Design and Performance of a Novel FAIMS Prototype Interface Mounted on High Conductance Sampling Mass Spectrometers

Michael Belford, Susan Abbatiello, Satendra Prasad, Thermo Fisher Scientific, 355 River Oaks Pkwy, San Jose, CA, USA 95134

ABSTRACT

Purpose: Increased protein and peptide identifications were demonstrated, at nanoflow rates with a new FAIMS prototype interface.

Methods: Optimal CV values were determined for analysis of HeLa digest standards.

Results: The new FAIMS prototype interface offers better than 50% sample transmission versus no FAIMS. The CV region between -30 V and -100 V shows selective transmission of multiply charged ions. Internally cycling between multiple CVs during MS acquisition increases protein identification versus no FAIMS. Interrogating multiple CVs increases both protein and peptide identification without the need of offline LC fractionation.

INTRODUCTION

FAIMS separates gas phase ions by subjecting them to tens of thousands of cycles of alternating high and low electric fields generated by applying an asymmetric waveform to an inner electrode counter to a grounded outer electrode.[1] Ions are carried through the gap between the electrodes, perpendicular to the electric field, within a flow of gas and are selectively transmitted into the inlet of a mass spectrometer by scanning a DC potential called compensation voltage, or CV. Since FAIMS separation is orthogonal to *m/z* separation, each CV setting selects a discrete ion population that can be further interrogated by the instrument. Here, a new FAIMS prototype interface is described with the ability to selectively introduce nanosprayed ions into a fast scanning Orbitrap™ instrument for the purpose of improving proteomic depth.

The FAIMS prototype interface comprises an electronics control box, a transformer box, and cylindrical electrode set designed to operate on a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer, shown in Figure 1. The electrodes, shown in Figure 2, are independently temperature controlled and sealed to the high capacity transfer tube (HCTT) of the mass spectrometer.[2] A dispersion voltage (DV) of -5000V is applied across the 1.5 mm electrode gap and nitrogen is supplied to the entrance of the electrodes to carry the ions through the FAIMS prototype interface and into the mass spectrometer, as shown in Figure 3.

Figure 1. FAIMS prototype interface installed on an Orbitrap Fusion Lumos Tribrid mass spectrometer with an EASY-nLC 1200 pump.

Figure 2. Exploded view of the FAIMS electrode set. Electrodes are assembled without tools and do not require a break in the mass spectrometer's vacuum system to install.

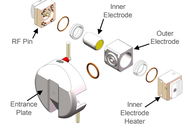
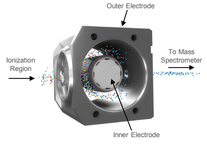


Figure 3. Simulation showing the flow of ions into the FAIMS electrode set. The CV and DV are applied to the Inner Electrode and ions flow around it during separation.



MATERIALS AND METHODS

Sample Preparation
Thermo Scientific™ Pierce™ HeLa digest protein standard was loaded onto a Thermo Scientific™ EASY-Spray™ 75 µm x 50 cm (C18, 2 µm) column, in 1 µg injections, and was driven by a Thermo Scientific™ EASY-nLC™ 1200 pump at 300 nL/min.

Test Method(s)
The flow rate of nitrogen supplied to the entrance of the electrode set is dictated by the mass spectrometer's inlet conductance and was set 5.5 L/min. A DC potential of 250 V was applied to the Entrance Plate to act as the counter electrode to the ionization source. Standard resolution mode, inner and outer electrodes set to 100 °C, and a DV of -5000 V were used for all experiments.

The FAIMS prototype interface was mounted to an APD-enabled Orbitrap Fusion Lumos Tribrid system for mass analysis. A data-dependent method selected precursors based on charge state, monoisotopic *m/z* assignment, and dynamic exclusion. ITMS2 spectra were collected using rapid scan rate with an injection time of 20 ms.

Data Analysis
Peptide and protein identifications were determined by running the result files through Thermo Scientific™ Proteome Discoverer™ 2.1 software.

RESULTS

Sensitivity and Charge State Separation

Improved FAIMS transmission using an optimized electrode geometry has been described in detail previously.[3] Here, this sensitivity improvement is demonstrated, in Figure 4, by running consecutive injections of a HeLa digest on a Orbitrap Fusion Lumos MS with and without the FAIMS prototype interface installed. For the injection with the FAIMS prototype interface, the CV was set to -60 V.

Scanning the CV while infusing a tryptic peptide standard demonstrates charge state separation with FAIMS and is shown in Figure 5. Although singly charged ions dominate the CV region between 0 V and -30 V, a region of multiply charged ions exists between -40 V and -100 V.

Figure 4. HeLa digest chromatograms run with Orbitrap Fusion Lumos MS (top trace) and with FAIMS-Orbitrap Fusion Lumos MS at 300 nL/min. Note that the signal with the FAIMS prototype interface installed is greater than 50% of the signal without it.

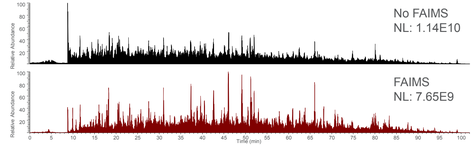
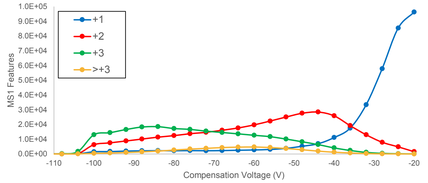


Figure 5. Scanning the CV from -20 V to -110 V while infusing a tryptic digest standard demonstrates charge state separation possible with FAIMS. Note that primarily multiply charged ions are transmitted at CVs less than -40 V.

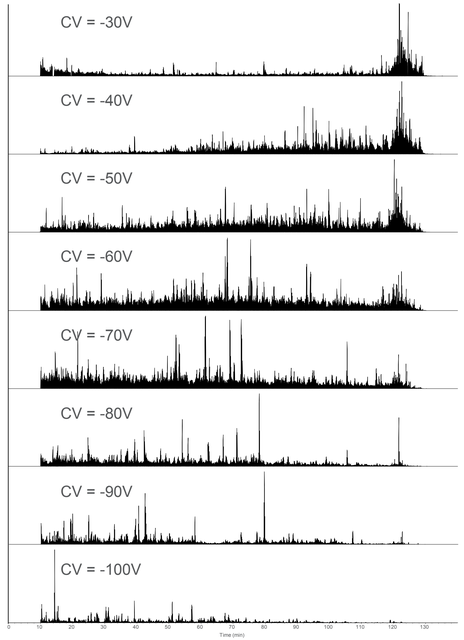


Gas Phase Fractionation

Offline LC fractionation is a preparation technique employed to enrich low abundance peptides in a complex sample. However, this method of sample prep requires extensive training and added pre-analysis time, up to one day, in addition to the added time needed to run each fraction separately. More importantly, the increase in sample handling can result in error and variability.

With FAIMS, each CV value transmits a different ion population to the mass spectrometer without the need for added sample prep. This gas phase fractionation occurs within the ion source housing, on a millisecond time scale, and is fully automated. To demonstrate this, the CV region showing an abundance of multiply charged ions was further interrogated by making a series of HeLa standard injections, 1 µg load, with FAIMS installed. A single CV was applied for the duration of the 140 minute LC gradient for each injection, starting at -30 V and stepped in 10 V steps through -100 V. The resulting chromatograms are shown in Figure 6, with each CV producing unique LCMS features across the gradient.

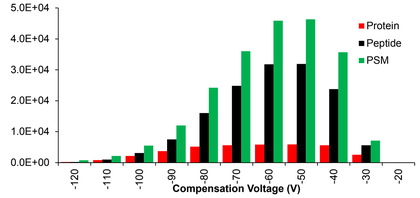
Figure 6. 1 µg injections of HeLa standard analyzed with discrete CV values, ranging from -30 V to -100 V. Note the unique chromatogram associated with each CV value.



Protein and Peptide Identification with FAIMS

To further characterize this CV region, the series of single CV chromatograms were further interrogated by post-acquisition software to determine their individual PSM, peptide, and protein distribution. This is summarized in Figure 7 and is described in greater detail in our companion poster.[4] As was suggested by the charge state plot, the majority of identifications are attributed to the CV range between -40 V and -80 V.

Figure 7. Protein, peptide, and PSM distribution across the CV range from -20 V to -120 V. Note that the region between -40 V and -80 V generates the most identifications.



Although unique identifications are made at each CV in this range, multiple CVs must be used to increase the number of identifications compared to not using FAIMS. This can be done by either processing runs with different CVs together or by internally switching between multiple CVs within the same run. For internally switching CVs, it is important to select voltages that are sufficiently spaced so that the gas phase fractions transmitted have limited overlap. For this experiment, -40 V, -60 V, and -80 V were selected.

Figures 8 and 9 show the number of peptide and protein identifications, respectively, for a 1 µg HeLa sample analyzed with the CV switching continually between -40 V, -60 V, and -80 V compared to a run without the FAIMS prototype interface installed. Although, there is only minimal improvement in peptide identification with FAIMS in this experiment, 12% more proteins are identified by FAIMS in the same amount of analysis time as analysis without FAIMS.

However, processing three injections (one at -40 V, one at -60 V, and one at -80 V) together yields 30% more peptides and 25% more proteins compared to analysis without FAIMS. This improvement in identification is even greater when all eight injections, between -30 V and -100 V, are processed together. This results in gains of 60% more peptides and 34% more proteins compared to analysis without FAIMS. This improvement in proteomic depth does require more analysis time, but without any added sample prep time.

Figure 8. Peptide identification determined by post-acquisition analysis of HeLa injections for No FAIMS installed (1st bar), internally cycled CVs (2nd bar), 3 CVs individually processed (3rd bar), and 8 CVs individually processed (4th bar). Note the increase in peptide identification as the number of CVs interrogated increases.

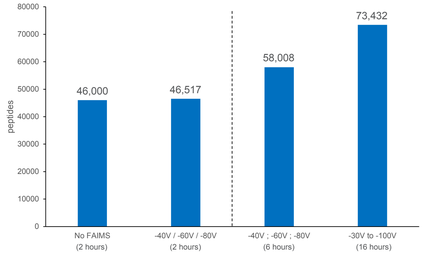
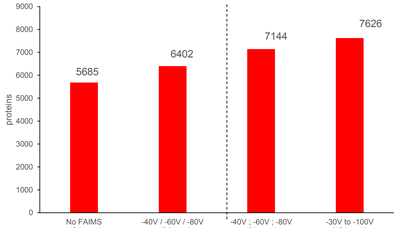


Figure 9. Protein identification determined by post-acquisition analysis of HeLa injections for No FAIMS prototype interface installed (1st bar), internally cycled CVs (2nd bar), 3 CVs individually processed (3rd bar), and 8 CVs individually processed (4th bar). Note the increase in protein identification as the number of CVs interrogated increases.



CONCLUSIONS

- The FAIMS prototype interface described here transmits over 50% of the ions detected by NSI-MS compared to when FAIMS not installed.
- Scanning across a range of CVs shows a region between -30 V and -100 V that is primarily multiply charged ions.
- The same sample interrogated at different CV values within this region allows for unique ions to be detected by the fast scanning Orbitrap MS. This is the effect of ion fractionation in the gas phase.
- CV values can be cycled within a single sample injection to produce an increase in protein identification versus no FAIMS prototype interface installed (in the same amount of analysis time).
- Multiple samples can be analyzed (each at a single CV) to produce an increase in peptide identification versus no FAIMS prototype interface installed without the need for benchtop LC fractionation.
- Further optimization of experimental parameters are described in TP684[5]

For Research Use Only. Not for use in diagnostic procedures.

REFERENCES

1. Purves, R. W.; Guevremont, R. *Anal. Chem.* **1999,** *71,* 2346–2357.
2. Belford, et al., 64th ASMS Conference on Mass Spectrometry and Allied Topics, San Antonio, TX
3. Belford, et al., 62nd ASMS Conference on Mass Spectrometry and Allied Topics, Baltimore, MD
4. Prasad, et al., 66th ASMS Conference on Mass Spectrometry and Allied Topics, San Diego, CA
5. Hebert, et al., 66th ASMS Conference on Mass Spectrometry and Allied Topics, San Diego, CA

ACKNOWLEDGEMENTS

The authors would like to acknowledge Romain Huguet, Josh Silveira, Philip Remes, Graeme McAlister, Eloy Wouters, and Jean-Jacques Dunyach from Thermo Fisher Scientific for help with data analysis and for insightful discussion.

TRADEMARKS/LICENSING

ThermoFisher SCIENTIFIC

PO65296-EN 0518S