



# BD<sup>®</sup> Spectrum Viewer User Guide and FAQs

The BD<sup>®</sup> Spectrum Viewer is an interactive, online tool to support flow cytometry fluorochrome comparison and selection. The tool enables you to:

- Analyze and compare excitation (Ex) and emission (Em) spectra of a broad library of dyes
- Detect spillover and optimize fluorochrome selection for your instrument configuration/filters setup
- Toggle between **Combined Ex/Em Graph** view or **Stacked Ex/Em Graphs** view for easier visibility between spectra
- Visualize and analyze emission profiles on any standard flow cytometer or use the BD FACSymphony™ A5 SE Spectral Cell Analyzer and access similarity and complexity indexes
- Visualize peak intensities of spectral signatures in a unique heatmap, which is especially helpful when looking at large fluorochrome combinations
- Save, export and/or share your panel

# User Guide

## Standard configuration pathway

### Instrument selection

- Select from a list of preconfigured BD instruments using the drop-down list available in the **Instrument Configuration** tab under the graph.
- Alternatively, build a unique configuration by clicking the blue **Add Laser(s) & Filters(s)** button.
- Save unique instrument configurations by clicking the **Save Configuration** button. You must be signed into your BD Biosciences account.

TIP: You can start with a standard configuration as a template, then adjust/add/delete detectors, lasers and filters in the table. Use the **Clear** button to reset.

TIP: Fluorochromes can be visualized without choosing an instrument configuration or by a specific laser through the **Add Laser(s) & Filters(s)** button from the **Instrument Configuration** tab. To start adding fluorochromes, see the **Fluorochrome selection** section below.

### Fluorochrome selection

- Fluorochromes can be added by name, laser, range of excitation or range of emission. Select multiple fluorochromes from one set of search results, or add additional fluorochromes by continuing to search and clicking **Add**.
- After a fluorochrome is selected, corresponding emission and excitation curves will be displayed. Deselect **Ex** or **Em** in the fluorochrome list to modify the view. Hover over an excitation or emission curve to highlight the individual spectrum.
- Save fluorochrome combinations by clicking the **Save Combination** button. You must be signed into your BD Biosciences account.
- Check back often as new fluorochromes will be added periodically.

TIP: Click on the information icon (ⓘ) in the fluorochrome list to view basic fluorochrome information and access our catalog.

### Visualization and manipulation

- Visualize fluorochromes in a single-laser stack by selecting **Stacked Ex/Em Graphs** in the drop-down menu above the graph. Default setting is normalized by laser.
- Use the scrollbar on the right to view any additional laser lines. Toggle back to **Combined Ex/Em Graph** using the same drop-down menu.
- To zoom in to a particular area on the graph, use your cursor (+) to click and drag over the area of interest. Click **Reset Zoom** above the graph to return to the default view.
- Additional selections can be made under **Graph Display Options** in the hamburger menu (≡).
  - Enable Mouseover Details
  - Show Background Grid
  - Normalize Emission to Laser Excitation
  - Show Fluorochrome Labels (hiding labels allows labels to be shown on hover)
  - Resolve Filter Overlap
- Hover your cursor over an emission profile to see fluorochrome label information.

TIP: You can export an image of the graph(s) by clicking on the hamburger menu (≡), expanding the **Export and Import** section and clicking **Export** next to **Graph**. To learn more about our export and import features, see the **Save and share** section below.

### Save and share

- Save and share spectral graph images, fluorochrome combinations and customized instrument configurations.

- Without logging in, you can export and import fluorochrome lists and customized instrument configurations as .csv files. These can be saved or shared locally and imported later using the **Import** option. Spectral graph images can be downloaded as a variety of file types that can be saved locally.
- If you are logged in to your BD Biosciences account, you can save fluorochrome lists and instrument configurations as user-specific files within your account. These can be loaded from the **Export and Import** section of the hamburger menu (☰).

## Spectral configuration pathway

### Instrument selection

- For spectral flow cytometry experiments using the BD FACSymphony™ A5 SE Flow Cytometer, select this instrument from the instrument drop-down list. Spectral instruments are not configurable, so customization is not possible, and lasers, filters and other options are greyed out. There is no option to save this configuration.
- The default view becomes the **Spectral Signature Graph**.

TIP: Fluorochromes can be visualized without choosing an instrument configuration or by a specific laser through the **Add Laser(s) & Filters(s)** button from the **Instrument Configuration** tab. To start adding fluorochromes, see the **Fluorochrome selection** section below.

### Fluorochrome selection

- Fluorochromes can be added by name, laser, range of excitation or range of emission. Select multiple fluorochromes from one set of search results, or add additional fluorochromes by continuing to search and clicking **Add**.
- After a fluorochrome is selected, corresponding emission and excitation curves will be displayed. Deselect **Ex** or **Em** in the fluorochrome list to modify the view. Hover over an excitation or emission curve to highlight the individual spectrum.
- Save fluorochrome combinations by clicking on the **Save Combination** button. You must be signed into your BD Biosciences account.
- Check back often as new fluorochromes will be added periodically.

TIP: Click on the information icon (ℹ) in the fluorochrome list to view basic fluorochrome information and access our catalog.

### Visualization and manipulation

The default view for spectral instrument configurations is the Spectral Signature Graph.

- Toggle to other views using the drop-down menu above the graph. You can choose between four available options: Combined Ex/Em Graph, Stacked Ex/Em Graphs, Spectral Signature Graph, and Spectral Signature Heat Map.
- Visualize fluorochromes in a single-laser stack by selecting **Stacked Ex/Em Graphs** in the drop-down menu above the graph. Default setting is normalized by laser. Use the scrollbar on the right to view any additional laser lines. You can also view a multi-laser view by selecting **Combined Ex/Em Graph**.
- The Spectral Signature Heatmap is an alternative way of visualizing the same information contained in the Spectral Signature Graph. The heatmap allows users to visualize and identify spectral signatures for all selected fluorochromes simultaneously and to assess areas of spectral overlap across the entire set. The heatmap is most useful for larger combinations of fluorochromes.
- When a spectral instrument configuration is selected, the **Similarity & Complexity** matrix option becomes available to the right of the drop-down menu located above the graph. Click to view the matrix in a pop-up window.
  - Tip: You can export the matrix for future reference in the available file formats by using the **Export** button at the bottom right of the pop-up window.
- Additional selections can be made under **Graph Display Options** in the hamburger menu (☰).
  - Enable Mouseover Details
  - Show Background Grid

TIP: You can export an image of the graph(s) by clicking on the hamburger menu (☰), expanding the **Export and Import** section and clicking **Export** next to **Graph**. To learn more about our export and import features, see the **Save and share** section below.

## Save and share

- Save and share spectral graph images and fluorochrome combinations.
  - Without logging in, you can export and import fluorochrome lists as .csv files. These can be saved or shared locally and imported later using the **Import** option. Spectral graph images can be downloaded as a variety of file types that can be saved locally.
  - If you are logged in to your BD Biosciences account, you can save fluorochrome lists as user-specific files within your account. These can be loaded from the **Export and Import** section of the hamburger menu (☰).

## Frequently Asked Questions

### General FAQs

#### **Q: Will more fluorochromes be added to the new BD® Spectrum Viewer?**

A: Yes, BD plans to expand the fluorochrome library continuously as new fluorochromes are released from BD and other vendors.

#### **Q: Will the new BD® Spectrum Viewer work on mobile devices?**

A: Yes. The new tool was designed to be a mobile-friendly web application, but it is best used on desktop computers.

#### **Q: Are there any recommended web browsers to use for the new BD® Spectrum Viewer?**

A: The new tool was tested successfully on multiple browsers on both PC and Mac™ platforms—it should work comparably on all common browsers.

#### **Q: Will instruments from other manufacturers be added to the new BD® Spectrum Viewer?**

A: At this time, the BD® Spectrum Viewer only lists pre-configured BD instruments, but you can create a custom configuration to match your instrument requirements.

### Standard configuration FAQs

#### **Q: A few of the excitation and emission spectra look different between the legacy tool and the new BD® Spectrum Viewer, why?**

A: To ensure that the next generation tool has the most complete and comparable data possible, BD collected new excitation and emission spectra for most of the fluorochromes in the library using state-of-the-art instruments and verified them against existing data sets to ensure consistency. Any differences in spectra may be attributable to differences in instrumentation, improvement in dye manufacturing over time or sample types (free dye vs conjugated fluorochrome).

### Spectral configuration FAQs

#### **Q: How were the BD FACSymphony™ A5 SE Cell Analyzer data generated?**

A: Fluorochrome spectral signatures were generated on the respective instrument platform using recommended set-up protocols. Spectral signatures are dependent on the cytometer configuration and settings used; therefore, results on your individual instrument may differ from what is depicted herein.

**Q: Why am I seeing an unexpected spectral signature with some fluorochromes when using the BD FACSymphony™ A5 SE Cell Analyzer configuration?**

A: You may notice an unexpected shape or peak laser/detector or a difference in the spectral signature compared to a different spectrum viewer when evaluating certain dyes. The exact shape of the spectral signature does not affect the final unmixed result so it should not be cause for concern if the spectrum looks different than expected. There could be several reasons for this:

- The measured spectral signature is a function not only of the dye's excitation and emission curves but also the power of each laser on the flow cytometer, the specific filter band used for each detector and the choice of detector voltage or gain. This has always been true, regardless of whether an instrument is conventional or spectral, but it is more apparent with a spectral instrument.
- The spectral filter bands on the BD FACSymphony™ A5 SE Cell Analyzer do not evenly split the spectrum but rather are designed to include a subset of conventional "peak" filters for commonly used dyes while filling in other parts of the spectrum.
- In many cases, the unexpected signature is observed with a tandem dye. Tandem dyes can be excited both by their primary laser, intended to excite the tandem donor, and by other lasers that may excite the tandem acceptor directly. If a laser that excites the acceptor directly has substantially higher power than a laser that excites the donor, then it is possible and expected that the signal on the acceptor-exciting laser will be higher.
- Even if spectral signatures look different than expected, we have determined that metrics related to spectral panel performance, such as similarity index, are consistent across BD FACSymphony™ A5 SE Cell Analyzer units.

**Q: When will the BD FACSDiscover™ S8 Cell Sorter be added to the new BD® Spectrum Viewer?**

A: BD is in the process of completing the work necessary to add the BD FACSDiscover™ S8 Cell Sorter to the list of pre-configured instruments included in the new BD® Spectrum Viewer; it is expected to be available later this year.

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