Quantitative Nanoplasmonics

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ABSTRACT: Plasmonics, the study of the interactions between photons and collective oscillations of electrons, has seen tremendous advances during the past decade. Controllable nanometer- and sub-nanometer-scale engineering in plasmonic resonance and electromagnetic field localization at the subwavelength scale have propelled diverse studies in optics, materials science, chemistry, biotechnology, energy science, and various applications in spectroscopy. However, for translation of these accomplishments from research into practice, major hurdles including low reproducibility and poor controllability in target structures must be overcome, particularly for reliable quantification of plasmonic signals and functionalities. This Outlook introduces and summarizes the recent attempts and findings of many groups toward more quantitative and reliable nanoplasmonics, and discusses the challenges and possible future directions.

I. INTRODUCTION

With the advent of nanotechnology, plasmonics that deals with the interaction between photons and the collective oscillations of electrons has seen remarkable advances across multiple fields of research.1–6 Plasmonically enhanced nanostructures generate an unprecedentedly strong electromagnetic field that enables the amplification of various plasmonic signals such as Raman scattering and fluorescence. Therefore, efforts have been made to apply these systems to a wide variety of applications including sensing, imaging,7–9 metamaterials,10 and energy applications.11,12 In particular, significant achievements have been made in understanding and harnessing the plasmonic effect to realize what had been previously difficult or impossible to achieve, such as an over 5000-fold fluorescence enhancement and the injection of hot electrons over a potential barrier.13,14 Even by changing the coupling distance between plasmonic nanostructures by ∼1 nm, the electromagnetic field from these structures can be varied by over 10-fold, and subsequent surface-enhanced spectroscopic signals such as surface-enhanced fluorescence (SEF) and surface-enhanced Raman scattering (SERS). This becomes highly critical when the gap size between plasmonic nanostructures becomes ∼1 nm since the detected signals can be enhanced by many orders of magnitude. The significantly enhanced strong electromagnetic field by silver nanoshells or nanoparticles led to the realization of single-molecule SERS and SERS-based chemical analysis.5,15–19 In addition to the optical enhancement from the strong electromagnetic field, surface plasmons convert the energy of photons into hot electrons which enables efficient plasmon-enhanced heterogeneous photocatalysis or energy applications.20–22 Nevertheless, the practical use of plasmonic nanostructures is still challenging, and the reliable, controllable strategies with plasmonically enhanced nanostructures for reproducible and quantifiable detection and analysis are lacking. More specifically, these are fundamentally related with the uncontrollable hot-spots on or between plasmonic nanostructures.23 Only a very small fraction of the molecules modified on plasmonic nanostructures experience such a hot-spot, and produce measurable and quantifiable signals. In particular, the distance between dye molecules and the surface of a plasmonic structure is key to reproducible surface-enhanced signals, but positioning dye molecules with sub-nm-level controllability is highly challenging and has not been well-addressed in most cases. Other issues include the heterogeneity of a single structure or assembled structures, and the density and orientation of modified molecules. It should be also noted that bulk sample analysis, single-particle-/sub-particle-level analysis, and single-molecule analysis should be all accompanied and compared to have full insights and test real potential for the developed plasmonic nanostructures and systems. Thus, the use of surface-enhanced signals has been mainly limited to qualitative analysis, and the quantitative analysis with SERS or SEF has not been widely adopted. These are key issues and challenges not only for surface-enhanced spectroscopy but also for nanoantennas, metamaterials, and sensing and imaging applications.

This Outlook aims to present an overview, the latest advances, and future directions for realizing quantitative nanoplasmonics, which can be defined as the research field related with designing, synthesizing, and tuning plasmonically functional nanostructures in a highly precise and reproducible manner to obtain highly reliable and quantifiable plasmonic signal outputs from these structures. To achieve this goal, precise synthesis/fabrication, nm-/sub-nm-level plasmonic control, molecular control, and highly reliable measurement/analysis should be considered and realized simultaneously with high accuracy...
II. PRECISE SYNTHESIS AND FABRICATION OF PLASMONIC NANOSTRUCTURES

Precise structural control, narrow morphological distribution, and scalable synthesis of the nanostructures of interest are the keys to fully exploiting the plasmonic properties and hence realizing quantitative plasmonics. Given the applicability of basic building-block nanostructures as templates for plasmonically enhanced and complex structures, their precise structural modulation has received a considerable amount of attention. Many studies on the precise structural control rely on wet chemistry or post-synthetic modifications, by which the synthesis of plasmonic nanoparticles is more controllable, and final structures can be more uniform. For example, oxidative etching can make extremely smooth, highly spherical single-crystalline gold nanoparticles by smoothing the surface features such as edges and vertices (Figure 2a). When plasmonic properties of ultrasmooth and randomly faceted gold nanoprisms coupled with a gold film were compared, the former exhibited a more uniform Rayleigh scattering (Figure 2b). The surface etching strategy can also be applied to prepare a variety of anisotropic nanostructures with a great monodispersity from highly monodisperse seeds. Instead of removing atoms from high energy facet, ultraprecise structural reshaping takes advantage of atomic displacement to create particles with a more stable energy facet. Treatment at 80 °C can lead to the formation of monodisperse seeds by thermal twinning, which increases the yield of bipyramids and nanorods to nearly 100%. In another effort, González-Rubio et al. utilized a femtosecond laser to anneal gold nanorods dispersed in a surfactant solution. What they found was that the irradiation on colloids with ultrafast pulses reshapes the nanoparticles to produce a uniform distribution of resonance lines that are nearly identical to single nanorods (Figure 2c). Additionally, Herrmann et al. observed the emergence of a junction due to atomic displacements when a pulsed laser was applied onto a gold interparticle gap of about 1 nm. Changing the wavelength and power of the laser or the size of the nanoparticles allowed them to control the width of the junction, thereby shifting plasmonic resonances. Therefore, when using light with nanoplasmonic control, the precise morphology changes should be adequately considered. All the achievements are encouraging and promising; however, while keeping the quality in structural control intact is still challenging, scalable synthesis, especially in a large scale, needs to be addressed for the widespread and practical use of these methods.

Thanks to the extensive research efforts, the architecture of plasmonically coupled and enhanced nanoparticles can be precisely manipulated, resulting in local field enhancements that far exceed the field enhancement for a single plasmonic nanoparticle. For example, core—gap—shell-type nanostructures allow for the plasmonic coupling between a core and a shell, and the control of the extent of coupling can be readily adjusted by controlling the gap thickness using a silica or sacrificial metal layer. However, since producing a ≤1 nm gap through such methods has been challenging, molecules have been used as interlayer fillers to obtain ultrasmall gaps. Lim et al. reported a method involving a shell formation on thiolated DNA-modified nanoparticles to produce gold nanobridged nanogap particles (Au-NNPs) with a uniform 1 nm interior gap, thereby generating strong and uniform local electromagnetic fields (Figure 2d). While a very small portion of the molecules exhibit strong Raman signals in most interparticle plasmonic coupling-based SERS systems, the enhancement factor of the Raman scattering from the molecules in the interior nanogap is uniform, with >90% of the SERS enhancement factors residing in >1.0 × 10^4. More recent study shows that interlayer-free dealloyed intra-nanogap particles (DIPs) with ~2 nm interior gap can generate the SERS enhancement factor distribution from 1.1 × 10^8 to 5.3 × 10^9 from 97.3% particles. Because of the highly robust, uniform, and stable SERS signals from DIPs, SERS-based target-specific cell imaging could be stably performed long-term with low laser power and short laser-exposure time. In addition, DIPs showed feasibility for SERS-based ultrasensitive DNA detection providing not only wider dynamic range and higher sensitivity but also reliability which is an essential factor for commercialization. Outer shell surface roughness also affects the electromagnetic field of the intra-nanogap and, hence, the

![Figure 1. Schematic illustration of the main components for quantitative nanoplasmonics.](image-url)
optical response of the structures, thereby providing additional room for enhancement by readily controlling the roughness. Nanocrevise particles with a conductive nanojunction are another type of plasmonically coupled structure with strong and controllable electromagnetic field. For Au−Ag hetero-nanostructures, salt-concentration-dependent kinetic control governs the structural morphology. In this case, a low salt concentration with the aid of polymers induces the asymmetric Ag deposition on the DNA-modified gold nanoparticles, resulting in the final morphology of Au−Ag head−body "nanosnowmen" rather than concentric core−shell nanoparticles. Furthermore, the junction area becomes extremely thin such that the charge transfer plasmon mode red-shifts, and a more enhanced local field is realized, particularly in the near-infrared region (Figure 2e). Precise junction control over the hetero-interfaces (i.e., Au−Ag or Cu−Ag) is also possible by varying the amount of polymer to control the morphology of the junction, which governs the balancing of far-field and near-field properties. Thus, high-precision chemical fabrication enables the development of well-designed nanostructures with desired plasmon modes and local fields, thereby making the systematic and reliable study of plasmonic nanostructures possible.

Moreover, site-dependent blockage of nanoparticles with a surfactant enables an anisotropic functionalization. The surfactant coverage density on the particular facets of the nanocrystal depends on the type and amount of surfactant, exposing less

Figure 2. Precise structural control of plasmonic nanoparticles. (a) Gradual transformation from octahedra to spheres by chemical etching. (b) Comparison of scattering spectra uniformity between ultrasmooth and randomly faceted gold nanospheres coupled with a gold film. (c) Extinction spectra (panel 1) and TEM images (panels 2, 3) of reshaped gold nanorods. In panel 1, red and blue correspond to before and after irradiation of 800 nm 50 fs pulses with 3.2 J/m² for 1 h, respectively. The normalized spectrum of the reshaped colloid (green) is almost identical to that calculated for a single particle (black). (d) TEM images showing growth intermediates and final structure of Au-NNPs (panels 1−4). Red arrows in panel 5 indicate nanobridges within the Au-NNP, and element line mapping showing ∼1 nm gap in the Au-NNP structure is shown in panel 6. (e) Au−Ag head−body nanosnowmen at 10, 3, and 0.7 mM salt concentrations (panels 1−3), respectively. (f) Polystyrene segregation on the isotropic gold nanoparticles surface in DMF and DMF/water mixture (panels 1, 2), respectively. Insets show the corresponding images of the individual nanospheres. Panel 3 shows gold nanosphere carrying photo-cross-linked thiol-terminated polystyrene-co-polyisoprene patches.

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covered surfaces for further deposition. As a result, organic materials or metals can be selectively grown onto high-curvature sites only.43−46 In addition, Choueiri et al. reported on the surface patterning with polymer patches on the isotropic nanoparticles, which is attributable to the solvent-mediated thermodynamic control of the polymer (Figure 2f).47

III. QUANTIFIABLE PLASMONIC CONTROL AND ENHANCEMENT IN NANOSTRUCTURES

Apart from the precise synthesis of plasmonic nanoparticles, groups of nanostructures should also be precisely arranged to obtain reproducible and quantifiable plasmonic properties from each structure. Notably, the degree of plasmonic properties between nanostructures varies greatly depending on the position in the order of a few molecular dimensions, i.e., as small as ∼1 nm (Figure 3a).48,49 Recent advances in top-down methods like an electron-beam lithography enable the production of <4 nm gaps,50−53 and top-down approaches54−56 have been exploited to realize various applications such as sensing,57 photocurrent conversion,58 and color generation.59 They are powerful tools to generate desired and intended nanostructures with high reproducibility, which is necessary for quantifiable plasmonic coupling. However, these approaches for the fabrication of plasmonic nanostructures are time-consuming, because of repetitive fabrication for making same pattern, and expensive, because of both high-cost fabricating equipment and wasted metal sources during operation, and have limitations in reliably tuning ∼1−2 nm gaps. On the contrary, bottom-up methods, wet chemistry in other words, are challenging to be realized for producing ultra-uniform nanostructures, but cost-effective and have consistently contributed to control the elaborate arrangement of nanoparticles.60,61 Ligands such as DNA and small molecules have helped researchers control the relative position of nanoparticles in the order of a nanometer.62,63 Another notable way to obtain strong and quantifiable plasmonic properties is locating a sharp tip in situ on a plasmonic nanostructure on the substrate. Tip-enhanced Raman spectroscopy is a representative example that exploits this strategy.64,65 It has apparent strengths in high spatial resolution and in situ measurement, but there are also limitations with respect to quantitative measurement and analysis because the signals from this platform are difficult to reproduce in a quantitative manner if different tips and conditions are used. Further, the implementation and measurement processes are serial and rather slow. For these reasons, bottom-up approaches can be quite efficient and powerful for quantitative plasmonics.

One of the most studied plasmonically coupled structures is a nanosphere dimer—a pair of plasmonic nanospheres separated by a small gap. A single dye-DNA-tethered nanodimer features a Raman dye in the nanogap hot-spot, providing nano-scale-gap engineering between two nanoparticles (Figure 3b).56 This nanostructure fabrication enables structurally and optically reproducible nanostructure-based single-molecule detection, and the distribution of the enhancement factor of the Raman signal from the structure is narrow (Figure 3c),57 helping it to be used in various applications. Moreover, DNA origamis, rigid DNA-based architectures with well-defined geometries, offer programmable templates for nanoparticle assemblies.58,66 Since specific oligonucleotide sequences can be
exposed at a designed position of the DNA origami, the position and gap size between two nanoparticles can be tuned in a rather quantitative manner.70–75 Such powerful controllability enables the active control of plasmonic interparticle systems such as the DNA walker and active chirality, and also provides an atomic-scale placement accuracy of molecules.76–78 A single barrel-like cucurbit[8]uril (CB[8]) molecule can be another linker for a fixed interparticle junction of 0.9 nm.79,80 Because of the selective host–guest chemistry, CB[8] hosts specific guest molecules at the center of the junction, thereby generating a reliable molecular-recognition-based SERS substrate. Thus, once proficiency in arranging the plasmonic nanoparticles and positioning the molecules in a desired manner is achieved, simultaneous quantification and signal amplification can be accomplished. This subtle controllability for a large number of structures still remains to be seen and is a major hurdle for these methods to be fruitful in many applications.

Angle of incidence and polarization of light should not be omitted in the nanoplasmonics that deals with the interaction between nanostructures and light. In particular, asymmetric nanostructures such as dimers respond differently to light depending on the incident angle81 or polarized direction82 of the electromagnetic wave. Even the emission direction of photoluminescence enhanced by plasmonic coupling can be controlled in this manner.83 Thus, these aspects are indispensable in the discussion of quantitative nanoplasmonics. A uniform signal from plasmonic nanostructures is another key aspect to realize quantitative nanoplasmonics. The quantitative property can be achieved from the highly localized electromagnetic field near synthesized nanostructures with nanometer-scale structural uniformity and the precise positioning and orientation control of molecules of interest in these structures.84 An example of this case is the gold nanoparticle coated with a thin silica shell, which keeps the probed material uniformly separated from the nanoparticles, thus providing a straightforward means to analyze the organic molecules and biological structures.85 A similar concept of the uniform SERS system was also reported by Chen et al.86 They found out that when molecules are placed in the hot-zone rather than the hot-spots, relatively uniform Raman signals are observed. This implies that if a uniform electric field is used to detect a single molecule, the relatively homogeneous Raman signal over a large area can be obtained. Furthermore, these quantifiable features have the potential to transform plasmonics into a next-generation analytical tool requiring ultrahigh sensitivity and selectivity in a quantitative manner.

IV. ACCURATE MOLECULAR CONTROL FOR QUANTITATIVE PLASMONICS

In addition to the precise synthesis of plasmonic nanoparticles for generating reproducible signals and the rational design and accurate plasmonic control of nanostructures for enhancing/controlling plasmonic properties, the molecular-level control (e.g., position, orientation, density, and resonance/chemical effect of molecules) is another essential element for quantitative plasmonics. Typically, plasmonic hot-spots are generated only on a very small part of the surface of an entire plasmonic nanostructure.87 This causes a significant variation in the signal intensity depending on the location of molecules. Importantly, this uncertainty obviously indicates that every single plasmonic structure may exhibit different signal intensities; thus, any information obtained from the part of the whole nanoparticles cannot represent the entire characteristics of the system of interest, causing serious limitations in quantitative plasmonics. Therefore, precisely positioning the molecules at the hot-spot should be a key issue when it comes down to obtaining a uniform signal from each molecule on each nanostructure. Several attempts to realize site-selective adsorption have been conducted, including the introduction of molecules at the precise locations where hot-spots are formed (i.e., the center of the dimeric structure) using DNA or the allowed access of molecules only at the hot-spot regions by blocking the remaining surface using surfactants.15,88 Because the signal intensity is highly dependent on not only site-selective functionalization of molecules but also the number (density) of molecules introduced on the nanostructures,35 adsorbing the “same” amounts of molecules “identically” to hot-spots of “all” individual plasmonic nanostructures is crucial to realize quantitative plasmonics.

The orientation of the molecules can also affect the plasmonic outcomes. Recently, Chikkaraddy et al. discovered that the alignment of the emitter (Raman analytes) placed in the plasmonic nanocavities affects the strong plasmon-exciton coupling.88 To create plasmonic nanocavities, they employed a nanoparticle-on-mirror (NPoM) geometry, with the emitters being placed in the nanogap between the nanoparticles and a mirror using the host–guest chemistry of CB[n] molecules (Figure 4a). It was found that when the transition dipole moment of the emitter is oriented parallel to the mirror, the resonant scattering plasmonic peak is same as that of NPoM without the emitter, thereby indicating the weak coupling (Figure 4b,c). In contrast, when the transition dipole moment of the emitter is oriented perpendicular to the mirror with the use of CB[n] molecules, the scattering spectra show two split peaks due to the strong interaction between the emitters and the plasmon within the nanocavities (Figure 4b,c). Indeed, since the scattering and amplification of the electromagnetic field by the plasmon are related to the component of the electric field that is perpendicular to the metal surface, the coupling between the molecule and plasmon becomes stronger when the molecular polarization is vertical to the metal surface. Notably, the orientation of the molecules is highly related to the surface coverage of the molecules on the plasmonic nanostructures.89 When a low amount of pyridine is adsorbed on the silver surface, it tends to lie in a direction parallel to the metal surface. However, as the number of pyridine molecules increases, the ring plane of pyridine is oriented in a direction perpendicular to the metal surface. This, in turn, shows the enhanced scattering signal, which is caused by the larger in-plane polarizability of the Raman molecules. As a result, surface coverage or density of molecules on the surface of plasmonic metal nanostructures as well as orientation and polarization of molecules should be carefully considered and precisely controlled in quantitative plasmonics.

The local electric field enhancement near nanostructures by light irradiation with a particular wavelength is highly dependent on their shape, size, and composition. Therefore, an appropriate matching between nanostructure’s plasmon mode and the excitation wavelength is critical to realize optimal local field enhancement. Moreover, a proper choice of molecules should be also carefully considered because optical signals such as SERS and SEF fundamentally come from the interaction between the molecules and light.90,91 In general, the SERS enhancement is strongly affected by the molecular resonance effect between Raman molecules and excitation source.92–94 If the frequency of the electronic transition in Raman molecules is close to the frequency of the excitation laser,
the Raman scattering signals can be additionally enhanced\textsuperscript{89,92} which is referred to as a surface-enhanced “resonance” Raman scattering (SERRS).\textsuperscript{89,92—94} Since SERRS has a larger cross-section than SERS, it provides lower detection limits. For example, the SERS enhancement factor for pyridine, a non-resonant analyte, is estimated to be about $10^6$, whereas that for Rhodamine, a resonant analyte, is more than $10^{13}$\textsuperscript{89}. In addition, SERRS can reduce the interferences from contaminants and selectively detect only the targeted Raman modes because only specific Raman modes are resonant with the excitation light.\textsuperscript{91} In terms of multiplexing capability, the Raman scattering is superior to the conventional fluorescence since it gives molecularly specific and largely varying signals along with narrow signal bandwidths and multiple signature peaks. However, different Raman dyes on the plasmonic nanostructures from the same batch can produce widely diverse Raman signals under the same laser irradiation, largely because of the SERRS effect. For this reason, the quantitative signal of each Raman dye can be strongly affected by the difference in resonance conditions with excitation light. When certain Raman dyes generate a very strong signal by the SERRS effect in multiplexed detection, analysis based on the quantitative signal intensity can be misleading, or the quantitatively different signal intensities can be obtained even with the same Raman dye, depending on the excitation wavelength. Therefore, when practically using the SERRS in multiplexed detection, one should carefully consider the absorption maxima, SERRS cross-section of each analyte, degree of resonance with the excitation light, and surface adsorption properties.\textsuperscript{93} Furthermore, when applying the single-excitation laser, both the proper design of the nanostructures with excellent plasmonic properties and the resonance condition of the molecules should be simultaneously considered.\textsuperscript{92}

Moreover, the photoinduced metal–molecule charge transfer (CT) can “chemically” contribute to the overall SERS enhancement.\textsuperscript{90,93—99} The molecules bound to the metal surface form a CT complex through chemical interaction with the metal, which induces a change in the polarizability of the metal–molecule complex, formation of intermediate electronic states, or temporary electron transfer.\textsuperscript{100—103} Although the exact mechanism and contribution of the chemical enhancement of SERS is not fully clear, various experimental and theoretical studies have reported that the chemical enhancement effect plays an important role in SERS.\textsuperscript{100—103} Generally, because of the surface condition (e.g., defects or curvature), only a small fraction of the Raman molecules at the hot-spots are CT-effective, and the electromagnetic enhancement dominates the overall SERS enhancement. However, for realization of quantitative plasmonics, the chemical enhancement effect should be precisely controlled because it directly contributes to the magnitude and the on–off blinking of the SERS signals due to the spatial diffusion or reorientation of the CT-active molecules.\textsuperscript{99}

V. RELIABLE MEASUREMENTS AND ANALYSIS OF THE SIGNALS FROM PLASMONIC NANOSTRUCTURES

To adequately characterize and exploit the plasmonic properties of interest, reliable, precise, and reproducible measurements should be performed, often at the single-particle and single-molecule level. Even when all the molecules are oriented well into one direction, each plasmonic nanostructure can produce varying signals due to the atomic-scale heterogeneity of the nanostructures and nonuniform spatial localization of the near-field distribution on each nanostructure. Therefore, single-particle and single-molecule measurements are needed to address these issues, and these further offer the results and insights that cannot be obtained from ensemble measurements. For instance, the SERS enhancement factors (EFs) of individual nanoparticles can be obtained by atomic-force-microscopy-correlated (AFM-correlated) nano-Raman measurements, which reveal the distribution of the SERS EFs of all the particles (Figure 5a–c).\textsuperscript{35,37} Hence, in single-particle studies, the properties of the whole samples that can be derived from the outcomes of single-particle measurements over a large number of particles are achieved. Although the experimental observations of SERS-based single-molecule detection were reported,\textsuperscript{18,104,105} the single-molecule measurement has been largely dominated by fluorescence. Because a very small fractional surface of the SERS substrate contributes to hot-spots, poor or irreproducible...
Signal statistics with a large signal variation on the same substrate often becomes a significant problem. It was later shown that single-molecule SERS is indeed possible in a reliable manner with nm-level silver shell engineering to control an ultra-small nanogap of a gold nanodimer. Highly sensitive SERS-active nanostructures with oligonucleotides or DNA origami were developed as aforementioned in Section III. It should be noted that these single-particle and single-molecule measurements typically require tedious processes and complicated instrumentation, and rely on the limited number of particles or molecules. In contrast, characterizing the performance of plasmonic nanostructures in ensemble/bulk measurements is relatively straightforward. However, such methods provide the averaged response from the whole system, thereby leading to misleading conclusions in some cases. Therefore, characterizing nanoscale features at the single-particle or single-molecule level in a high-throughput or massively parallel manner is desirable to obtain reliable data for practical applications.

Recently, Lee et al. developed a platform that enables massively parallel observation of a myriad of individual plasmonic nanoparticles on a supported lipid bilayer. The strategy combines dark-field microscopy and a two-dimensional (2D) fluidic surface. In contrast to colorimetric analysis that monitors distinct solution color changes via target-induced interparticle clustering in an ensemble, nanoparticle interactions could be observed via a dark-field scattering signal at the single-particle level.

Figure 5. Measurements at the single-molecule level and single-particle level. (a) AFM-correlated nano-Raman spectroscopy for the detection of a Raman signal at a single-molecule level. (b) AFM images of the individual particles with a single molecule and (c) corresponding SERS spectra. The black arrow indicates the incident laser-light polarization direction. (d) Illustration of probing nanoparticle interactions on a supported lipid bilayer surface. Mobile and immobile plasmonic probes are tethered to a supported lipid bilayer and target DNA hybridization induces two-dimensional cluster formation and plasmonic coupling. (e) Dark-field microscopic images of plasmonic nanoparticle clusters. The 15-step trajectories (white solid lines) of mobile nanoparticles are represented from the starting position (red arrows) to an immobile probe site (white dashed circle). (f) Optokinetically encoded nanoprobes generate nine identifiable scattering signal changes due to the combinatorial plasmonic couplings with three different plasmonic nanostructures. Reproduced with permission from ref 15, copyright 2010, Springer Nature; ref 106, copyright 2014, American Chemical Society; and ref 108, copyright 2017, American Chemical Society.
enabling the quantitative monitoring of the assembly process in situ (Figure 5d,e). A massively parallel observation of particle clustering processes (as opposed to freely dispersed nanoparticles in solution) is possible only when the plasmonic nanoparticles are tethered on a 2D fluidic surface. On the basis of the in situ monitoring of the stepwise evolution of the scattering intensity and cluster color induced by the addition of DNA, one can quantify the formations of dimers, trimers, and tetrarmers and down to 30 fM of DNA by counting the binding events. In addition, it was possible to develop this platform to quantify with high sensitivity, high selectivity, and multiplexing capabilities with 9 different microRNA targets using optokinetically encoded nanoprobes with three different plasmonic nanostructures with three distinct scattering features (Figure 5f). The in situ observation of individual reactions at the single-nanoparticle level also provides a way to investigate complex biological processes. The method can be used for real-time visualization of nanoparticle clustering during intracellular transport and RNA splicing in living cells. Stable Rayleigh scattering signals from plasmonic nanoparticles facilitate long-term visualization and monitoring, which allows the study of complex physical, chemical, and biological processes without interference by photobleaching and photoblinking. Further, correlating the analyses at the single-particle and ensemble levels may provide detailed information about the entire analytes or structures in a more quantitative and thorough manner.

Quantitative analysis allows for accurate extraction of information from the obtained data. With the background continuum accompanying SERS spectra being a major obstacle to obtain quantitative information from the SERS spectra, several techniques have also been employed to investigate the origin of the Raman background. It was supposed that the background is generated by the involvement of interband transition, intraband transitions,110 and a Purcell-effect-enhanced radiative recombination of hot carriers.113 Alternatively, temperature-dependent SERS from the gold substrate of periodic inverted pyramids suggested that anti-Stokes backgrounds were shown to be related to the inelastic light scattering of electrons within the metal.114 Recently, theoretical analysis with a spherical gold nanodimer revealed that the signal-to-background ratio of the SERS spectrum is higher with smaller gaps at the same excitation wavelength.115 Along with efforts to find the nature of the SERS background, Lin et al. reported the approach to quantitatively analyze the SERS spectrum without internal references.116 The authors exploit the idea that the SERS background is induced by the plasmon-modulated photoluminescence of metal, which also shares the local field information of the SERS. Thus, post-treatment of the spectrum with elastic scattering and photoluminescence of bulk (i.e., the photoluminescence of a Au(111) single crystal) spectrum could give an intrinsic Raman signal of the molecules on a nanoparticle. These results suggest that the understanding of the physical origin of the spectral information enables more reliable and reproducible spectral analysis, thus providing an improved quantification.

VI. CHALLENGES AND FUTURE DIRECTIONS

Recent advances in plasmonic nanostructure synthesis/fabrication have enabled the realization of sub-nm gaps between the structures, where the onset of quantum mechanical effects (tunneling current and nonlocality of electrons) quenches the concentrated electromagnetic field and resonance effects.117 Tunneling currents are photoinduced currents in plasmonic nanogaps. When nanostructures approach a few nanometers or less in the sub-nanometer regime, electrons flow between the nanostructures with energies lower than the energy barrier.118 Moreover, actual plasmons behave differently from the classical description because of nonlocality from the Pauli exclusion principle. This occurs in the regime corresponding to gaps of several nanometers between two plasmonic structures.119 Therefore, in order to accurately and quantitatively predict and analyze the experimental outcomes, it would be necessary to include quantum mechanics, especially for the cases with ultrasmall plasmonic nanogaps. Even though a quantum-corrected model and nonlocal hydrodynamic model have been proposed, more experimental work is required to validate and further improve these models.120

An elaborate fabrication with atomic-level details is still needed to ultra-precisely control plasmonic effects. Several recent studies have shown that individual atomic features determine sub-nanometric near-field hot-spots, thereby enabling extreme optical confinement.121–123 Thus, for full control of the process, a deeper understanding of the nanostructure development is necessary. Recently, Ye et al. revealed short-lived tetrahedrons as the intermediate particles of nanorods by in situ observation with an electron microscope of the nanoparticle shape transformation.124 Furthermore, structure reconstruction conducted by time-resolved X-ray scattering analysis with modeling revealed the details of the three-dimensional structural evolution with a spatial resolution of ~5 Å.125 In the same vein, the ability to accurately control the position of molecules in surface modification would help achieve more reliable plasmonic control and reproducibility in output with nanostructures. When the localization volume at which light is focused is adjustable to the level of 1 nm3, precise molecular control over the entire nanostructures becomes critical since this is comparable to the dimension of molecules. One promising method to achieve this control is the use of a plasmonic optical tweezer that can be employed to control the molecular position.126 Using this approach, trapping and placing the molecules at specific locations on the metallic surface can be possible at the nanometer scale.

Indeed, the intensity of the output signal can be varied not only because of nanostructural and molecular effects on plasmonic properties, but also because of minute changes in the measurement method. Because this can lead to quite varying results with the same molecular and nanostructural configurations, especially in terms of data reproducibility and quantification, depending on the measurement method, condition, and user, the standardization and automation of the measurement method and condition should be fulfilled in the future to realize quantitative plasmonics. In particular, the calculation method for estimating enhancement factors, which present an absolute comparison between the plasmonic enhancements of different nanostructures in different systems, should be also unified and

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standardized to prevent the over-/underestimation and misinterpretation of plasmonic properties and to make a fair and reliable comparison. The evaluation on the size, shape, composition, and surface chemistry of plasmonic nanostructures should be also standardized with a list of widely reliable quantification data. One of the main hurdles for nanomaterials to be utilized and approved in medicine is their variations in size, shape, composition, and surface chemistry. All the experimental protocols and evaluation methods for each nanostructure are widely varying, and this is particularly frustrating for medical applications because the efficacy and toxicity of nanostructures in medicine are very sensitively dependent on even small changes in these parameters. In the field of catalysis, it usually uses nanoparticles on which reactant molecules directly approach and subsequently participate in light-induced reactions, or chemically reactive metals are deposited. In these cases, the reaction yield and rate and the selectivity of desired products would be augmented if the degree of plasmonic-enhanced electric field can be adjusted, and the site approaching or deposition are able to be handled with success, which are the aims in Sections III and IV, respectively. Ultraprecise control on plasmonics over a large area of nanomaterials could largely help advance this field. In metamaterials, top-down approaches have been used more dominantly in forming metamaterials than bottom-up approaches since their properties come mainly from the periodicity of meta-atoms, which can be readily achieved with top-down methods. In addition, threedimensional periodic structures, consisting of nanoparticles, in the virtue of programmable DNA ligands have been assembled. The precise synthesis of nanoparticles and molecular control, discussed in Sections II and IV, would help advance two pivotal fields of metamaterials—making three-dimensional metamaterials and fabricating them on a large scale.

By addressing the above-mentioned issues, researchers in a wide variety of different fields can understand and reliably utilize plasmonic nanostructures of interest for a specific application. On the basis of these efforts, it will be possible to construct a standardized database library on the nanostructure-, laser-, and molecule-dependent quantitative plasmonic characteristics, which eventually will lead to quantitative plasmonics that can be applied to various disciplines and help generalize next-generation analytical techniques. In particular, advanced techniques to optimize and maximize plasmonic properties of nanostructures, such as wavelength-scanned spectroscopy and a plasmon-scanned technique, can help build a library on the nanostructure- and laser-dependent quantitative plasmonic characteristics. With many possibilities to explore various sciences and technologies based on plasmonics in the future, we expect that the realization of quantitative nanoplasmonics will lead to more in-depth and correct understanding and evaluation of plasmonic nanostructures and the feasible and practical utilization of plasmonic systems in widely diverse fields with high reliability.

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