Grouping FTICR-MS data with xcms

J. Bargsten

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Introduction

This document describes how to use *xcms* for aligning multiple MS spectra against each other.

1 Prerequisites

Lots of Preprocessing has to be done before the data is ready for aligning. First of all *xcms* and *MassSpecWavelet* are needed for further processing.

```
> library(xcms)
> library(MassSpecWavelet)
```

This documentation uses raw mzdata files from *msdata* as example data set. Assuming that *msdata* is installed, we locate the path of the package and extract the datafiles.

```
> library(msdata)
> mzdatapath <- system.file("fticr", package = "msdata")
> mzdatafiles <- list.files(mzdatapath, recursive = TRUE, full.names = TRUE)
> cat("Starting xcmsDirect.Rnw")
```

```
Starting xcmsDirect.Rnw
```

The *xcmsSet*-Constructor parses the given files and applies peakpicking using the MassSpecWavelet algorithm, leading to a `xcmsSet` object with 2 sampleclasses, ham4 and ham5, and 5 samples, respectively.

```
> data.mean <- "data.mean"
> xs <- xcmsSet(method = "MSW", files = mzdatafiles, scales = c(1,
+     4, 9), nearbyPeak = T, verbose.columns = FALSE, winSize.noise = 500,
+     SNR.method = "data.mean", snthr = 10)
```

```
HAM004_641fE_14-11-07--Exp1.extracted:
HAM004_641fE_14-11-07--Exp2.extracted:
HAM004_641fE_14-11-07--Exp3.extracted:
HAM004_641fE_14-11-07--Exp4.extracted:
HAM004_641fE_14-11-07--Exp5.extracted:
HAM005_641fE_14-11-07--Exp1.extracted:
HAM005_641fE_14-11-07--Exp2.extracted:
HAM005_641fE_14-11-07--Exp3.extracted:
HAM005_641fE_14-11-07--Exp4.extracted:
HAM005_641fE_14-11-07--Exp5.extracted:
```

2 Calibration

calibrate can be used to correct the m/z values in a xcmsSet. It needs a xcmsSet and a list of m/z value which should be found in the object. To show this on a example a sample of ham4 is created and discalibrated a bit after getting some m/z:

```
> xs4 <- xcmsSet(method = "MSW", files = mzdatafiles[1], scales = c(1,
+     4, 9), nearbyPeak = T, verbose.columns = FALSE, winSize.noise = 500,
+     SNR.method = "data.mean", snthr = 10)
```

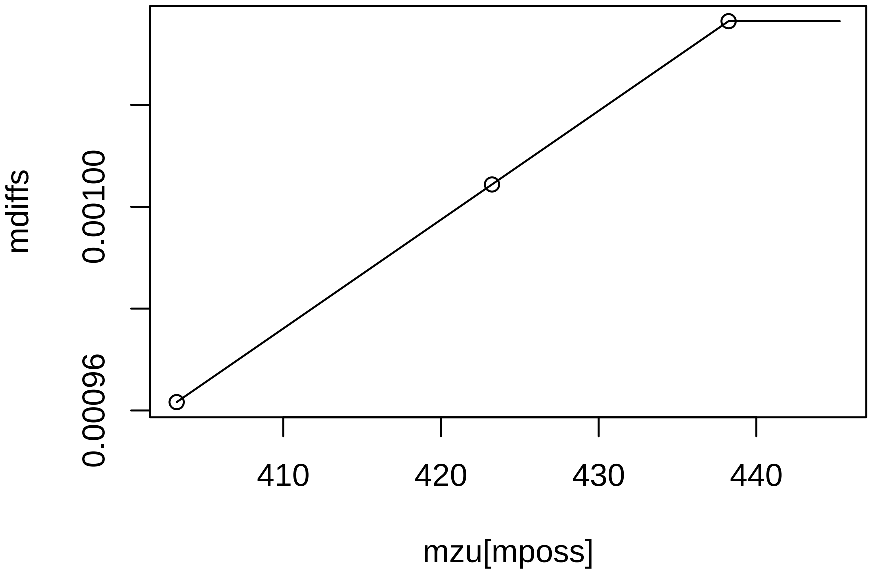
```
HAM004_641fE_14-11-07--Exp1.extracted:
```

```
> masslist <- xs4@peaks[c(1, 4, 7), "mz"]
> xs4@peaks[, "mz"] <- xs4@peaks[, "mz"] + 1e-05 * runif(1, 0,
+     0.4) * xs4@peaks[, "mz"] + 1e-04
```

The xcmsSet now can be calibrated again with the m/z from the masslist. The plot shows the reference masses with the distances to the found ones and the regression-line.

```
> xs4c <- calibrate(xs4, wishlist = masslist, method = "edgeshift",
+     mzabs = 1e-04, mzppm = 5, neighbours = 3, plotres = TRUE)
```

```
0.0009616446
0.001004380
0.001036441
```



The method ”shift” adds a value to each m/z, ”linear” does a regression and edgeshift does a regression but uses a shift before the smallest and after the biggest m/z from the wishlist.
These steps are necessary to create a usable input for *mzClust*. However, if you have already stored the data in a `xcmsSet`, you can skip the steps above.

3 Aligning

Now we can align `xs` with *mzClust*. The result is a clone of `xs` enhanced by the result of *mzClust*. For a description of the arguments *mzClust* takes, see helppage of the function.

```
> xsg <- group(xs, method = "mzClust")
```

```
1.14  6.82  17.05  21.59  27.27  31.82  37.50  38.64  42.05  47.73  53.41  54.55  61.36
```

```
> xsg
```

```
An "xcmsSet" object with 10 samples

Time range: -1--1 seconds (0-0 minutes)
Mass range: 400.1046-445.2931 m/z
Peaks: 88 (about 9 per sample)
```

```
Peak Groups: 18
Sample classes: ham4, ham5

Profile settings: method = bin
                  step = 0.1

Memory usage: 0.0182 MB
```

mzClust stores the grouping information like the standard *group* method of *xcms* suited for retrieval via *groups* and *groupidx*. An example is shown below.

```
> groups(xsg)[1:10, ]
```

```
         mzmed    mzmin    mzmax rtmed rtmin rtmax npeaks ham4 ham5
 [1,] 402.2854 402.2851 402.2859    -1    -1    -1      5    0    5
 [2,] 403.2365 403.2357 403.2367    -1    -1    -1      9    5    4
 [3,] 405.1089 405.1087 405.1095    -1    -1    -1      4    0    4
 [4,] 409.1844 409.1837 409.1845    -1    -1    -1      5    5    0
 [5,] 410.1444 410.1440 410.1448    -1    -1    -1      4    0    4
 [6,] 413.2672 413.2669 413.2677    -1    -1    -1      5    5    0
 [7,] 423.2374 423.2363 423.2398    -1    -1    -1      3    3    0
 [8,] 424.1611 424.1606 424.1615    -1    -1    -1      5    0    5
 [9,] 425.1346 425.1344 425.1353    -1    -1    -1      5    0    5
[10,] 427.2681 427.2679 427.2681    -1    -1    -1      6    5    1
```

```
> peaks(xsg)[groupidx(xsg)[[1]]]
```

```
[1] 402.2851 402.2851 402.2851 402.2859 402.2859
```

4 Postprocessing

In most cases not all samples are in one group. This can be the origin of serious problems in code, which is based on e.g. *groupval*. *groupval* sets missing peaks to NA. The solution is *fillPeaks*. It changes all NA values to random noise based on the raw data file.

```
> groupval(xsg)[1, ]
```

```
HAM004_641fE_14-11-07--Exp1.extracted HAM004_641fE_14-11-07--Exp2.extracted
                                   NA                                    NA
HAM004_641fE_14-11-07--Exp3.extracted HAM004_641fE_14-11-07--Exp4.extracted
                                   NA                                    NA
HAM004_641fE_14-11-07--Exp5.extracted HAM005_641fE_14-11-07--Exp1.extracted
                                   NA                                    37
```

```
HAM005_641fE_14-11-07--Exp2.extracted HAM005_641fE_14-11-07--Exp3.extracted
                                   49                                    60
HAM005_641fE_14-11-07--Exp4.extracted HAM005_641fE_14-11-07--Exp5.extracted
                                   70                                    80
```

```
> groupval(xsg, "medret", "into")[1:10, ]
```

```
          HAM004_641fE_14-11-07--Exp1.extracted
402.3/-1                                     NA
403.2/-1                               814693.1
405.1/-1                                     NA
409.2/-1                               732119.9
410.1/-1                                     NA
413.3/-1                              1018994.8
423.2/-1                               435858.5
424.2/-1                                     NA
425.1/-1                                     NA
427.3/-1                              1125644.3
          HAM004_641fE_14-11-07--Exp2.extracted
402.3/-1                                     NA
403.2/-1                              1046733.1
405.1/-1                                     NA
409.2/-1                               838774.7
410.1/-1                                     NA
413.3/-1                               771793.6
423.2/-1                                     NA
424.2/-1                                     NA
425.1/-1                                     NA
427.3/-1                              1007261.7
          HAM004_641fE_14-11-07--Exp3.extracted
402.3/-1                                     NA
403.2/-1                              1063981.3
405.1/-1                                     NA
409.2/-1                               802692.6
410.1/-1                                     NA
413.3/-1                               842933.1
423.2/-1                               411344.5
424.2/-1                                     NA
425.1/-1                                     NA
427.3/-1                               901531.0
          HAM004_641fE_14-11-07--Exp4.extracted
402.3/-1                                     NA
403.2/-1                               965765.8
```

```
405.1/-1                                    NA
409.2/-1                              764068.8
410.1/-1                                    NA
413.3/-1                              802110.5
423.2/-1                              357822.2
424.2/-1                                    NA
425.1/-1                                    NA
427.3/-1                              972574.2
         HAM004_641fE_14-11-07--Exp5.extracted
402.3/-1                                    NA
403.2/-1                             1127939.6
405.1/-1                                    NA
409.2/-1                              807672.1
410.1/-1                                    NA
413.3/-1                              744445.3
423.2/-1                                    NA
424.2/-1                                    NA
425.1/-1                                    NA
427.3/-1                              906765.5
         HAM005_641fE_14-11-07--Exp1.extracted
402.3/-1                              690820.7
403.2/-1                              813749.8
405.1/-1                              493671.4
409.2/-1                                    NA
410.1/-1                              460283.8
413.3/-1                                    NA
423.2/-1                                    NA
424.2/-1                              446598.9
425.1/-1                              768109.1
427.3/-1                              532297.9
         HAM005_641fE_14-11-07--Exp2.extracted
402.3/-1                              826545.2
403.2/-1                              390026.7
405.1/-1                              383127.7
409.2/-1                                    NA
410.1/-1                              340133.0
413.3/-1                                    NA
423.2/-1                                    NA
424.2/-1                              429362.0
425.1/-1                              648666.9
427.3/-1                                    NA
         HAM005_641fE_14-11-07--Exp3.extracted
```

```
402.3/-1                              825108.3
403.2/-1                              369377.0
405.1/-1                              520611.4
409.2/-1                                    NA
410.1/-1                              387501.5
413.3/-1                                    NA
423.2/-1                                    NA
424.2/-1                              411374.7
425.1/-1                              758610.4
427.3/-1                                    NA
         HAM005_641fE_14-11-07--Exp4.extracted
402.3/-1                              639126.8
403.2/-1                              437076.3
405.1/-1                                    NA
409.2/-1                                    NA
410.1/-1                              423966.5
413.3/-1                                    NA
423.2/-1                                    NA
424.2/-1                              458619.0
425.1/-1                              492720.7
427.3/-1                                    NA
         HAM005_641fE_14-11-07--Exp5.extracted
402.3/-1                              916502.6
403.2/-1                                    NA
405.1/-1                              461028.9
409.2/-1                                    NA
410.1/-1                                    NA
413.3/-1                                    NA
423.2/-1                                    NA
424.2/-1                              429517.4
425.1/-1                              578125.7
427.3/-1                                    NA
```

The results are suited for instance for heatmaps, etc.