Mass spectrometry

Discovery to validation at unprecedented scale

Stellar mass spectrometer

thermo scientific

Discovery to validation at unprecedented scale

Ultimate quantitative performance for a wide range of compound classes

Accelerate biomarker verification with confidence by quantifying more analytes with increased sensitivity, specificity, and throughput using the Thermo Scientific™ Stellar™ mass spectrometer. By synergistically combining the robust quantitative performance of triple-quadrupole technology with the sensitive, hyper-fast full-scan MS^n acquisition of dual-pressure linear ion trap technology, the Stellar mass spectrometer extends its unprecedented analytical capabilities to a wider range of compounds. Dynamic instrument control software simplifies instrument operation while maximizing sample throughput. Together, these capabilities enable researchers to gather the additional insight needed to make better informed decisions and move to the biomarker validation stage faster.

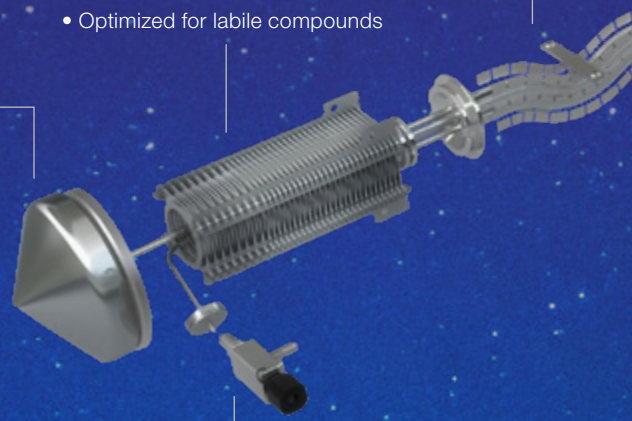
Stellar mass spectrometer coupled to the Thermo Scientific™ Vanquish™ Neo UHPLC system with the Tandem Direct Injection workflow

General omics research	• Increased target capacity • Wide dynamic range • Complex biological samples
Plasma proteomics	• Wide dynamic range • Increased target capacity • Method standardization
Low input/single cell	• Increased target capacity • High throughput • Data completeness
PTMs	• Low-level samples • Increased specificity • Sample complexity
Metabolites	• Broad compound classes • Method optimization • Higher throughput
Lipids	• Broad compound classes • Increased specificity • Higher throughput

Unrivaled quantitative productivity

Unrivaled translational research productivity for targeted quantitation

- Achieve biased systems biology analysis by confidently quantifying almost 10,000 peptides in an hour
- Increase quantitative study power using absolute quantitation on larger target panels with 4X the sample throughput
- Leverage enhanced sensitivity to extend targeted pathway analysis while mitigating missing values for input samples
- Overcome challenging background matrices using fast and sensitive full-scan Synchronous Precursor Selection (SPS) MS^3 acquisition
- Accelerate targeted method creation and implementation using a wide range of targeted and non-targeted data acquisition schemes



Innovative technology drives targeted quantitative productivity

The Stellar mass spectrometer combines two mass analyzers, a quadrupole mass analyzer for precursor ion selection and the hyper-fast dual-pressure linear ion trap mass analyzer. The ion concentrating routing multipole (ICRM) simultaneously manipulates ion packets in the two-ion traps. Synchronized ion management acquires MS^n data at rates up to 140 Hz with high sensitivity, wide dynamic range, and increased specificity, enabling scientists to confidently transition more putative biomarkers to validation in less time.

QR5 Plus segmented quadrupole mass filter with hyperbolic surfaces
Improved sensitivity and selectivity with 0.4 *m/z* precursor isolation widths

High-pressure linear ion trap
Efficient ion manipulation performing resonant RF ejection or isolation

Hyper-fast dual-pressure linear ion trap mass analyzer

Low-pressure linear ion trap
Scan speeds up to 140 Hz

Ion concentrating routing multipole
Efficient ion trapping and HCD fragmentation

Extended scale detector
Extremely sensitive photomultiplier detection with low noise and long lifetime

OptaMax Plus ion source
Increases ionization efficiency

Expanded analytical capabilities

The Stellar mass spectrometer is designed to redefine targeted quantitative analysis for a wide range of molecules. The instrument excels at many data acquisition strategies including data-dependent acquisition (DDA), data-independent acquisition (DIA), and MS^n acquisition with SPS for Tandem Mass Tag™ (TMT™) multiplexing as well as unlabeled MS^3 and higher tandem MS^n scan events. Leveraging synchronized ion packet management between the ICRM and the hyper-fast dual-pressure linear ion trap mass analyzer enables fast MS^2 and MS^3 acquisition of up to 140 and 40 Hz, respectively. Resonant RF applications in the high-pressure linear ion trap allow multi-notch ejection SPS or fragmentation at any MS^n level for targeted quantitative method development and confident study management.

Fast, sensitive, and specific MS^n data acquisition

The precursor ion beam is filtered in the QR5 Plus mass analyzer, transferred to the ICRM where precursor ions are dissociated, and then concentrated to reach the charge densities defined by the automatic gain control (AGC). The ion packet is then transferred to the hyper-fast dual-pressure linear ion trap mass analyzer for detection.

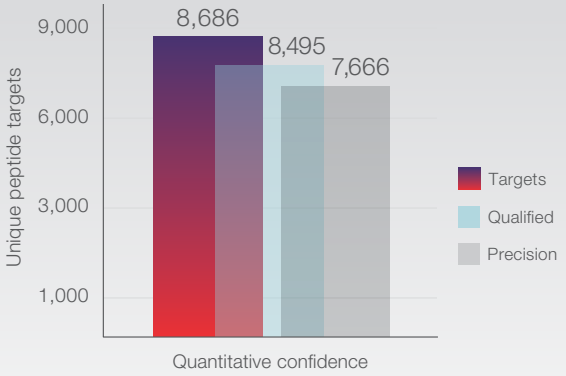
Synchronized ion trapping substantially increases acquisition speeds. While the first MS^2 ion packet is being excited out of the linear ion trap mass analyzer for detection, a second MS^2 ion packet is concentrated in the ICRM. Synchronized transfer to the high-pressure linear ion trap is performed following mass spectral acquisition of the previous product ion packet. The resulting ion packets are detected with a novel high-dynamic range detector that provides single-ion sensitivity, linearity over five orders of magnitude, low noise, and long detector lifetimes. A similar multiple ion packet management approach is used to provide fast MS^3 acquisition rates up to 40 Hz.

Expanded scale

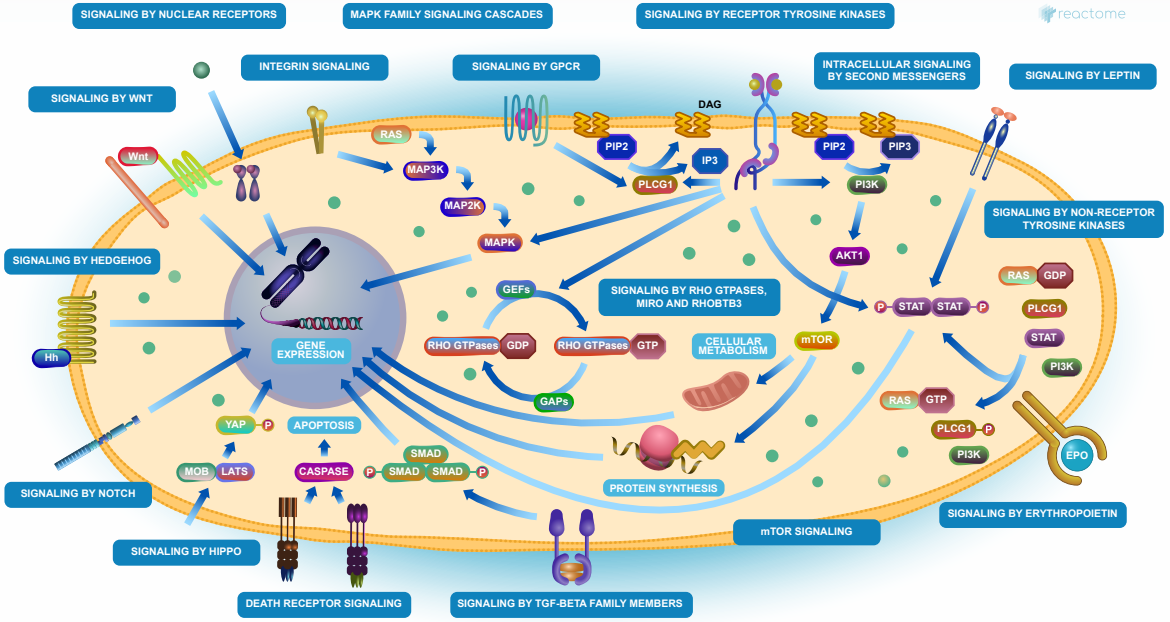
Transitioning putative biomarkers from discovery to validation requires an efficient workflow to verify the diagnostic value of each candidate, either individually or in a signaling pathway. To improve biomarker stratification, highly multiplexed targeted quantitation is performed to increase sensitivity and specificity as measured across the study's larger numbers of samples. The Stellar mass spectrometer expands experimental scale, permitting quantitative characterization of multiple signaling pathways in one experiment while maintaining the higher throughput needed to evaluate systems biology.

Quantitative performance at scale

With the Stellar mass spectrometer, almost 9,000 surrogate peptide markers representing almost 2,000 proteins can be reproducibly quantified in 60 minutes. Over 88% of these peptide markers can be confidently measured with CVs less than 20%, covering a wide dynamic of 4.5 orders of magnitude. Broad sample coverage with high data quality enables biased pathway analysis to determine biology as shown below in the pathway coverage report from the Reactome website tool.



The total number of targeted peptides reproducibly measured across technical replicates (red) as compared to the number of targeted peptides measured with CV values ≤50% (light blue), and the number of targeted peptides measured with CV values ≤20%.



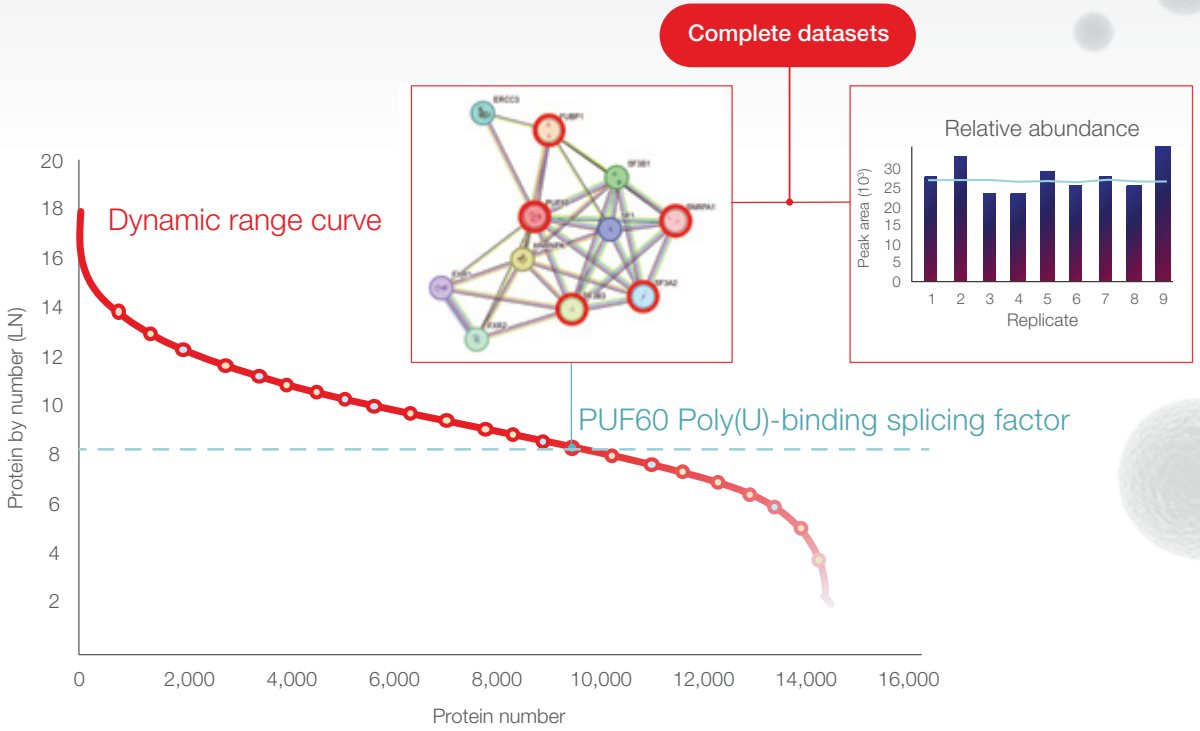
The Stellar mass spectrometer was used for the discovery and targeted quantitative analysis of the digested extracellular vesicles (EV) extracted from a donor pool with neurodegenerative decline. To create the targeted library, 500 ng of the EV digest was loaded on column and analyzed using LC-DIA MS using 100 sequential 2 Th-wide DIA scan events. Four replicate injections were performed over a 400–1,200 Da precursor *m/z* range. The resulting DIA data were processed using Thermo Scientific™ Proteome Discoverer™ software. The search results were uploaded into Skyline for filtering and targeted-MS2 (tMS2) method creation using the Thermo Scientific™ PRM Conductor tool, which filtered the 11,092 identified peptides to a final set of 8,686 based on user-defined LC and MS criteria. Replicates were analyzed to determine the reproducibility of the tMS2 method. Samples courtesy of Prof. Michael J. MacCoss, University of Washington.

Higher sensitivity

Increased method sensitivity is essential for targeted quantitative analysis of low-input samples such as single-cell and low-abundance analytes in bulk samples. Improved sensitivity enhances automated post-acquisition peak integration, enhancing quantitation quality and confidence when measuring smaller changes between biological groups or cells. The Stellar mass spectrometer provides high ion transmission efficiency throughout, efficient detection of ions ejected from the low-pressure linear ion trap, and increased sensitivity thanks to the extended scale detector. These features allow targeted signaling pathway analysis for single cells that cover a wide expression-level range. Lower detection limits minimize the risk of missing data, ensuring high confidence in the quantitative results obtained.

Extend targeted pathway quantitation to low-input sample analysis

Low-input sample analysis focuses on key biological pathways or markers that collectively can differentiate cell types, states, or functions. Targeted data acquisition increases selectivity and sensitivity, extending the dynamic range for reproducible measurements. Because data analysis is reduced to the presence or absence of targets based on the instrument's detection ranges and relative sample amounts, targeted acquisition ensures data completeness for the analysis of individual cells and cell types. The acquisition speed of the Stellar mass spectrometer broadens protein coverage, allowing scientists to capture a more complete biological picture of cellular activity.



A set of nine HeLa cells was isolated, digested, and analyzed using tMS2 acquisition for 395 peptides mapping to 383 proteins. The proteins were selected to cover a wide range of reported copy numbers, from more than 5,000,000 down to less than 5,000. Fifty samples per day (SPD) were analyzed to evaluate acquisition speed and data completeness. The example shown—PUF60 binding splicing factor—is estimated to be expressed in zeptomoles per HeLa cell. The inset bar chart shows the relative quantified abundance of PUF60 across the ten cell lysates. Additionally, the targeted proteins' signaling networks were mapped and evaluated using the String DB software. Measurements for all targeted peptides were reproducible across the ten cell lysates. Samples courtesy of Ryan Kelly, Associate Professor Brigham Young University.

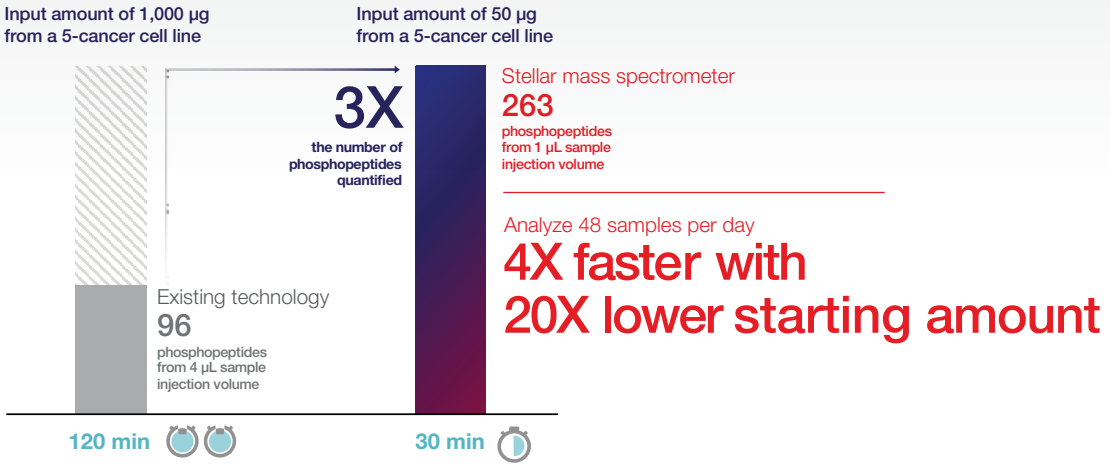
Greater specificity

Successful biomarker quantitation requires sensitivity and specificity to ensure measured signal is attributed to the targeted analytes. Complex biological matrices present quantitative challenges due to co-eluting isobaric/isomeric interference that MS^2 acquisition may not overcome. To reduce background interferences and provide greater specificity to differentiate isomeric compounds in complex matrices, MS^3 acquisition becomes a more efficient and effective solution.

The Stellar mass spectrometer offers rapid tMS3 acquisition speeds, up to 40 Hz, with automated method creation through the PRM Conductor software tool. The increased specificity of tMS3 acquisition can increase limits of detection (LOD) and limits of quantitation (LOQ) as well as enable simultaneous quantitation of multiple analytes in one spectrum—all while using fast ultra-high performance liquid chromatography (UHPLC) gradients.

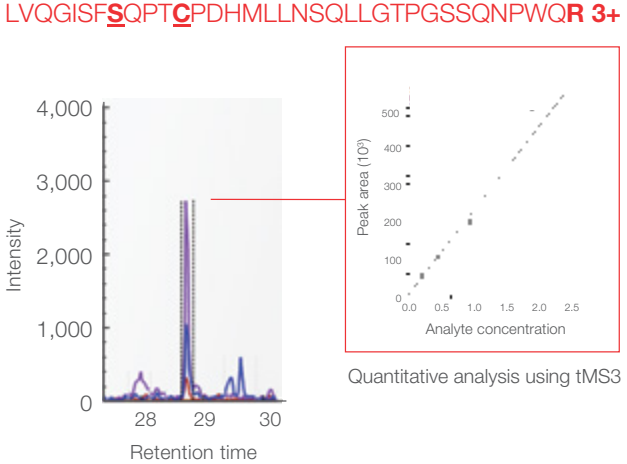
Overcome the challenges of biological complexity with targeted-MS3 acquisition

The Stellar mass spectrometer leverages dual-ion packet management, and extended instrument capabilities to boost sensitivity and specificity for highly multiplexed phosphopeptide quantitation. The high-pressure linear ion trap can isolate multiple MS^2 product ions using SPS. The resulting MS^2 product ions can be accelerated back to the ICRM to generate MS^3 product ions, which are subsequently transferred back to the hyper-fast dual-pressure linear ion trap mass analyzer for fast and sensitive detection. Fast acquisition rates enable quantitation of 263 endogenous phosphopeptides and their corresponding heavy labeled standards in a 30-minute method. Current published methods rely on significantly longer 120-minute gradients.



The PRM Conductor software uses initial library building experiments to automatically create the tMS3 acquisition method used to quantify the set of 263 heavy and light phosphopeptides extracted from a 5-cancer cell line mixture. To demonstrate instrument performance, the set of heavy labeled standards were spiked into samples enriched from different starting amounts of cell line material, from 50 to 1,000 µg. The number of phosphopeptide analogues detected by the Stellar mass spectrometer was compared to published results acquired using existing technology. The figure at the right shows the tMS3 extracted ion chromatogram (XIC) for the phosphopeptide LVQGI<u>S</u>FSQPT<u>C</u>PDHMLLNSQLLGTPGSSQNPWQR 3+** across the starting amounts analyzed.**

Samples courtesy of Steven Carr, Senior Director of Proteomics at the Broad Institute of MIT.

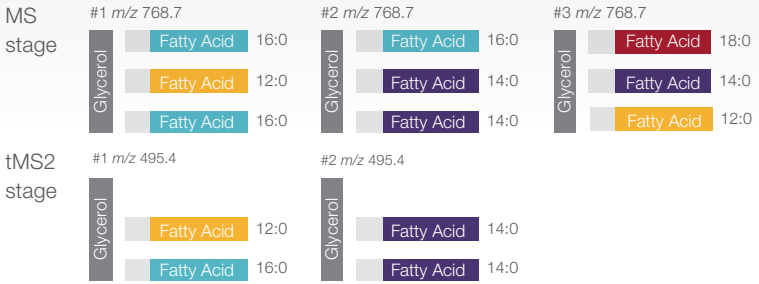


Quantitative analysis using tMS3

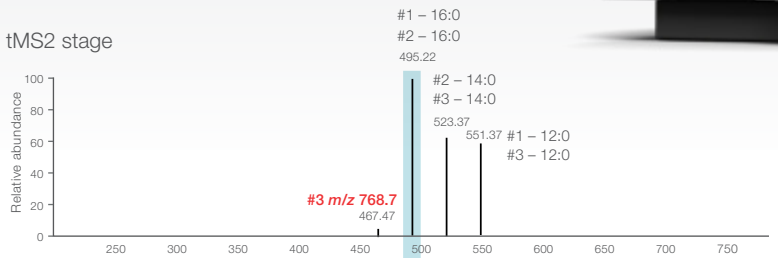
Characterization of isomeric triglycerides online using tMS3 acquisition

Triacylglycerides (TAG) are critical lipid classes involved in metabolism that are quantified for health assessment. The limitations of standard chromatographic separations can result in co-eluting isomeric TAGs, making their quantitation difficult. Employing MS^3 fragmentation introduces the specificity necessary to separate and quantify each isomer. The Stellar mass spectrometer rapidly acquires tMS2 and tMS3 data up to 140 and 40 Hz, respectively, enabling qualitative and quantitative TAG analysis. Acquiring full-scan MS^3 spectra permits simultaneous quantitation of each TAG from one scan event, increasing the target capacity per unit time, making it possible to use UHPLC separations to reduce sample turnaround time.

Existing technology

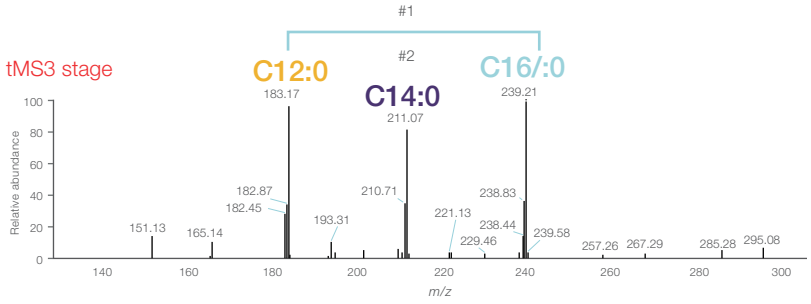


Existing technology



Stellar mass spectrometer

Stellar mass spectrometer



Initial sample characterization used DDA followed by tMS2 acquisition of TAG extracted from a commercially available plasma sample. Analysis of the tMS2 spectra was performed to set up the tMS3 data acquisition method for use with a 30-minute UHPLC gradient.

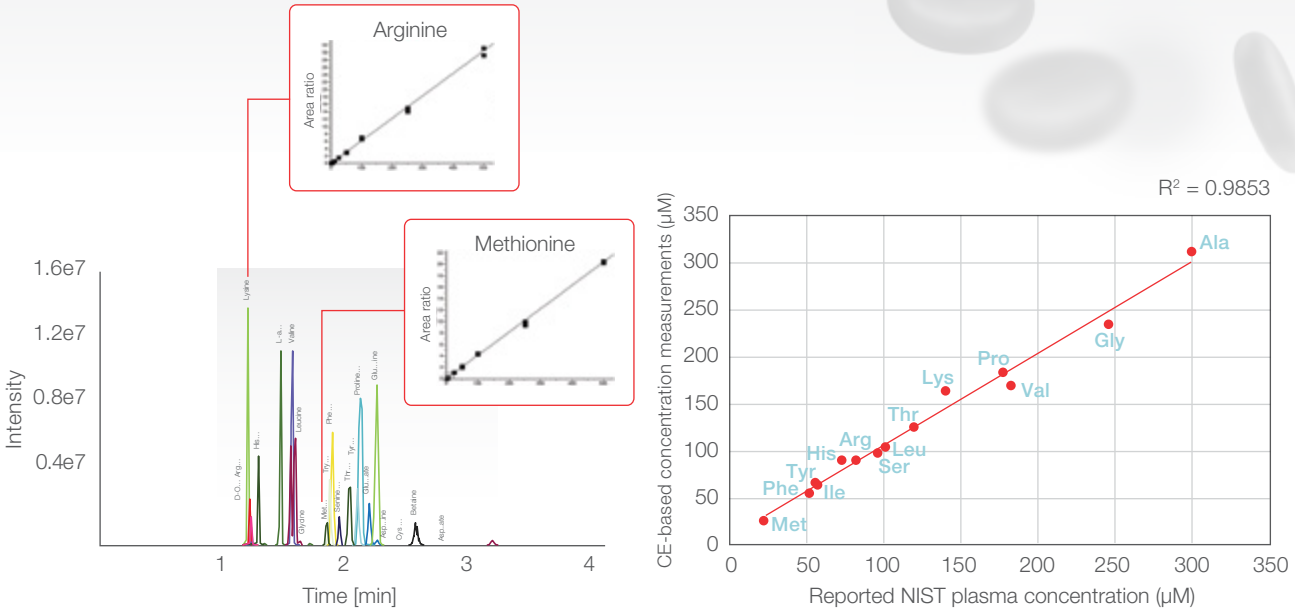
Samples courtesy of Julijana Ivanisevic, Head of Metabolomics / Senior Lecturer at University of Lausanne, Switzerland.

Massive throughput

Relative to label-free quantitation (LFQ)-DIA, targeted quantitation increases sensitivity and throughput, both of which combine to improve study power to the level needed to accurately verify putative biomarkers. The Stellar mass spectrometer makes it possible to accelerate biomarker verification, while maintaining analyte capacity, sensitivity, selectivity, and specificity. In addition, the acquisition speed of the Stellar mass spectrometer enables simultaneous accurate quantitation of more stable isotope labeled (SILs) analogues per experiment, permitting researchers to analyze multiple 96-well plates with greater confidence and in less time. This capability is particularly advantageous when developing standardized methods for transfer to validation.

Increase throughput without experimental compromise

The Stellar mass spectrometer delivers fast, full-scan tMS2 acquisition with excellent sensitivity, enabling researchers to increase sample throughput by 4X. Faster MS^2 acquisition speeds allow accurate quantitation of more than 100 endogenous amino acids and acylcarnitines in two minutes and processing of 96-well plates in nine hours. Full-scan MS^2 acquisition maintains specificity across the calibration range, overcoming the need to increase dwell times as the number of transitions monitored increases. Avoiding this experimental compromise is important because though SIL analogues are commonly used to enhance quantitative accuracy and method standardization against reference samples, they increase the number of transitions that must be monitored.



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A new paradigm for clinical sample analysis

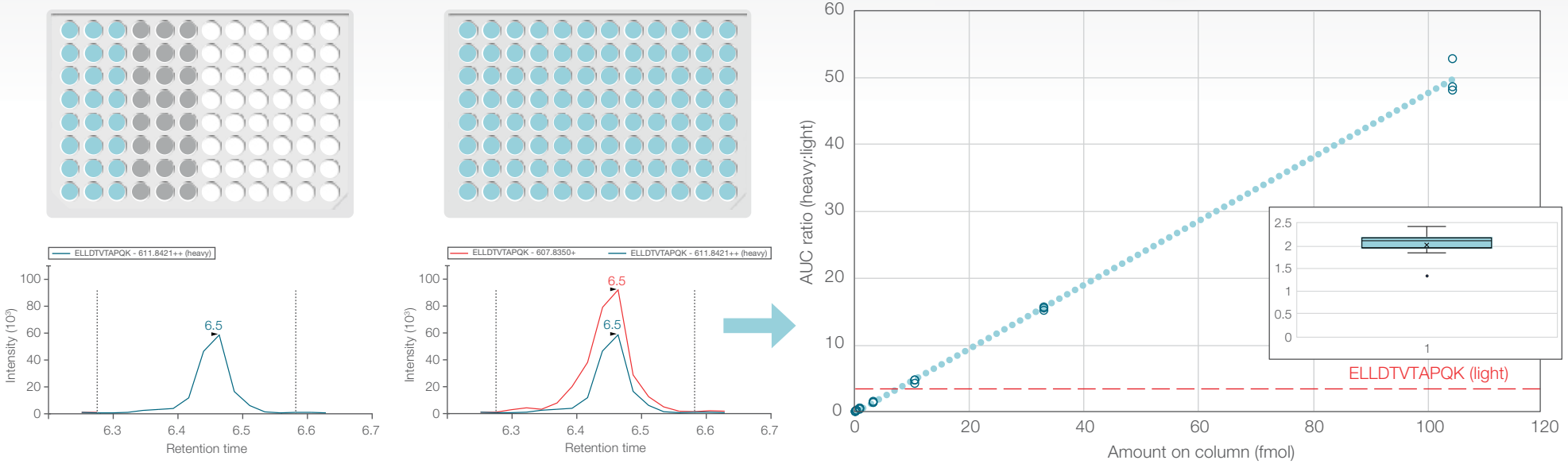
The LC-MS^n methods deployed in clinical studies must be robust and reproducible, particularly when fast chromatography is used to increase sample throughput. For targeted protein quantitation based on surrogate peptide biomarkers, use of SIL peptides improves method standardization, data confidence, and absolute quantitation, but can limit sample throughput by doubling concurrent scan events per experiment. The Stellar mass spectrometer introduces novel data acquisition strategies to increase throughput for large panels that rely on a greater number of SIL peptides. The Adaptive RT feature automatically manages real-time retention time adjustment for each tMS2 acquisition cycle. As a result, acquisition methods can use narrower tMS2 windows to ensure 5–10 ms MS^2 product ion accumulation, maintaining high data quality and quantitative accuracy across a wide dynamic range.

Existing technology

48 samples/day*

Stellar mass spectrometer

100 samples/day



The PQ500™ reference kit (Biognosys AG) is a reference method for the quantitation of 583 endogenous plasma proteins. The set of 804 stab SIL peptides are spiked into digested human plasma from 0.01 to 100 fmol and analyzed using targeted quantitation to evaluate instrument performance. *A previously published report showed quantitative analysis of the calibration range with a 30-minute sample-to-sample analysis time. The high-resolution accurate MS^2 method, however, only quantified 804 SIL peptides, limiting its potential to provide biological insight. Other published results have used targeted methods to quantify both endogenous and SIL peptides at 24 SPD. The Stellar mass spectrometer leverages fast acquisition speeds and sensitive tMS2 in an 18-second acquisition window–managed by the Adaptive RT routine–to perform absolute quantitation using a 100 SPD method. The endogenous peptide analysis above shows the relative measured response for both peptide analogues when using the Stellar mass spectrometer. The integrated peak area ratios per PQ500 kit spiking level for the targeted peptide pair shows a linear response from 0.03 to 100 fmol on column. The whisker plot shows the data quality obtained, demonstrating that the Stellar mass spectrometer reproducibly measures peptide expression regardless of PQ500 spiking amount. Samples courtesy of Bruce Wilcox, CTO of PrognomIQ, Inc.

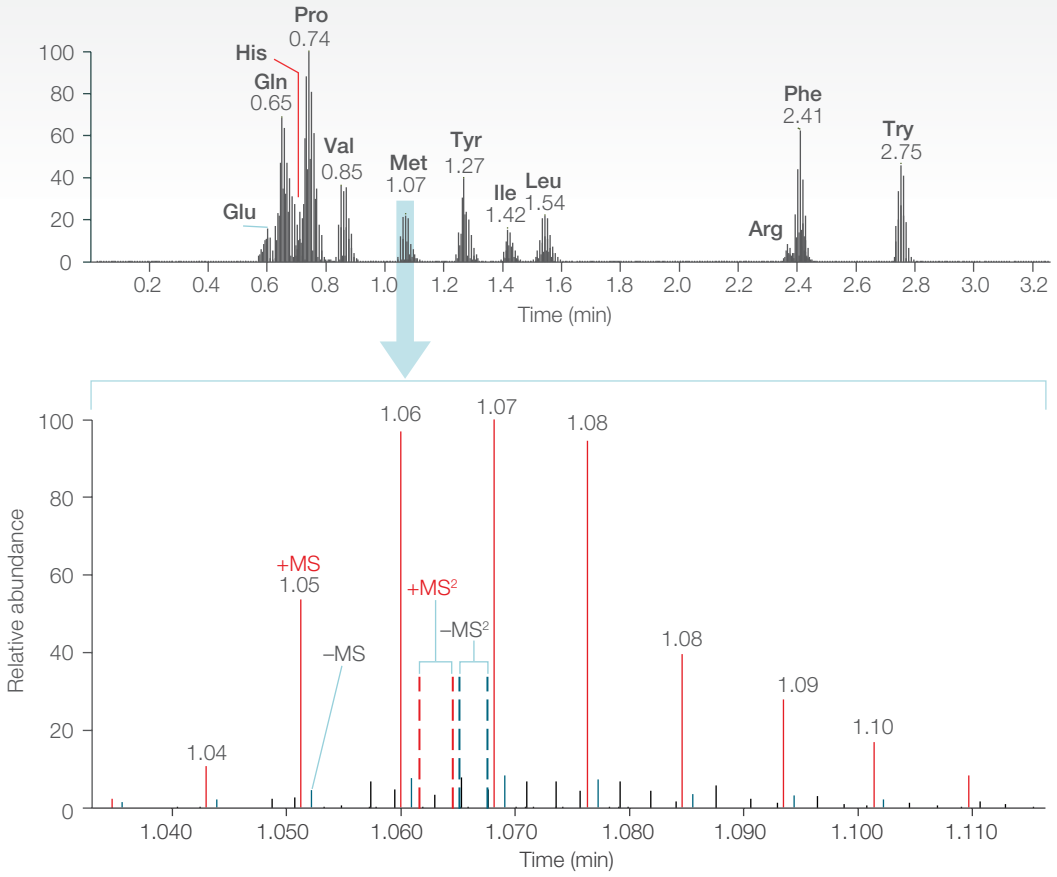
Unrivaled productivity

Method optimization for small-molecule assays becomes the most time-consuming step when performing selected reaction monitoring (SRM) quantitation. SRM acquisition parameters must be determined for each analyte, including ionization polarity, precursor and product ion *m/z* values, and their corresponding collision energies and dwell times. Standards are typically analyzed by flow injection analysis (FIA) or direct infusion to determine SRM parameters followed by matrix-matched analysis to evaluate potential background interference.

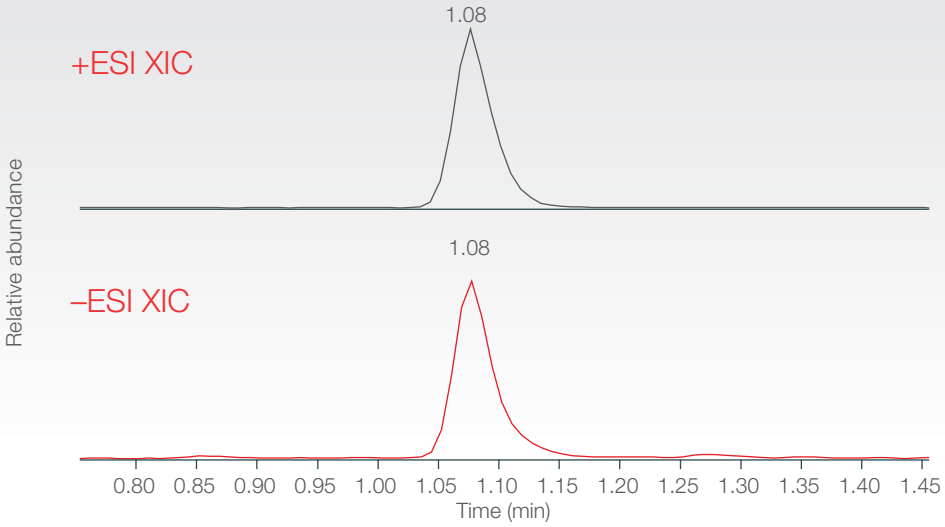
The Stellar mass spectrometer combines analytical flexibility with fast polarity switching to acquire full-scan MS and MS^2 acquisition from chromatographic separation. Complete method optimization can be performed in hours instead of days or weeks allowing researchers to begin analyzing critical samples and complete experimental studies faster with greater confidence.

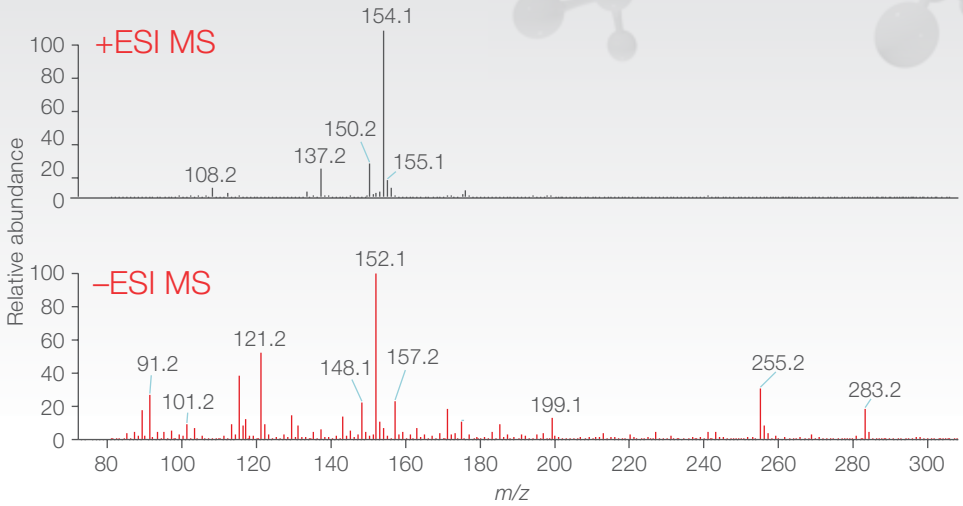
Expedite comprehensive method creation to boost laboratory capacity

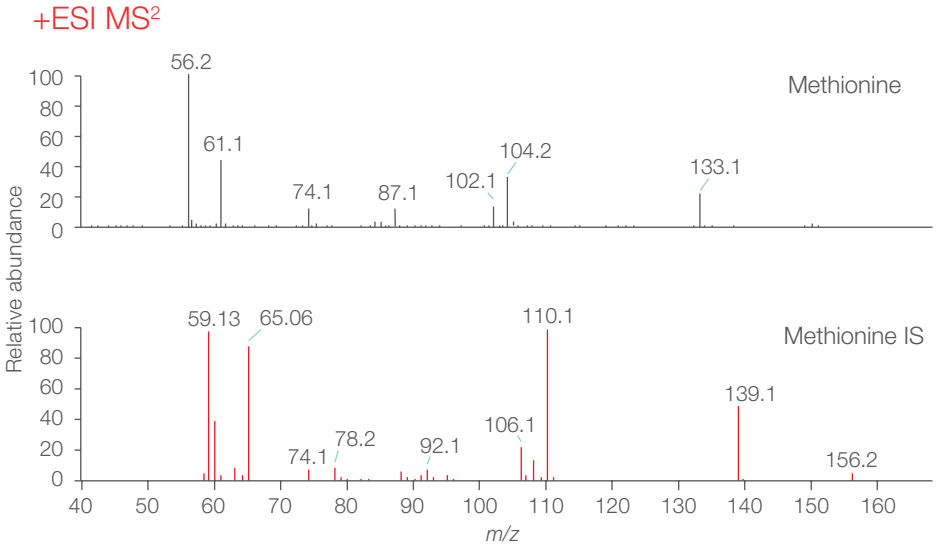
The Stellar mass spectrometer rapidly acquires full-scan MS^n data, expediting method creation. Using a targeted precursor mass list, the instrument can step between full-scan MS and MS^2 acquisition in positive and negative ionization (+ESI and –ESI) modes with only five millisecond polarity switching. Multiple full-scan MS^2 spectra can be acquired between MS acquisition events using higher-energy collisional dissociation (HCD) beam-type fragmentation and/or resonance RF collision induced dissociation (CID), in either ionization mode. Normalized collision energy (NCE) and Stepped CE settings eliminate the need for tedious CE optimization. In addition, the Stellar mass spectrometer leverages dynamic AGC to eliminate the need to set dwell times for each SRM transition.

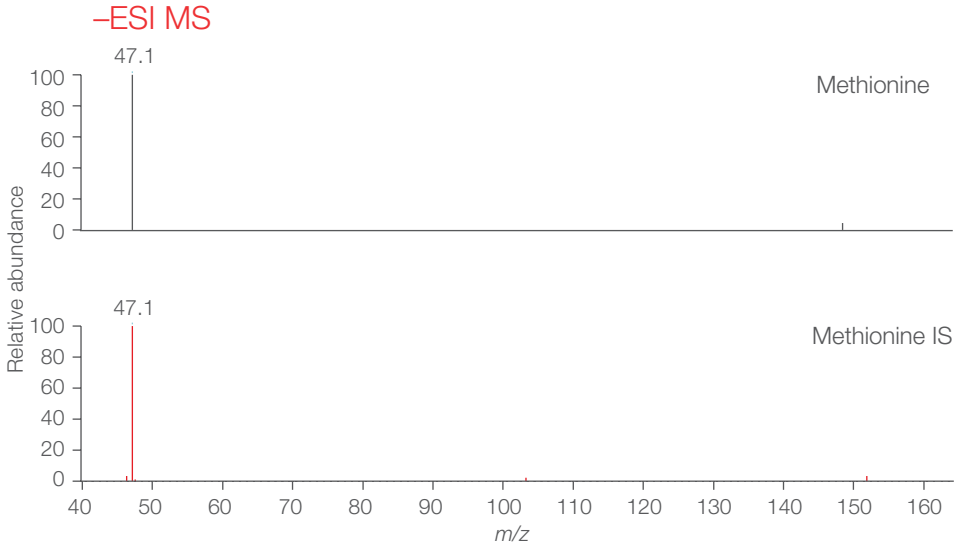


A set of 15 amino acids with corresponding internal standards were analyzed using a 7-minute method. The figure shows the data acquisition timing used for comprehensive Methionine analysis. The solid lines represent full-scan MS acquisition in +ESI (red) and –ESI (blue) modes. Following MS acquisition, a set of ten full-scan MS^2 spectra were collected and plotted within the dashed lines representing each polarity. In 3.5 seconds, a total of nine full-scan MS, and 120 full-scan MS^2, spectra were acquired for each polarity. Data courtesy of Metabolon Inc.





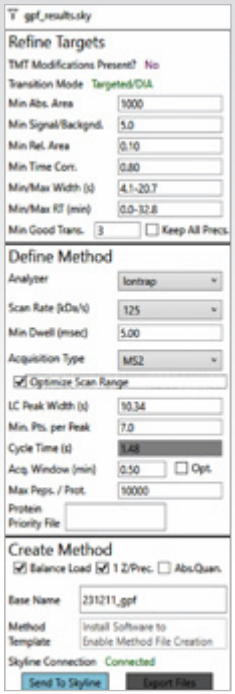


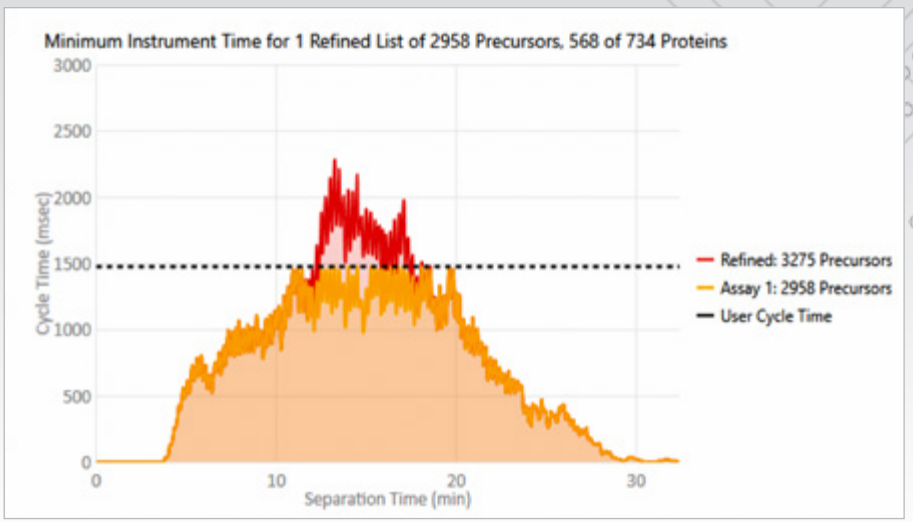


The comprehensive data produced by the Stellar mass spectrometer provides significant advantages compared to SRM method development. Full-scan MS^n acquisition enables researchers to determine potential background interference in the MS scan, which particular ionized form of the precursor provides the greatest sensitivity (e.g., protonated vs. ammonium adduct vs. sodium), and which product ions to consider for use in targeted data processing methods. The resulting data can help the scientist determine which target analytes will present challenges at the validation stage, potentially necessitating the use of multiple methods to analyze all or subsets of the target analytes, or if all the analytes can be easily validated in one method.

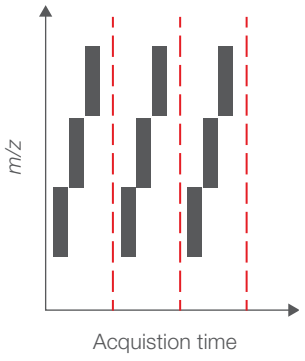
Automated intelligence streamlines method creation and manages data acquisition

The challenges researchers face when developing efficient and reliable multiplexed targeted quantitative experiments are inversely proportional to their scale. Determining the LC and MS^n parameters for each analyte is crucial for confident data acquisition. Expanding the number of target analytes or using faster gradients can introduce compromises in coverage, selectivity, or confidence in data. The intelligence provided by the novel, on-board PRM Conductor tool leverages discovery data to streamline targeted method creation. In three easy steps, the software aligns the Thermo Scientific™ Vanquish™ UHPLC system and Stellar mass spectrometer operation and manages data acquisition to ensure optimal LC-MS^n performance.

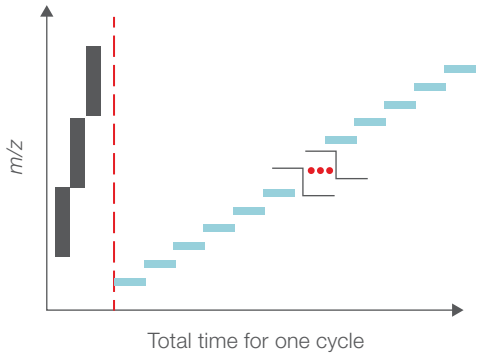




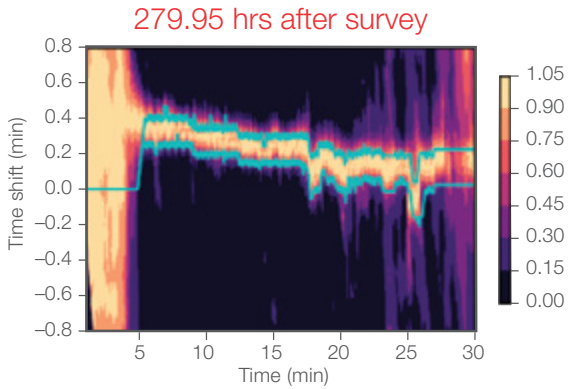
Step 1: Load the spectral library generated from a reference sample analysis into Skyline, open the PRM Conductor Tool, and set the values in the Refine Target, Define Method, and Create Method sections of the window. The PRM Conductor software identifies the optimum surrogate peptide candidates per protein that fit within the automatically determined cycle time, maximizing chromatographic utilization. The PRM Conductor software also uses the spectral library information as "look-up tables" that drive the Adaptive RT routine.



Step 2: Acquire the Alignment Map file that will be used to manage dynamic retention time adjustments. To map the key features in the reference file as a function of retention time, the acquisition method repeatedly steps across twelve 50 Th DIA windows acquired using a scan rate of 200 kDa/S.



Step 3: Analyze the study sample using the targeted data acquisition method. The acquisition method acquires the twelve DIA scan events (*ca*. 100 ms) followed by the tMSn events. The DIA acquisition sequence is acquired at the beginning of every other cycle.



Adaptive RT routine in action: An example of the acquisition cycle outlined using the DIA mapping function to detect, evaluate, and align the retention time windows following the completion of every other cycle. The teal lines show the leading and tailing edges of the tMSn events with automatically determined time shifts. The various colors are associated to the dot product overlap of RT features mapped in real time versus the Alignment Map file.

End-to-end workflows for targeted quantitative simplicity

Translational research requires workflows that can support the expanded scale needed to identify and transfer promising biomarker candidates to validation. With best-in-class components covering everything from targeted sample preparation, maximum chromatographic performance, orthogonal selectivity, to data acquisition and processing, the Stellar mass spectrometer is an integral part of the Thermo Scientific™ workflow solution designed to increase laboratory productivity and expand experimental capacity. Direct integration into the Thermo Scientific™ Xcalibur™ software ecosystem ensures seamless operation to confidently stratify biomarkers during verification.

Thermo Scientific™ KingFisher™ Flex system

Tremendous flexibility with 96-well plate format

Thermo Scientific™ Vanquish™ UHPLC systems

High performance from nano- to analytical flow rates

Thermo Scientific™ FAIMS Pro Duo interface

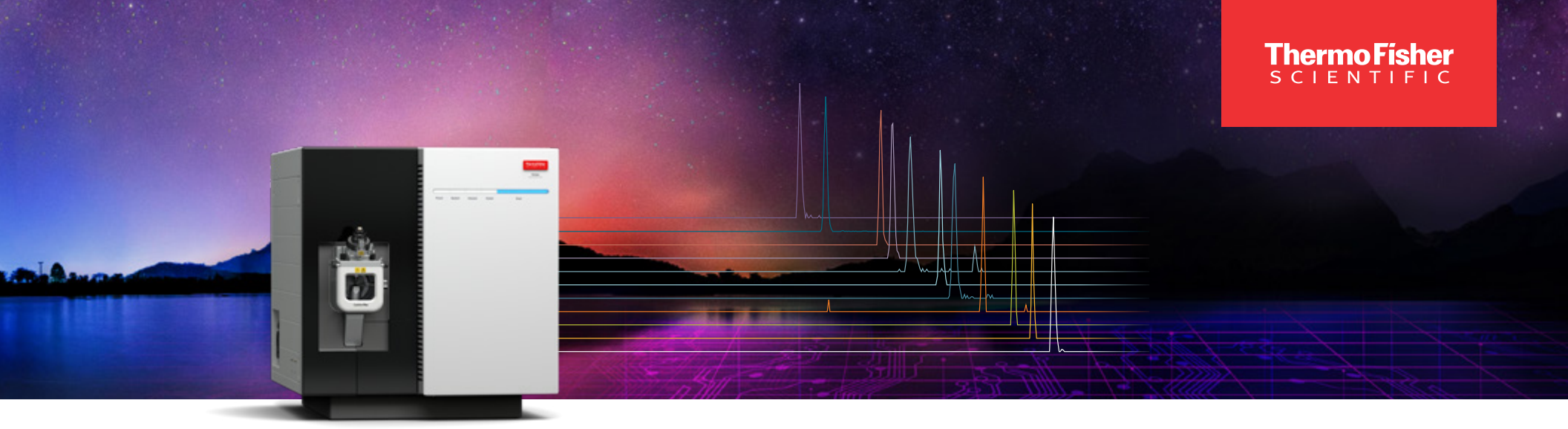
Reduces matrix inferences, increases workflow robustness

Thermo Scientific™ Stellar™ mass spectrometer

A new paradigm for targeted quantitation analyses

Thermo Scientific™ Ardia™ Platform

Central data storage, automated data processing, and tools to manage instruments, data, and users



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