NA Mass Spec Users Meeting

November, 2017

ThermoFisher SCIENTIFIC

Pushing the Leading Edge in Protein Quantitation:

Integrated, Precise, and Reproducible Protein Quantitation Workflow Solutions

Amy Rosenthal

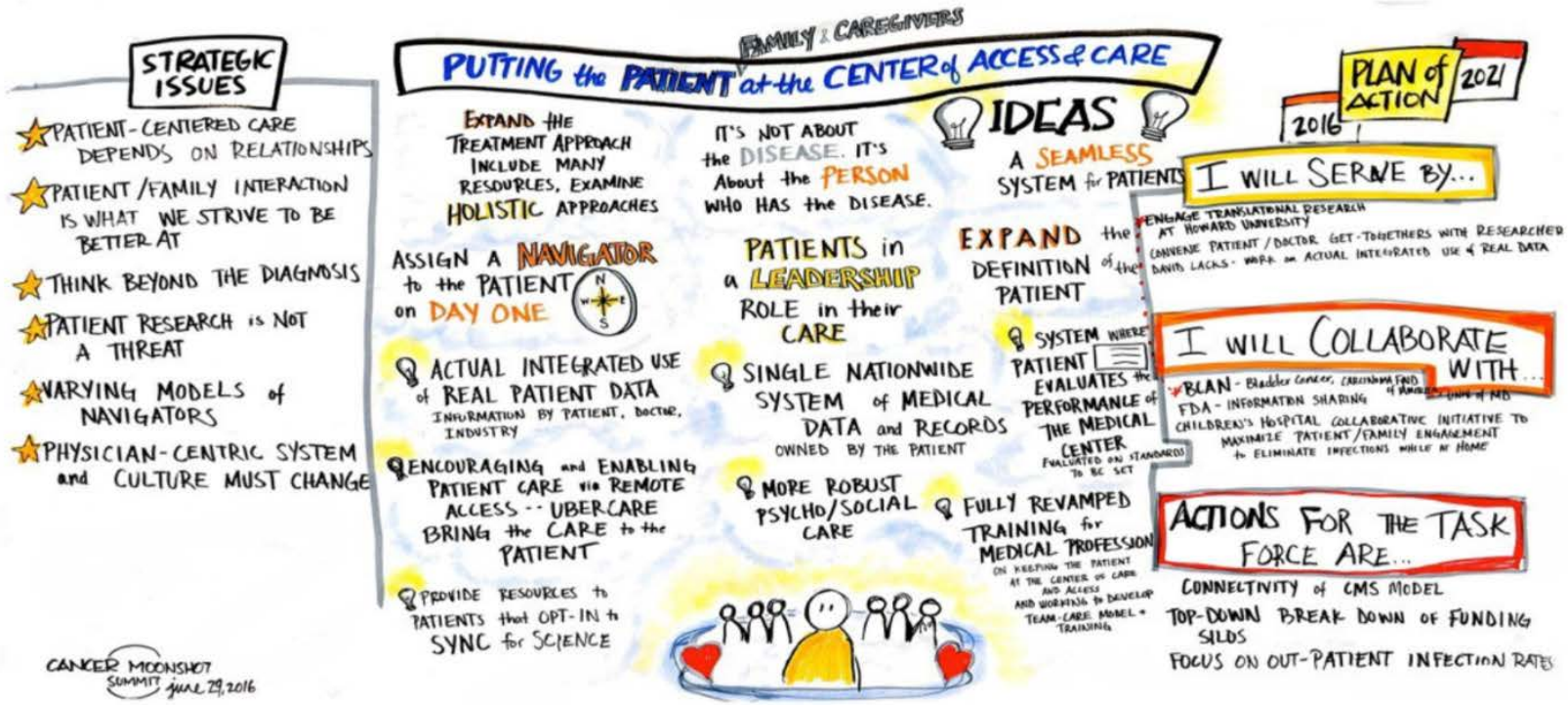
You may want to marry my husband

New York Times, 03/03/2017


Cancer Moonshot
Congress passed 21st Century Cures Act: $1.8 billion over seven years
For 2017: $300 million to fund Moonshot Initiatives

Cancer Moonshot: Precision Medicine, Immunotherapy, and Omics

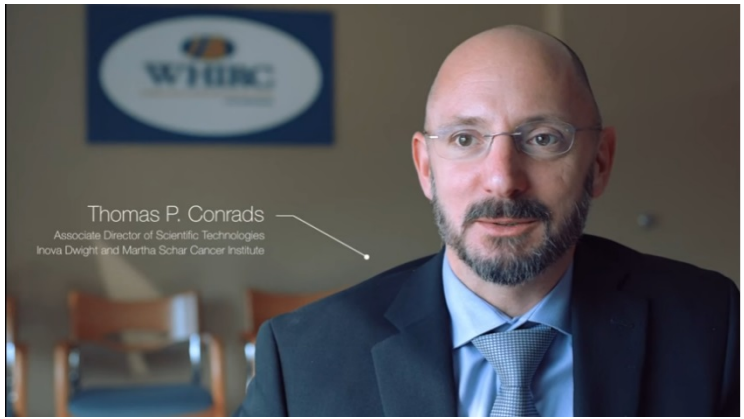
Goal: To detect cancer at an early stage while providing additional therapies to more patients.



Proteins Are the Machinery, Markers, and Targets for Cancer

"…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."

Thomas Conrads et al. 2016 Clinical Cancer Research

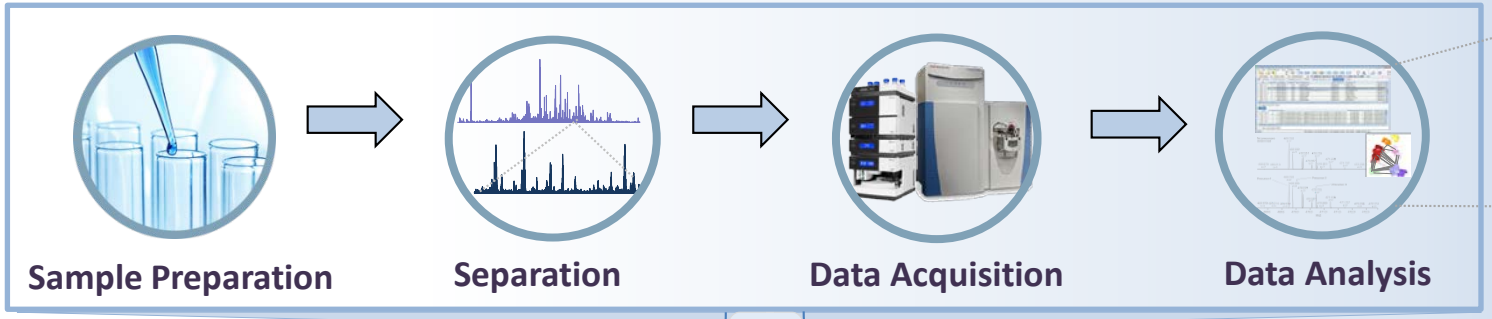


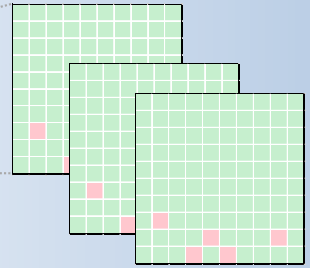
ThermoFisher SCIENTIFIC

INOVA®
Schar Cancer Institute

The Goal: Standardized, High Throughput Proteomics

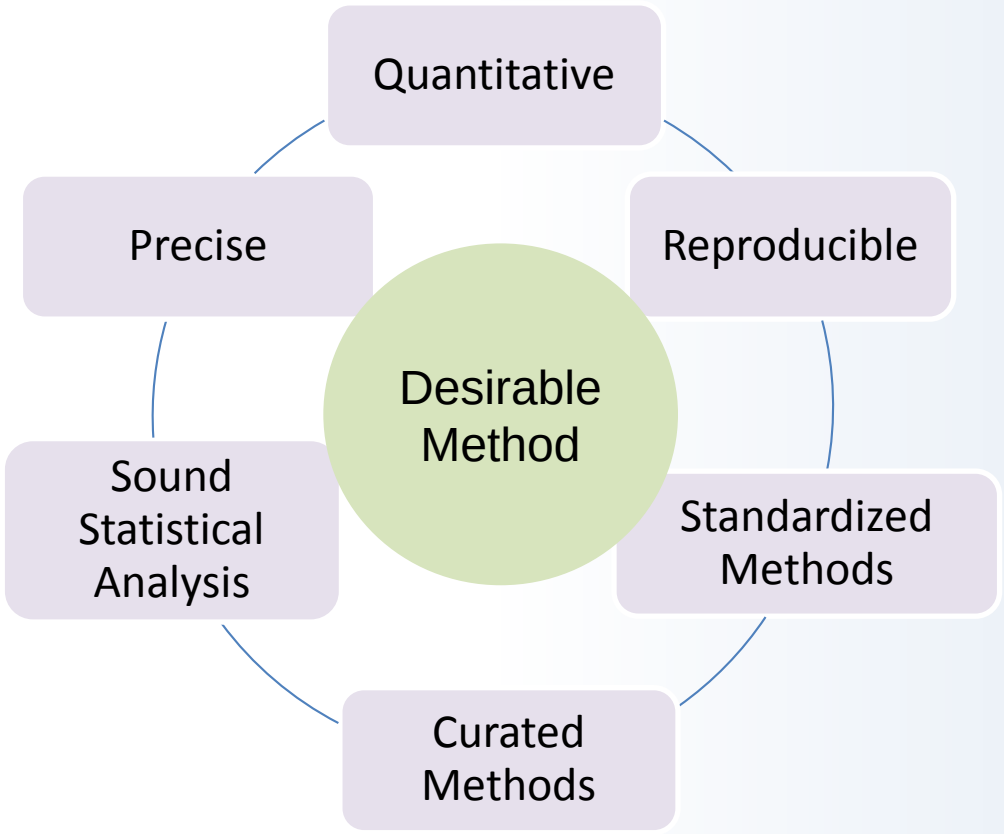
Large Scale Proteomics





- QUANTITATIVE
- PRECISE
- REPRODUCIBLE
- STANDARDIZED

Versatile Workflow Solutions For Your Needs



Our Solution

A new standard in quantitative, sensitivity, accuracy and precision

- HR DIA
- DDA+

Workflows

Flexible Quantitative Workflow Solutions

HR 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

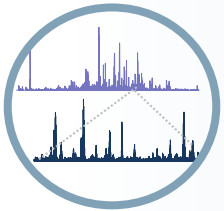
- Unrivaled precision in precursor quantitation
- Maximize complete, reproducible quantitation across samples
- Minimize ‘missing values’ among samples

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

HR DIA Workflow

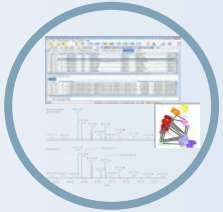
HR DIA Workflow

Sample Preparation



Separation

Data Acquisition



Data Analysis

- QUANTITATIVE
- PRECISE
- REPRODUCIBLE
- STANDARDIZED

HR DIA : Unparalleled Proteome Coverage and Dynamic R ge

new

Workflow

Chromatography

- *UltiMate 3000 RSLCnano*
- *Direct inject or preconcentration mode*
- *Dionex Viper fittings*

Easy-Spray Column

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

Q Exactive HF-X

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

Spectronaut™

Designed for Speed and Coverage

Spectronaut by Biognosys

Key Benefits

- Spectronaut™ software is specifically developed for the analysis of DIA 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 Proteome Discoverer™ 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

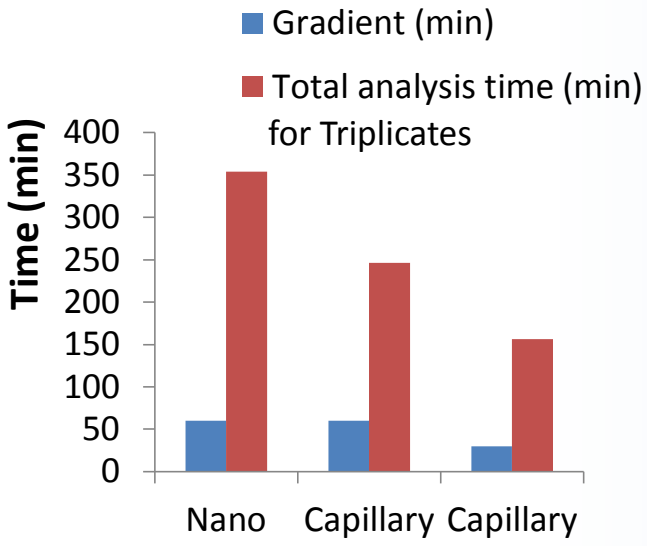
Balancing Efficiency Without Sacrificing Performance

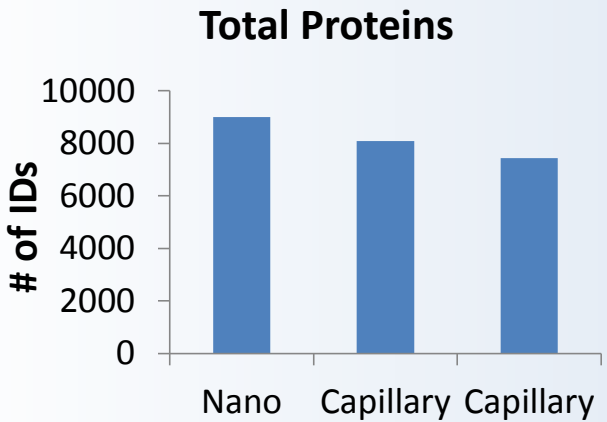
Nanoflow (300nL/min)

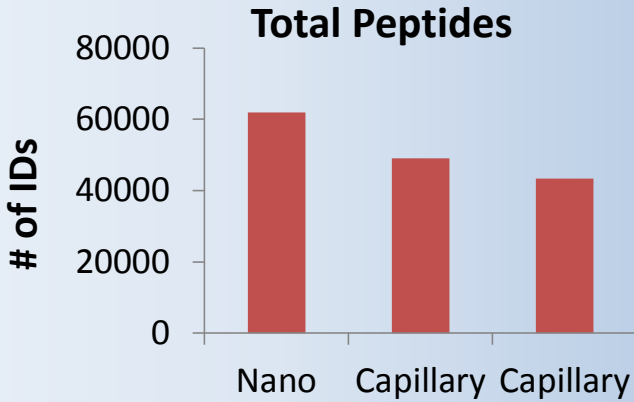
- Greater # of proteins
- Greater # of peptides
- Greater sensitivity
- *Longer total run times*

Capillary flow (4uL/min)

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

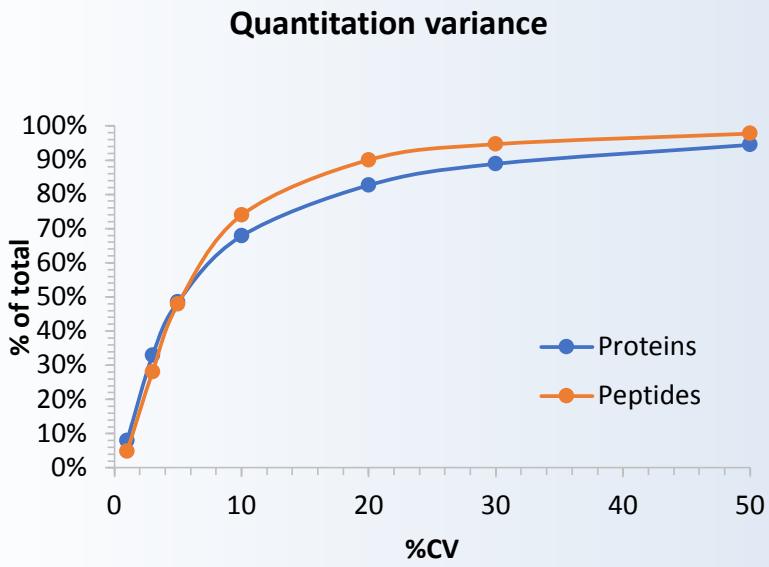




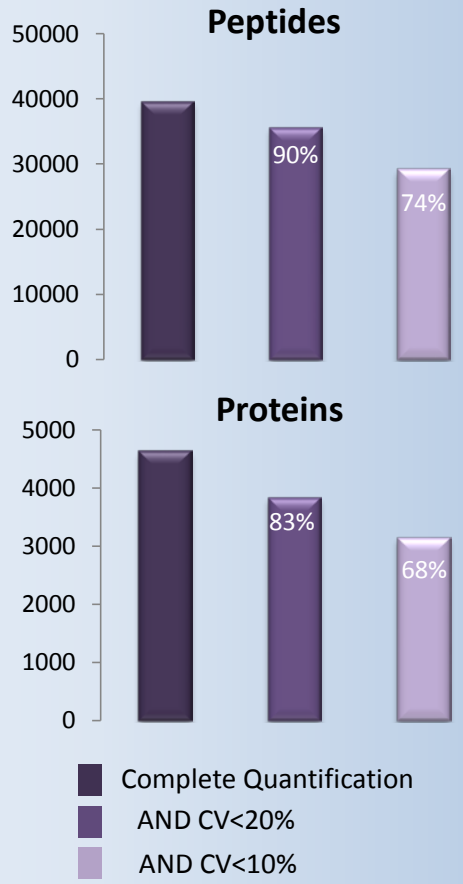


HR DIA Workflow: Highly Precise Proteome Quantitation

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



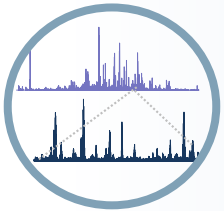
CapLC DIA, 4ug HeLa, 60min, 120K,
80 windows spanning 400-1200 m/z, Spectronaut Analysis



DDA+ Workflow

DDA+ Workflow

Sample Preparation



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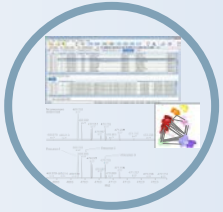

Data Analysis

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DDA+ Workflow: Quantitative Precision and Reproducibili

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Proteome Discoverer 2.2

Designed for Precision and Reproducibility

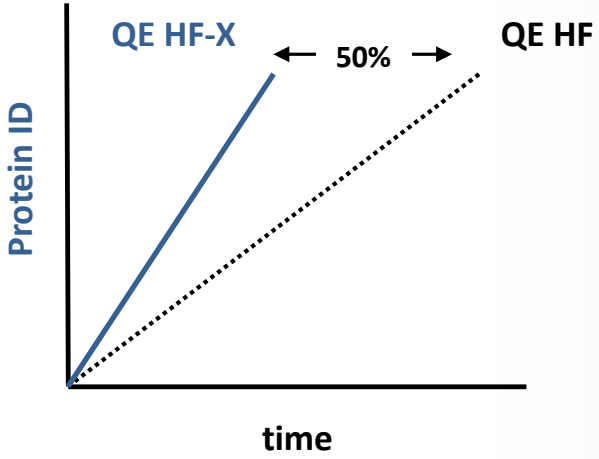
Proteome Discoverer 2.2

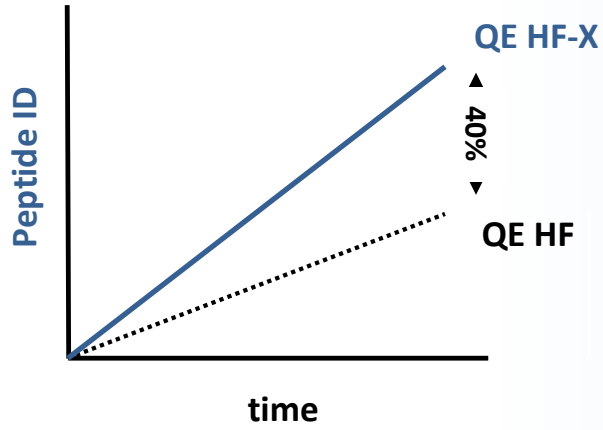
Key Benefits

- 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

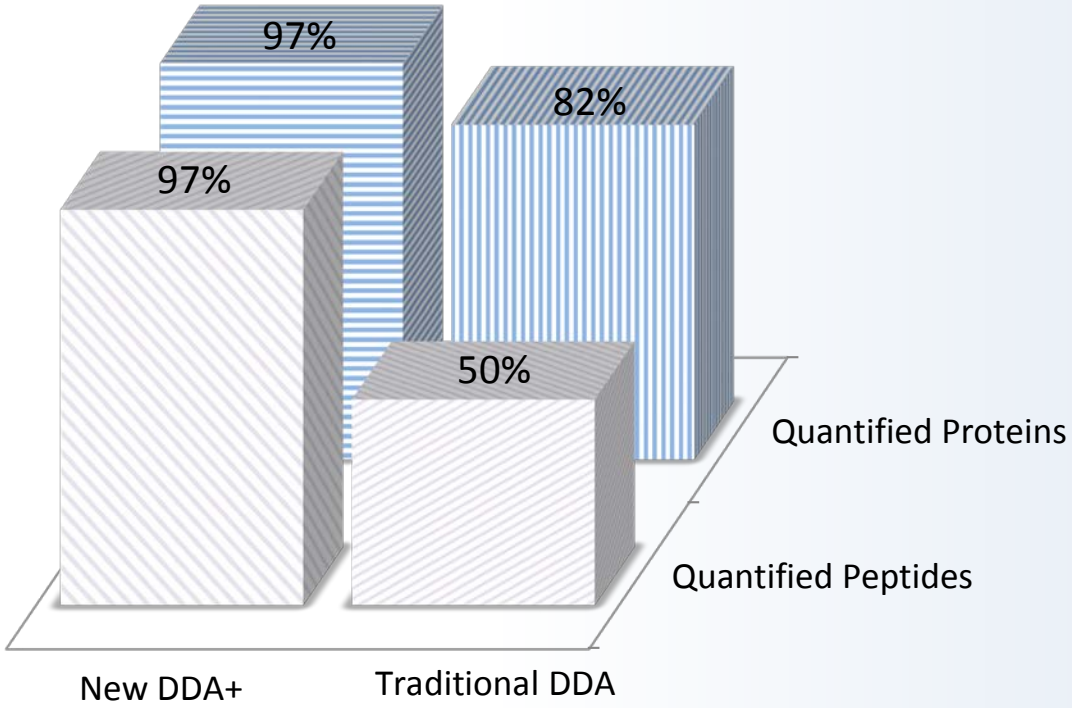
- Quick screening of complex samples
- Quality control of complex samples
- Assessment of sample concentration

Maximizing peptide identifications

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

Saves time and samples in large-scale proteomics efforts

Real Benefit of Using DDA+ Workflow



DDA+ workflow compared to DDA

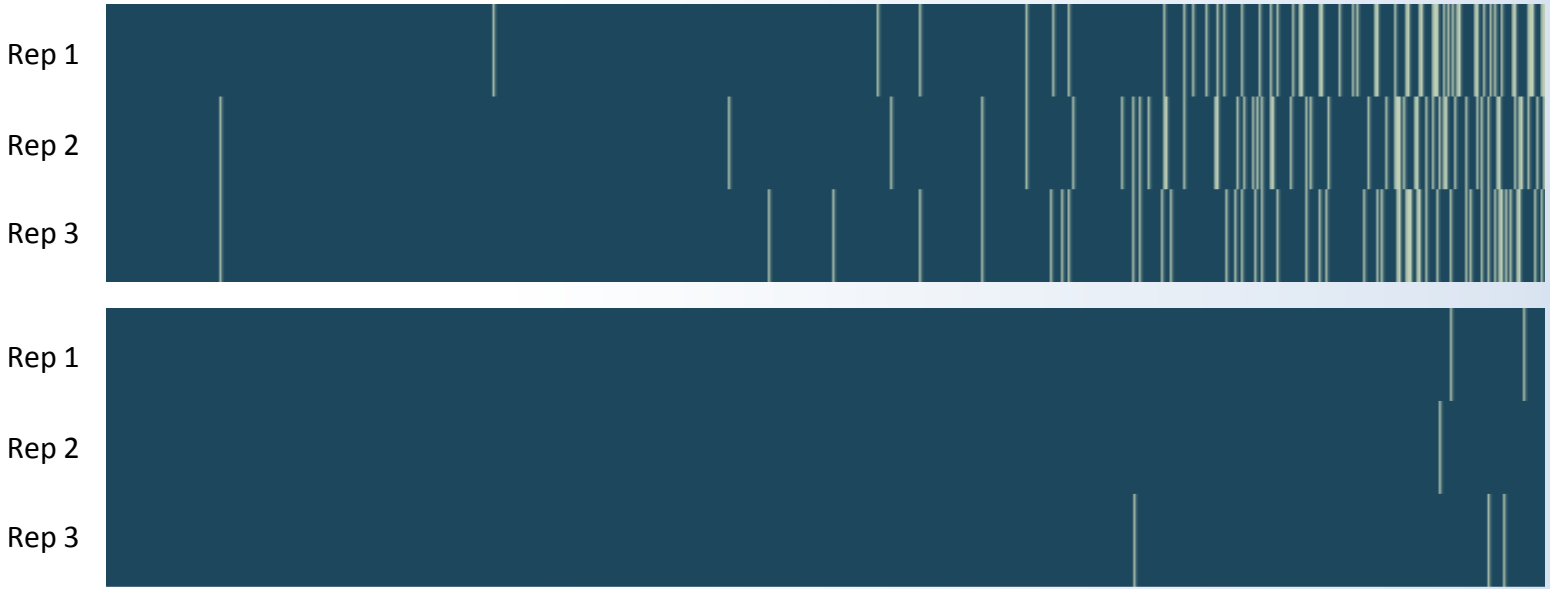
- 15% gain in completely quantified proteins
- 47% gain in completely quantified peptides
- Maximizes quantitation

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

DDA+ Workflow: Protein Quantification

new

Completely quantified proteins



Missing data = sparse quantitation

Complete quantitation

82% quantified during MS

97% quantified from MS and PD 2.2

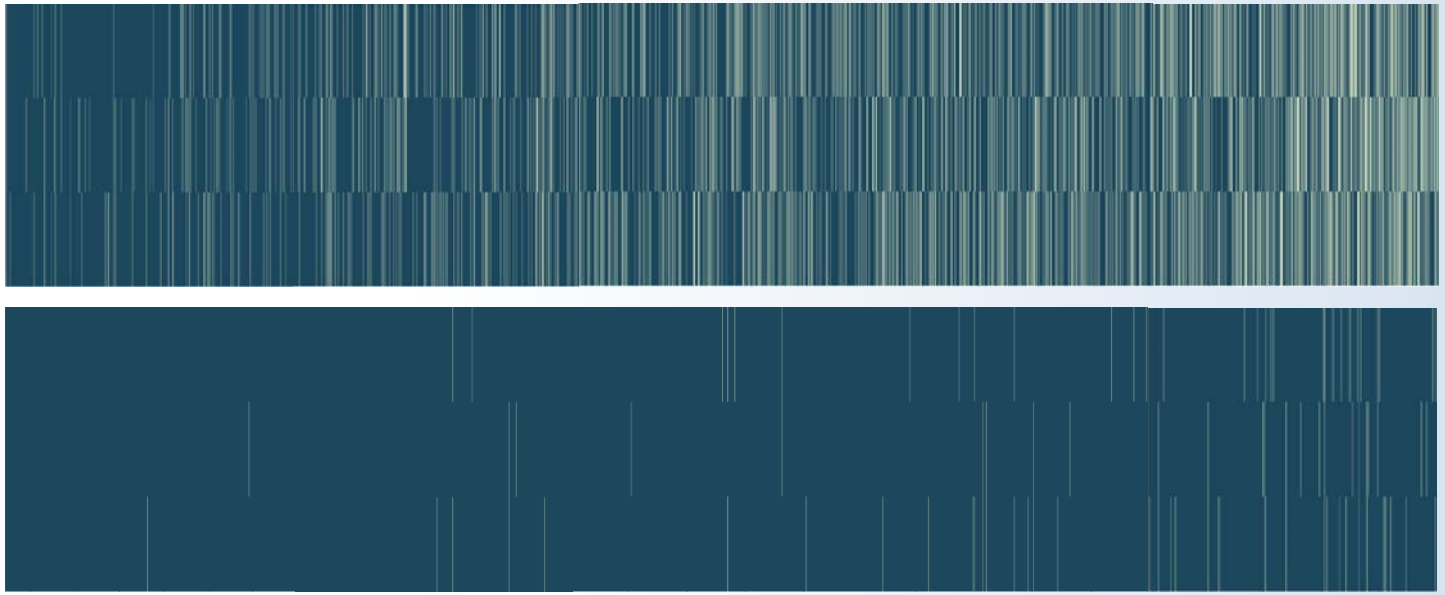
capLC DDA+, 4ug HeLa, 60min, 120K/7.5K, 19ms, Top 40 -> PD 2.2 Label-free Quant

Quantitation | Precision | Reproducibility | Standardization

DDA+ Workflow: Near Complete Peptide Quantification

new

Completely quantified peptides



Missing data = sparse quantitation

Complete quantitation

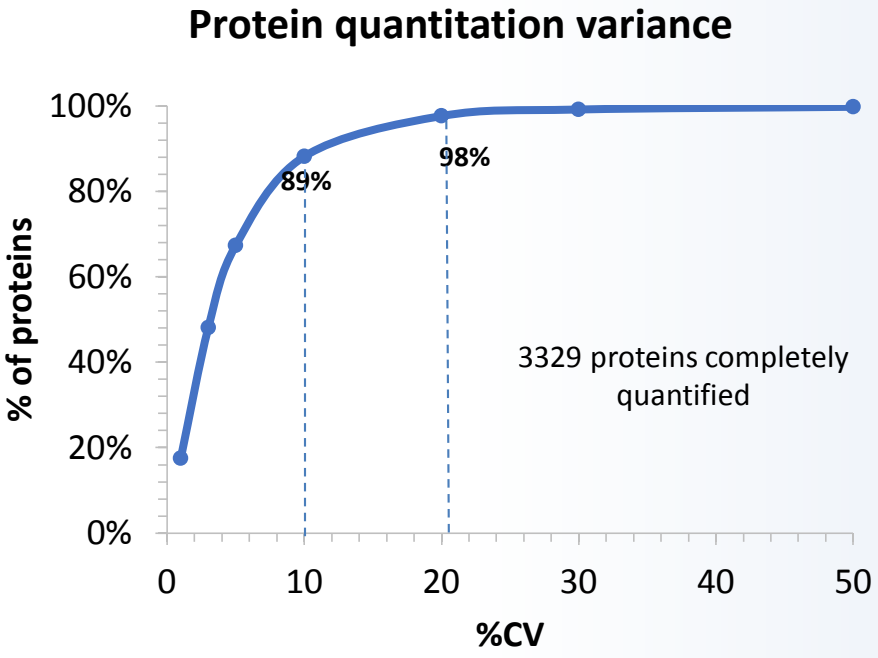
50% quantified during MS

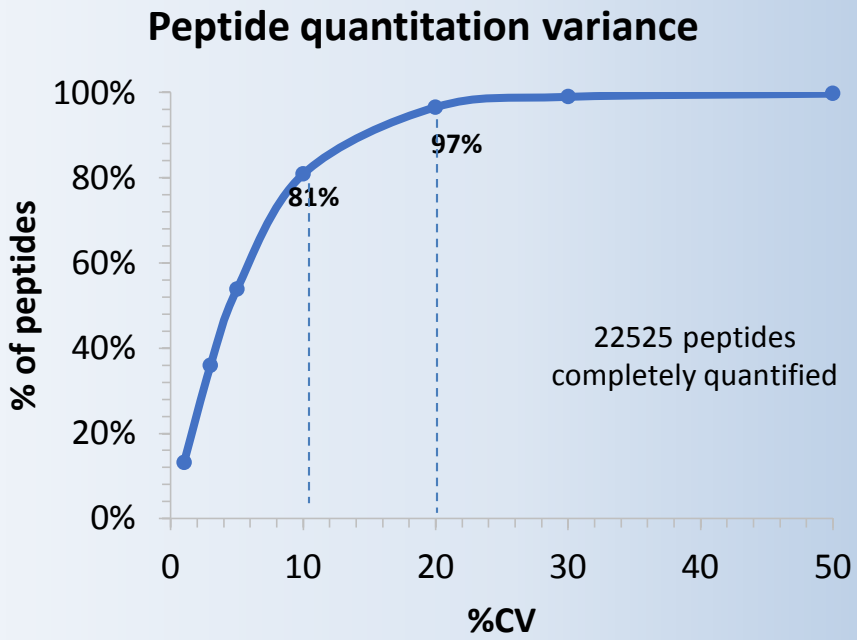
97% quantified from MS and PD 2.2

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

Quantitation | Precision | Reproducibility | Standardization

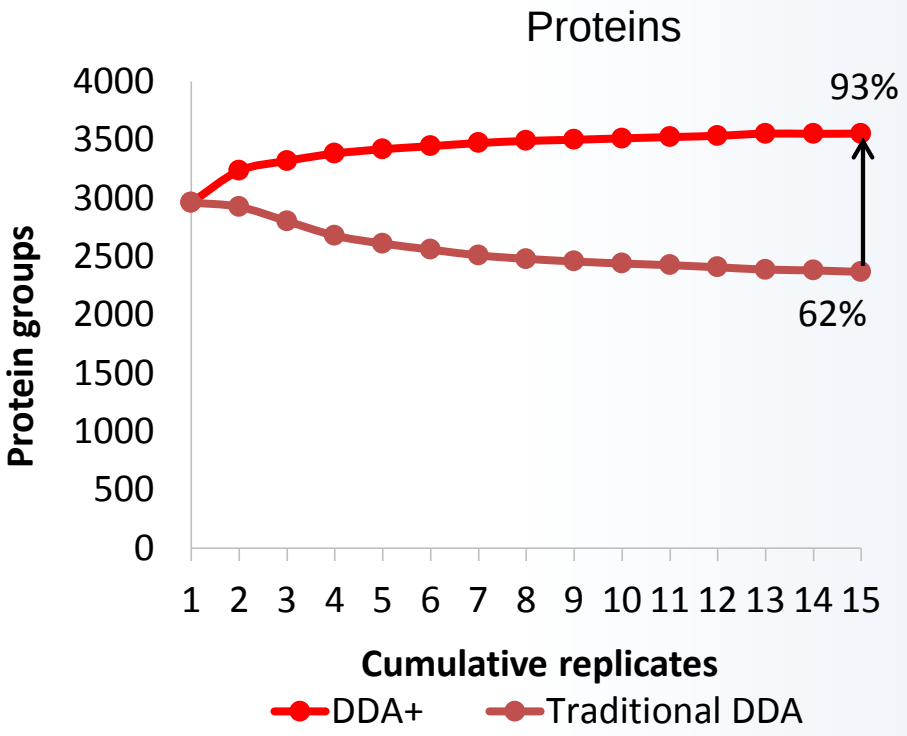
DDA+ Enables Unrivaled Quantitative Precision

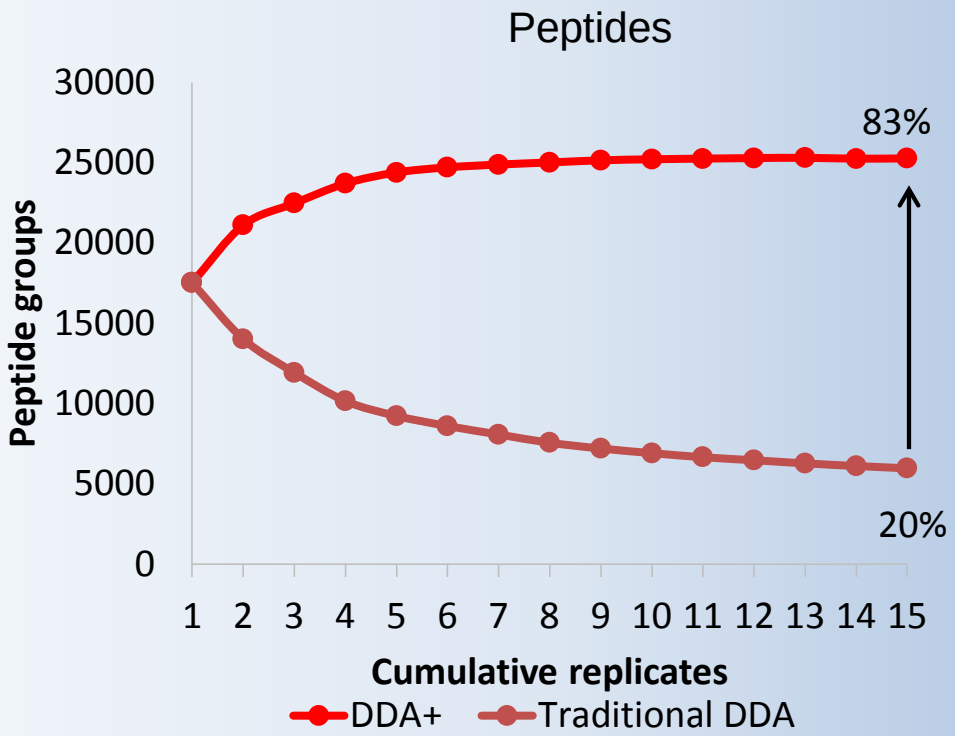




Quantitation | **Precision** | Reproducibility | Standardization

DDA+ Workflow: Greater Reproducibility Between Samples



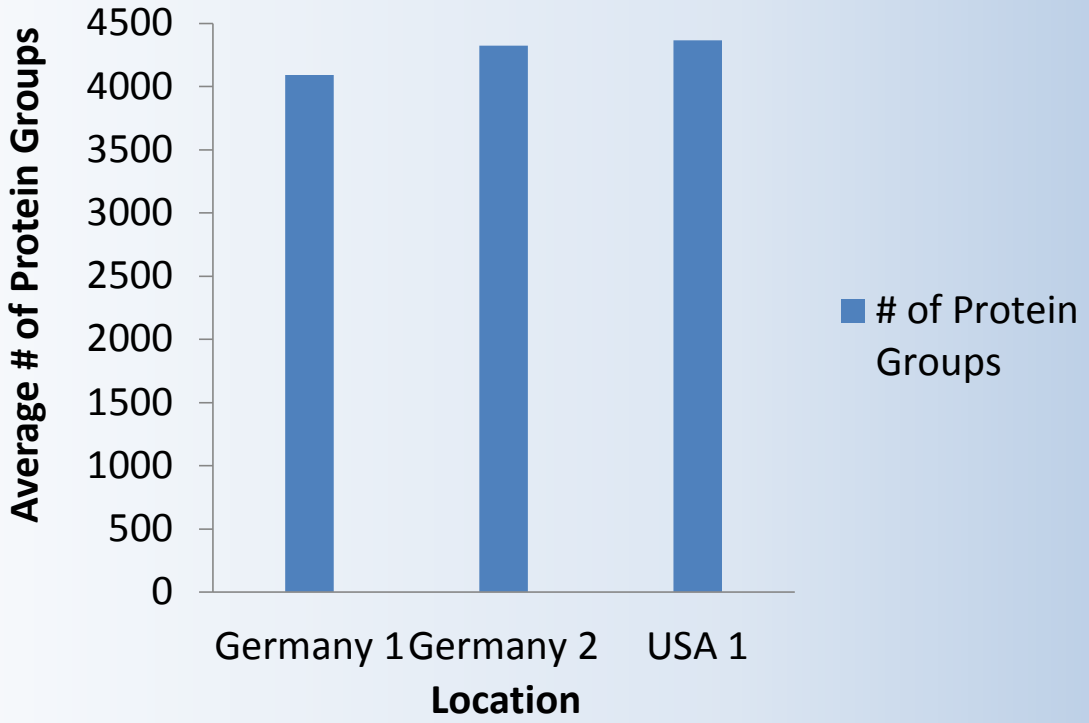


Quantitation → Precision → **Reproducibility** → Standardization

Inter-Site Consistency Across Different Instruments

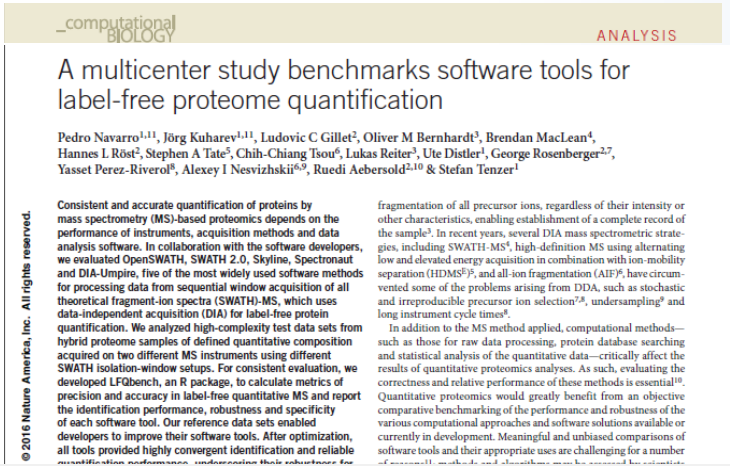
Instrument Standardization Test

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



Quantitation → Precision → Reproducibility → **Standardization**

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 Gillet[2], Oliver M Bernhardt[3], Brendan MacLean[4], Hannes L Röst[5], Stephen A Tate[5], Chih-Chiang Tsou[6], Lukas Reiter[3], Ute Distler[1], George Rosenberger[2,7], Yasset Perez-Riverol[8], Alexey I Nesvizhskii[6,9], Ruedi Aebersold[2,10] & Stefan Tenzer[1]

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

fragmentation of all precursor ions, regardless of their intensity or other characteristics, enabling establishment of a complete record of the sample[3]. In recent years, several DIA mass spectrometric strategies, including SWATH-MS[4], high-definition MS using alternating low and elevated energy acquisition in combination with ion-mobility separation (HDMS^E)[5], and all-ion fragmentation (AIF)[6], have circumvented some of the problems arising from DDA, such as stochastic and irreproducible precursor ion selection[7,8], undersampling[9] and long instrument cycle times[8].

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 essential[10]. 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

Thermo Fisher BRIMS Center

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

Phase 1
Labs identified, resources secured, SOP established

Phases 2
Study design, study setup, data collection

Phase 3
Data review, report-out


Cancer Moonshot


Questions?