Enabling scalable single-cell proteomics by utilizing the unique analytical properties of the Evotip Pure with new Whisper Zoom methods

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Highlights

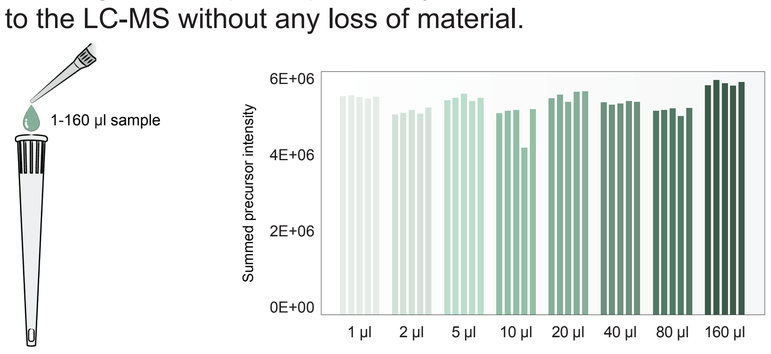
- New Whisper Zoom methods improve performance, robustness and reproducibility;

  Whisper Zoom 120 SPD -> New method!
  Whisper Zoom 80 SPD -> 100% improvement.
  Whisper Zoom 40 SPD -> 20% improvement

- Evotip Pure brings innovative sample storage and introduction to high-sensitivity and scalable workflows

Samples are efficiently concentrated on the Evotip

The Evotip can be loaded with large volumes enabling the concentration of dilute samples, allowing the Evotip to reproducibly handle and transfer minute sample amounts to the LC-MS without any loss of material.

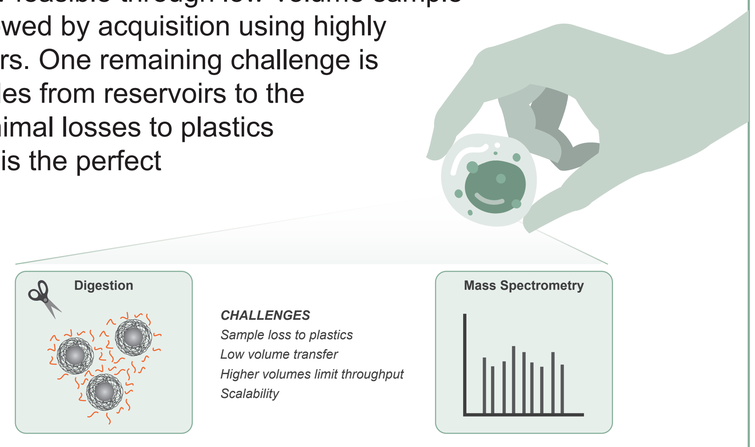


Summed precursor intensities of 250 pg HeLa loaded on Evotips in 1-160 µl solvent A. Evotips loaded with volumes lower than 20 µl had solvent A already deposited in them for a total loading volume of 20 µl. Samples were analyzed with Whisper 40 SPD.

High efficient sample capture and recovery

The Evotip solves analytical challenges

The advancements of modern mass spectrometers and cell sorters like the cellenONE have enabled high-sensitivity proteomics workflows. Analyzing single cells is now feasible through low-volume sample handling and digestion, followed by acquisition using highly sensitive mass spectrometers. One remaining challenge is efficiently transferring samples from reservoirs to the mass spectrometer with minimal losses to plastics and pipette tips. The Evotip is the perfect solution as it allows higher-volume, low-loss sample handling without sacrificing throughput.



Analytical challenges in single-cell proteomics solved by the Evotip.

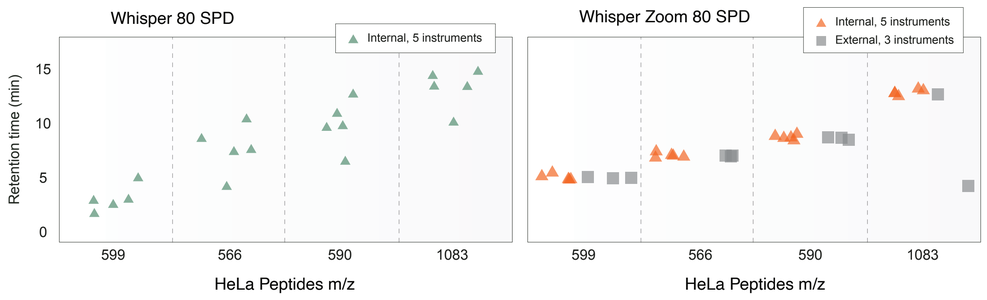
Methods

All samples were analyzed on a timsTOF Pro 2 mass spectrometer using data-independent acquisition parallel accumulation serial fragmentation (dia-PASEF). Data were processed with DIA-NN v1.8.1 library-free against the human proteome (Swiss-Prot) without isoforms (UniProt, downloaded Oct. 2020). Conditions were searched separately with match-between-runs (MBR).

Whisper Zoom drives scalable workflows

Unmatched robustness and reproducibility

The Whisper Zoom 80 SPD method significantly enhances retention time stability and inter-instrument reproducibility, while delivering sharper peaks and more reproducible peak widths across different Evosep instruments compared to the legacy method. This was compared from internal and external instruments.

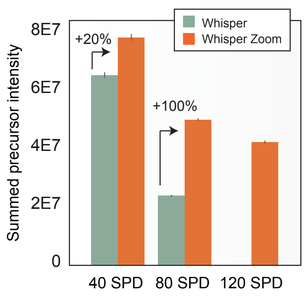


Improvement in inter-instrument retention time (RT) reproducibility of Whisper Zoom 80 SPD, internally (n=5) and externally (n=3). RT from four peptides were monitored from 5 ng injections (n=4).

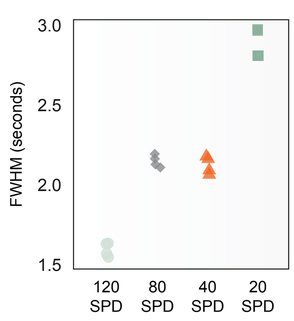
Significantly improved performance with Whisper Zoom

Whisper Zoom methods utilize the shortest possible flowpath in the Evosep. This enables sharp symmetric peaks as exemplified with an average FWHM of ~1.5 seconds with the Whisper Zoom 120 SPD method and ~2.2 seconds using the Whisper Zoom 40 SPD method.

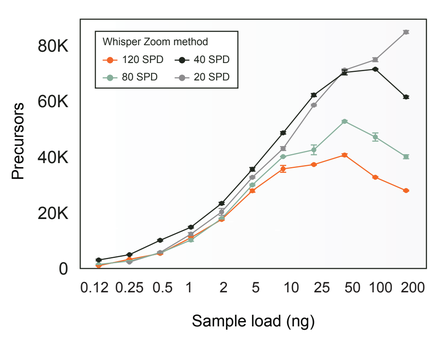
The sensitivity of the four Whisper Zoom methods was evaluated with a dilution series of 0.125 pg to 200 ng HeLa peptides using the timsTOF Pro 2. The Whisper Zoom 120 SPD shows saturating levels of precursor identifications at 10 ng load where additional peptide input does not improve analysis depth. For the Whisper Zoom 40 and 80 SPD methods, saturation occurs at 50 ng, while the Whisper Zoom 20 SPD method is saturated at or above 200 ng input.



Summed precursor intensity from 5 ng HeLa peptide digest analyzed with legacy Whisper 40 and 80 SPD and Whisper Zoom 40, 80, and 120 SPD methods, n = 4.



Average FWHM of chromatographic peaks from 5 ng HeLa peptide digest analyzed with Whisper Zoom methods, n = 4.

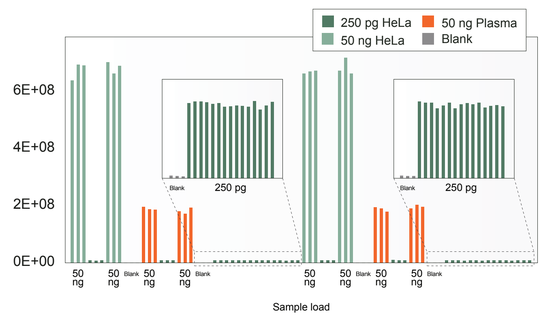


Dilution curve of HeLa digest analyze with Whisper Zoom methods, n = 4.

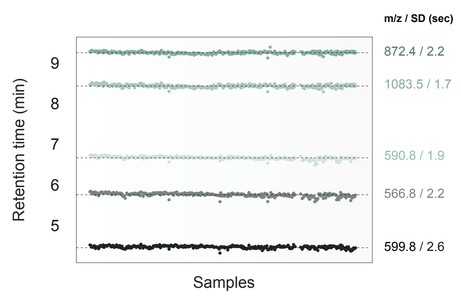
Disposable trap column increases system robustness

Low carryover enhances versatility and scalability

The high-sensitivity Evotip Pure workflow was evaluated by injecting 250 pg HeLa consecutively with 50 ng plasma and 50 ng HeLa. The analyzed samples were mixed with blank Evotips to monitor carryover using the Whisper Zoom 80 SPD method. Additionally, 250 pg HeLa were analyzed with Whisper Zoom 120 SPD across 340 samples with retention time standard deviations of less than 2.6 seconds for all monitored peptides. These data shows that Evosep One can be used for versatile samples without compromising the performance.



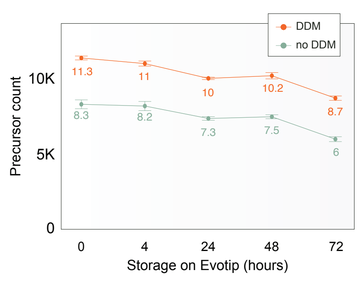
Summed precursor intensity of 50 ng HeLa, 50 ng plasma, 250 pg HeLa and blanks. Samples were analyzed with Whisper Zoom 80 SPD.



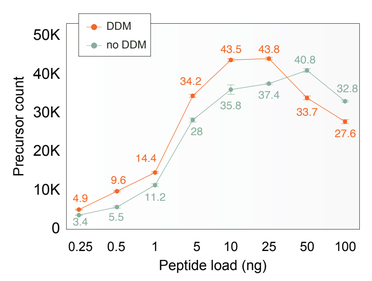
Pressure traces across 340 runs with the Whisper Zoom 120 SPD method and retention times of 5 monitored HeLa peptides.

Sensitive sample storage on the Evotip

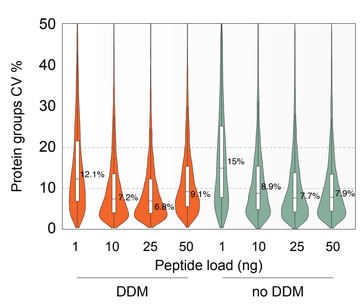
Sample storage is essential for robust and scalable single-cell proteomics, as it allows for the preparation and execution of experiments over extended periods. Storing samples on Evotips for up to 72 hours showed that over 90% of precursor identifications are preserved after 48 hours, with nearly 75% preserved after 72 hours. The impact of DDM on sample loading was evaluated and overall, DDM enhances precursor identifications and quantitative precision for low loads without affecting storage capability, demonstrating its beneficial impact on sample preparation workflows upstream of the Evotip.



Storage of 250 pg HeLa loaded +/- DDM. Samples were analyzed with Whisper Zoom 120 SPD, n = 4.



Precursor identifications from a HeLa dilution series loaded +/- DDM. Samples were analyzed with Whisper Zoom 120 SPD, n = 4.



Precursors and protein group CVs from HeLa dilution series loaded +/- DDM. Samples were analyzed with Whisper Zoom 120 SPD, n = 4.