

Processing data

Mike Blazanin

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Where are we so far?

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2. Importing and transforming data: `vignette("gc02_import_reshape")`
3. Incorporating experimental designs: `vignette("gc03_incorporate_designs")`
4. Pre-processing and plotting your data: `vignette("gc04_preprocess_plot")`
5. **Processing your data:** `vignette("gc05_process")`
6. Analyzing your data: `vignette("gc06_analyze")`
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So far, we've imported and transformed our measures, combined them with our design information, and pre-processed and plotted our data. Now we're going to do some processing of our raw data: calculating derivatives.

If you haven't already, load the necessary packages.

```
library(gcplyr)
library(dplyr)
library(ggplot2)
```

```

# This code was previously explained
# Here we're re-running it so it's available for us to work with
example_tidydata <- trans_wide_to_tidy(example_widedata_noiseless,
                                     id_cols = "Time")

example_design <- make_design(
  pattern_split = ",", nrows = 8, ncols = 12,
  "Bacteria_strain" = make_designpattern(
    values = paste("Strain", 1:48),
    rows = 1:8, cols = 1:6, pattern = 1:48, byrow = TRUE),
  "Bacteria_strain" = make_designpattern(
    values = paste("Strain", 1:48),
    rows = 1:8, cols = 7:12, pattern = 1:48, 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"))
ex_dat_mrg <- merge_dfs(example_tidydata, example_design)
#> Joining, by = "Well"
ex_dat_mrg$Well <-
  factor(ex_dat_mrg$Well,
         levels = paste(rep(LETTERS[1:8], each = 12), 1:12, sep = ""))
#Convert time to hours
ex_dat_mrg$Time <- ex_dat_mrg$Time/3600

```

How to process and analyze your data

With your data and design information pre-processed, **your dataset is now organized in a way that's easy to export and analyze.**

Broadly speaking, there are two main approaches to analyzing growth curves data:

1. directly quantify attributes of the growth dynamics (what **gcplyr** does)
2. fit the growth dynamics with a mathematical model, then extract parameters from the fitted model (what **other packages** do, see vignette("08_conclusion"))

At this point, since the data is now well-organized, advanced users may also decide they want to write their own custom analyses (in lieu of, or alongside, **gcplyr**-based and/or fitting-based analyses).

So, how do we directly quantify attributes of growth curves? Generally, we find features of the density data and its derivatives. Different projects may desire different analyses, so this article and the Analyzing Data and Dealing with Noise articles are written to highlight common analyses, rather than prescribing what everyone should do.

Below I list common metrics that require derivatives to be calculated. If you intend to calculate any of those metrics, or just want to calculate or plot the derivatives, continue reading. Otherwise, feel free to skip right to the Analyzing Data article.

Metrics requiring derivatives:

- the lag time
- the time to reach some growth rate
- the maximum per-capita growth rate (i.e. minimum doubling time)
- the inflection point

- the density and time when a diauxic shift occurs
- the maximum per-capita growth rate during diauxie

Before we dig into calculating derivatives, we first need to familiarize ourselves with the `dplyr` package and its functions `group_by` and `mutate`. Why? Because the upcoming `gcplyr` processing functions are *best* used **within** `dplyr::mutate`. **If you're already familiar with `dplyr`, feel free to skip straight to Calculating Derivatives.** If you're not familiar yet, the primer will teach you all you need to know to use `gcplyr`.

A brief primer on `dplyr`

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 `gcplyr`!) For our purposes, we're going to focus on two functions: `group_by` and `mutate`.

The `mutate` function in `dplyr` allows users to easily create new columns in their `data.frame`'s. For us, we're going to use `mutate` to create columns with the derivatives we calculate. However, we want to make sure that derivative-calculating is done on *each* unique well independently. In order to do that, we're first going to use the `group_by` function, which allows users to group the rows of their `data.frame`'s into groups that `mutate` will then treat independently.

For growth curves, this means we will:

1. `group_by` our data so that every unique well is a group
2. `mutate` to create new columns with our calculated derivatives

For `group_by`, we need to specify the `data.frame` to be grouped, and then we want to list all the columns needed to identify each unique well in our dataset. Typically, this includes all of our design columns along with the plate name and well name. 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.

To use `mutate`, we simply have to specify:

1. the name of the variable we want results saved to
2. the function that calculates the new column

If you want additional columns, you simply add them to the `mutate`.

As you'll see throughout the rest of this article, we'll be using `group_by` and `mutate` to calculate derivatives. If you want to learn more, `dplyr` has extensive documentation and examples of its own online, but this primer and the coming examples should be sufficient to calculate derivatives with `gcplyr`.

Processing data: calculating derivatives

There are two derivatives we are primarily interested in calculating:

- the plain derivative - the slope of the original density data
- the per-capita derivative - the growth rate of the cells

`gcplyr` includes a `calc_deriv` to calculate both of these.

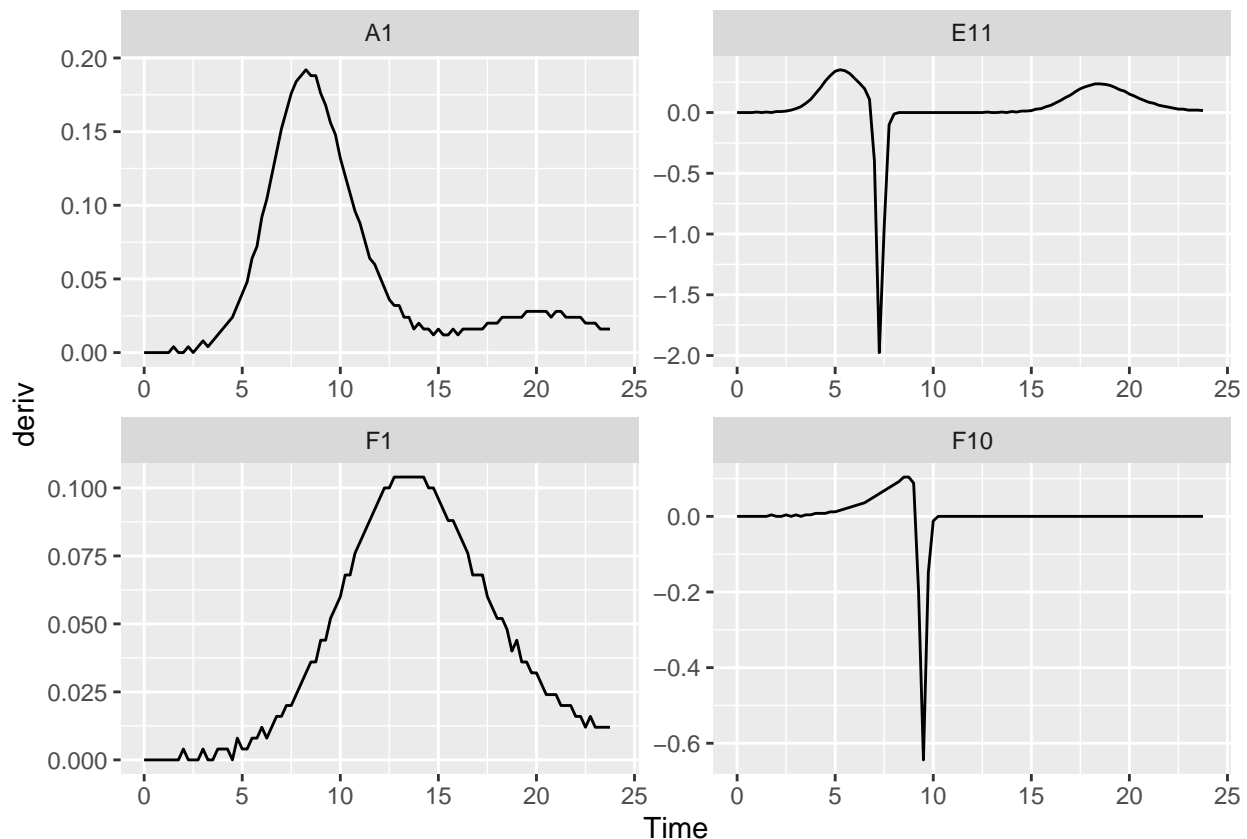
A simple derivative

To calculate a simple derivative (the slope of our original data) using `calc_deriv`, we simply have to provide the x and y values. Note that this is **not** the growth rate of the cells, but rather is a measure of how quickly the whole population was growing at each time point.

```
ex_dat_mrg <- mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage),  
  deriv = calc_deriv(x = Time, y = Measurements))
```

To visualize these results, let's look at a few wells that are representative of the overall diversity of dynamics in our example data. (In your own code, you should visualize all your data).

```
sample_wells <- c("A1", "F1", "F10", "E11")  
  
# Now let's plot the derivative  
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),  
  aes(x = Time, y = deriv)) +  
  geom_line() +  
  facet_wrap(~Well, scales = "free")  
#> Warning: Removed 1 row containing missing values (`geom_line()`).
```



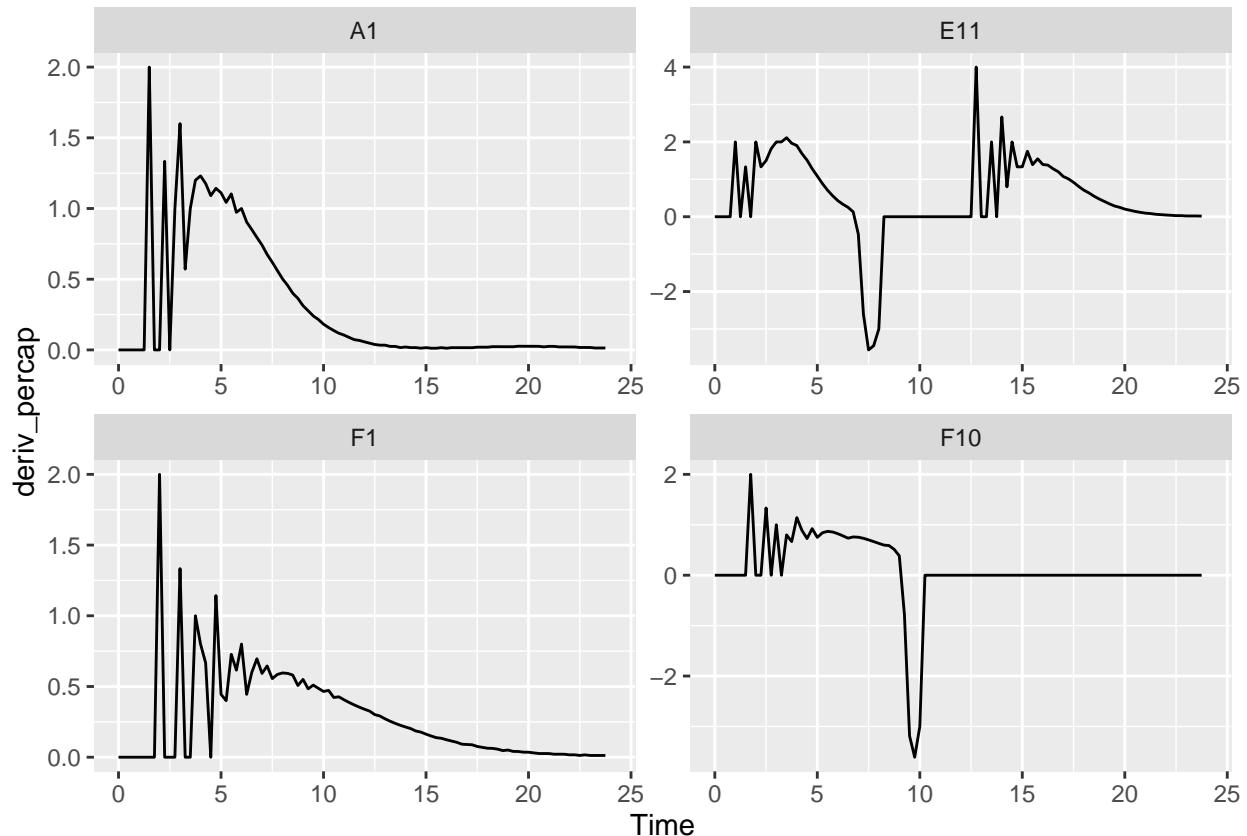
You might notice that these lines aren't super smooth. Why? The plate reader data has a limited resolution, only to the nearest 0.001, causing the derivative to "jump" when the reading increases.

Per-capita derivative

To calculate the per-capita derivative, we simply modify our use of `calc_deriv` with the argument `percapita = TRUE`. Note that in this case, you are required to specify a blank value, i.e. the value of your `Measurements` that corresponds to a population density of 0. If your data have already been normalized, simply add `blank = 0`.

```
ex_dat_mrg <- mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage),
                     deriv_percap = calc_deriv(x = Time, y = Measurements,
                                                percapita = TRUE, blank = 0))

# Now let's plot the per-capita derivative
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = deriv_percap)) +
  geom_line() +
  facet_wrap(~Well, scales = "free")
#> Warning: Removed 1 row containing missing values (`geom_line()`).
```



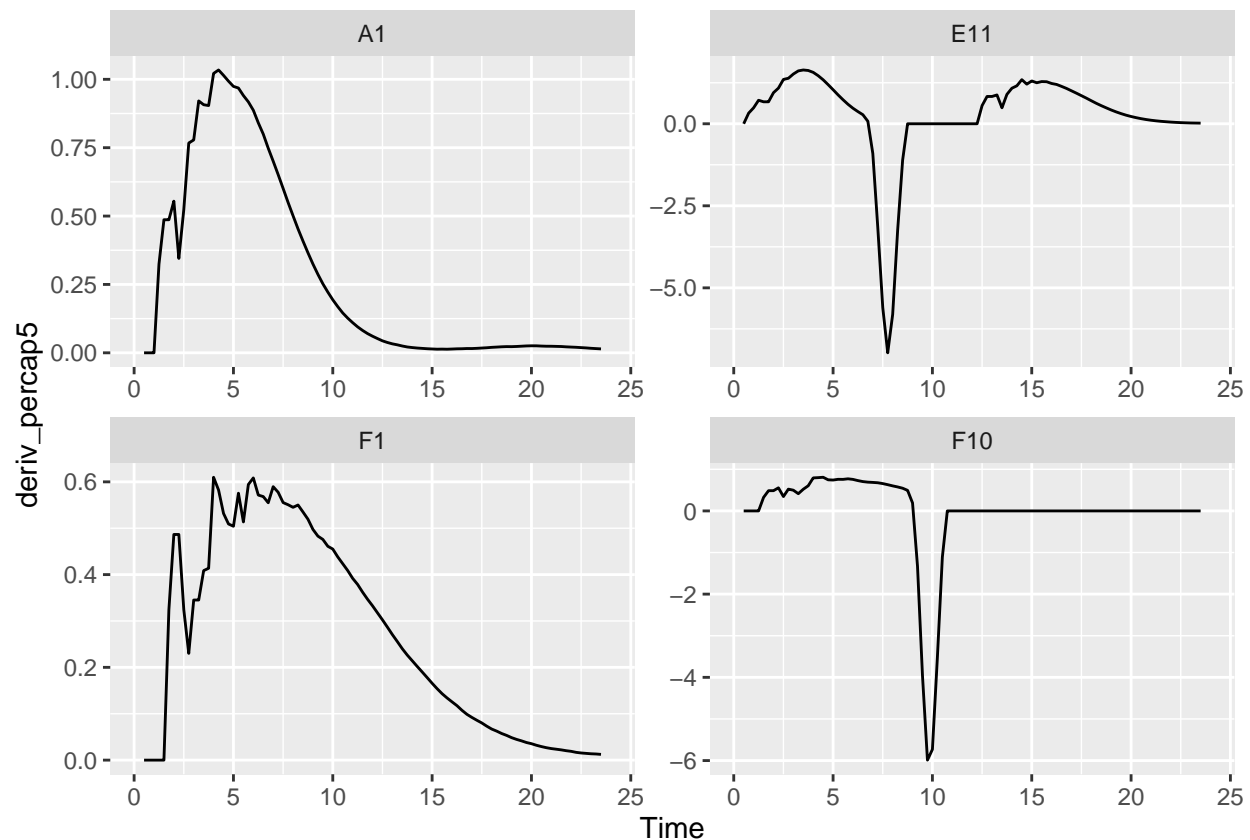
These derivatives are very jumpy. Why? The same limited resolution of the plate reader has an amplified effect on the per-capita derivative when densities are very close to 0. Luckily, `calc_deriv` can calculate derivatives by fitting a linear regression to multiple points, reducing this jumpiness in the derivative.

To use this fitting functionality of `calc_deriv`, specify either the `window_width` or `window_width_n` parameter. `window_width` specifies how wide the window used to include points for the fitting is in units of `x`, while `window_width_n` specifies it in number of data points. I recommend trying a `window_width_n` of three or five data points, since that works for most cases.

For best practice, I recommend doing this fitting on log-transformed y values, since exponentially growing density values are linear when log-transformed. You can achieve this simply by setting `trans_y = 'log'`. With log-transformation, note that `calc_deriv` will return NA for any data points where the reading is equal to or below your `blank` value.

```
ex_dat_mrg <- mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage),
                     deriv_percap5 = calc_deriv(x = Time, y = Measurements,
                                                percapita = TRUE, blank = 0,
                                                window_width_n = 5, trans_y = "log"))

# Now let's plot the derivative
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = deriv_percap5)) +
  geom_line() +
  facet_wrap(~Well, scales = "free")
#> Warning: Removed 4 rows containing missing values (`geom_line()`).
```



Great! The jumpiness has been reduced immensely.

Converting per-capita growth rates into doubling times

If you'd rather express your per-capita growth rates as a doubling time, simply use the `doubling_time` function to convert your per-capita growth rates into equivalent doubling times.

```

ex_dat_mrg <- mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage),
  deriv_percap5 = calc_deriv(x = Time, y = Measurements,
    percapita = TRUE, blank = 0,
    window_width_n = 5, trans_y = "log"),
  doub_time = doubling_time(y = deriv_percap5))
head(ex_dat_mrg)
#> # A tibble: 6 x 9
#> # Groups:   Well, Bacteria_strain, Phage [4]
#>   Time Well Measurements Bacteria_strain Phage      deriv deriv~1 deriv~2 doub~3
#>   <dbl> <fct>          <dbl> <chr>      <chr>      <dbl>    <dbl>    <dbl>    <dbl>
#> 1  0    A1            0.002 Strain 1    No Phage      0         0        NA        NA
#> 2  0    F1            0.002 Strain 31    No Phage      0         0        NA        NA
#> 3  0    F10          0.002 Strain 34    Phage Added   0         0        NA        NA
#> 4  0    E11          0.002 Strain 29    Phage Added   0         0        NA        NA
#> 5  0.25 A1          0.002 Strain 1    No Phage      0         0        NA        NA
#> 6  0.25 F1          0.002 Strain 31    No Phage      0         0        NA        NA
#> # ... with abbreviated variable names 1: deriv_percap, 2: deriv_percap5,
#> #   3: doub_time

```

What's next?

Now that you've processed your data, you're ready to analyze it!

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