

Spectronaut 14 Release Notes

New Features

directDIA 2.0

- Deep learning augmented spectrum-centric DIA analysis

Ion Mobility support

- Improved Bruker dia-PASEF support
- Improved Thermo FAIMS Pro support
- Waters HDMS^E support
- directDIA support for ion mobility data
- Improved PTM localization in ion mobility data

SNE combine

- Analyze huge experiments by analysis of partial datasets and merging of multiple .sne files into a single report file

Quant 3.0

- Improved differential abundance analysis using MS1 and MS2 level quantification
- New unpaired t-test option is new default

Deep-learning-augmented library generation and peptide identification

- Deep learning predicted decoys is new default
- Deep learning assisted iRT regression is new default
- Deep learning augmented scoring in Pulsar

New Visualizations

- Protein coverage plot
- LFQbench plot
- Ion Mobility method overview
- Ion Mobility calibration
- MS1 base peak chromatogram
- MS2 base peak and TIC chromatogram per window
- Digest specificity bar plot

New method support

- RTwinDIA [\[Li, 2019\]](#)
- Demultiplexing of overlapping windows (via HTRMS converter) [\[Amodei, 2019\]](#)
- dia-PASEF with multiple MS1 full scans in a cycle

Library Generation

- View library settings in library perspective (for new libraries only)
- Pulsar
 - Improved identifications for DDA and DIA runs
 - Improved speed of many modifications search when using high-resolution DDA data
 - ETD/ETHcD support
 - Faster PASEF and dia-PASEF database search
- Proteome Discoverer
 - PD 2.4 support
 - Library generation for FAIMS DDA results
 - Protein group FDR is taken into account for library generation

Other features

- All proteins protein inference strategy
- Protein quantity reported separately for each channel in labelling experiments
- XIC Graph plot improvements
- iBAQ protein quantities
- Updated downloadable content: FASTA protein databases

Other Changes

General Improvements

- Improved identifications in targeted analysis, especially for body fluid samples
- Improved sparse, percentile and complete filtering for PTM filtering
- Improved loading/saving speed for SNE files
- Improved CPU affinity selection settings box
- Support for CPUs with more than 64 logical cores
- Improved pipeline report settings option box
- Option to override mass tolerances during calibration
- Option to override ion mobility tolerances
- Improved folder selection framework
- Option to generate search archives from a directDIA experiment
- Improved reporting for labeled experiments

Report Perspective Changes

Standard Report

Removed Columns

- FG.IsotopeLabelType

New Columns

- PG.MS1ChannelQuantities
- PG.MS2ChannelQuantities
- PEP.MS1ChannelQuantities
- PEP.MS2ChannelQuantities
- E.AVGModifiedSequencesIdentified
- E.AVGPrecursorsIdentified
- E.AVGProteinGroupsIdentified
- E.AVGStrippedSequencesIdentified
- PG.IBAQ
- PEP.DigestType
- FG.MS1IsotopeIntensities (Measured)
- FG.MS1IsotopeQuantity
- FG.MS1IsotopeRelativeIntensities (Measured)
- FG.MS1IsotopeRelativeIntensities (Predicted)

Renamed Columns

- R.Gradient length → R.Gradient Length
- Analysis Version → R.Analysis Version
- FG.DriftTime → FG.IonMobility

Pivot Report

New Columns

- PEP.DigestType
- EG.IsDecoy
- PG.IBAQ
- EG.PTMPositions
- EG.PTMProbabilities
- EG.PTMSites

Changed Columns

- PG.IsSingleHit is a row column now

DIA Analysis Settings Changes

Changes in default settings

- Post Analysis → Differential Abundance Grouping → Smallest Quantitative Unit [Precursor Ion (Quantification Settings) → Major Group (Quantification Settings)]
- Post Analysis → Differential Abundance Testing [Student's t-test → Un-Paired t-test]

New settings

- XIC Extraction → XIC IM Extraction Window [Dynamic]
- XIC Extraction → XIC IM Extraction Window [Dynamic] → Correction Factor [1]
- Calibration → MS1 Mass Tolerance Strategy [System Default]
- Calibration → MS2 Mass Tolerance Strategy [System Default]
- Identification → Generate Decoys → Decoy Method → Preferred Fragment Source [NN Predicted Fragments]
- Protein Inference → Protein Inference Workflow → Inference Algorithm [IDPicker]
- Post Analysis → Differential Abundance Testing [Un-Paired t-test]
- Post Analysis → Differential Abundance Testing [Un-Paired t-test] → Assume Equal Variance [false]

- Post Analysis → Differential Abundance Grouping → Smallest Quantitative Unit → Use All MS-Level Quantities [true]
- Post Analysis → Differential Abundance Grouping → Run Clustering → Order Runs by Clustering

Renamed settings

- Post Analysis → Differential Abundance Testing [Student's t-test → Paired t-test]

Global Settings Changes

New settings

- Reporting → Generate Search Archives from directDIA