Biosensors and “lab-on-a-chip” devices with an optical detection system allow for a label-free, very sensitive real-time detection of many different molecules. They are often based on binding of an analyte on silicon transducer surface during an immunoassay using specific antigen-antibody reaction. However, this method of an analytes detection requires a multi-step functionalization of silicon surface with different molecules. This functionalization most often involves modification with monolayer of self-assembly molecules, immobilization of detecting molecules and blocking of free surface sites. The most popular and the easiest approach to proteins immobilization on such surfaces is physical adsorption.

An application of the multi-step procedure of functionalization prior to detection requires control of surface molecular composition after each subsequent step. However, so far such an analysis was hardly provided. Moreover, an increase of total surface density neglecting a possible desorption of detecting protein during subsequent immunoassay steps is a signal in optical biosensors. This approach could lead to inaccurate determination of an amount of bonded antigen and unreliable biosensors results. Another important problem is an orientation of antibodies immobilized on surface which is crucial for the ability of antigens binding. Despite a great number of research articles concerning this issue so far only few of them investigate a relation between immunoassay efficiency and antibodies orientation. What is more, in such research both the antibodies orientation and bounded antigens amount are determined indirectly based on an increase of total biomolecular surface density during subsequent functionalization and detection steps. Both these methods are inaccurate if ones takes into account the system multi-molecularity, desorption of molecules observed during these steps as well as the ambiguities of antibodies orientations for some ranges of surface density.

The aim of the proposed project is an analysis of multi-molecular overlayers on silicon surface by application of the time of flight secondary ion mass spectrometry TOF-SIMS and a model optical biosensor. The TOF-SIMS technique is characterized by unique surface sensitivity, limited to the outermost regions of immobilized proteins, and excellent mass resolution allowing for resolving mass signals of different amino acids. These features of TOF-SIMS spectrometry will enable a direct analysis of molecular surface composition and a direct determination of antibodies orientation in such multi-molecular system. The proposed approach will be demonstrated for fundamental problems concerning an immunoassay on silicon biosensing surfaces.

In the framework of the proposed project an orientation of antibodies immobilized on modified silicon surfaces by physical adsorption and chemical bonding and their dependence on surface density will be determined. Another research task will be a comparison of the functionalization of biosensing surface involving antigens immobilization and blocking free surface sites for physical adsorption and chemical bonding of proteins. Moreover, the influence of antigens surface density on multi-molecular surface composition will be investigated. The final stage of the proposed project will be an analysis of a model immunoassay. In particular an application of immobilization by physical adsorption and chemical bonding of proteins will be compared and the influence of antibodies orientation on antigens binding efficiency will be determined.