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Robust Label Free Proteomics Performance with New Orbitrap Exploris 240 with Improved Separation

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INTRODUCTION

LC-MS-based proteomics analysis shown to be a powerful analytical tool for identification and quantification of thousands of proteins in complex biological samples. There has been a need for a robust mass spectrometry system that provide robustness needed to analyze 1000s of samples without compromising on coverage and quantitation performance and provide ease of use for any level of analytical expertise. Here we present a new quadrupole orbitrap hybrid mass spectrometer, Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer, coupled to the High-Field Asymmetric Waveform Ion Mobility Spectrometry System (Thermo Scientific™ FAIMS Pro™ interface) as a robust workflow without compromising on performance. The peptide and protein identification performance in a data-dependent acquisition (DDA) mode on four different instruments across three different sites were evaluated for instrument readiness for large scale, cross laboratories studies. Untargeted label-free quantitation of peptides in different ratio and with mixed proteome showed great quantitation accuracy with the optimized label-free quantitation (LFQ) method template. In summary the new Orbitrap Exploris 240 mass spectrometer has shown great sensitivity and data reproducibility across multiple instrument with the optimized method templates for variety of proteomics applications available on this system providing versality and flexibility to navigate in between different proteomics application in a multidisciplinary proteomics laboratory with ease of use for different level of user's expertise.

MATERIALS AND METHODS

Sample Preparation: Thermo Scientific™ Pierce™ HeLa Protein Digest Standard was dissolved in sample loading buffer containing 2% Acetonitrile in 0.1% TFA and 0.1% FA with 30 second of vortexing and spinning down in concentration range of 5-500 ng/ul and are transferred to an autosampler vial for LC-MS analysis.

Methods: Instrument performance across different laboratories was compared with a 60 min LC-MS method with EASY-nLC 1200 and EASY source and a Thermo Scientific™ 50 cm EASY-Spray™ PepMap™ RSLC C18 column at 250 nL/min flow rate in direct injection mode with 1ug of Pierce HeLa Protein Digest Standard without FAIMS Pro Interface.

For LFQ methods, with HeLa Protein Digest Standard; an EASY-nLC 1200 was used with 25 cm Aurora column (Ionopticks) at 300 nL/min flow rate in direct injection mode, injecting 2ul of sample for total load on column (10-1000ng), following four optimized LC gradients (30, 60, 90 and 120 min), supporting different sample analysis throughput. FAIMS Pro Interface in user defined mode (FAIMS inner electrode temperature of 100°C and outer electrode temperature of 80°C) with 2 compensation voltage (CV) switching of -70 and -50 and Top 66, DDA method.

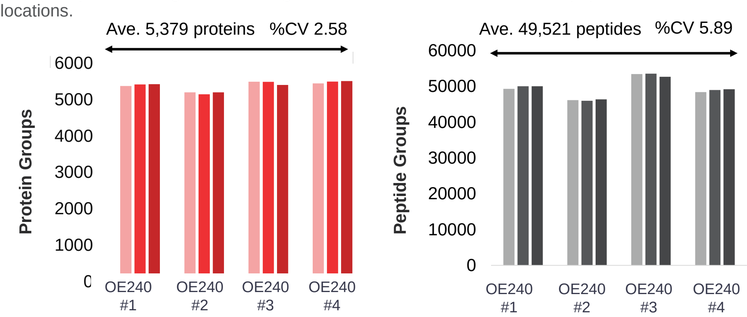
Figure 1.LC-MS setup for cross laboratory performance evaluation and HeLa curve Study.

Data Analysis: The raw files were processed using Thermo Scientific™ Proteome Discoverer™ 2.4 software with a 4-stage Search including, 3-stage SEQUEST search parameter including tryptic and semi tryptic and PTMs in addition to 1-stage MSPep Search against NIST HCD MS^2 spectral library with percolator used between each search to calculate the false discovery rate (FDR) and only report those spectra with q-values lower than 0.01.

RESULTS

To evaluate instrument reproducibility, four different mass spectrometer operators with different level of expertise at three different locations on four different instruments analyzed 1 µg of Thermo Scientific™ Pierce™ HeLa Protein Digest Standard (n=3). The Orbitrap Exploris 280 mass spectrometer data provided high reliability across instruments, a mandatory requirement for large-scale, cross-laboratory studies.

Figure 1. Orbitrap Exploris 240 MS Standard Benchmark. Instrument performance across different sites were evaluated with 1 µg of Pierce Hela Digest; in 60 min LC gradient and Top20 Data Dependent (DDA) MS data acquisition. Left figure indicates the number of proteins identified at 1% false discovery rate (FDR) and the figure on the right shows the number of identified peptides for each of the four locations.



Advantage of High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS). FAIMS Pro Interface provides separation based on a combination of factors, like charge state, shape, conformation, and size of gas phase ions. It had previously shown to improve dynamic range and peak capacity[1,2].

Figure 2. Increase Peptide and Protein Coverage with FAIMS Pro Interface. Instrument performance were evaluated with (red) and without FAIMS Pro Interface(grey) with 1ug of Pierce Hela Digest in a 60 minutes LC gradient and Top 20 Data Dependent (DDA) data acquisition mode. Improvement in both peptide and protein coverage were observed with 2 CV (-70, -50) fractionation in a 60 min gradient length. A single CV method will need to be optimized based on required peptide or protein coverage improvement.

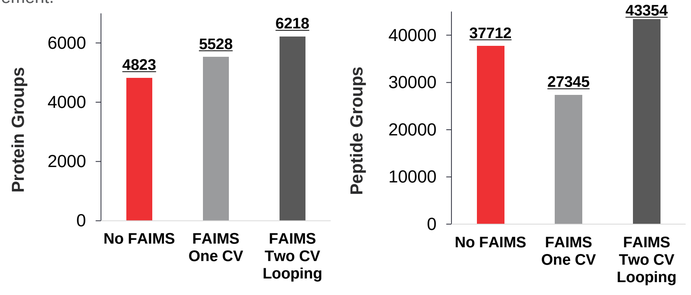


Figure 3. Optimized LFQ Methods with Different Throughput. The Orbitrap Exploris 280 mass spectrometer with the FAIMS Pro interface provides a great peptide and protein coverage from analysis of 200 ng HeLa digest over a 30-120 minutes gradients with two intra-analysis CV steps (–70V and –50V,) approximately 6400 proteins were identified with MS^2 only with 200ng Sample input in 2hrs.

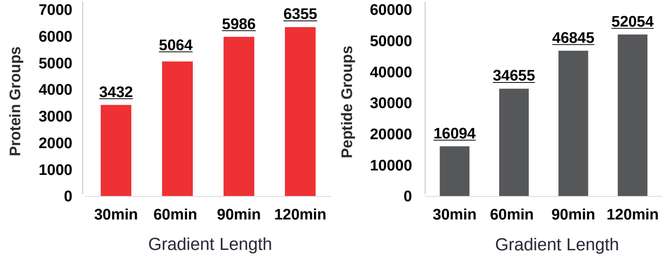


Figure 4. High-performance Peptide and Protein Identification. The Orbitrap Exploris 240 mass spectrometer with the FAIMS Pro interface provides maximum coverage for analysis of a wide range sample input 10-1000 ng HeLa digest) over 90 minutes gradient with multiple intra-analysis CV steps (–70V and –50V,) on average approximately 6700 proteins were identified with MS2 only with 1ug HeLa Digest.

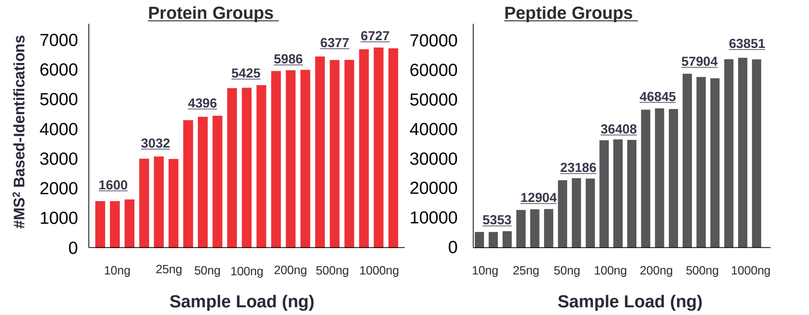
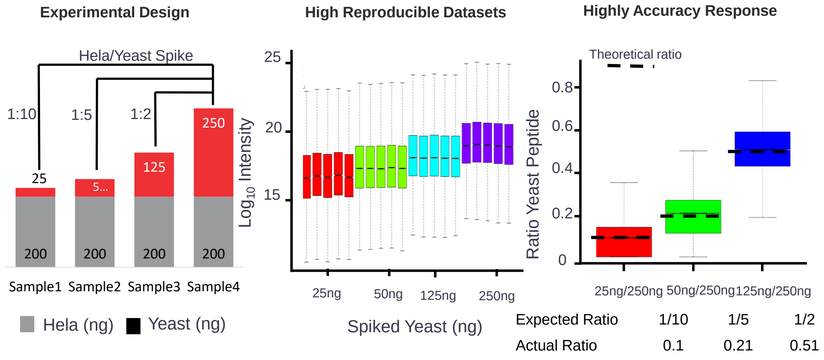


Figure 5. Label-Free Quantitation Accuracy. The Orbitrap Exploris 240 mass spectrometer provides sensitivity from low-ng to micrograms of sample inputs with exceptional quantitation accuracy. The mixed proteome (Left)) label-free DDA analysis shows great replicate reproducibly (middle) and quantitation accuracy across 1:10 ratio (right), instrument quantitative accuracy across a wide range of 1:100 ratio was also evaluated, data shown at ASMS Exploris 240 Mass spectrometer presentation.



CONCLUSIONS

- The Orbitrap Exploris 240 mass spectrometer defines next-generation performance for protein and peptide identifications and label-free quantitation in its class with very high reliability across instruments and multiple sites, a mandatory requirement for large-scale studies.
- The FAIMS Pro interface together with the Orbitrap Exploris 240 mass spectrometer provides high selectivity and sensitivity required to dig into complex proteome analysis.
- The easy to use optimized method templates for variety of proteomics applications, including gel spots and IP (Immuno-Precipitation) identification, LFQ methods beside TMT multiplexing with and targeted PRM method templates and provide versatility for a multidisciplinary core labs with variety of proteomics applications to support.

REFERENCES

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2. Schweppe D.K. et. al. Anal. Chem. 91,6, 4010-4016 (2019)

TRADEMARKS/LICENSING

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