SERS activity of silver and gold nanostructured thin films deposited by pulsed laser ablation

N. R. Agarwal · M. Tommasini · E. Fazio · F. Neri · R. C. Ponterio · S. Trusso · P. M. Ossi

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N. R. Agarwal · M. Tommasini
Dipartimento di Chimica Materiali e Ingegneria Chimica "G. Natta", Politecnico di Milano, P.zza L. Da Vinci 32, 20133 Milano, Italy

E. Fazio · F. Neri
Dipartimento di Fisica e di Scienze della Terra, Università di Messina, V.le F. Stagno d'Alcontres 31, Messina, Italy

R. C. Ponterio (✉) · S. Trusso
IPCF-CNR, Istituto per i Processi Chimico Fisici, V.le F. Stagno d'Alcontres 37, 98158 Messina, Italy
e-mail: ponterio@ipcf.cnr.it

P. M. Ossi
Dipartimento di Energia & NEMAS, Politecnico di Milano, Via Ponzio 34-3, 20133 Milano, Italy

1 Introduction

Surface-enhanced Raman scattering (SERS) has become a well-established analytical technique for the detection and recognition of molecular species at very low concentration [1]. Nevertheless, the search for production methods of SERS active substrates with high and reproducible Raman enhancement factors and high spatial uniformity to be used as active elements in biosensors is still extremely active [2, 3]. In this work, we report about SERS activity of gold and silver nanostructured films deposited onto glass substrates realized by laser ablation of silver and gold targets in a controlled Ar atmosphere [4, 5, 6]. Such a technique allows for the production of SERS active substrates also in a liquid environment [7] that will result free from contaminants, at difference with respect to the Lee–Meisel chemical method [8]. In such a case the experimental parameters to be taken into account for the process control are different from the ones typical of the deposition in presence of a controlled low pressure Ar atmosphere.

We looked at two distinct application fields, namely, cultural heritage and biomedical, for the identification of specific molecular species. Spatial uniformity of the substrate was tested by acquiring Raman spectra of two different chromophores (purpurin and alizarin) on large substrate areas. Garanza Lake belonging to anthraquinone dyes fam-ily, is a mix of these chromophores and it is one of the most commonly encountered pigments in art production from the antiquity to the nineteenth century [9, 10]. Raman maps were acquired on areas of the order of 120 μm^2 both on silver and

gold substrates. The sensitivity of the substrates was tested also in presence of biomolecules, to this aim we have chosen lysozyme: a protein whose deficiency or excessive production is indicative of disease [11, 12].

2 Experimental section

All the samples were deposited in a vacuum chamber with a base pressure lower than 10^{-4} Pa. A KrF excimer laser (wavelength 248 nm, pulse width 25 ns, repetition rate 10 Hz) was focused onto pure silver and gold targets mounted on a rotating holder. Substrates, a-C covered copper grids, c-Si and glass slides, were positioned at a distance of 35 mm from the target and held at room temperature. SEM images of the surface of the samples were acquired using a Zeiss Supra 40 field ion microscope. Raman spectra were collected after soaking the substrates into alizarin or, respectively, purpurin aqueous solutions (10^{-4} and 10^{-6} M concentration) for 40 min. Substrates were then rinsed in deionized water and dried in air. Enhancement factors were about 10^7 and 6×10^4 for silver and gold, respectively, estimated comparing the Raman scattering intensity of methylene blue (10^{-5} M) adsorbed on flat metallic surface versus the Raman scattering recorded from the same solution interacting with the PLD SERS substrate. Raman mapping of alizarin and purpurin on gold and silver substrates was carried out using a Jobin-Yvon XploRA system, equipped with a computer controlled X–Y stage. Spectra were excited using the 638 nm line from a solid state laser and integrated for 60 s, using a 50× long working distance microscope objective. The measured laser power at the sample surface was <1 mW on the illuminated area of 1.9 μm^2. Raman measurements of lysozyme were carried out on a gold substrate, (deposited at 70 Pa of Ar and with 10^4 laser shots) following the same procedure reported above. The spectra were acquired with a HR800 Jobin-Yvon micro-Raman spectrometer using a solid state laser emitting at 785 nm, to avoid any kind of fluorescence that might be produced by traces of impurities present in the biological sample. Spectra were collected with a 50× microscope objective and integrated for 600 s. Laser power at the sample surface, about 1.1 μm^2, was 0.8 mW. As a rule, laser power at the sample surface was kept as low as possible to avoid molecular degradation. Integration times and spectra accumulation were varied to optimize the signal-to-noise ratio.

3 Results and discussion

Spot-to-spot reproducibility over a defined area is one of the requirements for a good SERS substrate for biological application. A reproducibility of measurements better than 20 % over micrometer-sized area must be ensured. In Fig. 1, the pictures of the surface of a gold substrate

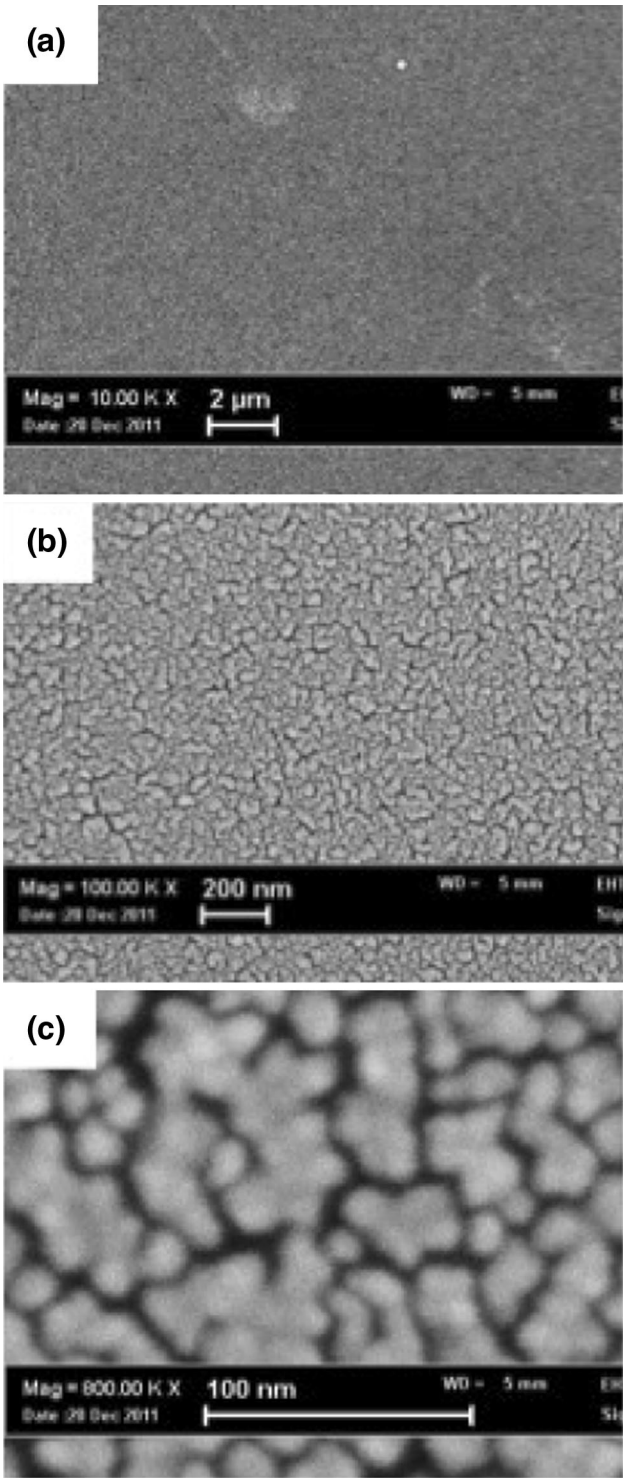

Fig. 1 SEM pictures of the surface of a gold SERS active substrate at different magnification degree. The substrate was deposited at 100 Pa of Ar with 10^4 laser shots

deposited by PLD using 10^4 laser shots and 100 Pa of Ar are shown. The picture taken at the lowest magnification factor, see Fig. 1a shows a spatial homogeneity of the surface morphology over lengths of the order of tens of micrometer. The picture in Fig. 1c, acquired at an higher magnification factor, shows a morphology characterized by the presence of islands with spatial dimension of some tenths of μm^2, and no evidence of percolation among islands. All the samples tested as SERS substrates, both silver and gold ones, showed nearly the same morphology, but we are aware that even small differences in the surface morphology can lead to strong variation of the Raman signal [13]. The spatial homogeneity of silver and gold substrates deposited by pulsed laser deposition (PLD) was tested by acquiring micro-Raman maps over areas between 50 and 120 μm^2 with a step of 1 μm. In Fig. 2 are reported Raman spectra collected on a silver substrate exposed to alizarin over an area of 10×12 μm^2. As can be seen all the spectra are similar when both Raman features and overall intensity are considered. To quantify the intensity variation we reported the intensity of a given Raman peak as a function of the position on the substrate surface. The peak was chosen in such a way to be representative of the dye (alizarin or purpurin) and to be far from other Raman peaks to better evaluate its height above the background level. To this purpose peaks observed at about 1,046 and 1,065 cm^{-1} were selected for alizarin and purpurin, (respectively), even though they were not among the most intense.

In Fig. 3, we report a representative Raman spectrum of purpurin recorded on the surface of a silver substrate. All the Raman peaks previously reported in the literature can be

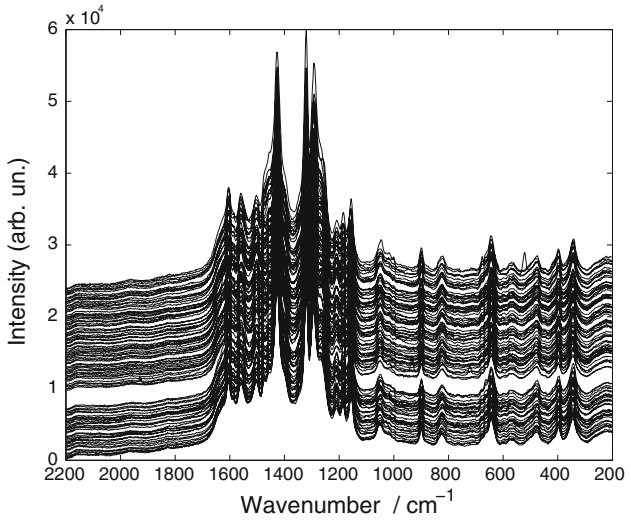

Fig. 2 Raman spectra acquired at different positions on a 10×12 μm^2 area on the surface of a silver substrate soaked into a 10^{-4} M alizarin aqueous solution

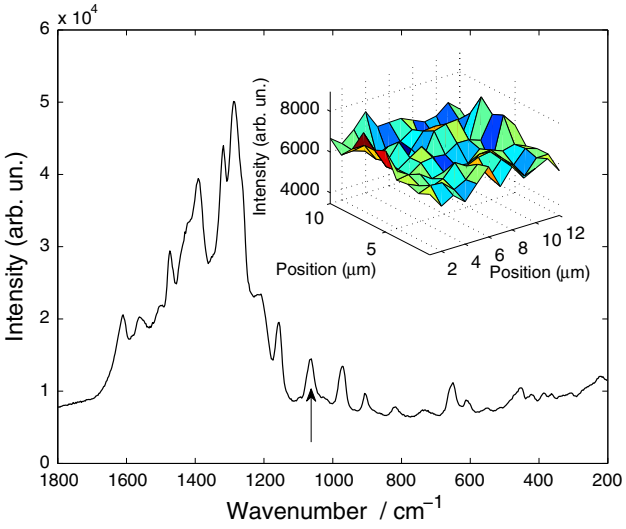

Fig. 3 SERS spectrum acquired on the surface of a silver substrate deposited at 70 Pa of Ar with 3×10^4 laser shots. The substrate was soaked into a 10^{-4} M purpurin solution. In the *inset* the peak intensity at 1,065 cm^{-1} as a function of the position on the substrate surface is reported

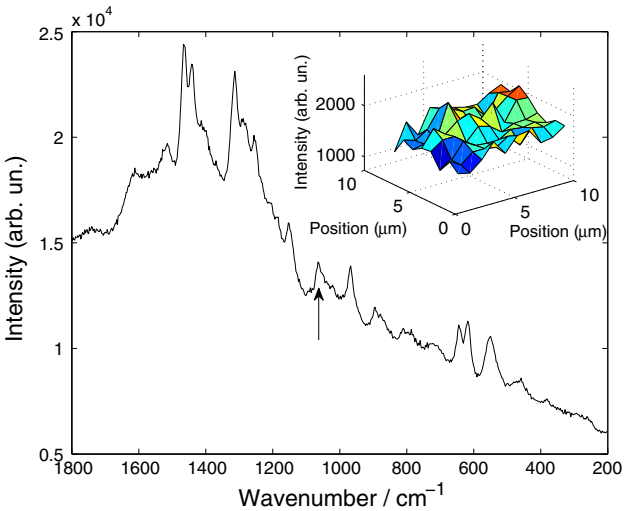

Fig. 4 SERS spectrum acquired on the surface of a gold substrate deposited at 100 Pa of Ar with 10^4 laser shots. The substrate was soaked into a 10^{-4} M purpurin solution. *Inset* description as for Fig. 3

clearly observed [9]. The arrow indicates the peak whose intensity was used to produce the map reported in the inset. As can be seen from the Raman map the intensity of the peak fluctuates around 6,200 counts s^{-1}. We report in Fig. 4 the spectrum of purpurin collected on the surface of a gold substrate. Comparing the spectrum with the one acquired on the silver surface the differences are evident. Even if most of the peaks observed in Fig. 3 are still recognizable in Fig. 4 their intensity ratios are clearly different

Table 1 Raman mapping results

Dye	Substr.	c_0(M)	Mean (counts)	σ (counts) (%)	Area μm^2
Purpurine	Ag	10^{-4}	6220	550 (9)	10 × 12
Purpurine	Au	10^{-4}	1670	187 (11)	10 × 8
Alizarin	Ag	10^{-4}	2024	305 (15)	10 × 12
Alizarin	Au	10^{-4}	983	90 (9)	7 × 7

pointing out for a different arrangement or interaction of the chromophore on silver and on gold. On gold substrates, Raman intensity is lower with respect to the intensities recorded on silver substrates, but the most intense Raman peaks are still clearly recognizable. Considering the intensity fluctuations as a function of the position on the substrate surface a good uniformity is observed also on gold, as can bee seen in the inset of Fig. 4.

In Table 1 we report the mean intensity of the peaks observed at 1,046 and 1,065 cm^{-1} for alizarin and purpurin, respectively, together with standard deviations calculated over all the spectra acquired on the substrates from solutions at the same concentration c_0 . It can be clearly seen that both on silver and on gold the standard deviation on large areas is between 10 and 15 %. The rms roughness of the substrates, as measured by AFM is below 2 %. The films, in fact, do not completely cover the glass substrate and their thickness is no larger than typical dimension of the NPs (few nm). Such an occurrence allows the acquisition of Raman maps over large areas, although it is well known that surface roughness can play a role in the enhancement of the Raman signal [14]. Hence for the present case, we believe that the observed Raman enhancement is associated with the presence of small gaps between adjacent NPs or islands, rather than to the film roughness, as we reported in a previous paper [13]. Moreover, a not uniform distribution of *hot spots* where the Raman amplification is enhanced by several orders of magnitude can lead to a strong dependence of the SERS signal as a function of the spatial distribution of such *hot spots* on the surface. The results presented in Figs. 3 and 4 demonstrate that this is not an issue for our substrates. The observed good uniformity of the Raman response of the substrates makes them suitable for use as active surfaces in SERS-based sensors.

To this purpose, we used Au substrates deposited in Ar atmosphere at 70 Pa with 10^4 laser pulses to test SERS for a prototype complex system such as a protein. To this aim the medium weight (molecular weight 14.3 kDa) lysozyme from chicken egg (Sigma-Aldrich) was chosen. The substrate was immersed in lysozyme solution and SERS spectra were accumulated over 600 s in acidic solution ($3 < pH < 4.5$) using the excitation line at 785 nm from a solid-state semiconductor laser, depositing a power of

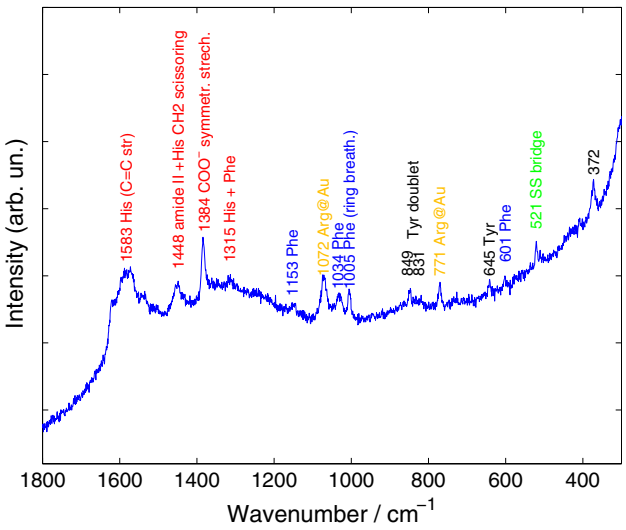

Fig. 5 SERS spectrum acquired on the surface of a gold substrate deposited at 70 Pa of Ar with 10^4 laser shots. The substrate was soaked into a 10^{-5} M lysozyme concentration solution

0.8 mW at the sample surface. The corresponding Raman spectrum is shown in Fig. 5. The Raman spectrum of a protein is significantly contributed by the aromatic residues of its amino acids that being π-conjugated have large Raman cross section. The Raman features of lysozyme have been assigned mainly to aromatic residues, besides specific amide vibrations. A strong peak at 1,384 cm^{-1} has been assigned to COO^- symmetric stretching in SERS. We have assigned several peaks in the high wavenumber region from 1,300 to 1,700 cm^{-1} to histidine in the SERS while only one histidine peak is evident in the normal Raman spectrum. Interestingly, a couple of strong peaks have emerged in the SERS spectrum at 1,072 and 771 cm^{-1} which have been assigned [15] to arginine residues bonded to gold and are not present in the Raman. Also, a broad band is assigned in the Raman to the stretching of disulphide bridge (due to cysteine–cysteine binding), peaked at 510 cm^{-1}, whose broadness is due to various conformations at the S–S bond. This signal changes into a sharp, narrow peak at 521 cm^{-1} in the SERS spectrum. Another peak in the low wavenumber region at 372 cm^{-1} holds a potential as a possible marker for ascertaining the binding process. This peak has been assigned to the proline amino acid which is evidenced on binding of proline with gold [16].

4 Conclusions

In conclusion we tested the spatial homogeneity of the SERS response of nanostructured silver and gold substrates prepared by pulsed laser ablation in presence of a controlled inert atmosphere. Aqueous solutions of alizarin and

purpurin, both being chromophores of interest for cultural heritage, were used to this purpose. The results showed that over micrometric-sized areas the intensity fluctuations of selected peaks are between 16 and 9 % of their corresponding average values. It was also shown that SERS measurements were able to detect the presence of lysozyme at concentration level of 10^{-5} M in a *label free* configuration. Preliminary analysis of the observed SERS signals evidenced the presence of two peaks, absent in the normal Raman spectrum, related to arginine residues bonded to gold, probing the interaction between lysozyme and the gold surface.

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