



**ChromaTOF Tile – uživatelsky přívětivé  
vyhledávání  
rozdílů v „omických“ datech měřených v GC×GC**

# Comaparison of large GCxGC-TOF MS sets/records

## Omics

Human metabol-



Bacteria metabol-



Food-



Petrole-



## Others

Forensic



Environmental



# Intended for Sample Group Differentiation

- The data comparison tool is intended to find differences between 2 or more groups of samples
- For example ...

Biomarkers to distinguish healthy and various states of diseases



Off-odor detection in food samples that pass vs fail QC



Water samples up and down stream from an outflow source



Differentiate petroleum samples from various geographical origins

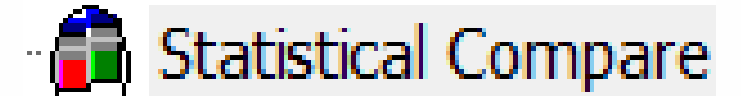


# Available GCxGC Data “Comparison” Tools in LECO SW

1) Target search for selected analytes via REFERENCES



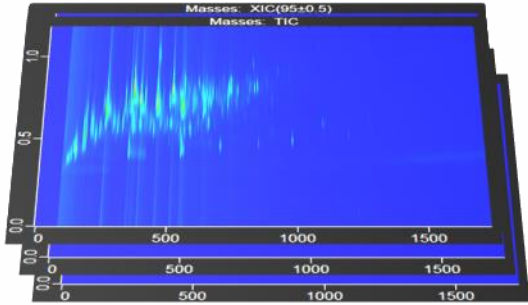
2) Non-Target search via STATISTICAL COMPARE



# Work with REFERENCES



GCxGC data



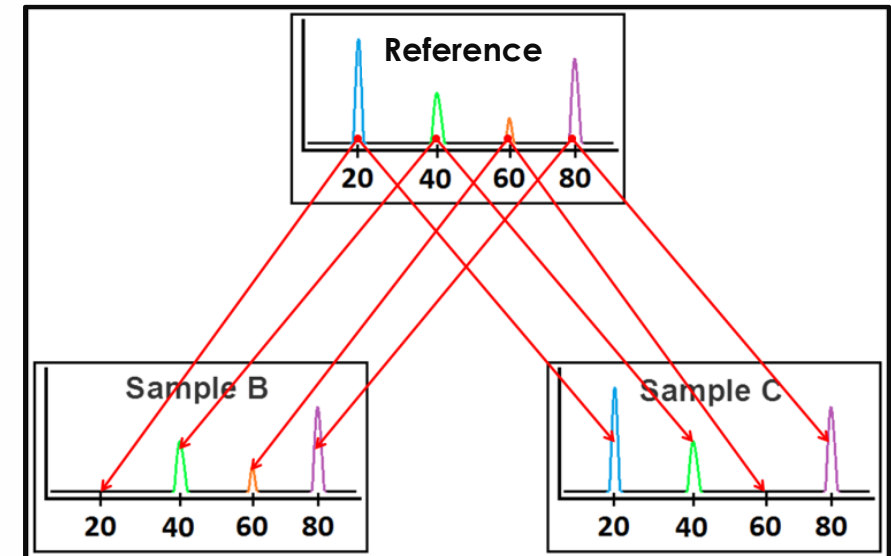
- Comparing of list of Selected analytes incl. RT & spectral information with (other) samples
- One way only!!!
- Work based on
  - Peak Find
  - **Deconvoluted** spectra

Resulting in 4 different peak types in each individual sample

Peak Table - Reference Unknown

Peak #	Name	Type
5	Dodecane	Match
	Chrysene/Lab 5 Reference Methods v1	Not Found
4	Naphthalene	Out of Toleran
1	Sulfurous acid, 2-ethylhexyl nonyl ester	Unknown

Type	Peak present	Spectral match	Area
Match	✓	✓	✓
Out of tolerance	✓	✓	X
Not Found	X or	X or	X
Unknown	Not present in original reference		

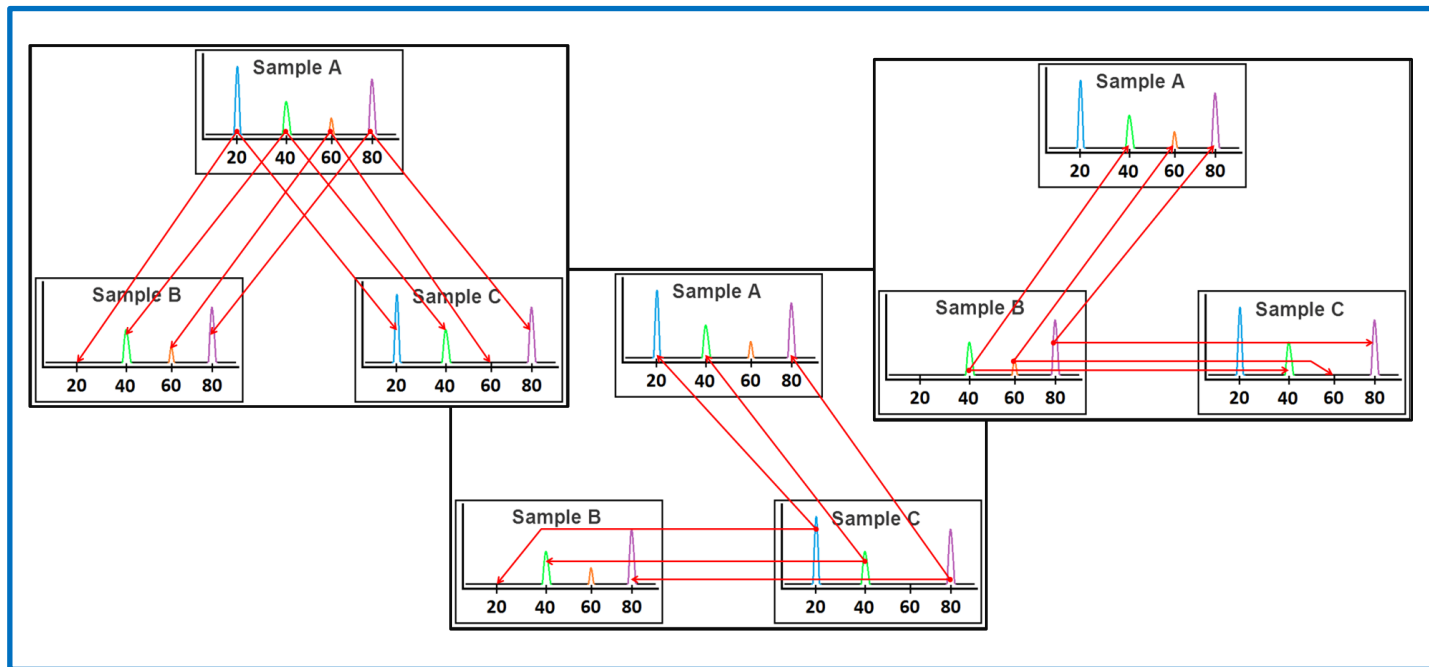
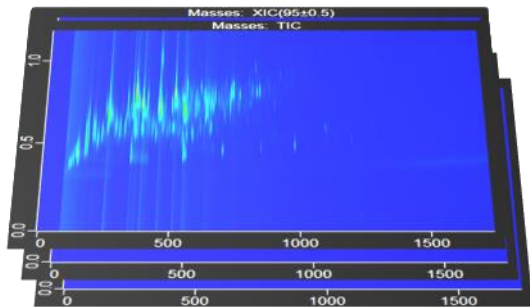


# Work with STATISTICAL COMPARE



Statistical Compare

GCxGC data



## Pair-wise comparison

Compares each peak found within a given sample's peak table to all the peaks found in every other sample's peak tables.



Analyte	Name	Count	Mass	Fisher Ratio
34	Cyclohexane, methyl-	12	83	3.8941
35*	Valproic Acid	9	102	21.080
36	Cyclopentane, ethyl-	12	68	18.754
37	Trimethylene oxide	10	57	Undefined

ID	Name	Count	Area Average	Area %RSD
35-1	C4	2	207059.154	16.172
35-2	C7	2	243043.217	36.875
35-3	C10	2	242928.734	1.771
35-4*	C55	3	533722.969	9.785
35-4-10	C55-1		474095.493	
35-4-11	C55-2		555752.444	
35-4-12	C55-3		571320.969	
35	All Classes	9	331914.568	47.591

- Export to .csv
- Work with 3rd party statistical tool





# New data comparison tool available from 2021



# What is this tool ?

- The algorithm is based on R Synovec et al, [TILE BASED FISCHER RATIOS \(TBFR\)](#)



# Based on Fundamental Research of Prof Synovec

Contents lists available at SciVerse ScienceDirect

**Talanta**

journal homepage: [www.elsevier.com/locate/talanta](http://www.elsevier.com/locate/talanta)

Tile-based Fisher-ratio software for improved feature selection analysis of comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry data

Luke C. Marney<sup>a</sup>, W. Christopher Siegler<sup>a,1</sup>, Brendon A. Parsons<sup>a</sup>, Jamin C. Hoggard<sup>†</sup>, Bob W. Wright<sup>b</sup>, Robert E. Synovec<sup>a,\*</sup>

<sup>a</sup> Department of Chemistry, University of Washington, P.O. Box 351700, Seattle 98198, WA, USA  
<sup>b</sup> Pacific Northwest National Laboratory, Battelle Boulevard, P.O. Box 999, Richland 99352, WA, USA

Contents lists available at ScienceDirect

**Journal of Chromatography**

journal homepage: [www.elsevier.com/locate/jchrom](http://www.elsevier.com/locate/jchrom)

Chemical characterization of the acid alteration of diesel fuel by non-targeted analysis by two-dimensional gas chromatography coupled with time-of-flight mass spectrometry with tile-based Fisher-ratio and combinatorial threshold determination

Brendon A. Parsons<sup>a</sup>, David K. Pinkerton<sup>a</sup>, Bob W. Wright<sup>b</sup>, Robert E. Synovec<sup>a,\*</sup>

<sup>a</sup> Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98198, USA  
<sup>b</sup> Pacific Northwest National Laboratory, Battelle Boulevard, P.O. Box 999, Richland, WA 99352, USA

**analytical chemistry**

pubs.acs.org/ac

**Tile-Based Fisher Ratio Analysis of Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GC × GC-TOFMS) Data Using a Null Distribution Approach**

Brendon A. Parsons<sup>†</sup>, Luke C. Marney<sup>†</sup>, W. Christopher Siegler<sup>†,‡</sup>, Jamin C. Hoggard<sup>†</sup>, Bob W. Wright<sup>‡</sup>, and Robert E. Synovec<sup>\*,†</sup>

<sup>†</sup>Department of Chemistry, University of Washington, Box 351700, Seattle, Washington 98198, United States  
<sup>‡</sup>Pacific Northwest National Laboratory, Battelle Boulevard, P.O. Box 999, Richland, Washington 99352, United States

Contents lists available at ScienceDirect

**Journal of Chromatography**

journal homepage: [www.elsevier.com/locate/jchrom](http://www.elsevier.com/locate/jchrom)

Performance evaluation of tile-based Fisher Ratio analysis using a benchmark yeast metabolome dataset

Nathanial E. Watson<sup>a,b</sup>, Brendon A. Parsons<sup>a</sup>, Robert E. Synovec<sup>a,\*</sup>

<sup>a</sup> Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195, USA  
<sup>b</sup> Department of Chemistry and Life Science, United States Military Academy, West Point, NY 10996, USA

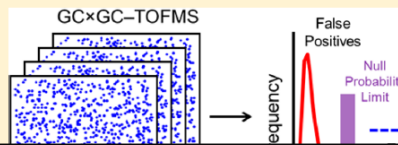
ARTICLE INFO

ABSTRACT

The illicit chemical alteration of petroleum fuels is of keen interest, particularly to regulate fuel specifications, or taxes/credits based on those specifications. One type of alteration is the acid alteration of diesel fuel with concentrated sulfuric acid.

Supporting Information

**ABSTRACT:** Comprehensive two-dimensional (2D) gas chromatography coupled with time-of-flight mass spectrometry (GC × GC-TOFMS) is a versatile instrumental platform capable of collecting highly informative, yet highly complex, chemical data for a variety of samples. Fisher-ratio (F-ratio) analysis applied to the supervised comparison of sample classes algorithmically reduces complex GC × GC-TOFMS data to a single dimension. The resulting data are analyzed using a null distribution approach to identify significant peaks. This approach reduces the number of false positives and improves the detection of significant peaks. The resulting data are analyzed using a null distribution approach to identify significant peaks. The resulting data are analyzed using a null distribution approach to identify significant peaks.



ARTICLE INFO

ABSTRACT

Performance of tile-based Fisher Ratio (F-ratio) data analysis, recently developed studies using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC × GC-TOFMS), is evaluated with a metabolomics dataset that was analyzed in great detail, but while taking a brute force approach. The previously published data (herein as the benchmark dataset) were intracellular extracts from *Saccharomyces cerevisiae* either metabolizing glucose (repressed) or ethanol (derepressed), which define the benchmark dataset.

**analytical chemistry**

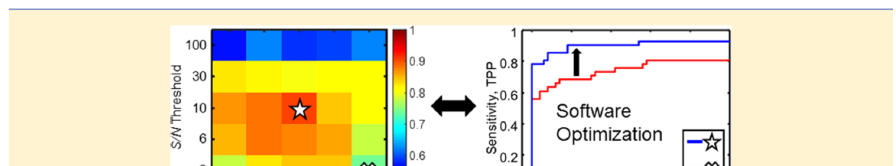
pubs.acs.org/ac

**Using Receiver Operating Characteristic Curves To Optimize Discovery-Based Software with Comprehensive Two-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometry**

Brooke C. Reaser<sup>†</sup>, Bob W. Wright<sup>‡</sup>, and Robert E. Synovec<sup>\*,†</sup>

<sup>†</sup>Department of Chemistry, University of Washington, Box 351700, Seattle, Washington 98198, United States  
<sup>‡</sup>Pacific Northwest National Laboratory, Battelle Boulevard, P.O. Box 999, Richland, Washington 99352, United States

Supporting Information



SULTS

# What is this tool ?

- The algorithm is based on R Synovec et al, **TILE BASED FISCHER RATIOS (TBFR)**
- Prof Synovec was partnered to develop a fully functional application usable by all LECO customers / HW+SW **GCxGC** platforms – Pegasus HT, BT, HRT



# How does ChromaTOF Tile work?

- **What it does:**

- Compares GCxGC data files to quickly and easily identify significant differences between classes of sample.
- Applies tiling across datasets and determines statistically significant differences in the sample groupings for every nominal mass.
- Nominalizes data to make it fast
- Presents this information in an intuitive and highly graphical interface to allow users to understand where differences exist and also allows them to begin to identify what those differences are.

- **What it does not:**

- Not performing deconvolution
- Not aligning the data sets

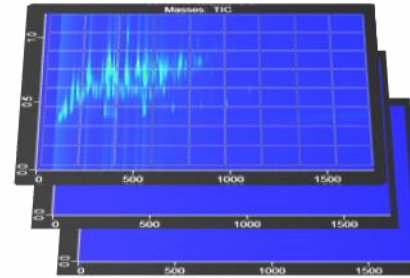


The data **must be** comparable in terms of retention time stability

# Principle of work with CHT-Tile

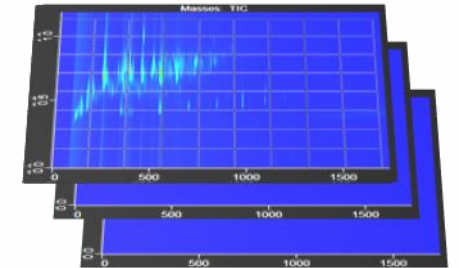
## 1) Definition of sample groups - USER

*e.g., Costa Rica vs. French Roast Coffee*



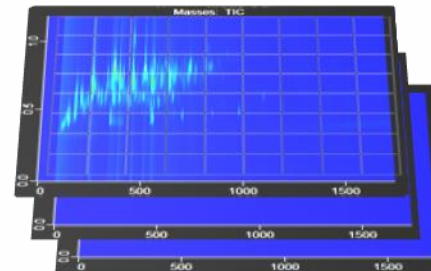
Costa Rica

$\times$



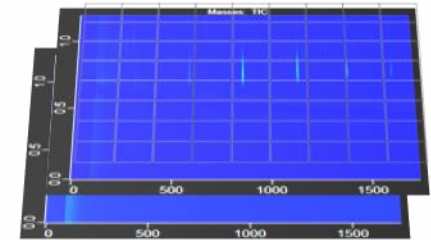
French Roast

*Or samples (all coffee samples) vs. Blank(s)*



Coffee samples

$\times$



SPME blanks

# Principle of work with CHT-Tile

## 1) Definition of sample groups - USER

*e.g., Blanks vs. different types of samples*

Samples

Intersecting mass range: 33 : 500  
Number of spectra: 552036  
Modulation period (spectra): 400  
Acquisition rate (spectra/sec): 200.012800819252  
Acquisition delay (sec): 0

Location	Name	Date	Class	Scale factor
Imported samples	0.001 2D, new vial	20-Nov-19 3:32:32 AM	Blank	1
Imported samples	0.01 2D, new vial	20-Nov-19 4:49:14 AM	Blank	1
Imported samples	0.1 2D, new vial	20-Nov-19 6:05:57 AM	Blank	1
Imported samples	Blackberry Chai, 50x 2D	23-Nov-19 8:29:06 AM	Blackberry Chai	1
Imported samples	Blackberry Chai, 50x 2D_2	23-Nov-19 9:45:45 AM	Blackberry Chai	1
Imported samples	Blackberry Chai, 50x 2D_3	23-Nov-19 11:02:27 AM	Blackberry Chai	1
Imported samples	Peach Ginger, 50x 2D	23-Nov-19 4:09:12 PM	Peach Ginger	1
Imported samples	Peach Ginger, 50x 2D_2	23-Nov-19 5:25:54 PM	Peach Ginger	1
Imported samples	Peach Ginger, 50x 2D_3	23-Nov-19 6:42:38 PM	Peach Ginger	1
Imported samples	Pom Hibiscus, 50x 2D	23-Nov-19 12:19:08 PM	Pom Hibiscus	1
Imported samples	Pom Hibiscus, 50x 2D_2	23-Nov-19 1:35:50 PM	Pom Hibiscus	1
Imported samples	Pom Hibiscus, 50x 2D_3	23-Nov-19 2:52:29 PM	Pom Hibiscus	1



# Principle of work with CHT-Tile

## 1) Definition of sample groups - USER

*e.g., Blanks vs. samples*

Sample set (12 samples, 2 classes)

Databases

Samples to compare:

	Location	Name	Date	Class	Scale factor
OK	Imported samples	0.001 2D, new vial	20-Nov-19 3:32:32 AM	Blank	1
OK	Imported samples	0.01 2D, new vial	20-Nov-19 4:49:14 AM	Blank	1
OK	Imported samples	0.1 2D, new vial	20-Nov-19 6:05:57 AM	Blank	1
OK	Imported samples	Blackberry Chai, 50x 2D	23-Nov-19 8:29:06 AM	Samples	1
OK	Imported samples	Blackberry Chai, 50x 2D_2	23-Nov-19 9:45:45 AM	Samples	1
OK	Imported samples	Blackberry Chai, 50x 2D_3	23-Nov-19 11:02:27 AM	Samples	1
OK	Imported samples	Peach Ginger, 50x 2D	23-Nov-19 4:09:12 PM	Samples	1
OK	Imported samples	Peach Ginger, 50x 2D_2	23-Nov-19 5:25:54 PM	Samples	1
OK	Imported samples	Peach Ginger, 50x 2D_3	23-Nov-19 6:42:38 PM	Samples	1
OK	Imported samples	Pom Hibiscus, 50x 2D	23-Nov-19 12:19:08 PM	Samples	1
OK	Imported samples	Pom Hibiscus, 50x 2D_2	23-Nov-19 1:35:50 PM	Samples	1
OK	Imported samples	Pom Hibiscus, 50x 2D_3	23-Nov-19 2:52:29 PM	Samples	1

For all selected samples

Class:  Apply

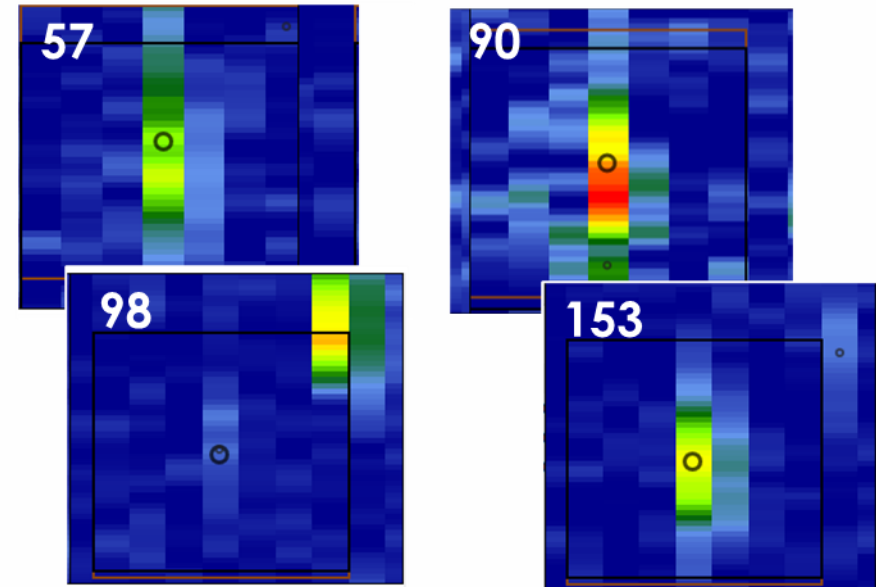
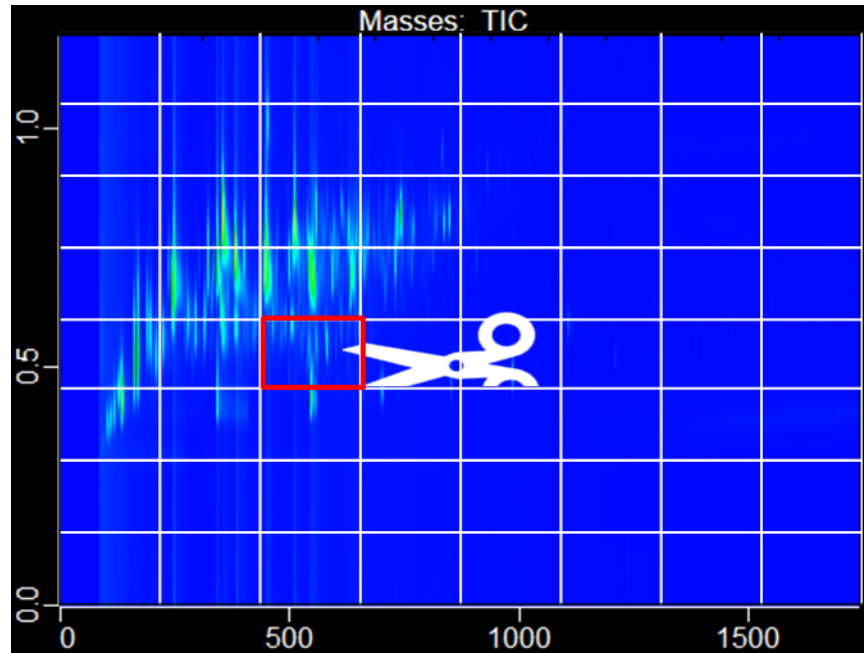
Scale factor:  Apply

Close

Rate: 200ss Mass range: 32 : 501.1

# Principle of work with CHT-Tile

2) Definition of tile size (wizard exists) - USER



# Principle of work with CHT-Tile

## 2) Definition of tile size (wizard exists) and other processing parameters - USER

[Click here to auto-calculate suggested tile size](#)

Tile size D1 (modulations):

Tile size D2 (spectra):

S/N threshold:

Samples that must exceed S/N threshold:  ?

Mass F-ratios to average:  ?

Threshold type to apply:

F-ratio threshold:  ?

Minimum masses per tile:  ?

Minimum mass:  ?

Maximum mass:  ?

Masses to ignore:  ?

Perform one-point normalization:

Auto-calculate tile size

typical peak width (sec)

D1:  at  50%  Base

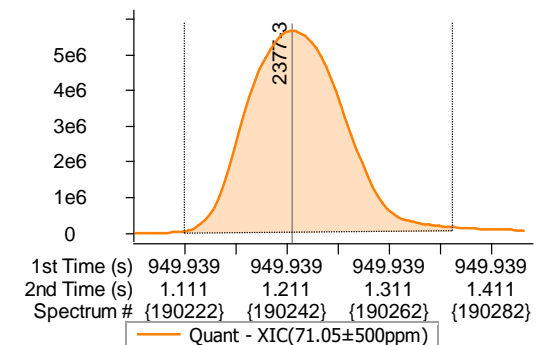
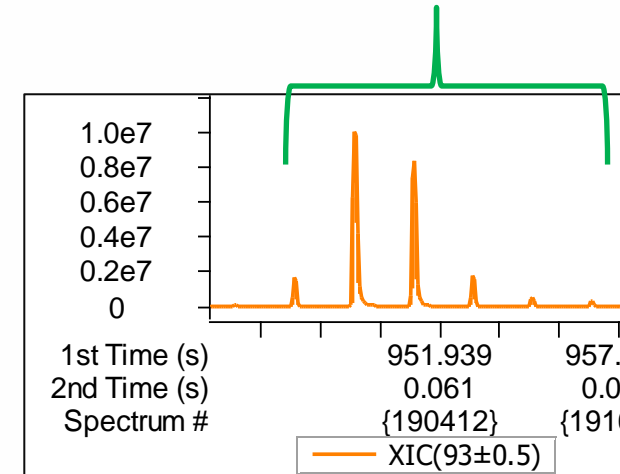
D2:  at  50%  Base

Max expected retention shift (sec)

D1:

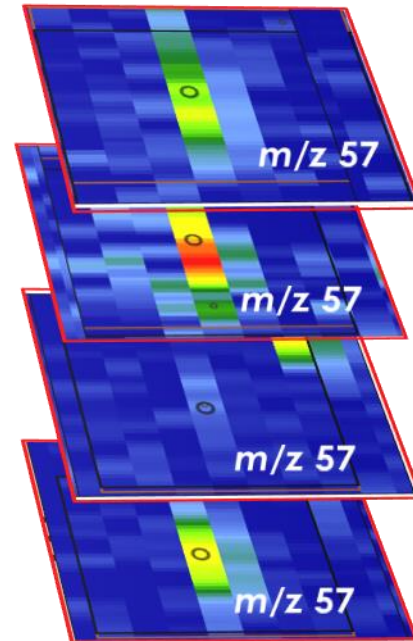
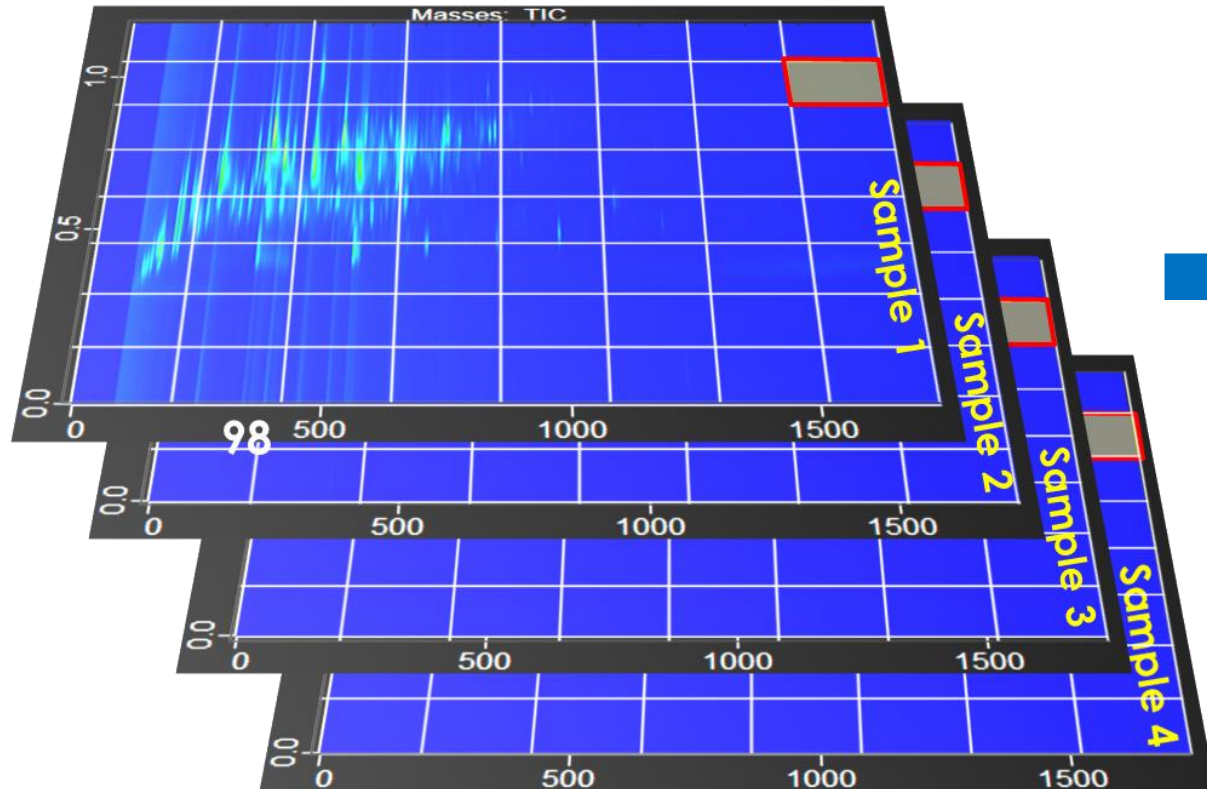
D2:

Note: If you switch to samples with different timing parameters, you will need to redo this calculation.



# Principle of work with CHT-Tile

- 3) Dividing chromatogram into retention windows/tiles - SW



Determination of differences



Output is tile locations and list of m/z with significant Fisher Ratio leading to analytes that differ between the samples

# How differences are determined

- Comparison of **raw data** windows (tiles)
- Based on variance, which necessitates replicates per group
- Calculated by Fisher Ratio,

$$\text{Fisher Ratio} = \frac{\sigma_{cl}^2}{\sigma_{err}^2} = \frac{\text{class to class variation}}{\text{within class variation}}$$

- Objective is to emphasize differences between sample groups/classes and ignore differences in data not related to pre-defined groups  
~ Supervised analysis



# Principle of work with CHT-Tile

- 4) Data (F-Ratio hits) review

# F-Ratio Hits Pane

F-ratio hits | Chemical features | Summary

Tile hits:

#	Avg F-ratio	Top mass	RT1	RT2	Masses	Blank	Blackbe	Peach C	Pom Hi
001	48241159.62	93	1231.92	1.22	284	1795431	1279474	5452074	5063141
002	5201502.36	134	865.90	1.14	288	4581488	4240945	1309222	1292904
003	756782.76	204	1495.90	1.20	269	348687	2931466	862394	803864
004	588412.18	143	1517.90	1.29	196	358525	1131322	649089	693147
005	431857.48	93	1365.91	1.11	276	135192	311772	315469	397619
006	430746.29	94	1299.92	1.13	385	640974	7314304	270175	253375
007	360365.83	94	1471.91	1.15	284	138497	275932	281464	289271
008	333543.56	105	1461.91	1.19	217	330787	108763	684634	664876
009	309333.16	121	1429.91	1.14	279	119488	247731	269002	194550
010	305152.43	120	1507.90	1.22	233	146833	622276	106734	562062
011	291551.48	157	1501.90	1.25	166	194705	630064	450422	354332
012	252842.67	122	791.95	1.11	306	100534	650254	372223	181317
013	179405.72	153	1523.90	1.32	139	440197	309869	321128	288001
014	158234.25	161	1427.91	1.19	214	158168	330216	435423	194021
015	154678.77	106	1131.93	1.22	276	199502	122523	212505	111569
016	148277.16	179	1387.91	1.16	267	662630	418514	164881	128285
017	141720.10	119	1379.91	1.15	255	904809	400919	242589	317999
018	141240.72	140	667.96	1.20	165	78203.5	673376	694230	485246
019	133312.34	91	947.94	1.23	297	110332	240721	288615	818031
020	122937.37	78	1453.91	1.16	185	264740	188183	134297	154740
021	113166.36	109	931.94	1.29	192	710912	143140	364247	379025
022	107064.39	136	1411.91	1.17	248	216802	281744	513386	512446
023	104807.78	105	1121.93	1.18	268	419895	163727	145456	242093
024	102912.94	134	1495.90	1.31	90	399020	348374	181056	101203
025	99546.42	142	1703.89	1.42	259	283437	436767	117121	123508
026	91723.36	121	1481.91	1.13	123	276532	156723	219693	261490
027	72503.32	94	1495.90	1.49	144	254350	191698	561647	218979
028	69441.72	92	1335.91	1.13	328	154225	184788	818934	976594
029	58280.78	122	1521.90	1.22	218	402102	343517	100846	123529
030	53721.52	136	1481.91	1.21	201	623977	305920	589934	665900

Class average for "Blank" (93)

Class average for "Blackberry Chai"

Class average for "Peach Ginger"

Class average for "Pom Hibiscus"

Mass hits for selected:

#	Mass	F-ratio	RT1	RT2	Area diff	Blank	Blackbe	Peach C	Pom Hi
1	93	48241159.62	1231.92	1.22	7678623.80	1795431	1279474	5452074	5063141
181	181	13.39	1233.92	1.20	21549.40	26547.9	16472.4	35491.1	38021.8
182	274	13.01	1231.92	1.24	12757.54	20313.2	10262.8	23020.4	19468.5
183	149	12.83	1233.92	1.23	1856934.63	154814	201174	361504	361227
184	142	12.25	1233.92	1.23	2794373.10	421924	129752	174154	321629
185	397	12.22	1233.92	1.20	25043.84	71697.6	81032.1	58719.6	55988.3
186	413	12.17	1233.92	1.18	51762.27	163486	197710	150306	145408
187	262	12.17	1233.92	1.20	9575.65	4959.59	261.38	9837.03	4799.85

- List of tile hits
- For each hit there is a list of masses for that tile (incl. calculated F-Ratio)
- Immediate feedback on class distinction based on:

- Heat map on calculated intensities to draw your eye
- Contour plots

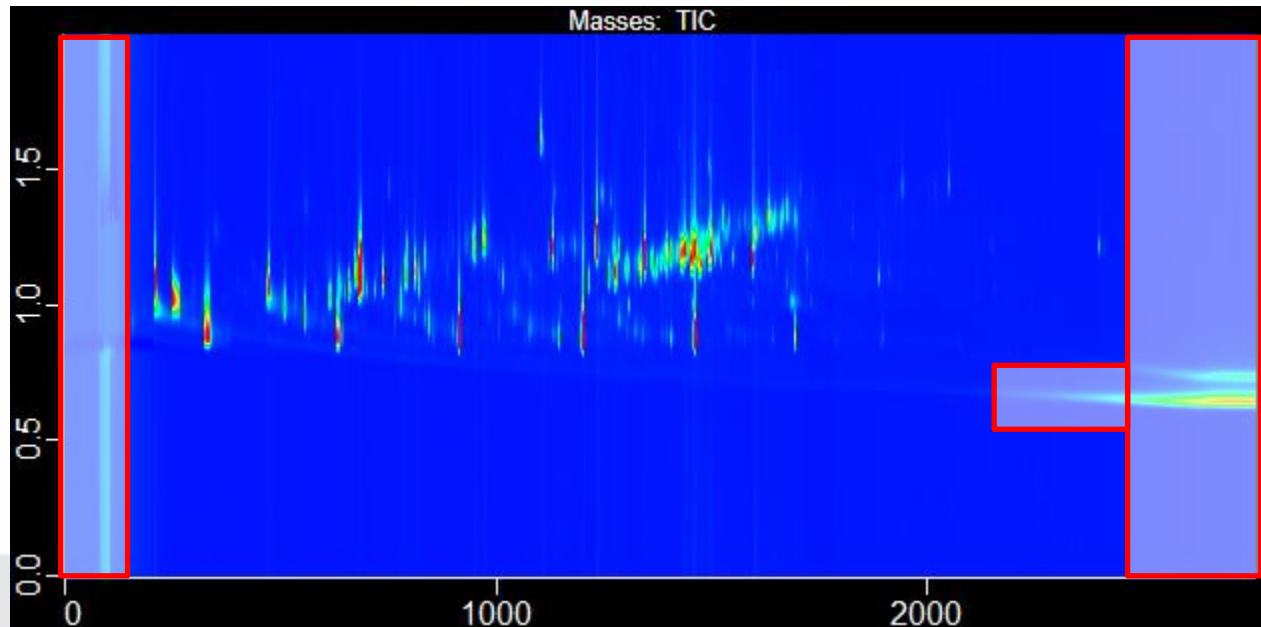
 - Tile

 - Zone for area calculation

# F-Ratio Hits Pane - Filters



- Filters allow to exclude/include only several zones (bleed, solvent, unmodulated area, etc.)
- When applied → immediately removing tiles from unwanted zone from an overview



# F-Ratio Hits Pane – curation



## Manual approach

- For each file can be selected one or more masses
- Each proposed file can be accepted or rejected

LECO ChromaTOF Tile (v1.0.2.0)

Home » Processings » Beverages with CBD #1

All selected tiles: [Reject](#) | [Unreviewed](#)

F-ratio hits | Chemical features | Summary

Tile hits:

#	Avg F-ratio	Top mass	RT1	RT2	Masses	Blank	Blackbe	Peach C	Pom Hi
001	48241159.62	93	1231.92	1.22	284	1795431	1279474	5452074	5063141
002	5201502.56	154	685.96	1.14	288	4381481	4206724	1369222	1995661
003	756782.76	204	1495.90	1.20	269	3486874	2931461	8623994	8038641
004	588412.18	143	1517.90	1.29	196	3585254	1131322	6490891	6931471
005	431857.48	93	1365.91	1.11	276	1351921	3117721	3154691	3976191
006	430746.29	94	1299.92	1.13	385	6409741	7314304	2701751	2533751
007	360365.83	94	1471.91	1.15	284	1384971	2759321	2814641	2892711
008	333543.56	105	1461.91	1.19	217	3307871	1087631	6846341	6648761
009	309333.16	121	1429.91	1.14	279	1194881	2477311	2690021	1945501
010	305152.43	120	1507.90	1.22	233	1468331	6222761	1067341	5620621
011	291551.48	157	1501.90	1.25	166	1947051	6300641	4504221	3543321
012	252842.67	122	791.95	1.11	306	1005341	6502541	3722231	1813171
013	179405.72	153	1523.90	1.32	139	4401971	3098691	2311281	2880011
014	158234.25	161	1427.91	1.19	214	1581681	3302161	4354231	1940211
015	147191.91	109	1429.91	1.23	192	1705121	1431401	5042171	3120211
016	147191.91	109	1429.91	1.23	192	1705121	1431401	5042171	3120211
017	147191.91	109	1429.91	1.23	192	1705121	1431401	5042171	3120211
018	147191.91	109	1429.91	1.23	192	1705121	1431401	5042171	3120211
019	147191.91	109	1429.91	1.23	192	1705121	1431401	5042171	3120211
020	147191.91	109	1429.91	1.23	192	1705121	1431401	5042171	3120211
021	113100.36	109	1429.91	1.23	192	1705121	1431401	5042171	3120211
022	107064.39	136	1411.91	1.17	248	2168021	2817441	5133861	5124461
023	104807.78	105	1121.93	1.18	268	4198951	1637271	1454561	2420931
024	102912.94	134	1495.90	1.31	90	3990201	3483741	1810561	1012031
025	99546.42	142	1703.89	1.42	259	2834371	4367671	1171211	1235081
026	91723.36	121	1481.91	1.13	123	2765321	1567231	2196931	2614901
027	72503.32	94	1495.90	1.49	144	2543501	1916981	5616471	2189791
028	69441.72	92	1335.91	1.13	328	1542251	1847881	8189341	9765941
029	58280.78	122	1521.90	1.22	218	4021021	3435171	1008461	1235291
030	53721.52	136	1481.91	1.21	201	6239771	3059201	5899341	6659001

Class average for "Blank" (93)

Class average for "Blackberry Chai"

Class average for "Pom Hibiscus"

#	Mass	F-ratio	RT1	RT2	Area diff	Blank	Blackbe	Peach C	Pom Hi
1	93	48241159.62	1231.92	1.22	7678623.80	1795431	1279474	5452074	5063141
181	429	13.39	1233.92	1.20	21549.40	26547.9	16472.4	35491.1	38021.8
182	274	13.01	1231.92	1.24	12757.54	20313.2	10262.8	23020.4	19468.5
183	149	12.83	1233.92	1.23	1856934.63	1548141	2011741	3615041	3612271
184	142	12.25	1233.92	1.23	2794373.10	4219241	1297521	1741541	3216291
185	397	12.22	1233.92	1.20	25043.84	71697.6	81032.1	58719.6	55988.3
186	413	12.17	1233.92	1.18	51762.27	1634861	1977101	1503061	1454101
187	262	12.17	1233.92	1.20	9575.65	4959.59	261.38	9837.03	4799.85

- Selected tiles/masses are converted to **Features**

# F-Ratio Hits Pane – curation



## Autocuration

- All or selected tile hits will be converted to Features
- Acceptance based on
  - Area difference between classes
  - F-ratio
- Possible filtering based on F-ratio value

### Autocuration

For:

All tile hits  
 Selected tile hits

Auto-accept the mass with the maximum:

Area difference between classes  
 F-ratio

If its F-ratio is at least:

Autocurate

LECO ChromaTOF Tile (v1.0.2.0)

Home >> Processings >> Beverages with CBD #1

All selected tiles: Reject | Unreviewed

F-ratio hits | Chemical features | Summary

Tile hits:

#	Avg F-ratio	Top mass	RT1	RT2	Masses	Blank	Blackbe	Peach C	Pom Hi
001	48241159.62	93	1231.92	1.22	284	1795431	1279474	5452074	5063141
002	5201502.56	154	685.96	1.14	288	4381481	4206724	1369222	1995661
003	756782.76	204	1495.90	1.20	269	3486874	2931461	8623994	8038641
004	588412.18	143	1517.90	1.29	196	3585254	1131322	6490891	6931474
005	431857.48	93	1365.91	1.11	276	1351921	3117721	3154691	3976191
006	430746.29	94	1299.92	1.13	385	6409744	7314304	2701751	2533751
007	360365.83	94	1471.91	1.15	284	1384971	2759325	2814644	2892711
008	333543.56	105	1461.91	1.19	217	3307871	1087631	6846344	6648761
009	309333.16	121	1429.91	1.14	279	1194881	2477311	2690021	1945501
010	305152.43	120	1507.90	1.22	233	1468331	6222761	1067344	5620621
011	291551.48	157	1501.90	1.25	166	1947051	6300644	4504221	3543321
012	252842.67	122	791.95	1.11	306	1005341	6502544	3722231	1813171
013	179405.72	153	1523.90	1.32	139	4401971	3098691	2311281	2880011
014	158234.25	161	1427.91	1.19	214	1581681	3302161	4354231	1940211
015	154678.77	106	1131.93	1.22	276	1995021	1225231	2125051	1115691
016	148277.16	179	1387.91	1.16	267	6626301	4185141	1648811	1282851
017	141720.10	119	1379.91	1.15	255	9048091	4009191	2425891	3179991
018	141240.72	140	667.96	1.20	165	7820351	6733761	6942301	4852461
019	133312.34	91	947.94	1.23	297	1103321	2407211	2886151	8180311
020	122937.37	78	1453.91	1.16	185	2647401	1881831	1342971	1547401
021	113166.36	109	931.94	1.29	192	7109121	1431401	3642471	3790251
022	107064.39	136	1411.91	1.17	248	2168021	2817441	5133861	5124461
023	104807.78	105	1121.93	1.18	268	4198951	1637271	1454561	2420931
024	102912.94	134	1495.90	1.31	90	3990201	3483741	1810561	1012031
025	99546.42	142	1703.89	1.42	259	2834371	4367671	1171211	1235081
026	91723.36	121	1481.91	1.13	123	2765321	1567231	2196931	2614901
027	72503.32	94	1495.90	1.49	144	2543501	1916981	5616471	2189791
028	69441.72	92	1335.91	1.13	328	1542251	1847881	8189341	9765941
029	58280.78	122	1521.90	1.22	218	4021021	3435171	1008461	1235291
030	53721.52	136	1481.91	1.21	201	6239771	3059201	5899341	6659001

Class average for "Blank" 93

Class average for "Blackberry Chai"

Class average for "Peach Ginger"

Class average for "Pom Hibiscus"

Mass hits for selected tile:

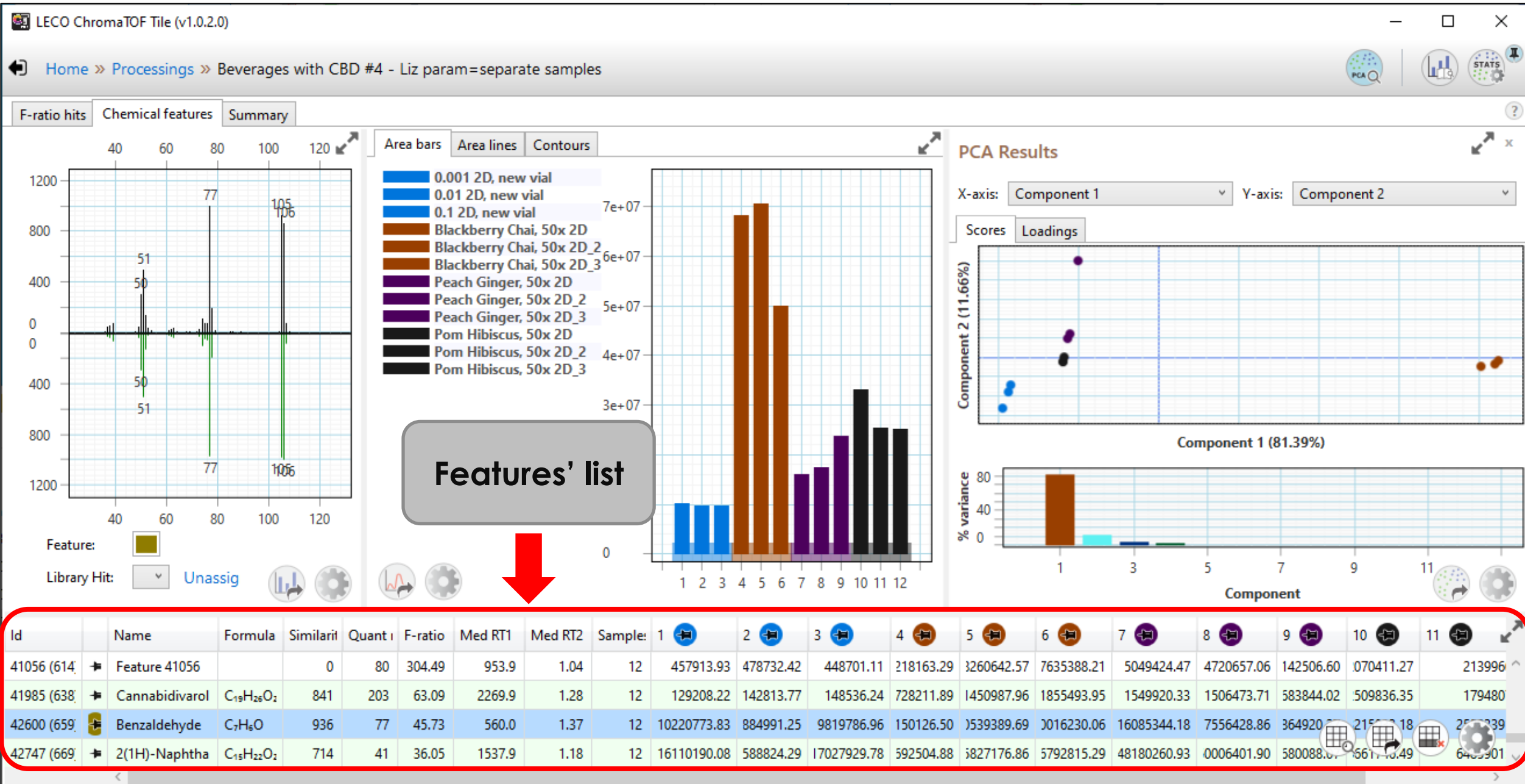
#	Mass	F-ratio	RT1	RT2	Area diff	Blank	Blackbe	Peach C	Pom Hi
1	93	48241159.62	1231.92	1.22	7678623.80	1795431	1279474	5452074	5063141
181	429	13.39	1233.92	1.20	21549.40	26547.9	16472.4	35491.1	38021.8
182	274	13.01	1231.92	1.24	12757.54	20313.2	10262.8	23020.4	19468.5
183	149	12.83	1233.92	1.23	1856934.63	154814.	201174.	361504.	361227.
184	142	12.25	1233.92	1.23	2794373.10	421924.	129752.	174154.	321629.
185	397	12.22	1233.92	1.20	25043.84	71697.6	81032.1	58719.6	55988.3
186	413	12.17	1233.92	1.18	51762.27	163486.	197710.	150306.	145446.
187	262	12.17	1233.92	1.20	9575.65	4959.59	261.38	9837.03	4799.85



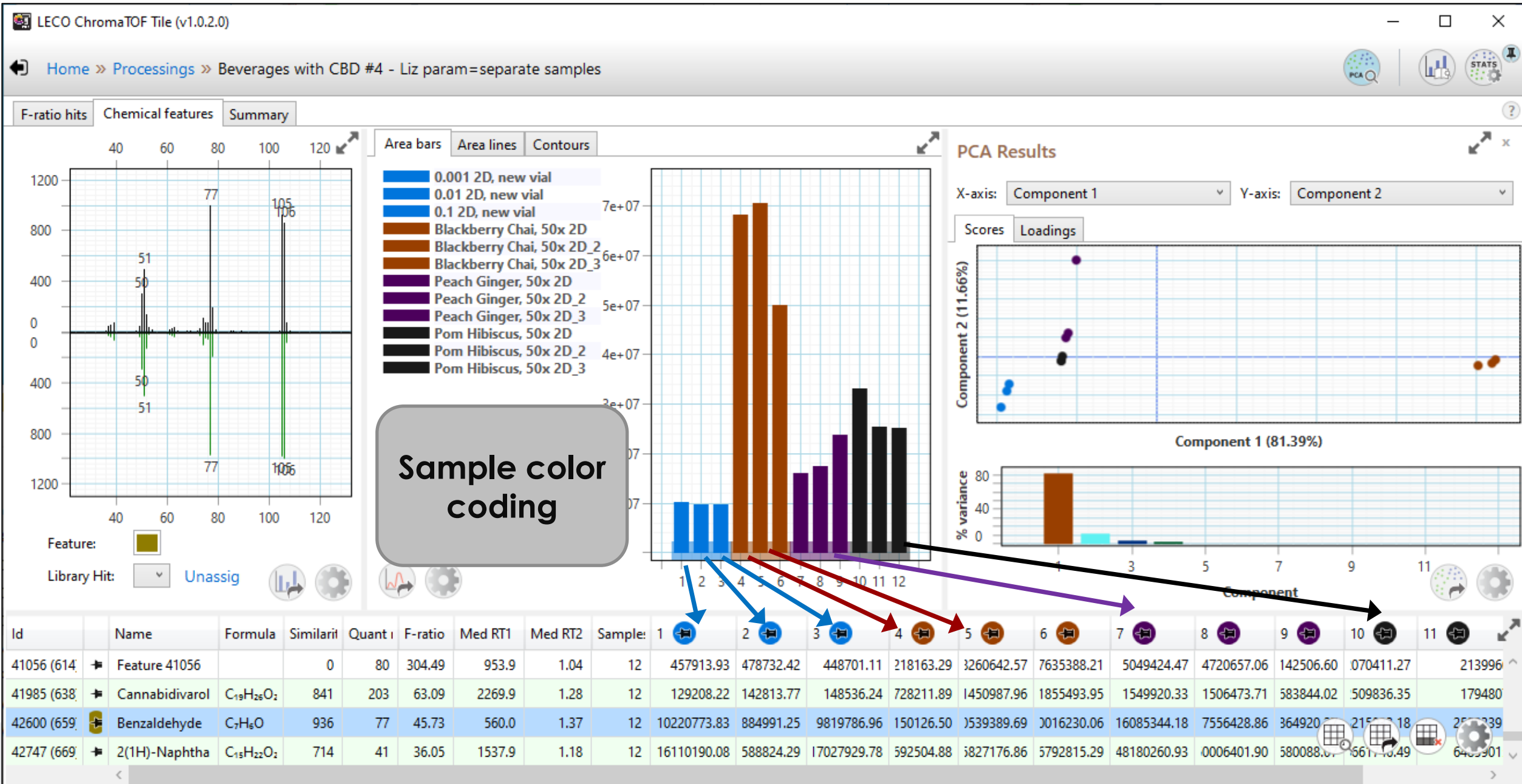
# Principle of work with CHT-Tile

- 5) Chemical features review

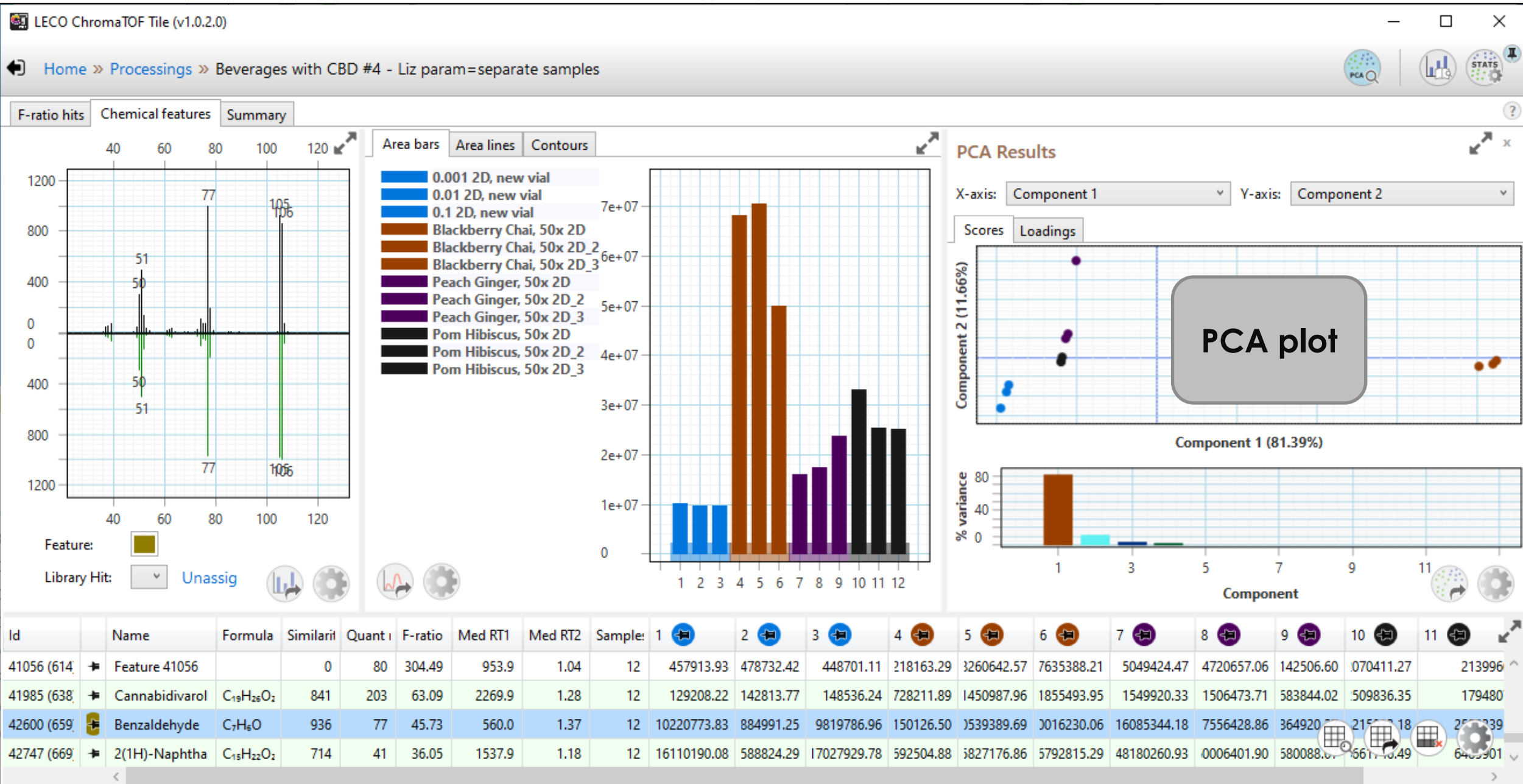
# Chemical Features Pane



# Chemical Features Pane



# Chemical Features Pane



# Chemical Features Pane

LECO ChromaTOF Tile (v1.0.2.0)

4 - Liz param=separate samples

**MS spectrum vs. library hit**

Area bars Area lines Contours

- 0.001 2D, new vial
- 0.01 2D, new vial
- 0.1 2D, new vial
- Blackberry Chai, 50x 2D
- Blackberry Chai, 50x 2D\_2
- Blackberry Chai, 50x 2D\_3
- Peach Ginger, 50x 2D
- Peach Ginger, 50x 2D\_2
- Peach Ginger, 50x 2D\_3
- Pom Hibiscus, 50x 2D
- Pom Hibiscus, 50x 2D\_2
- Pom Hibiscus, 50x 2D\_3

**PCA Results**

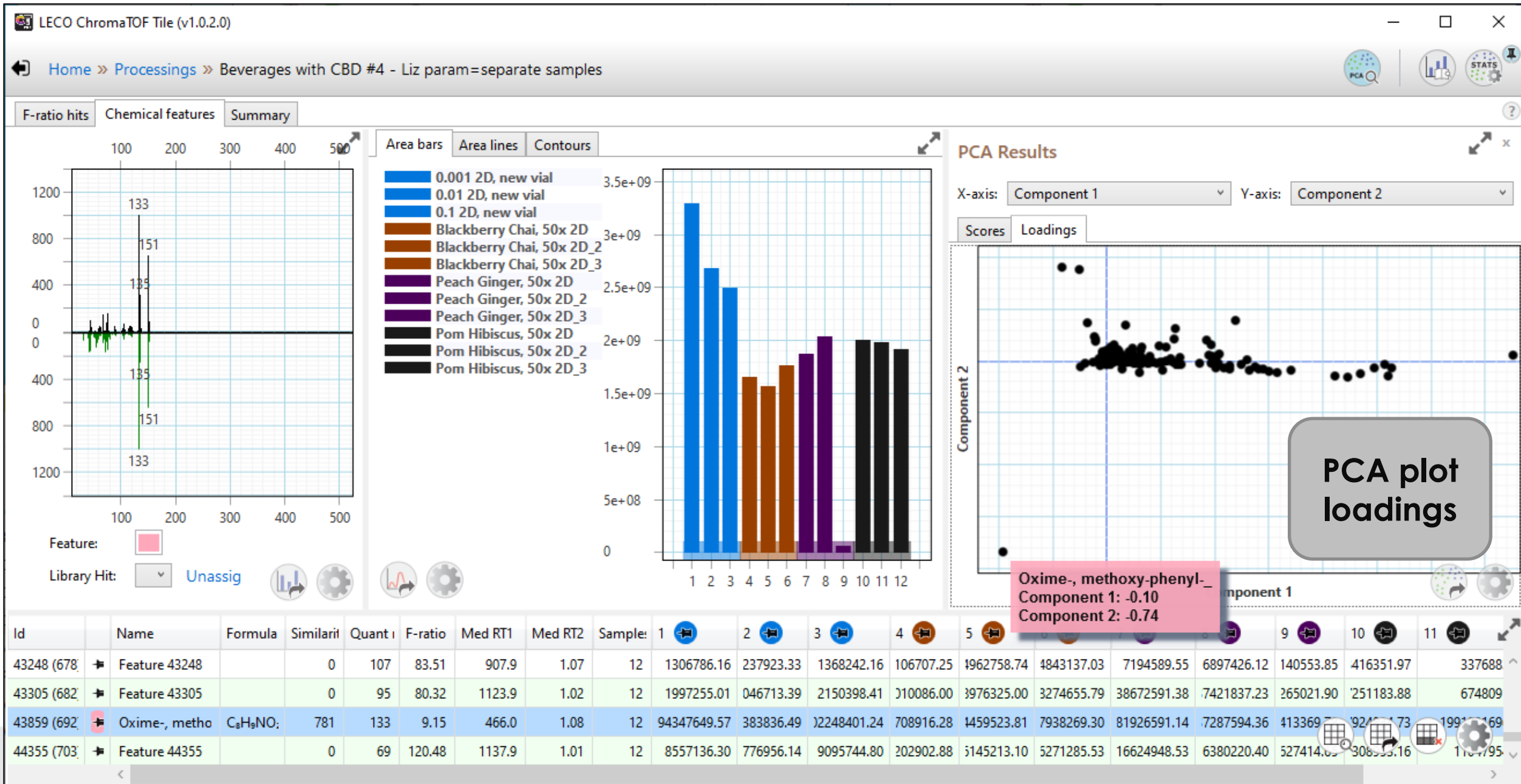
X-axis: Component 1 Y-axis: Component 2

Scores Loadings

Id	Name	Formula	Similarit	Quant	F-ratio	Med RT1	Med RT2	Sample:	1	2	3	4	5	6	7	8	9	10	11
41056 (614)	Feature 41056		0	80	304.49	953.9	1.04	12	457913.93	478732.42	448701.11	218163.29	3260642.57	7635388.21	5049424.47	4720657.06	142506.60	070411.27	213996
41985 (638)	Cannabidivanol	C <sub>19</sub> H <sub>26</sub> O <sub>2</sub>	841	203	63.09	2269.9	1.28	12	129208.22	142813.77	148536.24	728211.89	1450987.96	1855493.95	1549920.33	1506473.71	583844.02	509836.35	179480
42600 (659)	Benzaldehyde	C <sub>7</sub> H <sub>6</sub> O	936	77	45.73	560.0	1.37	12	10220773.83	884991.25	9819786.96	150126.50	3539389.69	3016230.06	16085344.18	7556428.86	364920	215	25
42747 (669)	2(1H)-Naphtha	C <sub>15</sub> H <sub>22</sub> O <sub>2</sub>	714	41	36.05	1537.9	1.18	12	16110190.08	588824.29	17027929.78	592504.88	3827176.86	5792815.29	48180260.93	0006401.90	580088.07	661	64

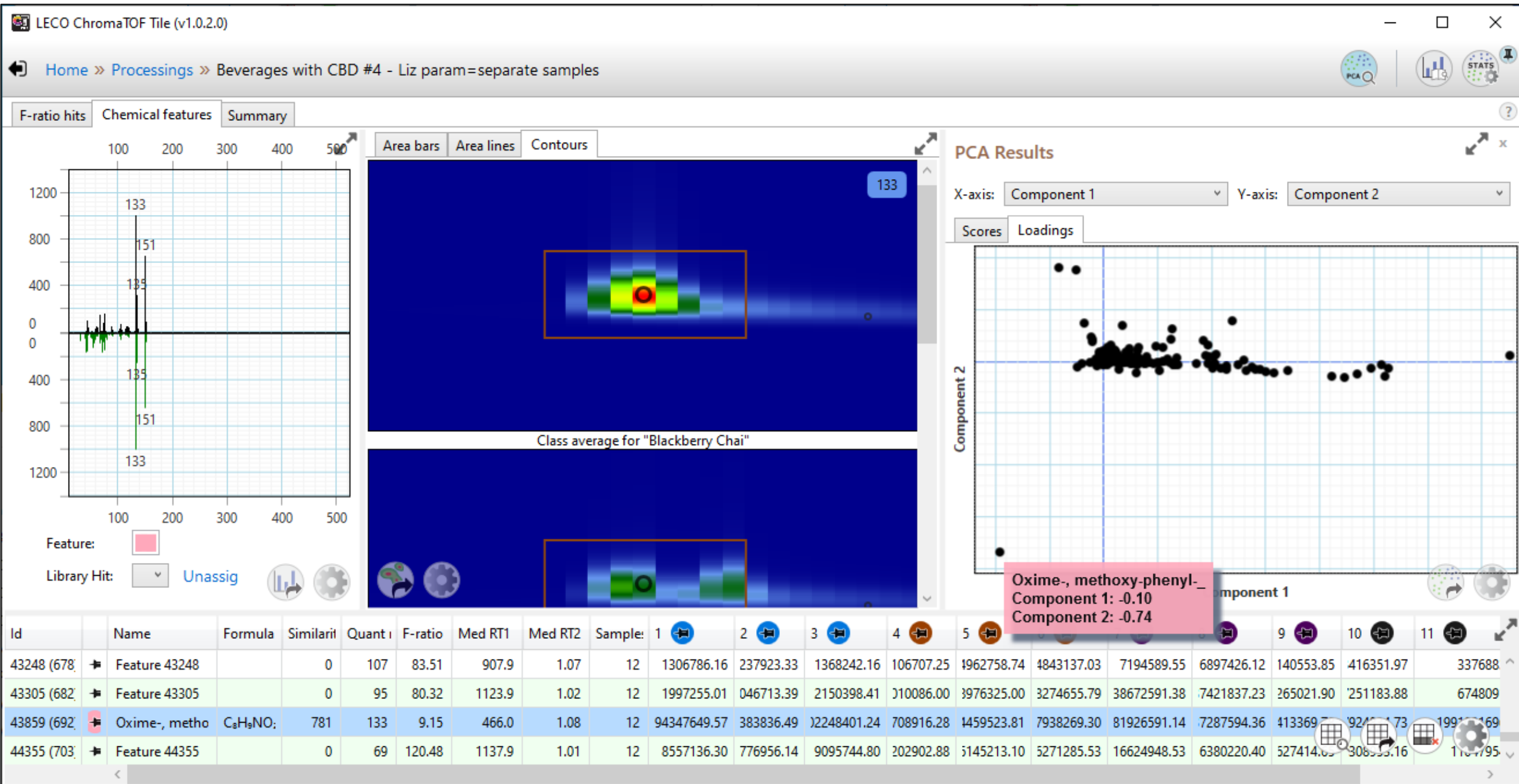


# Chemical Features Pane

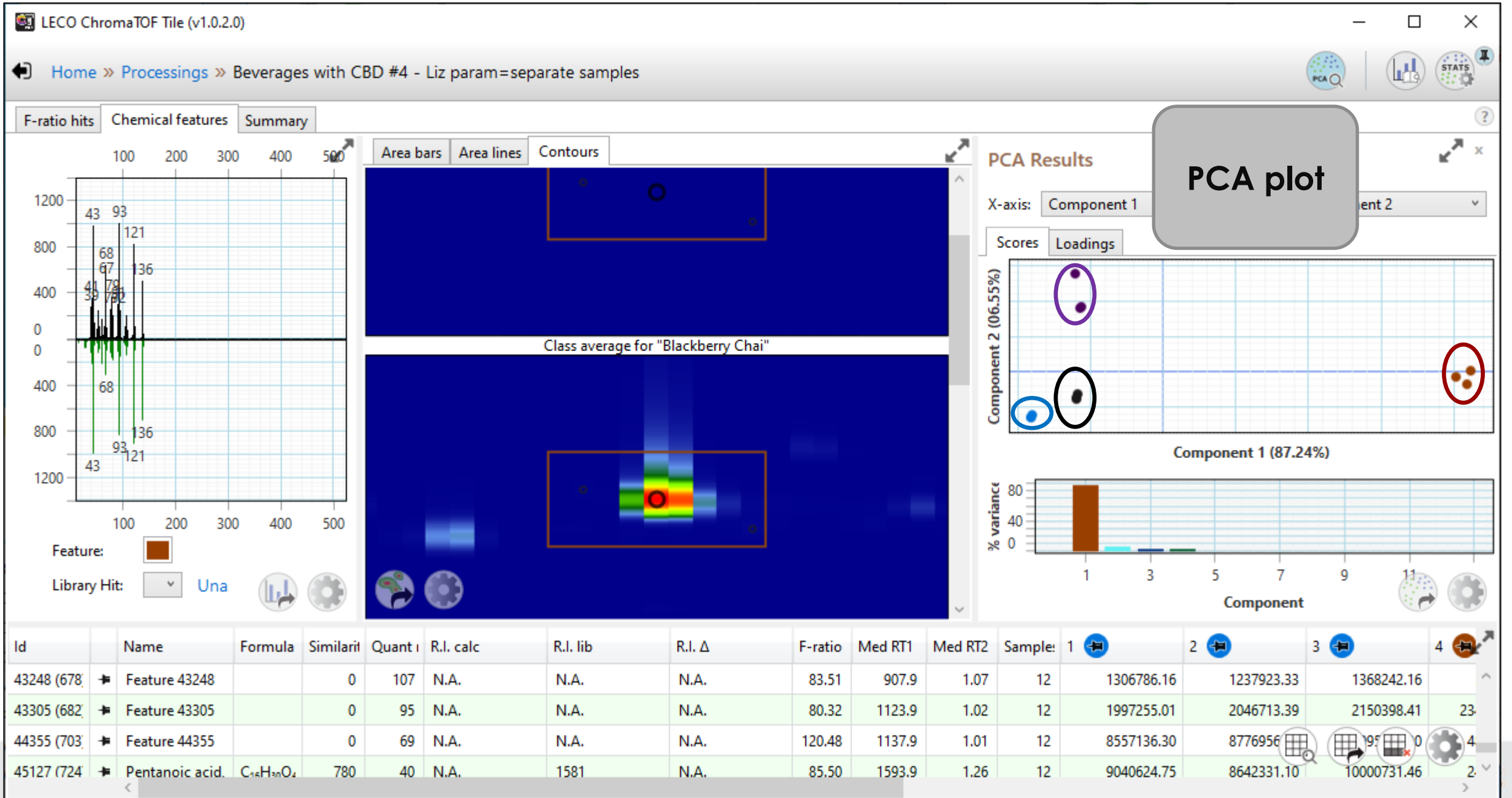




# Chemical Features Pane



# Chemical Features Pane



# Chemical Features Pane

LECO ChromaTOF Tile (v1.0.2.0)

Home » Processings » Beverages with CBD #4 - Liz param=separate samples

F-ratio hits Chemical features Summary

Area bars Area lines Contours

Class average for "Blackberry Chai"

PCA Results

X-axis: Component 1 Component 2

Scores Loadings

Component 2 (06.55%)

Component 1 (87.24%)

% variance




Component

Blanks

Samples A

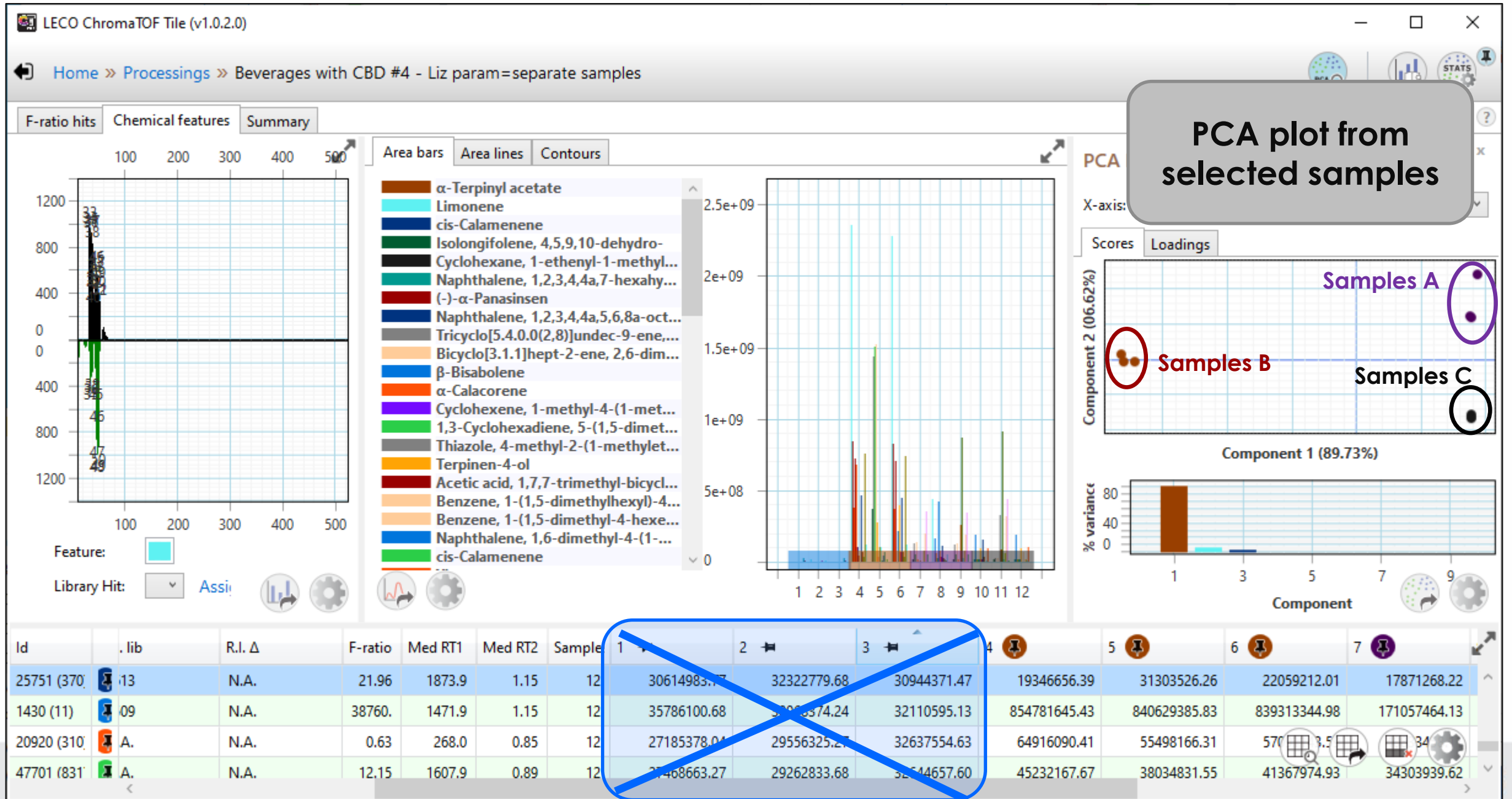
Samples B

Samples C

Feature:  Library Hit:   

Id	Name	Formula	Similarit	Quant	R.I. calc	R.I. lib	R.I. Δ	F-ratio	Med RT1	Med RT2	Sample: 1	2	3	4
43248 (678)	Feature 43248		0	107	N.A.	N.A.	N.A.	83.51	907.9	1.07	12	1306786.16	1237923.33	1368242.16
43305 (682)	Feature 43305		0	95	N.A.	N.A.	N.A.	80.32	1123.9	1.02	12	1997255.01	2046713.39	2150398.41
44355 (703)	Feature 44355		0	69	N.A.	N.A.	N.A.	120.48	1137.9	1.01	12	8557136.30	8776956.10	9500000.00
45127 (724)	Pentanoic acid.	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	780	40	N.A.	1581	N.A.	85.50	1593.9	1.26	12	9040624.75	8642331.10	10000731.46

# Chemical Features Pane



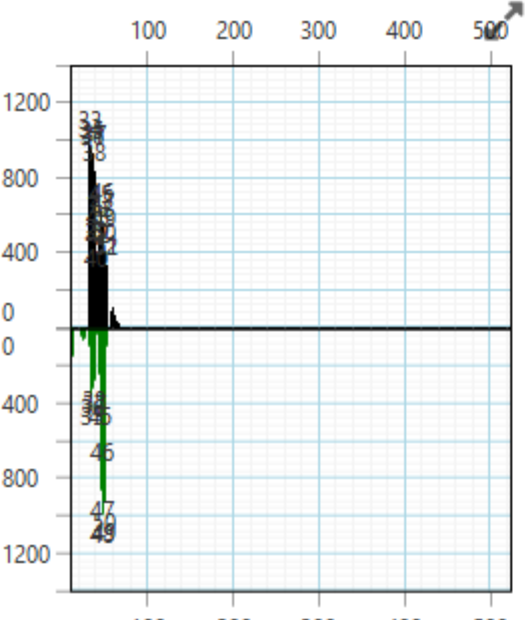
# Export to 3<sup>rd</sup> party SW

LECO ChromaTOF Tile (v1.0.2.0)

Home » Processings » Beverages with CBD #4 - Liz param=separate samples

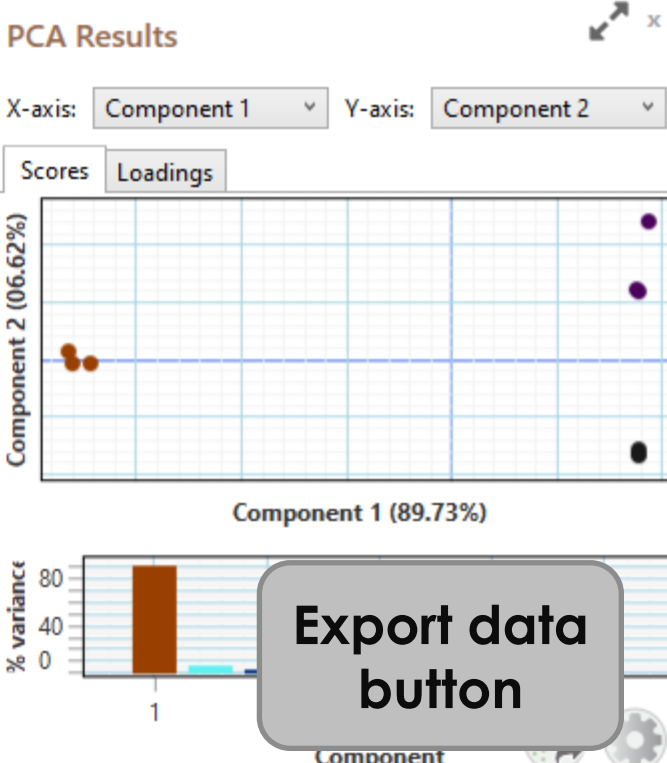
F-ratio hits | Chemical features | Summary

Area bars | Area lines | Contours



Feature:  Library Hit:  Assi

- α-Terpinyl acetate
- Limonene
- cis-Calamenene
- Isolongifolene, 4,5,9,10-dehydro-
- Cyclohexane, 1-ethenyl-1-methyl...
- Naphthalene, 1,2,3,4,4a,7-hexahy...
- (-)-α-Panasinsen
- Naphthalene, 1,2,3,4,4a,5,6,8a-oct...
- Tricyclo[5.4.0.0(2,8)]undec-9-ene,...
- Bicyclo[3.1.1]hept-2-ene, 2,6-dim...
- β-Bisabolene
- α-Calacorene
- Cyclohexene, 1-methyl-4-(1-met...
- 1,3-Cyclohexadiene, 5-(1,5-dimet...
- Thiazole, 4-methyl-2-(1-methylet...
- Terpinen-4-ol
- Acetic acid, 1,7,7-trimethyl-bicycl...
- Benzene, 1-(1,5-dimethylhexyl)-4...
- Benzene, 1-(1,5-dimethyl-4-hexe...
- Naphthalene, 1,6-dimethyl-4-(1-...
- cis-Calamenene



PCA Results

X-axis: Component 1 Y-axis: Component 2

Scores Loadings

Component 2 (06.62%)

Component 1 (89.73%)

% variance

Component

**Export data button**

Id	.lib	R.I. Δ	F-ratio	Med RT1	Med RT2	Sample:	1	2	3	4	5	6	7	8	9	10	11	12	
25751 (370)	13	N.A.	21.96	1873.9	1.15	12	30614983.77	32322779.68	30944371.47	19346656.39	31303526.26	22059212.01	17871268.22						
1430 (11)	09	N.A.	38760.	1471.9	1.15	12	35786100.68	30068374.24	32110595.13	854781645.43	840629385.83	839313344.98	171057464.13						
20920 (310)	A.	N.A.	0.63	268.0	0.85	12	27185378.04	29556325.27	32637554.63	64916090.41	55498166.31	57033333.33	34303939.62						
47701 (831)	A.	N.A.	12.15	1607.9	0.89	12	27468663.27	29262833.68	32644657.60	45232167.67	38034831.55	41367974.93	34303939.62						



# Export to 3<sup>rd</sup> party SW

LECO ChromaTOF Tile (v1.0.2.0)

Home » Processings » Beverages with CBD #4 - Liz param=separate samples

F-ratio hits | Chemical features | Summary

Area bars | Area lines | Contours

PCA Results

X-axis: Component 1 | Y-axis: Component 2

Scores | Loadings

Export data

Export:

- All rows
- Pinned/selected rows

When numeric data is missing:

- Write an empty cell
- Write zero

Include nominal mass spectra

Save/copy format:

- Comma-separated values (CSV)
- Tab-separated values (TSV)

Save to file

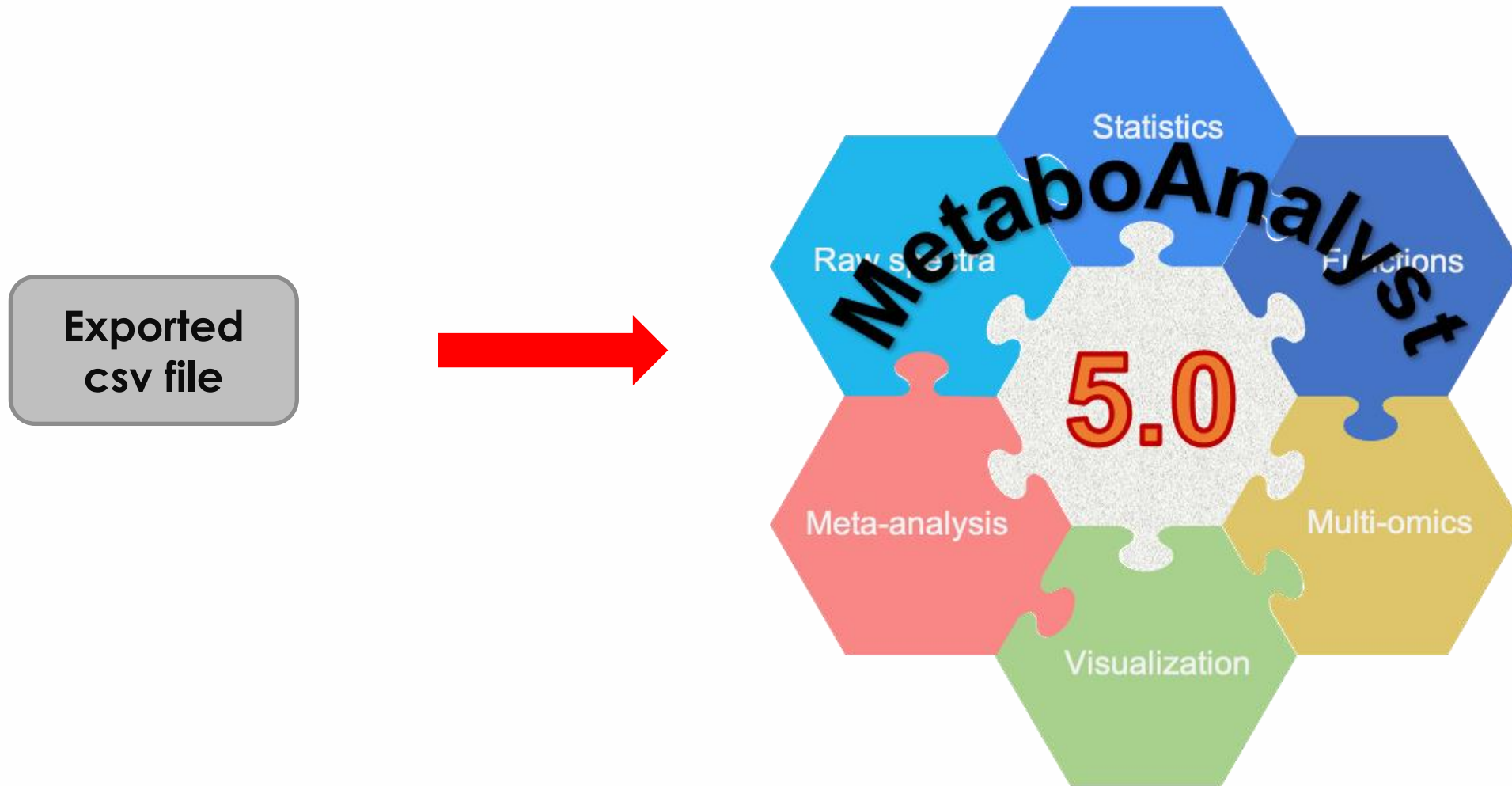
Copy to clipboard

The screenshot displays the LECO ChromaTOF software interface. On the left, a mass spectrum plot shows intensity versus m/z, with peaks labeled at 33, 35, 37, 39, 41, 43, 45, 47, and 49. Below the plot is a legend for 'Feature' (cyan square) and 'Library Hit' (dropdown menu). The central panel contains a list of chemical features with corresponding colored bars, including α-Terpinyl acetate, Limonene, cis-Calamenene, Isolongifolene, Cyclohexane, Naphthalene, (-)-α-Panasinsen, β-Bisabolene, α-Calacorene, Cyclohexene, 1,3-Cyclohexadiene, Thiazole, Terpinen-4-ol, Acetic acid, Benzene, and cis-Calamenene. To the right, a PCA Results window shows a scatter plot of Component 2 (06.62%) versus Component 1, with a bar chart below it showing the percentage of variance explained by each component. An 'Export data' dialog box is open in the bottom right corner, allowing the user to select export options such as 'All rows', 'Write an empty cell', 'Comma-separated values (CSV)', and 'Copy to clipboard'.

Id	.lib	R.I. Δ	F-ratio	Med RT1	Med RT2	Sample:	1	2	3	4	5	6
25751 (370)	13	N.A.	21.96	1873.9	1.15	12	30614983.77	32322779.68	30944371.47	19346656.39	31303526.26	
1430 (11)	09	N.A.	38760.	1471.9	1.15	12	35786100.68	30068374.24	32110595.13	854781645.43	840629385.83	
20920 (310)	A.	N.A.	0.63	268.0	0.85	12	27185378.04	29556325.27	32637554.63	64916090.41	55498166.31	570
47701 (831)	A.	N.A.	12.15	1607.9	0.89	12	27468663.27	29262833.68	32644657.60	45232167.67	38034831.55	41367974.93



# Export to 3<sup>rd</sup> party SW (e.g., Metaboanalyst)



# User manual or “How to” available?

LECO ChromaTOF Tile (v1.0.2.0)

Home

Begin new processing  
Load existing processing

**ChromatoF**  
Tile

LECO  
EMPOWERING RESULTS

[User manual](#) | [Create report for service](#) | [Change database location \(set default\)](#) | [Change NIST location \(set default\)](#) | [Graphics options](#) | [License info](#)



# User manual and “How to” available!

## ChromaTOF® Tile Guidance Document

Part Number 200-816  
December 2020

Patent Pending in U.S.A. and other countries  
© 2020 LECO Corporation  
Minor revisions may not be reflected in this manual.



**LECO**  
EMPOWERING RESULTS

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The first dimension tile size is marginally too small in Figure 7, following; it fails to span the whole peak in at least one sample (B4). Such incomplete integration in some samples confounds significance calculations, chemical fea

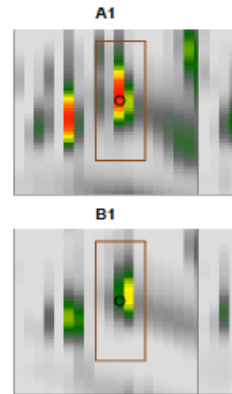


Figure 7. First

The first dim  
in Figure 8, f  
minimizing in  
retention tim

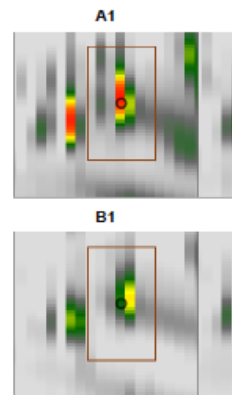


Figure 8. App

### How to Review Features



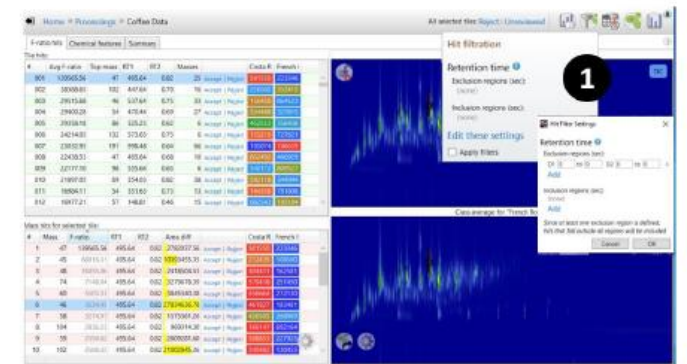
#### 1. View tentative chem

The spectrum for ea  
This feature spectru  
subtracting the aver  
feature is least inter  
wherein the feature  
several columns in t  
Similarity, RI (lib), e


#### 2. View relative trends

Relative trends betw  
specified quant mas  
autocoruration). The  
has the same displa  
F-ratio Hits tab. The  
table. They are also  
(shown in the previous screenshot) or Area Lines chart (second tab).

### How to Filter F-Ratio Hits



#### 1. OPTIONAL: Filter Tile Hits

Retention window filters can be applied (through the  icon) to selectively hide or display hits from the Tile hits table. The filter regions are defined as a retention window box by entering a time range (in seconds) in D1 and a time range (in seconds) in D2. If entered as an exclusion region, any hit within this defined window will be hidden/removed from the Tile hits table. If entered as an inclusion region, only hits within this defined window will remain in the Tile hits table. If both inclusion and exclusion regions are entered, the behavior is to include everything outside of the exclusion region and include everything inside the inclusion region, which allows for including sections of an exclusion region, if desired.

# We should remember that ...

## ... fundamentally its all about Statistics

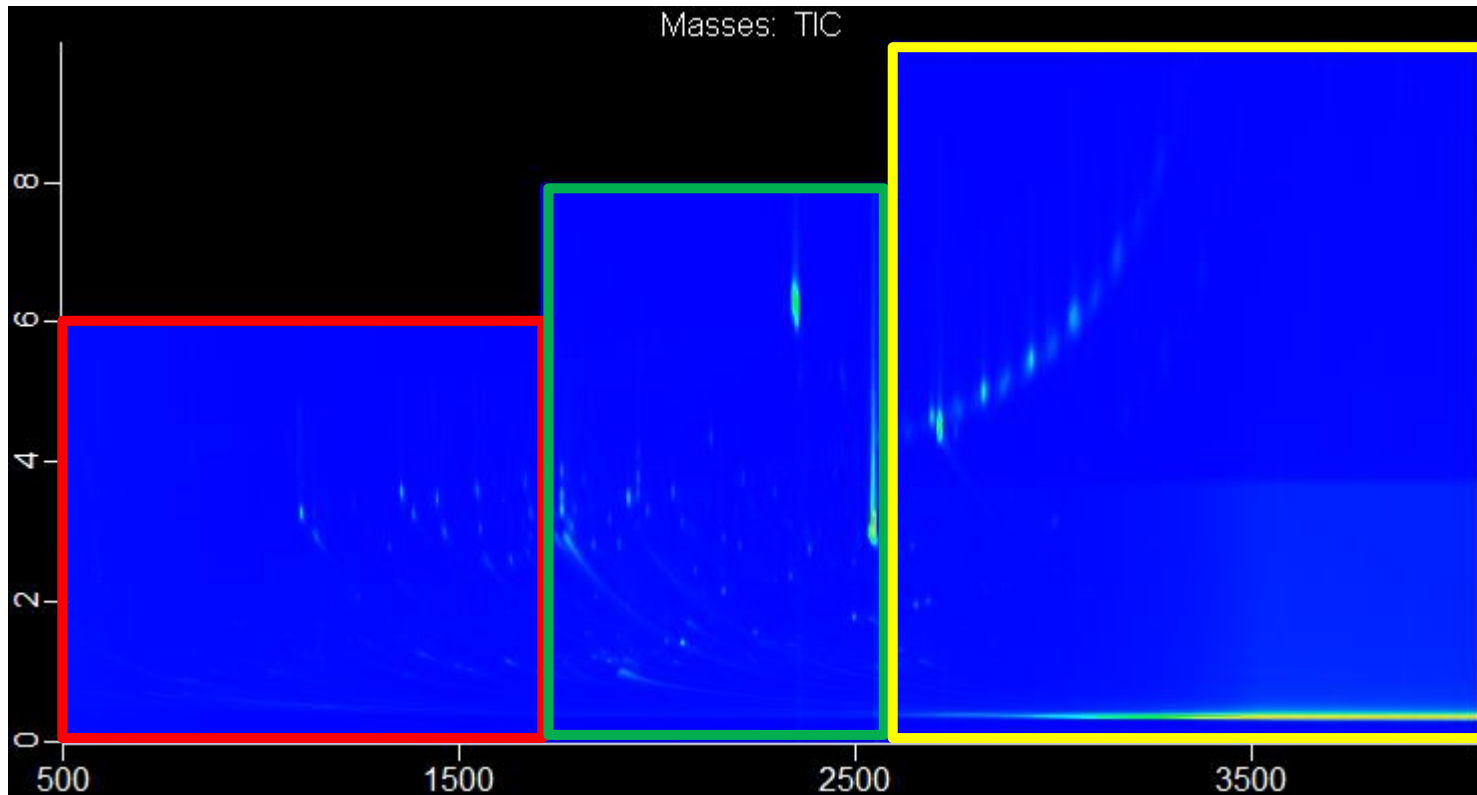
- Replicates are good & many classified samples are good.
- Random number of unclassified samples is VERY bad = UN-USEABLE.

# Practical hints based on beta-testers review:

# Practical hints based on beta-testers review:

Support variable modulation

YES, by cutting to areas with the same MP





# Conclusion



- Revolutionary data analysis software for GCxGC-TOF MS data
- Versatile tool for data comparison identifying statistically significant differences between classes of samples
- GCxGC-TOF MS data alignment tool with fast & effective processing
- Over traditional approaches reduces days-to- weeks of work down to hours, or even minutes
- Algorithm based on prof. Synovec's tile-based Fisher ratio analysis
- Compatible with with all of LECO's GCxGC TOF MS instruments - *Pegasus® BT 4D*, the *Pegasus GC-HRT+ 4D*, and the original *Pegasus 4D*
- [A free 90-day](#) trial of the software (for all current LECO GCxGC customers).

**Thank you for your attention 😊**

