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Polarization-resolved extinction and scattering cross-sections of individual gold nanoparticles measured by wide-field microscopy on a large ensemble

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We report a simple, rapid, and quantitative wide-field technique to measure the optical extinction $\sigma_{\text{ext}}$ and scattering $\sigma_{\text{sca}}$ cross-section of single nanoparticles using wide-field microscopy enabling simultaneous acquisition of hundreds of nanoparticles for statistical analysis. As a proof-of-principle, we measured gold nanoparticles of 40 nm and 100 nm diameter and found mean values and standard deviations of $\sigma_{\text{ext}}$ and $\sigma_{\text{sca}}$ consistent with the literature. Switching from unpolarized to linearly polarized excitation, we measured $\sigma_{\text{ext}}$ as a function of the polarization direction and characterized the nanoparticle asphericity. The method can be implemented cost-effectively on any conventional wide-field microscope and is applicable to any nanoparticles. © 2013 American Institute of Physics. [http://dx.doi.org/10.1063/1.4800564]

Metallic nanoparticles (NPs) exhibit morphology-dependent localized surface plasmon resonances (LSPRs), which couple to propagating light and manifest as an increased particle polarisability at the LSPR frequency. Besides fundamental interest, these local optical resonances can be exploited to image metallic NPs with high sensitivity and to probe nanoscale regions in the NP vicinity via the local field enhancement effect, with possible applications ranging from sub-wavelength optical devices,1 catalysis2 and photovoltaics3 to biomedical imaging4 and sensing.6–8

Important physical quantities characterizing the linear optical properties of a NP are the absorption cross-section $\sigma_{\text{abs}}$, the scattering cross-section $\sigma_{\text{sca}}$, and the resulting extinction cross-section $\sigma_{\text{ext}} = \sigma_{\text{abs}} + \sigma_{\text{sca}}$. Beyond traditional ensemble average measurements, a number of approaches have been developed recently to measure $\sigma_{\text{ext}}$ at the single particle level, showing that the optical properties of individual NPs can significantly differ from the ensemble average owing to inhomogeneities in NP size and shape. It is, therefore, particularly important to develop a technique that is able to rapidly quantify the cross-sections at the single NP level and to perform a statistical analysis over many NPs, providing a relevant sample characterization. Quantitative values of $\sigma_{\text{ext}}$, $\sigma_{\text{abs}}$, and/or $\sigma_{\text{sca}}$ of single NPs have been reported using dark-field micro-spectroscopy,9 photothermal imaging,10 and spatial modulation micro-spectroscopy.11 However, in order to provide cross-section values in absolute units, dark-field micro-spectroscopy and photothermal imaging require a calibration reference (e.g., by comparison with $\sigma_{\text{ext}}$ known from theory), while spatial modulation micro-spectroscopy needs a precise measurement of the beam profile at the sample. Moreover, photothermal imaging and spatial modulation micro-spectroscopy are beam-scanning techniques, therefore, costly and less amenable to the rapid characterization of a large number of NPs compared to wide-field techniques. Additionally, they are modulation-based which requires specialized equipments such as acousto-optical modulators and lock-in detection.

In this work, we report a simple and quantitative wide-field technique to measure $\sigma_{\text{ext}}$ and $\sigma_{\text{sca}}$ on single NPs using conventional bright and dark-field microscopy without the need for calibration standards and with a field of view enabling simultaneous acquisition of hundreds of NPs.

The experimental set-up consists of an inverted microscope (Nikon Ti-U) equipped with a white-light illumination (halogen lamp 100 W with Nikon neutral color balance filter), a oil condenser of 1.4 numerical aperture (NA) with a removable home-built dark-field illumination of 1.1–1.4 NA, a 40× 0.95 NA dry objective, a 1.5× intermediate magnification, and a Canon EOS 40D color camera attached to the left port of the microscope. Images were taken in Canon 14-bit RAW format with 10.1 megapixel resolution. The RAW images were converted using the DCRAW plugin in IMAGEJ, providing 16bit RGB images with a linear response to intensity and no color balancing. The investigated samples were gold NPs (GNPs) of nominal 40 nm and 100 nm diameter (BBInternational) covalently bound onto a glass coverslip functionalised with (3-mercapto) triethoxysilane, covered in silicone oil (refractive index $n = 1.518$) and sealed with a glass slide.

Dark-field microscopy was performed initially to confirm the presence of metallic NPs appearing as colored scatterers, distinguishable from the white scattering of other debris or glass roughness. The color camera enables a coarse spectroscopic detection separating the three wavelength ranges of red (R) 570–650 nm, green (G) 480–580 nm, and blue (B) 420–510 nm. Once a suitable region was located and focussed, a dark-field image was taken. Subsequent bright-field microscopy was performed by removing the dark field ring and adjusting the condenser numerical aperture NA of the objective to match the objective NA. To quantitatively measure the extinction cross-sections, two bright-field transmission images were taken, one with the NPs in the objective focus and the second one out-of-focus, moving the objective by approximately $d = 15 \mu m$ axially away from the sample. Background images were taken for blocked illumination. To achieve the lowest shot noise, the lowest camera sensitivity was used (100 ISO), for which the full-well capacity of the

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In the dipole approximation, the standard deviation for the G channel, using a constant written in extinction image, we developed an image analysis program written in image macro language. We split the raw images into RGB channels, subtract the background, average the multiple acquisitions of $I_I$ and $I_d$, and calculate $\Delta$. We then determine the particle locations as the maxima of $\Delta$ with values in a range adjusted to reject noise and large aggregates. For each maximum, we choose $A_i$ given by a centered disk of radius $r_i = 3\lambda/(2NA)$ = 837 nm and calculate $\sigma_{ext}$.

We select individual NPs using their extinction color, retaining NPs with $\sigma_{ext}$ largest in the color channel corresponding to the excited plasmon resonance, i.e., green (red) for 40 nm (100 nm) spherical GNPs having a LSPR at 540 nm (590 nm) in a surrounding medium of 1.5 refractive index. NPs which likely correspond to aggregates, debris, or largely non-spherical GNPs are excluded in this way. The statistical results over 104 individual GNPs of nominally 40 nm diameter are summarised in Fig. 2. The distribution of $\sigma_{ext}$ in the G channel corresponding to the plasmon resonance has a mean of $\bar{\sigma}_{ext} = 4000$ nm$^2$, which is consistent with experimental and theoretical values found in the literature. The standard deviation $\sigma_{total} = 1300$ nm$^2$ of $\sigma_{ext}$ contains a part $\sigma_{noise}$ due to measurement noise. This part is determined from the distribution of $\sigma_{ext}$ in image regions not containing NPs, which has a zero mean and a standard deviation $\sigma_{noise}$. We find $\sigma_{noise} = 590$ nm$^2$ for the G channel. The standard deviation $\sigma_{ext}$ arising from the NPs is accordingly determined by $\sigma_{ext}^2 = \sigma_{total}^2 - \sigma_{noise}^2$, resulting in $\bar{\sigma}_{ext} = 960$ nm$^2$ for the G channel. $\sigma_{ext}$ can be attributed to a size distribution of the GNPs as follows. The scaling of $\sigma_{ext} \propto R^\gamma$ for spherical particles of radius $R$ is known from Mie theory. In the dipole approximation, $\gamma \approx 3$ for small particles, where the extinction is dominated by absorption and increases towards $\gamma = 6$ for larger particles, where the extinction is dominated by scattering. We found $\gamma \approx 3$ for 40 nm diameter at 532 nm wavelength using calculated absorption and scattering cross-sections. This scaling allows

![FIG. 1](image-url)  
*(a) Full color (FC) dark-field image of 40 nm diameter gold NPs. (b) Corresponding FC extinction image $\Delta$ from 0 (black) to 0.043 (white). (c) Zoom of dark-field image. (d) Zoom of the FC bright-field transmission with NP in focus $I_I$ (d) and out-of-focus $I_d$ (e). (f) Zoom of FC extinction image.*

![FIG. 2](image-url)  
*Distributions of $\sigma_{ext}$ for 104 GNPs of nominal 40 nm diameter in the R, G, and B color channels as indicated. The grey histograms show the noise distribution obtained by measuring $\sigma_{ext}$ in 183 randomly selected regions without NPs.*
us to estimate the relative standard deviation of the radius \( \Delta R/R = \sigma_{\text{ext}}/(\sigma_{\text{ext}}) \approx 0.08 \). The manufacturer specifies \( \Delta R/R < 0.08 \) for 40 nm and 100 nm particles determined by electron microscopy. Thus, \( \sigma_{\text{ext}} \) is on the upper limit of what expected from the size distribution of spherical particles in a constant dielectric environment. It has been shown in the literature that additional factors influencing \( \sigma_{\text{ext}} \) might be the NP non perfect sphericity, as well as fluctuations in the measured light.

Conversely, scattering dominates for larger particles such that \( \sigma_{\text{ext}} \) is proportional to the scattering cross-section \( \sigma_{\text{sc}} \), given as circles. The dotted line gives the fitted scaling as labeled. The limit of our camera is known, \( r_{\text{ext}} = 589 \text{ nm} \), we used exposure times in the order of 5 s.

For the green channel of Fig. 2, we have \( N_{\text{ph}} = 4 \times 10^4 \), \( N_2 = 36 \), \( M = 60 \), \( d_{\text{px}} = 5.7 \text{ m} \mu \text{m} \), \( NA = 0.95 \), \( \lambda = 0.53 \mu \text{m} \), and \( \nu = 1/2 \), yielding \( \sigma_{\text{noise}} = 589 \text{ nm}^2 \), in agreement with the measured noise. The blue and red channels have a factor of \( \sqrt{2} \) larger noise due to the smaller \( \nu \). This detection limit could be improved by using an oil immersion objective with NA 1.45 NA, \( M = 150 \), and \( N_2 = 1800 \) possible in a 60 s video, for which Eq. (1) yields \( \sigma_{\text{noise}} = 43 \text{ nm}^2 \), which would allow measuring single GNPs down to 10 nm diameter.

We note that the finite angular range of the objective implies that it collects also a fraction of the scattered light, leading to an underestimate of the extinction. The solid angle in the sample with 1.5 refractive index collected by the objective is 1.6 sr, which for isotropic scattering is 13% of the scattered light. We could correct for this by adding 13% of the measured \( \sigma_{\text{sc}} \) to \( \sigma_{\text{ext}} \). We also note that \( \sigma_{\text{sc}} \) is determined using the scattering of the dark-field excitation into the objective, which also has a certain angular range that needs to be considered if the scattering is sufficiently anisotropic.

Furthermore, we measured the dependence of \( \sigma_{\text{ext}} \) on the polarization angle \( \theta \) of the excitation light, which is a sensitive probe of NP asphericity, by inserting a linear polariser in the illumination beam path before the condenser. The resulting \( \sigma_{\text{ext}}(\theta) \) is shown in Fig. 4 (right) for \( \theta \) between 0° and 180° in steps of 10° for two selected GNPs in the red channel. We analyze these results by fitting the expression \( \sigma_{\text{ext}}(\theta) = \sigma_0 (1 + z \cos (2(\theta - \theta_0))) \), where \( \sigma_0 \) is the polarization-averaged \( \sigma_{\text{ext}} \), \( z \geq 0 \) is the amplitude of the polarization dependence, and \( 0 \geq \theta_0 \geq \pi \) is an angular offset, indicating the direction of the NP asymmetry. To estimate the influence of the measurement noise on the fit parameters, we calculated their distribution over Gaussian random fluctuations of the fitted \( \sigma_{\text{ext}}(\theta) \) with a standard deviation \( \sigma_{\text{noise}} \). GNP1 has a fitted \( z = 0.07 \), and its distribution has a mean value \( \bar{z} = 0.15 \) and a standard deviation
\( \tilde{\alpha} = 0.08 \). GNP2 instead is significantly non-spherical with a fitted \( \tilde{\alpha} = 0.75 \) and a distribution with \( \tilde{\alpha} = 0.75 \) well above \( \tilde{\alpha} = 0.06 \). The red channel is used here, as it is most sensitive to LSPR shifts due to asphericity.

The distribution of \( \alpha \) over the NP ensemble is shown in Fig. 4 (left) for 40 nm and 100 nm GNPs for different color channels. For comparison, the simulated distribution of \( \alpha \) for GNPs having \( \sigma_{\text{ext}}(\theta) \) given by the fit function is shown for \( \alpha = 0, 0.1, 0.2, 0.5, 0.8 \) in black lines using \( \sigma_\alpha = \sigma_{\text{ext}} \) of the color channel. The comparison shows that the polarization dependence can identify non-spherical GNPs through the distinct values of \( \alpha \). To further infer the NP geometrical aspect ratio from these data, a comparison with theory is needed which will be reported in a forthcoming work.

In conclusion, we have shown that conventional wide-field microscopy can be implemented with a consumer camera to extract quantitative values of polarization-resolved extinction, scattering, and absorption cross-sections of individual nanoparticles and generate histograms for statistical characterization of large numbers of particles. Although quasi-spherical gold nanoparticles of 40 nm and 100 nm diameter were used in this work for proof of principle, the technique is applicable to any nanoparticles, with a detection sensitivity limit in the order of 100 nm\(^2\). Importantly, this technique can be adopted by any laboratory equipped with conventional wide-field microscopy as a tool to quantify the linear optical response of a statistically significant number of individual nanoparticles.

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