cosmiq - COmbining Single Masses Into Quantities

David Fischer
Christian Panse*
Endre Laczko

October 17, 2016

Contents

*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 [**?**, 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 `cosmiq` wrapper function shows a typical usage:

```
> library(cosmiq)
> cdfpath <- file.path(find.package("faahKO"), "cdf")
> my.input.files <- dir(c(paste(cdfpath, "WT", sep='/'),
+         paste(cdfpath, "KO", sep='/')), full.names=TRUE)
> # run cosmiq wrapper function
> #
> x <- cosmiq(files=my.input.files, mzbin=0.25, SNR.Th=0, linear=TRUE)
> #
>
> # graph result
> image(t(x$eicmatrix), main='mz versus RT map')
> head(x$xs@peaks)
```

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* [**?**] 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 [**?**] will be used as input.

```
> library(cosmiq)
> cdfpath <- file.path(find.package("faahKO"), "cdf")
> my.input.files <- dir(c(paste(cdfpath, "WT", sep='/'),
+         paste(cdfpath, "KO", sep='/')), full.names=TRUE)
> #
> # create xcmsSet object
> # todo
> xs <- new("xcmsSet")
> xs@filepaths <- my.input.files
```

Define the `phenoData`. This is usually done by the unexported method `xcms:::phenoDataFromPaths`.

```
> class <- as.data.frame(c(rep("KO",6),rep("WT", 6)))
> rownames(class) <- basename(my.input.files)
> 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                            KO
ko16.CDF                            KO
ko18.CDF                            KO
ko19.CDF                            KO
ko21.CDF                            KO
ko22.CDF                            KO
wt15.CDF                            WT
wt16.CDF                            WT
wt18.CDF                            WT
wt19.CDF                            WT
```

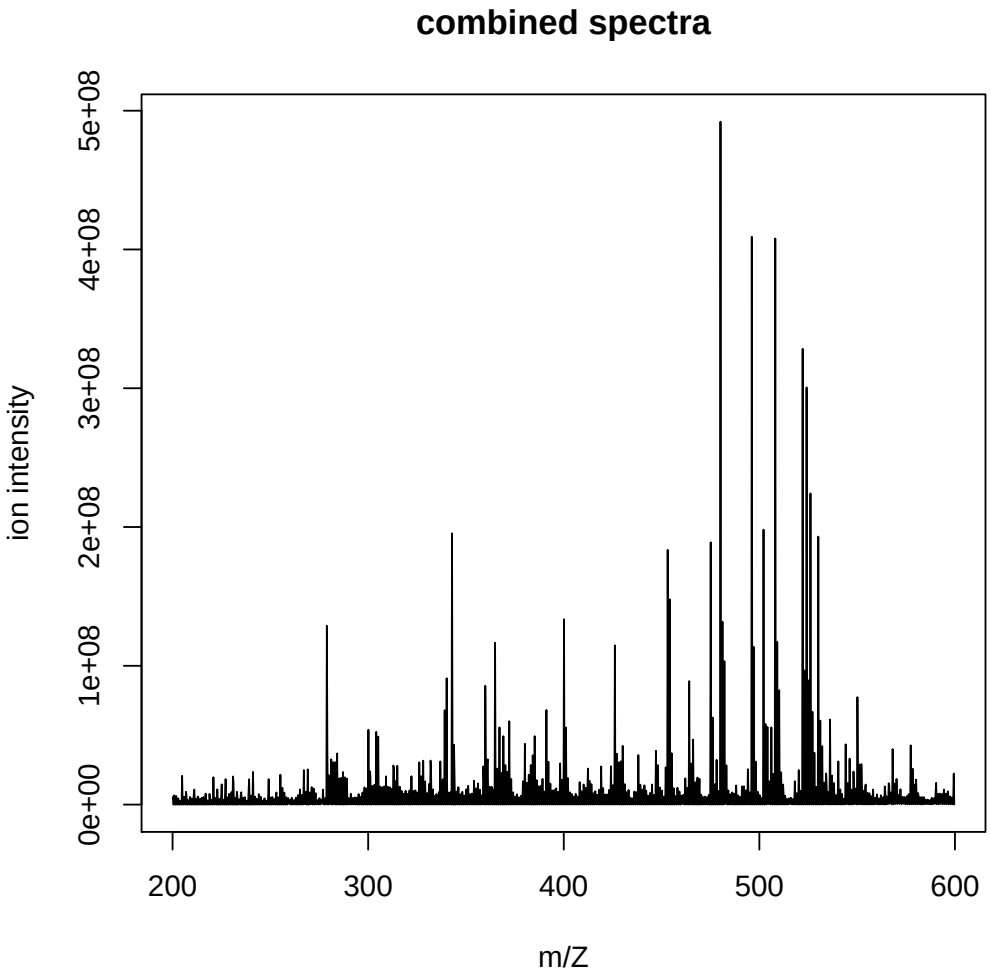
```
wt21.CDF                                WT
wt22.CDF                                WT

$rt
list()

$filepaths
 [1] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/KO/ko15.CDF
 [2] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/KO/ko16.CDF
 [3] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/KO/ko18.CDF
 [4] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/KO/ko19.CDF
 [5] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/KO/ko21.CDF
 [6] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/KO/ko22.CDF
 [7] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/WT/wt15.CDF
 [8] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/WT/wt16.CDF
 [9] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/WT/wt18.CDF
[10] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/WT/wt19.CDF
[11] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/WT/wt21.CDF
[12] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/faahKO/cdf/WT/wt22.CDF

$profinfo
list()

$dataCorrection
integer(0)

$polarity
character(0)

$progressInfo
list()

$mslevel
numeric(0)

$scanrange
numeric(0)

$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='l',
+         main='combined spectra',
+         xlab='m/Z', ylab='ion intensity')
>
```

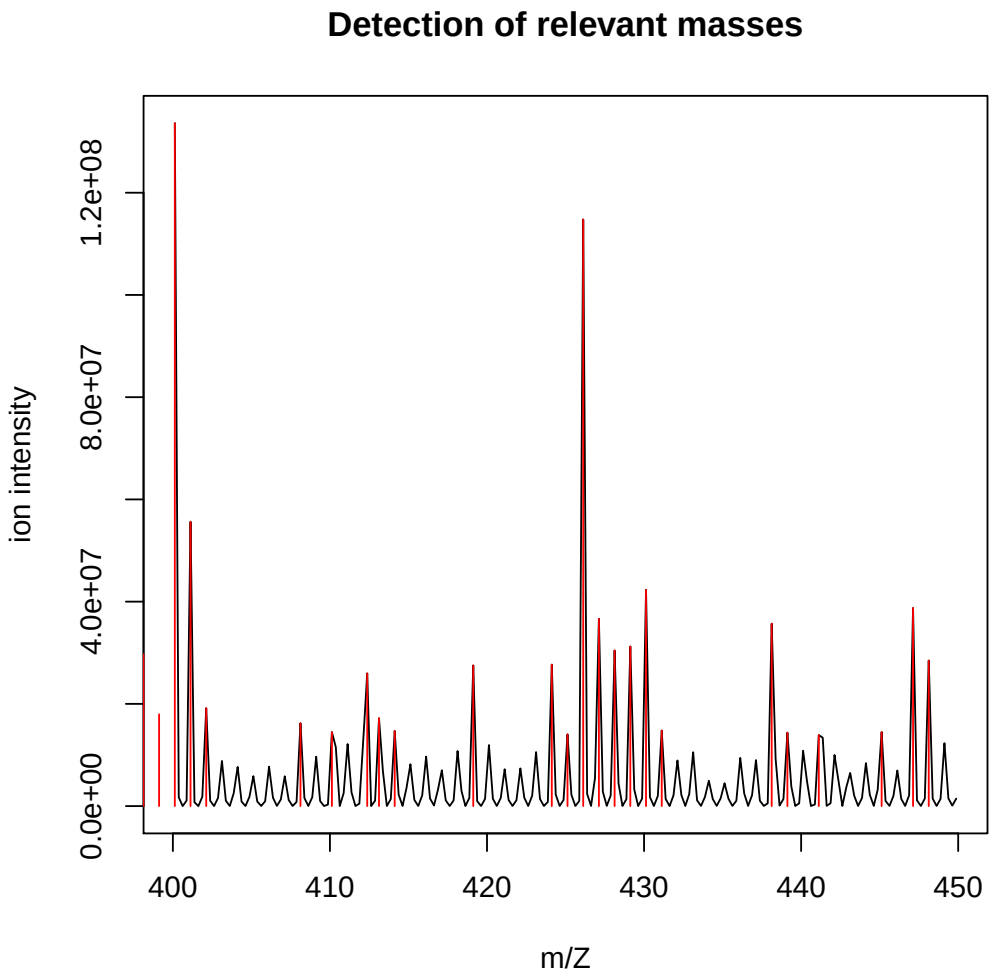


2.3 Detection of relevant masses

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 [**?**]. 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 [**?**].

```
> 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]
```

```
> filter.mz <- 400 < x$mz & x$mz < 450
> plot(x$mz[filter.mz], x$intensity[filter.mz],
+     main='Detection of relevant masses',
+     type='l',
+     xlab='m/Z',
+     ylab='ion intensity')
> points(x$mz[id.peakcenter],
+     x$intensity[id.peakcenter],
+     col='red', type='h')
>
```



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",
+         "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  wt18.CDF
```

```
>
```

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)
> #
> #  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
```

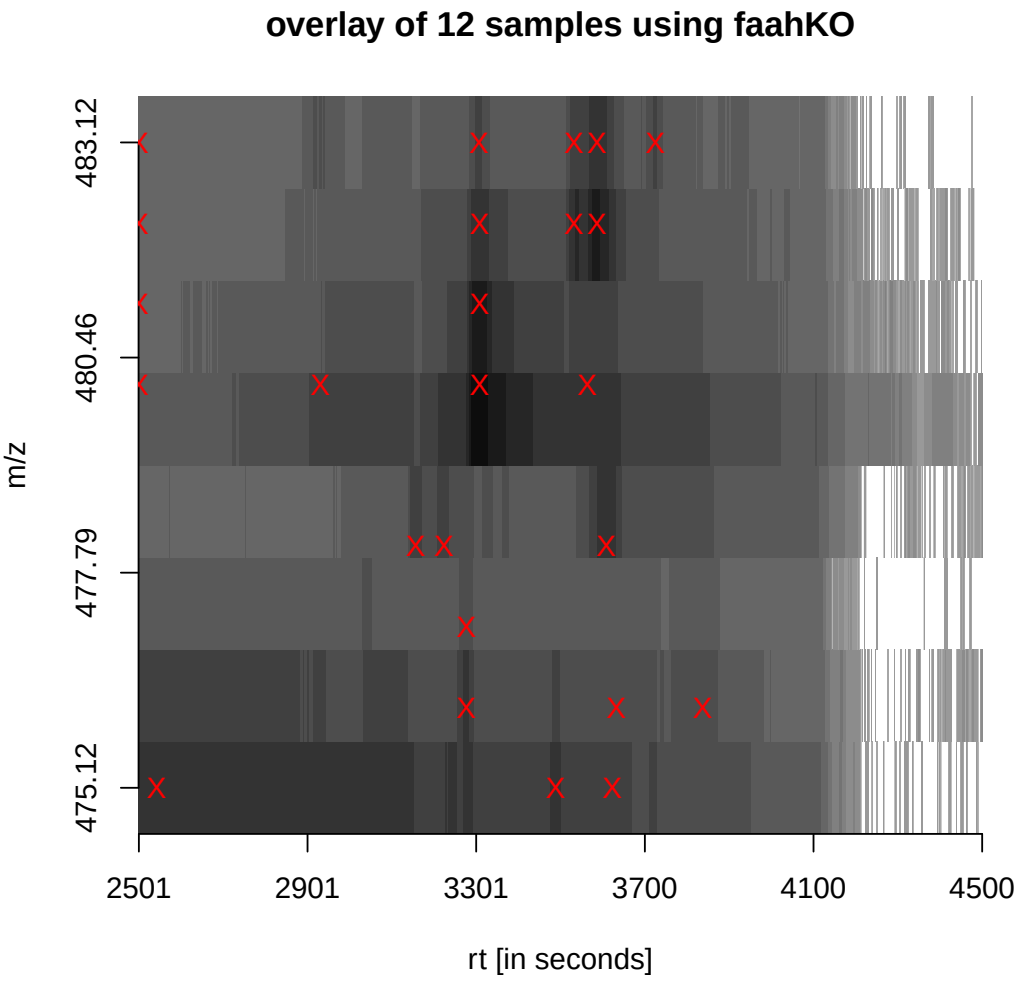
```
> eicmat.filter <- eicmat[eicmat.mz.range[1]:eicmat.mz.range[2],]
> xy.filter <- xy[eicmat.mz.range[1]:eicmat.mz.range[2],]
> #
> # determine the new range and plot the mz versus RT map
> (rt.range <- range(as.double(colnames(eicmat.filter))))
```

```
[1] 2501.378 4499.824
```

```
> (mz.range<-range(as.double(row.names(eicmat.filter))))
```

```
[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))
> #
> # determine the chromatographic peaks
> rxy <- retention_time(xs=xs,
+     RTscales=c(1:10, seq(12,32, by=2)),
+     xy=xy.filter,
+     eicmatrix=eicmat.filter,
+     RTSNR.Th=120, RTSNR.area=20)
> rxy.rt <- (rxy[,4] - rt.range[1]) / diff(rt.range)
> rxy.mz <- (rxy[,1] - mz.range[1]) / diff(mz.range)
> points(rxy.rt, rxy.mz, pch="X", lwd=2, col="red")
>
```



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)
```

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).

```
> peaktable <- xcms::peakTable(xs)
> idx <- order(rowSums(peaktable[,8:19]), decreasing=TRUE)
> head(peaktable[idx,])
```

```
        mz   mzmin   mzmax       rt    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
1  475.125 474.625 475.625 2543.632 2501.378 2620.315     12  5186949
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  8722476  8416403  7386527.2 13329889 13761044.0
20  9808221.2  9208821  5944353  6643533  4673246.6  6213538  9478365.3
1    487408.5  5335581  6752928  1041640   325511.9  5199330   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
20  8806739  7065545  6784720  5304007.7
1   4955608  7390373  1091445   332135.6
14  3778748  2791484  2661820  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 3.3.1 (2016-06-21), `x86_64-apple-darwin13.4.0`
- Locale: `C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8`
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: Biobase 2.34.0, BiocGenerics 0.20.0, ProtGenerics 1.6.0, Rcpp 0.12.7, cosmiq 1.8.0, mzR 2.8.0, xcms 1.50.0
- Loaded via a namespace (and not attached): BiocParallel 1.8.0, BiocStyle 2.2.0, MASS 7.3-45, MassSpecWavelet 1.40.0, Matrix 1.2-7.1, RANN 2.5, RColorBrewer 1.1-2, S4Vectors 0.12.0,

codetools 0.2-15, faahKO 1.13.0, grid 3.3.1, lattice 0.20-34, multtest 2.30.0, plyr 1.8.4, pracma 1.9.5, quadprog 1.5-5, splines 3.3.1, stats4 3.3.1, survival 2.39-5, tools 3.3.1