P-I-0138

Evaluation of high dynamic range MS1 acquisition methods on a Hybrid Orbitrap mass spectrometer.

Hanno Resemann[1], Markus Kellmann[1], Catharina Crone[1], Julia Kraegenbring[1], Christian Thoeing[1], Claire Dauly[1], Heiner Koch[1]

[1]Thermo Fisher Scientific, Bremen, GERMANY

Abstract

Purpose: Evaluation of a new scan matrix/scheme for improving the dynamic range in MS1 scans on an Orbitrap Exploris mass spectrometer

Methods: DIA-based shotgun proteomics applying MS1 quantification by utilizing a high dynamic range MS1 scan mode

Results: Improved accuracy of quantification for low and mid abundant peptides and proteins when using MS1 quantification

Introduction

Advancements in MS-based proteomics have enabled the analysis of the proteome comprehensively and quickly. However, accurately identifying and quantifying low-abundant human proteins remains difficult due to the wide range of protein abundance found in tissues and body fluids. Gas phase fractionation methods like BoxCar[1] as well as BoxCar Assisted MS Fragmentation (BAMF)[2] have addressed this challenge by adding multiple narrow mass-to-charge segments to fill the Orbitrap more efficiently. In our study, we have further developed these ideas. We made modifications to an Orbitrap ™ Exploris 480 mass spectrometer to allow gas phase fractionation and intelligent MS1 multiplexing schemes with optimized injection times for high dynamic range (HDR) Orbitrap MS1 scans.

Materials and methods

Materials and methods

Thermo Scientific™ Pierce™ HeLa Digest Standard 20 µg/vial was reconstituted by adding 200 µL of 0.1 % Formic acid. The vial was subsequently sonicated for 5 min, followed by multiple sample aspiration and release cycles with a pipette to dissolve it completely.

Samples were separated on a 25 cm x 75 µm Aurora Ultimate column using a Vanquish Neo UHPLC system. The Vanquish Neo system was operated in a direct injection configuration. Flow rate was kept at 250 nl/min in a 52.5 min gradient (6% B to 28% C for 40 min, 28% B to 50% B for 12.5 min) with 400 nL/min flow rate for column wash (99% B). The column with internal emitter was inserted into an EASY-Spray™ Source.

For direct comparison of regular MS1 and HDR MS1, two alternating full scan experiments were run within one method. One full scan experiment was set to regular MS1, the other to HDR scan mode. Both full scan experiments were set to a resolution of 120,000 m/z. The number of HDR windows was set to 12.

For proteomics analysis, the Orbitrap™ Exploris 480 MS was operated in a data-independent acquisition (DIA) mode at a MS1-resolution of 90,000 and MS2-resolution of 30,000. Isolation window was set to 12 m/z, window overlap was set to 1 m/z. Precursor mass range was set to 400-900 m/z, MS2 scan range was set to 145-1450 m/z. Data was matched against the Uniprot human proteome (as of September 2024). Quantification was calculated using MS1 signals only.

High dynamic range (HDR) orbitrap MS1 scans

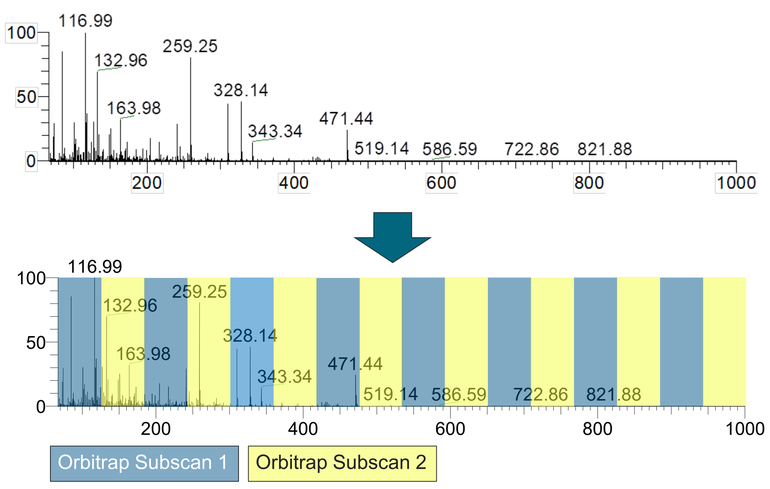

Figure 1. Illustration of the HDR principle. The full MS mass range is disected into two orbitrap subscans, each covering 8 windows, achieving gas phase fractionation and efficient ion delivery to the orbitrap. The stitched HRD Orbitrap MS1 spectrum is achieved automatically and fully embedded in the data structure. The number of windows can be adjusted according to individual needs.

A regular MS1 full scan covers the entire mass range in one orbitrap scan. In the high dynamic range (HDR) MS1 scan, the mass range is instead divided into two separate orbitrap subscans that cover different mass ranges in alternating mass range windows (see Figure 1). Each subscan is a multiplexed scan of n injections with individual injection times. A survey scan determines the required injection times for the individual mass ranges beforehand, resulting in scans with lower ion count receiving higher injection times, while scans with higher ion count receiving lower injection times. This principle of gas-phase fractionation comes with the advantage of a higher dynamic range while using the c-trap ion capacity more efficiently.

The two orbitrap subscans are then stitched together for one multiplexed MS1 full scan. The stitching is done via fixed overlap regions between two scans (e.g. 5 m/z). The overlap regions are then automatically rescaled, so the signal intensities over the whole MS 1 spectrum are not affected by the multiplexing. In contrast to previous techniques like BoxCar[1], this is done automatically during the scan and does not require post-processing tools. The resulting stitched MS1 multiplexed scan is then treated like any regular MS1.

Results

First, we investigated if the HDR mode works in principle, similar to previously described methods like BoxCar[1]. For HDR MS1 scans, we found a gain in signal to noise (S/N) for low abundant ion signals compared to regular MS1 scans (see Figure 2). Signals with very high abundance ion signal in contrast showed a slight reduction in S/N compared to regular MS1 scans but retained a high S/N regardless (Data not shown).

Next, we conducted a DIA experiment with either regular MS1 scans or HDR MS1 scans used. The HDR DIA showed a strong increase in detected number of signals (116% increase) and LCMS features (119 % increase). The detected LCMS features also contained a higher number of features with a charge state of larger than one (72% increase) (see Figure 3). This indicates that a higher number of peptide signals (z>1) were recovered above noise by increasing collection time and ion statistics.

Since HDR MS1 scans combine two MS1 subscans into one, the resulting time to acquire the stitched full scan increases. However, the decrease of MS1 and MS2 spectra of 6% across the run is neglectable and does not have negative effects on the number of identified peptide and proteins (see Figure 3).

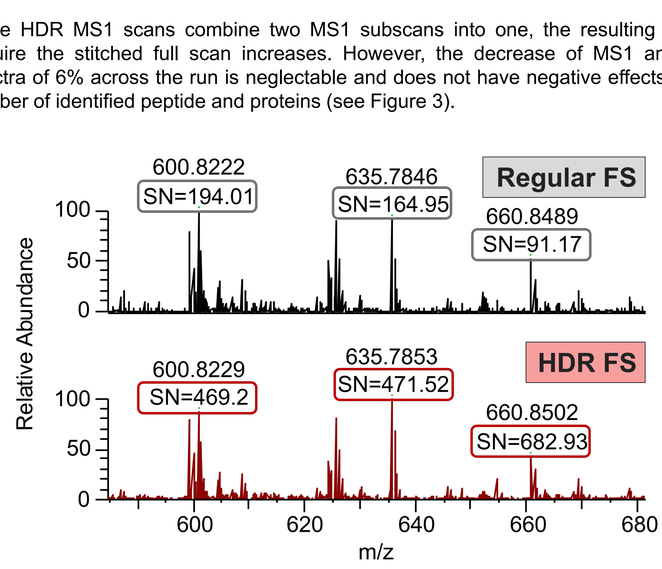

Figure 2. Comparison of parallel run MS1 spectra at 585-680 m/z, one with and one without HDR scan mode. Ions of lower abundance get the highest boost in signal to noise (S/N) with the HDR scan mode.

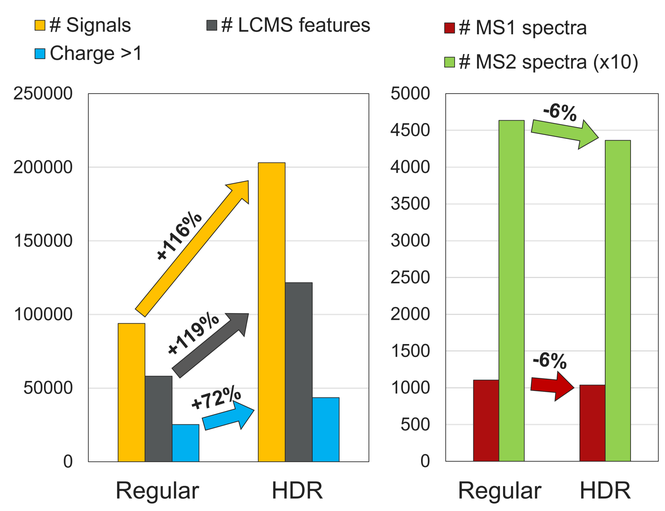

Figure 3. Comparison of DIA experiment with regular MS1 fullscan or HDR MS1 fullscan. Shown metrics are: Number of total signals detected, number of LCMS features detected, number of LCMS features with a charge state of > 1, number of MS1 spectra, and number of MS2 spectra (n=3). HDR MS1 full scans contain about double the numbers of signals, LCMS features and features with charges > 1. At the same time, the number of MS1 and MS2 scans per run drops slightly but insignificantly due to the time needed to acquire MS1 HDR scans.

HDR DIA does not affect the total number of identified precursors, peptides or protein groups, and the slightly lower number of MS1 and MS2 scans does not affect the results negatively (see Figure 4 A). However, when comparing the number of identifications quantitatively, HDR scans reveal their clear benefit. For quantification of peptides, HDR DIA shows a clear improvement over regular DIA, with the numbers of peptides in these categories increasing between by 17% for identities with CV < 20% and 15% for identities with CV < 10%, respectively. For protein groups, the effect is similar with increases of identities by 13% (CV < 20%) and 19% (CV < 10%) (see Figure 4 B). This is within expectation, since the improvement of S/N for signals should lead directly to an improvement in CVs when the same signals are compared between different runs.

When analyzing the improvements of CVs for protein groups and peptides, it also became clear that the strongest improvement of CV is achieved for peptides and proteins with low signal intensity while peptides with higher signal intensity do not benefit from HDR. Separating peptides and protein groups into bins of signal intensities reveals that the lower the signal intensity is, the stronger is the reduction of the CV at peptide and protein level, respectively (see Figure 5). This agrees with the principle of the HDR scan, S/N in windows with low ion current is boosted by longer ion injection times to the c-trap, resulting in more accurate quantification and lower CVs of corresponding peptides.

Conclusions and Outlook

We could demonstrate that using high dynamic range (HDR) Orbitrap MS1 scans improve S/N several fold for low and mid intensity signals, enables detection of up to 72% more features (z>1) in a 1h LCMS proteomics HeLa run, and improve the quantification of proteomics DIA results when using the MS1 trace for quantification. The improvement in quantification of low abundant peptide signals is particularly interesting as it extends the opportunity to accurately quantify proteins that are of low abundance and only identified by two or a single peptide. At the same time, it can also benefit all applications where accurate quantitation of low abundant peptides is needed e.g. samples where limited sample amount is available or in single cell proteomics.

References

1. Meier, F., Geyer, P.E., Virreira Winter, S. et al. **BoxCar acquisition method enables single-shot proteomics at a depth of 10,000 proteins in 100 minutes**. Nat Methods 15, 440–448 (2018). https://doi.org/10.1038/s41592-018-0003-5

2. Jenkins, C., Orsburn, B. **BoxCar Assisted MS Fragmentation (BAMF).** BioRxiv (2019). https://doi.org/10.1101/860858

Trademarks/licensing

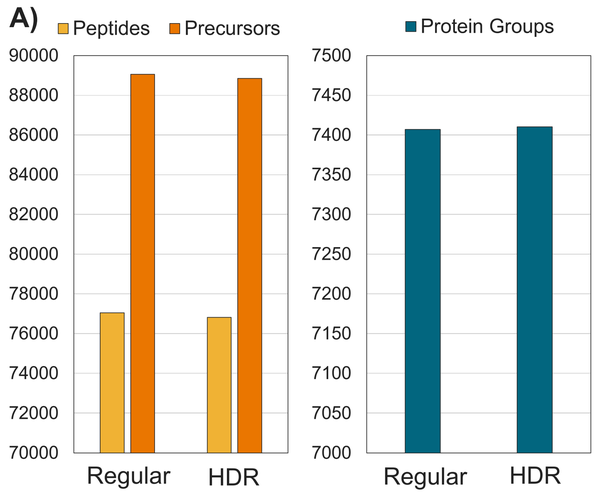

Figure 4. Analysis of 100ng Hela samples with a DIA method on a 60 min gradient (n=3). Datasets were generated using either a DIA method with a regular MS1 fullscan, or with a HDR MS1 fullscan instead. Data was analyzed utilizing MS1 quantification.
A) Comparison of identifications of peptides, precursors, and protein groups. Using HDR MS1 full scans instead of regular full scans has no significant effect on the number of identifications.
B) Comparison of coefficient of variation (CV) below X for peptides and protein groups. DIA runs with HDR MS1 achieve a higher number of identifications with CVs of <20% and <10% when using MS1 quantification than DIA with regular MS1.

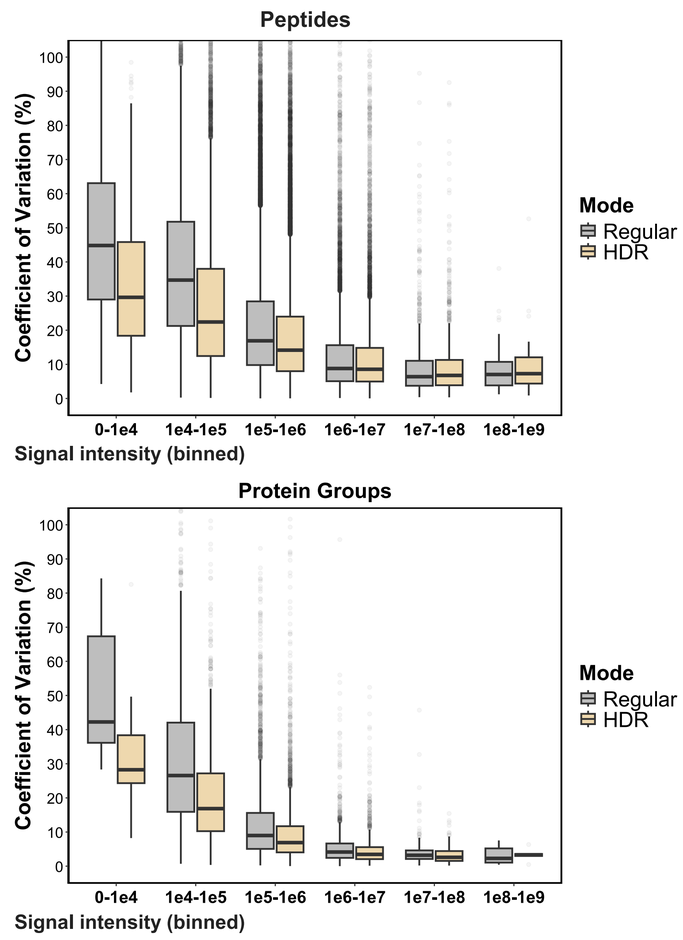

Figure 5. Comparison of number of features (protein groups, peptides), sorted into bins of signal intensity (0-1e4, 1e4-1e5, 1e5-1e6, 1e6-1e7, 1e7-1e8, 1e8-1e9). DIA runs with HDR MS1 full scans display significantly lower CVs for features with lower signal intensity.