Dealing with noise

Mike Blazanin

Contents

Where are we so far? 1

Introduction 2

Summarizing on subsets of derivatives 5

Fitting during derivative calculation 10

Smoothing raw data 12

Smoothing with moving-median 13

Smoothing with moving-average 14

Combining multiple smoothing methods 15

Smoothing with other methods 17

Choosing tuning parameter values 18

Calculating derivatives of smoothed data 20

What's next? 22

Where are we so far?

1. Introduction: `vignette("gc01_gcplyr")`
2. Importing and reshaping 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")`
7. **Dealing with noise:** `vignette("gc07_noise")`
8. Best practices and other tips: `vignette("gc08_conclusion")`
9. Working with multiple plates: `vignette("gc09_multiple_plates")`
10. Using make_design to generate experimental designs: `vignette("gc10_using_make_design")`

So far, we've imported and transformed our measures, combined them with our design information, pre-processed, 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.

```r
library(gcplyr)

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

Introduction

Oftentimes, growth curve data produced by a plate reader will have some noise in 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.

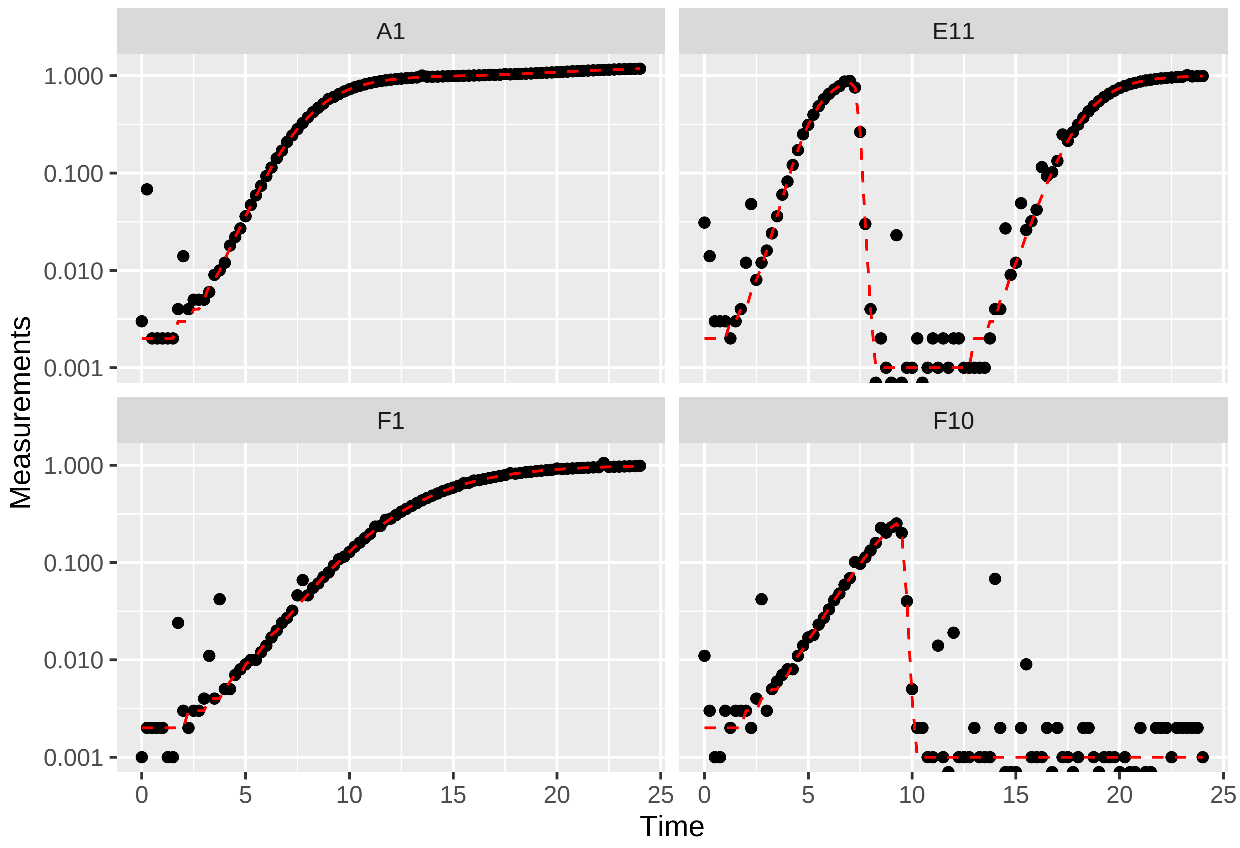
1. Excluding low-density data points
2. Using fitting during derivative calculations
3. Smoothing the raw data

Each approach is progressively more involved, so I recommend trying them out in that order. Typically, noise is most problematic in the derivatives, and all three approaches will help with that. However, if you're dealing with substantial noise in your raw density data, only approach #3 will help.

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.

```r
ex_dat_mrg <- make_example(vignette = 7, example = 1)

# Plot with a log y-axis
ggplot(data = dplyr::filter(ex_dat_mrg, noise == "Yes"),
       aes(x = Time, y = Measurements)) +
  geom_point() +
  geom_line(data = dplyr::filter(ex_dat_mrg, noise == "No"),
            lty = 2, color = "red") +
  facet_wrap(~Well) +
  scale_y_continuous(trans = "log10")
#> Warning in scale_y_continuous(trans = "log10"): log-10 transformation introduced
#> infinite values.
```

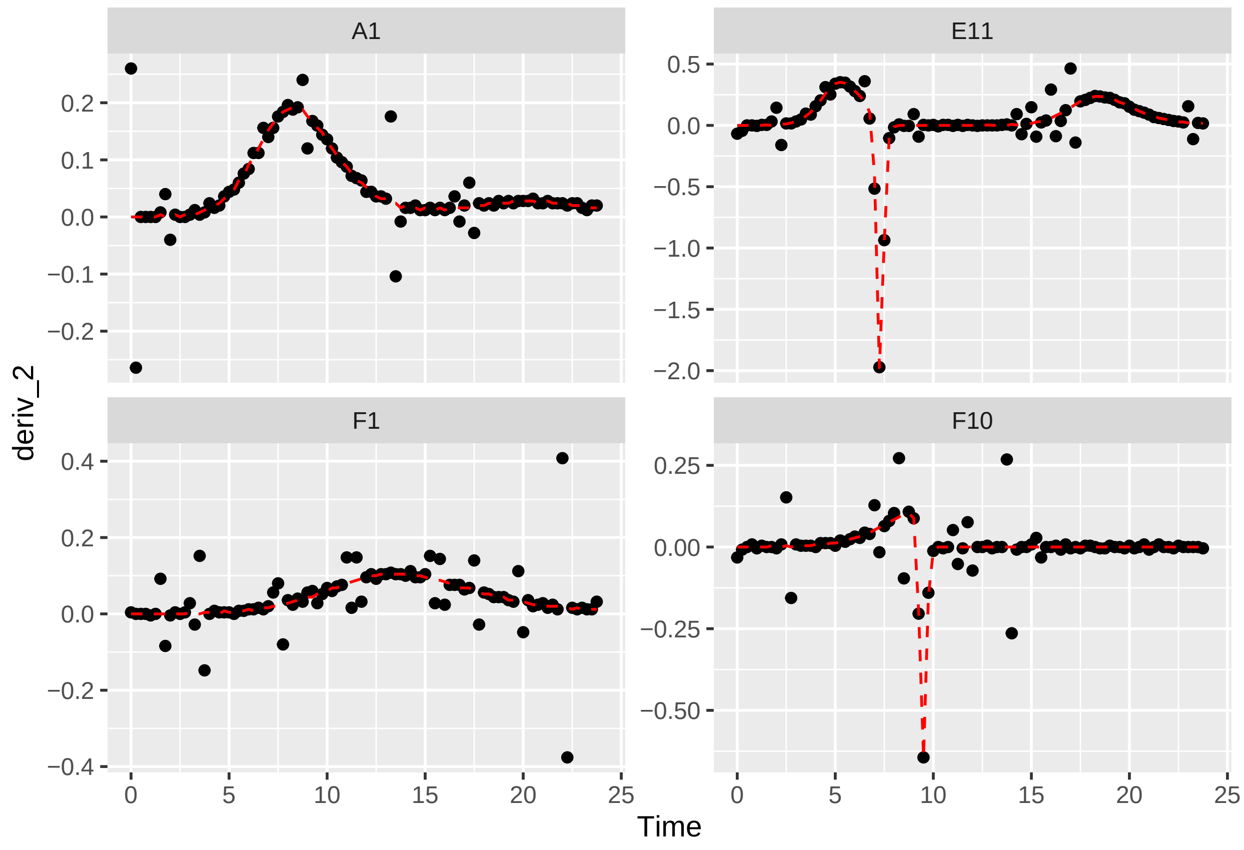


Great! Here we can see how the noisy (points) and noiseless (red line) data compare. We've plotted our data with log-transformed y-axes, which are useful because exponential growth is a straight line when plotted on a log scale. log axes also reveal another common pattern: **random noise tends to have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

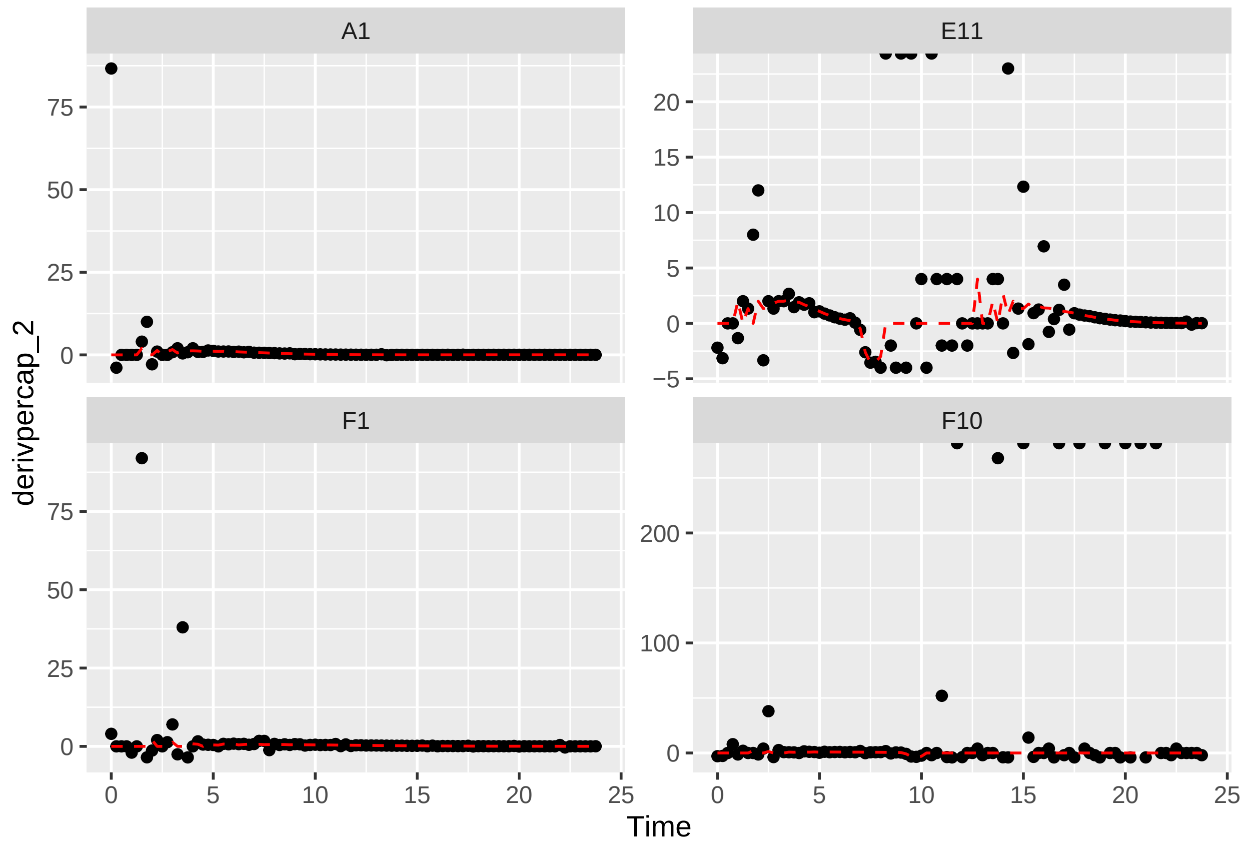
This level of noise doesn't seem like it would mess up calculations of maximum density or area under the curve much, so that's not enough of a reason to smooth. But let's look at what our derivatives look like.

```r
ex_dat_mrg <-
  mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage, noise),
         deriv_2 = calc_deriv(x = Time, y = Measurements),
         derivpercap_2 = calc_deriv(x = Time, y = Measurements,
                                    percapita = TRUE, blank = 0))

# Plot derivative
ggplot(data = dplyr::filter(ex_dat_mrg, noise == "Yes"),
       aes(x = Time, y = deriv_2)) +
  geom_point() +
  geom_line(data = dplyr::filter(ex_dat_mrg, noise == "No"),
            lty = 2, color = "red") +
  facet_wrap(~Well, scales = "free_y")
#> Warning: Removed 4 rows containing missing values or values outside the scale range
#> (`geom_point()`).
#> Warning: Removed 1 row containing missing values or values outside the scale range
#> (`geom_line()`).
```



```
# Plot per-capita derivative
ggplot(data = dplyr::filter(ex_dat_mrg, noise == "Yes"),
       aes(x = Time, y = derivpercap_2)) +
  geom_point() +
  geom_line(data = dplyr::filter(ex_dat_mrg, noise == "No"),
            lty = 2, color = "red") +
  facet_wrap(~Well, scales = "free_y")
#> Warning: Removed 8 rows containing missing values or values outside the scale range
#> (`geom_point()`).
#> Removed 1 row containing missing values or values outside the scale range
#> (`geom_line()`).
```

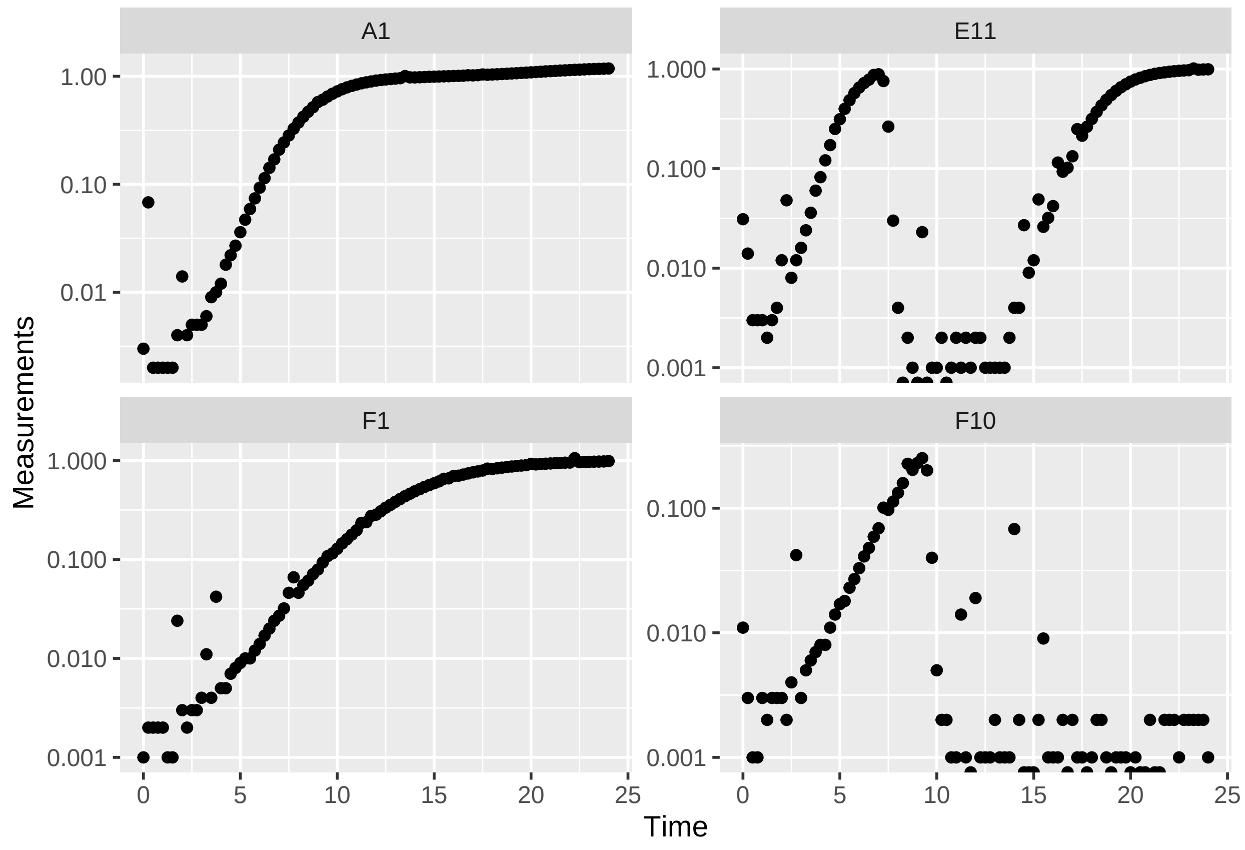


Those values are jumping all over the place! Let's see what we can do to address this.

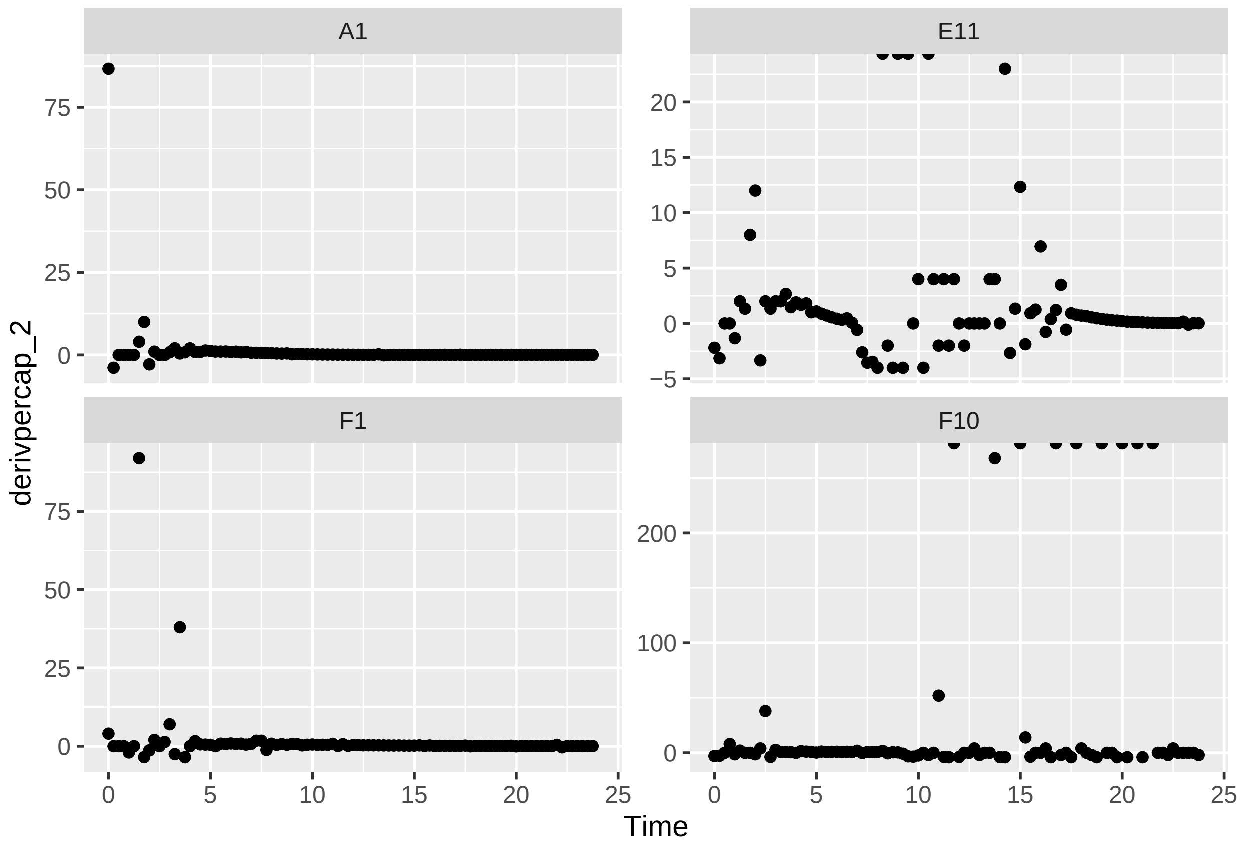
Summarizing on subsets of derivatives

One strategy we can employ when dealing with noisy data is 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:

```r
#Plot density
ggplot(data = dplyr::filter(ex_dat_mrg, noise == "Yes"),
       aes(x = Time, y = Measurements)) +
  geom_point() +
  facet_wrap(~Well, scales = "free_y") +
  scale_y_log10()
#> Warning in scale_y_log10(): log-10 transformation introduced infinite values.
```

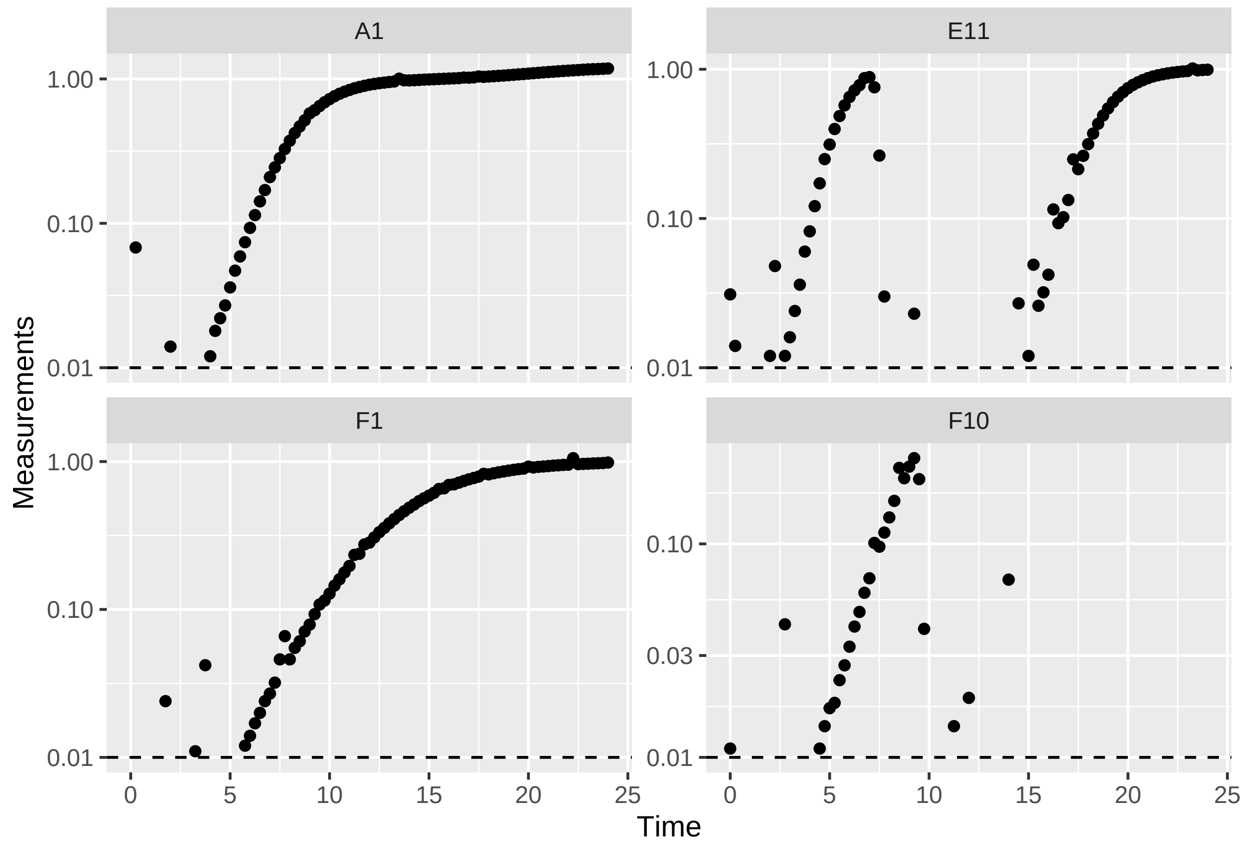


```
# Plot per-capita derivative
ggplot(data = dplyr::filter(ex_dat_mrg, noise == "Yes"),
       aes(x = Time, y = derivpercap_2)) +
  geom_point() +
  facet_wrap(~Well, scales = "free_y")
#> Warning: Removed 8 rows containing missing values or values outside the scale range
#> (`geom_point()`).
```

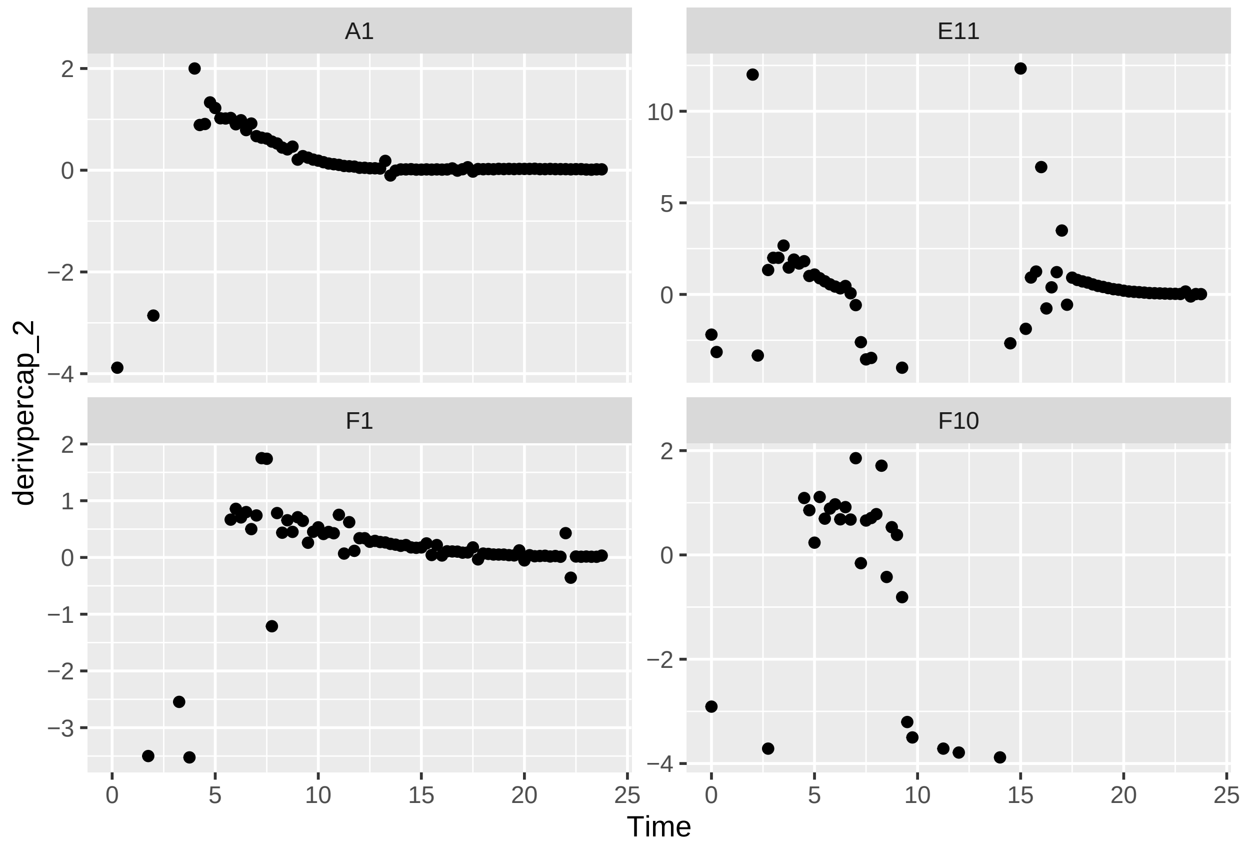


Per-capita growth rates are often very noisy when the density is close to 0, so it can make sense to simply exclude those data points.

```r
#Plot density
ggplot(data = dplyr::filter(ex_dat_mrg, noise == "Yes", Measurements > 0.01),
       aes(x = Time, y = Measurements)) +
  geom_point() +
  facet_wrap(~Well, scales = "free_y") +
  geom_hline(yintercept = 0.01, lty = 2) +
  scale_y_log10()
```



```
# Plot per-capita derivative
ggplot(data = dplyr::filter(ex_dat_mrg, noise == "Yes", Measurements > 0.01),
       aes(x = Time, y = derivpercap_2)) +
  geom_point() +
  facet_wrap(~Well, scales = "free_y")
#> Warning: Removed 3 rows containing missing values or values outside the scale range
#> (`geom_point()`).
```



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_2[Measurements > 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               1.23
#> 2 A1    Strain 1        No Phage    Yes              2
#> 3 E11   Strain 29       Phage Added No               2.11
#> 4 E11   Strain 29       Phage Added Yes             12.3
#> 5 F1    Strain 31       No Phage    No               0.8
#> 6 F1    Strain 31       No Phage    Yes              1.75
```

Fitting during derivative calculation

The next approach to try is to calculate derivatives by fitting a line to multiple points. (You might recall we previously used this in the Calculating Derivatives article `vignette("gc05_process")`.)

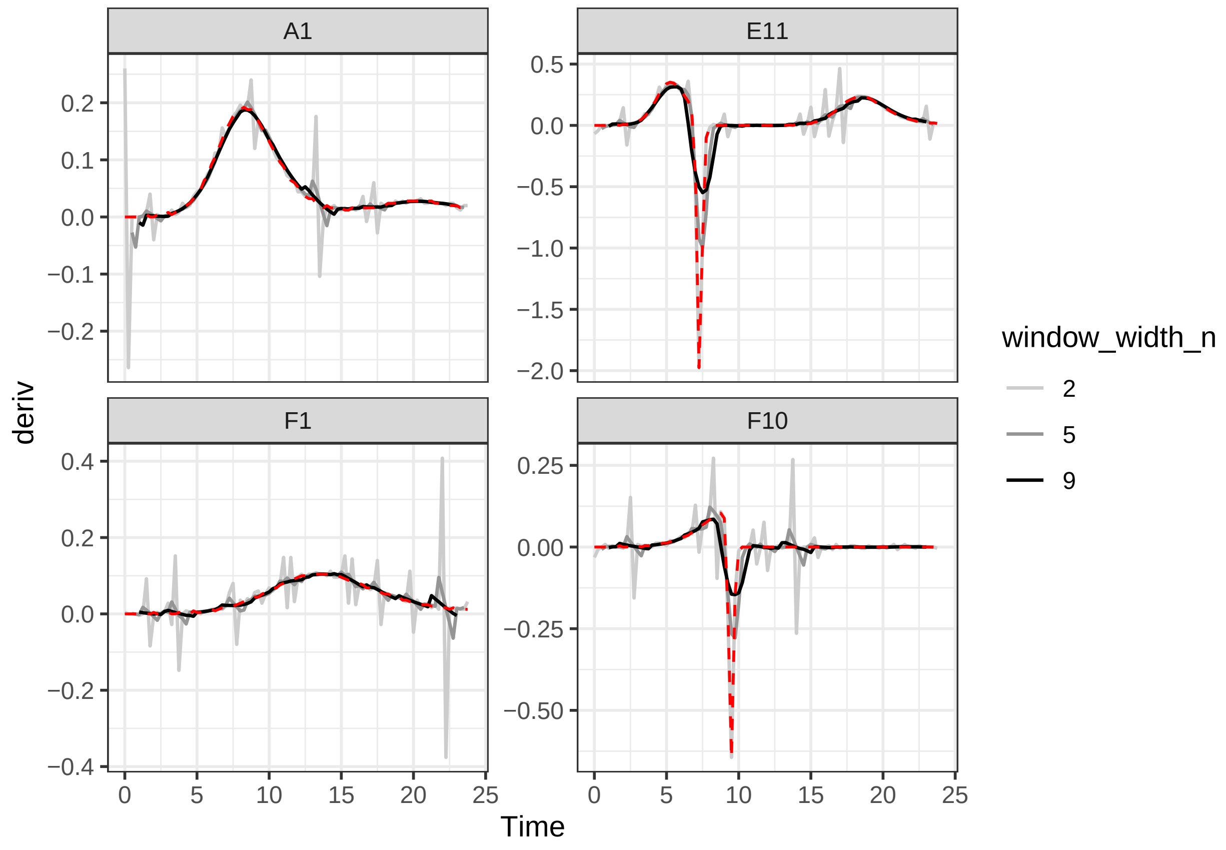
To use the fitting functionality of `calc_deriv`, specify the width of the moving window using `window_width`, `window_width_n`, `window_width_frac`, or `window_width_n_frac`. Wider windows will be more smoothed.

Fitting using a moving window is subject to a trade off between the amount of variance (noise) that remains and the amount of bias added to your data. A small window will allow higher variance but add little bias, while a wide window will allow less variance but add more bias.

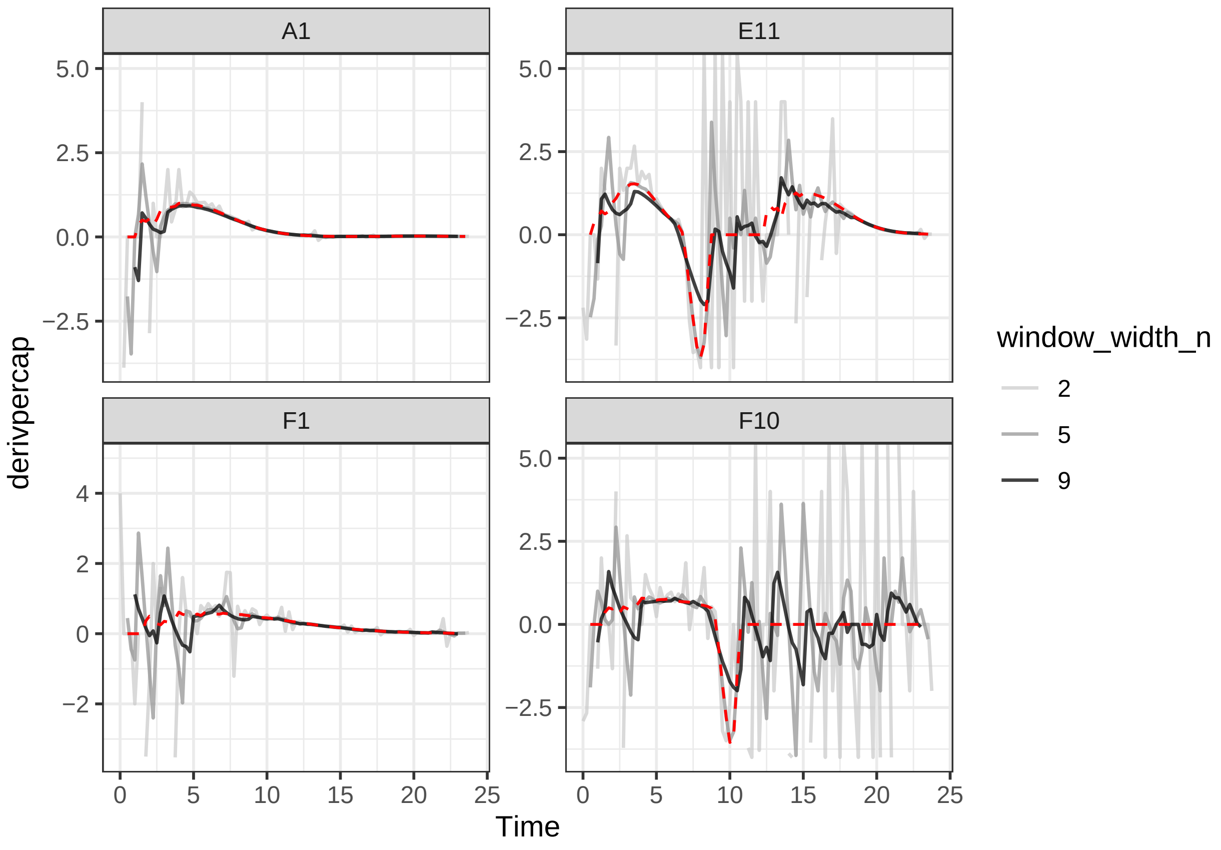
Thus, I recommend using as small a window as possible, like `window_width_n = 3` or `window_width_n = 5`. The best way to figure out what values to use for your data are to try different window widths and plot the results, then choose the smallest one that is sufficient for your analyses to succeed.

```r
ex_dat_mrg <-
  mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage, noise),
         deriv_5 = calc_deriv(x = Time, y = Measurements,
                              window_width_n = 5),
         derivpercap_5 = calc_deriv(x = Time, y = Measurements,
                                    percapita = TRUE, blank = 0,
                                    window_width_n = 5),
         deriv_9 = calc_deriv(x = Time, y = Measurements,
                              window_width_n = 9),
         derivpercap_9 = calc_deriv(x = Time, y = Measurements,
                                    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), linewidth = 0.6) +
  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 = 0.8, end = 0) +
  theme_bw()
#> Warning: Removed 13 rows containing missing values or values outside the scale range
#> (`geom_line()`).
#> Warning: Removed 1 row containing missing values or values outside the scale range
#> (`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), linewidth = 0.6, alpha = 0.75) +
  facet_wrap(~Well, scales = "free_y") +
  geom_line(data = dplyr::filter(ex_dat_mrg_wide,
                                 noise == "No", window_width_n == 5),
            lty = 2, color = "red") +
  scale_color_grey(start = 0.8, end = 0) +
  ylim(NA, 5) +
  theme_bw()
#> Warning: Removed 14 rows containing missing values or values outside the scale range
#> (`geom_line()`).
#> Warning: Removed 4 rows containing missing values or values outside the scale range
#> (`geom_line()`).
```



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, it also starts making peaks shorter and valleys shallower. Finding the right width that balances those two effects is the key to choosing the right `window_width`.

Smoothing raw data

The final approach to dealing with noise is to smooth the raw density data. `gcplyr` has a `smooth_data` function that can carry out such smoothing. `smooth_data` has five different smoothing algorithms to choose from: `moving-average`, `moving-median`, `loess`, `gam`, and `smooth.spline`.

- `moving-average` and `moving-median` are simple smoothing algorithms that primarily act to reduce the effects of outliers on the data
- `loess`, `gam`, and `smooth.spline` are spline-fitting approaches that use polynomial-like curves, which produces curves with smoothly changing derivatives, but can in some cases create curvature artifacts not present in the original data

Generally, I recommend sticking to the first two algorithms, alone or in combination, since the others tend to add artifacts to the derivatives of growth curve data.

Regardless of which smoothing algorithm you use, you will need to set a tuning parameter that controls how "smoothed" the data are. That choice is subject to a trade off between the amount of variance (noise) that remains and the amount of bias added to your data. Choosing a value that smooths your data less will allow higher variance but will add little bias to your data, while choosing a value that smooths your data more will allow less variance but add more bias to your data. Because of this, I generally recommend choosing the value that smooths your data as little as necessary for your analyses to work. For more details, see the Choosing tuning parameter values section.

Additionally, when sharing your findings, it's important to be transparent by sharing the raw data and smoothing methods, rather than presenting 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.

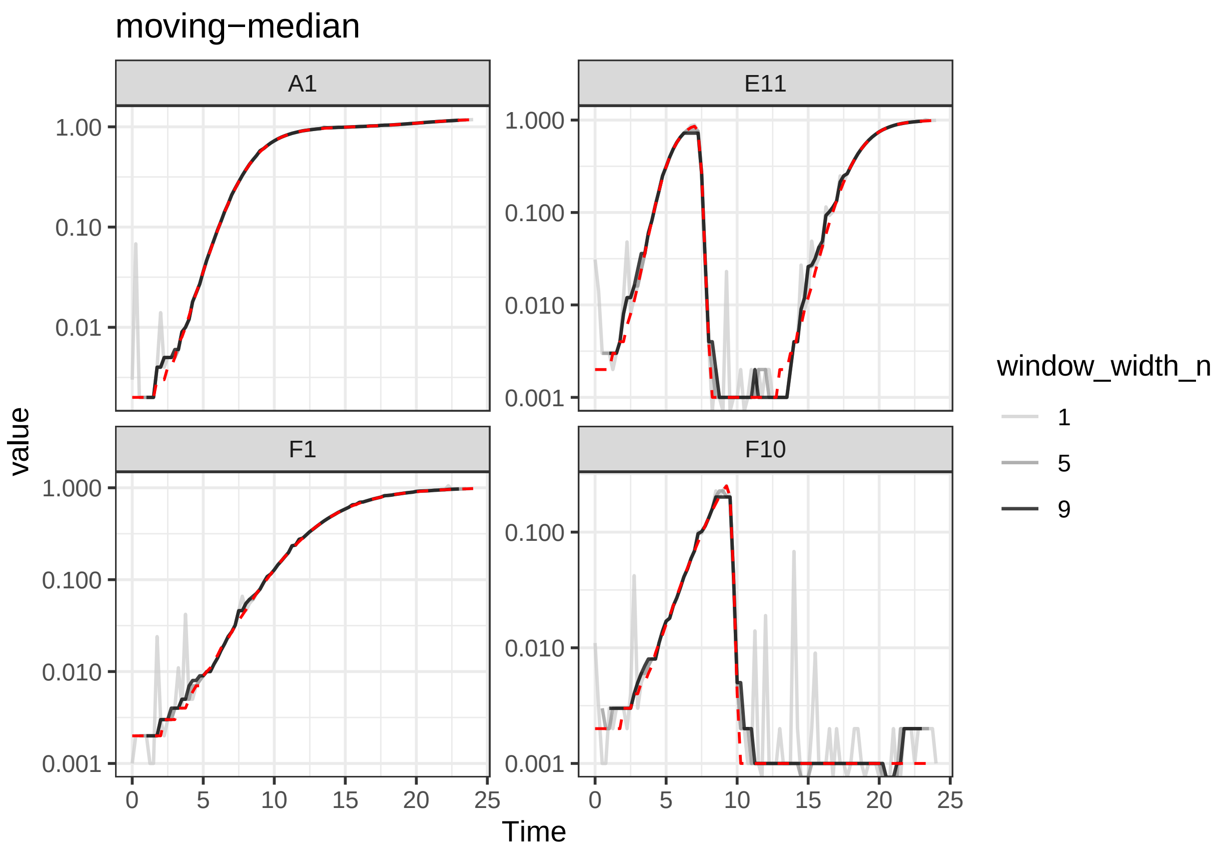
Smoothing with moving-median

For `moving-median`, specify the width of the moving window using `window_width`, `window_width_n`, `window_width_frac`, or `window_width_n_frac`. Wider windows will be more smoothed. Here, we'll show moving medians with windows that are 5 and 9 data points wide (`movmed_1` is just our raw, unsmoothed data).

```r
ex_dat_mrg <-
  mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage, noise),
         movmed_1 = Measurements,
         movmed_5 =
           smooth_data(x = Time, y = Measurements,
                       sm_method = "moving-median", window_width_n = 5),
         movmed_9 =
           smooth_data(x = Time, y = Measurements,
                       sm_method = "moving-median", window_width_n = 9))

#Reshape our data for plotting purposes
ex_dat_mrg_wide <-
  pivot_longer(ex_dat_mrg, cols = starts_with("movmed"),
               names_prefix = "movmed_", 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), linewidth = 0.6, 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 = 0.8, end = 0) +
  scale_y_log10() +
  ggtitle("moving-median") +
  theme_bw()
#> Warning in scale_y_log10(): log-10 transformation introduced infinite values.
#> Warning: Removed 12 rows containing missing values or values outside the scale range
#> (`geom_line()`).
```



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.

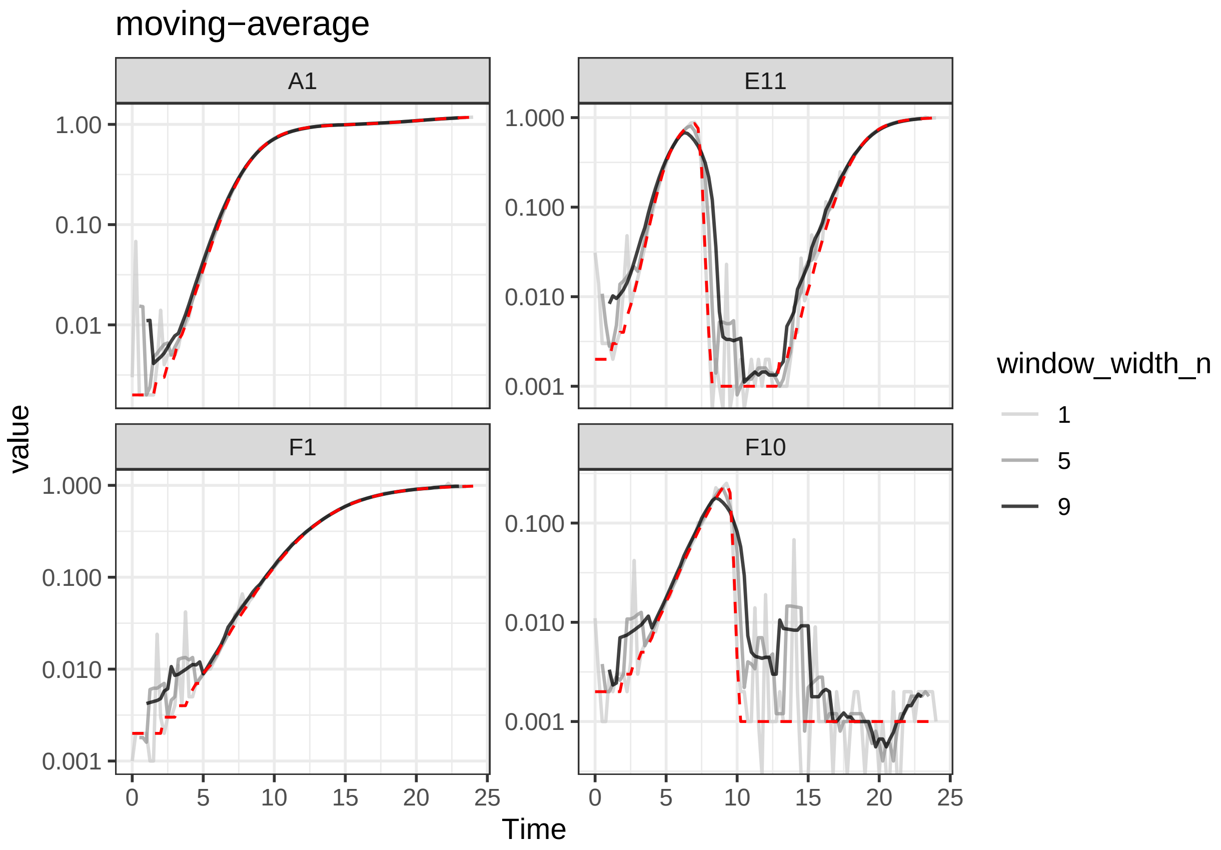
Smoothing with moving-average

For `moving-average`, specify the width of the moving window using `window_width`, `window_width_n`, `window_width_frac`, or `window_width_n_frac`. 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).

```r
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")
```

```r
#Plot data
ggplot(data = dplyr::filter(ex_dat_mrg_wide, noise == "Yes"),
       aes(x = Time, y = value)) +
  geom_line(aes(color = window_width_n), linewidth = 0.6, 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 = 0.8, end = 0) +
  scale_y_log10() +
  ggtitle("moving-average") +
  theme_bw()
#> Warning in scale_y_log10(): log-10 transformation introduced infinite values.
#> Warning: Removed 12 rows containing missing values or values outside the scale range
#> (`geom_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 starts biasing our data more strongly [e.g. underrepresenting the maximum density peaks relative to the true value (red line)].

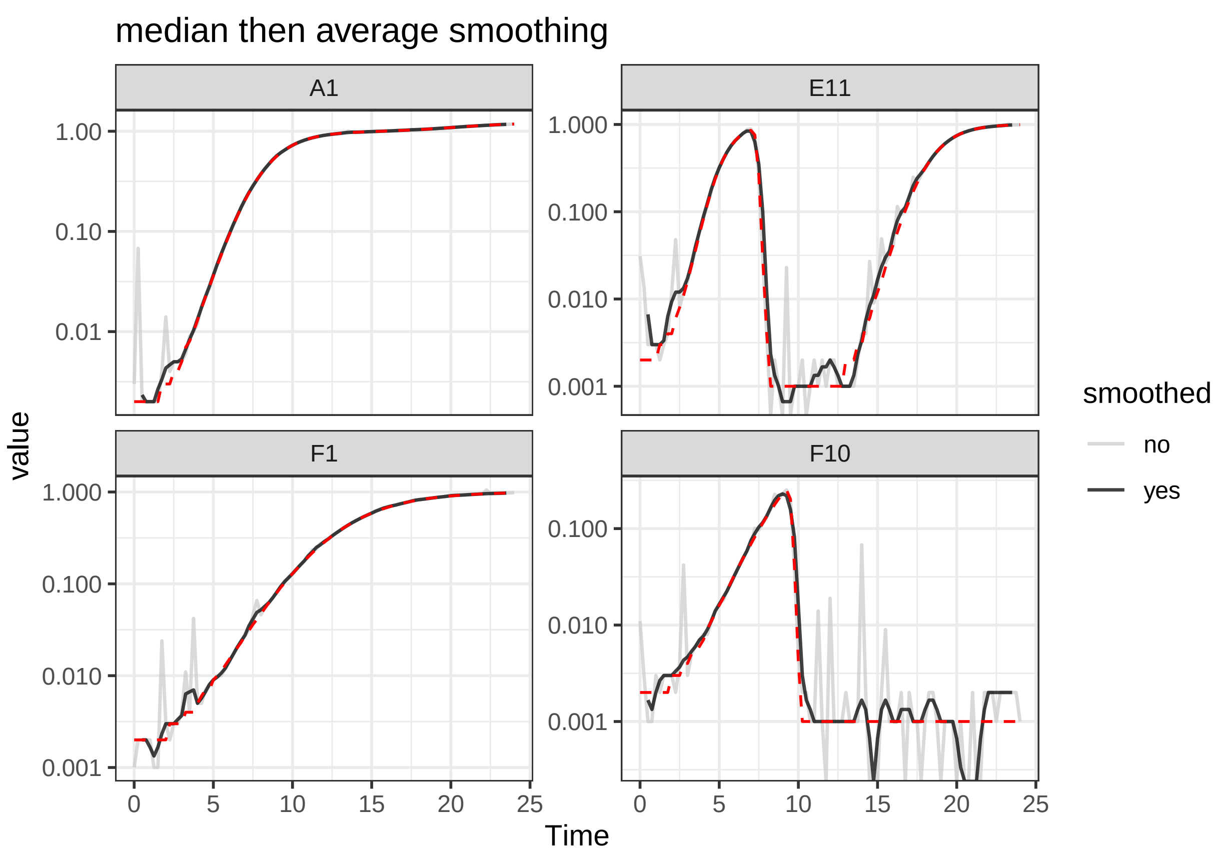
Combining multiple smoothing methods

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 smoothly changing data. In contrast, `moving-average` works well at producing smoothly changing data, but isn't as good at removing outliers. Often, we can get the best of both by using `moving-median` first, followed by `moving-average`:

```r
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(linewidth = 0.6, alpha = 0.75) +
  scale_color_grey(start = 0.8, end = 0) +
  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") +
  theme_bw()
#> Warning in scale_y_log10(): log-10 transformation introduced infinite values.
#> Warning: Removed 4 rows containing missing values or values outside the scale range
#> (`geom_line()`).
```



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).

Smoothing with other methods

I typically recommend against using other smoothing algorithms, since they have a tendency to add artifacts to the derivatives of growth curve data. However, they may work for your analyses, so if you are considering using them make sure to plot the smoothed output to check for any introduced artifacts.

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. `span` is the width of the window, as a fraction of all data points. `span` values typically are between 0 and 1, and larger values are more "smoothed". The fits can be linear (`degree = 1`) or polynomial (typically `degree = 2`).

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". However, unlike earlier methods, `k` values that are too large are also problematic, as they will tend to 'overfit' the data.

For `smooth.spline`, the primary tuning parameters are `df` and `spar`. `df` is the number of degrees of freedom, and must be between 1 and the number of unique x values. `spar` is the smoothing parameter, and is typically between 0 and 1.

Choosing tuning parameter values

Given that smoothing reduces the variance of your data but adds bias, how can you know the right value of the tuning 'smoothness' parameter for your data?

One way you can choose is by trying smoothing with different tuning parameter values, plotting the results, and then choosing the one that smooths your data as little as necessary for your analyses to work. This approach is especially useful if you're trying to deal with noise that is most strongly affecting your derivatives.

An alternative way is to use cross-validation to attempt to estimate the best smoothing parameter value. Cross-validation works by randomly splitting the data into blocks of roughly equal size, leaving out one block and smoothing the data, then seeing how close the smoothed values are to the left-out data, reporting the average error across all the blocks. We can then repeat this whole process at different values of the smoothing parameter, testing which smoothing parameter value gives us the lowest error. Note, however, that this approach doesn't quantify how well the smoothing improved your derivatives.

To do cross-validation of smoothing parameters, use `train_smooth_data`. `train_smooth_data` works similar to `smooth_data`, except that instead of returning the smoothed data values, it returns the average accuracy metrics. Because of this, we have to use `train_smooth_data` within the `dplyr` function `reframe`, which works like `summarize` except that it allows multiple rows to be returned.

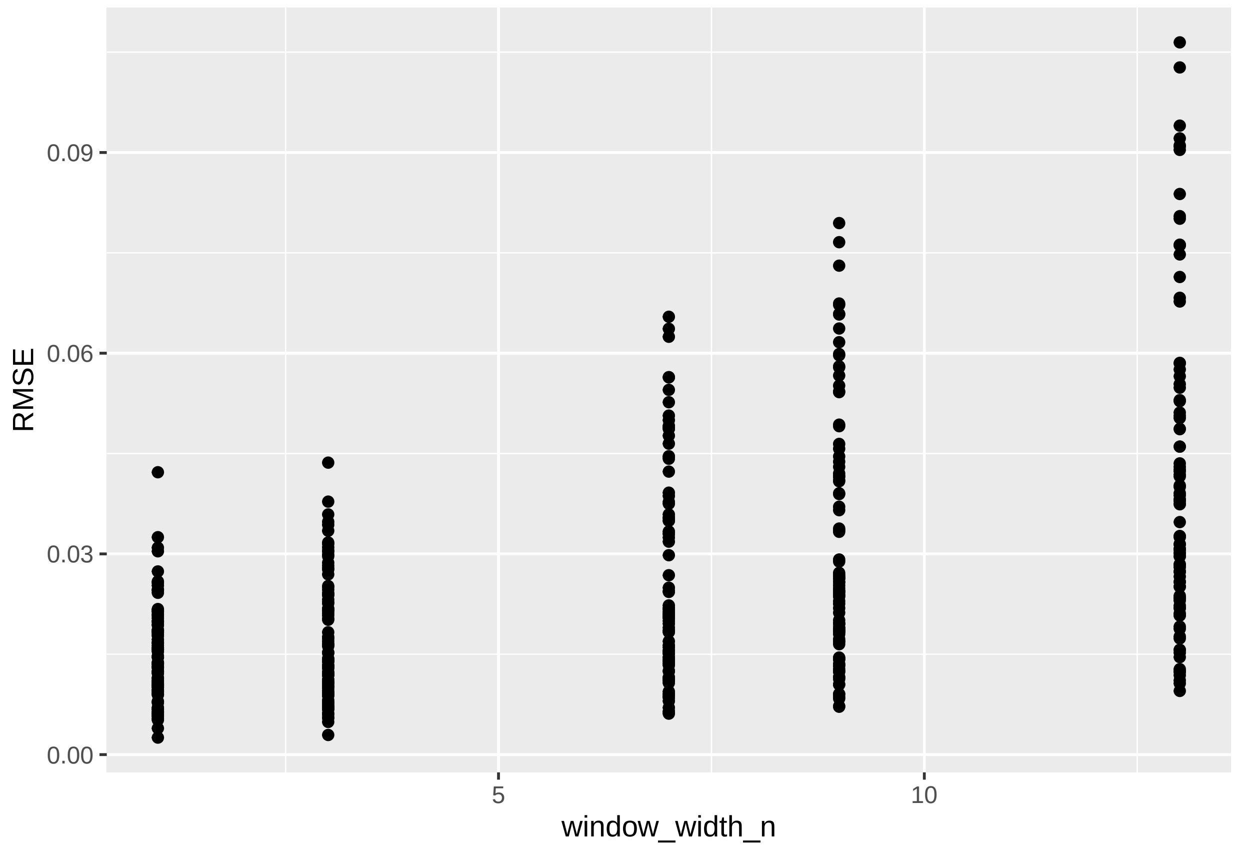
By default, `train_smooth_data` will test an evenly spaced grid of three values of the smoothing parameter. Usually, that's not enough values, so you'll want to set the number of values to test with `tuneLength` (more values will take longer to calculate).

```
ex_dat_mrg_fortraining <- make_example(vignette = 7, example = 2)

training_results <-
  reframe(group_by(ex_dat_mrg_fortraining, Bacteria_strain, Phage, Well),
          train_smooth_data(x = Time, y = Measurements,
                            sm_method = "moving-average",
                            tuneLength = 5))
#> Loading required package: lattice
head(training_results)
#> # A tibble: 6 x 10
#>   Bacteria_strain Phage  Well  window_width_n    RMSE Rsquared     MAE  RMSESD RsquaredSD
#>   <chr>           <chr>  <fct>          <dbl>   <dbl>    <dbl>   <dbl>   <dbl>      <dbl>
#> 1 Strain 1        No Ph~ A1                 1 0.00639    1.00  0.00327 0.00661   0.000630
#> 2 Strain 1        No Ph~ A1                 3 0.00674    1.00  0.00418 0.00648   0.000681
#> 3 Strain 1        No Ph~ A1                 7 0.00903    1.00  0.00619 0.00617   0.000831
#> 4 Strain 1        No Ph~ A1                 9 0.0113     0.999 0.00778 0.00583   0.000916
#> 5 Strain 1        No Ph~ A1                13 0.0174     0.999 0.0122  0.00525   0.00114
#> 6 Strain 1        Phage~ A7                 1 0.0172     0.911 0.00837 0.0135    0.141
#> # i 1 more variable: MAESD <dbl>
```

We can see that `train_smooth_data` has given us a number of accuracy metrics. The first one is 'RMSE', which stands for "root mean squared error" and is often a good default value to use (where lower values are better) to decide what the best parameter value is.

```
ggplot(data = training_results,
       aes(x = window_width_n, y = RMSE)) +
  geom_point()
```

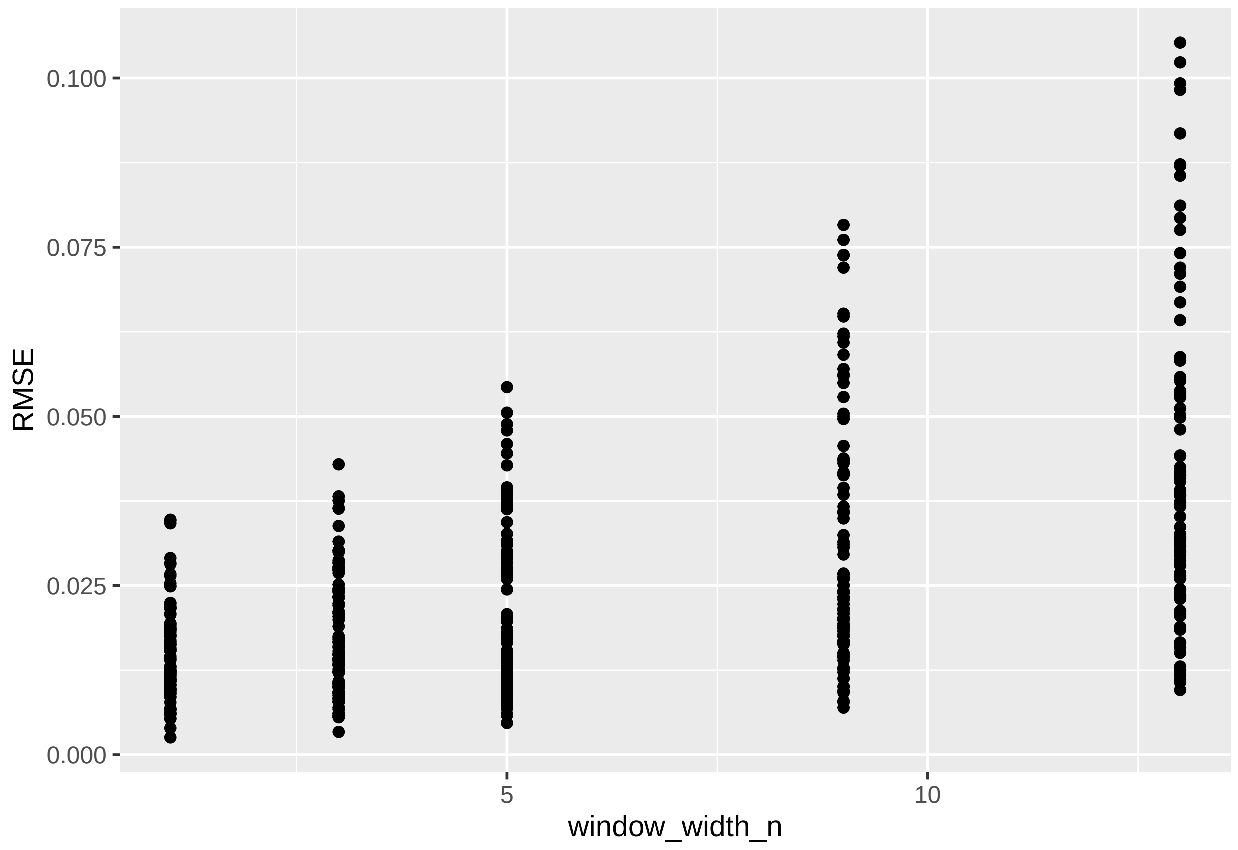


Based on this plot, a window_width_n of 1 has the lowest average error of all the values we tested.

However, often you'll want to test different values from the defaults in `train_smooth_data`. To do so, specify exactly what values you want to test with the `tuneGrid` argument:

```
training_results <-
  reframe(group_by(ex_dat_mrg_fortraining, Bacteria_strain, Phage, Well),
          train_smooth_data(x = Time, y = Measurements,
                   sm_method = "moving-average",
                   tuneGrid = list(
                     "window_width_n" = c(1, 3, 5, 9, 13))))

ggplot(data = training_results,
       aes(x = window_width_n, y = RMSE)) +
  geom_point()
```



Here we can see for our example data that cross-validation suggests little smoothing is necessary. This makes sense, since our example data has noise that is most problematic for our derivatives, and cross-validation doesn't consider the derivatives. For your data, I suggest combining cross-validation and manual observation of different smoothings to decide the best smoothing parameter value to use.

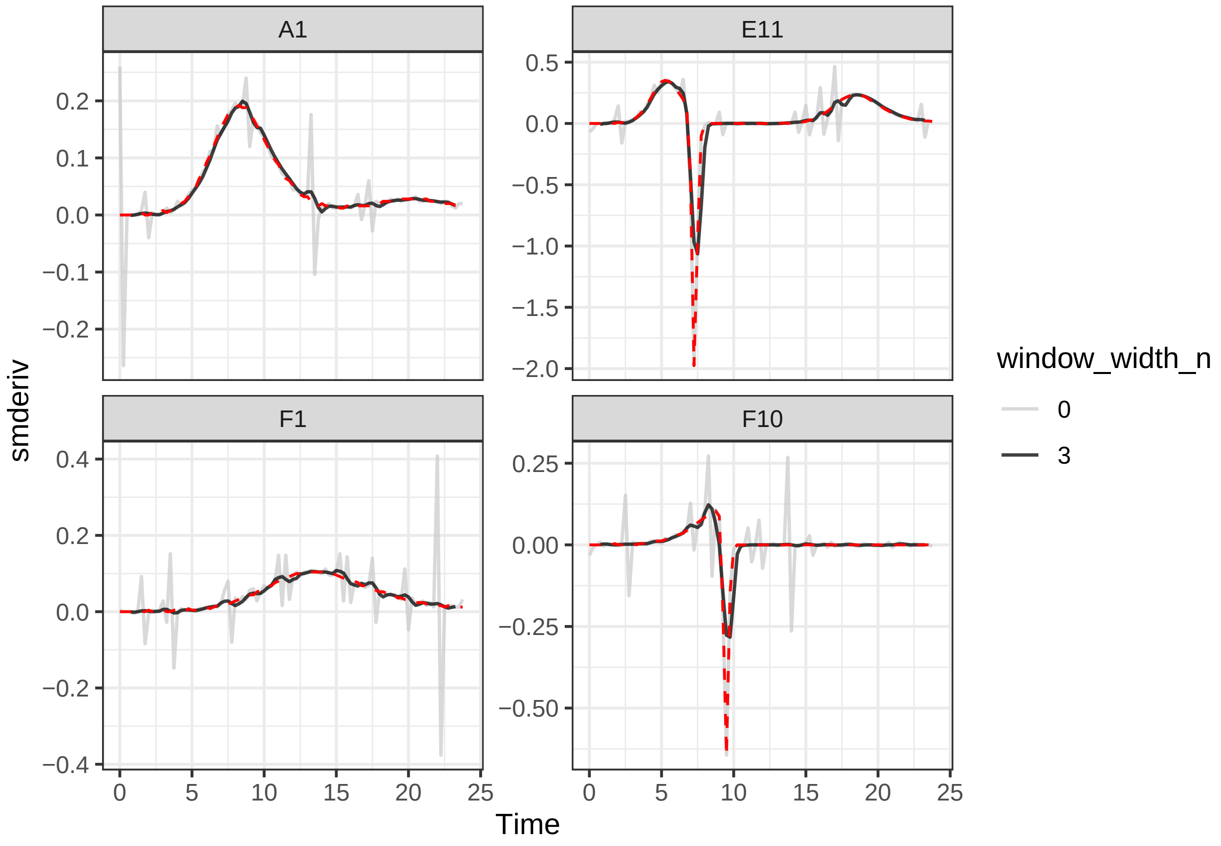
Calculating derivatives of smoothed data

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.

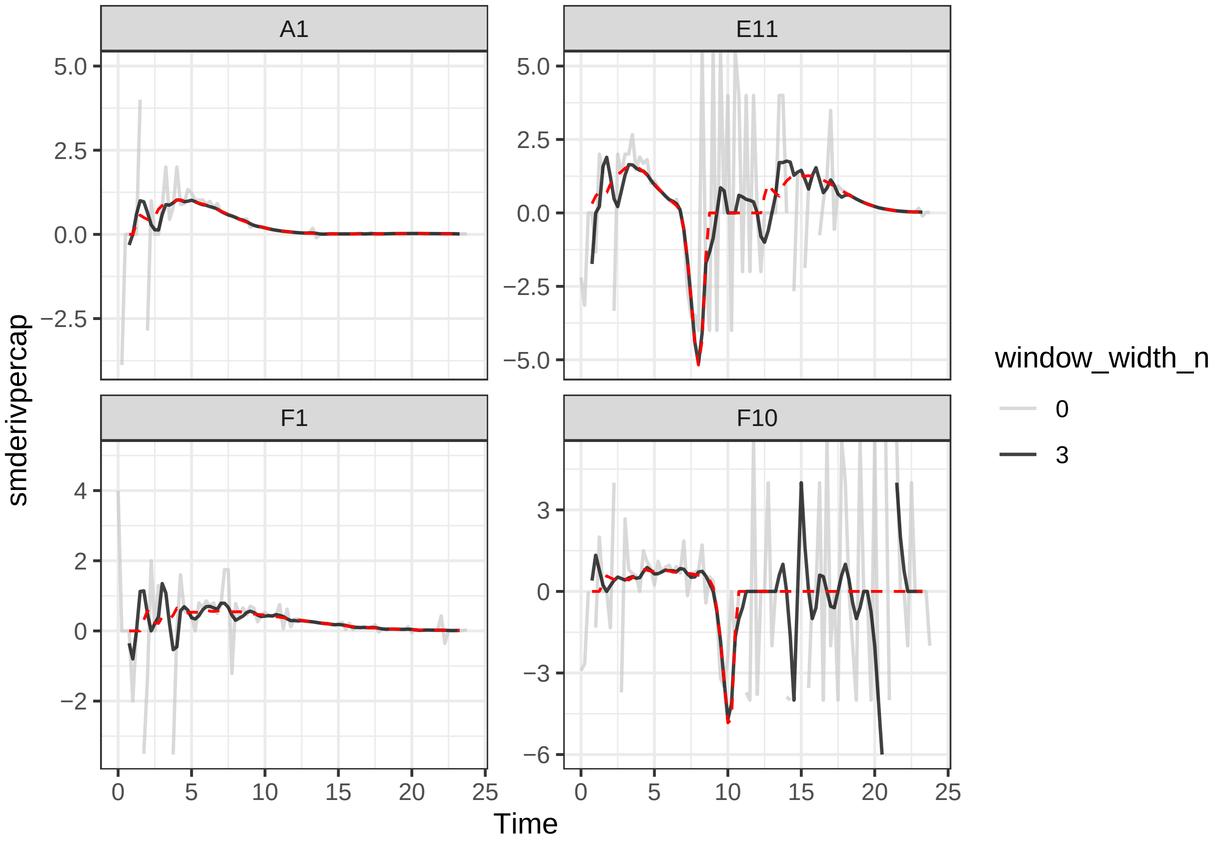
```r
# Note here that we're calculating derivatives of the smoothed column generated
#  in the previous section by combining moving median and moving average smoothing
ex_dat_mrg <-
  mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage, noise),
         smderiv_0 = calc_deriv(x = Time, y = Measurements),
         smderivpercap_0 = calc_deriv(x = Time, y = Measurements,
                                      percapita = TRUE, blank = 0),
         smderiv_3 = calc_deriv(x = Time, y = smoothed_yes, window_width_n = 3),
         smderivpercap_3 = calc_deriv(x = Time, y = smoothed_yes, percapita = TRUE,
                                   blank = 0, window_width_n = 3))

#Reshape our data for plotting purposes
ex_dat_mrg_wide <-
  pivot_longer(ex_dat_mrg, cols = starts_with("smderiv"),
               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 = smderiv, color = window_width_n)) +
  geom_line(linewidth = 0.6, alpha = 0.75) +
  scale_color_grey(start = 0.8, end = 0) +
  facet_wrap(~Well, scales = "free_y") +
  geom_line(data = dplyr::filter(ex_dat_mrg_wide,
                                 noise == "No", window_width_n == 0),
            lty = 2, color = "red") +
  theme_bw()
#> Warning: Removed 7 rows containing missing values or values outside the scale range
#> (`geom_line()`).
#> Warning: Removed 1 row containing missing values or values outside the scale range
#> (`geom_line()`).
```



```
#Plot per-capita derivative
ggplot(data = dplyr::filter(ex_dat_mrg_wide, noise == "Yes"),
       aes(x = Time, y = smderivpercap, color = window_width_n)) +
  geom_line(linewidth = 0.6, alpha = 0.75) +
  scale_color_grey(start = 0.8, end = 0) +
  facet_wrap(~Well, scales = "free_y") +
  geom_line(data = dplyr::filter(ex_dat_mrg_wide,
                                  noise == "No", window_width_n == 3),
            lty = 2, color = "red") +
  ylim(NA, 5) +
  theme_bw()
#> Warning: Removed 8 rows containing missing values or values outside the scale range
#> (`geom_line()`).
#> Warning: Removed 6 rows containing missing values or values outside the scale range
#> (`geom_line()`).
```



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

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.

1. Introduction: `vignette("gc01_gcplyr")`
2. Importing and reshaping 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")`

7. Dealing with noise: `vignette("gc07_noise")`
8. **Best practices and other tips:** `vignette("gc08_conclusion")`
9. Working with multiple plates: `vignette("gc09_multiple_plates")`
10. Using make_design to generate experimental designs: `vignette("gc10_using_make_design")`