Ion Pre-Accumulation for High Speed Orbitrap Exploris Operation

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ABSTRACT

Purpose: Implementation of an ion trapping and accumulation stage in parallel to the regular C-Trap operation of a modified Orbitrap Exploris 480 mass spectrometer, allowing 100% duty cycle at high repetition rates.

Methods: Sensitivity comparisons were made between standard operation and pre-accumulation modes, both for infused FlexMix calibration solution and chromatographic separations of HeLa digest.

Results: Instrument sensitivity was found to double at the normally allowed maximum repetition rate, and repetition rate could be increased above 70Hz without loss of duty cycle. Considerable increases in peptide ID rates were observed for proteomics application, with the most dramatic improvements recorded for short gradient / high throughput experiments.

INTRODUCTION

Orbitrap instruments have played a tremendous role in advancing MS driven scientific research. However, Orbitrap instruments have been to date limited to maximum acquisition rates <50Hz, primarily due to time constraints imposed not by the Orbitrap analyzer itself, but by timing overheads imposed by the operation of the C-Trap and its conjoined Ion Routing Multipole (IRM) that prepare and inject ions into the analyzer.

Figure 1 shows the layout of ion optical devices within a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer, along with the timings of operations running in series and in parallel. Before ions generated by the electrospray ion source may be analyzed by the orbitrap, they are first transferred into vacuum, quadrupole isolated, and injected into the IRM before being cooled and transferred to the C-Trap for orthogonal ejection to the Orbitrap analyzer. The preparation of ions within the IRM and the C-Trap, as well as the ejection process and reset, takes ~10ms, a dead time during which no further ions may be accumulated. The ion beam is normally dumped at the charge detector during this period and lost, though a portion of the period is used to adjust voltages of the ion guides and switch to the next target ion.

This fixed 10ms operation time runs in series with ion injection time, and faster operation rapidly leads to loss of duty cycle as only the latter may be cut. For the fastest normally allowed Orbitrap acquisition cycles resolution setting of 7500 (16ms transient), the overall cycle time is >20ms but only 10ms may be used for ion beam acquisition, a loss of >50%. Faster operation still is perfectly possible and might be preferred for high throughput applications with many species eluting over very short separations, except that the duty cycle penalty grows explosively and renders such a method non-viable for most real experiments.

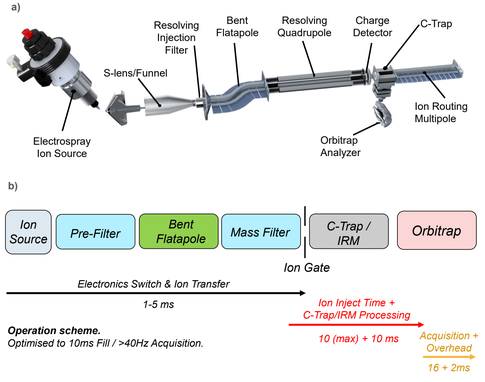

Figure 1. a) Ion optical layout of an Orbitrap Exploris mass spectrometer. b) Illustration of instrument operations that run in series and parallel.

One of the ion guides that serves to transfer ions across vacuum stages, between the ion source and C-Trap/IRM, is called the Bent Flatapole. This has a quadrupole structure, curved to separate ions from neutral gas, and incorporates a superimposed DC gradient, generated by a series of PCB printed DC electrodes. An exit lens aperture with an independent voltage separates the device from the quadrupole mass filter. Structurally this makes for an excellent ion trap, where trapping or release of ions may be controlled by switching the DC voltage applied to the exit lens.

A suitable method to circumvent the C-Trap dead time would be to trap ions within the bent flatapole during this period, performed by switching the bent flatapole exit lens to a trapping potential (+10V) during the dead period, and back to a transmitting potential (-35V) at the start of ion injection. This is shown in Figure 2 and represents the basic pre-accumulation method.

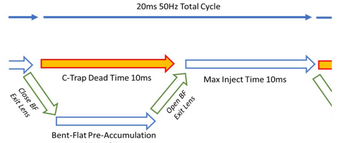

Figure 2) Illustration of pre-accumulation running in parallel to C-Trap unavailability.

MATERIALS AND METHODS

Instrument Set-Up

The Orbitrap Exploris 480 mass spectrometer's firmware files were modified so that when performing an injection to the IRM, the bent flatapole exit lens would be set to transmitting mode, but switch to trapping mode at the end of the injection. This gave the crudest possible implementation of a pre-accumulation method, incompatible with automatic gain control and forcing use of a fixed injection time.

For >70Hz operation, the Orbitrap transient was reduced to 8ms, equivalent to resolution of 3750 at m/z 200, and the injection time reduced from 10 to 3ms.

To enable tandem MS methods, the method control file was altered to allow 8ms Orbitrap transients, and a legacy C-Trap only injection matrix applied for full mass scans, which bypassed the pre-accumulation mode. This way pre-accumulation could be applied specifically to MS/MS spectra, where the added sensitivity mattered most, and not for full MS spectra where ion population control was more essential.

Sample Preparation

Thermo Scientific™ Pierce™ HeLa Digest Standard (20 µg/vial) was reconstituted by adding 200 µL 5% ACN / 0.1% FA to obtain a final concentration of 100 ng\µL. 2 µL of the sample were injected via an autosampler onto a trapping column and separated on a 15 cm PepMap Reversed Phase (RP) using a Thermo Scientific™ Vanquish™ Neo UHPLC system or a Thermo Scientific™ EASY-nLC™ system. Different gradients lengths were used to separate the samples. The eluting peptides were sprayed onto the mass spectrometer and measured. The mass spectrometer was operated in a Data Dependent Acquisition (DDA) mode, selecting the 10 most intense precursors for MSMS.

Data Analysis

The acquired raw data files were using Thermo Scientific™ Proteome Discoverer™ software 3.0 with Sequest HT search engine. PSM and Peptide validation is performed using Percolator.

RESULTS

Sensitivity with FlexMix Infusion

Before modifying the Orbitrap Exploris480 mass spectrometer software to perform pre-accumulation, Thermo Scientific™ Pierce™ FlexMix™ Calibration Solution was directly infused into the MS instrument via a syringe pump running at 5 ul\min. An MS/MS spectrum of the isolated MRFA peptide (524 Da) was acquired, using a fixed injection time of 10 ms, a 16ms Orbitrap transient, and a mass range from 150-600 Da. The intensities of this spectrum were compared to those acquired with pre-accumulation active, both of which are shown in Figure 3. The results show a >2x improvement in signal intensities when pre-accumulation is switched on, with preservation of relative intensities between fragments. The "normalized largest" (NL) measurement of signal current more than doubles, a consequence of the doubled effective injection time.

A second experiment was performed whereby the Orbitrap acquisition was set to 8ms, so that inject time limited the repetition rate. The inject time was scanned from 10 down to 2 ms, varying the repetition rate from 47 to 76 Hz, and the signal to noise ratio of the isolated MRFA peak recorded for pre-accumulation on and off. The ion current was calculated, and the proportional losses determined relative to the 10ms inject time. This is plotted in Figure 4 and shows the collapse in duty cycle without pre-accumulation, as inject time becomes a smaller and smaller proportion of the instrument cycle. Conversely when pre-accumulation was active, only slight losses were observed.

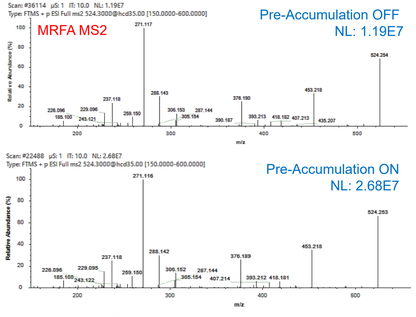

Figure 3) Comparison of MRFA MS/MS spectra with pre-accumulation disabled (top) and enabled (bottom).

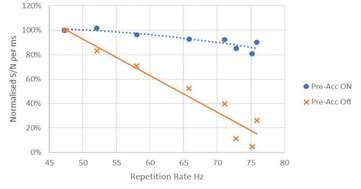

Figure 4) Relative MRFA ion current with increasing repetition rate (decreasing inject time), for pre-accumulation active and disabled.

HeLa DDA Analysis at 22 Hz

To further investigate this concept, we performed a data dependent acquisition using 200ng HeLa digest. In the experimental design a standard method was performed with pre-accumulation Off. In a follow-up experiment pre-accumulation was used. Because the instrument was running with the same scan frequency, the number of acquired MS2 for pre-accumulation On and Off was the same. However, we observed an increase in the number of spectra that were matched to peptides (PSM), increased by approximately 7 %. This translate to approximately the same percentage increase in unique peptides. We believe this increase in identification is coming from increase in the ion statistics because of the pre-accumulation.

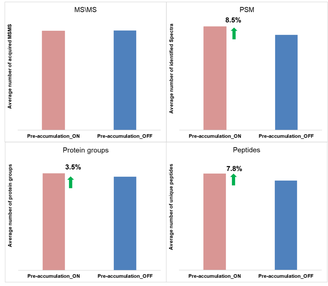

Figure 5). Comparative results obtained on a modified Orbitrap Exploris 480 with Pre-accumulation On and Off for a 60 min gradient.
FS resolution setting: 120k, AGC target: 250 %, max inject time: 20ms, ddMS2 resolution setting: 15k, AGC target: 50%, max inject time: 23ms,TopN:20.

In a second experiment we dropped the max injection time in MS2, from 23 ms to 12 ms. Changing the max injection time in MS2 ensures that any observed benefits are likely attributable to the pre-accumulation. As expected, there is no difference in the number of acquired MS2 scans, but an increase in the number of identified spectra (9%), peptides (8%) and proteins (5%).

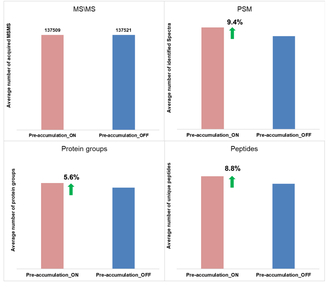

Figure 6). Comparative results obtained on a modified Orbitrap Exploris 480 with Pre-accumulation On and Off for a 60 min gradient.
FS resolution setting: 60k, AGC target: 250 %, max inject time: 20ms, ddMS2 resolution setting: 7.5k, AGC target: 50%, max inject time: 12ms,TopN:40

RESULTS

To investigate the ability to achieve higher scan rates, we decided to go for shorter gradients: 7.6, 14.4- and 24.6-mins total run time. The results were then compared to results with the standard 40 Hz operational scan frequency. Figure 7A below shows a plot of the scan frequency of the modified Orbitrap Exploris reaching 70 Hz for the 3 gradients length. The increase in scan speed is also seen the total number of acquired MS/MS spectra in figure 7B and identified spectra (figure 7C). Apart from the 24.6 min gradient, all other gradients showed an increase in the number of identified peptide and protein groups.

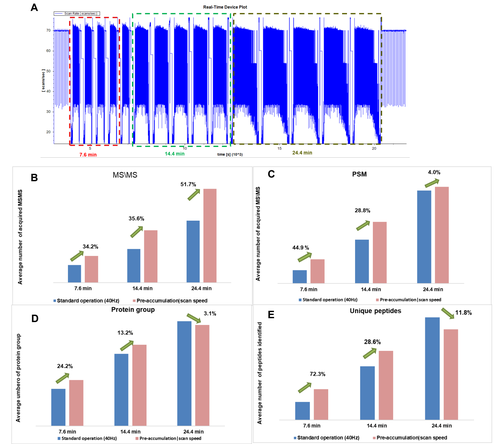

Figure 7). Evaluation of higher scan rate on a modified Orbitrap Exploris 480. A) Real time plot showing the scan rate at different gradient lengths. BCDE) comparative results between 40Hz and 70 Hz acquisition.
FS resolution setting: 45k, AGC target: 250 %, max inject time: 20ms, ddMS2 resolution setting: 3.75k\7.5k, AGC target: 1000%, max inject time: 3ms,TopN:70.

CONCLUSIONS

- A pre-accumulation method has been introduced to a modified Orbitrap Exploris 480 instrument,
- Standard 22 and 40Hz HeLa analyses with substantial sample load show modest improvements when pre-accumulation is used on a modified Orbitrap Exploris 480 instrument.
- A new 70Hz acquisition method shows considerable analytical gains for high throughput HeLa experiments on a modified Orbitrap Exploris 480.

TRADEMARKS/LICENSING

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