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Targeting improved reproducibility in surface-enhanced Raman spectroscopy with planar substrates using 3D printed alignment holders

Buddini Iroshika Karawdeniya; Robert B. Chevalier; Y. M. Nuwan D. Y. Bandara; ... et. al



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Buddini Iroshika Karawdeniya,¹ Robert B. Chevalier,² Y. M. Nuwan D. Y. Bandara,³
and Jason R. Dwyer^{2,a)}

AFFILIATIONS

¹Department of Electronic Materials Engineering, Research School of Physics, The Australian National University, Canberra ACT 2601, Australia

²Department of Chemistry, University of Rhode Island, 140 Flagg Road, Kingston, Rhode Island 02881, USA

³Lyle School of Engineering, Southern Methodist University, 3101 Dyer St., Dallas, Texas 75205, USA

^{a)}Author to whom correspondence should be addressed: jason_dwyer@uri.edu

ABSTRACT

Drop-casting is frequently used to deliver a sample for surface-enhanced Raman spectroscopy (SERS) and can result in inhomogeneous sample distribution during solvent evaporation. While soaking can provide better analyte homogeneity, it may require more sample than is available. Failure to optically sample analyte-rich substrate locations can compromise measurement outcomes. We developed and tested 3D printed SERS substrate holders that provided spatial registry of the dried sample droplet center for subsequent optical measurements. We found that deliberate and controlled spatial offsets (0–900 μm) between the analyte drop center and the laser excitation prevented signal intensity drops of as much as $\sim 3\times$ and improved reproducibility. Thus, the use of offset-controlled 3D printed holders provided a quick and inexpensive way to improve the reliability of SERS measurements when using the convenient and popular choice of sample drop-casting.

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I. INTRODUCTION

Failure to account for sampling and sample distribution inhomogeneities in surface-enhanced Raman spectroscopy (SERS) can have profound consequences on measurement reliability.^{1–5} Drop-casting is often preferred to soaking as a quick and easy means of adding a sample to a SERS substrate, especially when analyte availability is limited. Failure to consider analyte redistribution during solvent evaporation can undermine quantitative determinations and preclude its advantageous use for local analyte concentration.^{3,6–8} We wanted to provide a simple yet robust solution to ensure that spectroscopic sampling errors could be minimized and that the advantages of effects such as coffee-ring formation—analyte enrichment away from the drop center—could be realized. We fabricated holders to allow controlled spatial registry of sample delivery and

optical probing. 3D-printing is increasingly important in analytical science,^{9,10} and its use here is important for two reasons: (1) unconventional SERS substrate dimensions¹¹ can be easily accommodated without the need for significant retooling and (2) lateral offsets between drop-cast and optical probing center points could be quickly explored and controlled to improve measurement reproducibility or enhancement.^{6–8}

We tested our alignment tools using commercially available SERS substrates (from Silmeco ApS, Copenhagen, Denmark) that have been reported to have favorable spectral enhancement uniformity across their area.⁴ These substrates consist of metallized high aspect ratio silicon nanopillars that lean together during solvent evaporation to form (self-assembled) SERS hotspots.^{2,4} Signal spatial variability after sample delivery by substrate soaking has been previously reported as $\sim 8\%$ (coefficient of variation)

across a 10 mm-long substrate section.⁴ The spatially uniform enhancement factor would allow for greater ease of investigating the effect of spatial offset between drop-cast and laser spot centers.

II. EXPERIMENTAL

A. Materials

All custom components were 3D printed by a Makerbot Replicator (MakerBot Industries, Brooklyn, NY) using PLA plastic (MP06103, MakerBot Industries, Brooklyn, NY). A 1.00×10^{-4} M standard solution of 4-nitrobenzenethiol (NBT) was used for test measurements with an R3000QE Raman Systems spectrometer with the excitation power at 785 nm set to ~ 57 mW to avoid signal saturation. All reported spectra were averaged from three measurements at the same substrate location, except where noted otherwise. Background subtractions were performed using a wavelet transform method,¹² and the intensity of the most prominent peak (~ 1330 cm⁻¹) corresponding to the presence of the nitro group of NBT was used for all quantitative comparisons. Gold-coated SERS substrates (item ID 15G) were purchased from Silmeco ApS (Copenhagen, Denmark).

B. 3D Holders

Two types of holders were 3D printed [see Figs. 1(a) and 1(b)] to hold the SERS substrates for sample delivery and for SERS measurements [see Figs. 1(c)–1(e)]. SERS substrates were inserted into loose-fit openings in each holder type and reproducibly aligned by contact against back and side walls. The tapered close-fit sleeve for the pipet tip [Figs. 1(a) and 1(b)] fixes the pipet tip location vertically and laterally. This defines a reproducible lateral position for the sample droplet center and protects the delicate substrate from direct contact with the tip. The cylindrical axis of the holder is thus a spatial reference line for positioning the subsequent optical measurements relative to the analyte delivery center point. The optical probing holders [Figs. 1(c)–1(e)] had an integrated physical stop [$\varnothing 7.50$ mm opening in Fig. 1(d)] to set a reproducible $\sim$ mm-scale optical working distance from the sample. While pressing the probe head flat against the substrate would have the same result—albeit without the ability to tune the working distance (thus the spot size) by 3D printing, the holder prevented damage to the delicate surface of the SERS substrate. The recess height could be designed to allow a reproducible vertical separation by friction-fit to the substrate thickness with or without shimming or by resting the substrate flat against the recess bottom with the metal probe vertical in Fig. 1(e). The holders were designed so that, with a substrate present, the 3D printed components were not in the optical beam path. The use of opaque materials may be beneficial in reducing stray light. Four holders for SERS measurements were used with 0, 300, 600, and 900 μ m lateral offsets. The offsets were measured from the center where each aliquot was drop-cast using the holder in Fig. 1(b) and where the substrate would be optically interrogated using the holder in Fig. 1(d). The small surface area of the substrate and the desire to deliver the sample to the center to avoid edge effects during solvent flow and evaporation constrained the magnitude of lateral offset for the 4×4 mm² substrates. The offset magnitudes exceed the nominal

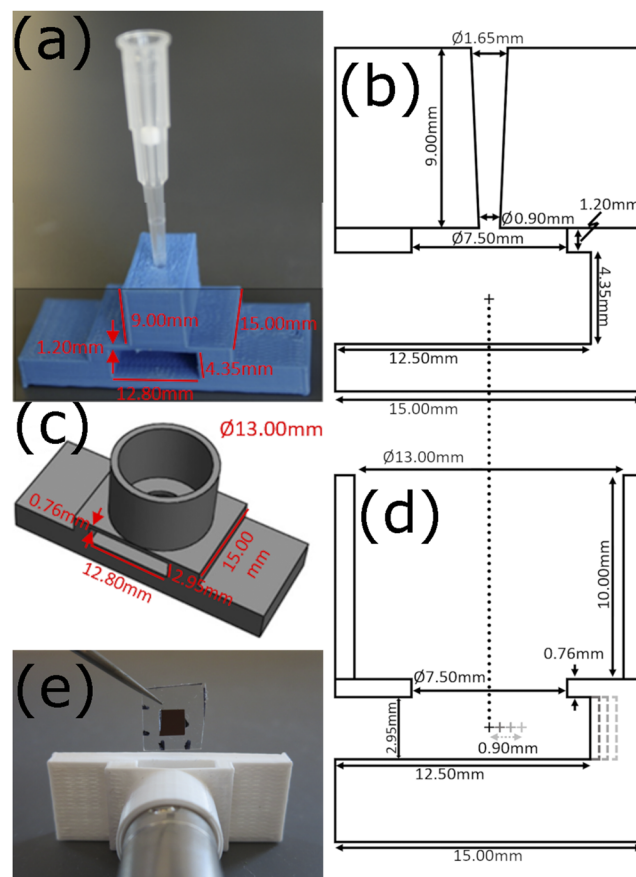


FIG. 1. SERS substrate holder with a tapered through-hole close-fit to a pipet tip for drop-casting the sample onto the substrate, shown (a) 3D printed and (b) a side-view cutaway CAD diagram. A separate holder was designed (c) in relative spatial registry [dotted vertical line in (d) with the pipet alignment tool in (b)]. The central close-fit sleeve for the SERS spectrometer probe [(d) and (e)] was fixed relative to this axis, while the substrate recess edge was shifted laterally in fixed increments [gray dashed vertical lines in (d); the center of each recess is shown by the “+” symbol in matching color]. A ~ 4 mm-wide, ~ 750 μ m thick SERS substrate was mounted to a $\sim 1 \times 1$ cm² segment of a glass slide. The manufactured edges of the slide were positioned by contact with the walls of the recess [in particular, the back denoted by vertical gray dashed lines in (d)].

~ 100 μ m laser spot size,¹¹ and alignment by contact and friction fit has excellent positional reproducibility.¹³ We achieved better than 100 μ m accuracy (<50 μ m standard deviation) in the lateral offset using pinprick tests of the sample delivery and lateral offset holders. A fifth holder was designed to allow a manually variable range of limited offsets between the sample center and probe location. This allowed the effect of only casual alignment to be compared to the deliberate controlled offset case. With the center axis at 0 μ m offset (the droplet center), the sleeve was loose-fit with a 900 μ m greater radius than for the close-fitting $\varnothing 13.00$ mm sleeves. For convenience of handling, the small SERS substrates were mounted on larger backing plates: cleaved glass slides with two regular as-supplied edges for alignment.

III. RESULTS AND DISCUSSION

Aliquots of $5\ \mu\text{l}$ of the $1.00 \times 10^{-4}\text{M}$ NBT solution were drop-cast onto the center of the substrates using the pipet tip alignment tool [Figs. 1(a) and 1(b)] and were allowed to air dry for $\sim 5\text{ min}$ before spectral acquisition. The aliquot volume was limited to an amount that would cover the substrate surface without the risk of overflow. Aggressive solvents may require a change in the holder material to ensure chemical compatibility and avoid substrate contamination if the droplet volume is not well-matched to the substrate. As a general precaution, we recommend minimizing the time between substrate preparation for sample delivery and measurement to avoid substrate surface contamination by a variety of ambient sources.¹⁴ When delivering the sample by soaking, the substrates were immersed in $>2\text{ ml}$ of $1.00 \times 10^{-4}\text{M}$ solution for 300 s and dried in seconds under a pressurized air stream.

In routine implementation, SERS measurements are unlikely to achieve the low spatial signal variability achieved in an experiment rigorously designed to assay substrate uniformity only. We use analyte delivery by substrate soaking as a benchmark for the uniformity of response by drop-casting.⁴ In Fig. 2, we used the loose-fit sleeve for the SERS spectrometer probe to allow for the manual displacement of the laser spot no more than $900\ \mu\text{m}$ from its (central) $0\ \mu\text{m}$ offset position. This loose-fit sleeve was designed to mimic the variability of freehand positioning. In general, it would not be expected that delivering the analyte by drop-casting and soaking from the same solution would result in the same surface concentrations, since the absolute amount of the analyte available to the surface by each method (also with the soaking time used) would strongly affect the outcomes. Here, the signal levels are similar, although the spatial sampling shows greater variability for the drop-cast case than for the soaking case. The spatial signal variability was higher with drop-casting, with a coefficient of variation (CV) of 22.5% vs the benchmark value of 12.3% by soaking. From this simulation of a casual approach to optical sampling, we moved to testing the close-fit holders of Figs. 1(c)–1(e). Deliberate but controlled misalignment would

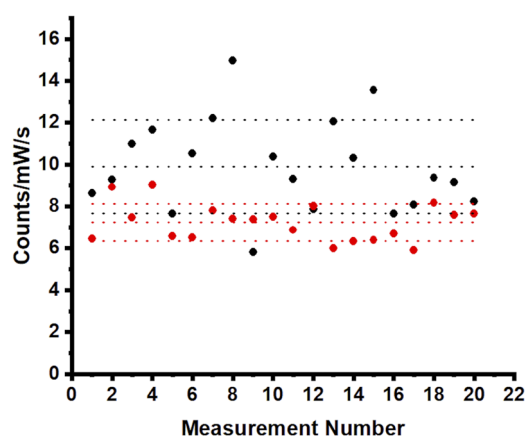


FIG. 2. Intensity of the $\sim 1330\text{ cm}^{-1}$ peak in 20 randomly positioned single measurements using $1.00 \times 10^{-4}\text{M}$ NBT and the loose-fit alignment sleeve after drop-casting (black, CV 22.5%) and soaking (red, CV 12.3%). Dotted lines show the mean and one standard deviation on either side of the mean.

be required to exploit known effects such as analyte redistribution and concentration by the coffee-stain-ring phenomenon.^{3,6–8}

We used drop-casting for sample delivery to exacerbate the potential for spatial signal variation arising from inhomogeneous analyte distribution (Fig. 2).^{3,6–8} Close-fit alignment holders with fixed offsets were used to take triplicate measurements from three unique SERS substrates without moving the SERS probe at each offset. Figure 3 plots the means and individual spectra of each replicate measurement at the specified offset. Several features of the data should be highlighted. First, the replicate measurements show very good agreement for a given substrate and constant offset. Second, while the data from one substrate show little variability with the lateral offset, the data from the other two substrates show significant variability with the offset. While this variability is not consistent, the data show that an appropriate deliberate alignment using only a low-cost 3D printed holder has the potential to be used to increase the measured signal intensity when drop-casting the sample. Similarly, inadequate attention to alignment can compromise the analytical performance, especially for generally low signal levels. This change in signal intensity with offset is important to consider in the context of the increasing popularity of orbital raster scanning (ORS).^{15–17} This optical sampling method in SERS [also referred to as raster

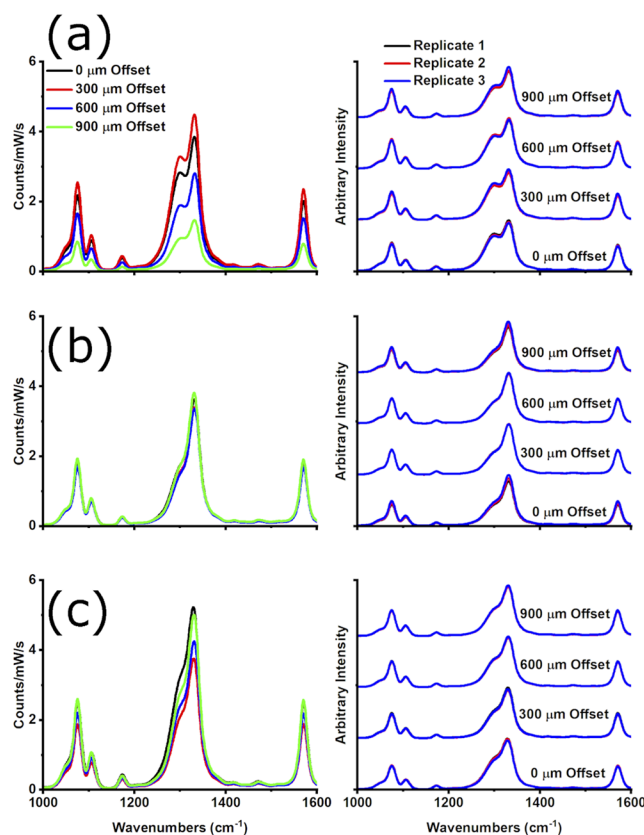


FIG. 3. SERS spectra of $5\ \mu\text{l}$ of $1.00 \times 10^{-4}\text{M}$ NBT drop-cast at the center ($0\ \mu\text{m}$ offset) and 300, 600, and $900\ \mu\text{m}$ offsets for three independent SERS substrates. (a)–(c) are from unique substrates with the average (first column) across the three replicated measurements (second column) at each offset.

orbital scanning (ROS)] rasters the excitation laser across a subset of the substrate.

ORS can be used to compensate by averaging over many substrate locations for the probability that a tightly focused excitation laser may overlap with analyte-poor or low-enhancement regions of a substrate. Figure 3(a) shows a $2\times$ change in the signal intensity with only a $900\text{ }\mu\text{m}$ change in offset. An exemplary ORS sampling radius of $\sim 1\text{ mm}$ would average across this $2\times$ change. Inexpensive 3D printed substrate holders can be used to screen for analyte-bearing substrates where the use of ORS could compromise the analytical reliability of a measurement or to identify where to apply it on a substrate (e.g., within the analyte-rich region of a coffee-stain ring).

IV. CONCLUSIONS

The 3D printed holders provided a built-in stop to ensure fixed working distances and protection of the delicate SERS substrates against damage despite repeated handling. While comprehensive Raman mapping of sample-exposed substrates and the use of substrate soaking when sufficient sample volumes are available would offer benefits, drop-casting remains a popular, rapid, and expedient approach whose reliability can be increased by the adoption of the simple approach outlined here. The analyte delivered here by drop-casting showed greater spatial signal inhomogeneities than by soaking and, on a length scale ($\sim 900\text{ }\mu\text{m}$) is of importance to ORS-based instruments ($\sim 1\text{ mm}$). Controlled offsets could increase signal magnitudes beyond the variability between replicate measurements of drop-cast samples. Controlled alignment could be particularly beneficial when using small volume aliquots of precious samples that contact only a small part of the substrate. The 3D printed components could be used to improve the reliability of SERS measurements.

AUTHORS' CONTRIBUTIONS

This manuscript was written through contributions of all authors. All authors have given approval to the final version of this manuscript. B.I.K. and R.B.C. contributed equally to this work.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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