Dealing with noise

Mike Blazanin

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Where are we so far?

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So far, we've imported and transformed our measures, combined them with our design information, preprocessed, processed, plotted, and analyzed our data. Here, we're going to learn potential strategies for dealing with noise in our growth curve data.

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

```
library(gcplyr)

library(dplyr)
library(ggplot2)
library(tidyr)
```

```
# This code was previously explained
# Here we're re-running it so it's available for us to work with
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"))</pre>
```

Introduction

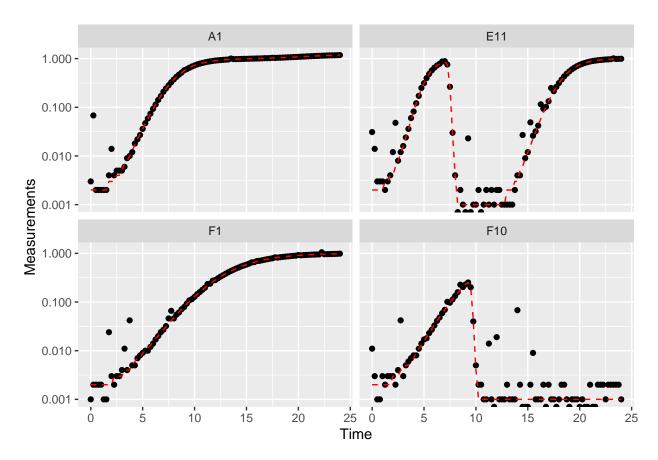
Oftentimes, growth curve data produced by a plate reader will have some noise it it. Since gcplyr does model-free analyses, our approach can sometimes be sensitive to noise, necessitating steps to reduce the effects of noise.

When assessing the effects of noise in our data, one of the first steps is simply to visualize our data, including both the raw data and any derivatives we'll be analyzing. This is especially important because per-capita derivatives can be very sensitive to noise, especially when density is low. By visualizing our data, we can assess whether the noise we see is likely to throw off our analyses.

Broadly speaking, there are three strategies we can use to deal with noise:

- Using fitting during derivative calculations
- Smooth the raw data
- Exclude low-density data points

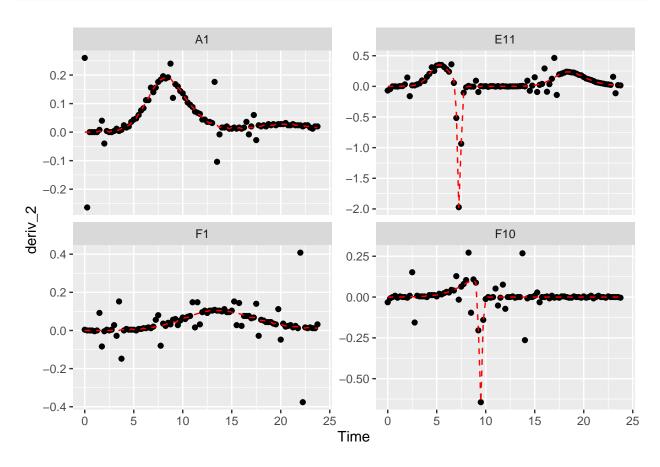
Let's start by pulling out some example data. The example data here is the same example data we've been working with, along with a version of that data with simulated noise added to it.

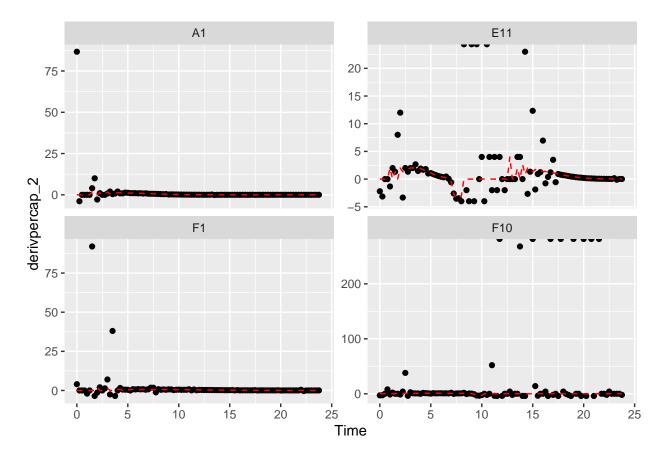


Great! Here we can see how the noisy (points) and noiseless (red line) data compare. We've plotted our data with log-transformed y-axes, which are useful because exponential growth is a straight line when plotted on a log scale. log axes also reveal another common pattern: random noise tends to have a much larger effect at low densities.

This level of noise doesn't seem like it would mess up calculations of maximum density or area under the curve much, so that's not enough of a reason to smooth. But let's look at what our derivatives look like.

```
lty = 2, color = "red") +
facet_wrap(~Well, scales = "free_y")
#> Warning: Removed 4 rows containing missing values (`geom_point()`).
#> Warning: Removed 1 row containing missing values (`geom_line()`).
```





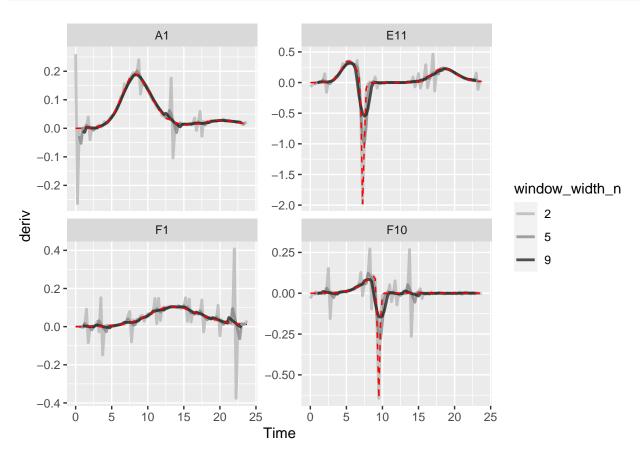
Those values are jumping all over the place, including some where the growth rate was calculated as infinite! Let's see what we can do to address this.

Fitting during derivative calculation

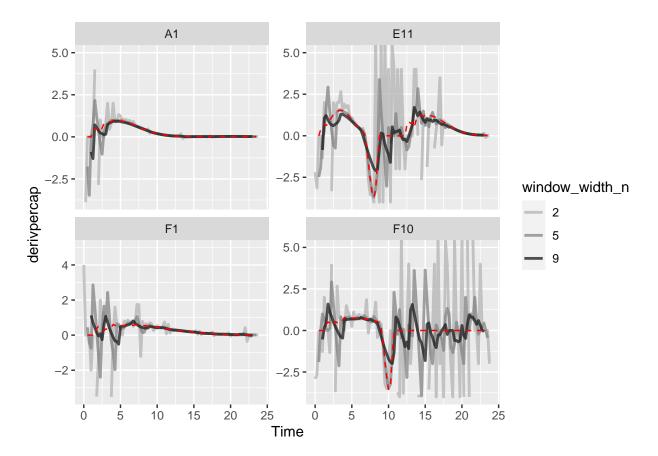
One thing we can do is calculate derivatives by fitting a line to a moving window of multiple points. (You might recall we previously used this in the Calculating Derivatives article vignette("gc05_process").)

To use the fitting functionality of calc_deriv, specify either the window_width or the 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. Wider windows will be more smoothed. Note that when using calc_deriv in this way, you should use as few points as is necessary for your analyses to work, so you should visualize different window widths and choose the smallest one that is sufficient for your analyses to succeed.

```
percapita = TRUE, blank = 0,
                                   window_width_n = 9))
#Reshape our data for plotting purposes
ex_dat_mrg_wide <-
  pivot_longer(ex_dat_mrg, cols = starts_with("deriv"),
               names_to = c("deriv", "window_width_n"), names_sep = "_")
ex_dat_mrg_wide <-
  pivot_wider(ex_dat_mrg_wide, names_from = deriv, values_from = value)
#Plot derivative
ggplot(data = dplyr::filter(ex_dat_mrg_wide, noise == "Yes"),
       aes(x = Time, y = deriv)) +
  geom_line(aes(color = window_width_n), lwd = 1, alpha = 0.75) +
  facet_wrap(~Well, scales = "free_y") +
  geom_line(data = dplyr::filter(ex_dat_mrg_wide,
                                 noise == "No", window_width_n == 2),
            lty = 2, color = "red") +
  scale_color_grey(start = 0.7, end = 0.1)
#> Warning: Removed 13 rows containing missing values (`geom_line()`).
#> Warning: Removed 1 row containing missing values (`geom_line()`).
```



```
#Plot per-capita derivative
ggplot(data = dplyr::filter(ex_dat_mrg_wide, noise == "Yes"),
    aes(x = Time, y = derivpercap)) +
geom_line(aes(color = window_width_n), lwd = 1, alpha = 0.75) +
```



As we can see, increasing the width of the window reduces the effects of noise, getting us closer to the noiseless data (red line). However, if we go too far (like window_width_n = 9 for the plain derivative), we'll start over-smoothing our data, making peaks shorter and valleys shallower.

Smoothing raw data

Smoothing raw density data is a straightforward approach to reduce the effects of noise. gcplyr has a smooth_data function that can carry out such smoothing. 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.

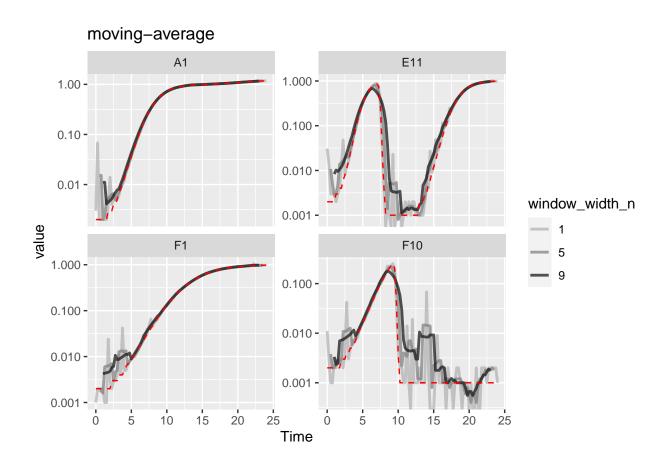
Smoothing data is a step that alters the values you will analyze. Because of that it can be rife with pitfalls. You should strive to do as little smoothing as is necessary for your analyses to work. To do so, run smoothing with different tuning parameter values and plot the results. Then, choose the parameter value that smooths the data as little as necessary. 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.

Smoothing with moving-average

For moving-average, there are two tuning parameters to choose between: 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. Wider windows will be more smoothed. Here, we'll show moving averages with window_width_n values of 5 or 9 data points wide (movavg_1 is just our raw, unsmoothed data).

```
ex_dat_mrg <-
  mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage, noise),
         movavg_1 = Measurements,
         movavg_5 = smooth_data(x = Time, y = Measurements,
              sm_method = "moving-average", window_width_n = 5),
         movavg_9 = smooth_data(x = Time, y = Measurements,
              sm_method = "moving-average", window_width_n = 9))
#Reshape our data for plotting purposes
ex dat mrg wide <-
  pivot_longer(ex_dat_mrg, cols = starts_with("movavg"),
               names_prefix = "movavg_", names_to = "window_width_n")
#Plot data
ggplot(data = dplyr::filter(ex_dat_mrg_wide, noise == "Yes"),
       aes(x = Time, y = value)) +
  geom_line(aes(color = window_width_n), lwd = 1, alpha = 0.75) +
  facet_wrap(~Well, scales = "free_y") +
  geom_line(data = dplyr::filter(ex_dat_mrg_wide,
                                 noise == "No", window_width_n == 1),
            lty = 2, color = "red") +
  scale_color_grey(start = 0.7, end = 0.1) +
  scale_y_log10() +
  ggtitle("moving-average")
#> Warning: Transformation introduced infinite values in continuous y-axis
#> Warning: Removed 12 rows containing missing values (`qeom line()`).
```

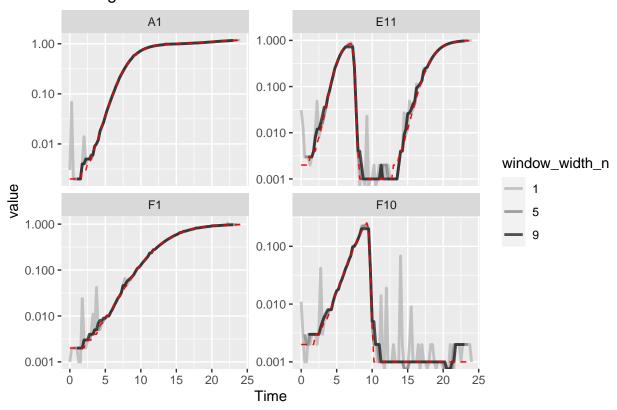


Here we can see that moving-average has helped reduce the effects of some of that early noise. However, as the window width gets larger, it also starts underrepresenting the maximum density peaks relative to the true value (red line).

Smoothing with moving-median

For moving-median, there are the same two tuning parameters: 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. Wider windows will be more smoothed. Here, we'll show moving medians with windows that are 5 and 9 data points wide (movemed_1 is just our raw, unsmoothed data).

moving-median



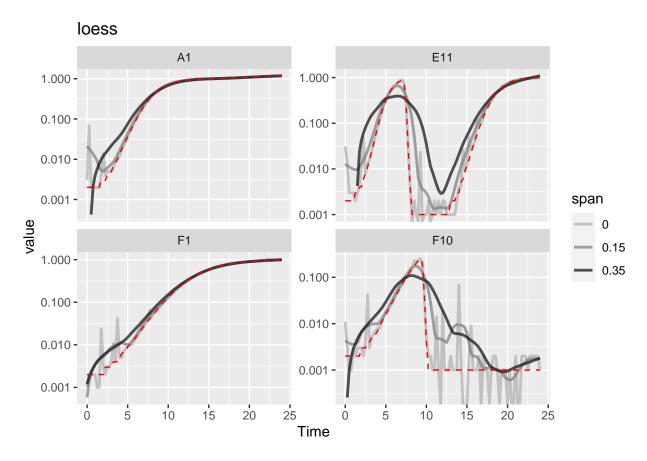
Here we can see that moving-median has done a great job excluding noise without biasing our data very far from the true values (red line). However, it has produced a smoothed density that is fairly "jumpy", something that is common with moving-median. To address this, you often will need to combine moving-median with other smoothing 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 0.15 and 0.35 and degree = 1 (loess_0 is just our raw, unsmoothed data).

```
ex_dat_mrg <-
  mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage, noise),
         loess_0 = Measurements,
         loess_15 = smooth_data(x = Time, y = Measurements,
                                 sm_method = "loess", span = .15, degree = 1),
         loess_35 = smooth_data(x = Time, y = Measurements,
                                 sm_method = "loess", span = .35, degree = 1))
#Reshape our data for plotting purposes
ex dat mrg wide <-
 pivot_longer(ex_dat_mrg, cols = starts_with("loess"),
               names_prefix = "loess_", names_to = "span")
#Plot data
ggplot(data = dplyr::filter(ex_dat_mrg_wide, noise == "Yes"),
       aes(x = Time, y = value)) +
  geom_line(aes(color = as.factor(as.numeric(span)/100)), lwd = 1, alpha = 0.75) +
  facet_wrap(~Well, scales = "free_y") +
  geom_line(data = dplyr::filter(ex_dat_mrg_wide,
                                 noise == "No", span == 0),
            lty = 2, color = "red") +
  scale_color_grey(name = "span", start = 0.7, end = 0.1) +
  scale_y_log10() +
  ggtitle("loess")
#> Warning in self$trans$transform(x): NaNs produced
#> Warning: Transformation introduced infinite values in continuous y-axis
#> Warning: Removed 2 rows containing missing values (`qeom line()`).
```

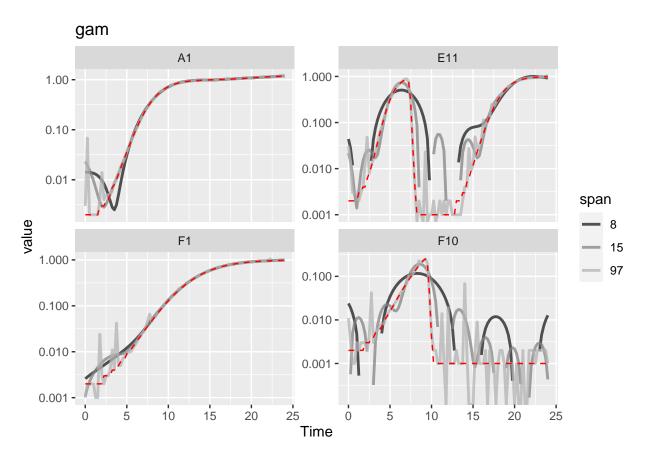


Here we can see that loess with smaller spans have smoothed the data somewhat but are still sensitive to outliers. However, loess with a larger span has introduced significant bias relative to the true values (red line). It doesn't seem like loess is working well for this data.

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 8 and 15 (gam_97 is just our raw, unsmoothed data).

```
ex_dat_mrg_wide <-
  pivot_longer(ex_dat_mrg, cols = starts_with("gam"),
               names_prefix = "gam_", names_to = "k")
#Plot data
ggplot(data = dplyr::filter(ex_dat_mrg_wide, noise == "Yes"),
       aes(x = Time, y = value)) +
  geom_line(aes(color = as.factor(as.numeric(k))), lwd = 1, alpha = 0.75) +
  facet_wrap(~Well, scales = "free_y") +
  geom_line(data = dplyr::filter(ex_dat_mrg_wide,
                                 noise == "No", k == 97),
            lty = 2, color = "red") +
  scale_color_grey(name = "span", start = 0.1, end = 0.7) +
  scale_y_log10() +
  ggtitle("gam")
#> Warning in self$trans$transform(x): NaNs produced
#> Warning: Transformation introduced infinite values in continuous y-axis
```



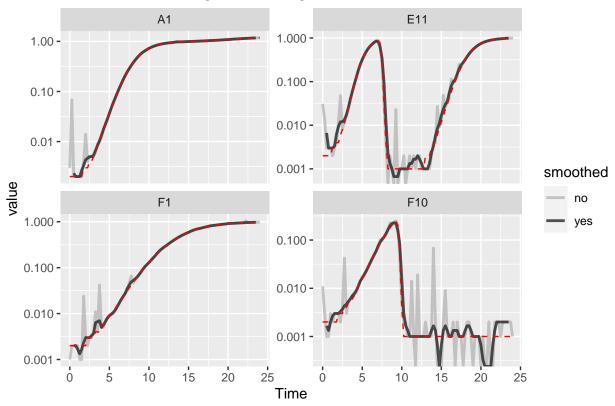
Here we can see that gam with lower values of k increasingly smooths the data. However, at basically all values of k it introduces significant bias and artifacts, so it doesn't seem like gam is working well for this data.

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.

```
ex_dat_mrg <-
  mutate(group by(ex dat mrg, Well, Bacteria strain, Phage, noise),
         smoothed_no = Measurements,
         sm 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_yes =
           smooth_data(x = Time, y = sm_med3,
                       sm_method = "moving-average", window_width_n = 3))
#Reshape our data for plotting purposes
ex_dat_mrg_wide <-
  pivot_longer(ex_dat_mrg, cols = starts_with("smoothed"),
               names_to = "smoothed", names_prefix = "smoothed_")
#Plot data
ggplot(data = dplyr::filter(ex_dat_mrg_wide, noise == "Yes"),
       aes(x = Time, y = value, color = smoothed)) +
  geom_line(lwd = 1, alpha = 0.75) +
  scale_color_grey(start = 0.7, end = 0.1) +
  facet wrap(~Well, scales = "free y") +
  geom_line(data = dplyr::filter(ex_dat_mrg_wide,
                                 noise == "No", smoothed == "no"),
            lty = 2, color = "red") +
  scale_y_log10() +
  ggtitle("median then average smoothing")
#> Warning: Transformation introduced infinite values in continuous y-axis
#> Warning: Removed 4 rows containing missing values (`qeom_line()`).
```

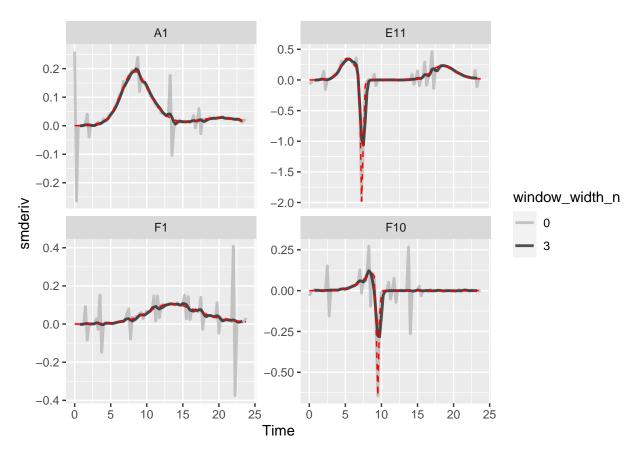
median then average smoothing



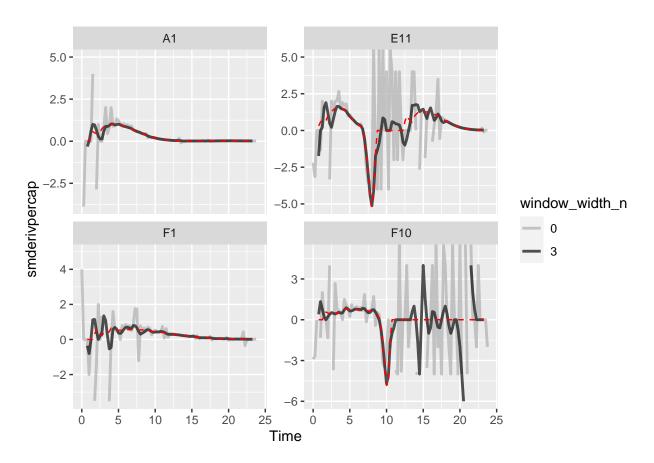
Here we can see that the combination of minimal moving-median and moving-average smoothing has produced a curve that has most of the noise removed with minimal introduction of bias relative to the true values (red line).

Calculating derivatives of smoothed data

Once you've smoothed your data, you can calculate derivatives using the smoothed data. Combining smoothing of raw data and fitting using multiple points for calculating derivatives can be a powerful combination for reducing the effects of noise while minimizing the introduction of bias.



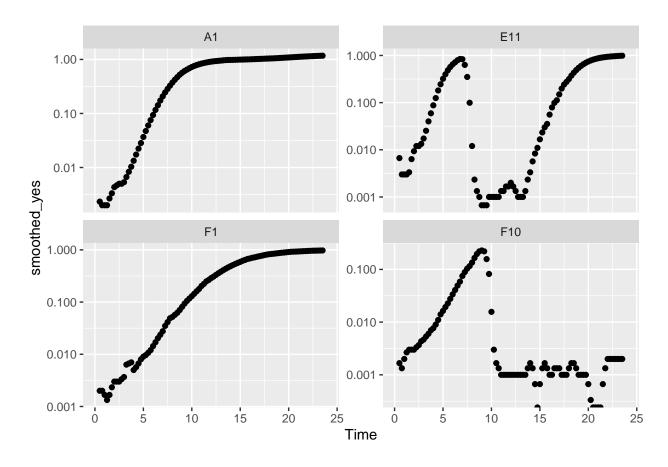
```
#> Warning: Removed 8 rows containing missing values (`geom_line()`).
#> Warning: Removed 6 rows containing missing values (`geom_line()`).
```

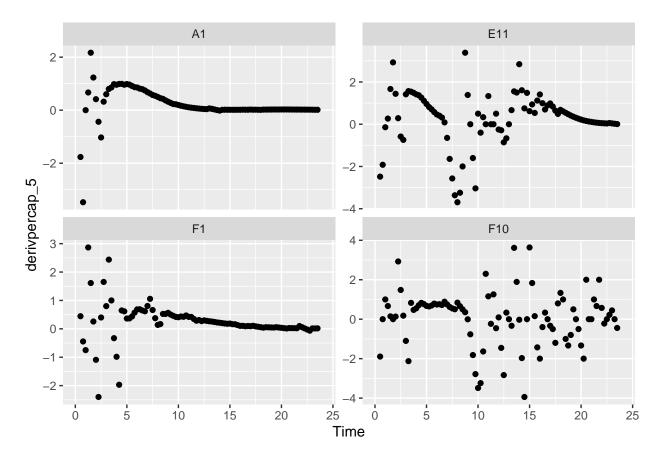


Here we can see that calculating derivatives from smoothed raw data can be a powerfully useful combination.

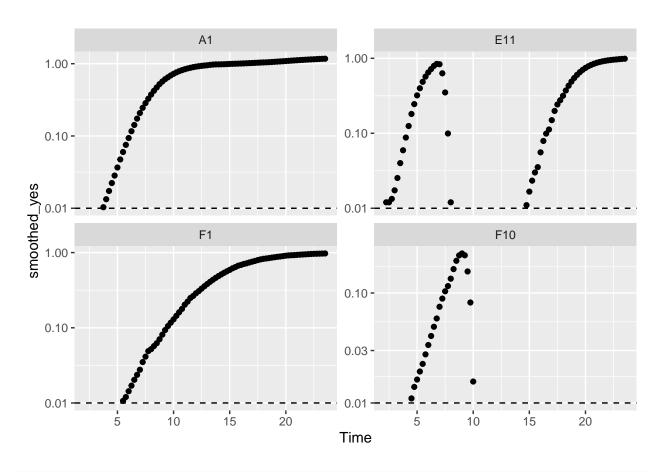
Summarizing on subsets of derivatives

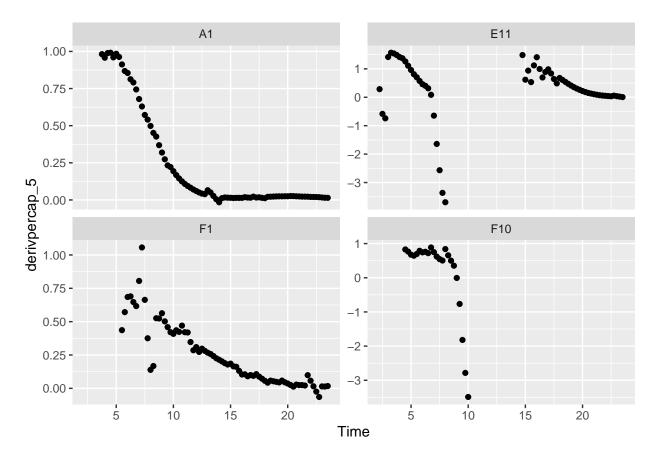
There is one final strategy we can employ when dealing with noisy data: excluding data points where the density is near 0. If we compare our per-capita growth rates and our density plots, we'll see that most of the noise occurs when the density is very close to 0:





Per-capita growth rates are often very noisy when the density is close to 0, so it can make sense to simply exclude those data points.





When we limit our analysis to data points where the density is not too close to 0, much of the noise in our per-capita derivative disappears.

To take this to the final step, we can use these cutoffs in our **summarize** commands to calculate the maximum growth rate of the bacteria when their density is at least 0.01.

```
ex dat mrg sum <-
  summarize(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage, noise),
            max_growth_rate = max(derivpercap_5[smoothed_yes > 0.01],
                                   na.rm = TRUE))
#> `summarise()` has grouped output by 'Well', 'Bacteria_strain', 'Phage'. You can override
#> using the `.groups` argument.
head(ex_dat_mrg_sum)
#> # A tibble: 6 x 5
#> # Groups:
               Well, Bacteria_strain, Phage [3]
     Well Bacteria_strain Phage
                                        noise max_growth_rate
     <fct> <chr>
                            <chr>
                                        <chr>
                                                        <dbl>
#>
#> 1 A1
           Strain 1
                           No Phage
                                        No
                                                        1.00
#> 2 A1
           Strain 1
                           No Phage
                                        Yes
                                                        0.991
#> 3 E11
           Strain 29
                           Phage Added No
                                                        1.53
#> 4 E11
           Strain 29
                           Phage Added Yes
                                                        1.57
#> 5 F1
           Strain 31
                           No Phage
                                        No
                                                        0.597
#> 6 F1
           Strain 31
                           No Phage
                                        Yes
                                                        1.06
```

What's next?

Now that you've analyzed your data and dealt with any noise, there's just some concluding notes on best practices for running statistics, merging growth curve analyses with other data, and additional resources for analyzing growth curves.

- Introduction: vignette("gc01_gcplyr")
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