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Evaluation of diagonal-PASEF for Data Independent Acquisition in Proteomics

Markus Lubeck[1], Stephanie Kaspar-Schoenefeld[1], Christoph Krisp[1], Andreas Schmidt[1], Silke Neustifter[1]

[1]Bruker Daltonics GmbH & Co. KG, Bremen, Germany

Introduction

Data-Independent Acquisition (DIA) is widely used for proteomics as it usually outperforms Data-Dependent Acquisition (DDA) for protein identification and quantitation, due to its higher ion usage and reproducibility. In contrast to DDA, DIA approaches record fragment ion information for each precursor in an unbiased way resulting in highly reproducible data sets. This advantage can be further increased by combining it with trapped ion mobility spectrometry (TIMS). Here, the additional separation dimension reduces complexity, enables ion focusing, and a sequential elution of condensed ion packages from the TIMS device for highly efficient and sensitive ion utilization (dia-PASEF, [1]). To increase the analytical performance of the timsTOF, researchers have recently proposed diagonal dia-PASEF acquisition methods with quadrupole selection windows following the ion mobility scan. These new acquisition schemes are termed synchro- or midia-PASEF and attempt to acquire the ion cloud more efficiently ([2], [3]). They survey seamlessly and continuously the observed diagonal shape of the precursor ion density in the ion mobility (1/K0) – mass over charge (m/z) space. The new scan mode has been fully implemented in Bruker's data acquisition software.

Material & Methods

Tryptic in-house digests of a human cell lysate, yeast, and E. coli were used to evaluate the performance of diagonal-PASEF on the timsTOF HT system.

Measurements were done using 5, 15, and 35-minute gradients. Optimized methods were used for overlapping and non-overlapping diagonal-PASEF, dia-PASEF and PASEF measurements. Non-overlapping diagonal-PASEF as well as dia-PASEF data were processed in Spectronaut (v19, Biognosys). Overlapping diagonal-PASEF data were processed through the newly developed MIDIAID pipeline and the generated mgf was directly searched in SAGE (forked by Bruker from official 0.13.2 version). PASEF data was also processed in SAGE.

Results

Different diagonal-PASEF method schemes can be easily set up with the newly developed method editor implemented in timsControl (Bruker). Optimal coverage of the ion cloud of interest is controlled via the the m/z and mobility range as well as the number of scans, isolation width and overlap , which collectively define cycle time estimate. Non-overlapping diagonal-PASEF methods greatly benefit from very short cycle times and initial results show performance in the range of dia-PASEF but with a greater percentage of protein groups at highest precision (CV<10%). Using overlapping diagonal-PASEF schemes combined with sophisticated data processing yields in high-quality deconvoluted MS/MS DDA-like spectra that can be analyzed with standard database search engines as well as with de novo sequencing tools. Initial results show depth of coverage, and throughput in the range of DIA and interpretability of DDA data.

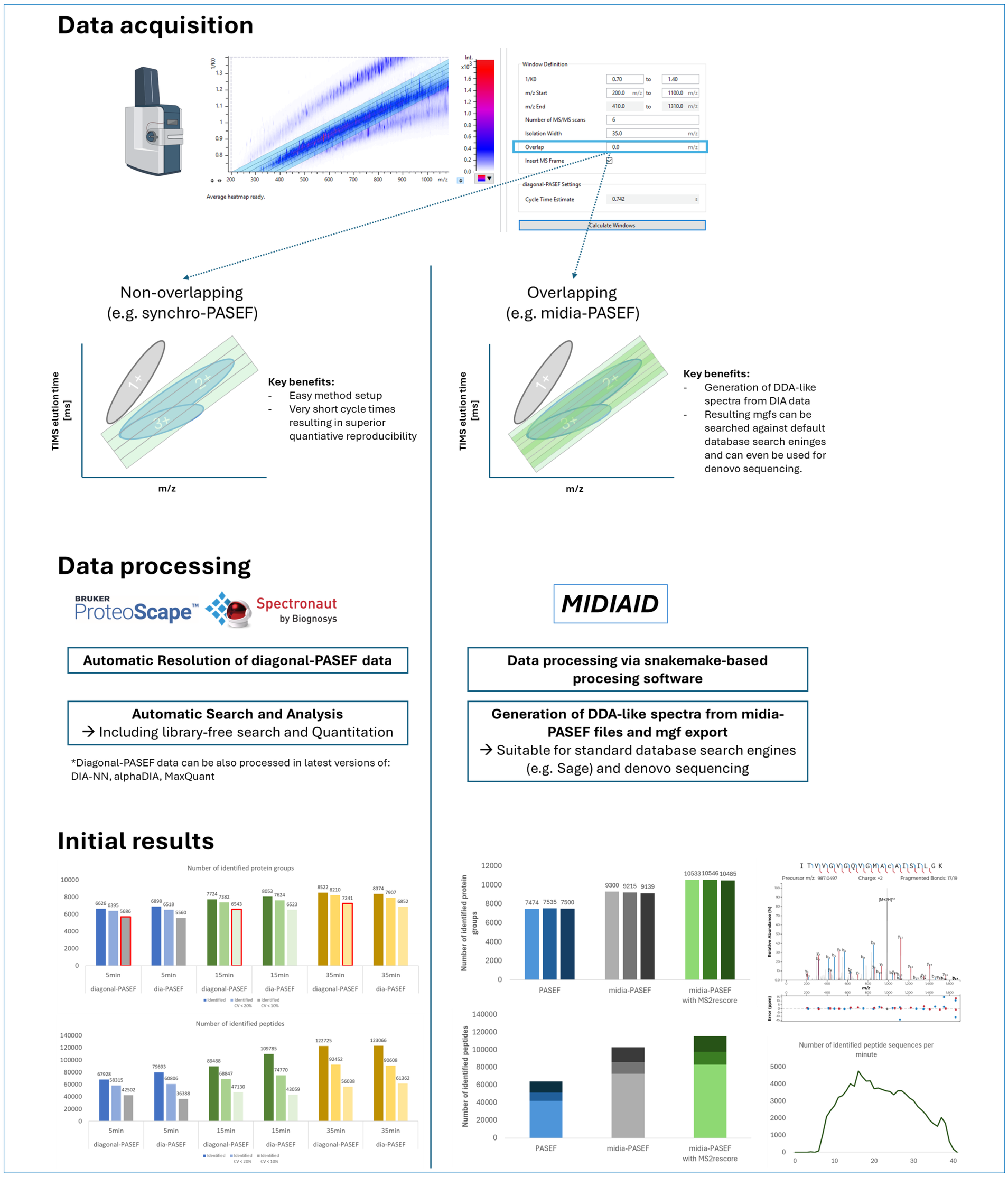

Figure 1: Overview about diagonal-PASEF using overlapping and non-overlapping window schemes.

- Diagonal-PASEF data acquisition is fully implemented in Bruker's data acquisition software timsControl and supports non-overlapping (e.g. synchro-PASEF) as well as overlapping (e.g. midia-PASEF, license required) acquisition schemes.
- Future improvements are expected both on data acquisition as well as on data processing site making use of the benefits of the tight precursor to fragment correlation.

nanoElute 2 + timsTOF HT

[1] Meier et al. (2020), Nature Methods. **17**:1229-1236.
[2] Skowronek et al. (2023), Mol Cell Proteomics. **22**(2).
[3] Distler et al., bioRxiv, doi.org/10.1101/2023.01.30.526204.

Conflict of interest: ML, SK-S, CK, AS and SN are employees of Bruker Daltonics.