Analyzing data

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So far, we've imported and transformed our measures, combined them with our design information, preprocessed, processed, and plotted our data. Now we're going to analyze our data by summarizing our growth curves into a number of metrics.

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

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

```
#This code was previously explained
#Here we're re-running it so it's available for us to work with
example tidydata <- trans wide to tidy(example widedata noiseless,
                                       id cols = "Time")
example design <- make design(
  pattern_split = ",", nrows = 8, ncols = 12,
  "Bacteria_strain" = make_designpattern(
   values = paste("Strain", 1:48),
   rows = 1:8, cols = 1:6, pattern = 1:48, byrow = TRUE),
  "Bacteria_strain" = make_designpattern(
   values = paste("Strain", 1:48),
   rows = 1:8, cols = 7:12, pattern = 1:48, byrow = TRUE),
  "Phage" = make_designpattern(
    values = c("No Phage"), rows = 1:8, cols = 1:6, pattern = "1"),
  "Phage" = make_designpattern(
    values = c("Phage Added"), rows = 1:8, cols = 7:12, pattern = "1"))
ex_dat_mrg <- merge_dfs(example_tidydata, example_design)</pre>
#> Joining, by = "Well"
ex_dat_mrg$Well <-
  factor(ex dat mrg$Well,
         levels = paste(rep(LETTERS[1:8], each = 12), 1:12, sep = ""))
ex_dat_mrg <- group_by(ex_dat_mrg, Well, Bacteria_strain, Phage)</pre>
ex_dat_mrg <-
  mutate(ex_dat_mrg,
         deriv = calc_deriv(x = Time, y = Measurements, x_scale = 3600),
         deriv_percap5 = calc_deriv(x = Time, y = Measurements,
                                        percapita = TRUE, blank = 0,
                                         window_width_n = 5, trans_y = "log",
sample_wells <- c("A1", "F1", "F10", "E11")</pre>
```

Analyzing data with summarize

Ultimately, analyzing growth curves requires summarizing the entire time series of data by some metric or metrics. For instance, we may calculate metrics like:

- the maximum density
- the total area under the curve
- the lag time (approximated as the time from the start until maximum per-capita growth rate is achieved)
- the maximum per-capita growth rate

- the density when a diauxic shift occurs
- the time until diauxic shift occurs
- the peak per-capita growth rate after a diauxic shift
- the peak density before a decline from phage predation
- the time when bacteria drop below some density because of phage predation

gcplyr contains a number of functions that make it easier to carry out these calculations. Additionally, gcplyr functions are flexible enough that you can use them in designing your own metric calculations. The following sections highlight general-use gcplyr functions and provide examples to calculate the common metrics above.

But first, we need to familiarize ourselves with one more dplyr function: summarize. Why? Because the upcoming gcplyr analysis functions *must* be used *within* dplyr::summarize. If you're already familiar with dplyr's summarize, feel free to skip the primer in the next section. If you're not familiar yet, don't worry! Continue to the next section, where I provide a primer that will teach you all you need to know on using summarize with gcplyr functions.

Another brief primer on dplyr: summarize

Here we're going to focus on the summarize function from dplyr, which must be used with the group_by function we covered in our first primer: A brief primer on dplyr. summarize carries out user-specified calculations on each group in a grouped data.frame independently, producing a new data.frame where each group is now just a single row.

For growth curves, this means we will:

- 1. group_by our data so that every well is a group
- 2. summarize each well with calculations like maximum density or area under the curve

Since summarize will drop columns that the data aren't grouped by and that aren't summarized, we will typically want to list all of our design columns for group_by, along with the plate name and well. Again, 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.

In the next section, I provide a simple example of how the max function is used with group_by and summarize to calculate lag time and the maximum per-capita growth rate. 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 remaining gcplyr functions.

Summarizing with simple base functions: maximum density and growth rate

One of the most common steps is calculating global maxima (or minima) of data. For instance, with bacterial growth, maximum density or growth rate are some of the most commonly measured traits. Here, we'll show how to find them using the built-in max function.

First, we need to group our data. As before, group_by simply requires the data.frame to be grouped, and the names of the columns we want to group by.

Then, we run summarize. Just like for mutate, we specify:

- 1. the name of the variable we want results saved to
- 2. the function that calculates the summarized results

In this case, the function should return just a single value for each group. For instance, in the code below we've calculated the maximum of the Measurements column, and saved it in a column named max_dens (note that we need to specify na.rm = TRUE to tell max to ignore all NA values). We've saved the output from summarize to a new data.frame: ex_dat_mrg_sum, short for example_data_merged_summarized.

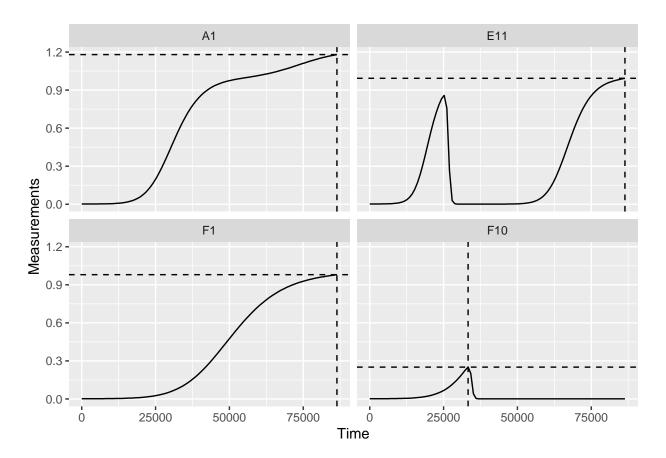
```
ex_dat_mrg_sum <- summarize(ex_dat_mrg,</pre>
                            max_dens = max(Measurements, na.rm = TRUE))
#> `summarise()` has grouped output by 'Bacteria_strain', 'Phage'. You can override using the
#> `.groups` argument.
head(ex_dat_mrg_sum)
#> # A tibble: 6 x 4
#> # Groups: Bacteria_strain, Phage [6]
   Bacteria_strain Phage
                                 Well max_dens
    <chr>
                                 <fct>
#>
                     <chr>
                                           <db1>
#> 1 Strain 1
                    No Phage
                                 A1
                                          1.18
#> 2 Strain 1
                    Phage Added A7
                                          0.499
#> 3 Strain 10
                    No Phage
                                 B4
                                          1.21
#> 4 Strain 10
                     Phage Added B10
                                          0.962
#> 5 Strain 11
                     No Phage
                                 B5
                                          1.21
#> 6 Strain 11
                     Phage Added B11
                                          1.03
```

If you want additional characteristics, you simply add them to the summarize. For instance, if we want the time when the maximum density occurs, you just add that as a second argument. In this case, we use the which.max function, which returns the index of the maximum value, to get the index of the Time when the maximum occurs, and save it to a column titled max_time:

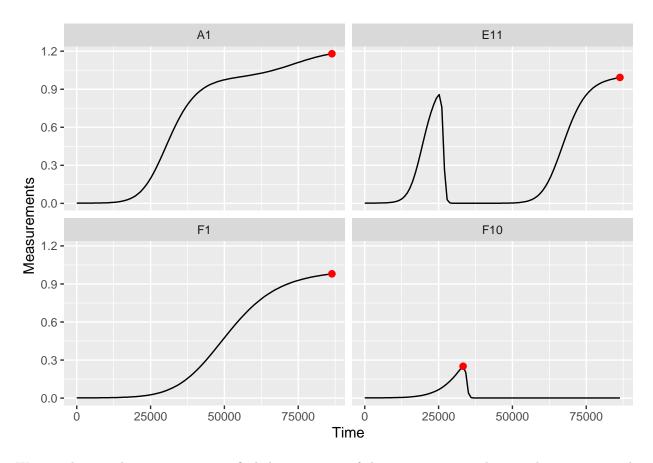
```
ex_dat_mrg_sum <- summarize(ex_dat_mrg,</pre>
                            max_dens = max(Measurements, na.rm = TRUE),
                            max_time = Time[which.max(Measurements)])
#> `summarise()` has grouped output by 'Bacteria_strain', 'Phage'. You can override using the
#> `.groups` argument.
head(ex_dat_mrg_sum)
#> # A tibble: 6 x 5
#> # Groups: Bacteria_strain, Phage [6]
   Bacteria_strain Phage
                                 Well max_dens max_time
#>
    <chr>
                     <chr>
                                 <fct>
                                          <dbl>
                                                    <db1>
#> 1 Strain 1
                     No Phage
                                                    86400
                                 A1
                                          1.18
#> 2 Strain 1
                    Phage Added A7
                                                    31500
                                          0.499
#> 3 Strain 10
                    No Phage
                                          1.21
                                 B4
                                                    85500
#> 4 Strain 10
                    Phage Added B10
                                          0.962
                                                    30600
```

```
#> 5 Strain 11 No Phage B5 1.21 70200
#> 6 Strain 11 Phage Added B11 1.03 86400
```

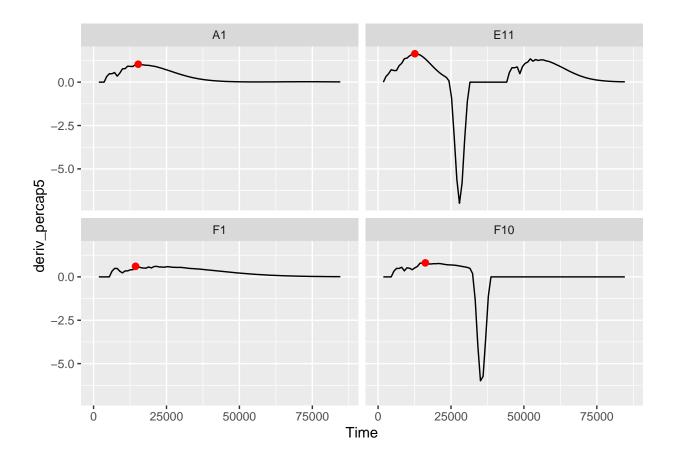
And we can quite easily plot such summarized values as a horizontal line or vertical line on top of our original growth curves data with the geom_hline or geom_vline functions:



Alternatively, we could plot these summary points as a point:



We can also use the same process to find the maximum of the per-capita growth rates that we previously calculated:



Summarizing with simple gcplyr functions: area under the curve

One common metric of growth curves is the total area under the curve. gcplyr has an auc function to easily calculate this area. Just like min and max, it needs to be used inside summarize on a data.frame that has been grouped.

To use auc, simply specify the x and y data whose area-under-the-curve you want to calculate. Here, we calculate the area-under-the-curve of the Measurements column and save it to a column titled auc.

```
ex_dat_mrg_sum <-
  summarize(ex_dat_mrg,
            auc = auc(x = Time, y = Measurements))
#> `summarise()` has grouped output by 'Bacteria_strain', 'Phage'. You can override using the
#> `.groups` argument.
head(ex_dat_mrg_sum)
#> # A tibble: 6 x 4
#> # Groups: Bacteria_strain, Phage [6]
     Bacteria_strain Phage
                                  Well
                                           auc
#>
     <chr>
                     <chr>
                                  <fct>
                                         <dbl>
#> 1 Strain 1
                     No Phage
                                  A1
                                        57291.
#> 2 Strain 1
                     Phage Added A7
                                         3856.
#> 3 Strain 10
                     No Phage
                                  B4
                                        73505.
#> 4 Strain 10
                     Phage Added B10
                                        22156.
#> 5 Strain 11
                     No Phage
                                        75289.
                                  B5
#> 6 Strain 11
                     Phage Added B11
                                        27966.
```

Finding local extrema: peak density, maximum growth rate, lag time, and diauxic shifts

We've previously shown how you can use max and min to find the global maxima and minima in data. However, what about *local* maxima or minima? That is, peaks and valleys that are obvious to the eye but aren't the highest or smallest values in the entire time series. In this section, we'll show how you can use the gcplyr functions first_peak and find_local_extrema to find points that are local maxima or minima in your data.

Finding the first peak: peak density, maximum growth rate, and lag time

One particular special case we're often interested in is the first peak in some set of data. For instance, when bacteria are grown with phages, the density they reach before they start declining due to phage predation is a measure of their susceptibility to the phage. Alternatively, in the previous section we found the global maximum per-capita growth rate, but what if some of these maxima happened after near-extinction and recovery and we wanted to only find the peak growth rate before near-extinction?

Peak density

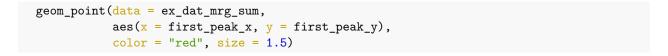
Let's start with the former example: finding the peak of density.

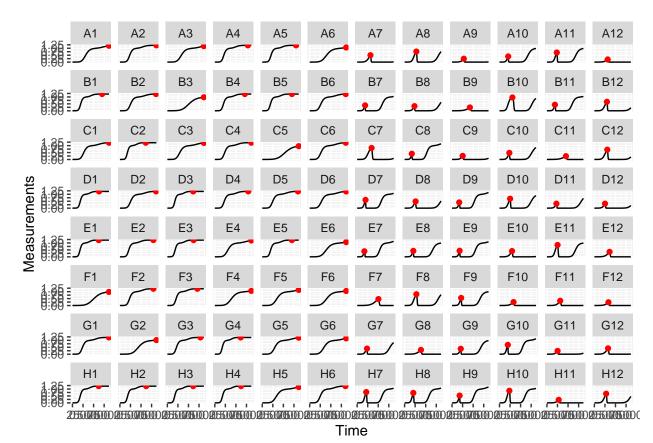
To identify the first peak, we can use the gcplyr function first_peak. first_peak simply requires the y data you want to identify the peak in. In this case, that's Measurements. We also need to specify whether we want the function to return the index of the first peak, the x value of the peak, or the y value of the peak. We'll get the x and y values, saving them in columns first_peak_x and first_peak_y, respectively. As usual, first_peak needs to be used inside of a summarize command on data that has already been grouped.

```
ex_dat_mrg_sum <-
  summarize(ex_dat_mrg,
            first_peak_x = first_peak(x = Time, y = Measurements, return = "x"),
            first_peak_y = first_peak(x = Time, y = Measurements, return = "y"))
#> `summarise()` has grouped output by 'Bacteria strain', 'Phage'. You can override using the
#> `.groups` argument.
head(ex_dat_mrg_sum)
#> # A tibble: 6 x 5
#> # Groups: Bacteria_strain, Phage [6]
   Bacteria_strain Phage
                                  Well first_peak_x first_peak_y
                                  \langle fct \rangle
#>
     <chr>
                      <chr>
                                                <dbl>
                                                             <db1>
#> 1 Strain 1
                     No Phage
                                  A1
                                                86400
                                                             1.18
#> 2 Strain 1
                     Phage Added A7
                                                31500
                                                             0.499
#> 3 Strain 10
                     No Phage
                                  B4
                                                71100
                                                             1.21
#> 4 Strain 10
                     Phage Added B10
                                                30600
                                                             0.962
#> 5 Strain 11
                     No Phage
                                  B5
                                                70200
                                                             1.21
#> 6 Strain 11
                     Phage Added B11
                                                18900
                                                             0.439
```

Let's plot these points on all the wells to confirm they are what we expect:

```
ggplot(data = ex_dat_mrg, aes(x = Time, y = Measurements)) +
  geom_line() +
  facet_wrap(~Well, nrow = 8, ncol = 12) +
```





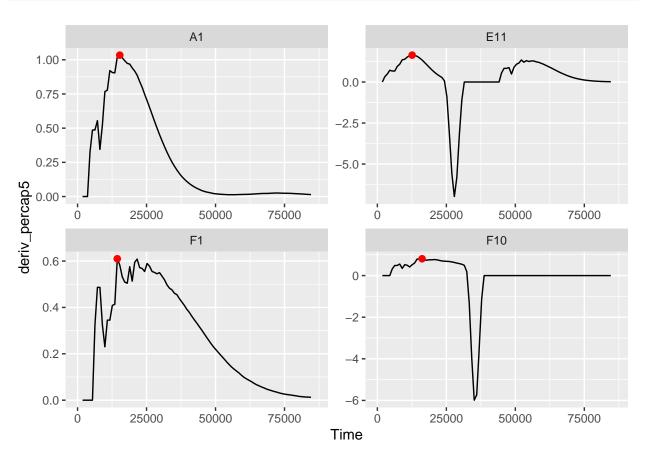
That worked great! In some cases, you might find that first_peak is not sensitive enough, or is too sensitive, for your data. In those cases, you can adjust the tuning parameters to make first_peak more or less sensitive to small peaks and valleys. For first_peak, the tuning parameters are window_width, window_width_n, and window_height:

- window_width determines the width of the window used to search for peaks and valleys, in units of x
- window_width_n determines the width of the window, in units of number of data points
- window_height determines the shortest peak or shallowest valley the window will cross, in units of y

Maximum growth rate and lag time

Now let's look at the other example: using first_peak to find the first peak in per-capita growth rate. Finding this point tells us both what the maximum growth rate is, and how long it took the cells to reach that rate (a measure of lag time).

```
#> `summarise()` has grouped output by 'Bacteria_strain', 'Phage'. You can override using the
#> `.groups` argument.
head(ex_dat_mrg_sum)
#> # A tibble: 6 x 5
#> # Groups:
               Bacteria_strain, Phage [6]
#>
     Bacteria_strain Phage
                                  Well max_growth_rate lag_time
#>
     <chr>
                     <chr>
                                  <fct>
                                                  <db1>
                                                            <db1>
                     No Phage
#> 1 Strain 1
                                  A1
                                                   1.03
                                                           15300
                     Phage Added A7
#> 2 Strain 1
                                                   1.03
                                                           15300
#> 3 Strain 10
                     No Phage
                                                   1.59
                                  B4
                                                           12600
#> 4 Strain 10
                     Phage Added B10
                                                   1.59
                                                           12600
#> 5 Strain 11
                     No Phage
                                                   1.65
                                  B5
                                                           12600
#> 6 Strain 11
                                                   1.65
                     Phage Added B11
                                                           12600
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = deriv_percap5)) +
  geom_line() +
  facet_wrap(~Well, scales = "free") +
  geom_point(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(x = lag_time, y = max_growth_rate),
             color = "red", size = 2)
#> Warning: Removed 4 row(s) containing missing values (geom_path).
```



But what if you want to find an extrema that's *not* the first peak? In the next section, we'll learn how to use find_local_extrema to identify all kinds of local extrema.

Finding any kind of local extrema: diauxic shifts

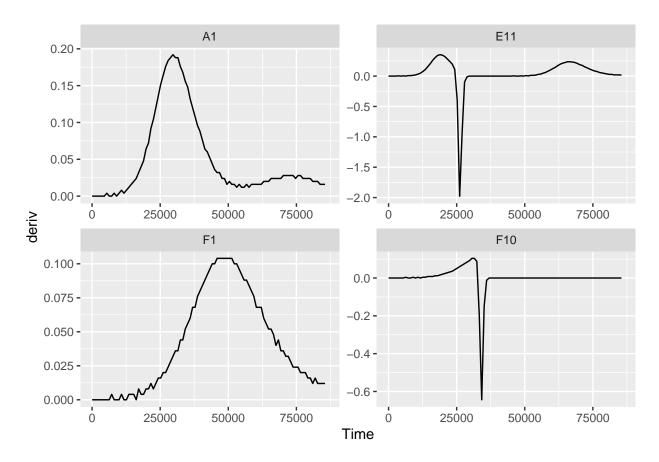
We've seen how first_peak can be used to identify the first peak in data. But what about other kinds of local extrema? The first minimum? The second peak?

In order to identify these kinds of extrema, we can use the more-general function find_local_extrema. In fact, first_peak is really just a special case of find_local_extrema. Just like first_peak, find_local_extrema only requires a vector of y data in which to find the local extrema, and can return the index, x value, or y value of the extrema it finds.

Unlike first_peak, find_local_extrema returns a vector containing all of the local extrema found under the given settings. Users can alter which kinds of local extrema are reported using the arguments return_maxima, return_minima, and return_endpoints. However, find_local_extrema will always return a vector of all the extrema found, so users must use brackets to select which one they want summarize to save.

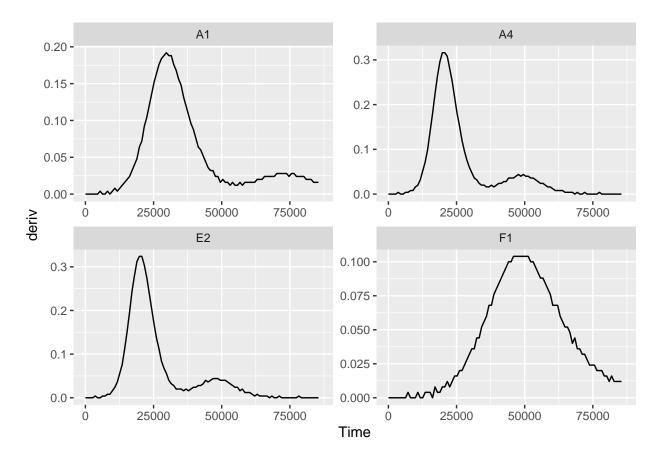
Let's dig into an example: identifying diauxic shifts. To refresh your memory on what we saw in the derivatives article, here's a plot of the derivative of some of the wells over time.

```
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
        aes(x = Time, y = deriv)) +
   geom_line() +
   facet_wrap(~Well, scales = "free")
#> Warning: Removed 1 row(s) containing missing values (geom_path).
```



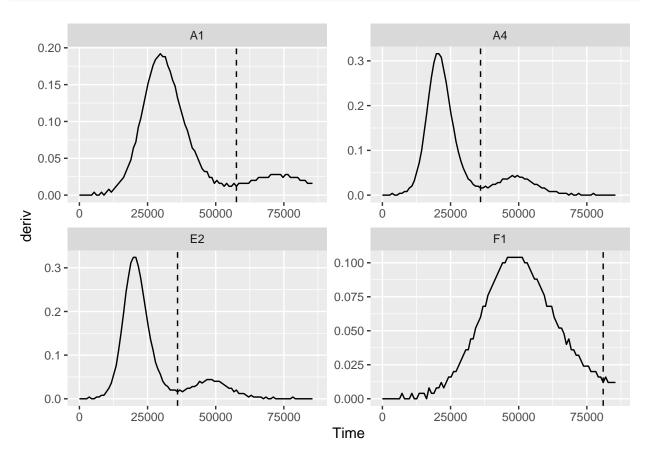
In fact, if we look at some more of the wells with no phage added, we'll see a similar pattern repeatedly.

```
sample_wells <- c("A1", "A4", "E2", "F1")
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
        aes(x = Time, y = deriv)) +
   geom_line() +
   facet_wrap(~Well, scales = "free")
#> Warning: Removed 1 row(s) containing missing values (geom_path).
```

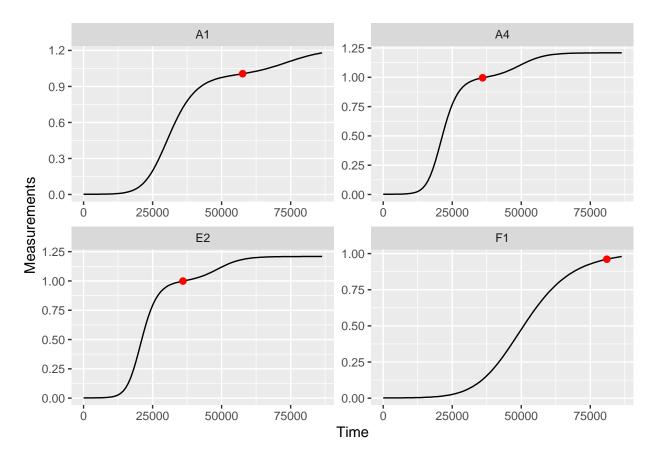


This second, slower, burst of growth after the first wave of growth is common in bacterial growth curves and is called *diauxic growth*.

How could we identify the time when the bacteria switch from their first burst of growth to their second? We can find the second minima in the deriv values (where the first minima is going to be at the start of the growth curve). To do so, we specify to find_local_extrema that we want return = "x" and we don't want maxima returned:



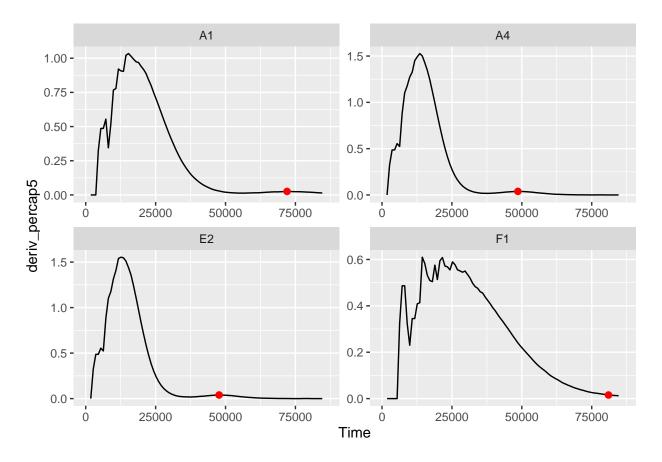
Now that we've found the point where the bacteria switch, let's identify the density where the diauxic shift occurs. First, we'll save the *index* where the diauxic shift occurs to a column titled diaxuie_idx. Then, we can get the Measurements value at that index. (Note that it wouldn't work to just specify return = "y", because the y values in this case are the deriv values).



Something that was hard to see on the density plot has now been easily quantified and can be visualized exactly where the shift occurs.

Combining subsets and local extrema: diauxic growth rate

In the previous section we identified when the bacteria shifted into their second burst of growth. Can we find out what the peak per-capita growth rate was during that second burst? Yes, we just have to put together some of the things we've learned already. In particular, we're going to combine our use of find_local_extrema, max, and subsets to find the max(deriv_percap_hr) during the times after the diauxic shift:



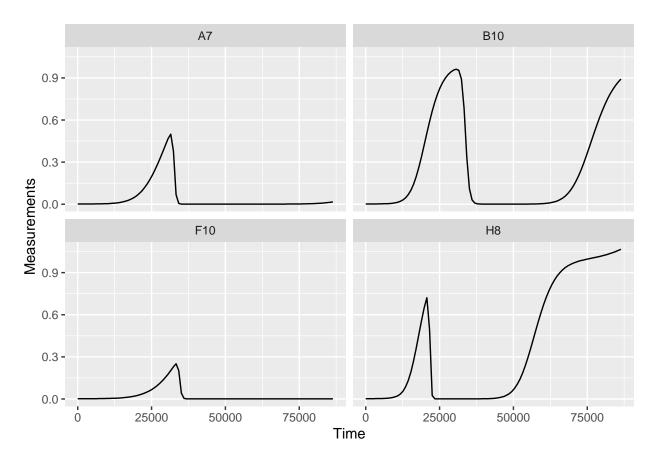
Finding threshold-crossings: extinction time and time to density

We've previously shown how you can find local and global extrema in data, but what if you just want to find when the data passes some threshold value? In this section, we'll show how you can use the gcplyr functions first_below and find_threshold_crosses to find the points when your data crosses user-defined thresholds.

Finding the first point below a threshold: extinction time

One common case of threshold-crossing we might be interested in is the first point that our data falls below some threshold density. For instance, when bacteria are grown with phages, the amount of time it takes before the bacterial population falls below some threshold can be a proxy metric for how sensitive the bacteria are to that phage.

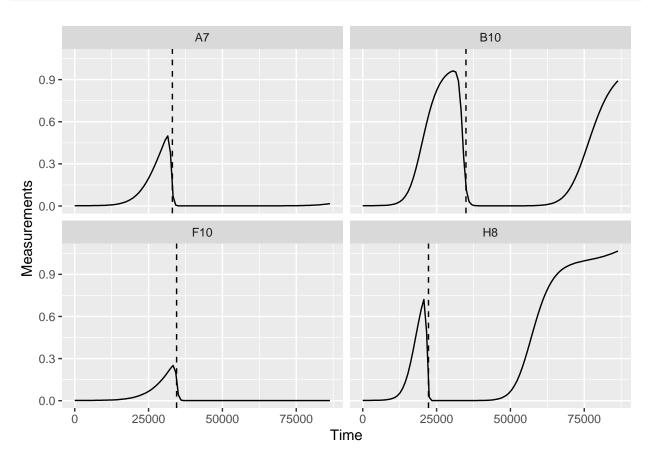
Let's take a look at the absorbance values in some example wells with both bacteria and phages:



Ok great. Now let's suppose that I think that an absorbance of 0.15 is a good threshold for extinction in my experiment. How could we use first_below to calculate the time when that first occurs across all our different wells? Well, primarily, first_below simply needs our x and y values, the threshold we want to use, as well as whether we want it to return the index of the first point below the threshold, or the x value of that point (since we care about the time it happened here, we'll do the latter). Additionally, we'll specify that we don't care if the startpoint is below the threshold: we only care when the data goes from above to below it.

```
ex_dat_mrg_sum <-
summarize(
```

```
ex_dat_mrg,
    extin_time = first_below(x = Time, y = Measurements, threshold = 0.15,
                             return = "x", return_endpoints = FALSE))
#> `summarise()` has grouped output by 'Bacteria_strain', 'Phage'. You can override using the
#> `.groups` argument.
head(ex_dat_mrg_sum)
#> # A tibble: 6 x 4
#> # Groups: Bacteria_strain, Phage [6]
     Bacteria_strain Phage
                                  Well
                                        extin\_time
     <chr>
                     <chr>
                                  <fct>
                                             <db1>
#> 1 Strain 1
                     No Phage
                                  A1
                                               NA
#> 2 Strain 1
                     Phage Added A7
                                            33063.
#> 3 Strain 10
                     No Phage
                                  B4
                                               NA
#> 4 Strain 10
                     Phage Added B10
                                            34946.
                     No Phage
#> 5 Strain 11
                                  B5
                                               NA
#> 6 Strain 11
                     Phage Added B11
                                            20319.
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = Measurements)) +
  geom_line() +
 facet_wrap(~Well) +
  geom_vline(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(xintercept = extin_time), lty = 2)
```



All the phage-added wells have a time when the bacteria drop below that threshold, and the plot clearly shows that it's right where we'd expect it.

Finding any kind of threshold-crossing: time to density

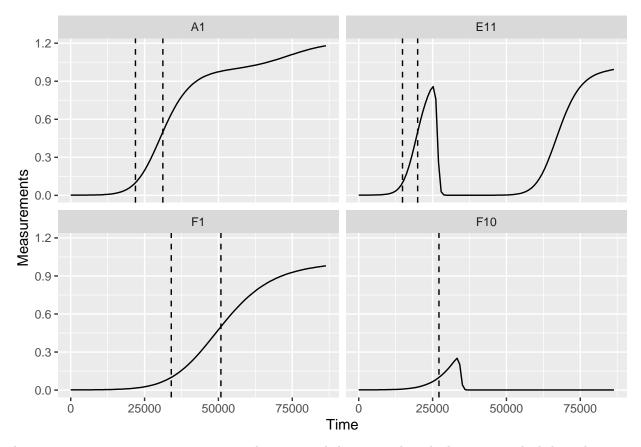
We've seen how first_below can be used to identify the first point some data crosses below a threshold. But what about other kinds of threshold-crossing events? The first point it passes above a threshold? The first point it's ever below a threshold, including at the start?

In order to identify these kinds of extrema, we can use the more-general function find_threshold_crosses. In fact, first_below is really just a special case of find_threshold_crosses. Just like first_below, find_threshold_crosses only requires a threshold and a vector of y data in which to find the threshold crosses, and can return the index or x value of the crossing events it finds.

However, unlike first_below, find_threshold_crosses returns a vector containing all of the threshold crossings found under the given settings. Users can alter which kinds of threshold crossings are reported using the arguments return_rising, return_falling, and return_endpoints. However, find_threshold_crosses will always return a vector of all the extrema found, so users must use brackets to select which one they want summarize to save.

Let's dig into an example: identifying the first time the bacteria reach some density, including if they start at that density

```
sample_wells <- c("A1", "F1", "F10", "E11")</pre>
ex_dat_mrg_sum <-
  summarize(
    ex_dat_mrg,
    time_to_01 = find_threshold_crosses(x = Time, y = Measurements,
                                         threshold = 0.1, return = "x",
                                         return_endpoints = TRUE,
                                         return_falling = FALSE)[1],
   time_to_05 = find_threshold_crosses(x = Time, y = Measurements,
                                         threshold = 0.5, return = "x",
                                         return endpoints = TRUE,
                                         return falling = FALSE)[1])
#> `summarise()` has grouped output by 'Bacteria_strain', 'Phage'. You can override using the
#> `.groups` argument.
head(ex_dat_mrg_sum)
#> # A tibble: 6 x 5
#> # Groups: Bacteria strain, Phage [6]
   Bacteria_strain Phage
#>
                                  Well time_to_01 time_to_05
#>
     \langle chr \rangle
                     <chr>
                                  <fct>
                                            <dbl>
                                                        <dbl>
#> 1 Strain 1
                     No Phage
                                 A1
                                            21913.
                                                       31194.
                     Phage Added A7
#> 2 Strain 1
                                            21913.
                                                          NA
#> 3 Strain 10
                     No Phage
                                            15300
                                                       20624.
                                 В4
#> 4 Strain 10
                     Phage Added B10
                                            15300
                                                       20624.
#> 5 Strain 11
                     No Phage
                                 B5
                                            14543.
                                                       19490
#> 6 Strain 11
                     Phage Added B11
                                            14543.
                                                       59955
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = Measurements)) +
  geom line() +
  facet_wrap(~Well) +
  geom_vline(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(xintercept = time_to_01), lty = 2) +
  geom vline(data = dplyr::filter(ex dat mrg sum, Well %in% sample wells),
             aes(xintercept = time_to_05), lty = 2)
#> Warning: Removed 1 rows containing missing values (geom_vline).
```



As we can see, find_threshold_crosses has returned the times when the bacteria reached those densities. We can see that some bacteria (e.g. those in Well F10) never reached 0.5, so they have an NA value for time_to_05. By comparing the times it took each strain to reach an absorbance of 0.1, we could learn something about how soon the bacteria started growing and how quickly they grew.

What's next?

Now that you've analyzed your data, you can read about approaches to deal with noise in your growth curve data, or you can read 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("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")
- 7. **Dealing with noise:** vignette("noise")
- 8. Statistics, merging other data, and other resources: vignette("conclusion")