

# High dynamic range proteome analysis with BoxCar DIA and super-resolution Orbitrap mass spectrometry

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## ABSTRACT

**Purpose:** Proteomics with BoxCar and data independent acquisition (DIA).

**Methods:** Real-time  $\Phi$ SDM (Phase-Constrained Spectrum Deconvolution Method) processing of full range mass spectra

**Results:** Two-fold scan speed allows rapid proteome analysis and efficient implementation of BoxCar DIA.

## INTRODUCTION

Phase-Constrained Spectrum Deconvolution Method ( $\Phi$ SDM) [1] is a signal processing approach that uses the phase information in Orbitrap<sup>TM</sup> mass analyzers to improve resolution on a refined frequency grid. It translates into increased mass resolution without penalizing acquisition time. Due to its high computational cost, it has so far only been applied for offline calculations or in narrow  $m/z$  regions of interest [2].

## MATERIALS AND METHODS

### Sample Preparation

Whole-cell lysates and human blood samples were digested with trypsin/LysC and analyzed in single runs.

### Test Method(s)

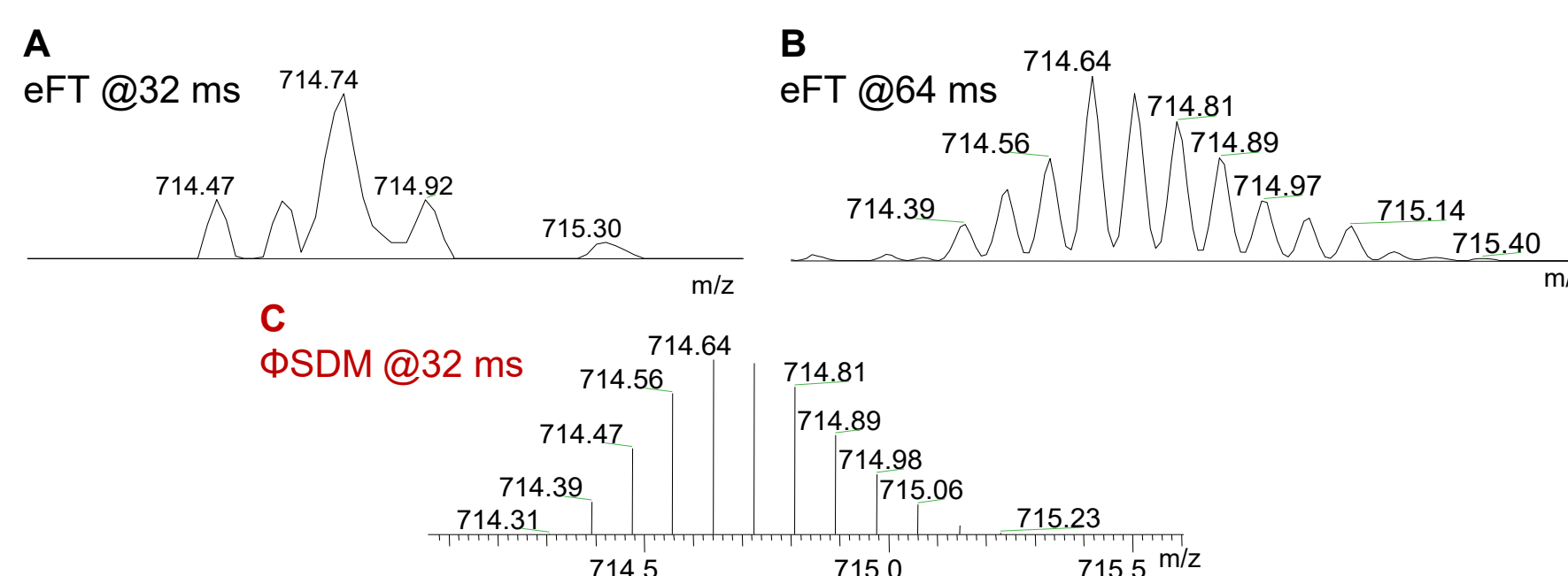
Four full range mass spectra were processed in parallel with a CUDA C++ implementation of the  $\Phi$ SDM algorithm on an auxiliary computer equipped with Nvidia TITAN X GPU cards.

### Data Analysis

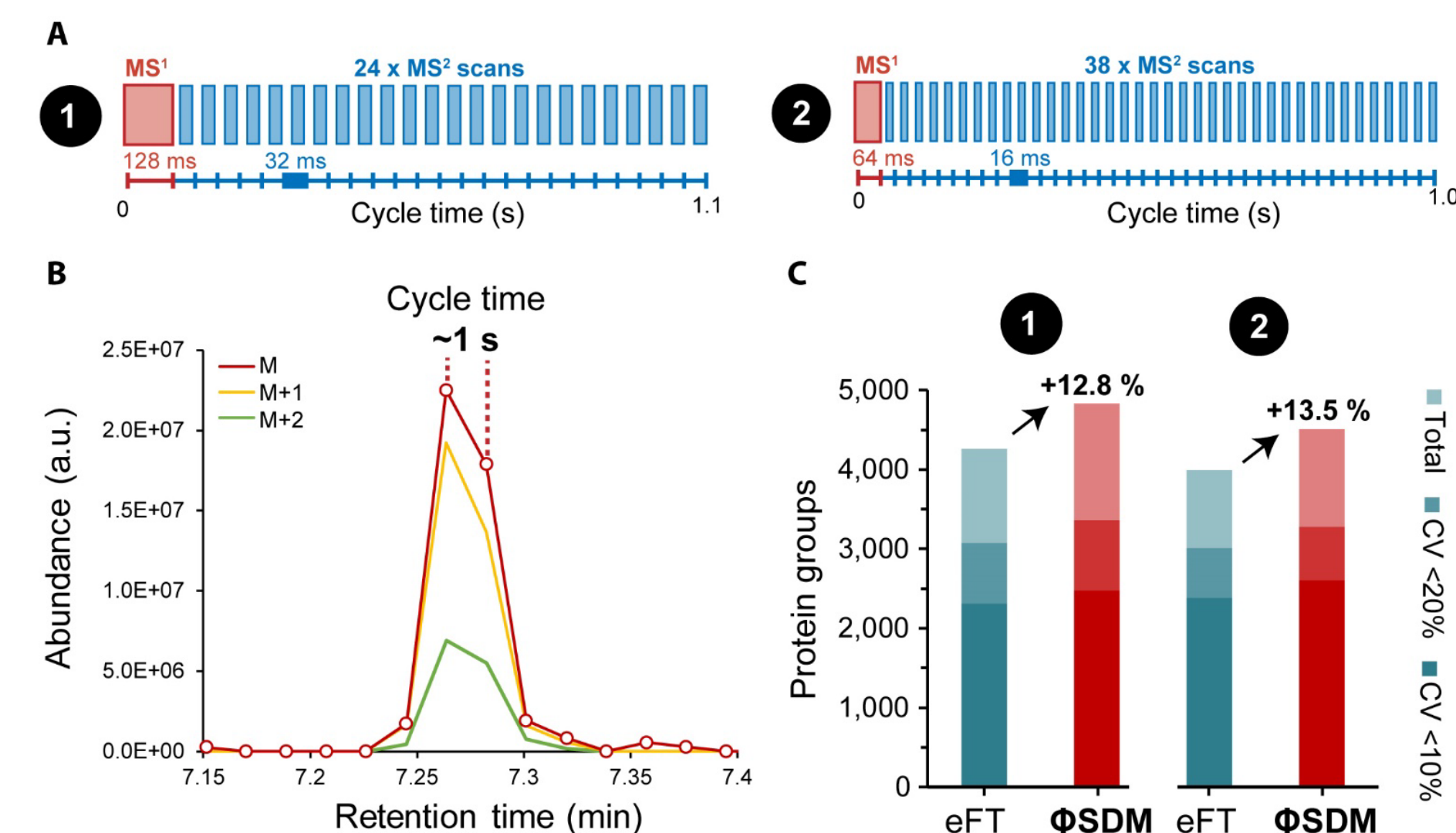
All data were analyzed with Spectronaut<sup>TM</sup> software (Biognosys).

## RESULTS

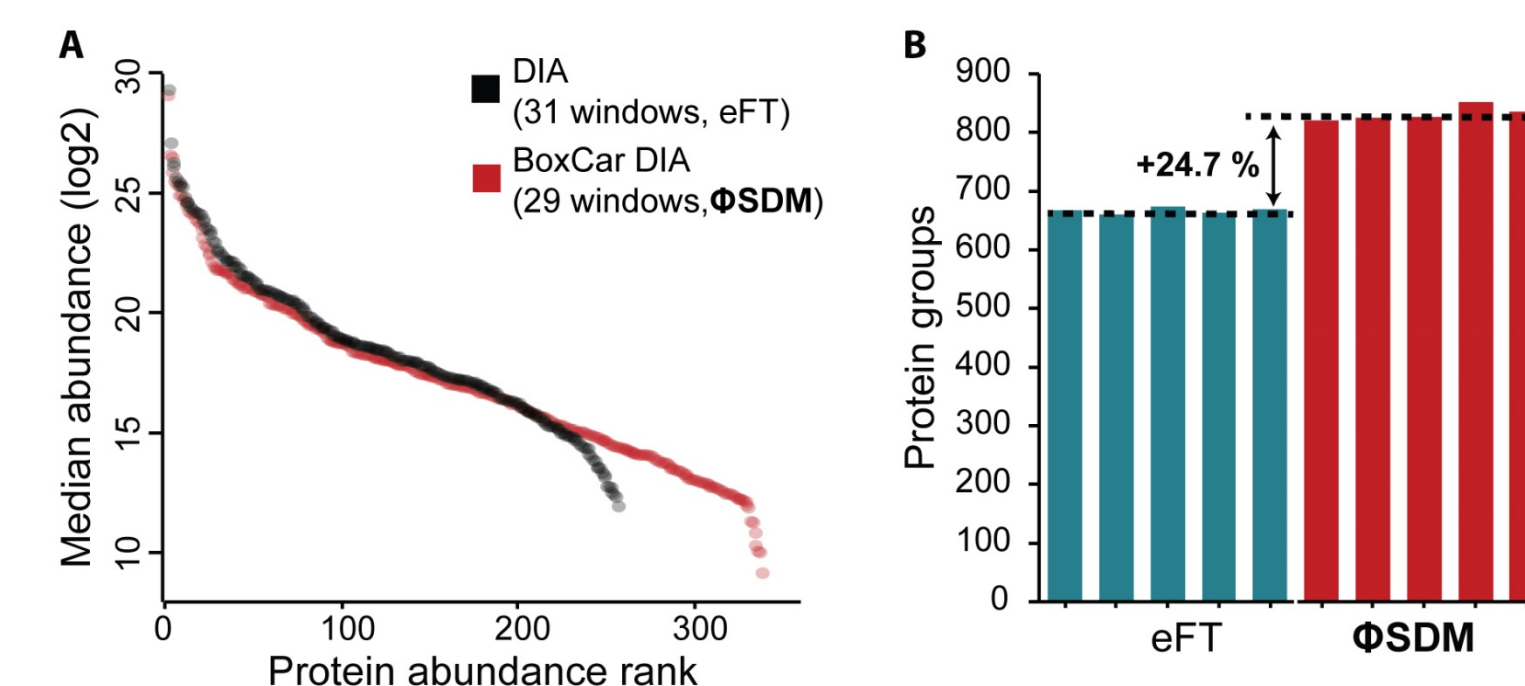
**Figure 1.** Real-time Full MS spectra of charge state 12+ of ubiquitin with **A, B** enhanced Fourier Transformation (eFT, 32 ms and 64 ms transients) and **C**  $\Phi$ SDM (32 ms transient) signal processing.



**Figure 2.** Comparison of eFT and  $\Phi$ SDM signal processing for DIA of a HeLa proteome digest with fast cycle times (**A, B**). Triplicates with the 100 samples/day method on an Evosep One LC coupled to a Thermo Scientific<sup>TM</sup> Orbitrap Exploris<sup>TM</sup> MS 480 with FAIMS (CV -45 V) (**C**).



**Figure 3.** Rapid DIA analysis of human blood samples (30 samples/day). **A**, Dynamic range coverage in human plasma. **B**, Proteins quantified in five whole blood samples with BoxCar [3] DIA using eFT or  $\Phi$ SDM signal processing.



## CONCLUSIONS

Super-resolution Orbitrap mass spectrometry has a great potential for proteomics research with its increasing demand for high-throughput technologies.

## REFERENCES & ACKNOWLEDGEMENTS

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## TRADEMARKS/LICENSING

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