HelmholtzZentrum münchen
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Working with MS^2 data in XCMS3

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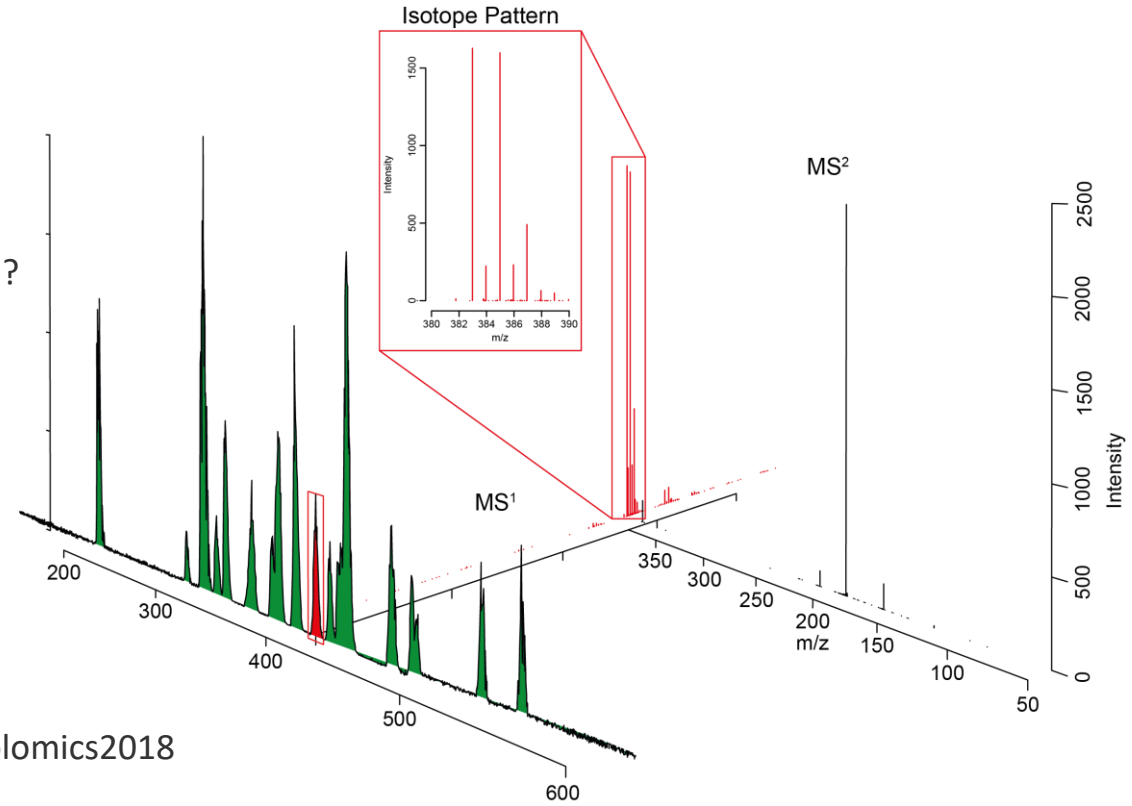
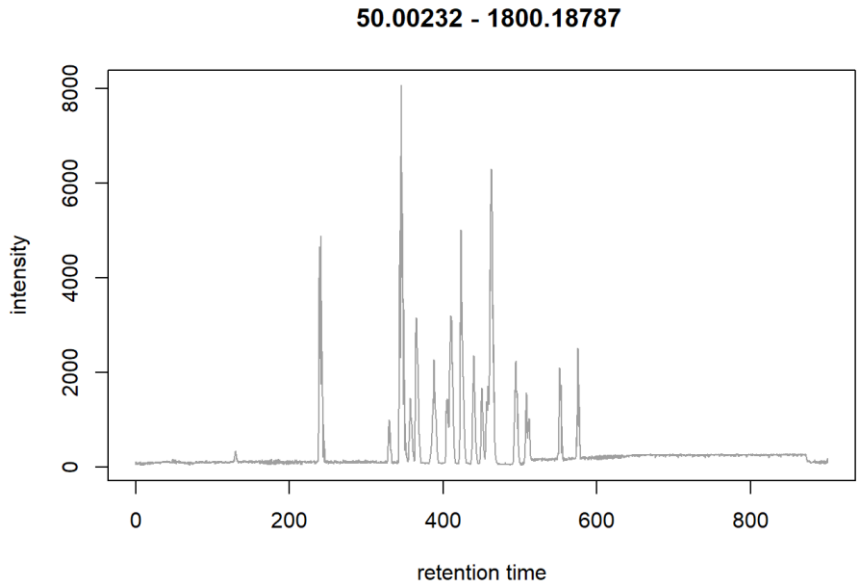
Accessing MS^2 data in XCMS3

- Important Questions:
- How to access the MS^2 data?
- What is noise? What is a real MS^2 spectrum?
- How to further analyze?
- Data available under:
  https://github.com/michaelwitting/metabolomics2018



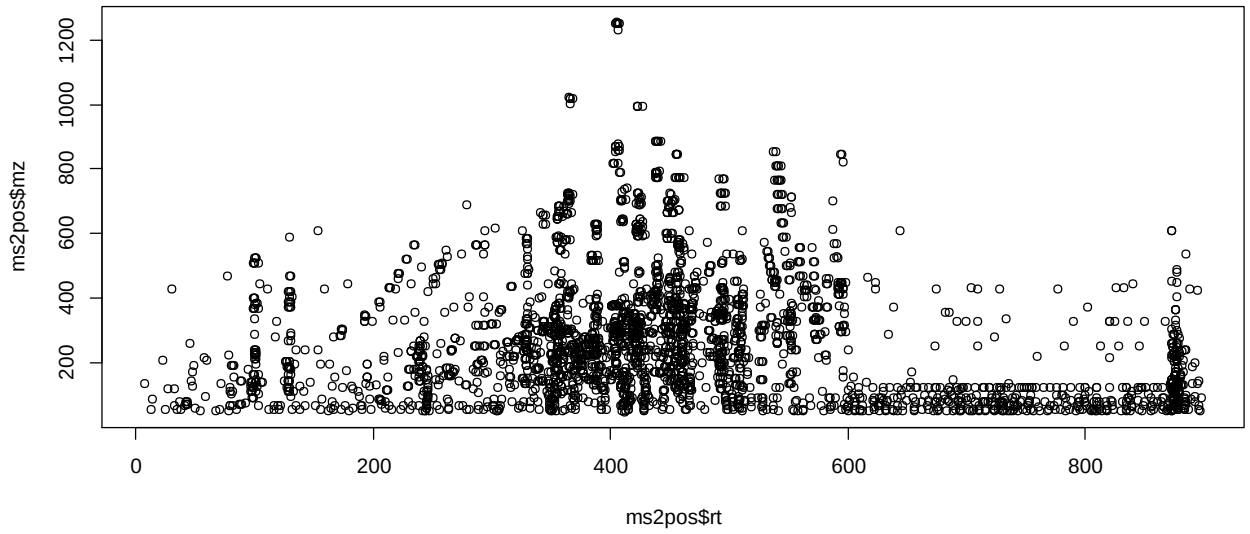
Step 1: Reading the raw data

```
## MS1 and MS2 data and file has to be read only once.
xrms <- readMSData("data\\PestMix1_DDA.mzML", mode = "onDisk", centroided = TRUE)

#plot BPC
bpis <- chromatogram(xrms, aggregationFun = "max") plot(bpis)
```



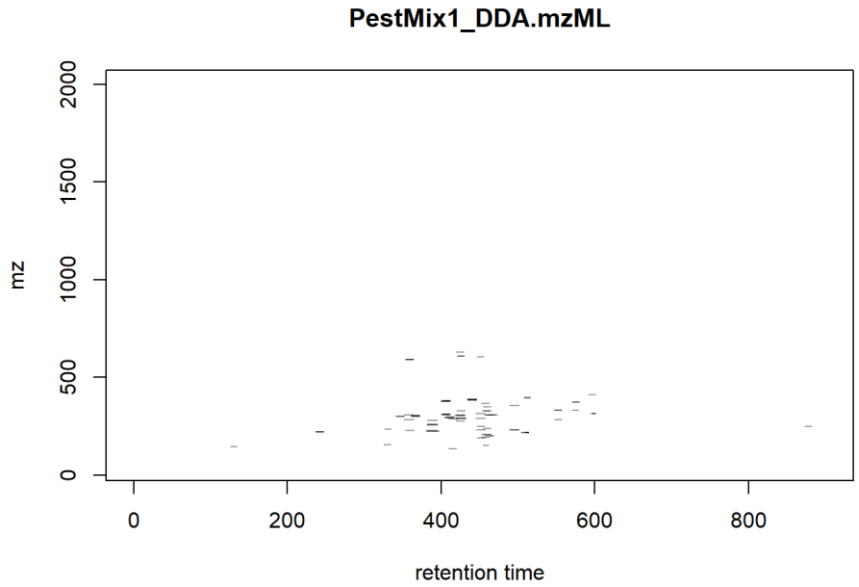
Step 2: Isolate MS^2 spectra

```
#get all MS2 spectra from DDA experiment
ms2spectra <- spectra(filterMsLevel(xrms, msLevel = 2))

#plot position of all acquired MS2 spectra
ms2pos <- data.frame(rt = unlist(lapply(ms2spectra, function(x) {return(x@rt)})),
                     mz = unlist(lapply(ms2spectra, function(x) {return(x@precursorMz)})))
plot(ms2pos$rt, ms2pos$mz)
```



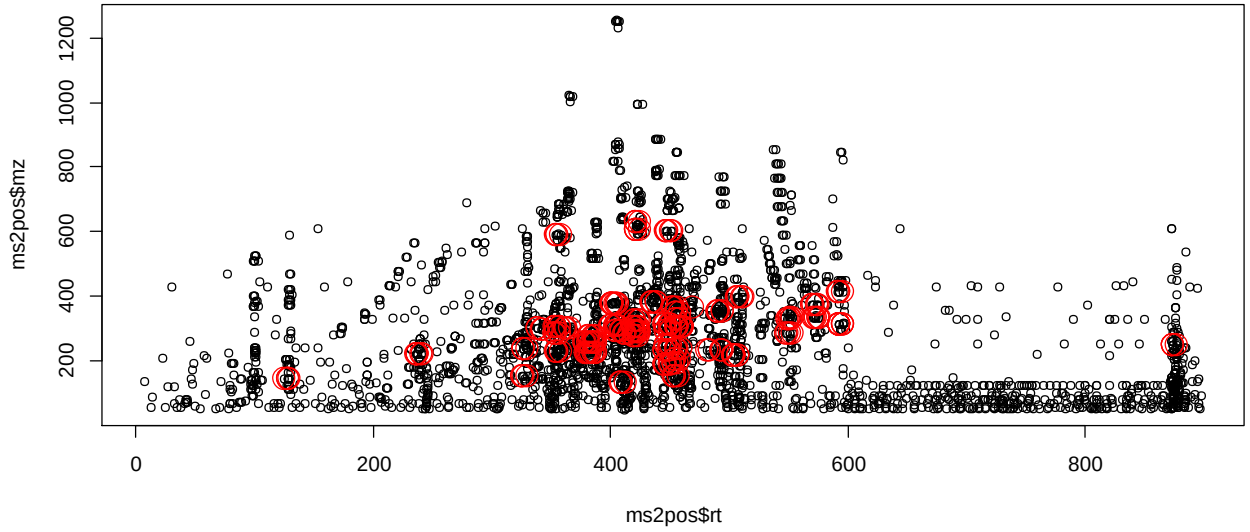
Step 3: Detect chromatographic peaks

```
#set parameters and find chromatographic peaks
ms1cwp <- CentWaveParam(snthresh = 5, noise = 100, ppm = 10, peakwidth = c(3,30))
ms1data <- findChromPeaks(xrms, param = ms1cwp, msLevel = 1)

#get all peaks
chromPeaks <- chromPeaks(ms1data)

#check detected peaks
plotChromPeaks(ms1data)
```

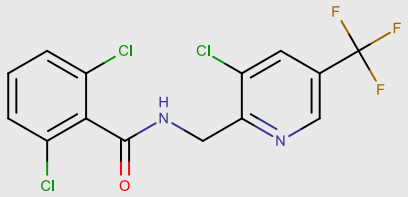


Step 4: Filter MS^2 spectra

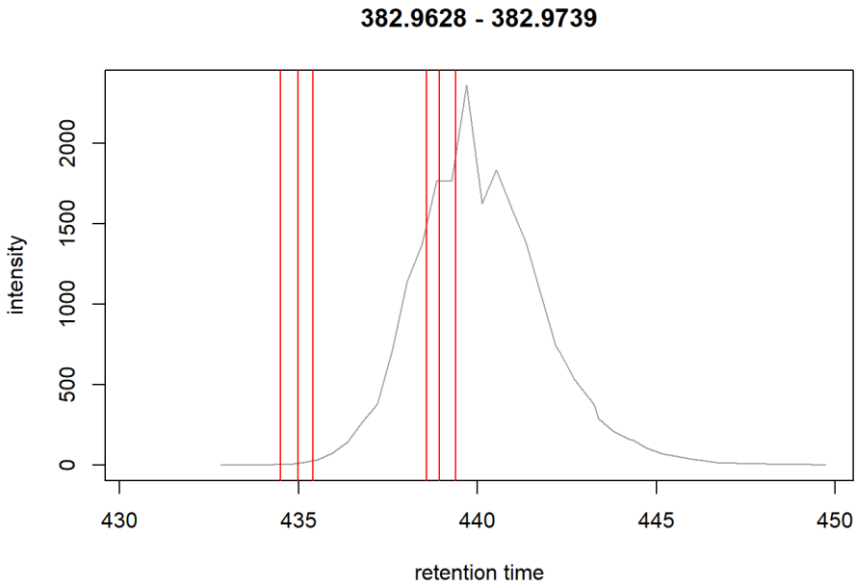
```
#create empty list
filteredMs2spectra <- list()

# interate over all chromatographic peaks and get only spectra in range of peaks
for(i in 1:nrow(chromPeaks)) {
            chromPeak <- chromPeaks[i,]
            filteredMs2spectra_clipboard <- getDdaMS2Scans(chromPeak, ms2spectra)
            filteredMs2spectra <- c(filteredMs2spectra, filteredMs2spectra_clipboard)
}

#plot the filtered spectra as red circles
ms2pos_filtered <- data.frame(rt = unlist(lapply(filteredMs2spectra, function(x) {return(x@rt)})),
                              mz = unlist(lapply(filteredMs2spectra, function(x) {return(x@precursorMz)})))

points(ms2pos_filtered$rt, ms2pos_filtered$mz, col = "red", cex = 2.5)
```



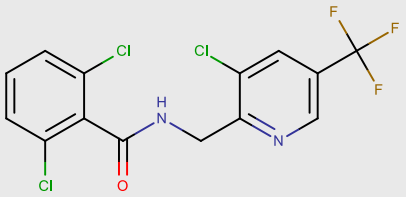
DDA example: Fluopicolide




```
#isolate Fluopicolide
chromPeak <- chromPeaks[57,]

#Fluopicolide, exact mass = 381.965430576, [M+H]+ = 382.972706
eic <- chromatogram(xrms, aggregationFun = "max", mz = c(382.96, 382.98), rt = c(430,450))
plot(eic)

#filter out fitting spectra
filteredMs2spectra <- getDdaMS2Scans(chromPeak, ms2spectra)

#mark position in EIC
abline(v = unlist(lapply(filteredMs2spectra, function(x) {return(x@rt)})), col = "red")
```

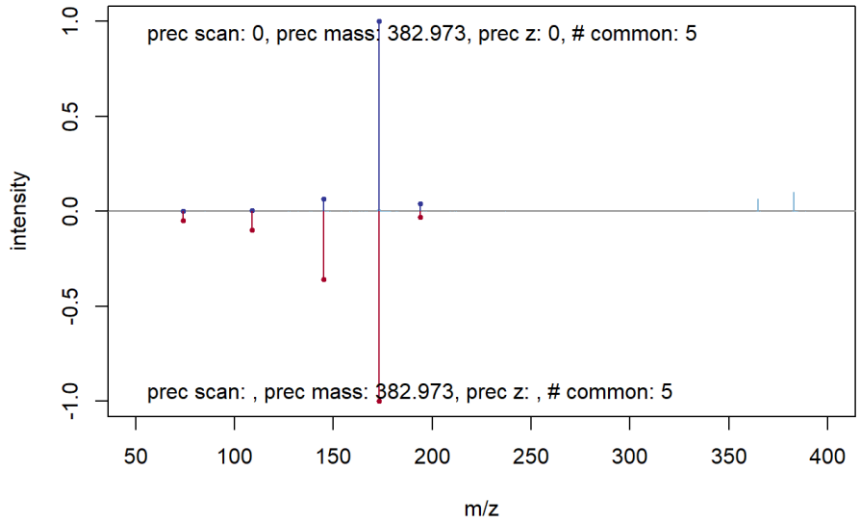


DDA example: Fluopicolide

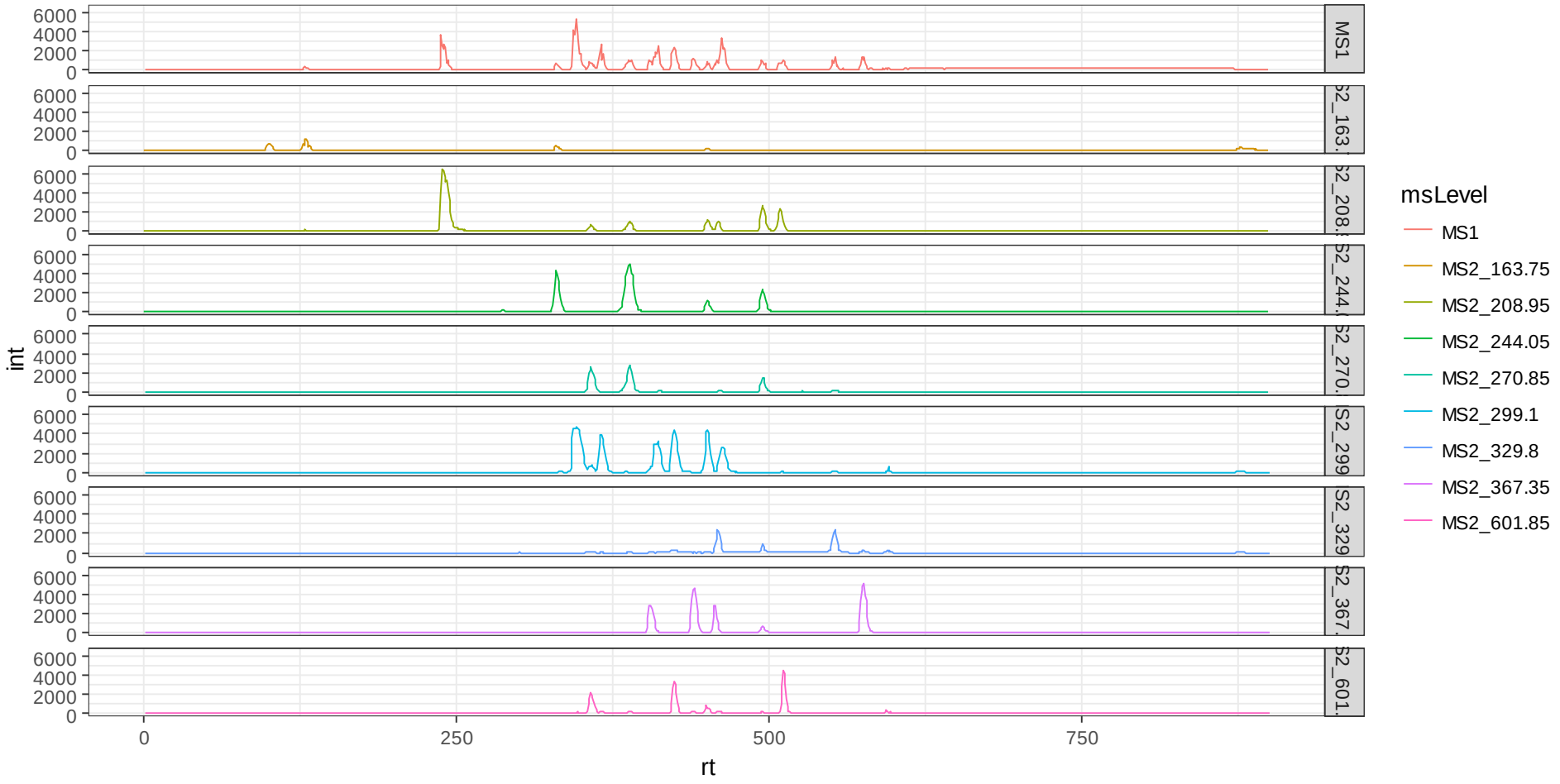



```
#example spectrum (taken from Metlin MID 72270, ESI-Q-ToF)
librarySpectrum <- new("Spectrum2",
                       precursorMz = 382.9727,
                       mz = c(193.9949, 172.9555, 144.9603, 108.9841, 74.0161),
                       intensity = c(3, 100, 36, 10, 5), centroided = TRUE)

# make mirror plot of two spectra
plot(filteredMs2spectra[[4]], librarySpectrum)

# calculate dot product
compareSpectra(filteredMs2spectra[[4]], librarySpectrum, binSize = 0.01, fun = "dotproduct")
```

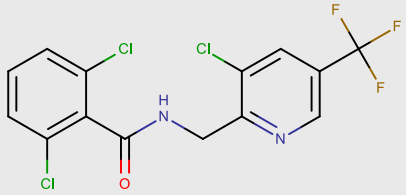
```
## [1] 0.9488386
```



Preview: DIA data in XCMS



DIA example: Fluopicolide – MS2



- Reconstruction of MS^2 spectra from DIA
- Step 1: Splitting of data into MS^1 and MS^2 data sets
- Step 2: Splitting of MS^2 data into defined SWATH pockets
- Step 3: Chromatographic peak detection
- Step 4: Deconvolution of MS^2 data using EICs correlation
- Step 5: Reconstruction of MS^2 spectrum