cosmiq - COmbining Single Masses Into Quantities

David Fischer Christian Panse* Endre Laczko

October 13, 2014

Contents

1	I Introduction				2		
2		LCMS feature detection step by step using cosmiq					2
	2.1 The Input						3
	2.2 Combination of ma	ass spectra					5
	2.3 Detection of releva	ant masses					6
	2.4 Generation and co	mbination of extracted ion chromatograms					7
	2.5 Detection of chron	matographic peaks					8
	2.6 Localisation and q	uantification of detected peaks					9
	2.7 The Output						10
3	3 Session information						10

^{*}cp@fgcz.ethz.ch

1 Introduction

cosmiq is a tool for the preprocessing of liquid- or gas-chromatography mass spectrometry (LCMS/GCMS) data with a focus on metabolomics or lipidomics applications. cosmiq has been developed and has shown to be effective using liquid ultra performance capillary chromatography coupled with high accuracy mass data (full width at half maximun > 20000), e.g. using TOF or Q-TOF type mass spectrometer. The data we have used consists of one hundreds files having a size of approx. 500MBytes each (see also [1, to be published]).

Because those high resolution data are too huge for beeing included in the package we will demonstate the usage of the *cosmiq* package using the smaller *faahKO* data set which is already available on Bioconductor.

The following code of the cosmig wrapper function shows a typical usage:

The cosmiq function is composed of the following steps:

- Combining spectra
- Detecting mz peaks on master spectrum
- Quantifying masses
- RT correction
- Computing the EIC matrix
- Detecting chromatographic peaks from EIC matrix
- Quantifying mz/RT features

cosmiq uses the xcms [2] object structur for handling the data. The following pages of this vignette are indented to demonstrate how all the steps can be run manually using the faahKO data set.

2 LCMS feature detection step by step using cosmiq

2.1 The Input

```
The faah knockout dataset [3] will be used as input.
```

```
> library(cosmiq)
> cdfpath <- file.path(find.package("faahKO"), "cdf")</pre>
> my.input.files <- dir(c(paste(cdfpath, "WT", sep='/'),
           paste(cdfpath, "KO", sep='/')), full.names=TRUE)
> #
> # create xcmsSet object
> # todo
> xs <- new("xcmsSet")</pre>
> xs@filepaths <- my.input.files
Define the phenoData. This is usually done by the unexported method xcms:::phenoDataFromPaths.
> class \leftarrow as.data.frame(c(rep("KO",6),rep("WT", 6)))
> rownames(class) <- basename(my.input.files)</pre>
> xs@phenoData <- class
The xcms object xs will be used as container to keep all the data.
> attributes(xs)
$peaks
<0 x 0 matrix>
$groups
<0 x 0 matrix>
$groupidx
list()
$filled
integer(0)
$phenoData
          c(rep("KO", 6), rep("WT", 6))
ko15.CDF
                                       ΚO
ko16.CDF
                                       ΚO
ko18.CDF
                                       ΚO
ko19.CDF
                                       KO
ko21.CDF
                                       ΚO
ko22.CDF
                                       ΚO
                                       WT
wt15.CDF
wt16.CDF
                                       WT
wt18.CDF
                                       WT
wt19.CDF
                                       WT
```

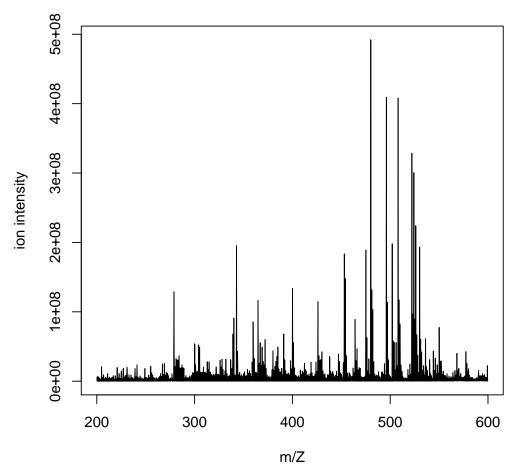
```
wt21.CDF
                                     WT
wt22.CDF
                                     WT
$rt
list()
$filepaths
 [1] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/KO/ko15.CDF"
 [2] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/KO/ko16.CDF"
 [3] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/KO/ko18.CDF"
 [4] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/KO/ko19.CDF"
 [5] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/KO/ko21.CDF"
 [6] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/KO/ko22.CDF"
 [7] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/WT/wt15.CDF"
 [8] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/WT/wt16.CDF"
 [9] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/WT/wt18.CDF"
[10] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/WT/wt19.CDF"
[11] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/WT/wt21.CDF"
[12] "/home/biocbuild/bbs-3.0-bioc/R/library/faahKO/cdf/WT/wt22.CDF"
$profinfo
list()
$dataCorrection
integer(0)
$polarity
character(0)
$progressInfo
list()
$progressCallback
function (progress)
NULL
<environment: namespace:xcms>
$class
[1] "xcmsSet"
attr(,"package")
[1] "xcms"
```

2.2 Combination of mass spectra

The first two processing steps search for relevant mass bins in the dataset. In order to select for optimal bins, we first calculate a combined spectrum. This approach of overlaying and summing intensities of single scans together is usual for applications in flow injection mass spectrometry and aims to improve ion statistics. Not only are mass spectra from all scans from a single LCMS run combined but from all acquired datasets. As a result, signal to noise ratio increases for each additional LCMS run and a master list of observed mass is generated.

```
> x <- combine_spectra(xs=xs, mzbin=0.25,
+ linear=TRUE, continuum=FALSE)
> plot(x$mz, x$intensity, type='1',
+ main='combined spectra',
+ xlab='m/Z', ylab='ion intensity')
>
```

combined spectra

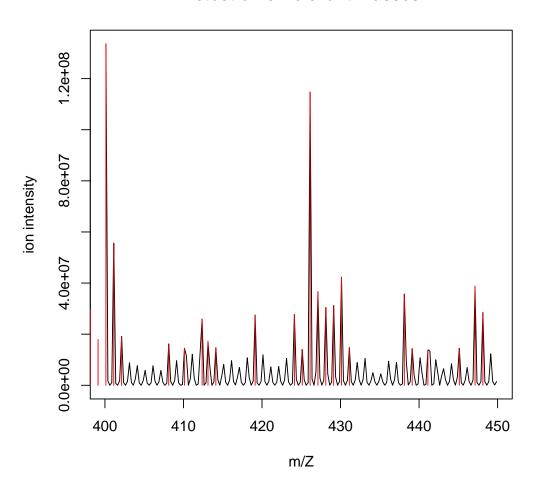


2.3 Detection of relevant masses

Based on this combined master mass spectrum we then determine location and boundaries of each observed mass. A modified peak detection algorithm based on continuous wavelet transformation (CWT) is used for this step [4]. Peak detection based on CWT has the advantage that a sliding scale of wavelets instead of a single filter function with fixed wavelength is used. This allows for a flexible and automatic approximation of the peak width. As a result it is possible to locate both narrow and broad peaks within a given dynamic range. The CWT algorithm was modified in order to consider overlapping peaks [1].

```
> xy <- peakdetection(x=x$mz, y=x$intensity,
      scales=1:10,
      SNR.Th=1.0,
      SNR.area=20, mintr=0.5)
> id.peakcenter<-xy[,4]</pre>
> filter.mz <- 400 < x$mz & x$mz < 450
> plot(x$mz[filter.mz], x$intensity[filter.mz],
      main='Detection of relevant masses',
      type='1',
+
      xlab='m/Z',
      ylab='ion intensity')
> points(x$mz[id.peakcenter],
      x$intensity[id.peakcenter],
      col='red', type='h')
>
```

Detection of relevant masses



2.4 Generation and combination of extracted ion chromatograms

Until now only the mz information was considered. In the following processing steps the chromatographic information will be added. For the comparison of different LCMS datasets it is important to consider RT shifts. These shifts are typically caused by technical variations and need to be corrected before chromatographic peaks between different LCMS runs are aligned. For this purpose cosmiq implements xcms retention time alignment using the obiwarp algorithm. For each detected mass in step 2.3 we calculate an extracted ion chromatogram (EIC). In order to determine the elution time for each detected mass, the EICs of every mass are combined between all acquired runs. Again, this combination approach aims for an improvement of the signal-to-noise ratio (SNR).

```
> # create dummy object
> xs@peaks <- matrix(c(rep(1, length(my.input.files) * 6),
+ 1:length(my.input.files)), ncol=7)
> colnames(xs@peaks) <- c("mz", "mzmin", "mzmax", "rt",</pre>
```

```
+ "rtmin", "rtmax", "sample")
> xs <- xcms::retcor(xs, method="obiwarp", profStep=1,
+ distFunc="cor", center=1)

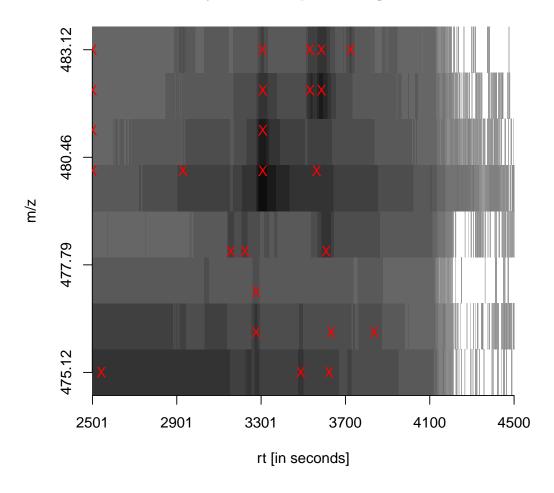
center sample: ko15.CDF
Processing: ko16.CDF ko18.CDF ko19.CDF ko21.CDF ko22.CDF wt15.CDF wt16.CDF</pre>
```

2.5 Detection of chromatographic peaks

Based on the combined EICs there is another peak detection step to be performed. The algorithm as described for the peak picking of m/z signals in Step 2.3 is used also for peak picking in the retention time domain. The final result is a peak table with location and boundaries of each mz/RT feature. This information will be further used to locate the relevant position in every single LCMS dataset in order to quantify sample specific feature intensities. Because the mz/RT features were detected on the combined mass spectra or EICs of all samples it is not necessary to align features between different LCMS runs as for a typical raw data processing workflow. Instead, a data matrix with intensity values for every mz/RT feature and every sample can be immediately calculated.

```
> eicmat <- eicmatrix(xs=xs, xy=xy, center=1)</pre>
> #
> # process a reduced mz range for a better package build performance
> (eicmat.mz.range <- range(which(475 < xy[,1] & xy[,1] < 485)))
[1] 136 143
> eicmat.filter <- eicmat[eicmat.mz.range[1]:eicmat.mz.range[2],]</pre>
> xy.filter <- xy[eicmat.mz.range[1]:eicmat.mz.range[2],]</pre>
> #
> # determine the new range and plot the mz versus RT map
> (rt.range <- range(as.double(colnames(eicmat.filter))))</pre>
[1] 2501.378 4499.824
> (mz.range<-range(as.double(row.names(eicmat.filter))))</pre>
[1] 475.125 483.125
> image(log(t(eicmat.filter))/log(2),
      main='overlay of 12 samples using faahKO',
+
      col=rev(gray(1:20/20)),
      xlab='rt [in seconds]',
+
      ylab='m/z', axes=FALSE)
> axis(1, seq(0,1, length=6),
      round(seq(rt.range[1], rt.range[2], length=6)))
> axis(2, seq(0,1, length=4),
      round(seq(mz.range[1], mz.range[2], length=4), 2))
```

overlay of 12 samples using faahKO



2.6 Localisation and quantification of detected peaks

With the information about their position in the combined datasets, each individual mz/RT feature is then located in the raw data. Due to the retention time correction, each feature is expected at the

same RT position as in the combined EIC. However small shifts in retention time still occur for most of the peaks. In order to locate the correct position of each feature, the EIC of the selected mass is calculated for the whole retention time. This EIC is filtered with CWT using only the scale where the feature was optimally located on the combined EIC in step 3. Local maxima are calculated on this transformed data and the maximum with the closest position to the expected retention time is chosen.

```
> xs <- create_datamatrix(xs=xs, rxy=rxy)</pre>
```

> peaktable <- xcms::peakTable(xs)</pre>

2.7 The Output

The output is a xcmsSet object including all necessary information (peak location and peak area), for further data analysis (statistics, metabolite database information).

```
> idx <- order(rowSums(peaktable[,8:19]), decreasing=TRUE)</pre>
> head(peaktable[idx,])
                                  rt
        mz
             mzmin
                     mzmax
                                        rtmin
                                                 rtmax npeaks ko15.CDF
13 480.125 479.625 480.625 3308.894 3269.770 3346.453
                                                            12 50056575
16 481.125 480.625 481.625 3308.894 3269.770 3346.453
                                                            12 13099239
20 482.125 481.625 482.625 3587.456 3556.157 3625.015
                                                            12
                                                                9236049
  475.125 474.625 475.625 2543.632 2501.378 2620.315
                                                                5186949
                                                            12
14 480.125 479.625 480.625 3563.981 3517.033 3599.975
                                                            12
                                                                3793218
18 482.125 481.625 482.625 3308.894 3269.770 3346.453
                                                            12
                                                                2436222
     ko16.CDF ko18.CDF ko19.CDF ko21.CDF
                                            ko22.CDF wt15.CDF
                                                                 wt16.CDF
13 49188674.0 42604200 32851699 32167083 28232603.9 50843962 53491143.1
16 12892410.4 11239241
                                           7386527.2 13329889 13761044.0
                        8722476
                                 8416403
20
    9808221.2
               9208821
                        5944353
                                  6643533
                                           4673246.6
                                                      6213538
                                                                9478365.3
     487408.5
               5335581
                                  1041640
                                            325511.9
                                                      5199330
1
                        6752928
                                                                 390509.4
14
   3609194.9
               3262888
                        2278957
                                  2281626
                                           1919592.2
                                                      3233217
                                                                3710765.2
18
   2411749.0
               2178509
                        1850595
                                  1760885
                                           1513305.6 2430864
                                                                2558690.9
   wt18.CDF wt19.CDF wt21.CDF
                                 wt22.CDF
13 44170198 31030988 33940727 26817322.5
16 11389325
            8178813
                      8871975
                                7162358.7
   8806739
20
             7065545
                      6784720
                                5304007.7
1
    4955608
            7390373
                      1091445
                                 332135.6
                      2661820
14
   3778748
             2791484
                                1700321.2
18
   2246852
            1680231 1852049
                                1488797.2
>
```

3 Session information

An overview of the package versions used to produce this document are shown below.

- R version 3.1.1 Patched (2014-09-25 r66681), x86_64-unknown-linux-gnu
- Locale: LC_CTYPE=en_US.UTF-8, LC_NUMERIC=C, LC_TIME=en_US.UTF-8, LC_COLLATE=C, LC_MONETARY=en_US.UTF-8, LC_MESSAGES=en_US.UTF-8, LC_PAPER=en_US.UTF-8, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=en_US.UTF-8, LC_IDENTIFICATION=C
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: Biobase 2.26.0, BiocGenerics 0.12.0, Rcpp 0.11.3, cosmiq 1.0.0, mzR 2.0.0, xcms 1.42.0
- Loaded via a namespace (and not attached): BiocStyle 1.4.0, MassSpecWavelet 1.32.0, codetools 0.2-9, faahKO 1.5.5, pracma 1.7.3, tools 3.1.1

References

- [1] David Fischer. Analysis of Metabolic Changes during Colorectal Cancer Development. PhD thesis, University Zurich, Switzerland, May 2014. URL: http://www.dissertationen.uzh.ch/.
- [2] Ralf Tautenhahn, Christoph Boettcher, and Steffen Neumann. Highly sensitive feature detection for high resolution lc/ms. *BMC Bioinformatics*, 9:504, 2008.
- [3] Colin A. Smith. faahKO: Saghatelian et al. (2004) FAAH knockout LC/MS data, 2012. R package version 1.2.17. URL: http://dx.doi.org/10.1021/bi0480335.
- [4] Pan Du, Warren A. Kibbe, and Simon M. Lin. Improved peak detection in mass spectrum by incorporating continuous wavelet transform-based pattern matching. *Bioinformatics*, 22:2059–2065, 2006.