Knowledge Assessment
Flow Cytometry Workshop, Part 1
April 20, 2015

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

1. **Tandem dyes are**
    a. highly stable fluorophores after fixation
    b. two covalently-linked fluorophores
    c. do not need to be compensated because there is no spectral overlap between donor and acceptor dye
    d. can dissociate due to heat, light or chemical treatment

2. **Tandem dyes can be sensitive to**
    a) Light
    b) High flow rate
    c) Temperature
    d) Fixation
    e) Plastic or glass tubes
    f) Vigorous vortexing

3. **A bandpass filter of 585/42 will allow light of the following wavelengths to pass through**
    a) 564
    b) 543
    c) 220
    d) 600
    e) 627
    f) 1007
    g) 300

4. **Excitation light, compared to emitted light, is**
    a. lower energy and shorter wavelength
    b. higher energy and longer wavelength
    c. lower energy and longer wavelength
    d. higher energy and shorter wavelength

5. **What is the FIRST consideration for assembling a multicolor panel:**
    a. Fluorophores
    b. Lasers and filter set of the instrument
    c. Clones of the antibody
    d. Titer of the reagents

6. **Titer of antibody should be expressed as:**
    a. Amount to use per number of cells (eg 5 ul per million cells)
    b. Amount to use per volume staining (eg 5 ul per 200 ul staining)
    c. Amount to use per test (eg 5 ul/test)
    d. MFI per cell

7. **The same antibody reagent may have different titers depending on the:**
    a. Staining protocol (intracellular or surface)
    b. Staining temperature
    c. Cell type or species (e.g., macaque)
    d. Fresh or frozen samples

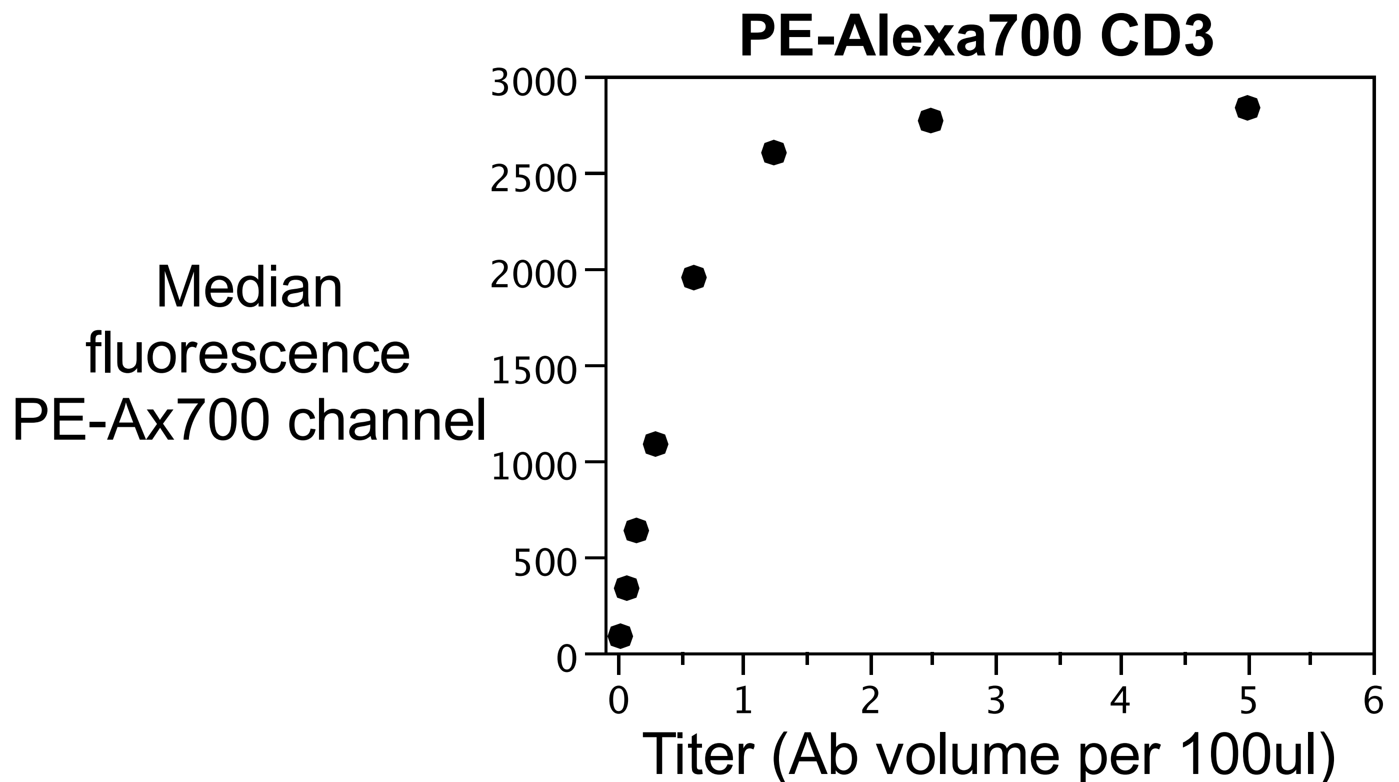
8. **If you were to create a common database of antibody staining reagents for your lab, which of the following should be included:**
    a. Antibody Specificity
    b. Antibody Clone
    c. Saturation Titer
    d. Lot Number
    e. The CV of the negative population

9. **Which of the following can be part of the titer procedure:**
    a. Titer multiple reagents at the same time on the same cells.
    b. Test 1 to 2 dilutions
    c. Titer single reagents on beads
    d. None of the above

10. **True/false: Saturation titer is the lowest titer that gives near maximal fluorescence.**

11. **What is the FIRST step in instrument set-up and standardization:**
    a. Set PMT voltages
    b. Ensure instrument is aligned properly
    c. Set the compensation percentages
    d. Run each of your singly-stained compensation control samples

12. **Where is the saturation titer on this graph (circle the data point):**



13. **Which of the following panels of fluorochromes would not work on a FACS Calibur?**
    a. FITC, PE, APC, PerCP
    b. Alexa 488, PE, PerCP-Cy5.5
    c. Pacific Blue, PE, PE-Cy5.5, APC
    d. FITC, PE, PE-Cy5

14. **In a Beckton Dickinson LSR instrument (or ARIA or Fortessa), excited photons are transmitted to the collection octagon by what medium?**
    a. Air
    b. Sheath fluid
    c. Fiber optic cables
    d. Band pass filters
    e. Long Pass Filters

15. **What parameter is the most commonly used to trigger signals (threshold) in the flow cytometer?**
    a. Forward scatter
    b. 90 degree scatter
    c. PE detector area
    d. APC detector height

16. **What is the name of the detector used to convert photons to photoelectrons?**
    a. Forward scatter
    b. PMT – (photomultiplier tube)
    c. Cascade tube
    d. DAQ board

17. **True/false: BD CS&T beads perform instrument standardization by setting laser delays and PMT voltages and also check for instrument alignment.**

18. **True/false: Single peak "rainbow" beads can be used as an alternative to BD CS&T beads to standardize the instrument.**

19. **The "gain" setting of a PMT is the same as which of the following?**
    a. Area
    b. Height
    c. Voltage
    d. Pulse width

20. **Which of the following are true of voltage settings:**
    a. Always adjust the voltages after running comp beads because of daily variation
    b. FSC and SSC voltages can be changed within an experiment
    c. If signals drop during acquisition, increase voltages to standardize MFI
    d. After doing your QC, the machine is stable for at least 48 hours so no need to further standardize
    e. None of the above

21. **True or False:**
    a. You have to prime between acquiring each sample tube
    b. High, Medium and Low fluidics settings will change laser strength and therefore brightness
    c. It is not recommended to leave the cytometer on 'Run' while not acquiring, as this will waste sheath fluid

22. **What is the angle of the longpass filters in a typical BD collection octagon?**
    a. 11.25°C
    b. 11.25°
    c. 90%
    d. 90°

23. **What is true of the *threshold*?**
    a. It is an electronic gate
    b. Often needs to be changed between cells and beads due to fluorescence differences
    c. Without setting it correctly you may acquire few of your cells of interest and lots of debris
    d. Events below the threshold are counted and recorded

24. **What is the purpose of longpass filters/dichroic mirrors?**
    a. To allow light above particular wavelengths to pass into the PMTs
    b. To direct laser light to the flow cell
    c. To reflect shorter wavelengths of light
    d. To allow light within a certain defined range of wavelengths through

25. **True/false: Hydrodynamic focusing occurs within the flow cell. This is affected by the flow rate, higher flow rates are associated with narrower focus and lower CV.**

26. **Monitoring of your instrument is critical. Typically a standard bead particle(s) is run on the instrument <u>each day or before each experiment</u>. For each channel of interest, it is important to monitor which of the following (choose one or more):**
    a. Robust CV
    b. MFI
    c. Linearity
    d. PMT voltage

27. What is true regarding laser delays:
    a. Excitation of dyes on the cell of interest is separated in time due to the laser delay.
    b. If a dye is excited by only one laser, but its emission overlaps the emission of another dye excited solely by a different laser, then there is no compensation requirement between those dyes because of the laser delay.
    c. The laser delay is the reason cross-laser compensation is required.
    d. The laser delay settings are adjusted each time BD CS&T beads are run.

28. **Number the following lasers in order from lowest to highest wavelengths**
    a. UV ______
    b. Violet ______
    c. Blue ______
    d. Red ______
    e. Green______

29. Assign the following fluorochromes to their appropriate excitation laser on the FACScalibur

FITC, PE, PerCP, APC, Pacific Blue, Qdot605, Alexa647, Alexa488, PE-Cy5, PE-Cy7

Blue	Red	Not usable on the FACScalibur

Fluorescent dyes are commonly used to label monoclonal antibodies specific for cell antigens of interest. Information about both the excitation and emission spectra of these dyes are needed to determine whether a particular dye can be used on a particular flow cytometer instrument.

30. True/false: The emission spectrum determines whether that dye is excited by one or more of the lasers in the instrument.

31. True/false: The excitation spectrum determines whether there is a detector in the instrument that will detect the appropriate wavelength of light from that dye.

32. True/false: Cross-laser compensation may be required when a dye is excited by more than one laser.