Increasing throughput while maintaining coverage depths in single cell proteomics using the timsTOF Ultra2

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Introduction

For single cell proteome analysis, ultra-high sensitivity mass spectrometry is a key to reach a proteome coverage necessary for understanding the cellular heterogeneity on a cell-by-cell level. The latest enhancements in ion transfer with a larger transfer capillary, an additional higher-pressure segment for more effective ion collection and two orthogonal deflections, to maintain robustness, pushes the limits of detection to the single cell level. Combined with automated single cell isolation and sample preparation using the cellenONE® platform for protein-loss reduced preparation and transfer with the proteoCHIP format leads to deep proteome coverage and high reproducibility.

Methods

HeLa cells at different cell counts were isolated into the LF48 proteoCHIP®, directly lysed and digested using the cellenONE platform. Samples were either dried down in the chip or manually transferred into 96-well plates. Peripheral blood mononuclear cells (PBMCs) were FACS sorted into T-Cells (CD4+, CD8+) B-Cells (CD19+) and monocytes (CD14+), isolated with the cellenONE and prepared as the HeLa cells above. The LF48 proteoCHIP was placed into the nanoElute® 2 autosampler. Sample were picked up with the dissolve sample function and injection onto a 5 cm Aurora Rapid 75 or a 25 cm Aurora Ultimate C18 column (IonOpticks) and eluted into a timsTOF Ultra2 at 80 or 32 SPD. dia-PASEF® data were processed with Spectronaut 19 (Biognosys) using directDIA+.

Results

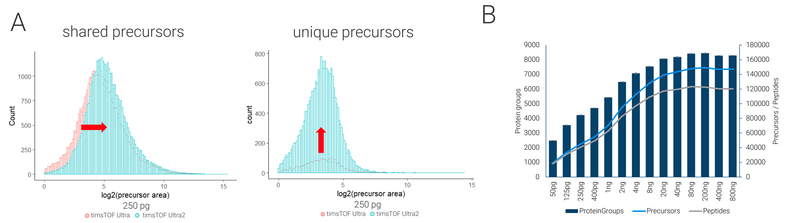

Figure 2
A) Histogram comparing precursor quantification information on the timsTOF Ultra and timsTOF Ultra2 obtained from 250 pg K562 digest. Precursor quantification of precursors identified by both systems demonstrate a consistent shift towards higher values achievable on the timsTOF Ultra2. Large number of uniquely identified precursors with the timsTOF Ultra2 with highest counts in the lower third of the precursor abundance range. B) K562 peptide digest dilution series form 800 ng to 50 pg with the new Ion charge control (ICC2) demonstrating consistent increase in protein group, peptide and precursor identifications (Spectronaut 19 directDIA+ per concentration) reaching a plateau at 148,000 precursors from 124,000 peptides derived from 8,500 protein groups between 40 and 80 ng and is maintained up to the highest load of 800 ng.

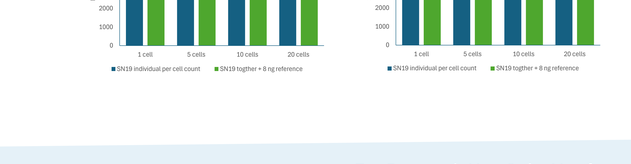

Figure 3:
A) Protein group identification rates of a K562 peptide dilution series from 16 ng to 62.5 pg loaded on column analyzed at A) 32 SPD (Ion Opticks Aurora Ultimate) or at B) 80 SPD (Ion Opticks Aurora Rapid 75), acquired in dia-PASEF mode and analyzed in Spectronaut 19 with directDIA+ either within a concentration group or processed together. Data indicate similar proteome coverage with the 80 SPD and 32 SPD methods at loads of less than 1 ng.

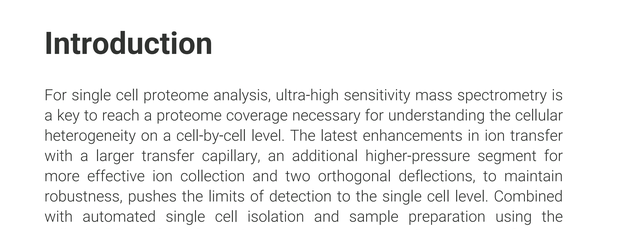

Figure 1:
Single cell proteomics workflow with the nanoElute 2 dissolve sample function for pickup of lyophilized samples from the label-free proteoCHIP or 384 or 96-well plate with acquisition on **timsTOF Ultra2** using a pyDIAid [1] optimized dia-PASEF data with data analysis in Spectronaut 19 .

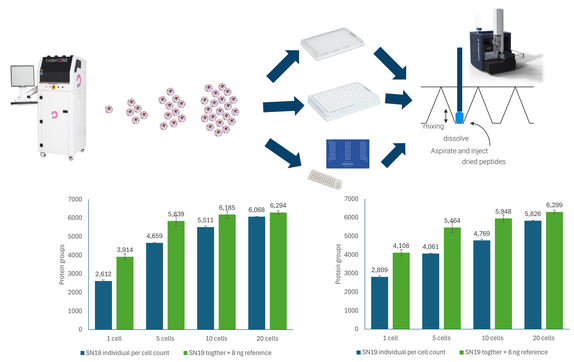

Figure 4:
Protein group and peptide identification rates of HeLa cells isolated at counts of 1, 5, 10, and 20 cells per well (n = 3). Samples were prepared in the proteoCHIP LF 48, A) transferred into a 96-well plate, dried, dissolved immediately prior to injection and analyzed at 32 SPD or B) kept in the proteoCHIP LF 48 dried, dissolved immediately prior to injection and analyzed at 80 SPD. The entire sample was loaded onto column, acquired in dia-PASEF mode and the resulting data were analyzed in directDIA+ using Spectronaut 19 either each cell count individually or with a 8 ng K562 peptide loads in 32 or 80 SPD as reference runs.

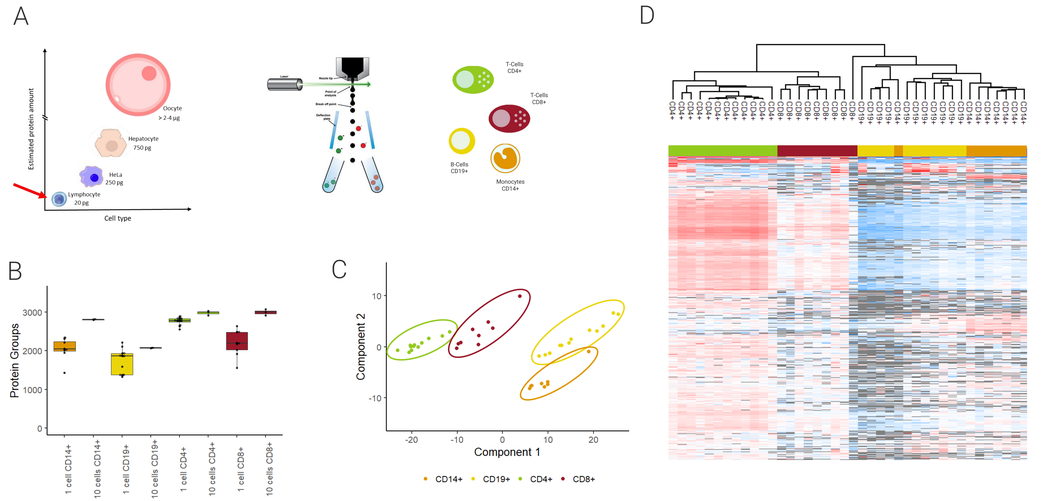

Figure 5:
A) PBMC analysis Workflow from FACS sorting of T-Cells (CD4+, CD8+) B-Cells (CD19+) and monocytes (CD14+), single cell and 10 cell isolation with the cellenONE into LF proteoCHIP, mass spectrometric analysis on the timsTOF Ultra2 using the 10 min gradient on IO5 (80 SPD) and data analysis in Spectronaut 19 in directDIA+ processing all samples together. B) Box plot of protein group identification rates across the 4 different cell types demonstrating good protein identification rate reproducibility for each cell type group. C) Differentiation on protein abundance of the 4 cell types in a PCA projection plot. D) Heatmap of protein group abundance pattern shows distinct clustering by cell type with good reproducibility withing a cell type group.

Conclusion

- Hands-free and pipetting-free workflow using the dissolve sample function of the nanoElute 2 for resuspension of lyophilized peptide pickup from the LF proteoCHIP
- High sensitivity with good chromatographic reproducibility and robustness with 10 min (80SPD) or 30 min (32 SPD) run time
- Good quantification accuracy at single cell level with good single cell to single cell reproducibility on protein level with protein depth of more than 4,000 protein groups per single HeLa cell on the timsTOF Ultra2
- Sorted PBMC analysis workflow shows good proteome coverage with distinct protein abundance profiles for different the 4 cell types

timsTOF Ultra2

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

Conflict of Interest

CK, ML, GK are employees at Bruker Daltonics GmbH & Co. KG; AS, DH, GT are employees at Cellenion.