Bacterial analysis in flowcytometry

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Sample preparation

- To be able to differentiate between bacteria and background we highly recommend using a DNA dye
- It is very important to standardize the staining of your samples. Both incubation temperature and incubation time will affect the outcome. See figure 1.

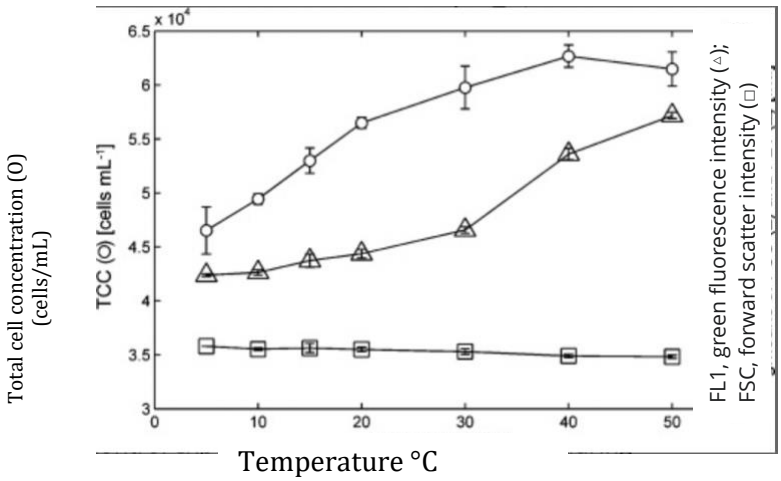

Figure 1: Effect of different incubation times and incubation temperature on bacteria count
Frederik Hammes, https://onlinelibrary.wiley.com/doi/10.1002/cyto.a.22048

Analysis

- The NovoCyte Quanteon and the BigFoot cell sorter are the best instruments for bacteria analysis and sorting in the FACS Core Facility.
- Use height (not area) for fluorescence as well as scatter signals.
  Because of the small size of bacteria, there is less variation on the height parameter compared to area.
- Use log scales on both forward and side scatter.
- You may want to set your threshold on a fluorescence parameter (DNA stain). If this is not possible, a side scatter threshold is often better than a forward scatter threshold.
- Acquire as slow as possible to increase signal to noise ratio and decrease
- If sorting on the FACS Aria III remember the possibility to remove the ND filter.

- Make sure you look at single events
  - Start out using the slowest speed possible (not just “slow”, but move the slider to the far left)
  - Determine the median fluorescence intensity (MFI) for the bacteria population.
  - Repeat this step but on a diluted version of your first sample.
  - The count-rate should decrease, and the MFI should stay the same as in the undiluted sample. If this is not the case, you are exposed to swarming (many events are being detected as one event). In this case you should make a dilution series to determine the dilution where you are detecting single events. See figure 2.

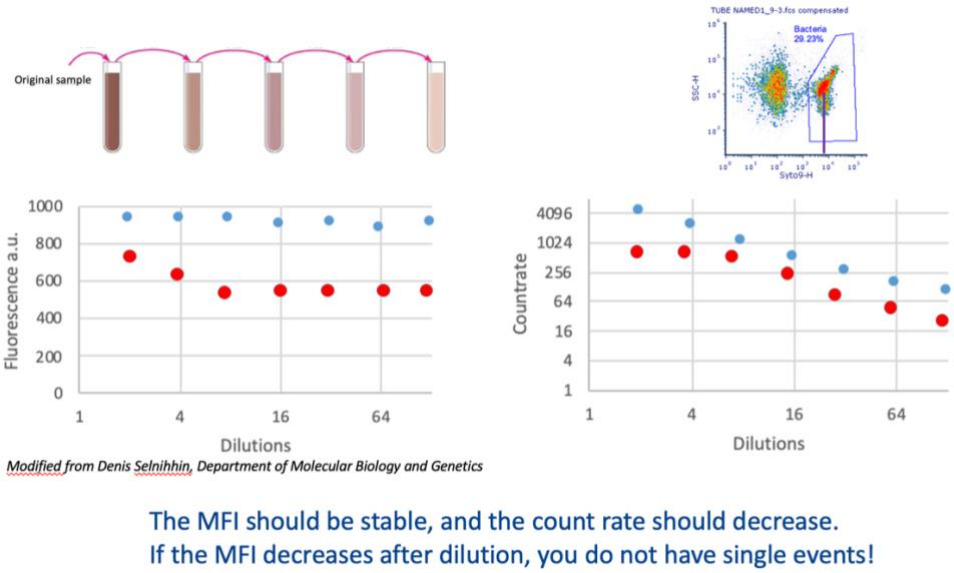

Figure 2: Make a dilution series of your bacterial sample. If dilution does not affect fluorescence intensity and your count rate decreases corresponding to dilution (as illustrated with the blue dots) you have single events. If fluorescence intensity decreases (as illustrated with the red dots) you do not have single events in the first two samples.
Ref: Denis Selnihhin, Department of Molecular Biology and Genetics - Geneexpression and Genemedicine, Aarhus University and Anja Bille Bohn, FACS Core Facility