We are under constant exposure to many threats - including viruses, bacteria, parasites, and the formation of new cancer cells. To protect us from those enemies, the evolution has equipped us with the amazingly powerful immune system. It consists of a complex network of a multitude of molecules, cells, and tissues that work together in an organized way throughout the entire body to fight against infections and cancers. At the same time, to avoid overreacting and induction of autoimmune diseases, the immune system has to be well regulated and stabilized. How to achieve such a balance? The secret to the success lies is in proper communication between all cells. Cells 'talk' to each other by sending dozens of the signal molecules. One of such molecule is interferon gamma (IFNγ), which is a protein, involved in a broad range of immune reactions. Cells receiving the signal are then informed about the current situation. Those signals can also operate as stimuli, what initiates the action of cells directed to fight various threats. Each threat is fought by the different set of stimuli.

One might expect, therefore, that the perception (sensing) of those signals (stimuli) is precisely regulated, and a specific concentration of one stimulus leads to a very reproducible strength of the action (cellular response) in all cells. It is therefore interesting, that in the identical cells (by 'identical' we understand, by our best knowledge, identical genetically and derived from the same cell type) and in the identical environmental conditions in the *in vitro* cultures, we have observed high variability of cellular responses to stimulation by given dosage of IFNγ. **The variability was so broad, that unstimulated cells exhibit similar responses to the cells stimulated by the highest IFNγ concentration.** This phenomenon is known as the cell-to-cell heterogeneity and is described in terms of many biological processes. **However, very little is known about the sources of high heterogeneity of responses IFNγ.** How the well-organized immune functions can be performed, when the seemingly identical cells respond to the identical signal in such a various way? Is the response heterogeneity biologically important? To what extent it is purely random? To answer these questions, the nature of the heterogeneity of IFNγ stimulated responses should be understood.

The main goal of this project is to reveal the sources of the observed high heterogeneity and find, which of the cellular processes or factors influence the response to IFNγ the most.

To do that, I will investigate the role of the cell cycle and circadian clock, two of the superior cellular processes, on the IFNγ stimulated cellular response, all using microscopy with the automated analysis of single cells *in vitro* cultures. In addition, I will check, to what extent the cellular proteins influences the total IFNγ response. The cellular response to IFNγ is commonly represented by the STAT1 protein activation and its transport to the cellular nucleus. Therefore, in my studies, the IFNγ response will be measured as concentration of activated STAT1 protein in the cell nuclei.

In the **first task,** I will discriminate cells which are in different states of the cell cycle and compare the heterogeneity of IFNγ responses among such dissected cells. In the **second task**, I will synchronize the circadian clock in the whole cell population and compare the IFNγ response between synchronized and unsynchronized cell populations. In the **third task**, I will fuse two identical cells (with the above-mentioned definition of identity), merging their cytoplasm component but not the nuclei. In such cell (called syncytium), all the cytoplasm- and membrane- related components will be shared for both nuclei. I will prepare a population of such syncytia and check, whether the IFNγ stimulated response is the same in each of the two nuclei. In the end, as the task **fourth**, together with my supervisors, I will build a mathematical model based on the gathered experimental results, which will simulate the stimulation of cells by IFNγ and explain the observed high heterogeneity of IFNγ response.

The research on the immune response heterogeneity is especially important, as malfunctions in signal molecules sensing and responding to the immune stimuli are commonly observed in cancer and immune diseases. In addition, treatments trials related to IFNγ faced severe issues like side effects or the uncertainty which dosage should be used. Some of those obstacles could be avoided, and personalized medicine could be more precise if the nature of cell-to-cell heterogeneity would be resolved. It can further help in more precise controlling of the patients' immune systems by targeting the IFNγ with the proper dosage at the right place.