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Drop-coating deposition surface-enhanced Raman spectroscopy on silver substrates for biofluid analysis

Maciej S. Wróbel*[a], Zufang Huang[b,c], Soumik Siddhanta[c], Ishan Barman[c, d]

[a]Faculty of Electronics, Telecommunications and Informatics, Gdansk University of Technology, G. Narutowicza 11/12, 80-233 Gdansk, Poland;
[b]Key Laboratory of Optoelectronic Science and Technology for Medicine of Ministry of Education, Fujian Provincial Key Laboratory of Photonics Technology, Fujian Normal University, Fuzhou 350007, China;
[c]Department of Mechanical Engineering, Johns Hopkins University, Latrobe Hall 223, 3400 N Charles St, Baltimore, MD 21218, USA;
[d]Department of Oncology, Johns Hopkins School of Medicine, Baltimore, USA

ABSTRACT

Utilization of surface-enhanced Raman spectroscopy as a measurement technique is of particular interest in biodetection due to its superb chemical specificity and high sensitivity. The use of SERS substrates further improve this method by massive enhancement of the molecule Raman spectrum, permitting very low levels of detection. Therefore it is important to seek for new ways to develop reliable substrates, which are quickly and easily manufactured at a low cost. This paper describes the development of a simple and cost-effective substrate for the SERS detection. The substrate is synthesized from a silver ink on the glass, and its utilization for biodetection is shown. The hydrophobicity of the substrate permits the pre-concentration benefit of the drop-coating deposition, by the formation of the coffee-ring. This allows to achieve lower limits of detection, by effectively measuring areas with higher concentration of measured molecules than the initial sample. However, the different properties of the medium, such as the influence of protein types and amounts, may influence the ring formation mechanics, thus effectively changing the pre-concentration of the target analyte.

Keywords: surface-enhanced Raman spectroscopy, nanotechnology, metrology, biophotonics, biofluid analysis, drop-coating deposition.

1. INTRODUCTION

Analysis of biological fluids is of utmost importance in a medical setting, especially for the detection of illicit substances[1] and disease biomarkers[2,3]. It is often crucial for the well-being of the patients to monitor their blood drug levels or levels or continuous monitoring of blood molecules, such as glucose[4,5]. The surface-enhanced Raman spectroscopy has emerged as one of the most promising tools for this purpose due to its superb chemical specificity and high sensitivity. The emergence of reproducible materials has enabled the use of SERS due to the high enhancement factors achievable with highly engineered nanomaterials, in the form of nanoparticles[6,7] or as substrates[8–11].

Both approaches to achieve SERS are vastly described throughout the literature. Nanoparticles are being favored when higher enhancement is needed due to high aggregation of nanoparticles creating many hot-spots causing massive EM enhancement. The substrates excel in uniformity due to precise nanofabrication, but offer usually lower enhancement factors and are often much more expansive. In the case of nanoparticles, the sample loading is achieved by simply spiking the nanoparticle solution with the sample to be tested, and introduction of thus prepared sample under the optics of the Raman spectrometer. In case of the substrates, however, the simpler act of sampling of the investigated specimen is vastly different. Many effects can happen during the sample loading, some of which can be utilized for the benefit of the method.

*maciej.wrobel@pg.edu.pl; phone: +48 58 347 2482

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The evaporation of the sample may cause two vastly different effects due to the difference in the substrate wetting angle, that is whether the substrate is hydrophilic or hydrophobic. In the first case, the drop of the sample is spread evenly over the area of the substrate, and the various molecules form mostly uniform layer over the area. In the latter case, the so-called coffee-ring pattern emerges[12]. It originates from the capillary flow due to different evaporation rates over the drop, with the ring drying faster, and the molecules from the center of the ring flow outwards, eventually forming a ring-pattern.

The benefit of such effect for the SERS can be the pre-concentration of the analyte in the ring area. Multiple layers of the molecules overlaps on the small area of the substrate, which can be the point of measurement by the probe. This means that the effective number of molecules in the investigated volume is much larger than in the original sample. The difference in composition of the sample could greatly influence the dynamics of ring creation due to the change in wetting angle of the sample on the substrate, and the formed protein layers. Thus the problem of this technique is the formation of coffee-ring in a more complicated, especially biological environments, such as blood plasma.

This paper presents the development of a silver-coating on glass surface utilized as a SERS substrate. With the added benefit of its hydrophobicity, to exploit the coffee-ring effect as a method for pre-concentration of the analyte. The substrate fabrication is presented, as well as its material properties, and evaluation of its performance is presented in combination with the drop-coating deposition. This substrate is intended to be used for the detection of drug molecules in a human plasma.

2. MATERIALS AND METHODS

The fabrication of the substrate is quick and simple, and the total time of fabrication is about 30 minutes. We have optimized the fabrication method to obtain a coverage of a whole standard 22 x 22 mm glass microscopic coverslip, but other materials as well as larger substrates can be easily utilized as well. First, the Ag-ink stock solution[13] is prepared, by mixing the isopropyl alcohol with the precursor silver ink, and then sonication for 15 min in the dark under ambient conditions. Subsequently, specific concentrations of the Ag-ink solution were created by diluting the stock solution with required volumes of isopropyl alcohol. To achieve the uniform coating of silver nanoparticles (AgNPs) on glass substrate, 30 μL of Ag-ink solution was pipetted onto the coverslip and spun for 3 min. at the speed of 6000 rpm, then heated inside the oven at 135°C for 10 min. The annealing of the substrate causes the ink to form a multitude of nano-islands, which give rise to the SERS effect.

The most important parameter characterizing the SERS substrate is the position of its plasmon resonance. Typical plasmon resonance for silver is about 400 nm, with a notable red-shift of the resonance with the bigger size of the nanoparticles[14]. The absorption spectra of Ag-ink coverslip were recorded on a Perkin-Elmer Lambda 950 UV/Visible spectrometer.

The SEM images of AgNPs formed on the Ag-ink treated glass substrate were acquired with field-emission scanning electron microscopy (JEOL, 6700F SEM) at an accelerating voltage of 10kV. before the measurements, the substrate was coated with a thin layer of gold, due to poor conductivity of the sample. The contact angles were analyzed using a goniometer (Ramé-Hart, Inc., Model 200).

The surface-enhanced Raman spectra were recorded on a Raman spectrometer with CCD camera (PIXIS, Princeton Instruments) the 1/8f imaging spectrograph (Holospec, Kaiser) with a VPH grating and a fiber-optic probe (EmVision, LLC.) with large collection area, and a 830 nm excitation laser. The use of a large collection area probe allowed to average the signal over the probed area.

The primary experiments were carried out on the substrates to assess their viability for the SERS enhancement, as well as to investigate the hydrophobicity of the substrate to allow for the coffee-ring formation on aqueous samples as well as blood plasma. The common test molecule for SERS studies, the Rhodamine 6g was utilized in this study. A drop-coated sample was placed on the substrate, forming a drop on the surface with a high contact angle. This was allowed to dry, after which the ring formation was observed. The substrate was measured at three most important areas, that is in the middle of the formed ring structure, labeled as 'edge', at the center of the drop labeled 'center', and in a midpoint between the two, labeled 'middle'. Comparison of the signal strength between these areas allows to prove the benefit of measuring the pre-concentrated molecules at the coffee-ring.

3. RESULTS

The material parameters of the fabricated substrates are shown in the figure 1. The SEM image (fig. 1a) presents the surface morphology of the substrate, where a multitude of nano-islands are clearly seen. The variation of shapes and sizes of the islands gives rise to a very broadband UV-VIS spectrum (fig. 1b) peaking at about 420 nm and extending further beyond the VIS into the NIR range. This characteristics makes the substrate a good candidate for a multi-wavelength substrate, capable of enhancement in the whole typical range of most commercially available Raman systems. The gray area marked in the figure encompasses the range of wavelengths at which the Raman-scattered light is emitted, when excited with an 830 nm laser source, as used in this study. The existence of quite uniform plasmon resonance at these wavelengths indicates that the emitted signal will be also almost uniformly enhanced in the whole range it covers.

Additionally, the contact angle measurement is shown as an inset, presenting the angle of 103°, which confirms that the substrate is indeed hydrophobic. This feature is crucial for the formation of the coffee-ring pattern, utilized further in the study.

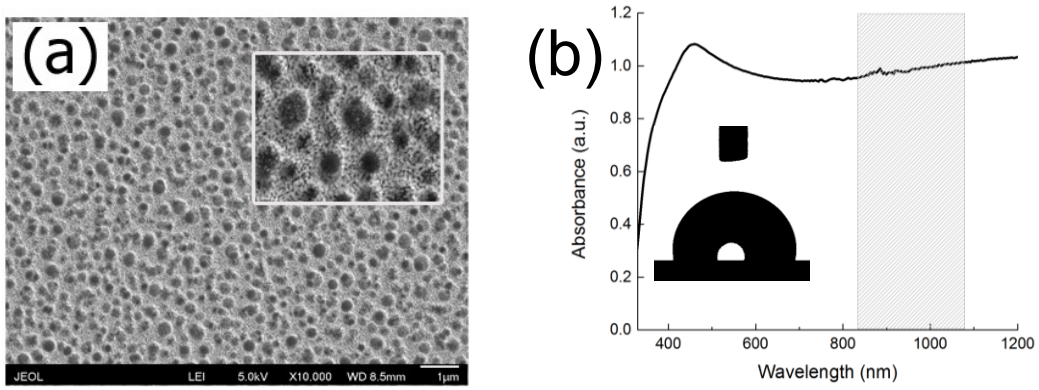

Figure 1. The SEM image of the substrate area with zoom-in (a). UV-VIS spectra with the gray area showing the and emission spectral region at 830 nm excitation, the inset shows the contact angle 103° (b).

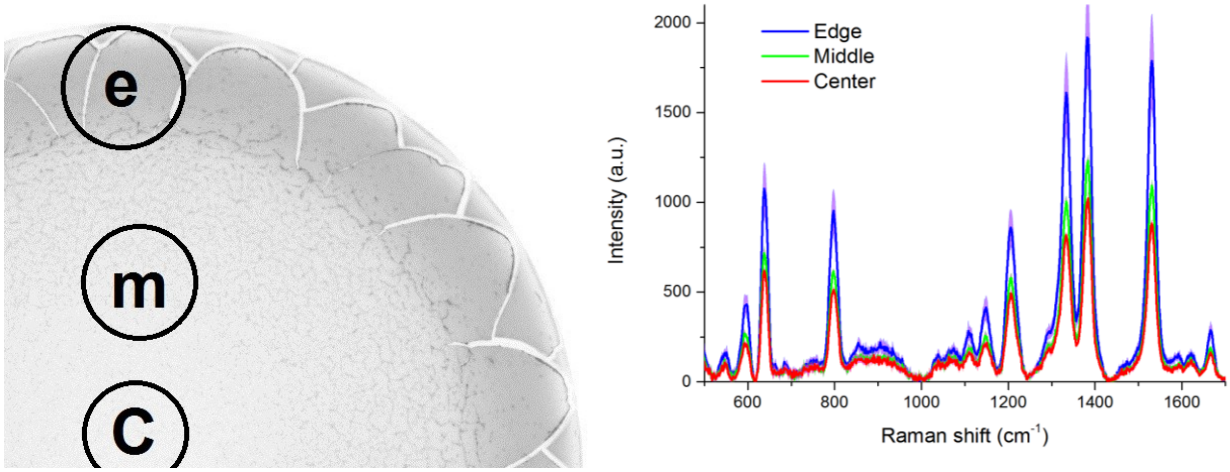

Figure 2. (left) The optical image of the blood plasma formulating a coffee-ring, the edge center and middle areas are indicated. (right) The resulting SERS spectra of R6G on the same substrate recorded for different areas as indicated on (a).

The figure 2. shows the formation of the coffee-ring of human plasma recorded under microscope, and the resulting SERS spectra of the test R6G molecules deposited on the substrate and measured at different spots (centre, middle, edge) of the dried area. The results indicate almost doubling the amount of signal counts for the edge measurement as compared to the central area. There is a small increase in signal in the middle point, of about 15% more than the edge signal. Thus, the molecules forming the coffee-ring are spread out from the middle towards the edge, forming a thin layer gradually increasing in thickness until the edge of the ring. At the ring a dense monolayer of the molecules is formed, thus confirming the pre-concentration benefit. It is worth to note, that even though the layer in the middle is thin, it is readily enhanced by the substrate, due to the close contact between the molecules and the metallic surface. The upper layers at the edge are much further from the surface, so even though the layer seems to be much thicker than at the centre, yet the signal is only doubled in strength. Such non-linear, or logistic, dependence of signal strength on the concentration may cause some additional challenges for the establishment of good calibration models for such types of substrates.

4. CONCLUSIONS

The results prove that the molecules have formed a densely packed ring, and that a much thinner layer still exists at the central location. This indicates the possibility of utilization of the silver ink-coated substrates for a NIR excitation wavelength SERS biomolcule detection on a hydrophobic substrate surface. The additional benefit is the drop-coating deposition resulting in the coffee-ring effect, which enables the use of this substrates for a pre-concentration of the analyte at the edge of the ring. The substrates are to be later utilized for a drug detection in human plasma, which is a difficult media for detection due to the multitude of other molecules, proteins etc. which influence the coffee-ring formation.

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