Investigation of cellular enzymatic activity through single cell analysis using DropMap

When researchers study cells and their functions, the studies tend to be done in bulk, i.e. on millions of cells of the same type. In this way the researchers assume that the cells will behave more or less the same, following a normal distribution. But during the latest years more and more techniques for studying cells have been developed and differences within several cell populations have been unveiled. This speaks for the importance of investigating cells on a single-cell level rather than in bulk. Studying cells one by one is not an entirely new field of science, there is a number of such techniques already on the commercial market, some of them even have very high throughput. However, these techniques analyze the cells based on features of the cells momentaneously. A well-studied, reliable technique studying cells over time is still lacking. Also, the most commonly used techniques study proteins on the cells or within the cells and have no possibility to study the proteins and other substances that are secreted by the cells.

The DropMap technology, first introduced by Eyer et al. in 2017 was used to study activity of secreted proteins from individual cells. This technology creates a water-in-oil emulsion utilizing a microfluidic system that produces aqueous 50 pL size droplets in an oil phase. The cells are diluted in the aqueous to a concentration that generates droplets containing one single cell. The droplets are loaded onto an observation chip achieving only one layer of droplets. These are then observed under microscope over a period of time, up to 24h. Around 1500 cells were analyzed in each measurement providing a more precise picture of the cell type that was investigated. Even within cell types where all the cells stemmed from one mother cell, cell lines, big differences from cell to cell could be proved.

Three cell types were tested under different conditions. First a cell type considered to be very homogenous in a population was tested with a viability dye, a dye that is supposed to mark the cells that are alive. The intensity of the dye could be measured and the difference from cell to cell was observed. This experiment was repeated with several sets of cells that had been thawed on different occasions. Here we could see that the population was extremely heterogenous the closer the experiment was to the thawing date. The further away from the thawing date the experiment was conducted, the more it resembled a normal distribution.

The second cell type, also considered to behave homogeneously in a population, was tested with a slightly more complex system. A substrate and an activator molecule were added and they were supposed to make the cell produce a certain product. To a different set of cells only the substrate was added. These experiments were repeated with numerous sets of cells. The cells were investigated individually and differences between the sets with the activator and without the activator were noticed. In this system the DropMap technology also proved its usefulness in terms of debugging, since the results can be reviewed manually.

The third and last cell type had been proved to show some signs of heterogeneity within the cell population in earlier studies. This cell line was expected to secrete a protein and the activity of that protein from each single cell was measured. The results were extremely heterogenous and showed that only 10% of the population were secreting the active protein. Also here the images generated during measuring could be used to draw conclusions on what had happened with the cells that showed a positive signal.

This not only showed that cell populations are more heterogenous than we think, but also equips the researcher with a tool that can characterize and quantify these heterogeneities. The information gain that these studies will lead to can help improve the knowledge of all the enzymatic activities keeping up life, like digesting food, muscle and nerve function, and respiration just to name three out of thousands of functions. It will also help us improve the treatments of enzymatic disregulations, like blood clotting, or to circumvent metabolic enzyme expressed by cancer cells that affect the ability of immune cells to infiltrate tumors.