

CCS-Aware DIA Data Analysis in PaSER

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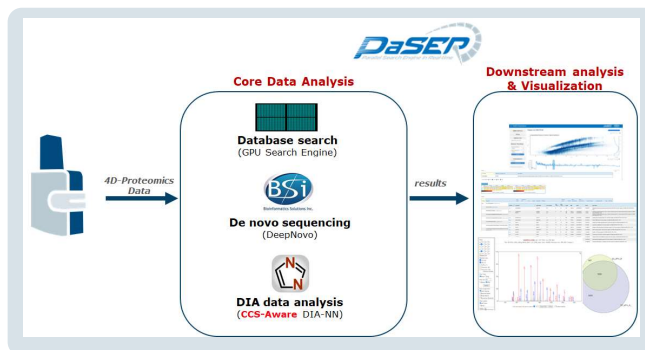


Introduction

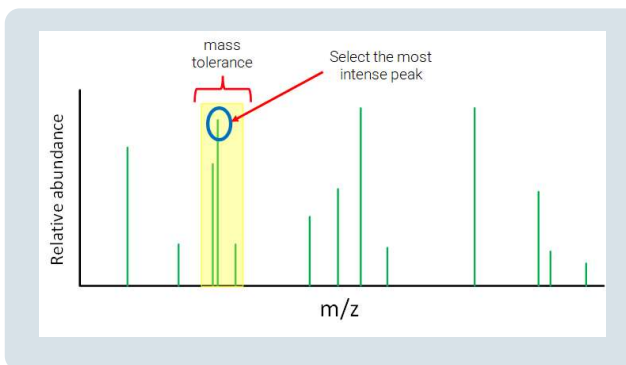
Since Venable et al. first introduced data-independent acquisition (DIA) in 2004, DIA acquisition and data analysis tools have been continuously improved, making DIA a vital technology to identify and quantify thousands of proteins with high reproducibility and deep proteomics coverage. DIA data analysis, in general, relies on a spectral library constructed from data-dependent acquisition (DDA). Alternatively, the library-free approach searches DIA data directly against a fasta database. We combined a recently developed CCS-aware ProLuCID-4D search engine using ion mobility and a spectral library-based DIA approach to increase coverage.

To build spectral libraries, we ran ProLuCID 4D GPU search engine of PaSER on DDA data. DTASelect filtered the scored spectra by 1% protein-level FDR using discriminant analysis. We used DTASelect output files as input to EasyPQP to generate spectral libraries. We ran DIA-NN to analyze dia-PASEF data using a spectral library, while we ran ProLuCID 4D GPU search engine for library-free database search.

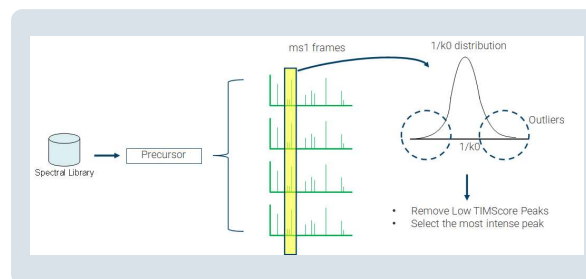
PaSER Data Analysis Platform Overview



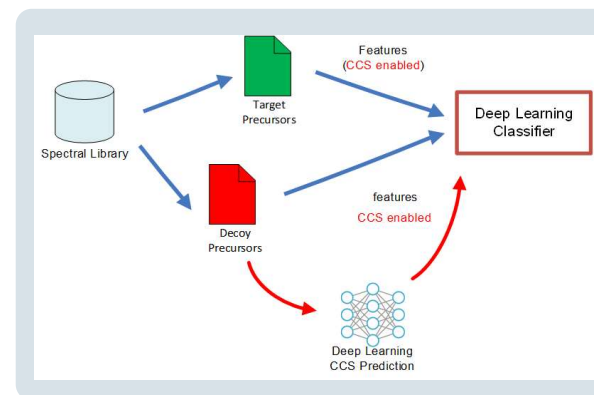
Conventional Peak Selection Strategy



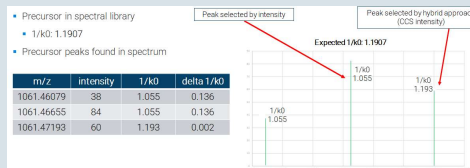
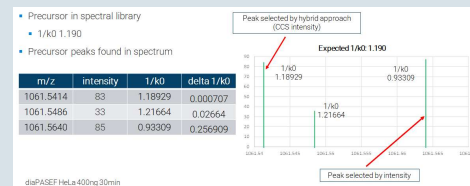
Hybrid Approach for Peak Picking with TIMScore



TIMScore Applications to DIA Data Analysis



Peak Selection Strategy with CCS



CCS Application to DIA Analysis

	DIA-NN open source (1.7.12)	DIA-NN CCS-enabled	Percent
Protein group	6,617	7,941	20%
Modified Peptides	63,770	95,451	50%
Stripped Peptides	53,783	75,174	40%

timsTOF Pro HeLa 30min 400 ng, triplicates