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Single cell proteome analysis with ultra-high sensitivity using timsTOF SCP and timsTOF Ultra

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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.

Single cell proteomics Ecosystem

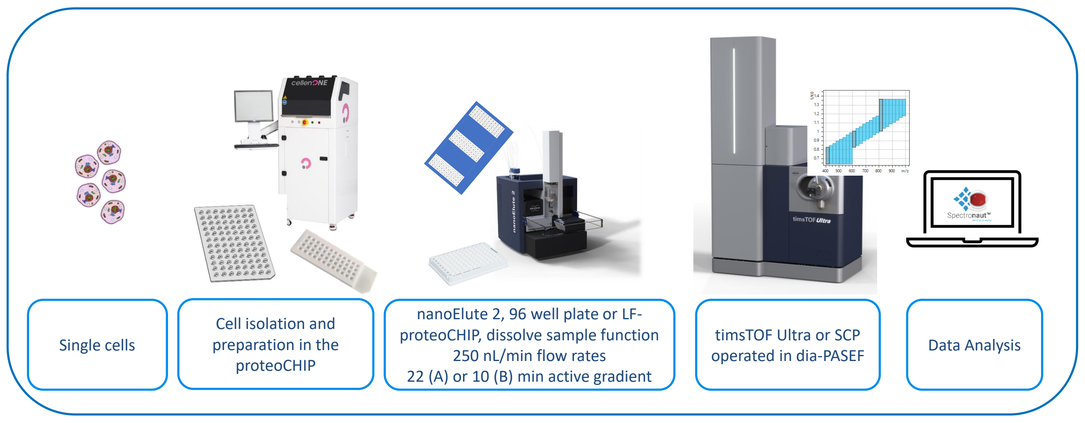

Figure 1:
Single cell proteomics workflow with the nanoElute 2 dissolve sample function for pickup of lyophilized samples from the label-free proteoCHIP or a 96-well plate on the **timsTOF SCP** or the **timsTOF Ultra**.

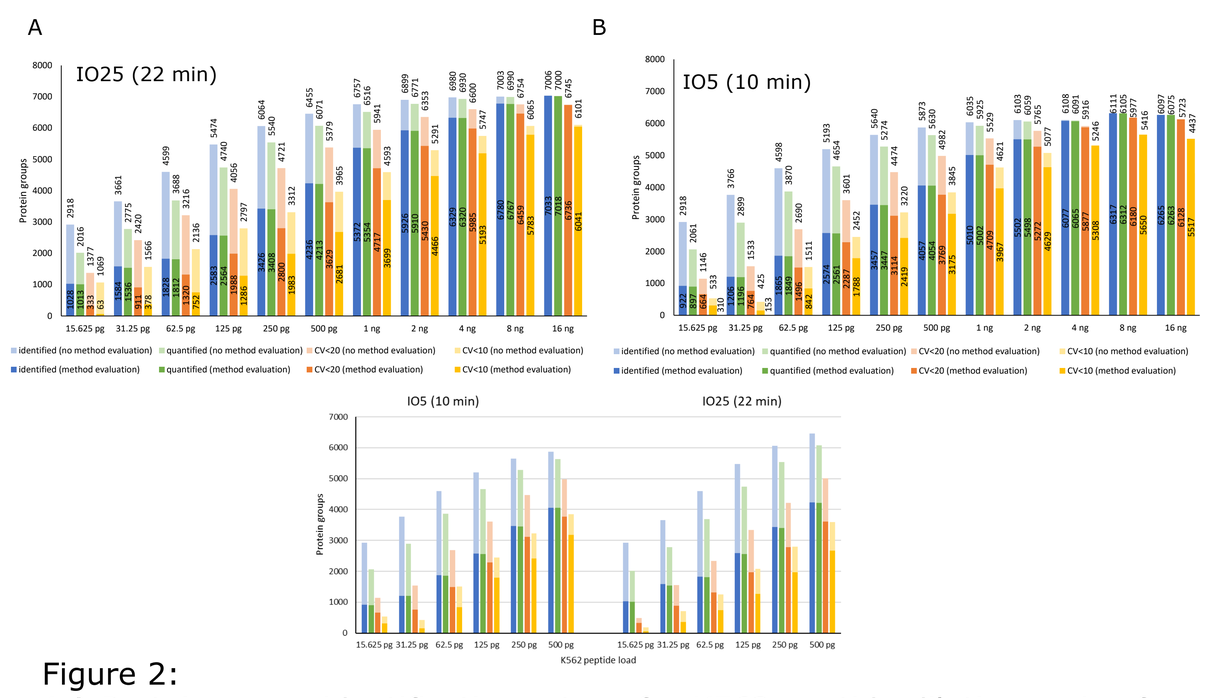

Figure 2:
A) Protein group identification rates of a K562 peptide dilution series from 16 ng to 15.625 pg loaded on column, acquired in dia-PASEF mode and analyzed in Spectronaut 18 with directDIA+, grouped by IDs total, quantified, CV < 20 and CV < 10 either with method evaluation enabled for each concentration group or without method evaluation (no method evaluation) using a Human protein sequence data base (20,598 entries). B) Same coverage using a 10 minute (80 SPD) method with 5 cm Aurora Rapid 75 column (IonOpticks).

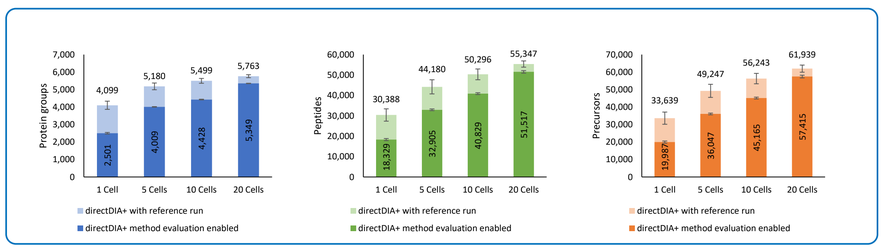

Figure 3:
A) Protein group, peptide and precursor identification rates of HeLa cells isolated at counts of 1, 5, 10, and 20 (n = 3 each) cells per well, acquired in dia-PASEF mode and analyzed in Spectronaut 18 with directDIA+ with method evaluation enabled for each cell count group or with additional reference run of 16 ng K562 bulk using a Human protein sequence data base (20,598 entries). B) Protein abundance correlation of 1 vs. 5 cells, 1 vs. 10 cells and 1 vs. 20 cells.

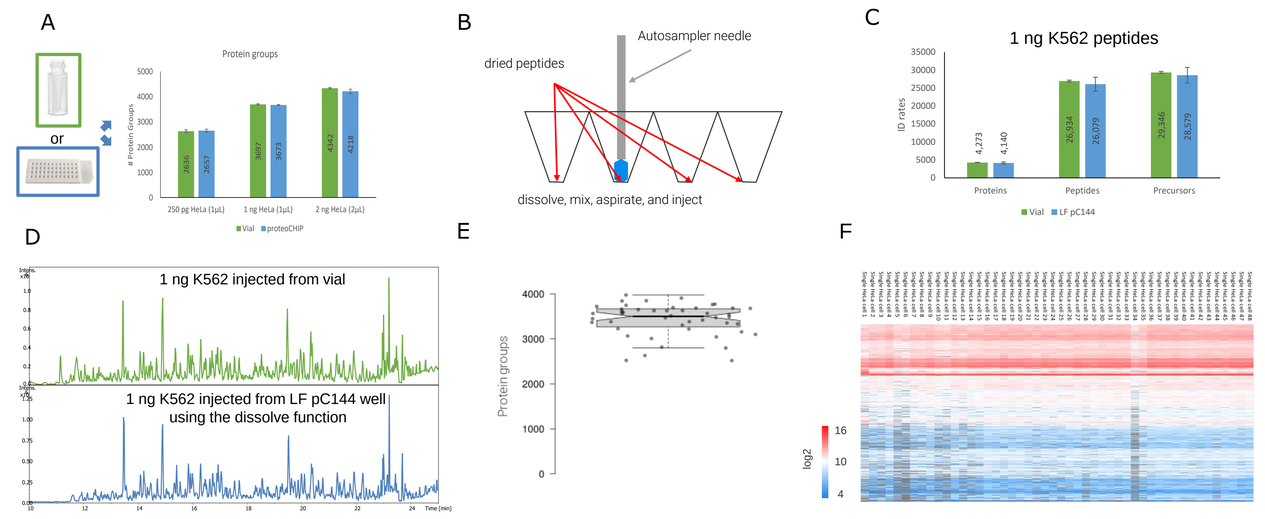

Figure 4:
A) Comparison on protein group identification rates of different volumes and sample amounts injected of bulk HeLa digest dilutions either from autosampler vial or LF proteoCHIP. B) schematic of the dissolve sample function using the nanoElute 2 CTC autosampler arm. C) Comparison on protein group, peptide, and precursor identification rates of 1 ng K562 cell digest run on a **timsTOF SCP** either injected from vial or resuspended with the dissolve sample function from the LF proteoCHIP. D) BPC of 1 ng K562 cell digest either injected from vial or resuspended with the dissolve sample function from the LF proteoCHIP. E) Plot of protein groups identified across 48 single HeLa cells injected from the LF proteoCHIP using the dissolve sample function using the nanoElute 2 CTC autosampler arm using Spectronaut 18 in directDIA+ with 4 ng HeLa reference runs. F) Heatmap showing protein group abundance pattern of protein identified in at least 24 of 48 single HeLa cell samples with good run to run reproducibility.

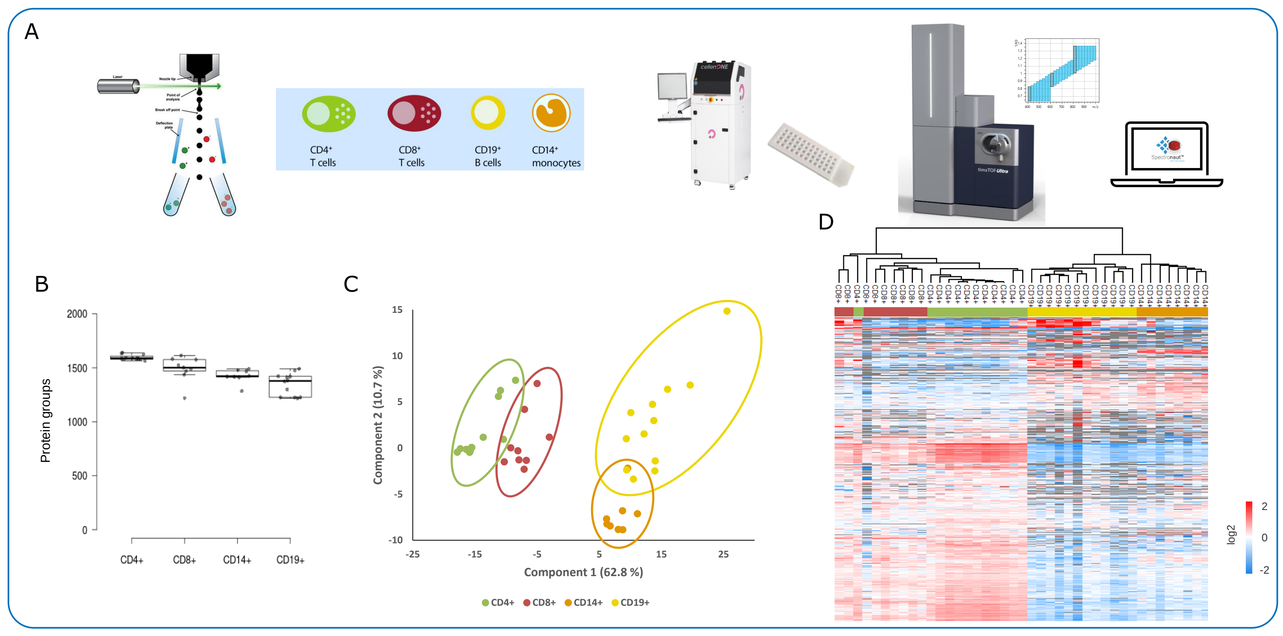

Figure 4:
A) PBMC analysis Workflow from FACS sorting of T-Cells (CD4+, CD8+) B-Cells (CD19+) and monocytes (CD14+), single cell isolation with the cellenONE into LF proteoCHIP, mass spectrometric analysis on the **timsTOF Ultra** using the 10 min gradient on IO5 and data analysis in Spectronaut in directDIA+ identifying in total 1,713 protein groups. 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.

Conclusions

- Hands-free and pipetting-free workflows using the proteoCHIP Evo-96 for transfer by centrifugation into 96-well plates or with resuspension of lyophilized peptides using the dissolve sample function of the nanoElute 2 for sample pickup from the LF proteoCHIP
- High sensitivity with good chromatographic reproducibility and robustness 10, 25 – 30 min run time
- Good quantification accuracy at single cell level with good single cell to single cell reproducibility on protein level with protein depth of 3,200 proteins on the timsTOF SCP and more than 4,000 protein groups per single cell HeLa cell on the timsTOF Ultra
- Sorted PBMC analysis workflow shows good proteome coverage with distinct protein abundance profiles for different the 4 cell types

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