Experiments at the single cell level have revolutionized the last few years of research in many areas of biology. The most popular assay in this field is RNA sequencing, which provides information about the activity of a cell and its physiological state at a given moment. Analysis of a transcriptome (collection of all the RNA molecules) at the level of individual cells, allows to obtain a precise description of the physiology of groups of cells, which is especially valuable for the analysis of the structure and functions of complex tissues and processes such as cancer development and therapy mechanisms, immune response or cell differentiation. Scientists can use information from RNA sequencing of single cells to precisely determine the cellular composition of a given tissue, the functions and roles of particular groups of cells, and to study communication between cells. Currently, the most popular method of analysing single cell transcriptomes is the use of a droplet microfluidics. During this assay, a single cell is encapsulated with the necessary reagents in ultra-small microdroplets with volume of a few hundred picolitres, i.e. less than one billionth of a litre. Then, inside microdroplets, each of the RNA molecules is combined with a special molecular barcode, unique for each of tens of thousands of cells, and then the RNA molecules are reverse transcribed into more stable cDNA molecules. In the next step, the reaction products can be released from the droplets, and the sequences of hundreds of millions of barcoded cDNA molecules are read by next-generation sequencing methods. The single-cell sequencing results are then analysed using bioinformatics to create graphical visualizations depicting the groups of cells in the tissues and the gene expression profiles of each. On this basis, new research hypotheses are built, and reference databases are created and included into the Human Cell Atlas.

As part of this project, we propose to go one step further and develop and validate methods not only for the analysis of single cells, but also for the study of gene expression in cells that are physically connected to each other. This type of interaction is very common in living organisms - e.g., antigen presenting cells interact with lymphocytes and trigger them to an immune response. Another example are cancer cells which, by physical interacting with the immune cells, deactivate them, thus unfortunately allowing for a tumour growth and spread in the organism. A more detailed study of these processes at the level of single cells and their interacting pairs would reveal new mechanisms in both healthy and diseased tissues and could lead to the development of new therapies.

In order to develop a method for analysing interacting cells, we will develop new microfluidic technologies for the selection and isolation of cell pairs based on multi-colour fluorescence measurement and ultrafast image analysis. In addition, we will develop new microfluidic devices and molecular methods to label RNAs derived not only from one, but also from several cells enclosed in a microdroplet. In the next stages of the project, we will optimize the model of interacting fibroblasts with cancer cell lines. These cells will be designed in such a way that at the time of interaction, the fluorescent signal will be activated, and the newly produced RNA will have a slightly different molecular structure. Such a solution will not only allow for a better understanding of the physiology and dynamics of changes in gene expression as a result of interactions between cells, but also will be a good strategy for assessing the research capabilities of the newly developed method.

Physical interactions between cells are one of the most common mechanisms of communicating and transmitting signals between cells in the organism, so our approach could become an important part of the technology arsenal for single cell research in the future. We believe that the newly developed method for the study of both single cells and their pairs will be used in many biomedical and clinical projects, especially in the field of immunology, oncology or infectious diseases.