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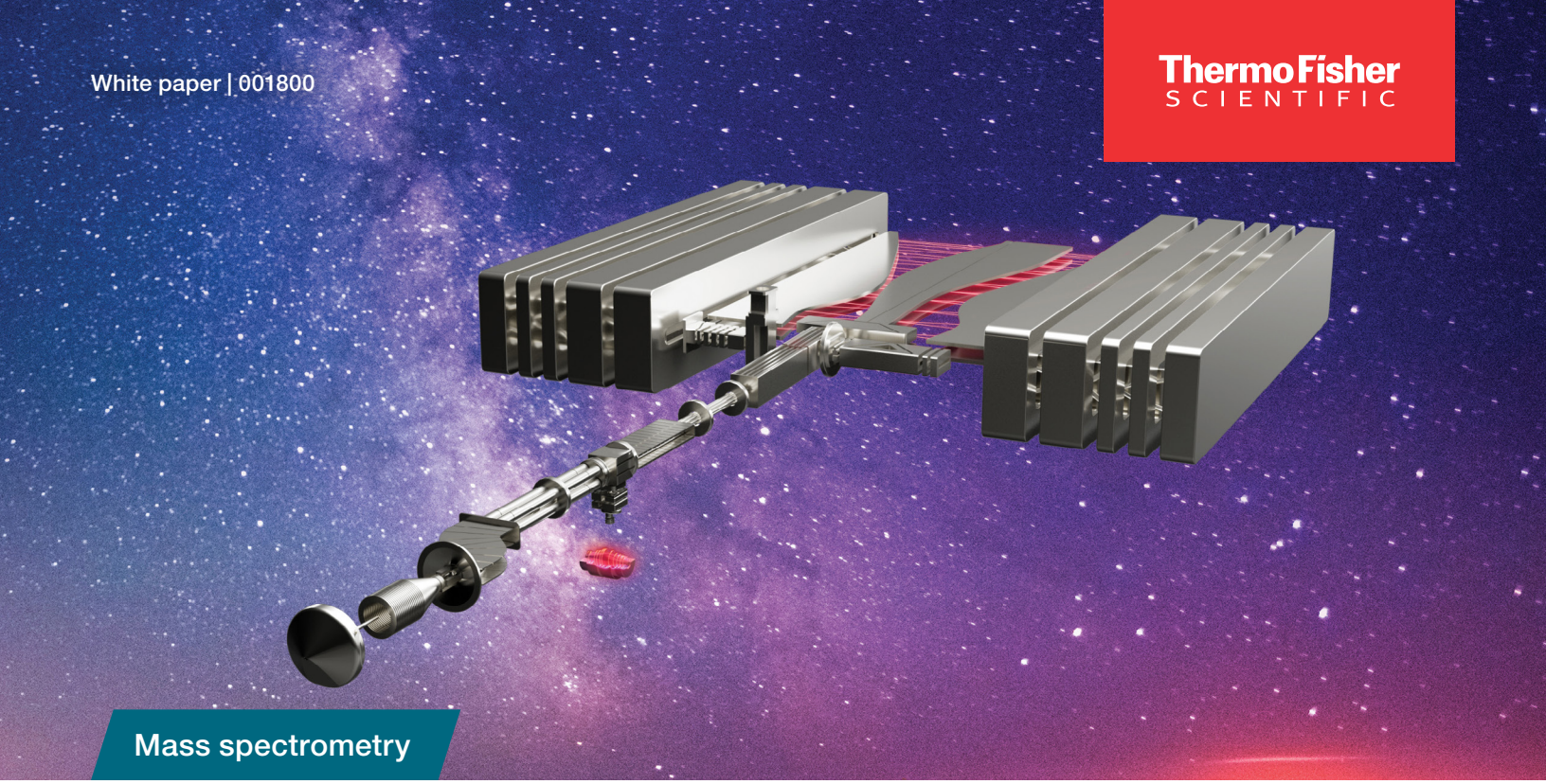

Mass spectrometry

Rethink what is possible with the Orbitrap Astral mass spectrometer

Faster throughput, deeper coverage, and higher sensitivity with accurate and precise quantitation

Authors

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Keywords

Orbitrap Astral mass spectrometer, general proteomics, translational proteomics, single-cell proteomics, DIA, TMT, biopharma, pharma research, disease biology, academic, government, life science research, mass spectrometry, grant writing

Introduction

Powered by the synergy of a high-resolution quadrupole mass filter, the Thermo Scientific™ Orbitrap™ mass analyzer and the novel Thermo Scientific™ Astral™ mass analyzer, this revolutionary new instrument achieves unsurpassed performance and experimental flexibility. The combination of the three mass analyzers enables the rapid acquisition of exceptional quality high resolution accurate mass (HRAM) spectra with high sensitivity and dynamic range. The new performance characteristics of the Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer make it ideally suited for accurate and precise quantitation at an unprecedented depth of coverage and throughput for samples from single cells to body fluids to bulk tissues.

The instrument

Orbitrap Astral mass spectrometer architecture

The front end, from the ion source up to the segmented quadrupole mass filter, is designed to maximize instrument sensitivity and robustness. Ions are trapped and concentrated in the ion routing multipole, which facilitates the transfer of ions to either of the HRAM analyzers. Synchronization of ion transfer and processing throughout the instrument enables the parallel handling of five separate ion packets simultaneously. This synchronization enables each mass analyzer to be used in combination to optimize performance and experimental flexibility. The Orbitrap analyzer is ideally suited for the acquisition of high dynamic range HRAM MS and MS^2 spectra, including an optional Biopharma mode to extend the mass range up to *m/z* 8,000. The Astral mass analyzer delivers high acquisition rates up to 200 Hz, single ion detection sensitivity, and high dynamic range HRAM SIM and MS^2 measurements that are fully synchronized with parallel Orbitrap analyzer operation. Due to this synchronization the Orbitrap Astral mass spectrometer excels at many data acquisition strategies including High Resolution Data-Independent Acquisition (HR-DIA) and data-dependent acquisition (DDA). These acquisition strategies combined with the high resolution and dynamic range of the Orbitrap and Astral analyzers enable accurate and precise quantitation using either Label-Free Quantitation (LFQ) or multiplexing with TMT™-labeled peptides. The schematic of the Orbitrap Astral mass spectrometer is shown in Figure 2.

Figure 1. The Orbitrap Astral mass spectrometer

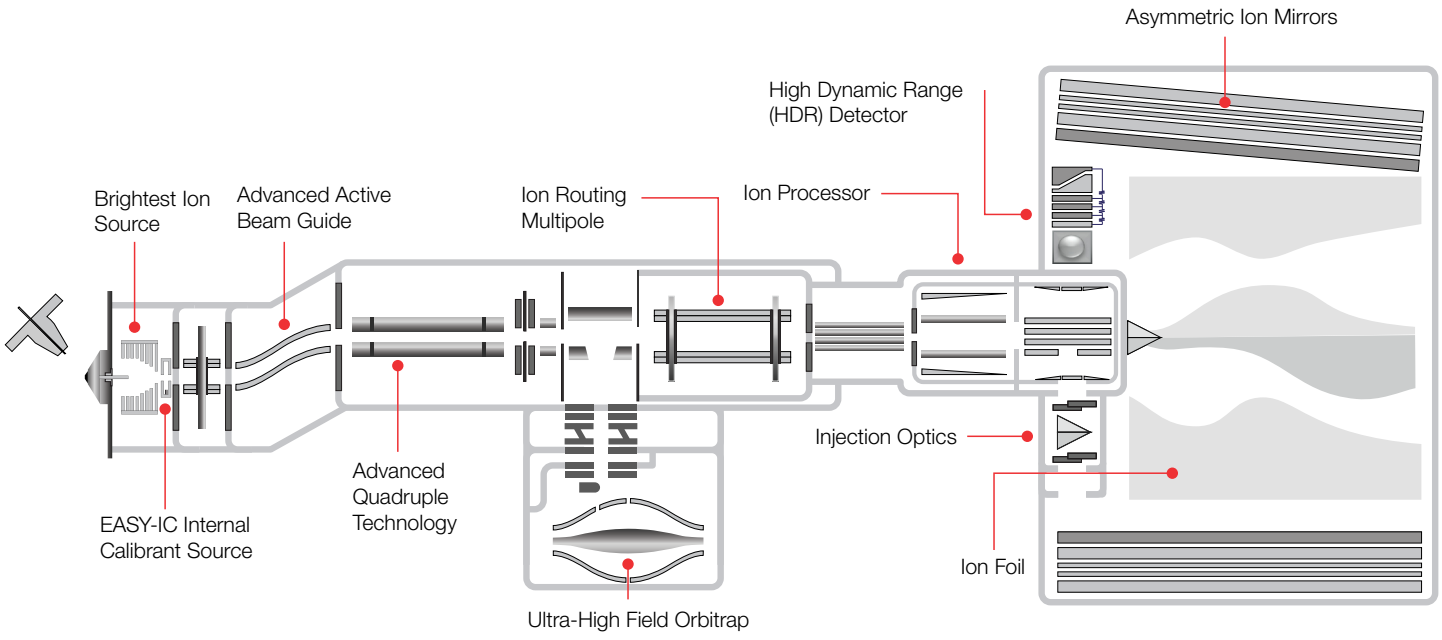

Figure 2. Instrument diagram of the Orbitrap Astral mass spectrometer

Astral mass analyzer operation

The Astral mass analyzer is a novel HRAM analyzer that is designed to deliver sensitive high dynamic range spectra at a high acquisition rate. The combined performance characteristics of the Astral and Orbitrap analyzers enable unprecedented sample throughput, deeper coverage and higher sensitivity including when working with low level samples, such as single cells, without sacrificing quantitative accuracy and precision.

For MS^2 acquisition in the Astral analyzer, the incoming ion beam is filtered in the Quadrupole to a specific isolation window, precursor ions are trapped and concentrated in the Ion Routing Multipole, and then transferred into the Ion Processor where they are trapped and fragmented at rates up to 200 Hz. The fragment ions are then carefully prepared for extraction in the low-pressure region of the Ion Processor and ejected through a series of Ion Optics that precisely align the ion packet to improve sensitivity. The ion packet travels through an open electrostatic trap and follows an over 30-meter-long asymmetric track, guided by the Asymmetric Ion Mirrors and Ion Foil. The Ion Foil consists of electrodes that shape and actively focus the ions in 3-dimensions during their zig-zag-like transversal asymmetric oscillations between the Asymmetric Mirrors, maximizing transmission throughout the analyzer while maintaining high resolution. The over 30-meter-long asymmetric track is achieved by a small tilt of one of the mirrors. This asymmetry of the mirrors generates an electrostatic force that returns the ions back on the asymmetric track to the High Dynamic Range Detector. The long track length allows for spectral resolution of 80,000 FWHM at *m/z* 524, sufficient to resolve the TMTpro™ 18plex isobaric reporter ions. The novel 2-channel High Dynamic Range Detector provides sensitive single ion detection with excellent linearity over multiple orders of magnitude, low noise, and a long lifetime due to an isolated vacuum chamber where it is located. Table 1 describes some of the key characteristics of the Astral analyzer.

Table 1

Key characteristics of the Astral analyzer	
Sensitivity	Single ion detection
HRAM scan rate	Up to 200 Hz
Intrascan dynamic range	>1,000 with a single microscan in a single spectrum at 200 Hz
Resolution	80,000 at *m/z* 524
Mass accuracy	RMS <5 ppm

Proteomics performance

Faster throughput

With fast and sensitive HRAM MS^2 capabilities, the Orbitrap Astral mass spectrometer enables identification of over 8,000 protein groups (Figure 3) from a complex Human whole cell lysate sample with a 5.5-minute gradient and an injection-to-injection cycle time of 8 minutes, resulting in a throughput of 180 samples per day (SPD). Now, it is possible to analyze four samples in the time it used to take to measure one with an equivalent or better depth of coverage. This breaks through the existing bottleneck of sample throughput to empower large-scale proteomics studies.

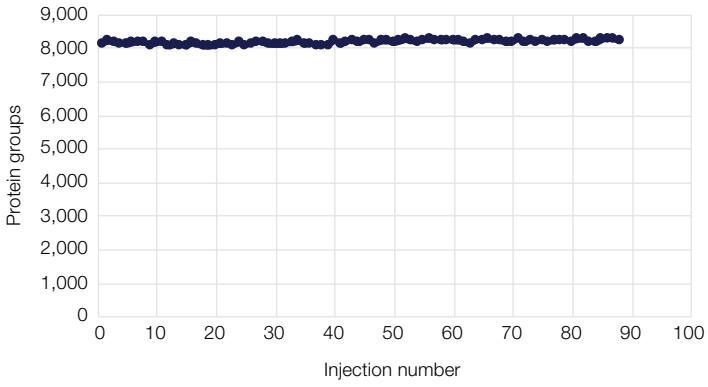

Figure 3. Faster throughput with the Orbitrap Astral mass spectrometer identifies over 8,000 proteins from every single injection of 200 ng of HeLa with a run-to-run time of 8 minutes demonstrating reproducible performance with continuous acquisition at a high sample throughput.

Experimental conditions

Thermo Scientific™ Pierce™ HeLa digest standard (200 ng) was analyzed using an 8-minute long method (injection-to-injection) on a Thermo Scientific™ Vanquish™ Neo UHPLC system (operated in a Trap and Elute mode) and a Thermo Scientific™ EASY-Spray™ PepMap™ 15 cm × 150 µm column. The Orbitrap Astral mass spectrometer was operated with 240,000 FWHM full MS in the Orbitrap analyzer and DIA *m/z* scan range of 380 to 980 using quadrupole isolation window widths of 2 Th with the Astral analyzer, normalized AGC target of 500%, 3 ms maximum injection time, and 40% source-RF. Data was processed with the CHIMERYS™ intelligent search algorithm using Thermo Scientific™ Proteome Discoverer™ 3.1 software and filtered to <1% FDR.

Deeper single-shot coverage

The Orbitrap Astral mass spectrometer delivers incredibly deep coverage from single-shot proteomics experiments, identifying over 9,000 proteins in an 11-minute gradient (100 SPD) or over 12,000 proteins in 60 minutes (24 SPD). The flexibility of the system allows a balancing of throughput and depth, based on the experimental needs (Figure 4).

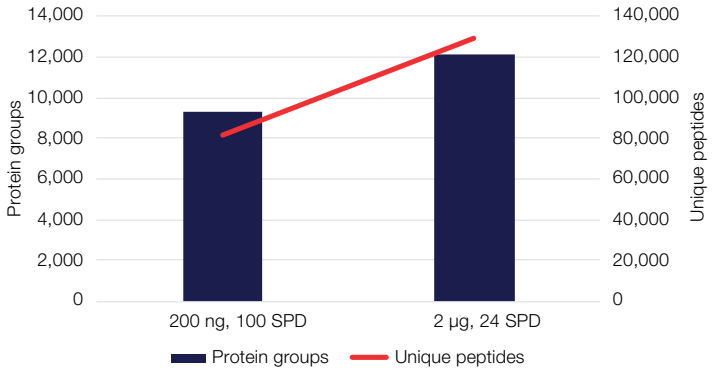

Figure 4. Exceptionally high proteome coverage using single-shot methods at 100 SPD and 24 SPD using HeLa digest.

Experimental conditions

HeLa digest (0.2–2 µg) was separated using various gradient lengths on a Vanquish Neo UHPLC system and an EASY-Spray PepMap 15 cm × 150 µm column (for 100 SPD) or a Thermo Scientific™ µPAC™ Neo 110 cm column (for 24 SPD). The Orbitrap Astral mass spectrometer was operated with 240,000 FWHM full MS in the Orbitrap analyzer, normalized AGC target of 500%, and DIA *m/z* scan range of 380 to 980, isolation window width of 2 Th in the Astral analyzer, normalized AGC target of 500%, 3.5 ms maximum injection time, and 40% source-RF. The data was further processed with CHIMERYS using Proteome Discoverer 3.1 software and filtered to <1% FDR.

Deeper multi-shot coverage

While essentially complete genomes can be readily sequenced, complete proteomes have so far eluded comprehensive coverage. Using multi-shot approaches with fractionated samples enables deep proteome coverage[1], however, at a low throughput of 30+ hours per sample. With the Orbitrap Astral mass spectrometer, it is possible to identify over 15,000 protein groups from a high-pH reversed-phase fractionated Human cell line digest sample using such multi-shot approaches in just 4.5 hours. This depth of coverage and faster throughput allows for the acquisition of up to eight nearly complete proteomes per day, unlocking a new era of whole proteome sequencing.

Higher sensitivity for single-cell proteomics

Higher sensitivity is essential for proteomics experiments, especially when sample amounts are extremely low. The Orbitrap Astral mass spectrometer delivers higher sensitivity and higher dynamic range HRAM MS^2 due to the high transmission and sensitivity of the Astral analyzer, allowing deep proteome coverage that was previously impossible to achieve with any methods. With a low input sample of 250 pg of standard HeLa digest, the Orbitrap Astral mass spectrometer enables reproducible identification of over 5,800 proteins with a throughput of 80 SPD using a spectral library and over 4,300 proteins with a directDIA approach—nearly double the number of identifications possible with current instruments while simultaneously doubling the throughput (Figure 5). The increased depth of coverage empowers a greater understanding of single-cell heterogeneity through the measurement of additional biologically critical proteins. At the same time, the throughput is doubled to measure more single cells in each study in a shorter period of time to increase statistical power.

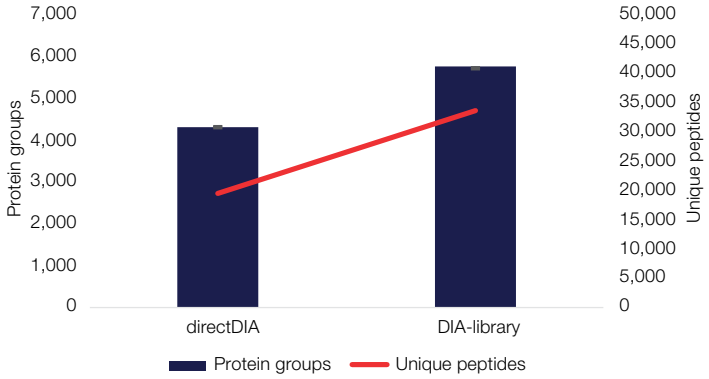

Figure 5. The analysis (n = 3) of 250 pg of standard HeLa digest with the Orbitrap Astral mass spectrometer run at a throughput of 80 SPD analyzed with directDIA identifies over 4,000 proteins and over 5,800 proteins when using a spectral library approach using Spectronaut 17 software.

Experimental conditions

HeLa digest (250 pg) was analyzed in triplicates with a throughput of 80 samples per day using 18 minute methods (injection to injection) on a Vanquish Neo UHPLC system and a 50 cm Thermo Scientific™ µPAC™ Neo low load column. The MS was operated with 240,000 FWHM full MS using the Orbitrap analyzer, 500% AGC target and DIA MS^2 with the Astral analyzer using a scan range of 400 to 800, 800% AGC target, isolation widths of 20 Th, 40 ms maximum injection time, 40% source-RF and Thermo Scientific™ FAIMS Pro Duo interface with a CV of –50V. The data was processed using Spectronaut™ 17 software (Biognosys) with a directDIA workflow or using a spectral library generated from 3 replicates of 250 pg, 500 pg, 1 ng, 2.5 ng, 5 ng and 10 ng HeLa DIA runs acquired on the Orbitrap Astral mass spectrometer using the 80 SPD method as described above. Match-between runs (MBR) was enabled.

Accurate and Precise Label-Free Quantitation (LFQ)

The Orbitrap Astral mass spectrometer significantly extends the dynamic range of quantitation due to the high acquisition speed and sensitivity of the Astral analyzer paired with the high resolution and dynamic range of the Orbitrap analyzer. When working with 3-proteome mixtures of Human, *E. coli*, and Yeast mixed with a constant amount of Human proteome and varying amounts of *E. coli* and Yeast proteomes, accurate and precise relative quantities can be obtained while identifying over 13,000 proteins, including nearly the entire Yeast and *E. coli* proteomes (Figure 6). Over 87% of identified proteins had a CV<20% and over 72% had a CV<10% across the 3 proteomes from a 500 ng total mixed proteome sample per injection. The median CV of all proteins was 4.7% in sample mix A and 4.5% in sample mix B (Figure 7). Both the accuracy and precision of quantitation were very high. The obtained ratio of average abundances for Human proteome was 1.017 (median value), while the expected ratio was 1 (based on 7,632 Human proteins; FDR 1%). For Yeast proteome, the obtained ratio of average abundances was 0.491 (median value), while the expected ratio was 0.5 (based on 4,094 yeast proteins; FDR 1%). For *E. coli* proteome, the obtained ratio of average abundances was 1.998 (median value), while the expected ratio was 2 (based on 1,402 *E. coli* proteins; FDR 1%). Run-to-run reproducibility of quantitation was higher than 99% for the triplicate runs.

This quantitative accuracy and precision is sufficient to differentiate protein amount changes as small as 1.5-fold in complex mixtures for both high and low abundance proteins, increasing statistical power and providing confidence in measuring small changes in complex systems.

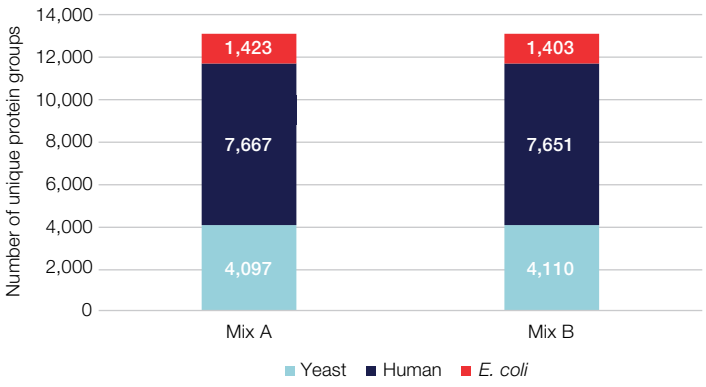

Figure 6. Accurate and precise quantitative performance of the Orbitrap Astral mass spectrometer. Over 13,000 proteins were identified across the three proteomes, including nearly complete Yeast and *E. coli* proteomes (1% FDR).

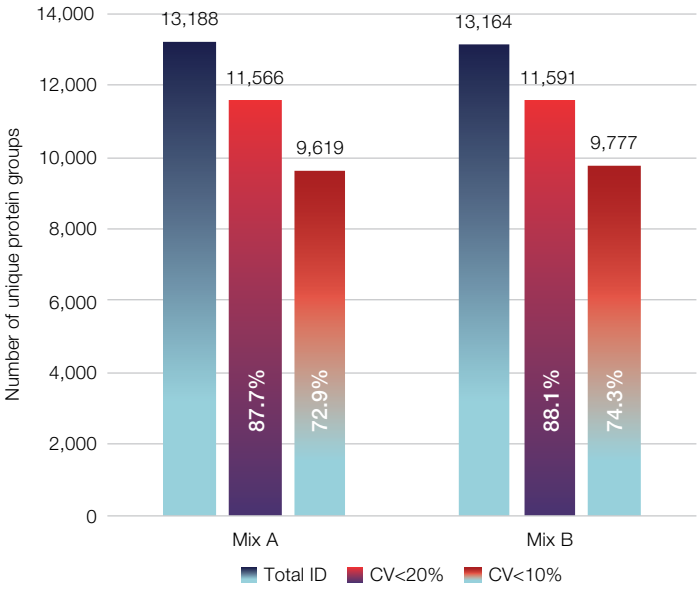

Figure 7. In addition to the deep proteome coverage, over 87% of identified proteins had CV<20% and over 72% of identified proteins had CV<10%.

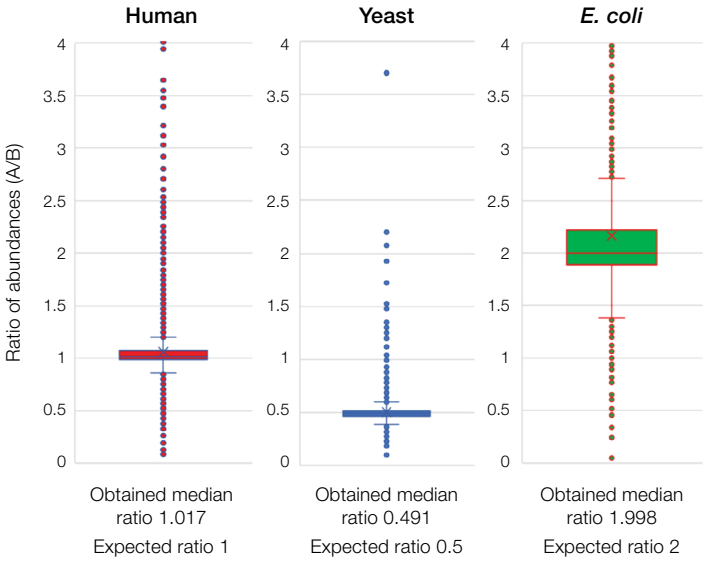

Figure 8. Proteome ratios calculation for 3-proteome mixtures A (36% *E. coli*, 46% HeLa, and 18% Yeast) and B (18% *E. coli*, 46% HeLa, and 36% Yeast). The obtained ratio of average abundances for Human proteome was 1.017 (median value), while the expected ratio was 1 (based on 7,632 Human proteins identified out of a total of 13,227; FDR 1%). For Yeast proteome, the obtained ratio of average abundances was 0.491 (median value), while the expected ratio was 0.5 (based on 4,094 Yeast proteins identified out of a total of 13,227; FDR 1%). For *E. coli* proteome, the obtained ratio of average abundances was 1.998 (median value), while the expected ratio was 2 (based on 1402 *E. coli* proteins identified out of a total of 13,227; FDR 1%).

Experimental details

A 3-proteome mix sample containing digests of HeLa, *E. coli* and Yeast (S. *cerevisiae*) cells was mixed in two different ratios (v/v), as follows: **Mixture A:** 36% *E. coli*, 46% HeLa and 18% Yeast, and **Mixture B:** 18% *E. coli*, 46% HeLa and 18% Yeast. Each mixture was analyzed using a 20-minute gradient on a Vanquish Neo UHPLC operated in direct injection mode, on a 50 cm Thermo Scientific™ µPAC™ Neo column, at a 250 nL/min analytical flow rate. The Orbitrap Astral mass spectrometer was operated with 240,000 FWHM full MS using the Orbitrap analyzer, normalized AGC target of 500%, and DIA MS^2 using the Astral analyzer with a *m/z* bin range of 380 to 980, isolation window width of 2 Th, normalized AGC target of 500%, 3 ms maximum injection time, MS^2 *m/z* range of 150 to 2,000 and 40% source-RF. The data was processed with Spectronaut 17 software using DirectDIA.

TMTpro 18plex isobaric labeling multiplexed quantitation

The Orbitrap Astral mass spectrometer also improves the throughput and depth of coverage with isobaric tag labeling techniques, such as TMT. Since the Astral analyzer combines high speed and sensitivity with high resolution and accurate mass, it can readily resolve the isobaric TMT reporter ions around *m/z* 130 (see inset example spectrum in Figure 9). The analyzer is designed to transmit and detect ions over a wide *m/z* range, such that both TMT reporter ions and MS^2 peptide fragment ions are measured in the same spectrum. The parallelization of acquisition in the Orbitrap and Astral analyzers enables the synchronization of high resolution and high dynamic range full MS in the Orbitrap analyzer with simultaneous acquisition of fast and sensitive Astral MS^2 spectra, transforming TMT-based experiments by significantly increasing the depth of coverage and throughput. With fractionated samples (Figure 9), the Orbitrap Astral mass spectrometer can quantify over 8,000 18-plexed proteins in only two fractions, each analyzed with a 90 minute LC/MS run (excluding sample loading time), equivalent to an LFQ throughput of 144 samples per day. With four fractions nearly 10,000 proteins are quantified at a throughput of 72 samples per day, and with 8 fractions 11,000 proteins are quantified at a rate of 36 samples per day, per proteome. The increased MS^2 sensitivity and spectral acquisition rate facilitate deep quantitative proteome coverage at unprecedented throughput (data courtesy Steve Gygi, Harvard Medical School, Cambridge, MA).

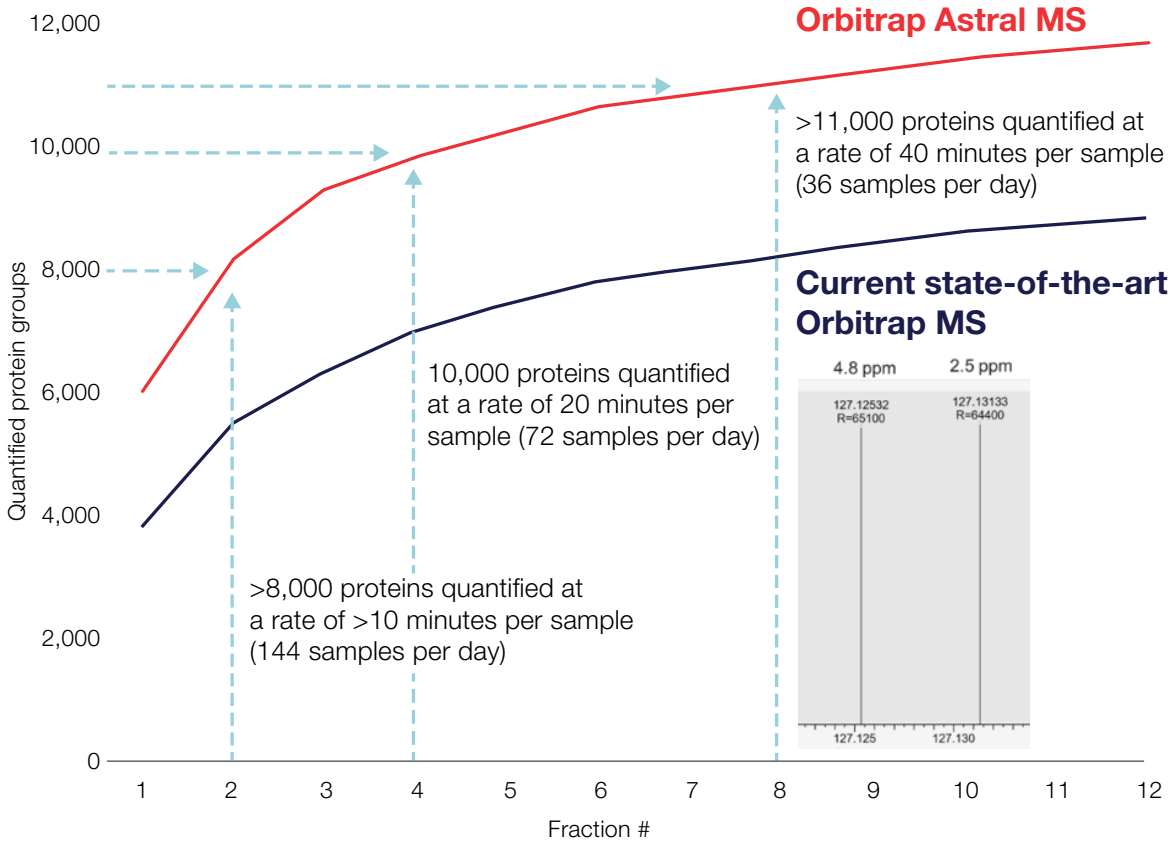

Figure 9. The Orbitrap Astral mass spectrometer quantified over 8,000 proteins using TMTpro 18-plex tags with only 2 fractions analyzed using 90-minute gradients, equivalent to a throughput of 144 samples per day. With 4 fractions over 10,000 proteins are quantified at a throughput of 72 samples per day, and with 8 fractions over 11,000 proteins are quantified at a rate of 36 samples per day. The enhanced depth and throughput of the Orbitrap Astral mass spectrometer combined with TMT multiplexing facilitates deep quantitative proteome coverage at speed. Inset: the Astral analyzer combines high speed and sensitivity with high resolution and accurate mass, it can readily resolve the isobaric TMT reporter ions around *m/z* 130.

Experimental details

Preparation of cell line samples: Samples preparation was as described by Navarrete-Perea and co-workers.[2]

Mass spectrometry: The sample was separated using a Vanquish Neo UHPLC system, applying a 90-minute gradient onto a 75 µm ID × 75 cm EASY-Spray PepMap column with direct injection mode. The ESI generated ions were further fractionated with a FAIMS Pro Duo interface with 3 CV's (–40 V, –60 V, –80 V). The Orbitrap Astral mass spectrometer was operated in DDA mode with full MS detected in the Orbitrap analyzer at 240,000 FWHM resolution from *m/z* 350–1,500. Precursors were selected in data dependent manner using a 0.5 Th isolation width and 50% purity threshold/APD off; MS^2 were detected with the Astral analyzer with a maximum injection time of 20 ms.

Bioinformatics: Data collected for library construction were searched using the open-source Comet search engine (ver. 2021.01.0).[3] Depending on the sample type involved, the UniProt Human proteome database (downloaded December 21, 2018) was used with contaminants and decoy sequences appended. Precursor error tolerance was set to 50 ppm, and fragment error tolerance was 0.02 Da. Static modifications were searched including carboxyamidomethylation of Cys (+57.0215), as well as TMTpro16 (+304.2071) on Lys side chains and peptide N-termini. A maximum of 3 methionine oxidation (+15.9949) events were allowed as variable modification. Search results were first filtered to a 1% peptide FDR using linear discriminant analysis employing a target-decoy strategy and further filtered to obtain a protein level FDR 1%.[4-6]

Phosphopeptide analysis

Genomic sequencing does not capture the entire complexity of biology because proteins can undergo additional post-translational modifications or exist in complexes, just two examples that can dramatically alter the function of a protein among other non-genetically coded changes. The Orbitrap Astral mass spectrometer improves the throughput and depth of coverage for studying such post-translationally modified proteins. For example (Figure 10), it enables double the depth of coverage vs. current state-of-the-art Orbitrap mass spectrometry analysis for both phosphopeptides and Class I phosphosites, reaching nearly 20,000 Class I phosphopeptides in just 8 minutes using 50 ng of enriched sample. Remarkably, this is a deeper coverage than achievable with the current best technology in 30 minutes, enabling a 4× improvement in the throughput.[7] In a 30-minute gradient, nearly maximum phosphopeptide and Class I phosphosite identification was achieved even when using just 50 ng of input material (data courtesy Josh Coon, University of Wisconsin–Madison, WI).

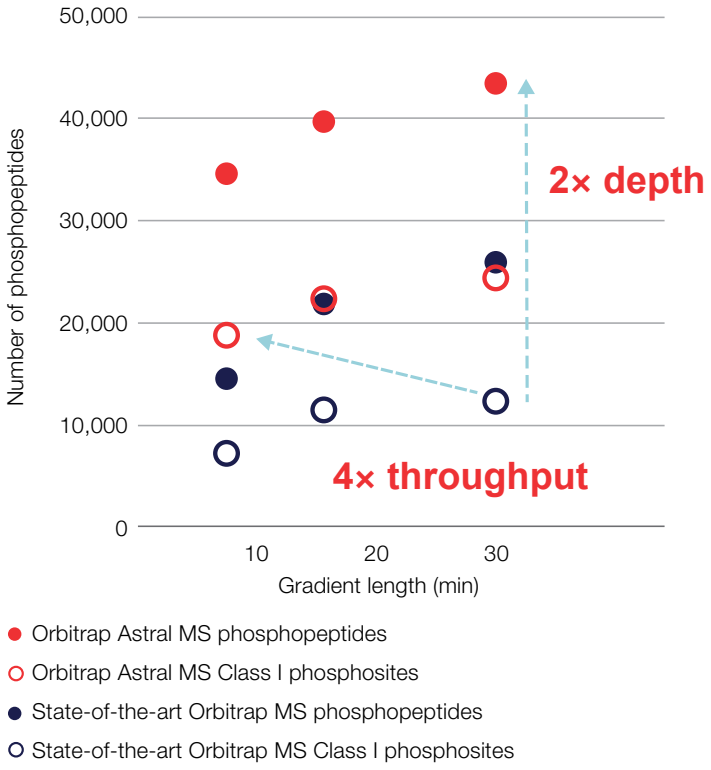

Figure 10. The Orbitrap Astral mass spectrometer doubles the depth of coverage vs. current state-of-the-art Orbitrap mass spectrometer analysis for both phosphopeptides and Class I phosphosites, reaching nearly 20,000 Class I phosphopeptides in just 8 minutes using 50 ng of enriched sample. Remarkably, this is a deeper coverage than achievable with the current state-of-the-art Orbitrap mass spectrometer in 30 minutes, enabling a 4× improvement in the throughput.

Experimental details

Protein purification and digestion: Phosphopeptide samples were prepared using HEK293T cells according to Batth and co-workers.[8]

LC/MS conditions: A reverse-phase column was made in-house using a 75 µm ID, 360 µm OD bare fused silica capillary and packed with 1.7 µm diameter, 130 Å pore size ethylene bridged hybrid C18 particles (Waters) to a length of 40 cm. The column was installed on a Vanquish Neo UHPLC system and heated to 50°C. Active gradients were adjusted from 7 minute to 30 minute and data was collected using both data independent acquisition (DIA) and data dependent acquisition (DDA) schemes. For DIA, all MS survey spectra were collected at a resolving power of 240,000 at 200 *m/z* in the Orbitrap analyzer with a scan range of 380 to 980 *m/z*, an AGC target of 100% and a maximum injection time of 50 ms. DIA scans were collected in the Astral analyzer with fixed isolation widths of 1 to 4 Th, bin range from 380 to 980 *m/z*, with MS^2 AGC target of 500%, and maximum injection

time ranging from 3.5 ms to 20 ms, with 0.6 s cycle times between MS scans. DDA experiments were performed with MS scans in the Orbitrap analyzer of resolving power 120,000 at 200 *m/z*, MS^2 scans in the Astral analyzer, maximum injection time of 3.5 ms to 20 ms, quadrupole isolation width of 0.7 Th, and a cycle time of 0.6 s for z = 2–5 precursors.

Bioinformatics: The data was processed using Spectronaut 17 software.

Science at scale for translational proteomics

With the ability to achieve much higher throughput and generate the highest-quality data from notoriously difficult plasma samples, the applications of the Orbitrap Astral mass spectrometer extend from early-stage discovery through to translational research and beyond. While plasma is one of the most common types of samples analyzed in clinical studies, the ability to screen large cohorts of these samples has been limited by the detection and quantitation limits of analysis. In particular, the high prevalence of albumin and a few other proteins create a large dynamic range and, as a result, difficulties achieving high protein coverage, especially at high throughput. The Orbitrap Astral mass spectrometer offers significant analytical advancements for neat plasma analysis, achieving reproducible coverage of over 600 proteins in 8 minute experiments, due to its exceptional MS^2 sensitivity and dynamic range through the use of narrower DIA isolation windows to decrease co-isolation of very abundant peptides. A longer experiment enables identification of over 1,000 protein groups from neat plasma, opening new possibilities for the discovery of clinically relevant biomarkers that were previously inaccessible (Figure 11).

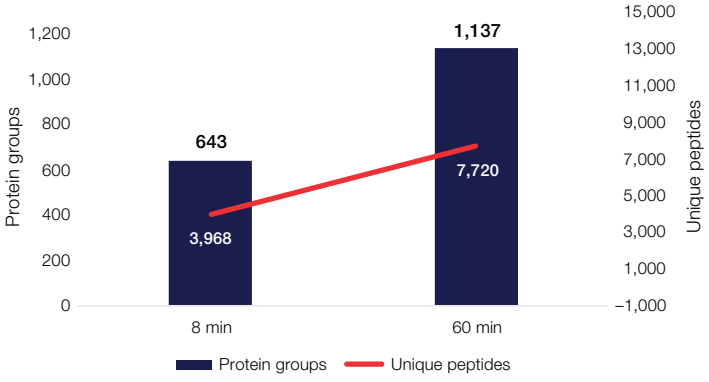

Figure 11. The Orbitrap Astral mass spectrometer offers significant analytical advancements for neat plasma analysis, achieving reproducible coverage of over 600 proteins in 8 minute experiments with just a 5.5-minute active gradient, due to its exceptional MS^2 sensitivity and dynamic range through the ability to use narrower DIA isolation windows to decrease co-isolation of very abundant peptides. A longer experiment enables identification of over 1,000 protein groups.

Experimental details

A tryptic digest of neat Human plasma was analyzed with an 8-minute method (injection-to-injection) using a Vanquish Neo UHPLC system (operated in a Trap and Elute mode) and an EASY-Spray PepMap 15 cm × 150 µm column or a uPAC Neo 110 cm × 75 µm column using a 60-minute method (injection to injection) in direct injection mode. Two hundred ng of neat plasma was loaded on the column for the 8-minute method and 1 µg for the 60-minute method. The MS was operated with 240,000 FWHM full MS using the Orbitrap analyzer and DIA MS^2 scans were collected with the Astral analyzer using a *m/z* bin range of 380 to 980 with an isolation window width of 3 Th, and 7 ms maximum injection time for the 8-minute method and 2 Th, and 7 ms maximum injection time for the 60-minute method. AGC target was set to 500% with 40% source-RF. The data was processed using the CHIMERYS intelligent search algorithm in Proteome Discoverer 3.1 software and filtered to <1% FDR.

Improved environmental impact

In addition to the improved performance delivered by the Orbitrap Astral mass spectrometer, it has been designed to also lower the environmental impact from manufacturing to operation by our customers. The Orbitrap Astral mass spectrometer utilizes just one oil-free dry pump, meaning it consumes at least 30% less energy than today's state-of-the art Orbitrap mass spectrometers. The Orbitrap Astral mass spectrometer's higher throughput also means that it can replace the workload of 3 to 4 state-of-the-art Orbitrap instruments to analyze the same number of samples to the same depth over the same time. This amounts to 77% less power consumed per sample. The Orbitrap Astral mass spectrometer is manufactured in a facility that uses 100% green energy, certified by Harz Energie GmbH (Osterode am Harz, Germany), including an 80 kwp solar energy system. The facility also utilizes 4 heat pumps and 2 electric heaters to repurpose the heat from the production process to further minimize energy consumption. Waste reduction practices ensure that the waste prevention, reuse, and recycling rate is 95.3%.

Summary of instrument specifications

Orbitrap analyzer

Orbitrap mass range: standard mass range *m/z* 40–6,000, *m/z* 40–8,000 with the BioPharma option

Orbitrap resolution: resolution settings range from 7,500 to 480,000 (FWHM) at *m/z* 200, with isotopic fidelity up to 240,000 (FWHM)

Acquisition rate*: Orbitrap MS and MS^2 acquisition rates up to 40 Hz when measured using a data-dependent experiment with Pierce™ FlexMix™ calibration solution, MS and HCD MS^2 resolution settings of 7,500 @ *m/z* 200

Orbitrap mass accuracy*: External calibration achieves <3 ppm RMS drift over 24 hours, measured with FlexMix solution; internal calibration achieves <1 ppm RMS drift over 24 hours, measured with FlexMix solution

MS^2 Electrospray Ionization (ESI) Orbitrap MS^2 sensitivity: 50 fg reserpine on column S/N 100:1; SIM: 50 fg reserpine on column S/N 150:1

Intra-spectrum dynamic range*: >5,000 within a single Orbitrap mass spectrum

Astral analyzer

Astral mass range: standard mass range *m/z* 40–6,000

Astral resolution*: 80,000 FWHM at *m/z* 524

Acquisition rate*: Astral MS^2 acquisition rates up to 200 Hz with a maximum injection time of 3 ms

Astral mass accuracy*: external calibration achieves <5 ppm RMS over 24 hours, measured with FlexMix solution

Intra-spectrum dynamic range*: >1,000 within a single Astral mass spectrum at 200 Hz

* Under defined conditions

References

1. Bekker-Jensen D.B. *et al.* An Optimized Shotgun Strategy for the Rapid Generation of Comprehensive Human Proteomes. *Cell Syst.* **2017** *4*(6), 587–599.
2. Navarrete-Perea, J.; Yu, Q.; Gygi, S. P.; Paulo, J. A. Streamlined Tandem Mass Tag (SL-TMT) Protocol: An Efficient Strategy for Quantitative (Phospho) Proteome Profiling Using Tandem Mass Tag-Synchronous Precursor Selection-MS3. *J. Proteome Res.* **2018**, *17* (6), 2226–2236.
3. Eng, J. K.; Jahan, T. A.; Hoopmann, M. R. Comet: An Open-Source MS/MS Sequence Database Search Tool. *PROTEOMICS* **2013**, *13* (1), 22–24.
4. Elias, J. E.; Gygi, S. P. Target-Decoy Search Strategy for Increased Confidence in Large-Scale Protein Identifications by Mass Spectrometry. *Nat. Methods* **2007**, *4* (3), 207–214.
5. Huttlin, E. L.; Jedrychowski, M. P.; Elias, J. E.; Goswami, T.; Rad, R.; Beausoleil, S. A.; Villén, J.; Haas, W.; Sowa, M. E.; Gygi, S. P. A Tissue-Specific Atlas of Mouse Protein Phosphorylation and Expression. *Cell* **2010**, *143* (7), 1174–1189.
6. Savitski, M. M.; Wilhelm, M.; Hahne, H.; Kuster, B.; Bantscheff, M. A Scalable Approach for Protein False Discovery Rate Estimation in Large Proteomic Data Sets [S]. *Mol. Cell. Proteomics* **2015**, *14* (9), 2394–2404.
7. He and co-workers, **2023**, *Anal. Chem* in review.
8. Batth, S.; Francavilla C.; Olsen, J. Off-Line High-pH Reversed-Phase Fractionation for In-Depth Phosphoproteomics. *J. Proteome Res.* **2014**, *13*(12), 6176–6186.

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