

Guide to the FCS Express™ BD Accuri Software™ Importer

Instruction Manual

FCS Express 6 is an advanced flow cytometry data analysis software package that allows advanced data analysis and presentation including color dot plots, unlimited overlays, heat maps, extended statistics, custom reporting, and batch processing.

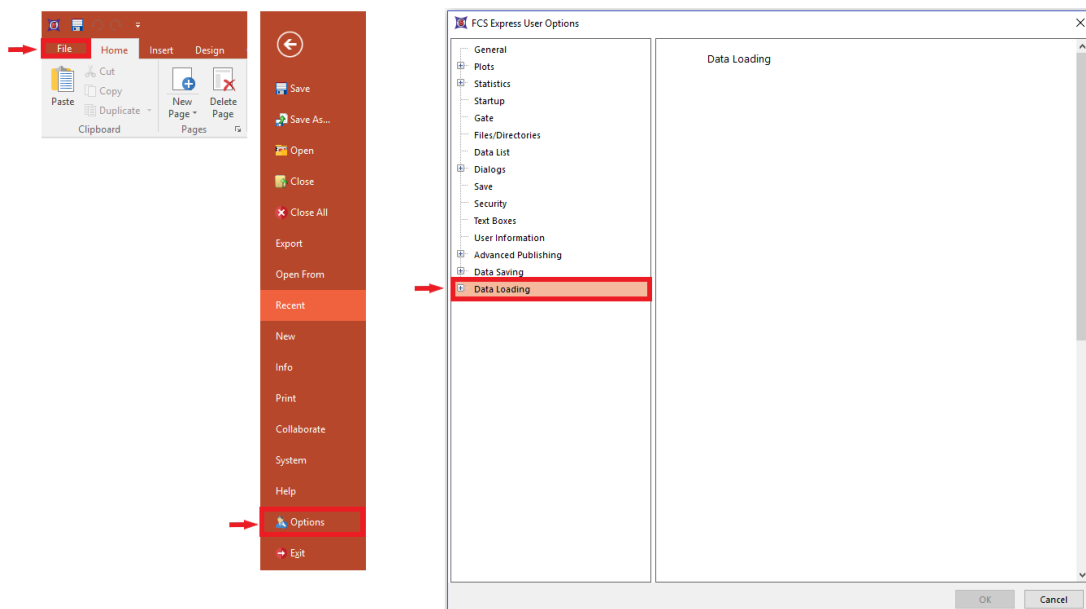
The FCS Express BD Accuri Software Importer, available in FCS Express 6, allows you to automatically recreate any analysis scheme from BD Accuri Software in FCS Express. After you have collected data on the BD Accuri™ C6 flow cytometer, you can easily convert the BD Accuri Software workspace to a FCS Express layout by following these directions.

We recommend that you update to the most recent version of FCS Express to ensure the best compatibility. Visit <http://www.denovosoftware.com> for more information.

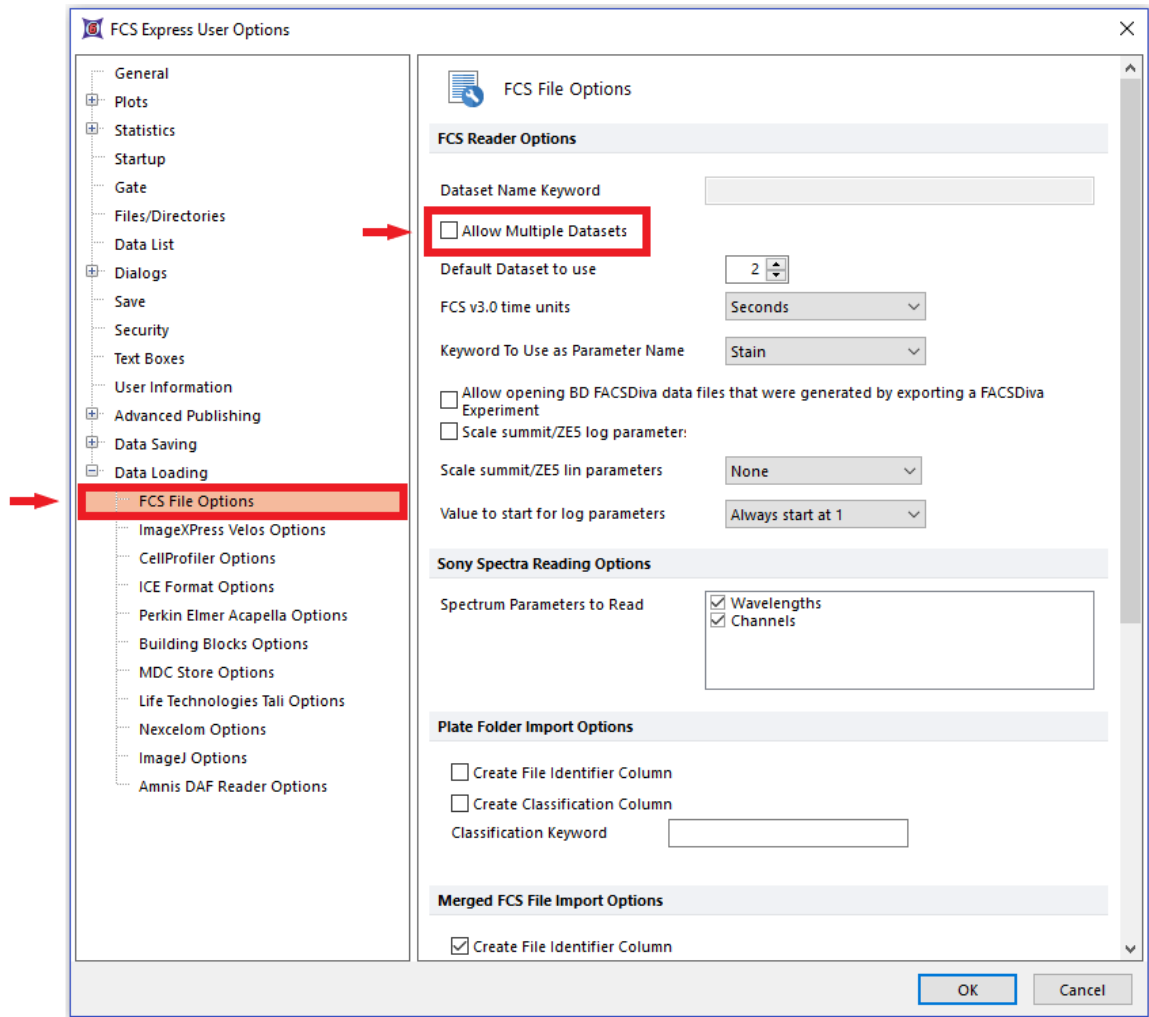
Setting Preferences

First set the user options in FCS Express for optimal performance.

1. Open FCS Express.
2. Select **Options** from the **File** tab.
3. Expand the **Data Loading** category in the **User Options** window by clicking the **+** next to **Data Loading**.



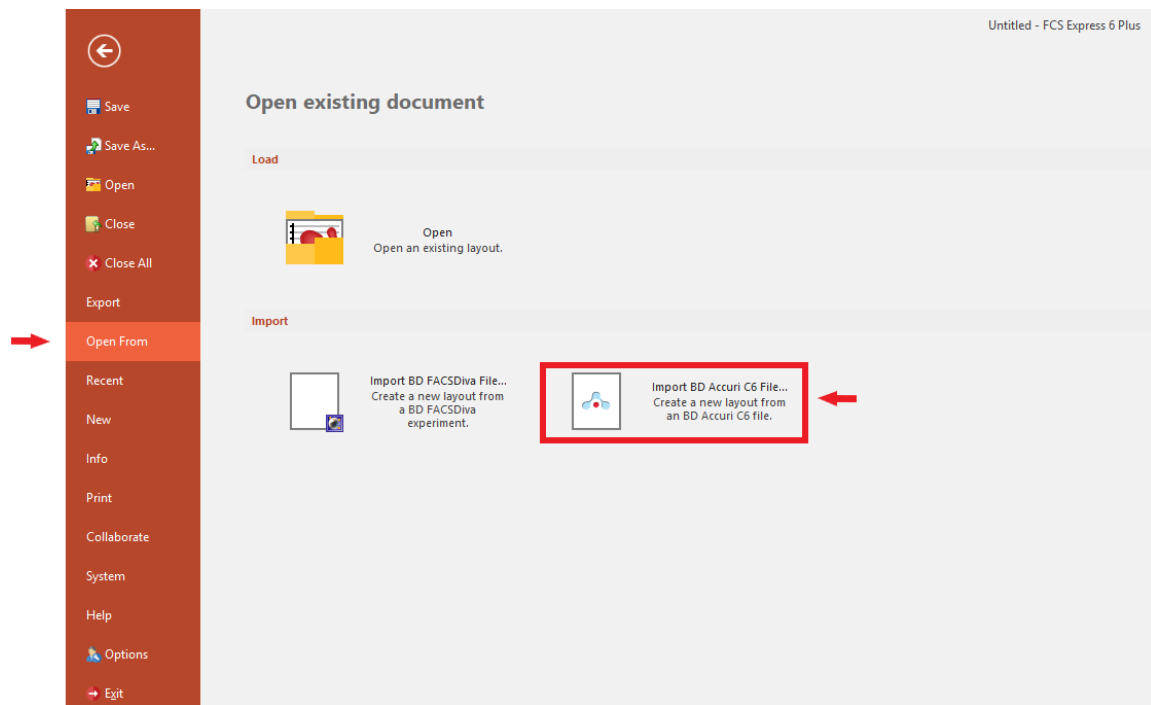
4. Select **FCS File Options** under **Data Loading** to update the window.



5. Clear the **Allow Multiple Datasets** checkbox.
6. Click **OK** to save the new user options. You are now ready to begin using FCS Express to view data collected with BD Accuri Software.

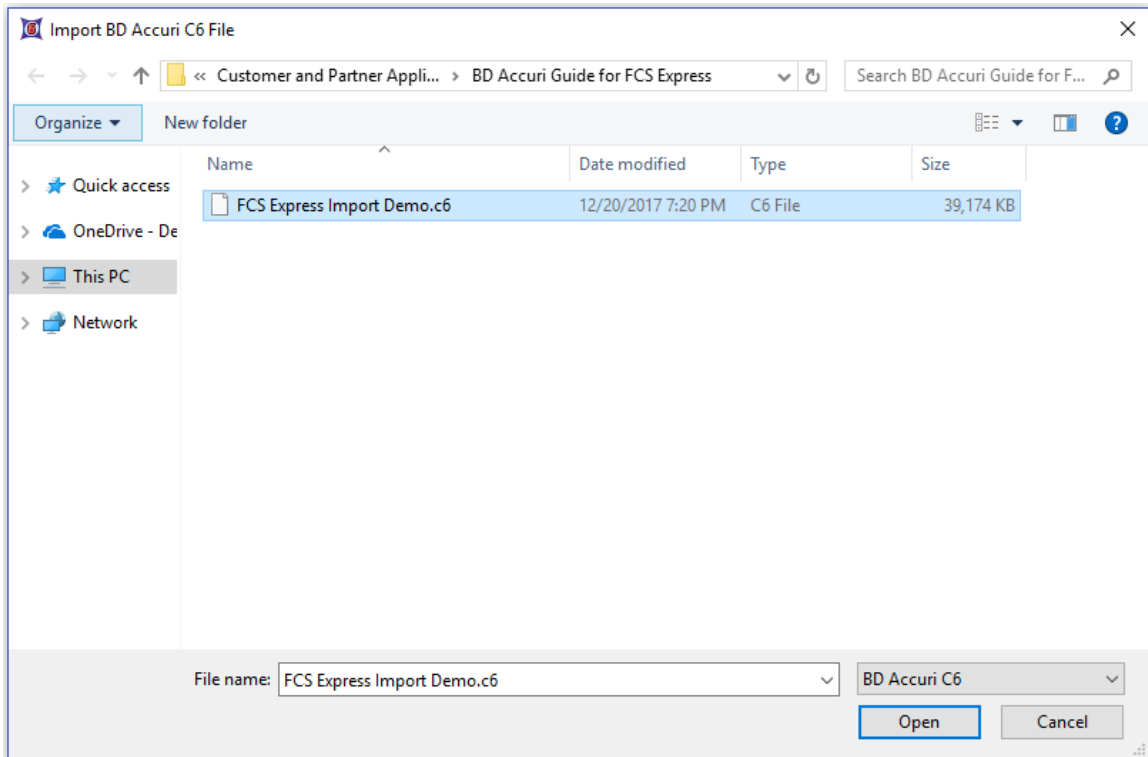
Importing a BD Accuri Software Layout into FCS Express

1. Open FCS Express.
2. From the **File** tab, select **Open From > Import BD Accuri C6 File**.



3. Select the BD Accuri Software file (.c6) that contains the data you want to view with FCS Express. (In this example, the file is FCS Express Import Demo.c6.)

NOTE: The BD Accuri Software Importer reads the FCS data directly from the BD Accuri Software file, so you do not have to export individual files.



4. Click **Open**.

FCS Express prompts you for a location from where to import plots: the **Collect** or **Analyze** tabs. It also displays a list of all the sample wells (FCS files) contained within the BD Accuri Software file.

5. Select whether you want to recreate the plots from the **Collect** and/or **Analyze** tabs in BD Accuri Software.

The screenshot shows a software window titled "Import BD Accuri C6 File Wizard". Inside, the "Import Selections" section is active. Below this, a "C6 Tab Selection" header is followed by the question "Which C6 tabs do you want to import plots from?". There are two checkboxes: "Import from the Collect tab" (checked) and "Import from the Analyze tab" (checked). Below the "Collect" checkbox, another question asks "Which data file would you like to display on the Collect tab plots?", with a dropdown menu showing "Sample A09.fcs". At the bottom right, there are three buttons: "< Back", "Next >" (highlighted with a blue border), and "Cancel".

NOTE: If you choose to import plots from the **Collect** tab, you can choose which data well you wish to display in the FCS Express layout.

Import BD Accuri C6 File Wizard

Import Selections

C6 Tab Selection

Which C6 tabs do you want to import plots from?

☒ Import from the Collect tab

Which data file would you like to display on the Collect tab plots?

☒ Import

Sample A09.fcs

Sample A04.fcs

Sample A05.fcs

Sample A06.fcs

Sample A07.fcs

Sample A08.fcs

Sample A09.fcs

Sample A10.fcs

Sample A11.fcs

< Back

Next >


Cancel

6. Click **Next**.

7. Select how you want the FCS Express layout to look. (The example has chosen to view the plots as they were displayed in BD Accuri Software: four rows and three columns per page in a landscape orientation).

Import BD Accuri C6 File Wizard

Page Layout Options

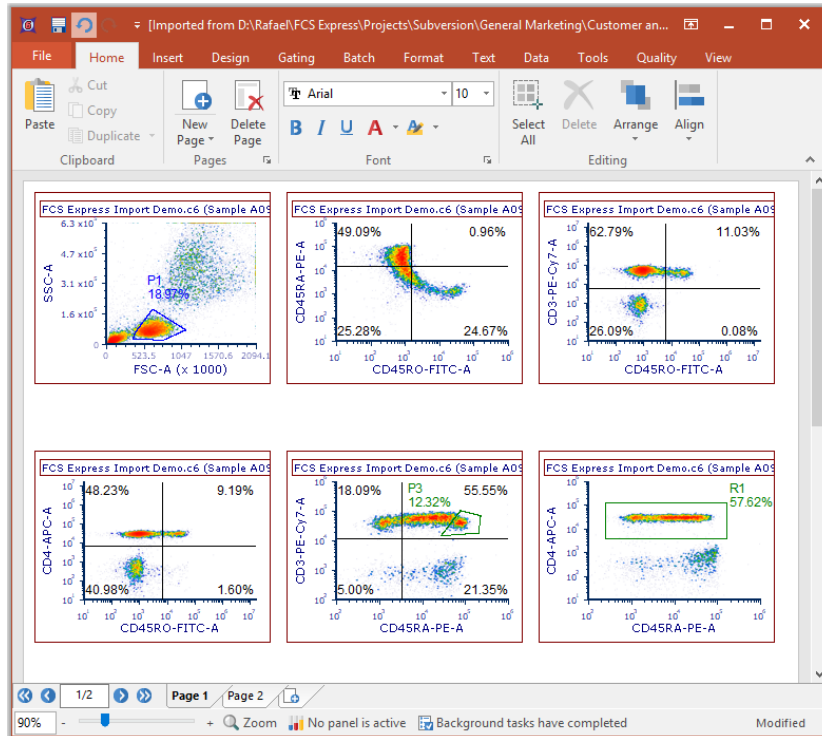
Page Size	Page Orientation	Preview
Letter Width: 8.50 Height: 11.00	<input checked="" type="radio"/> Portrait <input type="radio"/> Landscape	
Margins None Left Margin: 0.00 Right Margin: 0.00 Top Margin: 0.00 Bottom Margin: 0.00	Working Area Width: 8.5" Height: 11.0"	
Plot Layout Options Plot width: 200 Plot height: 200 <input checked="" type="checkbox"/> Include space for plots even if they are blank in the BD Accuri C6 software		
Resize Options <input checked="" type="checkbox"/> Maintain the size of all objects on the layout		

< Back **Finish** Cancel

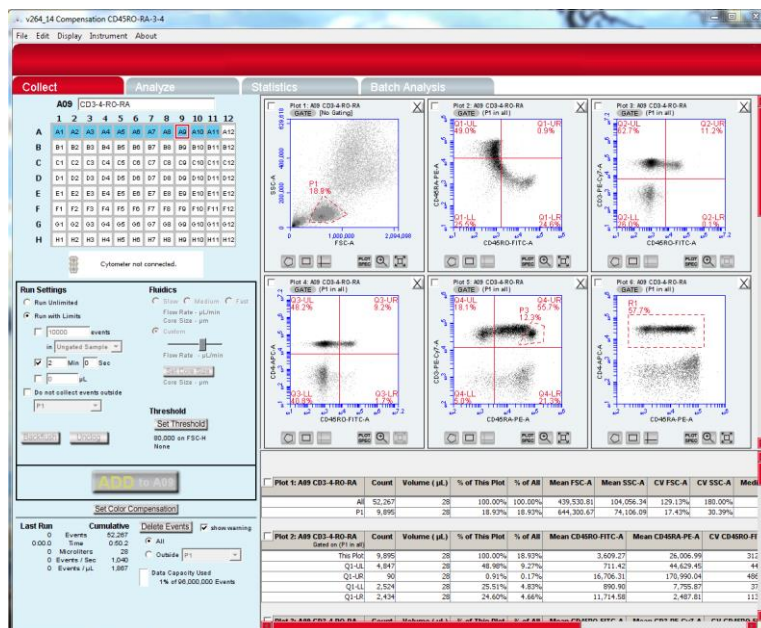
8. Click **Finish**.

FCS Express creates an exact copy of the BD Accuri Software workspace displayed as an FCS Express layout. The plots are all scaled and zoomed exactly the same way in FCS Express 6 as in BD Accuri Software. Furthermore, all regions, markers, and gating are retained. Parameter names and compensation settings are also applied.

Following is a comparison of data viewed in FCS Express and in BD Accuri Software.



Data displayed in FCS Express 6.



Original BD Accuri Software data.

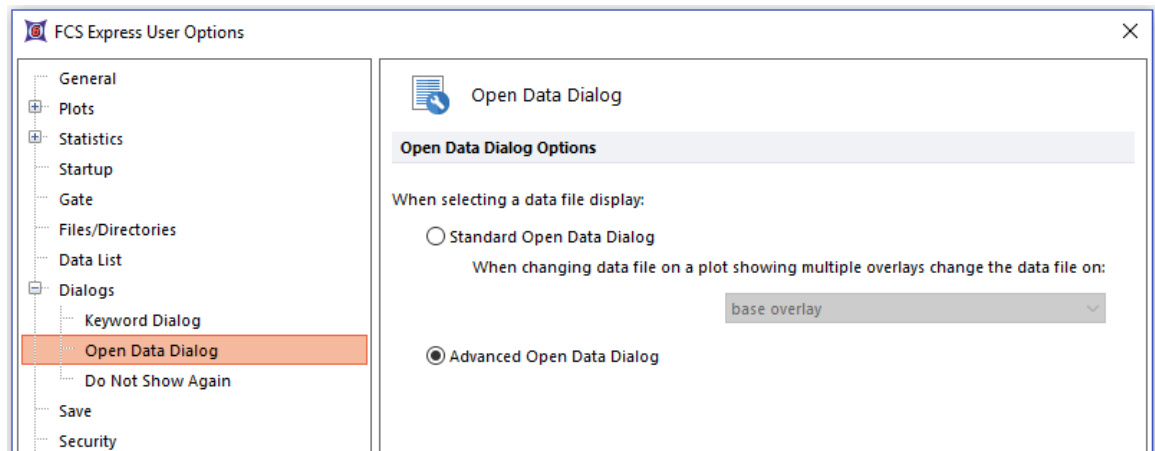
9. At this point, you can use any of the analysis features in FCS Express. De Novo Software has an excellent set of tutorials to help guide you through some of the more common features in FCS Express.

Switching BD Accuri Software Wells (FCS Files) Within FCS Express

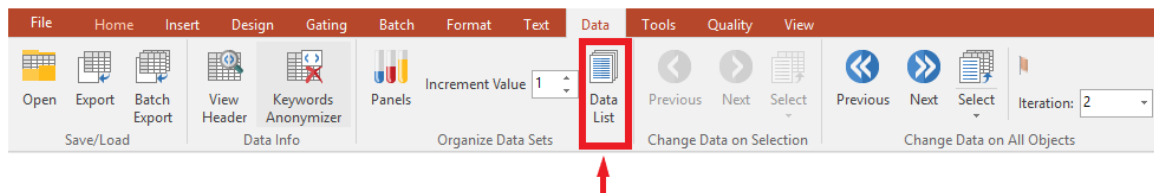
This feature allows you to navigate within the sample wells of a BD Accuri Software file without exporting the FCS files.

NOTE: If you selected to import plots from the BD Accuri Software **Collect** tab you have already selected the Sample Well (FCS file) you are viewing. This feature allows you to easily change Sample Wells (FCS files) within the BD Accuri Software file without leaving FCS Express.

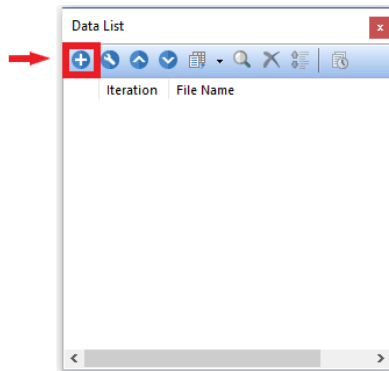
1. Select **Options** from the **File** tab.
2. Expand the **Dialogs** category in the **User Options** window by clicking the **+** next to **Dialogs**.
3. Select the **Open Data Dialog** tab then click the **Advanced Open Data Dialog** radio button.



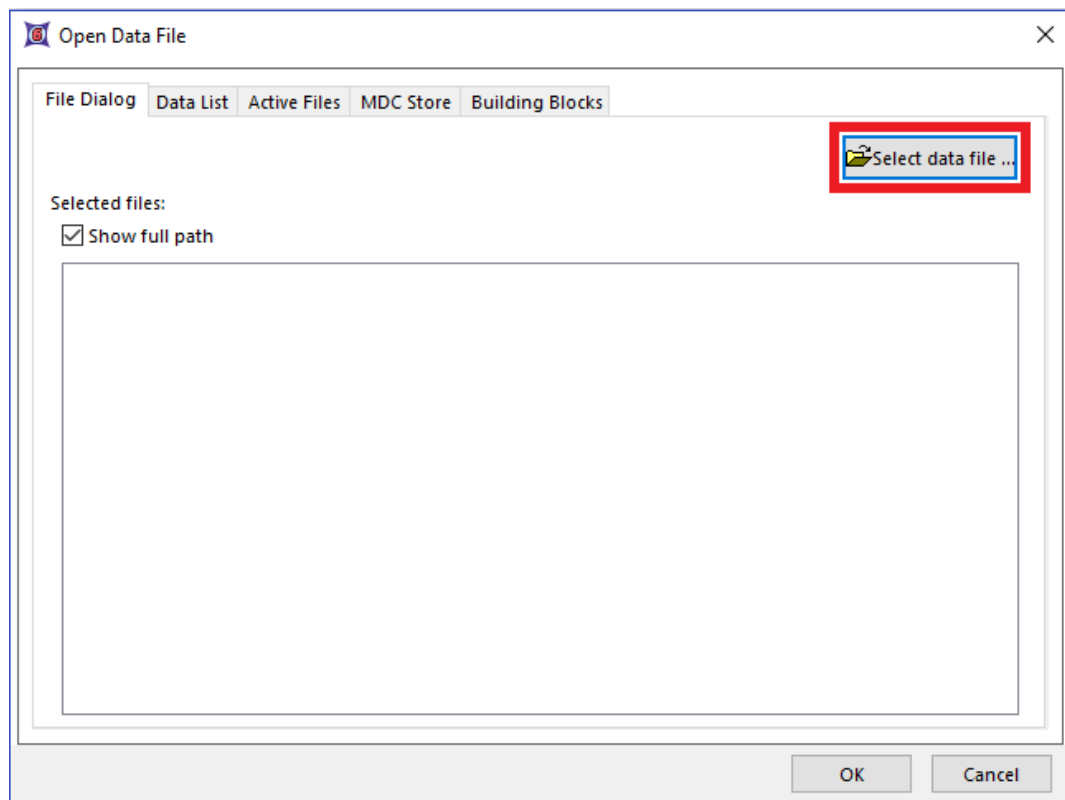
4. Click **OK** to save the new user options. You now have access to the Advanced Open Data Dialog to view and open sample well data collected with the BD Accuri Software.
5. Once you have loaded your BD Accuri Software Layout or created a new plot in FCS Express, Select **Data Tab>Organize Data Sets>Data List** from the ribbon.



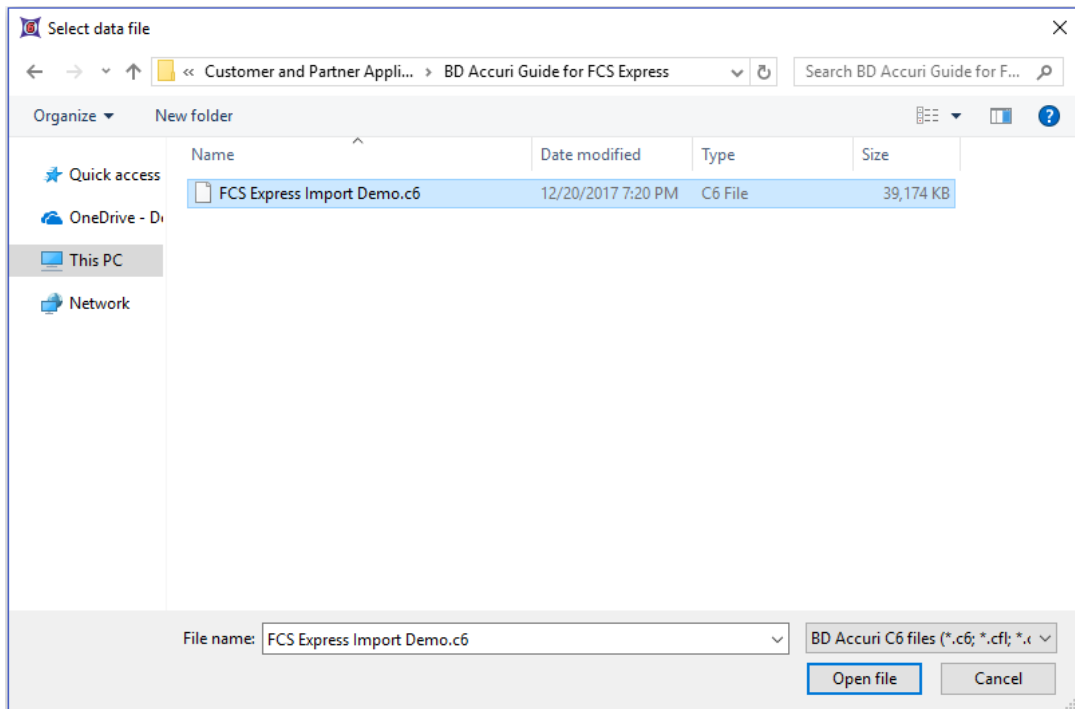
6. Click the green plus button to add data files.



7. Click on the **Select data file...** button when the Open Data File dialog opens.



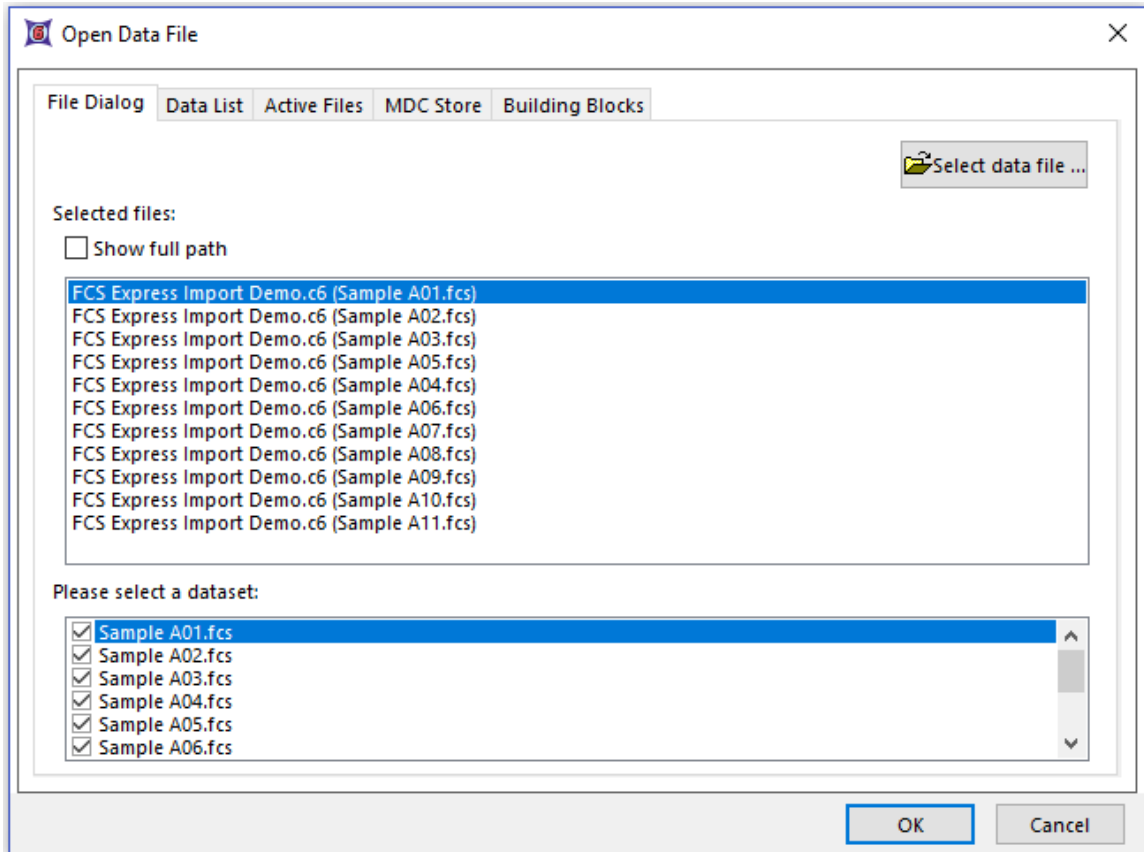
8. Change the file extension type field to **BD Accuri C6 files (*.c6, *.cfl)** when the Select data file dialog opens.



9. Select the BD Accuri Software file that contains the data you want to view.
10. Click **Open file**.

FCS Express now populates the Open Data File dialog with all of the sample wells that are contained within the BD Accuri Software file.

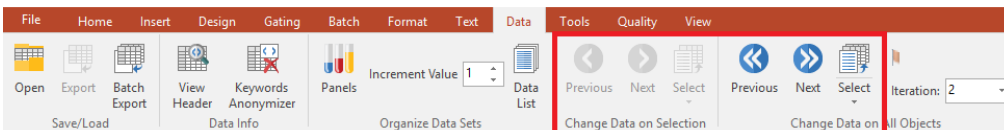
11. Check the box next to the files that you want to be included in the data list.



12. Click **OK**.

The data list is now populated with the selected files.

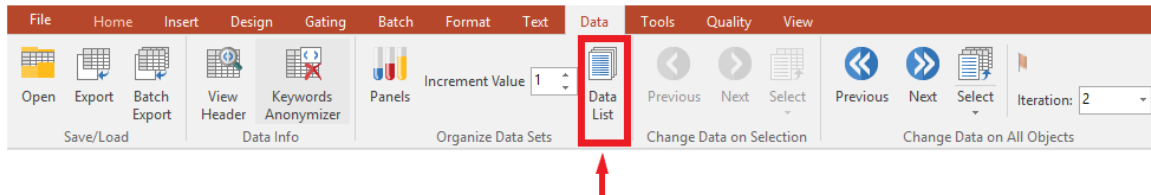
13. You can now navigate the sample wells by using either the **Data List** or **Next/Previous** commands in the FCS file menu.



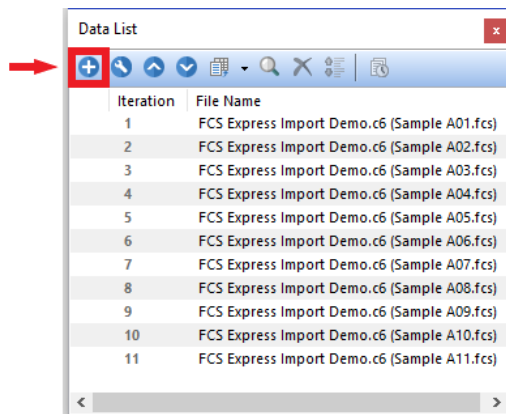
Using the High Content Add-on with BD Accuri Software in FCS Express

The high content add-on in FCS Express allows you to display, gate, and further utilize your plate-based assay acquired with BD Accuri Software.

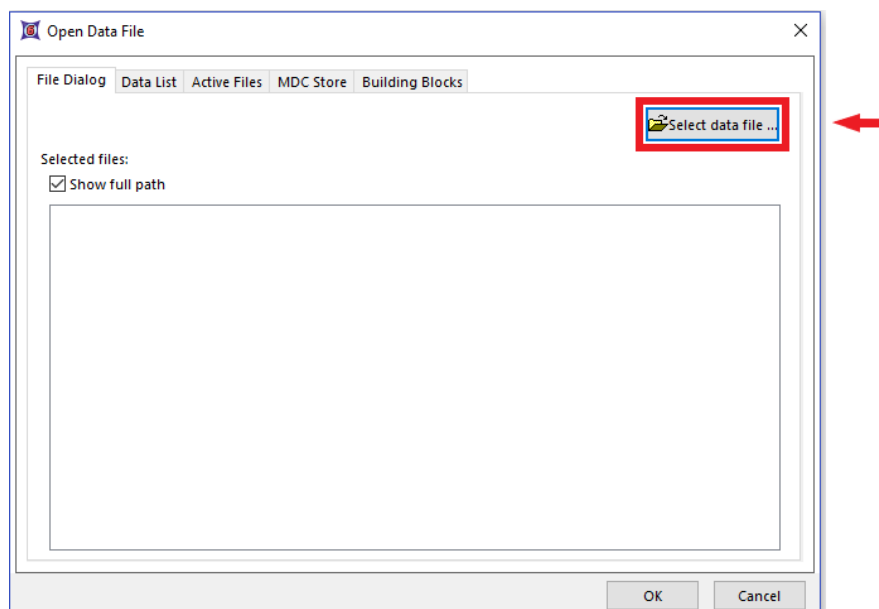
1. Once you have loaded your BD Accuri Software layout in FCS Express, select the **Data Tab>Organize Data Sets>Data List**.



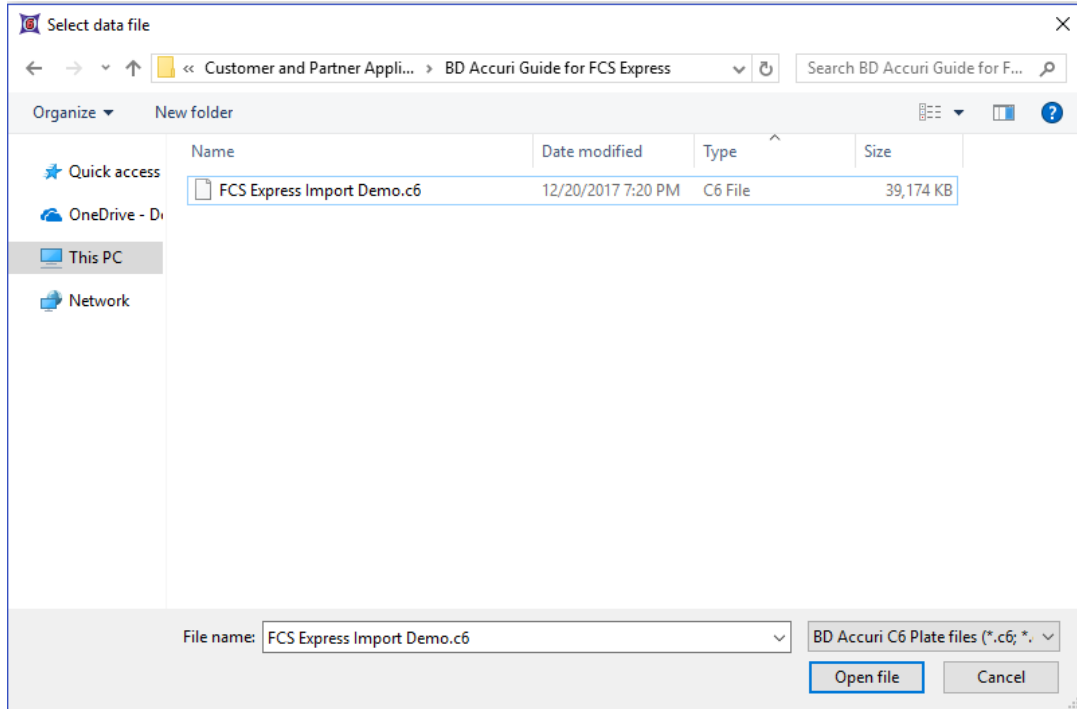
2. Click the green plus button to add data files.



3. Click on the **Select data file...** button when the Open Data File dialog appears.

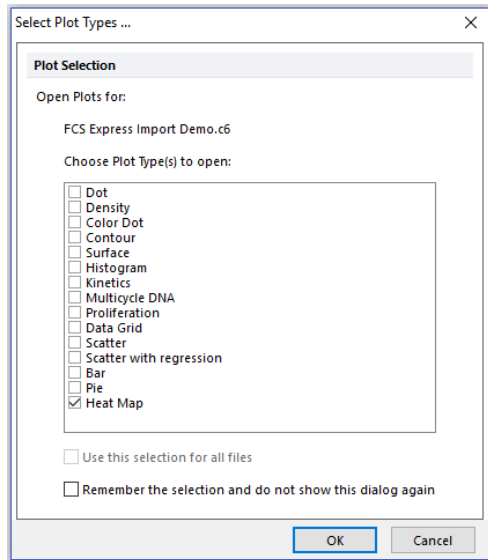


4. When the **Select data file** dialog opens, change the file type field to **BD Accuri C6 Plate files (*.c6, *.cfl)**.
5. Select the BD Accuri Software file that contains the data you want to view.

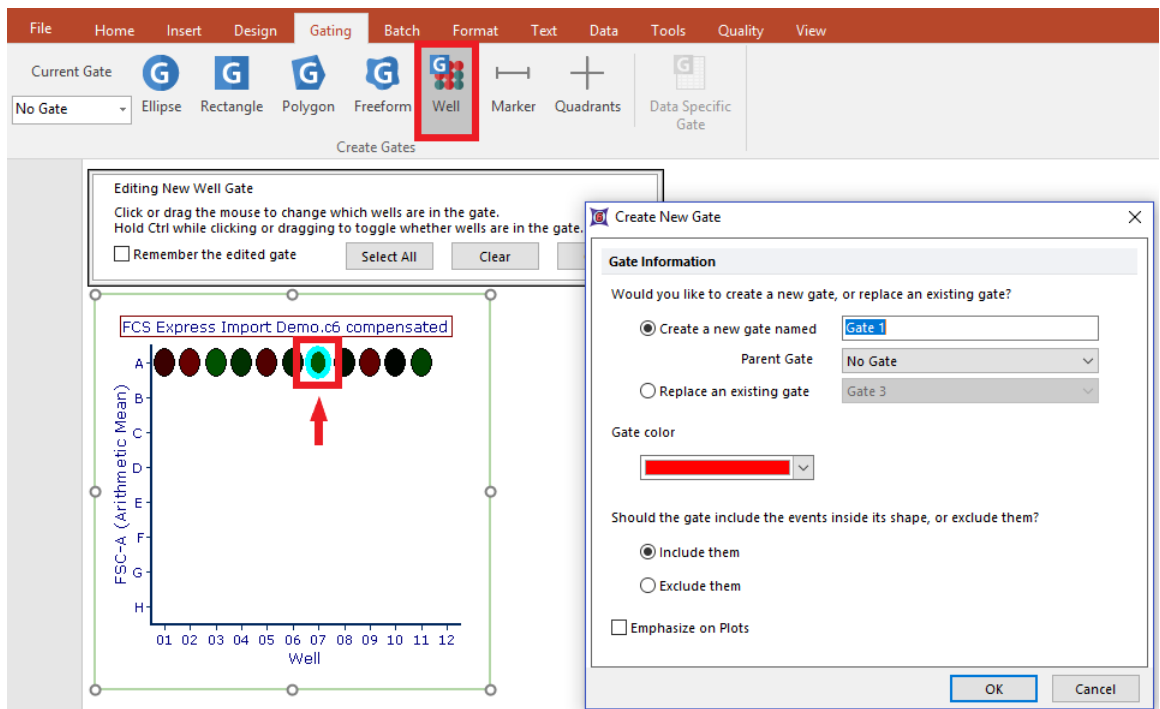


6. Click **Open file**, then click **OK** when the Open Data File dialog reappears.
The data list will now be populated with the plate-based file.
7. To insert the heat map, from the data list drag and drop the C6 Plate file anywhere on the layout.

8. When the **Select Plot Types...** dialog appears, check the Heat Map then click **OK**.



9. You can now choose and gate on individual or multiple wells from the **Gating Tab>Create Gates>Well** command from the ribbon.



NOTE: You may also insert a heat map from the Insert tab. This method will produce an error message; however, the error can be safely ignored. When the error message appears, click on **Continue**. You will need to replace the data on the heat map with the C6 Plate File loaded on the data list. To do so, simply drag and drop the C6 Plate File from the data list onto the heat map. All the sample wells will now appear on the plot.