Grouping FTICR-MS data with xcms

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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
+ )
```

2 Calibration

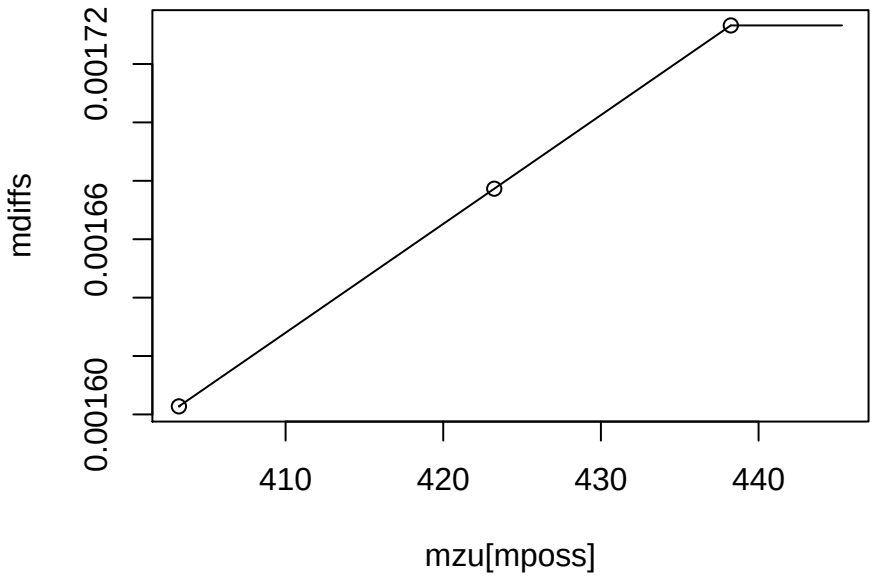
calibrate can be used to correct the m/z values in a `xcmsSet`. It needs a xcmsSet and a list of m/z value which should be found in the object. To show this on a example a sample of ham4 is created and discalibrated a bit after getting some m/z:

```
> 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)
```

```
> masslist <- xs4@peaks[c(1,4,7),"mz"]
> xs4@peaks[,"mz"] <- xs4@peaks[,"mz"] + 0.00001*runif(1,0,0.4)*xs4@peaks[,"mz"] + 0.
```

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,
+                  calibrants=masslist,
+                  method="edgeshift",
+                  mzabs=0.0001,
+                  mzppm=5,
+                  neighbours=3,
+                  plotres=TRUE
+                  )
```



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 calibrants.
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: 0.0221 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
```

```
> xsgf <- fillPeaks(xsg, method="MSW")
```

```
HAM004_641fE_14-11-07--Exp1.extracted HAM004_641fE_14-11-07--Exp2.extracted HAM004_641f
```

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

```
         HAM004_641fE_14-11-07--Exp1.extracted
402.3/-1                               768754.0
403.2/-1                              4735257.5
405.1/-1                               761632.1
409.2/-1                              4158404.5
410.1/-1                               726003.9
413.3/-1                              6099006.3
423.2/-1                              2708391.1
424.2/-1                               772516.1
425.1/-1                               885238.7
427.3/-1                              6302089.0
         HAM004_641fE_14-11-07--Exp2.extracted
402.3/-1                              1230140.4
403.2/-1                              6202417.6
405.1/-1                               491944.3
409.2/-1                              5004546.3
410.1/-1                               532868.8
413.3/-1                              4950641.7
423.2/-1                              1801494.2
424.2/-1                               521511.4
425.1/-1                               948516.6
427.3/-1                              5884065.2
         HAM004_641fE_14-11-07--Exp3.extracted
402.3/-1                               810120.4
403.2/-1                              6117414.1
405.1/-1                               650391.6
409.2/-1                              4403588.2
410.1/-1                              1182671.8
413.3/-1                              5517709.5
423.2/-1                              2826896.2
424.2/-1                               347349.0
425.1/-1                               376380.5
```

```
427.3/-1                              5354053.7
         HAM004_641fE_14-11-07--Exp4.extracted
402.3/-1                               568660.8
403.2/-1                              5328574.1
405.1/-1                               950315.1
409.2/-1                              4336554.2
410.1/-1                               805050.9
413.3/-1                              5008541.7
423.2/-1                              2427532.8
424.2/-1                               481549.3
425.1/-1                               961748.7
427.3/-1                              5654936.8
         HAM004_641fE_14-11-07--Exp5.extracted
402.3/-1                               572090.2
403.2/-1                              6429028.9
405.1/-1                              1452332.4
409.2/-1                              4580892.8
410.1/-1                              1128403.7
413.3/-1                              4856606.0
423.2/-1                              1856867.6
424.2/-1                               499037.6
425.1/-1                               662997.4
427.3/-1                              5248273.8
         HAM005_641fE_14-11-07--Exp1.extracted
402.3/-1                                4095293
403.2/-1                                4811391
405.1/-1                                2982453
409.2/-1                                1196232
410.1/-1                                2872023
413.3/-1                                1786533
423.2/-1                                1064349
424.2/-1                                2995850
425.1/-1                                4431535
427.3/-1                                3761371
         HAM005_641fE_14-11-07--Exp2.extracted
402.3/-1                              4804762.5
403.2/-1                              2581183.1
405.1/-1                              2268984.5
409.2/-1                              1210941.2
410.1/-1                              2133219.4
413.3/-1                              1061103.2
423.2/-1                               688353.7
```

```
424.2/-1                                2556865.3
425.1/-1                                3821099.0
427.3/-1                                1456574.4
         HAM005_641fE_14-11-07--Exp3.extracted
402.3/-1                                4657726.8
403.2/-1                                2727237.5
405.1/-1                                2971705.2
409.2/-1                                 544048.4
410.1/-1                                2466625.6
413.3/-1                                 892797.1
423.2/-1                                 897205.4
424.2/-1                                2567877.2
425.1/-1                                4246330.7
427.3/-1                                1196915.1
         HAM005_641fE_14-11-07--Exp4.extracted
402.3/-1                                3755889.7
403.2/-1                                2496858.9
405.1/-1                                2291624.1
409.2/-1                                1346778.3
410.1/-1                                2980996.6
413.3/-1                                 982491.3
423.2/-1                                1209006.2
424.2/-1                                2857624.1
425.1/-1                                2977003.1
427.3/-1                                1227957.6
         HAM005_641fE_14-11-07--Exp5.extracted
402.3/-1                                  5265972
403.2/-1                                  2165162
405.1/-1                                  3009065
409.2/-1                                  1187547
410.1/-1                                  2296774
413.3/-1                                  1027673
423.2/-1                                  1136440
424.2/-1                                  2892810
425.1/-1                                  3301529
427.3/-1                                  1682024
```

The results are suited for instance for heatmaps, etc.

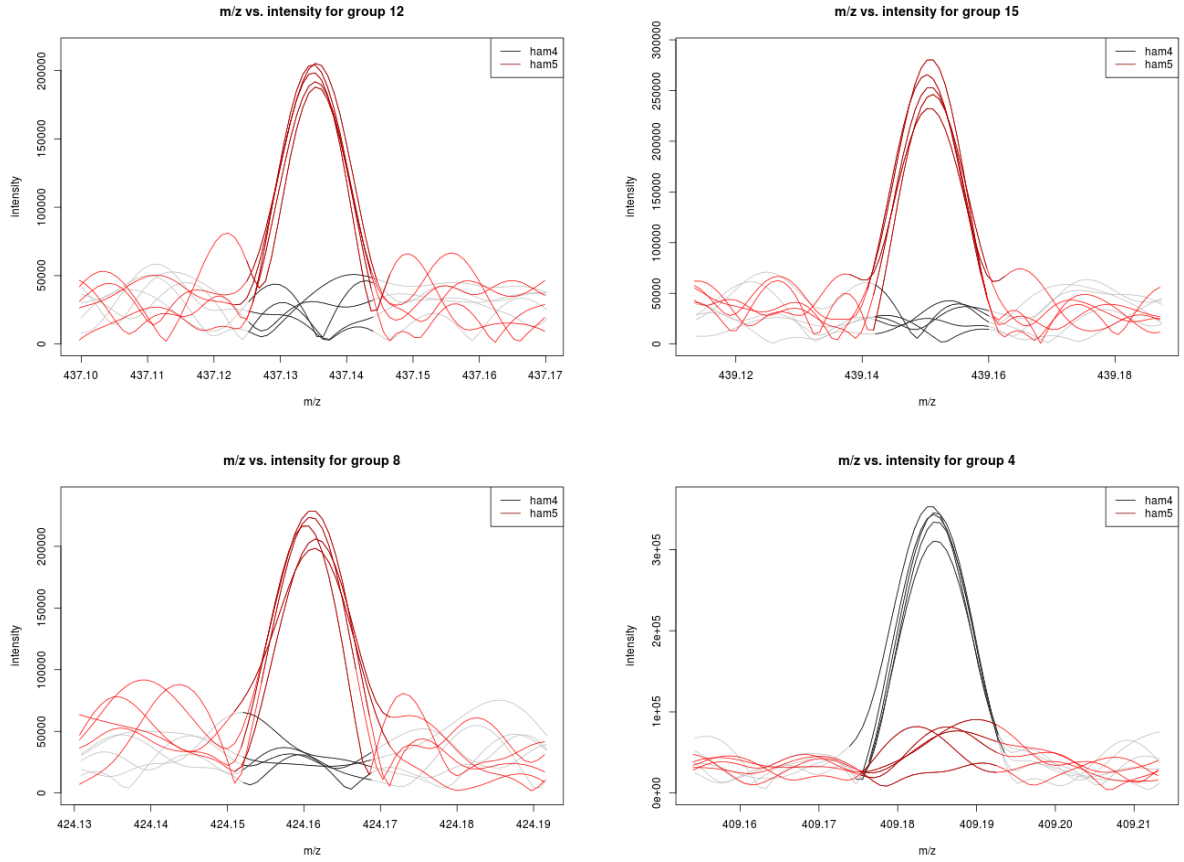


Figure 1: Auto-generated extracted spectra for the top three differentially regulated ions. Darkened lines indicate where the peaks were integrated for quantitation.

5 Analyzing and Visualizing Results

A report showing the most statistically significant differences in analyte intensities can be generated with the *diffreport* method. It will automatically sho wthe superimposed peaks in the spectra for a given number of them, in this case 10. Several of those chromatograms are shown in Figure 1.

```
> reporttab <- diffreport(xsgf, "ham4", "ham5", "example", eicmax=4,
+                         h=480, w=640)

Processing data from sample:   1  2  3  4  5  6  7  8  9  10

group:   12  15  8  4

> reporttab[1:4,]
```

```
      name     fold     tstat       pvalue    mzmed    mzmin    mzmax rtmed
1 M437T-1_1 5.144529  20.00921 4.258336e-08 437.1353 437.1353 437.1353    -1
2   M439T-1 6.877120  25.31711 4.886837e-08 439.1508 439.1503 439.1512    -1
3   M424T-1 5.290320  19.91077 8.868895e-08 424.1611 424.1606 424.1615    -1
4   M409T-1 4.098768 -16.86272 1.555750e-07 409.1844 409.1837 409.1845    -1
  rtmin rtmax npeaks ham4 ham5 HAM004_641fE_14-11-07--Exp1.extracted
1    -1    -1      4    0    4                                665915.1
2    -1    -1      5    0    5                                595855.3
3    -1    -1      5    0    5                                772516.1
4    -1    -1      5    5    0                               4158404.5
  HAM004_641fE_14-11-07--Exp2.extracted HAM004_641fE_14-11-07--Exp3.extracted
1                              368064.3                              303954.7
2                              645369.3                              378272.6
3                              521511.4                              347349.0
4                             5004546.3                             4403588.2
  HAM004_641fE_14-11-07--Exp4.extracted HAM004_641fE_14-11-07--Exp5.extracted
1                              683672.7                              511603.0
2                              320544.9                              533959.4
3                              481549.3                              499037.6
4                             4336554.2                             4580892.8
  HAM005_641fE_14-11-07--Exp1.extracted HAM005_641fE_14-11-07--Exp2.extracted
1                               2619631                               2432116
2                               3586827                               3224767
3                               2995850                               2556865
4                               1196232                               1210941
  HAM005_641fE_14-11-07--Exp3.extracted HAM005_641fE_14-11-07--Exp4.extracted
1                             2470892.7                               2826523
2                             3606573.9                               3129954
3                             2567877.2                               2857624
4                              544048.4                               1346778
  HAM005_641fE_14-11-07--Exp5.extracted
1                               2683008
2                               3465884
3                               2892810
4                               1187547
```