Dealing with noise

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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.

```
library(gcplyr)

library(dplyr)
library(ggplot2)
library(tidyr)
#>
#> Attaching package: 'tidyr'
#> The following object is masked from 'package:testthat':
#>
#>     matches
```

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.

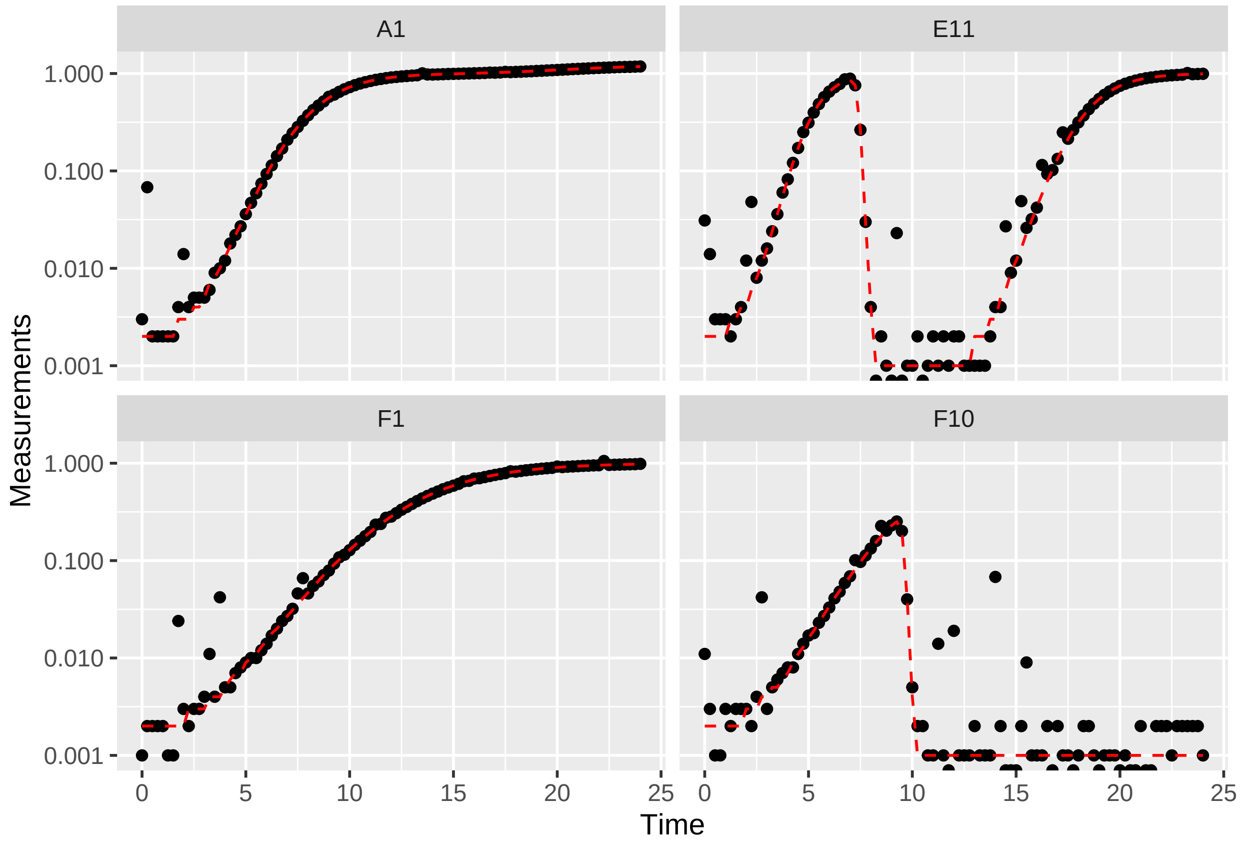
1. Excluding low-density data points
2. Using fitting during derivative calculations
3. Smoothing the raw data

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.

```
ex_dat_mrg <- make_example(vignette = 7, example = 1)
#> Joining, by = c("Time", "Well", "Measurements", "noise")
#> Warning in merge_dfs(noisy_data, noiseless_data):
#> merged_df has more rows than x and than y, this may indicate
#>                mis-matched values in the shared column(s) used to merge
#>               (e.g. 'Well')
#> Joining, by = "Well"

# 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: Transformation introduced infinite values in continuous y-axis
```

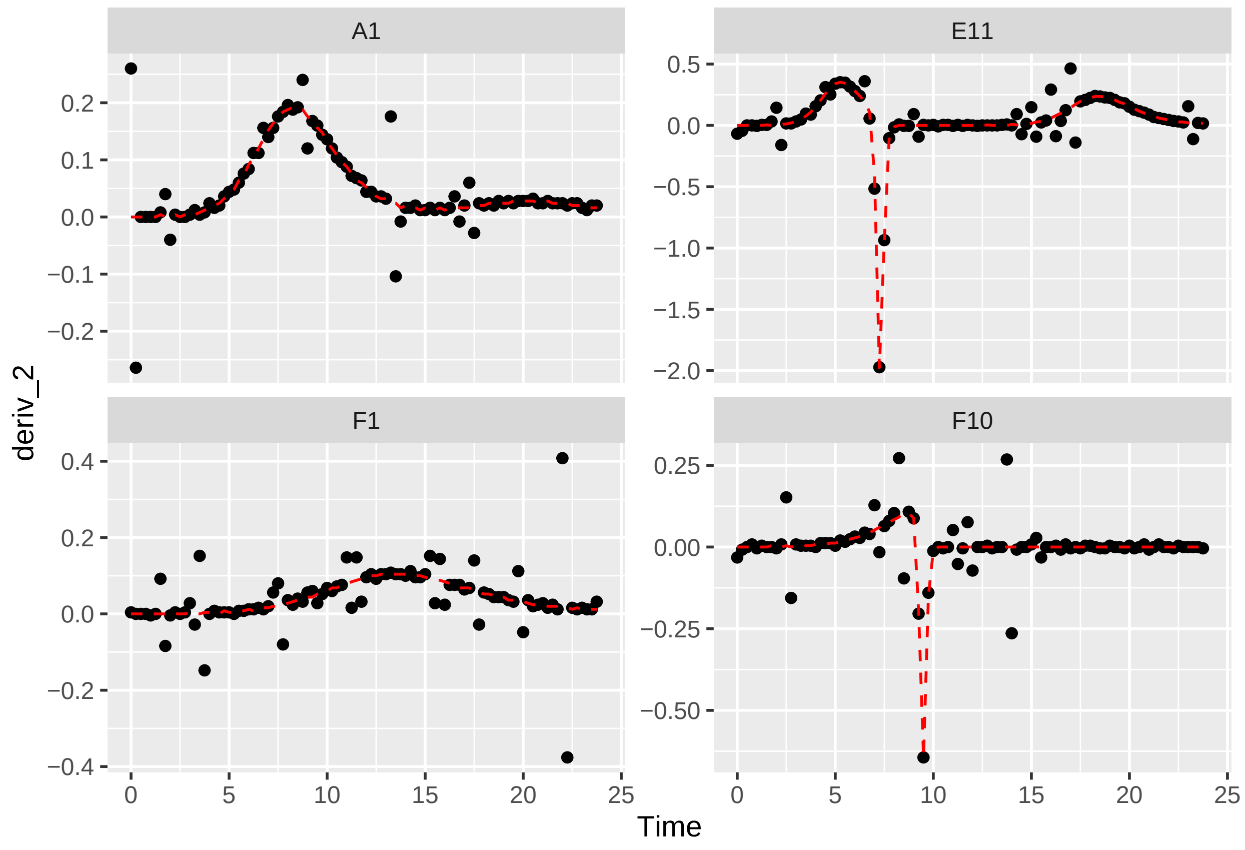


Great! Here we can see how the noisy (points) and noiseless (red line) data compare. We've plotted our data with log-transformed y-axes, which are useful because exponential growth is a straight line when plotted on a log scale. log axes also reveal another common pattern: **random noise tends to have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

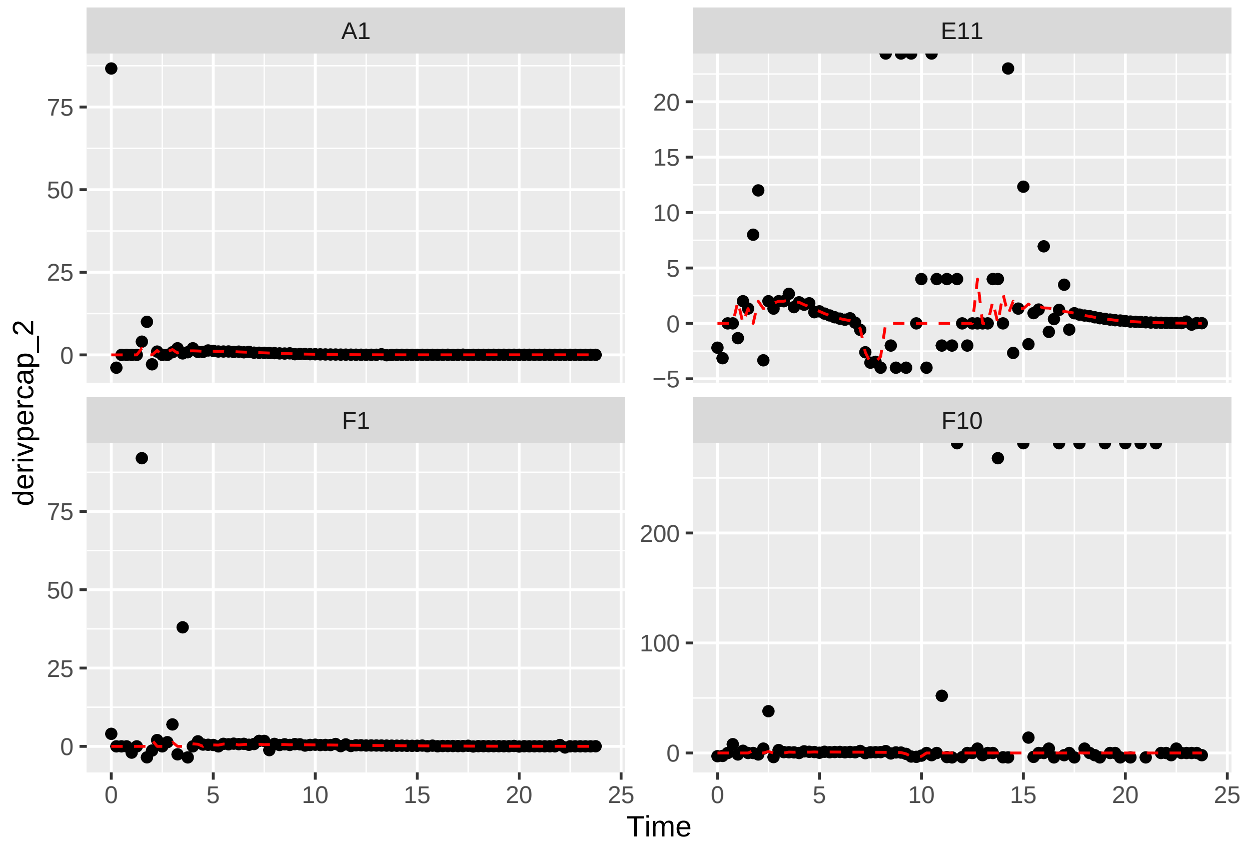
This level of noise doesn't seem like it would mess up calculations of maximum density or area under the curve much, so that's not enough of a reason to smooth. But let's look at what our derivatives look like.

```
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 (`geom_point()`).
#> Warning: Removed 1 row containing missing values (`geom_line()`).
```



```r
# 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 (`geom_point()`).
#> Removed 1 row containing missing values (`geom_line()`).
```

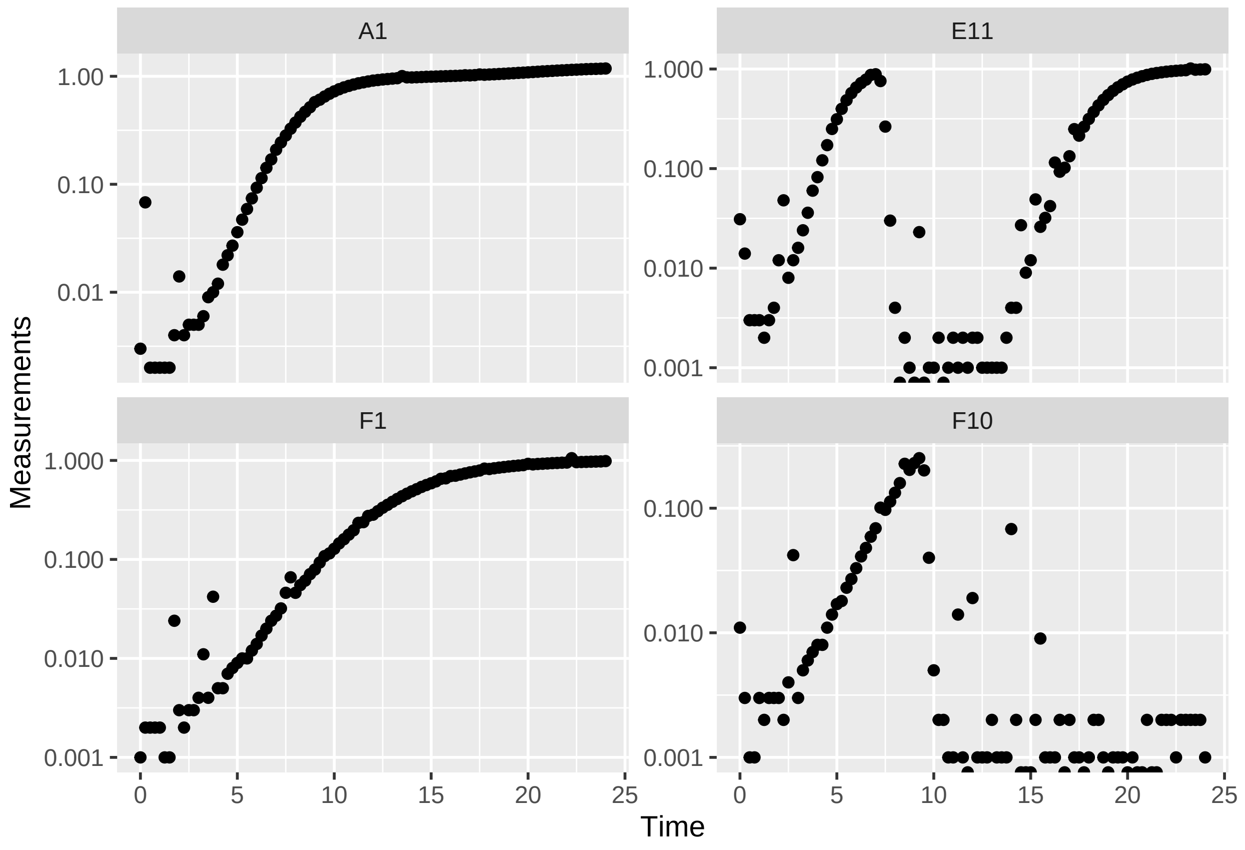


Those values are jumping all over the place! Let's see what we can do to address this.

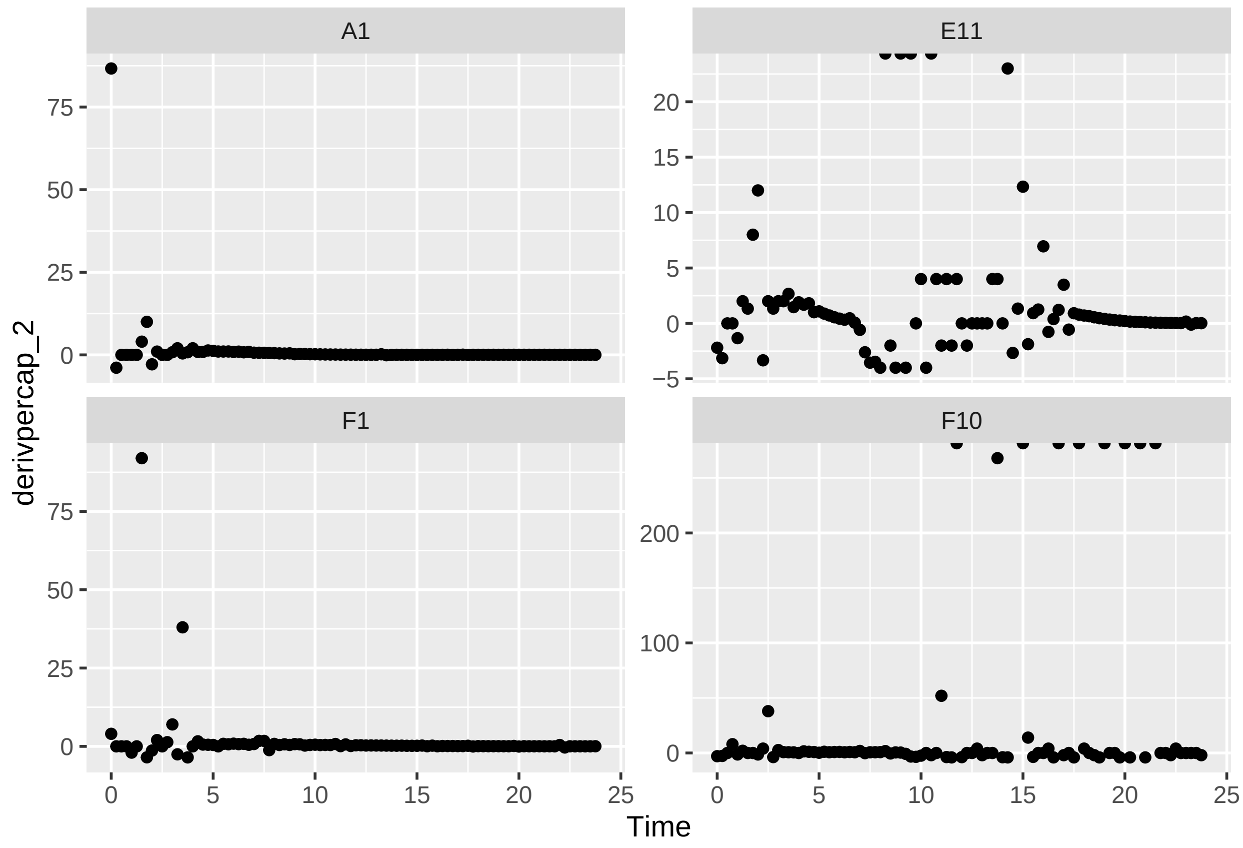
Summarizing on subsets of derivatives

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:

```
#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: Transformation introduced infinite values in continuous y-axis
```

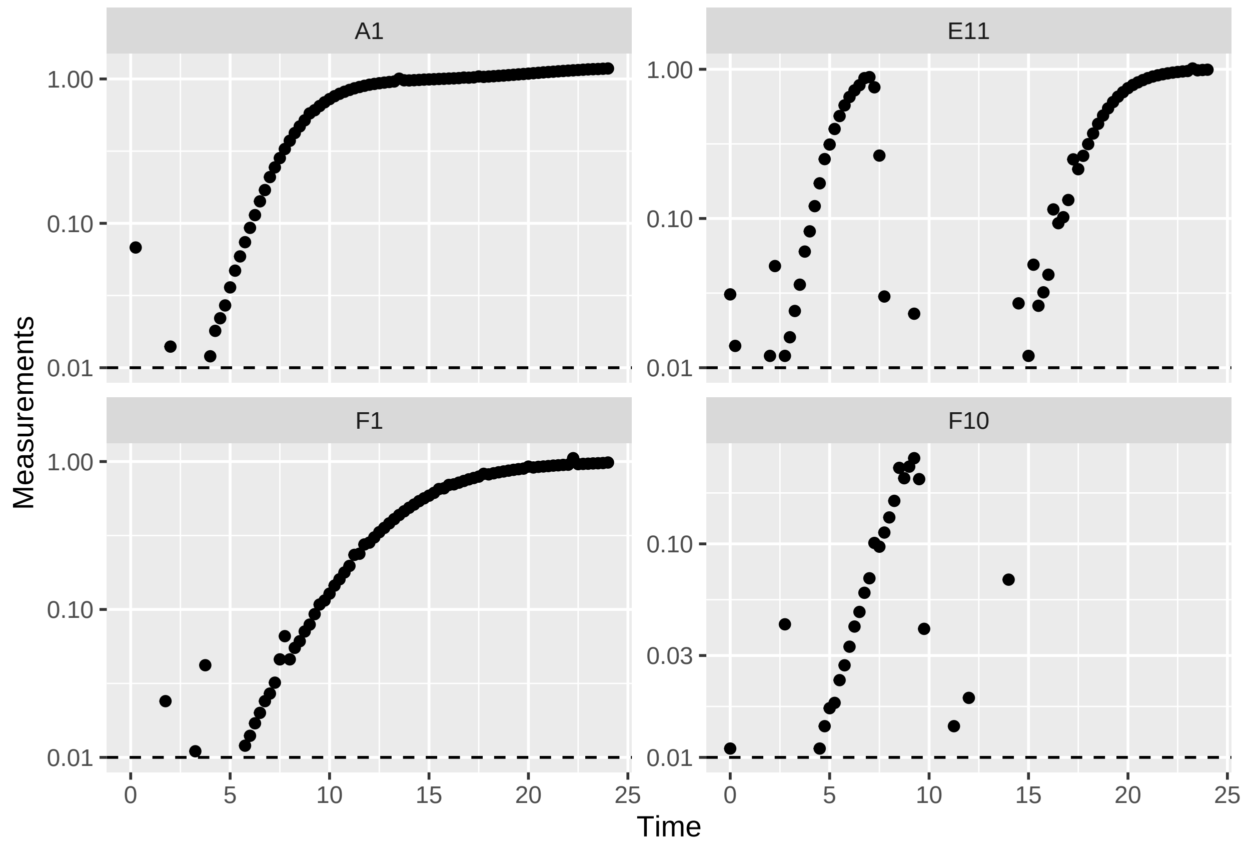


```
# Plot per-capita derivative
ggplot(data = dplyr::filter(ex_dat_mrg, noise == "Yes"),
       aes(x = Time, y = derivpercap_2)) +
  geom_point() +
  facet_wrap(~Well, scales = "free_y")
#> Warning: Removed 8 rows containing missing values (`geom_point()`).
```

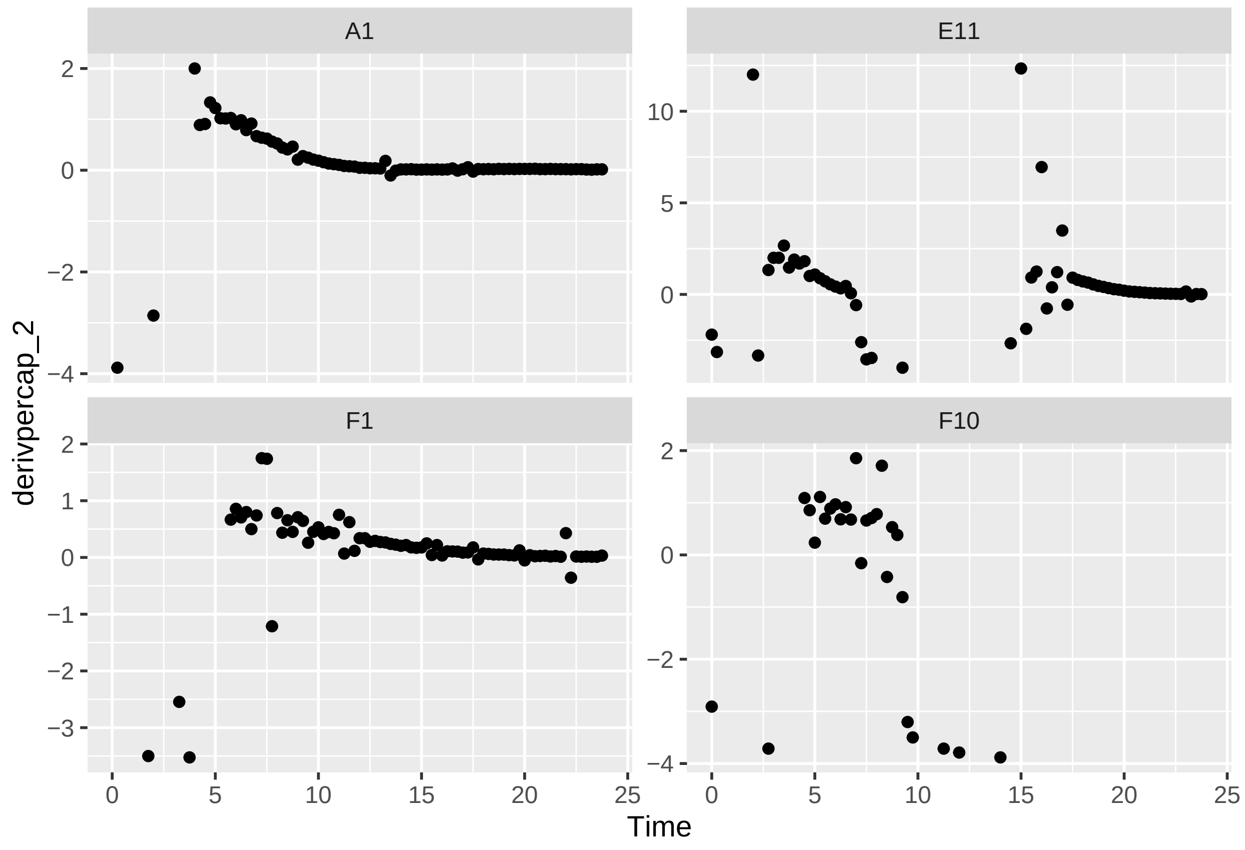


Per-capita growth rates are often very noisy when the density is close to 0, so it can make sense to simply exclude those data points.

```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 (`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
```

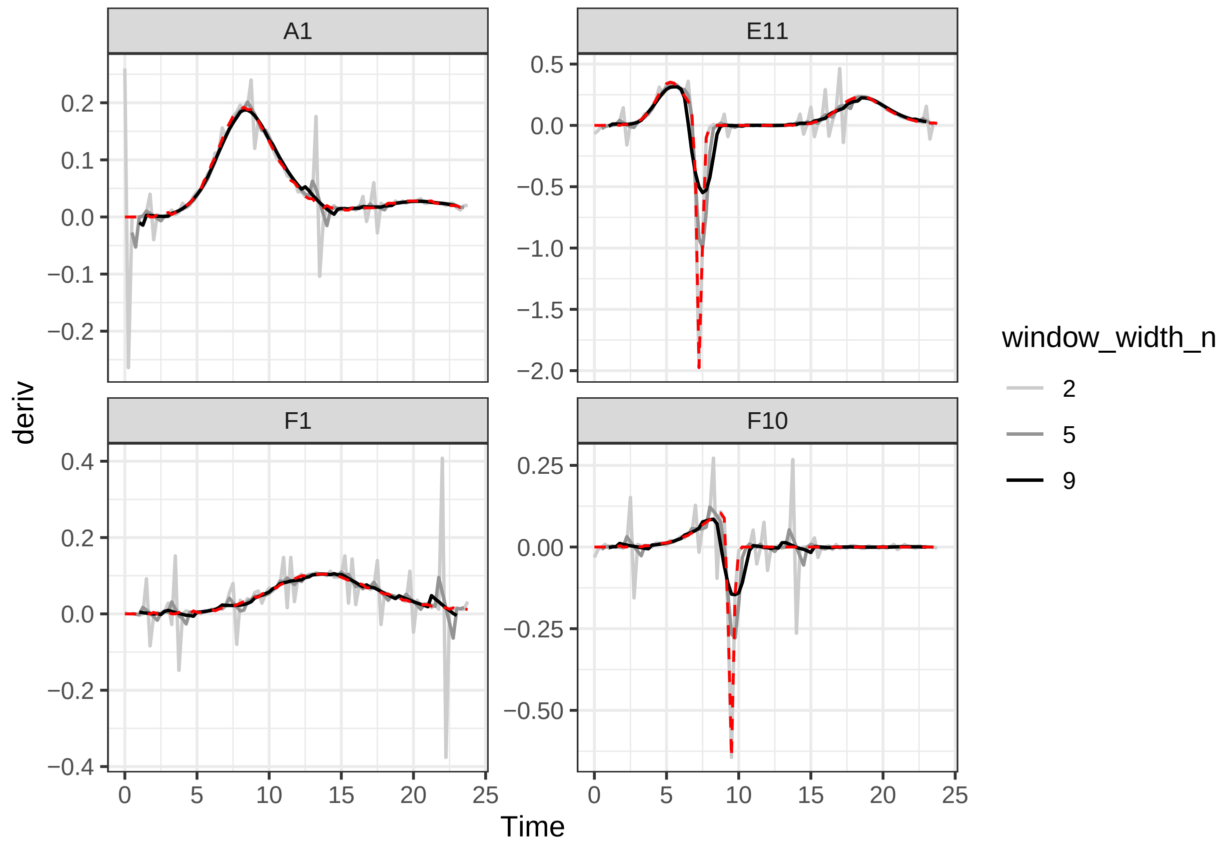
Fitting during derivative calculation

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")`.)

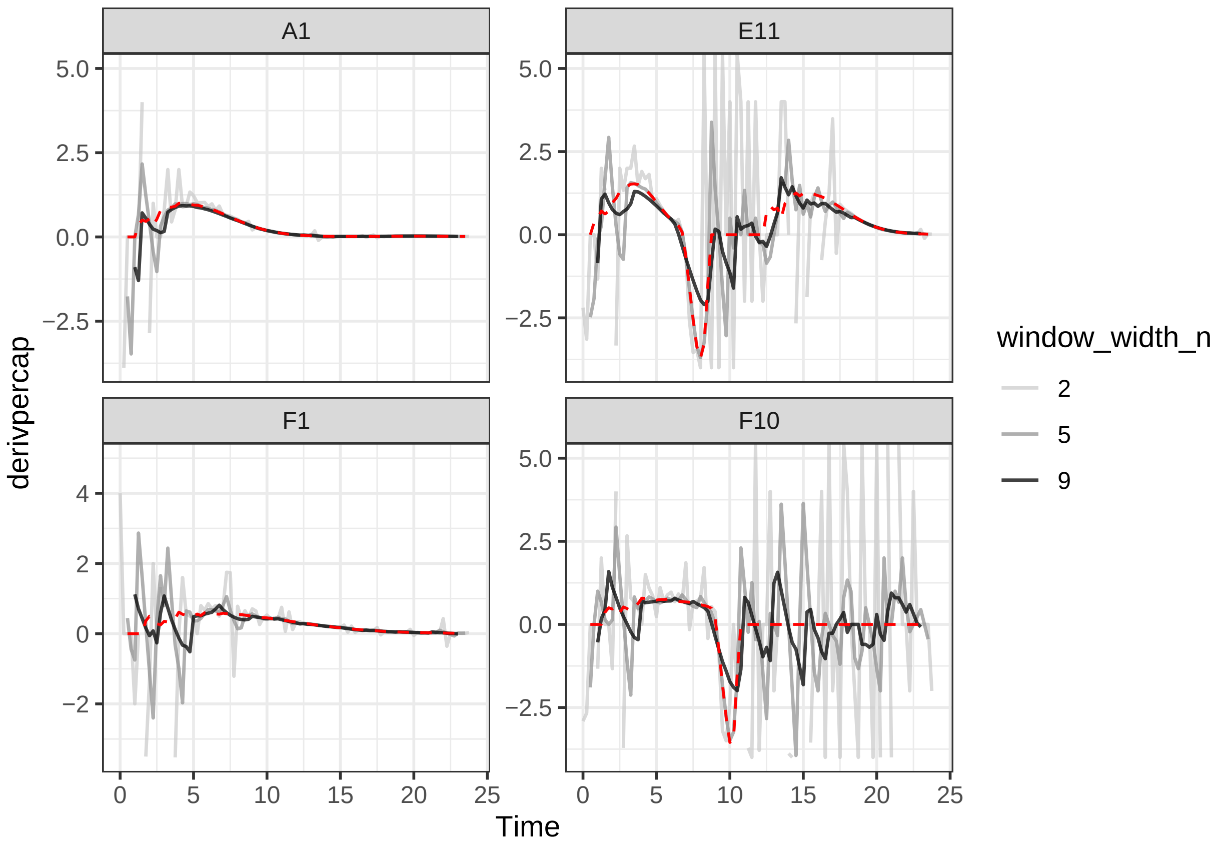
To use the fitting functionality of `calc_deriv`, specify either the `window_width` or the `window_width_n` parameter. `window_width` specifies how wide the window used to include points for the fitting is in units of `x`, while `window_width_n` specifies it in number of data points. Wider windows will be more smoothed. I recommend using as small a window as possible, like `window_width_n = 3` or `window_width_n = 5`. Visualize different window widths and 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 (`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), 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 (`geom_line()`).
#> Warning: Removed 4 rows containing missing values (`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

Finally, smoothing the raw density data can reduce the effects of noise. `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 when used on 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. Smoothing data is a step that alters the values you will analyze. Because of that it can be rife with pitfalls. I recommend choosing the value that smooths the data as little as necessary for your analyses to work. To do so, try smoothing with different tuning parameter values, plot the results, then choose the least smoothed one you can.

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.

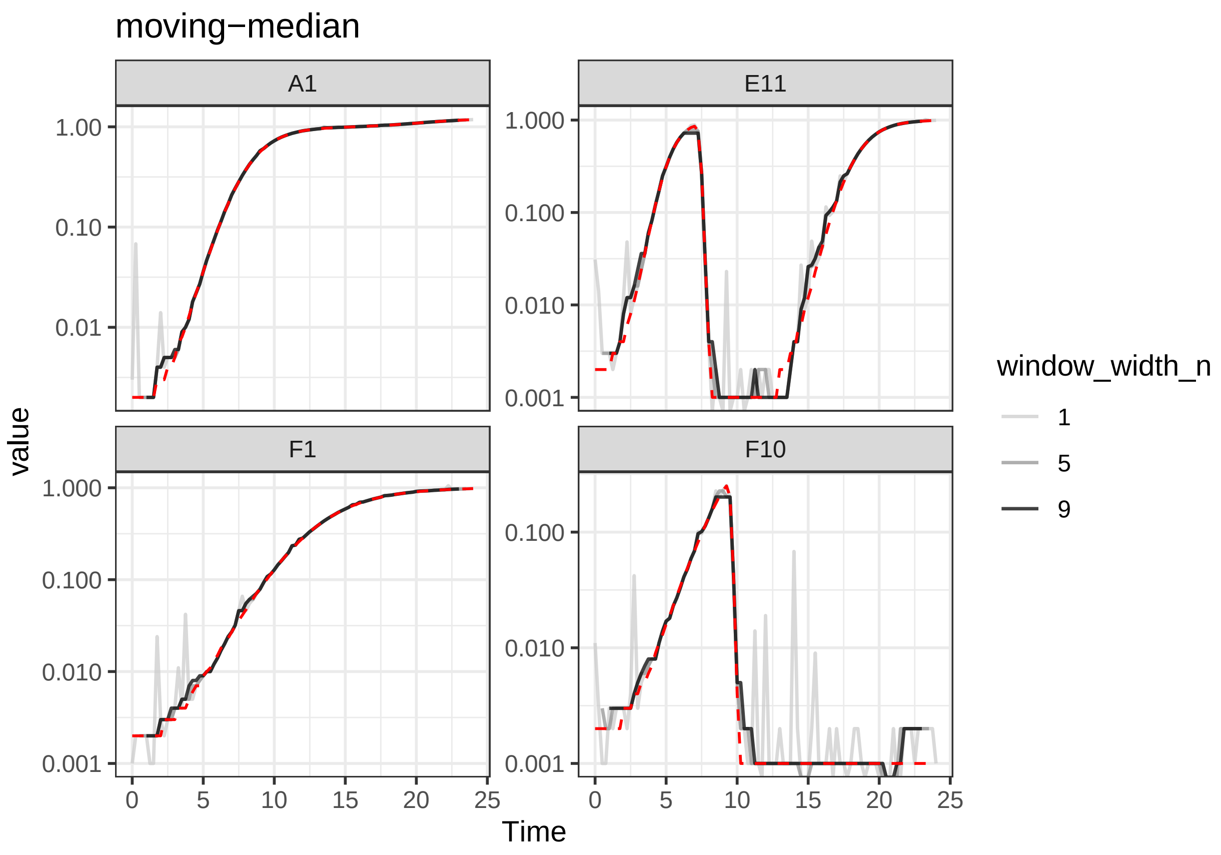
Smoothing with moving-median

For `moving-median`, 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 medians with windows that are 5 and 9 data points wide (`movemed_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: Transformation introduced infinite values in continuous y-axis
#> Warning: Removed 12 rows containing missing values (`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.

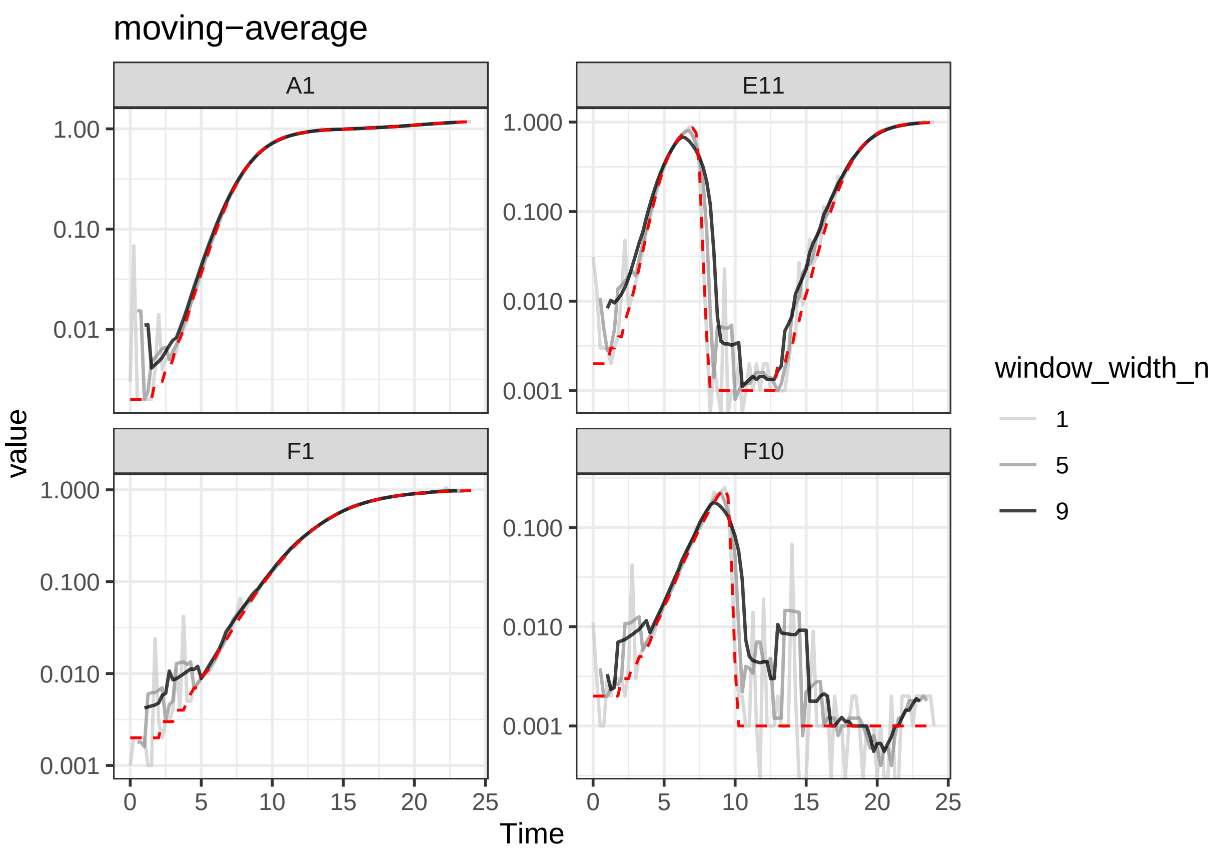
Smoothing with moving-average

For `moving-average`, 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. 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")
```

```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: Transformation introduced infinite values in continuous y-axis
#> Warning: Removed 12 rows containing missing values (`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 also starts underrepresenting the maximum density peaks relative to the true value (red line).

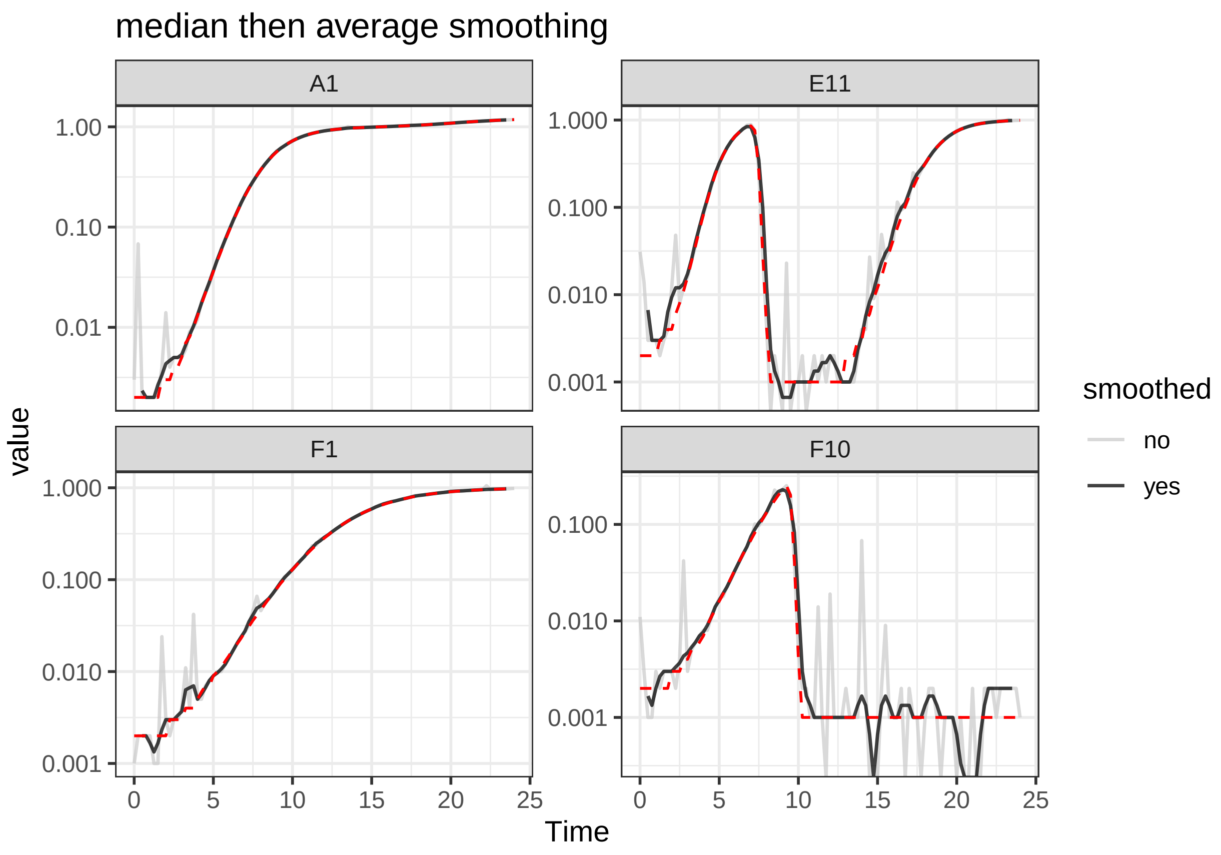
Combining multiple smoothing methods

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: Transformation introduced infinite values in continuous y-axis
#> Warning: Removed 4 rows containing missing values (`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 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.

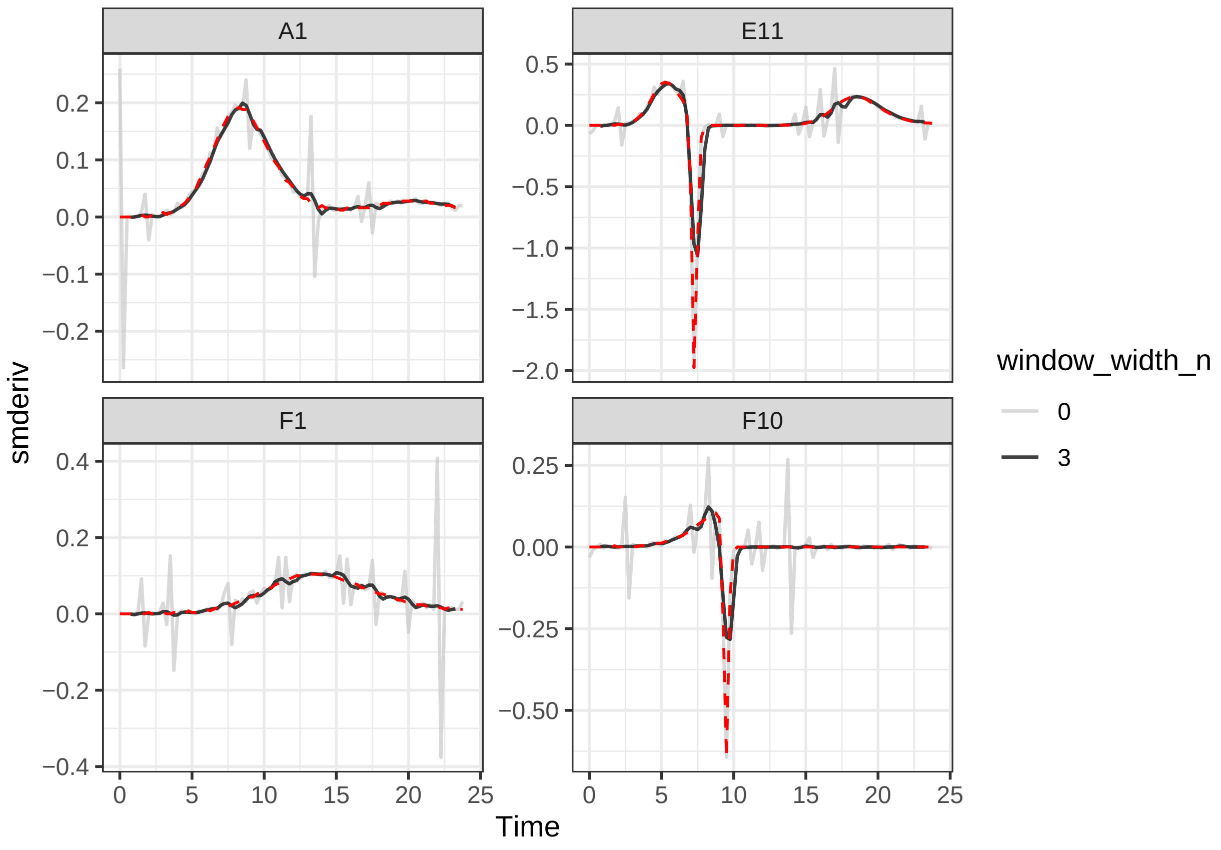
Calculating derivatives of smoothed data

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.

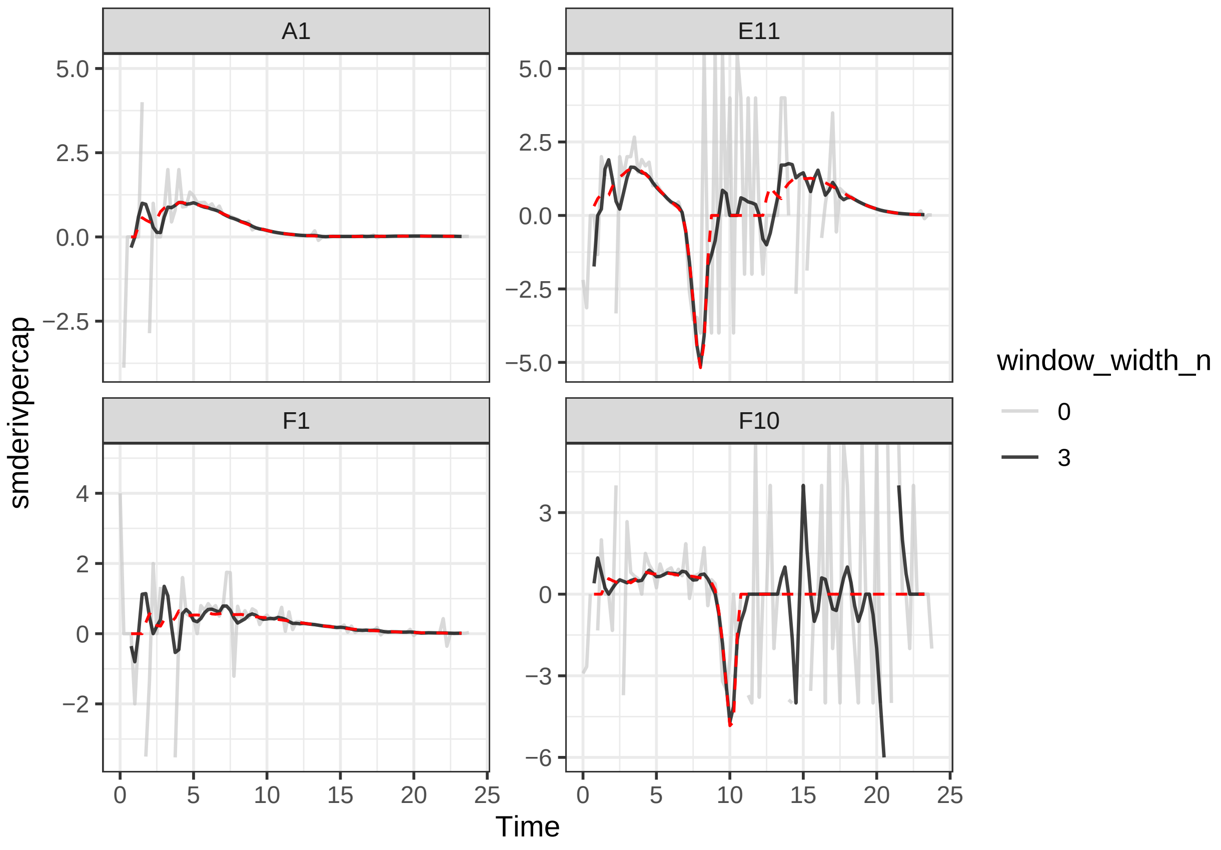
```r
# Note here that we're calculating derivatives of the smoothed column generated
#  in the previous section by combining moving median and moving average smoothing
ex_dat_mrg <-
  mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage, noise),
         smderiv_0 = calc_deriv(x = Time, y = Measurements),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

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. **Statistics, merging other data, and other resources:** `vignette("gc08_conclusion")`
9. Working with multiple plates: `vignette("gc09_multiple_plates")`