Patterned nanoporous gold as an effective SERS template

Yang Jiao, Judson D Ryckman, Peter N Ciesielski, Carlos A Escobar, G Kane Jennings and Sharon M Weiss

Department of Electrical Engineering and Computer Science, Vanderbilt University, Nashville, TN 37235, USA
Interdisciplinary Graduate Program in Materials Science, Vanderbilt University, Nashville, TN 37235, USA
Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN 37235, USA

E-mail: sharon.weiss@vanderbilt.edu

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Abstract

We demonstrate large area two-dimensional arrays of patterned nanoporous gold for use as easy-to-fabricate, cost-effective, and stable surface enhanced Raman scattering (SERS) templates. Using a simple one-step direct imprinting process, subwavelength nanoporous gold (NPG) gratings are defined by densifying appropriate regions of a NPG film. Both the densified NPG and the two-dimensional grating pattern are shown to contribute to the SERS enhancement. The resulting substrates exhibit uniform SERS enhancement factors of at least $10^7$ for a monolayer of adsorbed benzenethiol molecules.

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(Some figures in this article are in colour only in the electronic version)

1. Introduction

Surface enhanced Raman scattering (SERS) has been widely used for uniquely identifying molecules with very high detection sensitivities, and thus is an excellent platform for chemical and biological sensing. SERS provides a drastic enhancement in scattering efficiency over traditional Raman scattering, primarily due to the presence of intense electromagnetic fields localized at the metal surface where molecules are adsorbed [1–3]. Numerous SERS substrates with self-organized metallic nanoscale surface morphologies, including porous templates coated with metal films [4–6], electrochemically roughened metal surfaces [7, 8], colloidal metal nanoparticles [9, 10], metal nanoshells [11, 12], metal nanowires [13], and nanoporous gold (NPG) films [14–16], have been demonstrated to enable high detection sensitivity and even single-molecule detection [6, 17–19]. Highly reproducible nanofabrication techniques have been used to produce, in contrast to these SERS substrates that are characterized by random structures, SERS substrates that exhibit large enhancement factors at spatially defined locations, including nanoparticles of various shapes [20–22], gratings [23], and hole arrays [24, 25]. While nanofabricated SERS templates often obviate the need to search for a ‘hot spot’ where a maximum in the signal enhancement is observed, the required fabrication processes tend to be expensive and time-consuming, which limits cost-effectiveness and the potential for mass production.

In this work, we report an easy-to-fabricate, uniform, and sensitive SERS-active substrate that combines the self-organized and highly interacting nanoscale morphology of NPG with the advantages of reproducibly nanopatterned periodic structures. NPG is a low cost material ($\sim$6 cents cm$^{-2}$) that can be fabricated in a straightforward manner as a thin film chemically bound to glass and gold supports [26]. Although as-prepared NPG films have been reported for strong SERS intensity [14–16], we will show that utilizing a straightforward process to imprint two-dimensional square grating patterns in NPG films significantly enhances the SERS signal and further provides uniform SERS substrates without hot spots.
2. The experiment

2.1. Fabrication of nanoporous gold

We have reported the detailed fabrication of nanoporous gold (NPG) films elsewhere [26]. Briefly, a Ag\textsubscript{50}Au\textsubscript{50} film with thickness of approximately 160 nm is dealloyed in 70% HNO\textsubscript{3} for 15 min at room temperature to dissolve the silver. The dealloyed NPG is then transferred from the HNO\textsubscript{3} solution to deionized water for rinsing using a glass slide. Finally, the NPG is transferred to a 1,6-hexanediithiol-modified gold substrate on a silicon support for robust anchoring. The feature size of the NPG produced by this method can be controlled by adjusting the dealloying time. SEM imaging indicates that our NPG has pore openings of approximately 15 nm after the 15 min dealloying period at room temperature.

2.2. The imprinting method for nanoporous gold gratings

Our NPG SERS substrates are fabricated by imprinting as-prepared NPG films using a newly developed one-step stamping technique, DIPS (direct imprinting of porous substrates) [27, 28]. Figure 1(a) shows the schematic fabrication process of our patterned nanoporous gold (P-NPG) SERS substrate. Reusable silicon stamps (area = 9 mm\textsuperscript{2}) consisting of 2D gratings with variable periodicity were first fabricated using standard electron beam lithography and reactive-ion etching techniques, in a similar manner to what we reported previously [27, 28]. The silicon stamp was then pressed against the NPG substrate by applying a force in the range of 4.5 \times 10^2–2.7 \times 10^3 N to fully transfer the 2D grating pattern into the NPG. In addition to creating the 2D grating pattern, the DIPS process simultaneously forms a locally compressed NPG network, which we will show also contributes to the significantly enhanced SERS intensity that is observed. Figure 1(b) shows a plan view SEM image of a representative surface morphology of a P-NPG structure prepared at 1.5 \times 10^3 N with grating period \(\Lambda = 350\) nm and duty cycle \(f = 70\%\). The SEM image reveals that the pore openings on the grating ridges (unstamped region) are unaffected, while the pore openings on the grating grooves (stamped region) are reduced in size due to compression. The silicon stamp can be reused multiple times without any degradation of the pattern transfer [28], which makes our P-NPG SERS substrates highly reproducible and cost-effective compared to other nanoscale and microscale patterned SERS substrates.

2.3. SERS measurement

The SERS enhancement of our P-NPG substrate was investigated through the detection of a monolayer of adsorbed benzenethiol molecules. Each P-NPG sample was immersed in a 0.2 mM benzenethiol solution in ethanol for 1 h, and the samples were subsequently rinsed with ethanol and dried with nitrogen. SERS spectra were collected over an angular range of \(\approx 128^\circ\) using an XploRA 730 Raman microscope (Horiba-Jobin-Yvon) with 100 \times magnification, integration time of 20 s, and accumulations of five scans. The Raman microscope was run under low power of 0.9 mW from a 785 nm diode laser with a spot size on the order of 1 \(\mu\)m. Normal incidence of light was used in all SERS measurements, which enabled stable and reproducible SERS signals.

2.4. Reductive desorption measurements

Reductive desorption of benzenethiolate molecules in a deaerated phosphate buffer (pH 8.3) was performed in order to quantify the number of molecules per geometric area adsorbed to various SERS substrates. Using this technique, the accessible surface area provided by a P-NPG substrate as compared to a planar gold substrate and to Klarite\textsuperscript{\textregistered}, a commercially available gold standard substrate often employed in surface enhanced Raman spectroscopy, was determined. It is assumed that the area occupied by a benzenethiolate molecule is identical on the three substrates; hence, the integrated current measured by the reductive desorption technique scales with the relative surface area of the substrates. Voltammetric scans were performed with a Gamry Instruments CMS300 electrochemical system using a Ag/AgCl reference electrode, a gold counter electrode, and a working electrode that consisted of P-NPG that had been exposed to a 1 mM solution of benzenethiol
in ethanol for at least 1 h. Two independently prepared samples of P-NPG substrates were used for the experiments. For comparison, a gold-coated silicon wafer and a Klarite® substrate that were similarly exposed to a 1 mM ethanolic solution of benzenethiol were also used as working electrodes. Likewise, two independently prepared samples were used for each control. The potential was swept from 0 to $-1.2\, \text{V}$, with a scan rate of $200\, \text{mV} \, \text{s}^{-1}$.

### 3. Results and discussion

#### 3.1. SERS measurements from 2D P-NPG with various grating periods

Figure 2(a) shows the SERS spectrum from a P-NPG substrate with a grating period of 650 nm along with that from an as-prepared NPG film. A significant enhancement of the SERS signal intensity is observed for the P-NPG sample. Since the spot size of the laser beam used for the SERS measurements is larger than both the small pores of the NPG film and the imprinted grating squares, reproducible and uniform spectral intensity was observed across large areas ($>100 \, \mu\text{m}^2$) of the patterned film with <10% intensity variation (see supplementary information, figure S1 available at stacks.iop.org/Nano/22/295302/mmedia). The uniformity of the P-NPG substrate was limited primarily by the uniformity of the DIPS process across the substrate. The SERS intensities collected from three identically prepared P-NPG substrates varied by approximately 10–15%, again likely due to non-uniformity in the DIPS process, and repeated SERS measurements on a specific region of a P-NPG substrate exhibited no detectable SERS intensity change over the course of 15 days. In order to understand the origin of the enhancement due to the patterning of the NPG film, two sets of experiments were performed to isolate the influence of the grating pattern and the influence of the NPG film densification that occurs during the DIPS fabrication process. The experiment carried out to estimate the grating enhancement is described in this section and the experiment carried out to estimate the enhancement due to NPG densification is described in section 3.2.

In order to determine the influence of the grating period, P-NPG samples with various grating periods ranging from 350 to 750 nm were fabricated using the DIPS process (figure 2(c)). As shown in the inset of figure 2(a), a variation in the SERS response depending on grating pitch is observed. The SERS intensity reported for each grating period is the average SERS intensity measured on 8–10 different spots on each sample. It has been reported that a grating period-dependent SERS response from grating-based SERS substrates can be attributed to the activation of a surface plasmon resonance (SPR), which we believe also plays a role in the grating-dependent SERS response of the P-NPG substrates [1, 23]. Further evidence suggesting the important role of the SPR is found when comparing the SERS response of a simple 1D...
P-NPG grating ($\lambda = 550$ nm) under TE and TM excitation (figure 2(b)). Activation of a SPR is only expected for TM polarized light, and we do indeed observe a stronger SERS response under TM excitation. Note that the 1D gratings are utilized since the 2D gratings are polarization independent for the Raman laser excitation at normal incidence. 1D P-NPG substrates with other grating periods ranging from 450 to 950 nm are also investigated under both TE and TM excitations (see supplementary information, figure S2 available at stacks.iop.org/Nano/22/295302/mmedia). The maximum TM excited SERS intensity is achieved around $\lambda = 550$ nm. The SERS responses for larger grating periods of 750–950 nm are found to be comparable between TE and TM excitation, suggesting that these grating periods cannot satisfy the SPR excitation condition and that the anisotropy of the 1D structure is not the dominant factor in the polarization-dependent SERS intensity measured at shorter grating periods.

Generally, the maximum SERS signal is detected at an emission angle that corresponds to the plasmon resonance angle. However, since our measurement configuration utilizes a high magnification objective with a large numerical aperture, it is not possible to accommodate narrow angular selection for the observation of the highest SERS enhancement. Hence, our P-NPG structures were fabricated with relatively deep gratings ($\sim 60$ nm, as determined by AFM) that enable activation of SPR and collection of the SERS emitted signal over a broad angular range [23]. The SERS response trend shown in the inset of figure 2(a) suggests that activation of a plasmon is possible for grating periods between 450 and 750 nm, but not likely for 350 nm. We note that the measurable SERS response does depend on the grating depth, and further tuning of the grating height may lead to a stronger SERS signal. We also note that P-NPG substrates with smaller grating periods result in larger available surface area within the excitation laser beam, which leads to a $\sim 15\%$ difference in surface area available for benzenethiol adsorption between 350 and 750 nm gratings. The number of benzenethiol molecules adsorbed to a substrate contributes linearly to the SERS intensity; therefore, the trend shown in figure 2(a) cannot be solely due to the different numbers of adsorbed molecules on each substrate.

### 3.2. SERS measurements from densified NPG

The potential contribution of the densified NPG network surrounding the NPG grating squares to the SERS enhancement from P-NPG substrates was isolated by imprinting a NPG film with an unpatterned 9 mm$^2$ silicon square, which effectively densified the porous substrate to $\sim 70\%$ of its original thickness without imprinting any grating pattern. Figure 2(d) illustrates the SERS spectra of benzenethiol adsorbed on as-prepared NPG and on uniformly densified NPG. The inset shows the SEM image of the interface between the imprinted (right) and as-prepared NPG film (left). The surface topology and corresponding SERS response of the densified NPG film is significantly modified by the applied pressure during the DIPS process; the densified NPG shows SERS intensity $\sim 10$–15 times higher than that of the as-prepared NPG substrate. Although use of the unpatterned stamp results in a reduced imprint depth compared to the 1D or 2D grating due to the larger contact area, variations in the SERS intensity measured with slightly different imprint depths were far less than the one-order-of-magnitude SERS enhancement over as-prepared NPG substrates. We believe the enhancement produced by densified NPG can be partly attributed to nanoscale morphological changes. Under the pressure applied during the DIPS process, the pore size and distance between neighboring gold ligaments is decreased, promoting stronger localized fields at pores where SERS-active molecules are attached. Previous work similarly showed that NPG films with smaller pores [15, 16], and compressed metal pellets [29], generate stronger SERS signals due to the enhancement of localized electromagnetic fields.

By examining the peak heights of the SERS spectra in figures 2(a) and (d), we can compare the relative contributions of densified NPG and the 2D grating pattern to the overall SERS enhancement exhibited by our P-NPG substrates. While straightforward densification contributes approximately one-order-of-magnitude SERS enhancement over as-prepared NPG, the dominant contribution to the overall SERS enhancement is due to the imprinted grating patterns, providing an additional one-order-of-magnitude enhancement.

### 3.3. Calculation of SERS enhancement

In order to estimate an overall SERS enhancement factor relative to non-enhancing substrates, we compare the SERS signal from our $\lambda = 650$ nm grating period P-NPG substrate with a Klarite® commercial SERS substrate, as shown in figure 3. The Klarite® SERS substrate consists of gold-coated textured silicon (regular arrays of inverted pyramids $\sim 1.5$ μm wide and $\sim 1$ μm deep) mounted on a glass microscope slide. The enhancement factor for the inverted pyramid structure of Klarite® substrates relative to a non-enhancing surface is rated to have a lower bound of $\sim 10^6$ [30]. Using this lower bound
enhancement factor specified for Klarite® as a reference, we estimate the SERS enhancement factor of our \( \Lambda = 650 \) nm P-NPG substrates using the following equation:

\[
EF_{P-NPG} = EF_{Klarite} \frac{N_{Klarite} I_{P-NPG}}{N_{P-NPG} I_{Klarite}}
\]

where \( EF_{Klarite} \) is taken as \( 10^6 \), \( N_{Klarite} \) and \( N_{P-NPG} \) are the estimated number of benzenethiol molecules adsorbed on the Klarite® and P-NPG samples, respectively, and \( I_{P-NPG} \) and \( I_{Klarite} \) are the respective SERS intensities at the 1070 cm\(^{-1}\) band, one of the well-known and most distinct benzenethiol Raman signatures. Voltammetric scans for the reductive desorption of benzenethiol molecules were performed to estimate the number of adsorbed benzenethiol molecules on P-NPG and Klarite substrates, as well as on a planar gold electrode for comparison. As shown in figure 4, each voltammetric scan exhibits two cathodic current peaks that we ascribe to thiolates bound at different surface sites on these gold surfaces [31]. As compared to the planar gold electrode, the P-NPG electrode shows broader current peaks that we attribute to the different gold surface structures within this nanoporous metal film. Integration of these peaks is used to obtain a charge that is proportional to the number of benzenethiolate molecules desorbed from the surface. For planar gold, the integrated charge of the two peaks (56 ± 2 \( \mu \)C cm\(^{-2}\)) corresponds to an average of \( 3.5 \times 10^{14} \) mol cm\(^{-2}\), which is similar to the value of \( 3.2 \times 10^{14} \) mol cm\(^{-2}\) reported by Wan et al [32] and translates into a fractional coverage of 0.25. The integrated charges for the nanoporous gold and Klarite® were 108 ± 1 and 75 ± 3 \( \mu \)C cm\(^{-2}\), respectively. The average integrated charge ratio of stamped nanoporous gold to planar gold, both shown on a basis of 1 cm\(^2\) of geometric area, was approximately 1.9, meaning that the stamped electrode has ~90% more bound benzenethiolate molecules than the planar gold electrode does. The reductive desorption of benzenethiolate molecules from Klarite® exhibits two broad current peaks that are shifted to lower potentials than those on nanoporous gold or planar gold. The comparison between the stamped nanoporous gold substrate and Klarite® resulted in a current ratio of approximately 1.4, suggesting that 40% more molecules are adsorbed onto the P-NPG sample such that \( N_{Klarite}/N_{P-NPG} = 0.71 \). From figure 3, the measured SERS intensity ratio at 1070 cm\(^{-1}\) (\( I_{P-NPG}/I_{Klarite} \)) is 14. Therefore, we estimate that the lower bound enhancement factor of the P-NPG substrate is on the order of \( 10^7 \), which is one order of magnitude higher than that of the Klarite® commercial substrate.

4. Conclusions

In summary, we have used a simple one-step stamping technique, DIPS, to demonstrate the rapid, cost-effective nanofabrication of P-NPG SERS-active substrates. The P-NPG SERS templates exhibited efficient and reproducible SERS enhancement of at least \( 10^7 \). The well-organized 2D periodic NPG gratings and surrounding densified NPG film were both shown to contribute to the significantly enhanced SERS activity. At least one-order-of-magnitude higher SERS intensity was observed on the basis of our stamped 650 nm pitch, 60 nm deep grating structure compared to that of a commercial Klarite® substrate. Higher SERS intensity could be expected by properly tuning the pitch, depth, and shape of the gratings in the future.

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