MSnbase: labelled and label-free MS2 data pre-processing, visualisation and quantification.

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This vignette describes the functionality implemented in the MSnbase package. MSnbase aims at (1) facilitating the import, processing, visualisation and quantification of mass spectrometry data into the R environment (R Development Core Team, 2011) by providing specific data classes and methods and (2) enabling the utilisation of throughput-high data analysis pipelines provided by the Bioconductor (Gentleman et al., 2004) project.

Keywords: Mass Spectrometry (MS), proteomics, infrastructure, quantitative.

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Contents

Foreword

MSnbase is under active developed; current functionality is evolving and new features will be added. This software is free and open-source software. If you use it, please support the project by citing it in publications:

> Laurent Gatto and Kathryn S. Lilley. *MSnbase - an R/Bioconductor package for isobaric tagged mass spectrometry data visualization, processing and quantitation.* Bioinformatics 28, 288-289 (2011).

Questions and bugs

You are welcome to contact me directly about MSnbase. For bugs, typos, suggestions or other questions, please file an issue in our tracking system[1] providing as much information as possible as well as the output of `sessionInfo()`.

If you wish to reach a broader audience for general questions about proteomics analysis using `R`, you may want to use the Bioconductor mailing list[2].

[1] https://github.com/lgatto/MSnbase/issues
[2] https://stat.ethz.ch/mailman/listinfo/bioconductor

1 Introduction

MSnbase (Gatto and Lilley, 2012) aims are providing a reproducible research framework to proteomics data analysis. It should allow researcher to easily mine mass spectrometry data, explore the data and its statistical properties and visually display these.

MSnbase also aims at being compatible with the infrastructure implemented in Bioconductor, in particular Biobase. As such, classes developed specifically for proteomics mass spectrometry data are based on the `eSet` and `Expression` classes. The main goal is to assure seamless compatibility with existing meta data structure, accessor methods and normalisation techniques.

This vignette illustrates MSnbase utility using a dummy data sets provided with the package without describing the underlying data structures. More details can be found in the package, classes, method and function documentations. A description of the classes is provided in the `MSnbase-development` vignette.

Speed and memory requirements Raw mass spectrometry file are generally several hundreds of MB large and most of this is used for binary raw spectrum data. As such, data containers can easily grow very large and thus require large amounts of RAM. This requirement is being tackled by avoiding to load the raw data into memory and using on-disk random access to the content of `mzXML`/`mzML` data files on demand. When focusing on reporter ion quantitation, a direct solution for this is to trim the spectra using the `trimMz` method to select the area of interest and thus substantially reduce the size of the `Spectrum` objects. This is illustrated in section 6.2 on page 21 of the `MSnbase-demo` vignette.

The independent handling of spectra is ideally suited for parallel processing. The `quantify` method now performs reporter peaks quantitation in parallel. More functions are being updated.

2 Data structure and content

2.1 Importing experiments

MSnbase is able to import raw MS data stored in one of the `XML`-based formats as well as peak lists in the `mfg` format[3]

[3]Mascot Generic Format – http://www.matrixscience.com/help/data_file_help.html#GEN

Raw data The XML-based formats, mzXML (Pedrioli et al., 2004), mzData (Orchard et al., 2007) and mzML (Martens et al., 2010) can be imported with the readMSData function, as illstrated below (see ?readMSData for more details).

```
> file <- dir(system.file(package = "MSnbase", dir = "extdata"),
+             full.names = TRUE, pattern = "mzXML$")
> rawdata <- readMSData(file, msLevel = 2, verbose = FALSE)
```

Either MS1 or MS2 spectra can be loaded at a time by setting the msLevel parameter accordingly. In this document, we will use the itraqdata data set, provided with MSnbase. It includes feature metadata, accessible with the fData accessor. The metadata includes identification data for the 55 MS2 spectra.

Peak lists Peak lists can often be exported after spectrum processing from vendor-specific software and are also used as input to search engines. Peak lists in mgf format can be imported with the function readMgfData (see ?readMgfData for details) to create experiment objects. Experiments or individual spectra can be exported to an mgf file with the writeMgfData methods (see ?writeMgfData for details and examples).

Experiments with multiple runs Although it is possible to load and process multiple files serially and later merge the resulting quantitation data as show in section 12 (page 41), it is also feasible to load several raw data files at once. Here, we report the analysis of an LC-MSMS experiment were 14 liquid chromatography (LC) fractions were loaded using readMSData on a 32-cores servers with 128 Gb of RAM. It took about 90 minutes to read the 14 uncentroided mzXML raw files (4.9 Gb on disk in total) and create a 3.3 Gb raw data object (an MSnExp instance, see next section). Quantitation of 9 reporter ions (iTRAQ9 object, see 2.4) for 88690 features was performed in parallel on 16 processors and took 76 minutes. The resulting quantitation data was only 22.1 Mb and could easily be further processed and analysed on a standard laptop computer.

Since verions 1.13.5, parallel support is provided by the BiocParallel and various backends including multicore (forking), simple networf network of workstations (SNOW) using sockets, forking or MPI among others.

See also section 7.2 to import quantitative data stored in spreadsheets into R for further processing using MSnbase. The MSnbase-io vignette gives a general overview of MSnbase's input/ouput capabilites.

2.2 MS experiments

Raw data is contained in `MSnExp` objects, that stores all the spectra of an experiment, as defined by one or multiple raw data files.

```
> library("MSnbase")
> itraqdata

Object of class "MSnExp"
 Object size in memory: 1.87 Mb
- - - Spectra data - - -
 MS level(s): 2
 Number of MS1 acquisitions: 1
 Number of MSn scans: 55
 Number of precursor ions: 55
 55 unique MZs
 Precursor MZ's: 401.74 - 1236.1
 MSn M/Z range: 100 2069.27
 MSn retention times: 19:9 - 50:18 minutes
- - - Processing information - - -
Data loaded: Wed May 11 18:54:39 2011
 MSnbase version: 1.1.22
- - - Meta data  - - -
phenoData
  rowNames: 1
  varLabels: sampleNames sampleNumbers
  varMetadata: labelDescription
Loaded from:
  dummyiTRAQ.mzXML
protocolData: none
featureData
  featureNames: X1 X10 ... X9 (55 total)
  fvarLabels: spectrum ProteinAccession ProteinDescription
    PeptideSequence
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'

> head(fData(itraqdata))

    spectrum ProteinAccession
X1         1              BSA
```

```
X10       10          ECA1422
X11       11          ECA4030
X12       12          ECA3882
X13       13          ECA1364
X14       14          ECA0871
                          ProteinDescription PeptideSequence
X1                      bovine serum albumin          NYQEAK
X10 glucose-1-phosphate cytidylyltransferase VTLVDTGEHSMTGGR
X11         50S ribosomal subunit protein L4           SPIWR
X12                   chaperone protein DnaK        TAIDDALK
X13      succinyl-CoA synthetase alpha chain          SILINK
X14              NADP-dependent malic enzyme    DFEVVNNESDPR
```

As illustrated above, showing the experiment textually displays it's content:

- Information about the raw data, i.e. the spectra.
- Specific information about the experiment processing[4] and package version. This slot can be accessed with the `processingData` method.
- Other meta data, including experimental phenotype, file name(s) used to import the data, protocol data, information about features (individual spectra here) and experiment data. Most of these are implemented as in the `eSet` class and are described in more details in their respective manual pages. See `?MSnExp` and references therein for additional background information.

  The experiment meta data associated with an `MSnExp` experiment is of class `MIAPE`. It stores general information about the experiment as well as MIAPE (Minimum Information About a Proteomics Experiment) information (Taylor et al., 2007, 2008). This meta-data can be accessed with the `experimentData` method. When available, a summary of MIAPE-MS data can be printed with the `msInfo` method. See `?MIAPE` for more details.

2.3 Spectra objects

The raw data is composed of the 55 MS spectra. The spectra are named individually (X1, X10, X11, X12, X13, X14, ...) and stored in a `environment`. They can be accessed individually with `itraqdata[["X1"]]` or `itraqdata[[1]]`,

[4] this part will be automatically updated when the object is modified with it's *ad hoc* methods, as illustrated later

or as a list with `spectra(itraqdata)`. As we have loaded our experiment specifying `msLevel=2`, the spectra will all be of level 2 (or higher, if available).

```
> sp <- itraqdata[["X1"]]
> sp

Object of class "Spectrum2"
 Precursor: 520.7833
 Retention time: 19:9
 Charge: 2
 MSn level: 2
 Peaks count: 1922
 Total ion count: 26413754
```

Attributes of individual spectra or of all spectra of an experiment can be accessed with their respective methods: `precursorCharge` for the precursor charge, `rtime` for the retention time, `mz` for the MZ values, `intensity` for the intensities, ... see the `Spectrum`, `Spectrum1` and `Spectrum2` manuals for more details.

```
> peaksCount(sp)

[1] 1922

> head(peaksCount(itraqdata))

  X1  X10  X11  X12  X13  X14
1922 1376 1571 2397 2574 1829

> rtime(sp)

[1] 1149.31

> head(rtime(itraqdata))

     X1      X10      X11      X12      X13      X14
1149.31 1503.03 1663.61 1663.86 1664.08 1664.32
```

2.4 Reporter ions

Reporter ions are defined with the `ReporterIons` class. Specific peaks of interest are defined by a MZ value, a with around the expected MZ and a name

(and optionally a colour for plotting, see section 3). `ReporterIons` instances are required to quantify reporter peaks in `MSnExp` experiments. Instances for the most commonly used isobaric tags like iTRAQ 4-plex and 8-plex and TMT tags are already defined in MSnbase. See `?ReporterIons` for details about how to generate new `ReporterIons` objects.

```
> iTRAQ4

Object of class "ReporterIons"
iTRAQ4: '4-plex iTRAQ' with 4 reporter ions
 - 114.1 +/- 0.05 (red)
 - 115.1 +/- 0.05 (green)
 - 116.1 +/- 0.05 (blue)
 - 117.1 +/- 0.05 (yellow)
```

3 Plotting raw data

3.1 Default plots

Spectra can be plotted individually or as part of (subset) experiments with the `plot` method. Full spectra can be plotted (using `full=TRUE`), specific reporter ions of interest (by specifying with reporters with `reporters=iTRAQ4` for instance) or both (see figure 1).

It is also possible to plot all spectra of an experiment (figure 2). Lets start by subsetting the `itraqdata` experiment using the protein accession numbers included in the feature metadata, and keep the 6 from the *BSA* protein.

```
> sel <- fData(itraqdata)$ProteinAccession == "BSA"
> bsa <- itraqdata[sel]
> bsa

Object of class "MSnExp"
 Object size in memory: 0.1 Mb
- - - Spectra data - - -
 MS level(s): 2
 Number of MS1 acquisitions: 1
 Number of MSn scans: 3
 Number of precursor ions: 3
 3 unique MZs
```

```
> plot(sp, reporters = iTRAQ4, full = TRUE)
```

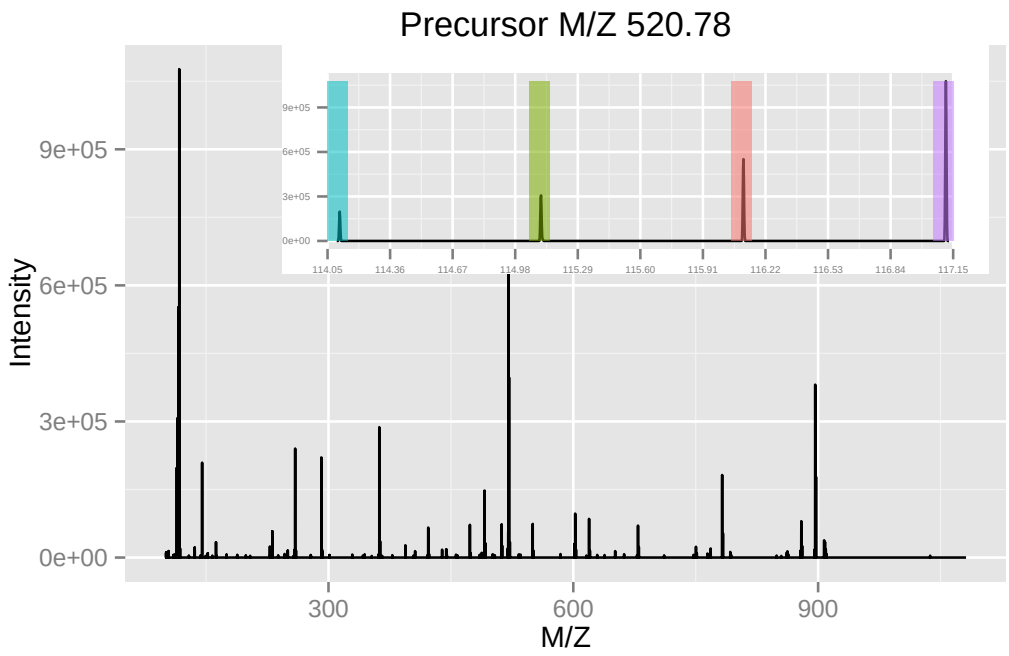


Figure 1: Raw MS2 spectrum with details about reporter ions.

```
 Precursor MZ's: 434.95 - 651.92
 MSn M/Z range: 100 1351.77
 MSn retention times: 19:9 - 36:17 minutes
- - - Processing information - - -
Data loaded: Wed May 11 18:54:39 2011
Data [logically] subsetted 3 spectra: Sat Mar 21 19:00:59 2015
 MSnbase version: 1.1.22
- - - Meta data  - - -
phenoData
  rowNames: 1
  varLabels: sampleNames sampleNumbers
  varMetadata: labelDescription
Loaded from:
  dummyiTRAQ.mzXML
protocolData: none
featureData
  featureNames: X1 X52 X53
  fvarLabels: spectrum ProteinAccession ProteinDescription
    PeptideSequence
```

```
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'

> as.character(fData(bsa)$ProteinAccession)

[1] "BSA" "BSA" "BSA"
```

These can then be visualised together by plotting the `MSnExp` object, as illustrated on figure 2.

3.2 Customising your plots

The MSnbase `plot` methods have a logical `plot` parameter (default is `TRUE`), that specifies if the plot should be printed to the current device. A plot object is also (invisibly) returned, so that it can be saved as a variable for later use or for customisation.

MSnbase uses the ggplot2 package to generate plots, which can subsequently easily be customised. More details about ggplot2 can be found in Wickham (2009) (especially chapter 8) and on http://had.co.nz/ggplot2/. Finally, if a plot object has been saved in a variable `p`, it is possible to obtain a summary of the object with `summary(p)`. To view the data frame used to generate the plot, use `p@data`.

```
> plot(bsa, reporters = iTRAQ4, full = FALSE) + theme_gray(8)
```

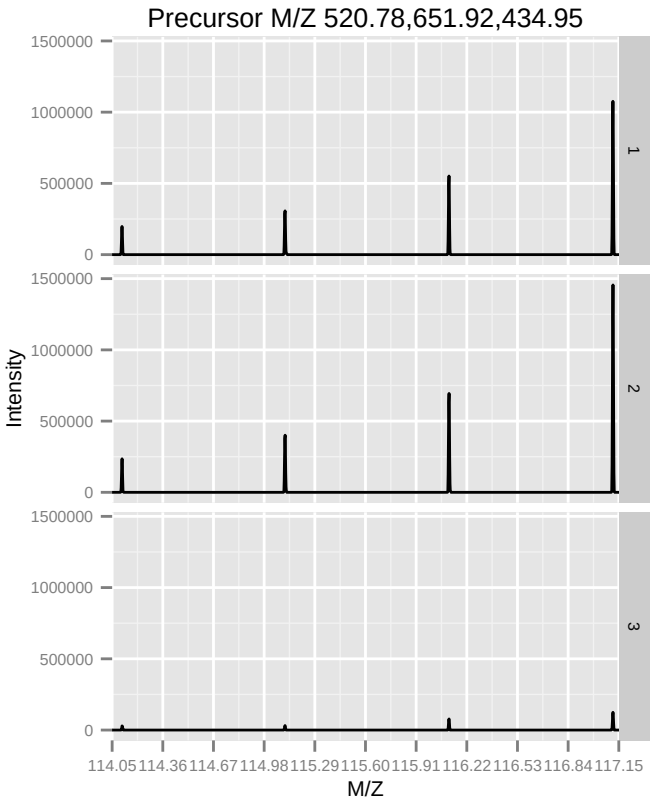


Figure 2: Experiment-wide raw MS2 spectra. The y-axes of the individual spectra are automatically rescaled to the same range. See section 7.4 to rescale peaks identically.

4 Tandem MS identification data

4.1 Adding identification data

MSnbase is able to integrate identification data from `mzIdentML` (Jones et al., 2012) files.

We first load two example files shipped with the MSnbase containing raw data (as above) and the corresponding identification results respectively. The raw data is read with the `readMSData`, as demonstrated above. As can be seen, the default feature data only contain spectra numbers[5].

```
> ## find path to a mzXML file
> quantFile <- dir(system.file(package = "MSnbase", dir = "extdata"),
+                  full.name = TRUE, pattern = "mzXML$")
> ## find path to a mzIdentML file
> identFile <- dir(system.file(package = "MSnbase", dir = "extdata"),
+                  full.name = TRUE, pattern = "mzid$")
> ## create basic MSnExp
> msexp <- readMSData(quantFile, verbose = FALSE)
> head(fData(msexp), n = 2)

     spectrum
X1.1        1
X2.1        2
```

The `addIdentificationData` method takes an `MSnExp` instance (or an `MSnSet` instance storing quantitation data, see section 7.1) as first argument and one or multiple `mzIdentML` file names (as a character vector) as second one and updates the `MSnExp` feature data using the identification data read from the `mzIdentML` file(s).

```
> ## add identification information
> msexp <- addIdentificationData(msexp, filenames = identFile,
+                                verbose = FALSE)
> head(fData(msexp), n = 2)

     spectrum scan number(s) passthreshold rank
X1.1        1             1          TRUE    1
X2.1        2             2          TRUE    1
```

[5] More data about the spectra is of course available in an `MSnExp` object, as illustrated in the previous sections. See also `?pSet` and `?MSnExp` for more details.

```
     calculatedmasstocharge experimentalmasstocharge chargestate
X1.1               645.0375                  645.3741           3
X2.1               546.9633                  546.9586           3
     ms-gf:denovoscore ms-gf:evalue ms-gf:rawscore ms-gf:specevalue
X1.1                77     79.36958            -39     5.527468e-05
X2.1                39     13.46615            -30     9.399048e-06
     assumeddissociationmethod isotopeerror isdecoy post pre end
X1.1                       CID            1   FALSE    A   R 186
X2.1                       CID            0   FALSE    A   K  62
     start       accession length
X1.1   170 ECA0984;ECA3829    231
X2.1    50         ECA1028    275
                                                                  description
X1.1 DNA mismatch repair protein;acetolactate synthase isozyme III large subunit
X2.1          2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
               pepseq modified modification
X1.1 VESITARHGEVLQLRPK    FALSE           NA
X2.1     IDGQWVTHQWLKK    FALSE           NA
                databaseFile identFile nprot npep.prot npsm.prot
X1.1 erwinia_carotovora.fasta         2     2         1         1
X2.1 erwinia_carotovora.fasta         2     1         1         1
     npsm.pep
X1.1        1
X2.1        1
```

Finally we can use `idSummary` to summarise the percentage of identified features per quantitation/identification pairs.

```
> idSummary(msexp)

  quantFile identFile coverage
1         1         2      0.6
```

4.2 Filtering identification data

One can remove the features that have not been identified using `removeNoId`. This function uses by default the `pepseq` feature variable to search the presence of missing data (`NA` values) and then filter these non-identified spectra.

```
> fData(msexp)$pepseq

[1] "VESITARHGEVLQLRPK" "IDGQWVTHQWLKK"     NA
[4] NA                  "LVILLFR"

> msexp <- removeNoId(msexp)
> fData(msexp)$pepseq

[1] "VESITARHGEVLQLRPK" "IDGQWVTHQWLKK"     "LVILLFR"

> idSummary(msexp)

  quantFile identFile coverage
1         1         2        1
```

Similarly, the `removeMultipleAssignment` method can be used to filter out non-unique features, i.e. that have been assigned to protein groups with more than one member. This function uses by default the `nprot` feature variable.

Note that `removeNoId` and `removeMultipleAssignment` methods can also be called on `MSnExp` instances.

4.3 Calculate Fragments

MSnbase is able to calculate theoretical peptide fragments via `calculateFragments`.

```
> calculateFragments("ACEK",
+                    type=c("a", "b", "c", "x", "y", "z"))

Modifications used:  C=160.030649

          mz ion type pos z  seq
1   44.04947  a1    a   1 1    A
2  204.08012  a2    a   2 1   AC
3  333.12271  a3    a   3 1  ACE
4  461.21767  a4    a   4 1 ACEK
5   72.04439  b1    b   1 1    A
6  232.07504  b2    b   2 1   AC
7  361.11762  b3    b   3 1  ACE
8  489.21258  b4    b   4 1 ACEK
9   89.07094  c1    c   1 1    A
10 249.10158  c2    c   2 1   AC
```

```
11 378.14417  c3    c   3 1  ACE
12 506.23913  c4    c   4 1 ACEK
13 173.09207  x1    x   1 1    K
14 302.13466  x2    x   2 1   EK
15 462.16530  x3    x   3 1  CEK
16 533.20242  x4    x   4 1 ACEK
17 147.11280  y1    y   1 1    K
18 276.15539  y2    y   2 1   EK
19 436.18604  y3    y   3 1  CEK
20 507.22315  y4    y   4 1 ACEK
21 130.08625  z1    z   1 1    K
22 259.12884  z2    z   2 1   EK
23 419.15949  z3    z   3 1  CEK
24 490.19660  z4    z   4 1 ACEK
```

It is also possible to match these fragments against an `Spectrum2` object.

```
> pepseq <- fData(msexp)$pepseq[1]
> calculateFragments(pepseq, msexp[[1]], type=c("b", "y"))

Modifications used:  C=160.030649

          mz ion type pos z        seq       error
1   100.0005  b1    b   1 1          V  0.07522824
4   429.2563  b4    b   4 1       VESI -0.02189010
21  513.3047  y4    y   4 1       LRPK  0.04598246
23  754.4504  y6    y   6 1     LQLRPK  0.04293155
25  982.5354  y8    y   8 1   EVLQLRPK  0.06897061
10 1080.5867 b10    b  10 1 VESITARHGE -0.04344392
```

5 Quality control

The current section is not executed dynamically for package size and processing time constrains. The figures and tables have been generated with the respective methods and included statically in the vignette for illustration purposes.

MSnbase allows easy and flexible access to the data, which allows to visualise data features to assess it's quality. Some methods are readily available, although many QC approaches will be experiment specific and users are encourage to explore their data.

The `plot2d` method takes one `MSnExp` instance as first argument to produce retention time *vs.* precursor MZ scatter plots. Points represent individual MS2 spectra and can be coloured based on precursor charge (with second argument `z="charge"`), total ion count (`z="ionCount"`), number of peaks in the MS2 spectra `z="peaks.count"`) or, when multiple data files were loaded, file `z="file"`), as illustrated on figure 3. The lower right panel is produced for only a subset of proteins. See the method documentation for more details.

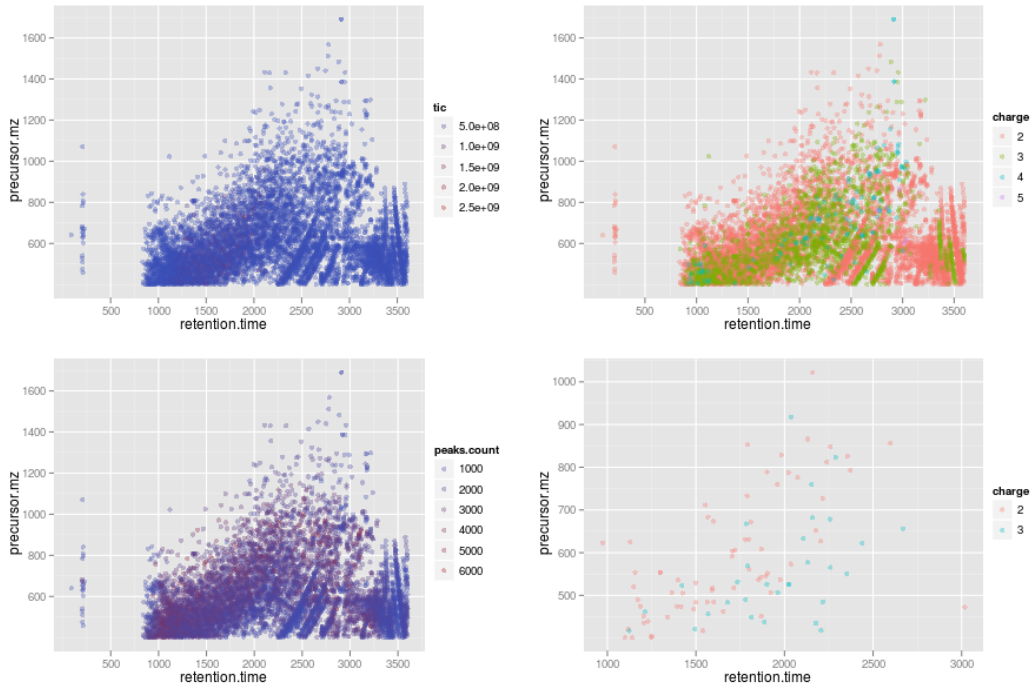


Figure 3: Illustration of the `plot2d` output.

The `plotDensity` method illustrates the distribution of several parameters of interest (see figure 4). Similarly to `plot2d`, the first argument is an `MSnExp` instance. The second is one of `precursor.mz`, `peaks.count` or `ionCount`, whose density will be plotted. An optional third argument specifies whether the x axes should be logged.

The `plotMzDelta` method[6] implements the M/Z delta plot from Foster et al. (2011) The M/Z delta plot illustrates the suitability of MS2 spectra for identification by plotting the M/Z differences of the most intense peaks. The resulting histogram should optimally shown outstanding bars at amino acid residu masses. More details and parameters are described in the method documentation (`?plotMzDelta`). Figure 5 has been generated using the PRIDE experiment 12011, as in Foster et al. (2011).

[6]The code to generate the histograms has been contributed by Guangchuang Yu from Jinan University, China.

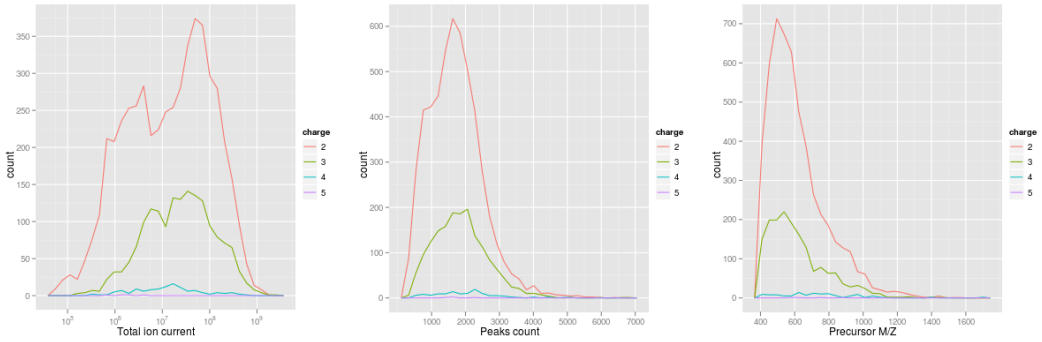


Figure 4: Illustration of the `plotDensity` output.

In section 11 on page 39, we illustrate how to assess incomplete reporter ion dissociation.

6 Data processing

6.1 Cleaning spectra

There are several methods implemented to perform basic data manipulation. Low intensity peaks can be set to 0 with the `removePeaks` method from spectra or whole experiments. The intensity threshold below which peaks are removed is defined by the `t` parameter. `t` can be specified directly as a numeric. The default value is the character `"min"`, that will remove all peaks equal to the lowest non null intensity in any spectrum. We observe the effect of the `removePeaks` method by comparing total ion count (i.e. the total intensity in a spectrum) with the `ionCount` method before (object `itraqdata`) and after (object `experiment`) for spectrum `X55`. The respective spectra are shown on figure 6 (page 20).

```
> experiment <- removePeaks(itraqdata, t = 400, verbose = FALSE)
> ## total ion current
> ionCount(itraqdata[["X55"]])

[1] 555408.8

> ionCount(experiment[["X55"]])

[1] 499769.6
```

Unlike the name might suggest, the `removePeaks` method does not actually remove peaks from the spectrum; they are set to 0. This can be checked using the `peaksCount` method, that returns the number of peaks (including 0

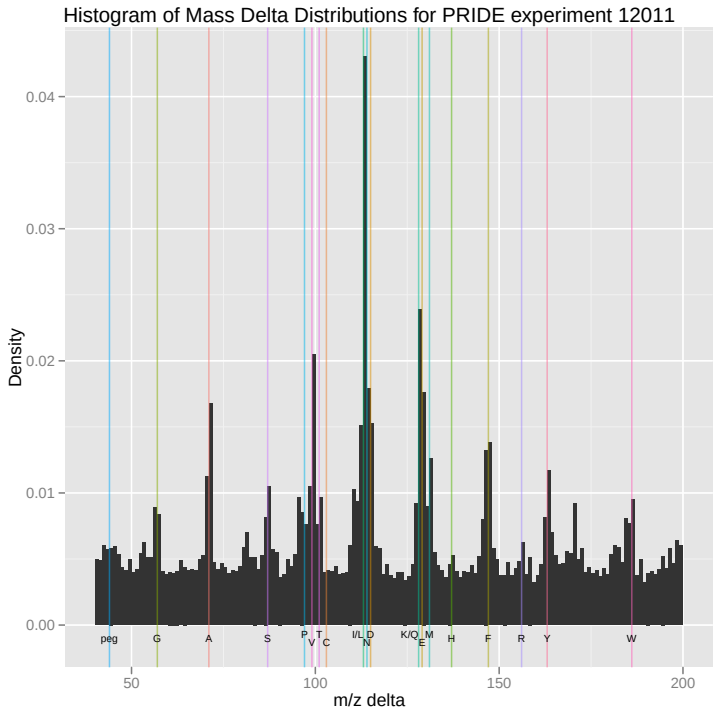


Figure 5: Illustration of the `plotMzDelta` output for the PRIDE experiment 12011, as in figure 4A from Foster et al. (2011).

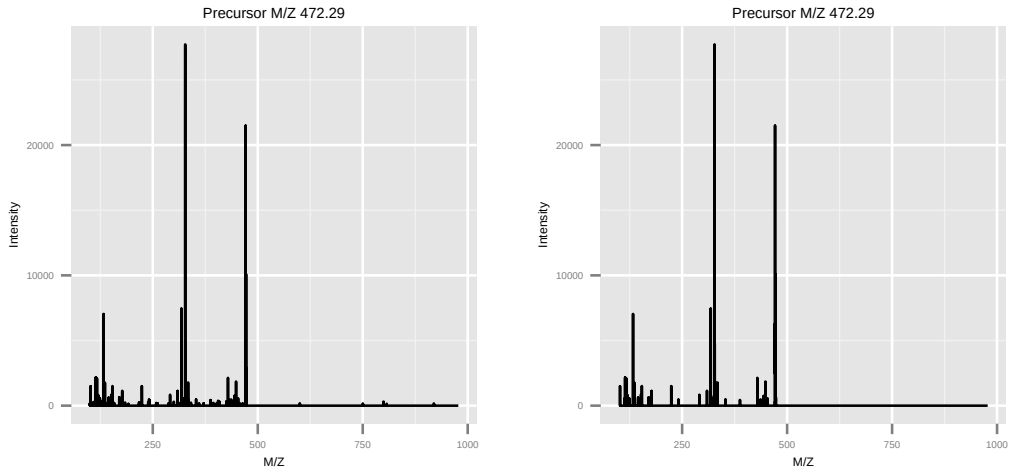


Figure 6: Same spectrum before (left) and after setting peaks ¡= 400 to 0.

intensity peaks) in a spectrum. To effectively remove 0 intensity peaks from spectra, and reduce the size of the data set, one can use the `clean` method. The effect of the `removePeaks` and `clean` methods are illustrated on figure 7 on page 22.

```
> ## number of peaks
> peaksCount(itraqdata[["X55"]])

[1] 1726

> peaksCount(experiment[["X55"]])

[1] 1726

> experiment <- clean(experiment, verbose = FALSE)
> peaksCount(experiment[["X55"]])

[1] 442
```

6.2 Focusing on specific MZ values

Another useful manipulation method is `trimMz`, that takes as parameters and `MSnExp` (or a `Spectrum`) and a numeric `mzlim`. MZ values smaller then `min(mzlim)` or greater then `max(mzmax)` are discarded. This method is particularly useful when one wants to concentrate on a specific MZ range, as for reporter ions quantification, and generally results in substantial reduction of data size. Compare the size of the full trimmed experiment to the original 1.87 Mb.

```
> range(mz(itraqdata[["X55"]]))

[1] 100.0002 977.6636

> experiment <- trimMz(experiment, mzlim = c(112,120))
> range(mz(experiment[["X55"]]))

[1] 113.0532 117.1219

> experiment

Object of class "MSnExp"
 Object size in memory: 0.29 Mb
- - - Spectra data - - -
 MS level(s): 2
 Number of MS1 acquisitions: 1
 Number of MSn scans: 55
 Number of precursor ions: 55
```

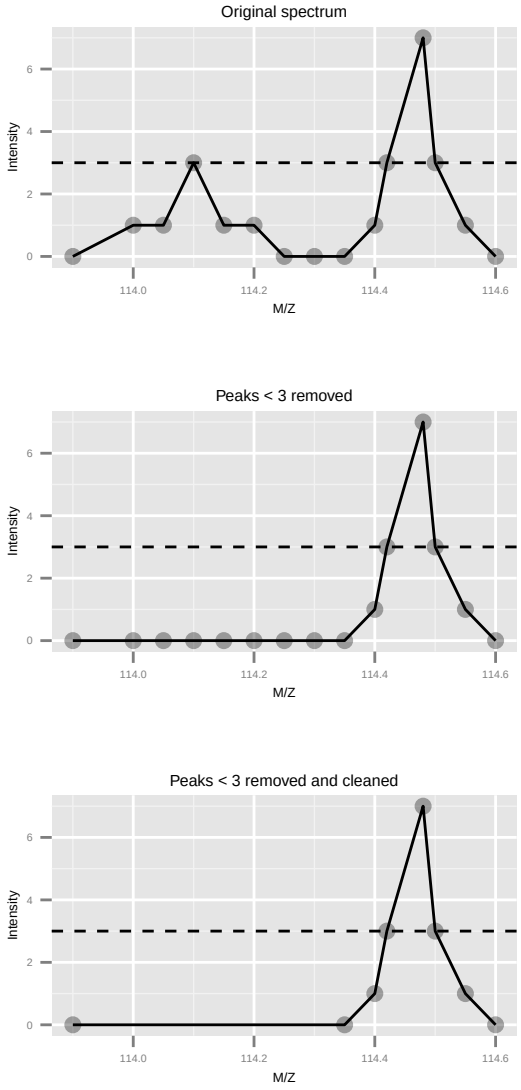


Figure 7: This figure illustrated the effect or the `removePeaks` and `clean` methods. The left-most spectrum displays two peaks, of max height 3 and 7 respectively. The middle spectrum shows the result of calling `removePeaks` with argument `t=3`, which sets all data points of the first peak, whose maximum height is smaller or equal to `t` to 0. The second peak is unaffected. Calling `clean` after `removePeaks` effectively deletes successive 0 intensities from the spectrum, as shown on the right plot.

```
 55 unique MZs
 Precursor MZ's: 401.74 - 1236.1
 MSn M/Z range: 112.04 119.87
 MSn retention times: 19:9 - 50:18 minutes
- - - Processing information - - -
Data loaded: Wed May 11 18:54:39 2011
Curves <= 400 set to '0': Sat Mar 21 19:01:02 2015
Spectra cleaned: Sat Mar 21 19:01:04 2015
MZ trimmed [112..120]
 MSnbase version: 1.1.22
- - - Meta data  - - -
phenoData
  rowNames: 1
  varLabels: sampleNames sampleNumbers
  varMetadata: labelDescription
Loaded from:
  dummyiTRAQ.mzXML
protocolData: none
featureData
  featureNames: X1 X10 ... X9 (55 total)
  fvarLabels: spectrum ProteinAccession ProteinDescription
    PeptideSequence
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
```

As can be seen above, all processing performed on the experiment is recorded and displayed as integral part of the experiment object.

7 MS^2 isobaric tagging quantitation

7.1 Reporter ions quantitation

Quantitation is performed on fixed peaks in the spectra, that are specified with an `ReporterIons` object. A specific peak is defined by it's expected `mz` value and is searched for within `mz` $\pm$ `width`. If no data is found, `NA` is returned.

```
> mz(iTRAQ4)

[1] 114.1 115.1 116.1 117.1
```

```
> width(iTRAQ4)

[1] 0.05
```

The `quantify` method takes the following parameters: an `MSnExp` experiment, a character describing the quantification `method`, the `reporters` to be quantified and a `strict` logical defining whether data points ranging outside of `mz` $\pm$ `width` should be considered for quantitation. Additionally, a progress bar can be displaying when setting the `verbose` parameter to `TRUE`. Three quantification methods are implemented, as illustrated on figure 8: `trapezoidation` returns the area under the peak of interest, `max` returns the apex of the peak and `sum` returns the sum of all intensities of the peak. See `?quantify` for more details.

The `quantify` method returns `MSnSet` objects, that extend the well-known `eSet` class defined in the Biobase package. `MSnSet` instances are very similar to `ExpressionSet` objects, except for the experiment meta-data that captures MIAPE specific information. The assay data is a matrix of dimensions $n \times m$, where m is the number of features/spectra originally in the `MSnExp` used as parameter in `quantify` and m is the number of reporter ions, that can be accessed with the `exprs` method. The meta data is directly inherited from the `MSnExp` instance.

```
> qnt <- quantify(experiment,
+                 method = "trap",
+                 reporters = iTRAQ4,
+                 strict = FALSE,
+                 verbose = FALSE)
> qnt

MSnSet (storageMode: lockedEnvironment)
assayData: 55 features, 4 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: iTRAQ4.114 iTRAQ4.115 iTRAQ4.116 iTRAQ4.117
  varLabels: mz reporters
  varMetadata: labelDescription
featureData
  featureNames: X1 X10 ... X9 (55 total)
```

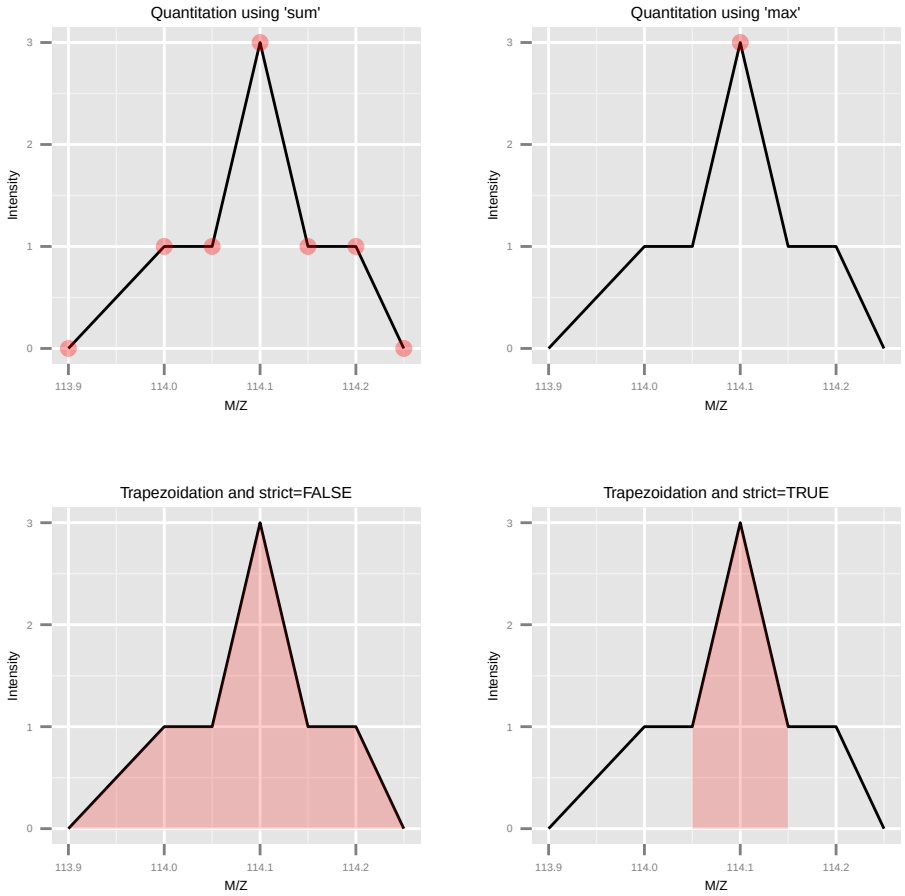


Figure 8: The different quantitation methods are illustrated above. Quantitation using `sum` sums all the data points in the peaks to produce, for this example, 7, whereas method `max` only uses the peak's maximum intensity, 3. `Trapezoidation` calculates the area under the peak taking the full with into account (using `strict=FALSE` gives 0.375) or only the width as defined by the reporter (using `strict=TRUE` gives 0.2).

```
  fvarLabels: spectrum ProteinAccession ... collision.energy
    (15 total)
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
Annotation: No annotation
- - - Processing information - - -
Data loaded: Wed May 11 18:54:39 2011
Curves <= 400 set to '0': Sat Mar 21 19:01:02 2015
Spectra cleaned: Sat Mar 21 19:01:04 2015
MZ trimmed [112..120]
iTRAQ4 quantification by trapezoidation: Sat Mar 21 19:01:07 2015
 MSnbase version: 1.1.22

> head(exprs(qnt))

    iTRAQ4.114 iTRAQ4.115 iTRAQ4.116 iTRAQ4.117
X1   1347.6158  2247.3097  3927.6931  7661.1463
X10   739.9861   799.3501   712.5983   940.6793
X11 27638.3582 33394.0252 32104.2879 26628.7278
X12 31892.8928 33634.6980 37674.7272 37227.7119
X13 26143.7542 29677.4781 29089.0593 27902.5608
X14  6448.0829  6234.1957  6902.8903  6437.2303
```

If no peak is detected for a reporter ion peak, the respective quantitation value is set to `NA`. In our case, there is 1 such case in row 41. We will remove the offending line using the `filterNA` method. The `pNA` argument defines the percentage of accepted missing values per feature. As we do not expect any missing peaks, we set it to be 0 (which is also the detault value).

```
> table(is.na(qnt))


FALSE  TRUE
  219     1

> qnt <- filterNA(qnt, pNA = 0)
> sum(is.na(qnt))

[1] 0
```

The filtering criteria for `filterNA` can also be defined as a pattern of columns

that can have missing values and columns that must not exhibit any. See `?filterNA` for details and examples.

The infrastructure around the `MSnSet` class allows flexible filtering using the `[` sub-setting operator. Below, we mimic the behaviour of `filterNA(, pNA = 0)` by calculating the row indices that should be removed, i.e. those that have at least on `NA` value and explicitly remove these row. This method allows one to devise and easily apply any filtering strategy.

```
> whichRow <- which(is.na((qnt))) %% nrow(qnt)
> qnt <- qnt[-whichRow, ]
```

See also the `plotNA` method to obtain a graphical overview of the completeness of a data set.

7.2 Importing quantitation data

If quantitation data is already available as a spreadsheet, it can be imported, along with additional optional feature and sample (pheno) meta data, with the `readMSnSet` function. This function takes the respective text-based spreadsheet (comma- or tab-separated) file names as argument to create a valid `MSnSet` instance.

Note that the quantitation data of `MSnSet` objects can also be exported to a text-based spreadsheet file using the `write.exps` method.

MSnbase also supports the `mzTab` format[7], a light-weight, tab-delimited file format for proteomics data. `mzTab` files can be read into `R` with `readMzTabData` to create and `MSnSet` instance. `MSnSet` objects can also be exported to `mzTab` with the `writeMzTabData` function.

See the `MSnbase-io` vignette for a general overview of MSnbase's input/ouput capabilites.

7.3 Peak adjustments

Single peak adjustment In certain cases, peak intensities need to be adjusted as a result of peak interferance. For example, the +1 peak of the phenylalanine (F, Phe) immonium ion (with m/z 120.03) inteferes with the 121.1 TMT reporter ion. Below, we calculate the relative intensity of the +1 peaks compared to the main peak using the Rdispo package.

[7] http://code.google.com/p/mztab/

```
> library(Rdisop)
> ## Phenylalanine immonium ion
> Fim <- getMolecule("C8H10N")
> getMass(Fim)

[1] 120.0813

> isotopes <- getIsotope(Fim)
> F1 <- isotopes[2, 2]
> F1

[1] 0.08573496
```

If desired, one can thus specifically quantify the F immonium ion in the MS2 spectrum, estimate the intensity of the +1 ion (0.0857% of the F peak) and substract this calculated value from the 121.1 TMT reporter intensity.

The above principle can also be generalised for a set of overlapping peaks, as described below.

Reporter ions purity correction Impurities in the reporter reagents can also bias the results and can be corrected when manufacturers provide correction coefficients. These generally come as percentages of each reporter ion that have masses differing by -2, -1, +1 and +2 Da from the nominal reporter ion mass due to isotopic variants. The `purityCorrect` method applies such correction to `MSnSet` instances. It also requires a square matrix as second argument, `impurities`, that defines the relative percentage of reporter in the quantified each peak. See `?purityCorrect` for more details.

```
> impurities <- matrix(c(0.929, 0.059, 0.002, 0.000,
+                        0.020, 0.923, 0.056, 0.001,
+                        0.000, 0.030, 0.924, 0.045,
+                        0.000, 0.001, 0.040, 0.923),
+                      nrow = 4)
> qnt.crct <- purityCorrect(qnt, impurities)
> head(exprs(qnt))

    iTRAQ4.114 iTRAQ4.115 iTRAQ4.116 iTRAQ4.117
X1   1347.6158  2247.3097  3927.6931  7661.1463
X10   739.9861   799.3501   712.5983   940.6793
X11 27638.3582 33394.0252 32104.2879 26628.7278
```

```
X12 31892.8928 33634.6980 37674.7272 37227.7119
X13 26143.7542 29677.4781 29089.0593 27902.5608
X14  6448.0829  6234.1957  6902.8903  6437.2303

> head(exprs(qnt.crct))

    iTRAQ4.114 iTRAQ4.115 iTRAQ4.116 iTRAQ4.117
X1   1402.9442  2214.0346  3762.2549  8114.4429
X10   779.4666   793.0792   678.8083   985.2003
X11 29034.3781 33271.0470 31484.7131 27279.1383
X12 33618.9092 33046.3075 37031.6133 38492.1376
X13 27508.0038 29440.9296 28390.4561 28814.2463
X14  6809.7600  6090.7894  6799.5030  6636.1450
```

The `makeImpuritiesMatrix` can be used to create impurity matrices. It opens a rudimentary spreadsheet that can be directly edited.

7.4 Normalisation

A `MSnSet` object is meant to be compatible with further downstream packages for data normalisation and statistical analysis. There is also a `normalise` (also available as `normalize`) method for expression sets. The method takes and instance of class `MSnSet` as first argument, and a character to describe the `method` to be used:

`quantiles` Applies quantile normalisation (Bolstad et al., 2003) as implemented in the `normalize.quantiles` function of the preprocessCore package.

`quantiles.robust` Applies robust quantile normalisation (Bolstad et al., 2003) as implemented in the `normalize.quantiles.robust` function of the preprocessCore package.

`vsn` Applies variance stabilisation normalization (Huber et al., 2002) as implemented in the `vsn2` function of the vsn package.

`max` Each feature's reporter intensity is divided by the maximum of the reporter ions intensities.

`sum` Each feature's reporter intensity is divided by the sum of the reporter ions intensities.

See `?normalise` for more methods. A `scale` method for `MSnSet` instances, that relies on the `base::scale` function.

```
> qnt.max <- normalise(qnt, "max")
> qnt.sum <- normalise(qnt, "sum")
> qnt.quant <- normalise(qnt, "quantiles")
> qnt.qrob <- normalise(qnt, "quantiles.robust")
> qnt.vsn <- normalise(qnt, "vsn")
```

The effect of these are illustrated on figure 9 and figure 10 reproduces figure 3 of Karp et al. (2010) that described the application of vsn on iTRAQ reporter data.

Note that it is also possible to normalise individual spectra or whole `MSnExp` experiments with the `normalise` method using the `max` method. This will rescale all peaks between 0 and 1. To visualise the relative reporter peaks, one should this first trim the spectra using method `trimMz` as illustrated in section 6, then normalise the `MSnExp` with `normalise` using `method="max"` as illustrated above and plot the data using `plot` (figure 11).

Additional dedicated normalisation method are available for MS^2 label-free quantitation, as described in section 9 and in the `quantify` documentation.

8 Feature aggregation

The above quantitation and normalisation has been performed on quantitative data obtained from individual spectra. However, the biological unit of interest is not the spectrum but the peptide or the protein. As such, it is important to be able to summarise features that belong to a same group, i.e. spectra from one peptide, peptides that originate from one protein, or directly combine all spectra that have been uniquely associated to one protein.

MSnbase provides one function, `combineFeatures`, that allows to aggregate features stored in an `MSnSet` using build-in or user defined summary function and return a new `MSnSet` instance. The three main arguments are described below. Additional details can be found in the method documentation.

`combineFeatures`'s first argument, `object`, is an instance of class `MSnSet`, as has been created in the section 7.1 for instance. The second argument, `groupBy`, is a `factor` than has as many elements as there are features in the `MSnSet object` argument. The features corresponding to the `groupBy` levels will be aggregated so that the resulting `MSnSet` output will have `length(levels(groupBy))` features. Here, we will combine individual MS2 spectra based on the protein

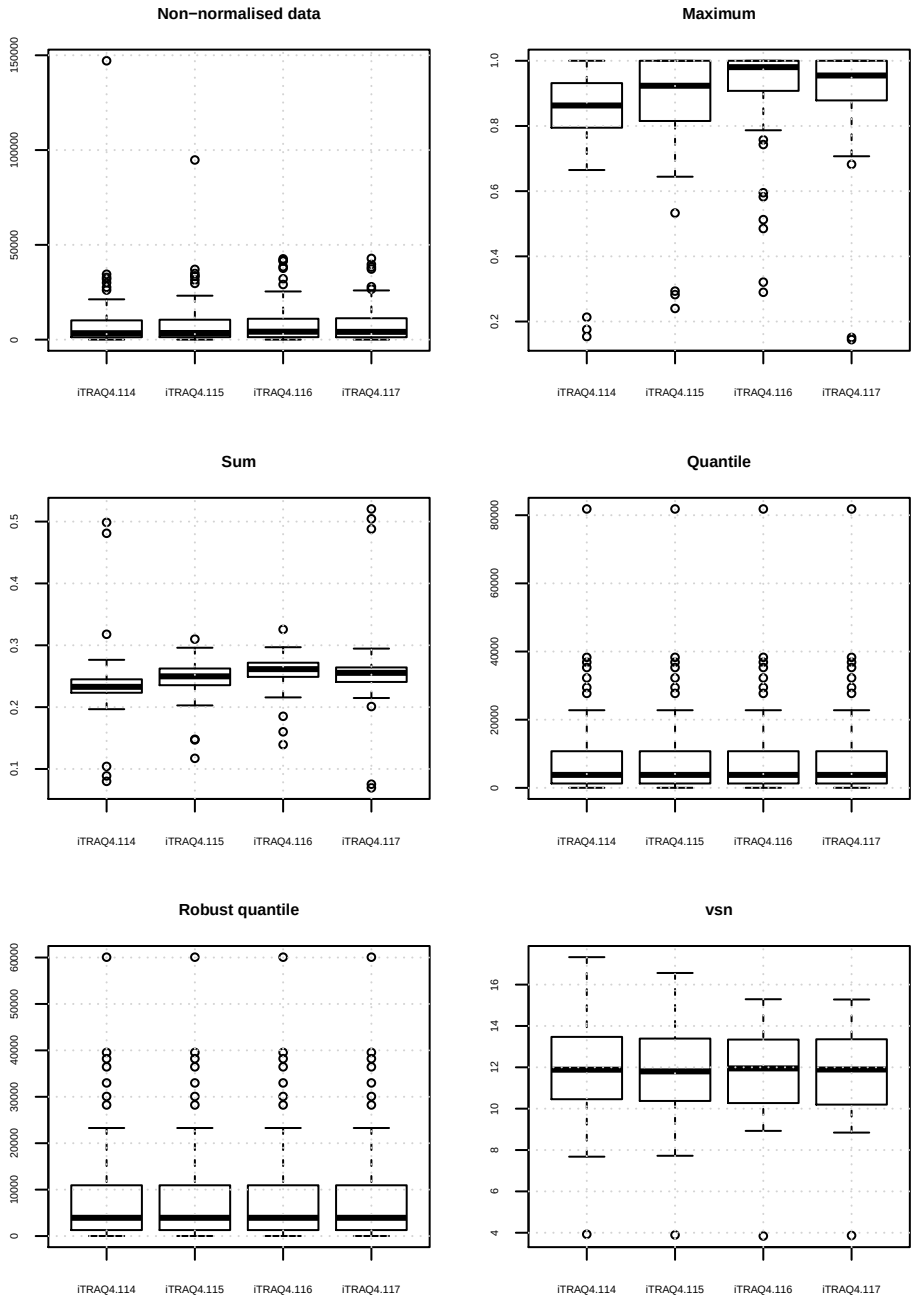


Figure 9: Comparison of the normalisation `MSnSet` methods. Note that vsn also glog-transforms the intensities.

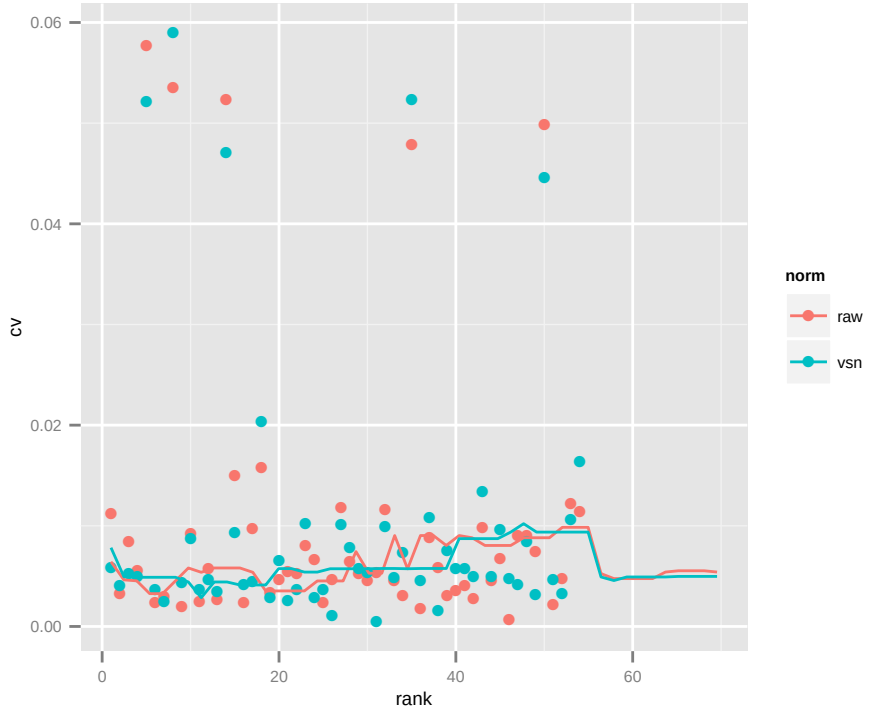


Figure 10: CV versus signal intensity comparison for log2 and vsn transformed data. Lines indicate running CV medians.

they originate from. As shown below, this will result in 40 new aggregated features.

```
> gb <- fData(qnt)$ProteinAccession
> table(gb)

gb
    BSA ECA0172 ECA0435 ECA0452 ECA0469 ECA0621 ECA0631 ECA0691
      3       1       2       1       2       1       1       1
ECA0871 ECA0978 ECA1032 ECA1093 ECA1104 ECA1294 ECA1362 ECA1363
      1       1       1       1       1       1       1       1
ECA1364 ECA1422 ECA1443 ECA2186 ECA2391 ECA2421 ECA2831 ECA3082
      1       1       1       1       1       1       1       1
ECA3175 ECA3349 ECA3356 ECA3377 ECA3566 ECA3882 ECA3929 ECA3969
      1       2       1       1       2       1       1       1
ECA4013 ECA4026 ECA4030 ECA4037 ECA4512 ECA4513 ECA4514     ENO
      1       2       1       1       1       1       6       3

> length(unique(gb))

[1] 40
```

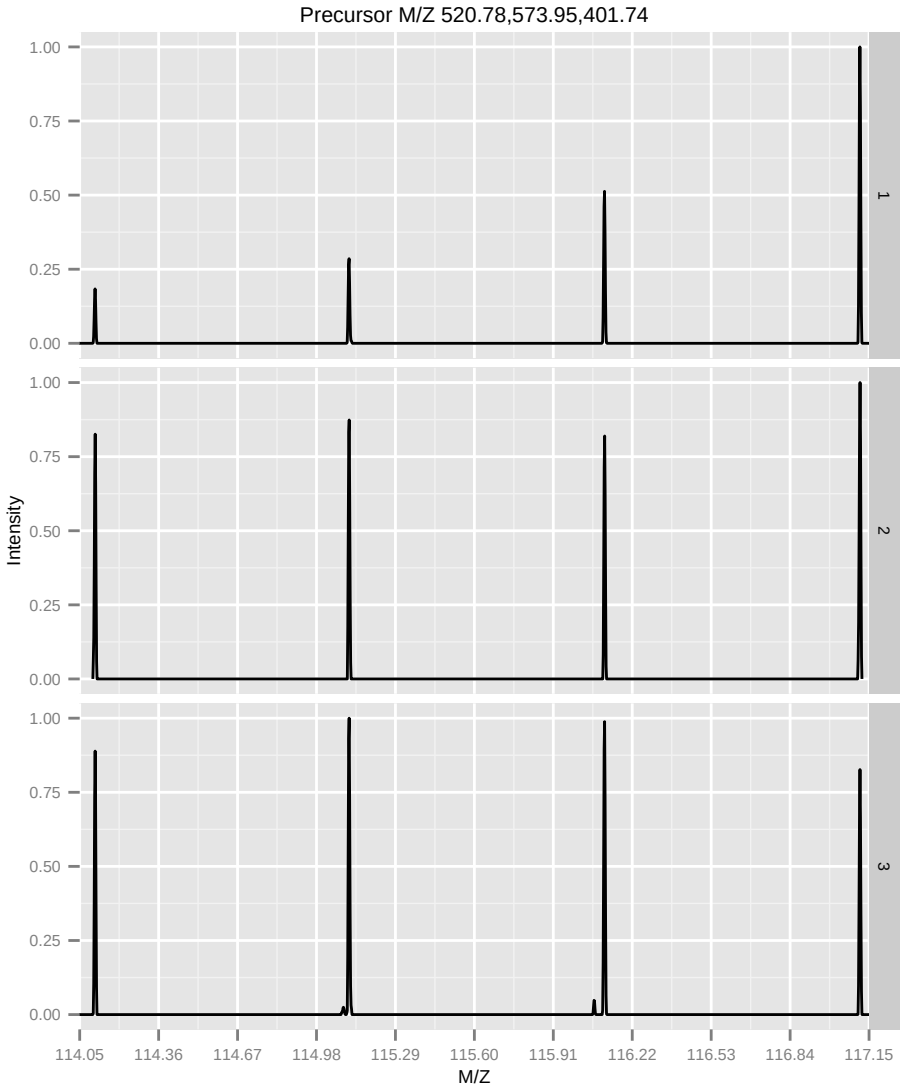


Figure 11: Experiment-wide normalised MS2 spectra. The y-axes of the individual spectra is now rescaled between 0 and 1 (highest peak), as opposed to figure 2.

The third argument, `fun`, defined how to combine the features. Predefined functions are readily available and can be specified as strings (`fun="mean"`, `fun="median"`, `fun="sum"`, `fun="weighted.mean"` or `fun="medianpolish"` to compute respectively the mean, media, sum, weighted mean or median polish of the features to be aggregated). Alternatively, is is possible to supply user defined functions with `fun=function(x) { ... }`. We will use the `median` here.

```
> qnt2 <- combineFeatures(qnt, groupBy = gb, fun = "median")

Combined 54 features into 40 using median

> qnt2

MSnSet (storageMode: lockedEnvironment)
assayData: 40 features, 4 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: iTRAQ4.114 iTRAQ4.115 iTRAQ4.116 iTRAQ4.117
  varLabels: mz reporters
  varMetadata: labelDescription
featureData
  featureNames: BSA ECA0172 ... ENO (40 total)
  fvarLabels: spectrum ProteinAccession ... CV.iTRAQ4.117
    (19 total)
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
Annotation: No annotation
- - - Processing information - - -
Data loaded: Wed May 11 18:54:39 2011
Curves <= 400 set to '0': Sat Mar 21 19:01:02 2015
Spectra cleaned: Sat Mar 21 19:01:04 2015
MZ trimmed [112..120]
iTRAQ4 quantification by trapezoidation: Sat Mar 21 19:01:07 2015
Subset [55,4][54,4] Sat Mar 21 19:01:07 2015
Removed features with more than 0 NAs: Sat Mar 21 19:01:07 2015
Dropped featureData's levels Sat Mar 21 19:01:07 2015
Combined 54 features into 40 using median: Sat Mar 21 19:01:10 2015
 MSnbase version: 1.1.22
```

9 Label-free MS^2 quantitation

9.1 Peptide counting

Note that if samples are not multiplexed, label-free MS^2 quantitation by spectral counting is possible using MSnbase. Once individual spectra have been assigned to peptides and proteins (see section 4), it becomes straightforward to estimate protein quantities using the simple peptide counting method, as illustrated in section 8.

```
> sc <- quantify(msexp, method = "count")
> ## lets modify out data for demonstration purposes
> fData(sc)$accession[1] <- fData(sc)$accession[2]
> fData(sc)$accession

[1] "ECA1028" "ECA1028" "ECA0510"

> sc <- combineFeatures(sc, groupBy = fData(sc)$accession,
+                       fun = "sum")

Combined 3 features into 2 using sum

> exprs(sc)

        1
ECA0510 1
ECA1028 2
```

Such count data could then be further analyses using dedicated count methods (originally developed for high-throughput sequencing) and directly available for `MSnSet` instances in the msmsTests Bioconductor package.

9.2 Spectral counting and intensity methods

The spectral abundance factor (SAF) and the normalised form (NSAF) (Paoletti et al., 2006) as well as the spectral index (SI) and other normalised variations (SI_{GI} and SI_N) (Griffin et al., 2010) are also available. Below, we illustrate how to apply the normalised SI_N to the experiment containing identification data produced in section 4.

The spectra that did not match any peptide have already been remove with the `removeNoId` method. As can be seen in the following code chunk, the first spectrum could not be matched to any single protein. Non-identified

spectra and those matching multiple proteins are removed automatically prior to any label-free quantitation. Once can also remove peptide that do not match uniquely to proteins (as defined by the `nprot` feature variable column) with the `removeMultipleAssignment` method.

```
> fData(msexp)[, c("accession", "nprot")]

           accession nprot
X1.1 ECA0984;ECA3829     2
X2.1         ECA1028     1
X5.1         ECA0510     1
```

Note that the label-free methods implicitely apply feature aggregation (section 8) and normalise (section 7.4) the quantitation values based on the total sample intensity and or the protein lengths (see Paoletti et al. (2006) and Griffin et al. (2010) for details).

Let's now proceed with the quantitation using the `quantify`, as in section 7.1, this time however specifying the method of interest, `SIn` (the `reporters` argument can of course be ignored here). The required peptide-protein mapping and protein lengths are extracted automatically from the feature metadata using the default `accession` and `length` feature variables.

```
> siquant <- quantify(msexp, method = "SIn")

Combined 2 features into 2 using sum

> processingData(siquant)

- - - Processing information - - -
Data loaded: Sat Mar 21 19:01:00 2015
Filtered 2 unidentified peptides out: Sat Mar 21 19:01:01 2015
Removed 1 features assigned to multiple proteins: Sat Mar 21 19:01:10 2015
Combined 2 features into 2 using sum: Sat Mar 21 19:01:10 2015
Quantification by SIn: Sat Mar 21 19:01:10 2015
 MSnbase version: 1.14.2

> exprs(siquant)

                  1
ECA0510 0.003588641
ECA1028 0.001470129
```

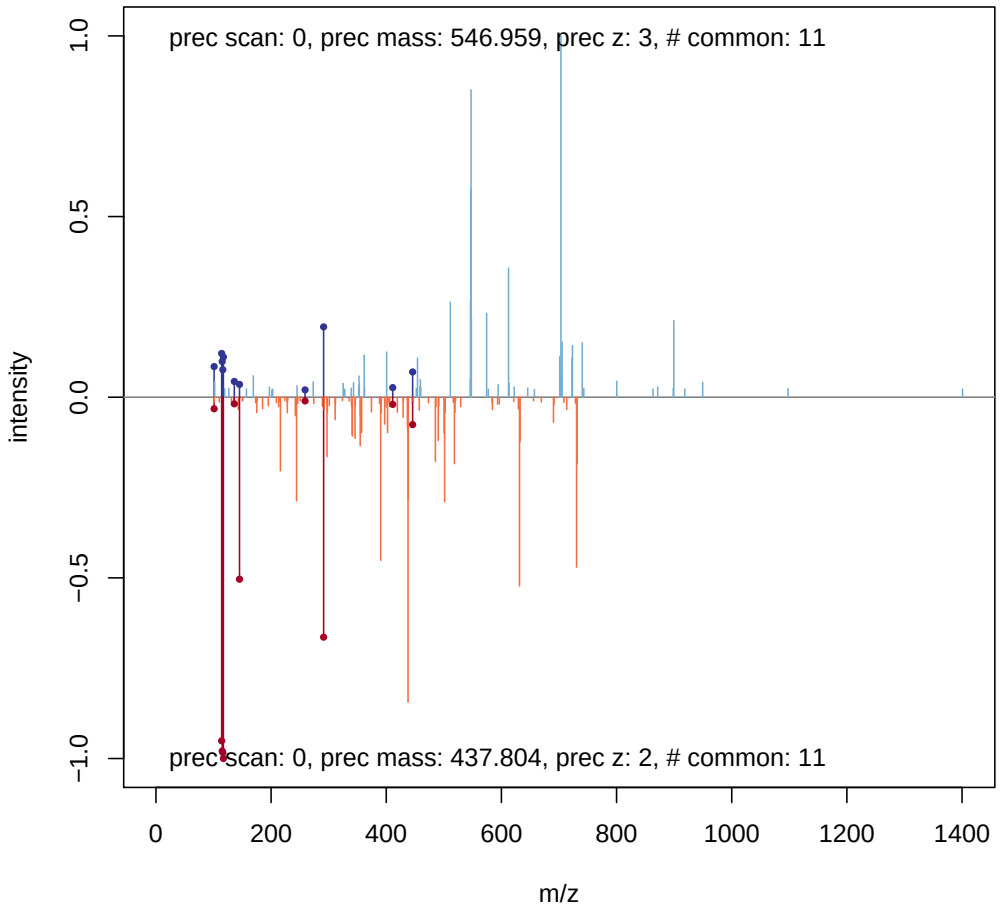
Other label-free methods can be applied by specifiying the appropriate `method` argument. See `?quantify` for more details.

10 Spectra comparison

10.1 Plotting two spectra

MSnbase provides functionality to compare spectra against each other. The first notable function is `plot`. If two `Spectrum2` objects are provided `plot` will draw two plots: the upper and lower panel contain respectively the first and second spectrum. Common peaks are drawn in a slightly darker colour.

```
> centroided <- pickPeaks(msexp, verbose=FALSE)
> plot(centroided[[2]], centroided[[3]])
```



10.2 Comparison metrics

Currently MSnbase supports three different metrics to compare spectra against each other: `common` to calculate the number of common peaks, `cor` to calculate the Pearson correlation and `dotproduct` to calculate the dot product. See `?compareSpectra` to apply other arbitrary metrics.

```
> compareSpectra(centroided[[2]], centroided[[3]],
+                fun = "common")

[1] 9

> compareSpectra(centroided[[2]], centroided[[3]],
+                fun = "cor")

[1] 0.006813591

> compareSpectra(centroided[[2]], centroided[[3]],
+                fun = "dotproduct")

[1] 0.008130662
```

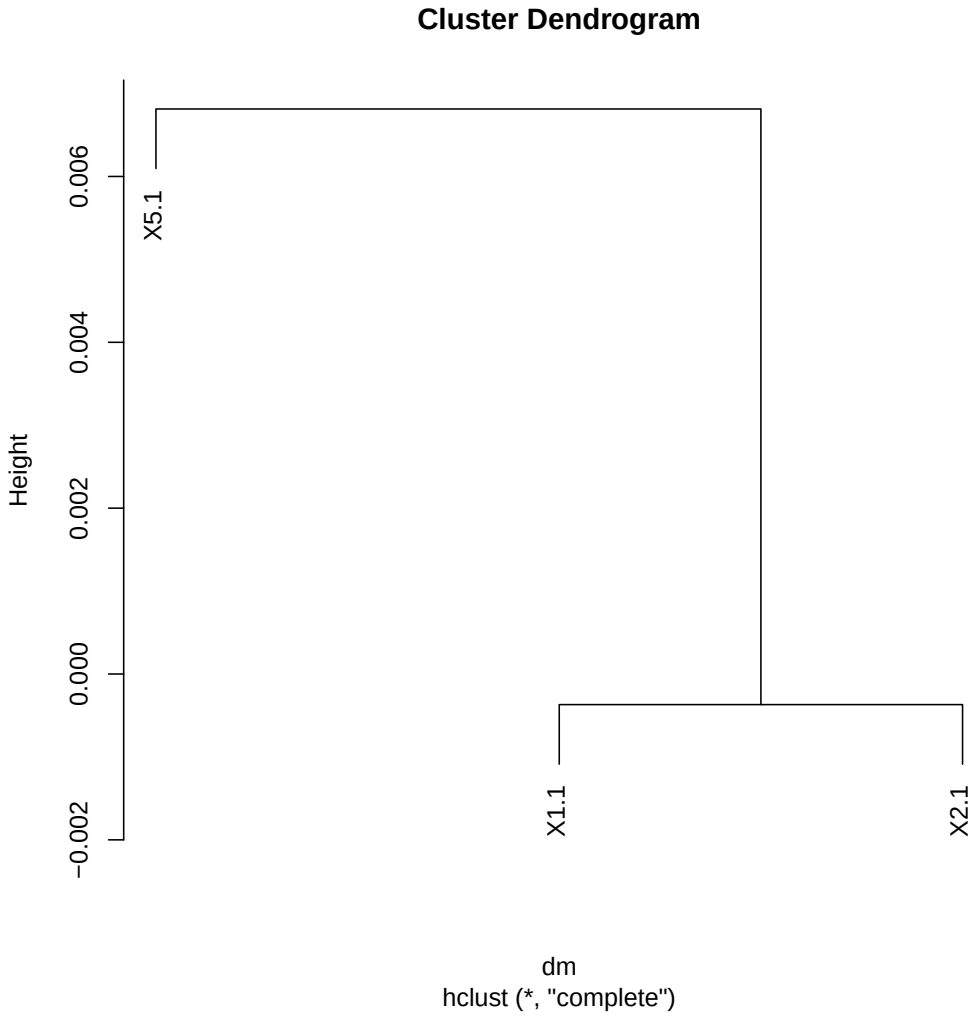
`compareSpectra` supports `MSnExp` objects as well.

```
> compareSpectra(centroided, fun="cor")

              X1.1          X2.1        X5.1
X1.1            NA -0.0003689564 0.001305087
X2.1 -0.0003689564            NA 0.006813591
X5.1  0.0013050868  0.0068135912          NA
```

Below, we illustrate how to compare a set of spectra using a hierarchical clustering.

```
> dm <- as.dist(compareSpectra(centroided, fun="cor"))
> plot(hclust(dm))
```



11 Quantitative assessment of incomplete dissociation

Quantitation using isobaric reporter tags assumes complete dissociation between the reporter group (red on figure 12), balance group (blue) and peptide (the peptide reactive group is drawn in green). However, incomplete dissociation does occur and results in an isobaric tag (i.e reporter and balance groups) specific peaks.

MSnbase provides, among others, a `ReporterIons` object for iTRAQ 4-plex that includes the 145 peaks, called `iTRAQ5`. This can then be used to quantify the experiment as show in section 7.1 to estimate incomplete dissociation for each spectrum.

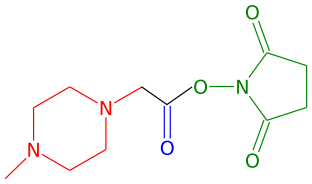


Figure 12: iTRAQ 4-plex isobaric tags reagent consist of three parts: (1) a charged reporter group (MZ of 114, 115, 116 and 117) that is unique to each of the four reagents (red), (2) an uncharged mass balance group (28-31 Da) (blue)and (3) a peptide reactive group (NHS ester) that binds to the peptide. In case of incomplete dissociation, the reporter and balance groups produce a specific peaks at MZ 145.

```
> iTRAQ5

Object of class "ReporterIons"
iTRAQ4: '4-plex iTRAQ and reporter + balance group' with 5 reporter ions
 - 114.1 +/- 0.05 (red)
 - 115.1 +/- 0.05 (green)
 - 116.1 +/- 0.05 (blue)
 - 117.1 +/- 0.05 (yellow)
 - 145.1 +/- 0.05 (grey)

> incompdiss <- quantify(itraqdata,
+                        method = "trap",
+                        reporters = iTRAQ5,
+                        strict = FALSE,
+                        verbose = FALSE)
> head(exprs(incompdiss))

    iTRAQ5.114 iTRAQ5.115 iTRAQ5.116 iTRAQ5.117 iTRAQ5.145
X1   1347.6158  2247.3097  3927.6931  7661.1463  2063.8947
X10   739.9861   799.3501   712.5983   940.6793   467.3615
X11 27638.3582 33394.0252 32104.2879 26628.7278 13543.4565
X12 31892.8928 33634.6980 37674.7272 37227.7119 11839.2558
X13 26143.7542 29677.4781 29089.0593 27902.5608 12206.5508
X14  6448.0829  6234.1957  6902.8903  6437.2303   427.6654
```

Figure 13 compares these intensities for the whole experiment.

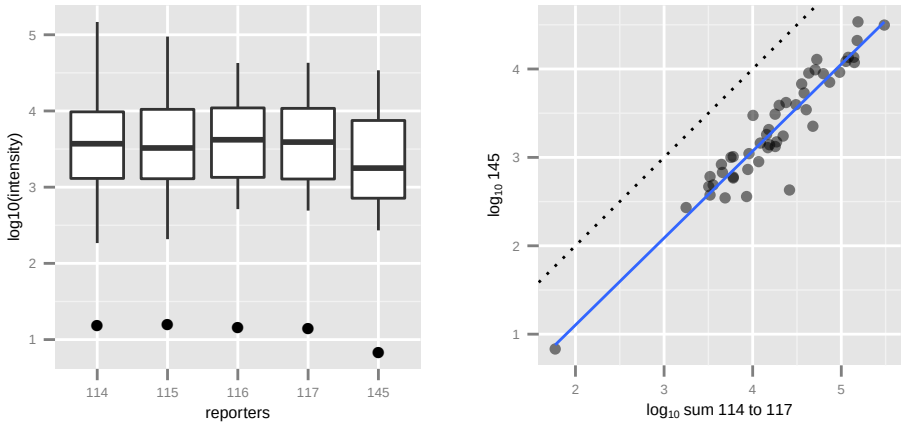


Figure 13: Boxplot and scatterplot comparing intensities of the 4 reporter ions (or their sum, on the right) and the incomplete dissociation specific peak.

12 Combining `MSnSet` instances

Combining mass spectrometry runs can be done in two different ways depending on the nature of these runs. If the runs represent repeated measures of identical samples, for instance multiple fractions, the data has to be combine along the row of the quantitation matrix: all the features (along the rows) represent measurements of the same set of samples (along the columns). In this situation, described in section 12.1, two experiments of dimensions n_1 (rows) by m (columns and n_2 by m will produce a new experiment of dimensions $n_1 + n_2$ by m.

When however, different sets of samples have been analysed in different mass spectrometry runs, the data has to be combined along the columns of the quantitation matrix: some features will be shared across experiments and should thus be aligned on a same row in the new data set, whereas unique features to one experiment should be set as missing in the other one. In this situation, described in section 12.2, two experiments of dimensions n_1 by m_1 and n_2 by m_2 will produce a new experiment of dimensions $unique_{n_1} + unique_{n_2} + shared_{n_1,n_2}$ by $m_1 + m_2$. The two first terms of the first dimension will be complemented by `NA` values.

Default `MSnSet` feature names (`X1`, `X2`, ...) and sample names (`iTRAQ4.114`, `iTRAQ4.115`, `iTRAQ4.116`, ...) are not informative. The features and samples of these anonymous quantitative data-sets should be updated before being combined, to guide how to meaningfully merge them.

12.1 Combining identical samples

To simulate this situation, let us use quantiation data from the `itraqdata` object that is provided with the package as experiment 1 and the data from the `rawdata` `MSnExp` instance created at the very beginning of this document. Both experiments share the *same* default iTRAQ 4-plex reporter names as default sample names, and will thus automatically be combined along rows.

```
> exp1 <- quantify(itraqdata, reporters = iTRAQ4,
+                  verbose = FALSE)
> sampleNames(exp1)

[1] "iTRAQ4.114" "iTRAQ4.115" "iTRAQ4.116" "iTRAQ4.117"

> exp2 <- quantify(rawdata, reporters = iTRAQ4,
+                  verbose = FALSE)
> sampleNames(exp2)

[1] "iTRAQ4.114" "iTRAQ4.115" "iTRAQ4.116" "iTRAQ4.117"
```

It important to note that the features of these independent experiments share the same default anonymous names: X1, X2, X3, ..., that however represent quantitation of distinct physical analytes. If the experiments were to be combined as is, it would result in an error because data points for the same *feature* name (say `X1`) and the same *sample name* (say `iTRAQ4.114`) have different values. We thus first update the feature names to explicitate that they originate from different experiment and represent quantitation from different spectra using the convenience function `updateFeatureNames`. Note that updating the names of one experiment would suffice here.

```
> head(featureNames(exp1))

[1] "X1"  "X10" "X11" "X12" "X13" "X14"

> exp1 <- updateFeatureNames(exp1)
> head(featureNames(exp1))

[1] "X1.exp1"  "X10.exp1" "X11.exp1" "X12.exp1" "X13.exp1"
[6] "X14.exp1"

> head(featureNames(exp2))

[1] "X1.1" "X2.1" "X3.1" "X4.1" "X5.1"
```

```
> exp2 <- updateFeatureNames(exp2)
> head(featureNames(exp2))

[1] "X1.1.exp2" "X2.1.exp2" "X3.1.exp2" "X4.1.exp2" "X5.1.exp2"
```

The two experiments now share the same sample names and have different feature names and will be combined along the row. Note that all meta-data is correctly combined along the quantitation values.

```
> exp12 <- combine(exp1, exp2)

Warning in combine(experimentData(x), experimentData(y)):
unknown or conflicting information in MIAPE field 'email'; using information
from first object 'x'

> dim(exp1)

[1] 55  4

> dim(exp2)

[1] 5 4

> dim(exp12)

[1] 60  4
```

12.2 Combine different samples

Lets now create two `MSnSets` from the same raw data to simulate two different independent experiments that share some features. As done previously (see section 8), we combine the spectra based on the proteins they have been identified to belong to. Features can thus naturally be named using protein accession numbers. Alternatively, if peptide sequences would have been used as grouping factor in `combineFeatures`, then these would be good feature name candidates.

```
> set.seed(1)
> i <- sample(length(itraqdata), 35)
> j <- sample(length(itraqdata), 35)
> exp1 <- quantify(itraqdata[i], reporters = iTRAQ4,
```

```
+                   verbose = FALSE)
> exp2 <- quantify(itraqdata[j], reporters = iTRAQ4,
+                   verbose = FALSE)
> exp1 <- droplevels(exp1)
> exp2 <- droplevels(exp2)
> table(featureNames(exp1) %in% featureNames(exp2))


FALSE  TRUE
   12    23

> exp1 <- combineFeatures(exp1,
+                         groupBy = fData(exp1)$ProteinAccession)

Combined 35 features into 27 using mean

> exp2 <- combineFeatures(exp2,
+                         groupBy = fData(exp2)$ProteinAccession)

Combined 35 features into 27 using mean

> head(featureNames(exp1))

[1] "BSA"     "ECA0435" "ECA0469" "ECA0621" "ECA0631" "ECA0978"

> head(featureNames(exp2))

[1] "BSA"     "ECA0172" "ECA0435" "ECA0452" "ECA0469" "ECA0621"
```

The `droplevels` drops the unused `featureData` levels. This is required to avoid passing absent levels as `groupBy` in `combineFeatures`. Alternatively, one could also use `factor(fData(exp1)$ProteinAccession)` as `groupBy` argument.

The feature names are updated automatically by `combineFeatures`, using the `groupBy` argument. Proper feature names, reflecting the nature of the features (spectra, peptides or proteins) is critical when multiple experiments are to be combined, as this is done using common features as defined by their names (see below).

Sample names should also be updated to replace anonymous reporter names with relevant identifiers; the individual reporter data is stored in the `phenoData` and is not lost. A convenience function `updateSampleNames` is provided to append the `MSnSet`'s variable name to the already defined names, although in

general, biologically relevant identifiers are preferred.

```
> sampleNames(exp1)

[1] "iTRAQ4.114" "iTRAQ4.115" "iTRAQ4.116" "iTRAQ4.117"

> exp1 <- updateSampleNames(exp1)
> sampleNames(exp1)

[1] "iTRAQ4.114.exp1" "iTRAQ4.115.exp1" "iTRAQ4.116.exp1"
[4] "iTRAQ4.117.exp1"

> sampleNames(exp1) <- c("Ctrl1", "Cond1", "Ctrl2", "Cond2")
> sampleNames(exp2) <- c("Ctrl3", "Cond3", "Ctrl4", "Cond4")
```

At this stage, it is not yet possible to combine the two experiments, because their feature data is not compatible yet; they share the same feature variable labels, i.e. the feature data column names (spectrum, ProteinAccession, ProteinDescription, ...), but the part of the content is different because the original data was (in particular all the spectrum centric data: identical peptides in different runs will have different retention times, precursor intensities, ...). Feature data with identical labels (columns in the data frame) and names (row in the data frame) are expected to have the same data and produce an error if not conform.

```
> stopifnot(all(fvarLabels(exp1) == fvarLabels(exp2)))
> fData(exp1)["BSA", 1:4]

    spectrum ProteinAccession   ProteinDescription PeptideSequence
BSA        1              BSA bovine serum albumin          NYQEAK

> fData(exp2)["BSA", 1:4]

    spectrum ProteinAccession   ProteinDescription PeptideSequence
BSA       52              BSA bovine serum albumin       QTALVELLK
```

Instead of removing these identical feature data columns, one can use a second convenience function, `updateFvarLabels`, to update feature labels based on the experiements variable name and maintain all the metadata.

```
> exp1 <- updateFvarLabels(exp1)
> exp2 <- updateFvarLabels(exp2)
> head(fvarLabels(exp1))

[1] "spectrum.exp1"           "ProteinAccession.exp1"
[3] "ProteinDescription.exp1" "PeptideSequence.exp1"
[5] "file.exp1"               "retention.time.exp1"

> head(fvarLabels(exp2))

[1] "spectrum.exp2"           "ProteinAccession.exp2"
[3] "ProteinDescription.exp2" "PeptideSequence.exp2"
[5] "file.exp2"               "retention.time.exp2"
```

It is now possible to combine `exp1` and `exp2`, including all the meta-data, with the `combine` method. The new experiment will contain the union of the feature names of the individual experiments with missing values inserted appropriately.

```
> exp12 <- combine(exp1, exp2)
> dim(exp12)

[1] 35  8

> pData(exp12)

         mz reporters
Ctrl1 114.1    iTRAQ4
Cond1 115.1    iTRAQ4
Ctrl2 116.1    iTRAQ4
Cond2 117.1    iTRAQ4
Ctrl3 114.1    iTRAQ4
Cond3 115.1    iTRAQ4
Ctrl4 116.1    iTRAQ4
Cond4 117.1    iTRAQ4

> exprs(exp12)[25:28, ]

           Ctrl1    Cond1    Ctrl2     Cond2    Ctrl3    Cond3
ECA4513 10154.95 10486.94 11018.19 11289.552       NA       NA
ECA4514 20396.49 20832.98 23280.82 23693.574 15965.52 16206.91
ENO     50826.03 31978.10       NA  7528.967 39965.73 24967.40
```

```
ECA0172       NA       NA       NA        NA 17593.55 18545.62
           Ctrl4     Cond4
ECA4513       NA        NA
ECA4514 18455.76 18704.058
ENO           NA  5925.663
ECA0172 19361.84 18328.237

> exp12

MSnSet (storageMode: lockedEnvironment)
assayData: 35 features, 8 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: Ctrl1 Cond1 ... Cond4 (8 total)
  varLabels: mz reporters
  varMetadata: labelDescription
featureData
  featureNames: BSA ECA0435 ... ECA4512 (35 total)
  fvarLabels: spectrum.exp1 ProteinAccession.exp1 ...
    CV.iTRAQ4.117.exp2 (38 total)
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
Annotation: No annotation
- - - Processing information - - -
Combined [35,8] and [27,4] MSnSets Sat Mar 21 19:01:19 2015
 MSnbase version: 1.1.22
```

In summary, when experiments with different samples need to be combined (along the columns), one needs to (1) clarify the sample names using `updateSampleNames` or better manually, for biological relevance and (2) update the feature data variable labels with `updateFvarLabels`. The individual experiments (there can be more than 2) can then easily be combined with the `combine` method while retaining the meta-data.

If runs for the same sample (different fractions for example) need to be combines, one needs to (1) differentiate the feature provenance with `updateFeatureNames` prior to use `combine`.

12.3 Averaging `MSnSet` instances

It is sometimes useful to average a set of replicated experiments to facilitate their visualisation. This can be easily achieved with the `averageMSnSet` function, which takes a list of valid `MSnSet` instances as input and creates a new object whose expression values are an average of the original values. A value of dispersion (`disp`) and a count of missing values (`nNA`) is recorded in the feature metadata slot. The average and dispersion are computed by default as the median and (non-parametric) coefficient of variation (see `?npcv` for details), although this can easily be parametrised, as described in `?averageMSnSet`.

The next code chunk illustrates the averaging function using three replicated experiments from Tan et al. (2009) available in the pRolocdata package.

```
> library("pRolocdata")
> data(tan2009r1)
> data(tan2009r2)
> data(tan2009r3)
> avgtan <- averageMSnSet(list(tan2009r1, tan2009r2, tan2009r3))
> head(exprs(avgtan))

            X114      X115      X116      X117
P20353 0.3605000 0.3035000 0.2095000 0.1265000
P53501 0.4299090 0.1779700 0.2068280 0.1852625
Q7KU78 0.1704443 0.1234443 0.1772223 0.5290000
P04412 0.2567500 0.2210000 0.3015000 0.2205000
Q7KJ73 0.2160000 0.1830000 0.3420000 0.2590000
Q7JZN0 0.0965000 0.2509443 0.4771667 0.1750557

> head(fData(avgtan)$disp)

              X114      X115        X116       X117
P20353 0.076083495 0.1099127 0.109691169 0.14650198
P53501 0.034172542 0.2640556 0.005139653 0.17104568
Q7KU78 0.023198743 0.4483795 0.027883087 0.04764499
P04412 0.053414021 0.2146751 0.090972139 0.27903810
Q7KJ73 0.000000000 0.0000000 0.000000000 0.00000000
Q7JZN0 0.007681865 0.1959534 0.097873350 0.06210542

> head(fData(avgtan)$nNA)

       X114 X115 X116 X117
```

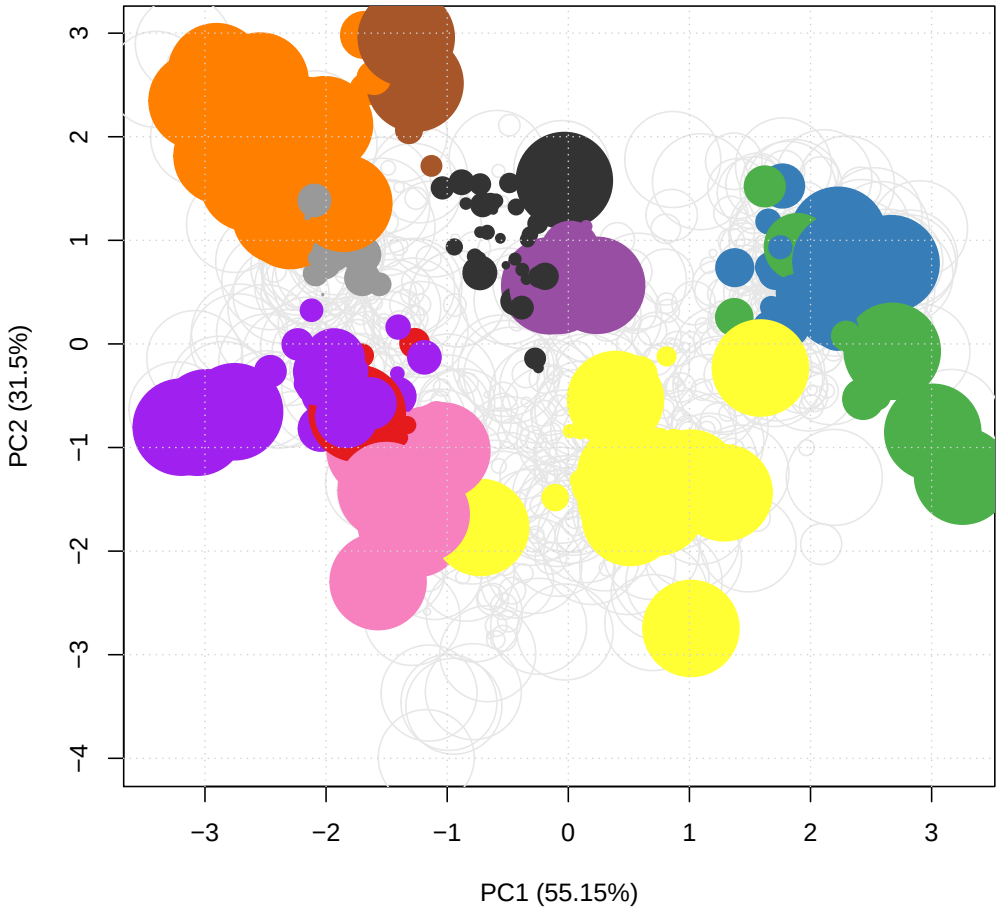
```
P20353    1    1    1    1
P53501    1    1    1    1
Q7KU78    0    0    0    0
P04412    1    1    1    1
Q7KJ73    2    2    2    2
Q7JZN0    0    0    0    0
```

We are going to visualise the average data on a principle component (PCA) plot using the `plot2D` function from the pRoloc package Gatto et al. (2014). In addition, we are going to use the measure of dispersion to highlight averages with high variability by taking, for each protein, the maximum observed dispersion in the 4 samples. Note that in the default implementation, dispersions estimated from a single measurement (i.e. that had 2 missing values in our example) are set to 0; we will set these to the overal maximum observed dispersion.

```
> disp <- rowMax(fData(avgtan)$disp)
> disp[disp == 0] <- max(disp)
> range(disp)

[1] 0.01152877 1.20888923

> library("pRoloc")
> plot2D(avgtan, cex = 7 * disp)
```



13 MS^E data processing

MSnbase can also be used for MS^E data independent acquisition from Waters instrument. The MS^E pipeline depends on the Bioconductor synapter package (Bond et al., 2013) that produces `MSnSet` instances for indvidual acquisitions. The MSnbase infrastructure can subsequently be used to further combine experiments, as shown in section 12.2 and apply *top3* quantitation using the `topN` method.

14 Session information

- R version 3.1.3 (2015-03-09), `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=en_US.UTF-8`, `LC_NAME=C`,

`LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`

- Base packages: base, datasets, grDevices, graphics, grid, methods, parallel, stats, stats4, utils
- Other packages: AnnotationDbi 1.28.2, Biobase 2.26.0, BiocGenerics 0.12.1, BiocParallel 1.0.3, GenomeInfoDb 1.2.4, IRanges 2.0.1, MLInterfaces 1.46.0, MSnbase 1.14.2, Rcpp 0.11.5, RcppClassic 0.9.6, Rdisop 1.26.0, S4Vectors 0.4.0, XML 3.98-1.1, annotate 1.44.0, cluster 2.0.1, ggplot2 1.0.1, knitr 1.9, mzR 2.0.0, pRoloc 1.6.2, pRolocdata 1.4.1, reshape2 1.4.1, zoo 1.7-12
- Loaded via a namespace (and not attached): BBmisc 1.9, BatchJobs 1.6, BiocInstaller 1.16.2, BradleyTerry2 1.0-6, DBI 0.3.1, FNN 1.1, MALDIquant 1.11, MASS 7.3-40, Matrix 1.1-5, RColorBrewer 1.1-2, RSQLite 1.0.0, SparseM 1.6, affy 1.44.0, affyio 1.34.0, base64enc 0.1-2, brew 1.0-6, brglm 0.5-9, car 2.0-25, caret 6.0-41, checkmate 1.5.2, class 7.3-12, codetools 0.2-11, colorspace 1.2-6, digest 0.6.8, doParallel 1.0.8, e1071 1.6-4, evaluate 0.5.5, fail 1.2, foreach 1.4.2, formatR 1.0, gdata 2.13.3, genefilter 1.48.1, gtable 0.1.2, gtools 3.4.1, highr 0.4, impute 1.40.0, iterators 1.0.7, kernlab 0.9-20, labeling 0.3, lattice 0.20-30, limma 3.22.7, lme4 1.1-7, lpSolve 5.6.10, mclust 4.4, mgcv 1.8-5, minqa 1.2.4, munsell 0.4.2, mvtnorm 1.0-2, mzID 1.4.1, nlme 3.1-120, nloptr 1.0.4, nnet 7.3-9, pbkrtest 0.4-2, pcaMethods 1.56.0, pls 2.4-3, plyr 1.8.1, preprocessCore 1.28.0, proto 0.3-10, proxy 0.4-14, quantreg 5.11, randomForest 4.6-10, rda 1.0.2-2, rpart 4.1-9, sampling 2.6, scales 0.2.4, sendmailR 1.2-1, sfsmisc 1.0-27, splines 3.1.3, stringr 0.6.2, survival 2.38-1, tools 3.1.3, vsn 3.34.0, xtable 1.7-4, zlibbioc 1.12.0

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