Translational Proteomics

Discovering hidden depths: High-throughput proteomics study for enhanced biomarker discovery on Orbitrap Astral mass spectrometer

Kevin Yang[1], Amarjeet Flora[2], Bhavin Patel[2], Khatereh Motamedchaboki[1], Stephanie Samra[1], and Amirmansoor Hakimi[1]
[1]Thermo Fisher Scientific, San Jose, California, U.S.A.
[2]Thermo Fisher Scientific, Rockford, Illinois, U.S.A.

Abstract

Purpose: To assessed the performance of a high-throughput, in-depth workflow for plasma proteomic analysis on the Orbitrap Astral mass spectrometer in a mini cohort of pre-classified diverse cancer and non-cancer samples, enabling larger-scale cancer cohort studies, and population-scale , translational research.

Methods: Proteomic analysis of plasma samples from a mini cancer cohort and age-matched healthy controls was conducted using the Proteograph XT Assay (Seer Inc.). The LC-MS analysis was performed on the Thermo Scientific Orbitrap Astral mass spectrometer, which was coupled with the Thermo Scientific Vanquish Neo UHPLC system. The throughput was set at either 60 or 16 samples per day.

Results: The Orbitrap Astral mass spectrometer coupled with the Proteograph XT Assay enables identification of nearly 10,000 protein groups. Furthermore, downstream analysis suggests that this workflow provides sensitivity to detect subtle changes in potential disease biomarkers in blood samples, distinguishing them from those in healthy individuals.

Introduction

Cancer claims millions of lives globally every year. An earlier and more timely diagnosis greatly improves the prognostic outcome, emphasizing the need to detect cancer at an earlier stage. Current tests, such as invasive biopsies or costly imaging scans can lack sensitivity or selectivity and are not readily available. For this reason, analyzing blood plasma is widely accepted as a promising technique for biomarker discovery, but the complex workflows involved in handling non-invasive blood-based samples collection have hindered the progress.

In the present study, we evaluated the performance of a high-throughput and in-depth workflow for discovery proteomic analysis using the Orbitrap Astral mass spectrometer on a small group of pre-classified diverse cancer and non-cancer samples. This initial assessment of the workflow paves the way for enabling larger-scale cancer cohort biomarker discovery studies and population scale translational research.

Materials and methods

Sample preparation

Plasma samples of various cancers, including B-cell lymphoma, colorectal, lung, ovarian and pancreatic cancer together with age- and ethnicity-matched healthy plasma samples were purchased from Discovery Life Sciences and processed from the same site (**Table 1**).

The neat plasma samples were prepared using the Thermo Scientific™ EasyPep™ Mini MS Sample Prep Kit (P/N A40006). Depletion was conducted on the Thermo Scientific High Select™ Depletion Spin Columns (P/N A36371) according to manufacture's instruction. For plasma protein enrichment, 240 µL of plasma sample was mixed with each of the two nanoparticles suspension (NPs) with the Proteograph XT Assay Kit (Seer Inc.) according to manufacturer's instruction .

LC-MS methods

Plasma samples were loaded onto a 15-cm Thermo Scientific™ EASY-Spray™ PepMap™ column for a throughput of 60 sample per day (SPD). To further improve proteome coverage, a 16 SPD method was separated with the 60-cm IonOpticks TS UHPLC column. The peptides were loaded onto column with trap and elute mode for 60 SPD or direct injection mode for 16 SPD by using a Thermo Scientific Vanquish™ Neo UHPLC system. The eluted peptides were analyzed on an Orbitrap Astral MS operated in narrow window DIA mode (**Figure 1**).

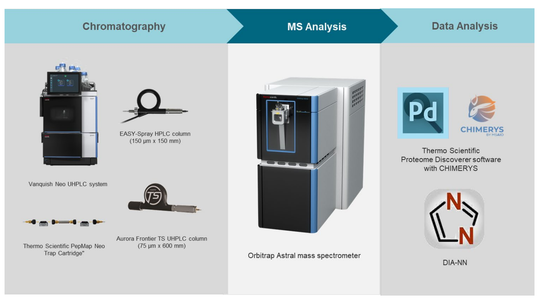
Data analysis

The LC-MS data has been processed by DIA-NN (v1.8.1) or Thermo Scientific™ Proteome Discoverer™ with CHIMERYS™ intelligent search algorithm by MSAID. The resulting tables were imported to Python for downstream data analysis and visualization.

Table 1. List of samples included in the mini cancer cohort study.

Plasma Sample	Ethnicity	Gender
Healthy	White	Male
	White	Female
	Black	Female
Colorectal Cancer	Black	Female
	White	Male
Lung Cancer	Black	Female
	White	Male
B Cell Lymphoma	White	Female
	Black	Female
Ovarian Cancer	Black	Female
	White	Female
Pancreatic Cancer	Black	Female
	White	Male

Figure 1. Complete 'end-to-end' LC-MS workflow.



LFQ-DIA workflow on Orbitrap Astral mass spectrometer for plasma proteomics analysis.

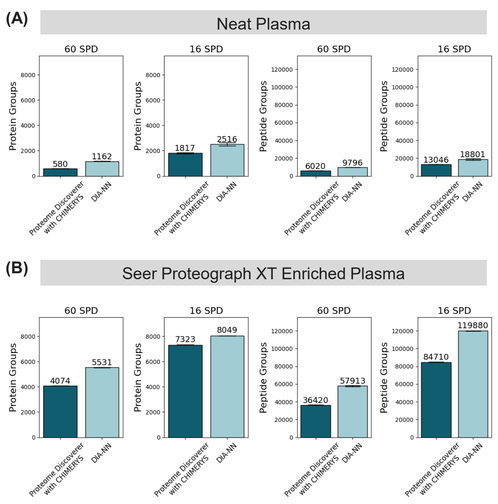
Results

High-throughput and maximized coverage LC-MS workflows on the Orbitrap Astral mass spectrometer

In-depth proteomic analysis has resulted in an increased number of features for precise and accurate sample classification. Here we demonstrate a proof-of-concept study on a workflow that provides statistical power for early biomarker discovery. In this mini cohort study, the plasma samples of various cancers, including B-cell lymphoma, colorectal, lung, ovarian and pancreatic cancer together with age-, gender- and ethnicity-matched healthy plasma samples were prepared and analyzed in an automated, high-throughput approach using the Proteograph XT Assay coupled with an Orbitrap Astral mass spectrometer for plasma proteomic analysis (**Figure 2**).

In this study, we employed the 60 SPD and 16 SPD workflows to meet the needs of plasma proteomics researchers for high-throughput and in-depth proteome analysis, respectively. To initially assess the suitability of these workflows for plasma proteomics analysis, we created a super mixture sample by combining plasma samples from a mini cancer cohort in equal volumes. The initial evaluation identified 1,162 protein groups and 9,796 peptide groups from neat plasma at 60 SPD (**Figure 2A**). Using the 16 SPD workflow, we improved the coverage to 2,516 protein groups and 18,800 peptide groups. When the samples were enriched using the Proteograph XT assay, we identified 5,531 protein groups and 57,913 peptide groups at 60 SPD, and 8,049 protein groups and 119,880 peptide groups at 16 SPD, demonstrating a 3 to 4-fold increase in coverage compared to neat digestion workflow (**Figure 2B**).

Figure 2. High-throughput and maximized coverage plasma proteomics analysis on the Orbitrap Astral mass spectrometer.

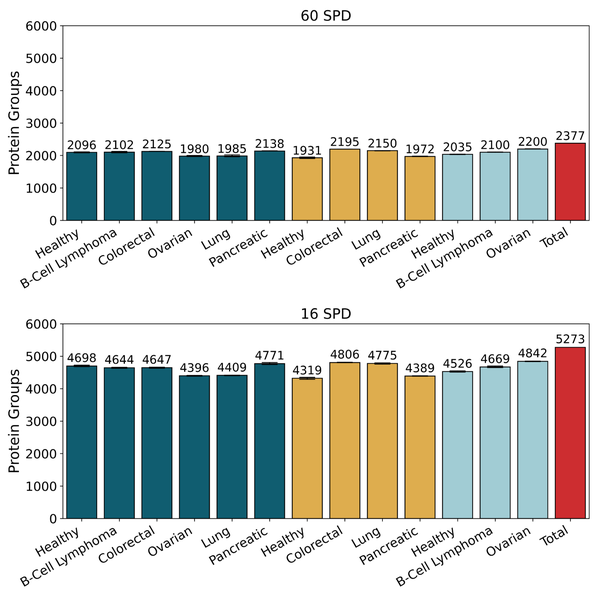


Bar charts showing the number of protein and peptide groups of **(A)** neat plasma and **(B)** Seer Proteograph XT enriched plasma from the high-throughput 60 SPD and maximized coverage 16 SPD methods.

The Orbitrap Astral mass spectrometer enables in-depth plasma proteome coverage and reproducible quantitation

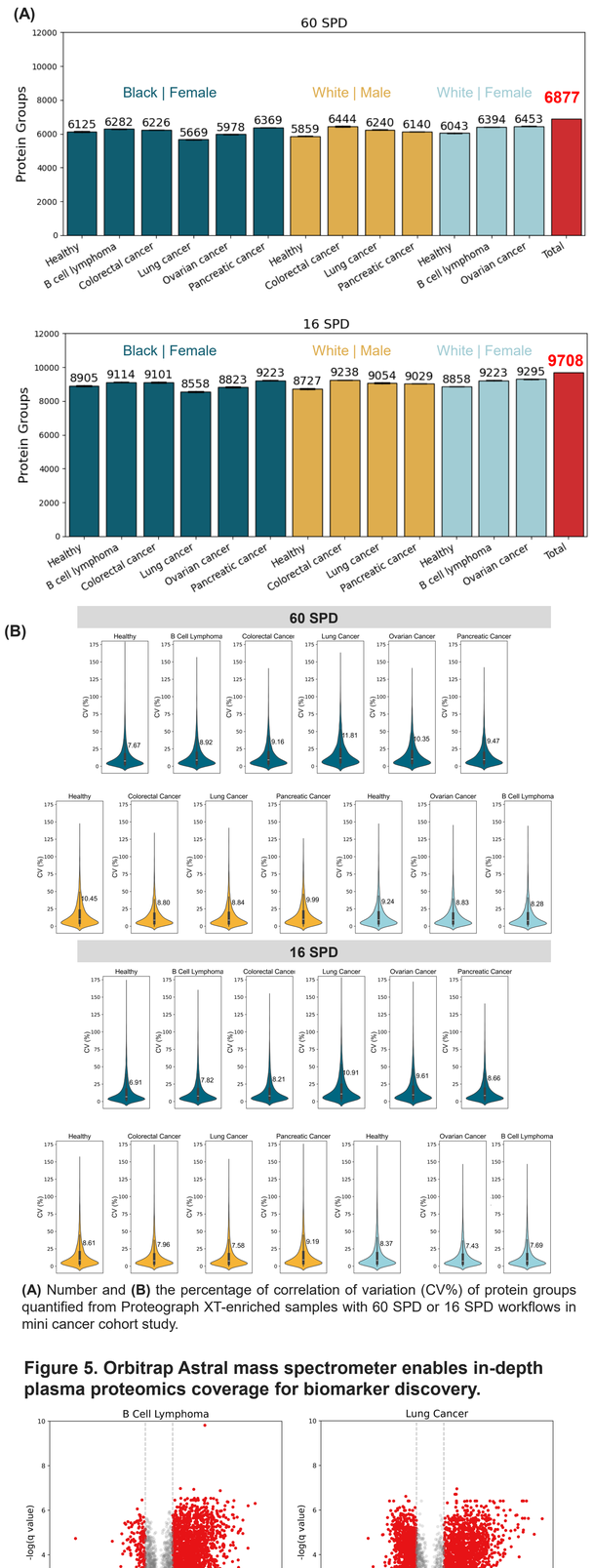
We further adapted the high-throughput 60 SPD and maximized coverage 16 SPD workflows to investigate plasma samples from a mini cancer cohort. We were able to identified 2,377 and 5,273 protein groups from depleted plasma samples with the 60 and 16 SPD methods, respectively (**Figure 3**). Utilizing enrichment from the Proteograph XT Assay, we identified 6,877 protein groups with the 60 SPD method and 9,708 protein groups with the 16 SPD method, showcasing the sensitivity of the Orbitrap Astral mass spectrometer (**Figure 4A**). Additionally, the coefficient of variation (CV) was less than 10 percent, underscoring the quantitation precision (**Figure 4B**). Collectively, these results demonstrate the excellent quantitation precision and accuracy of our plasma proteomics workflow, effectively supporting the high-throughput needs of translational research studies.

Figure 3. High-throughput and maximized coverage plasma proteomics analysis from High Select™ depletion spin columns.



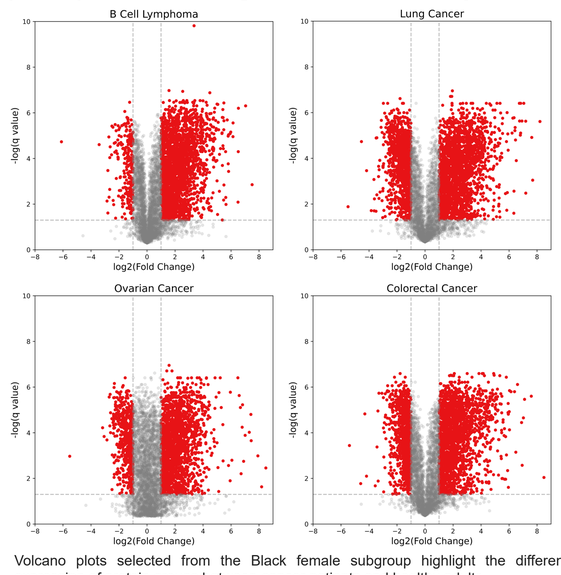
Number of protein groups quantified from depleted plasma samples with 60 SPD or 16 SPD workflows in mini cancer cohort study.

Figure 4. In-depth plasma proteome coverage and reproducible quantitation in mini cancer cohort study.



(A) Number and **(B)** the percentage of correlation of variation (CV%) of protein groups quantified from Proteograph XT-enriched samples with 60 SPD or 16 SPD workflows in mini cancer cohort study.

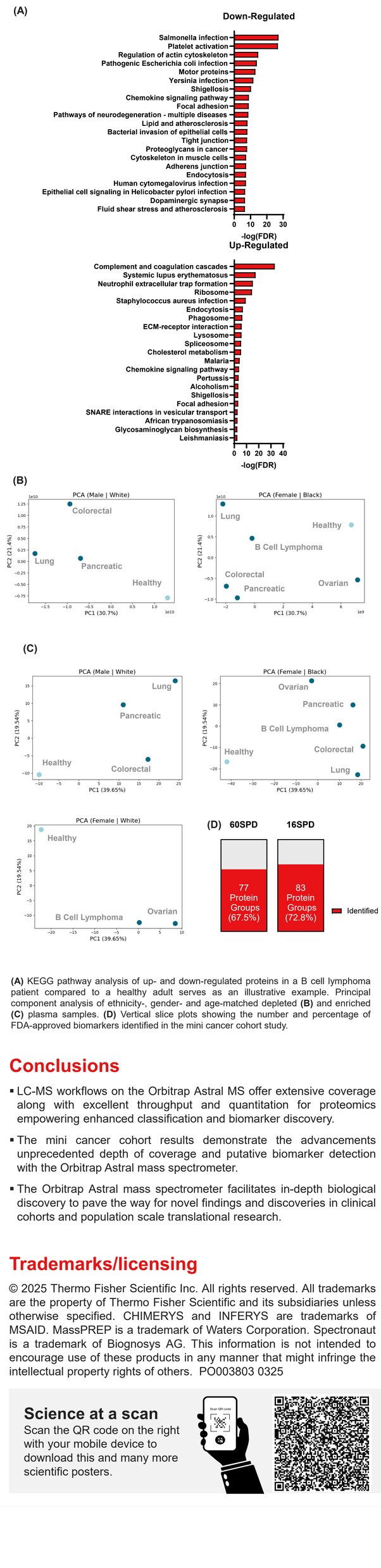
Figure 5. Orbitrap Astral mass spectrometer enables in-depth plasma proteomics coverage for biomarker discovery.



Volcano plots selected from the Black female subgroup highlight the differential expression of protein groups between cancer patients and healthy adults.

The plasma proteomics analysis of the mini cancer cohort study, utilizing the Orbitrap Astral mass spectrometer, provided sensitive identification and reproducible quantitation, enabling the detection of a substantial number of protein groups that are up- or down-regulated in cancer patients compared to healthy, age-, gender-, and ethnicity-matched adults (**Figure 5**). Furthermore, pathway and PCA analysis demonstrated that the in-depth coverage of the plasma proteome, powered by the Orbitrap Astral MS, enabled precise and well-informed classification of the samples (**Figure 6A-C**). Additionally, we identified 67.5% and 72.8% of the U.S. Food and Drug Administration (FDA)-approved oncology biomarkers in LFQ-DIA with direct DIA search (**Figure 6D**), further underscoring the Orbitrap Astral mass spectrometer's potential for highly successful biomarker validation in the future.

Figure 6. Biomarker discovery workflow empowered by the Orbitrap Astral mass spectrometer.



(A) KEGG pathway analysis of up- and down-regulated proteins in a B cell lymphoma patient compared to a healthy adult serves as an illustrative example. Principal component analysis of ethnicity-, gender- and age-matched depleted **(B)** and enriched **(C)** plasma samples. **(D)** Vertical slice plots showing the number and percentage of FDA-approved biomarkers identified in the mini cancer cohort study.

Conclusions

- LC-MS workflows on the Orbitrap Astral MS offer extensive coverage along with excellent throughput and quantitation for proteomics empowering enhanced classification and biomarker discovery.
- The mini cancer cohort results demonstrate the advancements unprecedented depth of coverage and putative biomarker detection with the Orbitrap Astral mass spectrometer.
- The Orbitrap Astral mass spectrometer facilitates in-depth biological discovery to pave the way for novel findings and discoveries in clinical cohorts and population scale translational research.

Trademarks/licensing

© 2025 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. CHIMERYS and INFERYS are trademarks of MSAID. MassPREP is a trademark of Waters Corporation. Spectronaut is a trademark of Biognosys AG. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. PO003803 0325

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.

Learn more at thermofisher.com/astral