Supplementary Information for

Characterization of Nanoparticles Using Coupled Gel Immobilization and Label-free Optical Imaging

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Experimental Section

Materials and reagents. The different sizes of citrate-stabilized AgNPs (20, 30, 40, 50, 60, 80, and 100 nm) (1 mg/mL) were purchased from Nanocomposix company (San Diego, USA). Glycerol Jelly Mounting Medium was obtained from Solarbio Science & Technology Co., Ltd. (Beijing, China). HCl solution, NaCl and NaOH were purchased from Sigma-Aldrich (Shanghai, China).

The immobilization of NPs into Gel. The dispersed AgNPs were immobilized by Glycerol Jelly Mounting Medium to inhibit their Brownian motion. First, Glycerol Jelly Mounting Medium (10 g gelatin dissolved into 60 mL water and mixed with 70 mL glycerol) was heated into liquid gel solution at 45 °C. Then AgNPs dilutions were mixed with Glycerol Jelly Mounting Medium (1:10 by volume) by vortex (30 s) and sonication (30 s) at 45 °C. The final concentration of AgNPs in the mixture are ranged from 0 to 10 μg/mL. Lastly, 50 μL gel containing AgNPs were transferred onto a culture dish with a glass bottom (Cellvis, Mountain View, USA), and cooled at room temperature. The solid and hemispheric gel was used for SLI analysis.

3D Scattered Light Imaging of AgNPs. 3D Scatter Light Imaging of NPs was performed on a laser scanning confocal microscrope equipped with longpass beamsplitters (Thorlabs, USA). The light source used was a 488 nm laser (Thorlabs) with a 63 × oil immersion microscope objective lens (1.0 NA W Plan-Apochromat, Zeiss, Germany). The key parameter of 3D imaging: scanning speed, 1000 hz; the wavelength range of receiving scatter light, 483 – 493 nm; z axis step size, 0.13 μm; the resolution of images, 1024 × 1024 or 2048 × 2048 pixels; frame average, 3 times. The glass bottom of the dish loading NPs immobilization gel is assumed to be the initiation point of z stack scanning in the vertical level. After the completement of z stack scanning, the images

series were imported into Image J software for 3D reconstruction, the maximum signal projection, number count and size measurement of NPs.

The measurement of AgNPs mass concentration by ICP-MS. The mass concentration of AgNPs in gel was measured by using inductively coupled plasma mass spectrometer (ICP-MS, Agilent 8800). AgNPs in the immobilization gel were transformed into silver ion by acidic digestion solution (8 mL 65% HNO_3 and 2 mL 30% H_2O_2). The digestion process was assisted by microwave heating (120 °C, 2 h). ^{115}In isotopes were used as internal standard. Calibrations were carried on by using ionic silver standards solution ranging from 0 to 20 ng/mL. Total amounts of silver in the gel were determined by ICP-MS in five independent replicates.

The characterization of AgNPs by dynamic light scattering. Hydrodynamic diameter of AgNPs was measured by using dynamic light scattering (DLS) (ZS90 Zetasizer, Malvern, UK). The NPs was suspended into water at about 10 μg/mL and analyzed by DLS. The size distribution of AgNPs were acquired using the Zetasizer Software v.7.02.

The characterization of AgNPs by transmission electron microscopy. The size and morphology of AgNPs were characterized using a transmission electron microscopy (TEM) (JEM-2100F, Toyota, Japan). The stock solution of each AgNPs was diluted to 10 μg/mL by ultrapure water. Then 5 μL of the diluent was pipetted onto an ultrafine carbon support film on a copper grid (200 mesh) and dried in vacuum drying oven at 55 °C. The size distribution of AgNPs in TEM images was analyzed and calculated by Image J and Origin 2018 software, respectively.

Statistical Analysis. All experimental results were analyzed by using GraphPad Prism 7.00 software and presented as means ± standard deviation (SD). Statistical significance of differences between groups was assessed by using Student's t test.

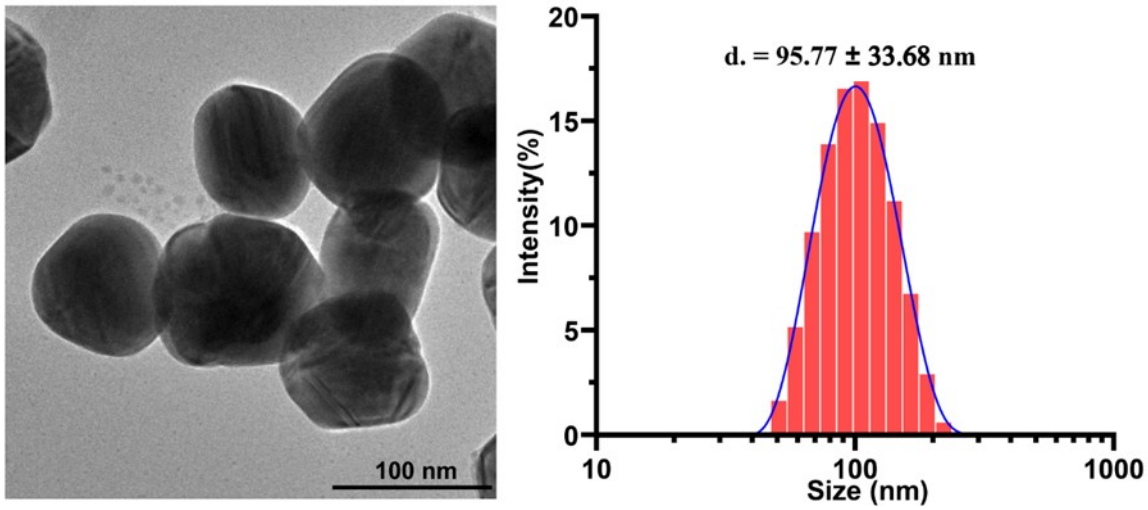


Fig. S1 The morphology and size of 100 nm citric acid-coated AgNPs were characterized using TEM (left) and DLS (right) respectively.

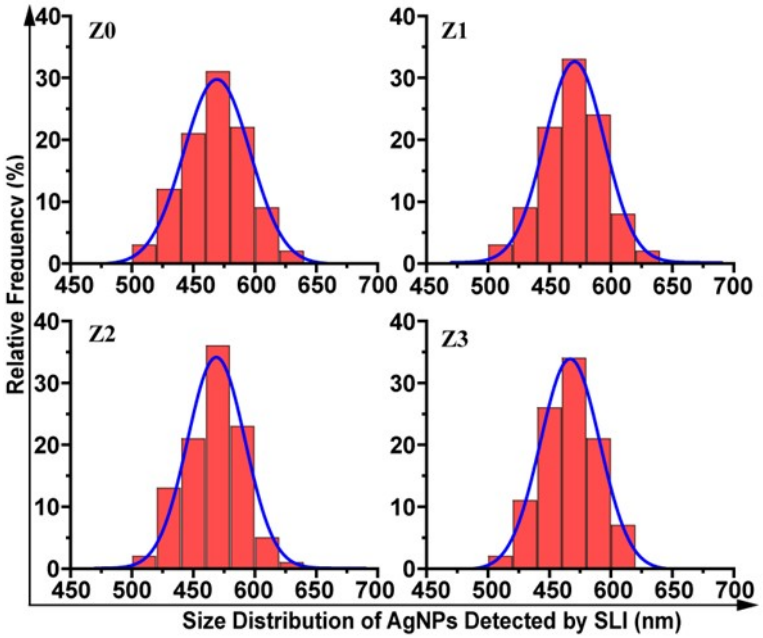


Fig. S2 The size distribution of AgNPs located at the different height of the gel (Z0 to Z3). The size distribution of AgNPs is determined by counting 100 single particle in SLI images at random.

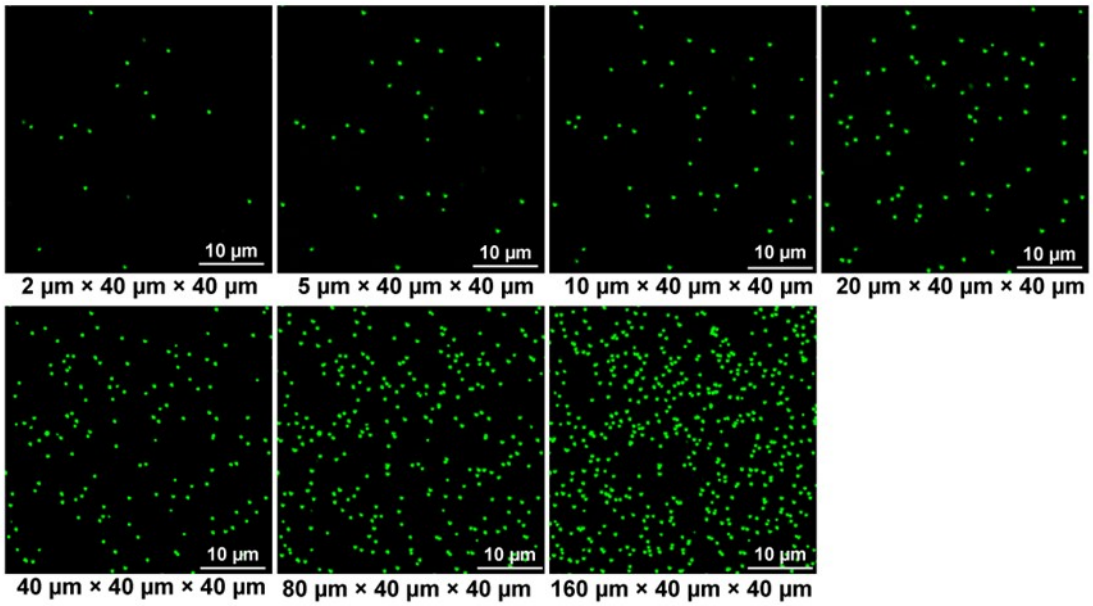


Fig. S3 The representative SLI images for planar projection of AgNPs in different scanning volume of 3D reconstruction. The total number of AgNPs in the planar images is automatically counted by using Image J software.

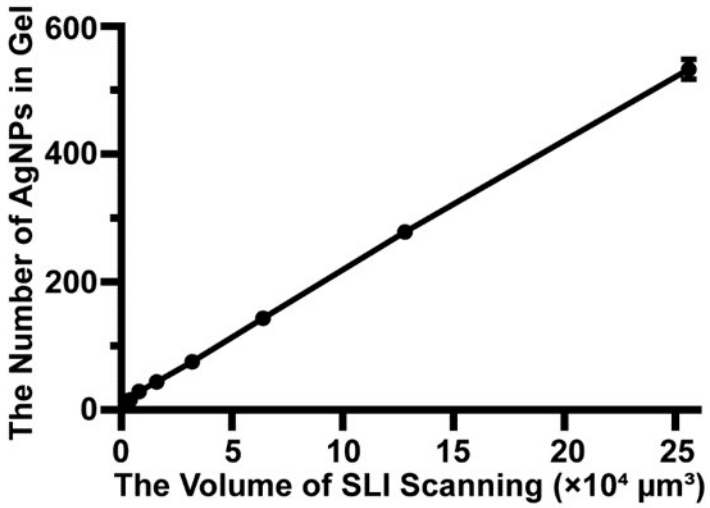


Fig. S4 Correlation between AgNP number and scanning volume. The total number of AgNPs in the planar images is automatically counted by using Image J software.

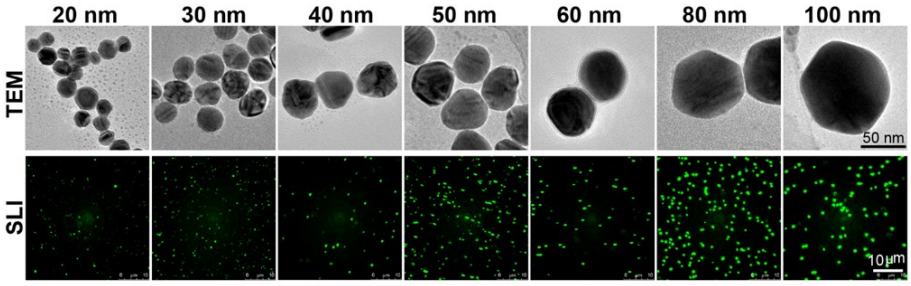


Fig. S5 Representative TEM (top) and SLI (bottom) images of AgNPs with different size.

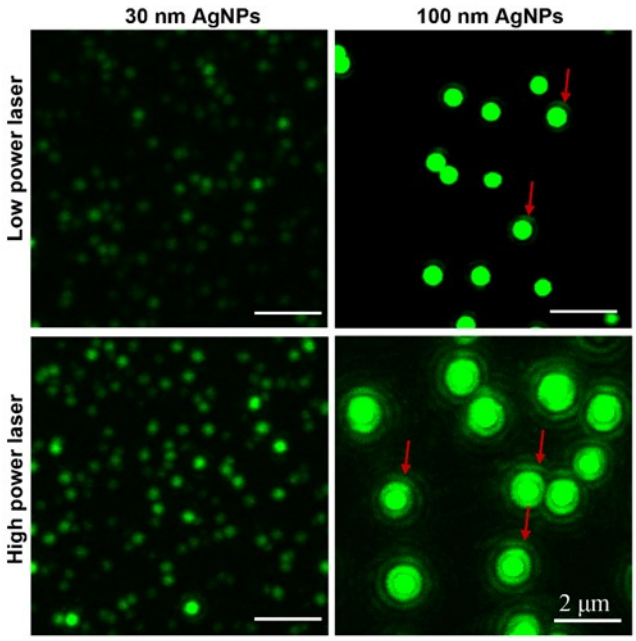


Fig. S6 Representative SLI images of 30 and 100 nm AgNPs using low or high power laser. Red arrows indicate the ghost of scattered light around AgNPs.

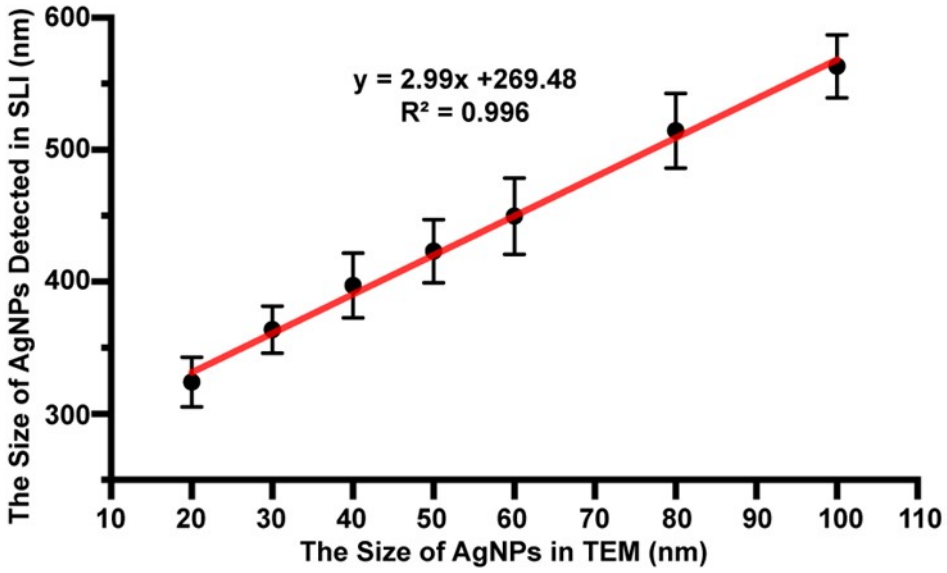


Fig. S7 Correlation of the AgNPs sizes between in SLI and TEM images. The average diameter of each size of AgNPs is determined by counting about 500 single particles in SLI and TEM images at random.

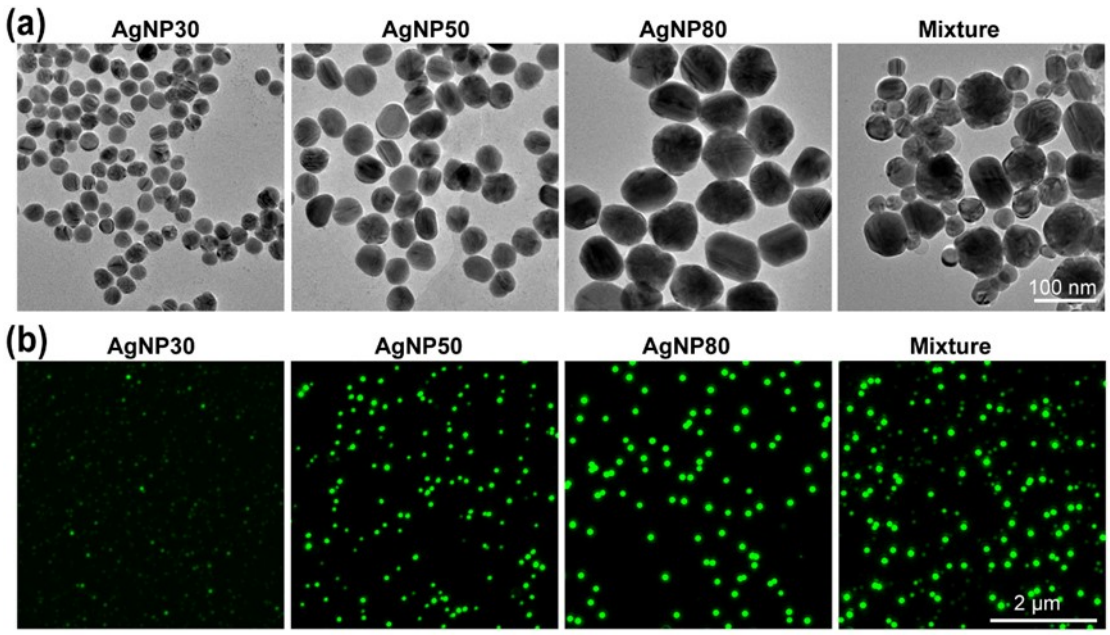
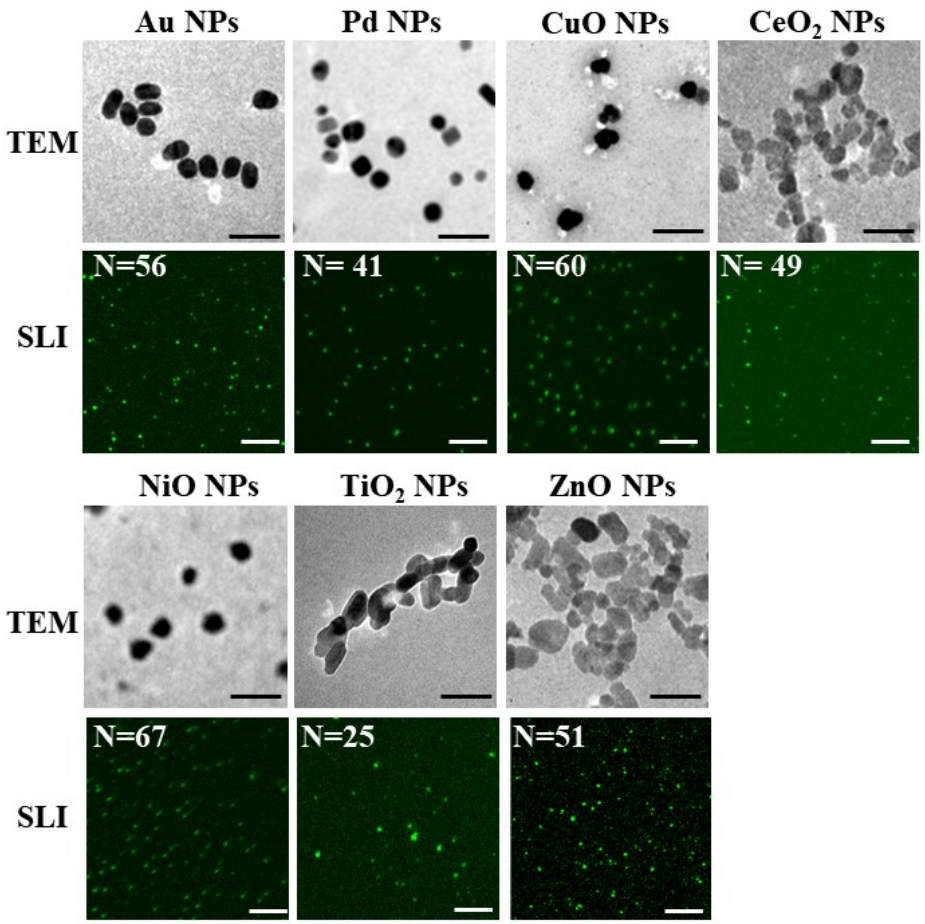


Fig. S8 Representative TEM (a) and SLI (b) images of three AgNPs (30, 50, and 80 nm) and their mixtures.



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95 **Fig. S9** Representative TEM and SLI images of AuNPs, PdNPs, CuO NPs, CeO_2 NPs, NiO NPs,
96 TiO_2 NPs and ZnO NPs with size about 20 nm. The scale bar, 50 nm (TEM), 2 μm (SLI).

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