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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.

<table>
<thead>
<tr>
<th>Kit name</th>
<th>Catalog number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Anaphylatoxin Kit</td>
<td>552363</td>
</tr>
<tr>
<td>Human Chemokine Kit</td>
<td>552990</td>
</tr>
<tr>
<td>Human Inflammatory Cytokines Kit</td>
<td>551811</td>
</tr>
<tr>
<td>Human Th1/Th2 Cytokine Kit</td>
<td>550749</td>
</tr>
<tr>
<td>Human Th1/Th2 Cytokine Kit II</td>
<td>551809</td>
</tr>
<tr>
<td>Human Th1/Th2/Th17 Cytokine Kit</td>
<td>560484</td>
</tr>
<tr>
<td>Mouse Inflammation Kit</td>
<td>552364</td>
</tr>
<tr>
<td>Mouse Th1/Th2 Cytokine Kit</td>
<td>551287</td>
</tr>
<tr>
<td>Mouse Th1/Th2/Th17 Cytokine Kit</td>
<td>560485</td>
</tr>
<tr>
<td>Non-Human Primate Th1/Th2 Cytokine Kit</td>
<td>557800</td>
</tr>
</tbody>
</table>

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

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

Creating a new FCAP Array experiment

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.

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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.

![Image of Bead Information window]

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 plex (page 7).

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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:

<table>
<thead>
<tr>
<th>Instrument name</th>
<th>Clustering parameters</th>
<th>Reporter parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD FACSAarray</td>
<td>Red-A and None</td>
<td>Yellow-A</td>
</tr>
<tr>
<td>BD FACSaria</td>
<td>APC-A and None</td>
<td>PE-A</td>
</tr>
<tr>
<td>BD FACSCanto</td>
<td>FL3-A and None</td>
<td>FL2-A</td>
</tr>
<tr>
<td>BD LSR II</td>
<td>FL4-A and None</td>
<td>FL2-A</td>
</tr>
</tbody>
</table>

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

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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.
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.

![Image of 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

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

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

<table>
<thead>
<tr>
<th>If...</th>
<th>Then...</th>
</tr>
</thead>
<tbody>
<tr>
<td>The order of data-file names exactly matches the order of experiment-sample names</td>
<td>Proceed to Performing one-step assignment</td>
</tr>
<tr>
<td>The order of data-file names does not match the order of experiment-sample names</td>
<td>Proceed to Performing manual assignment</td>
</tr>
</tbody>
</table>

Performing one-step assignment

To perform one-step assignment of data files:
1. Select the top item in the experiment-sample list and the top item in the data-file list.
2. 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:
1. Select the first experiment sample in the list in the left pane.
2. In the Data files pane, select the name of the corresponding data file (eg, Std01 should correspond to the 0 pg/mL standard).
3. Click the left arrow to assign the data file to the experiment sample.
4. Repeat steps 1 to 3 until data files have been assigned to all experiment samples.

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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.

![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.