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Reproducibility in Surface-Enhanced Raman Spectroscopy

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Abstract: Surface-enhanced Raman spectroscopy (SERS) is an intense ongoing hot topic because it is an attractive tool for sensing or detecting molecules in trace amounts. Despite its high specificity and sensitivity, the SERS technique has not been established as a routine analytic method most likely due to the low reproducibility of the SERS signal. This review considers the influence factors to produce the poor reproducibility during the SERS measurement. This review starts with the discussion of calculation of surface-enhanced Raman intensity in order to explain the reason why it is so difficult to achieve a high reproducibility of SERS measurement from the origin of enhancement mechanism. Then we focus on the fabrication of SERS substrates generally including two types: ① single particles and ② arrays on substrate that are directly used to detect molecules or other components. In addition, we discuss the molecule factors and optical system for the reproducibility for sample-to-sample or spot-to-spot on a substrate. In the final part of this review, some effects resulting in the irreproducibility of Raman bands' position from recent literatures are discussed.

Key words: surface-enhanced Raman spectroscopy (SERS), reproducibility, Raman intensity, Raman shift, plasmonic nanoparticle
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0 Introduction

Raman scattering, an inelastic scattering of incident light interacting with the molecular vibration, is typically very weak due to the small scattering cross-section of molecules. However, the surface-enhanced Raman scattering effect can result in the enhancement of Raman scattering more than 10^6 times by molecules adsorbed on rough metal surface. The discovery of surface-enhanced Raman spectroscopy (SERS) has been approximately 40 years[1-4]. Recent advances in physical technology and material chemistry as well as the increasing requirements for trace level analysis[5-8] and detection[9], especially the reports about the single molecular detection[10-11], have rekindled interest in this technique over the last two decades. According to the superiorities, SERS has the potential to be one of the most useful tools for sensing and detection. One of the most critical aspects of SERS effect is the metallic structure, namely called "SERS substrate", that is used to generate the surface plasmons due to the interactions with the incident visible or near-infrared (NIR) light. The plasmonic effect of metallic nanostructures is mainly responsible for the enhancement of the Raman signal. SERS has been extensively utilized in various fields such as ultrasensitive chemical and biochemical analytics. To realize the potential of SERS for the trace analysis and detection, it is necessary to engineer to maximize signal strength and ensure reproducibility for the surface plasmon resonances of the substrate. However, as a historical issue, the reproducibility in SERS has restricted its application into commercial market due to the fluctuation of the Raman signal intensity. Therefore, this review mainly focuses on the influence factors of the reproducibility in SERS measurement and we hope to bring more discussion about the possibility to overcome the reproducibility.

SERS combines the molecular fingerprint spectroscopy with an increased sensitivity due to an enhancement of the Raman signal intensity for molecules of interests on the metal surface or at least very close to it. The SERS signal intensity depends crucially on the enhancement factor (EF) and the wavelength of incident light. From the literatures, one representative way[12] that has been used to calculate the surface-enhanced Raman intensity contains three parts: ① the electric field at the substrate surface; ② the number of molecules and the differential Raman cross-section per molecule; ③ the optical system conditions such as the incident laser power, the area of the light focus, and the overall efficiency of the detection system including

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the collection efficiency of scattered light, transmission efficiency of the spectrograph and photon detection efficiency. These aspects that influence the Raman signal intensity are also the factors to determine the reproducibility of the SERS intensity that will be discussed later.

The first part of this review will be dominated by the discussion of calculation of surface-enhanced Raman signal intensity on some different substrates and explain the sensitivity which makes it much more difficult to achieve high uniformity and reproducibility. Then we will focus on the fabrication of SERS substrates generally including two types: ① single particles and ② arrays on substrate that are directly used to detect molecules or other components. In addition, we will discuss the molecule factors and optical system for the reproducibility for sample-to-sample or spot-to-spot on a substrate. As the fingerprint spectrum of a specific molecule, the position of its corresponding Raman bands (namely, the wavenumber of Raman shift) is typically constant. However, some effects may cause their reproducibility of the position of Raman bands. In the final part of this review, we will discuss the phenomena inducing the position shift of the Raman bands from recent literatures.

In order to make the problem more clearly, herein we think that we need to clarify the differences of substrate uniformity and reproducibility. As it is well known, the uniformity can be defined as the quality of lacking diversity or variation of surface-enhanced Raman signal intensity at spot-to-spot on the same substrate or on different samples that are made in one batch. In contrast, the reproducibility of SERS cares about the test results at different times or batches of samples. Although there are notable differences between uniformity and reproducibility from the definition, they are decided by the same mechanism to a large extent. Therefore, we do not make a distinction between them in this review unless we mention particularly.

1 Influencing Factors from Mechanism

In order to explain the theoretical background of SERS, two different enhancement mechanisms are widely accepted known as electromagnetic (EM) and chemical enhancement, with the first being awarded the main contribution. The interaction of light with metal surface results in the coupling between the free electrons of metal and the incident light, consequently yielding a coherent oscillation of the free electrons. This kind of surfaces can serve as substrates to enhance the Raman signal of (bio)molecular entities due to the EM field enhancement effects from plasmonic resonances[13]. The second chemical mechanism, sometimes called charge transfer, involves the excitation of charge transfer between analyte molecules and the metal surface to give rise to a resonance Raman enhancement process. We focus on the surface-enhanced Raman intensity calculations based on the EM enhancement factors from theory and experiments. During the discussion about the SERS mechanism in the following sections, we also point out the influence factors from the origins of SERS to demonstrate why it is difficult to achieve a high reproducible and uniform performance.

1.1 SERS Enhancement Factors

SERS EFs are the central to SERS both for research and applications. Its practical definition was summarized by Le Ru and Etchegoin[14], i.e. the ratio of SERS signal to the Raman signal that would be obtained for the same molecule in the absence of the SERS substrate, with all other conditions being identical. However there are a number of difficulties when one attempts to predict, measure, and compare EFs at different systems from different labs due to too many calculation methods in theory or experiments. Le Ru and Etchegoin have summarized different types of expressions to calculate the EFs for their characterizations at different conditions[14]. For instance, the EF for SERS can be described as a function of the frequency of laser radiation in theory[15]:

$$\mathrm{EF} = \frac{|E_{\mathrm{inc}}^2||E_{\mathrm{Stokes}}^2|}{E_0} \approx \left|\frac{E_{\mathrm{inc}}}{E_0}\right|^4, \qquad (1)$$

where, E_{inc} and E_{Stokes} are the electric fields at the wavelength of incident excitation and Stokes Raman shifts, respectively; E_0 is the electric field of the incident laser.

From the representative Eq. (1) above, although it is the function of the frequency of laser radiation, the most sensitive aspect to the reproducibility of surface-enhanced Raman signal intensity is the electric field E_{inc}/E_0, which is the biquadratic in the equation. For example, for a spherical particle whose radius is much smaller than the wavelength of visible or NIR light, the electric field is uniform across the particle and the electrostatic approximation is a good one. The field induced at the surface of the sphere is related to the applied, external field by

$$\frac{E_{\mathrm{inc}}}{E_0} = \frac{\varepsilon_1(\omega) - \varepsilon_2}{\varepsilon_1(\omega) + 2\varepsilon_2}, \qquad (2)$$

where $\varepsilon_1(\omega)$ is the complex, frequency-dependent dielectric function of the metal and ε_2 is the relative permittivity of the ambient phase. The resonance takes place at the frequency for $\mathrm{Re}(\varepsilon_1) = -2\varepsilon_2$ from Eq. (2), where $\mathrm{Re}(\varepsilon_1)$ is the real part of ε_1. But for other nonspherical particles, the resonant condition is changed enormously following the variation of particle size and shape and is really difficult to estimate it in theory. From this example of EF calculation of single nanoparticle, it is clear that EFs and the resonance condition are enormously sensitive to the particle size and shape which lead to a poor reproducibility for the little changed morphology of particles. In addition, if particles aggregate together, the resonance condition and induced electric field are changed tremendously so that the surface-enhanced Raman signal varies notably. So if one wants to obtain much more uniform and reproducible EFs, he has to make sure that the particle size, shape and distribution of nanoparticles must be uniform and reproducible in a higher degree.

Furthermore, since SERS is a near-field effect, the EM field distribution also has large effect on SERS intensity reproducibility for molecules attached on the noble metal surface. When there are some interactions among particles even if the particle distribution is uniform on the substrate, the EM field is not the same at spot-to-spot such as the example in Fig. 1[16], i.e. with a distribution of near-field in the space on the substrate. Indeed, their experiments and simulation results provide the clearest evidence to demonstrate that EM field affects SERS EFs and only a few regions are "hot". For this reason, in order to take full use of this kind of powerful tool of SERS, one has to find some novel methods to fabricate nanostructures controllably and precisely to realize a higher uniform and reproducible result. In a section later we will discuss the fabrication of nanostructures in detail to explain their influences on the SERS reproducibility.

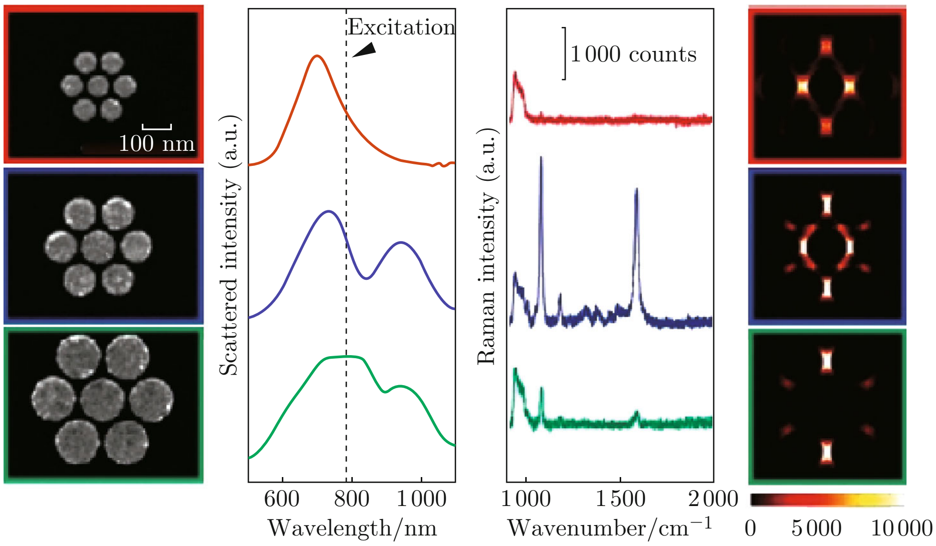


Fig. 1 Spatial dependence between SERS and near-field enhancement distribution for a single gold heptamer composed of different size gold nanodisks[16]

1.2 Raman Signal Intensity Calculation

From the application of SERS, the surface-enhanced Raman signal intensity is always measured by Raman instrument directly. Typically, researchers utilize obtained Raman signals to calculate EFs and Raman cross-section of molecules adsorbed on the metallic surface[14,17]. Here we discuss the calculation of surface-enhanced Raman signal intensity in an opposite way to demonstrate the influence factors of SERS reproducibility. In simple terms, the SERS intensity for a given vibrational mode of a given analyte should also be proportional to the laser intensity and to the normal Raman cross-section but affected heavily by EFs. For instance, the following expression which is adapted from the article of Le Ru and Etchegoin[14] is one of the classical equations to calculate surface-enhanced Raman signal intensity:

$$I_{\mathrm{SERS}} = \mathrm{EF}\frac{N_{\mathrm{Surf}}}{N_{\mathrm{Vol}}}I_{\mathrm{RS}}, \tag{3}$$

where, I_{SERS} and I_{RS} are the surface-enhanced Raman signal intensity and normal Raman intensity, respectively; N_{Surf} is the number of adsorbed molecules in the detection regions; N_{Vol} is the average number of molecules in the scattering volume. It is obvious that Eq. (3) is better suited to characterize substrate performance which contains the information about the EFs of the substrate and molecules adsorbed on it but presents much more challenges from an experimental point of view to achieve a high reproducibility of spot-to-spot on the same substrate or sample-to-sample. From the points of experiments, the real challenge here is to control rigorously and accurately the number of molecules on metallic surface in the detection regions, or equivalently the surface coverage which leads to the results that one can hardly make the surface-enhanced Raman signal intensity reproducibly for the molecule factors.

Furthermore, no matter what the expression really is in such kind of calculation of surface-enhanced Raman signal intensity, there is a similar result that the SERS intensity is proportional to EFs, laser power, the effective number of molecules adsorbed or molecule orientations[12] on metallic surface and efficiency of optical system. Because of so many influencing factors upon the surface-enhanced Raman signal intensity from substrates, molecules or optical system, these make it too difficult to achieve high uniformity and high reproducibility.

2 Structural Factors on Reproducibility

The EM field enhancement of the plasmon resonance is associated with the resonance condition that is determined by the surrounding medium and further by the size, shape, and material of the nanoparticle. From the discussion of mechanism above, it is essential to find some controllable methods to synthesize uniform and reproducible nanoparticles or arrays by chemical or physical techniques. Progress has been made in controlling the assembly of silver or gold nanoparticles into structures that would be employed as SERS substrates[18-21]. Electron beam lithography[22], nano sphere lithography[23], focused ion beam pattering[24], vacuum specific evaporation[25], and electrochemical deposition[26] are currently available physical techniques for fabrication of well ordered, periodic noble metallic nano particle arrays with desired geometries. The applicable chemical methods for synthesizing colloidal noble metallic nanoparticles are also on the way and that exhibit improved monodispersity and repeatability relative to previously published methods[27-28].

Moreover, analytical and numerical methods have been used to characterize and optimize nanostructures for SERS in experiments. Both kinds of these methods deal with the interaction of light with metallic nanostructure whose size is smaller than the applied excitation wavelength. These analytical methods were applied to describing nanostructures such as nanosphere or ellipsoids[29], gold dimer with a single particle in the gap[30], cylindrical protrusions or grooves on a flatmetallic surface[31]. For more complex structures, numerical methods like the finite difference time domain (FDTD) method[16,32-33], finite element method[34], or rigorous coupledwave analysis (RCWA)[35] can be utilized as well.

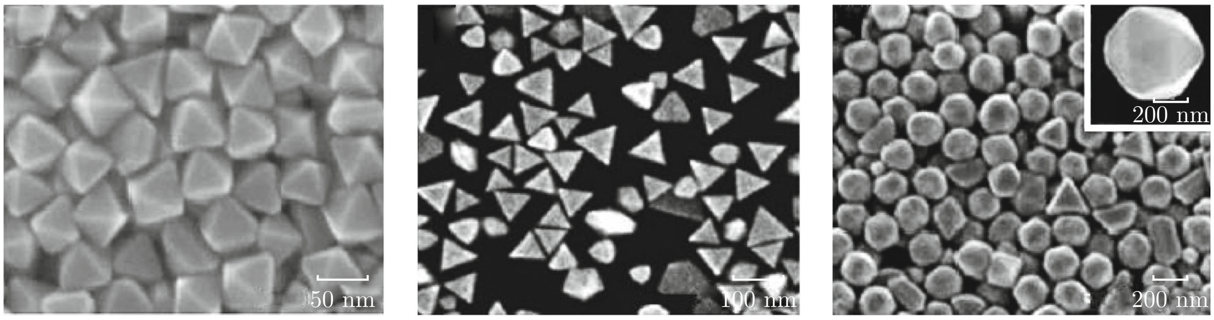
Here we discuss that when the morphology of the nanoparticles or arrays changes a little, the EM field and EFs demonstrated by the calculation of the FDTD simulations or some experimental results will vary enormously, consequently influencing the reproducibility of surface-enhanced Raman signal intensity.

2.1 Single Particles

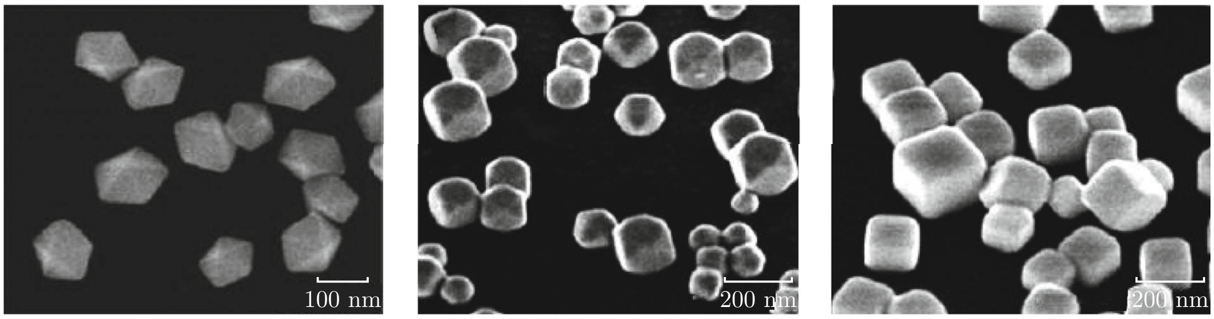
Metallic single nanoparticles are of major importance as SERS substrate. Much of what is known about the optical properties of shape-controlled metallic nanoparticles has been enabled by advances in synthesis, and now a range of shape-controlled nanostructures are prepared as high-quality SERS samples[36]. Shape ranges from Platonic solids such as cubes and octahedral to Archimedean solids and rhombic dodecahedra. There have been many demonstrated methods for controlling the shape of Au nanocrystals; the selection of reductant, reaction conditions, and stabilizer all are critical to form a particular shape. In addition, the methods for improving control of metallic nanoparticle size and shape as well as the properties of nanoparticles have been reviewed in many reports[15,36-38]. As expected, strong reducing agents, as typified by polyol reduction, facilitate the synthesis of thermodynamically favored polyhedral Au nanocrystals[39]. There are, however, notable differences of Raman signal intensity induced by the nanoparticles such as different shapes of nanoparticles which have a good reproducibility in the experiment progress in Fig. 2[36]. Although the geometry of metallic nanoparticles is much better in the formation from the scanning electron microscope (SEM) pictures, the size and shape still have some variations in some degree. These small variations bring notable difference of the SERS intensity and consequently lead to a poor reproducibility.

For example, in the works of Xia and his coworkers[40], they have demonstrated how the shape of an individual nanocube affects the SERS intensity. Figure 3 shows the SERS spectral intensity taken from four individual Ag particles of different geometries: (A) a right bipyramid, (B) a cube with sharpcorners, and (C, D) two truncated cubes. A similar result has been reported by Fang et al[41]. These absolutely demonstrate that with the change of the shape of nanoparticles considerable variations in intensity of the SERS signal take place.

Additionally, the aggregation of nanoparticles in solution or deposition on substrate is one of the notable questions for the application of SERS. From the discussion of EM enhancement mechanism above, we clearly show that the distribution of nanoparticles in solution or on substrate determines the resonance condition and EM field distribution to a great degree. The utility of this type of structure is limited by poor control of the nature and extent of particle aggregation and particle co-location, although strategies are available to improve reproducibility[42]. Because of these kinds of structure factors and its high sensitivity of surrounding environments, it usually leads to poor reproducibility between samples and also poor spatial repeatability when dried onto an inert surface[43]. To solve the problem that metal nanoparticles aggregate together, people investigate the dimers[30], clusters[16] and more complex structures including the following nanostructured arrays which we talk about in the next section.



(a) Octahedrons (b) Truncated tetrahedrons (c) Icosahedrons



(d) Decahedrons (e) Truncated nanocubes (f) Nanocubes

Fig. 2 SEM images of Au nanocrystals with different shapes[36]

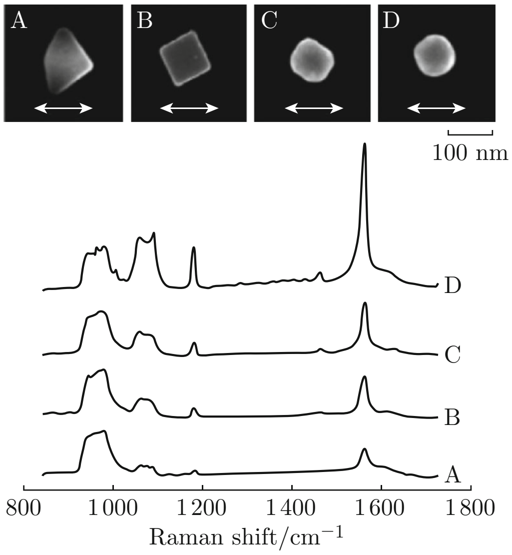
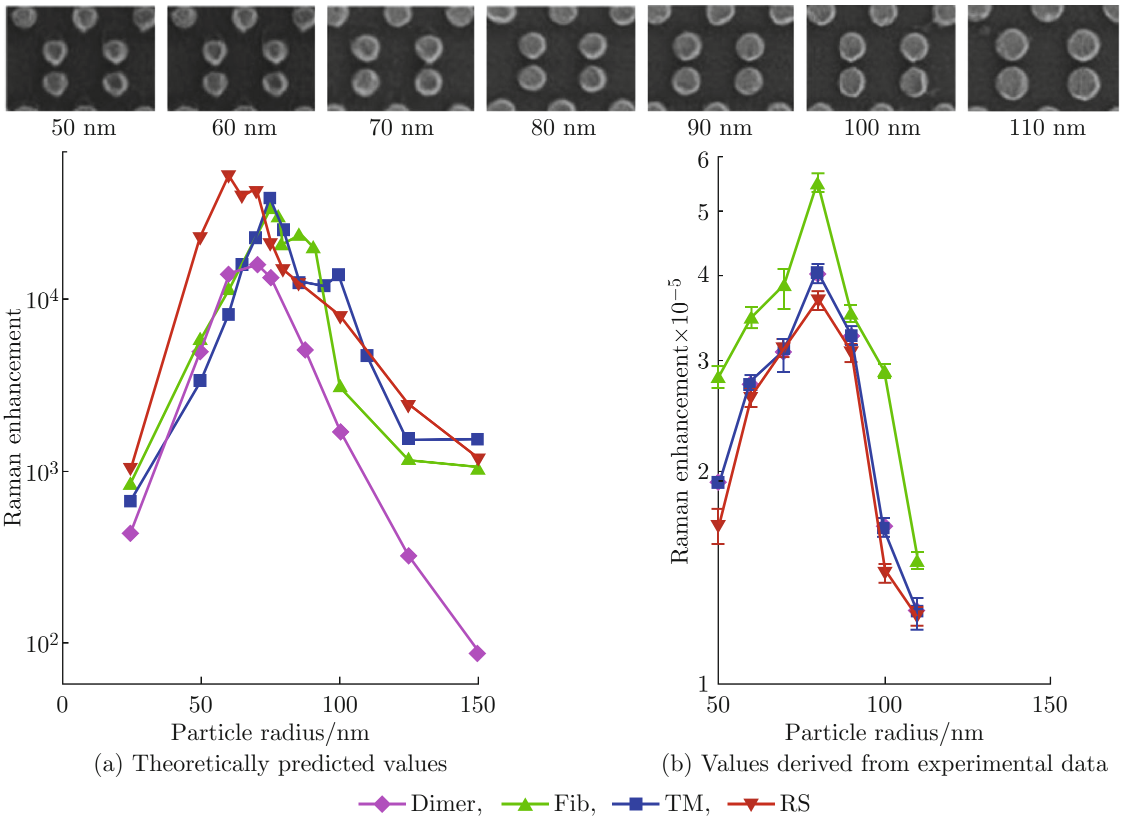


Fig. 3 SERS spectra of 1,4-benzenedithiol molecules adsorbed on single Ag nanoparticles with different morphologies in the accompanying SEM images[40]

2.2 Arrays on the Substrate

For the excellent optical properties of arrays of metallic nanoparticles in SERS application, lots of works[20,44-48] have been made to investigate its fabrication methods and optimize their morphology parameters such as shape, size and distribution on the substrates. Associated with single nanoparticle which is a basic unit of the arrays, there are nanohole arrays[46-52], nanorod arrays[53], nanocylinder arrays[53], nanotriangle arrays[54] and so on. Although physical techniques allow the fabrication of well-ordered and periodic silver or gold nanoparticle arrays with desired geometries, the formation of nanoparticle arrays from solution is generally the most straightforward way to synthesize an effective structure for SERS. This kind of work has been reviewed by Brown and Milton[45] who can acknowledge much more about the fabrication ways in solution. In addition, although the methods have been studied for many groups to achieve a higher uniform of arrays on the substrate, slight variation of nanoarray condition such as the size, aggregation state, and distribution of nanoparticles or nanoholes will lead to a notable difference of SERS intensity, therefore making it difficult to obtain a good reproducibility of SERS signal. Gopinath et al.[55] have investigated the nanocylinder arrays and nanotriangle arrays in detail. For example, the EFs and the surface-enhanced Raman intensity vary notably for the particle arrays with different sizes and morphologies (e.g. dimer, quasi-periodic (Fib), singular-continuous (TM) and absolutely-continuous (RS) structures) (see Fig. 4).

From a fundamental and practical point of view, we are interested in not only the high EFs but also the reproducibility of the SERS intensity. However, in some degree it is too much difficult for us to achieve excellent EFs and good reproducibility simultaneously. Some of endeavors[8,28] in the experiments have been made to obtain uniform and reproducible structures



(a) Theoretically predicted values (b) Values derived from experimental data

Fig. 4 Raman enhancement scaling with different nanoparticle radii in different arrays using generalized Mie theory (GMT)[55]

up to now. Many applications only focus on the average EF, i.e. for molecules with random positions on the substrate. Sometimes one is even eager to obtain much less-enhanced Raman intensity in order to minimize the signal variation. It is now believed that an EF value of 10^7 to 10^8 may be enough for single molecule detection[20,56-59], but problems with poor reproducibility of "hot" SERS active nanostructures, lack of quantitative SERS signals and generating a narrow distribution of high EF values remain unresolved. Qu et al.[8] used screen printing method to get the Ag nanoparticle arrays which exhibited high reproducibility and stability; the spot-to-spot SERS signals showed that the surface-enhanced Raman intensity variation was less than 10% and SERS performance could be maintained over 12 weeks. What is more, Cho et al.[60] used the similar method to fabricate ultrahigh-density array of silver nanoclusters for an SERS substrate showing a very high signal intensity with an SERS EF as high as 10^8, which is enough to detect a single molecule, and excellent reproducibility (less than 5% variation of the signal intensity).

3 Molecule Factors

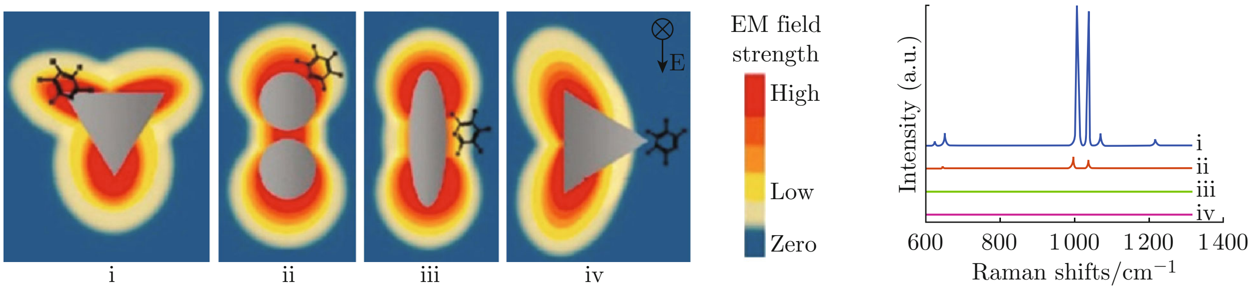
As known from the mechanism above, only a few positions around the nanoparticles are "hot" due to the localization and confinement of the near-field even though the size, shape and distribution of nanoparticles are uniform. Moreover, the SERS effect is distance-dependent and requires that the molecules must be adsorbed on the metal surface or at least is very close to it. From the practical points of SERS, we can expect that the average surface-enhanced Raman intensity is proportional to the average number of adsorbed molecules. But the average surface-enhanced Raman intensity is influenced by the effective number of molecules in "hot" regions and their orientation as well as Raman cross-section of molecule on metal surface.

3.1 Adsorption Efficiency/Quality of Monolayer of the Molecules on Metal Surface

SERS utilizes the large local field enhancements that exist at metallic surface to boost the Raman scattering signal of molecules adsorbed at the metallic surface. From the calculation of surface-enhanced Raman signal intensity above, it is obvious that the absorption quality of monolayer of molecules on the metallic surface is much more essential to test for calculation of EFs. Moreover the real challenge here is to determine rigorously and accurately the number of molecules on metal surface, or equivalently the surface coverage. Although the self-assembled monolayer (SAM) method has been extensively used to immobilize the molecules on the surface of fabrication of nanostructures, the actual number of the molecules may be affected by the adsorption efficiency of the molecules during the self-assembly process.

3.2 Effective Number of Molecules in “Hot” Region

Since the EM field around the nanoparticles or metal surface is highly localized, the actual number of the molecules in the “hot” region may vary remarkably. In the case of single molecule, the molecule is located somewhere in the “hot” spot around the nanoparticle and it may vary its location, for example by diffusion, consequently giving rise to spectral fluctuations[12] because SERS EFs in the presence of “hot” spots can vary by an order of magnitude over distances that are comparable to the size of typical molecules. It can be seen in Fig. 5 that not all the molecules adsorbed on the metallic surface can generate signal[61]. Expected SERS spectra for pyridine illustrate that signal enhancements are the greatest for molecules, while no enhancement is expected if the analyte is positioned outside the EM field. Secondly, the SERS signal of target molecules strongly depends on the molecular length of the linker (in many cases, a linker molecule between the metal surface and target molecules is necessary for detection) because the field enhancement decays with the distance to the substrate surface, even if the adsorption efficiency of the target molecules is quite high.



(a) Nanoparticle and its electromagnetic field

(b) Expected SERS spectra for pyridine on nanostructures i, ii, iii and iv

Fig. 5 Particle shape, size, and proximity to other particles affecting the EM field that forms around a metal nanoparticle[61]

3.3 Orientations and Cross-Section of Molecule on Metallic Surface

The signal enhancement of Raman modes depends additionally on the orientation of the molecule towards the metallic nanostructured surface[62]. A molecule is characterized by its Raman tensor and its orientation with respect to the surface. The enhancement of various Raman active modes is dependent on the field components parallel and perpendicular to the surface. Because of the dominant Raman tensor component perpendicular to the surface, vibrational modes with dynamic dipoles normal to the surface are detected. In general, Raman modes oriented parallel to the surface are weak in intensity or not detectable[2,63-64]. A direct demonstration of the surface selection rules was performed by applying a molecular monolayer onto a flat gold surface[65]. This detailed understanding allows the tracking of the molecular orientation by analyzing the variations in SERS intensities of molecules located at “hot” spots[66].

The most common situation in molecule spectra is the measurement of an ensemble of many random-oriented molecules. So the surface-enhanced Raman intensity we measured is normally an average signal over random orientations of the molecules. In spite of this, for improving the reproducibility of surface-enhanced Raman signal intensity, the orientations of all molecules still need to be uniform when the molecules are adsorbed on the substrates. What is more, the effective Raman cross-section of molecules is associated to their orientation closely though the Raman cross-section of a certain molecule is a constant value conventionally. In fact, the Raman cross-section simply relates the incident power direction and the power scattered by the molecule for the optical absorption process of molecules.

4 Optical System for the Reproducibility

From the practical point of view, the SERS measurement optical system is the most important component on which both the excitation and detection of the Raman signal have to rely. Typically in a measurement, we always try to keep the measurement conditions constant including the objective lens, laser frequency, incident power, integration time, polarization direction and so on. For example, from the results of Etchegoin et al.[67] we have observed that different laser powers can induce different SERS EFs. Besides these, other factors such as the attenuation of laser power, temperature-variation induced impact on the photon detection efficiency of charge-coupled device (CCD), and the alignment-dependent spot size of laser focus, excitation efficiency, and transmission efficiency of the spectrograph may strongly affect the reproducibility of SERS as well. In order to achieve high reproducibility of the surface-enhanced Raman signal intensity, one should keep the optical system as stable as possible.

5 Position Shifts of Raman Bands

It is well-known that SERS effect typically boots the Raman intensity dramatically without changing the position of Raman bands. Therefore, surface-enhanced Raman spectrum is commonly accepted as a fingerprint spectrum for the identification of a specific molecule. In the community of SERS study, one usually concerns about the reproducibility of the intensity of Raman bands instead of their positions (Stokes Raman shift). Although it is often seen that the position of Raman bands shifts to some extent in different measurements from the literature, this effect is nearly neglected for a small variation in some conditions. Several groups have explored the mechanism of these phenomena. Takahashi et al.[68] and Yaghobian et al.[69] have investigated the charge transfer effect induced shifts of Raman bands due to the electron donation and back-donation between molecules and the substrate. Yano et al.[70] have shown that an external pressure can induce the Raman shifts among those molecules that are pressurized. The similar pressure-induced mechanism has also been reported by Meléndez-Pagán and Ben-Amotz[71]. In addition, Kho et al.[72] have presented another mechanism, i.e. steric hindrance from the antibody which leads to the Raman shifts. Therefore, the position of Raman bands may vary for some reasons including the mechanical distortion of the sample such as pressure, steric hindrance from out space or the chemical effects and other aspects that may have not been discovered up to now.

6 Conclusion

Although the SERS technique is an attractive tool for sensing or detecting molecules in trace amounts, the reproducibility of SERS as a historical problem has limited its commercialization. From these studies, a number of guiding considerations including the aspects of substrates, molecules and optical system have emerged to direct us to achieve a highly reproducible surface-enhanced Raman signal intensity or the position shifts of Raman bands. Frist, the position and line width of SERS are governed by the plasmon enabled by the size, shape and distribution of nanoparticles. While synthesizing and optimizing nanoparticle shape, size, and distribution on substrate are certainly advantageous, it is not the entire solutions to extend SERS applications in commercial market. Even if the substrate is uniform in a very high degree, there are only a few “hot” positions around the nanoparticles. Second, the average surface-enhanced Raman signal intensity is influenced by the effective number of molecules in “hot” regions and their orientations as well as Raman cross-section of molecule on metallic surface. These make us to find a novel method to achieve a high uniform distribution of linker molecules at spot-to-spot. Third, we should keep the measurement conditions as constant as possible including the temperature, optics alignment, objective lens, laser frequency, incident power, integration time, polarization direction and so on.

Existing synthetic methods and physical technologies have achieved greater product uniformity in terms of size, shape and distribution of nanoparticles, and the molecular adsorption can be improved by optimizing the surface chemistry, but the field distribution of SERS substrate certainly exists. We hope the summary and discussion in this review will remind scientists to pay special attention during the SERS measurement to obtain a reproducible result and will pay the way to the development of novel reproducible SERS techniques.

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