ZhuLab

Lipid4DAnalyzer Help

Documentation, tutorial and configuration

Yandong Yin 1-25-2025

Contents

Lipid4DAnalyzer Documentation2	<u>'</u>
Introduction2	<u>)</u>
Interface2) -
Initial interface2	,
Function bar2)
Project and data management2) -
Data processing tools2) -
Go back to Met4DX2	,
Other functions	,
Parameter setting3	;
Load a parameter set	5
Parameter sets	5
Data processing	;
Run analysis	;
Other functions4	ļ
Library configuration4	ŀ
Interactive data viewer4	ŀ

Lipid4DAnalyzer Documentation

Introduction

Lipid4DAnalyzer is part of Met4DX software. It is a fast, robust, and user-friendly mass spectrometry data processing tool for lipidomics data analysis by rebuilding the online version of Lipid4DAnalyzer (http://lipid4danalyzer.zhulab.cn).

Interface

Initial interface

LapidiEtheadysee (Nestine 2.1.0)				- 0 X
Course Project Expert Results Par	anneler Set	Abort		Tools Likewise Cooly Hap
Project viewer	Fature Li V Vetose			Quantification box plot
Project file viewer			Interactive data viewer	EIC/EIM plot
Project file viewer				Spectrum plots

Function bar



Project and data management

- 1) Open a previously saved project or save the current project to a file.
- 2) Export data for reading by Lipid4DAnalyzer from backend.

Data processing tools

- 3) Load a parameter set with defined data analysis workflow or save parameters of current data analysis workflow to a parameter set.
- 4) Submit experiments for data processing using the current data analysis workflow or abort the processing for all submitted experiments.

Go back to Met4DX

5) Go back to Met4DX for raw data processing

Other functions

- 6) Config Met4DX software and lipidomics library.
- 7) View helps documents, check logs and report bugs.

Parameter setting

Lipid4DAnalyzer provides the most used parameter sets in Zhulab for different data processing tasks, which is highly recommended for inexperienced users, and even for all users. To start a data processing workflow, one should load a parameter set first, modifying parameters later, and submit experiment for data processing at last.

Load a parameter set

A parameter set contains multiple steps for data processing, which will be shown in the analysis workflow viewer after loading a parameter set. For lipids identification, the corresponding instrument type should be selected. One can also provide the RT calibration table for scoring RT. Fragmentation rules can be applied accordingly.



Parameter sets



Data processing

Run analysis

- 1) Left click "Run Analysis": submit all experiments for processing using the selected analysis workflow.
- Right click "Run Analysis": submit a single/all experiment(s) for data processing with selected analysis workflow.

		Left click						
	Left click	> pos	Processed					
Run Analysis		> neg	X Failed					
Run Analytis	Right click	Run Analysia pos All						

After submission, the experiments in project viewer will show the corresponding processing status.

- 💿 Processing current processing experiment.
- Submitted experiment, waiting for processing.
- 🖉 Processed already processed experiment.
- 🛛 🔯 Failed experiment.

NOTE:

• Please make sure the running experiments of the same ion mode with the selected parameter set.

Other functions

Library configuration

The lipids records in the library can be viewed in the library configuration. The reference RT calibration table and fragmentation rules can be checked by clicking the corresponding button.

braŋ	tpye lipidomic	s 🕤 Lib	rary name 3D li	pidomice ~	lon mode	positive ~					RT	Calibration Fragme	entation ru
omp	ound Info						Sp	ectrum records					
7						Total: 89178		labid	Adduct name	m/z	CCS	RT (RP lipidomics) RT (HIL
	Name	Formula	Exactmass	Smiles	Category	Classn	1	FA07070000	Car+H	260.186	185	77	360
	Car(4:0)	C11H22NO4	231.147	CCCC(=0)0	FA	Car							
	Car(5:0)	C12H24NO4	245.163	CCCCC(=0)	FA	Car							
	Car(6:0)	C13H26NO4	259.178	CCCCCC(=O	FA	Car							
	Car(6:1)	C13H24NO4	257.163	C\C=C\CCC(FA	Car							
	Car(6:2)	C13H22NO4	255.147	C\C=C\C=C\	FA	Car							
	Car(7:0)	C14H28NO4	273.194	CCCCCCC(=	FA	Car							
	Car(8:0)	C15H30NO4	287.21	CCCCCCCC(FA	Car	Sp	ectrum plot					
	Car(8:1)	C15H28NO4	285.194	CCC\C=C\C	FA	Car	1	1		caudutoa			na dan .
	Car(8:2)	C15H26NO4	283.178	CCC\C=C\C=	FA	Car		1000		C/HJMNO2			(H1471)+
)	Car(9:0)	C16H32NO4	301.225	cccccccc	FA	Car		800					
	Car(9:1)	C16H30NO4	299.21	CCCC\C=C\	FA	Car	4	600			IM-C2H10	NaHla	
2	Car(10:0)	C17H34NO4	315.241	cccccccc	FA	Car	Inter	000			[PPC3H10	and.	
3	Car(10:1)	C17H32NO4	313.225	CCCCC\C=C	FA	Car		400					
1	Car(10:2)	C17H30NO4	311.21	CC\C=C\C\C	FA	Car		200 FA Kete	1 FA Ketene+H]+ he-H2O+H]+				
5	Car(11:0)	C18H36NO4	329.257	cccccccc	FA	Car			100	150	200	25	0
											m/z		

Interactive data viewer

The interactive data viewer will load data from Met4DX for viewing by Lipid4DAnalyzer once the project is opened, which may take some time. One can check the general information for the features by selecting one line of the feature table. The details of the peaks from corresponding sample and identification results can be found in the lower panels via 'sample peak details' and 'identification details', respectively.

F	Features - pos											Quantification
Feab	ure table	• E Y	Verbose MSMS	assigned 🗌	Identified D Rule refined		-		-	To	tal: 5164	3500
	name	mz	rt	score	lipidMolecularSpecies Fir	naResult	Consistency	ConsistentAdducts	ConsistentName	nistplasma1_1	nistpl	3000
87	M341T563	341.304	562,666							2402.73	2104.	
88	M341T183	341.304	182.841							2439.18	2383.	18 2500
89	M342T56	342.263	56.3778							2498.93	2595.	2000
90	M342T103	342.335	102.852							1682.5	1702.	
91	M343T969	343.123	968.656							3767.76	3727.	Row
92	M343T56	343.294	56.4321							7497.44	6590.	anabiana
83	M348T211	348.287	211.202							1600.57	1727.	Sample groups
94	M349T114	349.211	113.653							4302.71	4045	EIC
36	M351T97	351.25	97.4627							17749.1	1861	800
96	M352T98_2	352.321	98.0373							1543.5	1454.	la l
97	M353T127	353.266	126.777							112064	1020	600
98	M354T48	354.127	48.1671							2492.71	2507.	1 400
99	M355T105	355.284	104.673							13772.3	1365/	I I I I I I I I I I I I I I I I I I I
100	M356T48	356.143	47.8822							1445.24	1471.	200
101	M356T270	356.352	270.442							2030.98	1961.	
102	M357T113	357.155	112.752							1225.74	1209.	202.5 265 267.6 270 272.5 275
103	M357T50	357.203	50.4092							6484.26	5956.	Retention time (s)
104	M357T134	357.3	133.944	0.8143	MG(18:1/0:0/0:0)					44695.4	4338-	MS/MS Spectrum
105	M358T113	358.274	112.757							144428	1370	
1												50
San	nple peak deta	ils identifica	tion details									40
m	viz	RT	Peak area (uniform)	Peak area	Peak area (w/o. baseline)	Peak height	SNR	Baseline	Sample			₹ 30
3 35	56.351	271.75	3234.56	3381.56	3332.94	745.994	63	4.50573	nistplasma2_1			inter .
4 35	56.352	270.415	2691.13	2892.72	2844.48	749.04	73	4.7731	nistplasma2_2			20
5 35	56.352	270.313	2415.61	2572.05	2515.69	676.611	59	5.46952	nistplasma3_1			10
6 35	56.352	270.47	2329.69	2595.54	2518.69	650.13	56	6.56827	nistplasma3_2			
7 35	56.352	270.024	1861.75	1897.96	1873.31	493.885	77	3.43245	nistplasma4_1			238 240 242 2 m/r (Da)

The panels in the right column show the corresponding figures as follows:

- 1) Quantification: The box plot of the peak area of different sample
- 2) EIC/EIM: EICs/EIMs of the peaks for belonging to the corresponding feature
- 3) MS/MS spectrum: MS/MS spectrum assigned to the feature

Clicking the peaks in 'sample peak details' panel will change the figures in the right column

- 1) EIC/EIM: EICs/EIMs of the peak. The red lines show the position where MS/MS spectrum was found. The corresponding MS/MS spectrum can be shown by clicking the line.
- 2) MS/MS spectrum: MS/MS spectrum under the peak. The MS/MS spectrum at the position of the solid red line in the EIC/EIM panel.



When the identification work is done, the 'MS/MS spectrum' panel of the identified features will be changed to 'MS/MS spectrum match' to show the mirror plot of the final identification and the 'identification details' panel is shown for checking the identification details. Clicking each identification, the corresponding mirror plot of identification is shown in the MS/MS spectrum match panel

Features - pos sature table	- K 7 OV	erbose 🗧 MSN	IS assigned 0	dentified 🧧 Rule refine	d					Total: 628	Quantification					
name	mz	rt	score	lipidMolecularSpecies	FinalResult	Consistency	ConsistentAdducts	ConsistentName	nistplasma1_1	nistpla ⁻	20000					
3 M679T629	678.677	629.457	0.8326; 0.8178	Cer(d20:1/24:0);	adduct{Cer+	Agreement	Cer+H	Cer(d44:1)	6250.91	5754.3	2 17500					
4 M687T493	686.575	492.578	0.6388; 0.638	DG(18:4/22:2/0:0);	adduct{DG+N	Unique	DG+NH4	DG(40:6)	4737.7	4369.4	¥ 15000	•				
5 M688T295	687.547	294.892	0.9941; 0.994	SM(d14:2/19:0);	adduct{SM+H	Unique	SM+H	SM(d33:2)	4232.85	4220.6	12500					
6 M689T520	688.591	520.49	0.6151; 0.6142	DG(18:4/22:1/0:0);	adduct{DG+N	Unique	DG+NH4	DG(40:5)	3762.09	3449.5	10000	_				
7 M689T678	688.608	677.827	1.0	CE(20:5)	adduct{CE+N	Unique	CE+NH4	CE(20:5)	1.05581e+06	1.0902		-				
8 M690T374	689.563	373.637	0.9941; 0.994	SM(d14:1/19:0);	adduct{SM+H	Unique	SM+H	SM(d33:1)	13409	12264.		Pos_nistplasma				
9 M690T349	689.564	348.851	1.0; 1.0; 1.0;	SM(d14:1/19:0);	adduct(SM+H	Unique	SM+H	SM(d33:1)	206636	19844	s	ample groups				
0 M691T393	690.51	393.222	0.9943; 0.994	PE(22:1/10:0);	adduct(PE+H	Unique	PE+H	PE(32:1)	4353.42	3770.6	EIC					
M691T424	690.546	423.727	0.9631; 0.950	PC(P-22:0/8:0);	adduct(PC(P)	SameClass	PC(P)+H; PC(0	PC(P-30:0);	12260.1	10634	ł.					
2 M691T698	690.624	698.26	1.0	CE(20:4)	adduct{CE+N	Unique	CE+NH4	CE(20:4)	1.68662e+07	1.6937	5000					
3 M692T374	691.578	373.637	1.0; 1.0; 1.0;	SM(d14:0/19:0);	adduct{SM+H	Unique	SM+H	SM(d33:0)	15572.8	15321.	4000	A				
4 M693T363	692.526	363.079	0.9923; 0.992	PC(2:0/27:0);	adduct{PC+H	Unique	PC+H	PC(29:0)	4177.86	3166.4	₹ 3000	M				
5 M693T437	692.563	437.015	0.9616; 0.957	PC(O-22:0/8:0);	adduct{PC(O	Unique	PC(O)+H	PC(O-30:0)	20511.6	17665	2000	2000				
6 M693T349	692.572	348.949	0.7151; 0.710	PC(0-22:0/8:0);	adduct{PC(O	Unique	PC(O)+H	PC(O-30:0)	3342.02	3444.0	1000	TAN				
7 M693T713	692.638	713.398	1.0	CE(20:3)	adduct{CE+N	Unique	CE+NH4	CE(20:3)	1.18076e+06	1.3201		N Inelle				
8 M696T259	695.513	259.377	0.612; 0.6043	SM(d16:1/16:1);	adduct{SM+N	Unique	SM+Na	SM(d32:2)	2086.33	1681.4	417.5 420 422.0	426 427.6 420 422.6				
9 M697T614	696.689	614.089	0.8886; 0.883	Cer(t17:0/27:0);	adduct{Phyto	Unique	PhytoCer+H	Cer(t44:0)	2170.56	2285.2	417.5 420 422.0 Re	ention time (s)				
0 M698T316	697.529	316.097	0.9472; 0.947	SM(d14:0/18:1);	adduct{SM+N	Unique	SM+Ne	SM(d32:1)	33316.7	32091	MS/MS spectrum match					
1 M700T341	699.546	340.757	0.7249; 0.724	SM(d14:0/18:0);	adduct{SM+N	Unique	SM+Na	SM(d32:0)	1634.22	1638.3	E	- Experiment spectrum				
										1	100	 Reference spectrum Matched fragments 				
iample peak detai	Is Identification of	letailis									Aig 50					
score	lipidMolecularSpe	cies adducts	mzError	rtError	rtScore	matchScore					a i	1				
0.9631	PC(P-22:0/8:0)	PC(P)+H	4	40	0.98	0.9552					nalize	1				
0.9508	PC(P-20:0/10:0)	PC(P)+H	4	41	0.96	0.9486					Ling -50					
0.9277	PC(O-20:1/10:0)	PC(O)+H	4	44	0.88	0.9486					-100					
0.9246	PC(O-22:1/8:0)	PC(O)+H	4	45	0.85	0.9552					200	400 600 8				
0.9228	PC(O-18:1/12:0)	PC(O)+H	4	44	0.88	0.9417						m/z (Da)				

Epprimert spectrum
 Reference spectrum
 Matched flagments

400 600 800 m/z (Da)

Sa	imple peak details	peak details identification details									
	score	lipidMolecularSpecies	adducts	mzError	rtError	rtScore	matchScore	100			
13		PE(P-16:0/17:0)	PE(P)+H	4	67	0.28		fils 50			
14		PE(P-18:0/15:0)	PE(P)+H	4	68	0.25		alized in			
15		PE(P-20:0/13:0)	PE(P)+H	4	68	0.25		-50 -50			
16		PE(P-22:0/11:0)	PE(P)+H	4	67	0.28		-100			
17		PE(O-16:1/17:0)	PE(O)+H	4	31	1		2	00		