7th Czech Mass Spectrometry Conference

April 11th, 2018

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Next Generation Technology for Reproducible and Precise Proteome Profiling

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Application and training specialist

Agenda

1) **Introduction and Q Exactive HF-X**

2) **High Resolution DIA Workflow**

3) **DDA+ Workflow (Label free quantification)**

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The Goal: Standardized, High Throughput Proteomics

Large Scale Proteomics

- QUANTITATIVE
- PRECISE
- REPRODUCIBLE
- STANDARDIZED

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Flexible Quantitative Workflow Solutions

High-Resolution DIA Workflow

Unparalleled proteome coverage and dynamic range

spectronaut pulsar

- Highest depth of proteome coverage and quantitative insight
- Robust quantitative precision

➢***Biospecimen profiling***
➢***Digital archiving***

DDA+ Workflow

Unsurpassed quantitative precision and reproducibility

- Unrivaled precision in precursor quantitation
- Maximize complete, reproducible quantitation across samples
- Minimize ‘missing values’ among samples

➢***Cellular signaling studies***
➢***Mechanism of action studies***
➢***PTM profiling***

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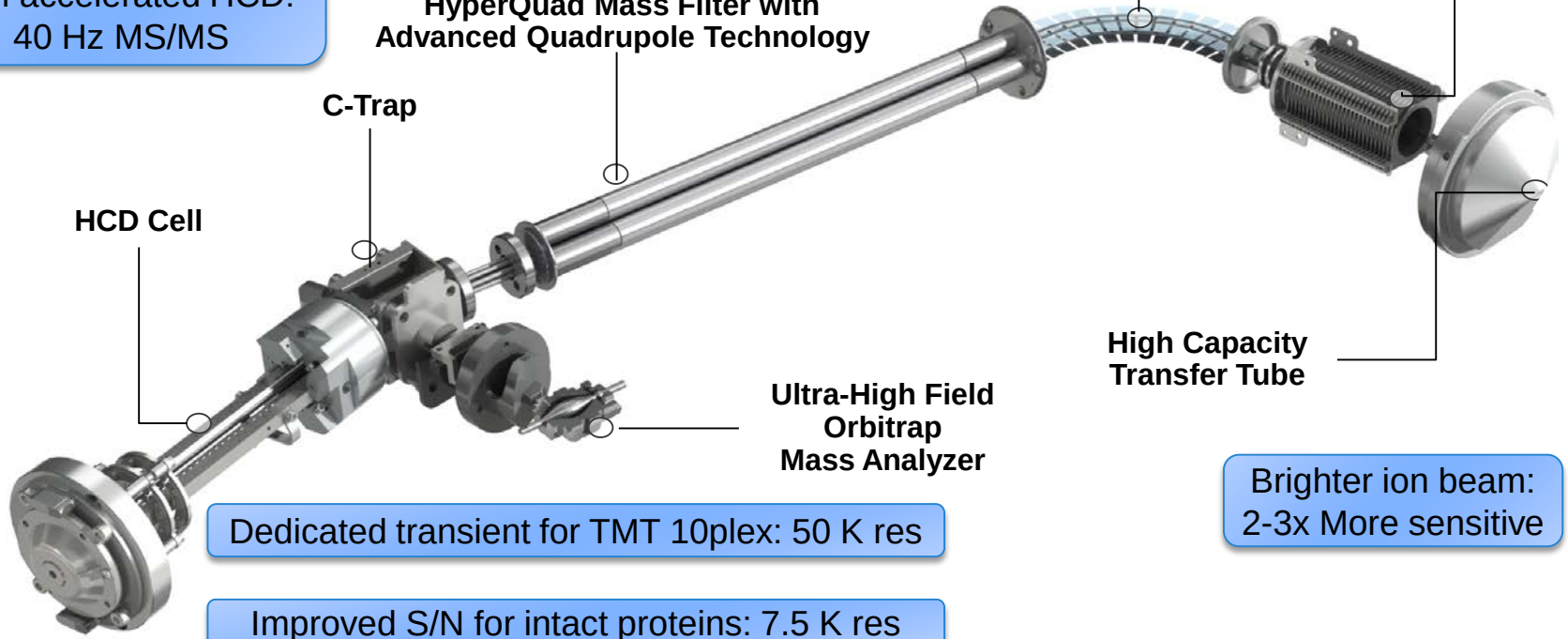
Q Exactive HF-X – New architecture

Advanced DDA for bottom-up and top-down: Advanced Peak Determination

Optimized Scan Matrix with accelerated HCD: 40 Hz MS/MS



Brighter ion beam: 2-3x More sensitive

Dedicated transient for TMT 10plex: 50 K res

Improved S/N for intact proteins: 7.5 K res

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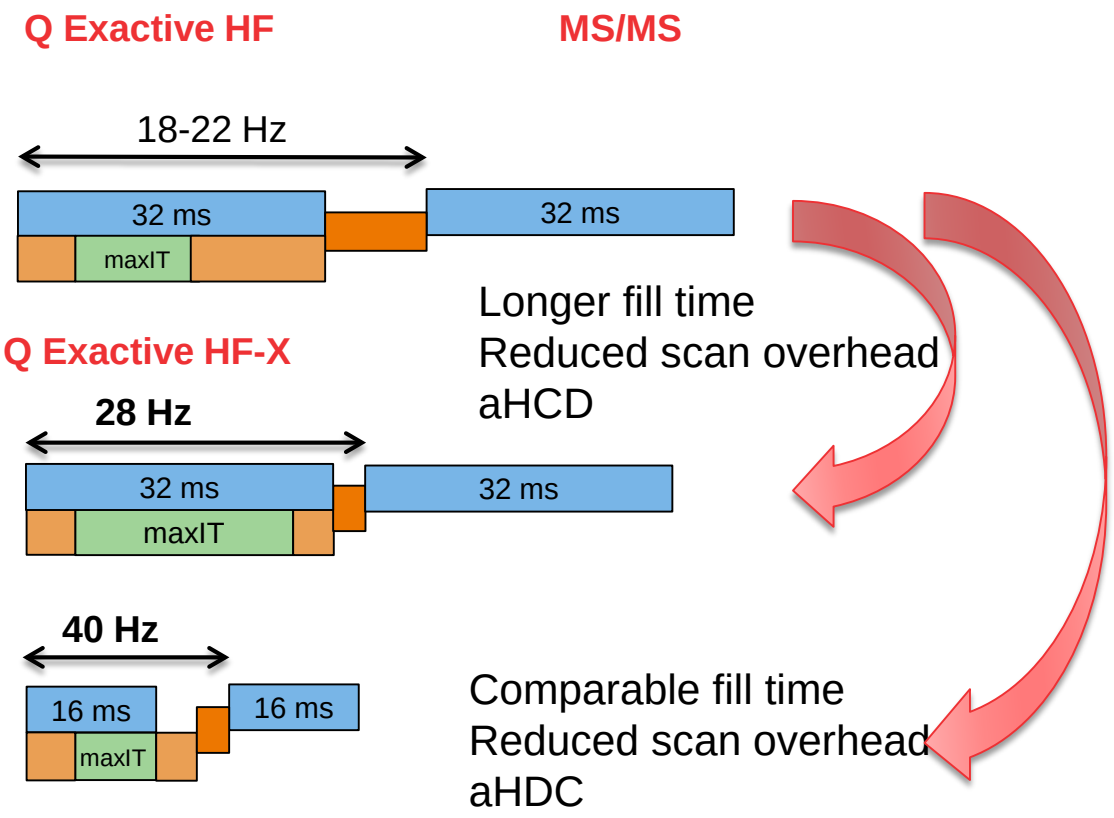
Q Exactive HF-X – Basis for increased speed and sensitivity



- Brighter ion beam (ion funnel), reduced scan overhead, and accelerated HCD (aHCD) is boosting acquisition speed
- Advantage for both MS and MS/MS mode
- Fast and high quality MS/MS acquisition up the 40 Hz with new 16 msec transient (7,500 resolution setting)

Orbitrap detection

Maximum fill time with precursor ions

Inter and intra scan overheads

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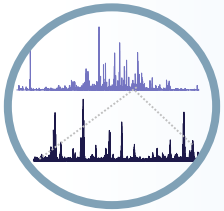
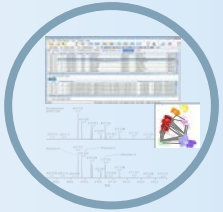
High-Resolution DIA Workflow

Sample Preparation



Separation

Data Acquisition



Data Analysis

- QUANTITATIVE
- PRECISE
- REPRODUCIBLE
- STANDARDIZED

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High-Resolution DIA: Unparalleled Proteome Coverage and Dynamic Range

new

Workflow

Thermo Scientific™ UHPLC Systems

- *Thermo Scientific™ UltiMate™ 3000 RSLCnano system*
- *Direct inject or pre-concentration mode*
- *Thermo Scientific™ Viper™ fittings*

Thermo Scientific™ EASY-Spray™ LC Column

- *150 µm ID x 150 mm,*
- *Sensitivity and robustness using Cap flow (≈ 1.2 µL/min)*
- *RT stability <1% observed for 350 injections*

Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap MS

- *Increased acquisition speed*
- *Advanced precursor determination*
- *Same # of protein IDs half the time*

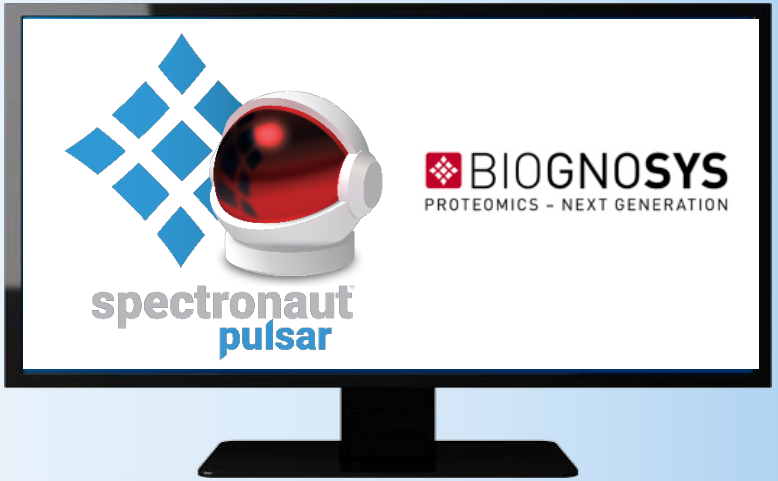
Spectronaut Pulsar™ software

Designed for Speed and Coverage

Spectronaut Pulsar Software

Key Benefits

- Spectronaut Pulsar™ is **specifically developed for the analysis of DIA & SWATH** data sets
- Data analysis with retention time correction based on spiked reference peptides using iRT Kit
- Spectral library generation from MaxQuant, Protein Pilot, and Thermo Scientific™ Proteome Discoverer™
- Direct visualization of qualitative and quantitative results on protein level including FDR and P-values
- Processing of very **large experiments** (1000s of runs)
- Fast data analysis speed in **less than 2 min per run**
- **DirectDIA™** library generation from DIA data (no need to create a DDA-based library)



Designed for high throughput DIA data analysis

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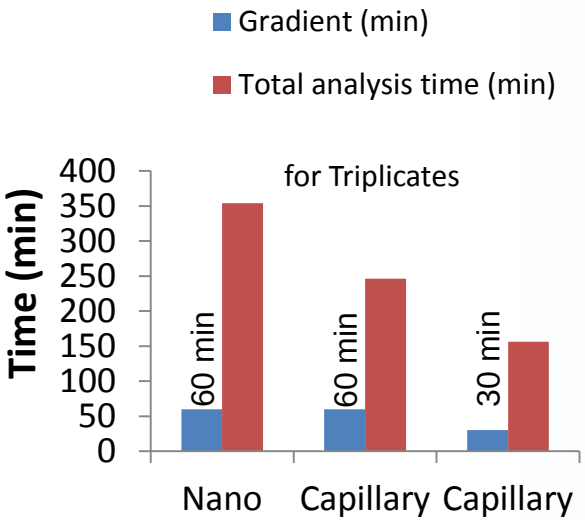
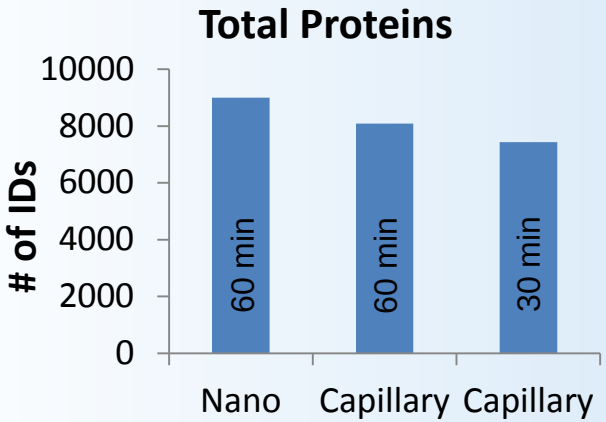
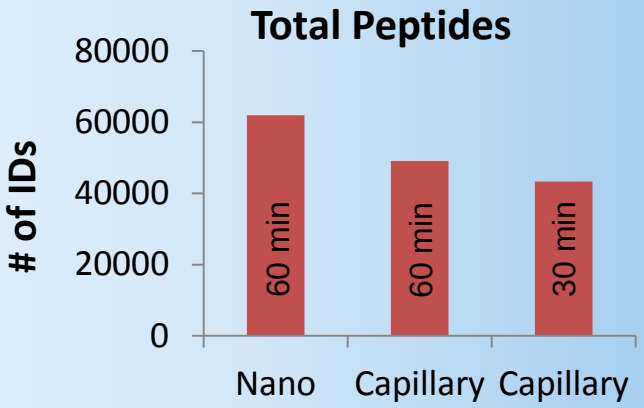
Balancing Efficiency Without Sacrificing Performance

Nanoflow
- Greater # of proteins
- Greater # of peptides
- Greater sensitivity
- Longer total run times

CapLC
- Greater Efficiency
- Shorter total run time (2X)
- Greater throughput
- More robust
- Less protein and peptide id's







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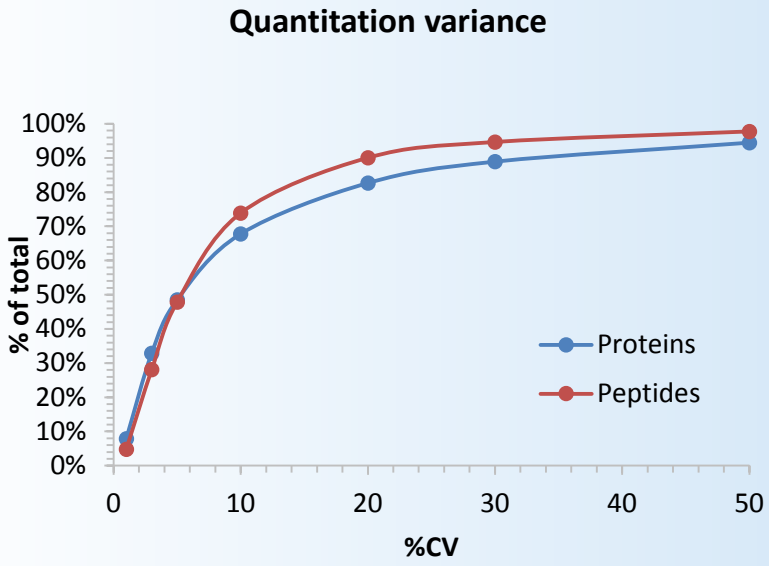
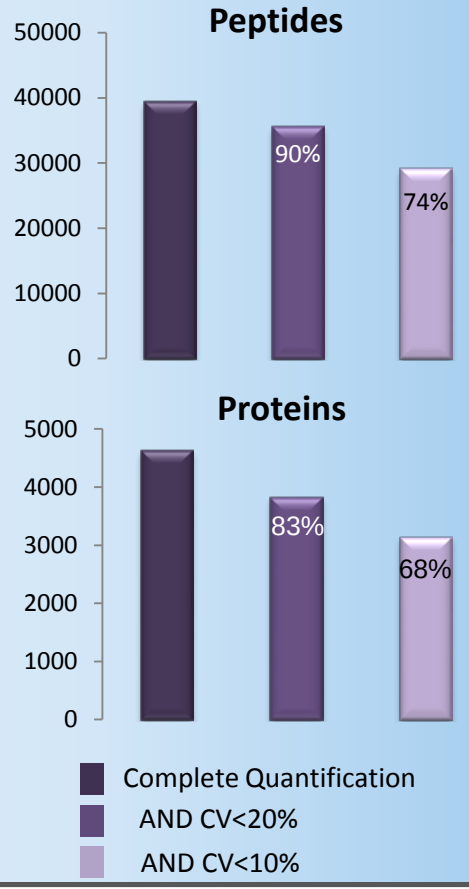
High-Resolution DIA Workflow: Highly Precise Proteome Quantitation

- Maximize depth of coverage
- Robust quantitative precision
- Confident in IDs
- Short analysis time



CapLC DIA, 4ug HeLa, 60min, 120K -> Spectronaut Analysis



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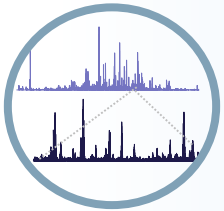
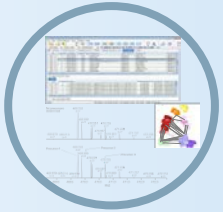
High-Resolution DDA+ Workflow

Sample Preparation



Separation

Data Acquisition



Data Analysis

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DDA+ Workflow: Quantitative Precision and Reproducibility

new

Workflow

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Q Exactive HF-X MS

- *Increased acquisition speed*
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Thermo Scientific™ Proteome Discoverer™ 2.2 software

Designed for Precision and Reproducibility

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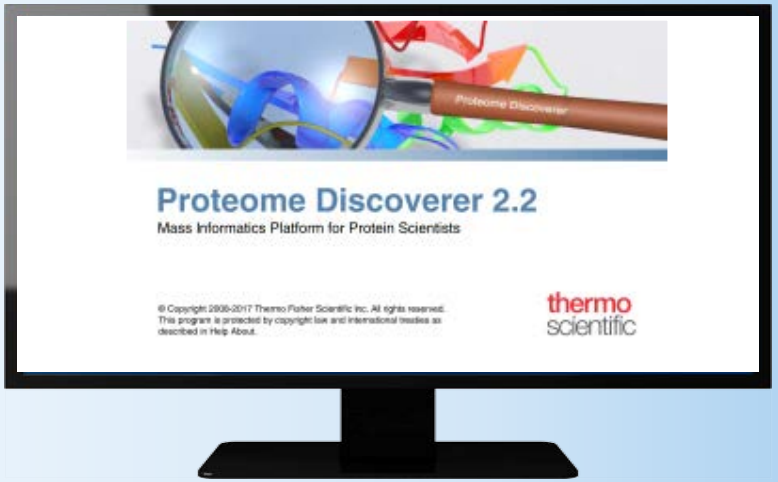
Proteome Discoverer 2.2 Software

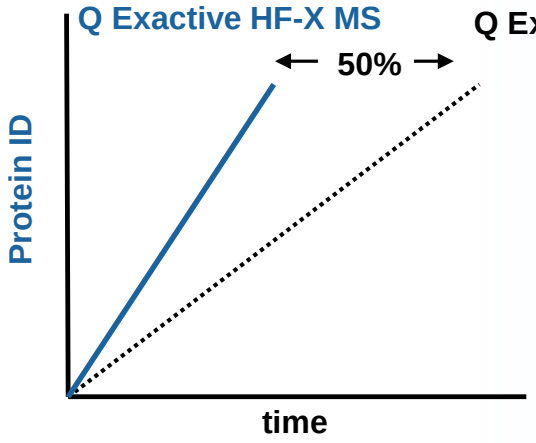
Key Benefits

- Enables large scale, multiplex proteomic studies (TMT 11-plex) and captures confident protein results which enables confident reproducibility
- Improved Label-free Quantitation
  - Feature mapping
    - Retention time alignment
    - Feature linking across files
- Minora Feature Detector node
  - Detects chromatographic peaks and features according to the specified quantification approach
- Minimizes ‘missing data points’ and maximizes quantitative insights



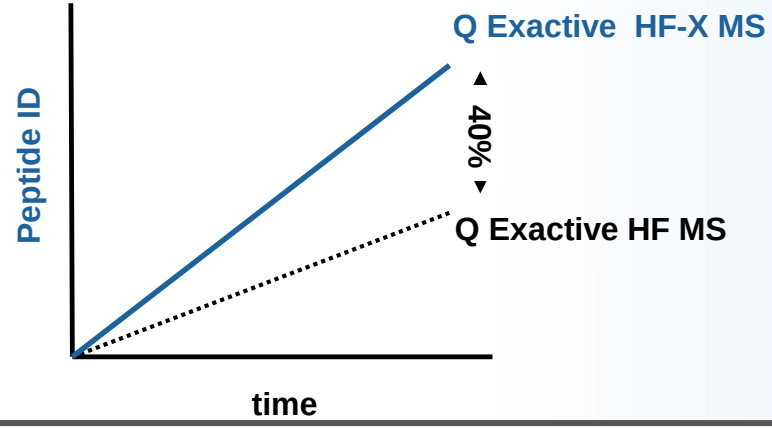
Most comprehensive data analysis platform for qualitative and quantitative proteomics research

Maximizing Efficiency for Large Scale Proteomics



Maximizing protein identifications

- Quick screening of complex samples
- Quality control of complex samples
- Assessment of sample concentration
- Same # of protein ID in half the time



Maximizing peptide identifications

- Highest peptide coverage
- Deep proteome analysis
- Spectral library building

Saves time and samples in large-scale proteomics efforts

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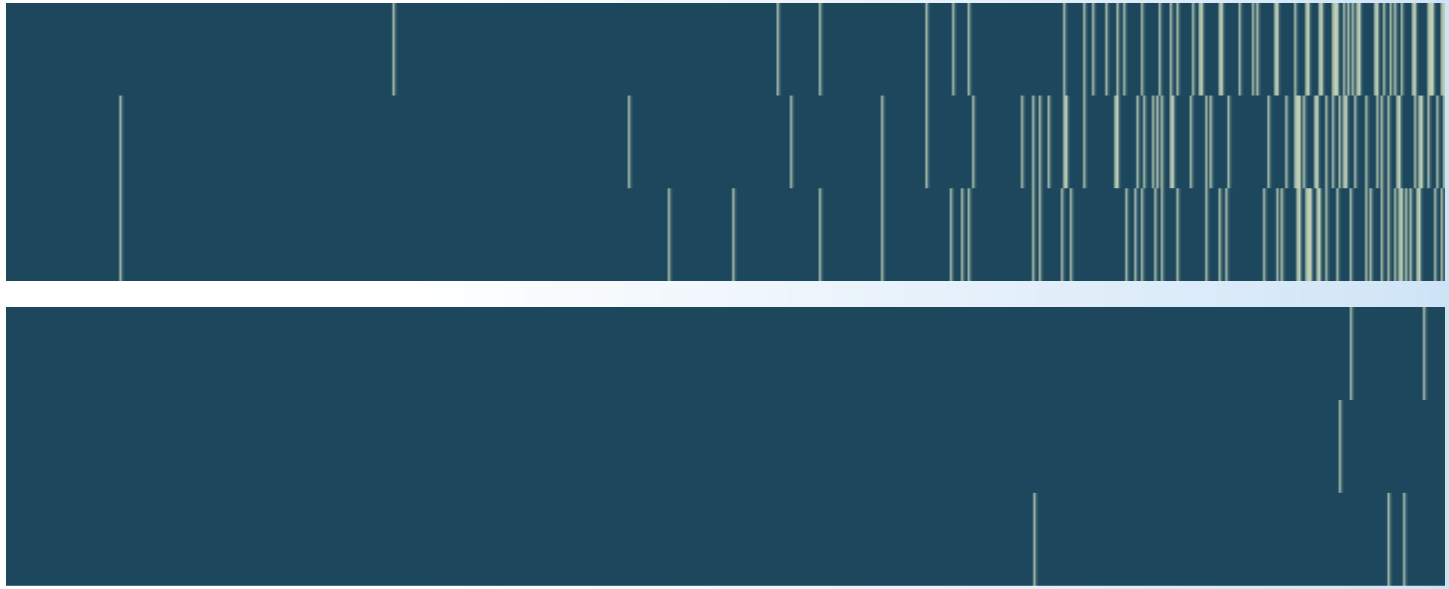
DDA+ Workflow: Protein Quantitation

new

Completely quantified proteins



Missing data = sparse quantitation

Complete quantitation

82% quantified during MS

97% quantified from MS and Protein Discoverer 2.2 software

capLC DDA+, 4ug HeLa, 60min, 120K/7.5K, 19ms, Top 40 -> PD 2.2 Label-free Quant

Quantitation → Precision → Reproducibility → Standardization

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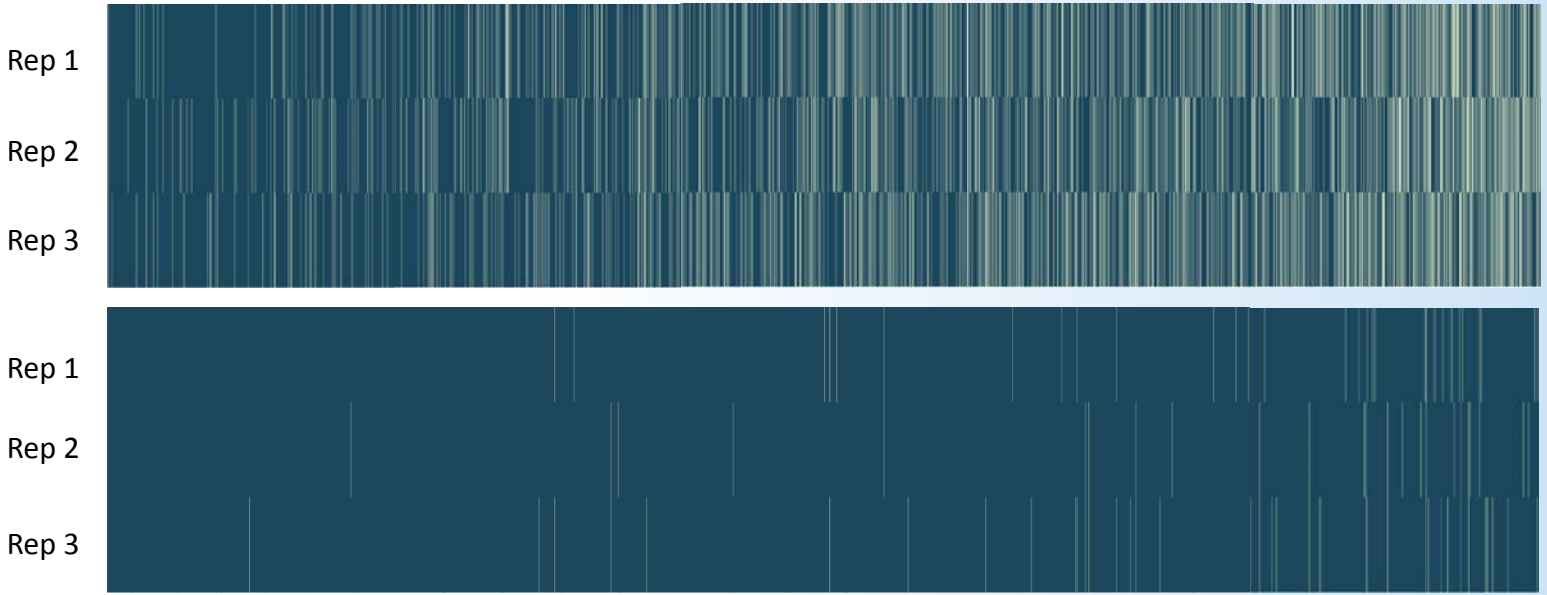
DDA+ Workflow: Near Complete Peptide Quantification

new

Completely quantified peptides



Missing data = sparse quantitation

Complete quantitation

50% quantified during MS

97% quantified from MS and PD 2.2

capLC DDA+, 4ug HeLa, 60min, 120K/7.5K, 19ms, Top 40 -> PD 2.2 Label-free Quant

Quantitation → Precision → Reproducibility → Standardization

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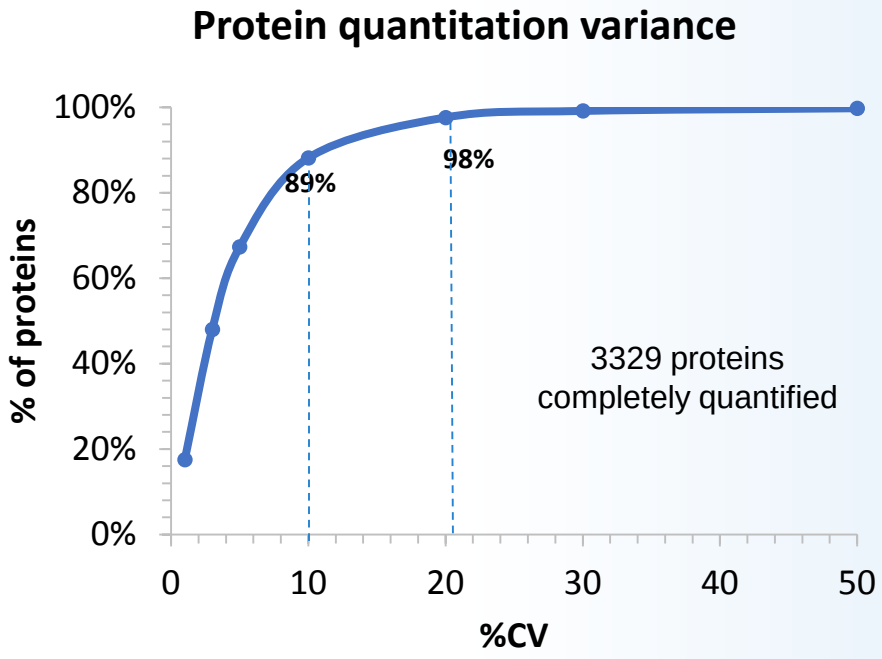
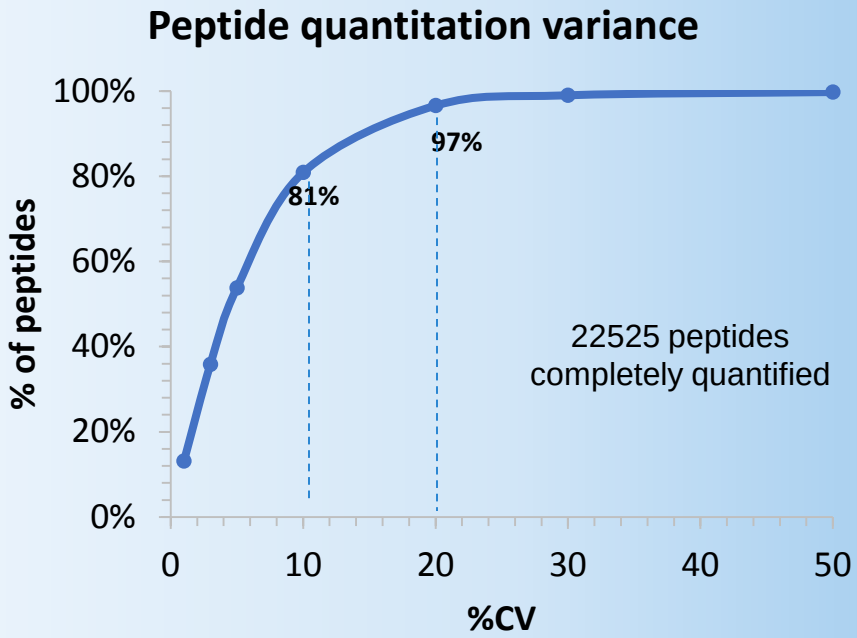
DDA+ Enable Unrivaled Quantitative Precision





Quantitation | **Precision** | Reproducibility | Standardization

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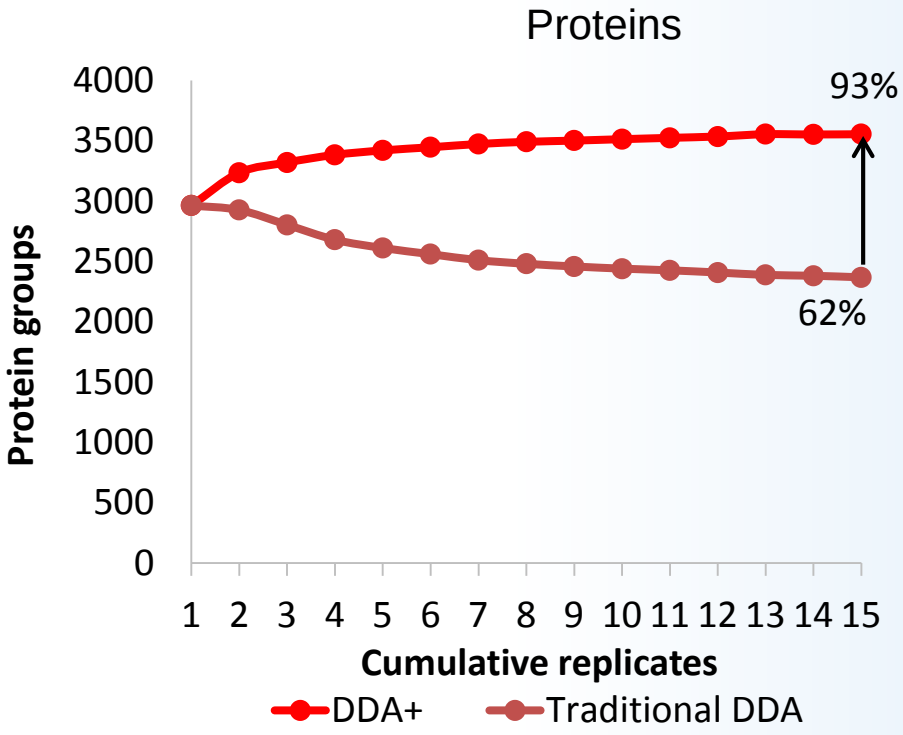
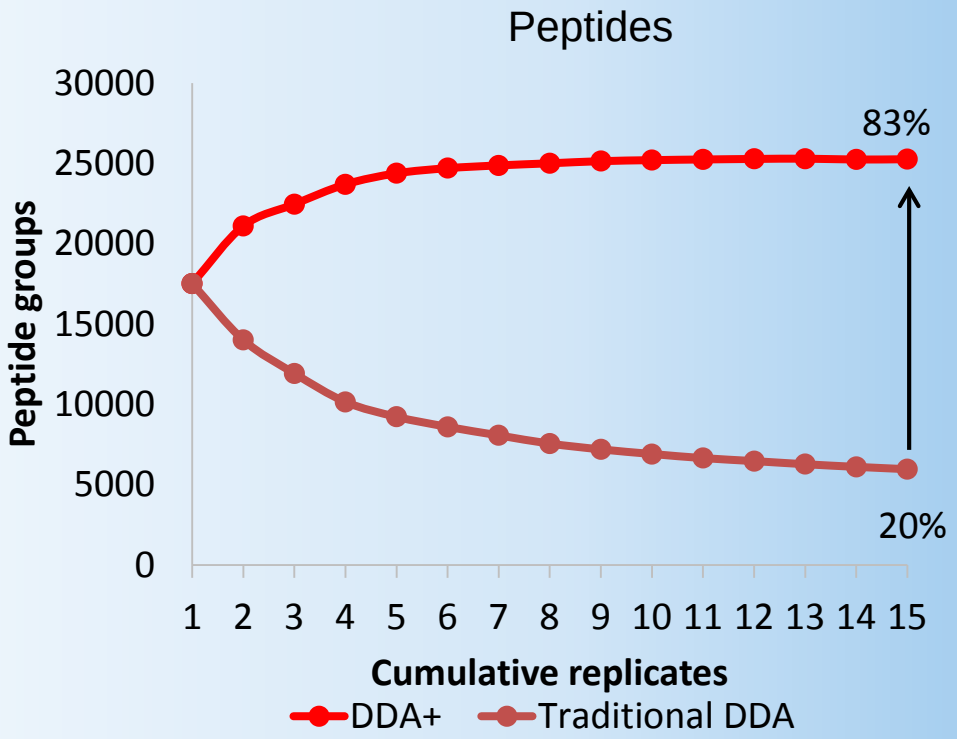
DDA+ Workflow: Greater Reproducibility Between Samples





Quantitation → Precision → **Reproducibility** → Standardization

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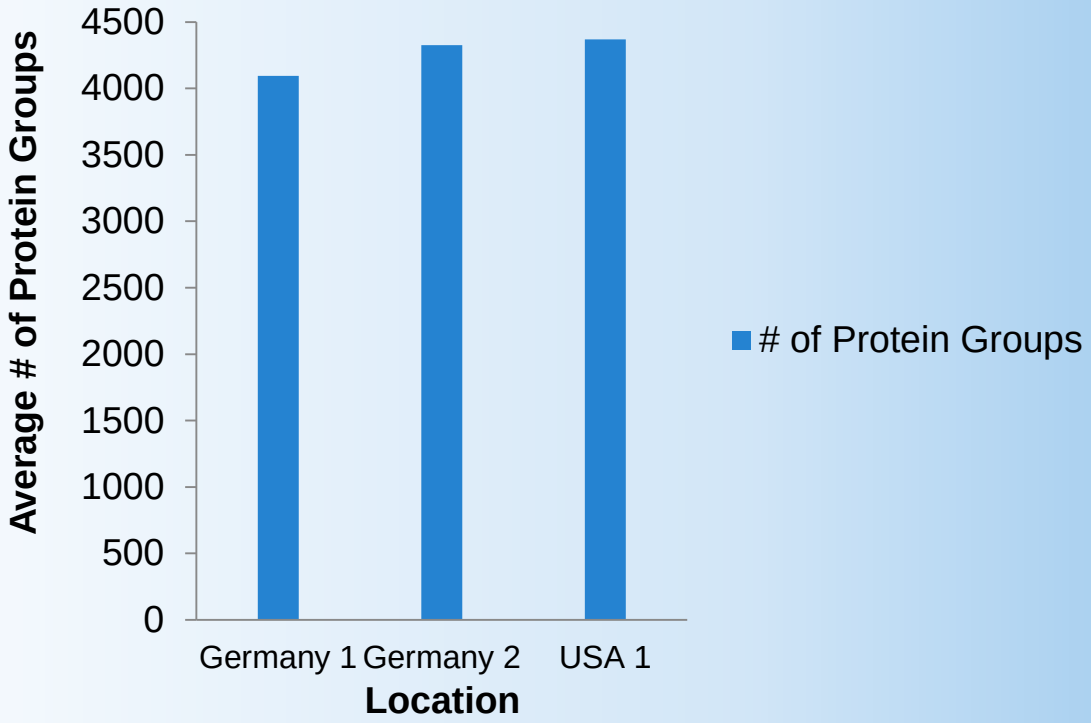
Inter-Site Consistency Across Different Instruments

Instrument Standardization Test

- Three Locations
- HeLa digest metrics
  - Protein
  - Peptide
  - PSMs
  - MS/MS
- 60 min gradient



Quantitation → Precision → Reproducibility → **Standardization**

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Questions?