Processing data

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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: smoothing and calculating derivatives.

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

```
library(gcplyr)

library(dplyr)
library(ggplot2)
```

```
#This code was explained in sections 2, 3, and 4
#Here we're re-running it so it's available for us to work with
example_tidydata <- trans_wide_to_tidy(example_widedata,
                                       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 = ""))
```

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**. It is also at this point that the next steps for what you can do diversify into many options.

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

1. directly quantify attributes of the growth dynamics
2. fit the growth dynamics with a mathematical model, then extract parameters from the fitted model

The remaining functions of `gcplyr` can facilitate analyses following the first approach: directly quantifying attributes of the observed dynamics. If you're interested in exploring model-fitting approaches, which can provide enormous analytical power, check out the **[Other growth curve analysis packages]** section. 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? First, we may need to carry out smoothing of our data to reduce the effect of noise. Then, we typically need to calculate derivatives of our (smoothed) data. The (smoothed) density and (smoothed) derivatives will be what we analyze to identify features of our growth curves. `gcplyr` has a number of functions that facilitate these steps.

However, unlike the import, transformation, and merging steps we've done so far, different projects may require different analyses, and not all users will have the same analysis steps. The **Smoothing**, **Calculating Derivatives** and **Analyzing** sections of this document, therefore, are written to highlight the functions available and provide examples of common analyses that you may want to run, rather than prescribing a set of analysis steps that everyone must do.

Before we dig into processing and analyzing our data, 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 this primer.** If you're not familiar yet, don't worry! This section provides a primer that will teach you all you need to know on using `group_by` and `mutate` with `gcplyr` functions.

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 this package!) For our purposes right now, we're going to focus on two particular 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 our smoothed data and the derivatives we calculate. However, we want to make sure that smoothing and derivative-calculating are 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 smoothed data and calculated derivatives

Let's walk through a simple example

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.

```
ex_dat_mrg <- group_by(ex_dat_mrg, Well, Bacteria_strain, Phage)

head(ex_dat_mrg)
#> # A tibble: 6 x 5
#> # Groups:   Well, Bacteria_strain, Phage [6]
#>    Time Well  Measurements Bacteria_strain Phage
#>   <dbl> <fct>        <dbl> <chr>           <chr>
#> 1     0 A1           0.003 Strain 1        No Phage
#> 2     0 B1           0.001 Strain 7        No Phage
#> 3     0 C1           0.002 Strain 13       No Phage
#> 4     0 D1           0.002 Strain 19       No Phage
#> 5     0 E1           0.002 Strain 25       No Phage
#> 6     0 F1           0.001 Strain 31       No Phage
```

Notice that this hasn't changed anything about our `data.frame`, but `R` now knows what the groups are. Now any calculations will be carried out on each unique well independently.

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

Note that the function has to return a vector that is as long as the number of data points in the group.

For a simple example, in the code below we've simply added one to the `Measurements` values and saved it in a column named `Measurements_plus1`:

```
ex_dat_mrg <-
  mutate(ex_dat_mrg,
         Measurements_plus1 = Measurements+1)

head(ex_dat_mrg)
#> # A tibble: 6 x 6
#> # Groups:   Well, Bacteria_strain, Phage [6]
#>    Time Well  Measurements Bacteria_strain Phage    Measurements_plus1
#>   <dbl> <fct>        <dbl> <chr>           <chr>                 <dbl>
#> 1     0 A1           0.003 Strain 1        No Phage               1.00
#> 2     0 B1           0.001 Strain 7        No Phage               1.00
#> 3     0 C1           0.002 Strain 13       No Phage               1.00
#> 4     0 D1           0.002 Strain 19       No Phage               1.00
#> 5     0 E1           0.002 Strain 25       No Phage               1.00
#> 6     0 F1           0.001 Strain 31       No Phage               1.00
```

If you want additional columns, you simply add them to the `mutate`. For instance, if we also want a column with the `Measurements` plus two, we just add that as a second argument:

```
ex_dat_mrg <-
  mutate(ex_dat_mrg,
         Measurements_plus1 = Measurements+1,
         Measurements_plus2 = Measurements+2)

head(ex_dat_mrg)
#> # A tibble: 6 x 7
#> # Groups:   Well, Bacteria_strain, Phage [6]
#>    Time Well  Measurements Bacteria_strain Phage    Measurements_plus1 Measurements_plus2
#>   <dbl> <fct>        <dbl> <chr>           <chr>                 <dbl>               <dbl>
#> 1     0 A1           0.003 Strain 1        No Phage               1.00                2.00
#> 2     0 B1           0.001 Strain 7        No Phage               1.00                2.00
#> 3     0 C1           0.002 Strain 13       No Phage               1.00                2.00
#> 4     0 D1           0.002 Strain 19       No Phage               1.00                2.00
#> 5     0 E1           0.002 Strain 25       No Phage               1.00                2.00
#> 6     0 F1           0.001 Strain 31       No Phage               1.00                2.00
```

This is a rather simple example, but in the next sections I show how we can use `mutate` with `smooth_data` and `calc_deriv` to create new columns containing smoothed data and derivatives. 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 and the coming example should be sufficient to use the `gcplyr` processing functions, which (as a reminder) are best used *within* `mutate`.

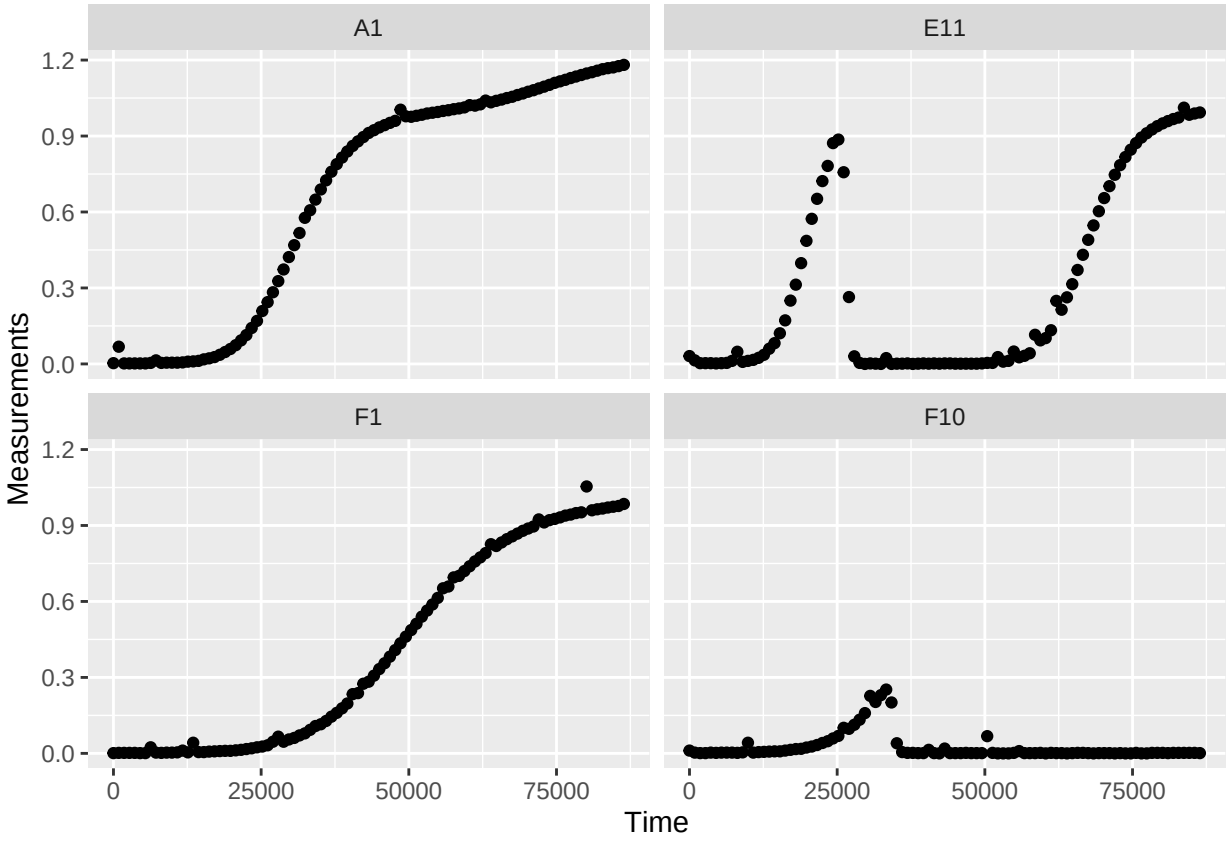
Processing data: smoothing

Oftentimes, growth curve data produced by a plate reader will have some noise it it. While sometimes this noise does not hinder analyses, sometimes it's necessary to smooth the data in each well for analyses to succeed. `gcplyr` has a `smooth_data` function that can carry out such smoothing. Generally you should carry out *as little* smoothing as is necessary for your analyses to work. That means that **right now you should skip this section** and go on to the **Calculating Derivatives** section, returning to this smoothing section if your derivatives are too noisy to analyze.

If you have returned in need of learning to use `smooth_data`, let's start by taking a look at a few wells from our example data, which have some noise.

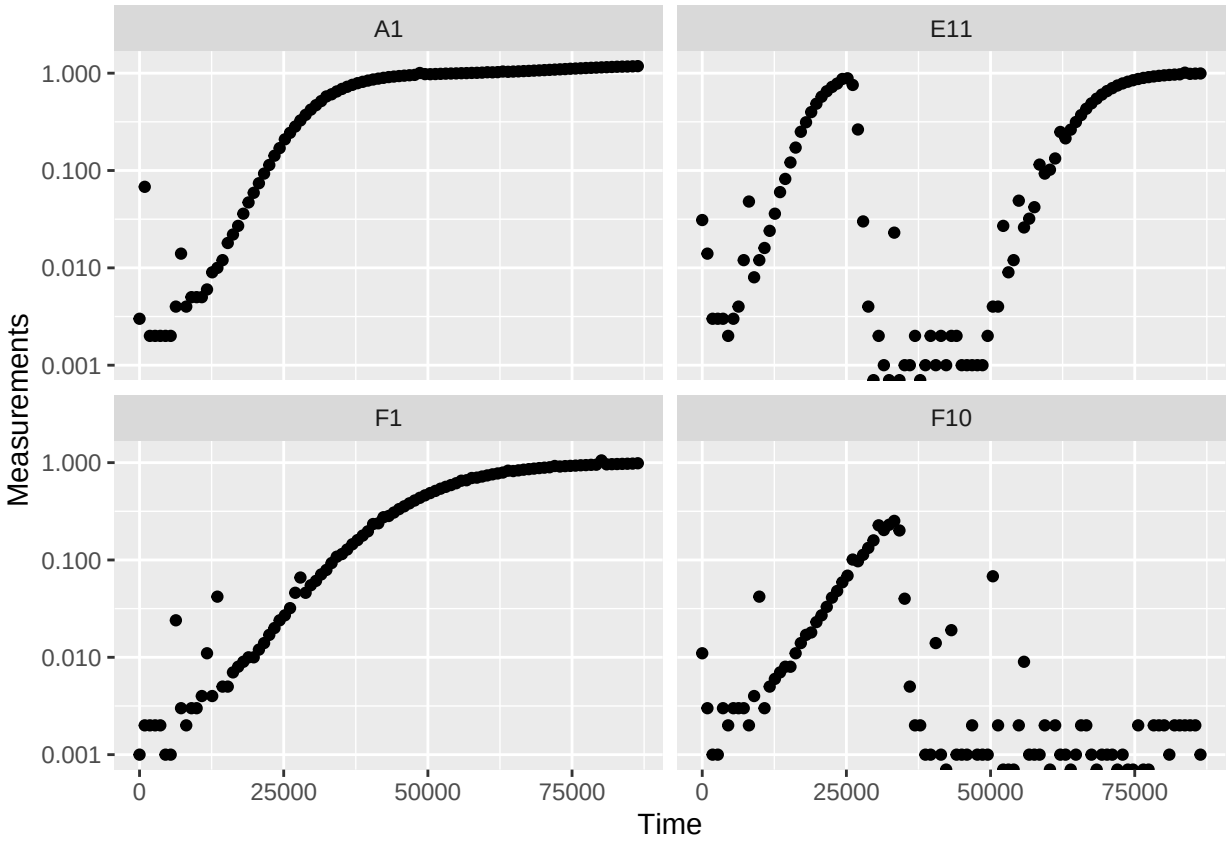
```
#Here we've chosen four wells that, from our previous plot, seem
# representative of the overall diversity of dynamics
#In your own code, you should visualize all your data
sample_wells <- c("A1", "F1", "F10", "E11")

#Plot with a linear y-axis
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = Measurements)) +
  geom_point() +
  facet_wrap(~Well)
```




```
#Plot with a log y-axis
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
```

```
      aes(x = Time, y = Measurements)) +
  geom_point() +
  facet_wrap(~Well) +
  scale_y_continuous(trans = "log10")
#> Warning: Transformation introduced infinite values in continuous y-axis
```



Plotting our data with a log scale for the y-axis is particularly useful for growth curves because exponential growth is a straight line when plotted on a log scale.

From the log plot especially we can see that at low densities there's a lot of noise relative to the density. In fact, this is a common occurrence: **at low densities, random noise tends to have a much larger effect** than at high densities. Unfortunately, calculating derivatives (especially the per-capita derivative) is very sensitive to such noise, so let's smooth our data.

`smooth_data` has four different smoothing algorithms to choose from: `moving-average`, `moving-median`, `loess`, and `gam`.

- `moving-average` is a simple smoothing algorithm that primarily acts to reduce the effects of outliers on the data
- `moving-median` is another simple smoothing algorithm that primarily acts to reduce the effects of outliers on the data
- `loess` is a spline-fitting approach that uses polynomial-like curves, which produces curves with smoothly changing derivatives, but can in some cases create curvature artifacts not present in the original data
- `gam` is also spline-fitting approach that uses polynomial-like curves, which produces curves with smoothly changing derivatives, but can in some cases create curvature artifacts not present in the original data

Additionally, all four smoothing algorithms have a tuning parameter that controls how "smoothed" the data are. For whichever smoothing method you're using, **you should plot smoothing with multiple different tuning parameter values**, then choose the value that smooths the data as little as is necessary to reduce noise. Make sure to plot the smoothing for every well in your data, so that you're choosing the best setting for all your data and not just one well.

Smoothing data is a step that alters the values you will analyze. Because of that, and because there are so many options for how to smooth your data, it is a step that can be rife with pitfalls. I recommend starting with the simplest and least "smoothed" smoothing, plotting your results, and only increasing your smoothing as much as is needed to enable downstream analyses. Additionally, when sharing your findings, it's important to be transparent by sharing the raw data and smoothing methods, rather than treating the smoothed data as your source.

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 we only want to smooth within each unique well, we'll first `group_by` our data:

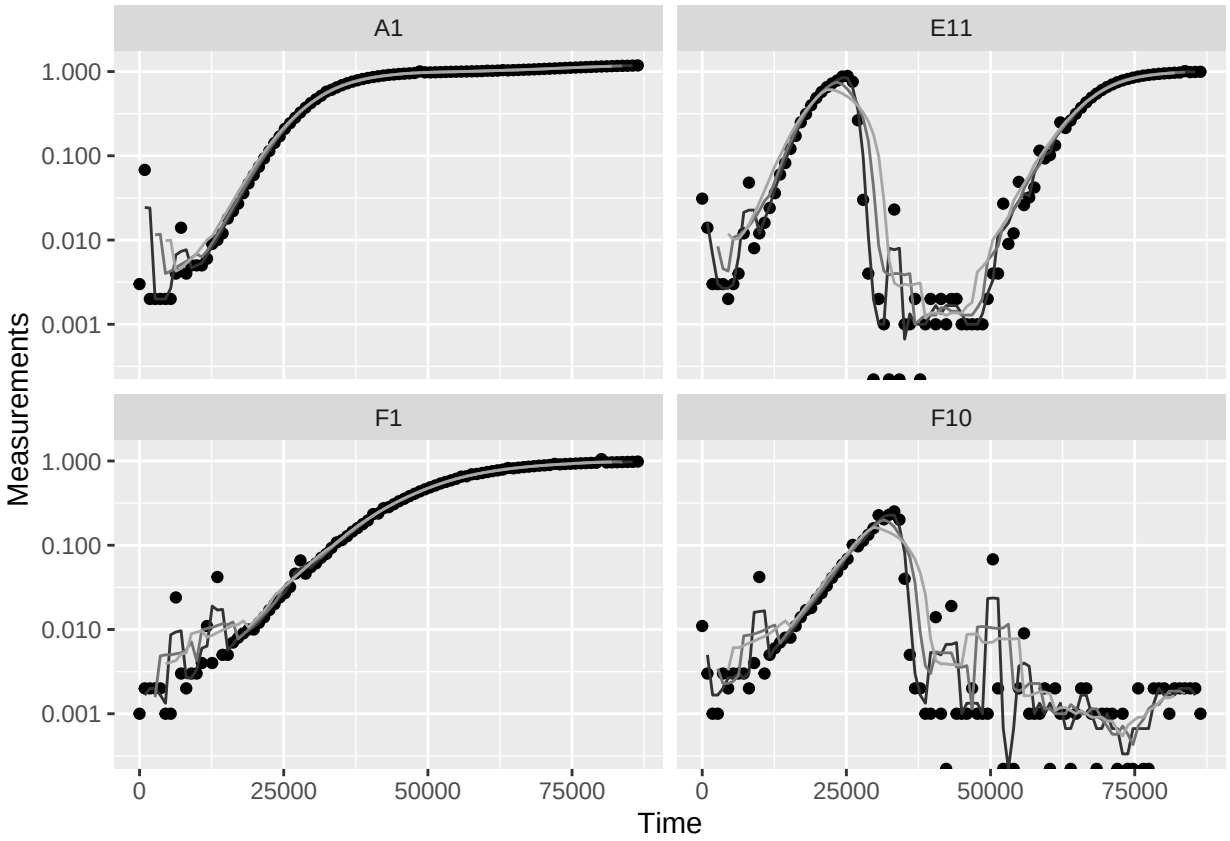
```
ex_dat_mrg <- group_by(ex_dat_mrg, Well, Bacteria_strain, Phage)
```

Smoothing with moving-average

For `moving-average`, the tuning parameter is `window_width_n`, which specifies how many data points wide the moving window used to calculate the average is. Specifying the `window_width_n` is required, and larger values will be more "smoothed". Here, we'll show moving averages with windows that are 3, 7, and 11 data points wide (because the window is centered on each data point, it must be an odd number of data points wide). Note that `moving-average` returns `NA` for the `window_width_n`/2 points at the start and end of your data.

```
ex_dat_mrg <-
  mutate(ex_dat_mrg,
         smoothed3 = smooth_data(x = Time, y = Measurements,
              sm_method = "moving-average", window_width_n = 3),
         smoothed7 = smooth_data(x = Time, y = Measurements,
              sm_method = "moving-average", window_width_n = 7),
         smoothed11 = smooth_data(x = Time, y = Measurements,
              sm_method = "moving-average", window_width_n = 11))

#What does the smoothed data look like compared to the noisy original?
#Lighter lines are wider window_width_n's and more "smoothed"
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time)) +
  geom_point(aes(y = Measurements)) +
  geom_line(aes(y = smoothed3), color = "gray20") +
  geom_line(aes(y = smoothed7), color = "gray45") +
  geom_line(aes(y = smoothed11), color = "gray65") +
  facet_wrap(~Well) +
  scale_y_continuous(trans = "log10")
#> Warning: Transformation introduced infinite values in continuous y-axis
#> Transformation introduced infinite values in continuous y-axis
#> Warning: Removed 2 row(s) containing missing values (geom_path).
#> Warning: Removed 6 row(s) containing missing values (geom_path).
#> Warning: Removed 10 row(s) containing missing values (geom_path).
```



Here we can see that `moving-average` has helped reduce the effects of some of that early noise. However, with `window_width_n = 11` (the lightest line), the smoothing has started biasing our medium-density data points to be higher than they actually are. Based on this, we'd probably want to use a `window_width_n` less than 11. Unfortunately, with smaller `window_width_n` our early data is still being affected by that early noise, so we should explore other smoothing methods, or try combining multiple smoothing methods.

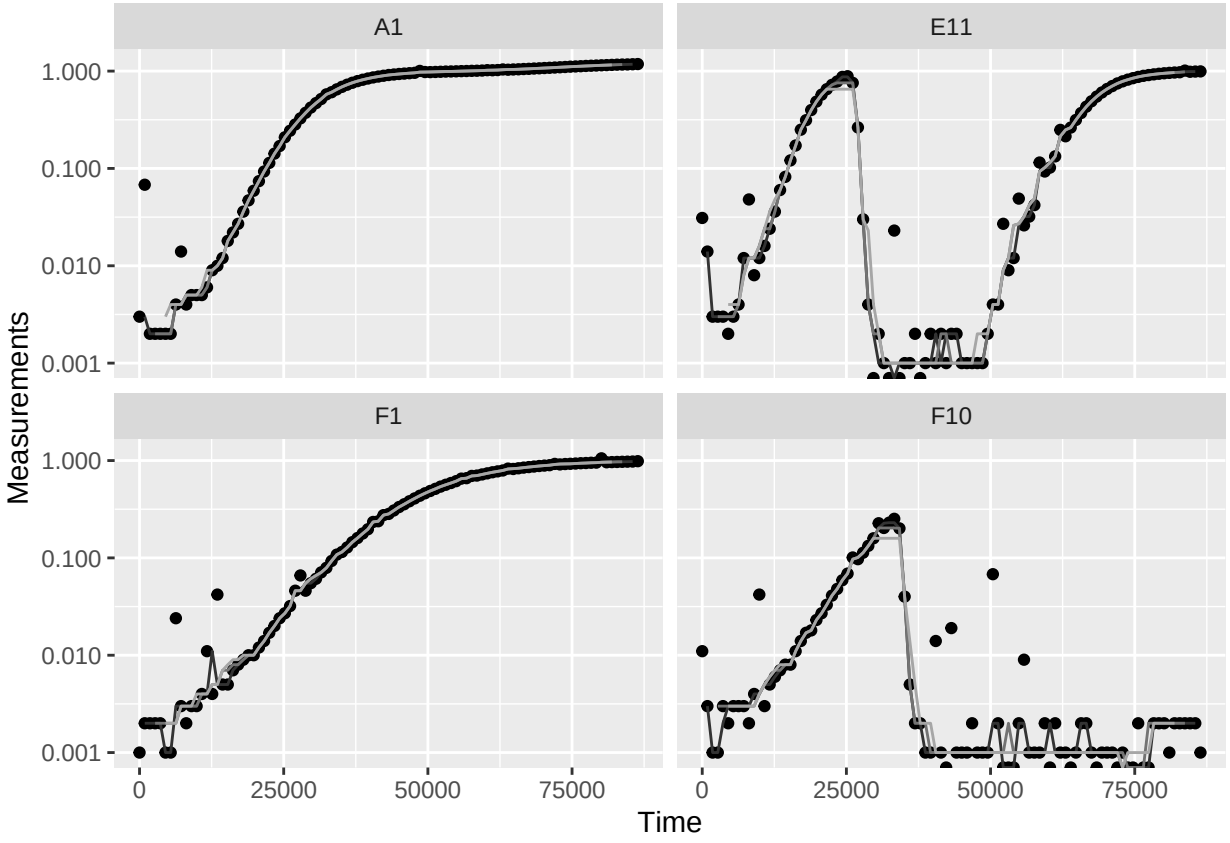
Smoothing with moving-median

For `moving-median`, the tuning parameter is also `window_width_n`, which specifies how many data points wide the moving window used to calculate the average is. Specifying the `window_width_n` is required, and larger values will be more "smoothed". Here, we'll show moving averages with windows that are 3, 7, and 11 data points wide (because the window is centered on each data point, it must be an odd number of data points wide). Note that `moving-median` returns `NA` for the `window_width_n`/2 points at the start and end of your data.

```
ex_dat_mrg <-
  mutate(ex_dat_mrg,
         smoothed3 =
           smooth_data(x = Time, y = Measurements,
                       sm_method = "moving-median", window_width_n = 3),
         smoothed7 =
           smooth_data(x = Time, y = Measurements,
                       sm_method = "moving-median", window_width_n = 7),
         smoothed11 =
           smooth_data(x = Time, y = Measurements,
```

```
                        sm_method = "moving-median", window_width_n = 11))

#What does the smoothed data look like compared to the noisy original?
#Lighter lines are wider window_width_n's and more "smoothed"
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time)) +
  geom_point(aes(y = Measurements)) +
  geom_line(aes(y = smoothed3), color = "gray20") +
  geom_line(aes(y = smoothed7), color = "gray45") +
  geom_line(aes(y = smoothed11), color = "gray65") +
  facet_wrap(~Well) +
  scale_y_continuous(trans = "log10")
#> Warning: Transformation introduced infinite values in continuous y-axis
#> Transformation introduced infinite values in continuous y-axis
#> Transformation introduced infinite values in continuous y-axis
#> Transformation introduced infinite values in continuous y-axis
#> Warning: Removed 2 row(s) containing missing values (geom_path).
#> Warning: Removed 6 row(s) containing missing values (geom_path).
#> Warning: Removed 10 row(s) containing missing values (geom_path).
```



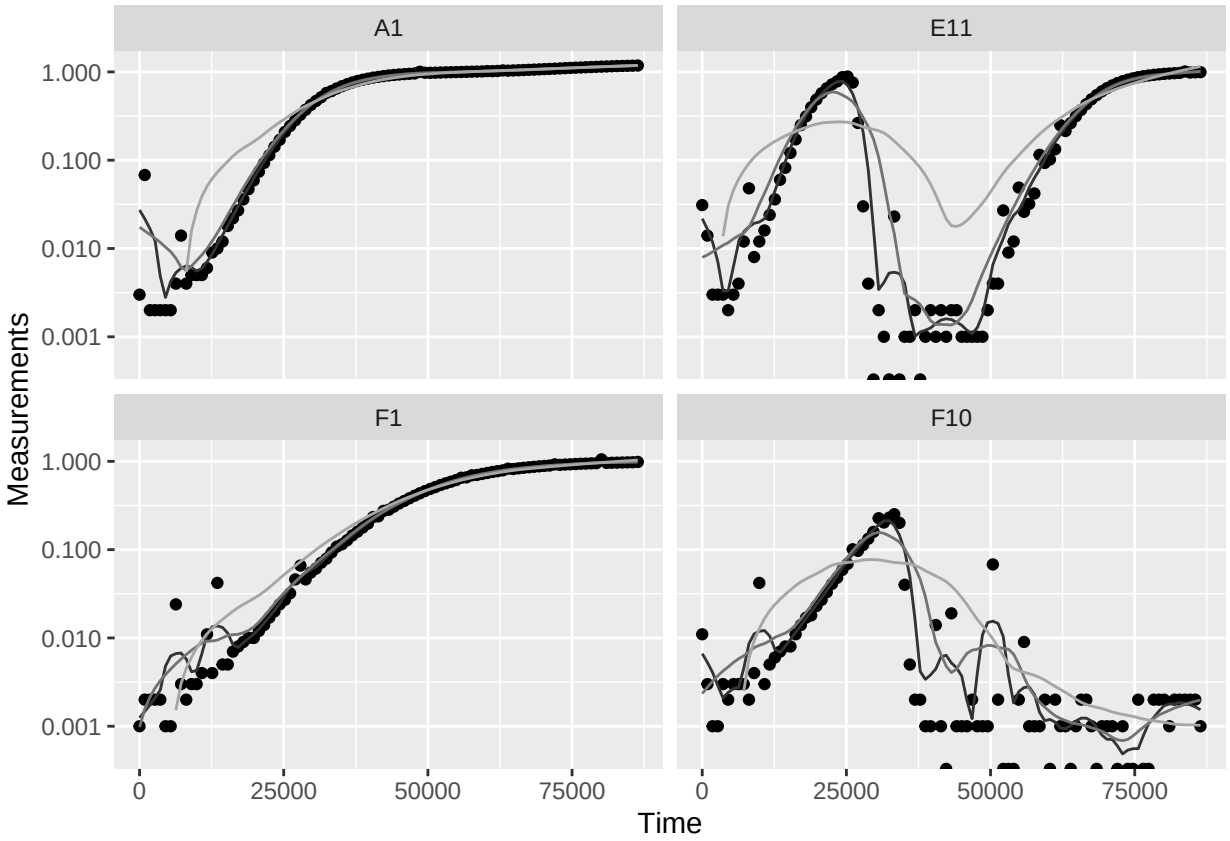
Here we can see that `moving-median` has really excluded that low-density noise, even with the smallest `window_width_n = 3`. Additionally, `moving-median` did not bias our larger data hardly at all, except with the widest `window_width_n`. However, it has produced a smoothed density that is fairly "jumpy", something that wider `window_width_n` did not fix. **This is common with moving-median**, so often you may need to try other smoothing methods or combining `moving-median` with other methods.

Smoothing with LOESS

For `loess`, the tuning parameter is the `span` argument. `loess` works by doing fits on subset windows of the data centered at each data point. These fits can be linear (`degree = 1`) or polynomial (typically `degree = 2`). `span` is the width of the window, as a fraction of all data points. For instance, with the default `span` of 0.75, 75% of the data points are included in each window. Thus, span values typically are between 0 and 1 (although see `?loess` for use of `span` values greater than 1), and larger values are more "smoothed". Here, we'll show `loess` smoothing with spans of 0.1, 0.2, and 0.5 and `degree = 1`.

```
ex_dat_mrg <-
  mutate(ex_dat_mrg,
         smoothed1 = smooth_data(x = Time, y = Measurements,
                                 sm_method = "loess", span = .1, degree = 1),
         smoothed2 = smooth_data(x = Time, y = Measurements,
                                 sm_method = "loess", span = .2, degree = 1),
         smoothed5 = smooth_data(x = Time, y = Measurements,
                                 sm_method = "loess", span = .5, degree = 1))

#What does the smoothed data look like compared to the noisy original?
#Lighter lines are larger span's and more "smoothed"
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time)) +
  geom_point(aes(y = Measurements)) +
  geom_line(aes(y = smoothed1), color = "gray20") +
  geom_line(aes(y = smoothed2), color = "gray45") +
  geom_line(aes(y = smoothed5), color = "gray65") +
  facet_wrap(~Well) +
  scale_y_continuous(trans = "log10")
#> Warning: Transformation introduced infinite values in continuous y-axis
#> Warning in self$trans$transform(x): NaNs produced
#> Warning: Transformation introduced infinite values in continuous y-axis
#> Warning: Removed 9 row(s) containing missing values (geom_path).
```



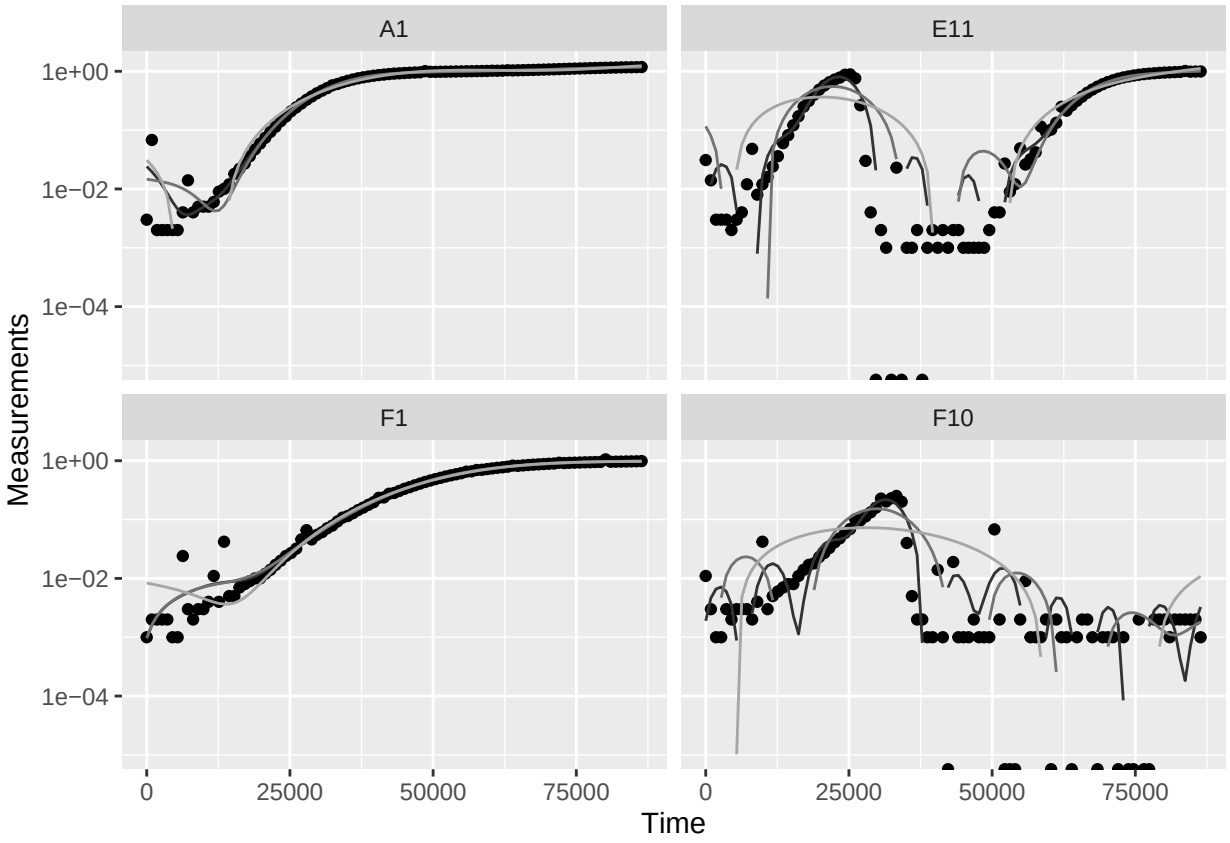
Here we can see that `loess` with smaller spans (darker lines) have smoothed the data somewhat but are still sensitive to outliers. However, `loess` with a larger span (lightest line) has introduced significant bias. To fix this, we might explore other smoothing methods, or combining `loess` with other smoothing methods.

Smoothing with GAM

For `gam`, the primary tuning parameter is the `k` argument. `gam` works by doing fits on subsets of the data and linking these fits together. `k` determines how many link points ("knots") it can use. If not specified, the default `k` value for smoothing a time series is 10, with **smaller values being more "smoothed"** (note this is opposite the trend with other smoothing methods). However, **unlike earlier methods, `k` values that are too large are also problematic**, as they will tend to 'overfit' the data. `k` cannot be larger than the number of data points, and should usually be substantially smaller than that. Also note that **`gam` can sometimes create artifacts**, especially oscillations in your density and derivatives. You should check that `gam` is not doing so before carrying on with your analyses. Here, we'll show `gam` smoothing with `k` values of 5, 10, and 20.

```
ex_dat_mrg <-
  mutate(ex_dat_mrg,
         smoothed20 = smooth_data(x = Time, y = Measurements,
                                  sm_method = "gam", k = 20),
         smoothed10 = smooth_data(x = Time, y = Measurements,
                                  sm_method = "gam", k = 10),
         smoothed5 = smooth_data(x = Time, y = Measurements,
                                 sm_method = "gam", k = 5))
```

```
#What does the smoothed data look like compared to the noisy original?
#Lighter lines are smaller k and more "smoothed"
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time)) +
  geom_point(aes(y = Measurements)) +
  geom_line(aes(y = smoothed20), color = "gray20") +
  geom_line(aes(y = smoothed10), color = "gray45") +
  geom_line(aes(y = smoothed5), color = "gray65") +
  facet_wrap(~Well) +
  scale_y_continuous(trans = "log10")
#> Warning: Transformation introduced infinite values in continuous y-axis
#> Warning in self$trans$transform(x): NaNs produced
#> Warning: Transformation introduced infinite values in continuous y-axis
#> Warning in self$trans$transform(x): NaNs produced
#> Warning: Transformation introduced infinite values in continuous y-axis
#> Warning in self$trans$transform(x): NaNs produced
#> Warning: Transformation introduced infinite values in continuous y-axis
```



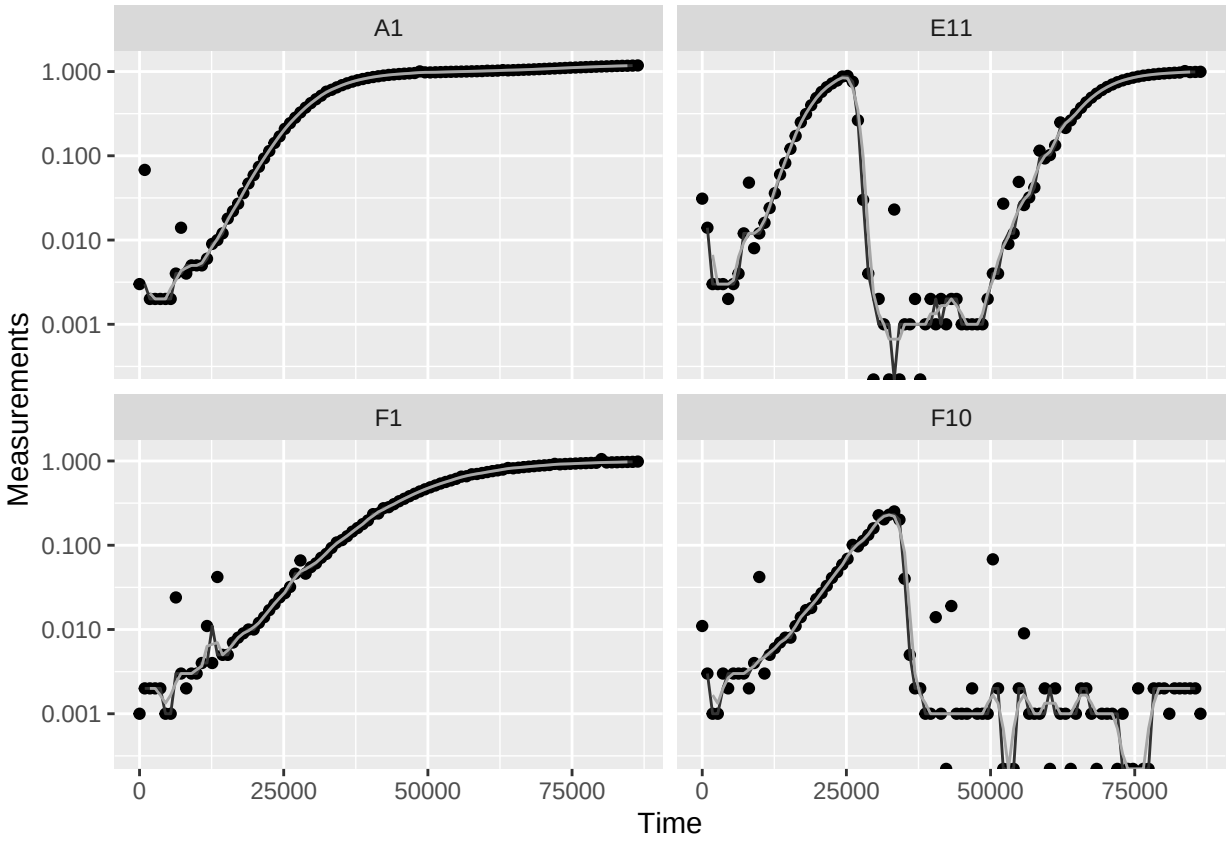
Here we can see that `gam` does alright when working with the no phage-added wells (A1 and F1): higher `k` values (darkest line) have smoothed the data but are still sensitive to those early outliers, while lower `k` values (lighter lines) have introduced significant bias. However, `gam` is struggling when phage have been added (E11 and F10). Across all the `k` values it has added many fluctuations and often dips into values of 0 or lower (plotted here as breaks in the line, since the log of numbers <= 0 are undefined). To fix this, we might explore other smoothing methods or combining `gam` with other smoothing methods.

Combining multiple smoothing methods

Often, combining multiple smoothing methods can provide improved results. For instance, `moving-median` is particularly good at removing outliers, but not very good at producing continuously smooth data. In contrast, `moving-average`, `loess`, and `gam` work better at producing continuously smooth data, but aren't as good at removing outliers. Here's an example using the strengths of both `moving-median` and `moving-average`. (Note that earlier columns created in `mutate` are available during creation of later columns, so both can be done in one step):

```
ex_dat_mrg <-
  mutate(ex_dat_mrg,
         smoothed_med3 =
           smooth_data(x = Time, y = Measurements,
                       sm_method = "moving-median", window_width_n = 3),
         #Note that for the second round, we're using the
         #first smoothing as the input y
         smoothed =
           smooth_data(x = Time, y = smoothed_med3,
                       sm_method = "moving-average", window_width_n = 3))

#What does the smoothed data look like compared to the noisy original?
#The first round of smoothing with moving-median is plotted in lighter colors
#The second round of smoothing with moving-average is plotted in darker colors
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time)) +
  geom_point(aes(y = Measurements)) +
  geom_line(aes(y = smoothed_med3), color = "gray20") +
  geom_line(aes(y = smoothed), color = "gray65") +
  facet_wrap(~Well) +
  scale_y_continuous(trans = "log10")
#> Warning: Transformation introduced infinite values in continuous y-axis
#> Transformation introduced infinite values in continuous y-axis
#> Transformation introduced infinite values in continuous y-axis
#> Warning: Removed 2 row(s) containing missing values (geom_path).
#> Warning: Removed 4 row(s) containing missing values (geom_path).
```



Here we can see that the combination of minimal `moving-median` and `moving-average` smoothing has produced a curve that has most of the noise removed with minimal introduction of bias. (Note that the first and last 2 data points are now `NA` because of the smoothing)

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!

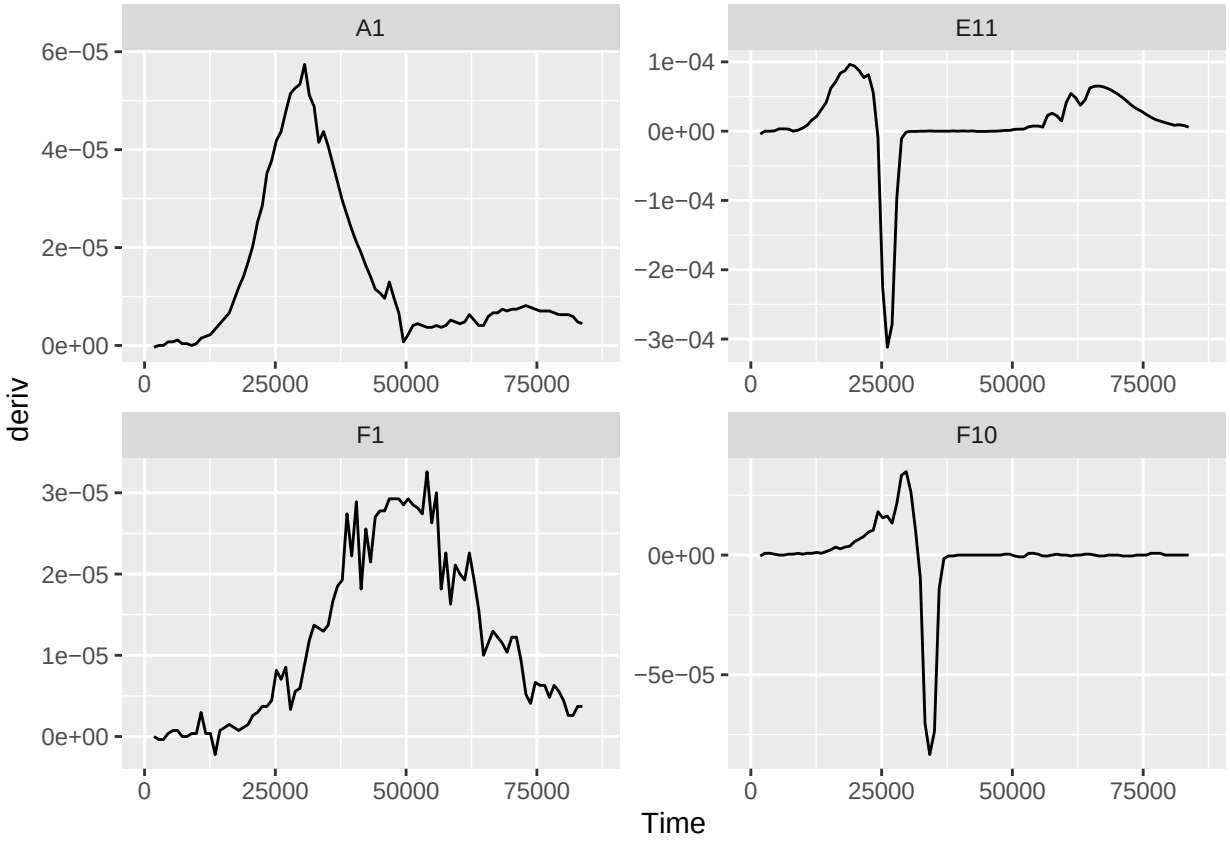
A simple derivative

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. This is useful for identifying events like population declines, or multiple rounds of growth.

```
ex_dat_mrg <- mutate(ex_dat_mrg,
                     deriv = calc_deriv(x = Time, y = smoothed))

#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 5 row(s) containing missing values (geom_path).
```



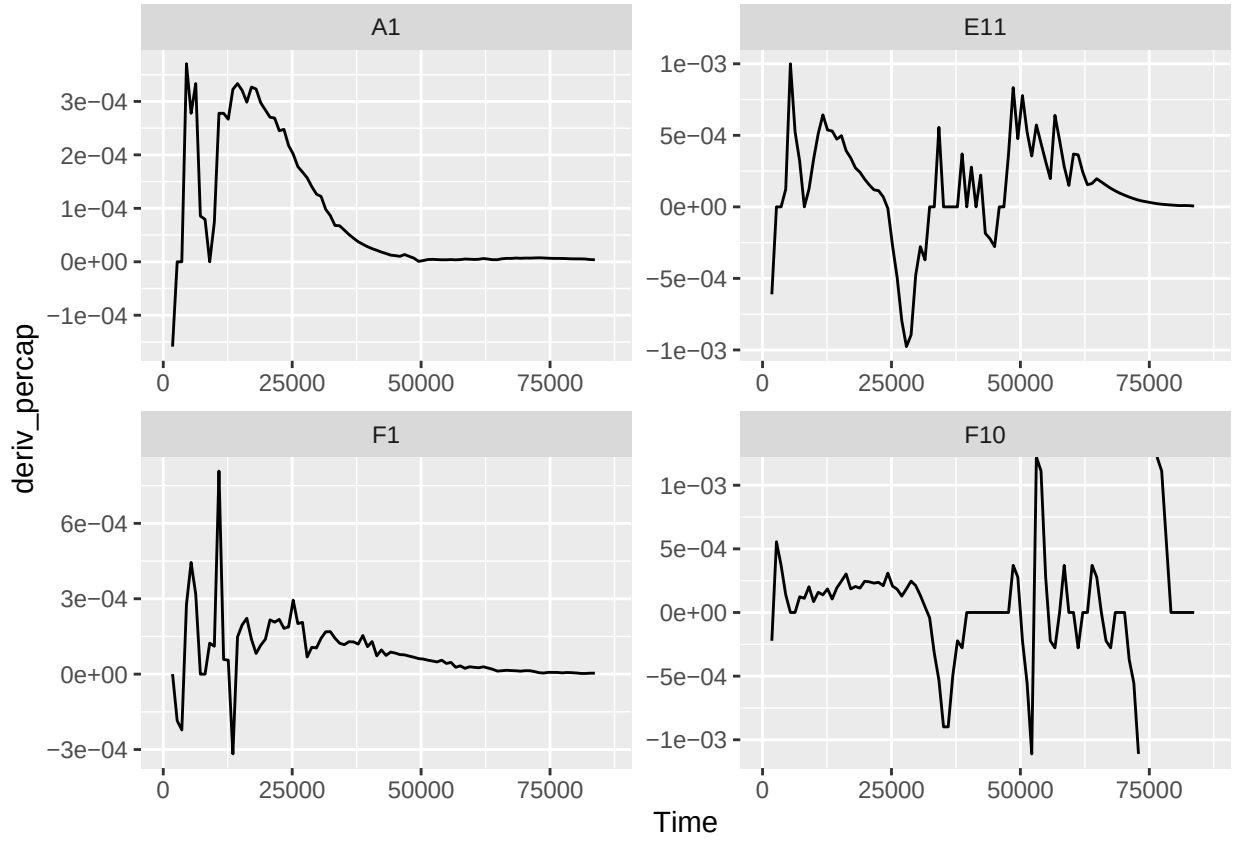
Here we can clearly see when the slope of the total population was increasing the fastest, and when it declines in the phage-added wells. But we can also see something surprising in Well A1 that may not have been immediately apparent visually: there is a second, slower, burst of growth later on. Such a pattern is common in bacterial growth curves and is called *diauxic growth*. Additionally, we can see in Well E11 when the bacteria start to grow again following near-extinction by phages, presumably after evolving resistance to the phage. (Note that the last value in the time series always becomes `NA` with `calc_deriv` under default settings)

Per-capita derivative

If we want to calculate the growth rate of the cells, we need to use `calc_deriv` to return the **per-capita** derivative. Just as before, provide the x and y values, but now set `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(ex_dat_mrg,
                     deriv_percap = calc_deriv(x = Time, y = smoothed,
                                               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 5 row(s) containing missing values (geom_path).
```



Here we can see that, in Well A1, the per-capita growth rate peaked much earlier in the time-series than might appear from the density dynamics or non per-capita derivative. We can also see that there was clearly a lag phase at the beginning before the bacteria started growing rapidly.

However, the other wells seem to have a lot of noise obscuring their per-capita growth rates. What happened? Why hasn't our smoothing been sufficient? As I explore later, per-capita growth rates can be strongly affected by even small noise at very low densities, something that can be excluded simply by only analyzing per-capita growth when densities are above some minimum value.

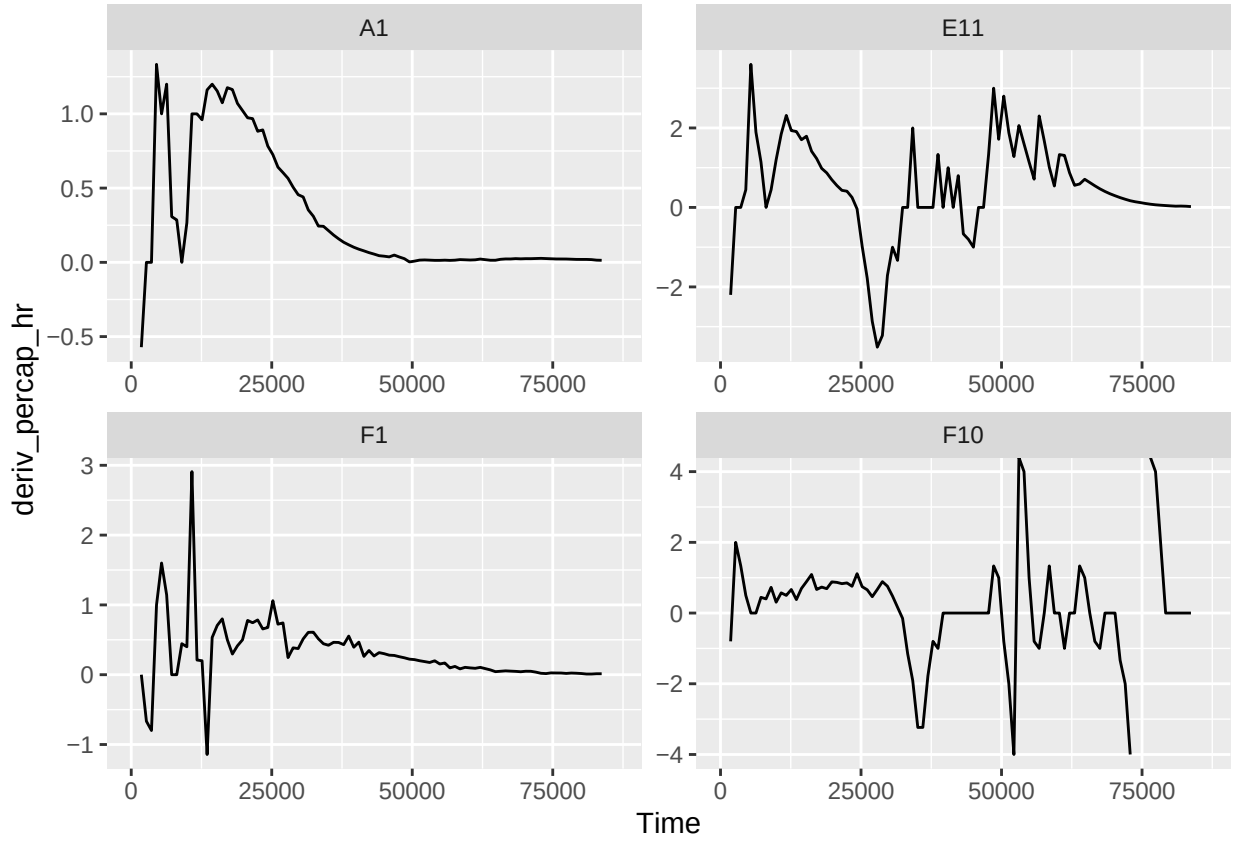
Changing the derivative units

To convert your x-axis (time) units in your derivative calculations to a different unit, use the `x_scale` argument. Simply specify the ratio of your x units to the desired units. 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`

```
ex_dat_mrg <-
  mutate(ex_dat_mrg,
```

```
        deriv_percap_hr = calc_deriv(x = Time, y = smoothed,
                                     percapita = TRUE, blank = 0,
                                     x_scale = 3600))

#Now let's plot the derivative in units of Abs/hour
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = deriv_percap_hr)) +
  geom_line() +
  facet_wrap(~Well, scales = "free")
#> Warning: Removed 5 row(s) containing missing values (geom_path).
```



Now we can see the bacterial growth rate in more-understandable units: peak growth rates are often around 1-2 times/hour (when ignoring the points that seem likely to be noise).

What's next?

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

1. Introduction: `vignette("gcplyr")`
2. Importing and transforming data: `vignette("import_transform")`
3. Incorporating design information: `vignette("incorporate_designs")`
4. Pre-processing and plotting your data: `vignette("preprocess_plot")`
5. Processing your data: `vignette("process")` **6. Analyzing your data: `vignette("analyze")`**
6. Statistics, merging other data, and other resources: `vignette("conclusion")`