SYNTHETIC INTERMEDIATE RESEARCH REPORT 2015

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Report on project achievement in the period November 10th, 2015 – December 31th, 2015

O1). A) The label-free character of the developed detection protocol was assured by the unspecific receptor immobilization procedure of the tested microorganisms. In this regard, the sample supports used in this preliminary step, were only glass- and MgF2-based. In the future investigations, we will elaborate a surface chemistry that will enhance the efficiency of the immobilization process and will enable it without the use of antibodies or other specific receptors.

B) An innovative detection and identification protocol for pathogens at single-cell level was optimized by employing most common pathogens and several distinct SERS-active substrates synthesis approaches:

- Leopold-Lendl AgNPs synthesized in the classical fashion;
- *in situ* synthesized Leopold-Lendl AgNPs (Bacteria@AgNPs approach);
- Lee-Meisel AgNPs (most commly used approach);
- PEG200 layered AuNPs
- concentrated AgNPs and AuNPs by centrifugation.

The most reliable and reproducible SERS results were obtained when using the *in situ* Bacteria@AgNPs approach and by exciting with the 633 nm laser line for detection. The optimization steps included determining the minimum irradiation time and the lowest effective laser power for avoiding thermal decomposition of the biomass, monitoring the influence of the bacterial growth media used for cultivation, investigating both Gram-positive and –negative microorganisms in order to ensure an extended use of the optimized methodology and demonstrating intra-species (strains) specificity. Two different culture media were tested (Luria Broth and classical nutrient broth) and at least two bacterial species of each Gram type were investigated. Moreover, the commonly encountered *E. coli* was selected as model for the intra-species study by using three strains. Finally, by achieving label-free SERS detection at single-cell level, the ultra-sensitivity of the optimized protocol was demonstrated.

As indicators of the accuracy detection method, the SERS marker bands were monitored in each experimental attempt. Their relative intensities and Raman shifting were considered qualitative parameters of the detection process. The obtained SERS results represent a starting point in achieving the objective of being able to identify and discriminated pathogens at strain level. In particular, distinctive spectral features specific to pathogenic strains were identified and will be analyzed in the forward investigations.

Continuing to pursue of the project's objectives, in our next investigations we will monitor the influence of the chosen excitation laser line and the growth phase of the pathogens, and we will reduce the reagents volume and the sample volume, respectively, at minimum (between 3-100µl).

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