

## Supplementary Information:

### Experimental

**Fabrication of SERS-Active Substrates:** A film of ZEP 520A was spin coated onto the surface of a 2" silicon wafer (Wafer World, Florida).<sup>1</sup> The wafer was then softbaked and placed under vacuum in the Jeol JBX-9300 FS/E EBL system (100 keV thermal field emission gun). Individual patterns were exposed to a  $420 \mu\text{C}/\text{cm}^2$  dose for writing, yielding unique disc shaped patterns with diameters ranging from 100-300 nm. Each disc had  $20 \mu\text{m}$  of space between other discs. Following beam exposure, the wafer was developed and then introduced to an  $\text{O}_2$  plasma (Technics RIE). The liftoff process is completed by deposition of 10 nm of Cr with an Electron-beam dual gun evaporation chamber (Thermonics Laboratory, VE- 240) onto the surface followed by an acetone bath and rinse. The patterns were etched to known heights in the range of 75-375 nm using an Oxford RIE prior to Cr removal. Finally, 20 nm of  $\text{SiO}_2$  was deposited onto the entire surface of the wafer using an Oxford Plasma Enhanced Chemical Vapor Deposition System. A representation of the fabrication process can be seen in Figure S1.

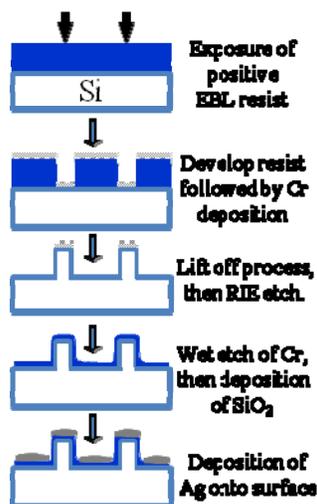
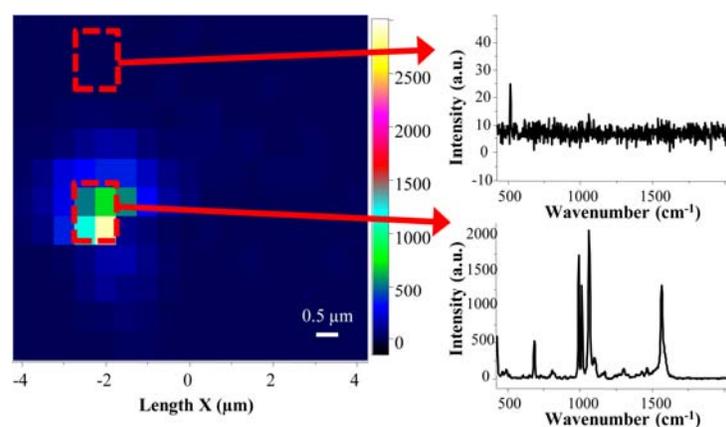


Figure S1. Representation of the EBL liftoff/RIE process.

**SERS-Active Substrate Preparation:** The fabricated substrates were made SERS-active by deposition of Ag using an Electron-beam dual gun evaporation chamber. Samples

were mounted 50 cm above and normal to the source. Average deposition rates and mass thicknesses were 1 Å/s and 20-40 nm, respectively.

**Raman Spectroscopy:** All SERS spectra were collected using a JY-Horiba LabRam Spectrograph. Neutral density filters were used to deliver 0.67-2.7 mW of the 633 nm laser line of a thermoelectrically cooled HeNe laser. The spectra were collected with a 50X (0.45 NA,  $\infty$ ) microscope objective, having a corresponding spot size of approximately 3  $\mu\text{m}$ , in a 180° scattering geometry. Similar wafer orientation was used for all DOP substrates. Sample acquisition times varied based on the study, but initial trials were set to 3 seconds. Also, SERS spectra are manually corrected for background scatter using the LabSpec 4.12 software available on our Raman instrument. An example of a raster plot from an initial trial is shown in Figure S2 with corresponding on and off pillar spectra.



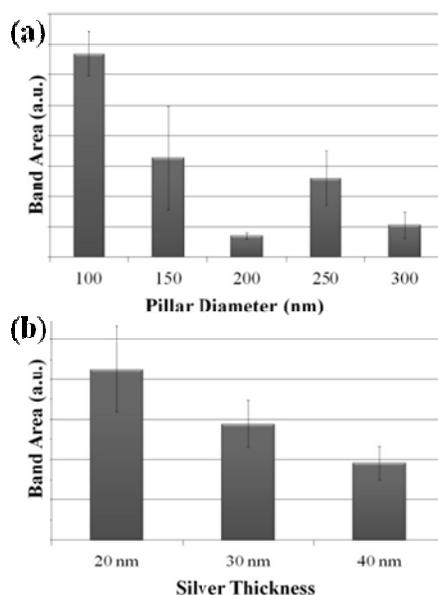
**Figure S2.** Raster plot for a single, 150 nm diameter DOP at optimal height.

## Supplementary Data

**Enhancement Factor Calculation for DOP:** A conventional Raman measurement (area under band 1575  $\text{cm}^{-1}$ ) was made of neat BT with a density of 1.073 g/mL and a focused volume of 30 nL. This amount is based on a 3  $\mu\text{m}$  laser spot size that has been estimated based on images of substrate degradation and is used to calculate  $N_{vol}$ . The band area of 4323 a.u. was used to calculate  $I_{vol}$  while the SERS band area was used for  $I_{surf}$ . The known packing density of the benzenethiol SAM ( $6.8 \times 10^{14}$  molecules/ $\text{cm}^2$ )<sup>2-3</sup> and the surface area of the DOP was used to calculate  $N_{surf}$ . The surface area used was determined using actual dimensions (diameter, height, etc.) from microscopy images of the given DOP structures with the Ag disc

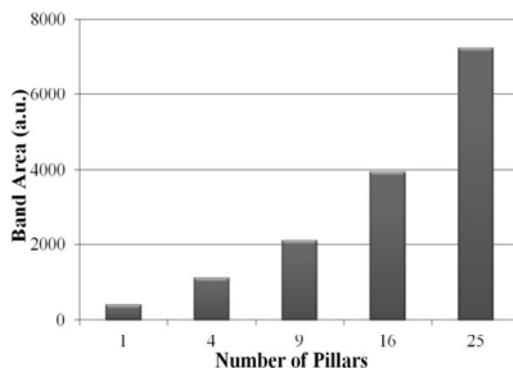
top and side area counting as the majority of the surface area. This area was then arbitrarily doubled to account for the nanoannulus and added area due to roughness on the surface of the DOP. While this is an estimated value, the likelihood is that it is an overestimation of surface area which would undervalue the actual EF. During the raster process, there was no discernable Raman signal in the absence of the DOP.

**Continued DOP Optimization:** Once the optimum height for the DOP system had been established to be 175 nm, other aspects of the system were tested to determine the best possible substrate for our Raman setup. It was found that the 100 nm pillar performed about twice as well, in raw signal, as the initial diameter of 150 nm. The 100 nm diameter DOP system also had an improved EF of  $4 \times 10^9$  along with an improved RSD of 18% (compared to  $1 \times 10^9$  and 24% for initial trials of the 150 nm diameter). The overall trends for each diameter can be seen in Figure S3a. The second parameter tested was the thickness of the silver. As seen in Figure S3b, the thinner film (20 nm) had the strongest overall signal but worst reproducibility. As such, we continued all subsequent experiments at the 25 nm thickness used initially to take advantage of both sensitivity and reproducibility.



**Figure S3.** Based on the optimum pillar height of 175 nm, (a) various pillar diameters are examined, and (b) three different silver deposition thicknesses are compared for the 100 nm diameter pillars.

**DOP Arrays:** For array studies, individual DOPs were arranged into squares of N-by-N pillars ranging from 2-by-2 to 5-by-5. In each array, each pillar was placed 220 nm from each other as to eliminate any variations due to spacing. As shown in Figure S4, there is a consistent increase in overall signal as the number of pillars increases. The raw signal for the 25 pillar 5-by-5 array was ~18 times greater than that of a single DOP.



**Figure S4.** Average band area for the  $1060\text{ cm}^{-1}$  band of BT for varying numbers of pillar ranging from a single DOP to a 5-by-5 array of pillars. All arrays have a gap of 220 nm between each pillar in a row and column and have a height of 250 nm while also having 20  $\mu\text{m}$  of space surrounding to avoid long range effects.

1. S. M. Wells, S. D. Retterer, J. M. Oran and M. J. Sepaniak, *Acs Nano*, 2009, **3**, 3845-3853.
2. L. J. Wan, M. Terashima, H. Noda and M. Osawa, *Journal of Physical Chemistry B*, 2000, **104**, 3563-3569.
3. C. M. Whelan, M. R. Smyth and C. J. Barnes, *Langmuir*, 1999, **15**, 116-126.