Translational Proteomics

Uncovering biological differences at scale: high-throughput and in-depth plasma proteomics with the Seer Proteograph ONE workflow and Orbitrap Astral Zoom Mass Spectrometer

Sudipa Maity[1], Jared Deyarmin[1], Jolene Duda[1], Kevin Yang[1], Xiaoyan Zhao[2], Taylor Page[2], Lee Cantrell[2], Aaron Gajadhar[2], Asim Siddiqui[2], Amirmansoor Hakimi[1], Stephanie Samra[1]

[1]Thermo Fisher Scientific, San Jose, CA; [2]Seer Inc., Redwood City, CA

Abstract

Purpose: Demonstrate capability of detecting comprehensive biological insights from clinically diverse plasma samples through deep and reproducible proteome profiling, enabled by the Seer® Proteograph® ONE workflow and the Thermo Scientific™ Orbitrap™ Astral™ Zoom Mass Spectrometer, supporting robust biomarker discovery workflows.

Methods: The Seer Proteograph ONE workflow was used to prepare plasma samples from a small disease cohort with matched healthy controls to evaluate its performance and feasibility in detecting biologically meaningful differences. Samples were then separated and analyzed using the Thermo Scientific™ Vanquish™ Neo UHPLC system coupled to the Orbitrap Astral Zoom mass spectrometer, enabling deep and reproducible proteome coverage. Two analytical approaches—a high-throughput method and a Max-ID workflow—were tested to demonstrate the versatility and throughput for comprehensive biomarker discovery in clinical cohort studies.

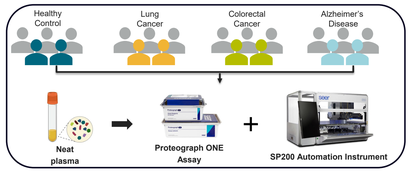
Results: The Seer Proteograph ONE workflow with Orbitrap Astral Zoom mass spectrometer demonstrated that both high-throughput and Max-ID workflow were able to detect differences in biology in diseased states from healthy controls. The high-throughput workflow enabled rapid, reproducible analysis, while the Max-ID approach delivered deeper proteome coverage. The synergy between the Seer Proteograph workflow and Orbitrap Astral Zoom mass spectrometer supports scalable, high-resolution biomarker discovery, making it suitable for large clinical studies and precision medicine applications.

Introduction

Plasma proteomics enables minimally invasive biomarker discovery by capturing disease-related molecular signatures. However, its effectiveness is limited by the wide dynamic range of protein concentrations. To address this, we combined the Seer Proteograph ONE workflow with the Orbitrap Astral Zoom mass spectrometer to enable a high-throughput, high-precision platform that delivers deep plasma profiling and actionable insights for large-scale clinical and translational research (**Figure 1**).

Experimental Design

Sample Processing



Data Acquisition And Analysis

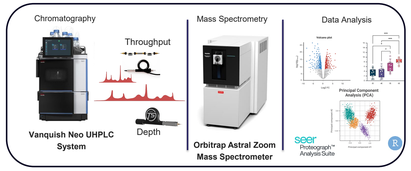


Figure 1. Transforming plasma proteomics with the Seer Proteograph ONE workflow and Orbitrap Astral Zoom mass spectrometer and unveiling biological insights. This streamlined workflow delivers unprecedented depth, precision, and throughput in plasma proteomics, enabling large-scale studies and meaningful discoveries for translational research, including comparisons of samples from three different disease states against healthy controls. Created with Biorender.com.

Experimental Procedure

Sample preparation

Age, gender, and ethnicity matched biologically diverse human K_2EDTA plasma samples from healthy control, lung cancer, colorectal cancer and Alzheimer's disease patients (n=5 patients per condition; n= 20 total samples) were obtained from BioIVT. Samples were processed using the Seer Proteograph ONE workflow. Pooled samples from healthy donors were processed in triplicate to evaluate preparative technical reproducibility. Digested plasma samples were resuspended in 98% Optima grade Water, 2% Optima grade Acetonitrile in 0.1% Formic Acid. Peptides were quantified using the Pierce™ Peptide Fluorometric Quantitative Assay, then normalized to 50 ng/uL.

LC separation

Sample separation was performed using the Vanquish Neo UHPLC. LC gradient conditions for different samples per day (SPD) throughputs and different columns are summarized in **Table 1**.

EASY-Spray™ HPLC ES906 column (150µm x 15cm, 2µm particle size)

IonOpticks™ Aurora Frontier™ column (60cm ×75 µm, 1.7µm particle size)

60SPD			
Gradient	Time (min)	% Mobile Phase B	Flow (µl/min)
	0	10	2.0
	0.3	10	2.0
	0.6	10	0.8
	13.6	22.5	0.8
	20.5	35.0	0.8
	20.9	55.0	2.0
	20.95	99.0	2.0
	22.35	99.0	2.0

16 SPD			
Gradient	Time (min)	% Mobile Phase B	Flow (µl/min)
	0	8	0.25
	1.0	8	0.25
	1.5	8	0.2
	61.5	28.0	0.2
	78.5	50.0	0.2
	79.0	99.0	0.4
	81.5	99.0	0.4
	83.5	99.0	0.4

Table 1. LC gradients for 60 and 16 SPD. 60 SPD and 16 SPD sample separation was performed with a trap-elute and direct configurations, respectively.

MS data acquisition and data analysis

The full scan MS1 and narrow range data independent acquisition (nDIA) MS2 mass spectrometer parameters are shown in **Table 2**. Five hundred nanograms of eluted peptides were analyzed on an Orbitrap Astral Zoom MS. Raw files were processed using Proteograph Analysis Suite with library free search (DIA-NN 1.8.1) without MBR, separated by throughput (60 SPD & 16SPD) using recommended parameters from Seer.

Global Parameters (Source & MS)	
Positive Ion Voltage	2100 Volts
Ion Transfer Tube Temperature	290° C
Expected Peak Width	10 seconds
Default Charge State	2
Lock Mass Correction	Off

MS1 Full Scan Experiment Parameters	
Orbitrap Resolution	240K
Scan Range (*m/z*)	380-980
Normalized AGC Target (%) / Absolute AGC Value	500% / 5.00e6
Maximum Injection Time (ms)	5 (16 SPD) or 3 (60SPD)
Microscans	1

MS2 DIA Scan Experiment Parameters	
Precursor Mass Range (*m/z*)	380-980
DIA Isolation Window (*m/z*)	2.5 (16 SPD) or 3 (60 SPD)
Window Placement Optimization	On
AGC Target	Custom
Normalized AGC Target (%) / Absolute AGC Value	500% / 5.00e4
Maximum Injection Time	7 milliseconds
DIA Scan Range (*m/z*)	150-2000
HCD Collision Energy (%)	25
RF Lens (%)	40
Pre-Accumulation	On
Loop Control	Time
Time	0.6 seconds

Table 2. MS methods for 60 and 16 SPD (global, MS1 full scan and MS2 DIA parameters).

Results

Reproducible and robust sample preparation and measurement precision with the Proteograph ONE workflow and the Orbitrap Astral Zoom mass spectrometer

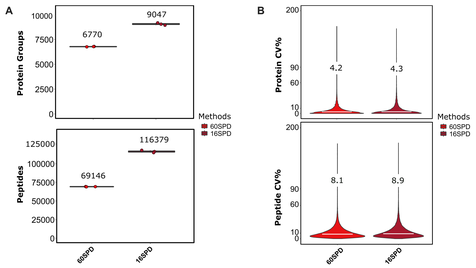


Figure 2. Measurement precision from plasma sample preparation technical replicates. (**A**) Box plots show protein (top) and peptide (bottom) identifications from triplicate preparations of pooled healthy plasma with single injections. Conditions are ordered left to right from high throughput (60 SPD, red) to maximum depth (16 SPD, dark red), with medians indicated by black lines and values. Circles represent individual sample preparation technical replicate injections. (**B**) Violin plots show protein (top) and peptide (bottom) %CV across the same triplicates, with median %CVs indicated.

Protein and peptide identifications across diseased samples demonstrate the groundbreaking proteomic depth and platform flexibility tailored to research needs

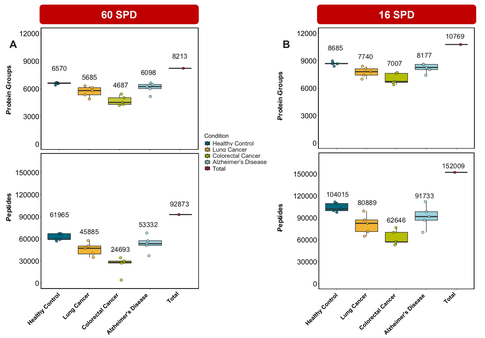


Figure 3. Protein group and peptide Identifications across biological sample groups. (**A**) High throughput (60 SPD) method and (**B**) maximum identification throughput (16 SPD) method. Median protein and peptide numbers are indicated by values above box plots.

Exceptional dynamic range captures 7-8 orders of magnitude in both high throughput and Max-ID throughputs

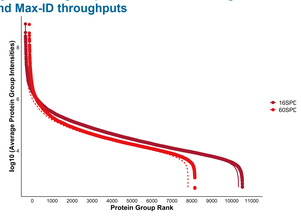


Figure 4. Rank plots across different throughputs. Protein groups from the 60 SPD (red) and 16 SPD (dark red) workflows were ranked on the x-axis, with the y-axis showing $\log_{10}$ of average protein group intensities, illustrating relative protein abundance.

Balancing speed and depth: high overlap in protein coverage between high throughput and Max-ID throughputs while identifying biologically relevant proteins

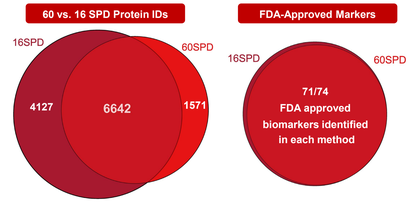


Figure 5. Protein overlaps between both SPDs. Euler diagram (left) shows that the high-throughput 60 SPD workflow covers 64% of proteins identified in the deeper 16 SPD run, including 96% (71/74) of FDA-approved biomarker proteins (right).

Distinct proteomic signatures: PCA highlights patient variability and sample preparation and instrument analytical precision

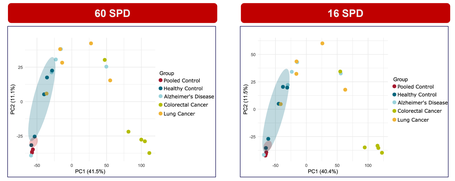


Figure 6. Principal Component Analysis (PCA) reveals greater variance among patient-derived samples compared to healthy controls. Healthy controls (dark blue) show minimal variance (11% along PC2), with tightly clustered technical replicates (red), indicating strong instrument reproducibility. In contrast, patient samples show broader PCA dispersion, reflecting biological heterogeneity and potential disease-associated proteomic shifts, consistent across both 60 (left) and 16 (right) SPD workflows.

Enhanced capability to detect and analyze biological signatures linked to variations in protein abundance in lung cancer samples

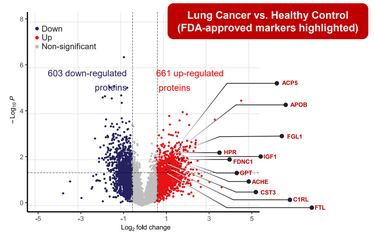


Figure 7. Differentially expressed proteins across lung cancer vs. healthy controls. Over 1,200 proteins were differentially expressed (DEPs) in plasma from lung cancer vs. healthy samples, with 603 downregulated and 661 upregulated. Notably, some of the key upregulated proteins—highlighted in dark red—include FDA-approved protein biomarkers.

Deep functional insights from differentially abundant proteins

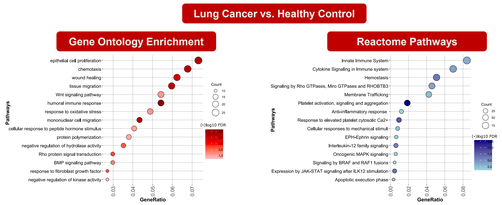


Figure 8. Pathway enrichment analysis. FDR enriched 15 Gene Ontology (GO, left) and Reactome pathway (right) enrichment analyses of differentially expressed proteins (DEPs) in lung cancer vs. healthy controls highlight dysregulated pathways, with statistical significance (-$\log_{10}$ FDR) shown by color and protein count by bubble size.

Conclusions

- The Seer Proteograph ONE workflow combined with Orbitrap Astral Zoom mass spectrometer identified >10,800 protein groups using ultradeep profiling, compared to >8,200 with the high-throughput method—demonstrating scalable, reproducible plasma proteomics with clear detection of biological differences for biomarker discovery.
- The Protoeograph ONE workflow combined with the Orbitrap Astral Zoom Mass Spectrometer captures exceptional dynamic range of 7-8 orders of magnitude in plasma proteome
- >1,000 differentially abundant proteins detected with biologically relevant dysregulated pathways such as immune signaling or tissue remodeling underscores the complex biology of lung cancer
- Identification of FDA markers as differentially expressed showcases the workflow's strength for capturing biologically relevant plasma proteins

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