Deep Proteomic Coverage Using Fast and Sensitive FAIMS Device Coupled to a Thermo Scientific Orbitrap Fusion Lumos Tribid Mass Spectrometer

Satendra Prasad, Michael W. Belford, Derek Bailey, Joshua A. Silveira, Romain Huguet, Eloy R. Wouters, Jean-Jacques Dunyach; Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA

ABSTRACT

Purpose: Assess FAIMS ion transit time and its impact on Mass Spectrometers (MS) tailored for speed with parallelized mass analyzers (Orbitrap and ion trap) and data acquisition scheme.

Methods: Protein and peptide IDs were monitored for HeLa sample (1µg sample load) over a 2hr LC gradient for 40 ms and 0.1 ms ion transit time.

Results: Shortening the ion transit time from 40 ms to 0.1 ms increased the MS/MS scans, PSM, peptide and protein IDs. Ion transit time adds no time delay to MS/MS acquisition and can be eliminated between CV switches. The CVs can be switched at a rate permissible by the DC power supply. This allowed for several CVs at no performance cost and produced 53000 unique peptides and 6600 proteins on an Orbitrap Fusion (+) APD in a single LC injection.

INTRODUCTION

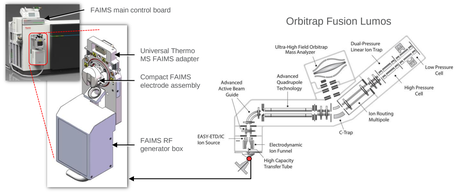
The new FAIMS electrode is improved to deliver speed and sensitivity, making the device well-suited for deep proteomic analysis with parallelized mass analyzers and a rapid MS2 acquisition scheme. Two examples of such MS are the Thermo Scientific™ Orbitrap Fusion™ and Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribid™ MS equipped with Orbitrap and IT mass analyzers that can be operated in parallel. The parallelized analysis and acquisition scheme make the MS popular for high throughput deep proteome analysis. For example, the Orbitrap Fusion Lumos can generate ~250,000 MS2 scans over a 2hr LC-MS/MS analysis of tryptic digest of HeLa and yield >45,000 unique peptide IDs.

However, the ability of the instrument to perform deep analysis on low abundance pre-cursor ions could be challenged by convolving chemical background. Interfacing a FAIMS device can reduce chemical background and likely produce more precursor enriched MS1 survey scan. The ability of FAIMS to enrich multiply charged ions have been understood to be partially dependent on the number of RF cycles the ions experience from the FAIMS ion separation waveform or dispersion voltage (DV). This in turn is dependent on how long the ions take to transit through FAIMS ion separation gap. The ion transit time is governed by the speed of the carrier gas which can vary depending on the conductance of the MS inlet. The transit time is 40 ms and 20 ms for an Orbitrap Fusion (standard 0.58 mm ID capillary) and an Orbitrap Fusion Lumos (0.6 mm × 1.6 mm slot shaped capillary), respectively. It has been understood that the ion transit time acts as a time overhead where the MS is unable to acquire survey scans or MS2 scans because on switch to a new CV, ions experience a 20 or 40 ms transit period. However, the consequence or shortening or eliminating the ion transit time on the quality of the MS1 and MS2 scans have never been explored. In this study, we explore the impact of removing the ion transit time on peptide and protein IDs in a HeLa sample over 1.5hr LC gradient and allow for switching of several CVs on an Orbitrap Fusion and an Orbitrap Lumos.

MATERIALS AND METHODS

Thermo Scientific™ Pierce™ HeLa digest protein standard was used to assess the acquisition speed of FAIMS interfaced to an Orbitrap Fusion and Orbitrap Fusion Lumos Tribid MS. Analysis with the two instruments used APD, a sample load of 1 µg was separated using a Thermo Scientific™ EASY-Spray™ 75 µm × 50 cm (C18, 2µm) column attached to a Thermo Scientific™ EASY-nLC™ 1000 system. A data-dependent method using multiple filter criteria (charge state, monoisotopic *m/z* assignment and dynamic exclusion) for precursors was used. ITMS2 spectra were collected using rapid and turbo scan rate over a range of injection time (10 ms to 35 ms). Compensation Voltages (CV) between -30V and -120V were interrogated to find precursor rich CVs. LC-MS and LC-FAIMS-MS data were searched using Thermo Scientific™ Proteome Discoverer™ 2.1 software.

Figure 1. A schematic showing implementation of FAIMS Pro hardware on an Orbitrap Fusion Lumos mass spectrometer.

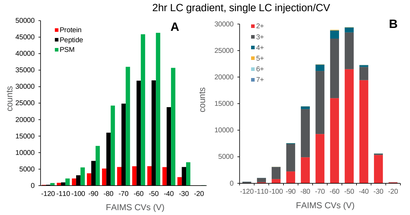


RESULTS

CVs Enriched with Multiply Charged Peptides

Predicting which CVs contain analytically useful information for a DDA experiment can be complicated by the non linear differential ion mobility behavior of peptides in the FAIMS ion separation gap. A one-time benchmark characterization was performed on FAIMS-Fusion Lumos MS by designing a pseudo targeted analysis OT HCD experiment where the mass was centered at *m/z* of 937.5 with a wide isolation window of *m/z* 1150. MS2 parameters were as follows: HCD activation, HCD collision energy (0), Orbitrap @ 60K, normal mass rage, scan range: 350-1550, RF lens 30%, AGC target $5x10^4$, IT 118 ms. MS1→MS2 acquisition were performed for CVs between -30V to -90V in steps of 5V.

Figure 2. Shows the PSM, peptide, and protein ID distribution across the CV range -120V to -20V (A) for a 1µg HeLa tryptic digest sample over a 2hr LC gradient (A). The data were processed to also show charge distribution across the same CV range (B).

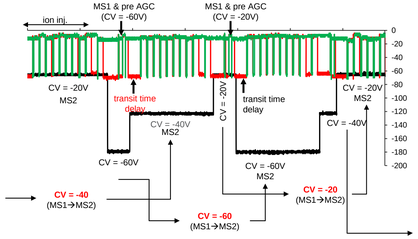


CVs -90V to -40 contained the most PSM counts and yielded the most peptide/protein IDs (left). This correlated with the charge state distribution histogram (right) that showed the same CV range enriched with multiply charged species (2+ to 7+). There was also correlation between charge state and CV bins; for example -50V was dominated by 2+ and -70V I -80V was dominated by 3+. This shows how charge enrichment occurs in FAIMS CVs and helps selection of CVs for a DDA.

FAIMS OTIT Analysis

Figure 3 shows a re-constructed oscilloscope trace of TK1 lens (green), TK2 (red), and FAIMS CV (black) during a DDA experiment where three CVs (-20l-40l-60) were switched internally. Each CV shows two events where in the first event an MS1 and pre-agc scan is acquired and in the second event MS2 are acquired. A CV setting correlating to a MS2 is followed by a delay: FAIMS ion transit time (20 ms). Current understanding is that during this period, the ions are in transit through the FAIMS device and the MS is deprived of an ion beam. Following the delay, a train of ion injection events occur (modulation of TK1/TK2 lens) for MS2 acquisition. Owing to a parallelized acquisition structure the MS1 and MS2 events for a CV are not adjacent in time.

Figure 3. Showing OTIT data acquisition scheme with FAIMS on a Fusion Lumos using a parallelized acquisition scheme between OT and IT mas analyzer.



Understanding FAIMS Function Towards Bottom Up Analysis

Figure 5A shows that although FAIMS acts as an ion filter, the device attenuates only 2 fold precursor ion intensity. This has been a differentiating feature of the new electrodes compared to the legacy electrodes. The ion filtering function is also anticipated to reduce chemical background and this is evident in Figure 5B. The plot shows average precursor purity (ratio of precursor abundance vs interference abundance in a *m/z* bin) against precursor intensity ranked by abundance. FAIMS produces superior peak purity for low abundance precursor.

Figure 5A. Shows difference in MS1 ion intensities without and with FAIMS (1s and 3s cycle time); 5B shows average precursor purity as a function precursor ion abundance. The cycle time was reduced to 1s to accommodate three CVs. For comparison a 3s cycle time was used also with three CVs and as expected less MS2 scans were collected.

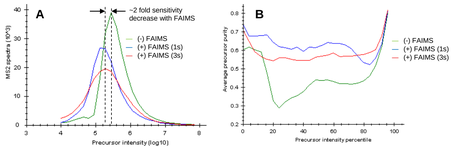


Figure 6A serves as a control plot showing that the median number of isotopic envelope between plot A and B are comparable without FAIMS but with FAIMS nearly 3 fold more precursors are available. This highlights the role and benefit of FAIMS as a gas phase enrichment tool for bottom up analysis.

Figure 6A and B demonstrate the increase in annotated isotopic envelopes or precursors with FAIMS using CV -40l-60l-80 for a 1µg HeLa analysis over a 2hr gradient over no FAIMS.

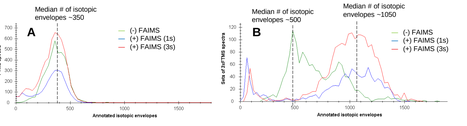
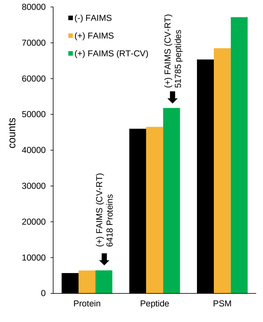


Figure 7. Protein and peptide comparison among (-) FAIMS and two (+) FAIMS when CV-RT involved dynamically adjusting CV as a function of peptide RT. Single LC injection 1µg HeLa, CV -40l-60l-80.



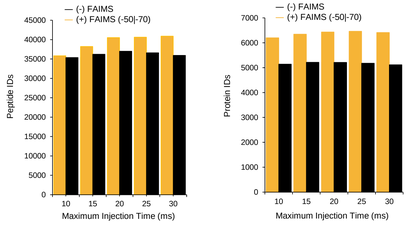
FAIMS-Orbitrap Lumos MS: Unique Peptides and Protein IDs

- (+) FAIMS yielded 6402 proteins and 46517 peptides compared to 5685 proteins and 46000 peptides from (-) FAIMS experiment. The increase in protein ID is seen as significant but the increase in peptide IDs are subtle.
- One explanation could be that select CVs during LC gradient could become redundant and may not pass much peptides.
- This was explored by dynamically adjusting the CVs as a function of retention time and was termed CV-RT.
- CV-RT yield 51785 peptides compared to (-) FAIMS peptide ID (46000). But there was a subtle increase in protein IDs.
- Another way to optimize peptide and protein IDs could involve shortening the ion transit time to reduce the time overhead and include more CVs to granulate the sampling of CV space that pass multiply charged species.

Optimize MS Parameters for CV -50l-70 and 1.5hr LC Gradient

FAIMS ion transit time is the longest (~40ms) when coupled to an Orbitrap Fusion owing to the MS being equipped with a capillary with the least gas conductance among the Tribrid MS fleet. Since the adverse effects of FAIMS ion transit time acting as a time overhead and slowing the MS acquisition will be most obvious on an Orbitrap Fusion, the instrument was selected for transit time study.

Figure 8. Optimization of ion injection time without and with FAIMS (-50|-70) on an Orbitrap Fusion (+) APD using a 1.5hr LC gradient and 1µg HeLa sample load.

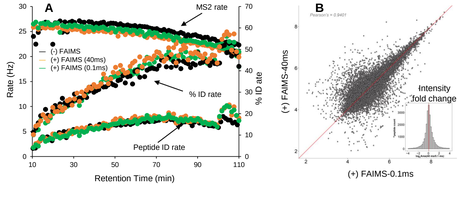


Peptide and protein IDs were used as metrics to optimize IT mass analyzer ion injection time between (-) FAIMS and (+) FAIMS experiments. Figure 8 shows the peptide IDs maximized at 20 ms injection time: 40523 peptides for (+) FAIMS and 37003 peptides for (-) FAIMS. Interestingly, the protein IDs were largely independent of injection time (10-30ms): (+) FAIMS produced ~6500 IDs compared to 5212 IDs for (-) FAIMS. APD was enabled in both studies. Optimum injection time of 20 ms was used to benchmark MS2, PSM, and peptide rate over the entire LC gradient for a 40 ms and 0.1ms FAIMS ion transit time with CVs -50|-70.

Figure 9 shows MS2, %ID rate (PSM/MS2), and peptide ID rate for (-) FAIMS (black), (+) FAIMS-40ms (orange) and (+) FAIMS-0.1ms (green). Comparison of the data shows that reduction of the ion transit time from 40ms to 0.1ms has little to no adverse impact on the acquisition or identification rate. This implies that the peptides in the CV -50 and -70 have very subtle spatial separation in the FAIMS gap meaning peptides that are not transmitting in a CV channel are likely lost to the exit region of the electrodes. When a transmitting CV is applied these ions travel only a short distance within 0.1ms to exit the FAIMS electrodes.

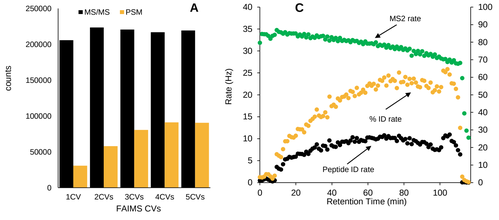
Also a correlation plot of MS1 intensities from 0.1ms and 40ms was used to assess if precursor ions were under sampled at 0.1ms. Figure 9B shows a good correlation and no fold change in MS1 intensities (insert, bottom right) between the two ion transit time studies. The findings clarify a historical misconception that FAIMS ion transit time imposes a duty cycle burden on fast scanning MS. With a 0.1ms transit time FAIMS adds no time overhead to MS acquisition.

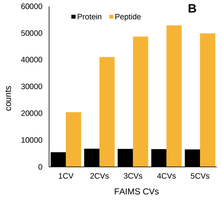
Figure 9A. Shows MS2, PSM, and Peptide ID rate across a 110 min LC gradient for a 1µg HeLa sample for (-) FAIMS (black) and (+) FAIMS-40ms (orange) and (+) FAIMS-0.1ms (green) on an Orbitrap Fusion with APD. Two CVs (-50 and -70) were internally switched with the transit time fixed at 40ms/CV. IT parameters were: auto scan range, rapid acquisition speed, first mass 100, AGC target $3x10^4$ and injection time of 20 ms. Figure 9B compares MS1 intensities between (+) FAIMS-40ms and (+) FAIMS-0.1ms.



Turbo CV Switching (4CVs) with Turbo IT MS2 Scans

Figure 10A and B show the effect of adding more CVs with turbo CV switching on MS/MS, PSM, Peptide and Protein IDs. Figure 10 C shows the rate of identification for the optimum 4CV turbo switching (-48V, -55V, -65V, -72V).





Unique Peptides and Protein IDs Turbo CV switching

(+) FAIMS-0.1ms with 4CVs produced 53000 peptides.

Protein IDs (+) FAIMS-0.1ms were largely unchanged between 2CVs and 5CVs: ~6600 protein, 1peptide/protein.

MS2 acquisition topped at 35 Hz, %ID rate at 67%, and peptide ID rate at ~12 peptide/s.

CONCLUSIONS

- The new FAIMS electrodes attenuated MS ion signal by 2 fold but generated higher purity MS1 and MS2 scans for low abundant ions compared to no FAIMS: gas phase enrichment of multiply charged peptides.
- Ability to operate FAIMS at 0.1ms transit time without performance penalty offers a way to operate FAIMS at near 100% duty cycle.
- Turbo FAIMS CV switch and Turbo MS2 scan combined allows use of move CVs at no performance cost and produced 53 000 unique peptides and 6600 proteins on an Orbitrap Fusion MS (+) APD, with a 1.5hr gradient, and single LC injection of 1µg HeLa.

ACKNOWLEDGEMENTS

We would like to thank Thermo Fisher Scientific colleagues Philip Remes, Graeme McAlister, Jesse Canterbury, Raman Mathur, and collaborator Sibylle Pfammatter from Institute for Research in Immunology and Cancer, H3T 1J4, Québec, Canada.

PO65276-EN 0518S

Thermo Fisher SCIENTIFIC