

Benchmarking diagonal-PASEF data acquisition for high-throughput proteomics applications

Christopher Below¹, Oliver M. Bernhardt¹, Stephanie Kaspar-Schoenefeld², Roland Bruderer¹, Shourjo Ghose³, Tejas Gandhi¹, Jonathan Krieger⁴, Markus Lubeck², Lukas Reiter¹

¹Biognosys AG, Zurich, Switzerland, ²Bruker Daltonics GmbH & Co KG, Bremen, Germany, ³Bruker Scientific, Billerica, MA ⁴Bruker Ltd., Milton, ON

More details:



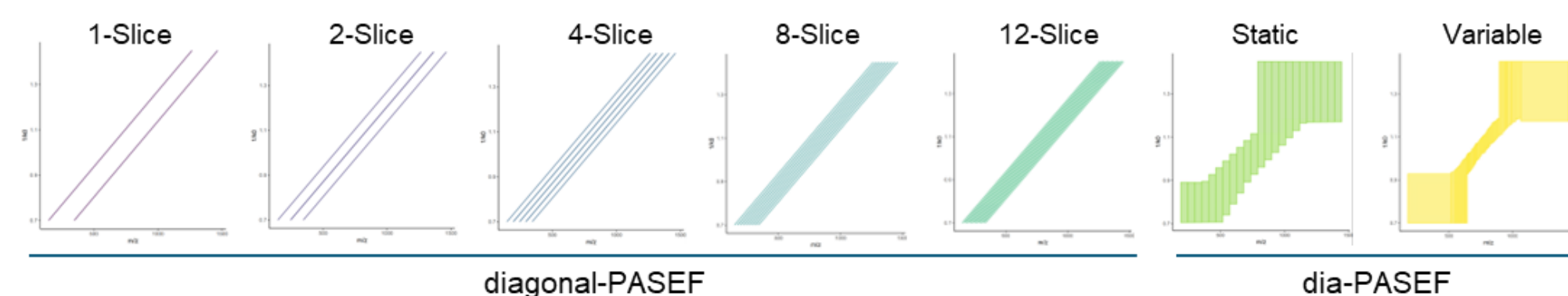
Introduction

Combining Data-Independent Acquisition (DIA) with Trapped Ion Mobility Separation (TIMS) reduces sample complexity by providing an additional dimension of separation, thereby increasing overall system peak capacity. This scan mode, known as dia-PASEF, uses fixed quadrupole isolation windows, which are applied for ion selection in the m/z and mobility panes. To further enhance the analytical performance, researchers have proposed diagonal-PASEF acquisition methods, termed synchro- or midia-PASEF, which aim to acquire the precursor ion cloud more efficiently. These methods operate by continuously following the diagonal shape of the precursor ion cloud. Several parameters can be adjusted to tailor the method for specific applications. Therefore, this study aims to optimize the efficiency of diagonal-PASEF for high throughput proteomics applications.

Methods

Peptide identifications and quantitative reproducibility among technical replicates were assessed using dilution series of human cell line digests (HeLa, in-house digest). Samples were separated by nanoHPLC at a flow rate of 300 nL/min, utilizing a 25 cm column (0.075 mm ID, IonOpticks, Melbourne) using a 17-minutes non-linear gradient (1-45% solvent B). Different diagonal-PASEF methods using a width of 200 m/z resolved by 1, 2, 4, 8 or 12 slices (Fig. 1) were compared to a “static” (using constant window widths of 55Da) and a “variable” (using variable window widths, minimum 9.5 Da, maximum 382.5 Da) dia-PASEF method. 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, IM sampling reduction was set to 3 and RT sampling reduction was selected according to the numbers of slices.

Data Acquisition



Data Processing

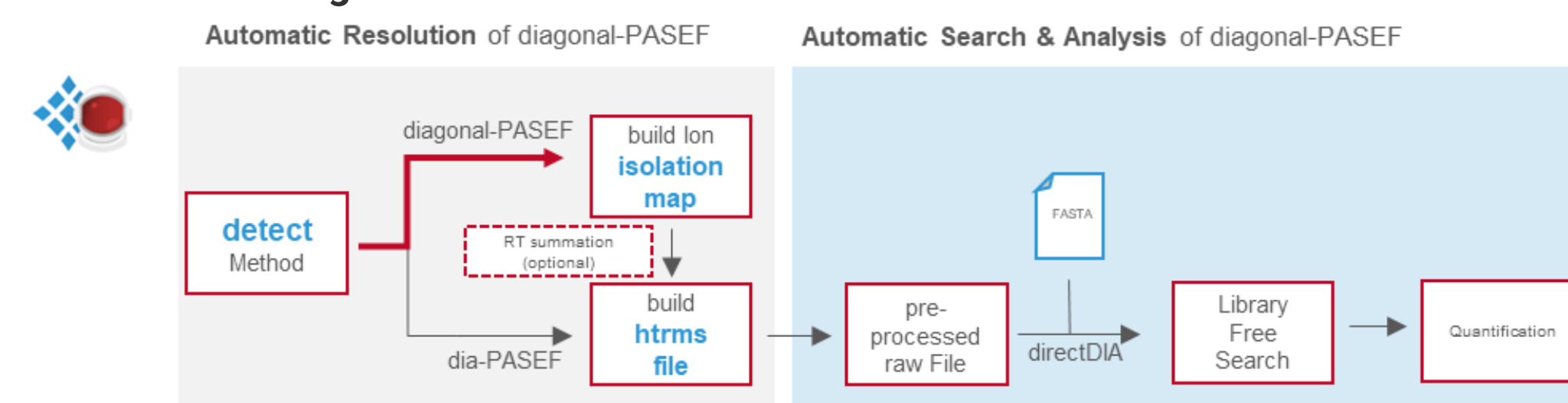


Fig. 1 diagonal-PASEF measurements using different acquisition schemes and subsequent data analysis with Spectronaut.

Results

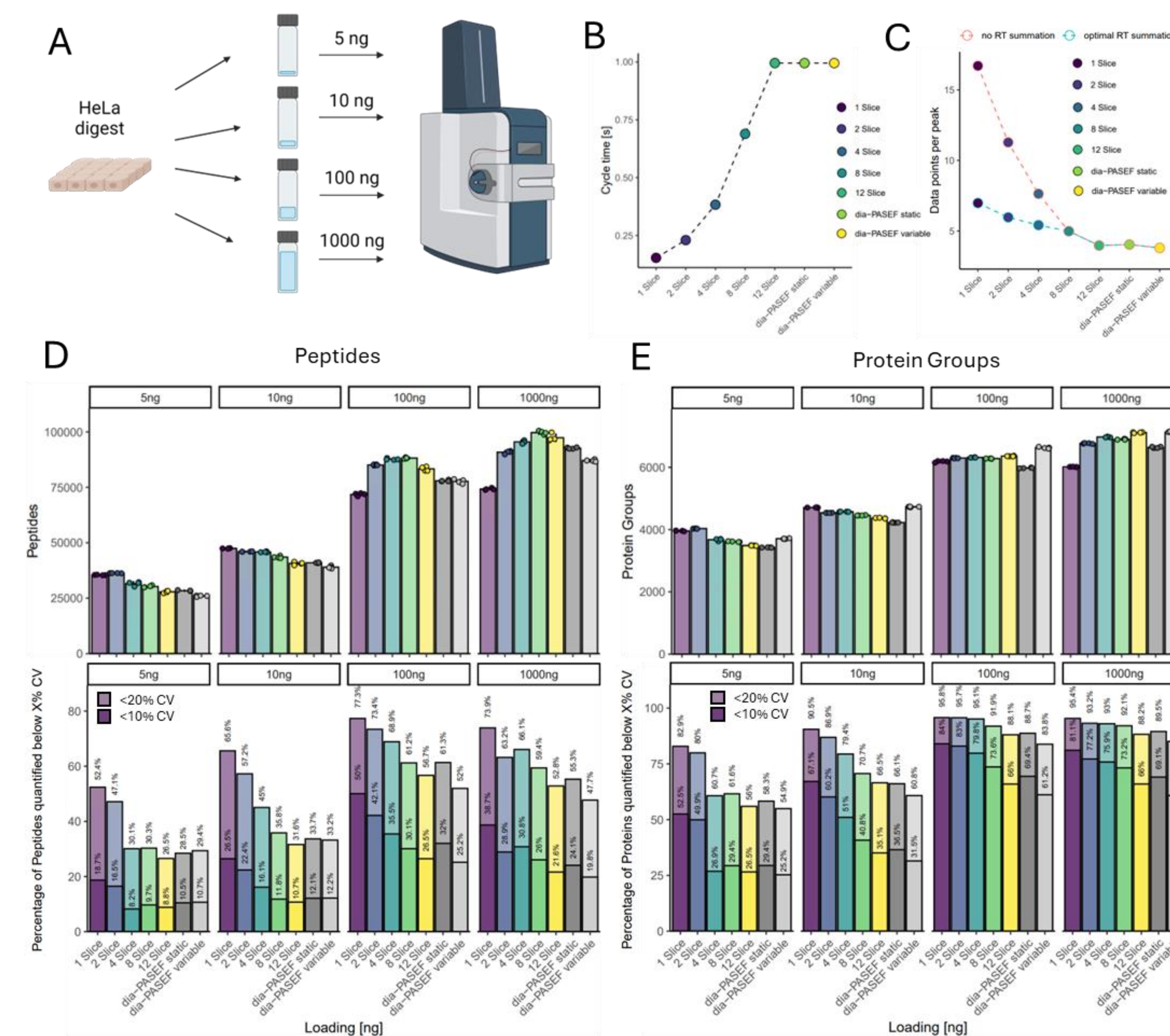


Fig. 2 Evaluation of the performance of diagonal-PASEF. A: Schematic of the experimental approach. B: Cycle time of tested methods for different loading ramps tested. C: Data points per peak. D-E: Results from evaluated loading ramps.

In this study we evaluated the performance of diagonal-PASEF compared to dia-PASEF. For this we injected a HeLa digest at different loading amounts from 5ng to 1000ng of net input material (Figure 2A) and analyzed them with diagonal-PASEF methods consisting of 1, 2, 4, 8 or 12 slices. On average, the tested diagonal-PASEF methods yielded similar peptide and protein group identifications to the dia-PASEF control methods across all tested loadings (Figure 2D, E). Diagonal-PASEF methods composed of <4 slices generally provided more peptide and protein group identifications at lower loadings. For example, at 5 ng of loading the 1-slice diagonal-PASEF method yielded 24.9% and 36.4% more peptides than the dia-PASEF static and variable methods, respectively. On the protein group level, the same method yielded 15.6% and 6.7% more protein groups than the dia-PASEF static or variable methods, respectively. In contrast, at the higher tested loadings of 100 and 1000 ng, the best diagonal-PASEF methods had more than 4 slices. The 12-slice diagonal-PASEF method yielded 2.8% or 18.3% more protein groups than the 1-slice method at 100 or 1000 ng respectively. This diagonal-PASEF method proved to be very competitive against dia-PASEF even at high loadings. At the highest tested loading of 1000 ng, the 12-slice method achieved 7.1% more protein groups and 5% more peptides than the dia-PASEF static method. Strikingly, the 12-slice diagonal-PASEF method only yielded 0.2% less protein groups but 11.8% more peptides than the highly optimized dia-PASEF variable method.

We additionally investigated the quantitative precision of the tested dia-PASEF and diagonal-PASEF methodologies. For this we computed the protein groups or peptides quantified below 20% or 10% CV. All tested diagonal-PASEF methods provided a very high quantitative precision. In general, we noticed that the relative quantitative precision was reduced with increasing number of diagonal-PASEF slices across the tested methods. For example, at 100 ng of loading 77.3% of all peptides were quantified below 20% CV using a 1-slice method whereas with the 12-slice method only 56.7% of all peptides were quantified below 20% CV (Figure 2, D). Despite this effect, the tested diagonal-PASEF had a notably higher quantitative precision compared to the investigated dia-PASEF methods. In summary, these data indicate that diagonal-PASEF methods not only provide improved overall identification rates but also allow for a better quantification of detected analytes.

Conclusion

- diagonal-PASEF acquisition methods continuously cover the observed diagonal shape of the peptide precursor distribution.
- Spectronaut 19 fully supports different diagonal-PASEF methods.
- Using optimized analysis strategies in Spectronaut results in improved overall identifications and quantitative reproducibility when compared to dia-PASEF.

timsTOF HT