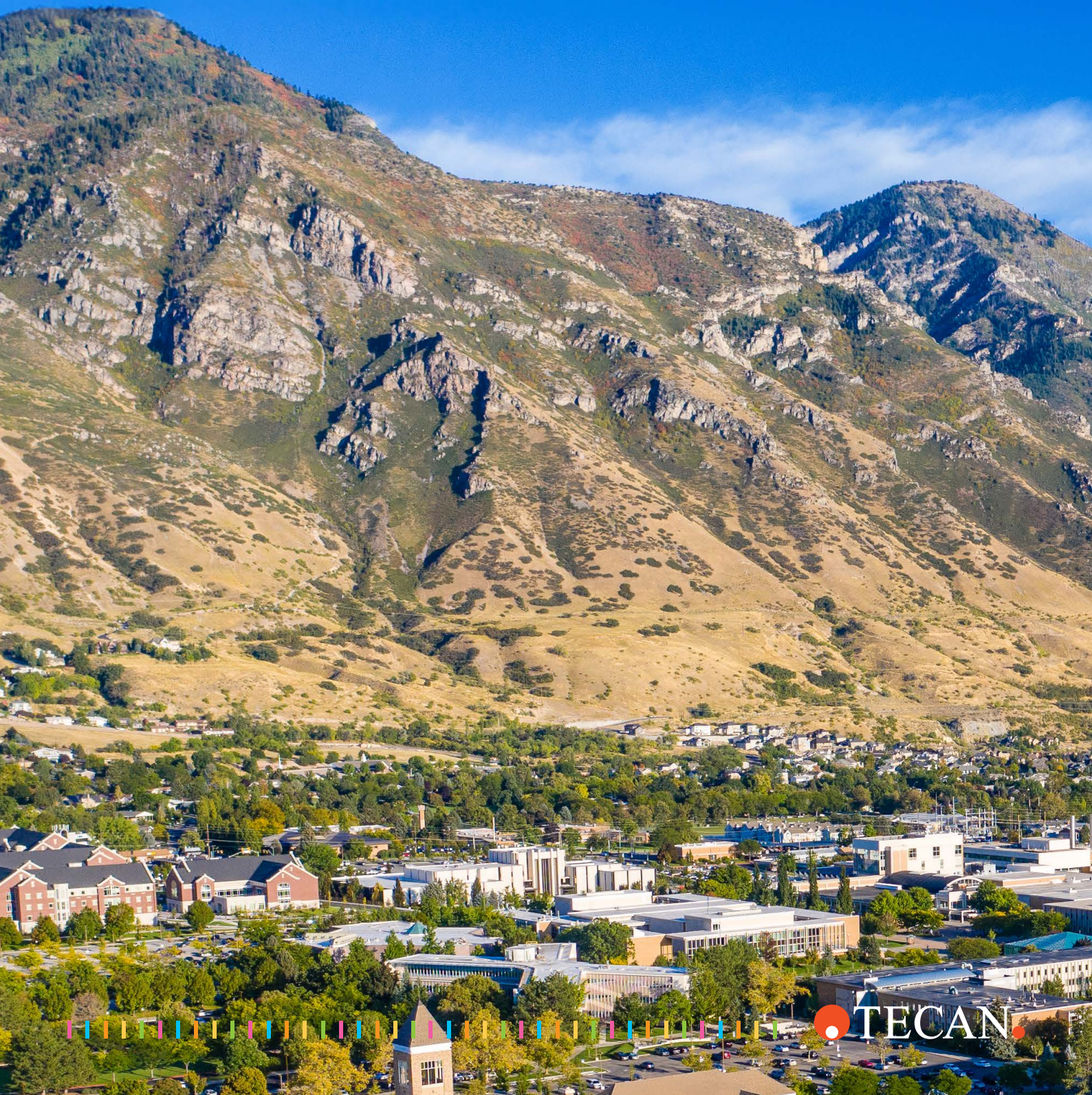
OPENING THE DOOR TO SINGLE-CELL PROTEOMICS.

Tecan Journal Article.



TECAN

The quantification of unique proteins within biological samples has traditionally required thousands, tens of thousands or even millions of cells, with no way to identify cellular heterogeneity within the populations. To overcome this issue, researchers at Brigham Young University have been evaluating the potential of single-cell dispensing to allow proteomic analysis of individual cells.

The Kelly Lab at Brigham Young University in Provo, Utah, focuses on ultrasensitive biochemical analysis. Single-cell and high-resolution spatial proteomics are of particular interest. Ryan Kelly, a Professor in the Department of Chemistry and Biochemistry, explained: "Cells are the building blocks of life, and we're looking to develop a more in-depth understanding of physiology – especially pathophysiology – and how diseases such as cancers originate and develop. For example, a solid tumor is not just a uniform collection of cells, but a complex arrangement of different cell types. The arrangement of these cells determines the tumor microenvironment during the initial stages of the cancer, and whether it will be targeted by the immune system for elimination, or protected to help it evade the body's defenses."

The Kelly Lab researchers are using single-cell dispensing to allow proteomic analysis of individual cells. The advent of single-cell proteomics (SCP) has enabled researchers to investigate cellular processes in unprecedented detail, providing information that is unattainable via bulk-scale protein measurements or single-cell profiling using other omics approaches. Unfortunately, commercially available platforms for single-cell isolation and sample preparation for SCP have a high cost, require technical expertise to operate, and often suffer from other system limitations, thereby constraining their accessibility. Ryan continued: "Our specialty is the analysis of proteins within individual cells.

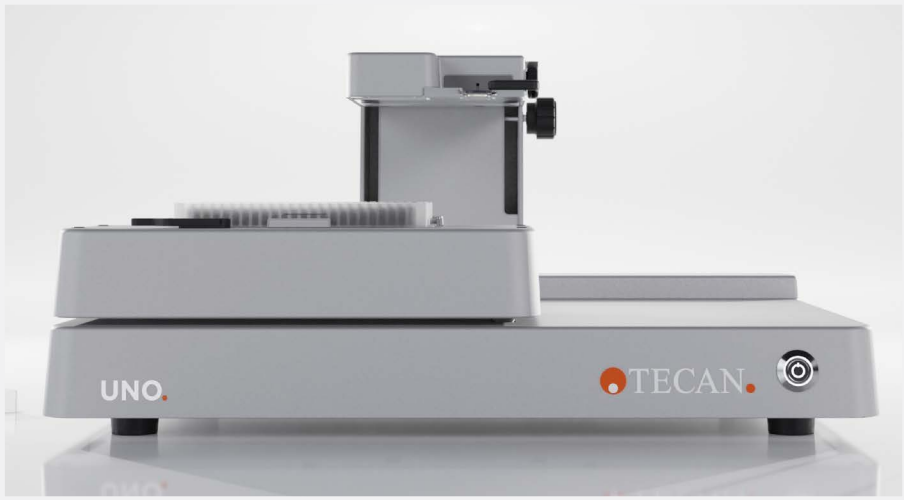
Single-cell sequencing approaches have been around for some time and provide a lot of unique insights. However, until recently, there hasn't been a way of directly profiling protein expression within these cells, rather than inferring it based on messenger RNA. Instead, we were performing bulk-level, in-depth measurements, which allowed quantification of thousands of unique proteins from each sample, but could not analyze single cells. To overcome this, we have now developed methods across the entire workflow – sample preparation, separation and mass spectrometry analysis – allowing us to broadly quantify proteins from single cells while increasing the speed of measurement. Instead of taking the whole tumor, putting it in a blender, then measuring an average of the protein expression within the entire sample, we now have the tools to dissect the tumor cell by cell, quantifying the proteins in each of the different cell types present, and sometimes obtain spatial information as well."

A major focus of the lab is making the methods it develops more accessible to other researchers, and the team started by building robotic nanopipetting platforms to pick up and deliver extremely small volumes of reagents to single cells, as well as fabricating custom nanowells out of microscope slides. Ryan added: "Business as usual approaches for proteomics simply can't be applied to single cells.
The volume of a single cell is about one picoliter, so if a single cell is placed into a standard reaction volume of, say, 100 microliters, the cell contents will be diluted by a factor of 100 million; it's like squishing a grape into a large swimming pool. Miniaturization was, therefore, clearly the way forward."

"We start with intact cells, and the aim is to end up with ready-to-analyze peptides. This requires cell lysis, protein extraction and, potentially, breaking the disulfide bonds that preserve proteins in their native structures. There may also be an alkylation step to prevent the disulfide bonds reforming. The proteins are then exposed to proteases – typically trypsin – to cleave them into smaller peptides that are easier to measure. Miniaturization allows us to keep peptide dilution and surface contact with the reaction vessel to a minimum, as well as to have higher protein concentrations, which makes the

"We now use the Uno routinely … it's just so easy and fast, and we know we can trust its accuracy."

Ryan Kelly, PhD, Professor, BYU Department of Chemistry and Biochemistry



kinetics of digestion more favorable. Fortunately, sample clean-up steps are not necessary, as contaminant levels are not sufficient to interfere with the downstream processes, and any gains would be outweighed by protein losses."

The miniaturized workflow, nanoPOTS – Nanodroplet Processing in One pot for Trace Samples – is now used by other SCP labs, with both label-free and multiplexed approaches being actively developed. "Although we use both approaches, our group mostly focuses on label-free proteomics," said Ryan. "We're interested in finding out what proteins are present, rather than targeting specific proteins with this technology. The technique is also quantitative; we can infer the protein abundances based on the mass spectral intensities of the peptides that we measure. Multiplexed approaches are also being explored, where additional reactions are performed to incorporate barcoded tags."

"About a year ago, our lab transitioned to using the Uno Single Cell Dispenser™ to enhance the accessibility of advanced techniques for biomedical researchers in fields like cancer and developmental biology. This automated benchtop device can isolate single cells into wells very effectively, then prepare them for proteomic analysis. This not only makes SCP easier for us, but also makes the technique more broadly accessible to the general community, due to the low cost of the platform relative to other commercially available solutions."

"It is obviously important that any new technology is fully validated before it is adopted for routine use, and we needed to be certain that a well didn't contain multiple cells when the system software reported the presence of a single cell. We therefore chose to validate the Uno system's single cell dispensing accuracy by mass spectrometry, and were able to demonstrate 97 % accuracy.[1] Reagent dispensing was also validated using fluorescence measurements, and was found to be accurate and reproducible from 200 nanoliters up to 2 microliters.[1] We now use the Uno routinely, as it's perfect for isolating cells from a suspension to give you an unbiased view of the whole cell population, and for low volume reagent dispensing. It's just so easy and fast, and we know we can trust its accuracy. Dispensing is contactless too, so we don't have to worry about cross-contamination. We love the Uno, and think it will have quite an impact in the field of single-cell proteomics," Ryan concluded.

For more information about the Kelly lab at Brigham Young University, visit https://chembio.byu.edu/kelly-lab.

1. Sanchez-Avila, X. *et al.* Easy and Accessible Workflow for Label-Free Single-Cell Proteomics. *J. Am. Soc. Mass Spectrom.* 2023, 34, 10, 2374-2380. https://doi.org/10.1021/jasms.3c00240

TO FIND OUT MORE about the Uno Single Cell Dispenser, visit www.tecan.com/uno

Australia +61 3 9647 4100 **Austria** +43 62 46 89 330 **Belgium** +32 15 42 13 19 **China** +86 21 220 63 206 **France** +33 4 72 76 04 80 **Germany** +49 79 51 94 170 **Italy** +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 17 4 **Nordic** +46 8 750 39 40 **Singapore** +65 644 41 886 **Spain** +34 93 595 25 31 **Switzerland** +41 44 922 89 22 **UK** +44 118 9300 300 **USA** +1 919 361 5200 **Other countries** +41 44 922 81 11

www.tecan.com