proteomics

thermoscientific

Proof of performance

Orbitrap Exploris 240 mass spectrometer

Differential ion mobility–maximizing proteome coverage, selectivity, and sensitivity with FAIMS technology

Summary

This document presents data that demonstrates the superior selectivity, sensitivity and greater proteome profiling of the Thermo Scientific™ FAIMS Pro™ interface on the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer.

Unlike drift tube or trapped ion mobility, the true value of FAIMS (high-field asymmetric waveform ion mobility spectrometry) technology comes from its role as an orthogonal filtering device of ions in the gas phase. FAIMS technology can also be tailored towards removing matrix interferences and reducing chemical noise while improving the dynamic range, sensitivity, as well as delivering confident quantitation of the analytes of interest.

In proteomics, gas-phase fractionation enabled by the FAIMS Pro interface increases depth of analysis without extra work, minimizing the time, expense, and variability of offline liquid chromatography (LC) fractionation by carrying out online gas-phase fractionation prior to ion introduction into the mass spectrometer.

1. Peptides are separated based upon their differential ion mobility, which primarily enriches for peptide precursors based upon their charge state. Importantly, for shotgun proteomics experiments, singly charged precursors are often contaminants, whereas using FAIMS technology can enrich for multiply charged precursors[1] that are common for peptides digested with trypsin.
2. Utilization of the FAIMS Pro interface delivers highly reproducible data, enabling accurate quantitation with enhanced sensitivity and selectivity.

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Best-in-class ion mobility: When the highest sensitivity, depth and coverage proteome data is key to obtain results that are actionable, the Orbitrap Exploris 240 MS with the FAIMS Pro interface delivers gas-phase fractionation thereby enabling FAIMS technology to increase unique peptides and proteins identified.

Sample

- Thermo Scientific™ Pierce™ HeLa Protein Digest Standard (Cat # 88329) 200 ng
- Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture (Cat # 88321) 10 fmol

LC method

- 25 cm IonOpticks™ Aurora™ series UHPLC emitter column (250 mm × 75 µm, 1.6 µm particle-integrated emitter)
- Flow rate 300 nL/min
- 30 min gradient
- Mobile phase A: Water/0.1% formic acid (FA), Mobile phase B: 80% acetonitrile (ACN) in 0.1% FA

Time (min)	B%
0	3
1	3
19	19
26	29
31	41
34	95
41	95

Instrumentation

- Thermo Scientific™ EASY-nLC™ 1200 system (Cat # LC140)
- Thermo Scientific™ Nanospray Flex™ ion source (Cat # ES071)
- Sonation Column Oven (PRSO-V2) operating at 40 ˚C
- FAIMS Pro interface (Cat # FMS02) (compensation voltage: -50 V/-70 V)

MS detection

- High-resolution, accurate-mass (HRAM) Orbitrap Exploris 240 mass spectrometer
- Data-dependent acquisition (DDA)

Software

- Thermo Scientific™ Proteome Discoverer™ software, version 2.4 with 1% PSM FDR

Data

Multiply charged ions (predominantly peptides)
Singly charged ions (predominantly chemical noise)

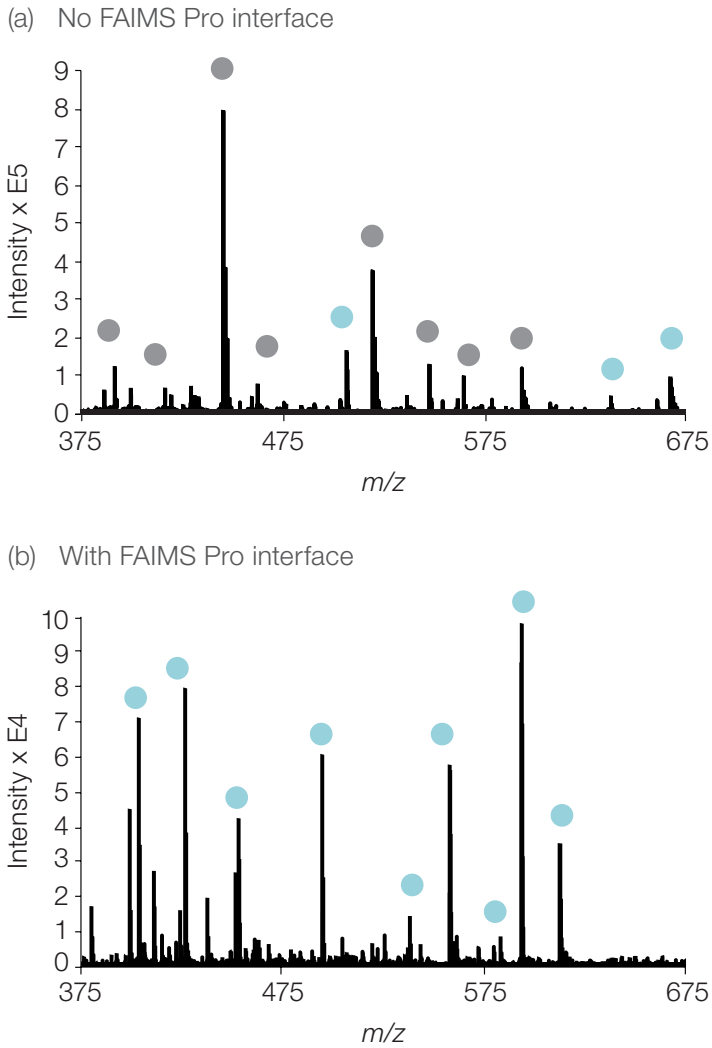

Figure 1. Gas-phase fractionation: FAIMS technology enables filtering out singly charge ions that typically correspond to chemical noise in shotgun proteomics experiments. (a) Proteomics analysis without the FAIMS Pro interface, where the majority of detected ions are +1. (b) Same analysis with the FAIMS Pro interface. All the +1 ions have been removed and the spectrum is populated by multi-charged ion species.

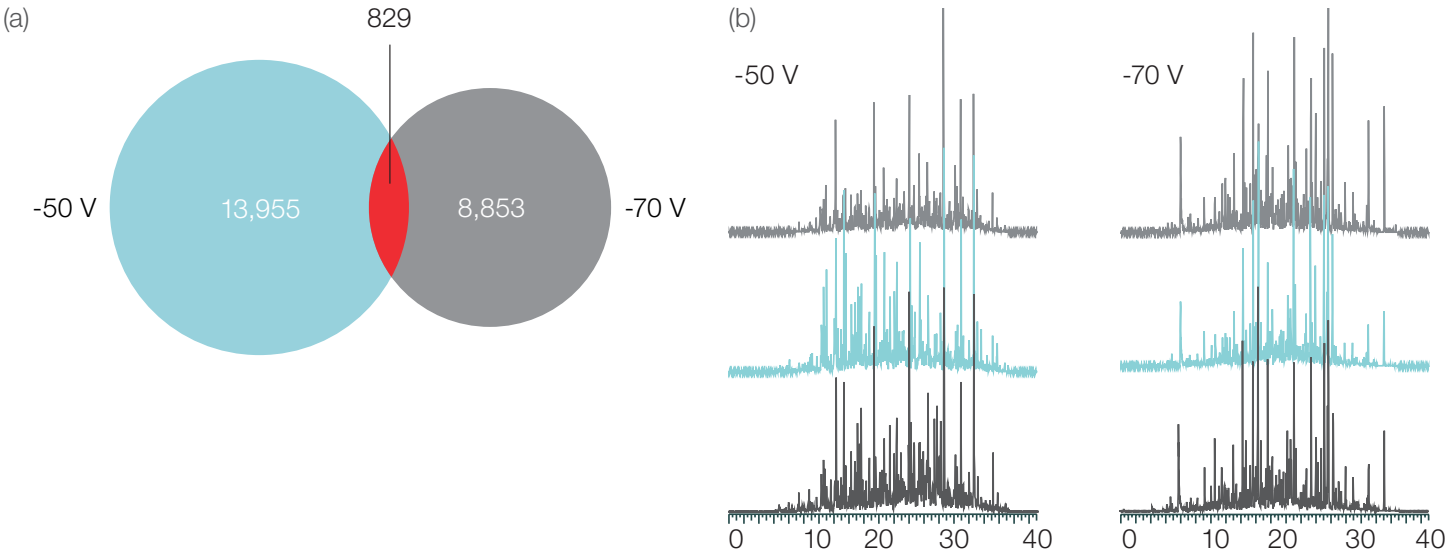

Figure 2. Three replicate injections of 200 ng Pierce HeLa Digest Standard was analyzed on a 30 min gradient, with two different compensation voltages (CVs) applied to the central FAIMS Pro interface electrode. The FAIMS Pro interface and intelligent peptide selection enables 90–95% peptide identification orthogonality. (a) Venn diagram of the identified peptides from a HeLa cell digest highlighting the minimal overlap when two CVs are used in the same analysis. Peptides were separated using a 30 min. gradient. (b) Highly reproducible chromatographic traces within the same CV. However, the peptide traces are significantly different within both CVs, demonstrating the capabilities of the FAIMS Pro interface for sorting out different ion populations in the gas phase.

Results

- The FAIMS Pro interface delivers increased precursor ion selectivity by removing unwanted containments and enriching for peptide precursors of interest based upon differential ion mobility and charge state (Figure 1).
- Reproducible gas-phase fractionation, yielding higher proteome coverage while using less sample than traditional high pH fractionation, has been demonstrated with the Orbitrap Exploris 240 mass spectrometer (Figure 2a & b).
- The combination of the FAIMS Pro Interface with intelligent dynamic exclusion enables almost 100% orthogonality among different compensation values.

Outlook

The FAIMS Pro interface maximizes sample profiling across wide dynamic loading amounts and gradient lengths to increase the productivity of proteomics. It also increases sensitivity and quantitative precision and accuracy, delivering more comprehensive insights that help you understand biology.

Conclusion

Gas-phase fractionation using the FAIMS Pro interface increases the depth and coverage for shotgun proteomics. It effortlessly fits into existing proteomics workflows.

References

1. Pfammatter et al. Molecular & Cellular Proteomics July 14, 2018

Find out more at
thermofisher.com/OrbitrapExploris240Proof

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