BD FACSVerse™ System Reference Guide

For Research Use Only

bdbiosciences.com
23-11879-00 Rev. 01
7/2011

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Patents

The BD FACSVerse™ flow cytometer is covered by one or more of the following US patents: 7,787,197; 7,129,505; 6,897,954; 6, 809,804; 6,683, 314; and 6,510,007.

Regulatory information

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Class I Laser Product.

FCC information

WARNING: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

NOTICE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his or her own expense. Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits. This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations. Cet appareil numérique de la classe A respecte toutes les exigences du Réglement sur le matériel brouilleur du Canada.

History

Revision	Date	Change made
23-11879-00 Rev. 01	7/2011	New document

Contents

1

Documentation and support

This section includes the following topics:

System documentation

Introduction

This topic describes the information that is available for this product.

Available documentation

- **Cytometer Safety and Limitations Guide.** This guide is included with your cytometer and describes any possible hazards or limitations.
- **User's Guide.** This guide provides information for setting up and running the BD FACSVerse with BD FACSVerse System using a typical workflow. Information includes basic instructions on the operation of the cytometer and basic instructions on how to set up, acquire, and analyze flow data using BD FACSVerse System. This document is available in print or as a PDF.
- **Reference system.** The information in this reference system is organized into the following parts:
  - **Introductory information.** This part contains information about system hardware and components, and a basic overview of BD FACSVerse System.
  - **Using the system.** This part contains sections that describe the tasks and provide typical workflows for the setup and operation of the system.
  - **Software reference.** This part contains information about the Library and describes the tools used for data visualization and analysis.
  - **System reference.** This part contains sections about the Loader, system options, cytometer configurations, system specifications, and troubleshooting.
- **Assay guide (RUO).** This document describes the BD-defined assays available for use with the system.
- **Technical data sheets.** These documents are included with reagents.

Safety symbols

Introduction

This topic describes that safety symbols used in this guide. For a complete description of all safety hazards, see the *BD FACSVerse System Safety and Limitations Guide*.

Safety symbols

The following table lists the safety symbols used in this guide to alert you to potential hazards.

Symbol	Meaning
	General warning. Risk of personal injury to operator.
	Biological hazard
	Electrical hazard
	Laser hazard
	Fire hazard
	Mechanical hazard, pinch points

System technical support

Introduction

This topic describes how to obtain assistance from BD Biosciences technical support.

Before contacting technical support

Try the following options for answering technical questions and solving problems:

- Read the section of this guide specific to the operation you are performing.
- Read topics of related information which are listed in the *More Information* section.
- Search the *BD FACSVerse System Reference* for a specific topic.

Contacting technical support

To contact technical support:

1. Go to www.bdbiosciences.com.
2. Select your region.
3. Click the **Support** link for details for your local region.

When contacting BD Biosciences, have the following information available:

- System health report that includes the Product name, part number, and serial number, and details of recent system performance. See Generating a system health report (page 481) for more information.
- Any error messages

More information

- System documentation (page 14)

Part 1: Introductory information

This part includes the following sections:

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About the system

This section includes the following topics:

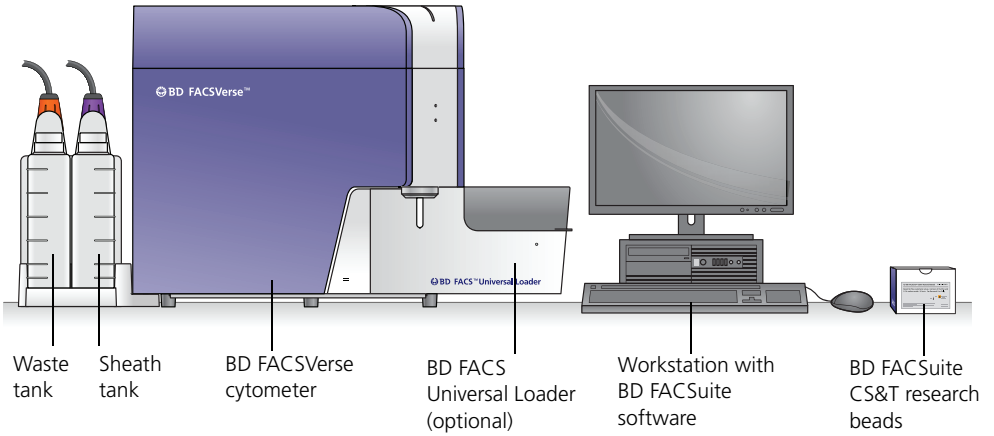
System overview

Introduction

This topic gives an overview of the BD FACSVerse system and provides a description of the main components.

About the system

The BD FACSVerse system includes the BD FACSVerse cytometer, the optional BD FACS Universal Loader, and BD FACSuite software running on the system workstation. The system also includes customized setup beads. All of these components combine to create an integrated system designed for use in a wide variety of multicolor research applications.



The BD FACSVerse flow cytometry system acquires and analyzes particles or cells in a liquid suspension. Antibodies to specific cell proteins are labeled with a fluorescent dye and incubated with the cell suspension. The suspension flows through the cytometer and is interrogated by a laser which excites the fluorescent antibodies. The fluorescence is captured and the resulting data is analyzed to reveal information about the cells. Multiple antibodies, each labeled with a different dye, can be used in a single tube to simultaneously identify different cell populations. This technique can be used in diverse research areas such as stem cell development, cell signaling pathways, and HIV.

BD FACSuite software is used to operate the instrument, acquire samples, and analyze the data. Quality control performance, tracking, and reporting are streamlined and automated. Routine tasks such as daily cleaning and shutdown can be programmed to occur automatically. Select from a variety of pre-defined research assay modules to quickly create experiments using existing BD reagent kits. The modular design of the software allows you to analyze data as samples are being acquired.

BD FACSVerse cytometer

The BD FACSVerse cytometer is a compact research flow cytometer. Several hardware options and upgrades can be used to customize the system for different applications.

The vacuum-driven fluidics along with a uniquely designed flow cell and sample injection tube provide reliability and good signal resolution. In addition to standard high, medium, and low flow rates, a special high-sensitivity fluidics mode makes it easier to detect dimly stained particles.

Three laser configurations provide the ability to analyze up to 8 colors (10 parameters). A unique heptagon detector array takes the guesswork out of changing filters and ensures that the correct filters and mirrors are installed.

BD FACS Universal Loader

The Loader is an optional automated loading system that delivers samples to the BD FACSVerse cytometer for acquisition. It is designed for walkaway operation.

The Loader offers various settings to resuspend and mix samples. It has the flexibility to draw from 12 x 75-mm tubes in 30- and 40-tube racks, and multiple types of microtiter plates, including deep-well plates. A barcode reader verifies the ID on tube racks, plates, and individual tubes. A built-in imaging system provides safety checks, such as verifying the correct rack type and tube orientation, and ensuring that tubes and plates were loaded correctly.

Workstation

The system is shipped with a workstation that includes a monitor, keyboard, and mouse. The workstation runs BD FACSuite software and other applications and controls the cytometer. The workstation comes equipped with these items:

- Microsoft® Windows® 7 operating system
- BD FACSuite software version 1.0 or later

The workstation requires a security key that plugs into a USB port to run the software.

Sheath and waste tanks

Several tank sizes are available depending on the sample throughput and needs of the individual laboratories. The standard 5-L capacity sheath and waste tanks are located to the left side of the cytometer in a dock. The dock can be disconnected from the cytometer. Optional 10-L extended-use tanks are also available. Level sensors alert you when fluid levels are low (sheath) or high (waste). Additionally, for high-volume labs, a BD FACSFlow cubitainer can be used to supply sheath fluid.

For more information on fluidics tank options, see System options and upgrades (page 31).

Beads, reagents, and assays

BD FACSuite CS&T research beads are used to check the cytometer performance and automatically make adjustments, ensuring consistent values from day to day and experiment to experiment.

BD FACSuite FC beads are used to set up compensation controls which are valid for 30 days.

BD research assays are available as predefined modules that can be used with standard BD reagents to support a wide range of applications. Worksheets with plots and gates are already set up for acquisition and analysis. The research assays can also be used as a starting point for creating customized experiments and assays.

More information

- Cytometer overview (page 23)
- System options and upgrades (page 31)
- BD FACSuite software overview (page 33)

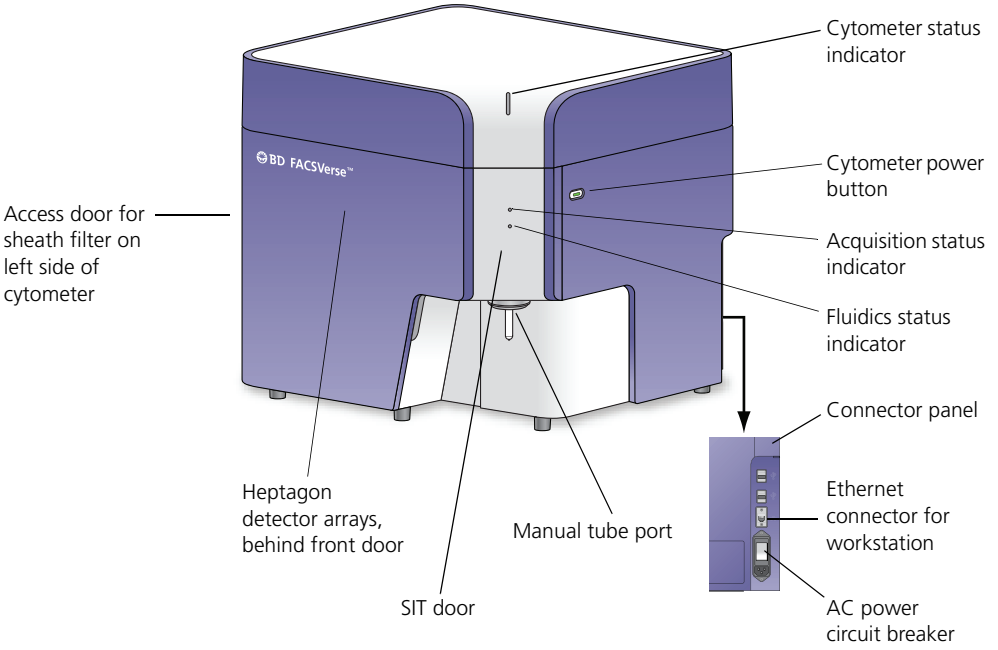
Cytometer overview

Introduction

This topic describes the main components of the BD FACSVerse cytometer, the location and functions of the status indicators, and the available cytometer configurations.

Main components

The locations of the main components of the cytometer, including the status indicators, are shown in the following figure.



Status indicators

When the system is started, status indicators display different conditions to indicate the system's status. The functions of the status indicators are described in the following table.

Indicator	Condition	Status
Cytometer status	Green	Ready for operation
	Solid amber	Fault condition
	Blinking amber	Warming up
	Red	System inoperable
Cytometer power button	Amber	Power is off to all major subsystems
	Green	Power is on
	Blinking green	Shutdown process has started
Acquisition status	Off	Not previewing or acquiring sample
	Blinking blue	Previewing or acquiring sample
Fluidics status	Off	Ready
	Blinking amber	• Sheath fluid low • Waste tank almost full
	Red	• Sheath fluid empty • Waste tank full • Waste tank disconnected

Cytometer configurations

The BD FACSVerse system is available in the following configurations.

Lasers	Number of colors
1 laser (blue)	4-color (4-0-0)
2 lasers (blue, red)	6-color (4-2-0)
3 lasers (blue, red, violet)	8-color (4-2-2)

More information

- Optical components (page 26)
- Fluidics components (page 28)
- System options and upgrades (page 31)
- BD FACSuite software overview (page 33)

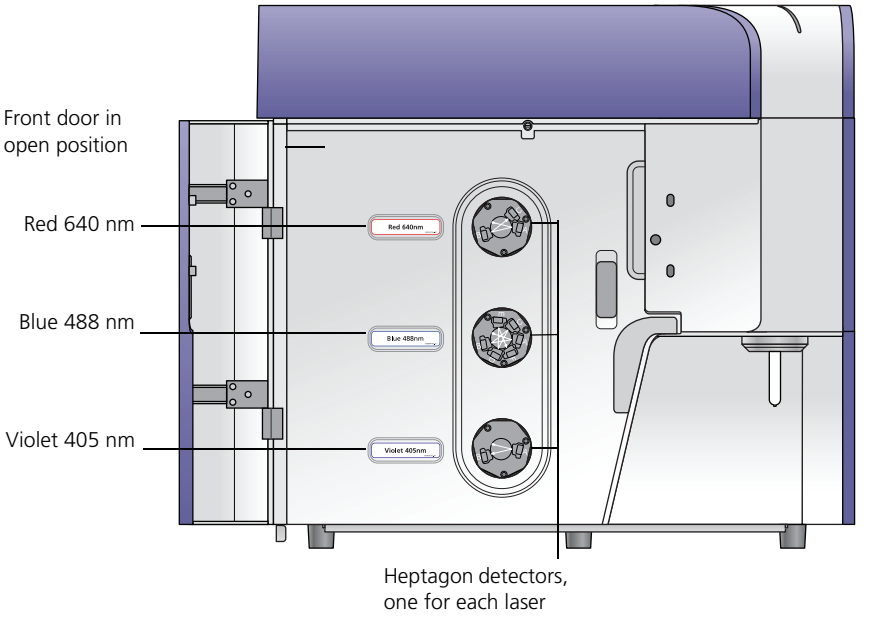
Optical components

Introduction

This topic describes the optical components, including the heptagon detectors and the filter holders.

Location of optical components

The optical compartment is located on the front of the cytometer, behind the front door. The heptagon arrays for each laser are accessible when the door is open. The following figure shows the locations of the optical components.



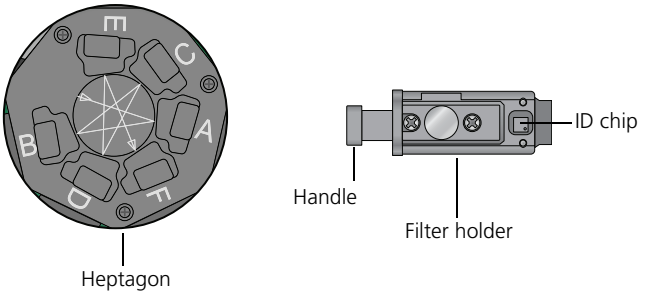
Heptagon detector arrays

The heptagon detector arrays contain the filters, mirrors, and photomultiplier tubes (PMTs) for each laser. There is a separate heptagon for each laser.

Filter holders

Each channel in a detector array has a removable filter holder that contains a bandpass filter and a mirror for that channel. The filter holder has an ID chip that identifies the holder to the system so the software can confirm that the correct filter holder is in place.

The following figure shows a heptagon and a filter holder.



Location of lasers

The system lasers and beam-steering optical components are located at the top of the cytometer, under the top cover. There is no user access to the laser area.

More information

- Fluidics components (page 28)
- System options and upgrades (page 31)
- Laser and detector configurations (page 538)
- Technical specifications (page 548)

Fluidics components

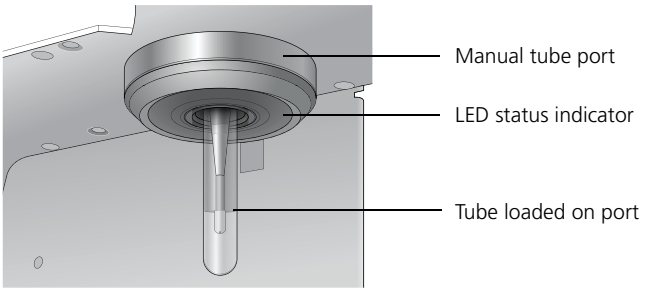
Introduction

This topic describes the main fluidics components of the BD FACSVerse system.

Manual tube port

The manual tube port is located on the right front of the cytometer. A circular LED indicator at the base of the port turns green when the system is ready to accept a tube.

The following figure shows the manual tube port.



The following table describes the conditions and status of the manual tube port indicator.

Condition	Status
Solid green	Ready to accept a tube
Blinking amber	SIT flush in progress, do not load a tube
Off	Tube is loaded

Sample injection tube (SIT)

The sample injection tube (SIT) is the tube that aspirates sample from a tube or a well and delivers it to the flow cell.

Qualified tubes

Only the following tubes have been qualified for use on the manual tube port on the cytometer.

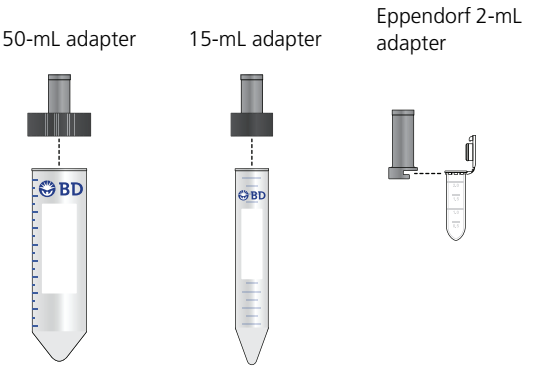
Tube type	Maximum volume
BD Falcon™ 5 mL (12 x 75-mm) polystyrene[a]	2 mL
BD Falcon 5 mL (12 x 75-mm) polypropylene[a]	2 mL
BD Trucount™ 5 mL (12 x 75-mm)[a]	2 mL
BD Falcon 15 mL (when used with adapter)	14 mL
BD Falcon 50 mL (when used with adapter)	45 mL
Eppendorf 2 mL (when used with adapter)	1.5 mL

a. For optimal performance in reducing carryover, fill to 0.5 mL or less, so the wash probe does not contact sample.

For information on BD part numbers for tubes, see the BD FACSVerse section of the BD Biosciences website.

Tube adapters

You can use 15-mL, 50-mL, and Eppendorf 2-mL tubes by installing a tube adapter. The 15-mL and 50-mL adapters screw onto the top of the tubes. The Eppendorf adapter slides onto the tube from the side.



Sheath filter

The sheath filter is located on the left side of the cytometer behind the access door. The sheath filter should be changed every three months.

More information

- Replacing the sheath filters (page 478)
- System options and upgrades (page 31)

System options and upgrades

Introduction This topic describes the BD FACSVerse system options and available upgrades.

Category	Option	Description
System hardware	BD FACS Universal Loader	The Loader is an optional automated loading system that mixes samples and delivers tube racks and plates to the BD FACSVerse system for acquisition. See BD FACS Universal Loader overview (page 502).
	Handheld barcode reader	The handheld barcode reader plugs into the USB port on the system computer workstation and reads most current barcode standards. See Using the handheld barcode reader (page 531).
	BD™ Flow Sensor	The optional Flow Sensor provides quick and accurate volume measurements for determining cell counts in a sample at medium and high flow rates. See About the BD Flow Sensor option (page 525).
Optics	Laser upgrades	Upgrade a 1-laser system to a 2-laser or 3-laser system. These upgrades can be done at the customer site.
Fluidics	Large fluidics tanks (10-L capacity)	The optional large volume sheath and waste tanks have a 10-L capacity. These tanks do not have a dock and are normally stored on the floor. See Fluidics tank options (page 532).
	Cubitainer	Sheath fluid can also be supplied from a BD FACSFlow cubitainer by using an optional adapter.

Category	Option	Description
Applications and assays	FCAP Array™ software version 3.0	FCAP Array software facilitates the data analysis of bead assays. These assays can detect the presence of, or determine concentrations for, multiple analytes (for example, proteins and peptides) in a sample.
	BD Assurity Linc™ software	BD Assurity Linc software is a highly secure remote systems management service that connects BD instruments and BD technical support personnel. Using the BD Assurity Linc Agent, BD support personnel can securely access your workstation through an enterprise server and diagnose problems remotely. See Using BD Assurity Linc software (page 534).
	BD assays	Contact your BD representative for a current list of available BD assays.

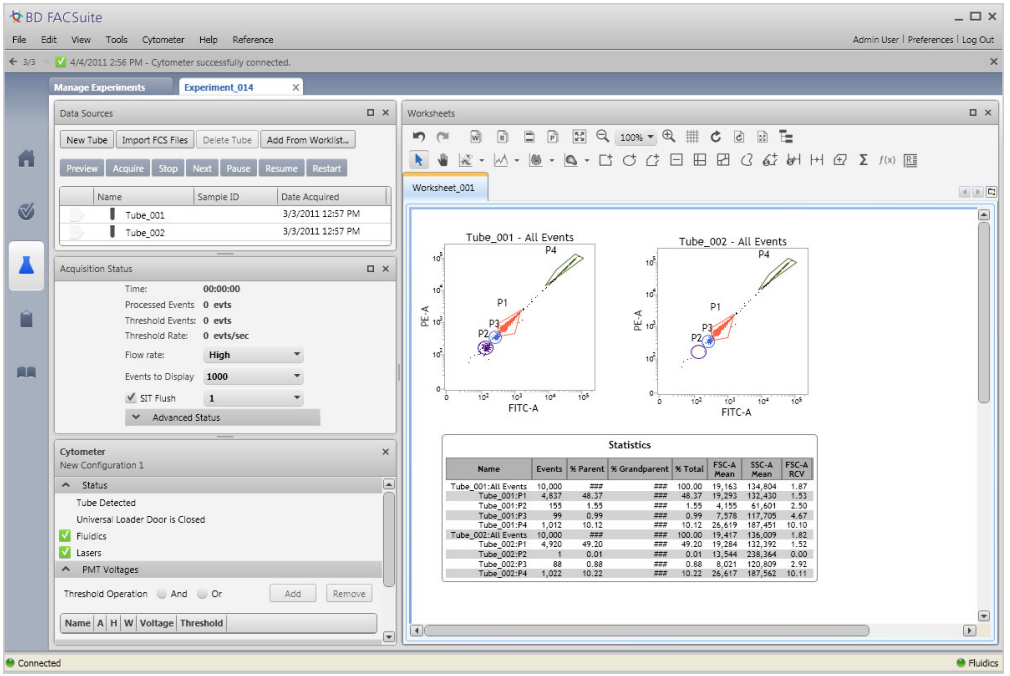
BD FACSuite software overview

Introduction

This topic provides an overview of the basic features and functionality of BD FACSuite software. BD FACSuite integrated multicolor flow cytometry software controls the BD FACSVerse cytometer and the optional BD FACS™ Universal Loader.

General software features

BD FACSuite software provides tools for data acquisition and analysis, and advanced setup and QC tools to maintain peak system performance. Using the software, you can customize the system hardware configuration and software interface to fit your specific workflow and application needs.



Use BD FACSuite software to build and refine experiments using the full array of BD reagents, to create assays from these experiments, and then to automatically acquire and analyze

samples from a list of entries in a worklist. Data can be displayed, saved, and exported based on your preferences.

Setup and QC

BD FACSuite software provides comprehensive tools to set up the cytometer, assays, and experiments and to run QC on a daily basis to maintain precise and reproducible results and ensure consistent performance.

The setup and QC procedures use BD FACSuite CS&T research beads and assays to measure and adjust cytometer PMT voltages. This ensures that target values for the cytometer and assays and experiments are maintained.

Levey-Jennings charts are generated. Use these charts to track and set acceptance criteria for various performance parameters. Use generated QC reports to document and track the system performance over time.

Experiments and assays

In BD FACSuite software, you can measure and analyze samples using either assays or experiments. Both formats organize and specify the conditions for acquisition and analysis for tubes.

Use assays when you want to run a specific protocol or analysis on samples repeatedly. Assays are run as entries in a worklist, which provides batch acquisition and analysis. Use experiments to test studies and different samples, and to develop protocols. Experiments are exploratory and highly customizable. You can define the properties of each tube and create custom tube settings and apply them to other tubes. You can refine an experiment and then save it as an assay to set the test protocols.

While assay protocols are pre-defined to target specific data (markers, populations, fluorochromes, etc) and look for specific results, you can modify plots, gates, and other worksheet or report elements. Worklists provide administrative control capabilities such as e-signature and audit trails. BD FACSuite software runs both BD-defined and user-defined assays.

In experiments, data can be displayed in a worksheet or report using any combination of plots or histograms. In BD FACSuite software, worksheets and reports are live data portals and continuously display current data. You can acquire data and then analyze it by gating populations of interest, displaying statistics, and adding expressions. You can create custom, formatted reports that include the details you want to analyze and present in a final lab report.

Worklist

The worklist is a list of tasks to be performed. It organizes multiple entries, which include tubes, tasks, status, and other information about the sample. Each entry task includes an assay or fluidics (cleaning or maintenance) procedure.

Using the worklist, you can acquire entries, display acquisition data, perform analysis on the acquired entries, and export data automatically based on your preferences.

With the BD FACS Universal Loader option, you can load tube racks or plates and run worklists in a more automated manner.

Library

The library stores and manages shared resources and assay properties. Resources include assays, beads, reagents, keywords, labels, and tube settings. You can import, add, and delete all resources. You can also edit keywords and assay properties.

Resources are used as elements in experiments, worklists, and setup and QC. For example, you can assign a tube setting to an experiment, or a keyword to an entry in a worklist.

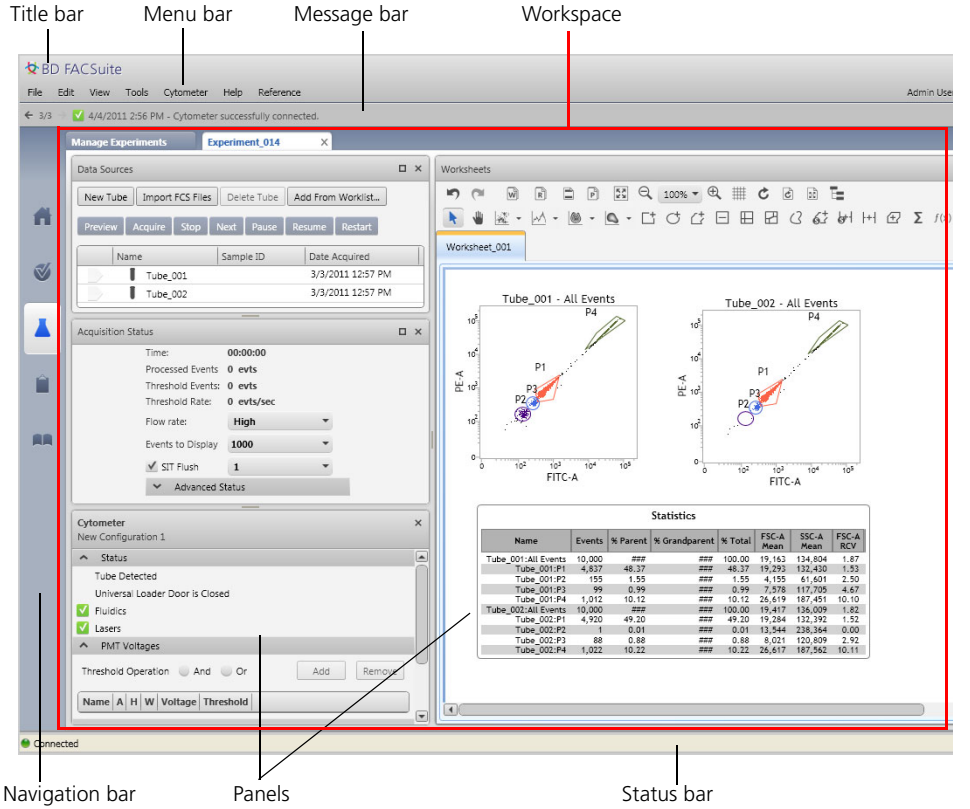
BD FACSuite software components

Introduction

This topic describes the components of BD FACSuite software windows and describes the menus, and shortcuts you can use to navigate the software and perform tasks.

Window components

The BD FACSuite windows consist of the following components.



- **Title bar.** Displays the BD FACSVerse System product name and the standard window controls (minimize, maximize, close).
- **Menu bar.** Displays the following software menus.

Menu	Description
File	This menu includes specific tools and items for the current window or workspace. Choices include importing, exporting, printing, saving, and managing specific workspaces (for example, creating a new experiment, opening a worklist).
Edit	This menu includes Cut, Copy, Paste, Delete, Undo, Redo, and other editing tools.
View	This menu includes display control items.
Tools	This menu includes user management, preferences, administration, tracking, and setup items.
Cytometer	This menu includes cytometer cleaning, information, and control items.
Help	This menu includes documentation in PDF format and basic software information.
Reference	This menu includes the BD FACSVerse System Reference. Use the BD FACSVerse System Reference to view and search for information on using the system.
User profile (username)	This menu item opens the My Profile dialog. Use this dialog to manage your login password and user profile information.
Preferences	This menu item opens the Preferences dialog. Use this dialog to set default system preferences.
Logout	This menu item log the current user out of BD FACSuite software.

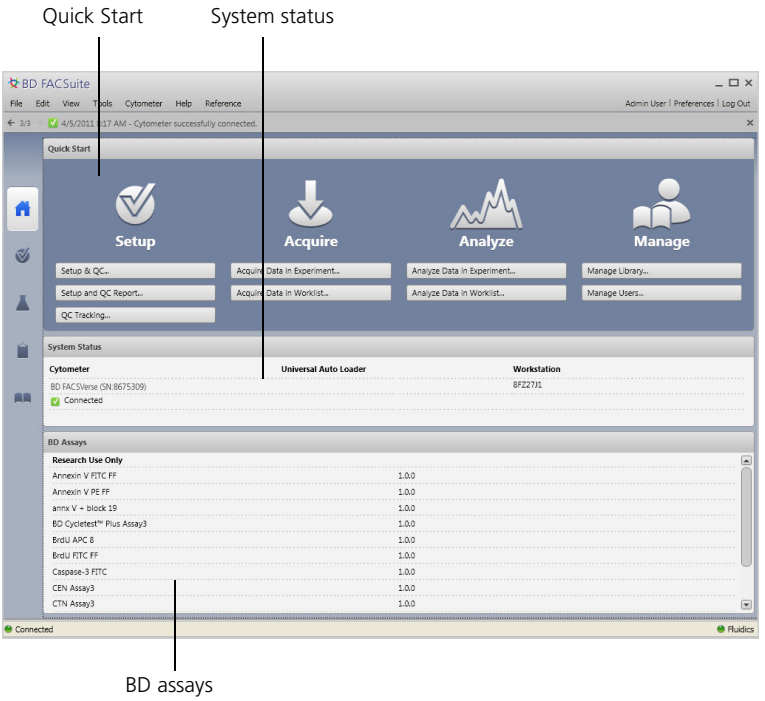
- **Message bar.** Located at the top of the window (below the Menu bar) and displays system messages.
- **Workspaces.** Contain the panels, fields, tables, and tools required for a specific function. Individual workspaces are provided for setup and QC, experiments, worklists, and the library.

- **Navigation bar.** Located at the left side of the window. Click the navigation bar icons to open the different workspaces.
- **Panels.** Contain the tools, fields, and options for performing specific and detailed functions required for a workspace. You can maximize, minimize, or reposition panels on the screen.
- **Status bar.** Located at the bottom of the window and displays the current cytometer connection status, fluidics status, and an acquisition progress bar.

About the Home page

The Home page is the default starting page. This page includes the following sections:

- **Quick Start.** Displays shortcuts for the most commonly used workflows or operations.
- **System Status.** Displays the current status and serial numbers of the system for the fluidics, lasers, and all system components, and the connection status. It also displays all installed options.
- **BD Assays.** Displays the list of currently installed BD-defined assays.



Quick Start shortcuts

The following table describes the available Quick Start shortcuts.

Shortcut	Task	Description
	Setup & QC	Opens the Setup & QC workspace and displays the Setup & QC tab.
	Setup & QC Report	Opens the Setup & QC workspace and displays the QC Report tab.
	QC Tracking	Opens the Setup & QC workspace and displays the QC Tracking tab.

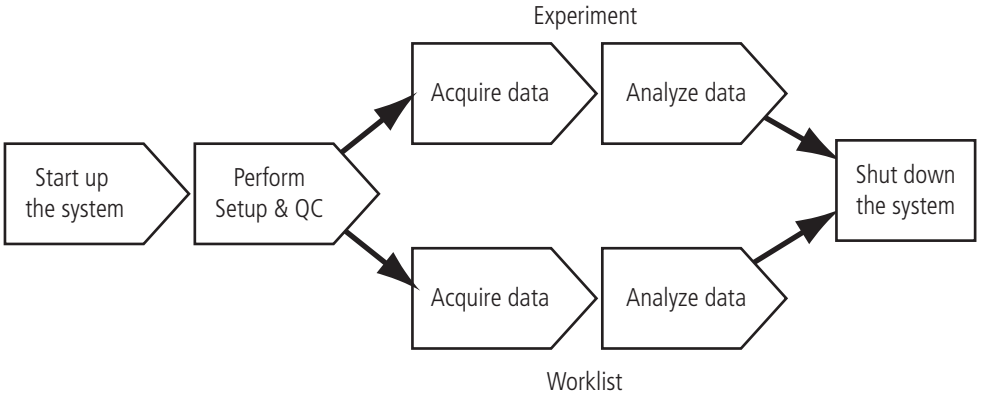
Shortcut	Task	Description
	Acquire Data in Experiment	Opens the Experiment workspace and displays a new experiment.
	Acquire Data in Worklist	Opens the Worklists workspace and displays a new worklist.
	Analyze Data in Experiment	Opens the Experiment workspace and displays the Experiment Management tab.
	Analyze Data in Worklist	Opens the Worklists workspace and displays the Worklists Management tab.
	Manage Library	Opens the Library workspace and displays the categories of library resources.
	Manage Users	(Administrators only) Opens the User Management dialog and displays the list of users.

More information

- System overview (page 20)
- Daily workflow (page 41)
- System documentation (page 14)

Daily workflow

The following diagram shows the typical daily workflow for the BD FACSVerse system.



More information

- System setup (page 43)
- System startup and shutdown (page 111)
- Daily setup and QC (page 117)
- Experiment acquisition and analysis (page 129)
- Worklist acquisition and analysis (page 221)
- Maintenance (page 467)

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3

System setup

This section includes the following topics:

Setting up the system

Introduction

This topic describes system setup and the setup tasks you can perform after system installation and configuration.

About system setup

Initial system installation and configuration are performed by your BD Service representative. After installation, the CS&T bead lot is imported, the system is characterized (characterization QC), and the default tube settings and references settings are created. A default administrator user account allows complete administrative access to your system.

BD FACSuite software includes default user accounts and preferences. You can operate the system using these default user accounts and preferences, or you can add user accounts (as needed) and modify preferences to suit your specific needs.

Setup tasks

Although you can perform many setup tasks in any order (as needed), the following tables describe the setup tasks and when they should be performed.

Initial setup tasks (performed after installation)

These tasks are typically performed after installation. Most tasks can also be performed at any time as needed.

Task	Purpose	Who	For more information
Import a CS&T bead lot	Adds a CS&T bead lot for a new installation, or replaces an expiring bead lot file. This is typically performed after installation, then whenever bead lots expire.	All users	See Importing or adding a CS&T bead lot (page 82).
Run characterization QC	Establishes a system baseline. This is typically performed after installation, then every six months.	Administrator only	See Running characterization QC (page 83).
Set administration preferences	Controls connected systems and software, and generates a system health report. This is typically performed after installation, but it can be performed at any time as needed.	Administrator only	See Setting administration preferences (page 50).
Manage users	Adds user accounts or manages users and passwords. This is typically performed after installation, but it can be performed at any time as needed.	Administrator only	See Managing user accounts (page 51).

Daily setup tasks

These tasks should be performed daily in the following order.

Task	Purpose	Who	For more information
Run performance QC	Measures daily system performance. These tasks should be performed daily, and as needed.	All users	See Running daily performance QC (page 123).
Run assay and tube setup	Prepares the system for daily use. These tasks should be performed daily, and as needed.	All users	See Running Assay and Tube Settings Setup (page 125).

Periodic setup tasks

These tasks can be performed as needed.

Task	Purpose	Who	For more information
Import a CS&T bead lot	Adds a CS&T bead lot for a new installation, or replaces an expiring bead lot file. This is typically performed after installation, then whenever bead lots expire.	All users	See Importing or adding a CS&T bead lot (page 82).
Run characterization QC	Establishes a system baseline. This is typically performed after installation, then every six months.	Administrator only	Running characterization QC (page 83).
Manage users	Adds user accounts or manages users and passwords. This is typically performed after installation, but it can be performed at any time as needed.	Administrator only	See Managing user accounts (page 51).
Set preferences	Defines how the system operates, displays, and organizes data. This can be performed at any time.	All users	See BD FACSuite preferences (page 61).
View the system optical configuration.	Displays the current laser, mirror, and filters. This can be performed at any time.	All users	See Cytometer configurations (page 537).

Task	Purpose	Who	For more information
View setup and QC reports.	Displays setup and QC results. This can be performed at any time.	All users	See About setup and QC reports (page 93).
Set up and view Levey-Jennings reports.	Determines how performance is tracked over time. This can be performed at any time.	All users	See QC tracking overview (page 97).
Manage My Profile	Manages your user profile and password. This can be performed at any time.	All users	See Managing my profile (page 60).

More information

- Setup and QC overview (page 79)
- About tube and reference settings (page 90)

Setting administration preferences

Introduction

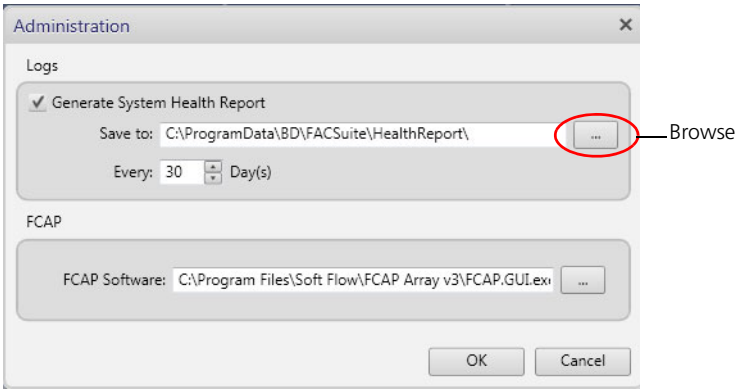
This topic describes how Administrators can set administration preferences. Administration preferences are global settings that specify where files are saved and when the system health reports are generated for the cytometer.

Procedure

To set administration preferences:

1. From the menu bar, select **Tools > Administration**.

   The **Administration** dialog opens.



2. Under **Logs**, select the **Generate System Health Report** checkbox to automatically create system health reports.
3. (Optional) Specify a different destination folder for system health reports.
   a. Click the **Browse** button to open the **Browse for Folder** dialog.
   b. Select a folder and click **OK**.
4. Enter or select a value in the **Every x Days** field to specify the frequency for generating system health reports.

   The default schedule is every 30 days.

5. If FCAP Array software is installed on your system, click the **FCAP Array Browse** button and navigate to the FCAP.exe file.
6. Click **OK** to save your administration preferences and close the dialog.

More information

- Setting up the system (page 45)
- Managing user accounts (page 51)

Managing user accounts

Introduction

This topic describes the user management tools in BD FACSuite software.

Administrators can use the User Management window to create and manage user accounts in BD FACSuite software, as well as manage and assign corresponding departments to user accounts and create passwords.

User account types

BD FACSuite software includes the following default user accounts:

- **Administrator.** This account can administer and manage all accounts (except BD Service) and has complete access to administrator and operator accounts.
- **Operator.** This account can only administer its own profile with certain limitations.

  See Managing my profile (page 60).

About the User Management window

The User Management window can be accessed only by Administrator user accounts. The window is divided into two panels: a Master panel that displays a table of current users and user information, and a Details panel for creating or editing information about the user.

User management tasks

The following table lists the user management tasks.

To...	See...
Add or edit the department that is associated to a user.	Managing departments (page 52)
Add or edit user profiles.	Managing users (page 54)
Set the password policy for your users.	Setting user login and password policies (page 57)
Export or import user accounts.	Importing and exporting users (page 58)

More information

- Setting up the system (page 45)
- Setting administration preferences (page 50)

Managing departments

Introduction

This topic describes how to manage departments by adding, editing, and deleting their information.

Departments must be created before you can assign users. This is an Administrator task.

Adding new departments

To add a new department:

1. From the menu bar, select **Tools > User Management**.

   The **User Management** panel opens.

2. In the **Departments** tab, click **New**.

   The **New Department** detail panel displays at the bottom of the tab.

3. Enter values in all required fields and optional fields, as needed.

   All values are alphanumeric text. All fields have a 30-character limit, except the **Address** field, which has a 40-character limit and the **URL** field, which has a 200-character limit.

4. (Optional) Add a custom department field if needed.

   a. Click the **Settings** tab.

   b. Under **Custom Department Fields**, click in a field and type a category (for example, *Supervisor*).

   c. Click the **Department** tab.

   The new department field is displayed in the **Department** detail panel.

5. Click **Done** to add the new department settings to the table.

Editing departments

To edit a department:

1. In the **Departments** tab, select a department to edit.

   The **Department** detail panel displays at the bottom of the tab.

2. Click **Edit**.

3. Edit the information as necessary.

4. Click **Done**.

Deleting a department

To delete a department:

1. In the **Departments** tab, select the department to delete.

   You can delete only one department at a time. Departments containing user accounts cannot be deleted.

2. Click **Delete**.

   The **Delete Department** dialog opens.

3. Click **Yes** to confirm the deletion.

The department is deleted.

More information

- Managing user accounts (page 51)
- Managing users (page 54)

Managing users

Introduction

This topic describes how Administrators can add a new user in BD FACSuite software and edit their information later.

Users must be assigned to a department in an institution. The value for the department can be None.

Adding a new user

To add a new user:

1. From the menu bar, select **Tools > User Management**.
2. In the **Users** tab, click **New**.

   The **User** detail panel opens at the bottom of the tab.

   Several fields in this panel use pre-defined values from other tabs in the **User Management** window (**Departments, Passwords,** and **Roles**).
3. Enter values for all required fields, and the optional fields as needed.

Values are alphanumeric text.

In the field...	Enter the value for...
First Name (Required)	First name for the user (1–20 characters).
Last Name (Required)	Last name for the user (1–20 characters).
User ID (Required)	A user ID for the user (1–25 characters).
Title	A work title for the user (1–30 characters).
Status	A status for the user: • **Active.** For users who are granted access to BD FACSuite software. • **Inactive.** For users who are no longer granted access to the software. • **Locked.** For active users with expired passwords, or users who have exceeded the maximum number of failed login attempts.
Department (Required)	A department for the user, as defined in the **Departments** tab. The value can be **None**.
Institution (Required)	An institution for the user, as defined in the **Departments** tab. If the Department value is **None**, then the Institution value is **None**.
Email	An email address for the user (must be 1–60 characters and include the @ symbol).
Role	A role for the user (for example, Administrator or Operator (default)).
Password Expiration Date	The date that the user password expires (for example, Password Expiration Date = today's date + Password Expiration Days). Password details are defined in the **Settings** tab.

In the field...	Enter the value for...
Phone	A phone number for the user.
Temporary Password (Required)	A temporary (initial login) password. Administrators can type specific passwords (case-sensitive, 4–16 characters, no spaces), or generate a random password by clicking **Generate Password**. At first login, the user is prompted to enter a new password.
Notes	Any notes to document history, or other descriptions of the new user (maximum of 250 characters).

4. (Optional) Add a custom user field if needed.
   a. Click the **Settings** tab.
   b. Under **Custom User Profile Fields**, click a field and type a category (for example, *Supervisor*).
   c. Click the **Users** tab.

   The new user profile field is displayed in the **User** detail panel.
5. Click **Done** to save the new user to the **Users** table.

Editing user details

To edit user details:

1. In the **Users** tab, select a row in the **Users** table.
2. Click **Edit**.
3. Edit the information as needed.
4. Click **Done**.

Resetting a user password

To reset a user password:

1. In the **Users** tab, select a row in the **Users** table.
2. Click **Edit**.
3. In the **User** detail panel, click **Reset Password** to generate a random password, or type a new password in the **Temporary Password** field.

4. In the **Password Expiration Date** field, reset the expiration date for the new password.
5. (Optional) Click the **Settings** tab to view the password policies.
6. Click **Done**.

Making users inactive

To make a user inactive:

1. In the **Users** tab, select a row in the **Users** table.
2. Click **Edit**.
3. In the **User** detail panel, select **Inactive** in the **Status** menu.
4. Click **Done**.

   The user status becomes inactive in the **Users** table and access is denied.

More information

- Managing user accounts (page 51)
- Managing departments (page 52)

Setting user login and password policies

Introduction

This topic describes how to set the user login and password policies for BD FACSuite users. These settings are global and changes affect all users. This is an Administrator task.

Procedure

To set user login and password policies:

1. From the menu bar, select **Tools > User Management**.
2. Click the **Settings** tab.
3. Enter values for all required fields and optional fields, as needed.

Values are alphanumeric text.

For the field...	Enter the value for...
Lockout Attempts	The maximum number of allowed invalid attempts (None, 3, 4, or 5) before login is locked. When None is selected there is no limit on the number of attempts.
Password Expiration	The number of days before a password expires.
Password Expiration Warning	The number of days before a password expires to start displaying the password expiration warning.
Custom User Profile Fields	The number of characters allowed for each required custom text (1–20 characters). If this field is blank, it is not added to the user profile.
Custom Department Fields	The number of characters allowed for each required custom text (1–20 characters). If this field is blank it is not added to the department profile.

More information

- Managing users (page 54)
- Importing and exporting users (page 58)

Importing and exporting users

Introduction

This topic describes how to import existing user management information into the User Management window. This allows you to transfer a repository of all user information. This is an Administrator task.

Importing users

Importing overwrites all current user management information in the User Management window. Logged in user and department information is not affected.

To import existing user management settings:

1. From the menu bar, select **Tools > User Management**.

   The **User Management** window opens.

2. Click **Import**.

   The **Import** dialog opens.

3. Navigate to the user management (XML) file you want to import, then click **Open**.

   All user management related data (user profile, department, and settings) are imported.

Exporting users

You can export (save) user management information as an XML file. You can then share this information with other BD FACSuite systems. You cannot export the BD Service account information.

To export user management information:

1. In the **User Management** window, click **Export**.

   The **Export** dialog opens.

2. Type a name for the file you want to export.

3. Navigate to the target folder, then click **Save**.

   The user management information (user profile, department, and settings) are exported as an XML file.

More information

- Managing user accounts (page 51)
- Managing my profile (page 60)

Managing my profile

Introduction

This topic describes how to manage your user profile and passwords. All users can manage profile information using the My Profile dialog. Only Administrators can create users and set an initial password in the User Management dialog.

See Managing user accounts (page 51) for administrator information about creating and managing user accounts.

Procedure

To manage your user profile:

1. Click the user name that appears in the right corner of the BD FACSuite title bar.

   The **My Profile** dialog opens.

   The name, user ID, and role appear in read-only fields.

2. Edit the personal profile information as needed.

Changing your password

To change your password:

1. In the **My Profile** dialog, click **Change Password**.

   The **Change Password** dialog opens.

   The **User ID** field displays the current User ID.

2. In the **Old Password** field, type your old password.
3. In the **New Password** field, type a new password.

   Passwords are case-sensitive and must be between 4–16 characters (with no spaces).

4. In the **Confirm New Password** field, type your new password again.
5. Click **OK** to set the new password.

More information

- Managing user accounts (page 51)
- BD FACSuite preferences (page 61)

BD FACSuite preferences

Introduction

This topic describes preferences: what they are and how they are managed.

About preferences

Preferences specify administration settings, display options, schedules for automatic actions, notifications, and other functions. They include settings for the System, Worklist, Experiment, Setup & QC, and BD FACS Universal Loader. Once set, preferences persist until modified.

All preferences, except system preferences, are specific to a user. Changes to system preferences affect all users while changes to other preferences affect only the logged-in user and his view.

The ability to edit preferences is defined by your assigned role. Administrators can set and edit preferences for all users but operators can set and edit only their user-defined preferences.

Preferences

The following table describes the various preferences.

Preference	Description
System	These global preferences set system startup and behavior, programmed startup and shutdown, and other general system settings. System preferences are applied to all users.
Setup & QC	These preferences set automatic printing for Setup and QC reports, exported file locations, QC expirations, and QC dot plot parameters for specific cytometer configurations. Setup and QC preferences are associated to each user ID, so you can customize them without affecting other users.
Experiments	These preferences set preview and acquisition and default tube settings. Experiment preferences are associated to each user ID, so you can customize them without affecting other users.
Worklist	These preferences set the acquisition and report delay timers, define exported file names and locations, and set printing options. Worklist preferences are associated to each user ID, so you can customize them without affecting other users.
Universal Loader	These preferences define how samples are loaded and mixed. Loader preferences are associated to each user ID, so you can customize them without affecting other users.

More information

- Setting system (global) preferences (page 63)
- Setting setup and QC preferences (page 66)
- Setting experiment preferences (page 70)
- Setting worklist preferences (page 71)
- Setting Loader preferences (page 76)

Setting system (global) preferences

Introduction

This topic describes how to set the system general preferences.

These preferences are global and are applied to all users. We recommend that only Administrators or lab supervisors set these preferences.

Setting system general preferences

This procedure describes how to set the system general preferences for the default startup view, a notification type to indicate completion of a task, language selection, and information that displays on assay and Setup and QC reports.

To set the system general preferences:

1. From the menu bar, select **Tools > Preferences**.

   The **Preferences** dialog opens with the **System** tab displayed**.**

2. Select **General** in the left panel.

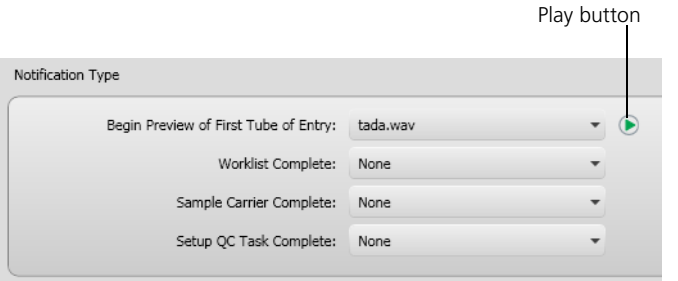
3. Under **Startup**, select a default startup view from the Default Startup View menu.

   The selected workspace is displayed when the software opens.

4. Under **Notification Type**, select a sound for each notification type.

   The value can be None.

   a. Click the **Play** button to hear the selected sound for each notification type.



5. Under **Reports,** enter values in the **Director** and **Address** fields.

   Values can be alphanumeric characters. The information entered here is displayed as a header or footer on all assay and setup and QC reports.

6. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

Setting cytometer schedule preferences

This procedure describes how to specify the cytometer shutdown and automatic startup schedules. Setting shutdown sets the time that the cytometer can stay idle before the system shuts down. Setting preprogrammed startup sets the times when the system automatically starts. When the schedules are set, the cytometer automatically starts at the scheduled time and shuts down after the defined idle time.

The default setting is unprogrammed (manual).

To set cytometer schedule preferences:

1. From the menu bar, select **Tools > Preferences**.

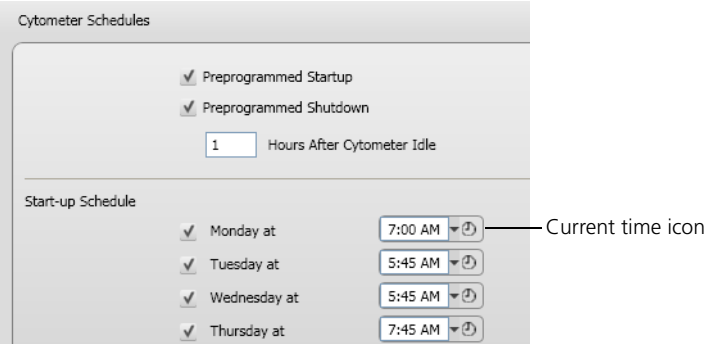
   The **Preferences** dialog opens with the **System** tab displayed**.**

2. In the **System** tab, select **Hardware** in the left panel.

3. Under **Cytometer Schedules**, select the **Pre-programmed Startup** checkbox.

   The **Start-up Schedule** fields are enabled.

4. Specify the startup days and times by selecting checkboxes for the days and then entering times. You can also use the current time icon to set the time to the current time.



   BD FACSuite software must remain running for automatic startup to begin. Do not turn BD FACSuite software off at the end of the day. The fluidics tanks need to be refilled and the waste emptied as in any other startup procedure.

5. Under **Cytometer Schedules**, select the **Pre-programmed Shutdown** checkbox.

   The **Hours After Cytometer Idle** field is enabled.

6. Specify the length of time that the system can be idle before shutting down (1–24 hours).

7. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

More information

- BD FACSuite preferences (page 61)
- Setting setup and QC preferences (page 66)

Setting setup and QC preferences

Introduction

This topic describes how to specify setup and QC preferences.

Since these preferences are associated to each user ID, you can customize them without affecting other users.

Specifying report preferences

This procedure describes how to specify setup and QC preferences for printing reports and including linearity charts in characterization QC reports.

To specify setup and QC report preferences:

1. From the menu bar, select **Tools > Preferences**.

   The **Preferences** dialog opens.

2. Click the **Setup & QC** tab.

   The **Setup & QC Preferences** dialog opens, displaying a left panel with a list of options and the **Reports** preferences in the right panel.

3. Select the **Automatically print Setup Report** checkbox to automatically print the setup and QC report, on the default printer when it is generated.

4. Select the **Include Linearity Charts in the Characterization QC Report** checkbox to include linearity charts in the characterization QC report.

   This selection is available to Administrators only.

5. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

Specifying file location preferences

This procedure describes how to specify the file location for the file types generated during setup and QC. Default locations can be specified for both automatically and manually exported files.

To specify file locations:

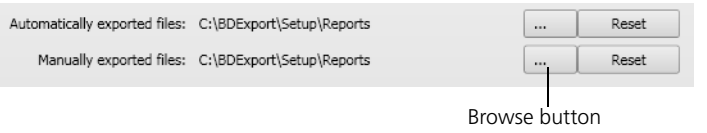
1. From the menu bar, select **Tools > Preferences**.

2. In the **Setup & QC** tab, select **File Locations** in the left panel.

   You can view or modify the destination folder location for exporting generated setup reports.

3. Specify a file location (storage) path for each generated file type. The default location is C:\BDExport\Setup\Reports.

   a. Click the **Browse** button to display the **Browse For Folder** dialog.



   b. Select a folder and click **OK**.

   The new file path with the new folder name is displayed.

4. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

Setting expiration preferences

This procedure describes how to specify the expiration preferences for characterization QC, performance QC, and LW/LNW and user-defined reference settings. This task is available only to Administrators.

To set expiration preferences:

1. From the menu bar, select **Tools > Preferences**.
2. In the **Setup & QC** tab, select the **Expiration** option.

3. Enter the expiration durations for characterization QC, performance QC, LW/LNW (default reference settings), and user-defined reference settings.

Item	Expiration limit
Performance QC	24 hours
Characterization QC	12 months
Lyse wash/Lyse no wash settings	190 days

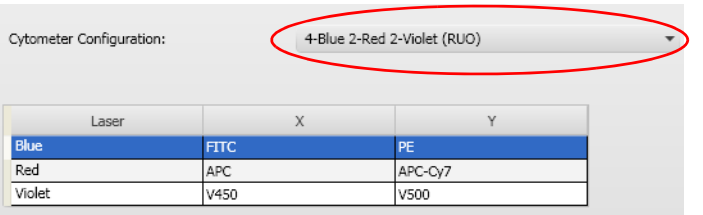
4. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

Setting dot plot parameter preferences

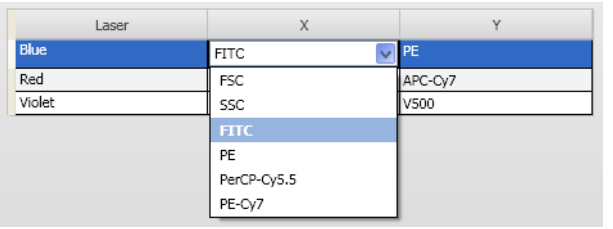
This procedure describes how to specify the default X and Y dot plot parameters for each installed laser on the system for viewing during setup and QC.

To set dot plot parameter preferences:

1. From the menu bar, select **Tools > Preferences**.
2. In the **Setup & QC** tab, select **Dot Plot Parameters** in the left panel.
3. In the **Cytometer Configuration** field, select a cytometer configuration from the list.



4. In the parameters table, double-click any parameter in the **X** or **Y** column to enable editing.



5. Select a different parameter for the X and Y axis.
6. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

   Plots display these parameters during characterization QC and performance QC for this configuration.

More information

- Setting system (global) preferences (page 63)
- Setting experiment preferences (page 70)

Setting experiment preferences

Introduction

This topic describes how to set experiment preferences. Experiment preferences can be set for each user and apply to all experiments created and run by that user.

Since these specific preferences are associated to each user ID, you can customize them without affecting other users.

Procedure

To set experiment preferences:

1. From the menu bar, select **Tools > Preferences**.

   The **Preferences** dialog opens.

2. Click the **Experiment** tab.

3. In the **Default Tube Settings for the new Experiment** field, select either **Lyse No Wash** or **Lyse Wash** from the list.

4. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

More information

- About tube and reference settings (page 90)
- Setting setup and QC preferences (page 66)
- Setting worklist preferences (page 71)

Setting worklist preferences

Introduction

This topic describes how to set preferences for running a worklist, details for exporting entry run packages, FCS files and results, and printing options.

Since these specific preferences are associated to each user ID, you can customize them without affecting other users.

Setting worklist acquisition preferences

To select worklist acquisition preferences:

1. From the menu bar, select **Tools > Preferences**.

   The **Preferences** dialog opens.

2. Select the **Worklist Preference** tab.

   The **Worklist Preference** dialog opens with a left panel that displays a list of options and a right panel that displays preferences.

3. Select **Acquisition** from the left panel.

4. Under **Acquisition Delay Timer:**

   a. Enter a value in the **Preview for** field to set how long to preview. This is the time to update PMT voltages and move gates before data is acquired.

   b. Select one of the following options:

   - **Use Acquisition Delay Timer and audible alarm for 1st tube in each entry**
   - **Use Acquisition Delay Timer for all tubes**

5. Under **Report Delay Timer**, in the **Preview for** field, enter a delay value.

   This time is the duration that the report is displayed before the next tube or entry acquisition is started.

   See Worklist tab overview (page 227) for more information about the Acquisition Delay Timer and the Report Delay Timer.

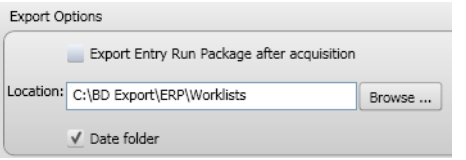
6. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

Setting entry run package export preferences

An entry run package includes all the information needed to replicate an entry in a different worklist. This includes acquired data.

To set entry run package export preferences:

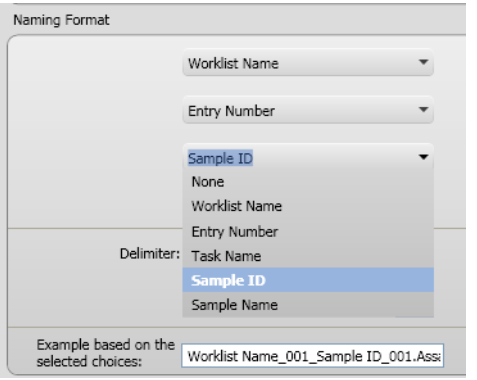
1. In the **Worklist Preference** tab, double-click **Export,** then click **Entry Run Package** to view the export options.
2. Under **Export Options**, select the **Export Entry Run Package after acquisition** checkbox to automatically export the entry run package, the FCS file, and metadata which are generated and saved to the specified folder location when the entry status is Approved.



3. In the **Location** field, specify the folder where the exported entry run packages are stored. Click the **Browse** button to display the **Browse for folder** dialog.
4. If you want to create separate dated folders for exported files, select the **Date folder** checkbox.

   A folder with the current date in *yyyymmdd* format is added to the **Location** field. For example, if the folder location field is *BDExport\ERP\Worklists* and the checkbox is selected, then the folder location becomes: *BDExport\ERP\Worklists\20110701*.
5. Under **Naming Format**, click one or more name fields, then select a naming element or type a name.

The naming elements you select are displayed in the **Example based on selected choices** field as an example. The selections are used in setting the name of the entry run package.



6. Click the **Delimiter** field and select a delimiter to display between naming elements in the file name.
7. Select the **Auto numbering Starting with** checkbox to add auto numbering to the file name.

   The example field shows an example of the resulting name (for example, *Worklist Name_001_Sample ID_001_AssayName*).
8. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

Setting FCS export preferences

An FCS file is a file that contains the raw data. In BD FACSuite software, the FCS standard is 3.0.

To set FCS export preferences:

1. In the **Worklist Preference** tab, select **Export > FCS**.
2. Under **Export Options**, select the **Export FCS after acquisition** checkbox to automatically export the FCS file that is generated when you run a worklist.

3. In the **Location** field, specify the folder where exported FCS files are exported. Click the **Browse** button to display the **Browse for folder** dialog.
4. If you want to create separate dated folders for exported files, select the **Date folder** checkbox.

   A folder with the current date in *yyyymmdd* format is added to the **Location** field. For example, if the folder location field is *BDExport\FCS\Worklists* and the checkbox is selected, then the folder location becomes: *BDExport\FCS\Worklists\20110701*.
5. Under **FCS Naming Format**, click one or more name fields, then select a naming element or type a name.

   The naming elements you select are displayed in the final name format as an example. The selections are used in setting the name of the FCS file.
6. Click the **Delimiter** field and select a delimiter to display between naming elements in the file name.

   The example field shows an example of the resulting name.
7. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

Setting result export preferences

Results are generated by running entry run packages in a worklist. One CSV file is created for the worklist. The file contains a column header row that contains the sample ID, assay name, export date, and the name of each exported result from all assay tasks in the worklist.

To set result export preferences:

1. In the **Worklist Preference** tab, select **Export > Result**.
2. Under **Export Options**, select the **Automatically export results** checkbox to automatically export the results.
3. In the **Location** field, specify the folder to export results to. Use the **Browse** button to display the **Browse for folder** dialog.

4. If you want to create separate dated folders for results files, select the **Apply date to folder** checkbox.
5. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

Setting worklist printing preferences

To set worklist printing preferences:

1. In the **Worklist Preference** tab, select **Print**.
2. Under **Print Options**, select the **Print Report for Entries** checkbox to automatically print a report when the entry state is approved.
3. Continue with additional preferences, or click **OK** to save your preferences and close the dialog.

More information

- Setting experiment preferences (page 70)
- Exporting entries and worklists (page 268)
- Setting Loader preferences (page 76)

Setting Loader preferences

Introduction

This topic describes how to set the loading, sample carrier, and mixing preferences for systems equipped with the BD Universal Loader option.

These preferences are user specific and are applied as the default each time you load sample carriers using the Loader. You can customize these settings for entries in a worklist and override these default preferences.

Setting loading options

To select loading options:

1. From the menu bar, select **Tools > Preferences**.

   The **Preferences** dialog opens.

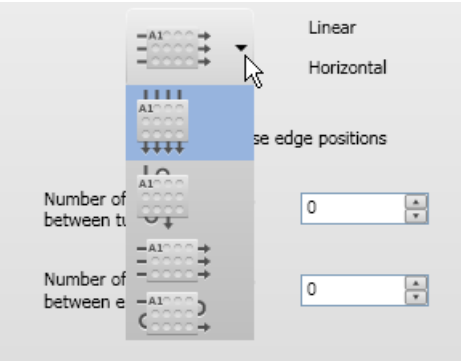
2. Click the **Loader** tab.
3. Select **Loading Options** in the left pane.
4. For **Loader type,** select *Loader.*
5. For **Carrier Type,** select a carrier (for example, *30 Tube Rack*).
6. (Optional) Select the **Unload Sample Carrier at end of Worklist Run** checkbox to have the Loader unload the sample carrier after each worklist run completes.
7. Click **OK** to save your preferences and close the dialog.

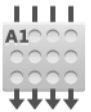
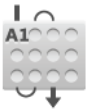
Setting carrier properties

To set carrier properties:

1. In the **Loader** tab, select **Carrier Properties** in the left pane.

2. Click the drop-down arrow and select a layout as shown in the following figure.



Layout option	Description
	Horizontal linear layout starts at A1 and loads each tube in the row. Once a row completes, the tubes in the next row load starting with B1.
	Vertical linear layout starts at A1 and loads each tube in the vertical row. Once a row completes, the tubes in the next vertical column load starting with A2.
	Horizontal serpentine layout starts at A1 and loads horizontal rows of tubes from the carrier left to right, then from right to left.
	Vertical serpentine layout starts at A1 and loads vertical columns of tubes from the carrier top to the bottom, then from bottom to top.

3. (Optional) Select the **Don't use edge positions** checkbox to ignore edge carrier positions.

4. (Optional) In the **Number of empty positions between tubes** field, specify the number of empty positions between tubes in the sample carrier.

   Type a value or click the up or down arrow in the field.

5. (Optional) In the **Number of empty positions between entries** field, specify the number of empty positions between entries.

   Type a value or click the up or down arrow in the field.

About mixing settings

You can set two types of mixing settings:

- **Initial mixing.** The first mix that occurs when the carrier is run. The initial mix setting is designed to re-suspend the samples. Because of this, the initial mix is more aggressive than the interim mix.
- **Interim mixing.** Any subsequent mixes that are defined by time or interval.

  These mixing settings apply to all tube racks and plates run on the system.

Setting initial mixing settings

To set initial mixing settings:

1. In the **Loader** tab, select **Mix settings** in the left pane.

   Under **Initial Mixing**, in the **Duration** (**sec**) field, type a value or click the up or down arrow.

2. In the **Intensity** (**rpm**) field, type a value or click the up or down arrow.

Setting interim mixing settings

To set interim mixing settings:

1. Under **Interim Mixing**, click the **Interim Type** field and select **Time** or **Interval**.
2. Set the interval according to the interim type you selected.
   - If you selected **Time**, set the time between mixes in the **Interval** (**sec**) **field**.
   - If you selected **Interval**, set the number of tubes between mixes in the **Interval** (**tubes**) field.
3. Set the interim mixing duration in the **Duration** (**sec**) field.
4. Set the interim mixing intensity in the **Intensity** (**rpm**) field.

5. Click **OK** to save your preferences and close the dialog.

More information

- Setting worklist printing preferences (page 75)
- Setup and QC overview (page 79)
- Defining custom sample carrier layouts (page 508)
- About mixing settings (page 512)

Setup and QC overview

Introduction

This topic describes periodic and daily setup and quality control (QC) procedures for the cytometer.

Use the Setup & QC workspace to perform these tasks. After you perform setup and QC tasks, you can view the results summary in the Setup & QC workspace, or view a detailed report of the task.

Pre-requisite tasks

The following tasks should be completed before performing setup and QC tasks (as needed).

Task	Description	For more information
Import or add a a CS&T bead lot	Verify that you have a current (non-expired) CS&T bead lot. You must have a current bead lot to characterize the system, perform daily quality control tasks, and create tube settings and reference settings. You can import bead lot ID information from the BD website or add bead lot ID information by scanning the bead lot file card in a kit.	See Importing or adding a CS&T bead lot (page 82).
Install assays	Install BD assays in BD FACSuite software. Follow the installation instructions included in the technical data sheet.	See System options and upgrades (page 31).

Daily setup and QC tasks

The following setup and QC tasks should be performed daily.

Setup and QC task	Description	For more information
Performance QC	Performance QC is a daily process that utilizes a set of automated software functions and calculations that measure the daily operating efficiency of the cytometer.	See Running daily performance QC (page 123).
Assay and tube settings setup	Assay and tube settings setup is a daily process that sets the tube settings and the daily performance QC. This task determines the correct PMT voltages needed that day for each fluorochrome to match median fluorescence intensity (MFI) determined by the tube target value (TTV) in the tube settings.	See Running Assay and Tube Settings Setup (page 125). See Running characterization QC (page 83)

Periodic setup and QC tasks

The following setup and QC tasks should be performed only as needed.

Setup and QC task	Description	For more information
Characterization QC	Characterization QC is only available to Administrators. This task establishes the measured cytometer performance baseline that is used for all subsequent performance QC runs. Characterization QC is performed at installation and every six months.	See Running characterization QC (page 83).
Laser setup	Laser setup is only available to Administrators. This task initiates an automatic re-alignment of the lasers, followed by performance QC to update settings. Run this procedure if the laser alignment check fails during performance QC, or if the % rCV is out of range.	See Running laser setup (page 86).
CS&T bead lot transfer	CS&T bead lot transfer transfers the associated ABD values from an existing bead lot to a new bead lot. This allows use of the tube target values and spillover values based on the new CS&T bead lot without having to re-characterize the system. In addition, CS&T bead lot transfer transfers the %rCV criteria from existing lot to a new lot for automatic laser realignment.	See Transferring CS&T bead lots (page 88).

More information

- Running characterization QC (page 83)
- About tube and reference settings (page 90)
- About setup and QC reports (page 93)

Importing or adding a CS&T bead lot

Introduction

This topic describes how to import or add a new CS&T bead lot when your existing lot has expired.

You must have a current bead lot to characterize the system, to perform daily quality control tasks, and to create tube settings and reference settings.

Importing CS&T bead lots

Import CS&T bead lots if you do not have the optional barcode reader. CS&T bead lot files can be downloaded from the BD website. See the information included in the CS&T bead kit for the specific URL and instructions for downloading bead lot files.

To import a CS&T bead lot:

1. On the navigation bar, click **Library**.

   The Library workspace opens.

2. In the **Browser**, double-click **Beads and Reagents,** then click **CS&T**.

   The CS&T bead information is displayed in the upper-right panel.

3. From the menu bar, select **File > Import**.

   The **Import** dialog opens.

4. Navigate to the CS&T bead lot file location and select the appropriate **CS&T** bead lot file.

5. Click **Open**.

   The new bead lot file is displayed in the table. The **Import** confirmation dialog opens if there are warnings or errors.

Adding a new CS&T bead lot

To add a new CS&T bead lot using the barcode scanner:

1. On the navigation bar, click **Library**.

   The Library workspace opens.

2. In the **Browser**, double-click **Beads and Reagents,** then click **CS&T**.

3. In the **CS&T Bead Lots** table, click **Scan barcode** and scan the new bead lot barcode card inside the CS&T bead kit.

   The information is automatically displayed in the **CS&T Bead Lots** table.

Running characterization QC

Introduction

This topic provides instructions for running characterization QC. This is an Administrator task.

About characterization QC

Characterization QC establishes initial values for measurements that are used to track cytometer performance. Characterization QC is performed using the normal and high-sensitivity fluidics modes. The measured values are specific to each cytometer configuration. Run characterization QC at installation (by BD Service), every 6 months, and when a new configuration is created.

Before you begin

- Prepare the CS&T beads according to the instructions in the technical data sheet.

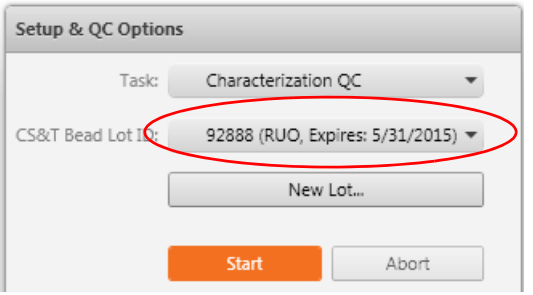
Procedure

To run characterization QC:

1. On the navigation bar, click **Setup & QC**.

   The **Setup & QC** workspace opens.

2. In the **Setup & QC Options** panel, verify that the BD FACSuite CS&T beads have not expired.

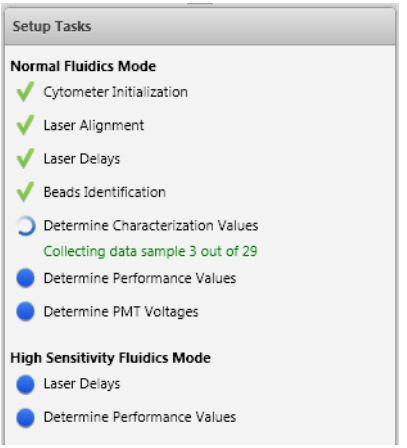


   - If the bead lot has expired, see Importing or adding a CS&T bead lot (page 82).
   - If your current bead lot is running low on beads, see Transferring CS&T bead lots (page 88).
3. Select **Characterization QC** from the **Task** menu.
4. Click **Start**.

   The **Load Tube** dialog opens.
5. Load the CS&T beads on the cytometer.

   The system detects the tube and setup task begins.

Characterization QC details for normal and high sensitivity fluidics modes are displayed in the **Setup Tasks** panel. A checkmark indicates that a step in the task has been completed.



When all tasks are complete, a dialog opens and indicates whether the task passed or failed.

6. Click **Yes** to view the report for the QC task, or click **No** to close the dialog.
7. Unload the tube.

More information

- Setup and QC overview (page 79)
- Running laser setup (page 86)
- Viewing a setup and QC report (page 94)

Running laser setup

Introduction

This topic describes how to perform laser setup using CS&T beads. This is an Administrator task.

About laser setup

Laser setup initiates an automatic re-alignment of the lasers, followed by performance QC to update the settings. Run this procedure if the laser alignment check fails during performance QC, or if the %rCV is out of range.

Before you begin

- Run the monthly clean procedure.
- Prepare the CS&T beads according to the instructions in the technical data sheet.

Procedure

To run laser setup:

1. In the navigation bar, click **Setup & QC**.

   The **Setup & QC** workspace opens.

2. In the **Setup & QC Options** panel, select **Laser Setup** from the **Task** menu.

3. In the **CS&T Bead Lot ID field**, select the appropriate bead lot.

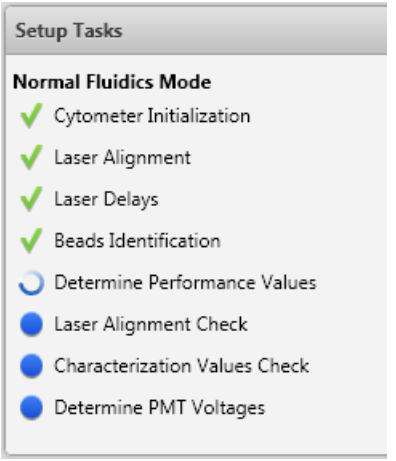
4. Click **Start**.

   The **Load Tube** dialog opens, indicating the lot of beads to use.

5. Load the tube of CS&T beads.

   The system detects the tube and setup task begins.

Laser setup details for normal and high sensitivity fluidics modes are displayed in the **Setup Tasks** panel. A checkmark indicates that a step in the task has been completed.



If the laser setup task completes successfully, a performance QC task begins. When all tasks are complete, a dialog opens and indicates whether the task passed or failed.

6. Click **Yes** to view the report for the QC task, or click **No** to close the dialog.
7. Unload the tube.

More information

- Setup and QC overview (page 79)
- Running characterization QC (page 83)
- Viewing a setup and QC report (page 94)
- Performing the monthly clean procedure (page 475)

Transferring CS&T bead lots

Introduction

This topic describes how to transfer to the current performance and characterization values used with a bead lot to a new CS&T bead lot when the existing lot has expired. This ensures consistency across bead lots. This is an Administrator task.

Before you begin

- Add the new CS&T bead lot to the library. See Importing or adding a CS&T bead lot (page 82) for instructions.
- Prepare the old and new lots of CS&T beads in separate tubes according to the directions in the technical data sheet.

Procedure

To transfer CS&T bead lots:

1. On the navigation bar, click **Setup & QC**.

   The **Setup & QC** workspace opens.

2. In the **Setup & QC Options** panel, click **CS&T Bead Lot Transfer**.

3. Select the old CS&T bead lot ID from the **Old CS&T Bead Lot ID** menu.

4. Select the new CS&T bead lot ID from the **new CS&T Bead Lot ID** menu.

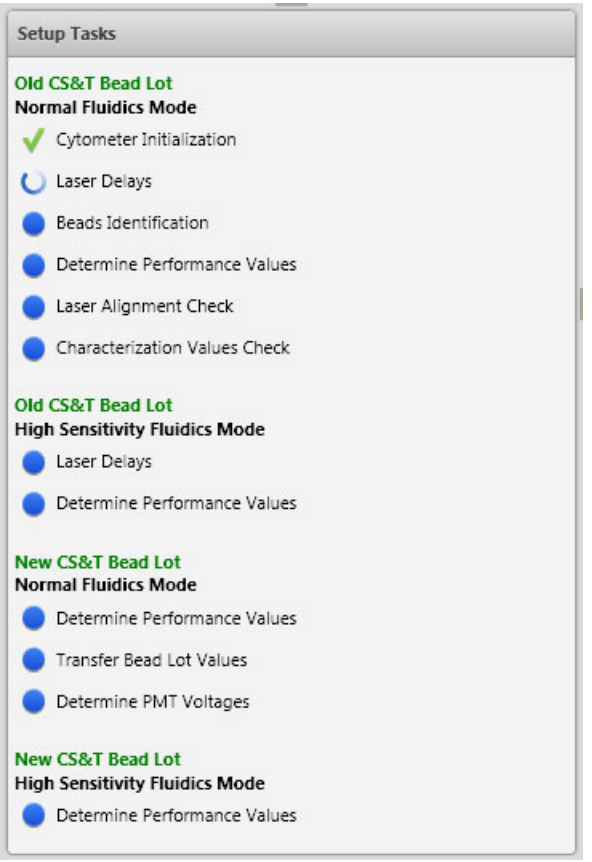
   If your current bead lot has expired and there are no new lots in the **New CS&T Bead Lot ID** list, then click **New Lot** to add a new bead lot. See Importing or adding a CS&T bead lot (page 82) for instructions.

5. In the **Setup & QC Options** panel, click **Start**.

   The **Load Tube** dialog opens.

6. Load a tube with the old CS&T bead lot indicated in the dialog.

The system detects the tube and setup task begins. Old CS&T bead lot details for normal and high sensitivity fluidics modes are displayed under **Old CS&T Bead Lot** in the **Setup Tasks** panel. A checkmark indicates that a step in the task has been completed.



When all of the old CS&T bead lot tasks have completed, the **Load Tube** dialog opens.

7. Unload the old CS&T tube and load the tube with the new CS&T bead lot.

New CS&T bead lot details for normal and high sensitivity fluidics modes are displayed under **New CS&T Bead Lot** in the **Setup Tasks** panel. A checkmark indicates that a step in the task has been completed.

When all tasks are complete, a dialog opens and indicates whether the task passed or failed.

8. Click **Yes** to view the report for the QC task, or click **No** to close the dialog.
9. Unload the tube and place a tube of DI water onto the port.

More information

- Setup and QC overview (page 79)
- Running characterization QC (page 83)
- Importing or adding a CS&T bead lot (page 82)
- Viewing a setup and QC report (page 94)

About tube and reference settings

Introduction

This topic describes tube settings and how they are used to ensure reproducible results from day to day and from system to system. This topic also describes reference settings and how they relate to tube settings.

About tube settings

Tube settings include a collection of attributes that are used to place the positive population at the same position (brightness) whenever the tube settings are applied to tubes. These values are called tube target values (TTVs). Tube settings allow the system to produce comparable results from day to day and from system to system.

Every tube includes an associated spillover value (SOV) matrix by default. If a tube setting has associated *measured* spillover values, the combination of the tube setting and the associated measured spillover values are referred to as reference settings. If there are no associated measured spillover values, the spillover values are automatically calculated based on the existing LW reference values and the current PMT voltages.

Tube settings include the following information.

Item	Description
Tube Target Values (TTVs)	Tube target values determine the median target channel of the positive population for each fluorescence and scatter parameter. These values are the a ratio of median fluorescence intensity (MFI) to assigned BD unit (ABD) for a tube.
Threshold	A level of discrimination to eliminate unwanted events. Only events with parameter values above the threshold are acquired.
Flow Rate	The rate that the sample flows through the flow cell in the cytometer (high, medium (default), low, or high sensitivity).
Window Extension	Adjusts the time the system measures the signal (pulse).
Area Scaling Factor ASF(R) ratio	Ratio of the area scaling factor set for the tube to the area scaling factor determined by the performance QC.

How tube settings are created and saved

You can create new tube settings after you create a tube and modify settings in an experiment. These tube settings are stored in the library and saved with the experiment. If you create a user-defined assay from the experiment, the tube settings are included with the assay. See Creating tube settings (page 184).

Tube settings are included when you export an experiment, a user-defined assay, or an entry run package (ERP) by running entries in a worklist.

When you import an exported experiment, user-defined assay, or ERP onto a different workstation, then run Assay and Tube Settings setup, the tube settings from these imported experiments,

assays, and entries ensure that the tubes run at the same intensity on each system and generate reproducible results.

How tube settings are used to ensure reproducible results

BD FACSuite software uses system measurements (collected during performance QC) and tube settings to ensure that experiments and worklists generate reproducible results on from day to day, over time, and on different systems.

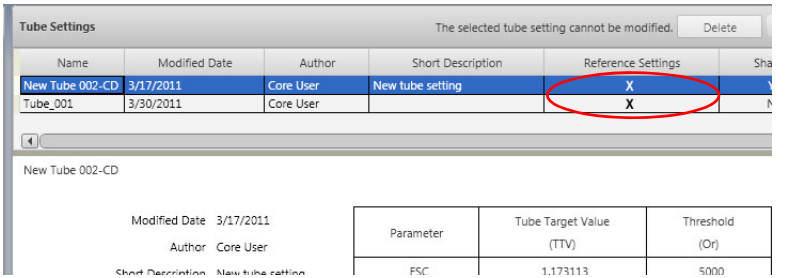
When you run performance QC and then run assay and tube settings setup in the Setup & QC workspace, PMT voltages and spillover values are set based on the tube settings.

This process:

- Reduces daily setup time
- Enables assays and experiments to be used on different systems and labs and produce equivalent results
- Sets initial PMT voltages and spillover values (SOVs) for BD-defined assays and any user-defined assays
- Provides the ability to add spillover values for a single fluorochrome without having to run each fluorochrome again

About reference settings

Reference settings are tube settings and the associated spillover values that have been measured using fluorescence control tubes. Tube settings that have associated measured spillover values are indicated by an *X* in the **Reference Settings** column of the **Tube Settings** table (in the BD FACSuite Library).



If a tube setting has associated measured spillover values, they are automatically applied to the tube when you preview or acquire the tube.

If your tube setting does not have associated measured spillover values, the spillover values are calculated. In this case, the spillover values applied to any tube using this tube setting are derived from the reference spillover values and actual MFI measurements from performance QC and assay and tube settings setup.

If you modify any values that are part of a tube setting, the software automatically calculates modified (star) tube settings and recalculates the spillover values based on the default LW reference setting and MFI and PMT voltages. Calculated spillover values are not saved and cannot be reused except by copying and pasting a tube from one experiment to another.

Using measured spillover values increases the accuracy of the compensation matrix compared to using the calculated settings.

More information

- Setup and QC overview (page 79)
- Running Assay and Tube Settings Setup (page 125)
- Creating tube settings (page 184)
- Creating reference settings (page 187)

About setup and QC reports

Introduction

This topic describes the content of the setup and QC reports. You can access these reports in the **Setup & QC** workspace, in the **Setup and QC Reports** tab.

About setup and QC reports

Setup and QC reports contain information about the system, detectors settings, lasers, setup bead lots, and cytometer settings. They are generated after characterization QC, performance QC, and laser setup. Each time a procedure is completed, two reports are generated, one for each fluidics mode: normal and high sensitivity. Information in the reports varies depending on the cytometer being used.

Viewing a setup and QC report

To view a setup and QC report:

1. In the **Setup & QC** workspace, click the **Setup and QC Report** tab.
2. In the **Report Browser**, click the report you want to view.

   The following sections describe report details.

System information

The top section displays the cytometer type, name, configuration, serial number, options, last characterization and QC date, and user and institution identity.

Summary

This section displays pass/fail status. Pass status is indicated by the word *PASSED*. Fail status in indicated by the word *FAILED*.

Warnings

Warnings are displayed when the current values are outside of the acceptable range. Red text indicates out-of-range or expired values.

Section or field	Description
Parameter	Laser power and/or laser current
Value	Current value of the parameter
Range	Expected value range for the parameter
Message	Reason for the warning

Detector settings

QC reports for characterization QC and performance QC are different. This table displays information that is displayed in a characterization QC report.

Section	Field	Description
Detector	Name	Name for the detector
	Mirror	Name of the mirror used with the detector
	Filter	Description of wavelengths transmitted
	Position	Location of the filter holder with mirror
PMT	Voltage	Measured PMT voltage
	Slope of Gain	Slope of the PMT voltage vs brightness for bright beads (log MFI vs log PMT voltages)
Bead (bright, mid, or dim)	Median	Median fluorescence intensity (MFI) value of the specific beads
	%rCV	Percent robust coefficient of variation of the bright beads
	Linearity Min Channel	Minimum value for the acceptable linear range of the detector
	Linearity Max Channel	Maximum value for the acceptable linear range of the detector
Resolution	Sensitivity	MFI of the bright bead to the two times the standard deviation of noise of a given detector.
	Electronic noise rSD	Robust standard deviation (rSD) of the electronic noise in the particular detector, used to predict the minimum acceptable signal levels required for the best attainable resolution and sensitivity for the system
	Qr	Relative fluorescence detection efficiency, used for describing the light collection efficiency of a detector
	Br	Relative optical background signal, used for tracking the optical background noise levels in a detector

Laser settings

The measurements shown in this section of the report are cytometer-dependent.

Measurement	Description
Laser	Laser name
Position	Location of each laser
Delay (Trigger on FSC)	Laser delay values when thresholding on FSC
Delay (Trigger on Fluorescence)	Laser delay values when thresholding on fluorescence
Area Scaling Factor	Area scaling factors that are determined by setting area and height values on the bright 3-µm beads
Power Actual (mW)	Laser power measured in milliwatts
Power Spec (mW)	Laser power specification in milliwatts
Current (mA)	Laser current measured in milliamperes
Current Spec (mA)	Laser current specification in milliamperes

Info

This section displays information on the setup beads and the cytometer settings that were used.

Field	Description
Bead Lot ID	Setup bead identifier on the kit label
Expiration date	Date after which the bead activity is not guaranteed
Windows extensions	The amount of time added to collect the signal pulse above the threshold
FSC area scaling factor	Area scaling factor that is determined by setting the FSC area and height values on the bright 3-µm beads

Comments

The **Comments** section displays comments that were previously added to the report. Click the **Comments** icon to add a new comment to the report.

Linearity results

This section of the report shows linearity plots for each detector.

A detector's linear range is determined by measuring the MFI ratio of bright beads to mid beads across the detector's dynamic range. The ratio values from the middle of the range, which is known to be linear, are averaged and compared against individual ratios. If the difference between the measured ratio and the averaged ratio is greater than 2%, the results are not considered linear.

More information

- Setup and QC overview (page 79)
- Running characterization QC (page 83)

QC tracking overview

Introduction

This topic describes how to use QC tracking tasks to set up Levey-Jennings charts.

About QC tracking

Using the QC Tracking tab, you can set the performance values you want to display in the Levey-Jennings (LJ) charts. Levey-Jennings charts and reports provide a visual display of instrument performance. Time is plotted on the x-axis. A data point is plotted, indicating its position relative to the mean. The distance from the mean is measured in standard deviations (SDs).

When the system is functioning at peak performance, the variability will be small (within one SD). If the performance declines, the variability and SD range will increase. The data should not fall more than 3 SD higher or lower than the mean.

Any user can specify scaling options and alarm boundaries, but only Administrators can customize the chart display.

QC tracking tasks

The following QC tasks can be performed as needed.

QC tracking task	Description	For more information
Set up Levey-Jennings chart preferences	These preferences determine which set of data will be displayed in LJ charts and reports based on different filters including bead lot, date range, filter status, and fluidics mode. These preferences are specific to each user ID.	See Setting Levey-Jennings charts preferences (page 99).
Setting alarms and scaling ranges	The alarm and scaling ranges for LJ charts and reports determine how the performance data is scaled and which alarm criteria is used in LJ charts and reports. This determines when to flag data points as out of range in reports.	See Setting alarms and scaling ranges (page 103).

More information

- About setup and QC reports (page 93)
- Levey-Jennings reports (page 105)

Setting Levey-Jennings charts preferences

Introduction

This topic describes how to set the data display and tracking preferences for Levey-Jennings (LJ) charts and reports. These preferences determine which set of data will be displayed in LJ charts and reports based on different filters including bead lot, date range, filter status, and fluidics mode. These preferences are specific to the user currently logged in.

Setting data display preferences

To set data display preferences for LJ reports:

1. On the navigation bar, select **Setup & QC**.

   The **Setup & QC** workspace opens.

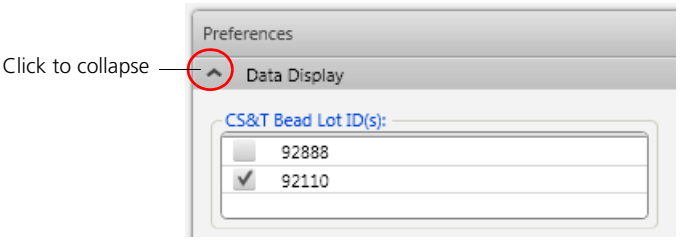
2. Click the **QC Tracking** tab.

3. In the **Cytometer Configuration** field, select an available cytometer configuration from the list.

4. Click the **LJ Charts** tab.

   The **LJ Charts** tab opens with a **Preferences** panel on the left and a **Charts** panel on the right.

5. In the **Preferences** panel, click and expand the **Data Display** box to view the list of preferences.



6. In the **CS&T Bead Lot ID(s)** field, select a bead lot ID.
7. Under **Fluidics Mode**, select Normal or High Sensitivity mode.
8. Under **Filter by Date Range**, select a date filtering preference.
9. Under **Filter By Status**, select a status filtering preference.
10. Under **X-Axis Label**, select a label preference.

Setting data tracking preferences

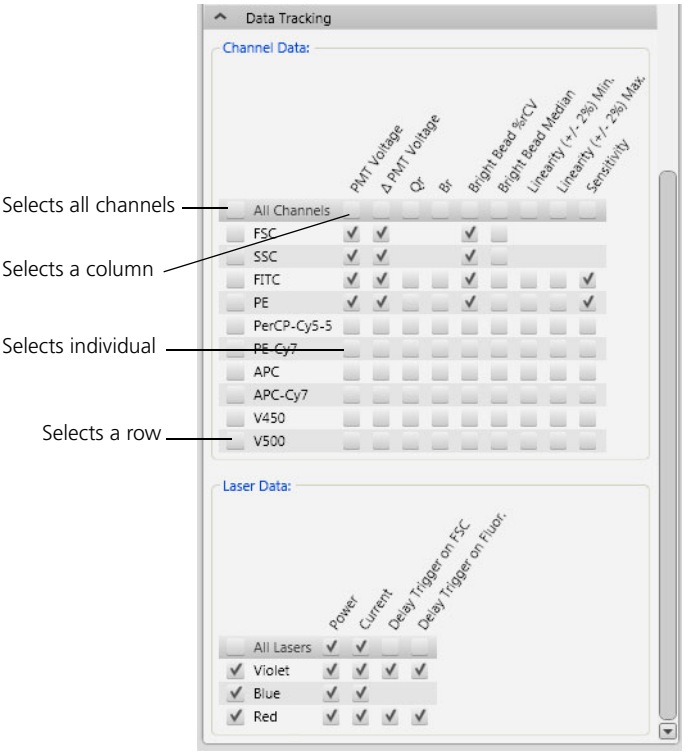
To set data tracking preferences for LJ reports:

1. On the navigation bar, select **Setup & QC**.

   The **Setup & QC** workspace opens.

2. In the left panel, click and expand the **Data Tracking** section to view the list of performance measurements.

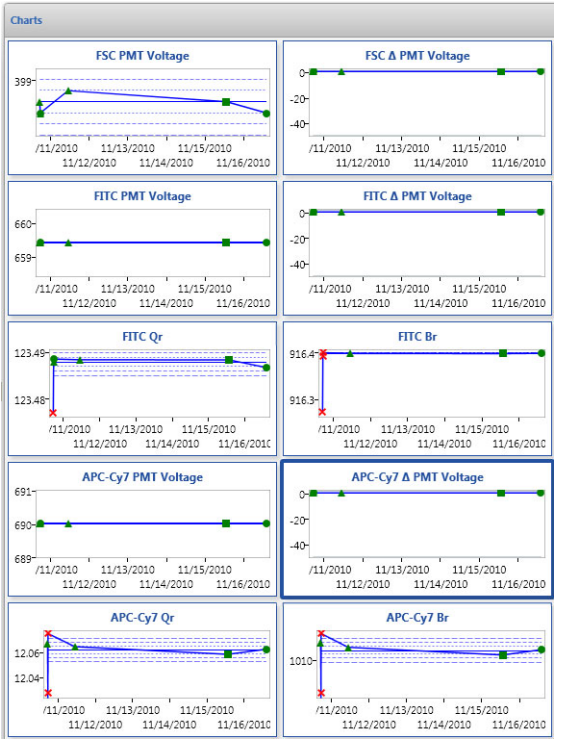
See Measurements (page 106) for information about the measurement categories.



3. Under **Channel Data**, select an option checkbox to display the channel data you want to track in the LJ chart, either using the controls for selecting all channels, columns, rows, or individual checkboxes.

4. Under **Laser Data**, select an option checkbox to display the channel data you want to track in the LJ chart, either using the controls for selecting all channels, columns, rows, or individual checkboxes.

As measurements are selected for display, their corresponding LJ charts are displayed in the **Charts** panel.



5. Complete any of the following actions as needed:

   - Click **View Report** to view the selections in a sample report.
   - Click **Comments** to add a comment to the report.
   - Use the icons on the top right of each chart to copy or zoom in on the charts individually.
   - Use the slider to the right of the LJ report to vary the size of the report.

More information

- About setup and QC reports (page 93)
- Levey-Jennings reports (page 105)
- Glossary definition: fluidic mode (page 578)

Setting alarms and scaling ranges

Introduction

This topic describes how to set the alarm and scaling ranges for Levey-Jennings (LJ) charts and reports.

These preferences determine how the performance data will be scaled and what alarm criteria will be used in the LJ charts and reports, and impact when data points are flagged as out of range in reports.

Procedure

To set alarms and scaling ranges for LJ charts:

1. On the navigation bar, select **Setup & QC**.

   The **Setup & QC** workspace opens.

2. In the **QC Tracking** tab, click the **Alarm Ranges & Scale** tab.

3. In the **Cytometer Configuration** field, select an available cytometer configuration from the list.

   The cytometer configuration should be the same as the one used for setting up the LJ charts.

4. In the left panel, click and expand the **Tracked Data** section to view the list of data types, channel data, and laser data, if necessary.

5. Under **Channel Data**, select a measurement in the left panel (for example, select *PMT*).

6. In the **Channel** column in the right panel, select a channel in one of the groups, either **ΔPMT** or **PMT** (for example, select *FSC*).
7. In the **Scale** column, select a scaling option.
    a. Select **Auto** to set an automatic scale on the y-axis based on the data. Select the checkboxes for each channel you want to autoscale. The min value is set to zero and the max value is set to a specific value based on the channel.
    b. Clear the **Auto** checkbox for any channels you want to adjust manually. Click a **Min** or **Max** field and type a minimum or maximum value.
8. Under **Alarm Ranges**, in the **Boundary column**, click a row and select a boundary type. The boundary is set to Mean +/-3 standard deviations.
9. Under **Alarm Ranges**, in the **Min** and **Max** columns, double-click in a **Min** or **Max** field and type a minimum or maximum value.

More information

- Levey-Jennings reports (page 105)

Levey-Jennings reports

Introduction

This topic describes the content of Levey-Jennings reports. You can access these reports in the **Setup & QC** workspace, in the **QC Tracking** tab.

About Levey-Jennings reports

Levey-Jennings reports contain information about the system, detectors settings, lasers, setup bead lots, and cytometer settings. They are generated for each fluidics mode that is selected. Levey-Jennings reports are organized into sections that display different system details, which are dependent on the preferences selected by the Administrator.

Viewing Levey-Jennings reports

To view a Levey-Jennings report:

1. In the **Setup & QC** workspace, click the **QC Tracking** tab.
2. In the **LJ Charts** tab, click **View Report**.

   The report opens.

System information

The top section of the report displays the cytometer type, cytometer name, serial number, options, last characterization QC date, user, and institution.

Warnings

Warnings appear when the current values are outside the acceptable range. Red text indicates out-of-range or expired values.

Section or field	Description
Parameter	Fluorochrome name
Value	Current value of parameter
Range	Expected value range for the parameter
Warning	Reason for the warning

Measurements

Following is a list of possible measurements you can track in the Levey-Jennings chart. For each graph, there is a legend.

Legend	Description
CST Lot	Setup bead identifier on the kit label
Setup Status	Current setup status of pass, warning, or fail
Setup Type	Task performed to generate a report: performance QC, characterization QC, or CS&T bead lot transfer

Measurement	Description
PMT Voltage	Photomultiplier tube voltage (PMTV) required to place the bright bead population
Δ PMT Voltage	Difference between PMT voltage value for the characterization check and current performance check
Qr	Relative fluorescence detection efficiency, a measurement used for tracking the light-collection efficiency of a detector
Br	Relative optical background signal, a measurement used for tracking optical background levels in a detector
Bright Bead %rCV	Percent robust coefficient of variation of the bright beads, a measurement used in the calculation of photon detection efficiency (Qr)
Bright Bead Median	MFI value of bright beads, a measurement used in the calculation of photon detection efficiency (Qr) and linearity
Linearity	The acceptable linear range is the ratio of bright beads to dim beads across the detector response. If the mean of the ratio is greater than 2%, the results are not considered linear.

Measurement	Description
Linearity (±2%) Min	Minimum value (±2%) for the acceptable linear range of the detector
Linearity (±2%) Max	Maximum value (±2%) for the acceptable linear range of the detector
Sensitivity	Instrument sensitivity (IS) is a fluorescence-normalized, detector-specific measure of signal to noise. For a given detector, the value is defined as the median fluorescence intensity (MFI) of the CS&T bright bead divided by twice the standard deviation of combined noise.
Power (mW)	Laser power, measured in milliwatts
Current (mA)	Laser current, measured in milliamperes
Delay trigger to FSC	Laser delay values when thresholding on FSC
Delay trigger to fluor	Laser delay values when thresholding on fluorescence

More information

- QC tracking overview (page 97)
- Setting Levey-Jennings charts preferences (page 99)
- Setting alarms and scaling ranges (page 103)

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Part 2: Using the system

This part includes the following sections:

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4

System startup and shutdown

This section includes the following topics:

Performing system startup

Introduction

This topic describes how to perform the normal system startup procedure. You can also set up a pre-programmed time and day to start the system automatically.

See Setting system (global) preferences (page 63) for more information.

Daily consumables

The following table describes the required consumables for daily operation of the system.

Item	Requirement	Supplied by
Bulk fluids	• BD FACSFlow sheath solution • Bleach (for the waste tank) • Deionized (DI) water	• BD • User • User
Setup beads	• BD FACSuite CS&T research beads	• BD

Procedure

To start up the system:

1. Turn on the power to the system by pressing the Power button.

   The Power button turns green when system power is on. Allow 20 minutes for the lasers to warm up before starting any acquisition work.

2. Log in to BD FACSuite software.

   a. Double-click the **BD FACSuite** icon to start BD FACSuite software.

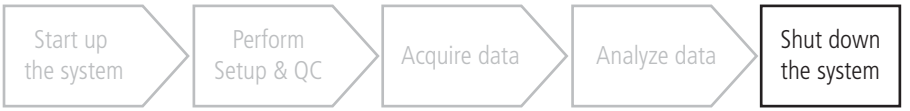
   b. Enter a username and password to log in, then click **OK**.

3. Verify that the software is connected to the cytometer by looking for the green **Connected** status icon in the lower-left corner of the workspace.
4. Check the fluid levels.
    a. Check the sheath tank to ensure that there is enough sheath fluid to perform your work.
    b. Check the waste tank to ensure there is adequate capacity.
5. Verify that the fluidics system is ready by looking for the green **Fluidics** status icon in the lower-right corner of the workspace.

More information

- Refilling the sheath tank (page 471)
- Emptying the waste tank (page 473)

Performing manual system shutdown



Introduction

This topic describes the manual system shutdown procedure. Use this procedure to manually perform the daily cleaning and shutdown of the system.

Alternatively, you can program the system to shut down automatically. See Performing automated system shutdown (page 115).

Required materials

- 1 tube containing 2 mL of 10% bleach solution
- 1 tube containing approximately 3 mL of DI water
- Disposable towels or wipes.

Shutting down the system

To manually shut down the system:

1. From the menu bar, select **Cytometer > Daily Clean**.

   The **Daily Clean** dialog opens.

2. Place a tube containing 2 mL of 10% bleach solution on the manual tube port, then click **Continue**.

3. When prompted, place a tube containing approximately 3 mL of DI water on the manual tube port, then click **Continue**.

   The dialog closes when the process is complete.

4. Leave a tube containing 2 mL of DI water on the manual tube port.

5. Clean external surfaces.

   a. (Optional) Wipe down the external surfaces of the cytometer and work area.

   b. Dispose of the used cleaning materials in biohazard containers.

6. From the menu bar, select **Cytometer > Shutdown**.

   The **Cytometer Shutdown** dialog opens.

7. Click **Yes**.

   The Power button blinks green for a few seconds, then power to the system turns off and the Power button turns amber.

   Note that a tube of DI water should be loaded on the manual tube port whenever the system is not in use.

Shutting down the software (optional)

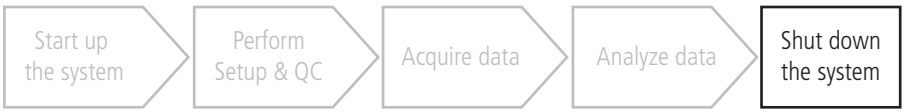
If you are using the pre-programmed startup feature, do not perform the following steps. The software must remain running to enable startup at the defined day and time.

1. Log out of the software.
   a. From the right side of the menu bar, click the **Log Out** button.
   b. In the confirmation dialog, click **Yes**.
2. Exit the software.
   a. From the main menu, select **File > Exit**.

More information

- Performing system startup (page 112)
- Performing automated system shutdown (page 115)

Performing automated system shutdown



Introduction

This topic describes the process for shutting down the system by running a worklist on the Loader.

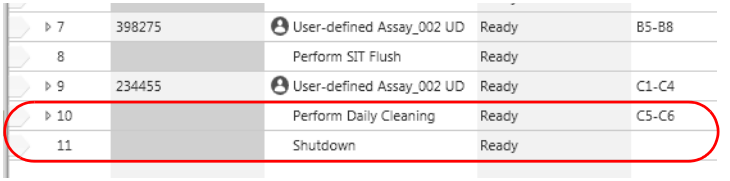
Automated shutdown using the worklist

Automate the shutdown process using the worklist by adding cleaning and shutdown entries to a worklist and then running that worklist using the Loader.

For this process, you need to prepare a tube with a 2 mL of 10% bleach solution and a tube containing approximately 3 mL of DI water. Place the tubes in a 30- or 40-tube rack (plates cannot be used). Next, create new worklist, or open an existing worklist, then add cleaning and shutdown entries to the end of the worklist.

For details about creating cleaning and shutdown entries in a worklist, see Adding fluidics cleaning or shutdown to a worklist (page 239).

The following figure shows a sample worklist with cleaning and shutdown entries added.



Once the entries are added to a worklist, you can open the worklist and run it.

Running an automated shutdown using a worklist

To run an automated shutdown using a worklist:

1. Open the shutdown worklist.
2. Run the worklist.

   When the worklist is finished, the system power turns off.
3. Place a tube of DI water on the manual tube port.

   Note that a tube containing 2 mL of DI water should be loaded on the manual tube port whenever the system is not in use.

More information

- Acquiring data in a worklist (page 249)

5

Daily setup and QC

This section includes the following topics:

About the Setup and QC workspace

Introduction

This topic describes the tabs in the Setup and QC workspace.

The Setup and QC workspace includes multiple tabs that you use to perform different setup and quality control tasks, view reports, track QC over time, and view or modify the cytometer optical configuration.

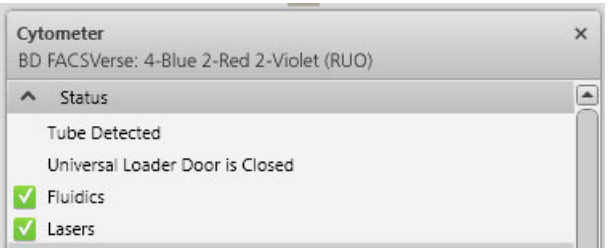
To open the Setup and QC workspace, click Setup & QC on the navigation bar.

Setup & QC tab

The Setup and QC tab includes the following panels:

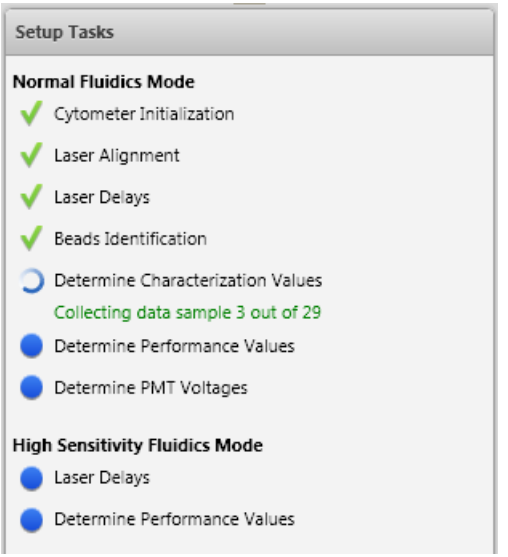
- **Setup & QC Options.** Use this panel to select setup and QC tasks, select CS&T bead lots, and start or abort setup and QC operations.

- **Cytometer.** This panel displays the current cytometer configuration and the current cytometer status. **Status** displays the system status (including real-time status for the SIT), fluidics, and lasers. This box also reminds you when you need to run system cleaning protocols.



You can click the arrow icon in the Status title bar to expand or collapse this section.

- **Setup Tasks.** This panel displays real-time status of setup and QC task steps. Green checkmarks indicate completed steps.



Setup and QC Reports tab

In the Setup and QC Reports tab, the Reports Browser table lists all of the reports that are generated when you perform a characterization, performance, or laser setup QC task. Separate reports for each fluidics mode (normal and high sensitivity) are generated for each setup and QC task.

Reports contain details about the system, detector settings, lasers, setup bead lots, and cytometer settings. Click a report in the table to view, print, or export the report.

QC Tracking tab

Use the QC Tracking tab to set the performance values that you want to display in Levey-Jennings charts and the alarm ranges and scales. Levey-Jennings charts are used to track the instrument performance over time.

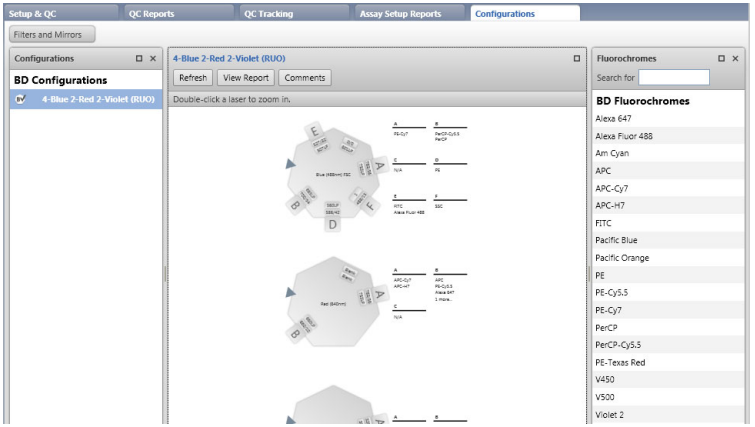
Assay Setup Reports tab

In the Assay Setup Reports tab, the Assay Setup Reports table lists all of the reports that are generated when you perform assay and tube settings setup.

Reports contain details about the assay, cytometer configuration, setup bead lot, and user. Click a report in the table to view, print, or export the report.

Configuration tab

Use the Configuration tab to view the current cytometer configuration and the details for each laser and detector.

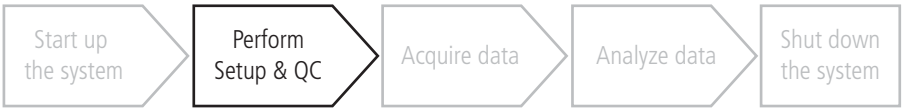


You can assign fluorochromes to detectors, and view, print, and export configuration reports.

More information

- QC tracking overview (page 97)
- Daily setup and QC workflow (page 122)
- About setup and QC reports (page 93)

Daily setup and QC workflow



Introduction

This topic describes the setup and QC tasks you need to complete before you acquire and analyze data using experiments or worklists.

Daily setup and QC tasks

Perform the following setup and QC tasks each day.

Stage	Description
1	Run performance QC to measure the daily operating efficiency of the cytometer. See Running daily performance QC (page 123).
2	Run Assay and Tube settings setup to determine the PMT voltages needed to meet the median fluorescence determined by the tube target values in the tube settings. See Running Assay and Tube Settings Setup (page 125).

More information

- About the Setup and QC workspace (page 118)
- Setup and QC overview (page 79)

Running daily performance QC

Introduction

This topic describes how to run daily performance QC in the Setup & QC workspace.

A typical performance QC should take approximately 10 minutes if the CS&T beads have already been prepared.

Before you begin

- Prepare a tube with BD FACSuite Research CS&T beads according to the directions in the technical data sheet.
- If you are planning to run QC with a new bead lot, add the new bead lot to the library first.

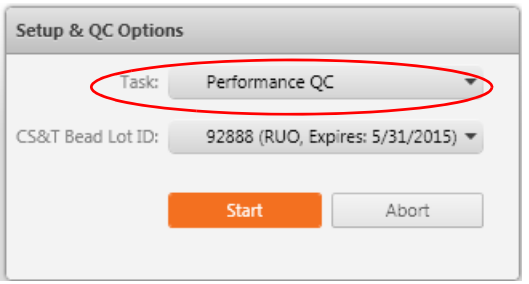
Procedure

To run daily performance QC:

1. On the navigation bar, click **Setup & QC**.

   The **Setup & QC** workspace opens.

2. In the **Setup & QC Options** panel, verify that **Performance QC** is selected.



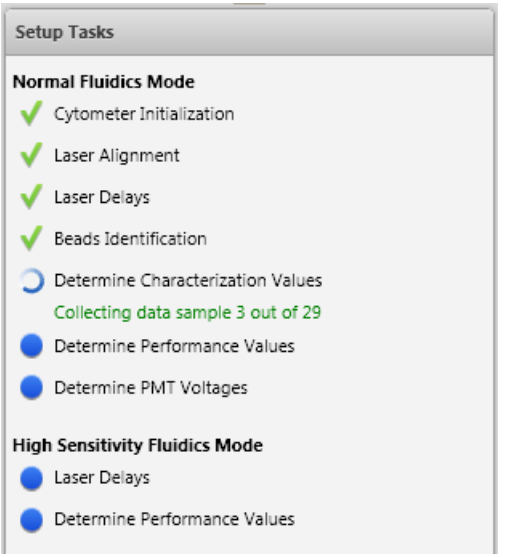
3. Verify that the correct CS&T bead lot ID is selected.
4. Click **Start**.

   The **Load Tube** dialog opens.

5. Load the tube of CS&T beads onto the manual tube port.

   The system detects the tube and setup task begins.

The details for normal and high sensitivity fluidics modes are displayed in the **Setup Tasks** panel. A checkmark indicates that a step in the task has been completed.



When all tasks are complete a dialog opens and indicates whether the task passed or failed.

6. Click **Yes** to view the report for the QC task or click **No** to close the dialog.
7. Unload the tube.

Next step Running Assay and Tube Settings Setup (page 125)

More information

- Setup and QC overview (page 79)
- Viewing a setup and QC report (page 94)
- About the Setup and QC workspace (page 118)
- Daily setup and QC workflow (page 122)
- Importing or adding a CS&T bead lot (page 82)

Running Assay and Tube Settings Setup

Introduction

This topic describes how to run daily assay and tube settings setup in the Setup & QC workspace.

Daily assay and tube settings setup is used to ensure that assays and experiments run properly. Use this procedure to select the assays and tube settings that you want to update for use today.

Before you begin

Run daily performance QC.

Procedure

To run assay and tube settings setup:

1. On the navigation bar, click **Setup & QC**.

   The **Setup & QC** workspace opens.

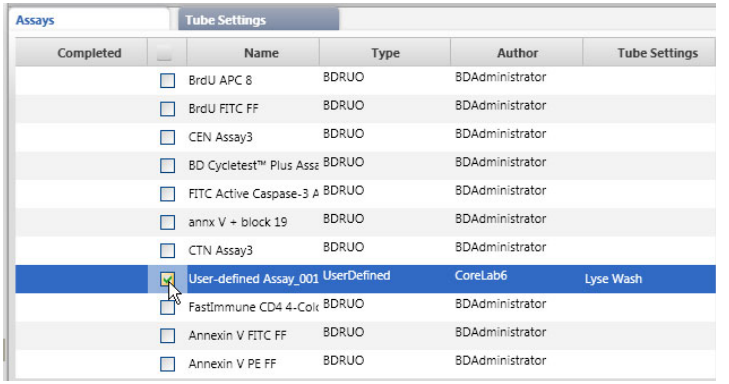
2. In the **Setup & QC Options** panel, select **Assay & Tube Settings Setup**.

3. Click **Select**.

   The **Assays** and **Tube Settings** tabs open in the right panel.

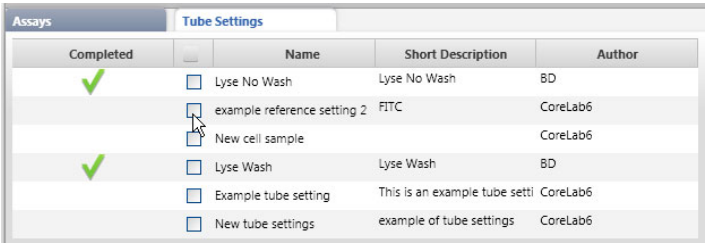
4. In the **Assays** tab, from the **Assays** list, select the checkboxes for the assays that you want to set up or select the checkbox in the header row to select all assays.

Selecting an assay automatically selects the appropriate tube settings.



5. If you want to update a different tube setting, click the **Tube Settings** tab and select a checkbox.

   Note that if you run performance QC, the lyse/wash and lyse/no-wash tube settings are automatically updated.



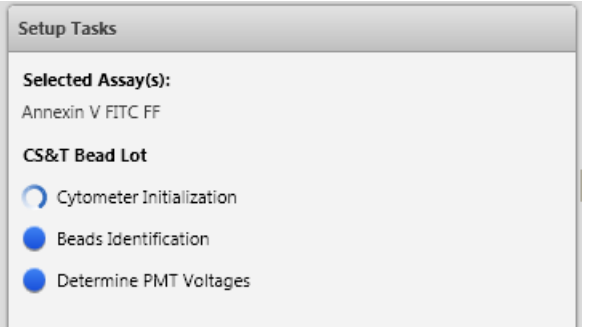
6. In the **Setup & QC Options** panel, click **Start.**

   The **Load Tube** dialog opens.

7. Load the tube of CS&T research beads.

   The system detects the tube and setup task begins.

The details for normal and high sensitivity fluidics modes are displayed in the **Setup Tasks** panel. A checkmark indicates that a step in the task has been completed.

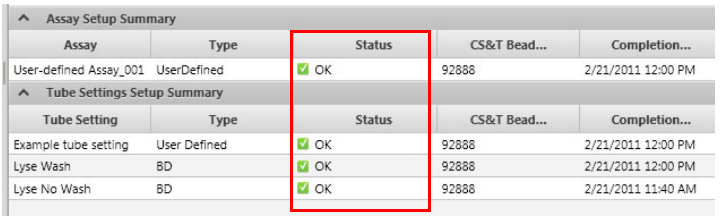


When all tasks are complete, a dialog opens and indicates whether the task passed or failed.

8. Click **Yes** to view the report for the QC task, or click **No** to close the dialog.

9. Unload the tube.

   The assays that were selected for assay and tube settings setup are now displayed on the opening page of the **Setup & QC** workspace with a status of **OK**.



More information

- About the Setup and QC workspace (page 118)
- Setup and QC overview (page 79)
- About setup and QC reports (page 93)
- Viewing a setup and QC report (page 94)
- Running characterization QC (page 83)
- Experiment overview (page 131)

6

Experiment acquisition and analysis

This section includes the following topics:

Experiment overview

Introduction

This topic describes experiments, the Experiment workspace, and provides typical workflow steps for creating, editing, and saving experiments. This basic workflow includes only the basic required elements for creating and acquiring data in an experiment using BD default settings.

See Using the Manage Experiments tab (page 134) for more information about optional features and functions.

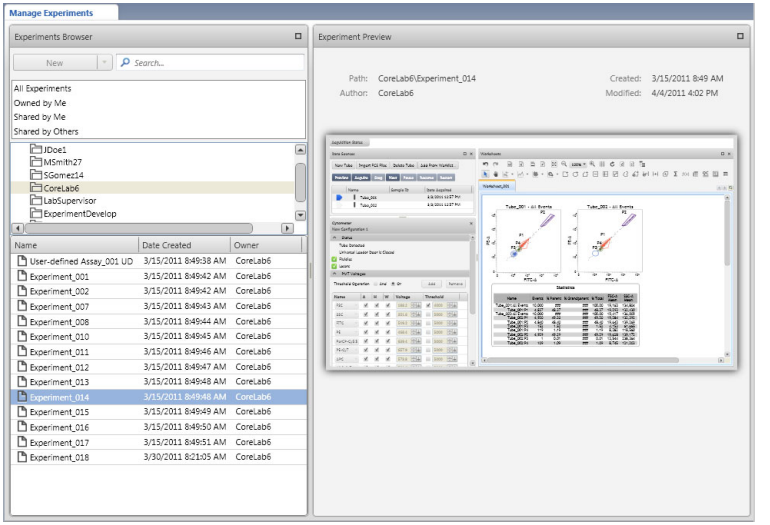
About the Experiment workspace

The Experiment workspace includes the Manage Experiments tab and Experiment tab which represents an open experiment.

To open the Experiment workspace, click Experiments on the navigation bar.

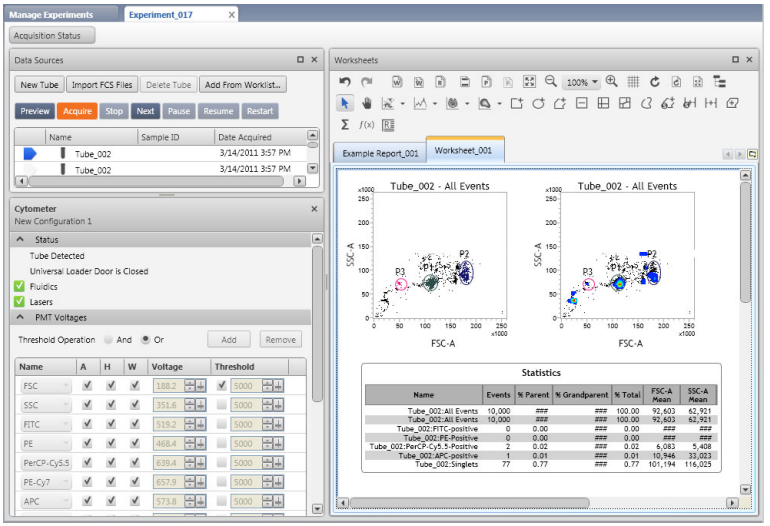
Use the Manage Experiments tab to create new experiments, preview and open existing experiments, organize experiments, filter and search for experiments, and share them with other users. Use the BD FACSuite menus to rename, import, or export experiments.

Manage Experiments tab



Use the Experiment tab to develop your experiment, adjust settings, set properties, acquire and visualize tube data, and analyze the data using different analysis tools. A separate tab is created for each open experiment.

Experiment tab



More information

- Using the Experiment tab (page 146)
- Using the Manage Experiments tab (page 134)
- Experiment acquisition workflow (page 141)

Using the Manage Experiments tab

Introduction

This topic describes experiment folders, how to open and preview existing experiments, and how to manage experiments using the Manage Experiments tab.

About experiment folders

The Experiments Browser panel in the Manage Experiments tab displays experiment folders. Each user has a default experiment folder identified by the user login name. You can create an unlimited number of subfolders and organize different experiments within your default experiment folder.

You can rename experiments and folders, move experiments from one subfolder to another, and delete, share, or make experiments private.

Opening the Experiment workspace

To open the Experiment workspace:

1. On the navigation bar, click **Experiments**.

   The **Manage Experiments** tab opens in the **Experiment** workspace.

Renaming experiments

When you create a new experiment, the experiment is identified by the default name *Experiment_nnn*, where nnn is a three-digit number (starting from 001).

A unique or meaningful experiment name is useful when locating or categorizing experiments by purpose, fluorochrome, bead type, cell type, author, or other attributes that are meaningful to your laboratory.

To rename an existing experiment:

1. In the **Experiments Browser**, right-click an experiment name, then select **Rename Experiment**.
2. Type a new name, then click outside the field or press **Enter**.

Saving an experiment as a different experiment

To save an experiment (Save As) as a different experiment:

1. In the **Experiments Browser**, right-click an experiment, then select **Save As**.

   A **Save Experiment As** dialog opens.

2. In the **Experiment Name** field, type a new experiment name.

3. (Optional) If you want to save the experiment properties, plots, gates, and other worksheet elements, but not the tube data, select the **Save Experiment without Data** checkbox.

4. Click **OK** to save a copy of this experiment with a new name.

Renaming an experiment subfolder

To rename an experiment subfolder:

1. Right-click an experiment or folder name, then select **Rename Folder**.

2. Type a new name, then click outside the field or press **Enter.**

Creating an experiment in a new subfolder

To create an experiment in a new experiment subfolder:

1. In the **Experiments Browser**, right-click your default user folder and select **New Folder.**

   A new subfolder is created with the default name *Folder_nnn*, where nnn is a three-digit number (starting from 001).

2. Click **New.**

   A new experiment opens.

Moving an experiment

To move an experiment to a different folder:

1. Click an experiment folder, then click an experiment.

2. Drag the experiment from one folder into a different folder.

Changing sharing settings

Experiments are private by default. Only Administrators or experiment owners (authors) can change share settings. Once an experiment is shared by the author, all users can modify, rename, or export the experiment. Changes are saved to the original, shared experiment. Only Administrators or experiment owners (authors) can delete private or shared experiments.

Shared experiments are listed in all *shared by* folders. Private experiments are listed only in the All Experiments and Owned by Me experiment folders.

To change the share setting for an experiment:

1. In the **Experiments Browser**, right-click an experiment and select **Make Private** or **Share.**

Deleting experiments

To delete an experiment:

1. In the **Experiments Browser**, right-click an experiment name, then select **Delete Experiment**.

   A confirmation dialog opens.

2. Click **Yes**.

Deleting experiment subfolders

To delete an experiment subfolder:

1. In the **Experiments Browser**, right-click an experiment subfolder, then select **Delete Folder**.

More information

- Locating existing experiments (page 137)
- Experiment acquisition and analysis (page 129)
- Creating and opening experiments (page 142)

Locating existing experiments

Introduction

This topic describes how to use the experiment search field to filter experiments and folders, and to search to locate specific experiments (both private and shared).

About locating experiments

Experiments are located in your user folder(s) and are listed by experiment name and creation date. Private experiments are accessible only by their author. Shared experiments are accessible by all users.

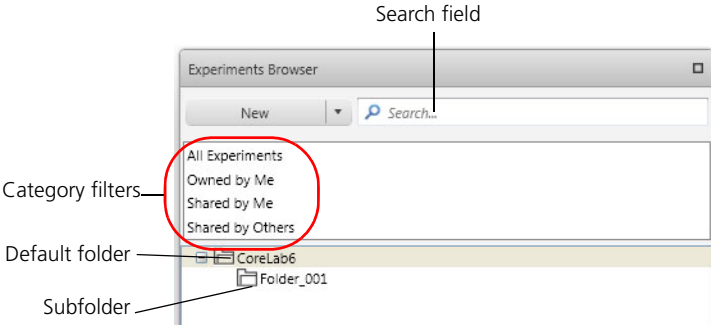
If your folders include numerous subfolders with many experiments, you can search individual folders to locate experiments.

If you cannot find a specific experiment, the experiment might not be shared. See your Administrator or the experiment author for more information.

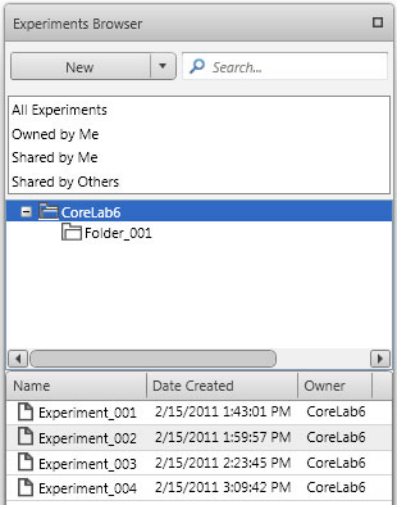
Procedure

To locate experiments:

1. In the **Experiment** workspace, click the **Manage Experiments** tab.
2. In the **Experiments Browser**, click your default experiment folder, subfolder, or an experiment category.



A filtered list of experiments is displayed.



3. If your experiment is not included in the list, you can type an experiment name, number, date, or owner in the **Search** field, then press **Enter**.

More information

- Experiment overview (page 131)
- Importing and exporting experiments (page 139)

Importing and exporting experiments

Introduction

This topic describes how to import and export BD FACSuite experiments using the Manage Experiments tab.

Importing experiments

To import an experiment:

1. In the **Experiment** workspace, click the **Manage Experiments** tab.
2. In the **Experiments Browser**, click an experiment folder.
3. From the menu bar, select **File > Import Experiments**.

   The **Import Experiments** dialog opens.
4. Navigate to the folder that contains the experiment you want to import (for example, *C:\BDExport\BDFSExperiment\myexperiment.Experiment.)*
5. Click **Open**.

   The experiment is imported and is displayed in the selected experiment folder.

Exporting experiments

Export experiments so that you can import them onto a different BD FACSuite workstation or to backup experiments as a part of your specific data management process.

To export an experiment:

1. In the **Experiment** workspace, click the **Manage Experiments** tab.
2. In the **Experiments Browser**, click an experiment folder.
3. Right-click an experiment.
4. Select **Export Experiments**, then select one of the following options:

   - **With Data.** Saves all tube properties, instrument settings, worksheets, reports, and acquired data.

- **Without Data**. Saves tube properties, worksheets, and reports. Select this option when you are creating tubes you want to reuse.

The **Browse For Folder** dialog opens.

5. Navigate to a target export folder (for example, *C:\BDExport\BDFSExperiment*).
6. Click **OK** to export the file.
7. View the status bar to confirm that the files have exported successfully.

More information

- Experiment overview (page 131)
- Locating existing experiments (page 137)

Experiment acquisition workflow

Introduction

This topic describes the typical workflow stages you need to complete to build an experiment and acquire data.

Typical workflow

Perform the following typical workflow stages for acquiring data in an experiment.

Stage	Description
1	Create a new experiment or open an existing experiment. See Creating and opening experiments (page 142).
2	Build an experiment. See Building experiments (page 155).
3	Acquire data. See Acquiring data in an experiment (page 202).
4	(Optional) Save, export, or print the experiment. See Using the Manage Experiments tab (page 134).
5	(Optional) Create a user-defined assay from the experiment. See Creating a user-defined assay from an experiment (page 218).

More information

- Daily workflow (page 41)
- Performing system startup (page 112)
- Creating and opening experiments (page 142)

Creating and opening experiments

Introduction

This topic describes the default experiment folder, how to create a new experiment in the Manage Experiments tab, and how to open an existing experiment.

About the default experiment folder

A default experiment folder is automatically created for each user ID. All experiments that you create are stored in the default folder associated with your user ID. Within this folder, you can create custom subfolders using your own method for storing experiments (for example, by category, by date, or by sample).

See Using the Manage Experiments tab (page 134) for more information about custom subfolders.

Creating a new experiment

To create a new experiment:

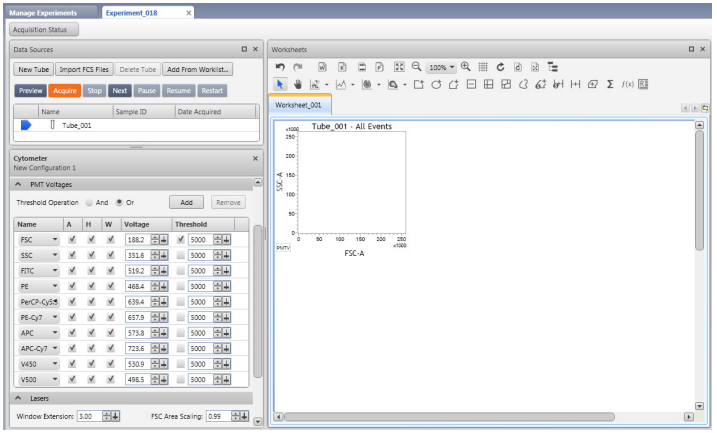
1. On the navigation bar, click **Experiments**.

   The **Manage Experiments** tab opens in the **Experiment** workspace.

2. In the **Experiments Browser** panel, click your default folder or subfolder.

3. Click **New**.

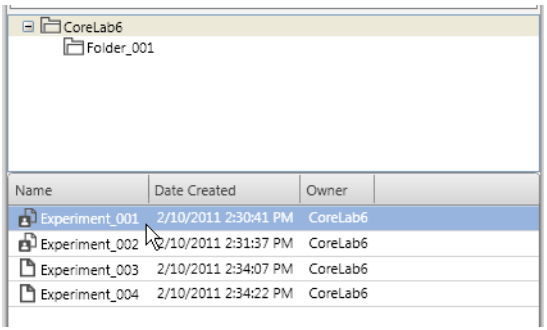
A new experiment opens. The new experiment name and creation date are displayed in the **Experiments Browser**, and a new tab opens in the **Experiment** workspace.



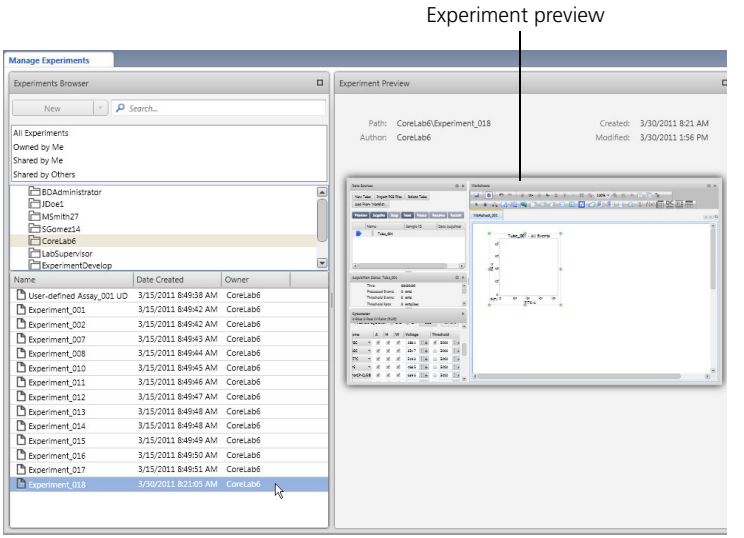
Opening an existing experiment

To open an existing experiment:

1. In the **Experiments Browser**, click your experiment folder or click an experiment subfolder to display the available experiments.



2. (Optional) Click an experiment to display a snapshot of the experiment in the **Experiment Preview** panel.



3. Double-click an experiment to open the experiment in the **Experiment** workspace.

Opening multiple experiments

To open multiple experiments at the same time:

1. In an experiments folder, Ctrl+click each experiment that you want to open.
2. Right-click, then select **Open Experiment**.

   All selected experiments open as individual tabs.

Creating a new experiment from an assay

If you want to modify a current BD-defined (RUO) assay or a user-defined assay, you can create a new experiment from the assay, modify the experiment, then create an assay from the modified experiment.

To create a new experiment from an assay:

1. Open the **Manage Experiments** tab.

2. From the menu bar, select **File > New experiment from assay**.

   The **Select an Assay** dialog opens.

3. Click an assay in the list, then click **OK**.

   The new experiment is displayed in the **Experiments Browser** and the new experiment opens.

   Note that when BD-defined assays are converted to experiments, they do not include any reports or worksheets that are present in the original assay.

If you intend to modify an existing user-defined assay and want to retain the same assay name, you can overwrite the original by creating an assay with the same name.

See Creating a user-defined assay from an experiment (page 218).

Next steps

- If you want to create new tubes or delete existing tubes in your experiment, continue with Creating tubes (page 156).
- If your experiment has the correct tubes, plots, gates and properties, you can acquire tubes, re-acquire existing tubes, or analyze the data. See Acquiring data in an experiment (page 202).

More information

- Using the Experiment tab (page 146)
- Modifying tube properties (page 167)
- Using the Manage Experiments tab (page 134)

Using the Experiment tab

Introduction

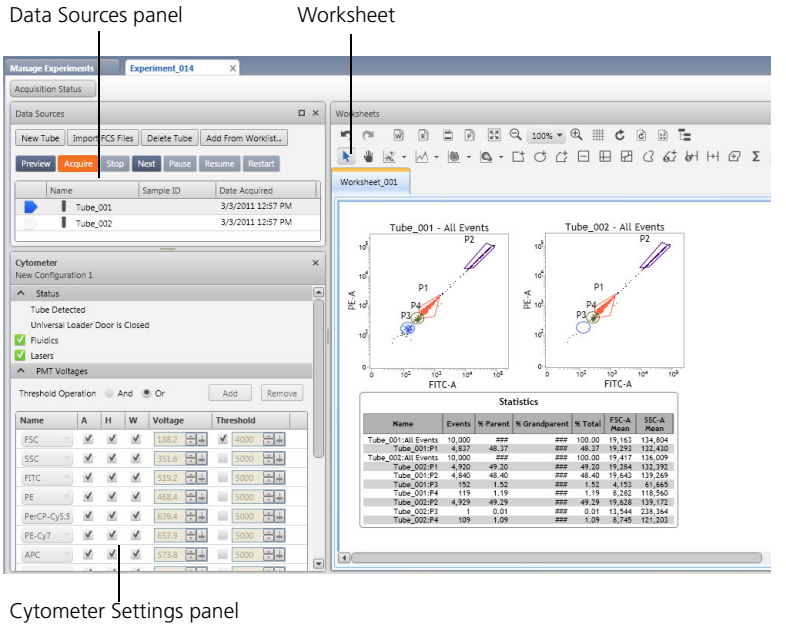
This topic describes the different panels in the Experiment tab and how to use them to build, modify, and run experiments.

About the Experiment tab

When you create a new experiment or open an existing experiment in the Manage Experiments tab, a new Experiment tab opens. The Experiment tab represents an experiment and includes the following panels:

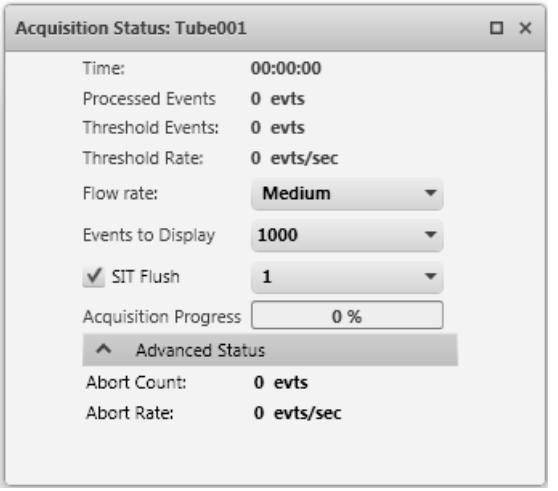
- Acquisition Status
- Data Sources
- Cytometer Settings
- Worksheets and reports

  You can drag the panels to organize them in any order within the workspace, or click to minimize panels to maximize display space.



About the Acquisition Status panel

Use the Acquisition Status panel to view real-time status for time, event counts, and aborts. You can also set flow rate and SIT flush options specific to an acquisition.



This panel is open by default. You can show or hide this panel. When you hide (close) the panel, an Acquisition Status button is displayed in a toolbar at the top of the Experiment tab. Click the button to display the panel.

Acquisition data displays each time you preview or acquire data. The display is refreshed (cleared) each time you preview or acquire a tube. You can also click to expand Advanced Status to display the acquisition abort count and abort rate.

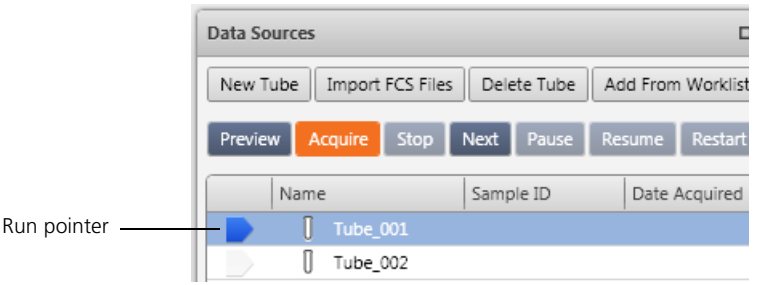
You can select the following options in this panel.

Option	Description
Flow rate	Select the rate (low, medium, or high) that the sample flows through the flow cell in the instrument. High and medium flow rates are typically used for immunophenotyping experiments and to increase event throughput. Lower flow rates are typically used when high precision is required (for example, DNA experiments) to measure slight variations in fluorescence. The fluidic mode (normal or high-sensitivity) varies the speed that the sheath and sample passes through the flow cell in the instrument. The high-sensitivity fluidics mode slows the sample and sheath flow, and is used to obtain better separation between the negative and positive fluorescence populations.
Events to display	Select the maximum number of events to display in plots.
SIT flush	The SIT Flush checkbox is selected by default to perform one SIT flush after the acquisition completes and after you remove the tube from the manual tube port. Increase the number of flushes when extra cleaning is required between acquisitions to reduce sample carryover. To specify a different number of SIT flushes: 1. In the list, select the number of times you want to flush the SIT (1–6).

These settings are applied to all subsequent tubes in an experiment unless you change them.

About the Data Sources panel

Use the Data Sources panel to add and delete tubes and FCS files for acquisition and analysis. You can right-click tubes to set tube properties, duplicate tubes, create tube settings, and create or update reference settings. The run pointer indicates which tube is being previewed or acquired, and which tube's data is applied to a plot.



The Data Sources table displays the tube name, sample ID, and acquisition date. The following table describes the buttons in the Data Sources panel.

Button	Description
New Tube	Click to add a new tube to the list. A new default tube has the default lyse/wash (LW), lyse/no-wash (LNW) properties (based on the cytometer optical configuration and experiment preferences). Lyse/wash provides a starting point for the majority of sample types. Lyse/no-wash provides a starting point for lysed whole blood prepared using a no-wash method.
Import FCS Files	Click to display a dialog where you can select FCS files to import files for analysis.
Delete Tube	Click a tube in the list, then click this button to delete the tube.
Add From Worklist	Click to select tubes from a worklist and add them as tubes in an experiment. See Adding tubes from entries in a worklist (page 157).
Preview	Click to start the sample flow and to populate plots with event data. This does not record event data.
Acquire	Click to start the sample flow and record event data to an FCS file.
Stop	Click to stop the sample flow and the current preview or acquisition.

Button	Description
Next	Click to set the run pointer to the next tube in the Data Sources table. You can also click this button to add tubes. Note that this is the equivalent of duplicating without data. See Adding tubes using the Next button (page 157).
Pause	Click during preview to pause the sample flow and event counters and timers.
Resume	Click to resume a paused preview.
Restart	During preview, click to clear the counters and timers. This clears the acquisition data without pausing the fluid flow. During acquisition, click to delete acquired events, clear counters and timers, and clear the progress bar. This clears the acquisition data without pausing the fluid flow.

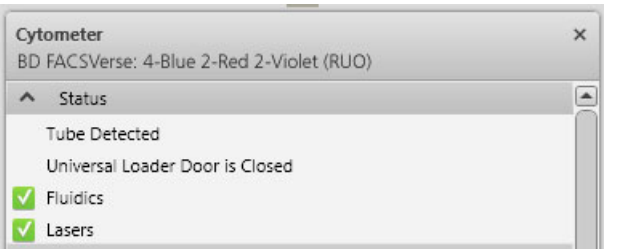
About the Cytometer Settings panel

Use the Cytometer Settings panel to view system status, run cleaning protocols, adjust PMT voltages, and view laser delay and area scaling. This panel includes the following sections:

- Status
- PMT Voltages
- Lasers

Status

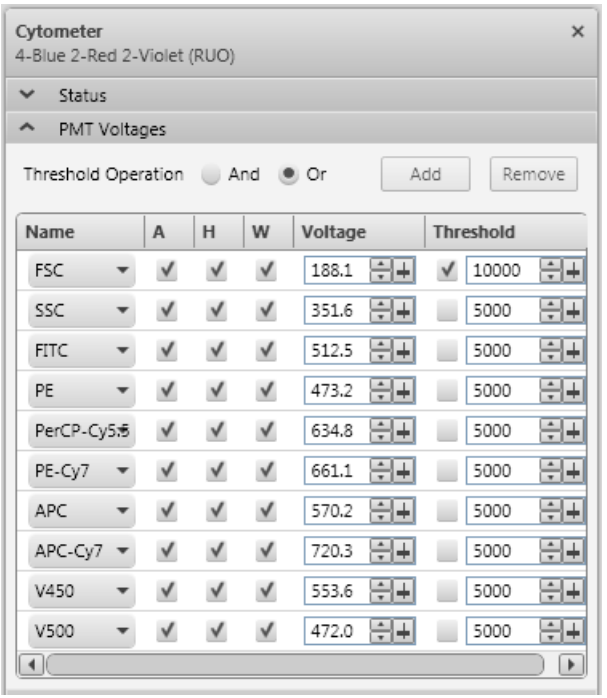
The Status section displays the system status, including real-time status for the SIT, fluidics, and lasers. This section also indicates when you need to run system cleaning protocols.



A checkmark indicates a *Ready* status. You can click the arrow icon in the Status title bar to expand or collapse this section.

PMT Voltages

Use the PMT Voltages section during preview to select the area, height, width, and adjust the voltage and threshold for scatter or fluorescence parameters. You can also add or remove parameters.



If you are using the default tube settings and then adjust PMT voltages or other cytometer settings, the changes apply only to the current tube. If you want to reuse adjusted settings for additional tubes, create a new tube setting.

In the PMT Voltages section, you can perform the following actions.

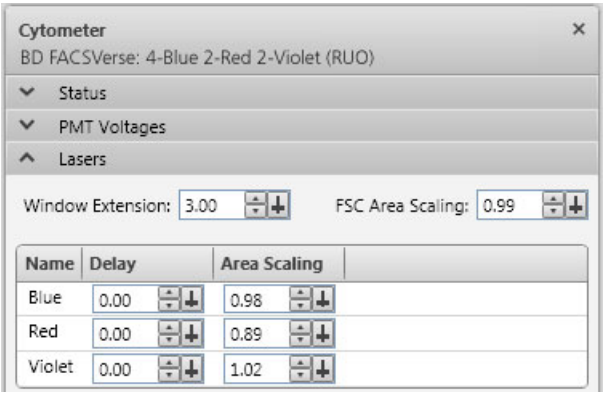
Button	Description
Threshold Operation	1. Select **And** or **Or** to specify how multiple thresholds are combined logically.
Add	1. Click **Add** to add a new parameter. 2. Click the **Name** field in the blank row and select a fluorochrome.
Remove	1. Select a parameter in the PMT Voltage table. 2. Click **Remove** to remove the selected parameter.
Parameter (Name column)	1. Click any fluorescence parameter in the table. 2. Select an available parameter and select a fluorochrome.
A (Area), H (Height), and W (Width)	A (Area) is the default parameter that measures the entire voltage pulse. H (Height) is the peak of the voltage pulse, and W (Width) is the amount of time taken for the event to pass through the laser (multiplied by a constant). 1. Select these checkboxes to specify which parameters to include in the acquisition.
Voltage	Adjusting the voltage changes the amount of sensitivity used by the PMT to view events. • Click the up and down arrows to adjust the value in increments of 1 V. • Ctrl+click the up and down arrows to adjust the value in increments of 10 V. • Drag the slider to adjust the voltage value in any increment.

Button	Description
Threshold	An electronic threshold on a parameter to eliminate unwanted events. Only events with parameter values above the threshold are acquired. 1. Select the checkbox to enable threshold for the parameter. 2. Adjust the value. – Click the up and down arrows to adjust the value in increments of 100. – Ctrl+click the up and down arrows to adjust the value in increments of 10. – Drag the slider to adjust the voltage value in any increment.

You can click the arrow icon in the PMT Voltages title bar to expand or collapse this section.

Lasers

Use the Lasers section to set the area scaling and delay values for each configured laser. If you performed daily performance QC, the laser delay settings are set appropriately. The laser delay values should not need adjustment. The area scaling factor might require adjustment depending on the experiment particle size.



These settings are only temporary and do not affect tube or reference settings in the library. Note that changes to the delay or area scaling affect all tubes in the experiment. These settings are only saved with the experiment.

In the Lasers section, you can perform the following actions.

Button	Description
Window Extension	This is time added to the pulse duration above the threshold to give the total time during which a pulse is sampled. The window extension can be set from 0–25 µs. The default is 3 µs (normal mode) and 5 µs (high-sensitivity mode).
FSC Area Scaling	Adjusts area measurements to be the same magnitude as height measurements for signals from the FSC detector.
Name	(Read-only) Indicates the name of the laser in the current configuration.
Delay	Adjusts the amount of time between processing signals from different laser intercepts to align the signals from multiple lasers (±162.5 µs). The delay in normal mode is typically 35 µs, and high-sensitivity mode is typically 70 µs.
Area Scaling	Adjusts area measurements relative to height measurements for signals from the corresponding laser. For optimal data, the magnitude of area and height measurements should match.

You can click the arrow icon in the Lasers title bar to expand or collapse this section.

About worksheets and reports

Worksheets are used to develop experiments and assays to visualize data. You can display plots, statistics, populations, and expressions to help visualize and calculate tube data.

Reports are used to display results and associated information and can include all the details you want to analyze and present in a final lab report.

You can create multiple worksheets and reports. Each worksheet or report can include multiple pages.

More information

- Experiment overview (page 131)
- Creating tubes (page 156)
- Creating plots in a worksheet (page 158)

Building experiments

Introduction

This topic provides the basic workflow for building an experiment.

Typical workflow

Perform the following typical workflow stages for building an experiment. Note that this basic workflow uses default tubes and does not require the modification of tube properties or create tube settings.

Stage	Description
1	Add tubes (if needed) with default tube settings, or apply existing tube settings. See Creating tubes (page 156).
2	Create and modify worksheet, reports, plots, and create gates and statistics views. See Creating plots in a worksheet (page 158), Previewing data in plots (page 161), and Drawing gates in plots (page 164).

Stage	Description
3	Adjust cytometer settings as needed. See Using the Experiment tab (page 146) for more information about the panels in the Experiment tab that you use to adjust cytometer settings.
4	(Optional) Create tube settings and reference settings as needed. See Creating tube settings (page 184) and Creating reference settings (page 187).
5	(Optional) Modify the tube properties for each tube (if you do not want to use the default tube properties). See Modifying tube properties (page 167).

More information

- Creating and opening experiments (page 142)

Creating tubes

Introduction

This topic describes how to create tubes in the Data Sources panel by adding or duplicating tubes. This topic also describes how to delete tubes from an experiment.

Adding tubes to an experiment

When you create a new experiment, one default tube is displayed in the **Data Sources** panel.

To add a tube to an experiment:

1. In the **Data Sources** panel, click **New Tube**.

   A new default tube is displayed. The Lyse Wash (LW) or Lyse No Wash (LNW) tube settings are applied to all default tubes.

Duplicating tubes with or without data

To duplicate tubes with acquired data:

1. Before you can duplicate with data, acquire the tube.

   See Acquiring data in an experiment (page 202).

2. In the tube list, right-click the tube you want to duplicate.
3. Select one of the following:

   - **Duplicate with data**. A duplicate tube is added to the list with the same tube name and includes all data that is associated with the source tube.
   - **Duplicate without data**. A duplicate tube is displayed with a new name and includes all tube properties and settings except the acquired data.

Adding tubes from entries in a worklist

To add tubes from entries in a worklist:

1. In the **Data Sources** panel, click **Add from Worklist.**

   The **Add Tubes From Worklist** dialog opens.

2. Select one of the following:

   - Under **Entries**, click a worklist entry, then click **Add Selected Entry Tubes**.
   - Under **Entries**, click a worklist entry. Under **Tubes**, click individual tubes that are displayed in the entry, then click **Add Selected Tubes**.

   The tubes are added to the **Data Sources** panel.

Adding tubes using the Next button

To add new tubes using the Next button:

1. In the **Data Sources** panel, click **Next** to move the run pointer to the next tube.

   If you click the last tube in the list, and then click **Next**, a new duplicate tube without data is created and the run pointer moves to the tube.

Clearing data in a tube

To clear existing data from a tube:

1. In the **Data Sources** panel, right-click a tube and select **Clear Tube**.

   All associated tube data is deleted and associated plots or statistics are cleared.

Deleting tubes from an experiment

To delete a tube from an experiment:

1. In the **Data Sources** panel, click a tube in the list.
2. Click **Delete Tube**.

Next step

After you add tubes to the experiment, create plots on a worksheet to visualize tube data.

More information

- Creating plots in a worksheet (page 158)
- Modifying tube properties (page 167)
- Understanding keywords (page 297)

Creating plots in a worksheet

Introduction

This topic describes how to create plots in an experiment worksheet.

Before you begin

- Be sure that you have created tubes before you create plots associated to the tubes.
- (Optional) You can also set tube properties before you begin, or at any time before you acquire the tube.

Creating a plot

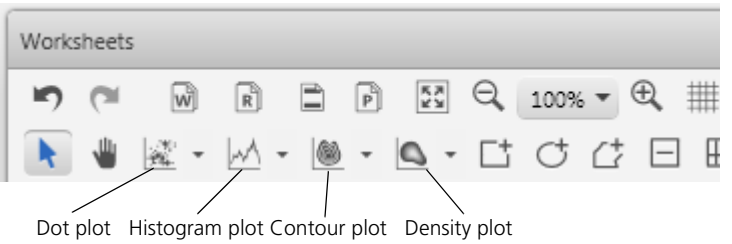
When you create a new experiment, one default tube is displayed in the Data Sources panel and one default plot is displayed in the worksheet.

When you create additional plots, the plots display the default plot title and parameter names. You can modify the plot title and select different parameters for each axis after you create the plot.

To create a plot:

1. Open an experiment.
2. (Optional) Click **Toggle Grid** on the **Worksheet** toolbar to enable the grid.

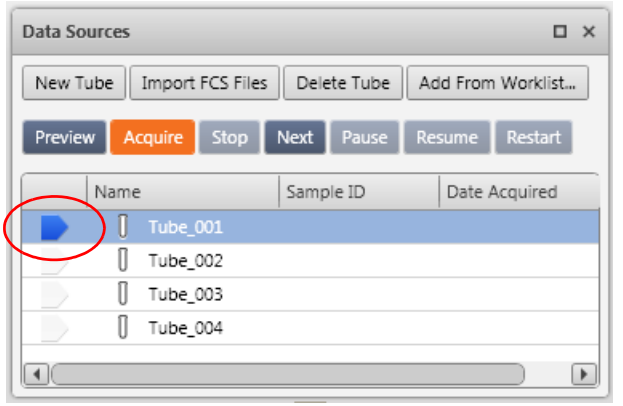
   A grid on the worksheet provides guidelines for plot size and placement.
3. Click a plot tool on the **Plot** toolbar.



4. Click in the worksheet to create the plot.
5. Continue to add plots for your scatter and fluorescence parameters as needed.

   The primary data source determines which tube is associated to a plot or plots in the worksheet. If the run pointer is selected

as the primary data source (default), all plots display data from the tube that is indicated by the run pointer.



You can change the primary data source for a plot from the run pointer to a specific tube if needed. See Changing the primary data source (page 320) for information.

Modifying plot parameters

To modify plot parameters:

1. Select a plot in a worksheet.
2. Right-click the x-axis parameter label, then select a parameter from the list.
3. Right-click the y-axis parameter label, then select a parameter from the list.

Next step

- If you are working with un-acquired tubes, plots remain empty until you preview or acquire the tube to populate the plots with data. Continue by previewing or acquiring data.
- If a tube contains acquired data, new plots automatically display data. Continue by drawing gates in the plots.

More information

- Modifying tube properties (page 167)
- Previewing data in plots (page 161)
- Acquiring data in an experiment (page 202)
- Drawing gates in plots (page 164)
- Plots (page 307)
- Plot types (page 309)

Previewing data in plots

Introduction

This topic describes how to preview data in plots and how to change the data display behavior for plots.

Before you begin

Create plots in the worksheet before you preview tube data.

About previewing data

Previewing data is the process of starting the sample flow and displaying event data. Previewing does not record data. While previewing, you can adjust the PMT voltages and modify the tube properties.

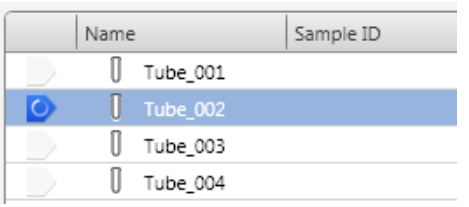
This example describes previewing data from a single (manually loaded) tube using the run pointer as the primary data source. See Changing the primary data source (page 320) for more information about the primary data source.

Procedure

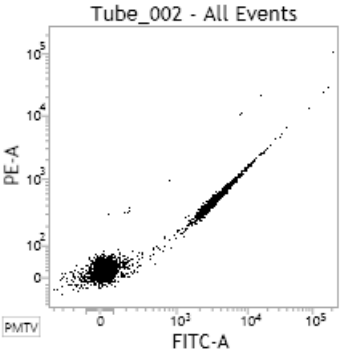
To preview data in a specific tube:

1. Load a tube on the manual tube port.
2. In the **Data Sources** panel, set the run pointer to the tube you want to preview (for example, *Tube_002)*.
3. Click **Preview.**

   During preview, the run pointer remains blue and displays an activity indicator.



Data is displayed in the plots.



Next step

Once data is displayed in plots, you can adjust the cytometer settings and draw gates to identify populations of interest.

More information

- Drawing gates in plots (page 164)

Adjusting the cytometer settings for a tube

Introduction

This topic describes how to adjust the cytometer settings for a tube to optimize the sample brightness and place the events on scale.

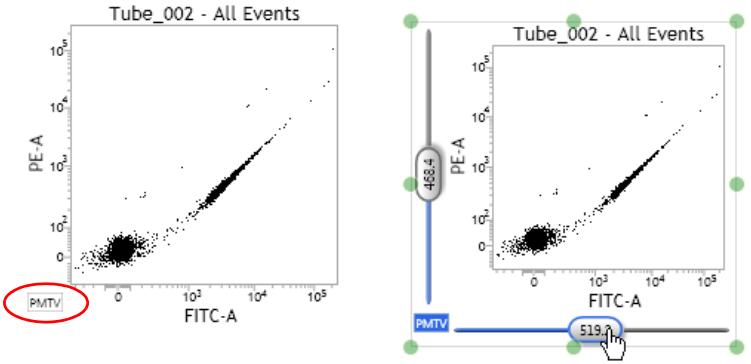
Procedure

Perform the following adjustments as needed before or after you create gates in a plot.

To adjust the cytometer settings:

1. Adjust the PMT voltages as needed to put all populations on scale.

   a. In a plot, click the **PMTV** button in the lower-left corner of the plot to enable the data sliders.



   b. Drag the slider control for each axis parameter in the plot.

      The PMTV value is displayed on the slider control.

   c. Click the **PMTV** button again to disable the slider control.

   See About the Cytometer Settings panel (page 150) for details about adjusting PMT voltage using the PMT Voltages section of the Cytometer Settings panel.

2. Select a threshold operation as needed.

3. Select a checkbox to enable a threshold, then adjust the threshold value as needed.

4. Select area, height, and width parameters as needed.

   See About the Cytometer Settings panel (page 150) for details about selecting parameters and adjusting values using the Lasers section of the Cytometer Settings panel.

5. Click **Stop** to stop previewing.

More information

- Changing the primary data source (page 320)
- Drawing gates in plots (page 164)
- Gates and populations (page 343)
- Acquiring data in an experiment (page 202)

Drawing gates in plots

Introduction

This topic describes four basic gate types and how to draw different types of gates to define populations in plots.

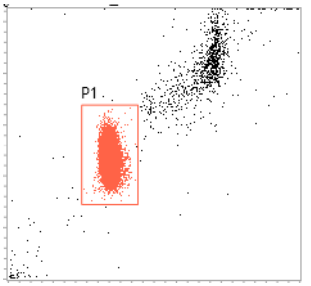
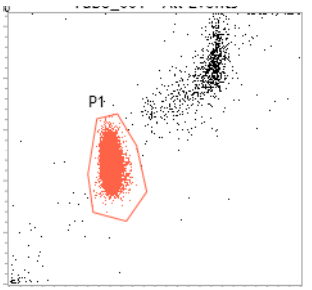
See Gates and populations (page 343) for descriptions and details about additional gate types including logical, interval, adaptive, and quadrant gates.

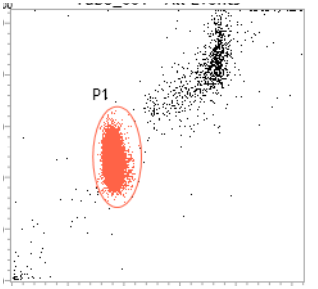
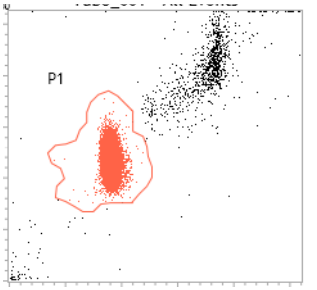
Before you begin

Create plots for tubes and populate the plots with data before creating gates.

Drawing gates

The following table describes how to draw basic gates in plots.

To draw a...	Then do this...
Rectangle gate 	1. Click this tool on the **Worksheet** toolbar. 2. Click in the plot and drag diagonally to create and size the rectangle around specific events in the plot. 3. Release the mouse button to set the gate. 
Polygon gate 	1. Click this tool on the **Worksheet** toolbar. 2. Click on the plot to specify a starting point. A vertex is displayed. 3. Move the cursor to another position and click to add another vertex. Repeat this step to create a minimum of three vertices around specific events in the plot. 4. Click the first vertex or double-click to set the last vertex to close the gate. 

To draw a...	Then do this...
Ellipse gate 	1. Click this tool on the **Worksheet** toolbar. 2. Click in the plot and drag diagonally to create and size the ellipse around specific events in the plot. 3. Release the mouse button to set the gate. 
Freehand gate 	1. Click this tool on the **Worksheet** toolbar. 2. In the plot, click and hold the mouse button, then move the cursor to draw a freehand shape around specific events. 3. Release the mouse button to set the gate. 

New gates are added to the hierarchy and are applied to all tubes within the experiment. The population hierarchy is updated to identify the new population.

Next step Experiment analysis (page 204)

More information

- Gating tools (page 347)
- About gates and population hierarchies (page 359)
- Creating logical gates (page 371)
- Creating adaptive (snap-to) gates (page 374)
- Creating auto gates (page 379)
- Creating quad gates (page 388)
- Creating staggered quad gates (page 394)

Modifying tube properties

Introduction

This topic describes how to modify the default properties for the current tube.

About tube properties

Tube properties define the identity and details for each tube and determine how tube data is acquired and displayed. Tube properties can be modified at any time before you acquire a tube. It can be useful to set some tube properties before you preview data (for example, selecting tube settings or adding labels), while other properties should be modified after plots and gates are created (for example, acquisition stopping rules).

Tube properties

The following table describes the tabs in the Tube Properties dialog.

Tab	Description
General	Use this tab to view or modify the tube name, ID, or sample ID, select tube settings, and view other descriptive information about the tube. See Setting general tube properties (page 169)

Tab	Description
Parameters	Use this tab to view the current parameter names, area, height, and width, PMT voltages, and thresholds for the tube. Use the Cytometer Status panel to modify these parameters. See Viewing tube parameters (page 171)
Compensation	Use this tab to view or modify the compensation matrix for the current tube (either calculated or measured). You do not need to modify this matrix for typical daily use. See Modifying the compensation matrix (page 172)
Reagents	Use this tab to select labels for fluorochromes used in this tube. See Editing reagent labels (page 174)
Keywords	Use this tab to view and assign keywords to the selected tube. See Working with keywords (page 176)
Acquisition	Use this tab to set acquisition stopping rules. See Setting acquisition stopping rules (page 179)

More information

- Building experiments (page 155)
- Setting general tube properties (page 169)

Setting general tube properties

Introduction

This topic describes how to set general tube properties using the General tab in the Tube Properties dialog.

Setting general tube properties

To set general properties for a tube:

1. In the **Data Sources** panel, double-click the run pointer, or right-click a tube and select **Properties** to open the **Tube Properties** dialog.

   The **Tube Properties** dialog opens in the **General** tab.

2. In the **Tube Name** field, use the current default name or type a new tube name.

3. (Optional) In the **Tube ID** field, type a tube ID or click in the field and scan a barcode.

4. (Optional) In the **Sample ID** field, type a sample ID or click in the field and scan a barcode.

Information for the following read-only fields is displayed when you acquire a tube:

- Total Events (acquired for this tube)
- Acquisition Date
- Cytometer Name (the cytometer that was used for acquisition)

Selecting existing tube settings

When you create a new tube, the default tube settings are applied (Lyse/Wash (LW), or Lyse/No-Wash (LNW), based on the Experiment preferences). This section describes how to apply a different tube setting from the library.

If you want to create an assay from the experiment or reuse the settings in other tubes, you need to create tube settings.

See Creating tube settings (page 184).

To select existing tube settings:

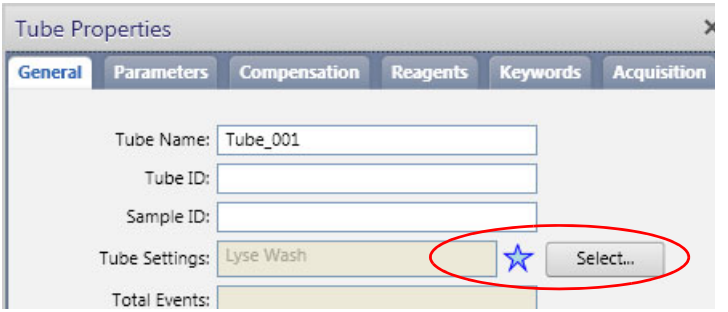
1. In the **General** tab, click **Select** in the **Tube Settings** field.

The **Select Tube Setting** dialog opens.

2. Select a tube setting in the list.
3. Click **OK**.

   The tube settings are applied to the current tube.
4. Continue with the next tab or click **OK**.

   If you modify any values that are part of a tube setting, a star icon is displayed in the **Tube Settings** field. The software automatically calculates modified TTV* (star) tube settings and recalculates the spillover values based on the default LW reference setting and MFI and PMT voltages.



If you select a different tube setting, you will undo your current modified star values. Calculated spillover values are not saved and cannot be reused except by copying and pasting a tube from one experiment to another. If you want to save these modified settings for future use, create tube settings.

See Creating tube settings (page 184).

More information

- Modifying tube properties (page 167)
- Viewing tube parameters (page 171)
- Creating tube settings (page 184)

Viewing tube parameters

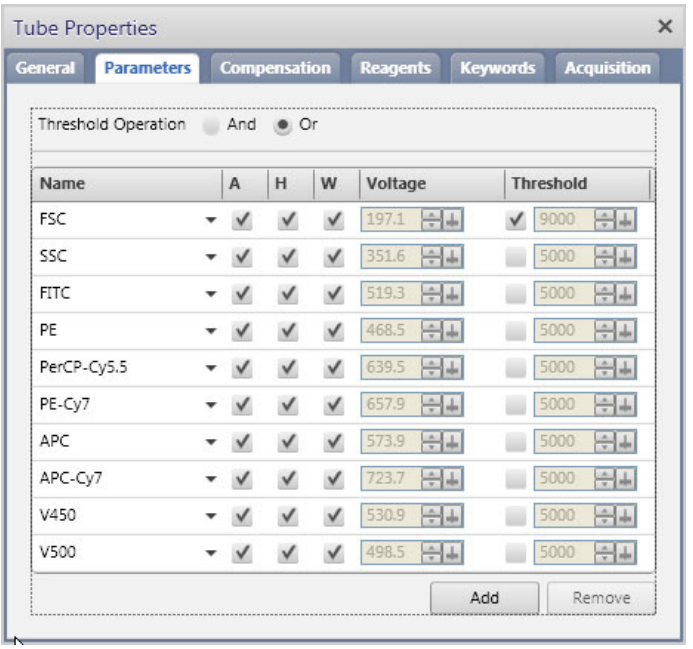
Introduction

This topic describes how to view the tube parameters, area, height, and width selections, and the PMT voltage and threshold settings.

Procedure

To view tube parameters for a tube:

1. In the **Data Sources** panel, set the run pointer to the tube you want to modify (for example, *Tube_001)*.
2. Double-click the run pointer, or right-click the tube and select **Properties** to open the **Tube Properties** dialog.
3. Click the **Parameters** tab.



This read-only tab displays only the parameters that are set for the current tube or an acquired tube.

More information

- PMT Voltages (page 151)
- Setting general tube properties (page 169)
- Modifying the compensation matrix (page 172)

Modifying the compensation matrix

Introduction

This topic describes how to view or modify the compensation matrix for your tube using the Compensation tab in the Tube Properties dialog.

About the compensation matrix

The compensation matrix is automatically recalculated any time you adjust the PMT voltages in the PMT Voltages section in the Cytometer Settings panel, or in a plot.

If you apply tube settings that do not have associated reference settings, the matrix reflects calculated compensation settings.

If you apply tube settings that have an associated reference setting, the matrix reflects measured compensation settings.

If the calculated spillover values work for your samples, you do not need to modify this matrix.

Procedure

To modify the compensation matrix for a tube:

1. In the **Data Sources** panel, set the run pointer to the tube you want to modify (for example, *Tube_001)*.
2. Double-click the run pointer, or right-click the tube and select **Properties** to open the **Tube Properties** dialog.

3. Click the **Compensation** tab.

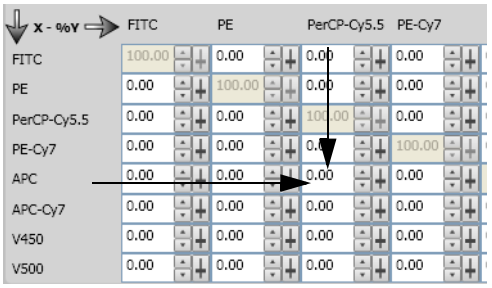
   The **Compensation Matrix** displays the spillover values associated with the acquired tube or the values that will be used when a tube is acquired. The table shows how much of the column (for example, FITC parameter) is spilling over into the row (for example, PE parameter). For example, *PE - 14.37%FITC*.

4. Select the **Enable Compensation** checkbox to analyze with compensated data, or clear the checkbox to analyze with uncompensated data.

5. (Optional) Edit the spillover values as needed.

   The goal is to ensure that a single positive population and the negative population are centered. For example for a plot with FITC vs PE, the FITC mean on the y axis should not be higher or lower than the negative population mean.

   a. Locate the row on the left, then move right across the table until you locate the intersecting column.



   b. Type a new value (0.00 to 1000.00) in the table, or click the up and down arrows to adjust the value.

   If you adjust the values in this matrix, the values are applied to the current tube. If you want to reuse these values, you need to save modified reference settings.

More information

- Saving modified reference settings (page 201)
- Viewing tube parameters (page 171)
- Editing reagent labels (page 174)

Editing reagent labels

Introduction

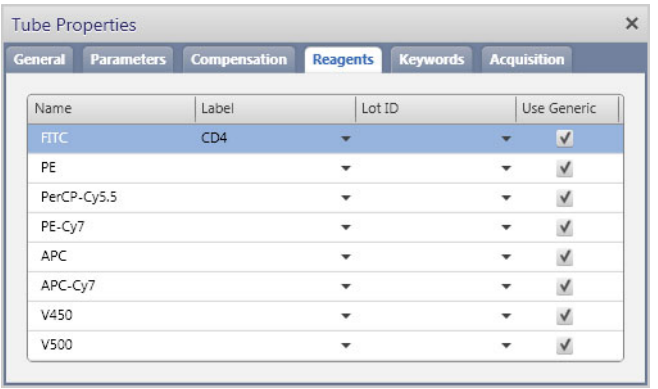
This topic describes how to view or edit reagent labels for available fluorochrome parameters in a tube.

Procedure

To edit reagent labels for available fluorochrome parameters in a tube:

1. In the **Data Sources** panel, set the run pointer to the tube you want to modify (for example, *Tube_001)*.
2. Double-click the run pointer, or right-click the tube and select **Properties** to open the **Tube Properties** dialog.

   Click the **Reagents** tab.
3. In the **Label** column for each parameter, click and select an available reagent label.



- If you use reagents that require either lot-specific or lot- and label-specific spillover values (tandem fluorophores), select either a label or a label and a lot ID, and clear the **Use Generic** checkbox in order to apply the correct spillover to the tube.
- If the label is not lot-specific, select the **Use Generic** checkbox for the fluorochrome.

  New lots and labels for tandem fluorophores must be added to the library before they are available as options in this tab.

More information

- Working with labels in the library (page 302)
- Adding fluorochromes to a reference setting (page 195)
- Modifying the compensation matrix (page 172)
- Working with keywords (page 176)

Working with keywords

Introduction

This topic describes how to view or assign keywords to a tube and how to modify keyword values using the Keywords tab in the Tube Properties dialog.

About keywords

Keywords are unique fields for storing information in files. They are used to identify particular data elements, both required and optional, and can be added to tubes in an experiment. Keywords can include information such as patient information, dilutions, and cell or sample types.

See Understanding keywords (page 297) for a description of keywords and how they are used in BD FACSuite software.

Before you begin

In the library, create any new keywords that you plan to assign to tubes.

See Working with keywords in the library (page 299) for more information about creating keywords.

Assigning new keywords to a tube

To assign a new (user-defined) keyword to a tube:

1. In the **Data Sources** panel, set the run pointer to the tube you want to modify (for example, *Tube_001)*.
2. Double-click the run pointer, or right-click the tube and select **Properties** to open the **Tube Properties** dialog.
3. Click the **Keywords** tab.
4. Click **Add**.

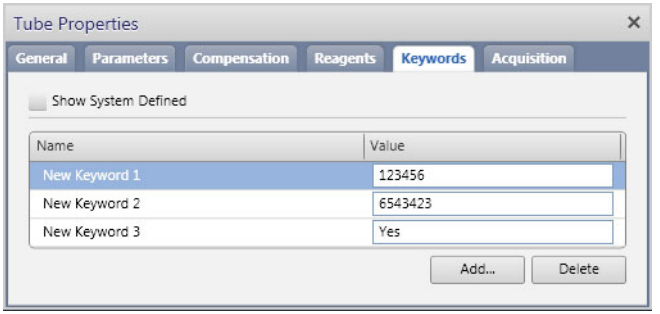
   The **Add Keywords to Tube(s)** dialog opens.
5. In the **Assign Keywords** column, select the checkboxes for keywords you want to assign to the tube.

To locate or view detailed information on the keywords, you can perform the actions in the following table.

To...	Then do this...
Filter keywords by name or type	In the **Keyword Filter** field, type the name or type criteria, then press **Enter**.
Show user-defined and system-defined keywords in the list	Select the **Show System Defined Keywords** checkbox.
View the properties of a keyword	1. Click **Keyword Properties**. 2. Click a keyword in the list to view its properties.

6. Click **OK**.

   The assigned user-defined keywords are displayed in the tab.



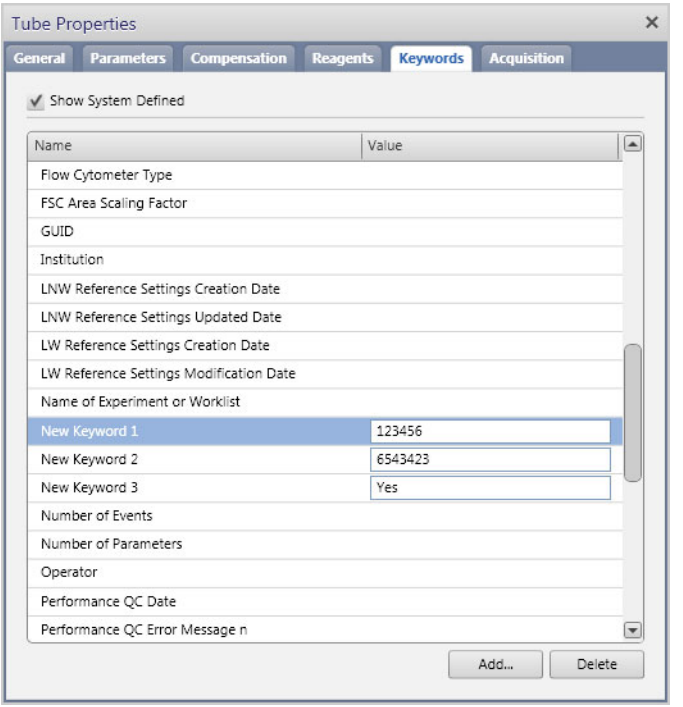
Changing values for assigned keywords

To change values for keywords assigned to a tube:

1. In the **Tube Properties** dialog, click the **Keywords** tab.

   Any currently assigned user-defined keywords are displayed.

2. Select the **Show System Defined** checkbox to display all user-defined and system-defined keywords that are assigned to this tube.



3. Click the user-defined keyword you want to modify.
4. Click the **Value** field for that keyword and type a new value.

More information

- Understanding keywords (page 297)
- Working with keywords in the library (page 299)
- Editing reagent labels (page 174)
- Setting acquisition stopping rules (page 179)

Setting acquisition stopping rules

Introduction

This topic describes how to set acquisition stopping rules using the Acquisition tab in the Tube Properties dialog.

If you want to assign a specific gate or gates to use as storage or stopping criteria, these gates must first be created before setting acquisition properties.

Setting acquisition stopping rules for a tube

To set acquisition stopping rules for a tube:

1. In the **Data Sources** panel, set the run pointer on the tube you want to modify (for example, *Tube_001)*.
2. Double-click the run pointer, or right-click the tube and select **Properties** to open the **Tube Properties** dialog.
3. Click the **Acquisition** tab.
4. If the experiment has multiple worksheets, click and select a worksheet in the **Worksheet to Display during Acquisition** field.
5. In the **Storage Gate** field, click and select an existing gated population if you want to use a gate as the storage gate.

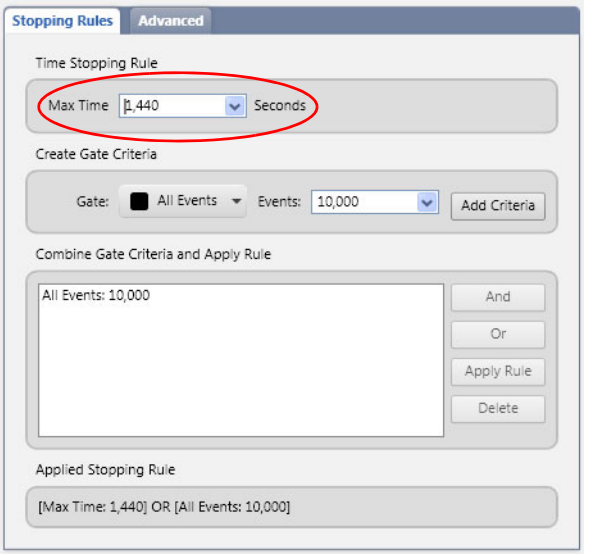
   A storage gate identifies which data is stored when the tube is acquired. If you select a gate for a subpopulation (P1, P2, etc), only events from that subpopulation are stored in the FCS file.
6. In the **Stopping Rules** tab, define the acquisition stopping rules.

   BD FACSuite software always uses a combination of a time stopping rule and a gate criteria rule using an OR operator. Use **Time Stopping Rule** to change the time criteria. Use **Create Gate Criteria** and **Combine Gate Criteria and Apply Rule** to change and apply the gate criteria. The current acquisition stopping rules are displayed under **Applied Stopping Rule**.

Defining time-based stopping rules

To define time-based stopping rules:

1. Under **Time Stopping Rule**, select a maximum acquisition time (seconds). Do not select *Infinite*.



2. Under **Create Gate Criteria**, make sure that the event count is high to ensure that it does not interfere with the time stopping rule.

   Time stopping rules are always applied with *Or* criteria. If the event count is set too low, the acquisition might stop before the time stopping rule is satisfied.

   The time stopping rule is automatically applied and is displayed under **Applied Stopping Rule.**

Defining event-based stopping rules

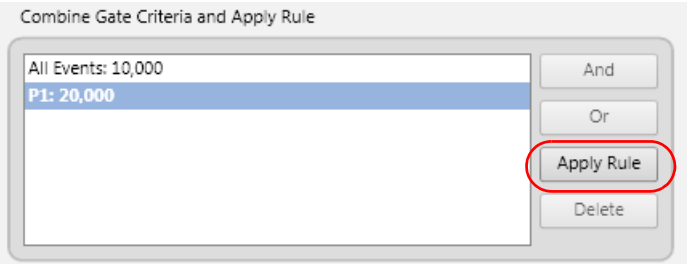
To define event-based stopping rules:

1. Under **Time Stopping Rule**, select *Infinite* as the maximum acquisition time.

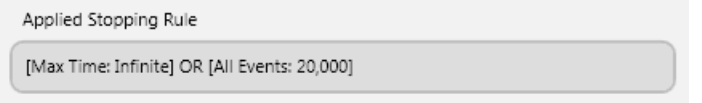
   This effectively eliminates time as a stopping trigger.

Single, event-based rules are always applied with *Or* criteria. If the max time is set too low, the acquisition might stop before the event stopping rule is satisfied.

2. Under **Create Gate Criteria,** select a stopping gate, then select the minimum number of required events that you want to acquire for the gate.
3. Click **Add Criteria** to add event criteria to the **Combined Gate Criteria and Apply Rule** box.
4. Click the new event criteria in the list, then click **Apply Rule**.



5. The event stopping rule is displayed under **Applied Stopping Rule.**



Defining combined stopping rules

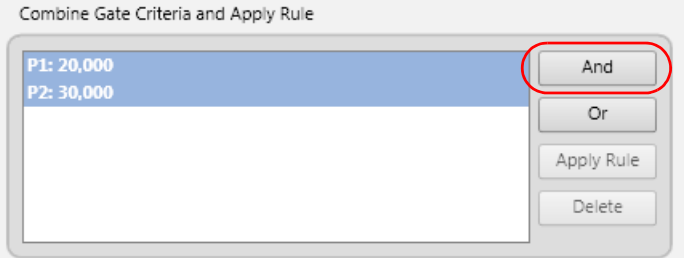
To define combined event-based stopping rules:

1. Under **Time Stopping Rule**, select *Infinite* as the maximum acquisition time.

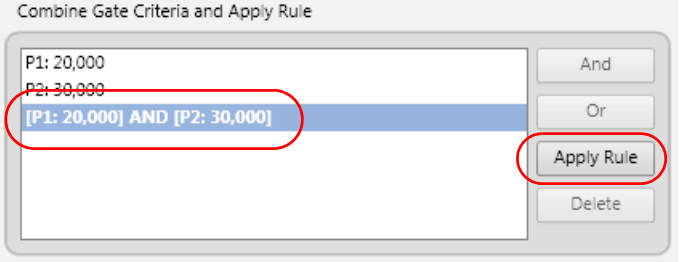
   This effectively eliminates time as a stopping trigger.
2. Under **Create Gate Criteria,** select a stopping gate, then select the minimum number of required events that you want to acquire for the gate.
3. Click **Add Criteria** to add event criteria to the **Combine Gate Criteria and Apply Rule** box.

4. Select additional stopping gates for additional populations, then select the minimum number of required events that you want to acquire for each gate.
5. Click **Add Criteria** to add event criteria to the **Combine Gate Criteria and Apply Rule** box.
6. In the **Combine Gate Criteria and Apply Rule** box, click all event criteria that you want to combine, then click an option:
   - **And**. Requires that all event criteria are satisfied before acquisition stops.
   - **Or.** Requires that one or more event criteria are satisfied before acquisition stops.

     Note that you can also click **Delete** to remove event criteria from the list.



The combined event-based criteria are added to the list.

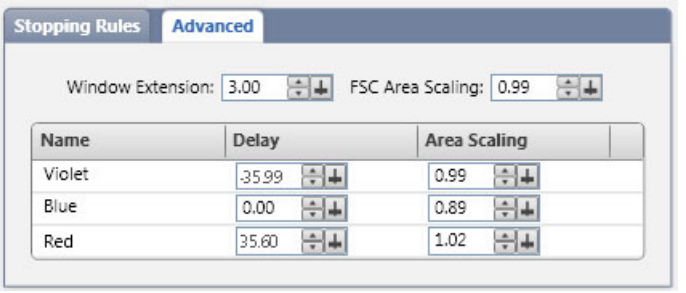


7. Click the new event criteria in the list, then click **Apply Rule**.

8. The combined event stopping rule is displayed under **Applied Stopping Rule.**

Using the Advanced tab

Click the **Advanced** tab to view window extension, area scaling factors, and laser delay values for the tube.



These fields are read-only. You can modify these values using the **Cytometer Settings** panel.

Note that these values are global and affect all experiments and data. To prevent errant data, these values should not be modified after the daily performance QC or Laser setup.

More information

- Lasers (page 153)
- Working with keywords in the library (page 299)
- Using the Manage Experiments tab (page 134)
- Drawing gates in plots (page 164)

Creating tube settings

Introduction

This topic describes how to adjust the default tube settings to optimize the brightness of the positive population, then create user-defined tube settings in an experiment.

About tube settings

If you want to create measured spillover values for a tube setting, you can:

- Create a new tube setting by creating a new reference setting with measured SOVs from an existing tube setting with reference settings.
- Append an existing tube setting with reference settings.

See Creating reference settings (page 187).

When you create a new (default) tube in an experiment, the default LW (lyse/wash) or LNW (lyse/no-wash) tube settings are automatically applied to the tube. The default is determined by your cytometer optical configuration and based on the experiment preferences setting.

When you create tube settings, you are creating new target values based on the current tube parameters and instrument settings (PMT voltages, thresholds, etc).

When to create tube settings

Create tube settings when:

- You want positive stained cells or beads to be either brighter or dimmer in the fluorescence parameter than the default tube setting for LW or LNW and you want to re-use these optimized settings repeatedly.
- You plan to create a user-defined assay from an experiment that includes modified tube settings.

Important note

If you modify the cytometer settings (PMT voltages, thresholds, area scaling, or window extension) to adjust the position or brightness of the positive populations, you are only modifying the cytometer settings for the selected tube.

If you do not create a new tube setting, these modified cytometer settings are saved only with the tube in the current experiment. The modified cytometer settings are identified as instrument settings (not tube settings) in the FCS file.

If you want to create additional tubes with the adjusted cytometer settings, you must manually duplicate the tube within the experiment. Note that you cannot create an assay from the experiment unless you create tube settings for modified tubes.

Before you begin

- Prepare a tube of CS&T beads according to the instructions in the technical data sheet.
- Run performance QC.

Optimizing the positive population

To optimize the position and brightness of the positive population:

1. Create a new experiment.
2. In the **Data Sources** panel, select a tube, or add a new tube.
3. Load a tube of your sample onto the manual tube port.
4. Click **Preview** to start the sample flow and preview the data.
5. In the **Acquisition Status** panel, adjust the flow rate or fluidic mode as needed.
6. (Optional) In the **Laser** section of the **Cytometer** panel, adjust the following as needed:
    a. Adjust the window extension to add or reduce the sampling time above the threshold.
    b. Adjust the FSC area scaling so that the area measurements reflect an appropriate area vs height measurements for the FSC parameter. Note that this is automatically adjusted during daily performance QC.
    c. For each laser that is used, adjust the area scaling factor to reflect an appropriate magnitude vs height measurement from the corresponding laser.

7. In the **PMT Voltages** panel, adjust the PMT voltages and threshold as needed.

Creating user-defined tube settings

The following procedure describes the typical method for creating tube settings without measured spillover values or acquired data.

To create user-defined tube settings:

1. Right-click the tube and select **Create Tube Settings**.

   The **Create Tube Settings** wizard opens.

2. The **CS&T lot ID** field displays the CS&T bead lot to be used for this tube setting.

   If you want to use a different bead lot, click and select a different CS&T bead lot. If the appropriate lot is not available, add the CS&T lot to the library.

   See Working with bead lots in the library (page 289).

3. Load a tube of CS&T beads onto the manual tube port.

4. Click **Acquire**.

   When acquisition completes, the **Name and Description** dialog opens.

5. In the **Tube Settings name** field, type a meaningful name.

6. (Optional) In the **Description** field, type a meaningful description that helps to differentiate between similarly named tube settings.

7. Click **Finish.**

   The new tube settings are saved in the library.

More information

- About tube and reference settings (page 90)
- Creating reference settings (page 187)
- About setup and QC reports (page 93)

Creating reference settings

Introduction

This topic describes how to create reference settings using the Create Reference Settings wizard.

Note that if your system uses multiple user-defined configurations (optical configurations), each different optical configuration requires its own default reference settings.

About default and user-defined reference settings

Default reference settings (LW or LNW) are created for the optical configuration when the system is initially installed or anytime the system is re-configured. Default reference settings are initially applied to all default tubes. These are used to calculate spillover values.

User-defined reference settings are created by measuring spillover values using control tubes.

When to create reference settings

Create reference settings when:

- You need a reproducible system setup from day to day and over time.
- You want to create reusable tube settings and measured spillover values for your samples that are different than the default reference settings provided with the system.

Methods for creating reference settings

There are two methods for creating reference settings:

- Creating reference settings when the default tube settings are applied to a tube.
- Creating reference settings when user-defined tube settings are applied to a tube.

If you start with the default tube settings, modify the cytometer settings, then create the reference settings. A new tube setting is created in the library with the same name as the reference settings. This also creates measured spillover values for the new tube setting.

If you start with a user-defined tube setting applied to the tube, and then create the reference settings, the measured spillover values are associated to the existing user-defined tube setting.

Before you begin

- Prepare the CS&T beads according to the instructions in the technical data sheet.
- Prepare the BD FACSuite FC beads or other single-color control according to the instructions in the technical data sheet.
- When using CompBeads, use 3 drops of CompBeads with 40µl of staining label fluorescence conjugates (for example, CD4).
- Make sure the kit information is entered into the FC Bead reagent section in the library.
- Ensure that the performance QC is current.

Preparing a tube for creating reference settings

To prepare a tube for creating reference settings:

1. Create a new experiment.
2. In the **Data Sources** panel, click an existing tube or add a new tube.
3. Right-click the tube and select **Properties**.

   The **Tube Properties** dialog opens.
4. In the **Tube Settings** field, verify that the correct tube settings have been applied to the tube.

   If not, click **Select** to select the correct tube settings. If you have not created tube settings yet, make sure the LW or LNW default is selected.
5. Close the **Tube Properties** dialog.
6. (Optional) Preview the tube data and modify the cytometer settings.

   If you do not need to adjust the cytometer settings, you can associate the spillover values (measured by this process) with the existing tube settings.

If you need to adjust the cytometer settings, you can create a new tube settings to capture the modified target values.

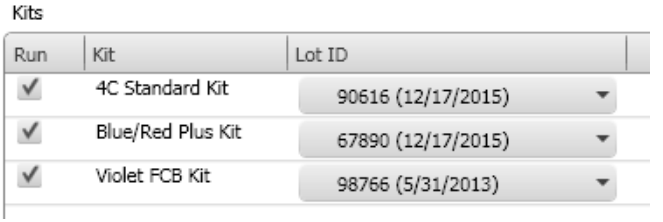
7. Right-click the tube and select **Create Reference Settings.**

   The **Create Reference Settings** wizard opens.

8. The **CS&T lot ID** field displays the bead lot used for the latest performance QC. If you want to use a different bead lot, click and select a different CS&T bead lot.

Selecting kits to run

The **Kits** table displays the available BD FACSuite FC beads that are stored in the library.



To select kits to run:

1. In the **Run** column, select the **Run** checkbox for the FC bead kit you want to run.
2. In the **Lot ID** column, select a lot ID if you want to use a different BD FACSuite FC bead lot.

Selecting control tubes

The Control Tubes table displays the fluorochromes of the beads included in each FC Bead kit. Control types include FC beads, BD™ CompBead particles, and fluorescence controls. The fluorescence control (FC) is useful when the fluorochrome/dye of interest is stained on a specific particle other than FC beads or CompBeads.

To select control tubes:

1. In the **Lot ID** column, select a lot ID if you want to use a different FC bead lot.
2. (Optional) If you want to add new fluorochromes or control types:
   a. Under **Control Tubes**, click **Add** to add a new fluorescence control.

      Available controls are stored in the library.

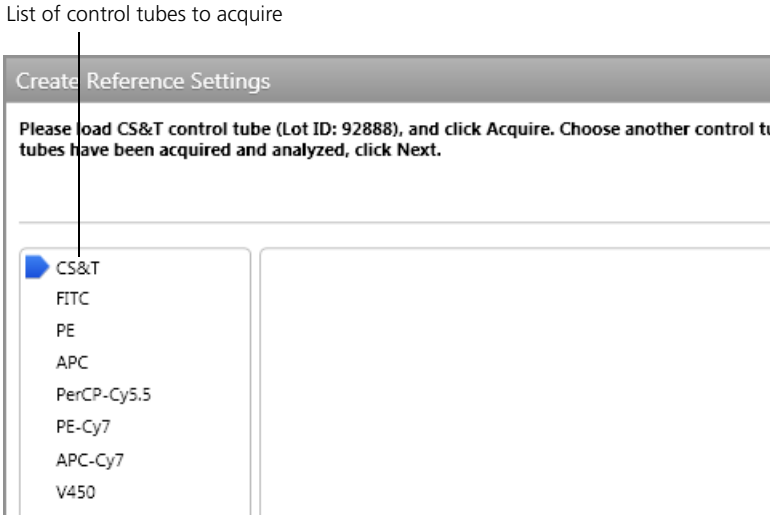
   b. In the **Fluorochrome** column, select a fluorochrome for the new control tube.
   c. In the **Control Type** column, select the control type (FC beads, or FC and CompBeads).
3. In the **Label** column, select to use a generic or specific label.

   Generic labels apply to any antibody or label. Use Specific labels when compensation requirements are different between labels (for example, for tandem dyes). Note that specific labels must be created in the library before you can select them.
4. In the **Lot ID** column, select a lot ID if you want to use a different bead lot.
5. In the **Unstained** column, select a separate tube for the unstained control.

   If the field is blank, the unstained control is in the same tube as the fluorescence control.

6. Click **Next**.

   The next wizard page displays a list of control tubes to acquire.



Acquiring control tubes

To acquire the control tubes:

1. Follow the instructions in the wizard.

   a. Load a tube of CS&T beads onto the manual tube port.

      The run pointer is set to the first control tube in the list and indicates which tube to acquire.

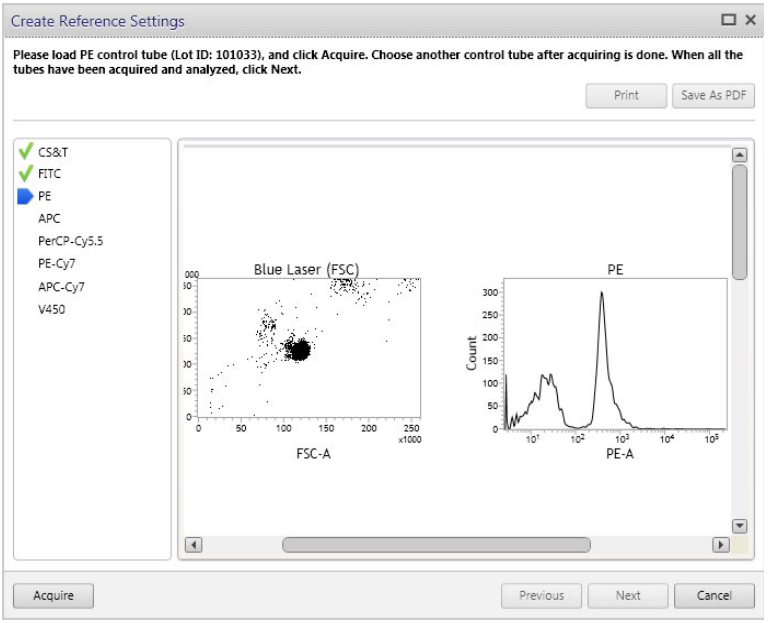
   b. Click **Acquire**.

      During acquisition, plots display data for the CS&T beads. When the CS&T beads are acquired, the run pointer moves to the next tube.

   c. Remove the control tube, then load the next control tube onto the manual tube port (for example, FITC).

   d. Click **Acquire**.

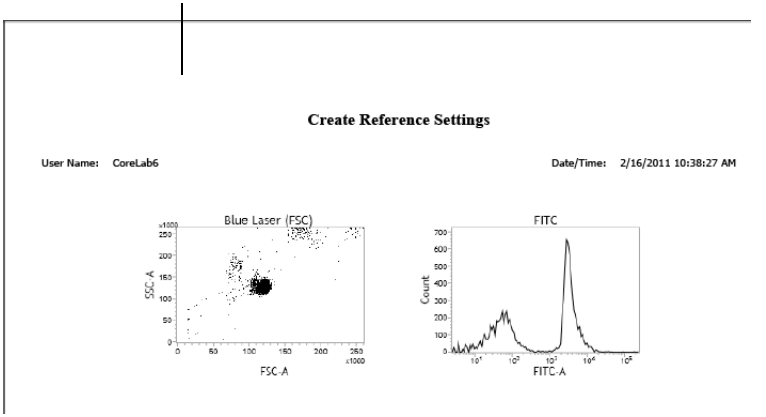
During acquisition, plots display data for each control tube.



2. When the acquisition of the tube completes, a green checkmark is displayed. Follow the instructions on the wizard and repeat step 1 for the remaining control tubes.

3. (Optional) Click **Print** to print the plots for each tube (in a single file) or click **Save as PDF** to save the file.

Example of the reference settings report



4. Click **Next**.

Saving the new reference settings

To save the reference settings:

1. Follow the instructions in the **Reference Setting Name** wizard.
   a. Type a name for a new tube setting that includes the new reference settings.
      - To create a new tube setting with this reference setting, type a new name in the **Reference Setting Name** field.
      - To create a tube setting with this reference setting and replace an existing tube setting with the same name, type the name of an existing tube setting. This overwrites the existing tube setting in the library.

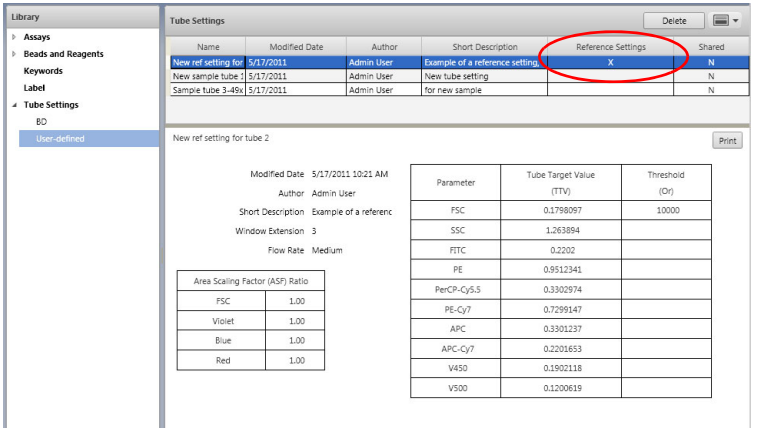
   b. (Optional) In the **Description** field, type a meaningful description that helps to differentiate between similarly named tube settings.
2. Click **Finish.**

   The new tube settings are saved in the library with the associated reference settings.
3. (Optional) View the tube settings:
   a. On the navigation bar, click **Library**.

b. In the **Library** panel, double-click **Tube Settings,** then click **User-defined**.

c. Click a tube setting in the **Tube Settings** table.

The tube settings and reference settings are displayed.



Tube settings that include reference settings are indicated by an *X* in the **Reference Settings** column.

4. (Optional) Print tube settings or reference settings:

   a. Click a tube setting in the **Tube Settings** table.

      The tube settings and reference settings are displayed.

   b. Click **Print**.

More information

- About tube and reference settings (page 90)
- Creating tube settings (page 184)
- Importing or adding a CS&T bead lot (page 82)
- Working with labels in the library (page 302)
- About setup and QC reports (page 93)
- Running characterization QC (page 83)

Adding fluorochromes to a reference setting

Introduction

This topic describes how to add fluorochromes to the current reference settings. This procedure updates a selected tube setting (with an associated reference setting) with measured spillover values from the new fluorochrome.

Before you begin

- Verify that the fluorochrome you want to add to a reference setting has already been added to the current optical configuration.

  See Working with configuration reports (page 543).

- For tandem dyes, you must first add the new lot to the library.

  See Working with labels in the library (page 302).

- Prepare the CS&T beads according to the instructions in the technical data sheet.
- Prepare the BD FACSuite FC beads or other single-color control according to the instructions in the technical data sheet.
- When using CompBeads, use 3 drops of CompBeads with 40µl of staining label fluorescence conjugates (for example, CD4).

Procedure

To add a fluorochrome:

1. Create a new experiment.
2. In the **Data Sources** panel, click a tube or add a new tube.
3. Load a tube of CS&T beads onto the manual tube port.
4. Right-click the tube and select **Add Fluorochromes**.

   The **Add Fluorochromes** wizard opens.
5. If necessary, select a different CS&T bead lot.
6. Under **Kits**, select a lot ID if you want to use a different FC bead lot or a lot of a tandem dye (that was previously added to the library).

7. Under **Reference Settings**, select a checkbox to add fluorochromes to a specific reference setting.

   The **Control Tubes** table displays the fluorochromes of the beads included in each FC Bead kit. Control types include FC beads, BD CompBeads, and fluorescence controls.

8. To add new fluorochromes:

   a. Under **Control Tubes**, click **Add** to add a new fluorescence control tube, FC beads, or BD CompBeads.

   b. In the **Fluorochrome** column, select a fluorochrome for the new control tube.

   c. In the **Control Type** column, select the control type.

   d. In the **Label** column, select a generic or specific label.

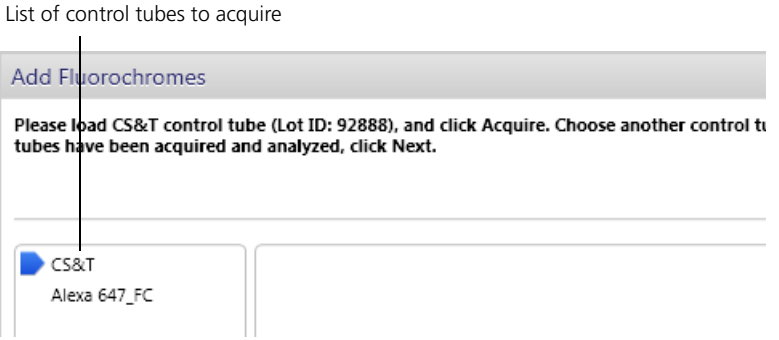
   e. In the **Lot ID** column, select a lot ID if you want to use a different FC bead lot.

   f. In the **Unstained** column, select a separate tube for the unstained control.

      If the field is blank, the unstained control is in the same tube as the fluorescence control.

9. Click **Next**.

   The wizard displays a list of control tubes to acquire.



   The run pointer is set to the first control tube in the list and indicates which tube to acquire.

10. Follow the instructions in the wizard.

    a. Load a tube of CS&T beads onto the manual tube port.

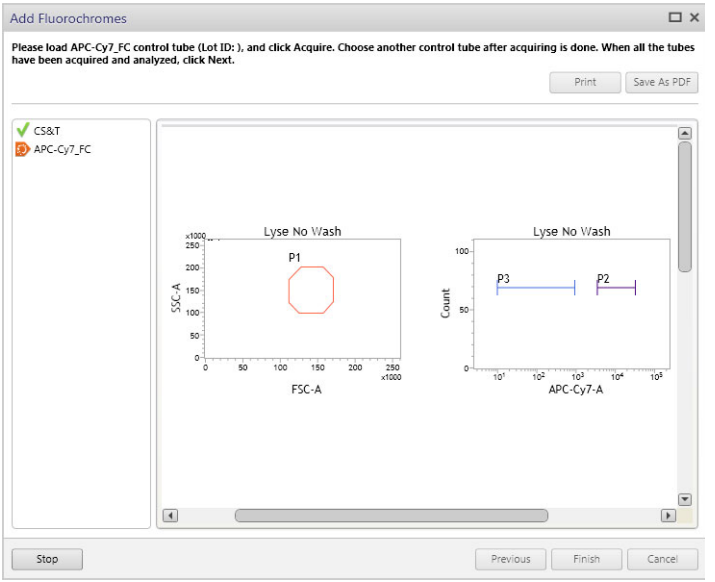
    b. Click **Acquire**.

       When the CS&T beads are acquired, the run pointer moves to the next tube.

    c. Remove the control tube, then load the next control tube onto the manual tube port (for example, APC-Cy7).

    d. Click **Acquire**.

    During acquisition, plots display data for each fluorochrome.



11. When the acquisition of the tube completes, a green checkmark is displayed. Follow the instructions on the wizard and repeat step 10 for the remaining fluorochromes.

12. (Optional) Click **Print** to print the plots for each tube (in a single file) or click **Save as PDF** to save the file.

13. Click **Finish**.

The new fluorochrome is added to the existing tube settings and the reference settings are updated to include the measured SOVs for the new fluorochrome.

More information

- Updating reference settings in an experiment (page 198)
- Saving modified reference settings (page 201)

Updating reference settings in an experiment

Introduction

This topic describes when and how to update the existing reference settings.

Reference settings must be updated every 30 days, and whenever you run characterization QC, change the optical configuration, or modify the fluorochromes.

If you modified reference settings by adjusting cytometer settings (PMT voltages, thresholds, window extension, flow rate, or area scaling) but did not modify the control tubes or fluorochromes, see Saving modified reference settings (page 201) for a simplified procedure.

Before you begin

- Prepare the CS&T beads according to the instructions in the technical data sheet.
- Prepare the BD FACSuite FC beads or other single-color control according to the instructions in the technical data sheet.
- When using CompBeads, use 3 drops of CompBeads with 40µl of staining label fluorescence conjugates (for example, CD4).

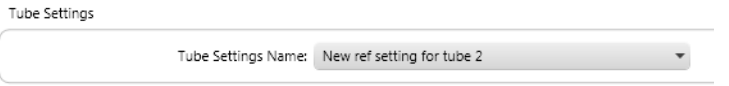
Procedure

To update reference settings in an experiment:

1. In the **Data Sources** panel, right-click the tube and select **Update Reference Settings.**

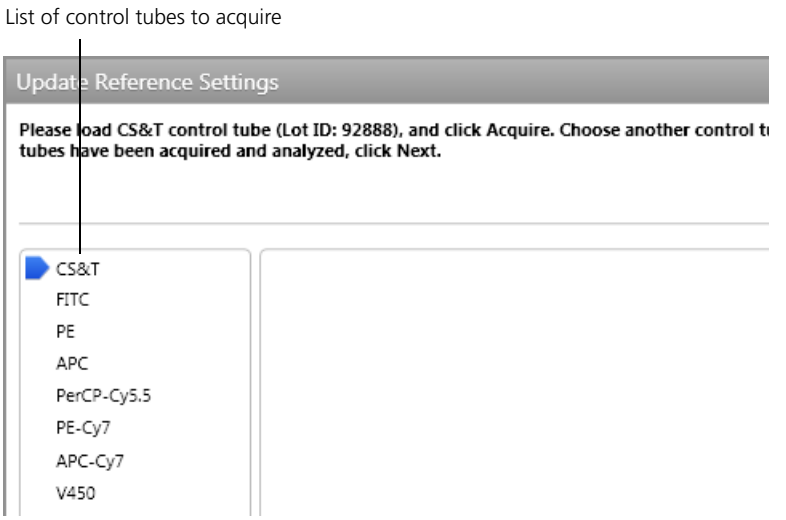
   The **Update Reference Settings** wizard opens.

The **Tube Settings name** field displays the tube settings currently associated with the tube. These tube settings include reference settings.



2. (Optional) Select a different tube setting if needed.
3. If necessary, select a different CS&T bead lot.
4. Under **Kits**, select a lot ID if you want to use a different FC bead lot.
5. Under **Control Tubes**, select the checkboxes for control tubes you want to use for measuring SOVs.
6. Click **Next**.

   The wizard displays a list of control tubes to acquire.



   The run pointer is set to the first control tube in the list and indicates which tube to acquire.

7. Follow the instructions in the wizard.

a. Load the tube of CS&T beads onto the manual tube port.

b. Click **Acquire**.

   During acquisition, plots display data for the CS&T beads. When the CS&T beads are acquired, the run pointer moves to the next tube.

c. Remove the control tube, then load the next control tube onto the manual tube port (for example, PE).

d. Click **Acquire**.

8. When the acquisition of the tube completes, a green checkmark is displayed. Follow the instructions on the wizard and repeat step 7 for the remaining control tubes.

9. (Optional) Click **Print** to print the plots for each tube (in a single file) or click **Save as PDF** to save the file.

10. Click **Finish**.

    The updated reference settings are saved with the tube setting in the library.

More information

- Creating reference settings (page 187)
- Creating tube settings (page 184)
- About setup and QC reports (page 93)

Saving modified reference settings

Introduction

This topic describes how to save modified reference settings when you adjusted cytometer settings (PMT voltages, thresholds, window extension, flow rate, or area scaling) but did not modify the control tubes or fluorochromes.

If your reference settings have expired or if you have changed the optical configuration, modified the fluorochromes, or have run a new characterization QC, see Updating reference settings in an experiment (page 198) for the complete updating procedure.

Procedure

To save modified reference settings:

1. In the **Data Sources** panel, click a tube that is associated to the tube setting (with reference settings) and adjust the settings as needed.

   See Optimizing the positive population (page 185).

2. Right-click the tube, then select **Save Modified Reference Settings**.

   The **Save Modified Reference Settings** dialog opens.

3. In the **Reference Setting Name** field, type a name for the tube setting that includes the modified reference settings.

   - To create new tube settings with this modified reference setting, type a new name in the **Name** field.
   - To create tube settings with this reference setting and replace an existing tube setting with the same name, type the name of an existing tube setting. This overwrites the existing tube setting in the library.

4. (Optional) In the **Description** field, type a meaningful description to help differentiate between similarly named tube settings.

5. Click **Finish.**

   If you typed an existing name to replace an existing tube setting with the same name, the **Save Settings** dialog opens.

6. Click **Yes** to update (overwrite) the existing tube setting.

   The modified reference settings are saved in the library with their associated tube settings and calculated SOVs.

More information

- Updating reference settings in an experiment (page 198)
- Creating tube settings (page 184)

Acquiring data in an experiment

Introduction

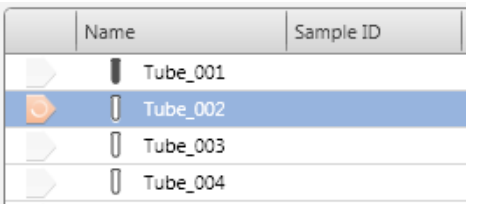
This topic describes how to acquire data in an experiment. You can acquire data in an experiment as long as the experiment has at least one tube.

Procedure

To acquire data in an experiment:

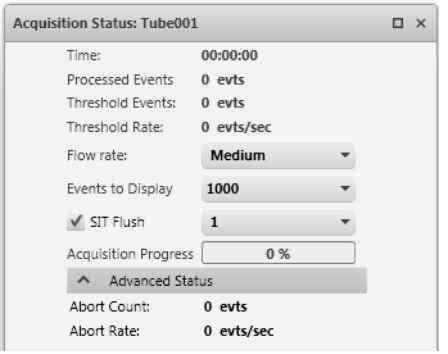
1. Load a tube onto the manual tube port.
2. In the **Data Sources** panel, set the run pointer to the tube you want to acquire (for example, *Tube_001)*.
3. Click **Acquire.**

   During acquisition, the run pointer turns orange and displays an activity indicator.



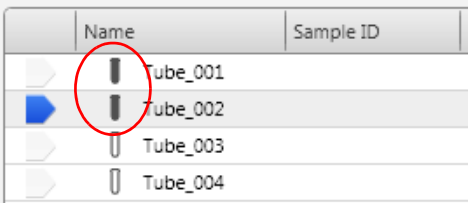
Acquisition continues until the stopping rules (defined in the **Tube Properties** dialog) are satisfied.

The acquisition status is displayed in the **Acquisition Status** panel.



During acquisition, you can click **Stop** to manually stop acquisition, or click **Restart** to clear the current acquisition.

When acquisition is complete, the tube icon displays as a filled tube to indicate that data has been acquired.



4. Click **Next** to move the run pointer to the next tube.

   If no next tube exists, a new tube (duplicate without data) is created and the run pointer moves to the tube.

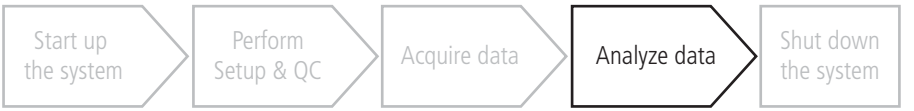
Next step

After you acquire data, you can analyze the data immediately, or open the experiment later to analyze it.

More information

- Experiment analysis (page 204)
- Creating and opening experiments (page 142)
- Experiment overview (page 131)

Experiment analysis



Introduction

This topic describes the workflow for analyzing data acquired in an experiment.

About analyzing experiments

When you create an experiment, you can preview data and perform basic analysis by adding plots and drawing gates to define populations.

A more detailed analysis might include additional plots or histograms, specific fluorochromes, different types of gates, custom statistics views, expressions, or custom reports.

You can perform analysis on the workstation that is connected to the cytometer or from a remote workstation with BD FACSuite software and exported FCS files.

Typical workflow

Perform the following the tasks to analyze data in an experiment.

Stage	Description
1	Open an existing experiment or import an experiment on a remote workstation. See Creating and opening experiments (page 142).
2	Create and modify reports. See Creating experiment analysis reports (page 206) and Modifying experiment worksheets and reports (page 208).
3	(Optional) Export or print reports. See Exporting experiment reports as PDFs (page 217).

More information

- Data analysis example (page 433)
- Creating plots in a worksheet (page 158)
- Drawing gates in plots (page 164)

Creating experiment analysis reports

Introduction

This topic describes how to create custom experiment analysis reports. If you plan to create and run a user-defined assay, create and format a report when you create an experiment.

About analysis reports

In experiments, reports are used to report results and associated information and can include the details you want to present in a final lab report. You can include any of the worksheet elements (for example, plots, gates, and statistics) and create a format that includes company logos and custom text in the headers and footers. Reports are associated and saved with the current experiment.

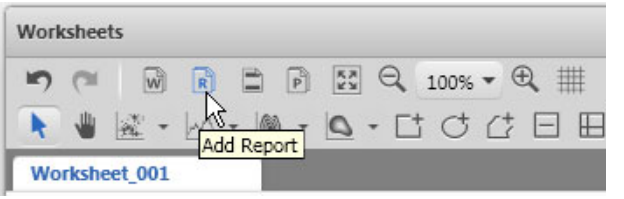
Reports are similar to worksheets in functionality. However, only reports can display an ESignature Box for electronic signature or approval. ESignature is enabled when you edit assay details.

In assays, the report displays the results after acquisition. BD-defined assays include a pre-defined report. Reports update when you run an analysis.

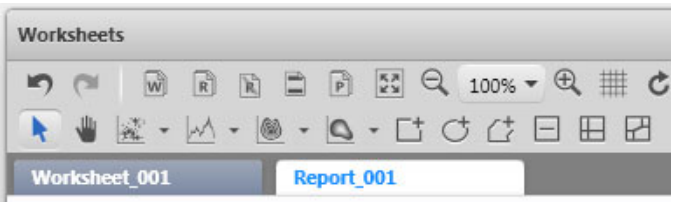
Creating a new report

To create a report from a blank report sheet:

1. In an experiment, click the **Add Report** tool on the **Worksheet** toolbar.



A new **Report** tab opens.



2. Click the text in the **Report** tab, then type a new name (for example, *Analysis Report*).
3. Create plots, statistics views, and other analysis elements.

   The report is automatically saved with the experiment.

More information

- Experiment analysis (page 204)
- Exporting experiment reports as PDFs (page 217)
- Formatting and printing a report (page 213)

Modifying experiment worksheets and reports

Introduction

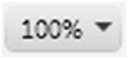
This topic describes how to modify the experiment worksheet or report layout, and how to modify the individual items on the worksheet or report.

Worksheet and report items include plots, statistic views, population hierarchies, expressions, and text boxes.

Worksheet and report tools

The following table describes tools you can use to create and modify the format of worksheets and reports.

Tool	Description
	Click this tool to create a new worksheet. The worksheet displays as a tab.
	Click this tool to create a new report. The report displays as a tab.
	Click a worksheet or report tab, then click the Delete Worksheet or Delete Report tool to delete the worksheet or report.
	Click a worksheet or report tab, then click the Header & Footer tool to open the Header/Footer dialog. Use this dialog to add text to headers and footers, display dividing lines, add logos, and modify font formats. See Formatting and printing a report (page 213).
	Click this tool to add a page to the current worksheet or report. To effectively manage the pages (for future printing or report generation), make sure to only add new pages as needed.
	Click this tool to fit the entire worksheet or report page to the window.

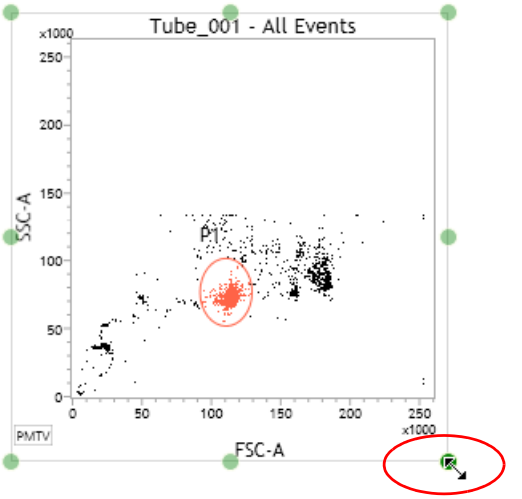
Tool	Description
	Click the magnification tools to increase or decrease magnification for the current worksheet or report. You can also Ctrl+scroll to zoom the worksheet or report.
	Click this tool to select a page view percentage (from 10% to 350%).
	Click this tool to toggle the grid on or off for the current worksheet or report.
	Click this tool to select vertical or horizontal layout. In vertical layout, you can view two pages of a worksheet side by side.
	Click this tool to toggle the orientation of a single worksheet or report page between portrait and landscape page orientation for printing.
	Click this tool to change the page size.
	Click this tool to add a text box to the worksheet or report. You can resize or move the text box and format the text. The text box can include text, keywords, expressions, and results. See Adding text to a worksheet or report (page 211).

Resizing one item on a worksheet or report

To resize worksheet or report items:

1. Open an experiment that includes at least one worksheet or report.
2. Click an item in the worksheet (for example, a plot or statistics view).

3. Click on the vertical or horizontal handles and drag vertically or horizontally to stretch the element, or click one of the corner handles and drag diagonally to proportionally resize.



Resizing multiple items

To resize multiple items on a worksheet or report:

1. Click an item, then Ctrl+click additional items in the worksheet.
2. Click an item handle in one of the items and drag vertically or horizontally.

   This resizes all items proportionally.
3. Click in the worksheet to ungroup the items.

Moving items

To move items in a worksheet or report:

1. Click a plot or Ctrl+click multiple plots.
2. Drag the selected objects to a new location on the worksheet.

Adding text to a worksheet or report

To add text to a worksheet or report:

1. On the **Worksheet** toolbar, click the **Add Rich Text** tool.

   A text box is displayed on the worksheet or report.

2. Type text in the text box, or right-click in the text box and select **Insert** to insert operands from any of the following categories.

Operand category	Description
Worksheet	Select statistics by worksheet > plot > tube > population > parameter > statistic.
Statistics	Select statistics by statistic > tube > population > parameter.
Populations	Select statistics by population > tube > keyword or event.
Expression	Select existing expressions.
Constants	Select pi, or e constants.

3. (Optional) To modify the font, size, and style of the text, right-click and select **Format**.
4. (Optional) To align the text within the text box, right-click and select **Justify**.
5. (Optional) To move the text to a new location within the worksheet or report, drag the text box.

About copying and pasting items

You can copy worksheet or report items and paste them:

- Within the same worksheet or report
- Onto a different page of the worksheet or report
- Onto a different worksheet in the same experiment
- Onto a worksheet or report in a different experiment
- Into a third-party application (outside BD FACSuite software) as an editable, enhanced metafile

Copying and pasting within BD FACSuite software

To copy and paste worksheet or report items within BD FACSuite software:

1. Click an existing item in a worksheet.
2. Right-click and select **Copy.**
3. Locate your paste target.
   - To paste within a worksheet or report, move to a new location in the worksheet.
   - To paste into a different worksheet or report page, navigate to the target page and click to select the page.
   - To paste into a worksheet in a different experiment, open the experiment and click the worksheet to select the page.
4. Right-click and select **Paste**.

Copying and pasting into third-party software

To copy and paste worksheet or report items into third-party software:

1. Click on an existing item in a worksheet.
2. Right-click and select **Copy**.
3. Open the third-party application (for example, Microsoft® Word or PowerPoint®).
4. Click in the target document page.
5. Paste the item. For example, select **Edit > Paste Special**.
6. Select to paste the item as an enhanced metafile.

   After you paste the item, you can ungroup it and click on image elements to modify text, lines, colors, and sizes.

Changing the orientation of single worksheet or report page

To change the orientation of a single page:

1. On the **Worksheet** toolbar, click **Change Page Orientation** to change to portrait or landscape page orientation for printing.

Printing worksheets

To print the currently active experiment worksheet page:

1. From the menu bar, select **File > Print.**
2. Perform your typical printing process.

More information

- Editing assay properties (page 284)
- Formatting and printing a report (page 213)
- About worksheets and reports (page 154)

Formatting and printing a report

Introduction

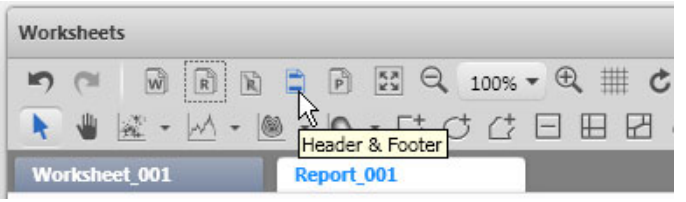
This topic describes how to format a report and set header and footer properties.

You can customize the headers and footers to include specific information and images, and create a custom report layout. Once you have an appropriate format, you can print the report.

Setting experiment report properties

To set report header and footer properties:

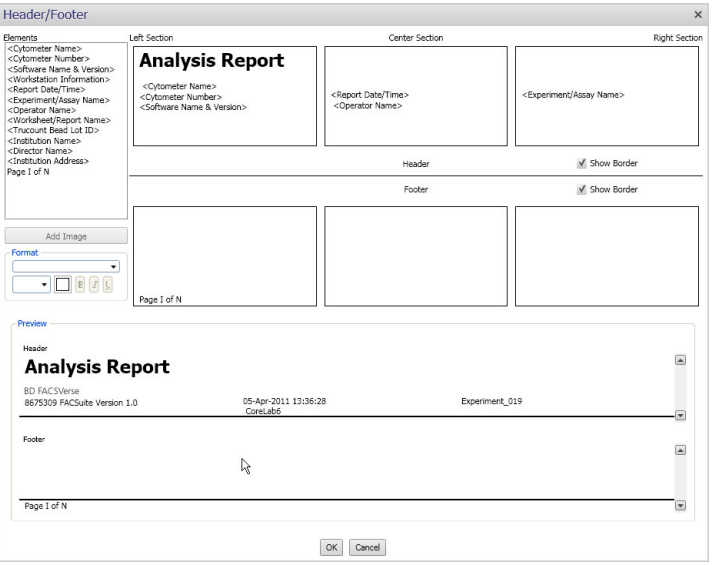
1. Open an experiment or assay report.
2. On the **Worksheet** toolbar, click the **Header & Footer** tool.



The **Header/Footer** dialog opens.

3. Set the cursor in the upper-left corner of a header (left, center, or right) section.
4. Under **Elements,** double-click an element you want to add to the header section (for example, *Cytometer Name)*.
5. Add additional header elements to each section as needed.
6. Set the cursor in lower-left corner of a footer (left, center, or right) section.
7. Add additional footer elements to each section as needed.

   The **Preview** box shows the layout of the elements you selected.



8. Once you have added elements to the report, you can perform the following optional actions.

To...	Then do this...
Move elements from one section to another	Drag elements from one section to another.
Delete elements from the report header or footer	Click an element in a section and press **Delete** to delete it from the section.

9. You can also perform the following optional formatting actions.

To...	Then do this...
Display a border around the elements in the header or footer	In the Header or Footer section of the dialog, select the **Show Border** checkbox.
Add a logo or other image to the report	1. Click **Add Image**. The **Add Image** dialog opens. 2. Navigate to the folder that contains the image, then click **Add**. The image is added to the section and the header or footer is displayed under **Preview**.
Format the report text	Select a font, font size, color, or style for all text in the report.

10. Click **OK**.

    The formatted report opens.

11. Rename the report as appropriate for the experiment.

    a. Click the report title.

    b. Type a new name, then press **Enter**.

Printing the analysis report

To print the analysis report:

1. Click the **Report** tab.
2. From the menu bar, select **File > Print**.
3. Preview the report and make sure that all analysis items are in the print area of the page.
4. Select **Print** on the **Print Preview** menu bar.
5. Complete your typical printing process.

More information

- Experiment analysis (page 204)
- Creating plots in a worksheet (page 158)
- Drawing gates in plots (page 164)

Exporting experiment reports as PDFs

Introduction

This topic describes how to export reports as PDFs.

Exporting reports in PDF format allows you to archive a report separately from the experiment. If your experiment includes multiple report tabs, you must select each and export them separately.

Procedure

To export a report:

1. Open an experiment.
2. Click the **Report** tab.
3. From the menu bar, select **File > Export To PDF**.

   The **Save As** dialog opens.
4. Navigate to the folder where you want to export your report.
5. Type a file name in the **Name** field.
6. Click **OK**.

More information

- Modifying experiment worksheets and reports (page 208)

Creating a user-defined assay from an experiment

Introduction

This topic describes how to create a user-defined assay from an experiment.

About saving experiments

Experiments are automatically saved as you make changes to them. Creating a user-defined assay from an experiment is useful when an experiment must be repeated often by you or others. The user-defined assays provide uniform cytometer settings and format.

When you create a user-defined assay from an experiment, all properties, parameters, and preferences become permanent in the assay. You cannot edit these properties and parameters in the worklist.

If you want to edit properties, you must create an experiment from the assay, modify it, then save it as a user-defined assay again with the same name.

Procedure

To create a user-defined assay from an experiment:

1. Build or open an experiment in the **Experiment** workspace.

   See Creating and opening experiments (page 142).

2. From the menu bar, select **File > Create Assay**.

   The **Create Assay** dialog opens.

3. In the **Name** field, type a name for the new user-defined assay.

   If you intend to modify an existing user-defined assay and want to retain the same assay name, you can overwrite the original by creating an assay with the same name. Note that you cannot overwrite BD assays.

   See Creating a new experiment from an assay (page 144) for more information about modifying an existing assay.

4. (Optional) In the **Description** field, type a description of any details you want to document for the assay.

5. (Optional) Select the **Share** checkbox if you want this user-defined assay to be shared with all users.

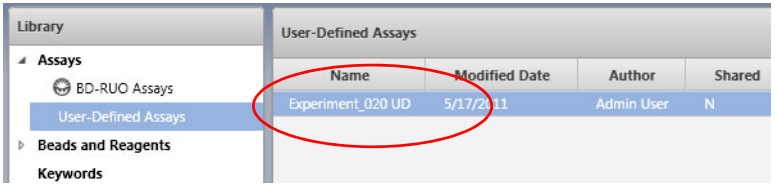
   You can also make the assay shared from within the library after you save it.

6. (Optional) Select a report to display in the **Report to Display After Entry Run** list.

   This sets the default report that appears after the worklist runs.

7. Click **OK**.

   The user-defined assay is added to the library.



More information

- Building a worklist (page 237)
- Changing resource sharing settings (page 306)

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7

Worklist acquisition and analysis

This section includes the following topics:

Worklist overview

Introduction

This topic describes the Manage and Worklist tabs in the Worklist workspace.

A worklist is a list of tasks to be performed for sample acquisition and analysis. The worklist organizes multiple entries, which include sample IDs, tubes, tasks, status, and other information about the sample.

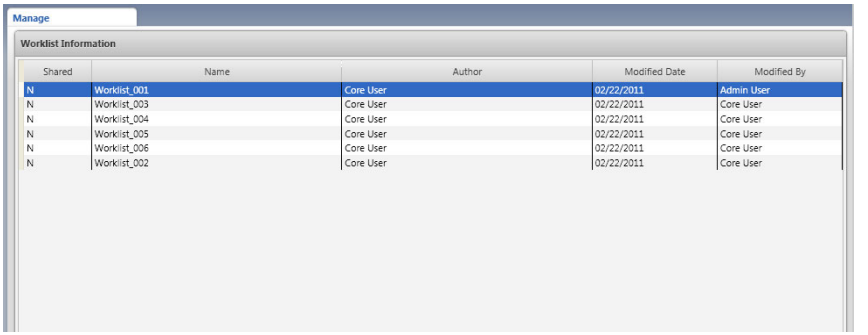
About the Worklist workspace

The Worklist workspace includes the Manage tab and Worklist tabs which represent open worklists.

To open the Worklist workspace, click Worklists in the navigation bar, or click a worklist shortcut on the Home page.

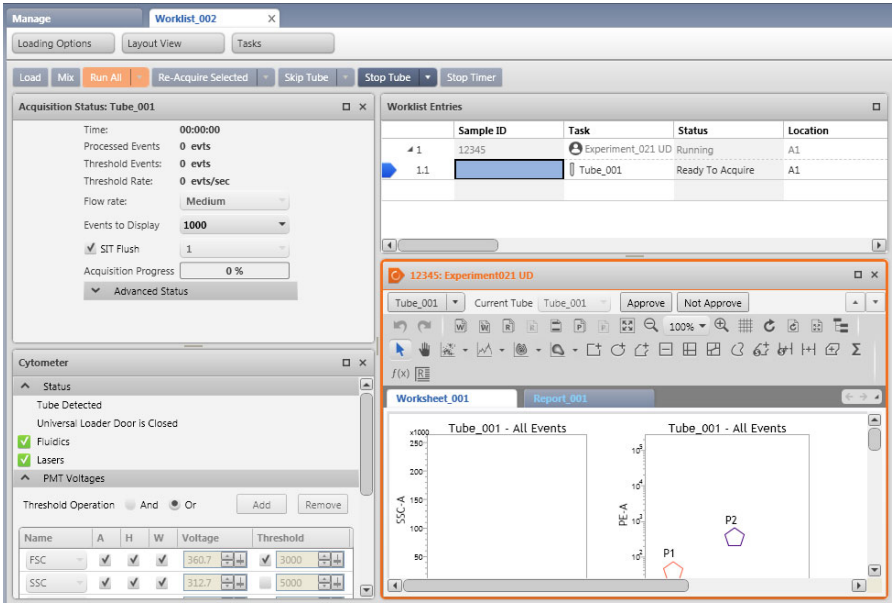
Use the Manage tab to create new worklists, open existing worklists, and filter, search, and share worklists with other users. Use the BD FACSuite menus to create, rename, import, and export worklists.

Manage tab



Use the Worklist tab to develop your worklist and acquire and analyze the data using different analysis tools.

Worklist tab



Using the Manage tab

Introduction

This topic describes how to use the worklist Manage tab to create, open, or import a worklist, and how to change the share settings, sort, filter, and delete worklists.

Creating a worklist

To create a worklist:

1. On the navigation bar, click **Worklists**.

   The **Manage** tab opens.

2. From the menu bar, select **File > New Worklist**.

   The new worklist opens as a new tab in the **Worklist** workspace.

Opening an existing worklist

To open an existing worklist:

1. In the **Manage** tab, double-click a worklist in the **Worklist Information** table.

Importing a worklist

To import a worklist from a folder:

1. From the menu bar, select **File > Import Worklist**.

   The **Import Worklist** dialog opens.

2. Navigate to the folder that contains the worklist you want to import and select the worklist.

3. Click **Open**.

   The worklist is displayed in the **Worklist Information** table.

Changing worklist share settings

Worklist owners (authors) can change or delete only their worklists. Administrators can change or delete all worklists.

Worklists are private by default, but can be shared with other users. The Shared column in the Worklists table indicates whether a worklist is shared (Y) or private (N).

To change the share setting for a worklist:

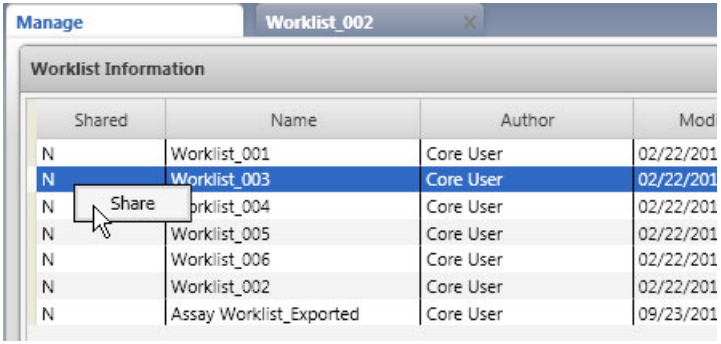
1. On the navigation bar, click **Worklists**.

   The **Manage** tab opens.

2. In the **Worklist Information** table, right-click a worklist in the **Shared** column.

   - If a **Y** is displayed in the **Shared** column, select **Make Private** to make the worklist private.

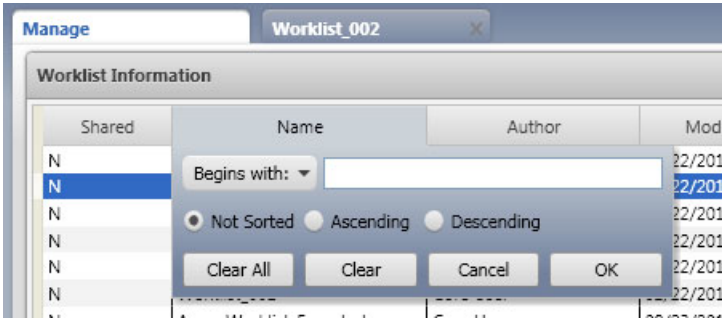
- If an **N** is displayed in the **Shared** column, select **Share** to share the worklist.



Sorting worklists in the table

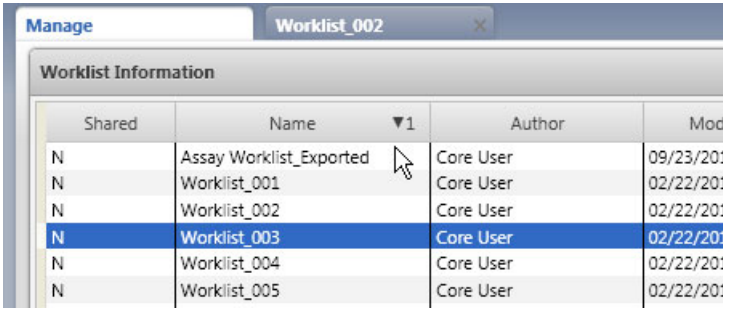
To sort worklist files in the table:

1. In the **Worklist Information** table, click any column header.
2. Select a sort mode option (Ascending or Descending) and click **OK**.



The worklists are sorted by category. Names (text) are sorted in alphabetical order. Numeric values are sorted in ascending or descending order. An icon is displayed in the column header

that indicates ascending (down arrow), or descending (up arrow) order.



You can click the icon to toggle between ascending and descending sort modes.

Deleting worklists

Deleting a worklist makes the data files associated with the worklist inaccessible in the database. When you delete a worklist, the entry run packages (ERPs) for the worklist are automatically exported to the default export folder (as defined in worklist preferences). You can navigate to the export folder to locate and import the ERPs.

To delete a worklist:

1. In the worklist **Manage** tab, click a worklist in the **Worklist Information** table.
2. From the menu bar, select **Edit > Delete**.

   The worklist is deleted.

More information

- Worklist tab overview (page 227)
- Setting worklist preferences (page 71)
- Building a worklist (page 237)
- Setting worklist preferences (page 71)

Worklist tab overview

Introduction

This topic describes the different panels in the Worklist tab and how you use them to build and run worklists.

About the Worklist tab

When you create a new worklist or open an existing worklist in the Manage tab, a new Worklist tab opens. Use the Worklist tab to build and run a worklist. This tab includes the following controls, panels, and tables:

- Worklist controls
- Layout View
- Acquisition Status
- Tasks
- Loading Options
- Cytometer
- Worklist Entries table (worklist)
- Entry Details

About the Worklist Controls

The Worklist Controls bar is displayed at the top of the Worklist tab and includes options for different worklist acquisition, re-acquisition, analysis, and stopping conditions. The following table describes the worklist controls.

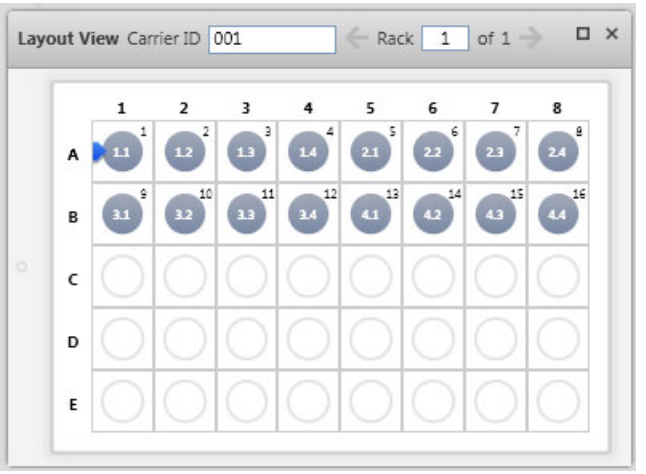
Control	Description
Load	Click this button to load the sample carrier (tube rack or plate) with the optional Loader.
Unload	Click this button to manually unload the current sample carrier with the optional Loader.
Mix	Click this button to perform any mix actions on the sample carrier (based on default or custom sample carrier preferences).
Run All	Click this button to acquire unacquired entries, tubes, or wells, or analyze acquired entries. Click the arrow button to select additional options. A worklist run begins with preview mode, then begins acquiring after the Acquisition Delay Timer expires.

Control	Description
Re-Acquire Selected	Click this button to reacquire any acquired entries, tubes, or wells. Click the arrow button to select additional options.
Skip Tube	Click this button to skip tubes, entries, or sample carriers during an acquisition or analysis run. Click the arrow button to select additional options.
Stop Tube	Click this button to stop a tube (immediately). Click the arrow button to select to stop the run after a tube completes, or stop the run after an entry completes.
Stop Timer Resume	**Before acquisition** During preview mode, the Stop Timer button controls the Acquisition Delay Timer. Click this button to manually stop the timer countdown for a specific duration (defined by the Acquisition Delay Timer) and pause the worklist before acquisition begins. You can stop the timer if you need to adjust PMT voltages, thresholds, or modify gates and statistics markers. If you make changes, a dialog opens. In this dialog, select how you want to apply these changes to the worklist. The next tube or entry in the worklist automatically starts when the pre-defined Acquisition Delay Timer time expires, or if you click Resume. **After acquisition** After acquisition, the Stop Timer button controls the Report Delay Timer. The report displays data from an acquired entry until the timer expires. You can click Stop Timer (before time expires) to continue viewing the report and adjust gates and statistics markers. Click Resume to resume acquisition and display the next entry in the report. You can adjust the duration of the Acquisition and Report delay timers in the Preferences dialog. See Setting worklist preferences (page 71) for more information.

See Worklist run options (page 254) for more information about the Worklist controls.

About the Layout View panel

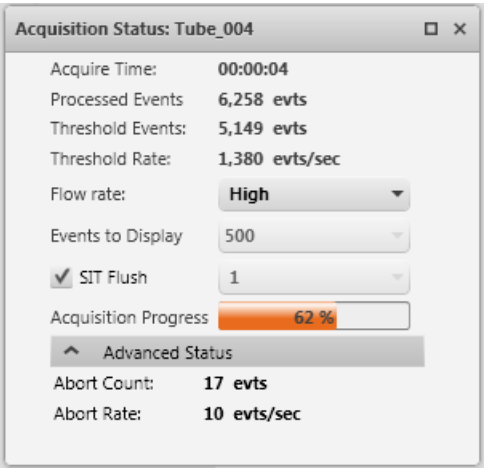
The Layout View panel displays the carrier ID of the tube rack or plate and the tube or well layout. The order of tubes is based on where they are displayed in the worklist.



Right-click a tube or well in the layout to display properties of the tube or well.

About the Acquisition Status panel

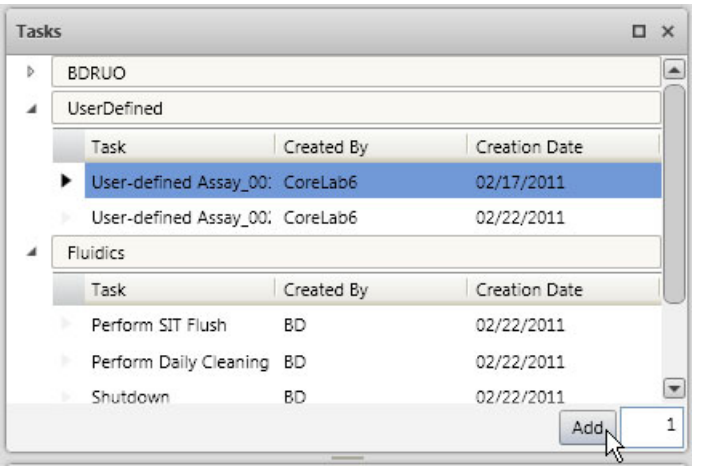
Use the Acquisition Status panel to view real-time status for time, event counts, and aborts. You can also set flow rate, events to display, and SIT flush options specific to an acquisition.



The settings apply to the entire worklist, not individual tubes. For some BD-defined assays, the assay controls the flow rate, events to display, and the SIT flush settings.

About the Tasks panel

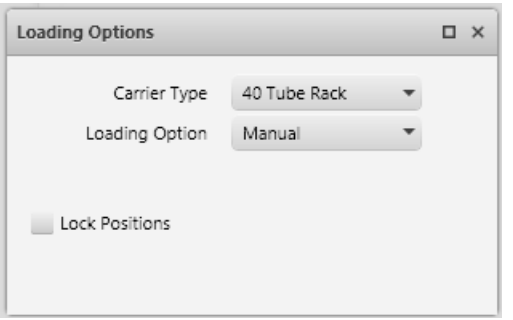
The Tasks panel lists the available BD-defined and user-defined assays, and fluidics tasks you can add to a worklist.



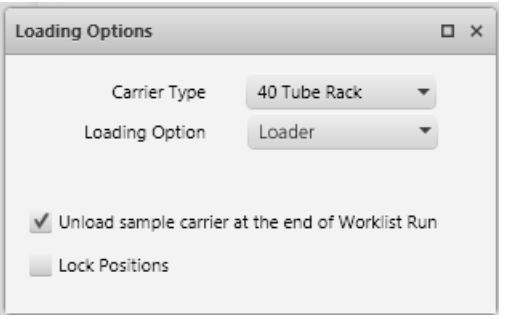
You can select one or more tasks, then click Add. You can also select a task, then add the task multiple times by typing a number in the field, then clicking Add.

About the Loading Options panel

Use the Loading Options panel to select the carrier type and manual or automated loading mode. The default is 40 Tube Rack, Loader (automatic mode).



If you do not have the Loader option installed, the default is Manual.



In automatic mode, you can select the Unload sample carrier at the end of Worklist Run checkbox if you want to automatically unload the sample carrier after acquisition.

Select the Lock Positions checkbox if you want to maintain the physical location of the target tubes or wells—even if you re-order or add new entries in the worklist. This can be helpful when you

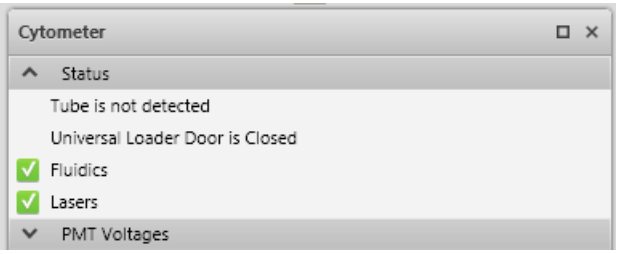
are manually preparing samples to run using the Loader. Note that you cannot unlock the positions once you lock them.

About the Cytometer panel

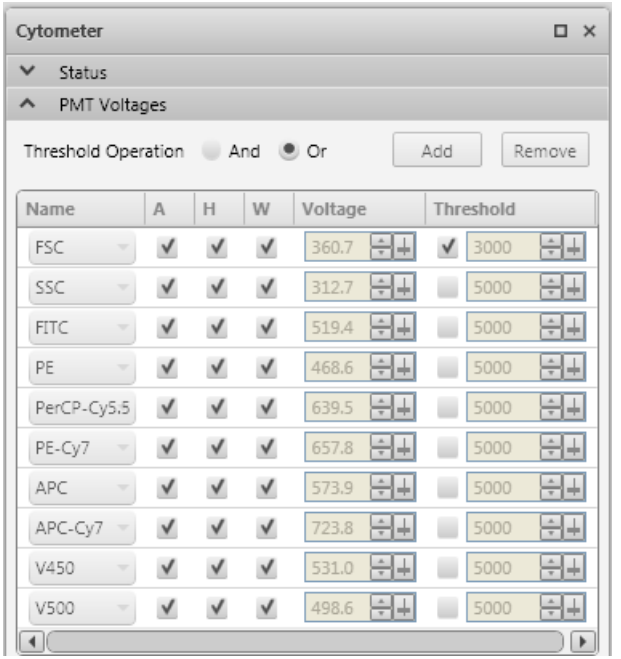
Use the Cytometer panel to view system status, run cleaning protocols, and adjust PMT voltages.

The Status section displays the system status, including real-time status for the manual load port and Loader, fluidics, and lasers.

A checkmark indicates a ready status. This tab also reminds you when you need to run system cleaning protocols.



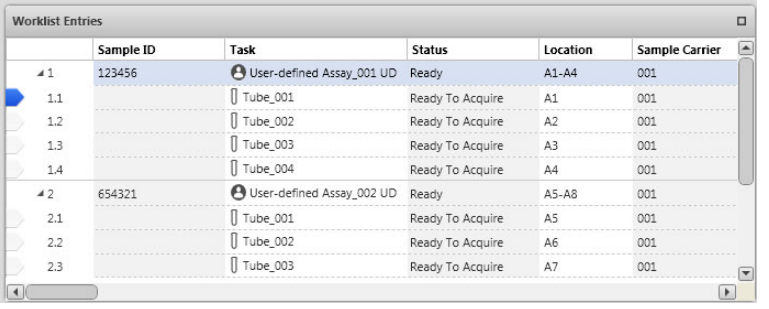
Use the PMT Voltages section to view and adjust the PMT voltages, area, height, width, and enable and adjust thresholds for scatter or fluorescence parameters. You can also add or remove parameters before you run a worklist.



During preview mode, you can adjust threshold and PMT voltages and the system will automatically adjust spillover values for the current worklist only.

About the Worklist Entries table

The Worklist Entries table (worklist) organizes multiple entries to be acquired or analyzed, and displays status and other information about the entry.



Worklists include the following elements.

Worklist elements	Description
Entries	An entry includes a sample ID, tubes, and one task. You can start a new worklist with blank entries and add new tasks. You can also start by importing saved entry run packages into the worklist. An entry run package includes all information needed to replicate an entry in a different worklist. This includes acquired data. You can acquire individual entries, tubes, or an entire worklist, then perform individual sample analysis or batch analysis (entire worklist). Each entry has an ID. Each tube within an entry is a child of the entry. For example, if a entry number is 1, then the tube IDs are 1.1, 1.2, and 1.3.
Sample ID	Each entry requires a sample ID. You can specify a sample ID by typing in the Sample ID column for an entry, or by clicking in the column and scanning the barcode that contains the information.
Task	A task is an action that is performed when you run a worklist. Tasks identify the assay (BD-defined or user-defined) or a fluidics action. Assays are a collection of tubes. There are two types of assays that you can run in a worklist: • BD-defined assays • User-defined assays Fluidics tasks include: • Daily clean • SIT flush • Shutdown When you add a task in the Task column, all tubes associated with the task are added to the entry.

Worklist elements	Description
Status	The current entry status is displayed in the Status column. • **Ready.** Indicates that the entry has a sample ID and a task. • **Not Ready.** Indicates that the entry does not have a sample ID or task. • **Ready for Acquisition.** Indicates that the tube is ready to be acquired and has all required information. • **Complete.** Indicates that the entry or tube has been acquired. • **Ready For Approval.** Indicates that the entry or tube has been acquired and requires approval. This is displayed when Automatically Approve is not enabled for the assay (default). • **Approved.** Indicates that the entry or tube has been approved. This is displayed when Automatically Approve is enabled for the assay. • **Needs Review.** Indicates that changes were made to an audited entry. This is displayed when an audit trail is enabled.
Location	The Location column identifies the tube or well location used for this entry.
Sample Carrier	The Sample Carrier column identifies the tube rack or plate for this entry. You can select different sample carrier types using the Loading Options panel. You can select a specific carrier using the Layout View panel.

More information

- Building a worklist (page 237)
- Defining custom sample carrier layouts (page 508)
- Using the layout view with worklists (page 259)
- BD FACS Universal Loader (page 501)
- Assigning keywords to entries and tubes (page 243)

Worklist acquisition workflow

Introduction

This topic describes the typical workflow stages you need to complete to build a worklist and acquire data.

Workflow stages

Perform the following typical workflow stages for acquiring entries and tubes in a worklist.

Stage	Description
1	Open an existing worklist, or create a new worklist and create new entries. See Building a worklist (page 237).
2	Run the worklist. See Acquiring data in a worklist (page 249).
3	(Optional) Modify worksheet elements or instrument settings (as permitted). You can only modify elements based on user privileges and assay type. See Worklist tab overview (page 227).
4	(Optional) Approve the changes and/or results. See Approving entries in a worklist (page 264).
5	Save, print, or export results, FCS files, or entry run packages. See Exporting entries and worklists (page 268) and Working with assay reports (page 273).

More information

- Worklist overview (page 222)

Building a worklist

Introduction

This topic describes how to build a worklist using default preferences. It includes information about creating or opening a worklist and adding entries. Once you build the worklist, you can save it, export it, or begin acquiring data. This topic also includes information about creating entries by importing entry run packages, adding fluidics entries, and setting acquisition options for an entry.

Building a worklist

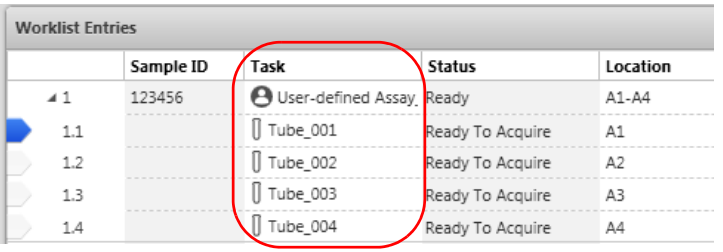
You can set worklist preferences for acquisition delay and report delay timers, manual tube loading, exporting, and printing.

To build a worklist:

1. Create a new worklist or open an existing worklist.
2. In the first blank row in the worklist, click in the **Sample ID** column, type a sample ID for an entry or scan a barcode, and then press **Enter**.

   A new entry is displayed in the worklist.
3. Click in the **Task** column and select an assay or fluidics task.

   All tubes associated with the task are added to the entry.



If you want to add multiple tasks to a worklist (at the same time), you can add tasks using the Tasks panel. See About the Tasks panel (page 230).

When you add a sample ID and a task, the **Status** column displays *Ready*. Each associated tube displays *Ready to Acquire*.

4. (Optional) Once you add an entry, assign keywords to an entry or tube, or enable an audit trail for the entry.

   See Assigning keywords to entries and tubes (page 243) or Working with audit trails (page 245).

5. When you are finished adding entries you can do any of the following:

   - Modify the default loading options.
   - Modify worksheet or report elements, or instrument settings (as permitted by the assay type or your user privileges).
   - Begin acquisition.
   - Close and save the worklist.

Adding entries by importing an entry run package

Individual worklist entries can be exported as entry run packages (ERPs). You can import these ERP files into any worklist and analyze the entry. Note that you cannot import an ERP into a worklist if an identical entry already exists in the worklist.

To import an existing entry run package as an entry in your worklist:

1. Click to highlight an entry in the worklist.
2. Select **File > Import > Entry Run Package**.

   The **Import Entry Run Package** dialog opens.

3. Select an available ERP file, then click **Open**.

   The ERP populates the entry in the worklist.

Adding fluidics cleaning or shutdown to a worklist

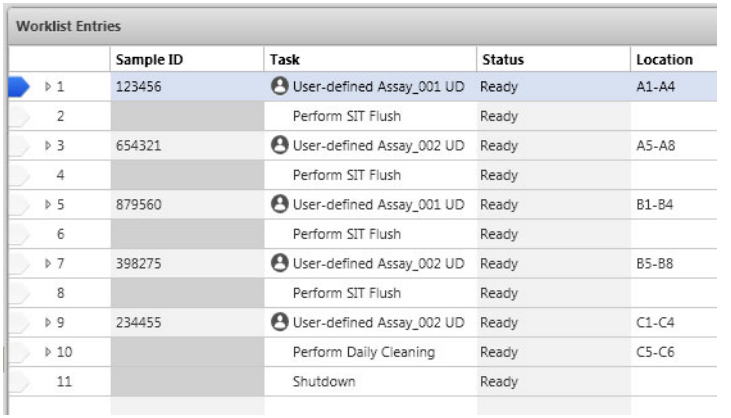
You can create entries in an existing worklist to automate fluidics cleaning and shutdown tasks, or create a standalone cleaning or clean and shutdown worklist that you can run at the end of each day.

To add fluidics cleaning or shutdown entries to an existing worklist:

1. In an existing worklist, navigate to the first blank row at the bottom of the worklist.
2. Click in the **Task** column and select a fluidics task:
   - **Perform Daily Cleaning**
   - **Perform SIT Flush**
   - **Shutdown**

   The new entry is displayed at the end of the worklist.
3. If you want to insert a SIT flush task between other entries in the worklist, drag the SIT flush entry before or after another entry in the list.
4. If your workflow requires additional steps, add additional entries and select the task. For example, add entries for daily cleaning and shutdown to shut down the system after the cleaning task completes.

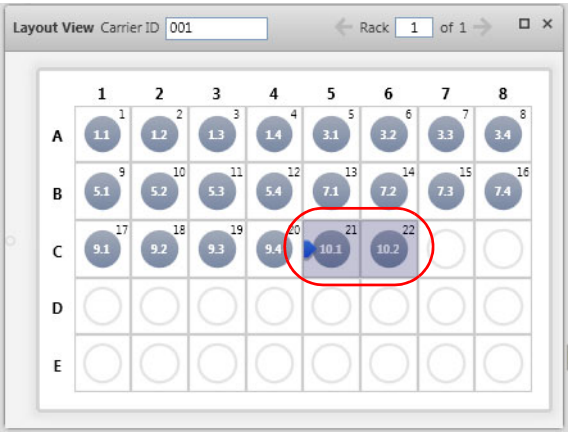
The following figure shows a SIT flush task after each assay task entry. The daily cleaning and shutdown tasks are displayed at the end of the worklist.



Worklist Entries

	Sample ID	Task	Status	Location
1	123456	User-defined Assay_001 UD	Ready	A1-A4
2		Perform SIT Flush	Ready	
3	654321	User-defined Assay_002 UD	Ready	A5-A8
4		Perform SIT Flush	Ready	
5	879560	User-defined Assay_001 UD	Ready	B1-B4
6		Perform SIT Flush	Ready	
7	398275	User-defined Assay_002 UD	Ready	B5-B8
8		Perform SIT Flush	Ready	
9	234455	User-defined Assay_002 UD	Ready	C1-C4
10		Perform Daily Cleaning	Ready	C5-C6
11		Shutdown	Ready	

The **Layout View** automatically displays the tube locations for the new entries. The following figure shows the tube locations for the daily cleaning task.



For cleaning tasks, make sure that your tube racks include a tube with 10% bleach and a tube with DI water in the locations identified in the **Layout View**.

5. Save the worklist.

If you are creating a standalone fluidics cleaning worklist, make sure to name the worklist as a cleaning or clean and shutdown worklist when you save it.

Setting a fluidic mode or flow rate

The fluidic mode controls the speed that the combination of sheath and sample passes through the flow cell in the instrument. These modes include:

- Normal (uses low, medium, or high flow rates)
- High-sensitivity (uses a flow rate that obtains a better separation between the negative and positive fluorescence populations).

Flow rates (low, medium, or high) determine the rate that the sample flows through the flow cell in the instrument.

Note that you cannot apply different fluidic modes to separate entries within the same worklist. Changing from one fluidic mode to another causes the cytometer settings to recalculate and requires time for the fluidics to stabilize. However, you can change from one flow rate to another without recalculation.

To select a different sample flow rate or fluidic mode:

1. In the **Acquisition Status** panel, select a different flow rate than the default (medium). This setting applies to all entries in the worklist.

   Note that if you are running a BD-defined assay, this setting might be controlled by the assay.

Setting the number of events to display

To set the number of events to display:

1. In the **Acquisition Status** panel, select the number of events to display. This setting applies to all entries in the worklist.

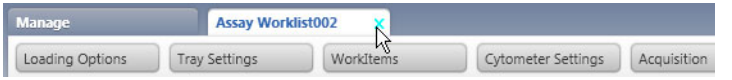
   Note that if you are running a BD-defined assay, this setting might be controlled by the assay.

Saving a worklist

Worklists are automatically saved as you add entries or make changes. You can also manually save the worklist at any time. All worklist entries and acquired data are saved only with the worklist (not as separate entities).

To save a worklist when you close it:

1. Click the **X** on the worklist tab to close the worklist.



   The **Save Worklist** dialog opens.

2. In the **Worklist Name** field, type a name and click the **Save** button.

To save the worklist at any time:

1. From the menu bar, select **File > Save**.

Next step

Acquiring data in a worklist (page 249)

More information

- Using the Manage tab (page 223)
- Defining custom sample carrier layouts (page 508)
- Using the layout view with worklists (page 259)
- Exporting entries and worklists (page 268)
- Running the daily clean procedure (page 469)

Assigning keywords to entries and tubes

Introduction

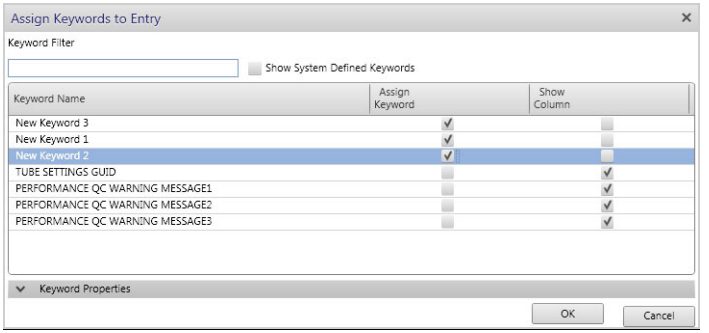
This topic describes how to assign keywords to entries in a worklist.

Assigning keywords to entries

To assign keywords to a worklist entry:

1. In the **Worklist Entries** table, right-click in the first column of a worklist entry, then select **Assign Keywords**.

   The **Assign Keywords to Entry** dialog opens.

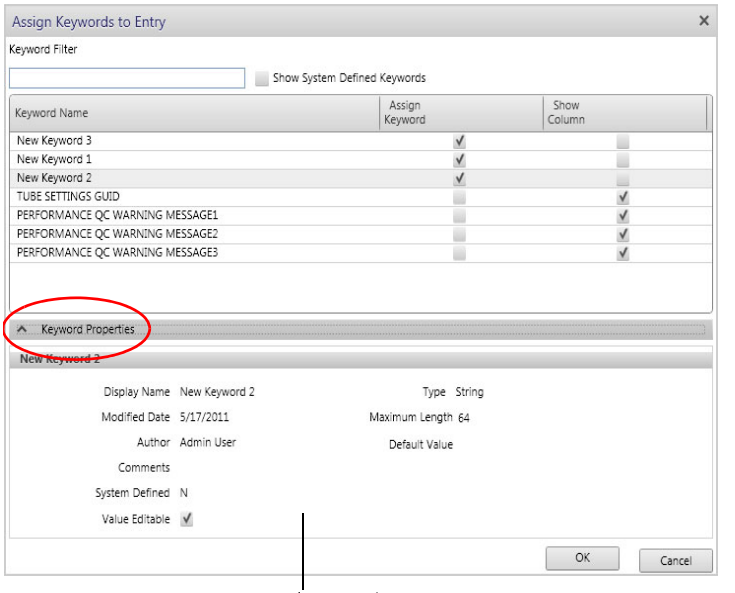


2. (Optional) In the **Keyword Filter** field, type a partial or complete keyword name or identifier**.**

   The table filters keywords by name or identifier.

3. (Optional) Select the **Show System Defined Keywords** checkbox to include system-defined keywords in the list.

4. (Optional) Click **Keyword Properties,** then click a keyword in the list to display its properties.



5. In the **Assign Keyword** column, select the checkbox for all keywords you want to assign to the entry.
6. (Optional) In the **Show Column** column, select the checkbox for any keyword that you want to include as columns in the worklist.
7. Click **OK** to apply your assignments and close the dialog.

Assigning keywords to tubes

To assign keywords to tubes or wells:

1. In the **Worklist Entries** table, right-click a tube in an entry, then select **Assign Keywords**.

   The **Assign Keywords to Tube** dialog opens.

2. In the **Assign Keyword** column, select the checkbox for each keyword you want to assign to the tube.

3. (Optional) In the **Show Column** column, select the checkbox for any keyword you want to include as a column in the worklist.
4. Click **OK** to apply your assignments and close the dialog.

More information

- Using the Manage tab (page 223)
- Understanding keywords (page 297)
- Working with keywords in the library (page 299)
- Defining custom sample carrier layouts (page 508)

Working with audit trails

Introduction

This topic describes how to enable audit trails for entries in worklists, how to work with audited entries, and how to view, print, and export the audit trail log.

About audit trails

Audit trails track changes to entries. Any changes that affect the data (for example, instrument settings, worksheet, reports, plots, gates, and statistics markers) are listed as changes in the audit trail log.

When an audit trail is enabled, the following information is tracked:

- Date and time of changes
- User ID
- Reason for the changes
- Details of the changes

If you enable an audit trail for entries and then modify the audited entries, you must provide reasons for the changes for each entry. This helps to provide a more accurate history of the changes.

Once you enable an audit trail, you cannot disable it. The audit trail is saved as part of the entry run package.

Enabling an audit trail

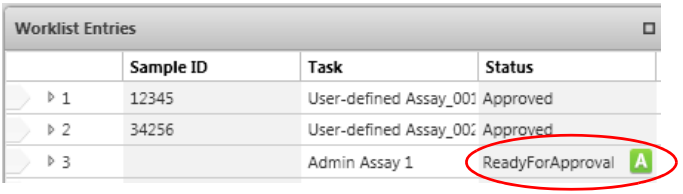
To enable an audit trail:

1. Right-click the entry number, then select **Enable Audit Trail**.

   A confirmation dialog opens.

2. Click **OK**.

   A green audit icon is displayed in the **Status** column.

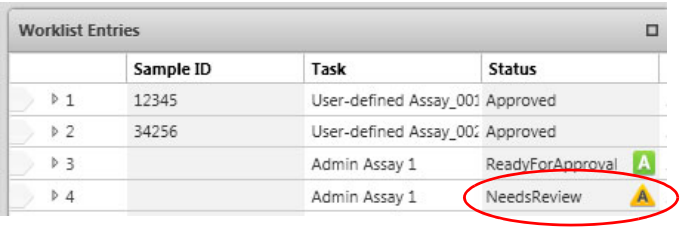


Modifying audited entries

To modify audited entries:

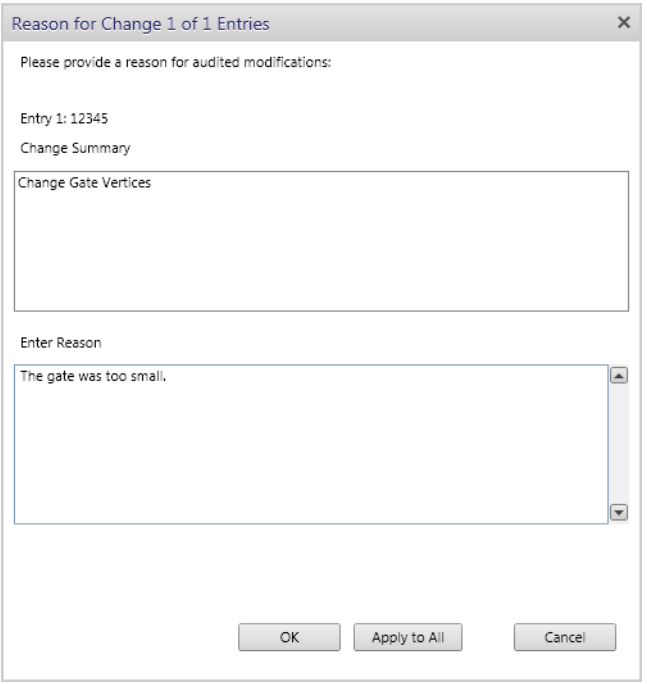
1. Modify the entry (for example, the worksheet or report).

   A yellow audit icon is displayed and remains yellow until you provide a reason for the change.



2. Right-click the entry and select **Provide Reason for Change**.

The **Reason for Change** ***(number of changes)*** **Entries** dialog opens.



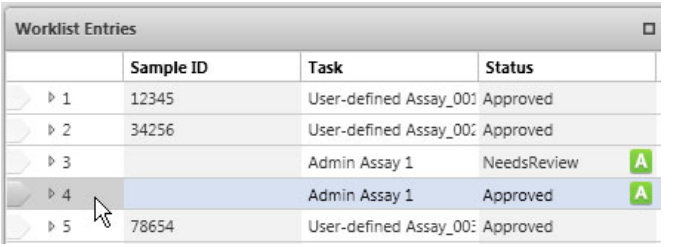
3. Under **Change Summary,** select an entry.
4. Under **Enter Reason,** type a reason for the changing the selected entry.
5. Click **OK.**

   The reason is added to the audit trail log and the yellow audit icon turns green.

Reviewing the audit trail log

To review the audit trail log:

1. Click an audited entry in the worklist to select it.



2. Right-click the entry and select **View Audit Trail**.

   The **Audit Trail Log** dialog opens.

   The task name is displayed in the title bar. The audit trail log displays the history of changes and the reason for each change.

Printing the audit trail log

To print the audit trail log:

1. Click **Print** in the lower-right corner of the dialog.

   The **Audit Trail Viewer Print View** dialog opens.

2. Click the **Print** icon.

   The **Print** dialog opens.

3. Complete your typical printing procedure.

Exporting the audit trail log

To export the audit trail log as a PDF file:

1. Click **Export** in the lower-right corner of the dialog.

   The **Export Audit Trail** dialog opens.

2. Click **Save**.

   The PDF file is exported to the default audit trail folder.

More information

- Approving entries in a worklist (page 264)
- Using ESignature (page 266)

Acquiring data in a worklist

Introduction

This topic describes the worklist controls and how to load tubes or plates and start acquisition for assays in a worklist.

Acquisition is performed only on entries that do not have an associated FCS file. If you want to reacquire an entry, tubes, or a worklist, see Reacquiring entries in a worklist (page 257).

Before you begin

- Run performance QC and assay and tube settings setup.
- Build a worklist.

Loading or unloading tubes or plates

If you have the Loader option, you load prepared sample carriers onto the Loader, then the sample carriers are automatically loaded into the cytometer. If you do not have the Loader option, you need to load each tube manually.

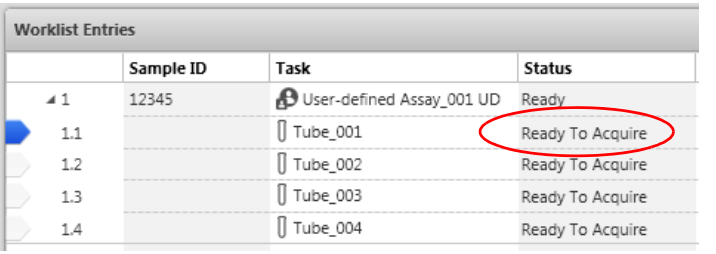
1. Complete one of the actions in the following table.

To...	Then do this...
Manually load a single tube	1. Click **Run All**. 2. Follow the system prompts for loading and unloading tubes. 3. When the LED ring light turns green, place a tube onto the manual tube port and press the top of the tube onto the gasket until you feel a click. The LED ring light turns off. 4. Repeat for each tube as needed.
Manually unload a single tube	Carefully pull the tube down from manual tube port.
Automatically load a tube rack or plate	1. Make sure the sample carrier is loaded onto the Loader tray. 2. Click **Load** or **Run All** on the **Worklist Controls** bar.
Unload a tube rack or plate	If Unload sample carrier at the end of the run is selected in the Loading Options panel, the sample carrier automatically unloads after the acquisition completes. If the checkbox is not selected, the sample carrier remains in position until you click Unload in the Worklist Controls bar.

Running entries in the worklist

To run entries in a worklist:

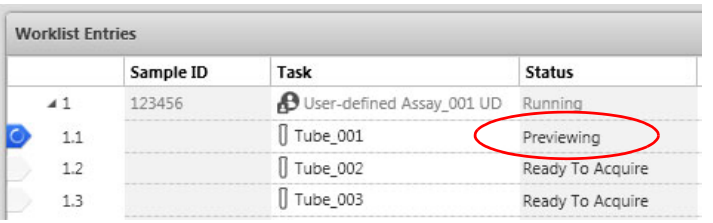
1. Make sure that the entry is ready to be acquired. The Status column should display *Ready* (entry) and *Ready to Acquire* (tubes).



2. In the **Worklist Controls** bar, click **Run All.**

3. Follow the instructions on the dialog that opens.

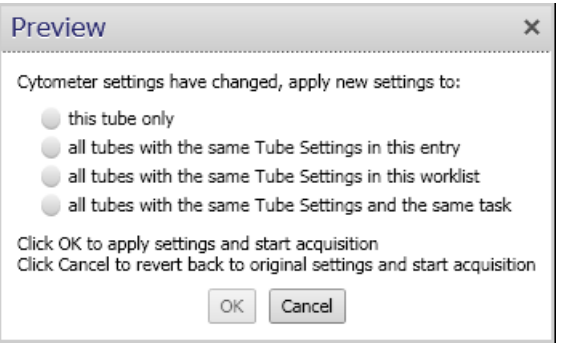
   The worklist run starts by previewing the first unacquired tube.



4. (Optional) Pause the worklist in preview mode.

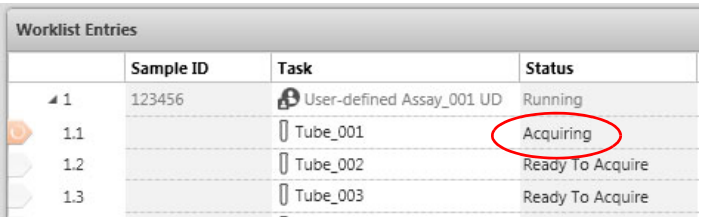
   a. Click **Stop Timer** to manually stop the acquisition delay timer countdown and pause the worklist for a specific duration before acquisition begins.

      Stop the timer if you need to adjust PMT voltages, thresholds, or modify gates and statistics markers. If you make changes, the **Preview** dialog opens.



   b. Select how you want to apply these changes to the worklist, then click **OK**.

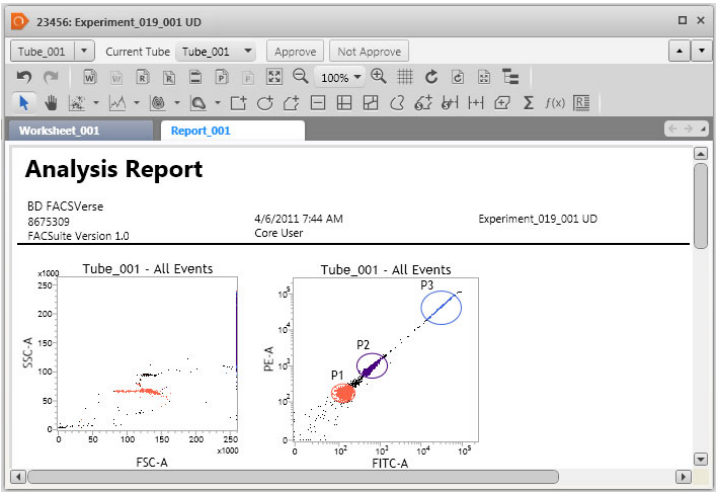
c. Click **Resume**. Acquisition of the tube automatically starts.



As acquisition progresses, the **Acquisition Status** panel displays the time, events, and an acquisition progress bar. The run pointer moves to the next entry as each entry completes, until each entry is completely acquired.

5. (Optional) Review the results during assay acquisition.

   During acquisition, the results are displayed on the assay worksheet in the **Entry Detail** panel. Data is displayed in the report after the entry is acquired.



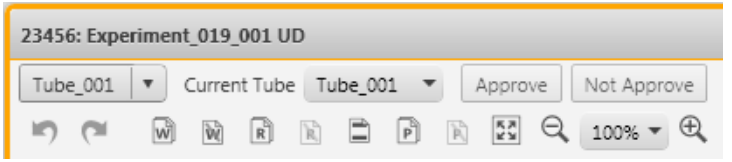
6. (Optional) Modify gates or statistics on the report once the report is populated with data.

   After acquisition, the **Stop Timer** button controls the report delay timer. The report displays data from an acquired entry until the timer expires. You can click **Stop Timer** (before time expires) to continue viewing the report and adjust gates and statistics markers. Click **Resume** to resume acquisition and display the next entry or tube in the report.

   See Setting worklist preferences (page 71) for information about changing the timer settings.

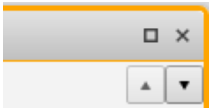
7. (Optional) After acquisition, select a row in the worklist to manually display the worksheets and reports of tubes and entries.

8. (Optional) View the tube properties for any tube in the entry by selecting a tube in the **Entry Detail** toolbar.



9. (Optional) View acquired data for specific tubes by selecting a current tube for this entry in the **Entry Detail** toolbar.

   You can also click the arrow buttons on the right side of the toolbar to view results for the previous entry or the next entry.



Next steps

You can continue by approving acquired entries, re-acquiring specific entries in a worklist, exporting entry run packages, or performing analysis on the acquired entries.

More information

- Worklist analysis (page 270)
- Worklist run options (page 254)
- Loading or unloading tubes or plates (page 249)
- Reacquiring entries in a worklist (page 257)
- Modifying tube properties (page 167)
- Running daily performance QC (page 123)
- Worklist tab overview (page 227)
- Approving entries in a worklist (page 264)

Worklist run options

Introduction

This topic describes worklist run options.

About worklist run order

Acquisition can be performed in different run order depending on the acquisition status of the tubes and where you want to start in a worklist. Once you start a worklist run, you cannot re-order tubes or entries during acquisition. You can add entries to the end of the worklist as it is running. However, the entry is assigned to a new sample carrier.

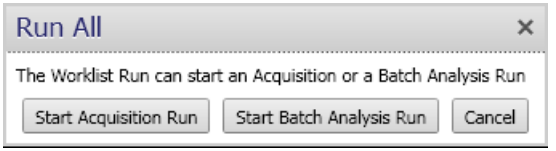
If you are using the Loader option, you can use the Layout View panel to view wells and tubes as they are ordered in the worklist. If you re-order entries or tubes in the worklist, the tray layout updates to match the worklist. If you prefer, you can lock the tray layout for a sample carrier, so that worklist changes do not affect the current layout. Any new entries are assigned to a different sample carrier. If you lock the tray, you cannot unlock it.

Running all entries

To run all entries:

1. Click **Run All** in the **Worklist Control** bar.

   If your worklist includes acquired and unacquired entries, a dialog opens.



   - Click **Start Acquisition Run** to acquire any unacquired tubes or entries.
   - Click **Start Batch Analysis Run** to start analyzing acquired tubes or entries.

   If your worklist includes acquired entries or tubes (FCS files exist for the entry or tube), you can reacquire. See Reacquiring entries in a worklist (page 257).

If you do not want to run the entire worklist from start to finish, you can perform one of the actions in the following sections.

Running one or multiple entries

To run one or multiple entries in a worklist:

1. Complete one of the actions in the following table.

To...	Then do this...
Run from a specific entry or tube and all subsequent tubes	1. Click a specific entry or tube in the worklist to set the run pointer. 2. Click the arrow next to the **Run All** button and select **Run from pointer**. The worklist starts with the specified tube, then runs all subsequent tubes in the worklist.
Run a specific entry or tube in the worklist.	1. Ctrl+click entries or tubes anywhere in the worklist. The tubes do not need to be adjacent in the worklist. 2. Click the arrow next to the **Run All** button and select **Run selected**. The worklist starts with the specified tube and continues with the next selected entry or tube in the worklist.

Skipping tubes, entries, and carriers

To skip tubes, entries, and sample carriers:

1. Complete one of the actions in the following table.

To...	Then do this...
Skip a tube in a worklist	1. Click a specific tube in the worklist. 2. Click **Skip Tube**.
Skip an entry	1. Click a specific entry in the worklist. 2. Click the arrow next to the **Skip Tube** button and click **Skip Entry**.
Skip a carrier type	This applies only to systems using the optional Loader hardware. 1. In the **Sample Carrier ID** column, click a specific carrier for a tube. 2. Click the arrow next to the **Skip Tube** button and click **Skip Sample Carrier**.

Stopping a worklist run

To stop the worklist run:

1. Complete one of the actions in the following table.

To...	Then do this...
Stop the current tube immediately	1. Click a specific tube in the worklist. 2. Click **Stop Tube**.
Stop the worklist after the current tube completes	1. Click a specific tube in the worklist. 2. Click the arrow next to the **Stop Tube** button and click **Stop After Tube Completes**.
Stop the worklist after the current entry completes	1. Click a specific tube in the worklist. 2. Click the arrow next to the **Stop Tube** button and click **Stop After Entry Completes**.

More information

- Worklist overview (page 222)
- Acquiring data in a worklist (page 249)
- Reacquiring entries in a worklist (page 257)

Reacquiring entries in a worklist

Introduction

This topic describes when you can reacquire entries, tubes, or the entire worklist and how to reacquire them.

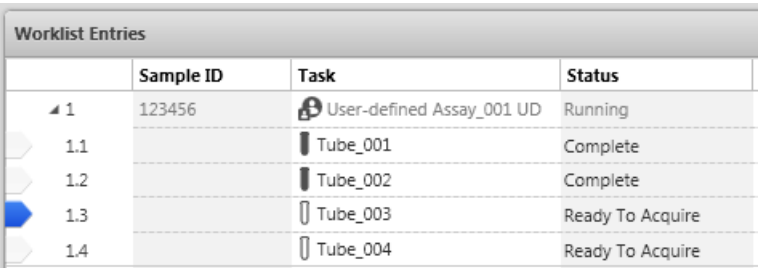
You can only reacquire if FCS files exist for the entry or worklist.

How you know when FCS files exist

After an entry or tube is acquired, a message is displayed in the Status column of the worklist.

- If the Status column displays *Complete*, or if the tube icon is displayed as a filled tube, then an FCS file exists and you can reacquire if needed.

- If the Status column displays *Ready To Acquire*, you have not acquired the entry or tube and no FCS file exists.



Reacquiring in a worklist

To reacquire in a worklist:

1. Complete one of the actions in the following table.

To...	Then do this...
Reacquire an entire worklist	1. Select the first tube in the worklist. 2. Click **Re-Acquire All**. This reacquires all tubes in the worklist that have an FCS file. If your worklist includes acquired and unacquired entries, a dialog opens. Use this dialog to select how to run acquired or unacquired entries.
Reacquire from a specific starting point	1. Set the run pointer at a specific tube. 2. Click the arrow next to the **Re-Acquire All** button, then click **Re-Acquire from Pointer**. This reacquires all subsequent tubes in the worklist that have an FCS file.
Reacquire specific entries or tubes	1. Ctrl+click to select specific tubes that have an FCS file. The selected entries or tubes do not have to be adjacent. 2. Click the arrow next to the **Re-Acquire All** button, then click **Re-Acquire Selected**.
Restart a partially acquired tube	1. Select the tube that was stopped. 2. Click the arrow next to the **Re-Acquire All** button, then click **Re-Acquire Selected**.

More information

- Worklist analysis (page 270)
- Worklist run options (page 254)

Using the layout view with worklists

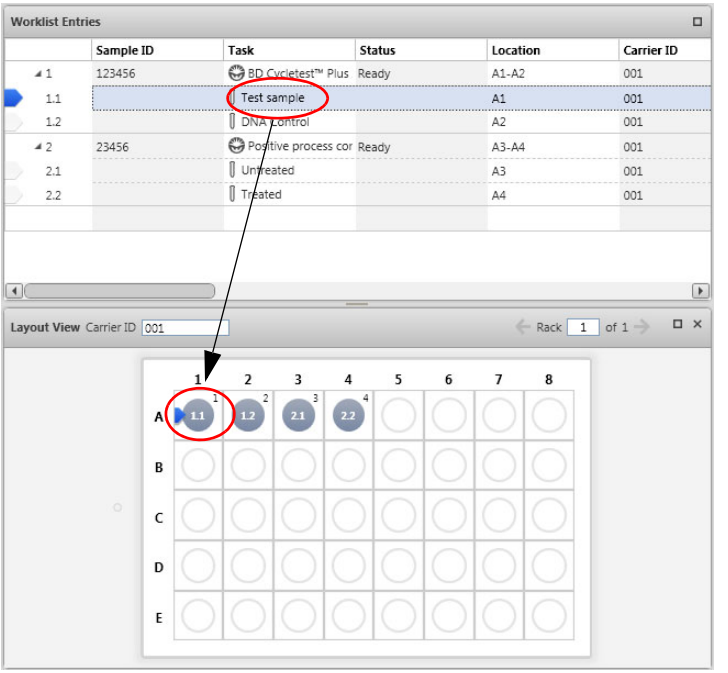
Introduction

This topic describes how entries are assigned in the Layout View panel, how to view well details, and how to monitor the status of an acquisition. This topic also provides definitions for status indicators.

How entries populate the tray layout

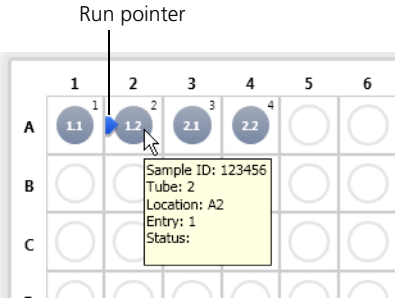
As you add entries in a worklist, each tube in the entry is added to the layout view based on the loading options and run pattern you selected when you defined the layout. The entries populate the layout view starting with A1.

The following example shows *Entry #1, Test sample (tube 1)* mapping to the *A1* tube in a tube rack. This example uses a linear-horizontal run pattern.



Viewing tube or well details

You can view tube or well details by moving the mouse pointer over any populated well or tube. Note that the run pointer moves to the next well or tube after the well or tube is acquired or analyzed. You can manually set the run pointer to a different well or tube.



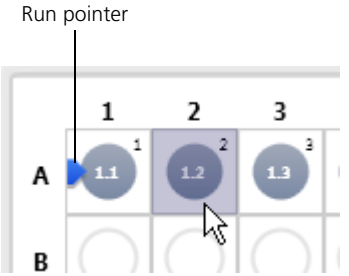
Setting the run pointer in the layout view

The run pointer in the layout view is synchronized with the run pointer in the worklist. It automatically moves to the next well or tube after the well or tube is acquired or analyzed.

In the layout view, you can manually set the run pointer on a different well or tube, then click Run from pointer in the worklist controls.

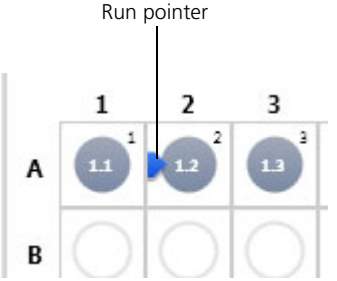
To manually set the run pointer to a different well or tube:

1. In the layout view, click a target well or tube.



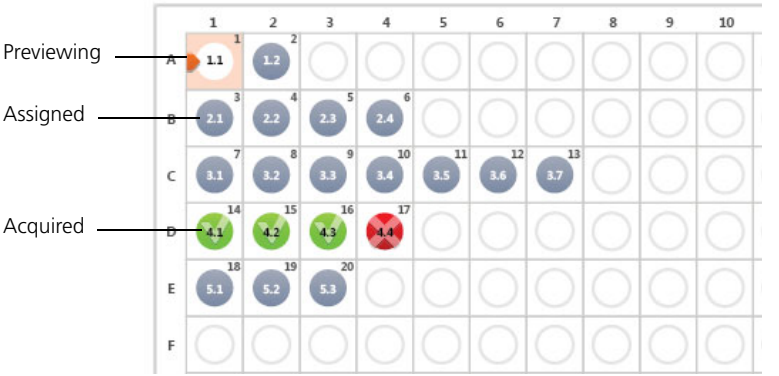
2. Right-click the target well or tube and select **Set Run Pointer**.

   The run pointer is set to the target well or tube.



Monitoring worklist acquisition and analysis

You can monitor the status of a worklist run by viewing the color of each tube or well in the Layout View. The following illustration shows the color indicators.



The following table describes the layout view indicators and their corresponding meanings in the worklist

Indicator	Description	Worklist equivalent
	Location not assigned.	No tubes in an entry.
	Tube or well has a sample.	Tubes exist in a worklist entry.
	Tube or well (with sample) is selected by the run pointer.	The run pointer is set to a tube in the worklist.
	Tube or well is previewing.	*Previewing* is displayed in the worklist Status column.

Indicator	Description	Worklist equivalent
	Tube or well acquisition is in progress.	*Acquiring* is displayed in the worklist Status column.
	Acquisition is complete.	*Complete* is displayed in the worklist Status column.
	Tube or well acquisition error.	*Needs Review* is displayed in the worklist Status column.

More information

- Exporting entries and worklists (page 268)
- Approving entries in a worklist (page 264)

Approving entries in a worklist

Introduction

This topic describes how to approve entries after an acquisition or analysis.

About entry approval

After an entry is acquired in a worklist, the Status column displays one of the following status messages.

Status message	Condition
Approved	This message is displayed when Automatically Approve is enabled for the assay, or when you manually approve the entry.
ReadyForApproval	This message is displayed when Automatically Approve is not enabled for the assay (default) and when no errors are reported for this entry.
NeedsReview	This message is displayed when an audit trail is enabled and you make changes to the plots, gates, statistics, or expressions in an assay and when no errors are reported for the entry.

If your laboratory workflow requires manual approval of an entry before work can continue, you can manually approve or reject it. You can also save a worklist without approving, then return to the worklist at a later time and finalize the status.

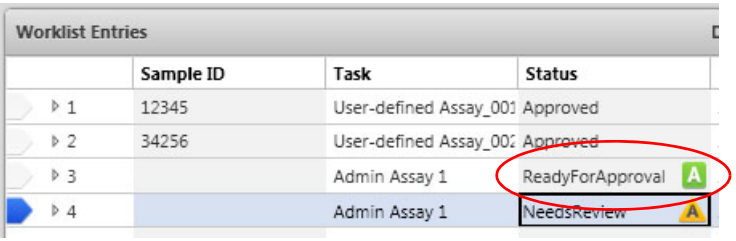
Approving an entry

Note that *Approve* and *Sign* have different functions and are controlled by user permissions. Approving an entry automatically releases results to the LIS.

To approve an entry:

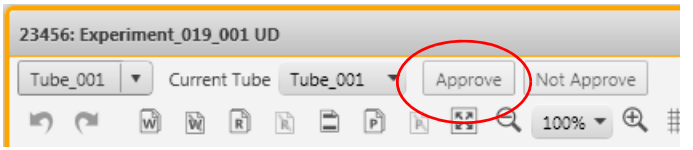
1. Run an assay in a worklist.

   When the worklist run completes, a *ReadyForApproval* or *NeedsReview* message is displayed in the **Status** column.



2. Approve the entry by doing one of the following.

   - Right-click the entry number, then select **Approve**.
   - Ctrl+right-click to select multiple entries, then select **Approve**.
   - In the **Entry Detail**, click **Approve** in the toolbar.



   If you do not want to approve the entry, you can click **Not Approve**. The status changes to *Not Approved*.

Next step

Export entry run packages or analyze entries in a worklist.

More information

- Using ESignature (page 266)
- Exporting entries and worklists (page 268)
- Working with audit trails (page 245)
- Worklist analysis (page 270)

Using ESignature

Introduction

This topic describes how to electronically sign a report after an acquisition or analysis.

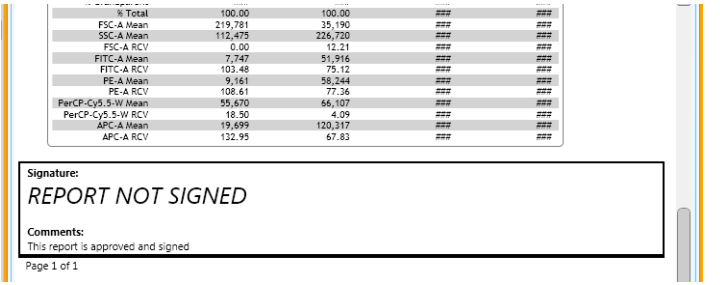
E-signing reports

To E-sign reports:

1. View the report to see if the report requires an e-signature.

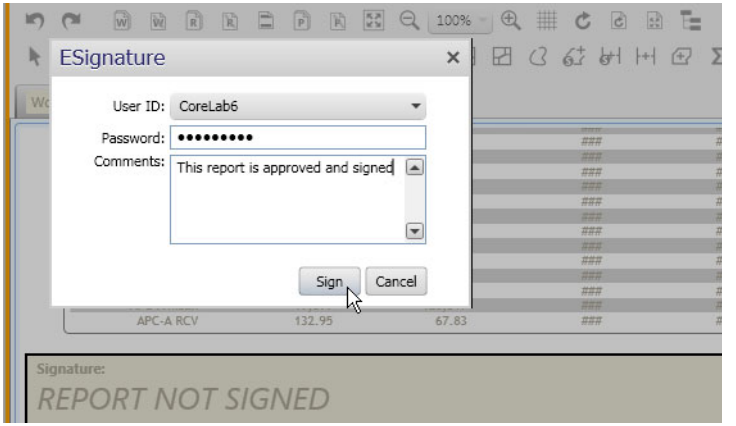
   If e-signature is enabled for the report, the signature box is displayed at the bottom of the report.

   E-signature can be enabled for a user-defined assay in the library. See Editing assay properties (page 284).



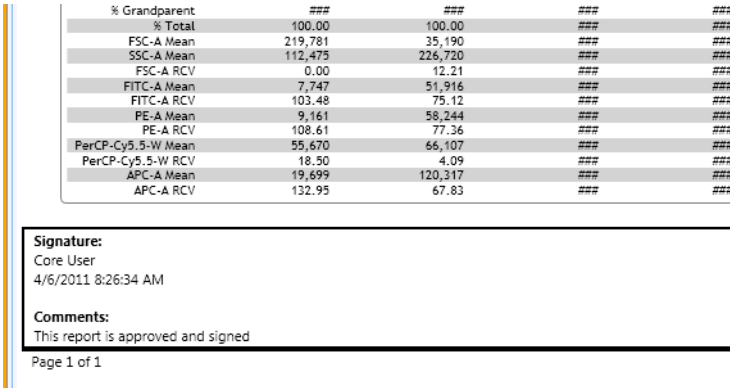
2. In the **Worklist Entries** table, right-click the entry number that corresponds to the report, then select **e-Sign**.

The **ESignature** dialog opens.



3. Select a user ID.
4. Type your password.
5. (Optional) Enter any comments.
6. Click **Sign**.

   The e-signature box displays the signers user id, date and time, and comments that were entered.



If you modify the worksheet or report layout, or any other elements or settings that affect the data after you e-sign the

report, the report is automatically un-signed and must be e-signed again.

More information

- Approving entries in a worklist (page 264)
- Working with audit trails (page 245)

Exporting entries and worklists

Introduction

This topic describes how to export individual worklist entries as entry run packages (ERPs) and how to export complete worklists.

You can build a base of specific re-usable worklists, and archive, share, and import ERPs into other worklists.

About entry run packages

Entry run packages are standalone, individual entries from a worklist that include everything that is required to recreate the entry in a different worklist. You can set export preferences to automatically export ERPs after each worklist acquisition, or you can manually export specific entries.

ERP data includes:

- The assay
- Worksheets and reports
- Audit trails
- Tube settings
- Tube properties
- Assigned keywords
- Acquired data

Manually exporting entries as ERPs

To manually export entries as ERPs:

1. In the navigation bar, click **Worklists**.

   The **Worklist** workspace opens.

2. In the worklist **Manage** tab, click a worklist in the **Worklist Information** table.

   The worklist opens.

3. Click to highlight an entry in the worklist.

4. From the menu bar, select **File > Export > Entry Run Package**.

   The **Browse for Folder** dialog opens.

5. Navigate to an export target folder (for example, *C:\BDExport\ERP\Worklists)*.

6. Click **OK**.

   The entry is exported as an ERP file.

Exporting worklists

To export a worklist:

1. In the worklist **Manage** tab, click a worklist in the **Worklist Information** table.

2. From the menu bar, select **File > Export Worklist > With Data**.

   The **Export Worklist path** dialog opens.

3. Navigate to an export target folder (for example, *C:\BDExport\AssayWorklists*).

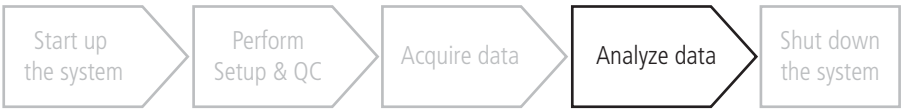
4. (Optional) Before you click **Save**, modify the name of the worklist if needed.

5. Click **Save**.

More information

- Building a worklist (page 237)
- Setting worklist preferences (page 71)
- Assigning keywords to entries and tubes (page 243)

Worklist analysis



Introduction

This topic describes what you can analyze in a worklist and how worklist analysis works. It also provides a worklist analysis workflow and instructions for analyzing individual entries and entire worklists.

About worklist analysis

Analyzing worklists is an automated process that results in an automatically generated report and/or automatically exported statistics files.

You can analyze any individual entry or tube that has been acquired, or use batch analysis to analyze all acquired entries or tubes in a worklist. Batch analysis allows you to increment files, pause between data sets, and print automatically.

Acquisition and batch analysis cannot run simultaneously for the same worklist. However, a worklist acquisition can be running while a different worklist batch analysis is running.

You can start an acquisition run and simultaneously analyze the results of a selected tube or entry in the same worklist.

See topics in the following sections about analyzing entries and tubes in a worklist, and working with assay reports.

Typical analysis workflow

The following table describes the typical worklist analysis workflow stages.

Stage	Description
1	Create a new worklist and import entry run packages, or open an existing worklist. See Using the Manage tab (page 223) and Building a worklist (page 237).
2	Modify worksheet or report elements, or instrument settings (as permitted). See Worklist tab overview (page 227).
3	Run the worklist. See Worklist run options (page 254).
4	Approve the results. See Approving entries in a worklist (page 264).
5	Print or export results, FCS files, or entry run packages. See Exporting entries and worklists (page 268).

Before you begin

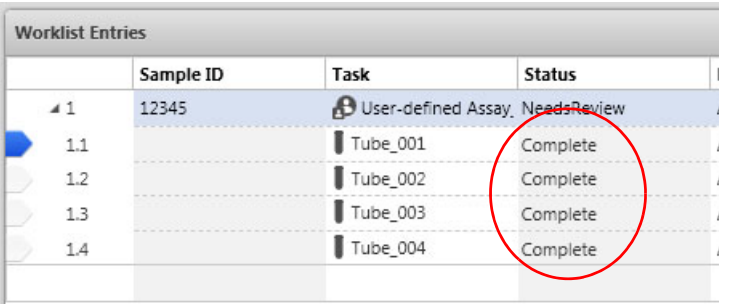
You need to have acquired data from at least one entry or tube. Batch analysis is performed only on entries or tubes that have an associated FCS file. A worklist batch analysis run skips tubes that do not have an FCS file.

Analyzing selected entries or tubes in a worklist

To run analysis on selected entries or tubes in the worklist:

1. Click an entry or tube in the worklist that has been acquired and includes an FCS file.

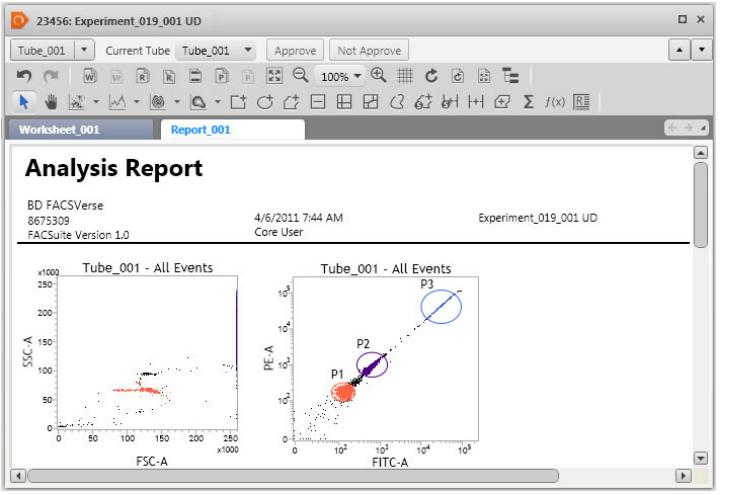
   This is indicated by a *Complete* status in the **Status** column for the entry or tube.



2. Click **Run Selected** on the **Worklist control** bar.

   Analysis begins.

3. The analysis preview opens in the assay worksheet.



Performing analysis on an entire worklist

To run analysis on an entire worklist:

1. Click **Run All** on the **Worklist control** bar.

   Batch analysis begins and analyzes entries and tubes with acquired data.

More information

- Acquiring data in a worklist (page 249)
- Working with assay reports (page 273)

Working with assay reports

Introduction

This topic describes how to view assay reports and how to modify report elements and save the assay as a new user-defined assay.

About assay reports

Reports must be created in an experiment. When you create a user-defined assay from an experiment, the report is included in the new user-defined assay. If your user-defined assay includes a report, the plots and statistics automatically display data when you acquire or analyze a worklist.

If you are using a BD-defined assay, reports are included in the assay. The plots and statistics automatically populate with data when you acquire or analyze a worklist.

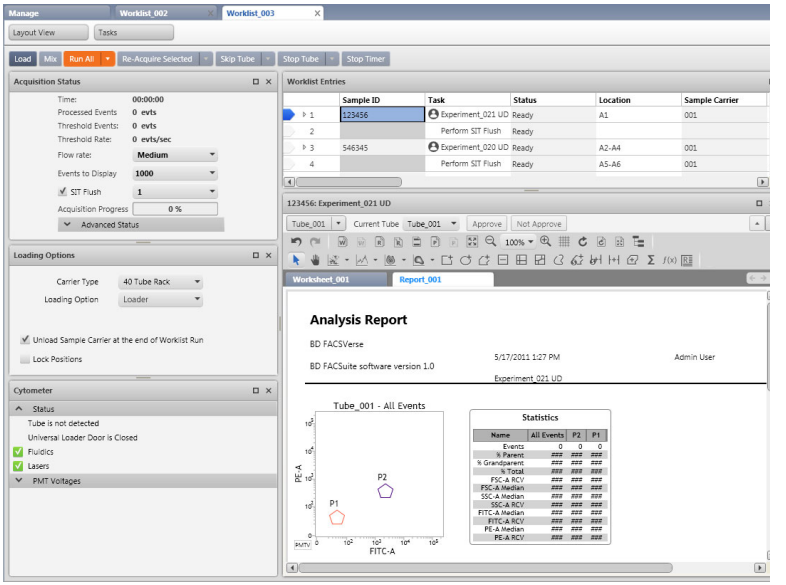
If you want to modify an assay report, you must create a new experiment from the assay, modify the report, then create a new user-defined assay from the modified experiment. When you create an experiment from a BD-defined assay, the reports and data are removed. When you create an experiment from a user-defined assay, the report and data is included.

See Creating a new experiment from an assay (page 144) for information about modifying assays.

Reports are automatically saved with the worklist.

Viewing reports in the worklist

After you run a worklist, the report is displayed in the **Entry Details** panel. The plots and statistics are populated with acquired data.



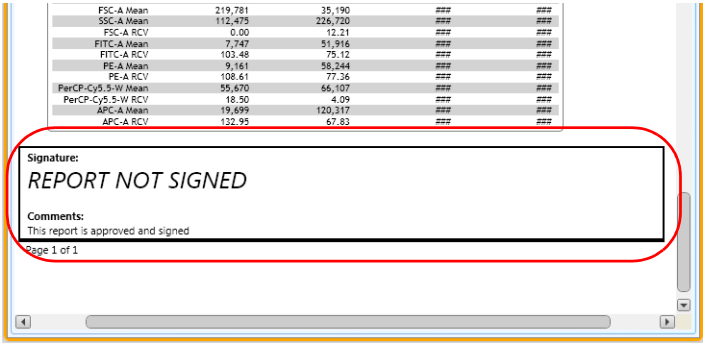
Viewing saved reports

To review a saved assay report:

1. In the navigation bar, click **Worklists**.
2. In the **Manage** tab, double-click a worklist in the **Worklist Information** table.

   The saved worklist opens and the saved report is displayed in the **Entry Detail** panel.

3. If an **E-signature** box is displayed at the bottom of the report, the report might require approval or electronic signature. Perform your typical procedure for approval.



Modifying user-defined assay report elements

See Creating experiment analysis reports (page 206) and Formatting and printing a report (page 213).

More information

- Worklist analysis (page 270)
- Editing assay properties (page 284)
- Approving entries in a worklist (page 264)
- Using ESignature (page 266)

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Part 3: Software reference

This part includes the following sections:

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8

Library

This section includes detailed information about the library and procedures that are not typically part of the daily workflow. Use this section to learn about adding, editing, importing, exporting, and managing shared resources in the library.

This section includes the following topics:

Library overview

Introduction

This topic describes the Library workspace.

About the library

The library serves as the repository for shared and frequently used resources. You can add resources to the library, manage them, and modify details.

Most resources are shared and visible to all users. Some library resources are editable within the library and others are defined in different workspaces and are read-only in the library.

The library workspace stores the following resources:

- BD-defined and user-defined assays
- Bead and reagent information
- BD-defined (LW/LNW) and user-defined tube settings
- All keywords
- Labels

The following table describes where library resources are created and used by other functions in BD FACSuite software.

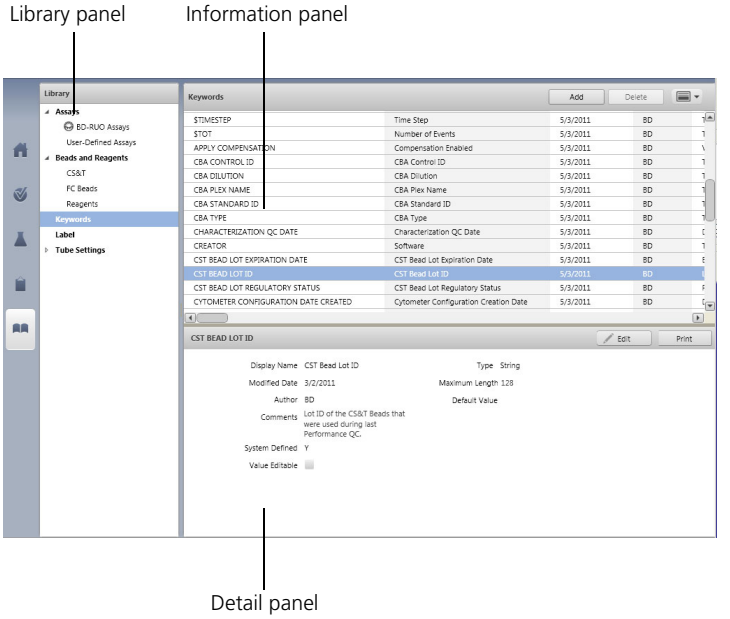
Resource	Created in	Used with
Assays	BD-defined assays are installed. User-defined assays are created from experiments.	• Assay and Tube settings setup (Setup & QC) • Worklist tasks (Worklist) • Assay properties (Library)
Beads and Reagents	Bead lots and reagents are imported or installed.	• Characterization QC • Performance QC • Assay and Tube settings setup (all in Setup & QC)

Resource	Created in	Used with
Keywords	Library	• Tube properties (Experiment) • Entry and tube details (Worklist)
Labels	BD labels are included with BD FACSuite software. User-defined labels are created in the library.	• Tube properties (Experiment) • Creating reference settings (Experiment)
Tube settings	Experiments	• Assay and Tube settings setup (Setup & QC) • Tube properties (Experiment) • Creating reference settings (Experiment)

The Library workspace includes the following panels:

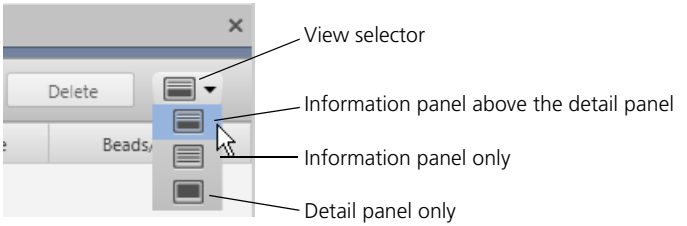
- **Library.** This panel includes the library tree which lists the categories of library resources that you can view in the workspace.
- **Information.** When you select a category in the Library panel, this panel displays a table that lists all items in the category.

- **Detail.** When you select an item in the table, the Detail panel displays the details for the selected item.



Library viewing options

You can click the view selector to change how you view information and detail panels for library resources.



More information

- Importing and exporting assays (page 283)
- Working with bead lots in the library (page 289)

Importing and exporting assays

Introduction

This topic describes how to import and export assays.

About importing and exporting assays

You can import assays from other folders or network locations into the BD FACSuite library. Imported assays include all pre-defined properties, tube-settings, worksheets, reports, keywords, expressions, statistics, and results that were saved with the original assay.

You can export user-defined assays from the library to other folders or network locations. You cannot import or export BD-defined assays. BD-defined assays are installed using installer software that is included with the assay or kit.

Importing an assay

To import an assay:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, double-click **Assays**, then click **User-defined**.
3. From the menu bar, select **File > Import**.

   The **Import Assay** dialog opens.
4. Navigate to the folder or network location that contains the assay you want to import.
5. Select the assay and click **Open**.

   If an assay with the same name already exists in the library, a dialog opens and prompts you to name the imported assay.

The assay is imported and is displayed in the **Assays** table.

Exporting an assay

To export an assay:

1. In the **Library** panel, double-click **Assays,** then click **User-defined**.

   Available assays are displayed in the **Assays** table.

2. In the **Assays** table, click the user-defined assay you want to export.

3. From the menu bar, select **File > Export**.

   The **Export Assay** dialog opens.

4. Navigate to the folder that contains your exported assays (for example, C:\BDExport\Library\Assays).

5. Click **Save**.

   The assay is exported.

More information

- Library overview (page 280)
- Editing assay properties (page 284)

Editing assay properties

Introduction

This topic describes how to edit assay properties in the library. You can modify properties for user-defined assays only.

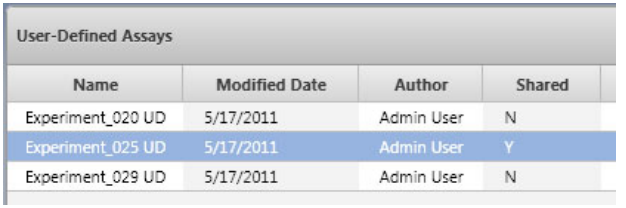
Renaming user-defined assays

To rename user-defined assays in the Assays table:

1. On the navigation bar, click **Library**.

2. In the **Library** panel, double-click **Assays**, then click **User-defined**.

3. Right-click a user-defined assay in the **Assays** table, then select **Rename** to enable editing.



4. Type a new name over the existing name, then click the field to apply the new name.

Editing assay results approval criteria

To edit assay results approval criteria:

1. Click an assay in the **Assays** table.

   The assay details are displayed in the **Details** panel.

2. Click the **General** tab.

3. Under **Approve Results,** select the **Automatically Approve** checkbox to automatically approve results when acquisition completes and there are no acquisition errors.

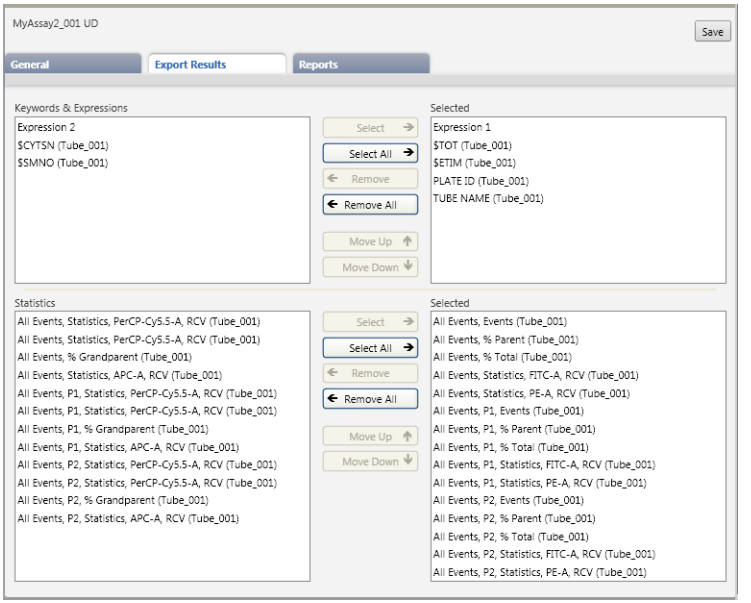
4. Click **Save**.

Editing results exporting properties

You can automatically export assay results and keywords as CSV files.

To edit results exporting properties:

1. Click an assay in the **Assays** table, then click the **Export Results** tab.



2. In the **Keywords & Expressions, Statistics,** and **Selected** boxes, complete one of the following options.

To...	Then do this...
Add one item to the **Selected** box	Click the item in the list, then click **Select**.
Add all available items to the **Selected** box	Ctrl+click each item, then click **Select All.**
Remove one item from the **Selected** box	Click an item in the **Selected** list, then click **Remove**.
Remove all items from the **Selected** box.	Ctrl+click each item in the **Selected** list, then click **Remove All.**
Reorganize the list of selected items	Click an item in the **Selected** list, then click **Move Up** or **Move Down** to move the item.

The results file displays the results in the same order as they appear in the **Export Results** tab.

Important note about results data

The results data displayed in the Export Results tab must be defined when you create a worksheet or report.

See Exporting statistics from statistics views (page 416) for instructions about including this results data in the user-defined assay.

Editing assay report preferences

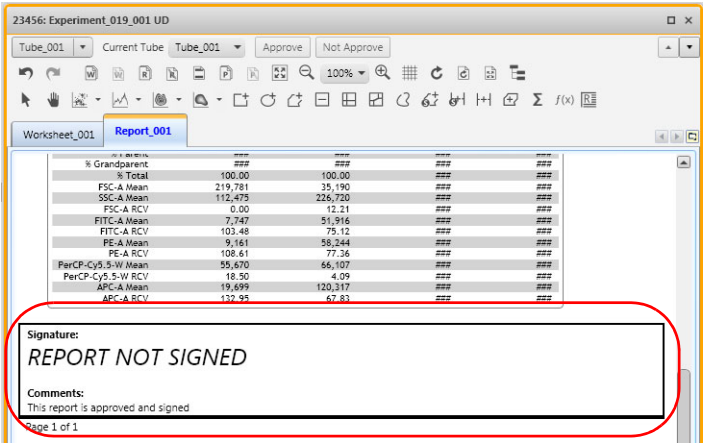
BD FACSuite software generates user-defined reports based on the report layout. You can specify which assay reports are automatically printed or e-signed, and whether to display a report when running a worklist or export a report after running a worklist.

If you select the checkboxes in this tab, you override the default worklist preferences for printing and exporting.

To edit assay report preferences:

1. Click an assay in the **Assays** table, then click the **Reports** tab.
2. Select the **Print report** checkbox to automatically print the entire report after you run the assay.
3. Select the **Include e-Signatures** checkbox to enable electronic signatures in the worklist entry and the signature box on the report.

You can electronically sign entries in the worklist after data has been acquired. The signature box is displayed on the report.



This is an optional report element. However, you should include it for your assay report if supervisor or administrator signature is required in your laboratory.

4. Select the **Display report when running assay** checkbox to display the report and worksheets while the assay runs.
5. Select the **Export report to** checkbox, then click **Change** to select an export location.
6. Click **Save**.

More information

- Modifying experiment worksheets and reports (page 208)

Working with bead lots in the library

Introduction

This topic describes how to add, edit, and delete bead lots in the library. To import or add a CS&T bead lot into the library, see Importing or adding a CS&T bead lot (page 82).

Importing FC bead lots

You need to import or add bead lots when you get a new bead lot or when beads expire. Beads are shared resources and are visible to all users.

To import FC beads:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, double-click **Beads and Reagents**, then click **FC Beads**.
3. From the main menu, select **File > Import**.

   The **Import** dialog opens.
4. Navigate to the folder that contains the bead lot files.
5. Click **Open**.

   A table for the selected subcategory displays the lot or file.

Adding a new FC bead lot

To add a new FC bead lot using the barcode scanner:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, double-click **Beads and Reagents**, then click **FC Beads**.
3. In the **FC Bead Lots** table, click **Scan Barcode** and scan the new bead lot barcode.

   The information is automatically displayed in the FC Bead Lots table.

Deleting bead lots

To delete bead lots:

1. In the **Library** panel, double-click **Beads and Reagents**, then click **CS&T** or **FC Beads**.
2. Click a bead lot in the **Bead Lots** table.
3. In the table title bar, click **Delete**.

   A delete confirmation dialog opens.
4. Click **Yes**.

   The bead lot is deleted from the **Bead Lots** table.

More information

- Library overview (page 280)
- Working with reagents in the library (page 290)

Working with reagents in the library

Introduction

This topic describes how to add, edit, and delete reagents in the library. Reagents are shared resources and are visible to all users.

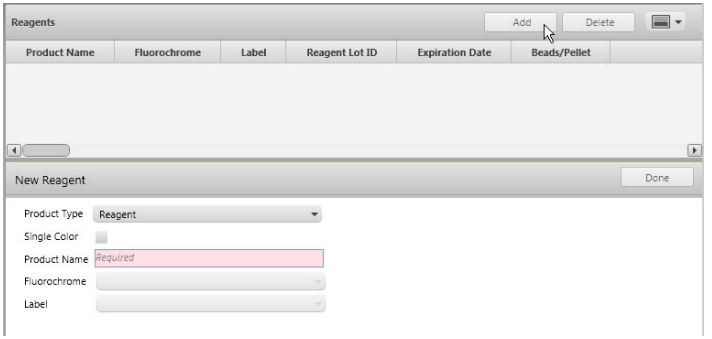
Adding reagents in the library

You need to import existing reagents or manually add reagents when required by a BD-defined assay, when reagents expire, or when you want to use specific reagents with an experiment.

To manually add reagents:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, double-click **Beads and Reagents**, then click **Reagents**.
3. In the **Reagents** table, click **Add**.

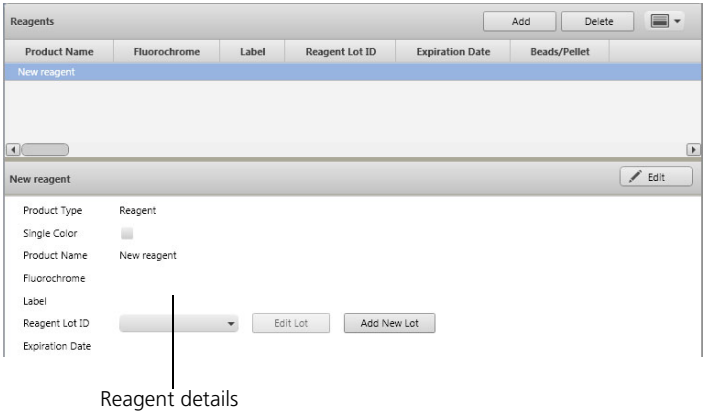
The **New Reagent** details panel is displayed.



4. In the **New Reagent** details panel, type the reagent information in the fields, then click **Done**.

   See your reagent package insert or data sheet for the information.

   The new reagent is displayed in the **Reagent** table.



Adding a new reagent lot

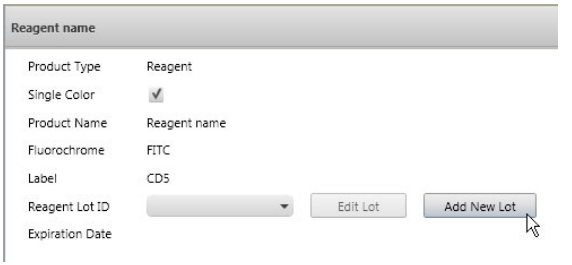
Create a new reagent lot ID as needed after you add a new reagent.

To add a new reagent lot:

1. Click a reagent in the **Reagents** table.

   The reagent details are displayed in the **Details** panel.

2. Click **Add New Lot**.



   The **Add/Edit Lot ID** dialog opens.

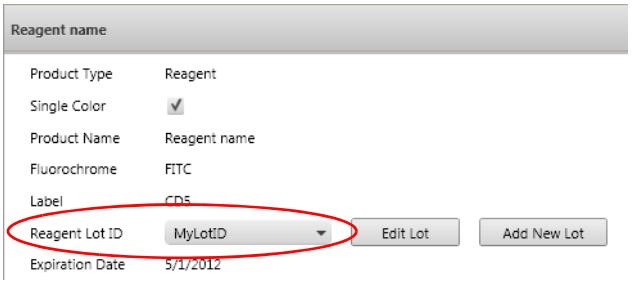
3. If the reagent is a single-color reagent, select the **Single Color** checkbox.

4. In the **Lot ID** field, type a new lot ID.

5. In the **Expiration Date** field, click the calendar and select an expiration date for the new reagent lot. The expiration date for reagents is listed on the vial or on the box.

6. Click **OK**.

   The reagent lot ID is displayed.



Editing reagent lot ID expiration dates

To edit the expiration date for an existing reagent lot ID:

1. In the **Details** panel, click **Edit Lot**.

   The **Add/Edit Lot ID** dialog opens.

2. In the **Expiration Date** field, click the calendar and select an expiration date for the new reagent lot.

3. Click **OK**.

Editing reagent details

To edit existing reagent details:

1. Click a reagent in the **Reagents** table.

   The reagent details are displayed in the **Details** panel.

2. Click **Edit** to enable editing in the fields.

3. Edit the fields as needed, then click **Done.**

Deleting reagents

To delete reagents:

1. Click a reagent in the **Reagents** table.

2. In the table title bar, click **Delete**.

   A delete confirmation dialog opens.

3. Click **Yes**.

   The reagent is deleted from the **Reagents** table.

Exporting reagent information

You can export reagent information so that it can be imported by other users on different workstations. This can be helpful in reducing the amount of data entry if you are adding numerous reagents to the library.

To export reagent information:

1. In the **Reagents** table, click a reagent.

2. From the menu bar, select **File > Export**.

The **Export** dialog opens.

3. Navigate to the folder that contains your exported tube settings (for example, C:\BDExport).
4. Click **Save**.

   The reagent information is exported as an XML file.

Importing reagent information

To import reagent information:

1. From the menu bar, select **File > Import**.

   The **Import** dialog opens.

2. Navigate to the folder that contains your exported reagent information.xml file and select a file to import.
3. Click **Open**.

   The reagent information is imported into the **Reagents** table.

More information

- Working with bead lots in the library (page 289)
- Working with tube settings in the library (page 296)

Importing and exporting tube settings

Introduction

This topic describes how to import and export tube settings in the library.

Tube settings are defined in the Experiment workspace. By default, tube settings are visible to authors only. You can change the sharing preference to share tube settings with all users.

Importing tube settings

To import tube settings:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, double-click **Tube Settings**, then click **User-defined**.
3. From the menu bar, select **File > Import.**

   The **Import Tube Setting** dialog opens.
4. Navigate to the folder that contains previously exported tube settings files (for example, C:\BDExport\Library\TubeSettings).
5. Select the tube setting you want to import, then click **Open**.

   The table for the selected subcategory displays the tube settings file.

Exporting tube settings

To export tube settings:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, double-click **Tube Settings**, then click **User-defined**.
3. In the table, click a tube setting you want to export.
4. From the menu bar, select **File > Export.**

   The **Export Tube Setting** dialog opens.
5. Navigate to the folder that contains your exported tube settings (for example, C:\BDExport\Library\TubeSettings).
6. Click **Save**.

   The tube settings file is exported as an XML file.

More information

- Working with tube settings in the library (page 296)

Working with tube settings in the library

Introduction

This topic describes how to view, print, or delete tube settings files in the library.

Tube settings are defined in the Experiment workspace. By default, tube settings are visible only to authors. You can change the sharing preference to share tube settings with all users.

Viewing and printing tube settings details

To view and print tube settings details:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, double-click **Tube Settings**, then click **BD** or **User-defined**.
3. In the table, click the tube settings you want to view.

   The **Tube Settings** detail panel is displayed.
4. Click **Print** to print the current tube settings details.

Deleting user-defined tube settings

Authors can delete only their own user-defined tube settings. You cannot delete BD-defined or LW/LNW default tube settings.

To delete a user-defined tube setting:

1. Click a tube setting in the **Tube Settings** table.
2. In the table title bar, click **Delete**.

   A delete confirmation dialog opens.
3. Click **Yes**.

   The tube setting is deleted from the **Tube Settings** table.

   Any tube that used the deleted tube settings will have the default tube settings and any associated reference settings applied to it.

More information

- Working with reagents in the library (page 290)
- Working with keywords in the library (page 299)

Understanding keywords

Introduction

This topic provides a description of keywords, how they are used in BD FACSuite software, and keyword rules.

About keywords

Keywords provide additional information (metadata) about your sample. This information can identify samples, differentiate the samples from each other, or group them based on a similar trait. Keywords can be system-defined (which include FCS standard keywords) or user-defined. Keywords are shared resources and are stored in the library.

When you assign keywords to a tube (in an experiment) or an entry or tube (in a worklist), they are displayed in the text header of exported FCS files and have a name (for example, $DATE) and a value (for example, Jan-1-00). The form of a keyword value depends on the nature of the keyword (for example, a character string or a number).

You can also display any keywords that are already defined and assigned to the assay as a column in the worklist. The column displays specific values that apply to the entry or tube. You can modify values for many keywords.

Using keywords

You can do the following with keywords:

- Assign keywords to one, several, or all tubes in an experiment or worklist entry.
- Assign keywords to tubes in an experiment and use them in statistical expressions within the experiment.
- Add keywords to a statistics view header.

- Specify keyword values in a column in the worklist or the Tube Properties dialog in an experiment.

Keyword rules

- All keywords are stored in the library. However, the keyword values are associated to the tube or entry. Once you assign a keyword and set a value for a tube or entry, it is disconnected from the keyword in the library (it's a snapshot of the keyword and value). Any changes to the keyword in the shared library do not affect the keyword that is assigned to the tube or entry.
- If you want to change the definition of an assigned keyword (for example, if you change the type from numeric to string) you need to unassign it in the tube or entry, reassign it.

More information

- Working with keywords (page 176)
- Assigning keywords to entries and tubes (page 243)
- Importing and exporting keywords (page 298)
- Working with keywords in the library (page 299)

Importing and exporting keywords

Introduction

This topic describes how to import and export keywords in the library.

Importing keywords

To import keywords into the library:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, click **Keywords**.
3. From the menu bar, select **File > Import**.

   The **Import Keywords** dialog opens.
4. Navigate to the folder that contains previously exported keywords files (for example, C:\BDExport\Library\Keywords).

5. Select the keyword you want to import and click **Open**.

   The keyword is added to the **Keywords** table.

Exporting keywords

Only user-defined keywords can be exported.

To export keywords:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, click **Keywords**.
3. From the menu bar, select **File > Export.**

   The **Export Keywords** dialog opens.
4. Navigate to the folder that contains your exported keywords (for example, C:\BDExport\Library\Keywords).
5. Click **Save**.

   The user-defined keywords are exported as an XML file.

More information

- Understanding keywords (page 297)
- Working with keywords in the library (page 299)

Working with keywords in the library

Introduction

This topic describes how to add, edit, and delete user-defined keywords in the library.

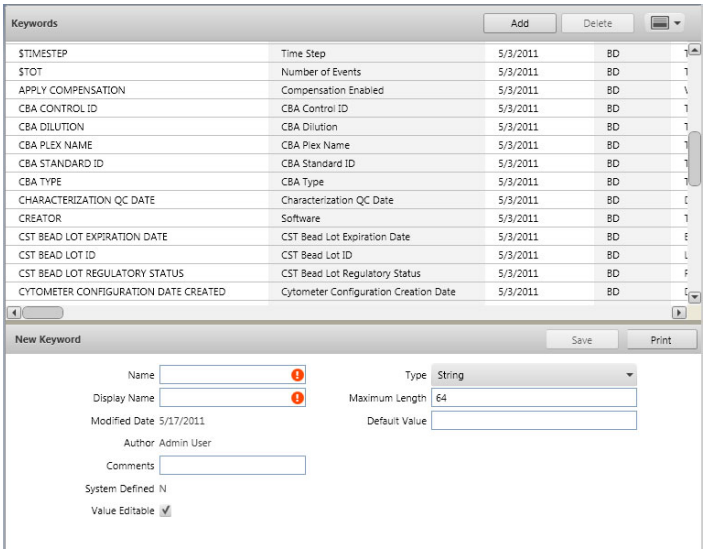
System-defined and user-defined keywords are shared resources and visible to all users. Only keyword authors can edit their user-defined keywords.

Creating a new keyword

To add a new keyword to the Keywords table:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, click **Keywords**.
3. In the **Keywords** table, click **Add** on the table title bar.

   The **New Keyword** details panel is displayed.



4. Type the appropriate information into each field.
5. Click **Save**.

   The new keyword is added to the table.

Editing an existing keyword

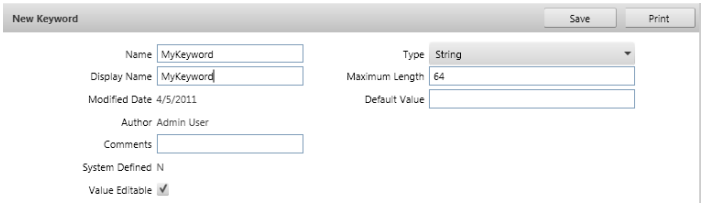
To edit an existing keyword:

1. In the table, click the keyword you want to edit.

   The **Keyword** detail panel is displayed.

2. Click **Edit** to enable the fields.

3. Edit the fields as needed.



4. Click **Save.**

   The edited keyword is displayed in the table.

5. (Optional) Click **Print** to print the current keyword details.

Deleting user-defined keywords

Authors can delete only their own user-defined keyword. System-defined keywords cannot be deleted.

To delete a user-defined keyword:

1. Click a keyword in the **Keywords** table.
2. In the table title bar, click **Delete**.

   A delete confirmation dialog opens.

3. Click **Yes**.

   The keyword is deleted from the **Keywords** table.

More information

- Understanding keywords (page 297)
- Working with tube settings in the library (page 296)
- Working with labels in the library (page 302)

Working with labels in the library

Introduction

This topic describes how to import, create, and delete fluorochrome labels (for example, CD3) in the library.

Labels are shared resources and are visible to all users. BD labels are authored by BD. User-defined labels can be created, edited, or deleted only by the label author.

Importing labels

To import the list of labels into the library:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, click **Label**.
3. From the menu bar, select **File > Import**.

   The **Import Labels** dialog opens.
4. Navigate to the folder that contains label files (for example, C:\BDImport\Library\Labels).
5. Click **Open**.

   The labels are added to the **Labels** table.

Creating a user-defined label

To create a user-defined label:

1. In the table title bar, click **Add**.

   A new line is added to the bottom of the **Labels** table.
2. In the **Name** column, type the label name, then press **Enter.**

   The new label is added to the table. Your user ID is displayed as the author in the **Author** column.

Deleting a user-defined label

Authors can delete only their own user-defined labels. BD labels cannot be deleted.

To delete a user-defined label:

1. Click a label in the **Labels** table.

2. In the table title bar, click **Delete**.

   A delete confirmation dialog opens.

3. Click **Yes**.

   The label is deleted from the **Labels** table.

Exporting labels

You can export a list of labels only if the list includes user-defined labels.

To export a list of labels:

1. From the menu bar, select **File > Export**.

   The **Export Labels** dialog opens.

2. Navigate to the folder that contains your export labels (for example, C:\BDExport\Library\Labels).

3. Click **Save**.

   The file is exported as an XML file.

More information

- Working with keywords in the library (page 299)
- Locating library resources (page 303)

Locating library resources

Introduction

This topic describes how to sort, search to locate resources, and print lists of resources after you locate them. The following procedures apply to all library resources.

Sorting items in the table

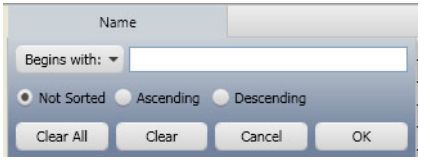
To sort items in the resource table:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, click a category.

In any resource table, click any column header.

3. Select a sort mode button.

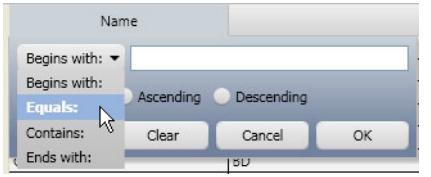
   The resources are sorted by category. Names (text) are sorted in alphabetical order. Numeric values are sorted in ascending or descending order.



Defining search criteria

To define search criteria:

1. Click the search filter mode button to define the search.



2. Select one of the following options.

   - **Begins with.** This mode is not case sensitive. This searches by the first letter of the word, or the number in a numeric value.
   - **Equals.** This mode is case sensitive. This searches for the exact form of the word (for example, FSC, Assay 1, Br), or the exact form of the numeric item (for example, 12, 14.5, 12,056).
   - **Contains.** This mode is not case sensitive. This searches for text or numeric value that might be included in a file name (for example, assay, 126, tube).
   - **Ends with.** This mode is not case sensitive. This searches for the last letter of the word, number in a numeric value, or a string.

3. In the **Search** field, type the text or number you want to use as the search keyword.
4. Click **OK** to filter.

   The table displays the filtered items in the column.

Printing lists of library resources

To print a list of library resources:

1. Display the resource table that contains the resource you want to print.

   - To print a single row of a resource table, right-click the row, then select **Print**.
   - To print multiple rows of a resource table, Ctrl+click multiple rows, then select **Print**.

   A print preview is displayed.
2. Click the **Print** icon, then complete your typical printing procedure.

More information

- Library overview (page 280)

Changing resource sharing settings

Introduction

This topic describes how to change the sharing setting for assays and tube settings in the library.

About library resource sharing settings

Library resources are private by default. Only administrators or resource owners (authors) can change a resource from private to shared, or rename or delete private or shared resources.

- Private library resources are unique only within the library resources for a specific user. Resources can have the same name if they are not shared.
- Shared library resources are unique and cannot have the same name as other public resources.

Procedure

To change the sharing setting for a resource:

1. On the navigation bar, click **Library**.
2. In the **Library** panel, double-click **Assays** or **Tube Settings**.
3. Display the assay or tube setting table that contains the resource you want to change.
4. Right-click a row in the resource table, then select a share setting.
   - For assays, select **Share Assay** or **Make Private**.
   - For tube settings, select **Share Tube Setting**.

     Note that you can share tube settings, but you cannot make them private once you have shared them.

More information

- Locating library resources (page 303)
- Library overview (page 280)

9

Plots

This section includes detailed information about plots and plot procedures that are not typically part of the daily workflow. Use this section to learn about different plot types and how to change plot properties.

This section includes the following topics:

Other related sections:

- See Experiment acquisition and analysis (page 129) for information about creating plots in an experiment.

Plot overview

Introduction

This topic describes plots and plot parameters.

About plots

Plots graphically represent the data acquired from tubes and are used to compare and analyze acquired data. Each plot is associated to a specific tube or to the run pointer.

Plot parameters

BD FACSuite software automatically selects X and Y parameters for all newly created plots. If you draw a new 2D plot, FSC-A is automatically selected as the X parameter and SSC-A is automatically selected as the Y parameter. If you draw a histogram plot, FSC-A is automatically selected as the X parameter. You can change these parameters as needed.

More information

- Modifying plot parameters (page 160)
- Changing plot axis parameters (page 324)
- Plot types (page 309)
- Statistics (page 399)
- Expressions (page 419)

Plot types

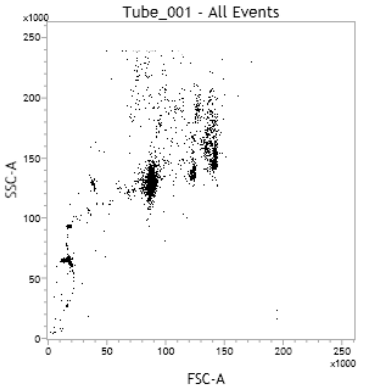
Introduction

This topic describes the plot tools and the different plot types you can use to visualize and analyze data.

Plot types

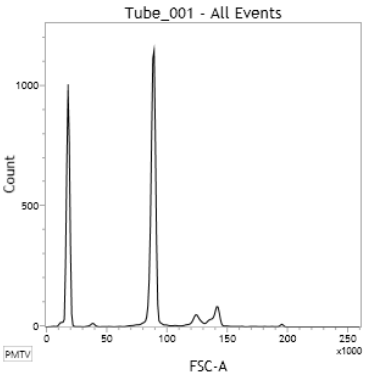
There are four basic plot types, as well as overlays and hybrids.

- **Dot plot.** This plot is a graphical representation of two-parameter data, where each axis displays the signal intensity of one parameter and each dot represents one or more events (cells or particles).



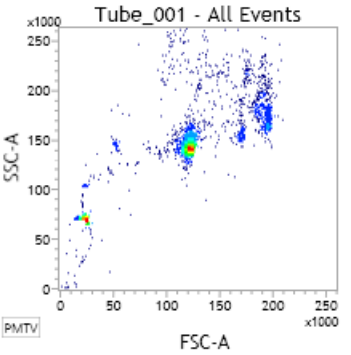
- **Histogram.** This plot is a graphical representation of single-parameter data, where the horizontal axis represents the

increasing signal intensity of the parameter, and the vertical axis represents the number of events (count).



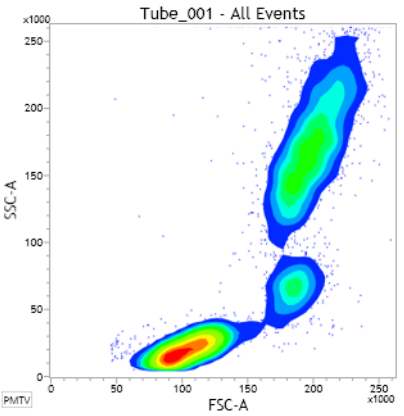
- **Density plot.** This plot is a graphical representation of two-parameter data where each axis displays the signal intensity of one parameter and colors indicate the number of events in a cluster.

  Density plots are similar to dot plots, except colors are used to represent the accumulation of events (density) for events with the same signal intensity. A density plot simulates three-dimensional event display.



- **Contour plots.** This plot is a graphical representation of two-parameter data, where each event has a position in the plot according to its intensity values for both parameters.

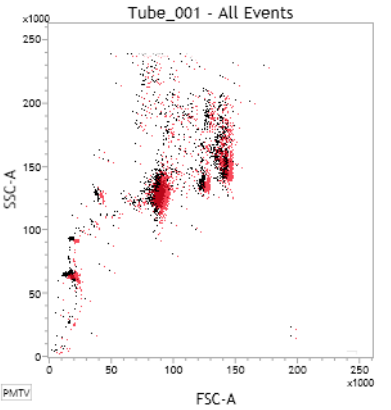
  Contour lines provide a third dimension by joining x- and y-coordinates with similar event counts. These plots are similar to topographic maps, which use contour lines to show points at the same elevation.



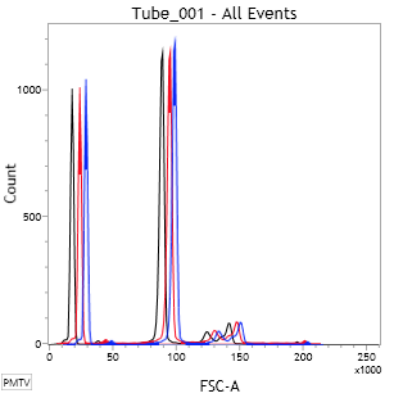
- **Overlay plots.** Overlays are used to compare similar data. When the scales for all overlay layers are the same, then tick-

marks are displayed. When the scales are not the same for all layers, the tick-marks are not displayed.

Dot plot overlay



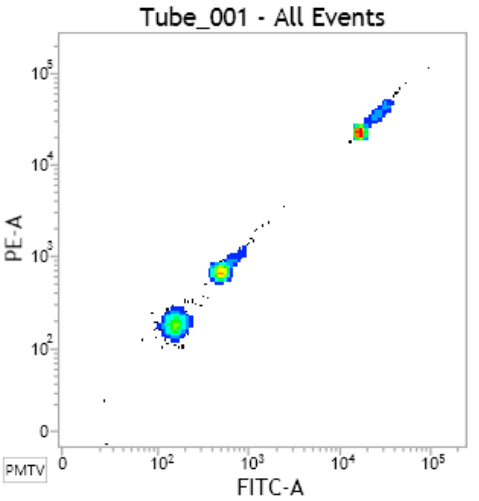
Histogram overlay



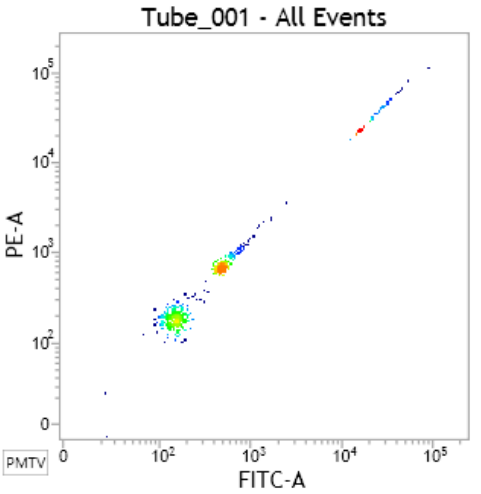
- **Hybrid plots.** These plots combine dot plots with density or contour plots. See Density plot display properties (page 335) and Contour plot properties (page 337) for information about how to create hybrid plots.

In a hybrid plot, the individual dots are displayed like a dot plot, but the dots display the color gradients from a density or contour plot.

Dot + density hybrid plot



Dot + contour hybrid plot



More information

- Drawing plots (page 314)
- Previewing data in plots (page 161)
- Modifying experiment worksheets and reports (page 208)

Drawing plots

Introduction

This topic describes how to use the plot tools to create plots with default parameters or with specified parameters.

Creating plots with default parameters

Use the plot tools on the Worksheet toolbar to create plots on a worksheet or report with the default (scatter) parameters.

Tool	Description
Dot plot 	Click this tool to create a dot plot on a worksheet or report.
Histogram plot 	Click this tool to create a histogram plot on a worksheet or report.

Tool	Description
Contour plot 	Click this tool to create a contour plot on a worksheet or report.
Density plot 	Click this tool to create a density plot on a worksheet or report.

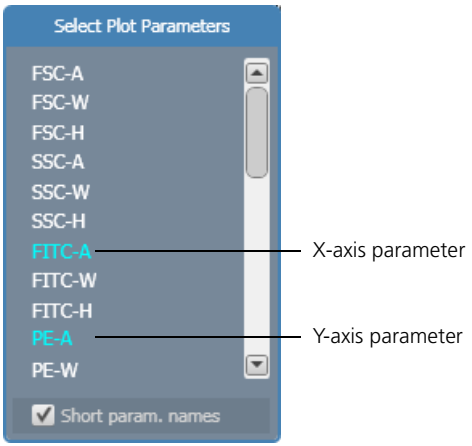
You can change the x- and y-axis parameters if needed. See Changing plot axis parameters (page 324) for more information.

Creating plots with specific parameters

To create plots with specific parameters:

1. Click a plot tool on the **Worksheet** toolbar.
2. Right-click in the worksheet or report.

   The **Select Plot Parameters** dialog opens.



3. Select an x-axis parameter, then select a y-axis parameter from the list (for example, FITC-A and PE-A). Note that the first parameter you select is the x-axis parameter.

   The new plot displays in the worksheet or report.

More information

- Plot types (page 309)
- Viewing plot coordinates (page 316)
- Changing plot axis parameters (page 324)
- Setting plot display properties (page 328)

Viewing plot coordinates

Introduction

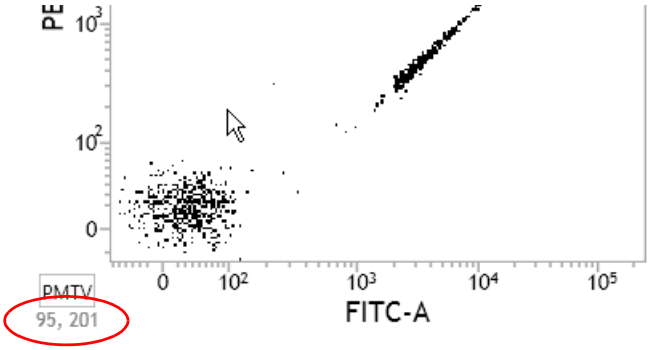
This topic describes how to view plot coordinates.

Procedure

To view the coordinates of the current mouse position in a plot:

1. Click in the plot.
2. Move the mouse within the plot.

Plot x- and y-coordinates for the current mouse position are displayed in the lower-left corner of the plot. The coordinates are displayed as rounded integers.



More information

- Creating plots in a worksheet (page 158)
- Previewing data in plots (page 161)

Setting general plot properties

Introduction

This topic describes how to set general properties for plots after you add them to a worksheet.

General plot properties define the plot type and which data categories are displayed in the plot title.

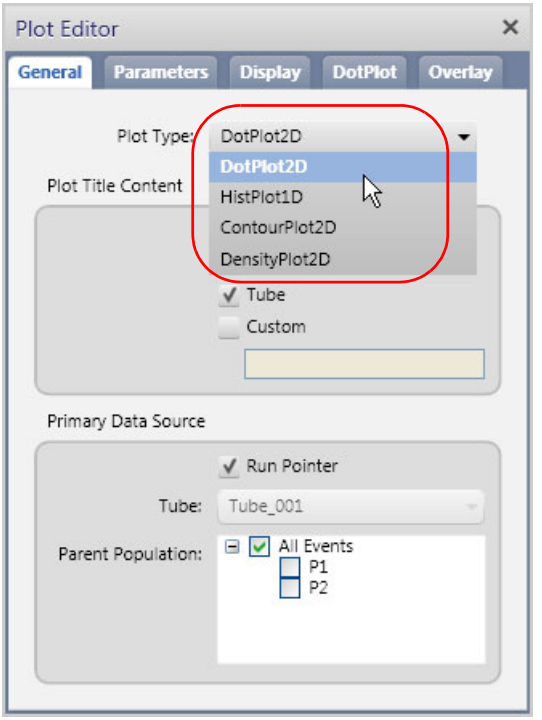
Changing the plot type

To change the plot type:

1. Right-click a plot and select **Properties**.

   The **Plot Editor** dialog opens and displays the **General** tab.

2. Click the **Plot Type** field and select a different plot type.

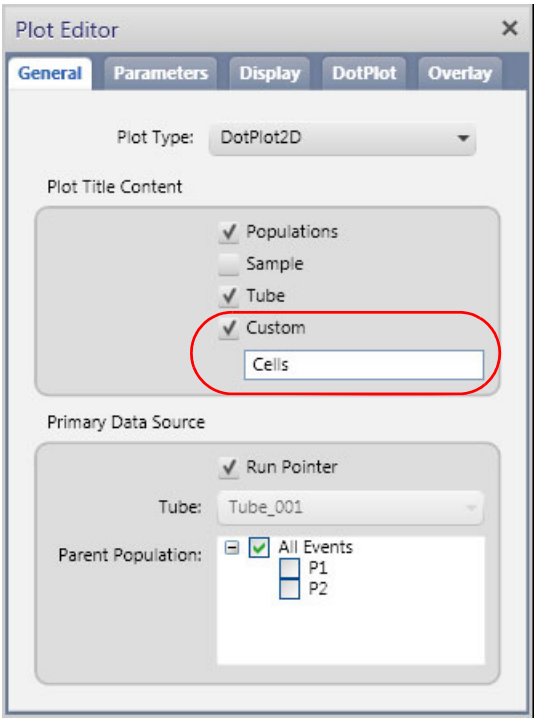


Modifying the plot title

To modify the plot title:

1. Under **Plot Title Content**, select the checkboxes for the elements you want to include in the plot title.

The default plot title is the name of the primary data source (tube) that you selected and the current population(s).



2. (Optional) Select the **Custom** checkbox to enable a custom title, then type a custom title in the field.

More information

- Changing the primary data source (page 320)
- Creating plots in a worksheet (page 158)
- Previewing data in plots (page 161)

Changing the primary data source

Introduction

This topic describes how to change the primary data source for a plot.

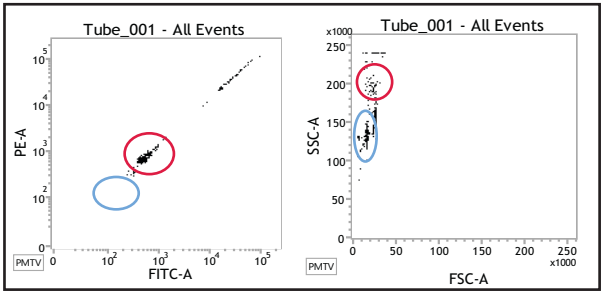
The primary data source determines which tube is associated to a plot or plots in the worksheet.

About data sources

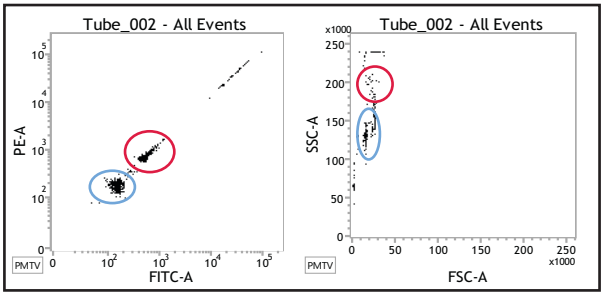
You can change the data display behavior of plots in a worksheet by assigning the run pointer or a specific tube as the primary data source for the plot.

For example, when the run pointer points to *Tube_001*, all plots that are assigned to the run pointer display data from *Tube_001*. The plot titles change to match the tube that is selected by the run pointer. When the run pointer points to *Tube_002*, all plots that are assigned to the run pointer display data from *Tube_002*.

Run pointer on Tube 001



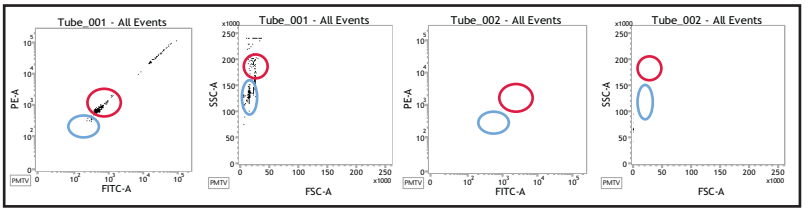
Run pointer on Tube 002



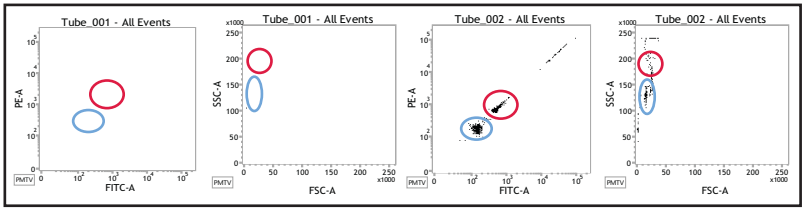
If you have more than one tube in an experiment, you can assign a specific tube as the primary data source for one plot and display one plot per tube within the same worksheet and compare plots side-by-side on the same worksheet.

The following illustration shows plots for two separate tubes in the same worksheet. When *Tube_001* is assigned as the primary data source, only the data for plots assigned to *Tube_001* is displayed.

Primary data source set as Tube 001



Primary data source set as Tube 002



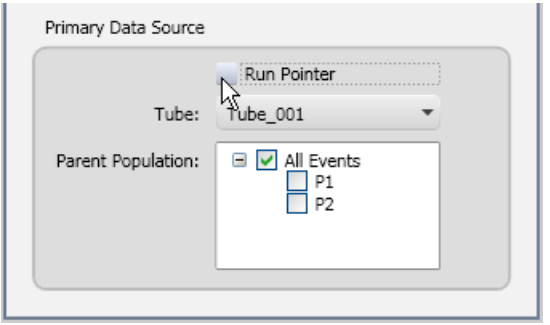
Assigning the primary data source

To assign a specific tube as the primary data source:

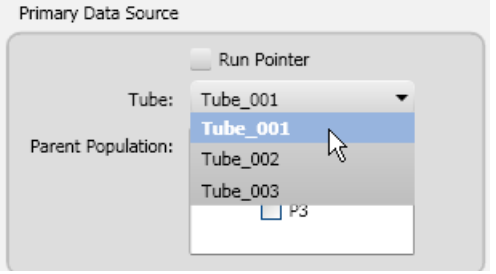
1. In the **Data Sources** panel, set the run pointer on a tube (for example, *Tube_001)*.
2. In the worksheet, right-click a plot displaying *Tube_001* data, then select **Properties**.

   The **Plot Editor** dialog opens.

3. Under **Primary Data Source**, clear the **Run Pointer** checkbox.

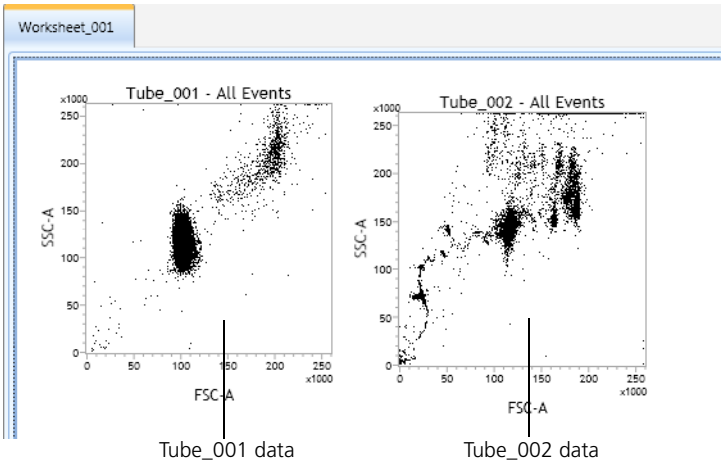


4. Select the tube you want to assign as the primary data source for this plot (for example, *Tube_001)*.



5. Close the dialog.
6. Set the run pointer to a different tube.

Notice that the data does not update in the plot because the plot primary data source has been assigned to *Tube_001*.



Note that even though the run pointer is no longer assigned as the primary data source, you use the run pointer in the **Data Sources** panel to indicate the tube you want to select or preview.

You can re-assign the run pointer as the primary data source at any time.

Displaying specific populations

To display a specific population:

1. Under **Primary Data Source**, click one or more populations in the **Population(s)** tree.

   The updated plot displays the selected populations for the current data source. If the plot included overlays, then only the primary data source updates. The overlays are not affected.

See **Working with gates (page 352)** for additional topics related to creating gates and defining populations.

More information

- Creating plots in a worksheet (page 158)
- Drawing gates in plots (page 164)
- Changing plot axis parameters (page 324)

Changing plot axis parameters

Introduction

This topic describes how to set the parameters and scale for x- and y-axes.

About plot axis parameters and scaling

Most often flow cytometry data exhibits a log-normal distribution and is displayed using a logarithmic scale. However, when data is compensated, the data might display event distributions that extend below zero. Logarithmic displays can not accommodate zero or negative values. Zero or negative values cause the events to pile-up on the baselines of the plots (commonly referred to as the "log artifact").

Linear scaling is more appropriate for displaying data that is close to zero or below zero. However, linear scaling does not accommodate data that is much brighter (which should be displayed using a log scale).

To address the log artifact, BD FACSuite software plots utilize a data transformation for scaling purposes. This allows the use of a linear scale for near zero or below zero data and a logarithmic scale for data with larger signals on the same plot. The transformation is for display only and does not affect the raw data.

Autoscaling automatically determines the extent of negativity based on the compensated data for all events in the data file. Advanced users can select manual scaling and adjust the range of the negative scale using the Biexponential R values. This is referred to as manual biexponential scaling. The minimum and maximum values for R are 0 and 50000 respectively.

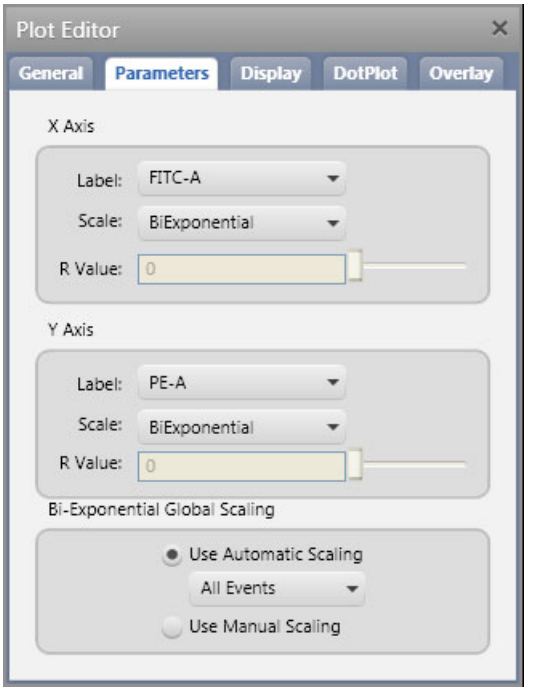
Procedure

To change the parameter and scale for an axis:

1. Right-click a plot and select **Properties**.

   The **Plot Editor** dialog opens.

2. Click the **Parameters** tab.



3. In the **Label** fields, select a parameter for each axis.

   Note that you can also change axis parameters by right-clicking a parameter label in a plot and selecting a new parameter.

4. In the **Scale** fields, select **Log**, **Linear**, or **Biexponential** scale for each axis.

   The default for scatter parameters is linear. The default for fluorescence parameters is biexponential.

Setting automatic biexponential scaling

To set automatic biexponential scaling:

1. In the **Parameters** tab, under **Bi-exponential Global Scaling**, select **Use Automatic Scaling.**
2. Select a population to scale.

   This is the default biexponential scaling for all tubes that automatically scales the selected events.

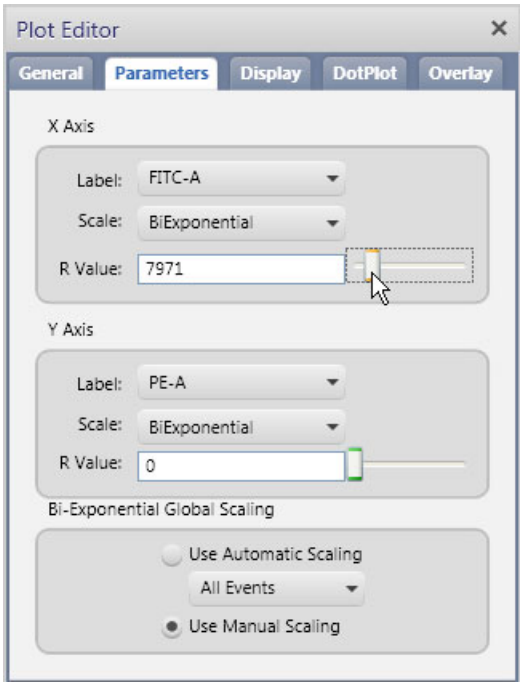
Setting manual biexponential scaling for a tube

To set manual biexponential scaling for a tube:

1. In the **Data Sources** panel, set the run pointer on the tube you want to scale.
2. Right-click a plot and select **Properties**.

   The **Plot Editor** dialog opens.

3. Click the **Parameters** tab.



4. Under **Bi-exponential Global Scaling**, select **Use Manual Scaling** to enable the R Value data slider.

5. Use the data slider to set an R value for the selected tube. Note that manual scaling is tube specific.

Changing log display

The log scale can be displayed using 4 or 5 decades. A 4 decades log display removes the first decade (0 to 10) of data from display. A 5 decade log display includes the first decade.

To change log display:

1. From the menu bar, select **View > Change Log Display** to toggle between 4 and 5 decade log display.

   This selection applies to all plots.

More information

- Creating plots in a worksheet (page 158)
- Managing layers for a plot overlay (page 338)

Setting plot display properties

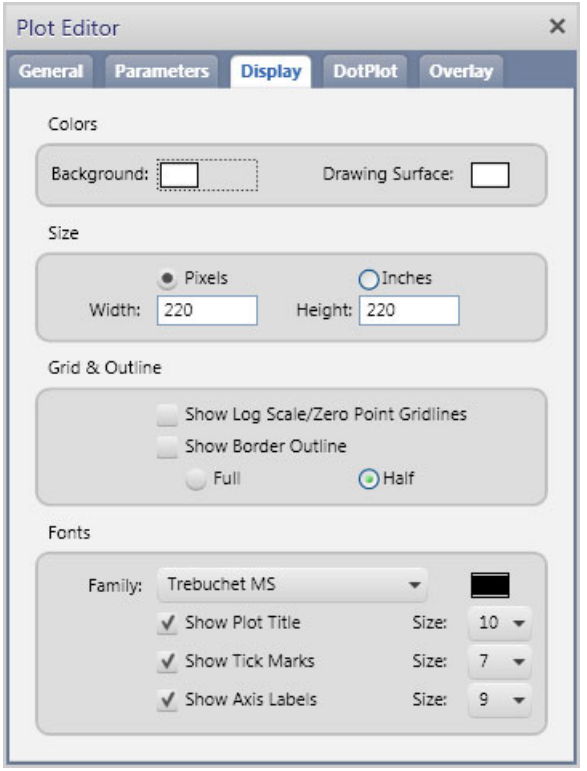
Introduction

This topic describes how to set plot display properties which determine how plot colors, fonts, labels, gridlines, and tick marks are displayed.

Procedure

To set plot display properties:

1. In the **Plot Editor** dialog, click the **Display** tab.



2. Under **Colors**, click and select colors for the plot background or drawing surface.
3. Under **Size**, select the measurement units and set the size for the selected dot plot.
4. Under **Grid & Outline**, select the **Show Log Scale/Zero Point Gridlines** checkbox to display gridlines for the log scale parameters and a line on zero for the biexponential parameters.

5. Select the **Show Border Outline** checkbox, then select **Full** or **Half** to display a plot border outline.
6. Under **Fonts**, select the font family, color, and the font sizes for the plot title, tick marks, and axis labels.

   You can also select show or hide plot titles, tick marks, or axis labels.

More information

- Creating plots in a worksheet (page 158)
- Density plot display properties (page 335)

Setting plot-specific display properties

Introduction

This topic describes how to set the display properties for specific plot types. These properties control how the population data and plot display in a worksheet.

Procedure

To set plot-specific display properties:

1. Right-click a plot and select **Properties**.

   The **Plot Editor** dialog opens. The tabs that open are based on the type of plot you selected (for example, dot, histogram, contour, or density plot).
2. Select the plot-specific tab (for example, **DotPlot, Histogram, Contour,** or **Density**).
3. Set the properties for the plot type.

Dot plot properties

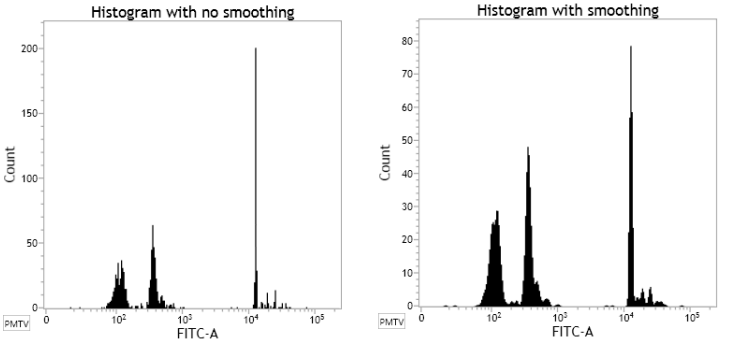
Use the DotPlot tab to set dot plot properties.

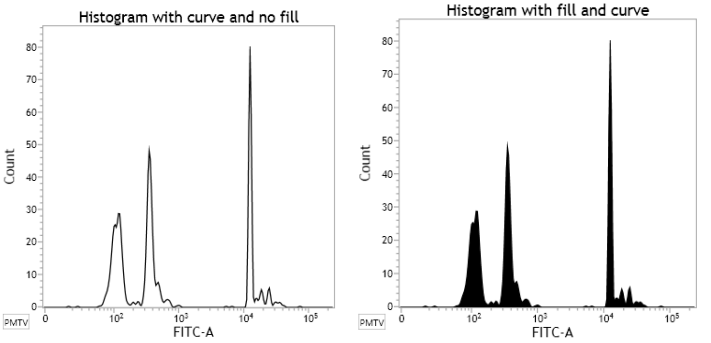
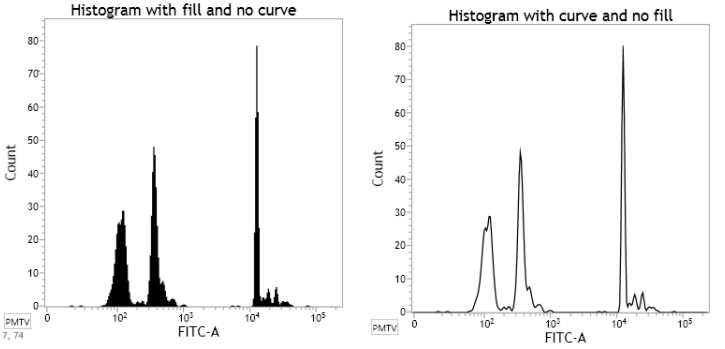
To...	Then do this...
Change the dot size	Under **Plot Properties**, click the **Dot Size** field and select a dot size.
Change which data is displayed in the plot	Under **Show Data**, click **% Events** or **# Events** to enable the display of this data. Type a percentage or number of events in the field for the enabled data.

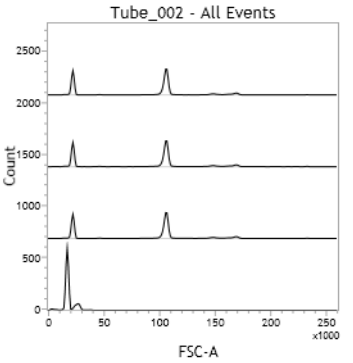
Histogram properties

Use the **Histogram** tab to set histogram properties.

To...	Then do this...
Change the histogram resolution	In the **Resolution** field, select a resolution of 64, 128, 256, or 512. The default is 256. Higher resolution displays a more solid (less pixelated) image. You can combine higher resolution with higher smoothing to generate the most detailed histogram plot.
Set the y-axis scale	Under **Y Axis**, click the **Y Axis Scale** field and select a logarithmic, linear, percent, or normalized percent scale. The default is linear. You can select a percent scale when using overlays. If you select **Percentage**, the following steps are performed: • The highest peak in each layer is identified. • A percentage is calculated. The number of events in the peak are divided by the total number of events in the layer. • A maximum percentage among all the layers is calculated and used as the maximum value for the y-axis.

To...	Then do this...
Set y-axis max height	Under **Y Axis**, select one of the following: • If you selected multiple populations using the primary data source population (in the **General** tab), then select **Global Maximum** to normalize each of the populations. • Select **Use Manual Y Height** to apply the y-axis maximum value in the **Max. Height** field. You can modify the max height value manually by typing a new value.
Smooth the data	Under **Smoothing**, select the **Use Smoothed Data** checkbox to enable both the **Clear Smooth Edges** checkbox and the Smoothing Level controls. Smoothing captures trends and patterns from the individual data bins and applies it to a curve—while filtering noise or outliers. Higher smoothing levels filter out more noise. 
Hide edges of data	Under **Smoothing**, select the **Clear Smooth Edges** checkbox to make the edges of the rendered data invisible.
Adjust smoothing	Under **Smoothing**, drag the **Smoothing Level** slider to select a higher or lower smoothing level (1–8), or type a value. The default value is 4.

To...	Then do this...
Set how the histogram is displayed	• Under **Histogram Visualization**, select the **Fill Histogram** checkbox to fill the histogram area. 
	• Select the **Draw Curve** checkbox to draw a curve instead of individual bins. 

To...	Then do this...
	• Select the **Display Stacked View** checkbox to display data in vertical stacks.  You can stack data to show multiple populations in separate sections in the same histogram. For example, if you display stacked view for a plot that includes three populations, each population is displayed separately within the histogram. When stacked view is disabled, the three populations display on top of each other. Note that in an overlay, different datasets are layered on top of each other.

To...	Then do this...
	• In the **Edge Percent Ignore** field, enter the percentage of the histogram edge that you want to ignore. This helps to reduce edge effects. The default value is 5%. Ignoring edge effects excludes negative events when using automated scaling of the y-axis. For example, if you display a histogram with a log parameter, many negative events can accumulate at "0" on the y-axis. You can ignore these events on the extreme edges of the scale so that accumulated negative data does not influence the overall data results.
Set population properties	Under **Population Properties:** • In the **Population** field, select a population. • In the **Line Style** field, select a line style. This style is applied to the gate frame that surrounds the population. • In the **Transparency** field, enter a transparency value between 0 and 100 (0 = transparent, 100 = opaque).

Density plot display properties

Use the **Density** tab to set density plot properties.

To...	Then do this...
Change the density resolution	In the **Resolution** field, select a resolution of 64, 128, 256, or 512. The default is 128. Higher resolution displays a more solid (less pixelated) image. You can combine higher resolution with higher smoothing to generate the most detailed density plot.
Smooth the data	Under **Smoothing**, select the **Use Smoothed Data** checkbox to enable both the **Clear Smooth Edges** checkbox and the **Smoothing Level controls**. Smoothing captures trends and patterns in the data and applies it to density level while filtering noise or outliers. Higher smoothing levels filter out more noise.
Hide edges of data	Under **Smoothing**, select the **Clear Smooth Edges** checkbox to make the edges of the rendered data invisible.

To...	Then do this...
Adjust smoothing	Under **Smoothing,** drag the **Smoothing Level** slider to select a smoothing level (1–8), or type a value. The default is 4.
Change how density is displayed	Under **Density Visualization**, in the **Scale Mode Type** field, select either **Logarithmic**, **Linear,** or **Probability.**
Change the level of density to display	Under **Density Visualization**, in the **Percentage** field, enter a value between 2 and 90. The default is 10. When displaying density, the values of the density levels must be determined. Depending on the algorithm, the levels are calculated differently. The percentage value is the input into the algorithm. Values include the following: **Linear.** Percentage of peak height of density levels with equal spacing between levels. **Log.** Percentage of peak height of density levels with log spacing. **Probability.** Percentage of matching events in each density level.
Change the color of the density data	Under **Density Visualization**, in the **Color Type** field, select **MultiColor**, **Grayscale**, or **Single Color.**
Show outlier events	Under **Density Visualization**, select the **Show Outliers** checkbox.
Show a dot/density hybrid plot	Under **Density Visualization**, select the **Show as Dot/Density Hybrid** checkbox to display data as a hybrid plot. Clear this checkbox to display data as a normal density plot. You can use this option to see a dot plot applied with a mask of the density plot. The density plot obscures the individual event dots while the hybrid view shows just the event dots colored by the density mask colors.

Contour plot properties

Use the **Contour** tab to set contour plot properties.

To...	Then do this...
Change the density resolution	In the **Resolution** field, select a resolution of 64, 128, 256, or 512. The default is 128. Higher resolution displays a more solid (less pixelated) image. You can combine higher resolution with higher smoothing to generate the most detailed contour plot.
Smooth the data	Under **Smoothing**, select the **Use Smoothed Data** checkbox to enable both the **Clear Smooth Edges** checkbox and the **Smoothing Level controls**. Smoothing captures trends and patterns in the data and applies it to a contour level while filtering noise or outliers. Higher smoothing levels filter out more noise.
Hide edges of data	Under **Smoothing**, select the **Clear Smooth Edges** checkbox to make the edges of the rendered data invisible.
Adjust smoothing	Under **Smoothing**, drag the **Smoothing Level** slider to select a smoothing level (1–8), or type in a value. The default value is 4.
Change how contour data is displayed	Under **Contour Visualization**, in the **Scale Mode Type** field, select either **Logarithmic**, **Linear**, or **Probability**.
Change the level of contour to display	Under **Contour Visualization**, in the **Percentage** field, enter a value between 2 and 90. The default value is 10. When drawing a contour, the values of the contour levels must be determined. Depending on the algorithm, the levels are calculated differently. The percentage value is the input into the algorithm. Values include the following: **Linear.** Percentage of peak height of contour levels with equal spacing between levels. **Log.** Percentage of peak height of contour levels with log spacing. **Probability.** Percentage of matching events in each contour level.
Change the color of the contour data	Under **Contour Visualization**, in the **Color Type** field, select **Multicolor**, **Grayscale**, or **SingleColor**.

To...	Then do this...
Show outlier events	Under **Contour Visualization**, select the **Show Outliers** checkbox
Fill the contours	Under **Contour Visualization**, select the **Fill Contours** checkbox.
Show contour lines in the plot	Under **Contour Visualization**, select the **Show Contour Lines** checkbox. Select the **Is Contour Line Colored** checkbox to apply the contour color to the contour outline.
Show a dot/contour hybrid plot	Under **Contour Visualization**, select the **Show as Dot/Contour Hybrid** checkbox to display data as a hybrid plot. Clear this checkbox to display data as a normal contour plot. You can use this option to see a dot plot applied with a mask of the contour plot. The contour plot obscures the individual event dots while the hybrid view shows just the event dots colored by the contour mask colors.

More information

- Creating plots in a worksheet (page 158)

Managing layers for a plot overlay

Introduction

This topic describes how to add, organize, define, or remove the layers of data that you can overlay on a dot plot or histogram.

About layers and overlays

Layers are individual data sets from tubes or FCS files (for example, all events and gates populations displayed in a plot). You can layer data from different tubes or FCS files to create a quick visual comparison of data.

All layers in the Layers table are applied to the plot. In a dot plot, the layer with the most events is always the back-most layer. The layer with the fewest events becomes the front-most layer. All other layers are sorted according to event count and positioned between the front and back layers.

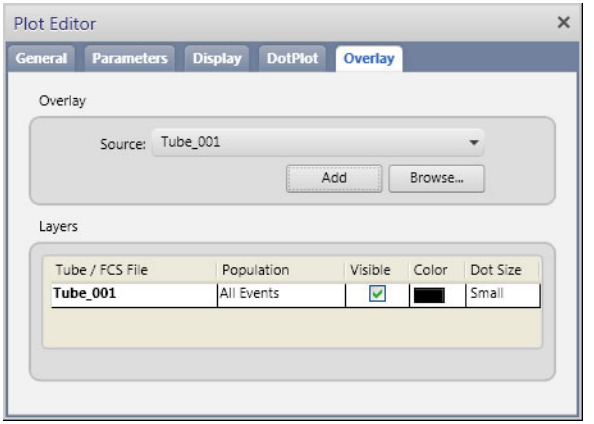
In a histogram, the overlay sequence is based on the order in which the data source was added.

The primary data source populations are the base of the plot and are not displayed as a layer. You specify which layers are visible, the percentage of transparency, and the color of the population data.

Adding overlay layers

To add layers to a plot overlay:

1. In the **Plot Editor,** click the **Overlay** tab.
2. Under **Overlay**, select a tube and click **Add**.



The new overlay layer is displayed in the **Layers** table. You can drag rows in the table to organize where layers appear in the table. The plot updates to display the layers.

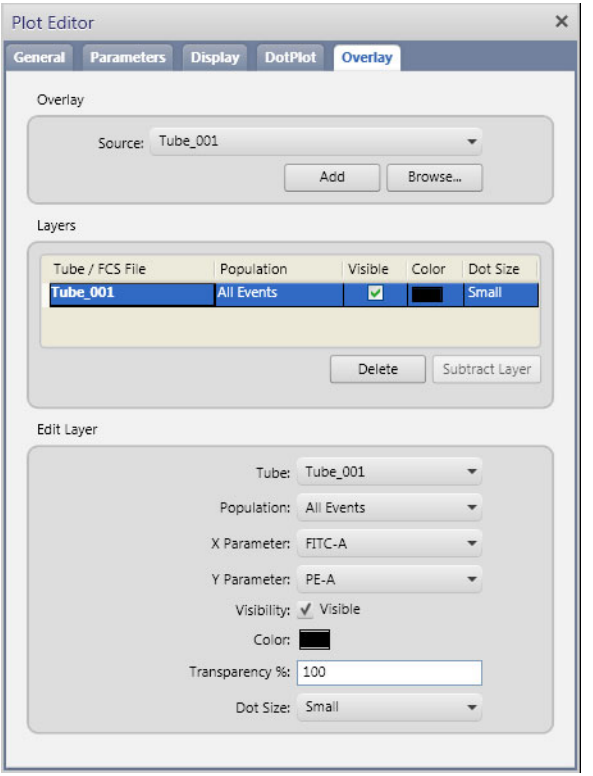
Modifying layer properties

To modify overlay layer properties:

1. In the **Layers** table, select a layer.

   The **Edit Layer** group is displayed.

2. To change the tube for a layer, click a layer in the table, then under **Edit Layer**, click **Tube** and select a different tube for the layer.



3. Click the **Population** field and select a population for this layer.
4. Click the **X Parameter** field and select an X parameter for the layer.
5. Select the layer in the table, then select the **Visible** checkbox to make the overlay visible or clear the checkbox to make the layer invisible.
6. Click the **Color** box to select a color for the data in this overlay.

7. In the **Transparency %** field, enter a transparency value between 0 and 100 (0 = transparent, 100 = opaque).
8. For dot plots, click the **Dot Size** field and select a dot size for the events in the layer. For histogram overlays, select an option from the **Line Style** field.

Deleting a layer from an overlay

To delete a layer from an overlay:

1. In the **Layers** table, select a layer.
2. Click **Delete**.

   The layer is removed from the **Layer** table and the overlay.

Subtracting a histogram layer

Subtracting a histogram layer shows the difference between the two histogram lines. This is enabled only when you select two layers with the same scale.

To subtract a histogram layer:

1. In the **Layers** table, click a histogram row.
2. Ctrl+click a different histogram row, then click **Subtract Layer**.

More information

- Creating plots in a worksheet (page 158)
- Setting plot display properties (page 328)

Displaying the legend for an overlay plot

Introduction

This topic describes how to display a plot legend in the worksheet.

The following overlay options are available for dot plot and histogram overlay layers. The overlay legend displays the list of tubes (data sources) that are assigned to the layer.

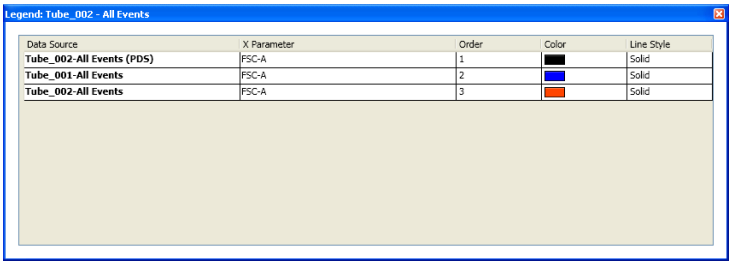
Procedure

To display the plot legend:

1. Use the **Plot Editor** dialog to create at least two layers in an overlay.
2. Click on a histogram in the worksheet.
3. Right-click and select **Legend**.

   The **Legend** dialog opens.

   The overlay legend displays the list of tubes (data sources) that represent layers, the x-axis parameter, the layer order of appearance, color, and line style.



More information

- Managing layers for a plot overlay (page 338)

10

Gates and populations

This section includes detailed information about gates and populations. Use this section to learn about gating guidelines, different gate types, and how to add or modify the different gate types. This section also describes gating and population hierarchies.

This section includes the following topics:

Introduction to gates

Introduction

This topic provides an overview of the different gate types.

About gates

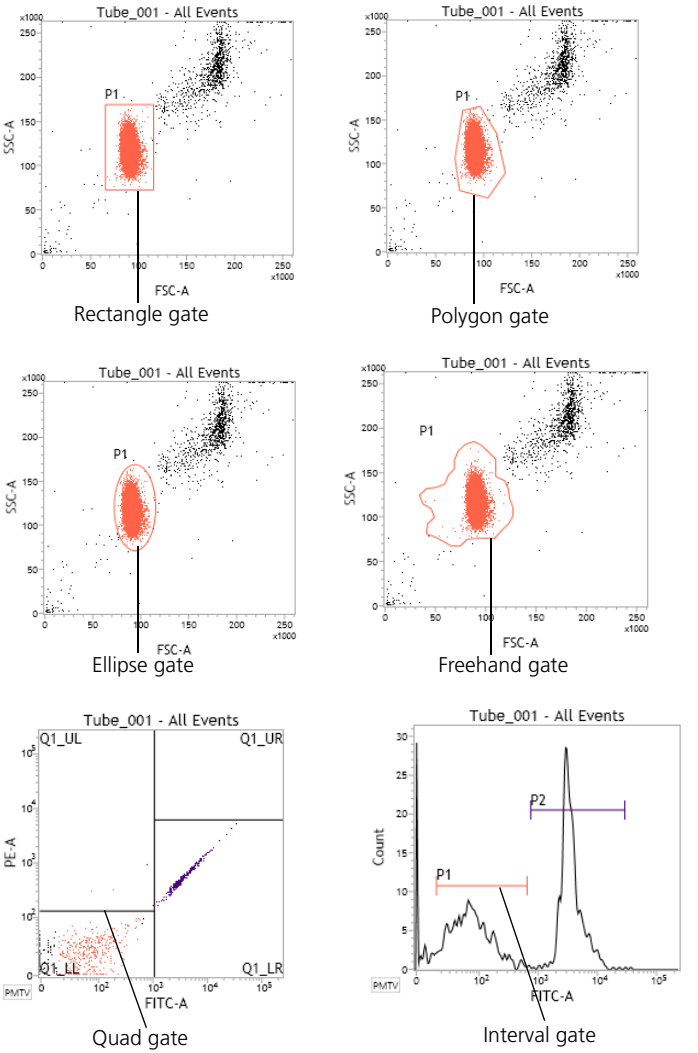
A gate is a boundary in a plot that defines a subset of the total sample population. Gating identifies cells of interest, classifies events in the gated population, and calculates population statistics.

Gate types

You can create the following gates within dot, density, or contour plots:

- Rectangle
- Ellipse
- Polygon, snap-to polygon, and auto polygon
- Quad and staggered quad
- Freehand
- Interval, auto-interval, snap-to interval
- Logical (AND, NOT, OR)

You can create interval gates within histograms.



More information

- Drawing gates in plots (page 164)
- Gating guidelines (page 350)

Gating tools

Introduction

This topic describes the gating tools on the Worksheet toolbar.

Worksheet tools

The following table describes the gating tools on the Worksheet toolbar and how to use them to draw gates in plots.

To draw a...	Then do this...
Rectangle gate 	1. Click this tool on the **Worksheet** toolbar. 2. Click in the plot and drag diagonally to create and size the rectangle around specific events in the plot. 3. Release the mouse button to set the gate.
Polygon gate 	1. Click this tool on the **Worksheet** toolbar. 2. Click on the plot to specify a starting point to display a vertex. 3. Move the cursor to another position and click to add another vertex. Repeat this step to create a minimum of three vertices around specific events in the plot. 4. Click the first vertex or double-click to set the last vertex and close the gate.
Ellipse gate 	1. Click this tool on the **Worksheet** toolbar. 2. Click in the plot and drag diagonally to create and size the ellipse around specific events in the plot. 3. Release the mouse button to set the gate.
Interval gate 	1. Click this tool on the **Worksheet** toolbar. 2. Click on a cluster of events in the plot to display the interval gate. See Creating and adjusting interval gates (page 380) for more information.

To draw a...	Then do this...
Quadrant gate 	1. Click this tool on the **Worksheet** toolbar. 2. In the plot, position the cursor to specify the quadrant intersection point. 3. Click to set the quad gate. See Creating quad gates (page 388) for more information.
Staggered quadrant gate 	1. Click this tool on the **Worksheet** toolbar. 2. In the plot, position the cursor to specify the quadrant intersection point. 3. Click to set the staggered quad. 4. Click the center point in the quad and drag to create a staggered quadrant. 5. See Creating staggered quad gates (page 394) for more information.
Freehand gate 	1. Click this tool on the **Worksheet** toolbar. 2. In the plot, click and hold the mouse button, then move the cursor to draw a freehand shape around specific events. 3. Release the mouse button to set and close the gate.
Snap-to polygon gate 	1. Click this tool on the **Worksheet** toolbar. 2. Click on a cluster of events in the plot. The polygon snaps to the nearest cluster of events in the population. See Creating adaptive (snap-to) gates (page 374) for more information.

To draw a...	Then do this...
Snap-to interval gate 	1. Click this tool on the **Worksheet** toolbar. 2. Click on a cluster of events in the plot. The polygon snaps to the nearest cluster of events in the population. See Creating adaptive (snap-to) gates (page 374) for more information.
Auto interval gate 	1. Click this tool on the **Worksheet** toolbar. 2. Click on a specific population in the plot. The auto gate adapts to the population data, then becomes a fixed interval. See Creating auto gates (page 379) for more information.
Auto polygon gate 	1. Click this tool on the **Worksheet** toolbar. 2. Click on a specific population in the plot. The auto gate adapts to the population data, then becomes a fixed polygon. See Creating auto gates (page 379) for more information.

More information

- Gating guidelines (page 350)
- Working with gates (page 352)
- Resizing and reshaping gates (page 354)
- Modifying gate properties (page 357)

Gating guidelines

Introduction

This topic describes the rules and guidelines for general gating procedures.

What is affected by modifying gates

When you create, delete, or modify gates, the gates and populations are updated in the following panels and fields:

- Gate hierarchy
- Population view
- Experiment
- Stopping Rules
- Statistics view
- Expression Editor
- Plots

You can undo all gating selections at any time.

Guidelines for deleting gates

You can delete a gate in a plot or within the hierarchy view.

- If the gate is unique, you cannot delete it. Use Remove Unique to convert the gate back to a regular gate.
- If the selected gate is not unique and the population has subpopulations or dependents, (for example, it is used to form a logical gate), then a confirmation message is displayed. When you delete a gate that is not unique:
  - The gated population and its descendants are deleted from the plot(s).
  - All gates that depend on the deleted entity are removed from the plot and hierarchy.
  - The population and gate hierarchy are updated.
  - The ungated events revert to the parent event colors.

You can delete quads. However, you cannot delete individual quadrants and bins.

Guidelines for copying and pasting gates

The following guidelines apply when you copy and paste single gates in a plot:

- If you paste a gate into a plot, a new gate is created (with the same shape) using the gate properties. The gate has the same definition (except for its parameters, which reflect the parameters of the plot). The gate's dependents are not added.
- If a gate with the same name exists, the new gate is renamed with the next available number in the naming sequence.
- If a gate with the same color exists, the new gate is assigned a new color and is displayed with its outline shown in the plot. In the hierarchy view, it is placed under the parent population displayed in the plot.
- If the plot has a different scale (log, linear, or biexponential), parameter space, file resolution, or histogram resolution, the gate shape is preserved and the gate maintains the same relative size and location in the plot. Gates are not converted if the scaling changes.
- After you paste the gate and a new gate is created, the gate hierarchy is updated and the new gate definition is applied to all tubes in the panel.

Guidelines for moving gates

The following guidelines apply when moving gates:

- After you move a gate, the population updates and all events are re-classified.
- If you move a snap-to polygon or snap-to interval gate, it does not automatically snap to the new closest cluster.

  If you want the snap-to gate to revert back to its snap-to behavior after moving it, right-click the gate and select Recalculate.
- The gate labels move with the gate, but you can move the labels anywhere in the plot if you prefer.

More information

- Introduction to gates (page 345)
- Working with gates (page 352)

Working with gates

Introduction

This topic describes how to perform basic gating procedures that you can use for most gate types.

Deleting gates

To delete gates from a plot:

1. In the worksheet, right-click a gate in a plot then select **Delete**.

Copying, cutting, and pasting gates

To copy, cut, or paste a gate in a plot:

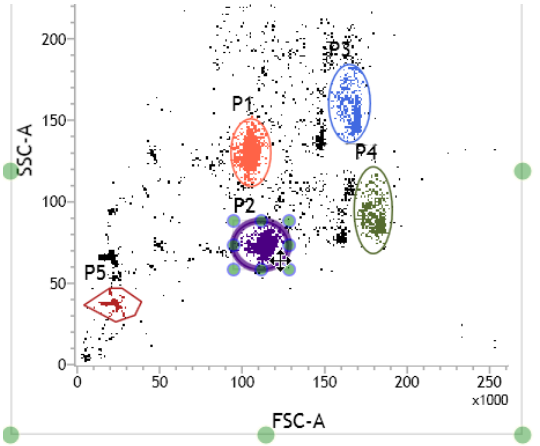
1. Right-click a gate in a plot.
2. Select **Copy, Cut,** or **Paste.**

Moving gates

You can move a gate within a plot without altering its shape.

To move a gate:

1. Click on a gate in a plot to highlight the gate boundary.



2. Click the gate boundary and drag it to a new location within the same plot.

More information

- Gating guidelines (page 350)
- Resizing and reshaping gates (page 354)

Resizing and reshaping gates

Introduction

This topic describes how to resize, reshape, and rotate gates.

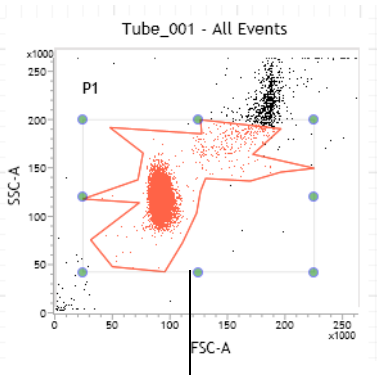
You can resize or reshape a gate by adjusting the boundary that encloses it.

Toggling between gate modes

Use bounds mode (boxed image area) for resizing rectangular and elliptical gates. Toggle to vertex mode when you want to modify any of vertices for a polygon or freehand gate.

To toggle between bounds mode and vertex mode:

1. Ctrl+click on a polygon or freehand gate in the plot.



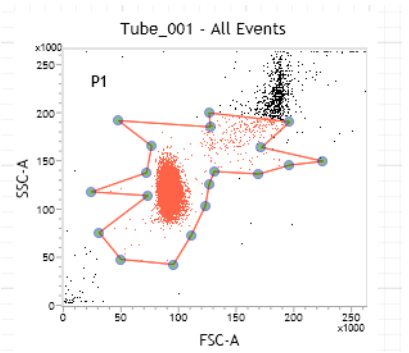
A polygon gate in bounds mode.

A polygon gate in vertex mode.

Resizing or reshaping in vertex mode

To resize or reshape a gate in vertex mode:

1. Toggle to vertex mode.



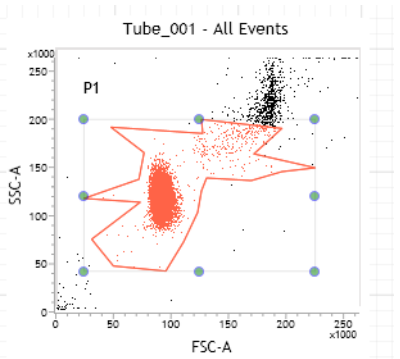
   Click on any vertices on the gate outline and drag to resize or reshape.

   The gate remains in vertex mode after you resize or reshape it. If you need to make more adjustments, toggle to bounds mode and resize or reshape the gate.

Proportional resizing in bounds mode

To proportionally resize the gate in bounds mode:

1. Toggle to bounds mode.



2. Click and drag any of the handles in the box to proportionally resize the gate.

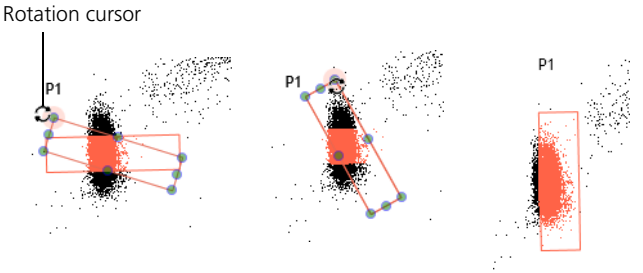
3. Click in the gate to set the edited gate.

   After you resize or reshape a gate, the gates update and re-classify all events within the same panel.

Rotating a gate

To rotate a gate:

1. Click on a gate in a plot.
2. Move the cursor over a gate handle until the rotation cursor is displayed.



3. Drag the handle in the desired direction.
4. Release the mouse button to set the gate position.

More information

- Working with gates (page 352)
- Making gates unique (page 373)

Modifying gate properties

Introduction

This topic describes how to modify gate properties and how gate modifications are applied to gates.

About modifying gate properties

Gates are associated with a tube data source. When you create or modify a gate, modifications are applied to all plots that are assigned to the tube data source and all tubes that include this gate. For example, if you have multiple plots on a worksheet and the run pointer is the primary data source, all plots display a modified gate.

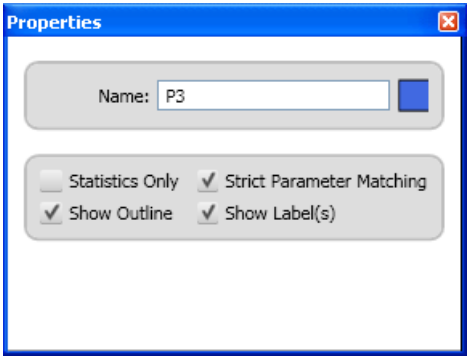
Use the gate Properties dialog to inspect and modify properties of a selected gate.

Procedure

To modify gate properties:

1. Right-click on a gate in a plot and select **Properties**.

   The **Properties** dialog opens.



2. The current name is displayed in the **Name** field.

   You can type a different name if you prefer.

3. Click the color block next to the **Name** field and select a color for the gate outline.

4. Select any of the following gate properties.

Option	Description
Statistics Only	Select this checkbox to display gate statistics only. If you select this checkbox, you cannot use this gate as the source population for another plot or use it in a logical gate. Events in this gate are not colored. This is the default for quad gates only.
Show Outline	Select this checkbox to display the outline of the gate in the plot. This is the default for all gates.
Strict Parameter Matching	Select this checkbox to apply the gate across all plots and tubes that match the long parameter name. Clear this checkbox to use the less restrictive short parameter name in the matching. Strict parameter matching is the default for all gates.
Show Label(s)	Select this checkbox to display labels on all plots containing the gate. This is the default for all gates.

More information

- Making gates unique (page 373)
- About gates and population hierarchies (page 359)

About gates and population hierarchies

Introduction

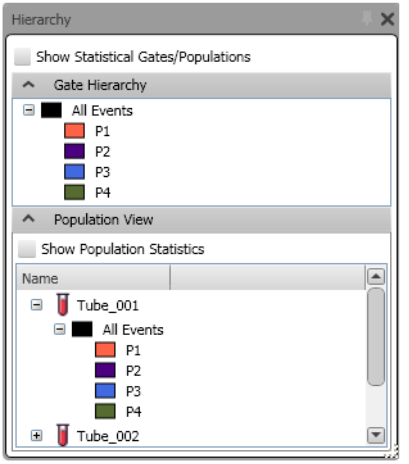
This topic describes gate and population hierarchy information and gate relationships.

Viewing a gate hierarchy

To view a gate hierarchy:

1. Click **Display Hierarchy** on the **Worksheet** toolbar.

   The hierarchy view opens.



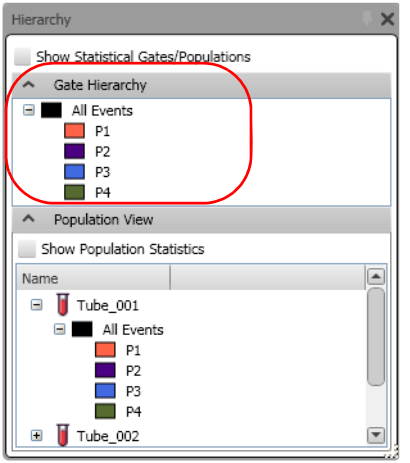
2. Under **Gate Hierarchy**, expand **All Events** to view all gates from the experiment in the hierarchy.

   By default, gated populations are identified in the hierarchy by color boxes and population labels (P1, P2, P3, etc.). You can rename these labels to include any name or description.

Gate and population hierarchy statistics

The hierarchy view includes the following information:

- **Gate Hierarchy.** Contains the hierarchy of all gates for the selected experiment. The hierarchy shows the name, color, and relationships with other gates.

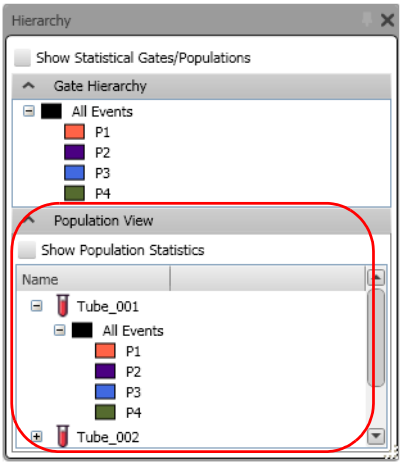


Each experiment has a gate hierarchy. The All Events gate is always the root in the hierarchy.

If you select a single gate in the hierarchy, the corresponding gate on the plot is displayed in bounds mode.

If you select a single gate on a plot, the corresponding gate in the hierarchy is highlighted.

- **Population View.** This section displays the hierarchy of populations that applies to each tube for the selected experiment.

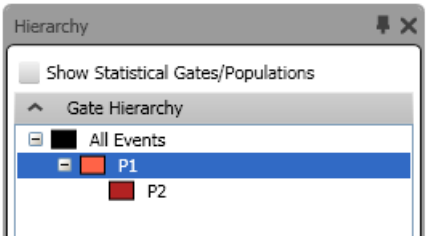


The Gate Hierarchy and Population View update when you create, delete, and modify gates.

Relationship of gates in the hierarchy

The relative position of each gate in a hierarchy can be expressed in terms of its relationship to other gates within the same hierarchy.

- **All Events.** This is the parent gate. This gate is the parent population of all populations.
- **P1.** In this example, this gate defines a subset of events as the population, P1. P1 is a child population of All Events.
- **P2.** In this example, this gate defines the population P2. P2 is subset of events of the population P1, thus making P2 a child population of P1 and a descendant of All Events.



More information

- Working with the gate hierarchy (page 363)

Working with the gate hierarchy

Introduction

This topic describes how to copy, paste, reorder, and delete gates within the hierarchy view.

About copying and pasting in the gate hierarchy

You can only copy, cut, and paste regular (not unique) gates from the gate hierarchy. Note the following:

- If you copy and paste a gate into a gate hierarchy, a new gate is created with a new name (using the next available number in the naming sequence) and new color.
- If you cut and paste a gate into the gate hierarchy, a new gate is created with the same name and color. The gate's children are also added to the hierarchy.

Copying gates

To copy gates:

1. Under **Gate Hierarchy**, right-click a gate, then select **Copy**.

Pasting gates into the hierarchy

To paste gates into the hierarchy view:

1. Under **Gate Hierarchy**, click the parent gate.
2. Right-click to display a menu, then click **Paste**.

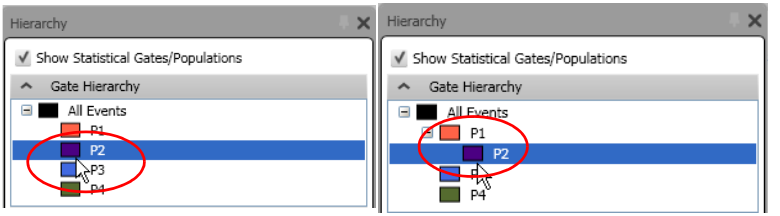
   The gate is pasted as a child of the selected gate.

Changing the relationship of gates

To change the relationship of gates in the Gate Hierarchy:

1. Under **Gate Hierarchy**, create a child gate by dragging one gate onto another gate.

   For example, if you want to make the P2 gate a child of the P1 gate, drag P2 onto P1.



When changing the relationship of gates:

- You can change the relationship of quads in the gate hierarchy by dragging the parent quadrant gate. You cannot change the relationship of individual quadrants and bins.
- You cannot place any gate above All Events (the parent gate) in the gate hierarchy.

Deleting gates from the hierarchy

To delete gates from the hierarchy view:

1. Under **Gate Hierarchy**, right-click the gate you want to delete, then select **Delete**. If the gate is unique, right-click and select **Remove Unique**.

Showing statistical gates or populations

Statistical gates display statistics for all events within the gate. All events appear as the same color. Typically, quad gates are used for statistics gates. However, you can display statistics for any gate by modifying the gate properties. See Modifying gate properties (page 357) for more information.

To show statistical gates or populations in a hierarchy:

1. Under **Gate Hierarchy,** select the **Show Statistical Gates/ Populations** checkbox.

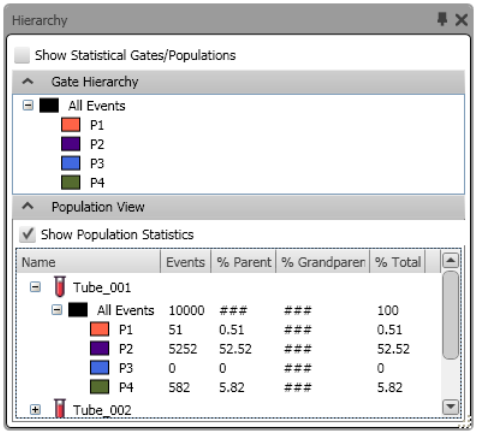
   You can toggle this checkbox to show or hide gates or populations that are used for statistics only.

Showing population statistics

To show population statistics in the population view:

1. Under **Population View**, select the **Show Population Statistics** checkbox.

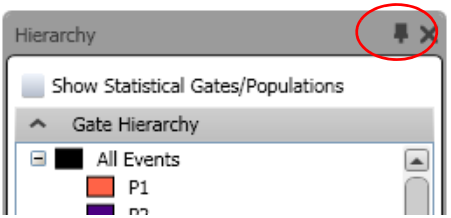
The hierarchy view expands and displays the population statistics.



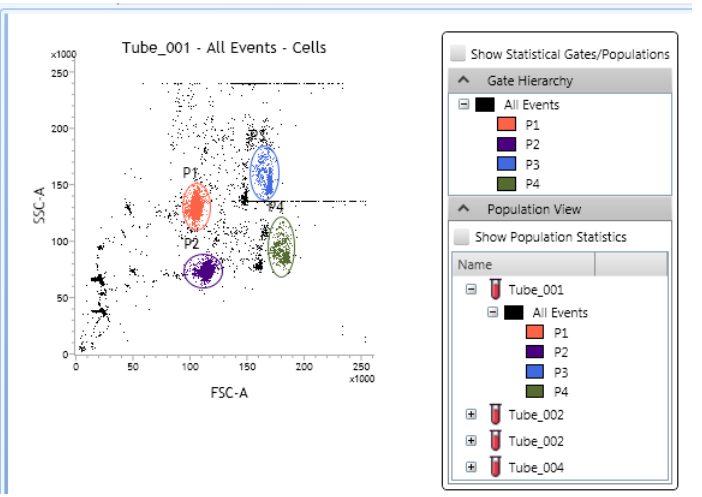
Pinning the hierarchy to a worksheet or report

To pin the hierarchy to a worksheet or report:

1. Move the hierarchy view inside the worksheet or report.
2. Click the **Pin** icon at the top of the hierarchy view.



The hierarchy pins to the worksheet.



You cannot unpin a hierarchy. To remove a pinned hierarchy, right-click in the hierarchy, then select **Delete**.

More information

- Creating adaptive (snap-to) gates (page 374)

Finding and highlighting populations

Introduction

This topic describes how to find and highlight populations in dot plots.

About finding events

You can find and graphically isolate a particular population in one or more dot plots. You can also highlight populations to make the population easier to identify.

You can find events for only one population at a time across all dot plots in an experiment. If you want to focus on a particular population without making other populations unavailable, you can highlight all events in tubes that contain a specific population. You can highlight multiple populations at the same time within an experiment.

Finding events

To find events across all plots:

1. Click **Display Hierarchy** on the **Worksheet** toolbar.

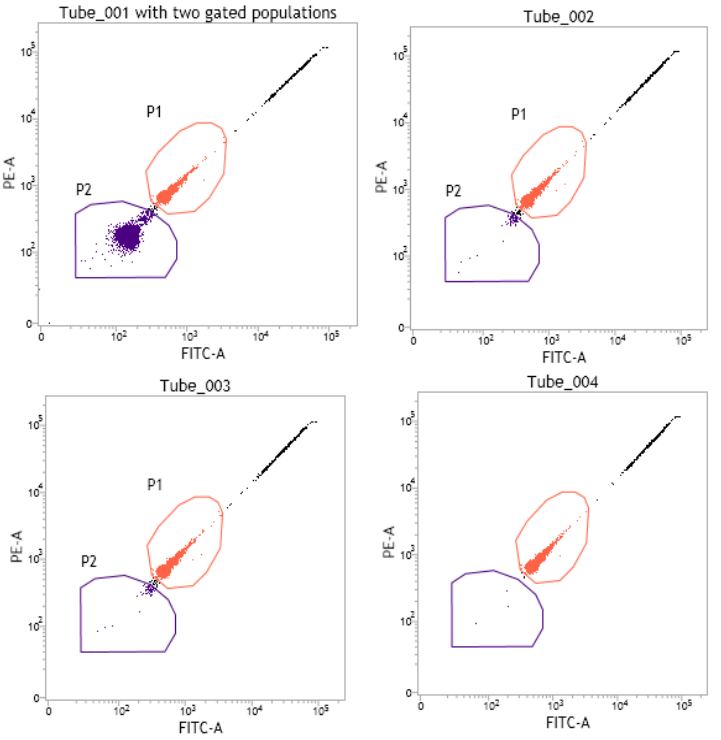
   The hierarchy view opens

2. If you want to find events for a population in all tubes in an experiment, under **Gate Hierarchy**, right-click a population and select **Find Events**.

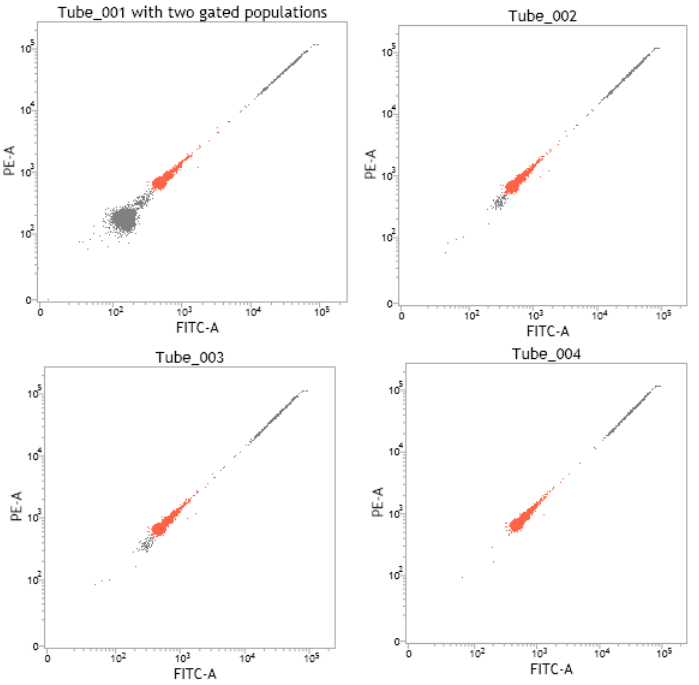
   The selected events are isolated and displayed in the population color.

   The following figures show plots for four tubes before Find Events is performed and after the P1 population is isolated in the plots.

Before Find Events is performed



After Find Events is performed



If you use Find Events, then delete the specific population, all events in the plot revert to the default population colors.

Turning off Find Events

To turn off Find Events:

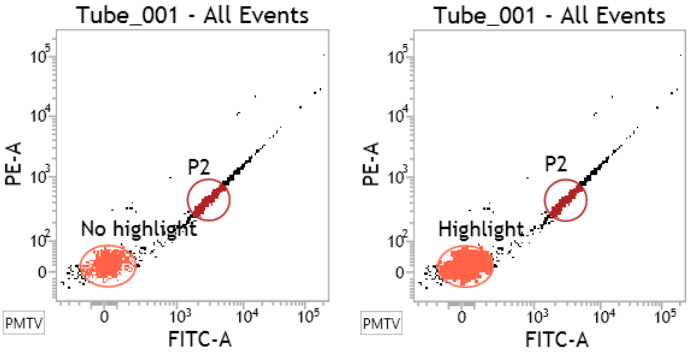
1. In the hierarchy view, under **Gate Hierarchy,** right-click a gate and select **Turn Off Find Events**.

Highlighting a specific population

If you want to focus on a particular population without making other populations unavailable, you can highlight all events in tubes that contain a specific population.

To highlight a specific population in a dot plot:

1. Under **Gate Hierarchy**, right-click a gate and select **Highlight**.



The populations are highlighted. During highlighting, gate and population hierarchies do not change, but the events of the highlighted populations display larger in the plot.

Turning off event highlights

To remove highlighting:

1. Under **Gate Hierarchy**, right-click a highlighted gate and select **Turn Off Highlight**.

More information

- Modifying gate properties (page 357)
- About gates and population hierarchies (page 359)

Creating logical gates

Introduction

This topic describes the different types of logical gates and how they are applied to the population hierarchy. It also describes how to create logical gates by combining existing gates from the gate hierarchy to form a single gate.

Types of logical gates

Logical gates are created by combining non-statistical gated populations to form gates based on AND, OR, NOT, or REST OF criteria.

Gate type	Description
AND	This gate contains the intersection of two or more existing gates. This gate is added to the hierarchy under the first common ancestor of the selected gates.
OR	This gate joins two or more existing gates to include events in either gate. This gate is added to the hierarchy under the first common ancestor of the selected gates.
NOT	This gate contains all events that are in the parent population, but not in an gate itself. This gate is added to the hierarchy at the same level as the previously selected gate.
REST OF	This gate contains all events that are in the selected gate, but not in any of its children. This gate is updated when a child is added or removed from its parent.

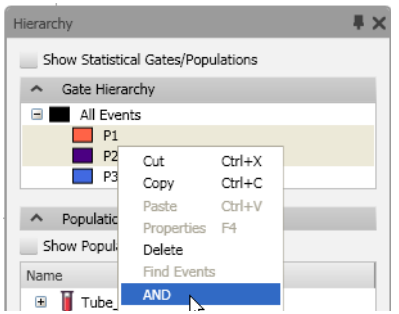
Procedure

To create a logical gate:

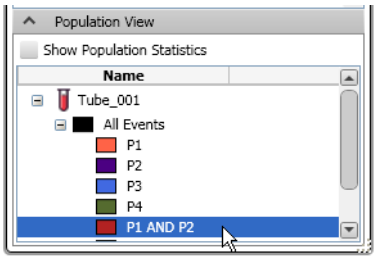
1. View a plot with gates.
2. Select **Display Hierarchy** on the **Worksheet** toolbar.
3. Under **Gate Hierarchy**, select one or more descendant gates under **All Events** (for example, Ctrl+click *P1* and *P2*).
    - For AND and OR gates, select at least two gates.

- For NOT and REST OF gates, select only one gate. REST OF gates are only available when a child gate exists for a gate.

4. Right-click the selected gates and select an available logical gate option (for example, select *AND)*.



A new gate definition is created based on your specified existing gate(s) and it is added to the gate hierarchy under the first common ancestor. The new gate definition is applied to all tubes, and the population hierarchies are updated to identify the new logical population.



The plots (that contain the existing gates) are updated to graphically show the logical gate with no outline.

More information

- Creating adaptive (snap-to) gates (page 374)
- Quad gates overview (page 384)

Making gates unique

Introduction

This topic describes how to make gates unique to a specific tube.

About making gates unique

When gates are made unique, the population hierarchy is updated and the unique gate is identified by a tube icon. The associated gate is also marked in the plot as unique.

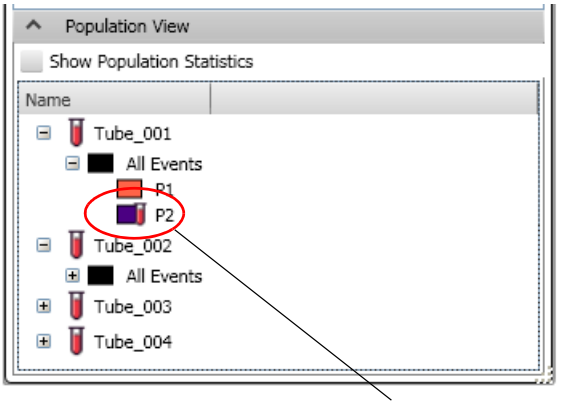
Since a unique gate is specific to one tube, any modifications are not applied to other tubes within the experiment.

Making a gate unique

To make a gate unique:

1. In the hierarchy view, under **Population View**, select a tube.
2. Right-click the gate that you want to make unique to the tube, then select **Make Unique**.

   The gate is defined as unique in the **Population View** of the Hierarchy.



Removing unique

To remove unique from a gate:

1. In the hierarchy view, under **Population View,** right-click the unique gate, then select **Remove Unique**.

The gate reverts to its original definition (before the gate was made unique). The tube icon is removed from the plot and population view.

More information

- Resizing and reshaping gates (page 354)
- Modifying gate properties (page 357)

Creating adaptive (snap-to) gates

Introduction

This topic describes how to create snap-to polygon and snap-to interval gates, and describes how to recalculate adaptive gates when the event data changes.

Adaptive gate behavior

Adaptive gates include snap-to polygon and snap-to interval gates. An adaptive gate conforms its shape and location to the underlying data on the plot, and applies the same start point, sizing factor, and movement factor to all tubes or files that use this adaptive gate.

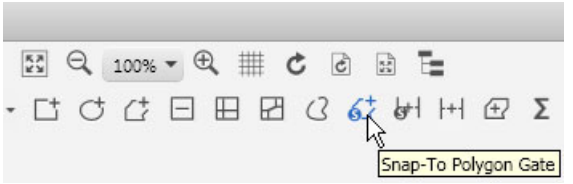
If you move, resize, or reshape an adaptive gate, it stops adjusting to the underlying data. After you resize or reshape an adaptive gate, you must recalculate to restart the adaptive behavior.

If the gate snaps to another cluster after it is recalculated, then any plots that use the recalculated snap-to gate, a descendent, or dependent logical gate are updated.

Creating a snap-to polygon gate

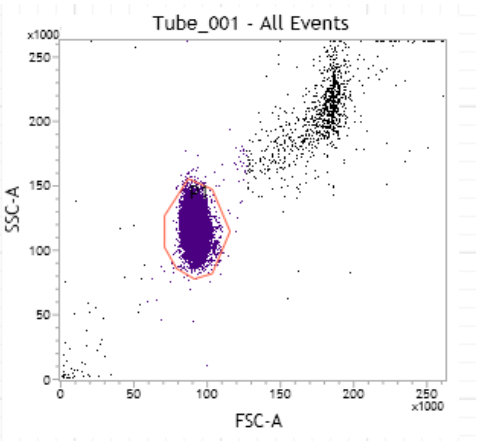
To create a snap-to polygon gate:

1. Click on a plot.
2. Click the **Snap-To Polygon Gate** tool on the **Worksheet** toolbar.



3. Click on a cluster of events in the plot.

   The polygon snaps to the nearest cluster of events in the population.



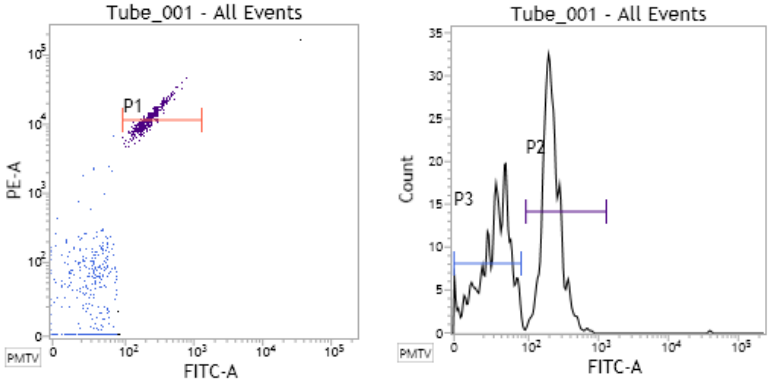
   If a cluster is not found, a message is displayed and a default small pentagon gate is created in the plot. If this occurs, try using an ellipse or polygon gate instead.

Creating a snap-to interval gate

To create a snap-to interval gate:

1. Click on a plot.
2. Click the **Snap-To Interval** tool on the **Worksheet** toolbar.
3. Click on a specific population in the plot.

   A snap-to interval gate is displayed around the closest cluster that is within the default range.



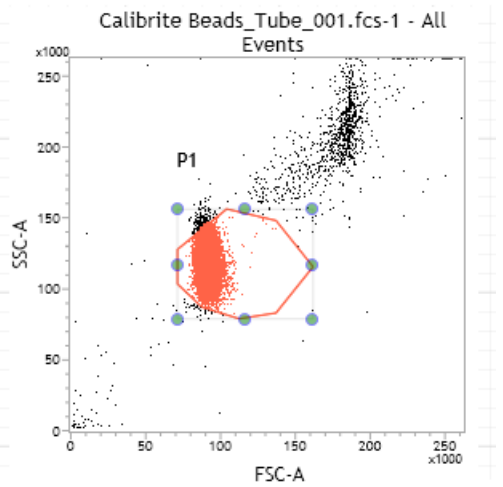
If a cluster is not found, a message is displayed and a default narrow interval gate is created. If this occurs, try using an auto interval gate instead.

Re-calculating (restarting) adaptive gate behavior

To re-calculate adaptive gate behavior after the gate has been resized or reshaped:

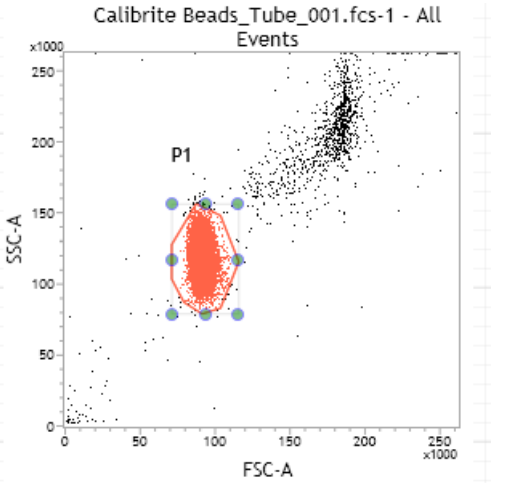
1. Right-click the adaptive gate in a plot and select **Recalculate**.

Resized snap-to gate before recalculation



If the gate snaps to another cluster after it is recalculated, then any plots that use the recalculated snap-to gate, a descendant, or dependent logical gate is updated.

Resized snap-to gate after recalculation



More information

- Creating auto gates (page 379)

Creating auto gates

Introduction

This topic describes auto gate behavior and how to create auto gates. Auto gates include auto polygon and auto interval gates.

Auto gate behavior

Auto gates adapt to the underlying data only during creation. After you create an auto gate, it behaves like a regular polygon or interval and does not adapt.

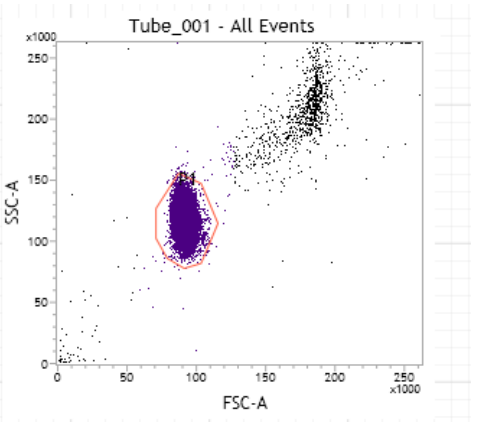
When an auto polygon gate is created on an overlay, it adapts to the top layer only.

Procedure

To create an auto gate:

1. Click on a plot.
2. Click the **Auto-polygon Gate** or **Auto Interval Gate** tool on the **Worksheet** toolbar.
3. Click on a specific population in the plot.

   The auto gate adapts to the population data, then becomes a polygon or interval.



More information

- Creating adaptive (snap-to) gates (page 374)
- Creating and adjusting interval gates (page 380)

Creating and adjusting interval gates

Introduction

This topic describes how to create interval gates in plots and adjust their size and position.

Use interval gates to indicate and measure an x-axis data interval in a plot.

Interval gate behavior

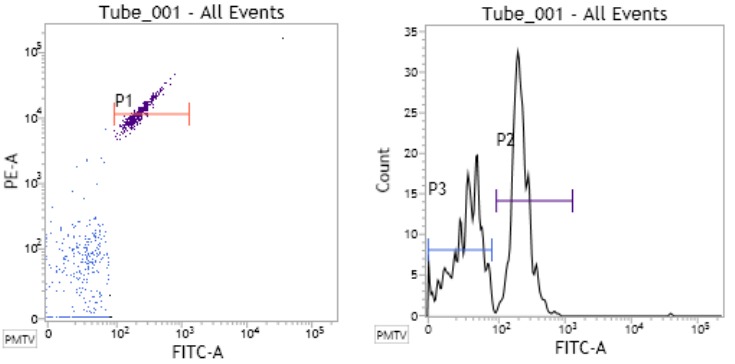
When you create an interval gate on a histogram for a tube, all events in related dot plots for the tube are displayed with the population color (with no visible outline).

When you create an interval gate on a dot plot, all events on related histograms are not displayed until you configure the plots to display the newly created gate. Once the histogram is configured, the histogram curve is displayed in the population color.

Creating an interval gate

To create an interval gate:

1. Click on a plot.
2. Click the **Interval Gate, Auto Interval Gate** or **Snap-to Interval Gate** tool on the **Worksheet** toolbar.
3. Click on a cluster of events in the plot. The interval gate is displayed.

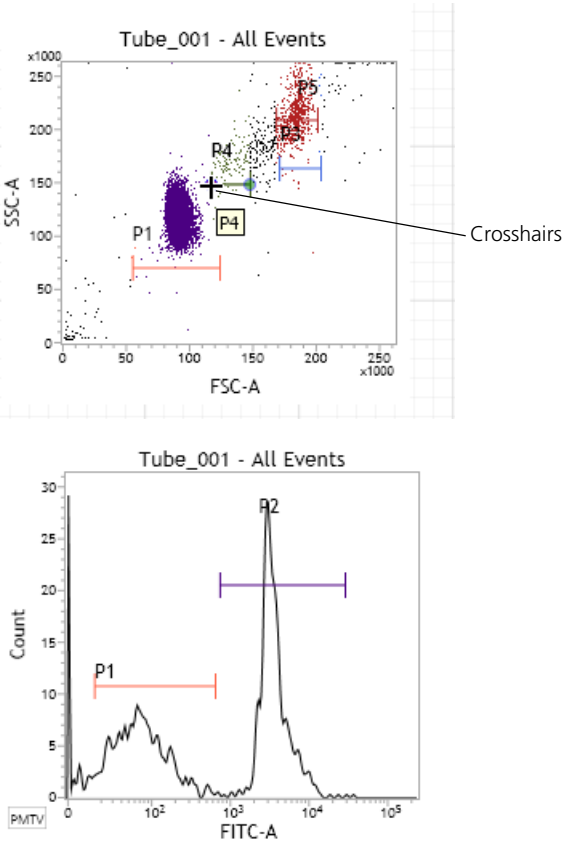


Adjusting an interval gate

To adjust an interval gate:

1. Click on the left or right boundary in an interval gate.

   Crosshairs are displayed.



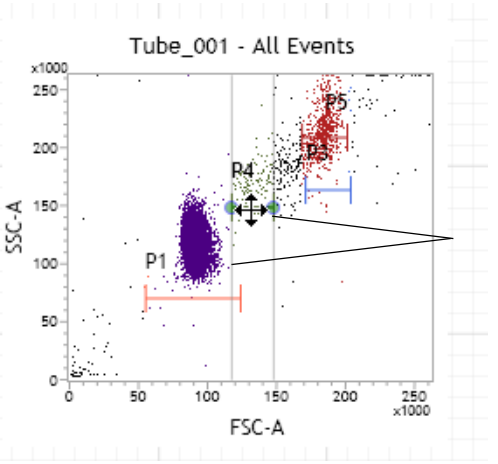
2. Drag the boundary of the interval (to increase or decrease the interval size).

Moving an interval gate

To move an interval gate:

1. Click and drag the entire gate to the left or right.

   The directional cursor and vertical location indicators are displayed.



More information

- Creating auto gates (page 379)
- Creating logical gates (page 371)

Quad gates overview

Introduction

This topic describes quad gates, different quad gate modes, and how to align, pivot, and reset the gates.

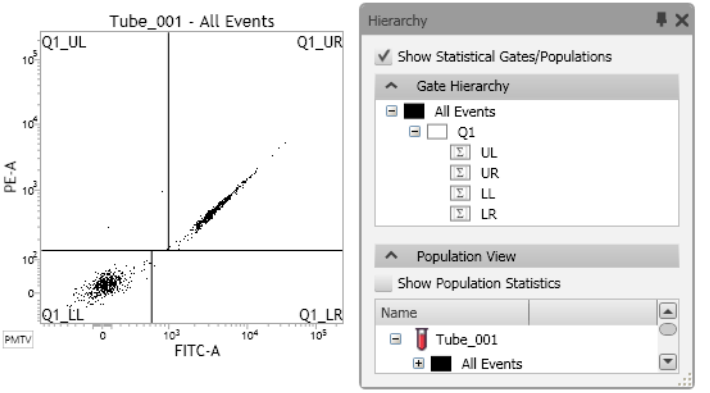
In addition to the quad gates described in this topic, you can create staggered quad gates. Staggered quad gates are an alternate type of basic quad gate, with two center points (or handles). See Staggered quad gate overview (page 392) for more information.

About quad gates

A quad gate consists of four segments that divide a plot into four quadrants (sections). The default name for a quads is *Qn*, where *n* is the quad number.

The default names for its quadrants are:

- UL (upper-left quadrant)
- UR (upper-right quadrant)
- LL (lower-left quadrant)
- LR (lower-right quadrant)



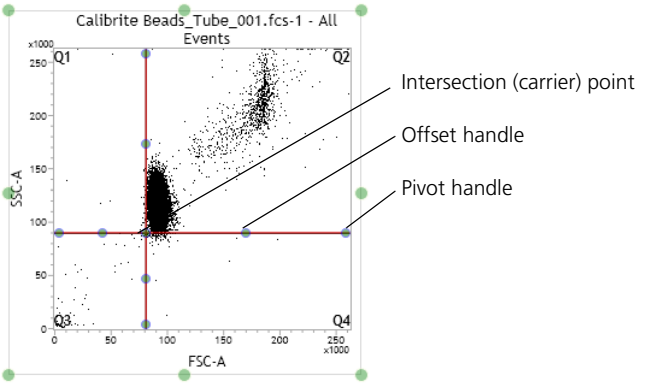
By default, the gate is created as a statistics-only gate.

There are four different quad gate modes:

- Basic
- Vertical offset
- Horizontal offset
- Hinged

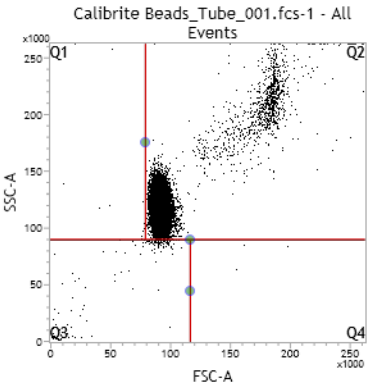
Basic mode

All new quad gates start in basic mode. This mode has a single center point, four offset handles, and four pivot handles. Any segment in the quad can move in the x or y direction (using the offset handles). You can use the pivot handles to change the slope of any segments.



Vertical offset mode

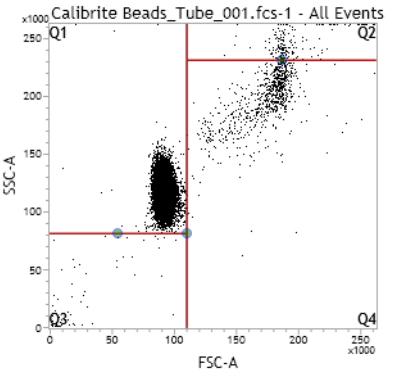
Enable this mode by dragging the offset handle on a vertical segment. You can move the vertical segments individually in the x-dimension, but you cannot move the individual horizontal segments or pivot them in the plot.



If you want to enable horizontal offset or hinged mode, you need to reset the quad to basic mode. Resetting re-aligns the vertical and horizontal segments.

Horizontal offset mode

Enable this mode by dragging the offset handle on a horizontal segment. You can move the horizontal segments individually in the y-dimension, but you cannot move the individual horizontal segments or pivot them in the plot.

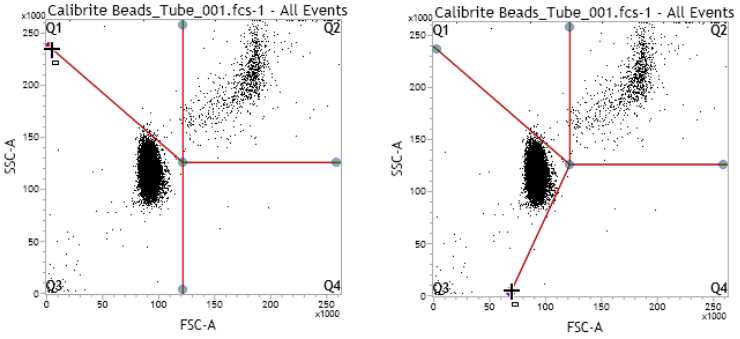


If you want to enable vertical offset or hinged mode, you need to reset the quad to basic mode. Resetting re-aligns the vertical and horizontal segments.

Hinged mode

Enable this mode by dragging a pivot handle to change the slope of any segment. A segment cannot be pivoted past another segment in either direction. The pivot stops when it reaches the bounds of another segment.

No segments can be offset in this mode. The left and bottom segments are attached to their respective sides. The left segment can only be moved on the left edge and the bottom segment can only be moved along the bottom edge. The other segments can be moved to all sides.



More information

- Creating quad gates (page 388)
- Staggered quad gate overview (page 392)
- Creating staggered quad gates (page 394)
- Creating logical gates (page 371)
- Resetting quads to basic mode (page 390)

Creating quad gates

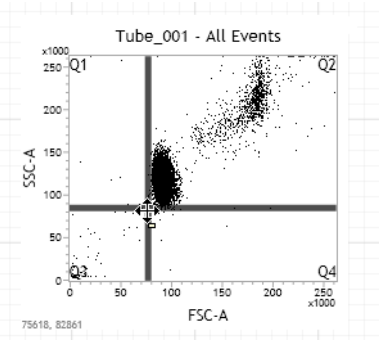
Introduction

This topic describes how to create a basic quad gate and how to modify the quad segments.

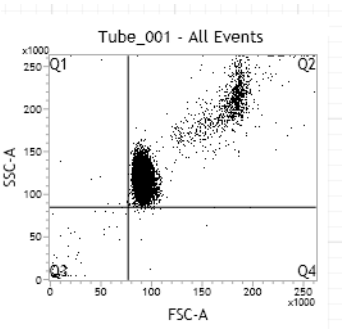
Creating a quad gate

To create a quad gate:

1. Click a plot.
2. Select the **Quad** tool on the **Worksheet** toolbar.
3. In the plot, position the cursor to specify the quadrant intersection point.



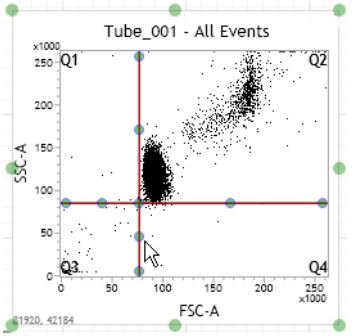
4. Click to set the quad.



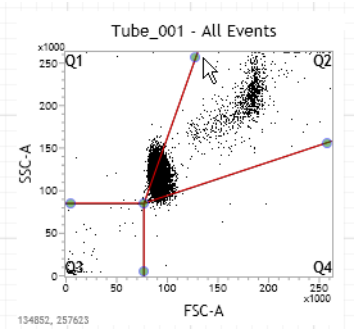
Modifying quad segments

To modify quad segments:

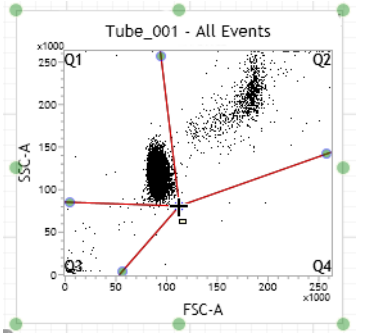
1. Click any quad segment to display the outline and handles for the current quad mode.



2. Drag the segment.



The intersection (center) point of the gate is shown in each mode. You can reposition the intersection at any time by clicking and dragging in the plot area.



Resetting quads to basic mode

To reset modified quads:

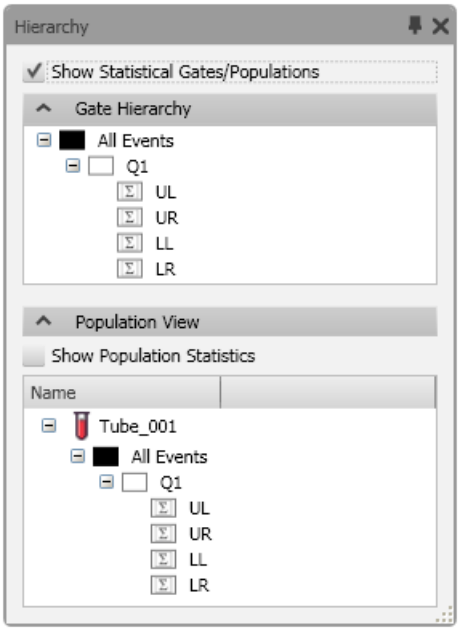
1. Double-click the quad in the plot.

Viewing quads in the hierarchy

To view quads in the hierarchy:

1. In a worksheet, select a plot with quad gates.
2. Click **Display Hierarchy** on the **Worksheet** toolbar.
3. Select the **Show Statistical Gates/Populations** checkbox.

4. Under **Gate Hierarchy**, expand **All Events** to view all quads in the hierarchy.



Quads are identified in the hierarchy as UL (upper left), UR (upper right), LL (lower left), and LR (lower right).

More information

- Quad gates overview (page 384)
- Creating staggered quad gates (page 394)
- Statistical quad gates (page 395)

Staggered quad gate overview

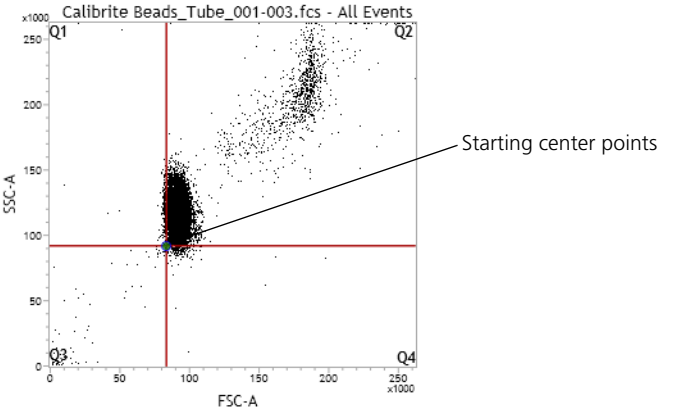
Introduction

This topic describes staggered quad gates.

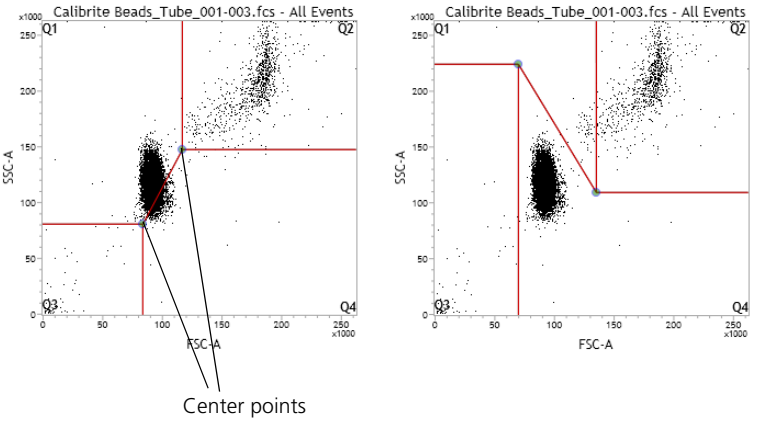
About staggered quad gates

A staggered quad gate is an alternate version of the basic quad gate, with two center points (or handles).

All staggered quads start in basic mode with the first center point positioned on top of its second center point.



New staggered quads look similar to a basic quad until you drag one of the two center points in the plot.



The right and top segment form a right angle with the first center point, while its left and bottom segment form a right angle with the second center point.

A line connects the two center points. You can click on any segment to display the outline and handles for the current mode.

More information

- Creating staggered quad gates (page 394)
- Quad gates overview (page 384)
- Statistical quad gates (page 395)

Creating staggered quad gates

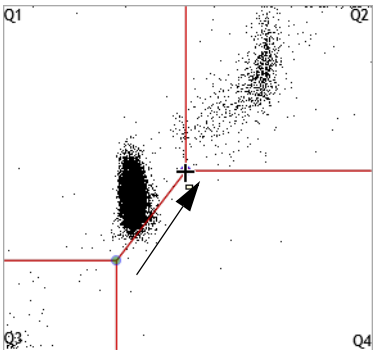
Introduction

This topic describes how to create staggered quads in plots and how to reset them from staggered mode to basic mode.

Creating a staggered quad gate

To create a staggered quad gate:

1. Click the **Staggered Quad** tool on the **Worksheet** toolbar.
2. In the plot, position the cursor to specify the quadrant intersection point.
3. Click to set the staggered quad.
4. Click a center point in the quad and drag it to create a staggered quadrant.



By default, the gate is created as statistics-only. The intersection (center) points of the gate are shown in each mode, and can be repositioned at any time by clicking and dragging in the plot area.

Resetting a staggered quad gate

To reset a staggered quad gate:

1. Double-click the gate in the plot.

   Resetting a staggered quad moves the bottom center point to the top center point.

More information

- Resizing and reshaping gates (page 354)
- Statistical quad gates (page 395)

Statistical quad gates

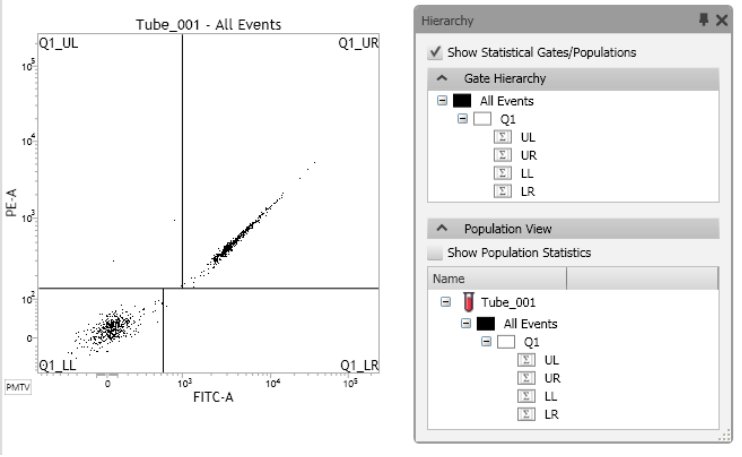
Introduction

This topic describes statistical quad gates and how to display statistics in them.

About statistical quad gates

Quad gates are statistical gates by default. Statistical quad gates display statistics for all events in the plot. All events are displayed in the same color and cannot be used as a parent population. These gates are only used for statistics are not used for other gating functions (for example, to gate another plot or as part of a logical gate).

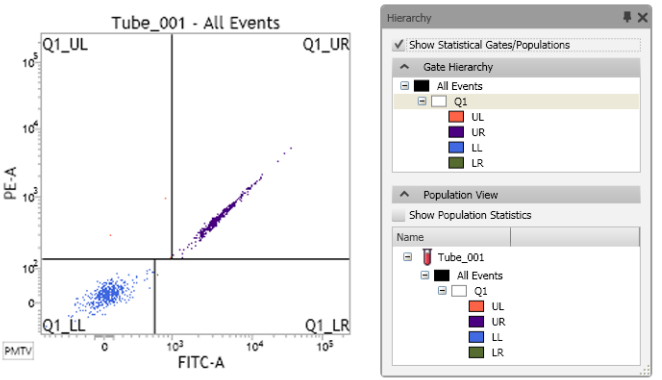
Statistical quad gate



Non-statistical quad gates display statistics and event counts for populations in each quadrant. Each quadrant displays a different

population color and can be used as parent populations or in logical gates.

Non-statistical quad gate

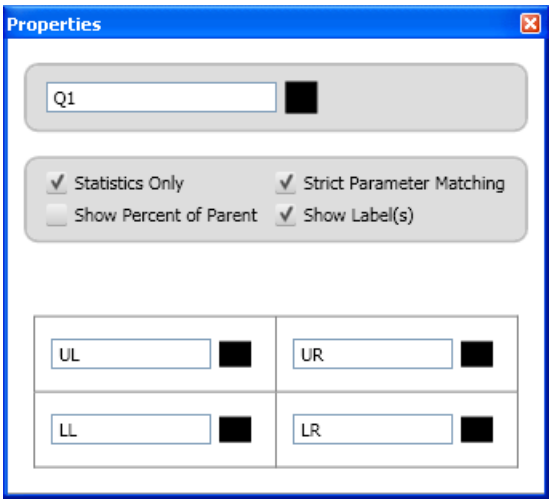


Procedure

To show statistics in quad gates:

1. Right-click a quad gate in a plot and select **Properties**.

   The **Properties** dialog opens.



2. Select the **Statistics Only** checkbox to display a statistical quad gate or clear this checkbox to display a non-statistical quad.
3. (Optional) Select the **Strict Parameter Matching** checkbox to apply the gate across all plots that match the long parameter name (full name of the parameter or fluorochrome).

   Clear this checkbox to use the less restrictive short parameter name (less specific parameter name, for example, FITC) in the matching.
4. (Optional) Select the **Show Percent of Parent** checkbox to show the percent of the parent population for each quadrant.
5. (Optional) Select the **Show Label(s)** checkbox to display bin labels on all plots containing the gate.

More information

- Creating staggered quad gates (page 394)
- Gating guidelines (page 350)

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11

Statistics

This section includes detailed information about statistics views. Use this section to learn about creating, modifying, and exporting statistics views.

This section includes the following topics:

Working with statistics views

Introduction

This topic describes how to create, rename, and delete statistics views.

About statistics views

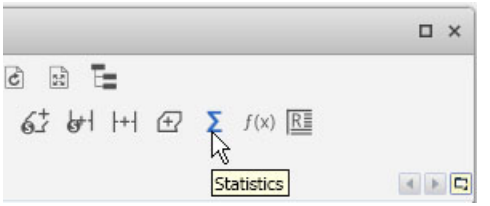
Statistics are associated with all gates and populations in plots on a worksheet. The calculated statistics are displayed in a view that includes a title, keywords, expressions, and statistics for one or more populations and/or parameters.

You need to set the statistics properties to display the worksheet statistics in this view.

Creating statistics views

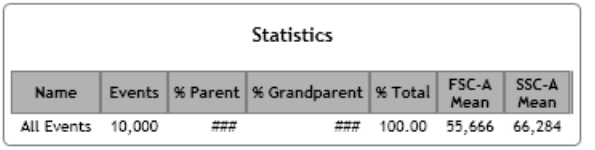
To create a statistics view:

1. Click **Statistics** on the **Worksheet** toolbar.



2. On the worksheet, click to draw the statistics view.

   A new statistics view is displayed in the worksheet.



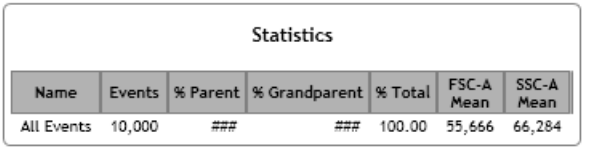
Statistics

Name	Events	% Parent	% Grandparent	% Total	FSC-A Mean	SSC-A Mean
All Events	10,000	###	###	100.00	55,666	66,284

Renaming statistic views

To rename a statistics view:

1. Double-click in the statistics title in the header to enable edit mode.



2. Type a new title for the statistics view.
3. Click the header to exit edit mode.

Deleting statistics views

To delete a statistics view from a worksheet:

1. Right-click the statistics view and select **Delete**.

   The statistics view is deleted.

More information

- Setting statistics view properties (page 401)

Setting statistics view properties

Introduction

This topic describes how to set properties for a specific statistics view. You must set the properties for each statistics view after you create it.

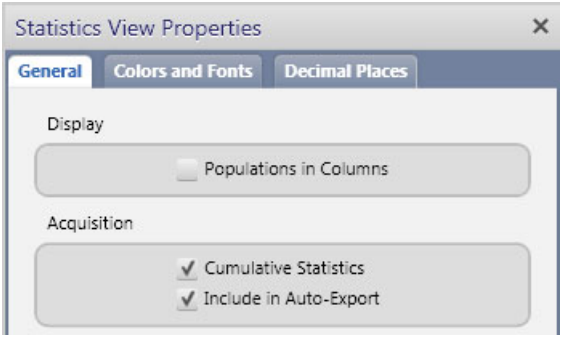
All modifications to statistics view properties are automatically applied. No additional saving or applying is required.

Selecting statistics view options

To set statistics view properties:

1. Right-click the statistics view and select **Properties.**

The **Statistics View Properties** dialog opens.



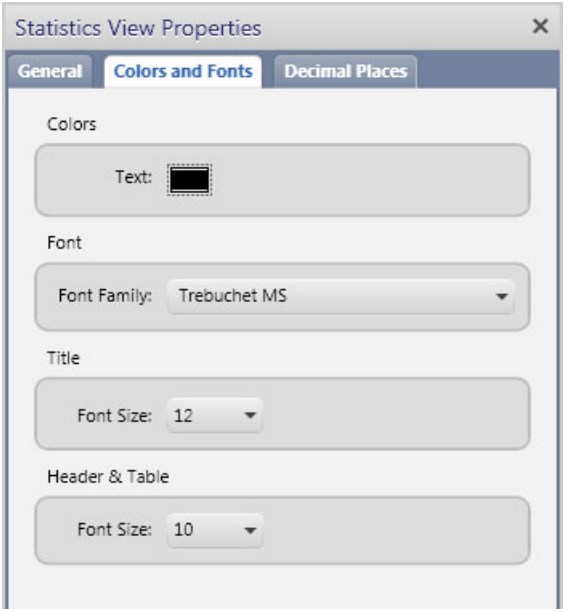
2. In the **General** tab, under **Display**, select the checkbox to display populations in columns.
3. Under **Acquisition**, select the checkboxes to acquire cumulative statistics and include the statistics in the auto-exported file.

   See Exporting statistics from statistics views (page 416) for more information.

Setting font properties

To select font properties for a statistics view:

1. Click the **Colors and Fonts** tab.

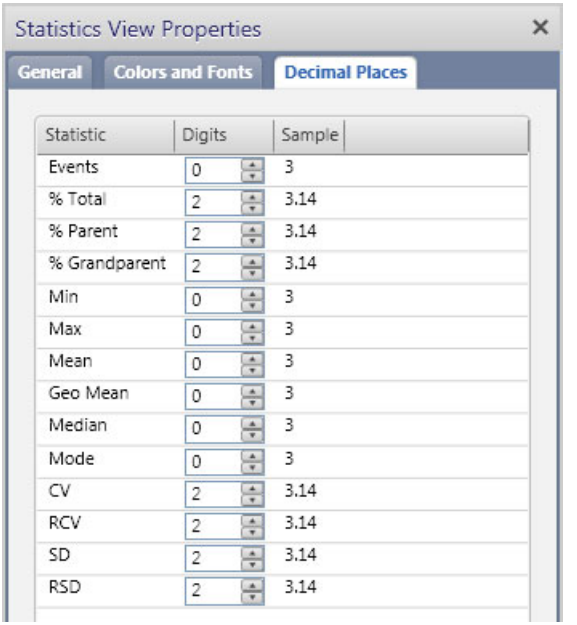


2. Set the color, font family, title, and font size properties for the statistics view.

Setting statistics formats

To set the decimal place format for statistics:

1. Click the **Decimal Places** tab.



2. In the **Digits** column, specify the decimal place for each statistic in the table.

More information

- Working with statistics views (page 400)
- Selecting populations for statistics views (page 405)

Selecting populations for statistics views

Introduction

This topic describes how to select populations for statistics views.

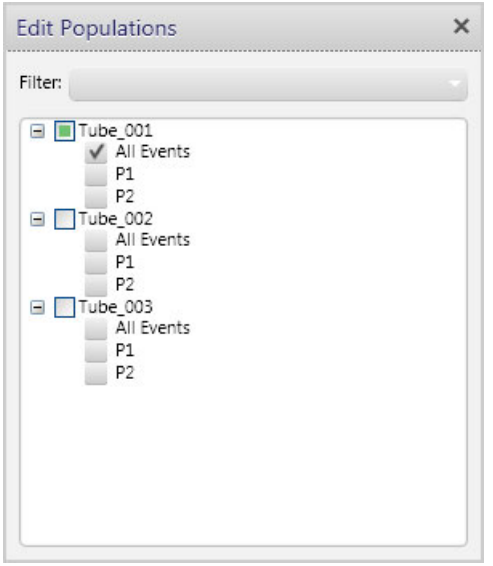
Make sure to add tubes to your experiment first so that they are available for selection.

Procedure

To select populations for a statistics view:

1. Right-click the statistics view and select **Edit Populations.**

   The **Edit Populations** dialog opens.



   This dialog includes all tubes and all populations (all events and gated events).

2. Click a tube in the tree to expand a population category.

3. Select the checkboxes for all population categories you want to display in the statistics view (for example, select *All Events)*.

   If the dialog includes numerous tubes, files, and populations, type a tube, file, or population name in the **Filter** field to locate

a specific tube, file, or population (for example, type %). All population names that contain % are displayed in the list.

More information

- Setting statistics view properties (page 401)
- Selecting statistics for statistics views (page 406)

Selecting statistics for statistics views

Introduction

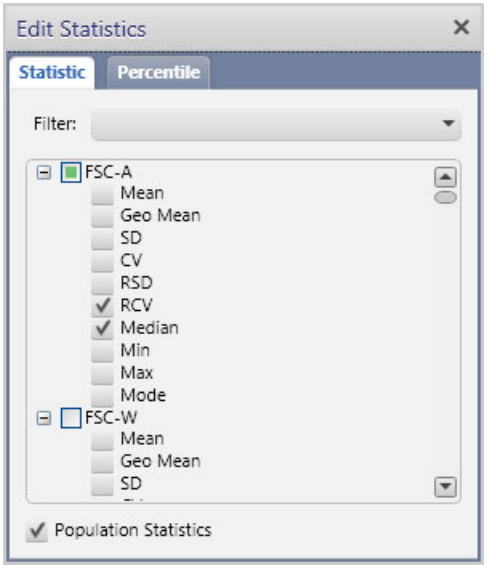
This topic describes how to select which statistics display in statistics views.

Procedure

To select which statistics to display in a statistics view:

1. Right-click the statistics view and select **Edit Statistics**.

   The **Edit Statistics** dialog opens.



2. Click the **Statistic** tab.

   Top-level parameters include parameters contained in the experiment.

3. Click a parameter in the tree to expand a parameter statistics category, then select the checkboxes for all of the statistics you want to display in the statistics view.

   You can filter the populations by typing a population name or part of a name, or multiple words in the **Filter** field. For example, if you type *Min,* all population names that contain *Min* are displayed in the list.

4. At the bottom of the dialog, select the **Population Statistics** checkbox to display the population statistics (Events, % Total, % Parent, % Grandparent) in the statistics view.

5. (Optional) Click the **Percentile** tab to edit the percentile formulas.

More information

- Selecting populations for statistics views (page 405)
- Editing and deleting percentile formulas for statistics (page 407)

Editing and deleting percentile formulas for statistics

Introduction

This topic describes how to edit or delete percentile formulas for a statistics view.

Use the Percentile tab to set a value for a percentile of event distribution in a plot.

Editing percentile formulas

To edit percentile formulas for percentile statistics:

1. Right-click the statistics view and select **Edit Statistics**.

2. Click the **Percentile** tab.
3. Click **Add** to add a new percentile formula.

4. In the **Percentile** column, type a value for the percentile (for example, *95)*.
5. (Optional) In the **Decimals** column, add or remove decimal places.

The new percentile is now available as a statistics function for all parameters. To display the percentile, click the **Statistic** tab and select the new percentile statistic.

Deleting existing percentile formulas

To delete an existing percentile formula from the table:

1. Click a row in the **Percentile** table.
2. Click **Delete**.

   The percentile formula is deleted.

More information

- Selecting statistics for statistics views (page 406)
- Adding expressions to statistics views (page 409)

Adding expressions to statistics views

Introduction

This topic describes how to display existing expressions in the statistics view header.

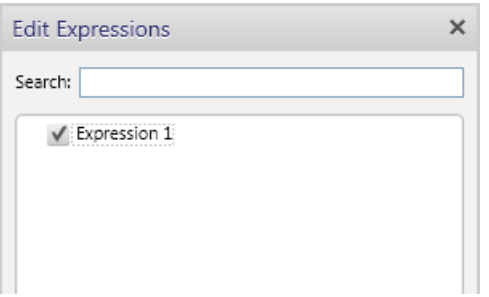
You must define expressions before you can add them to the statistics view.

Procedure

To display existing expressions in the statistics view header:

1. Right-click the statistics view and select **Edit Expressions**.

   The **Edit Expressions** dialog opens.

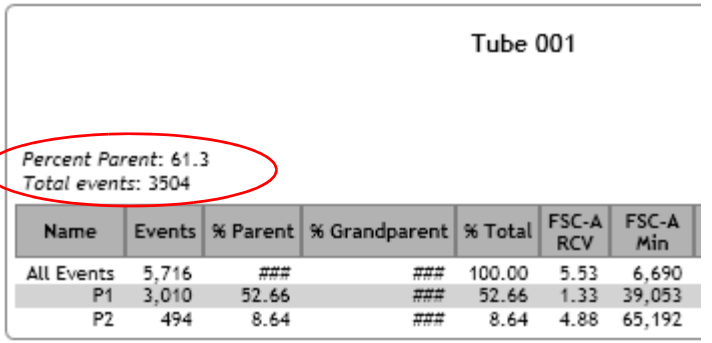


2. Select the checkboxes for all expressions you want to display in the statistics view header.

   You can filter the expressions by typing a name, part of a name, or multiple words in the **Filter** field.

3. Close the dialog to apply the changes.

The expressions are added to the statistics view.



Name	Events	% Parent	% Grandparent	% Total	FSC-A RCV	FSC-A Min
All Events	5,716	###	###	100.00	5.53	6,690
P1	3,010	52.66	###	52.66	1.33	39,053
P2	494	8.64	###	8.64	4.88	65,192

More information

- Modifying expressions (page 430)
- Editing and deleting percentile formulas for statistics (page 407)
- Adding keywords to statistics views (page 411)

Adding keywords to statistics views

Introduction

This topic describes how to display existing keywords in the statistics view header.

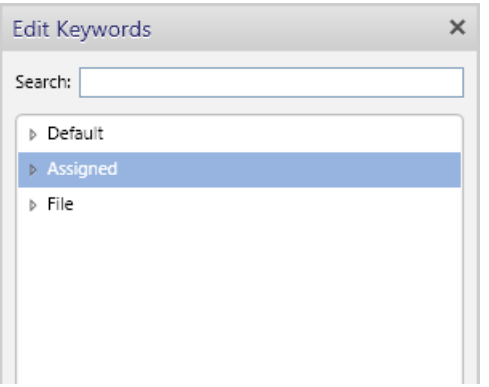
These keywords provide descriptions which help you identify the statistics on the worksheet or in a final report.

Procedure

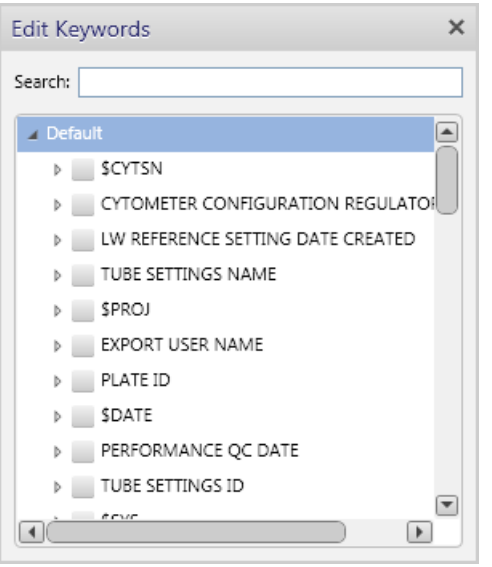
To select keywords to display in a statistics view:

1. Right-click the statistics view and select **Edit Keywords**.

   The **Edit Keywords** dialog opens.



2. Click a parameter in the tree to expand a keyword statistics category.

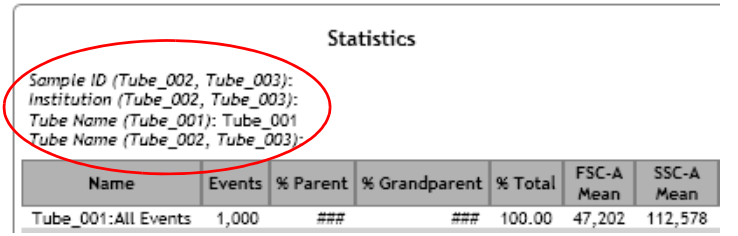


You can search the keywords by typing a name, part of a name, or multiple words in the **Search** field.

3. Select the checkboxes for all of the keywords you want to display in the statistics view header.

4. Close the dialog to apply the changes.

The keywords are added to the statistics view.



More information

- Adding expressions to statistics views (page 409)
- How gate modifications affect statistics views (page 413)

How gate modifications affect statistics views

Introduction

This topic describes how statistics for populations are affected when you make modifications to gates.

Effects of modifying gates

- If you modify the size, shape, or position of a gate, all statistics views (containing the population) display the new computed statistics for the modified gate, and dependent and descendant populations.
- If you modify a gate hierarchy, the statistics view updates to reflect the new hierarchical structure and all included populations.
- If you delete a gate from a plot, the population and its statistical data are removed from the statistics view.
- If you add or remove a layer from an overlay plot, or change the order of layers, the associated statistics are displayed in the same order as the layers displayed in the plot.

More information

- Adding keywords to statistics views (page 411)

Rearranging elements in a statistics view

Introduction

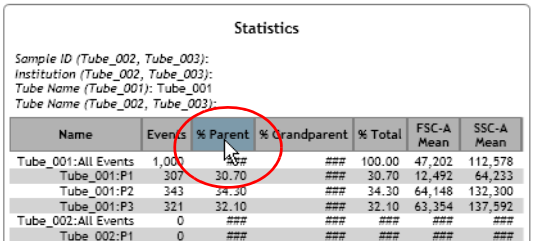
This topic describes how to rearrange elements in a statistics view.

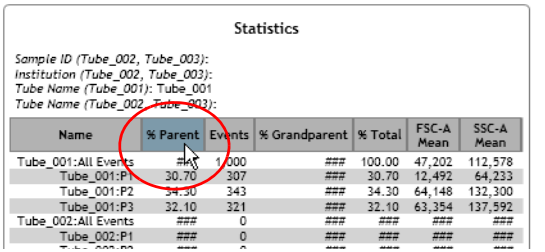
You can rearrange all columns except the Name column (when populations are displayed in the row headers).

Rearranging columns

To rearrange columns in a statistics view:

1. Drag a column header to a new location in the table and release the mouse button.



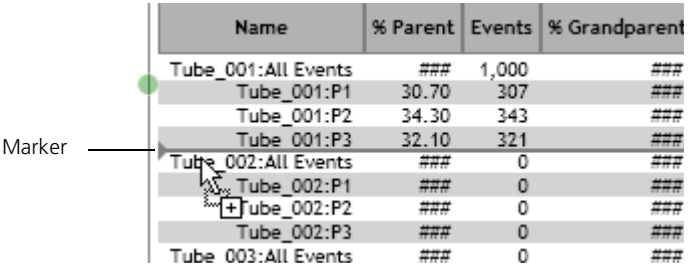


Rearranging rows

To rearrange the rows in a statistics view:

1. Click a row in the table and drag it to a new location.

A marker indicates the new target location.



2. Release the mouse button.

Rearranging header elements

To rearrange header elements:

1. Click an item in the header and drag it to a new position in the header.

   A marker indicates the target position.

2. Release the mouse to drop the item.

Displaying header elements in side-by-side lists

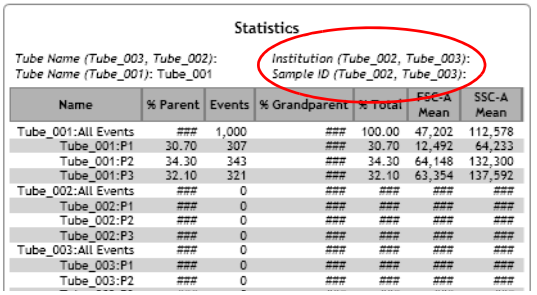
To display expressions and keywords in two side-by-side lists:

1. Click an item in the header and drag it to the right.

   A marker indicates the target position in the right list.

2. Release the mouse button.

   The header elements are moved to a second column.



More information

- Exporting statistics from statistics views (page 416)

Exporting statistics from statistics views

Introduction

This topic describes how to export statistics from a statistics view to a file. You can open the file in third-party software (for example, a spreadsheet application) and format the data as needed.

Procedure

To export statistics:

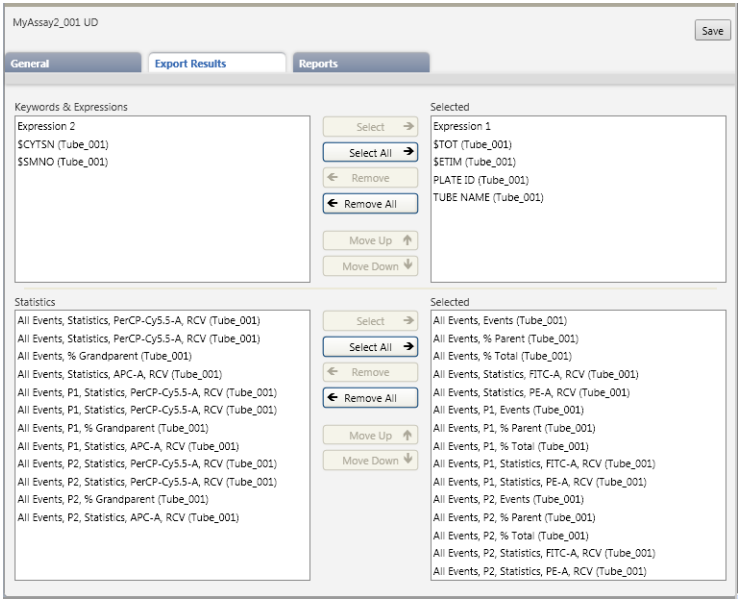
1. Click the statistics view on the worksheet (or report).
2. Right-click the statistics view and select **Export Statistics**.

   The **Save As** dialog opens.
3. In the **File name** field, type a specific export file name.
4. In the **Save as type** field, select a CSV (default) or XML file type.
5. Click **Save**.

Including statistics in user-defined assays

The exported results data for user-defined assays is displayed in the Library. You can view this data by opening the Library workspace, then selecting Assays > User-defined > Export Results tab.

The following figure shows the Export Results tab in a user defined assay in the Library.



The exported data that appears in the Keywords and Expressions and Statistics boxes must be defined in a statistics view in an experiment before you create the user-defined assay.

The following table describes the steps you need to perform to generate the keywords, expressions, and statistics data.

Step	Description
1	In an experiment worksheet or report, add a statistics view. See Working with statistics views (page 400) for more information.
2	In the statistics view, select the statistics and populations as needed. See Selecting populations for statistics views (page 405) and Selecting statistics for statistics views (page 406) for more information.
3	Add expressions and keywords to the statistics view header. See Adding expressions to statistics views (page 409) and Adding keywords to statistics views (page 411) for more information.
4	In the Statistics View Properties dialog, select the Include in Auto-Export checkbox. See Setting statistics view properties (page 401) for more information.
5	Create an assay from the experiment. See Creating a user-defined assay from an experiment (page 218) for more information.
6	Manage the exported results in the user-defined assay. See Editing results exporting properties (page 285) for more information.

More information

- Rearranging elements in a statistics view (page 414)
- Working with statistics views (page 400)

12

Expressions

This section includes detailed information about expressions and procedures that are not part of the daily workflow. Use this section to learn about building expressions.

This section includes the following topics:

Other related topics:

Expressions overview

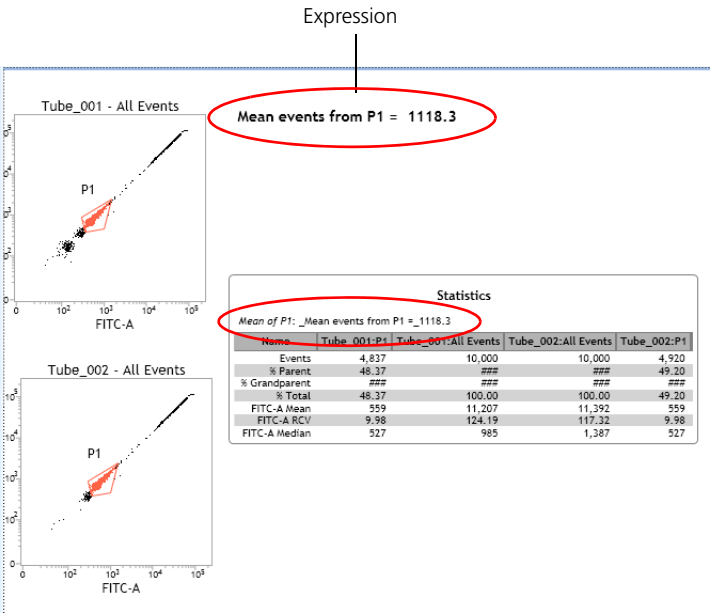
Introduction

This topic describes the BD FACSuite tools used to create custom expressions and add them to a worksheet or report.

About expressions

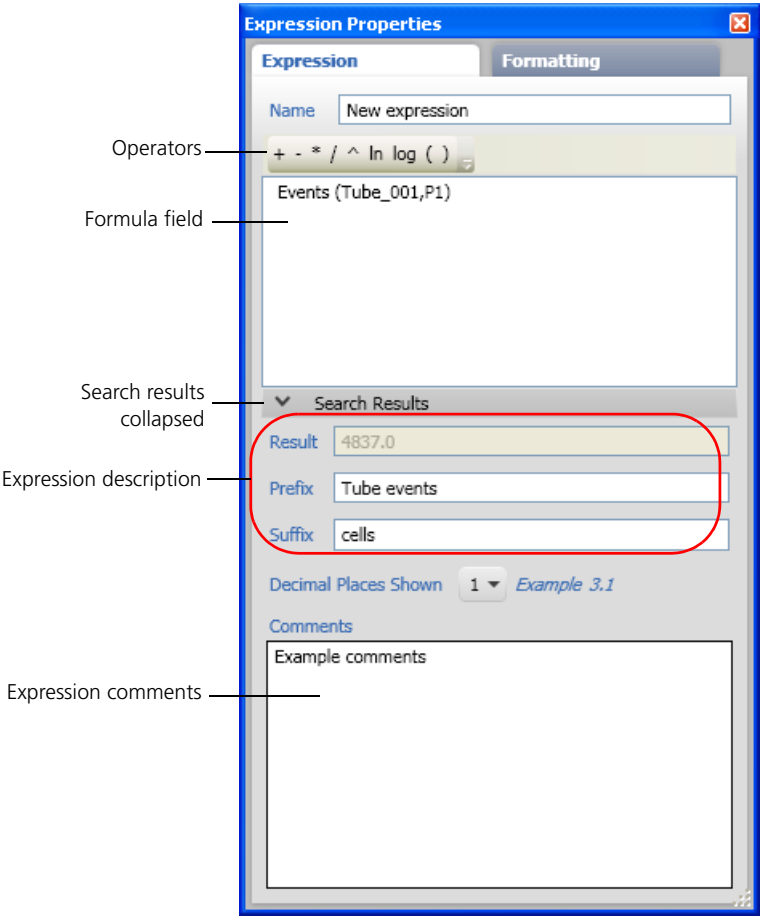
An expression is a mathematical formula made up of results and operators, including acquisition or analysis statistics, populations, keywords, constants, and other expressions.

Expressions are items on the worksheet, such as plots and statistics, and include a prefix, result, suffix, and a unique name (the key identifier of the expression). You can also add expressions to the header of the Statistics view or add them to a text box.



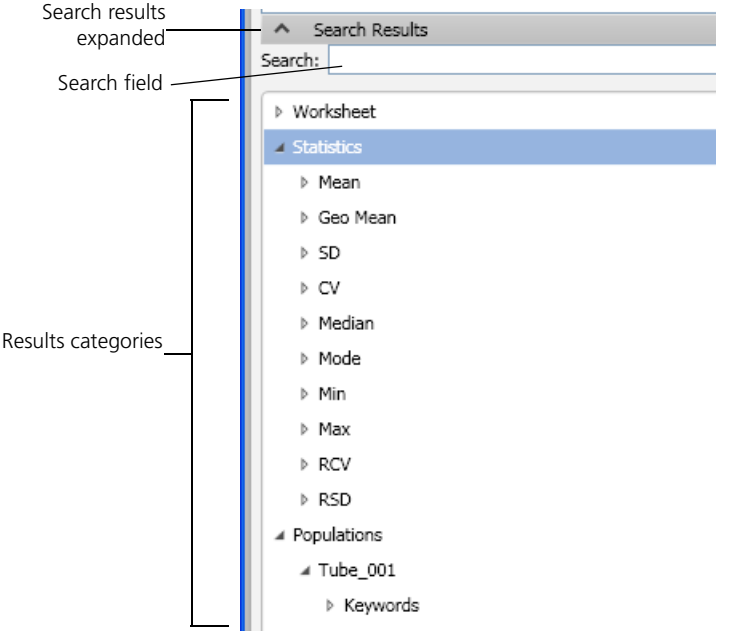
About the Expression Properties dialog

Use the Expression Properties dialog to create and edit expressions. The following figure shows the Expression Properties dialog with the Search Results panel collapsed.



The following figure shows the Expression Properties dialog with the Search Results panel expanded. Click to expand each category

to view and select options. You can also type a name in the Search field to locate specific results.



You can create and edit expressions in the worksheet to calculate results. When you create an expression, you can use results from following different categories.

Result category	Description
Worksheet	Displays the analysis elements that appear on the current worksheet or report. Results are grouped first by existing element type (expression, statistics, plots), then by specific elements that exist on the current worksheet or report. Note that you can also select statistics and expressions from their own results categories.
Statistics	Displays the analysis results grouped first by statistic (for example, mean, max, CV), then by tube, then by events parameter.
Populations	Displays the analysis results grouped first by population, then by tube, then by events.
Expressions	Displays the existing expressions in a worksheet or report (for example, Expression 1).
Constants	Displays constants (for example, pi and e).

More information

- Modifying expressions (page 430)
- Adding expressions to statistics views (page 409)

Building expressions

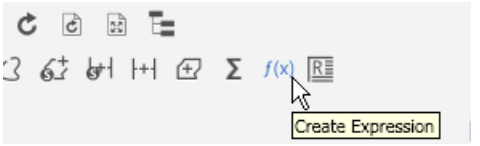
Introduction

This topic describes different ways to build expressions.

Building expressions from results

To build expressions from results:

1. In a worksheet, click **Create Expression** on the **Worksheet** toolbar.

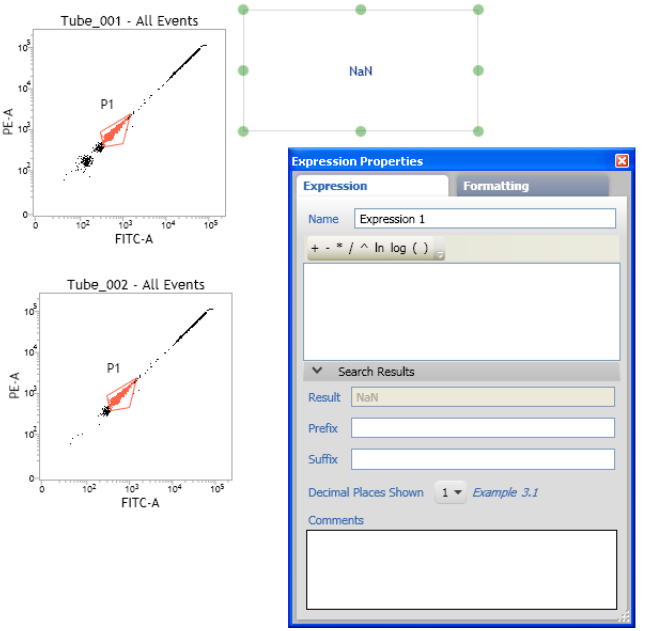


2. Click in the worksheet to create a new blank expression.

   The **Expression Properties** dialog opens.

   The default expression formula is empty and its result displays as *NaN* (not a number). If a formula is invalid and cannot be

calculated, the expression is displayed on the worksheet with a red background.

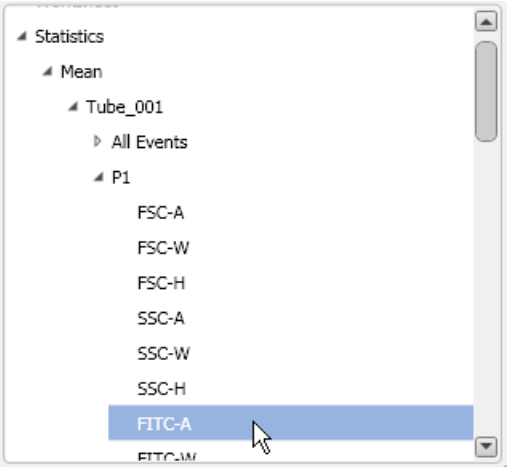


3. In the **Expression Properties** dialog, click **Search Results**.

4. Locate available results by performing one of the following actions:

   - Type your search string into the **Search** field.

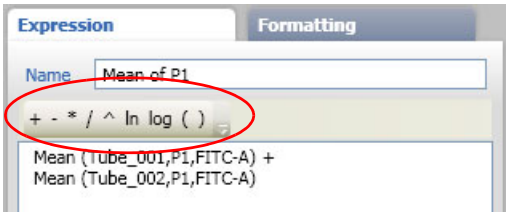
- Select a category from the **Results** tree, then expand the tree items to locate and select tubes, parameters, and populations.



- Locate specific statistics in a statistics view.

5. Create a formula using one of the following methods:
   - Select a result from the **Results** tree to add it to the **Formula** field.
   - Type numerical values and operators in the **Formula** field.
   - Drag statistics from a row in a statistics view into the **Formula** field.
6. Use operands on the toolbar to perform specific calculations.

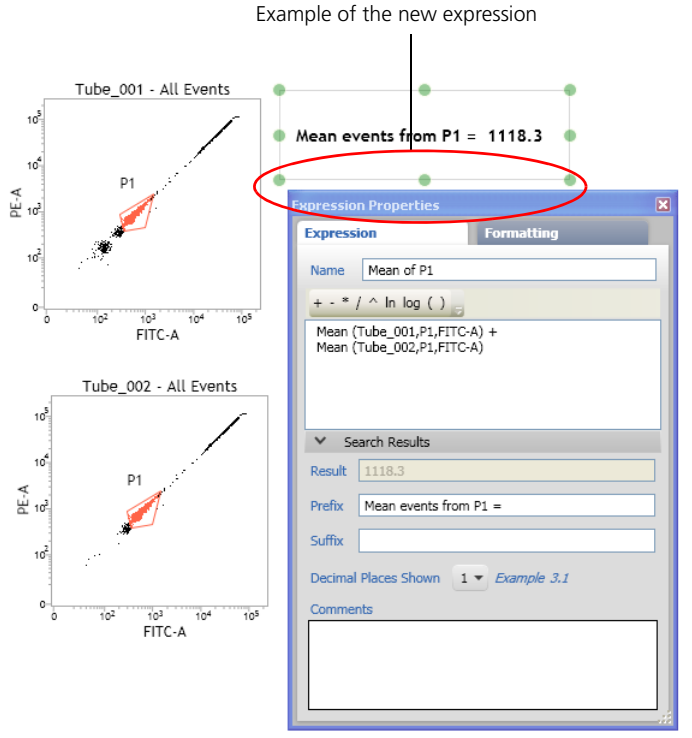
   Click the operator buttons to add them to the **Formula** field.



The calculated expression is displayed in the worksheet.

7. Click the **Search Results** arrow to display the expression details.
8. In the **Name** field, specify a name for this expression.

   The name must be unique within the experiment.
9. (Optional) Type in the **Prefix** or **Suffix** field to create a displayed name for the expression.
10. Specify the decimal place display in the **Decimal Places Shown** field.
11. Under **Comments**, add any comments that describe this expression.



12. Close the **Expression Properties** dialog to save the expression.

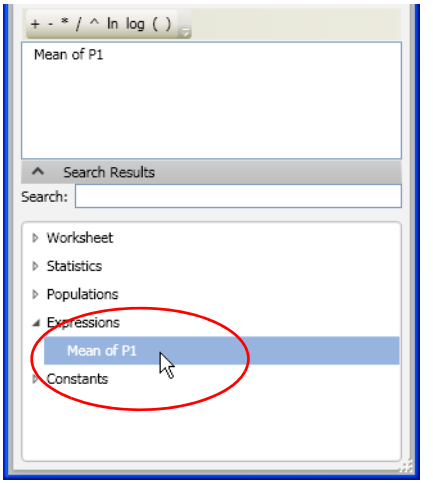
The process for building expressions from results is the same for all categories. The following examples provide more specific instructions.

Example: Building a compound expression formula

The following example describes how to create a basic expression that adds the mean of P1 from Tube 1 and Tube 2.

To build a compound expression by using results of another calculated expression:

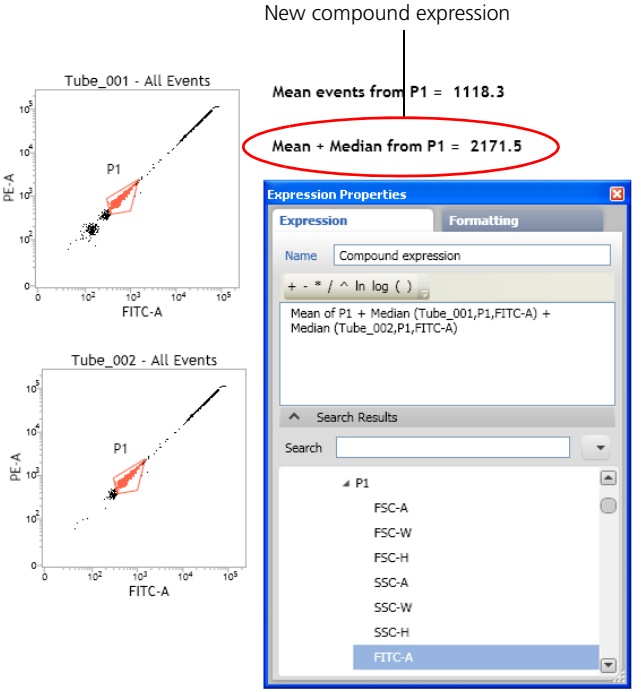
1. In the **Name** field, create a unique name for your expression.
2. In the **Prefix** field, type the text that you want to display on the worksheet (for example, *Mean + Median from P1)*.
3. Click **Search Results**.
4. In the **Results** tree, select **Expressions**.
5. Select an existing expression (for example, select *Mean of P1)*.



6. Select an operand (for example, click the plus sign (+)).
7. In the **Results** tree, select **Statistics**.

8. Select a statistical category, tube, parameter, and population (for example, select *Median* > *Tube_001* > *FITC-A* > *P1)*.
9. Repeat for additional tubes if needed.

   The selected result is displayed in the **Formula** field. The calculated compound expression is displayed in the worksheet.



   The formula updates each time the individual statistic changes and updates the expression.

More information

- Modifying expressions (page 430)

Modifying expressions

Introduction

This topic describes how to modify existing expression properties and how to resize, move, and delete expressions. This topic also describes how to modify text font, size, and weight.

Modifying existing expression properties

To edit existing expression properties:

1. Right-click an expression and select **Properties** to open the **Expression Properties** dialog.
2. Edit the fields.
3. Click **X** to save the properties and close the dialog.

Resizing an expression

To resize the expression display:

1. In a worksheet, click an expression.
2. Drag a handle to re-size the expression.

Moving an expression

To move an expression within a worksheet:

1. In a worksheet, drag the expression to a new location in the worksheet.

Deleting an expression

Before you delete an expression, make sure that it is not used elsewhere. Deleting an expression can invalidate all references in formulas, reports, and statistics views that depend on the deleted expression. This can include references that might exist in other worksheets.

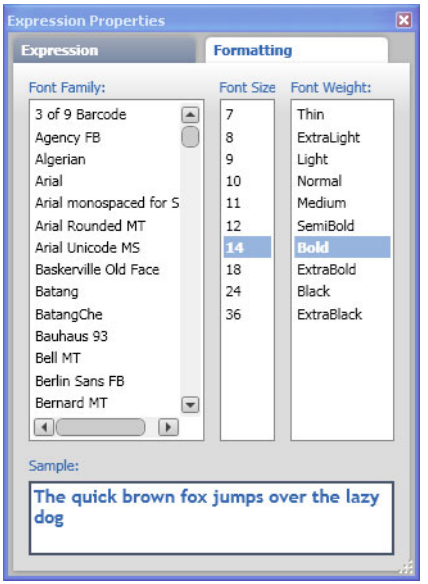
To delete an existing expression:

1. Right-click an expression and select **Delete Expression**.

Modifying the expression format

To modify the expression format:

1. In the **Expression Properties** dialog, click the **Formatting** tab.



2. Select a font, size, and weight for the expression.

More information

- Expressions overview (page 420)
- Building expressions (page 424)

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13

Data analysis example

This section includes the following topics:

Analyzing data acquired in an experiment

Introduction

This topic describes how to use BD FACSVerse System analysis tools to create plots, gates, and statistics views on an experiment worksheet or report and analyze the acquired data.

In this example

Since experiment analysis and gating strategies can be unique to each sample, laboratory, or operator, this example does not suggest an analysis strategy. Instead, it demonstrates how to use specific BD FACSVerse System tools to create a basic analysis worksheet and report.

This example uses one FCS file and fluorescence data collected from beads. This example compares and analyzes fluorescence data from four different parameters. You can substitute cells and different fluorescence parameters as needed.

Example workflow

The following table describes a basic example of an analysis workflow.

Stage	Description
1	Create or open an experiment in the Experiment workspace. See Creating and opening experiments (page 142) for more information.
2	Create a new report in the experiment. See Creating a new analysis report (page 436) for more information.
3	Acquire tubes, or add existing FCS files to the Data Source panel. See Selecting an FCS data source (page 437) for more information.
4	Create plots in the worksheet or report for each scatter or fluorescence parameter you want to analyze. See Creating plots for analysis (page 438) for more information.

Stage	Description
5	Draw gates to define the populations you want to analyze. See Defining populations for analysis (page 440) for more information.
6	Display or isolate specific populations. See Showing the populations of interest (page 444) for more information.
7	Create a Statistics view in the worksheet or report and add statistics and populations. See Creating a statistics view (page 446) for more information.
8	Format the report by adding headers, footers, text, then print or export the report. See Formatting and printing a report (page 448) for more information.

More information

- Selecting an FCS data source (page 437)
- Analyzing multiple FCS files in an experiment (page 451)

Creating a new analysis report

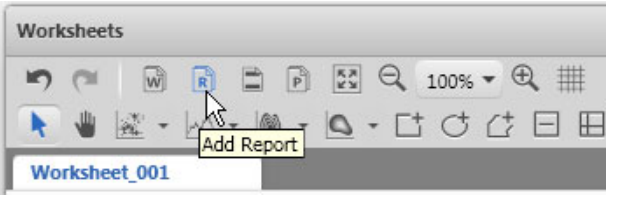
Introduction

This topic describes how to create a new analysis report and rename it.

Procedure

To create a new analysis report:

1. In an experiment, click the **Add Report** tool on the **Worksheet** toolbar.



   A new **Report** tab opens.

2. Click the text in the **Report** tab, then type a new name (for example, *Example Report_001*).

   The report is automatically saved with the experiment.

Next step

Selecting an FCS data source (page 437)

More information

- Creating plots for analysis (page 438)
- Analyzing data acquired in an experiment (page 434)

Selecting an FCS data source

Introduction

This topic describes how to select an FCS file as the data source for plots in a report. This example uses an FCS file created by acquiring a tube of beads. You can substitute any FCS file as needed.

Procedure

To select an FCS data source:

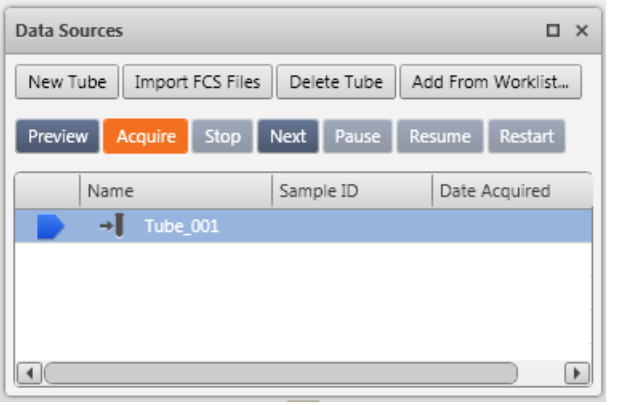
1. In the **Data Sources** panel, click **Import FCS Files**.

   The **Open** dialog opens.

2. Navigate to the folder that contains your FCS files.

3. Select an FCS file, then click **Open**.

   A new (filled) tube is created for the FCS file in the **Data Sources** panel.



   The run pointer indicates the current (selected) FCS file.

Next step

Creating plots for analysis (page 438)

More information

- Creating a new analysis report (page 436)
- Analyzing data acquired in an experiment (page 434)

Creating plots for analysis

Introduction

This topic describes how to create plots for analysis in a report.

Procedure

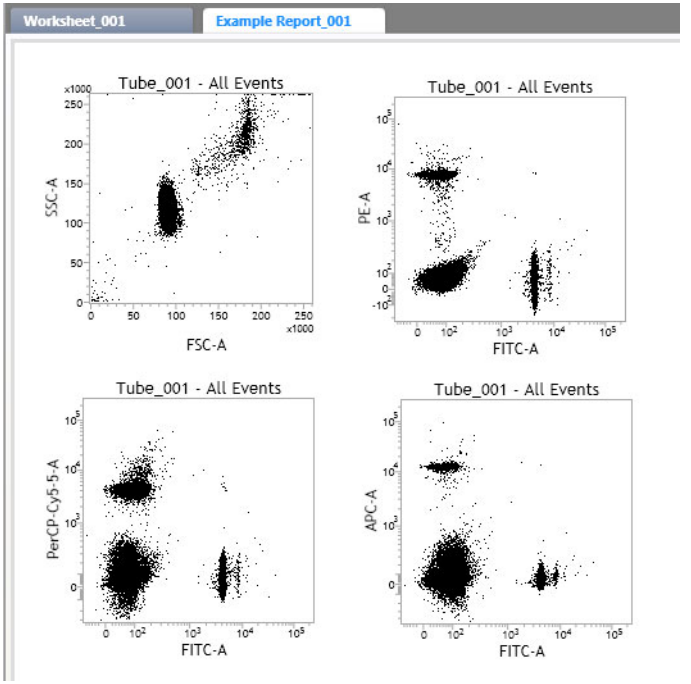
To create plots for analysis:

1. Create four dot plots on the report.
   a. Click the **Dot Plot** tool on the **Worksheet** toolbar.
   b. Click in the report to create a plot.
2. Change the X and Y parameters for each plot.

   In this example, the following parameters are used for the plots on the analysis report:

   - FSC vs SSC
   - FITC vs PE
   - FITC vs PerCP-Cy5.5
   - FITC vs APC

Your report should look like the following example.



Note that the FSC vs SSC plot is displayed in linear scale (by default). Subsequent plots are displayed in biexponential scale (by default).

Next step

After you create the plots with data, you need to draw gates to define the populations of interest. See Defining populations for analysis (page 440).

More information

- Analyzing data acquired in an experiment (page 434)
- Selecting an FCS data source (page 437)

Defining populations for analysis

Introduction

This topic describes how to draw and name gates to define populations in an analysis plot in a report.

Before you begin

You need to create the plots with data so you can draw gates to define and identify the populations of interest.

Procedure

To define populations for analysis:

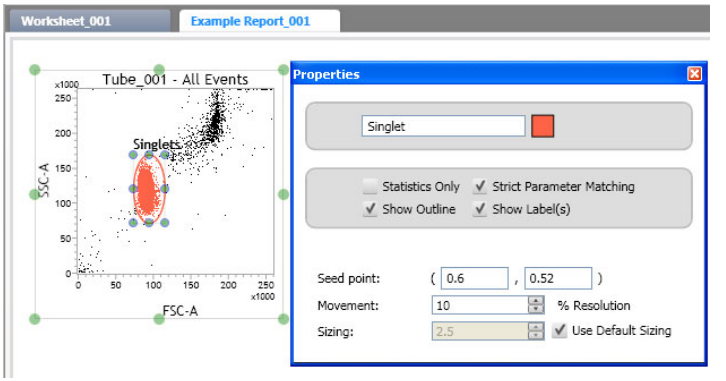
1. In the FSC vs SSC plot, draw a gate around the singlets.

   See Drawing gates in plots (page 164) for more information.

2. Right-click the gate and select **Properties**.

   The **Properties** dialog opens.

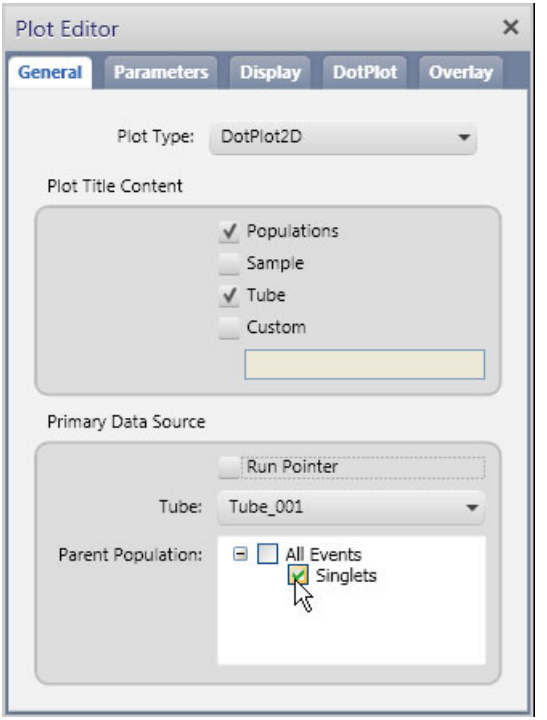
3. In the **Name** field, type *Singlets*, then press **Enter**.



4. Right-click the plot and select **Properties**.

   The **Plot Editor** dialog opens.

5. In the **General** tab, under **Parent Population**, select the **Singlets** checkbox to display only singlet events in the selected plot.



6. Close the **Plot Editor** dialog.
7. Repeat steps 4 through 6 for the remaining plots.
8. In the FITC vs PE plot, create gates to define the FITC-positive and PE-positive populations, then name the populations.

   For example, complete the following steps:

   a. Select the plot, then click a gate tool on the **Worksheet** toolbar and draw a gate around a positive population.

   b. Right-click the gate and select **Properties**.

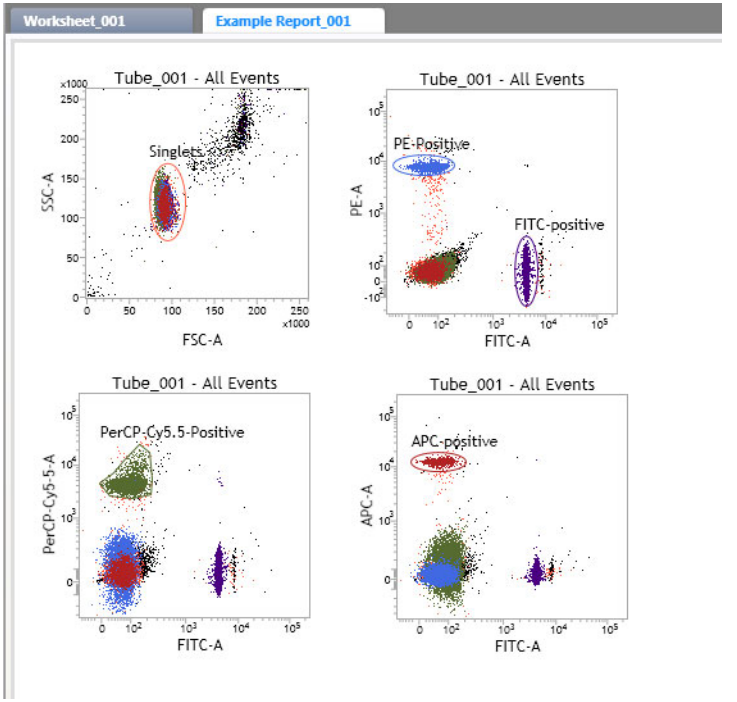
   c. Type a name (for example, *FITC-positive*), then close the dialog to rename the population.

d. Repeat steps a through c for the other positive populations.

9. In the FITC vs PerCP-Cy5.5 plot, create gates to define the PerCP-Cy5.5 positive population, then name the population.

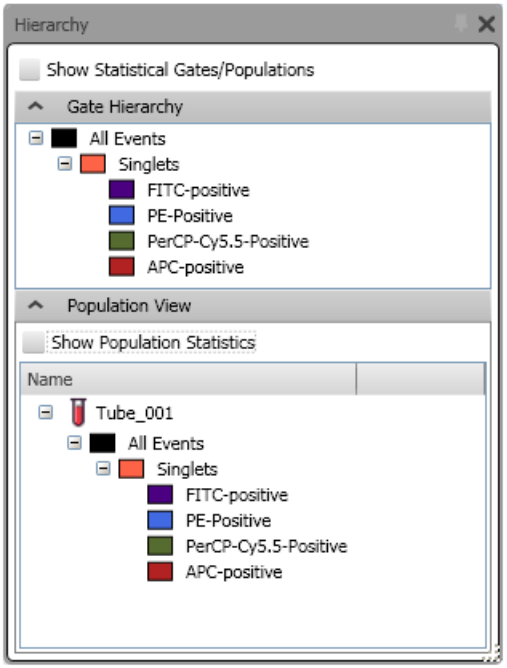
10. In the FITC vs APC plot, create gates to define the APC-positive population, then name the population.

    Your report should look like the following example.



11. Click **Display Hierarchy** on the **Worksheet** toolbar.

The gate hierarchy should look like the following example.



Next step

After you draw gates, define, and identify the populations, you can isolate the populations in each plot. See Showing the populations of interest (page 444).

More information

- Analyzing data acquired in an experiment (page 434)
- Creating plots for analysis (page 438)
- Drawing gates in plots (page 164)

Showing the populations of interest

Introduction

This topic describes how to show the populations of interest in a report. Note that if you want to show both a negative and a positive population, you need to create gates for both populations.

Before you begin

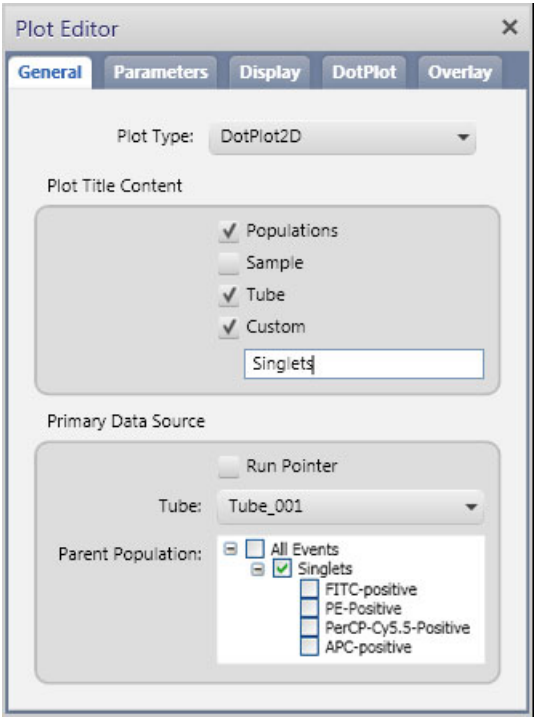
You need to draw gates, define, and identify the populations, before you can isolate the populations in each plot.

Procedure

To show the populations of interest:

1. Right-click the FSC vs SSC plot, then select **Properties**.

   The **Plot Editor** dialog opens.



2. Under **Plot Title Content,** verify that the **Tube** and **Populations** checkboxes are selected.

   This displays their names in the plot titles.

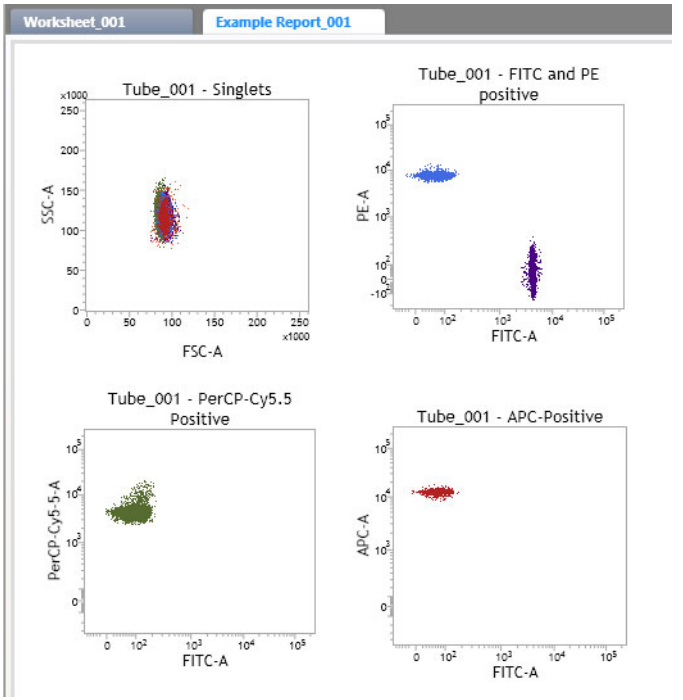
3. Under **Primary Data Source,** select the **Singlets** checkbox in the **Population**(s) tree.

   This displays only the singlet population in the selected plot and statistics view.

4. Close the **Plot Editor** dialog.

5. Repeat steps 2 and 3 for each plot to select only the positive population to display in each plot.

   Your report should look like the following example.



Next step

Create a statistics view for each plot to display the population statistics. See Creating a statistics view (page 446).

More information

- Analyzing data acquired in an experiment (page 434)
- Defining populations for analysis (page 440)

Creating a statistics view

Introduction

This topic describes how to create a statistics view in a report and how to view the gate and population hierarchies.

Before you begin

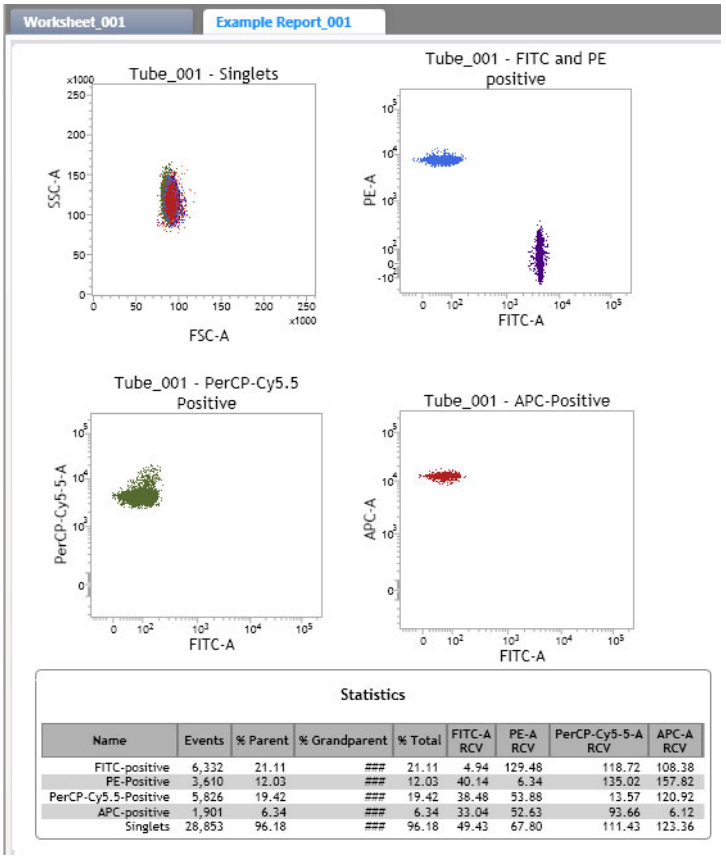
You should define and isolate populations in each plot before you create a statistics view to display the population statistics for all plots.

Creating a statistics view

To create statistics views for all plots in the report:

1. Click the **Statistics** tool on the **Worksheet** toolbar.
2. Click in the worksheet or report to add the statistics view.
3. Drag the statistics view below a plot on the report.
4. Right-click the statistics view and select **Edit Populations**.
5. In the **Edit Populations** dialog, select all of the populations you want to include in the statistics view, then close the dialog.
6. Right-click the statistics view and select **Edit Statistics**.
7. In the **Edit Statistics** dialog, select the statistics you want to display for each parameter (for example, RCV), then close the dialog.

The statistics view updates and displays the selected populations and statistics.



Statistics

Name	Events	% Parent	% Grandparent	% Total	FITC-A RCV	PE-A RCV	PerCP-Cy5-5-A RCV	APC-A RCV
FITC-positive	6,332	21.11	###	21.11	4.94	129.48	118.72	108.38
PE-Positive	3,610	12.03	###	12.03	40.14	6.34	135.02	157.82
PerCP-Cy5.5-Positive	5,826	19.42	###	19.42	38.48	53.88	13.57	120.92
APC-positive	1,901	6.34	###	6.34	33.04	52.63	93.66	6.12
Singlets	28,853	96.18	###	96.18	49.43	67.80	111.43	123.36

Next step

Add report headers and footers, and format the report before you print it. See Formatting and printing a report (page 448).

More information

- Analyzing data acquired in an experiment (page 434)
- Showing the populations of interest (page 444)

Formatting and printing a report

Introduction

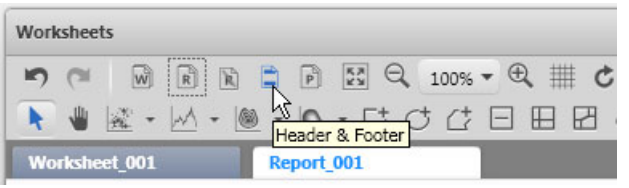
This topic describes how to format a report and set header and footer properties.

You can customize the header and footers to include specific information and images, and create a custom report layout. Once you have an appropriate format, you can print the report.

Formatting headers and footers

To format report headers and footers:

1. Open an experiment or assay report.
2. Click the **Header & Footer** tool on the **Worksheet** toolbar.



   The **Header/Footer** dialog opens.
3. Set the cursor in the upper left corner of a header (Left, Center, or Right section).
4. Under **Elements,** double-click an element you want to add to the header section (for example, *Cytometer Name)*.

   Note that you can type custom text in any of the sections if you prefer.
5. Continue to add header elements or type text in each section as needed.
6. Set the cursor in the bottom left corner of a footer (Left, Center, or Right section).
7. Continue to add footer elements or type text in each section as needed.

The **Preview** box shows the layout of the elements you selected.

8. Once you have added elements to the report, you can perform the following optional actions.

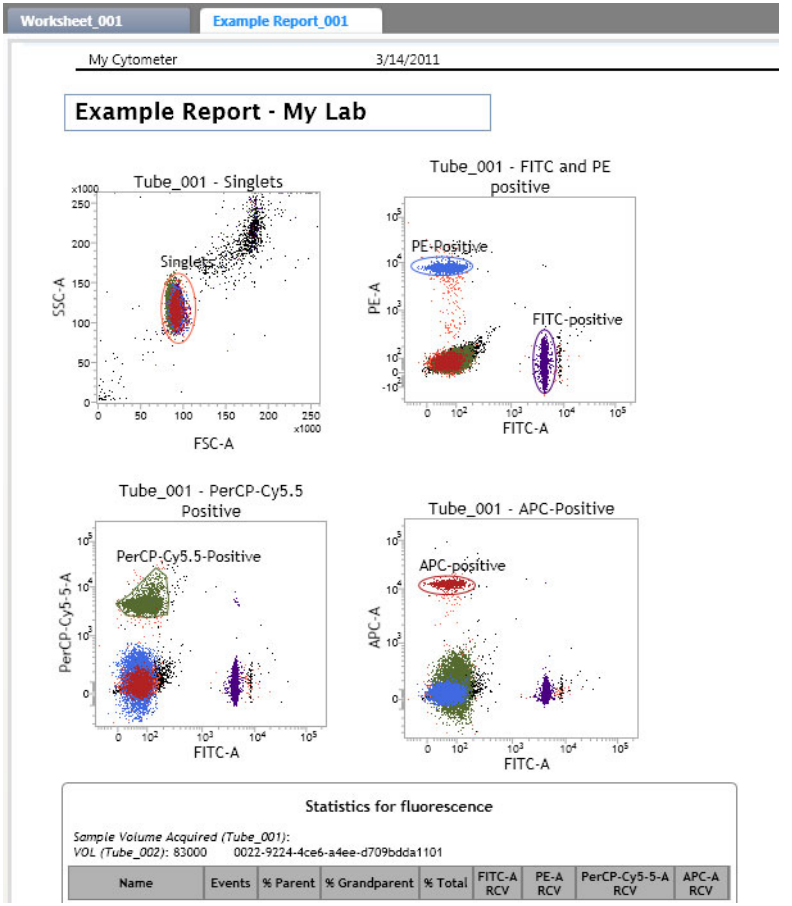
To...	Then do this...
Move elements from one section to another	Drag elements from one section to another section.
Delete elements from the report header or footer	Click an element in a section and press **Delete** to delete it from the section.

9. You can also perform the following optional formatting actions.

To...	Then do this...
Display a border around the elements in the header or footer	Select the **Show Border** checkbox.
Add a logo or other image to the report	1. Click **Add Image.** The **Add Image** dialog opens. 2. Navigate to the folder that contains the image, then click **Add**. The image is added to the section, and the header or footer is displayed under **Preview**.
Format the report text	Select a font, font size, color, or style for all the text in the report.

10. Click **OK**.

The report layout is displayed.



Printing the analysis report

To print the analysis report:

1. Click the **Report** tab.
2. From the menu bar, select **File > Print**.
3. Preview the report and make sure that all analysis items are in the print area of the page.
4. Click **Print** on the **Print Preview** bar.
5. Complete your typical printing process.

More information

- Analyzing data acquired in an experiment (page 434)
- Creating plots in a worksheet (page 158)
- Drawing gates in plots (page 164)
- Analyzing multiple FCS files in an experiment (page 451)

Analyzing multiple FCS files in an experiment

Introduction

This topic describes how to perform analysis on data from multiple acquired tubes (FCS files) in a single experiment by creating separate report pages for each tube.

Before you begin

You need to have acquired data from more than one tube.

Procedure

To analyze data from multiple FCS files in an experiment:

1. Open an experiment and create and format a report.
2. In the **Data Sources** panel, click **Import FCS Files**.

   The **Open** dialog opens.
3. Select the FCS files you want to add, then click **Open**.

   New (filled) tubes are added for the FCS files in the **Data Sources** panel.
4. Click the **Report** tab.
5. Click **Add Page** on the **Worksheet** toolbar.
6. Set the run pointer to a tube in the **Data Sources** panel.
7. On a report page, create plots based on the new FCS file.
   - If you are using the same fluorochromes in all tubes (FCS files), create plots on the same page of the report.

- If you are using different fluorochromes for each tube, set up each plot with the specific fluorochrome parameters that match the FCS file parameters. Consider adding report pages and organizing plots by fluorochrome or by tube.

After you add a report page for each FCS file you want to analyze, you can optimize the report display by adjusting the display percentage so you can view the pages side by side, magnifying the view, or changing the report orientation to view reports vertically.

See Modifying experiment worksheets and reports (page 208) for more information.

More information

- Creating a new analysis report (page 436)
- Formatting and printing a report (page 448)
- Creating plots in a worksheet (page 158)
- Drawing gates in plots (page 164)
- Analyzing data acquired in an experiment (page 434)

14

Keywords

This section includes the following topics:

BD FACSuite generated FCS keywords

Introduction

This topic describes the FCS keywords generated by BD FACSVerse System. These keywords are used with the FCS 3.0 format.

Keywords

Keyword	Description
$BEGINANALYSIS	Begin analysis segment offset
$BEGINDATA	Begin data segment offset
$BEGINSTEXT	Begin text segment offset
$BYTEORD	Byte order
$DATATYPE	Floating point data type
$ENDANALYSIS	End analysis segment offset
$ENDDATA	End data segment offset
$ENDTEXT	End text segment offset
$MODE	List-mode data
$NEXTDATA	Offset to next data set
$PAR	Number of parameters for acquired data
$PnB	Bits for parameter *n*
$PnE	Amplification type for parameter *n*
$PnR	Range of parameter *n*
$TOT	Number of events

More information

- Modifying tube properties (page 167)
- Assigning keywords to entries and tubes (page 243)
- Understanding keywords (page 297)

- Importing and exporting keywords (page 298)
- Working with keywords in the library (page 299)

Optional FCS keywords

Introduction

This topic describes FCS 3.0 keywords that are optional.

Keywords

Keyword	Description
$BTIM	Time at beginning of acquisition
$CYT	Flow cytometer type
$CYTSN	Flow cytometer serial number
$DATE	Acquisition date
$ETIM	Time at end of acquisition
$EXP	Creator of experiment or worklist
$FIL	FCS file name
$INST	Institution
$OP	Operator
$PnN	Short name of parameter *n*
$PnS	Name of parameter *n*
$PnV	Voltage of parameter *n*
$PROJ	Name of experiment or worklist
$SMNO	Tube ID
$SRC	Sample ID
$SYS	Computer type and operating system
$TIMESTEP	Time step

More information

- Modifying tube properties (page 167)
- Assigning keywords to entries and tubes (page 243)
- Understanding keywords (page 297)
- Importing and exporting keywords (page 298)
- Working with keywords in the library (page 299)

BD created keywords

Introduction

This topic describes the BD created keywords.

Keywords

Keyword	Description
ADDRESS1	Address (for example, patient, or institution)
ADDRESS2	Address (for example, patient, or institution)
ADDRESS3	Address (for example, patient, or institution)
ADDRESS4	Address (for example, patient, or institution)
APPLY COMPENSATION	Compensation enabled
CHARSET	Character set
CREATOR	Application name and version number that created the data file
DIRECTOR	Director name (for example, for the institution, department, or study)
EXPORT TIME	Export time stamp

Keyword	Description
EXPORT USER NAME	Export user name
FLUIDICS MODE	Current fluidics mode
FSC ASF	FSC area scaling factor
GUID	Unique ID for an exported FCS file
LASERnNAME	Name of laser *n*
LASERnDELAY	Laser delay for laser *n*
LASERnASF	Area scaling factor for laser *n*
PLATE ID	Plate ID for manually loaded tube (keyword is not written to the FCS file)
AUTOBS	Auto Biexponential Scale enabled
PnBS	R-value for parameter *n*
PnDISPLAY	Display for parameter *n*
PnMS	Manual R-values for parameter *n*
SPILL	Spillover matrix
THRESHOLD	Threshold
TUBE NAME	Tube name
VOL	Sample volume consumed during acquisition (using the BD Flow Sensor)
WELL ID	Well ID for manually loaded tube (keyword is not written to the FCS file)
WINDOW EXTENSION	Window extension value

More information

- Modifying tube properties (page 167)
- Assigning keywords to entries and tubes (page 243)
- Understanding keywords (page 297)
- Importing and exporting keywords (page 298)
- Working with keywords in the library (page 299)

Setup and QC keywords

Introduction

This topic describes the setup and QC keywords.

Keywords

Keyword	Description
TUBE SETTINGS NAME	Name of the tube settings that were used when data was acquired
TUBE SETTINGS ID	ID of the tube settings that were used when data was acquired
CYTOMETER CONFIGURATION NAME	Name of the cytometer configuration that was current at the time data was acquired
CYTOMETER CONFIGURATION DATE CREATED	Date and time when the cytometer configuration was created
CYTOMETER CONFIGURATION DATE MODIFIED	Date and time when the cytometer configuration was last modified
CYTOMETER CONFIGURATION REGULATORY STATUS	Regulatory status of the current cytometer configuration at the time data was acquired
CST BEAD LOT ID	Lot ID of the CS&T beads that were used during the last performance QC

Keyword	Description
CST BEAD LOT EXPIRATION DATE	Expiration date of the CS&T beads that were used during the last performance QC
CST BEAD LOT REGULATORY STATUS	Regulatory status of the CS&T beads that were used during the last performance QC
CHARACTERIZATION QC DATE	Date and time when characterization QC was last run for the cytometer configuration that was current at the time data was acquired
PERFORMANCE QC DATE	Date and time when performance QC was last run for the cytometer configuration that was current at the time data was acquired
PERFORMANCE QC STATUS	Status of the last performance QC for the cytometer configuration that was current at the time data was acquired. Possible values: Pass, Fail.
PERFORMANCE QC ERROR MESSAGEn	Performance QC Error Message *n*
PERFORMANCE QC WARNING MESSAGEn	Performance QC Warning Message *n*
LW REFERENCE SETTING DATE CREATED	Date and time when lyse/wash reference settings were created
LW REFERENCE SETTING DATE MODIFIED	Date and time when lyse/wash reference settings were last updated
LNW REFERENCE SETTING DATE CREATED	Date and time when lyse/no-wash reference settings were created
LNW REFERENCE SETTING DATE MODIFIED	Date and time when lyse/no-wash reference settings were last updated

More information

- Modifying tube properties (page 167)
- Assigning keywords to entries and tubes (page 243)
- Understanding keywords (page 297)
- Importing and exporting keywords (page 298)
- Working with keywords in the library (page 299)

CBA keywords

Introduction

This topic describes the keywords used when you run CBA assays with BD FACSuite software. These keywords are used with FCAP Array software to build plexes.

See the *FCAP Array Software User's Guide* for more information.

Keywords

Keyword	Description
CBA PLEX NAME	The name of a plex. Used as part of the information transfer to FCAP Array software.
CBA TYPE	Identifies the sample type as either standard or control. Used as part of the information transfer to FCAP Array software.
CBA STANDARD ID	The number of the standard. The value can also be "Pos" or "Neg" to identify positive or negative populations for qualitative CBA assays. Used as part of the information transfer to FCAP Array software.

Keyword	Description
CBA CONTROL ID	ID of the control in the tube or well. Used as part of the information transfer to FCAP Array software.
CBA DILUTION	Used to specify the dilution of the sample. Used as part of the information transfer to FCAP Array software.

More information

- Modifying tube properties (page 167)
- Assigning keywords to entries and tubes (page 243)
- Understanding keywords (page 297)
- Importing and exporting keywords (page 298)
- Working with keywords in the library (page 299)

Keywords used by BD FACSuite software

Introduction

This topic describes the keywords used by BD FACSuite software.

Keywords

Keyword	Description
$SRC	The value for this keyword is assigned to the sample ID of the entry.
$SMNO	The value for this keyword is assigned to the tube ID of the entry. If this keyword is not available, the Tube ID of the entry is blank.
$PnN	The value for this keyword is parsed and assigned to the parameter name. If this keyword is not available, the parameters is named “Pn.”

Keyword	Description
$PnS	The value for this keyword is parsed and used to determine the labels of the parameters. If this keyword is not available, the labels for the parameters are blank.
$PnV	The value for this keyword is parsed and assigned to the voltage of parameter. If this keyword is not available, the voltage for the parameter is 0.
$TIMESTEP	This value is used to determine the step for the time parameter. If this keyword is not available, BD FACSuite software assumes a time step of 0.01.
APPLY COMPENSATION	This value for this keyword is parsed and assigned to the flag for applying compensation for the tube. If this keyword is not available, compensation for the tube is disabled.
AUTOBS	Auto Biexponential Scale enabled
CHARSET-AUTOBS	Character set for Auto Biexponential Scale
PnBS	R-value for parameter *n*
PnDISPLAY	Display for parameter *n*
PnMS	Manual R-values for parameter *n*

Keyword	Description
SPILL	The value for this keyword is parsed and assigned to the spillover matrix for the tube. If this keyword is not available in an FCS 3.0 file, the spillover matrix for the tube uses the default matrix.
THRESHOLD	The value for this keyword is parsed and assigned to the threshold for the tube. If this keyword is not available, the threshold for the tube is disabled.
TUBE NAME	The value if this keyword is assigned to the tube name of the entry. If this keyword is not available, the tube name of the entry uses the default tube name creation for the entries.

More information

- Modifying tube properties (page 167)
- Assigning keywords to entries and tubes (page 243)
- Understanding keywords (page 297)
- Importing and exporting keywords (page 298)
- Working with keywords in the library (page 299)

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Part 4: System reference

This part includes the following sections:

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Maintenance

This section includes the following topics:

Maintenance overview

Introduction

This topic provides a list of daily, unscheduled, and scheduled maintenance.

Daily maintenance

Daily maintenance is part of the shutdown procedure.

Procedure	When
Running the daily clean procedure (page 469)	Daily
Performing manual system shutdown (page 113)	Daily

Unscheduled maintenance

The following table lists unscheduled maintenance that you might have to perform.

Procedure	When
Refilling the sheath tank (page 471)	As needed
Emptying the waste tank (page 473)	As needed
Using the fluidics commands (page 482)	As needed
Cleaning the manual tube port (page 483)	As needed
Preparing for long term shutdown (page 484)	As needed
Replacing the sample line (page 485)	As needed
Replacing the sample line in a system with a flow sensor (page 491)	As needed

Scheduled maintenance

Scheduled maintenance should be performed according to the following table.

Procedure	When
Performing the monthly clean procedure (page 475)	Monthly
Replacing the sheath filters (page 478)	Every 3 months

Running the daily clean procedure

Introduction

This topic describes how to run the daily clean procedure. This procedure is included when you perform system shutdown. You can also use this procedure to clean the system whenever it is needed.

Required materials

- 2 mL of 10% bleach solution
- 3 mL of DI water

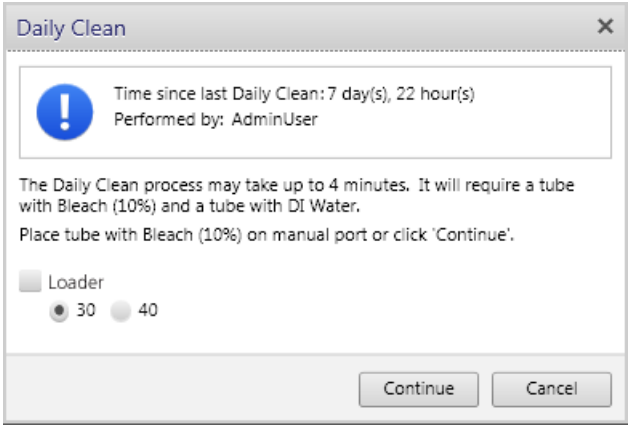
Procedure

Caution! Do not use the same tube repeatedly for DI water or bleach during the daily clean procedure. Repeated use can cause wear on the tube, and resulting particles can damage the tube sensor in the manual tube port.

To run the daily clean procedure:

1. From the menu bar, select **Cytometer > Daily Clean**.

The following dialog opens.



2. Place a tube containing 2 mL of 10% bleach solution on the manual tube port, then click **Continue**.
3. When prompted, place a tube containing approximately 3 mL of DI water on the manual tube port, then click **Continue**.

   The dialog closes when the process is complete.

Note: You must complete this entire procedure. If the procedure is interrupted or not completed, the system prevents any other actions from happening. This is to avoid the possibility of bleach remaining in the fluidics path.

More information

- Fluidics components (page 28)
- Performing manual system shutdown (page 113)

Refilling the sheath tank

Introduction

This topic describes how to check the sheath fluid level, illustrates sheath tank components, and describes how to refill the sheath tank.

The sheath and waste tanks must be placed at a level even with, or below, the cytometer. Placing the tanks higher than the cytometer can cause uncontrolled siphoning.

Checking the sheath fluid level

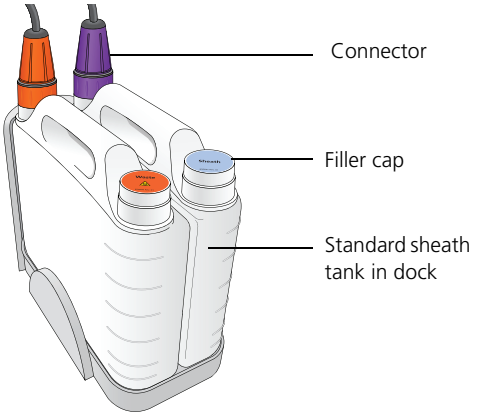
The standard sheath tank is translucent so you can visually check the fluid level. In addition, a message in the software alerts you when the tank is close to empty and starts a 10-minute timer. You must refill the tank before the 10 minutes elapses to avoid acquisition being interrupted. The system stops operation when the timer expires.

Required materials

- BD FACSFlow sheath fluid to fill the sheath tank (5 L or 10 L, depending on which tank is being used)

Sheath tank components

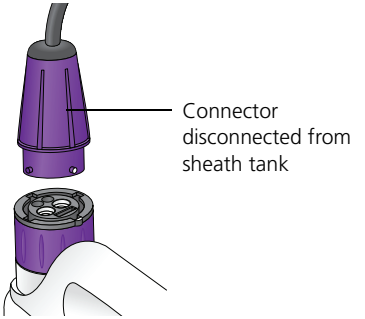
The following figure shows the parts of the standard sheath tank.



Procedure

To refill the sheath tank:

1. Disconnect the connector from the sheath tank by turning it counter-clockwise.



2. Remove the sheath tank from the dock and take it to a filling station.
3. Remove the filler cap and fill the tank with BD FACSFlow sheath fluid.

   Do not use sheath fluid with surfactant.
4. Re-install the filler cap and place the tank in the dock.
5. Re-install the connector and turn clockwise to tighten it.

More information

- Replacing the sheath filters (page 478)
- Fluidics components (page 28)
- Emptying the waste tank (page 473)

Emptying the waste tank

Introduction

This topic describes how to check the waste tank level, illustrates the waste tank components, and describes how to empty the waste tank.

The sheath and waste tanks must be placed at a level even with, or below, the cytometer. Placing the tanks higher than the cytometer can cause uncontrolled siphoning.

Checking waste tank level

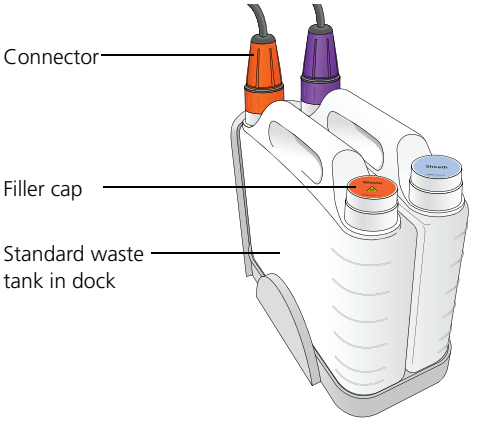
The standard waste tank is translucent so you can visually check the fluid level. In addition, a message in the software alerts you when the tank is close to full, and starts a 10-minute timer. If the tank is not emptied within 10 minutes, the system prevents further operation.

Required materials

- Enough bleach solution to equal 10% of volume of waste tank

Waste tank components

The following figure shows the parts of the standard waste tank.

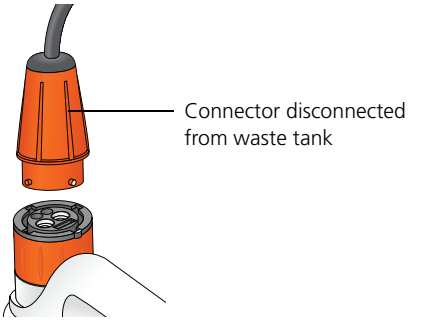


Caution! All biological specimens and materials can transmit potentially fatal disease. To prevent exposure to biohazardous agents, expose waste container contents to bleach (10% of total volume) before disposal. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.

Procedure

To empty the waste tank:

1. Verify that the system is not processing any samples.
2. Disconnect the connector from the waste tank by turning it counter-clockwise.



3. Remove the tank from the dock and take it to a dumping station.

4. Remove the filler cap and empty the tank.

   Hold the tank at an angle as you empty it and pour slowly to avoid splashing the contents.

5. Add bleach to the tank to equal 10% of the volume.
6. Re-install the filler cap and install the tank in the dock.
7. Re-install the connector and turn clockwise to tighten it.

More information

- Fluidics components (page 28)
- Refilling the sheath tank (page 471)

Performing the monthly clean procedure

Introduction

This topic describes how to perform the monthly clean procedure. This procedure should be performed at least once per month. It can be performed more often if the system is heavily used or if any contamination is suspected.

Description

The monthly clean procedure rinses the fluidics system with a 10% bleach solution, followed by another rinse with DI water and sheath fluid. The procedure takes about 20 minutes to complete.

Caution! All biological specimens and materials can transmit potentially fatal disease. To prevent exposure to biohazardous agents, expose waste container contents to bleach (10% of total volume) before disposal. Dispose of waste in accordance with local regulations. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.

Required materials

- 2 mL of 10% bleach solution
- 3 mL of DI water
- 2 L of 10% bleach solution

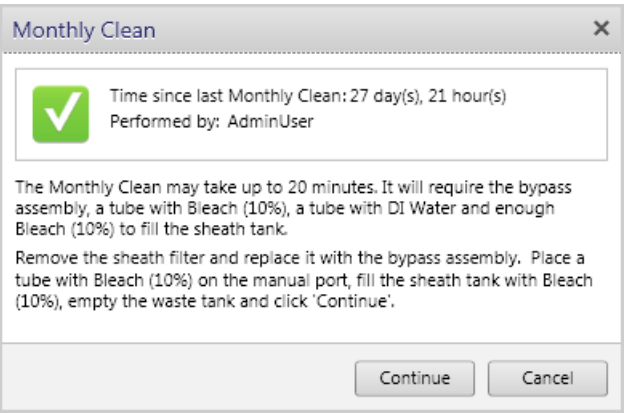
- Sheath filter bypass assembly
- BD FACSFlow sheath fluid to fill the sheath tank (5 L or 10 L)

Procedure

To perform the monthly clean procedure:

1. From the menu bar, select **Cytometer > Monthly Clean.**

   The following dialog opens.



2. Load a tube with 2 mL of 10% bleach onto the manual tube port.
3. Fill a tank with 2 L of 10% bleach solution.
   - We recommend using an extra tank dedicated for 10% bleach for this procedure. If you have this tank, remove the connector from the sheath tank and install it on the dedicated bleach tank.
   - If you do not have a dedicated bleach tank, then empty the sheath fluid from the sheath tank and fill it with 10% bleach solution.
4. Empty the waste tank.
5. Remove the sheath filter and store it carefully for replacement at the end of this procedure. See Replacing the sheath filters (page 478) for a figure showing the details.
   a. Open the door on the left side of the chassis.

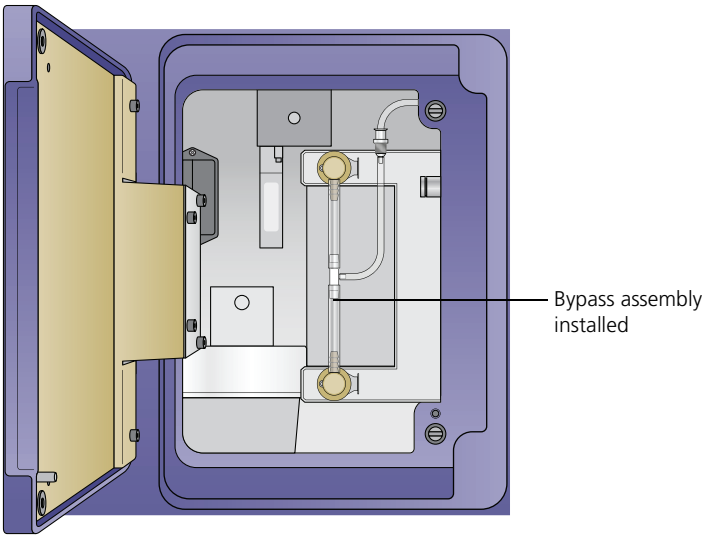
b. Disconnect the vent line on the top of the filter by unscrewing the connector nut.

c. Press the quick-disconnect tabs at the top and bottom of the filter and remove the filter from the chassis.

6. Install the sheath filter bypass assembly onto the two quick-connects and the vent line connector.

Caution! Installing the bypass assembly is a critical step. Failure to do this can damage the system.

The bypass assembly is shown installed in the following figure.



7. Click **Continue** in the dialog to start the cleaning process.

   A progress bar in the dialog shows the status of the process.

8. When the bleach cycle is done, remove the tube that contained bleach and replace it with a tube containing 3 mL of DI water.

9. Remove the bleach tank and connect the sheath tank.

   - If you are using a dedicated bleach tank, disconnect it and install the connector on the sheath tank.

- If you are not using a dedicated bleach tank, empty any remaining bleach from the sheath tank, rinse it thoroughly with DI water, and refill it with sheath fluid.

10. Click **Continue** to continue the cleaning process.

    A message is displayed when the process is complete, and the software records the time and date of the completed procedure.

11. Remove the bypass assembly and re-install the sheath filter.

12. Select **Cytometer > Fluidics > Purge Sheath Filter** and run this command twice to remove any air bubbles that might have formed during the process.

More information

- Fluidics components (page 28)
- Replacing the sheath filters (page 478)

Replacing the sheath filters

Introduction

This topic describes how to replace the sheath filter on the side of the cytometer. It also describes how to replace the sheath supply-line filter in the sheath tank. You should replace these filters every three months.

Required materials

- 1 new sheath filter
- 1 new sheath supply-line filter

Replacing the sheath filter

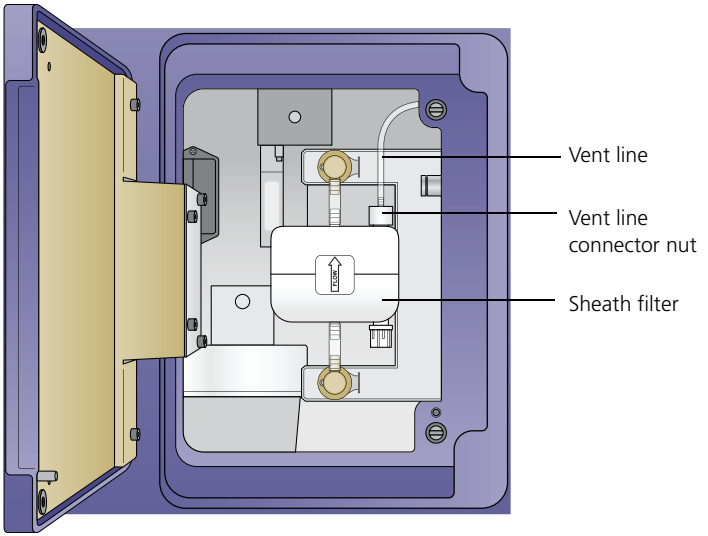
To change the sheath filter:

1. From the menu bar, select **Cytometer > Shutdown** to turn off power to the cytometer.

2. Open the door on the left side of the chassis.

   You might have to move the fluidics tanks dock if it is positioned next to the cytometer.

3. Disconnect the vent line on the top of the filter by unscrewing the connector nut.



4. Press the quick-disconnect tabs at the top and bottom of the filter and remove the filter from the chassis.
5. Discard the used filter.
6. Install a new filter assembly, with the flow arrow pointing up, by inserting each end into the connectors.
7. Reconnect the vent line on the top of the filter by screwing on the connector nut.
8. Select **Cytometer > Fluidics > Purge Sheath Filter** to bring sheath fluid into the new filter.

   This process takes about one minute to complete.
9. Repeat step 8 to fill the filter.

   You should see fluid in the vent line when it is done.
10. Close the door and resume normal operation.
11. Select **Cytometer > Maintenance > Replace Sheath Filter.**

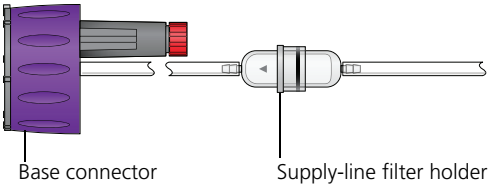
12. Enter the information about the new filter, then click **OK**.

Replacing the sheath supply-line filter

The sheath supply-line filter is located inside the sheath tank in the tube that draws up the sheath fluid.

To change the sheath supply-line filter:

1. Disconnect the connector from the sheath tank by turning it counter-clockwise.
2. Remove the base connector from the sheath tank by unscrewing it and pulling out the connector and supply-line assembly that includes the supply tube and the filter.
3. Place the connector and supply-line assembly on clean, lint-free disposable towels so that you can work on it.
4. Twist open the supply-line filter holder and pull it apart to access the filter. See the following figure.



5. Remove the used filter and install a new filter.
6. Push the two halves of the filter holder back together and twist to close it.
7. Place the supply-line assembly back into the sheath tank and screw on the base connector until it is secure.

More information

- Fluidics components (page 28)
- Refilling the sheath tank (page 471)

Generating a system health report

Introduction

This topic describes how to generate a system health report. This report is needed when contacting BD technical support for assistance.

About the system health report

The system health report consists of a text file containing the current status of the system. It also includes a ZIP file containing hardware and software configuration information and error logs that can be used that can be used by a BD technical support representative to analyze and troubleshoot the system.

Procedure

To generate a system health report:

1. Select **Cytometer > Maintenance > Generate System Health Report**.

   A dialog opens showing where the text and ZIP files are saved.

2. Open the text file to see the current information about the system.

More information

- System technical support (page 16)

Using the fluidics commands

Introduction

The topic describes when to use the fluidics commands. They include Clean Cuvette, Drain and Fill Flow Cell, Purge Sheath Filter, and SIT Flush.

Command descriptions

Select **Cytometer > Fluidics** to access these commands.

Command	Description
Clean Cuvette	Use this command to run cleaning solution through the flow cell. This action is also included during the shutdown process.
Drain and Fill Flow Cell	Use this command to remove persistent air bubbles from the flow cell. Place a tube of DI water on the manual tube port before starting this command.
Purge Sheath Filter	Use this command whenever you suspect air bubbles in the sheath path. This command is also executed twice after changing the sheath filter to fill the filter and remove air bubbles.
SIT Flush	Use this command to clean the sample path by backflushing sheath fluid through the SIT. This command is also executed by default after each sample is acquired. The automatic SIT flush can be disabled by clearing the checkbox in the Acquisition Status panel.

More information

- Replacing the sheath filters (page 478)
- Cleaning the manual tube port (page 483)

Cleaning the manual tube port

Introduction

This topic describes the procedure for cleaning the manual tube port and the wash probe.

Perform this action to keep the tube sensor from getting clogged due to sample collecting around the top of the manual tube port.

Required materials

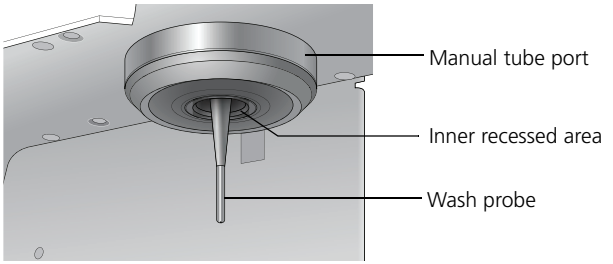
- Lint-free, small-tipped cleaning swabs
- Lint-free tissues
- Cleaning solution (DI water, ethanol or bleach)

Procedure

Caution! All biological specimens and materials can transmit potentially fatal disease. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.

To clean the manual tube port and the wash probe:

1. Remove any tubes from the manual tube port.



2. Use a lint-free, small-tipped cleaning swab dipped in DI water, ethanol, or bleach to clean the inner recessed area around the top of the manual tube port.
3. Use a lint-free tissue dipped in DI water, ethanol, or bleach to wipe down the exterior surface of the wash probe.
4. Dispose of all cleaning materials following biohazard precautions.

More information

- Replacing the sheath filters (page 478)
- Replacing the sample line (page 485)

Preparing for long term shutdown

Introduction

This topic describes how to prepare the cytometer for long term shutdown (one or more weeks).

Description

The preparation process consists of running the monthly clean procedure and substituting DI water for sheath fluid near the end of the procedure.

Avoid storing a system filled with fluid in a location where it can encounter freezing temperatures. Fluid expansion due to freezing temperatures can damage fluidic components.

Procedure

To prepare the cytometer:

1. Start the monthly clean procedure, see Performing the monthly clean procedure (page 475).
2. Stop when you reach the step that instructs you to refill the sheath tank with sheath fluid.
3. Instead, fill the sheath tank with DI water, then complete the procedure.

   This will leave DI water in the system to prevent salt accumulation that can result if a sheath fluid-filled system is left idle for an extended period of time.
4. Wipe down the exterior surfaces of the cytometer with a 10% bleach solution, then wipe it down again with DI water.
5. Dispose of all cleaning materials following biohazard precautions.

More information

- Maintenance overview (page 468)

Replacing the sample line

Introduction

This topic describes how to remove the existing sample line, assemble the sample line for the flow cell, and re-install the new sample line. This procedure is required only if the sample line has become kinked from improper handling or clogged from running a sticky sample.

If your system has a Flow Sensor, see Replacing the sample line in a system with a flow sensor (page 491).

Required materials

- Replacement sample line
- Two plastic nuts, two lock rings, and two ferrules
- SIT length tool
- Lint-free tissue

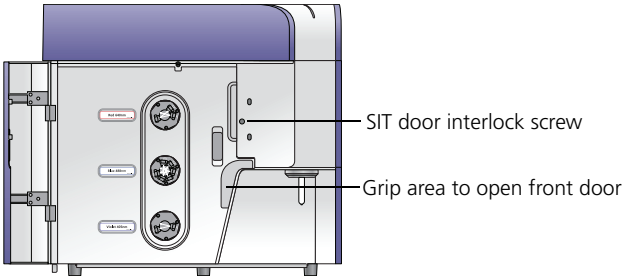
Removing the existing sample line

Caution! All biological specimens and materials can transmit potentially fatal disease. To prevent exposure to biohazardous agents, use proper precautions and wear suitable protective clothing, eyewear, and gloves.

To remove the existing sample line:

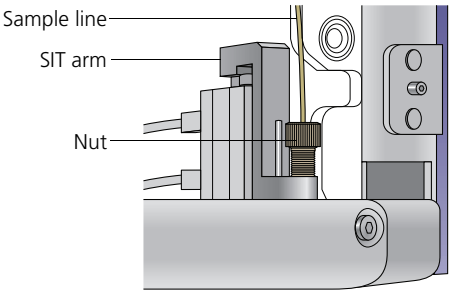
1. Turn off the power by pressing the Power button, then turn off the circuit breaker.

2. Open the front door by pulling the grip area at the right side of the door.

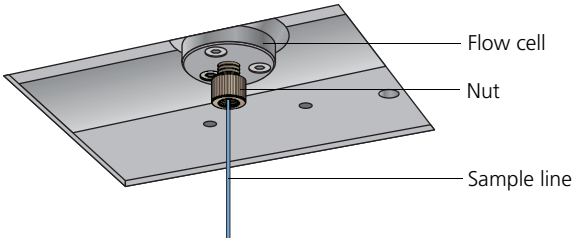


3. Open the SIT door by using a Phillips screwdriver to release the SIT door interlock screw.

4. Remove the sample line from the SIT arm by unscrewing the nut and carefully pulling the nut and sample line out.



5. Remove the sample line from the bottom of the flow cell by unscrewing the nut and carefully pulling the nut and sample line out.



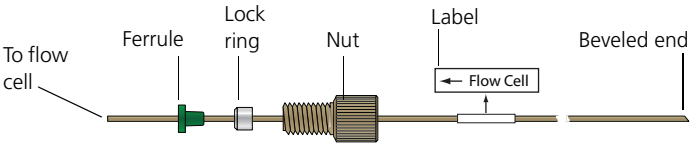
6. Discard the used sample line, nut, and fittings following biohazard precautions.

Assembling the new sample line

Handle the new sample line carefully and do not bend it at sharp angles or kink it. The interior of the line is fused silica and it will break internally if not handled correctly.

To assemble the new sample line:

1. Lay the new sample line on a clean surface.
2. Install a nut, lock ring, and ferrule on the sample line, as shown in the following figure.



The sample line has a label near the end of the line that goes to the flow cell. The other end of the line has a beveled cut, and that end must go to the SIT arm.

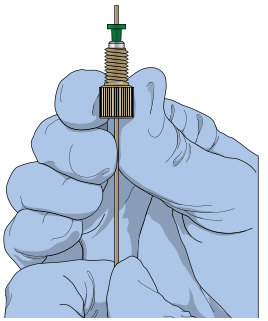
The tapered edge of the lock ring faces toward the ferrule.

Installing the sample line into the flow cell

During this process, you install the sample line into the bottom of the flow cell. This process establishes a critical junction between the flow cell and the sample line and must be performed carefully to ensure proper operation of the system.

To install the new sample line into the flow cell:

1. Position the end of the sample line underneath the flow cell while holding it with both hands as shown in the following figure.



2. Slide the sample line up into the bottom of the flow cell until it contacts the inside surface, while holding the nut assembly with the other hand.

3. Slowly screw the nut up into the flow cell while holding the sample line steady against the inside surface.

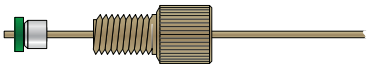
4. Tighten the nut until it is securely seated in the flow cell.

   As the nut is tightened, the ferrule is pushed into the lock ring. It is normal to feel extra resistance while turning the nut during this action. Once the nut is tightened, the lock ring and ferrule are bonded together into a single assembly and secured onto the sample line at a precise location. This action creates the proper seal between the flow cell and sample line.

5. Confirm that the sample line has been installed correctly by unscrewing the nut from the flow cell and visually inspecting the location of the lock ring and ferrule on the sample line.

   The following figure shows a correct installation. Notice that the lock ring and ferrule are pressed together and the sample

line extends a short distance (approximately 1.5 mm) beyond the end of the ferrule.



6. Re-install the sample line by screwing the nut back into the flow cell until it is secure.

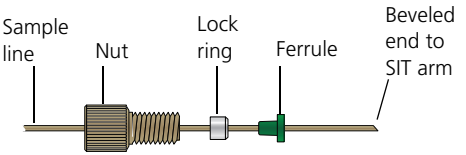
Installing the sample line into the SIT arm

During this process, you install the sample line into the SIT arm and down into the wash probe. This process sets the exact length of the sample line. This is a critical step for ensuring proper installation and minimizing dead volume in the sample tube.

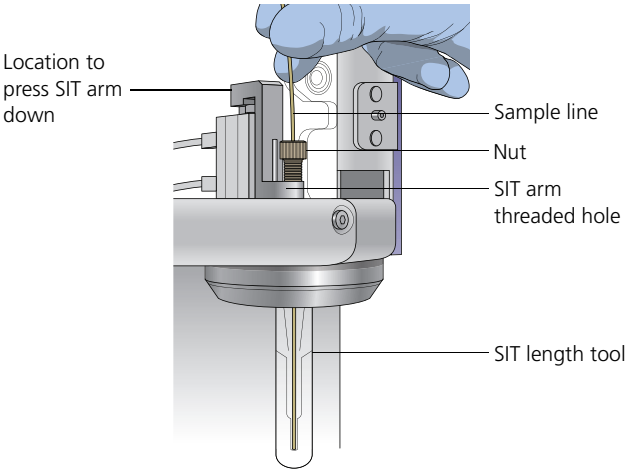
To install the sample line into the SIT arm:

1. Wipe off the wash probe with a lint-free tissue, then place the SIT length tool onto the manual tube port.
2. Install a nut, lock ring, and ferrule on the other end of the sample line, as shown in the following figure.

   The tapered edge of the lock ring faces toward the ferrule.



3. Slide the sample line (with the nut, lock ring, and ferrule in place) down into the threaded hole in the SIT arm.



4. Press the SIT arm down to its lowest position and hold it there, then slide the sample line down into the SIT length tool until it touches the bottom of the opening in the tool.

   Pushing the sample line down too hard could displace the SIT length tool slightly. Make sure the SIT length tool is pushed all the way up into the manual tube port before proceeding to the next step.

5. Route the length of sample line to the left side of the SIT arm as it goes up towards the flow cell, so that it does not contact anything inside the chassis.

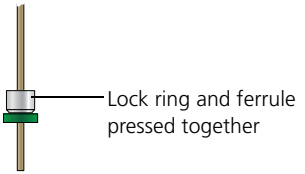
   This is especially important if the system has a Loader installed.

6. While holding the SIT arm down and the sample line in place, tighten the nut in the SIT arm hole until it is securely seated in the SIT arm.

   Perform this action carefully so the sample line is installed in the correct position.

7. Confirm that the sample line has been installed correctly by unscrewing the nut from the SIT arm and visually inspecting the location of the lock ring and ferrule on the sample line.

   The following figure shows a correct installation. Notice that the lock ring and ferrule are pressed together.



8. Re-install the sample line by screwing the nut back into the SIT arm until it is secure.
9. Remove the SIT length tool from the manual tube port.
10. Close the SIT door and tighten the interlock screw, then close the front door.

More information

- Cleaning the manual tube port (page 483)
- Running the daily clean procedure (page 469)

Replacing the sample line in a system with a flow sensor

Introduction

This topic describes how to replace the sample line in a system that contains a BD Flow Sensor (the flow sensor). This procedure is required only if the sample line has become kinked from improper handling or clogged from running a sticky sample.

The sample line is divided into lower and upper sections. The lower section is between the flow sensor and the SIT arm. The upper section is between the flow sensor and the flow cell. You

should replace only the lower section to see if that solves the problem.

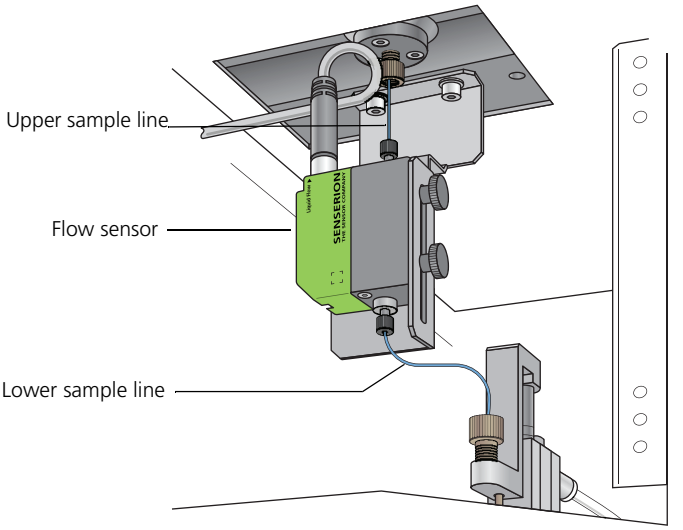
If you suspect a clog or kink in the upper sample line, contact your BD service representative. Do not attempt to replace the upper section of the sample line.

Required materials

- Replacement sample line for the lower section (make sure to use a sample line labeled for use with a flow sensor)
- One plastic nut, lock ring, and ferrule
- One nut for the flow sensor
- SIT length tool
- Lint-free tissue

Location of upper and lower sections

The following figure shows the flow sensor and the upper and lower sections of the sample line.

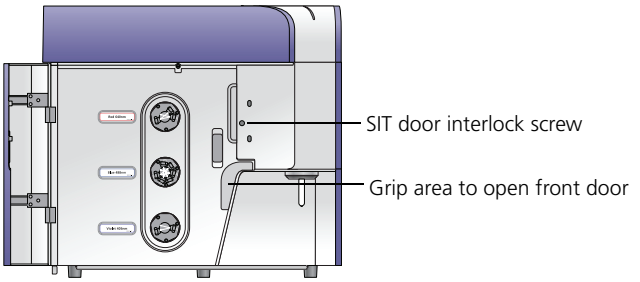


Removing the lower sample line

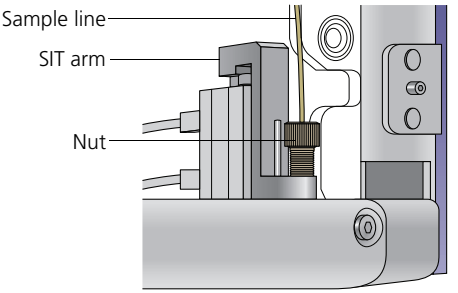
Caution! All biological specimens and materials can transmit potentially fatal disease. To prevent exposure to biohazardous agents, use proper precautions and wear suitable protective clothing, eyewear, and gloves.

To remove the lower sample line:

1. Turn off the power by pressing the Power button, then turn off the circuit breaker.
2. Open the front door by pulling the grip area at the right side of the door.



3. Open the SIT door by using a Phillips screwdriver to release the SIT door interlock screw.
4. Remove the lower sample line from the SIT arm by unscrewing the nut and carefully pulling the nut and sample line out.



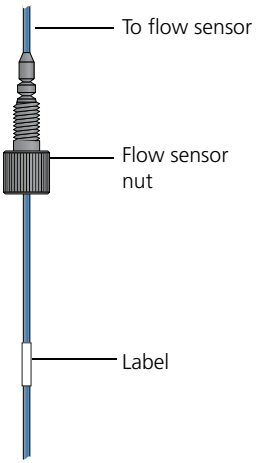
5. Remove the lower sample line from the bottom of the flow sensor by unscrewing the nut and carefully pulling the nut and sample line out.
6. Discard the used sample line, nut, and fittings, following biohazard precautions.

Installing the lower sample line into the flow sensor

Handle the new sample line carefully and do not bend it at sharp angles or kink it. The interior of the line is fused silica and it will break internally if not handled correctly.

During this process you install the sample line into the bottom of the flow sensor. This process establishes a critical junction between the flow sensor and the sample line and must be performed carefully to ensure proper operation of the system.

1. Lay the long piece of new sample line on a clean surface.
2. Install a black flow sensor nut on the sample line, as shown in the following figure.



The sample line has a label near the end of the line that goes to the flow sensor. The other end of the line has a beveled cut, and that end must go to the SIT arm.

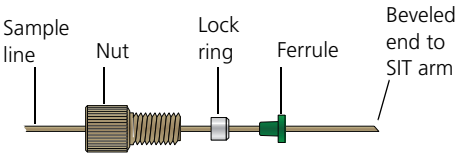
3. Slide the sample line up into the bottom of the flow sensor until it contacts the inside surface, while holding the nut assembly with the other hand.
4. Slowly tighten the flow sensor nut while holding the sample line steady against the inside surface of the flow sensor.
5. Tighten the nut until it is securely seated in the flow sensor.

Installing the sample line into the SIT arm

During this process, you install the sample line into the SIT arm and down into the wash probe. This process sets the exact length of the sample line. This is a critical step for ensuring proper installation and minimizing dead volume in the sample tube.

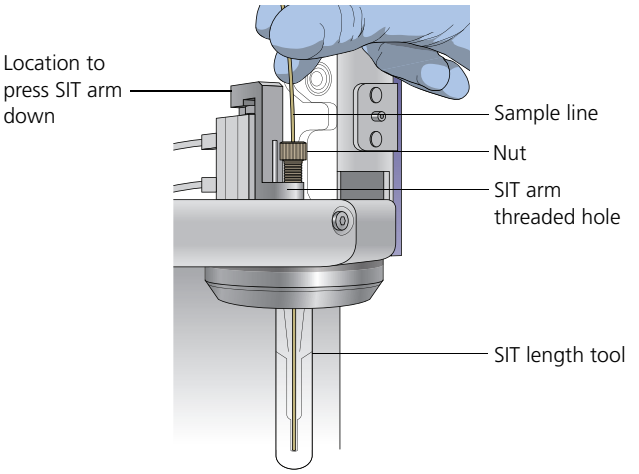
To install the sample line into the SIT arm:

1. Wipe off the wash probe with a lint-free tissue, then place the SIT length tool onto the manual tube port.
2. Install a nut, lock ring, and ferrule on the other end of the sample line, as shown in the following figure.



The tapered edge of the lock ring faces toward the ferrule.

3. Slide the sample line (with the nut, lock ring, and ferrule in place) down into the threaded hole in the SIT arm.



4. Press the SIT arm down to its lowest position and hold it there, then slide the sample line down into the SIT length tool until it touches the bottom of the opening in the tool.

   Pushing the sample line down too hard could displace the SIT length tool slightly. Make sure the SIT length tool is pushed all the way up into the manual tube port before proceeding to the next step.

5. Route the length of sample line to the left side of the SIT arm as it goes up towards the flow cell, so that it does not contact anything inside the chassis.

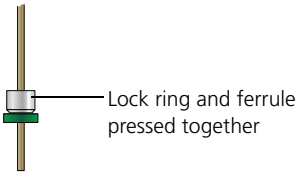
   This is especially important if the system has a Loader installed.

6. While holding the SIT arm down and the sample line in place, tighten the nut in the SIT arm hole until it is securely seated in the SIT arm.

   Perform this action carefully so the sample line is installed in the correct position.

7. Confirm that the sample line has been installed correctly by unscrewing the nut from the SIT arm and visually inspecting the lock ring and ferrule on the sample line.

   The following figure shows a correct installation. Notice that the lock ring and ferrule are pressed together.



8. Re-install the sample line by screwing the nut back into the SIT arm until it is secure.
9. Remove the SIT length tool from the manual tube port.
10. Close the SIT door and tighten the interlock screw, then close the front door.

More information

- Cleaning the manual tube port (page 483)
- Running the daily clean procedure (page 469)

Managing your database

Introduction

This topic describes the tasks involved in managing your database. The BD FACSuite Backup and Restore utility allows you to back up and restore your database.

About the BD FACSVerse System Backup and Restore utility

Use the BD FACSuite Backup and Restore utility to back up or restore all data that is stored in the BD FACSuite database, along with all FCS files located in the application-defined directories.

With this utility, you can create a single backup set which contains a backup of the database, along with experiment and worklist FCS files. The utility maintains backup sets indefinitely. It displays how much disk space each set is taking and how much disk space is left. You can discard existing backup sets to free up space.

Since the Experiment and Worklist workspaces save FCS files to different locations, these folders are handled separately when the backup is created.

You can also use any backup set to restore the BD FACSuite database and FCS files. When you restore, you erase any new data created since the backup was created.

Creating a new backup set

To create a new backup set:

1. Start the **BD FACSuite Backup and Restore** utility from the icon on the desktop.

   The **BD FACSuite Backup and Restore** window opens.

2. Click **Back Up**.

   The **Back Up** window opens, indicating the required hard disk space. If the estimated space required is greater than the amount available, the software prompts you to free up additional space and try again.

3. Verify that adequate hard disk space is available and click **Back Up**.

The backup process starts and displays a progress bar. A completion dialog is displayed and indicates success or failure. If the backup succeeds, the timestamp of the new backup is provided. If the backup fails, the reason is indicated.

4. Click **Finish** to close the window.

Restoring a backup set

To restore a backup set:

1. Click the **BD FACSuite Backup and Restore** utility icon on the desktop.

   The **BD FACSuite Backup and Restore** window opens.

2. Select the backup set to restore.

3. Click **Restore**.

   The **Restore** window opens, indicating the timestamp of the selected backup set and the required disk space. If the estimated space required is greater than the amount available, the system prompts you to free up additional space and try again.

   The estimated space required takes into account the files that will be removed during the process, and it is possible that this number could be negative. In that case, 0 KB is used.

4. Click **Restore**.

   A confirmation dialog is displayed.

5. Click **OK**.

   The restore process begins and displays a progress bar. A completion dialog is displayed and indicates success or failure.

6. Click **Finish** to close the window.

Deleting a backup set

To delete a backup set:

1. Click the **BD FACSuite Backup and Restore** utility icon on the desktop.

   The **BD FACSuite Backup and Restore** window opens.

2. Select the backup set to delete.
3. Click **Delete**.

   A confirmation dialog is displayed to verify that the selected backup set needs to be deleted.

4. Click **OK**.

Changing settings

To change settings:

1. Click the **BD FACSuite Backup and Restore** utility icon on the desktop.

   The **BD FACSuite Backup and Restore** window opens.

2. Click **Change Settings**.

   The **Settings** window opens.

3. Click **Browse** and navigate to the backup directory.

   The **Browse for folder** dialog opens.

4. In the **Browse for folder** dialog, click **OK**.

   The **Backup Directory** value is updated to the selected location.

5. In the **Settings** window, click **OK**.

   The changes are saved and the window closes.

More information

- Maintenance overview (page 468)

16

BD FACS Universal Loader

This section includes the following topics:

BD FACS Universal Loader overview

Introduction

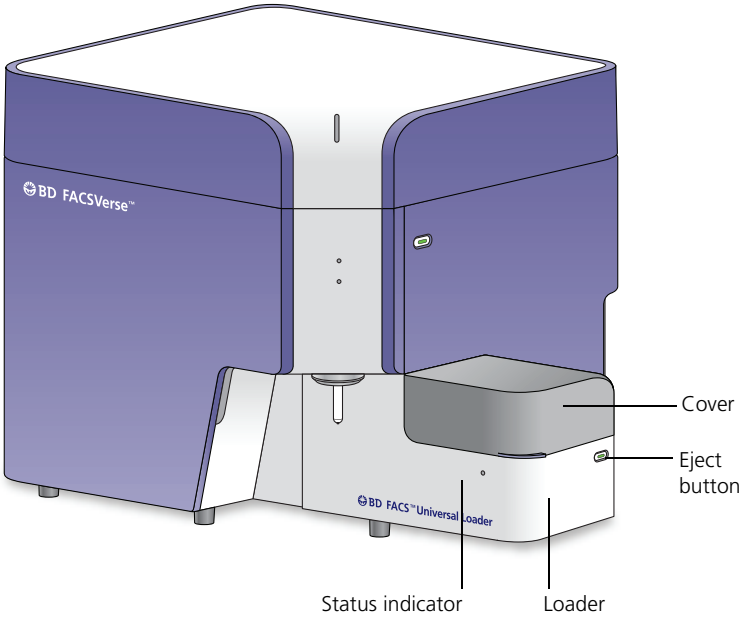
This topic gives an overview of the components of the BD FACS Universal Loader (the Loader).

About the Loader

The Loader is an optional automated loading system that mixes samples and delivers tube racks and plates to the BD FACSVerse cytometer for acquisition. The Loader can be included as an option on a new system or it can be ordered and installed at a later time by a BD field service engineer.

External components

The following figure shows the location of the Loader's external components.



Status indicator

The status indicator uses illumination and color to show the status of the Loader.

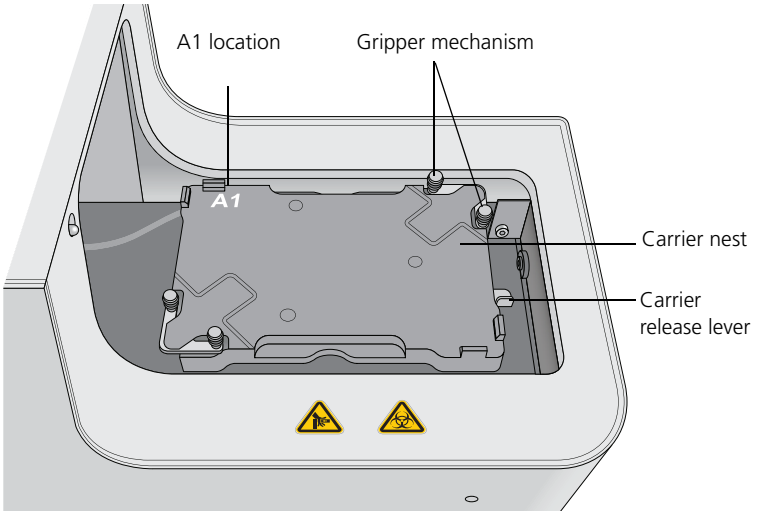
Condition	Status
Off	Ready to operate Note: If main power to the system is off, this indicator is off.
Blue	Cover is locked and system is running
Blinking blue	Loading or unloading
Red	Error condition

Eject button

The Eject button should be used if there is a problem during operation. Pressing this button stops acquisition and moves the carrier out to the loading position.

Internal components

The following figure shows the location of the internal components.



Overhead imaging system

The Loader has an internal overhead imaging system that can detect:

- The presence and location of tubes in racks
- The correct type and orientation of carriers
- Lids on plates

Only compatible carriers can be used for this system to work.

Recommendations for using the Loader

Follow these recommendations to ensure that the Loader operates correctly.

- Do not use any tubes, racks, or plates that are not listed as compatible carriers. See Sample carrier specifications (page 505).
- Do not use black plates.
- Keep the top surface of tube racks clean so that the camera imaging system works properly.
- Inspect the flange, upper lip, and barcode label on all tube racks for signs of wear and replace it if excess wear is found. See Placing carriers into the Loader (page 506).
- Inspect the numbers on the top surface of tube racks to make sure they are legible and not faded.
- Keep all barcode labels clean and dry.
- Do not use CONTRAD® detergents for any cleaning procedures when using the Loader.
- Do not autoclave tube racks.

More information

- Worklist overview (page 222)

Sample carrier specifications

Introduction

This topic describes the types of sample carriers (tube racks and plates) that are compatible with the Loader.

Carrier type compatibility

The following tables list the carrier types that are compatible with the Loader. The tube racks are available only from BD.

For information on part numbers and additional details on compatible carriers, see the BD FACSVerse section of the BD Biosciences website.

The minimum and maximum volumes for tubes and wells are shown. Volumes below the minimum may need additional mixing to resuspend the sample. Using volumes above the maximum could result in cross-contamination and spillage during mixing.

Carrier type for tubes[a]	Recommended minimum volume (µL)	Maximum volume (µL)
30-tube rack (12 x 75 mm)	100	2,000
40-tube rack (12 x 75 mm)	100	2,000

a. For polystyrene, polypropylene, and BD Trucount tubes.

Carrier type for plates	Bottom geometry	Material[a]	Recommended minimum volume (µL)	Maximum volume (µL)
BD 96 standard height	Round	PS	55	200
BD 96 standard height	Flat	PS	55	200
BD 96 standard height	Round	PP	55	200
BD 96 standard height	Conical	PP	55	200
384 standard height	Flat	PS	40	75
96 half deep	Conical	PP	55	500

Carrier type for plates	Bottom geometry	Material[a]	Recommended minimum volume (µL)	Maximum volume (µL)
96 deep	Conical	PP	55	1,000
96 matrix tube rack	N/A	N/A	55	700
96 filter bottom	Filter	PP	150	200

a. PS = polystyrene, PP = polypropylene

Barcode reading

The system can read barcodes on plates, tube racks, and individual tubes in 30-tube racks. To confirm the identification and correct location of tubes in racks, the barcodes must first be entered into a worklist with the handheld barcode reader or entered manually. Then the readers in the Loader can confirm that the correct barcode has been recognized.

Placing carriers into the Loader

Introduction

This topic describes how to place sample carriers into the Loader.

Before you begin

Confirm that the carrier type you are using is compatible with the Loader. See Carrier type compatibility (page 505).

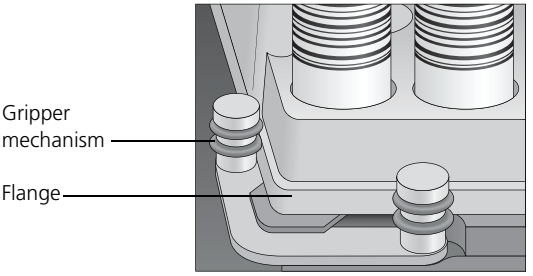
Procedure

To place a carrier into the Loader:

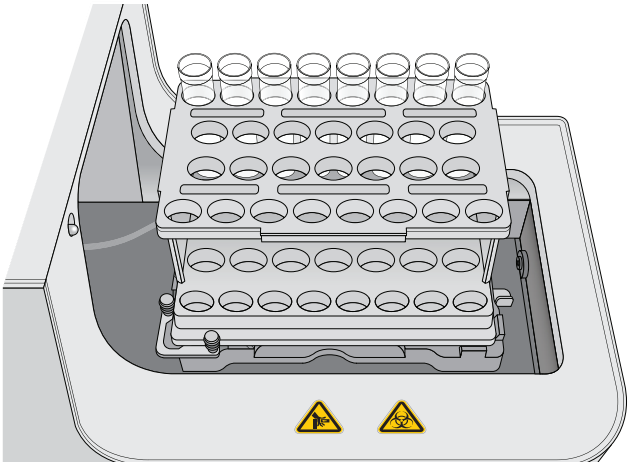
1. Open the cover.
2. Push the carrier release lever toward the back and hold it open.
3. Install the carrier into the carrier nest with the carrier centered on the nest, then release the lever as you continue to hold the carrier centered.

   This is especially important with heavier carriers such as tube racks, deep-well plates, and matrix tube racks. Make sure that

the flange along the perimeter of the carrier is held securely in the gripper mechanisms, as shown in the following figure.



The following figure shows a tube rack loaded onto the nest.



More information

- Sample carrier specifications (page 505)

Defining custom sample carrier layouts

Introduction

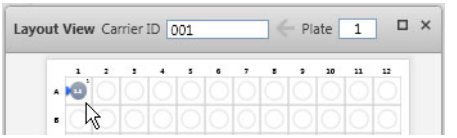
This topic describes how to define custom sample carrier layouts for a worklist. This applies only to systems that include the Loader option.

Custom layouts are saved with a worklist. You need to perform this procedure only if you want to define a layout different from the default. The default is set as a preference in the Preferences dialog.

Defining a plate layout

To define a plate layout:

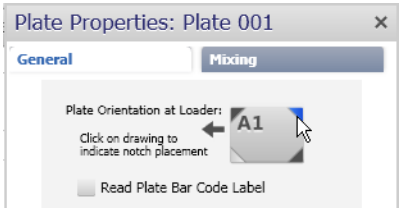
1. Create a new worklist or open an existing worklist.
2. Navigate to the **Loading Options** panel.
3. Select a carrier type. For example, *96 Well Plate Standard flat bottom*.
4. In the **Layout View** panel, right-click a tube or well and select **Display Properties**.



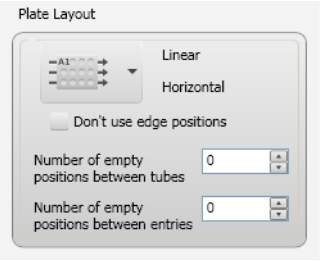
The **Plate Properties** dialog opens.

5. (Optional) In the **General** tab, click to select the location of the plate notches to set the plate orientation at the Loader.

This setting is only required if you are using a plate that is not on the list of BD-qualified plates.

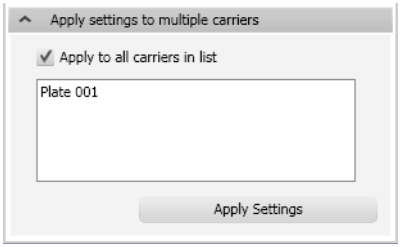


6. (Optional) To read the plate barcode label during loading, select the **Read Plate Bar Code Label** checkbox.
7. Under **Plate Layout,** click the arrow and select a linear horizontal, linear vertical, serpentine horizontal, or serpentine vertical layout.



8. (Optional) Make the following selections:
   - If you do not want to include the wells on the horizontal or vertical edges, select the **Don't use edge positions** checkbox.
   - Select the number of empty positions in the layout between each well.
   - Select the number of empty positions in the layout between worklist entries.
9. (Optional) If you want to apply these settings to multiple plates:
   a. Click **Apply settings to multiple carriers**.

b. Select the **Apply to all carriers in list** checkbox or select specific carriers in the list.

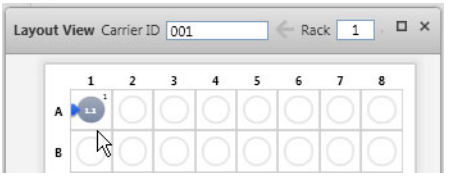


10. Click **Apply Settings**.

Defining a tube rack layout

To define a tube rack layout:

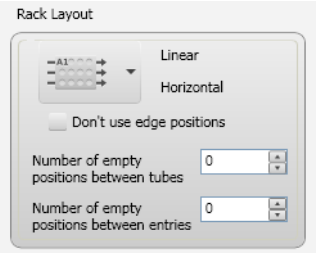
1. In the **Loading Options** panel, in the **Carrier Type** field, click and select a tube rack type. For example, *40 Tube Rack*.
2. In the **Tray Layout** panel, right-click a tube and select **Display Properties**.



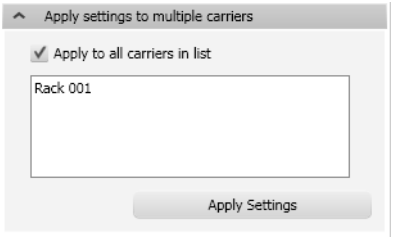
The **Rack Properties** dialog opens.

3. (Optional) To read the rack barcode label during loading, select the **Read Rack Bar Code Label** checkbox.

4. Under **Rack Layout,** click the arrow and select a linear horizontal, linear vertical, serpentine horizontal, or serpentine vertical layout.



5. (Optional) Make the following selections:
   - If you do not want to include the tubes on the horizontal or vertical edges, select the **Don't use edge positions** checkbox.
   - Select the number of empty positions in the layout between each tube.
   - Select the number of empty positions in the layout between worklist entries.
6. (Optional) If you want to apply these settings to multiple racks:
   a. Click **Apply settings to multiple carriers**.
   b. Select the **Apply to all carriers in list** checkbox or select specific carriers in the list.



7. Click **Apply Settings**.

About mixing settings

Introduction

This topic describes the mixing settings for systems equipped with the Loader.

Types of mixing settings

You can set two types of mixing settings:

- **Initial mixing.** The first mix that happens when the carrier is run. The initial mixing setting is designed to resuspend the samples, even if they have been sitting overnight. Because of this, the initial mix is more aggressive than the interim mix.
- **Interim mixing.** Any subsequent mixes that are defined by time or interval.

Mix settings specifications

For information on mixing specifications and ranges for compatible carriers, see the BD FACSVerse section of the BD Biosciences website.

More information

- Selecting custom mixing settings (page 513)
- Sample carrier specifications (page 505)

Selecting custom mixing settings

Introduction

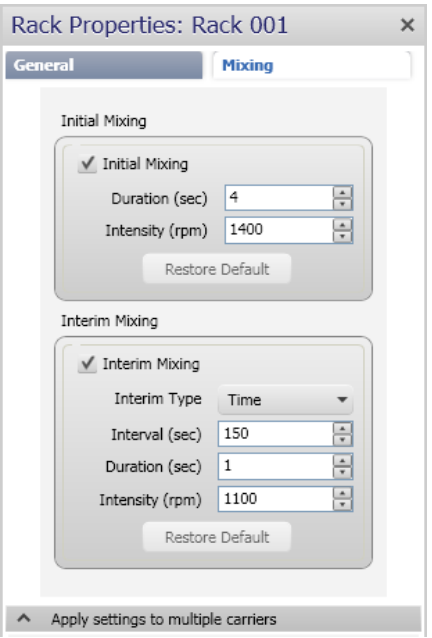
This topic describes how to select custom mixing settings.

Procedure

Default mixing preferences are set in the Preferences dialog. You can specify custom mixing settings using the Rack Properties or Plate Properties dialog in the Worklist workspace.

To select custom mixing settings:

1. In the **Rack Properties** or **Plate Properties** dialog, click the **Mixing** tab.



2. Under **Initial Mixing**, select the **Initial Mixing** checkbox to enable initial mixing.
3. Set a custom mixing duration in the **Duration** (**sec**) field.
4. Set a custom mixing intensity in the **Intensity** (**rpm**) field.

5. Under **Interim Mixing,** select the **Interim Mixing** checkbox to enable interim mixing.
6. Select a time or interval-based interim mixing type.
   - If you select **Time,** then set a time interval between mixes in the **Interval** (**sec**) field.
   - If you select **Interval,** then set a tube or well interval in the **Interval** (**tubes** or **wells**) field.
7. Set a custom interim mixing duration in the **Duration** (**sec**) field.
8. Set a custom interim mixing intensity in the **Intensity** (**rpm**) field.
9. (Optional) If you want to apply these settings to multiple racks:
   a. Click **Apply settings to multiple carriers**.
   b. Select the **Apply to all carriers in list** checkbox or select specific carriers in the list.
10. Click **Apply Settings**.

More information

- About mixing settings (page 512)

Cleaning the Loader

Introduction

This topic describes how to clean the Loader. It is a good practice to perform this cleaning daily.

Required materials

- 10% bleach solution in a squirt-type bottle
- DI water
- Disposable towels or wipes.

Caution

Caution! Do not use a spray bottle to spray the 10% bleach solution because the mist can get into areas that can cause problems. Instead, use a squirt-type (squeeze) bottle to distribute the solution.

Caution! All biological specimens and materials can transmit potentially fatal disease. Use proper precautions and wear suitable protective clothing, eye wear, and gloves. Dispose of waste in accordance with local regulations.

Procedure

To clean the Loader:

1. Apply the 10% bleach solution to a disposable towel, then wipe down the following areas:
   - Top surface of the carrier nest
   - Inside surfaces of the cover
   - Outside surfaces of the cover
   - Outside surfaces of the Loader chassis
2. Use the DI water on the same areas to remove the bleach, then wipe them dry with a towel.
3. Dispose of used cleaning materials following biohazard precautions.

For any major spills of liquids down into the interior of the Loader, contact BD technical support.

More information

- Performing manual system shutdown (page 113)

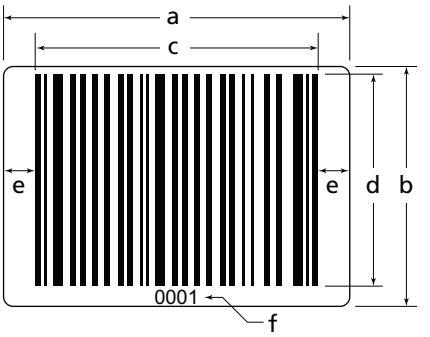
Barcode label specifications

Introduction

This topic describes the specifications for barcode labels for tubes, tube racks, and plates.

Tube label specifications

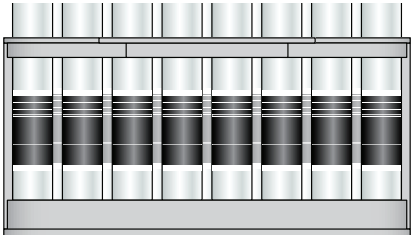
The following table lists the details for barcode labels that are used on tubes.

Specification	Description
Supported symbologies (types)	Code 128, Code 39, Codabar, Interleaved 2 of 5 You do not need to specify the type of symbols for labels on tubes. The barcode reader can sense differences automatically.
Narrow element (width of the narrowest bar in a label)	10 mil (0.25 mm, 0.01 in.) or greater
Dimensions 	a - Max label length: 44.45 mm (1.75 in.) This length should include the barcode symbol and quiet zone. b - Max label height: Cannot exceed 10 mm more than the circumference of the tube. c - Max symbol length: 37.45 mm (1.47 in.) d - Minimum symbol height: 19.05 mm (0.75 in.). We recommend the maximum symbol height possible because this makes it easier to orient tubes within a rack. e - Minimum quiet zone: 3.5 mm (0.14 in.) at each end of the symbol.
Human readable	f - Size and placement of the human readable to be determined by the user.

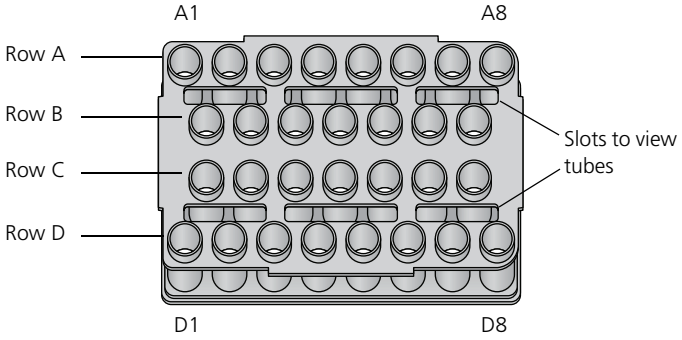
Specification	Description
Label thickness	Thickness of label and adhesive not to exceed 3.9 mil. (0.099 mm, 0.0039 in.)
Finish	Print labels on material with a matte finish.
Placement of label on tube	The label must be placed a minimum of 12 mm (0.47 in.) from the bottom of the tube. The label must be placed so the bars are perpendicular to the length of the body of the tube.

Tube rack loading requirements

Tubes in the 30-tube rack are arranged in a staggered configuration, providing a direct line of sight between tube barcode readers in the Loader and tube labels (tube barcode scanning is not available using the 40-tube rack). See the following figure.



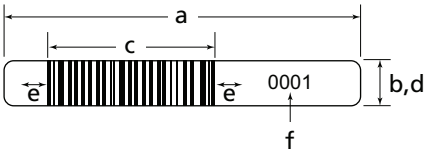
Barcode labeled tubes in a rack should be oriented so that the labels on tubes in rows A and B are facing the long side of the tube rack between A1 and A8. The labels on tubes in rows C and D are facing the long side of the tube rack between D1 and D8, as shown in the following figure.



Slots on the top surface of the rack can be used to help orient tubes in rows C and D.

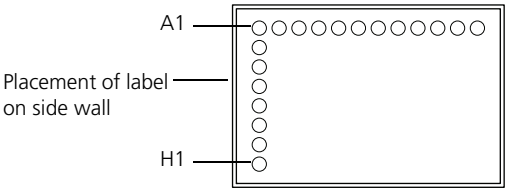
Plate label specifications

The following table lists the details for barcode labels that are used on plates.

Specification	Description
Supported symbologies (types)	Code 128, Code 39, Codabar, Interleaved 2 of 5 You do not need to specify the type of symbol for labels on plates. The barcode reader can sense symbols automatically.
Narrow element (width of the narrowest bar in a label)	10 mil (0.25 mm, 0.01 in.) or greater
Dimensions  	a - Max label length: 47.63 mm (1.88 in.). This length should include the barcode symbol and quiet zone. b - Label height: 6.35 mm (0.25 in.) c - Max symbol length: 40.63 mm (1.60 in.) without human readable value. d - Symbol height: 6.35 mm (0.25 in.). The symbol should bleed off the bottom and top of the label because this increases the likelihood of the label being decoded. e - Quiet zone: 3.5 mm (0.14 in.) at each end of the symbol
Human readable	f - Size and placement of human readable to be determined by the user
Label thickness	Thickness of label and adhesive not to exceed 3.9 mil. (0.099 mm, 0.0039 in.)
Finish	Print labels on material with a matte finish.

Placement of label on plate

The label should be placed on the side wall of the plate along the short side of the plate between A1 and H1/P1.



The bottom edge of the label must be placed directly above the plate flange. Use this edge to guide placement of the label.

The label must be placed so that the bars are perpendicular the plate flange.

Label recommendations

Observe these recommendations for optimal performance.

- Labels must be clean and not yellowed.
- Use labels prior to expiration date.
- Label must not have defects such as spots, lines, missing sections, cuts, folds, or density problems.
- Bars must be well defined and bar edges must not be irregular.

Barcode error rate guidelines

- Code 128 and 39 are more accurate and have lower error rates than Codabar and Interleaved 2 of 5.
- CLSI recommends Code 128 because of its accuracy, compact form, and self-checking capabilities.
- A checksum greatly increases accuracy. If possible, use a checksum with Codabar and Interleaved 2 of 5 because they are less accurate symbologies.
- If available, select the fixed length option since this is more accurate than variable length.

More information

- Barcode reading (page 506)

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17

BD FACSVerse system options

This section includes the following topics:

BD FACSVerse system options overview

Introduction

This topic lists the available options and upgrades for the BD FACSVerse system.

About options

System options and upgrades described in this section have been validated and qualified for use with the BD FACSVerse system. If you plan to use other third-party hardware (for example, venting hood, or handlers) or software (for example, analysis software) that is not included in this list, you are responsible for validating the system and verifying the results.

Options

The following table lists available options and upgrades.

Category	Option	For more information
System hardware	BD FACS Universal Loader	See BD FACS Universal Loader overview (page 502).
	Handheld barcode reader	See Using the handheld barcode reader (page 531).
	BD™ Flow Sensor	See About the BD Flow Sensor option (page 525).
Optics	Laser upgrades	Contact your BD representative.
Fluidics	Large fluidics tanks (10-L capacity)	See Fluidics tank options (page 532).
	BD FACSFlow cubitainer adapter	See Installing a cubitainer adapter (page 533).
Applications and assays	FCAP Array version 3.0 software	See documentation included with FCAP Array software.
	BD Assurity Linc software	See Using BD Assurity Linc software (page 534).
	BD assays	Contact your BD representative for a current list of available BD assays.

About the BD Flow Sensor option

Introduction

This topic describes the BD Flow Sensor option and how it measures flow volume.

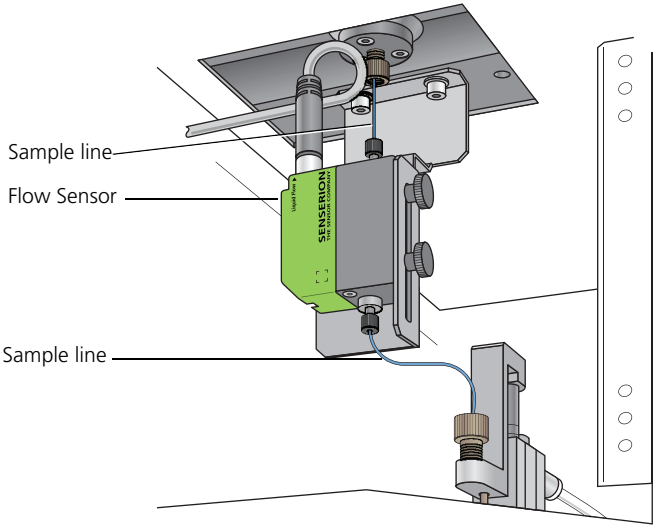
The Flow Sensor is intended for Research Use Only (RUO). Not for use in diagnostic or therapeutic procedures.

About volumetric measurement

Volumetric measurement is performed by the optional Flow Sensor installed in the sample flow line. The Flow Sensor provides quick and accurate volume measurements for determining cell counts in a sample at medium and high flow rates.

The Flow Sensor works by adding a minimal amount of heat at a specific point along the flow path. The sample temperature is measured at the beginning and end of the flow path. The temperature difference is used to calculate the volumetric flow rate. The total volume appears in the software as a keyword.

The following figure shows the location of the Flow Sensor.



Volumetric measurement conditions

For proper reading, the Flow Sensor should be used only with the medium and high flow rates.

More information

- Using the BD Flow Sensor (page 526)
- Using an expression for volumetric measurement (page 528)

Using the BD Flow Sensor

Introduction

This topic describes how to use the BD Flow Sensor on the BD FACSVerse system. The Flow Sensor works with any research application.

Setup

No setup is necessary. When purchased with the cytometer, the Flow Sensor comes installed on the instrument. If the Flow Sensor is purchased after the cytometer, a BD service representative installs it.

The Flow Sensor turns on when the cytometer is on and automatically turns off when the cytometer is shut down. No adjustments to the Flow Sensor are necessary. The volume is automatically reported as a keyword for all acquisitions.

About sample flow rates

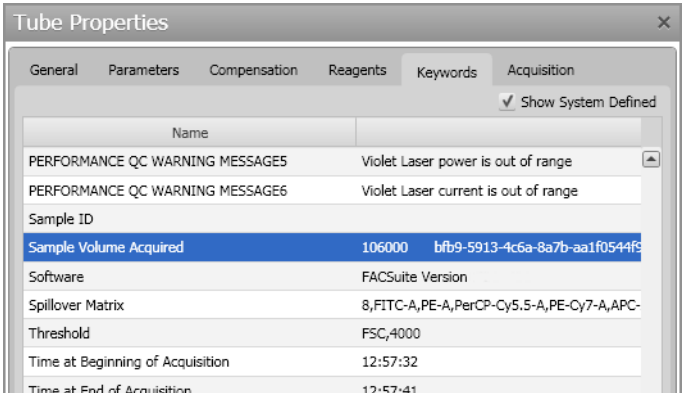
We recommend running samples at a high flow rate when using the Flow Sensor. The flow rate can be changed in the Acquisition Status panel before acquisition.

Reading the sample volume

To read the sample volume after data acquisition:

1. Right-click a tube and select **Properties.**
2. Click the **Keywords** tab and select the **Show System Defined** checkbox to see all keywords.

The volume is reported as a value (nL/min) next to the Sample Volume Acquired keyword.



If the value is *NA*, you might need to adjust the flow rate or decrease the viscosity of the sample before re-acquiring the sample. Flow issues such as air bubbles, clogs, or a low sample volume may result in an *NA* value.

You can add the **Sample Volume Acquired** keyword to statistics views so you don't have to open the **Tube Properties** dialog to read the volume.

More information

- About the BD Flow Sensor option (page 525)
- Using an expression for volumetric measurement (page 528)
- Adding keywords to statistics views (page 411)

Using an expression for volumetric measurement

Introduction

This topic describes how to create an expression to display volumetric measurement data. Volumetric measurement uses a standard keyword to generate this data.

Procedure

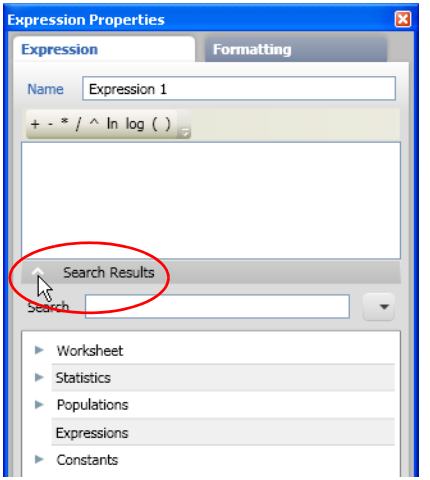
To build expression formulas from results:

1. In a worksheet, click **Create Expression** on the **Worksheet** toolbar.

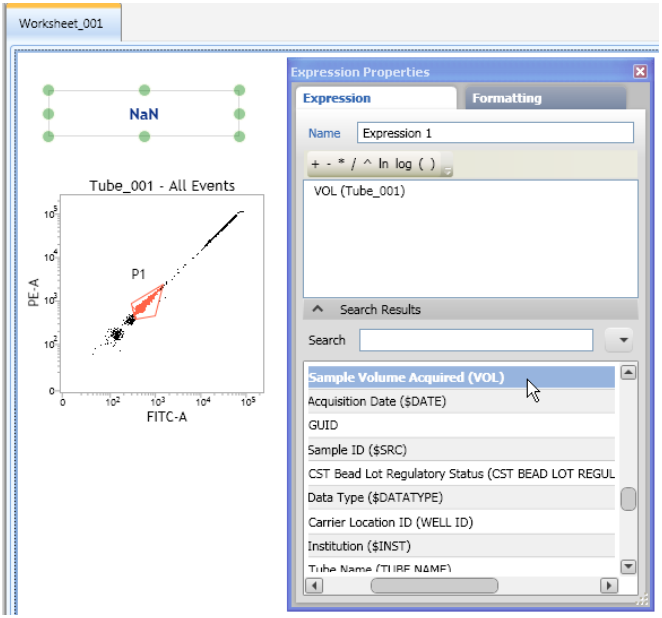
2. Click in the worksheet to create a new blank expression.

   The **Expression Properties** dialog opens.

3. In the **Expression Properties** dialog, click **Search Results**.



4. In the Results tree, select **Populations > Tube_0x > Keywords > Default > Sample Volume Acquired (VOL)**.



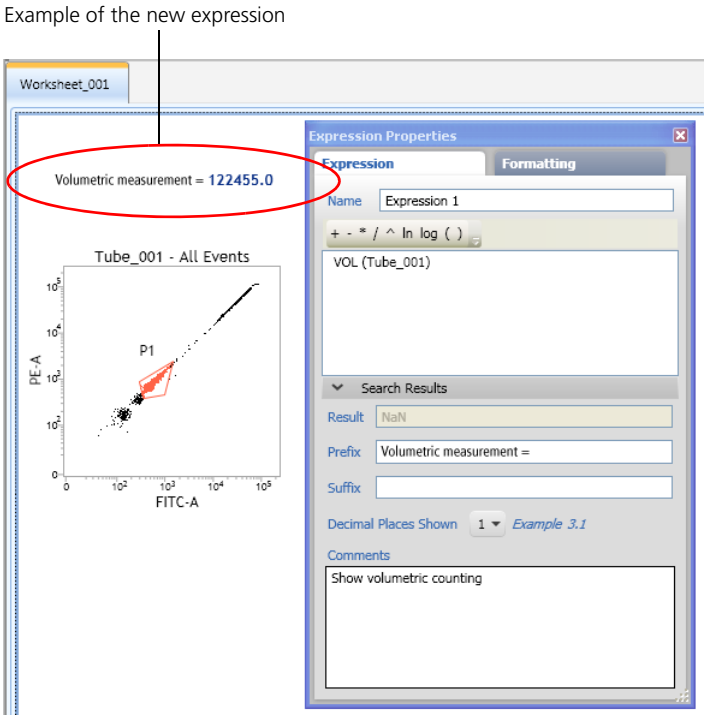
The selected result is displayed in the **Formula** box above the search results. The calculated expression is displayed in the worksheet.

5. Click the **Search Results** arrow to display the expression details.

6. In the **Name** field, specify a name for the expression.

   The name must be unique within an experiment.

7. (Optional) Make entries in the **Prefix** or **Suffix** fields to create a displayed name for the expression.



8. Specify the decimal place display in the **Decimal Places Shown** field.
9. Under **Comments**, add any comments that describe the expression.
10. Close the **Expression Properties** dialog to save the expression.

More information

- About the BD Flow Sensor option (page 525)
- Using the BD Flow Sensor (page 526)
- Expressions overview (page 420)

Using the handheld barcode reader

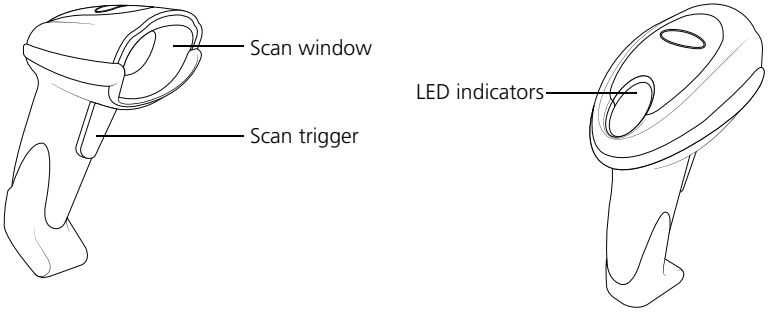
Introduction

This topic describes the optional handheld barcode reader. See the manufacturer's documentation for details on safety and how to use the reader.

Description

The handheld barcode reader plugs into the USB port on the computer workstation.

The barcode reader reads most current barcode standards. See the manufacturer's documentation for the complete list of standards.



When to use the reader

You can use the reader to scan the following labels when using BD FACSVerse system.

- Lot file labels on a CS&T bead vial
- Sample ID label on a sample tube
- ID label on a 30- or 40-tube rack
- ID label on a well plate

More information

- Barcode label specifications (page 516)

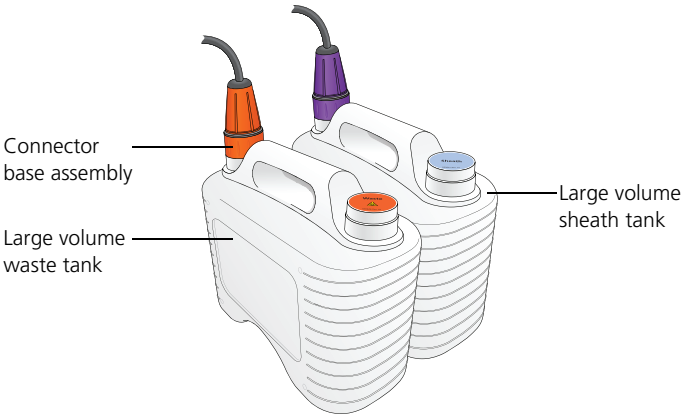
Fluidics tank options

Introduction

This topic describes the fluidics tank options for the system. These include the large volume sheath and waste tanks and the cubitainer adapter. The large volume tanks give you extended running times for the system.

Large volume fluidics tanks

The large volume fluidics tanks have a 10-L capacity and are available as an option to replace the standard tanks. The large volume tanks use the same connectors as the standard tanks and are normally placed on the floor near the cytometer.



The sheath and waste tanks must be placed at a level even with, or below, the cytometer. Placing the tanks higher than the cytometer can cause uncontrolled siphoning.

Installing large volume fluidics tanks

You can switch from a standard tank to a large tank by removing the connector base assembly from the standard tank and installing it on the large tank.

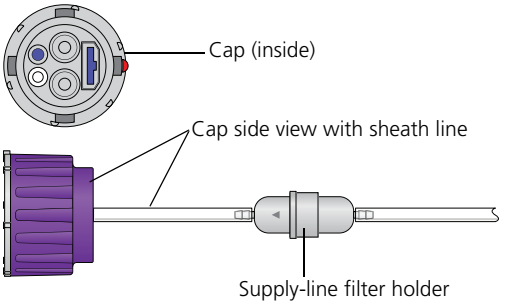
To install a large volume tank:

1. Disconnect the connector from the standard tank.
2. Unscrew the connector base assembly from the standard tank.

3. Install the connector base assembly into the large tank and screw it in until it is secure.
4. Re-install the connector from the cytometer into the large tank and turn it to secure it.

Installing a cubitainer adapter

The cubitainer adapter can be installed on a BD FACSFlow cubitainer to serve as a higher volume sheath tank.



The adapter can only be used for sheath fluid. It cannot be used on an empty cubitainer for waste.

To install a cubitainer adapter:

1. Remove the cap from a cubitainer.
2. Insert the adapter assembly into the opening in the cubitainer.
3. Screw the adapter onto the cubitainer.
4. Install the sheath line connector from the cytometer onto the adapter and turn it one-quarter turn to secure it.

Using BD Assurity Linc software

Introduction

This topic describes how to establish a remote session with a BD technical representative using BD Assurity Linc™ software.

About BD Assurity Linc software

BD Assurity Linc is a highly secure remote systems management service that connects BD instruments to BD technical support personnel. Using the BD Assurity Linc Agent, BD support personnel can securely access your workstation through an enterprise server and diagnose problems remotely. You must grant access to the instrument to enable this remote diagnostics feature.

Description of functionality

BD Assurity Linc can continually monitor the health of your instrument and automatically communicate any changes to the BD Technical Support Server. When problems or questions arise, key data is already available for diagnosis by BD, which can speed up troubleshooting efforts.

With your explicit authorization, the BD support representative can see what you see on-screen, and in many cases, can make adjustments or suggestions that prevent downtime and the need for a service call.

When an on-site visit is needed from a BD Field Service or Technical Applications Support engineer, the system logs and alarms can be checked before they leave the BD office, helping to ensure that the right personnel and the right parts are dispatched to your site.

Procedure

To grant access to a BD technical support representative:

1. Ensure that your workstation is connected to the internet.
2. Contact your local BD technical support representative.

   If a remote session is required, the BD representative will initiate a session through a secure link.

   A dialog opens once the connection is established.

3. Acknowledge the request.

   The BD representative can now assist you.

More information

- Generating a system health report (page 481)
- Setting administration preferences (page 50)

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18

Cytometer configurations

This section includes the following topics:

Laser and detector configurations

Introduction

This topic describes the laser and detector configurations that are available and the optics and intended fluorochromes for each laser.

Description

There are two types of cytometer configurations: BD-defined and user-defined. The BD-defined configurations are based on the actual hardware components, including lasers and optics, that are installed on the system. The possible configurations are:

- 1-Laser (blue), 4-color (4-0-0)
- 2-Laser (blue, red), 6-color (4-2-0)
- 3-Laser (blue, red, violet), 8-color (4-2-2)

User-defined configurations are created when you install a new BD-approved filter holder. The system recognizes the new holder and automatically creates a new user-defined configuration. Only Administrators can create and manage user-defined configurations.

Default detector array setup

The following table shows the default setup for the detector arrays.

Laser	Wavelength (nm)	Detector	Mirror	Filter	Intended fluorochromes
Blue	488	A	752	783/56	PE-Cy7
		B	665	700/54	PerCP-Cy5.5, PerCP, 7-AAD
		C	605	Blank	N/A
		D	560	586/42	PE, Propidium Iodide
		E	507	527/32	FITC, Alexa Fluor® 488
		F	N/A	488/15[a]	SSC

Laser	Wavelength (nm)	Detector	Mirror	Filter	Intended fluorochromes
Red	640	A	752	783/56	APC-Cy7, APC-H7
		B	660/10	660/10	APC, AlexaFluor® 647
		C	Blank	Blank	N/A
Violet	405	A	500	528/45	V500, AmCyan, Pacific Orange™
		B	448/45	448/45	V450, Pacific Blue™

a. There is a 10% neutral density filter installed in front of the SSC filter.

More information

- Cytometer overview (page 23)
- Creating a new optical configuration (page 540)

Creating a new optical configuration

Introduction

This topic describes how to create a new optical configuration.

About creating configurations

You can create a new optical configuration by removing one of the existing filter holders from a heptagon and replacing it with an optional BD-approved filter holder. You must have administrator permissions to create new configurations.

Creating a new configuration

To create a new optical configuration:

1. In the **Setup & QC** workspace, click the **Configurations** tab.
2. Open the front door to access the heptagon detectors.
3. Remove the filter holder that you want to replace by pulling the holder out of the heptagon.

   See the figure in Optical components (page 26).
4. Install a BD-approved filter holder by inserting it into the empty position in the heptagon.

   The software creates a new configuration.
5. Close the front door.
6. Add the new fluorochromes to the fluorochrome list in the **Configuration** tab if necessary.
7. Assign the new fluorochrome to the correct detector by selecting the name from the fluorochrome list at the right and dragging it into the correct detector list.

   The configuration is displayed in the list on the left under **User** configurations. Icons are displayed indicating that characterization QC and Reference Settings have not been performed for this configuration.
8. Select the **Setup & QC** tab and select the **Characterization QC** task, then follow the software prompts.

   See Running characterization QC (page 83).

9. Select the **Performance QC** task and follow the software prompts.
10. Create reference settings for the new configuration.

    See Creating reference settings for a new configuration (page 541).

Restoring the original configuration

To restore the original configuration:

1. Remove the new filter holder from the heptagon.
2. Install the original filter holder in the heptagon.

   The system detects the original holder and restores the configuration.

More information

- Laser and detector configurations (page 538)
- Cytometer overview (page 23)

Creating reference settings for a new configuration

Introduction

This topic describes how to create LW/LNW reference settings after you have created a new optical configuration.

Before you begin

Prepare the FC beads single-color controls according to the instructions in the technical data sheet. Make sure the kit information is entered into the FC Bead reagent section in the library.

Procedure

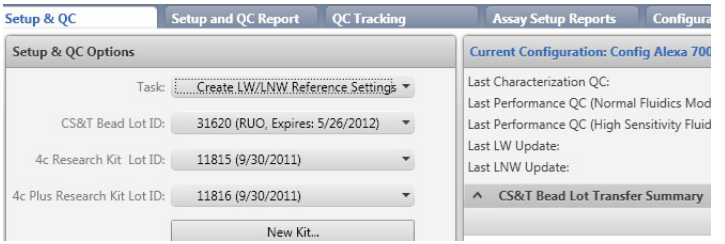
To create LW/LNW reference settings:

1. Log in to the software as Administrator.
2. Select the **Setup & QC** tab.

3. In the **Task** menu select **Create LW/LNW Reference Settings**.

   The **Create LW/LNW Reference Settings** task is only available to administrators after a new configuration has been created.

4. Select the correct lot IDs for the CS&T beads and the FC bead kits.



5. Click **Start,** follow all software prompts, and load the correct bead tube as required.

   Progress is indicated in the **Setup Tasks** panel as shown in the following figure.

6. Click **OK** in the confirmation dialog.

   Note that if the new fluorochrome has been added to the Blue detector array in position B when LW/LNW Reference Settings are created, only LW reference settings are created. LNW reference settings use PerCP, detector B, as the primary threshold. Since this PerCP will not be added to detector B for use with the new filter/mirror pair, the software cannot set the threshold required for LNW.

Working with configuration reports

Introduction

This topic describes how to use the Configuration tab to view optical configurations, modify the display, and view configuration reports.

Viewing a configuration

To view the configuration:

1. In the **Setup & QC** workspace, click the **Configurations** tab.

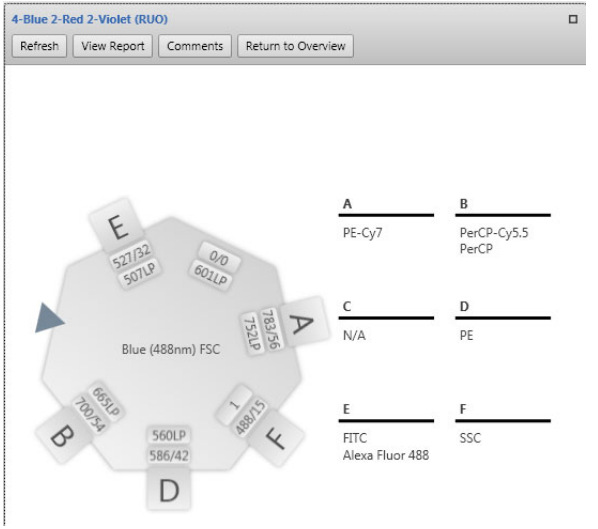
   The **Configurations** tab includes the following panels and dialogs:

   - **Configurations.** This panel lists the current cytometer optical configuration and any user-defined configurations.
   - **Configuration Detail.** This panel displays the optical configuration and shows the assigned detectors and fluorochromes.
   - **Fluorochromes.** This panel lists the available fluorochromes. You can select fluorochromes from this list. This is for administrators only, not available to operators.
   - **Filters and Mirrors.** This panel lists the filters and mirrors available in the system. This is for administrators only, not available to operators.

Zooming in on a configuration

To zoom in on the Configuration Detail panel:

1. Double-click a heptagon to zoom in on it.



2. Click **Return to Overview** to return to the default size.

Adding comments to a configuration report

To add comments to a configuration report:

1. Click **Comments** on the **Configuration Detail** toolbar.

   The **Comments** dialog opens.

2. In the **Comments** field, type a comment (up to 300 characters).

3. Click **Save** to save your comment and close the dialog.

   The comment is saved to the report. It cannot be modified or deleted.

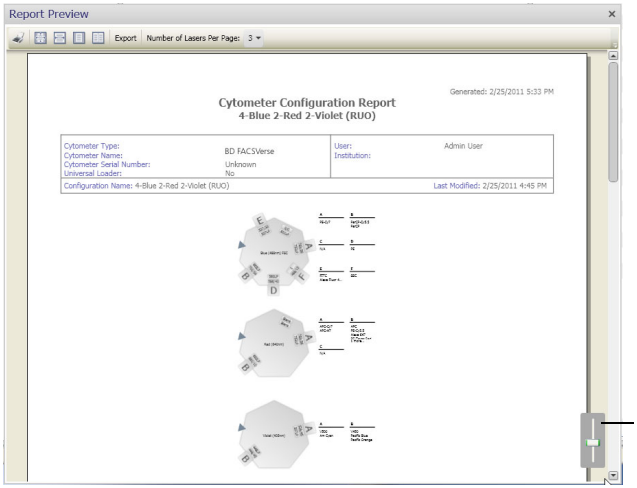
Viewing a cytometer configuration report

To view a cytometer configuration report:

1. In the **Configuration Detail** panel, click **View Report**.

   The **Report Preview** dialog opens and displays the current configuration report.

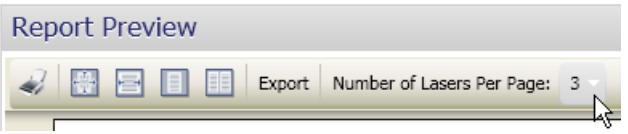
2. To zoom the report in or out, use the slider at the bottom right of the dialog.



Selecting the number of lasers per page

To select the number of lasers to show on each page of the report:

1. Open a report in the **Configuration Detail** panel.
2. On the **Report Preview** toolbar, click in the **Number of Lasers Per Page** field and select 1, 2, or 3.



The report displays the selected number of lasers on each page of the report.

Exporting a report

To export a report:

1. Open a report in the **Configuration Detail** panel.
2. On the **Report Preview** toolbar, click **Export**.

   The **Save As** dialog opens.
3. Type a name for the file you want to export.
4. Navigate to the target folder, then click **Save**.

   The report is saved in PDF format.

More information

- Laser and detector configurations (page 538)
- Creating a new optical configuration (page 540)

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BD FACSVerse technical specifications

This section includes the following topics:

Technical specifications

Optical specifications

Parameter	Value
Excitation optics	
Excitation optical platform	Possible system configurations: • 1-Laser (blue), 4-color (4-0-0) • 2-Laser (blue, red), 6-color (4-2-0) • 3-Laser (blue, red, violet), 8-color (4-2-2)
Laser specifications	Blue laser • Wavelength: 488 nm • Optical power: 20 mW • Beam spot size: 9 µm x 63 µm Red laser • Wavelength: 640 nm • Optical power: 40 mW • Beam spot size: 9 µm x 63 µm Violet laser • Wavelength: 405 nm • Optical power: 40 mW • Beam spot size: 9 µm x 63 µm
Laser alignment	Fixed, with auto-alignment on demand
Emission optics	
Forward scatter detection	• Si-photodiode with built-in 488/10 band pass filter

Parameter	Value
Fluorescence and side scatter detection	• Reflective optics with single transmission bandpass filter in front of each PMT • High performance customized PMT modules for all fluorescence and SSC channels • Light collected by objective lens is delivered by fiber optics to specially designed Heptagon detector arrays • The cuvette flow cell is gel-coupled by refractive index-matching optical gel to the fluorescence objective lens (1.2 NA) for optimal collection efficiency.
Forward and Side Scatter Sensitivity	Enables separation of fixed platelets from noise.
Forward and Side Scatter Resolution	Scatter performance is optimized for resolving lymphocytes, monocytes, and granulocytes.
Side Scatter Resolution	Enables separation of 0.2-µm beads from noise.

Performance specifications

Parameter	Value	
Nominal acquisition rate	10,000 events per second	
Carryover	less than or equal to 0.5%	
Nominal fluorescence sensitivity in Normal mode	Channel	Qr (x 1000)[a]
	FITC	20
	PE	133
	PerCP-Cy5.5	13
	PE-Cy7	17
	APC	10
	APC-Cy7	7
	V450	47
	V500	17
Fluorescence resolution	Coefficient of variation PI: Area of <3%, full G_0/G_1 peak for propidium iodide (PI)-stained chicken erythrocyte nuclei (CEN)	
Fluorescence linearity	Doublet/singlet ratio of 1.95–2.05 for CEN stained with PI and excited with the 488-nm (blue) laser	

a. Qr is the relative fluorescence detection efficiency, used for describing the light collection efficiency of a detector, measured in assigned BD units (ABD units). One ABD unit, for a given fluorochrome, is defined as the fluorescence of one antibody bound to a $CD4^+$ cell.

Fluidics specifications

Parameter	Value
Flow cell	Stainless steel with low coefficient of thermal expansion for predictable, stable performance
Cuvette internal cross-section	430 µm x 180 µm
Sample flow rates	• Low: 12 µL/min • Medium: 60 µL/min • High: 120 µL/min • High sensitivity: 50 µL/min
Fluid capacity	Standard 5-L tanks, optional 10-L tanks, 20-L sheath cubitainer adapter available
Sheath core stream fluid velocity	• Normal: 5.4 m/s • High sensitivity: 2.7 m/s
Sheath fluid consumption	• Normal: 13.6 mL/min • High sensitivity: 6.6 mL/min
Integrated cleaning cycles	Daily Clean, Monthly Clean, SIT flush
BD Flow Sensor option	Used for volumetric measurement

Sample input formats

For information on BD part numbers for tubes, racks, and plates, see the BD FACSVerse section of the BD Biosciences website.

Parameter	Value
For use on the manual tube port	
Tubes	• BD Falcon 5 mL (12 x 75-mm) polystyrene • BD Falcon 5 mL (12 x 75-mm) polypropylene • BD Trucount™ 5 mL (12 x 75-mm) • BD Falcon 15 mL[a] • BD Falcon 50 mL[a] • Microcentrifuge 2 mL[a]
For use with the Loader	
Tube racks	• 30-tube rack (12 x 75-mm tubes) • 40-tube rack (12 x 75-mm tubes)
Plates	• 96 standard height, round, polystyrene • 96 standard height, flat, polystyrene • 96 standard height, round, polypropylene • 96 standard height, conical, polypropylene • 384 standard height, flat, polystyrene • 96, half deep, conical, polypropylene • 96, deep, conical, polypropylene • 96, matrix tube • 96, filter bottom, polypropylene

a. When used with an adapter

Electronics and software specifications

Parameter	Value
Software	BD FACSuite software version 1.0 or later
Operating system	Windows 7 Professional
Data resolution	Uncompensated data has a range of 0 to 262143, which is 18 bits
FCS format	FCS 3.0 for export FCS 2.0 and 3.0 for import

Physical specifications

Parameter	Value
Operating temperature	The cytometer has an operating range between 15°C (59°F) and 30°C (86°F). We recommend that the lab temperature fluctuate less than 5°C within a day for best operation.
Humidity	The operating humidity tolerance is between 5% and 95% relative humidity (non-condensing).
Dimensions (W x D x H)	
Cytometer	63.3 x 57.9 x 57.9 cm (24.93 x 22.8 x 22.8 in.)
Cytometer with standard tanks	85.2 x 57.9 x 57.9 cm (33.5 x 22.8 x 22.8 in.)
Cytometer with standard tanks and Loader	107.2 x 57.9 x 57.9 cm (42.2 x 22.8 x 22.8 in.)
See the *BD FACVerse System Site Preparation Guide* for additional information on dimensions and clearances.	
Weight	Cytometer: 55.0 kg (121 lb) Loader: 13.2 kg (29 lb)

Parameter	Value
Voltage	100–240 ±10% VAC
Frequency	50–60 ±10% Hz
Current	2 A
Power	150 W
Heat dissipation	Less than 430 BTU/hour at ambient temperature with the cytometer and Loader running.
Noise	Less than 65 dBA over 8 hours under normal operating conditions with the cytometer and Loader running.

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Troubleshooting

This section includes the following topics:

Cytometer troubleshooting

Introduction

This topic describes possible problems and recommended solutions for cytometer issues.

Cytometer will not turn on

Possible causes	Recommended solutions
Power cord disconnected from wall socket or cytometer	Reconnect the power cord to the wall socket or cytometer.
Cytometer circuit breaker off	Switch on the cytometer circuit breaker, followed by the cytometer power. See Cytometer overview (page 23) for a figure showing the location of the circuit breaker.

Cytometer on but no response to software commands

Possible causes	Recommended solutions
Bad keyboard or mouse connection	Check the keyboard or mouse connections to computer.
Communication failure between computer and cytometer	1. Turn off the computer and the cytometer. 2. Reseat the Ethernet cable, located above the power cord on the right side of the cytometer. 3. Turn on the cytometer, then the computer.
User-installed firewall	• Contact your system administrator to open a port in the firewall for the cytometer. • Contact BD Biosciences technical support.
Cytometer initialization failed.	Run the **Cytometer > Initialize** command.

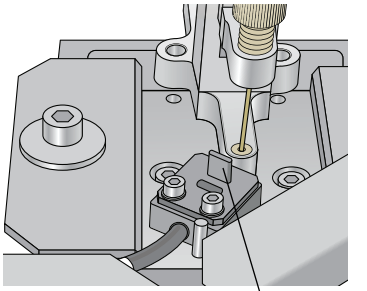
Cytometer did not complete initialization when using pre-programmed start up.

Possible causes	Recommended solutions
BD FACSuite software was turned off	Leave BD FACSuite software running at the end of the day.
Cytometer circuit breaker off	Switch on the cytometer circuit breaker, followed by the cytometer power. See Cytometer overview (page 23) for a figure showing the location of the circuit breaker.

Tube does not fit securely on manual tube port

Possible causes	Recommended solutions
Wrong tubes used	Use only qualified tubes. See Qualified tubes (page 29).
Tube not seated properly	Push the tube all the way up into the manual tube port.
Defective or cracked tube adapter	Replace the tube adapter.
Manual tube port is dirty	Clean manual tube port. See Cleaning the manual tube port (page 483).

Status indicator on manual tube port stays off when tube is removed

Possible causes	Recommended solutions
Tube sensor could be stuck	• Remove and reinsert the tube several times. • Clean the manual tube port. See Cleaning the manual tube port (page 483).
	1. Turn off the cytometer power. 2. Use a Phillips screwdriver to open the SIT door. 3. Push down gently on the tube detector tab to free it. See the following figure. 

Low event rate

Possible causes	Recommended solutions
Threshold too high	Lower the threshold.

Possible causes	Recommended solutions
Improperly mixed sample	Mix the sample to suspend cells.
Diluted sample	• Concentrate the sample • Increase the flow rate
Clogged or kinked sample injection tube	Perform tasks individually in this order, checking after each to see if the problem is resolved. • Run a SIT flush several times. • Run Drain and Fill Flow Cell. • Run Daily Clean. • Run Monthly Clean. • Replace sample line. See Replacing the sample line (page 485).

High event rate

Possible causes	Recommended solutions
Threshold too low	Increase the threshold.
Threshold is set as OR instead of AND	Adjust the threshold, or select AND instead of OR.
Concentrated sample or flow rate too high	Decrease the sample flow rate or dilute the sample.

Erratic event rate

Possible causes	Recommended solutions
Air bubble in flow cell	• Run a SIT flush (multiple times if needed) • Run Clean Cuvette. • Run drain and fill flow cell.
Sheath filter is dirty	Replace the sheath filter.
Clogged, bent, or kinked sample injection tube	Perform tasks individually in this order, checking after each to see if the problem is resolved. • Run SIT flush several times. • Run Drain and Fill Flow Cell. • Run Daily Clean. • Run Monthly Clean. • Replace the sample line. See Replacing the sample line (page 485).

High CVs

Possible causes	Recommended solutions
Air bubble in flow cell	Perform tasks individually in this order, checking after each to see if the problem is resolved. • Run a SIT flush (multiple times if needed) • Run Clean Cuvette. • Run Drain and Fill Flow Cell.
Sample flow rate too high	Decrease the flow rate.
Dirty flow cell	Perform tasks individually in this order, checking after each to see if the problem is resolved. • Run clean cuvette. • Run Daily Clean. • Run Drain and Fill Flow Cell. • Run Monthly Clean.
Improper sample preparation	Verify the sample preparation technique.
Air in sheath filter	Run Purge Sheath Filter.
Problem with sheath pressure	Verify that the bypass assembly was not left in place after running Monthly Clean.
Laser alignment needed	Run Laser Setup.

Low laser power indication

Possible causes	Recommended solutions
Laser power output is below requirement	Contact BD Biosciences technical support.

Performance QC fails after doing monthly clean

Possible causes	Recommended solutions
Bypass assembly still in place	Remove the bypass assembly and install the sheath filter.
Residual bleach in system because tank not rinsed thoroughly	Make sure to thoroughly rinse the bleach from the sheath tank during the procedure.

Fluidics error after replacing sheath fluid or emptying waste

Possible causes	Recommended solutions
Status change not yet detected by software	• After replacing or emptying fluids, wait until the software detects a status change before restarting acquisition. • Check the waste tank connection.

Droplet forms at tip of SIT

Possible causes	Recommended solutions
Air bubble in flow cell	Perform tasks individually in this order, checking after each to see if the problem is resolved. • Run a SIT flush (multiple times if needed) • Run Drain and Fill Flow Cell.

Liquid leakage around cytometer base

Possible causes	Recommended solutions
Sheath filter connections not secure after changing filter.	Check connections at top and bottom of filter.
Interior valve failure	1. Turn off the cytometer power. 2. Clean up the liquid, using proper precautions. 3. Contact BD Biosciences technical support.

Barcode reader error

Possible causes	Recommended solutions
Dirty barcode reader window	Clean the barcode reader window with isopropyl or ethyl alcohol and try again.
Blurred or damaged barcode label	Try scanning with a duplicate label (if available), or enter the data manually.

More information

- BD FACSuite software troubleshooting (page 564)
- BD FACS Universal Loader troubleshooting (page 573)

BD FACSuite software troubleshooting

Introduction

This topic describes possible problems and recommended solutions for software issues.

Software does not connect to cytometer

Possible causes	Recommended solutions
Cytometer not turned on	Turn on the cytometer.
Cytometer initialization failed	Run **Cytometer > Initialize.**
Internal firmware error	Switch the cytometer power off and then on again.
Waste tank not connected properly	Check to see that waste tank connector is securely installed on the tank.
Undetermined cause	Exit the software, shut down the computer and cytometer, and then restart them.

Software message "Security key not accessible..."

Possible causes	Recommended solutions
Security key not installed in USB port	Install the security key in the USB port, then restart the software.
Security key damaged	Contact BD Biosciences technical support.

Software not responding

Possible causes	Recommended solutions
Saving or loading large data file	Look for screen activity. If there is no activity, wait 1–2 minutes, and then restart the software.
Calculating large number of statistics	Calculating statistics is memory intensive. When calculating many statistics on a large number of displayed events, wait 1–2 minutes before using the software.
Waiting for response from cytometer	Wait until the cytometer action is complete. If after 2 minutes no time-out is received, restart the cytometer and software.
Software frozen	1. Press Ctrl+Shift+Esc. Locate BD FACSuite software in the Windows Task Manager. Click **End Task**. If acquisition is in progress, data will be lost when you click **End Task**. 2. Restart the software.

Excessive amount of debris in plots

Possible causes	Recommended solutions
Threshold too low	Increase the threshold.
Stained sample too old	Improper sample preparation. Verify the sample preparation technique.

Distorted populations or unexpected pattern in plot

Possible causes	Recommended solutions
Cytometer settings adjusted incorrectly	Optimize the scatter or fluorescence parameters.
Air bubbles in flow cell	Perform tasks individually in this order, checking after each to see if the problem is resolved. • Run SIT flush (multiple times if needed) • Run clean cuvette. • Run drain and fill flow cell.
Flow cell dirty	Perform tasks individually in this order, checking after each to see if the problem is resolved. • Run clean cuvette. • Run daily clean. • Run drain and fill flow cell. • Run monthly clean.
Flow rate is too low (sample is viscous or fluidics is overcoming an air bubble).	Raise to next highest flow rate until events are seen, then switch back to the desired rate.
Flow rate is too high	Lower the flow rate.
Incorrect spillover values	Verify that correct tube settings and reference settings are applied.
Incorrect gating	Verify the gating.

No events in plots after clicking Preview, acquisition indicator light is blinking as expected

Possible causes	Recommended solutions
Current run pointer is not set on current tube	Set the run pointer on the appropriate tube.
Viewing plots for a different tube	The primary data source is set to a different tube in Plot Properties dialog. Set it to the correct tube or set the run pointer as the primary data source.
Threshold not set to correct parameter	Set the threshold to the correct parameter for your application.
PMT voltage too low or too high	Adjust the voltage to bring events back on scale.
Threshold too high	Adjust the threshold.
Improper sample preparation	Verify the sample preparation technique.

Fluorescent signal missing

Possible causes	Recommended solutions
Area scaling set incorrectly	• Re-run performance QC. • Adjust area scaling.
Laser delay incorrect	Re-run performance QC.
Incorrect sample preparation	Re-stain and re-run samples.

Fewer events than expected in gated population

Possible causes	Recommended solutions
On-axis events left out of gate	Include events on the axis.
Gates improperly set	Check the gating strategy.
Stopping criteria not set correctly	Verify that the stopping criteria are set correctly.

Possible causes	Recommended solutions
Dirty flow cell	Perform tasks individually in this order, checking after each to see if the problem is resolved. • Run clean cuvette. • Run drain and fill flow cell. • Run daily clean. • Run monthly clean.
Sheath filter is dirty	Replace the sheath filter.
Incorrect sample preparation	Verify the sample preparation is correct.

High electronic abort rate (>10% of system event rate)

Possible causes	Recommended solutions
Event rate too high	Decrease the flow rate.
Sample too concentrated	Dilute the sample.
Threshold set too low	Increase the threshold.
Window extension could be set too high	Adjust the window extension.

Increasing threshold results in decreased area signal

Possible causes	Recommended solutions
Window extension set too low	Increase the window extension.

Area or height measurement off scale, while the other is on scale

Possible causes	Recommended solutions
Area scaling not set correctly	Adjust the area scaling so area and height are equal.

Optical filter holder not recognized

Possible causes	Recommended solutions
Filter holder not installed completely after removal	Press the holder firmly into the heptagon to ensure that it is installed correctly.
Incorrect filter holder used	Check the filter holder to confirm that it is an approved BD filter holder.
Filter holder broken	Contact BD Biosciences technical support.

FCS files or Entry Run Packages are not exported automatically when running a Worklist

Possible causes	Recommended solutions
Action not enabled in Preferences	• Ensure you enabled the preference to export files automatically through **Tools > Preferences > Worklist Preferences > Export.** • Ensure the location you selected for the files exists and you have write access to it.
The entry is not approved	Ensure that the entry is Approved. Files are only exported automatically when they are approved. You can enable automatic approval for your assays in the Library.

Results are not exported automatically when running a worklist

Possible causes	Recommended solutions
Various causes	• Ensure that Include in Auto-Export is enabled for one of the statistics views in the experiment that is used to create the assay. You can enable this option through the General Tab in the Properties view of the Statistics view. • Ensure you selected results to export for your assay in the assay properties in the Library. • Ensure you enabled the preference to export results automatically through **Tools > Preferences > Worklist Preferences > Export > Result**. • Ensure the location you selected for the result files exists and you have write access to it. • Ensure that the entries are Approved. Results are only exported automatically when they are approved. You can enable automatic approval for your assays in the Library.

Failed CRC value check error message when importing an FCS file

Possible causes	Recommended solutions
BD FACSuite software uses a CRC value mechanism that is specified in the FCS 3.0 standard to ensure that the FCS file has not been modified through file transfers.	When a BD FACSuite FCS file has been modified, the file cannot be read by BD FACSuite software. You must return to the original FCS file.
The FCS file does not adhere to the FCS 3.0 standard, which requires a CRC value to be written with the data.	Export the FCS file from an FCS 3.0 compliant application.

Incorrect password message

Possible causes	Recommended solutions
Password has been forgotten	Contact your administrator to change your password. Your administrator can provide you with a new temporary password through **Tools > User Management**. When you log in again, you will be asked to provide your new password.

Incorrect cytometer settings for an imported assay

Possible causes	Recommended solutions
Missing Assay Setup settings for your cytometer	After importing an assay you need to run Assay Setup for that assay using the **Setup & QC** workspace.

Fluorochrome is not assigned to a configuration message during data acquisition

Possible causes	Recommended solutions
The fluorochrome is not assigned to a detector in the current configuration.	1. Assign the fluorochrome to the configuration through **Setup & QC > Configurations**. This is an administrator-only task. 2. In an experiment, add the fluorochrome to the reference settings.

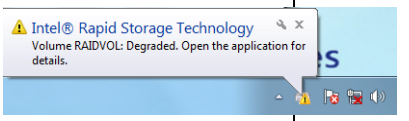
Cannot move an individual point of a polygon gate

Possible causes	Recommended solutions
Not selecting the gate correctly	Use Ctrl+click on the gate. This selects the gate and shows the individual points. Now you can drag a point to a different location.

Cannot paste the picture of statistics view into Excel

Possible causes	Recommended solutions
Wrong paste option	In Microsoft Excel, use Paste Special and select to paste a bitmap.

Non-BD FACSuite message displayed in system tray

Possible causes	Recommended solutions
A message similar to this appears: 	There could be a problem with one of the hard drives. Call BD Biosciences.

More information

- Cytometer troubleshooting (page 556)
- BD FACS Universal Loader troubleshooting (page 573)

BD FACS Universal Loader troubleshooting

Introduction

This topic describes possible problems and recommended solutions for Loader issues.

Carrier does not load

Possible causes	Recommended solutions
Cover not closed	Verify that the cover is fully closed.
Tube not fully seated in rack	Check all tubes to see they are seated in the rack and that the cover is fully closed.
Rack or plate not seated properly in the nest	Check that the rack or plate is centered in the nest and gripped securely at the corners.
Lid on plate	Remove the lid and retry.
Barcode label on rack or plate is dirty or wet, causing misread	Verify the barcode label is clean, dry, and able to be read.

System fails to initialize or nest does not return to home position

Possible causes	Recommended solutions
Stray object inside Loader preventing movement	Check the interior region around the nest for stray objects (tubes, tube caps, etc).

Tube not detected

Possible causes	Recommended solutions
Top of tube rack is dirty	Clean the top of the rack.
Top of tube is dirty	Verify that top edge of the tube is clean.
Incorrect tube	Verify that the tube is in the correct position.

Tube runs dry, Loader not advancing to next sample

Possible causes	Recommended solutions
Dilute sample or rare events	Set the acquisition max time or number of events to a lower value in software.
Bubble in flow cell diverts stream	• Run SIT flush several times. • Run daily clean. • Run drain and fill flow cell.

More information

- Cytometer troubleshooting (page 556)
- BD FACSuite software troubleshooting (page 564)

Glossary

A

ABD unit An acronym for assigned BD trackable fluorescence unit assigned to CS&T beads. One ABD unit is defined as 1/40,000th of the fluorescence of a lymphocyte stained with anti-CD4 for a particular detector/fluorochrome.

acquisition The process of collecting data from a flow cytometer and storing it in an electronic flow cytometry standard (FCS) file.

acquisition delay timer (worklist) A set lag time between previewing and acquiring a tube in a worklist. Before the time expires, a stop timer can be used to continue previewing and adjusting settings, such as voltage and threshold. The delay value is set as a worklist preference.

Administrator For BD FACSuite software, an assignable user role. Administrators can set up the instrument, create user accounts, and manage and save elements in all workspaces.

area scaling factor (ASF) A control used to determine the magnitude of the area signal. Usually used to set the area equal to the height for the particles of interest. During setup, the ASF determined is based on 3-µm beads.

analysis The use of gating strategies to define the populations in the data and display statistics about populations.

assay In BD FACSuite software, a set number of tubes that share the same tube settings, worksheets, and reports. Assays are reusable and are run by worklists using the Loader. The two types of assays include BD-defined assays and user-defined assays.

audit trail A software function that tracks changes to entries. Any changes that affect the data (for example, worksheet, reports, plots, gates, statistics) are listed as changes in the audit trail log.

B

batch analysis The automated analysis of a set of samples.

bead lot file The CS&T and FC bead identifier that can be scanned or downloaded.

BD Flow Sensor An optional hardware device that measures the acquired volume, which is used to determine cell counts in a sample.

BD FACS Universal Loader (Loader) An optional automated system that mixes samples and delivers tube racks and plates to the BD FACSVerse for acquisition.

BD FACSuite CS&T research beads Particles that are hard-dyed at three different intensities to emit in a broad spectrum that is detectable by all channels of the BD FACSVerse cytometer. BD FACSuite CS&T research beads are used with the software to automatically set up tube settings and assays, and characterize and track performance of the cytometer.

Br A relative optical background signal used for tracking optical background levels in a detector.

C

characterization QC The process that establishes target cytometer settings and sets pass or fail values for linearity, sensitivity, and %rCV.

coefficient of variation (CV) The standard deviation of the data divided by the mean of the data, typically expressed as a percentage (also known as relative standard deviation). When applied to channel data measured on a population of cells, the CV is a measure of variation independent of the population mean.

compensation A mathematical or electronic correction applied to flow cytometric data to account for the overlapping fluorescence emission from one fluorochrome into the wavelength region where the second is measured.

configuration A representation of the lasers, detectors, and mirrors that make up the cytometer's optics.

cytometer settings See *Instrument settings*.

D

detector A device that converts light signals into electronic signals. Photodiodes and photomultiplier tubes are the two types of detectors used in BD cytometers.

E

electronic noise (rSD) A statistical value in a particular detector used to predict the minimum acceptable signal levels required for the best attainable resolution and sensitivity for the system.

entry (worklist) A row in a worklist. Each entry includes a sample ID, tubes, and one task.

entry run package (ERP) A file containing all information needed to replicate an entry in a different worklist, including acquired data. Individual worklist entries can be exported as entry run packages (ERPs). You can import ERP files into any worklist and analyze the entry.

E-sig (e-signature) An electronic signature that can be used on reports to verify and approve an entry after acquisition or analysis.

event rate The number of particles per second detected by the cytometer.

experiment In BD FACSuite software, a group of elements used to acquire and analyze data from the flow cytometer. An experiment is flexible and can be saved as an assay for use in a worklist.

expression A mathematical equation included in the system, or created by the user, to produce a statistic derived from existing statistics.

F

FC beads Particles that are used to create or update reference settings for BD standard fluorochromes.

FCS The flow cytometry standard file format used to save flow cytometry data.

filter (optical) A device used to selectively block light of particular wavelengths or frequencies while allowing other wavelengths to pass through.

filter (fluidic) A device that removes particles from the sheath fluid.

flow rate The volume per second of the sheath and the sample that passes through the flow cell. The rates are high, medium, and low. High and medium flow rates typically can be used for immunophenotyping and to increase event throughput. The low flow rate is typically used when high precision is required (for example, DNA studies) to measure slight variations in fluorescence.

fluidic mode Variation in the speed that the sheath and the sample pass through the flow cell to obtain different separation. The normal sensitivity fluidics mode has three flow rates: high, medium and low. The high-sensitivity fluidics mode has only one flow rate and slows the sample and sheath flow to obtain better separation between negative and positive fluorescence populations.

fluorescence The emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength. In most cases, emitted light has a longer wavelength, and therefore lower energy, than the absorbed light.

forward scatter (FSC) A measurement of light refracting at less than 10° to the laser beam as a particle passes through the beam. The FSC measurement is an indicator of cell size.

G

gate A boundary in a plot that defines a subset of the total sample population. Gating identifies cells of interest, classifies events in the gated population, and calculates population statistics.

gate hierarchy A representation of the relationship of gates in an experiment or assay. The hierarchy shows the name, color, and relationships with other gates.

H

heptagon In the BD FACSVerse system, the assembly of mirrors, filters, and detectors in the cytometer.

I

instrument settings A collection of values describing the state of the cytometer for acquisition. These settings include photomultiplier (PMT) voltages, thresholds, delays, window extensions, area scaling, spillover values, sheath flow rates, and sample flow rate.

J

K

keyword A unique field used to identify particular data attributes in data files created by the flow cytometer. Keywords are used in tubes, entries, statistics, and expressions.

keyword value The alphanumeric text entered in the Value field.

L

label A marker associated with a fluorochrome. For example, for CD4-FITC, CD4 is the label.

laser delay A time adjustment in the software representing the amount of time it takes a particle to travel between lasers. This value is used to synchronize the processing and display of data for a single particle from multiple lasers. This value is measured and adjusted automatically during performance QC.

Levey-Jennings charts A graphical presentation of data points from multiple runs for a single parameter, intended for analysis of trends in cytometer performance.

library In BD FACSuite software, a workspace for storing and managing shared system resources. Resources include assays, beads, reagents, keywords, labels, and tube settings.

Linearity The acceptable linear range is the ratio of bright beads to dim beads across the detector response. If the mean of the ratio is greater than 2%, the results are not considered linear.

LW reference settings Cytometer settings that roughly place normal lysed whole blood lymphocytes on scale for FSC, SSC, and fluorescence parameters using an FSC threshold.

LNW reference settings Cytometer settings that roughly place normal lysed, unwashed whole blood lymphocytes on scale for FSC, SSC, and fluorescence parameters using a PerCP or PerCP-Cy5.5 threshold.

M

manual tube port A loading area that accepts individual tubes for acquisition. This port is located on the right front of the cytometer.

median fluorescence intensity (MFI) The fluorescence intensity value of the event in a defined population that has an equal number of events with fluorescence intensities higher and lower than it.

N

O

operator For BD FACSuite software, an assignable user role. Operators can use the instrument, and create, modify, and save their own elements in the software.

P

panel In BD FACSuite software, objects that contain buttons, fields, and selections to perform specific functions required for a workspace. You can maximize, minimize, or reposition a panel on the screen.

parameter Tube and plot properties that define the data to be acquired from each particle and the display of data in plots.

performance QC The set of automated software functions used to measure and track cytometer operation and to set up consistent LW and LNW tube settings.

plot A graphic representation of data acquired by a flow cytometer.

PMT voltage (PMTV) Acronym for photomultiplier tube voltage, the voltage for each detector in a cytometer configuration.

population A subset of events defined by a gate.

population view (hierarchy) A display of the relationship between groups (subsets) defined in each tube based on the gating strategy.

pre-programmed startup and shutdown (idle based shutdown) Settings that allow you to assign times for the system to automatically start and shut down after being idle. BD FACSuite software must remain on for preprogrammed startup to occur.

preview The process of receiving and displaying live data on a worksheet without saving an FCS file. You can modify instrument settings during preview.

process control A control sample run exactly as a patient specimen. Normally produces an expected result.

Q

QC A group of procedures that measure cytometer performance and prepare the system for daily use.

Qr (relative fluorescence) The detector efficiency defined as the number of photoelectrons measured per ABD unit.

R

rCV (%rCV) The percent robust coefficient of variation of a population. Used in the calculation of photon detection efficiency (Qr).

reagent A chemically reactive substance used to label, detect, measure, or produce other substances.

reference settings Tube settings and the associated spillover values that have been measured using fluorescence control tubes.

report A display of results and associated information. Reports can be saved or exported.

report delay timer (worklist) A set lag time between the acquisition of two entries in a worklist for viewing the report of the first entry. Before the time expires, use the stop timer to continue viewing the report and adjusting gates and statistics markers. The value is set as a worklist preference.

S

sample A single unit of material to be analyzed on a flow cytometer. Samples can be associated with one or more assays, divided into multiple tubes or wells, and mixed with substances such as reagents.

sample carrier A compatible tube rack or multiwell plate used to organize samples for increased throughput acquisition on the Loader.

setup & QC The group of functions and activities in BD FACSuite software that prepares the sample, control, and/or cytometer for operation.

sheath fluid A solution used in flow cytometers that carries and hydrodynamically focuses sample in the flow cell.

SIT (sample injection tube) The component that aspirates sample from a tube or a well and delivers it to the flow cell.

side scatter (SSC) The light scattered at a right angle to the incident light beam.

slope of gain The slope of the brightness versus voltage for bright beads (log MFI vs log PMT voltages) used in setup and QC.

spectral overlap The phenomenon of different fluorochromes emitting light within the same detection range.

spillover values (SOVs) The amount of spectral overlap calculated as the ratio of the primary signal in the channel to any interfering signal from another channel.

statistics view The calculated statistics displayed in a format that includes a title, keywords, expressions, and statistics for one or more populations and/or parameters. Statistics are associated with all gates and populations in plots on a worksheet.

stopping rules The criteria used by the system to automatically stop acquisition, (for example, time, number of total events, and number of target population events).

stop timer A countdown mechanism that allows you to pause an action (acquisition or reporting) to make changes to settings before resuming the action. The duration of the counter can be set as a worklist preference. See *acquisition delay timer* or *report delay timer*.

storage gate A boundary that defines which population will be saved in an FCS file.

system health report A text file containing the current status of the system. The report also includes a ZIP file containing log files and other files that can be used by a BD technical support representative to analyze and troubleshoot the system.

T

task An action that the cytometer performs when you run a worklist. Tasks include the assay (BD-defined or user-defined), or a fluidics action.

threshold A trigger signal and level of discrimination to eliminate unwanted events. Only events with parameter values above the threshold will be analyzed.

tube In BD FACSuite software, a representation of a physical tube that contains saved properties (for example, reagents, labels, FCS data, stopping rules, tube settings, worksheets, and keywords).

tube settings A collection of values that place the positive population at the same position (brightness) whenever the tube settings are applied. Tube settings allow the system to produce comparable results from day to day and from system to system.

tube target values (TTV) The ratio of median fluorescence intensity (MFI) to assigned BD unit (ABD) for a tube. TTVs determine the brightness of the positive population for each fluorescence parameter.

U

V

W

window extension The time added to the pulse duration above the threshold to give the total time during which a pulse is sampled.

worklist A series of tasks to be performed. A worklist organizes multiple entries, which include tubes, tasks, status, and other information about the sample.

worksheet An area within the software where plots, gates, statistics, and other elements are created and modified.

workspace A functional area within the software that contains panels, fields, tables, and tools required for a specific function. Individual workspaces are provided for setup and QC, experiments, worklists, and the library.

X

Y

Z