

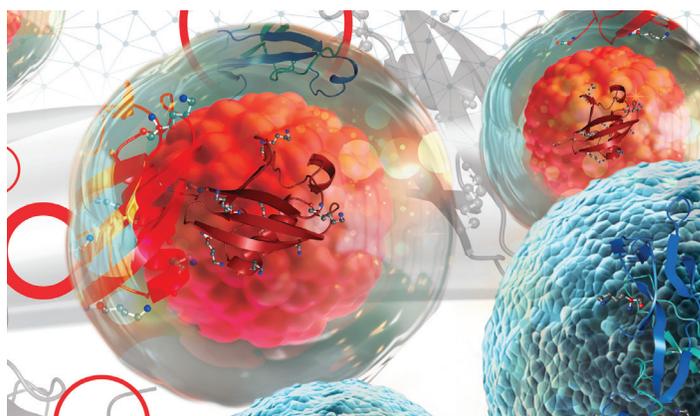
Label-free proteomics performance with the Orbitrap Exploris 480 mass spectrometer with single-cell sensitivity

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Abstract

LC-MS-based proteomics analysis has shown to be a powerful analytical tool for identification and quantification of thousands of proteins in complex biological samples. Moving forward from discovery to targeted quantitation in proteomics, there is a need for a robust mass spectrometry system and methods that provide reproducibility needed to analyze thousands of samples without compromising on coverage and quantitation performance with ease of use for any level of analytical expertise. Here we present the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer coupled to the Thermo Scientific™ FAIMS Pro™ Interface, a high-field asymmetric waveform ion mobility spectrometry (FAIMS) system for proteomics applications. The performance



of this small benchtop mass spectrometer is evaluated in data-dependent acquisition (DDA) mode for sample injection amounts of just a single HeLa cell up to 5000 HeLa cells (~1 ug), providing the sensitivity and performance required for analysis of large numbers of samples without compromising on sensitivity and proteome coverage. To demonstrate the sensitivity of the instrument, we analyzed proteins from a single HeLa cell, as well as bulk digest equal to single-cell protein levels. This instrument sensitivity enables identification of ~7000 protein groups from only a 200 ng of bulk HeLa digest and ~800 protein groups from a single HeLa cell in a 2 hour LC gradient LC-MS analysis. The method performance at 1 ug sample injection was also evaluated across different instruments located in different laboratories.

Materials and methods

Sample preparation

Single HeLa cells were isolated and processed on the nanoPOTS (Nanodroplet Processing in One-pot for Trace Samples) platform¹. 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 seconds of vortexing and spinning down in the concentration range of 0.5-500 ng/μl and was 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 a Thermo Scientific™ EASY-nLC™ 1200 LC system, a Thermo Scientific™ EASY-Spray™ source and a 50 cm Thermo Scientific™ Acclaim™ PepMap™ RSLC C18 column at 250 nL/min flow rate in direct injection mode with 1 μg of HeLa Protein Digest Standard without the FAIMS Pro interface.

For HeLa Protein Digest Standard work, an EASY-nLC 1200 LC system was used with a 25 cm Aurora column (IonOpticks) at 300 nL/min flow rate in direct injection mode, injecting 2 μl of sample for total load on column (10-1000 ng), following four optimized LC gradients (30, 60, 90 and 120 min), supporting different sample analysis throughput.

Single HeLa cell tryptic digest (200 nL total volume) and single-cell level HeLa digest (0.5 - 2 ng) were individually transferred to a short (4 cm) capillary tube and peptides were loaded to a 5 cm solid phase extraction (SPE) trap and analytical column (20 μm i.d, 3 μ, 50 cm) from CoAnn Technologies for peptide trapping with minimum sample loss, followed by analytical peptide separation on a Thermo Scientific™ UltiMate™ 3000 RSLCnano system coupled to a PRSO-V2 Sonation column oven (Sonation GmbH) and an Orbitrap Exploris 480 MS with the FAIMS Pro interface in standard resolution mode. The ultra-low nanoLC flow rate of 20 nL/min for single-cell analysis was achieved through split flow set up¹.



Figure 1. LC-MS setup for cross-laboratory performance evaluation and sample load study

Data analysis

For single-cell data analysis, the raw files were processed using Thermo Scientific™ Proteome Discoverer™ 2.4 software with 2-stage SEQUEST search parameters including tryptic and semi tryptic search, and Percolator was used between each search to calculate the false discovery rate (FDR). Only those spectra with q-values lower than 0.01 were sent to the subsequent search filter and MaxQuant software for match between runs to estimate proteins in the blank sample run. Label-free quantitation (LFQ) data from the HeLa Protein Digest Standard runs was analyzed using a 4-stage search consisting on an MSPep Search against the NIST HCD MS² spectral library and three Sequest database searches with a combination of tryptic, semi tryptic and expected amino acid modifications. Each search was analyzed with Percolator and only those spectra with q-values lower than 0.01 were counted as a positive identification.

Results

To evaluate instrument reproducibility, three operators at two different locations on three different instruments analyzed the 1 µg of HeLa Protein Digest Standard (n=3). The Orbitrap Exploris 480 mass spectrometer data provided high reliability across instruments, a mandatory requirement for large-scale, cross-laboratory studies.

Advantage of FAIMS

The FAIMS Pro interface provides separation based on a combination of factors, like charge state, shape, conformation, and size of gas phase ions. It has been previously shown to improve dynamic range and peak capacity.²

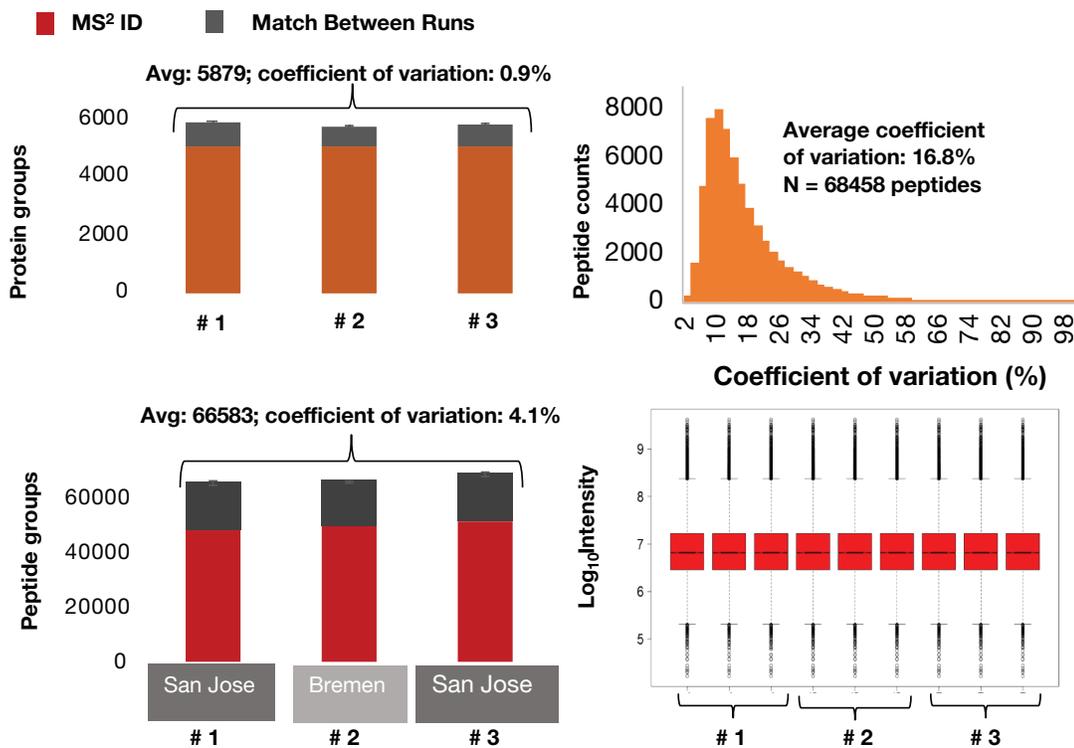


Figure 1. Orbitrap Exploris 480 MS standard benchmark. Instrument performance across different sites was evaluated with 1 µg of HeLa Protein Digest Standard, in 60 min LC gradient and Top20 DDA MS data acquisition. The top left figure indicates the number of proteins identified at 1% FDR, and the lower left figure shows the number of identified peptides for each of the three locations.

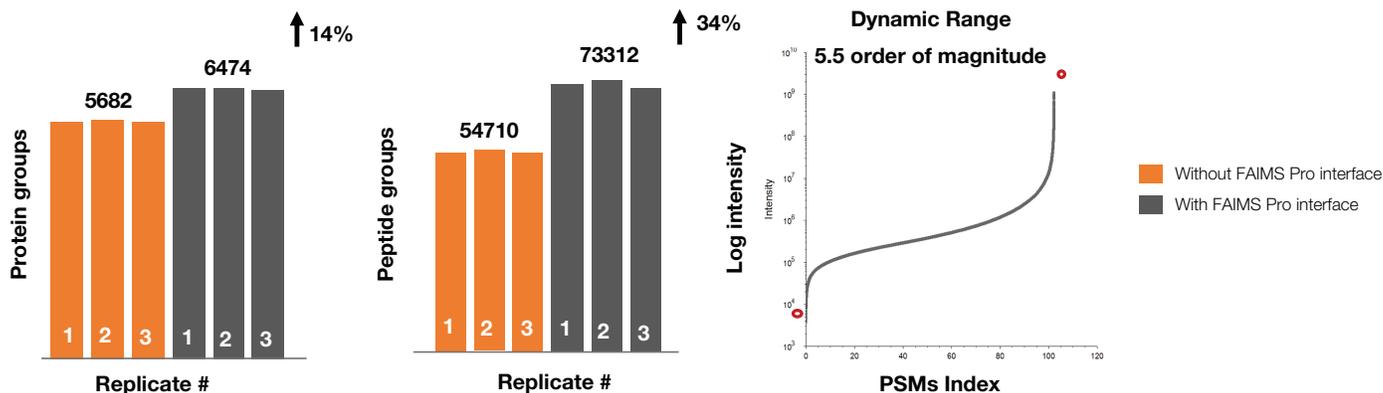


Figure 2. Increase peptide and protein coverage. Instrument performance was evaluated with (grey) and without the FAIMS Pro Interface (orange) using 200 ng of HeLa Protein Digest Standard with a 120 min LC gradient and Top 66 DDA mode. Improvements in both peptide and protein coverage were observed with the FAIMS Pro interface with 5.5 order of magnitude dynamic range (right).

Table 1. Replicate reproducibility. Instrument performance at 10 ng sample load with the same LC-MS method used for 200 ng sample load without any further method optimization was evaluated. The data generated showed great reproducibility and low coefficient of variation across replicate analysis at 10 ng as well as the higher sample load.

	10 ng HeLa Digest		200 ng HeLa Digest	
	Mean (n=3)	Coefficient of variation (%)	Mean (n=3)	Coefficient of variation (%)
Protein groups	2937	1.74	6703	0.90
Peptide groups	13224	2.44	64311	2.00
PSMs	17388	2.09	99780	1.96
MS/MS	83396	0.66	169082	0.49

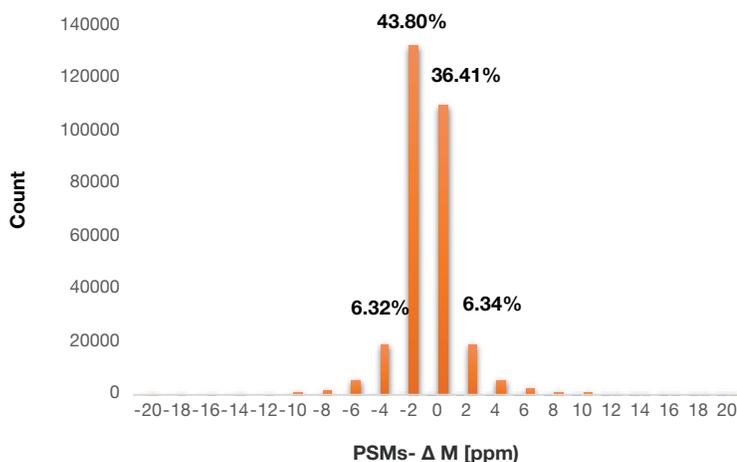


Figure 3. Mass accuracy with external calibration. Reliable mass accuracy of < 3 ppm was achieved for ~90% PSMs across a wide range of sample load and gradients for two weeks with external mass calibration.

Protein Groups

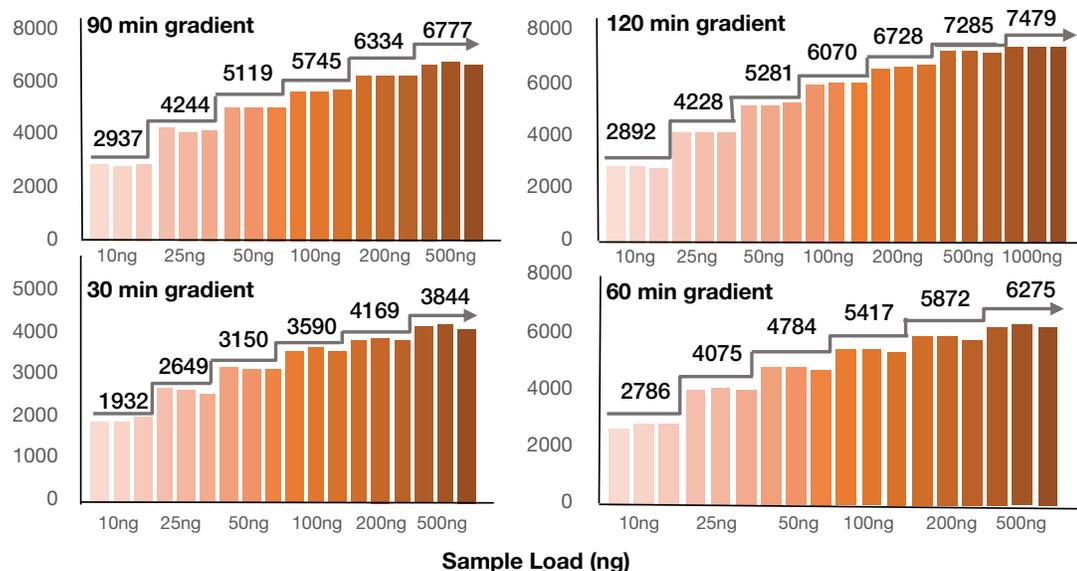


Figure 4. High-performance peptide and protein identification. The Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface provides maximum coverage for the analysis of wide range of sample inputs (10-500 ng HeLa digest) over 30-120 minutes gradients with multiple intra-analysis CV steps (-70V and -50V). Approximately 6700 proteins were identified with MS² using only a 200 ng sample input.

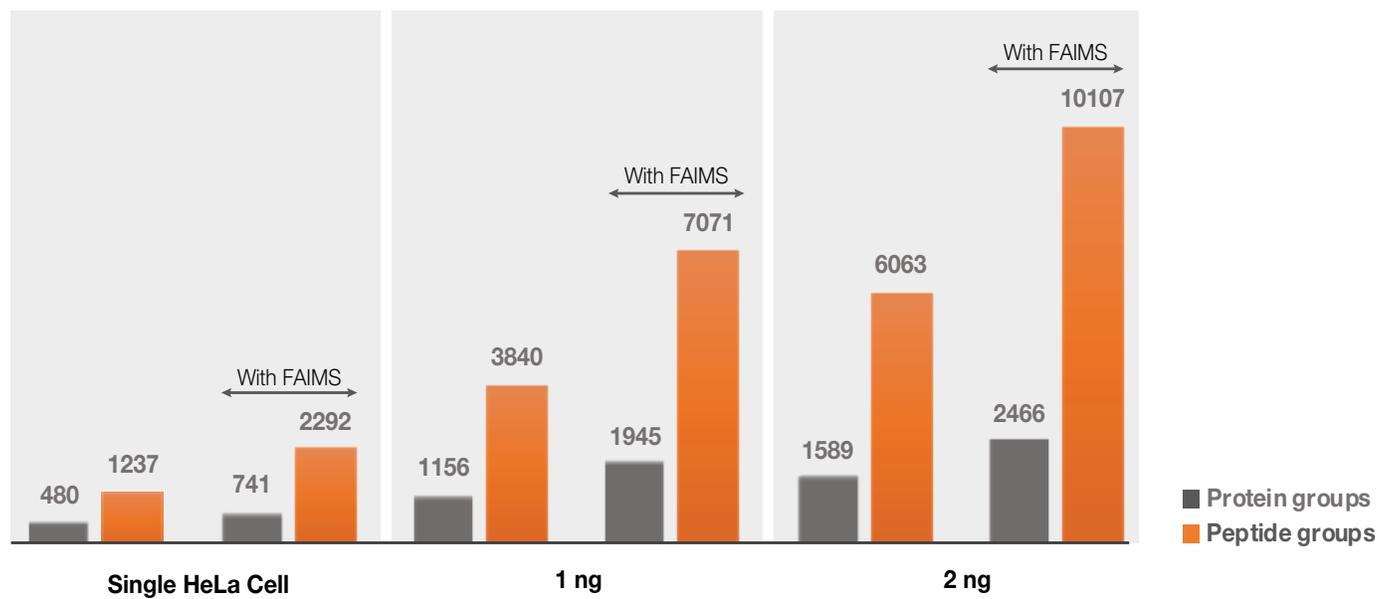


Figure 5. Sensitivity provided at low nanogram level. The Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface provides sensitivity at a low nanogram to single-cell level (~0.2 ng/single HeLa cell). The instrument peptide ID (grey) and protein ID performance (orange) with and without the FAIMS Pro interface is shown at low nanogram levels of HeLa Digest Protein Standard.

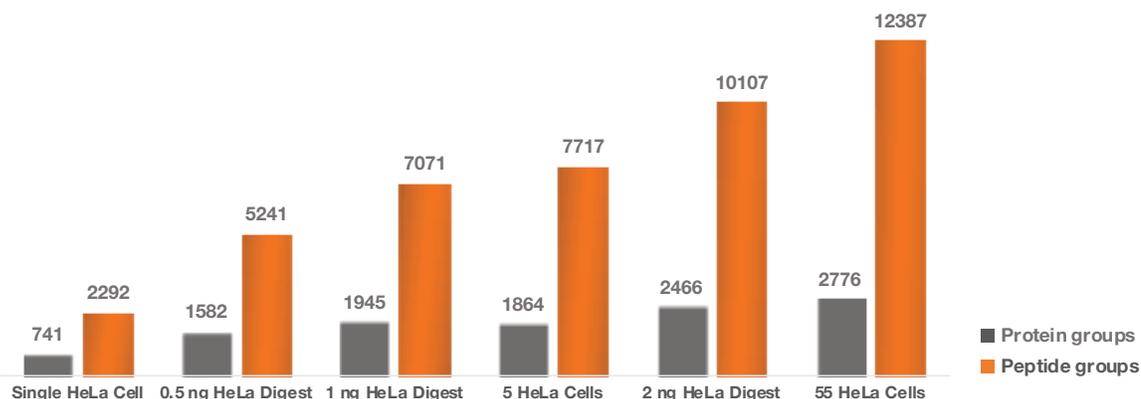


Figure 6. Sensitivity provided for single-cell proteomics analysis. The Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface provides sensitivity from low-ng to single-cell levels. The instrument peptide ID (grey) and protein ID performance (orange) is shown at low nanogram levels (0.5-2 ng HeLa Digest Protein Standard), and at single-cell levels (1, 5, and 55 HeLa cells).

Conclusions

The FAIMS Pro interface coupled to the Orbitrap Exploris 480 mass spectrometer with enhanced selectivity and sensitivity enables digging deeper into the proteome in a variety of experimental conditions. Significant performance enhancements have been demonstrated for different sample amount inputs ranging from one single cell (~0.2 ng) up to 1000 ng of HeLa digest in a variety of chromatography gradients over 30 to 120 minutes. Approximately 6700 proteins were identified with MS² with only 200 ng sample input and ~7500 peptide groups from 1 ng of HeLa digest in a 2-hour single injection analysis without the need for peptide liquid chromatography fractionation to achieve such wide dynamic range and protein coverage.

Approximately 750 proteins were identified with MS² with only a single nanoPOTS digested HeLa cell and ~2000 protein groups from 1 ng of HeLa digest in 2 hours. The ultra-low nanoflow chromatography on the 50 cm, 20 μm ID column¹ with the FAIMS Pro interface has proven to provide the sensitivity required for analysis of low-ng to single-cell.

In summary, the Orbitrap Exploris 480 mass spectrometer defines next-generation performance for protein and peptide identifications in its class with very high reliability across instruments and sites, an essential requirement for large-scale studies without compromising on sensitivity and performance.

References

1. Kelly, R.T. et. al. Anal. Chem. 92,3, 2665-2671 (2020)
2. Sibylle Pfammatter et al. Molecular & Cellular Proteomics July 14 (2018)

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