## IsoMS User Manual For FT-ICR-MS (version 1.0; March 9, 2014)

- IsoMS is a program written in R for processing data generated by differential chemical isotopic labeling LC-MS. It consists of two scripts, IsoMS and IsoMS-align. IsoMS is for peak picking, peak pairing and peak ratio calculation from an LC-MS dataset. IsoMS-align is for alignment of peak pairs and their peak ratios from multiple datasets. These two scripts are freely available for non-commercial use from www.mycompoundid.org/IsoMS.
- The overall workflow of IsoMS for processing multiple LC-MS datasets produced from running multiple samples in a typical metabolomics study includes three steps: 1) converting the raw LC-MS data into a centroid peak list file (CSV format) and storing all the converted files in a folder, 2) using IsoMS to process all the files automatically, and 3) using IsoMS-align to align the peak pairs and their ratios from all the datasets to produce the final CSV file that can be exported for data and statistics analysis.
- The instruction for data conversion in Step 1 and the script (BD-convert) used to convert the data file into a format compatible with IsoMS can be downloaded from the MyCompoundID website.
- The instructions for installing and using the IsoMS (i.e., IsoMSFT for this instruction) and IsoMS-align scripts are given below.

## **1. Installation of IsoMS**

- 1) Two versions of IsoMS, IsoMSFT and IsoMSTOF, have been developed for processing the FT-ICR-MS data and TOF- or QTOF-MS data, respectively. For processing the FT-ICR-MS data, download the IsoMSFT version (zipped file).
- R graphic user interface (RGui) should be installed in a computer with Windows XP or Windows 7. RGui can be downloaded from the following URL: <u>http://www.r-project.org/</u>. Since IsoMS has been packaged with RGui 3.0, it is recommended to install the same version to run IsoMS.
- 3) If a different version of RGui is installed, R-tools is required for packaging the IsoMS source files which can be found in the src folder within the IsoMS unzipped folder (Figure 1). R-tools can be downloaded from the URL: <u>http://cran.rstudio.com/</u>.

Name	Date modified	Туре	Size
퉬 input	2/10/2014 5:59 PM	File folder	
🐌 output	2/10/2014 5:59 PM	File folder	
퉬 src	2/11/2014 2:06 PM	File folder	
🖼 derivatives	7/15/2013 11:57 PM	Microsoft Office E	1 KB
IsoMSFT_1.0	2/10/2014 3:49 PM	WinRAR ZIP archive	33 KB
IsoMSMain	2/10/2014 5:10 PM	R File	12 KB
🐴 mzBackground	7/15/2013 11:57 PM	Microsoft Office E	1 KB
🖼 mzCutoff	7/15/2013 11:57 PM	Microsoft Office E	1 KB

Figure 1. Unzipped IsoMS folder (for FT-MS version).

4) During the installation of R-tools, the environmental variables should be checked (see Figure 2). Otherwise a user needs to edit the R environmental variables after installation by right clicking Computer → Properties → Advance system properties → Advance → Environmental variables.

Setup - Rtools	
Select Additional Tasks Which additional tasks should be performed?	<u>1</u>
Select the additional tasks you would like Setup to perform while installing Rtools, th dick Next.	ien
Edit the system PATH.	~
Current value: PATH=C:\Program Files (x86)\CambridgeSoft\ChemOffice2005\Common\DLLs;c:\Rtools\bin; c:\Rtools\gcc-4.6.3\bin;%SystemRoot%\system32;%SystemRoot%;	E
%SystemRoot%\System32\Wbem;%SYSTEMROOT%\System32\WindowsP C:\Program Files\WIDCOMM\Bluetooth Software\; C:\Program Files\WIDCOMM\Bluetooth Software\syswow64; C:\Program Files (x86)\Common Files\Bruker Daltonik\DLLs\; C:\Program Files (x86)\Common Files\Bruker Daltonik\DLLs; C:\Program Files (x86)\Common Files\Bruker Daltonik\DLLs;	
C:/PROGRA~1/R/R-30~1.1/bin/i386	-
< <u>B</u> ack <u>N</u> ext > C	ancel

Figure 2. Select/check the environmental variables during the installation of R-tools.

- 5) Windows 7 users must change "user account control" to the lowest level in order to package the IsoMS scripts by opening Control panel  $\rightarrow$  User account  $\rightarrow$  User account settings.
- 6) After downloading IsoMSFT.zip, unzip it. In the unzipped file folder (Figure 1), there is another zipped file, IsoMSFT\_1.0; DO NOT unzip it. Install it in RGui by clicking Packages → Install packages(s) from local zip files (Figure 3), and then select the zipped IsoMS package named as "IsoMSFT\_1.0".

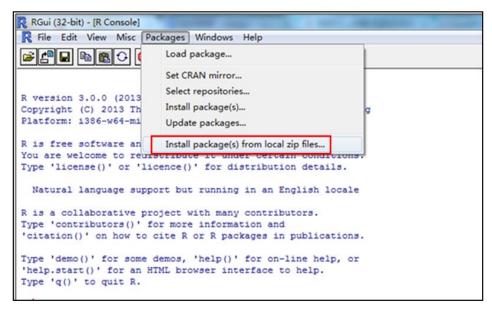


Figure 3. Install the IsoMS package in RGui.

 Assign the unzipped folder of IsoMS (Figure 1) as the working folder of RGui by clicking: File → Change dir..... (Figure 4).

File Edit View	Misc Packages Windows Help
Source R coo New script Open script. Display file(s	
Load Works Save Works Load History Save History	, s with ABSOLUTELY NO WARRANTY. bute it under certain conditions. ( e()' for distribution details.
Change dir	but running in an English locale
Print Save to File	t with many contributors. ore information and R or R packages in publications.
Exit	s, 'help()' for on-line help, or

Figure 4. Change the working folder of RGui.

## 2. Running IsoMS

1) Run IsoMS by clicking: File  $\rightarrow$  Source R code... to open the working folder (Figure 5), and then click "IsoMSMain".

Source R code	
New script	
Open script	
Display file(s)	) "Masked Marvel"
	undation for Statistical Computing
Load Workspa	ce 1386 (32-bit)
Save Workspa	ce s with ABSOLUTELY NO WARRANTY.
Load History	bute it under certain conditions.
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Change dir	Suo running in un Engrion rooure
Print	t with many contributors.
	ore information and
Save to File	R or R packages in publications.
Exit	s, 'help()' for on-line help, or
o.start()' fo	r an HTML browser interface to help.

Figure 5. Run IsoMSMain in RGui.

 A user has the option to change the IsoMS parameters based on the LC-MS data acquired and the desired output. To open the parameter page in RGui, click File → Open scripts → IsoMSMain (Figure 6A). A partial list of the parameter page is shown in Figure 6B.

RGui (32-bit) File Edit View Misc Pa	ackages Windows Help
Source R code New script	
Open script	
Display file(s)	
Load Workspace Save Workspace	4-03) "Masked Marvel" R Foundation for Statistical Computing w32/i386 (32-bit)
Load History Save History	comes with ABSOLUTELY NO WARRANTY. stribute it under certain conditions. cence()' for distribution details.
Change dir	and hur supprise in an English legals
Print	ort but running in an English locale
Save to File	oject with many contributors. or more information and
Exit	cite R or R packages in publications.

**(B)** 

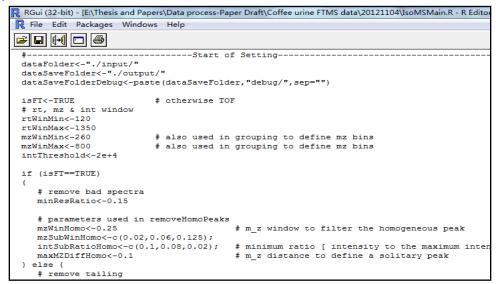


Figure 6. (A) Open IsoMSMain script. (B) Partial list of the parameter page of IsoMSMain.

3) Change the parameters. Table 1 lists the parameters and their functions. These parameters should be adjusted according to the LC-MS data obtained.

Parameter	Function
dataFolder	Location of source data files. Default folder is "input" in the working folder (see Figure 1).
	Note: if a user opts to use a folder containing all the peak list files outside this working folder, replace "input" with the directory of the folder containing the peak list files.
dataSaveFolder	Location of resultant data files. Default folder is "output" in the working folder (see Figure 1).
	Note: if a user opts to use a folder outside this working folder, replace "output" with the directory of the folder to contain all the resultant data files.
rtWinMin	Chromatogram cutoff window: Spectra before this retention time are not processed.
	Note: use this parameter to exclude any mass spectra showing only background peaks at the beginning of the chromatogram where no metabolite peak pairs are expected.
rtWinMax	Chromatogram cutoff window: Spectra after this retention time are not processed.
	Note: use this parameter to exclude any mass spectra collected after metabolite elution where no metabolite peak pairs are expected.
mzWinMin	Mass spectrum cutoff window: Spectral peaks with lower m/z than the

 Table 1. IsoMS parameters that need to be changed according to the LC-MS data obtained.

	cutoff value are not processed.	
	Note: this window can be useful to exclude peak pairs are expected (e.g., below the m/z	
mzWinMax	Mass spectrum cutoff window: Spectral p cutoff value are not processed.	eaks with higher m/z than the
intThreshold	Intensity threshold. Spectral peaks with lowe	er intensity are not processed.
intSaturated	Upper limit of intensity or ion count to defin	e the peak saturation.
	Note: the value of this limit is dependen instrument. It can be determined by inspec under the experimental conditions; the upp ion count where the peak saturation occurs.	ting the mass spectra obtained
isMZBackground	No change is needed. Always select = mzBackground csv file to filter out the back be placed in the working folder (see Figure 1	kground peaks. This file should
	Note 1: The mzBackground.csv file contain peaks of known background peak pairs to l An example of the mzBackground csv file Users should change the mass values background peak pairs found in their own w Note 2: Any other peaks a user wants to	be excluded in the final results. is given in the working folder. in this file according to the ork.
	Note 2: Any other peaks a user wants to remove can be added to this mzBackground file. For example, if a blank sample is run and the blank dataset has been processed by IsoMS, the m/z_light masses of the peak pairs found in the blank can be added to the mass column in the mzBackground file. Only the m/z_light masses need to be added. The program automatically removes the corresponding m/z_heavy peaks.	
	Note 3: If there is no background peak to b csv file should still contain "m/z" in row blank.	-
mzTolGroup	Mass tolerance to group the same peak pairs from the neighboring spectra.	Note: the mass tolerance value used is depending on
mzTolIso	Mass tolerance to search <sup>13</sup> C isotopologues within a pair.	the mass measurement accuracy of the instrument
mz_tol_dt	Mass tolerance to filter derivative pairs.	used. There is no need adjust the individual ma
mzTolChargeVec	Mass tolerance to calculate the charge number of an ion.	tolerance values (i.e., use the default values already given),
mzTolFixLev3	Mass tolerance to pair the peaks for level 3 pairs.	unless a user wants to fine- tune these values to see if any additional improvement on
mzTolDimer	Mass tolerance to filter dimer or other multimer pairs.	the specificity and sensitivity of IsoMS can be made.
mzTolBackground	Mass tolerance to filter background noises.	

mz_tol_ctfilter	Mass tolerance to filter multiply charged ions.	
minIntensity	Lowest intensity of exported peak pairs.	
minRatio	The peak intensity ratio range of exported peak pairs.	
MaxRatio		
minSN	Only for FTMS data: the lowest S/N of exported peak pairs.	

## **3. Installing and running IsoMS-align**

- 1) Download the IsoMS-align script from the MyCompoundID website.
- Assign the folder of IsoMS-align as the working folder of RGui by cliking: File → Change dir...(Figure 7).

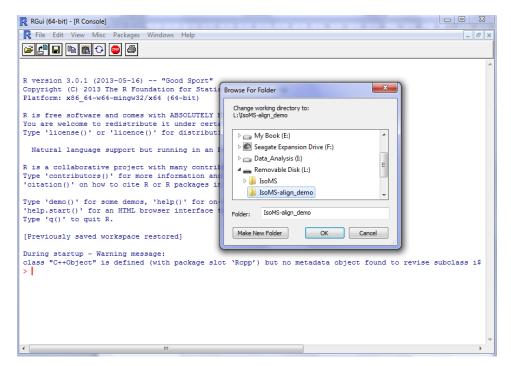


Figure 7. Select the alignment script.

3) Open the IsoMS-align script (Figure 8) and change the parameters therein (see Table 2 for the explanation of these parameters).

RGui (64-bit) - [I:\20140205alignment\alignment20140205.R - R Editor]	<u> </u>
R File Edit Packages Windows Help 🖉	×
# This is a script to make the alignment of multiple datasets generated by the data processin # Tao Huan, Feb 05, 2014 # Copyright @ 2014 University of Alberta	u 🔺
* Copyright © 2014 University of Alberta	
<pre># This is the setting part data.path &lt;- "L:/IsoMS-align_demo/"</pre>	E
Alignment.level <- 2 # "1" for align level 1, "2" for align level 1 and 2, "3" for align level 1, 2, and 3. rt.tol <- 10 $\#$ default retention time tolerance is 10 s	
mz.tol <- 8 # default mz tolerance is 8 ppm	

Figure 8. Change the parameters in IsoMS-align.

Parameter	Function
data.path	Set the data path to the folder that contains all the peak pair csv files.
Alignment.level	Select the levels of peak pairs to perform the alignment.
rt.tol	Set the retention time tolerance during the alignment process.
mz.tol	Set the mz tolerance during the alignment process.

4) Save the parameter changes to the script. Type in the command code in RGui as shown in red in Figure 9 and press enter to run the script.

🥂 RGui (64-bit) - [R Console]
R File Edit View Misc Packages Windows Help
A
R version 3.0.1 (2013-05-16) "Good Sport" Copyright (C) 2013 The R Foundation for Statistical Computing
Platform: x86_64-w64-mingw32/x64 (64-bit)
R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions. Type 'license()' or 'licence()' for distribution details.
Natural language support but running in an English locale
R is a collaborative project with many contributors.
Type 'contributors()' for more information and 'citation()' on how to cite R or R packages in publications.
Type 'demo()' for some demos, 'help()' for on-line help, or 'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.
[Previously saved workspace restored]
During startup - Warning message:
<pre>class "C++Object" is defined (with package slot 'Rcpp') but no metadata object found\$ &gt; source("IsoMS-align")</pre>
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Figure 9. Type in the command code in RGui and then run the script.

5) After running the script, a new csv file named "Alignment\_result.csv" will be created. This csv file contains the alignment result.