

Scaling-up low input spatial proteomics using Evosep Eno on the timsUltra AIP

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Introduction

Throughput and scalability are important factors for ultra-low input spatial tissue proteomic. Enhanced sample throughput is key to understand spatial proteome heterogeneity for cohort size profiling. For reliable data acquisition with statistical power in ultra-high sensitivity spatial proteomics, fast chromatography with low overhead time with fast scanning ultra-sensitive mass spectrometry is required to enable scalability in spatial proteome analyses of ultra-low input applications. Here, we demonstrate applicability of the Evosep Whisper Zoom methods for speeding up label-free spatial tissue proteomics on FFPE preserved tissue analysis of up to 120 samples per day (SPD) with data acquisition on the timsUltra AIP

Methods

FFPE preserved tissue contours (8,000 μm^2) 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 lyzied followed by LysC/trypsin digestion on deck of a cellenONE system (proteoCHIP EVO-96). Samples were transferred by centrifugation onto Evtotips, 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® and diagonal-PASEF® mode and processed with Spectronaut 19 using directDIA+.

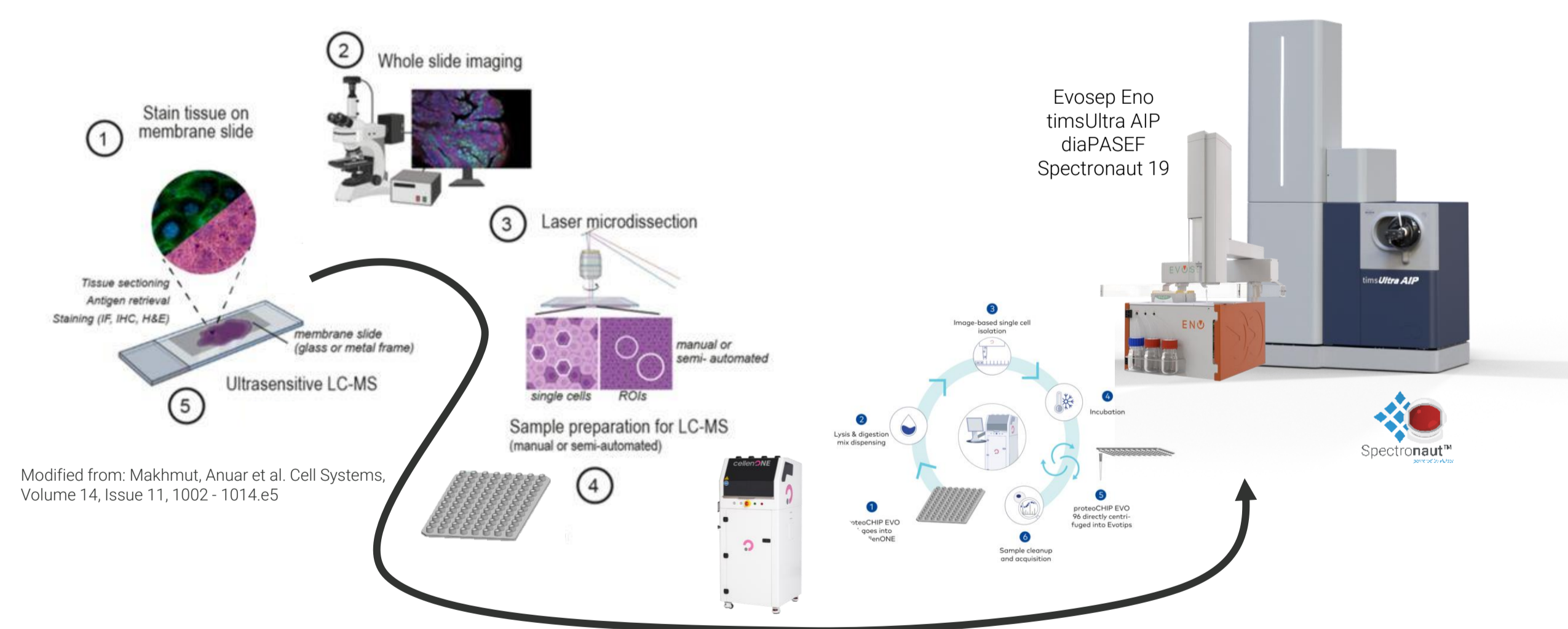


Figure 1: Single cell and Spheroid proteomics workflow with the Evosep One with Whisper Zoom 120, 80, and 40SPD using the proteoCHIP EVO96 for pipetting-free sample transfer onto Evtotips Pure and pyDIAid [1] optimized dia-PASEF data acquisition on **timsUltra AIP** with data analysis in Spectronaut 19.

Results

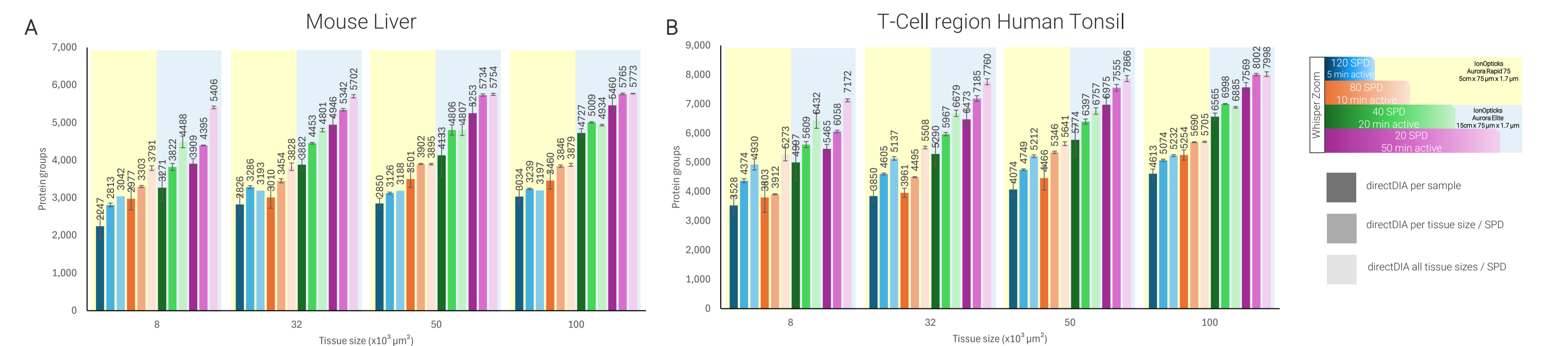


Figure 2: Protein group identification rates of 5 μm thin A) mouse FFPE liver tissue and B) T-Cell regions of human FFPE tonsil tissue at tissue sizes of 8,000, 32,000, 50,000 and 100,000 μm^2 analyzed with Whisper Zoom 120, 80, 40 and 20 SPD. The homogeneous but high dynamic range liver tissue identified ~2,800 protein groups at 120 SPD for 8k μm^2 and ~4,400 protein groups at 20 SPD, ~3,200 protein groups at 120 SPD for 100k for 8k μm^2 and ~5,800 protein groups at 20 SPD. The more heterogeneous tonsil tissue identified ~4,400 protein groups at 120 SPD for 8k μm^2 and ~4,400 protein groups at 20 SPD, ~3,200 protein groups at 120 SPD for 100k for 8k μm^2 and ~5,800 protein groups at 20 SPD.

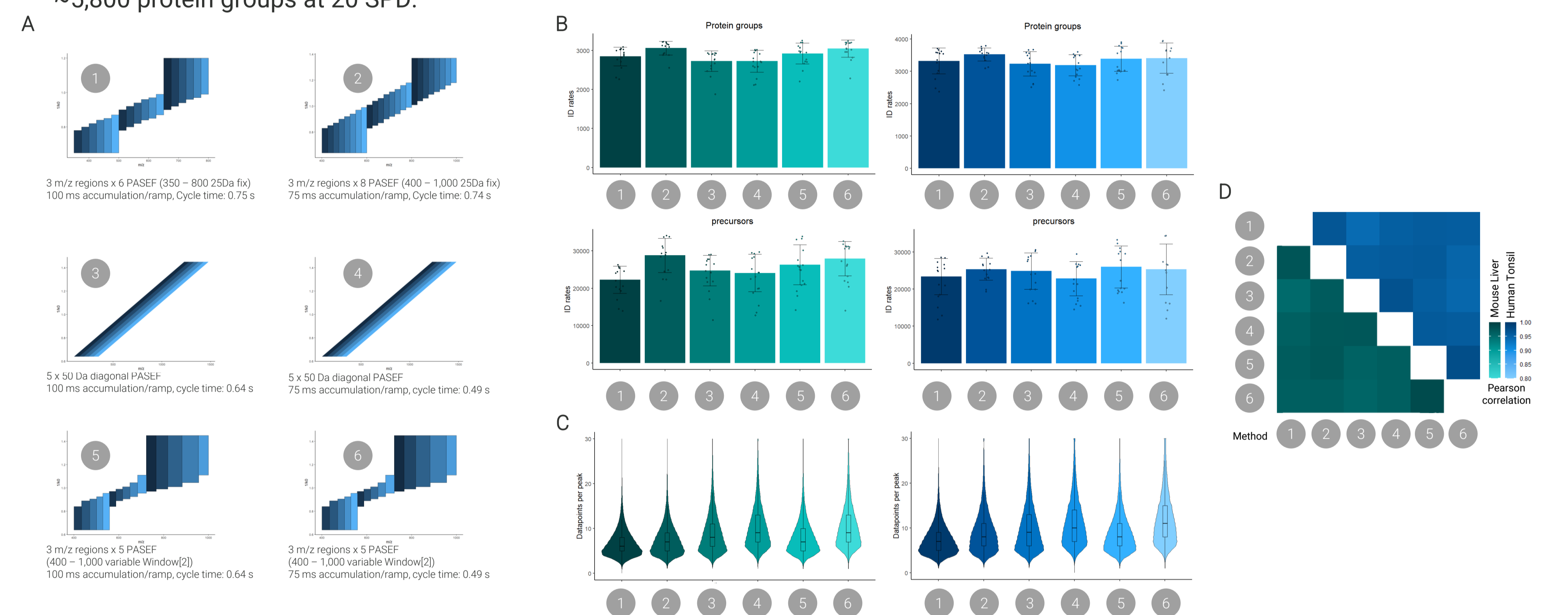


Figure 3: A) dia-PASEF and diagonal-PASEF acquisition parameter used for method evaluation analyzing 8,000 μm^2 mouse FFPE liver tissue (green) and T-Cell region of human tonsil tissue (blue) with Whisper Zoom 120SPD. B) Protein groups and precursor identification rates across the 6 methods for with method 2 and 6 showing the highest protein group and precursor identifications. C) Data point per peak (DPPP) distribution per acquisition method with all methods excellent sampling rates needed for quantification, highest number of data points per peak reached with method 6 ~11 DPPP. D) Pearson correlation across the different method shows excellent correlation scores (>0.9) for all acquisition methods.

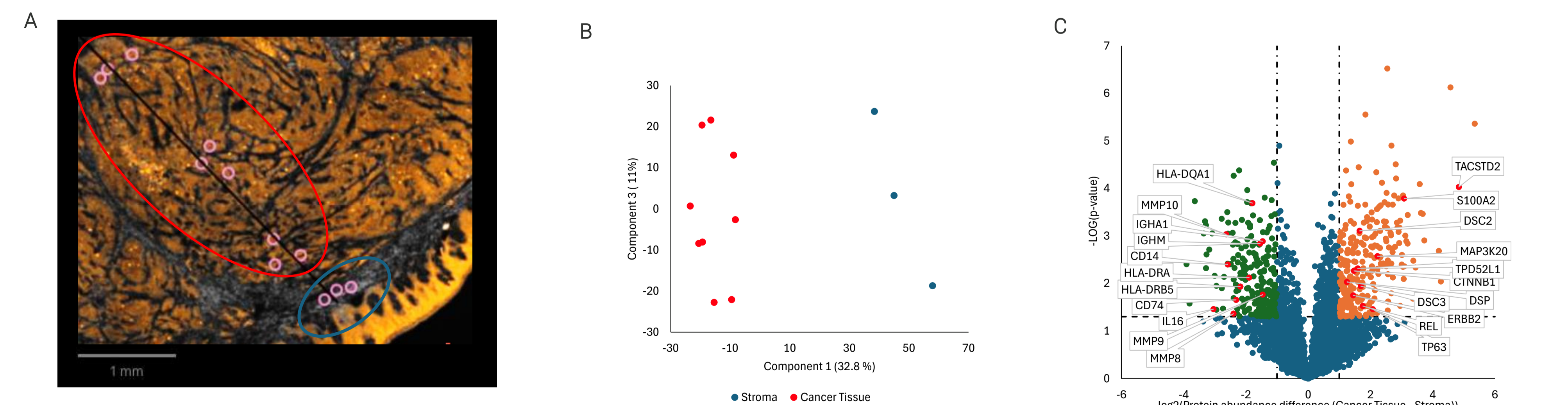


Figure 4: A) Image of a 5 μm thin human FFPE head and neck squamous cell carcinoma (HNSCC) with marked regions of 8,000 μm^2 slices sampled from the cancer region (circled in red) and the stroma region (circled in blue). B) Principal component analysis clearly separates tissue areas samples from the cancer region from the stroma region. C) Volcano plot comparing quantified proteins (after downshifted data imputation) showing classical immune cells surface marker, HLA molecules and extra-cellular matrix related proteins to be more abundant in the stroma region (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.

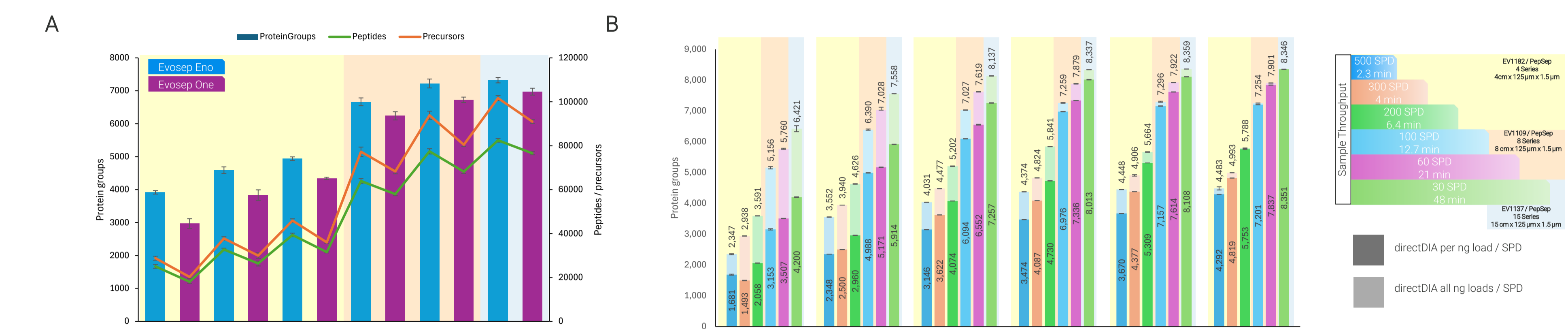


Figure 5: A) Comparison of **Evosep One** standard methods and the new **Evosep Eno** standard methods of 500 to 30 SPD acquired on a **timsTOF Ultra 2** demonstrating for the fastest method 500 SPD a 30 % increase in protein group and 40 % increase in peptide identifications and around 10 % for 100 to 30 SPD. B) 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 evtotips 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.

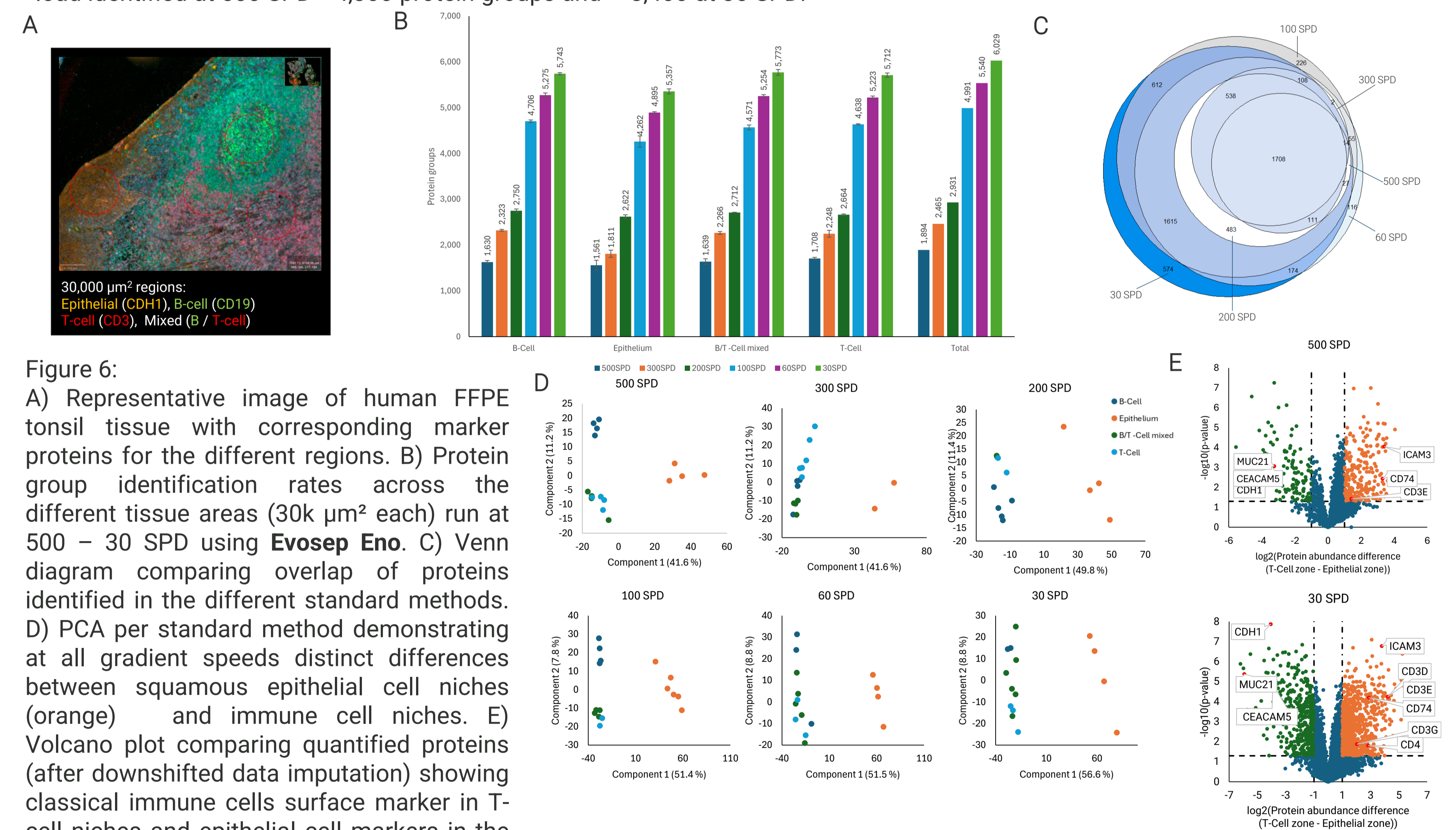


Figure 6: A) Representative image of human FFPE tonsil tissue with corresponding marker proteins for the different regions. B) Protein group identification rates across the different tissue areas (30k μm^2 each) run at 500 – 30 SPD using **Evosep Eno**. C) Venn diagram comparing overlap of proteins identified 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)

References

- [1] Modified from: Makhmut, Anuar et al. Cell Systems, Volume 14, Issue 11, 1002 - 1014.e5
- [2] 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
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Application Note, Bruker Daltonics, LCMS-238, 1918674, 2025

Conflict of Interest

CK, GK are employees at Bruker, AS is employee at Cellenion; DBJ, OBH, NB are employees at Evosep.

Conclusion

- Excellent proteome coverage from small pieces (8,000 μm^2) of mouse FFPE liver tissue and human FFPE tonsil tissue using **Whisper Zoom 120 – 20 SPD** on the **timsUltra AIP**.
- Accurate differentiation of stroma tissue regions from HNSCC cancer regions in a **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 μm^2) at all standard gradient speeds (500 – 30 SPD) on the new **Evosep Eno** on the **timsUltra AIP**

timsUltra AIP