gcplyr-workflow

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# Getting started

gcplyr is a package that implements a number of functions to make it easier to import, manipulate, and analyze bacterial growth from data collected in multiwell plate readers (“growth curves”). This document gives a walkthrough of how to use gcplyr’s most common functions.

To get started, all you need is the data file with the growth curve measures saved in a tabular format (.csv, .xls, or .xlsx) to your computer.

Users often want to combine their data with some information on experimental design elements of their growth curve plate(s). For instance, this might include which strains went into which wells. You can save this information into a tabular file as well, or you can just keep it handy to enter it directly through a function later on.

Let’s get started by loading gcplyr

library(gcplyr)

# Data layouts

Growth curve data and design elements can be organized in one of three different tabular layouts: block-shaped, wide-shaped, and tidy-shaped, described below.

Tidy-shaped data is the best layout for analyses, but most plate readers output block-shaped or wide-shaped data, and most user-created design files will be block-shaped. Thus, gcplyr works by reshaping block-shaped into wide-shaped data, and wide-shaped data into tidy-shaped data, then running any analyses.

So, what are these three data layouts, and how can you tell which of them your data is in?

**Block-shaped**

In block-shaped data, the organization of the data corresponds directly with the layout of the physical multi-well plate it was generated from. For instance, a data point from the third row and fourth column of the data.frame will be from the well in the third row and fourth column in the physical plate. Because of this, a timeseries of growth curve data that is block-shaped will consist of many separate block-shaped data.frames, each corresponding to a single timepoint.

For example, here is a block-shaped data.frame of a 96-well plate (with “…” indicating Columns 4 - 10, not shown). In this example, all the data shown would be from a single timepoint.

|  | Column 1 | Column 2 | Column 3 | … | Column 11 | Column 12 |
| --- | --- | --- | --- | --- | --- | --- |
| **Row A** | 0.060 | 0.083 | 0.086 | … | 0.082 | 0.085 |
| **Row B** | 0.099 | 0.069 | 0.065 | … | 0.066 | 0.078 |
| **Row C** | 0.081 | 0.071 | 0.070 | … | 0.064 | 0.084 |
| **Row D** | 0.094 | 0.075 | 0.065 | … | 0.067 | 0.087 |
| **Row E** | 0.052 | 0.054 | 0.072 | … | 0.079 | 0.065 |
| **Row F** | 0.087 | 0.095 | 0.091 | … | 0.075 | 0.058 |
| **Row G** | 0.095 | 0.079 | 0.099 | … | 0.063 | 0.075 |
| **Row H** | 0.056 | 0.069 | 0.070 | … | 0.053 | 0.078 |

**Wide-shaped**

In wide-shaped data, each column of the dataframe corresponds to a single well from the plate, and each row of the dataframe corresponds to a single timepoint. Typically, headers contain the well names.

For example, here is a wide-shaped dataframe of a 96-well plate (here, “…” indicates the 91 columns A4 - H10, not shown). Each row of this dataframe corresponds to a single timepoint.

| Time | A1 | A2 | A3 | … | H11 | H12 |
| --- | --- | --- | --- | --- | --- | --- |
| 0 | 0.060 | 0.083 | 0.086 | … | 0.053 | 0.078 |
| 1 | 0.012 | 0.166 | 0.172 | … | 0.106 | 0.156 |
| 2 | 0.024 | 0.332 | 0.344 | … | 0.212 | 0.312 |
| 3 | 0.048 | 0.664 | 0.688 | … | 0.424 | 0.624 |
| 4 | 0.096 | 1.128 | 0.976 | … | 0.848 | 1.148 |
| 5 | 0.162 | 1.256 | 1.152 | … | 1.096 | 1.296 |
| 6 | 0.181 | 1.292 | 1.204 | … | 1.192 | 1.352 |
| 7 | 0.197 | 1.324 | 1.288 | … | 1.234 | 1.394 |

**Tidy-shaped**

In tidy-shaped data, there is a single column that contains all the plate reader measurements, with each unique measurement having its own row. Additional columns specify the timepoint, which well the data comes from, and any other design elements.

Note that, in tidy-shaped data, the number of rows equals the number of wells times the number of timepoints. For instance, with a 96 well plate and 100 timepoints, that will be 9600 rows. (Yes, that’s a lot of rows! But don’t worry, tidy-shaped data is the best format for downstream analyses.) Tidy-shaped data is common in a number of R packages, including ggplot where it’s sometimes called a “long” format. If you want to read more about tidy-shaped data and why it’s ideal for analyses, see: Wickham, Hadley. Tidy data. The Journal of Statistical Software, vol. 59, 2014.

| Timepoint | Well | Measurement |
| --- | --- | --- |
| 1 | A1 | 0.060 |
| 1 | A2 | 0.083 |
| 1 | A3 | 0.086 |
| … | … | … |
| 7 | H10 | 1.113 |
| 7 | H11 | 1.234 |
| 7 | H12 | 1.394 |

# Importing data

Once you’ve determined what format your data is in, you can begin importing it using the read\_\* functions of gcplyr.

If your data is block-shaped, you’ll use read\_blocks and you can start in the next section.

If your data is wide-shaped, you’ll use read\_wides and you can skip down to the **Importing wide-shaped data** section.

In the unlikely event your data is already tidy, you can simply read it using the built-in R function read.table.

## Importing block-shaped data

To import block-shaped data, use the read\_blocks function. read\_blocks only requires a list of filenames (or relative file paths) and will return a list of data.frames, with each data.frame corresponding to a single block.

### The simplest example

Here’s a simple example. First, we need to create a series of example block-shaped .csv files. **Don’t worry how this code works**. When working with real growth curve data, these files would be output by the plate reader. All you need to do is put the file names in R in a vector, here we’ve stored the file names in temp\_filenames.

#This code just creates a series of block-shaped example files  
#Don't worry about how it works - when working with real growth  
#curves data, all these files would be created by the plate reader  
temp\_filenames <- tempfile(  
 pattern = paste(as.character(example\_widedata$Time), "\_", sep = ""),  
 fileext = ".csv")  
for (i in 1:length(temp\_filenames)) {  
 temp\_filenames[i] <- strsplit(temp\_filenames[i], split = "\\\\")[[1]][  
 length(strsplit(temp\_filenames[i], split = "\\\\")[[1]])]  
}  
for (i in 1:length(temp\_filenames)) {  
 write.table(  
 cbind(matrix(c("", "A", "B", "C", "D", "E", "F", "G", "H"), nrow = 9),  
 rbind(  
 matrix(1:12, ncol = 12),  
 matrix(  
 (example\_widedata[i, 2:ncol(example\_widedata)]/(5\*10\*\*8)),  
 ncol = 12)  
 )  
 ),   
 file = temp\_filenames[i], quote = FALSE, row.names = FALSE, sep = ",",  
 col.names = FALSE)  
}

Here’s what one of the files looks like (where the values are absorbance/optical density):

print\_df(read.csv(temp\_filenames[10], header = FALSE,   
 colClasses = "character"))  
#> 1 2 3 4 5 6 7 8 9 10 11 12  
#> A 0 2e-12 0 2e-12 2e-12 0 0 2e-12 0 2e-12 2e-12 0  
#> B 2e-12 2e-12 0 2e-12 2e-12 2e-12 2e-12 2e-12 0 2e-12 2e-12 2e-12  
#> C 2e-12 4e-12 0 2e-12 0 2e-12 2e-12 4e-12 0 2e-12 0 2e-12  
#> D 2e-12 2e-12 4e-12 2e-12 2e-12 2e-12 2e-12 2e-12 4e-12 2e-12 2e-12 0  
#> E 4e-12 2e-12 4e-12 0 2e-12 0 4e-12 2e-12 2e-12 0 2e-12 0  
#> F 0 2e-12 2e-12 0 0 0 0 2e-12 2e-12 0 0 0  
#> G 2e-12 0 2e-12 4e-12 0 0 2e-12 0 2e-12 4e-12 0 0  
#> H 4e-12 4e-12 4e-12 4e-12 0 2e-12 2e-12 4e-12 4e-12 4e-12 0 2e-12

This would correspond to all the reads for a single plate taken at the very first timepoint. We can see that the first row contains column headers, and the first column contains row names. The absorbances look small here because R doesn’t know that the first row is a header yet.

If we want to read these files into R, we simply provide read\_blocks with the vector of file names.

imported\_blockdata <- read\_blocks(files = temp\_filenames)

### Specifying the location of your block-shaped data

However, running read\_blocks with only the filenames only works if the data in your block-shaped files starts in the first row and column (or has column names in the first row and/or rownames in the first column). If your data starts elsewhere, read\_blocks needs to know what row/column to start reading on (if your data isn’t the last thing in the file, read\_blocks also needs to know where your data ends).

To show how this works, first let’s create some example files where the data doesn’t begin in the first row/column. In these example files, the plate reader saved the time that each plate was read in the 2nd row of the file, and started saving the data itself with a header in the 4th row.

Again, **don’t worry how this code works**. When working with real growth curve data, these files would be output by the plate reader. All you need to do is put the file names in R in a vector, here we’ve stored the file names in temp\_filenames2.

#This code just creates a series of block-shaped example files  
#Don't worry about how it works - when working with real growth  
#curves data, all these files would be created by the plate reader  
temp\_filenames2 <-   
 tempfile(pattern = paste(as.character(example\_widedata$Time), "\_2\_", sep = ""),  
 fileext = ".csv")  
for (i in 1:length(temp\_filenames2)) {  
 temp\_filenames2[i] <- strsplit(temp\_filenames2[i], split = "\\\\")[[1]][  
 length(strsplit(temp\_filenames2[i], split = "\\\\")[[1]])]  
}  
for (i in 1:length(temp\_filenames2)) {  
 write.table(  
 cbind(  
 matrix(c("", "", "", "", "A", "B", "C", "D", "E", "F", "G", "H"),   
 nrow = 12),  
 rbind(  
 rep("", 12),  
 matrix(c("Time", example\_widedata$Time[i], rep("", 10)), ncol = 12),  
 rep("", 12),  
 matrix(1:12, ncol = 12),  
 matrix(  
 (example\_widedata[i, 2:ncol(example\_widedata)]/(5\*10\*\*8)),  
 ncol = 12)  
 )  
 ),   
 file = temp\_filenames2[i], quote = FALSE, row.names = FALSE, sep = ",",  
 col.names = FALSE)  
}

Let’s take a look at one of the files:

print\_df(read.csv(temp\_filenames2[10], header = FALSE,  
 colClasses = "character"))  
#>   
#> Time 8100   
#>   
#> 1 2 3 4 5 6 7 8 9 10 11 12  
#> A 0 2e-12 0 2e-12 2e-12 0 0 2e-12 0 2e-12 2e-12 0  
#> B 2e-12 2e-12 0 2e-12 2e-12 2e-12 2e-12 2e-12 0 2e-12 2e-12 2e-12  
#> C 2e-12 4e-12 0 2e-12 0 2e-12 2e-12 4e-12 0 2e-12 0 2e-12  
#> D 2e-12 2e-12 4e-12 2e-12 2e-12 2e-12 2e-12 2e-12 4e-12 2e-12 2e-12 0  
#> E 4e-12 2e-12 4e-12 0 2e-12 0 4e-12 2e-12 2e-12 0 2e-12 0  
#> F 0 2e-12 2e-12 0 0 0 0 2e-12 2e-12 0 0 0  
#> G 2e-12 0 2e-12 4e-12 0 0 2e-12 0 2e-12 4e-12 0 0  
#> H 4e-12 4e-12 4e-12 4e-12 0 2e-12 2e-12 4e-12 4e-12 4e-12 0 2e-12

In the above example, the column names are in row 4 and the rownames are in column 1. To specify that to read\_blocks, we simply do:

#Now let's read it with read\_blocks  
imported\_blockdata <- read\_blocks(  
 files = temp\_filenames2,  
 startrow = 4, startcol = 1)

If you’re looking at your data in Excel or a similar spreadsheet program, you’ll notice that the columns aren’t nicely numbered. Instead, they’re coded by letter. Rather than have to count by hand what columns your data starts and ends on, just specify the column by letter and read\_blocks will translate that to a number for you!

#Now let's read it with read\_blocks  
imported\_blockdata <- read\_blocks(  
 files = temp\_filenames2,  
 startrow = 4, startcol = "A")

Additionally, some plate readers might output growth curve data in a block shape but in a single file. For instance, the file may contain the block from lines 1 - 8, then an empty line, then the next block from lines 10 - 17, etc. Since read\_blocks is vectorized on most of its input arguments, including startrow, startcol, endrow, and endcol, such a layout can be specified by passing a vector of startrows and endrows to read\_blocks:

imported\_blockdata <- read\_blocks(  
 files = "example\_file.csv",  
 startrow = c(1, 10, 19, 28, 37, 46, 55),  
 endrow = c(8, 17, 26, 35, 44, 53, 62))

### Specifying metadata

Sometimes, your input files will have information you want to import that’s not included in the main block of data. For instance, with block-shaped data the timepoint is nearly always specified somewhere in the input file. read\_blocks can include that information as well via the metadata argument.

For example, let’s return to our most-recent example files:

print\_df(read.csv(temp\_filenames2[10], header = FALSE,  
 colClasses = "character"))  
#>   
#> Time 8100   
#>   
#> 1 2 3 4 5 6 7 8 9 10 11 12  
#> A 0 2e-12 0 2e-12 2e-12 0 0 2e-12 0 2e-12 2e-12 0  
#> B 2e-12 2e-12 0 2e-12 2e-12 2e-12 2e-12 2e-12 0 2e-12 2e-12 2e-12  
#> C 2e-12 4e-12 0 2e-12 0 2e-12 2e-12 4e-12 0 2e-12 0 2e-12  
#> D 2e-12 2e-12 4e-12 2e-12 2e-12 2e-12 2e-12 2e-12 4e-12 2e-12 2e-12 0  
#> E 4e-12 2e-12 4e-12 0 2e-12 0 4e-12 2e-12 2e-12 0 2e-12 0  
#> F 0 2e-12 2e-12 0 0 0 0 2e-12 2e-12 0 0 0  
#> G 2e-12 0 2e-12 4e-12 0 0 2e-12 0 2e-12 4e-12 0 0  
#> H 4e-12 4e-12 4e-12 4e-12 0 2e-12 2e-12 4e-12 4e-12 4e-12 0 2e-12

In these files, the timepoint information was located in the 2nd row and 3rd column. Here’s how we could specify that metadata in our read\_blocks command:

#Reading the blockcurves files with metadata included  
imported\_blockdata <- read\_blocks(  
 files = temp\_filenames2,  
 startrow = 4, startcol = "A",  
 metadata = list("time" = c(2, 3)))

You can see that the metadata argument must be a list of named vectors. Each vector should have two elements specifying the location of the metadata in the input files: the first element is the row, the second element is the column.

And just like how you can specify startrow, startcol, etc. with Excel-style lettering, the location of metadata can also be specified with Excel-style lettering.

#Reading the blockcurves files with metadata included  
imported\_blockdata <- read\_blocks(  
 files = temp\_filenames2,  
 startrow = 4, startcol = "A",  
 metadata = list("time" = c(2, "C")))

### What to do next

Now that you’ve imported your block-shaped data, you’ll need to transform it for later analyses. Skip the next section, **Importing wide-shaped data**, and instead jump to the **Transforming data** section.

## Importing wide-shaped data

To import wide-shaped data, use the read\_wides function. read\_wides only requires a filename (or vector of filenames, or relative file paths) and will return a data.frame (or list of data.frames).

### The simplest example

Here’s a simple example. First, we need to create an example wide-shaped .csv file. **Don’t worry how this code works**. when working with real growth curve data, these files would be output by the plate reader. All you need to do is put the file name(s) in R, here we’ve stored the file name in temp\_filename.

#This code just creates a wide-shaped example file  
#Don't worry about how it works - when working with real growth  
#curves data, this file would be created by the plate reader  
temp\_filename <- paste(tempfile(), ".csv", sep = "")  
temp\_filename <- strsplit(temp\_filename, split = "\\\\")[[1]][  
 length(strsplit(temp\_filename, split = "\\\\")[[1]])]  
write.csv(example\_widedata, file = temp\_filename, row.names = FALSE)

Here’s what the start of the file looks like (where the values are absorbance/optical density):

print\_df(head(read.csv(temp\_filename, header = FALSE),   
 c(10, 4), row.names = FALSE))  
#> Time A1 B1 C1  
#> 0 0 0 0  
#> 900 0 0 0  
#> 1800 0 0 0  
#> 2700 0 0 0  
#> 3600 0 0 0  
#> 4500 0 0.001 0  
#> 5400 0 0.001 0  
#> 6300 0 0.001 0  
#> 7200 0 0.001 0.001

This would correspond to all the reads for a single plate taken across all timepoints. For instance, we can see that the first column contains the timepoint information, and each subsequent column corresponds to a well in the plate.

If we want to read these files into R, we simply provide read\_wides with the file name.

#Now let's use read\_wides to import our wide-shaped data  
imported\_widedata <- read\_wides(files = temp\_filename)

The resulting data.frame looks like this:

print\_df(head(imported\_widedata, c(10, 6)))  
#> filed2868132fb4 0 0 0 0 0  
#> filed2868132fb4 900 0 0 0 0  
#> filed2868132fb4 1800 0 0 0 0  
#> filed2868132fb4 2700 0 0 0 0  
#> filed2868132fb4 3600 0 0 0 0  
#> filed2868132fb4 4500 0 0.001 0 0.001  
#> filed2868132fb4 5400 0 0.001 0 0.001  
#> filed2868132fb4 6300 0 0.001 0 0.001  
#> filed2868132fb4 7200 0 0.001 0.001 0.001  
#> filed2868132fb4 8100 0 0.001 0.001 0.001

Note that *read\_wides automatically saves the filename* the data was imported from into the first column of the output data.frame. This is done to ensure that later on, data.frames from multiple plates can be combined without fear of losing the identity of each plate.

Note that if you have multiple files you’d like to read in, you can do so directly with a single read\_wides command. In this case, read\_wides will return a list containing all the data.frames:

#If we had multiple wide-shaped data files to import  
imported\_widedata <- read\_wides(files = c(temp\_filename, temp\_filename))

### Specifying the location of your wide-shaped data

However, running read\_wides with only the filename(s) only works if the data in your wide-shaped files starts in the first row and column (or has column names in the first row and/or rownames in the first column). If your data starts elsewhere, read\_wides needs to know what row/column to start reading on (if your data isn’t the last thing in the file, read\_wides also needs to know where your data ends).

To show how this works, first let’s create an example file where the data doesn’t begin in the first row/column. In this example file, the plate reader started saving the data itself with a header in the 5th row.

Again, **don’t worry how this code works**. When working with real growth curve data, these files would be output by the plate reader. All you need to do is put the file names in R in a vector, here we’ve stored the file name in temp\_filename2.

#This code just creates a wide-shaped example file where the data doesn't  
#start on the first row.  
#Don't worry about how it works - when working with real growth  
#curves data, this file would be created by the plate reader  
temp\_filename2 <- tempfile(fileext = ".csv")  
temp\_filename2 <- strsplit(temp\_filename2, split = "\\\\")[[1]][  
 length(strsplit(temp\_filename2, split = "\\\\")[[1]])]  
temp\_example\_widedata <- example\_widedata  
colnames(temp\_example\_widedata) <- paste("V", 1:ncol(temp\_example\_widedata),  
 sep = "")  
modified\_example\_widedata <-  
 rbind(  
 as.data.frame(matrix("", nrow = 4, ncol = ncol(example\_widedata))),  
 colnames(example\_widedata),  
 temp\_example\_widedata)  
modified\_example\_widedata[1:2, 1:2] <-   
 c("Experiment name", "Start date", "Experiment\_1", as.character(Sys.Date()))  
  
write.table(modified\_example\_widedata, file = temp\_filename2,   
 row.names = FALSE, col.names = FALSE, sep = ",")

Let’s take a look at the file:

#Let's take a peek at what this file looks like  
print\_df(head(read.csv(temp\_filename2, header = FALSE), c(10, 6)))  
#> Experiment name Experiment\_1   
#> Start date 2022-03-25   
#>   
#>   
#> Time A1 B1 C1 D1 E1  
#> 0 0 0 0 0 0  
#> 900 0 0 0 0 0  
#> 1800 0 0 0 0 0  
#> 2700 0 0 0 0 0  
#> 3600 0 0 0 0 0.001

Thus, we can see the data header is in row 5, and the data begins in row 6. To specify that to read\_wides, we simply do (note that header = TRUE by default):

imported\_widedata <- read\_wides(files = temp\_filename2,  
 startrow = 5)  
print\_df(head(imported\_widedata, c(10, 6)))  
#> filed281bda20b7 0 0 0 0 0  
#> filed281bda20b7 900 0 0 0 0  
#> filed281bda20b7 1800 0 0 0 0  
#> filed281bda20b7 2700 0 0 0 0  
#> filed281bda20b7 3600 0 0 0 0  
#> filed281bda20b7 4500 0 0.001 0 0.001  
#> filed281bda20b7 5400 0 0.001 0 0.001  
#> filed281bda20b7 6300 0 0.001 0 0.001  
#> filed281bda20b7 7200 0 0.001 0.001 0.001  
#> filed281bda20b7 8100 0 0.001 0.001 0.001

If you’re looking at your data in Excel or a similar spreadsheet program, you’ll notice that the columns aren’t nicely numbered. Instead, they’re coded by letter. Rather than have to count by hand what columns your data starts and ends on, just specify the column by letter and read\_wides will translate that to a number for you! (in this example we don’t have to specify a start column, since the data starts in the first column, but we do so just to show this letter-style functionality).

imported\_widedata <- read\_wides(files = temp\_filename2,  
 startrow = 5, startcol = "A")

### Specifying metadata

Sometimes, your input files will have information you want to import that’s not included in the main block of data. For instance, many readers will output information like the experiment name and date into a header in the file. read\_wides can include that information as well via the metadata argument.

The metadata argument should be a list of named vectors. Each vector should be of length 2, with the first entry specifying the row and the second entry specifying the column where the metadata is located.

For example, in our previous example files, the experiment name was located in the 2nd row, 2nd column, and the start date was located in the 3rd row, 2nd column. Here’s how we could specify that metadata:

imported\_widedata <- read\_wides(files = temp\_filename2,  
 startrow = 5,  
 metadata = list("experiment\_name" = c(1, 2),  
 "start\_date" = c(2, 2)))  
print\_df(head(imported\_widedata, c(6, 3)))  
#> filed281bda20b7 Experiment\_1 2022-03-25  
#> filed281bda20b7 Experiment\_1 2022-03-25  
#> filed281bda20b7 Experiment\_1 2022-03-25  
#> filed281bda20b7 Experiment\_1 2022-03-25  
#> filed281bda20b7 Experiment\_1 2022-03-25  
#> filed281bda20b7 Experiment\_1 2022-03-25

And just like how you can specify startrow, startcol, etc. with Excel-style lettering, the location of metadata can also be specified with Excel-style lettering.

imported\_widedata <- read\_wides(files = temp\_filename2,  
 startrow = 5,  
 metadata = list("experiment\_name" = c(1, "B"),  
 "start\_date" = c(2, "B")))

### What to do next

Now that you’ve imported your wide-shaped data, you’ll need to transform it for later analyses. Continue on to the **Transforming data** section.

# Transforming data

Now that you’ve gotten your data into the R environment, we need to transform it before we can do analyses. To reiterate, this is necessary because most plate readers that generate growth curve data outputs it in block-shaped or wide-shaped files, but tidy-shaped data.frames are the best shape for analyses and required by gcplyr.

You can transform your data.frames using the trans\_\* functions in gcplyr.

## Transforming from block-shaped to wide-shaped

If the data you’ve read into the R environment is block-shaped, you’ll need to transform it from block-shaped to wide-shaped, and then wide-shaped to tidy-shaped. For the first step, you’ll use trans\_block\_to\_wide. All you need to do is provide trans\_block\_to\_wide with the R object you saved when you used read\_blocks.

imported\_blocks\_now\_wide <- trans\_block\_to\_wide(imported\_blockdata)  
#> Warning in trans\_block\_to\_wide(imported\_blockdata): Inferring nested\_metadata to be  
#> TRUE

Note that trans\_block\_to\_wide automatically detected the metadata that read\_blocks had pulled from our files, and has stored each piece of metadata as a column in our output file.

print(head(imported\_blocks\_now\_wide, c(6, 12)), row.names = FALSE)  
#> block\_name time A\_1 A\_2 A\_3 A\_4 A\_5 A\_6 A\_7 A\_8 A\_9 A\_10  
#> 0\_2\_d2828a82cf6 0 0 0 0 0 0 0 0 0 0 0  
#> 900\_2\_d287e14600f 900 0 0 0 0 0 0 0 0 0 0  
#> 1800\_2\_d284a044c4a 1800 0 0 0 0 0 0 0 0 0 0  
#> 2700\_2\_d2836453433 2700 0 0 0 0 0 0 0 0 0 0  
#> 3600\_2\_d284c8b6e1f 3600 0 0 0 0 0 0 0 0 0 0  
#> 4500\_2\_d28212e1ecb 4500 0 0 0 0 0 0 0 0 0 0

Now that your block-shaped data has been transformed to wide-shaped data, you can use trans\_wide\_to\_tidy (below) to further transform it into the tidy-shaped data we need for our analyses.

## Transforming from wide-shaped to tidy-shaped

If the data you’ve read into the R environment is wide-shaped (or you’ve gotten wide-shaped data by transforming your originally block-shaped data), you’ll transform it to tidy-shaped using trans\_wide\_to\_tidy.

First, you need to provide trans\_wide\_to\_tidy with the R object created by read\_wides or by trans\_block\_to\_wide.

Then, you have to specify one of: \* the columns your data (the spectrophotometric measures) are in via data\_cols \* what columns your non-data (e.g. time and other information) are in via id\_cols

imported\_blocks\_now\_tidy <- trans\_wide\_to\_tidy(  
 wides = imported\_blocks\_now\_wide,  
 id\_cols = c("block\_name", "time"))  
  
imported\_wides\_now\_tidy <- trans\_wide\_to\_tidy(  
 wides = imported\_widedata,  
 id\_cols = c("file", "experiment\_name", "start\_date", "Time"))  
  
print(head(imported\_blocks\_now\_tidy), row.names = FALSE)  
#> block\_name time Well Measurements  
#> 0\_2\_d2828a82cf6 0 A\_1 0  
#> 0\_2\_d2828a82cf6 0 A\_2 0  
#> 0\_2\_d2828a82cf6 0 A\_3 0  
#> 0\_2\_d2828a82cf6 0 A\_4 0  
#> 0\_2\_d2828a82cf6 0 A\_5 0  
#> 0\_2\_d2828a82cf6 0 A\_6 0

# Including design elements

Often during analysis of growth curve data, we’d like to incorporate information on the experimental design. For example, which bacteria are present in which wells, or which wells have received some treatment. gcplyr enables incorporation of design elements in two ways: 1. Design elements can be imported from tidy-shaped files using read\_table functions and merged with previously-imported data 2. Design elements can be generated programmatically using make\_tidydesign

## Reading design elements from files

Just like spectrophotometric data, design elements that are saved in tidy-shaped tabular data files can be read using the read\_table function.

Once these design elements have been read into the R environment, you can merge them with your data. See the next section for details.

## Generating tidy-shaped design elements programmatically

If you don’t have your experimental design information saved in a file, you can directly create such a data.frame using the gcplyr function make\_tidydesign. make\_tidydesign uses the spatial location of design elements in a multiwell plate as input arguments, but outputs a tidy-shaped data.frame that can be easily merged with your tidy-shaped data.

### An example with a single design

Let’s start with a simple example demonstrating the basic use of make\_tidydesign (we’ll move on to more complicated designs afterwards).

For example, let’s imagine a growth curve experiment where a 96 well plate (12 columns and 8 rows) has a different bacterial strain in each row, but the first and last columns and first and last rows were left empty.

| Row names | Column 1 | Column 2 | Column 3 | … | Column 11 | Column 12 |
| --- | --- | --- | --- | --- | --- | --- |
| Row A | Blank | Blank | Blank | … | Blank | Blank |
| Row B | Blank | Strain #1 | Strain #1 | … | Strain #1 | Blank |
| Row B | Blank | Strain #2 | Strain #2 | … | Strain #2 | Blank |
| … | … | … | … | … | … | … |
| Row G | Blank | Strain #5 | Strain #5 | … | Strain #5 | Blank |
| Row G | Blank | Strain #6 | Strain #6 | … | Strain #6 | Blank |
| Row H | Blank | Blank | Blank | … | Blank | Blank |

To generate a tidy-shaped design data.frame representing this information, we can use make\_tidydesign:

my\_design <- make\_tidydesign(  
 nrows = 8, ncols = 12,  
 Bacteria = list(  
 c("Strain 1", "Strain 2", "Strain 3",   
 "Strain 4", "Strain 5", "Strain 6"),  
 2:7,  
 2:11,  
 "123456",  
 FALSE)  
)

Now, what are each of the things we’ve specified for our “Bacteria” design component?

Well, make\_tidydesign expects five things for each design component: \* a vector containing the possible values \* a vector containing all the rows these values should be applied to \* a vector containing all the columns these values should be applied to \* a string of the pattern itself within those rows and columns \* a Boolean for whether this pattern should be filled byrow (defaults to TRUE)

So for our example above, we can see: \* the possible values are c("Strain 1", "Strain 2", "Strain 3", "Strain 4", "Strain 5", "Strain 6") \* the rows these values should be applied to are rows 2:7 \* the columns these values should be applied to are columns 2:11 \* the pattern these values should be filled in by is "123456" \* and these values should *not* be filled byrow

This entire list is passed with a name (here, “Bacteria”), that will be used as the resulting column header.

What does the resulting data.frame look like?

head(my\_design, 20)  
#> Well Bacteria  
#> 1 A1 <NA>  
#> 2 A2 <NA>  
#> 3 A3 <NA>  
#> 4 A4 <NA>  
#> 5 A5 <NA>  
#> 6 A6 <NA>  
#> 7 A7 <NA>  
#> 8 A8 <NA>  
#> 9 A9 <NA>  
#> 10 A10 <NA>  
#> 11 A11 <NA>  
#> 12 A12 <NA>  
#> 13 B1 <NA>  
#> 14 B2 Strain 1  
#> 15 B3 Strain 1  
#> 16 B4 Strain 1  
#> 17 B5 Strain 1  
#> 18 B6 Strain 1  
#> 19 B7 Strain 1  
#> 20 B8 Strain 1

### A few notes on the pattern string

The fourth element of every argument passed to make\_tidydesign is the string specifying the pattern of values.

Oftentimes, it will be most convenient to simply use single-characters to correspond to the values. This is the default behavior of make\_tidydesign, which splits the pattern string into individual characters, and then uses those characters to correspond to the indices of the values you provided.

For instance, in our example above, we used the numbers 1 through 6 to correspond to the values "Strain 1", "Strain 2", "Strain 3", "Strain 4", "Strain 5", "Strain 6".

It’s important to **note that the “0” character is reserved for NA values.** There is an example of this later.

If you have more than 9 values, you can use letters (uppercase and/or lowercase) and specify to make\_tidydesign what letter you’d like the indices to start with. By default, the order goes from 1 to 9, then A to Z (uppercase), then a to z (lowercase). For instance, in the previous example, we could have done:

my\_design <- make\_tidydesign(  
 nrows = 8, ncols = 12, lookup\_tbl\_start = "A",  
 Bacteria = list(  
 c("Strain 1", "Strain 2", "Strain 3", "Strain 4", "Strain 5", "Strain 6"),  
 2:7,  
 2:11,  
 "ABCDEF",  
 FALSE)  
)

Or we could have done:

my\_design <- make\_tidydesign(  
 nrows = 8, ncols = 12, lookup\_tbl\_start = "a",  
 Bacteria = list(  
 c("Strain 1", "Strain 2", "Strain 3", "Strain 4", "Strain 5", "Strain 6"),  
 2:7,  
 2:11,  
 "abcdef",  
 FALSE)  
)

Alternatively, you can use a separating character like a comma to delineate your indices. If you are doing so in order to use multicharacter indices (like numbers with more than one digit), all your indices will have to be numeric.

my\_design <- make\_tidydesign(  
 nrows = 8, ncols = 12, pattern\_split = ",",  
 Bacteria = list(  
 c("Strain 1", "Strain 2", "Strain 3", "Strain 4", "Strain 5", "Strain 6"),  
 2:7,  
 2:11,  
 "1,2,3,4,5,6",  
 FALSE)  
)

### Continuing with the example: multiple designs

Now let’s return to our example growth curve experiment. Imagine that now, in addition to having a different bacterial strain in each row, we also have a different media in each column in the plate.

| Row names | Column 1 | Column 2 | Column 3 | … | Column 11 | Column 12 |
| --- | --- | --- | --- | --- | --- | --- |
| Row A | Blank | Blank | Blank | … | Blank | Blank |
| Row B | Blank | Media #1 | Media #2 | … | Media #10 | Blank |
| … | … | … | … | … | … | … |
| Row G | Blank | Media #1 | Media #2 | … | Media #10 | Blank |
| Row H | Blank | Blank | Blank | … | Blank | Blank |

We can generate that design by adding an additional argument to our make\_tidydesign call.

my\_design <- make\_tidydesign(  
 nrows = 8, ncols = 12, lookup\_tbl\_start = "a",  
 Bacteria = list(c("Strain 1", "Strain 2", "Strain 3",  
 "Strain 4", "Strain 5", "Strain 6"),  
 2:7,  
 2:11,  
 "abcdef",  
 FALSE),  
 Media = list(c("Media 1", "Media 2", "Media 3",  
 "Media 4", "Media 5", "Media 6",  
 "Media 7", "Media 8", "Media 9",  
 "Media 10", "Media 11", "Media 12"),  
 2:7,  
 2:11,  
 "abcdefghij")  
 )  
head(my\_design, 20)  
#> Well Bacteria Media  
#> 1 A1 <NA> <NA>  
#> 2 A2 <NA> <NA>  
#> 3 A3 <NA> <NA>  
#> 4 A4 <NA> <NA>  
#> 5 A5 <NA> <NA>  
#> 6 A6 <NA> <NA>  
#> 7 A7 <NA> <NA>  
#> 8 A8 <NA> <NA>  
#> 9 A9 <NA> <NA>  
#> 10 A10 <NA> <NA>  
#> 11 A11 <NA> <NA>  
#> 12 A12 <NA> <NA>  
#> 13 B1 <NA> <NA>  
#> 14 B2 Strain 1 Media 1  
#> 15 B3 Strain 1 Media 2  
#> 16 B4 Strain 1 Media 3  
#> 17 B5 Strain 1 Media 4  
#> 18 B6 Strain 1 Media 5  
#> 19 B7 Strain 1 Media 6  
#> 20 B8 Strain 1 Media 7

Now, imagine after the experiment we discover that Bacterial Strain 4 and Media #6 were contaminated, and we’d like to exclude them from our analyses by marking them as NA in the design. We can simply modify our pattern string, placing a 0 anywhere we would like an NA to be filled in.

my\_design <- make\_tidydesign(  
 nrows = 8, ncols = 12, lookup\_tbl\_start = "a",  
 Media = list(c("Media 1", "Media 2", "Media 3",  
 "Media 4", "Media 5", "Media 6",  
 "Media 7", "Media 8", "Media 9",  
 "Media 10", "Media 11", "Media 12"),  
 2:7,  
 2:11,  
 "abcde0ghij"),  
 Bacteria = list(c("Strain 1", "Strain 2", "Strain 3",  
 "Strain 4", "Strain 5", "Strain 6"),  
 2:7,  
 2:11,  
 "abc0ef",  
 FALSE))  
head(my\_design, 20)  
#> Well Media Bacteria  
#> 1 A1 <NA> <NA>  
#> 2 A2 <NA> <NA>  
#> 3 A3 <NA> <NA>  
#> 4 A4 <NA> <NA>  
#> 5 A5 <NA> <NA>  
#> 6 A6 <NA> <NA>  
#> 7 A7 <NA> <NA>  
#> 8 A8 <NA> <NA>  
#> 9 A9 <NA> <NA>  
#> 10 A10 <NA> <NA>  
#> 11 A11 <NA> <NA>  
#> 12 A12 <NA> <NA>  
#> 13 B1 <NA> <NA>  
#> 14 B2 Media 1 Strain 1  
#> 15 B3 Media 2 Strain 1  
#> 16 B4 Media 3 Strain 1  
#> 17 B5 Media 4 Strain 1  
#> 18 B6 Media 5 Strain 1  
#> 19 B7 <NA> Strain 1  
#> 20 B8 Media 7 Strain 1

Note that make\_tidydesign is not limited to simple alternating patterns. The pattern string specified can be any pattern, which make\_tidydesign will replicate sufficient times to cover the entire set of listed wells.

my\_design <- make\_tidydesign(  
 nrows = 8, ncols = 12, lookup\_tbl\_start = "a",  
 Media = list(c("Media 1", "Media 2", "Media 3"),  
 2:7,  
 2:11,  
 "aabbbc000abc"),  
 Bacteria = list(c("Strain 1", "Strain 2"),  
 2:7,  
 2:11,  
 "abaaabbbab",  
 FALSE))  
head(my\_design, 20)  
#> Well Media Bacteria  
#> 1 A1 <NA> <NA>  
#> 2 A2 <NA> <NA>  
#> 3 A3 <NA> <NA>  
#> 4 A4 <NA> <NA>  
#> 5 A5 <NA> <NA>  
#> 6 A6 <NA> <NA>  
#> 7 A7 <NA> <NA>  
#> 8 A8 <NA> <NA>  
#> 9 A9 <NA> <NA>  
#> 10 A10 <NA> <NA>  
#> 11 A11 <NA> <NA>  
#> 12 A12 <NA> <NA>  
#> 13 B1 <NA> <NA>  
#> 14 B2 Media 1 Strain 1  
#> 15 B3 Media 1 Strain 2  
#> 16 B4 Media 2 Strain 1  
#> 17 B5 Media 2 Strain 1  
#> 18 B6 Media 2 Strain 1  
#> 19 B7 Media 3 Strain 1  
#> 20 B8 <NA> Strain 2

gcplyr also includes an optional helper function for make\_tidydesign called make\_designpattern. make\_designpattern just helps by reminding the user what arguments are necessary for each design and ensuring they’re in the correct order. For example, the following produces the same data.frame as the above code:

my\_design <- make\_tidydesign(  
 nrows = 8, ncols = 12, lookup\_tbl\_start = "a",  
 Media = make\_designpattern(  
 values = c("Media 1", "Media 2", "Media 3",  
 "Media 4", "Media 5", "Media 6",  
 "Media 7", "Media 8", "Media 9",  
 "Media 10", "Media 11", "Media 12"),  
 rows = 2:7, cols = 2:11, pattern = "abcde0ghij"),  
 Bacteria = make\_designpattern(  
 values = c("Strain 1", "Strain 2", "Strain 3",  
 "Strain 4", "Strain 5", "Strain 6"),  
 rows = 2:7, cols = 2:11, pattern = "abc0ef",  
 byrow = FALSE))  
head(my\_design, 20)  
#> Well Media Bacteria  
#> 1 A1 <NA> <NA>  
#> 2 A2 <NA> <NA>  
#> 3 A3 <NA> <NA>  
#> 4 A4 <NA> <NA>  
#> 5 A5 <NA> <NA>  
#> 6 A6 <NA> <NA>  
#> 7 A7 <NA> <NA>  
#> 8 A8 <NA> <NA>  
#> 9 A9 <NA> <NA>  
#> 10 A10 <NA> <NA>  
#> 11 A11 <NA> <NA>  
#> 12 A12 <NA> <NA>  
#> 13 B1 <NA> <NA>  
#> 14 B2 Media 1 Strain 1  
#> 15 B3 Media 2 Strain 1  
#> 16 B4 Media 3 Strain 1  
#> 17 B5 Media 4 Strain 1  
#> 18 B6 Media 5 Strain 1  
#> 19 B7 <NA> Strain 1  
#> 20 B8 Media 7 Strain 1

# Merging spectrophotometric and design data

Once we have both our design and data in the R environment, we can merge them using merge\_dfs.

For this, we’ll use the data in the example\_widedata dataset that is included with gcplyr, and which was the source for our previous examples with read\_blocks and read\_wides.

In the example\_widedata dataset, we have 48 different bacterial strains. The left side of the plate has all 48 strains in a single well each, and the right side of the plate also has all 48 strains in a single well each:

| Row names | Column 1 | … | Column 6 | Column 7 | … | Column 12 |
| --- | --- | --- | --- | --- | --- | --- |
| Row A | Strain #1 | … | Strain #6 | Strain #1 | … | Strain #6 |
| Row B | Strain #7 | … | Strain #12 | Strain #7 | … | Strain #12 |
| … | … | … | … | … | … | … |
| Row G | Strain #37 | … | Strain #42 | Strain #37 | … | Strain #42 |
| Row H | Strain #43 | … | Strain #48 | Strain #43 | … | Strain #48 |

Then, on the right hand side of the plate a phage was also inoculated (while the left hand side remained bacteria-only):

| Row names | Column 1 | … | Column 6 | Column 7 | … | Column 12 |
| --- | --- | --- | --- | --- | --- | --- |
| Row A | No Phage | … | No Phage | Phage Added | … | Phage Added |
| Row B | No Phage | … | No Phage | Phage Added | … | Phage Added |
| … | … | … | … | … | … | … |
| Row G | No Phage | … | No Phage | Phage Added | … | Phage Added |
| Row H | No Phage | … | No Phage | Phage Added | … | Phage Added |

Let’s generate our design:

example\_design <- make\_tidydesign(  
 pattern\_split = ",", nrows = 8, ncols = 12,  
 "Bacteria\_strain" = make\_designpattern(  
 values = paste("Strain", 1:48),  
 rows = 1:8, cols = 1:6,  
 pattern = paste(1:48, collapse = ","),  
 byrow = TRUE),  
 "Bacteria\_strain" = make\_designpattern(  
 values = paste("Strain", 1:48),  
 rows = 1:8, cols = 7:12,  
 pattern = paste(1:48, collapse = ","),  
 byrow = TRUE),  
 "Phage" = make\_designpattern(  
 values = c("No Phage"),  
 rows = 1:8, cols = 1:6,  
 pattern = "1"),  
 "Phage" = make\_designpattern(  
 values = c("Phage Added"),  
 rows = 1:8, cols = 7:12,  
 pattern = "1"))

Now let’s transform the example\_widedata to tidy-shaped.

example\_tidydata <- trans\_wide\_to\_tidy(example\_widedata,  
 id\_cols = "Time")

And finally, we merge the two using merge\_dfs:

example\_data\_and\_designs <-  
 merge\_dfs(example\_tidydata,  
 example\_design)  
#> Joining, by = "Well"  
  
head(example\_data\_and\_designs)  
#> Time Well Measurements Bacteria\_strain Phage  
#> 1 0 A1 0 Strain 1 No Phage  
#> 2 0 B1 0 Strain 7 No Phage  
#> 3 0 C1 0 Strain 13 No Phage  
#> 4 0 D1 0 Strain 19 No Phage  
#> 5 0 E1 0 Strain 25 No Phage  
#> 6 0 F1 0 Strain 31 No Phage

# A note on pre-processing, processing, and analyzing your data

Once you have your spectrophotometric and design data merged, you’re ready to move on to the next steps: pre-processing, processing, and analyzing.

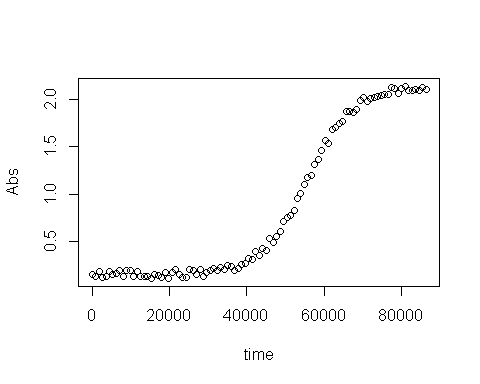
There are a number of functions in gcplyr that can help pre-process, process, and analyze growth curves data. However, unlike the import and transformation steps we’ve done so far, different projects may require different analyses, and not all users will have the same analysis steps. The **Pre-processing**, **Processing**, and **Analyzing** sections of this document, therefore, are written to highlight the functions available for analysis in gcplyr, rather than prescribing a certain series of analysis steps.

# Pre-processing: smoothing

Oftentimes, growth curve data produced by a plate reader will be noisy, and some degree of smoothing before analysis is necessary to reduce this noise and improve the accuracy of analyses. gcplyr has a smooth\_data function that can carry out such smoothing.

First, let’s add some noise to the example data we’ve been working with:

#First let's add some simulated noise to our example data  
example\_data\_and\_designs$Measurements <-  
 example\_data\_and\_designs$Measurements +   
 runif(nrow(example\_data\_and\_designs), min = 0.1, max = 0.2)  
  
#What does this noisy data look like?  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$Measurements[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "Abs")



Now, we can see how our smoothing works. smooth\_data has four different smoothing algorithms to choose from: moving average, moving median, loess, and gam. Moving average and moving median are simple smoothing algorithms that primarily act to reduce the effects of outliers on the data. loess and gam are both spline-fitting approaches that smooth data. loess uses polynomial-like curves, which produce curves with smoothly changing derivatives, but can in some cases create curvature artifacts not present in the original data. gam uses additive curves with less smoothly changing derivatives, but tends to better avoid the creation of curvature artifacts.

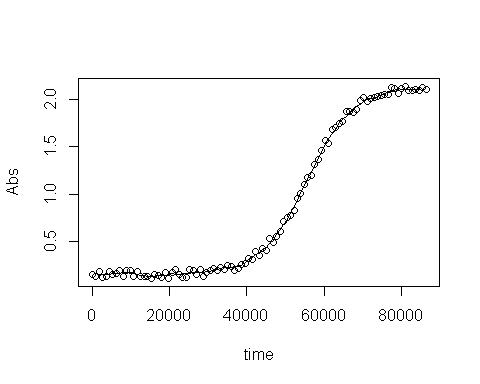
To use smooth\_data, pass your x and y values, your method of choice, and any additional arguments needed for the method. It will return a vector of your smoothed y values.

Since your dataframe likely includes data from multiple wells (or even plates), we’ll want to only smooth within each of those subsets. You can specify the groupings using the subset\_by argument, which should be a vector as long as y, whose unique values denote the subset groups. (Note: if you’re using an approach like dplyr::mutate, smooth\_data will work within mutate on your groups with no need for the subset\_by argument)

**A note on tuning parameters:** All four smoothing algorithms require a tuning parameter that controls how “smoothed” the data are. \* For moving-average and moving-median, this is the window\_width parameter, which controls how wide the moving windows used to calculate the median and average is. \* For loess, this is primarily determined by the span argument, which can be passed to smooth\_data via the ... argument. \* For gam, see mgcv::gam for details, where tuning would require passing formula and data to smooth\_data via the ... argument, and altered tuning parameters (e.g. k, sp, bs) would be included in formula.

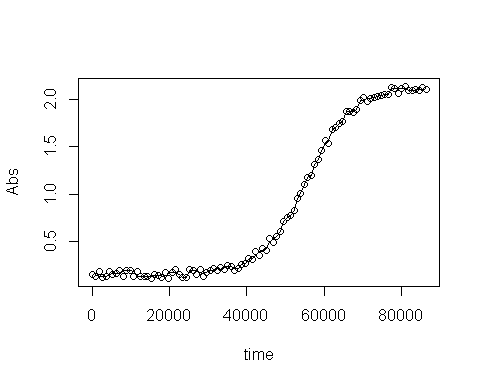
## Smoothing with moving-average

example\_data\_and\_designs$smoothed <-  
 smooth\_data(x = example\_data\_and\_designs$Time,  
 y = example\_data\_and\_designs$Measurements,  
 method = "moving-average",  
 subset\_by = example\_data\_and\_designs$Well,  
 window\_width = 5)  
  
#What does the smoothed data look like compared to the noisy original?  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$Measurements[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "Abs")  
lines(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$smoothed[  
 example\_data\_and\_designs$Well == "A2"])



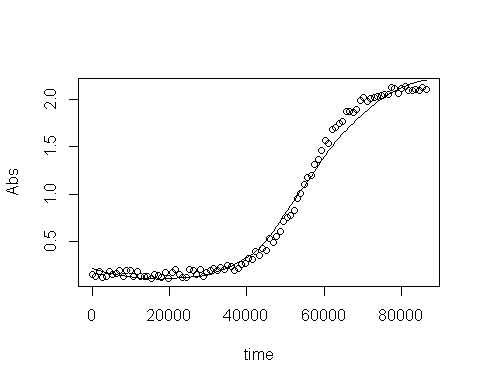
## Smoothing with moving-median

example\_data\_and\_designs$smoothed <-  
 smooth\_data(x = example\_data\_and\_designs$Time,  
 y = example\_data\_and\_designs$Measurements,  
 method = "moving-median",  
 subset\_by = example\_data\_and\_designs$Well,  
 window\_width = 3)  
  
#What does the smoothed data look like compared to the noisy original?  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$Measurements[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "Abs")  
lines(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$smoothed[  
 example\_data\_and\_designs$Well == "A2"])



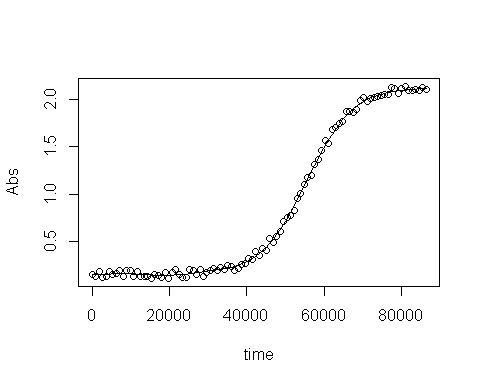
## Smoothing with LOESS

example\_data\_and\_designs$smoothed <-  
 smooth\_data(x = example\_data\_and\_designs$Time,  
 y = example\_data\_and\_designs$Measurements,  
 method = "loess",  
 subset\_by = example\_data\_and\_designs$Well)  
  
#What does the smoothed data look like compared to the noisy original?  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$Measurements[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "Abs")  
lines(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$smoothed[  
 example\_data\_and\_designs$Well == "A2"])



## Smoothing with GAM

example\_data\_and\_designs$smoothed <-  
 smooth\_data(x = example\_data\_and\_designs$Time,  
 y = example\_data\_and\_designs$Measurements,  
 method = "gam",  
 subset\_by = example\_data\_and\_designs$Well)  
  
#What does the smoothed data look like compared to the noisy original?  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$Measurements[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "Abs")  
lines(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$smoothed[  
 example\_data\_and\_designs$Well == "A2"])



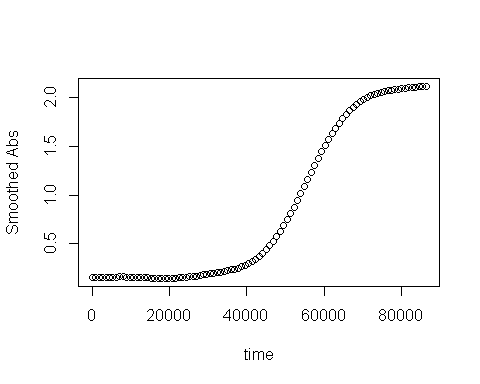
# Processing data: calculating derivatives

In many cases, identifying features of a growth curve requires looking not only at the absorbance data over time, but the slope of the absorbance data over time. gcplyr includes a calc\_deriv function that can be used to calculate the empirical derivative (slope) of absorbance data over time.

*If you’ve previously smoothed your absorbance data, remember to use those smoothed values rather than the original values!*

**Here’s the smoothed absorbance data we’ll be getting the derivatives of:**

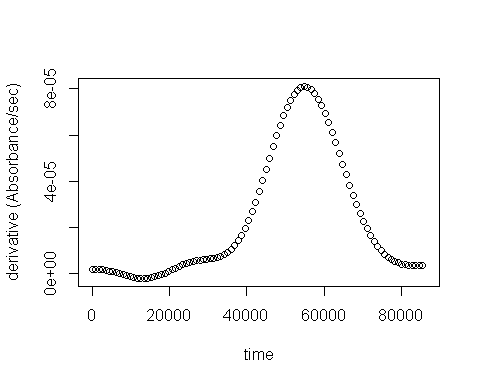
#Let's plot the smoothed absorbance to remind ourselves what it looks like  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$smoothed[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "Smoothed Abs")



## A simple derivative

To calculate a simple derivative using calc\_deriv, we simply have to provide the x and y values, along with a vector of subset\_by values differentiating our unique growth curves (here, the different wells). (Note: if you’re using calc\_deriv within dplyr::mutate, there’s no need to use the subset\_by argument)

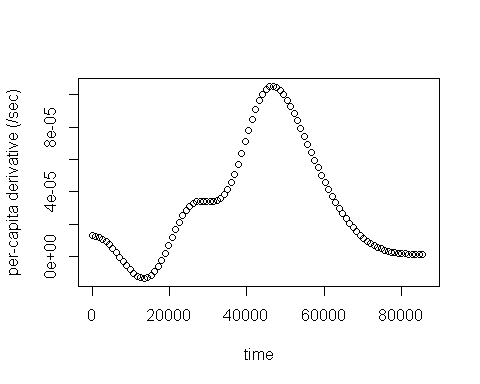
example\_data\_and\_designs$deriv <-  
 calc\_deriv(x = example\_data\_and\_designs$Time,  
 y = example\_data\_and\_designs$smoothed,  
 subset\_by = example\_data\_and\_designs$Well)  
  
#Now let's plot the derivative  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$deriv[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "derivative (Absorbance/sec)")



## Per-capita derivative

calc\_deriv can also return the per-capita derivative. Just as before, provide the x and y values, along with a vector of subset\_by values (as needed), but now set percapita = TRUE

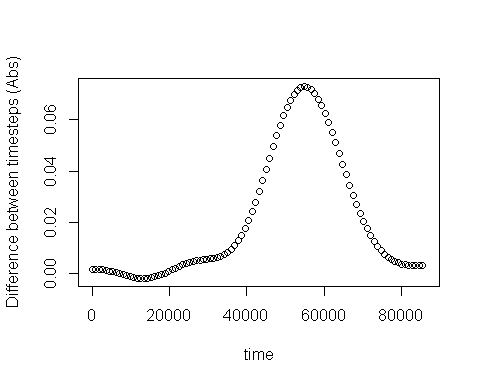
example\_data\_and\_designs$deriv\_percap <-  
 calc\_deriv(x = example\_data\_and\_designs$Time,  
 y = example\_data\_and\_designs$smoothed,  
 subset\_by = example\_data\_and\_designs$Well,  
 percapita = TRUE)  
  
#Now let's plot the per-capita derivative  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$deriv\_percap[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "per-capita derivative (/sec)")



## Finite differences

If, instead of derivatives, you simply want the difference between each subsequent y value, you can set scale\_x = NA (in which case, you also don’t need to provide the x values). (This looks very similar to our original derivative plot because in the example data all timepoints are equally spaced)

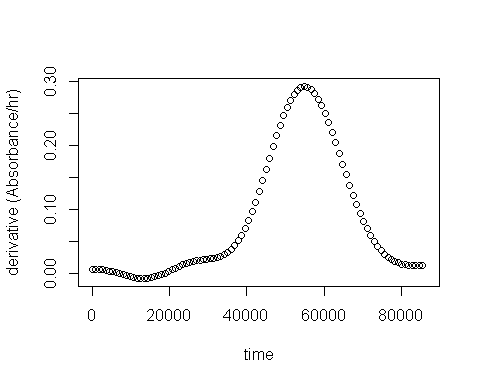
example\_data\_and\_designs$difference <-  
 calc\_deriv(y = example\_data\_and\_designs$smoothed,  
 subset\_by = example\_data\_and\_designs$Well,  
 x\_scale = NA)  
  
#Now let's plot the finite differences  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$difference[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "Difference between timesteps (Abs)")



## Changing the derivative units

Finally, if you want your derivative in units different from those that x is provided in, you can specify the ratio of your x units to the desired units with x\_scale as well. For instance, in our example data x is the number of seconds since the growth curve began. What if we wanted growth rate in per-hour? There are 3600 seconds in an hour, so we set x\_scale = 3600

example\_data\_and\_designs$deriv\_hr <-  
 calc\_deriv(x = example\_data\_and\_designs$Time,  
 y = example\_data\_and\_designs$smoothed,  
 subset\_by = example\_data\_and\_designs$Well,  
 x\_scale = 3600)  
  
#Now let's plot the derivative in units of Abs/hour  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$deriv\_hr[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "derivative (Absorbance/hr)")



# Analyzing data with summarize

Ultimately, analyzing growth curves requires summarizing the entire time series of data by some metric or metrics. For instance, we may calculate the maximum density, maximum per-capita growth rate, or total area under the curve. gcplyr contains a number of functions to assist with these calculations.

However, before we can explore how to use those functions, we need to familiarize ourselves with the dplyr functions group\_by and summarize. Why? Because the upcoming gcplyr functions need to be used *within* dplyr::summarize. **If you’re already familiar with dplyr, feel free to skip the next section.** If you’re not familiar yet, don’t worry! Continue to the next section, where we provide a primer on using group\_by and summarize that will teach you all you need to know for.

## A brief primer on dplyr: grouping and summarize

The R package dplyr provides a “grammar of data manipulation” that is useful for a broad array of data analysis tasks (in fact, dplyr is the direct inspiration for the name of this package!) For our purposes, we’re going to focus on two particular functions: group\_by and summarize (also available as summarise).

The group\_by functions in dplyr allow users to group the rows of their data.frame’s into groups. Then, summarize will carry out user-specified calculations on *each* group independently, producing a new data.frame where each group is a single row. For growth curves, this means we will group\_by our data so that every well is a group, and then we’ll summarize each well with calculations like maximum density or area under the curve.

Let’s work through an example. First, we need to group our data. group\_by simply requires the data.frame to be grouped, and the names of the columns we want to group by.

library(dplyr)  
grouped\_example\_data\_and\_designs <-   
 group\_by(example\_data\_and\_designs,  
 Bacteria\_strain, Phage, Well)

Since dplyr will drop any columns that the data aren’t grouped by, we will typically want to list all of our design columns, and the plate name and well. Make sure you’re *not* grouping by Time, Absorbance, or anything else that varies *within* a well, since if you do dplyr will group timepoints within a well separately.

Then, we run summarize, specifying the name of the summarized column and the function that calculates the summary output. For instance, in the code below we’ve calculated the minimum smoothed absorbance each well reached at any point in its growth.

example\_data\_and\_designs\_sum <-  
 summarize(grouped\_example\_data\_and\_designs,  
 min\_abs = min(smoothed))  
#> `summarise()` has grouped output by 'Bacteria\_strain', 'Phage'. You can override using the `.groups` argument.  
head(example\_data\_and\_designs\_sum)  
#> # A tibble: 6 x 4  
#> # Groups: Bacteria\_strain, Phage [6]  
#> Bacteria\_strain Phage Well min\_abs  
#> <chr> <chr> <chr> <dbl>  
#> 1 Strain 1 No Phage A1 0.152   
#> 2 Strain 1 Phage Added A7 0.151   
#> 3 Strain 10 No Phage B4 0.124   
#> 4 Strain 10 Phage Added B10 0.134   
#> 5 Strain 11 No Phage B5 0.132   
#> 6 Strain 11 Phage Added B11 0.0741

If you want additional characteristics, you simply add them to the summarize. For instance, we could get the maximum of the per-capita growth rate (note that na.rm is needed to tell max to ignore NA values):

example\_data\_and\_designs\_sum <-  
 summarize(grouped\_example\_data\_and\_designs,  
 min\_abs = min(smoothed),  
 max\_percap\_deriv = max(deriv\_percap, na.rm = TRUE))  
#> `summarise()` has grouped output by 'Bacteria\_strain', 'Phage'. You can override using the `.groups` argument.  
head(example\_data\_and\_designs\_sum)  
#> # A tibble: 6 x 5  
#> # Groups: Bacteria\_strain, Phage [6]  
#> Bacteria\_strain Phage Well min\_abs max\_percap\_deriv  
#> <chr> <chr> <chr> <dbl> <dbl>  
#> 1 Strain 1 No Phage A1 0.152 0.0000594  
#> 2 Strain 1 Phage Added A7 0.151 0.0000607  
#> 3 Strain 10 No Phage B4 0.124 0.000134   
#> 4 Strain 10 Phage Added B10 0.134 0.000129   
#> 5 Strain 11 No Phage B5 0.132 0.000151   
#> 6 Strain 11 Phage Added B11 0.0741 0.000172

That’s all you need to know for now! If you want to learn more, dplyr has extensive documentation and examples of its own online. Feel free to explore them as desired, but this primer should be sufficient to use the remaining gcplyr functions, which have to be used *within* summarize to work correctly.

## Finding local extrema

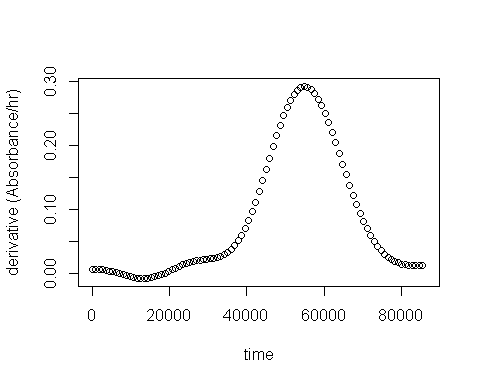
One common analysis step is identifying peaks and valleys in growth curve data, whether it be in the original absorbance data, or in one of the derivatives in the curve. gcplyr has several functions to facilitate identifying these local extrema.

### A common use-case: the first peak

One of the main peaks or valleys users are interested in identifying is the first peak. For instance, in absorbance data, the first peak could be the maximum absorbance reached before the population begins to decline as a result of phages or antibiotics. Whereas in derivative data, the first peak could show the maximum growth rate of the bacteria.

To identify the first peak, use first\_peak. first\_peak simply requires the y data you want to identify the peak in. Let’s use the derivative we calculated in the previous section, since it has a clear peak we might want to identify.

#Let's plot the derivative in units of Abs/hour again  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$deriv\_hr[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "derivative (Absorbance/hr)")



Now let’s identify the peak in our data. First, we’ll group our data using dplyr::group\_by, then use first\_peak inside our summarize command. (Remember to load dplyr with library(dplyr) if you haven’t already)

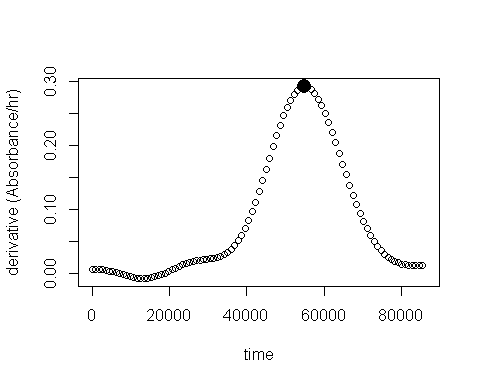
example\_data\_and\_designs\_grouped <-   
 group\_by(example\_data\_and\_designs,  
 Bacteria\_strain, Phage, Well)  
example\_data\_and\_designs\_sum <-  
 summarize(example\_data\_and\_designs\_grouped,  
 first\_peak\_index = first\_peak(deriv\_hr))  
#> `summarise()` has grouped output by 'Bacteria\_strain', 'Phage'. You can override using the `.groups` argument.  
head(example\_data\_and\_designs\_sum)  
#> # A tibble: 6 x 4  
#> # Groups: Bacteria\_strain, Phage [6]  
#> Bacteria\_strain Phage Well first\_peak\_index  
#> <chr> <chr> <chr> <dbl>  
#> 1 Strain 1 No Phage A1 25  
#> 2 Strain 1 Phage Added A7 15  
#> 3 Strain 10 No Phage B4 50  
#> 4 Strain 10 Phage Added B10 50  
#> 5 Strain 11 No Phage B5 46  
#> 6 Strain 11 Phage Added B11 46

By default, first\_peak returns the index of the timepoint where the first peak is located *within* the group. If you want the x or y of the first peak, simply set return = "x" or return = "y". Note that if return = "x", you must specify the x values to first\_peak

example\_data\_and\_designs\_sum <-  
 summarize(example\_data\_and\_designs\_grouped,  
 first\_peak\_x = first\_peak(deriv\_hr, x = Time, return = "x"),  
 first\_peak\_y = first\_peak(deriv\_hr, return = "y"))  
#> `summarise()` has grouped output by 'Bacteria\_strain', 'Phage'. You can override using the `.groups` argument.  
head(example\_data\_and\_designs\_sum)  
#> # A tibble: 6 x 5  
#> # Groups: Bacteria\_strain, Phage [6]  
#> Bacteria\_strain Phage Well first\_peak\_x first\_peak\_y  
#> <chr> <chr> <chr> <dbl> <dbl>  
#> 1 Strain 1 No Phage A1 21600 0.00660  
#> 2 Strain 1 Phage Added A7 12600 0.00386  
#> 3 Strain 10 No Phage B4 44100 0.358   
#> 4 Strain 10 Phage Added B10 44100 0.357   
#> 5 Strain 11 No Phage B5 40500 0.382   
#> 6 Strain 11 Phage Added B11 40500 0.432

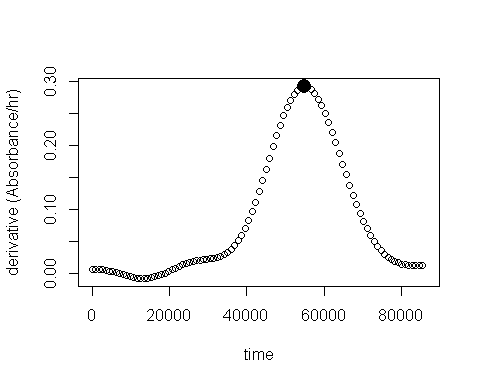
And now that we have x and y values, we can plot them to confirm that first\_peak finds what we expect.

plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$deriv\_hr[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "derivative (Absorbance/hr)")  
points(x = example\_data\_and\_designs\_sum$first\_peak\_x[  
 example\_data\_and\_designs\_sum$Well == "A2"],  
 y = example\_data\_and\_designs\_sum$first\_peak\_y[  
 example\_data\_and\_designs\_sum$Well == "A2"],  
 pch = 16, cex = 2)



Here we can see that first\_peak has found *a* peak, but perhaps not the large one we’re primarily interested in. If we want first\_peak to be less sensitive to local peaks, we can increase the width\_limit argument (which defaults to 20% of the length of y, in this case = 19).

example\_data\_and\_designs\_sum <-  
 summarize(example\_data\_and\_designs\_grouped,  
 first\_peak\_x = first\_peak(deriv\_hr, x = Time, return = "x",  
 width\_limit = 39),  
 first\_peak\_y = first\_peak(deriv\_hr, return = "y",  
 width\_limit = 39))  
#> `summarise()` has grouped output by 'Bacteria\_strain', 'Phage'. You can override using the `.groups` argument.  
head(example\_data\_and\_designs\_sum)  
#> # A tibble: 6 x 5  
#> # Groups: Bacteria\_strain, Phage [6]  
#> Bacteria\_strain Phage Well first\_peak\_x first\_peak\_y  
#> <chr> <chr> <chr> <dbl> <dbl>  
#> 1 Strain 1 No Phage A1 21600 0.00660  
#> 2 Strain 1 Phage Added A7 12600 0.00386  
#> 3 Strain 10 No Phage B4 44100 0.358   
#> 4 Strain 10 Phage Added B10 44100 0.357   
#> 5 Strain 11 No Phage B5 40500 0.382   
#> 6 Strain 11 Phage Added B11 40500 0.432  
plot(example\_data\_and\_designs$Time[  
 example\_data\_and\_designs$Well == "A2"],  
 example\_data\_and\_designs$deriv\_hr[  
 example\_data\_and\_designs$Well == "A2"],  
 xlab = "time", ylab = "derivative (Absorbance/hr)")  
points(x = example\_data\_and\_designs\_sum$first\_peak\_x[  
 example\_data\_and\_designs\_sum$Well == "A2"],  
 y = example\_data\_and\_designs\_sum$first\_peak\_y[  
 example\_data\_and\_designs\_sum$Well == "A2"],  
 pch = 16, cex = 2)

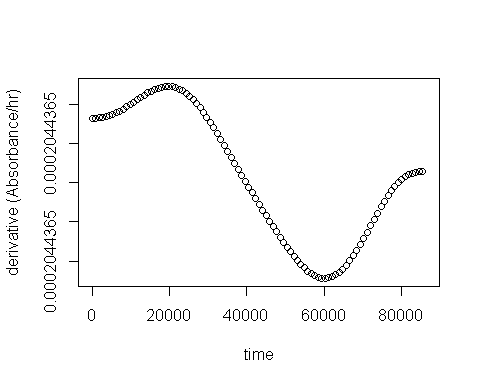


Great! However, if we look at all the summarized data, some of the curves have NA for their first peak information.

head(example\_data\_and\_designs\_sum[  
 is.na(example\_data\_and\_designs\_sum$first\_peak\_x), ])  
#> # A tibble: 6 x 5  
#> # Groups: Bacteria\_strain, Phage [6]  
#> Bacteria\_strain Phage Well first\_peak\_x first\_peak\_y  
#> <chr> <chr> <chr> <dbl> <dbl>  
#> 1 Strain 15 Phage Added C9 NA NA  
#> 2 Strain 17 No Phage C5 NA NA  
#> 3 Strain 23 Phage Added D11 NA NA  
#> 4 Strain 24 Phage Added D12 NA NA  
#> 5 Strain 28 No Phage E4 NA NA  
#> 6 Strain 30 Phage Added E12 NA NA

Why is that? Let’s take a look at one of them:

#Let's plot the absorbance  
plot(example\_data\_and\_designs$Time[example\_data\_and\_designs$Well == "F1"],  
 example\_data\_and\_designs$deriv\_hr[example\_data\_and\_designs$Well == "F1"],  
 xlab = "time", ylab = "derivative (Absorbance/hr)")



Here, we can see that this derivative curve doesn’t have a local peak in the data. By design, first\_peak only identifies peaks *within* the data, ignoring any peaks at the endpoints of the data. In the next section, we’ll learn how to use find\_local\_extrema to identify other kinds of local extrema, including peaks at the beginning or end of the growth curve.

### Finding any kind of local extrema

We’ve seen how first\_peak can be used to identify the first peak. But what about other extrema in the data? The first minimum? Or peaks that occur at the beginning or end of the data? The *second* peak? Etc.

In order to identify these kinds of extrema, we can use the more-general function find\_local\_extrema. find\_local\_extrema works very similarly to first\_peak, but with a few additional options that users can specify to get exactly the kinds of peaks and valleys they want.

Just like first\_peak, find\_local\_extrema only requires a vector of y data in which to find the local extrema, and will return the index of the extrema *within* the current group. By altering the return argument to return = "x" or return = "y", find\_local\_extrema will return x and y values rather than indices.

Unlike first\_peak, find\_local\_extrema returns a vector containing *all* of the local extrema found under the given settings. Users can alter which kinds of local extrema are reported using the arguments return\_maxima, return\_minima, and return\_endpoints.

Additionally, with find\_local\_extrema, users must specify at least one of the tuning parameters: width\_limit or height\_limit. These parameters control how sensitive the function is to smaller local peaks and valleys. width\_limit is the number of data points wide the algorithm will search at each step, meaning that a smaller width\_limit will be more sensitive to narrow peaks and valleys. height\_limit (in units of y) limits the depth of the peaks and valleys the algorithm will search over at each step, meaning that a smaller height\_limit will be more sensitive to shallow peaks and valleys.

## Threshold identification

## Area under the curve