# Paving the Way to Industrially Fabricated Disposable and Customizable Surface-Enhanced Raman Scattering Microfluidic Chips for Diagnostic Applications

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Both surface-enhanced Raman scattering (SERS) spectroscopy and microfluidics are increasing their presence in industry. The integration of SERS into microfluidic chips is relevant for label-free biosensing and can expand the function of microfluidics to provide an efficient platform for on-site biochemical analysis equipped with the powerful sensing capability of SERS. Herein, the design and industry manufacturing of reliable and cost-effective disposable SERS microfluidic chips using high throughput technologies of injection molding and roll-to-roll are demonstrated. The SERS microfluidic chip is made of cyclic olein copolymers and of an ultrathin nanostructured polyethylene terephthalate foil covered by a plasmonic nanostructured Ag film, enabling Raman readout through it. This ultrathin plasmonic Ag-coated PET layer overcomes the drawbacks of SERS through thick polymeric microfluidic cells, i.e., it avoids strong fluorescence signals interfering with the Raman measurements and avoids high working distances. The achieved SERS enhancement in the range 10<sup>5</sup>-10<sup>6</sup> is demonstrated by probing 100 nl of 1 M rhodamine and of  $10^{-12}$  M crystal violet with a > 90% reproducible signal, with large-area uniformity on all the SERS area of 20 mm 2 and good stability of the signal over months. Therefore, this approach of SERS microfluidic chips provides an industrially viable customizable and cost-effective route toward disposable, point-of-care diagnostic applications.

of commercially available, compact, robust, Raman systems that can be used in-field are contributing to expanding SERS to point-of-care diagnostics, environmental protection, and food safety applications.<sup>[3]</sup> Concurrently, this has required considerable advances in the area of SERS-active substrates design and fabrication, not only for laboratory research but also for industrial applications.<sup>[4]</sup> SERS substrates are characterized by the incorporation of metallic nanostructures that support the excitation of localized surface plasmon resonances (LSPRs). LSPRs excited in metallic nanostructures are able of focusing light into nanometer scale volumes and magnify the local electromagnetic field in regions called hot spots.

The design and manufacturing of SERS plasmonic substrates is still a challenge<sup>[5]</sup> at both laboratory research and industrial manufacturing scale to overcome the following limitations: 1) the interaction between the SERS surface and the analyte causing the degradation of the SERS substrates with time and resulting in a decrease of the signal; 2) poor selectivity

# 1. Introduction

The concurrent rise of surface-enhanced Raman spectroscopy (SERS) and microfluidic technologies has enabled in recent years the detection of a wide range of chemical and biological species at low concentrations down to single molecule level.<sup>[1,2]</sup> A number

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of the substrates for a given analyte; 3) limited reusability of substrates; and 4) nonhomogeneity and nonreproducibility of the SERS signal within a substrate. Therefore, the fabrication of SERS substrates is a topic of continuous research and improvement, with several paths advanced at the lab scale, but with their mass production facing the challenge of exploring large-scale

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manufacturing technologies that can result in customizable, reproducible, and cost-effective SERS substrates.

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Various approaches are being investigated to enhance the Raman signal using plasmonic colloidal metallic nanoparticles,<sup>[6]</sup> sharp nanotips (nanostars, nanourchins, nanorods, etc.),<sup>[7,8]</sup> roughened,<sup>[9,10]</sup> or nanostructured metal surfaces.<sup>[11]</sup> In order to overcome the disadvantages of dispersed plasmonic nanoparticles and take advantage of the hot spots, several strategies have been employed to immobilize those nanoparticles on solid substrates,<sup>[12]</sup> such as the controlled self-assembly of plasmonic NPs,<sup>[13]</sup> and the use of nanolithography fabrication.<sup>[14]</sup>

Meanwhile, the design, fabrication, and use of microfluidic devices have enabled high precision and reproducibility in analysis. The integration of plasmonic components into microfluidics led to the so-called microfluidics SERS (MF-SERS) approaches.<sup>[15]</sup>

MF-SERS has been explored by mixing Au or Ag nanoparticles within the sample to analyze,<sup>[16]</sup> which has the drawbacks of nanoparticles batch-to-batch reproducibility, as well as of nonhomogeneous spatial SERS signal depending on nanoparticle aggregation in the microfluidic channel. Alternatively, SERS-active patterns have been fabricated by directly writing 2D periodic metal nanostructures inside polydimethylsiloxane (PDMS) or glass microfluidic channels.<sup>[17]</sup> On the other hand, solid-state SERS substrates deposited on the microfluidic channels have enabled more reusable microfluidic SERS chips by reducing "memory effect."<sup>[18]</sup> Nevertheless, when coupling to the plasmonic component, microfluidic chip materials alternative to PDMS and glass must also be considered for the SERS chip fabrication. In this regard, injection molding of cyclic olefin copolymers (COP) provides an alternative to realizing microfluidic devices<sup>[19]</sup> overcoming the drawbacks of PDMS or glass. The fabrication of COP SERS flow cells by injection molding was shown in 2018, via multiple and complicated manufacturing steps including processes like milling,<sup>[20]</sup> resulting in microfluidic chips with a thick backplane, whose fluorescence could interfere with the SERS signal of analytes.

Therefore, challenges still exist in the large scale and industrial fabrication of microfluidics SERS substrates.<sup>[21]</sup> Pros and cons of MF-SERS have recently been reviewed,<sup>[4]</sup> showing that MF-SERS is attracting increasing attention but also encountering difficulties in transition from the laboratory level to the industrial scale.

Here, we demonstrate the fabrication at industrial scale of fully customizable polymeric SERS microfluidic chips that combine injection molding for the fabrication of the COP microfluidic chip and roll-to-roll thin film technology to fabricate the ultrathin plasmonic silver (Ag)/polyethylene terephthalate (PET) nanopatterned component, which is coupled to the COP through a feasible and cost-efficient process. The plasmonic effect of the nanopatterned Ag/PET component is designed and rationalized by finite-difference time-domain (FDTD) calculations. The advantage of the present MF-SERS chip is that the plasmonic component can be customized and designed to tune the wavelength of the plasmon resonance to match specific resonant absorption of any analyte molecule of interest, to couple any microfluidic chip of any shape, and to be used in any commercially available Raman system, thereby paving the way to fully customizable MF-SERS chips fabrication. Specifically, we demonstrate that the proposed nanostructures can be designed to support localized surface plasmon resonances (LSPRs) in the range spanning from 1.8 to 2.7 eV, and the manufacturing at industrial level of substrates with resonances at 1.9, 2.25, and 2.7 eV as examples of the fact that the chips' plasmon resonance wavelength can be customized according to the customer's need.

Here, provided that the plasmon resonance can be tuned all over the visible spectral range, we focus on the nanostructures with resonance on the blue because many organic compounds such as small pharmaceuticals and biomolecules have absorption bands in that spectral range.<sup>[22]</sup> The SERS effect is demonstrated by probing rhodamine (R6G) and crystal violet (CV). CV is a synthetic dye widely used in printing inks and paints and for coloring textiles, and traces of CV are being found as water pollutant and in aquaculture products, such as fish.<sup>[23]</sup> Currently, the detection at very low limit of CV in food, not allowed in the European Union, is a challenge. The probing of 1  $\mu$ M R6G and 10<sup>-12</sup>  $\mu$ CV by the MF-SERS chip using solid-state lasers demonstrates that a plasmonic Ag/PET nanopatterned ultrathin tape that can be externally combined to any microfluidic device results in an enhancement factor (EF) in the  $10^5$ – $10^6$  range, reproducible at 90% and stable over months, providing a promising route to an industrial scale fabrication of cost-effective high precision, scalable, and customizable SERS microfluidic chips.

# 2. Microfluidic Chip Fabrication

Figure 1 shows the scheme of the fabricated MF-SERS. It consists mainly of two parts as shown in Figure 1a, i.e., the roll-to-roll Ag/PET ultrathin film with an overall thickness of 50  $\mu$ m, and the injection molded COP flow cell; the height of the flow channel is 60  $\mu$ m; the injection molded part has an overall thickness of 1 mm giving stability to the final assembled chip. The picture of the final assembled MF-SERS chip is shown in Figure 1b.

#### 2.1. Injection Molding for Microfluidic Flow Cell

Injection molding is an efficient and cost-effective technique to generate microfluidic components with a flatness of  $1 \,\mu m \,mm^{-2}$ . The flow cell template was manufactured with a lithography process generating a 60 µm high flow cell. Minimizing the depth of the flow cell to 60 µm is important to enable a fast binding and interaction of the surface with molecules of interest, also keeping at the same time a controlled flow through the cell. This lithographic master is copied into nickel by electroplating, serving as an insert for the injection molding process. Here, a former Blu-ray Disc and DVD manufacturing process was adapted to generate injection molded parts from COP. Further details on the injection molding process have been reported in Prinz et al.<sup>[5]</sup> Although injection molding is also capable of realizing nanostructures for the SERS effect, it typically generates components with thicknesses above 600 µm, not suitable for fabrication of ultrathin backplanes as those show in next paragraph.







**Figure 1.** Schematic of the MF-SERS chip. a) It consists of the nanostructured thin ( $50 \mu m$ ) PET film (gray part) with a 50 nm Ag coating (dark gray line) realizing the SERS effect and the injection molded COP part (blue part) defining the inlet/outlet of the chip. b) Picture of the assembled MF-SERS chip with the bonded parts, showing the inlet and outlet. The central orange square (color comes from the light reflection) is the nanostructured Ag area.



**Figure 2.** a) Picture of a Silicon Master fabricated by e-beam lithography; b) SEM picture of the nanostructured array imprinted in the PET; c) picture of rolled ultrathin Ag/PET layer including the patterned areas indicated by the arrows; d) SEM picture of the final Ag coated patterned field (from b) on the roll film with a tilted view of 45°.

### 2.2. Ag/PET Thin Film Roll-to-Roll Technology

The SERS active area consists of a  $2.5 \times 8 \text{ mm}^2$  in size array of nanopatterned squared pillars fabricated at the Leibniz IPHT Jena and copied into nickel by electroplating at Stratec Consumables. The period of the array is 250 nm, and the squared pillars have widths and heights of 125 nm. Roll-to-roll processes were performed at KURZ realizing the structures onto a PET thin film with an ultrathin (50 nm) Ag coating, as shown in **Figure 2**.

Specifically, Figure 2b demonstrates that the nanostructures imprinted in the PET are well maintained in the bending state.

### 2.3. MF-SERS Chip Assembly

Figure 3 schematizes all the steps at industrial scale to get the SERS Ag/PET ultrathin tape. Specifically, to combine the thin film technology (roll-to-roll, R2R, based process) and injection molding (batch process), the thin film was converted into label







Figure 3. Scheme of R2R thin film manufacturing including a) Shim manufacturing, b) R2R embossing, c) PVD coating of Ag, and d) adhesive coating, which is applied to enable an easy assembly of converted labels e) to the injection molded flow cell.

at KURZ Group. Therefore, first a cylindrical shim was manufactured for R2R replication. Then negative structures on the shim were embossed into a resin which is coated on PET carrier film. The Ag coating was deposited via physical vapor deposition (PVD). The final step included adhesive coating on the backside and die cutting, which is called converting, to enable a simple lamination process of the final thin film to the injection molded microfluidic chip, as shown in Figure 1b.

# 3. Design and Rationale for the Plasmonic Enhancement of the Ag/PET Pattern and SERS Effect

The dominant contribution to the SERS effect is the electromagnetic enhancement mechanism produced by LSPRs in metallic nanostructures.<sup>[2]</sup> In order to design an Ag array supporting LSPRs in the visible spectral range, we designed as proof-of-concept the structure sketched in **Figure 4a**. It consists of an ultrathin (50  $\mu$ m) and flexible nanopatterned PET surface with a 250 nm period bidimensional grid of cubes with size 125 nm covered by a 50 nm ultrathin layer of Ag, as shown by the scanning electron microscopy image (SEM) of the fabricated SERS substrate reported in Figure 2d. The 50 nm thickness assured a continuous film with good adhesion to PET. This design of the SERS substrate was input to electromagnetic simulations using the FDTD method implemented in the commercial software FDTD Lumerical

(see Experimental Section). Figure 4b shows the calculated reflectance for normal incidence k and electric field E polarization parallel to the cubes array (see Figure 4a). The reflectance spectrum shows a minimum at 2.7 eV (460 nm) due to the excitation of LSPRs on the periodic nanopattern. The near-field enhancement  $(EF = |E|^2/|E_0|^2)$  maps at 2.7 eV are shown in Figure 4c–f, plotted at different planes of the nanostructure such as in the plane containing k and E (Figure 4c), and several planes parallel to the substrate but at different heights (i.e., z = 0, z = h/2, and z = h, being *h* the height of the cubes). In our case,  $|E_0| = 1$ , therefore, for now on and for simplicity we will just use  $EF = |E|^2$ . Those near-field enhancement maps (see Figure 4c-f) show that the maximum near-field enhancement regions (hot spots) are at the edges of the cubes, consistently with the lightning rod effect,<sup>[24]</sup> reaching a maximum value of the near-field enhancement  $|E|^2$  of 30. The maps clearly show that the hot spots where R6G or CV molecules could sense an enhancement of the electric field upon adsorption to result in a very high SERS EF account for a portion of the total area of the SERS substrate. This makes statistically difficult that target molecules adsorb exactly at the hot spots.

Side views in Figure 4c indicate that the probability that the target molecule experiences a high  $|E|^2$  increases if we consider also the gaps between consecutive cubes in the array. In these regions, where cooperative effects between LSPRs excited in adjacent cubes are produced, there is also a high near-field enhancement, whose magnitude also depends on the *z*-displacement. As shown in Figure 4d–f, the EF decreases when going down







**Figure 4.** Design and electromagnetic characterization of the SERS substrate. a) Sketch of the design SERS substrate. The green and blue arrows indicate the wavevector (*k*) and electric field polarization vector (*E*) used in the electromagnetic simulations. b) Calculated reflectance spectrum of the studied system. The minimum at 2.7 eV is attributed to LSPRs excited nanostructures. c,g) Near-field distribution map over the nanostructures excited with a photon energy (2.8 eV) coincident with the dip appreciated in (b) on different planes: c) in the plane containing the incident beam wave vector and polarization, and in planes parallel to the substrate d) at z = h (interface with the substrate), e) z = h/2, and f) z = 0. Complex dielectric function  $\langle \varepsilon \rangle = \langle \varepsilon_1 \rangle + i \langle \varepsilon_2 \rangle$  spectra measured on g) a flat and h) nanopatterned region of the Ag on PET film.

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parallel to the *z*-direction from the top surface of the cubes toward the substrate.

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The experimental optical response of the SERS substrates was investigated by spectroscopic ellipsometry (SE). Ellipsometric spectra were acquired both at a flat region of the Ag/PET (Figure 4g) and at the nanopatterned area (Figure 4h). The complex pseudodielectric function  $\langle \varepsilon \rangle = \langle \varepsilon_1 \rangle + i \langle \varepsilon_2 \rangle$  spectra of the Ag/ PET were fitted using Ag optical constants from the literature assuming a surface roughness of 2 nm.<sup>[25]</sup> Whereas the real part,  $\langle \varepsilon_1 \rangle$ , shows a good fit, the imaginary part,  $\langle \varepsilon_2 \rangle$ , shows deviations from the fitting curve in the spectral region below 3 eV due to the scattering produced by the roughness of the Ag/PET film. Conversely, the imaginary part,  $\langle \varepsilon_2 \rangle$ , of the pseudidielectric function spectra of the nanopatterned area shows peaks at 1.5 and  $\approx$ 2.7 eV. The more prominent peak at  $\approx$ 2.7 eV can be attributed to the LSPR produced in the array, showing a very good agreement with the electromagnetic behavior predicted by the FDTD simulation (see Figure 4b). The peak at lower energy can be attributed to LSPR originated from the inhomogeneous granular texture of the deposited Ag film that can be appreciated in Figure 2. Above 3.5 eV both the Ag unpatterned film and the array are characterized by the Ag interband transitions. In Figure 4h, several spectra measured in different points of the array (with a microspot) are shown, indicating a high reproducibility of the main LSPR peak. On the other hand, the good stability of the Ag/PET was checked by chemical analysis performed by X-ray photoelectron spectroscopy that indicated that there was no significant oxidation of the Ag film (see Figure S1, Supporting Information).

In order to present the versatility of the design, as well as the impact of the geometry on the electromagnetic enhancement EF, **Figure 5**a shows that, by tailoring the size of the cubes and the period of the array, the plasmonic resonance can be tuned to longer wavelengths. As the size of the cube, *h*, is increased along with the period of the array, *P*, the LSPR is redshifted. This has also been confirmed experimentally by measuring the plasmon resonance by SE of gratings with a period of 300 and 350 nm, showing the LSPR at  $\approx$ 1.9 and 2.25 eV, respectively (see right  $\langle \varepsilon_2 \rangle$  plot in Figure 5a) in agreement with the simulations. The broader experimental resonance peaks, again, can be ascribed to the some imperfection of the grating geometry as also shown in Figure 2d.

Figure 5b-e shows equivalent near-field maps as in Figure 4c-f but for a nanopatterned array with a size of the cubes of h = 175nm and period P = 300 nm resonant in the visible (at 2.3 eV/ 540 nm). Comparing this geometry with the one in Figure 4 (h = 125 nm and period P = 250 nm), it can be inferred that the EF distribution is very similar, but the maximum EF at the hot spots is one order of magnitude higher ( $\approx$ 300 vs previous  $\approx$ 30). Nevertheless, although the maximum EF is higher, it is worth noting that the surface density of hot spots (i.e., the ratio of the hot spot area to the total surface area of the array) is lower, therefore reducing the probability of a target molecule being adsorbed at the hot spots. For instance, if we consider a laser spot of  $1 \,\mu\text{m}^2$ , for the  $P = 300 \,\text{nm}$  array, 9 cubes are illuminated by the laser spot, while for the P = 250 nm array, 16 cubes are excited. Therefore, in considering the EF for SERS, two competing effects must be considered, i.e., the electromagnetic enhancement at the hot spots but also the density of the hot spots and their accessibility to molecules. On one side, we have shown that tuning the resonant wavelength to the green-red where most

commercial Raman instruments operate leads to an increase in the EF at the hot spots. On the other side, increasing the period to move the resonances to higher wavelengths reduces the density of hot spots, thereby reducing the probability of target molecules being absorbed at hot spots.

As proof-of-concept of the SERS effectiveness of the designed Ag/PET substrate, a droplet of 100 nL of R6G 1 µM has been deposited on both the flat and nanopatterned areas of the Ag/ PET film. The Raman and SERS spectra with excitation wavelengths of 633 nm (red laser: 1.95 eV) and 479 nm (blue laser: 2.63 eV) were taken from both the Ag and PET sides for the patterned and unpatterned areas, as shown in Figure 6. For a relative comparison of spectra, they were taken sequentially under the same experimental conditions to avoid/minimize perturbation from ambient conditions. Noteworthy, the blue laser excitation wavelength is resonant with the energy of the LSPR peak in the  $\langle \varepsilon_2 \rangle$  spectrum of Figure 4h and the simulation in Figure 4b. The measured SERS spectra are shown in Figure 6 along with the ordinary spectra (OR) of R6G (see also Figure S2 and S3, Supporting Information) and the PET substrate. The list of the R6G Raman bands observed in the OR spectra as well as in the SERS spectra is reported in Table S1, Supporting Information.

When measuring in the Ag pattern from the Ag side (configuration 1 in Figure 6 and S4, Supporting Information), very intense Raman spectra with sharp R6G lines were acquired with the blue laser (479 nm) resonant with the LSPR peak. Conversely, spectra of R6G measured with the off-resonance red laser (633 nm) show orders of magnitude lower intensity. This excitation wavelength dependence supports the electromagnetic enhancement produced by the excitation of the LSPRs in the substrate upon blue laser irradiation. When measuring in the unpatterned Ag film from the Ag side (configuration 2 in Figure 6 and S4, Supporting Information), the resonant blue laser results in R6G Raman signals at least two order of magnitude less intense than in the Ag patterned area, and the signal recorded is probably due to the electromagnetic enhancement produced at the inhomogeneous granular texture of the deposited Ag film; conversely, in the unpatterned Ag film, no Raman bands of R6G were observed with the off-resonance red laser.

Interestingly, even when probing the Ag pattern from the PET side (configuration 3 in Figure 6 and S4, Supporting Information), a SERS effect is observed, although the enhancement is lower due to the decay in intensity of the electromagnetic film through the PET thin film and back reflection at the PET/Ag interface. Therefore, it is important to have a thin plasmonic film (like the thin Ag/PET in the present case) to probe signal through it. Thus, Figure 6 (and Figure S4, Supporting Information) clearly demonstrates a SERS effect at  $\approx$ 2.7 eV produced by hot spots at the Ag/PET array when illuminated at resonance (blue laser), consistently with previous electromagnetic modeling and ellipsometric measurements of the Ag LSPR. Noteworthy, no functionalization of the Ag array has been applied in this case (differently from the various functionalization procedures in colloidal gold nanoparticles with capping agents replaced by functionalized layer of polyethylene glycol [PEG] or similar), simplifying thereby the overall fabrication and use of the Ag/PET MF-SERS chip.

We have calculated a SERS enhancement factor (EF)<sup>[26]</sup> in the  $10^{5}$ – $10^{6}$  range by comparing the intensity of the Raman signal at 1650 cm<sup>-1</sup> obtained dropping  $\approx 100$  nL of 1  $\mu$ M R6G on the Ag-





**Figure 5.** a) Calculated reflectance spectra (left plot) and experimental reflectance ellipsometric spectra (right plot) of the pseudodielectric function,  $\langle \varepsilon_2 \rangle$ , showing the tunability of the plasmon resonance peak. b) Near-field distribution map over the nanostructures excited with a photon energy (2.3 eV-540 nm) coincident with the dip in reflectance appreciated in (a) on different planes for the structure with P = 300 nm and h = 175 nm: c) in the plane containing the incident beam wave vector and polarization, and in planes parallel to the substrate at c) z f (interface with the substrate), d) z = h/2, and e) z = 0.

array and on a bare PET substrate. The SERS EF in the  $10^5-10^6$  range can be explained by the electromagnetic near-field enhancement maps in Figure 4c, being most likely an averaged

value in the area probed by the laser spot, considering that the R6G molecules could adsorb on the large top surface of the cube as well as in the dip and along the sidewall of the cubes. This

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**Figure 6.** Ordinary spectra of a) Rhodamine 6G and (c) PET. b) Raman spectra of R6G measured with 472 nm blue (blue lines) and 633 nm red lasers (red lines) on the Ag patterned area (configuration 1), on the Ag unpatterned film (configuration 2) and on the patterned region from the PET side (configuration 3) as indicated by the corresponding sketches. Dotted lines indicate R6G Raman band that clearly appear in the SERS spectra on Ag patterning and cannot be confused with any of the PET bands.

heterogeneity and dimensions of the plasmonic hot spots affects the average EF. Specifically, it has to be considered that SERS EF as high as 10<sup>8</sup> has been reported at the center of hot spots<sup>[26]</sup> and that in the framework of the  $|\mathbf{E}|^4$  approximation, the SERS enhancement decays drastically with the increase in distance from the peak value at the corner hot spots.

We also point out here that those SERS EF values are meant as proof-of-concept of the whole industrial fabrication process and readout through the PET film, which is highly reproducible on several points and from several fabricated microfluidic chips from different fabrication batches, as discussed more in the next paragraph; they are not meant to represent any record of EF, which could be improved by the array design, which is ongoing customized work.

# 4. SERS Measurements in the Channel of the Assembled Microfluidic Chip

SERS R6G has also been probed in the assembled microfluidic chip by filling it with the 1  $\mu$ M R6G aqueous solution as shown in Figure 7a. The red rectangle visible at the center in the figure corresponds to the Ag/PET array. Measurements with a







**Figure 7.** SERS measurements in the channel of the assembled microfluidic chip. a) R6G solution injected in the assembled flow cell. The dark-red rectangle area corresponds to the plasmonic Ag nanopatterned area. b) Sketch of the measurement configurations from both the COP and PET side, and in and out the Ag array; with c) the  $\times$ 50 LWD and d)  $\times$ 100 microscope objectives. Spectra measured with the e)  $\times$ 50 LWD and f)  $\times$ 100 microscope objectives on the patterned (blue lines) and unpatterned (green lines) areas of the Ag/PET film from the COP and PET sides. The OR spectra of R6G are also shown for comparison. The dotted lines highlight all the R6G Raman bands observed in the SERS spectra. For all measurements, a 474 nm excitation wavelength was used.



479 nm laser in various configurations have been performed: measurements in and out the Ag array through both the thick COP side and the thin PET side and using  $a \times 50$  long working distance (LWD) microscope objective and  $a \times 100$  microscopy objective as shown in Figure 7b–d.

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The SERS enhancement in the Ag/PET array can be clearly seen when comparing measurements of R6G in and out Ag pattern (compare blue-in Ag pattern and green-out Ag pattern spectra). We have also considered effects of measuring through the thick COP and the thin PET sides. When acquiring spectra with a standard ×100 objective, SERS enhancement of the R6G Raman peaks is observed only by the thin PET side while measuring from the COP side results in just the COP Raman signature as light only probes COP not reaching the Ag array as shown in the sketch of Figure 7d. Therefore, the importance of developing ultrathin Ag/PET film technology is demonstrated as the working distance of the ×100 microscopy objective cannot sample the plasmonic array through the COP layer. When using  $a \times 50$  LWD microscope objective (see Figure 7c), excitation of the Ag array from both COP and PET sides can be fulfilled. Nevertheless, by comparing ×50 LWD measurements from the COP and PET sides without the background subtraction (see Figure S5, Supporting Information), it can be noted the higher background fluorescence of COP interfering with the R6G signal. The ratio of the Raman peak intensities to the fluorescence background signal is lower when measuring from COP side, especially above 1000 cm<sup>-1</sup>, thus increasing the possibility of the COP fluorescence background overshadowing low Raman peaks coming from probed molecule. Therefore, the ultrathin Ag/PET films provide the advantages of a SERS enhancement with a reduced fluorescence background. Noteworthy, contrary to the  $\times 50$  LWD,  $\times 100$  microscope objectives are standardly mounted in commercial Raman setups, posing an additional advantage to choosing the ultrathin Ag/PET for the SERS measurements.

The spatial stability of the SERS signal for the microfluidic chip has been demonstrated by measuring 40 random points at the Ag array of  $2.5 \text{ mm} \times 8 \text{ mm}$  area and at the Ag

unpatterned channel as shown in **Figure 8**. The stability of the SERS signal and the very low standard deviation (the shadowed area in Figure 8c) in the Ag array proves the reproducibility of the averaged SERS signal and EF factor.

As for the stability and reliability of the manufactured SERS microfluidic chips, we have analyzed randomly ten slides from various fabrication batches both after fabrication and after 7 months to check the time stability and any aging effect. Over ten microfluidic chips, a variation of the intensity peak at 617 cm<sup>-1</sup> (with a negligible fluorescent background) of  $\pm 2\%$  has been found. The SERS signal of 10  $\mu$ M R6G did not change significantly after 7 months, with an EF still >10<sup>5</sup>, proving the evidence that the Ag /PET MF-SERS chip is stable for months.

Being the rapid and high-sensitivity detection and identification of poisonous substances in the environment one of the most important tasks for SERS analysis, we have tested our SERS microfluidic chip for detection of  $10^{-12}$  M CV using MF-SERS chips produced 7 months ago. The SERS CV spectra acquired in ten random point of the Ag pattern are shown in Figure 9. The thick line is the mean value over ten measurements, with the shadow being the plus/minus standard deviation. The assignment of the CV Raman bands is in Table S2, Supporting Information. Also in this case, no signal was measured in the unpatterned microfluidic channel, while the measured signal in the Ag array gave an EF in the range  $10^5-10^6$ . Noteworthy, this level of signal and EF for the CV would allow to still lower the detection limit of CV approaching the fM detection of CV achieved so far only in laboratories fabricated complex SERS substrates such as composite mesh of silver and gold nanoparticles.<sup>[27]</sup>

# 5. Conclusions

We have shown that the combination of roll-to-roll thin film technology with injection molding enables the industrial fabrication of customizable SERS microfluidic chips. The thin film technology presents advantages in the production of



**Figure 8.** Statistical SERS measurements of R6G in the assembled MF-SERS chip. SERS spectra of  $10 \,\mu$ M R6G injected in the assembled microfluidic chip. a) Spectra taken in 40 random points at the Ag array in the microfluidic channel; y-scale is 15 000 with a D2 filter. b) Spectra taken in 40 random points of the unpatterned area inside the microchannel: no R6G is detected; y-scale is 100. c) Mean value of the SERS intensity over the 40 taken spectra of R6G on the Ag patterned and unpatterned regions; the shadow is the mean plus/minus the standard deviation, demonstrating the good reproducibility of the signal.





**Figure 9.** Statistical SERS measurements of CV in the assembled microfluidic chip. SERS spectra of  $10^{-12}$  M CV injected in the assembled microfluidic chip. Spectra have been taken in ten random points of the Ag plasmonic pattern; the shadow is the mean plus/minus the standard deviation. The line shows the absence of CV signal in any region out of the Ag plasmonic array.

flexible nanopatterned Ag/PET films with Ag arrays of any customized design that can be externally coupled to any microfluidic chip to be used in any commercial Raman system, resulting in a plasmonic resonance that can be tuned by the grating design. We have demonstrated by experiment and design that Ag nanopatterned tapes can be tailored to various plasmonic and SERS frequencies and, therefore, customized to specific needs to match specific target absorbing molecules or resonant laser on available Raman systems. In the present case, the Ag/ PET pattern assembled in the microfluidic chip presents a plasmon resonance at  $\approx$ 2.7 eV, which induces electromagnetic enhancement and hot spots upon blue laser excitation. The plasmonic thin Ag/PET films proposed here, when assembled in the flow cell, helps to simplify the measurement setups decreasing possible working distance to the molecules of interest while reducing the interference from the COP or PET background fluorescence. The averaged electromagnetic enhancement results in a reproducible and stable SERS signal enhancement in the range  $10^5-10^6$  as demonstrated by detection of R6G and CV probe molecules, with detection of  $10^{-12}$  M for the CV. The SERS microfluidic chips have been demonstrated to have a reproducibility better than 90% in terms of samples random points with large-area uniformity on all the SERS area of  $2.5 \text{ mm} \times 8 \text{ mm}$ , and to be stable over 7 months so far. Therefore, this approach of SERS microfluidic chips can provide an industrially viable customizable and cost-effective route toward disposable, point-of-care diagnostic applications.

## 6. Experimental Section

*Electromagnetic Simulations*: We performed FDTD calculations to simulate the electromagnetic response of the proposed SERS substrate. The FDTD method allows us to solve the Maxwell's equations for an arbitrary geometry. In this study, the software FDTD Lumerical 2020a was employed. Periodic



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boundary conditions were used along the x- and y-directions, whereas perfectly matched layer boundary conditions were set in the z-direction. An illuminating linearly polarized plane-wave polarized along the x-direction was set to propagate perpendicular to the substrate (z-direction). A nonuniform mesh was used in the simulation region. A finer mesh was defined in the vicinity of the nanostructures. In this region, the mesh step was fixed to dx = dy = dz= 1 nm. The reflectance spectrum was obtained through a frequency-domain field and power monitor perpendicular to the propagation direction. The nearfield maps were obtained through a frequency-domain field and power monitor containing the propagation and polarization directions. The incident electromagnetic wave was set to have an intensity of  $|E_0|^2 = 1$ .

SE: Ellipsometric spectra were acquired using a phase-modulated spectroscopic ellipsometer (UVISEL, Horiba) in the 0.75–6.5 eV spectral range with 0.01 eV resolution at an angle of incidence of  $70^{\circ}$ .

Raman Spectroscopy: Raman spectra were collected using a LabRAM HR Horiba-Jobin Yvon spectrometer with the 479 and 633 nm excitation lasers to probe SERS effects on the designed substrates. Raman and SERS measurements were run under ambient conditions at low laser power (0.5 mW for 633 nm and 0.2 mW for 479 nm laser) to avoid laser-induced heating and damage.  $50 \times$  long working distance (numerica aperture NA = 0.75) and  $100 \times$  (NA = 0.9) microscope objectives were uesd for the measurements. The Raman band of a silicon wafer at  $520 \text{ cm}^{-1}$  was used to calibrate the spectrometer, and the accuracy of the spectral measurement was estimated to be better than 1 cm<sup>-1</sup>.

# **Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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# **Conflict of Interest**

The authors declare no conflict of interest.

# Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

# **Keywords**

biosensors, flow cells, in vitro diagnostics, injection molding, microfluidic, roll-to-roll, surface-enhanced Raman spectroscopy

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[2] B. Sharma, R. R. Frontiera, A.-I. Henry, E. Ringe, R. P. Van Duyne, Mater. Today 2012, 15, 16.

S.-Y. Ding, J. Yi, J.-F. Li, B. Ren, D.-Y. Wu, R. Panneerselvam, Z.-Q. Tian, Nat. Rev. Mater. 2016, 1, 16021.

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- [3] J. Langer, D. Jimenez de Aberasturi, J. Aizpurua, R. A. Alvarez-Puebla, B. Auguié, J. J. Baumberg, G. C. Bazan, S. E. J. Bell, A. Boisen, A. G. Brolo, J. Choo, D. Cialla-May, V. Deckert, L. Fabris, K. Faulds, F. J. G. de Abajo, R. Goodacre, D. Graham, A. J. Haes, C. L. Haynes, C. Huck, T. Itoh, M. Käll, J. Kneipp, N. A. Kotov, H. Kuang, E. C. Le Ru, H. K. Lee, J.-F. Li, X. Y. Ling, et al., ACS Nano 2020, 14, 28.
- [4] J. Guo, F. Zeng, J. Guo, X. Ma, J. Mater. Sci. Technol. 2020, 37, 96.
- [5] I. Prinz, M. J. Haslinger, M. Mühlberger, G. Reiter, A. Prinz, M. M. Schmidt, T. Schaller, M. Bauer, M. Musso, G. Bauer, *J. Appl. Phys.* **2021**, *129*, 130902.
- [6] F. Tian, F. Bonnier, A. Casey, A. E. Shanahan, H. J. Byrne, Anal. Methods 2014, 6, 9116.
- [7] A. Taguchi, Opt. Rev. 2017, 24, 462.
- [8] T. Deckert-Gaudig, A. Taguchi, S. Kawata, V. Deckert, Chem. Soc. Rev. 2017, 46, 4077.
- [9] M. Fleischmann, P. J. Hendra, A. J. McQuillan, Chem. Phys. Lett. 1974, 26, 163.
- [10] C. S. Allen, R. P. Van Duyne, J. Am. Chem. Soc. 1981, 103, 7497.
- [11] B. Sharma, M. Fernanda Cardinal, S. L. Kleinman, N. G. Greeneltch, R. R. Frontiera, M. G. Blaber, G. C. Schatz, R. P. Van Duyne, *MRS Bull.* 2013, *38*, 615.
- [12] A. R. Ferhan, D.-H. Kim, Nano Today 2016, 11, 415.
- [13] S. Gómez-Graña, C. Fernández-López, L. Polavarapu, J.-B. Salmon, J. Leng, I. Pastoriza-Santos, J. Pérez-Juste, *Chem. Mater.* 2015, *27*, 8310.

- [14] Q. Zhang, Y. H. Lee, I. Y. Phang, C. K. Lee, X. Y. Ling, Small 2014, 10, 2703.
- [15] K. K. Reza, A. A. I. Sina, A. Wuethrich, Y. S. Grewal, C. B. Howard, D. Korbie, M. Trau, Biosens. Bioelectron. 2019, 126, 178.
- [16] N. D. Kline, A. Tripathi, R. Mirsafavi, I. Pardoe, M. Moskovits, C. Meinhart, J. A. Guicheteau, S. D. Christesen, A. W. Fountain, *Anal. Chem.* 2016, *88*, 10513.
- [17] J.-A. Huang, Y.-L. Zhang, H. Ding, H.-B. Sun, *Adv. Opt. Mater.* 2015, 3, 618.
- [18] H. Pu, W. Xiao, D.-W. Sun, Trends Food Sci. Technol. 2017, 70, 114.
- [19] G. S. Fiorini, D. T. Chiu, BioTechniques 2005, 38, 429.
- [20] M. Viehrig, A. H. Thilsted, M. Matteucci, K. Wu, D. Catak, M. S. Schmidt, K. Zór, A. Boisen, ACS Appl. Mater. Interfaces 2018, 10, 37417.
- [21] S. F. Berlanda, M. Breitfeld, C. L. Dietsche, P. S. Dittrich, *Anal. Chem.* **2021**, *93*, 311.
- [22] M. Taniguchi, J. S. Lindsey, Photochem. Photobiol. 2018, 94, 290.
- [23] S. Fateixa, H. I. S. Nogueira, T. Trindade, Phys. Chem. Chem. Phys. 2015, 17, 21046.
- [24] P. F. Liao, J. Chem. Phys. 1982, 76, 751.
- [25] E. D. Palik, Handbook of Optical Constants of Solids, Academic Press, Boston, MA 1998.
- [26] P. G. Etchegoin, E. C. Le Ru, Phys. Chem. Chem. Phys. 2008, 10, 6079.
- [27] X. Gao, H. Zhang, X. Fan, C. Zhang, Y. Sun, C. Liu, Z. Li, S. Jiang, B. Man, C. Yang, *Opt. Express* 2019, *27*, 25091.