Comparison between Wener setting in FAcs with mine

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Title : Comparison between the fAcs dettings used for Werner in 2017 with the ones I am using currently.

Date

13022020-14022020

Objective

To understand what happen to the output fluorescence signal if the settings are slightly changed, and perhaps we can understand why in 2017 when he measured with Marit they measured 6-7 fold between 0-2% gal while I am only measuring 1.5-2.5 fold change, when taking the geometric means of the histograms.

- From @fig:marit-data we can observe that already the sugar source it was used is different than mine experiments. The used glucose as a second sugar source for the

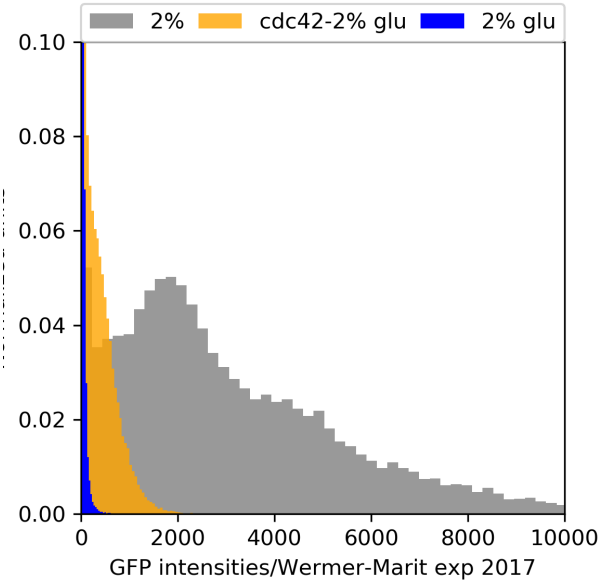


Figure 1: Marit-Werner data 2017

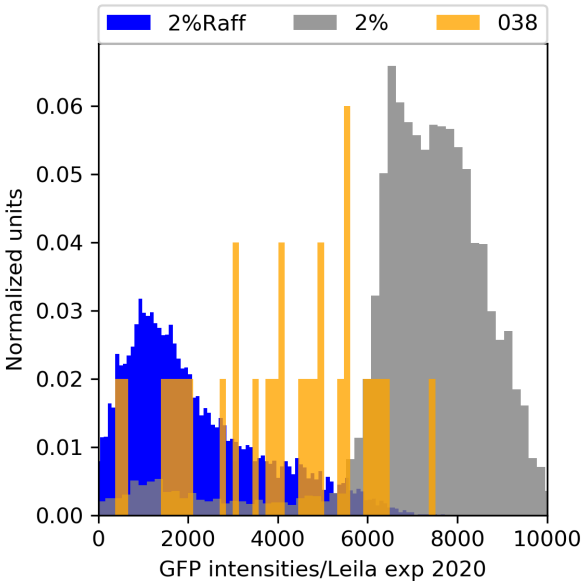


Figure 2: Leila Data 06022020

galactose promoter , and all the reamining controls are in incbated in glucose. The cells here were incubated in 30C and I incubated them in 36C (as Ilse measured in the bulk growth). I used raffinose as a second sugar source , and also for the controls without galactose promoter, as Ilse used in her bulk growth experiments.

- From the histograms, we can see that @fig:marit-data have a long tail for 2% gal (gray curve), going until 25000.
- The 2% Raff in my case(@fig:leila-data) is more spread than the 2% glu for @fig:marit-data. maybe because Raffinose has some galactose???
- Looking from peak to peak in @fig:leila-data, we can see that the fold change is around 8, from 1000 to 8000.

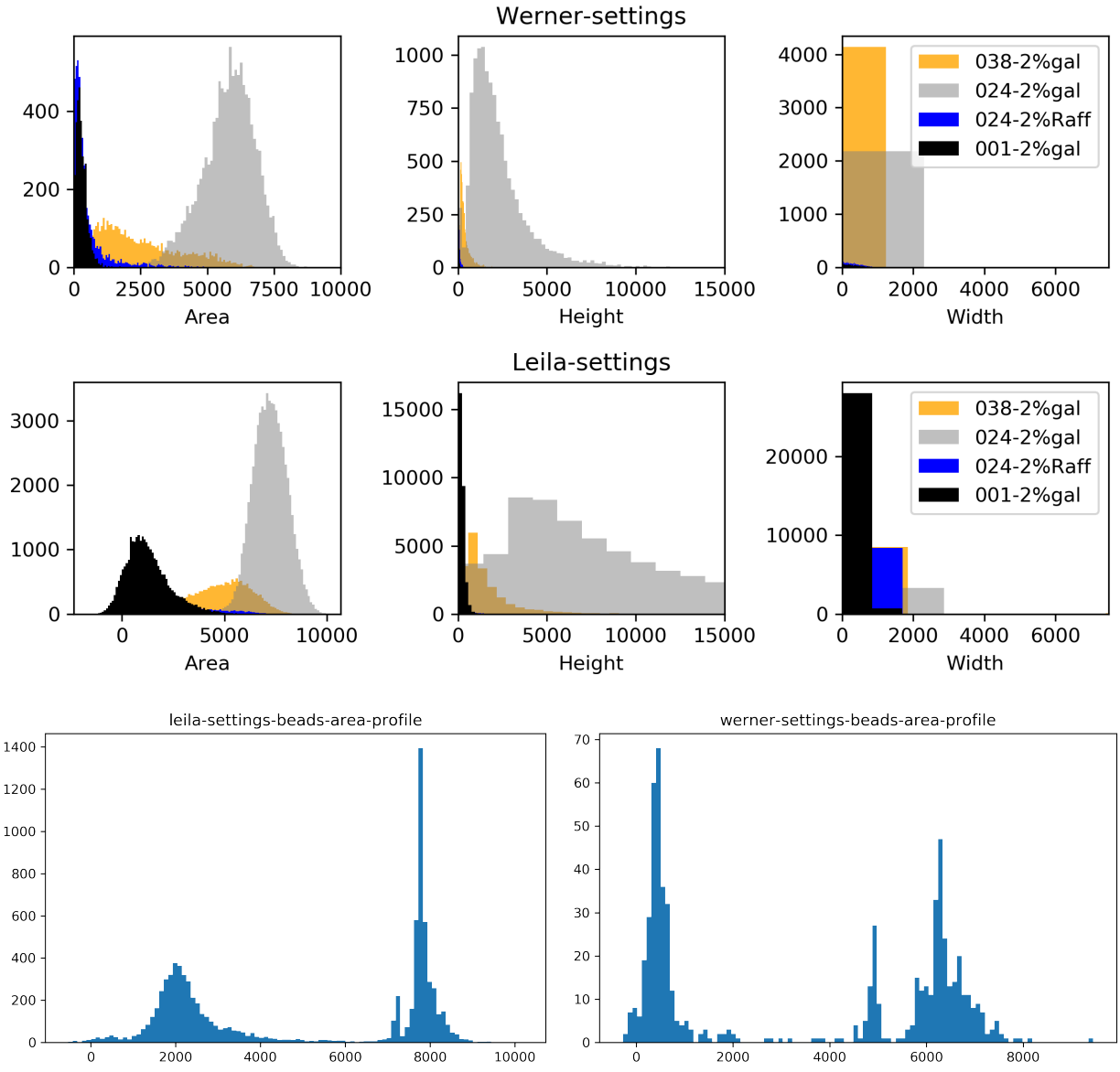
Method

- I am going to use only the following strains:
  - ywkd024 : RWS119 Wedlich-Söldner Lab collection a W303 *can 1 1-100 his3 11,15 Galpr-myc-**GFP**-CDC42 YipLac204-MET-CLN2 cln1Δ::HisG, cln2Δ, cln3Δ::HisG* (**strain to compare with ywkd065(sfGFP)**)
  - ywkd038: RWS1421 Wedlich-Söldner Lab collection a W303 *can1 1-100 his3 11,15 CDC42pr-myc-**GFP**-CDC42 YipLac204-MET-CLN2 cln1Δ::HisG, cln2Δ, cln3Δ::HisG* (**Reference for the native CDC42 expression**)
  - ywkd001: α W303 *can 1 1-100 his3 11,15 leu2 3,112* S288C ,yLL3a -Laan Lab collection (**Reference for the background fluorescence**)
- The incubation is going to be for 15 hours from glycerol stock to the measurement point, in 0% and 2% Gal.
  - Incubation started at 8:50 am on 13022020

Different setting used for Werner (2017) and for me (2020)

- **Equipment-Model**: BDFACSCelesta
- **Lasers-488nm**: 375V (Werner) // 495V (Leila)
- **FSC threshold**: 50000 (Werner) // 20000 (Leila)
- **FSC voltage**: 375V (Werner) // 407V (Leila)
- **SSC volatge**: 233V (Werner) // 275V (Leila)

Results



Conclusion

- The settings of Werner , what does is to shift the signal to the left including the signal from the beads.
- After the analysis of the height and the area output from the GFP output pulse , we can say that we can see 7 fold variation among the means of intensities when taking the height measure as the intensity signal.

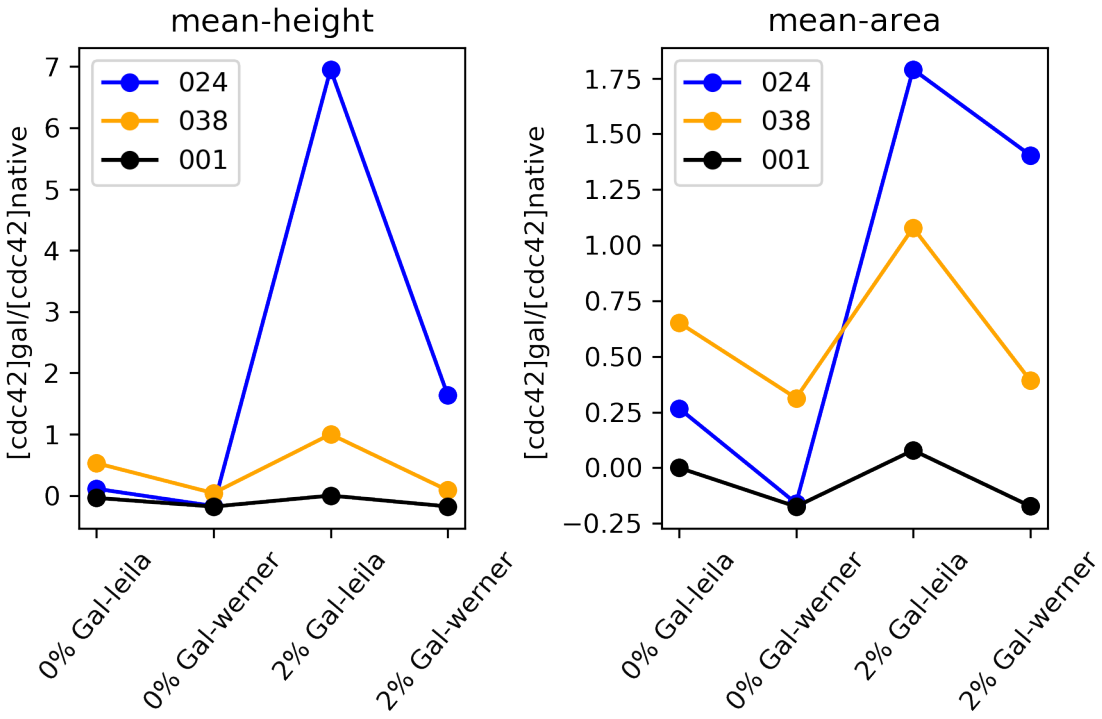


Figure 3: Considering Leila settings to normalize

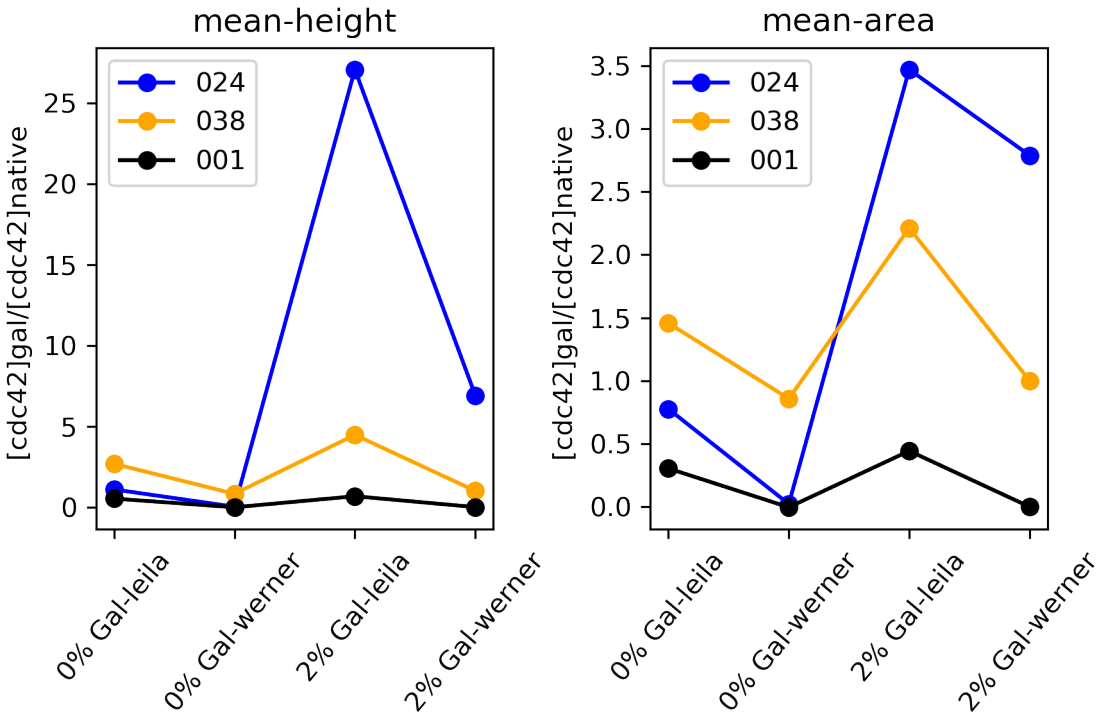


Figure 4: Considering Werner settings to normalize