# Implementation and application of an external high performance data acquisition system on Orbitrap mass spectrometers

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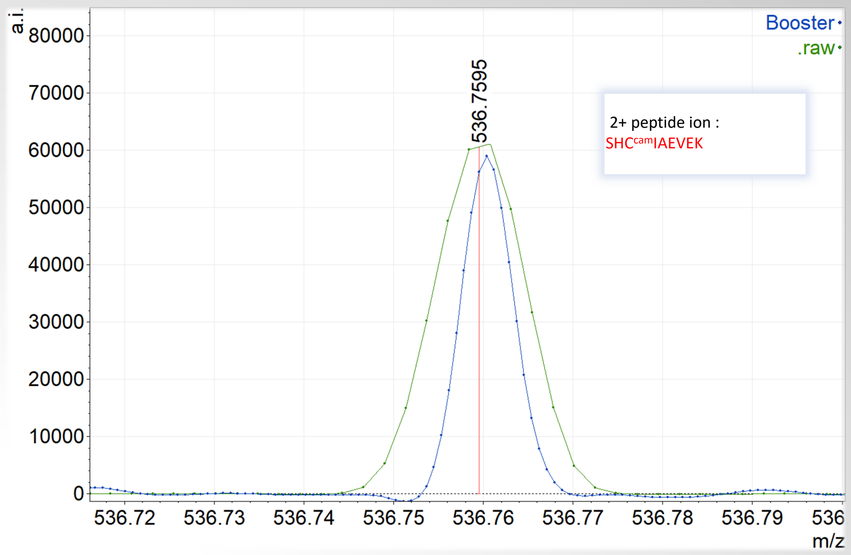
## FTMS Booster

FTMS Booster is a innovative device designed for the improvement of the performance of the Orbitrap™ and FT-ICR instruments. This device collects raw time domain data (before Fourier transform) in parallel with the original data acquisition system (to .raw files) and enable to process them independently. The benefits of using the FTMS Booster on an LTQ Orbitrap FTMS as available at ICCVS include the following: 1) resolving power increase (up to 2 times) in comparison to standard processing and 2) signal-to-noise ratio improvements.

The expected improvements will be of a great importance for applications involving analysis of trace amounts of the samples or requiring high mass accuracy. It is the case in field of cancer vaccine research, where usually the sample amount is limited, and de novo peptide sequencing required for identification of potential cancer neoantigens benefits from the highest possible mass accuracy.

Here we report an example of in-house tests of the performance of the FTMS Booster device.

## Increased resolving power




Example of an increase of resolving power due to processing the transients in absorption mode FT. In this case the resolving power increased from 43200 to 63800. In our hands, the Booster allows to generate spectra with 150-200% of the original resolution. Furthermore, the more data points per peak in spectrum leads to better definition of the peak centroid (top of the peak).

## Enhanced peptide identification

To test the efficiency improvement of peptide identification, we have analyzed the model sample(tryptic digest of BSA). MS/MS spectra of peptides were collected from 3ng/ul sample for 5 minutes. The data were converted to .mgf peak lists and searched using Mascot.

**Booster**

**Protein sequence coverage: 59%**

Matched peptides shown in ***bold red***.

```
  1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
 51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
601 STQTALA
```

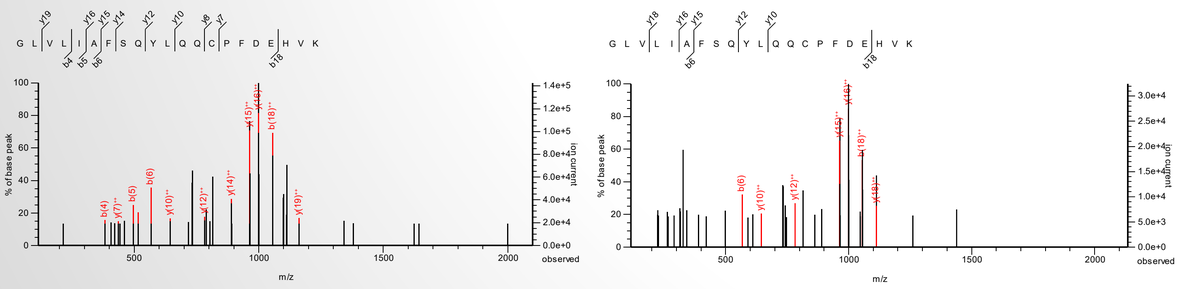
**.raw**

**Protein sequence coverage: 41%**

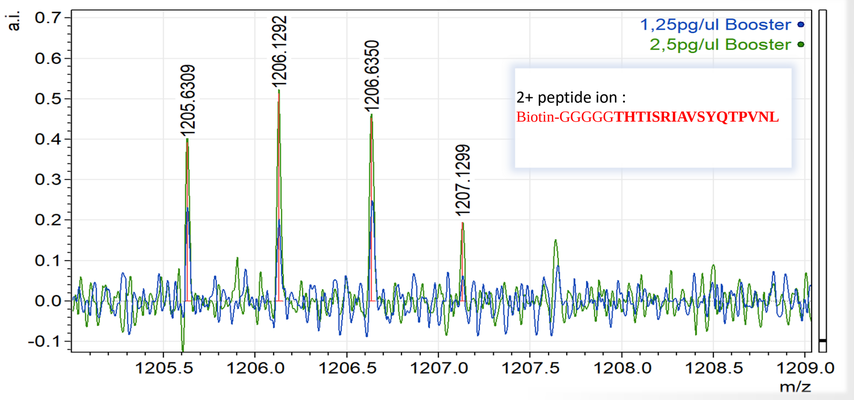
Matched peptides shown in ***bold red***.

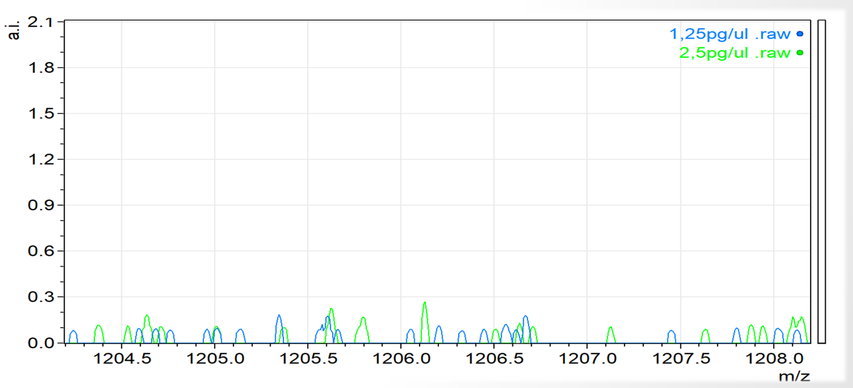
```
  1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
 51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
601 STQTALA
```

The peak lists obtained from Booster dataset resulted in clearly higher sequence coverage. We have investigated the data to determine the exact cause of the improvement.




## Increased sensitivity



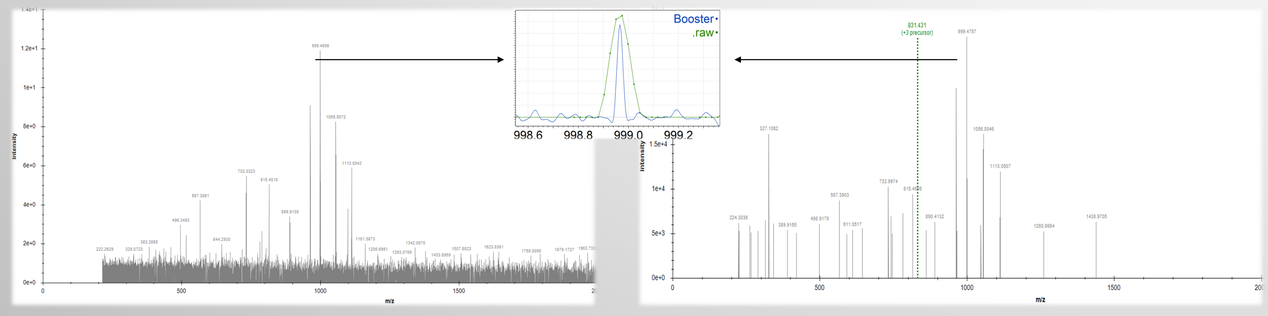




Transient averaging leads to improvement of sensitivity. On the panel above, we have compared result of averaging a 120 transients an 120 .raw spectra of the same mixture( 1,25 and 2,5 pg/ul of the synthetic peptide in a matrix of BSA digest). While averaging transients leads to recovery of a signal of 2+ peptide ion, averaged .raw spectra does not show anything above the noise level.

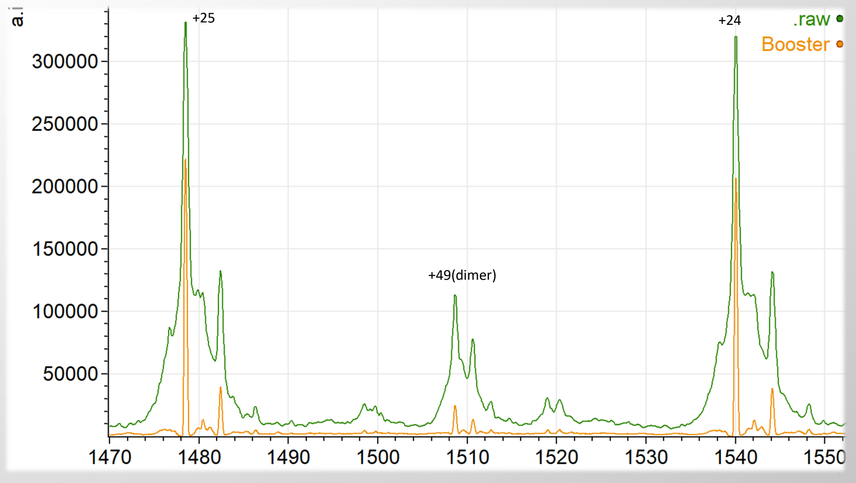
## Better mass spectra of large molecules

An example of MS/MS spectra obtained from the same scan from the Booster and .raw dataset. More fragments can be annotated in the former spectrum, and the obtained mass accuracy is about twice as high.

**Booster** **.raw**




A direct comparison of raw data reveals the cause of the mass accuracy and sensitivity improvement. Firstly the right spectra(Booster) shows much better separation between signals and the noise background, so the noise level can be adjusted to match the actual data. Furthermore, as can be seen on the insert, the resolution and mass accuracy in the case of low-intensity fragment ion peak is improved dramatically, which leads to higher mass accuracy.




Acquisition of a intact protein mass spectra is a demanding task. On a panel above, we see the comparison of spectra obtained by averaging of 5 minute injection of 37kDa protein. Average .raw spectrum (green) shows multiple artifacts and baseline distortion on the bottom of the peaks, while averaged aFT spectra (orange) show much better baseline correction and proteoform separation.

## References:

**Producing absorption mode Fourier transform ion cyclotron resonance mass spectra with non-quadratic phase correction functions.** Kilgour DP, Nagornov KO, Kozhinov AN, Zhurov KO, Tsybin YO Rapid Commun Mass Spectrom. 2015 Jun 15;29(11):1087-93

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