User Manual for *Lipid Wizard*

Installation

1. Environment

Users must have Python 3.9.0 or later installed before download the Lipid Wizard software.
Python 3.9.0 can be downloaded from https://www.python.org/downloads/release/python-390/

2. Download

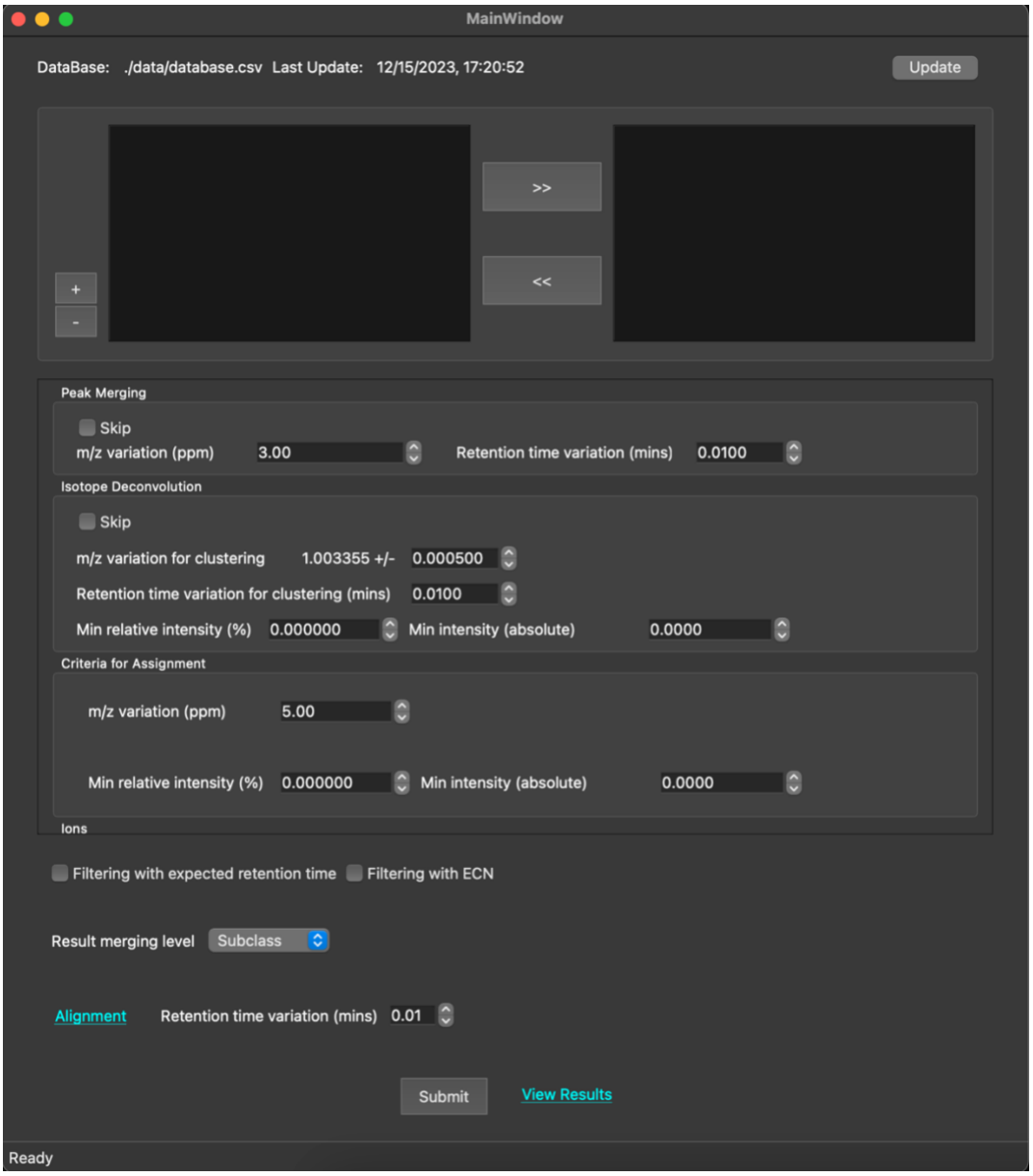
Lipid Wizard can be downloaded from https://github.com/RaoboXu/Lipidwizard.
Unzip the downloaded package.

3. Run

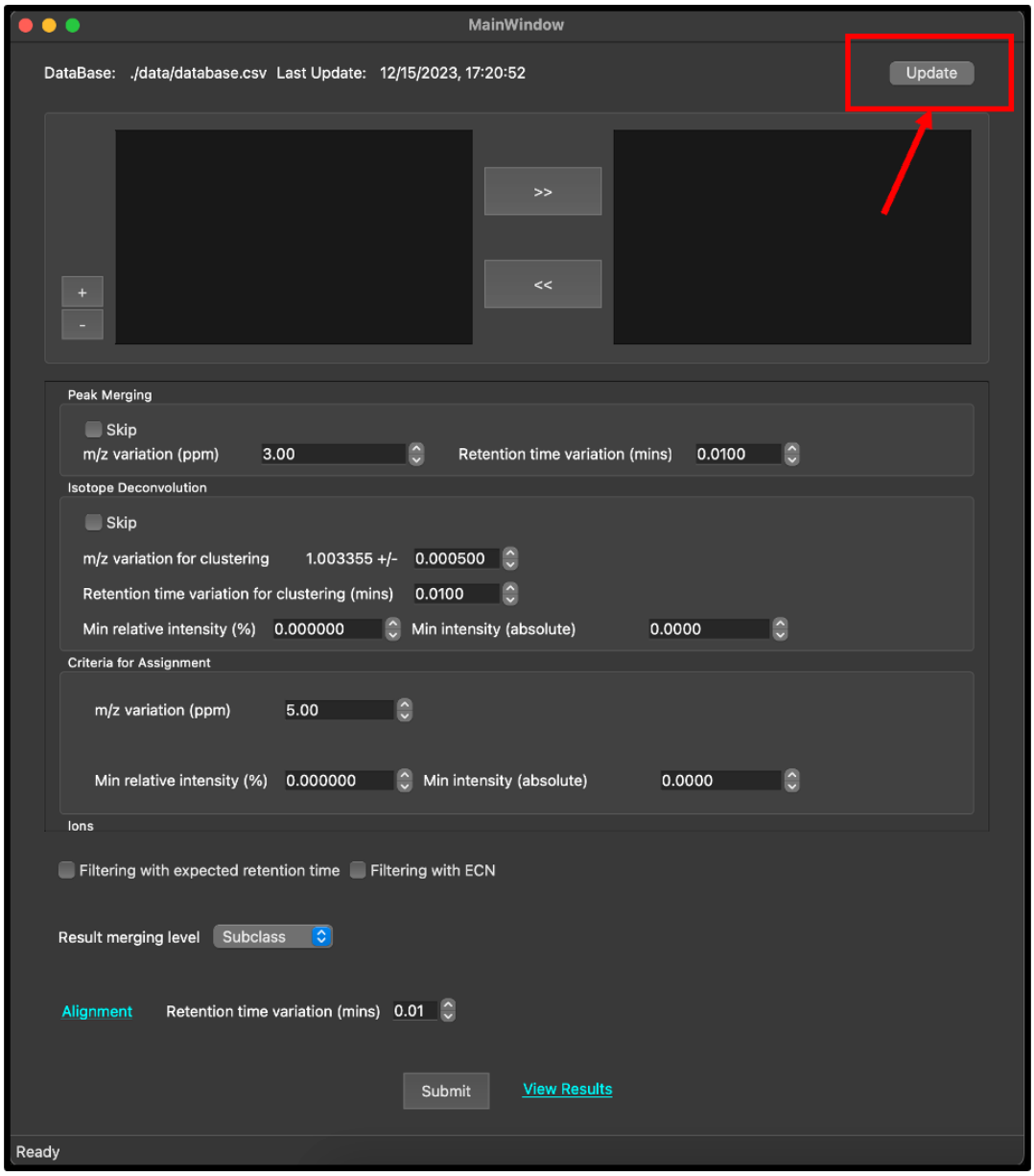
3.1 For Windows users, double click the *LipidWizard.exe*
3.2 For Ubuntu Users, double click the *LipidWizard*
3.3 For Apple OSX Users, you must open the *Terminal* and change the directory to the unzipped folder. Then, run the command: ./LipidWizard to launch the software.

Step-by-step Guidance for Lipid Wizard

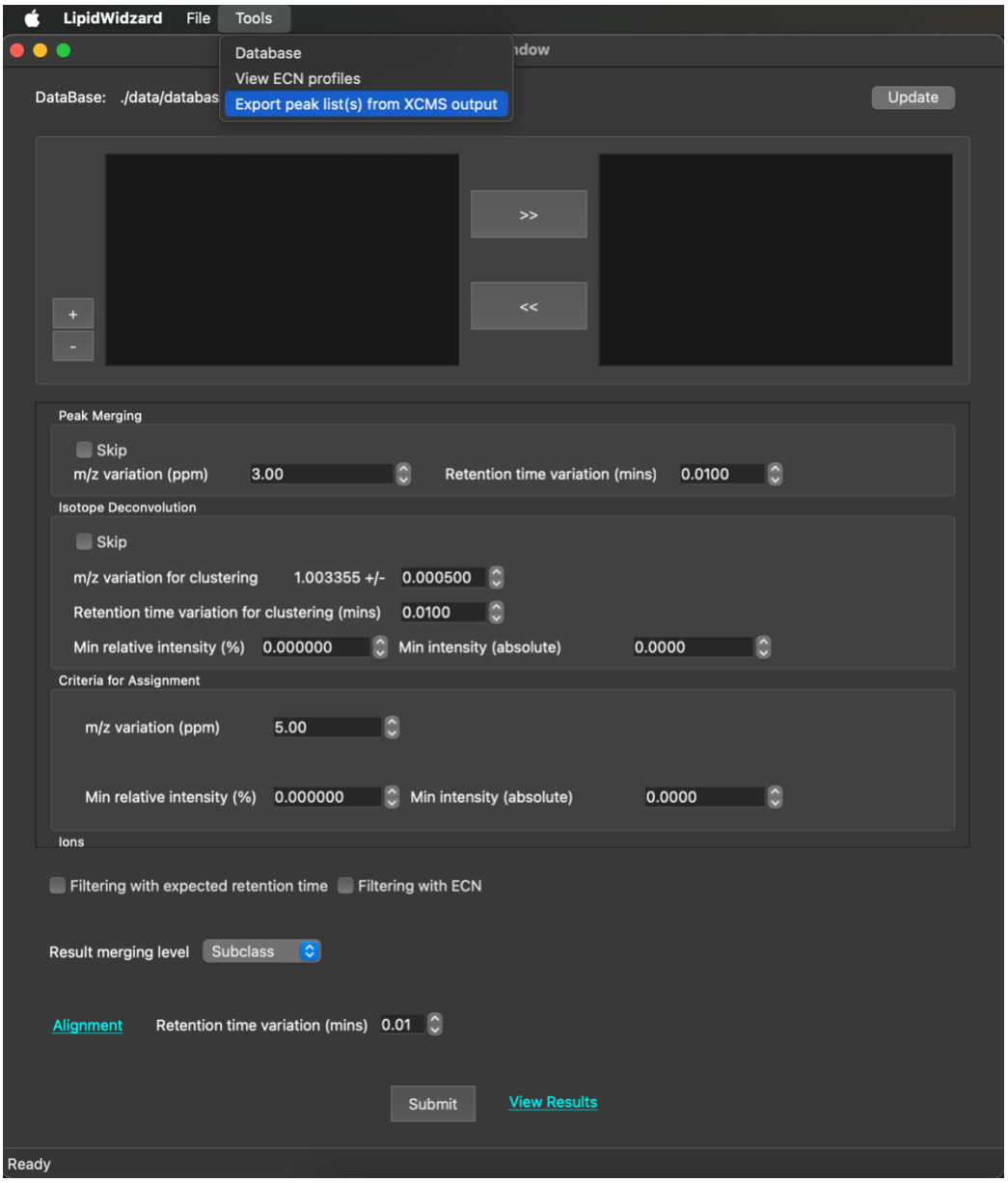
1. User Interface



2. Updating database from Lipid Maps

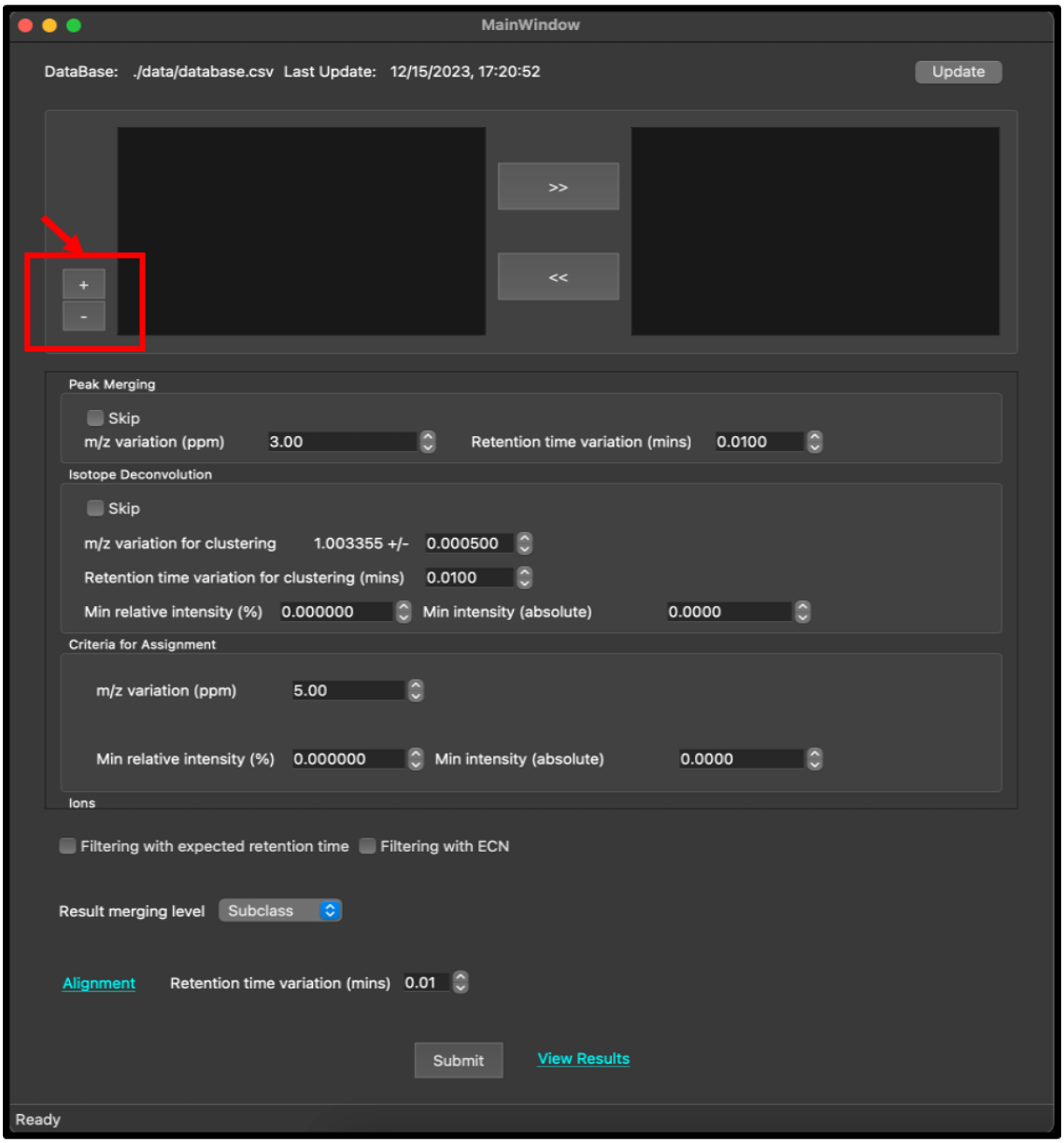


3. Split Peak List from XCMS platform

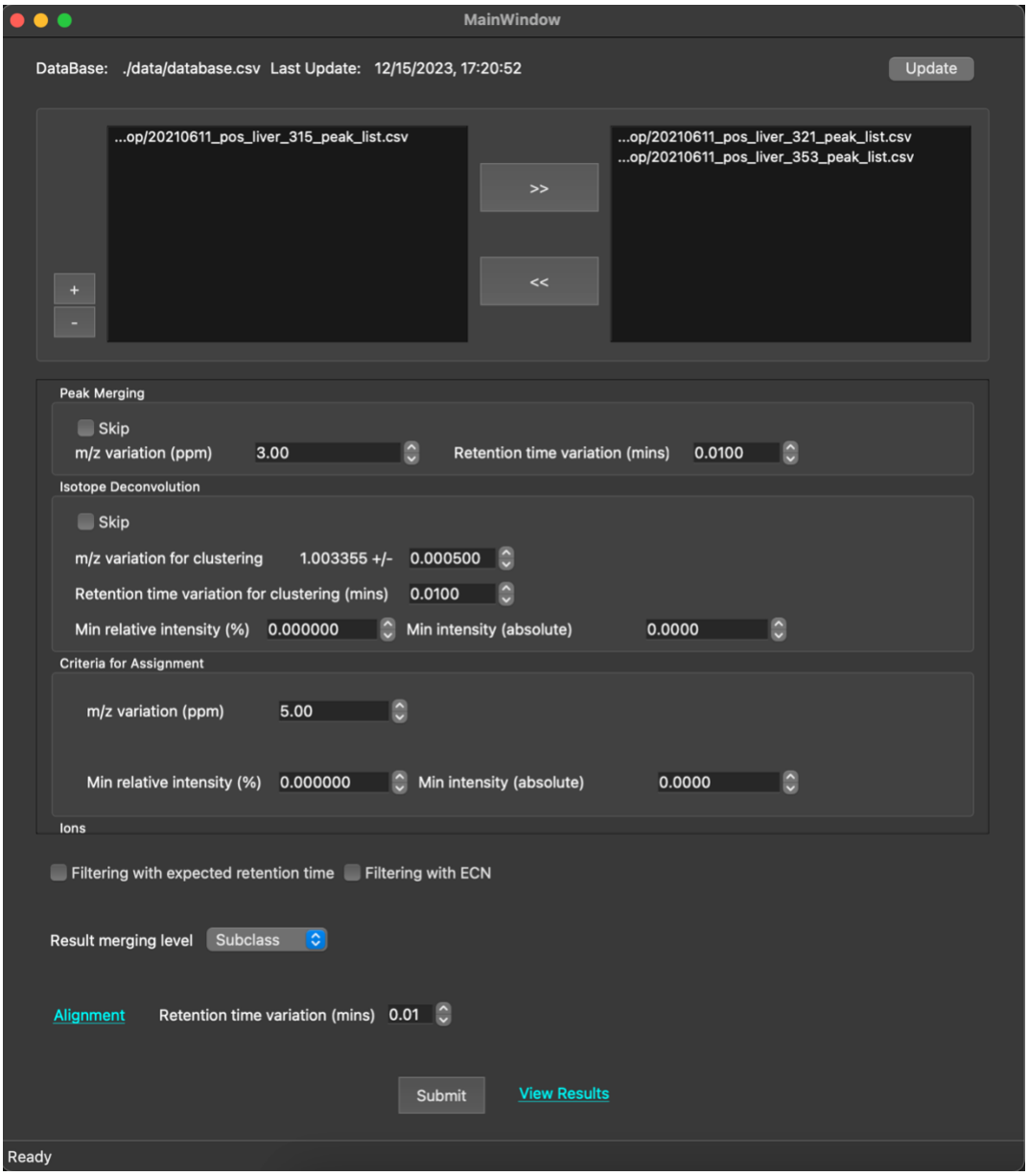


By click the "export peak list(s) from *XCMS* output", select your combined peak lists file from XCMS platform in the pop-up window. After clicking OK, *Lipid Wizard* automatically splits the combined peak list file into individual sample-dependent peak lists.

4. Sample peak list loading



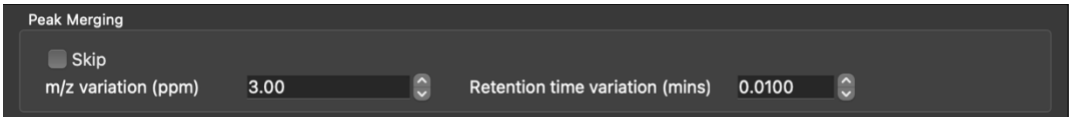
Use “+” and “-” button to select and unselect the individual sample peak lists which users want to analyze. After selecting the peak lists, click the arrow button to confirm.



5. Parameter selection for analysis

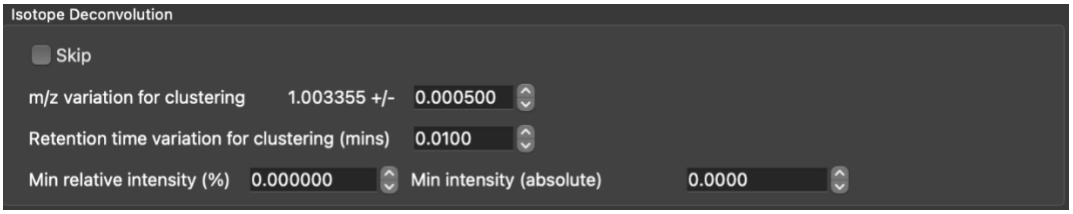
5.1 Peak Merging

Configure m/z variation and retention time variation for peak merging to get rid of the potential peak picking problem from XCMS.



5.2 Isotope Deconvolution

Configure parameters for isotopic peak clustering and stripping to quantitatively deconvolute the potential overlapping peaks.



5.3 Criteria for Assignment

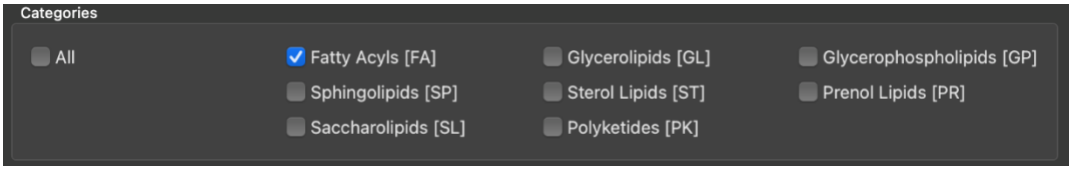
Configure parameters for assigning the lipids with database including the m/z variation in ppm, minimum relative or absolute intensity.

5.4 Ions

Configure parameters for ionization modes and possible adduct ions.

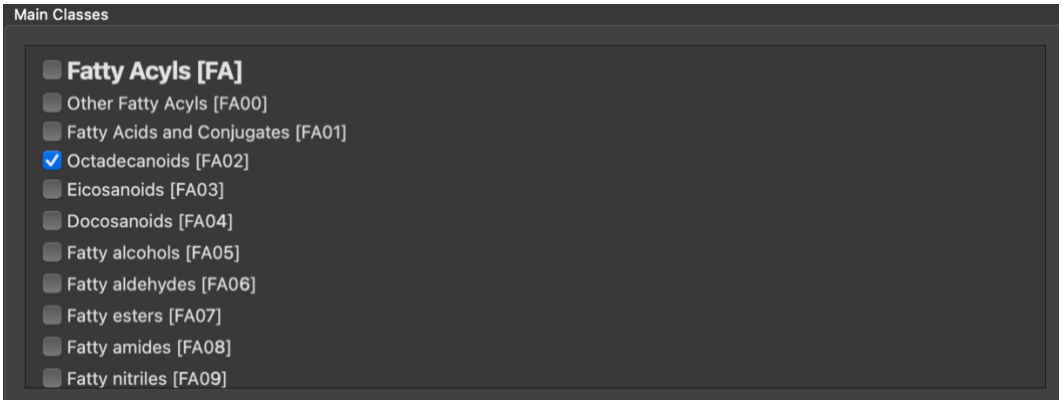
5.5 Categories

Configure parameters for interested lipid categories need to be assigned, e.g., check Fatty Acyl [FA] if users only focus on fatty acyl.



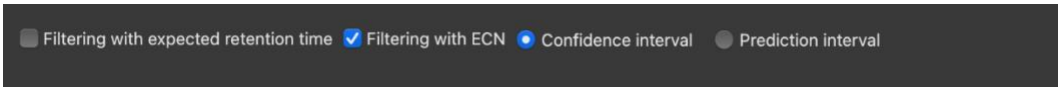
5.6 Main Classes

Configure parameters for interested lipid main classes, e.g., check Octadecanoids [FA01] if users only focus on Octadecanoids.



5.7 Enable retention time filters.

Filtering with expected retention time is to perform the ^{1}D RT filtering; Filtering with ECN is to perform the ^{2}D RT filtering.



5.8 Result merging

User can select the extra result output by merging the assigned lipid with same selected level in one row, e.g., users only focus on sub-class level lipid alteration, select sub-class to achieve an output file which merged all assigned lipids only with different subclass in the same row in the exported outputs.

5.9 Alignment

Configure Retention time variation parameter for cross-sample alignment.

6. Submit Task

7. Configuration Files Update for 1D and 2D RT Filtering

ExpectedRTRangle.xlsx (File path data/ERT_DATA) is used for 1D RT filtering. Users have to determine 1D expected RT for each lipid class based on their instrument configuration.

Row A is class, the format is Categories.Main class.Subclass. Row B and C are defined RT time ranges.

A	B	C	D
class	r.t.start	r.t.end	comment
Fatty Acyls [FA]	1	15	LCFA
Glycerolipids [GL]	1	15	DG/MG/TG
Sphingolipids [SP].Ceramides [SP02]	1	15	Cer
Glycerophospholipids [GP].Glycerophosphoglycerols [GP04].Diacylglycerophosphoglycerols [GP0401]	15	73	PG
Glycerophospholipids [GP].Glycerophosphoglycerols [GP04].Monoacylglycerophosphoglycerols [GP0405]	32	90	LPG
Glycerophospholipids [GP].Glycerophosphoinositols [GP06].Monoacylglycerophosphoinositols [GP0605]	32	90	LPI
Glycerophospholipids [GP].Glycerophosphoethanolamines [GP02].Monoacylglycerophosphoethanolamines [GP0205]	55	90	LPE
Glycerophospholipids [GP].Glycerophosphocholines [GP01].Monoacylglycerophosphocholines [GP0105]	72	90	LPC
Glycerophospholipids [GP].Glycerophosphates [GP10].Monoacylglycerophosphates [GP1005]	72	90	LPA
Sphingolipids [SP].Sphingoid bases [SP01].Lysosphingomyelins and lysoglycosphingolipids [SP0106]	72	90	LSM
Sphingolipids [SP].Phosphosphingolipids [SP03].Ceramide phosphocholines (sphingomyelins) [SP0301]	72	90	SM
Glycerophospholipids [GP].Glycerophosphoserines [GP03].Diacylglycerophosphoserines [GP0301]	73	90	PS
Glycerophospholipids [GP].Glycerophosphoinositols [GP06].Diacylglycerophosphoinositols [GP0601]	32	90	PI
Glycerophospholipids [GP].Glycerophosphoethanolamines [GP02].Diacylglycerophosphoethanolamines [GP0201]	32	90	PE
Glycerophospholipids [GP].Glycerophosphocholines [GP01].Diacylglycerophosphocholines [GP0101]	55	90	PC
Glycerophospholipids [GP].Glycerophosphates [GP10].Diacylglycerophosphates [GP1001]	72	90	PA
Glycerophospholipids [GP].Glycerophosphoglycerophosphoglycerols [GP12].Monoacylglycerophosphoglycerophosphomonoradylglycerols [GP1207]	35	90	CL
Glycerophospholipids [GP].Glycerophosphoglycerophosphoglycerols [GP12].Diacylglycerophosphoglycerophosphomonoradylglycerols [GP1202]	35	90	CL
Glycerophospholipids [GP].Glycerophosphoglycerophosphoglycerols [GP12].Diacylglycerophosphoglycerophosphodiradylglycerols [GP1201]	35	90	CL

“Lipid category”.xlsx (File path: data/ECN_DATA) is used for 2D RT filtering. It may contain up to eight .xlsx files where the file name should be exact same as the lipid category determined by Lipid Maps. In each file, users can generate new sheet for different lipid class under the same categories. Sheet name has not format, e.g., under Glycerophospholipids GP.xlsx, Lipid Wizard provides Cardiolipin (CL), Phosphatidylglycerol (PG), Phosphatidylethanolamine (PE), Phosphatidylcholine (PC) in several sheets. In each sheet, users can determine the confidence level (0% to 100%) for 2D RT filtering, and update the name (no format) in row A, lipid name at species level in row B, and its experimental RT in row C.

A	B	C	D	E	F	G	H	I	J
confidence(0.0-1.0)	0.95								
name	abbr	R.T							
14:0 (4) CL	CL 56:0	47.44							
16:0 (2)-18:1 (2) CL	CL 68:2	48.60							
16:1 (4) CL	CL 64:4	47.62							
14:1 (4) CL	CL 56:4	44.35							
18:1 (4) CL	CL 72:4	48.60							
18:0 (4) CL	CL 72:0	48.70							
16:0 (4) CL	CL 64:0	48.60							
18:2 (4) CL-d5 (deuterated)	CL 72:8	48.06							
14:1 (3)-15:1 (1) CL	CL 57:4	44.82							
15:0 (3) - 16:1 (1) CL	CL 61:1	48.48							
22:1 (3) - 14:1 (1) CL	CL 80:4	48.68							
24:1 (3) - 14:1 (1) CL	CL 86:4	48.74							

CL1 | CL2 | CL3 | PG1 | PG2 | PG3 | PG4 | PS1 | PE1 | PE1 lowDB | PE2 | PE3 | PC1 | PC1 lowDB | PC2