In situ dynamic measurements of the enhanced SERS signal using an optoelectrofluidic SERS platform

Hyundoo Hwang,†a,b Dongsik Han,†,a Young-Jae Oh,a Yoon-Kyoung Cho,b Ki-Hun Jeong,a and Je-Kyun Park*a

Received 1st April 2011, Accepted 17th May 2011
DOI: 10.1039/c1lc20277d

A novel active surface-enhanced Raman scattering (SERS) platform for dynamic on-demand generation of SERS active sites based on optoelectrofluidics is presented in this paper. When a laser source is projected into a sample solution containing metal nanoparticles in an optoelectrofluidic device and an alternating current (ac) electric field is applied, the metal nanoparticles are spontaneously concentrated and assembled within the laser spot, form SERS-active sites, and enhance the Raman signal significantly, allowing dynamic and more sensitive SERS detection. In this simple platform, in which a glass slide-like optoelectrofluidic device is integrated into a conventional SERS detection system, both dynamic concentration of metal nanoparticles and in situ detection of SERS signal are simultaneously possible with only a single laser source. This optoelectrofluidic SERS spectroscopy allows on-demand generation of ‘hot spots’ at specific regions of interest, and highly sensitive, reliable, and stable SERS measurements of the target molecules in a tiny volume (~500 nL) of liquid sample without any fluidic components and complicated systems.

Introduction

Surface-enhanced Raman scattering (SERS) spectroscopy can provide great advantages for probing the fingerprints of miscellaneous chemical and biological compounds. SERS spectroscopy allows us to obtain specific structural information of the target of interest with highly resolved spectroscopic bands. These characteristics of SERS enable us to perform label-free detection of specific target molecules or simultaneous detection of multiple species. In addition, the SERS-based sensing technologies provide high sensitivity comparable with fluorescence-based detection technologies with reduced photobleaching problem.1,2

There are two approaches in microfluidic platforms for SERS-based detection: (i) colloid-based systems manipulate metallic nanoparticles within liquid samples; and (ii) structure-based systems provide identical SERS-active sites in nanoscopically defined nanostructures. The former has advantages such as ease of implementation and facile synthesis of nanoparticles. In this case, uniform and controlled mixing of reagents is essential and there have been efforts to utilize the microfluidic systems. For example, Park et al. applied a microfluidic passive mixer for efficient mixing of reagents and well-controlled aggregation of metal colloids, which result in reproducible and quantitative SERS detection.3 Strehle et al. developed a droplet-based microfluidic system for high-throughput online SERS detection and reduction of nanoparticle adhesion onto channel walls.4 However, these colloid-based approaches are disadvantageous to some applications which require relatively high sensitivity and stability because the metal nanoparticles which work as the Raman enhancers are dispersed in solution.

To overcome the limitation of the solution-based optofluidic platforms, several microfluidic technologies were used to prepare reliable and stable SERS substrates and to concentrate the target molecules. Wang et al. applied a nanogap in a microfluidic device to induce the accumulation of metal nanoparticles and the target molecules at the microchannel–nanochannel junction, resulting in the enhancement of SERS signal intensity.5 Choi et al. developed a centrifugal microfluidic platform, in which controlled precipitation of metal nanoparticles and sample pre-concentration through a repetitive filling-drying cycle were achieved.6 However, these approaches have not been very effective in terms of sensitivity, reliability, and stability, compared to the conventional structure-based systems, which require complicated fabrication processes but provide well-defined nanostructures. In addition, these methods cannot take the intrinsic advantages of colloid-based systems such as facile implementation and high-throughput analysis.

Recently, active SERS platforms, in which the aggregation of metal nanoparticles can be precisely controlled by external forces, have attracted much attention. Tong et al. applied optical tweezers for trapping and inducing aggregation of colloidal particles.7 However, complicated optical systems with two laser
sources for Raman excitation and optical tweezing, respectively, were always required. In addition, the colloidal solution should be continuously injected through a microchannel to form the metal nanoparticle aggregate in the optical trap, because the particles could be trapped only within a local area, where an axial optical gradient force was dominant. On the other hand, Huh et al. demonstrated electrokinetic concentration and dispersion of metal nanoparticles using patterned microelectrodes for efficient mixing of samples and enhancement of SERS signal intensity. In this electrokinetic platform, however, the SERS-active sites were formed only in the pre-designed positions as well as complicated fabrication processes for patterning microelectrodes were required.

In this paper, we present a novel active SERS platform based on optoelectrofluidics, in which metal nanoparticles, as the Raman enhancers, are spontaneously concentrated and assembled within the laser spot, which is originally for the Raman excitation, by optically induced electrokinetic mechanisms, resulting in the in situ measurement and the enhancement of SERS signal. Here a simple glass slide-like optoelectrofluidic device without any fluidic components or complicated electrode patterns is integrated into the conventional Raman detection system. Only one laser source is required for both the Raman excitation and the aggregation of gold nanoparticles. This optoelectrofluidic SERS spectroscopy allows us the enhancement and in situ measurement of SERS from the target molecules in a specific region of interest at a specific time interval with a tiny volume of liquid sample.

Principles of optoelectrofluidic SERS spectroscopy

Optoelectrofluidics is a phenomenon of the electrokinetic motions of particles or fluids under an optically induced electric field. Among many different kinds of optoelectrofluidic platforms which have been reported, the most widely used one is an optoelectronic tweezer (OET) device based on a photoconductive material deposited on a plate electrode. The OET device is composed of three layers: (i) the photoconductive layer on a transparent electrode, (ii) the liquid chamber, and (iii) the ground electrode. When a light is projected onto the photoconductive layer, the current flows through only the partially illuminated area, forming a nonuniform electric field in the liquid chamber. This optically induced electric field exerts forces on the particles or fluids, resulting in their movements by several electrokinetic mechanisms such as ac electroosmosis (ACEO), electrothermal (ET) flows, and dielectrophoresis (DEP). The optoelectrofluidic platforms have been applied to manipulate various biological materials such as blood cells, oocytes, motile bacteria, and molecules. Recently, this optoelectrofluidic manipulation technique has been utilized in analytical applications such as molecular diffusion measurement and sandwich immunoassays. Concentration of micro- and nanoparticles using the optoelectrofluidic technology has also been reported.

A schematic diagram of the optoelectrofluidic SERS spectroscopy, in which an optoelectrofluidic device is integrated into a conventional SERS detection system, is shown in Fig. 1. When the laser source for Raman scattering excitation is projected into the device, a nonuniform electric field is formed in the liquid solution and the metal nanoparticles, which were dispersed in the liquid sample, start to be concentrated into the illuminated area by several electrokinetic forces—ACEO, ET flows, and DEP. Due to the extremely tiny volume of metal nanoparticles, the drag forces due to the optically induced ACEO and ET flows more dominantly influence the nanoparticle behavior than the DEP force, which is proportional to the particle volume.

The ACEO is a fluidic motion generated by the motion of ions within the electric double layer due to the tangential electric field, , along the surface of the photoconductive layer, and the rectified slip velocity is defined as:

\[
\langle v_{\text{lip}} \rangle = \frac{1}{2} \frac{\lambda_D}{\eta} \text{Re} \left[ \sigma q E_t \right], \tag{1}
\]

where \(\lambda_D\) is the Debye length; \(\eta\) is the fluid viscosity; and \(\sigma q\) is the charges contained in the Debye layer. This ACEO flow becomes dominant as the ac frequency lowers in general. The ET flow due to a temperature gradient in the liquid phase also affects the metal nanoparticle concentration. In an optoelectrofluidic device, the thermal gradients can be generated by Joule heating or by highly focused light in the application of an ac signal which has relatively high amplitude and frequency. The thermal gradient in the fluid results in a gradient in the fluid permittivity and conductivity, thus a fluidic motion is induced by a body force due to an electric field, which is defined by:

\[
\langle j_{\text{ET}} \rangle_t = \frac{1}{2} \text{Re} \left[ \frac{\sigma_m e_m}{\sigma_m + i\sigma_\infty} \frac{\kappa e_m}{\kappa_m - \kappa e_m} |\nabla T| E^* \right], \tag{2}
\]

Gold nanoparticles

Photoconductive layer

Electrode

AC

Electrode

Objective lens

Fig. 1 Schematic diagram of optoelectrofluidic SERS spectroscopy. Metal nanoparticles dispersed in a sample solution are concentrated into a laser spot for Raman scattering excitation by optically induced electrokinetic mechanisms, allowing on-demand generation of SERS-active sites and in situ measurement of the enhanced SERS signal.
where \( \sigma_m \) is the fluid conductivity; \( \kappa_e = (1/\epsilon)(\partial E/\partial T) \) and \( \kappa_p = (1/\epsilon) (\partial E/\partial T) \) are the variations of the electrical properties according to the temperature; \( T \) is the temperature; and \( E^* \) is the complex conjugate of the electric field.\(^{21} \) Both ACEO and ET flows occur globally in the direction toward the illuminated area, resulting in the rapid concentration of metal nanoparticles into the area. The concentrated nanoparticles are trapped within the illuminated area, in which a stagnation region is formed due to the converging flows around the light pattern. The DEP force, which also contributes to trap the concentrated particles, is given by:

\[
F_{\text{DEP}} = 2\pi r^3 \epsilon_m \text{Re}[f_{\text{CM}}] \nabla |E|^2, \tag{3}
\]

where \( r \) is the radius of the particles; \( \epsilon_m \) is the permittivity of the suspending medium. \( \text{Re}[f_{\text{CM}}] \) is the real part of the Clausius–Mossotti factor, which is described as below:

\[
f_{\text{CM}} = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*}, \tag{4}
\]

where \( \epsilon_p^* \) and \( \epsilon_m^* \) are the complex permittivities of the particle and the medium, respectively. In the case of highly conductive materials such as gold particles, the value of \( \text{Re}[f_{\text{CM}}] \) usually has a positive value, thus the particles move toward the light pattern when they are exposed to a relatively high electric field gradient near the surface of the photoconductive layer.\(^{18} \) Some electrostatic interactions generated by induced dipole of dielectric particles also affect the assembly of the concentrated metal nanoparticles in an optoelectrofluidic device.\(^{22} \)

Finally, the concentrated metal nanoparticles efficiently form the SERS-active sites in a reliable and stable manner, thus the SERS signal intensity from the target molecules around the aggregated metal nanoparticles gradually increases, resulting in a more sensitive and reproducible SERS measurement in solution phase. This method allows on-demand generation of ‘hot spots’ at a specific site in a tiny volume (~500 nL) of sample solution without any fluidic components, facilitating the enhancement and \textit{in situ} measurement of SERS from the target molecules.

### Experimental

Rhodamine 6G and adenine were obtained from Sigma-Aldrich Corporation (St Louis, MO, USA) and diluted with deionized water. Gold nanoparticles with diameter of 40 nm as the Raman enhancers were purchased from BB International (Cardiff, UK). Fluorescent polystyrene nanoparticles with a diameter of 50 nm as a model particle for visualization were purchased from Polysciences, Inc. (Warrington, PA, USA). The sample solution, of which total volume was ~500 nL (volume ratio of rhodamine 6G or adenine solution: gold nanoparticles = 1 : 1), was injected into a 30 \( \mu \)m-height liquid chamber between a photoconductive layer and a ground layer of the optoelectrofluidic device.

The photoconductive layer of the optoelectrofluidic device was fabricated using a plasma enhanced chemical vapor deposition method. A triple layer of a 50 nm thick heavily doped hydrogenated amorphous silicon, an 1 \( \mu \)m-thick intrinsic hydrogenated amorphous silicon, and a 20 nm-thick silicon nitride were sequentially deposited on indium tin oxide (ITO) electrode coated on a glass substrate in a single chamber reactor. Then, a region was etched by reactive ion etch to expose the ITO electrode for electrical connection. The detailed fabrication method has been described in the previous literature.\(^{11} \)

The experimental setup is illustrated in Fig. 2. A transparent ground layer, which is a bare ITO electrode, of the optoelectrofluidic device was faced down to the objective lens of an inverted microscope. A 50\( \times \) objective lens, of which numerical aperture (NA) and working distance were 0.5 and 7 mm, respectively, was used. An ac voltage produced from a function generator (AFG310; Tektronix, OR, USA) was applied across the ITO electrodes of the photoconductive and the ground layers. The microscope was equipped with a 5 mW He–Ne laser (Thorlabs, Inc., Newton, NJ, USA) at \( \lambda = 632.8 \) nm, which serves as an excitation source for Raman scattering as well as a light source for generating a nonuniform electric field in the device. Raman scattering was measured by using a monochromator (Spectra 2500i; Princeton Instruments, Trenton, NJ, USA) with a charge-coupled device camera at a spectral resolution of 2 cm\(^{-1} \). All Raman spectra were measured for 1 s in the range of 509–1732 cm\(^{-1} \).

**Results**

First of all, the effect of the frequency of the applied electric field on the concentration distribution of rhodamine 6G was investigated as shown in Fig. 3. The fluorescence intensity of rhodamine 6G decreased as the frequency decreased from 100 kHz to 100 Hz. These results are consistent with the previous works, in which the similar frequency-dependent concentration change was observed for the case of small fluorophores such as bisbenzimide and fluorescein.\(^{14} \) Although the mechanisms of these phenomena could be still debatable, it is clear that the concentration distribution of rhodamine 6G does not change significantly by the projected light in the application of an ac signal of 100 kHz. At the same condition, the fluorescent polystyrene nanoparticles were effectively concentrated into the illuminated...
area, thus the fluorescence signal intensity within the area was significantly increased as shown in Fig. 4a. The fluorescence signal intensity, which was calculated by integrating the fluorescence intensity within the concentration area, exceeded 38-fold of the initial value within 25 s as shown in Fig. 4b. These results show that we can concentrate only the nanoparticles into the illuminated area, which means the detection area, without perturbing the concentration distribution of molecules at the ac frequency of 100 kHz.

For simple demonstration of on-demand generation of SERS-active sites based on the optoelectrofluidic concentration of metal nanoparticles in a solution phase, we applied a mixture of rhodamine 6G molecules and gold nanoparticles as a SERS-active target molecule and a metal colloidal SERS substrate, respectively. The Raman scattering signal from extremely high concentration around 1 mM of rhodamine 6G was not detectable without the assistance of the surface-enhancement effect by SERS-active substrates. When we mixed the 100 μM rhodamine 6G solution with gold nanoparticles for the SERS substrates, some distinctive peaks indicating rhodamine 6G could be much more easily determined due to the enhancement of Raman scattering signal. Without the voltage application, however, the SERS signal of the rhodamine 6G was very weak and not reproducible, thus it was difficult to distinguish meaningful peaks. After an ac voltage of 20 V_{pp} at 100 kHz was applied to the optoelectrofluidic device, the intensity of the SERS signal was significantly increased as shown in Fig. 5a. This enhancement of the SERS signal might be due to the concentration of gold nanoparticles and the formation of the SERS-active sites in the focal volume of the laser source. When we turned off the voltage after 2 min, the SERS signal gradually decreased. This signal decrease might be caused by the diffusion out of the concentrated gold nanoparticles from the focused laser spot, resulting in the decrease of the ‘hot spot’ density in the region. We could quantitatively explore the optoelectrofluidic enhancement of the SERS signal by integrating the area in the range of 1118.5–1229.5 cm\(^{-1}\) of the SERS spectra shown in Fig. 5b after the background reduction. The SERS intensity increased continuously until it reaches the limit of a detector when we kept the voltage on. As soon as the voltage was turned off, the intensity started to decrease as shown in Fig. 5c. Other typical peaks in the ranges of 547.2–672.8, 731.1–820.4, and 1225.5–1413.7 cm\(^{-1}\) also showed almost the same tendency with the peak in the range of 1118.5–1229.5 cm\(^{-1}\) shown in Fig. 5b.

The optoelectrofluidic enhancement and measurement of SERS from 250 μM adenine as a target molecule was demonstrated as shown in Fig. 6a and b. It is noted that the SERS signal from excessively high concentration around 10 mM of adenine molecules was not detectable without any processes for inducing molecular adsorption or particle aggregation, even if the sample was mixed with gold nanoparticles which are the SERS-active substrates. In the application of a voltage of 20 V_{pp} at 100 kHz, significant enhancement of the SERS signal from adenine was...
observed. The SERS signal intensity increased continuously until the value was saturated into the steady state after about 2 min. This signal saturation might be due to the complete filling and assembly of the concentrated gold nanoparticles within the laser spot. This kind of phenomenon could be observable with polymer microparticles as well in the previous work. Here we investigated the effect of the applied voltage on the enhancement rate of the SERS signal intensity. The larger the ac voltage applied, the faster and larger the enhancement of the SERS signal intensity observed as shown in Fig. 7. This result is in good agreement with previous reports. 

Fig. 5 Optoelectrofluidic on-demand generation of SERS-active sites and in situ measurement of SERS from 100 μM rhodamine 6G. (a) The SERS intensity map and (b) the SERS spectra of rhodamine 6G according to the time of application of an ac voltage of 20 V_{pp} at 100 kHz. (c) The SERS intensity, which was calculated by integrating the area in the ranges of 1118.5–1229.5 cm\(^{-1}\) of the spectra after background reduction, increased according to the time. The SERS intensity gradually decreased when the voltage was turned off after 2 min due to the diffusion of gold nanoparticles.

Fig. 6 Optoelectrofluidic enhancement and in situ measurement of SERS from 250 μM adenine. (a) The SERS intensity map and (b) the SERS spectra of adenine according to the time of application of an ac voltage of 20 V_{pp} at 100 kHz.

Fig. 7 Voltage-dependent signal enhancement rate in the optoelectrofluidic SERS enhancement and in situ measurement of 250 μM adenine. The enhancement rate for SERS intensity, which was calculated by integrating the area in the ranges of 687.1–777.9 cm\(^{-1}\) of the spectra after background reduction, increased as the applied ac voltage increased from 10 V_{pp} to 20 V_{pp}.
agreement with theoretical expectations because all the mechanisms such as ACEO, ET flows, and DEP, which dominantly affect the behavior of metal nanoparticles in the optoelectrofluidic device, are proportional to the strength of an electric field or its gradient, which were induced by a light source. The enhancement of SERS due to the optoelectrofluidic concentration of gold nanoparticles occurred in the application of a voltage larger than 15 V_{pp}, while there was no change in the signal intensity below the critical voltage. These results are very similar to the tendency of particle velocity or fluorescence intensity changes when we concentrated microparticles or fluorescent molecules using an optoelectrofluidic device. This consistency with the previous works also indicates that the enhancement of SERS signal has arisen due to the optoelectrofluidic concentration of gold nanoparticles forming ‘hot spots’.

Dynamic control of the SERS-active sites was demonstrated by adjusting the position of a laser spot during the voltage application as shown in Fig. 8a. After the increase of the SERS signal intensity for 60 s, we immediately moved the stage to change the position of the laser spot toward a certain region within the sample solution in the optoelectrofluidic device. Due to the immediate movement of the laser spot, wherein the metal nanoparticles were concentrated, the SERS signal significantly decreased, but it started to increase again when we stopped the device as shown in Fig. 8b and c. Based on this approach, we could detect the SERS signal from the target molecules, adenine, in real-time with the enhanced sensitivity through the instant local concentration of the metal nanoparticles—on-demand ‘hot spot’ generation—at several places of interest in a tiny volume of sample solution.

**Discussion**

In this research, we utilized a fluorescent dye, rhodamine 6G, as one of the target molecules because we can determine their spatial distribution through a fluorescence microscope as well. When we applied a voltage to the optoelectrofluidic device, the fluorescence intensity of the fluorescent dye within the illuminated area was decreased when the ac frequency was lower than 100 kHz as shown in Fig. 3. Such phenomena have also been observed in the previous research based on other fluorescent dyes such as fluorescein. Thus we applied an ac voltage of fixed frequency of 100 kHz, at which the concentration of the target has the maximum value.

We simply characterized the optoelectrofluidic particle concentration at 100 kHz using fluorescent polystyrene nanoparticles as shown in Fig. 4 although dielectric particles may behave differently from metal particles. However, it is clear that the nano-sized particles usually have a tendency to be concentrated into the illuminated area in the optoelectrofluidic device, resulting in the increased density within the laser spot, although the internal assembly structure of the concentrated nanoparticles can be dependent on the type of materials, which affect the ACEO along the particle surface or the electrostatic interactions. This tendency is caused by the fact that the hydrodynamic drag forces due to the optically induced ACEO or ET flows, which converge into the illuminated area, dominantly act on such nano-sized particles at the frequency condition. The previous work, in which the optoelectrofluidic concentration of gold nanoparticles was observed at the frequencies ranged from 10 to 100 kHz, also supports this statement.

We have also investigated the SERS signals from the target molecules—rhodamine 6G and adenine—according to the applied ac frequencies ranging from 100 Hz to 100 kHz to directly determine the influence of the frequency-dependent behavior of small molecules and metal nanoparticles on the SERS signals (data not shown). At 100 Hz and 1 kHz, the SERS intensity was significantly decreased within a few seconds after the voltage application for both molecules. This signal decrease
might be due to the depletion of target molecules from the laser spot as shown in Fig. 3. At 10 kHz, the SERS signal was slightly enhanced, but the signal severely fluctuated. The signal enhancement in spite of a little decrease of molecular concentration within the laser spot (Fig. 3) might be due to the gold nanoparticle concentration forming SERS-active sites, but the strong flow vortices around the laser spot might continuously transport the nanoparticles resulting in the severe fluctuation of SERS by the in-and-out motion of particles. At 100 kHz, on the other hand, more significant and stable signal enhancement could be observed. This result might be due to the efficient concentration, trapping, and assembly of gold nanoparticles like the fluorescent nanoparticles shown in Fig. 4 and the settled—not depleted—molecular concentration over the whole sample solution at the frequency condition (Fig. 3). Therefore, we decided that frequencies around 100 kHz were the most well-optimized frequency range for measuring SERS from small molecules such as rhodamine 6G and adenine in an active manner using this optoelectrofluidic platform.

In the case of macromolecules such as proteins or polysaccharides, however, their concentration was generally increased in the illuminated area as the frequency was decreased in the range of 1–100 kHz. It has been shown that micro-/nanoparticles dispersed in deionized water were also concentrated into the illuminated area at frequencies below 100 kHz in general. Therefore, both metal nanoparticles and target molecules would be concentrated into the illuminated area at relatively low frequencies around 1 to 10 kHz. If our target samples are macromolecules such as proteins, the sensitivity of this optoelectrofluidic SERS detection platform could be enhanced even more, because the local concentration of the target molecule around the concentrated metal nanoparticles could also be increased. Of course, if someone needs to measure the natural concentration of the molecules at the specific region in the solution, 100 kHz frequency, at which the molecular concentration is not changed, would be applicable.

The minimum concentration of adenine solution that we could measure using this optoelectrofluidic “hot spot” generation scheme with 40 nm-sized gold nanoparticles and a 632.8 nm-wavelength laser source was 50 μM. This value is relatively high compared to the previous literature, in which 1 μM adenine could be detected using colloidal gold nanoparticles as the SERS-active substrates. In these studies, however, most of them have applied some additives such as NaCl or H₂SO₄ to induce the aggregation of the nanoparticles and the adsorption of the target molecules on them. In most cases, they assumed that all the target molecules were adsorbed onto the aggregated metal nanoparticle clusters, which are the SERS-active substrates. They should usually dry the sample solution for complete adsorption and concentration of the target molecules onto the SERS substrates, resulting in a much lower detection limit. In our active SERS system, we maintained the molecular concentration over the whole region of the solution by applying 100 kHz, and concentrated only the metal nanoparticles to generate ‘hot spots’. The ‘hot spots’ disappeared without the voltage or the laser spot by diffusion of the nanoparticles, returning to the initial state, at which the nanoparticles were uniformly distributed in the solution, again. We could decrease the detection limit by utilizing silver nanoparticles or other SERS-active substrate materials instead of gold nanoparticles, or by adding some additives for inducing efficient molecular adsorption onto the substrates. In addition, the ac frequencies, at which not only the nanoparticles, but also the molecules are concentrated into the laser spot, would be applicable in the case of macromolecules.

The tendency of the optoelectrofluidic SERS enhancement might depend on the initial amount of molecules and gold nanoparticles around the light spot. In our experimental condition—100 kHz frequency, the molecular concentration distribution was the same as that of the bulk sample solution as shown in Fig. 3. Thus, the enhanced SERS signal was almost linearly proportional to the molecular concentration. For example, when we applied a lower concentration of rhodamine 6G, the signal became smaller and was saturated before overcoming the limit of the detector. On the other hand, the concentration of gold nanoparticles affected not only the intensity of the SERS signal, but also its enhancement rate. For example, if there is a smaller amount of gold nanoparticles around the light spot, the concentration rate—the number of particles per unit time—as well as the total amount of the concentrated nanoparticles—the number of particles per unit area—decreased, resulting in not only the reduction of the enhanced SERS intensity but also the decreased enhancement rate.

The SERS enhancement factor, which is closely related to the localized surface plasmon resonance (LSPR) effect, is affected by not only the number of the concentrated metal nanoparticles, but also their size distribution, shape, density, and nanoscopic assembly structures. When microparticles are concentrated and assembled in the illuminated area by the optoelectrofluidic mechanism, it is known that they are assembled in a crystalline structure with different center-to-center spacings at each frequency condition. However, such characteristics have never been investigated in the case of the nanoparticle assembly formed by optoelectrofluidics due to the lack of effective visualization methods. Also in our experimental study, a little shift of band positions during the optoelectrofluidic nanoparticle concentration has been observed. Such a change of band positions was neither reproducible nor consistent, it might be due to the structural change of assembled nanoparticles. If some tools for characterization of the particle assembly at the nanoscale are developed or utilized to this system, it could be very useful. For example, if we couple this optoelectrofluidic SERS platform with an LSPR analysis system, it would be possible to analyze the LSPR characteristics of the concentrated metal nanoparticles according to several parameters such as their size distribution, the particle shape, and the applied voltage conditions to find the optimal conditions for the maximum SERS enhancement factor.

In the optoelectrofluidic device, several electrokinetic and electrostatic forces, which depend on the physical and electrical properties of the target objects as well as the applied ac voltage conditions, cause the movement of target objects in concert, as we mentioned above. Therefore, we can selectively concentrate specific target molecules in a mixture based on their own physical and electrical properties at certain ac frequency conditions. For example, an optoelectrofluidic separation of microparticles of specific size from a mixture has been demonstrated and applied for sandwich immunoassays. If we apply the concept to this optoelectrofluidic SERS spectroscopy, it would also be
applicable for SERS-based direct immunoassays based on the selective concentration of antibody-coated metal nanoparticles for capturing specific targets and SERS-based detection without interferences.

The key advantage of this optoelectrofluidic SERS platform is that highly sensitive detection of SERS from the targets at a specific region of interest with exact timing is possible. If the optical system is stable, the reliability and stability of the SERS measurement are also guaranteed. This platform requires no fluidic components and complicated fabrication processes. The electrical system is also very simple compared to the previous electrokinetically active SERS platform because of the nature of the optoelectrofluidic platform, in which the virtual electrodes induced by an optical method are used for controlling an electric field.

Conclusions

A novel active SERS platform, where an optoelectrofluidic device is combined with a conventional SERS detection system, was presented. When a laser source was projected into a sample solution containing gold nanoparticles in an optoelectrofluidic device in the application of an ac voltage at 100 kHz frequency, the gold nanoparticles were concentrated into the laser spot and formed SERS-active sites without any change of the concentration distribution of Raman-active target molecules. In this optoelectrofluidic SERS platform, both the concentration of metal nanoparticles and the detection of SERS signal from the target molecules could be simultaneously achieved with only a single laser source. Here we demonstrated on-demand generation of ‘hot spots’ at specific regions of interest in a sample solution and in situ measurement of Raman scattering signals from rhodamine 6G and adenine molecules using this optoelectrofluidic SERS platform. The voltage-dependent characteristic of the optoelectrofluidic enhancement of SERS was also investigated. This optoelectrofluidic SERS spectroscopy could provide a new way to realize highly sensitive, reliable, and stable SERS measurements in an active manner without any fluidic components and complicated optical or electrical systems. In addition, this novel technique for active SERS substrate formation should be more useful for the applications which require label-free real-time monitoring or unintentional detection in both a spatial and temporal manner.

Acknowledgements

This research was supported by the Nano/Bio Science and Technology Program grant (2008-00771) and by the National Research Laboratory (NRL) Program grant (R0A-2008-000-20109-0, 2010-0017693) through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (MEST). The authors at UNIST were partially supported by World Class University (WCU) program (R32-2008-000-20054-0).

Notes and references