Integrated AFM-Raman and AFM-IR systems as novel tools in radiation damages analysis

All living organisms are exposed to radiation all the time through natural sources. Since radiation has direct impact on tissues and cells, it has been successfully applied for cancer treatment and diagnosis. Indeed, radiation shows a negative effect on some molecules such as DNA causing serious damages. Thus, in the proposed project, we would like to shed new light on the problem of molecular damages and repairs in cells caused by ionising radiation. Our research will be mainly focused on changes in DNA, but also other cellular components such as lipids and proteins. Studies will be performed on PC-3 cell line, which is a prostate cancer line. The main objective of this research project is, therefore, to study the basic mechanisms underlying the biological effects brought about by charged particles that are of relevance for the functions of cancer and healthy cells. Furthermore, the project aim is to investigate low dose ionising radiation influence on biological systems at the cellular, sub-cellular and molecular level by means of selected non-destructive spectroscopic methods. Due to rapid development of vibrational spectroscopy techniques, it is an excellent chance to get new information about radiation-induced damages in cells using systems with nanometric scale resolutions. Additionally, TERS (Tip Enhanced Raman Spectroscopy) technique seems to be a potential tool for studying local damages in small cell components like DNA.

In the proposed project experimental spectroscopy methods (in micrometric and nanometric scales) will be combined with theoretical ones to achieve deeper insight into processes of radiation-induced damages in living systems at the molecular and cellular levels. Research methodology includes several tasks to be performed in order to investigate damages in PC-3 cells using vibrational spectroscopy methods. Firstly, cell culture will be done to generate the main research objects, *i.e.* cells. Secondly, mature cells will be irradiated using two kinds of the charged particles, *i.e.* X-rays and protons. Furthermore, two ways of irradiation will be applied, *i.e.* normal beam and microbeam, to irradiate the whole cell colony and the individual cells, respectively. Thirdly, the number and kind of radiation-induced damages in cells will be studied by biological tests, *i.e.* micronucleus and comet assays. Finally, spectroscopic studies of irradiated cells will be carried out using Raman and Infrared (IR) spectroscopy techniques. Living and fixed cells as well as isolated cell nuclei will be studied at the micrometric and nanometric scale to recognize and identify radiation-induced damages in DNA, lipids, proteins, and other cellular components. Micrometric measurements will be performed under microscopes to obtain more general information about radiation-induced damages, *i.e.* dominant type of the damages and distribution of damages within the cell colony. On the other hand, nanometric studies will be carried out under an AFM (Atomic Force Microscopy) microscope to show more detailed information about the individual damages, *i.e.* type of the individual damages and distribution of damages in the individual cells. Apart from single point measurements, fast screening of the sample (mapping, 3D imaging) will be applied to get more information from the bigger area of the cell. Additionally, due to metal-generated tip enhancement, TERS measurements will be performed to study DNA damages in cell nuclei. Since dealing with biological samples requires a large sample number, all obtained results from the spectroscopic studies will be subjected to statistical analysis using different kinds of multivariate data analysis. Experimental studies will be supported by theoretical calculations such as Monte Carlo simulations to get information on radiation deposited dose in the studied cells as well as quantum-chemical calculations to predict vibrational spectra of different biomolecules, which can be damaged after irradiation.

Since ionising radiation can damage the structure of DNA and other cellular biomolecules, it may result in transformations and, as a consequence, lead to cancerous changes. It is obvious that human beings have always been in the range of the natural, low dose ionising radiation. However, nowadays we are especially exposed to additional exposure due to commonly used medical diagnosis and frequent airplane flights. Furthermore, employees of research centres, nuclear power facilities, extractive and processing industries, and military facilities are of particular risk of this kind of exposure. Due to increasing probability of radiation exposure, it is of great importance to continue basic research on the influence of low dose ionising radiation on biological systems. Although application of vibrational spectroscopy techniques for studies on cancer lines have been already reported, it is still a strong need for a comprehensive and detailed study on radiation influence on cells, especially in nanometric scale. Since all radiation-induced processes take place at the molecular level, it is extremely important to investigate small areas in order to get better insight into mechanisms that are responsible for the observed damages and repairs. Research methods working in the micrometric scale show the average information from a huge, in comparison to single molecules, region. Consequently, obtained results do not show single changes in molecules, but average response of the system.