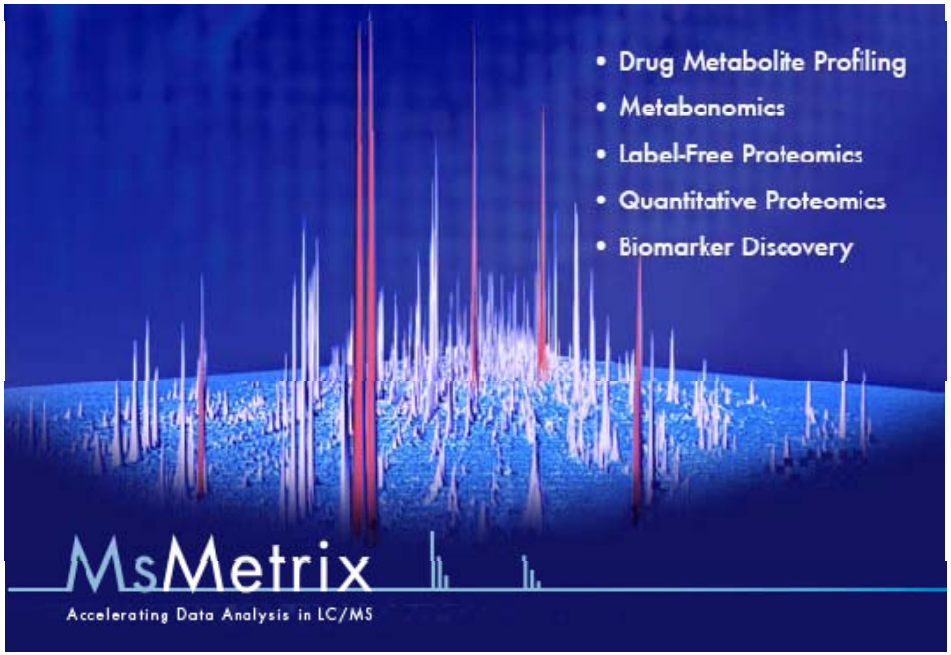
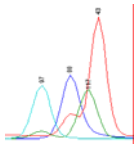
MsXelerator™: LC/MS & GC/MS Data Processing



Accelerating Data Analysis in LC-MS Profiling Studies

- **Impurity Profiling**
- **Metabolite Profiling**
- **Degradation Profiling**
- **Differential Analysis**
- **Metabonomics (LCMS & GCMS)**
- **Proteomics**
- **Biomarker Discovery**

MsMetrix



MsMetrix: Introduction

MsMetrix

Maarssen, the Netherlands
founded 2006

Training/Consultancy

Customization/Dedicated Software Solutions

MsXelerator Software

Develop Software Solutions for LC/MS and GC/MS Data Analysis
Our mission is to be the premier provider of fast, affordable, user-friendly and reliable software in the following application fields

MsXelerator™

Metabolite Profiling

Johnson & Johnson
Bristol-Myers Squibb Company
Schering-Plough
NOVARTIS
Roche

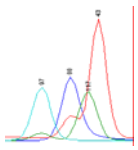
Metabolomics

Universiteit Utrecht
HZPC
evolva

GC/MS Data Processing

Proteomics/BioMarkers

PHILIP MORRIS
rivm
LUMC
Erasmus MC



MsXelerator: Overview

MsXelerator™

One Program: All Vendors – All Tools

The Browser

Import of all Vendor Formats
Explorative Analysis

MsCompare

Multi Sample Data Processing:
Metabolomics / BioMarker
Discovery / Targeted Analysis

MPeaks

Full Dataset Peak Picking
Differential Analysis
Identification

IPeaks

Isotope Pattern Filtering
Differential Analysis
Identification, Quant Proteomics

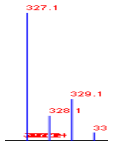
GCMS QC

Quality Control for GCMS and
LCMS in Metabolomics:
Calibration, Instrument
Performance

GC-Analyzer

GCMS Sample / Control
Comparison, Deconvolution and
Identification

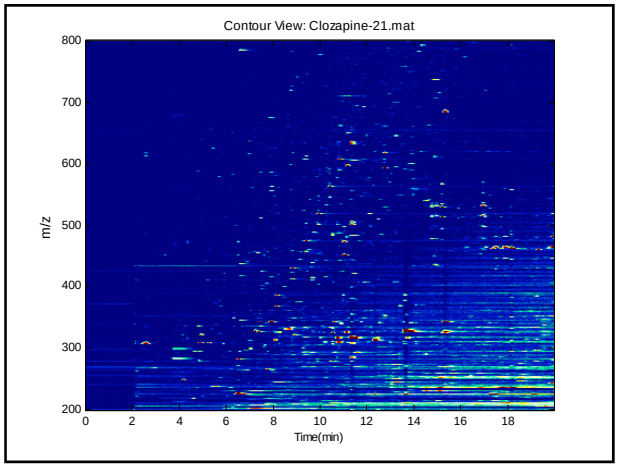
All Modules are Linked and highly Interactive



MPeaks / IPeaks / MsCompare

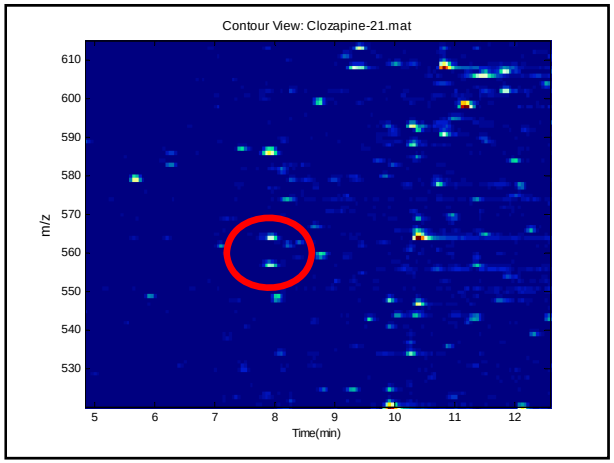
The Browser

MPeaks



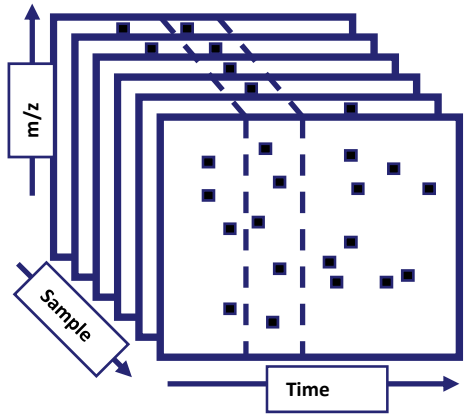
- Detect all (significant) Peaks at any resolution
- Identification
- Differential Analysis: Sample/Control

IPeaks



- Isotope Peak Finding & Quantitation
- predefined labeling patterns, any pattern
- Reactive Metabolite Detection

MS Compare

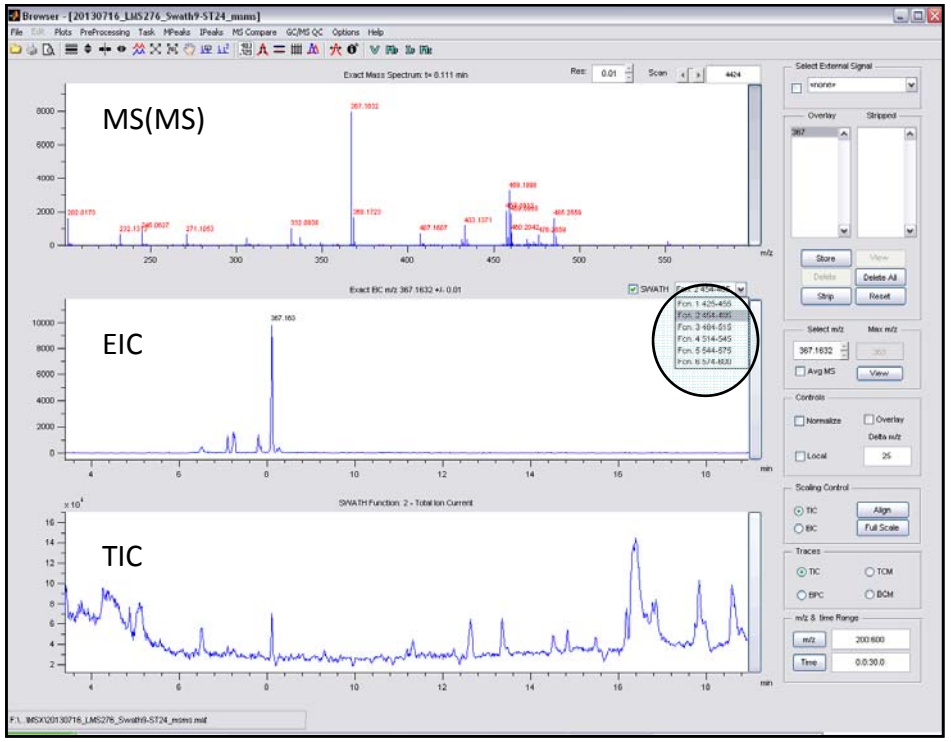


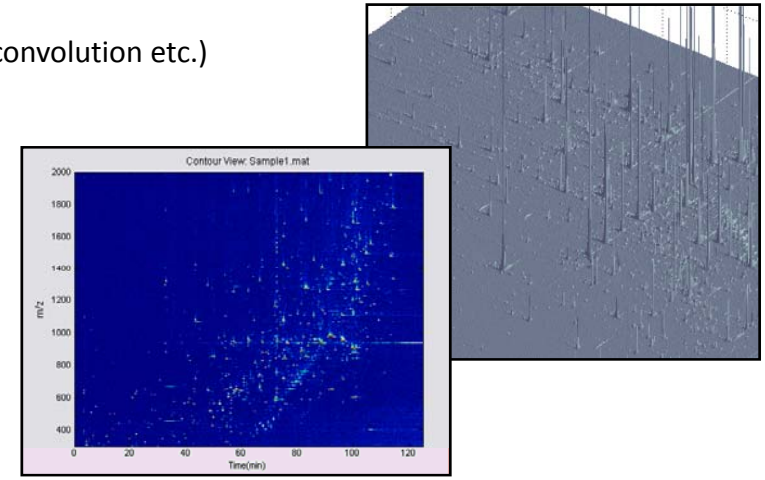
- Handling series of samples
- BioMarkers, Metabolomics
- Chromatographic Alignment
- Statistics, Multivariate Data Analysis

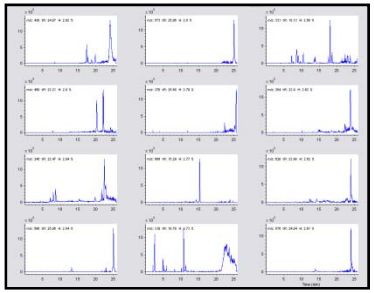
MsMetrix

The Browser: Data Exploration

- Import of All Vendor Data Formats: Thermo, Bruker, Waters, Sciex (SWATH), Agilent, NetCDF, mzXML
- Data Exploration and Data Mining (Noise Levels, Peak Widths, etc.)
- TIC / BPC, MS & MSMS, Extracted Ion Currents, 3D & Contour Plots, Interactive
- Basic Data Pre-Processing Tools (Smoothing, Baseline, MDF, Deconvolution etc.)







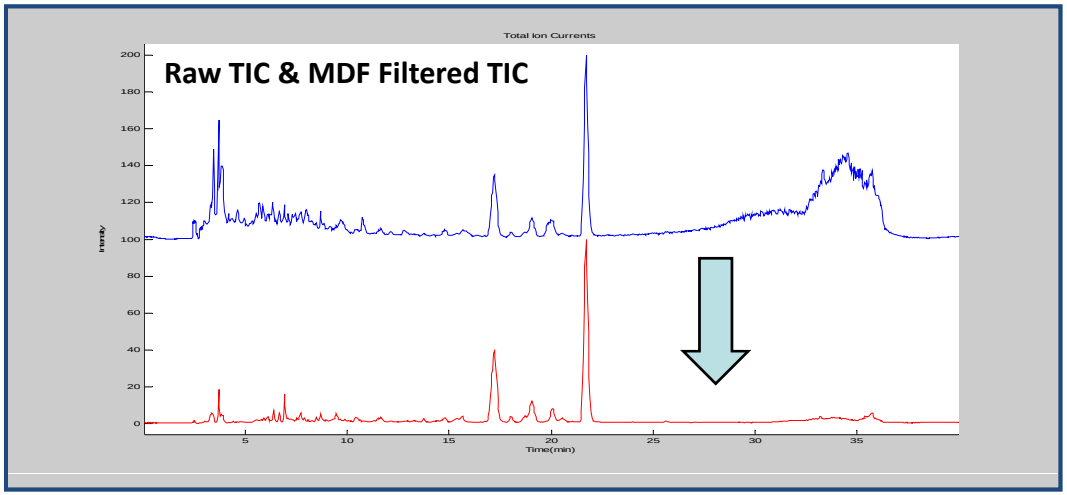
MsMetrix

Browser: Examples of Data Filters

Mass Defect Filtering

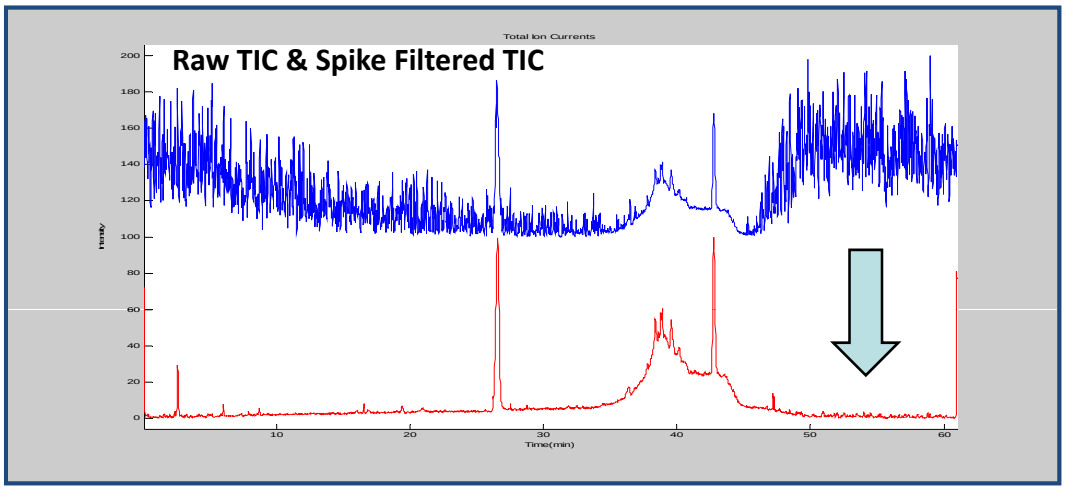
Remove ions outside **Mass Defect Window**: 0.05 Dalton from Parent Ion

E.g. m/z XXX.2356 +/- 0.05 Da



Spike Filtering

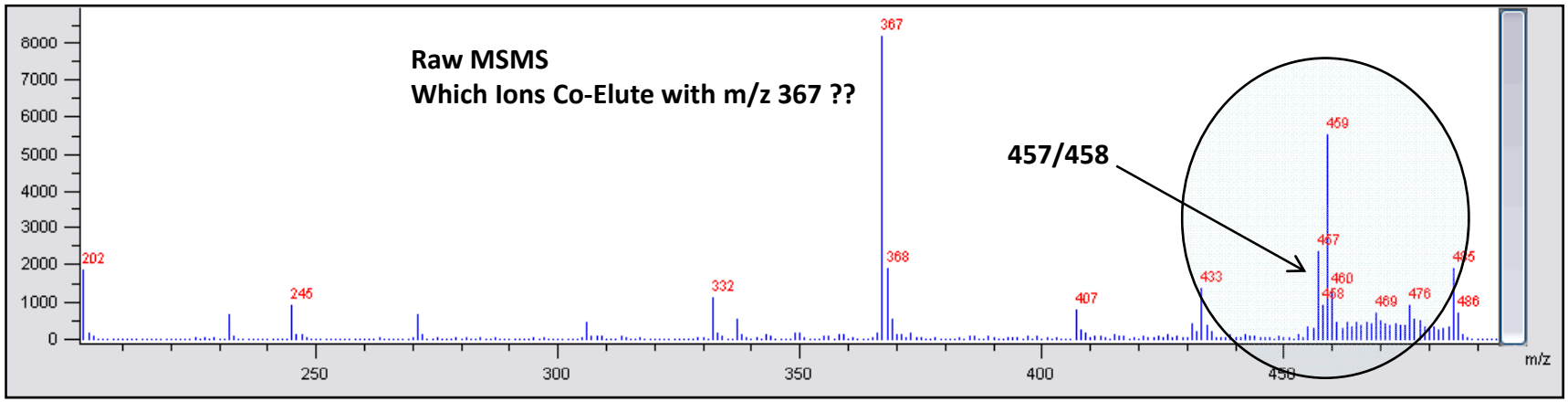
Remove ions < x scans wide

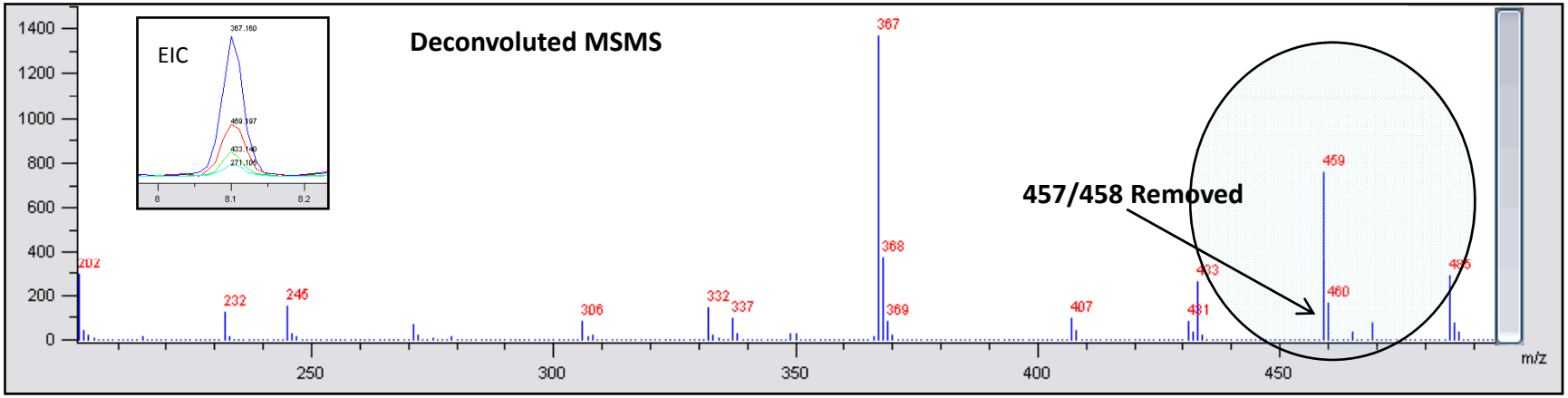


MsMetrix

The Browser: Deconvoluting SWATH MSMS Data

The Browser has functionality to determine which MSMS ions are co-eluting (Deconvolution)

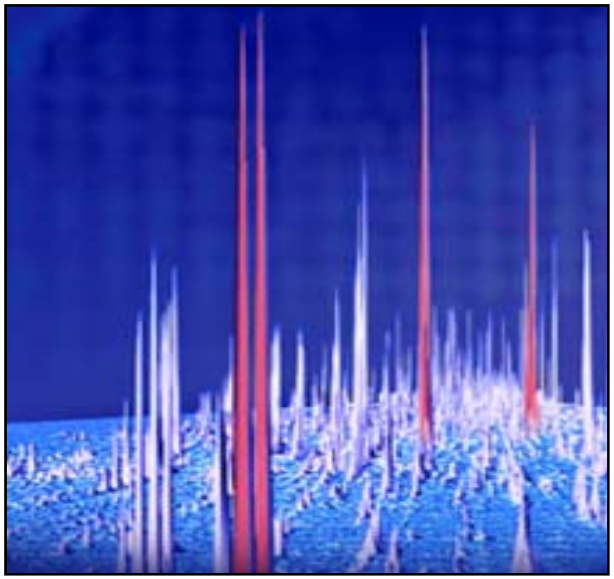


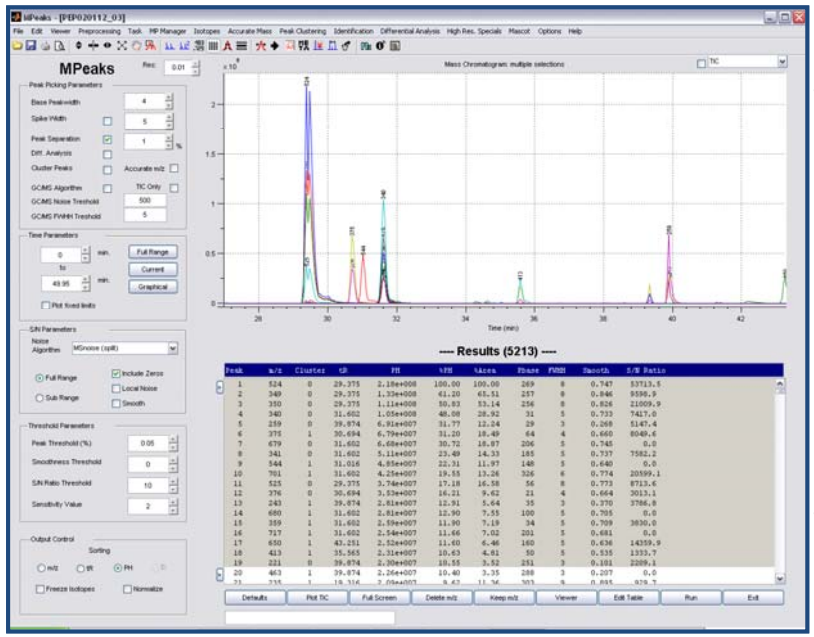


MsMetrix

MPeaks: Peak Picking Features

- ❑ Peak Picking
- ❑ Conversion of Peaks to Components
- ❑ Mono-isotopic Peak Conversion, Charge States
- ❑ Accurate Mass Utilities, Detect Adducts, Fragments
- ❑ **Differential Analysis** at any Resolution
- ❑ Identification of Metabolites and Degradation Products





TIC
EIC
MS

Peak
Picking
Table

Peak	m/z	Cluster	tR	PH	%PH	%Area	Degradation ID MW(534)	
1	535	0	44.73	1981952.0	100.00	100.00	[+0] Parent Molecule	----------
2	557	0	44.72	360256.0	18.18	17.60	[+22] Adduct Na	H -> Na
3	573	1	44.79	7751.0	0.39	0.21	[+38] Adduct K	H -> K
4	573	1	44.53	6528.0	0.33	0.11	[+38] Adduct K	H -> K
5	549	1	50.31	4768.0	0.24	0.15	[+14] Ketone *	CH2 -> C=O
6	533	1	43.92	2253.0	0.11	0.04	[-2] Oxidation	Ring aromatisation
7	533	1	43.58	2030.0	0.10	0.02	[-2] Oxidation	Ring aromatisation
8	489	0	44.65	169600.0	8.56	8.32		
9	579	1	45.29	14846.0	0.75	0.31		

- Remove ^{13}C Isotopes
- Conversion of Peaks to Components
- Find adducts, fragments etc.
- Identify Peaks: Search in MS compound libraries

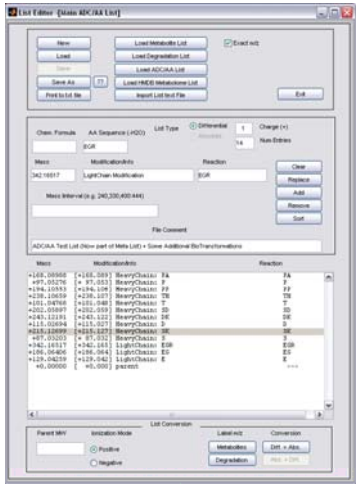
MsMetrix

MPeaks: Identification Tools

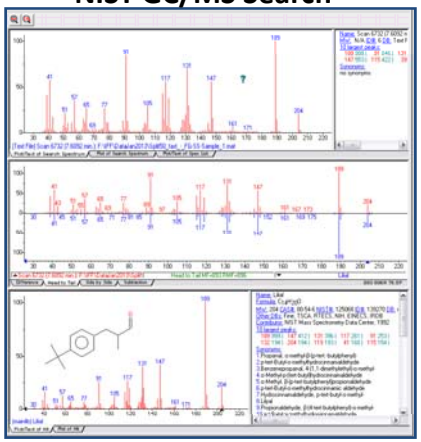
- Direct identification of modifications based on common Biotransformation lists. List of modifications can be easily adapted by user.
- Molecular Formula Determination, including Isotopic Pattern Matching.
- Link Peak Picking Results with Common Search Libraries: Human Metabolite Database, NIST, User Libraries, etc.

Common BioTransformations

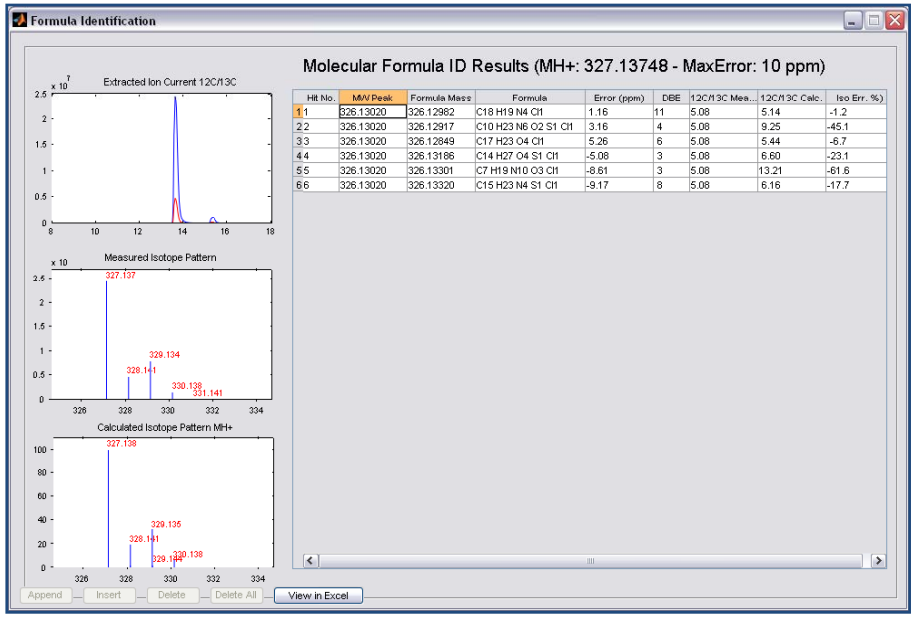
Peak	m/z	Charge	tR	PH	VPH	%Area	PPM	Metabolite ID MW(326.1302)
1	327.13740	1	13.622	24413878.0	68.95	10.00	-0.001	ppm [+0.000] parent
2	313.12180	1	12.327	6185452.5	17.47	10.00	-0.276	ppm [-14.016] demethylation
3	343.13226	1	15.291	5735514.5	16.20	10.00	-0.346	ppm [+15.995] oxidation
4	343.13239	1	7.904	3168463.0	8.95	10.00	0.009	ppm [+15.995] oxidation
5	632.20599	1	11.336	3021142.5	8.53	10.00	0.809	ppm [+305.068] glutathion conjugation
6	309.17130	1	4.871	1914943.3	5.41	10.00	-0.281	ppm [-17.966] oxidative dechlorination
7	503.16342	1	11.336	930848.9	2.65	10.00	-12.044	ppm [+176.032] glucuronidation
8	343.13223	1	10.593	746256.2	2.11	10.00	-0.435	ppm [+15.995] oxidation
9	506.16690	1	11.336	739748.9	2.09	10.00	8.730	ppm [+179.025] oxidation+acetylcysteination
10	329.11670	1	7.242	700395.5	1.98	10.00	-0.252	ppm [+1.979] oxidation + desethylation



NIST GC/MS Search



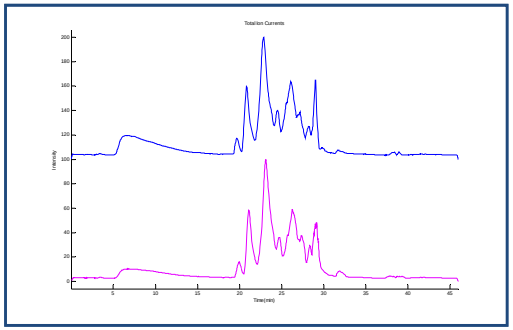
Molecular Formula Finder

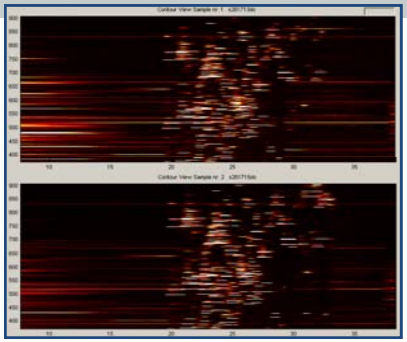


Hit No.	MW Peak	Formula Mass	Formula	Error (ppm)	DBE	12C/13C Mea...	12C/13C Calc.	Iso Err. %)
1 1	326.13020	326.12982	C18 H19 N4 Cl1	1.16	11	5.08	5.14	-1.2
2 2	326.13020	326.12917	C10 H23 N6 O2 S1 Cl1	3.16	4	5.08	9.25	-45.1
3 3	326.13020	326.12849	C17 H23 O4 Cl1	5.26	6	5.08	5.44	-6.7
4 4	326.13020	326.13186	C14 H27 O4 S1 Cl1	-5.08	3	5.08	6.60	-23.1
5 5	326.13020	326.13301	C7 H19 N10 O3 Cl1	-8.61	3	5.08	13.21	-61.6
6 6	326.13020	326.13320	C15 H23 N4 S1 Cl1	-9.17	8	5.08	6.16	-17.7

MPeaks: Differential Analysis – Sample/Control

FT-MS Data Example





Contour Plots

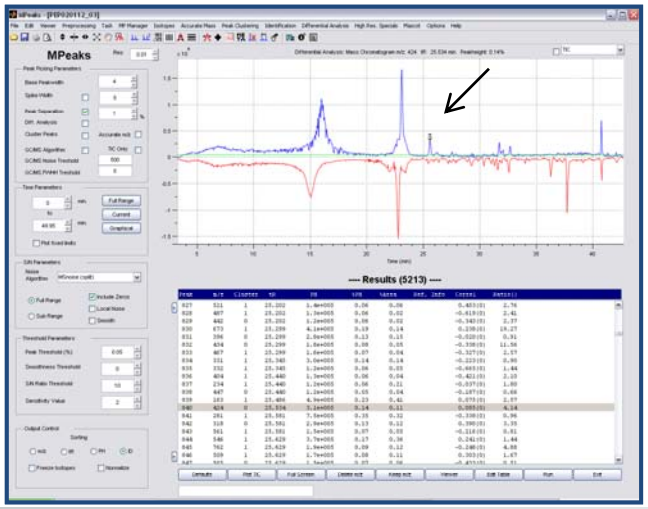
A **B**

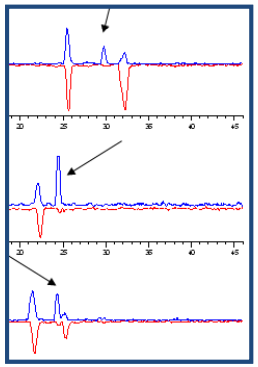
205	10.006	1161465869
206	10.006	277647129
207	10.006	108313685
241	10.376	391333360
243	10.376	127755738
266	14.445	80702799
266	14.664	102321396
267	14.445	57285816.5
267	14.664	97020320
297	15.471	993419167
298	15.471	209938883
299	15.471	790735740
300	12.377	644146385
300	15.454	134304249
301	12.377	97124140.5

205	10.006	1161465869
206	10.006	277647129
207	10.006	108313685
241	10.376	391333360
243	10.376	127755738
266	14.445	80702799
266	14.664	102321396
267	14.445	57285816.5
267	14.664	97020320
297	15.471	993419167
298	15.471	209938883
299	15.471	790735740
300	12.377	644146385
300	15.454	134304249
301	12.377	97124140.5

Peak Picking Tables

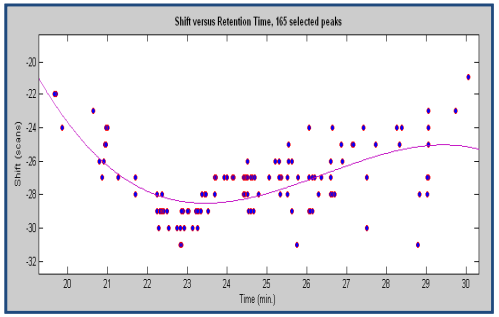
Differential Peak Table





Differential Peak Plots

Correlation Based
Local Alignment Correction

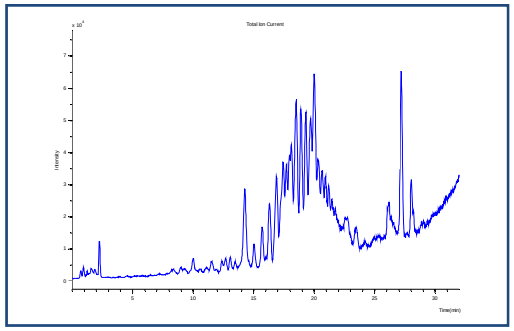


MsMetrix

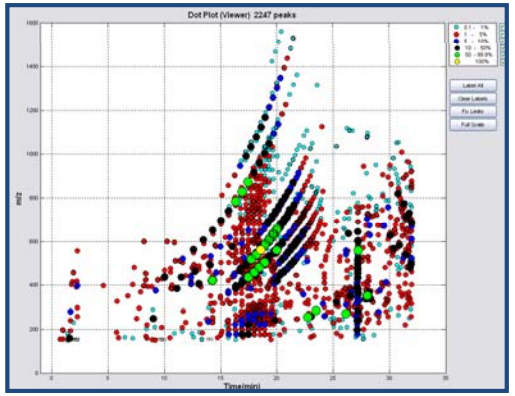
IPeaks: Isotope Pattern Matching

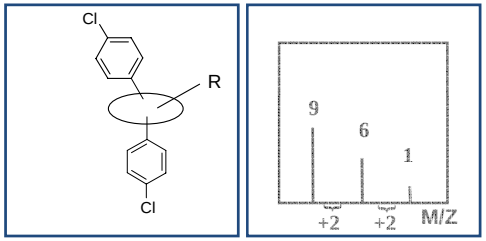
- Detect Peaks in Data File having a specific isotopic Pattern
- Predefined labeling patterns: ^{13}C, $^{12}C/^{14}C$, Cl, Br, $^{16}O/^{18}O$, SILAC, $^{14}N/^{15}N$, GSH, KCN
- Run at any Resolution – Combine with Differential Analysis and other Filters
- Create your own user pattern
- GSH Reactive Metabolites Search (labeling) combined with MSMS Neutral Loss Search

PEG
Contamination

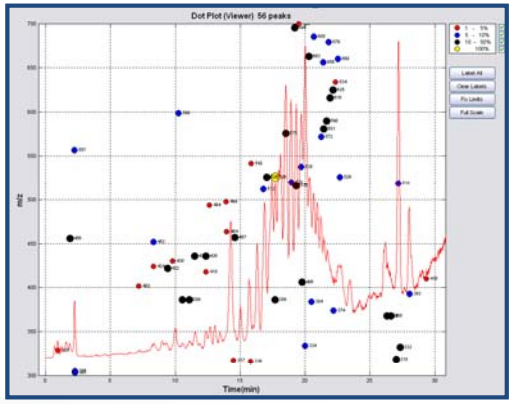


MPeaks: → 2553 Peaks (4 sec.)





IPeaks: → 56 Peaks (20 sec.)

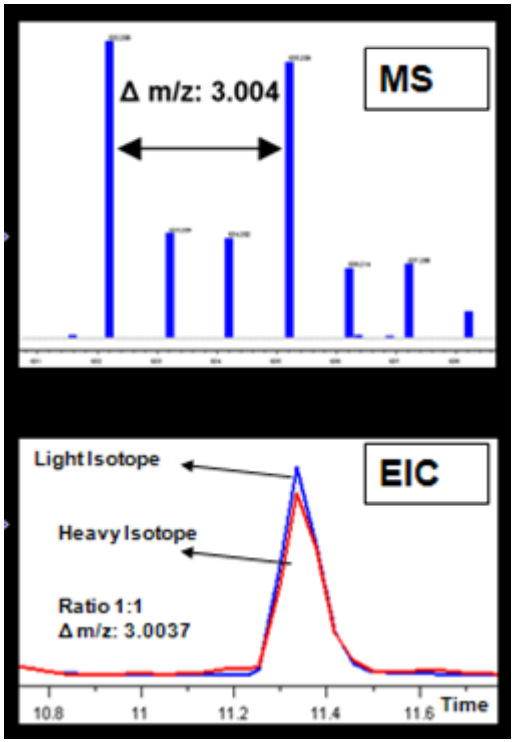


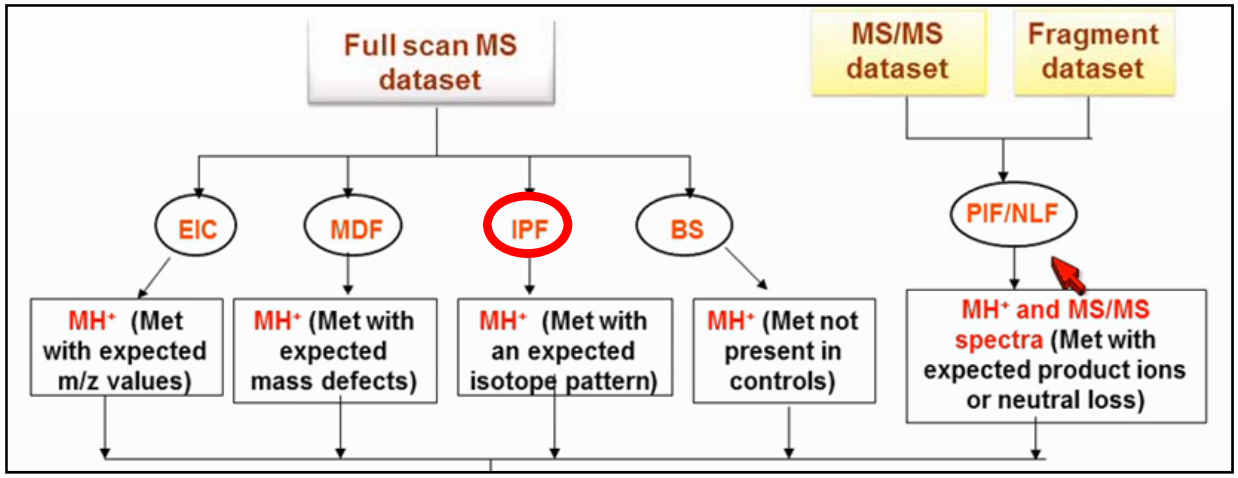
MsMetrix

IPeaks: Reactive Metabolite Detection using IPF II

Detect GSH Adducts using Labeled and non-labeled GSH as Trapping Agent (HRMS)

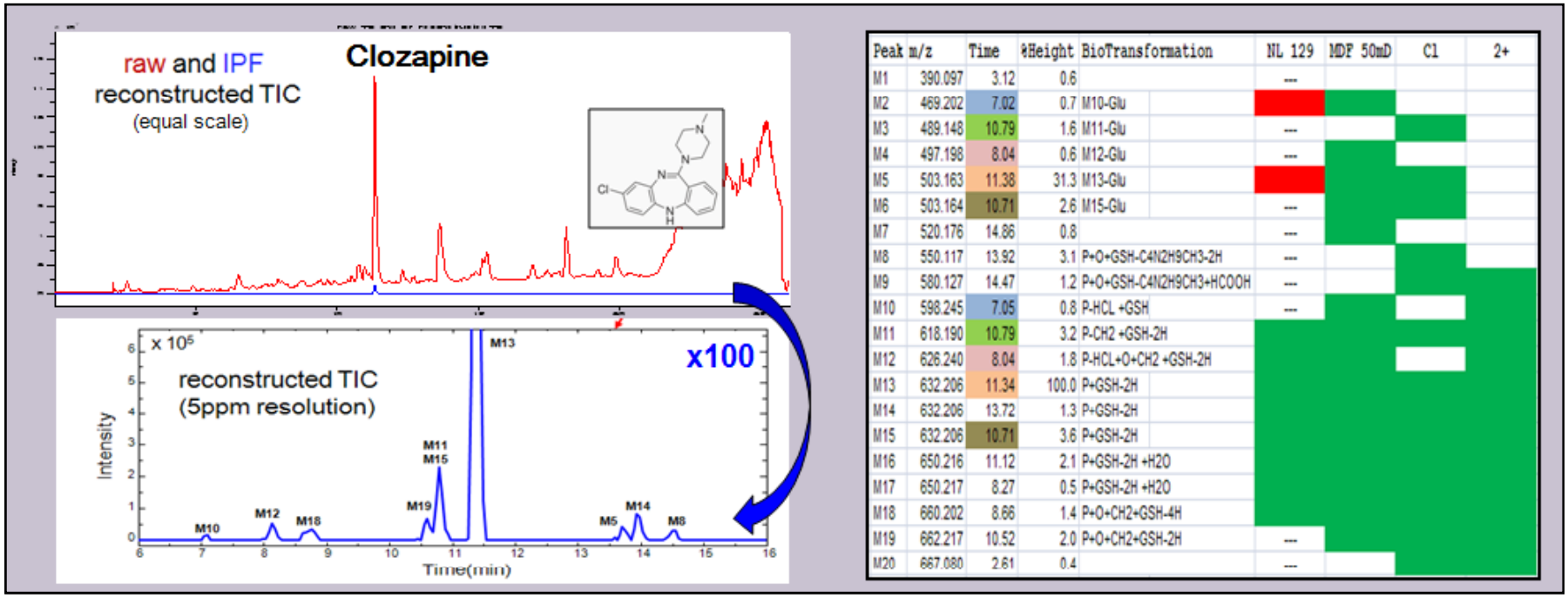
1. Labeled/non-Labeled Ratio 1:1
2. Mass Difference 3.0037 +/- 0.005
3. Light and Heavy EIC should co-elute
4. Additional Checks: Sample/Control Check (BS)
   Mass Defect Filter Check
   Remove ^{13}C isotopes and adducts
   Presence of ^{13}C or e.g. Chlorine
   Neutral Loss Check -129





MsMetrix

IPeaks: Reactive Metabolite Profiling - Clozapine

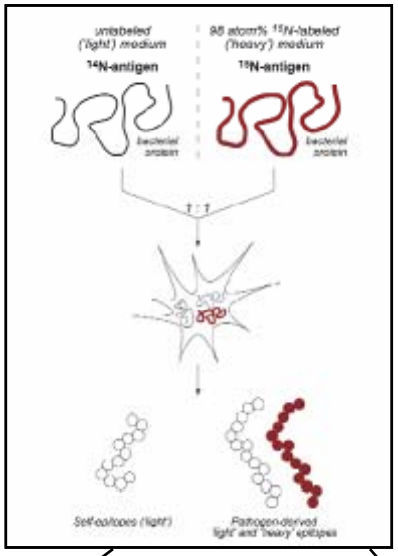


Peak	m/z	Time	%Height	BioTransformation	NL 129	MDF 50mD	Cl	2+
M1	390.097	3.12	0.6		---			
M2	469.202	7.02	0.7	M10-Glu				
M3	489.148	10.79	1.6	M11-Glu	---			
M4	497.198	8.04	0.6	M12-Glu	---			
M5	503.163	11.38	31.3	M13-Glu				
M6	503.164	10.71	2.6	M15-Glu	---			
M7	520.176	14.86	0.8		---			
M8	550.117	13.92	3.1	P+O+GSH-C4N2H9CH3-2H	---			
M9	580.127	14.47	1.2	P+O+GSH-C4N2H9CH3+HCOOH	---			
M10	598.245	7.05	0.8	P-HCL +GSH	---			
M11	618.190	10.79	3.2	P-CH2 +GSH-2H				
M12	626.240	8.04	1.8	P-HCL+O+CH2 +GSH-2H				
M13	632.206	11.34	100.0	P+GSH-2H				
M14	632.206	13.72	1.3	P+GSH-2H				
M15	632.206	10.71	3.6	P+GSH-2H				
M16	650.216	11.12	2.1	P+GSH-2H +H2O				
M17	650.217	8.27	0.5	P+GSH-2H +H2O				
M18	660.202	8.66	1.4	P+O+CH2+GSH-4H				
M19	662.217	10.52	2.0	P+O+CH2+GSH-2H	---			
M20	667.080	2.61	0.4		---			

1. Any Isotopic Pattern: GSH, KCN, MOA, Chlorine, $^{12}C/^{14}C$
2. Fast: < 1 minute, including High Resolution Post Processing
3. Sensitive: all ions checked
4. Interactive Graphics (MS/EIC) directly from Table
5. Hits confirmed by: Neutral Loss, Cl, MDF and 2+ ions

MsMetrix

IPeaks: Stable Isotope Tagging of Epitopes



^{14}N / ^{15}N Labeling Rules:

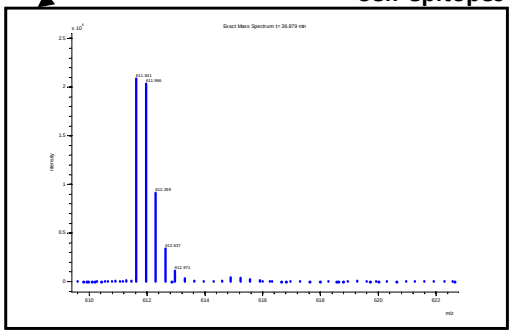
1. **Ratio 1:1**
2. **Δ m/z ~ 1.2%**
3. **98 atom% ^{15}N labeled medium**

Published in: Current Protocols in Immunology

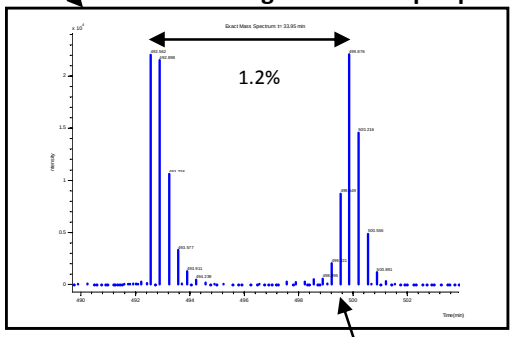
Averagine
$C_{4.94}\ H_{7.76}\ N_{1.36}\ O_{1.48}\ S_{0.042}$

SCX fractionation (26 fractions)

Self-epitopes



Patogen-derived epitopes

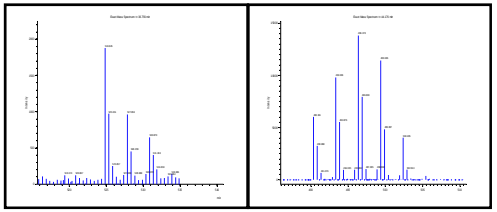


98 atom% labeling

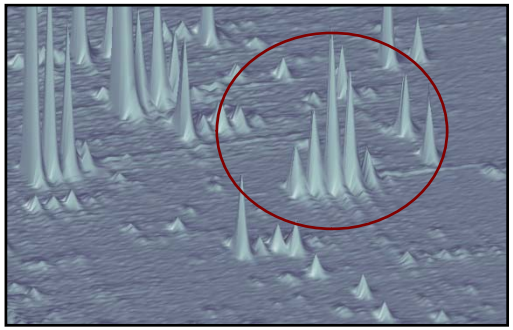
Binomial Pattern Search

Self-Peptide

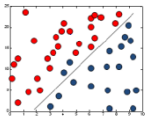
Infection associated Self-Peptide



Binomial Pattern Matching



MsMetrix

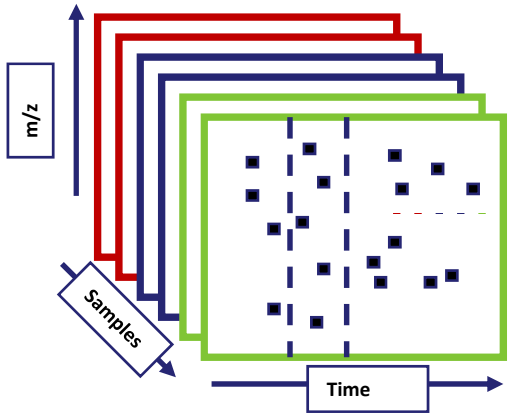


MsCompare: Metabolomics & BioMarker Discovery

MsCompare Features:

- ❑ Comparison and Statistical Analysis of series of Samples (LC/MS or GC/MS)
- ❑ Alignment: Cross Correlation, COW, **Reference Peak Warping**, Manual Shift Correction
- ❑ Pre-Processing: Smoothing, Normalization etc.
- ❑ Peak Matching and Peak Picking for all Samples: Low & High Resolution
- ❑ Visualization/Comparison techniques to view differences (EIC/MS)
- ❑ Univariate Data Analysis: Find unique or Differential Peaks, Time Series Analysis
- ❑ Multivariate Data Analysis: PCA, Clustering, Correlation Maps, Regression & Discriminant Analysis (PLS-DA), ECVA
- ❑ Combines Univariate and Multivariate Data Analysis

Stay in Touch with Your Data !!



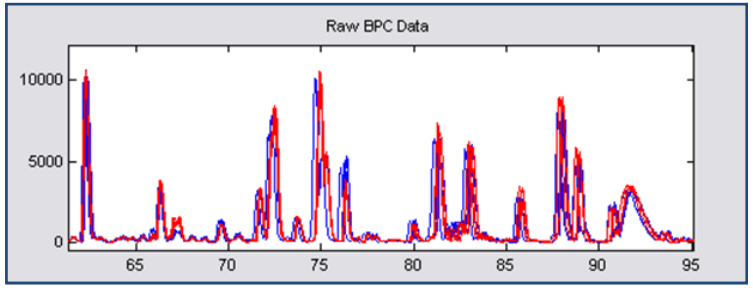
MsCompare Applications:

- Compare Impurity Profiles of Batches
- BioMarker Discovery – detect unique features
- Metabolomics – find differences between groups of samples
- Drug Metabolite Profiling – Species Comparison
- Targeted Quantitative Analysis on many Samples: Trending

MsMetrix

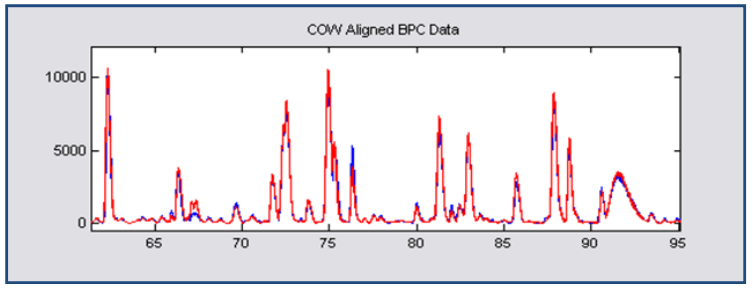
MsCompare – Chromatographic Alignment

Correlation Optimized Warping



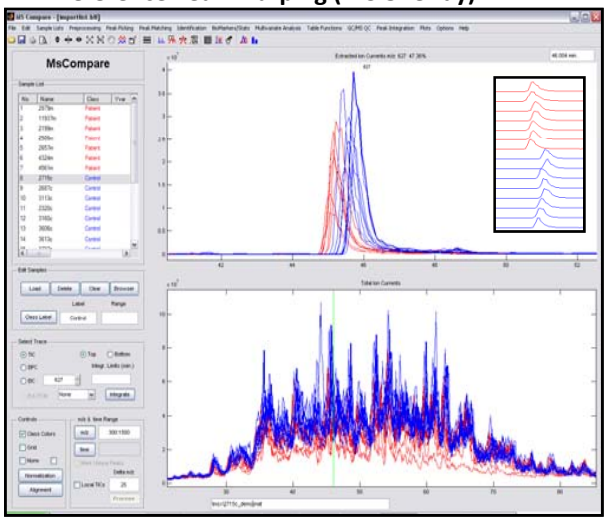
COW

COW Aligned BPC Traces

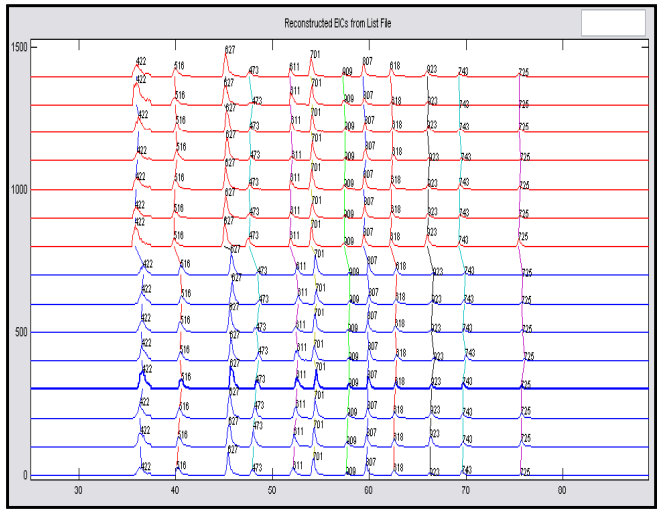


RPW

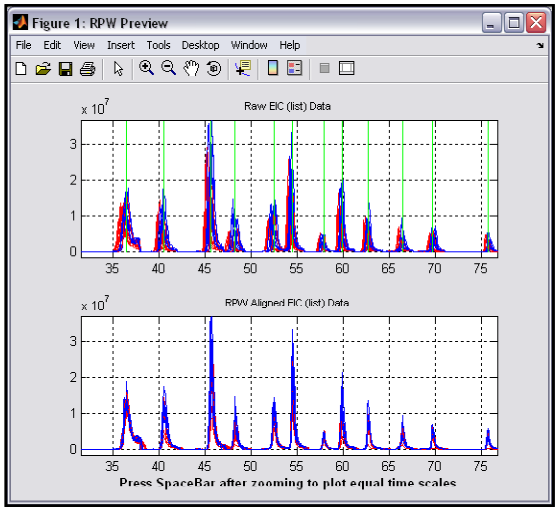
Reference Peak Warping (TIC Overlay)



Auto Reconstructed TICs based on 12 Reference Peaks



RPW: Before and after Alignment



MsMetrix

MsCompare - BioMarker Discovery Example

MS Compare

- **Compare Multiple Samples Interactively**
- **Click & Identify to view EIC/MS for all Samples**

Peak Picking vs. Peak Matching

- Peak Picking: 1. Detect Peaks in each sample individually
  2. Cluster all Peaks for all Samples
- Peak Matching: Use Target List of Ions (m/z, time) to perform HR Peak Picking in all Samples

Multivariate Analysis: Unsupervised Pattern Recognition

- Principal Component Analysis to detect Groups & Outliers (Dominant Features, often fails for LC & GC/MS)

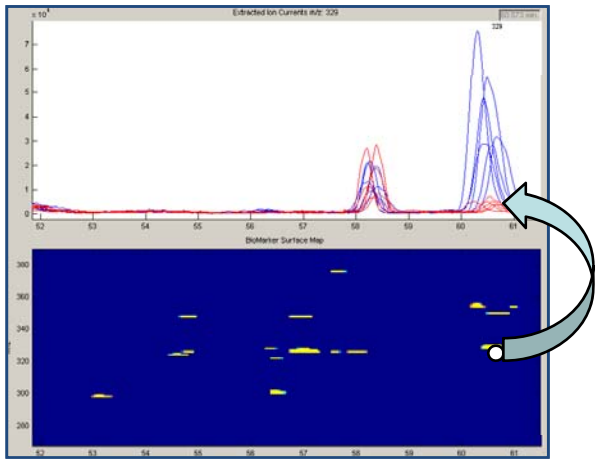
Multivariate Analysis: Supervised Pattern Recognition

- PLS-DA, ECVA, Use Class Information to separate Groups

Univariate Analysis

- Combine Univariate Statistics to find discriminating peaks: Class Ratio's, Fisher Discriminant Scores, Uniqueness Value T-test, P-test etc.

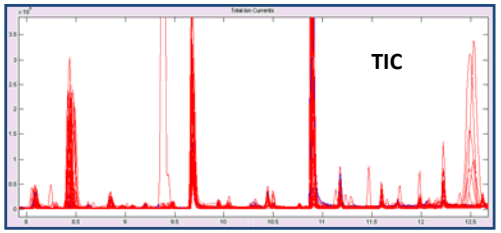
Detect Unique Class Discriminating Peaks

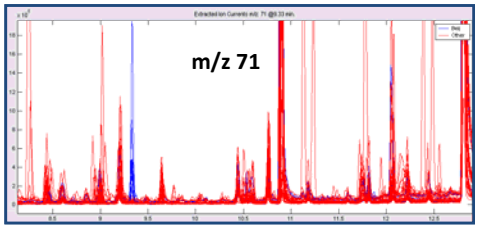


BioMarker Surface Plot

Bacteria Classification (50 Samples – 2 Groups) (6/44)

Multi. Analysis X
Uni. Analysis OK





MsMetrix

MsCompare - High Resolution Targeted Screening

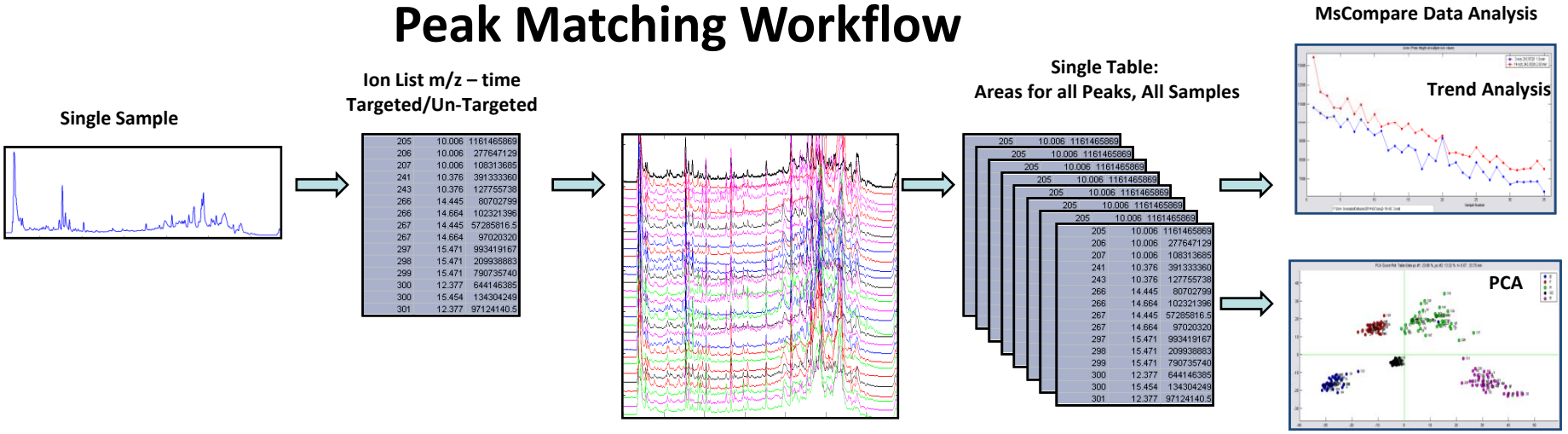
MS Compare

Run Peak Matching on Large Number of Samples using (Targeted) List of Compounds

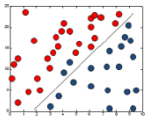
Input for MsCompare:

1. MPeaks Result File - Accurate Mass, tR
2. User Created List File – Accurate Mass, tR, Compound Info
3. Excel List file – Accurate Mass, tR, Compound Info
4. Chemical Formulas

Peak Matching Workflow



MsMetrix

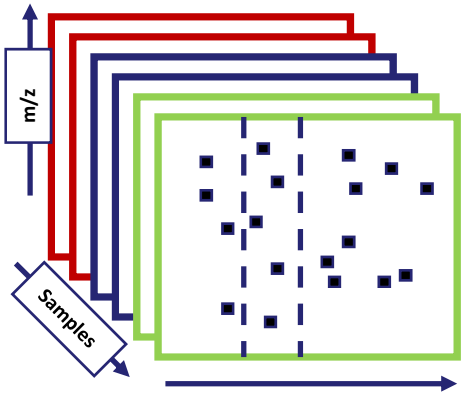


MsCompare – Metabolomics Example

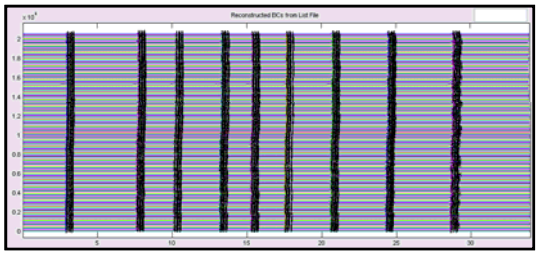
MsCompare Workflow

- Alignment
- Peak Picking, Normalization
- Multivariate Analysis :PCA
- Univariate Analysis;
  Find Class Separating Peaks
- Time Profiles

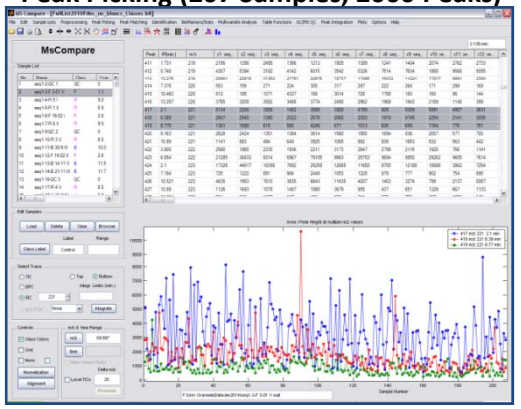
Sample Pooling → QC Sample



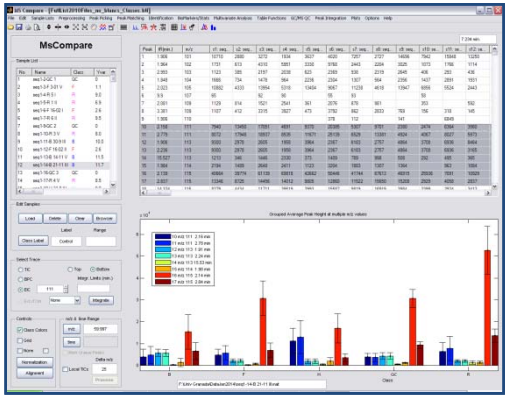
Alignment: RPW

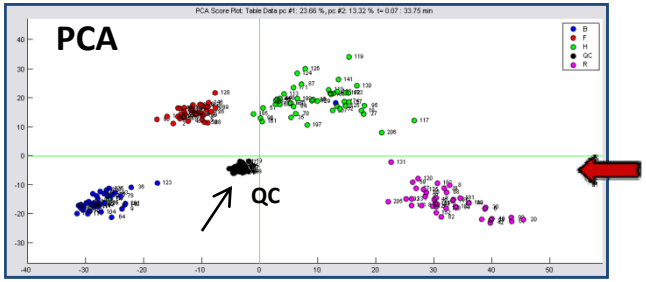


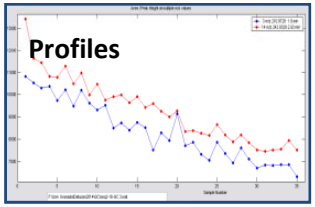
Peak Picking (207 Samples, 2000 Peaks)



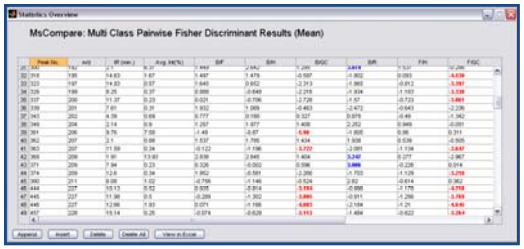
Statistical Plots







Up or Down Regulated Peaks (5 Groups)



MsXelerator: Custom Applications

MsXelerator: **Continuously add new (small) Applications**

Big(ger) Projects in co-operation with Customer

New Project are always added to Software

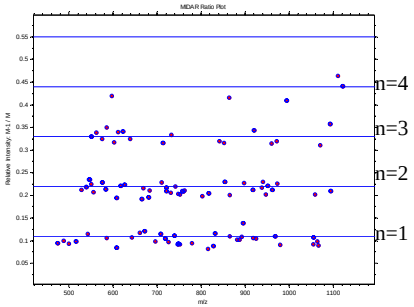
Examples:

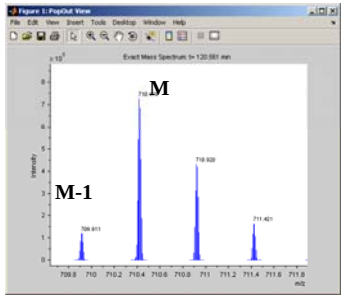
- ❑ Quantitative Proteomics
- ❑ GC/MS Quality Control and Quantitation in Metabolomics
- ❑ MIDAR: Relative Quantitation
- ❑ Import Metabolite Prediction Results from third Party Software
- ❑ Metabolite Deconvolution Radio Signal and MS
- ❑ GC/MS Product Control

Software Customization: Small Applications

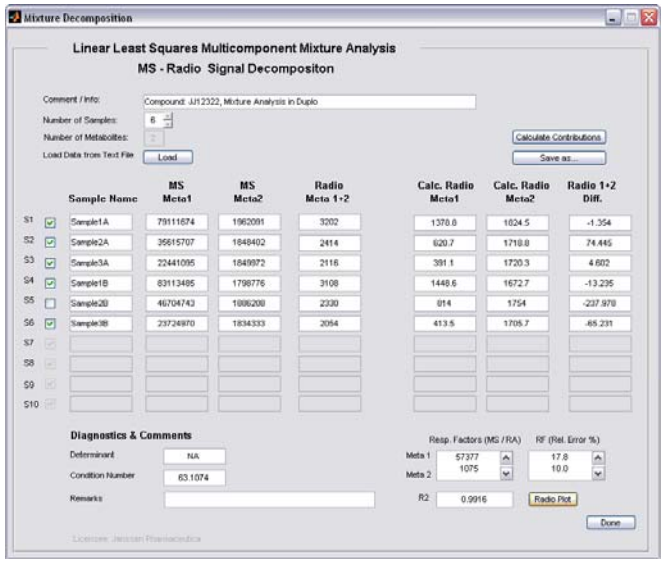
MIDAR
Identification based on Ratio Analysis of Labeled Peptides

Ratio (M-1)/M

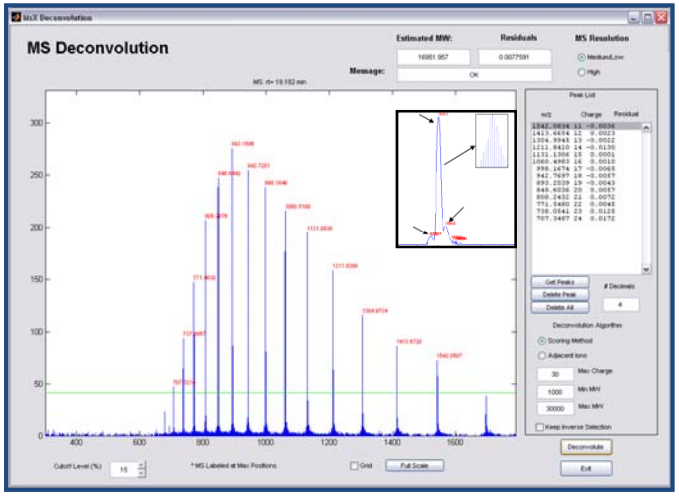




MS-Radio: 2-Component Mixture Analysis: Deconvolute Overlapping Radio Signal based on MS Signal



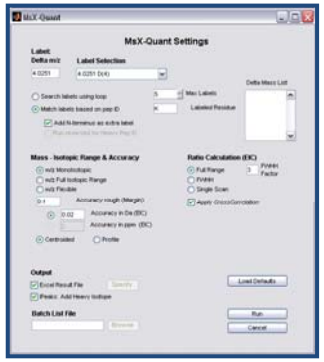
MS Multi Charged Protein Deconvolution

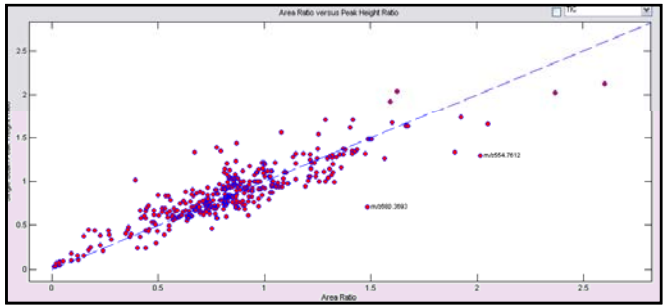


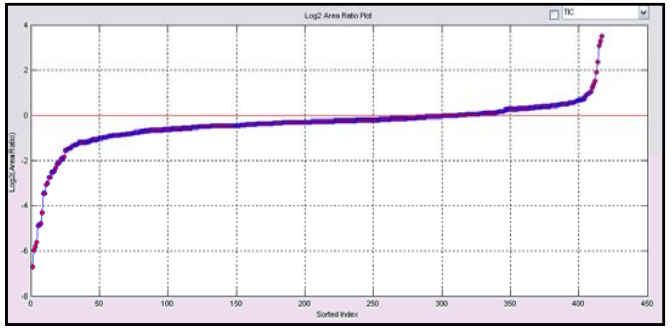
MsMetrix

MsX-Quant: High Resolution Isotope Search / Quant

Quantitative Proteomics:





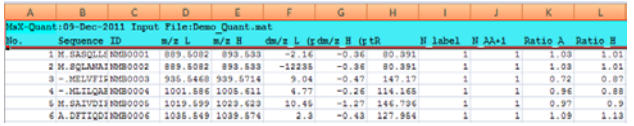


Input Files: Mascot Result Files
Peak Picking Tables from Mpeaks or IPeaks
Excel File, m/z, charge, sequences, ID
. . . . .

Labeling: Define any kind of labeling pattern
Number of labeled residues determined from sequence
Number of labeled residues determined in loop
$^{14}N/^{15}N$ Metabolic Labeling

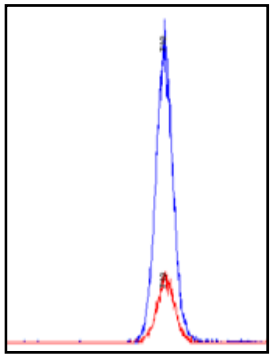
Features: Quantify or Search Peaks with specific Label
Filter Mascot Results (Score threshold etc.)
Operate on deconvoluted MS files
Accurate Peak Area (volume) and Peak Height
Error statistics: co-elution check and visualization of EIC and MS
Easy detection of Singles

Output: IPeaks, Excel, text files

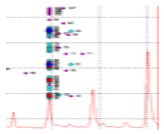


MsX-Quant:09-Dec-2011 Input File:Demo_Quant.mat

No.	Sequence	ID	m/z L	m/z H	dm/z L (p	dm/z H (pt	R	N label	N AA+1	Ratio A	Ratio H
1	M.SASQLLS	NMB0001	889.5082	893.533	-2.16	-0.36	80.391	1	1	1.03	1.01
2	M.SQLANAI	NMB0002	889.5082	893.533	-12235	-0.36	80.391	1	1	1.03	1.01
3	-.MELVFI	NMB0003	935.5468	939.5714	9.04	-0.47	147.17	1	1	0.72	0.87
4	-.MLILQA	NMB0004	1001.586	1005.611	4.77	-0.26	114.168	1	1	0.96	0.88
5	M.SAIVDI	NMB0005	1019.599	1023.623	19.45	-1.27	146.736	1	1	0.97	0.9
6	A.DFTIQD	NMB0006	1035.549	1039.574	2.3	-0.43	127.954	1	1	1.09	1.13



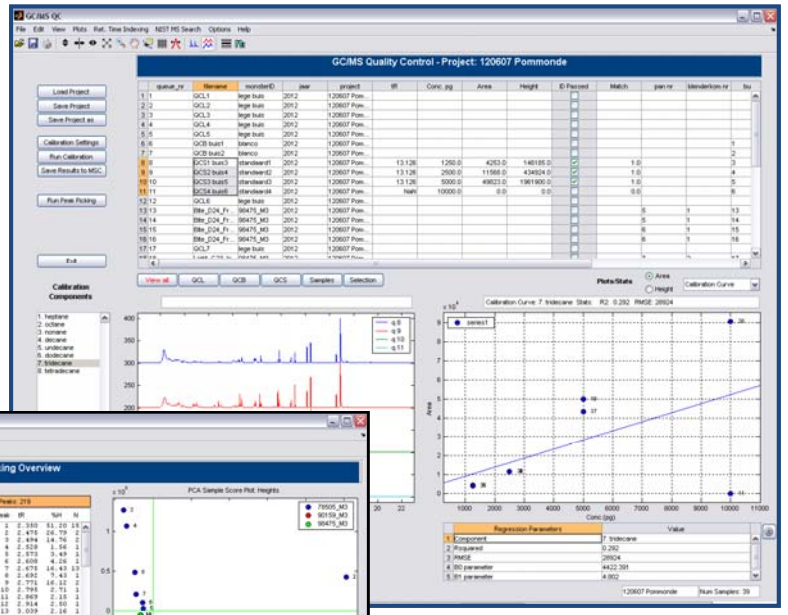
MsMetrix

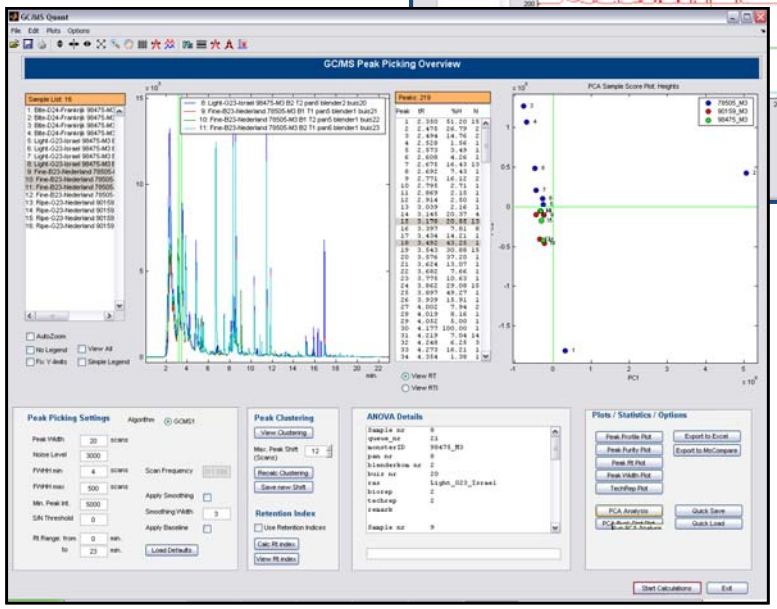


GCMS QC / Quant for Metabolomics

GC/MS Quality Control:

- ANOVA Workflow
- Blanc Signal Check
- Optimized GC/MS Peak Picking
- Calibration Curves/Statistics
- PCA Calibration Results
- Check Stability within Series
- Check Stability between Series
- Retention Time Index Conversion
- NIST MS Search





GC/MS Data Processing:

- Peak Picking & Deconvolution
- Alignment of chromatograms
- Univariate Analysis
- Multivariate Analysis
- PCA, combined with ANOVA
- PCA Distance & Influence Plots
- Peak Purity Analysis

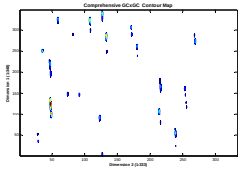
MsMetrix

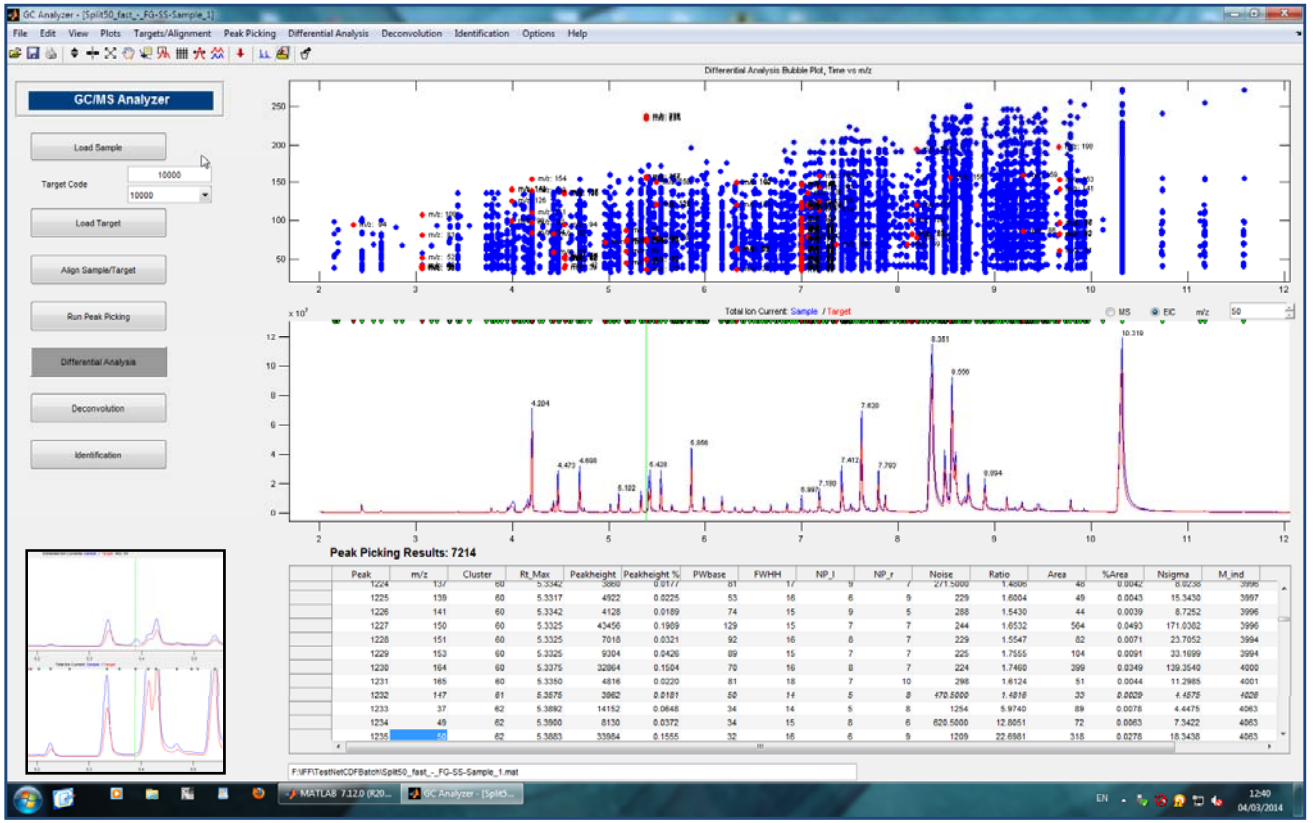
GC/MS Product Control: Abnormality Detection

GC/MS Product Control

- QC Lab Application
- 1000+ Complex Products
- Compare Sample / Target
- Detect Small Differences (+ -)
- Automated GC/MS Deconvolution
- Automated Identification NIST
- Link with Company Database
- Different User Levels

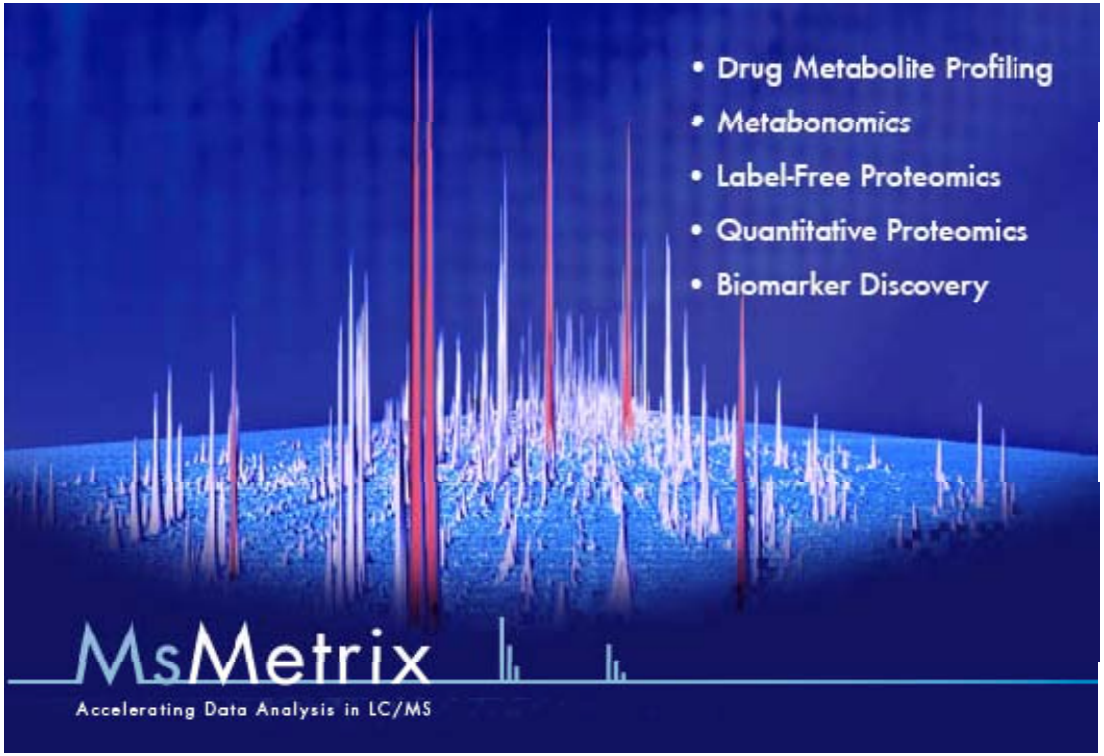
- Comprehensive GCxGCxMS





MsMetrix

MsXelerator for Windows



www.MsMetrix.com