Intracellular pH (pHi) is an important indicator of the cellular well-being, as almost all the substances crossing through the cellular membrane influence this value. Therefore, by monitoring the changes in pH we can assume the condition of cell. In cancer cells, pHi is increased compared to normal cells (~7.3–7.6 versus ~7.2), while extracellular pH (pHe) is decreased (~6.8–7.0 versus ~7.4). However, changes of pHi can be different in different organelles, moreover, for example cytosolic pHi can be different in distinct parts of the cell. Therefore, measurements of pHi separately in each cellular compartment can provide more information about cellular well-being comparing to average pHi of whole cell.

There are only a few techniques that can be employed for the determination of pHi such as pH-sensitive microelectrodes, nuclear magnetic resonance and fluorescence. Until now, fluorescence spectroscopy has been shown to be the most sensitive and the most commonly used technique to measure pHi. However, the signal from the fluorescent probe is relatively wide, so during the measurements the signal from different probes simultaneously can result in signal overlapping leading to high measurement error and unreliable pH estimations. Also, changes of pHi due to incubation of cells with fluorescent sensors limit the application of fluorescence microscopy. Therefore, SERS can be a good alternative due to stability of the probes, possibility of multiplexing and its very low limit of detection.

The aim of the project is to optimize the procedure of measurements of pHi simultaneously in several cell organelles (lysosomes, cytoplasm, mitochondria and nucleus) in cells with the use of surface enhanced Raman spectroscopy (SERS).

Gold nanoparticles labelled with pH-dependent molecules (e.g., 4-mercaptobenzoic acid) will be functionalized with organelle-targeting peptides and introduced to cells. Then, Raman mapping will be performed and pHi in lysosomes, mitochondria, nucleus and cytoplasm will be indicated by calculating the ratio of selected bands appearing in SERS spectra. In the project, fluorescence will be used as a gold standard as the methodology is set and the pH stains are commercially available. Electronic microscopy (TEM) will be used to confirm the position of nanoparticles in cells. Electronic absorption spectroscopy (UV-Vis) will be used to perform cells' viability tests and to control the synthesis of nanoparticles. TEM, UV-Vis and DLS (dynamic light scattering) will be used for nanoparticles' quality control. The methodology will be set on healthy and cancer keratinocytes, which represent 95% of the epidermal cells, playing the structural and barrier function of the epidermis. Then the methodology will be tested on well-known models (different healthy and cancer cell lines).

To our best knowledge, it's the first time that SERS spectroscopy will be optimized for detection of pHi in 4 different cellular organelles and tested on different cells. Moreover, SERS probes are more stable and narrow comparing to fluorescent ones, so reliability of the signal will be better and the information about cellular well-being will be provided on a large scale.

The results of the project can make critical insights into the spectral profiles of subcellular organelles, improve the state of knowledge regarding cancer and sub-cellular changes in stressful conditions as well as inspire people in the development of high-efficacy cancer therapeutic strategies by subcellular organelle-targeting drugs.