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

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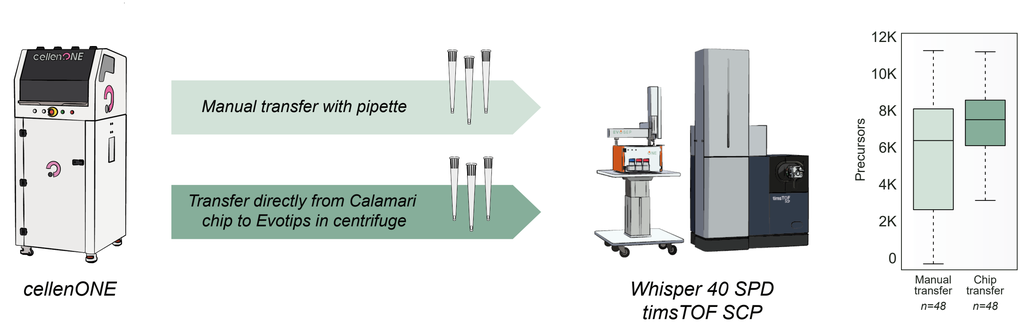
Highlights

- The combination of the Aurora Elite column, Whisper methods and dia-PASEF on the timsTOF SCP provides an excellent combination for robust and high-throughput single cell analysis at competitive sensitivity.
- The presented workflow maps single cell proteomes with an average coverage of 3,500 proteins using Whisper 40 SPD.
- A faster Whisper method with a throughput of 80 samples per day is developed to scale the throughput for single cell analysis.

Robust and reproducible single cell analysis

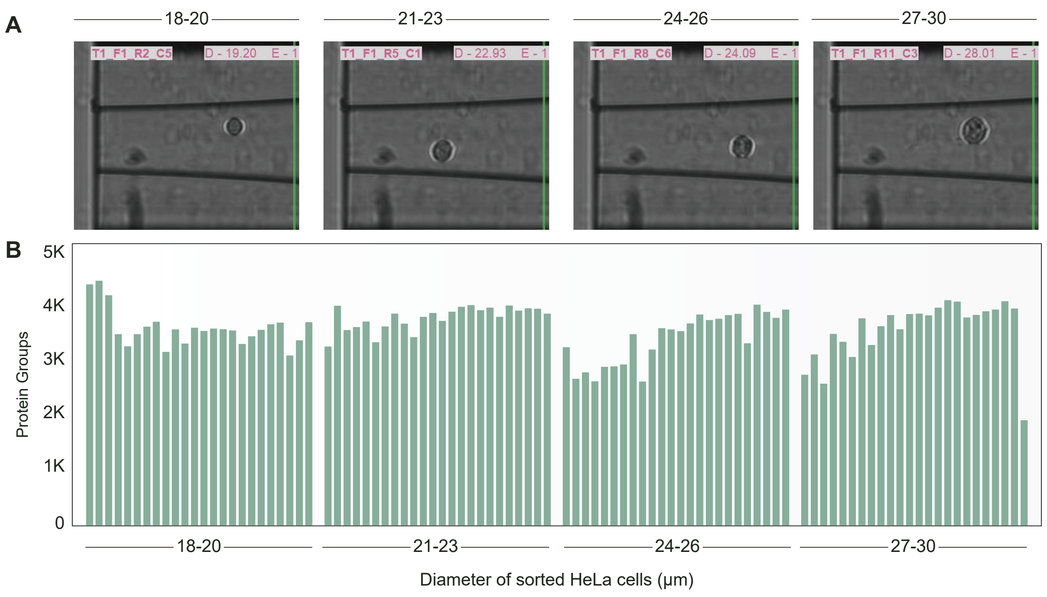
3,500 proteins identified from single HeLa cells

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 more tight distribution of precursors using direct centrifugation compared to transferring peptides with a pipette confirming that every step in a highly sensitive protocol affects performance and reproducibility.



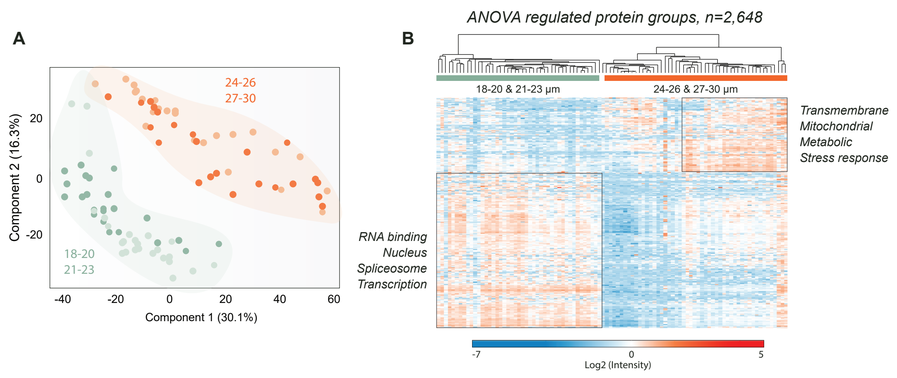
Workflow for robust and reproducible single cell analysis

We sorted cells in different groups dependent on size and digested these in the protoype chip 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 measument, the loaded Evotips were stored on the instrument at room temperature with no observed loss in proteome depth.



(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.

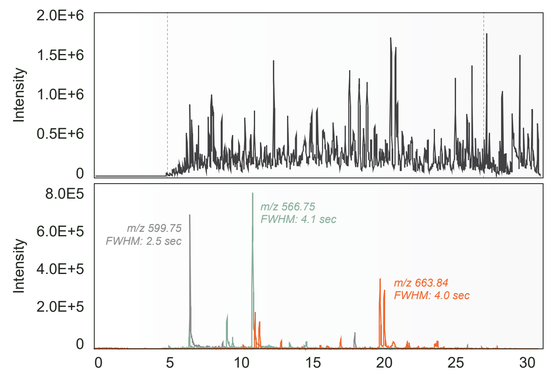


(A) Principal component analysis of the global proteome. (B) Hierarchical clustering analysis of ANOVA regulated protein groups.

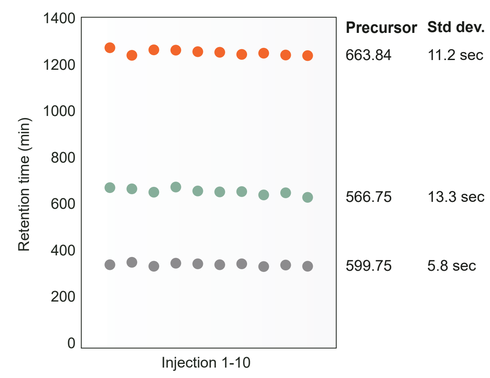
Chromatographic performance

Improved peak performance

Ongoing improvements of Whisper combined with a new generation Evotip provide efficient capture and recovery of peptides from very low sample amounts down to the single cell level. Additional optimizations include a new 15 cm column from IonOpticks (75 µm ID with 1.7 µm beads, Generation 3 Aurora Elite), that works well together with the single use and disposable trap columns with a slightly more hydrophobic material.



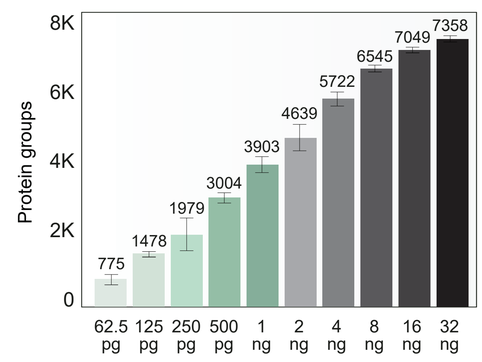
Base peak chromatogram and extracted ion chromatograms for m/z 599.75, 566.75 and 663.84 from 5 ng HeLa.



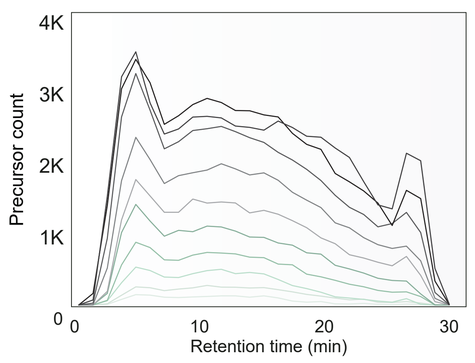
Reproducibility of retention times cross 10 replicate injections.

Robust dilution curves

We evaluated the sensitivity of our workflow with a dilution series of HeLa tryptic peptides analyzed in hexuplicate (Pierce), 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).



Protein group identifications from dilution series with 40 SPD.

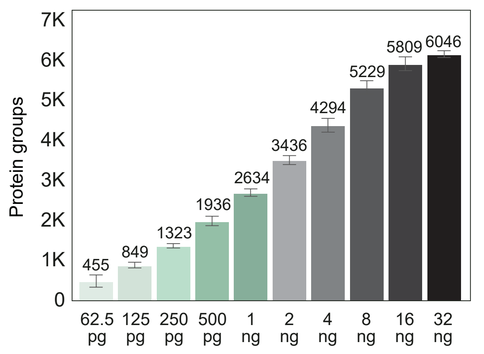


Precursors per minute throughout the gradient for all loads

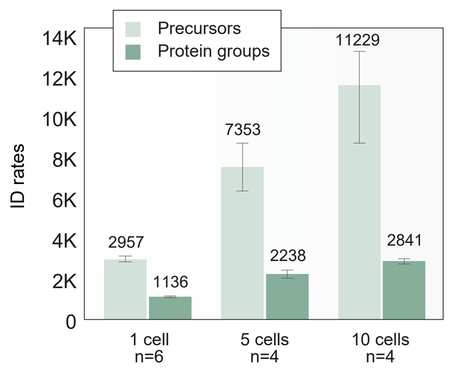
Speeding up single cell analysis

Faster throughput with Whisper 80 SPD

We have developed a faster Whisper method and evaluated the sensitivity using the new Aurora Rapid 75 column with a dilution series of HeLa tryptic peptides analyzed in eight replicates (Pierce), ranging from low loads of 62.5 pg up to saturating levels of 32 ng peptide material. Furthermore 1, 5 and 10 cells were analyzed.



Protein group identifications from dilution series with 80 SPD.



Precursor and protein identifications from 1, 5 and 10 cells with 80 SPD.