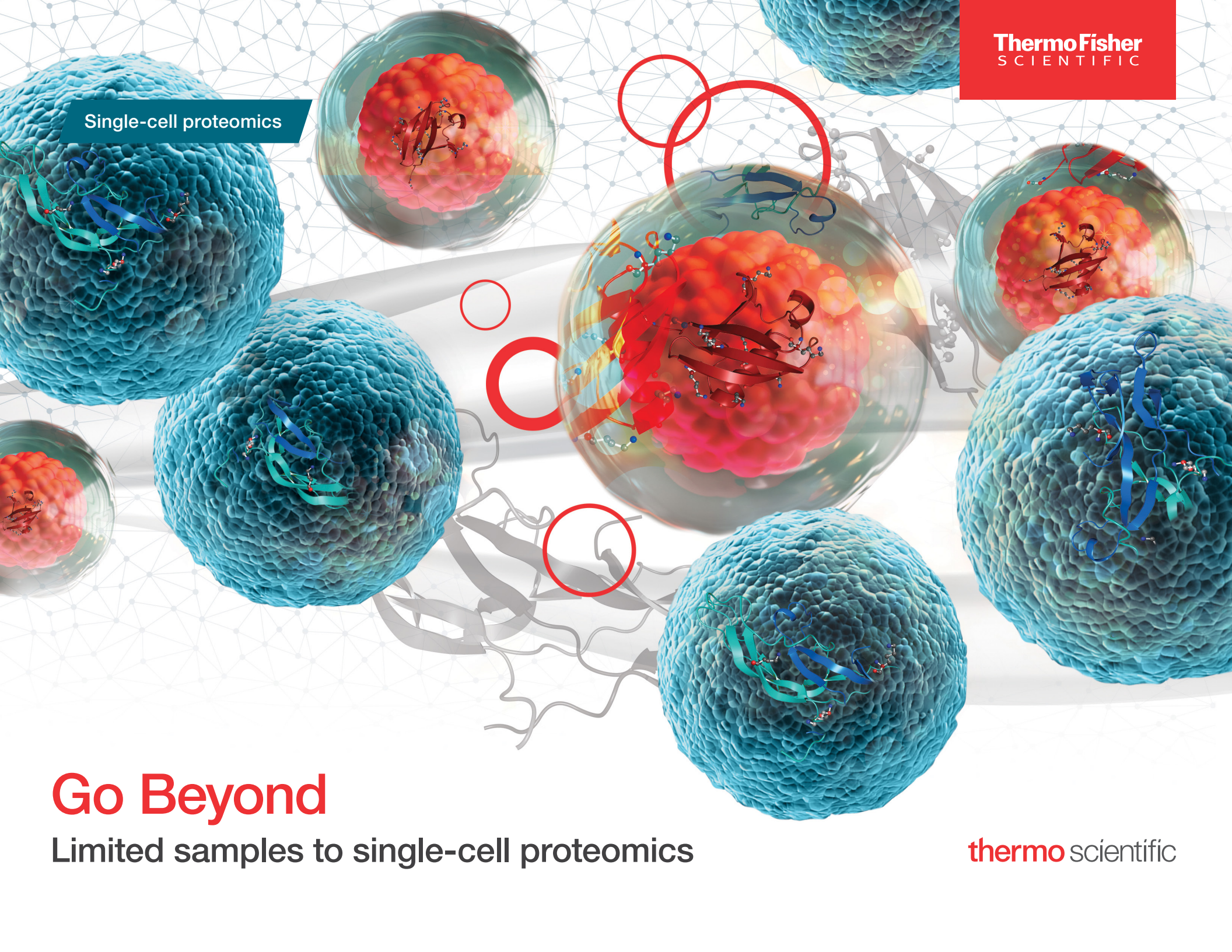


Single-cell proteomics

Go Beyond

Limited samples to single-cell proteomics

thermo scientific





A singular focus: One cell. One shared mission.

Protecting human health: It's a mission that drives us all—and one that's advancing at an unprecedented pace. Every day, we apply relentless focus and desire to use new approaches to ask the most difficult questions, to see things in new ways, and to arrive at new answers to benefit people everywhere.

At Thermo Fisher Scientific, we're proud to partner with life science innovators who are transforming the future of human health. Every single day.

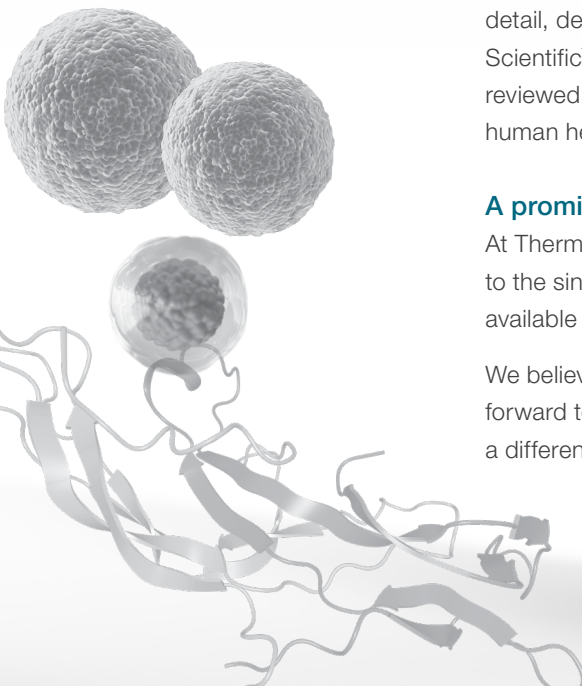
We believe in what's possible—and provide solutions to help

Mass spectrometry enables you to investigate different views on the same biological system to uncover new insights in cellular phenotypes, function and signaling status. Yet to advance proteomics, analytical techniques must become more sensitive, accurate and precise than ever before. They need to interrogate biological systems with much more detail, depth and specificity—areas that we have been innovating for more than 25 years. In fact, bringing Thermo Scientific™ Orbitrap™ mass spectrometry solutions to the scientific community has resulted in more than 75,000 peer-reviewed publications and contributed to insights in human disease that have led to numerous solutions impacting human healthcare.

A promising future awaits

At Thermo Fisher Scientific, we're dedicated to accelerating proteomics research, pushing the limits of sensitivity down to the single-cell level, streamlining workflows that maximize throughput, and making single-cell proteomics widely available to all scientific researchers.

We believe this is just the beginning. With dedicated focus and innovation from the life science community, we look forward to partnering with you to unlock the potential of single-cell proteomics so you can spend more time on making a difference.



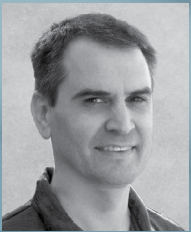
Innovation through collaboration—how we're driving progress

Leading the way in single-cell proteomics means collaborating with experts in the scientific community. By joining forces with single-cell proteomics leaders from around the world, we have taken a holistic approach to advancing this field.

We know it's crucial to ensure cells are viable and high quality—from sample handling and sample preparation, all the way through LC-MS analysis. The scientific progress

that has been made for limited sample analysis and single-cell proteomics is impressive, with many researchers and laboratories taking diverse approaches to reduce sample loss and maximize sensitivity.

With mass spectrometry at the heart of their research, see how various members of the scientific community are tackling these challenges in each part of the workflow.



Ryan Kelly
Associate Professor
Brigham Young
University, USA

“To achieve a successful single-cell proteomic analysis, each stage of the workflow prior to introduction of sample into the Orbitrap MS should be carefully considered. While the community has come a long way, further advances, particularly in ultrasensitive separations, will dramatically improve these analyses in coming years.”



Karl Mechtler,
Head of Protein Chemistry
Facility, Research
Institute of Molecular
Pathology (IMP) at
Campus-Vienna-Biocenter,
Austria

“One major problem is to adapt multi-step sample preparation to such small sample quantities. By collaborating with Cellenion, we have developed a single-cell proteomics chip for sample volumes <100 nL. Together with the Vanquish Neo HPLC system and the Orbitrap Exploris 480 MS with the FAIMS Pro interface, we have achieved remarkably sensitive and accurate TMT-based quantification of a large numbers of cells.”



Erwin Schoof,
Associate Professor,
Technical University of
Denmark

“Thanks to Orbitrap MS and TMTpro technology, we can in principle multiplex 18 single cells at the same time. With advanced chromatography, this enables the possibility of running multiple 10,000-cell projects per instrument, per year, leading to real impactful biological insights across life science research.”



Nikolai Slavov,
Allen Distinguished
Investigator and Associate
Professor, Northeastern
University, USA

“We need creative experimental designs and methods that make accurate single-cell proteomics broadly accessible, so that it can drive the next wave of single-cell biology. Mass spectrometry is well poised to provide such measurements while increasing the depth of proteome coverage and the versatility of the analysis.”

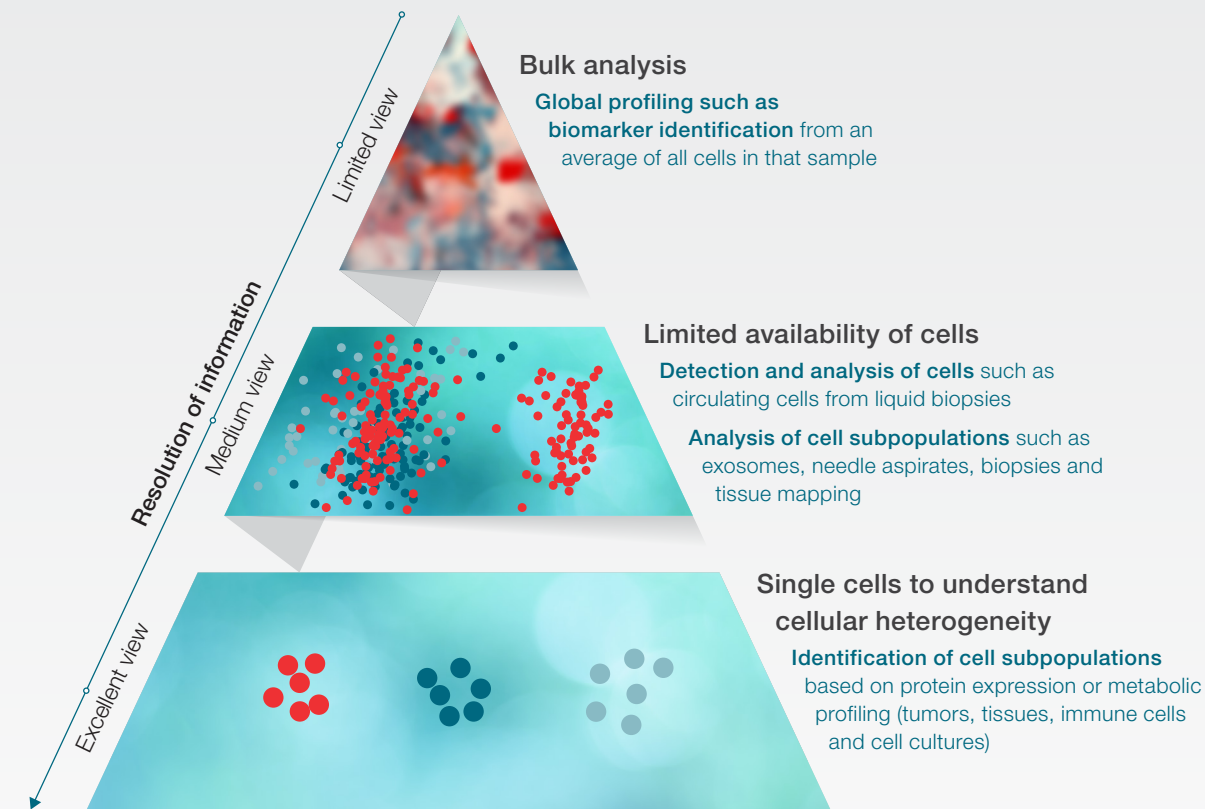
Expand your discovery to far-reaching applications

By collaborating with the scientific community, we've developed innovations in single-cell proteomics that allow you to move away from bulk sample to rare cells, single cells and spatial proteomics that reveal the full complexity of cellular diversity.

We enable you to generate maximum insights that assess cell heterogeneity and rare cell types across extensive applications from biological discovery to translational research.

Single-cell proteomics that deliver actionable insights

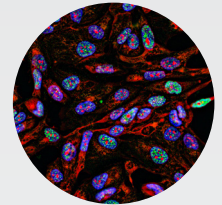
Because proteins mediate almost all of the cellular functional processes of a living organism, they are key to understanding biology. With depth and sensitivity that enables the study of single cells to be practical, we can now delve deeper in the understanding of complex cell heterogeneity, analysis of rare cells and spatial tissue mapping.



Different technologies facilitate varying levels of resolution for uncovering cellular complexity, from population-level metrics via bulk sample analysis to fine-grained aspects of cellular architecture with single-cell analysis.

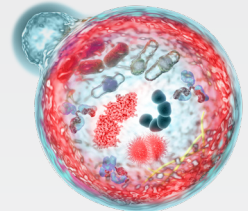
Characterize complex cell populations

Capture cellular heterogeneity through unbiased detection of cellular protein expression without prior knowledge of cell subtypes or cell markers.



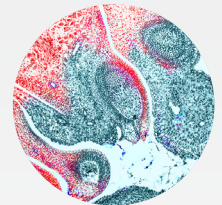
Unravel complex cellular processes

Profile the drivers of complex cellular processes in disease and development with full proteome analysis.



Discover novel cell types and states

Refine cell phenotypes and discover new biomarkers with protein expression profiling.



Scalable functional genomics

Explore gene function variation across diverse cell types with paired CRISPR knockdowns and rich single-cell phenotype readouts.



Single-cell proteomics enables biological studies.

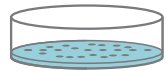
Every sample is precious—make every cell count

Proteomics provides access to the wealth of information inside every cell. With the Orbitrap mass spectrometer at the heart of the single-cell proteomics workflow, we can unlock this potential—from sample preparation, separations, mass spectrometry and

data analysis—by delivering more proteins per cell and from tens to hundreds of cells per day from cell populations, cell types and states, on a cell-to-cell basis.

Biology focused

Examining cell subpopulations and understanding complex systems

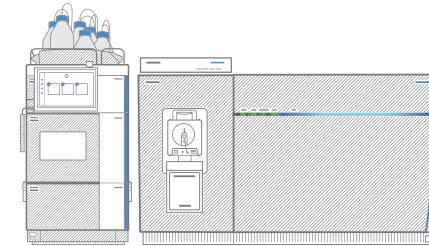
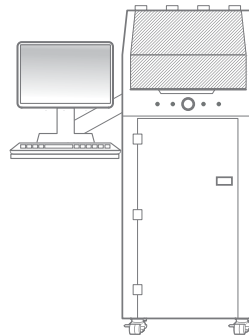


Sample procurement

Cell populations

Sample handling and preparation

Single-cell isolation, dispensing, digestion, and TMT labeling



LC-MS analysis

Spectra-based identification and TMT reporter ion quantitation



Data analysis

Data processing, batch normalization, and data interpretation

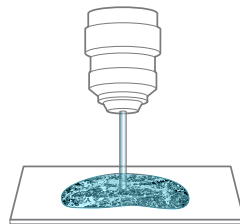
Clinically focused

Examining protein localization within tissue biopsies



Sample procurement

Tissue slide

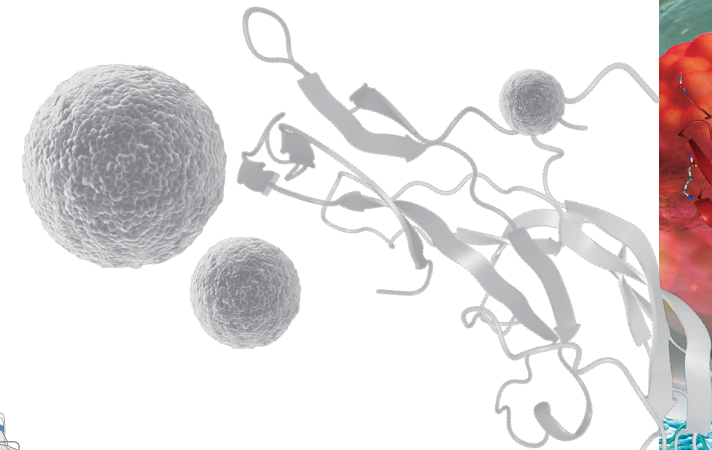


Sample handling

Deposit tissue voxels in 384 well plate

Sample preparation

Tissue voxel digestion and TMT labeling



Going beyond one cell at a time—maximizing throughput and sensitivity

Gaining comprehensive biological insights requires the ability to analyze a statistically significant number of cells. Multiplexing single cells using isobaric tags allows for up to 18 single cells per run when combined with the high-resolution and intelligent

Increasing throughput to >200 single cells per day

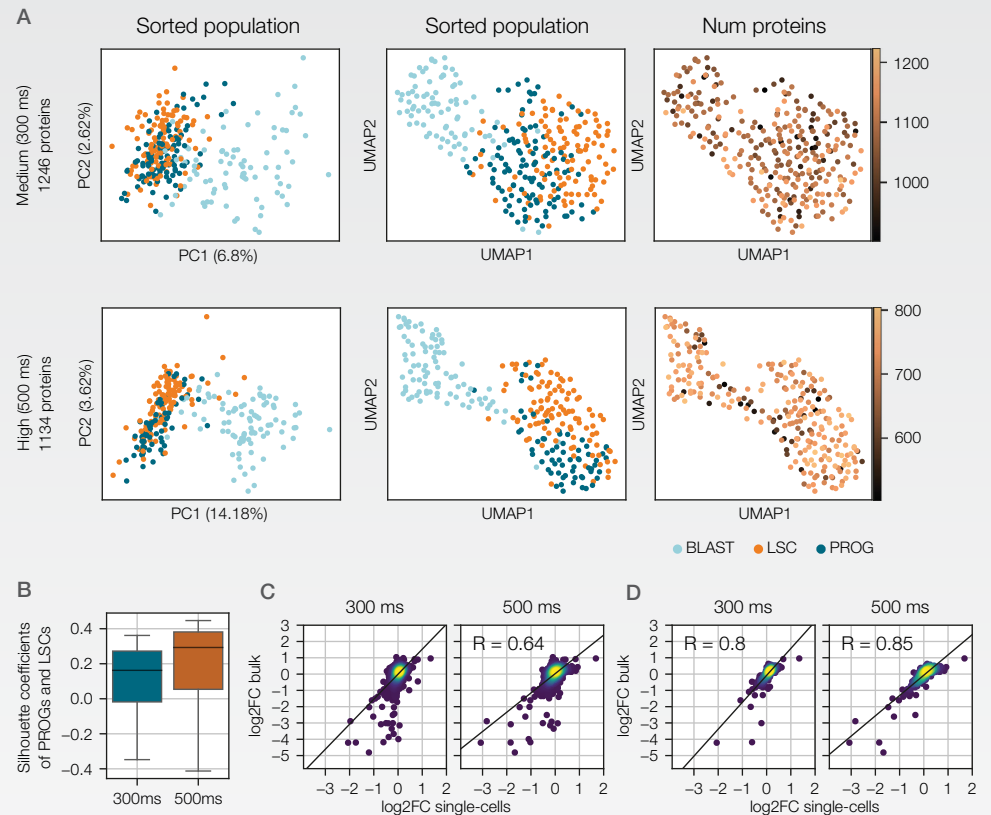
Single-cell proteomics approaches put significant strain on throughput requirements. Yet researchers need a high number of cells to gain confidence in biological observations or to identify rare subpopulations.

Multiplexing with TMT reagents in combination with Orbitrap mass spectrometers delivers throughput gains that go beyond one cell at a time. This unique approach enables the consistent quantification of thousands of proteins per cell across thousands of individual cells using limited instrument time, thereby unleashing the potential to democratize global single-cell proteomics studies.

“At a throughput of 20 samples per day, we could therefore in principle cover more than 300 cells per day using TMTpro 18-plex reagents, cutting down the total analysis time of 10,000 cells to just over one month. It’s become clear that the high-resolution Orbitrap technology is essential to really be able to multiplex 18 cells at a time.”

—Erwin Schoof

data acquisition delivered by the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer or the Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer.



(A) With Thermo Scientific™ TMTpro™ 16plex multiplexing reagents, generating data on more than 650 single cells takes less than one week of measurement time, resulting in biologically meaningful separation of cell types. (B) Higher MS² injection times are beneficial for more accurate separation of cell types. (C) Compared to bulk proteomics data, the quantitative accuracy of our single cell measurements correlate quite well, and very well for the 400 highest expressed proteins in (D).

Data courtesy of Schoof, E.M., et al., 2021. Nature communications, 12(1), pp. 1–15

Driving new applications that accelerate research

The ability to examine a biological system down to a single cell can pave new paths in research. Featured in more than 75,000 peer-reviewed scientific publications, Orbitrap mass spectrometry solutions have enabled high impact discoveries by the scientific community and will continue to do so. Single-cell proteomics is advancing very rapidly from unbiased identification and quantitation, to the targeted quantitation of protein

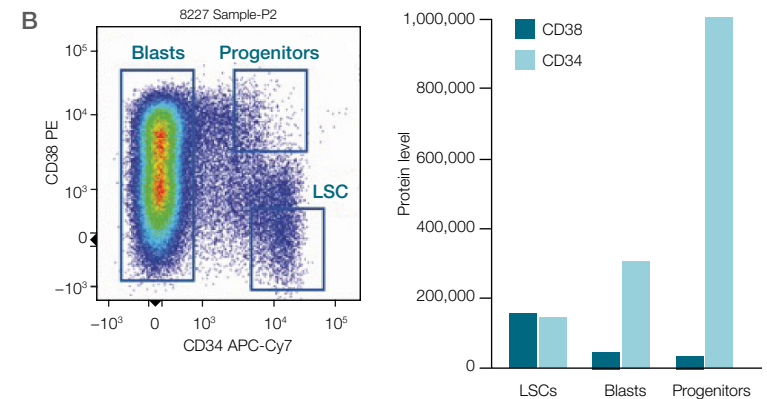
panels. We have progressed from large single-cell oocytes, cultured cells and small mammalian cells, to spatially resolved mammalian tissues. The future is bright with the scientific community driving new applications and technologies to enable these single-cell proteomics techniques to become powerful and widely used approaches in biomedical research.

High throughput targeted single-cell proteomics using the Thermo Scientific™ SureQuant™ workflow

Most biochemical functions of the cell are performed by proteins, and they're also the target of most current drugs. Enabling the power of high-throughput targeted proteomics to conduct analysis of specific panels of proteins, post-translational modifications (PTMs) and low abundance proteins, such as transcription factors and kinases in a single cell, can deliver new insights with the understanding of complex molecular mechanisms in the context of disease and cell development.

A

Cell stage	Targets	Targets detected and quantified by	
		g-scMS	t-scMS
LSCs	99	24	99
Progenitors	99	12	98
Blasts	99	20	96

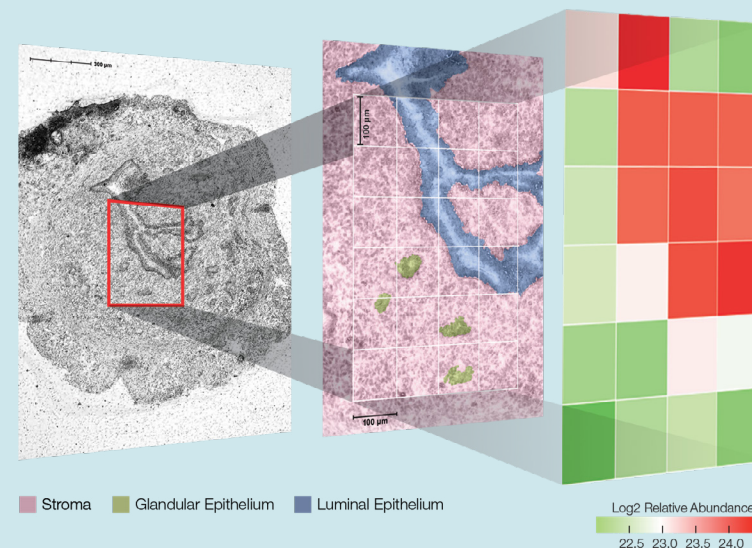


(A) Initial developments using sc-SureQuant reagents workflow for targeting a panel of 99 specific peptides in single cells isolated from our heterogeneous OCI-AML8227 primary culture system. A clear advantage can be seen when comparing global scMS with targeted scMS, with almost all targets being measured in the t-scMS setting. (B) Overview of CD34/CD38 FACS markers measured by flow cytometry, compared to t-scMS measurements of those same markers at the peptide level.

Data courtesy of Erwin Schoof (personal communication)

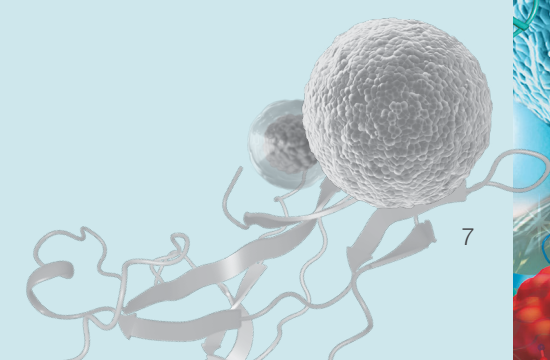
Spatial tissue profiling

Populations of cells are arranged into tissues with diverse functionalities. To better understand cell function and interaction *in situ*, it's critical to explore the cellular machinery and processes in these microenvironments. Spatial proteomics characterizes the abundance and distribution of proteins in this microenvironment. Leveraging the power of single-cell proteomics has enabled an automated approach to imaging that can generate quantitative cell type specific images with 100 µm resolution.



An automated approach to imaging that utilizes nanoproteomics to analyze tissue voxels, generating quantitative cell-type-specific images for over 2,000 proteins with 100-µm spatial resolution.

Data courtesy of Piehowski, P.D., et. al, 2020 Nature communications, 11 (1), pp. 1–12



Optimize your work with leading single-cell proteomics solutions

Thermo Fisher Scientific offers seamless single-cell proteomics workflow solutions that optimize your analysis from start to finish and make this widely available to all scientific researchers. As industry leaders and innovators of proteomics technologies and applications, we're here to deliver the experience and expertise to unlock the

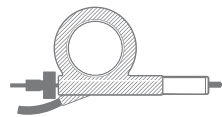
potential of single-cell proteomics. With a unified effort of experts in sample preparation, separations, mass spectrometry, bioinformatics and biologists, we continue to deliver leading workflows for single-cell proteomics to generate maximum insights that assess cell heterogeneity and rare cells, so you can speed discoveries in biology and medicine.

Sample preparation



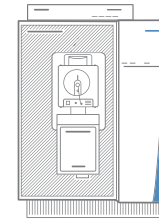
Thermo Scientific™ Pierce™ Trypsin Protease, MS Grade

Separations



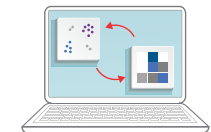
Thermo Scientific™ EASY-Spray™ PepMap™ Neo UHPLC column

Mass spectrometry analysis

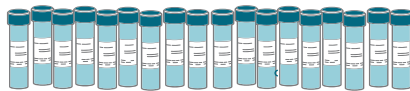


Thermo Scientific™ Orbitrap Exploris™ 480 MS with Thermo Scientific™ FAIMS Pro interface

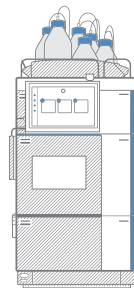
Data processing



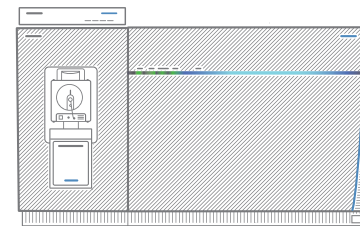
Thermo Scientific™ Proteome Discoverer™ software with MSAID GmbH's CHIMERYS search engine



Thermo Scientific™ Tandem Mass Tags™ Multiplexing Reagents



Thermo Scientific™ Vanquish™ Neo UHPLC system



Thermo Scientific™ Orbitrap Eclipse™ Tribid™ MS with FAIMS Pro interface

Learn more at thermofisher.com/singlecellproteomics

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