High throughput acquisition and processing of tandem mass spectra

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?

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```
> ##
> ## finish the faahKO data
> ##
>
> faahko <- group(faahko)
```

```
262 325 387 450 512 575
```

```
> faahko <- fillPeaks(faahko)
```

```
ko15 ko16 ko18 ko19 ko21 ko22 wt15 wt16 wt18 wt19 wt21 wt22
```

```
> ##
> ## Create the annotation for putative [M+H]+
> ##
> xa_pos <- xsAnnotate(faahko)
> xa_pos <- groupFWHM(xa_pos)
```

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```
Start grouping after retention time.
Created 133 pseudospectra.

> xa_pos <- findIsotopes(xa_pos)

Generating peak matrix!
Run isotope peak annotation
 % finished: 10  20  30  40  50  60  70  80  90  100
Found isotopes: 58

> xa_pos <- groupCorr(xa_pos)

Start grouping after correlation.
Generating EIC's ..

Calculating peak correlations in 133 Groups...
 % finished: 10  20  30  40  50  60  70  80  90  100

Calculating graph cross linking in 133 Groups...
 % finished: 10  20  30  40  50  60  70  80  90  100
New number of ps-groups:  321
xsAnnotate has now 321 groups, instead of 133

> xa_pos <- findAdducts(xa_pos,
+                        ppm=100, mzabs=0.1,
+                        polarity="positive")

Generating peak matrix for peak annotation!

Calculating possible adducts in 321 Groups...
 % finished: 10  20  30  40  50  60  70  80  90  100

> p <- getPeaklist(xa_pos)
> ## Find Interesting peaks
> dr <- diffreport(faahko, sortpval=FALSE)
> ## Interesting groups have to be
> ## 1) potential [M+H]
> ## 2) differental
> ## 3) with a minimum intensity
>
> targetgroups <- which ( grepl("[M+H]", p[,"adduct"], fixed=TRUE)
+                         & dr[,"fold"] > 1
+                         & dr[,"wt15"] > 1000 )
> if (length(targetgroups) == 0) {
+     message("Sorry, nothing of interest left :-(")
+ }
> priorities <- order(dr[targetgroups, "fold"])
> ##
> reporttab <- groups(faahko)[targetgroups[priorities],]
> ## Fix column names
> ## Need to fix code later ;-)
```

```
>
> colnames(reporttab) <- sub("^rt$", "rtmed", colnames(reporttab))
> colnames(reporttab) <- sub("^mz$", "mzmed", colnames(reporttab))
> ##
> ## The exclusion peakID list is for iterative
> ## method generation
> ##
>
> templateFile <- system.file("20minKalibpos_Startermethod_MSMS.m",
+                             package = "MetShot")
> collisionEnergy <- c(20)
> methodname <- paste(tempdir(), "/MSMS-faahKO-20eV", sep="")
> picklist <- xcms2method(reporttab, methodPrefix=methodname,
+                         widthFactor=1.5, minWidth=4,
+                         template=templateFile,
+                         MSMSManual_ListCollisionEnergy=collisionEnergy,
+                         MSmode="positive")
```

```
[1] 603
[1]  2  3  5  6  7  8 10
      mzmed   mzmin    mzmax    rtmed    rtmin    rtmax npeaks KO WT
7  315.0000 315.000 315.0000 2520.939 2498.248 2554.589      8  4  4
10 313.0012 313.000 313.0293 2787.765 2780.332 2796.762     12  6  6
4  267.1573 267.127 267.2000 3668.833 3654.749 3682.920     12  6  6
[1] 252
[1] 1 2 3
[1] mzmed  mzmin  mzmax  rtmed  rtmin  rtmax  npeaks KO     WT
<0 rows> (or 0-length row.names)
[[1]]
     mzmed    mzmin    mzmax    rtmed    rtmin    rtmax npeaks KO WT
2 219.0848 219.0488 219.1000 2524.852 2515.853 2532.286      9  4  5
5 343.0000 343.0000 343.0324 2686.042 2677.044 2693.478     12  6  6
1 279.0180 279.0000 279.0602 2791.676 2783.852 2797.939     12  6  6
8 506.1921 506.1000 506.2000 3393.401 3364.450 3453.652     13  6  6
6 330.1491 330.1054 330.2000 3494.342 3487.299 3506.078      6  2  4
3 255.1596 255.1118 255.1954 3677.440 3653.575 3698.179     12  6  6
9 564.2577 564.2424 564.3629 3876.972 3783.857 3901.229      4  1  3

[[2]]
      mzmed   mzmin    mzmax    rtmed    rtmin    rtmax npeaks KO WT
7  315.0000 315.000 315.0000 2520.939 2498.248 2554.589      8  4  4
10 313.0012 313.000 313.0293 2787.765 2780.332 2796.762     12  6  6
4  267.1573 267.127 267.2000 3668.833 3654.749 3682.920     12  6  6

>
>
```

The picklist

```
> picklist
```

```
[[1]]
     mzmed    mzmin    mzmax    rtmed    rtmin    rtmax npeaks KO WT
2 219.0848 219.0488 219.1000 2524.852 2515.853 2604.665      9  4  5
5 343.0000 343.0000 343.0324 2686.042 2604.665 2738.665     12  6  6
1 279.0180 279.0000 279.0602 2791.676 2738.665 3081.194     12  6  6
8 506.1921 506.1000 506.2000 3393.401 3081.194 3470.476     13  6  6
6 330.1491 330.1054 330.2000 3494.342 3470.476 3579.826      6  2  4
3 255.1596 255.1118 255.1954 3677.440 3579.826 3741.018     12  6  6
9 564.2577 564.2424 564.3629 3876.972 3741.018 3901.229      4  1  3

[[2]]
      mzmed   mzmin    mzmax    rtmed    rtmin    rtmax npeaks KO WT
7  315.0000 315.000 315.0000 2520.939 2498.248 2667.460      8  4  4
10 313.0012 313.000 313.0293 2787.765 2667.460 3225.756     12  6  6
4  267.1573 267.127 267.2000 3668.833 3225.756 3682.920     12  6  6
```

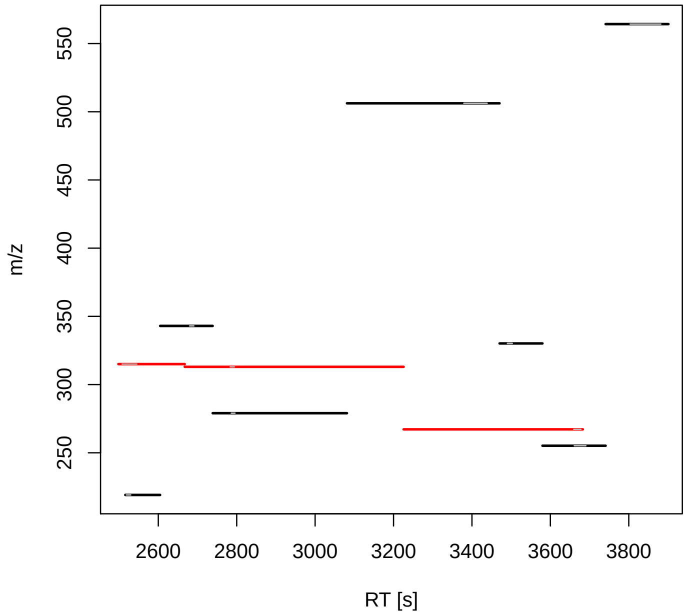
It is also possible to create manymany MSMS methods:

```
> collisionEnergies <- c(20)
> pickLists <- list()
> reporttabTargetRun <- rep(NA, nrow(reporttab))
> ## write bruker method
> for (j in seq(length = length(collisionEnergies))) {
+   methodname <- paste(tempdir(), "/MSMS-faahKO-20eV",
+                       collisionEnergies[j], ".m", sep="")
+
+   pickLists <- xcms2method(reporttab, method=methodname,
+                            widthFactor=1.5, minWidth=4,
+                            template=templateFile,
+                            MSMSManual_ListCollisionEnergy=collisionEnergies[j],
+                            MSmode="positive")
+
+   message(paste("Created ", methodname,
+                 "with", length(pickLists), "Runs"))
+
+   for (i in 1: length(pickLists)) {
+     filename <- paste(methodname,"_",i, ".csv", sep="")
+     write.csv(pickLists[[i]], file=filename)
+   }
+ }

[1] 603
[1]  2  3  5  6  7  8 10
      mzmed   mzmin    mzmax    rtmed    rtmin    rtmax npeaks KO WT
7  315.0000 315.000 315.0000 2520.939 2498.248 2554.589      8  4  4
10 313.0012 313.000 313.0293 2787.765 2780.332 2796.762     12  6  6
4  267.1573 267.127 267.2000 3668.833 3654.749 3682.920     12  6  6
[1] 252
[1] 1 2 3
[1] mzmed  mzmin  mzmax  rtmed  rtmin  rtmax  npeaks KO     WT
<0 rows> (or 0-length row.names)
```

```
[[1]]
     mzmed    mzmin    mzmax    rtmed    rtmin    rtmax npeaks KO WT
2 219.0848 219.0488 219.1000 2524.852 2515.853 2532.286      9  4  5
5 343.0000 343.0000 343.0324 2686.042 2677.044 2693.478     12  6  6
1 279.0180 279.0000 279.0602 2791.676 2783.852 2797.939     12  6  6
8 506.1921 506.1000 506.2000 3393.401 3364.450 3453.652     13  6  6
6 330.1491 330.1054 330.2000 3494.342 3487.299 3506.078      6  2  4
3 255.1596 255.1118 255.1954 3677.440 3653.575 3698.179     12  6  6
9 564.2577 564.2424 564.3629 3876.972 3783.857 3901.229      4  1  3

[[2]]
      mzmed   mzmin    mzmax    rtmed    rtmin    rtmax npeaks KO WT
7  315.0000 315.000 315.0000 2520.939 2498.248 2554.589      8  4  4
10 313.0012 313.000 313.0293 2787.765 2780.332 2796.762     12  6  6
4  267.1573 267.127 267.2000 3668.833 3654.749 3682.920     12  6  6

> for (j in 1:length(pickLists)) {
+   reporttabTargetRun[match(rownames(pickLists[[j]]), rownames(reporttab))] <- j
+ }
> reporttab<-cbind(reporttab, reporttabTargetRun)
>
```

Plot peaks and target windows superimposed.

```
> ## plot overview
>
> for (i in seq(1, length(pickLists))) {
+   plotMS2windows(reporttab, pickLists[[i]],
+                  peaks=ifelse(i==1, TRUE, FALSE),
+                  col=i)
+ }
>
```



3 Processing

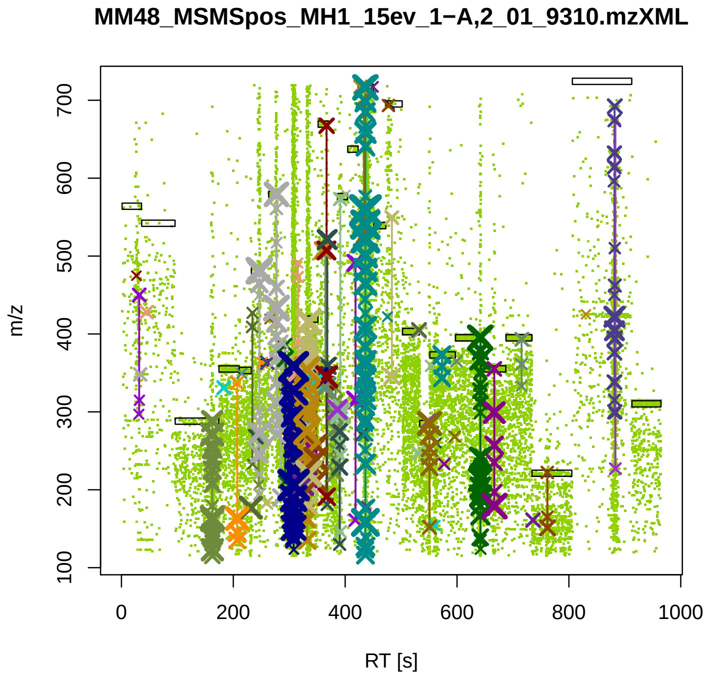
Now a file was measured, we need to extract the MS2 spectra. Instead if "just" taking a spectrum, we do peak picking and CAMERA grouping.

```
> ########################################
> ##
> ## Parameters common to MS1 and MS2 picking
> ##
>
> peakwidth=c(6,15)
> ppm=25
> snthresh=2
> prefilter=c(2,20)
> ########################################
> ##
> ## Read MS1 peaks
> ##
>
> ms1peaks <- faahko
> ########################################
> ##
> ## Read MS2 peaks
> ## Obtain MS2 peaks (ab)using normal peak picker
> ##
```

```
>
> snthresh=2
> ## Create a pseudo-MS1 xraw
> filename <- system.file("microtof/MM48_MSMSpos_MH1_15ev_1-A,2_01_9310.mzXML",
+                         package = "MetShot")
> xraw <- msn2xcms(xcmsRaw(filename, includeMSn=TRUE))
> ms2peaks <- findPeaks(xraw, method="centWave",
+                       snthresh=snthresh, prefilter=prefilter,
+                       peakwidth=peakwidth, ppm=ppm, verbose.columns=T)

 Detecting mass traces at 25 ppm ...
 % finished: 0 10 20 30 40 50 60 70 80 90 100
 817 m/z ROI's.

 Detecting chromatographic peaks ...
 % finished: 0 10 20 30 40 50 60 70 80 90 100
 726  Peaks.

> ## for each peak
> ms2precursor <- cbind(rt=xraw@msnRt, mz=xraw@msnPrecursorMz)
> ##
> ## CAMERA annotation of spectra
> ##
>
> xs <- new("xcmsSet")
> peaks(xs) <- ms2peaks
> filepaths(xs) <- c(filename)
> sampnames(xs) <- c("MM48_MSMSpos")
> xs@peaks <- cbind(xs@peaks, sample=1)
> xa <- xsAnnotate(xs)
> xa <- groupFWHM(xa)

Start grouping after retention time.
Created 82 pseudospectra.

> ## xa <- groupCorrhack(xa,xraw,cor_eic_th=0.75)
>
```

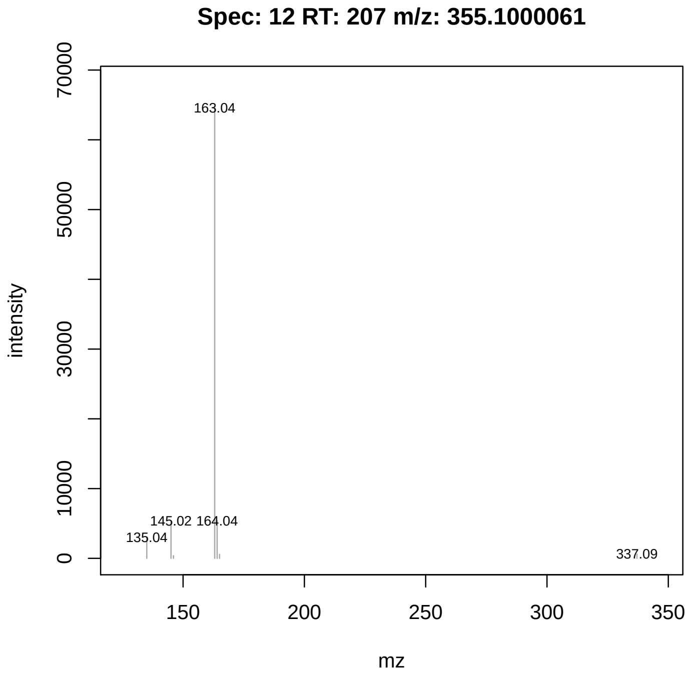
You can plot the overlaid MS2 raw data and the picked and grouped peaks:

```
> plotGroups(xraw, ms2precursor, xa)
>
```



You can plot the individual retrieved MS2 Spectrum:

```
> pspec <- 12
> rt <- median(getpspectra(xa, pspec)[,"rt"])
> mz <- ms2precursor[which(ms2precursor[,"rt"] == rt), "mz"]
> sp <- getpspectra(xa, grp=pspec)[,c("mz", "maxo")]
> plotPsSpectrum(xa, pspec=pspec,
+           maxlabel=5, log=FALSE, sleep=0,
+           title=paste("Spec:", pspec, "RT:", as.integer(rt), "m/z:", mz))
>
```



4 Session information

- R version 2.14.0 (2011-10-31), `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=C`, `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, stats, utils
- Other packages: Biobase 2.14.0, CAMERA 1.13.1, MetShot 0.1.18, XML 3.4-3, class 7.3-3, faahKO 1.2.8, multtest 2.10.0, xcms 1.30.3
- Loaded via a namespace (and not attached): Hmisc 3.9-0, MASS 7.3-16, RBGL 1.30.1, cluster 1.14.1, graph 1.32.0, grid 2.14.0, igraph 0.5.5-3, lattice 0.20-0, splines 2.14.0, survival 2.36-10, tools 2.14.0

References