Precision Mass Spectrometry: Advances and Applications

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Vice President, Omics Marketing
Life Sciences Business Unit

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50 Years of “Firsts” in MS - Thermo Fisher Scientific’s Legacy of Innovation

1967

First commercial triple quad MS
First benchtop MALDI-ToF MS
First LC ion trap MS
First high-res triple quadrupole MS
First radial ejection linear ion trap
First commercial ion trap FT-ICR MS
First commercial Orbitrap MS
First commercial Linear Trap ETD
First benchtop quadrupole-Orbitrap MS
First tribrid MS

Continuing the Legacy of MS Innovation in 2017...
Major Product Introductions at ASMS 2017

Thermo Scientific™ Instruments
- Orbitrap HRAM MS
- Orbitrap Fusion™ Lumos™ MS Options
  - UVPD
  - 1M
  - APD
- Q Exactive™ HF-X LC-MS
- Exactive™ GC Orbitrap™ GC-MS

Thermo Scientific™ Software
- Small molecule analysis
- Protein analysis
- Cloud Applications

Sample Prep, Kits and Consumables
- MS Crosslinking reagents
- Capillary flow HPLC columns
- Metabolomics Standards Kits

Biocorates AbsoluteIDQ p400HR Kit
DSSO and DSBU MS-Cleavable Linkers
Easy-Spray PepMap 150µm Column
Sensitivity and robustness without compromise

**TSQ Altis Triple Quad LC-MS**

- Ultimate sensitivity and speed
- Increased selectivity with H-SRM
- Unmatched analytical flexibility and reproducibility
- For the most demanding applications

**AIM+ TECHNOLOGY**

**Active Ion Management+**

Optimize ion management precision, reliability, speed, and reproducibility.

**TSQ Quantis Triple Quad LC-MS**

- Ultimate robustness
- Best in class sensitivity
- Increased selectivity with H-SRM
- Ease of use and maintenance

"......."With the new innovations in the Thermo Scientific TSQ Altis MS, my lab can develop quantitative methods for biotherapeutic proteins and target receptors with extreme sensitivity, selectivity, accuracy and precision. This is very exciting for our research since this capacity is very unique"

Jun Qu, University of Buffalo
Innovations that deliver new quantitative performance

- **Presenter:** JAKOB

**NEW Thermo Scientific TSQ Altis Triple Quadrupole Mass Spectrometers**

- High capacity Ion transfer tube (HCTT)
- Segmented quadrupoles for enhanced performance with both SRM and H-SRM
- Enhanced dual-mode electron multiplier detector ensuring excellent linearity and dynamic range
- Active collision cell facilitating more SRMs/sec
- Electrodynamic ion funnel (EDIF)
- Ion beam guide with neutral blocker
- OptaMax™ NG ion source with integrated APCI

**AIM+ TECHNOLOGY**
Thermo Scientific Q Exactive HF-X MS — New Innovations

Fastest, most powerful Q Exactive MS

- HyperQuad Mass Filter with Advanced Quadrupole Technology (AQT)
- Advanced Active Beam Guide (AABG)
- Electrodynamc Ion Funnel
- High Capacity Transfer Tube (HCTT)
- Ultra-High Field Orbitrap Analyzer (up to 40Hz acquisition speed)
- Optimized Scan Matrix with accelerated HCD 40 Hz MS/MS
- Accelerated HCD Cell
- Improved S/N for intact proteins / mAb
- Brighter ion beam 2-3 x more sensitive
- More ions
- Faster scanning

Fastest, most powerful Q Exactive MS
Thermo Scientific Q Exactive HF-X MS — Faster, More Sensitive

**Productivity increase in DDA**

- Q Exactive HF, 60 min
- Q Exactive HF-X, 30 min
- Q Exactive HF-X, 60 min

**Peptide quantitation in DIA**

- Peptide Precursors
  - ID
  - CV% <20%
  - CV% < 10%

**Sample:** 1ug Pierce Hela digest

**ASMS 2017: TP 389, T.N. Arrey et al.**

**Sample:** Pierce Hela digest, 60 min CapLC

**ASMS 2017: ThP237, Y. Xuan et al.**
“The 1 million resolution Orbitrap is a unique tool to allow fluxomics analysis of lipids with sensitivity comparable to radioactivity tracing.”
Matthew Mitsche, UT Southwestern Medical Center

“……UVPD-MS/MS and MS^n promises an unprecedented level of structural information for the improved ID and characterization of lipids, not possible to attain on any other commercial MS system”
Gavin Reid, University of Melbourne

“The new APD algorithm provides new peptide precursors to go after and amounts to a 25–35% identification bonus. This will be a key technology in achieving deeper coverage and higher throughput for proteome analyses.”
Josh Coon, University of Wisconsin-Madison
Thermo Scientific Orbitrap Fusion Lumos Tribrid Mass Spectrometer

Delivering even more power – UVPD Option

Integrated UVPD

Improves sequence coverage with new fragmentation technique. Over 20% unique bond cleavages. Lipid double bond localization.

21% unique coverage

79.7% total coverage

Glycosylation site locations

DUAL PRESSURE LINEAR ION TRAP MS² and sensitive mass analysis of fragments resulting from CID, HCD, ETD, EThcD and UVPD

Light Chain UVPD MS² scan was measured at 120,000 FWHM resolution after a UVPD activation time of 8 ms
Thermo Scientific Orbitrap Fusion Lumos Tribrid Mass Spectrometer

Delivering even more Identification power - APD

**Advanced Peak Determination**

Assigns more monoisotopic peaks and charge states in complex spectra which significantly increases the population of precursors available for data-dependent analysis to deliver more unique peptide identifications.

**Standard Peak Determination**

**Advanced Peak Determination**

![Graph showing comparison between Standard and Advanced Peak Determination](image)

- More IDs in the same time
- Equivalent IDs in half the time
Thermo Scientific Orbitrap Fusion Lumos Tribrid Mass Spectrometer
Delivering even more power – 1M Option

1M Resolution

Resolves fine isotope structure of small molecules and enables lipid metabolic flux analysis

Fine Isotope Structure of Irganox:
$^{18}$O and $^{213}$C isotopes resolved at 1M

Orbitrap resolution 500,000
(m/z 200 FWHM)

Orbitrap resolution 1,000,000
(m/z 200 FWHM)

Labeled deuterium and natural $^{13}$C isotopes resolved on LC timescale

Thermo Fisher Scientific
## Major Software Releases

### Unleashing the potential of MS data

<table>
<thead>
<tr>
<th>Proteomics and Protein Analysis</th>
<th>Instrument Control and Qualitative Analysis</th>
<th>Quantitative Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermo Scientific™ Proteome Discoverer™ 2.2 Software</td>
<td>Thermo Scientific™ FreeStyle™ 1.3 Software</td>
<td>Thermo Scientific™ TraceFinder™ 5.0 Software **</td>
</tr>
<tr>
<td>Thermo Scientific™ ProteinCenter™ 4.0 Software **</td>
<td>Thermo Scientific™ Xcalibur™ 4.1 Software</td>
<td>** preview</td>
</tr>
<tr>
<td>Thermo Scientific™ ProSightPC™ 4.0 Software</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Small Molecule Analysis</th>
<th>Biopharmaceutical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermo Scientific™ Compound Discoverer™ 2.1 Software</td>
<td>Thermo Scientific™ Bioparma Finder™ 3.0 Software **</td>
</tr>
</tbody>
</table>

- Maximizing investment in instrumentation
- Rigorous data processing and results

** preview
Cloud Applications

- **MS Instrument Connect**: Remote monitoring dashboard for multiple instruments
- **Sample Profiler**: Profiling of authentic products, add/compare new samples and determine trends and differences
- **Pathway Overrepresentation**: Find significant pathways from gene or protein lists
- **Omics Comparator**: See overlap and uniqueness comparing protein or gene lists

Addressing evolving needs to connect scientists, instruments, software and data
Supporting Segment Evolution

Enabling breakthrough research to fight cancer

Translational Research and Personalized Medicine

Standardized metabolic phenotyping

Metabolomics

Confident characterization of biologics

Biopharmaceutical Analysis and QA/QC

Next-generation structure analysis

Integrative Structural Biology

Tomorrow’s quantitation today

Food/Env Tox
Clinical apps pK
Proteome Profiling for Precision Medicine

From Sample to Read-Out In a Day

FFPE archive

Fresh/frozen tissue

SkBr3 (EGFR+)
U251 (EGFR++)
A431 (EGFR+++)

Proteome Signature
Classified histotypes

Features & Abundance

Samples

Biomarkers
Differentially regulated pathways
Dysregulated functional modules

QUANTITATIVE
REPRODUCIBLE
STANDARDIZED
SCALABLE
The Goal: Standardized, High Throughput Proteomics

Large Scale Proteomics

Sample Preparation ➔ QC ➔ Control ➔ Sample ➔ QC ➔ Separation ➔ Data Acquisition ➔ Data Analysis

> Hundreds of SAMPLES

Standardization Method
- Defined QC, optimized separation, efficient data acquisition
- Defined analysis criteria (# replicates, <1%FDR, < 20%CV)

Reproducible Results
- Highest specificity & confidence
- Optimal sensitivity & depth of coverage

Delivering comprehensive proteome profiles with reproducibility and precision

NOTE:
- **COMPLETE quantitation**
  Peptides reproducibly quantified across all repeats
- **SPARSE quantitation**
  Peptides identified, but not quantified across all repeats
Precursor Label Free Quantitation Workflows Built On HRAM Orbitrap MS

Data Dependent Acquisition DDA+
- Thermo Scientific™ Proteome Discoverer™ Software

Data independent Acquisition HR-DIA
- Thermo Scientific™ Q Exactive™ HF-X MS
- Biognosys Spectronaut Software

Standardization
- Complete quantification
- Designed for large sample cohorts
- Flexible workflows serving the community

Supports both
- Biospecimen profiling/digital archiving
- Mechanism of action studies high quantitative precision

Driving quantitative reproducibility, precision and standardized workflow solutions
Robust, Reproducible Proteome Profiling

Completely quantified 3,434 proteins

Of 3434 proteins (1%FDR) 3329 (97%) are quantified across all replicates with using PD 2.2 MS1 peak detection/aligment and label free quant capabilities

Proteomics can deliver very deep levels of analysis with complete quantitation
TMT Multiplexing Workflow for Precise Data in Less Time

Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS with Method Templates

Thermo Scientific™ TMT™ 11-Plex Reagents

Sample Labeling and Preparation → LC-MS/MS (SPS MS3) Analysis → Data Analysis

Number of protein quantifications per day

Recent Technology
3500 proteins/2 hours

Next Generation Technology
5000 proteins/2 hours

Advances in Tribrid MS technology

Unique Thermo Fisher Scientific workflow with potential for massive throughput
Driving Rigor and Standardization
A Comprehensive Solution for Metabolomics Analysis

Highest Quality Data
- Comprehensive untargeted discovery
- Unambiguous Orbitrap HRAM accuracy
- Robust for large cohort studies

Target Metabolites of Interest
- Full scan HRAM quantitation
- Biocrates Absolute/DQ p400 HR Kit
- Downstream targeted SRM triple quadrupole quantitation

Addressing Chemical Diversity
- UHPLC for broad coverage
- IC for polar compounds
- GC for volatiles, complements UHPLC

Turn complex data to knowledge
- Robust, rigorous statistical analysis
- Curated libraries for confident ID
- Interactive visualization of results

Core of successful metabolomics powered by Orbitrap technology
Complex Data into Knowledge – Software for the Total Solution

Customers Demanding Insights

- Go from “features” to confident identifications
- Reduce the risk of false positives
- Designed to mine rich Orbitrap data

Thermo Scientific™ Compound Discoverer™ 2.1 Software

Complete ID and Characterization Solution

- Differential analysis
- Rigorous statistical analysis
- Confident identification
- Structure elucidation
- Pathway mapping

Database and Spectral Libraries

High Quality Curation

- mzCloud* online fragmentation library
  - ~7,000 compounds
  - > 2 million spectra
- New Thermo Scientific™ Orbitrap™ GC-MS Metabolomics Library

* HighChem LLC

Significant investment to deliver robust and rigorous results that matter
Standardized Workflow for Targeted Metabolomics with Biocrates Kit

Delivering standards, throughput and reproducibility across studies
Thermo Fisher Scientific: A Global Partner in Precision Medicine

Multi-Omic profiling and data integration in practice
- Targeted NGS Cancer Panels
- Proteome & Metabolome profiling
- BRIMS Center: working lab

The Boston Globe
Thermo Fisher targets a growing niche: precision medicine

Pillars for Success
- Quantitative
- Reproducible
- Standardized
- Scalable
Disciplines for full biotherapeutic characterization

Higher Order Analysis
Intact Analysis
Aggregate Screening
Charge Variant Screening
Glycan Analysis
Sub-Unit Analysis
Released Glycan
Peptide Mapping
Thermo Scientific Q Exactive HF-X with Biopharma Option

Expanded functionality for complete characterization

- High Mass Range mode for intact native proteins and ADCs

- Protein Mode with high sensitivity and speed reveals mAb subunits even without LC separation 5-10X S/N improvements

- Brighter ion source and enhanced speed ensures comprehensive coverage for peptide mapping
  
  - Advancing MS technology for multi attribute measurements in QC
Peptide mapping of trastuzumab

Thermo Scientific Q Exactive HF-X MS with Biopharma Option

Sequence coverage map with color coded peptides

Modification Summary:
Quantification of glycoforms, deamidation, oxidation

Identified peptides highlighted by color coded peak shading in chromatogram

Sequence coverage 100%
Identification and quantitation of modifications

100% Sequence coverage based on MS/MS spectra

<table>
<thead>
<tr>
<th>Protein</th>
<th>Number of MS Peaks</th>
<th>MS Peak Area</th>
<th>Sequence Coverage</th>
<th>Abundance (mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Herceptin Heavy Chain</td>
<td>680</td>
<td>34.2%</td>
<td>100.0%</td>
<td>60.39%</td>
</tr>
<tr>
<td>2. Herceptin Light Chain</td>
<td>312</td>
<td>15.9%</td>
<td>100.0%</td>
<td>39.61%</td>
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<tr>
<td>Unidentified</td>
<td>2370</td>
<td>50.4%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Thermo Scientific Q Exactive HF-X MS with Biopharma Option

Intact mass analysis – Native and denatured proteins

Superior analysis of intact proteins under native and denaturing conditions

Resolves protein modifications such as glycosylation and adducts

Example: Intact infliximab

Deconvoluted spectrum of the complex glycoforms under native & denaturing conditions
Analysis of Intact Trastuzumab under Native Conditions in HMR Mode

S/N ratio improved by ~5-10 on the Q Exactive HF-X MS

SEC-LCMS analysis of intact trastuzumab monoclonal antibody using Acclaim SEC column, 4.6 x 300 mm, 300 μl flow, 50mM ammonium acetate.

Full MS, HMR mode, m/z 2500–8000, resolution setting 30k, 10 μscans. Spectra show an average of 3 μscans each.
Thermo Fisher Scientific — Clear Leadership in Structural Biology
Complimentary Leadership in Cryo-EM and Orbitrap MS

Publications in Nature and Science

The Nobel Prize in Chemistry 2017
The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2017 to
Jacques Dubochet, Joachim Frank, Richard Henderson
University of Lausanne, Switzerland
Columbia University, New York, USA
MMI Laboratory of Molecular Biogeodynamics, Cambridge, UK
“For developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution”
Cool microscope technology revolutionises biochemistry

Nature Methods\(^1\) names
Cryo-electron microscopy “Method of the Year”

“This represents a new era in imaging of proteins in humans with immense implications for drug design”
Francis Collins, M.D.

Launch Year 4 Year 10

Nature Methods, 12/30/2015

Orbitrap Publications

Integrative structural biology answers:
- 3D Structure
- Stoichiometry
- Composition
- Topology (binding partners, cofactors, messengers, etc.)
- Binding affinity
- Dynamics
- Aggregates
- Biological function
Understanding the structure-function context has game changing potential.

Thermo Fisher Scientific Integrative Structural Biology Solutions

Structure-function information based upon chemical information

3D structure-function information from single protein imaging

Crosslinking MS  Native MS  HDX  FPOP

Accelerating the path from structure to function through integrative structural biology solutions

Mass Spectrometry (MS)  Cryo-Electron Microscopy (Cryo-EM)
Combined methods provide structural basis to understand protein assembly
Crosslinking Mass Spectrometry Workflow

Sample Labeling and Enrichment → MS^n and Multiple Fragmentation Analysis → Data Analysis

Thermo Scientific™ DSSO and DBSU MS Cleavable Linkers
Thermo Scientific™ Orbitrap Fusion™ Lumos™ Trubrid™ MS with Method Templates
Thermo Scientific™ Proteome Discoverer™ Software with XlinkX Node

Protein Structure
Protein Complexes
Interactome

Unique workflow that is complete, easy to use and sensitive
Summary

New Product Introductions for Precision Mass Spectrometry – Continuing the legacy of innovation

- Extending leadership in HRAM Orbitrap MS
- Driving quantitation solutions for complex analyses with new QQQ’s and Orbitraps
- Major software releases that unleashing the potential of MS data

Leading the Way with Scientific Rigor and Precision in Key Application Areas

- Delivering standardized solutions for proteomics and metabolomics based translational research/precision medicine
- Game-changing structure function studies by integrative structural biology solutions
- Comprehensive and pioneering workflows for biopharma characterization and QA/QC