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Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Direct access to mass spectrometry data in R

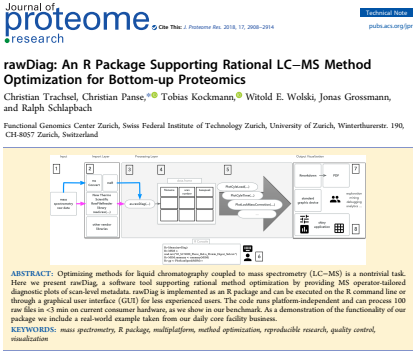
Christian Panse and Tobias Kockmann

European Biocondutor Meeting, de Duve Institute, UCLouvain, Campus de Woluwé Brussels, Belgium, Dez 2019

https://fgcz.ch/

Recently on the R for Mass Spectrometry community `-mtime -365`

- rawDiag: access Orbitrap data on the fast lane (European Bioconductor Meeting 2018)



Journal of proteome research

Cite This: J. Proteome Res. 2018, 17, 2908−2914

Technical Note

pubs.acs.org/jpr

rawDiag: An R Package Supporting Rational LC−MS Method Optimization for Bottom-up Proteomics

Christian Trachsel, Christian Panse,*[] Tobias Kockmann,[] Witold E. Wolski, Jonas Grossmann, and Ralph Schlapbach

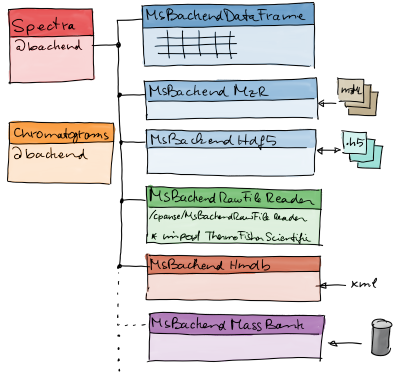
Functional Genomics Center Zurich, Swiss Federal Institute of Technology Zurich, University of Zurich, Winterthurerstr. 190, CH-8057 Zurich, Switzerland

ABSTRACT: Optimizing methods for liquid chromatography coupled to mass spectrometry (LC−MS) is a nontrivial task. Here we present rawDiag, a software tool supporting rational method optimization by providing MS operator-tailored diagnostic plots of scan-level metadata. rawDiag is implemented as an R package and can be executed on the R command line or through a graphical user interface (GUI) for less experienced users. The code runs platform-independent and can process 100 raw files in <3 min on current consumer hardware, as we show in our benchmark. As a demonstration of the functionality of our package we include a real-world example taken from our daily core facility business.

KEYWORDS: *mass spectrometry, R package, multiplatform, method optimization, reproducible research, quality control, visualization*

Recently on the R for Mass Spectrometry community `-mtime -365`

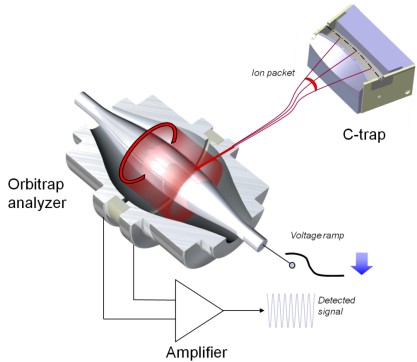
- rawDiag: access Orbitrap data on the fast lane (European Bioconductor Meeting 2018)
- rformassspectrometry.org



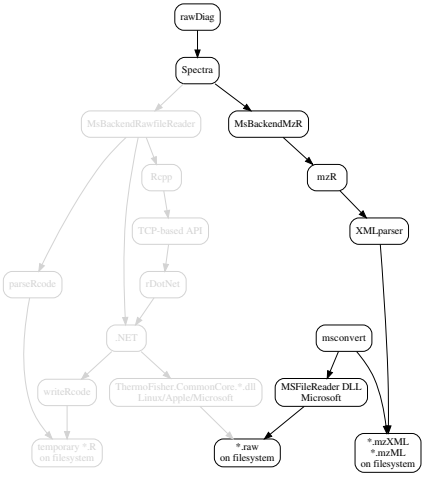
source: `https://github.com/jorainer/swemsa_2019`

Recently on the R for Mass Spectrometry community `-mtime -365`

- rawDiag: access Orbitrap data on the fast lane (European Bioconductor Meeting 2018)
- rformassspectrometry.org
- Why? ≈80% of todays mass spectrometer used in proteomics are Orbitraps (Thermo Fisher Scientific).

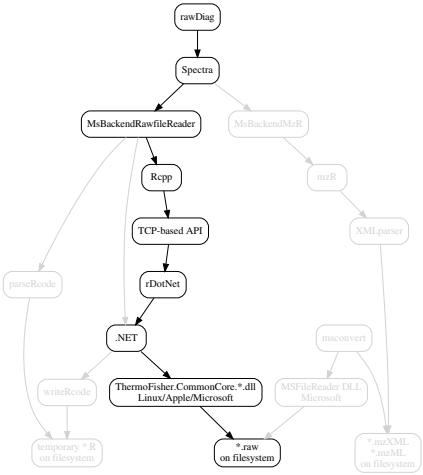


source: `https://en.wikipedia.org/wiki/Orbitrap`



- ☺ HUPO PSI
- ☹ conversion triggered by an external tool
- ☹ `ReAdW.exe` / `msconvert` work on Microsoft only
- ☹ mzXML file is not always mzXML - different converter; different options
- ☹ mzXML does not contain all the data provided by the instrument. (see poster)

MsBackendRawfileReader



Usage - Spectra::backendInitialize

```
R> library(MsBackendRawFileReader)
R> be <- backendInitialize( MsBackendRawFileReader(), files = rawfile)
```

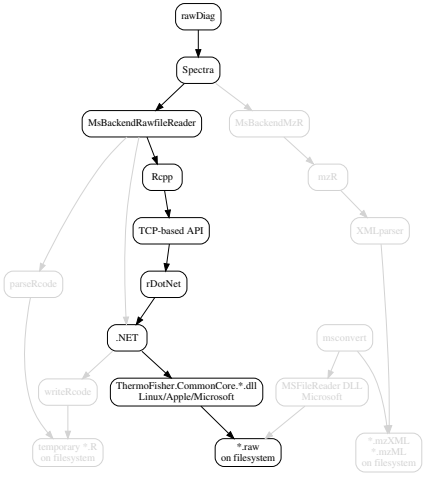
Usage - Spectra::backendInitialize

```
R> library(MsBackendRawFileReader)
R> be <- backendInitialize( MsBackendRawFileReader(), files = rawfile)

R> (s <- Spectra(be))

MSn data (Spectra) with 8742 spectra in a MsBackendRawFileReader backend:
filename:         /Users/cp/Library/Caches/ExperimentHub/24ab678291f6_3238.r
creation date:         7/16/2019 5:56:24 PM
first scan:         1
last scan:         8742
model:         Orbitrap Fusion Lumos
name:         Orbitrap Fusion Lumos
SerialNumber:         FSN20583

Processing:
```

Usage - R package `rawDiag` – now running on Spectra objects.

Fast and colorful diagnostic plot functions – designed by, and for, mass spectrometer experts

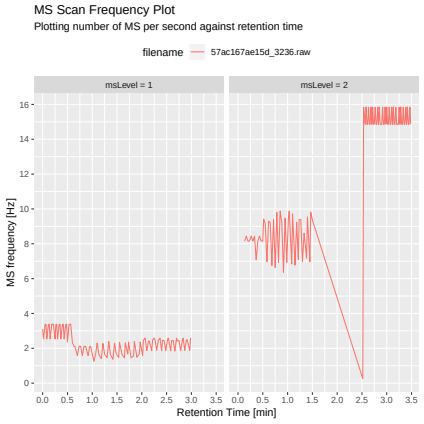
```
R> class(s)

[1] "Spectra"
attr(,"package")
[1] "Spectra"
```



Usage - R package rawDiag – now running on Spectra objects.

Fast and colorful diagnostic plot functions – designed by, and for, mass spectrometer experts

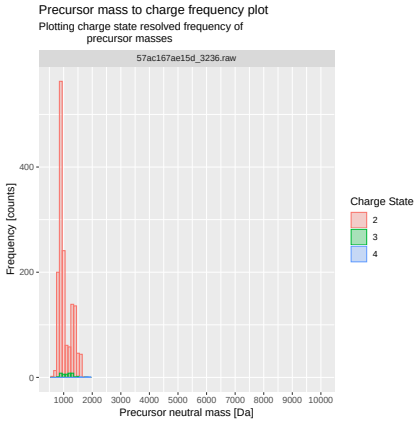
```
R> class(s)

[1] "Spectra"
attr(,"package")
[1] "Spectra"

R> library(rawDiag)
R> Plot(s, FUN='ScanFrequency')
```



Usage - R package rawDiag – now running on Spectra objects.

Fast and colorful diagnostic plot functions – designed by, and for, mass spectrometer experts

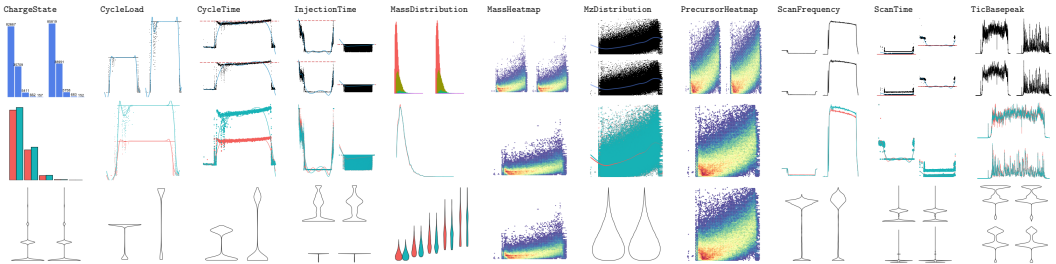
```
R> class(s)

[1] "Spectra"
attr(,"package")
[1] "Spectra"

R> library(rawDiag)
R> Plot(s, FUN='ScanFrequency')
R> Plot(s, FUN='MassDistribution')
...
```



Usage - R package `rawDiag` – now running on Spectra objects.

Fast and colorful diagnostic plot functions – designed by, and for, mass spectrometer experts

cheatsheet of plot methods:



PMID: 29978702 DOI: 10.1021/acs.jproteome.8b00173

C# listings – accessing .NET vendor libraries

https://github.com/cpanse/MsBackendRawFileReader/blob/Bioc3.10/src/MsBackendRawFileReader.cs

```
12 using System;
13 using System.Collections.Generic;
14 using System.Diagnostics;
15 using System.IO;
16 using System.Runtime.ExceptionServices;
17 using System.Collections;
18 using System.Linq;
19 using ThermoFisher.CommonCore.Data;
20 using ThermoFisher.CommonCore.Data.Business;
21 using ThermoFisher.CommonCore.Data.FilterEnums;
22 using ThermoFisher.CommonCore.Data.Interfaces;
23 using ThermoFisher.CommonCore.MassPrecisionEstimator;
24 using ThermoFisher.CommonCore.RawFileReader;
```

C# listings – accessing .NET vendor libraries

https://github.com/cpanse/MsBackendRawFileReader/blob/Bioc3.10/src/MsBackendRawFileReader.cs

```
27 namespace MsBackendRawFileReader
28 {
```

C# listings – accessing .NET vendor libraries

https://github.com/cpanse/MsBackendRawFileReader/blob/Bioc3.10/src/MsBackendRawFileReader.cs

```
27  namespace MsBackendRawFileReader
28  {

46      public class Rawfile
47      {
```

C# listings – accessing .NET vendor libraries

https://github.com/cpanse/MsBackendRawFileReader/blob/Bioc3.10/src/MsBackendRawFileReader.cs

```
27  namespace MsBackendRawFileReader
28  {

46      public class Rawfile
47      {

53          // ThermoRawFileParser accessing the RAW file
54          private IRawDataPlus rawFile;
55
56          public Rawfile(string rawfile)
57          {
```

C# listings – accessing .NET vendor libraries

https://github.com/cpanse/MsBackendRawFileReader/blob/Bioc3.10/src/MsBackendRawFileReader.cs

```
27  namespace MsBackendRawFileReader
28  {

46      public class Rawfile
47      {

53          // ThermoRawFileParser accessing the RAW file
54          private IRawDataPlus rawFile;
55
56          public Rawfile(string rawfile)
57          {

620         public double[] GetSpectrumMasses(int scanNumber)
621     {
622       var scanStatistics = rawFile.GetScanStatsForScanNumber(scanNumber);
623       var centroidStream = rawFile.GetCentroidStream(scanNumber, false);
624
625       if (scanStatistics.IsCentroidScan && centroidStream.Length > 0)
626       {
627         return centroidStream.Masses.ToArray();
628       }
629       else
630       {
631         var segmentedScan = rawFile.GetSegmentedScanFromScanNumber(scanNumber, scanStatistics);
632         return segmentedScan.Positions.ToArray();
633       }
634     }
```

Calling the C# methods from R – through using rDotNet

. . . running somewhere in the MsBackend

```
R> ##  https://CRAN.R-project.org/package=rDotNet
R> ## create an object and call a method
R> (x <- .cnew ("Rawfile", rawfile))

<dotnet obj: 459, class: MsBackendRawFileReader.Rawfile, value: "MsBackendR
```

Calling the C# methods from R – through using rDotNet

. . . running somewhere in the MsBackend

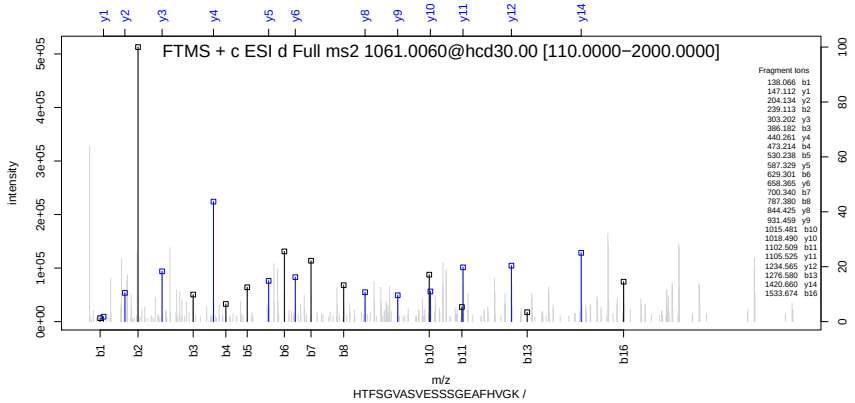
```
R> ##  https://CRAN.R-project.org/package=rDotNet
R> ## create an object and call a method
R> (x <- .cnew ("Rawfile", rawfile))

<dotnet obj: 459, class: MsBackendRawFileReader.Rawfile, value: "MsBackendR

R> ## getting a vector of mass values
R> head(mZ <- x$GetSpectrumMasses(4034))

[1] 110.0709 111.0744 114.2004 120.0805 129.1020 138.0659
```

Calling the C# methods from R – through using rDotNet

. . . running somewhere in the MsBackend

```
R> ##  https://CRAN.R-project.org/package=rDotNet
R> ## create an object and call a method
R> (x <- .cnew ("Rawfile", rawfile))

<dotnet obj: 459, class: MsBackendRawFileReader.Rawfile, value: "MsBackendR

R> ## getting a vector of mass values
R> head(mZ <- x$GetSpectrumMasses(4034))

[1] 110.0709 111.0744 114.2004 120.0805 129.1020 138.0659

R> ## getting a vector of intensities
R> head(intensity <- x$GetSpectrumIntensities(4034))

[1] 328132.188  11543.382   4682.475  20583.205   7574.656
[6]   6460.671
```

```
R> # https://CRAN.R-project.org/package=protViz
R> pp <- protViz::peakplot("HTFSGVASVESSSGEAFHVGK",
+     list(mZ = mZ, intensity = intensity),
+     FUN=function(b,y){cbind(b,y)}, itol=0.01, col='lightgrey')
R> legend("top", "", title=x$GetScanFilter(4034), cex=1.5, bty = "n")
```



Extracted Ion Chromatogram (XIC)

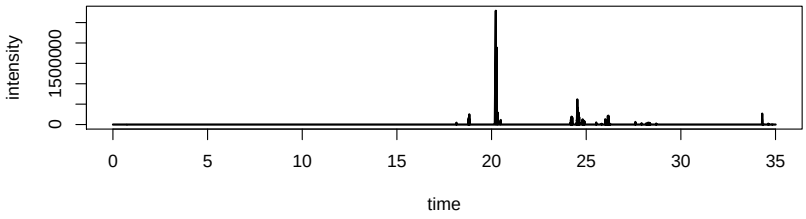
```
R> (mZ <- (protViz::parentIonMass("HTFSGVASVESSSGEAFHVGK") + 1.008) / 2)
[1] 1060.506
```

Extracted Ion Chromatogram (XIC)

```
R> (mZ <- (protViz::parentIonMass("HTFSGVASVESSSGEAFHVGK") + 1.008) / 2)
[1] 1060.506
R> X <- x$GetXIC(mZ, tol=5, filter="ms")
```

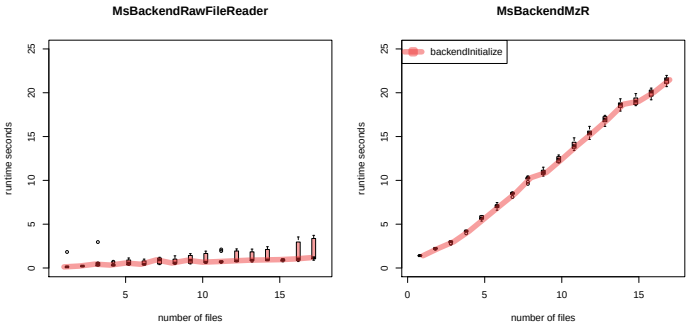
Extracted Ion Chromatogram (XIC)

```
R> (mZ <- (protViz::parentIonMass("HTFSGVASVESSSGEAFHVGK") + 1.008) / 2)
[1] 1060.506
R> X <- x$GetXIC(mZ, tol=5, filter="ms")
R> idx <- seq(1, length(X), by=2)
R> plot(X[idx], X[-idx],
+     lwd=2, type='l', xlab='time', ylab='intensity')
```



Benchmark

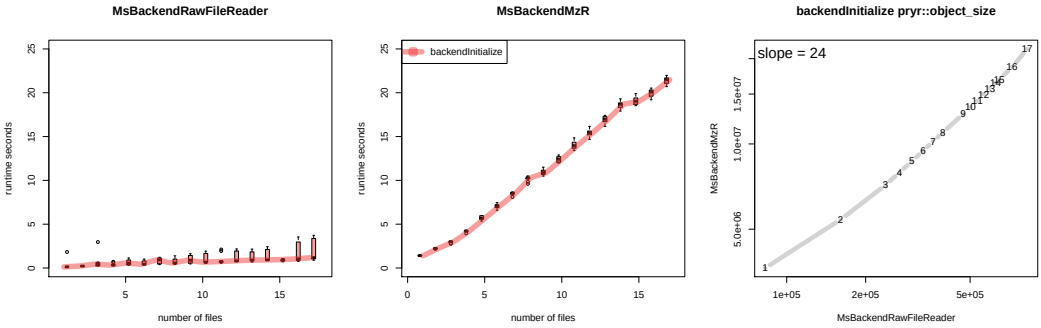
calling `backendInitialize`



single core; 17 mzXML files size 5.4GB; 17 raw files size 2.5GB

Benchmark

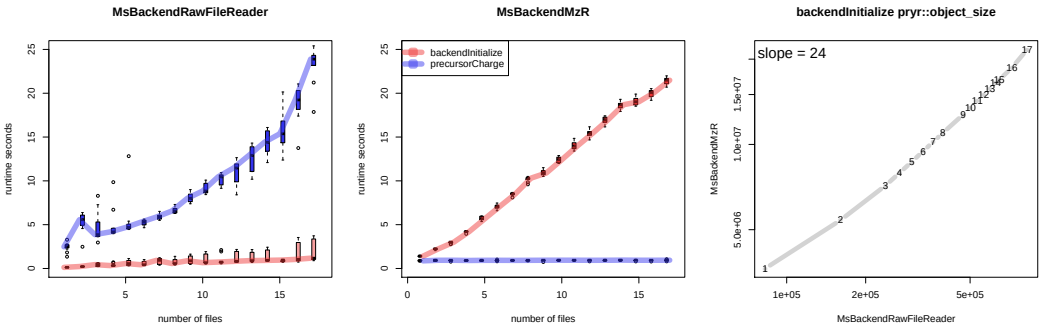
backendInitialize and estimate of the memory



single core; 17 mzXML files size 5.4GB; 17 raw files size 2.5GB

Benchmark

. . . calling `Spectra::precursorCharge`



single core; 17 mzXML files size 5.4GB; 17 raw files size 2.5GB

TODO

- synchronize with community development (`Spectra` and `Chromatograms`).

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when submitting the `MsBackendRawFileReader` to Bioconductor

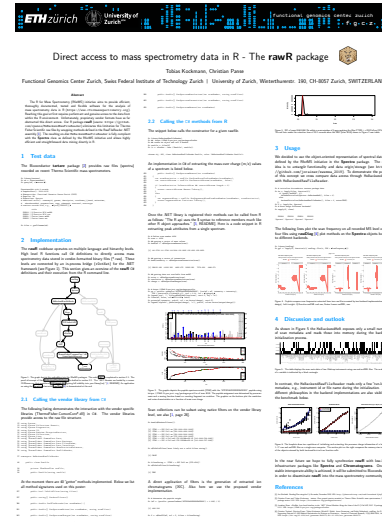
- How do we handle policy with vendor libraries?
- How do we handle the `rDotNet` webservice in the build system?

```
/usr/bin/mono \
  --nollvm /home/cp/R/x86_64-pc-linux-gnu-library/3.6/rDotNet/server/bin/Debug/CLRServer.exe \
  -url svc://localhost:56789/ \
  -dll /home/cp/R/x86_64-pc-linux-gnu-library/3.6/MsBackendRawFileReader/exec/MsBackendRawFileReader.dll
```

Thank you

Lori Shepherd; Jonathan Shore (rDotNet); Matthew E Monroe (pnnl.gov); Ralph Schlapbach (FGCZ); Antje Dittmann; Lilly van de Venn; Jonas Grossmann; Lucas Kook; Witold E. Wolski; ...

RforMassSpectrometry; Bioconductor; CRAN;



Bookmarks

- http://planetorbitrap.com/rawfilereader#.WjkqIUtJmL4
- https://CRAN.R-project.org/package=rDotNet
- https://github.com/rformassspectrometry/
- https://github.com/cpanse/MsBackendRawFileReader
- https://bioconductor.org/packages/tartare/

Session information

- R version 3.6.1 (2019-07-05), `x86_64-apple-darwin15.6.0`
- Locale: `en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8`
- Running under: `macOS Catalina 10.15.1`
- Matrix products: default
- BLAS: `/Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.dylib`
- LAPACK: `/Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRlapack.dylib`
- Base packages: base, datasets, graphics, grDevices, methods, parallel, stats, stats4, utils
- Other packages: BiocGenerics 0.32.0, BiocParallel 1.19.0, IRanges 2.19.10, MsBackendRawFileReader 0.0.1, ProtGenerics 1.17.4, protViz 0.5.1, rDotNet 0.9.1, S4Vectors 0.24.0, Spectra 0.3.0
- Loaded via a namespace (and not attached): codetools 0.2-16, compiler 3.6.1, magrittr 1.5, MsCoreUtils 0.0.2, R6 2.4.1, Rcpp 1.0.3, rlang 0.4.2, testthat 2.1.1, tools 3.6.1