The reactive oxygen species (ROS), reactive nitrogen species (RNS) as well as other free radical species may have damaging impact on our bodies on the cellular level, however their production is also an integral process necessary for many metabolic reactions. Blood play a special role in oxidative stress mechanism, as it is not only a connective tissue which supply oxygen and nutrients to tissues but also removes many wastes including oxidative species. Therefore all blood cells are exposed during oxidative stress to oxidative environments rich in ROS, NOS and free radical species. The foundation of the project is to investigate RBCs alternations. This is untypical approach, as previous studies were mostly focused on other blood fractions, mainly white blood cells. We believe, that biological understanding of biochemical changes due to oxidative stress *in situ* in isolated RBCs as well as RBCs in whole blood could expand or knowledge about oxidative stress action. RBCs exhibit oxidative damage through characteristic changes in the biochemical content of the hemoporphirins and cell membrane, the size and shape of cell morphology as well as mechanical properties such as for example stiffness and functional parameters. All those changes have impact or are connected with changes in other blood fractions and general blood properties like increased blood viscosity and impaired flow.

In this project we focus on comprehensive assessment of oxidative stress in red blood cells (RBCs) with the use of innovative technology. The use of combination of Raman spectroscopy (RS), Fourier transform infrared spectroscopy (FT-IR) and atomic force microscopy (AFM) supported with referenced techniques (UV-Vis, biochemical parameters, stainings, blood analysis and ektocytometry) will provide the knowledge about biochemical, mechanical and functional fingerprints of level of oxidative stress in RBCs. The recognition of such fingerprints due to oxidative stress *in situ* in RBCs can be a biomarker of general oxidative stress status. Project will allow to create the innovative methodology for sensitive and fast diagnosis of the oxidative stress level in RBCs which will be tested to be apply to in *vitro/ex vivo* description of oxidative stress level in RBCs obtained from mice models of different diseases of affluence. Such diseases as atherosclerosis, or diabetes share etiologic pathways wherein oxidative stress plays the contributing role. To the main aims of the project we may include:

1. ***In situ* characterization of oxidative stress chemically induced in isolated RBCs from healthy mice** with the focus on haemoglobin structure; alternations of the lipids and proteins inside membranes of RBCs; mechanical properties of RBCs such as stiffness, topography, adhesion; functional properties such as deformability and aggregation; biochemical content of the macrovesicles produced by RBCs in oxidative stress conditions.
2. **Definition and description of the possible biochemical, mechanical and functional fingerprints of oxidative stress in RBCs** will be carry out depending on type of chemically induce oxidative damage/pathology, type of RBCs and comparison between results obtained for isolated RBCs *versus* whole blood.
3. **Selection and testing of chosen fingerprints obtained with the use of IR or Raman or AFM for *in vitro/ex vivo* detection of the oxidative stress level in RBCs obtained from the different mice models of human disease of affluence** which share etiologic pathways wherein oxidative stress plays the contributing role.

We believe that the planed in our studies use of Raman, IR and AFM techniques allows not only for detection of oxidative stress level of RBCs but maybe also a potential new set of techniques for detection of some RBCs alternations in the first place. Raman technique uses the advantages of immersion confocal spectroscopy as well as provides information about the biochemical changes of the sample on the molecular level. IR has additional advantage of not only detection but also easy quantification of many biochemical changes. It was already shown that both, IR and RS have huge diagnostic potential also in RBCs studies. If specific molecular change of RBCs has specific marker bands the diagnosis could take even up to several second what gives additional possibility of the fast *in vitro*/*ex vivo* determination of the oxidative stress in RBCs. AFM can provide a mechanical fingerprint of a single-cell pathological changes also for RBCs what makes this a promising technique in cell alternation diagnosis. Moreover, those techniques allow for an analysis of the molecular and mechanical changes in a single RBC in small quantity of blood without previous staining or fixation. Obtained data (the set of Raman/IR/AFM results) which keep the molecular information, can be easily stored for future analysis.

It is expected that the new methodology used in this project will be developed for characterization of the oxidative stress level in RBCs. Results of the project will give physical output such as formation of unique research group which uses new applications of imaging biospectroscopy and AFM to new concept of RBCs investigation. Moreover, results will be published in international journals with a high Impact Factor, present on international and Polish conferences. The project contribution to a higher level goal is creation of the methodology of RS/IR/AFM diagnosis of the oxidation damage in RBCs which can be apply in studies on animal models of different human diseases of affluence.