All-Optical Imaging of Gold Nanoparticle Geometry Using Super-Resolution Microscopy

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ABSTRACT: We demonstrate the all-optical reconstruction of gold nanoparticle geometry using super-resolution microscopy. We employ DNA-PAINT to get exquisite control over the (un)binding kinetics by the number of complementary bases and salt concentration, leading to localization accuracies of ∼5 nm. We employ a dye with an emission spectrum strongly blue-shifted from the plasmon resonance to minimize mislocalization due to plasmon-fluorophore coupling. We correlate the all-optical reconstructions with atomic force microscopy images and find that reconstructed dimensions deviate by no more than ∼10%. Numerical modeling shows that this deviation is determined by the number of events per particle, and the signal-to-background ratio in our measurement. We further find good agreement between the reconstructed orientation and aspect ratio of the particles and single-particle scattering spectroscopy. This method may provide an approach to all-optically image the geometry of single particles in confined spaces such as microfluidic circuits and biological cells, where access with electron beams or tip-based probes is prohibited.

INTRODUCTION

As a consequence of their plasmon resonance, metal nanoparticles confine incident optical fields to subdiffraction limited volumes.1 Concentrating these optical fields enhances the linear and nonlinear optical response of nearby emitters,2,3 while binding of biomolecules in these high-field regions results in modifications of the plasmonic response.4−6 The optical properties and performance of metal particles in these applications strongly depend on their size and shape.7,8 Nanoparticle geometry is usually studied using methods such as atomic force microscopy (AFM) and electron microscopy (EM). Correlation between optical properties and nanoparticle geometry now requires colocalization schemes across different techniques to allow unambiguous comparisons.9,10 Electron microscopy usually requires the sample to be dried and exposed to ultrahigh vacuum, potentially perturbing surface functionalization. AFM on the other hand requires nanoparticles to be physically accessible to the tip, prohibiting studies in confined spaces such as microfluidic circuits and biological cells.

To overcome the limitations imposed by correlative techniques, there is a need to all-optically reconstruct the geometry of plasmonic nanoparticles and their assemblies. Pioneering studies employed PALM,11 ground-state depletion microscopy,12−14 or immobilized dyes combined with stochastic optical reconstruction microscopy.15,16 However, resonant coupling between the plasmon and the fluorophore resulted in a localization bias toward the center of the nanoparticle. Recently the mislocalization of a fluorophore that is resonantly coupled to a gold nanosphere was quantified17 to be up to 50 nm, depending on the particle-fluorophore distance. Lim et al.18 and Raab et al.19 performed similar experiments finding a fluorophore near a resonant nanoparticle can induce localization errors of up to 90 nm.

It was recently shown that this plasmon-fluorophore coupling can be minimized by choosing a fluorophore with an emission spectrum strongly blue-shifted from the plasmon resonance and by keeping the fluorophore at a distance of >6 nm from the gold surface to prevent quenching.20−22 Although clever design of the experiments have minimized plasmon−fluorophore coupling, these approaches employed fluorophores electrostatically stuck to a polyelectrolyte coated particle.23 This offers little control over the fluorophore density and location, and limits the localization accuracy to several tens of nanometers due to bleaching.

Here we overcome these limitations by employing DNA-PAINT (points accumulation for imaging nanoscale topography) to all-optically reconstruct gold nanoparticle geometry. The unique advantage of this method lies in the exquisite control over the (un)binding kinetics by the number of complementary bases and salt concentration, leading to localization accuracies of a few nanometers.23,24 We demonstrate all-optical reconstruction of the geometry of dozens of nanorods in parallel in a wide-field microscope. We correlate these super-resolution reconstructions to atomic force microscopy images and to single-particle white-light spectroscopy. We...
find excellent correlation between the orientation and plasmon wavelength obtained from super-resolution microscopy and single-particle spectroscopy. Numerical calculations show that particle dimensions can be reconstructed with an accuracy of a few nanometers, determined by the number of events per particle and the photon count per event.

### EXPERIMENTAL METHODS

The experimental layout is shown in Figure 1a–c, with immobilized gold nanorods excited in an objective-TIR configuration. After being spin-coated onto glass coverslips, gold nanorods are functionalized with thiolated dsDNA with a single-stranded toe-hold, so-called docking strands (Figure 1b). The toe-hold provides binding-sites for fluorophore-labeled imager strands. With the first 20 bases of the docking strand hybridized, a 10 nucleotide toe-hold is rigidly placed away from the gold surface which reduces quenching of the emission. Docking sites are deposited as a mixed monolayer with thiol functionalized PEG4 to control the docking site density.

Configuration. After being spin-coated onto glass coverslips, gold nanorods are functionalized with thiolated dsDNA with a single-stranded toe-hold, so-called docking strands (Figure 1b). The toe-hold provides binding-sites for fluorophore-labeled imager strands. With the first 20 bases of the docking strand hybridized, a 10 nucleotide toe-hold is rigidly placed away from the gold surface which reduces quenching of the emission. Docking sites are deposited as a mixed monolayer with thiol functionalized PEG4 to control docking site density. From the association rate of imager stands we deduce that each particle on average contains ∼145 docking strands (see Supporting Information). Two representative scattering spectra are plotted in Figure 2c. The particles exhibit a longitudinal surface plasmon resonance (LSPR) in the near-infrared with single-particle plasmon wavelengths of 790 ± 40 nm. We use the line shape and line width of the single-particle scattering spectra to identify clusters of particles, evidenced by multiple peaks in the near-infrared or line widths exceeding 190 meV. Clusters of particles were omitted from further analysis.

We employ imager strands functionalized with ATTO 532, its emission spectrum is plotted in dark green in Figure 2c. The emission is detuned by >150 nm to the blue from the longitudinal plasmon peak to reduce plasmon-fluorophore coupling. Fluorescence is excited at 532 nm (bright green line), and collected between 545 and 605 nm (gray shaded region). Although this filter bandwidth suppresses the blue and red tails of the fluorophore emission and reduces the photon count per event, it also minimizes spectral overlap between the detected emission and the transverse (∼520 nm) or longitudinal (>750 nm) plasmon resonance.
This spectral overlap between a plasmon resonance and fluorophore emission may induce mislocalization due to the plasmonic antenna effect, whereby the emission of the fluorophore is enhanced by coupling to plasmonic modes in the particle. This coupling results in modification of the radiative and nonradiative rates of the fluorophore. Mislocalization originates from enhancements in the radiative rate, $\xi_{rad}$, of the complex. We have quantified $\xi_{rad}$ using the boundary element method (BEM). For a range of dipole emission wavelengths (here approximated as a single-wavelength emitter) the orientation-averaged $\xi_{rad}$ values are plotted in Figure 2d. A detailed description of the calculation can be found in the Supporting Information.

For an emitter resonant with the LSPR of the nanorod, high $\xi_{rad}$ values of $\sim 90$ and 13 are found for respectively tip and side binding positions. This high $\xi_{rad}$ may explain previous underestimation of reconstructed nanorod dimensions using a resonant emitter, with largest mislocalization occurring for tip-bound emitters. To minimize fluorophore-plasmon coupling we choose a fluorophore emitting in the wavelength window that minimizes $\xi_{rad}$ occurring on the blue side of the LSPR for both for tip- and side-bound emitters. We therefore choose ATTO532 as fluorophore, for which we expect a reduction of $\xi_{rad}$ of one to 2 orders of magnitude compared to resonantly coupled emitters.

**RESULTS AND DISCUSSION**

An experimental time trace of fluorescent imager strands stochastically binding and unbinding to a single gold nanorod is shown in Figure 3a. Each spike corresponds to the photon counts of a single binding event, integrated over a region of interest of $3 \times 3$ pixels centered on the nanoparticle. The background signal is plotted in red, measured similarly in a $3 \times 3$ region displaced 4 pixels away from the nanorod emission center. This illustrates that imager strands bind predominantly to the functionalized nanorods, with minimal nonspecific binding to the coverslip. We find a distribution of residence times that follows a single exponential distribution with a mean binding time of 3.6 s (see Figure S3). Events longer than 15 s are discarded as multiple binding events (see Figure S3). The small offset between the baseline of the intensity measured on the nanorod and off the nanorod originates from the one-photon luminescence (1PL) of the gold nanorod.

An exemplary fluorescent binding event is marked by the green star, persisting above threshold for 5 frames. Merging these 5 frames together, and subtracting the contribution from the weak 1PL (see Supporting Information), returns the total emission from only the bound imager strand, which is depicted in the inset image. The binding location is then super-resolved by numerically fitting this intensity distribution with a Gaussian function using the maximum likelihood method. The binding location is extracted from the Gaussian centroid (red dot in the image).
inset), resolved here with a precision a $1/34$ of the pixel size or $\sim \lambda/100$.

Fitting all binding events on each single nanorod results in a set of spatially distributed points for each particle, which are corrected for drift. For a typical nanoparticle, these localizations are plotted as the red dots in Figure 3b. The mean integrated photon count per event is $\sim 3 \times 10^4$ counts, resulting in a mean localization precision of 6 nm (see Supporting Information). These localizations and reconstructed geometries are correlated with AFM measurements of the same particles. An exemplary AFM image of a single nanorod is shown in Figure 3b, along with localized binding events (red dots). Overlaid as the blue solid line is the numerically determined nanorod geometry, obtained by fitting an error ellipse to the localizations to extract out the nanorod length and width. A nanorod shape with this fitted length and width is then overlaid onto the localizations (see Supporting Information for full procedure to reconstruct geometry). Good agreement is observed between the AFM resolved and reconstructed nanorod geometry. Convolution with the AFM tip potentially causes the particle to appear slightly larger in the AFM images. The height in the AFM image is therefore a better estimate for the nanorod width, because it avoids tip convolution effects. Here a nanorod height of $33 \pm 2$ nm was measured while a width of $37 \pm 4$ nm was reconstructed. AFM measured heights are correlated with reconstructed widths for 6 single nanorods in Figure 3c, where we observe deviations less than 5 nm for all particles. We will discuss the accuracy of the reconstructions in more detail below.

In addition to AFM, we correlated the reconstructed geometry with the measured single-particle scattering spectra. Correlations are presented in Figure 4 for three nanoparticles, with reconstructions shown in Figure 4a, along with the polarization (Figure 4b) and spectral response (Figure 4c). In the top two rows (i and ii), single nanorods are reconstructed (Figure 4a, blue outline) with aspect ratios of 1.7 and 3.6. The polarization of the scattered light indicates the angle of the nanorod, which corresponds to within 20° with the angle obtained from the super-resolution reconstruction. As expected we find a clearly red-shifted plasmon wavelength for the longer aspect ratio nanorod. In the third row (iii), a different picture emerges, with localizations arranged in a T-shape, suggesting a cluster. The spectral response confirms the presence of a dimer because two peaks are resolved. These results indicate the ability of DNA-PAINT to resolve the underlying geometry and orientation all-optically, without the requirement of AFM or EM.

Figure 4. (a) Red dots show localized emitter positions on nanorod surface. Blue outlines in parts i and ii are computationally fitted using an error ellipse method, while blue outlines in part iii are fit by hand as a guide to the eye. (b) Polarization dependent scattering response and (c) single-particle scattering spectra of the respective particles, along with fit Lorentzian fits in parts i and ii (blue curves).
The wide-field detection strategy demonstrated here enables simultaneous super-resolution microscopy and spectroscopy of many particles and particle-assemblies. In Figure 5a, we depict the correlation between the measured LSPR energy and the calculated one. The LSPR energy for each nanorod is calculated using numerical simulations (BEM) with the reconstructed dimensions as input. Here a positive correlation is observed as expected, with the 76% of the nanorods having an LSPR energy that deviates by less than 0.1 eV from the calculated LSPR. Apart from errors in reconstructed dimensions, we attribute the residual spread in LSPR energy to effects of end-cap shape, which can significantly affect plasmon peak position but are not captured in our calculations that assume all nanorods have hemispherical end-caps. We observe a similar picture for the orientation of the particles (Figure 5c), with 88% of the measured nanorods having a reconstructed angle that closely resembles the orientation measured using scattering spectroscopy. We further plot the reconstructed lengths, widths, and aspect ratios of 25 nanorods in Figure 5c−e (blue bars) together with the size distribution obtained from TEM (yellow bars). We find that both the mean and standard deviation of the distribution matches the TEM dimensions to within 10%.

Interesting to note is that this agreement between mean reconstructed and TEM dimensions occurs despite the imager strand being bound an average of 7.5 nm from the gold surface. The deviations between the dimensions from TEM and super-resolution microscopy that we observe in Figure 5c−e could arise from two phenomena: (1) Reconstructions are made from points which each have a finite localization precision, and (2) reconstructions are made from a finite number of binding events. We now estimate the effect of both mechanisms to establish an achievable “resolution” considering the experimental conditions. Here we achieve this by simulating the stochastic reconstruction process of a single nanorod, over an experimentally relevant range of signal and background levels, for differing numbers of events per particle.

For each event, a binding location is randomly sampled from the 2d projected surface of a 120 nm × 38 nm nanorod. A Gaussian point-spread-function plus constant background is then generated, with the desired signal-to-background ratio (SBR). To this image shotnoise is added. The apparent emission center of this noise-affected signal is then estimated using the same procedure we used in the experiments. This is repeated for the desired number of events on the particle, after which the dimensions are extracted as we do in the experiments.

Three examples of the obtained spatial distributions are shown in Figure 6a, where the original (simulated) nanorod geometry is depicted by the black dotted line and the reconstructed geometry by the blue solid line. Cases i-iii depict how improving both the number of events and SBR improves the accuracy of reconstruction. With a low number of events and a low SBR, particle dimensions are overestimated and the orientation is poorly reconstructed (case i). Both effects are due to poor sampling the nanorod surface due to the low number of events, and the low SBR leading to high localization uncertainty. Increasing the number of events and the SBR leads to improved estimation of all parameters (case ii and iii). The absolute errors for both length and width are plotted as heat maps in Figure 6b, reflecting both the over and under-

Figure 5. (a) Correlation between measured LSPR peak energy, and the value calculated using BEM calculations that use the reconstructed dimensions as input. (b) Correlation of measured orientation angle of nanorods with reconstructed angle. (c)-(e) Histogram comparison between reconstructed (blue bars) and TEM measured (yellow bars) dimensions of nanorods, in terms of length (c), width (d), and aspect ratio (e).
estimation observed for case i and ii, and the near perfect reconstruction achieved for case iii. Reconstructing 120 nm × 38 nm nanorods with errors below 10% thus requires >100 events and a SBR > 5, while smaller nanorods will have more stringent requirements. Case ii reflects the average number of events and SBR in our experiments. The simulations indicate that the length is underestimated by ~10 nm, while the width is underestimated by ~5 nm due to the limited sampling of the edges of the particle. This explains why we reconstruct particle sizes that match the dimensions from AFM and EM to 5 nm (see Figure 5a), even though the spacing of 7.5 nm between particle and fluorophore should result in overestimation of the dimensions by 15 nm. The reconstruction errors observed here are thus dominated by the limited number of events per particle.

**CONCLUSIONS**

We have demonstrated the first method capable of all-optically reconstructing the dimensions of single metallic nanoparticles using DNA-PAINT, while correlating the particle geometry to single-particle spectroscopy. This correlation provides a method to validate the reconstructions, and a tool to study optical properties of single particles without requiring an electron microscope or atomic force microscopy. Numerical simulations of our experimental conditions reveal an underestimation of particle dimensions by 5–10 nm, caused by the limited sampling of the particle’s edges. This error can be reduced to below 1% with for SBR > 20 and more than 500 events per particle. This may be achieved through employing e.g. a FRET-PAINT approach that minimize background and allows one to work at higher imager strand concentration. The photon budget can then be maintained while reducing the binding duration, allowing for more binding events per second, yielding greater statistics and improved reconstructions. The presented method may provide an approach to all-optically image the geometry of single particles in confined spaces such as microfluidics and biological cells, where access with electron beams or tip-based probes is prohibited.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcc.7b12473.

Detailed description of sample preparation methods, including DNA sequences employed here, and how optical and AFM experiments were carried out, furthermore, the fitting procedure for events and nanoparticle reconstruction, and electromagnetic simulations discussed in more detail, and finally, fluorescence time traces and reconstructions for additionally nanorods (PDF)

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Notes

The authors declare no competing financial interest.

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