Analyzing data

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So far, we've imported and transformed our measures, combined them with our design information, pre-processed, 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")
ex_dat_mrg <- merge_dfs(example_tidydata, example_design_tidy)
#> Joining with `by = join_by(Well)`
ex_dat_mrg$Well <-
```

```
  factor(ex_dat_mrg$Well,
         levels = paste(rep(LETTERS[1:8], each = 12), 1:12, sep = ""))
ex_dat_mrg$Time <- ex_dat_mrg$Time/3600 #Convert time to hours
ex_dat_mrg <-
  mutate(group_by(ex_dat_mrg, Well, Bacteria_strain, Phage),
         deriv = calc_deriv(x = Time, y = Measurements),
         deriv_percap5 = calc_deriv(x = Time, y = Measurements,
                                    percapita = TRUE, blank = 0,
                                    window_width_n = 5, trans_y = "log"),
         doub_time = doubling_time(y = deriv_percap5))
sample_wells <- c("A1", "F1", "F10", "E11")
# Drop unneeded columns (optional, but makes things cleaner)
ex_dat_mrg <- dplyr::select(ex_dat_mrg,
                            Time, Well, Measurements, Bacteria_strain, Phage,
                            deriv, deriv_percap5)
```

Analyzing data with summarize

Ultimately, analyzing growth curves requires summarizing the entire time series of data by some metric or metrics. gcplyr makes it easy to calculate a number of metrics of interest, which I've grouped into categories:

Most common metrics

- the lag time
- the maximum cellular growth rate (i.e. minimum doubling time)
- the maximum density (e.g. carrying capacity)
- the area under the curve

Growth

- the initial density
- the lag time
- the time to reach some density
- the time to reach some growth rate
- the maximum per-capita growth rate (i.e. minimum doubling time)

Saturation

- the mid-point time or inflection point
- the maximum density (e.g. carrying capacity)

Total growth

- the area under the curve
- the centroid of area under the curve

Diauxic growth

- the density and time when a diauxic shift occurs
- the maximum per-capita growth rate during diauxie

Growth with antagonists (e.g. phages)

- the peak bacterial density before a decline (e.g. from phage predation)
- the extinction time (e.g. from phage predation)
- the area under the curve
- the centroid of area under the curve

The following sections show how you can use `gcplyr` functions to calculate these metrics.

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 into one or several metrics

As before, to use `group_by` we simply pass the `data.frame` to be grouped, and the names of the columns we want to group by. 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, Measurements, or anything else that varies *within* a well, since if you do `dplyr` will group timepoints within a well separately.

Then, we run `summarize`. `summarize` works much like `mutate` did, where we specify:

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

Just like `mutate`, if we want additional summary metrics, we simply add them to the summarize. However, unlike `mutate`, `summarize` functions return just a single value for each group.

As you'll see throughout the rest of this article, we'll be using `group_by` and `summarize` to calculate our metrics of interest. If you want to learn more, `dplyr` has extensive documentation and examples of its own online, but this primer and the coming example should be sufficient to analyze data with `gcplyr`.

Plotting summarized metrics

Once you've calculated your summarized metrics, you should plot them on the original data to make sure everything matches what you expect. We can plot summarized values right on top of our original data:

- density or rate metrics can be plotted as a horizontal line with `geom_hline`
- time metrics can be plotted as a vertical line with `geom_vline`
- pairs of metrics that correspond to both density/rate and time can be plotted as a point with `geom_point`

You'll see examples of these plots throughout this article.

The most common metrics

Lag time

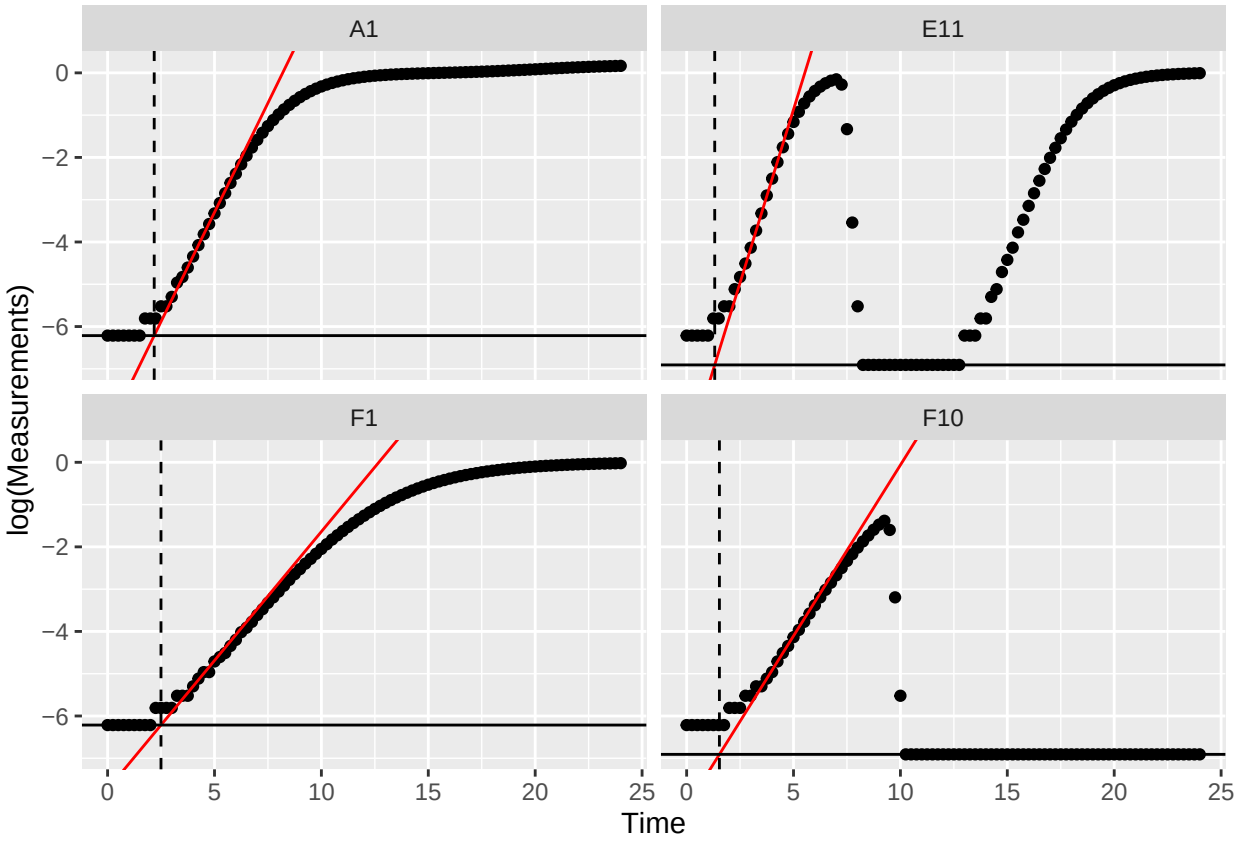
Bacteria often have a period of time before they reach their maximum growth rate. If you would like to quantify this lag time, you can use the `lag_time` function. `lag_time` needs the x and y values, as well as the (per-capita) derivative. It will find the maximum derivative, then project the tangent line with that slope back until it crosses the starting density.

Below, I calculate lag time. So that you can see a visualization of what this tangent-line calculation does, I also calculate the `max_percap`, `max_percap_time`, `max_percap_dens`, and `min_dens`, but you don't have to do that.

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            lag_time = lag_time(y = Measurements, x = Time,
                                deriv = deriv_percap5),
            max_percap = max_gc(deriv_percap5),
            max_percap_time = Time[which_max_gc(deriv_percap5)],
            max_percap_dens = Measurements[which_max_gc(deriv_percap5)],
            min_dens = min_gc(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 8
#> # Groups:   Bacteria_strain, Phage [6]
#>   Bacteria_strain Phage     Well  lag_time max_percap max_percap_time max_percap_dens
#>   <chr>           <chr>     <fct>    <dbl>      <dbl>           <dbl>           <dbl>
#> 1 Strain 1        No Phage  A1        2.18       1.03            4.25           0.017
#> 2 Strain 1        Phage Ad~ A7        1.51       1.03            4.25           0.017
#> 3 Strain 10       No Phage  B4        1.78       1.59            3.5            0.031
#> 4 Strain 10       Phage Ad~ B10       1.34       1.59            3.5            0.031
#> 5 Strain 11       No Phage  B5        1.67       1.65            3.5            0.041
#> 6 Strain 11       Phage Ad~ B11       1.24       1.65            3.5            0.041
#> # i 1 more variable: min_dens <dbl>

ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = log(Measurements))) +
  geom_point() +
  facet_wrap(~Well) +
```

```
  geom_abline(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
              color = "red",
              aes(slope = max_percap,
                  intercept = log(max_percap_dens) - max_percap*max_percap_time)) +
  geom_vline(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(xintercept = lag_time), lty = 2) +
  geom_hline(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(yintercept = log(min_dens)))
```

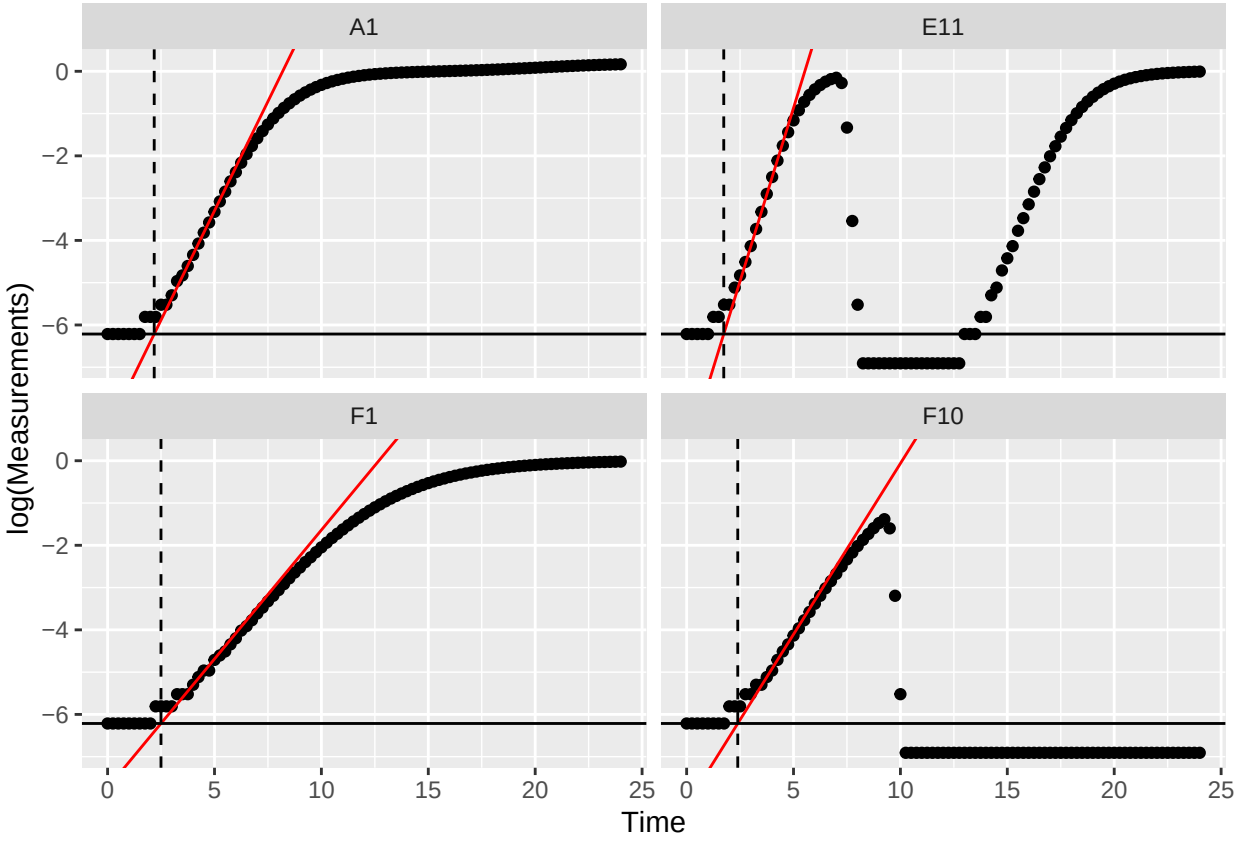


Notice how in some of the wells the minimum density value isn't the *initial* density? We can fix that by overriding the default minimum density calculation with `first_minima` via the `y0` argument of `lag_time`.

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            min_dens = first_minima(Measurements, return = "y"),
            lag_time = lag_time(y = Measurements, x = Time,
                                deriv = deriv_percap5, y0 = min_dens),
            max_percap = max_gc(deriv_percap5),
            max_percap_time = Time[which_max_gc(deriv_percap5)],
            max_percap_dens = Measurements[which_max_gc(deriv_percap5)])
#> `summarise()` has grouped output by 'Bacteria_strain', 'Phage'. You can override
#> using the `.groups` argument.
head(ex_dat_mrg_sum)
#> # A tibble: 6 x 8
#> # Groups:   Bacteria_strain, Phage [6]
#>   Bacteria_strain Phage       Well  min_dens lag_time max_percap max_percap_time
```

```
#>   <chr>           <chr>       <fct>    <dbl>    <dbl>      <dbl>           <dbl>
#> 1 Strain 1        No Phage    A1       0.002     2.18       1.03            4.25
#> 2 Strain 1        Phage Added A7       0.002     2.18       1.03            4.25
#> 3 Strain 10       No Phage    B4       0.002     1.78       1.59            3.5
#> 4 Strain 10       Phage Added B10      0.002     1.78       1.59            3.5
#> 5 Strain 11       No Phage    B5       0.002     1.67       1.65            3.5
#> 6 Strain 11       Phage Added B11      0.002     1.67       1.65            3.5
#> # i 1 more variable: max_percap_dens <dbl>
```

```
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = log(Measurements))) +
  geom_point() +
  facet_wrap(~Well) +
  geom_abline(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
              color = "red",
              aes(slope = max_percap,
                  intercept = log(max_percap_dens) - max_percap*max_percap_time)) +
  geom_vline(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(xintercept = lag_time), lty = 2) +
  geom_hline(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(yintercept = log(min_dens)))
```



Maximum growth rate and minimum doubling time

If you want to calculate the bacterial maximum growth rate (i.e. the minimum doubling time), it will often be sufficient to use `max_gc` on the per-capita derivatives we calculated in `vignette("gc05_process")`. (`max_gc` works just like `R`'s built-in `max`, but with better default settings for growth curve analyses with `summarize`).

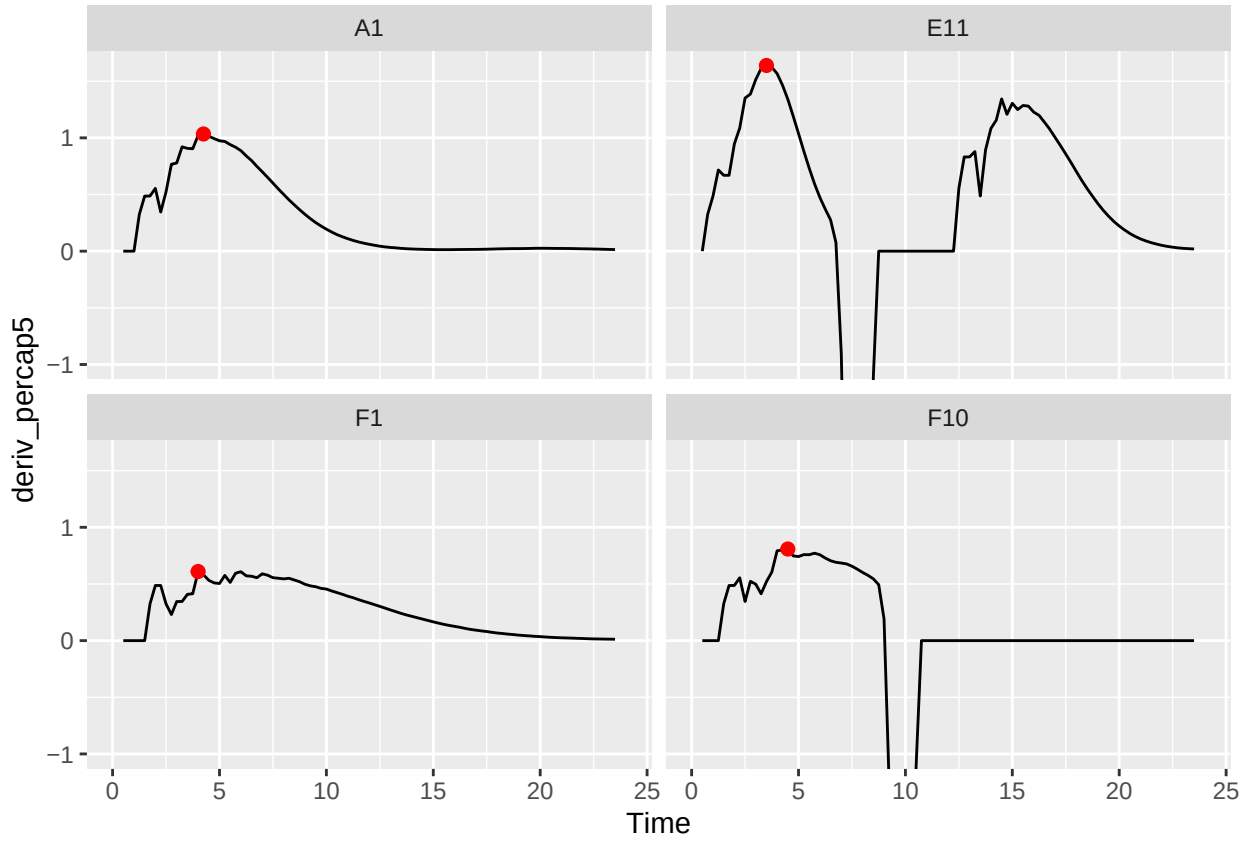
We can also save the time when this maximum occurs using the `which_max_gc` function. `which_max_gc` returns the *index* of the maximum value, so then we can get the `Time` value at that index and save it to a column titled `max_percap_time`. (`which_max_gc` and `extr_val` work just like `R`'s built-in `which.max` and `[`, but with better default settings for growth curve analyses with `summarize`)

If you would like the equivalent minimum doubling time, you can simply calculate the maximum growth rate as above and then convert that into the equivalent minimum doubling time using the `doubling_time` function.

It is important to note that `gcplyr` calculates the maximum *realized* growth rate during your growth curve. This is distinct from the intrinsic growth rate (the maximum *possible* growth rate), although the two will be quite similar if you started your growth curves at sufficiently low densities (see: Ghenu et al., 2024. Challenges and pitfalls of inferring microbial growth rates from lab cultures. Frontiers in Ecology and Evolution.)

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            max_percap = max_gc(deriv_percap5, na.rm = TRUE),
            max_percap_time = extr_val(Time, which_max_gc(deriv_percap5)),
            doub_time = doubling_time(y = max_percap))
#> `summarise()` has grouped output by 'Bacteria_strain', 'Phage'. You can override
#> using the `.groups` argument.
head(ex_dat_mrg_sum)
#> # A tibble: 6 x 6
#> # Groups:   Bacteria_strain, Phage [6]
#>   Bacteria_strain Phage       Well  max_percap max_percap_time doub_time
#>   <chr>           <chr>       <fct>      <dbl>           <dbl>     <dbl>
#> 1 Strain 1        No Phage    A1          1.03            4.25     0.670
#> 2 Strain 1        Phage Added A7          1.03            4.25     0.670
#> 3 Strain 10       No Phage    B4          1.59            3.5      0.436
#> 4 Strain 10       Phage Added B10         1.59            3.5      0.436
#> 5 Strain 11       No Phage    B5          1.65            3.5      0.421
#> 6 Strain 11       Phage Added B11         1.65            3.5      0.421

ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = deriv_percap5)) +
  geom_line() +
  facet_wrap(~Well) +
  geom_point(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(x = max_percap_time, y = max_percap),
             size = 2, color = "red") +
  coord_cartesian(ylim = c(-1, NA))
#> Warning: Removed 4 rows containing missing values (`geom_line()`).
```



Maximum density

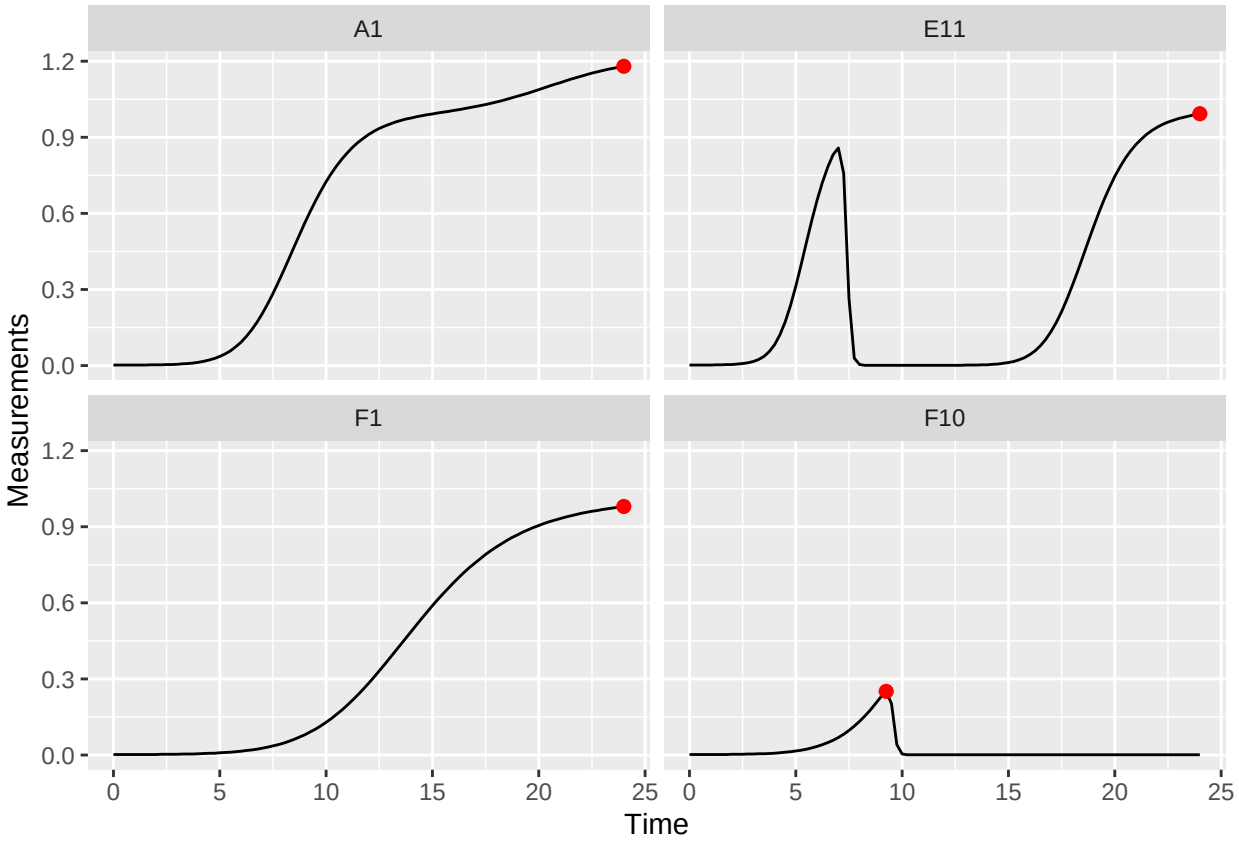
The maximum bacterial density can be a measure of bacterial growth yield/efficiency. If your bacteria plateau in density, the maximum density can also be a measure of bacterial carrying capacity. If you want to quantify the maximum bacterial density, we can use `max_gc` to get the global maxima of `Measurements` (`max_gc`, `which_max_gc`, and `extr_val` work just like `R`'s built-in `max`, `which.max`, and `[`, but with better default settings for growth curve analyses with `summarize`).

See Peak bacterial density for identifying *local* maxima of `Measurements` (e.g. if you wanted the first peak in Well E11 shown below).

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            max_dens = max_gc(Measurements, na.rm = TRUE),
            max_time = extr_val(Time, which_max_gc(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>    <dbl>
#> 1 Strain 1        No Phage    A1       1.18     24
#> 2 Strain 1        Phage Added A7       0.499     8.75
#> 3 Strain 10       No Phage    B4       1.21     23.8
```

```
#> 4 Strain 10       Phage Added B10      0.962     8.5
#> 5 Strain 11       No Phage    B5       1.21     19.5
#> 6 Strain 11       Phage Added B11      1.03     24
```

```
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = Measurements)) +
  geom_line() +
  facet_wrap(~Well) +
  geom_point(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(x = max_time, y = max_dens),
             size = 2, color = "red")
```



Area under the curve

The area under the curve is a common metric of total bacterial growth, for instance in the presence of antagonists like antibiotics or phages. If you want to calculate the area under the curve, you can use the `gcplyr` function `auc`. Simply specify Time as the x and Measurements as the y data whose area-under-the-curve you want to calculate.

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            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     15.9
#> 2 Strain 1        Phage Added A7      1.07
#> 3 Strain 10       No Phage    B4     20.4
#> 4 Strain 10       Phage Added B10     6.15
#> 5 Strain 11       No Phage    B5     20.9
#> 6 Strain 11       Phage Added B11     7.77
```

Growth metrics

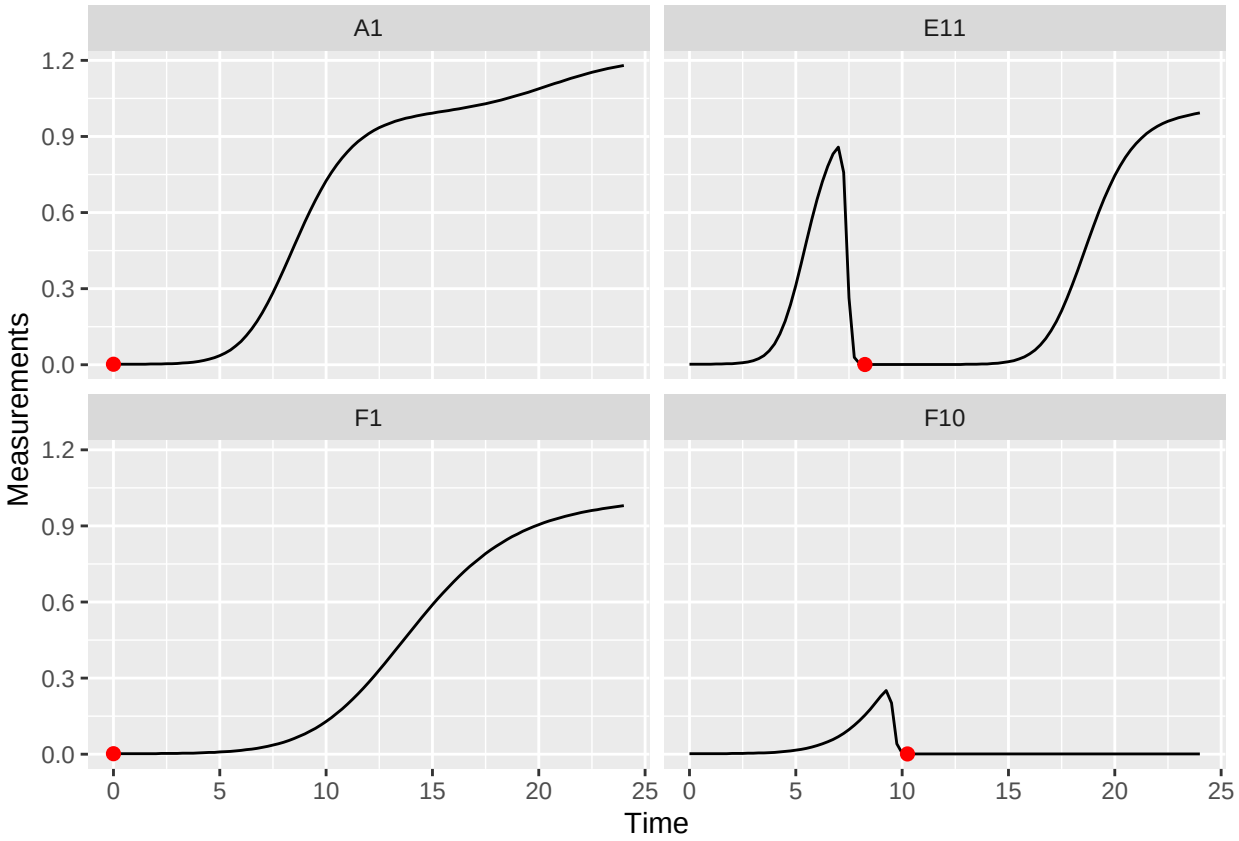
Initial density

If you want to identify the initial density of your bacteria, it will often be sufficient to use `min_gc` (this works just like R's built-in `min`, but with better default settings for growth curve analyses with `summarize`).

We can also save the time when this minimum occurs using the `which_min_gc` function. `which_min_gc` returns the *index* of the minimum value, so then we can get the `Time` value at that index and save it to a column titled `min_time`. (`which_min_gc` and `extr_val` work just like R's built-in `which.min` and `[`, but with better default settings for growth curve analyses with `summarize`)

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            min_dens = min_gc(Measurements, na.rm = TRUE),
            min_time = extr_val(Time, which_min_gc(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  min_dens min_time
#>   <chr>           <chr>       <fct>    <dbl>    <dbl>
#> 1 Strain 1        No Phage    A1       0.002     0
#> 2 Strain 1        Phage Added A7       0.001     9.75
#> 3 Strain 10       No Phage    B4       0.002     0
#> 4 Strain 10       Phage Added B10      0.001    11
#> 5 Strain 11       No Phage    B5       0.002     0
#> 6 Strain 11       Phage Added B11      0.001     6

ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = Measurements)) +
  geom_line() +
  facet_wrap(~Well) +
  geom_point(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(x = min_time, y = min_dens),
             size = 2, color = "red")
```



In some cases (e.g. growing with phages), bacteria may later drop to a lower density than they started in the growth curve. In this case, we want the first *local* minima of the Measurements data, rather than the global minima:

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            min_dens = first_minima(y = Measurements, x = Time, return = "y"),
            min_time = first_minima(y = Measurements, x = Time, return = "x"))
#> `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  min_dens min_time
#>   <chr>           <chr>       <fct>    <dbl>    <dbl>
#> 1 Strain 1        No Phage    A1       0.002        0
#> 2 Strain 1        Phage Added A7       0.002        0
#> 3 Strain 10       No Phage    B4       0.002        0
#> 4 Strain 10       Phage Added B10      0.002        0
#> 5 Strain 11       No Phage    B5       0.002        0
#> 6 Strain 11       Phage Added B11      0.002        0
```

Note that you can tune the sensitivity of `first_minima` to different heights and widths of peaks and valleys using the `window_width`, `window_width_n`, and `window_height` arguments. You should check that `first_minima` is working with your data by plotting it, although the default sensitivity works much of the time.

Lag time

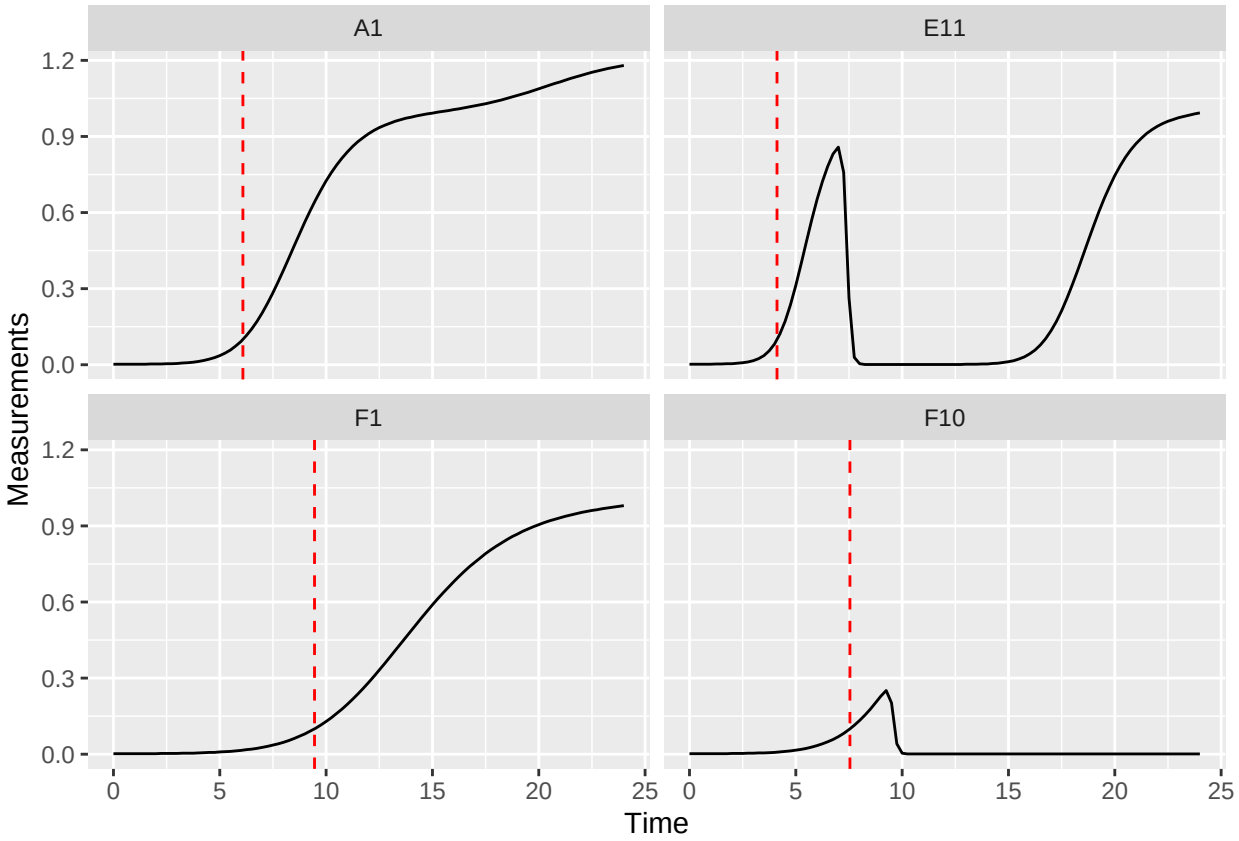
See the lag time section in the Most Common Metrics section

Time to reach threshold density

If you want to quantify how long it takes bacteria to reach some threshold density, you can use the `first_above` function. In this example, we'll use a Measurements value of 0.1 as our threshold.

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            above_01 = first_above(y = Measurements, x = Time,
                                   threshold = 0.1, return = "x"))
#> `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  above_01
#>   <chr>           <chr>       <fct>    <dbl>
#> 1 Strain 1        No Phage    A1        6.09
#> 2 Strain 1        Phage Added A7        6.09
#> 3 Strain 10       No Phage    B4        4.25
#> 4 Strain 10       Phage Added B10       4.25
#> 5 Strain 11       No Phage    B5        4.04
#> 6 Strain 11       Phage Added B11       4.04

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 = above_01), lty = 2, color = "red")
```



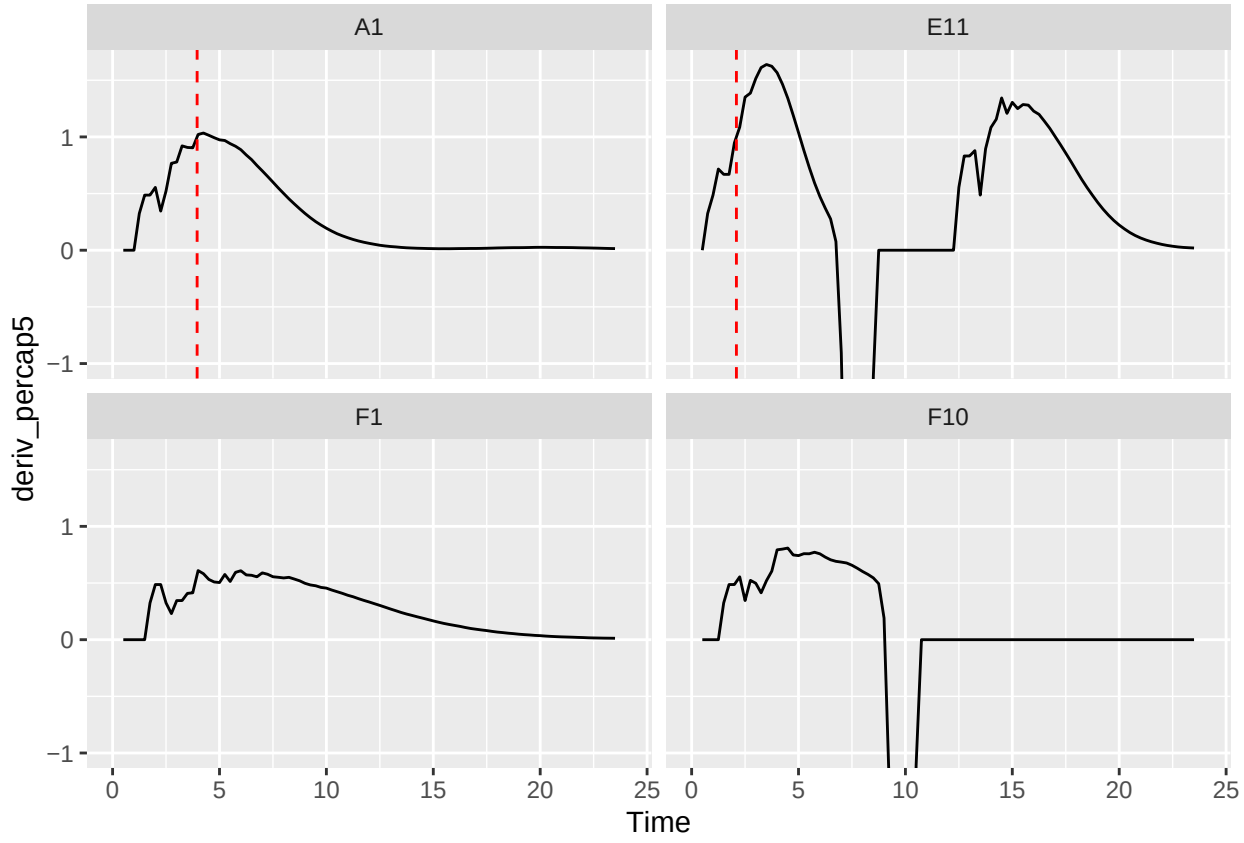
Time to reach threshold growth rate

If you want to quantify how long it takes bacteria to reach some threshold per-capita growth rate, you can use the `first_above` function. In this example, we'll use a per-capita derivative of 1 as our threshold.

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            percap_above_1 = first_above(y = deriv_percap5, x = Time,
                                         threshold = 1, return = "x"))
#> `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  percap_above_1
#>   <chr>           <chr>       <fct>          <dbl>
#> 1 Strain 1        No Phage    A1              3.95
#> 2 Strain 1        Phage Added A7              3.95
#> 3 Strain 10       No Phage    B4              2.28
#> 4 Strain 10       Phage Added B10             2.28
#> 5 Strain 11       No Phage    B5              2.11
#> 6 Strain 11       Phage Added B11             2.11

ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = deriv_percap5)) +
```

```
  geom_line() +
  facet_wrap(~Well) +
  geom_vline(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(xintercept = percap_above_1), lty = 2, color = "red") +
  coord_cartesian(ylim = c(-1, NA))
#> Warning: Removed 4 rows containing missing values (`geom_line()`).
#> Warning: Removed 2 rows containing missing values (`geom_vline()`).
```



Maximum growth rate and minimum doubling time

See the maximum growth rate section in the Most Common Metrics section

Saturation metrics

Mid-point time or inflection point

If you want to find the mid-point or inflection point of bacterial growth, there are two different approaches:

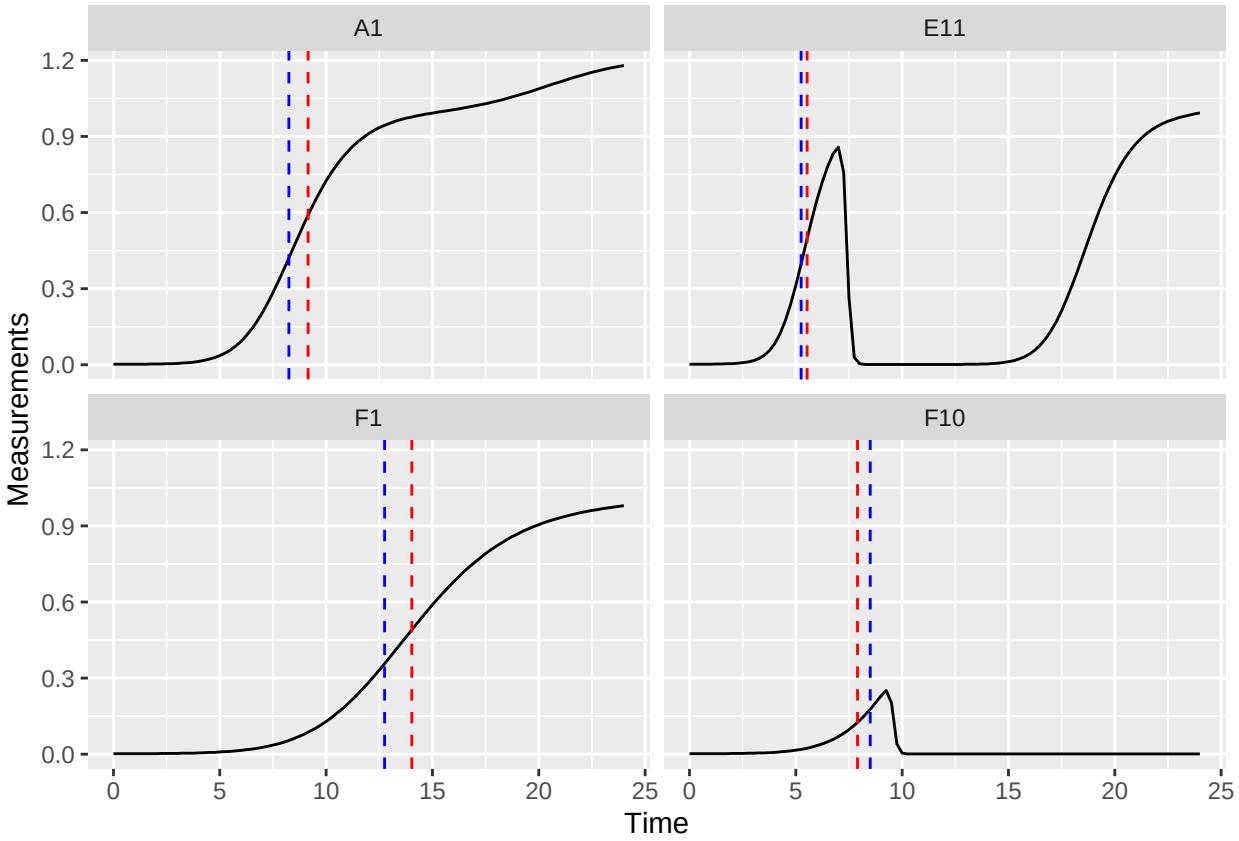
1. Mid-point: find the point when the density first reaches half the maximum density.
2. Inflection point: find the point when the derivative is at a maximum.

In growth curve analysis approaches using fitting of a symmetric function (e.g. when other R packages fit a logistic function to data), these two points will be equivalent. However, since gcplyr does model-free analyses, we do not assume symmetry, and so the points may be very similar or very different.

For the mid-point, we use the first_above function, with the threshold equal to the maximum bacterial density divided by 2. For the inflection point, we find the time when the deriv was at a maximum using which_max_gc (which_max_gc and extr_val work just like R's built-in which.max and [, but with better default settings for growth curve analyses with summarize).

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            mid_point = first_above(y = Measurements, x = Time, return = "x",
                                    threshold = max_gc(Measurements)/2),
            infl_point = extr_val(Time, which_max_gc(deriv)))
#> `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  mid_point infl_point
#>   <chr>           <chr>       <fct>     <dbl>      <dbl>
#> 1 Strain 1        No Phage    A1         9.15       8.25
#> 2 Strain 1        Phage Added A7         7.29       8.25
#> 3 Strain 10       No Phage    B4         6.06       5.5
#> 4 Strain 10       Phage Added B10        5.67       5.5
#> 5 Strain 11       No Phage    B5         5.71       5
#> 6 Strain 11       Phage Added B11       16.7        5

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 = mid_point), lty = 2, color = "red") +
  geom_vline(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(xintercept = infl_point), lty = 2, color = "blue")
```



Maximum density

See the maximum density section in the Most Common Metrics section

Total growth metrics

Area under the curve

See the area under the curve section in the Most Common Metrics section

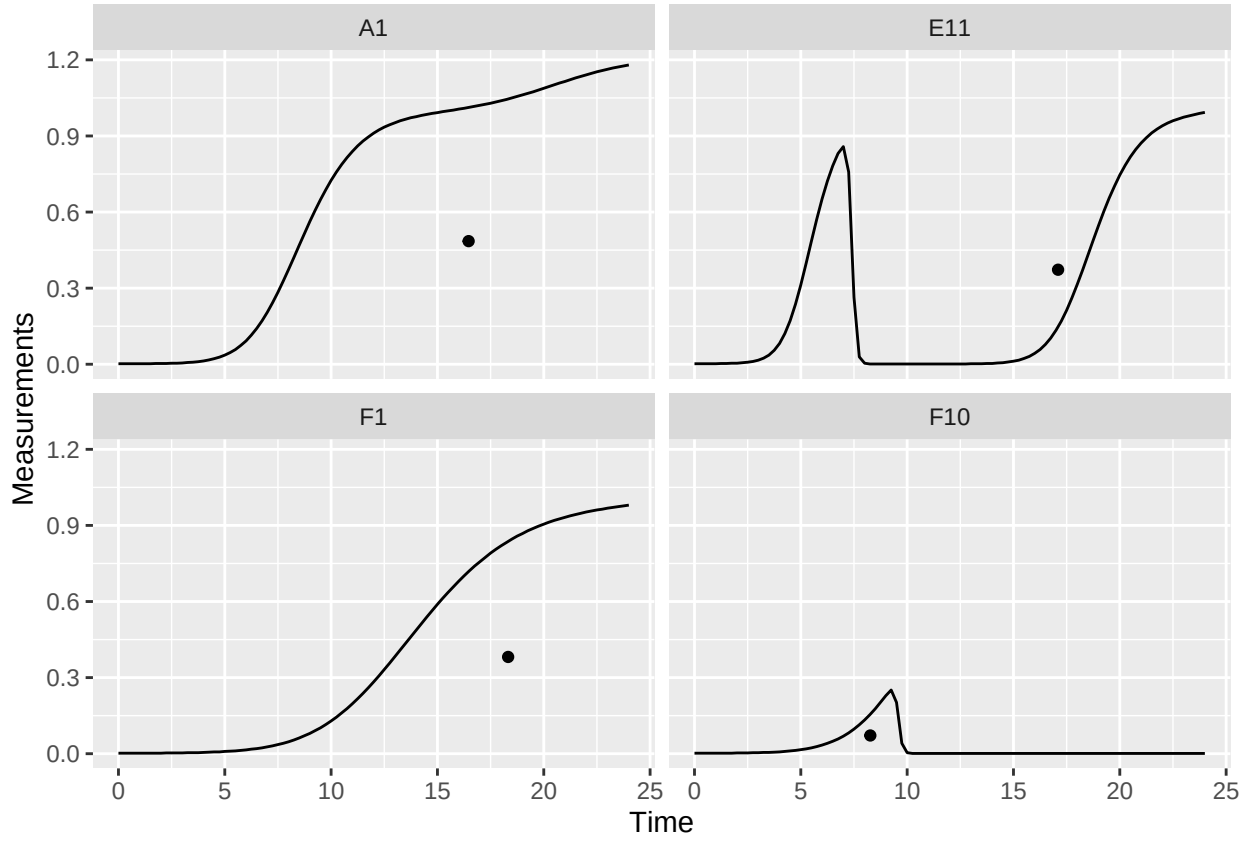
Centroid of area under the curve

The centroid, or center of mass, of the area under the curve can function as a metric of total microbial growth. If the area under the curve were a solid object, the centroid is the point where that object would balance perfectly. The centroid has both an x coordinate and a y coordinate. To calculate the centroid coordinates, you can use the `gcplyr` function `centroid_x` and `centroid_y`. Simply specify Time as the x and Measurements as the y data whose centroid you want to calculate.

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            centr_x = centroid_x(x = Time, y = Measurements),
            centr_y = centroid_y(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 5
#> # Groups:   Bacteria_strain, Phage [6]
#>   Bacteria_strain Phage       Well  centr_x centr_y
#>   <chr>           <chr>       <fct>   <dbl>   <dbl>
#> 1 Strain 1        No Phage    A1       16.5   0.485
#> 2 Strain 1        Phage Added A7        8.07  0.150
#> 3 Strain 10       No Phage    B4       15.2   0.546
#> 4 Strain 10       Phage Added B10      13.5   0.347
#> 5 Strain 11       No Phage    B5       15.1   0.552
#> 6 Strain 11       Phage Added B11      19.2   0.414

ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = Measurements)) +
  geom_line() +
  facet_wrap(~Well) +
  geom_point(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(x = centr_x, y = centr_y))
```



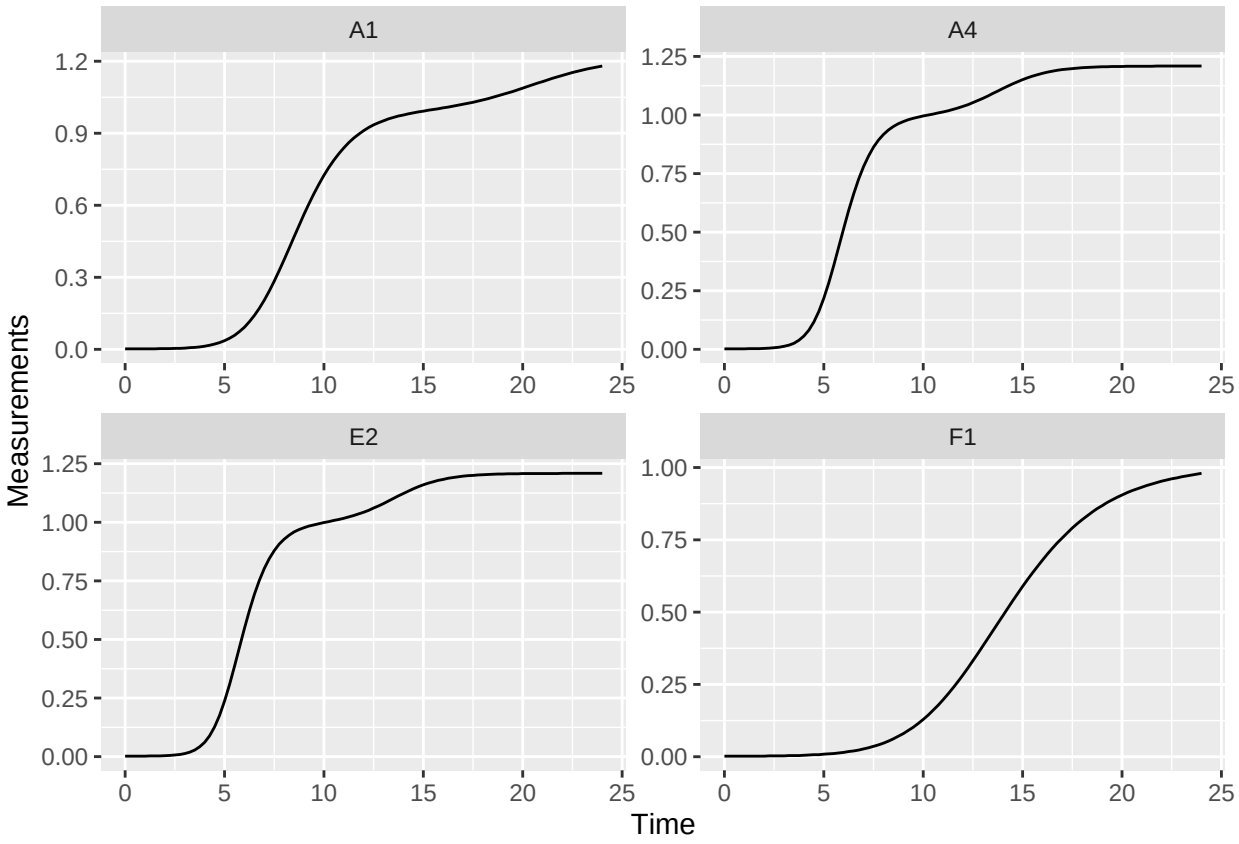
Diauxic growth metrics

Diauxic shifts

Bacteria frequently exhibit a second, slower, burst of growth after their first period of rapid growth. This is common in growth curves and is called *diauxic growth*.

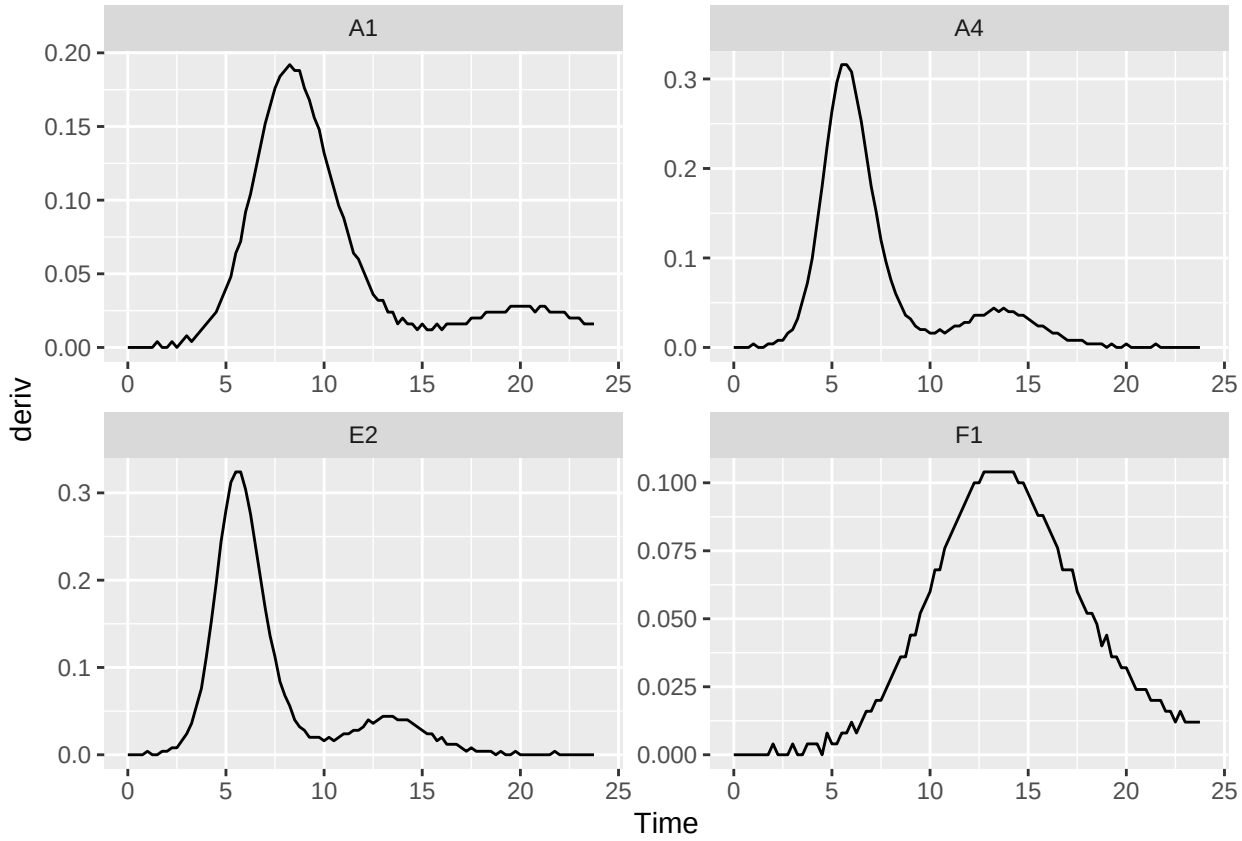
If we plot the data from some of our example data wells with no phage added, we'll see this pattern repeatedly:

```
nophage_wells <- c("A1", "A4", "E2", "F1")
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% nophage_wells),
       aes(x = Time, y = Measurements)) +
  geom_line() +
  facet_wrap(~Well, scales = "free")
```



We can identify the time when bacteria switch from their first period of rapid growth to their second period by finding a minima in the derivative values. Specifically, we want to identify the second minima (the first minima will occur at the beginning of the growth curve, when bacteria are just starting to grow). Let's look at some of the derivative values to see this.

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



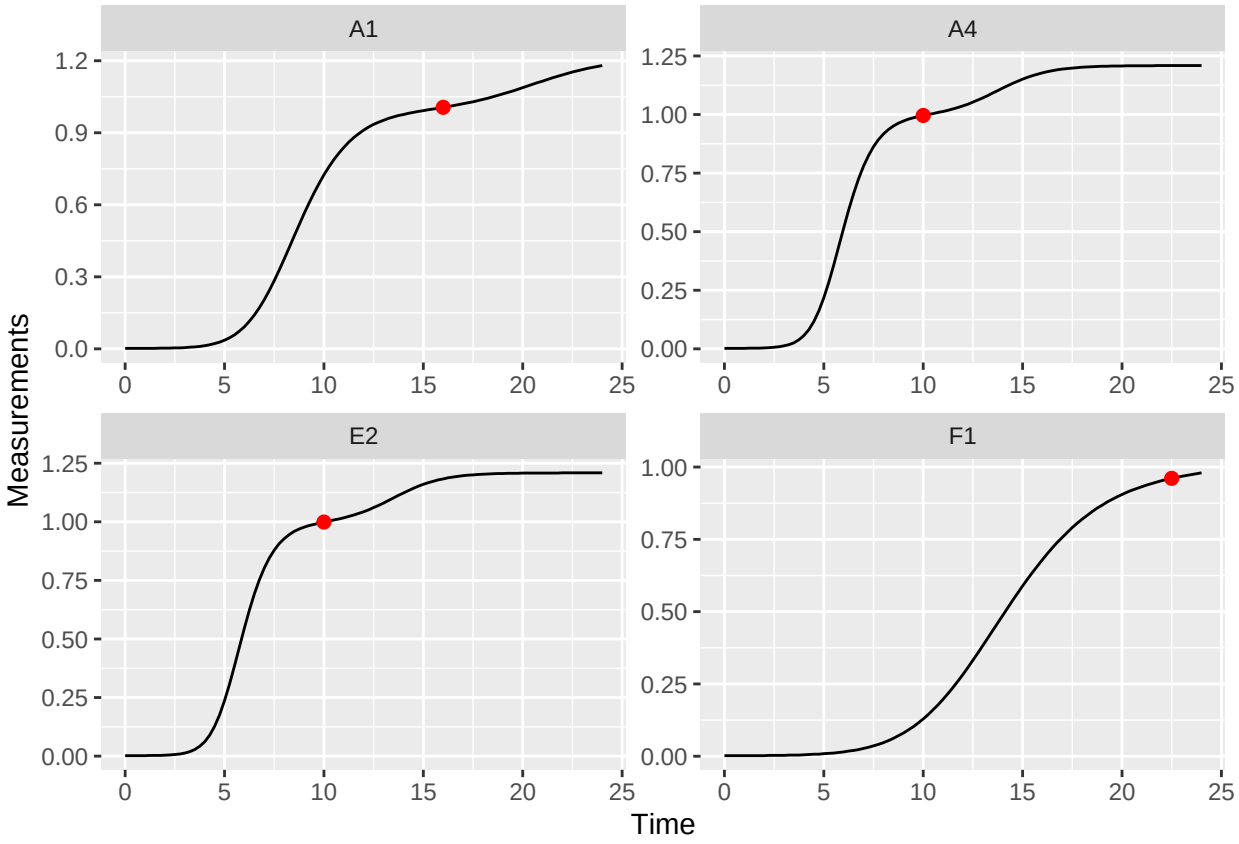
We can use the `gcplyr` function `find_local_extrema` to find that minima. Specify `deriv` as the y data and `Time` as the x data, and that we want `find_local_extrema` to `return` the `x` values associated with local minima. It will return a vector of those `x` values, and we're going to save just the second one.

At the same time, we're also going to save the density where the diauxic shift occurs. First, we'll use `find_local_extrema` again, but this time to save the *index* where the diauxic shift occurs to a column titled `diauxie_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). (`extr_val` works just like `R`'s built-in `[`, but with better default settings for growth curve analyses with `summarize`).

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
    diauxie_time = find_local_extrema(x = Time, y = deriv, return = "x",
                                      return_maxima = FALSE, return_minima = TRUE,
                                      window_width_n = 39)[2],
    diauxie_idx = find_local_extrema(x = Time, y = deriv, return = "index",
                                      return_maxima = FALSE, return_minima = TRUE,
                                      window_width_n = 39)[2],
    diauxie_dens = extr_val(Measurements, diauxie_idx))
#> `summarise()` has grouped output by 'Bacteria_strain', 'Phage'. You can override
#> using the `.groups` argument.
head(ex_dat_mrg_sum)
#> # A tibble: 6 x 6
#> # Groups:   Bacteria_strain, Phage [6]
#>   Bacteria_strain Phage       Well  diauxie_time diauxie_idx diauxie_dens
#>   <chr>           <chr>       <fct>        <dbl>       <int>        <dbl>
#> 1 Strain 1        No Phage    A1              16          65         1.01
```

```
#> 2 Strain 1        Phage Added A7          9            37        0.379
#> 3 Strain 10       No Phage    B4          9.75         40        0.999
#> 4 Strain 10       Phage Added B10         9.25         38        0.682
#> 5 Strain 11       No Phage    B5          9.5          39        1.01
#> 6 Strain 11       Phage Added B11         5.5          23        0.346

# Plot data with a point at the moment of diauxic shift
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% nophage_wells),
       aes(x = Time, y = Measurements)) +
  geom_line() +
  facet_wrap(~Well, scales = "free") +
  geom_point(data = dplyr::filter(ex_dat_mrg_sum, Well %in% nophage_wells),
             aes(x = diauxie_time, y = diauxie_dens),
             size = 2, color = "red")
```



If needed, you can tune the sensitivity of `find_local_extrema` to different heights and widths of peaks and valleys using the `window_width`, `window_width_n`, and `window_height` arguments.

Growth rate during diauxie

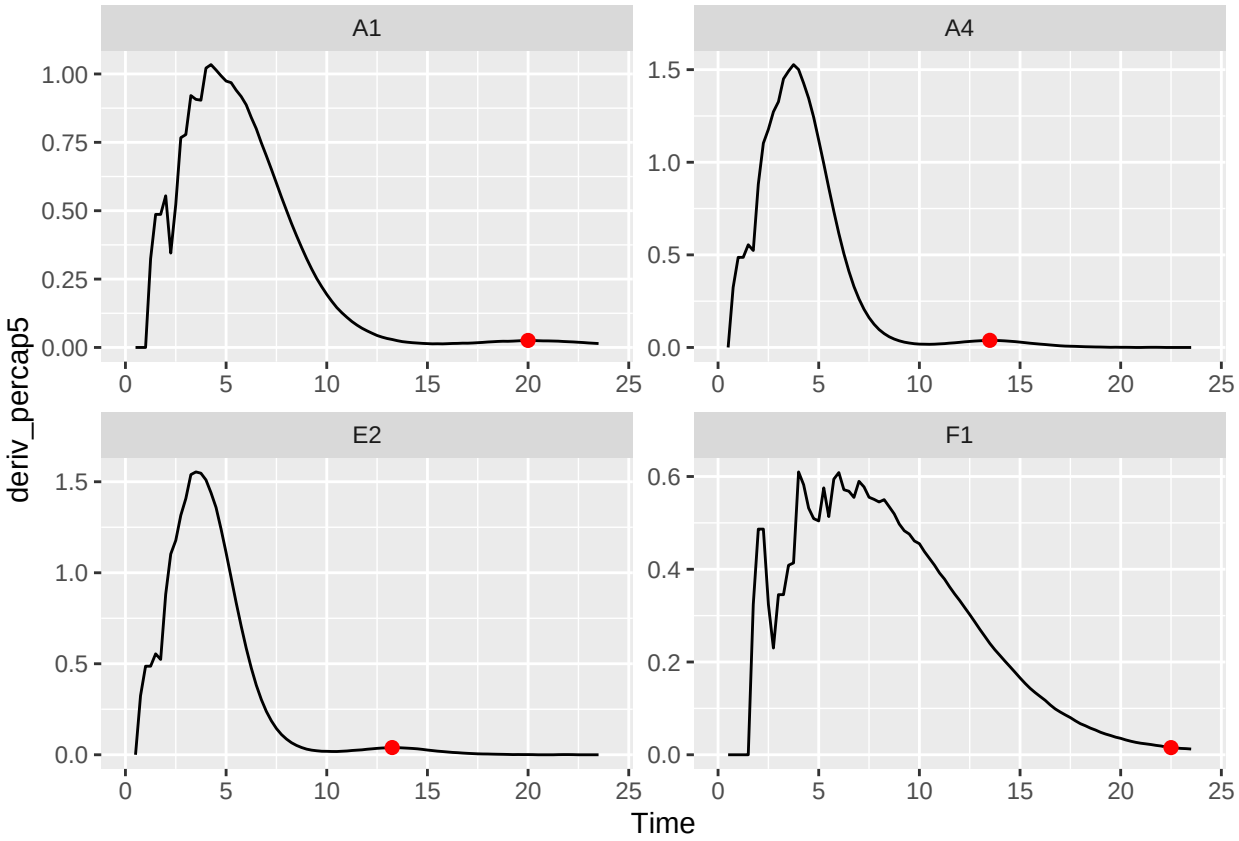
In the previous section we identified when bacteria shifted into their second period of rapid growth ('diauxic growth'). If you want to find out what the peak per-capita growth rate was during that second burst, we'll have to use `max` on the subset of data after the diauxic shift identified by `find_local_extrema`

Just as we did in the previous section, we'll use `find_local_extrema` to save the time when the diauxic shift occurs. Then, we'll find the maximum of the per-capita derivative after that shift occurs. Finally,

we'll find the time when that post-diauxie maximum growth rate occurs. Note that we're using `max_gc` and `which_max_gc`, which work just like R's built-in `max` and `which.max`, but with better default settings for growth curve analyses with `summarize`.

```
ex_dat_mrg_sum <-
  summarize(
    group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
    diauxie_time =
      find_local_extrema(x = Time, y = deriv, return = "x",
                         return_maxima = FALSE, return_minima = TRUE,
                         window_width_n = 39)[2],
    diauxie_percap = max_gc(deriv_percap5[Time >= diauxie_time]),
    diauxie_percap_time =
      extr_val(Time[Time >= diauxie_time],
               which_max_gc(deriv_percap5[Time >= diauxie_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 6
#> # Groups:   Bacteria_strain, Phage [6]
#>   Bacteria_strain Phage       Well  diauxie_time diauxie_percap diauxie_percap_time
#>   <chr>           <chr>       <fct>        <dbl>          <dbl>                <dbl>
#> 1 Strain 1        No Phage    A1              16         0.0257                 20
#> 2 Strain 1        Phage Added A7               9         0.832                  19.8
#> 3 Strain 10       No Phage    B4              9.75       0.0398                 13.2
#> 4 Strain 10       Phage Added B10             9.25       1.24                   17.8
#> 5 Strain 11       No Phage    B5              9.5        0.0438                 12.2
#> 6 Strain 11       Phage Added B11             5.5        1.43                   12.8

# Plot data with a point at the moment of peak diauxic growth rate
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% nophage_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% nophage_wells),
             aes(x = diauxie_percap_time, y = diauxie_percap),
             size = 2, color = "red")
#> Warning: Removed 4 rows containing missing values (`geom_line()`).
```



Metrics of growth with antagonists

Peak bacterial density

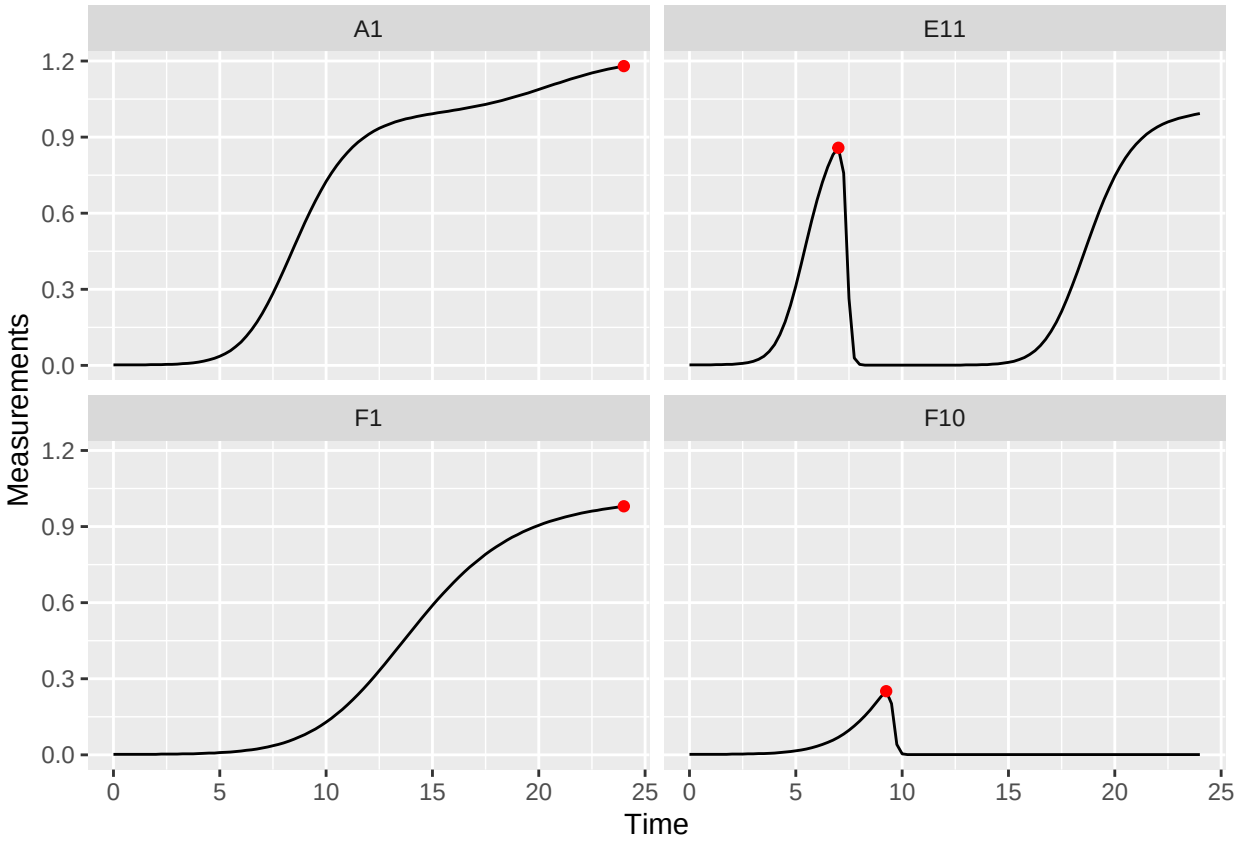
We previously found the global maximum in bacterial density using the simple `max_gc` and `which_max_gc` functions. The first *local* maxima can also be of interest. This is especially true when bacteria are grown with phages, where their first peak density can act as a proxy measure for their susceptibility to the phage. If you're interested in finding the first local maxima in bacterial density, you can use the `gcplyr` function `first_maxima`.

`first_maxima` simply requires the `y` data you want to identify the peak in. Specify `Measurements` as the y data and `Time` as the x data, and that we want `first_peak` to `return` the `x` and `y` values associated with the peak. We'll save those in columns `first_maxima_x` and `first_maxima_y`, respectively.

```
ex_dat_mrg_sum <-
  summarize(group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
            first_maxima_x = first_maxima(x = Time, y = Measurements,
                                          return = "x"),
            first_maxima_y = first_maxima(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_maxima_x first_maxima_y
#>   <chr>           <chr>       <fct>          <dbl>          <dbl>
#> 1 Strain 1        No Phage    A1             24             1.18
#> 2 Strain 1        Phage Added A7              8.75          0.499
#> 3 Strain 10       No Phage    B4             19.8           1.21
#> 4 Strain 10       Phage Added B10             8.5           0.962
#> 5 Strain 11       No Phage    B5             19.5           1.21
#> 6 Strain 11       Phage Added B11             5.25          0.439
```

```
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% sample_wells),
       aes(x = Time, y = Measurements)) +
  geom_line() +
  facet_wrap(~Well) +
  geom_point(data = dplyr::filter(ex_dat_mrg_sum, Well %in% sample_wells),
             aes(x = first_maxima_x, y = first_maxima_y),
             color = "red", size = 1.5)
```



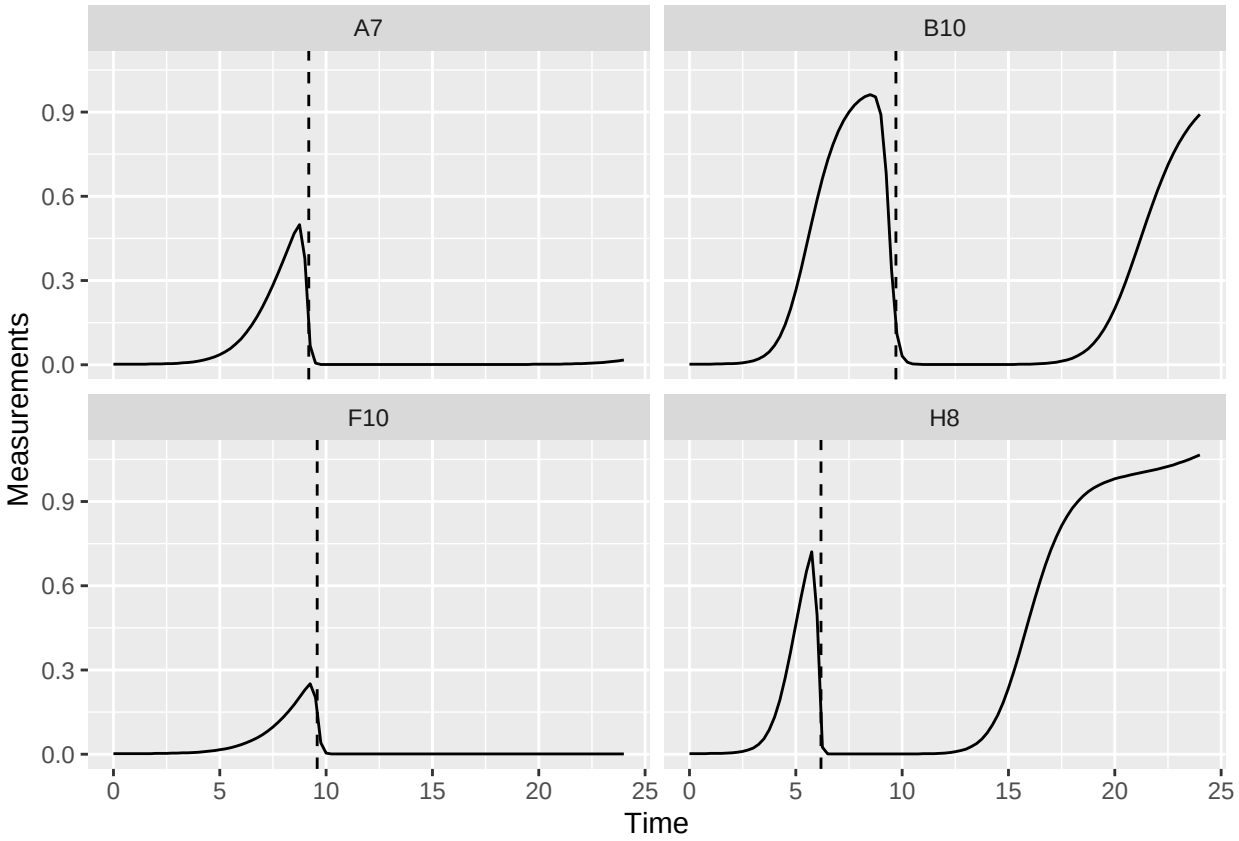
Note that you can tune the sensitivity of `first_maxima` to different heights and widths of peaks and valleys using the `window_width`, `window_width_n`, and `window_height` arguments, although the defaults work much of the time.

Extinction time

The time when bacterial density falls below some threshold can also be of interest. This is especially true when bacteria are grown with phages, where this 'extinction time' can act as a proxy measure for their susceptibility to the phage. If you're interested in finding the extinction time, you can use the `gcplyr` function `first_below`. In this example, we'll use a Measurements value of 0.15 as our threshold.

```
ex_dat_mrg_sum <-
  summarize(
    group_by(ex_dat_mrg, Bacteria_strain, Phage, Well),
    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>      <dbl>
#> 1 Strain 1        No Phage    A1         NA
#> 2 Strain 1        Phage Added A7          9.18
#> 3 Strain 10       No Phage    B4         NA
#> 4 Strain 10       Phage Added B10         9.71
#> 5 Strain 11       No Phage    B5         NA
#> 6 Strain 11       Phage Added B11         5.64

phage_wells <- c("A7", "B10", "F10", "H8")
ggplot(data = dplyr::filter(ex_dat_mrg, Well %in% phage_wells),
       aes(x = Time, y = Measurements)) +
  geom_line() +
  facet_wrap(~Well) +
  geom_vline(data = dplyr::filter(ex_dat_mrg_sum, Well %in% phage_wells),
             aes(xintercept = extin_time), lty = 2)
```



Area under the curve

See the area under the curve section in the Most Common Metrics section

Centroid of area under the curve

See the centroid section in the Total Growth Metrics section

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("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")`