### Introduction

To improve the detection of peptides and proteins, we performed comprehensive proteomic analysis using the FAIMS Pro Duo Interface with individual CV values allowing access to more peptides than multiple runs using single CV values with a FAIMS Pro Duo Interface. These results demonstrate that pairing optimized instrument acquisition strategies for both OT/OT and OT/IT leveraging UHPLC, ultra-high resolution separation columns, and high abundance analysis improves speed, sensitivity, and depth of coverage compared to current acquisition methods and workflows paired with a standard consensus workflow.

### Materials and Methods

Sample Preparation and Liquid Chromatography

The method set in the LabScribe method was used to prepare HeLa cell lysate digest samples. Samples were extracted and prepared using single 15 mm columns packed with 4.6 mm was.m. silica particles using a C18-LightningPrep system. Samples were lyophilized in a SpeedVac system. Student means between 0.05 and 0.05 were used to evaluate single shot proteome analysis.

Black Spectra Method

Data were collected using a Thermo Scientific EXACT source and Orbitrap Eclipse Tribrid mass spectrometer to collect data-dependent acquisition in model with 50% data collection, using Orbitrap detection and MS-MS for peptides. These strategies were repeated for a single and multireference strategies.

### Results

#### Optimisation of mass spectrometry

To determine the effects of acquisition strategies for both OT/OT and OT/IT leveraging UHPLC, ultra-high resolution separation columns, and high abundance analysis, improvements were made to improve performance compared to existing methods and workflows.

<table>
<thead>
<tr>
<th>Protein Groups</th>
<th>Unprocessed Proteome</th>
<th>CHIMERYS</th>
<th>CHIMERYS Intelligent Search</th>
</tr>
</thead>
<tbody>
<tr>
<td>4_Aurora_cv30_027</td>
<td>4.35E10</td>
<td>4.15E10</td>
<td>4.45E10</td>
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<td>7.65E10</td>
<td>7.45E10</td>
<td>7.95E10</td>
</tr>
</tbody>
</table>

#### Performance improvements for longer gradients

To determine if the number of unique peptides and proteins identified by OS/OT/OT data-dependent acquisition can be improved, we compared the number of unique peptides and proteins identified by OS/OT/OT data-dependent acquisition with these longer PD 3.0 2.0 2.0 MaxIT用自己的标准一致流程。

#### Spectra being collected with these longer PD 3.0 2.0 2.0 MaxIT

To determine if the number of unique peptides identified by OS/OT/OT data-dependent acquisition can be improved, we compared the number of unique peptides identified by OS/OT/OT data-dependent acquisition with these longer PD 3.0 2.0 2.0 MaxIT

#### CHIMERYS intelligent search algorithm

The performance of CHIMERYS intelligent search algorithm was assessed on a variety of datasets, including the HeLa cell lysate digest sample. The algorithm was able to identify a significant number of unique peptides and proteins, improving the overall performance compared to existing methods.

### Conclusion

These results demonstrate that pairing optimized instrument acquisition strategies for both OT/OT and OT/IT leveraging UHPLC, ultra-high resolution separation columns, and high abundance analysis improves speed, sensitivity, and depth of coverage compared to current acquisition methods and workflows.

#### TRADEMARKS LICENSING

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