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Label-Free Proteomics Performance with New Orbitrap Exploris 480 Mass Spectrometer with Single-Cell Sensitivity

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Abstract

Purpose

Evaluation of the label-free proteomics performance on Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer with Thermo Scientific™ FAIMS Pro™ interface with data dependent (DDA) label-free method with range of sample like just a single HeLa cell up to 5000 HeLa cells.

Methods

Instrument performance across different laboratories was compared with a 60 min LC-MS method with Thermo Scientific™ EASY-nLC™ 1200 system and Thermo Scientific™ EASY-Spray™ Source and a Thermo Scientific™ EASY-Spray™ Column (PepMap RSLC C18) at 250 nL/min flow rate in direct injection mode with 1ug of Thermo Scientific™ Pierce™ HeLa Protein Digest Standard without FAIMS Pro Interface. Single HeLa cells isolated via fluorescence-activated cell sorting, processed on Nanodroplet Processing in One Pot for Trace Samples (nanoPOTS) platform and Pierce HeLa digest in a range of 0.2-1000ng were analyzed to evaluate instrument sensitivity on either the Thermo Scientific™ UltiMate™ 3000 RSLCnano System (single cells) or EASY-nLC 1200 system coupled to an Orbitrap Exploris 480 MS with FAIMS Pro interface.

Results

The Orbitrap Exploris 480 mass spectrometer with FAIMS Pro interface defines next-generation performance for protein and peptide identifications in its class with very high reliability across instruments and multiple sites, designed for large-scale studies proteomics experiments and sensitivity to analyze single-cell proteomics or other sample limited applications such the analysis of CTCs or Exosomes.

Introduction

Challenge

Robustness and sensitivity required to analyze 1000s of samples in wide variety of proteomics applications like single-cell proteomics with optimized methods, easy to use for any level of analytical expertise.

Methods

Pierce HeLa Protein Digest Standard analyzed with EASY-nLC 1200 system, 25cm Aurora Column (Ionopticks) at 300 nL/min flow rate in direct inject mode with total load on column (0.2-1000 ng). Following four optimized LC gradients (30, 60, 90 and 120 min), for different analysis throughput. FAIMS Pro Interface was used in user defined mode with two compensation voltage (CV) switching of -70 and -50 and top 60, DDA method. Single HeLa cells isolated via fluorescence-activated cell sorting, processed on Nanodroplet Processing in One Pot for Trace Samples (nanoPOTS) platform were analyzed to evaluate instrument sensitivity on UltiMate 3000 RSLCnano system (single cells) coupled to an Orbitrap Exploris 480 MS with FAIMS Pro interface.

New Approach

The Orbitrap Exploris 480 mass spectrometer with FAIMS Pro Interface provide great performance for protein identification and label-free quantitation with high reliability across instruments and multiple sites, a mandatory requirement for large-scale studies. This robust and sensitive system, provides throughput and sensitivity needed for label-free proteomics analysis for wide variety of proteomics application, including single-cell proteomics analysis.

Materials and Methods

Sample Preparation

FACS sorted single HeLa cells were processed on nanoPOTS (nanodroplet Processing in One-pot for Trace Samples) platform. 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 0.5-1000 ng/uL and are transferred to an autosampler vial for LC-MS analysis.

Instrument Cross-Laboratory Performance

Instrument performance across different laboratories was compared with a 60 min LC-MS method with EASY-nLC 1200 system and EASY-Spray Source and EASY-Spray HPLC Columns (ES803A) at 250 nL/min flow rate in direct injection mode with 1ug of Pierce HeLa Protein Digest Standard without FAIMS Pro Interface.

Single-Cell Proteomics Analysis

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 packed 5 cm solid phase extraction (SPE) trap1 and analytical column (20 µm i.d, 3 u, 50cm) with integrated emitter for peptide trapping with minimum sample loss followed by analytical peptide separation on a UltiMate 3000 RSLCnano system coupled to a PRSO-V2 Sonation column oven (sonation lab solutions) and Orbitrap Exploris 480 MS with FAIMS Pro Interface in standard resolution mode.

The ultra low nanoLC flow rate of 20nL/min for single cell analysis was achieved through split flow set up.

Label-Free Quantitation

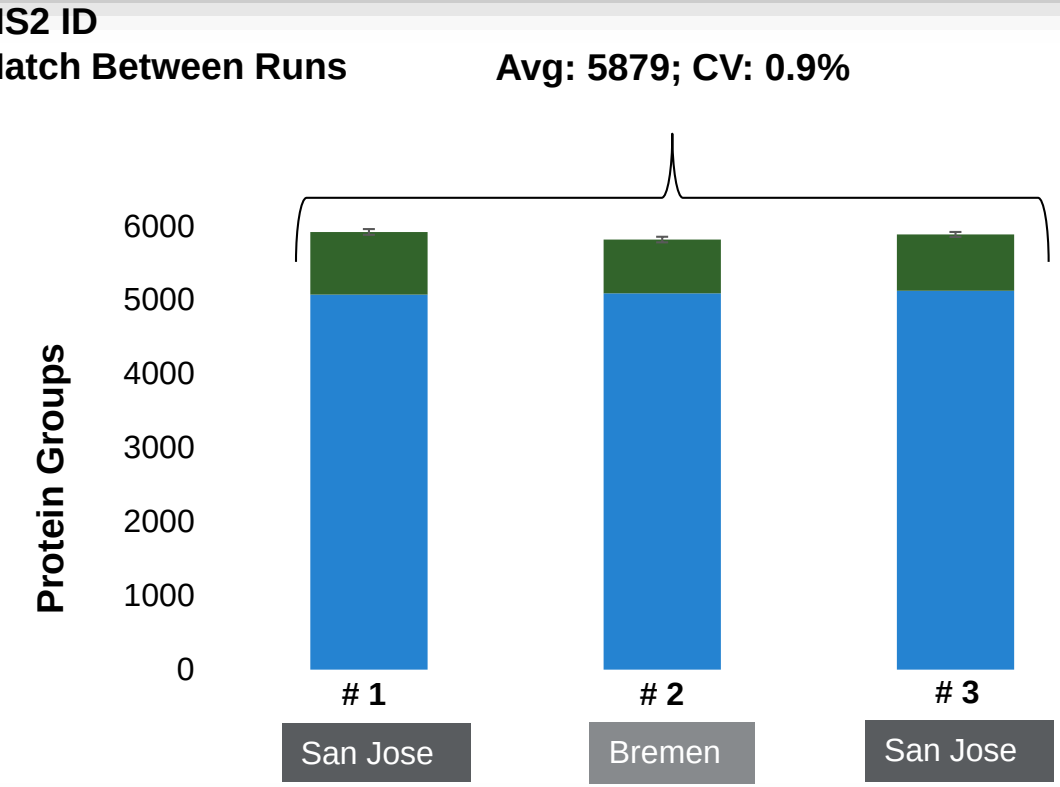
For Pierce HeLa Protein Digest Standard work an EASY-nLC 1200 was used with 25cm Aurora Series emitter column (Ionopticks) at 300 nL/min flow rate in direct injection mode, injecting 2ul of sample for total load on column (10-1000ng), following four optimized LC gradients (30, 60, 90 and 120 min), supporting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

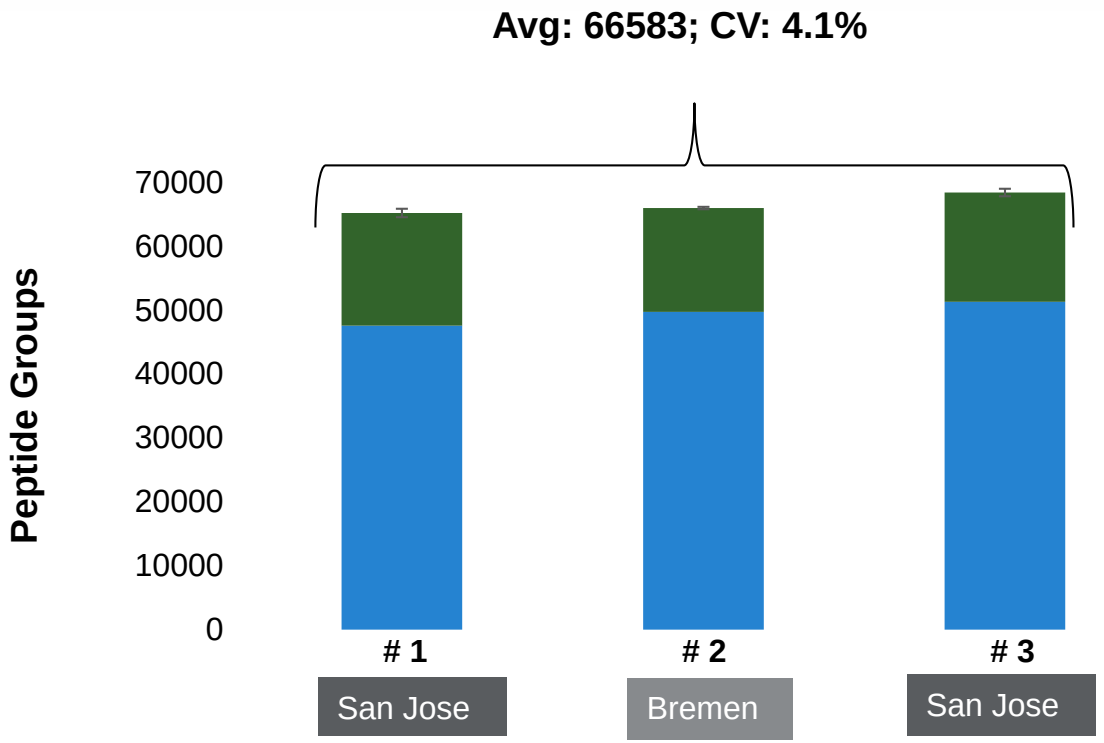
Data Analysis

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.

Orbitrap Exploris 480 MS Standard Performance Benchmark

Evaluating instrument performance reproducibility, across instruments and different sites

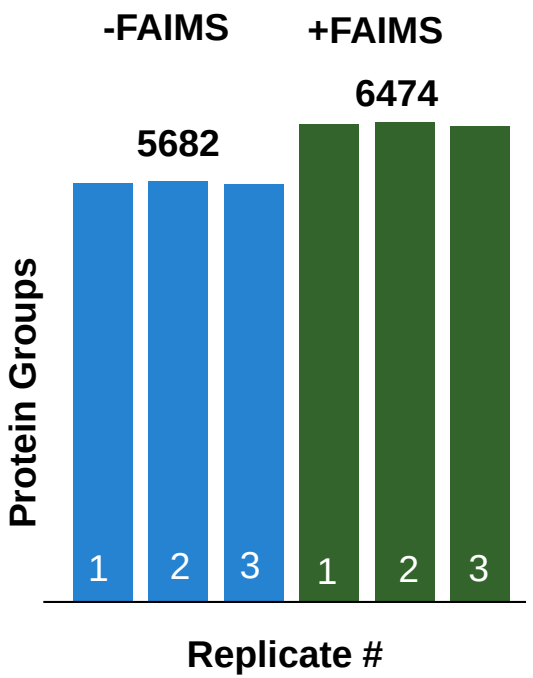


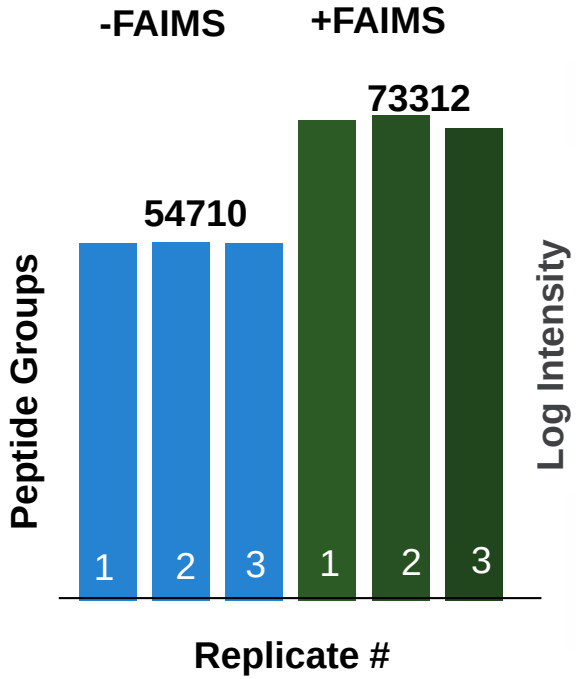


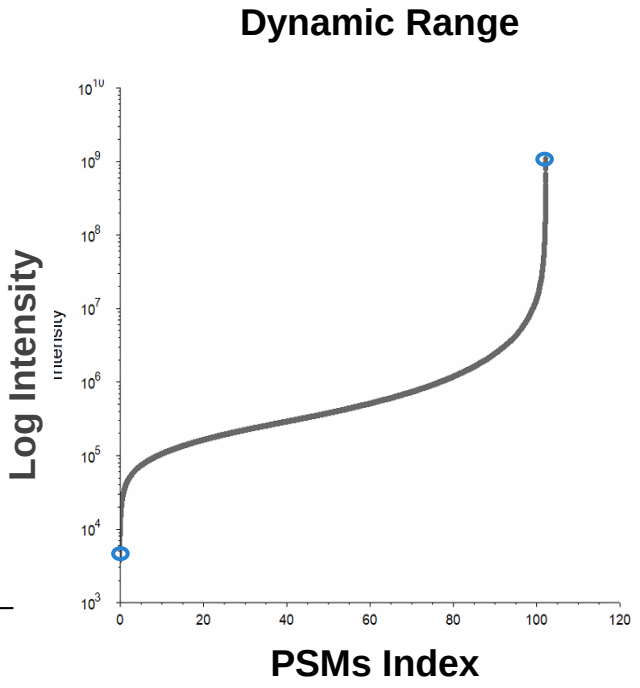
Instrument performance across different sites were evaluated with 1 µg of Pierce Hela Digest Standard; in 60 min LC gradient and Top20 Data Dependent (DDA) MS data acquisition. Left figure indicates the number of proteins identified at 1% false discovery rate (FDR) and the figure on the right shows the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

Optimized LFQ Methods with and without FAIMS Pro Interface

- The Orbitrap Exploris 480 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).
- Unique Protein and Peptide coverage and dynamic range covered in 120min gradient is shown here.
- Approximately 6500 proteins were identified with MS^2 only with 200ng Sample input in 2hrs.







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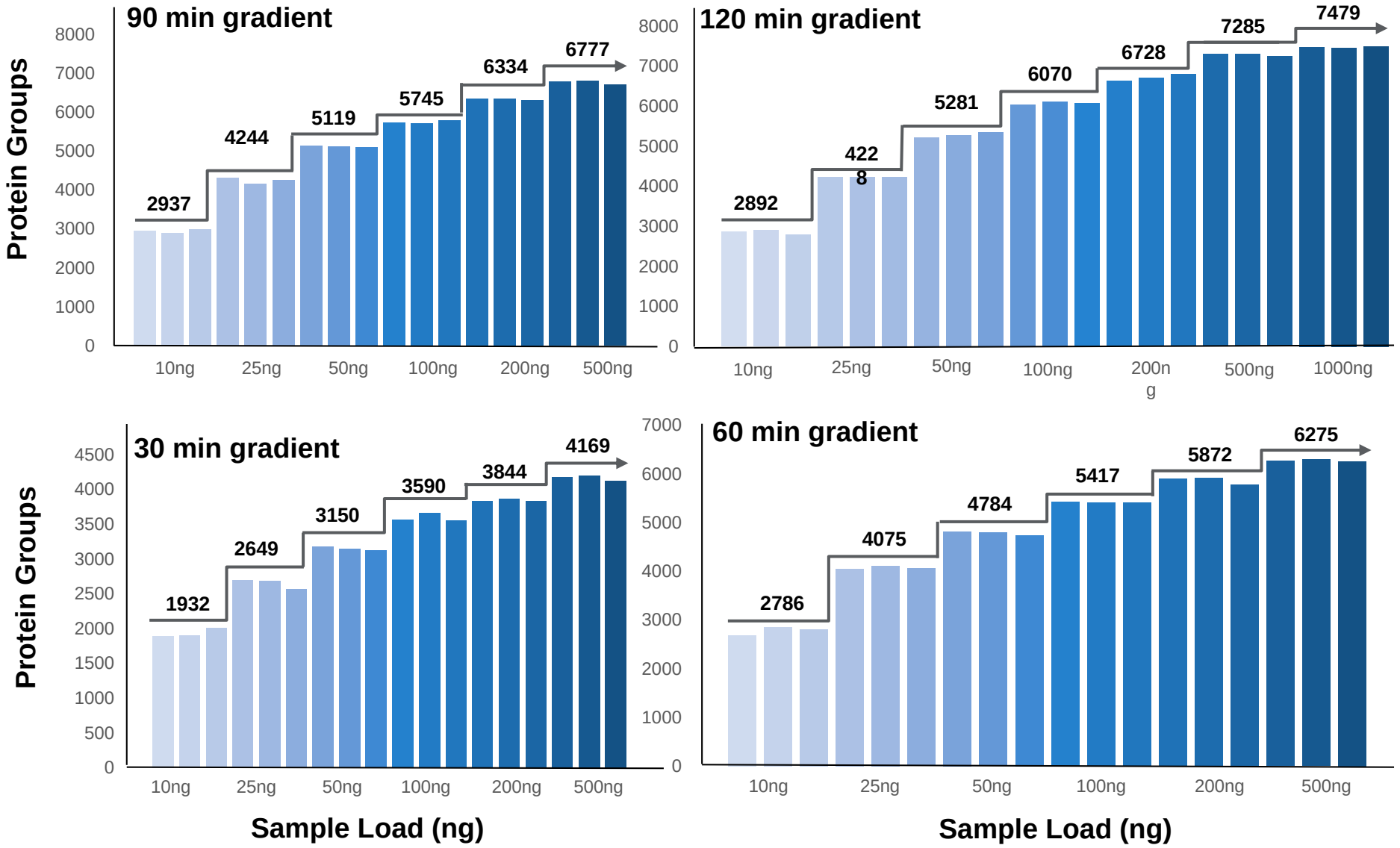
Orbitrap Exploris 480 MS: High-performance Peptide and Protein Identification

- The Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface provides maximum coverage for analysis of a wide range sample input 10-1000 ng HeLa digest) with four different analysis throughput (30-120 min).
- FAIMS Pro interface with multiple intra-analysis CV steps (–70V and –50V,) was used.
- On average approximately 7500 proteins were identified with MS^2 only with 1ug HeLa Digest with 2hrs gradient.



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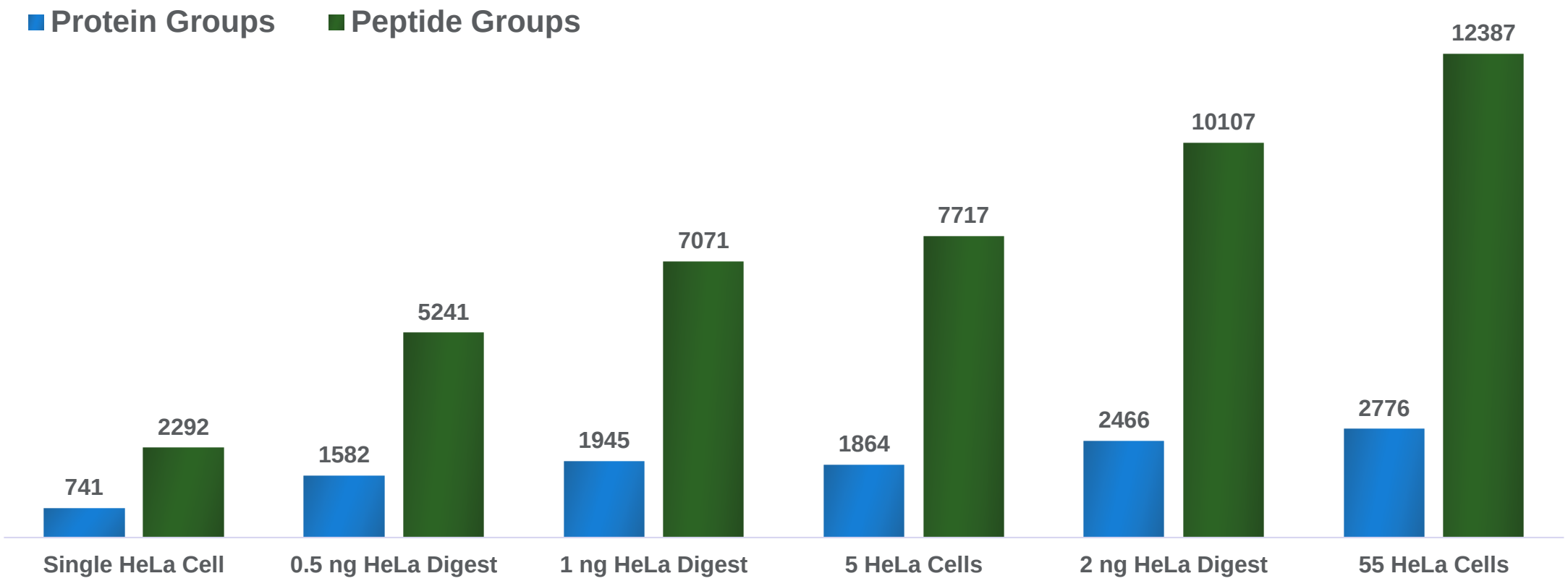
Ultra-sensitivity Provided for Single Cell Proteomics Analysis



The Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface provides sensitivity from low-ng to single-cell levels. The instrument peptide ID (green) and protein ID performance (blue) is shown at low nanogram levels (0.5-2 ng Pierce HeLa Protein Digest Standard), and at single-cell levels (1, 5, and 55 HeLa cell).

Conclusions

- 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, a mandatory requirement for large-scale studies.
- The FAIMS Pro interface together with the Orbitrap Exploris 480 mass spectrometer provides high selectivity and sensitivity required to dig into complex proteome analysis.
- The ultra-low flow workflow with FAIMS Pro Interface delivers sensitivity required for analysis of low-level proteins from rare and individual cells.

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INTRODUCTION

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 1000s of samples without compromising on coverage and quantitation performance with ease of use for any level of analytical expertise. Here we present Orbitrap Exploris 480™ mass spectrometer coupled to the High-Field Asymmetric Waveform Ion Mobility Spectrometry System (FAIMS Pro™ Interface) for proteomics application on this new quadrupole orbitrap hybrid mass spectrometer. The performance of this small benchtop mass spectrometer is evaluated in a data-dependent acquisition (DDA) mode for sample injection amounts of just a single Hela cell up to 5000 HeLa cells (~1ug); providing 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 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 2hr gradient. The method performance was also evaluated across different instruments located in different laboratories.

MATERIALS AND METHODS

Sample Preparation: Single HeLa cells were isolated and processed on nanoPOTS (nanodroplet Processing in One-pot for Trace Samples) platform[1]. 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 0.5-500 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-Spray™ Source and Thermo Scientific™ EASY-Spray™ LC Columns (ES803A) at 250 nL/min flow rate in direct injection mode with 1ug of Pierce HeLa Protein Digest Standard without FAIMS Pro Interface.

For HeLa Protein Digest Standard work an EASY-nLC 1200 was used with 25cm Aurora Series emitter 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.

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 packed 5 cm solid phase extraction (SPE) trap[1] and analytical column (20 µm i.d, 3 u, 50cm) with integrated emitter[1] 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 lab solutions) and Orbitrap Exploris 480 MS with FAIMS Pro Interface in standard resolution mode. The ultra low nanoLC flow rate of 20nL/min for single cell analysis was achieved through split flow set up[1].

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

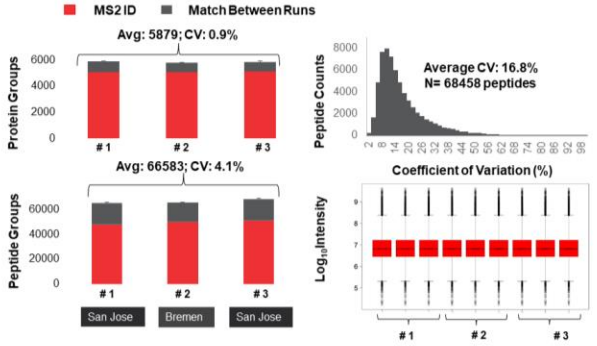
EASY-nLC™ 1200 | Orbitrap Exploris 480™ Mass Spectrometer with FAIMS Pro™ Interface | Proteome Discoverer™ Software 2.4

Data Analysis: Raw single cell and single cell level data files were processed using Thermo Scientific™ Proteome Discoverer™ 2.4 software with 2-stage SEQUEST search parameter including tryptic and semi tryptic search and percolator was used between each search to calculate the false discovery rate (FDR) and 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. For HeLa Protein Digest Standard data, 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, three operators at two different locations on three different instruments analyzed 1 µg of Thermo Scientific Pierce 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.

Figure 1. Orbitrap Exploris 480 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. Top left figure indicates the number of proteins identified at 1% false discovery rate (FDR) and the lower left figure shows the number of identified peptides for each of the three 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[3].

Figure 2. Increase peptide and protein coverage. Instrument performance were evaluated with (grey) and without FAIMS Pro Interface(red) with 200ng of Pierce Hela Digest in a 120 minutes LC gradient and Top 66 Data Dependent (DDA) data acquisition mode. Improvement in both peptide and protein coverage were observed with FAIMS Pro interface with 5.5 order of magnitude dynamic range (right).

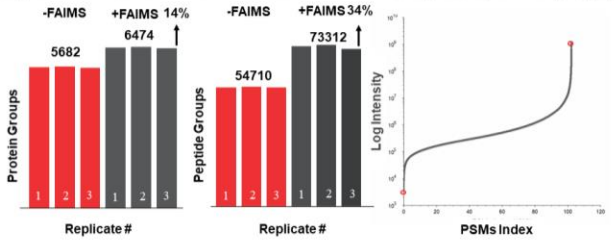
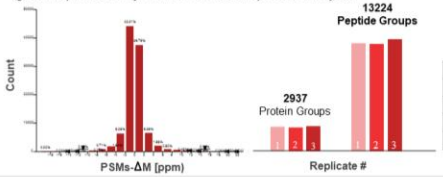


Figure 3. Replicate Reproducibility. Instrument performance at 10ng with same LC-MS method without any further method optimization was as reproducible as higher sample load. Reliable mass accuracy of < 3 ppm was achieved for ~90% PSMs across wide range of sample load and gradients with great reproducibility and CV across replicate analysis.



10ng HeLa Digest	Mean (n=3)	CV (%)
Protein Groups	2,937	1.74
Peptide Groups	13,224	2.44
PSMs	17,388	2.09
MS/MS	83,398	0.66

Figure 4. High-performance Peptide and Protein Identification. The Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface provides maximum coverage for analysis of a wide range sample input 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 MS^2 only with 200ng Sample input.

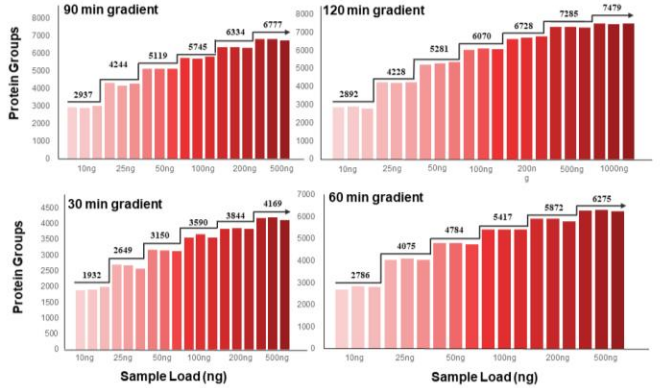
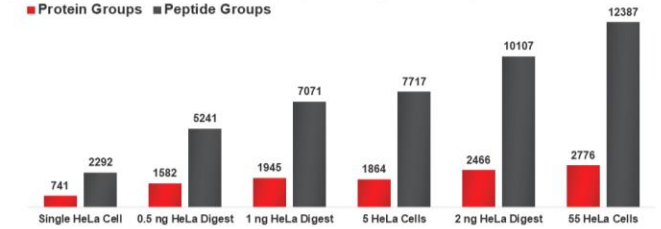


Figure 5. Ultra-sensitivity Provided for Single Cell Proteomics Analysis. The Orbitrap Exploris 480 mass spectrometer with the FAIMS Pro interface provides sensitivity from low-ng to single-cell levels. The instrument peptide ID (grey) and protein ID performance (red) is shown at low nanogram levels (0.5-2 ng Pierce HeLa Digest Protein Standard), and at single-cell levels (1, 5, and 55 HeLa cell).



CONCLUSIONS

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REFERENCES

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