DESCRIPTION FOR THE GENERAL PUBLIC

The phenomenon known as the Raman effect is inelastic scattering of photons ("particles of light") by the molecules of the examined substance. Inelasticity here means that the energy of some photons is lost or gained during its contact with the sample. In Raman experiment every substance produces characteristic system of bands (a spectrum) being a kind of fingerprint of the examined substance. As everything in this world, this method has also drawbacks. The most important con of the Raman spectroscopy is the fact that the Raman effect is very rare - only one out of million photons is scattered in the inelastic way. Things changed in 1974 when Martin Fleischmann, Patrick J. Hendra, and A. James McQuillan discovered that the Raman signal intensity rises up to million times when they placed the sample on a roughened surface of silver. The discovered technique was named surface-enhanced Raman spectroscopy (SERS) and the metal enhancing Raman signal was named the SERS platform or SERS substrate. At the beginning of 21st century this technique was reported as potential new rapid and facile method of bacteria identification. Although this method of bacteria detection and identification has increased very much in popularity, up to now there is no method to make the SERS spectra of bacteria reproducible. Result of every measurement is dependent on a variety of factors, and as a result the fingerprint of the bacterium is different every single time.

The aim of the project proposed by us is to verify which factors have a significant impact on SERS spectrum and on Raman signal enhancement of four bacteria species (*Escherichia coli, Salmonella enterica, Bacillus subtilis*, and *Listeria monocytogenes*). These factors can be grouped into these that are associated with the process of culture of micro-organisms and a method of sample preparation and in these which are related to the apparatus and the type of platforms used during SERS measurements.

Within the project we plan to check how the type of SERS substrate affects the spectrum of bacteria. The typical SERS platform consists of a core base material (e.g. zinc oxide, silicon, fluorine doped tin oxide), which gives an appropriate structure to the platform and of a metal shell (such as silver, gold and copper). We want to know how the type of base material, its structure and the type of a metal shell affect the spectral image of bacteria (Fig. 1). Silver is the metal most applicable in the surface-enhanced Raman spectroscopy. However, as is widely known, silver has antibacterial properties. Within the project we want to check whether the presence of silver on the SERS platform will change the result of the experiment, and if so, how quickly after the deposition of a sample on the substrate changes will occur. We want also to show how the spectrum of bacteria changes after using gold or copper as shell metal. Gold, in contrast to silver, does not darken in the presence of air and copper is the least expensive among these three metals. In our experiments, we also want to use a mix of these three types of metals.

Our next goal is to verify the influence of cell culture temperature, incubation time and the types of culture media on the structure and chemical composition of bacterial cells. Such a change in the composition could reveal the appearance of additional bands in the spectrum. The culture conditions are very important because the same bacteria under different conditions can produce various kinds of substances and then release them to the environment, e.g. *Serratia marcescens* bacteria at 30°C produce a red die, prodigiosin, which is not produced at 37°C. Moreover, some bacteria are capable of producing spores. Such bacterial products may have an additional contribution to the spectrum. At the end, we plan to determine how the death of bacteria affects the SERS spectral image. When the death of bacteria is caused by cell membrane damage the elements located in the cytoplasm, including nucleoid and other cellular organelles, are released. Their presence can appear in the form of additional bands in the spectrum. We want to involve bacteria in various processes leading to their death, so that we can determine whether this kind of treatment will give us more information about the examined species.

A detailed analysis of the project is an essential step towards understanding how bacteria interact with nanostructures localized on the SERS substrate and to determine which compounds of the bacterial cell are visible in the SERS spectrum and why. These are the basic research goals that may find many practical applications in the future. So far there were no attempts to optimize the conditions and factors that will lead to the development of a uniform, reproducible SERS spectra of bacterial cells. The huge differences between the spectra resulting from different cell culture conditions and SERS substrates lead to many ambiguities and to the inability to compare the obtained spectra. Sometimes differences between two spectra are limited to increase or decrease of the intensity of particulars bands, but sometimes we can observe completely dissimilar spectral images. Therefore SERS applications in medicine, forensics, food industry, and many fields of science are often very limited.

The results obtained in the project will reveal the factors that have a significant impact on the Raman spectra of bacterial cells. This is very important from the analytical, especially qualitative, point of view. This project will develop the current knowledge of biospectroscopy and may start the introduction of uniform methods used in SERS studies of bacterial cells in every field in which detection and identification of bacteria are important. Quick identification would allow for taking proper action towards the development of a new and rapid diagnostic method used, e.g., in hospitals and analytical and microbiological laboratories.