Paramounter.R User Manual

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- Paramounter.R is an R script to directly measure parameters for processing LC-MS-based metabolomics data
- The program is written in the language 'R' and is publicly available at https://github.com/HuanLab/Paramounter
- Please follow the instructions for Paramounter.R

1) Create a folder to store the files used for parameter optimization (**Figure 1**). Use 3-4 sample or QC LC-MS data files from the beginning, middle, and end of the sample analysis. For using mzML files, please change line 20 in the Paramounter R code part 1 and line 26 in part 2 to "filename <- list.files(pattern = ".mzML")".

Figure 1. The file organization used for Paramounter.

2) Download "Paramounter_part1 (V2).R" and "Paramounter_part2 (V2).R" from GitHub (https://github.com/HuanLab/Paramounter) (**Figure 2)**.

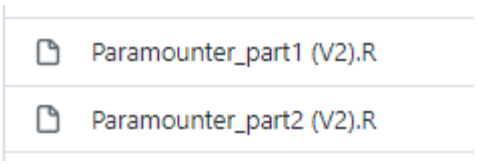


Figure 2. Paramounter part 1 and 2 on GitHub.

3) Use R Version 4.0 or newer. Install "xcms", "MSnbase", "dplyr", "ggplot2", and "gridExtra" libraries, if they were not installed previously. All packages should be updated to the newest available version.
4) Set the directory, massSDrange, smooth, and cutoff values in Paramounter part 1 (**Figure 3**).
   directory: The data path of the mzXML files.
   massSDrange: The range of standard deviation of the mass differences in each ZOI. Default value is 2 (95% confidence). Users can adjust this value (e.g., 3 for 99.7% confidence).
   smooth: Chromatographic smoothing level that is used in an embedded function to smooth out a chromatographic peak to better determine ZOIs. Default value is 0. For DDA data, use default value. For full-scan data with acquisition rate > 2 Hz, use a positive smooth value. For example, smooth = 4 for spectral rate = 4 Hz.
   cutoff: The ppm percentage for determining the cutoff line in the mass accuracy distribution. The default is 0.95 (95%). User can adjust this value based on their own discretion (e.g., 0.9 for less noise included in the parameter measurement).

```
1  ############################################################
2  #This is the script to perform parameters estimation
3  #Jian Guo, Tao Huan 2021-07-28
4  #Copyright @ University of British Columbia
5  ############################################################
6
7  library(xcms)
8  library(MSnbase)
9  library(dplyr)
10 library(ggplot2)
11 library(gridExtra)
12
13 # User input the directory and software to optimize parameters for (XCMS, MSDIAL, or MZMINE2)
14 directory <- "F:/Jian_Guo/SoftwareComparison_20211103/singleDatafilecompare20220207/New10datasetResults20220204/BrukerUrineRPdiluted10DDA"
15 massSDrange <- 2
16 smooth <- 0
17 cutoff <- 0.95
```

Figure 3. Example of parameters set up in Paramounter part 1.

5) Run Paramounter part 1 by clicking "Source" in the top right corner of the document window in RStudio. Paramounter part 1 generates a plot showing the distribution of mass accuracy in the dimension of *m/z* and the cutoff line (red line in **Figure 4**).

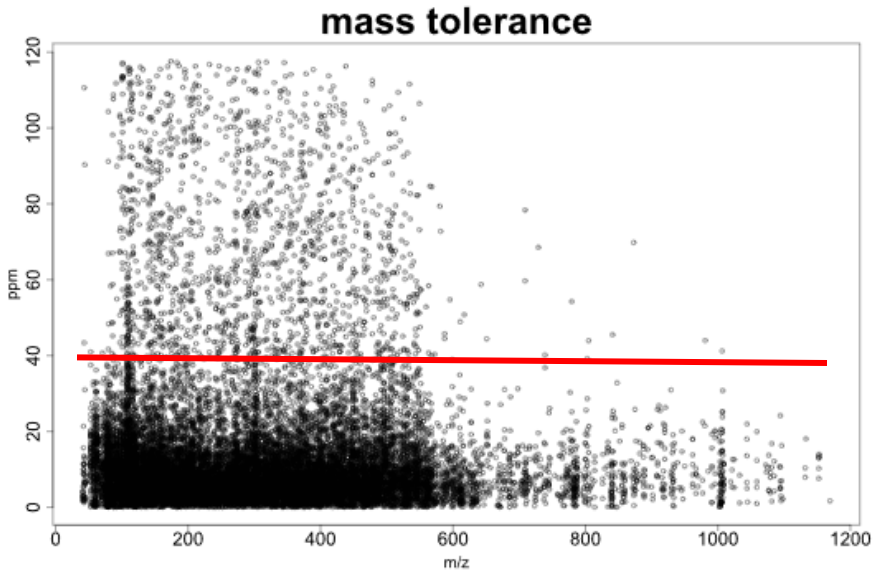


Figure 4. The mass accuracy distribution generated by Paramounter part 1.

6) Set the directory, Software, massSDrange, ppmCut, and smooth values in Paramounter part 2 (**Figure 5**).
   directory: The same as part 1. The data files used for part 2 should be the same as part 1.
   Software: The name of the software for parameter output. Select either XCMS, MSDIAL, MZMINE2, or ALL for any one of or all three software-specific parameters. The universal parameters are automatically generated together with the software-specific parameters.
   massSDrange: The same as part 1.
   ppmCut: the mass accuracy cutoff determined from Part 1.
   Smooth: The same as part 1.

```
#############################################################
#This is the script to perform parameters estimation
#Jian Guo, Tao Huan 2021-07-28
#Copyright @ University of British Columbia
#############################################################

library(xcms)
library(MSnbase)
library(dplyr)
library(ggplot2)
library(gridExtra)

directory <- "F:/Jian_Guo/Paramounter_paper_20210421/Response_20211219/10datasetREDOwithIPOAutoTuner_20220120/BrukerUrineHILICoriginalDDA/parameter"
# User input the directory and software to optimize parameters for (XCMS, MSDIAL, MZMINE2, or ALL)
# Available choices:
# "XCMS" for XCMS-based data processing
# "MSDIAL" for MSDIAL-based data processing
# "MZMINE2" for MZmine2-based data processing
# "ALL" for all three software data processing
Software <- "XCMS"
massSDrange <- 2
ppmCut <- 20
smooth <- 0
#############################################################################################
```

Figure 5. Example of parameters set up in Paramounter part 2.

7) Run Paramounter part 2 by clicking on “Source” in the top right corner of the document window of RStudio. Paramounter part 2 generates two folders. Each folder contains one .png file and one .pdf file (**Figure 6**).

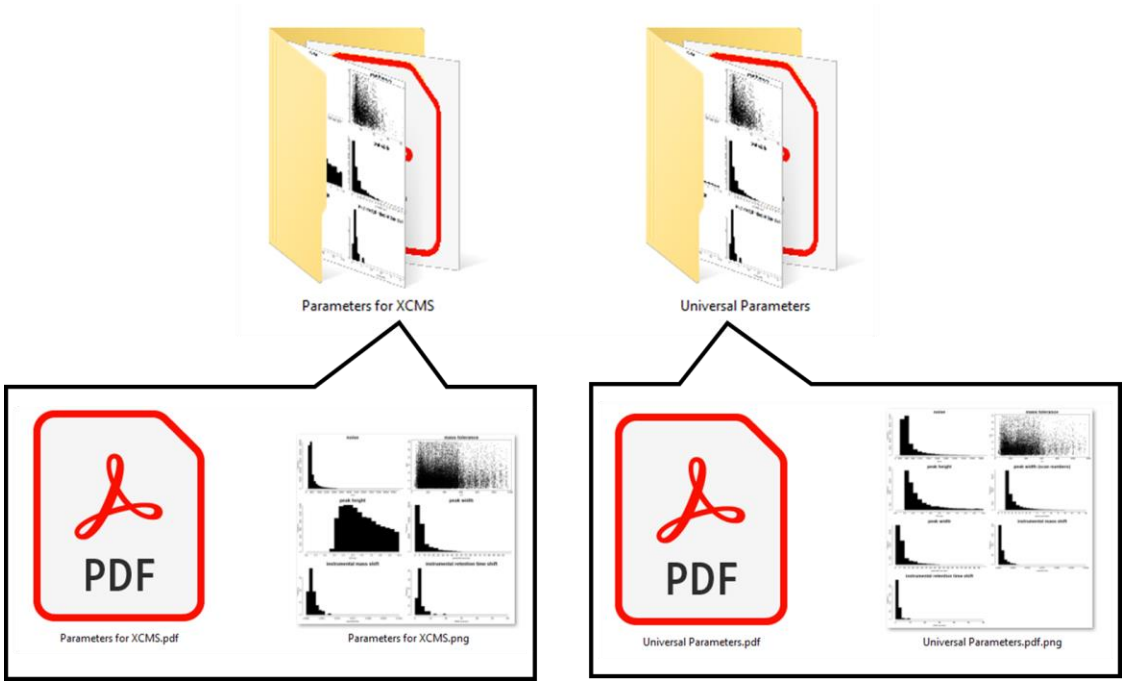
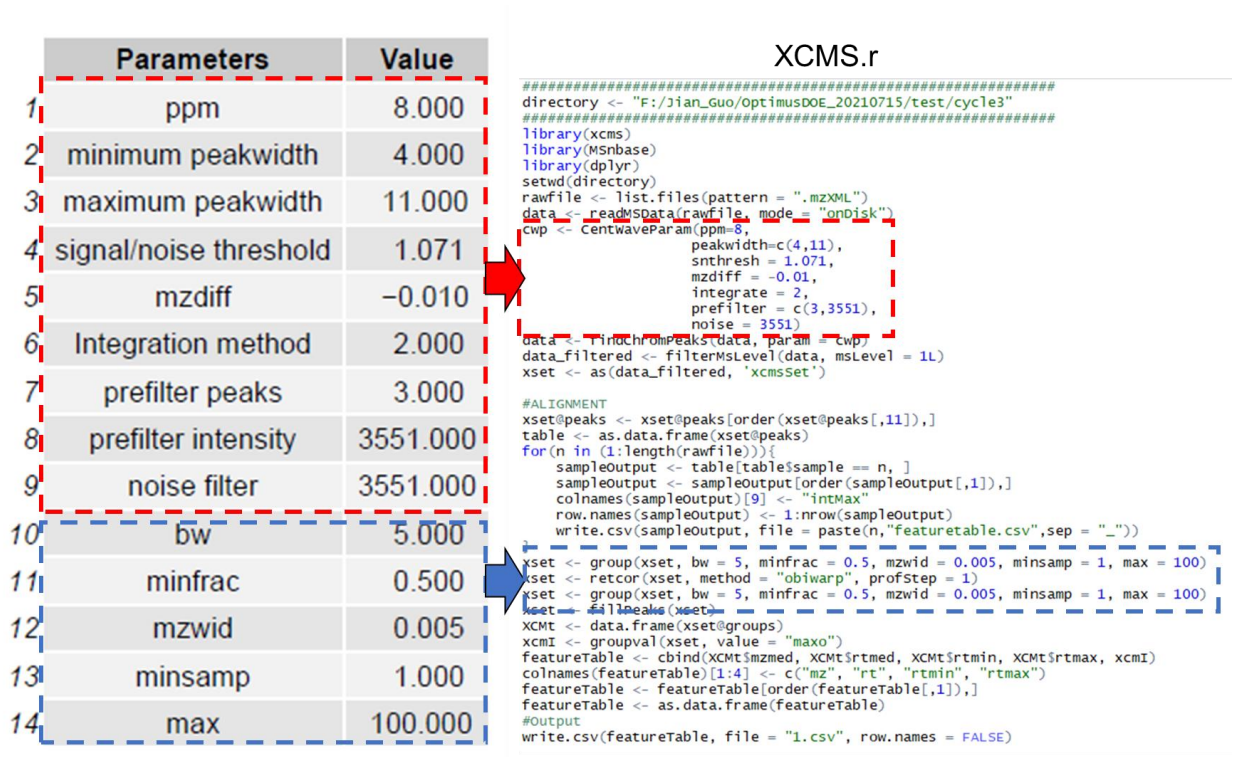


Figure 6. The output of Paramounter part 2.

8) Open the .pdf file and export the recommended values into the corresponding data processing software (e.g., XCMS.R in **Figure 7**). Open the complementary .png file to visualize the distribution of each parameter for the raw data.

Note: mzdiff is used as the mass tolerance to dereplicate the features (similar m/z values and retention times) extracted by XCMS *CentWave*. Suggested default value: 0.001 or 0.01. However, some true positive metabolic features with mass differences smaller than that value may be removed by mistake. Therefore, if a user wants to disable the dereplication function, set the mzdiff to be any negative value.

Note: Paramounter tunes an optimized peak height to maximize the number of true positive features. A drawback of that optimized value is the higher rate of false positive features and the likelihood of software crash. To address this issue, users can try a higher peak height threshold to reduce the number of false positive features (e.g., 2X the optimized peak height threshold).



	Parameters	Value
1	ppm	8.000
2	minimum peakwidth	4.000
3	maximum peakwidth	11.000
4	signal/noise threshold	1.071
5	mzdiff	−0.010
6	Integration method	2.000
7	prefilter peaks	3.000
8	prefilter intensity	3551.000
9	noise filter	3551.000
10	bw	5.000
11	minfrac	0.500
12	mzwid	0.005
13	minsamp	1.000
14	max	100.000

Figure 7. Where to copy the recommended parameters into XCMS.R.