



2017 Metabolomics Seminars

ThermoFisher
SCIENTIFIC

Pushing the Leading Edge in Protein Quantitation:

Integrated, Precise, and Reproducible Protein Quantitation Workflow Solutions

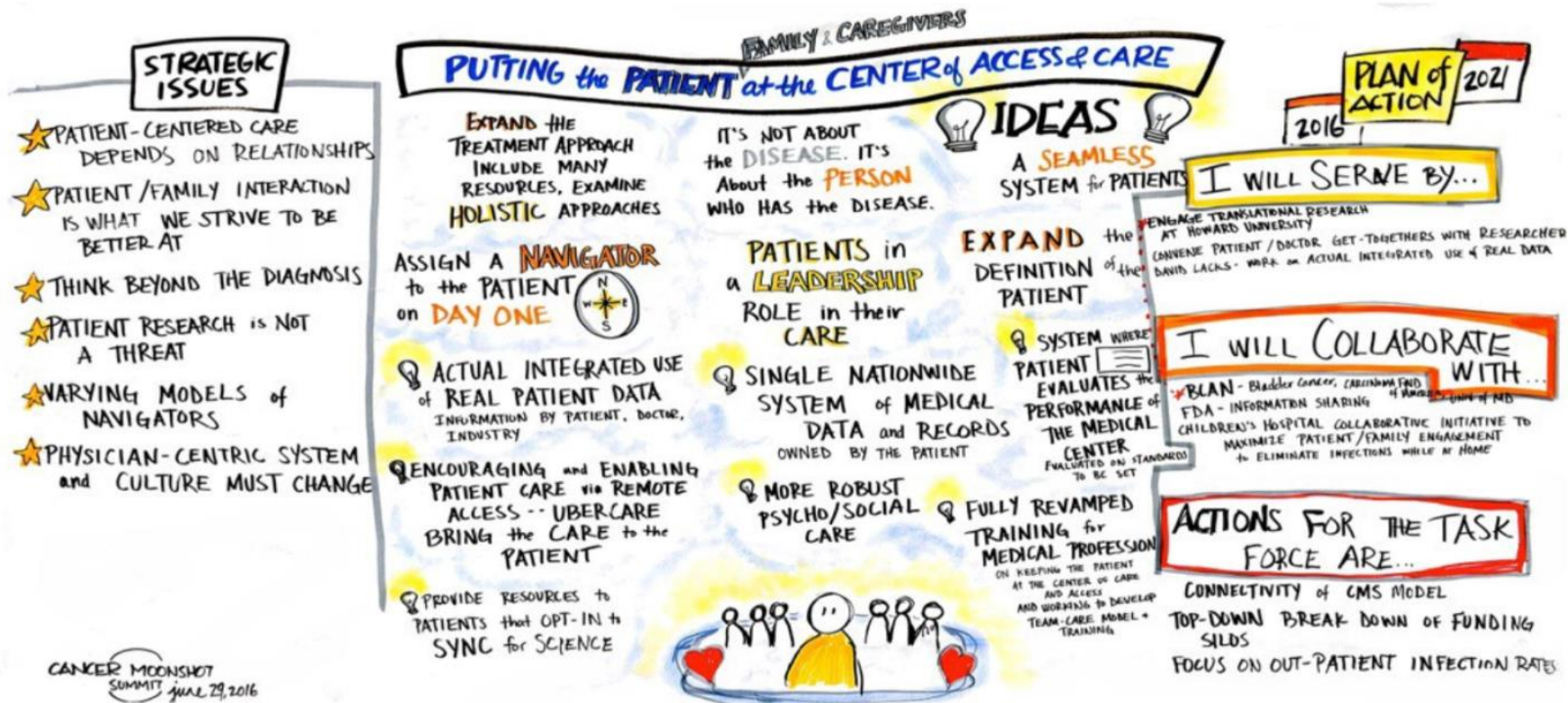
The world leader in serving science





Cancer Moonshot

Goal: To detect cancer at an early stage while providing additional therapies to more patients.



“...It is the proteins that comprise most of the biomarkers that are measured to detect cancers, constitute the antigens that drive immune response and inter and intracellular communications, and it is the proteins that are the drug targets for nearly every targeted therapy that is being evaluated in cancer trials today.”

Conrads et al. 2016 Clinical Cancer Research

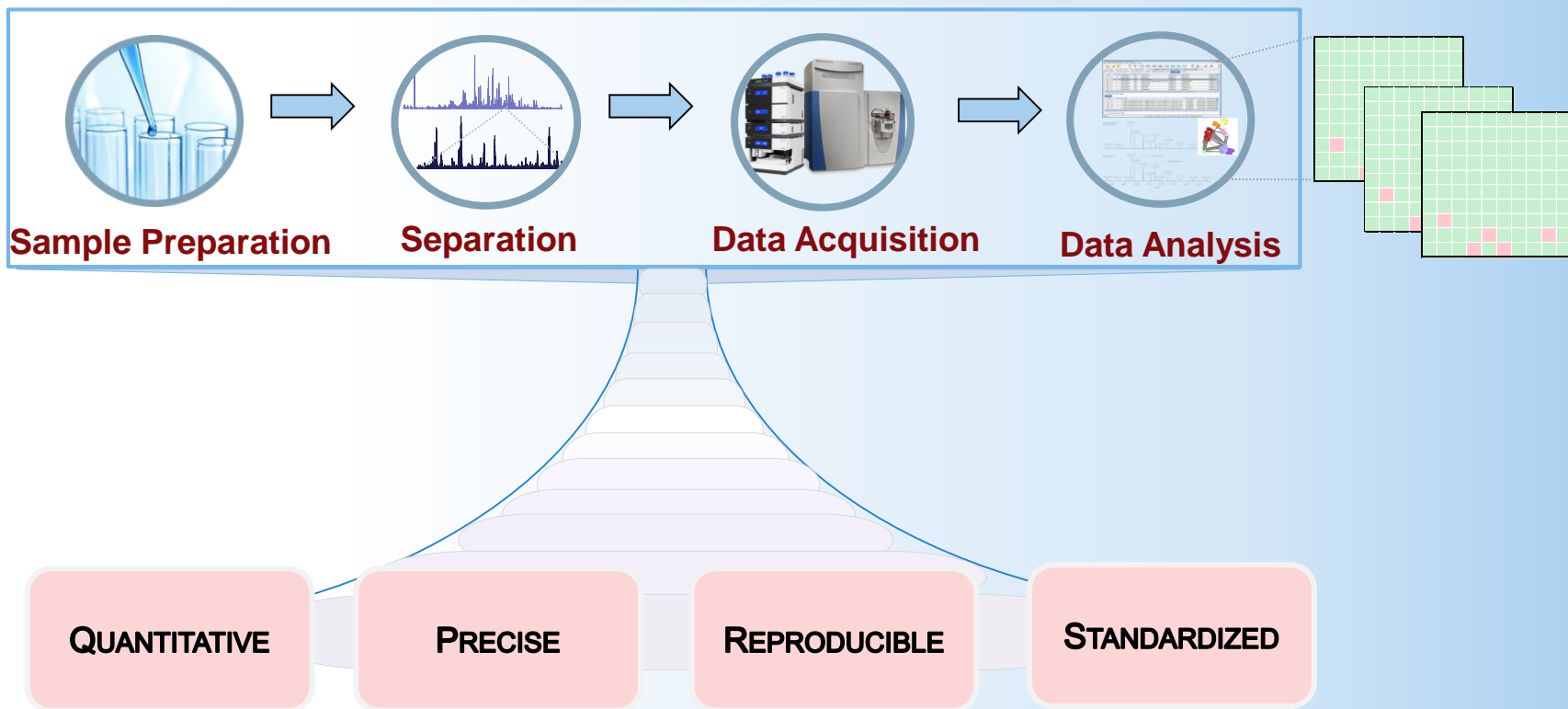


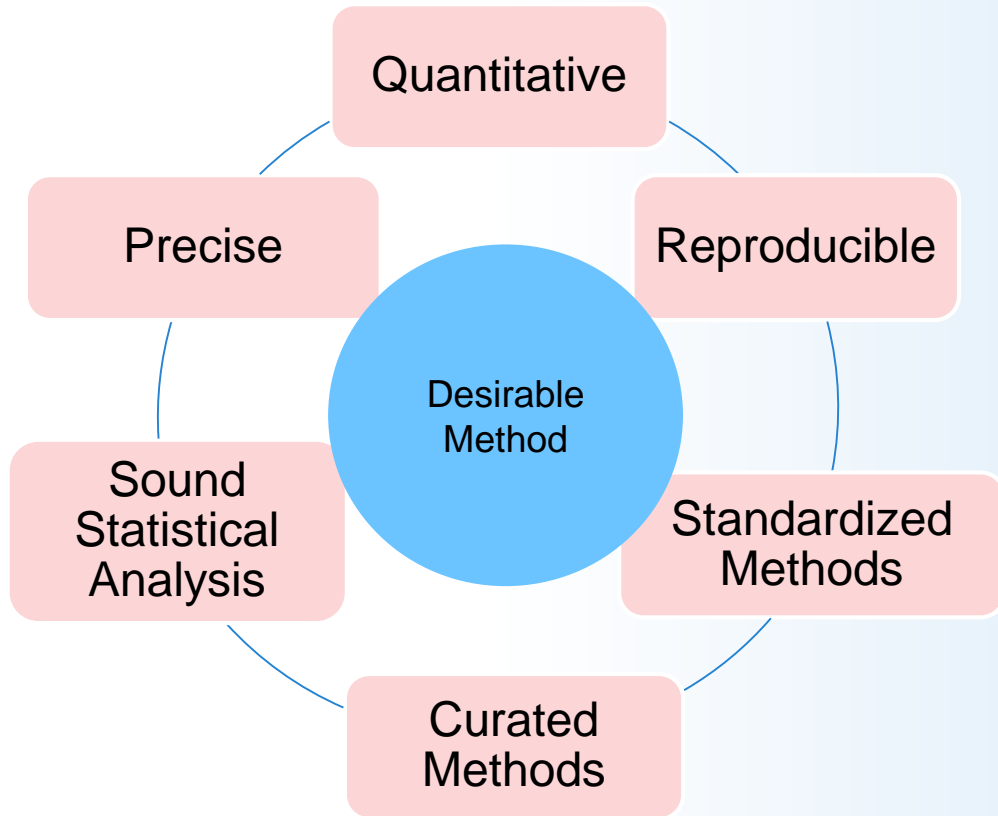
ThermoFisher
S C I E N T I F I C

 **INOVA**[®]
Schar Cancer Institute

The Goal: Standardized, High Throughput Proteomics

Large Scale Proteomics





Our Solution

A new standard in quantitative, sensitivity, accuracy and precision

- HR DIA
- DDA+

Workflows

High-Resolution DIA Workflow

Unparalleled proteome coverage and dynamic range



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

- *Biospecimen profiling*
- *Digital archiving*

DDA+ Workflow

Unsurpassed quantitative precision and reproducibility



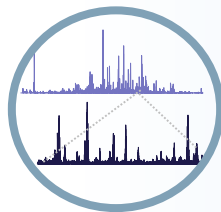
- Unrivaled precision in precursor quantitation
- Maximize complete, reproducible quantitation across samples
- Minimize 'missing values' among samples

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

High-Resolution DIA Workflow



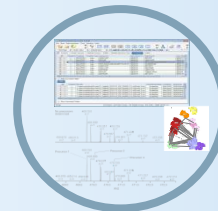
Sample Preparation



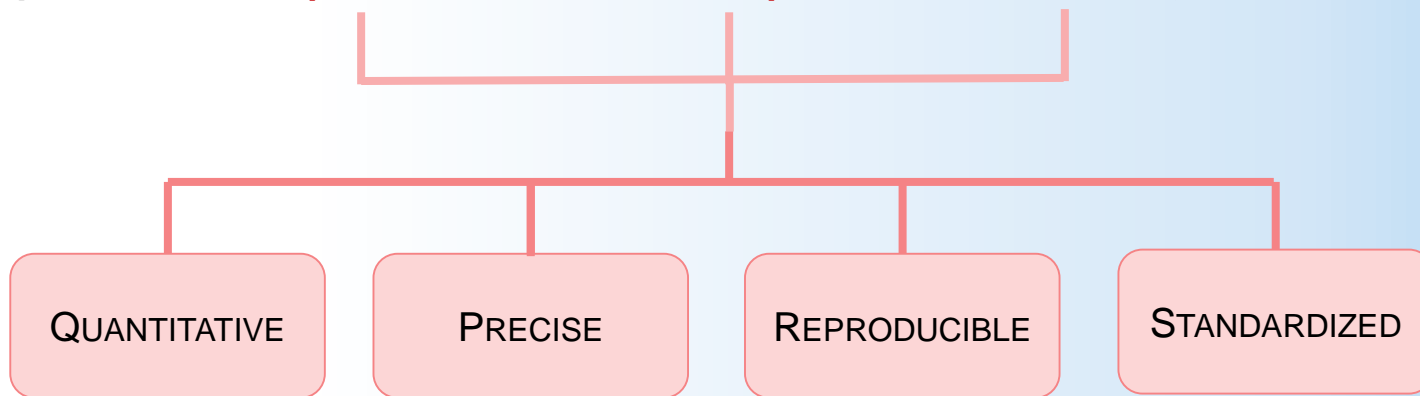
Separation



Data Acquisition



Data Analysis



QUANTITATIVE

PRECISE

REPRODUCIBLE

STANDARDIZED

Workflow



**Thermo Scientific™
UHPLC Systems**



**Thermo Scientific™
EASY-Spray™ LC Column**



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



**Spectronaut™
software**

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

- *150 μm ID x 150 mm,*
- *Sensitivity and robustness*
- *RT stability <1% observed for 350 injections*

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

Designed for Speed and Coverage

Key Benefits

- Spectronaut™ software is **specifically developed for the analysis of DIA & SWATH** data sets
- Data analysis with retention time correction based on spiked reference peptides-HRM calibration kit or iRT Kit
- Spectral library generation from MaxQuant and Thermo Scientific™ Proteome Discoverer™ software search results
- Direct visualization of qualitative and quantitative results on protein level
- Fast data analysis speed in **less than 2 min per run**



Designed for high throughput DIA data analysis

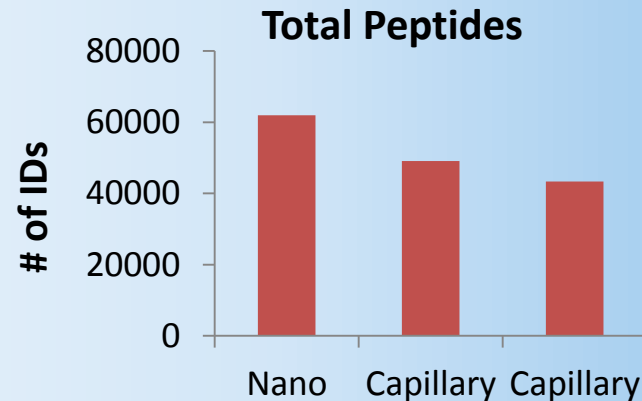
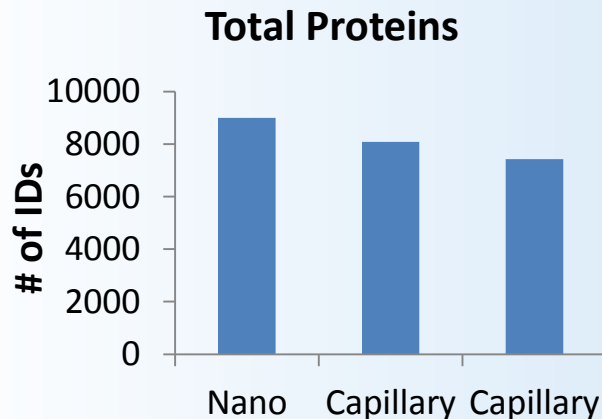
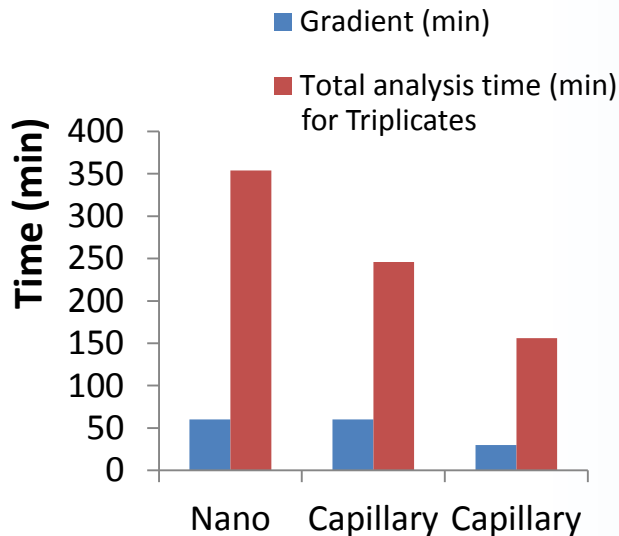
Balancing Efficiency Without Sacrificing Performance

Nanoflow

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

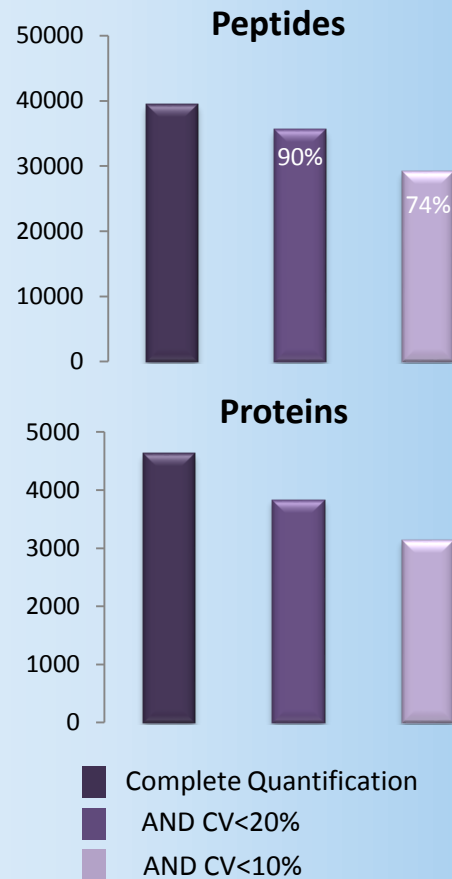
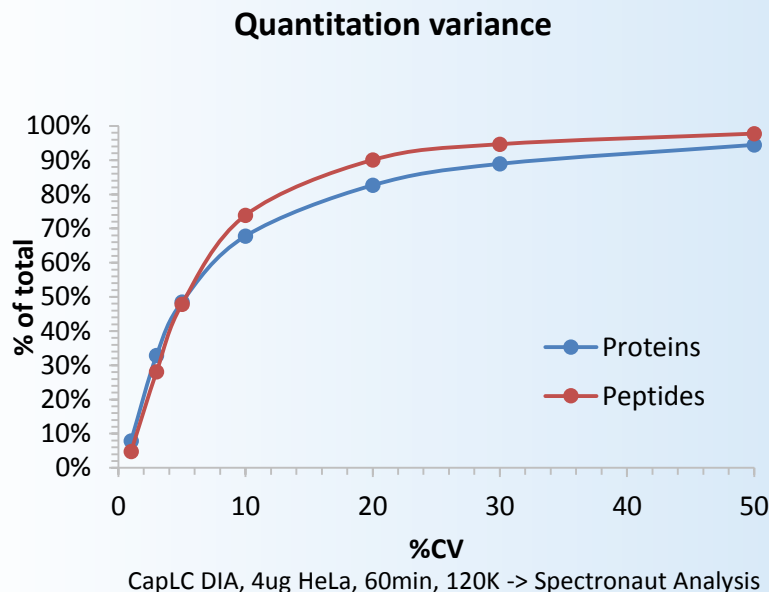
CapLC

- Greater Efficiency
- Shorter total run time (2X)
- Greater throughput
- *Comparable protein and peptide id's*

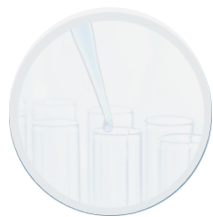


High-Resolution DIA Workflow: Highly Precise Proteome Quantitation

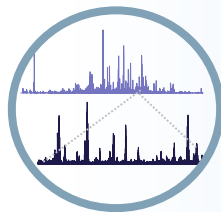
- Maximize depth of coverage
- Robust quantitative precision
- Confident in IDs
- Short analysis time



High-Resolution DDA+ Workflow



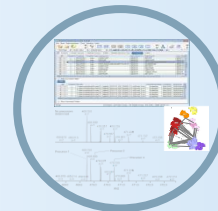
Sample Preparation



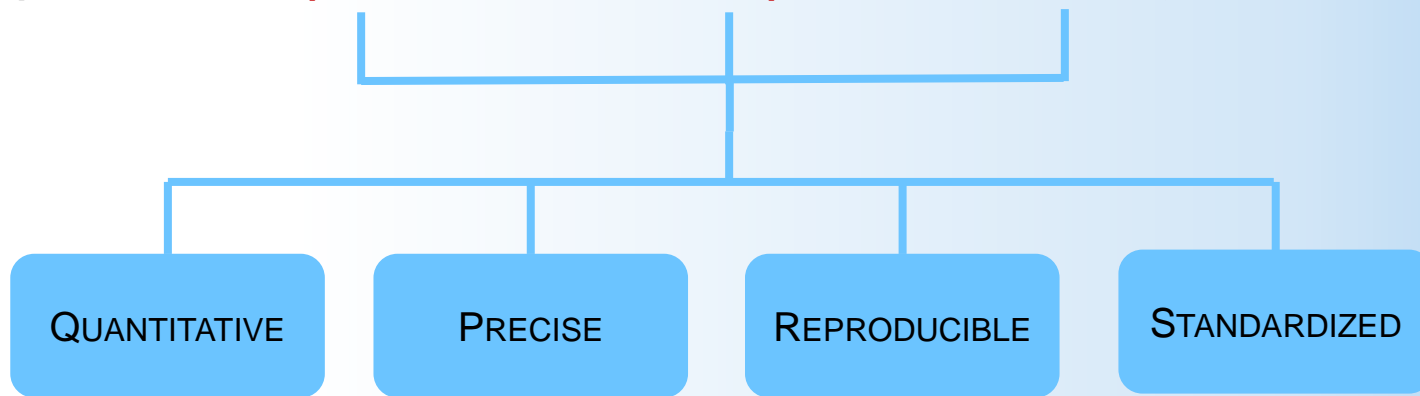
Separation



Data Acquisition



Data Analysis



Workflow



UHPLC Systems

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



EASY-Spray LC Column

- *150 μ m ID x 150 mm,*
- *Sensitivity and robustness*
- *RT stability <1% observed for 350 injections*



Q Exactive HF-X MS

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

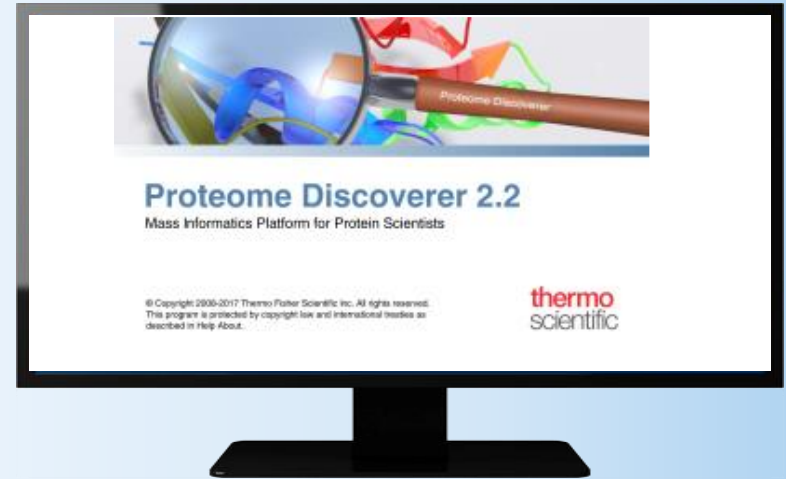


Thermo Scientific™ Proteome Discoverer™ 2.2 software

Designed for Precision and Reproducibility

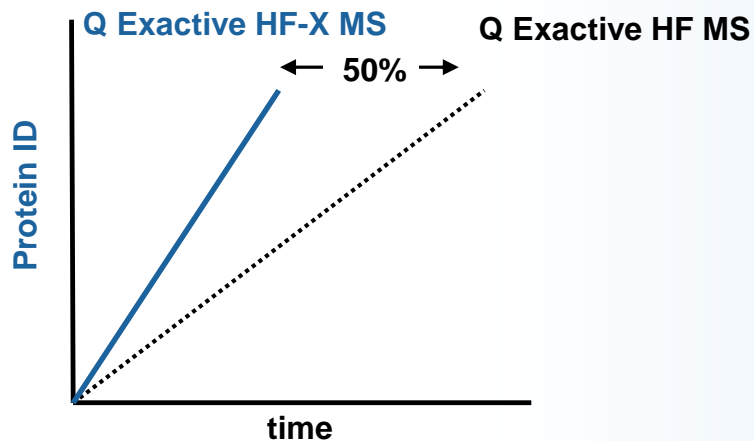
Key Benefits

- Enables large scale, multiplex proteomic studies 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



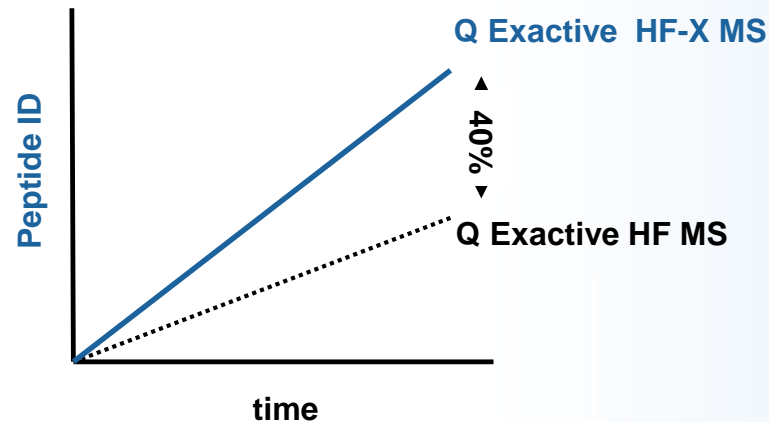
**Most comprehensive data analysis
platform for qualitative and
quantitative proteomics research**

Maximizing Efficiency for Large Scale Proteomics



Maximizing protein identifications

- Quick screening of complex samples
- Quality control of complex samples
- Assessment of sample concentration



Maximizing peptide identifications

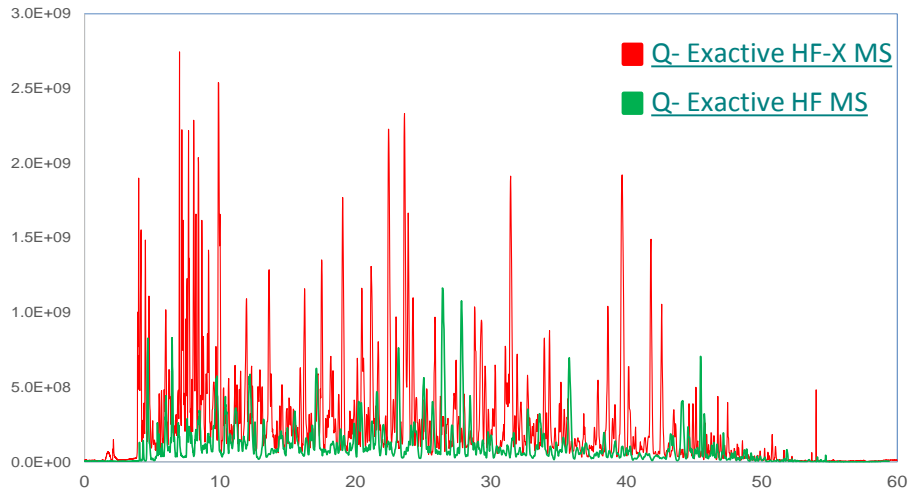
- Highest peptide coverage
- Deep proteome analysis
- Spectral library building

Saves time and samples in large-scale proteomics efforts

Maintaining Sensitivity at Increased Robustness – capLC with Q Exactive HF-X MS

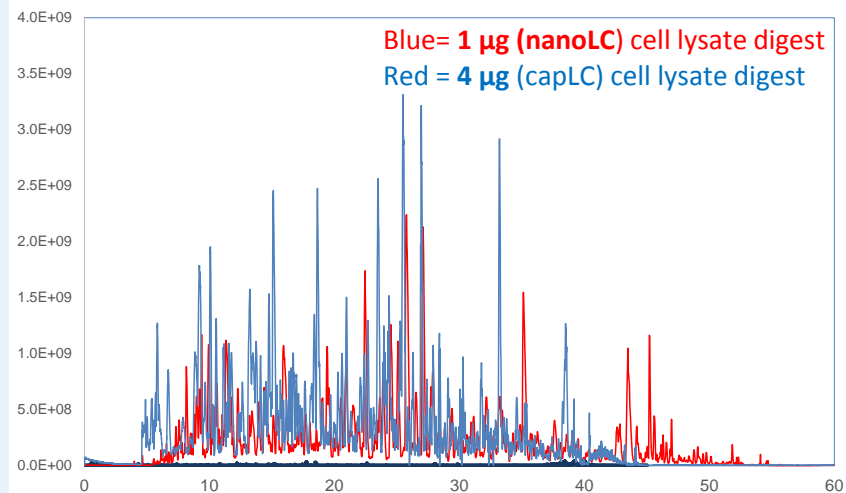
- Increased sensitivity on QE HF-X
- Increased peptide identifications at higher robustness
- Higher reproducible protein identifications with reduced total run times

Sensitivity



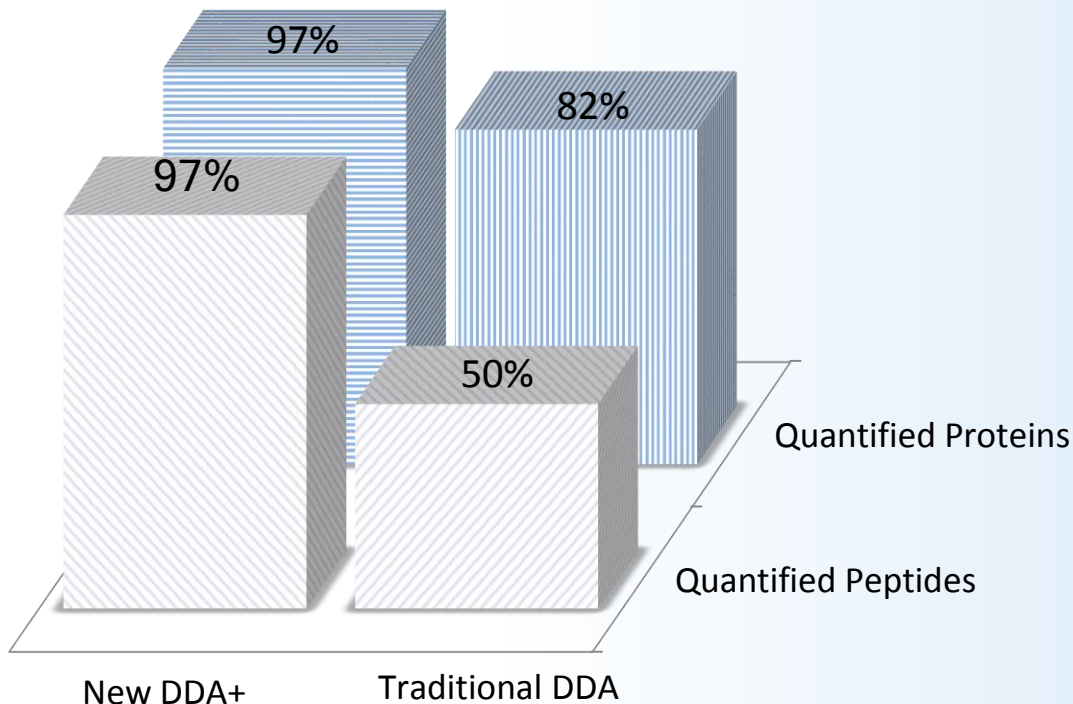
1 ug HeLa + 150 uM x 15 cm Easy Spray

Robustness



75 µm ID x 15 cm vs 150 µm ID x 15 cm

Real Benefit of Using DDA+ Workflow

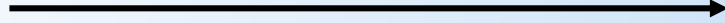


DDA+ workflow compared to DDA

- 15% gain in completely quantified proteins
- 47% gain in completely quantified peptides
- Maximizes quantitation

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

Completely quantified proteins



Missing data = sparse
— quantitation
— Complete quantitation



82%
quantified
during MS

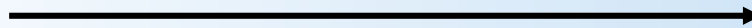


97%
quantified
from MS and
Protein
Discoverer
2.2 software

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



Completely quantified peptides

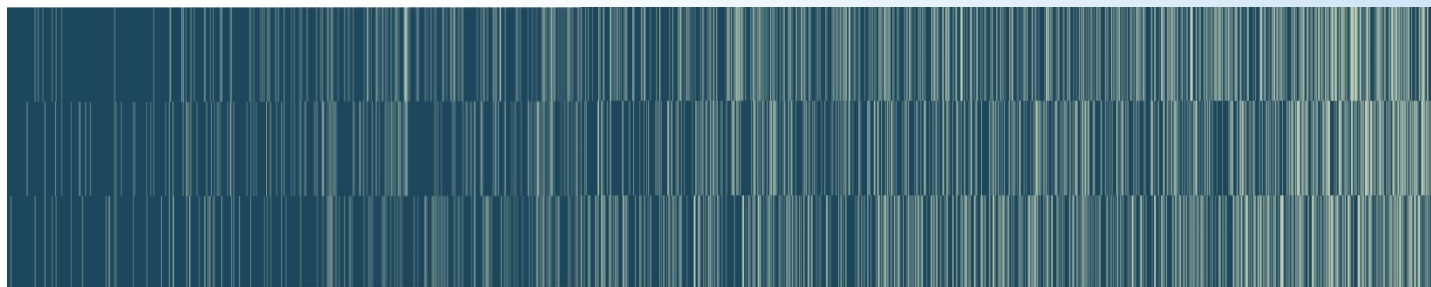


Missing data = sparse
— quantitation
— Complete quantitation

Rep 1

Rep 2

Rep 3



50%
quantified
during MS



97%
quantified
from MS and
PD 2.2

Rep 1

Rep 2

Rep 3



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

Quantitation

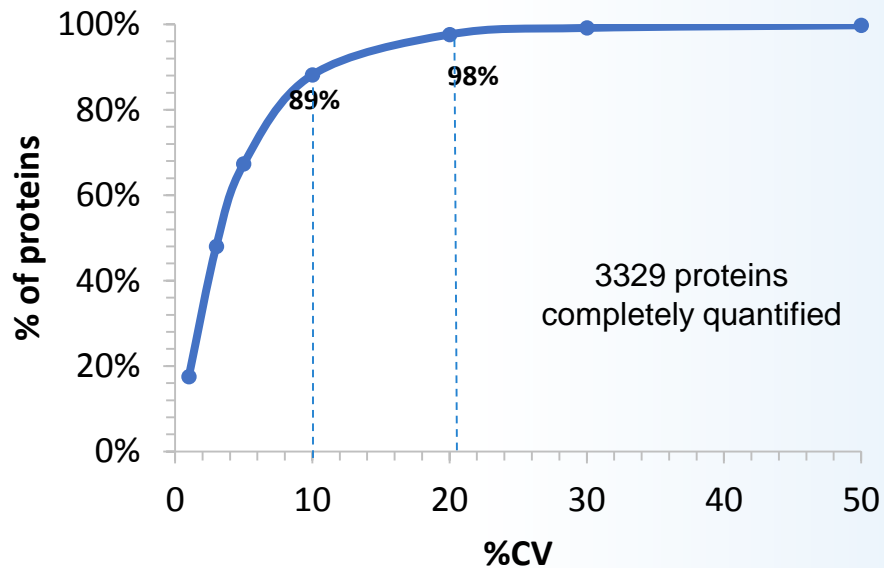
Precision

Reproducibility

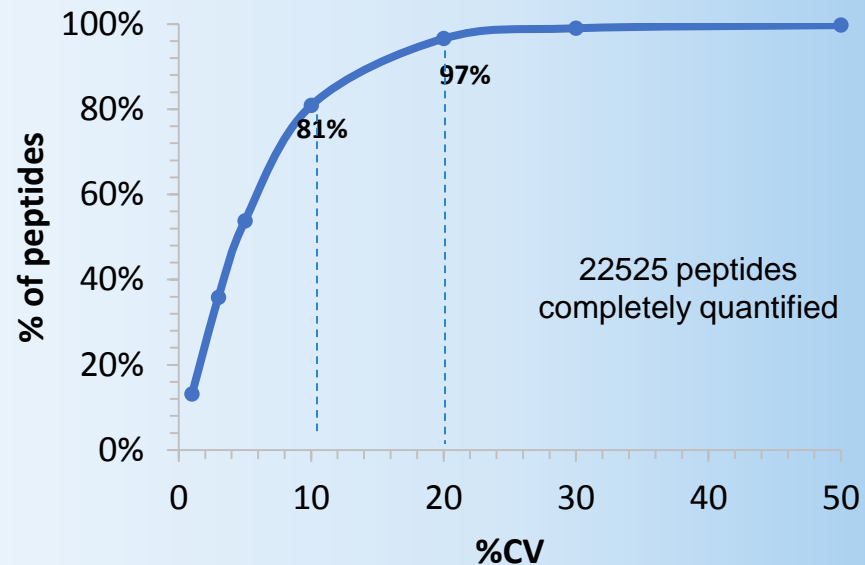
Standardization

DDA+ Enable Unrivalled Quantitative Precision

Protein quantitation variance



Peptide quantitation variance



Quantitation

Precision

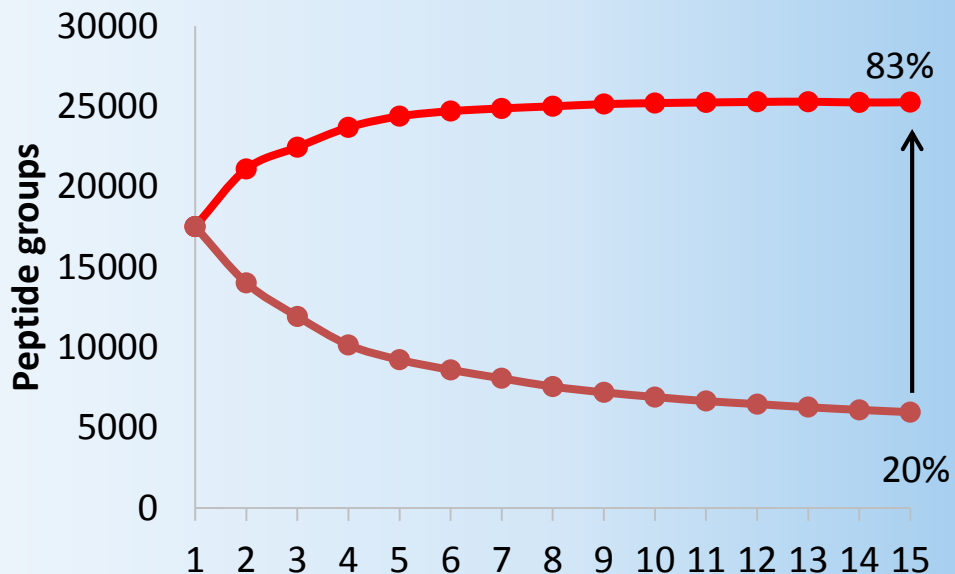
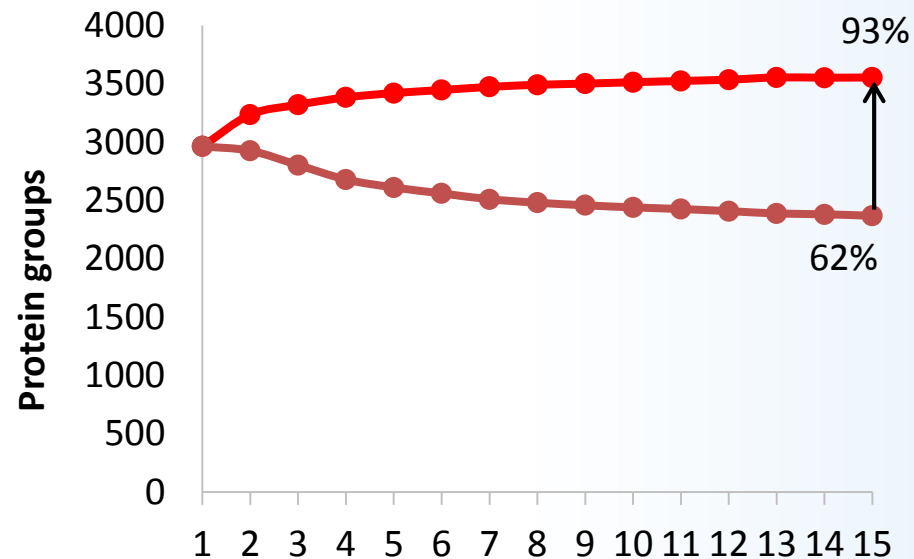
Reproducibility

Standardization

DDA+ Workflow: Greater Reproducibility Between Samples

Proteins

Peptides



Cumulative replicates

● DDA+ ● Traditional DDA

Cumulative replicates

● DDA+ ● Traditional DDA

Quantitation

Precision

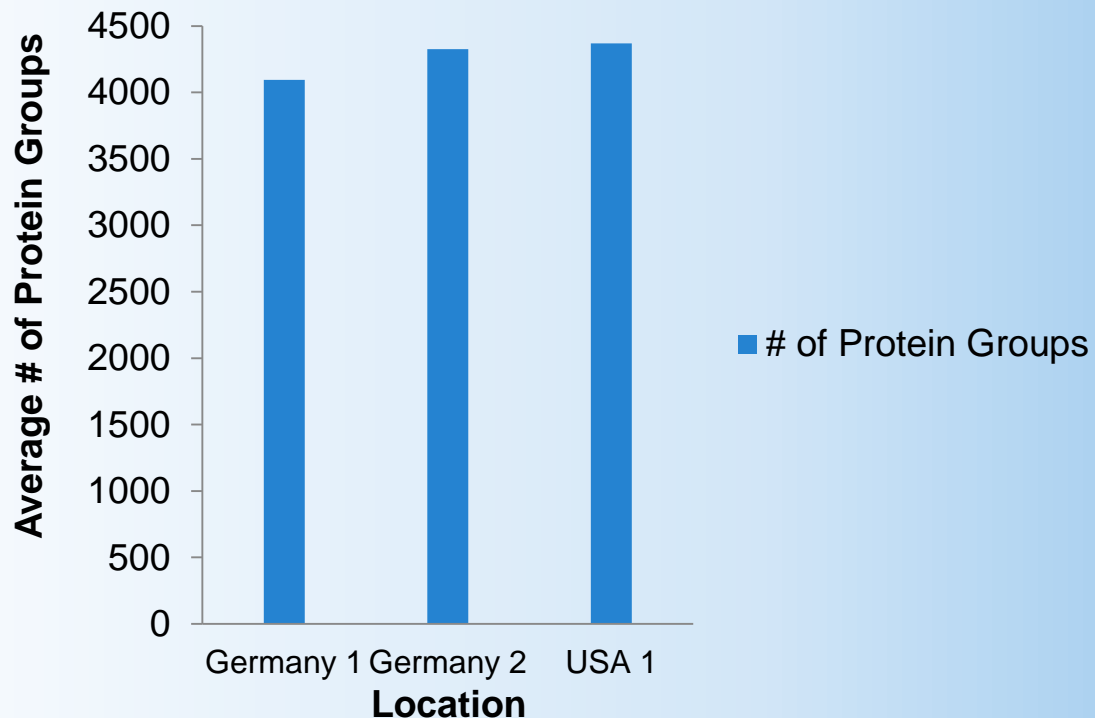
Reproducibility

Standardization

Inter-Site Consistency Across Different Instruments

Instrument Standardization Test

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



Multi-Center Study to Demonstrate Large-Scale Capabilities

_computational
BIOLOGY

ANALYSIS

A multicenter study benchmarks software tools for label-free proteome quantification

Pedro Navarro^{1,11}, Jörg Kuharev^{1,11}, Ludovic C Gillet², Oliver M Bernhardt³, Brendan MacLean⁴, Hannes L Röst⁵, Stephen A Tate⁵, Chih-Chiang Tsou⁶, Lukas Reiter⁷, Ute Distler⁸, George Rosenberger^{2,7}, Yasset Perez-Riverol⁸, Alexey I Nesvizhskii^{6,9}, Ruedi Aebersold^{2,10} & Stefan Tenzer¹

Consistent and accurate quantification of proteins by mass spectrometry (MS)-based proteomics depends on the performance of instruments, acquisition methods and data analysis software. In collaboration with the software developers, we evaluated OpenSWATH, SWATH 2.0, Skyline, Spectronaut and DIA-Umpire, five of the most widely used software methods for processing data from sequential window acquisition of all theoretical fragment-ion spectra (SWATH-MS, which uses data-independent acquisition (DIA) for label-free protein quantification. We analyzed high-complexity test data sets from hybrid proteome samples of defined quantitative composition acquired on two different MS instruments using different SWATH isolation-window setups. For consistent evaluation, we developed LFQbench, an R package, to calculate metrics of precision and accuracy in label-free quantitative MS and report the identification performance, robustness and specificity of each software tool. Our reference data sets enabled developers to improve their software tools. After optimization, all tools provided highly convergent identification and reliable quantification performance, underscoring their robustness for label-free quantitative proteomics.

MS-based quantitative proteomics is an essential tool to elucidate the

fragmentation of all precursor ions, regardless of their intensity or other characteristics, enabling establishment of a complete record of the sample². In recent years, several DIA mass spectrometric strategies, including SWATH-MS³, high-definition MS using alternating low and elevated energy acquisition in combination with ion-mobility separation (HDMS²)³, and all-ion fragmentation (AIF)⁴, have circumvented some of the problems arising from DDA, such as stochastic and irreproducible precursor ion selection^{5,6}, undersampling⁶ and long instrument cycle times⁶.

In addition to the MS method applied, computational methods—such as those for raw data processing, protein database searching and statistical analysis of the quantitative data—critically affect the results of quantitative proteomics analyses. As such, evaluating the correctness and relative performance of these methods is essential¹⁰. Quantitative proteomics would greatly benefit from an objective comparative benchmarking of the performance and robustness of the various computational approaches and software solutions available or currently in development. Meaningful and unbiased comparisons of software tools and their appropriate uses are challenging for a number of reasons¹¹: methods and algorithms may be assessed by scientists lacking relevant expertise, the tested method may suffer from insufficient documentation or the interpretation of the test results may be subjective^{12–15}. In addition, benchmarking requires high-quality

Objective: Determine analytical robustness and reliability between laboratories

- Comparability of measurements between laboratories and define critical parameters
- Ring trial participants adapt a system suitability test protocol to maintain analytical performance
- Determine the range of accuracy and precision that users can expect to achieve following the standardized product/assay
- The standardization enables the transfer of measurements between laboratories

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Thermo
Fisher
BRIMS
Center



Phase 1

Labs identified, resources secured,
SOP established



Phase 2

Study design, study setup, data
collection

Phase 3

Data review, report-out



Cancer Moonshot



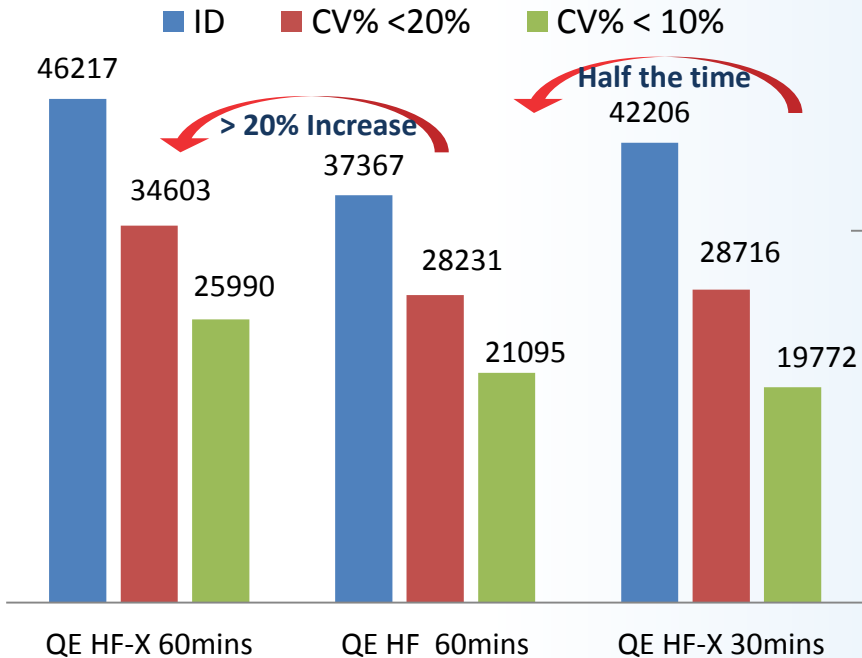


Questions?

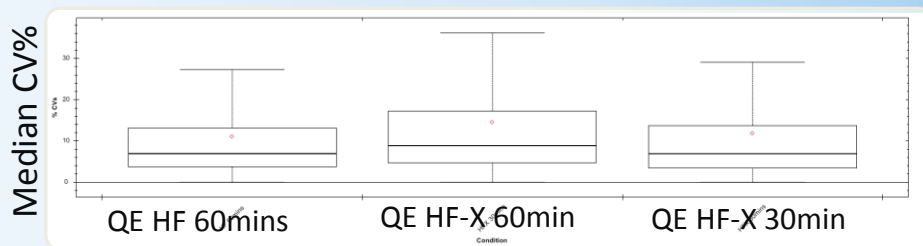
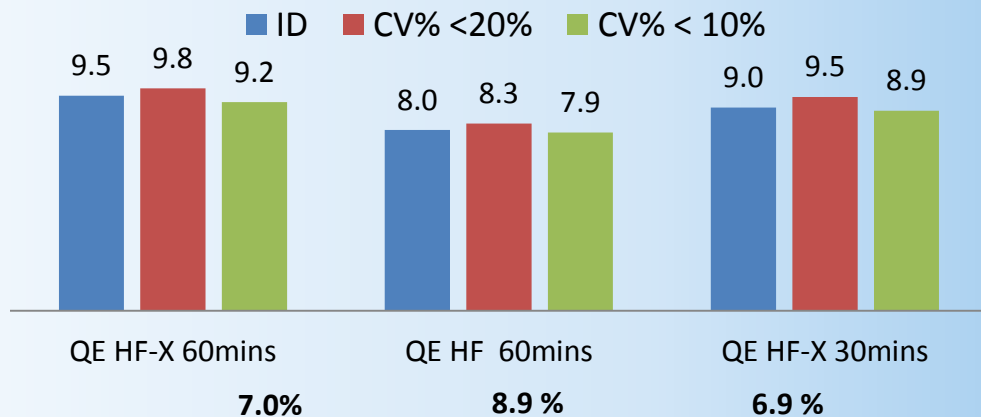
optional slides follow

Increased Throughput: Cap LC DIA – Q Exactive HF-X MS vs. Q Exactive HX MS

Peptide Precursors (1% FDR)



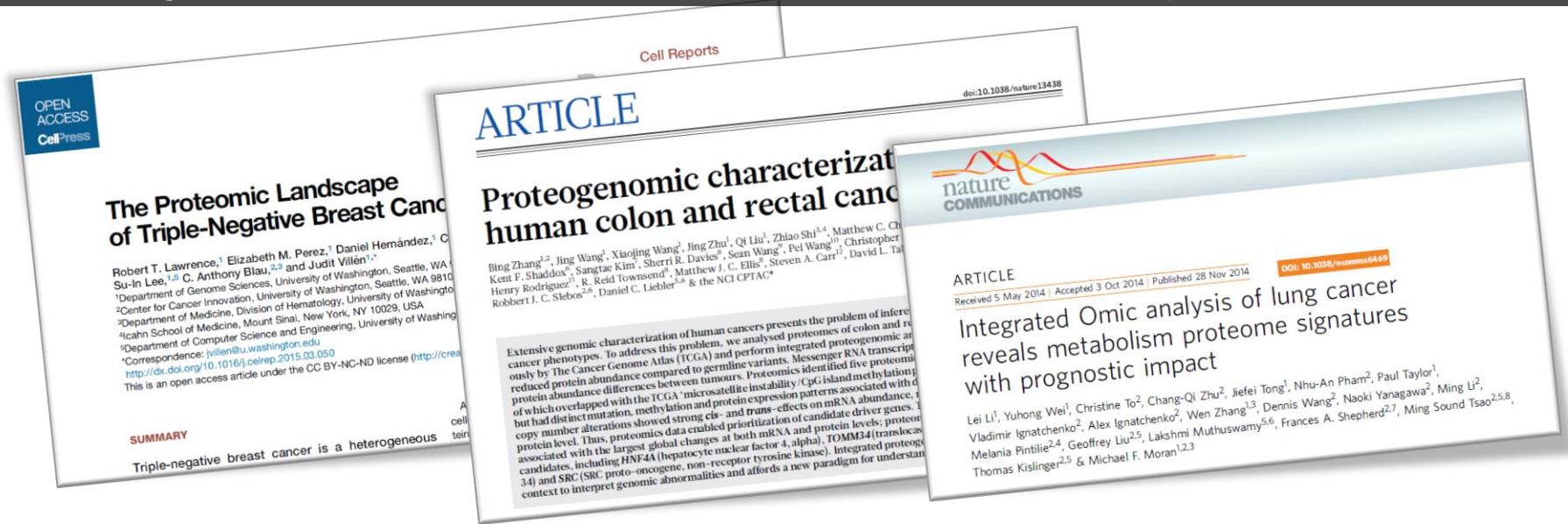
Peptide precursors per Protein Group



* 4ug HELA digest, 3 technical replicates, 120k @ MS1



The Age of Multi-Omics Is Here. Are We Ready?



- Proteomics is being used to discover and establish the protein landscape of cancer cells or tissues
- Proteomic measurements complement genomics/transcriptomic measurements by reducing the vast number of potentially actionable somatic mutations and identifying genomic variances that might be actionable

Proteomics Complements Genomics