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High Fidelity Nano-Hole Enhanced Raman Spectroscopy

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Abstract

Surface Enhanced Raman Spectroscopy (SERS) is a sensitive technique that can even detect single molecules. However, in many SERS applications, the strongly inhomogeneous distribution of intense local fields makes it very difficult for a quantitative assessment of the fidelity, or reproducibility of the signal, which limits the application of SERS. Herein we report the development of exceptionally high fidelity Hole-Enhanced Raman Spectroscopy (HERS) from ordered, two-dimensional hexagonal nanohole arrays. We take the fidelity f to be a measure of the percent deviation of the Raman peaks from measurement to measurement. Overall, area averaged fidelities for 12 gold array samples ranged from $f \sim 2\% - 15\%$ for HERS using aqueous R6G molecules. Furthermore, intensity modulations of the enhanced Raman spectra were measured for the first time as a function of polarization angle. The best of these measurements, which focus on static laser spots on the sample, could be consistent with even higher fidelities than the area-averaged results. Nanohole arrays in silver provided supporting polarization measurements and a more complete enhanced Raman fingerprint for phenylalanine molecules. We also carried out finite-difference time-domain calculations to assist in the interpretation of the experiments, identifying the polarization dependence as possibly arising from hole-hole interactions. Our results represent a step towards making quantitative and reproducible enhanced Raman measurements possible and also open new avenues for a large scale source of highly uniform hot spots.

Introduction

Although Raman spectroscopy is a powerful analytical tool due to its nondestructive nature and structural fingerprinting capability with very narrow and highly resolved bands (0.1 nm), the Raman effect is particularly weak. Typically only 1 in 10^8 incident photons are inelastically scattered. Therefore, obtaining a reliable Raman spectrum from just a few molecules requires collection times on the order of minutes, rather than seconds, and fairly high laser power. The Raman process can be enhanced by many orders of magnitude compared with the normal Raman scattering process by using a substrate (a metal particle or a rough metallic surface) for Surface Enhanced Raman Spectroscopy (SERS).^{1–5} The SERS enhancement is thought to be the result of a combination of metal-molecule chemical effects and intense, localized fields (hot spots) arising from surface plasmon resonances in and between metallic nanoparticles (junctions).^{6–12} Unfortunately, random and uncontrolled positioning of metallic nanoparticles

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precludes any direct control over the strength, polarization, and spatial distribution of these local fields and adversely affects the observed spectral fidelity, f , a figure of merit representing the percent deviation of the Raman signal from measurement to measurement. Recent work with nanopillar arrays¹³, inverted pits¹⁴, and nanostructured films¹⁵ have achieved $f \sim 15\%$, $\sim 10\%$, $> 5.5\%$ (respectively).

Nanohole structures also exhibit localized fields that can be exploited for Raman enhancements. Recently, we reported *non-resonant* Hole-Enhanced Raman Spectroscopy (HERS) from films containing randomly distributed nanoholes and the concept of an enhanced Raman spectroscopic medium containing stable 2-D hot spots.¹⁶ Holes in thin metal films have demonstrated a number of advantages over substrates based on metal colloids (particles). Holes tend to be more mechanically stable than junctions; therefore, they fluctuate less and have a high degree of spatial uniformity. Random holes also have a single broad plasmon resonance, making them less sensitive to excitation wavelength.¹⁷ Furthermore, plasmon resonance maxima can be tuned to the excitation wavelength by controlling the hole diameter, the average center-to-center (c-c) interhole distance (hole surface density) and the refractive index of adjoining dielectric surfaces.

It is well established that propagating surface plasmon polaritons (SPPs) are launched from the edges of holes in metal films along the incident polarization angle.^{18,19} It is also the case that near-field intensities in the proximity of these hole edges can be enhanced relative to the incident intensity owing to stationary or local surface plasmon (LSP) excitations.^{17,19} These excitations have been shown to be analogous to the corresponding excitations in metal nanoparticles.¹⁷ Three dimensional FDTD calculations (discussed below) for periodicities associated with 100 nm diameter holes in hexagonal hole arrays (200–346 nm c-c spacings) with an incident wavelength of 633 nm show that LSP excitations are expected to be the dominant source for the enhanced Raman spectra.

In this paper, we report the extension of HERS to an ordered nanohole array substrate, aimed at dramatically improving and bench-marking the fidelity for enhanced Raman spectroscopy, thus enabling quantitative substrate enhanced Raman characterization of molecules. The new results include: 1) fabrication of a novel HERS substrate composed of ordered, 2-D arrays of holes in gold or silver film using electron-beam lithography, 2) determination of excellent area-averaged spectral fidelities ($f \sim 2 - 15\%$) for HERS of aqueous R6G molecules 3) quantification of R6G Raman intensity modulations as a function of laser polarization, 4) improved spectral fingerprint for phenylalanine molecules and 5) 3-D finite-difference time-domain (FDTD) calculations to assist in the interpretation of our experimental results.

Experimental Methods

The nanohole arrays were fabricated by combining electron-beam lithography (EBL) with subsequent metal evaporation. As substrates we used regular glass ¼ circle cover slips with a thickness of $\sim 150 \mu\text{m}$. The fabrication procedure started with substrate cleaning (organic solvents followed by short intervals of reactive ion etching with CF_4 and O_2). A layer of ~ 200 nm thick PMMA (950K, 3 wt% PMMA in Anisole) was then spin-coated onto the etched side of the substrate and baked for 1 hr at $150\text{--}175^\circ\text{C}$. To reduce charging effects during the subsequent EBL, a thin layer (4 to 6 nm thickness) of Cr was then evaporated on top of the PMMA. A Hitachi S-2700 Scanning Electron Microscope (SEM) equipped with a Nabity pattern generator was used to write the array pattern into the PMMA. After removal of the Cr layer with etchant solution, the pattern was developed in MIBK solution (MIBK : IPA = 1 : 3) for 30 seconds and blown dry with grade 5 nitrogen.

To metalize the array pattern, we used oblique-angle evaporation. The sample was mounted on a rotating stage tilted 45° towards the metal source. To improve the sticking to the PMMA, we evaporated 4–6 nm thick Cr first, followed by 20–30 nm Au. The gold thickness varied with the diameter of the holes in the array. In general, we evaporated thicker Au films for bigger holes (see Table 1). Lift-off of the metal covering areas not exposed by the electron beam was achieved by soaking the sample in acetone. A similar procedure was used for silver metalization.

Nanohole array patterns on cover slips were prepared for recording liquid phase HERS spectra (aqueous R6G or phenylalanine molecules) by inverted placement onto clean 24×40 mm #1 cover slips to form a fixed gap width between glass surfaces sandwiching the hole array pattern. To mount each hole array sample, the gap spacing was fixed by applying a few μL of a fast drying, water immiscible, adhesive (Duco cement) to each of the three corners of the $\frac{1}{4}$ circle and allow to air dry completely. Small liquid samples ($< 1 \mu\text{L}$) were then carefully introduced by micropipette to the fixed gap by capillary action. With the aqueous liquid samples saturating the hole array pattern within the fixed gap, two of the three sides of the $\frac{1}{4}$ circle were sealed with adhesive and a larger volume of liquid sample ($\sim 10 \mu\text{L}$) was placed in contact with the gap of the third side to serve as a liquid reservoir and prevent the sample dry-out during spectral measurements.

The mounted liquid samples were placed on a previously described inverted microscope (Olympus IX-71) equipped with a 60×/1.2 water immersion objective and computer controlled X-Y stage and then coupled to a spectrograph (Jobin Yvon Triax 320) equipped with a liquid nitrogen cooled CCD detector.¹⁶ In general, area averaged HERS spectra were taken in epi-illumination configuration by scanning a focused laser beam (633 nm @ 1 mW, $I \sim 10^5 \text{ W/cm}^2$). We used fluorescence correlation analysis of standard dye solutions to confirm that the spot size or beam diameter was a diffraction-limited $\sim 0.64 \mu\text{m}$.

Each hole array *sample* we studied, corresponding to a given nanohole diameter, typically consisted of 4 individual ($40 \mu\text{m} \times 34.6 \mu\text{m}$) hexagonal nanohole *arrays* on a $\frac{1}{4}$ circle cover slip. Within each array we chose four random $100 \mu\text{m}^2$ *areas* and measured an area-averaged HERS spectra for each, giving rise to up to 16 HERS spectra per sample. The scanned areas were sampled by a square serpentine raster while collecting spectra at constant exposure time of 10 s (all other relevant instrumental parameters held constant; e.g. laser power, intensity, slit width etc.). All HERS spectra were based on intensity measurements evaluated at the strong xanthene ring stretching feature (1361 cm^{-1}) for R6G (or at the symmetric ring breathing mode (1000 cm^{-1}) for phenylalanine, see Table 2).

A fidelity for each of the four arrays within a given sample is determined for the area-averaged HERS measurements noted above as follows. We do not use the actual peak heights but the difference between the peak maximum and the average intensity at the line wings, or baseline, of the transition. Suppose we have a set of different measurements of this adjusted peak height, $\{I_j\}$, $j = 1, \dots, N$. One measure of fidelity is to find the two measured values of the set, I_b and I_a , that give the largest magnitude difference, $|I_b - I_a|$, and to normalize this maximum deviation by the mean value, $(I_b + I_a)/2$, representing this relative maximum deviation as a percentage:

$$f = \frac{2|I_b - I_a|}{(I_b + I_a)} \times 100\% \quad (1)$$

(The “highest” fidelity is of course given by the smallest percentage value, f .) This definition of fidelity parallels that used in Raman difference spectroscopy.²⁰ Now, within each of the 4 nanohole arrays of a sample, we have available four area-averaged HERS spectra. This

corresponds to 6 unique difference spectra I_1-I_2 , ..., I_3-I_4 , for each array upon which to determine the particular I_a and I_b that lead to the maximum adjusted peak difference and the fidelity, Eq. (1). (Each sample fidelity we report in Table 1 corresponds to the average of the four array fidelities for the sample.)

Results and Discussion

We used electron-beam lithography to prepare 12 gold samples with various uniform hole diameters, d : 2 samples with $d = 80$ nm, 4 samples each with $d = 100$ nm and 120 nm, and 2 samples with $d = 140$ nm. As discussed above, each sample consisted of 4 identical hole arrays (same hole diameters) with dimensions $40\ \mu\text{m} \times 34.6\ \mu\text{m}$. For example, in a sample composed of 100 nm diameter holes, where the shortest c-c distance is 200 nm, each array consisted of approximately 40,000 holes. Figure 1 shows a $10\ \mu\text{m} \times 10\ \mu\text{m}$ area of a hole array (inset $1.3\ \mu\text{m} \times 1.3\ \mu\text{m}$), demonstrating the very high degree of uniformity achievable by this fabrication process.

The average gain (counts/s) and fidelity of aqueous R6G on nanohole arrays in gold films as a function of hole diameter (at constant ratio of c-c spacing/diameter = 2) were measured with results given in Table 1. Background-subtracted peak heights (counts) were measured from each area-averaged spectrum and divided by the integration time of the measurement (all count rates reported herein may be corrected for total system through-put by multiplication by $1/0.013 \sim 77$). Area-averaged spectral data were compared for gain (counts/s) and fidelity. Note that the HERS gain of these substrates did not correlate strongly with either hole diameter or hole spacing, although the data suggest a trend to higher fidelity as the hole size is decreased from 120 nm to 80 nm (however this conclusion is tentative since the 140 nm holes had essentially the same fidelity as the 100 nm holes). The average count rate for all of the 80–140 nm hole samples in Table 1 was ~ 135 counts/s. The average fidelity measured was found to be $f = 8.2\%$ ($\pm 4.5\%$).

For each gold sample considered for Table 1, we also estimated the average enhancement factor, EF , as

$$EF = \frac{I_{\text{HERS}}(\text{counts/s})/N_{\text{HERS}}}{I_{\text{Raman}}(\text{counts/s})/N_{\text{Raman}}}, \quad (2)$$

where N denotes the number of molecules imaged. In general terms, for 100 nm diameter holes with a 200 nm c-c spacing, and taking the laser spot diameter to be $\sim 0.6\ \mu\text{m}$, the maximum number of holes illuminated is ~ 7 . The volume of each hole is $\sim 2.4 \times 10^{-19}$ L, thus for 70 μM R6G (4×10^{19} molecules/L), in solution there are ~ 10 molecules/hole or $10 \times 7 = 70$ molecules imaged by the optical system at the static laser spot. (We take 10 molecules/hole as an upper limit since it is assumed that the enhancement is uniform over the entire nanohole volume while FDTD calculations actually place the largest fields at the edges of the hole bottoms.) For R6G molecules on holes in gold, we typically observe ~ 200 counts/s (Table 1); thus, our optical system detects typically >3 counts/molecule-s. The Raman counting rate for R6G molecules with our system, under identical experimental conditions, is $\sim 2.4 \times 10^{-6}$ counts/molecules-s; thus, $EF \sim 3/2.4 \times 10^{-6} \sim 10^6$. This corresponds to a lower limit for the enhancement factor, which is near the threshold for single molecule detection.⁵

We also determined enhancement factors for the silver samples corresponding to uniform arrays with hole diameters of 60 and 80 nm: $EF \sim 3 \times 10^6$ for 60 nm holes (c-c = 100 nm) and $\sim 3 \times 10^5$ for 80 nm holes (c-c = 160 nm). The latter result is a factor of ~ 6 greater than for the

80 nm (c-c = 160 nm) gold substrates (Table 1), where the overall estimated enhancement factor measured for *all* gold samples was found to be $EF \sim 8 \times 10^4$.

In addition to R6G molecules, we also measured HERS spectra for aqueous (200 mM) phenylalanine from the silver hole arrays with 60 nm diameter holes at a 30 s integration time (see Figure 2a). In contrast, holes in gold film could not produce any spectral features for this amino acid in solution, even for the maximum exposure time permitted by these experiments (~ 100 s, limited by CCD detector saturation due to continuum emission). The subtracted phenylalanine spectrum, illustrated in Figure 2b, demonstrates a high degree of fidelity ($f \sim 50/685 \times 100\% \sim 7\%$ @ 1000 cm^{-1}). For this hole array sample, the measured count rate for phenylalanine molecules was rather low at $\sim 10^{-4}$ counts/molecule-s, but nevertheless the Raman features were quite reproducible. As shown in Table 2, comparison of the spectral assignments with published data from silver and gold colloids shows that the HERS spectrum is a more complete spectral characterization and reveals the presence of four previously unreported Stokes features at 588.6, 676.1, 1180.4, and 1324.3 cm^{-1} .

The area-averaged fidelity results demonstrate the high fidelity our HERS systems have with respect to spatial displacements along our hole arrays. Figure 3a shows two area-averaged HERS spectra of aqueous $70 \mu\text{M}$ R6G from different areas of a 100 nm diameter nanohole array on gold film and the result of subtraction (bottom trace) ($f \sim 4\%$ @ 1361 cm^{-1}). An advantageous consequence of having such high area-averaged fidelities is the reproducibility one gets with even static spectra, i.e. spectra taken from the same static laser spot (no area averaging). Figure 3b shows a series of static R6G HERS spectra, with different exposure times. The intensity was found to be linear with either laser power or integration time (in the range 0–1 mW and 0–80 s). Each of the 4 traces (a–d) provides nearly identical spectral features. The only differences appear in traces c and d, where two new features appear at ~ 1410 and $\sim 1470 \text{ cm}^{-1}$, possibly signifying R6G conformation change or dimer formation. In the absence of a metal film, the liquid $70 \mu\text{M}$ R6G was found to contribute ~ 1 count/s at 1361 cm^{-1} (not shown), while the smooth hole-free metal surface typically contributed ~ 20 counts/s (see bottom trace in Figure 3b).

It is interesting to note that the first Raman scattering enhancement factors for nanohole arrays were reported for benzenethiol molecules on a silver film by Rowlen and coworkers.²⁶ Their measured enhancement factor, $EF \sim (6 \pm 3) \times 10^7$, was attributed to two distinctly different sources: nm surface roughness ($EF \sim 10^5$) and nanoholes ($EF \sim 600$); thus only about 0.6% of the total enhancement was due to nanoholes. In contrast, the results Figure 3b) suggest the nanohole systems studied here show ~ 6 times *greater* enhancement from nanoholes than from surface roughness. Moreover, the FDTD calculations of Rowlen, *et al.* predict the highest electric field localization at the air/silver interface while the FDTD calculations reported here place it at the glass/gold interface.

To determine the extent to which short-range hole-hole coupling might contribute to the HERS gain, time-averaged HERS spectra of R6G were recorded as a function of excitation polarization angle in 20° increments (see Figure 4). In these measurements, the A-axis (c-c spacing = 200 nm) was set to be parallel with laser polarization at 0° . As the polarization angle of the laser increased, maxima were found to occur whenever the laser polarization angle was at 60° , 120° , etc. Thus, the maxima correlated roughly with the laser polarization parallel to the A-axis (conversely minima at 30° , 90° , ..., corresponded to alignment with the B-axis with c-c spacing = 346 nm.). The experimental data (e.g. Figure 4, symbols) were normalized to the average intensity and fit to the expression $y = a_1 + a_2\theta + a_3 \cos(a_4\theta + a_5)$ (e.g. Figure 4, curves). For holes in gold (# of samples = 12), the average measured intensity amplitude (a_3) was found to be $a_3 = 0.057$ (std. = 0.021). The average angular period (from a_4) was 62.7° (std. = 4.33). For silver hole arrays (# of samples = 6) the average amplitude was $a_3 = 0.097$ (std. = 0.024), with

an average period of 58.7° (std.=2.80). For all hole samples (# of samples = 18) the average amplitude and period was $a_3=0.066$ (std.=0.030) and 61.5° (std.=3.83). There was typically some overall drift ($\pm a_2$) in each data set since it required 3–5 minutes for each measurement. The drift was well represented by a term linear in θ (since θ was linear in time), coefficients (a_2) could be positive or negative (the source of such drifts is not yet established). It is worth noting that the symmetry reflected in these polarization experiments may be due primarily to hole-hole interactions, since the measured intensity modulations reflect the hexagonal symmetry of the arrays. See also the FDTD calculations below which further confirm this picture.

It is also worth noting that the data in Figure 4 would not be observable from a low fidelity substrate. In particular, the lowest intensity amplitude variations observed, 2–4% (Figure 4, blue), suggest that under some circumstances static HERS spectra with fidelities better than 2 % can be achieved. In related polarization experiments, Odom and coworkers performed polarization dependent enhanced optical transmission (EOT) measurements for microscale square arrays of nanoholes in gold film.²⁷ The measured EOT for circular holes at normal incidence and for polarization alignment with different lattice axes showed *no noticeable difference*. In other related work, Le Ru and coworkers²⁸ measured polarization resolved relative enhancements for R6G molecules on square arrays of oblate spherical as well as prolate elliptically shaped gold *nanoparticles* with the polarization in alignment with one or both of the two principal array axes (resolved both in excitation as well as detection). However, the measurements represent Raman enhancements due to particle shapes rather than interparticle (the complement to hole-hole) interactions.

To better understand our experimental results, we carried out 3-D finite-difference time-domain (FDTD) calculations.^{19,29,30} The technical details of the calculations were similar to those of prior work.^{19,30} The calculations employed periodic boundary conditions consistent with a hexagonal array of holes in a gold film with glass (refractive index $n = 1.5$) below and with water ($n = 1.33$) above. The incident light was normal from within the water, and we calculated the electromagnetic near-field enhancements consistent with an incident wavelength of $\lambda_0 = 633$ nm. Similar to the experimental setup, we used a gold film thickness of 26 nm, 100 nm diameter circular holes, and a hexagonal lattice constant of $a_0 = 200$ nm, which corresponds to nearest neighbor c-c separation of the holes along rows that are parallel to the A-axis of the hexagonal array. The c-c separation of holes in rows parallel to the B-axis is $\sqrt{3} a_0$. We carried out separate calculations with the incident light polarized along the A-axis and along the B-axis. To obtain reliable near-field amplitudes, we used 0.5 nm grid spacings.

Our FDTD calculations showed that the largest intensity enhancements, g^2 , where $g^2 = |E|^2/|E_0|^2$ with E being the total electric field and E_0 being the incident electric field, occur in the hole bottoms near the glass. Figure 5 contains a representative plot of g^2 for a two-dimensional slice of the unit cell within the hole ~ 2 nm above the glass bottom (note Figure 5 corresponds to polarization oriented along the A-axis, and values for $g^2 > 20$ are shown saturated so as to see the small hole-hole interactions). Typically, maximum values of g^2 are $\sim 10^3$ in these regions. This implies HERS enhancement factors of $g^4 \sim 10^6$. (This is a moderate enhancement factor. However, imperfections in the holes and film can lead to finer nanoscale surface variations and larger enhancements.)

While having incident light polarized along the A-axis and along the B-axis represent two distinct physical situations, we found only very small differences in the near-fields for the two cases and almost identical far-field zero-order transmission ($T \approx 0.65$ at $\lambda_0 = 633$ nm). This latter result is consistent with prior observations.³¹ Regarding the predicted HERS enhancements, we find that g^4 is approximately 2% higher for A compared to B. While these calculations do not result in fidelities (measures of experimental reproducibility), they do

suggest that high quality, and thus high fidelity, experiments should yield a slight dependence of HERS enhancement on polarization. This slight dependence is consistent with the smallest of the HERS experimental gains in Figure 4 (blue).

We attribute the similarities of the A-axis and B-axis polarization cases to the fact that, due to the relatively large hole separations, the individual hole surface plasmon excitations are largely confined to the hole edges and thus only weakly interact with neighboring holes. We also carried out similar FDTD calculations with $a_0 = 110$ nm, keeping all else the same, thereby reducing the distance between neighboring holes and found more significant asymmetry between the near-fields for A-axis and B-axis incident polarizations, with the A-axis incident polarization near-field intensities being typically 10% larger than the B-axis case near the hole bottoms. It should also be noted, for the $a_0 = 200$ nm lattice constant, that $\lambda_0 = 633$ nm is too large to excite any collective Bloch wave or Bragg surface plasmons.³²

While a nanohole array can be fabricated by other methodologies, such as ion beam milling³³, nanosphere lithography³⁴, nanoimprint³⁰, and PEEL (phase shifting photolithography etching electron-beam deposition and lift-off),³⁵ the electron-beam lithography (EBL) method described here provides several advantages. Compared to nanosphere lithography, EBL allows for the implementation of arbitrarily shaped two-dimensional patterns, is defect-free, and is not limited to the close-packed hole arrangement demonstrated here. At the same time, with EBL it is possible to produce very small feature sizes. Finally, the oblique angle evaporation on a rotating sample holder and the small film thickness lead to highly uniform metal coverage. As the inset to Fig. 1 shows, the metal films consist of densely packed grains, with typical sizes ~ 40 nm. While the new fabrication method reported herein uses PMMA, all detectable traces of this compound are removed. This provides a substrate with continuum emission, originating from the metallic structures and enhanced fluorescence, that is highly reproducible and therefore reliably subtracted (Figures 2b and 3a), as requisite for enabling enhanced Raman difference spectroscopy. Although nanoholes in gold films have less gain than for silver, they were better suited for this study for two reasons. Gold films were found to be more resistant to optical or chemical damage, and they have a longer shelf life. (Hole arrays in silver were easily damaged by optical intensities of $\sim 10^5$ W/cm². No optical damage threshold for holes in gold could be observed with our laser, but is at least three times higher than for silver. Optical damage appeared as thin, 1–2 μ m etched tracks in the silver hole arrays. Holes in silver were also observed to degrade in contact with aqueous samples, particularly when the liquid had a high ionic strength, such evidence of optical or chemical attack was not observed with any of the gold substrates. Thirty day storage of gold arrays in desiccators at room temperature typically had $\sim 30\%$ lower gain than fresh arrays; silver displayed significantly larger losses that were difficult to quantify.

Although Raman scattering has been enhanced to the degree that single molecule SERS is achievable, without adequate fidelity, full spectrum characterization (up to single molecule) and quantification by Raman spectroscopy remains challenging. Initial investigations of nanohole arrays as Raman substrates successfully detected small numbers of Oxa-720 dye molecules.³⁶ Since then there have been significant improvements in the gain of these substrates, e.g. through careful optimization of hole spacings and shapes.³⁷

Detailed comparisons by Parsons et al³⁸ of arrays of cylindrical holes with comparable disk-shaped particles indicate approximately two-fold larger LSP fields for particle arrays are possible. However, increased enhancement from hole arrays is possible through modification of the hole shape. For example, Koerkamp et al³⁹ observed 10-fold transmission enhancements when hole shapes are changed from circular to rectangular and Lesuffleur et al³⁷ observed a 12-fold increase in Raman from oxazine 720 molecules on double-hole apertures arrays versus arrays of single holes.

However, further improvements in fidelity are highly desirable. Our ordered nanohole array substrate aims at dramatically improving fidelity as well as enhancement factors while providing complete spectral characterization. The nominal fidelity obtained for well established Raman Difference Spectroscopy is $f \sim 0.1\%$.^{20,40} Thus, since the fidelity of these nanohole substrates lies within a factor of 10 of well established Raman difference spectroscopy methodology, the realization of “subtractive HERS” appears within reach. The factors limiting fidelity for these substrates are not known, however close inspection of hole array AFM images do show small irregularities which includes non-circular hole shapes,^{37, 39} crystalline or granular structures at the hole perimeters, and small height variations in film topography. Plausibly, the fidelity of these substrates can be further improved with optimization of hole shapes and spacings (and substrate composition) and further reductions in surface irregularities. In the near future, we anticipate new analytic applications for these substrates, in particular subtractive HERS, with further improvements in enhancement.

Conclusions

In conclusion, a novel new technique for preparing highly uniform 2-D nanohole arrays in gold and silver by EBL and oblique metal evaporation was established. Area-averaged HERS measurements for the gold hole array substrates indicate sufficient gain to detect small numbers of R6G molecules at area-averaged fidelities of $\sim 2\% - 15\%$. Spectra of aqueous phenylalanine from nanoholes in silver film provided a more complete enhanced Raman spectrum, including four previously unreported Raman features. Static HERS spectra exhibited interesting intensity modulations consistent with hole-hole interactions and possibly even better fidelities.

Nanohole array substrates and hole enhanced Raman spectroscopy not only make quantitative and reproducible enhanced Raman measurements possible, but also open new avenues for characterizing molecular orientation and hierarchical structures as the method is sensitive to the orientation of the sample on the substrate. Extending the nanohole arrays to the scale of inches is currently under experimental investigation.

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Figure 1. AFM image of nanohole array in thin gold film on a glass substrate (field of view is $10 \times 10 \mu\text{m}$). Inset shows a zoomed-in detail (field of view is $1.3 \times 1.3 \mu\text{m}$). The average hole diameter is 120 nm and the hole depth is 35 nm.

Figure 2.

Phenylalanine (200 mM aqueous) on 60 nm silver hole array a) Sequential 30 second HERS spectra (slit 0.2 mm) of aqueous phenylalanine on 60 nm nanohole array (spacing 100 nm) in silver film (see Table 2 for line positions and assignments). b) Difference HERS spectrum obtained by subtracting top and bottom traces of Figure 2a.

Figure 3.

a) Area-averaged HERS spectra of aqueous R6G molecules on holes in gold film (trace b was vertically displaced by -100 counts from trace a for clarity). Bottom trace: difference spectrum (trace a - trace b) illustrating high fidelity ($f \sim 4\%$) @ 1361 cm^{-1} ; b) HERS spectra, from ~ 7 nanoholes, of aqueous $70\text{ }\mu\text{M}$ R6G molecules on 100 nm diameter nanoholes in gold film. Bottom trace (not labeled): metal film only (no holes) (integration time 10 s). Trace (a) film with holes (10 s), trace (b) 20 s , trace (c) 40 s , and trace (d) 80 s .

Figure 4.

HERS intensity of R6G from ~ 7 holes versus polarization angle, illustrating nearest-neighbor inter-hole contributions to HERS gain (2–9 % amplitude modulation) for three data sets (intensities were normalized to the average intensity). The solid curves represent fits to the intensity data (symbols) from three different, representative array samples.

Figure 5.

Two-dimensional slice of g^2 for unit cell of hole-array corresponding to 100 nm diameter holes with nearest c-c distances = 200 nm and incident light polarized with the y-axis (A-axis). Values of $g^2 > 20$ are saturated so as to see the weak hole-hole interactions. Maximum values of g^2 were $\sim 10^3$ for the current setup.

Area-averaged HERS results for 12 separate nanohole samples in gold films based on intensity measurements at the 1361 cm⁻¹ line of R6G. Column 1: diameter, *d*, of holes in a given sample. Columns 2 – 5: counts/s and calculated fidelity (*f*) (Eq. 1) for each array in the sample. Column 6: average count/s, average *f*, and estimated enhancement factor [*EF*] (See Eq. 2.). Bottom row: overall averages. Counts can be corrected for total system through-put by dividing by 0.013.¹⁶ For each sample, the smaller c-c distance is twice the hole diameter. Typical chromium and gold film thicknesses were: (4.5, 20), (5.0, 25), (5.0, 30), and (5.0, 35), respectively.

Table 1

<i>d</i> (nm)	counts/s (<i>f</i>)				Averages, [<i>EF</i>]
80	49 (3.6%)	144 (2.1%)			97 (2.9%), [$\sim 5 \times 10^4$]
100	280 (1.7%)	160	(5.6%)	56 (13%)	97 (8.7%)
120	124 (1.3%)	120	(14%)	156 (15%)	148 (7.3%), [$\sim 10^5$]
140	206 (5.9%)	150	(9%)		119 (12%), [$\sim 7 \times 10^4$]
					178 (7.5%), [$\sim 10^5$]
					Overall Averages: 135 (8.2%) [$\sim 8 \times 10^4$]

Table 2Phenylalanine Stokes shifts (cm^{-1}) and assignments (from spectra of Figure 2).

Stokes Shift [this work] (Nanoholes/Ag)	Stokes Shift[21] (Ag surface)	Stokes Shift [22] (Ag colloid)	Stokes Shift[23] (Au colloid)	Assignment[21–25]
588.6				i.p. ring def.
611.7	620	620		COO ⁻ wag C-C twist
676.1				o.p. ring def.
739.9	722	745		COO ⁻ def.; o.p. CH (ring) wag; o.p. CH def.
769.3			762	o.p. CH (ring) wag
812.1		819	822	COO ⁻ rock
1000.7	1001	1002	998	symm. ring breath
1026.9	1034	1033	1025	i.p. CH def.
1156.8			1130	i.p. CH def.
1180.4				i.p. CH bend
1201.7		1207	1204	ring breath
1318.0			1306	CH ₂ wag
1324.3				o.p. CH ₂ bend
1359.9			1346	i.p. OH bend
1451.1	1411		1438	CH ₂ sciss.
1583.7	1586			ring C-C str.
1605.9	1602		1597	ring C-C str.