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Mass Accuracy and Dynamic Range in Ion Mobility-Mass Spectrometry Measurements

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Analog-to-Digital Conversion of IMS-TOF MS signals enhances both spectral dynamic range and mass measurement accuracy

Overview

- **The ion funnel trap (IFT) enables the delivery of ion currents that routinely exceed levels produced by a continuous electrospray ion beam**
- **Traditional ion recording mechanisms have a limited dynamic range and are prone to timing errors when large ion currents are delivered to the detector**
- **By incorporating analog-to-digital (ADC) recording of IMS-TOF MS signals, mass measurement accuracy (MMA) and dynamic range are significantly enhanced**

Introduction

Despite the strong analytical presence of ion mobility spectrometry (IMS) as a stand-alone gas phase separation technique, the levels of precision and accuracy of each separation may be significantly augmented by using a mass spectrometer (MS) as a detection system. A wide range of mass spectrometry systems have been interfaced with IMS, however, the speed of a time-of-flight mass spectrometer (TOF MS) is uniquely suited to examine the varying ion populations exiting and IMS drift cell.[1] Recent advances in ion trapping external to the IMS drift cell have allowed ion currents with significantly higher charge density to be injected into the IM-TOF MS system.[2] In order to assess the influence of these increased ion currents on system performance, we have quantitatively assessed the dynamic range and mass measurement accuracy (MMA) of time-to-digital conversion (TDC) and analog-to-digital conversion (ADC) data acquisition systems.

Instrumentation

A detailed description of the design and operation of the ion mobility—time-of-flight mass spectrometer shown in Figure 1 may be found in references 1 and 2. Using an electrospray ionization source (2000V, 300 nL/min) ions were accumulated in an IFT[2] and the frequency of each ejection pulse was ~16 Hz. The ion mobility drift cell (98 cm, 16 V/cm, and 26 °C) was continually purged with 4 Torr of dried N_2. Once through the drift cell, ions were refocused using a conventional ion funnel (520 kHz, 120 Vp-p) into a segmented RF-only quadrupole (700 kHz, 160 Vp-p) prior to entering the accepting octopole of the TOF-MS (Agilent Technologies Inc., Santa Clara, CA). In order to assess the performance of different acquisition systems data were recorded using either an Acqiris-AP240 SSR ADC (Agilent Technologies Inc., Monroe, NY), Ortec 9353 TDC (Ortec, Oak Ridge, TN), or an Ortec 9353 combined with an Ortec 935 constant fraction discriminator (CFD). All acquisition cards were interfaced to a custom software package written in C# and the data processed using a series of custom scripts written in IGOR Pro (Wavemetrics, Lake Oswego, OR).

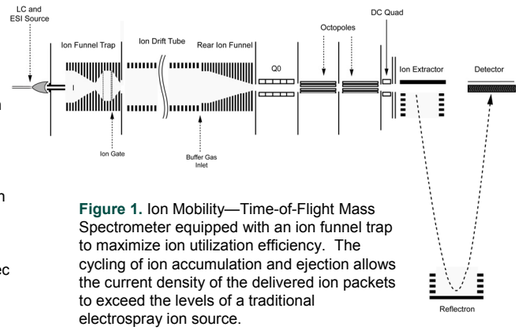

Figure 1. Ion Mobility—Time-of-Flight Mass Spectrometer equipped with an ion funnel trap to maximize ion utilization efficiency. The cycling of ion accumulation and ejection allows the current density of the delivered ion packets to exceed the levels of a traditional electrospray ion source.

Methods

Proteolytic digestions of bovine serum albumin (BSA) were conducted using sequencing grade trypsin. After digestion the concentration of each sample was determined using a BCA assay. The final concentration of each sample was diluted to 300 nM in the electrospray solvent (75:24:1 (ACN:$H_2$0:Formic Acid). Lyophilized peptides were reconstituted in the electrospray solution at a concentration of 100 nM.

Each IMS-TOF MS spectrum was a composite sum of 500 individual IMS experiments each lasting 60 ms. Prior to each IMS experiment, ions produced by the electrospray source were allowed to accumulate in the IFT for 4 ms before being released into the IMS drift cell over the period of 200 µs.

To accurately compare the three different acquisition systems the gain of the PMT was held constant at ~ 680 V. The full scale of the ADC was 50 mV and the offset for acquisition held at -30 mV. The same offset settings were used for both of the TDC-based acquisition configurations.

Data were acquired at a resolution of 0.8 ns for all TDC-based experiments and at 1 MHz with the ADC system. The spectra for each IMS-TOF MS dataset were deisotoped using a modified THRASH algorithm contained in the open source software package Decon2LS[3] developed at PNNL.

Results

- **Compared to traditional ion counting mechanisms, no observable MMA deviations are observed for ADC as a function of ion intensity (Figures 2 and 3).**
- **For both simple and complex mixtures, the dynamic range observed for ADC was consistently greater by a factor of at least 2 compared to TDC based systems.**
- **The combination of enhanced MMA and dynamic range afforded by ADC acquisition allows a significantly larger number of peptides to be observed for complex samples.**

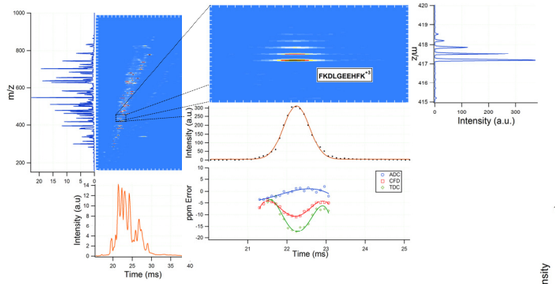

Figure 2. The zoomed subset highlights the tryptic BSA peptide FKDLGEEHFK. Shown below the ion mobility contour are the errors in MMA observed across an individual IMS peak. The average MMA using ADC was –0.630 ± 1.716 ppm.

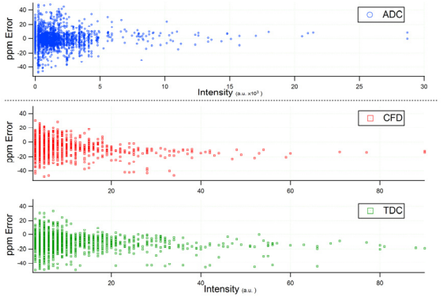

Figure 3. Ion intensity vs. MMA for the peptides derived from tryptically digested BSA. Due to the mechanism of ion counting, TDC-based systems routinely demonstrate a negative mass error shift for ion packets containing high charge density even when CFD is used.

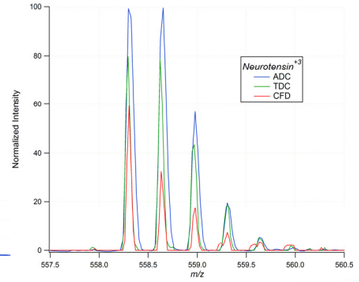

Figure 4. Under controlled conditions constant fraction discriminators can correct the negative intensity shift observed with high intensity ion packets.

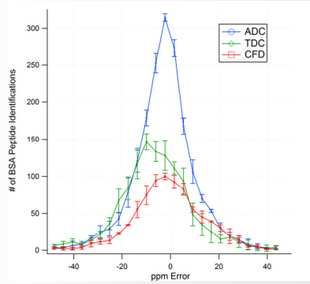

Figure 5. Histogram of BSA tryptic peptide identifications using ADC, TDC only, and TDC/ CFD. Matches against the BSA *in-silico* tryptic digest were performed at MMA of 50 ppm.

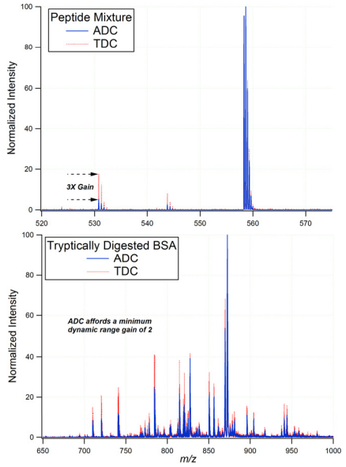

Figure 6. Comparison of dynamic range observed in a single spectrum using ADC and TDC. Because the ADC can record more than one ion event per spectrum the composite of multiple averages allows a greater dynamic range to be observed.

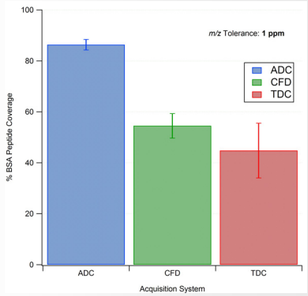

Figure 7. Percentage of tryptic BSA peptides observed using the different acquisition systems (within 1 ppm). The benefits of enhanced dynamic range and low MMA allow ADC based systems to identify ~30 % more peptides compared to TDC/CFD acquisition.

Conclusions

- **Despite providing higher degrees of resolution, traditional ion counting mechanisms such as TDC severely limit the quality of information that may be derived from IMS-TOF MS systems equipped with an IFT.**
- **Because multiple ion events may be recorded per spectrum using ADC, a greater dynamic range is routinely observed compared to TDC. The dynamic range of a single TOF spectrum extracted from the IMS domain routinely exhibits at least a 2 fold gain in dynamic range.**
- **Combining improvements in both MMA and dynamic range, ADC acquisition increases the number of peptides confidently identified by more than 30%.**
- **Given the importance of MMA to proteomics experiments, ADC is uniquely suited to record the high levels of pulsed ion currents produced by an IFT-equipped IMS-TOF MS instrument.**

Acknowledgements

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References

1. Baker, E.S.; Clowers, B.H.; Li, F.; Tang, K.; Tolmachev, A.V.; Prior, D.C.; Belov, M. E.; Smith, R.D. *JASMS* **2007** *18*:1176-1187.
2. Clowers, B.H.; Ibrahim, Y.M.; Prior, D.C.; Danielson III , W.F.; Belov, M.E.; Smith, R. D., *Anal. Chem.* **2008**, *80*:612 -623.
3. Decon2LS, Open-Source Software for Deisotoping of Mass Spectra. Available for download at: **http://ncrr.pnl.gov/software/**

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