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REVIEW PAPER

Optofluidic SERS: synergizing photonics and microfluidics for chemical and biological analysis

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Abstract Surface enhanced Raman spectroscopy (SERS) leverages the specificity of Raman scattering and the sensitivity provided by localized plasmonic effects for applications in chemical and biomolecular detection. However, nearly four decades after the first report of SERS, practical uses of the technique remain limited. Optofluidic SERS—the synergistic use of microfluidics to improve the performance of SERS—may finally lead to practical devices for chemical and biomolecular detection. In this review, we describe recent advances in optofluidic SERS microsystems that have been developed to improve the performance and applicability of SERS. These techniques include designs that improve the light–analyte interaction, that perform active or passive concentration of metal nanoparticles and/or analyte molecules, and that utilize microfluidic techniques to improve functionality. In addition, we present optofluidic SERS techniques that enable new applications that have not been possible before the advent of optofluidics. Finally, we project future advances in optofluidic SERS and present a vision for the disruptive technologies that will enable the translation of SERS from the research lab to practical uses.

Keywords Optofluidics · Surface enhanced Raman spectroscopy · Chemical sensors · Biosensors

1 Introduction

Photonic-based biological and chemical sensing techniques are often categorized either as label-free (e.g., refractive index transduction) or as labeled (e.g., using fluorophore-conjugated biorecognition molecules). It is commonly accepted that label-free techniques enable direct detection with fewer steps while fluorescent-based techniques provide improved detection limit. Surface enhanced Raman spectroscopy (SERS) has continued to gain in popularity as an alternative transduction method. In many implementations, SERS can offer the simplicity of label-free detection while providing the sensitivity of fluorescent-labeled techniques. When utilized in a labeled detection paradigm, such as when biorecognition molecules are conjugated to plasmonic nanostructures, SERS can offer significantly denser multiplexing as compared with fluorescence detection while requiring a simpler optical system.

Raman spectroscopy enables these advantages because of the specificity of the acquired signal. Upon laser light excitation, Raman-scattered photons from a molecule reveal the landscape of vibrational energy states of the molecule, which are unique to any molecule. Thus, detection of the Raman scattered photons provides a unique spectral fingerprint that can be used to identify the molecule and its characteristics. Unfortunately, Raman scattering is an extremely weak effect and thus it cannot generally be applied to detection of trace quantities of analytes in its conventional form. Nearly 40 years ago, however, it was discovered that noble metal nanostructures provide a boost of many orders of magnitude to the Raman signal for molecules interacting at the surface (Albrecht and Creighton 1977; Fleischmann et al. 1974; Jeanmaire and Van Duyne 1977; Moskovits 1978). This effect, surface enhanced Raman scattering, is the result of the combination of an electromagnetic enhancement provided by the localized surface plasmon resonances at the metal nanostructure surface (Kneipp et al. 1999; Moskovits 1985) as well as by a less understood chemical effect at the metal

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surface (Michaels et al. 1999). The power of the SERS technique was realized 15 years ago with the demonstration of single molecule identification using SERS (Kneipp et al. 1997, 1998; Michaels et al. 1999; Nie and Emory 1997).

In general, SERS is capable of performing label-free detection on small molecules, as the spectral bands can easily be identified and distinguished. For macromolecules, such as large protein and DNA molecules, it is more common to employ SERS in a labeled immunoassay (Grubisha et al. 2003; Han et al. 2009; Huh et al. 2009b; Huh and Erickson 2010) or hybridization format (Cao et al. 2002; Culha et al. 2003; Driskell et al. 2008; Fabris et al. 2007; Huh et al. 2009a, b; Isola et al. 1998; Lowe et al. 2010; Mahajan et al. 2008; Vo-Dinh 2008; Wabuyele and Vo-Dinh 2005). The labels, which are often fluorophores or other strong Raman scatterers, are referred to as Raman reporter probes (RRPs). As opposed to fluorescence-based transduction, however, the RRPs each generate a unique Raman spectral fingerprint upon laser excitation, which enables much denser multiplexing than fluorescence while utilizing only a single laser and a single filter set (Cao et al. 2002; Faulds et al. 2004). These conceptual advantages make SERS an intriguing potential choice for a number of translational applications in molecular detection.

2 Optofluidics: a toolkit for SERS microsystems

Despite years of research and a mounting number of published reports, SERS has had a relatively small impact outside of the research laboratory. Meanwhile, over the past decade a trend has emerged in photonic biosensing in which the detection head is integrated with microfluidic functions into a microsystem; this is generally thought to improve the practical use of the photonic device. Implementing SERS in a microfluidic environment provides the advantage of functional integration of automated sample processing and delivery. For example, a number of reports have shown on-chip mixing of metal nanoparticles and target analytes in a microfluidic channel immediately before SERS detection within the channel (Lee et al. 2007; Park et al. 2005; Quang et al. 2008; Yea et al. 2005). In addition, one report demonstrated the capability to synthesize SERS-active metal nanostructures within a microfluidic channel (Wilson et al. 2010), while another report leveraged optical tweezers within a microfluidic channel to construct metal nanoparticle aggregates to increase SERS activity (Tong et al. 2009).

While the integration of these and other microfluidic functions is an advantage, the performance of SERS can be limited when conducted within a microfluidic environment. Figure 1 compares a conventional SERS detection method with microfluidic-based SERS. Today, SERS measurements are commonly performed by fabricating a gold or silver nanostructured substrate and drying the sample onto the substrate (Fig. 1a). When SERS was initially implemented in microfluidics, two common approaches were utilized: mixing the sample with a silver colloid in a microfluidic channel (Fig. 1b), or passing the sample through a microfluidic channel that has a silver or gold nanostructured substrate at the bottom of the channel (Fig. 1c). When performing SERS detection with a colloid, the acquired Raman signal intensity can be reduced as compared with conventional SERS detection because there are fewer analyte molecules that have adsorbed onto or become bound to SERS-active surfaces within the detection volume (Fig. 1b vs. a). Likewise, when using a 2D SERS substrate at the bottom of a microfluidic channel, the acquired signal intensity is often worse because under laminar flow the analyte is transported to the SERS substrate only by diffusion, which is less effective than drying the sample onto the SERS-active surface (Fig. 1c vs. a).

Over the past few years, some in the SERS community have drawn upon *optofluidics* techniques to improve upon the performance of and add functionality to SERS microsystems. Optofluidics has emerged recently out of the emphasis on the integration of photonics into microsystems (Erickson et al. 2011b; Fainman et al. 2010; Fan and White 2011; Hawkins and Schmidt 2010; Monat et al. 2007; Psaltis et al. 2006; Schmidt and Hawkins 2011). In this article, we define optofluidics to imply a synergistic relationship between photonics and microfluidics in which microfluidics improves the function or performance of the photonics and/or photonics improves the function or performance of the microfluidics. Numerous examples of optofluidics-based molecular detection have been reported recently. In label-free refractive-index-based detection, the concept of micro/nanofluidic flow-through devices is replacing planar biosensors (Escobedo et al. 2010; Guo et al. 2011; Yanik et al. 2010). In these microsystems, the sample is passed through micro/nanochannels within the sensor head [e.g., nanohole arrays (Escobedo et al. 2010; Yanik et al. 2010) or Fabry–Perot cavities with nanochannels (Guo et al. 2011)], as opposed to being passed over the top of the sensor head, thus eliminating the strong dependence on diffusion and dramatically improving performance. One recently reported example of optofluidic-based fluorescence detection is the use of a capillary-based microfluidic channel in which the cross-section acts as a ring resonator (the optofluidic ring resonator, or OFRR). This device has been used in conjunction with Förster resonance energy transfer (FRET) to detect DNA hybridization (Sun et al. 2010).

To understand how optofluidic concepts can be applied to SERS microsystems to improve the performance, we

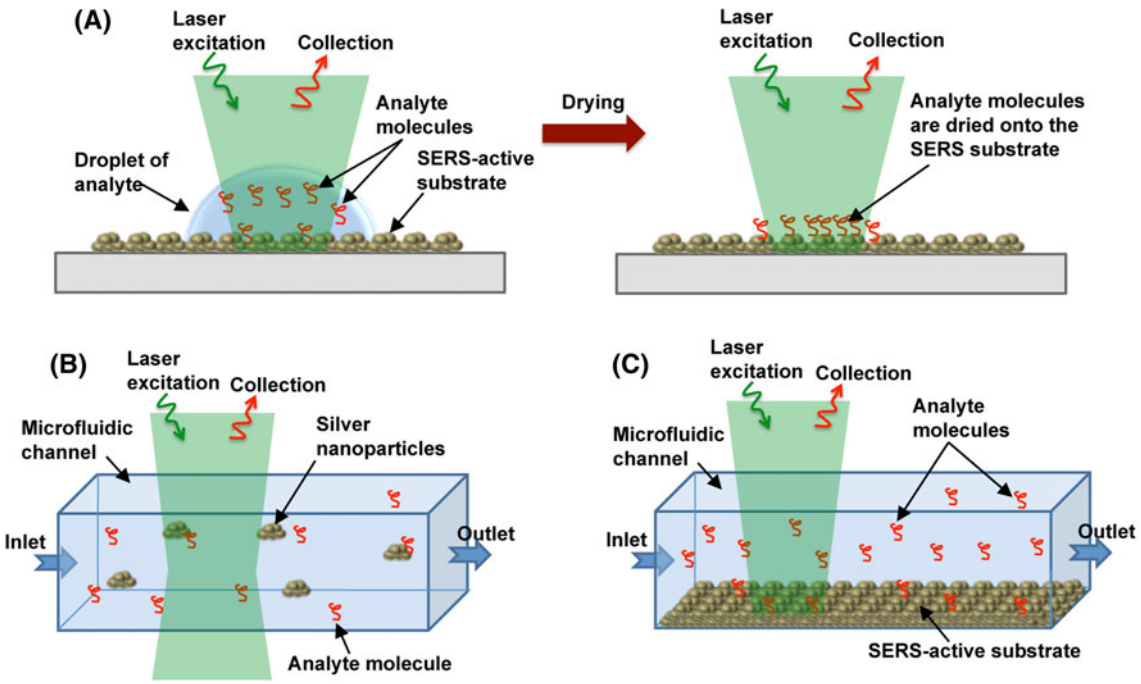

Fig. 1 **a** In conventional SERS, a sample droplet is dried onto a SERS-active substrate; target analyte molecules are concentrated onto the substrate after drying. **b** In one form of microfluidic SERS, analyte molecules are mixed with a solution of nanoparticles and passed through a microfluidic channel under laser illumination. **c** In another form of microfluidic SERS, a SERS-active substrate is incorporated as the bottom of a microfluidic channel. In **b** and **c**, the density of target analyte molecules adsorbed at SERS-active locations within the SERS detection region is much lower compared with conventional SERS techniques

consider the parameters related to the measured power of a Raman signal:

$$P_{\text{Raman}} \propto I \times \sigma \times N \tag{1}$$

where I is the laser excitation intensity in the detection volume, σ is the Raman scattering cross-section, and N is the number of analyte molecules within the detection volume. When considering SERS, N becomes the number of analyte molecules interacting with the metal nanostructures within the detection volume, while σ includes the enhancement (electromagnetic and chemical) provided by the metal nanostructures (in reality, the electromagnetic enhancement is locally increasing the intensity I, but for convenience, we group the enhancement into the cross-section term). For the special case of surface enhanced resonance Raman spectroscopy (SERRS), in which the excitation laser wavelength matches the optical absorption of the target molecule, the Raman scattering cross-section σ is further increased (Stacy and Van Duyne 1983). Taken together, Eq. 1 demonstrates that to maximize the measured SERS signal, a system should aim to maximize the intensity in the detection volume, the enhancement provided by the metal nanostructures, and the number of target molecules that interact with the nanostructures within the detection volume.

In this review, we evaluate how the concepts of optofluidics can be applied to improve the performance of SERS microsystems. Most reports published to date are focused on improving SERS performance by increasing the parameter N. We will first present three categories of optofluidic SERS techniques that aim to increase detection performance:

(i) Photonic structures that expand the detection volume, and thus increase N.
(ii) Active techniques that increase target analytes and their interaction with metal nanostructures, thereby increasing N.
(iii) Passive techniques that increase nanoparticle-analyte conjugates in the detection volume, and thus increase N.

We will then discuss the possibility for increasing the detection performance of SERS microsystems by incorporating optically resonant structures into the detection volume, which can increase the parameter I from Eq. 1. After reviewing the reported techniques for increasing performance of SERS microsystems, we will then describe new applications in chemical and biological analytics that have been enabled by the employment of optofluidic SERS. Finally, we will discuss two important trends that we predict will have a significant impact on future uses of optofluidic SERS: (i) ultra-low-cost fabrication of SERS-active substrates and (ii) optofluidic trapping and analysis of micro- and nano-particles.

3 Improving the performance of SERS microsystems with optofluidics

3.1 Photonic crystal waveguides for optofluidic SERS

Photonic crystal fiber (PCF) utilizes an array of longitudinal holes along the optical cable to impart special transmission properties, such as particular spectral characteristics or tolerance to nonlinearities. Recently, the air cavities in PCF have been assigned the additional function of microfluidic sample containment. Thus, for a properly designed PCF, light propagates along the fiber, interacting with the sample, which is contained throughout the entire length of the channel. In the case of SERS, the excitation light acts as a Raman pump along the fiber, and Raman-scattered photons are also guided along the fiber to the detector (Fig. 2a). As a result, the detection volume extends along the entire length of the PCF, as opposed to comprising only a small spot on a SERS-active substrate or a small volume in a cuvette at which the excitation laser is focused. This extension of the detection volume increases the number of analyte-nanostructure conjugates (N in Eq. 1), and thus improves the detection performance of SERS.

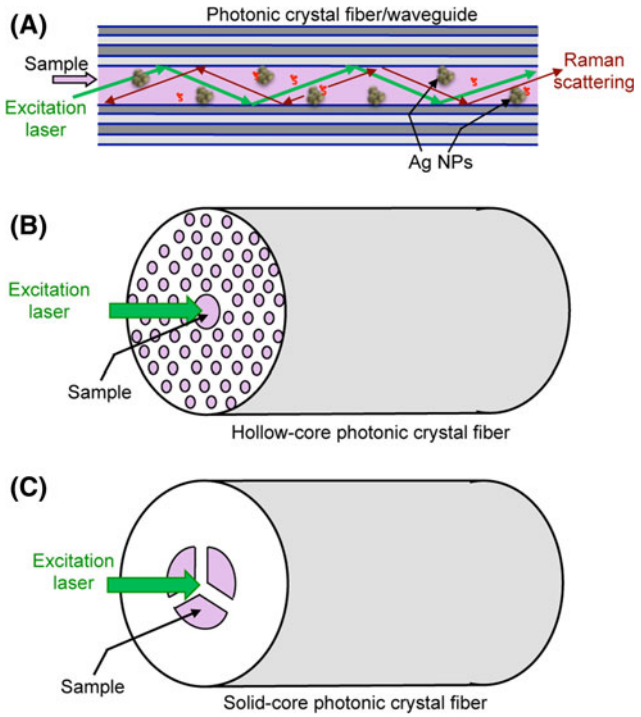

Fig. 2 Increasing N (Eq. 1) by extending the detection volume. **a** Photonic crystal structures can be used to confine and guide light along a microfluidic channel. **b** Excitation light guided along with the sample in the hollow core of a PCF, as in (Yang et al. 2010). **c** Excitation light guided in the solid core of a PCF, such that the evanescent field interacts with the sample, as in (Khaing Oo et al. 2010)

A number of published reports have demonstrated the concept of using PCF for optofluidic SERS (Khaing Oo et al. 2009, 2010; Yan et al. 2006; Yang et al. 2010). In general, there are two parallel approaches to form a SERS detection system from PCF. In work by Yang et al. (2010) the core of a hollow-core fiber is filled with the sample in a silver colloid, and thus the excitation light propagates directly along with the sample (Fig. 2b). A detection limit of 100 pM for Rhodamine 6G (R6G) was achieved. In an alternative approach, Khiang Oo et al. (2010) utilized a solid core fiber and loaded the sample into the hollow channels that form the cladding (Fig. 2c). In that work, metal nanoparticles were first immobilized onto the surface of the hollow channels, and then the sample was loaded. Because the fiber cable has a solid core, the evanescent field of the guided mode serves to excite the Raman scattered photons. A detection limit of 100 pM for R6G was also achieved with this structure.

The optofluidic concept of extending the sample volume along a liquid light-guiding structure has also been demonstrated on-chip. Measor et al. (2007) utilized an anti-resonant reflecting optical waveguide (ARROW) structure to create a liquid core waveguide on a chip. Light is coupled into the ARROW structure via an adjacent on-chip waveguide, while the liquid sample is also loaded into the ARROW structure from an on-chip microfluidic channel. While the on-chip nature of this structure limits the length of the detection volume as compared with the PCF, the on-chip implementation may have practical advantages when considering the benefits of system integration.

3.2 Concentration with active microfluidic techniques

For decades, a simple trick has been used to increase the parameter N when performing SERS in a bulk environment; the sample is dried onto a surface (Fig. 1a). Clearly, this is difficult to do within a microfluidic channel. However, alternative techniques have been developed to transport a large number of the target analyte molecules from the bulk solution to the detection volume. In one example, Cho et al. (2009) leveraged the conductive properties of a nanostructured SERS-active surface at the bottom of a microfluidic channel; it is used as an electrode to attract charged analyte molecules from the bulk solution, as illustrated in Fig. 3a. Using this approach, the authors were able to detect the Raman signal for 10 fM adenine, an improvement of eight orders of magnitude over the non-concentrated case in which diffusion is the only mass transfer mechanism to deliver the analyte molecules to the SERS substrate.

Active forces have also been used to concentrate analyte molecules bound to silver nanostructures into the detection volume as a method to improve the SERS detection

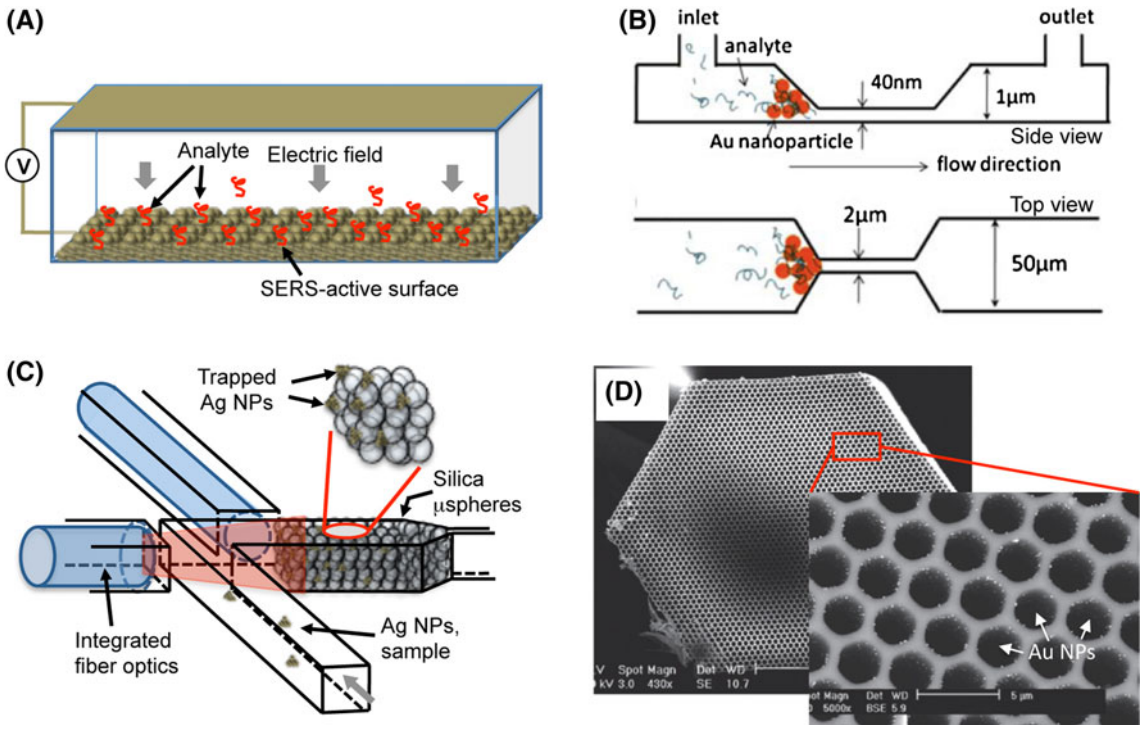

Fig. 3 Increasing *N* (Eq. 1) by concentrating the number of analyte molecules adsorbed to SERS-active hot spots in the detection volume. **a** Electro-active concentration of analyte molecules at a SERS-active surface, as in (Cho et al. 2009). **b** Concentration of silver nanoparticles and analyte molecules using a nanofluidic channel. Reprinted from (Wang et al. 2009b). **c** Concentration of silver nanoparticles and analyte molecules using nanoporous silica microspheres, as in (Yazdi and White 2012). **d** Microhole array for "flow-through" optofluidic SERS. Reprinted with permission from (Guo et al. 2012), Copyright 2012, American Chemical Society

performance. Huh et al. (2009a) fabricated electro-active microwells into a microfluidic chip in which opposing electrodes were located at the top and bottom of the well. Oligo-modified nanoparticles were loaded into the chip and electrokinetic forces between electrodes cycled the nanoparticles in the microwell, causing them to mix with the sample, which contained the target oligo sequence. After mixing, the nanoparticles were then driven to one of the electrodes and concentrated within the detection volume.

Using this method, the authors have reported a number of biologically significant results. In the initial demonstration, the authors were able to detect 30 pM TAMRA-labeled oligonucleotide sequences of the Dengue virus (Huh et al. 2009a). Later, the same research group utilized this optofluidic microsystem to detect single-nucleotide polymorphisms (SNPs) in DNA sequences using the ligase detection reaction to link the reporter molecule with the metal nanostructure in the case of an SNP (Lowe et al. 2010). Furthermore, the multiplexing capabilities of SERS were demonstrated in this work, as three K-Ras oncogene alleles were detected simultaneously at a concentration of 10 pM. Importantly, while many optofluidic SERS techniques presented to date use purified small molecules for characterization, the work presented for the electro-active microwell chip demonstrates biologically significant applications of SERS.

Similar to the electro-active microwell system, Hwang et al. (2011) recently reported the electrokinetic concentration of metal nanoparticles and adsorbed analyte molecules in a liquid sample sandwiched between two electrode plates. However, unlike other electrokinetic SERS devices, in this report one of the electrodes is formed from a photoconductive layer. As a result, when the excitation laser is focused onto the photoconductive plane and an AC voltage is applied across the electrodes, charged particles are concentrated at the activated location of the photoconductive plane (i.e., the exact location of the excitation laser focusing spot). As a result, nanoparticles and adsorbed analytes can be concentrated and analyzed at any desired location within the fluidic microcell. The authors have termed this technique as *optoelectrofluidic* SERS. While the initial report of optoelectrofluidic SERS presents a poor detection limit (50 μM adenine), we expect that optimization of the technique will lead to detection limits closer to that achieved by the electro-active microwell concentration device reported by Huh et al., described above.

In addition to electrokinetic forces, magnetic forces provide another mechanism for actively concentrating analyte molecules into the SERS detection volume, which again increases the term *N* in Eq. 1. Han et al. (2011) utilized magnetic beads coated with silver nanostructures

as a SERS-active substrate. Whereas conventional SERS techniques may use a static substrate, in this case mobile magnetic beads that can mix throughout the sample serve as the substrate; this provides an advantage in terms of mass transport of target analyte molecules to the SERS substrate. After mixing these mobile SERS-active substrates with the sample, a magnetic field concentrates the beads into a small detection volume. Malachite green was detected down to 10 ppb using this technique.

3.3 Concentration with passive micro/nanofluidic techniques

While active concentration of analytes at the SERS-active substrate is an effective optofluidic technique to increase the SERS signal, this improvement may come at the cost of increased fabrication steps to incorporate the active control elements. Therefore, it is also useful to consider the passive concentration of analyte-nanoparticle conjugates within nanofluidic elements. This concept was first reported by Wang et al. (2007) who formed a channel 40 nm in height in a glass substrate that bridged two microfluidic channels. Gold nanoparticles loaded into the microchannel become trapped at the inlet of the nanochannel, forming a high-enhancement detection zone (Fig. 3b). As the sample is driven through the channel, the analyte molecules are captured at the surfaces of the nanoparticles that are lodged at the channel inlet. The authors reported a detection limit of 10 pM adenine in these experiments. In later work, the same group used this concept to detect β-amyloid protein (Chou et al. 2008) and subsequently to obtain structural information from BSA and insulin proteins (Wang et al. 2009b). While these reports make clear the advantage of using a nanofluidic channel to trap and concentrate nanoparticles and adsorbed analyte molecules, the nanofabrication of microchannels into glass substrates can be difficult, and thus may not provide significant advantages as compared with integrating active control components. More recently, Park et al. (2009) demonstrated the fabrication of a nanofluidic channel using the controlled collapse of polydimethylsiloxane (PDMS), the most popular material for soft-lithography microfabrication. Oligo-labeled gold nanoparticles, which had been reacted with 3 nM of TAMRA-labeled complementary target, were loaded electrophoretically. As expected, the Raman signal for TAMRA increased significantly during the first minute of loading, showing that the nanoparticles and bound target molecules were being concentrated into the detection volume. In comparing the two aforementioned approaches, the PDMS device appears to be simpler to fabricate, but the hydrophobic nature of PDMS makes it difficult to load; as a result, electrophoretic pumping is required.

More recently, a nanofluidic SERS microsystem that can passively trap SERS-active nanoparticles and adsorbed analytes without the need for complex nanofabrication has been reported (Yazdi and White 2012). In this design, nanoporous silica microspheres are packed into a microfluidic channel to create a nanofluidic trapping matrix (Fig. 3c). When loading the sample mixed with silver colloid, the nanoparticles and adsorbed analyte molecules become trapped within the detection volume. Just as in the aforementioned schemes, this increases the number of analyte molecules in SERS-active hot spots within the detection volume as compared with conventional microfluidic SERS. Using this approach, the detection limit for the organophosphate pesticide malathion was improved by more than four orders of magnitude as compared with an open microfluidic channel.

3.4 Three-dimensional micro/nanofluidic SERS substrates

Figure 1c above illustrated a conventional technique for a SERS microsystem in which the sample is passed across a two-dimensional SERS-active substrate. The performance is limited by diffusion of analyte molecules to the substrate. Recently, Liu et al. (2011) have extended the microfluidic SERS-active substrate into three dimensions by trapping metal nanoparticles throughout a porous polymer monolith in a microfluidic channel. The nanoparticle-functionalized monolith presents a tortuous path through which the sample passes, creating nearly continuous opportunities for analyte molecules to adsorb to a metal nanoparticle. Thus, as opposed to relying on diffusion to deliver the analyte molecules to the SERS-active substrate (Fig. 1c), the analyte molecules are essentially passing through the SERS substrate. With this monolith-based three-dimensional SERS substrate, the authors reported a detection limit of 220 femtomoles of R6G.

In a similar but more ordered approach, Guo et al. (2012) created a three-dimensional SERS-active surface by fabricating a multihole capillary; the 190-μm glass capillary has nearly 3,000 “holes” (2.1 μm each) in the cross-section, which serve as a microfluidic array (Fig. 3d). Gold nanoparticles are attached to the walls of each channel, creating a three-dimensional SERS-active structure. A detection limit of <1 pM R6G is reported. This approach builds upon the new optofluidic trend of “flow-through” sensing, in which the sample flows through a nanofluidic biosensor instead of flowing over a planar sensor (Escobedo et al. 2010; Guo et al. 2011; Yanik et al. 2010). Just as with the monolith, the flow-through optofluidic device dramatically reduces the mass transport limitations of conventional open microfluidic channels.

3.5 Active versus passive optofluidic SERS techniques

In Fig. 1, we illustrated the challenges involved when translating SERS to microfluidic devices; compared with conventional sample application techniques, typical microfluidic techniques are hindered by poor interaction between target molecules and the metal colloid or nanostructured surface. Sections 3.2 through 3.4 presented two optofluidic paradigms (active and passive) to promote interaction between target molecules and the SERS-active materials. Both classes of optofluidic devices are able to overcome the challenges of microfluidic SERS devices. While it is premature to predict which technique will ultimately have a commercial impact, it is nonetheless instructional to compare the active and passive optofluidic SERS methodologies.

In general, the active techniques promote interactions between target molecules and the SERS-active nanostructures and concentrate the target molecules within the detection volume through the use of forces, such as electrokinetic forces. Thus, the devices require the incorporation of the active elements, such as electrodes and control circuitry in the case of electrokinetic devices. On the other hand, although the passive devices do not require the fabrication of the active elements, the nanofluidic devices may require complicated fabrication or additional fabrication steps to create the passive concentration elements. Furthermore, the devices based on single nanofluidic channels presented above may be more prone to clogging, are limited in sample throughput, and may have poor repeatability due to variations in aggregation at the nanofluidic channel. The three-dimensional nanofluidic devices presented above were shown to be repeatable, had good sample throughput, and were not prone to clogging (Liu et al. 2011; Yazdi and White 2012). However, they have not yet been evaluated with complex biological samples, which are believed to cause problems in channels with small dimensions. Assuming that the passive optofluidic devices can overcome the issues with sample throughput and clogging, we believe that ultimately the technology that leads to the devices with the lowest cost per chip will have the greatest impact in commercial applications, including environmental testing, defense, and clinical assays.

3.6 The next step: SERS excitation by optofluidic resonators

In the optofluidic approaches described above, the SERS detection performance is improved by increasing the number of analyte molecules in the SERS-active detection volume, either through the use of photonic crystal structures to extend the detection volume, or through microfluidic techniques that concentrate analyte molecules into the detection volume. We expect that published reports using either of these optofluidic SERS concepts will continue to appear in the future. However, returning to Eq. 1, we see that an alternative approach to improving the SERS performance is to increase the optical intensity that excites the sample. One method to increase the optical intensity within the detection volume is to leverage optically resonant structures. Examples of optical microresonators include microspheres, waveguide ring resonators, microtoroids, Fabry–Perot cavities, and photonic crystal structures (Baehr-Jones et al. 2005; Bog et al. 2008; Gorodetsky and Ilchenko 1999; Guo et al. 2011; Hossein-Zadeh and Vahala 2007; Vollmer and Arnold 2008; White et al. 2007). Optically resonant structures serve as photon traps, which leads to a dramatic increase in the optical intensity within the resonator as compared with the intensity that is incident upon the resonator. For example, using the well-established coupling theory of optical ring resonators (Cai et al. 2000), it can be shown that a ring resonator with a Q-factor of 10^6 can have an optical enhancement of two to three orders of magnitude when operated at critical coupling (White et al. 2007).

The first demonstration of the use of an optical resonator integrated into a microfluidic channel for SERS excitation was based on the optofluidic ring resonator (OFRR) (White et al. 2007); the cross-section of the capillary serves as a ring resonator while the sample is delivered through the microfluidic capillary. More recently, planar photonic crystal structures have been utilized as an optically resonant substrate for SERS excitation (Kim et al. 2008, 2010; Zhao et al. 2011). In each of these examples, the resonators create a high-intensity optical field at the surface of the resonator. As shown in Fig. 4, the photonic crystal resonator reported by Kim et al. showed a significant increase in SERS intensity when the analyte was excited by the resonant optical mode. We expect that the true value of using optical resonators as a substrate for high-intensity SERS excitation will be realized when the resonant structures are combined with the analyte-nanoparticle pre-concentration approaches described in Sects. 3.2 and 3.3. While the pre-concentration methods are able to increase the number of analytes (N in Eq. 1) in the SERS-active detection volume by many orders of magnitude, the optically resonant structures promise to increase the optical intensity (I in Eq. 1) by two to three orders of magnitude. Thus, as the optofluidic SERS approaches reported in this review exhibit detection limits on the order of picomolar to femtomolar concentrations, the synergistic combination of optical resonators with micro/nanofluidic concentration is expected to push the detection limits into the attomolar range.

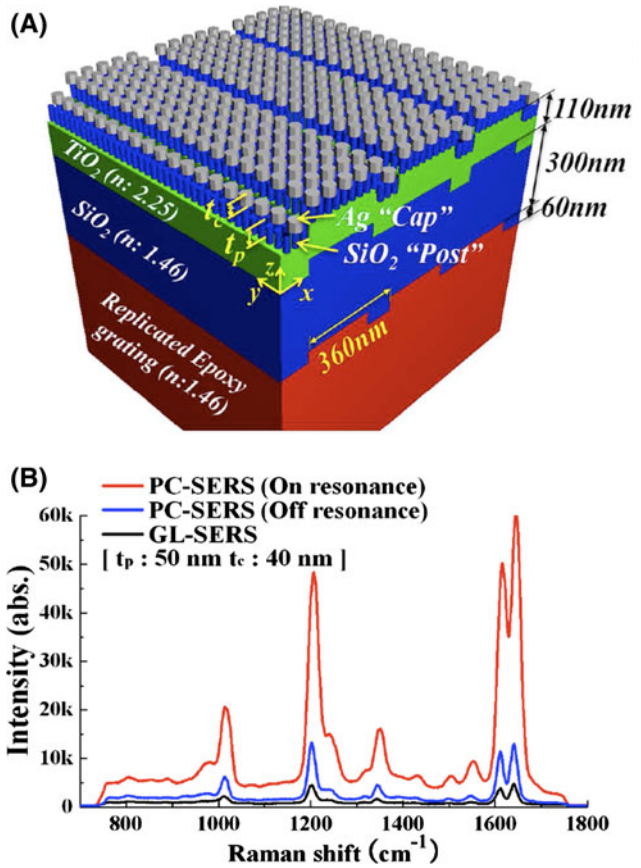

Fig. 4 Increasing *I* (Eq. 1) using resonant optical structures. **a** A one-dimensional photonic crystal structure enhances the excitation optical field at the SERS-active surface. **b** The measured SERS signal is significantly increased when the excitation light is on resonance. Reprinted with permission from (Kim et al. 2010), Copyright 2010, Optical Society of America

4 New applications enabled by optofluidic SERS

In addition to improvements in SERS detection performance, the synergistic integration of microfluidic functions with SERS detection is leading to a range of new applications for chemical and biomolecular analytics. In Sect. 3.3, the advantage of nanofluidic channels for trapping nanoparticles was presented. More recently, Choi et al. (2011) have utilized the nanofluidic channel for the trapping and SERS-based detection of protein aggregates, which may lead to a simple detection mechanism for disease-related protein aggregates, such as those involved in the formation of plaques that are related to Alzheimer's disease. In this example, the nanofluidic structure not only increases the sensitivity of the detector, but also enables the specific enrichment and detection of the target, namely protein aggregates. Similarly, Piorek et al. (2007) developed an optofluidic method of capturing airborne molecules into a microfluidic channel, thus concentrating them for SERS detection in the channel. The structure of this unique microsystem is illustrated in Fig. 5. One surface of the pressure-driven microfluidic channel is open to the air, but surface tension of the water holds it within the channel, even while the sample is being forced along the channel. The open surface enables the channel to collect water-soluble airborne molecules, which then react with the metal nanoparticles in the aqueous channel solution.

Another technique that leverages the fluidic properties of water at the microscale is droplet microfluidics. Recently, droplet microfluidics has been utilized in SERS detection systems, often for the purpose of improving the repeatability (Ackermann et al. 2007; Cecchini et al. 2011; Strehle et al. 2007; Wang et al. 2009a). A number of new applications of SERS are expected to result from this combination. For example, Walter et al. (2011) recently utilized droplet microfluidics for sample preparation and species-level identification of bacteria in a continuous flow microfluidic chip.

One additional innovative application of optofluidic SERS that was recently demonstrated is the integration of SERS with microfluidic chromatography. Lee and Moskovits (2011) incorporated an on-column SERS-based detector with chromatographic separation of metal ions. The inside surface of the capillary, which is coated with carboxylated gold nanoparticles, serves as the separation medium (due to selective adsorption properties of the metal ions) and also as the SERS-active surface. This eliminates the need for a post-separation detection mechanism, such as mass spectrometry.

In the future, we envision two important drivers for new applications that leverage optofluidic SERS: (i) ultra-low-cost SERS devices using new innovations in fabrication techniques and (ii) sophisticated analytics based on optical trapping and analysis within microfluidic channels. The development of ultra-low-cost microfluidic devices has been driven by the need for diagnostics in low resource settings. Today, the quintessential example in microsystems for low resource settings is paper microfluidics, or micro paper analysis devices (μPADs) (Martinez et al. 2010; Pelton 2009). Optofluidic SERS microsystems on paper are sure to follow, as SERS on paper-based substrates has already been reported (Lee et al. 2011; Yu and White 2010). Notably, SERS-active substrates have been fabricated using printing, including inkjet printing (Fig. 6a) (Yu and White 2010) and screen printing (Qu et al. 2012), both of which are candidates for the fabrication of paper-based microfluidic devices (Khan et al. 2010; Li et al. 2010; Martinez et al. 2010). Another innovative and promising fabrication method that has the potential to lead to ultra-low-cost optofluidic SERS devices is the so-called *Shrinky-Dink* lithography technique (Grimes et al. 2008). Metal nanostructures with localized surface plasmon resonances that were created on heat-shrinkable plastic films were reported recently (Fig. 6b) (Fu et al. 2010). We

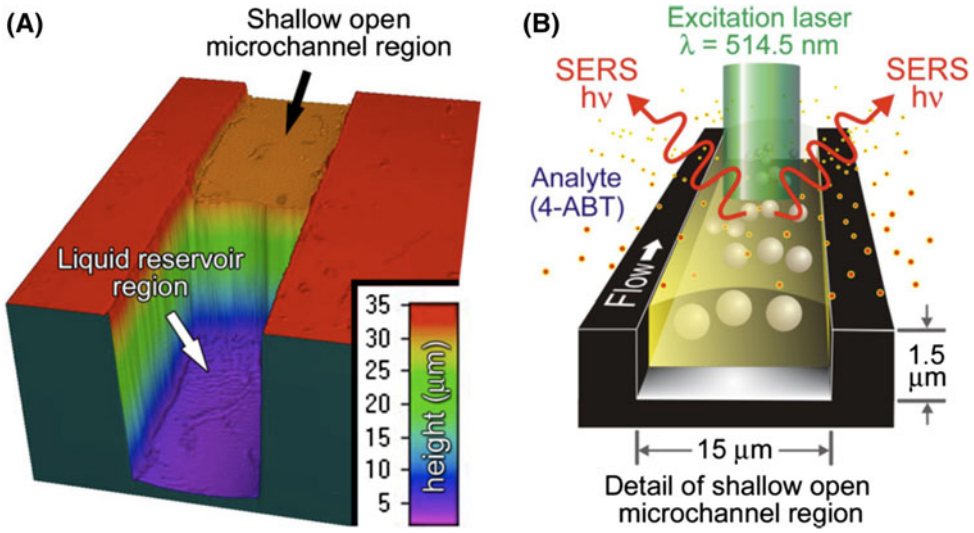

Fig. 5 A new application enabled by optofluidic SERS. **a** The microfluidic channel is designed to allow open-surface collection of analytes from the air. **b** This enables SERS detection of the analyte in a microfluidic channel. Reprinted with permission from (Piorek et al. 2007), Copyright 2007, The National Academy of Sciences

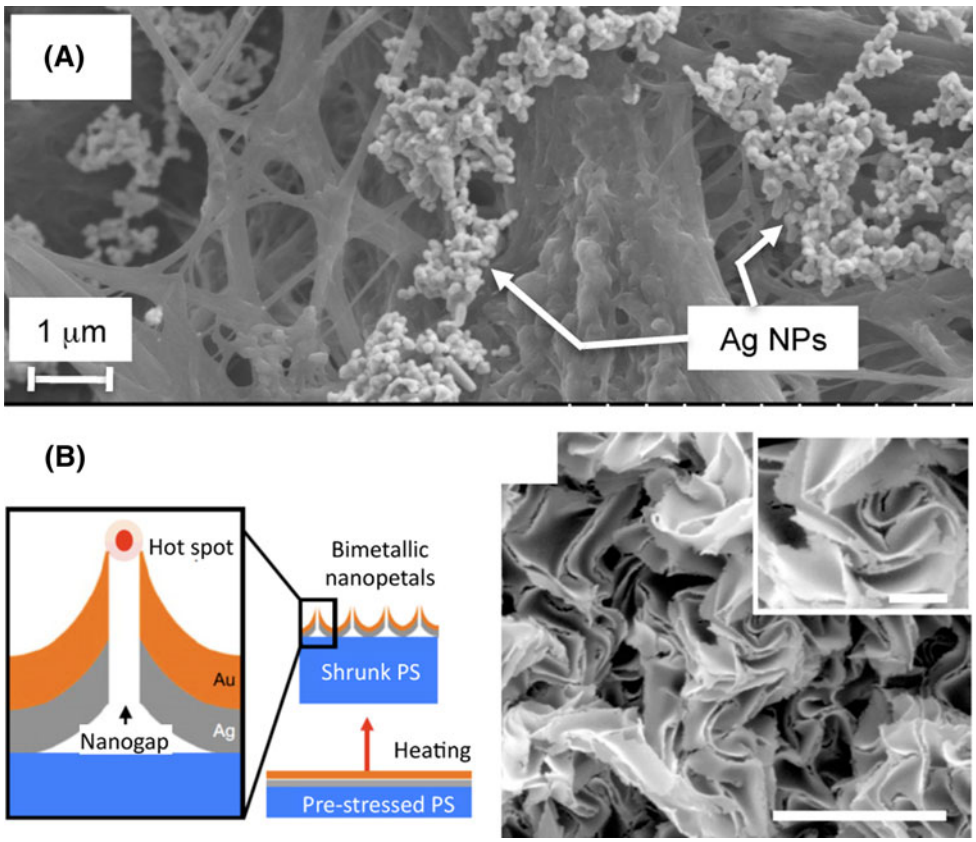

Fig. 6 SERS-active surfaces produced using ultra-low-cost methods. **a** Silver nanoparticles inkjet-printed onto cellulose paper (Yu and White 2010). **b** Plasmonic nanostructures created on heat-shrinkable plastic. Reprinted with permission from (Fu et al. 2010), Copyright 2010, American Institute of Physics

expect that ultra-low-cost optofluidic SERS devices will follow soon from this.

These ultra-low-cost techniques are expected to find applications in field-based sample analysis and routine laboratory screening. Alternatively, SERS may also find applications in the study of single molecule and single micro/nanoparticle studies. For these applications, advancements in optofluidic trapping may be combined with SERS for the analysis of (i) macromolecules, including DNA, (ii) microorganisms, such as virions and bacteria, and (iii) biofunctionalized micro/nanoparticles. A number of recent reports have demonstrated trapping of micro- and nano-particles using optical gradients in a fluidic environment (Arnold et al. 2009; Erickson et al. 2011a; Kühn et al. 2009, 2010; Lin et al. 2010; Mandal and Erickson 2008). These optofluidic systems can be more

compact and may use significantly lower optical power than traditional optical tweezer systems. Although today only fluorescence-based (Kühn et al. 2010) and refractive-index-based (Arnold et al. 2009; Mandal and Erickson 2008) analyses have been conducted with optofluidic trapping, we can envision that SERS will be combined with optofluidic trapping in the near future to provide rich information about the target system.

5 Summary and conclusions

While the dramatic signal enhancement of SERS was discovered nearly 40 years ago, today there are still only a small number of practical implementations for biological and chemical analytics. Microfluidic integration is often thought to be a common road to practical implementations for optical sensing technologies, but in the case of SERS, a simple microfluidic translation can be detrimental to the detection performance. However, the new paradigm of optofluidics, in which synergy between photonics and microfluidics increases performance and functionality, is enabling dramatic progress in SERS-based microsystems. A number of recent reports were reviewed here that demonstrate the use of optofluidic techniques to improve the detection performance of SERS by several orders of magnitude, thus matching or exceeding the performance of traditional SERS measurements while retaining the benefits of integrated microsystems. Although this trend represents a dramatic improvement as compared with just 5 years ago, we predict that the tipping point for wide use of SERS in biological and chemical analytics will occur when optofluidic approaches are combined with new ultra-low-cost fabrication techniques, such as paper microfluidics and heat-shrinkable plastics. Subsequently, as SERS becomes more familiar as an option for biological and chemical analytics, and as the cost of microsystem fabrication drops, we expect that the advantages of SERS, discovered decades ago, will finally be utilized in practical applications.

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