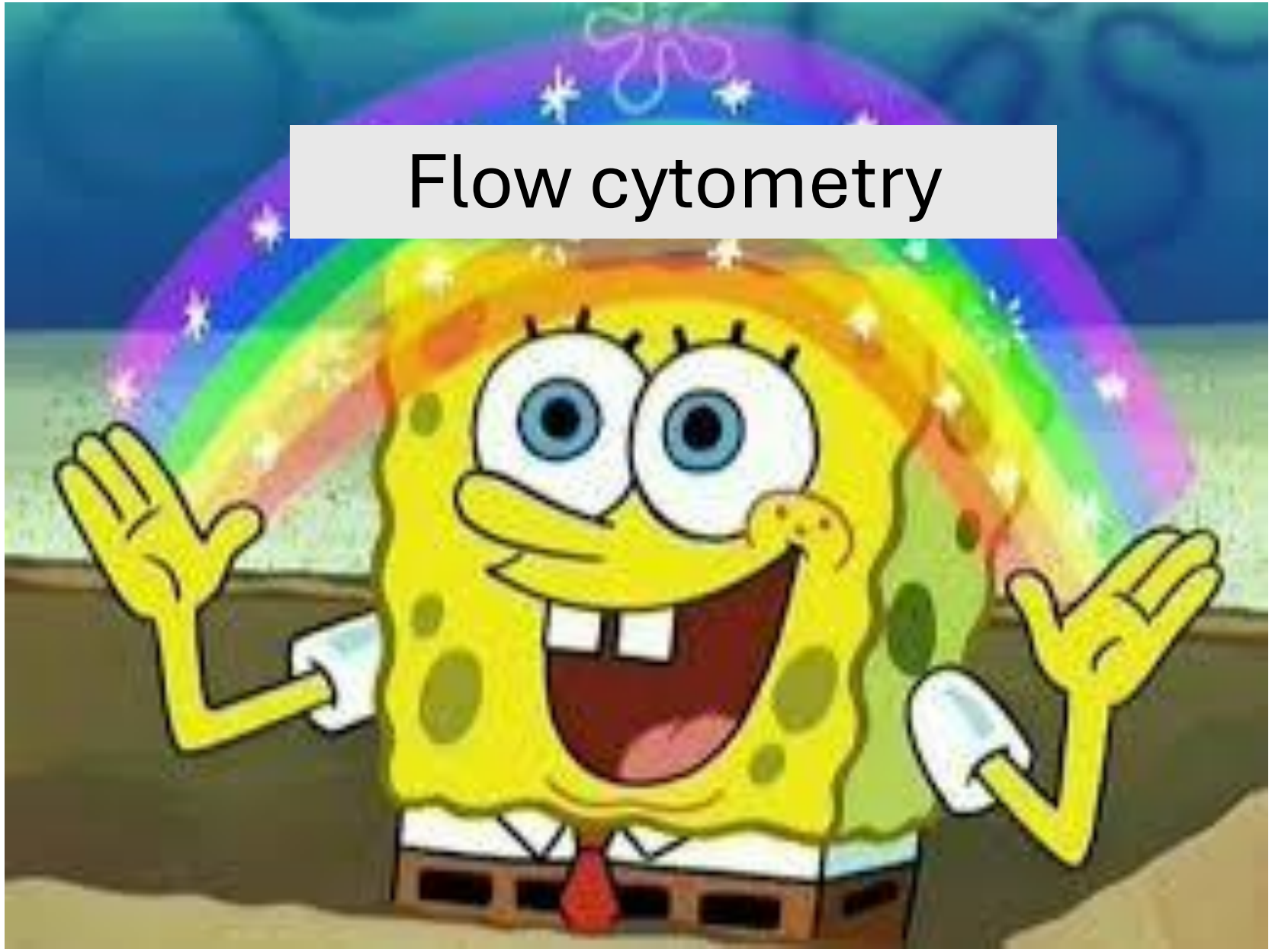
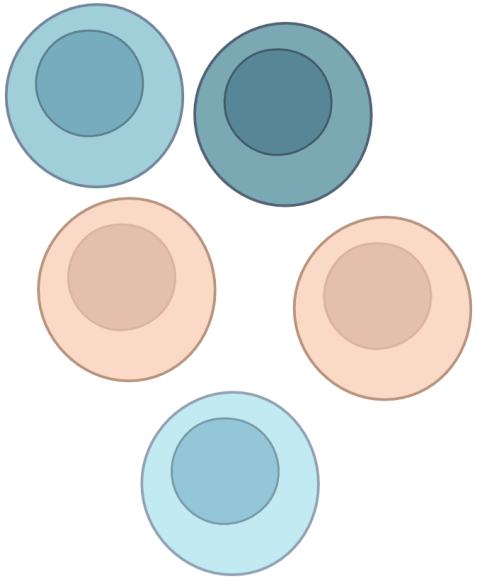
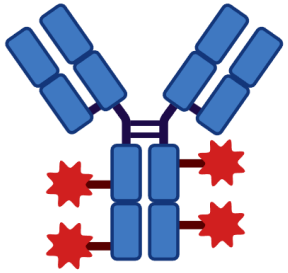
Flow cytometry

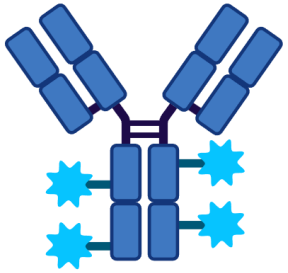


Flow staining

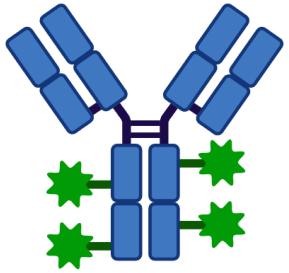




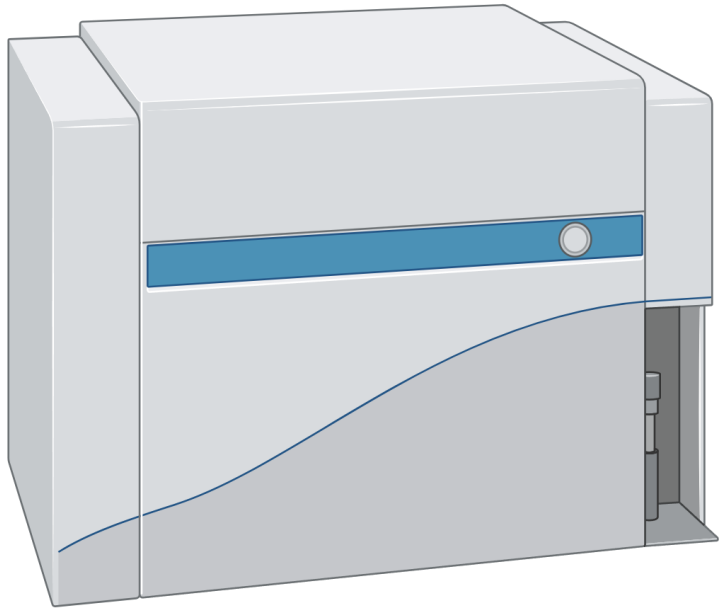
Anti-CD3

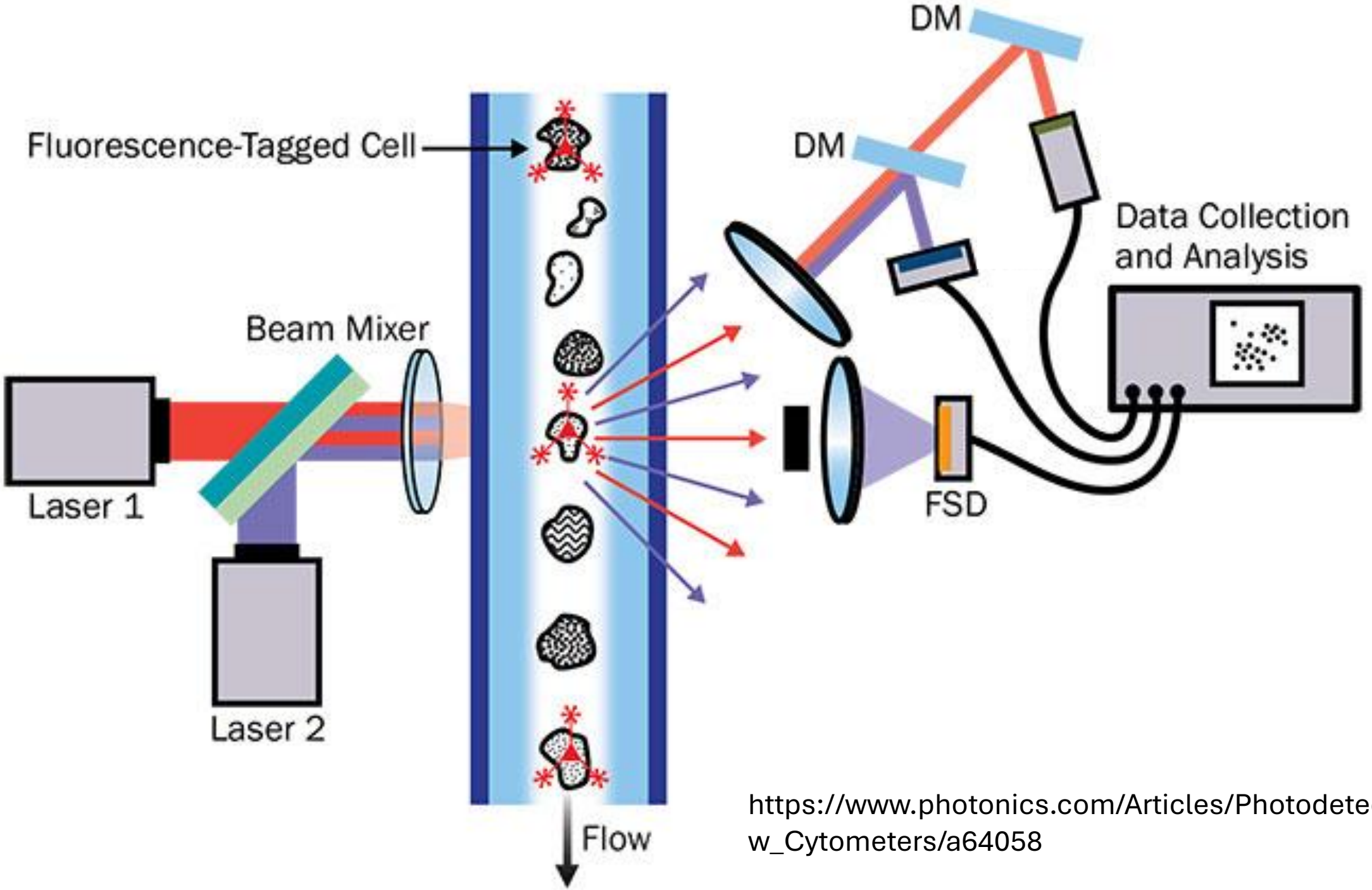


Anti-CD4



Anti-CD8



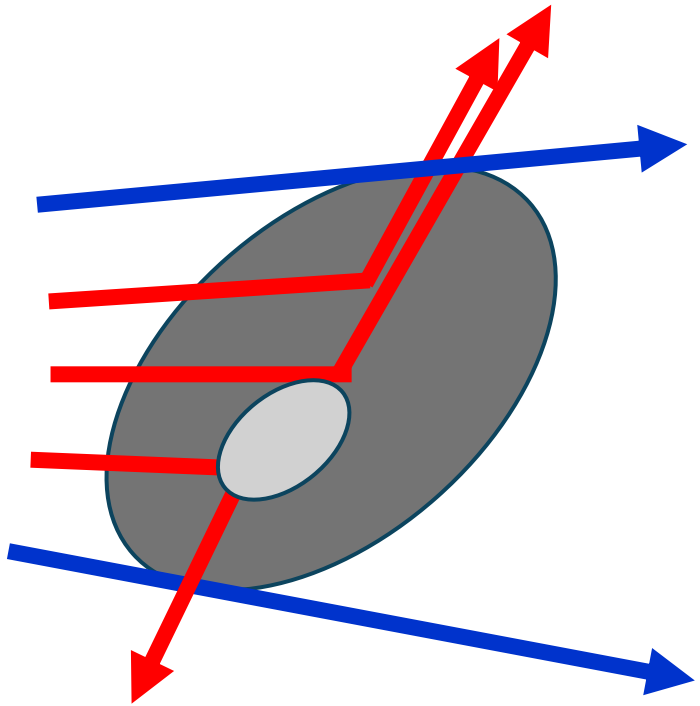


https://www.photonics.com/Articles/Photodetectors_in_Flow_Cytometers/a64058

SSC and FSC

Cell populations can be differentiated by:

- Cell size
- Cell internal complexity (granularity)
- Surface/intracellular Ag expression and fluorescence



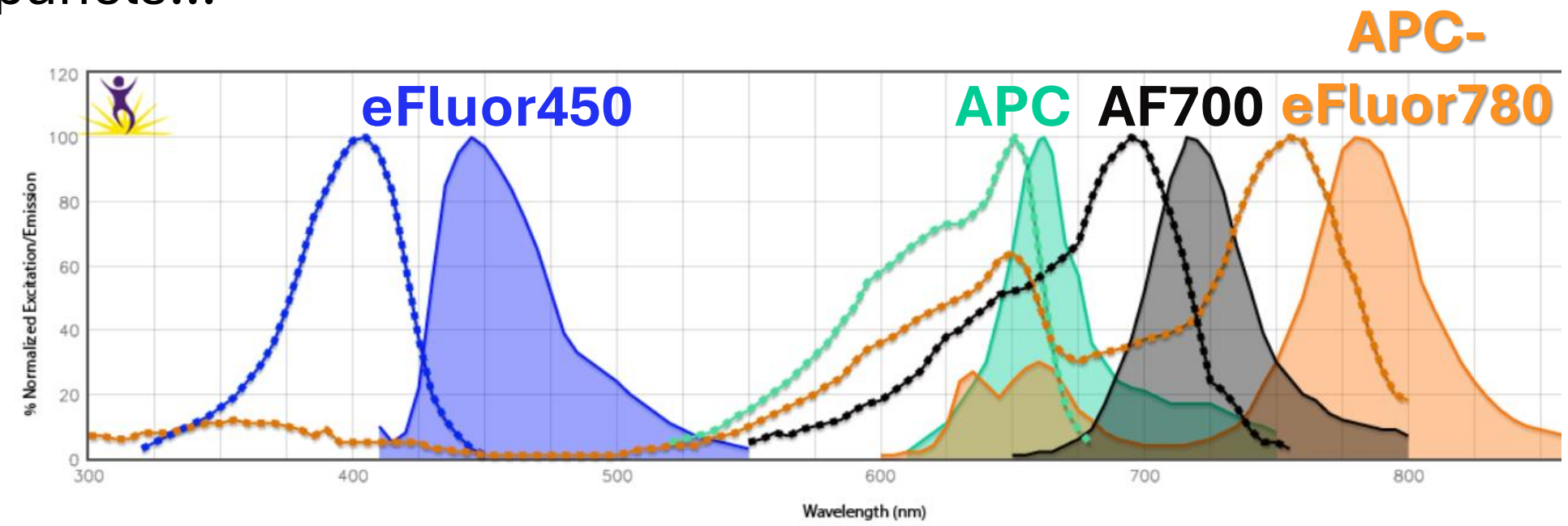
FSC (**F**orward **Sc**atter) – SIZE

SSC (**S**ide **Sc**atter) - GRANULARITY

Slide from Jessica Breznik for HTHSCI 4II3

Fluorophores

Up to 20 fluorophores may be incorporated into some flow cytometry staining panels…



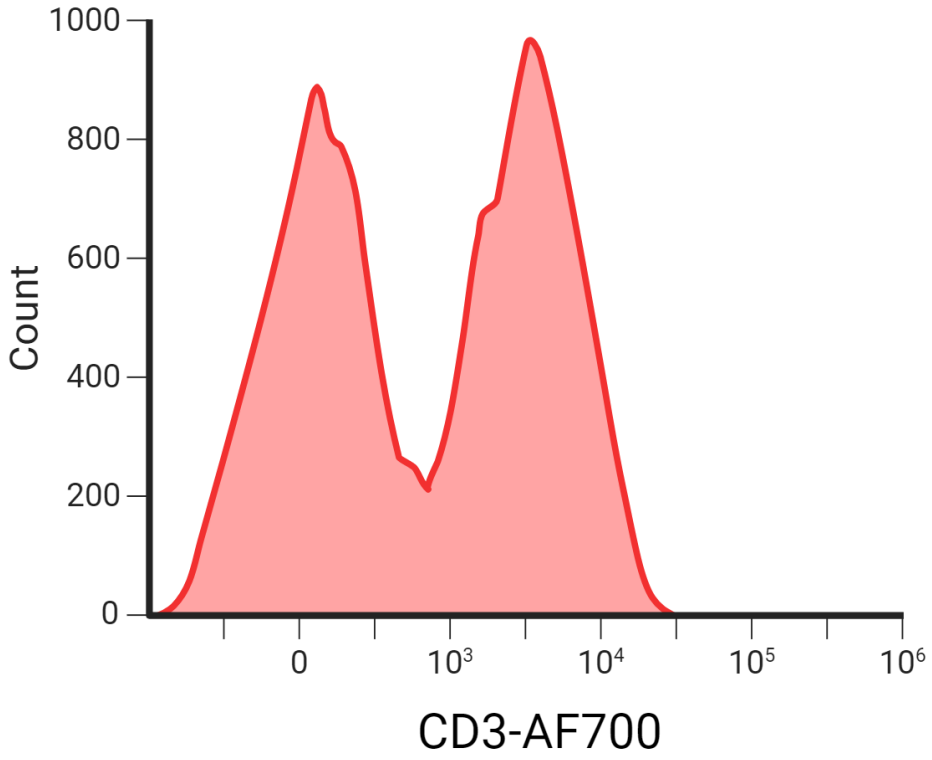
Some excitation/emission spectra overlap, but **emission spillover** can be corrected by *compensation*.

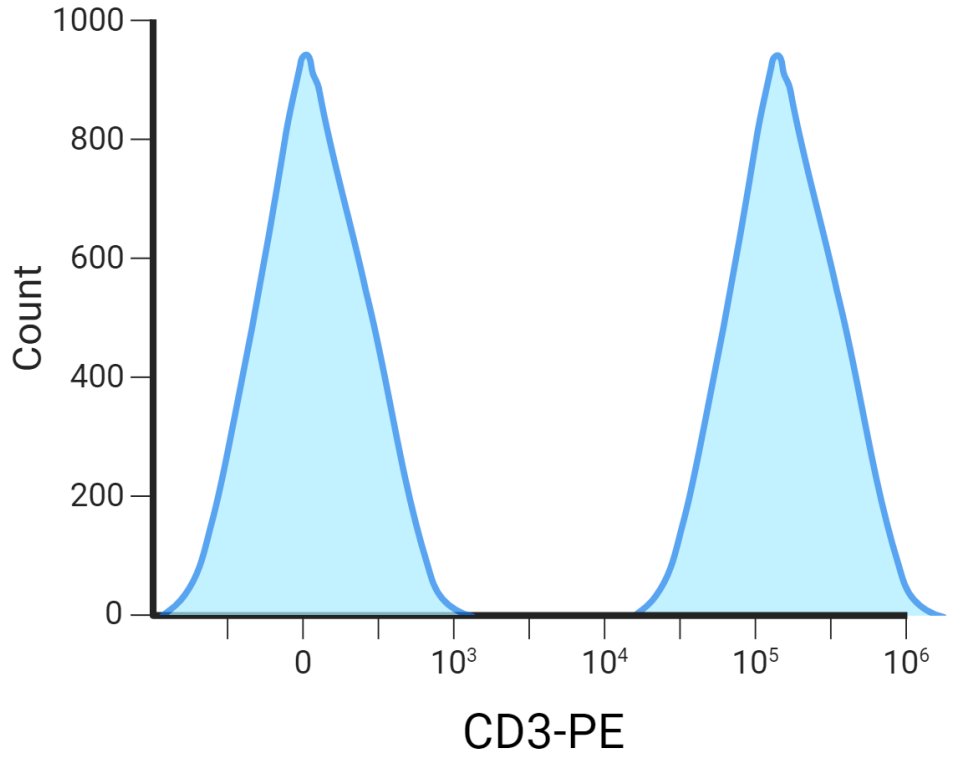
Slide adapted from Jessica Breznik for HTHSCI 4II3

Fluorophore Selection- Color side

Consider aspects intrinsic to fluorophore:

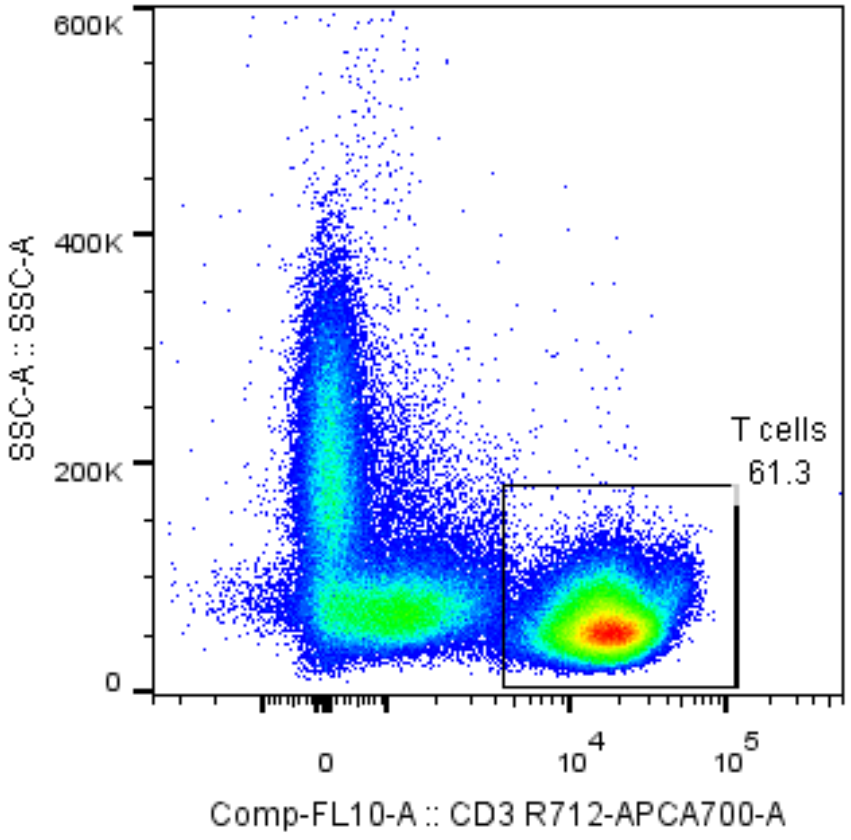
- Brightness- AF700 is dim. PE, APC, FITC are bright
- Separation – some colors, especially dim, may not separate as well
- Colors similar to one another in emission- requires careful compensation

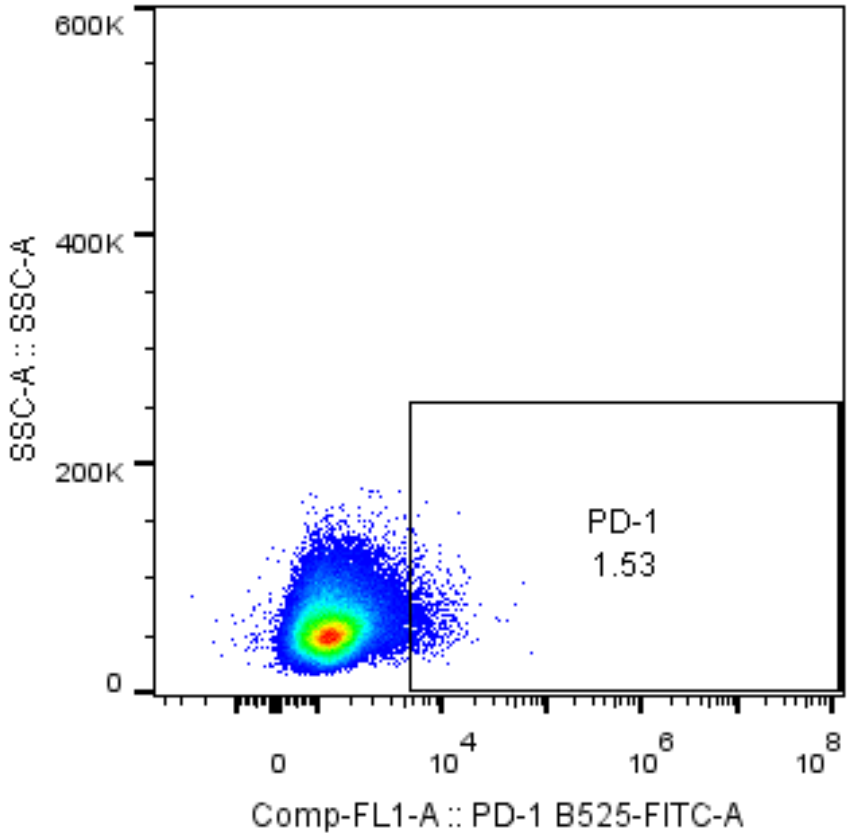


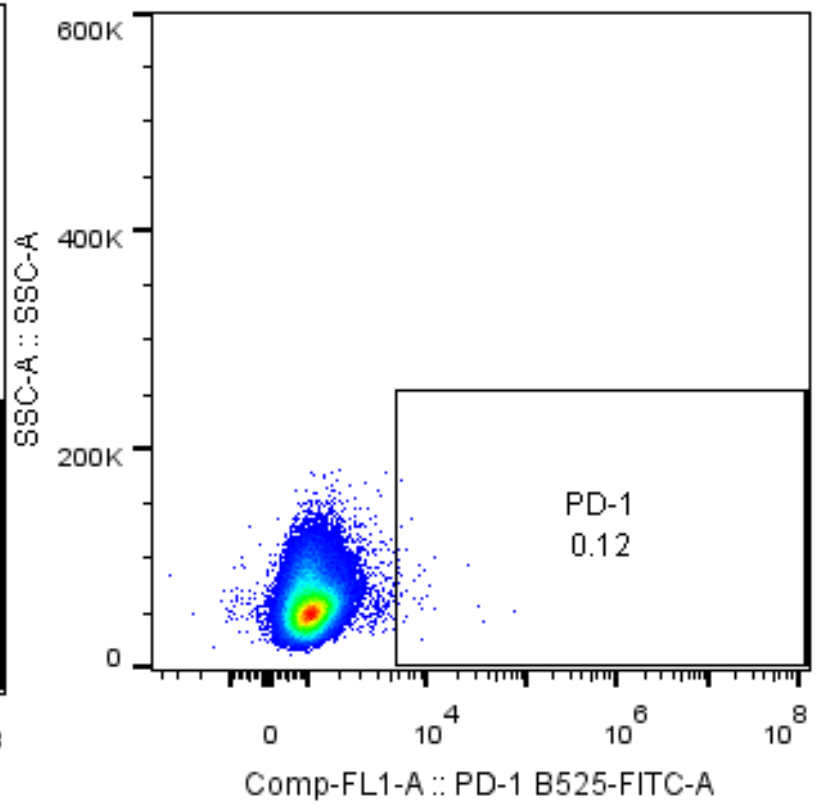


Fluorophore Selection- Cell side

- Clear positive and negative population?
- Many cells expressing the marker, or few cells expressing the marker?







Fluorophore Selection

Clear positive and negative populations, many cells expressing marker:

- Can use a dimmer fluorophore like AF700
- Think like CD3- a cell is either a T cell, or not a T cell, separates out well. There are generally a decent amount of T cells.

Positive and negative populations not well separated, or marker not commonly expressed:

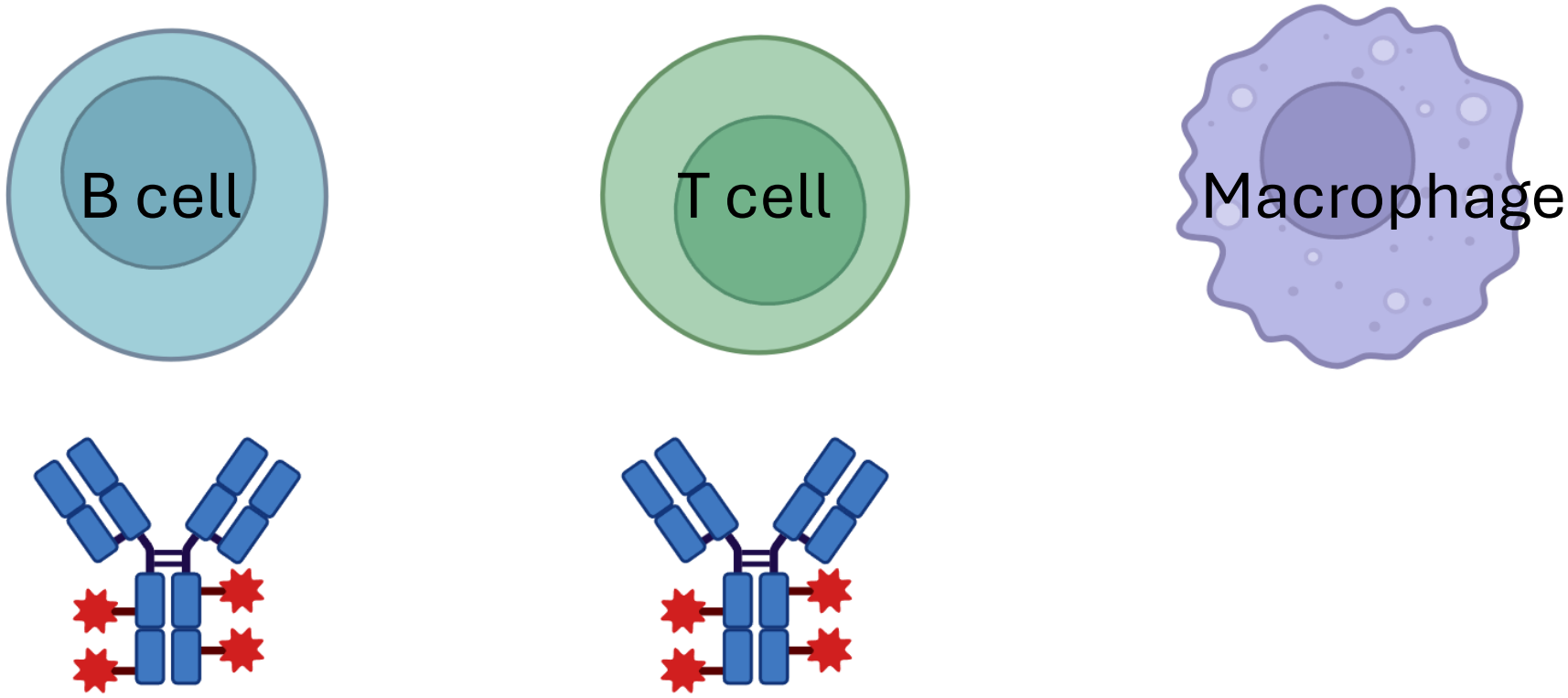
- Brighter fluorophore with better separation (APC, PE)

Consider colors to use together:

- If only need 5 colors and cytometer can use 16, pick colors “far apart” from one another in the spectrum to minimize overlap
- Pick good colors, avoid potential problem colors unless you later need them

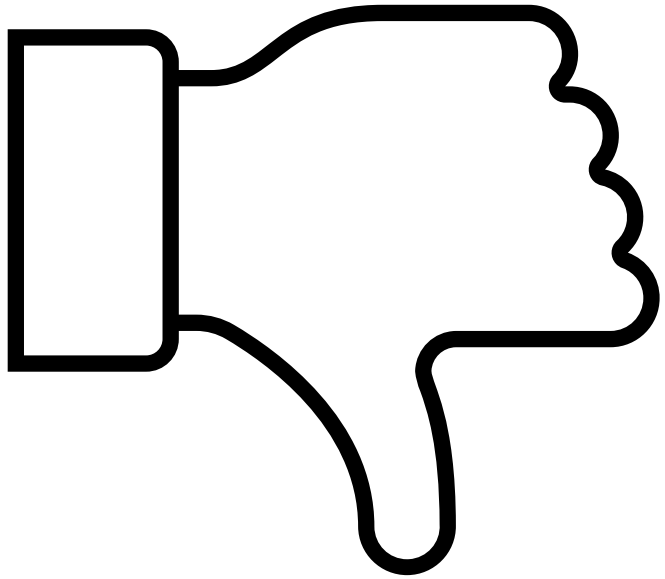
Dump Channel

- A way to “remove” cell types during gating to clean up later gates
- Multiple different antibodies, on the same color, because don’t care about the cell types they bind



Most important lesson

- PerCP is the devil
  - This applies to PerCP efluor780, PerCP Cy5, PerCP Cy5.5
  - PE-Cy5 and PE-Cy5.5 are walking on thin ice
- It interacts with everything, its dim even in its own channel
- Don’t use it unless you want to suffer



Fluorophores- Compensation

- Colors usually compensated using beads, which bind the antibodies
- Cannot use beads for viability dye unless using Thermo's ARC beads WITH thermos's dye
  - Biolegend zombie dyes do not bind thermos arc beads
  - Have to use cells to compensate viability dyes that don't have their own specific beads
  - Cannot use regular beads

- Beads typically bind mouse or rat isotype antibodies
  - If you have a weird isotype antibody like goat, it may not bind the versa comp beads. May need special comp beads

- Antibodies from Miltenyi may need to use Miltenyi comp beads, not VersaComp