

Job Aid

BD FACSDiscover™ Family: Creating a blank experiment

This job aid contains instructions for how to create a blank experiment in BD FACSDiscover™ Software. For additional information, see the *BD FACSDiscover™ A8 Cell Analyzer or BD FACSDiscover™ S8 Cell Sorter with BD CellView™ and BD SpectralFX™ Technology user's guide*.



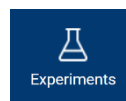
Before you begin

Start up the system and run a daily or extended fluidics startup procedure.

Creating an experiment

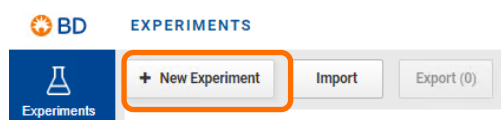
Adding an experiment

1. Click **Experiments** on the navigation bar.



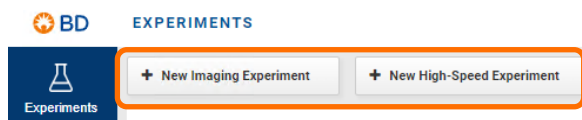
2. Create a new experiment.

BD FACSDiscover™ S8 Cell Sorter



Click + New Experiment.

BD FACSDiscover™ A8 Cell Analyzer

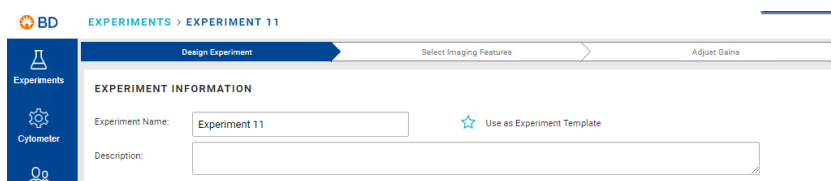


Click + New Imaging Experiment or + New High-Speed Experiment.

- If you have previously created templates, select **Blank Experiment** in the New Experiment dialog, then click **Create Experiment**.
- If you have not created templates, a new, default experiment is created.

Designing the experiment

1. Enter an experiment name.
2. (Optional) Enter a description, if needed.
3. (Optional) To designate the experiment as a template, click the **Use as Experiment Template** icon.



Designing the experiment, continued

4. In the Fluorochrome Selection panel, use the **Search Fluorochrome** tool to add your fluorochromes to the list. Enter the first letter or letters of the name of the fluorochrome.

FLUOROCROME SELECTION

Fluorochromes + AF: 00

Select the fluorochromes from the search for your experiment below. Fluorochromes are ordered by wavelength to assist with organization. All fluorochromes v "Add Fluorochrome" to add the custom fluorochrome to the fluorochrome list in the experiment.

Imaging fluorochromes must be excited by the blue laser. Some fluorochromes that are excitable by multiple lasers may not default to the blue laser for assignr Imaging features." The Unstained Controls and Autofluorescence Controls are created and run on the Set Up Single-Stain Controls page.

Fluorochromes cannot be changed during acquisition. Acquisition must be stopped prior to making any changes to Design Experiment.

Search Fluorochrome

Add New Fluorochrome

Name	Label	Remove
No fluorochromes are added to this experiment.		

5. From the dropdown list, select the fluorochrome names needed for your experiment. More than one fluorochrome can be selected simultaneously.

Search Fluorochrome

Add New Fluorochrome

☐

7-AAD

☐

Alexa Fluor 405

☐

Alexa Fluor 488

☐

Alexa Fluor 532

☐Alexa Fluor 561

Remove

TIP When adding an imaging fluorochrome, for example, PE, name it as "*Image PE*", which helps distinguish it easily while searching from the list

NOTE Fluorochromes must be added under the blue laser to be available for imaging.

NOTE Imaging fluorochromes must be excited by the blue laser. Some fluorochromes that are excitable by multiple lasers might not default to the blue laser for assignment to the imaging channels. In such cases, create a custom fluorochrome under the blue laser to use these fluorochromes for the imaging channels

6. (Optional) Click **Enter label** to add appropriate labels for each selected fluorochrome.

NOTE You do not need to add spectral dyes separately.

NOTE Label names must be unique within the experiment

Search Fluorochrome

Name	Label
^ VIOLET	
<div><div></div>FVS450</div>	viability
^ BLUE	
<div><div></div>eGFP</div>	GFP
<div><div></div>DRAQ5*</div>	DNA

7. If needed, click **Add New Fluorochrome**.

Search Fluorochrome

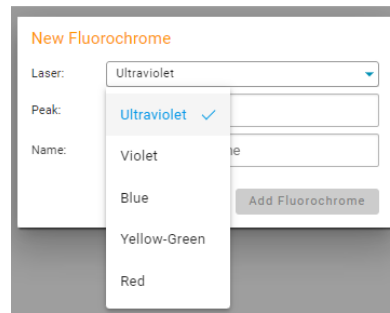
Add New Fluorochrome

Name	Label	Remove
^ VIOLET		

Designing the experiment, continued

8. Select the laser, enter the new (custom) fluorochrome name, and emission peak (wavelength) for the fluorochrome.

NOTE For a 5-laser instrument, the Ultraviolet laser is selected by default. For a 4- or 3-laser instrument, the Violet laser is selected by default.



The new fluorochrome is added under its respective laser and is marked with an asterisk.



Selecting imaging features

If your experiment contains any fluorochromes that are excited by the blue laser, you can choose to collect images and imaging feature data for those fluorochromes. For a non-imaging experiment, you can skip these steps.

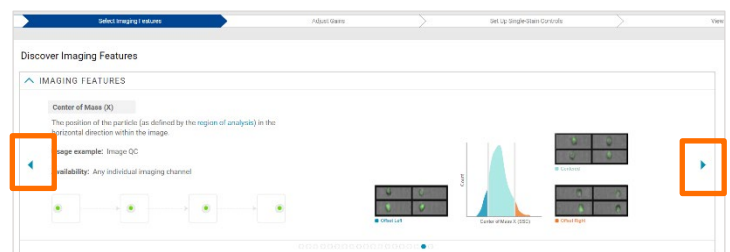
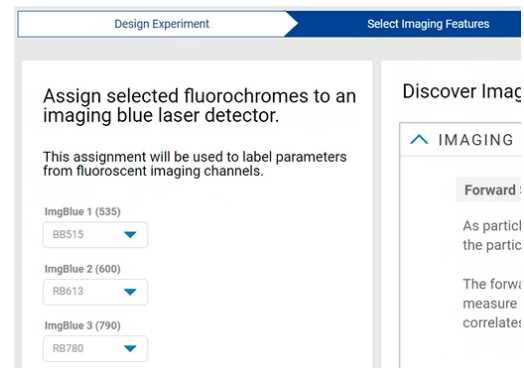
1. Click the **Select Imaging Features** tab.
2. In the drop-down menus, assign a selected fluorochrome to the appropriate imaging detector.
 - Select ImgBlue 1 (535) for a fluorochrome emitting between 511–557 nm.
 - Select ImgBlue 2 (600) for a fluorochrome emitting between 570–630 nm.
 - Select ImgBlue 3 (790) for a fluorochrome emitting between 675–900 nm.

NOTE When three imaging fluorochromes are added, they will appear in the dropdown list from all channels. Make sure to assign the correct image channel.

NOTE Forward Scatter (FSC), Side Scatter (SSC), and Light Loss (Imaging) detectors are available by default and cannot be deselected.

3. (Optional) Click the arrows on the carousel to explore the different imaging features.

Return to this page as often as needed throughout the experiment workflow to view these slides.



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