Raman reporters as a tool to evaluate *in vitro* myeloid precursors differentiation.

Hematopoiesis is a multi-level process that results in the formation and differentiation of the morphotic elements of the blood, occurring in the bone marrow. It is regulated by physicochemical conditions, allowing for the transformation from a hematopoietic stem cell into functional blood cells. Any abnormalities in the differentiation process and uncontrolled proliferation of precursors affect the blood count and thus disrupt important functions of the circulatory system, such as immune response, transport of nutrients and gases, etc. The process of blood cells precursor differentiation is characterized by changes in the expression of essential proteins, changes in chromatin condensation, or partial or complete loss of the cell compartment functions. Thus, the first signs of differentiation can be detected by staining accessing the organelles' state and ability to sustain their proper functions. The classical approach of single-cell imaging is an application of peripheral smear staining and fluorescence microscopy. **The aim of this project is to find unique biomarkers allowing to determine chemical and morphotic changes related to induced differentiation in three leukemia model cell lines towards erythrocytes, monocytes, macrophages, neutrophils and eosinophils using a number of spectroscopic methods such as: Raman imaging, imaging with molecular Raman reporters, fluorescence and biochemical tests and cytometry**. In particular, changes in the biochemical status of the mitochondrion, nucleus, endoplasmic reticulum and lysosomes will be assessed. Detailed characteristics of biochemical, morphological changes at the subcellular level will allow to characterize the changes in phenotype occurring during the induced differentiation of precursor cells into erythrocytes, monocytes, neutrophils and eosinophils, what in turn will allow for a better understanding of the differentiation process in the context of new drugs development for antileukemia clinical applications

The method of choice is Raman microscopy that allows non-destructive identification of sample components, as well as determining their spatial distribution. Despite many advantages, in particular related to the label-free detection of biochemical changes, the concept of molecular Raman reporters is gaining in importance. Raman probes are isotopically substituted compounds or molecules containing triple bonds in their chemical structure together with a selective group targeting appropriate subcellular compartments. Such a structure displays characteristic bands in the area that is nonspecific for a significant amount of biological compounds, which allows avoiding Raman bands overlapping. Of particular interest is the idea of multiple cell organelles imaging, using a wide range of well-designed Raman probes with a low bandwidth compared to conventionally used fluorescent markers. This is particularly important when considering the applicability of this type of probes in imaging using Raman reporters together with currently developed nonlinear techniques such as Stimulated Raman Scattering (SRS), enabling signal amplification. Combining label-free Raman imaging with molecular Raman probes provides a highly selective and sensitive platform for monitoring spectroscopic markers related to biochemical and morphological changes in single cells.

In addition to the selection of well-designed candidates of Raman reporters, within this project it is crucial to develop a complete Raman imaging methodology using Raman probes to obtain optimal conditions for rapid cellular differentiation assessment. The use of external compounds and reporters also requires comprehensive cytotoxicity studies, which will be carried out using a number of other modern analytical methods to determine changes at the biochemical and morphological level in the tested systems.