Single-Particle Correlation Study: Polarization-Dependent Differential Interference Contrast Imaging of Two-Dimensional Gold Nanoplates

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Abstract

Questions surrounding the optical properties of two-dimensional (2D) triangular single gold nanoplates (AuNPs) remain largely unanswered. Herein, a scanning-electron microscopy-correlated single-particle study was conducted to identify polarization-dependent optical properties of AuNPs under dark-field (DF) and differential interference contrast (DIC) microscopy. AuNPs with an aspect ratio of ~3 showed a single broad DF scattering spectrum without separation of the two dipole and quadrupole resonance modes present in 2D AuNPs. Polarization-sensitive interference properties of the individual AuNPs were revealed through periodic changes in the intensities and types of DIC images obtained. Dipole resonance mode was found to mainly contribute to the polarization-sensitive interference properties of AuNPs. Furthermore, DIC polarization anisotropy allowed us to track real-time orientation of a dipole resonance mode of a AuNP rotating on a live cell membrane.

Keywords, Gold nanoplate, surface plasmon resonance, polarization dependence, differential interference contrast microscopy, dark-field microscopy
Introduction

Plasmonic gold nanoparticles have attracted a great amount of interest because of their size- and shape-dependent localized surface plasmon resonance (LSPR) properties. Furthermore, there has been impressive progress in the synthesis of a variety of gold nanoparticles, such as nanospheres, nanostars, nanorods, nanocubes, and nanoplates. In this regard, gold nanoparticles are widely used in many research fields, including bio-sensing, photothermal cancer therapy, catalysis, and drug delivery mechanisms. Recently, two-dimensional (2D) triangular gold nanoplates (AuNPs) have been gaining considerable interest due to their unique optical properties and extremely high anisotropy. 2D anisotropic AuNPs have large surface areas, sharp corners, and edges which can greatly enhance any applied electric fields. Therefore, 2D AuNPs are of importance in various applications, including bio-imaging and surface-enhanced Raman scattering.

To date, the optical properties of 2D AuNPs have been mainly investigated at the ensemble level. However, ensemble measurements are oftentimes limited by heterogeneity issues related to preparation of the AuNPs. In this respect, single-particle measurements need to be carried out to have a deeper insight into the relationship between the structures and their unique optical properties without the effects of ensemble averaging. Recent advances in single-particle spectroscopy techniques have enabled us to avoid ensemble averaging in the optical properties of non-homogeneous AuNPs and elucidate their unique optical properties at the single-particle level. Increasingly, research efforts are geared toward better comprehending the optical properties in 2D single AuNPs with large surface areas under dark-field (DF) microscopy. For instance, 2D AuNPs with large surface areas were shown to present two characteristic dipole and quadrupole resonance modes in the spectral ranges between the visible and near-infrared.

Despite the recent advances in elucidating the scattering properties of the individual
AuNPs, details surrounding their optical properties remain largely ambiguous. For example, 2D AuNPs have not been studied under interference-based differential interference contrast (DIC) microscopy, as such, there are still unanswered questions regarding their interference properties. More specifically, the dependence of the DIC image patterns (or intensities) of these 2D AuNPs on polarization and wavelength of light is unanswered. Therefore, it is necessary to verify the optical properties of 2D AuNPs with DIC microscopy a powerful imaging technique to visualize and characterize plasmonic nanoparticles.

In the current study, we present the scattering- and interference properties of 2D AuNPs with mean edge length and thickness of 125.7 nm and 42.5 nm, respectively, under DF and DIC microscopy. Particularly, a 360° rotational study was conducted to elucidate the effect of polarized light on DIC image patterns and their intensities at the respective LSPR wavelengths. To the best of our knowledge, this is the first report to focus on the interference properties of triangular 2D AuNPs with large surfaces under a DIC microscope and the polarization-dependent DIC images and intensities of AuNPs. Last, we demonstrated that DIC polarization anisotropy can be used to determine the orientation of a dipole resonance mode of single AuNP rotating on a live cell membrane.
Experimental Section

Chemicals and Materials

HAuCl₄, cetyltrimethylammonium chloride (CTAC, solution in water, 25 wt.%), AgNO₃, poly (vinyl pyrrolidone) (MW = 55,000), cetyltrimethylammonium bromide, HCl (37%), sodium citrate dihydrate, and NaBH₄ were all obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Ascorbic acid and ammonia (NH₃) were obtained from DaeJung Chemicals & Metals Co. and Junsei, respectively. All chemicals were reagent grade. Ultrapure water was used in the preparation of all aqueous solutions.

Scattering-Based Dark-Field Imaging and Spectroscopy

We performed scattering-based DF microscopy using an inverted microscope (ECLIPSE Ti-U, NIKON, Japan). A Nikon Plan Fluor oil iris objective (100×) with an adjustable numerical aperture (NA, 0.5–1.3) and a Nikon DF condenser were used for DF imaging. To obtain high quality DF scattering images, we used an Andor EMCCD camera (iXon Ultra 897, UK). The collected DF scattering images were analyzed via Image J and Matlab. After obtaining the DF images, the DF scattering spectra of the individual gold nanoplates (AuNPs) were taken using an Andor spectrometer (SHAMROCK303i, SR-303I-A, UK) equipped with an Andor CCD camera (Newton DU920P-OE, UK). The light scattered from AuNPs was collected via an objective lens and directed to the spectrometer to obtain a spectrum. The scattered light was dispersed by a grating (300 l/mm) inside the spectrometer and detected using the Andor CCD camera (Newton DU920P-OE, UK). Finally, a background spectrum was taken at an area without nanoparticles.

Differential Interference Contrast Microscopy

Interference-based DIC microscopy was performed using an inverted microscope.
DIC microscopy consists of a set of two Nomarski prisms, two polarizers, and a quarter-waveplate. We illuminated the samples by an oil-immersion DIC condenser with an NA of 1.4. DIC signals coming from the sample were collected using a Plan Apo oil-immersion objective (100×, NA = 1.4). An Andor EMCCD camera (iXon Ultra 897, UK) was employed to obtain high quality DIC images. Image J and Matlab were used to analyze the DIC images that were collected.
Results and Discussion

The synthesized 2D AuNPs were first characterized via scanning electron microscopy (SEM). Fig. 1A shows an SEM image of 2D AuNPs chemically prepared. The average edge length and height of the AuNPs were 125.7 nm and 42.5 nm, respectively, which yielded an aspect ratio (AR, defined as the edge length/height) of ~3. In this study, we observed that, by chance, some of the AuNPs were vertical on the glass slide in their respective SEM images (Fig. 1A). Therefore, the vertical AuNPs on the glass slide was used to measure the nanoparticles’ thickness. An extinction spectrum was obtained in water by using a Varian Cary 300 UV-Vis spectrophotometer (Agilent, Fig. S1). The 2D AuNPs typically presented two LSPR peaks in the ranges from visible to near-infrared \(^{28-29, 33-34, 36}\). In this study, we observed that two LSPR peaks were not completely separated for 2D AuNPs and that the dipole and quadrupole resonance modes appeared at approximately 535 nm and 635 nm, respectively, as shown in Fig. S1. The two dipole and quadrupole LSPR resonances could be induced in the ensemble sample of heterogeneous 2D AuNPs, as shown in Fig. 1B.

A single-particle study was conducted to better understand the optical properties of 2D AuNPs (AR = ~3) under DF microscopy and spectroscopy (Fig. S2). The experimental setup for single-particle microscopy and spectroscopy is provided in Fig. S3. In this study, samples were obtained by spin casting a solution containing AuNPs onto a pre-cleaned glass slide. The concentration of AuNPs cast on the glass surface was controlled at 1 μm\(^{-2}\) to facilitate single-particle measurements without the occurrence of inter-particle LSPR coupling. Furthermore, we employed a correlation study between SEM and optical microscopy, as already demonstrated in our previous studies, to ensure single-particle characterization and measurements \(^{37-38}\). Fig. 2A shows a DF scattering image of the individual AuNPs, while the inset in Fig. 2A shows a correlated SEM image of AuNP1 obtained from the correlation study. The edge length of AuNP1 was determined to be 128 nm, as shown in Fig. 2A. Fig. 2B is the
single-particle scattering spectrum of AuNP1 squared in Fig. 2A. A single broad LSPR spectrum was observed for AuNP1, peaking at approximately 637 nm. The 637-nm LSPR peak could be assigned to the dipole resonance mode in 2D AuNP1. However, the quadrupole resonance mode was not observed in the single-particle spectra of 2D AuNPs with an AR of ~3; this was further supported by the scattering spectra obtained for other AuNPs (Fig. S4). The disappearance of quadrupole mode in this single particle study could be mainly ascribed to the low scattering signal from single 2D AuNPs.

Next, we performed a rotational study for 2D AuNPs at the dipole LSPR wavelength of 640 nm. A polarizer and a band-pass filter with a center wavelength of 640 (± 5) nm were placed in the light path of the lamp to confirm the polarization-dependent scattering properties of the individual AuNPs with an AR of ~3. Fig. S5 shows changes observed in the scattering intensities of the three AuNPs in Fig. 2A at 640 nm when the polarizer was rotated from 0° to 180° at increments of 10°. The DF scattering intensity, which changed periodically, was found utilizing the rotation angle for the three AuNPs. In addition, it is worthwhile noting that the three AuNPs had different phases of DF scattering intensities, which were dependent on their dipole orientation at the same rotational angle, as shown in Fig. S5. Therefore, the results support the polarization-dependent scattering properties on the dipole resonance mode of the individual AuNPs with an AR of ~3.

Besides the scattering property of 2D AuNPs, the characteristic interference is also a significant optical property of 2D AuNPs. However, there are no studies to date, which characterize the interference properties of the AuNPs, therefore, we attempted to elucidate the interference properties at their LSPR wavelength of 640 nm under DIC microscopy. In DIC microscopy, the incident light was split into two orthogonally polarized beams in the two bright and dark polarization directions by the first Nomarski prism (Fig. S6).39-40 These two beams were separated by a certain distance (a few hundred nanometers) along the shear direction. After
the beams passed through the sample, they were recombined by the second Nomarski prism and interfered with each other. This interference at recombination was dependent on the difference in their optical path lengths. After the two orthogonally polarized beams passed through the sample, they generated image contrasts that were dependent on optical path length gradients in the sample. In this study, the dipole orientation of an AuNP was defined as the orientation angle $\theta$ between the dipole axis of an AuNP and the dark axis, as shown in Fig. S7. The orientation angle $\theta$ increased in the counterclockwise direction toward the bright axis. Fig. 3A presents a DIC image of individual AuNPs, and the inset shows a correlated SEM image of AuNP4 with an edge length of 139 nm. A single-particle scattering spectrum of AuNP4 was then obtained, as seen in Fig. 3B, in which a single broad LSPR peak was observed at around 635 nm. Therefore, the AuNPs were measured individually to determine their characteristic interference properties under a DIC microscope. Notably, we observed polarization-dependent DIC images of two AuNPs (AuNP4 and AuNP5) fixed on a glass slide at 640 nm, in which different bright and dark DIC images were obtained, depending on their dipole orientations in Fig. 3A. The brightest intensity was noted for AuNP4, which meant that the dipole axis of AuNP4 was parallel to the bright polarization axis ($\theta = 90^\circ$, red-arrow). In contrast, the darkest intensity was observed for AuNP5, which meant that it was parallel to the dark polarization axis ($\theta = 0^\circ$, blue-arrow).

To better understand the polarization-dependent DIC imaging of the individual AuNPs, a rotational study was performed, in which the stage was rotated by 10° per step to position the AuNPs in different orientations. Interestingly, the DIC image patterns of AuNP4 were changed periodically as a function of the polarization direction (Fig. 4A). For example, a completely bright image pattern was observed at the rotational angle of 0°. However, when the rotational angle was 90°, a dark image pattern was observed. The polarization-dependent DIC images were also observed for AuNP 5 (Fig. S8). Moreover, it was worthwhile noting that the result showed a good agreement with the polarization-dependent DIC images of short gold nanorods.
with a single dipole character. DIC intensities from bright and dark polarization directions at 640 nm were then plotted as a function of rotational angle in Fig. 4B. The DIC intensities changed periodically in both polarization directions when the stage was rotated by 10° per step. The bright and dark intensity curves were anti-correlated with a shift of ~90°, that is, an increase in the bright intensity was accompanied by a decrease in the dark intensity and vice versa (Figs. 4B and S9). Therefore, we were able to demonstrate that the dipole resonance mode mainly contributes to the optical properties of AuNPs with an AR of ~3. Polarization-dependent interference properties of the individual AuNPs were also demonstrated under DIC microscopy.

Last, we used the DIC polarization anisotropy to track the rotational dynamics of 2D AuNPs rotating on a live cell membrane. The detailed information on the DIC polarization anisotropy is provided in the electronic supplementary information. We therefore recorded movies showing the dynamics of 2D AuNPs with a temporal resolution of 100 ms at 640 nm under DIC microscopy. Fig. S10 shows a DIC image of AuNPs rotating on a HeLa cell membrane. Fig. 5A presents consecutive DIC images of AuNP6 (Fig. S10) rotating on the live cell membrane as a function of time. In Fig. 5, 20 consecutive frames of AuNP6 were chosen from the recorded movies. We observed changes in the DIC image patterns over time (Fig. 5A), and the normalized DIC intensities for two bright (blue) and dark (red) polarization directions at 640 nm were plotted as a function of time in Fig. S11. We observed that a DIC signal of a rotating AuNP6 gives anti-correlated intensities in the two orthogonal polarization directions. We then calculated the DIC polarization anisotropy from the two orthogonal bright and dark DIC intensities as a function of frames, and the DIC polarization anisotropy was distributed between -1 and +1 (Fig. 5B). Therefore, DIC polarization anisotropy measurement allowed us to track real-time orientation of an anisotropic 2D AuNP during the dynamic process. To the best of our knowledge, this is the first report to present polarization-dependent interference properties of 2D AuNPs, and to track real-time rotational dynamics of the dipole resonance...
mode of AuNP with DIC microscopy.

Conclusions

In summary, we investigated the polarization-dependent optical properties of 2D AuNPs with large surfaces under DF and DIC microscopy. It was demonstrated that the dipole resonance mode of AuNPs with an AR of ~3 mainly contributed to their scattering and interference properties. More importantly, the polarization-dependent interference properties of the individual AuNPs were revealed via periodic changes in the intensities and types of DIC images obtained at their dipole LSPR wavelength of 640 nm. Last, we demonstrated that the orientation of a dipole resonance mode of a single AuNP rotating on the live cell membrane can be tracked with DIC polarization anisotropy at the temporal resolution of 100 ms. Therefore, we were able to provide a better understanding of the optical properties of 2D AuNPs and their possible uses in many biological and physical applications.
Acknowledgements

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Supporting Information

This document contains additional experimental methods and supporting figures (Figs. S1 to S11). This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.
References


Figure Captions

Fig. 1 (A) SEM image of the individual Au nanoplates. Note that some AuNPs are vertical. (B) Schematic depicting the formation of two typical dipole and quadrupole modes for 2D AuNPs.

Fig. 2 (A) DF scattering image of the individual AuNPs. SEM image of AuNP1 is also presented in the inset. (B) Single-particle scattering spectrum of AuNP1. The LSPR peak is observed at around 637 nm, which is close to a 640-nm excitation.

Fig. 3 (A) DIC image of individual AuNPs. SEM image of AuNP4 is also presented in the inset. (B) Single-particle scattering spectrum of AuNP4. The LSPR peak is observed at around 635 nm, which is close to a 640-nm excitation.

Fig. 4 (A) Polarization-dependent DIC images of AuNP4 as a function of rotational angle with 10° intervals. (B) Periodic changes in bright and dark DIC intensities of AuNP4 as a function of the rotational angle.

Fig. 5 (A) Consecutive DIC images of a AuNP6 rotating on a live cell membrane. (B) DIC polarization anisotropy for a AuNP6 as a function of time with a temporal resolution of 100 ms.
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Fig. 3 (A) DIC image of individual AuNPs. SEM image of AuNP4 is also presented in the inset. (B) Single-particle scattering spectrum of AuNP4. The LSPR peak is observed at around 635 nm, which is close to a 640-nm excitation.
Fig. 4 (A) Polarization-dependent DIC images of AuNP4 as a function of rotational angle with 10° intervals. (B) Periodic changes in bright and dark DIC intensities of AuNP4 as a function of the rotational angle.
Fig. 5 (A) Consecutive DIC images of a AuNP6 rotating on a live cell membrane. (B) DIC polarization anisotropy for a AuNP6 as a function of time with a temporal resolution of 100 ms.
Graphical Index

DIC Image of Au Nanoprism

Polarization Dependence

0° 10° 20° 30° 40° 50° 60° 70° 80° 90° 100° 110° 120° 130° 140° 150° 160° 170° 180°

500 nm