Lab-on-a-Chip Surface-Enhanced Raman Spectroscopy

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Abstract In the area of bioanalytics reliable and sensitive detection methods for the analysis of a variety of molecules like, for example, drugs and DNA are required. As Raman spectroscopy is characterized by a high specificity, it is well suited for bioanalytical issues. However, a major drawback concerning this technique is the low sensitivity due to the weakness of the Raman effect. A possibility to achieve the sensitivity needed for bioanalytics is the use of surface-enhanced Raman spectroscopy (SERS). The addition of nanostructured metal surfaces leads to an enhancement of the Raman signal and a more sensitive detection method which is even able to detect single molecules.

Since the analysis of small samples and minimal sample volumes as well as the detection of low analyte concentrations are requested in bioanalytics, the combination of SERS and a lab-on-a-chip device seems to be a promising way to offer a sensitive detection method with reproducible measurement conditions and a highly defined detection area specified by a chip system.

The range of lab-on-a-chip devices applied for SERS detection varies from microarray platforms to microfluidic systems equipped with, for example, implemented SERS substrates fabricated by lithography or the possibility of in situ synthesis of colloidal substrates. Besides a multiplicity of designs and application areas, a variety of different materials and fabrication procedures for lab-on-a-chip

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W. Fritzsche and J. Popp (eds.), *Optical Nano- and Microsystems for Bioanalytics*,
Springer Series on Chemical Sensors and Biosensors (2012) 10: 229–246
DOI 10.1007/978-3-642-25498-7_8,

systems are also established, for example, glass chips produced by wet etching or PDMS chips manufactured by pattern replication. Most of the devices are especially designed for a certain bioanalytical application. The combination of microarray platforms and SERS is, used for the detection of cancer, proteins, and microorganisms as well as for labelled and label-free detection of DNA and RNA. The applications of microfluidic systems vary from monitoring of proteins and drugs to the detection of cells. In addition to quantification using direct and indirect SERS measurements, multiplexing analysis in a microfluidic device is feasible.

The Lab-on-a-Chip Surface-Enhanced Raman Spectroscopy (LOC-SERS) as a reproducible and sensitive detection method enriches the field of bioanalytics.

Keywords Bioanalytic • Lab-on-a-chip (LOC) • Microarray • Microfluidic • SERS

Contents

1 Introduction

Lab-on-a-Chip Surface Enhanced Raman Spectroscopy (LOC-SERS) is becoming more and more important as a valuable and reliable detection method in the field of bioanalytics. The requirements needed for bioanalytical analysis like high sensitivity and specificity are provided by surface-enhanced Raman spectroscopy (SERS) whereas highly defined and reproducible measurement conditions are realized by the application of a lab-on-a-chip (LOC) device. This chapter gives an outline of the most common varieties and applications of LOC devices utilized in SERS. After a short summary of the basic principle of SERS, different types of LOC systems such as microfluidic devices, dielectrophoretic chips, microwell SERS chips, and microarrays as well as their applications are introduced.

2 Surface-Enhanced Raman Spectroscopy

Raman spectroscopy is based on the Raman effect, which is described as an inelastic light scattering between photons and molecules resulting in distinctive frequency-shifted scattered light. Even though Raman scattering is characterized by high specificity, a major drawback with respect to its application in bioanalytics is its low sensitivity as only one out of 10^6 photons is scattered inelastically. Considering the light-scattering effects, the incident laser radiation induces an electric dipole moment μ in the molecules, which can be described by

$$\mu = \alpha \cdot E_0 \tag{1}$$

with E_0 representing the electric field of the laser light and α the polarisability of the molecule. The intensity of a Raman signal is proportional to the square of the induced electric dipole moment. Therefore, an enhancement of the Raman intensity can be achieved according to (1) by an increase of the electric field as well as an increase of the polarisability α.

SERS takes advantage of the optical properties of nanostructured metal surfaces to achieve a Raman signal enhancement. Fleischmann et al. observed in 1974 an intense Raman signal of pyridine on a roughened silver electrode surface [1]. From that time, many researchers started to investigate this phenomenon to find an explanation for this Raman enhancement. Two mechanisms have been discussed to be responsible for the occurrence of an increased Raman signal intensity for molecules in close vicinity to a nanostructured metal surface. The first mechanism results from the long-range classical electromagnetic enhancement due to interaction of the incident laser light with the metal surface exciting surface plasmons leading to a local field enhancement. This electromagnetic enhancement mechanism shows a larger contribution to the overall enhancement of SERS as compared to the second mechanism, the so-called short-range chemical enhancement. The chemical enhancement mechanism is based on the interaction of a molecule with the metal surface causing the appearance of a new electronic charge transfer state due to chemisorption. This new electronic state acts as a resonant intermediate state and the enhancement can be explained according to electronic resonance Raman enhancement mechanism [2]. If both mechanisms appear simultaneously, the observed enhancement is composed multiplicative by both effects [3].

2.1 Electromagnetic Enhancement

The applied SERS active substrates vary in shape, size, and material. The largest enhancement factors have been achieved so far by applying gold, silver, and copper on a nanoscale of 10–100 nm. Concerning the shape of the substrate-roughened electrode surfaces, colloids, thin films, as well as lithographically produced

nanopatterns are feasible. Even though there is a large variation within size and shape of the used SERS substrate, a basic model can be applied for a general explanation of the electromagnetic enhancement mechanism: an isolated spherical particle much smaller than the incident laser wavelength is taken into account, so all processes taking place can be considered as quasi-static. The particle, characterized by a frequency-dependent dielectric function $\varepsilon_1(\omega)$, is surrounded by a medium or vacuum described by $\varepsilon_2(\omega)$. $\varepsilon_1(\omega)$ is supposed to be independent of the size of the particle and can be described by

$$\varepsilon_1(\omega) = n(\omega)^2, \tag{2}$$

$$n(\omega) = n'(\omega) + \mathrm{i}k(\omega), \tag{3}$$

where $n(\omega)$ represents the complex refractive index with $n'(\omega)$ (dispersion) as real and $k(\omega)$ (index of absorption) as imaginary part. The incident laser light with an electric field E_0 excites the surface plasmons within the metal nanoparticle inducing an electric field E_{out} outside the sphere according to the Lorenz–Mie theory [4]. The absolute square of E_{out} determines the intensity of the SERS signal and can be written as:

$$E_{\mathrm{out}}^2 = E_0^2 |g|^2 (1 + 3\cos^2\theta) \tag{4}$$

with θ being the angle along the polarisation direction and g given by:

$$g = \frac{\varepsilon_1(\omega) - \varepsilon_2(\omega)}{\varepsilon_1(\omega) + 2\varepsilon_2(\omega)}. \tag{5}$$

Equations (4) and (5) indicate that there are two factors which significantly influence the field intensity: first of all, a large field intensity is obtained when the angle θ is equal to 0° or 180°. Second, large g values lead to large field intensities. In order to maximize g, $\varepsilon_1(\omega)$ has to be approximately $-2\varepsilon_2(\omega)$. If the real part of $\varepsilon_1(\omega)$ is $-2\varepsilon_2(\omega)$, the imaginary part is small. This is the so-called plasmon resonance condition.

Considering now that the resulting electric field E_{out} induces an electric dipole moment within molecules in direct neighbourhood to the surface of the metal nanoparticle, the induced dipole radiates according to the Raman theory. This radiation, however, can now be enhanced too. The so-called second enhancement is much more complex and described in detail by Kerker et al [4]. To specify a rough overall enhancement, it is considered that the molecule is affected by the maximum enhancement and $|g| \gg 1$ with:

$$E_R = \frac{E_{\mathrm{out}}^2 {E'_{\mathrm{out}}}^2}{E_0^4}, \tag{6}$$

whereas $E'_{\text{out}}{}^2$ refers to the field appointed at the scattered frequency. For a more detailed description of the electromagnetic enhancement mechanism, the interested reader is referred to [4, 5].

2.2 Charge Transfer Enhancement

The charge transfer enhancement (CT) mechanism contributes much less to the overall enhancement of SERS than the electromagnetic mechanism. While the electromagnetic enhancement mechanism leads to typical enhancement factors of 10^6–10^8, the CT mechanism results in enhancement factors of 10–10^2 [2, 6, 7]. In order to explain the CT mechanism, the formation of a surface complex between the SERS active substrate M and the analyte molecule A (adsorbate) due to chemisorption is assumed:

$$\mathrm{M} + \mathrm{A} \rightarrow \mathrm{M} - \mathrm{A} \tag{7}$$

The formed metal–adsorbate complex M–A exhibits intermediate charge-transfer states similar to the ones known for inorganic complexes. If the energy of the incident laser light is in resonance with the energy of the newly formed charge-transfer transition according to

$$E_{\text{CT}} = E(S_1 - S_2) = h\nu, \tag{8}$$

an electronic resonance Raman excitation takes place. Since the resonance effect arises from the formation of the metal–adsorbate complex, the observed enhancement is comparable to the one observed for the well-known resonance Raman effect. The enhancement due to a resonance Raman effect is caused by an increase of the transition polarisability and, therefore, an increase of the Raman scattering [2, 3, 8]. For a more detailed description of the CT enhancement and resonance Raman effect, the interested reader is referred to [7].

2.3 Selection Rules

The selection rules for surface-enhanced Raman scattering are more relaxed compared to the ones for normal Raman scattering. It can be noticed that vibrational bands considered to be Raman inactive appear in a SERS spectrum. The selection rules for SERS take the interactions occurring between metal surface and analyte molecules into account. The CT enhancement mechanism originates from an adsorption of the molecule to the surface. The polarisability tensor α of a molecule adsorbed on a surface can be described as follows:

$$\alpha_{\text{eff}} = \frac{9}{(\varepsilon(\omega_0)+2)(\varepsilon(\omega_s)+2)} \begin{pmatrix} \alpha_{xx} & \alpha_{xy} & \varepsilon(\omega_s)\alpha_{xz} \\ \alpha_{yx} & \alpha_{yy} & \varepsilon(\omega_s)\alpha_{yz} \\ \varepsilon(\omega_0)\alpha_{zx} & \varepsilon(\omega_0)\alpha_{zy} & \varepsilon(\omega_0)\varepsilon(\omega_s)\alpha_{zz} \end{pmatrix} \quad (9)$$

with $\varepsilon(\omega_0)$ and $\varepsilon(\omega_s)$ representing the dielectric functions of the metal for incident laser frequency and the scattered frequency, respectively [9]. The tensor reveals that all vibrations parallel to the metal surface and, therefore, without contribution of α in z-direction are not enhanced. All vibrations with a contribution of α in z-direction are enhanced whereas vibrations oriented perpendicular to the surface experience the highest enhancement [10].

3 Lab-on-a-chip Devices

LOC devices applied in SERS vary in size, material, design, functionality, and application. Nevertheless, all LOC systems utilizing SERS have in common to achieve more reproducible SERS sample preparation and SERS measurement conditions. Before discussing the application of different LOC devices, the material and fabrication of these devices as well as the design and functions in particular of microfluidic devices concerning advantages, disadvantages, and innovations with respect to the implementation of such systems in SERS are introduced.

3.1 Materials and Fabrication

The two most common materials for LOC devices are polymers like polydimethylsiloxane (PDMS) and glass. They are either combined or applied as all-glass and all-polymer devices. Both materials offer advantages and disadvantages concerning the fabrication of LOC systems and their application in SERS.

Polymer devices (e.g., from polycarbonate (PC) or cyclo olefin copolymer(COC)) are suitable for mass production and can be used as disposables due to their low manufacturing costs. Another advantage of polymer devices is the fast availability of prototype devices made from PDMS. If the channel layout of a chip is designed, usually a moulding technique is utilized for the production of the device. Therefore a master of the chip design has to be created by, for example, photolithography, which is then used to mould the device in, for example, PDMS, and can be finally applied to produce a high quantity of the polymer-based chips with the same layout. A detailed description of the fabrication can be found in [11].

The polymer-based devices are usually used as disposable products as the material and fabrication is inexpensive and they are hard to regenerate after usage. However, a major drawback of polymer-based devices is the low chemical resistance of these devices. Some polymers exhibit moisture expansion by contact

with oil and are not resistant against organic solvents. Since most SERS experiments are carried out in an aqueous solution, this is not an issue to be concerned of. Another drawback is that polymers cause disturbing Raman signals when carrying out SERS measurement within purely polymer-based LOC devices [12]. In case the detection point is too close to the polymer material of the device or the excitation and scattered light has to pass through the polymer material, signals caused by the device material will appear in the spectrum. The combination of polymer-based devices with all-glass detection windows prevents this issue [13].

Glass as LOC material is more expensive and more difficult in terms of generating a large number of devices. One way to produce all-glass chips is the application of the wet-etching technique. Glass-wafers are coated with a microstructured nickel–chromium alloy mask, providing the channel layout of the LOC system. The structures are etched by using hydrofluoric acid. For the assembly of an all-glass microfluidic device, two mirror-inverted substrates with half channels are prepared and bonded (anodic) by using, for example, silicon as bond support layer [14]. All-glass devices provide a high chemical resistance and are reusable.

Most of the LOC devices applied for SERS are manufactured utilizing additional or individually modified productions steps to those mentioned above. Since the variety of LOC systems is quite high, just a brief overview concerning the used materials and assembly strategies is presented here.

3.2 Microfluidics and Their Functionalities

Microfluidic devices are often applied to work with solutions. For SERS to be implemented in microfluidic devices, special functionalities of microfluidic LOC systems are required to achieve reproducible sample preparation and measurement conditions and to allow the handling of small sample volumes. Lab-on-a-chip devices for SERS are designed to provide special functionalities for specific applications: for a SERS detection within solutions with colloids as SERS active substrate, an important issue is a reproducible mixing behaviour. To control the aggregation time and the mixing efficiency of colloid and analyte solution, the reagents are combined in situ. In general, the injection of the components into the fluidic device is realized by syringe pump systems, pressure-driven flow or capillary forces. An advantage of SERS measurements in a LOC fluidic device compared to cuvette measurements is a reproducible and high degree of mixing of the reagents by utilizing microstructures to initiate optimal mixing conditions. The group of Quang et al. introduced a pillar array PDMS microfluidic channel into their measurement arrangement (Fig. 1a) [15]. The pillar array fluidic channel is produced by a moulding technique. The pillars cause a stirring of the fluid rather than a turbulent flow. By doing so, a high mixing efficiency and a fast absorption of the analyte on the nanoparticles is achieved. Figure 1b represents another structured microfluidic channel applied by Lee et al. [16]. Here a zigzag-shaped PDMS microfluidic channel is created in order to obtain an efficient and fast mixing. The

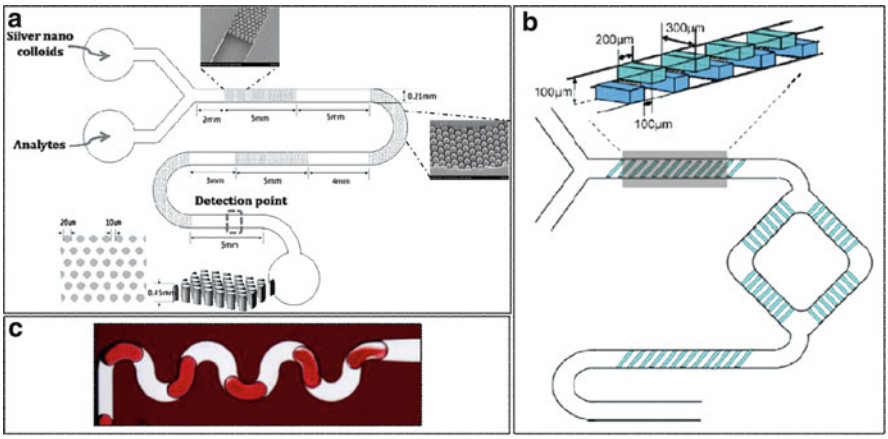

Fig. 1 Functionalities implemented in a microfluidic device to increase the mixing efficiency; (**a**) pillar array fluidic channel [15], (**b**) zigzag-shaped PDMS microfluidic channel [16], (**c**) all-glass chip with loops to provide mixing in droplets of the applied segmented flow. (Reprinted from Quang et al. Copyrights (2008) with permission from The Royal Society of Chemistry—Lab on a Chip, reprinted from Lee et al. Copyrights (2007) with permission from Elsevier—Analytica Chimica Acta)

zigzag shape is fabricated by stacking two PDMS layers. The layers with the upper and lower pattern of the zigzag channel are produced by a pattern replication method using a master and moulding it into the PDMS. Another possibility utilized to obtain a high mixing efficiency is the integration of loops into a microfluidic channel as shown in Fig. 1c. Both März et al. [18] who applied an all-glass chip as well as Wang et al. [17, 18] who employed a PDMS chip for their SERS measurements introduced mixing loops within their microfluidic devices. Both groups are working with the so-called segmented flow where the analyte and colloid solutions form droplets in an immiscible separation and transport fluid that usually is an oil. The application of a segmented flow prevents depositions in the microchannels and causes a high reproducibility of SERS measurements as "memory effects" are avoided [19]. The high degree of mixing initiated by the loops in combination with the application of a segmented flow can be proven by a visual experiment: two colourless components, iron chloride and ammonium thiocyanate, are injected into the LOC device. If these reagents coalesce a deep red complex, ferric thiocyanate, is formed. With a CCD camera, the mixing within the droplets is observed by investigating the build-up of the red complex in the droplets [17]. This simple visual experiment demonstrates the high mixing efficiency of integrated loops as functional element for mixing. Wilson et al. [20] implemented an in-situ colloidal synthesis within their microfluidic device, which requires an additional active mixing facility for the in situ colloidal synthesis. This operation is implemented by a stirring element. Besides the mentioned structures implemented in LOC devices, several others can be found in the literature all aimed at efficient mixing [13, 21].

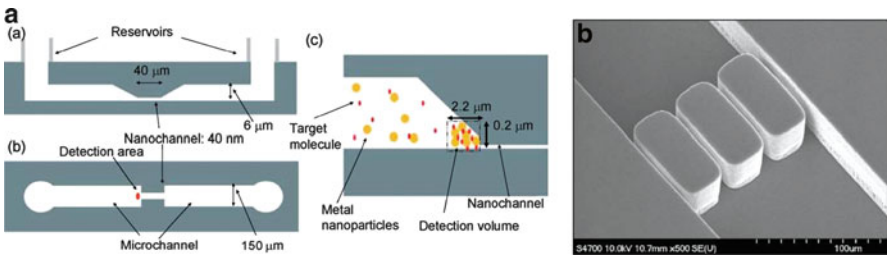

Fig. 2 Functionalities implemented into microfluidics for trapping of particles; (**a**) trapping of nanoparticles for the formation of a cluster and an increase of analyte concentration to create high detection sensitivity [23]; (**b**) PDMS posts in a microfluidic channel acting as membrane for trapping beads required for sample preparation steps like washing steps [24]. (Reprinted from Wang et al. Copyrights (2007) with permission from The Royal Society of Chemistry—Lab on Chip, reprinted from Monaghan et al. Copyrights (2007) with permission from American Chemical Society—Analytical Chemistry)

Besides introducing functional structures taking care of a high degree of mixing into a LOC fluidic device, there is the possibility of integrating some other functionalities: one function applied in LOC for SERS measurements is trapping of particles by membranes or reduction of channel diameters. Trapping of particles arises from different motivation, for example, some sample preparation steps require, trapping in advance to the SERS detection or the trapping is carried out in the area of the detection window to enhance the signal intensity. Wang et al. applied an optical device as shown in Fig. 2a where the micro-nanostructured fluidic channel allows the trapping of nanoparticles. A mixture of analyte and nanoparticle is added to one of the reservoirs (Fig. 2a) and drawn into the optical device by capillary forces. The nanoparticle size is larger than the diameter of the nanochannel; therefore they are trapped at the entrance resulting in the formation of a cluster. The capillary force still transports analyte through the cluster and an area of high analyte concentration is created achieving high detection sensitivity [22, 23]. Figure 2a presents in detail the parameters of the optical device used by Wang et al. like, for example, channel diameters, detection area, and detection volume. Monaghan et al. introduced a membrane into a microfluidic device for trapping microbeads needed for sample preparation (shown in Fig. 2b). The integrated membrane is fabricated by a moulding technique and consists of three 26 $\times$ 100 µm rectangular PDMS posts in a 110 µm-wide PDMS microchannel. The posts are spaced by 8 µm intervals and allow the passing of smaller particles. In this particular application, streptavidin-coated microspheres are trapped to provide the possibility of capturing biotinylated PCR products. The bead bed still enables washing steps and the flow of fluids containing the analyte molecules, which are supposed to be detected [24, 25]. Another approach for trapping nanoparticles and additionally controlling the aggregation is introduced by Tong et al. They apply a microfluidic device with implemented laser tweezers using an 830-nm laser for trapping metal nanoparticles and an excitation wavelength of 514 nm for the SERS detection. The trapping laser also induces inter-particle optical forces, which cause an agglomeration and are feasible for controlling the aggregation of nanoparticles [26].

Another functionality implemented by Huh et al. is the electrokinetic actuation of SERS nanoparticles at microwells, formed by three stacked layers: established of three stacked layers: the upper layer contains a gold electrode; the middle layer consists of a polyimide dielectric layer including microwells and an additional layer containing microchannels; the third layer includes again a gold electrode. This combination enables an active mixing to increase the binding efficiency of analyte and SERS substrate (nanoparticles) and rapid concentration of the product to be analysed in the detection area [27, 28].

The functionalities utilized with and within a microfluidic device are multifaceted. Besides the ones mentioned above, microfluidic devices implemented in SERS provide the transport of analytes to the detection area, the application of washing steps [29] or other more complex sample preparations like electrophoresis [30]. Other advantages offered by microfluidic LOC devices are the fast exchange of reagents within the detection window [31], online monitoring [32] of analyte concentration, multiplexing [33], stable and reproducible measurement conditions [19], as well as the possibility of high throughput. In terms of high throughput for sample preparation, Choi et al. designed a SERS-CD platform with the possibility of 12 different preparation steps on one LOC system [34].

3.3 Integrated Substrates in Microfluidic Devices

Beside the application of metallic nanoparticles as surface-enhanced Raman scattering active substrate in microfluidic devices, the direct integration of SERS substrates in the LOC devices for SERS measurements is more and more of interest. Several research groups are working on novel off-chip SERS substrates, which are also promising for on-chip applications [35–37]. However, there are already research groups that implement substrates into microfluidic channels. The group of Connatser et al. integrated nanostructures in microfluidic devices as shown in Fig. 3a. The periodic morphology is achieved by applying electron beam lithography on a polymer layer for the production of the structures. The dimension of the created patterns was 250 nm in height and 50–300 nm in diameter with a spacing of 50–200 nm. To achieve a Raman signal enhancement, a silver metal film with a thickness of 25 nm was evaporated on the completed pattern. Connatser et al. investigated the response factor of different types of patterns to compare the intensity of the enhanced Raman signals [38].

A new concept for the implementation of SERS active substrates in microfluidics was introduced by Vlasko-Vlasov et al. who integrated surface plasmon polariton (SPP) lenses in their device as shown in Fig. 3b. A silver layer with a thickness of 75 nm is applied to fabricate circular structures of eight nanoslits with a centre-to-centre distance of 460 nm and a width of each slit of approximately 110 nm in the flat metal surface by ion beam lithography. This structure provides high concentration of SPP energy in a subwavelength spot at the focal point as the surface plasmon polariton of the nanoslits excited with 633 nm, which corresponds to the SPP on the silver surface, interfere constructively. The combination of the SPP lens with a

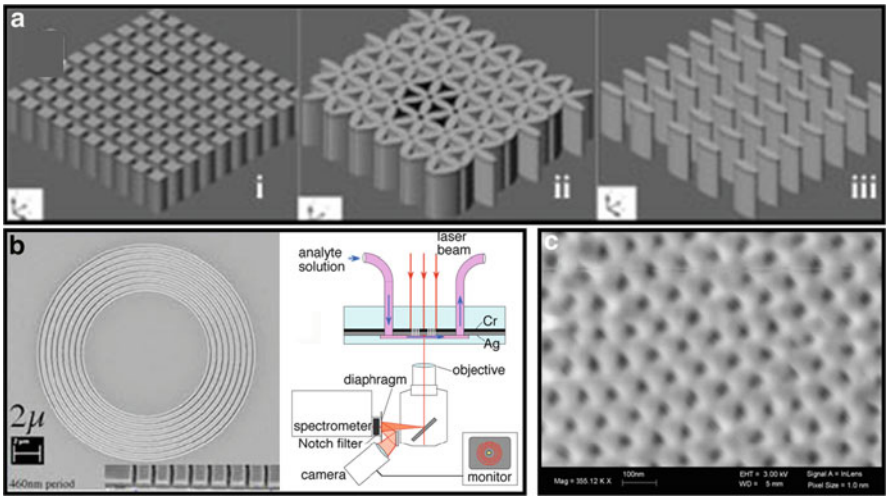

Fig. 3 Integrated structures used as SERS active substrate; (**a**) three patterns (pillars with a height of 250 nm capped with silver) of SERS active substrate integrated in a microfluidic device [38]; (**b**) surface plasmon polariton (SSP) lens—circles of nanoslits in a metal film applied for an enhancement of the electromagnetic field [39]; (**c**) nanoporous anodized aluminum oxide (AAO) on the top of a metallic aluminum substrate utilized as bio-compatible SERS substrate [40]. (Reprinted from Connatser et al. Copyrights (2008) with permission from Wiley-VCH Verlag GmbH & Co—Electrophoresis, reprinted from Vlasko-Vlasov et al. Copyrights (2010) with permission from American Institute of Physics—Applied Physics Letters, reprinted from Banerjee et al. Copyrights (2010) with permission from Elsevier—Chemical Physics Letters)

microfluidic device is also shown in Fig. 3b. Here, the analyte solution is pumped through a microfluidic channel passing the surface plasmon polariton lens. The excitation takes place from the top of the device and the scattered light is detected in transmission. Vlasko-Vlasov et al. demonstrate that the application of such a lens as SERS active substrate produces robust and stable spectra, which are comparable with spectra detected using colloidal solution for signal enhancement [39].

Another approach for integrated substrates in microfluidics is presented by Banerjee et al. who introduced a nanoporous anodic aluminum oxide (AAO) layer of hexagonally packed holes with a diameter of 20 nm and 50 nm in depth on the top of an aluminum substrate. The porous layer is shown in Fig. 3c. A microfluidic channel is placed on the substrate to provide the analyte solution and achieve a high throughput. The choice of AAO as substrate matrix is due to its high biocompatibility as this platform is applied in the area of bioanalytics for the monitoring of protein binding to a lipid bilayer [40].

There are several other research groups implementing SERS active substrates into microfluidic LOC devices. Huh et al. introduced ordered nanotube structures fabricated by metal evaporation through a shadow mask of AAO membrane [41]. Gordon et al. presents an array of nanoholes in a gold film applied as SERS substrate within a microfluidic device [42]. More detailed information and additional approaches concerning the implementation of nanostructures as SERS active substrate into a microfluidic device can be found in [42–46].

3.4 Microarrays

Considering LOC devices implemented in SERS, not just microfluidic systems but also microarrays have to be considered. Microarrays in SERS are applied as a chip-based assay with a spot-wise detection possibility for a specific issue. There are different approaches for this kind of LOC device; the microarray is either based on a chip with an integrated SERS active substrate or a functionalized platform is provided and labelled and label-free SERS active nanoparticles are introduced for the spectroscopic readout. Abell et al. presented a novel approach for a multiwell array SERS chip by introducing a PDMS array with integrated SERS active substrates of isolated microwells ensuring that no cross-contamination can occur. A high throughput and multiplexing can be achieved by the multiwall array [47]. Allain et al. also designed a microarray platform based on a silver-coated glass plate with microwells. They functionalized their SERS active substrate to bind labelled DNA for detection [48]. Another approach for a SERS array is presented by Wang et al. They applied a silver nanoparticle-coated Si nanowire array for the detection of pesticides [49].

Concerning the application of microarrays based on a functionalized platform, a lot of different approaches can be found in the literature: label-free microarray readout for microorganisms is introduced by Knauer et al. who present a chip device with a functionalized surface for a specific binding of microorganisms [50]. Label-free nanoparticles are added to achieve the signal enhancement for the detection [50]. Usually biomolecules are identified by using special SERS labels. Thereby a substrate is functionalized to bind the analyte molecule and nanoparticles equipped with specific antibodies and a Raman label used for the detection. The antibody sitting on the surface of the nanoparticle binds to the analyte molecule. The label is assigned to a specific antibody for the analyte molecule. The specific SERS spectrum of the label attached to the nanoparticle acting as SERS active substrate is used for the identification of the analyte molecule [51–55].

A novel approach regarding microarrays is presented by Islam et al. They demonstrate the great potential of an interdigitated array of electrodes for a LOC application [56, 57].

For more detailed and additional information on SERS mircoarrays, their fabrication, design, and application, the interested reader is referred to [58–61].

4 Application

The applications of LOC devices for surface-enhanced Raman scattering measurements in bioanalytics vary from investigations of cells, microorganisms, and DNA to quantitative and monitoring experiments of drugs and proteins. In the following section, some representative examples out of the broad variety of applications will be presented in more detail.

4.1 Quantitative and Monitoring Investigations

A major issue in SERS is the quantification and the monitoring of analyte molecules. Especially for monitoring experiments, the application of microfluidic devices is quite useful. A number of research groups utilized LOC-SERS measurements to perform quantitative analytics and monitoring experiments. The groups of Yea et al. and Lee et al. worked on sensitive trace analysis of cyanide water pollutant [62] and malachite green [16] applying PDMS microfluidic channels. Both groups [63] demonstrated the great potential of LOC-SERS for sensitive analyte quantification. Ackermann et al. presented investigations on online monitoring of concentration fluctuations of the drugs promethazine and mitoxantrone by applying a segmented flow in a microfluidic device [19, 32]. Subsequent studies by März et al. demonstrated that the additional implementation of an isotope-edited internal standard improves the monitoring of experiments by taking the influence of enhancement variations due to the applied colloid into account [17]. Abu-Hatab et al. applied a device with parallel microchannels for multiplex monitoring of influences of different pH values and different anions of the aggregation agent on the SERS spectra [33].

A different approach for quantitative studies on mercury (II) ions is presented by Wang et al. [18]. They demonstrate that quantification can be done indirectly: rhodamine B dye molecules in close vicinity to the surface of gold nanoparticles are replaced upon adding mercury (II) ions (Fig. 4) leading to decreasing SERS intensities of rhodamine B bands with an increase of the concentration of mercury (II) ions (see Fig. 4a). Figure 4b represents the change of the peak area of the rhodamine B band at 1,547 cm^{-1} for different mercury (II) ions concentration. Wang et al. applied segmented flow measurements in a PDMS microfluidic device for these investigations [18].

4.2 DNA and RNA Detection

The detection of DNA using LOC-based SERS is in the focus of several research groups. For example, Monaghan et al. developed a chip-based analysis for the detection of nucleic acid sequences of Chlamydia trachomatis [24]. Therefore, biotinylated PCR products are captured by polymer microspheres covered with streptavidine. The PCR products are subsequently hybridised against a labelled probe for detection. A sequence-specific DNA detection applying LOC-SERS is presented by Strelau et al. using a solid chip surface with immobilised capture DNAs, where target strands can be hybridised [29]. The target strands are labelled for SERS detection [29]. The detection of duplex dye-labelled DNA oligonucleotides applying SERS in a PDMS microfluidic chip was carried out by Park et al. [64]. Their method prevents the need of immobilisation steps and PCR amplification. A multiplexing of DNA and RNA has been carried out by Cao et al. by also applying

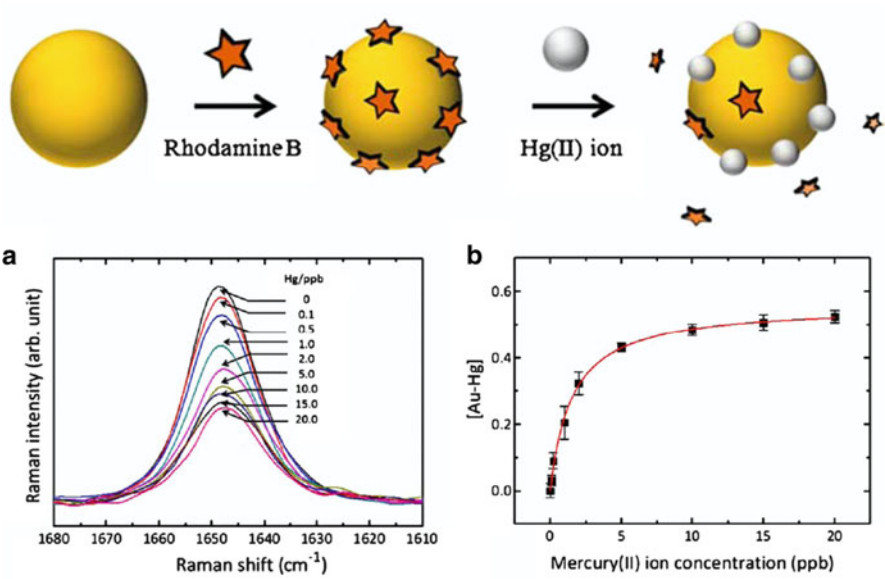

Fig. 4 Schematic mechanism of sensing of mercury (II) ion on gold nanoparticles based on the replacement of rhodamine B dye molecules; (**a**) change of SERS intensity of the band at 1,547 cm^{-1} referring to different concentrations of Hg (II) ion; (**b**) variation of the peak area with regard to the Hg (II) ion concentration for the band shown in Fig. 4a [18]. (Reprinted from Wang et al. Copyrights (2009) with permission from Springer—Analytical and Bioanalytical Chemistry)

a label-based method [51]. For more detailed information, the interested reader is referred to [65].

4.3 Investigations on Cells, Microorganisms, and Other Biomolecules

Furthermore, a lot of investigations are reported on cells, microorganisms, and other biomolecules utilizing SERS in a LOC system. The group of Zhang et al. successfully demonstrated the characterisation of a Chinese hamster ovary (CHO) cell in a microfluidic device by the means of SERS [66]. Figure 5a presents a picture of CHO cells in a microfluidic channel. By incubating the cells with gold nanoparticles, cellular SERS spectra can be recorded as shown in Fig. 5b (upper spectrum). Cells without gold nanoparticles do not exhibit any significant Raman information (lower spectrum). For a detailed cell characterization, a mapping experiment can be carried out with the applied setup.

A lot of other LOC-SERS studies to investigate microorganisms such as bacteria [50, 67, 68], peptides [25], proteins [22, 40, 69], pesticides [49], enzymes [28, 54,

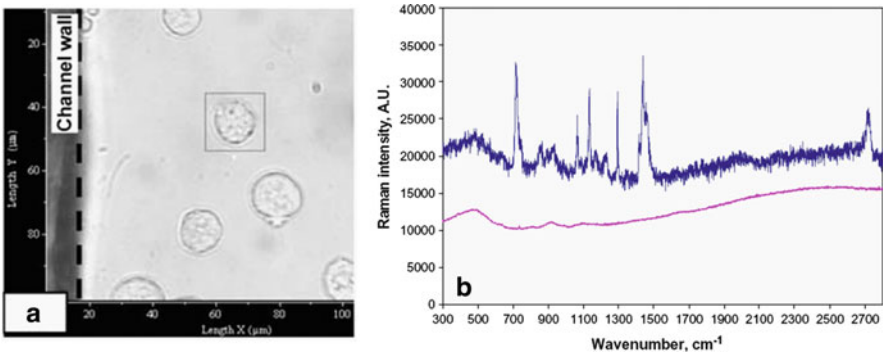

Fig. 5 Investigation of cells in a microfluidic device: (**a**) photograph of CHO cells in a microfluidic channel; (**b**) *upper graph*: SERS spectrum of CHO cells incubated with gold nanoparticles; lower spectrum: Raman spectrum of CHO cell without gold nanoparticles [66]. (Reprinted from Zhang et al. Copyrights (2008) with permission from Springer—Analytical and Bioanalytical Chemistry)

55, 70], and pathogens [52, 58] can be found. More detailed information concerning the application of LOC-SERS for molecular and cellular analysis can be found in the review article by Huh et al. [71].

5 Conclusion

SERS implemented in a LOC device is becoming more and more important in the area of bioanalytics. SERS as a detection method provides the sensitivity and specificity needed for the analysis of biomolecules, whereas the application of a LOC device allows the handling of small sample volumes, offers reproducible and highly definite measurement conditions, and exhibits a high analysis throughput.

The application of LOC systems allows the implementation of a variety of functionalities: for example, sample preparation like mixing, washing steps, separation, trapping, etc. can be integrated in microfluidic devices. Especially for small sample volumes the handling and the reproducibility of the sample preparation become much easier. Concerning the application of SERS, the integration of active substrates in flow-through devices is extremely promising to improve the LOC setups in terms of an even more reliable analysis. Microarrays utilized as LOC devices for SERS measurements provide the possibility of multiplexing as well as a high analysis throughput. The combination with fluidic channels seems to be even more effective as shown by several research groups [27–29].

Many research groups showed that the application of LOC-SERS exhibits a high potential for bioanalytics. Besides quantitative monitoring of drugs, sequence-specific analysis of DNA, investigations of microorganisms, as well as the

characterization of cells, many other biomolecules have been studied utilizing LOC-SERS demonstrating its versatility.

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