

Optimizing Selectivity and Ion Utilization for Trapped Ion Mobility Spectrometry for Enhanced DIA Performance

Markus Lubeck¹, Andreas Schmidt¹, Stephanie Kaspar-Schoenefeld¹, Matt Albano², Cory Lytle², Dijana Vitko², Matthew Willetts², Daniel Hornburg³

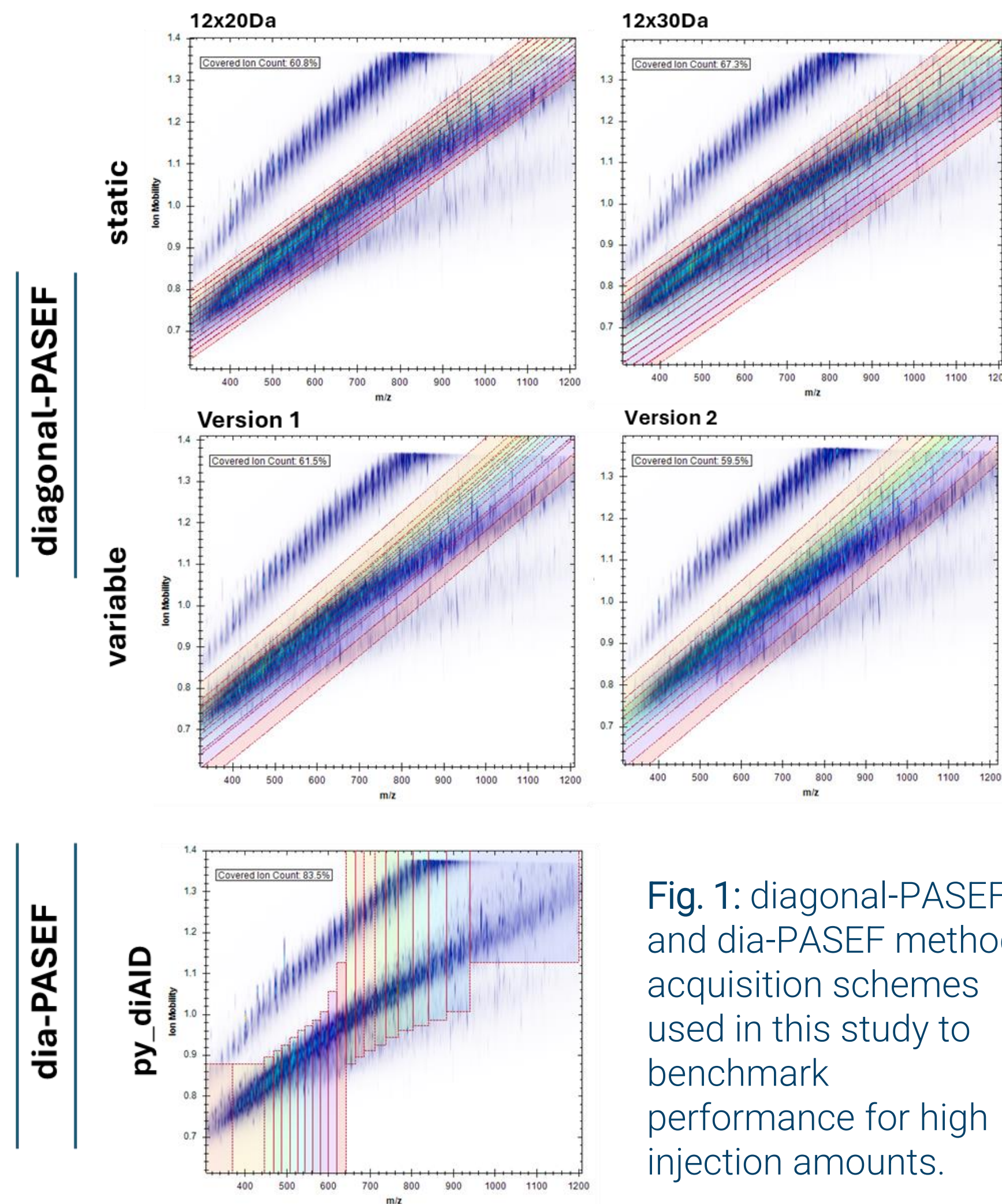
¹Bruker Daltonics GmbH & Co. KG, Bremen, Germany, ²Bruker Scientific, Billerica, MA, ³Bruker Daltonics GmbH & Co. KG, San Francisco, CA

Introduction

Data independent acquisition (DIA) with and without additional ion mobility-based separation or filtering became the most commonly used method for proteomics due to its high level of sensitivity, reproducibility, and data completeness. The combination with dispersive ion mobility separations like Trapped Ion Mobility (TIMS) prior to quadrupole isolation increases selectivity and ion usage, so that a variety of window placements in the mass- mobility panes were developed during the last years including slice-PASEF, diagonal-PASEF (synchro-PASEF and midia-PASEF), and py_diAID. Here we compare different diagonal-PASEF schemes with a py_diAID optimized dia-PASEF method for different sample types and separation times.

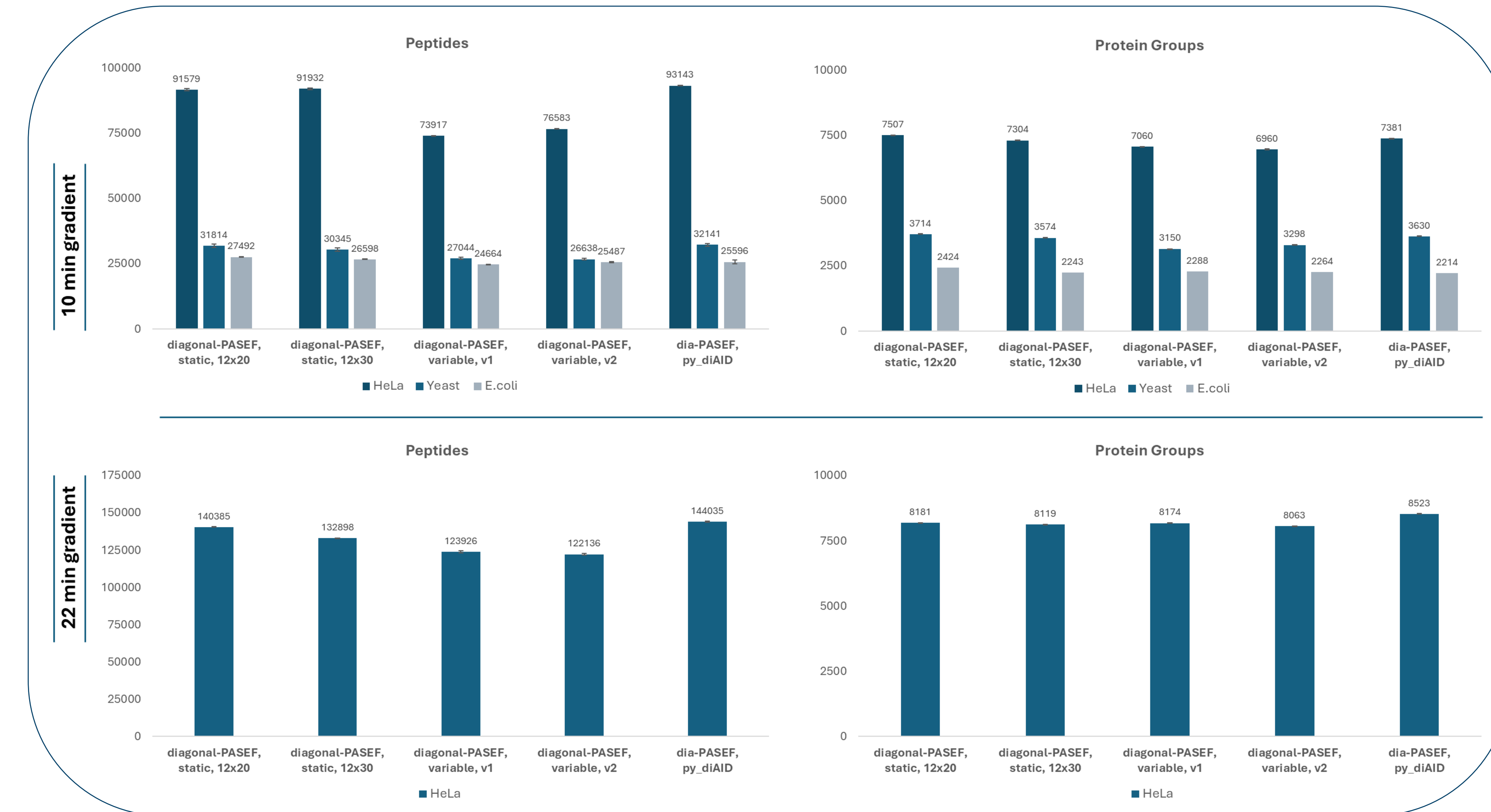
Methods

Proteomics performance of different DIA approaches were assessed using a human cell line digests (HeLa), E.coli, and Yeast digests (all in-house digests). Samples were separated by nanoHPLC at a flow rate of 300 nL/min, utilizing either an 8 cm or a 25 cm column (0.075 mm ID, IonOpticks, Melbourne) using a 10 and a 22-minutes gradient, respectively (1-35% solvent B). Different diagonal-PASEF methods using either fixed or variable slices were compared to a variable, py_diAID optimized dia-PASEF method (Fig.1). All measurements were done on a timsTOF HT (Bruker). Data were processed with Spectronaut (v19.8, Biognosys, Schlieren) using the directDIA pipeline. For diagonal-PASEF acquisitions, the preprocessing mode "Legacy (Spectronaut 18)" was used, and IM sampling reduction was set to 3.



Results

In this study we evaluated the performance of different diagonal-PASEF schemes compared to dia-PASEF. Diagonal-PASEF operates by seamlessly and continuously following the observed diagonal shape of the ion distribution in m/z and ion mobility dimension. Whereas previous results already prove superior performance of diagonal-PASEF for lower input amounts (Below et al, 2025), method optimization is still required to improve its performance for higher loads. Here, we generated different diagonal-PASEF methods using either fixed



slices (12x20Da and 12x30Da) and variable slices (with smaller slices placed in the denser ion region). Using varying slices resulted in shorter cycle times of 0.8s compared to 1.2 for the static window placement scheme. A py_diAID optimized dia-PASEF method was used as reference, also having a cycle time of 1.2. Using a 10 min gradient, the static 12x20 Da method yielded higher number of identified protein groups compared to dia-PASEF for the three proteomes analyzed (HeLa, Yeast, E.coli, 400ng per proteome, injected separately, Fig 2.). Interestingly, using a method with broader coverage of the

ion cloud (12x30Da) didn't improve number of identification, neither on precursor nor on peptide or protein group level indicating sufficient coverage already with a more focused method. The tested variable slice methods didn't show competitive performance. When analyzing the human cell line digest with a 22 min gradient, the static 12x20Da diagonal-PASEF method, resulted in comparable number of identified peptides, but lower number of protein groups compared to dia-PASEF. The presented study highlights the fact, that further method optimization aids improving diagonal-PASEF.

Conclusion

- diagonal-PASEF acquisition methods continuously cover the observed diagonal shape of the peptide precursor distribution and can be easily set up.
- Continuous improvements of diagonal-PASEF methods result in improved performance compared to dia-PASEF for short cycle times and high loads.

timsTOF HT