Spatial and low input proteomics at scale – evaluating proteome coverage at high run speed using the Evosep Eno and the timsUltra AIP

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Introduction

Characterizing the phenotypic diversity of cells from heterogeneous tissues or organoids requires single-cell analysis to resolve distinct cell types and subtypes. Achieving meaningful depth at this resolution demands fast, reproducible chromatography paired with highly sensitive and robust mass spectrometry. This study evaluated label-free workflows using the Evosep platform in combination with the ultrasensitive timsTOF system to assess proteomic coverage at single-cell resolution.

Method

Dilution series of HeLa and K562 digests were prepared to simulate both bulk and single-cell proteomes. FaDu cells, derived from head and neck cancer, were isolated into proteoCHIPs EVO96, lysed, and digested using the cellenONE platform. Samples were analyzed using Whisper Zoom and the new Evosep Eno standard gradients on timsTOF Ultra 2 and timsUltra AIP systems. Acquisition in dia-PASEF mode was used to evaluate performance across different throughput settings. FFPE preserved tissue contours (8,000 µm²) from 5 µm thick mouse liver and human tonsil were cut by laser microdissection and cut into a proteoCHIP EVO-96, heated to 65°C for antigen retrieval, reduced and alkylated, and lysed followed by LysC/trypsin digestion on deck of a cellenONE system (proteoCHIP EVO-96). Samples were transferred by centrifugation onto Evotips, separated in Whisper Zoom 120, 80, 40 and 20 SPD or in standard methods at 500, 300, 200, 100, 60, and 30 SPD using the new Evosep Eno with analysis on a timsUltra AIP in dia-PASEF in fixed and variable window [1] mode and processed with Spectronaut 20 using directDIA+.

Results

Bulk Digest Dilutions

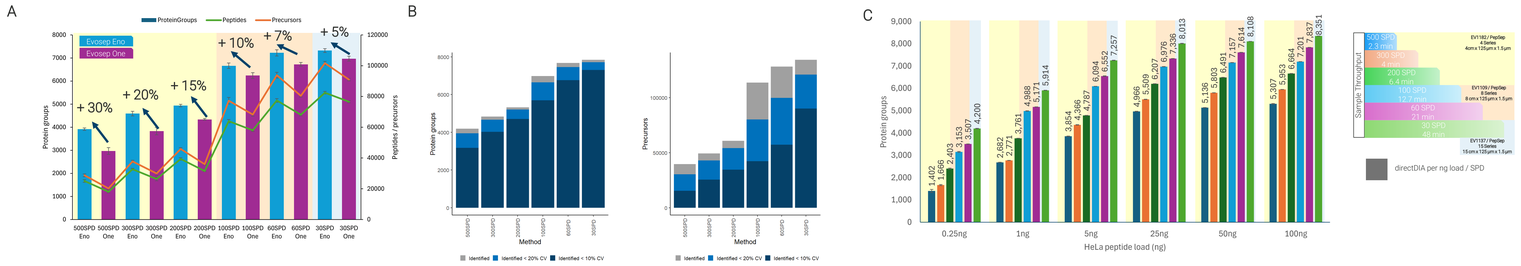

Figure 1
A) Comparison of Evosep One standard methods and the new Evosep Eno standard methods of 500 to 30 SPD acquired on a timsTOF Ultra 2 demonstrating for the fastest method 500 SPD a 30 % increase in protein group and 40 % increase in peptide identifications and around 10 % for 100 to 30 SPD). B) Bar chart showing CVs as an estimate of protein group and precursor level quantification reproducibility. C) HeLa digest dilution series from 100 ng to 0.25 ng run at 500 to 30 SPD using a Evosep Eno and a timsUltra AIP. Excellent sensitivity was achieved for 250pg loaded on Evotips and run at the various standard methods. >1,500 protein groups were identified at 500 SPD increasing to 4,200 protein groups at 30 SPD. The highest load identified at 500 SPD ~4,300 protein groups and ~8,400 at 30 SPD.

Scalable Single Cell Analysis

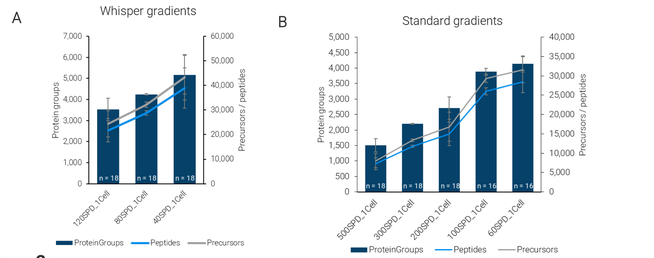

Figure 2:
Protein groups, peptide and precursor identification rates of single FaDu head and neck squamous cell carcinoma (HNSCC) cells analyzed A) with Evosep Whisper Zoom 120, 80 and 40SPD and B) standard gradients at 500, 300, 200, 100 and 60 SPD using the Evosep Eno and a timsUltra AIP.

100 Spatial Proteomes per Day

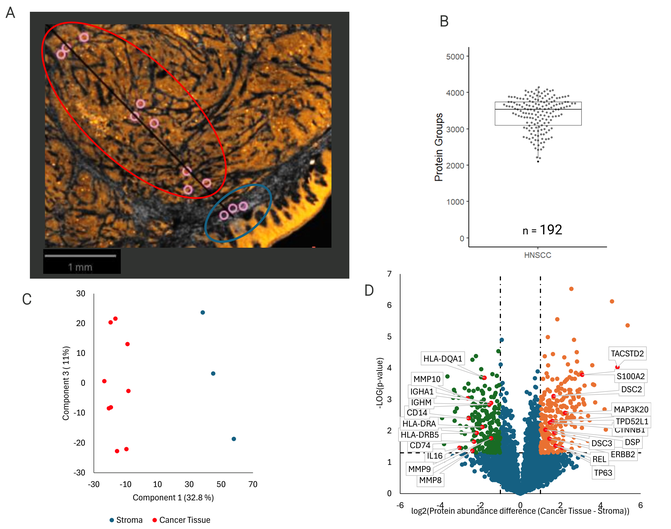

Figure 3:
A) Image of a **5 µm** thin human FFPE head and neck squamous cell carcinoma (HNSCC) with marked regions of **8,000 µm²** slices sampled from the cancer region (circled in red) and the stroma region (circled in blue). B) Boxplot of identified protein groups per sample (n = 192) using Evosep Whisper Zoom 120SPD on a timsUltra AIP. C) Principal component analysis clearly separates tissue areas samples from the cancer region from the stroma region. D) Volcano plot comparing quantified proteins (after downshifted data imputation) showing classical immune cell surface marker, HLA molecules and extra-cellular matrix related proteins to be more abundant in stroma (green) and cancer hallmark proteins and signaling pathway proteins to be more abundant in the cancer regions (orange). Selected proteins with label shown in red [2].

Scalable Tissue Proteomics

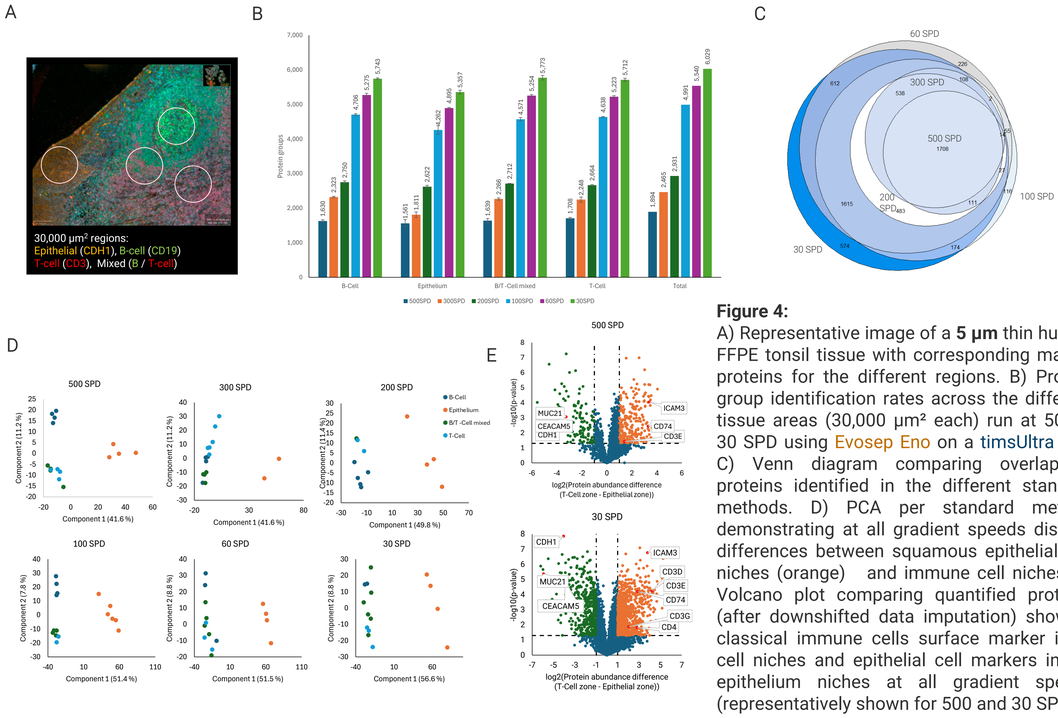

Figure 4:
A) Representative image of a **5 µm** thin human FFPE tonsil tissue with corresponding marker proteins for the different regions. B) Protein group identification rates across the different tissue areas (30,000 µm² each) run at 500 – 30 SPD using Evosep Eno on a timsUltra AIP. C) Venn diagram comparing overlap of proteins identified in the different standard methods. D) PCA per standard method demonstrating at all gradient speeds distinct differences between squamous epithelial cell niches (orange) and immune cell niches. E) Volcano plot comparing quantified proteins (after downshifted data imputation) showing classical immune cells surface marker in T-cell niches and epithelial cell markers in the epithelium niches at all gradient speeds (representatively shown for 500 and 30 SPD).

Conclusion

- Excellent proteome coverage from single FaDu cells at **Whisper Zoom 120 – 40 SPD** and **standard gradients at 500 – 60 SPD** on the **timsUltra AIP.**
- Accurate differentiation of stroma tissue regions from HNSCC cancer regions in **100 spatial tissue proteomics per day** from preparation to MS acquisition with **Whisper Zoom 120 SPD** with high proteome coverage.
- Exceptional performance increase with the **new Evosep Eno** at 500 – 200 SPD with good sensitivity at single cell equivalent sample loads.
- Accurate and consistent differentiation of epithelial and immune cell-rich regions in human FFPE tonsil tissue (30,000 µm²) at all standard gradient speeds (**500 – 30 SPD**) on the **new Evosep Eno** on the **timsUltra AIP**

References

- [1] P. Skowronek, M. Mann et al. *Mol Cell Proteomics*, 2022, 21, 9, 100279
- [2] M. Klingeberg, F Coscia et. al, *MCP*, 2026, Volume 25, 1101489

timsUltra AIP

COI: CK, DV, ML, and DH are employees of Bruker. MK, LS, SK, and FC are members of the MDC–Bruker CoE for Single-Cell Omics.

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