Reusable SERS active substrates for ultrasensitive molecular detection

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Abstract

Silver nanoparticles coated silicon nanopikes were investigated as efficient and reusable SERS active substrates. Femtosecond laser pulses fabricated silicon nanopikes were made SERS active through deposition of silver nanoparticles by reducing silver nitrate. **The prepared substrates offer good SERS reproducibility with high enhancement factors. The SERS detection limits of 1 pico-molar from Rhodamine 6G and 100 pico-molar from Methylene blue were demonstrated.** In addition, these SERS substrates are reusable by simple washing and no observable degradation of sensitivity was measured.

Keywords: SERS; Femtosecond; Nanostructures; Detectors

1. Introduction

Surface-enhanced Raman spectroscopy (SERS) is a powerful analytical tool for ultra-sensitive and selective detection of chemicals using nanostructured metallic materials.¹⁻³ Numerous research efforts have been devoted to develop SERS active substrates using facile and cost-effective fabrication approaches.⁴⁻⁵ Among all the SERS active substrates, gold and silver based nanostructures with sharp edges (“hot spots”) are commonly utilized due to their high sensitivity and good biocompatibility.^{6,7} Single molecule detection with the SERS substrates has been demonstrated.⁸ However, most of the approaches used so far are characterized by major challenges in stability and reproducibility of the Raman signal.^{9,10} The ‘hot-spots’ on those substrates are randomly distributed and consequently the SERS responses often vary from spot to spot over the substrate surface.^{11, 12} Recently, femtosecond (FS) laser machined silicon nanopike structures and their replicas were utilized as viable SERS substrates.^{13, 14} These patterned nanostructures prepared using FS-laser in conjunction with coating of metallic layers

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by evaporation,¹⁴ and chemical plating,¹⁵ yielded an enhancement factor (EF) of 10⁷. However, the practical use of these fabricated substrates was frequently limited by instability and poor reproducibility of the Raman signals.^{15,16} The lack of uniformity and persistent performance might be attributed to randomly distributed “hot spots” on the nanostructures. Moreover, it has been reported that the SERS substrates in general are lack of reproducibility for the Raman measurements and could not be reused.¹⁷ The absence of reproducibility and reusability has restricted these substrates as cost-effective SERS sensors for practical applications. In this communication, we report an efficient approach to fabricate uniform, large area, ultrasensitive and reusable SERS substrates that can be repeatedly used in Raman experiments without losing their sensitivity. **These prepared substrates provide good SERS reproducibility with high enhancement factors, enabling the ultra-sensitive detection of 1 pico-molar Rhodamine6G (R6G) and 100 pico-molar Methylene blue (MB) analytes adsorbed from aqueous solutions.** These substrates were reusable and no significant change was observed in the enhancement factor upon a number of sensing and cleaning cycles.

2. Experimental details

Silicon wafers were first cleaned with acetone to remove any possible contaminants and were then rinsed with water, and dried with nitrogen gas. An FS Ti:sapphire laser operating at 800 nm (100 FS, 1 kHz repetition rate and 1 W average power) was utilized to produce the nanostructures on the silicon wafer. The cleaned substrates were subjected to the focused FS laser beam at 400 nm (frequency-doubled) in methanol on a computer controlled X-Y stage. The nanospike structures with an area of 0.5 cm × 0.5 cm were fabricated. R6G, MB, silver nitrate, hydrazine monohydrate, Vitamin C, and citric acid were purchased from Sigma-Aldrich and used without further purification. All solutions were prepared with distilled water. The laser machined silicon nanostructures were first rinsed with distilled water. The cleaned nanostructured substrates were then dipped into the aqueous solution of silver nitrate (100 mM) for 30 min and followed by addition of several drops of aqueous hydrazine hydrate (3 M) onto the substrates. The treated silicon nanostructured substrate was then heated at 50 °C in a Vitamin C (0.3 M): citric acid (2.6 mM) aqueous solution (6:1) for 1 hour. The substrate was then rinsed with distilled water and dried with nitrogen gas. For comparison, a silicon wafer without the nanostructures was also coated with silver nanoparticles by the same reduction method. The nanospikes and the silver nanoparticles coated nanostructure were examined by scanning electron microscopy (SEM). Figure 1 shows the SEM images of nanospike structures with and without silver nanoparticles, and the silver nanoparticles coated flat silicon surface. As shown in Figure 1 (a), the nanospikes with height and

spacing of 200-300 nm were fabricated by the FS laser pulses. Silver nanoparticles with particle size about 50 nm were uniformly formed on the silicon nanostructures (Figure 1 (b)). Figure 1(c) shows the SEM image of the silver nanoparticles coated flat silicon surface. Fewer amounts of silver nanoparticles were loaded on the flat Si surface as compared to that of the nanostructured one.

R6G and MB (commonly used test analytes) solutions with concentrations of 10^{-5} M, 10^{-8} M, 10^{-10} M and 10^{-12} M were prepared in distilled water to examine the sensitivity of the fabricated SERS substrates. The Raman spectra were measured using a Bruker Senterra Raman microscope. Since the highest SERS sensitivity in detecting R6G and MB molecules were obtained by employing laser excitation at 532 nm,^{18,19} all Raman measurements with the fabricated SERS substrates were carried out using a green laser at 532 nm with 2 mW of incident power. The laser beam diameter on the sample surface was about 1 μ m achieved using a 50X microscope objective. The spectral resolution of the spectrometer was set at 3 cm^{-1} . All the Raman spectra were recorded with a data acquisition time of 2 seconds. The Raman spectra were background corrected. 2 μ l of the aqueous R6G or MB solution was directly spread on the SERS active area (0.5 cm $\times$ 0.5 cm) and was dried under ambient condition.

3. Results and discussion

Figure 2 shows the Raman spectra of R6G and MB molecules adsorbed on the SERS substrates prepared from the analyte solutions at 10^{-8} M. The characteristic Raman bands of R6G at 615, 775, 1127, 1182, 1310, 1362, 1508, 1573, and 1649 cm^{-1} (Figure 2 (1))^{13, 18} and of MB at 495, 497, 677, 768, 890, 1067, 1181, 1331, 1396, 1411, 1544, and 1620 cm^{-1} were measured (Figure 2(2))¹⁹ Raman spectra of R6G and MB prepared from the solutions with concentrations of 10^{-10} M and 10^{-9} M, were recorded over nine random spots to evaluate the uniformity in sensitivity of the SERS substrates, respectively. As displayed in Figure 3, the R6G and MB Raman peaks were detected on all nine spots with similar peak intensities. The relative standard deviations (RSDs) were evaluated from the SERS spectra of the randomly selected nine spots for R6G and MB molecules and the RSD values for the main Raman peaks are summarized in Table 1. The averaged RSD values were determined to be about 15.93% and 5.86% for R6G and MB respectively. The observation suggests that the analytes were uniformly distributed on the SERS substrates and the enhancement of the R6G and MB Raman signals with the substrates were highly reproducible over the SERS active area.

To determine the detection limits and the EFs of the fabricated SERS substrates, Raman spectra were measured using the SERS substrates and the silver nanoparticles coated silicon wafer (un-patterned)

with the prepared R6G and MB solutions. Figure 4 shows the SERS spectra of R6G and MB. The silver nanoparticles coated wafer without the nanospikes was only able to detect R6G and MB molecules at a concentration of 10^{-5} M. Figure 4 (1) depicts the SERS spectra of R6G at 10^{-5} and 10^{-12} M. All the characteristic R6G Raman bands were observed even with a solution of 10^{-12} M concentration for the fabricated substrate. This result indicates that the fabricated SERS substrates were able to sense about eight R6G molecules in the focal spot of the excitation laser beam assuming these molecules are spread uniformly over the substrate. Figure 4 (2) displays the SERS spectra of MB at 10^{-5} and 10^{-10} M. The EFs were estimated using the following expression:

where I_{SERS} represents the SERS intensities of the Raman peaks from the adsorbed analyte molecules on the SERS active substrate; I_{Ref} represents the normal Raman signals from the adsorbed analyte molecules on the silver nanoparticles coated flat silicon substrate; N_{SERS} and N_{Ref} represent the corresponding number of analyte molecules excited by the laser beam. Assuming there is no specific binding between the analyte and the sample surface, the number of adsorbent molecules on the surface is proportional to the concentration of the analyte solution.²⁰ Using the above equation and the assumptions, the EFs were determined to be about 3×10^7 , 4.3×10^7 , 5.3×10^7 , 7.7×10^7 , and 1.03×10^8 for the corresponding Raman peaks at 615, 775, 1362, 1508 and 1649 cm^{-1} , respectively. It is noted that a maximum EF approaching 10^8 was achieved for the Raman band at 1649 cm^{-1} . Similarly for MB, the EFs for the main Raman peaks at 768, 1067, 1181, 1396 and 1620 cm^{-1} were, estimated to be, 1.3×10^5 , 1.1×10^5 , 1.3×10^5 , 1.7×10^5 , and 2.3×10^6 , respectively. The measured high EFs clearly indicated that the silver nanoparticles (about 50 nm) formed on the nanostructures significantly enhanced the local electromagnetic fields in the visible spectral region. In addition, higher resonance Raman enhancement due to strong absorption of R6G molecules from the 532 nm laser excitation led to the higher EFs as compared to those of the MB. The high sensitivity of the fabricated SERS substrates suggests that these substrates could be used as efficient SERS sensors for other analytes as well.

The reusability of the fabricated SERS substrates was examined using the R6G and MB solutions at concentrations of 10^{-10} M and 10^{-9} M, respectively. After the Raman spectrum was taken, the substrates were cleaned by immersing in distilled water for 10 min to remove the adsorbed analytes and dried with nitrogen gas. Prior to re-adsorbing the analyte, the Raman spectrum of the cleaned substrate was recorded. Total 10 cycles of washing and re-adsorbing R6G and MB molecules on the SERS substrates

were performed. No trace of R6G and MB was detected in the Raman spectra after washing, indicating the analyte molecules were completely rinsed off. Almost similar signal strengths for all the Raman bands were measured for re-adsorbed R6G and MB on the same substrates for all the nine times, as compared to those of the analytes on the freshly fabricated substrates. Figure 5 shows the Raman spectra of R6G and MB measured with the freshly made substrates, the same substrates after washing, and at the fifth and tenth re-adsorptions. The result strongly demonstrated that the prepared SERS substrate is reusable. The reusability also suggests that the fabricated nanostructured SERS sensor would be cost-effective. To the best of our knowledge, no clear demonstration of such reusability of other nanostructured SERS substrates has been reported.

4. Conclusions

In summary, we have developed highly sensitive and reusable SERS substrates. The SERS substrates can sense R6G and MB at concentrations down to 10^{-12} M and 100 pico-molar, respectively. The sensitivity of the substrate is quite homogenous over the SERS active region. These sensors can be reused, allowing reliable and cost-effective means for sensitive detection of chemical. Further improvement of the sensitivity could be achieved by optimizing the coverage of silver particles on the silicon nanospikes. The developed SERS substrates in conjunction with portable Raman spectrometers would offer sensitive, reliable, and low-cost approaches to sense other chemicals and biological pathogens.

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Figures

Figure 1. SEM images of (a) nanospikes formed on a silicon wafer by the FS laser pulses, (b) the nanospikes coated with silver nanoparticles and (c) the flat silicon surface coated with silver nanoparticles.

Figure 3. Raman spectra over nine randomly selected spots from (1) a R6G molecules adsorbed SERS substrate from a 10^{-10} M solution and (2) a MB molecules adsorbed substrate from a 10^{-9} M solution.

Figure 4. Raman spectra of (1) R6G molecules adsorbed on (a) a SERS substrate with a 10^{-12} M solution, (b) a silver nanoparticles coated flat silicon surface using a solution at 10^{-5} M and (c) a silver nanoparticles coated flat silicon surface using a solution at 10^{-12} M. (2) MB molecules adsorbed on (a) a SERS substrate with a 10^{-10} M solution, (b) a silver nanoparticles coated flat silicon surface using a solution at 10^{-5} M and (c) a silver nanoparticles coated flat silicon surface using a solution at 10^{-10} M.

Table1: RSD values for the major peaks for the R6G and MB SERS spectrum.

Table1: Estimated RSD values of the main Raman peaks for R6G and MB molecules.

Peak position (cm ⁻¹) R6G	615	775	1127	1182	1310	1362	1508	1573	1649
RSD (%)	23.3	19.0	24.3	14.0	18.3	17.8	13.9	16.8	12.8
Peak position (cm ⁻¹) MB	445	677	768	890	1067	1181	1396	1544	1620
RSD (%)	5.11	4.43	8.30	5.60	6.30	4.45	5.60	4.80	8.21