Fabrication of Nanostructured Au Films as Promising SERS Substrates for the Detection of Pathogenic Bacteria

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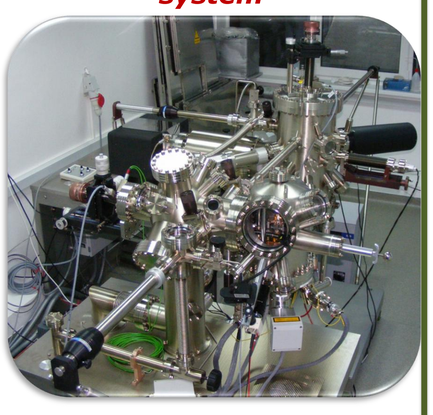
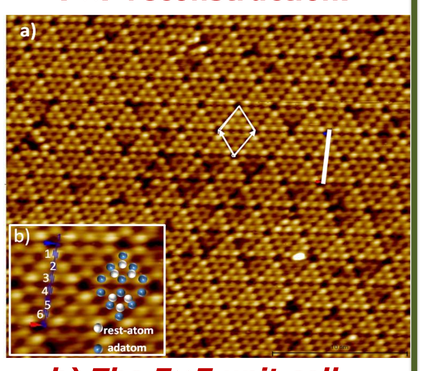
As there is a continuous emerging of new and multidrug-resistant pathogens, the need to develop faster, more accurate and multiplex detection methods is crucial. Having several unique characteristics, such as the ability to provide molecule-specific fingerprint-like spectra and the non-destructive, label-free, and highly sensitive nature of the measurement, SERS (Surface-Enhanced Raman Scattering) spectroscopy gain more and more popularity in problems related to ultrasenzitive detection. Our aim is to fabricate nanostructured metallic films for SERS-based, whole-bacteria detection applications. Thin Au/Si(111) films were deposited by molecular beam epitaxy under ultra-high vacuum (UHV) environment. The nanostructured surface topography, growth mode and controlled roughness of the Au films were assessed using Scanning Tunneling Microscopy (STM). The deposition process of the Au-coated Si substrates was optimized in order to exhibit a high SERS signal of the investigated bacteria. High resolution and reproducible SERS spectra of Rosetta strain of the Gram-negative bacteria *Escherichia coli* (*E. coli*) were obtained using the 632.8 nm and 532 nm laser lines with a power in the μW range, in order to avoid sample degradation.

Integrated MBE-SPM system



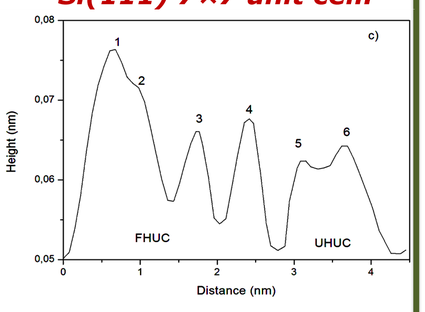
RHEED Si(111) 7×7 pattern obtained after annealing and flashing

a) STM image of Si 7×7 reconstruction.

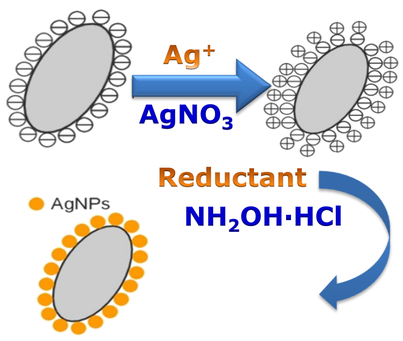


b) The 7×7 unit cell.

c) Height profile along the Si(111) 7×7 unit cell.



In situ Ag NPs synthesis (Bacteria@AgNPs) [1].



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Experimental

I. **Bacteria cultivation:** 2 ml of LB grown *E. coli (Rosetta strain)* was centrifuged at 6000 rpm for 10 minutes and washed 3 times in saline buffer.

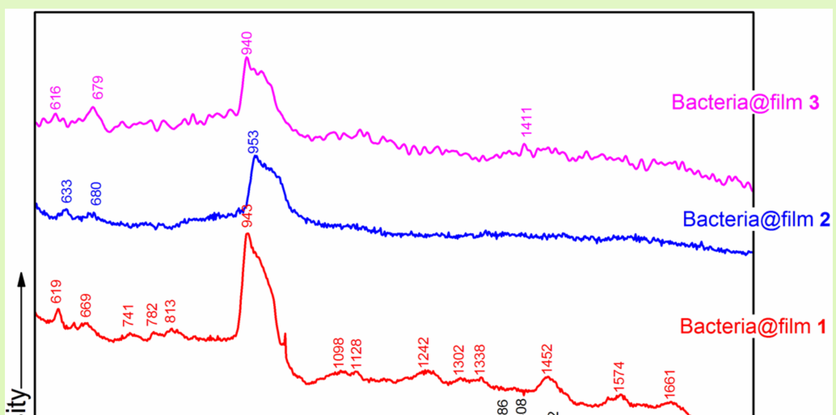
II. **UHV-MBE deposition of Au films:** Au pellets of 99.9995% purity (Premion, Alfa Germany); evaluation of deposition rates – beam flux monitor; substrate temperature – NiCrNi thermocouple; annealing at deposition temperature – 1 h.

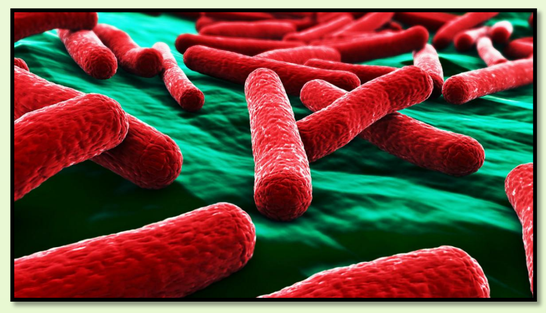
Deposited film	Deposition temperature (°C)	Deposition rate (nm/min)	Thickness (nm)
1	480	1.6	~ 100
2	580		
3		2.5	~ 150

III. **Sample preparation:** 3μl of centrifugated and saline resuspended bacteria were deposited on the Au/Si(111) films and let to dry in atmospheric conditions;

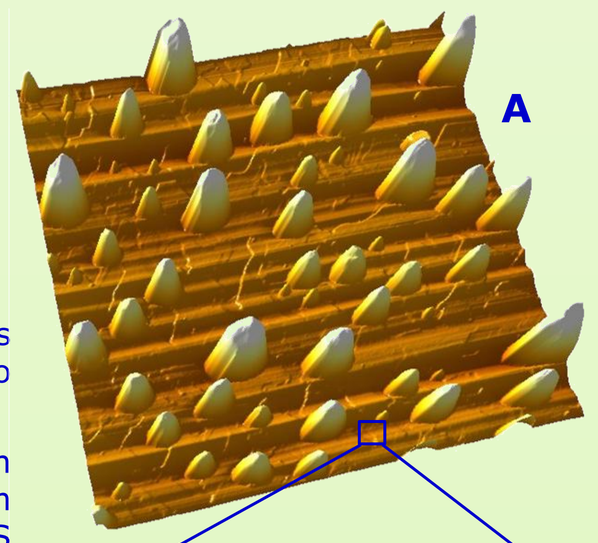
- ***in situ* Ag NPs synthesis (Bacteria@AgNPs):** as described in [1]. In this case, 5 μl of **Bacteria@AgNPs** were dried on glass substrate prior to measurement.

IV. **Characterization methods:** substrate cleaniness - **R**eflection **H**igh **E**nergy **E**lectron **D**iffraction (**RHEED**) system, STAIB Instruments GmbH, Germany; substrate and film topographic evaluation – UHV **S**canning **T**unneling **M**icroscopy (UHV-**STM**); SERS effect: Raman – AFM Ntegra platform, NT-MDT, lasers: 532 nm and 632.8 nm.



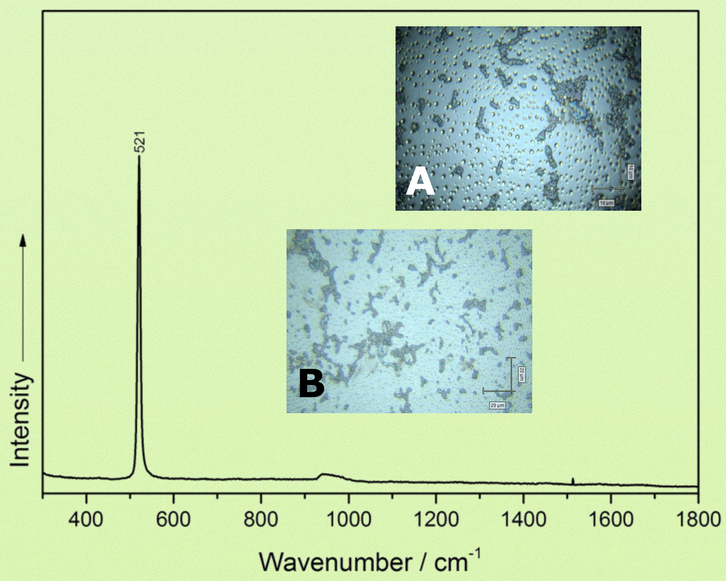


Visual aspect of E. coli http://playtech.ro



8 μm × 8 μm 3D STM images of the deposited thin films (A) and 1 μm × 1 μm 2D STM images of the Au surface between the bubbles (B). The STM images were recorded by sample bias voltage of 1.6V and tunneling current of 0.3 nA.

The influence of Si(111) substrate in the SERS spectrum of E. coli. Inset. Optical microscopy image of E. coli under 100× objective (A) and under 50× objective (B).



Conclusions

- **The great influence of the Si(111) substrate over the bacteria SERS spectrum minimizes the bacterial fingerprint.**
- **The SERS spectra of bacteria on Au/Si(111) films is dominated by a very broad peak, which could not be resolved.**
- **Several SERS bands can be observed only in case of Bacteria@film 1.**
- **With a further optimization of the film deposition parameters, comparable spectra to those obtained using Ag-based SERS active substrates is possible and could open an avenue for nanostructured films SERS-based biosensors.**

[1] H. Zhou et al., Anal. Chem. (2014) 86(3), 1525-1533.