



# Flow Cytometry Panel Design Journey

We're with you every step of the way

## Waterfall of Discovery

There are hundreds of research applications for flow cytometry.

### Viability Volcano

Beware of hitchhiking dead cells because they can skew your data. Be sure to identify them properly with a viability dye.



## Define your experimental hypothesis

**Step 1:** Defining your experimental hypothesis is the first step in panel design. Start with identifying:

- The biological information you are trying to achieve
- The population(s) of cells you wish to interrogate
- Whether targets are found on the cell surface or intracellularly

### Did you know?

BD launched the world's 1<sup>st</sup> commercially available flow cytometer in 1974 - kicking off a legacy of research innovation.

## Know your flow cytometer

**Step 3:** Knowing your instrument is essential.

Understanding your instrument's configuration will let you know how many markers and which fluorochromes your instrument can detect.

Elements to consider include:

- Laser wavelength for excitation
- Number of detectors off each laser
- Filters available to detect the fluorochromes



**Did you know?** BD offers over 40 fluorochromes and more than 1,500 specificities to provide researchers flexibility in panel design.

## Marker selection

**Step 2:** During the second step of the panel design process, you will need to identify which and how many markers you need to identify the population of interest.

Pay attention to:

- Marker expression levels
  - Primary antigen: Expressed at high density, often defining lineages
  - Secondary antigen: Often expressed over a continuum
  - Tertiary antigen: Critical markers expressed at low density
- Marker coexpression, especially of dim markers
- The gating strategy needed to identify the population(s) of cells you wish to interrogate



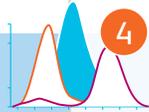
### Lake of Permeabilization

For smooth sailing, select the appropriate buffer system for your antibodies.

### Did you know?

Since the introduction of revolutionary Sirigen polymer dye technology, BD has launched 17 innovative dyes including BD Horizon Brilliant™ UV and BD Horizon Brilliant Violet™ Dyes.

**TIP:** For help choosing fluorochromes, check out our BD Spectrum Viewer: [bdbiosciences.com/spectrum-viewer](http://bdbiosciences.com/spectrum-viewer)



## Fluorochrome assignment

**Step 4:** Carefully select fluorochromes to resolve markers at all expression levels and minimize spectral overlap. Consider using tools like a fluorochrome resolution ranking and a spectrum viewer to help assess:

- Cross laser excitation
- Fluorochrome spillover

Remember to pair bright fluorochromes with low expressing antigens and dim fluorochromes with high expressors. Keep in mind that spread only impacts the resolution of coexpressed markers.

## Review panel

**Step 5:** Review your panel design and begin ordering your reagents.

Remember to titrate your mass size reagents and optimize your staining protocol. Include proper controls for compensation, FMO and biological controls to help ensure optimal panel performance.



Ready to begin workflow

## Check out our online flow cytometry panel design educational resources



[bdbiosciences.com/panel-design](http://bdbiosciences.com/panel-design)

### Spectral Mountain Passage

Map out the peaks and valleys of the dyes' spectra to minimize spillover and spread.

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