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*"I always said that single cell  
proteomics would not happen in  
my lifetime, but I'm happy to have  
been proven wrong."*



## **timsTOF SCP**

- Unbiased, quantitative true single cell proteomics (SCP) research

# timsTOF SCP

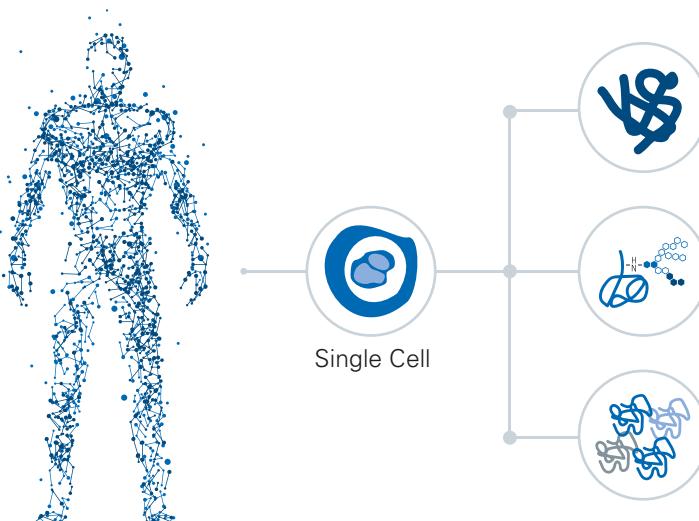
Expanding the horizons of single cell research



*Mass spectrometric proteomics has become a staple of modern research in understanding biological function and disease mechanisms. Healthy or diseased tissues that seem homogenous are composed of cells with a variety of different proteomes. Deciphering the proteome of each single cell is key to fully understanding its function and has traditionally presented a major challenge.*

## Harness the power of 4D-Proteomics for single cell research

After revolutionizing proteomics by the introduction of 4D-Proteomics, Bruker launches the timsTOF SCP. With a new ion source concept for 5 times greater ion-transfer together with the TIMS based time-focusing effect and higher fidelity separation of noise from signal as well as the proven acquisition speed in PASEF ( $> 100$  Hz), the timsTOF SCP is unique in its class. Taking single cell proteomics research, PTM analysis, and immunopeptidomics to the next level for a holistic approach in proteogenomics.



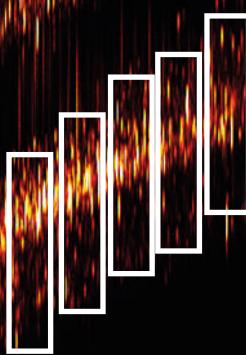
Genomic information is translated into a unique proteome spanning several orders of magnitude in abundance

>200 post translational modifications (PTMs) with hundreds of potential sites per protein driving protein activity and interactions, as well as cell function.

Cell function is driven by the exact protein composition such as protein abundance, PTMs, and interactions

# Ultra-high sensitivity 4D-Proteomics with dia-PASEF

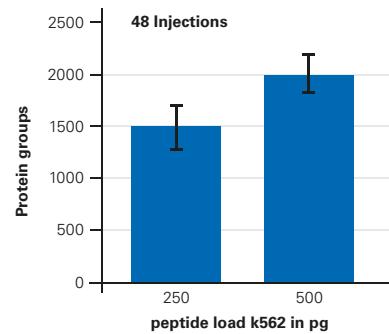
CCS-enabled analysis for  
confident identifications



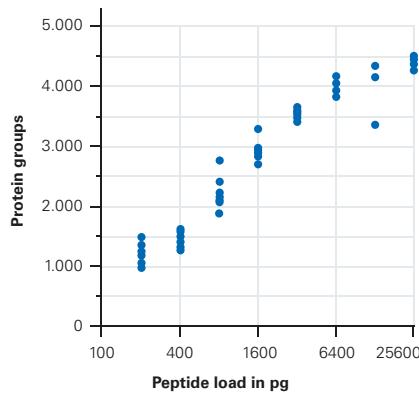
timControl allows customization of the dia-PASEF window scheme to focus on the ions of interest. Adjusting the mass isolation width, TIMS range and cycle time allows adaptation of dia-PASEF to different chromatography methods.

Combining low flow liquid chromatography on the EVOsep One system with Whisper with the high sensitivity of dia-PASEF on the timTOF SCP, over 2000 proteins were identified from 500 pg of cell digest and 1500 proteins were identified from 250 pg, demonstrating the sensitivity needed for true single cell proteomics.

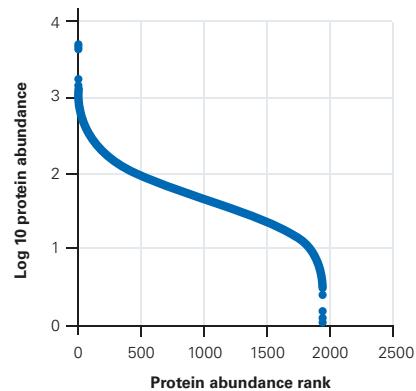
The proteins identified from 250 pg covered an abundance range of about 4 orders of magnitude, enabling quantitative proteome analysis at single level.



Number of identified proteins from 250 and 500 pg of k562 cell digest over 48 consecutive runs.



Identified proteins from a dilution series of HeLa digest ranging from 32 down to 0.4 ng of peptide load using the EVOsep Whisper method.



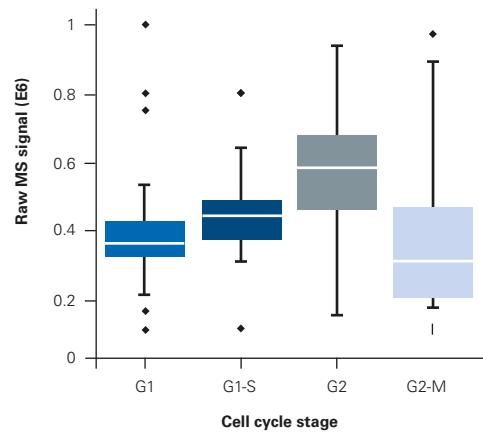
Protein distribution according to abundance rank shows a dynamic range of about 4 orders of magnitude

# Fast and reproducible low flow 4D-Proteomics

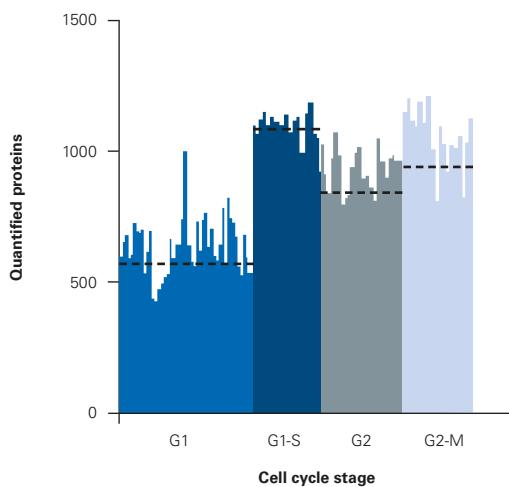
timstOF SCP, Evosep One and dia-PASEF for ultra sensitive single cell proteomics

With the raw sensitivity increase of the SCP instrument, the laboratories of Prof. Matthias Mann performed true single cell proteome analysis quantifying over 1200 proteins from a single cell.

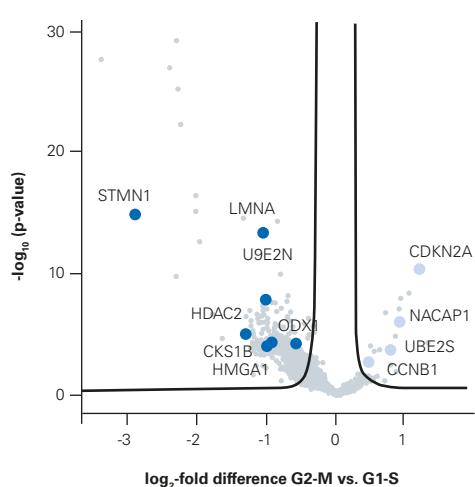
The timstOF SCP was coupled to the Evosep One using the low flow Whisper method. Analysis of cells arrested at different proliferation stages effectively showed cell heterogeneity depending on cell size and cycle stage. Proteins known to be involved in cell cycle regulation (e.g. CDKs, HDACs) are significantly differentially regulated. Data were kindly provided by Prof. Matthias Mann<sup>1</sup>.



*The MS signal intensity correctly reflects the cell proliferation state by correlation with protein amount.*



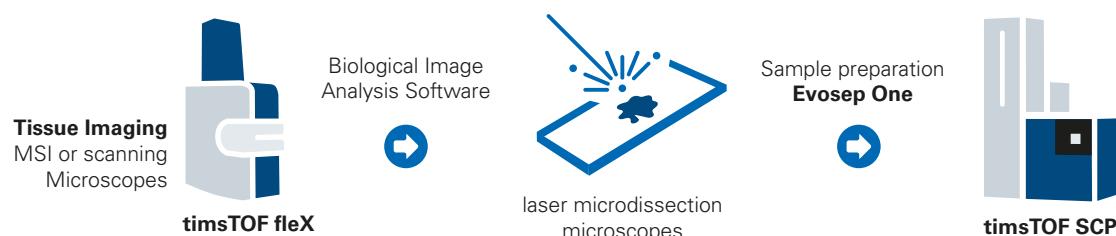
*Depending on the proliferation stage up to 1209 proteins were quantified from a single cell.*



*The biological significance of single cell proteomics is further demonstrated by the significantly regulated cell cycle regulating proteins.*

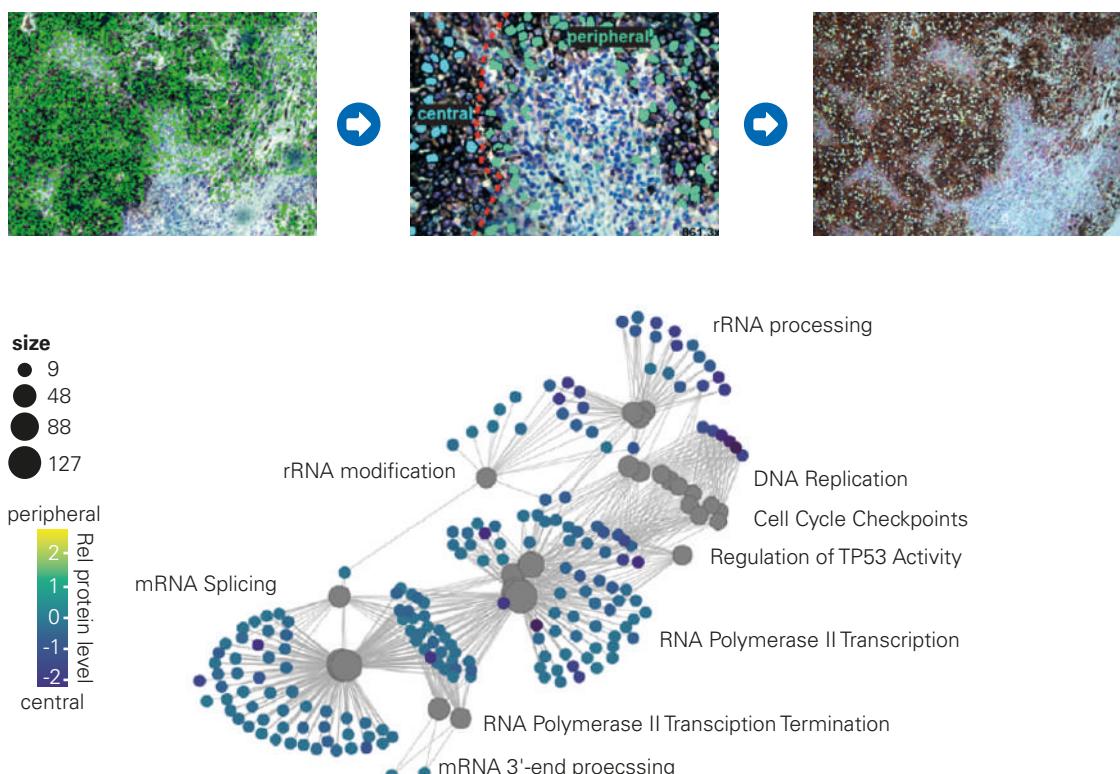
# Exploring the Tumor Microenvironment

Understanding the TME together with its infiltrating immune cells can be considered a crucial step to influence disease progression, treatment response and patient survival. The timsTOF SCP due to its high sensitivity enables sufficient proteome depth on cells excised by laser capture microdissection (LMD) from FFPE tissue samples. A typical workflow is shown in the figure below.



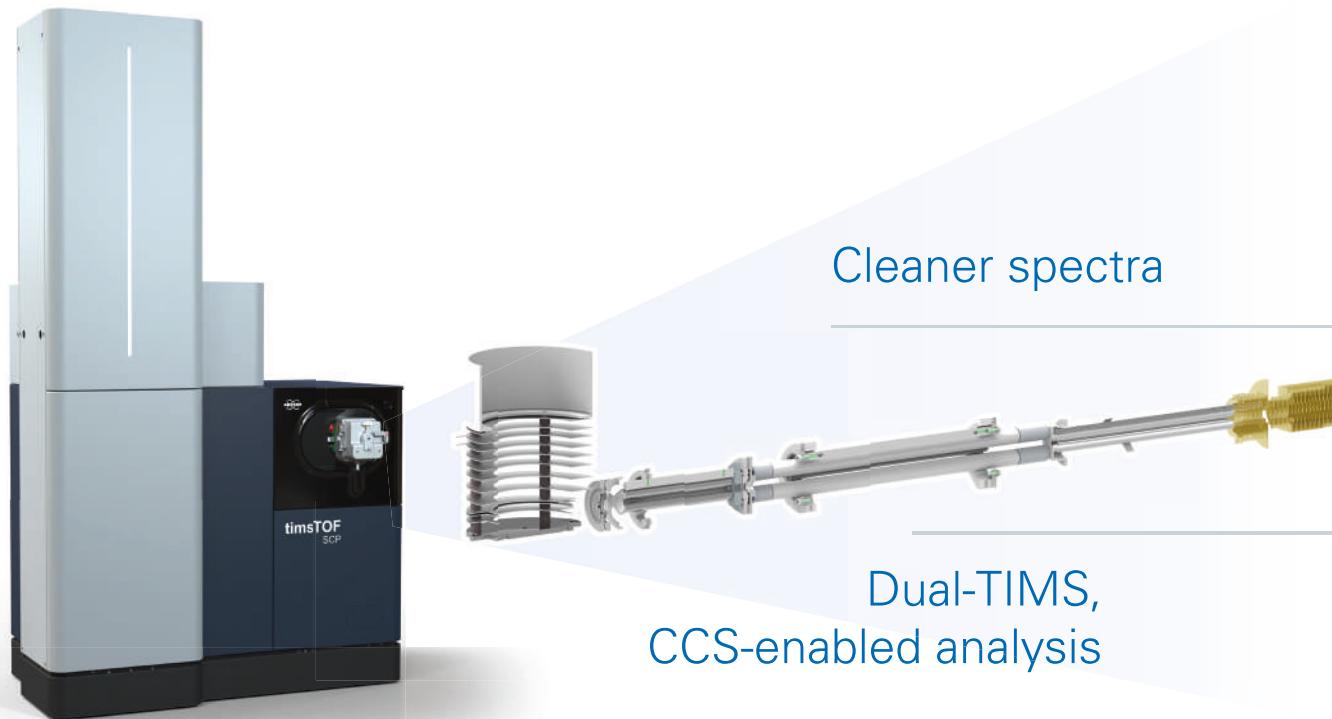
Cellular markers were used to identify melanoma cancer cells in the tumor mass and those closely related to the stroma for subsequent isolation of both populations by LMD and unbiased 4D-proteomics analysis on a timsTOF SCP instrument (Figure below).

**Key Finding:** Enrichment analysis revealed differentially regulated proteins between central and peripheral melanoma cells with potential for disease subtyping to guide clinical decision-making (Figure below). Results are provided in courtesy of Prof. Matthias Mann<sup>2</sup>.



# Unleashing PASEF on a cutting-edge ion-transfer geometry

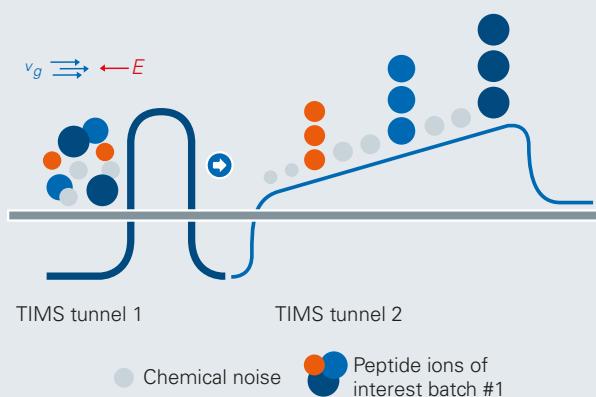
The timsTOF SCP mass spectrometer enables the full capabilities for ultra-high sensitivity analysis with the ground-breaking design of a novel ion source geometry. These functionalities are further enhanced by the speed and sensitivity of PASEF, dia-PASEF and prm-PASEF, making them ideally suited to comprehensive or targeted analysis in proteomics or biopharmaceutical characterization experiments.

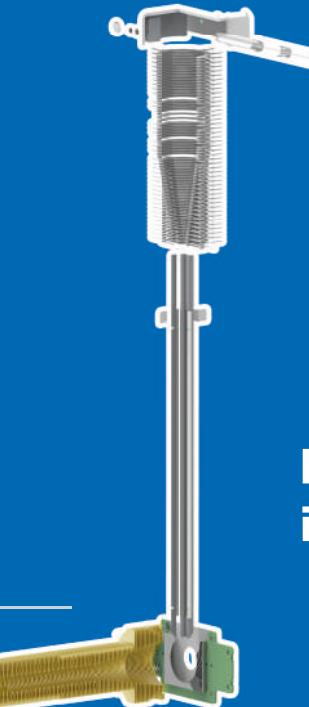


## Dual-TIMS and CCS-enabled analysis

TIMS (Trapped Ion Mobility Spectrometry) accumulates and concentrates ions of a given mass and mobility, while removing chemical noise, enabling an increase in sensitivity and speed.

A near 100% duty cycle can be achieved with the dual-TIMS technology accumulating ions in the TIMS tunnel 1, while ions in TIMS tunnel 2 are released sequentially. This process of parallel accumulation serial fragmentation (PASEF®) enables collisional cross section (CCS)-enabled analysis and acquisition at > 100 Hz.





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Radically improved ion-transfer

## Dramatic improvements in proteomics performance

The timsTOF SCP offers improvements in ion transmission into the source while maintaining robustness with an added higher pressure vacuum stage. This results in an almost 5x improvement in ion current (bottom left). When combined with the Evosep One Whisper method running at 100 nL/min flow rate, and the dia-PASEF method, sensitivity gains of about 100X over previous high throughput results with the Evosep One are achieved. This enables unbiased proteomics at the true single cell level with good reproducibility, robustness and coverage of about 1500 proteins per cell for the first time.

## Dual-TIMS, CCS-enabled analysis

Trapped Ion Mobility Spectrometry (TIMS) is first and foremost a separation technique in gas phase, which resolves sample complexity with an added dimension of separation in addition to HPLC and mass spectrometry, increasing peak capacity and confidence in compound characterization. Equally important, the TIMS device also serves to accumulate and concentrate ions

of a given mass and mobility, enabling a unique increase in sensitivity and speed along with the additional dimension of separation. Beside the sensitivity gain from concentration of ions, a near 100% duty cycle can be achieved with the dual-TIMS technology facilitating accumulation in the front section, while ions in the rear section released sequentially depending on their ion mobility. This process is called parallel accumulation serial fragmentation (PASEF) and it enables collisional cross section (CCS) analysis.

## Ideal for immunopeptidomics and other enrichment workflows

Besides unbiased true single cell proteomics applications, the timsTOF SCP also offers outstanding sensitivity for workflows that involve enrichment of peptides from the proteome. Immunopeptidomics studies start with purification of immunopeptides from plasma or tissue. Since immunopeptides are present at relatively low abundance in these samples, the timsTOF SCP is ideal for immunopeptidomics for neoantigen discovery where the available material is limited, as in needle biopsies. The timsTOF SCP also has the sensitivity to revolutionize the use of phosphoproteomics for the study of signalling pathways in cancer.

# timsTOF SCP

Expanding the horizons of single cell research



**4D-Proteomics** - CCS-enabled proteomics for confident IDs and isomer quantification

**PASEF acquisition** - PASEF enables acquisition at >100 Hz with ion focusing and removal of chemical noise for clean MS and MS/MS

**Single Cell Sensitivity** - New ion source concept coupled to the PASEF principle



**timsTOF Pro 2 and PaSER** (Parallel Search Engine in Real-time) is a combined hardware and software solution enabling fully integrated GPU based real-time database searches and results-based sample queue management. PaSER delivers results with uncompromising speed, including PTM searches. This uncompromised search speed of PaSER allows you to have results on hand, seconds after the acquisition ends, Run & Done!

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