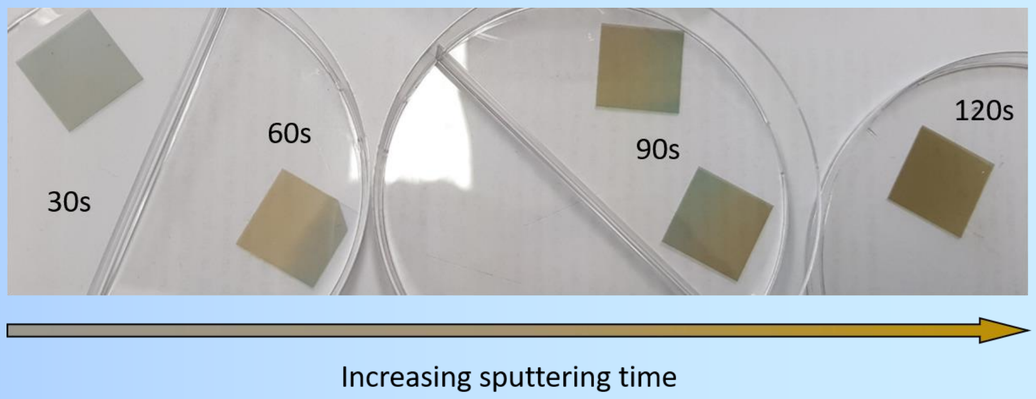
Thin layer gold-based substrate for pathogens SERS detection and quorum sensing monitoring

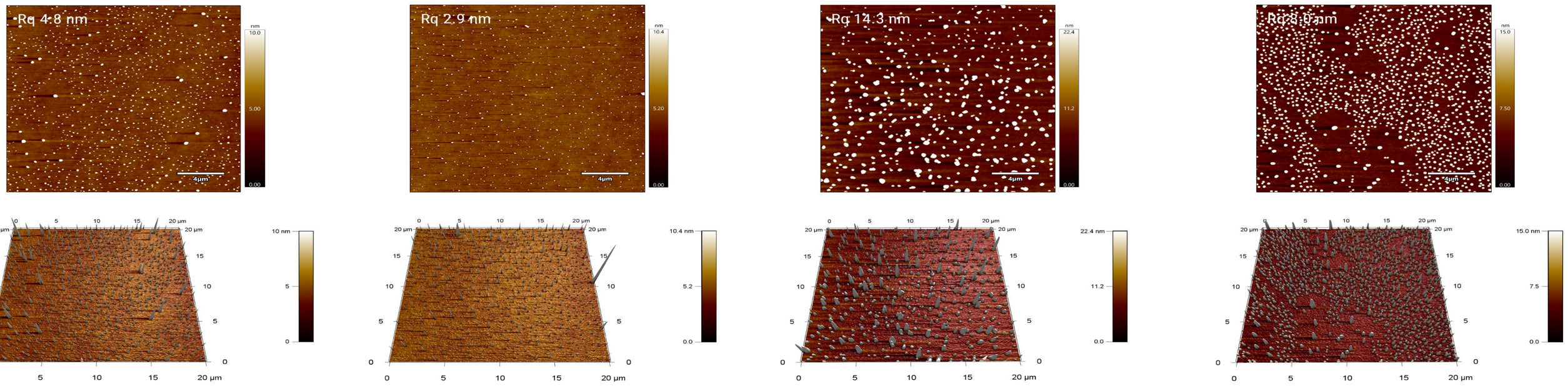
N.E. Dina, D. Marconi, A. Colniță and D. Bogdan

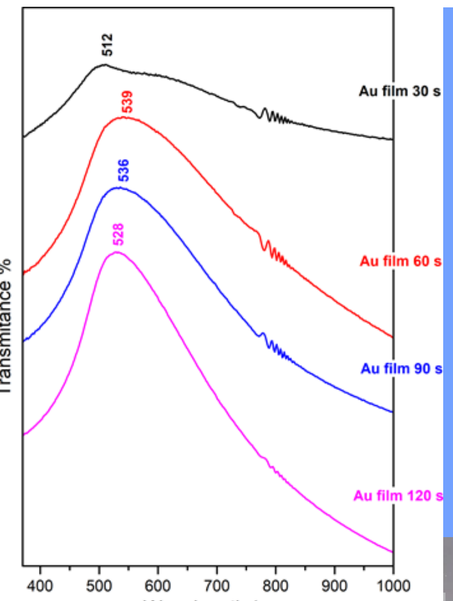
National Institute for Research & Development of Isotopic and Molecular Technologies, 67-103 Donat Street, RO-400293 Cluj-Napoca, Romania

Biosensing using **surface-enhanced Raman scattering (SERS)**-based platforms is developing towards **point-of-care (PoC)** solutions in more flexible, wearable, ready-to-use and affordable solid substrates. This work proposes the use of gold (Au) thin layer-based substrates for SERS sensing, in 'real-life' applications. Gold thin films with different thicknesses were deposited by magnetron sputtering technique on commercial glass slides. Their plasmonic properties were confirmed by AFM analysis which suggested that the sputtering process generates rough surfaces composed of globular clusters of a few nm ranges, smaller with respect to the wavelength of the exciting light. The analytical sensitivity of the substrates was tested by detecting bacteria in Raman and SERS effect conditions. The thickness of the Au layer was found to play a significant role in the SERS enhancement. Thus, we report on fabricating a SERS-active substrate appropriate for fast bacteria detection directly from a 3 µL droplet of sample. Ready-to-use, simple, cheap and the multi-assay capacity by using all ten microchannels for different analytes ultra-sensitive detection are key advantages of the herein proposed SERS substrates.

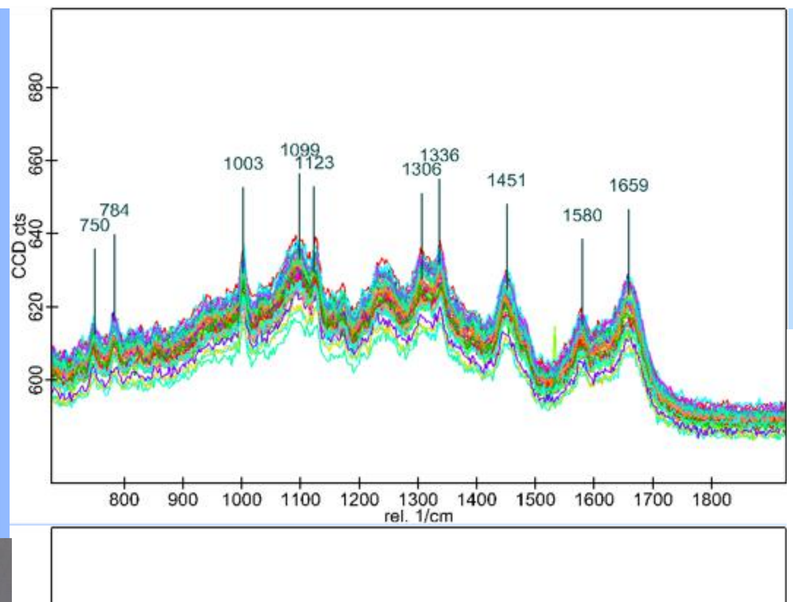
This work was supported by a grant of the Romanian the Ministry of Research, Innovation and Digitization, CNCS – UEFISCDI, project number PN-III-P1-1.1-PD-2016-0475, within PNCDI III.

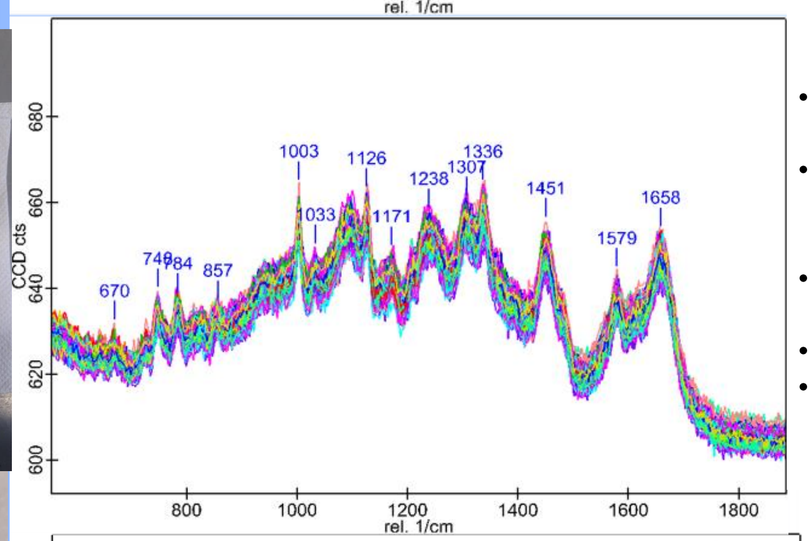




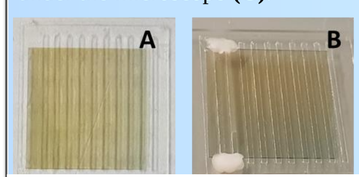


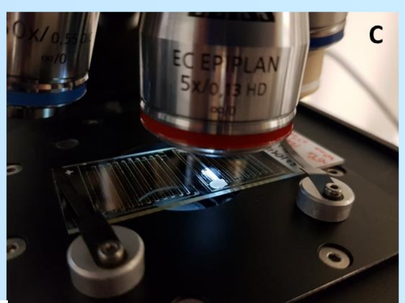
Transmittance spectra of the **thin gold films sputtered on glass substrate**, with different sputtering times. The decreasing of transmittance with increasing of deposition times is strongly related to the thickness of the films. All these films have a **transmittance maximum at λ ≈500 nm** which is directly influenced by the film's thickness. The position of this maximum is slightly red-shifted with increasing film thickness and it can be associated with a discontinuous structure of the film and observed at specific wavelengths, dependent on thickness.





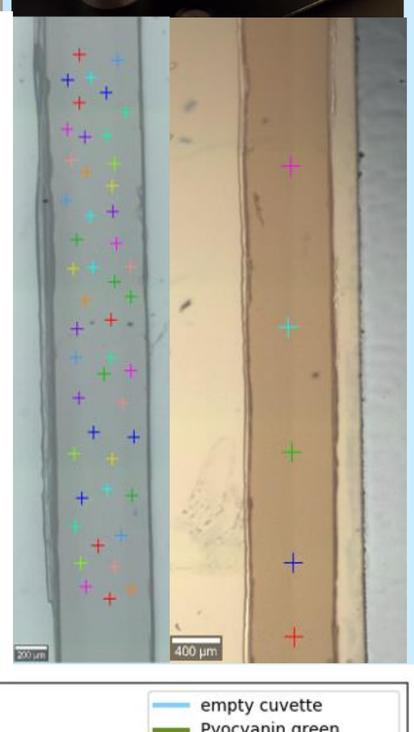
Microchannels cut into adhesive tape covered with thin gold films **(A)** and sealed with wax **(B)** for laser irradiation under the microscope **(C)**.





Experimental protocol:

- Channel design with Graphtec cutting plotter CE7000-40 and adhesive tape (Adhesivs research Inc. ARcare 90106)
- Place adhesive tape on microscope slide
- Cover channels with Au-sputtered glass cover slip
- Fill channels with *E. coli* in PBS buffer
- Seal channels with wax
- Raman/SERS measurements with 532 nm & 633 nm laser



Pyocyanin extraction and purification method: bacterial pigment produced by *P. aeruginosa* species

Pyocyanin purified using the aqueous extraction method described below:

- The agar was cut aseptically into 1-cm squares and placed in a sterile bottle.
- A 20-mL aliquot of dry chloroform was added.
- The mixture was shaken vigorously and the chloroform (**colored blue**), was removed by a pipette and filtered.
- The procedure was repeated to extract the remaining pigment.
- A 10 mL volume of 0.2M HCL was added to the chloroform solution and after shaking the **red pigment** was passed to the aqueous phase, leaving the **lemon-yellow** chloroform phase.
- The aqueous phase was removed and 0.2M NaOH was added to it drop by drop until the **blue color** was recovered.
- A volume of 20 mL of dry chloroform was then added to the aqueous solution and shaken vigorously until the **blue pigment** passed to the chloroform phase.
- The chloroform phase was removed and filtered; at the end of the procedure no pyocyanin crystals were formed, only a diluted solution. *(Abou Raji El Feghali P., Nawas T. Extraction and purification of pyocyanin: a simpler and more reliable method. MOJ Toxicol. 2018;4(6):417–422)*