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Scalable single cell analysis towards 1000 samples per day using the Evosep whisper zoom methods on the timsTOF Ultra2

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Introduction

Scalability is one of the crucial factors for single cell proteomics to allow meaningful data collection with reliable and robust statistical power for elucidating heterogeneity in ultra-high sensitive single cell and near single cell tissue input applications. Multiplexing as well as fast chromatography with low overhead time aids in upscaling proteome analyses. Here, we demonstrate applicability of the new Whisper Zoom methods on the Evosep system for speeding up label free sample analysis up to 120 samples per day (SPD) and exceeding >1,000 samples per day in a multiplexing approach with data acquisition on the timsTOF Ultra 2.

Results

Label-free Workflow

Figure 1:
Single cell proteomics workflow with the Evosep One with Whisper Zoom 120, 80, and 40SPD using the proteoCHIP EVO96 for pipetting-free sample transfer onto Evotips Pure and pyDIAid [1] optimized dia-PASEF data acquisition on **timsTOF Ultra 2** with data analysis in **Spectronaut 19**.

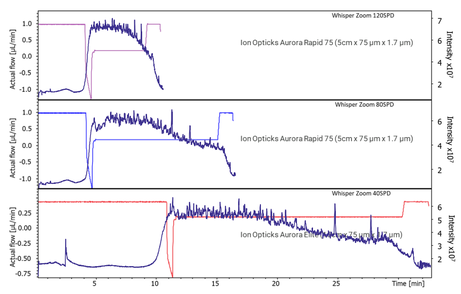

Figure 2:
A) Overview of isocratic high pressure pump flow rates (µL/min) in Whisper Zoom 120, 80 and 40 SPD with 200 nL/min during peptide elution and 1 µL/min (W120 and W80 SPD) or 450 nL/min (W40 SPD) during transfer of sample in pre-formed gradient to the analytical column overlayed with a typical total ion currents (TIC) MS1 of 5 ng HeLa peptide digest (Pierce).

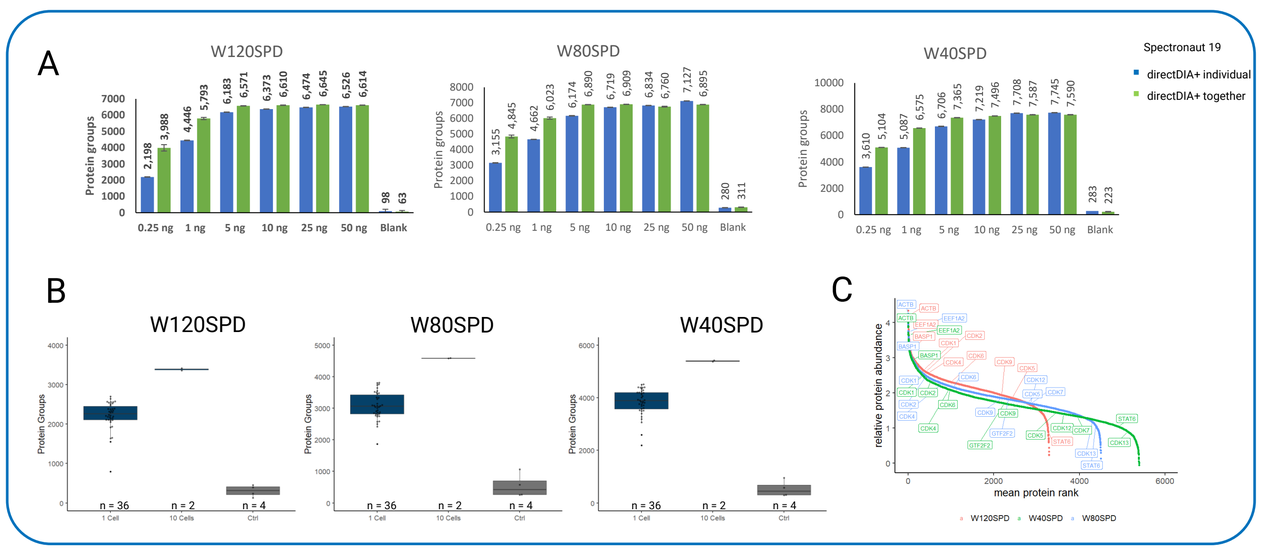

Figure 3:
A) Protein group identifications of HeLa peptide digest (Pierce) dilution series from 50 ng to 250 pg loaded on Tip, acquired in dia-PASEF mode (window placement optimized using pyDIAid [1]) and analyzed in Spectronaut with directDIA+ either processed individually per group or the entire dilution series together. Protein group identification at 250 pg increase from 2.200/4.000 (indv./together) at 120 SPD to 3.600/5.100 (indv./together) at 40SPD. B) Protein groups identified from single HeLa cells, 10 Hela cells and no-cell dispense controls analyzed in 3 Whisper Zoom methods 120, 80, 40 SPD using a spectral library generated from the respective 10 cell runs in directDIA+. Protein groups show a steady increase in identifications from 120 SPD (mean 2.300 protein groups) to 80 SPD (mean 3.000 protein groups) to 40 SPD (mean 4.000 protein groups). C) Mean protein group rank plots of proteins identified in single HeLa cells showing same dynamic range across the 3 Whisper Zoom methods with increasing proteome coverage correlating with the gradient lengths. Protein abundance rank remains comparable between the methods, as shown for selected structural proteins, transcription factors and cell cycle regulating proteins. Proteins gained with the longer gradient methods are from the lower abundance range.

Labeling based Workflow

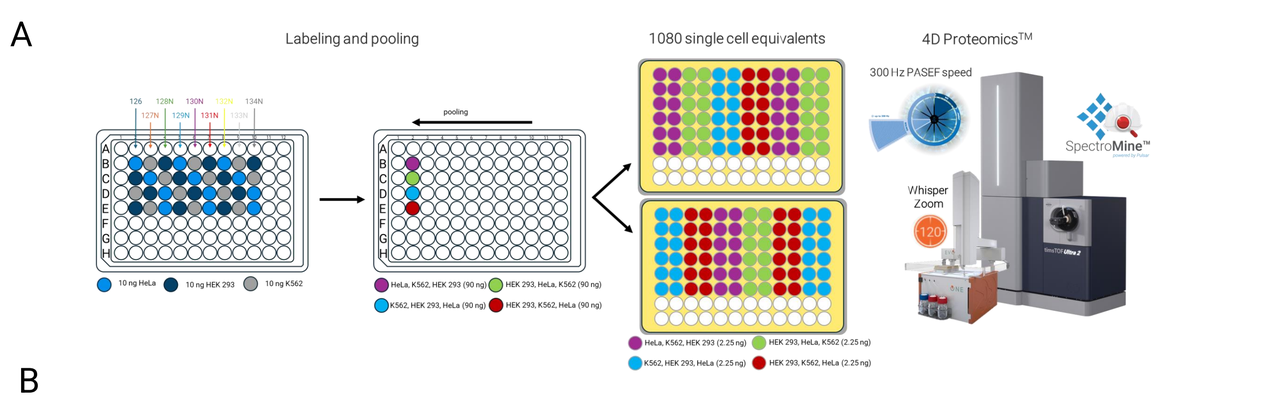

Figure 4:
A) Schematic depicting TMTpro subset sample labeling workflow to achieve 9-plexicity for LC-MS/MS based sample analysis using the Evosep One in Whisper Zoom 120 SPD and precursor detection with a timsTOF Ultra 2 in MS/MS stepping dda-PASEF mode followed by data processing in SpectroMine 4.5. B) schematic visualization of the precursor selection process for MS/MS stepping dda-PASEF acquisition. Precursor fragmentation with two different collision energy (CE), ion transfer and pre-pulse storage settings analyzed within two consecutive dda-PASEF ramps for optimal peptide fragment and TMTpro reporter detection.

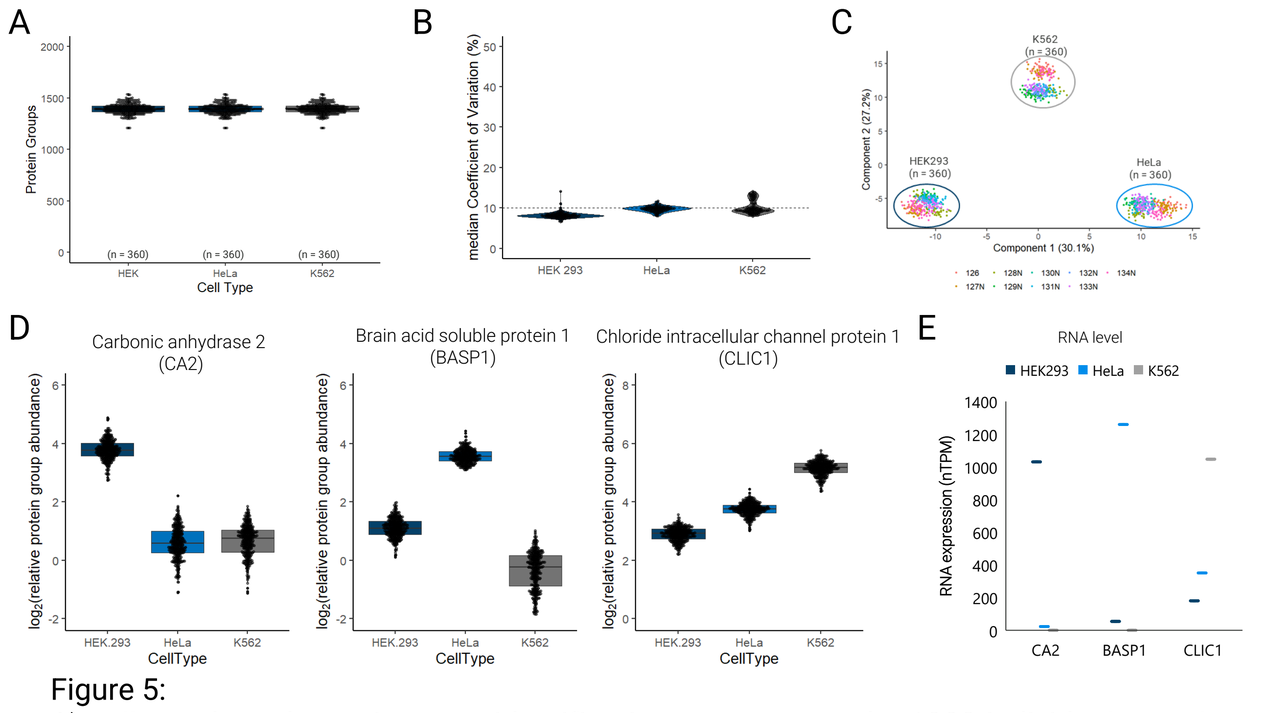

Figure 5:
A) cell type-based protein group identification rates across the 1080 individual samples. B) Median coefficient of variation distribution within the three preparation replicates per cell line and TMT batch with median values less than 10% for all three cell types. C) Sample projection in the first and second component of a principal component analysis of all proteins identified in all 1080 samples (676 protein groups) showing clear cell type separation. D)) Representative cytosolic protein abundance profiles of proteins among the most significantly different when comparing the three cell lines to each other; carbonic anhydrase 2 (CA2) for HEK 293, brain acid soluble protein 1 (BASP1) for HeLa, and chloride intracellular channel protein 1 (CLIC1). E) normalized RNA transcription level of CA2, BASP1 and CLIC1 as reported in the Human Protein Atlas (proteinatlas.org)

References

[1] P. Skowronek, Matthias Mann et al. Mol Cell Proteomics, 2022, 21, 9, 100279

Further reading

Application Note, Bruker Daltonics, LCMS-193, 1894933, 2022;
Application Note, Bruker Daltonics, LCMS-194, 1895627, 2022;
Application Note, Bruker Daltonics, LCMS-206, 1815135, 2023;
Application Note, Bruker Daltonics, LCMS-213, 1901456, 2023,
Application Note, Bruker Daltonics, LCMS-222, 1911577, 2024;
Application Note, Bruker Daltonics, LCMS-228, 1914261, 2024

Conflict of interest

CK, and ML are employees at Bruker Daltonics GmbH & Co. KG
DBJ, OBH, and NB are employees at Evosp Biosystems

Conclusion

- Highly reproducible and robust low sample amount analysis on the timsTOF Ultra 2 using Evosep Whisper Zoom methods in 120 SPD, 80 SPD and 40 SPD.
- Scalable single cell analysis with high sensitivity and reproducibility on identification and quantification demonstrated on HeLa cells with protein depth of more than 4,000 protein groups identified per single HeLa cell in label-free dia-PASEF mode.
- 1080 SPD on single cell equivalents of 9-plex TMTpro labeled cell line digests analyzed in Whisper Zoom 120 SPD demonstrate high degree of reproducibility using a TMTpro optimized MS/MS stepping dda-PASEF method.
- Labeling Workflow optimal for fast cell type screening for phenotyping – Cell type specific protein abundance profiles of selected proteins correlate well with RNA expression

timsTOF Ultra 2