

- Announcements
- Lab Quiz
- Pre-lab Lecture
  - ❖ [C401] dose response staining
  - ❖ FC analysis preview/review
  - ❖ Today in Lab (M2D7)

# Announcements

- Module 2 report due Wed 4/23 at 5 pm
  - more time to write cf M1 → because you need it!
  - individual reports
  - no formal revision

OH - Sun + Tue before? replace M PM  
Sat? W/F AM?

- If you visit the BE Writing Lab before Mon 4/21, you get a 24 hr extension!
  - make an appointment at [bewritinglab.mit.edu](http://bewritinglab.mit.edu)
- Notebook due by 10 pm tonight
  - email Su for time-stamp

(+) intros to Stellar for Leslie

# Staining approach for [C401] assay

## DAY 6

Plate irradiated K1  
with varying [C401]

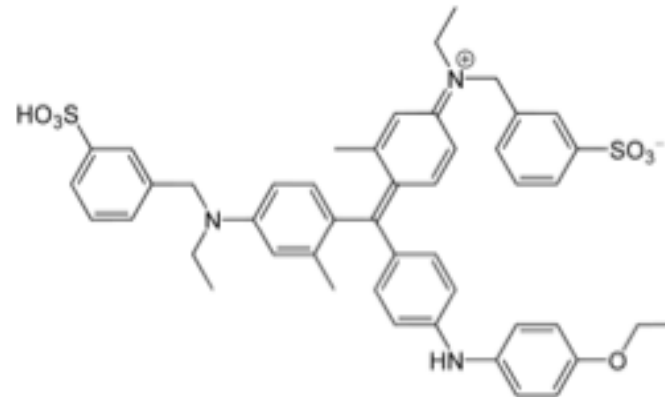
## DAY 7

Stain for colonies



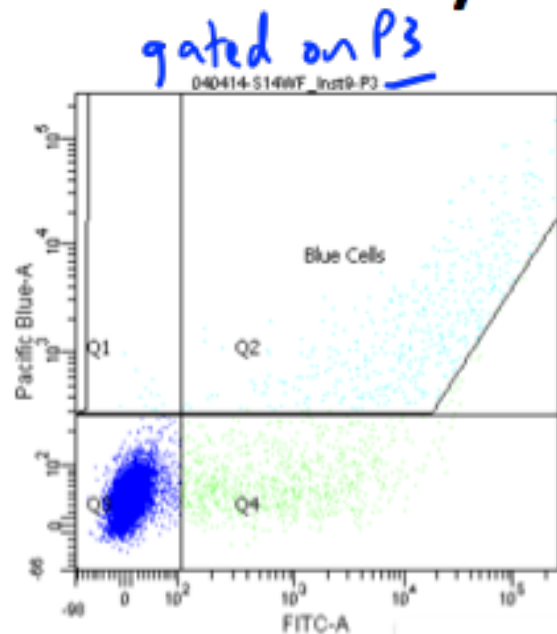
Coomassie dye (G variant) *in a little solvent*

Absorbs at 595 nm as binds protein – *orange/red light*  
Reflects therefore – *blue*



*binds mainly to basic, aromatic  
AA - especially Arg/R*

# Flow cytometry analysis p/review



① Raw XFP =  $(\% X) \cdot (MFI_X)$   
 X = blue ; green

② Norm condition replicate =  $\left( \frac{\text{Raw BFP}}{\text{Raw GFP}} \right)_{\text{replicate}}$

③ % repair pair =  $\frac{\text{Norm\_damaged}}{\text{Norm\_intact}}$   
 (NHETJ)

parent of P3 is probably P2 (or P1)

green gate: 22+24

Experiment Name: AgiStachowiak040414  
 Tube Name: S14WF\_Inst9  
 Record Date: Apr 4, 2014 2:47:01 PM  
 GUID: 5631ecdc-2e63-4112-8ec5-3600a0bff593

Population	#Events	% Parent	FITC-A Mean	FITC-A Median	Pacific Bl... Mean	Pacific Bl... Median
All Events	12,460	###	1,955	10	327	41
P3	10,233	94.9	1,712	10	290	39
Green Cells	1,323	12.9	13,195	1,631	1,980	104
Blue Cells	439	4.3	32,263	15,574	5,686	1,494

# What (kinds of) replicates do we have?

- technical = same sample (cell/reagent stocks)  
aliquotted → measured twice  
\* duplicates within each team
- biological = independent samples/stocks/days  
\* duplicates across sections (usually)

we will perform stats with all 4 reps  
but (!) 3-5 bio reps is ideal in "real" science

# A few more statistics notes

- Assumption for t-test: normal/Gaussian distribution  
(cf non-parametric tests)

- 95% CI vs t-test:

t-test compares  
two population means

"confidence level" or "p-value"



error bars relate  
to 95% C.I. - is  
about mean of one  
population

- Types of t-tests

1-tailed  $\leftarrow$  priori hypothesis ( $A > B$ ) - use half of distribution

when in  
doubt!

2-tailed

no hypothesis ( $A > B?$   
 $A < B?$ ) - full distribution.  
more conservative

# Today in Lab (M2D7)

- Decide whether you want to stain today or next time
  - start by 2 pm if you plan to try it today

- Flow cytometry analysis – this is it!

we will make master  
data sheet w pm RAM

- start with instructor data
- next inspect and quantify your own
- must email worksheet to 20109.submit@gmail.com before leaving

- Optional: stats practice

- heights:

M: 70, 69, 66, 63, 65, 63, 67

F: 70, 67, 67, 70, 64, 65, 64, 64, 67, 67, 67, 64, 60

- For next time
  - methods redux

Do you love science as much as this guy?

