Reproducible Research with R

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Overview

Aims for lecture

1. What are "knitted" documents?
2. How to create these with R
3. What is data pre-processing?
4. Creating reproducible data pre-processing protocols for your research

Running example

As a running example, we will use data preprocessing with the `xcms` package, available on Bioconductor.

Package description:

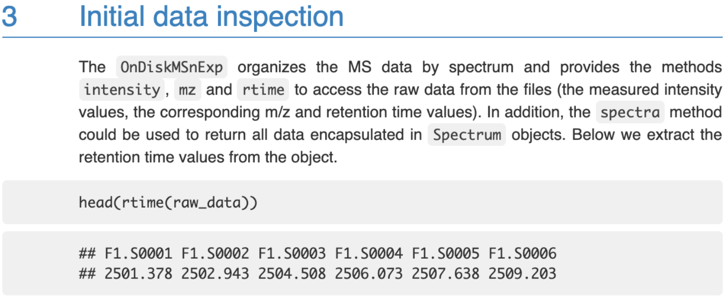
> *"Framework for processing and visualization of chromatographically separated and single-spectra* ***mass spectral data****. Imports from AIA/ANDI NetCDF, mzXML, mzData and mzML files.* ***Preprocesses data*** *for high-throughput, untargeted* ***analyte profiling****."*

Knitted documents

You already use knitted documents!

You have likely already seen and used examples of **knitted documents**.

Many tutorials for R or Python packages are written as knitted documents. For example, here's part of the `xcms` vignette:



3 Initial data inspection

The `OnDiskMSnExp` organizes the MS data by spectrum and provides the methods `intensity`, `mz` and `rtime` to access the raw data from the files (the measured intensity values, the corresponding m/z and retention time values). In addition, the `spectra` method could be used to return all data encapsulated in `Spectrum` objects. Below we extract the retention time values from the object.

```
head(rtime(raw_data))
```

```
## F1.S0001 F1.S0002 F1.S0003 F1.S0004 F1.S0005 F1.S0006
## 2501.378 2502.943 2504.508 2506.073 2507.638 2509.203
```

Definition of knitted documents

The defining characteristic of a knitted document is that it interweaves two elements:

1. Executable code
2. Formatted documentation meant for humans

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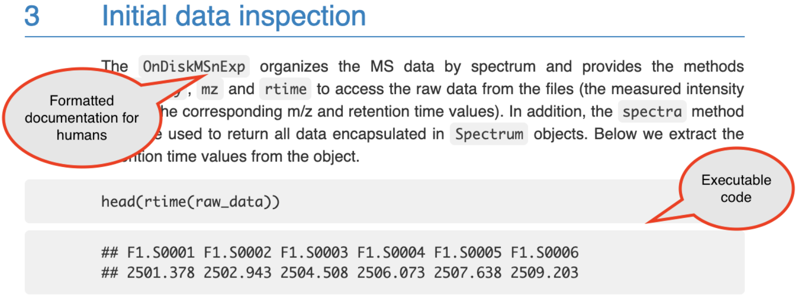
Example:



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How knitted documents work

1. Knitted documents start as plain text
2. A special section at the start of the document (**preamble**) gives some overall directions about the document
3. Special combinations of characters indicate where the executable code starts
4. Other special combinations show where the regular text starts (and the executable code section ends)
5. Formatting for the rest of the document is specified with a **markup language**
6. You create the final document by **rendering** the plain text document. This process runs through two software programs.
7. The final document is attractive and **read-only**—you should never make edits to this output, only to your initial plain text document.

How knitted documents work

1. Knitted documents start as plain text

For example:

```
# Initial data inspection

The `OnDiskMSExp` organizes the MS data ...
```

How knitted documents work

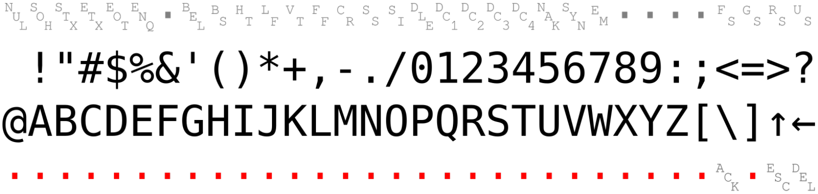
Writing plain text:

- Use a text editor (*not* Word or similar word processing programs)
- Only use character from the American Standard Code for Information Interchange (ASCII)
- White space is important (empty lines and spaces)
- Flexibility in file extension—choose based on the "knitting" software (for RMarkdown, ".Rmd")

ASCII

128 characters. Includes:

- Digits 0–9
- Lowercase and uppercase alphabet (a–z, A–Z)
- Punctuation: . . .
- Some control codes (e.g., new line, tab, ring a bell)



```
NUL SOH STX ETX EOT ENQ ■ BEL BS HT LF VT FF CR SO SI DLE DC1 DC2 DC3 DC4 NAK SYN EM ■ ■ ■ ■ FS GS RS US
 !"#$%&'()*+,-./0123456789:;<=>?
@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]↑←
■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ACK ■ ESC DEL
```

Image source: https://commons.wikimedia.org/wiki/File: ASCII1963-infobox-paths.svg

How knitted documents work

2. A special section at the start of the document (**preamble**) gives some overall directions about the document

In RMarkdown documents, this preamble is specified using **YAML**.

For example, here is the YAML for this presentation:

```
---
title: "Reproducible Research with R"
author: "Brooke Anderson"
date: "1/25/2021"
output: beamer_presentation
---
```

How knitted documents work

In this preamble, you can specify things using **keywords** and **values** [?].

For example, you can specify the title:

```
title: "Reproducible Research with R"
```

and the type of output:

```
output: beamer_presentation
```

How knitted documents work

There are other types of knitted documents, too—they might use other languages for the preamble and the markup. Examples of other Markup languages include LaTeX and HTML.

There are websites, cheatsheets, and other resources you can use to find out which keywords are available for the preamble in the type of document you're creating, as well as the range of values those keywords can take.

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For example:

~~~
Some text is here. And then some code:

```{r, eval=TRUE}
class_grades <- c(95, 98, 88)
mean(class_grades)
```
~~~

How knitted documents work

This combination indicates the start of executable code:

~~~
```{r}
~~~

How knitted documents work

This combination indicates the start of executable code:

~~~
```{r}
~~~

This combination indicates the start of regular documentation (that is, the end of executable code):

~~~
```
~~~

How knitted documents work

This combination indicates the start of executable code:

~~~
```{r}
~~~

This combination indicates the start of regular documentation (that is, the end of executable code):

~~~
```
~~~

In the starting combination, you can also add some specifications for how you want the code run and showed:

~~~
```{r echo = FALSE, fig.align = "center"}
~~~

How knitted documents work

5. Formatting for the rest of the document is specified with a **markup language**

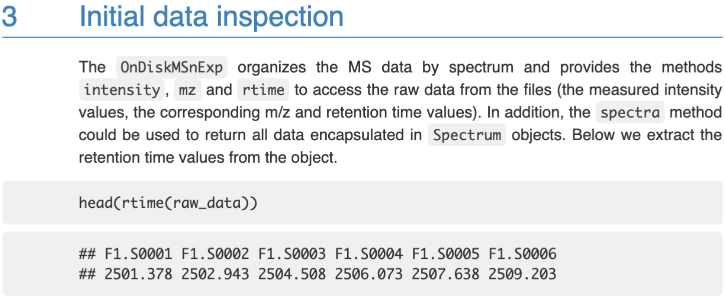
You do not have buttons to click for formatting like bold, italics, font size, and so on. Instead, you use **special characters or character combinations** to specify formatting in the final document.

For example, you'll surround a word or phrase in `**` to make it bold.

To write "**this**" in the final document, you'll write `"**this**"` in the plain text initial document.

How knitted documents work

The start of this document:



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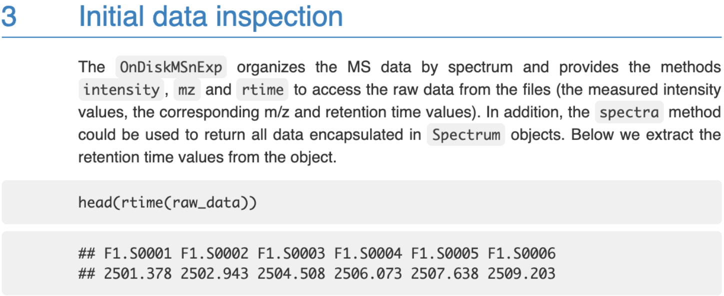

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```

Is written like this:

```
# Initial data inspection

The `OnDiskMSExp` organizes the MS data ...
```

How knitted documents work

Imagine yourself dictating everything to your computer—you have to say not just the words, but the formatting you want as each spot.

Source: The Churchill Project

How knitted documents work

6. You create the final document by **rendering** the plain text document. This process runs through two software programs.
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Why use knitted documents?

1. Code is checked every time you render the document
2. Code is formatted without special symbols
3. Code can be re-run with updated or new datasets
4. Document is in plain text, so it can be tracked well with version control

Why use knitted documents?

1. Code is checked every time you render the document

Why use knitted documents?

2. Code is formatted without special symbols

[Example of code symbols in Word that can mess up code]

Why use knitted documents?

3. Code can be re-run with updated or new datasets

Why use knitted documents?

4. Document is in plain text, so it can be tracked well with version control

[Picture of diff from git tracking]

Creating knitted documents in R

RMarkdown

R has a special format for creating knitted documents, **RMarkdown**.

- **RMarkdown** files are in plain text. They use **YAML** for the preamble and **Markdown** for the primary markup language.
- **Code sections** are marked with ` ```{r} ` at the beginning and ` ``` ` at the end
- **Executable code** can be in R, but also in a number of other languages

[Figure for RMarkdown, from cheatsheet?]

Pre-processing for research data

Preprocessing LC-MS data with code

- Want to get from data (direct measurements of something) to knowledge
- Sometimes direct measurements line up very closely with a research question (e.g., weight of research animal, to some degree CFUs), sometimes they need a lot of pre-processing to use to gain knowledge and test meaningful hypotheses
- Often lots of preprocessing required for data from complex equipment that do very clever things with chemistry or physics—leveraging cleverness to see a new angle, but need to work more to interpret the resulting measurements

Preprocessing LC-MS data with code

- Complex equipment will often come with its own, or have available through outside vendors, proprietary software
- This is typically based on a **GUI** [more on this]
- You can use this for your preprocessing, but there are some very good reasons not to if you can avoid it

Preprocessing LC-MS data with code

Liquid chromatography–mass spectrometry: [Type of equipment] [Kinds of data] [File formats: NetCDF, mzML/mzXML, mzData]

- Often used for chemical analysis, including biochemical molecules
- Used for metabolomics and proteomics
- Used in research, also in industry research (pharmaceutical, for example)
- Leverages principles from chemistry and physics [?] to identify "stuff" in a sample and how much of each type of "stuff" [?]

Preprocessing LC-MS data with code

- [More on chromatography]
- [More on mass spec]

Preprocessing LC-MS data with code

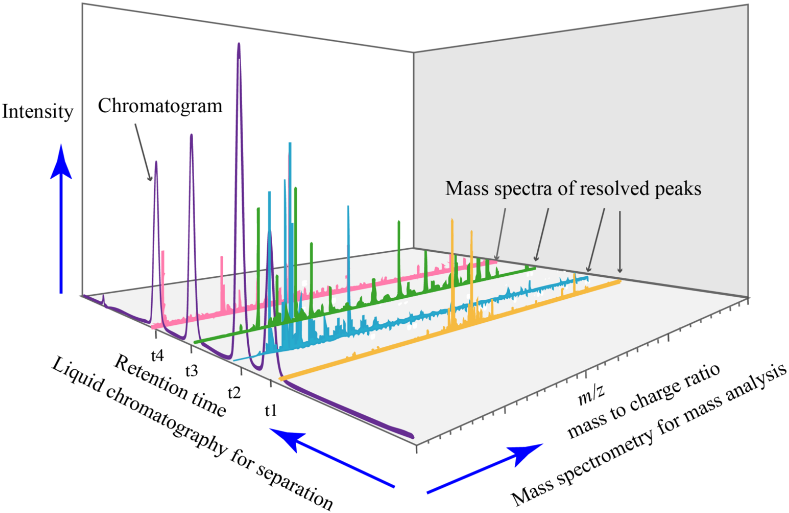
Intensity
Chromatogram
Mass spectra of resolved peaks
t4
t3
t2
t1
Retention time
Liquid chromatography for separation
m/z
mass to charge ratio
Mass spectrometry for mass analysis

Image source: Daniel Norena-Caro

Preprocessing LC-MS data with code

Common preprocessing steps:

- Import data (R has *loads* of packages for handling import of many file formats; `mzR` [?] helpful for common LC-MS file formats)
- Filter to a subset range of retention time [add why]
- Ensure files from different samples are comparable
- Identify and remove sample runs that failed or had other major problems
- Line up retention times across samples [?]
- Figure out the typical width of chromatographic peaks in a sample (to decide on parameters for chromatographic peak detection)
- Chromatographic peak detection
- Refine results from peak detection (e.g., diagnose and fix or remove overlapping peaks or incorrectly split peaks)
- Extract specific chromatographic peaks
- Peak alignment / retention time correction (adjusts raw retention times so that they're comparable across

Preprocessing LC-MS data with code

These preprocessing steps all come *before* any data analysis or visualization (other than exploratory data analysis). Data analysis *after* this preprocessing could include things like checking which features [?] / peaks [?] are significantly different between two experimental groups.

Preprocessing choices: GUI or script

You will have choices about how you preprocess the data. For example, when doing chromotographic peak detection, there will be choices in the algorithm that is used (the `centWave` algorithm in `xcms`). These include the expected range of chromatographic peak widths and the deviation of mass to charge values that will are expected, as a maximum, for chromatographic peaks [?].

In the `centWave` function, these two choices can be specified with `peakwidth` and `ppm`, respectively.

Preprocessing choices: GUI or script

- Reproducible—by you or by others.
- Much easier to write the Methods section later!

Reproducible data pre-processing protocols

In-course exercise

In-course exercise

- Recreate a document's formatting by looking up Markdown syntax (focuses on Markdown syntax to format text in document)
- Create data preprocessing protocol for CFU data—data at different dilutions, pick good dilution, convert back to bacterial load in original sample (Amy's package?)