Technical Overview

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Quick Tips and Tricks for Flow Cytometry

Flow cytometry doesn't have to be complicated. These tips and tricks will help you eliminate many of the causes of flow frustration.

Tip 1: Know your controls

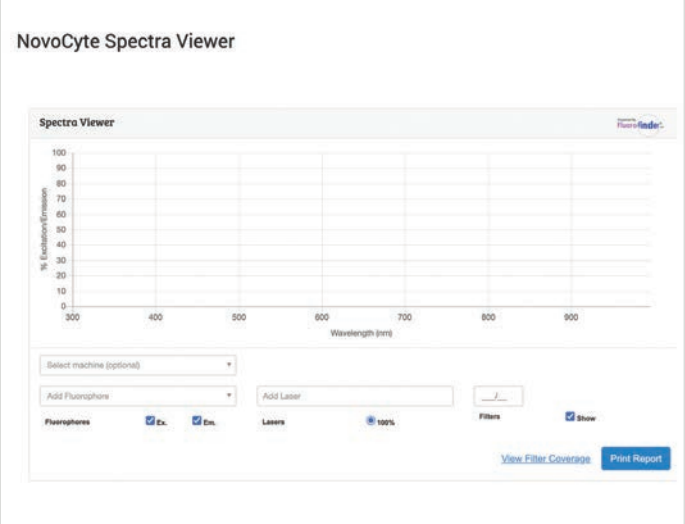
- Single-stain controls assess spillover between channels. There must be a single-stain control for every fluorescence parameter in your experiment.
  - Stain each single-stain control with the same dye or fluorochrome-conjugated antibody that is used to stain your experimental sample.
  - Compensation controls should ideally contain both negative and positive events in the same sample.
  - If each sample does not have an internal negative control, you can use a universal negative control. An example would be purified T cell lymphocytes using a single-stained control with anti-CD3-FITC antibody. Here, a universal negative control must be run (or compensation beads used), because there are not enough CD3-negative cells in the population.
  - When you create the single-stain control for a live/dead marker, there should be enough dead cells to ensure a sufficient number of positive events for spillover calculations.
- Fluorochrome-conjugated isotype controls assess nonspecific binding of the fluorochrome-conjugated antibody. Do not use isotype controls as gating controls, because they do not consider spillover from other channels.
- Fluorescence-minus-one (FMO) controls contain all fluorophores in the panel minus one marker, allowing you to properly gate populations of interest.

Tip 2: Brighter is better

- Single-stain controls should be as bright as, or brighter than, any experimental sample to which the compensation will be applied.
- For poorly expressed markers, such as certain intracellular cytokines, use compensation beads—rather than cells—for single-stain controls. Do not use compensation beads when your samples are auto fluorescent, or when you are using fluorescent dyes.

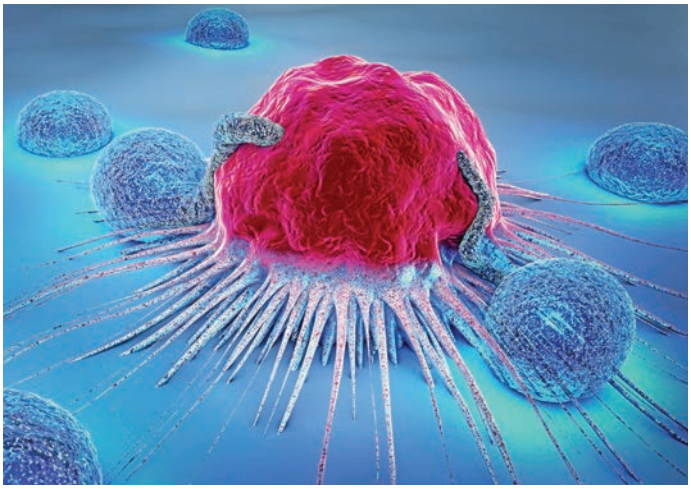
Tip 3: Find the perfect fluorochromes

- Minimize compensation by separating your fluorochromes as much as possible in the emission spectrum.
- To make designing your flow cytometry panel easier, use a spectral analyzer, such as the Agilent NovoCyte Spectra Viewer.



Tip 4: Antigen density matters

- The number of target molecules expressed on a cell affects the stain.
- To increase resolution, pair bright fluorophores with low antigen-density targets.
- To limit spillover, pair dim fluorophores with high antigen-density targets.



Tip 5: Use tandem dies properly

- Always run compensation controls using the same fluorophore for each experiment.
- Unlike single dyes, tandem dyes, such as PE-Cy5, APC-Cy7, and Brilliant Violet-650, are composed of two covalently attached fluorescent molecules. Tandem dyes are susceptible to degradation by exposure to light, so use strategies to minimize the potential negative impacts on your final data quality.
- Run a single-stain control for every lot of tandem dye used for experimental samples. Tandem dyes can have lot-to-lot differences, which can be exacerbated after the tubes are opened or exposed to light.

Tip 6: Take advantage of viability dyes

- Viability dyes eliminate dead cells, which can contaminate downstream gating and skew your results. Many viability dyes are available to suit any panel.

Tip 7: Treat samples and compensation controls equally

- Exposure to light and buffers during sample preparation may affect fluorescence. For this reason, you should treat single-stain controls, isotype controls, and FMO controls the same way you treat experimental samples.
    - For example, overnight staining/fixation for samples should also include overnight staining/fixation for compensation controls.

To learn more about reducing your flow frustration, visit www.agilent.com/chem/novocyte

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