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Isolating Surface-Enhanced Raman Scattering Hot Spots Using Multiphoton Lithography

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Molecular adsorption sites on macroscopic surface-enhanced Raman scattering (SERS) substrates exhibit a high variance in Raman enhancement factors.[1] As a direct consequence of this broad distribution, molecules adsorbed to the extremely small fraction of sites of extraordinary SERS enhancement ($>10^9$) contribute a large percentage of the total Raman signal measured. Random site adsorption over this distribution poses a problem for reliably measuring SERS signals from a number of molecules that form less than a monolayer of surface coverage. In this Communication, we present a method for isolating hot spots on large area (>25 mm^2) SERS substrates. We use multiphoton-induced exposure of a commercial photoresist on the substrate to uncover only the electromagnetic hot spots. The electromagnetic enhancement at SERS hot spots and the intensity-dependent nature of multiphoton absorption cause preferential isolated exposure of the photoresist at the hot spots. Removal of the exposed photoresist then yields a substrate for which only hot spots are available as adsorption sites. This process does not require prior knowledge of the location or SERS enhancement of hot spots. Compared to a randomly adsorbed submonolayer of analyte molecules on an unprocessed SERS substrate, the same number of molecules adsorbed on isolated hot spots exhibits a 27-fold improvement in average Raman scattering cross section.

The macroscopic SERS substrates used in this work are fabricated using a femtosecond laser-nanostructuring process described elsewhere.[2] The resulting substrate surface is comprised of a quasi-ordered array of cones with an average period of 500 nm, as shown on the left of Figure 1. The cones are covered with aggregates of silver nanoparticles, formed by thermal deposition. These substrates offer a spatially uniform and large ($>10^6$) average SERS enhancement factor over the visible and near-infrared spectral regions. The hot spot isolation (HSI) process is depicted on the right in Figure 1. Positive-tone photoresist (Shipley S1805, MicroChem) is diluted 10:1 in resist thinner (Shipley Thinner-P, MicroChem) and spun onto the substrate at 3000 rpm for 1 min. On a flat silver-coated silicon wafer, this spin speed yields a 30-nm thick layer after a soft bake (120 °C, 1 min). This layer of photoresist prevents analyte molecules from adsorbing to the silver surface of the SERS substrate. We use galvanometric scan mirrors to subject each point on the substrate to 100 pulses from a regeneratively amplified titanium:sapphire femtosecond laser system ($\tau = 60$ fs, $\lambda_{\text{center}} = 795$ nm, 100-kHz repetition rate). The laser pulses are focused by a single lens placed before the scan mirrors to achieve fluences at the substrate in the range 0−400 J/m ^{2}. After fs-laser exposure, a commercial aqueous alkaline developer (Shipley CD-30, MicroChem) is used to remove the exposed photoresist covering the hot spots.

To test the improvement of trace detection due to the HSI process, we dosed both an unprocessed SERS substrate and HSI-processed substrates processed at various exposure fluences with a submonolayer of benzenethiol molecules. We chose benzenethiol as a test molecule because it has a large nonresonant Raman cross section and a strong binding affinity to silver. To ensure submonolayer analyte coverage, we incubated each of the substrates in 4 mL of a 1 pM concentration solution of benzenethiol in ethanol (4 fmol of benzenethiol) for 24 h. Considering the dimensions of the substrate and the packing density of benzenethiol on a silver surface,[3] this dosage (2.4×10^9 molecules) represents a surface coverage of ∼0.001% on the unprocessed sample. We performed micro-Raman spectroscopy on all substrates using 12 mW of 785-nm excitation through a 0.40-NA microscope objective with an

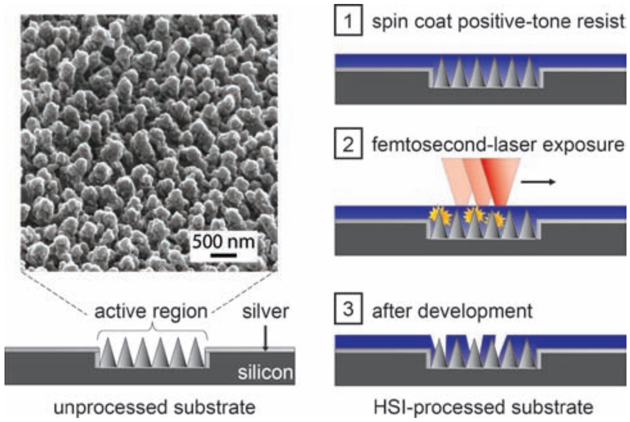

Figure 1. Helium ion microscope image (45°) and schematic diagram of SERS substrate (left). Hot spot isolation (HSI) process (right): (1) Diluted positive-tone photoresist is spin-coated onto a SERS substrate to cover the surface. (2) A femtosecond laser pulse train is scanned over the surface, selectively exposing the photoresist covering electromagnetic hot spots. (3) The photoresist is developed, and the SERS hot spots are uncovered.

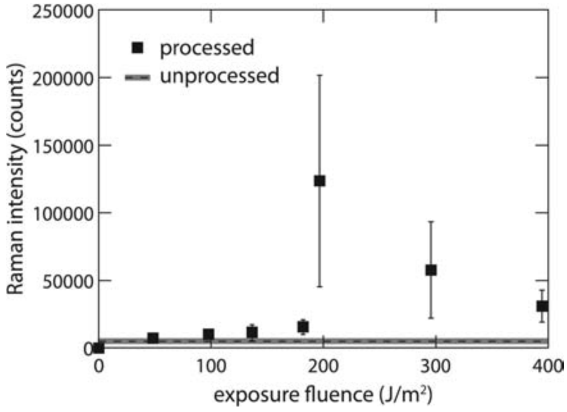

Figure 2. SERS signal as a function of exposure fluence. The average signal from the unprocessed sample is represented as the dashed line, with the standard deviations represented by the gray area. Error bars for the first 3 samples are smaller than the data marker. All data points are obtained by integrating the intensity of the 998 cm^{-1} Raman band of benzenethiol recorded at 10 random locations on each substrate. The signal from the sample processed using an exposure fluence of 200 J/m^2 is 27 times larger than that of the unprocessed sample.

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integration time of 30 s. We measured the full width at half-maximum of the 785-nm laser spot to be 4.2 μm. For each sample we measured the integrated signal-to-baseline intensity of the 998-cm^{-1} ring-breathing mode of benzenethiol at 10 random locations. The average integrated intensity of the 998-cm^{-1} ring-breathing mode of benzenethiol from these spectra is shown in Figure 2. The unprocessed sample's average integrated intensity is plotted as a dashed line, with the gray area representing the standard deviation of the distribution. We find that exposure of the photoresist using a fluence of 200 J/m^2 improves the signal intensity relative to the unprocessed substrate by a factor of 27. To illustrate this improvement, averaged spectra obtained from the sample processed at 200 J/m^2 and the unprocessed sample are shown in Figure 3.

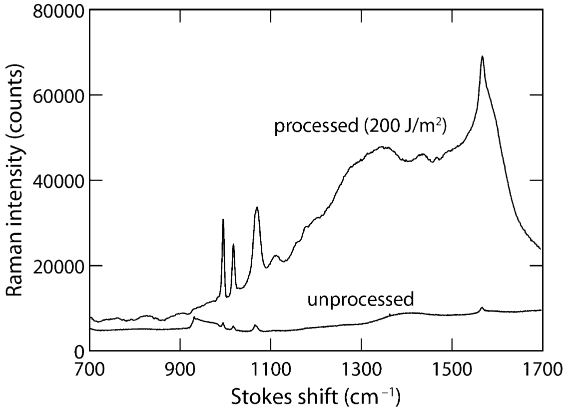

Figure 3. Comparison of Raman spectra from HSI-processed and unprocessed samples incubated with 4 fmol of benzenethiol. Each trace is the average of 10 spectra taken from the 200 J/m^2 and unprocessed sample. No baseline correction is applied to either spectrum; the background in the HSI spectrum is due to C$-$H vibrations in the undeveloped photoresist.

Qualitatively, the trend of the data shown in Figure 2 can be understood as follows. A resist covered sample that has not been exposed to fs-laser pulses exhibits no discernible benzenethiol SERS signal; the molecules are unable to adsorb to the silver surface covered by the photoresist. At low fluences, not enough hot spots are uncovered to generate large signals. At intermediate fluences, we record large improvements in signal relative to the unprocessed sample. Finally, at high fluence values, the photoresist covering areas of weaker enhancement is also exposed and the signal improvement declines, with signal levels approaching those of the unprocessed sample.

Given that our photoresist is optimized for G-line exposure (436 nm) and that our exposure laser wavelength is 795 nm, we attribute the selective photoresist exposure to multiphoton-induced luminescence from an interband electronic transition in silver.[4] The overlap of the luminescence spectrum and the absorption band of the photoresist enables its exposure. We rule out that harmonic generation at hot spots plays a significant role in the HSI process, as the spectrum of the scattered light in our experiment shows that luminescence from the silver is orders of magnitude more intense. Additionally, previous studies of polymerization using ultrafast optical excitation of metallic nanoparticle aggregates have ruled out field-enhanced multiphoton absorption in the photoresist as a dominant exposure mechanism.[5] Finally, we conclude that this effect is both intensity-dependent and nonthermal in nature, as no HSI is observed if the process is performed using comparable continuous-wave excitation. For these reasons, we attribute the selective exposure of the photoresist to multiphoton-induced luminescence, which is expected to be greatest near hot spots, due to its intensity-dependent nature.

Several factors influence the improvement obtained with the HSI process. First, the spatial distribution of hot spots in metallic nanoparticle aggregates depends strongly on the excitation frequency.[6,7] Therefore, the Raman excitation wavelength should be chosen to match the femtosecond exposure wavelength. In this work, the femtosecond pulses are centered at 795 nm, with a full width at half-maximum of 30 nm. The Raman excitation wavelength of 785 nm is within this bandwidth, allowing excitation of the same hot spots that were isolated by the femtosecond pulses. To confirm the need to match the Raman wavelength to the femtosecond exposure wavelength, we probed the same HSI-processed and unprocessed substrates using 632.8-nm excitation. At 632.8 nm, the maximum HSI improvement over the unprocessed substrate is reduced to a factor of 4. Second, the finite resolution of the photoresist influences the HSI performance. The most intense SERS hot spots are often of single or subnanometer dimension,[8] which is less than the current resolution of most photoresists. This finite resolution causes regions of the surface larger than the hot spots to be exposed during HSI, resulting in analyte adsorption both in and around hot spots. This lack of localization of the analyte adsorption and the range of enhancements associated with different hot spots[1] explain the large variance in the data points in Figure 2 at intermediate exposure fluences. Finally, the HSI process is useful only for submonolayer analyte coverages. Only when there are too few molecules to cover all surface sites on a SERS substrate does the HSI process provide an improvement over an unprocessed substrate.

In conclusion, we have presented a method for physically isolating hot spots on a macroscopic SERS substrate. We have demonstrated its effectiveness using one type of substrate only, but the HSI process can be applied to other metallic SERS substrates as well because most noble metals generate broad multiphoton luminescence.[9] Theoretically, we predict even better hot spot isolation using photoresists with absorption spectra that are confined to the ultraviolet spectral region. Such photoresists would be exposed directly by surface-enhanced multiphoton absorption,[10] producing more anisotropic exposure at the hot spots and minimizing the surrounding area that is uncovered during the HSI process. Further optimization of the HSI process in this fashion could eventually lead to substrates where every available molecular adsorption site has sufficient enhancement to generate single-molecule SERS.

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References

(1) Fang, Y.; Seong, N.-H.; Dlott, D. D. *Science* **2008**, *321*, 388–392.
(2) Diebold, E. D.; Mack, N. H.; Doom, S. K.; Mazur, E. *Langmuir* **2009**, *25*, 1790–1794.
(3) McFarland, A.; Young, M.; Dieringer, J.; Van Duyne, R. *J. Phys. Chem. B* **2005**, *109*, 11279–11285.
(4) Kempa, T.; Farrer, R. A.; Giersig, M.; Fourkas, J. T. *Plasmonics* **2006**, *1*, 45–51.
(5) Nah, S.; Li, L.; Fourkas, J. T. *J. Phys. Chem. A* **2009**, *113*, 4416–4422.
(6) Gresillon, S.; Aigouy, L.; Boccara, A.; Rivoal, J.; Quelin, X.; Desmarest, C.; Gadenne, P.; Shubin, V.; Sarychev, A.; Shalaev, V. *Phys. Rev. Lett.* **1999**, *82*, 4520–4523.
(7) Markel, V.; Shalaev, V.; Zhang, P.; Huynh, W.; Tay, L.; Haslett, T.; Moskovits, M. *Phys. Rev. B* **1999**, *59*, 10903–10909.
(8) Camden, J. P.; Dieringer, J. A.; Wang, Y.; Masiello, D. J.; Marks, L. D.; Schatz, G. C.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2008**, *130*, 12616.
(9) Boyd, G.; Yu, Z.; Shen, Y. *Phys. Rev. B* **1986**, *33*, 7923–7936.
(10) Sundaramurthy, A.; Schuck, P.; Conley, N.; Fromm, D.; Kino, G.; Moerner, W. *Nano Lett.* **2006**, *6*, 355–360.

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