

Working as a team to track down single cells

Researchers from the Nano Argovia program develop a system for analyzing single cells

In the Argovia project SCellNA (Single Cell NanoAnalytics), an interdisciplinary team of scientists led by Dr. Thomas Braun (C-CINA, Biozentrum, University of Basel) has developed a system that can be used to analyze a variety of parameters associated with single cells. The researchers focus on analyzing single cells as there can be considerable differences between the cells found in a tissue or cell culture. If a mixture containing thousands of cells is analyzed, as in a standard analysis, the researchers obtain only mean values and no information on individual differences. It is also impossible to observe interactions between cells in the measurement results. The analysis of single cells, however, yields a clear picture that permits a different set of statistical analyses and conclusions regarding different populations within the culture.

Means are not always meaningful

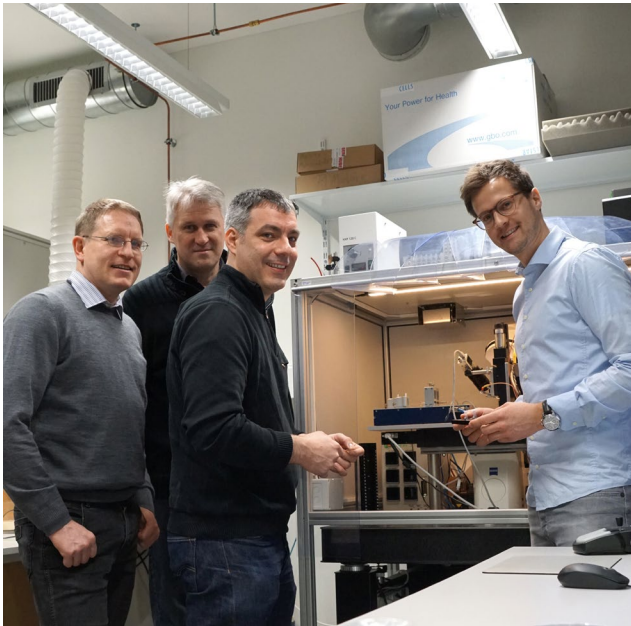
There are differences between the individual cells in a tissue or in a cell culture. For example, there are variations in the proteins that are formed, and individual cells can also vary widely in the level of low molecular weight compounds they contain. If all the cells in a cell culture are processed and analyzed together, these individual differences disappear and the researchers obtain nothing more than an overview of the mean values. Still, to understand how diseases develop, spread, and can be treated, it is essential to know how the individual cells behave and what metabolic processes are taking place inside them. It is also important to examine the interactions between the cells, for example to improve our understanding of how neurodegenerative diseases such as Parkinson's and Alzheimer's spread.

Therefore, the team of scientists involved in the SCellNA project set themselves the aim of constructing a system for the automated analysis of a range of variables relating to single cells. Obtaining meaningful answers to a specific problem requires the observation of a large

number of cells, so effective sample preparation and smooth transfer to the various analyzers is key.

A compact system for selecting and processing single cells

Stefan Arnold, who has been a PhD student at the Swiss Nanoscience Institute's PhD School since 2013, laid the foundations for this approach in his doctoral dissertation. He developed a compact system that allows him to grow cells in a cell culture, examine them under an optical microscope, and select single cells in a highly targeted manner. Subsequently, he uses an electrical field to make the cell membrane permeable before sucking out the entire cell contents, a few nanoliters in all, into a microcapillary in a matter of seconds. Depending on the planned analysis, this lysate is then applied to specific microscope slides or grids. "Unlike in the preparation of an entire cell culture, our approach allows us to obtain a precise overview of the contents of single cells. Our cells are also exposed to less stress, as they are living in their adherent cell culture and are interacting



with one another until a few seconds before processing,” Stefan Arnold explains.

Sample preparation is followed, for example, by examination on an electron or scanning force microscope, allowing the proteins contained in the cells to be visualized and identified. This analysis does not require labelling, but the samples must be desalted and partially treated with heavy metal salts to increase the contrast.

Viscosity indicates RNA binding

The researchers are interested not only in the proteins that the sample already contains but also in the nucleic acids (RNA) involved in the production of proteins. With this in mind, the scientists first measure the viscosity of the sample using a high-throughput microfluidic system that was developed in-house. Their plan is then to take complementary RNA sequences for the RNA fragments they want to detect and add these to the sample. If this results in binding and hence the formation of double strands, the resulting change in the sample’s viscosity can be measured precisely even in the case of small sample volumes.

Further analyses by the partners

Further analyses are conducted by the project partners. For example, Dr. Gregor Dernick of the Roche pRED Innovation Center Basel investigated how antibodies can be used to detect specific proteins in the cells. The system was adapted so that, within a period of 90 minutes, 192 single cells could be selected, lysed, and placed on a single coated slide for analysis. “With heat shock proteins, we showed that antibodies can be used to detect specific proteins reliably and in a short period of time,” Gregor Dernick reports.

Partners from the FHNW School of Life Sciences in Muttensz expand the range of investigations even further with mass spectrometric analyses. Professor Dr. Götz Schlotterbeck and Dr. Christian Berchtold face the problem of achieving the necessary sensitivity to detect various low molecular weight compounds in single cells. So far, they are able to search for specific substances in a targeted manner in a lysate mixture of about ten cells. It will therefore be necessary to further optimize the system’s sensitivity before information can be obtained on the low molecular weight metabolites of a single cell.

A successful conclusion

“We succeeded in developing an effective selection, preparation, and hand-over system for a wide range of single cell analyses. This system is largely automated, allowing a large number of single cells from an adherent cell culture to be processed in a short time,” concludes project leader Thomas Braun with regard to the results of the Argovia project SCeNa. Braun’s research group is working to further optimize various aspects of this single cell analysis, so refinements can be expected over the next few years and will most likely be followed by applications that are tailored to concrete scientific questions.