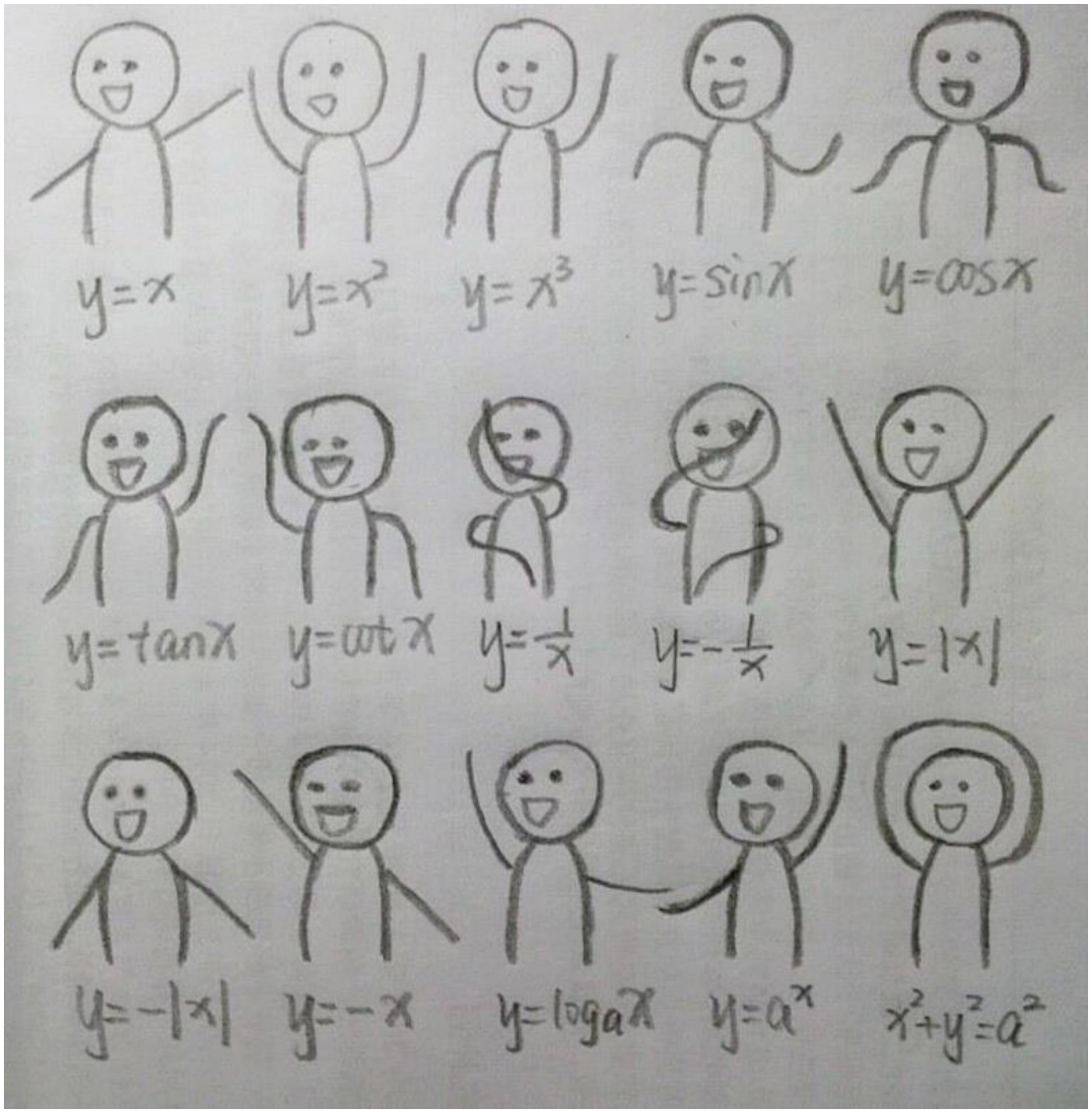
4/2/15

M2D5: DNA repair assays

Do the math dance!



Source: PhilipsShiu

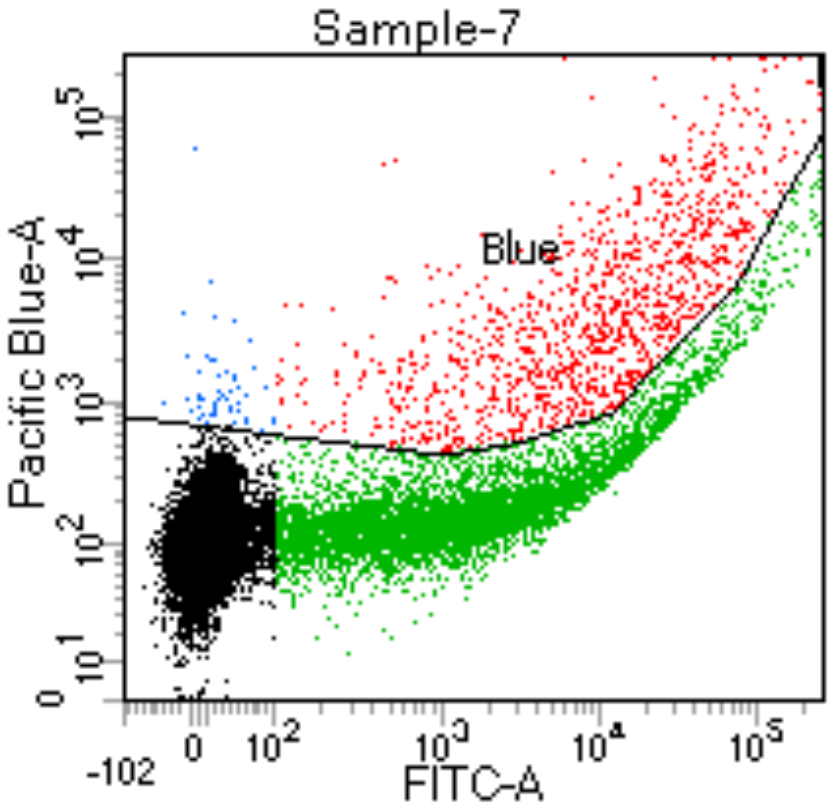
Today we do the tissue culture dance!

Announcements:

Mod1 Revision due **Sunday at 5pm**

Don't forget about your blog posts.

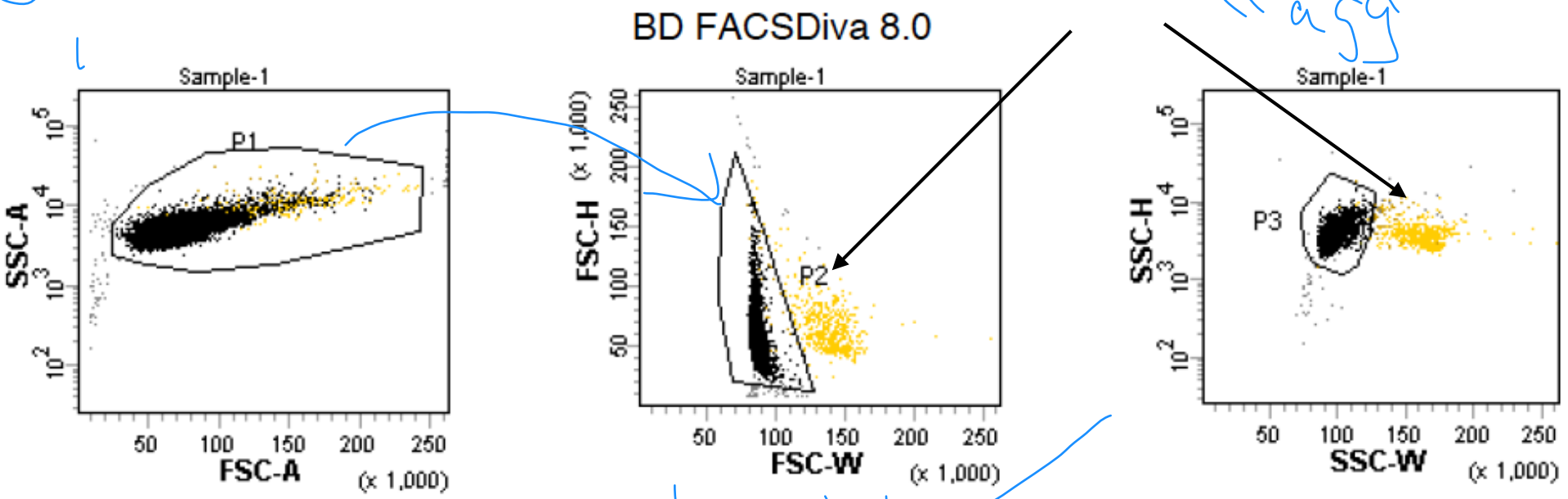
Flow cytometry: There are a lot of steps before we get to this plot!



1) Determine the 'relevant' cell population

Uninfluor cells – gate out dead, agg. debris

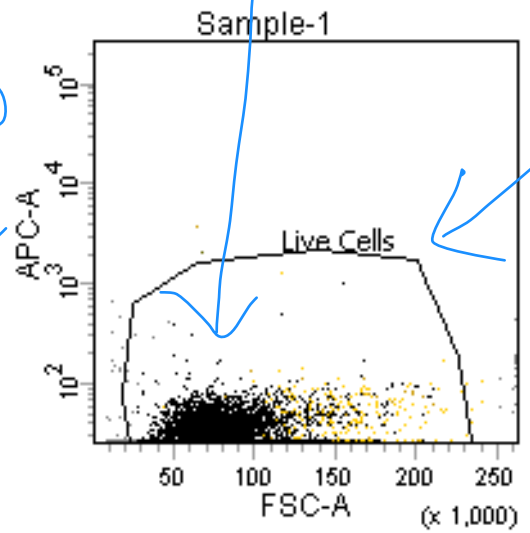
Doublets



relevant

height

width



toPro3 dead

Any cell with ToPro3 will have high far red fluorescence = dead cells

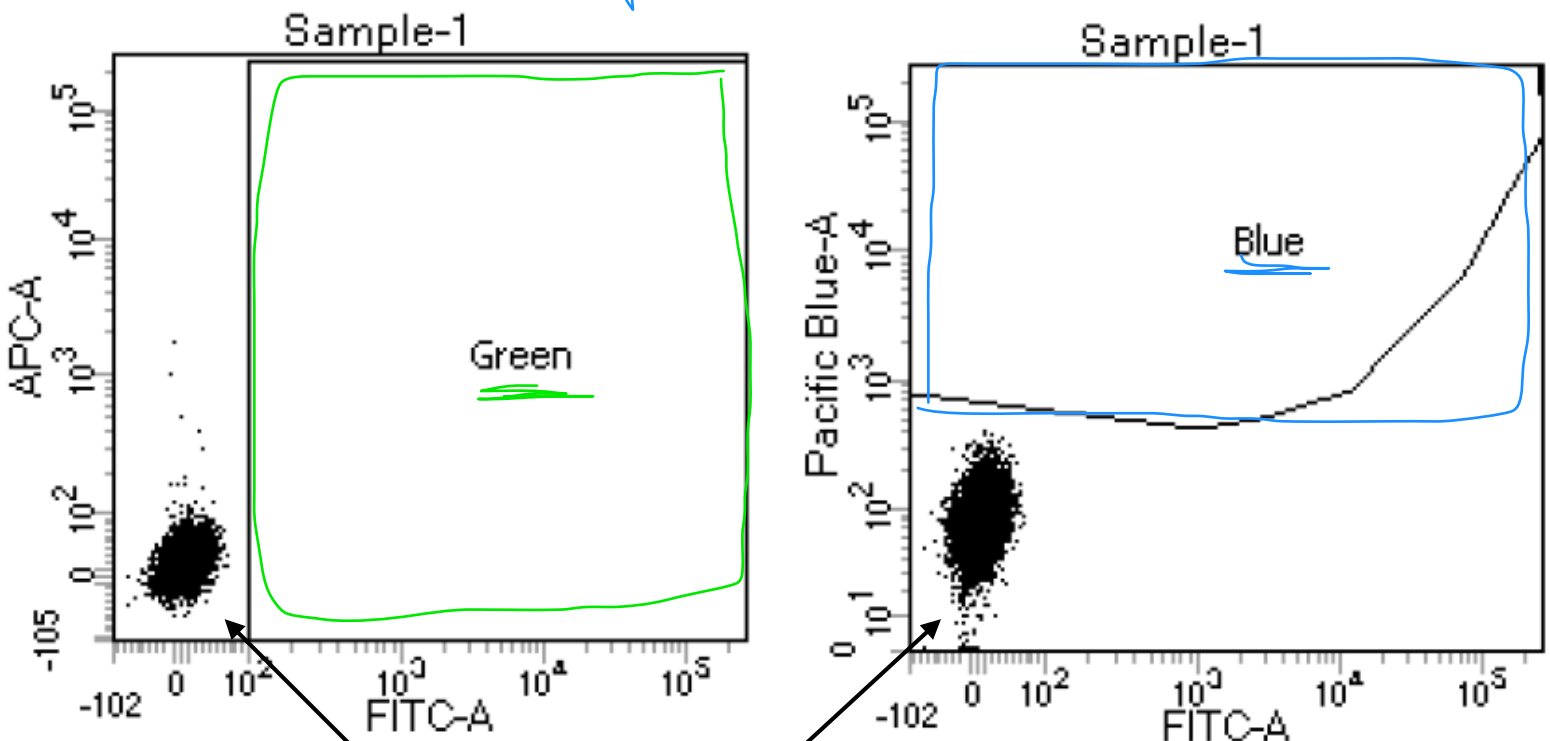
SSC = side scatter = shape

FSC = forward = size

good debris agg

2) Set 'negative' gates with untransfected cells

Unfluor. } set gate BFP, GFP

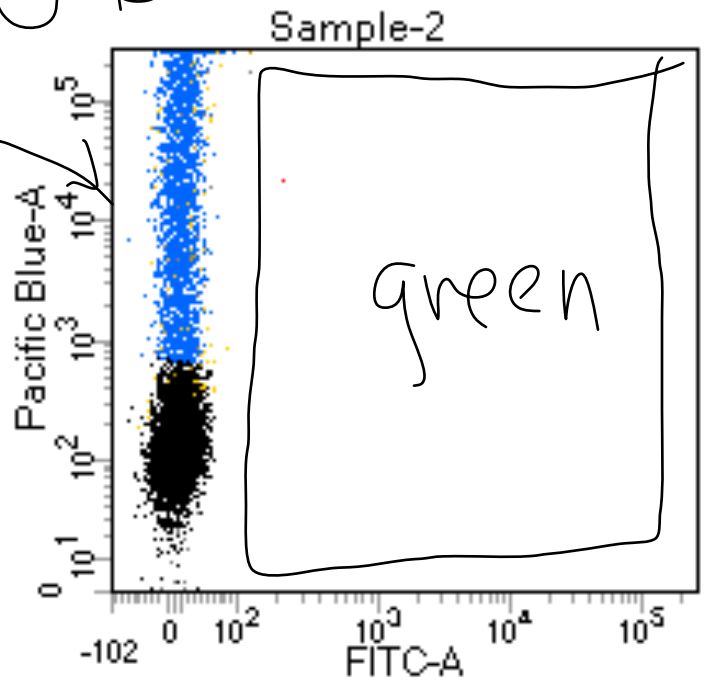


These are the "live cells"

relevant

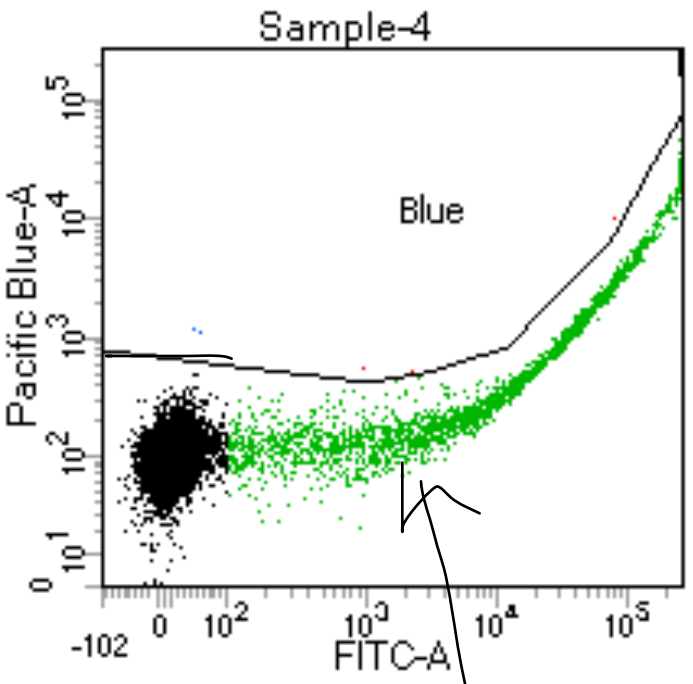
3) Set 'positive' gates with single color controls

U BFP positive



blue BFP only

U GFP alone

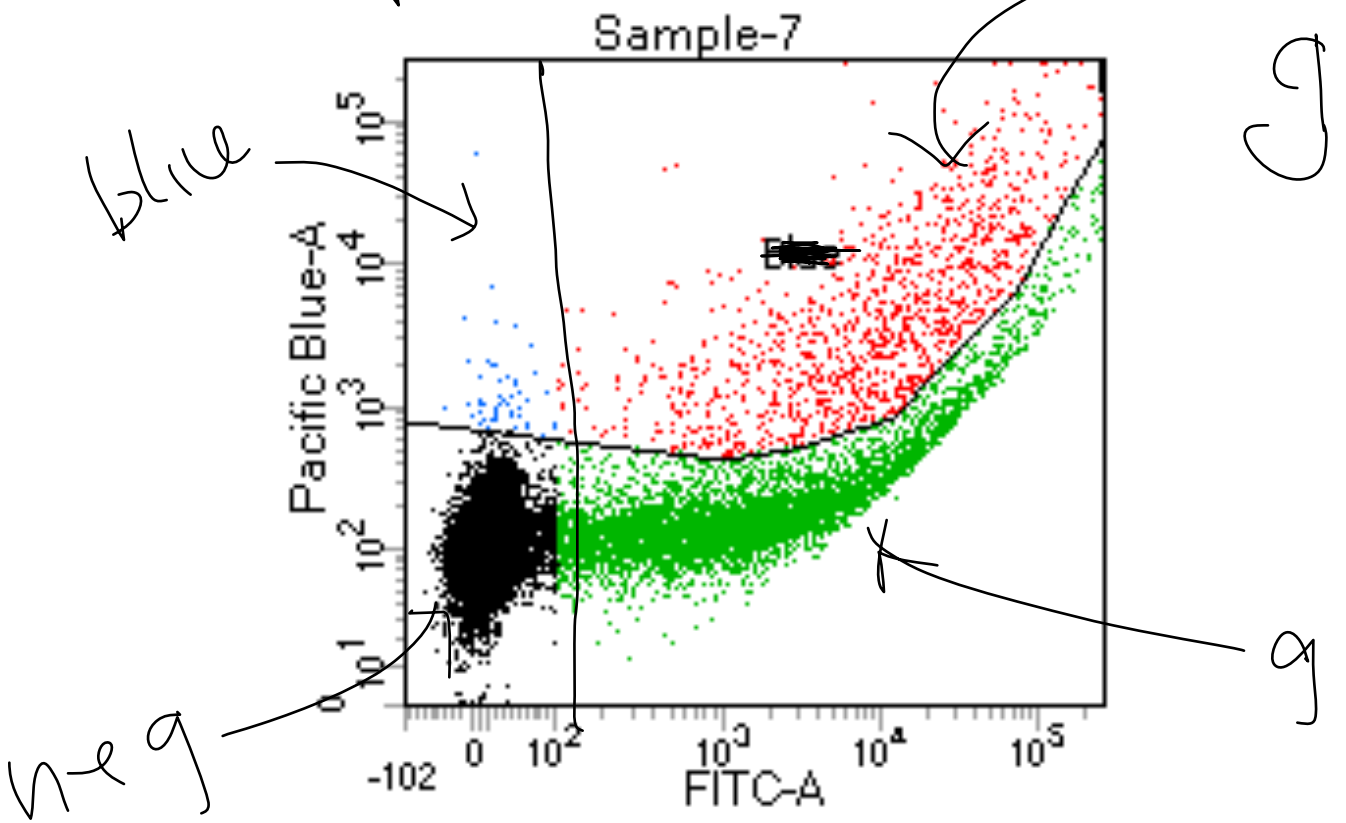


green bleeds blue

green GFP only

4) Quantify experimental conditions

UBFP+GFP



FACS data % cells blu + green (red)

and mean fluor. intensity ⇒ calculations

Part 1: Hints

1. Aspirating media — clean pipette tip between conditions
2. Correct tube labeling is key.

Condition	Orange	Yellow	Green	Blue	Purple
K1 Intact	5	17	29	41	53
K1 Intact	6	18	30	42	54
Drug Intact	7	19	31	43	55
Drug Intact	8	20	32	44	56
xrs6 Intact	9	21	33	45	57
xrs6 Intact	10	22	34	46	58
K1 damaged	11	23	35	47	59
K1 damaged	12	24	36	48	60
Drug damaged	13	25	37	49	61
Drug damaged	14	26	38	50	62
xrs6 damaged	15	27	39	51	63
xrs6 damaged	16	28	40	52	64

3. Mixing is very important — need single cell suspension!
4. Plan your workflow with partner BEFORE you start.

How will we know that the inhibitor works?

Protocol

1. We plated cells for you yesterday.
2. You make up drug dilutions (3ml/well)
3. You add drugs to cells
4. Marcus will irradiate cells with 4 Gy
5. After 7 days, stain colonies and count

NOTE: Teams using DMNB —
Vehicle = DMSO
The same volume of DMSO must be added to each well.

Today in the lab:

1. Orange, yellow, green in TC first to prep flow. Blue and purple will follow once hoods open up.
2. Work at a purposeful pace.
3. The TC room has a rotating door policy today:
    1. Prep for flow —> leave
    2. Next team prep for flow —> leave
    3. Prep for 'kill curve' —> leave
    4. etc.