Stacked and Tunable Large-Scale Plasmonic Nanoparticle Arrays for Surface-Enhanced Raman Spectroscopy

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ABSTRACT: Surface-enhanced Raman spectroscopy takes advantage of plasmonic substrates that sustain resonances at tunable frequencies with a reproducibly extraordinary field enhancement. Low-cost and large-scale fabrication of these substrates is further required. Here, we present stacked large-scale arrays of strongly coupled gold nanoparticles as promising candidates for such substrates. These arrays are fabricated by bottom-up techniques that fulfill the aforementioned requirements. The distance between adjacent arrays in the stack is controlled with high precision using a discrete number of monolayers of molecules that enable the spectral position of the plasmonic resonances to be tuned. Although the nanoparticles are randomly arranged in each array, the spatial proximity of the stacked arrays enables a strong coupling among nanoparticles to be achieved in adjacent arrays. The huge field enhancements due to these strongly coupled gold nanoparticles are shown to enhance the Raman signal. We show that effectively the optical response from these stacked arrays and the Raman signals can be understood in a simplifying picture where only one individual nanoparticle dimer is considered. The possibility to tune the plasmonic resonances of the substrate across the visible spectrum makes our material a plasmonic substrate of choice for many applications where light–matter interactions need to be intensified.

INTRODUCTION

Surface-enhanced Raman spectroscopy (SERS) has been established as a powerful analytical tool for the characterization of molecules. It is a further development of Raman spectroscopy where light is inelastically scattered by molecules. This Raman spectrum is unique to a given molecule. The serious drawback of conventional Raman spectroscopy is its relatively low cross section. This disadvantage prohibited many applications in the past, i.e., especially those where only a few molecules are required to be detected, such as, for example, for cancer diagnostics or the detection of bacteria.

To mitigate this problem, the exploitation of suitable enhancement mechanisms is necessary. The potential of plasmonic nanoparticles (NPs) to excite localized surface plasmon polariton resonances (LSPRs) in the visible is well-known to dramatically enhance the Raman cross section if the molecules are brought in close proximity to them. In principle, there are two mechanisms that cause this enhancement: the chemical and the electromagnetic enhancement mechanism. The first one stems from the chemical binding of the investigated molecules to the plasmonic NPs and the latter one from the enhanced local electromagnetic field due to the excited LSPRs in the NPs. Recent investigations have revealed that the chemical mechanism, generally, modifies the SERS spectra, and the electromagnetic one accounts for the enhancement. In the lowest-order approximation, the enhancement of the Raman cross section due to electromagnetic fields scales with the square of the local electric field at the excitation frequency \( E_{loc}^2(r, \omega_0) \) multiplied by the square of the local electric field at the Stokes frequency \( E_{loc}^2(r, \omega_s) \). An enhancement factor (EF) of the SERS cross section can be defined as \( EF = (1/V) \int dV \left| E_{loc}^2(r, \omega_0) E_{loc}^2(r, \omega_s) \right| \). Therefore, it is of major importance to design and fabricate nanostructures that provide strongly enhanced local electric fields.

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Spherical gold NPs, nanoshells, and dimers made of these entities were shown to cause highly increased SERS signals. Instead of spherical NPs, shaped objects of higher complexity such as nanoflowers or nanocubes provide strongly enhanced local electric fields. Complex clusters of NPs of different size or materials were also identified as possible SERS substrates. In most of the proposed SERS substrates the observed field enhancement is due to the excitation of LSPRs in gold or silver NPs. In particular, these local fields can be further enhanced by two effects: sharp geometrical features such as rough edges (e.g., for nanocubes) or the near-field coupling between neighboring NPs. Evidently, the combination of both effects is a particularly useful option. Recently, it has been demonstrated that complex resonances resulting from multiple NPs, such as Fano resonances, can exhibit huge local field enhancements yielding to high SERS EFs.

The fabrication of suitable SERS substrates that display high local field enhancements constitutes a major challenge. If the field enhancement stems from the coupling of NPs, appropriately small distances have to be controlled to ensure enhanced fields. Frequently, self-assembly techniques are applied to fabricate the single unit cells in combination with conventional top-down techniques to arrange them on an appropriate lattice. Then, the effect of the lattice on the optical response is known, and the most suitable lattice can be designed. Extraordinary small distances between NPs at the nanometer scale have been achieved by angle evaporation or by self-assembly techniques based on linkers, such as polymers, organic linkers, or DNA, to bind the NPs. Another possibility is to use block copolymer self-assembly to fabricate suitable SERS substrates. Recently, subnanometer separations have been achieved in the case of self-assembled nanorods. In general, all of these bottom-up techniques solve the issue of fabricating low-cost and large-scale substrates that dramatically enhance the SERS cross section. However, they usually suffer from an inability to tune the frequency of operation across an extended spectral domain, and they tend to work reliably only in a spectrally narrow frequency range.

In the present article we tackle this issue and introduce a self-assembled SERS substrate that is fabricated by exploiting electrostatic forces on large scales. The fabrication process with electrostatic forces is fast, robust, and inexpensive. However, most importantly, it offers the possibility to easily tune the resonances over a wide frequency range. This allows the application of SERS to a large variety of molecules.

The principal idea behind the bottom-up fabrication of the present SERS substrate is shown in Figure 1. The target structure consists of two amorphous plasmonic NP arrays that are brought in very close proximity. Here the spatial position of the NPs in the plane does not follow a periodic repetition but rather an amorphous pattern. Their distance is precisely adjusted by selecting a discrete number of polyelectrolyte polymer monolayers that exclude conductive coupling between adjacent arrays. The thickness of this spacer can be tuned with nanometric precision. The resonance frequencies of the strongly coupled NP arrays can be adjusted across an extended spectral domain by choosing a proper separation and exploiting the hybridization of plasmonic resonances sustained by the NPs in each array. This ability to fine-tune the plasmonic resonance is the key advantage of our method when compared to others presented in the literature. Eventually, the molecules to be analyzed can be infiltrated into the structure where they experience a huge electromagnetic field directly in the gap. This promotes a highly efficient SERS measurement. In passing we note that polyelectrolyte layers have been previously used in plasmonic studies to establish a well-defined distance on the...
nanoscale, e.g., with the purpose to enhance the two-photon absorption cross section of an organic chromophore in a deterministic manner. 22 They are, therefore, perfectly suitable material systems to enforce distances close to metallic nanostructures.

II EXPERIMENTAL METHODS

Fabrication of NP Arrays. Gold nanospheres with a radius of approximately 20 nm were prepared according to the well-known Turkevich method. 33 Briefly, 500 mL of an aqueous solution of HAuCl₄ (0.25 mM), under constant magnetic stirring, was heated to 100 °C in an oil bath. The gold was then reduced through the addition of 4 mL of an aqueous solution of sodium citrate (0.039 M). A series of color changes were observed before a deep-red solution was produced, and after 15 min the reaction vessel was removed from the oil bath and allowed to cool to room temperature.

Glass and silicon substrates, used for spectral measurements and electron microscopy, respectively, were prepared according to the same method. They were first rinsed with water and ethanol before being dried under a stream of compressed air. The surfaces were then cleaned and hydroxylated through immersion in a piranha solution (3:1 mixture of concentrated sulfuric acid to 30% hydrogen peroxide) for 30 min. Piranha solution is strongly acidic and highly oxidizing and should be handled with caution. The substrates were next rinsed with copious amounts of water and again dried under a stream of compressed air. Subsequently, the surface chemistry was altered through functionalization with an organosilane. 34 Here, the substrates were immersed in a 5% (v/v) solution of (trimethoxysilyl)propyl]ethylenediamine in ethanol for 30 min before being rinsed with water, dried, and set in an oven at 120 °C for a further 30 min.

Gold nanospheres were deposited on the substrates (silicon or glass) by immersing them in a solution of the nanoparticles for a period of two and a half hours. The substrates were then washed with water and dried under a stream of compressed air. The surface coverage of nanospheres was 15.6%.

PE solutions had a concentration of 5 mg/mL [in the case of poly(allyl amine hydrochloride) and poly(styrenesulfonate)] or 1 mg/mL [in the case of polyfluorescein isothiocyanate allylamine hydrochloride] in a 0.1 M aqueous solution of sodium chloride. Substrates were immersed in these solutions for 1 min before being rinsed with water and dried under a stream of compressed air. The electrostatic nature of the fabrication process allows for a cyclic build-up of multiple gold nanoparticle and PE layers.

SERS Measurements. To characterize the gold NP arrays, a 1 μL droplet of an aqueous solution of the analyte Nile blue with a concentration of 10⁻⁴ M is dried on the surface. Thus, the analyte molecules are allowed to bind to the metallic surface via the hetero atoms. The SERS spectra of the model analyte Nile blue were recorded using a conventional WITec Raman microscope (WITec GmbH, Ulm, Germany) equipped with a He–Ne laser (633 nm) as the excitation source. The laser power incident on the sample was 0.5 mW. A 100× objective (NA 0.95) was used for focusing the laser light onto the samples and further collecting the Raman signal. The Raman scattered light was diffracted by using a 600 lines/mm grating and finally detected by a CCD camera. For each sample, 20 scans with 20 single spectra of 0.5 s of integration and 50 × 50 μm² were recorded. For the analysis, mean value spectra for each scan were generated. In the case of the copolymer layers that contain fluorescein the same SERS-based measuring routine was employed, however, without coating with a special analyte molecule.

Numerical Simulations. The simulations of the optical response of two coupled spheres were performed based on an analytical solution of Maxwell’s equations, known as Mie theory. 35 Though introduced a century ago solely for single spheres, it can be extended toward arbitrary clusters of spheres. 36,37 The solution is based on an expansion of the electromagnetic fields into eigenfunctions, the vector spherical harmonics. For the presented results of two strongly coupled spheres, ten orders of this expansion were taken into account to ensure fully converged fields. The spheres with 20 nm radii were illuminated by a plane wave, and material parameters of gold were taken from the literature 38 with a size-dependent correction of the imaginary part. 39 The surrounding was assumed as a homogeneous dielectric with a constant permittivity ε = 2.2. This simplification is justified since the difference of the permittivities of the glass substrate (ε = 2.2) and the PE layers (ε = 2.25) is negligible.

The separation of two spheres was chosen according to the number of PE layers. The thickness of one PE layer was set to 0.9 nm according to previous work 24,25 Therewith the separation of the centers of the two spheres in Figures 4, 5, and 6 along the propagation direction is N·0.9 nm and parallel to the incident field 2r + N·0.9 nm, where N is the number of separating PE layers and r the radius of the spheres. This chosen geometry resembles the experimental situation where the NPs from the bottom NP array are fully covered by PE layers. The electromagnetic fields are simulated inside a three-dimensional box with fixed dimensions for all simulations. Respective slices through this box are shown in Figure 5.

RESULTS AND DISCUSSION

The fabrication starts with the functionalization of the glass substrate to attach negatively charged gold NPs to it, as shown on the top left of Figure 1. The net negative charge of the NPs is highly beneficial to prevent the NPs from aggregation and to ensure a suitable filling fraction on the surface. This results in an array of amorphously arranged NPs. In the first process step [labeled as (1) in Figure 1] this gold NP array is coated with a positively charged polymer [a polyelectrolyte (PE)] of about 1 nm thickness. This can be sequentially repeated with oppositely charged PE layers [step (2)] to define an effective polymer layer with a discrete thickness of only a few nanometers. Finally, in step (3) a second array of gold NPs is deposited on top of the PE layers, again mediated by electrostatic forces. To do this successfully, the last PE layer is required to be positively charged to attract the negatively charged NPs. This implies that only an odd number of PE layers can be used. The separation of both gold NP arrays is therefore defined by the number of PE layers in between. SERS measurements are performed on such samples after doping them with a solution including the molecules of interest [step (4)]. Figure 1 shows a SEM image of two stacked gold NP arrays that are fabricated in such a manner. The gold NP arrays are separated by 15 PE layers. The separation between the top and the bottom array can be clearly seen as well as the amorphous character of both arrays. More details on the fabrication process were described in the Methods section.

The investigation of the performance of the fabricated NP arrays for SERS measurements is carried out by doping them with Nile blue as a model analyte. Different samples consisting
of two stacked NP arrays were fabricated using the aforementioned bottom-up technique. The number of PE layers separating the NP arrays varied from 1 to 15 PE layers. The analyte molecule Nile blue is penetrating the PE layers and thus is bound via the hetero atoms to the metallic surface. For comparison, one sample consisting of a single NP array was also considered. The gold NPs exhibit a radius of 20 nm. The fabricated samples were illuminated by a laser beam at 633 nm with 0.5 mW incident on the sample. The Raman signal was detected by a conventional spectrometer. The measured SERS spectra are shown in Figure 2. The recorded Raman spectra are dominated by the contributions of the dye molecule Nile blue due to the resonance Raman effect.26,27 Thus, the other components of the PE layers have a smaller Raman cross section in comparison to Nile blue and are according to this not observable under these conditions. The expected pronounced Raman peak at 590 cm$^{-1}$ for Nile blue is visible for the single NP array (blue curve) as well as for all double NP arrays independent of the number of separating PE layers. However, the intensity of this peak with respect to the background varies tremendously. An optimum exists for three separating PE layers (red curve). For this sample a multitude of additional wavenumbers. To quantify this effect of more enhanced/pronounced Raman peaks in more detail, the integrated intensity of the prominent mode at 590 cm$^{-1}$ is taken as an indicator for the SERS EF. Figure 3 shows as a function of the number of PE layers the integrated intensity of the pronounced SERS peak at 590 cm$^{-1}$ to quantify the results in a more convenient manner. The error bars correspond to the variation of the signal due to the measurements at 20 different spatial locations at each sample. A clear optimum can be identified for three PE layers (red bar in the bar diagram) where the enhancement of the SERS signal increases by a factor of 3–4 compared to the single NP array (blue bar). In the following the physical origin of this resonance is revealed by simulations before the tuning capabilities of the samples are discussed.

Solving Maxwell’s equations for an amorphous single or double NP array [as shown in Figure 1] remains challenging since all NPs illuminated by the incident light need to be considered. It has been shown in the literature,24 and seems to be intuitively clear, that the optical response of a single NP array with a surface filling fraction below 30% should be dominated by the excitation of the LSPRs in isolated NPs. The reason is that these filling fractions ensure a mean distance between adjacent NPs that is larger than three times their radii. In other words, coupling between NPs in an amorphous array can be safely neglected. Thus, for the fabricated double NP arrays only NPs from different arrays can couple to each other. The thickness of a single PE layer is about 1 nm,28–30 which is small compared to the NP radius of 20 nm. From all these considerations it can be concluded that it is sufficient to describe the optical response of the amorphous NP arrays just by considering the coupling of one NP from the bottom and one from the top array. However, it is clear that not all NPs in adjacent arrays will form such a tight arrangement. These deviating configurations will not, however, contribute to a noticeable extent to the Raman signal since they sustain spectrally detuned resonances and the field enhancement they provide is inferior. Therefore, consideration of solely two NPs (as a simple dimer model) that will cause the highest SERS signal is fully sufficient. The distance and spatial separation between these two NPs is chosen with respect to the thickness of the PE layers, as shown in Figure 4(B). In simulations the PE layers are assumed to be a homogeneous dielectric medium with a permittivity of $\varepsilon = 2.2$ and a thickness of 0.9 nm. These parameters and the applied restriction to the coupling of just two spheres have been proven as fully sufficient and very effective means to describe the optical response of the fabricated double NP arrays.24,25

As can be seen in Figure 1, the NPs of the samples are almost spheres. In simulations perfect spheres are assumed, and the optical response is simulated by using an extension of Mie theory.57 Details thereof were given in the Methods section. The simulated extinction cross section of the coupling between one sphere from the bottom and one from the top NP array is shown in Figure 4(A). These simulated spectra are in good agreement with the measured plasmonic properties.24 One clearly observes the excitation of the LSPR for a single gold NP (blue curve) at around 550 nm. For one separating PE layer

Figure 2. (A) SERS spectra of Nile blue as a function of the wavenumber of the molecular vibrations for fabricated double gold NP arrays with different numbers of PE layers. The number of separating PE layers varies from bottom to top, where 0 corresponds to a single gold NP array. This notation is maintained from now on for all the following figures.

Figure 3. Integrated intensity of the pronounced SERS peak at 590 cm$^{-1}$ as a function of the number of separating PE layers.
(dark gray curve) the splitting of the extinction curve into two resonances is observed, where the dominant one is shifted to longer wavelengths. This resonance shifts back to shorter wavelengths if the number of PE layers, or in other words the distance between the two spheres, is increased [as sketched in Figure 4 (B)]. It is important to note that for three PE layers this resonance is at around 650 nm. This resonance position is inside the gray shaded area in Figure 4(A) that defines the wavelength range between the excitation (633 nm) and the Stokes (658 nm) wavelength in the experiments. (C) Amplitude of the extinction at the excitation wavelength of 633 nm of the SERS experiments as a function of the number of PE layers. (D) Simulated SERS EF as defined in the text as a function of the number of PE layers.

Figure 4. (A) Simulated extinction cross sections of two strongly coupled gold NPs, as sketched in (B). From bottom to top the number of PE layers is increased. The numbers shown in (B) correspond to the numbers of PE layers that define the distance between the NPs, where 0 corresponds to a single NP. The gray shaded area in (A) defines the wavelength range between the excitation (633 nm) and the Stokes (658 nm) wavelength in the experiments. (C) Amplitude of the extinction at the excitation wavelength of 633 nm of the SERS experiments as a function of the number of PE layers. (D) Simulated SERS EF as defined in the text as a function of the number of PE layers.
excitation and the Stokes wavelength; for the experimental condition this means $\text{EF} = \frac{1}{V} \int_V dV \frac{\overline{E}_{\text{loc}}^2}{V_{\text{loc}}^2}(633 \text{ nm}) \overline{E}_{\text{loc}}^2(658 \text{ nm})$. Exactly this EF can be obtained by simulating the local electromagnetic fields and integrating them over a volume $V$ that includes both NPs. The results are shown in Figure 4(D). Again, the maximal EF occurs for three PE layers identical to the experiments. The spatial distribution of the simulated local electromagnetic fields at 633 nm is shown for one selected plane in Figure 5. Although the highest amplitudes of the local fields appear for a single PE layer (middle image in first row), a greater spatial area is occupied by high local field amplitudes for three PE layers (right image in first row).

The coincidence between experiments and simulations is appealing. The number of PE layers that have to be chosen to get an optimum SERS enhancement can be safely predicted by simulations. Of course the simulations do not account for the amorphous arrangement of the NPs, and therefore the resonances appear much sharper when compared to the experiments. On the other hand, the simulations are only based on the coupling between two spheres which makes them incredibly fast and simple to perform. This constitutes an enormous advantage. If another incidence wavelength is used in the experiments, it is easily possible to predict the number of PE layers to be used to obtain a maximal SERS enhancement. From Figure 4(A) it is quite obvious that, in principle, every wavelength between 550 and 750 nm can be addressed by shifting the extinction resonance to the respective excitation wavelength of the molecule that should be investigated. Furthermore, instead of gold, silver NPs can be used, as demonstrated in the literature. This makes it possible to address the spectral domain down as low as 400 nm. In principle, the presented samples can offer huge local field enhancements across a wide spectral domain which underlines their tuning applicability. This precise tuning of the resonance positions is only possible because of the small thickness of one PE layer which is about 1 nm. Here, the advantage of using a bottom-up fabrication method is apparent since this thickness defines the distance and the coupling strength between both NP arrays. The precise control of the spatial separation in the nanometer range would be impossible with common top-down techniques. This confirms the argumentation that double NP arrays can be tuned for a desired application. A disadvantage of the present configuration, however, is the insufficient spatial alignment of the molecules to be detected. They are arranged over the entire substrate, and only a small fraction is directly at positions of the highest local field enhancements. Besides the amorphous character of the NP array, this very likely explains the difference in the quantitative signal strength between measurements and simulations. A large share of molecules that contribute to the SERS signal do not experience the huge fields directly in the junction.

To remove this limitation, we explored an alternative variant how SERS measurements could be performed with the present samples. Specifically, we studied the SERS spectra from fluorescein molecules. The advantage of fluorescein molecules is the possibility to directly embed them into the charged polymer layers as shown on the right panel in Figure 6. The positively charged polymer layers from Figure 1 are replaced by copolymer layers. This copolymer consists of a polyelectrolyte and a fluorescein unit with a ratio of 50:1. Therefore, the molecule of interest is directly involved in the fabrication process and has not been added in solution as done for Nile blue [c.f. process step (4) in Figure 1]. The advantage is the definite position of the molecule under investigation directly in between the NP arrays. Fluorescein has two dominant Raman modes at 1176 and 1321 cm$^{-1}$. If the excitation wavelength of 633 nm in the experiments is fixed, the respective Stokes modes should appear at 684 and 691 nm, respectively. From Figure 4(A) it is obvious that the three PE layer configurations should offer an extinction resonance that should enhance the first two resonances at 684 and 691 nm.

Samples of one single gold NP array (20 nm radius) as well as a double NP array with three PE layer separation were fabricated, identical to the previous experiments. The only difference (as shown in Figure 6) is the replacement of the positively charged PE layers by copolymer layers containing fluorescein units. The measured SERS spectra in Figure 6 reveal exactly the performance predicted by the simulations. The Raman modes appear at 1175 and 1318 cm$^{-1}$, which is close to the documented ones. and they get enhanced for the double NP array (red curve) with respect to the single NP array (blue curve). Indeed, for the isolated NP array they are barely visible on top of the background, but they get well pronounced in the stacked NP arrays.

**CONCLUSION**

In this article, we investigated large-scale tunable SERS substrates. The substrates consist of two amorphously arranged arrays of plasmonic NPs that are stacked one above the other. The arrays were fabricated by a bottom-up technique based on electrostatic forces. This allows for large-scale samples that can be fabricated in short time and at low cost. Furthermore, the
samples allow for an easy tuning of their operation wavelength just by varying the distance between the NP arrays in the nanometer range. For this purpose, we used charged monolayers of molecules that act as a reliable spacer.

The performance of the fabricated samples was proven for two different molecules and in two different configurations. Nile blue and fluorescein were the two selected molecules. Their Raman modes were remarkably enhanced when compared to samples where the molecules have been added on top of single NP arrays. Supporting simulations revealed that the performance of the samples could be predicted by a simple model based on two spheres. Therefore, the presented SERS samples serve as a universal, inexpensive, and easy to produce tool to investigate the Raman modes of a huge class of molecules. Beyond their usage as SERS substrates, we can safely anticipate that the present substrates will find applications in many other functional devices where the interaction of light with matter needs to be intensified at a specific wavelength. This concerns sensor applications, ultrafast nonlinear spectroscopy, or quantum applications where the lifetimes of atomic systems need to be controlled.

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Notes
The authors declare no competing financial interest.

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