Evaluation of a modified Orbitrap Astral Mass Spectrometer for label-free quantitation of proteomic samples

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Abstract

Purpose: Evaluation of label-free quantitation performance of the new Thermo Scientific™ Orbitrap™ Astral™ Zoom™ mass spectrometer using a multi-organism dataset of proteomes mixed in known ratios, with sample loads ranging from 100pg to 1µg. The results were compared to those from a standard Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer.

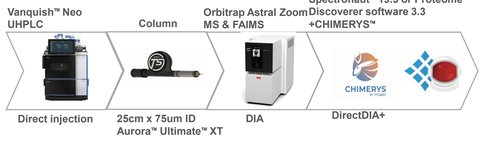
Methods: 30 and 50SPD, Orbitrap Astral MS and Orbitrap Astral Zoom MS

Results: Improved performance of Orbitrap Astral Zoom MS: on average, 5-7% more IDs on the protein level, 10-15% on the peptide level, 15% increase in the median number of data points per peak.

Introduction

The demand for high-throughput proteomics analysis of complex samples is continually increasing, focusing on greater analysis depth and improved precision and accuracy in quantitation. Label-free quantitation of very complex proteomic samples poses significant challenges for mass spectrometers, requiring faster and more efficient processing of eluting peptide ions and their fragments. The new Orbitrap Astral Zoom mass spectrometer addresses these challenges, offering a higher acquisition rate by improved ion optics switching times and faster ion transfer, higher sensitivity due to ion pre-accumulation, and enhanced spectral processing, leading to deeper coverage with higher confidence.

Analytical Workflow



Materials and methods

Sample: 3 proteome mix (HeLa, Yeast, E.coli), two mixtures: H65-Y15-E20 (mix A) and H65-Y30-E5 (mix B)
- High loads (10-100ng): lyophilized peptides re-dissolved in 0.015% DDM:0.1% FA (aq) to obtain 500 ng/µL stocks, used for Mix A and Mix B preparations. Low loads (100pg-10ng): 100 ng/µL stocks were used to prepare Mix A and Mix B, which were further diluted to 5 ng/µL before injection.

Vanquish Neo: Direct injection mode using 25cm x 75 µm ID Aurora Ultimate TS column (IonOpticks)
- High loads: 37min gradient; 4-45%B and injection-to-injection time 48min (30SPD)
- Low loads: 20min gradient; 1-40%B and injection-to-injection time 28min (50SPD)

Mass Spectrometer: Orbitrap Astral and Orbitrap Astral Zoom
- Orbitrap (MS1) and Astral (MS2) detectors
- Orbitrap: 240K; m/z range 380-980; AGC 500%; max IT 5ms
- NCE 25; Thermo Scientific™ FAIMS Pro Duo (low loads): -48V; gas 3.5 L/min
- Astral: DIA; window size depending on loads (in the tables below); m/z 150-2000; AGC 800% (low loads) or 100% (high loads); max IT depending on load; cycle time: 0.6s

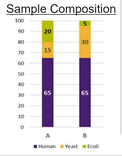
Data Processing
- Spectronaut® v19.5 Direct DIA, 6 files (2 x 3 replicates) processed together for each load; all other settings were default
- Thermo Scientific™ Proteome Discoverer™ software 3.3.0.54, CHIMERYS™ 4.0.21
- Human, E.coli, and Yeast FASTA (all w/o isoforms) from UniProt; normalization by Human FASTA

DIA Window Sizes and Injection Times:

Load per run	Astral DIA Isolation width, Th	Astral Injection Time, ms
10-20 ng	5	10
50 ng	4	8
100 ng	3	5
200-1000 ng	2	3

Load per run	Astral DIA Isolation width, Th	Astral Injection Time, ms
100 pg	20	60
250 pg	20	40
500 pg	15	40
1-2 ng	10	20
5-10 ng	5	10

Sample Composition



Results (High Loads)

The new Orbitrap Astral Zoom mass spectrometer takes advantage of ion pre-accumulation in the bent trap, increasing the effective MS2 injection time. Its reduced ion transfer overheads result in a higher MS2 acquisition rate of up to 270 scans per second. Additionally, an enhanced spectral processing algorithm was implemented to deconvolute overlapping spectral features in MS2, for better handling spectra with increased spectrum density.

Using Orbitrap Astral Zoom MS and Spectronaut® 19.5 software, there was a 12-17% increase in IDs at the precursor level, 10-15% at the peptide level (stripped sequence), and 4-9% at the protein groups level. With Proteome Discoverer software, the gain was 12-19% at the DIA precursor level, 12-17% at the peptide level, and 4-7% at the protein group level. The increase in identifications was primarily observed for low-abundant species. Orbitrap Astral Zoom mass spectrometer also yielded, on average, a 15% higher median number of MS2 data points per peak, while maintaining consistently high quantitation precision and accuracy at the MS2 level.

Figure 1. A comparison of Protein and Peptide IDs obtained with Orbitrap Astral Zoom MS and Orbitrap Astral MS (SN19.5 results)

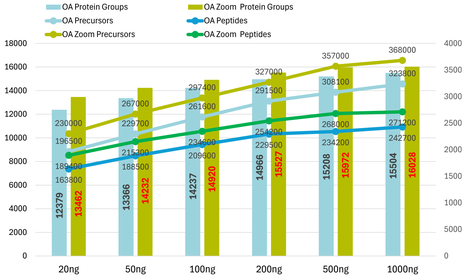
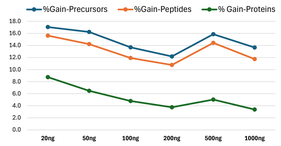
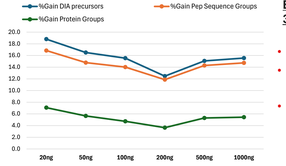


Figure 2. % ID Gain for Orbitrap Astral Zoom MS vs Orbitrap Astral MS at 30SPD and 20 - 1000ng of 3-proteome-mix samples



A. Spectronaut 19.5 results

- 12-17% increase at the precursor level
- 10-15% at the peptide level
- 4-9% at the protein level



B. Proteome Discoverer software 3.3/CHIMERYS 4.0 results

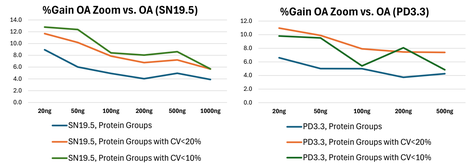
- 12-19% increase in DIA precursors
- 12-17% increase in peptide sequence groups
- 4-7% increase in protein groups

Figure 3. Median CV (%) at Protein Groups and Precursor levels obtained with Orbitrap Astral MS and Orbitrap Astral Zoom MS (triplicate runs, 3-proteome mix, SN19.5)

		SN Protein Groups			SN Peptide Groups		
		SN19.5, OA, %CV, Protein Groups mixA	SN19.5, %OA, CV, Protein Groups mixB	Median %CV, Average	SN19.5, Astral, %CV, Peptides mixA	SN19.5, Astral, %CV, Peptides mixB	Median %CV, Average
Orbitrap Astral MS	20ng	5.7	5.4	5.55	14.6	14.2	14.4
	50ng	4.9	4.9	4.9	13.2	13.4	13.3
	100ng	4.4	4.3	4.35	12.2	12.1	12.2
	200ng	4.3	4.2	4.25	12.7	12.9	12.8
	500ng	3.7	3.9	3.8	11.3	11.0	11.2
	1000ng	3.5	3.6	3.55	10.6	10.6	10.6
	Average All			**4.4**			**12.4**
		SN Protein Groups			**SN Peptide Groups**		
		SN19.5, OA Zoom, %CV, Protein Groups mixA	SN19.5, OA Zoom, %CV, Protein Groups mixB	Median %CV, Average	SN19.5, OA Zoom, %CV, Peptides mixA	SN19.5, OA Zoom, %CV, Peptides mixB	Median %CV, Average
Orbitrap Astral Zoom MS	20ng	5.4	5.2	5.3	14.6	14.7	14.7
	50ng	4.6	4.5	4.55	13.5	13.4	13.5
	100ng	4.4	4.3	4.35	13.4	13.2	13.3
	200ng	4.3	4.2	4.25	13.6	13.2	13.4
	500ng	3.9	3.9	3.9	12.3	12.7	12.5
	1000ng	4	4	4	13.1	12.9	13.0
	Average All			**4.4**			**13.4**

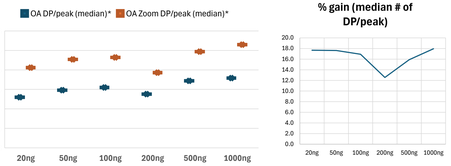
- For Orbitrap Astral Zoom MS, median CVs are the same as for Orbitrap Astral MS at the protein level, yet slightly higher at the precursor level. This can be explained by more low-abundant species picked up by Orbitrap Astral Zoom MS.
- The same trend was observed when the data was processed with SN and Proteome Discoverer software/ CHIMERYS software (data is available but not shown on this poster).

Figure 4. %Gain for Orbitrap Astral Zoom MS and Orbitrap Astral MS for proteins quantified with CV < 20% and 10% (SN and PD/CHIMERYLS results)



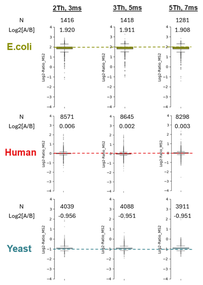
The % gain of Orbitrap Astral Zoom MS versus Orbitrap Astral MS is higher for proteins with CV<20% and CV<10% compared to the total protein ID gain, demonstrating that Orbitrap Astral Zoom produces higher-quality data. SN and PD yielded the same results in this context.

Figure 5. median number # of data points per peak for Orbitrap Astral Zoom MS vs. Orbitrap Astral MS in the (Proteome Discoverer software/CHIMERYS results)



*Statistics Insights, PD3.3

Figure 6. Impact of data points per peak on quan accuracy and precision in LFQ experiments at 30SPD



N corresponds to the numbers of Protein Groups quantified, filtered for missing values, and used for median Log2 ratio calculations.

Astral Settings (DIA Window, IT)	2Th; 3ms	3Th; 5ms	5Th; 10ms
Precursors	295157	297405	273648
Unique Peptides	236132	234610	215806
Protein Groups	14807	14940	14424
MS2 Data Points Per Peak, median*	3	4	5
CV (MixA-MixB), % (peptides), median*	14.9-14.8	13.4-13.2	13.5-13.2
CV (MixA-MixB), % (proteins), median*	4.7-4.8	4.3-4.4	4.5-4.6

*As reported in Spectronaut, Post Analysis

Astral Method	HUMAN Log2 Ratio	% Error	YEAST Log2 Ratio	% Error	ECOLI Log2 Ratio	% Error
2Th_3ms	0.006	**0.64**	-0.956	**-4.37**	1.920	**4.17**
3Th_5ms	0.002	**0.22**	-0.951	**-5.15**	1.911	**4.65**
5Th_10ms	0.003	**0.27**	-0.951	**-5.15**	1.908	**4.82**

- Three DIA methods were used for 100ng sample load, resulting in 3, 4, and 5 median data points per peak for peptide precursors. The chosen methods were reasonable for this load; using smaller or larger DIA windows or IT values would not be optimal for identifications.
- Method Evaluation: For a 100ng load, the DIA method with 3Th window and 5 ms IT was the best for obtaining maximum IDs and best CVs at peptide and protein group levels.
- Quantitation Accuracy: Based on the calculated median log2 values for the ratios of MS2 peak intensities of mixtures A and B, there was no significant difference between the three methods. All methods yielded results within 10% of the theoretical ratios.
- Quantitation Precision: All three methods yielded MS2 peak intensities of mixtures A and B with median CV less than 5% on the protein level, and the difference between the results was minimal.

Results (Low Loads)

Figure 7. A comparison of Protein Groups and Peptide IDs obtained with Orbitrap Astral Zoom MS and Orbitrap Astral MS at low loads with FAIMS (SN 19.5 results)

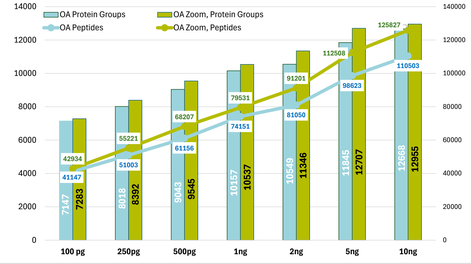
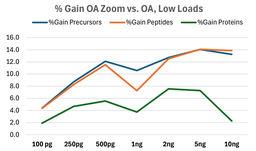


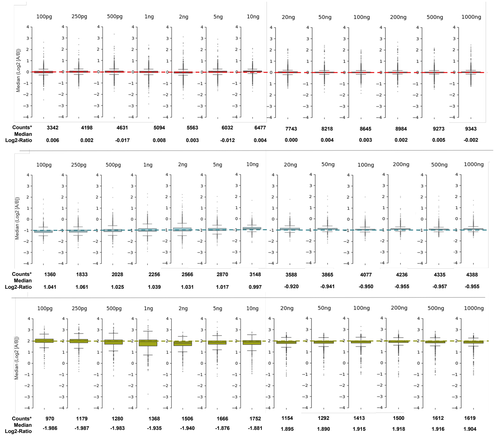
Figure 8. % ID Gain for Orbitrap Astral Zoom MS vs Orbitrap Astral MS at 50SPD and100pg-10ng loads of 3-proteome-mix samples



- 4-14% increase at the precursor and peptide level
- 2-8% increase at the protein level

With the new low input application mode, the single ion detection probability is increased from 85% to 95%, thereby improving peptide and protein identifications.

Figure 9. Label-Free Quantitation accuracy achieved with Orbitrap Astral Zoom MS at a load range spanning four orders of magnitude (100pg-1µg)



Conclusions

The novel Orbitrap Astral Zoom mass spectrometer demonstrates improved performance for qualitative and quantitative analysis of complex proteomics samples over a wide range of sample loads spanning four orders of magnitude, from 100 pg to 1µg.

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