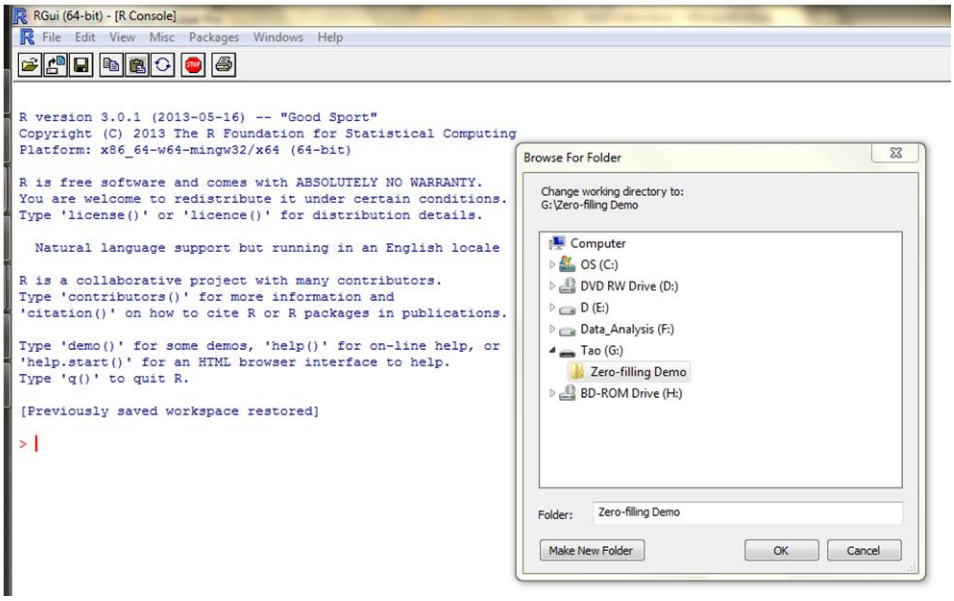
Zero-fill User Manual (Version 1.0; Dec 12, 2014)

- Zero-fill is a program written in R for retrieving missing intensity ratio values in an metabolite-intensity data file initially generated by IsoMS. This program is part of the data processing software used for the chemical isotope labeling (CIL) LC-MS metabolomics platform.
- The zero-fill script is freely available for non-commercial use from www.mycompoundid.org.
- The instruction for using the zero-filling program is given below.

1) Download the zero-fill script from MyCompoundID.org.
2) Assign the folder of zero-fill as the working folder of RGui by clicking: File → Change dir… (see below).



3) Open the zero-fill script (see below) and change the parameters therein (see Table 1 for the explanation of these parameters).

```
########################################
# This is a script to do zero filling for data file after alignment
# Tao Huan, Mar 19, 2014
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########################################
# This is the setting part
aligned.file.path <- "G:/20140812 tumor tissues/IsoMS/IsoMS/"
raw.file.path <- "G:/20140812 tumor tissues/peak list/"
mz.tol = 5 # default 5 ppm
rt.tol = 30 # default 30 seconds
########################################


setwd(aligned.file.path)
aligned.file <- read.csv("Alignment_result.csv")
after.zerofill.file <- aligned.file
setwd(raw.file.path)
raw.file.list <- list.files(pattern = ".csv")
for (i in 1:(ncol(aligned.file) - 9)){
  print(paste("processing file", i))
```
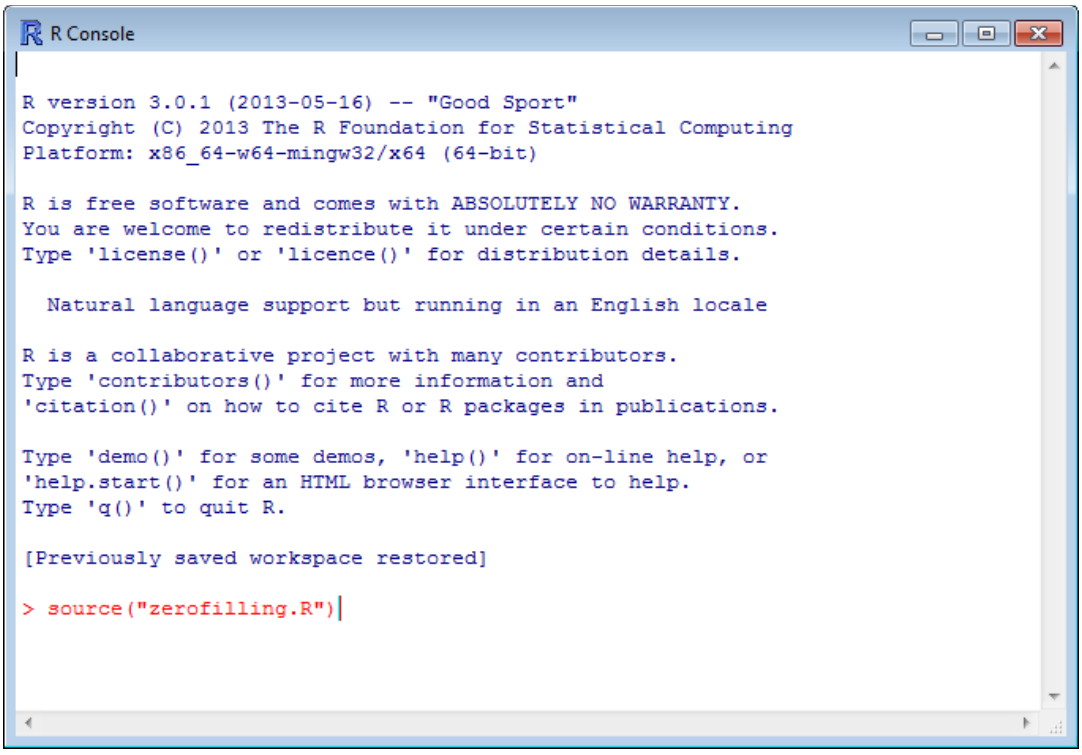

Table 1. Zero-fill parameters that need to be changed according to the user's LC-MS instrumental conditions.

Parameter	Function
aligned.file.path	Set the data path to the folder that contains the aligned dataset
raw.file.path	Set the data path to the folder that contains all the raw LC-MS data containing all the peak information
mz.tol	Set the mz tolerance during the zero-filling process
rt.tol	Set the retention time tolerance during the zero-filling process

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



5) After running the script, a new csv file named “After zerofilling.csv” will be created. This csv file contains the zero-fill result.