Platinum-coated Core-Shell Gold Nanorods as Multifunctional Orientation Sensors in Differential Interference Contrast Microscopy

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We characterized the optical properties of single platinum-coated core-shell gold nanorods (Pt-AuNRs) under dark-field (DF) and differential interference contrast (DIC) microscopy. Furthermore, we examined their potential use as multifunctional orientation probes. Longitudinal surface plasmon resonance damping was observed for single Pt-AuNRs due to Pt metals coated on the AuNR surface under single-particle scattering spectroscopy. Despite the strong plasmon damping with a much-decreased scattering intensity, DIC microscopy allowed us to detect single Pt-AuNRs with much higher sensitivity. We found polarization-dependent DIC images and intensities of single Pt-AuNRs, which allowed us to determine their orientation angle under DIC microscopy. Therefore, we report that single Pt-AuNRs can be used to develop multifunctional orientation probes under DIC microscopy.

Keywords Bimetallic core-shell nanorods, platinum-coated gold nanorods, differential interference contrast microscopy, surface plasmon resonance, orientation probes

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DIC image patterns and intensities of single Pt-AuNRs at their LSPR wavelengths, and checked their potential use for developing multifunctional orientation probes under a DIC microscope.

**Experimental**

**Sample preparation and characterization**

Bare AuNRs (25 × 73 nm) without other metals on the surface were obtained from Nanopartz (Loveland, CO, USA) (Fig. S1), and Pt-AuNRs with an average size of 25 × 73 nm were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Pt-AuNR colloid solution was first diluted with 18.2-MΩ pure water to a proper concentration. The diluted solution was then sonicated for 15 min at room temperature. Samples were prepared by spin casting the solution on the pre-cleaned glass slide. Then, a 22 × 22 mm No. 1.5 coverslip (Corning, NY) was covered on the glass slide. In this study, the concentration of Pt-AuNRs on the glass surface was controlled to be ~1 μm⁻² in order to facilitate single-particle characterization and to minimize inter-particle SPR coupling, resulting in a spectral shift. Structural characterization was carried out using a transmission electron microscope (TEM) (H-8100, Hitachi, Japan).

**Single particle scattering microscopy and spectroscopy**

DF microscopy imaging was performed under a Nikon inverted microscope (ECLIPSE Ti-U) in this study. In the DF mode, the microscope utilized a Nikon Plan Fluor 100× 0.5 – 1.3 oil iris objective and a Nikon DF condenser. An Andor iXon EM+ CCD camera (iXon Ultra 897) was employed to record DF images of Pt-AuNRs. The collected images were analyzed with Image J. Furthermore, DF scattering spectra were acquired with an Andor spectrometer (SHAMROCK 303i, SR-303I-A) and an Andor CCD camera (Newton DU920P-OE). When taking a spectrum, the scanning stage moved the sample to the desired location so that only the scattered light from the selected location would be collected by the objective. The scattered light was directed to the entrance of the spectrometer, dispersed by a grating (300 l/mm, center wavelength, 700 nm), and detected by the Newton CCD camera. The background was measured at a region without any particles. Data analysis was performed with specially designed Matlab programs.

**Results and Discussion**

Figure 1A shows a transmission electron microscopy (TEM) image of the Pt-AuNRs with a mean length and diameter of 73 and 25 nm, respectively. The thickness of Pt coated on the AuNR surface was estimated to be about 3 – 7 nm, as shown in a magnified TEM image (Fig. 1B). More TEM images are provided in Supporting Information (Fig. S2). We then obtained UV-Vis absorption spectra of both bare AuNRs and Pt-AuNRs dispersed in water (Fig. S3). Anisotropic AuNRs typically have two distinct LSPR absorption bands associated with transverse plasmon band and longitudinal plasmon band, corresponding to electron oscillation along the short and long axes of the AuNR, respectively. As shown in Fig. S3, the transverse SPR mode of bare AuNRs appeared at around 521 nm, and the prominent longitudinal SPR mode was observed at 714 nm. However, the longitudinal SPR peak was strongly damped with an increased SPR linewidth for the Pt-AuNRs due to Pt clusters coated on the AuNR surface. More notably, Pt-AuNRs caused stronger damping in the longitudinal SPR peak than palladium (Pd)-coated AuNRs of similar size.34

To have a better understanding of the scattering properties and
plasmon damping of Pt-AuNRs, we performed single-particle measurements that eliminated ensemble averaging under DF scattering microscopy and spectroscopy. This single particle study to elucidate their optical properties is also necessary to develop multifunctional orientation probes using bimetallic Pt-AuNRs. The experimental setup used in this study is provided in Fig. S4. A sample was prepared by spin casting a solution containing Pt-AuNRs on a pre-cleaned glass slide, and the Pt-AuNRs were positioned relatively flat to the surface when they were fixed to the slide. The concentration of Pt-AuNRs on the glass surface was controlled to be 1 μm⁻² in order to facilitate single-particle characterization and to avoid inter-particle SPR coupling, resulting in a spectral shift. Figure 2A shows a DF scattering image of Pt-AuNRs deposited on a glass slide. We then obtained single-particle scattering spectra of a Pt-AuNR1 highlighted with a yellow square in Fig. 2A and a bare AuNR without Pt (Fig. 2B). It is worthwhile noting that Pt metals coated on the AuNR surface resulted in longitudinal plasmon damping, which decreased scattering intensity and increased the SPR linewidth as shown in the overlaid, normalized scattering spectra (Fig. 2B). This result from single-particle measurements is consistent with the result from ensemble experiments, as shown in Fig. S3. Furthermore, it should be noted that scattering-based DF microscopy is not a good choice for the single-particle rotation tracking of bimetallic Pt-AuNRs, which show strong plasmon damping and greatly reduced scattering intensity.

On the other hand, interference-based DIC microscopy is better suited to decipher the orientation and rotational motion of Pt-AuNRs than scattering-based DF microscopy, which is significantly affected by plasmon damping. In a DIC microscope, the incident plane-polarized beam is separated into two orthogonally polarized mutually coherent beams pertaining to the bright (blue-line) and dark (red-line) polarization directions by the first Nomarski prism (Fig. S5). After the beams pass through the sample, they are recombined by the second Nomarski prism and interfere with each other. This interference at recombination is sensitive to their optical path-length difference. When two beams pass through the sample, they generate image contrasts for optical path-length gradients in the sample. Therefore, each of the two orthogonally polarized beams generates an independent intermediate image. For an anisotropic shape of AuNRs, two intermediate images are different compared to the isotropic shape of gold nanospheres.

So far, the optical properties of single Pt-AuNRs have not been studied under DIC microscopy, and their DIC images and intensities as a function of the orientation angle, ϕ, of Pt-AuNR with the respect to the dark polarization axis. (B) Magnified DIC images of two Pt-AuNRs highlighted in (A). DIC image patterns are different for the two Pt-AuNRs, depending on their orientations on a glass.
orientation on the glass slide. For example, a totally dark image pattern is observed when a Pt-AuNR is aligned parallel to the dark polarization axis ($\phi = 0^\circ$, red-arrow). Therefore, orientation-dependent DIC image patterns of single Pt-AuNRs were observed with high contrast and sensitivity under DIC microscopy.

We further performed polarization-dependent DIC imaging at 700-nm excitation by rotating the rotational stage by $10^\circ$ per step for the fixed Pt-AuNR2 marked by the yellow square in Fig. 3A. When we rotated, the Pt-AuNR was positioned in different orientations under DIC microscopy. Figure 4A shows the complete set of DIC images of Pt-AuNR2 from 0 to 180° with an interval of 10°. As shown in Fig. 4A, we found that DIC image patterns are periodically changed as a function of the rotation angle. To gain a deeper insight into the polarization dependence, the normalized DIC intensities for two bright (blue) and dark (red) polarization directions are plotted as a function of the rotation angle in Fig. 4B. The DIC intensities changed periodically in both polarization directions when the stage rotated by $10^\circ$ per step. The bright (blue) and dark (red) intensity curves were anti-correlated; that is, an increase in the bright intensity was accompanied by a decrease in the dark intensity, and vice versa (Fig. 4B, top). Furthermore, the normalized bright and dark DIC intensities for Pt-AuNR2 were well fitted with functions of $\sin^4(\phi)$ and $\cos^4(\phi)$, respectively (Fig. 4B, top). Therefore, we found polarization-sensitive, periodic DIC images and intensities of bimetallic Pt-AuNRs at the single-particle level. Furthermore, this result indicates that we can determine the spatial orientation of single Pt-AuNRs by measuring their DIC images and intensities. Note that this is challenging for DF scattering microscopy strongly affected by plasmon damping, which results in greatly decreased scattering intensities. To the best of our knowledge, this is the first report to present the polarization-sensitive optical properties of single Pt-AuNRs under DIC microscopy.

DIC polarization anisotropy using an intensity ratio instead of absolute intensities is less affected by any intensity instabilities, so that it can lead to more accurate, reproducible and reliable angle measurements. We therefore attempted to calculate the DIC polarization anisotropy, $P$, which can be conveniently obtained from the bright and dark intensities of a single DIC image of the nanorod. DIC polarization anisotropy is defined by

$$P = \frac{I_{BN} - I_{DN}}{I_{BN} + I_{DN}},$$

where $I_{BN}$ and $I_{DN}$ are the normalized bright and dark intensities, respectively. Figure 4B shows the DIC polarization anisotropy, $P$, for Pt-AuNR2 as a function of orientation angle $\phi$. For the Pt-AuNR2 the DIC polarization anisotropy $P$ was changed between the minimum and maximum values of $-1$ and $+1$ depending on its orientation. When the Pt-AuNR2 is aligned parallel to the bright axis, $P$ is going to be the maximum value of 1, as illustrated in Fig. S6. Note that DIC polarization anisotropy, $P$, can be related to the orientation angle, $\phi$, as shown in the following.

$$\phi = \arccos \left( \frac{A - \sqrt{A^2 - 2A}}{2} \right), \quad P < 0$$

$$\phi = \arccos \left( \frac{A + \sqrt{A^2 - 2A}}{2} \right), \quad P > 0$$

where $A$ is defined as $(P - 1)/P$. The detailed calculation to get the above equation is described in Supporting Information.

Fig. 4 (A) Polarization-dependent DIC images of single Pt-AuNR2 obtained by rotating a rotational stage with an increment of $10^\circ$. The Pt-AuNR was excited at 700 nm close to its longitudinal SPR mode. (B) DIC intensity profiles (top) and DIC polarization anisotropy (bottom) of the Pt-AuNR2 as a function of rotation angle. The normalized bright and dark DIC intensities were well fitted with functions of $\sin^4(\phi)$ and $\cos^4(\phi)$, respectively. Furthermore, the experimental $P$ values (pink dots) were well fitted with the calculated $P$ values (green curve).
Therefore, the orientation angle, $\phi$, can be deduced backward from the measurement of the DIC polarization anisotropy, $P$. In the present study, we found that DIC polarization anisotropy can be used to determine the orientation angles of single Pt-AuNRs.

Last, it is necessary to compare the Pt-AuNR probes with bare AuNRs conventionally used as orientation probes. We can determine the orientation angles of both Pt-AuNRs and bare AuNRs with high sensitivity under DIC microscopy. However, a major advantage of using the Pt-AuNRs is that we can add more functions to the bare AuNR probe. For example, the Pt-AuNRs can be used as multifunctional orientation probes that can provide more functions because of the synergetic effect of different metals that are capable of sensing of hydrogen gas, humidity, etc. Furthermore, the Pt-AuNRs can be used as more efficient photocatalysts than bare AuNRs.

**Conclusions**

In summary, we present the optical properties of platinum-coated core-shell gold nanorods (Pt-AuNRs) under scattering-based DF and DIC microscopy at the single-particle level. The longitudinal SPR peak was strongly damped with greatly decreased scattering intensity and increased LSPR linewidth. Despite the strong plasmon damping observed in DF microscopy, interference-based DIC microscopy enabled the detection of single Pt-AuNRs with much higher contrast and sensitivity. We then examined their use as orientation probes, and observed polarization-sensitive DIC images and intensities of single Pt-AuNRs. The normalized DIC intensities from bright and dark polarization directions at the longitudinal SPR wavelength of 700 nm were anti-correlated and well fitted with $\sin^4(\phi)$ and $\cos^4(\phi)$, respectively. Furthermore, we found that the DIC polarization anisotropy, $P$, computed from the orthogonally polarized bright and dark intensities can be used for more accurate and reliable angle measurements of single Pt-AuNRs under DIC microscopy. Therefore, the results support that single Pt-AuNRs with two different metals could be used for developing multifunctional orientation probes that can be applied to biological and physical studies in combination with a DIC microscope.

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**Supporting Information**

This document contains additional experimental methods and supporting figures (Figs. S1 to S6). This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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