Multicolor flow cytometry panel design

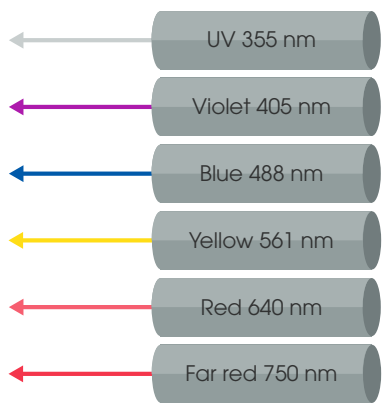
abcam

Our guide to help you build successful multi-color flow cytometry panels

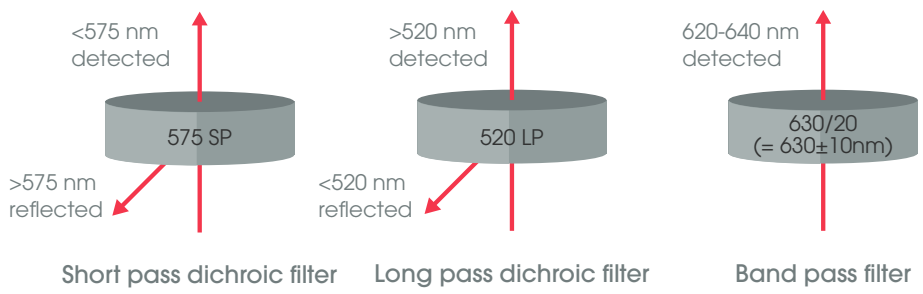
1. Know your flow cytometer

Lasers: only fluorochromes excited by the corresponding wavelength of light from the laser can be used

To ensure optimal detection, make sure you understand the combination of lasers /filters on your machine. Refer to your instrument's manual or speak to your core facility



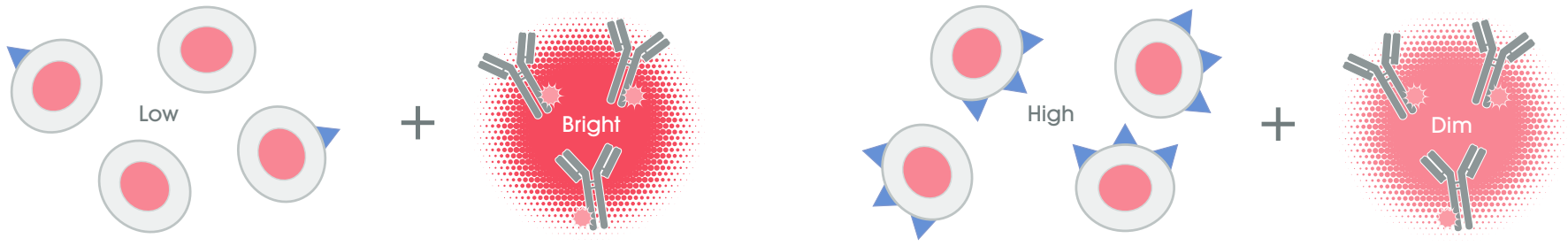
Filters: detection of light emitted from fluorochromes is controlled by filters



2. Know your cell population, antigens, and fluorochromes

Low/unknown antigen expression and/or low cell populations
= use brighter fluorochromes, eg PE

High antigen expression and/or high cell populations
= use dimmer fluorochromes, eg PerCP

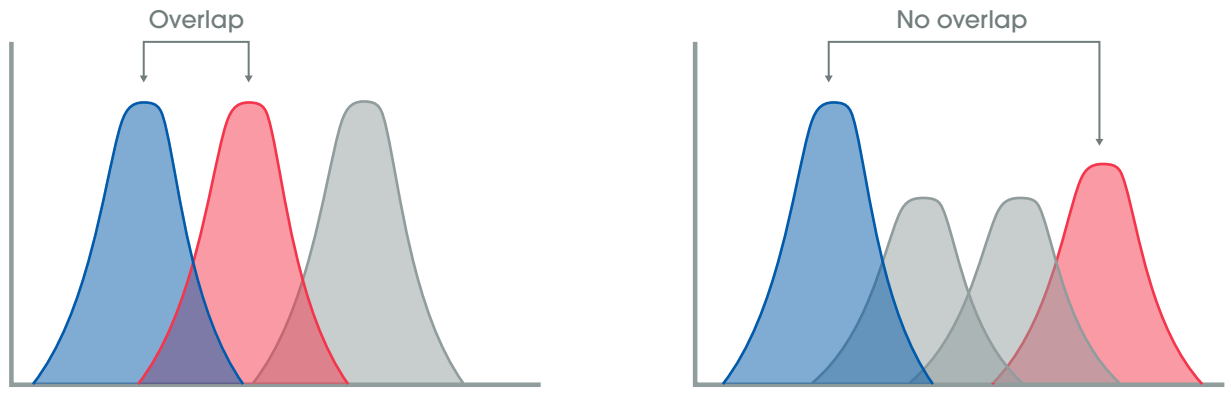


Check the relative brightness of your fluorochromes at **www.abcam.com/fluorochrome-chart**

3. Minimize spectral overlap

Minimize emission spectra overlap

- Sacrifice bright fluorochromes to avoid overlap
- Compensation can be used to control the effects of spectral overlap



4. Include controls

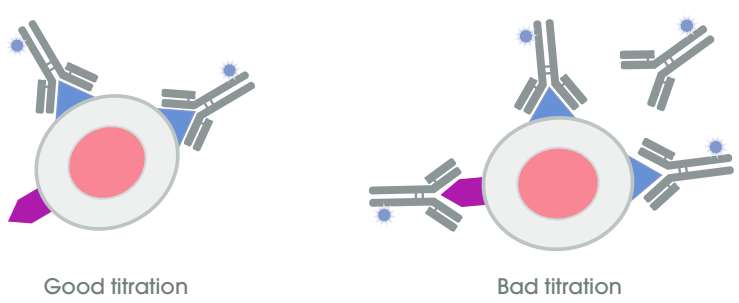
Unstained cells for defining negative populations, cell size, and granularity

Live/dead markers to isolate healthy cells

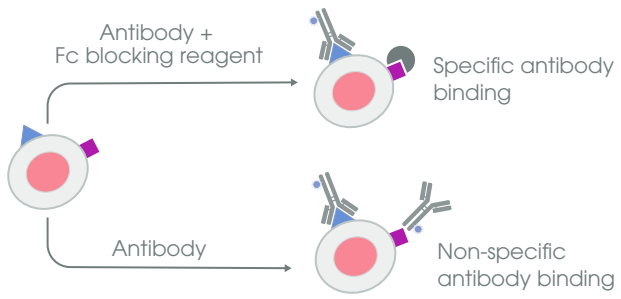
Single-stained positive controls for setting compensation

Fluorescence minus one staining to define positive populations

5. Optimize your staining protocol



Antibody concentration: titrate your antibodies to avoid non-specific binding or reduced sensitivity



Fc blocking reagents:
Human IgG for human
Anti-CD16+ CD32 for mouse

Fc blocking: use Fc blocking reagents in cells with high content of Fc receptors (eg phagocytic cells) to avoid non-specific binding