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Efficient SERS substrates based on laterally ordered gold nanostructures made using interference lithography

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Abstract. Laterally ordered gold nanoisland arrays made using interference lithography has been investigated in this work as SERS substrates. The developed substrates demonstrate performance in enhancing Raman signal of common dye analyte (crystal violet (CV)) comparable to commercial SERS substrates, with the lowest detectable concentration of CV ~10⁻⁶...10⁻⁷ M. They can be reused after heat treatment at 300 °C without any drop of performance. Furthermore, we found a principal difference in the effect of annealing of SERS substrates before deposition of the analyte molecules and additional *in situ* annealing (with molecules) on the intensity of Raman signal. Possible physical origin of the latter effect has been discussed.

Keywords: SERS, gold nanostructures, interference lithography.

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1. Introduction

Over the recent decades, surface-enhanced Raman spectroscopy (SERS) has been intensively developing and has proved itself as effective analytical method. Due to its capabilities, it finds its application in chemistry, materials science, medicine, biology, pharmacology, ecology, forensic science and other fields [1-7]. The examples of its particular use are measuring blood sugar level [2], early diagnostics of cancer [3], detection of pesticides in water, soil and vegetables [4], as wells as of air contamination [5], *etc.* [6, 7].

Using SERS allows analysis and detection of ultralow quantity of molecules (down to single molecule detection) in highly diluted solutions mixed with plasmonic nanoparticles (NPs) or when deposited in a single or sub-monolayer on nanostructured metal substrates [8, 9]. SERS, as well as traditional Raman spectroscopy, is a non-destructive express technique that allows identification of the chemical composition of substances and their molecular structure. It also allows investigation of photochemical reactions under laser irradiation, as well as numerous other *in situ* and *in operando* studies of physical and chemical processes in functional micro- and nanostructures, and (micro)devices.

The theory explains the emergence of SERS effect based on two mechanisms: electromagnetic and chemical ones [10]. The first mechanism is due to the collective oscillations of electrons (localized surface plasmons) in metallic nanostructures that create an electric field near the surface, enhancing Raman scattering from molecules or semiconductor nanostructures deposited on this surface. The second mechanism involves formation of chemical bond between the molecules and metallic nanostructures that involves resonant energy or charge transfer between the molecules and metal, subject to excitation by laser light. In case of charge transfer, in particular, the increased intensity of the Raman scattering by molecular vibrations is caused by a change in the polarizability of molecule.



Fig. 1. AFM image of initial SERS substrate obtained using the interference lithography method. Inset: enlarged image of a single island.

The effectiveness of the SERS effect depends on morphology (form-factor) of the nanostructured metal surface, parameters of the exciting laser radiation, properties of the investigated substance and conditions of its deposition, as well as on the distance between the molecule and metal surface [11].

For effective application of SERS, affordable and reproducible SERS substrates are needed, usually designed as a thin nanostructured film of gold or silver on Si or glass substrate [12-17]. Further requirements to SERS substrates are as follows: significant enhancement of the Raman signal from the substances deposited on them, the stability of the enhancement over time and their lateral homogeneity.

Most of the technologies used to fabricate the ordered nanostructures, such as electron-beam lithography and molecular-beam epitaxy [18], are expensive, making these substrates economically not attractive. Therefore, substrates made using such techniques are produced in limited quantities and are intended solely for scientific research.

A promising alternative can be provided by the technique based on interference lithography. Therewith, the produced SERS substrates are featured by lateral ordering of nanostructures, reproducibility, relatively low production cost, the possibility to control the size and shape of nanostructures, as well as the ability to choose a metal, usually gold or silver [19, 20]. It should also be noted that SERS substrates made according to this method can serve as patterns of nanostructures, which in the long run will accelerate their commercialization and reduce the cost of manufacturing other substrates.

Along with the above practical aspects for this type of nanostructures, there are a number of physical problems, the solution of which will improve the efficiency of the SERS substrates and brings deeper understanding of physical processes behind SERS effect. Particularly, the relationship between the degree of morphological homogeneity of plasmon nanostructures, the sharpness of the plasmon resonance, and the magnitude of the SERS enhancement.



Fig. 2. AFM image of the same SERS substrate as in Fig. 1 but after annealing at 420 °C. Inset: enlarged image of a single island.

2. Experimental

Fabrication of SERS substrates. SERS substrates with gold nanostructures were prepared by successive thermal vacuum deposition of 3-nm-thick Cr adhesive layer, a 20...120-nm-thick Au layer and As₂S₃ photoresist layer onto polished glass substrates at the residual pressure $2 \cdot 10^{-5}$ Pa. The photoresist thickness was varied from 50 to 300 nm between the samples. Raman/SERS results described in this paper were obtained on the sample fabricated with 55 nm gold layer and 50 nm photoresist layer, which were found to be optimum combination to form the most efficient SERS substrates. The recording of periodic structures on chalcogenide films was carried out using the interference pattern formed with a He-Cd laser ($\lambda = 442$ nm) at the exposition value of 0.3 to 0.75 J/cm². Two-dimensional periodic structures were formed by double exposure with rotation of the sample between expositions by 90° around the normal. After exposure, the samples were chemically treated in nonwater alkaline organic solutions to form a resistive mask in photoresist layer, through which the metal film was etched. After removing the photoresist residues in alkaline solution, washing and drying, the metal periodic structure was obtained. A part of substrates obtained in this way were subjected to thermal annealing (in vacuum) at 420 °C for 5 min. This technology of interference lithography based on vacuum chalcogenide photoresist is described in more detail in previous works [19, 20].

Characterization methods. The spatial frequency of the periodic structures was determined using the optical stand equipped with the goniometer G5M providing the measurement accuracy close to ± 5 line·mm⁻¹. Morphology of SERS substrates was studied using atomic force microscopy (AFM, Dimension 3000 Scanning Probe Digital Instruments Inc., Tonawanda, NY, USA), scanning electron microscopy (SEM, Tescan Mira 3 LMU). Optical absorption spectra were measured by doublebeam UV-VIS-IR Shimadzu UV-3600 spectrophotometer. Raman spectra were excited with 532-nm solidstate laser and registered by double monochromator MDR-23 (LOMO) equipped with TE-cooled CCD detector (Andor).

3. Results and discussion

The performance of SERS substrates crucially depends on the form-factor (morphology) of the metal nanostructure, since it determines the optical properties of the substrates. The morphology of the laterally ordered array of nanostructures formed in this work by interference lithography was investigated using the AFM and SEM methods. Fig. 1 shows the AFM image of the as-prepared SERS substrate being a laterally squareordered array of Au nanoislands with an average height of 55 nm and lateral dimensions at the base of 200 nm and 150...200-nm distance between the islands. The period of this array is 296.6 ± 0.5 nm (spatial frequency $v = 3372 \text{ mm}^{-1}$). Interestingly, the islands have not a single apex but a "crone" with 4 to 7 separate peaks.

In order to improve crystallinity of the as-formed nanoislands, they were subjected to thermal annealing.

Fig. 2 shows the same SERS substrate as in Fig. 1, but after annealing at 420 °C in vacuum. As seen from the AFM images, both the shape and size of islands have been significantly changed after annealing. Their apex becomes rounded, with a single peak, their average height increased from 55 to 67 nm, the lateral dimensions at the base decreased from 200 to 180 nm, and the distance between them increases by 20% (Fig. 3). Moreover, numerous metal connections between the adjacent islands of as-grown sample (Fig. 3a), caused by incomplete photoresist removal, disappear after annealing (Fig. 3b).

The results of SEM studies of the annealed substrates (Fig. 4) make it possible to more accurately estimate the lateral dimensions of the nanostructures, giving ~155 nm, and the distances between adjacent islands $-L_1$ ~140 nm and L_2 ~ 270 nm (see marks in Fig. 4).



Fig. 3. Top-view AFM image (a,b) and set of reprentative profiles of individual islands of the films before (c) and after annealing (d).



Fig. 4. SEM images of nanostructured annealed gold films taken at different magnification.



Fig. 5. Optical absorption spectra of laterally ordered gold nanostructures: 1 -as-prepared, 2 -annealed.

Fig. 5 shows the optical absorption spectra of fabricated nanostructured SERS substrates before (curve *1*) and after annealing (curve *2*). Upon annealing, the plasmon absorption band shifts significantly to the blue, 605 vs 671 nm, and its width gets notably smaller, 128 vs 264 nm. The larger width of the band in the initial sample is obviously due to the large distribution of the size of islands and distance between them, as well as by interconnections between some islands. The latter causes increase in the propagation length of plasmon oscillations in metallic nanostructures, contributing to the scatter of plasmon energies. Finally, a less dense crystalline structure of as-deposited gold is supposed to contribute to the increase of plasmon band width. From sharpening of plasmon resonance, one can expect improvement of the resonant conditions of SERS enhancement from studied molecules.

The enhancement of Raman scattering by the obtained gold nanostructures was tested using crystalline violet (CV) as analyte. The dye molecules were deposited on SERS substrates from solution, by immersing the substrate in a 10^{-6} M aqueous solution of CV for 1 hour. After that they were rinsed with distilled water, assuming that only molecules adsorbed on the surface remain, and dried in atmosphere of air. Fig. 6 schematically illustrates the SERS study of CV molecules on SERS substrates with ordered arrays of golden nanoislands.



Fig. 6. Schematic illustration of SERS spectroscopy of CV molecules.

The reliable Raman signal of CV down to concentrations of 10⁻⁶ M could be detected on the annealed SERS substrates (Fig. 7, curve 1), showing comparable to commercial substrates investigated in the same conditions (Fig. 7, curve 3). Based in Fig. 7 and the enhancement factor of 10^6 of these commercial substrates claimed by the manufacturer (AtoID $^{\text{TM}}$, Lithuania), we can conclude that the enhancement factor for our substrates is of the same order of magnitude. The minor disadvantage of our substrates is the broad band \sim 1100 cm⁻¹ due to Raman scattering from glass substrate. However, because of its large half-width and significant difference of CV bands frequency, it does not substantially affect acquisition and interpretation of the actual SERS spectra of the molecules and can be subtracted as a part of background.

It should be noted that the multi-peak shape of the gold nanoislands apexes in as-deposited SERS substrates (Fig. 1) can be expected to be a source of additional enhancement via "hot spots", as compared to the single rounded apex of the islands after annealing (Fig. 2). However, this factor is obviously compensated by sharpening of the plasmon resonance after annealing, leading to a stronger SERS signal in the pre-annealed structures (Fig. 7).

We further investigated the effect of an additional thermal annealing of the obtained SERS substrates with deposited dye molecules. Comparison of the Raman spectra of such substrates that were pre-annealed at 420 °C before CV deposition (Fig. 8) and not pre-annealed (Fig. 9) gives rather unexpected result. Additional annealing of the pre-annealed substrates leads to disappearance of Raman peaks of CV. In case of not pre-annealed substrates, which did not have measurable Raman signal, CV peaks appear after additional annealing at 100 °C, get their maximum intensity at 200...250 °C and then disappear at higher temperatures (Fig. 9). It is important to note that the maximum signal in this case is 2-3 times higher than that achieved for pre-annealed substrates (Fig. 7).



Fig. 7. Representative Raman spectra of CV molecules deposited on pre-annealed SERS substrate (1), not pre-annealed (2), and on the commercial substrate RandaS from AtoIDTM (3).



Fig. 8. Raman spectra of CV molecules (10^{-6} M) deposited on SERS substrates *annealed* at 420 °C and subjected to additional annealing between 100 to 300 °C after CV deposition.

The above observations indicate a complex effect of temperature on the interaction between the nanostructured gold surface and dye molecule. We can assume that adsorption of molecules on pre-annealed gold nanosurface is weaker than in case of *in situ* annealing of such gold nanostructures *with* molecules. Probably, in the latter case, during restructuring the gold surface, the molecules can find more energetically stable adsorption sites. The sharp decrease in the intensity of the Raman signal from CV molecules after annealing at 250 °C (Fig. 9) is due to thermal decomposition of CV molecules at 215 °C. In case of pre-annealed substrates, with presumably weaker bonding of molecules, we obviously loose them after additional annealing already at the lowest temperature (100 °C, Fig. 8).



Fig. 9. Raman spectra of CV molecules deposited on *non-annealed* SERS substrates subjected to additional annealing between 100 to 300 $^{\circ}$ C, with the step 50 $^{\circ}$ C, after CV deposition.



Fig. 10. Raman spectra of CV molecules deposited on the previously used SERS substrate, which was annealed at 300 °C, at various concentrations of molecules on their surface.

After annealing of the substrates with CV at the temperature 300 °C, the molecules on the sample surface "burn out" completely and the SERS substrate can be used again. To test this, CV molecules were repeatedly deposited by drop-casting onto previously used non-annealed SERS substrates after additional annealing (at 300 °C for 15 min). For this repeated use of substrate, it was possible to register SERS signal from CV molecules down to the concentration of 10^{-7} M (Fig. 10).

Comparing the Raman bands of CV presented in Figs. 9 and 10, one can notice that there is some redistribution of Raman intensity between two bands with the frequencies 1575 and 1607 cm⁻¹. It can be an evidence of chemical enhancement in case of annealing the substrate with CV molecules on it (Fig. 9), due to formation of chemical bonds between molecules and metal nanoparticles. Formation of chemical bonds can

cause resonant energy transfer between molecule and metal nanoparticle, as well as change in polarizability of the molecule and thus the change in the relative intensity of Raman bands related to different vibrations. Besides, there is also some difference in the frequency position of in-plane aromatic C–C vibrations, from 1575 to 1580 cm⁻¹ and 1607 to 1612 cm⁻¹, for annealed and non-annealed substrates, respectively. This effect was noticed by other authors [21, 22], but in their experiment they compare their custom SERS substrates with commercial ones, without any additional thermal annealing, and concluded that "weak interaction occurs between crystal violet molecules and Q-SERS or Klarite, which was consistent to other studies for large dye molecule like to crystal violet" [21, 22].

It should be noted that the efficiency of Raman signal enhancement from molecules on SERS substrates, developed by interference lithography method, can be further improved by optimizing the size and density of nanoislands on the substrate surface and the resonance conditions of localized surface plasmon excitation. Despite slight resonant condition of laser excitation with surface plasmons, we have obtained quite well Raman signal enhancement of CV molecules. After further optimization of morphology, much better detection of minimum concentration of analyte could be obtained. In particular, further improvement of Raman intensity (and thus of the detection level) can be obtained using popular lasers emitting at the wavelengths 633 or 671 nm, which will be much closer to resonant excitation of plasmons in our SERS substrates (peaked at 671 nm before and at 605 nm after preliminary annealing).

4. Conclusions

The morphological, optical and Raman enhancement properties of SERS substrates made using the interference lithography method have been investigated in this work. The resulting nanostructured substrates are laterally square-ordered arrays of Au nanoislands. It has been shown that after annealing, morphological homogeneity of nanostructures is improved, and the maximum of plasmonic absorption band shifts from 670 down to 600 nm. The developed substrates demonstrate performance in enhancing Raman signal of common dye analyte (crystal violet) comparable to commercial SERS substrates. We found opposite effects of an additional in situ (with dye molecules on their surface) annealing at 150...200 °C of initially pre-annealed and preliminary non-annealed substrates, with improvement of the SERS signal only in the latter case. It is assumed that gold adsorption of molecules on pre-annealed nanosurface is weaker than in the case of in situ annealing of these gold nanostructures with molecules. Probably, in the latter case, during restructuring the gold surface, the molecules can find more energetically stable adsorption sites. It is shown that the developed substrates can be reused after heat treatment at 300 °C for 15 min. The minimum concentration of CV molecules that can be registered on the re-used substrate is as low as 10^{-7} M.

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