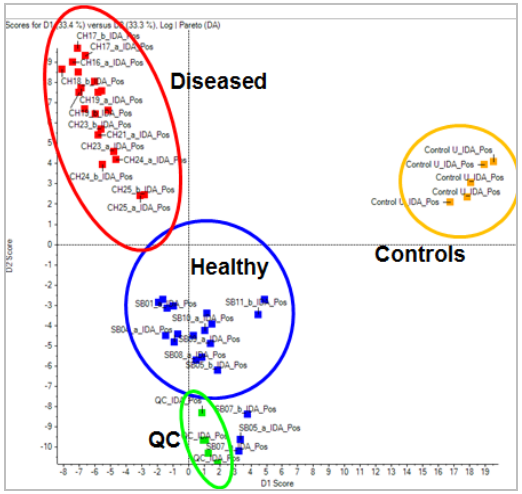
Answers for Science.
Knowledge for Life.™

MarkerView™ Software 1.3

New Features

MarkerView™ Software Overview

- A data visualization tool designed for scientists who wish to visualize their data in terms of sample groupings and apply statistics in order to gain valuable insight into any trends within their mass spectral data.
- MarkerView is unique in that SCIEX users can explore statistical correlations with direct connections back to the raw data. This allows them to find meaningful relationships much more quickly.
- Target Applications:
  - Metabolomics
  - Lipidomics
  - Proteomics
  - Food Authenticity
  - Water Testing



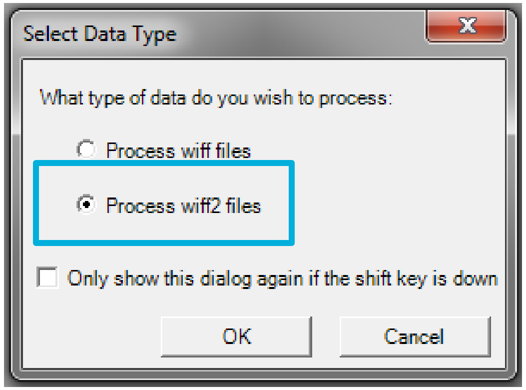
MarkerView™ Software 1.3

New Features

- Support for *.wiff2 format
  - X500R QTOF System
- Import wizard improves ease of learning
- Changes to t-test view
- Box and whiskers plots
- Infusion MS/MSALL Support
- SWATH® Acquisition support
- Most likely ratio (MLR) normalisation
- Custom sample columns
- ‘Set Names’ script
- Speed and other small improvements

Support for *.wiff2 Format

- Supports *.wiff2 format enabling Metabolomics and Food/Environmental workflows on the X500R QTOF System



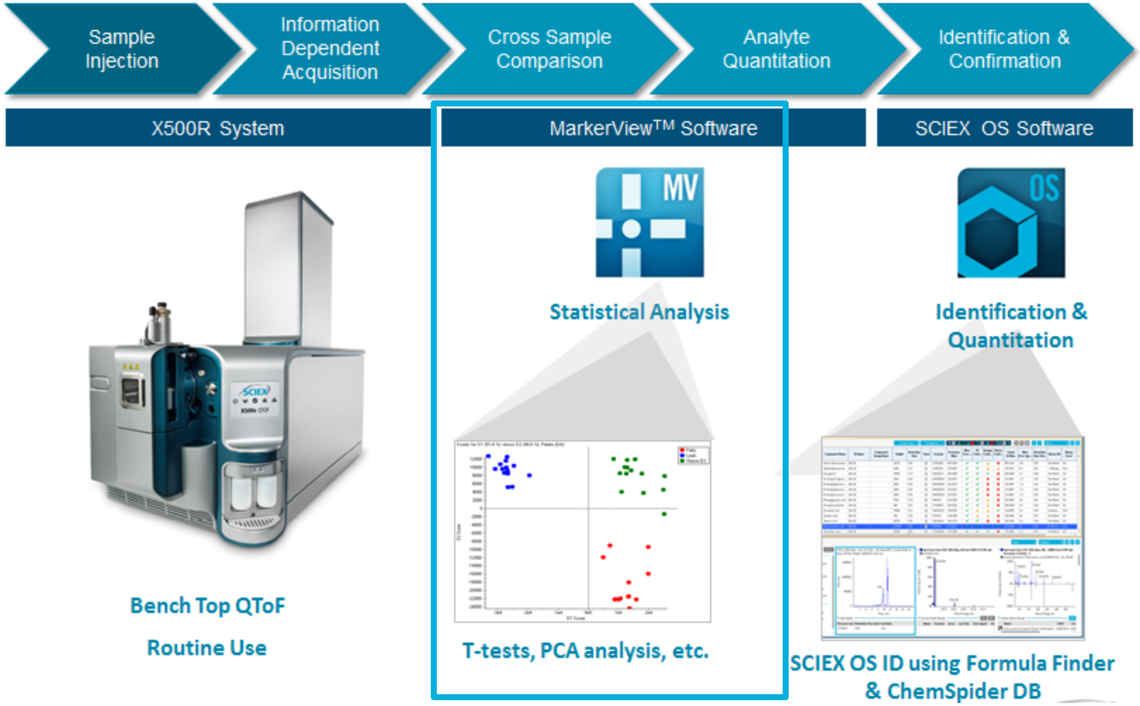
Select Data Type

What type of data do you wish to process:

○ Process wiff files

● Process wiff2 files

☐ Only show this dialog again if the shift key is down



Sample Injection → Information Dependent Acquisition → Cross Sample Comparison → Analyte Quantitation → Identification & Confirmation

X500R System

MarkerView™ Software

SCIEX OS Software

Bench Top QToF

Routine Use

Statistical Analysis

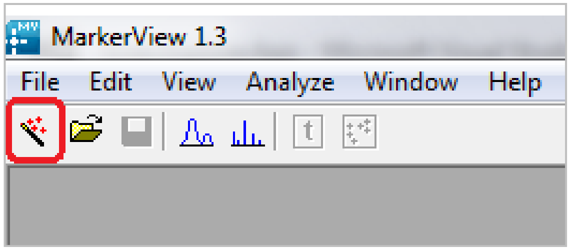
T-tests, PCA analysis, etc.

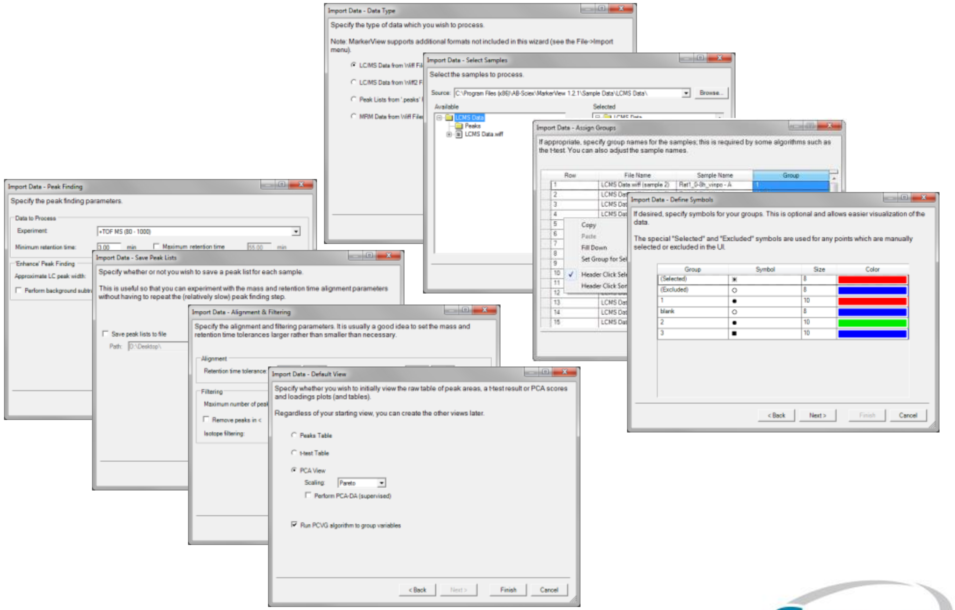
Identification & Quantitation

SCIEX OS ID using Formula Finder & ChemSpider DB

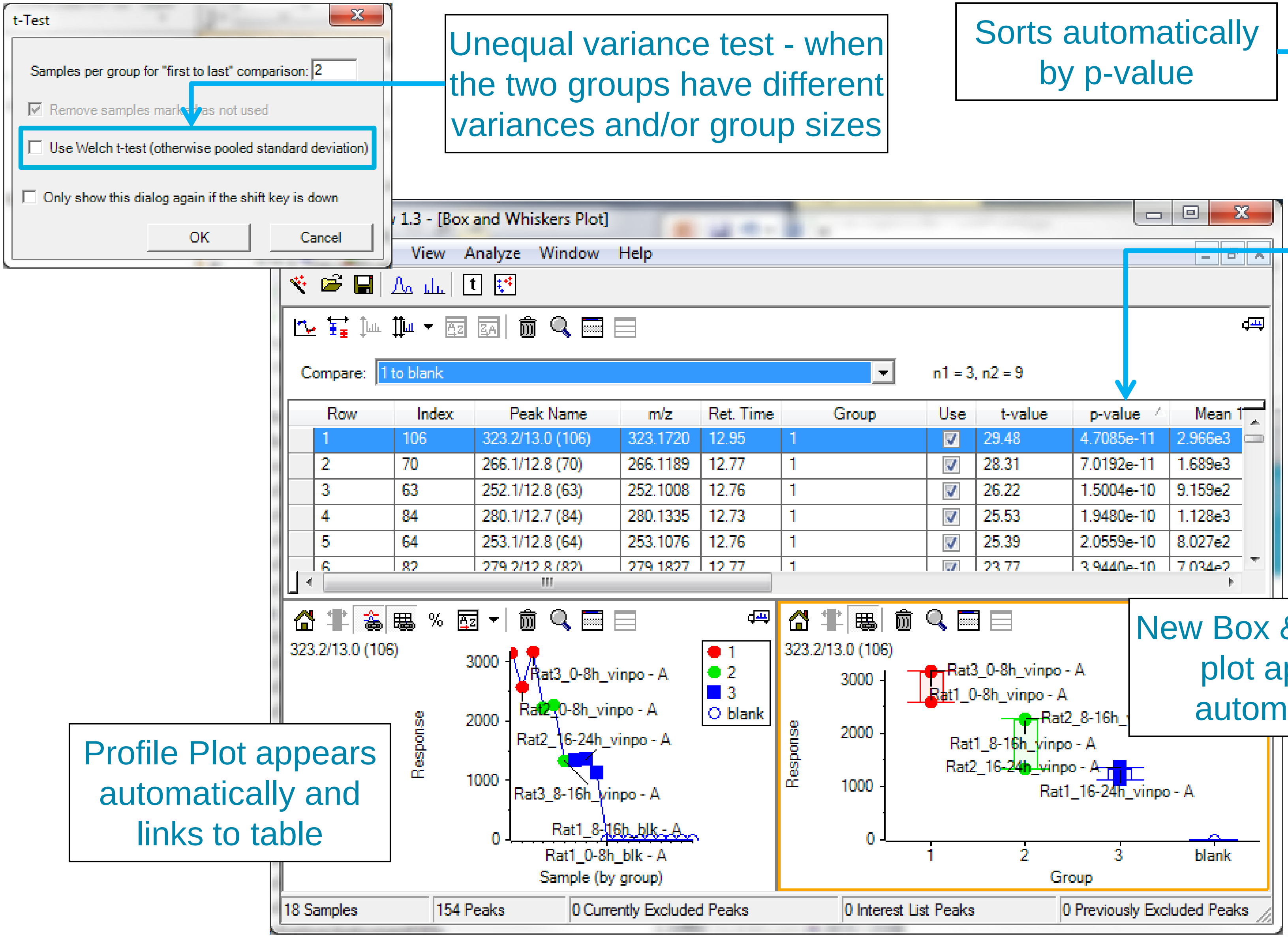
Import Wizard

- A wizard enables several steps to be combined:
  - Selecting input files
  - Peak finding and alignment parameters
  - Sample group definition (previously set in Samples Table)
  - Point symbols for groups (previously set in Options)
  - Automatic processing such as PCA or t-test (previously done explicitly)





Updated T-tests



Samples per group for "first to last" comparison: 2

☑ Remove samples marked as not used

☐ Use Welch t-test (otherwise pooled standard deviation)

☐ Only show this dialog again if the shift key is down

OK Cancel

Compare: 1 to blank

n1 = 3, n2 = 9

Row	Index	Peak Name	m/z	Ret. Time	Group	Use	t-value	p-value	Mean 1
1	106	323.2/13.0 (106)	323.1720	12.95	1	☑	29.48	4.7085e-11	2.966e3
2	70	266.1/12.8 (70)	266.1189	12.77	1	☑	28.31	7.0192e-11	1.689e3
3	63	252.1/12.8 (63)	252.1008	12.76	1	☑	26.22	1.5004e-10	9.159e2
4	84	280.1/12.7 (84)	280.1335	12.73	1	☑	25.53	1.9480e-10	1.128e3
5	64	253.1/12.8 (64)	253.1076	12.76	1	☑	25.39	2.0559e-10	8.027e2
6	82	279.2/12.8 (82)	279.1827	12.77	1	☑	23.77	3.9440e-10	7.034e2

18 Samples | 154 Peaks | 0 Currently Excluded Peaks | 0 Interest List Peaks | 0 Previously Excluded Peaks

Unequal variance test - when the two groups have different variances and/or group sizes

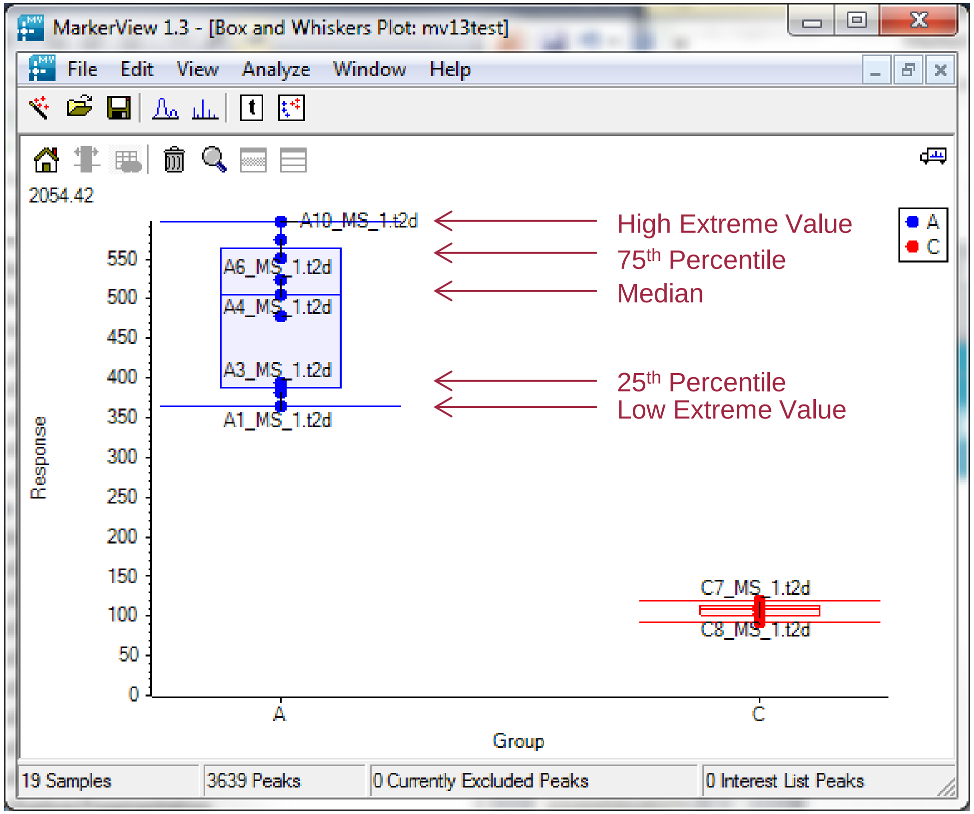
Sorts automatically by p-value

New Box & Whiskers plot appears automatically

Profile Plot appears automatically and links to table

Box and Whiskers Plots

- Standard way of visualizing statistical data across groups
- Spacing between the different parts of the box indicate the degree of spread and skewness in the data
- Highlights outliers
- Automatically generated after t-test



High Extreme Value

75th Percentile

Median

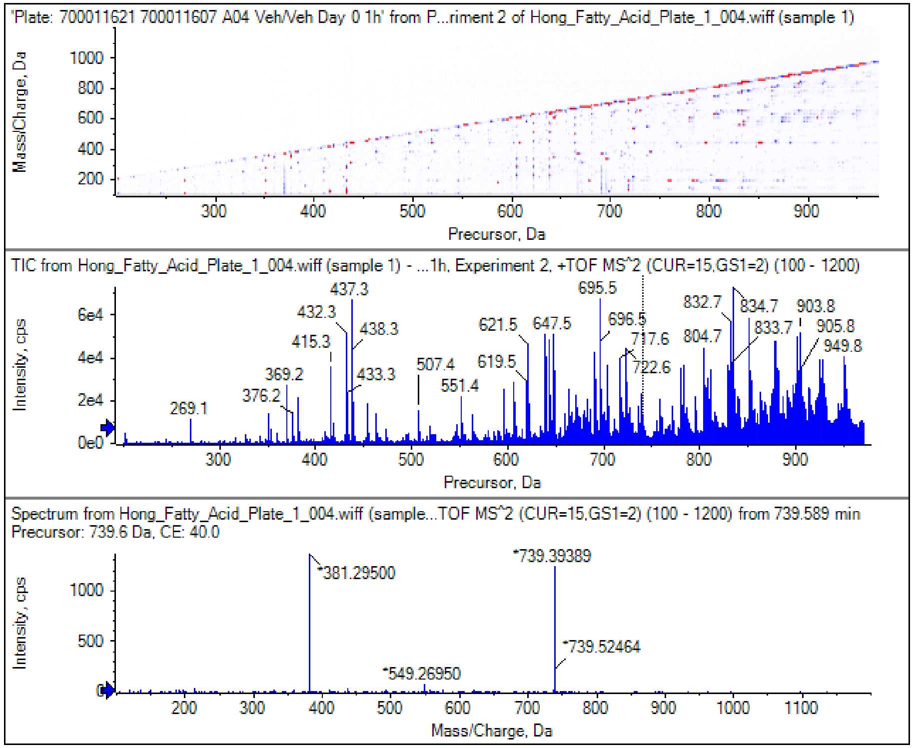
25th Percentile

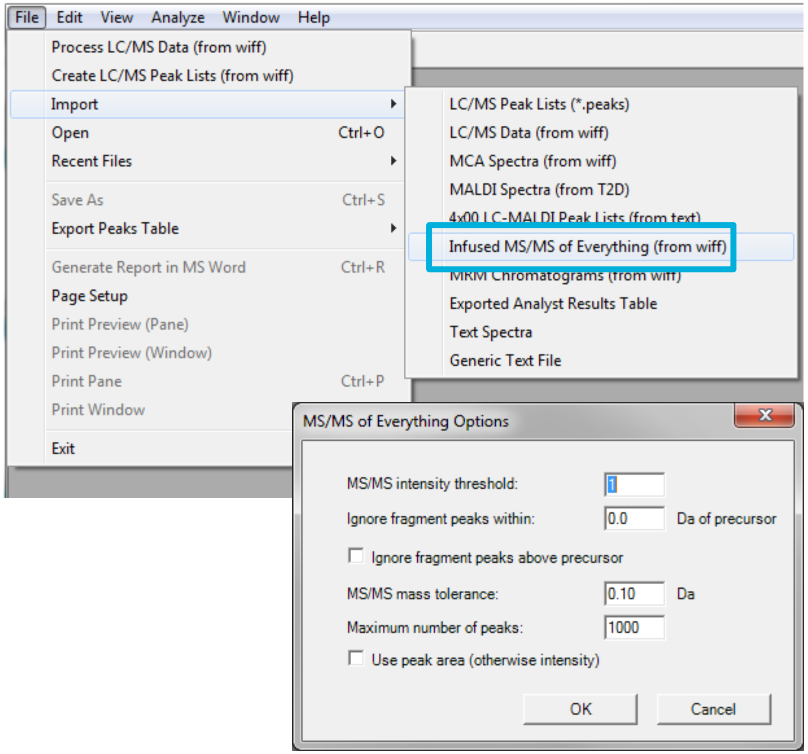
Low Extreme Value

19 Samples | 3639 Peaks | 0 Currently Excluded Peaks | 0 Interest List Peaks

Infusion MS/MS^{ALL} Support

- Infusion MS/MS^{ALL} acquisition is an technique in which a sample is infused and MS/MS is acquired for each precursor (at unit resolution) over a wide mass range
  - Typically used for lipidomics





MS/MS of Everything Options

MS/MS intensity threshold: 1

Ignore fragment peaks within: 0.0 Da of precursor

☐ Ignore fragment peaks above precursor

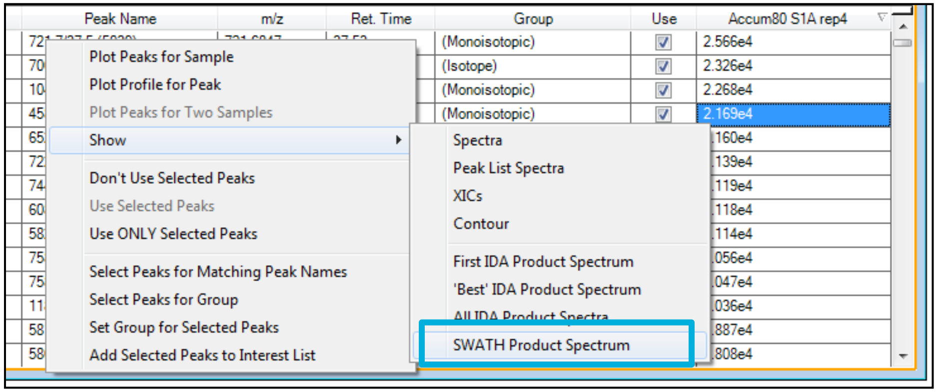
MS/MS mass tolerance: 0.10 Da

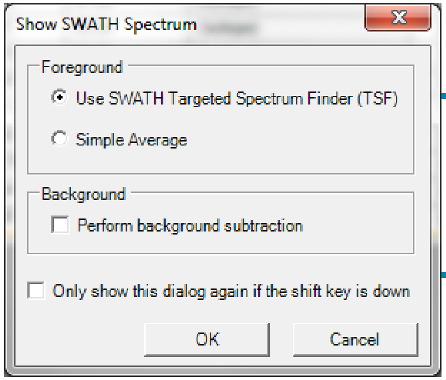
Maximum number of peaks: 1000

☐ Use peak area (otherwise intensity)

SWATH® Acquisition Support

- Import raw SWATH acquisition datafiles
- MS/MS spectra can be viewed for selected features (similar to IDA functionality).
- Targeted Spectrum Finder removes fragments with mis-matching LC peak profiles

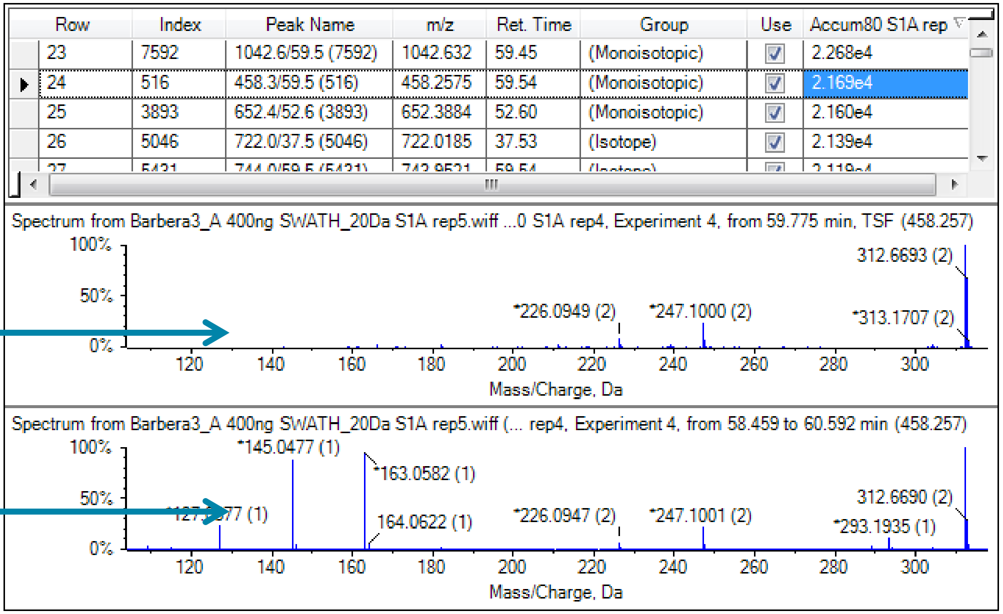



Show SWATH Spectrum

Foreground
● Use SWATH Targeted Spectrum Finder (TSF)
○ Simple Average

Background
☐ Perform background subtraction

☐ Only show this dialog again if the shift key is down



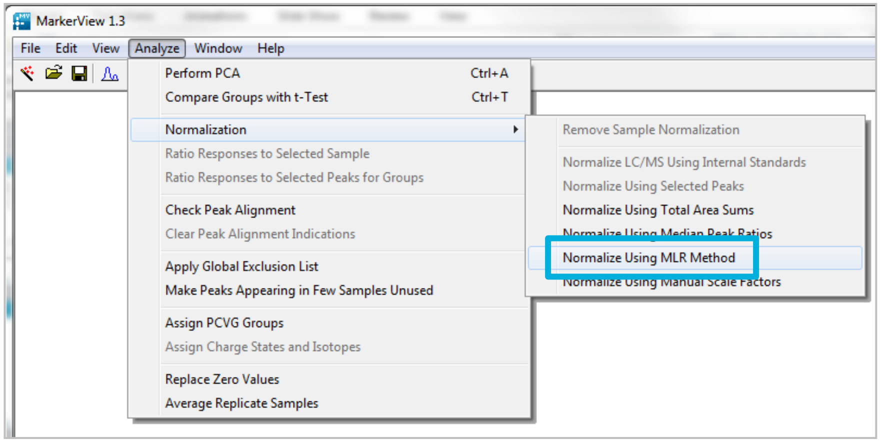
Row	Index	Peak Name	m/z	Ret. Time	Group	Use	Accum80 S1A rep
23	7592	1042.6/59.5 (7592)	1042.632	59.45	(Monoisotopic)	☑	2.268e4
24	516	458.3/59.5 (516)	458.2575	59.54	(Monoisotopic)	☑	2.169e4
25	3893	652.4/52.6 (3893)	652.3884	52.60	(Monoisotopic)	☑	2.160e4
26	5046	722.0/37.5 (5046)	722.0185	37.53	(Isotope)	☑	2.139e4

Spectrum from Barbera3_A 400ng SWATH_20Da S1A rep5.wiff ...0 S1A rep4, Experiment 4, from 59.775 min, TSF (458.257)

Spectrum from Barbera3_A 400ng SWATH_20Da S1A rep5.wiff (... rep4, Experiment 4, from 58.459 to 60.592 min (458.257)

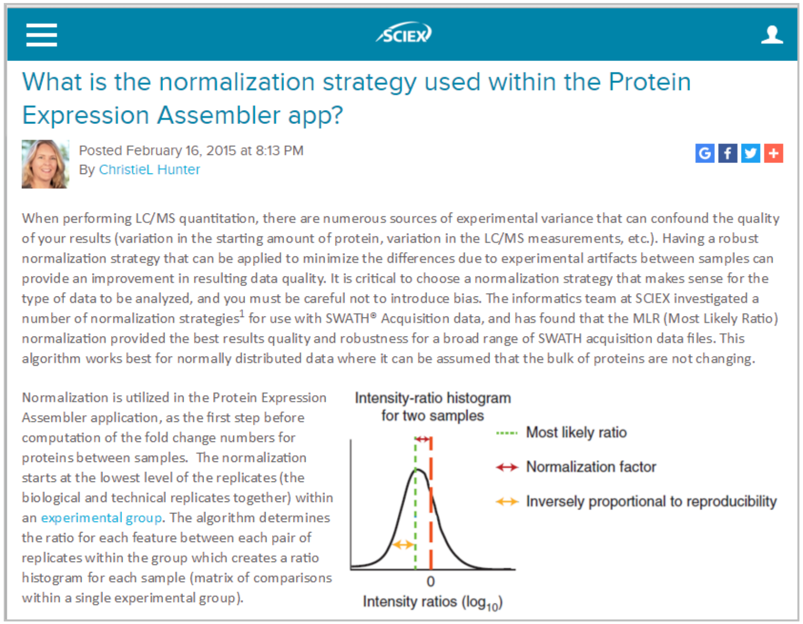
Most Likely Ratio (MLR) Normalisation

- Often need to normalize responses for a sample to allow for the fact that the *absolute* sample amount might not be constant (due to different starting amounts, sample prep differences, etc.)
- Typically used for protein/peptide normalization using large numbers of endogenous peptides (features)



For more information on the normalization strategy:

- Lambert et al. (2013) Nature Methods, 10, 1239-1245
- SCIEX Community discussion



What is the normalization strategy used within the Protein Expression Assembler app?

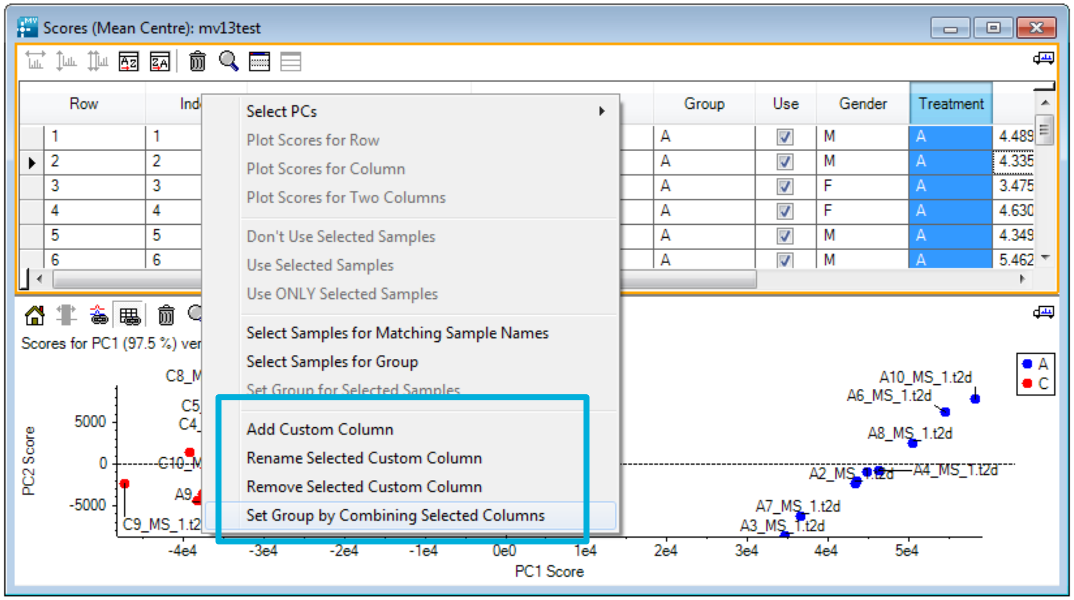
Posted February 16, 2015 at 8:13 PM
By ChristieL Hunter

When performing LC/MS quantitation, there are numerous sources of experimental variance that can confound the quality of your results (variation in the starting amount of protein, variation in the LC/MS measurements, etc.). Having a robust normalization strategy that can be applied to minimize the differences due to experimental artifacts between samples can provide an improvement in resulting data quality. It is critical to choose a normalization strategy that makes sense for the type of data to be analyzed, and you must be careful not to introduce bias. The informatics team at SCIEX investigated a number of normalization strategies[1] for use with SWATH® Acquisition data, and has found that the MLR (Most Likely Ratio) normalization provided the best results quality and robustness for a broad range of SWATH acquisition data files. This algorithm works best for normally distributed data where it can be assumed that the bulk of proteins are not changing.

Normalization is utilized in the Protein Expression Assembler application, as the first step before computation of the fold change numbers for proteins between samples. The normalization starts at the lowest level of the replicates (the biological and technical replicates together) within an experimental group. The algorithm determines the ratio for each feature between each pair of replicates within the group which creates a ratio histogram for each sample (matrix of comparisons within a single experimental group).

Custom Sample Columns

- Ability to create arbitrary columns to allow metadata entry
- Allows switching between different ways of grouping data for visualisation or supervised algorithms (t-test, PCA-DA).
- Populated manually in MarkerView™ Software or by adding custom fields to Analyst® Software batch.

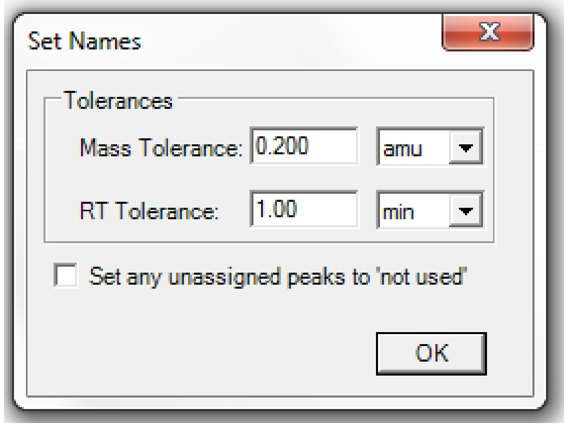


Scores (Mean Centre): mv13test

Row	Ind	Group	Use	Gender	Treatment	
1	1	A	☑	M	A	4.489
2	2	A	☑	M	A	4.335
3	3	A	☑	F	A	3.475
4	4	A	☑	F	A	4.630
5	5	A	☑	M	A	4.349
6	6	A	☑	M	A	5.462

‘Set Names’ Feature

- Utility which allows variables to be named based on their mass and retention time.
- Assuming you have a list of such masses and retention times for known compounds
  - More useful than a list of m/z-RT ion pairs
- Unknown compounds can be excluded, otherwise they remain with the default names.



Set Names

Tolerances

Mass Tolerance: 0.200 amu

RT Tolerance: 1.00 min

☐ Set any unassigned peaks to 'not used'

Other New Features

- Peak-finding has been made ‘multi-threaded’
  - On a multiple-core computer, processing will be faster (when there are multiple samples)
- ‘Impact’ column in PCA Loadings Table
  - Measure of the importance of each variable to separation

Trademarks/Licensing

RUO-MKT-11-4893-A

Answers for Science.
Knowledge for Life.™

SCIEX