Background

We previously observed that after diluting o/n culture grown without shaking in 96-well plates into 1xPBS (~1:20), the measured intensity drifts up such that the first well and the last well may differ by more than 3 fold even though they contain exactly the same strain. My hypothesis is that cells grown without shaking settle very early on and due to the lack of mixing, cells remained in a metabolically inactive mode that suppressed either transcription or translation. After being "released" into PBS, which doesn't contain any nutrient but does give the cells access to oxygen and phosphate, may result in the cells restarting their metabolism and thus increasing the protein reporter we measure.

Goal

Test the above hypothesis, namely after diluting the o/n culture 1:20 into PBS, the fluorescent signal measured will continue to increase and reach a plateau after some time.

Approach

yH156 and JZ-B18 (ScPho4 with Pho2) were inoculated into SC or SD-leu in the morning, grown to early log phase in the afternoon, diluted 1:5 to 1:10 into 96-well plates (standard) and left at 30C without shaking. The next morning, the o/n culture was diluted 1:20 into PBS (~1 mL total) and were measured by flow cytometry over the course of 3 hours.

For comparison, another sample was made from o/n culture of the same B18 strain grown in deep well plate (300 uL) with a 3 mm glass bead, shaken at 500 rpm.

Data

The flow data was processed using the Attune software to export the median fluorescence after gating for single cells. The CSV file is lightly edited and stored in this folder.

For the sample from the shaken culture, the raw FCS files were collected along with the plate and filed under the 20220104-deep-weel folder. The FCS files were processed using an R script (with flowCore etc) and the MFI were exported and written to a separate CSV file in the current folder.

Results

Load libraries and data

```
require(tidyverse)
```

```
## Loading required package: tidyverse
```

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.1 --
```

```
## v ggplot2 3.3.5     v purrr   0.3.4
## v tibble  3.1.6     v dplyr   1.0.7
## v tidyr   1.1.4     v stringr 1.4.0
## v readr   2.1.1     v forcats 0.5.1
```

```
## -- Conflicts ---------------------------------------- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
require(cowplot)
```

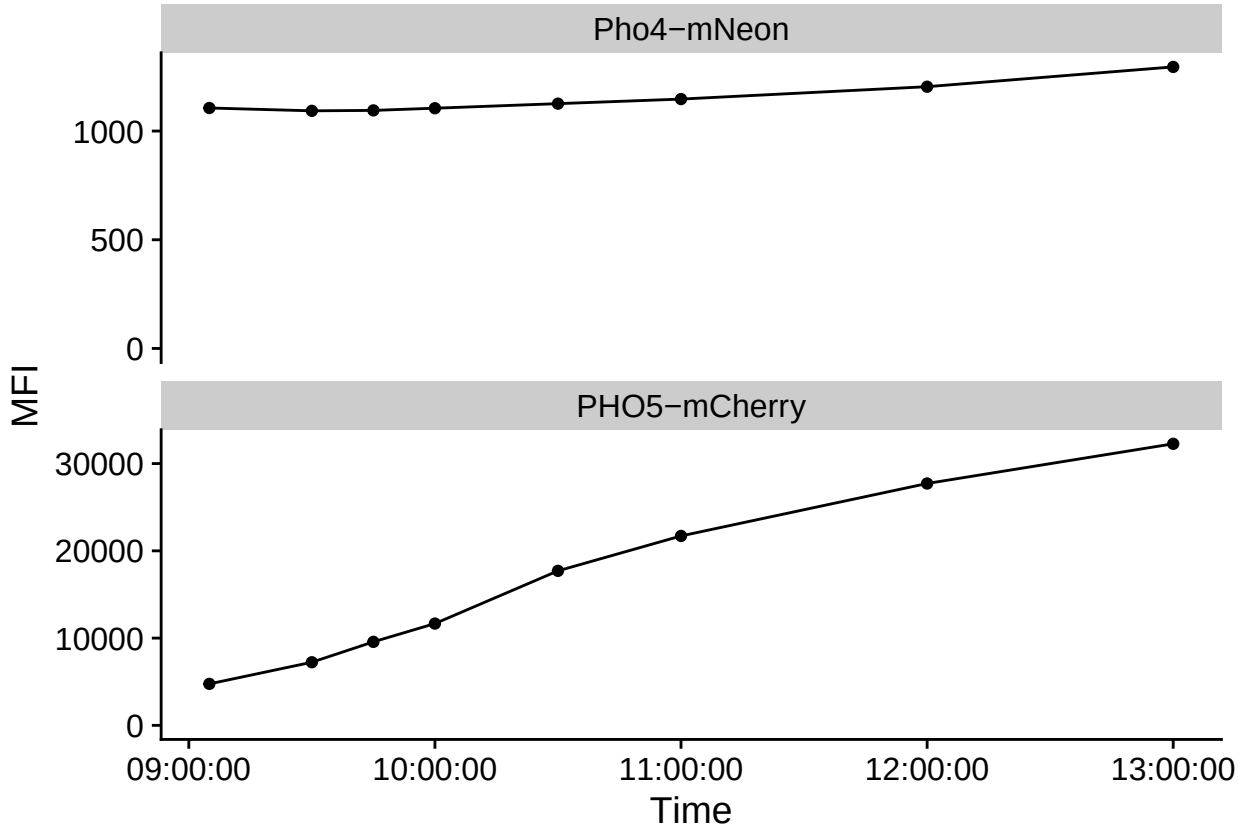
```
## Loading required package: cowplot
```

```
dat <- read_csv("20220104-signal-change.csv", col_types = "ctcidi") %>%
  mutate(Parameter = factor(Parameter, levels = c("Pho4-GFP-GFP-H","PHO5pr-mCherry-mCherry-H"),
                            labels = c("Pho4-mNeon", "PHO5-mCherry")))
dat1 <- read_csv("20220104-signal-change-shaking.csv", col_types = "ctiddddd")
```

Plot change in signal over time

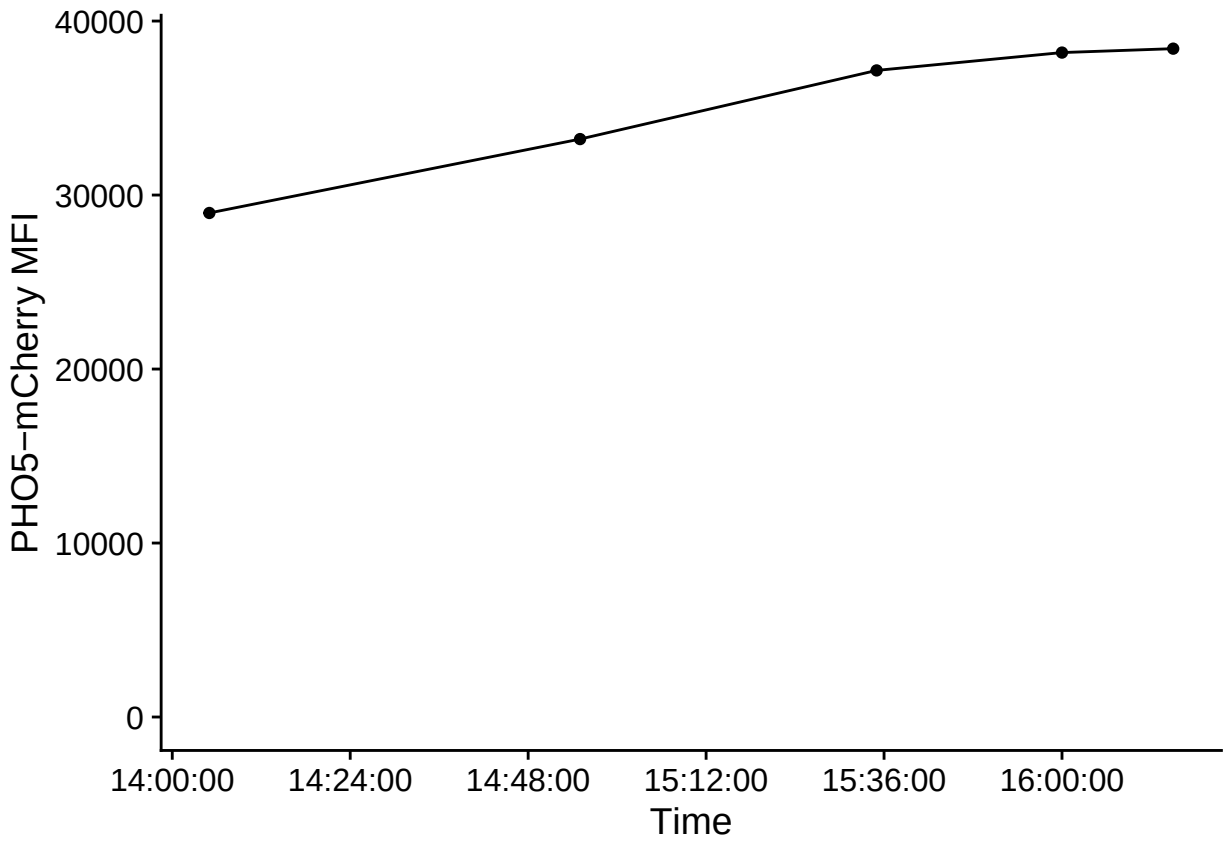
Without shaking

```
dat %>%
  filter(Sample == "Pos") %>%
  ggplot(aes(x = Time, y = MFI)) + geom_line() + geom_point() +
  expand_limits(y = 0) +
  facet_wrap(~Parameter, scales = "free_y", ncol = 1) +
  theme_cowplot()
```



With shaking

```
ggplot(dat1, aes(x = Time, y = YL2.H)) + geom_point() + geom_line() +
  ylab("PHO5-mCherry MFI") + expand_limits(y = 0) + theme_cowplot()
```



Conclusion

- The signal kept rising even after 4 hours for the o/n sample grown without shaking. It is not a good idea to use the no-shaking method.
- With shaking, the signal only rose modestly (~30k -> ~38k). Still substantial. By 2 hours the level is stabilized. Need to repeat this (potentially by measuring several samples right after dilution and then two hours after dilution)