

Guide to Analyzing Data from BD Cytometric Bead Array (CBA) Kits Using FCAP Array Software



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History

Revision	Date	Change Made
647966 Rev A	7/09	New document

Contents

About this guide	1
Workflow for data analysis	2
Creating a new FCAP Array experiment	3
Creating a new bead group	5
Defining the beads in the FCAP Array plex	7
Defining the standards in the FCAP Array plex	10
Finishing experiment setup	12
Assigning data files to experiment samples	13
Analyzing the experiment	15

About this guide

About this topic To help you determine whether you should read this guide, this topic explains the purpose of the guide, and tells you where to find related information.

Purpose of this guide The purpose of this guide is to provide the instructions you will need for using FCAP Array™ software to analyze data obtained with a BD™ Cytometric Bead Array (CBA) kit.

BD CBA kits The following table lists examples of BD CBA kits from which data can be analyzed following these instructions.

Kit name	Catalog number
Human Anaphylatoxin Kit	552363
Human Chemokine Kit	552990
Human Inflammatory Cytokines Kit	551811
Human Th1/Th2 Cytokine Kit	550749
Human Th1/Th2 Cytokine Kit II	551809
Human Th1/Th2/Th17 Cytokine Kit	560484
Mouse Inflammation Kit	552364
Mouse Th1/Th2 Cytokine Kit	551287
Mouse Th1/Th2/Th17 Cytokine Kit	560485
Non-Human Primate Th1/Th2 Cytokine Kit	557800

More information For instructions on how to prepare standards and samples for a BD CBA assay, see the instruction manual for your BD CBA kit.

The procedures for application setup and acquisition differ depending upon whether you are using a BD FACSArry™ flow cytometer, a BD FACSCalibur™ flow cytometer, or a BD FACSTM digital flow cytometer. See one of the following documents for setup and acquisition instructions:

- The instruction manual for your BD CBA kit
- The *Guide to Using BD FACSDiva Software with BD Cytometric Bead Array (CBA) Kits*.

Related topics

- [Workflow for data analysis \(page 2\)](#)
-

Workflow for data analysis

About this topic This topic provides an overview of the steps involved in using FCAP Array software to analyze data from a BD CBA assay.

Before you begin To perform the procedures in this guide, you will need to have access to FCS 2.0 data files from a BD CBA assay.

Transfer the data files that you wish to analyze into a single folder.

Workflow overview

The following table contains an overview of the steps involved in data analysis:

Stage	Description
1	Creating a new FCAP Array experiment (page 3)
2	Creating a new bead group (page 5)
3	Defining the beads in the FCAP Array plex (page 7)
4	Defining the standards in the FCAP Array plex (page 11)
5	Finishing experiment setup (page 12)
6	Assigning data files to experiment samples (page 13)
7	Analyzing the experiment (page 15)

Related topics

- [About this guide \(page 1\)](#)

Creating a new FCAP Array experiment

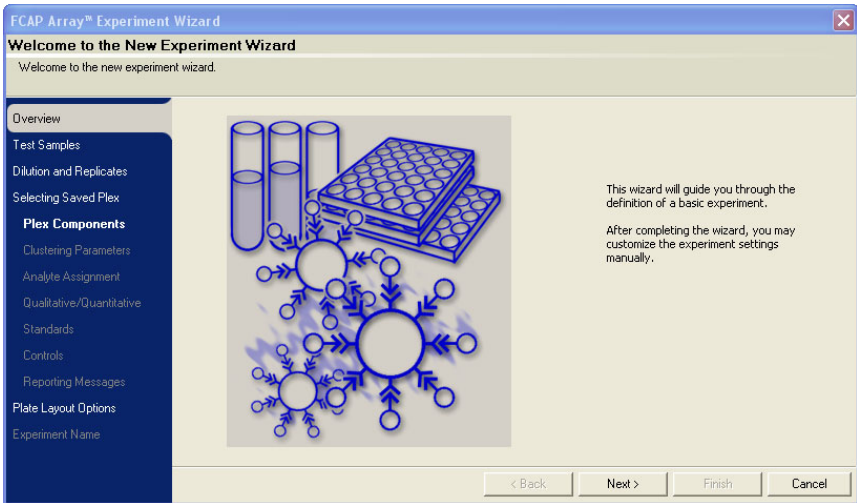
About this topic This topic describes the first set of steps you must take to create a new FCAP Array experiment for analyzing data from a BD CBA assay.

Procedure

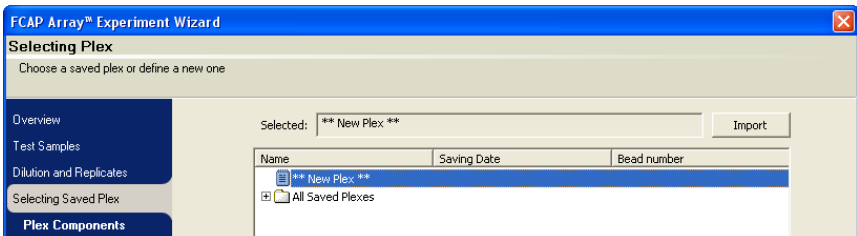
To create an experiment:

1. Start FCAP Array software.
2. Select File > New Experiment Wizard.

The FCAP Array™ Experiment Wizard appears.



3. Click **Next**.
4. In the **Test Samples** view, specify the number of samples in your assay, then click **Next** to advance to the next view.
5. In the **Dilution and Replicates** view, specify the dilution factor (leave the value at 1.00 if you did not dilute your samples), then click **Next**.
6. In the **Selecting Saved Plex** view, select ****New Plex**** (top line), then click **Next**.



7. In the **Plex Components** view, click **Edit**.

The **Bead Library** window appears.

Next step

If this is the first time you are analyzing data from a particular BD CBA kit, proceed to [Creating a new bead group \(page 5\)](#).

If you have already created a bead group for your BD CBA kit, proceed to [Defining the beads in the FCAP Array plex \(page 7\)](#).

Creating a new bead group

About this topic This topic describes how to create a new bead group in FCAP Array software that is specific to your BD CBA kit.

Before you begin Complete the steps described in [Creating a new FCAP Array experiment \(page 3\)](#).

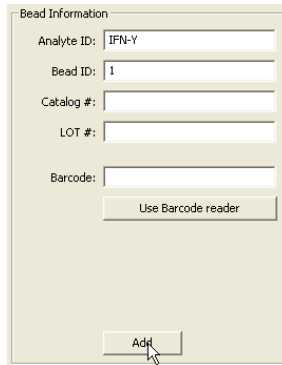
Using Figure 1 in your BD CBA kit instruction manual, look up the analyte of each of the beads in your kit according to bead brightness.

Procedure

To create a new bead group:

1. In the **Bead Library** window, click **Edit Groups**, then click **New Group**.
2. In the **New Bead Group** window, enter the name of your kit, then click **OK**.
3. Click **OK** to return to the **Bead Groups** window, then click **OK** again to return to the **Bead Library** window.

4. Enter the **Analyte ID** for the dimmest bead in your kit, specify a **Bead ID** of 1, then click **Add** to add the bead to the library.



5. Enter the **Analyte ID** for the next brightest bead in your kit, specify a **Bead ID** of 2, then click **Add**.
6. In the same manner for all of the beads in your kit, enter the **Analyte ID** and **Bead ID** (increasing with increasing brightness), then add each bead to the library.
7. Click **Edit Groups**.
8. In the **Bead Groups** window, select the bead group you just created, then click **Modify Group**.
9. Select the beads you want to add to the bead group by clicking the appropriate checkboxes, then click **OK**.
10. Click **OK** to close the **Bead Groups** window, then click **OK** to close the **Bead Library** window.

Next step

Proceed to [Defining the beads in the FCAP Array plx \(page 7\)](#).

Defining the beads in the FCAP Array plex

About this topic This topic describes how to define the beads in a new plex within your FCAP Array experiment.

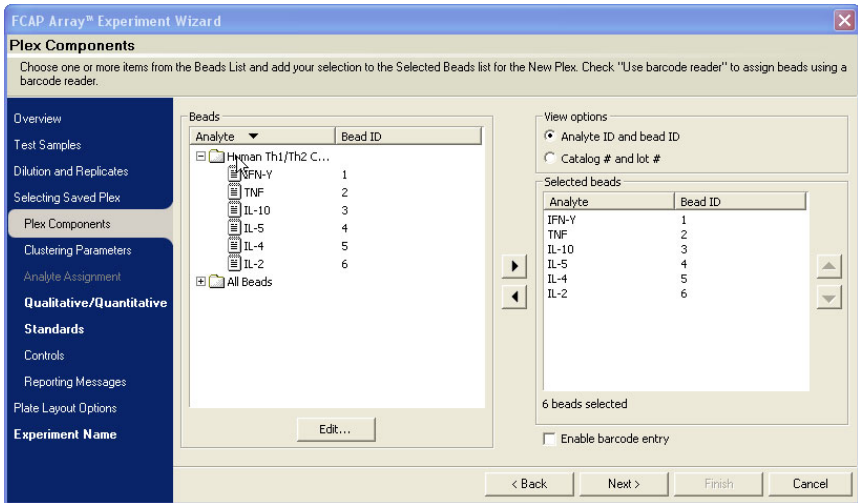
This procedure associates the distinct fluorescence characteristics of your CBA beads with the appropriate analyte.

Before you begin Complete the steps described in [Creating a new FCAP Array experiment \(page 3\)](#).

If this is the first time you are analyzing data from this BD CBA kit, complete the steps described in [Creating a new bead group \(page 5\)](#).

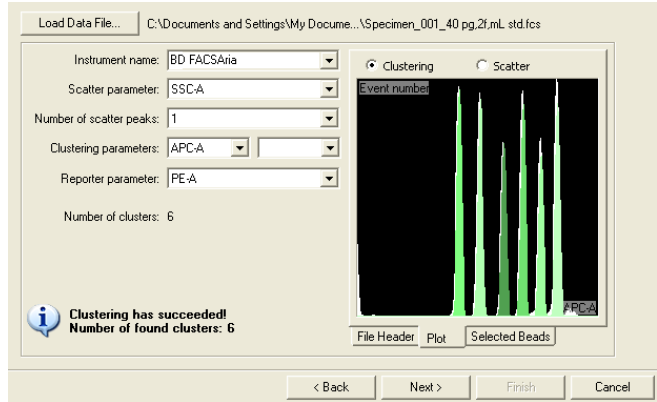
Procedure To define the beads in your new plex:

1. In the **Beads** list box of the **Plex Components** view, double-click the name of the appropriate bead group to move its components to the **Selected Beads** list box.



2. Click **Next** to display the **Clustering Parameters** view.
3. Click **Load Data File** and navigate to the folder containing your FCS 2.0 data files. If your FCS 2.0 files were acquired on a BD FACSCalibur flow cytometer, specify **All files** in the **Files of type** field to display the list of files.
4. Select any one FCS file and click **Select**.
5. Select your flow cytometer from the menu in the **Instrument name** field.

The beads appear as a histogram in the data plot. If clustering was successful, the software displays a message in the bottom-left corner of the window.



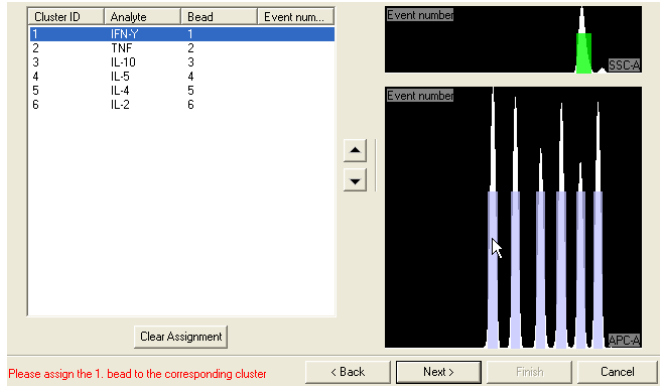
If clustering was not successful, see your kit manual for troubleshooting tips.

6. Specify SSC-A in the **Scatter parameter** field and 1 in the **Number of scatter peaks** field.
7. Specify clustering and reporter parameters as follows:

Instrument name	Clustering parameters	Reporter parameter
BD FACSAria	Red-A and None	Yellow-A
BD FACSCanto BD LSR II	APC-A and None	PE-A
BD FACSCalibur, single-laser	FL3-A and None	FL2-A
BD FACSCalibur, dual-laser	FL4-A and None	FL2-A

8. Click **Next** to advance to the **Analyte Assignment** view.

9. Select bead 1 in the bead list, then double-click the dimmest (left-most) peak in the histogram to assign Bead 1 to that peak.



10. Repeat step 9 to assign the remaining beads to peaks in order of increasing brightness.
11. Click Next to advance to the **Qualitative/Quantitative** view.
12. For all analytes, specify a fitting equation. BD recommends the 4- or 5-parameter logistic models.
13. Click **Next** to advance to the **Standards** view.

Next step

Proceed to [Defining the standards in the FCAP Array plex \(page 11\)](#).

Defining the standards in the FCAP Array plex

About this topic This topic describes how to define the standards in your FCAP Array plex.

This procedure associates reporter fluorescence with the known concentrations of the standards to enable quantitative analysis of the analytes in your samples.

Before you begin Complete the steps described in [Defining the beads in the FCAP Array plex \(page 7\)](#).

Procedure

To define the standards in your plex:

1. In the **Standards** view, specify the **Number of standard samples** for your BD CBA assay.
2. Select **pg/ml** from the menu in the top row of the **CC** column.
3. For each standard, specify the concentration in the **CC** column.
Std01 should be 0 pg/mL. See your BD CBA kit instruction manual for the concentrations of your other standards.
4. If your kit has concentrations that vary across analytes, clear the **Uniform concentrations for all analytes** checkbox, then modify concentrations as necessary.
5. Specify the appropriate number of replicates in the **Number of replicates for each sample** field.
6. If you are not using the optional **Controls** and **Reporting Messages** views, click **Next** three times to advance to the **Plate Layout Options** view.

Next step Proceed to [Finishing experiment setup \(page 12\)](#).

Finishing experiment setup

About this topic This topic describes how to finish setting up your FCAP Array experiment in preparation for analysis.

You can reduce the amount of effort required to set up future analyses by saving the plex at this stage. If you choose to save the plex, all of the information you have entered will be saved except for the number of samples and the dilution factor.

Before you begin Complete the steps described in [Defining the standards in the FCAP Array plex \(page 11\)](#).

Procedure **To complete experiment setup:**

1. Click the **Place samples at the end** checkbox.
2. Click **Next** to advance to the **Experiment Name** view.
3. Enter a name in the **Experiment Name** field.
4. If you want to save the plex, select **Save plex** and enter a name in the **Plex name** field.
5. Click **Finish** to complete experiment setup and close the FCAP Array™ Experiment Wizard.

Next step Proceed to [Assigning data files to experiment samples \(page 13\)](#).

Assigning data files to experiment samples

About this topic This topic describes how to assign FCS 2.0 data files to the samples you defined in your FCAP Array experiment.

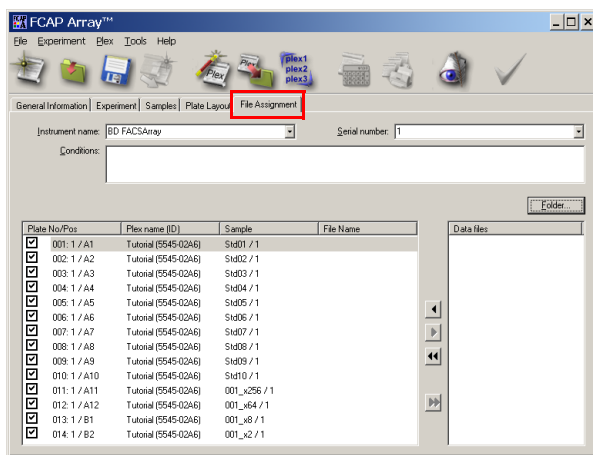
The procedure consists of two steps: preparing to assign data, then performing either one-step or manual data assignment.

Before you begin Complete the steps described in [Finishing experiment setup](#) (page 12).

Preparing to assign data

To prepare to assign data files:

1. Select the File Assignment tab.



2. Enter the appropriate information for your flow cytometer in the **Instrument name** and **Serial number** fields.

If the instrument name does not exactly match the instrument name specified in the **Clustering**

Parameters view, data analysis cannot be completed. See [Defining the beads in the FCAP Array plex on page 7](#).

- Inspect the list of experiment samples in the left pane and the list of FCS 2.0 data files in the right pane:

If...	Then...
The order of data-file names exactly matches the order of experiment-sample names	Proceed to Performing one-step assignment
The order of data-file names does not match the order of experiment-sample names	Proceed to Performing manual assignment

Performing one-step assignment

To perform one-step assignment of data files:

- Select the top item in the experiment-sample list and the top item in the data-file list.
- Click the double left arrow.

The view changes to indicate that all file names are assigned.

Performing manual assignment

To perform manual assignment of data files:

- Select the first experiment sample in the list in the left pane.
- In the **Data files** pane, select the name of the corresponding data file (eg, **Std01** should correspond to the 0 pg/mL standard).
- Click the left arrow to assign the data file to the experiment sample.
- Repeat steps 1 to 3 until data files have been assigned to all experiment samples.

Next step

Proceed to [Analyzing the experiment \(page 15\)](#).

Analyzing the experiment

About this topic This topic describes how to analyze the FCAP Array experiment that you prepared in the previous stages of this workflow.

Before you begin Complete the steps described in [Assigning data files to experiment samples \(page 13\)](#).

The **Start analyzing this experiment** icon becomes active as soon as you finish assigning data files to experiment samples.

If the icon does not become active, check to be sure you have entered the correct instrument name and serial number in the **File Assignment** tab.

Procedure

To analyze the experiment:

1. Click the **Start analyzing this experiment** icon.



2. Review the messages in the **Analysis Messages** dialog, then click **OK**.
3. When the analysis is complete, view the experiment report by clicking the **Report Printout** tab.

Related topics

- [About this guide \(page 1\)](#)
- [Workflow for data analysis \(page 2\)](#)

More information

See the *FCAP Array Software User's Guide* for instructions on printing the experiment report, viewing the standard curves, and exporting raw data, or for more information about any of the software features.

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