Conclusions from last experiment 20190115:

* We learned from Lau that the thresholds are always on H, so when DIVA software records both H and W it seems to mislabel these columns. In the future we will only record H, then the labelling is correct.
* We think that (FSC,900).AND.(SSC,200) would be a good threshold for us
  + We found that (FSC,200).AND.(SSC,200) is too low (too many noise and debris events if you compare to (FSC,1200).AND.(SSC,200)) but by plotting FSC-H SSC-H we could see the two populations (maybe debris vs cells) and think FSC-H 900 would still kill almost all debris, but leave a bit more room for cells
* To reproduce the Nature paper gating we will record APC-Cy7-A in the future and I will try to test their gating on it.
* We should always have some control samples on all our counting plates!!

We also collected a lot of other channels, but neither: FITC-A, PE-A, APC-A, "PerCP-Cy5-5-A, PE-Cy7-A, AmCyan-A, APC-Cy7-A combined with Pacific-Blue-A was better than FSC-A x Pacific-Blue-A, from <https://www.bdbiosciences.com/documents/Multicolor_Fluorochrome_Guide.pdf> I can see that the one closest to the >670 nm filter from VAndeputte is: PerCP-Cy5-5-A with 695/40 so from 675 to 715 nm, and indeed that one looked also best

Tresholding lessons:

* (FSC,200).AND.(SSC,200) already gives only ca 4000 el noise events in Buffer only in 100 seconds
* (FSC,1200).AND.(SSC,200) gives only ca 3000 el noise events in Buffer only in 100 seconds
* (FSC,200).AND.(Pacific Blue,200) gives only ca 3000 el noise events in Buffer
* (FSC,200).AND.(SSC,200) seems to allow too much debris noise because > 10% of sample events have negative FSC-A, SSC-A, >>>> This threshold might be too low
* Also (FSC,200).AND.(Pacific Blue,200) allows 10% of events in samples to be negative, so also this threshold seems too low
* Another reason why I would prefer (FSC,1200).AND.(SSC,200) over (FSC,200).AND.(Pacific Blue,200) is that more of the el noise events that pass the threshold lie in our Pacific\_Blue-A FSC-A gate!
* (FSC,200).AND.(Pacific Blue,200) does not allow a clear gate between untained and stained samples, so it’s out
* Currently: I would vote for (FSC,1200).AND.(SSC,200) or please try (FSC,800).AND.(SSC,200)

For Friday:

* Record FSC-A, FSC-H, SSC-A, SSC-H, Pacific-Blue-A, PE-A, APC-Cy7-A
* Threshold: (FSC,900).AND.(SSC,200)
* Dilutions:
  + 200x, 300x, 8500x (a la nature paper)
* Samples:
  + All 5 persons, only Buffer stained,
* Maybe run a pure e.coli sample once
* For future: Think about a pooled mixed sample that serves as control on all plates!