

Intro to RaMS

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Welcome to RaMS! This vignette is designed to provide examples using the package at various levels of complexity. Let's jump right in.

If you have your own data, feel free to load it here. If not, there's a couple small example files you're welcome to use in the "extdata" folder. I'll be using these throughout.

```
library(RaMS)
library(data.table)

# Locate the file directory
msdata_dir <- system.file("extdata", package = "RaMS")

# Identify the files of interest
data_files <- list.files(msdata_dir, pattern = "HL.*mzML", full.names = TRUE)

# Check that the files identified are the ones expected
basename(data_files)
#> [1] "LB12HL_AB.mzML.gz" "LB12HL_CD.mzML.gz" "LB12HL_EF.mzML.gz"
```

There's only one function to worry about in RaMS: the aptly named `grabMSdata`. This function has a couple arguments with sensible defaults, but you'll always need to tell it two things: one, which files you'd like to process; and two, the data you'd like to obtain from those files.

Let's start simple, with a single file and the most basic information about it.

Basic RaMS usage

TICs, BPCs, and metadata

A TIC reports the total intensity measured by the mass analyzer during each scan, so the data is parsed into two columns: retention time (rt) and intensity (int). This makes it easy to read and simple to plot:

```
single_file <- data_files[1]

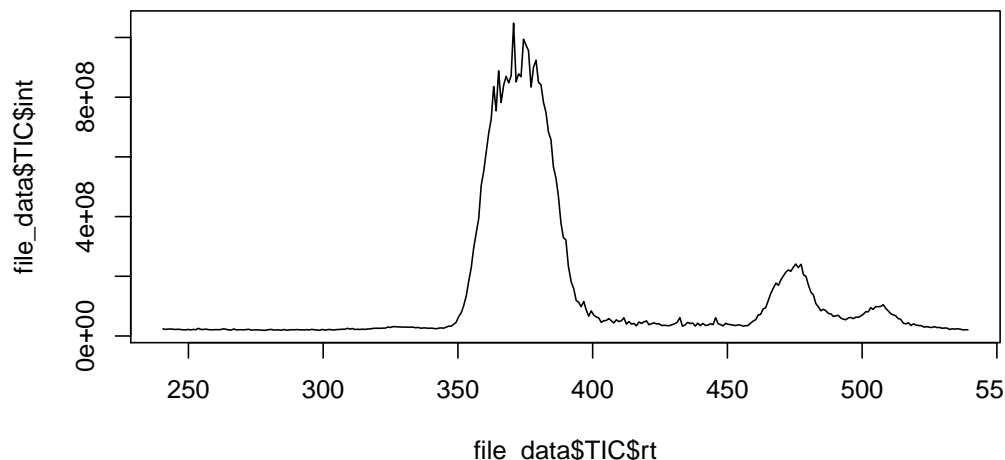
file_data <- grabMSdata(single_file, grab_what = "TIC")

knitr::kable(head(file_data$TIC, 3))
```

rt	int	filename
240.540	23813853	LB12HL_AB.mzML.gz
241.472	22097972	LB12HL_AB.mzML.gz
242.408	23141649	LB12HL_AB.mzML.gz

Since we asked for a single thing, the TIC, our `file_data` object is a list with a single entry: the TIC. Let's plot that data:

```
par(mar=c(4.1, 4.1, 0.1, 0.1))
plot(file_data$TIC$rt, file_data$TIC$int, type = "l")
```



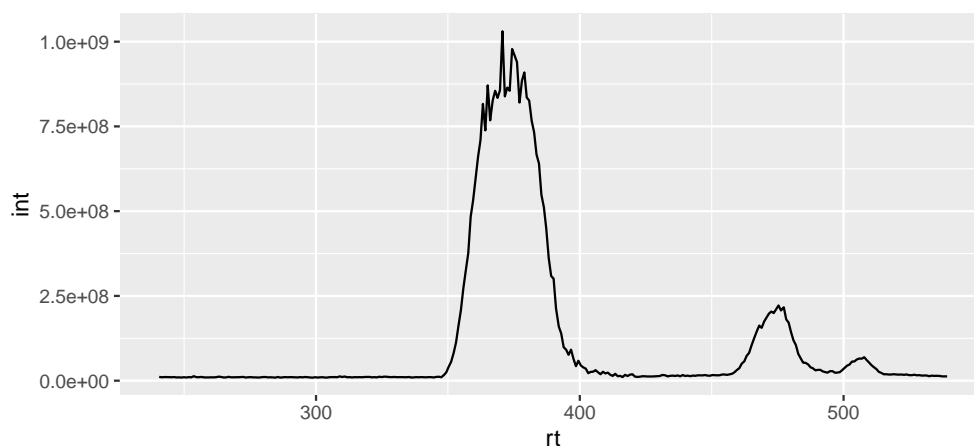
Simple enough!

A BPC is just like a TIC except that it records the *maximum* intensity measured, rather than the sum of all intensities. This data is also collected by the mass analyzer and doesn't need to be calculated.

```
file_data <- grabMSdata(single_file, grab_what = "BPC")
```

Since the data is parsed in a “tidy” format, it plays nicely with popular packages such as `ggplot2`. Let's use that to plot our BPC instead of the base R plotting system:

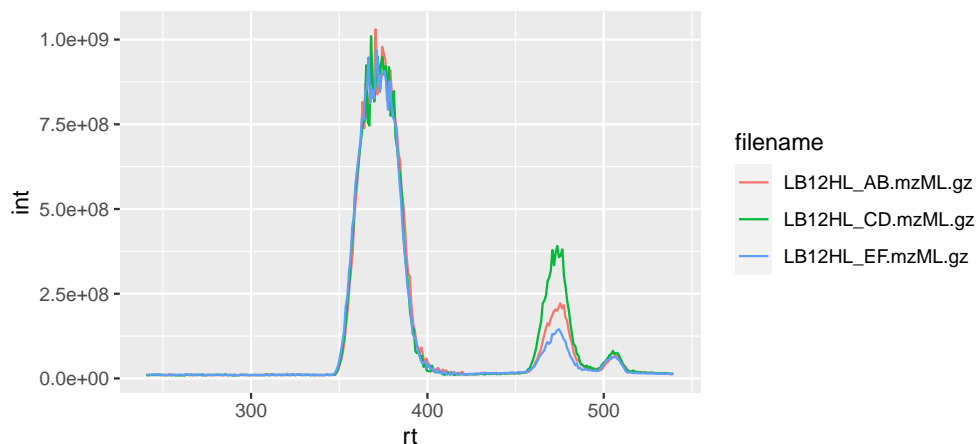
```
suppressPackageStartupMessages(library(tidyverse))
ggplot(file_data$BPC) + geom_line(aes(x=rt, y=int))
```



The advantages of tidy data and `ggplot` become clear when we load more than one file at a time because we can group and color by the third column, the name of the file from which the data was read. Here I've also enabled a progress bar with the argument `verbosity="minimal"` because it's nice to see progress when multiple files are being read.

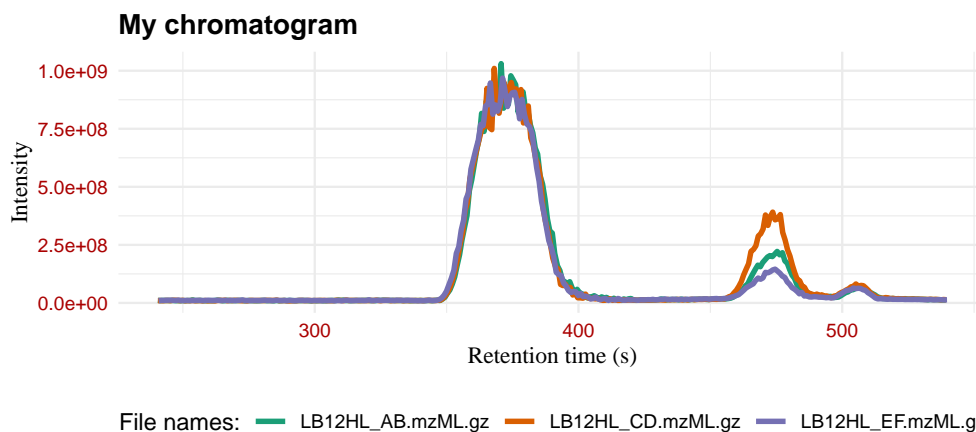
```
file_data <- grabMSdata(data_files, grab_what = "BPC", verbosity = "minimal")
#> /
#> Total time: 0.25 s

ggplot(file_data$BPC) + geom_line(aes(x=rt, y=int, color=filename))
```



And of course, this means that all of `ggplot`'s aesthetic power can be brought to your chromatograms as well, so customize away!

```
ggplot(file_data$BPC) +
  geom_line(aes(x=rt, y=int, color=filename), lwd=1.2) +
  theme_minimal() +
  theme(legend.position = "bottom",
        axis.text = element_text(color = "#AA0000"),
        axis.title = element_text(family = "serif"),
        plot.title = element_text(face = "bold")) +
  scale_colour_brewer(palette = "Dark2") +
  labs(x="Retention time (s)", y="Intensity",
        title = "My chromatogram", color="File names:")
```



RaMS also provides some basic file metadata extraction capability, although the focus for this package is on the actual data and other MS packages handle file metadata much more elegantly. This is one area where

there are major differences between mzML and mzXML file types - the mzXML file type simply doesn't encode as much metadata as the mzML filetype, so RaMS can't extract it.

```
# Since the minification process strips some metadata, I use the
# less-minified DDA files here
metadata_files <- list.files(msdata_dir, pattern = "DDA", full.names = TRUE)
grabMSdata(metadata_files, grab_what = "metadata")
#> $metadata
#>
#> 1: 170223_Poo_AllCyanoAqExtracts_DDAPos_2.raw
#> 2: 170223_Poo_AllCyanoAqExtracts_DDAPos_2.raw
#>
#> 1:
#> 2: Thermo Scientific,Q Exactive,electrospray ionization,quadrupole,inductive detector
#>
#> 1: <data.frame[1x3]>
#> 2:
```

Adding a column: MS1 data

MS1 data can be extracted just as easily, by supplying “MS1” to the `grab_what` argument of `grabMSdata` function.

```
file_data <- grabMSdata(data_files, grab_what = "MS1")
knitr::kable(head(file_data$MS1, 3))
```

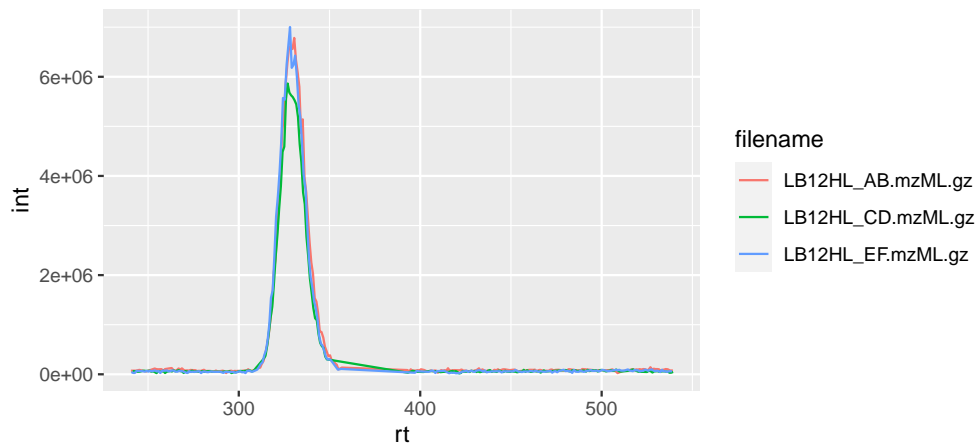
rt	mz	int	filename
240.54	104.0710	1297755.00	LB12HL_AB.mzML.gz
240.54	104.1075	140668.12	LB12HL_AB.mzML.gz
240.54	112.0509	67452.86	LB12HL_AB.mzML.gz

So we've now got the *mz* column, corresponding to the mass-to-charge ratio (m/z) of an ion. This means that we can now filter our data for specific masses and separate out molecules with different masses.

Note that this also makes the data much larger in R's memory - so don't go loading hundreds of files simultaneously. If that's necessary, check out the section below on saving space.

Because RaMS returns `data.tables` rather than normal `data.frames`, indexing is super-fast and a bit more intuitive than with base R. Below, I also use the `pmppm` function from RaMS to produce a mass range from an initial mass and spectrometer accuracy (here, 5 parts-per-million).

```
adenine_mz <- 136.06232
adenine_data <- file_data$MS1[mz%between%pmppm(adenine_mz, ppm=5)]
ggplot(adenine_data) + geom_line(aes(x=rt, y=int, color=filename))
```

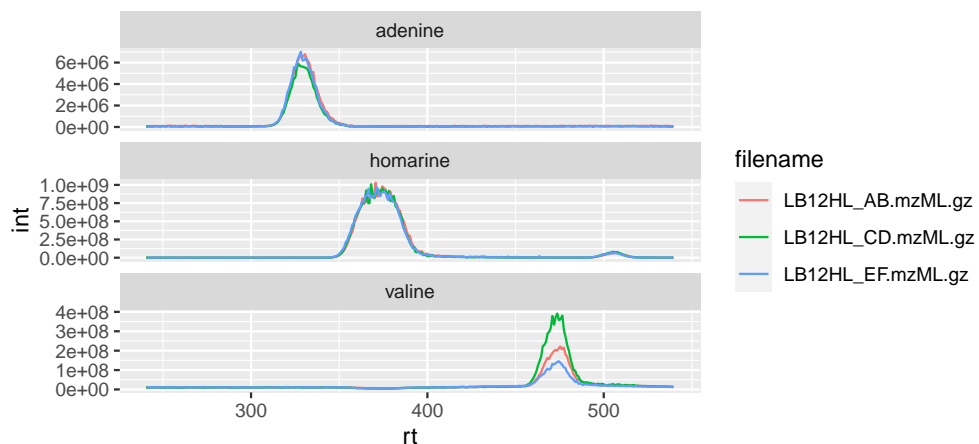


This makes it easy to grab the data for multiple compounds of interest with a simple loop, provided here by the `purrr` package of the tidyverse:

```
mzs_of_interest <- c(adenine=136.06232, valine=118.0865, homarine=138.055503)

mass_data <- imap(mzs_of_interest, function(mz_i, name){
  cbind(file_data$MS1[mz%between%ppm(mz_i, ppm=10)], name)
}) %>% rbindlist()

ggplot(mass_data) +
  geom_line(aes(x=rt, y=int, color=filename)) +
  facet_wrap(~name, ncol = 1, scales = "free_y")
```



Moving along: MS2 data

RaMS also handles MS2 data elegantly. Request it with the “MS2” option for `grab_what`, although it’s often a good idea to grab the MS1 data alongside.

```
DDA_file <- list.files(msdata_dir, pattern = "DDA.*mzML", full.names = TRUE)
DDA_data <- grabMSdata(DDA_file, grab_what = c("MS2"))
knitr::kable(head(DDA_data$MS2, 3))
```

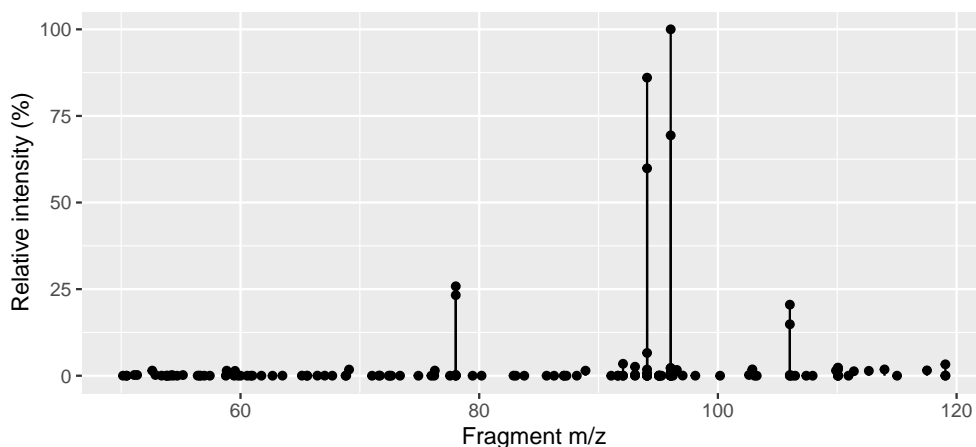
rt	premz	fragmz	int	voltage	filename
240.184	127.0325	56.05023	17019.613	35	DDApos_2.mzML.gz
240.184	127.0325	59.46344	1003.812	35	DDApos_2.mzML.gz
240.184	127.0325	70.06580	1521.526	35	DDApos_2.mzML.gz

DDA data can be plotted nicely with `ggplot2` as well. Typically it makes sense to filter for a precursor mass, then render the fragments obtained.

```
homarine_mz <- 137.047678+1.007276

homarine_MS2 <- DDA_data$MS2[premz%between%pmppm(homarine_mz, 5)]
homarine_MS2$int <- homarine_MS2$int/max(homarine_MS2$int)*100

ggplot(homarine_MS2) +
  geom_point(aes(x=fragmz, y=int)) +
  geom_segment(aes(x=fragmz, xend=fragmz, y=int, yend=0)) +
  labs(x="Fragment m/z", y="Relative intensity (%)")
```



This is also the perfect place to enable some interactivity with packages such as `plotly`, making data exploration not only simple but also enjoyable.

```
## Not run to save space in the vignette:
library(plotly)
data_files <- list.files(msdata_dir, pattern = "mzML", full.names = TRUE)
file_data <- grabMSdata(data_files, grab_what = c("MS1", "MS2", "BPC"))

clean_MS2 <- file_data$MS2 %>%
  filter(premz%between%pmppm(homarine_mz, 10)) %>%
  group_by(rt) %>%
  arrange(desc(int)) %>%
  summarise(fragmz=paste(
    paste(round(fragmz, digits = 3), round(int), sep = ": "), collapse = "\n"),
    .groups="drop"
  )
file_data$MS1 %>%
  filter(mz%between%pmppm(homarine_mz, 10)) %>%
```

```

filter(!str_detect(filename, "DDA")) %>%
plot_ly() %>%
add_trace(type="scatter", mode="lines", x=~rt, y=~int, color=~filename,
          hoverinfo="none") %>%
add_trace(type="scatter", mode="markers", x=~rt, y=0,
          text=~frags, hoverinfo="text", showlegend=FALSE,
          marker=list(color="black"), data = clean_MS2) %>%
layout(annotations=list(x=min(clean_MS2$rt), y=0,
                          text="Mouse over to see\nMSMS fragments"))

```

Easy access to MS2 data also allows us to rapidly perform simple operations such as searching for a specific fragment mass. For example, if we know that homarine typically produces a fragment with a mass of 94.06567, we simply subset the MS2 data for fragments in a range around that mass:

```

homarine_frag_mz <- 94.06567
DDA_data$MS2[fragmz%between%pmppm(homarine_frag_mz, ppm = 5)] %>%
  head() %>% knitr::kable()

```

rt	premz	fragmz	int	voltage	filename
240.759	111.09200	94.06557	4905.352	35	DDApos_2.mzML.gz
242.776	94.04554	94.06561	488163.750	35	DDApos_2.mzML.gz
251.146	152.94838	94.06570	1254.113	35	DDApos_2.mzML.gz
252.136	138.05498	94.06548	2639.737	35	DDApos_2.mzML.gz
252.477	94.06559	94.06562	431732.594	35	DDApos_2.mzML.gz
257.887	112.08720	94.06561	1621.821	35	DDApos_2.mzML.gz

We find that there's not only homarine that produces that fragment, but several other compounds. Precursor masses like this can then be searched manually in online databases or, since the data is already in R, passed to a script that automatically searches them.

Similarly, we can easily search instead for neutral losses with this method. If we suspect other molecules are producing a similar neutral loss as homarine:

```

homarine_neutral_loss <- homarine_mz - homarine_frag_mz

file_data$MS2 <- mutate(DDA_data$MS2, neutral_loss=premz-fragmz) %>%
  select("rt", "premz", "fragmz", "neutral_loss", "int", "voltage", "filename")
file_data$MS2[neutral_loss%between%pmppm(homarine_neutral_loss, ppm = 5)] %>%
  arrange(desc(int)) %>% head() %>% knitr::kable()

```

rt	premz	fragmz	neutral_loss	int	voltage	filename
363.822	138.0549	94.06567	43.98918	15944193.0	35	DDApos_2.mzML.gz
383.996	138.0549	94.06569	43.98921	11094394.0	35	DDApos_2.mzML.gz
506.290	138.0549	94.06567	43.98921	1218680.0	35	DDApos_2.mzML.gz
404.379	138.0550	94.06568	43.98931	334129.3	35	DDApos_2.mzML.gz
365.863	137.0470	93.05766	43.98934	161222.3	35	DDApos_2.mzML.gz
424.803	138.0550	94.06566	43.98932	126193.9	35	DDApos_2.mzML.gz

We can again confirm our suspicions that there's another signal with a similar neutral loss: one with a mass

of 137.0470.

Advanced RaMS usage

Saving space: EICs and rtrange

Mass-spec files are typically tens or hundreds of megabytes in size, which means that the simultaneous analysis of many files can easily exceed a computer's memory. Since RaMS stores all data in R's working memory, this can become a problem for large analyses.

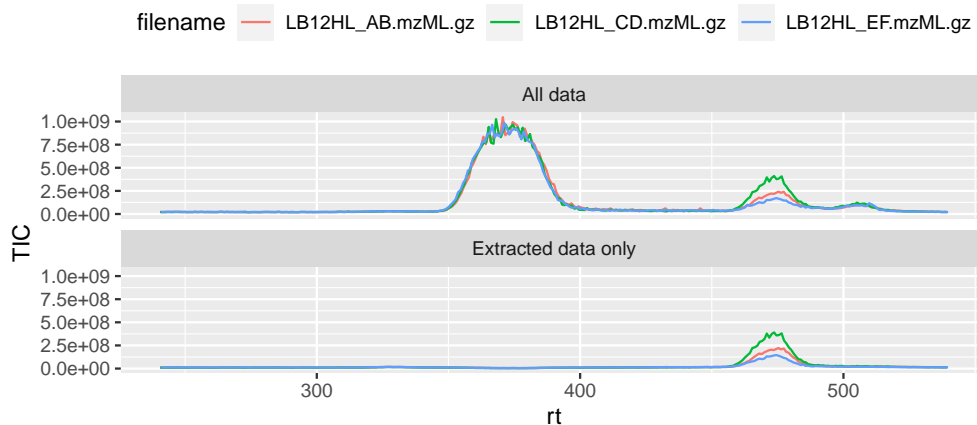
However, much of the usage envisioned for RaMS on this scale doesn't require access to the entire file, the entire time. Instead, users are typically interested in a few masses of interest or a specific time window. This means that while each file still needs to be read into R in full to find the data of interest, extraneous data can be discarded before the next file is loaded.

This functionality can be enabled by passing "EIC" and/or "EIC_MS2" to the `grab_what` argument of `grabMSdata`, along with a vector of masses to extract (`mz`) and the instrument's ppm accuracy. When this is enabled, files are read into R's memory sequentially, the mass window is extracted, and the rest of the data is discarded.

```
data_files <- list.files(msdata_dir, pattern = "mzML", full.names = TRUE)
all_data <- grabMSdata(data_files, grab_what = c("MS1", "MS2"))

mzs_of_interest <- c(adenine=136.06232, valine=118.0865)
small_data <- grabMSdata(data_files, grab_what = c("EIC", "EIC_MS2"),
                        mz=mzs_of_interest, ppm = 5)

all_data$MS1 %>%
  mutate(type="All data") %>%
  rbind(small_data$EIC %>% mutate(type="Extracted data only")) %>%
  filter(!str_detect(filename, "DDA")) %>%
  group_by(rt, filename, type) %>%
  summarise(TIC=sum(int), .groups="drop") %>%
  ggplot() +
  geom_line(aes(x=rt, y=TIC, color=filename)) +
  facet_wrap(~type, ncol = 1) +
  theme(legend.position = "top")
```



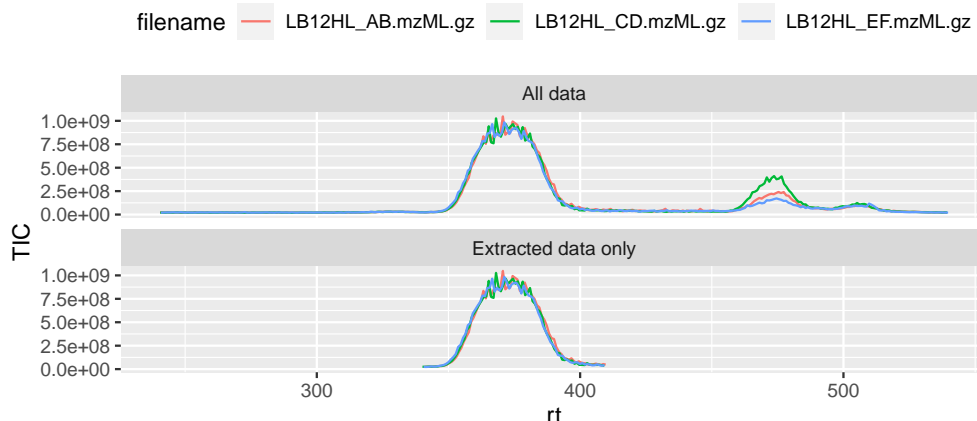

```
# Size reduction factor:
as.numeric(object.size(all_data)/object.size(small_data))
#> [1] 50.13485
```

As expected, the size of the `small_data` object is much smaller than the `all_data` object, here by a factor of nearly 50x. For files that haven't already been "minified", that size reduction will be even more significant. Of course, this comes with the cost of needing to re-load the data a second time if a new mass feature becomes of interest but this shrinkage is especially valuable for targeted analyses where the analytes of interest are known in advance.

A second way of reducing file size is to constrain the retention time dimension rather than the m/z dimension. This can be done with the `rtrange` argument, which expects a length-two vector corresponding to the upper and lower bounds on retention time. This is useful when a small time window is of interest, and only the data between those bounds is relevant.

```
small_data <- grabMSdata(data_files, grab_what = c("MS1", "MS2"), rtrange = c(340, 410))

all_data$MS1 %>%
  mutate(type="All data") %>%
  rbind(small_data$MS1 %>% mutate(type="Extracted data only")) %>%
  filter(!str_detect(filename, "DDA")) %>%
  group_by(rt, filename, type) %>%
  summarise(TIC=sum(int), .groups="drop") %>%
  ggplot() +
  geom_line(aes(x=rt, y=TIC, color=filename)) +
  facet_wrap(~type, ncol = 1) +
  theme(legend.position = "top")
```



```
# Size reduction factor:
as.numeric(object.size(all_data)/object.size(small_data))
#> [1] 5.409828
```

The savings are more modest here, but constraining the retention time actually speeds up data retrieval slightly. Since m/z and intensity data is encoded in `mzML` and `mzXML` files while retention time information is not, eliminating scans with a retention time window removes the need to decode the intensity and m/z information in those scans.

However, decoding is rarely the rate-limiting step and for more information about speeding things up, continue to the next section.

Speeding things up

So, **RaMS** isn't fast enough for you? Let's see what we can do to improve that. The first step in speeding things up is discovering what's slow. Typically, this is the process of reading in the **mzML**/**mzXML** file rather than any processing that occurs afterward, but this is not always the case. To examine bottlenecks, **RaMS** includes timing information that's produced if the **verbosity** argument is set to "very".

```
all_data <- grabMSdata(data_files, grab_what = c("MS1", "MS2"), verbosity = "very")
#> |
#> Reading file DDAPos_2.mzML.gz... 0.55054 s
#> Reading MS1 data...0.21543 s
#> Reading MS2 data...0.499007 s
#> |
#> Reading file LB12HL_AB.mzML.gz... 0.05285692 s
#> Reading MS1 data...0.05585098 s
#> Reading MS2 data...0.009979963 s
#> |
#> Reading file LB12HL_CD.mzML.gz... 0.04886603 s
#> Reading MS1 data...0.05188608 s
#> Reading MS2 data...0.008976936 s
#> |
#> Reading file LB12HL_EF.mzML.gz... 0.05287004 s
#> Reading MS1 data...0.06483388 s
#> Reading MS2 data...0.007972956 s
#> |
#> Total time: 1.62 s
```

These minified demo files are pretty quick to load, but it looks like reading the file in takes about as long as extracting **MS1** information, with **MS2** extraction taking a fraction of that time.

In general, slow file read times can be improved by compressing the data. **mzML** files are highly compressible, with options to compress both the data itself using the **zlib** method and the files as a whole using **gzip**.

gzip is the simplest one to use, as a plethora of methods exist to compress files this way, including online sites. **RaMS** can read the data directly from a gzipped file, no decompression necessary, so this is an easy way to reduce file size and read times. Sharp-eyed users will have noticed that the demo files are already gzipped, which is part of the reason they are so small.

zlib compression is slightly trickier, and is most often performed with tools such as Proteowizard's **msconvert** tool with the option "-z".

Read times may also be slow if files are being accessed via the Internet, either through a VPN or network drive. If your files are stored elsewhere, consider first moving those files somewhere more local before reading data from them.

If the bottleneck appears when reading **MS1** data, consider restricting the retention time range with the **rtrange** argument or using more detailed profiling tools such as RStudio's "Profile" options or the **profvis** package. Pull requests that improve data processing speed are always welcome!

While the package is not currently set up for parallel processing, this is a potential future feature if a strong need is demonstrated.

Finer control: grabMzmlData, grabMzxmlData

While there's only one main function (`grabMSdata`) in `RaMS`, you may have noticed that two other functions have been exposed that perform similar tasks: `grabMzmlData` and `grabMzxmlData`. The main function `grabMSdata` serves as a wrapper around these two functions, which detects the file type, adds the "filename" column to the data, and loops over multiple files if provided. However, there's often reason to use these internal functions separately.

For one, the objects themselves are smaller because they don't have the filename column attached yet. You as the user will need to keep track of which data belongs to which files in this case.

Another use case might be applying functions to each file individually, perhaps aligning to a reference chromatogram or identifying peaks. Rather than spending the time to bind the files together and immediately separate them again, these functions have been exposed to skip that step.

Finally, these functions are useful for parallelization. Because iterating over each mass-spec file is often the largest reasonable chunk, these functions can be passed directly to parallel processes like `mclapply` or `doParallel`. However, parallelization is a beast best handled by individual users because its actual implementation often differs wildly and its utility depends strongly on individual setups (remember that parallelization won't help with slow I/O times, so it may not always improve data processing speed.)

```
## Not run:
data_files <- list.files(msdata_dir, pattern = "mzML", full.names = TRUE)

library(parallel)
output_data <- mclapply(data_files, grabMzmlData, mc.cores = detectCores()-1)

library(foreach)
library(doParallel)
registerDoParallel(numCores)
output_data <- foreach (i=data_files) %dopar% {
  grabMzmlData(i)
}
stopImplicitCluster()
```

The nitty-gritty details

`RaMS` is possible because `mzML` and `mzXML` documents are fundamentally XML-based. This means that we can leverage speedy and robust XML parsing packages such as `xml2` to extract the data. Fundamentally, `RaMS` relies on XPath navigation to collect various bits of mass-spec data, and the format of `mzML` and `mzXML` files provides the tags necessary. That means a lot of `RaMS` code consists of lines like the following:

```
## Not run:
data_nodes <- xml2::xml_find_all(mzML_nodes, xpath="//d1:precursorMz")
raw_data <- xml2::xml_attr(data_nodes, "value")
```

...plus a lot of data handling to get the output into the tidy format.

The other tricky bit of data extraction is converting the (possibly compressed) binary data into R-friendly objects. This is usually handled with code like that shown below. Many of the settings can be deduced from the file, but sometimes compression types need to be guessed at and will throw a warning if so.

```
## Not run:
decoded_binary <- base64enc::base64decode(binary)
raw_binary <- as.raw(decoded_binary)
```

```

decomp_binary <- memDecompress(raw_binary, type = file_metadata$compression)
final_binary <- readBin(decomp_binary, what = "double",
                        n=length(decomp_binary)/file_metadata$mz_precision,
                        size = file_metadata$mz_precision)

# See https://github.com/ProteoWizard/pwiz/issues/1301

```

Fundamentally, mass-spectrometry data is formatted as a ragged array, with an unknown number of m/z and intensity values for a given scan. This makes it difficult to encode neatly without interpolating, but tidy data provides a solution by stacking those arrays rather than aligning them into some sort of matrix.

This ragged shape is also the reason that subsetting mass-spec data by retention time is trivial - grab the scans that correspond to the desired retention times and you're done. Subsetting by mass, on the other hand, requires decoding each and every scan's m/z and intensity data. If you're reading a book and only want a couple chapters, it's easy to flip to those sections. If you're looking instead for every time a specific word shows up, you've gotta read the whole thing.

For more information about the mzML data format and its history, check out the specification at <http://www.psdev.info/mzML>.

Minified file details

While many RaMS users will arrive at this vignette with their own data, several small files are provided for demonstration. These files originate from the Ingalls Lab at the University of Washington, USA and are published in the manuscript **“Metabolic consequences of cobalamin scarcity in diatoms as revealed through metabolomics”**. Files were downloaded from the corresponding Metabolights repository.

Due to CRAN's restrictions on package size, the original files cannot be included. Instead, MS1 data was compressed by extracting EICs for each compound in the Ingalls Lab list of targeted compounds as of August 24, 2020. EIC width was set with a ppm value of 10 and time data were filtered to fall between 240 and 540 seconds. These compressed files were then written out using MSnbase's `writeMSdata` function after coercing the existing data frame into an MSnExp object. Finally, they were gzip-compressed with R.utils's `gzip` function and default parameters.

MS2 data was processed via Proteowizard's `msconvert` tool instead. Files were converted with the flags `-gz` and `--noindex` to save space, and three filters were used: `--filter "scanTime [240,540]" --filter "mzWindow [0,120]" --filter "absolute 100000 most-intense"`

These filters reduced the MS2 data to a sufficiently limited size that they could be published with the package. The mzXML file was created identically, but the `--mzXML` flag was added.