Pushing the boundaries for robust and high-throughput single cell analysis with Whisper Flow Technology powered by dia-PASEF

Dorte B. Bekker-Jensen[1], Christoph Krisp[2], David Hartlmayr[3], Anjali Seth[3], Ole B. Hørning[1], Magnus Huusfeldt[1], Andreia Almeida[4], Markus Lubeck[2], Gary Kruppa[5], Jarrod Sandow[4] & Nicolai Bache[1]

[1] Evosep Biosystems, Denmark. [2] Bruker Daltonics GmBH & Co. KG, Bremen, Germany. [3]Cellenion, Lyon, France. [4]IonOpticks, Melbourne, Australia. [5]Bruker s.r.o, Brno, Czech Republic.

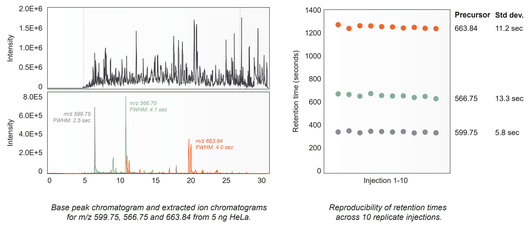
Highlights

- The combination of the Aurora Elite column, the Whisper 40 SPD method and dia-PASEF on the timsTOF SCP maps single cell proteomes with an average coverage of 3,500 proteins.
- A faster Whisper method with a throughput of 80 samples per day is developed to scale the throughput of single cell analysis.

Chromatographic performance

Excellent peak performance

The Aurora Elite column (IonOpticks) is recommended for the Whisper 40 and 20 SPD methods, running with a gradient flow of 100 nl/min. The pulled emitter provides sharp chromatographic peaks, even at low flow rates.

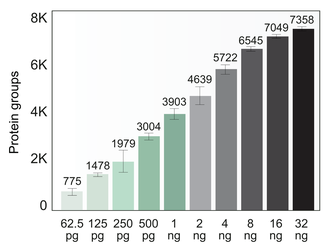


Base peak chromatogram and extracted ion chromatograms for m/z 599.75, 566.75 and 663.84 from 5 ng HeLa.

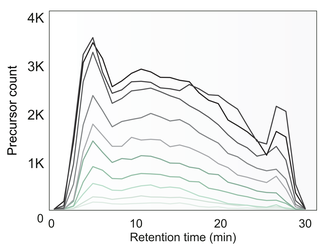
Reproducibility of retention times across 10 replicate injections.

Robust dilution curves

We evaluated the sensitivity of our workflow with a dilution series of tryptic HeLa peptides (Pierce) analyzed in hexuplicate, ranging from low loads of 62.5 pg up to saturating levels of 32 ng peptide material.
We used our Whisper 40 SPD method in combination with the fast and sensitive timsTOF SCP mass spectrometer using data-independent acquisition parallel accumulation serial fragmentation (dia-PASEF). Data were analyzed with DIA-NN v1.8 with a predicted library of a human Swiss-Prot fasta file including isoforms (UniProt, downloaded November 2021) without match between runs. Identifications represent protein groups and precursors as stated in the pr_matrix.tsv and pg_matrix.tsv files.



Protein group identifications from dilution series with Whisper 40 SPD.

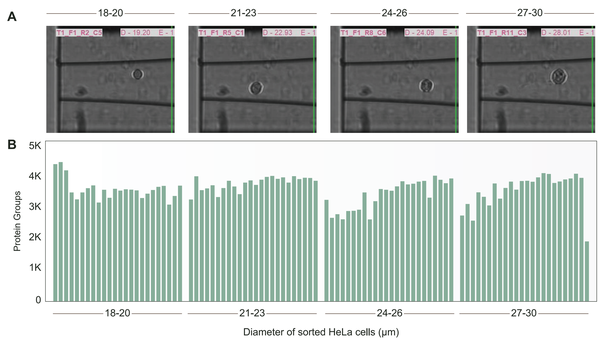


Precursors per minute throughout the gradient for all loads.

Robust and reproducible single cell analysis

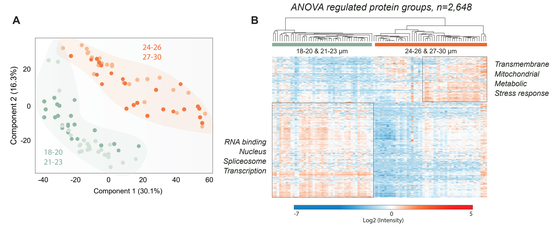
3,500 proteins identified from single HeLa cells

We sorted cells in different groups depending on size and digested these in a protoype chip (Cellenion) and transferred peptides into Evotips via centrifugation. The single-cell proteomes revealed excellent coverage with an average of 3,500 protein groups per cell and close to 5,500 proteins identified in the entire dataset. During the 2.5 days of measurement, the loaded Evotips were stored on the instrument at room temperature ith no observed loss in proteome depth. Data analysis was performed with DIA-NN 1.8.1 using a spectral library generated by DDA from a deeply fractionated human cell line containing 573,610 precursors from 13,679 proteins. Match between runs (MBR) was enabled and a 5 ng HeLa sample was added as a reference run for MBR-related spectral library generation.



(A) Images of sorted HeLa cells representing the four groups of cells sorted. (B) Protein group identifications from 96 cells analyzed with Whisper 40 SPD with dia-PASEF on a timsTOF SCP and analyzed with DIA-NN.

Interestingly, the number of proteins identified does not correlate with cell size in this experiment, but indeed we see a nice separation of the cell size groups, where the bigger cells 24-26 µm and 27-30 µm, are nicely separated from the 18-20 µm and 21-23 µm cells in a principal component analysis. From an ANOVA analysis, we find more than 2,500 proteins to be significantly regulated between the groups.

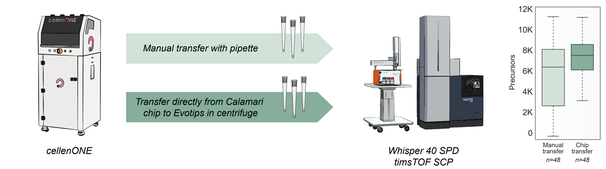


(A) Principal component analysis of the global proteome. (B) Hierarchical clustering analysis of ANOVA regulated protein groups.

Seamless transfer via proteoCHIP for Evotips

Pipetting-free transfer increases reproducibility

We have developed a prototype chip for seamless integration between sample preparation on the cellenONE and transfer of peptides directly into Evotips. Across 48 replicate injections, we observe a tighter distribution of the number of identified precursors using direct centrifugation compared to transferring peptides with a pipette confirming that every step in a highly sensitive protocol affects performance and reproducibility.

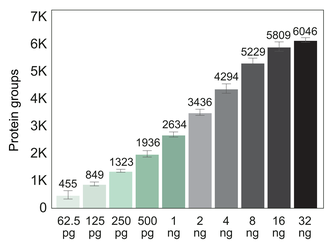


Workflow for robust and reproducible single cell analysis.

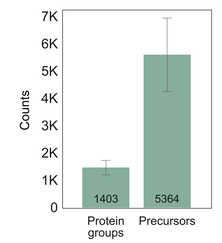
Scalable single cell analysis

Faster throughput with Whisper 80 samples per day

We have developed a faster Whisper method and evaluated its sensitivity using the new Aurora Rapid 75 column (IonOpticks) with a dilution series of HeLa tryptic peptides analyzed in eight replicates, ranging from low loads of 62.5 pg up to saturating levels of 32 ng peptide material. Data were analyzed with DIA-NN v1.8 with a predicted library of a human Swiss-Prot fasta file including isoforms (UniProt, downloaded November 2021) without match between runs.
Furthermore, A549 cells were isolated and digested using the prototype chip and 80 single cells were analyzed with the Whisper 80 SPD method on the timsTOF SCP. Data analysis was performed with DIA-NN 1.8.1 using a spectral library generated by DDA from a deeply fractionated human cell line containing 573,610 precursors from 13,679 proteins. This resulted in the average identification of 1,400 proteins per cell.



Protein group identifications from dilution series with 80 SPD.



Identifications from A549 single cells with 80 SPD.