Knowledge Assessment
Flow Cytometry Workshop, Part 2
April 27, 2015

Each question may have MULTIPLE correct answers. Select all that are correct.

1. **Compensation corrects for:**
    a. problems with your achilles heel/itchy feet
    b. fluorescence spillover
    c. high optical background from certain molecules
    d. overlapping spectral emission
    e. overlapping spectral excitation
    f. area under the curve of lost light

2. **True or False: Compensation subtracts the contaminating light from the primary detector**

3. **Which of the following are true for compensation:**
    a. You should match stained comp beads with unstained beads from the same lot
    b. You should match stained cells with unstained beads
    c. The comp signal must be as bright or brighter than signal detection on cells
    d. You only need to run single stained tubes for tandem dyes

4. **What is the purpose of clicking “H” (height) under the FSC parameter?**
    a. FSC-H allows you to run beads as compensation controls
    b. FSC-H in combination with FSC area allows you to discriminate doublets from singlets
    c. FSC-H allows you to compensate after acquiring
    d. FSC-H allows you to lift your cells for better compensation

5. **What is the reason(s) why partial or complete compensation at the time of collection (“on-line”) on a Calibur is more accurate than collecting samples uncompensated and then compensating in external software such as FlowJo?**
    a. The software compensation is not as accurate because the mathematical compensation algorithms differ between the external software and the Calibur, and the Calibur algorithm is more mathematically correct
    b. The data is analog and the log transformers on a Calibur may not be accurate across all 4 decades of the scale. Compensation at the time of

collection is performed on the non-transformed data, whereas compensation after collection is performed on the log-transformed data.

c. There is actually no difference between on-line and software compensation for Calibur data.
d. Data that is exported from the Calibur cannot be imported accurately into external software.

6. **Some of the differences between the Calibur and LSRII include:**
    a. The data from the Calibur is analog and the data from the LSRII is digital.
    b. The acquisition software for the Calibur is CellQuest and the acquisition software for the LSRII is DiVa.
    c. The Calibur uses a flow cell but the LSRII uses a nozzle and stream in air
    d. Data from the Calibur can only be analyzed in CellQuest, but data from the LSRII can be analyzed in any FACS analysis software.

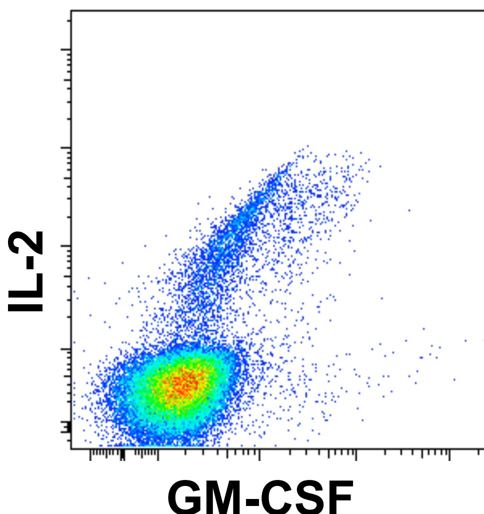
7. **True/false: Compensation controls should always be as bright or brighter than the fluorochrome-matched antibody staining of your sample.**

8. **True/false: It is acceptable to use the CD8 PE-Cy7 Ab to compensate the IFNg PE-Cy7 in your panel.**

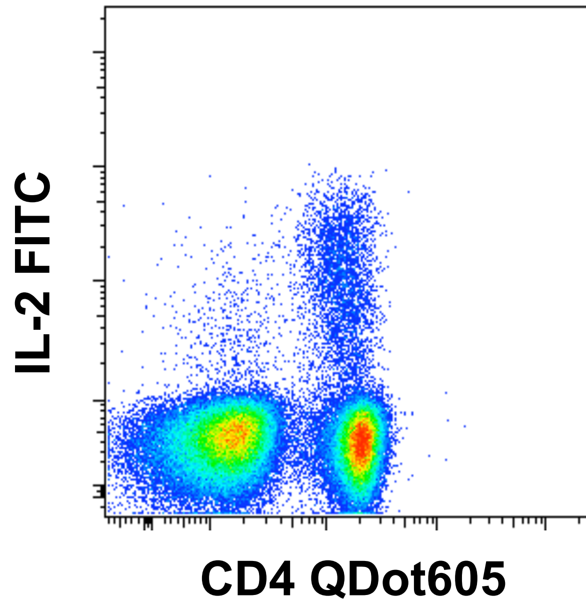
9. **True/false: It is acceptable to use the CD8-FITC Ab to compensate the IFNg-FITC in your panel.**

Indicate whether the fluorophores shown in the following samples are correctly compensated by circling your selection.

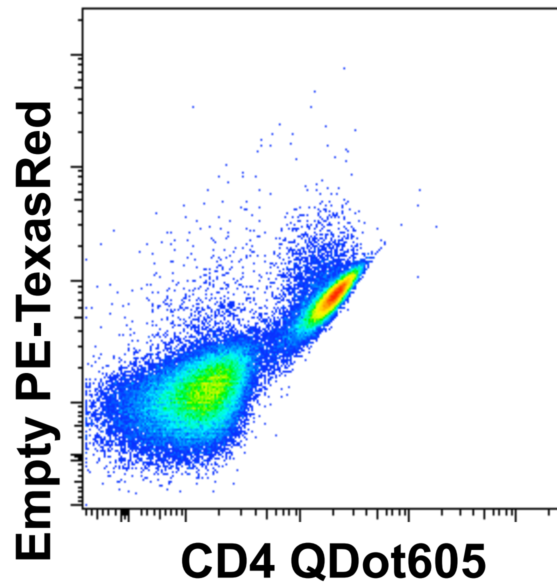
10. **Correct Incorrect Cannot be determined with the data shown**



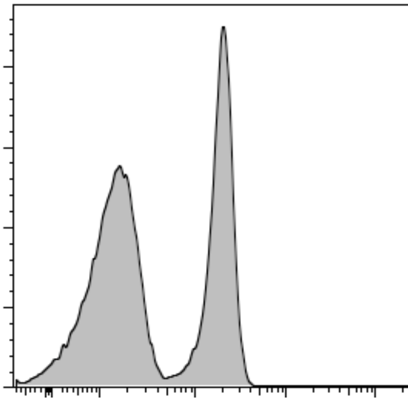
11. Correct   Incorrect   Cannot be determined with the data shown



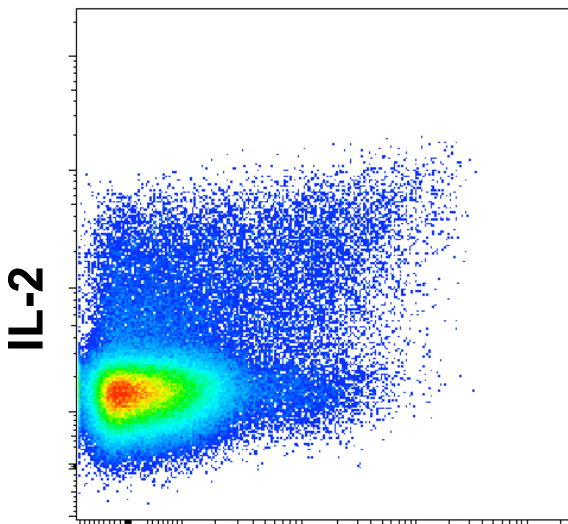
12. Correct   Incorrect   Cannot be determined with the data shown



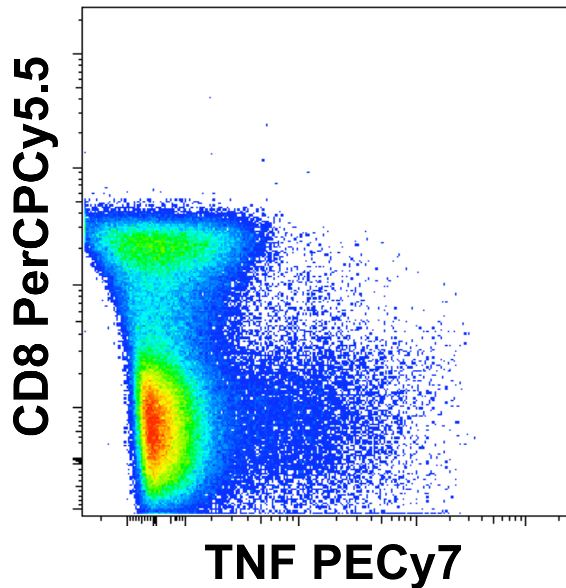
13. Correct   Incorrect   Cannot be determined with the data shown



14. Correct   Incorrect   Cannot be determined with the data shown



15. Correct Incorrect Cannot be determined with the data shown



16. True/false: A log scale allows you to display negative values

17. What is true regarding fluorescence minus one controls:

a. These are useful for optimizing a new antibody panel
b. Determine the proper position for gating positive events
c. Gates may be placed higher than that determined by the FMO
d. Gates may be placed lower than that determined by the FMO
e. FMO controls are typically only performed during panel development/optimization, but can be included in every experiment for selected markers

18. While running your FMO control you see that CD3 Qdot655 has huge spillover into the PE-Cy5 channel. What steps can you take to solve this?

a. Cry
b. Choose an antigen that is expressed at lower level as the Qdot655 reagent
c. Drop Qdot655 from the panel
d. Drop PE-Cy5 from the panel
e. Re-gate the FlowJo plot
f. Drop the PMT voltage in the PE-Cy5 channel

19. **When data are properly compensated:**

    a. The median fluorescence intensity (MFI) of the positive and negative cells (or beads) in the compensation sample are equivalent in the primary channel (the channel for that comp sample)
    b. The MFI of the positive and negative cells or beads in the compensation sample are equivalent in the secondary channels
    c. The MFI of the negative cells (or beads) is zero in all channels
    d. The stain index is equivalent in all channels

20. **Which of the following are correct for performing proper compensation:**

    a. Using comp beads and cells in the same experiment for compensation, as long as you run unstained beads and unstained cells for each
    b. For amine-binding viability dyes, cells or amine beads can be used for compensation
    c. For compensation of CFSE, FITC-stained comp beads can be used
    d. Only one negative bead control is required if monoclonal antibodies are of rat or mouse origin, but it must be the same lot
    e. Fluorescent monoclonal antibodies bind to comp beads by capturing photoelectrons
    f. When compensating a tandem dye such as PE-Cy5.5, you need to set up single tubes for both PE and Cy5.5

21. **True/false: Compensation does not introduce error, but only reveals the measurement error.**

22. **Which of the following is true of bi-exponential transformation of flow data:**

    a. It affects the visualization of the data only but not the data itself
    b. It allows visualization of negative values
    c. It helps in determining whether the data are properly compensated
    d. It changes the entire axis scale from log to linear
    e. All of the above

23. **The fluorescence minus one control (FMO) is useful for:**

    a. Determining where to place the gate to identify positive cells
    b. Determining the amount of non-specific binding of a staining reagent
    c. Determining whether a new staining panel will work well
    d. Optimizing the target MFI settings on your instrument

24. **Cross-laser compensation is only a problem when:**

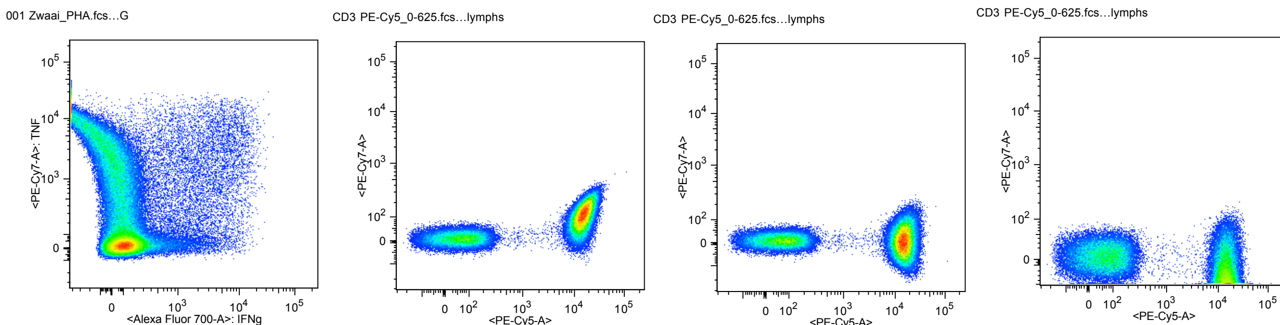
    a. Fluorochromes detected off different lasers have similar emission spectra but each is excited by only one laser
    b. A fluorochrome is excited by more than one laser and there are bandpass filters in the detectors from the different lasers that overlap

c. This problem is limited to Qdots; other fluorochromes never have this problem
d. An example is Qdot655 detected in the APC channel

25. Related to the issue of "spreading" in the data following compensation:

a. This can be corrected by increasing the compensation percentage
b. This is due to error in the measurement and has contributions from errors in all the channels that have spillover into the channel of interest
c. Any gate for positive events must be placed above the upper level of the spreading
d. This phenomenon only occurs between fluorochromes detected from different lasers and not between those detected from the same laser

26. Indicate which of the plots below are correctly compensated.



Intracellular cytokine staining (ICS) is a commonly-used method for measuring and characterizing antigen-specific T cells. Positivity testing is often useful. Antigen-stimulated response is often compared against the response without stimulation (the negative control). Positivity methods include:

- Use of a fold increase of the stimulated over the unstimulated (typically 3-fold or greater).
- Fisher's exact test comparing event counts of cells gated as cytokine positive or negative in the stimulated and unstimulated samples.
- Adjustment for multiple testing for multiple different antigens used for stimulation.
- In addition to the requirement for a fold increase, inclusion of a minimum threshold (as a means to reduce false positivity).
- MIMOSA also compares stimulated with unstimulated but uses a Bayesian approach.

27. True/false: Because the Fisher's method uses a p-value threshold, the sensitivity to detect a positive response is not affected strongly when small numbers of cells are collected for a sample.

28. True/false: As a Bayesian approach that borrows information across samples, a minimum sample size is needed to use this approach.