

LC-MS/MS data handling with **xcms**

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LC-MS data analysis with `xcms`

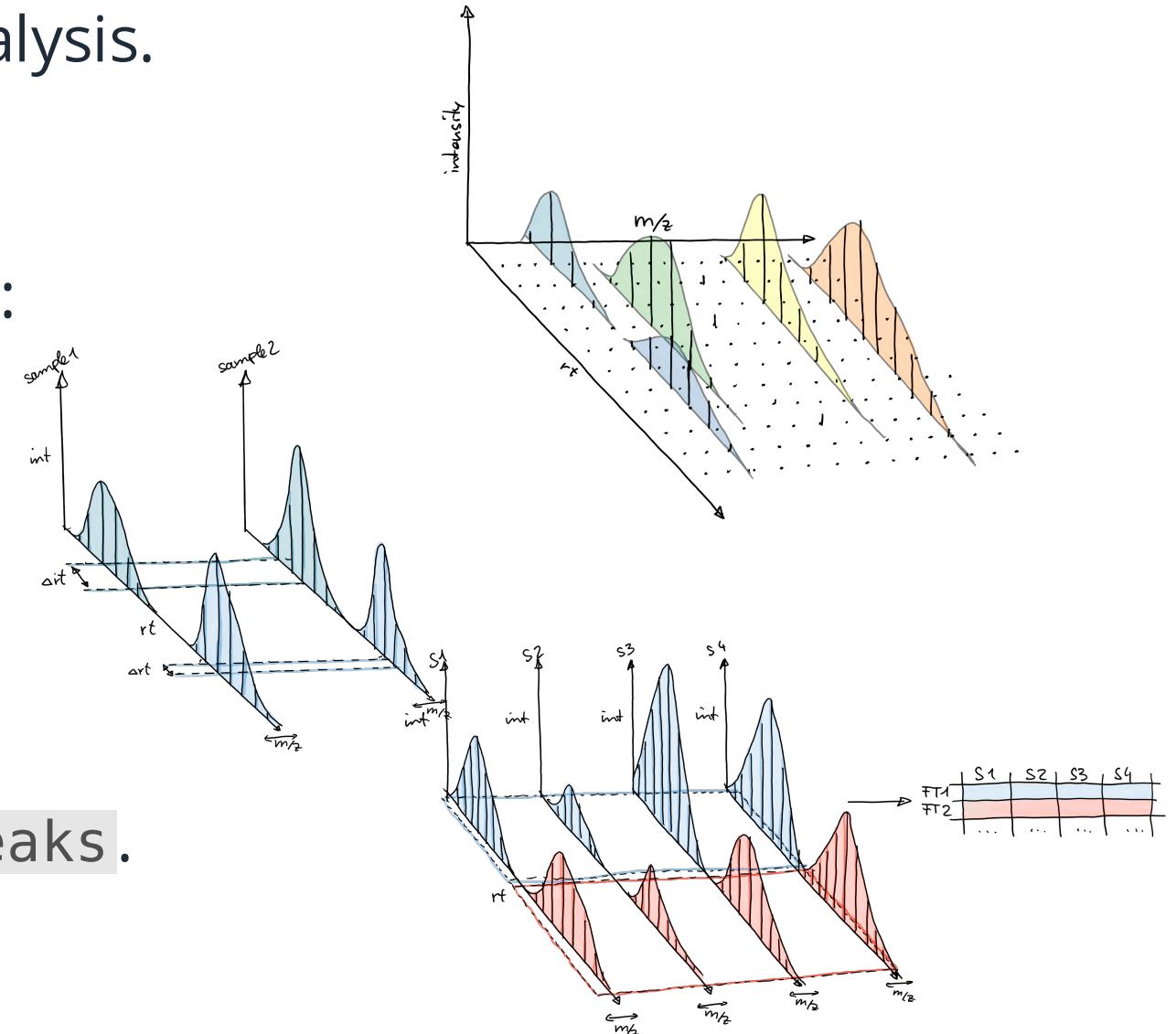
- `xcms`: toolbox for LC-MS data analysis.

- Chromatographic peak detection:
`findChromPeaks`.

- Alignment: `adjustRtime`.

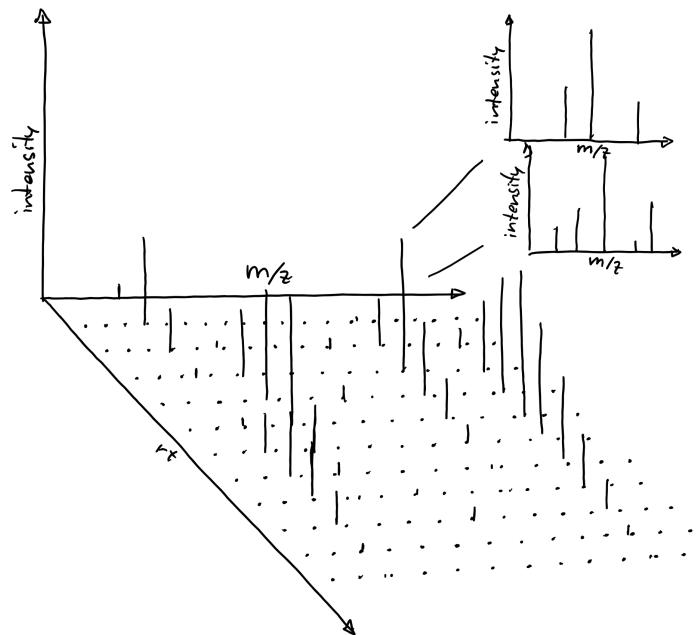
- Correspondence: `groupChromPeaks`.

- **Don't use defaults!**
see [Metabolomics data pre-processing using xcms workshop](#).



- Result: `matrix` with feature abundances in samples.
- Normalization and identification of features of interest: use any functionality available in `R`.
- Annotation of features from LC-MS experiments challenging.
- LC-MS/MS data: MS2 spectra assist in annotation.
- Added support for LC-MS/MS data analysis in `xcms`.
- Possible because we re-use functionality and classes from the `MSnbase` package.

Analyzing DDA data with **xcms**



```
dda_file <- system.file("TripleTOF-SWATH/PestMix1_DDA.mzML",
                        package = "msdata")
dda_data <- readMSData(dda_file, mode = "onDisk")
table(msLevel(dda_data))
```

```
##
##    1    2
## 4627 2975
```

Analyzing DDA data with **xcms**

- MS1 chromatographic peak detection:

```
cwp <- CentWaveParam(snthresh = 5, noise = 100, ppm = 10,  
                      peakwidth = c(3, 30))  
dda_data <- findChromPeaks(dda_data, param = cwp)
```

```
dda_spectra <- chromPeakSpectra(dda_data)  
dda_spectra
```

```
## Spectra with 158 spectra and 1 metadata column(s):  
##          msLevel      rtime peaksCount |    peak_id  
##          <integer> <numeric> <integer> | <character>  
## CP01.F1.S1000        2   128.237       16 |    CP01  
## CP01.F1.S1008        2   128.737       40 |    CP01  
## ...                 ...     ...       ... |    ...  
## CP98.F1.S5266        2   596.054       88 |    CP98  
## CP99.F1.S7344        2   873.714       20 |    CP99
```

Analyzing DDA data with **xcms**

- Example: annotate chrom peak with an m/z of 304.1131.

```
chromPeaks(dda_data, mz = 304.1131, ppm = 20)
```

```
##          mz      mzmin      mzmax       rt     rtmin     rtmax     into     intb
## CP53 304.1133 304.1126 304.1143 424.614 417.985 430.784 13709.7 13658.01
##          maxo sn sample
## CP53 3978.987 74      1
```

- Get MS2 spectra associated with that peak

```
ex_spectra <- dda_spectra[mcols(dda_spectra)$peak_id == "CP53"]
```

Analyzing DDA data with **xcms**

```
## Spectra with 5 spectra and 1 metadata column(s):
##          msLevel      rtime peaksCount |    peak_id
##          <integer> <numeric> <integer> | <character>
## CP53.F1.S3505      2   418.926      10 |    CP53
## CP53.F1.S3510      2   419.306      30 |    CP53
## CP53.F1.S3582      2   423.036     694 |    CP53
## CP53.F1.S3603      2   423.966     783 |    CP53
## CP53.F1.S3609      2   424.296     753 |    CP53
```

- Build *consensus* spectrum.

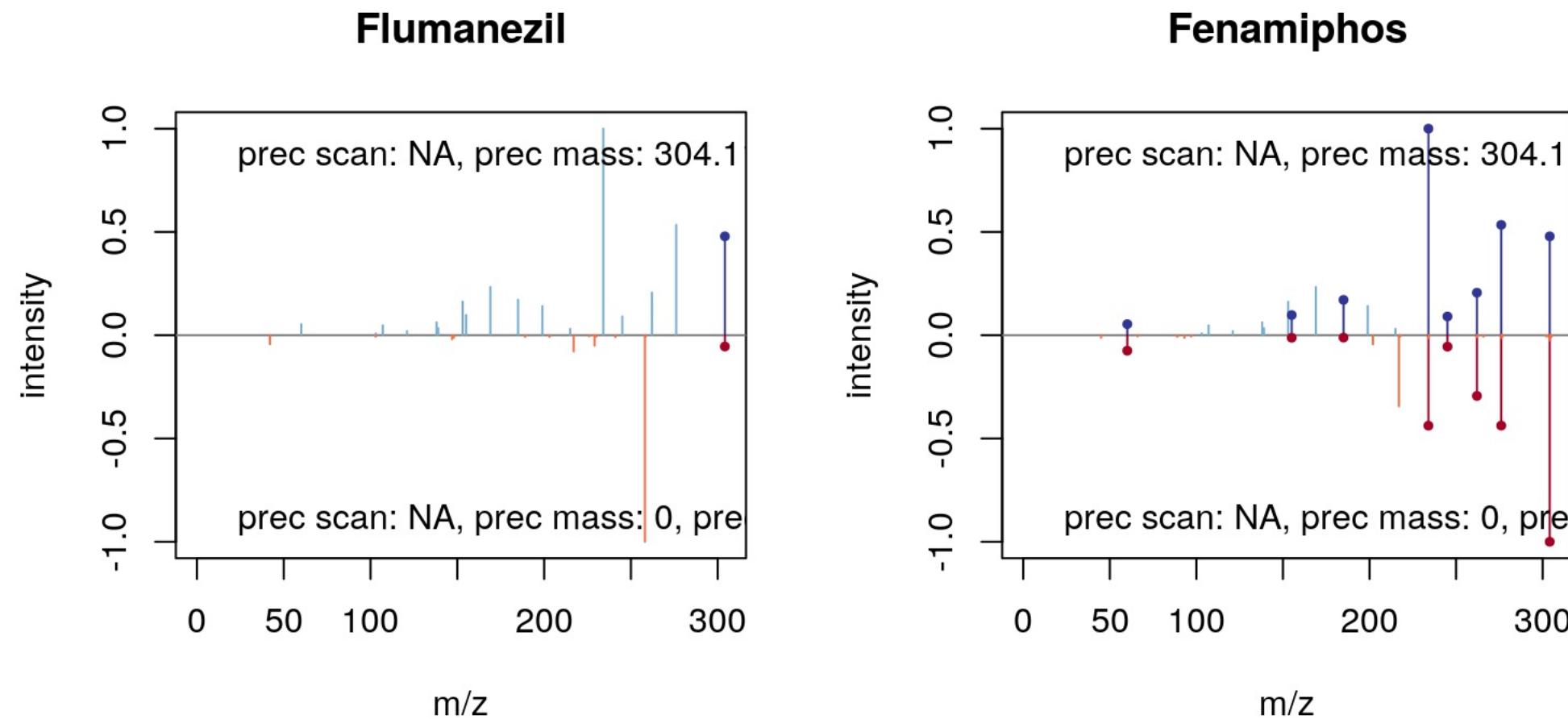
```
ex_spectrum <- combineSpectra(ex_spectra, method = consensusSpectrum,
                                ppm = 10, minProp = 0.8)
ex_spectrum
```

```
## Spectra with 1 spectra and 1 metadata column(s):
##          msLevel      rtime peaksCount |    peak_id
##          <integer> <numeric> <integer> | <character>
## CP53.F1.S3505      2   418.926      17 |    CP53
```

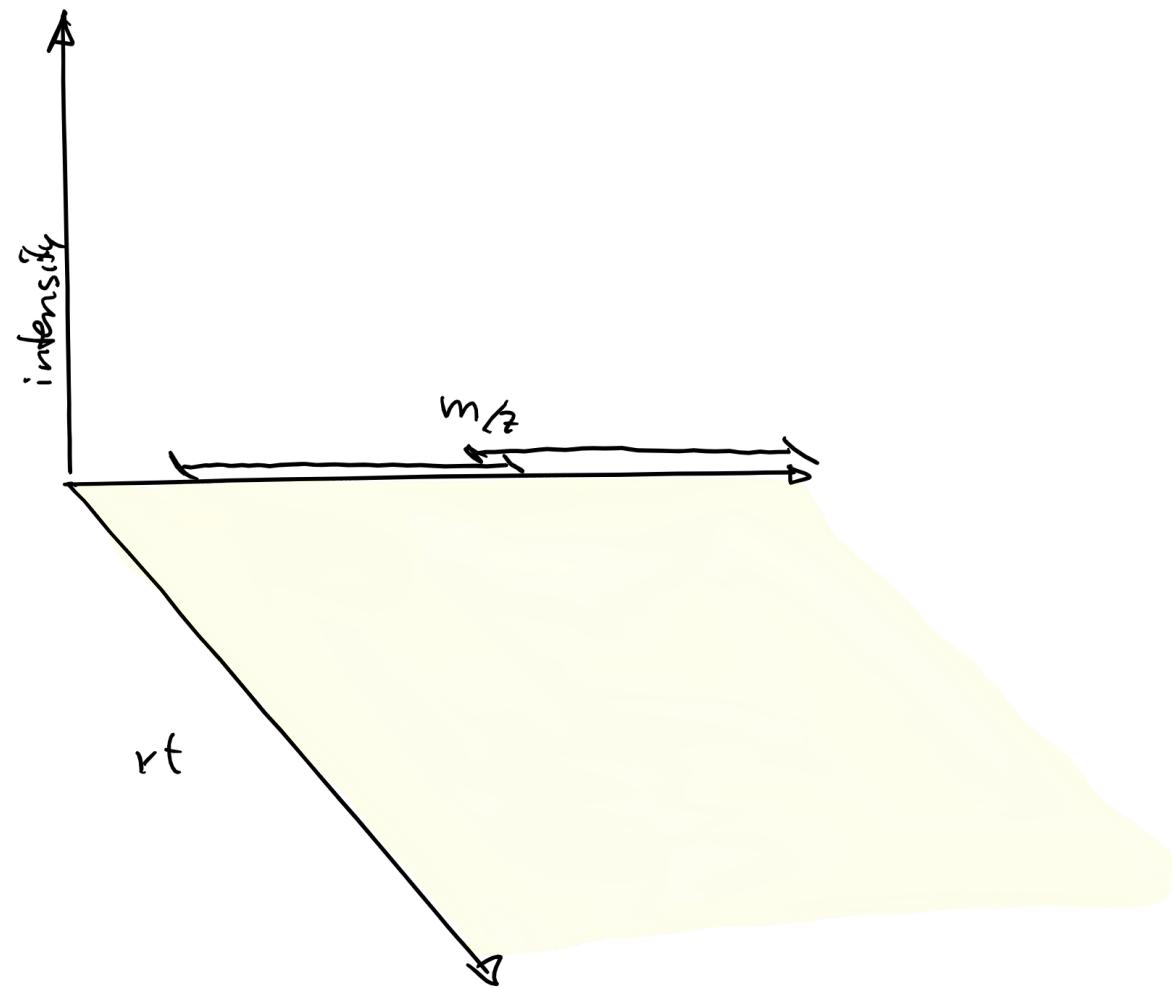
Analyzing DDA data with **xcms**

- Compare the consensus spectrum against 2 candidates with same m/z.

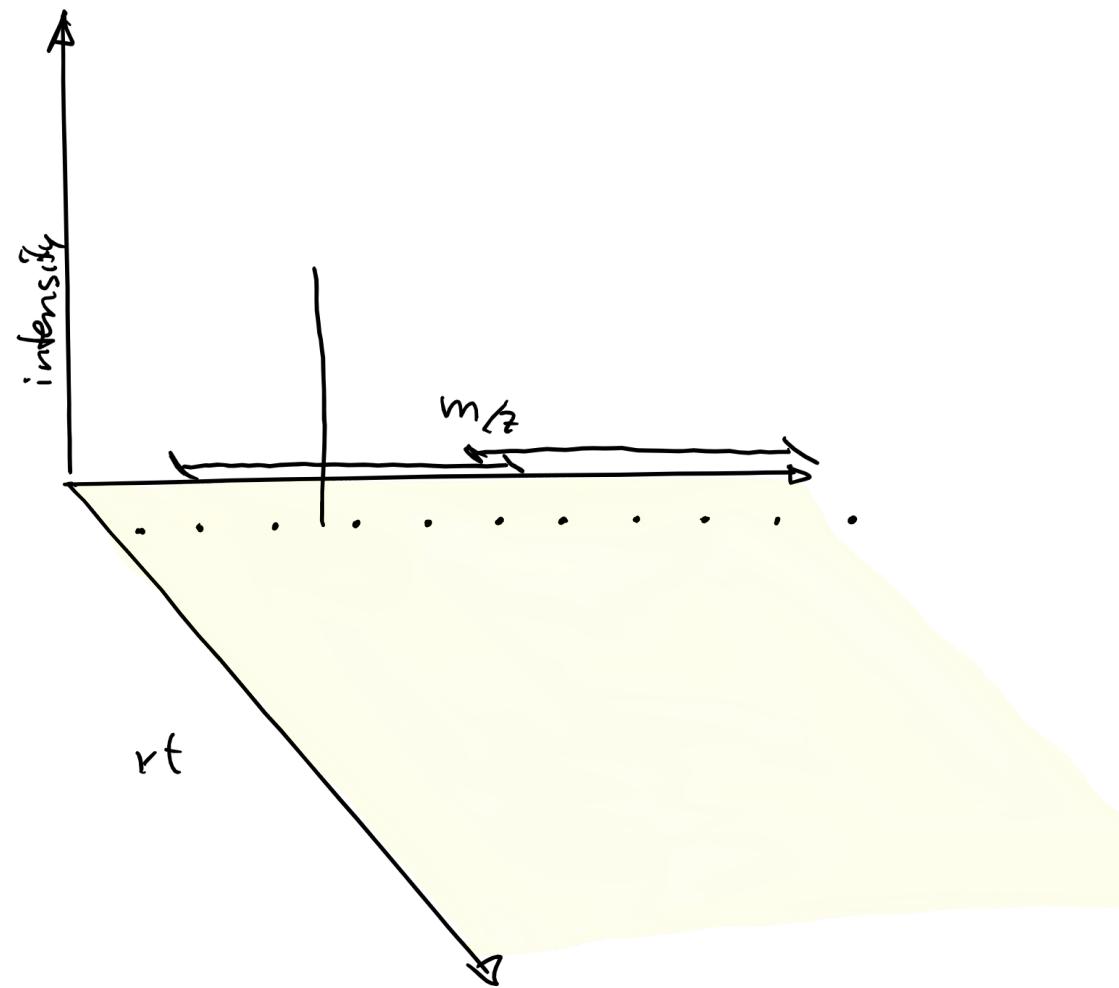
```
par(mfrow = c(1, 2))
plot(ex_spectrum, flumanezil, main = "Flumanezil", tolerance = 40e-6)
plot(ex_spectrum, fenamiphos, main = "Fenamiphos", tolerance = 40e-6)
```



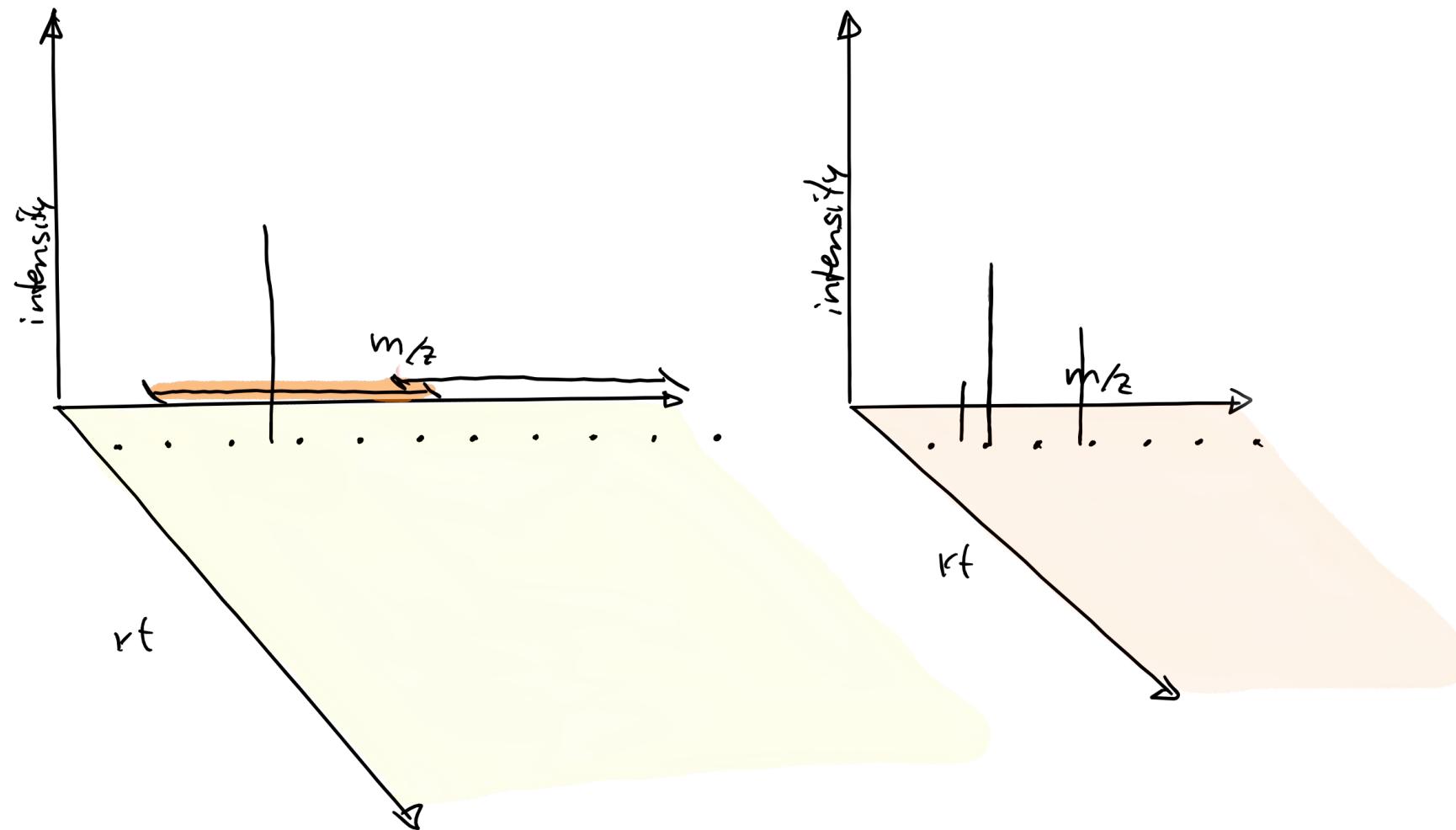
SWATH data



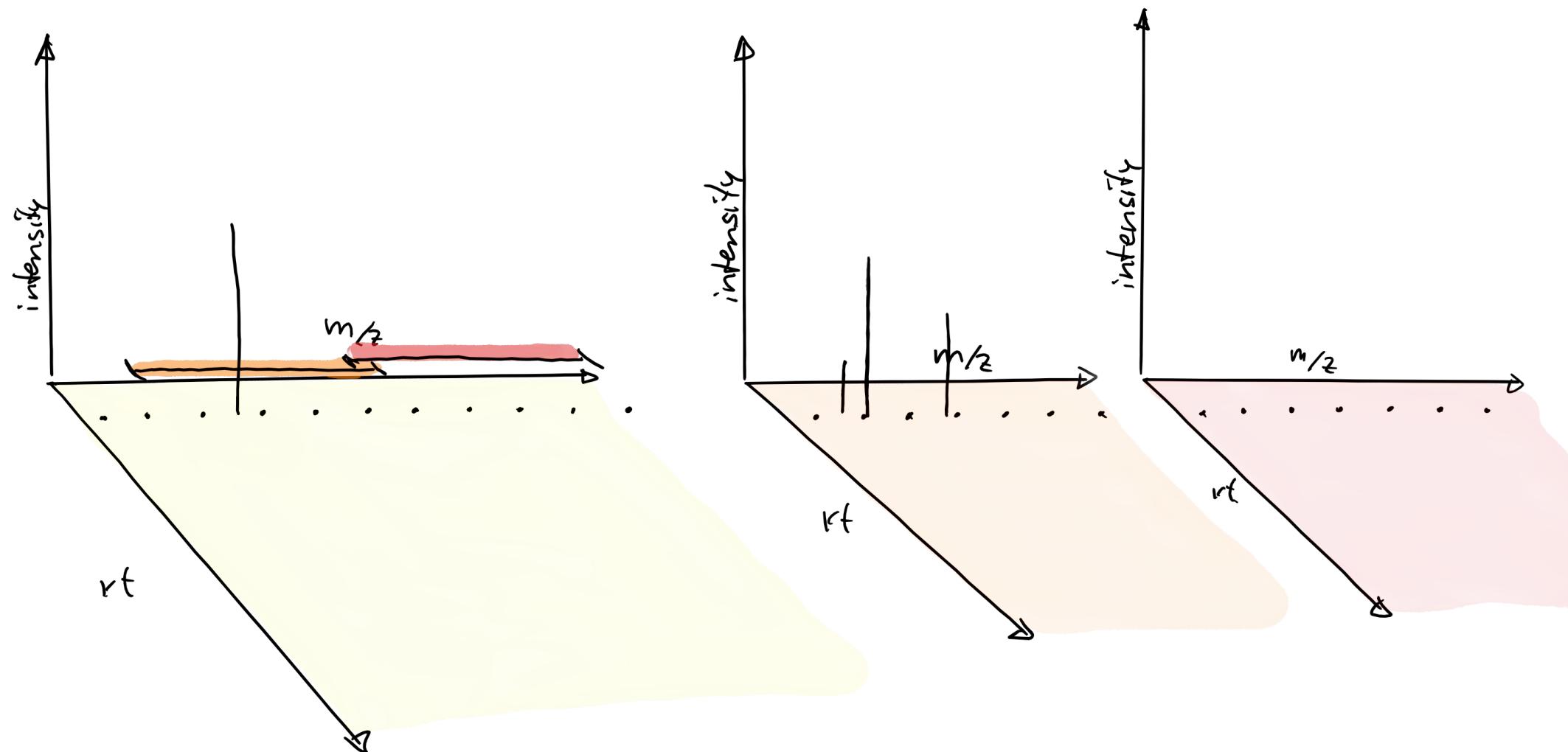
SWATH data



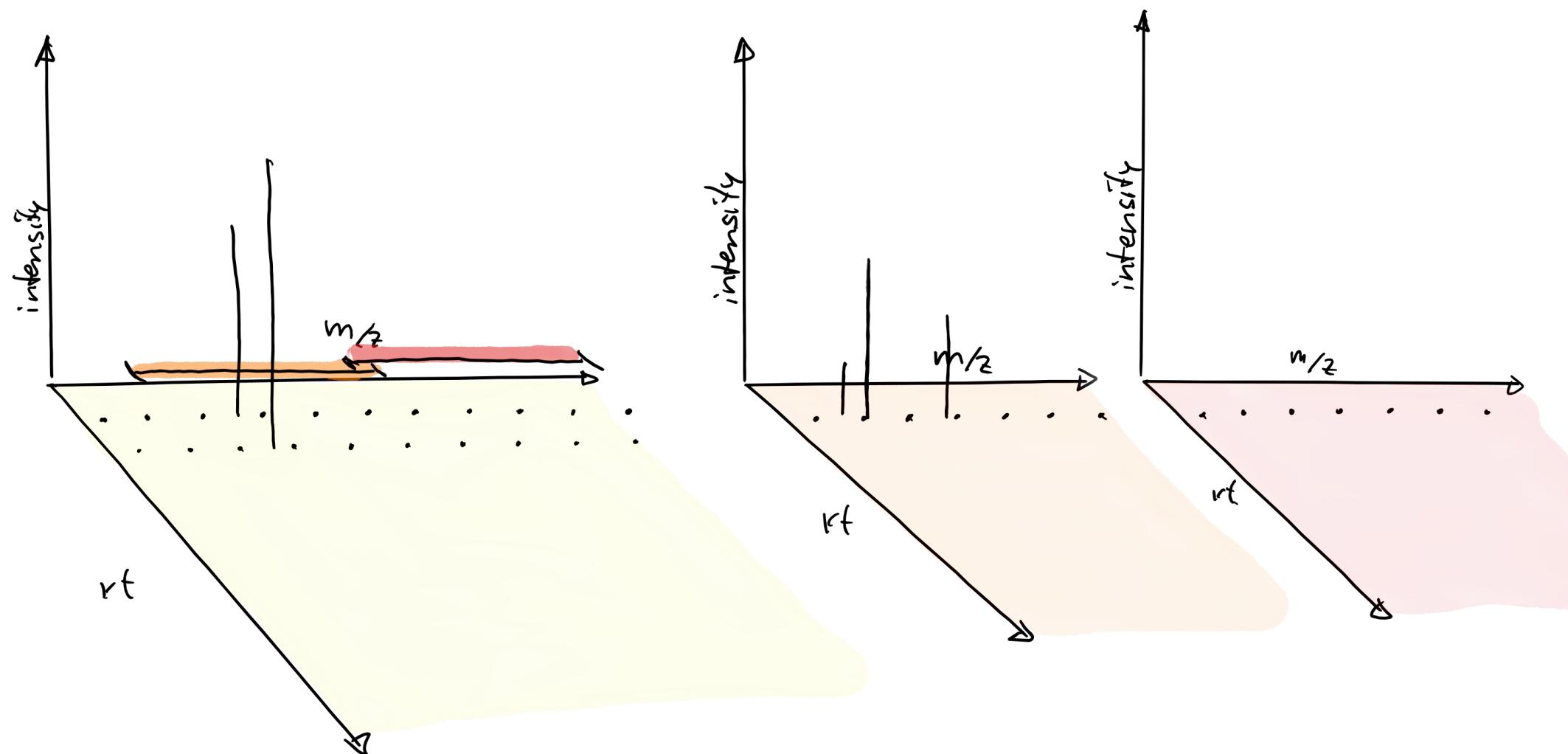
SWATH data



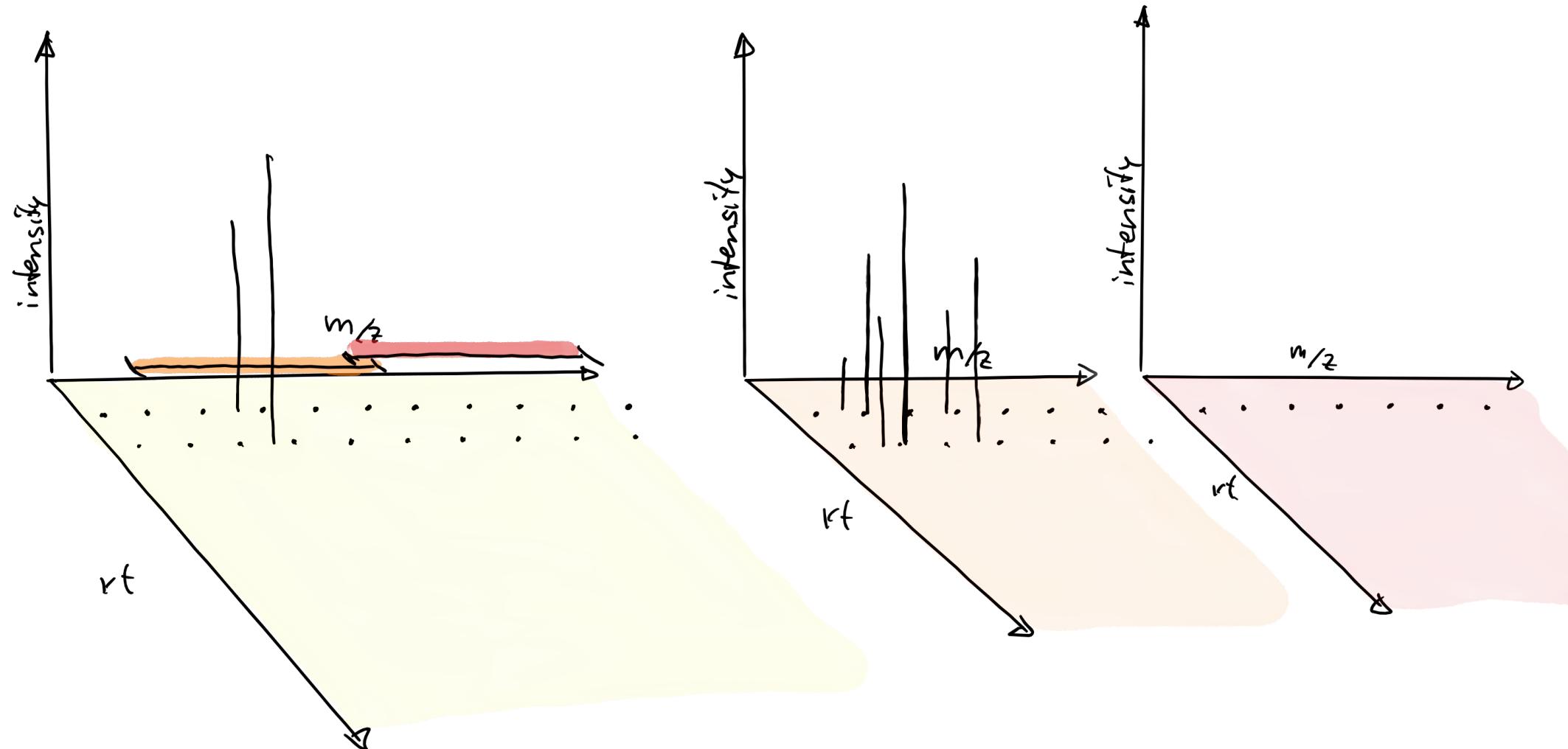
SWATH data



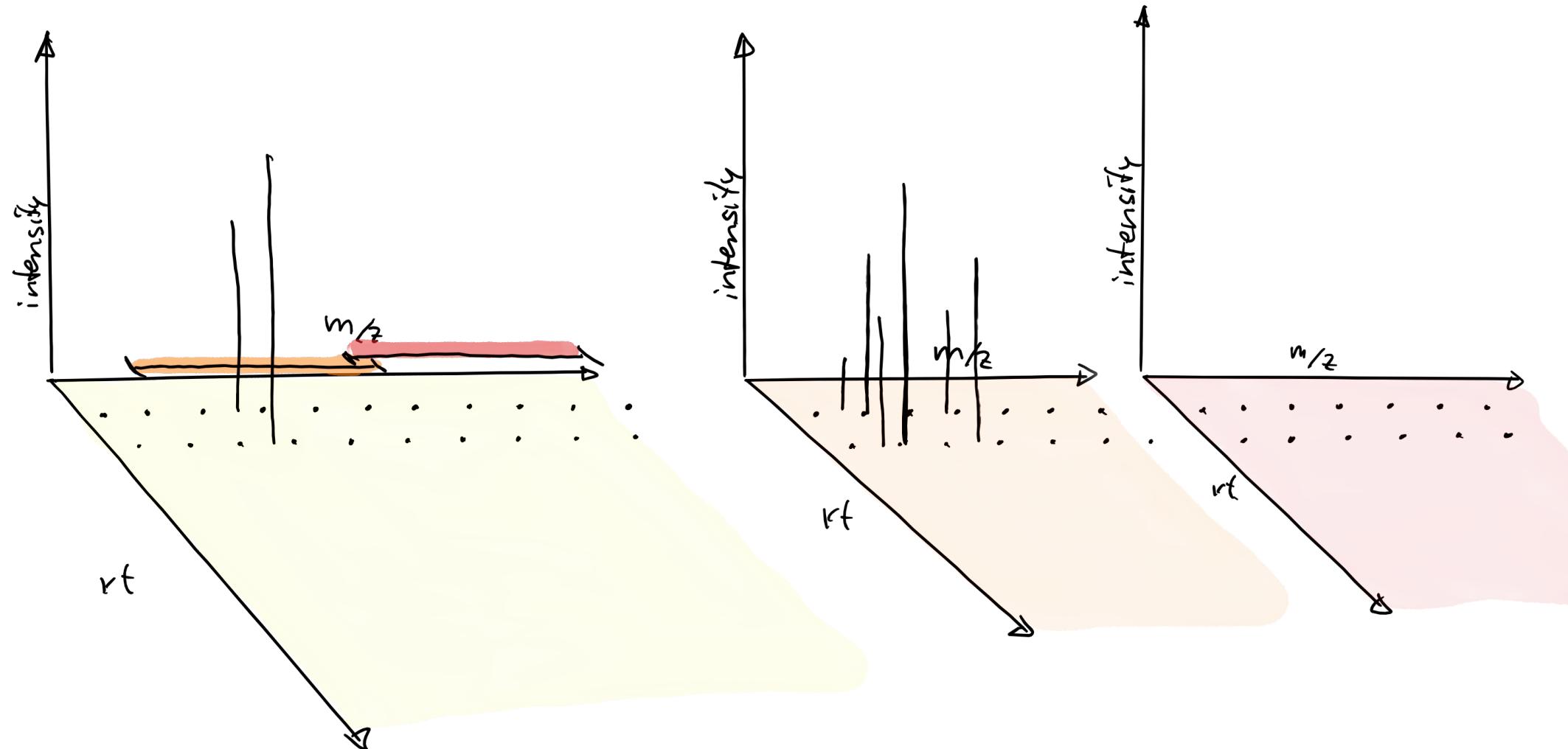
SWATH data



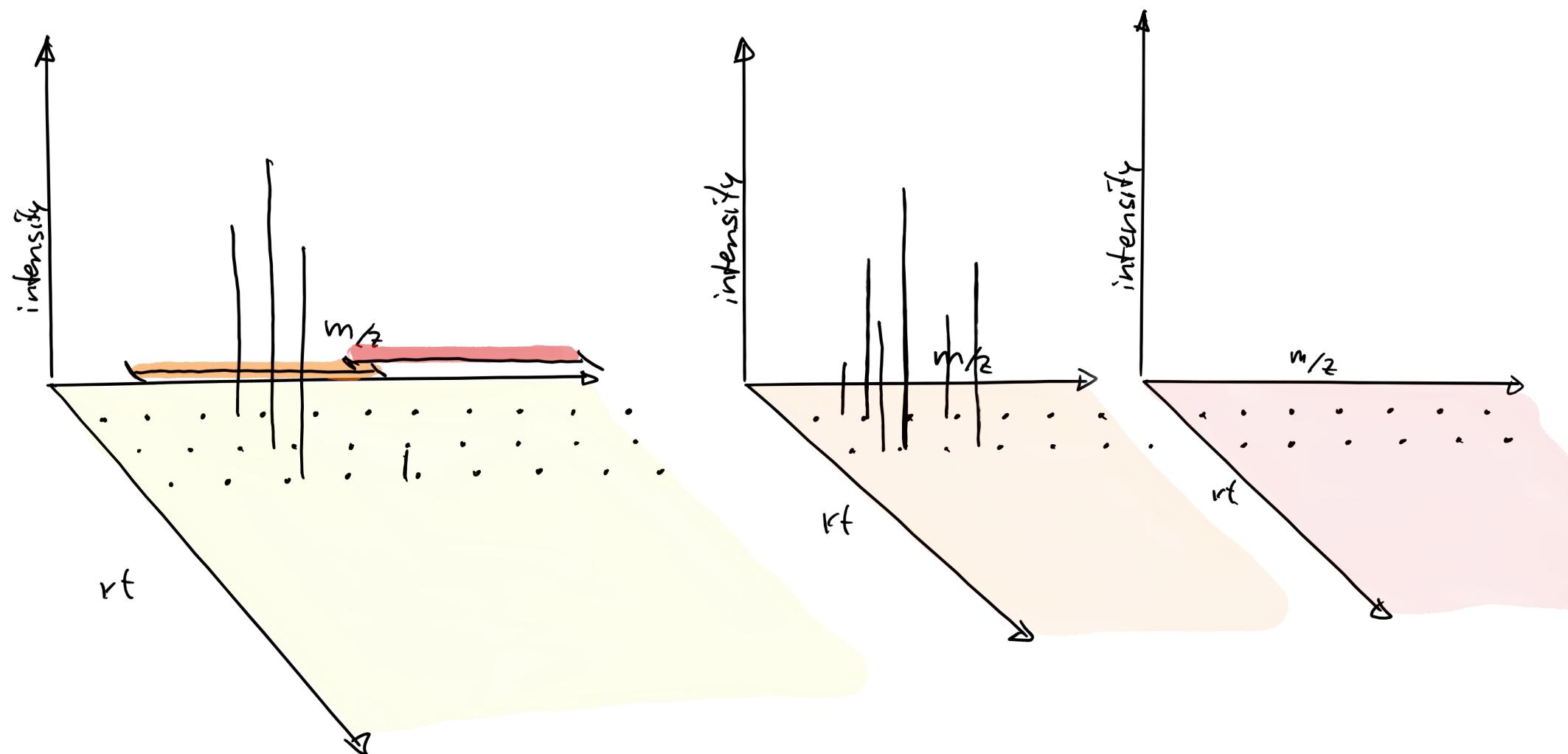
SWATH data



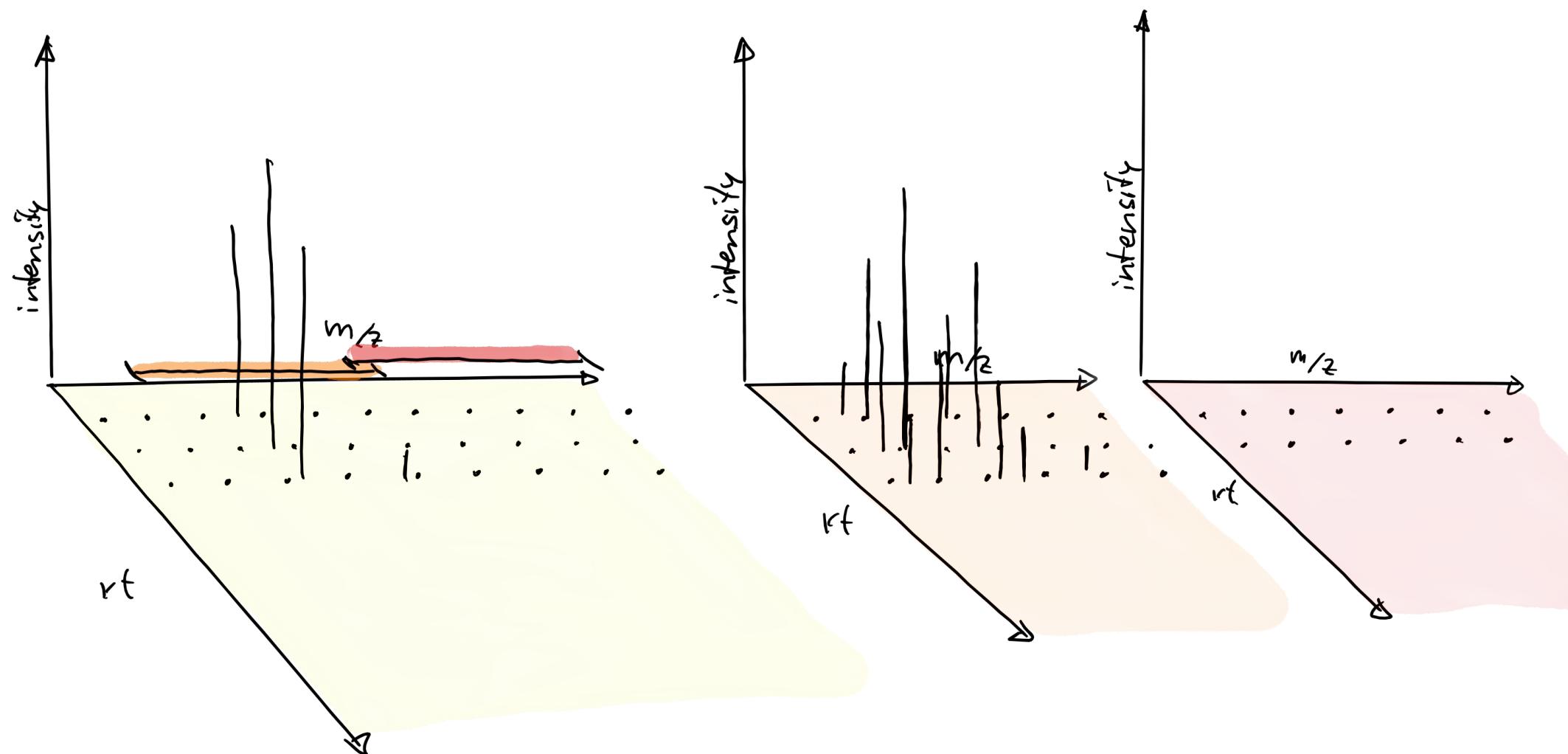
SWATH data



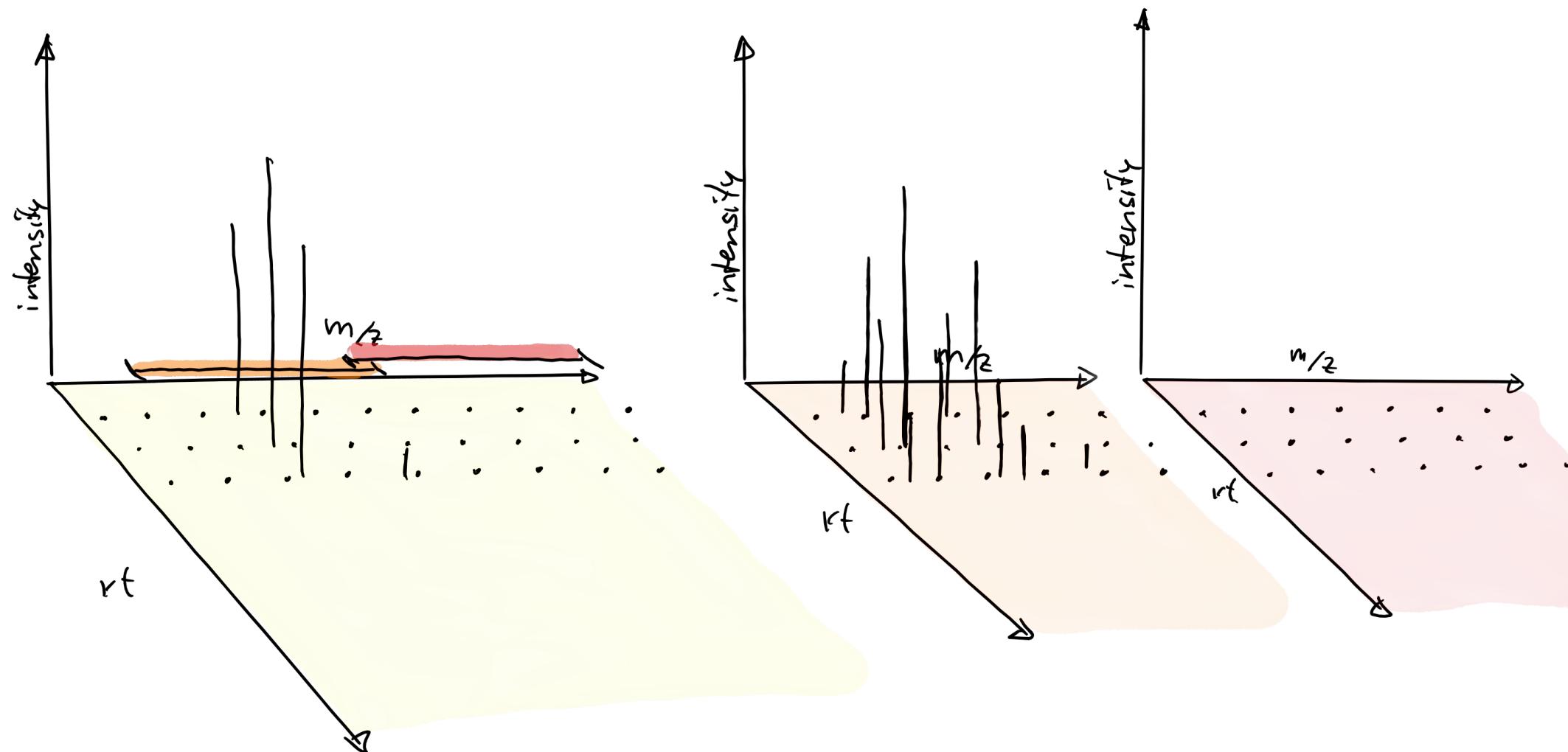
SWATH data



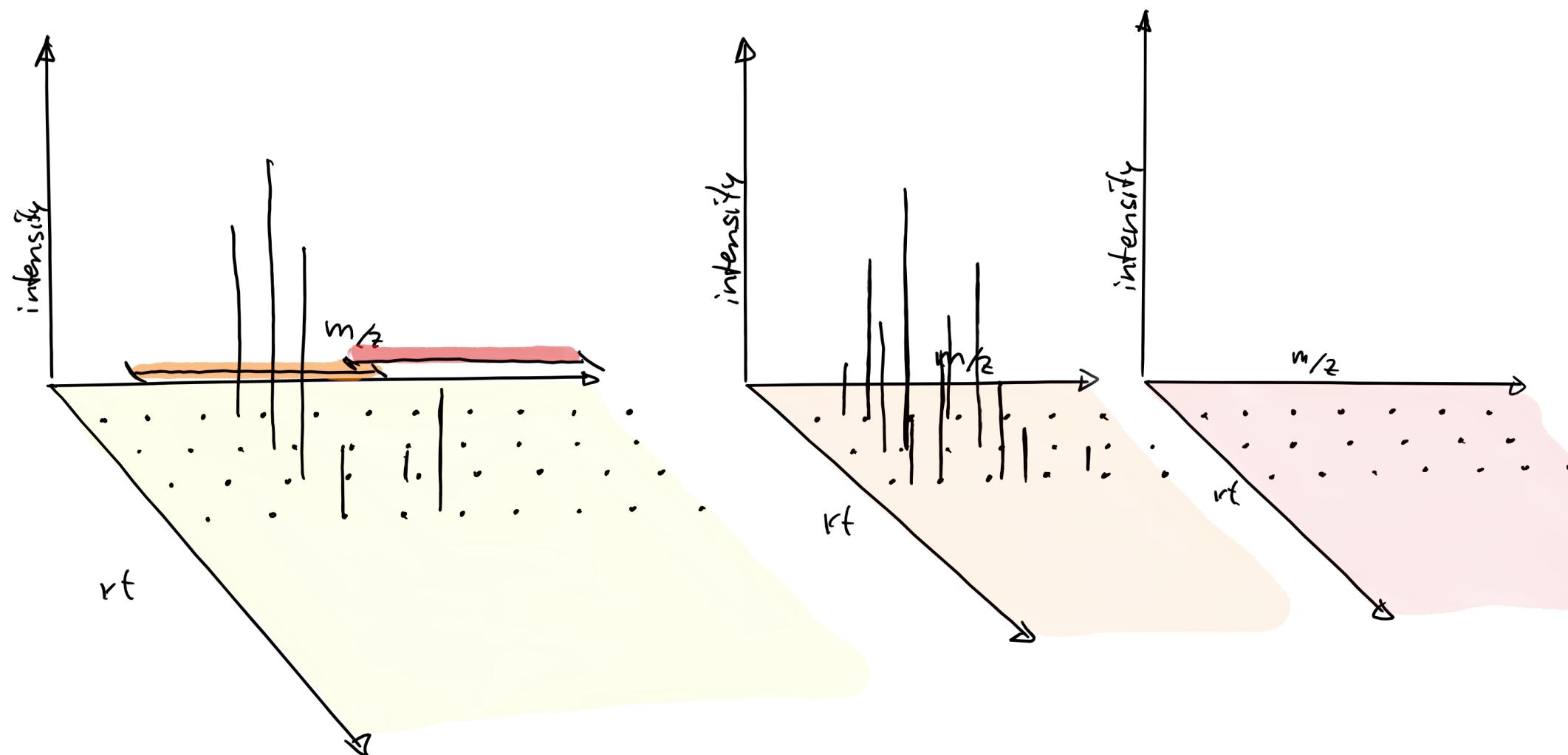
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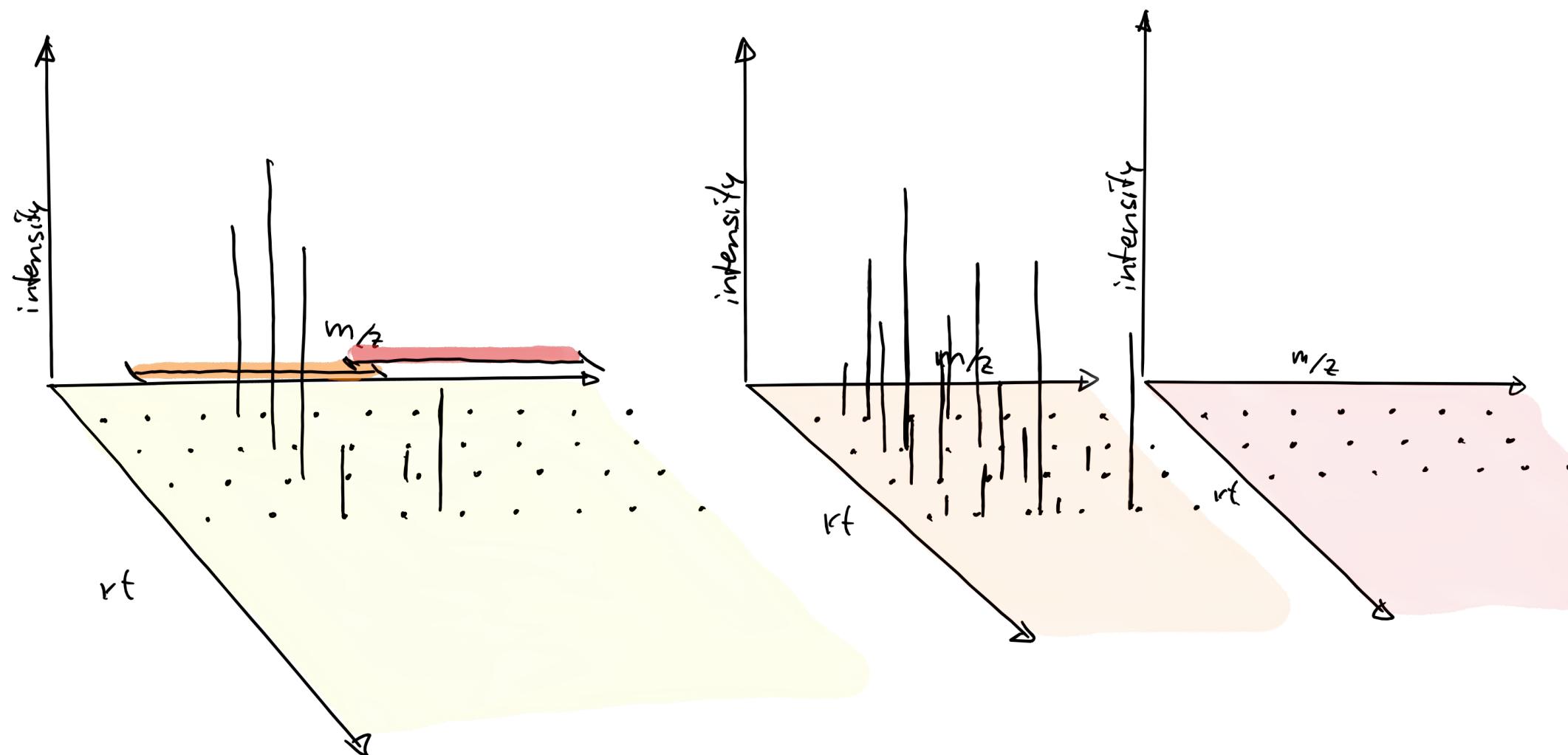
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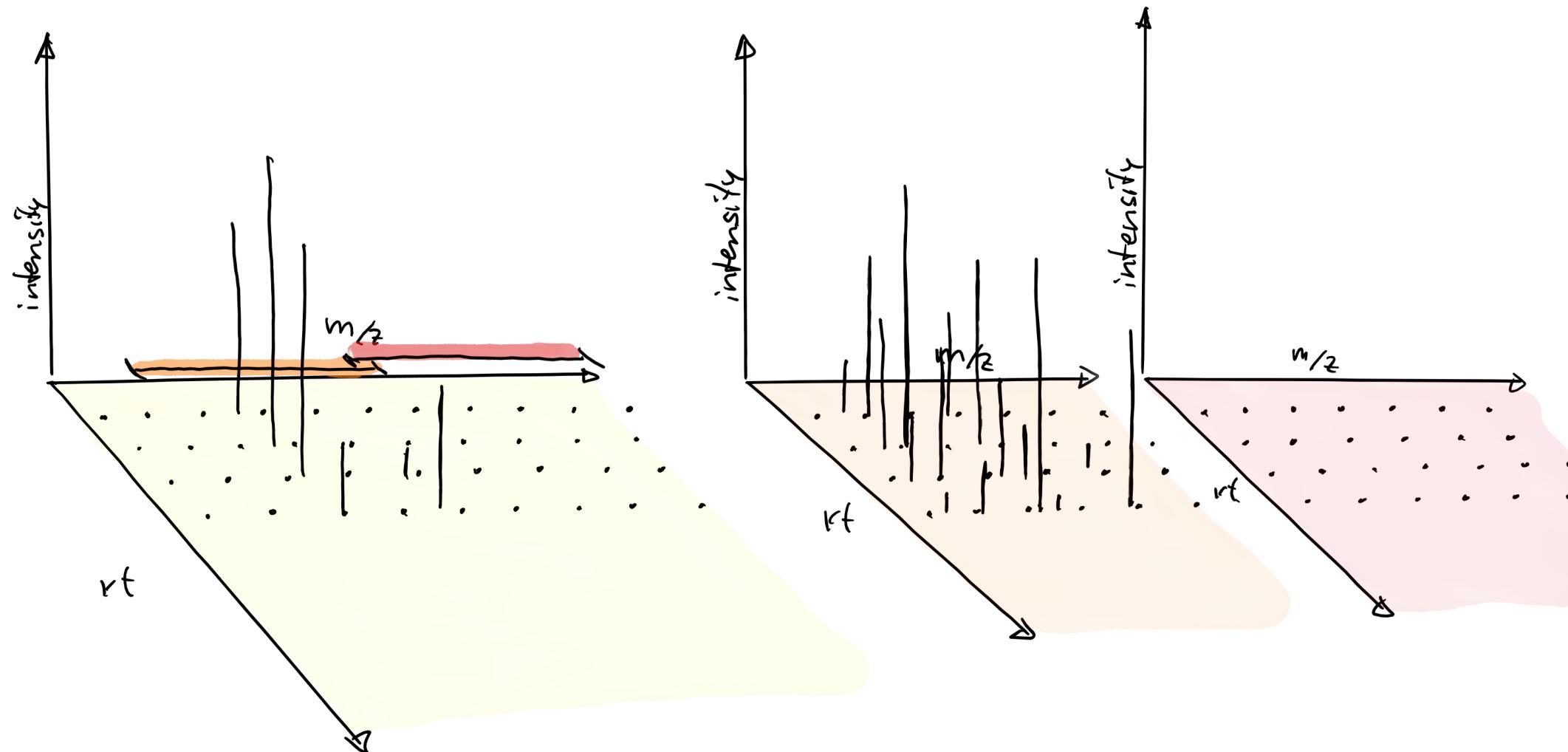
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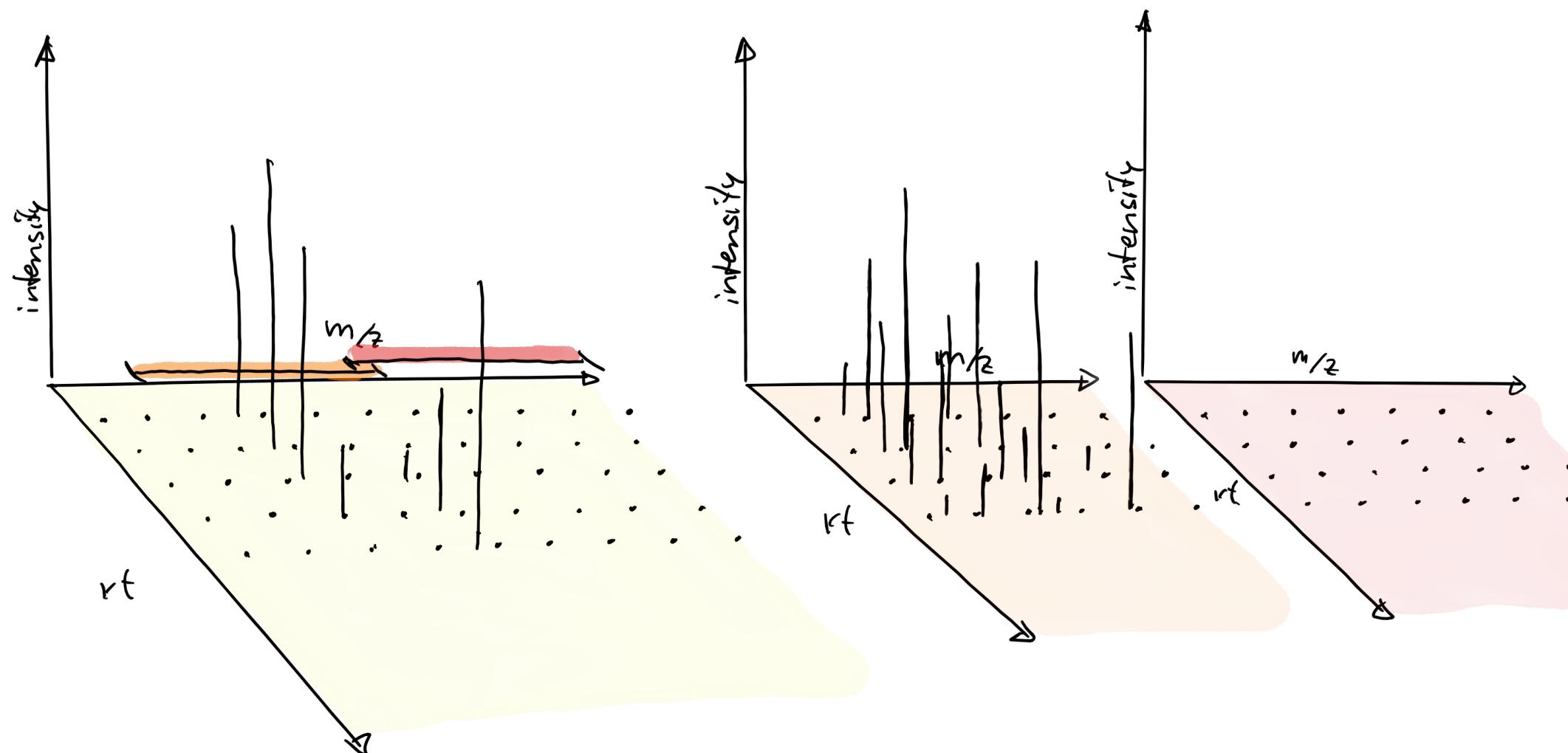
SWATH data



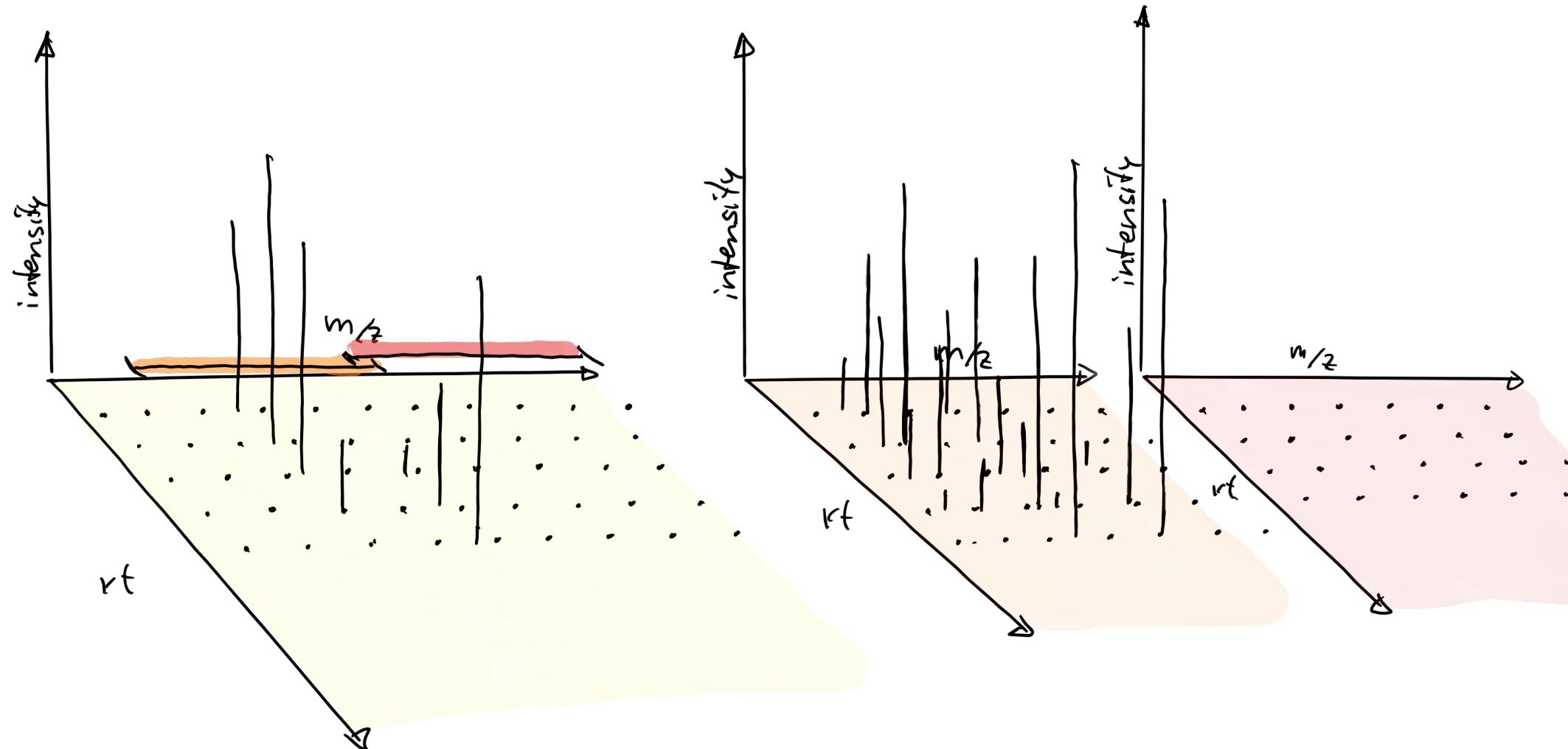
SWATH data



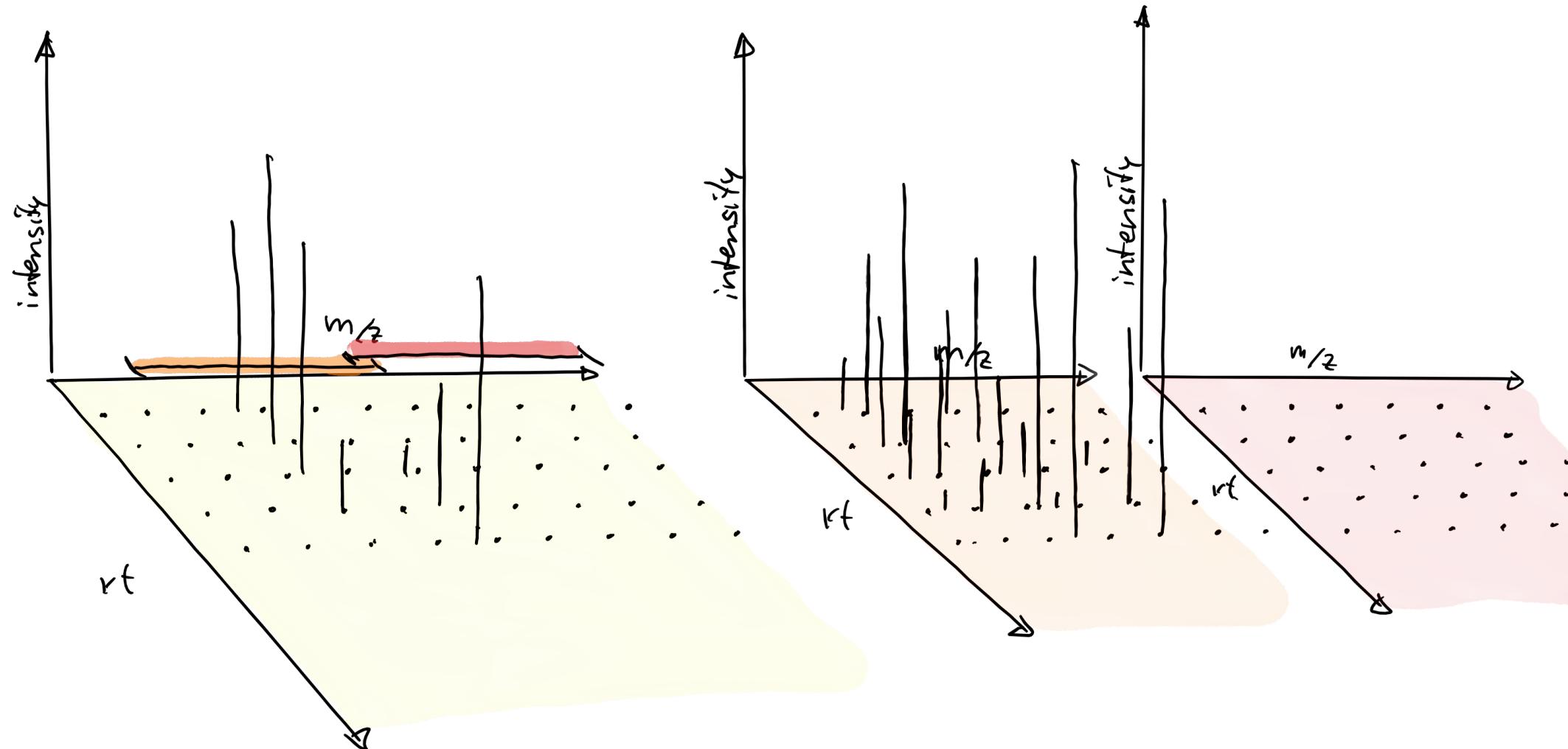
SWATH data



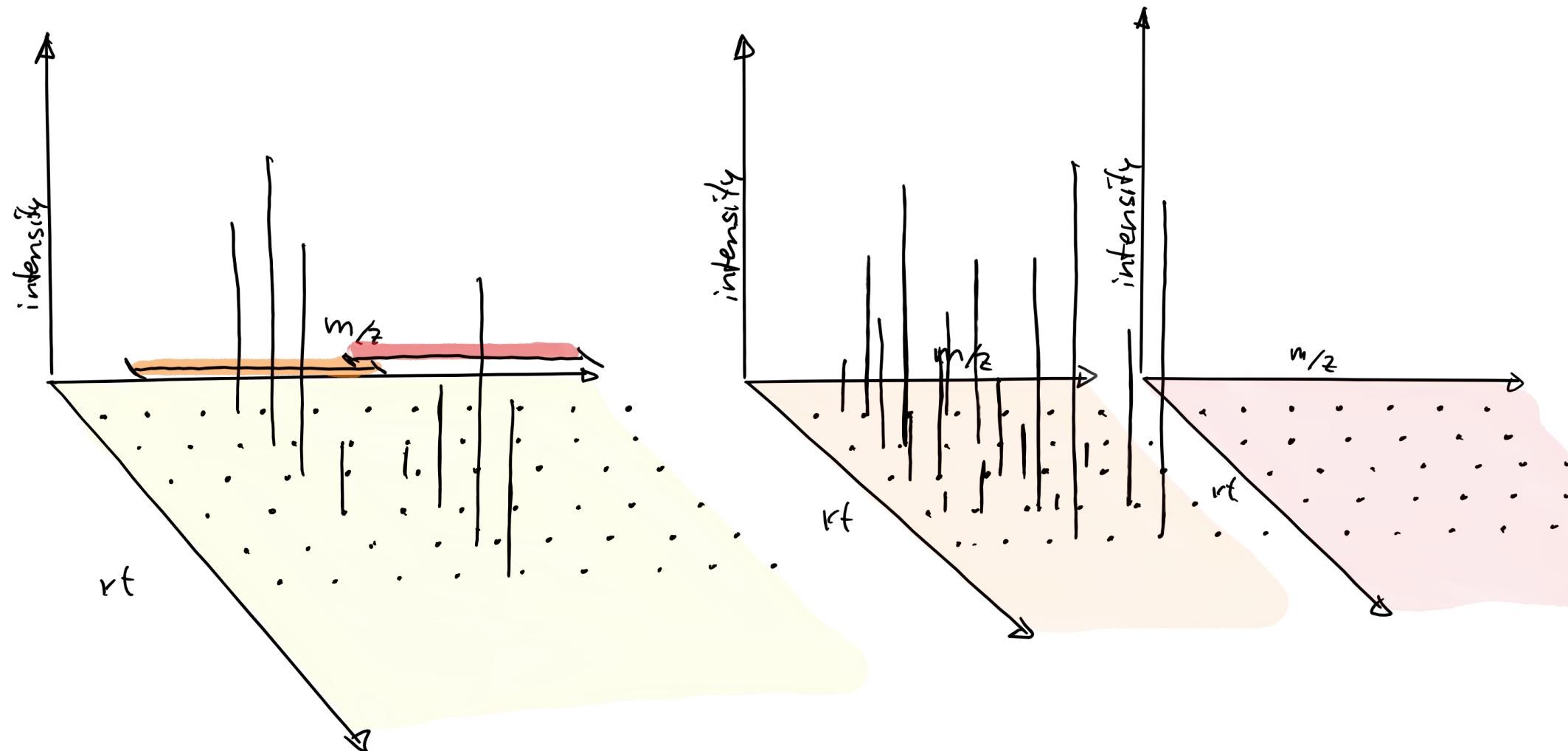
SWATH data



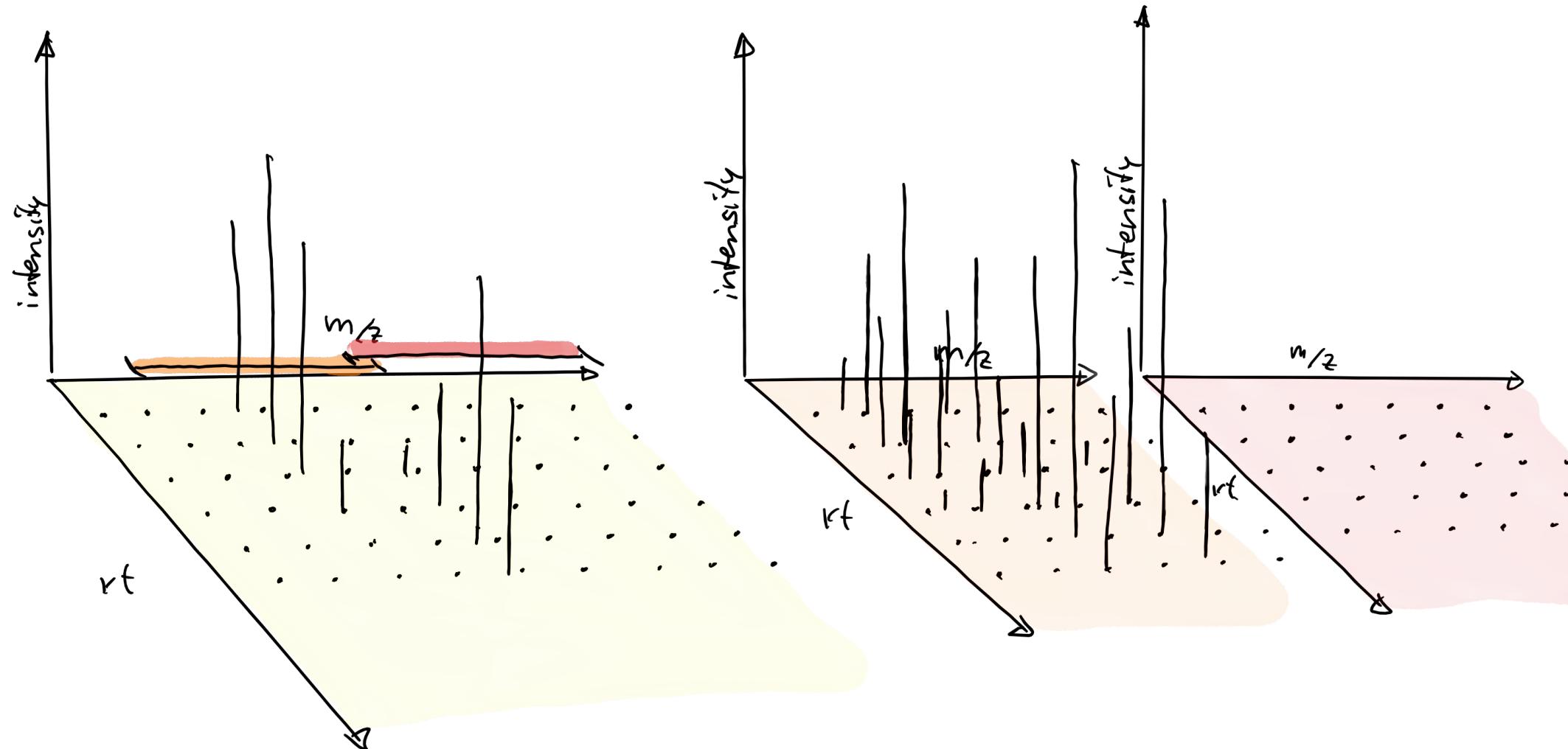
SWATH data



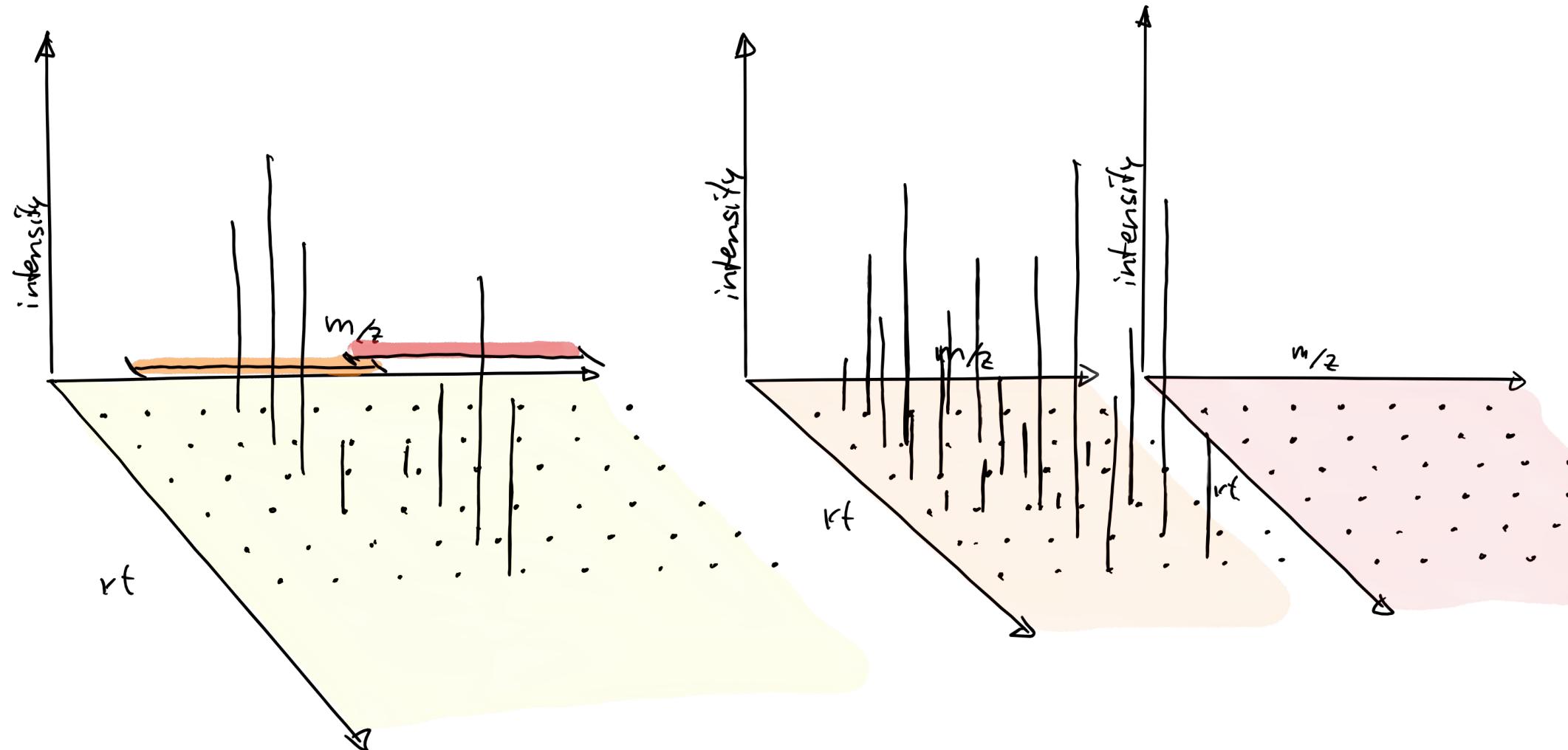
SWATH data



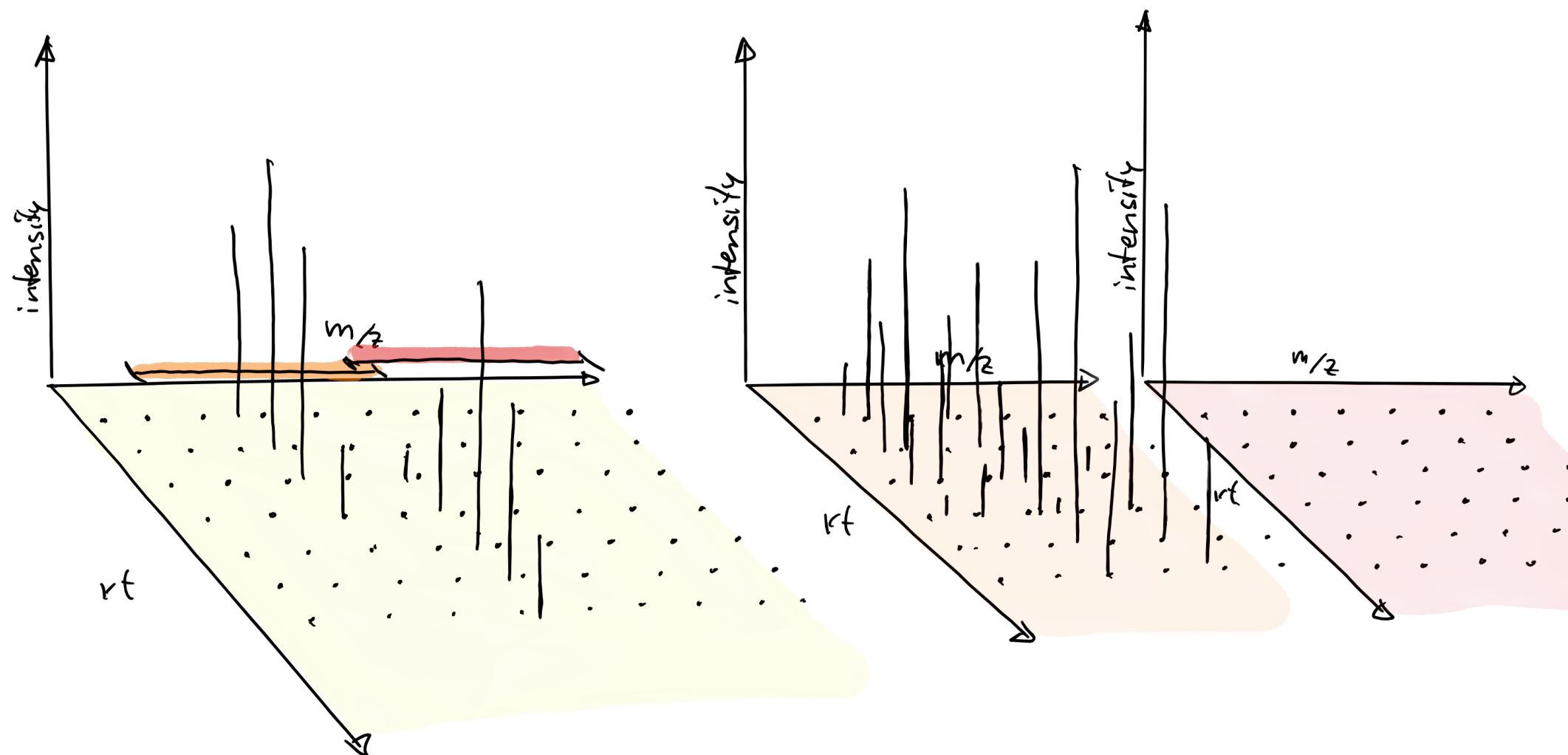
SWATH data



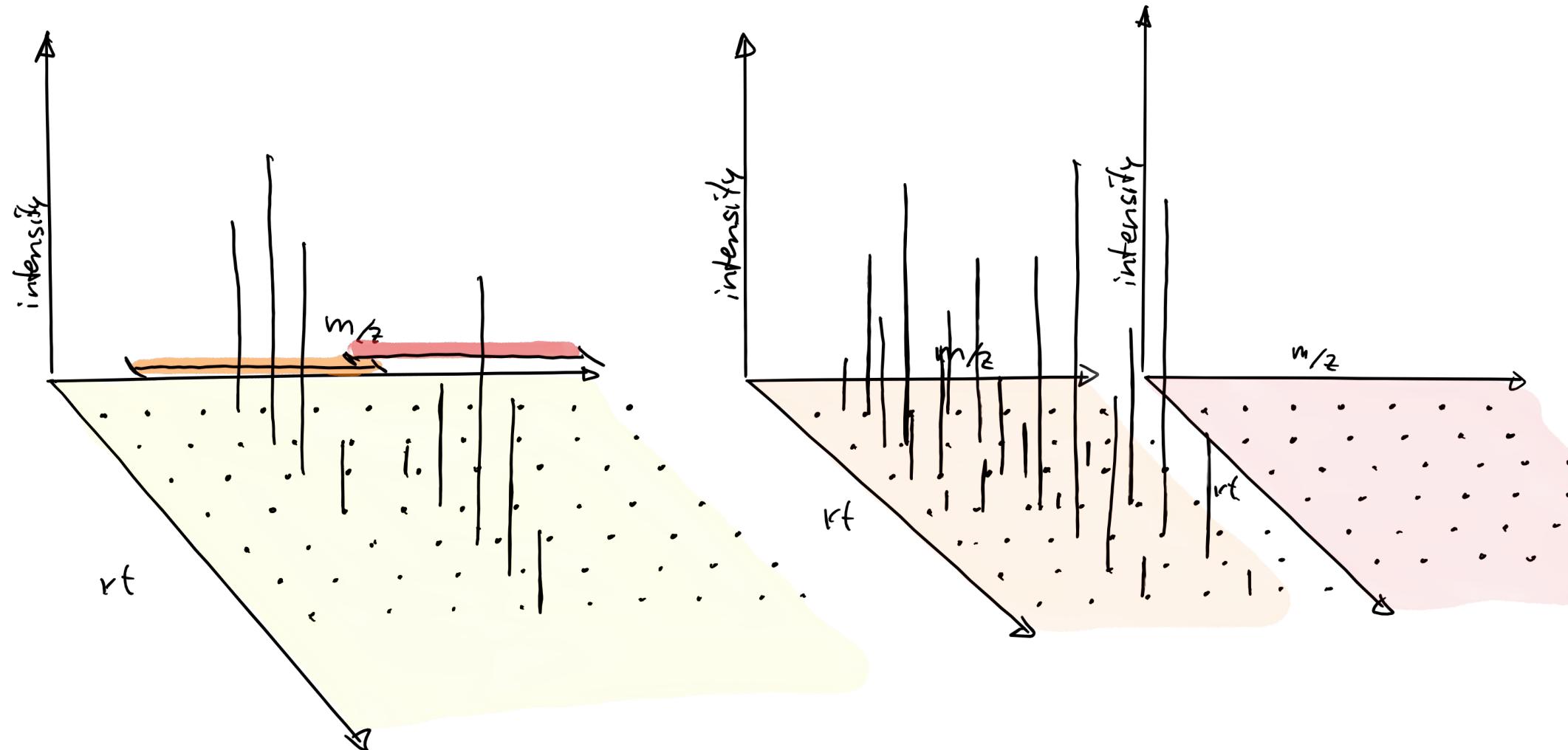
SWATH data



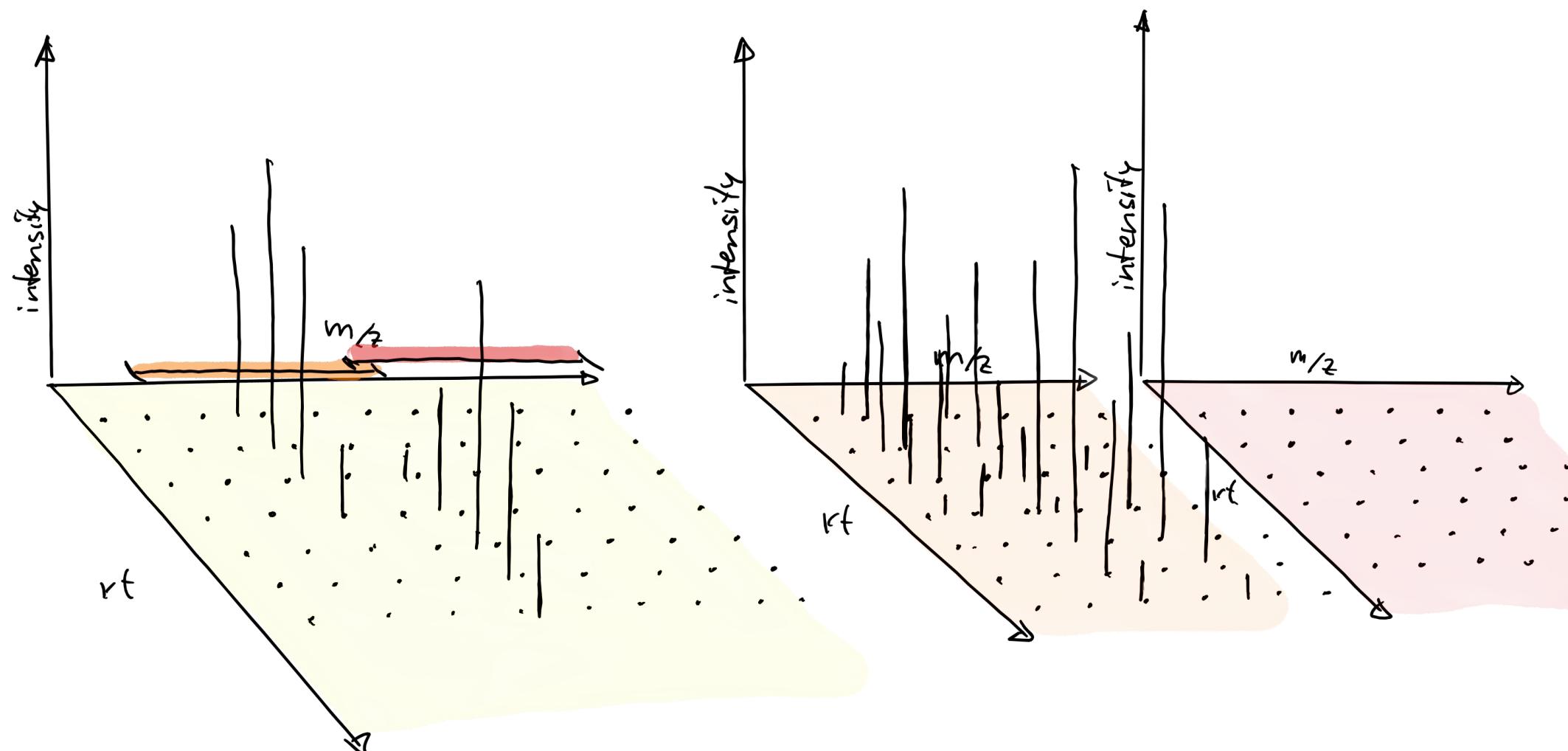
SWATH data



SWATH data



SWATH data

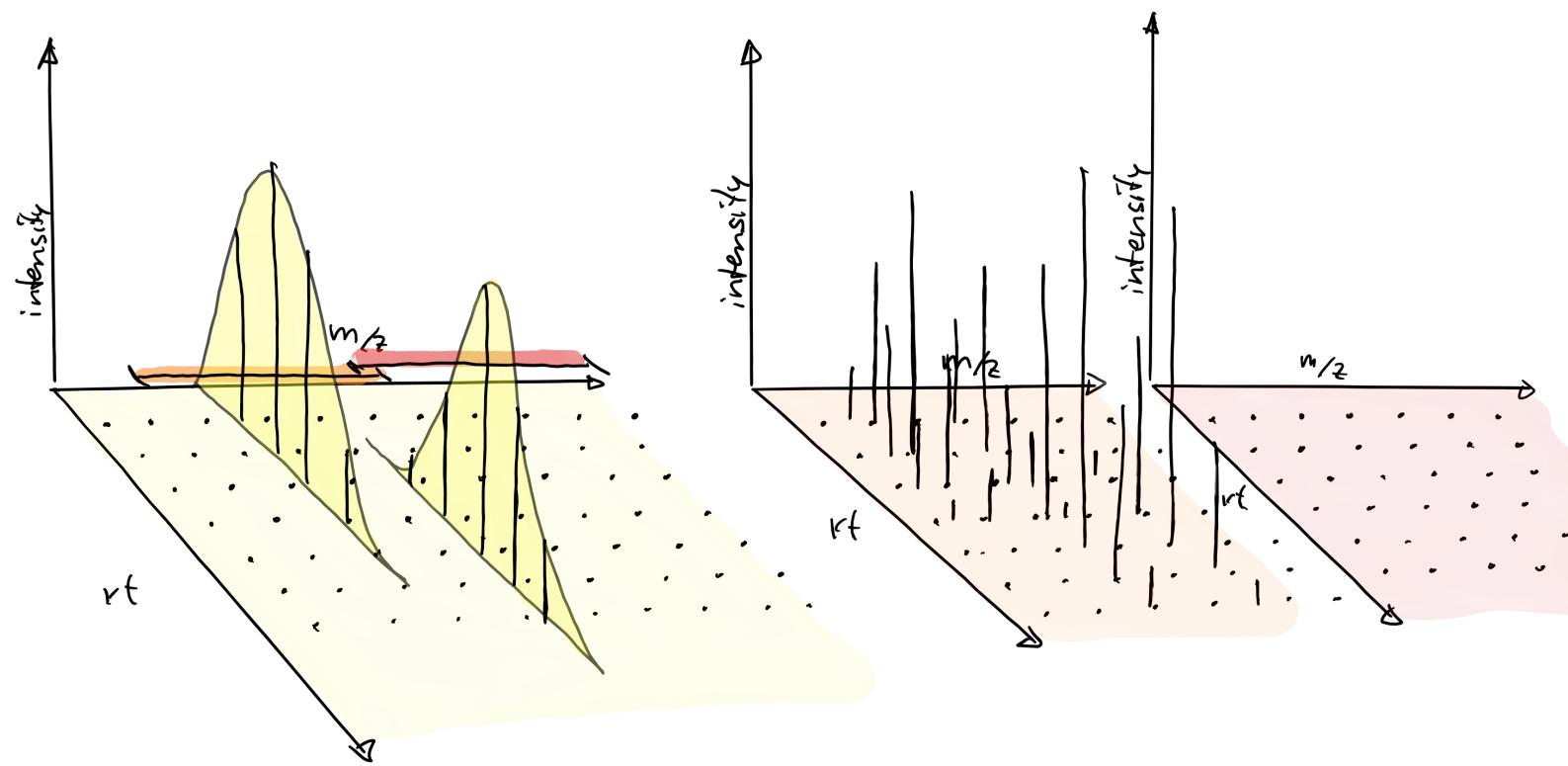


```
swath_data <- readMSData("PestMix1_SWATH.mzML", mode = "onDisk")
```

Analyzing SWATH data with **xcms**

- Chromatographic peak detection in MS1.

```
cwp <- CentWaveParam(snthresh = 5, noise = 100, ppm = 10,  
                      peakwidth = c(3, 30))  
swath_data <- findChromPeaks(swath_data, param = cwp)
```



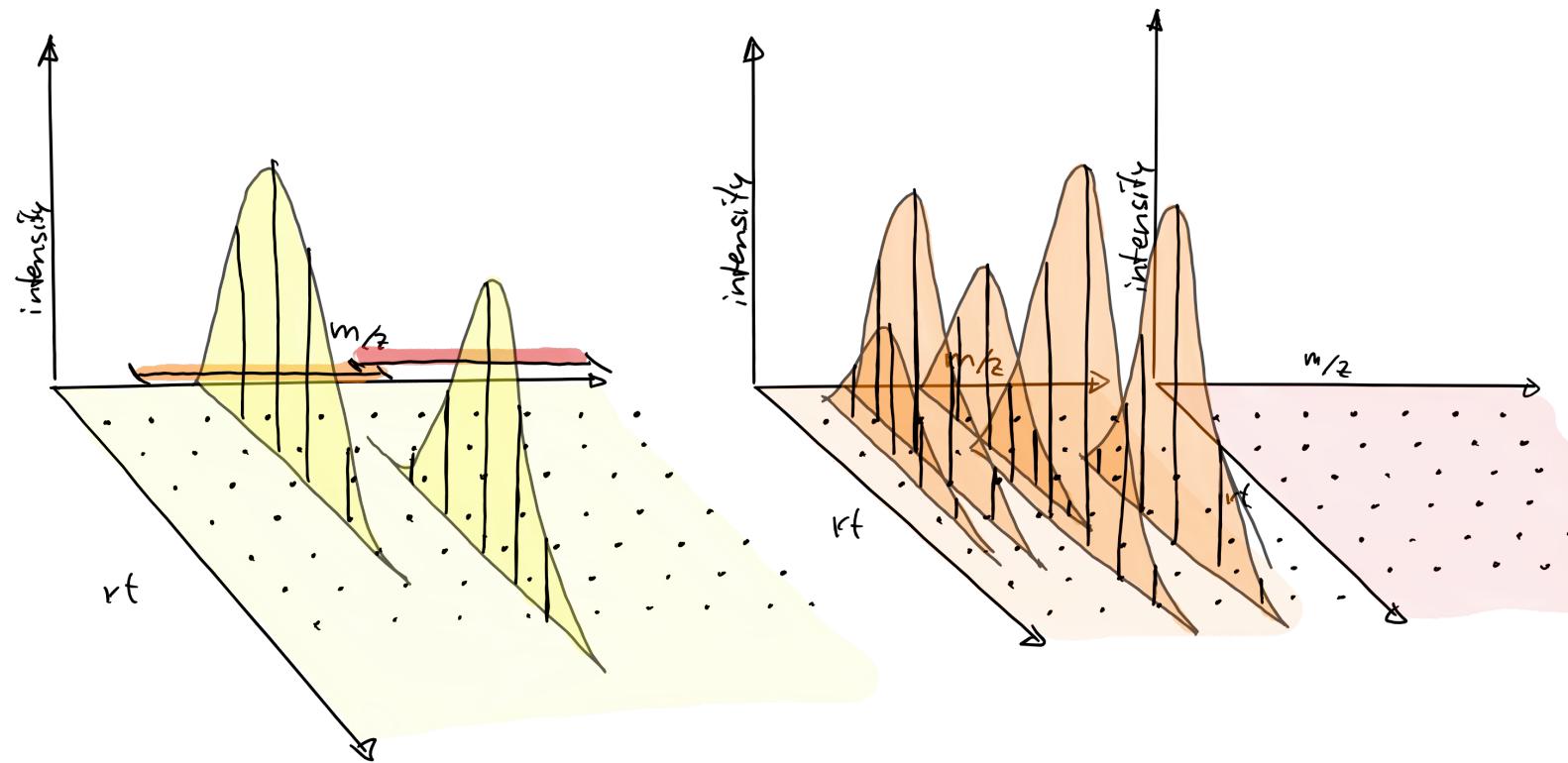
Analyzing SWATH data with **xcms**

- Chromatographic peak detection in MS1.

```
cwp <- CentWaveParam(snthresh = 5, noise = 100, ppm = 10,  
                      peakwidth = c(3, 30))  
swath_data <- findChromPeaks(swath_data, param = cwp)
```

- Chromatographic peak detection in MS2 (within each isolation window).

```
swath_data <- findChromPeaksIsolationWindow(swath_data, param = cwp)
```



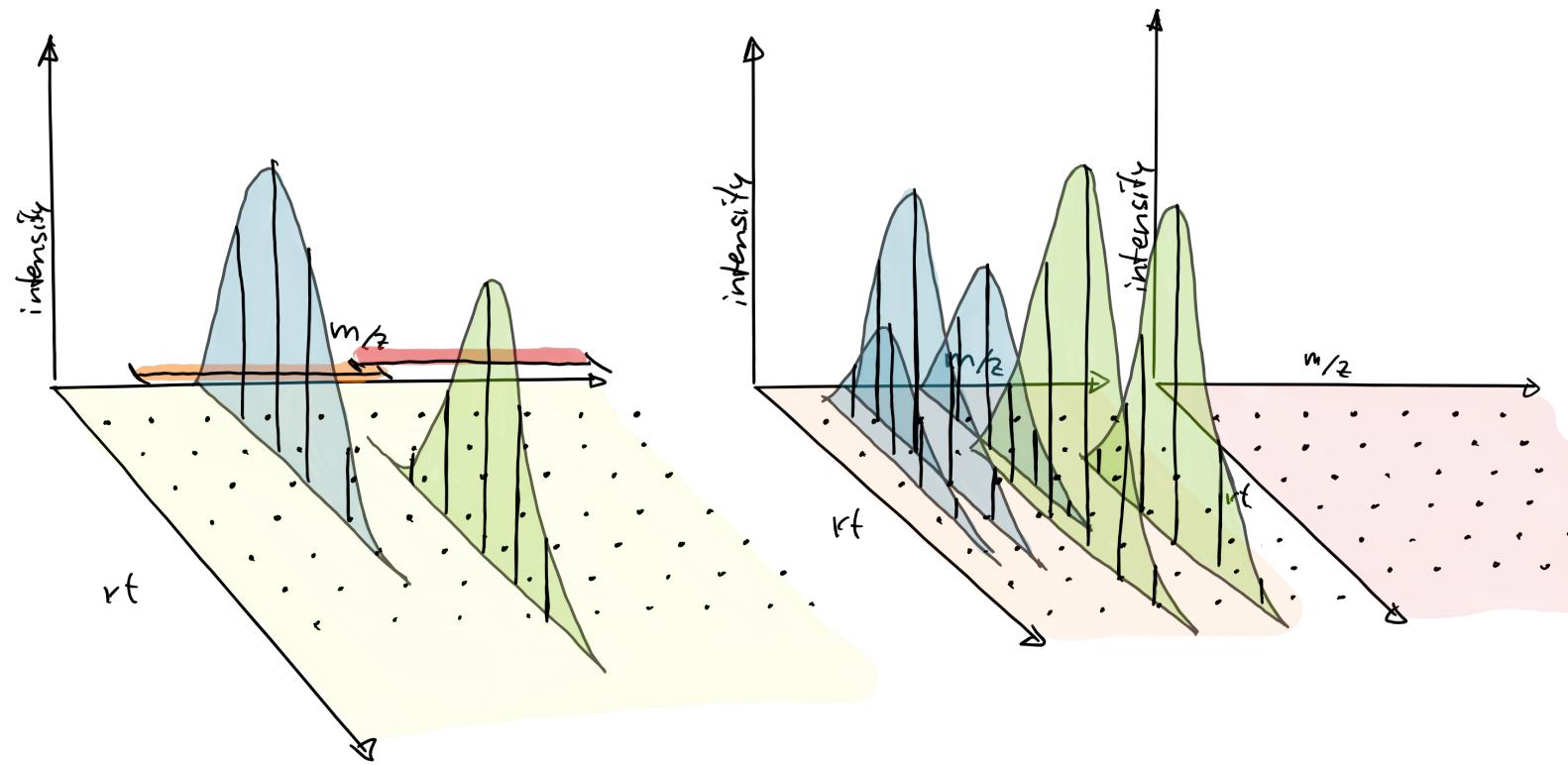
Analyzing SWATH data with **xcms**

- Chromatographic peak detection in MS1.

```
cwp <- CentWaveParam(snthresh = 5, noise = 100, ppm = 10,  
                      peakwidth = c(3, 30))  
swath_data <- findChromPeaks(swath_data, param = cwp)
```

- Chromatographic peak detection in MS2 (within each isolation window).

```
swath_data <- findChromPeaksIsolationWindow(swath_data, param = cwp)
```



Analyzing SWATH data with **xcms**

- Reconstructing MS2 spectrum from SWATH data:

```
swath_spectra <- reconstructChromPeakSpectra(swath_data, minCor = 0.9)
```

- For each MS1 chromatographic peak:
 - Find MS2 peaks (within the correct isolation window) with similar retention time.
 - Correlate peak shape of MS1 and candidate MS2 peaks.
 - Reconstruct the MS2 spectra based on matching MS2 peaks' m/z and intensity.

Analyzing SWATH data with **xcms**

- Example: reconstructed MS2 spectrum for Fenamiphos.

```
chromPeaks(swath_data, mz = 304.1131, ppm = 20, msLevel = 1L)
```

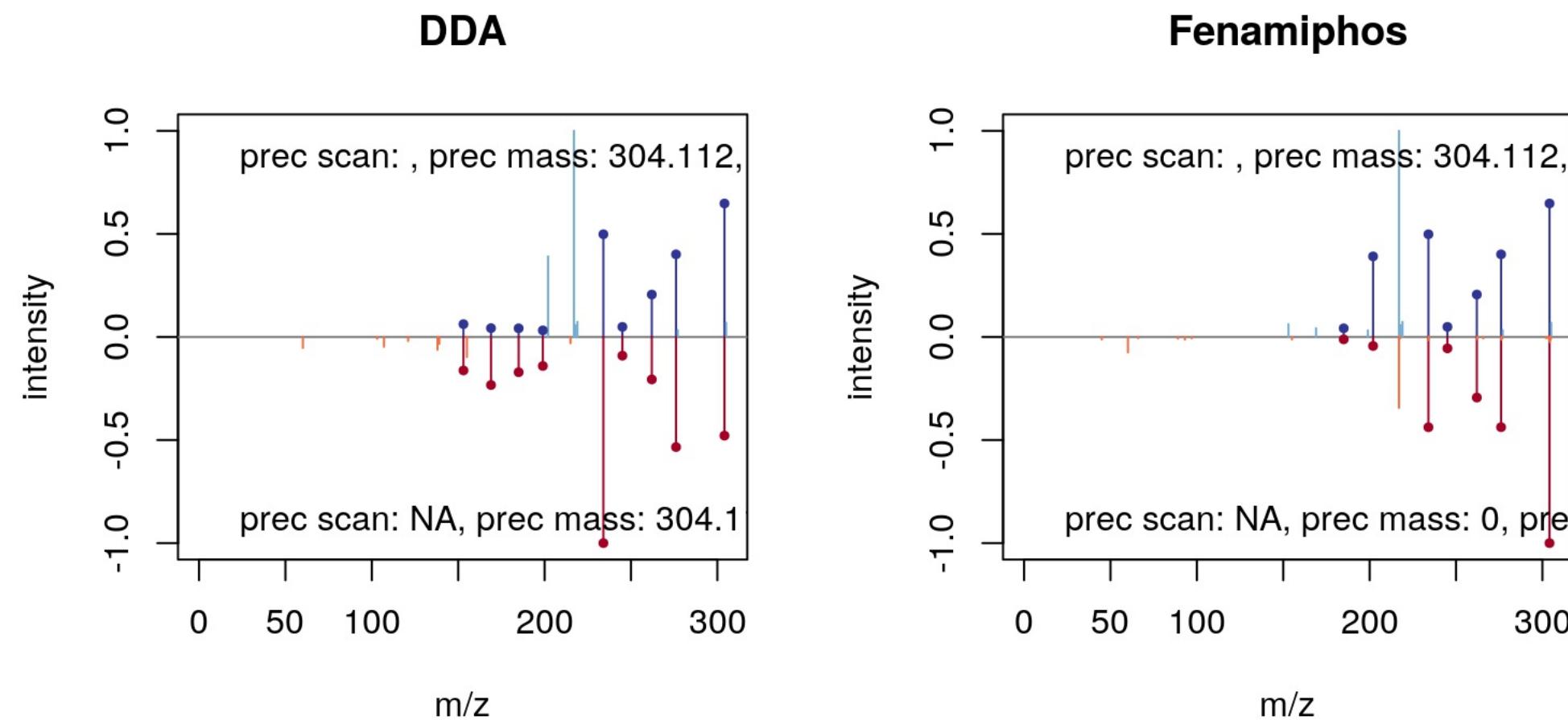
```
##          mz      mzmin      mzmax       rt      rtmin      rtmax      into      intb
## CP35 304.1124 304.1121 304.1126 423.945 419.445 428.444 10697.34 10688.34
##          maxo     sn sample
## CP35 2401.849 618       1
```

```
swath_sp <- swath_spectra[mcols(swath_spectra)$peak_id == "CP35"]
swath_sp
```

```
## Spectra with 1 spectra and 3 metadata column(s):
##      msLevel      rtime peaksCount |           ms2_peak_id
##      <integer> <numeric> <integer> |           <CharacterList>
##      1          2        NA         15 | CP205,CP207,CP217,....
##                                         ms2_peak_cor      peak_id
##                                         <NumericList> <character>
##      1 0.999787099100833,0.964862731008839,0.980288982893133,...      CP35
```

Analyzing SWATH data with **xcms**

```
par(mfrow = c(1, 2))
plot(swath_sp[[1]], ex_spectrum, main = "DDA", tolerance = 40e-6)
plot(swath_sp[[1]], fenamiphos, main = "Fenamiphos", tolerance = 40e-6)
```



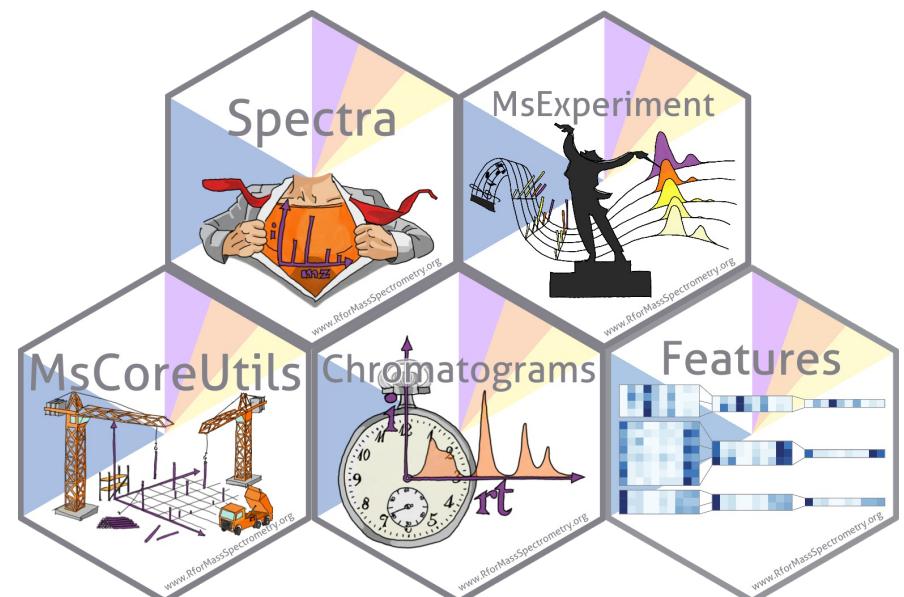
Annotating MS2 spectra

- Compare `Spectra` against reference `Spectra`:
 - import from mgf file(s).
 - ... (future developments...)
- Export spectra and peak/feature intensities for Feature-Based Molecular Networking

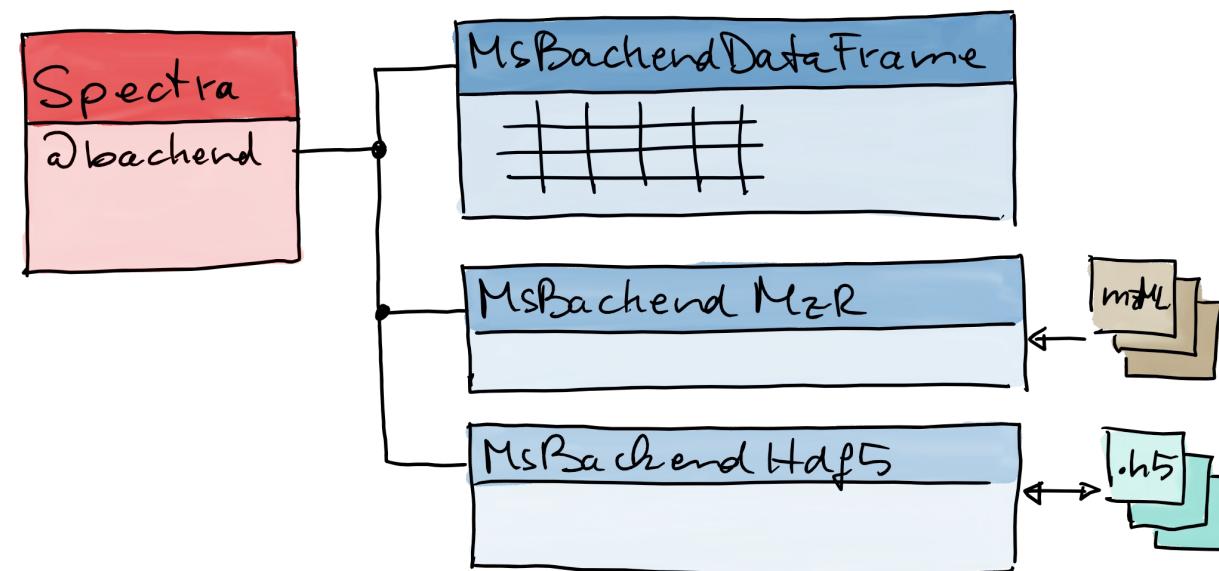


Future developments

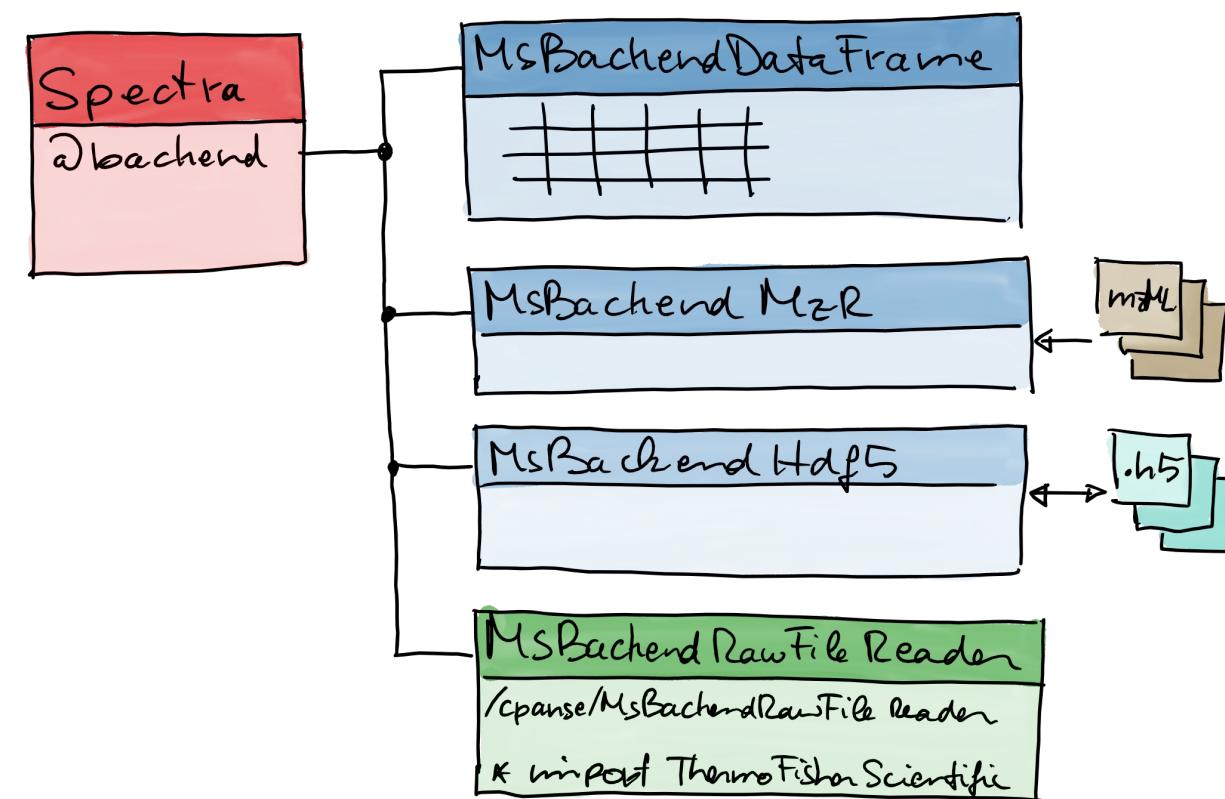
- <https://RforMassSpectrometry.org>
- Define an efficient, flexible and well documented infrastructure for Mass Spectrometry data in R.
- Provide core functionality.
- Provide core data representations.
- Reusable in other packages.



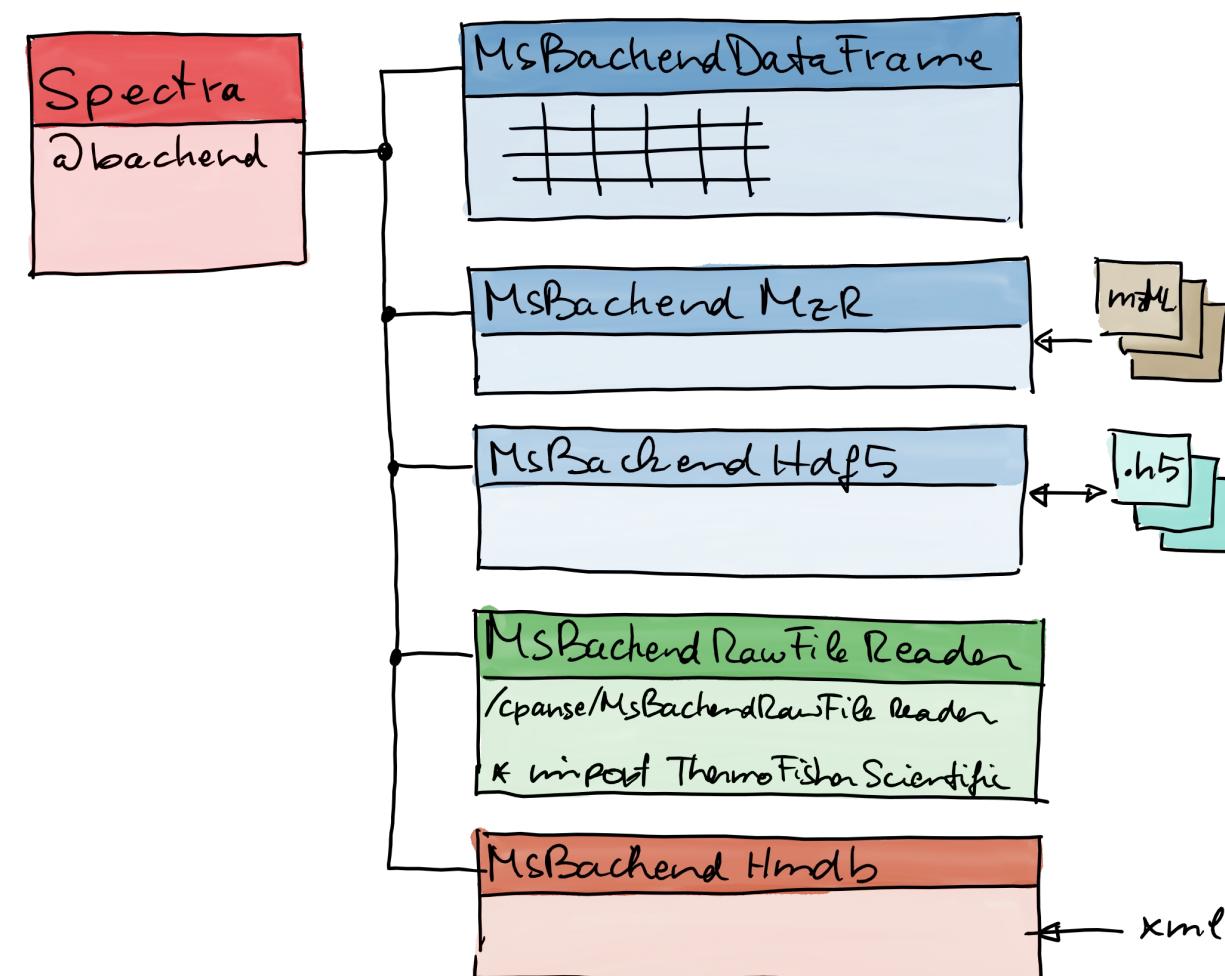
- Introduction of *backends*: independence between MS functionality and data origin/storage.



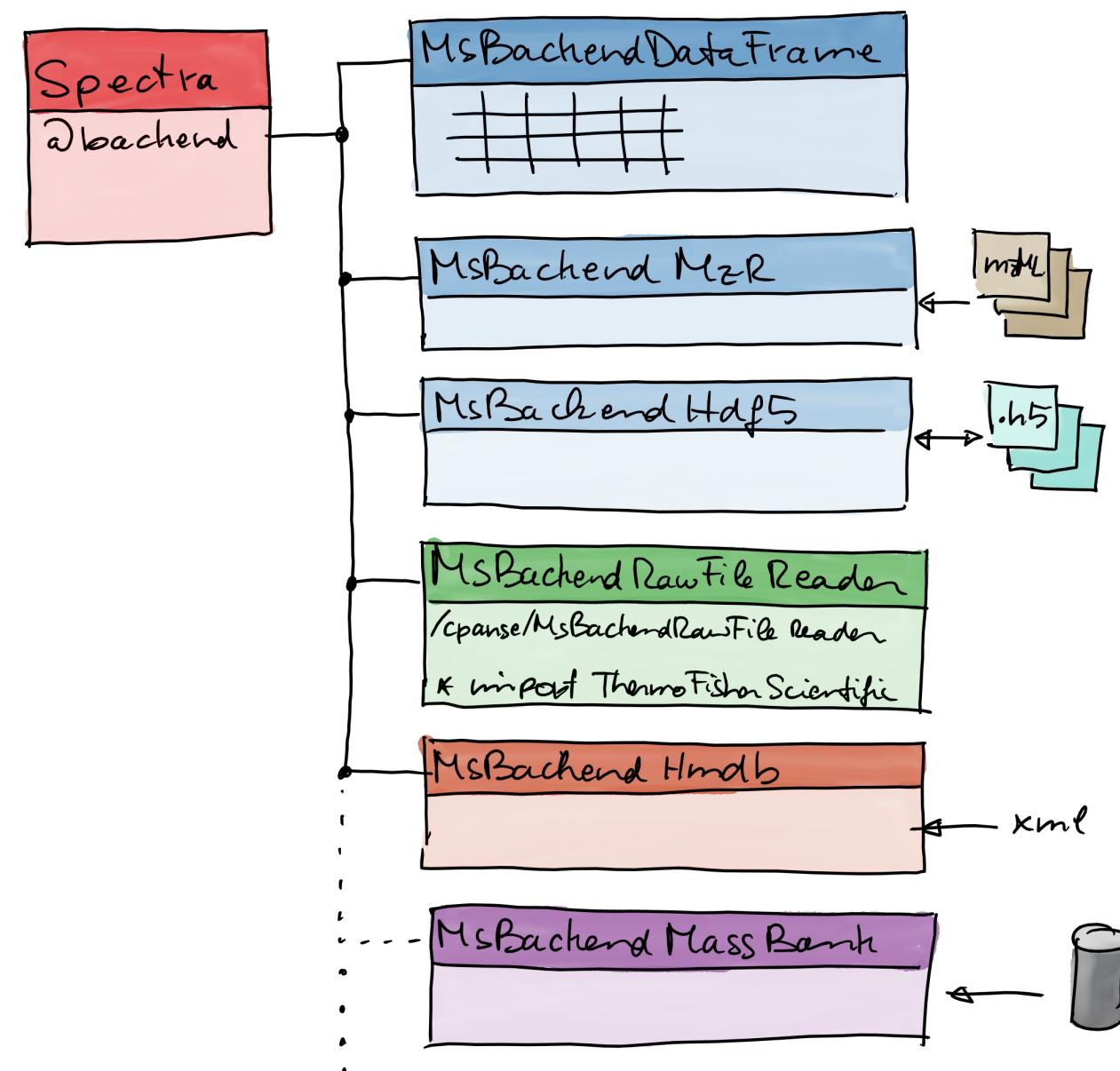
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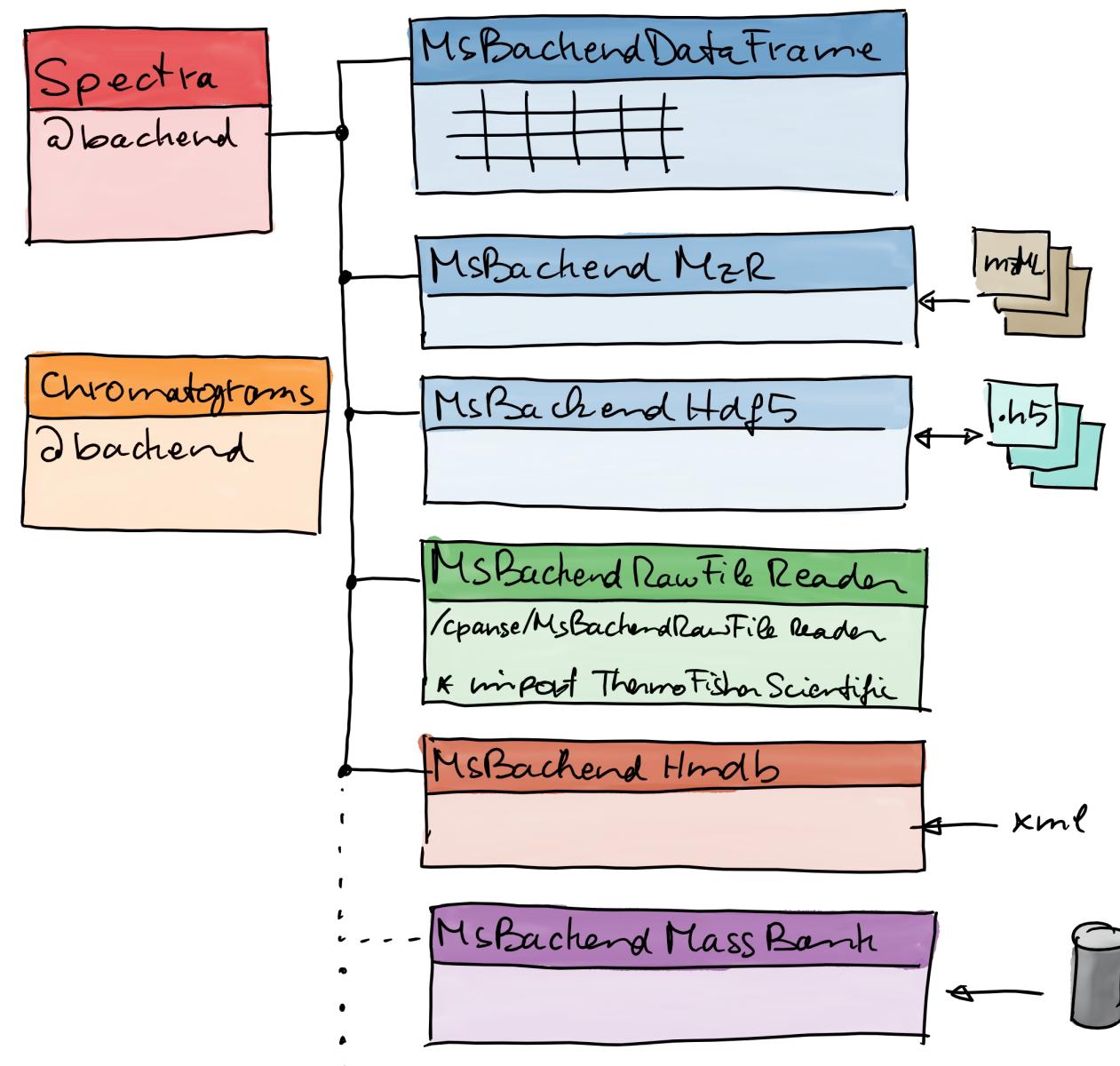
- Introduction of *backends*: independence between MS functionality and data origin/storage.



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Thank you for your attention

Also thanks to: Michael Witting, Jan Stanstrup, Steffen Neumann, Sebastian Gibb, Laurent Gatto

Ideas? Comments? <https://github.com/sneumann/xcms> or
<https://github.com/RforMassSpectrometry>