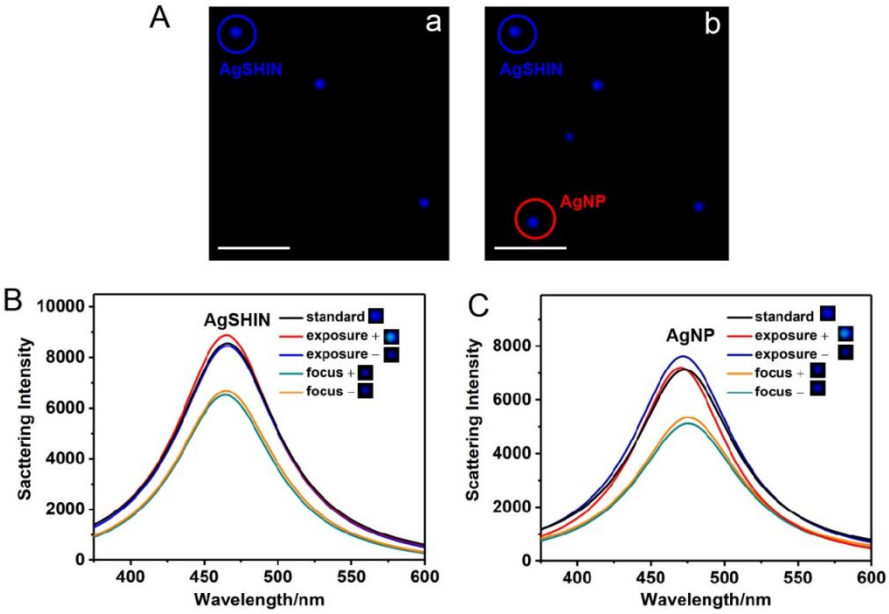

Figure S6. Effect of operation on scattering spectrums (AgSHIN is represented by blue circle and AgNP is represented by red circle). (A) AgSHINs are deposited (a) first and AgNPs are deposited (b) later. (B) the scattering spectrums of AgSHIN at difference exposure time and focus planes. (C) the spectrums of AgNP at difference exposure time and focus planes. The scale bar is 8 μm for all DFM images.

Summary of α and β.

Table S1. Summary of α and β at different conditions in Figure 3A c-f.

calibration factor	c (exposure time 526 ms)	d (exposure time 191 ms)	e (focus +)	f (focus -)
α	1.511	0.567	0.634	0.717
β	1.367	0.565	0.715	0.781

The calculation progress of calibration factors at c (exposure time 526 ms):

$$\alpha(\mathrm{c}) = \frac{I_{\mathrm{IR}}(\mathrm{c})}{I_{\mathrm{IR}}^{0}} = \frac{6212}{4110} = 1.511; \quad \beta(\mathrm{c}) = \frac{V_{\mathrm{IR}}(\mathrm{c})}{V_{\mathrm{IR}}^{0}} = \frac{14784}{10805} = 1.367$$

the scattering intensity and RGB values of nanoparticles are obtained by Image-Pro Plus 6.0 (IPP) software (Media Cybernetics, USA).