Electronic supporting information:

Testing gold nanostructures fabricated by hole-mask colloidal lithography as potential substrates for SERS sensor: Sensitivity, signal variability, and the aspect of adsorbate deposition

Vlastimil Peksa*¹, Petra Lebrušková², Hana Šípová², Josef Štěpánek¹, Jiří Bok¹, Jiří Homola², Marek Procházka*¹

¹Charles University in Prague, Faculty of Mathematics and Physics, Institute of Physics, Ke Karlovu 5, 12 16, Prague 2, Czech Republic
²Institute of Photonics and Electronics, Academy of Sciences of the Czech Republic, Prague, Chaberská 57, Prague 8, Czech Republic

*CORRESPONDING AUTHORS: V. Peksa: peksav@karlov.mff.cuni.cz, M. Procházka: prochaz@karlov.mff.cuni.cz

1. Schematic representation of fabrication of the Au nanostructures by HCL (Fig. S1).

2. Details and results of additional mapping of the SERS signal (Fig. S2).

3. Details of the computer simulation performed to estimate the contribution to the SERS signal variation caused by the fluctuations in the number of nanoparticles inside the laser spot.

4. Details and results of the fit of measured RSD values for different objectives (Fig. S3).
1. Schematic representation of fabrication of the Au nanostructures by HCL (Fig. S1).

**A**
- Spin-coating of PMMA
- PMMA charged layer (+)
- Deposition of gold mask

**B**
- Polystyrene particles
- Hole-mask evaporation
- PP lift-off + reactive ion etching
- Lift-off in acetone

**C**
- Reactive ion etching
- Angle gold deposition
- Lift-off in acetone

**Fig. S1** Schematic representation of fabrication of the Au nanostructures by HCL. A – Basic fabrication process, with 50 nm high discs as a resulting substrate. B – Alteration to basic fabrication process to produce cones: The thickness of layer of deposited gold is 260 nm instead of 50 nm, resulting in sharpened structures. C – Alteration to basic fabrication process to produce dimers: Reactive ion etching is 10 minutes long instead of 5 minutes. Gold is then deposited at two different angles.
2. Details and results of additional mapping of the SERS signal (Fig. S2).

In addition to three 7×7 point maps measured on a substrate with discs incubated in MB, three additional maps with 300 points in total were measured on a substrate with discs incubated in MB to determine the influence of increased number of points on the RSD of SERS signal. The maps were 10×10 points measured under the same conditions as described in Experimental section, using 50× air objective. The RSD of map (450 points in total) was 19.78% (rounded to 20%), the RSD of combined additional maps was 19.33%. RSD of combined original and additional maps was 19.95%.

![Fig. S2 Baseline-corrected integral intensity of 1620 cm⁻¹ MB peak measured from 450 points on a substrate with Au discs.](image)

3. Details of the computer simulation performed to estimate the contribution to the SERS signal variation caused by the fluctuations in the number of nanoparticles inside the laser spot.

A 20×20 µm square was covered with nonoverlapping nanocircles, the diameters of which corresponded to the bases of the nanostructures, i.e. 180 nm for the nanodiscs and 230 nm for the nanocones. The average nanoparticle density was set at the values of 11.1 and 9.6 particles/µm², which corresponds to the density determined from AFM images. After that, a circle representing the
laser spot (1.4 µm diameter for the 50×, 0.86 µm diameter for the 100× and 3.09 µm diameter for the 10× objective) was randomly placed to various positions inside the square and the number of particle centers within the laser spot circle was calculated. The RSD of the number of particles calculated from 10000 placements was averaged for 75 particular iterations.

The simulations were performed for two types of particle distributions, random distribution of circles, which cannot overlap, and regular distribution of circles with fixed distance between the neighboring circles. Resulting RSD values (RSD$_{numb}$) were 13% (nanodiscs) and 11% (nanocones) for the random distribution, while 6% (nanodiscs) and 7% (nanocones) for the regular one.

4. Details and results of the fit of measured RSD values for different objectives (Fig. S3).

For the fit, the basic equation for RSD

$$RSD(d_f) = \sqrt{RSD_{dens}^2 + RSD_{numb}(d_f)^2 + RSD_N(d_f)^2 + RSD_{ap}^2}$$

was transformed to

$$\sqrt{RSD^2(d_f) - RSD_{numb}(d_f)^2 - RSD_{dens}^2} = \sqrt{RSD_i^2/N + RSD_{ap}^2}$$

A least square fit of the right side of eq. s2 with RSD$_i$ and RSD$_{ap}$ as free parameters to the left side was performed for data obtained from short-range maps of methylene blue deposited on nanocone surface. The results are shown in Fig. S3.
Fig. S3 Circles: RSD of the SERS signal in short-range maps of the nanocone substrate incubated in methylene blue aqueous solution. RSD is reduced by subtraction the contributions of the nanoparticle density variations (RSD$_{dens}$) and variations of the number of nanoparticles inside the laser spot (RSD$_{numb}$). Lines: Result of a least square fit according to eq. s2. Red and brown colors denote the solutions for RSD$_{numb}$ estimated from simulation for uniformly and randomly distributed nanoparticles, respectively.