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Increasing throughput while maintaining coverage depths in single cell proteomics using the timsTOF Ultra 2

Verena Tellstroem[1]; Christoph Krisp[1]; David Hartlmayr[2]; Anjali Seth[2]; Guilhem Tourniaire[2]; Léo Laboubi[3]; Thorsten Ledertheil[1]; Torsten Mueller[1], and Markus Lubeck[1]

1 Bruker Daltonics GmbH & Co. KG, Bremen, Germany; 2 Cellenion, Lyon, France; 3 CISTAR, Cancer Research Centre of Lyon, Lyon, France

Introduction

Scalability while maintaining coverage depths is one of the bottlenecks in the field of single cell proteomics. Therefore, fast scanning, 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 across large sample cohorts. Previously, we demonstrated that with protein extract digests (K562, Promega) dilution series of single cell equivalents as well as single HeLa cells, we can obtain comparable proteome depths when more than doubling the sample throughput from about 30 samples per day (SPD, 22min active gradient) to 80 SPD (10 min active gradient). Here we use the cellenONE® platform with oil-free LF48 proteoCHIP for direct sample pickup with the nanoElute 2 and detection with the timsTOF Ultra 2 to investigate peripheral blood mononuclear cells (PBMCs).

Results

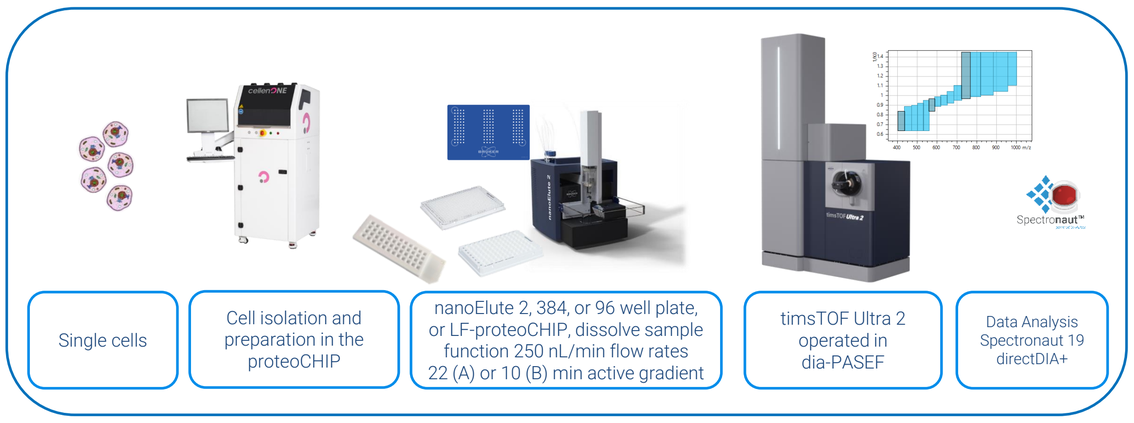

Figure 1:
Single cell proteomics workflow with the nanoElute 2 dissolve sample function for pickup of lyophilized samples from the label-free proteoCHIP or 384 or 96-well plate with acquisition on **timsTOF Ultra2** using a pyDIAid [1] optimized dia-PASEF data with data analysis in Spectronaut 19.

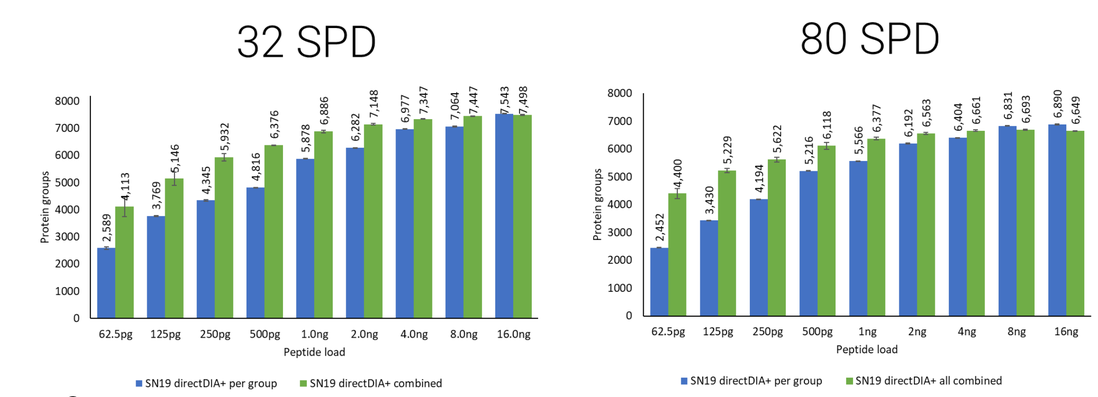

Figure 2:
A) Protein group identification rates of a K562 peptide dilution series from 16 ng to 62.5 pg loaded on column analyzed at A) 32 SPD (Ion Opticks Aurora Ultimate) or at B) 80 SPD (Ion Opticks Aurora Rapid 75), acquired in dia-PASEF mode and analyzed in Spectronaut 19 with directDIA+ either 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.

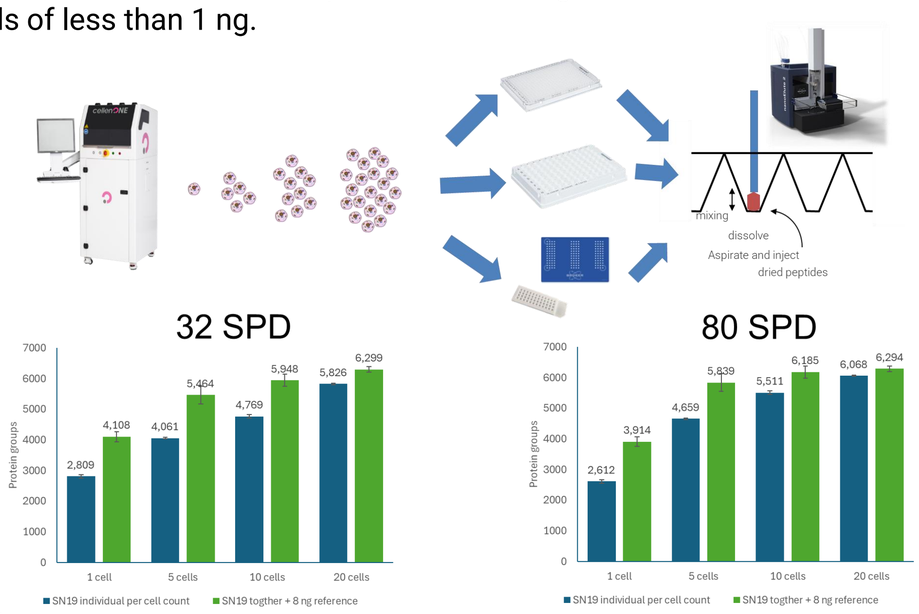

Figure 3:
Protein group and peptide identification rates of HeLa cells isolated at counts of 1, 5, 10, and 20 cells per well (n = 3). Samples were prepared in the proteoCHIP LF 48, A) transferred into a 96-well plate, dried, dissolved immediately prior to injection and analyzed at 32 SPD or B) kept in 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.

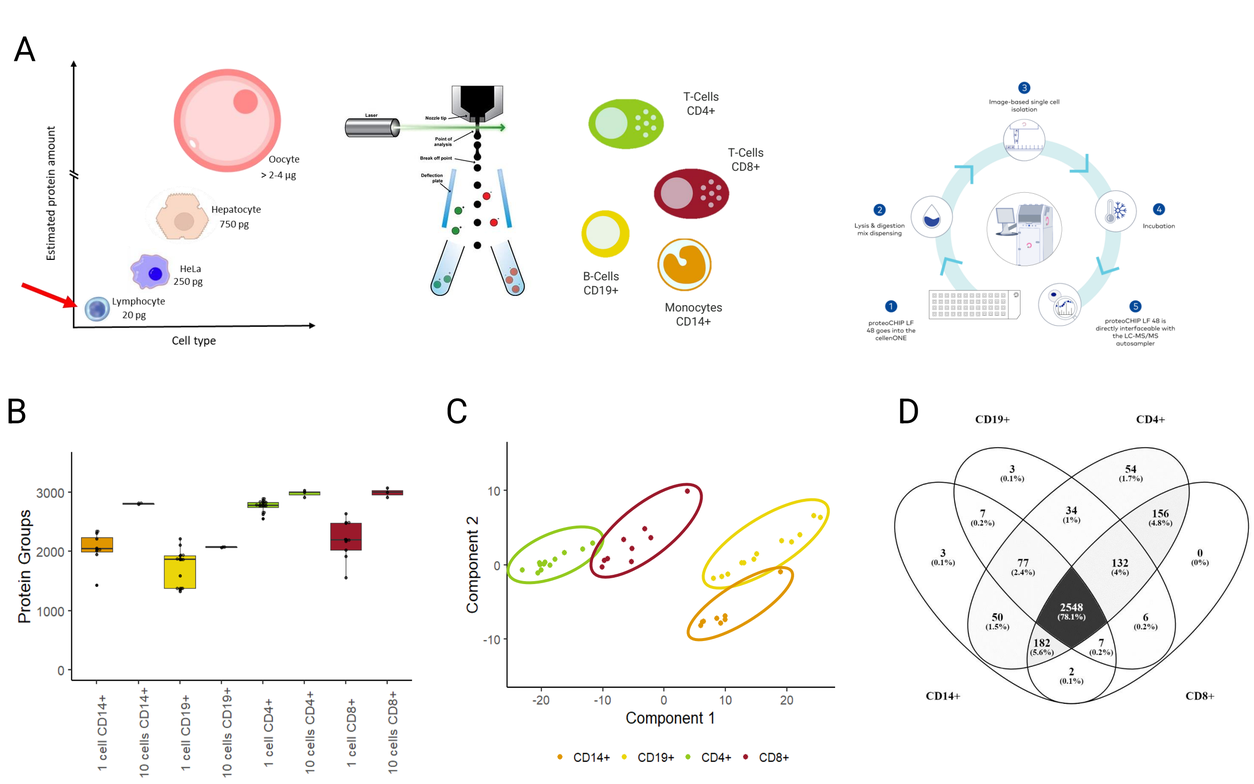

Figure 4:
A) PBMC analysis Workflow from FACS sorting of T-Cells (CD4+, CD8+) B-Cells (CD19+) and monocytes (CD14+), single cell and 10 cell isolation with the cellenONE into LF proteoCHIP, mass spectrometric analysis on the timsTOF Ultra2 using the 10 min gradient on IO5 (80 SPD) and data analysis in 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) Venn diagram depicting overlap of proteins identified across the 4 cell types, 54 exclusively identified in CD4+ T-cells and in total 210 exclusive to T-cells (CD4+/CD8+) only a proteins exclusive to monocytes (CD14+) or B-cells (CD19+)

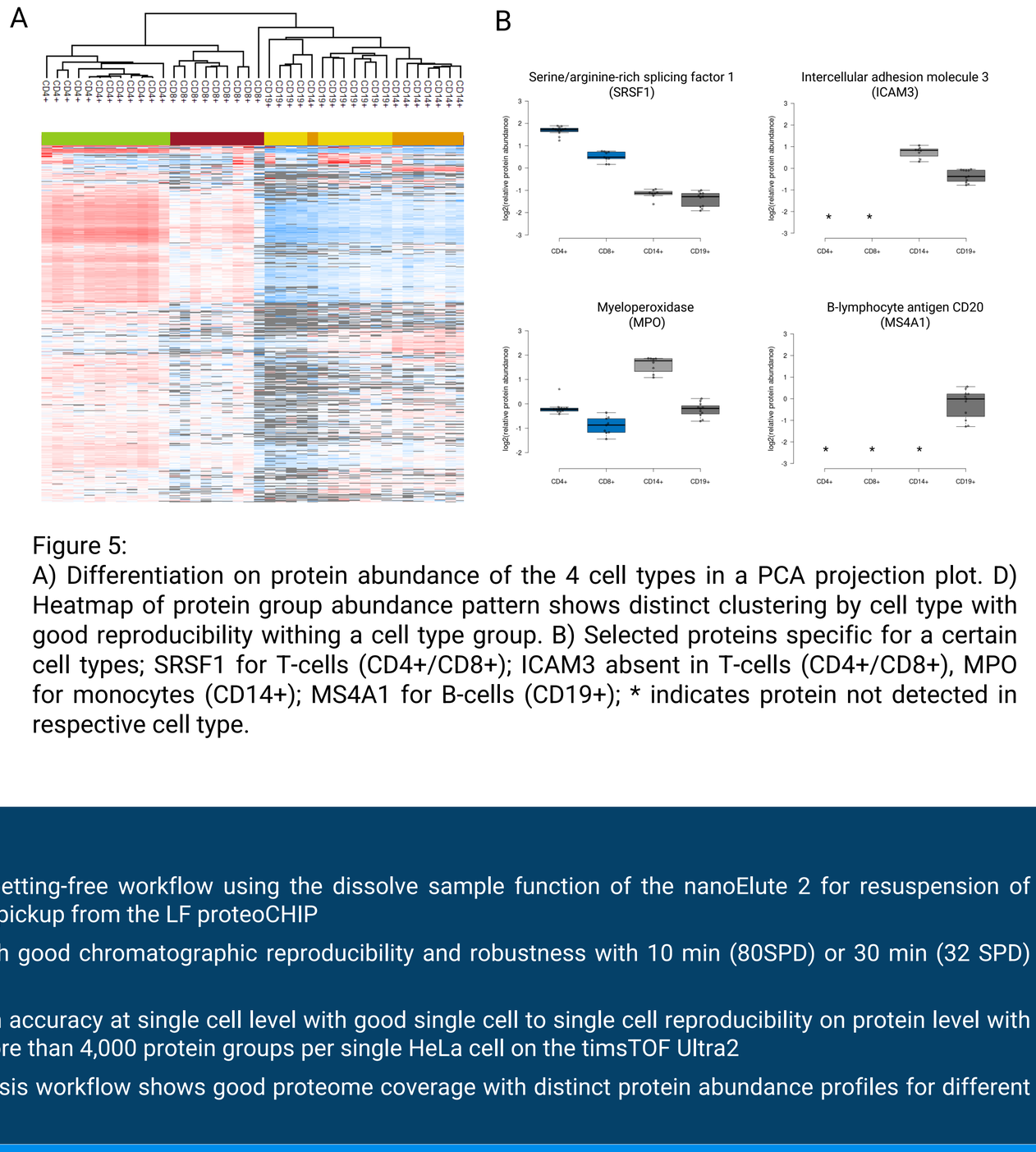

Figure 5:
A) Differentiation on protein abundance of the 4 cell types in a PCA projection plot. D) Heatmap of protein group abundance pattern shows distinct clustering by cell type with good reproducibility withing a cell type group. B) Selected proteins specific for a certain cell types; SRSF1 for T-cells (CD4+/CD8+); ICAM3 absent in T-cells (CD4+/CD8+), MPO for monocytes (CD14+); MS4A1 for B-cells (CD19+); * indicates protein not detected in respective cell type.

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, TL,TM, and ML are employees at Bruker Daltonics GmbH & Co. KG
DH, AS, and GT are employees at Cellenion SASU

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 Ultra 2

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