Flow Cytometry Controls

- unstained cells
- compensation controls
- Fluorescence minus one (FMO) controls
- isotype controls
Unstained Cells

- Unstained cells are used to assess cell autofluorescence.
- Unstained cells should not be used to set the optimal PMT voltage.
  - Adjusting the PMT voltage to place highly autofluorescent cells such as cell lines in the first decade may result in a loss of resolution of dimly positive cells.
  - Set PMT voltages within the linear range of the PMT, with bright populations completely on scale, and the voltage balanced in relationship to other PMT voltages to allow for compensation.

![Graphs showing autofluorescence at different PMT voltages (400, 450, 500 Volts)]
Compensation Controls

Compensation controls are used to correct for spectral overlap of fluorochromes.

Properties of Good Compensation Controls:
- The cells should include negatives & positives with matching autofluorescence levels.
- Use the same fluorochromes as you use for the test samples.
- Include a separate control for each tandem dye.
- The positive cells should be as bright or brighter than the test samples.
Question:
What’s best to use for compensation, beads or cells?

Answer:
Beads if you can, because they are.....
• bright
• auto-fluorescence matches between negative & positive
• not rare events
### FMO controls

#### WHAT is a “Fluorescence Minus One” control?
- A control that includes all the assay colors except one

#### WHY do I need a FMO control?
- Properly compensated data frequently spreads into the other colors
- In multicolor flow the addition of this background from the other colors is usually greater than the contribution from the color of interest

#### WHEN do I need a FMO control?
- where separation of negative and positive is crucial and not already easily identified
Isotype controls

An isotype control is a fluorochrome tagged antibody of the same Ig isotype as the target antibody, but with no specificity for the target.

Isotype controls are NOT negative controls
- They are staining controls
- They are meant to rule out non-specific or specific (FC receptor) binding
- They must be matched for species, isotype, protein:fluorochrome ratio, and manufacturer
Identifying CD4 cells with 4 colors

PBMC were stained as shown in a 4-color experiment. Compensation was properly set for all spillovers.

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<th>FITC</th>
<th>PE</th>
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<tbody>
<tr>
<td>Unstained Control</td>
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<tr>
<td>FMO Control</td>
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<td>CD8</td>
<td>CD4</td>
<td>CD45RO</td>
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<tr>
<td>Fully Stained</td>
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