Analysis of signal variability in surface-enhanced Raman spectroscopy

Raman spectroscopy is an optical spectral measurement technique utilizing the Raman effect, where the incident light is scattered causing the generation of new waves, with energy dependent on the energy of a specific molecular bond in the irradiated sample. These waves, called Stokes and anti-Stokes waves, where one's energy is a sum of the energy of laser and of the molecular bond, while for the other one it is a difference in the energies, are what is called a Stokes shift which carries the information on the chemical (molecular) composition of the object. Because the difference in energies is always the same for a specific chemical bond. Thus the Raman spectrum comprises a set of the specific chemical bonds of the molecule, which provides a so-called *chemical fingerprint* of said molecule. The effect is in general non-destructive, thus this method is greatly appreciated in chemical research for studying structure of molecules, chemical processes, in material science, physics, life sciences and in the industry for product quality, etc. However, one of the limiting factors of the method is the low quantum efficiency of the Raman effect itself, which means that only one in about a million photons undergoes a Raman scattering event. This causes a low signal-to-noise ratio (SNR) in this technique. Along with noise from various sources and interference from other molecules in the investigated sample, the limit of detection (minimal detectable concentration of the compound) is in practice severely hindered.

One of the approaches for increasing the efficiency of this method is the utilization of so-called localized surface plasmon phenomena (local oscillations of the electron cloud located near the vicinity of a noble metal surface), in a surface-enhanced Raman spectroscopy (SERS). When the molecule is close to the metal surface the electromagnetic field generated by the laser gets amplified due to resonance with the plasmons. In SERS, the strength of the signal is much higher than in regular Raman spectroscopy, achieving the enhancement factor usually of the $10^6 – 10^8$ order. This allows a highly-sensitive detection of extremely small quantities of analytes, as has been reported for various substances such as drugs, proteins, disease biomarkers, etc. A surface-enhanced Raman spectroscopy combines the exquisite molecular specificity of vibrational spectroscopy with the ultrahigh sensitivity emanating from surface plasmon resonance offering e.g. a promising label-free tool for medical diagnostics. To get enhancement, metallic materials must be used whose properties allow get the highest enhancement. However, despite the rapid development of nanotechnology and the emergence of a wide range of substrates and nanoparticles that cause SERS, there is a serious problem of reproducibility of measurement results. Nowadays, this is not just a matter of inaccurate materials but a specific feature of the SERS phenomenon.

We anticipate that in order to improve the sensitivity and repeatability of the SERS method, we will look closely at the mechanisms responsible for the amplification and examine their effect on signal stability and hence on the accuracy and reproducibility of measurements. We will look closely at the characteristics and properties of signal fluctuations depending on the various parameters of materials, molecules studied and measurement parameters, as they affect the variation of the SERS measurement signal. We anticipate the use of both nanoparticles of silver and gold as well as silver nano-porous substrates. Both of the presented materials allow for very reproducible results, so the observed variability is mainly due to the mechanisms that govern the formation of the reinforcement. The nature of variability is determined using a number of statistical methods and processing of measurements, and we develop mathematical models that explain variation in the SERS method. This new knowledge will enable the development of tools for optimizing the measurement conditions in the SERS method for selected research objects.

The planned research is interdisciplinary and their results will affect the following areas: optoelectronics, chemistry, physics, nanotechnology and biomedical engineering. We try to solve the presented research problem because of the importance of this research for the development of the SERS method and to improve its sensitivity and selectivity, also for measurements in complex systems such as biological objects. This can be the basis for the development of much more accurate medical diagnostics based on the SERS effect, allowing rapid and accurate screening of many civilization diseases.