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Correlation and Characterization of 3D Morphological Dependent Localized Surface Plasmon Resonance Spectra of Single Silver Nanoparticles Using Dark-field Optical Microscopy and Spectroscopy and AFM

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Abstract

We have developed a new and effective methodology to correlate optical and AFM images of single Ag nanoparticles (NPs), allowing us to study 3D-morphological dependent localized surface plasmon resonance (LSPR) spectra of individual Ag NPs. We fabricated arrays of distinctive microwindows on glass coverslips using photo-lithography method, and created well-isolated individual Ag NPs with a wide variety of shapes and morphologies on the glass coverslips using a modified nanosphere lithography method (NSL). Using distinctive geometries of microwindows, we located individual Ag NPs of interest in their optical and AFM images, enabling us to correlate and characterize the LSPR spectra and 3D morphologies of the same single NPs using dark-field optical microscopy and spectroscopy (DFOMS) and AFM, respectively. We found that LSPR spectra of single Ag NPs, with nearly equal volume $[(8.6 \pm 0.4) \times 10^3 \text{ nm}^3]$, cross-section $[(2.2 \pm 0.2) \times 10^2 \text{ nm}^2]$, and height $(39.6 \pm 3.6 \text{ nm})$, highly depend on their shapes, showing the red shift of peak wavelength to 629 nm (quasi trapezoidal cylindrical NP) from that of 506 nm (quasi circular cylindrical NP). LSPR spectra of single Ag NPs simulated using discrete dipole approximation (DDA) agree well with those measured experimentally when their shapes and morphologies can be accurately described in both methods, but differ when they are not. Furthermore, we found location-dependent LSPR spectra on and around a single NP, offering a unique opportunity to characterize multi-mode plasmonic NPs at nanometer resolution for better understanding their plasmonic optical properties and for rational design of single NP optics.

Keywords

Single silver nanoparticles; single nanoparticle optics; localized surface plasmon resonance spectrum; microfabrication; discrete dipole approximation (DDA) simulation

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Supporting Information Available. This material is available free of charge via the internet at <http://pubs.asc.org>

1. Introduction

Noble metal nanoparticles (NPs) (e.g., Ag, Au) possess unique optical, electronic and catalytic properties, offering numerous opportunities for fundamental studies and for developing new technologies for a wide variety of applications.^{1–5} Noble metal (e.g., Ag) NPs show exceptionally high quantum yield of Rayleigh scattering that are orders of magnitude higher than fluorophores (e.g., R6G), allowing individual NPs with diameters as small as 2 nm to be directly imaged and characterized using dark-field optical microscopy and spectroscopy (DFOMS).⁶ Unlike fluorescent probes and semiconductor quantum dots (QDs), these noble metal NPs exhibit superior photostability.^{6–8} They do not suffer photobleaching and do not blink under white-light dark-field optical illumination, which allows them to serve as photostable nanophotonic optical probes for tracking dynamic events of interest in living cells and living embryos for desired period of time.^{6–13} In our previous studies, we have utilized single Ag NPs as plasmonic nanophotonic probes to image transport kinetics of single living cells and single living embryos, and to map single ligand-receptor interactions on single living cells in real-time at nanometer scale resolution, as well as to sense single protein molecules (e.g., tumor markers) and their binding reactions in real time.^{6–13}

Studies have demonstrated that it is critical to further investigate high dependence of optical properties of individual noble metal NPs upon their morphologies and surrounding environments in order to address fundamental questions in single NP optics and to enable rational design of single NP photonics for a wide variety of applications.^{1,2,4,5,14,15} These applications include: (i) development of single NP optical probes for imaging and sensing of biomolecules of interest in solution,^{6,16–18} single living cells and single embryos,^{7–9} as well as for design of plasmonic light sources;^{1,19} (ii) study of dependence of Raman scattering of surface adsorbates on surface and morphological properties of NPs to address fundamental questions and explore new applications of surface enhanced Raman scattering (SERS);^{20–22} (iii) development of nanoscale opto-electronic and electro-optical devices;^{1,5} and (iv) study of dependence of chemical catalytic properties of NPs on their sizes, morphologies and surface properties.^{23,24}

These potential applications have inspired numerous studies on LSPR of noble metal NPs.^{1–5} LSPR of metal NPs are generated by coherent oscillations of their surface free electrons, as the NPs are radiated by light (electromagnetic waves). The LSPR spectra of single NPs exhibit high dependence on their size, shape and dielectric environments, as well as interparticle interactions.^{3,25–27} Therefore, identification of well-isolated individual NPs and correlation of their morphologies with their unique LSPR responses are essential to better understanding of the dependence of LSPR on their morphologies and interparticle coupling.

Unfortunately, due to lacking of effective correlation means of single NPs among a wide variety of characterization systems at sub-nanometer, micro- and macro- scale, most current studies investigate physical and chemical properties of ensemble NPs, providing average results of many individual NPs (bulk), instead of single NPs.^{3,16,17,28} Ensemble NPs are heterogeneous, because morphologies of individual NPs prepared by chemical synthesis or fabrication are rarely exactly identical at sub-nanometer resolution.^{15,29} Therefore, the effective means to correlate and characterize 3D morphologies of same single NPs with their optical properties is essential to address fundamental and practical questions related to single NP plasmonic optics.

Morphologies of NPs have been widely characterized using transmission electron microscopy (TEM), scanning electron microscopy (SEM), scanning tunneling microscopy (STM), and atomic force microscopy (AFM) at nanometer or sub-nanometer spatial resolution.^{14–16} In contrast, characterization of LSPR spectra of single NPs is mostly carried out using optical

microscopy and spectroscopy at micrometer and sub-micrometer resolution, which is unable to offer morphological details of the NPs at nanometer and sub-nanometer spatial resolution. Therefore, to characterize the morphologies and optical properties of individual NPs correspondingly, one will need to either develop an instrumentation that can measure both optical properties and morphologies of the same individual NPs, or design a means to identify same individual NPs and allow their optical properties and morphologies to be characterized using desired instrumentation. In fact, AFM has been reportedly installed onto an inverted microscope, enabling the characterization of morphology and the optical properties of a single NP *in-situ*.³⁰ However, the scattering of AFM tips and their interactions with the NPs could affect the measurements of optical properties of NPs. Furthermore, such a method cannot achieve characterization of same individual NPs using other means (e.g., TEM, SEM).

Correlation of optical and TEM images of individual NPs using enumerated TEM grids, position markers, or unique patterns formed by several NPs, has also been reported.^{31–33} However, such approaches typically rely upon unique patterns randomly formed by a group of NPs that can be recognized and imaged using both optical and electron microscope (EM) to identify single NPs of interest. Thus, it cannot study any desired NPs that may not form a recognizable pattern or that are located below optical diffraction limit because they cannot be resolved using optical microscopy. Furthermore, TEM and SEM have to be operated in high vacuum, which prevents them from being used to characterize single NPs in living organisms. Although EM equipped with EDS capabilities can offer chemical characterization of individual NPs, EM does not provide quantitative 3D morphologies of individual NPs, as AFM and STM does. Therefore, it is important to develop a simple and effective method to precisely locate and identify single NPs of interest and characterize their morphologies and optical properties (e.g., LSPR) using desired instrumentation.

In this study, we have developed a methodology to identify the location and orientation of single Ag NPs using a multi-hierarchy array of micro-windows fabricated on glass coverslips. This methodology allows us to characterize 3D morphologies of single NPs using AFM and their LSPR spectra using DFOMS, and enables us to examine 3D morphological-dependent LSPR spectra of given size and shape of single NPs using both experimental and simulation approaches (discrete dipole approximation, DDA). We found high-dependence of LSPR spectra of single NPs upon their morphologies and various locations on single NPs.

2. Experimental and Simulation Section

2.1. Chemicals and Materials

Polystyrene nanospheres (290 nm in diameter with coefficient of variation < 5.0 % and 4.0 wt %; Duke Scientific Corporation), silver (99.999%) and chromium (99.99%) shots (Alfa Aesa), graphite crucibles (POCO Graphite), borosilicate glass slides and glass coverslips (18 mm in diameter, Fisher Scientific), positive photoresist (S1816) and developer (MF-321) (Shipley Microposit), and Cr etchant (Cr-7, Cyantek), were purchased and used as they were provided. Deionized water (18 MΩ, Nanopure Barnstead) was used to rinse glass slides and coverslips and to prepare solutions, including 5-10x diluted polystyrene nanospheres.

2.2. Design and Fabrication of Arrays of Distinctive Micro-windows on Glass Coverslips

We designed a photomask (Figure 1) which includes a 5×5 array of circles (18 mm in diameter). Each circle matches with that of a glass coverslip, and each circle consists of a 7×7 array of squares (each length of the square = 1383.3 μm). Each square includes a 10×10 array of assemblies of distinctive micro-windows in a small square (Figure 2A). Each assembly has five distinctive shapes of micro-windows (triangle, trapezium, circle, hexagon, and parallelogram) (Figures 2B–C). This design allows us to create 122,500 assemblies of five

distinctive micro-patterned windows simultaneously, enabling potential high-throughput fabrication. The photomask was fabricated and characterized by CNF (Cornell NanoScale Science & Technology Facility).

We used the photomask to print the array of micro-windows on each glass coverslip using a photo-lithography method, as illustrated in Figure 1S in supporting information and as described in the following. We first soaked the glass coverslips in a piranha solution (30% H_2O_2 : $\text{H}_2\text{SO}_4 = 1:3$ v/v) at 80°C for 30 min, well rinsed them with nanopure water and ethanol, and dried them with N_2 . The well-cleaned coverslips were placed in the e-beam vacuum evaporation deposition chamber. A 15–20 nm thickness of Cr (99.99%) layer was deposited on the coverslips at a deposition rate of $1\text{--}2 \text{ \AA/s}$ under high vacuum ($<10^{-7}$ torr) using electron beam evaporation. The $1\text{--}2 \mu\text{m}$ thickness of positive photoresist layer was then spin-coated on the top of the Cr layer (3500–4500 rpm for 20–30 s). The photomask was carefully aligned directly on the top of the photoresist layer so that each circle on the photomask (Figure 1) matched with each coverslip. We then illuminated the photoresist through the micro-patterned windows of mask using UV light, and removed the exposed areas of photoresist using the positive photoresist developing reagents, which revealed the Cr layer. The Cr areas without photoresist protection were removed by incubating it with a Cr etcher solution for 100–150 s, which created an array of 5 distinct micro-patterned transparent glass windows. The undeveloped photoresist was finally washed away using acetone, generating the Cr layer etched with an array of 5 distinct micro-patterned transparent glass windows (Figure 2D).

The shapes of patterned micro-windows (Figure 2D) are distorted from those originally designed in the photomask (Figure 2C) due to the optical resolution limitation of photolithography methods. Nonetheless, their shapes are still distinctive, allowing us to locate individual NPs of interest fabricated in each window using both DFOMS and AFM.

2.3. Fabrication of Ag NPs Using Modified Nanosphere Lithography Methods

We modified a standard nanosphere lithography (NSL) method developed by Van Duyne and his co-workers,³⁴ to generate well isolated individual NPs, aiming to avoid potential plasmonic coupling among NPs and to study optical properties of individual NPs. In our experiments, we packed a mono-layer of polystyrene nanospheres on the glass coverslip by adding $1.5\text{--}3.0 \mu\text{L}$ of 5-10x diluted nanosphere solutions onto the coverslips, slightly tilting the coverslip to evenly spread polystyrene nanospheres, and allowing it to be dried overnight. We mounted the samples onto a E-beam deposition system (Consolidated Vacuum Corporation), which was equipped with quartz crystal microbalance (QCM, XTM/2, Leybold Inficon) to monitor the thickness of the Ag film deposited through the gaps among polystyrene nanospheres. This approach allows us to control the desired thickness of Ag films at 10–200 nm (height of Ag NPs fabricated on the coverslip via the gaps among polystyrene nanospheres). We completely removed well-packed polystyrene nanospheres from the coverslip surface by sonicating the entire coverslip in absolute ethanol for 5–10 min and rinsing the coverslip with nanopure water. This approach typically allows us to generate an array of triangular Ag NPs, as shown in Figure 2S in supporting information, and as reported previously.^{17,34}

We finally removed majority of Ag NPs from the surface of coverslips to create well-isolated individual NPs with various shapes by sonicating the entire samples in a 10% HCl solution for 0.5–2 h and then in nanopure water for 3–30 s, rinsing them with tetrahydrofuran (THF), nanopure water, and ethanol, and drying them with N_2 . In some cases, we also annealed the NPs at $100\text{--}300^\circ\text{C}$ under nitrogen atmosphere for 2–8 h, or refreshed the surface of Ag NPs using 5% HNO_3 for 3–60 s, rinsed the samples with nanopure water and ethanol, and dried them with N_2 . The samples were then immediately characterized, or carefully wrapped with Al foil to prevent them from exposing to the light, and stored in a vacuum desiccator for the future use. This precaution aims to prevent any possible reactions of Ag NPs with the air and

moisture, and to preserve the samples. However, despite such efforts, we found that the samples degraded over time (months). Thus, the experiments were carried out with freshly-prepared samples.

2.4. Correlation and Characterization of 3D Morphologies and LSPR Spectra of Single Ag NPs

The 3D morphologies of individual Ag NPs of interest were imaged and characterized at ambient conditions using Atomic Force Microscope (AFM, Nanoscope IV, Dimension 3100, Digital Instruments) via a tapping mode and etched Si nanoprobe tips (RTESP, radius < 10 nm and cone angle of 20°). Optical properties of individual Ag NPs of interest were investigated in a microchannel filled with nanopure water using our single NP dark-field optical microscopy and spectroscopy (SN-DFOMS). We have fully described the design and construction of our microchannel and dark-field optical microscopy and spectroscopy (DFOMS) (also named as SNOMS by us) in our previous studies for imaging and characterization of LSPR spectra of single Ag and Au NPs in solution, in single living cells, in zebrafish embryos, and for single molecule detection.^{6-13,35,36} In this study, a high-resolution CCD camera (Micromax, 5 MHz Interline) (Roper Scientific), EMCCD camera (PhotonMAX) coupled with a SpectraPro-2150 (Roper Scientific), and a color camera were used for imaging and characterization of LSPR spectra of single Ag NPs. The locations of individual Ag NPs of interest were identified using the address and distinctive geometries of micro-patterned windows, allowing us to correlate their 3D morphologies characterized by AFM with their optical properties determined using DFOMS, as illustrated in Figure 2.

2.5. Simulation of LSPR Spectra of Single Ag NPs

The LSPR spectra of well-isolated individual Ag NPs (diameter $\ll$ wavelength of the incident light) can be simulated using Maxwell's equations. Mie theory is an accurate analytical solution of Maxwell's equations, which is used to describe spherical shape of well-isolated individual NPs.²⁷ For all other shapes of NPs, Maxwell's equations have not analytical solutions. Therefore, several numerical methods, such as discrete dipole approximation (DDA),^{2,17,37} the multiple multipole method,³⁸ finite differences in the time domain (FDTD),³⁹ and the boundary element method (BEM),⁴⁰⁻⁴¹ have been developed to provide approximate solutions of Maxwell equations and to simulate the LSPR spectrum of individual noble metal NPs. Currently, DDA is one of most popular methods for computing LSPR spectra of single Ag NPs.^{2,17,38}

In this study, we utilized DDA method and an open-source Fortran-90 code, DDSCAT 7.0, developed by Draine and Flatau,^{37,42} to calculate the LSPR spectra (wavelength-dependent scattering intensity) of well-isolated individual Ag NPs. We adapted wavelength-dependent dielectric constants of Ag reported by Palik ($n = 0.209-0.143$, for wavelengths of 350–800 nm),⁴³ and the refractive index of water ($n = 1.33$).^{44,45} Note that Ag NPs fabricated on the surface of glass coverslips were completely immersed in nanopure water in the microchannel as their LSPR spectra were acquired. We used 5 nm as an inter-dipole separation distance to build a cubic lattice of polarized points (dipoles) for each NP in Figures 5A–E. Their effective radii were 0.0595, 0.0592, 0.0591, 0.0600 and 0.0573 μm , respectively, calculated as instructed by the program. Bi-conjugate gradient with stabilization (PBCGS2) and general prime factor algorithm fast Fourier transform (GPFA FFT) were selected as the iteration algorithm and the FFT algorithm, respectively. The angular resolution of 0.5 and the iteration error tolerance of 10^{-5} were set for all calculations.

3. Results and Discussion

3.1. Correlation of AFM Images of Single Ag NPs with their Optical Images

We have designed and fabricated an array of distinctive micro-windows (Figure 2), as described in Experimental Section. Each basic unit of the array includes five distinctive shapes of micro-windows (triangle, trapezium, circle, hexagon, and parallelogram) (Figures 2B–C), allowing us to identify and characterize the same single NPs of interest using both AFM and DFOMS.

As illustrated in Figure 3, single Ag NPs fabricated on the surface of coverslip within the hexagonal microwindow were identified and characterized using AFM (Figure 3A:a) and DFOMS equipped with color camera (Figure 3A: b) and CCD camera (Figure 3A:c). Note that CCD camera offers higher spatial resolution than color camera, while the color camera provides the true colors of individual Ag NPs that are generated by LSPR. The zoom-in images of these individual NPs as squared in Figure 3A are shown in Figure 3B. We located the center of individual NPs in optical images at single pixel resolution (125 nm) of the CCD camera by determining the address of pixel with the highest intensity of the NPs. The positions of individual NPs of interest within the microwindow in optical images (Figure 3A: b–c) were determined with spatial resolution of optical diffraction limit (~ 200 nm) and orientation angle resolution of 0.5 degree, which were compared with those of individual NPs in AFM images (Figure 2A: a). This approach allows us to correlate AFM images of individual NPs (as one circled in Figure 2B: a) with the same NP shown in optical images (as circled in Figure 2B: b–c), and to investigate their 3D morphological-dependent LSPR spectra.

3.2. Shape-Dependent LSPR of Single Ag NPs

This new method allows us to select different shapes of individual NPs with nearly equal volume and cross-section area, and characterize their 3D morphologies and LSPR spectra using AFM and DFOMS, respectively, aiming to determine their shape-dependent LSPR spectra. Representative top-view of AFM images of individual NPs (Figure 4A–E: a) illustrates different shapes of individual NPs, including (A:a) quasi-circular cylindrical; (B:a) oblate spheroid; (C:a) irregular-hexagonal cylindrical; (D:a) quasi-pentagonal cylindrical, and (E:a) quasi-trapezoidal cylindrical NPs. Their optical images and LSPR spectra acquired using DFOMS (Figure 4A: b–c-i) show a peak wavelength ($\lambda_{p,i}$) of 506, 537, 548, 603, and 629 nm with a shoulder peak wavelength of 470 nm, respectively. Note that optical color images of single Ag NPs (Figure 4A–E: b) appear larger than their actual physical sizes due to optical diffraction limit and light scattering of NPs. Nonetheless, the true colors of individual Ag NPs reflect their LSPR responses, as illustrated by their LSPR spectra (Figure 4A–E: c).

The experimental results in Figure 4 and Table 1 clearly demonstrate shape-dependent LSPR spectra of single Ag NPs. Peak wavelength of LSPR spectra of single Ag NPs gradually shifts to the longer wavelength (red-shift) as the shape of the NPs deviates significantly from a circle and the tips of the NPs become sharper.

To further understand shape-dependent LSPR spectra of single Ag NPs, we used DDA simulation approach, as described in Simulation Section, to compute the shape-dependent LSPR spectra of single NPs. The simulation results in Figure 4A–E: c-ii and Table I show that the peak wavelength ($\lambda_{p,ii}$) of individual NPs calculated using DDA is similar to those measured using experimental method (DFOMS), and the effectiveness of simulation depends on regularity of given shapes of individual NPs and ability to accurately describe their actual shapes. For example, the peak wavelength ($\lambda_{p,ii} = 489$ nm) and FWHM (96 nm) of LSPR spectrum of the quasi-circular cylindrical NP in Figure 4A computed using DDA is in excellent agreement with the experimental data ($\lambda_{p,i} = 506$ nm; FWHM = 82 nm). The DDA simulation result for irregular-hexagonal cylindrical NP ($\lambda_{p,ii} = 510$ nm; FWHM = 117 nm) (Figure 4C:

c) shows a certain degree of agreement with the experimental measurement ($\lambda_{p,i} = 548$ nm; FWHM = 75 nm), but not as well as those for the quasi-circular cylindrical NP. The deviation may be attributed to using a simplified model by assuming the same height for the entire hexagonal cylindrical NP, while the height of the NP varies through the boundaries and the center of the NP, as measured by AFM.

The simulation results for oblate spheroid (Figure 4B) and quasi-pentagonal cylindrical NP (Figure 4D) show a shoulder peak of LSPR spectra at 414 nm (FWHM = 72 nm) and 457 nm (FWHM = 47 nm), respectively, which are not observed experimentally. The shoulder peaks of LSPR spectra are generated by an in-plane quadrupole resonance (transverse collective oscillation of the surface electrons between the two edges) of the NPs, which may be too weak to be observed experimentally, because the edges of the NPs in Figures 4B and 4D exhibit round curvature, and they are not as sharp and straight as they are assumed in the simulation. For single Ag NPs with sharper corners, as the one shown in Figure 3E, we observed a weak shoulder peak at 470 nm (FWHM = 85 nm) experimentally, which is weaker than the simulated shoulder peak wavelength at 526 nm (FWHM = 47 nm). The corners of the NP in Figure 4E are not as sharp as they are assumed in the simulation, which may attribute to the slightly blue-shift of shoulder peak wavelength with the lower intensity as shown in experimental result (Figure 4E-c-i). The larger FWHM of LSPR spectra are observed in simulation than those acquired experimentally, which may also attribute to the sharper corners and edges of NPs assumed for simulation study (Figure 4A–E: c). Note that other DDA calculations by Schatz and co-workers have found that the sharper tips (corners) attribute to the broader line width of LSPR spectra²⁹, which is consistent with what we observed in this study.

The results in Figure 4 and Table 1 show that the LSPR spectra of single Ag NP of similar sizes (volumes) and cross-sections are highly sensitive to the shape of the NPs, demonstrating possibilities of tuning optical properties (LSPR, colors) of single Ag NPs using their shapes.

3.3 3D Morphological and Location-Dependent LSPR Spectra of Single Ag NPs

To further investigate the effects of the 3D morphology of single Ag NPs on their LSPR spectra, we characterized 3D morphology of a selected single NP using AFM. The AFM images in Figure 5A show a NP with one sharp tip and three curvature corners with the diameter of cross-section (from the tip to the opposite edge) of NP at 210 nm and a variety of heights ranging from 10 to 50 nm. The optical image of the NP acquired using DFOMS (Figure 5B: a) and 3D plot of distribution of scattering intensity of the NP vs its x-y dimension (Figure 5B: b) illustrate the high dependence of scattering intensity of NP upon different locations on and surrounding the NP.

The color image and LSPR spectrum of the NP in Figure 5C show the primary peak wavelength at 630 nm (red color), and two weak shoulder peaks at 524 nm and 465 nm, respectively. Comparing this result with the DDA simulation on a snipped triangle nanoprisms,^{3,29} we suggest that the peak at 630 nm is attributed to the in-plane dipole plasmon resonance, while the peak at 524 nm and 465 nm is generated by the in-plane and out-plane quadrupole plasmon resonance, respectively. Since the intensity of the in-plane and the out-plane quadrupole plasmon resonance (524 and 465 nm) are much weaker than in-plane dipole plasmon resonance (630 nm), the NP exhibits the red color, which is attributed by the primary peak of LSPR spectra (Figure 5C:a).

Note that the optical images of NP (Figure 5B and C: a) look larger than its actual physical size, and show much lower resolution than the one determined by AFM (Figure 5A), due to optical diffraction limit. However, using the method described in Figure 3, AFM and optical images in Figures 5 are well correlated and aligned on the same orientation using the unique features of microwindows. Using the position of pixel with the highest scattering intensity and

intensity distribution profile of the NP (Figure 5B), we determined the physical location of the NP in optical image as marked in pixel (i) and its surrounding areas (pixel ii-vi), which allows us to investigate the LSPR spectra at different locations of the NP. The LSPR spectra taken at each pixel location of (i-iv) are presented in Figure 6(A-F), respectively, and their peak wavelengths and FWHMs are summarized in Table 2. The result clearly illustrates that the LSPR spectrum of a single Ag NP depends on its locations within and surrounding the NP. We found that the primary peak wavelengths varied from 612–630 nm with their FWHMs ranging from 47 to 57 nm, and the first shoulder peak wavelengths varied from 512–524 nm for locations of ii–v, and the second very weak shoulder peak wavelengths were observed at 410, 451, and 465 nm for locations of ii, iv and v, respectively. Interestingly, no obvious shoulder peaks were observed in location (i) and (vi), where is on the center of the NP and the surrounding area next to the round curvature of the NP, respectively. Notably, the LSPR spectrum of location (vi) exhibits high symmetry, which may primarily attribute to the in-plane dipole plasmon resonance.

4. Summary

In summary, we have developed a new method to effectively correlate the optical image of single Ag NPs with their AFM images, which enables us to characterize optical properties (e.g., LSPR spectra) of single Ag NPs at sub-micrometer resolution (> 200 nm) using DFOMS and their 3D morphologies at nanometer resolution using AFM. This approach allows us to investigate shape-dependent LSPR spectra of single Ag NPs. We found that LSPR spectra of single Ag NPs with nearly equal volume, cross-section and height, exhibit high-dependence on their shapes. In general, the experimental measurements of shape-dependent LSPR spectra of single NPs agree well with DDA simulation results. Nonetheless, the simulation data of regular shaped NPs fit better with experimental results than those of irregular shaped NPs, suggesting that the simulation method needs to be further developed in order to better describe LSPR spectra of irregular shaped NPs. Furthermore, we can locate the position of a single NP in its optical image, by correlating its optical image with its AFM image, which permits us to measure LSPR spectra at different locations of a single NP, and its surrounding area. The result shows location-dependent LSPR spectra of a single NP on its surrounding area, offering a unique opportunity to characterize individual multi-mode plasmonic NPs at nanometer resolution for better understanding of optical properties of single NPs and rational design of single NP plasmonic probes. This study provides effective means to investigate 3D morphological dependent LSPR spectra of single NPs, offering valid experimental data for better understanding of effects of 3D morphologies on optical properties of individual NPs, and for further developing simulation methods that can accurately describe optical properties of NPs with irregular morphologies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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research project, and personally involved in every experimental detail, especially design of the photomask, all data analysis, interpretation, and presentation.

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Figure 1.

Photo of a photolithograph mask shows a 5×5 array of circles (18 mm in diameter). Each circle matches with the size of a glass coverslip and each circle consists of a 7×7 array of squares (each length of the square = $1383.3 \mu\text{m}$). Each square includes a 10×10 array of assemblies of distinctive micro-windows in a small square as shown in Figure 2A. This photomask allows us to create 122,500 assemblies of five distinctive micro-patterned windows simultaneously, enabling potential high-throughput fabrication. Scale bar is 18 mm.

Figure 2. Design and fabrication of an array of micro-patterned windows (each unit includes five distinctive shapes of micro-windows) on a glass coverslip using a photolithograph approach, for correlation and characterization of individual NPs at micrometer scale using DFOMS and at nanometer scale using AFM

(A) A bright-field optical image of a 10×10 array on the smallest square of photomask as illustrated in Figure 1;

(B) Zoom-in of each unit on the array in (A) shows five distinctive micro-windows;

(C) Close-up of the micro-windows in (B) shows five distinctive shapes: triangular, trapezium, circular, hexagonal, and parallelogram patterns.

(D) A dark-field image of five micro-windows patterned on a glass coverslip using a standard photolithography procedure, showing five distorted shapes with distinctive features. Scale bar is 500 μm in (A), 50 μm (B) and 10 μm in (C–D).

Figure 3. Correlation of AFM and optical images of single Ag NPs using distinctive micro-patterned windows

(A): (a) AFM images, (b) optical true-color images and (c) CCD images of single Ag NPs in a hexagon micro-patterned window;

(B) Zoom-in images of individual NPs squared in (A), showing the correlation of (a) AFM images of given single NPs at nanometer scale with (b) optical true-color images and (c) CCD images of single Ag NPs at sub-micrometer scale, as illustrated by a circled NP. Scale bar is 5 μm in (A: a–c) and 0.5 μm in (B: a–c). Note that the scale bars in (b–c) show the distances among individual NPs, but not the sizes of single NPs because they are imaged under optical diffraction limit.

Figure 4.

Characterization of shape-dependent optical properties (LSPR spectra) of single Ag NPs determined using experimental measurements and theoretical calculations. (A–E): (a) AFM images, (b) dark-field optical color images, and (c) normalized LSPR spectra: (i) experimental measurements and (ii) theoretical calculations of single Ag NPs. All scale bars are 100 nm. Note that the scale bars in (b) are used to measure the size of image arrays, but not the sizes of single NPs because NPs are imaged under optical diffraction limit.

Figure 5. Characterization of 3D morphological and optical properties of a single Ag NP
(A): (a) AFM image and (b) 3D-plot of AFM image shows a quasi-pentagonal cylindrical NP with various heights.
(B): (a) dark-field optical CCD image of the single Ag NP and (b) its 3D plot of scattering intensity in z-axis versus x-y spatial dimension. The grid in (a) represents the CCD pixel array with each square corresponding to a single CCD pixel.
(C): (a) dark-field optical color image of the single Ag NP and (b) its LSPR spectrum, showing a red NP with a primary peak wavelength of LSPR spectra at 626 nm and two shoulder peaks at 524 and 465 nm. Scale bar is 100 nm in (A: a) and 250 nm in (B and C: a). Note that

the scale bars in (B and C: a) are used to measure the size of image arrays, but not the sizes of single NPs because of optical diffraction limit.

Figure 6.

Characterization of location-dependent LSPR spectra of a single NP. (A–F): normalized LSPR spectra of various locations on and surrounding the single NP at single-pixel resolution, as those marked as (i–vi) in Figure 5B-a, respectively. Correlation and Characterization of 3D Morphological Dependent LSPR Spectra of Single Ag NPs Using Dark-field Optical Microscopy and AFM

Table 1

Summary of Shape-Dependent LSPR Spectra of Single Ag NPs

NP*	Shape of cross-section area	V (nm ³)	A (nm ²)	H (nm)	λ_{Exp} (nm)		$\lambda_{Theoretic}$ (nm)	
					$\lambda_{p,i}$	$\lambda_{s,i}$	$\lambda_{p,ii}$	$\lambda_{s,ii}$
					FWHM	FWHM	FWHM	FWHM
A	Quasi circle	8.8×10 ³	2.3×10 ³	39	506		489	
					82		96	
B	Oblate spheroid	8.7×10 ³	2.2×10 ³	39	537		576	414
					68		86	57
C	Irregular hexagon	8.6×10 ³	2.5×10 ³	35	548		510	
					75		117	
D	Quasi pentagon	9.0×10 ³	2.0×10 ³	45	603		649	457
					83		92	72
E	Quasi trapezoid	7.9×10 ³	2.0×10 ³	40	629	470	646	526
					85	N/A	91	47

* V, A, and H represents physical volume, cross-section area and average height (± 5 nm) of individual NPs measured by AFM in Figure 4A–E-a, respectively. The $\lambda_{p,i}$ and $\lambda_{s,i}$ represents the primary and shoulder peak wavelength of LSPR spectra of these individual NPs determined using DFOMS, and $\lambda_{p,ii}$ and $\lambda_{s,ii}$ represents the simulation results calculated using DDA. N/A represents undetermined FWHM (full width at half maximum).

Table 2

Summary of Location-Dependent LSPR Spectra of Single Ag NPs

Location *	λ_p (nm)	$\lambda_{s,1}$ (nm)	$\lambda_{s,2}$ (nm)
	FWHM (nm)	FWHM (nm)	FWHM (nm)
i	612		
	57		
ii	616	512	410
	47	45	N/A
iii	624	522	
	50	43	
iv	630	524	451
	51	43	N/A
v	625	512	465
	54	35	N/A
vi	619		
	54		

* Locations are marked in the CCD pixel array in Figure 5B-a. λ_p , $\lambda_{s,1}$ and $\lambda_{s,2}$ are primary, and the first and second shoulder peak wavelength of LSPR spectra in Figure 6. N/A represents undetermined FWHM.