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Title of the doctoral dissertation: **Detection of microorganisms in clinical samples *via* surface-enhanced Raman spectroscopy**

Abstract

The doctoral dissertation presents a series of studies that aim to find a spectral image of selected disease entities using surface-enhanced Raman spectroscopy (SERS) and chemometric methods. SERS analyses performed in a label-free variant provided information about spectral changes caused by a given type of infection directly from the recorded SERS signal of a clinical sample. The reagentless procedure that does not require additional modification of the analyte can significantly reduce the risk of errors and the work and time involved.

As part of the research, one established the procedure for preparing clinical materials and the SERS measurement conditions using pre-optimised plasmonic nanostructures. An important element of the work was the comprehensive spectral characterization of the control and experimental groups in relation to the considered disease entity. The assistance of chemometric methods provided insight into the correlation between data and identification of marker bands having diagnostic significance. Calibration models were created based on the properly defined classes, and their performance was tested at the stage of external validation simulating actual diagnostic conditions.

The first part of the research was devoted to the SERS analysis of bacteria from *Neisseria* spp., and urethral discharge in the context of gonorrhea recognition. The obtained data constituted a catalog of SERS spectra of microorganisms (reference data). Then, based on it, the unknown strain cultured from the clinical sample was correctly identified as *Neisseria gonorrhoeae* using its unique spectral image (the so-called 'fingerprint'). In the analysis of the urethral discharge, the experimental group related to the control one, exhibited different spectral character in the entire range. The appearance of new bands (especially the one at 724 cm^{-1}) indicated the certain contribution of pathogens in SERS signal formation. These differences are significant for identifying infections directly from SERS spectra of clinical samples. The chemometric analysis

also indicated high accuracy in classifying external samples, *i.e.* 89% for SIMCA and 100% for PLS-DA.

The next section concerns the spectral characterization of *Candida* spp. and bacteria of particular importance for the development of vaginal infections. The SERS spectra of microorganisms present the contribution of individual components, *e.g.* chitin, glucagon, and mannoproteins for *Candida* spp. and lipids, phospholipids, and proteins for bacteria. The spectral variability was particularly observed for the clinical samples taken from women with abnormalities within microflora and biochemistry of the swab. The comprehensive analysis resulted in establishing the control group that provides an excellent basis for the diagnosis of VVC infection from the SERS spectra of the analysed clinical sample. The PLSR method visualized the clear differences between the two considered classes (control and VVC). It also revealed marker bands identified as those belonging to microorganisms (727, 1003, 1125, 1258, 1331, 1455, and 1670 cm^{-1}) and components of the swab, *i.e.* amino acids, proteins, saccharides, lactic acid (669, 891, and 1047 cm^{-1}).

The third section of the study concerns the analysis of saliva and nasopharyngeal swabs in terms of COVID-19 diagnosis. The chemometric methods extracted differential spectral information such as: for CoV(+) saliva – the elevated levels of methionine, nucleic acids, proteins (*e.g.*, ferritin) and immunoglobulins and for CoV(+) nasopharyngeal swabs – neopterin. Among all the tested methods, SVMC provided the best performance in the classification of external samples, achieving 90 % accuracy and 100 % sensitivity for saliva and 75 % accuracy and 88 % sensitivity for nasopharyngeal swabs.

The results presented in the doctoral dissertation indicated the competitiveness of the SERS technique compared to those currently accepted methods. It is also worth noting that the research was performed using a portable Raman spectrometer, which is a great advantage for point-of-care diagnostics.