

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Hybrid nanostructured supports for surface enhanced Raman scattering

Elena Froner^a, Francesco Baschera^a, Francesco Tessarolo^a, Paolo Bettotti^a, Lorenzo Pavesi^a, Barbara Rossi^a, Marina Scarpa^{a,*}, Gino Mariotto^b, Adelio Rigo^c

^a Dipartimento di Fisica, Università di Trento, Via Sommarive 14, 38050 Povo-Trento, Italy

^b Dipartimento di Informatica, Università di Verona, strada le Grazie, 15-37134 Verona, Italy

^c Dipartimento di Chimica Biologica, Università di Padova, Via G. Colombo 3, 35100 Padova, Italy

ARTICLE INFO

Article history:

Received 2 December 2008

Received in revised form 9 February 2009

Accepted 15 April 2009

Available online 21 April 2009

PACS:

81.07.–b

Keywords:

SERS

Porous silicon

Metal nanoparticles

Silanization

Rhodamine 6G

ABSTRACT

Porous silicon solid supports with pore diameter 0.5–1 μm , infiltrated with Ag nanostructures for surface enhanced Raman scattering (SERS) were prepared according to two procedures: spontaneous Ag^+ reduction on the surface of freshly etched porous silicon immersed in Ag^+ aqueous solutions, or anchoring colloidal Ag nanoparticles on the surface previously functionalized by aminosilane. Using Rhodamine 6G (RH6G) as analyte the detection limits were of the order of 20 μM and 20 nM with porous silicon metalized by the first and second procedure, respectively. This large increase of sensitivity notwithstanding a reduced surface density of Rhodamine 6G obtained on porous silicon metalized by the second procedure is discussed in terms of better hot spot efficiency and reduced aspecific binding out of the hot regions obtained depositing the colloids on the aminosilane functionalized surface.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Surface enhanced Raman scattering (SERS) offers the advantages of Raman spectroscopy overcoming its sensitivity drawback [1]. In fact Raman spectra are the fingerprint of the scattering molecules so they provide compound specificity and are suitable for multi-component analysis. Unfortunately the high detection limits of Raman spectroscopy are usually not compatible with analytical applications. Conversely SERS performances appear promising if high specificity and very low detection limit requirements must be met.

SERS occurs when a molecule is in very close proximity to metallic surfaces with roughness on the 10–100 nm scale. This condition can be successfully achieved by 2D rough metallic surfaces or by insertion of metal nanostructures on a patterned substrate. The arrangement of these structures in sites with high enhancement efficiency (“hot spots”) is hypothesized and the signal from the molecules adsorbed in these sites dominates the spectrum. Rough surfaces usually offer high sensitivity due to large surface area, while the highest enhancement factors are obtained by metal colloids [2,3], nanosphere lithography [4] and

films grown over nanospheres [5]. Recent progress was obtained by electron beam lithography since it allows forming a number of differently shaped structures and gratings [6], characterized by chemical and temporal stability and good reproducibility. However, these techniques are not of widespread use, and bioanalysis laboratories usually are not familiar with them. A further limitation to the analytical application is due to the low effective detection sensitivity which is still a “bottleneck” in spite of the very large number of studies dealing with single molecule detection by SERS [7]. In fact, though the single molecule regime makes it possible to monitor specific and not averaged molecular events through the related vibration modes and conformational changes, it is not straightforwardly evident that this approach is of utility to detect low concentrations of chemicals in biofluids. For analytical applications an interesting parameter to be taken into account is the concentration of the analyte in the solution left to interact with the solid substrate before its detection. In literature, when SERS is applied, this concentration appears usually higher than the level of the most important markers of disease in biofluids. An approach to increase sensitivity consists on the use of rough substrates which provide larger active surface area. To this regard pSi offers an open structure which can be tailored to accommodate metal nanostructures and analytes. pSi was suggested as the starting material for nanocomposite SERS support by Chan et al. [8] who

* Corresponding author. Tel.: +39 0461 882029; fax: +39 0461 881696.
E-mail address: marina.scarpa@unitn.it (M. Scarpa).

used AgNO_3 solutions to obtain silver-coated pSi and Lin et al. [9] who grew silver film over pSi with randomly spaced dendritic structure. In both cases immersion plating was used for Ag deposition thanks to the reductive properties of just prepared pSi surface. In this paper we report about the formation of highly active nanocomposite materials starting from pSi coated by Ag nanostructures. The feasibility of obtaining pSi–Ag nanostructures by Ag^+ reduction or by anchoring colloidal particles of Ag on a hybrid nanomaterial is assessed by optical techniques and scanning electron microscopy. In addition the possibility of using pSi as robust and very active SERS substrate for the detection of a low concentration of a probe analyte in solution is demonstrated. Finally, we demonstrate that the tailoring of the surface with organic functionalization makes the surface itself more selective toward the analyte binding reducing the unspecific adsorption out of the hot spots.

2. Materials and methods

2.1. pSi production and metalization by Ag^+ reduction

P-type, $\langle 100 \rangle$ oriented silicon wafers, with 10–20 Ω cm resistivity, were purchased from Universitywafer (Boston, MA). All solvents and reagents were purchased from Sigma-Aldrich (Milan, Italy), except for Ag_2SO_4 and Sodium Citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) which were purchased from Carlo Erba (Milan, Italy).

Macroporous silicon with pore diameter 0.5–1 μm , was obtained by electrochemical etching in a home-made Teflon cell, in a 4 M HF solution in dimethyl sulfoxide (DMSO)[10,11], using a current density of 10 mA/cm^2 and an etching time ranging from 2 to 30 min. After etching, macroporous samples were rinsed in ethanol and doubly distilled water, dried under N_2 flux and then

under vacuum in order to remove solvent traces from the pores. The porous area was a circle of about 1 cm^2 .

pSi metalized by Ag^+ reduction (Ag–pSi) was obtained by dipping freshly prepared pSi [10] into Ag_2SO_4 aqueous solution, in the dark, at various temperatures and times followed by thoroughly rinsing with water.

2.2. Silanization procedure and silver colloids deposition

Silanization was carried on macro pSi after oxidation for 48–72 h in H_2O_2 10% (v/v), to induce silanols on the silicon surface. The oxidized samples were sonicated in solvents of decreasing polarity: water, water and methanol 1:1, methanol, methanol and toluene 1:1 and toluene. Silanization was carried out in a 2% (3-aminopropyl)-triethoxysilane (APTES) toluene solution degassed under mild vacuum, at RT for 10 min. Then the temperature was raised to 70 $^\circ\text{C}$ under stirring for 10 min. After thoroughly rinsing in solvents of increasing polarity (from toluene to water) the samples were dried in a N_2 stream and stored in air until use. The silanized pSi (NH_2 -pSi) was metalized by silver colloid deposition (Ag– NH_2 -pSi). Silver colloids were prepared according to Lee and Meisel procedure [2]: 1 ml of 1% sodium citrate solution was added to 50 ml of boiling aqueous solution of 0.18 mg/ml AgNO_3 under vigorous stirring. Silanized samples were soaked in cold filtered silver colloidal solution for 1–4 h, in the dark and under mild stirring, and then rinsed in water and dried under N_2 .

2.3. Rhodamine 6G deposition and detaching procedure

Rhodamine 6G (RH6G) was deposited by dipping the silver plated pSi into aqueous dye solution for 1 h and thoroughly rinsing them in water (dip and dry procedure).

RH6G, stuck on bare, silanized or metalized pSi, by dip and dry procedure, was brought into solution with 1% CTAB-containing HF

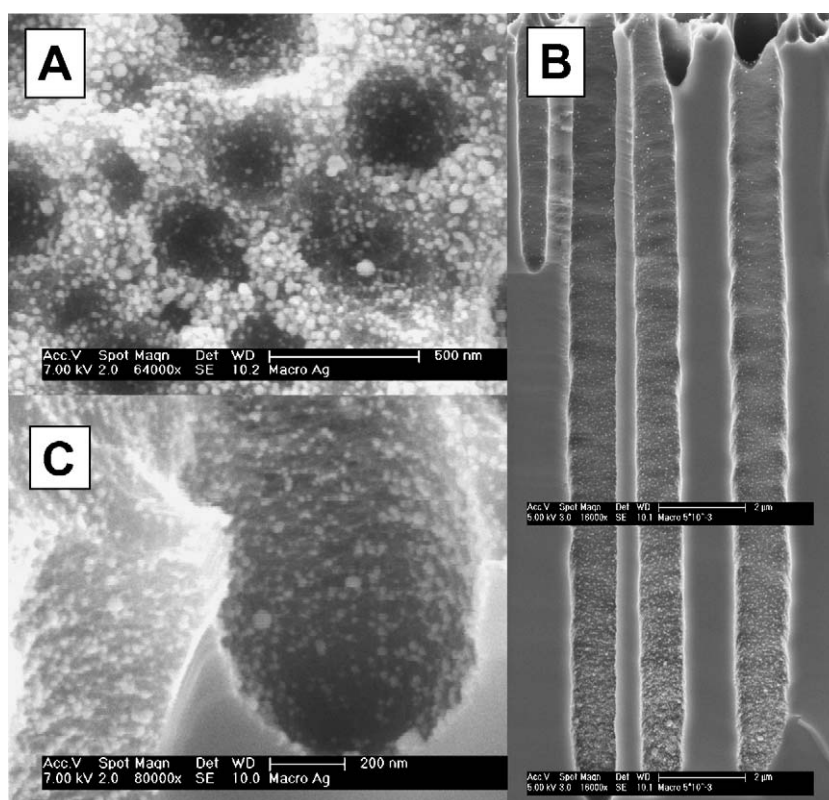


Fig. 1. SEM images of macroporous silicon metalized by immersion into 5 mM Ag_2SO_4 for 60 min at RT: (A) plan view, (B) cross-section: pore depth of about 30 μm , (C) a cross-section: detail of the mouth of a 5 μm -deep pore. Note: images are presented at different magnifications to better appreciate the specific features.

aqueous solution (4%, v/v) [12]. The detaching HF solution was then neutralized with 8 M KOH and the fluorescence was measured (Varian Cary Eclipse, excitation wavelength: 526 nm, emission wavelength: 550 nm) and the RH6G molecules were quantified on the basis of calibration curves obtained under the same experimental conditions.

2.4. SEM images and optical characterization

Scanning electron microscopy (SEM) images were obtained with an ESEM-FEG XL 30 (Fei Company) in high-vacuum mode by fixing silicon dices onto the stub holder with double bonding carbon tape. No gold sputtering was needed operating in the range of 5–15 keV electron beam energy. Top view and cross-section representative images were obtained by 0° and 30° stage-tilt, respectively.

Optical reflection spectra were carried out with a Cary 100 UV–vis spectrophotometer (spectral range: 300–800 nm, spectral resolution: 2 nm), using a Labsphere DRA-CA-30I integrating sphere for the total (diffuse and specular at 8°) reflectance measurements.

2.5. Raman spectroscopy measurements

Raman scattering measurements in fully backscattering geometry were conducted at RT on samples brought in air, using a microprobe setup (Horiba-Jobin-Yvon, Labram HR), consisting of a He–Ne laser (excitation wavelength: 632.8 nm), of a narrow-band notch filter (Holographic super-notch filter of the Kaiser Optical Systems Inc.) of a 80 cm focal length spectrograph, mounting a 1800 grooves/mm grating, and of a charge coupled device detector cooled by liquid nitrogen. The laser beam was focused onto the sample surface, with a spot size of about 2 μm (focal spot area of about 3 μm^2), by means of a 100 \times objective with numerical aperture NA = 0.9. At the occurrence, neutral filters of different optical densities were used to avoid unwanted effects due to laser heating. The spectral resolution was about 2 cm^{-1} /pixel.

3. Results and discussion

3.1. Ag–pSi

A uniform layer of Ag nanoparticles was obtained by immersion of pSi (pore diameter 0.5–1 μm) in Ag^+ solutions. The SEM images (top view and cross-section) of Ag–pSi substrates are reported in Fig. 1. As we can see from Fig. 1A and C, the macro pSi surface is crowded by globular-shaped silver nanoparticles with diameters ranging approximately from 20 nm to less than 100 nm. These nanoparticles appear infiltrated into the porous network even in the case of 30 μm -deep pores, as shown in Fig. 1B. However we also observed the presence of silver particles with diameters ranging from 100 nm to 1 μm (not visible in Fig. 1). These macro particles could give only a non-electromagnetic contribution to SERS [13]. Washing under stirring or sonication was not successful in detaching the large particles. The absorption spectrum of the nanostructured silver layer formed on these pSi samples consists of a weak and flat plasmon resonance falling in the region 420–480 nm, as can be seen from Fig. 2 (upper panel, Trace C). Both the final morphology and the reflectance spectrum of the deposited silver layers partially depend on the experimental conditions of deposition. In order to optimize the metal deposition procedure the pSi samples were immersed into Ag_2SO_4 solutions at various immersion times, temperature and Ag^+ concentrations. After each experiment the particle density and size was checked on the basis of the SEM image and absorption band position and intensity. The highest small-particle density was obtained after a 1 h immersion

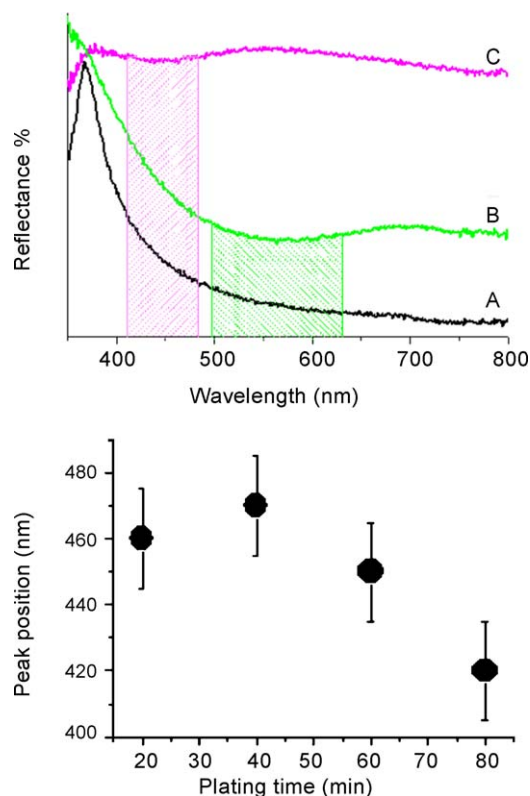


Fig. 2. Upper panel: reflectance spectra of: (A) freshly etched macroporous silicon sample, (B) Ag–NH₂–pSi sample and (C) Ag–pSi sample obtained by 1 h immersion into 5 mM Ag_2SO_4 at RT. The respective absorption bands are highlighted by the shadowed areas under the traces. The spectral traces have been vertically shifted for clearness sake. Lower panel: wavelength at minimum of reflectance band for Ag–pSi samples obtained by immersion into 5 mM Ag_2SO_4 at RT for different times (error bars have been estimated from testing various samples).

into 5 mM Ag_2SO_4 at RT. The absorption band was more clearly distinguishable when the samples were dipped into 5 mM Ag_2SO_4 at RT and was approximately blue-shifted at increasing immersion time, being at about 450 nm for 1 h immersion, as shown in the lower panel of Fig. 2.

3.2. Immobilization of Ag nanoparticles on pSi by aminosilane

The diameter of Ag nanoparticles was within the range 20–100 nm with a population density peak around 50–60 nm, according to the SEM images, obtained for the colloidal particles deposited on flat silicon slides. These particles, deposited onto NH₂–pSi appeared strongly bound probably by chemical interaction with amino groups. In fact a 20 min sonication at ultrasound power of about 100 W could not detach them from NH₂–pSi while a mild washing completely removed silver particles from not silanized pSi. The reflectance spectrum of these silver particles deposited onto silanized pSi showed a very large band centred around 500–550 nm, as shown in the upper panel of Fig. 2 (Trace B). The SEM images in Fig. 3 show the plan and cross-section of a representative Ag–NH₂–pSi sample (Fig. 3A and B): it appears that its surface is uniformly covered by interspaced silver nanoparticles, while some nanoparticle clusters are visible mainly around sharp interpoles edges. The particle size distribution was in the range 60–80 nm and turned out narrower with respect to that obtained by Ag^+ reduction. The macroporous support seemed to affect the particle spatial distribution on the surface, since uniform distribution was observed on macro pSi sample while large clusters of particles were present on flat silanized surfaces (images not shown). Immersion time had no effect on the resulting deposition.

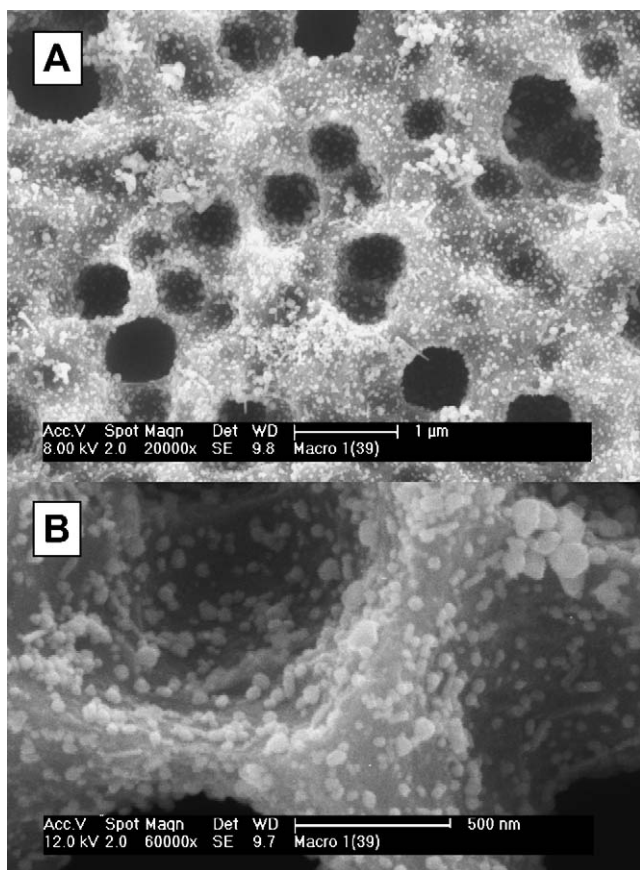


Fig. 3. SEM images of Ag colloidal nanoparticles deposited onto silanized pSi. The nanoparticles were deposited by immersion into Ag colloid solution for 4 h at RT: (A) top view (original magnification 20,000 \times), (B) detail of pore mouths (original magnification 60,000 \times).

3.3. SERS test experiments

Nanostructured Ag–pSi and Ag–NH₂–pSi were tested as SERS supports with RH6G as probe molecule deposited by the dip and dry procedure. Spectra were carried out also on pSi metalized by both the procedures where no RH6G had been deposited and onto bare pSi and NH₂–pSi where the dye was deposited by spotting. Fig. 4 reports the Raman spectra, normalized so that they show comparable noise amplitude. Several typical Raman peaks of RH6G in the spectral region between 1080 and 1680 cm⁻¹ were easily identified in the samples containing the metal nanostructures (see traces 3 in Fig. 4A and 4–6 in Fig. 4B). Conversely, the Raman spectra of bare pSi (see Trace 1 in Fig. 4B) and NH₂–pSi presented no features in the range where RH6G peaks lie, even after spotting on them of a relatively large quantity of dye (30 μ l of 20 μ M RH6G solution were spotted and then left to dry out, see Fig. 4B, Trace 2 for NH₂–pSi). This means that both kinds of metalized substrates are SERS active. However, the main Raman bands of RH6G [14] (around 1185, 1315, 1365, 1515, 1570 and 1650 cm⁻¹) were clearly recognizable only on Ag–NH₂–pSi after dipping in 20 nM to 20 μ M RH6G solution or on Ag⁺ plated pSi after immersion into 20 μ M RH6G.

To test the reproducibility of the results, the spectra were acquired on three sets of samples and the prominent Raman bands of RH6G, adsorbed from 20 μ M dye solution by the dip and dry procedure, were always clearly recognizable on all the spectra recorded, independently of the area under examination. A certain variability of the S/N ratio was observed.

The representative spectra reported in Fig. 4A (Trace 3) and B (Trace 6) show more intense characteristic RH6G peaks in the

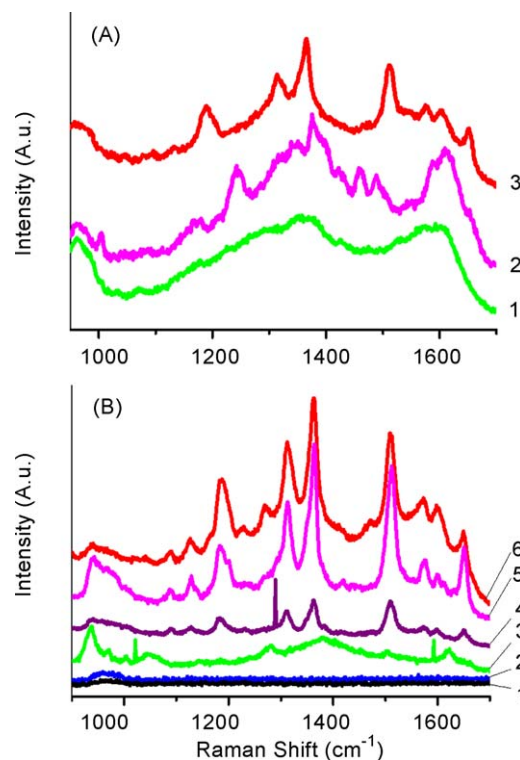


Fig. 4. Raman spectra of RH6G on Ag nanostructures deposited on pSi. Part A: Ag nanostructures obtained by immersion into 5 mM Ag₂SO₄ for 1 h: Trace 1: barely metalized sample; Trace 2: metalized sample immersed into RH6G 200 nM solution; Trace 3: metalized immersed into 20 μ M RH6G solution; Part B: Ag nanostructures obtained by colloid deposition. Trace 1: silanized sample; Trace 2: silanized sample on which 30 μ l of RH6G 20 μ M were dropped and left to dry out; Trace 3: sample silanized and immersed into Ag colloids for 4 h; Trace 4: sample silanized, immersed into Ag colloids for 4 h and with RH6G deposited from 20 nM solution; Trace 5: sample silanized, immersed into Ag colloids for 4 h and with RH6G deposited from 200 nM solution; Trace 6: sample silanized, immersed into Ag colloids for 4 h and with RH6G deposited from 20 μ M solution; Signals of non metalized non-silanized samples both with and without RH6G deposited on them are identical to traces 2 e 1 in Part B, respectively. The scale on y axis of both Parts A and B is the same, and the spectra are normalized in order to present a comparable noise level.

Ag–NH₂–pSi samples. This increased peak intensity could be due to an increase of the enhancement and/or of surface density of RH6G molecules. This density was measured by fluorescence [15] on two sets of differently metalized samples, incubated into 20 μ M RH6G solution, then immersed into 1%CTAB–4%HF aqueous solution (see Section 2.3). Table 1 reports the density of RH6G molecules removed from both sets of metalized macro pSi (columns 1–2) and of RH6G molecules removed from the correspondent non

Table 1

Surface density of RH6G molecules deposited on silicon samples by dip and dry procedure. The RH6G molecules were deposited by dipping the samples into 3 ml of 20 μ M RH6G solutions for 1 h and then thoroughly washed. The deposited dye was removed and quantified by comparison with a calibration curve. The deposition area was about 1 cm². The lightened molecules can be calculated assuming homogeneous molecule distribution and considering that the focal spot area is about 3 μ m². The RH6G adsorbed over both kinds of metalized samples by dipping into 200 and 20 nM solution was not detectable by this fluorimetric method. Numbers in columns 1, 2, 3 and 4 have been obtained by experimental fluorescence data after background signal subtraction.

| RH6G (molecules/ μ m ²) detached from | | | |
|---|-------------------------|-------------------------------|----------------------|
| Metalized macroporous pSi | | Non-metalized macroporous pSi | |
| Ag–pSi | Ag–NH ₂ –pSi | BarepSi | NH ₂ –pSi |
| 1.2×10^7 | 6×10^5 | 2×10^6 | 4×10^4 |

metalized pSi substrate. In this case the Raman spectra did not present any RH6G feature. Both deposition procedures produced an increased number of RH6G molecules, with respect to non-metalized samples, indicating that RH6G molecules interact directly with the silver nanostructures. Bare pSi samples retain almost 50 times more RH6G than NH₂-pSi, as expected since both silane and RH6G bear a positive charge at the pH of deposition. The peak intensity of the Raman spectra obtained after incubation of Ag-NH₂-pSi in 20 μM and 200 nM was the same and decreased by about a factor of 5 by incubation into 20 nM RH6G. The surface concentration of this compound was found to be about 10⁻¹⁸ mol/μm² in the first case while in the other cases it was undetectable by our fluorimetric method. Parallel experiments performed on Ag-pSi showed a strongly reduced peak intensity after incubation into 20 μM RH6G, notwithstanding the surface density of 2 × 10⁻¹⁷ mol/μm², that is 20 times higher than that measured in a parallel experiment performed on Ag-NH₂-pSi. This behaviour may indicate a higher efficiency of the hot spots present on Ag-NH₂-pSi and their saturation by the fluorescent probe at concentrations equal or higher than 200 nM. Since the RH6G fluorescence detection limit in the detaching solution is 3 nM, an upper limit of the surface density of 9 × 10⁻²⁰ mol/μm² was estimated for the Ag-NH₂-pSi incubated into 200 nM RH6G. This upper limit is expected to decrease by about a factor of 5, that is RH6G < 2 × 10⁻²⁰ mol/μm² for the sample obtained by dipping into 20 nM solution, in the hypothesis of a linear dependence of SERS intensity on RH6G surface density below hot spots saturation. The number of RH6G molecules lightened by the laser beam can be inferred from this upper estimated value by assuming homogeneous dye distribution and considering that the focal spot area on the samples is about 3 μm². This number was found to be about 40,000 molecules. This detection limit appears due to the binding constant between the dye and the hot spots, since decreasing the concentration of the dye in the deposition solution the Raman spectrum was undetectable. Then the detection limit is expected to decrease by introducing inside the hot spots anchoring groups with high affinity for the analyte. However in view of the possible application to small volume samples, as an example for criminal investigations, we can argue that to obtain a density of 40,000 molecules over 3 μm² spot area, we need less than 5 × 10⁻¹² L of 20 nM solution.

The most probable cause of better enhancement obtained on Ag-NH₂-pSi is the presence of clusters of Ag nanoparticles on silanized substrate, which are recognizable in the SEM image, combined with the lower affinity of silanized silicon surfaces for RH6G molecules. The Ag clusters could host more efficient hot spots, and RH6G molecules could be pushed into them due to the electrostatic repulsion from positively charged silanized pSi surface. The more efficient enhancement on colloid-metalized samples could also be explained by the best matching between plasmonic absorption band and laser wavelength for this kind of samples. Furthermore, there could be a partial adaptability of silver nanostructures bound on the flexible aminosilane layer. Drachev et al. [16] pointed out that a certain degree of adaptability of the nanostructured silver layer, following biomolecules deposition is necessary in order to obtain the enhancement of Raman signal. However we could not obtain straightforward experimental evidence by SEM of a reorganization of the silver layer structure after the RH6G deposition.

Finally, the detection limit we obtained represents an improvement over previous solid pSi-based SERS active support set-up [8], suggesting that macro pSi after silanization and colloid deposition is a well-suited nanostructured material for ultra-sensitive measurement in small volumes. Finally, a suitable functionalization of the support surrounding the hot sites reduces

the aspecific adsorption, which is fundamental in the design of sensors for multiplexed analysis.

4. Conclusions

We tested and compared the SERS efficiency of two different metalization procedures able to generate silver nanostructures on macroporous silicon substrates. By one procedure silver nanoparticles are grown on pSi by Ag⁺ reduction and by the other one silver nanoparticles are first synthesized in solution and successively deposited onto silanized pSi. Both procedures cover pSi surfaces with silver particles whose sizes fall in the range that elicits SERS effect when properly lighted. SERS effect was tested on both kinds of metalized samples using RH6G as a probe molecule, and we estimate that about 13,000 molecules μm⁻² can be seen by Raman measurements, using an excitation wavelength of 632.8 nm on silanized-metalized pSi supports. The RH6G surface density was independently measured by fluorescence and, taking into account some relationship between concentration and SERS signal intensity, it appears that the detected molecules can be located inside a limited number of hot spots. Finally we showed that a suitable functionalization must be the first step to design the surface, not only to increase the hot spot number or affinity but also to decrease the unspecific adsorption of the target molecules in Raman-silent regions.

Acknowledgments

We would like to thank FBK Foundation (Istituto per la Ricerca Scientifica e Tecnologica) and Azienda Provinciale per i Servizi Sanitari di Trento (Section of Electron Microscopy) for SEM analysis. This work was partially supported by MIUR (Prin 2005), Provincia Autonoma di Trento (Progetti PAT-CRS2008) and Fondazione CARITRO (CELTIC Project).

References

- [1] R. Aroca, Surface Enhanced Vibrational Spectroscopy, Wiley, Chichester, 2006; K. Kneipp, M. Moskovits, H. Kneipp, Surface Enhanced Raman Scattering Physics and Applications, Springer-Verlag, Heidelberg, Berlin, 2006.
- [2] P.C. Lee, D. Meisel, J. Phys. Chem. 86 (1982) 3391.
- [3] R.F. Aroca, R.A. Alvarez-Puebla, N. Pieczonka, S. Sanchez-Cortez, J.V. Garcia-Ramos, Adv. Colloid Interface Sci. 116 (2005) 45.
- [4] C.L. Haynes, R.P. VanDuynne, J. Phys. Chem. B 105 (2001) 5611.
- [5] G.A. Baker, D.S. Moore, Anal. Bioanal. Chem. 382 (2005) 1751; T-Vo-Dinh, Trends Anal. Chem. 17 (1998) 582.
- [6] E.C. LeRu, P.G. Etchegoin, J. Grand, N. Félidj, J. Aubard, G. Lévi, A. Hohenau, J.R. Krenn, Curr. Appl. Phys. 8 (2008) 467; G. Laurent, N. Félidj, J. Grand, J. Aubard, G. Lévi, A. Hohenau, J.R. Krenn, F.R. Aussenegg, J. Microsc. 229 (2) (2008) 189.
- [7] (a) S. Nie, S.R. Emory, Science 275 (1997) 1102–1106; (b) K. Kneipp, Y. Wang, H. Kneipp, L.T. Perelman, I. Itzkan, R.R. Dasari, M.S. Feld, Phys. Rev. Lett. 78 (9) (1997) 1667; (c) M. Moskovits, L.L. Tay, J. Yang, T. Haslett, Optical Properties of Nanostructure Random Media, Springer, Heidelberg, 2002, p215; (d) A. Otto, J. Raman, J. Raman Spectr. 33 (2002) 593.
- [8] S. Chan, S. Kwon, T.W. Koo, L.P. Lee, A.A. Berlin, Adv. Mater. 15 (19) (2003) 1595.
- [9] H. Lin, J. Mock, D. Smith, T. Gao, M.J. Sailor, J. Phys. Chem. B 108 (2004) 11654.
- [10] F.A. Harraz, K. Kamada, K. Kobayashi, T. Sakka, Y.H. Ogata, J. Electrochem. Soc. 152 (4) (2005) C220.
- [11] P. Bettotti, L. Dal Negro, Z. Gaburro, L. Pavesi, A. Lui, M. Galli, M. Patrini, F. Marabelli, J. Appl. Phys. 92 (12) (2002) 6966.
- [12] F. Vianello, L. Zennaro, M.L. Di Paolo, A. Rigo, C. Malacarne, M. Scarpa, Biotechnol. Bioeng. 68 (5) (2000) 488.
- [13] K. Kim, H.S. Lee, N.H. Kim, Anal. Bioanal. Chem. 388 (2007) 81.
- [14] W.-H. Li, X.Y. Li, N.-T. Yu, Chem. Phys. Lett. 312 (1999) 28.
- [15] As residual silver nanoparticles, removed from the surface, could quench fluorescence in solution, we centrifuged the solutions. But we found no difference in fluorescence intensities of centrifuged and non-centrifuged solutions. We inferred that either HF can brake Ag-RH6G linkage or it can destroy silver particles.
- [16] V.P. Drachev, M.D. Thoreson, E.N. Khaliullin, V. Jo Davison, V.M. Shalaev, J. Phys. Chem. B 108 (2004) 18052.