Pre-processing and plotting data

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Contents

Where are we so far?

1. Introduction: `vignette("gc01_gcplyr")`
2. Importing and reshaping data: `vignette("gc02_import_reshape")`
3. Incorporating experimental designs: `vignette("gc03_incorporate_designs")`
4. **Pre-processing and plotting your data:** `vignette("gc04_preprocess_plot")`
5. Processing your data: `vignette("gc05_process")`
6. Analyzing your data: `vignette("gc06_analyze")`
7. Dealing with noise: `vignette("gc07_noise")`
8. Best practices and other tips: `vignette("gc08_conclusion")`
9. Working with multiple plates: `vignette("gc09_multiple_plates")`
10. Using make_design to generate experimental designs: `vignette("gc10_using_make_design")`

So far, we've imported and transformed our measures, then combined them with our design information. Now we're going to do some final pre-processing steps and show how to easily plot our data with `ggplot`.

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

```
library(gcplyr)

library(dplyr)
library(ggplot2)
library(lubridate)
#>
#> Attaching package: 'lubridate'
#> The following objects are masked from 'package:base':
```

```r
#>
#>     date, intersect, setdiff, union
```

```r
# 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)`
```

Pre-processing

Now that we have our data and designs merged, we're almost ready to start processing and analyzing them. However, first we need to carry out any necessary pre-processing steps, like excluding wells that were contaminated or empty, converting time formats to numeric, and subtracting blanks.

Pre-processing: excluding data

In some cases, we want to remove some of the wells from our growth curves data before we carry on with downstream analyses. For instance, they may have been left empty, contained negative controls, or were contaminated. We can use `dplyr`'s `filter` function to remove those wells that meet criteria we want to exclude.

For instance, let's imagine that we realized that we put the wrong media into Well B1, and that strain 13 was contaminated. To exclude them from our analyses, we can simply:

```r
example_data_and_designs_filtered <-
  dplyr::filter(ex_dat_mrg,
         Well != "B1", Bacteria_strain != "Strain 13")
head(example_data_and_designs_filtered)
#>   Time Well Measurements Bacteria_strain    Phage
#> 1    0   A1        0.002        Strain 1 No Phage
#> 2    0   D1        0.002       Strain 19 No Phage
#> 3    0   E1        0.002       Strain 25 No Phage
#> 4    0   F1        0.002       Strain 31 No Phage
#> 5    0   G1        0.002       Strain 37 No Phage
#> 6    0   H1        0.002       Strain 43 No Phage
```

Pre-processing: converting dates & times into numeric

Growth curve data produced by a plate reader often encodes the timestamp information as a string (e.g. "2:45:11" for 2 hours, 45 minutes, and 11 seconds), while downstream analyses need timestamp information as a numeric (e.g. number of seconds elapsed). Luckily, others have written great packages that make it easy to convert from common date-time text formats into plain numeric formats. Here, we'll see how to use `lubridate` to do so:

First we have to create a data frame with time saved as it might be by a plate reader.

```r
ex_dat_mrg <- make_example(vignette = 4, example = 1)

head(ex_dat_mrg)
#>      Time Well Measurements Bacteria_strain    Phage
#> 1 0:00:00   A1        0.002        Strain 1 No Phage
#> 2 0:00:00   B1        0.002        Strain 7 No Phage
#> 3 0:00:00   C1        0.002       Strain 13 No Phage
#> 4 0:00:00   D1        0.002       Strain 19 No Phage
#> 5 0:00:00   E1        0.002       Strain 25 No Phage
#> 6 0:00:00   F1        0.002       Strain 31 No Phage
```

We can see that our `Time` aren't written in an easy numeric. Instead, they're in a format that's easy for a human to understand (but unfortunately not very usable for analysis).

Let's use `lubridate` to convert this text into a usable format. `lubridate` has a whole family of functions that can parse text with hour, minute, and/or second components. You can use `hms` if your text contains hour, minute, and second information, `hm` if it only contains hour and minute information, and `ms` if it only contains minute and second information.

Once `hms` has parsed the text, we'll use `time_length` to convert the output of `hms` into a pure numeric value. By default, `time_length` returns in units of seconds, but you can change that by changing the `unit` argument to `time_length`.

```r
# We have previously loaded lubridate, but if you haven't already then
# make sure to add the line:
#    library(lubridate)

ex_dat_mrg$Time <- time_length(hms(ex_dat_mrg$Time), unit = "hour")

head(ex_dat_mrg)
#>   Time Well Measurements Bacteria_strain    Phage
#> 1    0   A1        0.002        Strain 1 No Phage
#> 2    0   B1        0.002        Strain 7 No Phage
#> 3    0   C1        0.002       Strain 13 No Phage
#> 4    0   D1        0.002       Strain 19 No Phage
#> 5    0   E1        0.002       Strain 25 No Phage
#> 6    0   F1        0.002       Strain 31 No Phage
```

And now we can see that we've gotten nice numeric `Time` values!

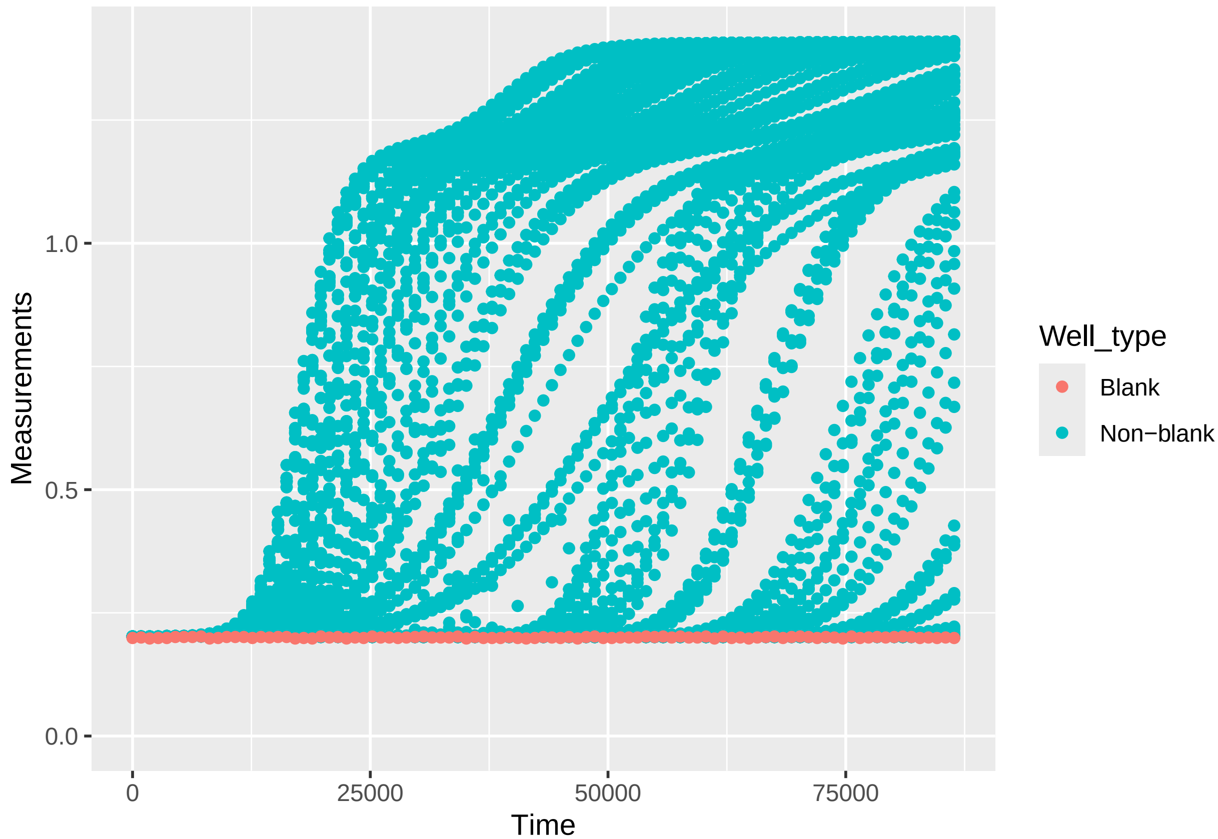
Pre-processing: subtracting blanks

Many growth curves are collected by measuring the absorbance or optical density of a culture. However, with such data an absorbance value of 0 is not equal to a cell density of 0, since components of the media often absorb some light. It's best practice to have at least one 'blank' well in your plate containing only media and no cells, so that you can subtract out this difference from your data so that the values you are working with are scaled correctly.

Here we have some data including a blank well. The first thing you should always do is plot your blank wells data to ensure they look correct:

```r
ex_dat_mrg <- make_example(vignette = 4, example = 2)
ggplot(data = ex_dat_mrg,
```

```
       aes(x = Time, y = Measurements, color = Well_type)) +
  geom_point() +
  ylim(0, NA)
```

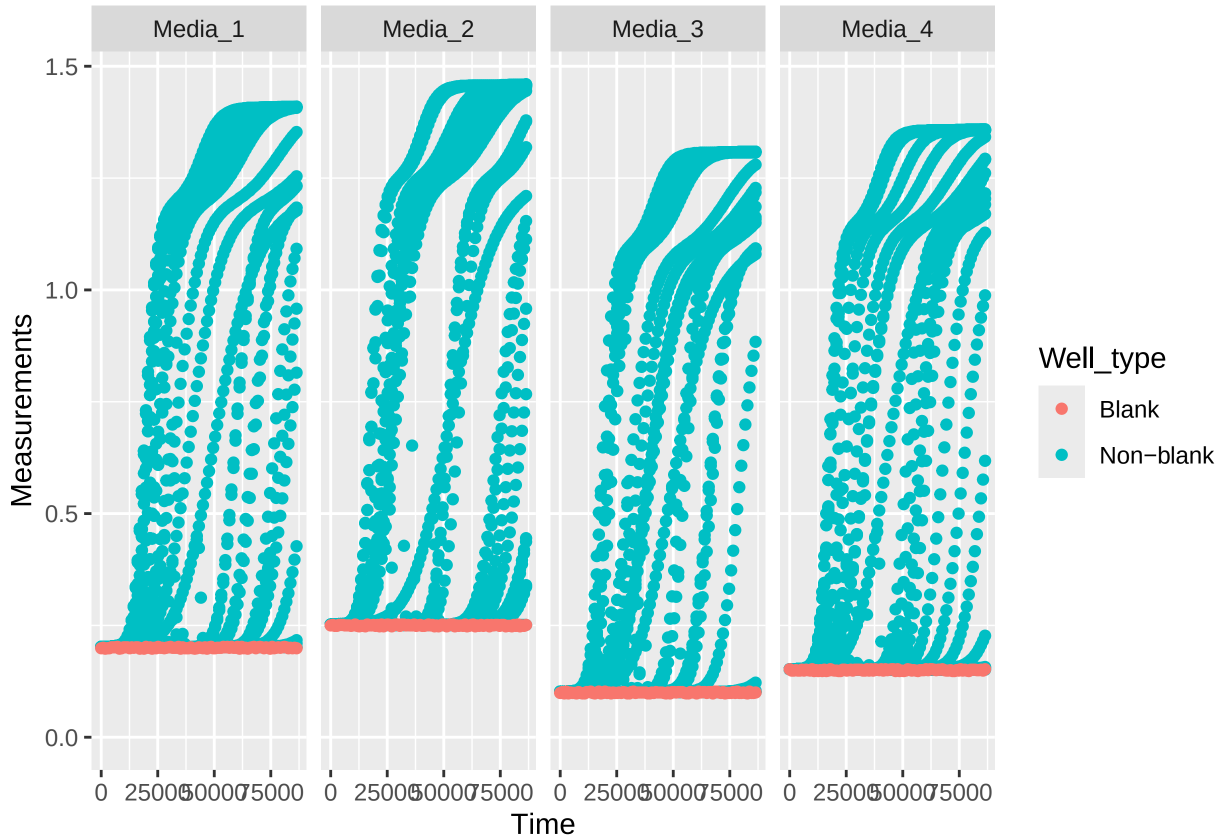


Once you've confirmed your blank wells weren't contaminated, one simple way to subtract blanks is to calculate the average value of your blank well(s) across all timepoints and subtract that from your `Measurements`:

```
mean_blank <- mean(dplyr::filter(ex_dat_mrg, Well_type == "Blank")$Measurements)
mean_blank
#> [1] 0.2000928
ex_dat_mrg$Meas_norm <- ex_dat_mrg$Measurements - mean_blank
```

Note that if you have different blanks for different wells (e.g. you have multiple medias), you'll have to calculate different blank values for each [`vignette("gc06_analyze")` has a primer on the `summarize` function used here, if you'd like to learn more]:

```
ex_dat_mrg <- make_example(vignette = 4, example = 3)
ggplot(data = ex_dat_mrg,
       aes(x = Time, y = Measurements, color = Well_type)) +
  geom_point() +
  facet_grid(~Media)  +
  ylim(0, NA)
```




```
blank_data <- dplyr::filter(ex_dat_mrg, Well_type == "Blank")
blank_data <- group_by(blank_data, Media)
ex_dat_sum <- summarize(blank_data,
                        mean_blank = mean(Measurements))
head(ex_dat_sum)
#> # A tibble: 4 x 2
#>   Media   mean_blank
#>   <chr>        <dbl>
#> 1 Media_1     0.200
#> 2 Media_2     0.250
#> 3 Media_3     0.0997
#> 4 Media_4     0.150
ex_dat_mrg <- merge_dfs(ex_dat_mrg, ex_dat_sum)
#> Joining with `by = join_by(Media)`
ex_dat_mrg$Meas_norm <- ex_dat_mrg$Measurements - ex_dat_mrg$mean_blank
```

Plotting your data

Once your data has been merged and times have been converted to numeric, we can easily plot our data using the `ggplot2` package. That's because `ggplot2` was specifically built on the assumption that data would be tidy-shaped, which ours is! We won't go into depth on how to use `ggplot` here, but there are three main commands to the plot below:

- `ggplot` - the ggplot function is where you specify the `data.frame` you would like to use and the

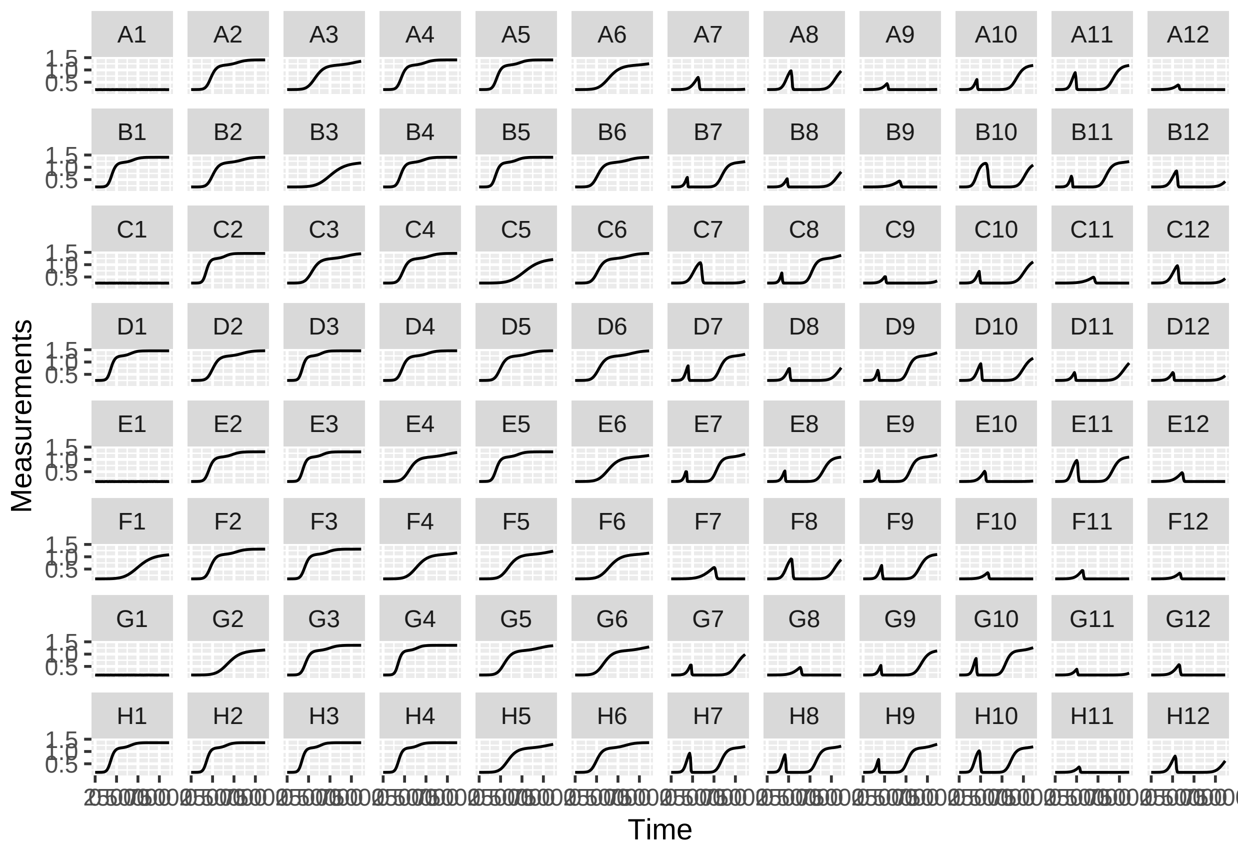
*aes*thetics of the plot (the x and y axes you would like)
- `geom_line` - tells `ggplot` how we would like to plot the data, in this case with a line (another common `geom` for time-series data is `geom_point`)
- `facet_wrap` - tells `ggplot` to plot each Well in a separate facet

We'll be using this format to plot our data throughout the remainder of this vignette

```
# We have previously loaded ggplot2, but if you haven't already then
# make sure to add the line:
#     library(ggplot2)

# First, we'll reorder the Well levels so they plot in the correct order
ex_dat_mrg$Well <-
  factor(ex_dat_mrg$Well,
         levels = paste0(rep(LETTERS[1:8], each = 12), 1:12))

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



Generally speaking, **from here on you should plot your data frequently**, and in every way you can think of! **After every processing and analysis step, visualize both the input data and output data** to understand what the processing and analysis steps are doing and whether they are the right choices for your particular data (this vignette will be doing that too!)

What's next?

Now that you've pre-processed and visualized your data, it's time to process (in most cases) and analyze (pretty much always) it!

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