Supercharging super-localization microscopy: how electrochemical charging of plasmonic nanostructures uncovers hidden heterogeneity

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Abstract. Super-localization microscopy allows the position of single plasmonic nanoparticles to be determined with <25 nm precision, enabling single nanoparticle tracking and super-resolution imaging experiments to be conducted with sub-diffraction-limited spatial resolution. In many of these applications, the super-localized position of the nanoparticle is assumed to correspond to the geometric center of the nanoparticle. However, recent work from Wang *et al* in *ACS Nano* suggests that this assumption is incorrect, based on studies in which electrochemically charging a nanoparticle leads to reproducible shifts in its scattering center. The shift is believed to originate from non-uniform charge accumulation in the nanoparticle, due to the inherent heterogeneity in nanoparticle surface properties. This Perspective explores the implication of this result, both for using this shift to probe dynamic changes in nanoparticle surface chemistry, as well as exploiting non-uniform charge accumulation to promote site-specific chemical reactions.

Noble metal nanoparticles (silver, gold, and beyond) have generated sizable interest from researchers, due to their ability to interact with light through the excitation of localized surface plasmons.[1] Plasmons, which are the collective oscillation of surface conduction electrons in materials with a negative real and small positive dielectric constant, lead to nanoparticles having brilliant colors, due to Rayleigh scattering at the localized surface plasmon resonance. The color can be tuned by varying the size, shape, composition, and external environment of the nanoparticles, allowing structures to be designed with resonances across the near UV, visible, and near infrared. While early studies classified the optical properties of these materials through measurements in bulk solution, advances in optical microscopy techniques (such as dark field scattering) have allowed plasmonic nanostructures to be interrogated at the single particle level. These studies enable researchers to understand how heterogeneity across a nanoparticle population affects the overall performance, impacting applications as broad ranging as sensing, optoelectronics, photothermal heating, and energy harvesting.[2]

Even with the single nanoparticle sensitivity that is now routinely achieved for studying plasmonic nanoparticles, optical microscopy is fundamentally limited by the diffraction limit of light, which limits the spatial information that is attainable by optical techniques. According to the Rayleigh criterion, structures that are smaller than $0.61*\lambda/NA$ (where λ is the wavelength of light and NA is the numerical aperture of the imaging optic) will appear as diffraction-limited spots in an optical microscope, with diameter of twice the Rayleigh criterion. Consider what this means for a gold nanorod with length and width of 70 nm and 30 nm, respectively: if the nanorod scatters light at 680 nm and the light is collected with a 0.7 NA objective, the nanorod will appear as a bright, spatially symmetric dot with diameter of ~1.2 μm in an optical microscope image, much larger than the actual size of the nanorod and with no indication of its underlying anisotropic structure. The implication is that both nanoscale structure and surface information, which are critical to understanding the optical properties of plasmonic nanoparticles, are hidden by traditional diffraction-limited optical microscopy techniques.

Recently super-resolution imaging has emerged as a suite of techniques capable of defeating the optical diffraction limit and providing spatial resolution better than 20 nm.[3] Rather than summarizing all of the variations of super-resolution imaging and their applications to plasmonics (having done so recently elsewhere),[4] in this Perspective, we will focus on localization-based super-resolution imaging. In this approach, diffraction-limited emission/scattering from an object is analyzed to extract the position of brightest intensity (e.g. the peak of the point spread function), which is then assigned as the location of the object. For example, diffraction-limited scattering from a single nanorod can be fit to a 2-dimensional Gaussian, shown as equation 1 below, where z is the background intensity, I_0 is the peak intensity, x_0 and y_0 are the peak of the distribution (e.g. the position associated with the brightest intensity), and s_x and s_y are the widths of the distribution. By extracting the values of x_0 and y_0, the position of the nanorod can be localized, with precision better than 25 nm (depending both on signal-to-noise and experimental considerations).

$$I(x,y) = z_0 + I_0 e^{\left[-\frac{1}{2}\left[\left(\frac{x-x_0}{s_x}\right)^2 + \left(\frac{y-y_0}{s_y}\right)^2\right]\right]} \tag{1}$$

A key misconception of super-resolution imaging is that we can simply shine light on a plasmonic sample and see the underlying shape revealed (e.g. our 70 nm x 30 nm gold nanorod described above will suddenly appear rod-like and correctly sized in the super-resolution microscope). Instead, localization-based super-resolution techniques only allow us to determine where individual objects are located. In the case of plasmonic nanostructures, the assumption is that the scattering center that we extract by analyzing diffraction-limited emission (e.g. x_0, y_0) corresponds to the geometric center of the nanorod. This assumption has been applied across a number of applications, from single particle tracking experiments to using nanoparticles as stationary spatial fiducials for super-resolution studies.[4-5]

However, in this issue of *ACS Nano*, Wang and coworkers show that the scattering center of a single gold nanorod can be manipulated by electrochemical charging, suggesting that the region of brightest scattering is not necessarily linked to the geometric center of the nanorod.[6] In their experiments, charge is added and removed from nanoparticles via a sinusoidally-oscillating potential waveform, leading to modulation of the scattering intensity at the same frequency, a well-known effect during charging/discharging.[7] Fitting the diffraction-limited scattering from a single nanorod to equation 1 reveals no obvious potential-dependent modulation in the x- and y-center positions; however, when the data is Fourier transformed, a clear potential-dependent shift in the center position is observed, modulating at the same frequency of the applied potential waveform. This effect is unique to plasmonic nanoparticles, with controls on polymer beads showing no such modulation in the center position. The magnitude of the shift in the scattering (which we will refer to as Δ_{scat} through the remainder of this Perspective) is linearly dependent on the applied potential. The authors attribute the effect to non-uniform charging/discharging of the nanorod, where injected electrons accumulate within specific regions of the nanorod, affecting how the light is radiated out into the far field (as illustrated schematically in Figure 1).[6] Interestingly, the direction of the shift of the center position of the nanorod scattering does not correspond to any geometric features of the nanorod (for example, its orientation), leading the authors to speculate that nanoscale variations in the local capacitance of the nanorod are responsible for the observed effect. These variations could be due to structural variations across the nanorod (facets, curvature, defects) or non-uniformity in the surface chemistry and/or ligand shell. While the exact origin of the effect requires more exploration, one key result is that the potential-dependent modulation of the scattering center is reproducible and stable across multiple potential cycles (and many hours), indicating that it corresponds to an intrinsic property of the nanoparticle, rather than transient surface restructuring of atomic-level defects or dynamic ligand rearrangements.

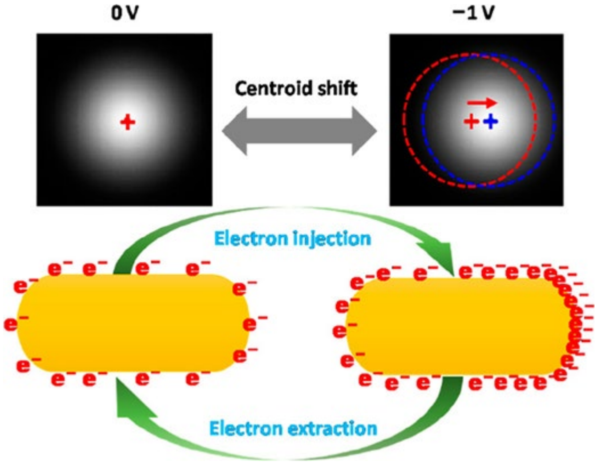

Figure 1. Schematic of the shift in the center of the diffraction-limited scattering from a single gold nanorod under electrochemical charging. Reprinted with permission from ref. 6. Copyright 2019 American Chemical Society.

The remainder of this Perspective explores the potential implications of a non-centrosymmetric, manipulable scattering center from a plasmonic nanoparticle. The intent of this discussion is to be speculative in nature, proposing new ideas and encouraging new avenues of exploration, rather than summarizing what is already known. Comprehensive reviews on plasmonic nanoparticles used in both super-resolution imaging and electrochemical charging/discharging experiments can be found elsewhere.[4, 7]

Where is the geometric center of the nanoparticle? Many super-resolution studies of plasmonic nanoparticles involve localizing fluorescently-labeled molecules at or near the surface of the nanostructure, in order to probe the interaction between the molecular emission and the plasmon modes of the nanoparticle and to gain insight into heterogeneity in the binding interactions at the interface.[4] Inherent to these studies is the need to address the background contribution from the supporting plasmonic nanostructure. Both gold and silver are well-known to produce a luminescent background (the origin of which remains under some debate), which must be removed in order to isolate and localize the contribution of the fluorescent molecules.[4] Much like nanoparticle scattering, the luminescent background from the plasmonic metal is assumed to correspond to the geometric center of the nanoparticle, and is often used as a spatial fiducial, or a fixed reference point, for super-resolved images.

In our own work,[8] we have seen evidence that the position of the luminescent background may not correlate with the geometric center of the particle, consistent with the work of Wang *et al*.[6] Figure 2A shows an example in which fluorescently-labeled DNA is attached to the surface of gold nanorods.[8] The emission from the attached fluorophores is modulated using a triplet-state mediated photoswitching strategy, allowing individual molecules to be localized by fitting their diffraction-limited emission to equation 1. As the fluorescent labels transition between emissive and non-emissive states, each one is localized, allowing us to build up a distribution that reveals the underlying shape of the gold nanorod as well as heterogeneity in where the DNA molecules are bound. Figure 2, B – C, shows scatter plots of the localized events associated with two different nanorods, in which the blue points represent individually localized fluorescence events from the fluorophore-labeled DNA and the red “x” shows the average location of the gold luminescence. While the data from the DNA maps out the expected shape and orientation of each nanorod (confirmed by AFM), we see that the gold luminescence does not fall within the center of the localized DNA, an effect we have observed in multiple cases and could never satisfactorily explain.

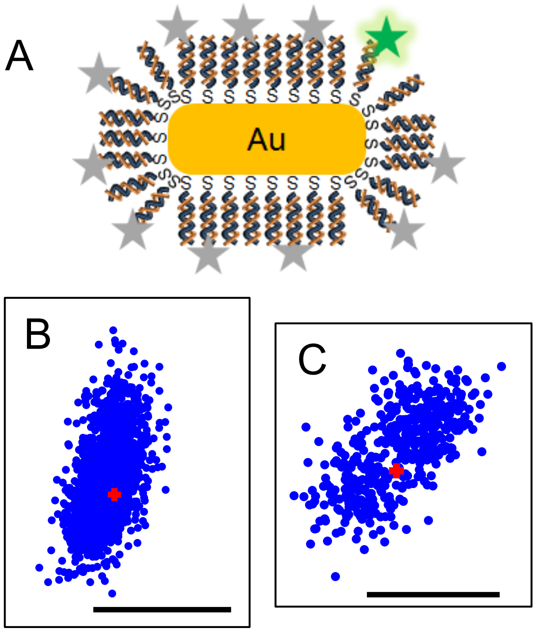

Figure 2. (A) Schematic of a single gold nanorod functionalized with DNA (not to scale). Some of the DNA carry fluorescent labels (stars), which can be toggled between a non-emissive (gray) and emissive form (green). As each molecule transitions into its emissive form, the emission is fit to equation 1 and the molecule is localized. (B – C) Scatter plots showing (blue) localized emission events from individual fluorescently-labeled DNA molecules and (red) the localized emission from the gold nanorod. Scale bars = 50 nm. Reprinted with permission from ref. 8. Copyright 2014 John Wiley & Sons.

In light of the results from Wang and coworkers,[6] however, we now consider the possibility that the gold luminescence is shifted from the geometric center of the nanorod by some intrinsic property of the nanorod itself (either structural or surface heterogeneity). This suggests that we do not need to inject electrons in order to induce an asymmetric distribution of charge; rather, the charge may be asymmetrically distributed in the as-prepared nanorod. The good news is that this will have limited impact on single nanoparticle tracking experiments or in super-resolution imaging (where the background is often treated as static and can therefore be subtracted away in order to isolate the fluorophore contribution). Put another way, even though the localized emission/scattering center may not correlate with the geometric center of the particle, as long as it originates from a stable position, then our current strategies for using the localization data remain valid (the question as to whether it remains stable under certain conditions associated with super-resolution imaging is a topic for another discussion).[9] However, the deviation of the scattering center from the geometric center does offer intriguing possibilities for using Δ_{scat} as a readout to understand nanoscale heterogeneity or even manipulate local charges at the nanoscale, as will be discussed in more detail below.

The role of surface chemistry. One interesting result from the electrochemical modulation studies of Wang and coworkers is how ligand identity impacts the measured shift in the center of mass of the nanorod scattering.[6] Δ_{scat} is twice as large when cetyl trimethyl ammonium bromide (CTAB) is used as a nanorod surfactant, compared to sodium dodecyl sulfate (SDS). The authors attribute this to differences in charge between the two ligands (positive and negative, respectively) and suggest that different ligands affect how charge is able to accumulate at the surface of the gold, thereby affecting the magnitude of the shift.

A question that arises from this result is the sensitivity of Δ_{scat} to ligand identity beyond simple charge considerations. In the case of gold-thiol or gold-amine interactions, the ligands have the ability to perturb the electronic structure of the surface gold, which would presumably have a significant impact on local charge accumulation. Beyond the binding chemistry, the long range nature of the effect remains an interesting unknown. Plasmonic nanoparticles are well-known to be responsive to the refractive index of the local environment, essentially reporting on how deeply the oscillating electrons penetrate into their surroundings. Thus, if the bulk solvent environment is changed, the magnitude of Δ_{scat} may also change.

Where these ideas become particularly compelling is the case when nanoparticles experience non-uniformity in their environments, for example, if ligands bind asymmetrically or heterogeneous surface reactions (such as surface oxide formation) occur on the nanoparticle surface. Wang and coworkers note in their study that the direction of Δ_{scat} does not correlate with geometric features of the nanoparticles (e.g. the long axis of the nanorod), and SEM data suggests no significant defects/asymmetry in the overall shape of the nanorods. This suggests that the nanoscale chemical makeup at the surface may play a strong role in driving the direction of Δ_{scat}. This finding opens the possibility that both the magnitude and direction of Δ_{scat} could change if the region of charge accumulation shifts in response to a changing environment (as illustrated schematically in Figure 3A). In our previous work, we showed directional changes in the scattering center from single nanoparticles undergoing oxidation, which we explained by heterogeneous surface oxides leading to asymmetric electrodissolution (Figure 3, B - D).[10] However, in our work, we were electrochemically etching the nanoparticle, fundamentally changing its size and morphology, which led to a permanent shift in the scattering center. By using the charging/discharging strategy outlined by Wang and coworkers, similar insight into the local nanoscale surface chemistry may be gained, but in a manner that is non-destructive to the particle. This opens the door for an additional parameter for probing dynamic surface chemistry effects by performing charging/discharging experiments before and after (or even during) surface modification and monitoring not only the magnitude of Δ_{scat} but also the direction.

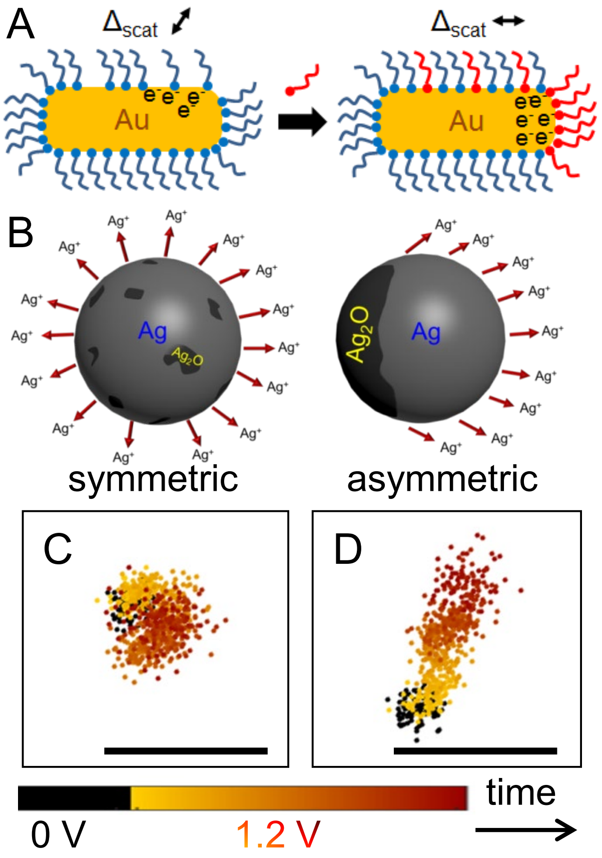

Figure 3. (A) Schematic showing how changes in the ligand shell could shift the region of enhanced charge accumulation and thus the direction of Δ_{scat}. (B - D) Silver electrodissolution data in which the scattering center remains (C) stationary or (D) shows a time-dependent shift under an oxidizing potential. The effect is due to (B) heterogeneity in the surface chemistry, which leads to either symmetric or asymmetric dissolution. Reprinted with permission from ref. 10. Copyright 2018 American Chemical Society.

Controlling local charge accumulation. With increasing interest in using plasmon-generated hot charge carriers to promote charge transfer reactions involving molecules near nanoparticle surfaces,[11] having the ability to tune the site of charge extraction would provide a powerful strategy for optimizing reaction efficiency. Calculations have shown that electrons "spill out" of the nanoparticle and into the local environment to varying extents during charging/discharging, as illustrated in Figure 4A.[7, 12] To model this effect, Nordlander and coworkers treated a gold nanoparticle as having shells of different charge density (Figure 4B, bottom row), which introduced new plasmon resonances as compared to a homogenous gold nanosphere (Figure B, top row).[12] This picture of local surface regions of charge accumulation is consistent with the themes developed in the previous section, although the model shown here treats the charge accumulation as homogenous across the surface. Calculations from Borisov show that electrons can even escape from the nanoparticle and into the surrounding medium if the particle is sufficiently charged (Figure 4C).[13] However, in all of the calculations shown here, the system is treated as spatially symmetric, with charges uniformly penetrating into the surrounding medium.

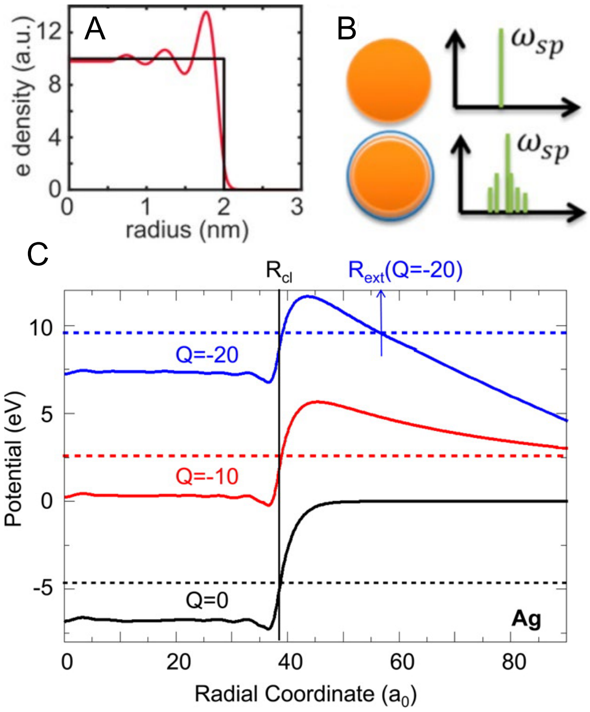

Figure 4. (A) (Black) The classical picture of charge density within a 4 nm diameter nanoparticle, with zero charge spilling past the boundary (e.g. at the nanoparticle radius of 2 nm). (red) The quantum mechanical picture shows a build up of charge density near the surface, with some charge spilling out past the nanoparticle boundary. (B) Treating charge density as non-uniform (bottom) leads to side bands in the plasmon resonance (ω_{sp}). Reprinted with permission from ref. 12. Copyright 2017 American Chemical Society. (C) Electron potential (solid line) relative to the Fermi level (dashed line) for 7.78 nm diameter Ag nanoparticles as charge (Q) is added to the particle. Zero corresponds to the vacuum level and R_{cl} is the

nanoparticle radius. Reprinted with permission from ref. 13. Copyright 2016 American Chemical Society.

Returning to plasmon-driven reactivity, it is interesting to consider whether regions of non-uniform charge accumulation within a nanoparticle could lead to enhanced reactivity at specific surface sites. In this case, there may be value in designing structures that can effectively funnel more of the charges to a specific location on the nanoparticle surface to enhance reactivity. Previous work on light-mediated silver nanoprism growth suggests that a combination of plasmon-driven photovoltages and site-specific ligand binding can drive anisotropic growth.[14] Here, we ask if this can be further tuned by controlling how local charge density builds up on the nanoparticle surface, allowing anisotropic reactivity to be engineered into the particle. For example, if ligands can help promote charge accumulation at a specific site as well as promote binding and/or capture of target molecules of interest, it may be possible to design synergistic hybrid organic-metallic catalysts to increase reaction efficiency.

Another interesting idea associated with regions of local charge accumulation is the concept of creating a potential drop across a single nanoparticle, even allowing it to behave as a bipolar electrode.[15] Even though the voltage difference might be small, the electric field associated with this potential drop would be enormous, given the small dimensions of the nanostructure. By understanding the factors that lead to asymmetric charge accumulation, it may be possible to design nanoparticles with large potential differences across them. One would expect this to have a large impact on the nanoscale charge distribution at the surface, such as local perturbations to the electrical double layer, but could also lead to emergent electrochemical phenomena at nanoscale interfaces.

Embrace the heterogeneity. It is well recognized that single nanoparticle measurements offer insight well beyond their bulk counterparts because they report the heterogeneity that is inherent to nanoparticle populations. Often the results of single particle measurements are complemented by theory, in which observable structural differences (for example, sharp vs. rounded tips in nanoprisms) are used to understand how plasmon resonances or peak widths are affected by the overall nanoparticle structure. However, as experimental techniques become more granular, providing insight into nanoscale heterogeneity associated with surface chemistry or local charging effects, we must challenge theorists to expand their calculations to account for these subtle differences. As seen in Figure 4, many calculations assume a uniform environment around the nanoparticle, providing insight into global effects (e.g. spectral broadening) but limited insight into what is happening at specific sites on the nanoparticle, particularly when local environments are non-uniform. As experimental tools become more sensitive to nanoscale differences across the nanoparticle surface, we need theoretical calculations that help us relate experimental measurables to specific surface features. In the plasmonic super-resolution imaging community, work has already been done to start addressing these ideas, with theorists placing fluorescent molecules at different positions along the nanoparticle surface and calculating how this affects the far field radiation pattern.[4, 9, 16] But less attention has been paid to how heterogeneity in local surface chemistry might impact measured properties of single nanoparticles, from spectral features to local charge density. One challenge that we need to address is how to design these non-uniform environments: do we use Monte Carlo style approaches and allow them to emerge at random sites, or do we require a more "intelligent design" strategy, where we strategically place heterogeneous defects at sites most likely to have an impact (e.g. near sharp corners)? While this remains a computational challenge (in part due to the associated computational expense), understanding how heterogeneous surface chemistry plays a role in dictating nanoparticle properties will allow new and as-yet-unclear design strategies to be explored for harnessing emergent phenomena, such as regions of enhanced charge accumulation.

Conclusions. As super-localization microscopy approaches become more widely used in the field of plasmonics, it is important to consider what hidden features can be revealed through careful analysis of the localized emission and/or scattering. As seen here, using strategies such as time-dependent electrochemical charging/discharging in combination with super-localization microscopy provides a unique handle for understanding possible heterogeneous charge accumulation within a nanoparticle. This Perspective has discussed several ways in which charging/discharging experiments can be used to learn about surface heterogeneity in plasmonic nanostructures, as well as considered how asymmetric charge accumulation may be useful in driving site-specific reactivity. Beyond the ideas discussed herein, it is also worth considering other ways in which nanoparticles can be reversibly manipulated,[17] such as in photothermal imaging experiments (where heating lasers are turned on and off)[18] or experiments in which nanoparticle spacing is dynamically tuned.[19] Combining other time-dependent manipulations with the spatial resolution afforded by super-localization techniques may yield additional new insight into nanoscale heterogeneity that is as-yet unexpected or unknown. Thus, the work from Wang and coworkers serves as a compelling inspiration to explore these other modalities in order to continue uncovering hidden heterogeneity in nanoscale systems.

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