



Agilent's NEW MassHunter Profinder

The Most Advanced Batch Feature
Extraction Software for Metabolomics

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MassHunter Profinder

A Batch Feature Extraction and Review Tool

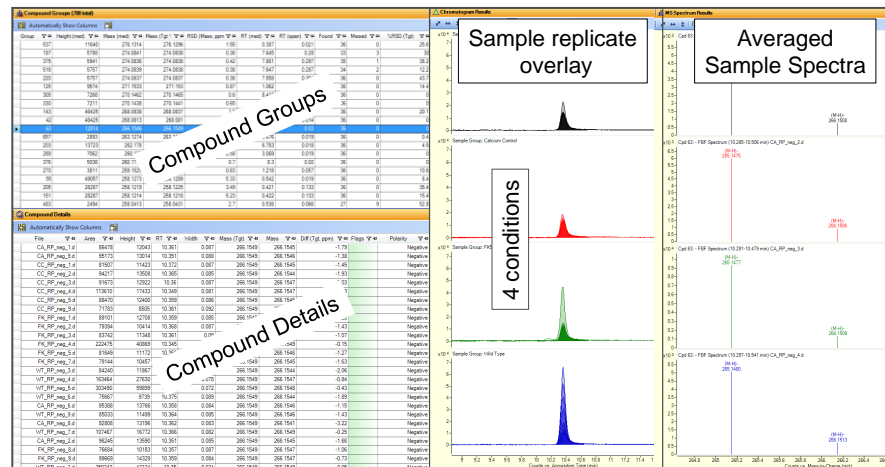


FREE

INTRODUCING THE NEW MASSHUNTER PROFINDER SOFTWARE FOR ACCURATE MASS LC/MS

- Only commercially available batch processing tool
- Fast and robust feature extraction from TOF/Q-TOF raw data
- Optimized manual re-integration for single or batch files

The Measure of Confidence



Which Metabolomics Customers can benefit from using MassHunter Profinder?

Biological Research

- Basic, clinical Research
- Drug Development

Food

- Food process development
- Food quality/ adulterants
- Nutrition

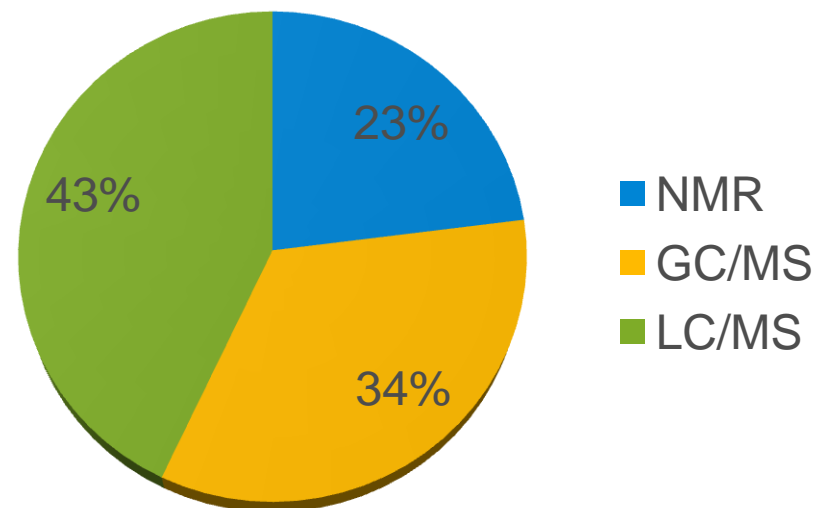
Ag Chem

- Crop development
- Pesticide / herbicide development

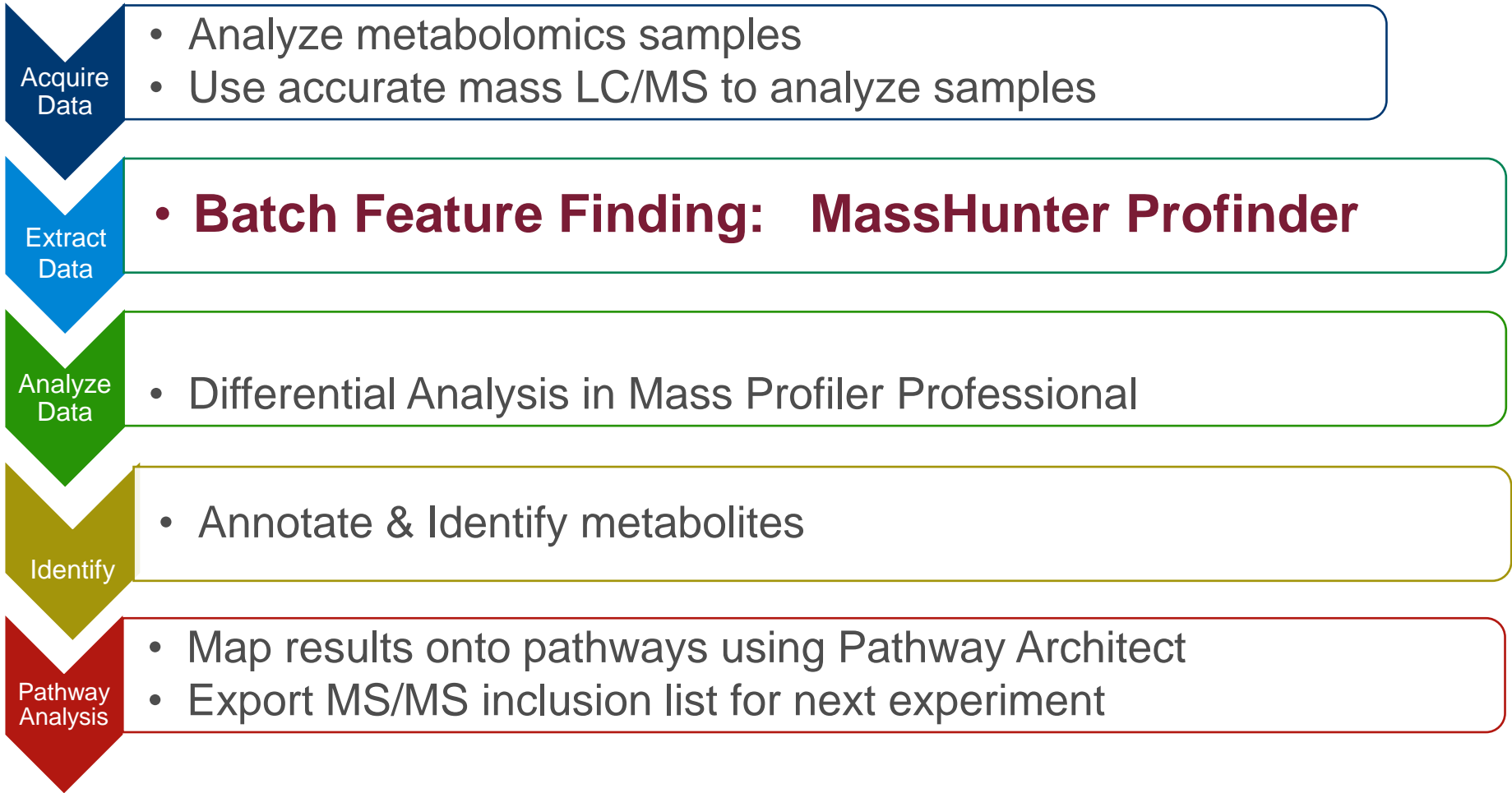
Synthetic Biology

- Biofuels

Toxicology

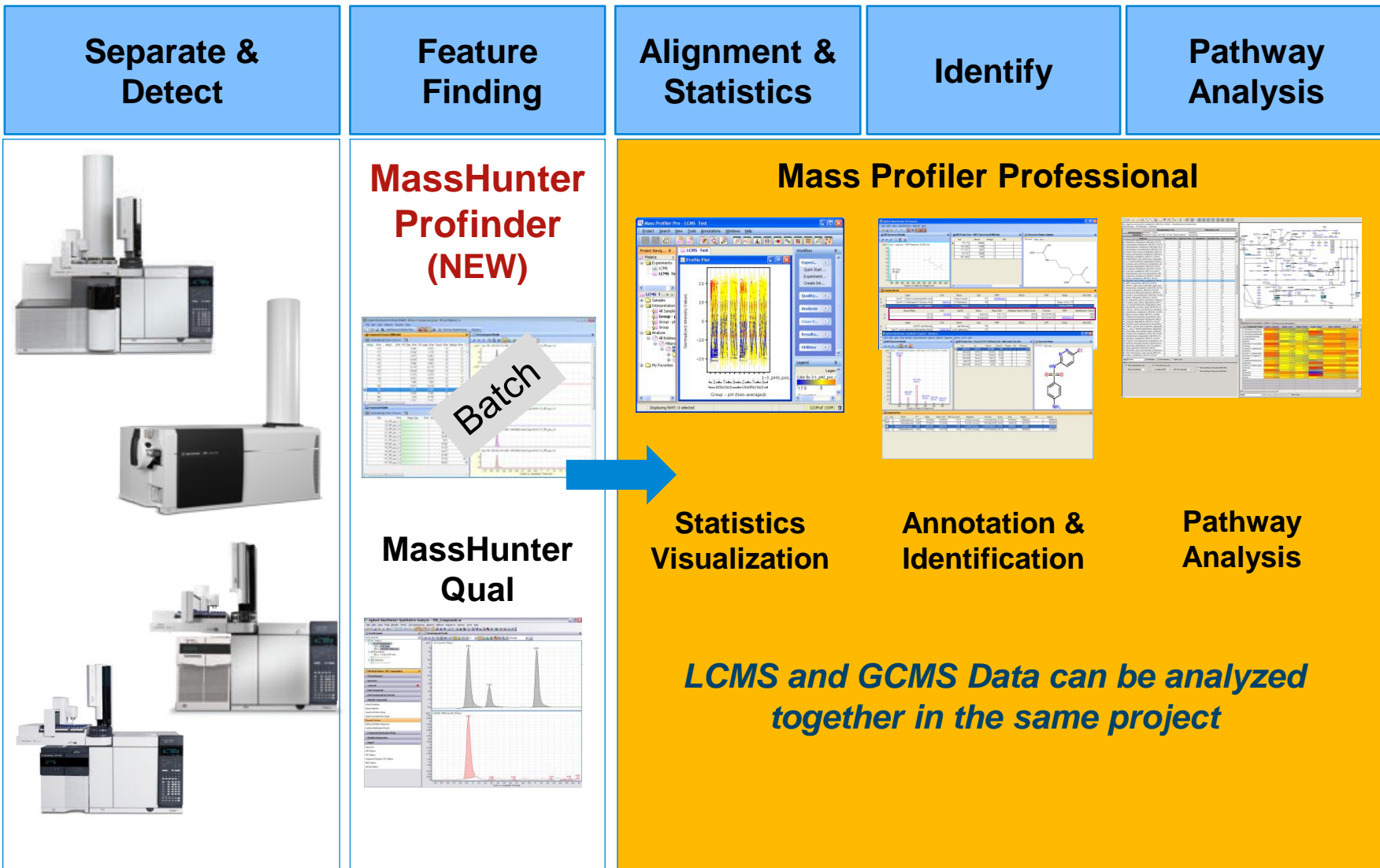


Metabolomics Workflow



Agilent's *Comprehensive* Metabolomics Solution

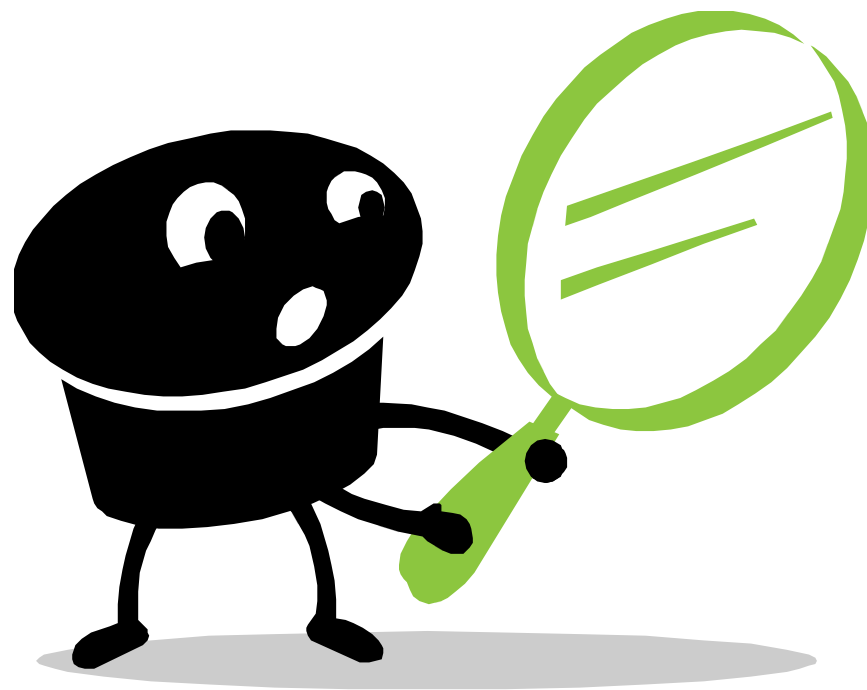
Agilent LCMS and GCMS



Customer Goal: Find and correctly extract all chromatographic peaks in a sample

Challenges:

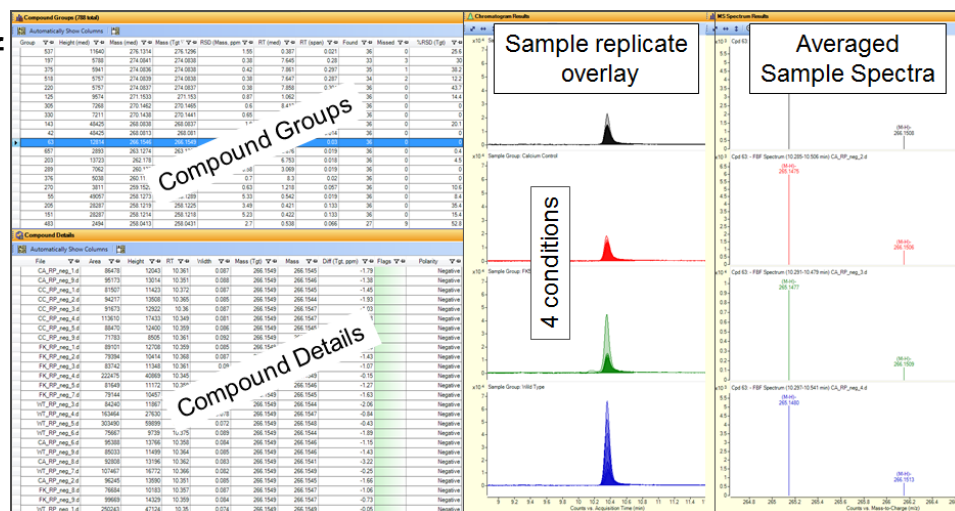
- Incomplete peak separation
- Unresolved peaks contribute to an increase in:
 - False peak detection, excessive missing values, incorrect identifications
 - Misdirected and wasted efforts
 - Missed or inaccurate biomarkers
 - Decreased productivity



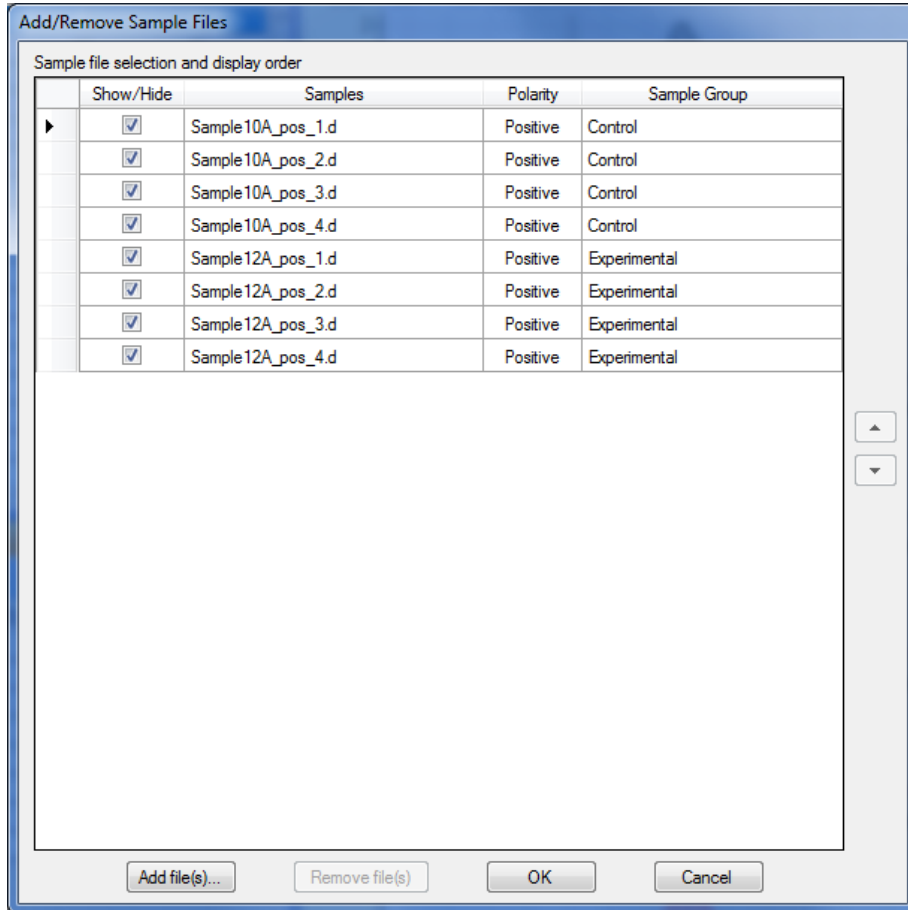
MassHunter Profinder Workflow solution: So what's new?

- A one-shot process for untargeted and targeted feature extraction
- Designed specifically for the needs of the metabolomics user
- Processes many samples!
- Recursive analysis
- Compound Group Centric: new manual review and editing functionalities
- Major reduction in processing time
- It's FREE!!

MassHunter Profinder

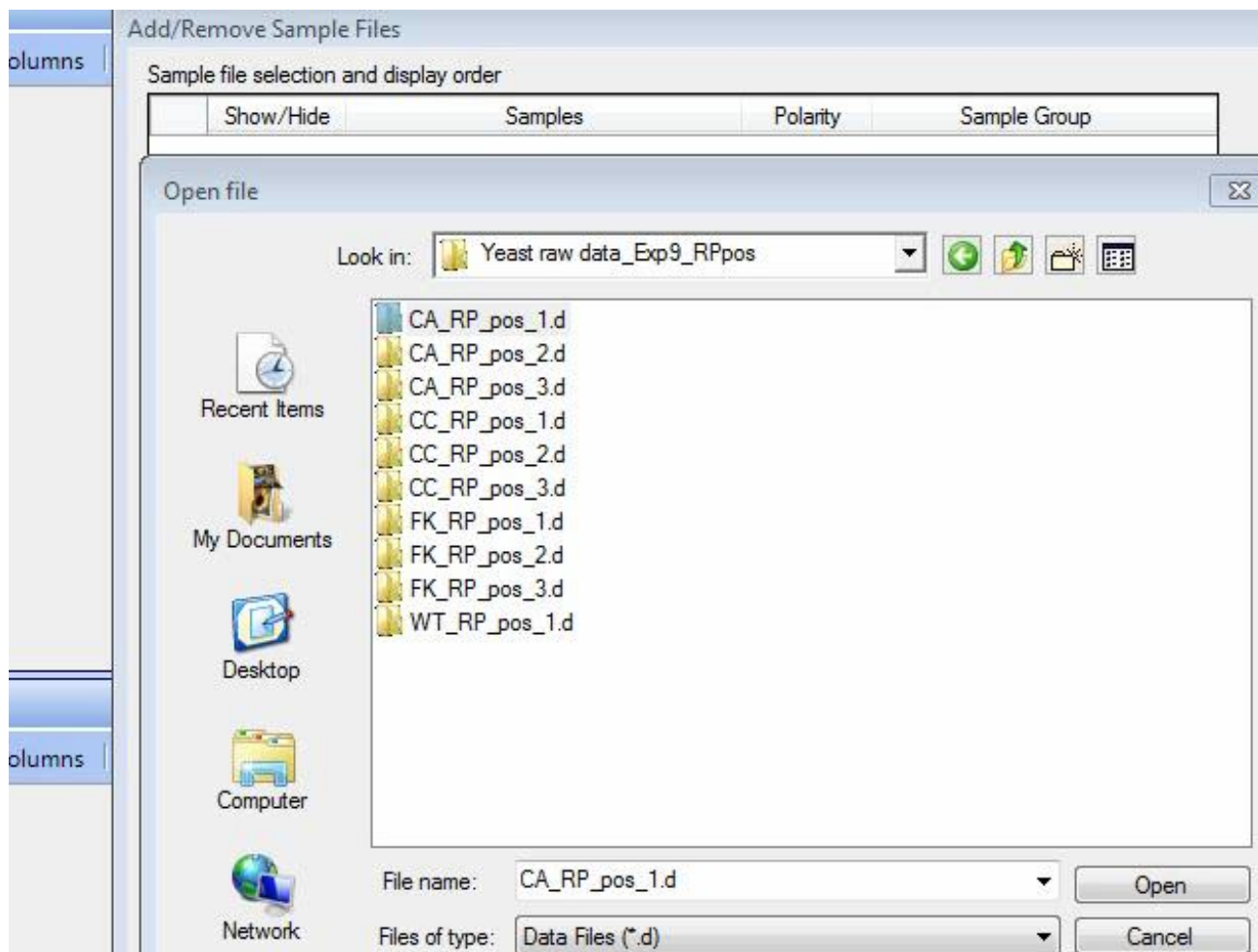


First step: Add/Remove Sample Files and Grouping



- Projects consist of multiple data (.d) files
- One sample group column, but multiple sample groups may be assigned
- This helps with filtering, and plot overlay of the results

Profinder software demo1: Add/Remove Sample Files and Grouping



Profinder's **Three** Feature Extraction Algorithms

1. Batch **Molecular** Feature Extraction



- Recursive MFE reduces false positives

2. Batch **Recursive** Feature Extraction

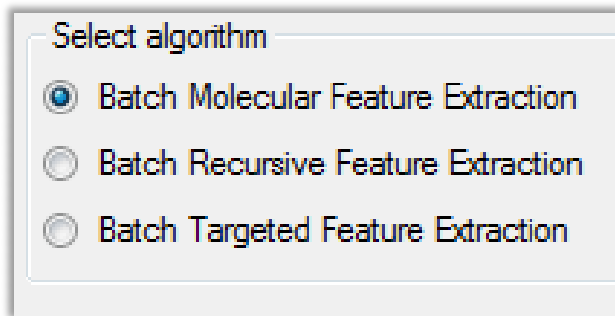


- Greater “missing feature” recovery
- Allows manual editing of compounds

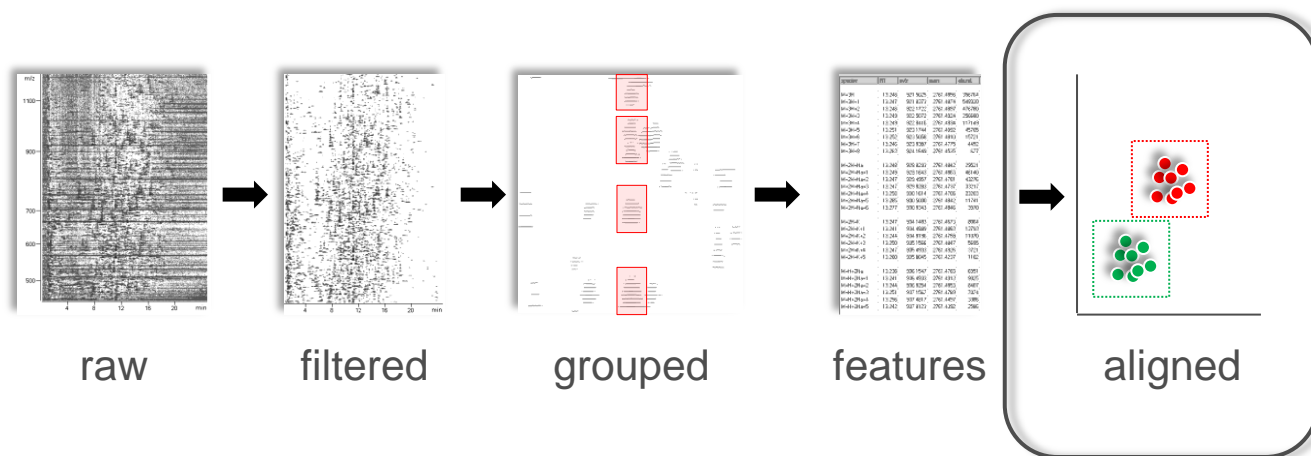
3. Batch **Targeted** Feature Extraction



- Also allows manual editing
- Data source

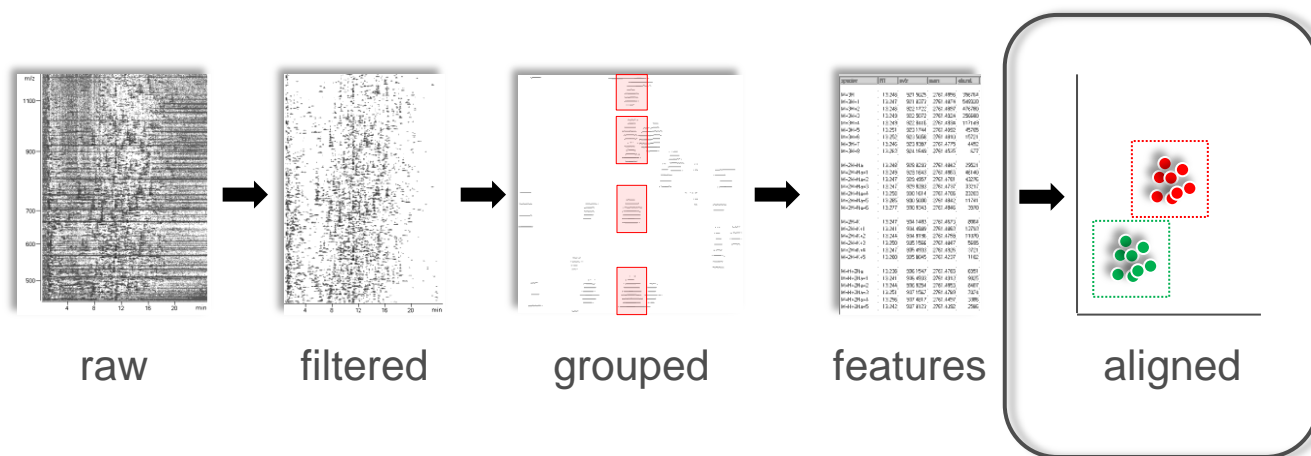


What is Batch Molecular Feature Extraction?



- Batch Molecular Feature Extraction begins with MFE on each data file:
 - From the raw data it finds co-eluting ions that are related:
 - isotopes, adducts (such as Na^+ / K^+), and dimers
 - Filters noise
 - Creates a compound chromatogram for the group of ions
 - Sums all ion signals into one value: one Feature = one compound

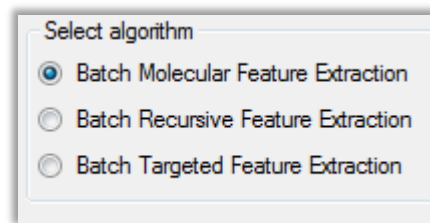
What is Batch Molecular Feature Extraction?



- In MassHunter Qualitative Analysis, features are extracted one file at a time
- However, in MassHunter Profinder Batch MFE aligns features across all sample files to build a consensus spectrum for each compound group, enabling recursive, re-extraction of the batch files

When should I use Batch MFE?

- Regular MFE doesn't work across multiple data files
- Batch MFE is based on recursive analysis (rMFE) across multiple data files
- This results in a an averaged consensus spectrum, that is used for re-extracting all the data files
- Use Batch MFE to optimize settings first, prior to using Batch Recursive Feature Extraction



Profinder's "Batch Recursive Feature Extraction"

1. Batch **Molecular** Feature Extraction



- Recursive MFE reduces false positives

2. Batch **Recursive** Feature Extraction

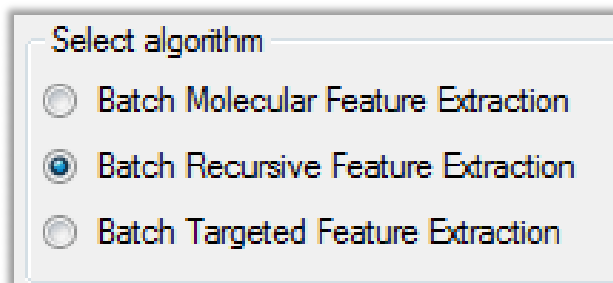


- Find by Ion reduces false negatives
- Allows manual editing of compounds

3. Batch **Targeted** Feature Extraction



- Also allows manual editing
- Data source can be .CEF files, a .CSV file



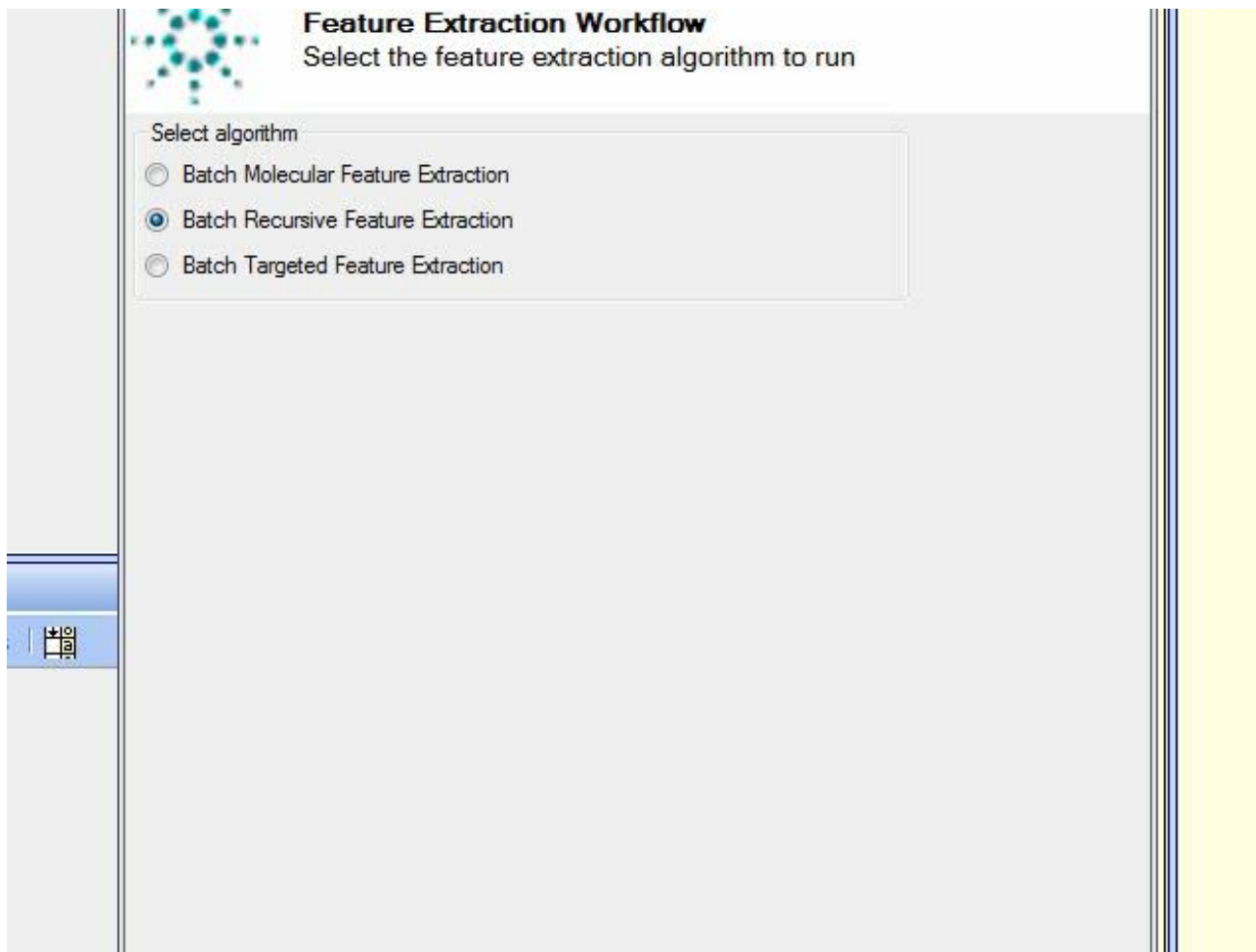
Profinder's "Batch Recursive Feature Extraction": Post-alignment filtering after rMFE and Find by Ion



There are two post-alignment filters:

1. **After rMFE** → improves the quality of your target list for Find by Ion
2. **After Find by Ion** → improves the quality of your final compound group list, reducing the amount of manual cleanup

Profinder software demo2: Batch Recursive Feature Extraction Wizard



The screenshot displays the 'Feature Extraction Workflow' wizard. At the top left is a logo consisting of a cluster of green dots. To its right, the title 'Feature Extraction Workflow' is followed by the instruction 'Select the feature extraction algorithm to run'. Below this, a section titled 'Select algorithm' contains three radio button options: 'Batch Molecular Feature Extraction', 'Batch Recursive Feature Extraction' (which is selected), and 'Batch Targeted Feature Extraction'. The interface includes a vertical sidebar on the left with a blue button at the bottom, and a vertical yellow bar on the right side.

Four Profinder Windows:

Compound centric visualization and editing of results

Compound Groups (788 total)

Group	Height (med)	Mass (med)	Mass (Tgt)	RSD (Mass, ppm)	RT (med)	RT (span)	Found	Missed	%RSD (Tgt)
537	11640	276.1314	276.1296	1.55	0.387	0.021	36	0	25.6
197	5788	274.0841	274.0838	0.38	7.645	0.28	33	3	30
375	5941	274.0836	274.0838	0.42	7.861	0.297	35	1	38.2
518	5757	274.0839	274.0838	0.38	7.647	0.287	34	2	12.2
220	5757	274.0837	274.0837	0.38	7.858	0.36	36	0	43.7
125	9574	271.1533	271.153	0.87	1.062	0.03	36	0	14.4
305	7268	270.1462	270.1465	0.6	8.41	0.03	36	0	0
330	7211	270.1438	270.1441	0.65	0.014	0.03	36	0	0
143	48425	268.0838	268.0837	1	0.014	0.03	36	0	20.1
42	48425	268.0813	268.081	0.03	0.014	0.03	36	0	0
63	12814	266.1546	266.1549	0.03	0.014	0.03	36	0	0
657	2893	263.1274	263.1274	0.019	0.019	0.019	36	0	0.4
203	13723	262.178	262.178	6.753	0.018	0.018	36	0	4.5
289	7062	260.11	260.11	3.069	0.019	0.019	36	0	0
376	5038	260.11	260.11	0.7	8.3	0.02	36	0	0
270	3811	259.152	259.152	0.63	1.218	0.057	36	0	10.6
55	49057	258.1273	258.1273	5.33	0.542	0.019	36	0	8.4
205	28287	258.1219	258.1225	3.49	0.421	0.133	36	0	35.4
151	28287	258.1214	258.1218	5.23	0.422	0.133	36	0	15.4
483	2494	258.0413	258.0431	2.7	0.538	0.066	27	9	52.8

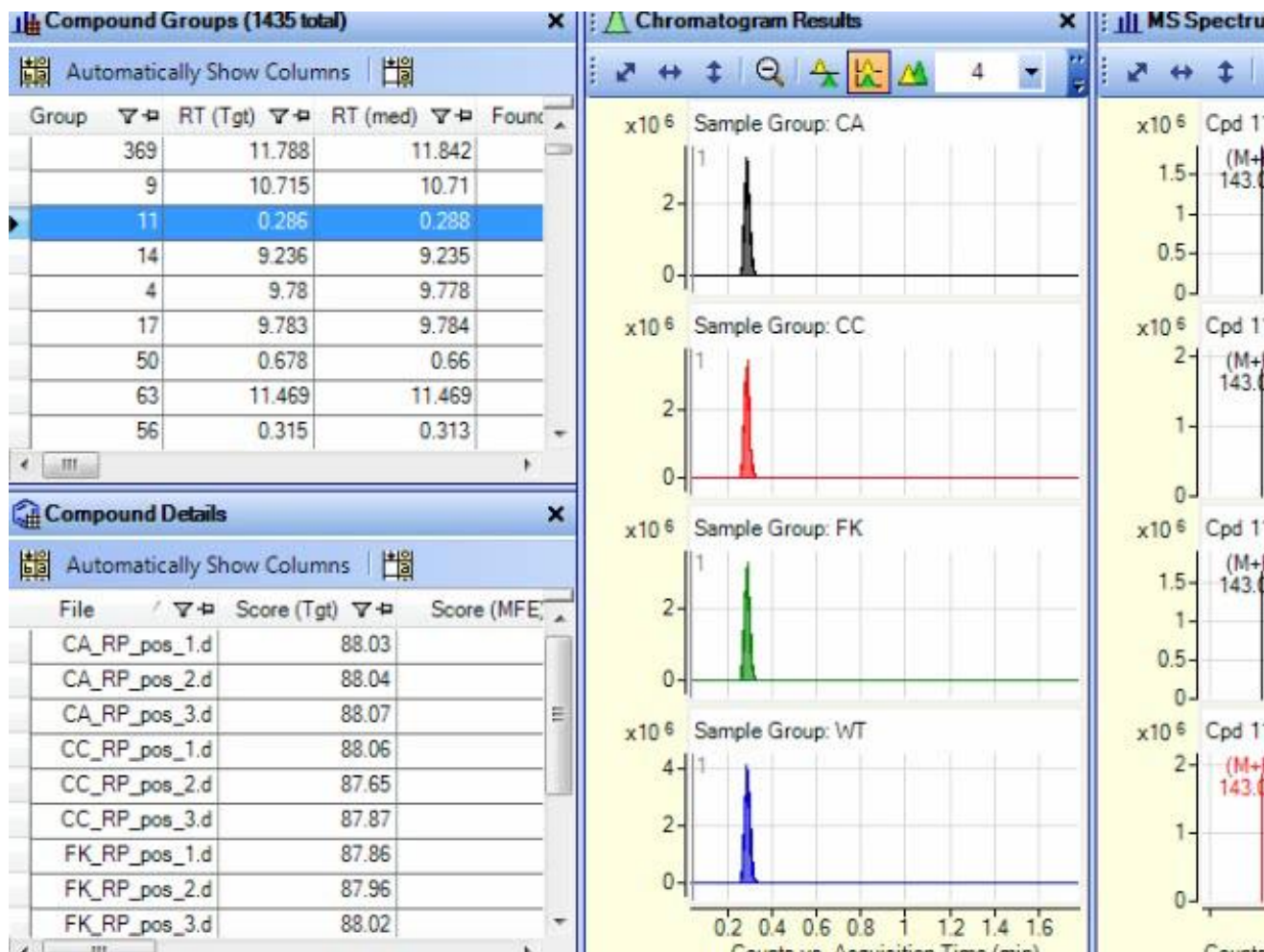
Compound Details

File	Area	Height	RT	Width	Mass (Tgt)	Mass	Diff (Tgt, ppm)	Flags	Polarity
CA_RP_neg_1.d	86478	12043	10.361	0.087	266.1549	266.1545	-1.79		Negative
CA_RP_neg_9.d	95173	13014	10.351	0.088	266.1549	266.1546	-1.38		Negative
CC_RP_neg_1.d	81507	11423	10.372	0.087	266.1549	266.1545	-1.45		Negative
CC_RP_neg_2.d	94217	13508	10.365	0.085	266.1549	266.1544	-1.93		Negative
CC_RP_neg_3.d	91673	12922	10.36	0.087	266.1549	266.1547	-0.03		Negative
CC_RP_neg_4.d	113610	17433	10.349	0.081	266.1549	266.1547	-0.03		Negative
CC_RP_neg_5.d	88470	12400	10.359	0.086	266.1549	266.1544	-1.63		Negative
CC_RP_neg_9.d	71783	8505	10.361	0.092	266.1549	266.1544	-1.63		Negative
FK_RP_neg_1.d	89101	12708	10.359	0.085	266.1549	266.1544	-1.63		Negative
FK_RP_neg_2.d	79394	10414	10.368	0.087	266.1549	266.1544	-1.43		Negative
FK_RP_neg_3.d	83742	11348	10.361	0.087	266.1549	266.1544	-1.07		Negative
FK_RP_neg_4.d	222475	40869	10.345	0.087	266.1549	266.1544	-0.15		Negative
FK_RP_neg_5.d	81649	11172	10.359	0.087	266.1549	266.1544	-1.27		Negative
FK_RP_neg_7.d	79144	10457	10.359	0.087	266.1549	266.1544	-1.63		Negative
WT_RP_neg_3.d	84240	11867	10.359	0.087	266.1549	266.1544	-2.06		Negative
WT_RP_neg_4.d	163464	27630	10.359	0.087	266.1549	266.1547	-0.84		Negative
WT_RP_neg_5.d	303490	59899	10.359	0.072	266.1549	266.1548	-0.43		Negative
WT_RP_neg_6.d	75667	9739	10.375	0.089	266.1549	266.1544	-1.89		Negative
CA_RP_neg_6.d	95388	13766	10.358	0.084	266.1549	266.1546	-1.15		Negative
WT_RP_neg_9.d	85033	11499	10.364	0.085	266.1549	266.1546	-1.43		Negative
CA_RP_neg_8.d	92808	13196	10.362	0.083	266.1549	266.1541	-3.22		Negative
WT_RP_neg_7.d	107467	16772	10.366	0.082	266.1549	266.1549	-0.25		Negative
CA_RP_neg_2.d	96245	13590	10.351	0.085	266.1549	266.1545	-1.66		Negative
FK_RP_neg_8.d	76684	10183	10.357	0.087	266.1549	266.1547	-1.06		Negative
FK_RP_neg_9.d	99669	14329	10.359	0.084	266.1549	266.1547	-0.73		Negative
WT_RP_neg_1.d	250243	47124	10.35	0.074	266.1549	266.1549	-0.05		Negative

Chromatogram Results

MS Spectrum Results

Profunder software demo3: Batch Recursive Feature Extraction



Profinder's "Batch Targeted Feature Extraction"

1. Batch **Molecular** Feature Extraction



- Recursive MFE reduces false positives

2. Batch **Recursive** Feature Extraction

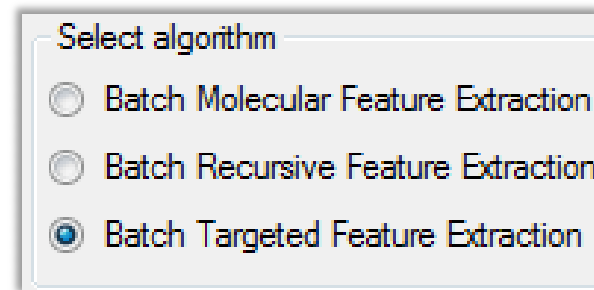


- Find by Ion reduces false negatives
- Allows manual editing of compounds

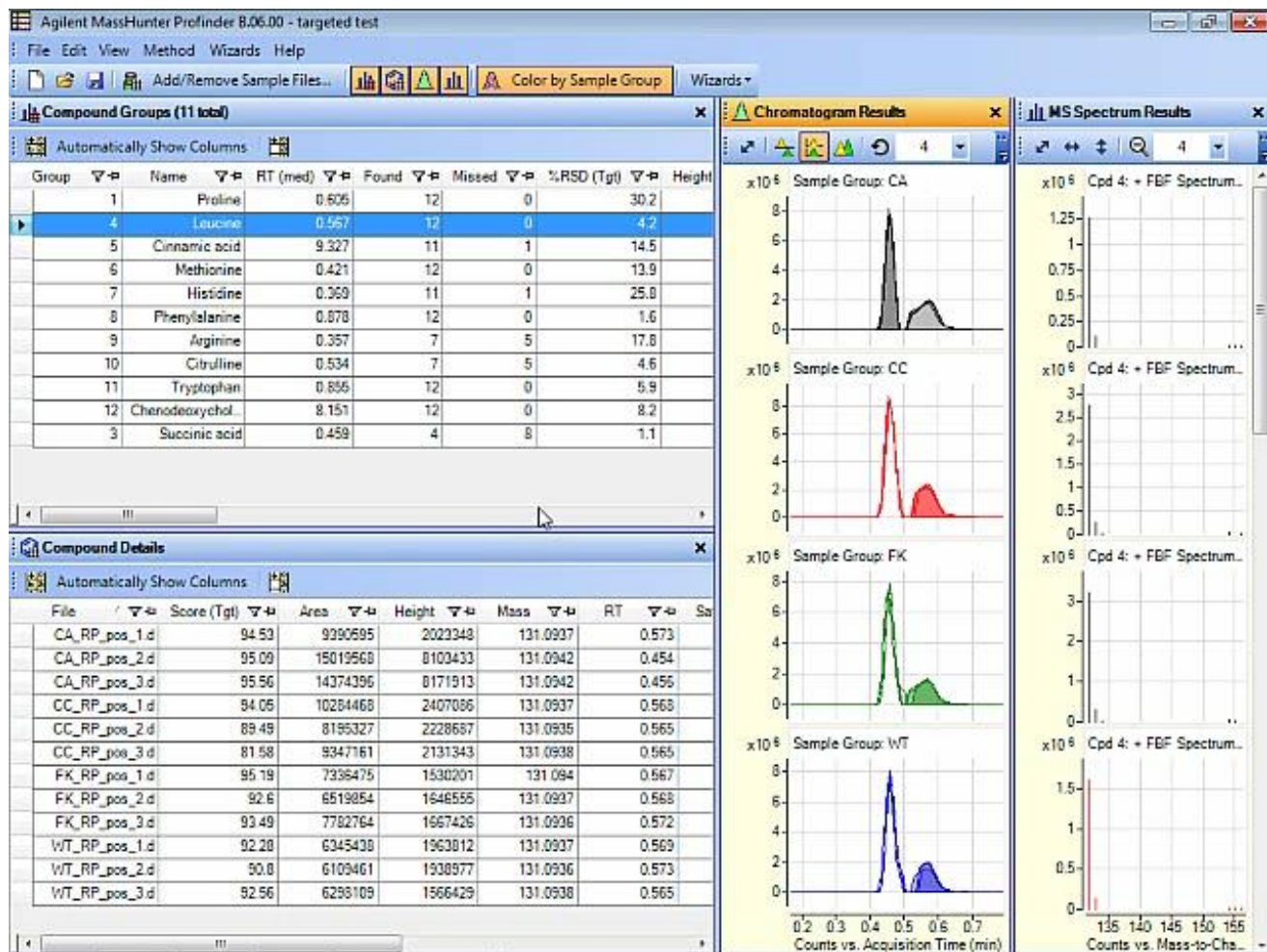
3. Batch **Targeted** Feature Extraction



- Also allows manual editing
- Formula source can be .CEF files, a .CSV file or a PCD/L database



Profinder software demo4: Batch Targeted Feature Extraction



The Profinder Advantage:

A Complete Solution

Features	Agilent Profinder	Customer Benefits
Supported instruments	LC/TOF, and LC/Q-TOF GC/MS planned	Load raw accurate mass LC/MS data without any conversion
Feature finding	Untargeted and targeted	Maximum flexibility for discovery profiling
Ion grouping	Compound-centric	Let's the software determine which ions are related
Recursive analysis	Yes	Higher quality results
Feature quality score	Q-score	Helps in ranking which compound groups to review
Feature visualization and editing	Yes	Quickly re-integrate or remove compounds
Peak alignment	Yes, by compound	Takes advantage of two separate peak alignment steps

How Can You Obtain a copy of MassHunter Profinder?

- MassHunter Profinder is FREE for MPP customers
- It will be placed on the MPP Supplemental DVD
- It will also be available on Agilent SubscribeNet in the near future to update existing customers
- Please contact your local product specialist for further information

