Fabrication of large-area ordered and reproducible nanostructures for SERS biosensor application

Gobind Das,* Niranjan Patra, Anisha Gopalakrishnan, Remo Proietti Zaccaria, Andrea Toma, Sanjay Thorat, Enzo Di Fabrizio, Alberto Diaspro, and Marco Salerno

Section 1
SERS substrate fabrication based on APA template:

![Process flow diagram of AuAPA SERS substrate.](image)

**Fig. S1:** Process flow diagram of AuAPA SERS substrate.

APA template with different pore size and wall thickness were produced using different electrolyte solution (acid). This phenomenon is called anodization of Al plate to become aluminium oxide with pores within it. Thereafter, the APA templates were gold-coated of 25 nm. SERS substrate fabrication procedure is shown in **Fig. S1**.

Section 2

**Cross-section SEM image:** The cross section SEM measurements of AuAPA2 substrate were performed in order to understand the gold grain present in the depth of the AuAPA2 pore. The measurements were performed on 45° sample inclination from the plane. SEM image, shown in **Fig. S2**, for sample AuAPA2 indicate that these gold nanoparticles are present up to 150-200 nm from the surface of AuAPA2 substrate. The pore height is found to be 50-80 μm. To be noted, that these interior pore area out of focus and so contributing negligible in the observed SERS signal.
Fig. S2: Cross-sectional image of AuAPA2 substrate. The gold grain can be observed down to 100-150 nm from the surface.

Section 3

SERS substrate reproducibility verification: An optical image of large area mapping for AuAPA1 and AuAPA3 substrates are shown in Fig. S3. These measurements were performed to verify the average-area SERS reproducible enhancement substrate with CV deposited over it by means of chemical adsorption technique. The chemical analysis is made for the reference band at 591 cm⁻¹ and can be observed that the SERS signals at different points are almost equal (±300 counts). In the below of the SERS image, SERS spectrum of CV deposited on AuAPA1 and AuAPA3 are also reported with standard deviation.

Fig. S3: Optical image of the sample (AuAPA1 (P=27.5 μW, t=1 sec) in the left side and AuAPA3 ((P=27.5 μW, t=1 sec)) in the right side) and the crossed area is the place where the mapping was performed, respectively. The analysis is performed for the Raman band of CV centred at 591 cm⁻¹. SERS spectrum with the standard deviation is also reported.
Section 4

SERS spectrum of CV deposited over APA$_{\text{commercial}}$ substrate: The SERS measurements on APA$_{\text{commercial}}$ based substrate with CV molecule deposited over it.

Fig. S4: SERS spectrum of bare device (background) (black line) and of CV deposited on AuAPA$_{\text{commercial}}$ substrate