

# Stanford BD Discover S8 User Guide

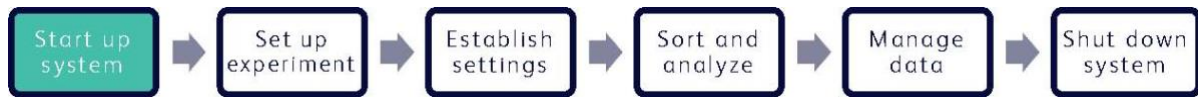
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## Read and Follow Before Starting the System

- If a daily shutdown was performed within the past 24 hours, perform a **daily fluidics startup** See page 2).
- If a daily shutdown was performed and a daily fluidics startup was not completed within 24 hours, the system recommends an **extended fluidics startup** (see Page 8).
- If the daily System Startup has been performed but the stream is off, insert a proper sort nozzle for your experiment, turn the stream on in the stream window. If sorting is needed, run drop delay as follows: go to **Cytometer** navigator > click **System Start Up** > skip until you see **Run Drop Delay** > load a tube of Accudrop beads onto the loading port > hit **Run Drop Delay**. When Accudrop is done, if performing aseptic sort, follow Preparation for Aseptic Sorting on page 10. If not, skip to page 11.

# Daily Fluidics Startup



## Before you begin

- Ensure that the in-house external air is supplied to the system between 5.5–5.9 Bar (80 and 95 psi).

## Starting up the system

### Turning on the system

1. Turn on the blower at least 3 minutes before powering on the cell sorter.
2. Set the sash to the correct height.
3. Verify that the laminar air flow is working.
4. Turn the AMS to Low during analyzing and sorting.
5. Press the power button on the right side of the upper cell sorter unit.

**NOTE** Ensure the instrument is switched on for at least 30 minutes before use.

6. Press the power button on the PC workstation, and log into Microsoft® Windows® with username “facsusers” and password “BDIS#2”.
7. Open BD FACSCorus™ Software by double-clicking the shortcut on the desktop and logging in with your username and password.



The software connects with the cell sorter within 5 minutes. The Connected status in the Status Indicator panel will initially be yellow when the software connects with the instrument. After the connection is established successfully, the Connected status turns green.

### Filling the sheath tank

1. Disconnect the clear airline by holding the top of the air line and pulling up on the lower air connector port to release the connection.
2. Vent the air pressure from the sheath tank by pulling the metal ring on the pressure-relief valve.
3. Unscrew the sheath tank cover knob and remove the cover.

4. Fill the sheath tank to the weld line with sheath fluid (DILUENT 2).

**NOTE** Do not overfill the sheath tank because it can cause incorrect sample flow rates.

5. Replace the cover and tighten the knob.

**NOTE** To avoid damage to the O-ring, do not over-tighten the knob to seal the lid.

6. Reconnect the air line by pushing the air line into the connector until you hear a click.



### Emptying the waste tank if it's 50% full or more

1. Disconnect the waste sensor and liquid waste line from the fluid storage cart's respective labeled ports and remove the tank.
2. Unscrew the disposable waste cap (large cap) and the attached waste trap from the tank. Place the assembly on the bench with the label side up.



**NOTE** Do not wet the cap on top of the trap.

3. Remove the waste tank from the cart, move it to the sink, wear a face shield or eye protection, empty the waste into the sink, turn on the tap water to rinse the sink for a few minutes.
4. Add approximately 1 L of undiluted bleach to the waste tank to deactivate biohazardous waste or enough so that 10% of the total volume is bleach.
5. Replace the disposable waste cap (large cap) and the attached waste trap, then tighten them by hand until they are closed.



**NOTE** Do not overtighten the trap or attached filter cap. Tighten each component only until it is hand-tight. Do not use sealants or adhesives.

6. Reconnect the waste liquid line and waste sensor to the respective ports on the fluid storage cart.

### Cleaning the workspace and machine

Decontaminate the front area inside the hood, the sample loading pot, sort chamber, collection chamber, the collection device and corresponding adapter, the splash guard (when sorting onto a plate) by wiping with Cavicide-wet paper towel.

Clean the deflection plates with Cavicide-wet Kimwipe. Full them out for cleaning if necessary.

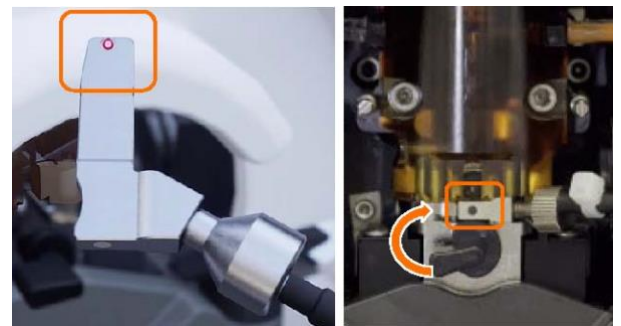
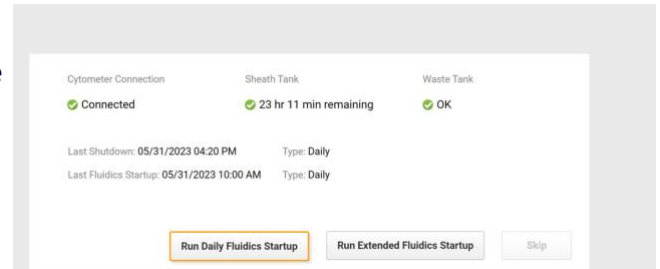
## Running a Daily Fluidics Startup

### Initiating a fluidics startup

1. Click **Run Daily Fluidics Startup**.

**NOTE** If no one used the machine in the past 24 hours, the system recommends an extended fluidics startup (see Page 8).

2. Follow the prompts on the screen through each numbered step.
  - a. At prompt 2, to prepare for cleaning, insert the closed-loop nozzle into the bottom of the flow cell cuvette with the O-ring facing up.
  - b. Close the sort chamber doors, then click **Continue**.
3. Click **Close** and then **Continue** to proceed to the next step in the Startup workflow: Cleaning.

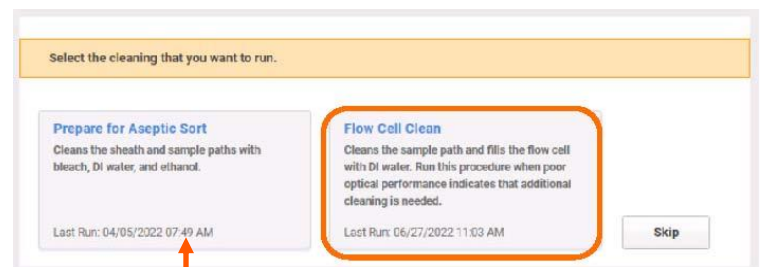


### Cleaning the flow cell

1. Click **Flow Cell Clean** and follow each numbered prompt in the guided workflow.

**NOTE** For additional cleaning, use 1.5% BD<sup>®</sup> Detergent Solution instead of DI water.

2. After cleaning is complete, click **Close**, then **Continue** to proceed to the next step in the Startup workflow: Sort Nozzle.



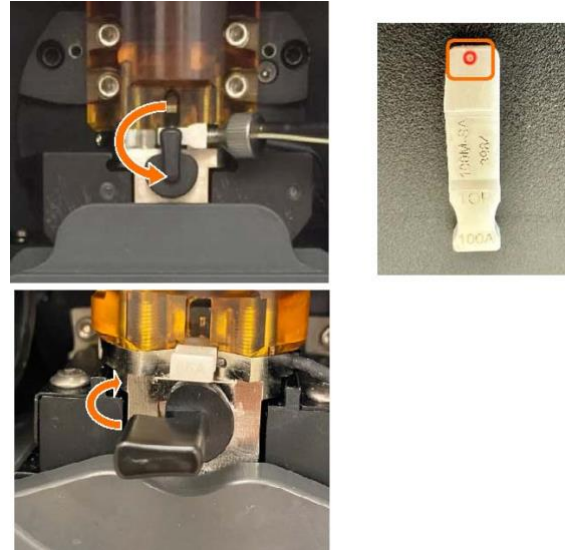
This function is not in use. If prepare for aseptic sort, follow page 16 after QC and drop delay calibration.

## Inserting the Sort Nozzle

1. Remove the closed-loop nozzle by turning the nozzle-locking lever counterclockwise to the 9 o'clock position, pull the nozzle straight out and place it in the holder.
2. Insert a sort nozzle straight into the bottom of the flow cell cuvette with the orange O-ring facing up.

### NOTE

- Choose a nozzle at least 4~5 times larger than your cells.
  - If your cells have low viability, use a larger nozzle.
  - If your cells are clumpy, use a larger nozzle and filter more times.
  - Always check the nozzle under the microscope before use, sonicate if necessary.
  - **Do not use 85um nozzle if sorting onto a plate because the overall yield may be low with 85um.**
  - **If preparing for aseptic sorting, soak the nozzle in a tube of 70% Ethanol for 5~10min to disinfect. Do not soak it over 30min, otherwise the O-ring will be trashed. Use a 70% Ethanol-wet green Q-tip to gently wipe the nozzle insertion before inserting.**
3. Turn the nozzle-locking lever clockwise to the 12 o'clock position, click **Continue** to start the stream, and proceed to the next step in the Startup workflow, Setup and QC.



## Running a Setup and QC

**Staff run Baseline:** A baseline setup must be run every 6 months, when changed to a new bead lot, or after a major service. A baseline run takes more than 10 minutes to complete.

**Users run Daily Performance:** A daily performance run should be performed each day you run the system to ensure that your cell sorter performs consistently over time. A daily performance run takes about 7–10 minutes to complete.

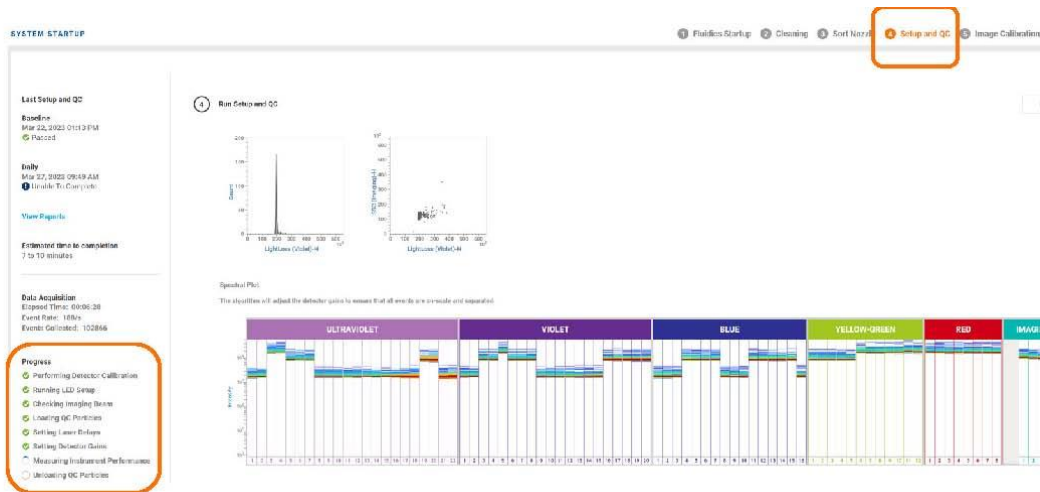
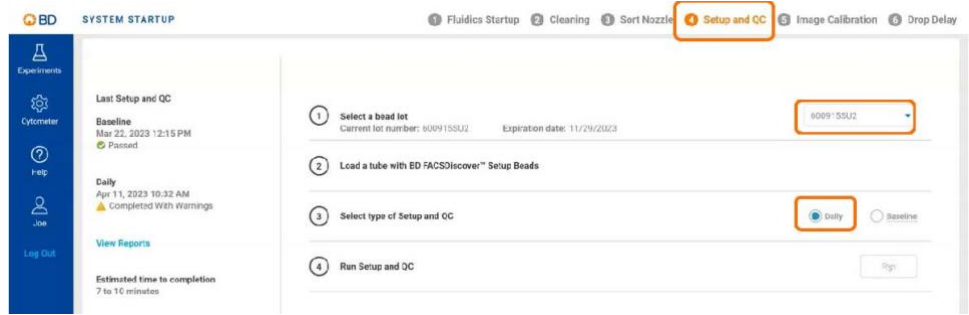
1. Prepare a tube of BD FACSDiscover™ Setup Beads: add 2 drops of beads into 0.5mL sheath fluid in a 5mL FACS tube. A tube of freshly made beads is good through 48h.

2. Check the bead lot to the top right, select the correct bead lot from the drop-down if needed.

3. Load the tube of BD FACSDiscover™ Setup Beads.

4. Close the sample loading and sort collection chamber door.

5. Select **Daily** and click **Run**.



The steps progress sequentially, and a spinning circle indicates the current step. When Setting Laser Delays begins, events start appearing on the plots. The system unloads the tube after the steps are completed or if there is an error during the setup process. If there's an error, contact the staff.

6. Click **Continue** to proceed to Startup workflow: Image Calibration.

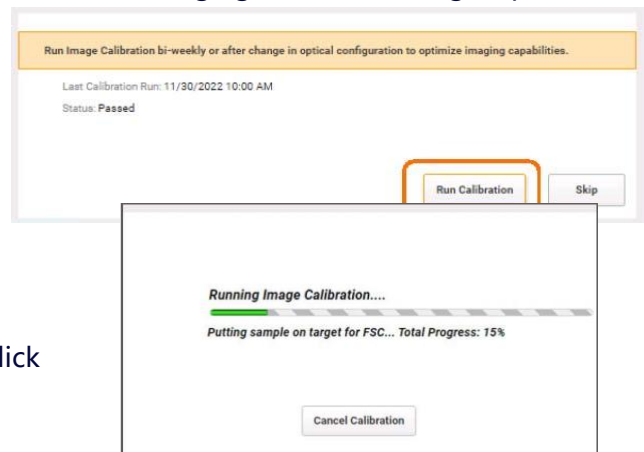
## Running the Image Calibration

BD CellView™ Calibration Beads are used to optimize the imaging capability of the BD FACSDiscover™ S8 Cell Sorter. Run the Image Calibration every two weeks or for certain imaging troubleshooting steps.

1. Prepare the BD CellView™ Calibration Beads: add 2 drops of beads into 0.5mL sheath fluid in a 5mL FACS tube.

2. Click **Run Calibration**.

3. Load a tube of BD CellView™ Calibration Beads and click **Continue**.



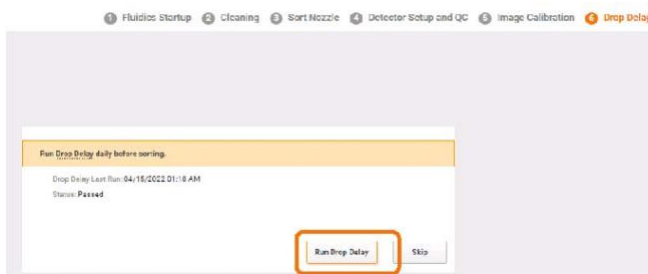
4. The system displays a progress bar and identifies the various stages of the process.

When the image calibration is completed successfully, a dialog is displayed; click **Continue** to proceed to Startup workflow: Drop Delay. If there's an error, contact the staff.

## Setting the Drop Delay

BD FACS™ Accudrop Beads automatically set an accurate drop delay value. **Run drop delay daily, or when you change the nozzle before a sort, or restart the stream.** This step can be skipped if no sorting is needed.

1. Prepare the BD FACS™ Accudrop Beads: add 1 drop of beads into 0.5mL sheath fluid in a 5mL FACS tube.
2. Click **Run Drop Delay**.
3. Load a tube with BD FACS™ Accudrop Beads and click **Continue**.



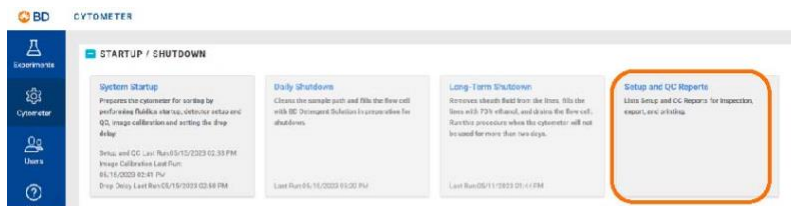
4. The system displays a progress bar and identifies the various stages of the process as they progress.

When the drop delay is completed, a completion dialog is displayed. If there's an error, contact the staff.

5. Click **Continue**.

## Viewing the Reports (Optional)

1. On the **Cytometer** page, click **Setup & QC Reports**.



**NOTE** A report is not generated if the Setup and QC fail to complete.

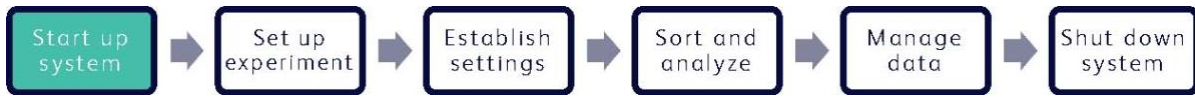
2. From the list of reports, click a report to open it.
3. Click **Export** to export the report as a PDF.
4. Select a folder to save the report and click **Save**.

Completed (DD/MM/YYYY)	Type	Sample Size	Run# (mID)	Status
04/18/2022 01:18 AM	Daily Check	100	6012E	Passed
04/07/2022 03:03 AM	Daily Check	100	6012E	Passed
04/07/2022 03:41 AM	Daily Check	100	6012E	Passed
01/24/2022 04:00 AM	Buickline Setup	100	6012E	Passed



**If aseptic sort is needed, perform the Preparation for Aseptic Sorting in page 10. If not, skip to page 11.**

# Extended Fluidics Startup



If a daily shutdown was performed and a daily fluidics startup was not completed within 24 hours, the system recommends an extended fluidics startup.

## Before you begin

- Ensure that the in-house external air is supplied to the system between 5.5–5.9 Bar (80 and 95 psi).

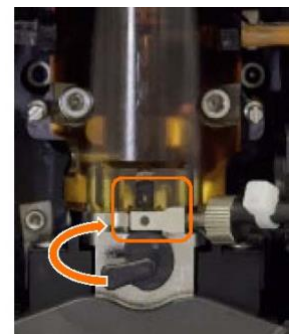
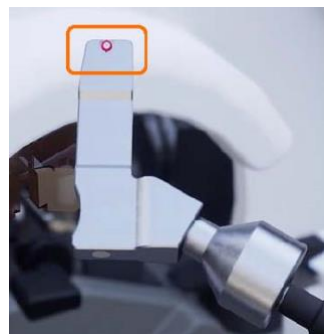
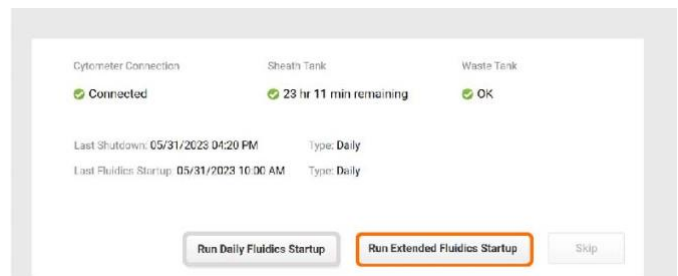
## Starting up the system

Follow [page 2 to page 3](#) to turn on the system, fill the sheath tank, empty the waste tank, clean the workspace and machine.

## Running An Extended System Startup

### Initiating a fluidics startup

1. Click **Run Extended Fluidics Startup**.
2. Follow the prompts on the screen through each numbered step.
  - a. At prompt 2, to prepare for cleaning, insert the closed-loop nozzle into the bottom of the flow cell cuvette with the O-ring facing up.
  - b. Close the sort chamber door, then click **Continue**.

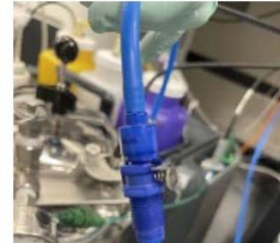


c. At prompt 3, to prepare the bottles, tanks and tubing connections for the extended cleaning cycle by doing the following:

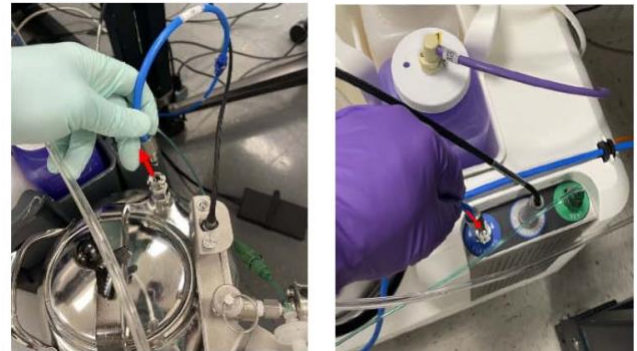
- i. Fill the white DI water bottle on the fluid cart with 1.2 L of sterile DI water.
- ii. Bypass the sheath filter by disconnecting the blue sheath line connectors from the top and bottom of the filter and connecting them together. Use a piece of foil to cover the sheath filter to prevent from dust.

**3** Prepare the tanks and tubing connections for the extended cleaning cycle

- Fill the DI water bottle with 1.2L of sterile DI water
- Bypass the sheath filter by disconnecting the sheath line connectors from the filter and connecting them together
- Disconnect the sheath line from the sheath tank and connect it to the Cleaning port on the fluid storage cart
- Empty the waste tank



- iii. Disconnect the blue sheath line from the sheath tank and connect it to the cleaning port of the fluid storage cart.
- iv. Double check to make sure the waste tank is emptied and 10% bleach is in.
- v. Click **Continue**.



d. At prompt 4, to prepare the tanks and tubing connections for normal operation.

- i. Make sure the sheath tank is filled with sheath fluid (DILUENT2).
- ii. Disconnect the sheath line from the cleaning port and reconnect it to the sheath tank.
- iii. Re-install the sheath filter into the sheath line.
- iv. Click **Continue**.

**4** Prepare the tanks and tubing connections for operation

- Fill the sheath tank with sterile, 1X PBS
- Disconnect the sheath line from the Cleaning port and reconnect to the sheath tank
- Re-install the sheath filter into the sheath line
- Empty the waste tank

e. At prompt 5, click **Continue** to start a sheath filter purge and prime the fluidics.

f. Click **Close** and then **Continue** to proceed to the next step in the System Startup workflow: Cleaning.

**Follow [page 4 to page 7](#) to complete cleaning the flow cell, inserting the Sort Nozzle, running a Setup and QC, running the Image Calibration, setting the Drop Delay.**

**If aseptic sort is needed, perform the Preparation for Aseptic Sorting in page 10. If not, skip to page 11.**

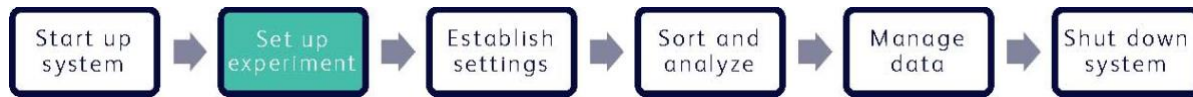
# Preparation for Aseptic Sorting

[Skip this procedure if not doing aseptic sort.](#)

1. Clean the sample line.
  - a. Load a tube containing 3 mL of a 10% bleach solution onto the sample loading port.
  - b. Click **Experiment** navigator, create a new experiment or open an existing experiment, from the **View Data** tab, set the Flow rate to 20, click **Load Sample**.
  - c. After 10 minutes, click **Unload Sample**.
  - d. Load a tube containing 3 mL of FACS Rinse onto the sample loading port, click **Load Sample**.
  - e. After 1 minutes, click **Unload Sample**.
  - f. Load a tube containing 3 mL of Sterile DI water onto the sample loading port, click **Load Sample**.
  - g. After 5 minutes, click **Unload Sample**.
2. Click **BACKFLUSH** to back flush the sample line: Click **Start** in the pop-out window, when it finishes, close the window.

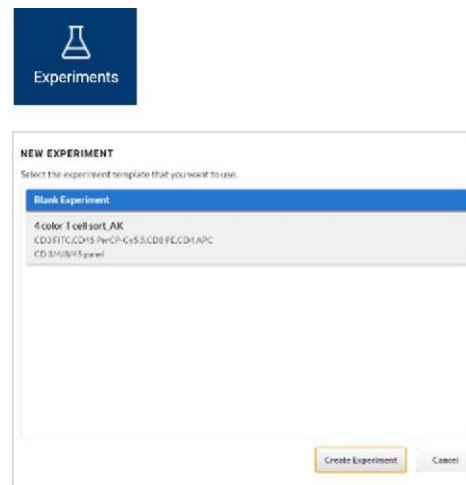


# Create An Experiment in BD FACSCorus™ Software



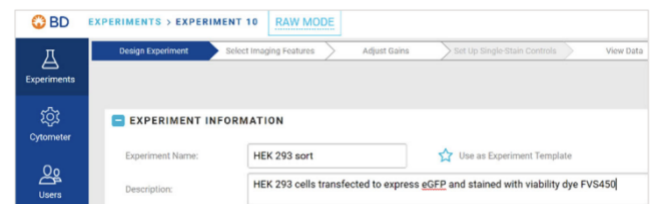
## Adding an experiment

1. Click **Experiments** on the navigation bar.
2. Click **+New Experiment**.
  - If you have not created templates, a new, default experiment is created.
  - If you have previously created templates, a New Experiment dialog with a list of templates appears. Select **that template you want to use**, then click **Create Experiment**.
  - If you don't want to use any template, select **Blank Experiment**, then click **Create Experiment**.



## Designing the experiment

1. Enter an experiment name under Experiment Information.
2. Enter a description, if needed.
3. In the **Select Your Dyes panel**, expand the laser rows to view the fluorochromes.
4. Select the fluorochrome names needed for your experiment.



**TIP** You can add additional fluorochromes at the bottom of the list. Click the **+** icon and enter the new fluorochrome name and maximum emission.

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

**NOTE** A maximum of 64 total fluorochrome and auto fluorescence controls can be unmixed.

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

**NOTE** You do not need to add spectral dyes separately.

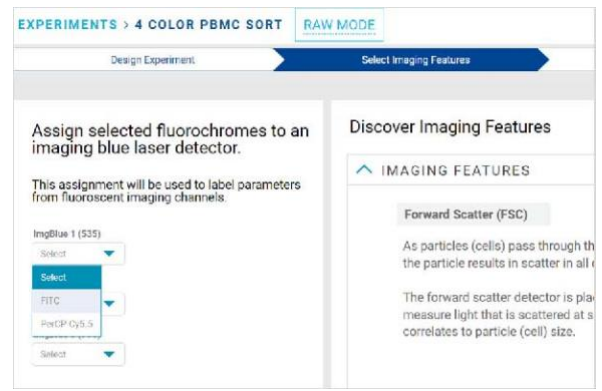


**NOTE** Label names must be unique within the experiment.

## Selecting imaging features

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

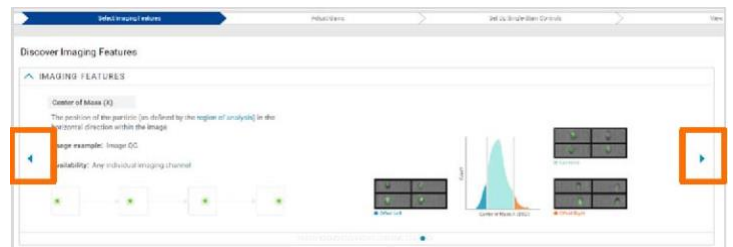
1. Click the **Select Imaging Features** tab.
2. In the drop-down menus, assign a selected fluorochrome to the appropriate imaging detector.
  - a. Select ImgBlue 1 (535) for a fluorochrome emitting between 511–557 nm.
  - b. Select ImgBlue 2 (600) for a fluorochrome emitting between 570–630 nm.
  -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



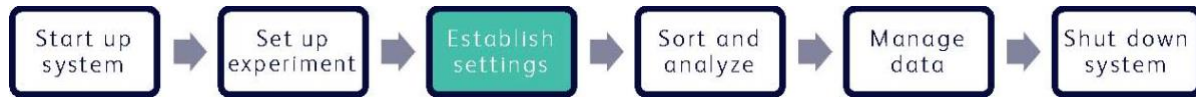
**NOTE** Forward Scatter (FSC), Side Scatter (SSC), and Light Loss (Imaging) detectors are available by default and cannot be deselected.

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

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



# Adjust the Settings for An Experiment



## Adjusting the plots, gains, and gates

1. Click the **Adjust Gains** tab.

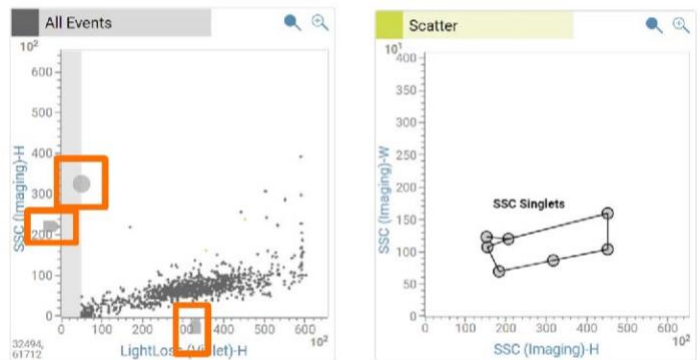


2. Load a fully stained sample on the sample loading port.
3. In the dashboard, click **Load**.
4. Adjust the plot zoom, scatter gains, threshold, and gates to encompass cells of interest.

- a. Zoom in on a plot by clicking the plot, then rolling the mouse scroll wheel upwards. Roll downwards to zoom out.

- b. Adjust the scatter gains, if needed. Hover over the scatter plots axes then drag the gain sliders along the axes.

- c. Adjust the threshold by hovering over the y-axis of the first default plot and then dragging the threshold marker horizontally.

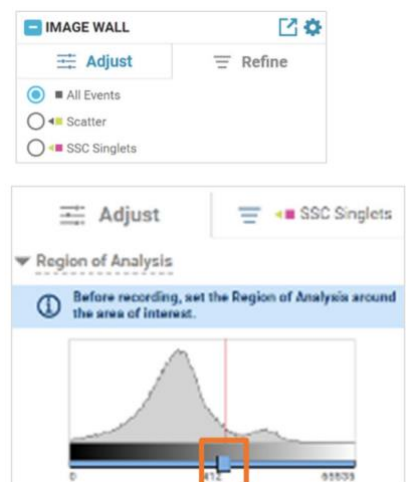


- d. Adjust the gates by dragging the vertices to encompass populations.

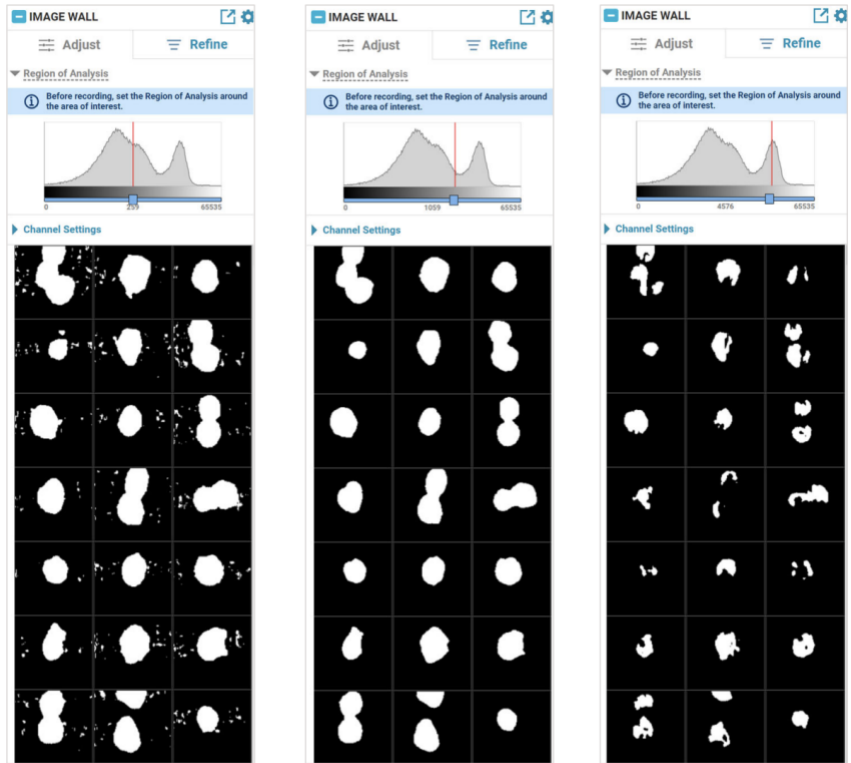
## Adjusting Region of Analysis (ROA) and spectral gains

1. (Optional) To conserve the sample, click **Pause** in the dashboard.
2. Adjust the **Region of Analysis (ROA)** in the Image Wall.

- a. In the **Refine** tab, select **SSC Singlets**.
- b. In the **Adjust** tab, move the ROA slider slowly until the white area on the images completely encompasses the events of interest while minimizing background.



**TIP** To adjust the slider in smaller increments, click the slider then press the arrow keys.



Example: ROA set too low (left), accurately (center) and too high (right).

3. If needed, click **Resume** to continue sample acquisition.
4. Verify that no channels are saturated on the spectral plot. Saturated channels are indicated by a colored box with the (!) icon, which displays at the bottom of the affected channel.

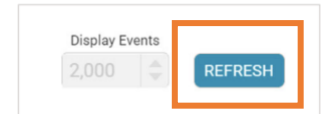


Lower the gains of any saturated channel.

- a. Click the down arrow on the top left corner of the spectral plot panel.
- b. Adjust the spectral plot gains under Gain Value:

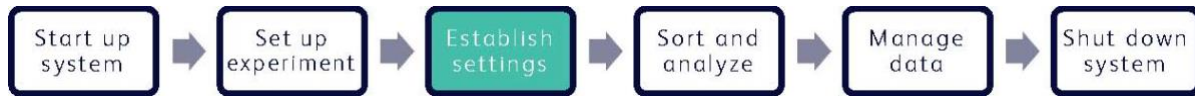
- Enter a new value for the channel, then press Enter.
- Select a channel, then scroll using your mouse scroll wheel.
- Click the + and – buttons to adjust gains for all channels in that laser.
- Click **Reset All** to set the gains of all channels to the default settings.

**TIP** After adjusting the gain, click **Refresh** in the dashboard to visualize the change.



5. Click **Unload** in the dashboard.

# Set Up and Record Single-stained Controls



## Before you begin

- You have adjusted your scatter and spectral gains and Region of Analysis (ROA) for your sample.
- Ensure that the ROA has been set up on the Adjust Gains page for the specific particles used in your fluorochrome controls.

## Working with the Set Up Single-Stain Controls tab

### Setting up single-stained controls

1. Click the **Set Up Single-Stain Controls** tab.

The page is automatically populated with control tubes based on the fluorochromes selected in the **Select Your Dyes panel**.

**NOTE** Autofluorescence can be added later using any unstained control.

2. Load a control tube on the sample loading port.

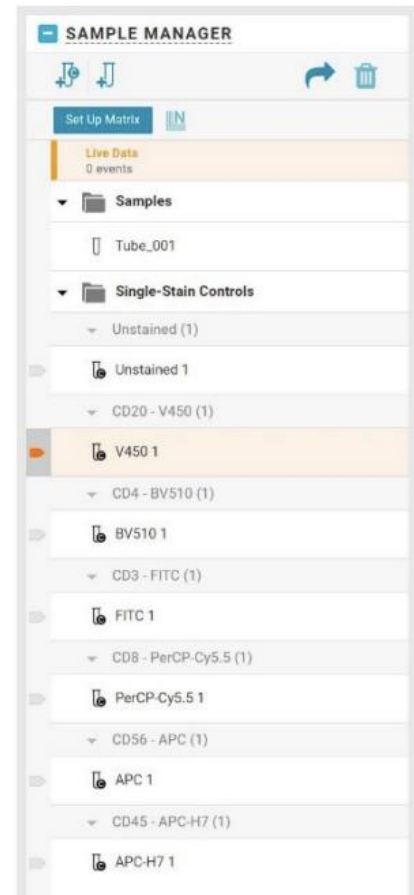
**NOTE** Control tubes can be acquired in any order.

**NOTE** Data does not be recorded in the unstained tube in order to calculate unmixing, however only unstained tubes can be used for autofluorescence.

3. Select the appropriate tube from the list and click **Load** in the dashboard.

### Adjusting settings

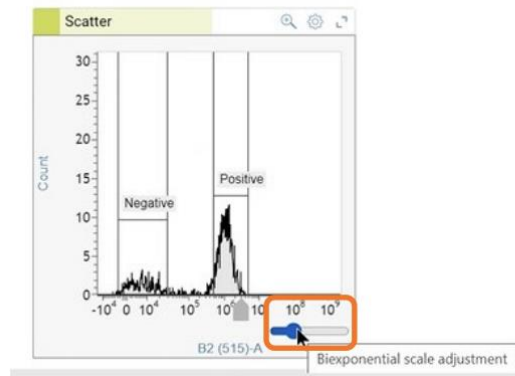
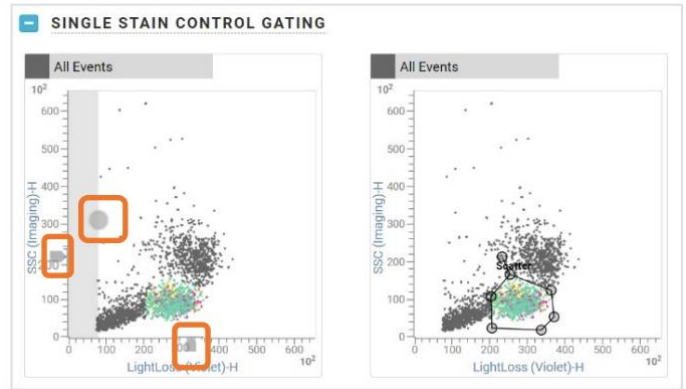
1. Adjust the plot zoom, scatter gains, threshold, plot scaling, and gates to encompass cells of interest.
  - a. Zoom in on a plot by clicking the plot then rolling the mouse scroll wheel upwards. Roll downwards to zoom out.



- b. Adjust the scatter gains, if needed. Hover over the scatter plots axes then drag the gain sliders along the axes.

**NOTE** If the SSC-Imaging gain is changed, then the ROA must be reset for the control particles before recording data.

- c. Adjust the threshold by hovering over the gray portion of the first default plot and then dragging the threshold marker (gray dot) horizontally.
- d. Adjust the plot scaling by clicking the biexponential scale adjustment slider.
- e. Adjust the gates by dragging the vertices to encompass populations.



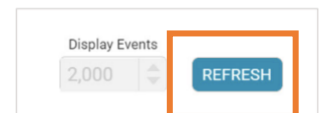
2. Verify that no channels are saturated on the spectral plot.

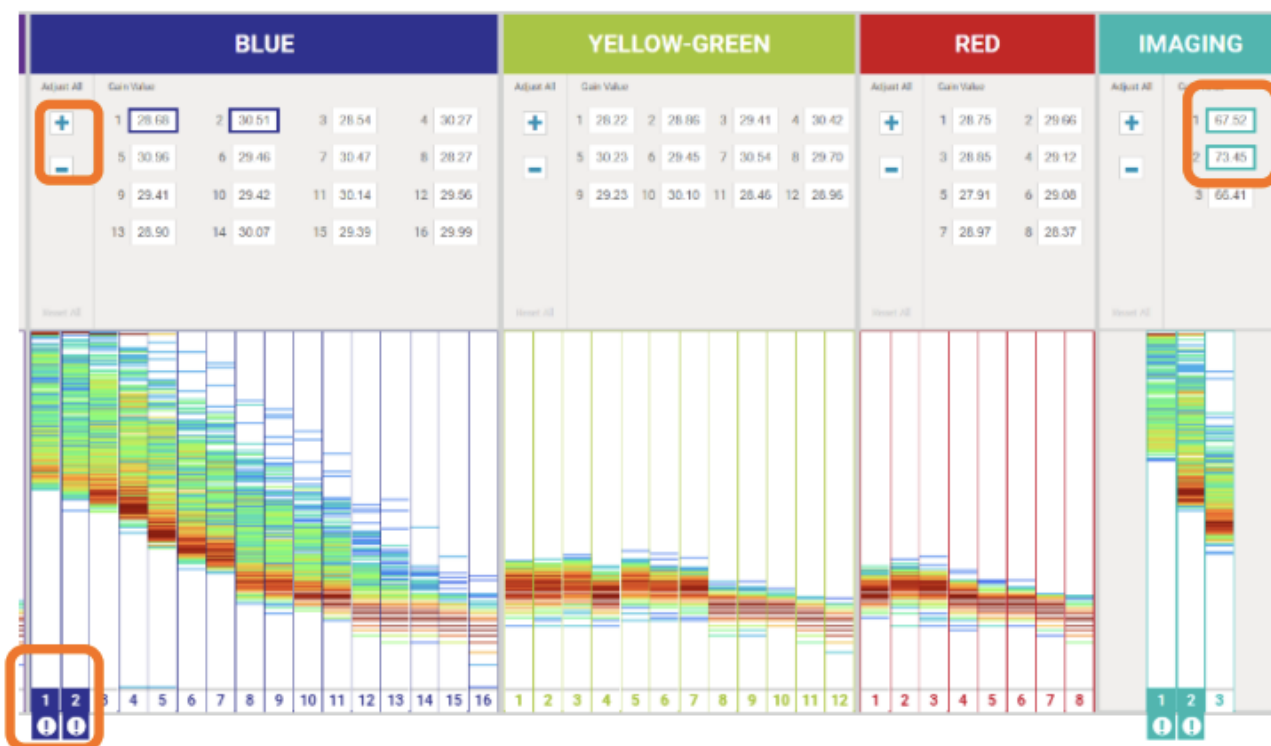
Saturated channels are indicated by a colored box with the (!) icon, which displays at the bottom of the affected channel.

Lower the gains of any saturated channel.

- a. Click the down arrow on the top left corner of the spectral plot panel.
- b. Adjust the spectral plot gains under Gain Value:
  - Enter a new value for the channel, then press Enter.
  - Or select a channel, then scroll using your mouse scroll wheel.
  - Or click the + and – buttons to adjust gains for all channels in that laser.

**TIP** After adjusting the gain, click **Refresh** in the dashboard to visualize the change.





Click **Reset All** to set the gains of all channels to the default settings.

## Recording controls

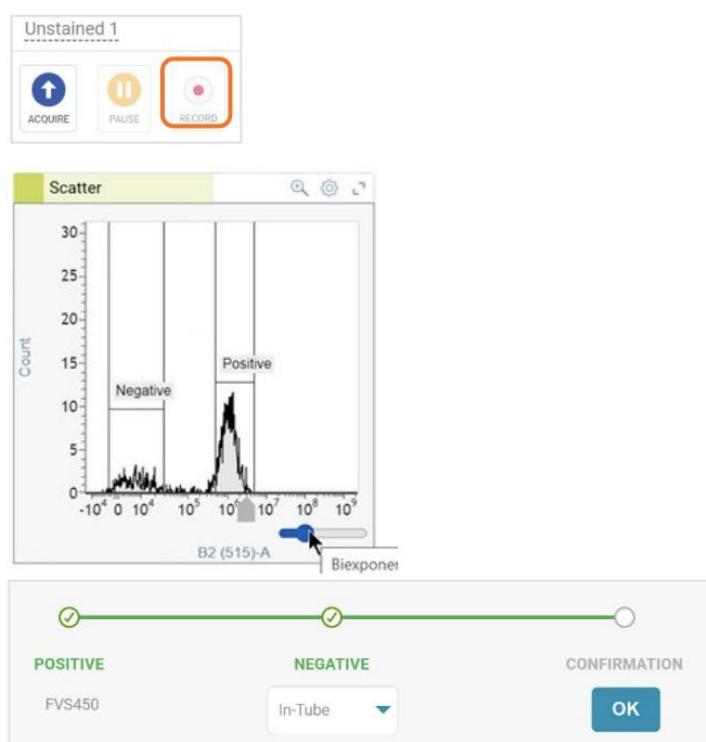
1. (Optional) Change the number of events to record in the FCS Stopping Criteria field.

**NOTE** The default is 10,000.

2. Click **Record** in the dashboard.
3. Adjust the scatter and histogram (interval) gates to encompass positive and negative populations of interest.

**NOTE** As an alternative for the negative population in your single-stained controls, you can select a previously recorded unstained tube as your negative control.

**NOTE** Gates can be adjusted at any time before, during, or after recording.



**TIP** In order to add multiple autofluorescence controls, gate the population(s) in the unstained tube.

4. Click **OK** under **Confirmation**.

**NOTE** This step is not necessary for the Unstained control tubes.

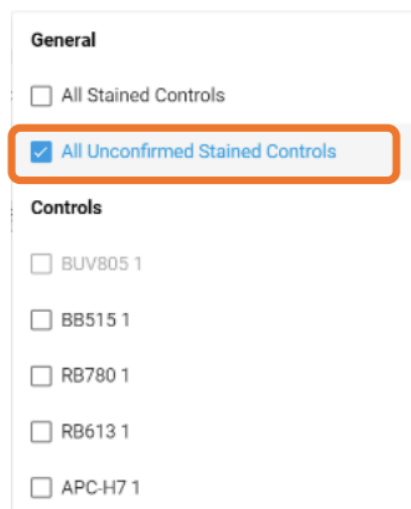
5. Load the next control tube on the sample loading port.

6. Select the appropriate tube from the list and click **Load** in the dashboard.

7. Repeat steps 1 through 6 for all the remaining controls.

**TIP** If single-stain controls are not all the same particle type (for example, some control tubes contain beads and some contain cells), we recommend first verifying ROA for one particle type, and recording all tubes containing that particle type. Then verify ROA for the second particle type, and record all tubes containing that particle type.

**TIP** Click the **Select recordings** dropdown menu to apply the plots, gates and negative populations to the remaining applicable control tubes by selecting **All Unconfirmed Stained Controls**, then click **Apply**.



## Performing spectral unmixing

Use the **Set Up Matrix** icon in the **Sample Manager** panel to perform spectral unmixing of the recorded fluorescence data which generates the spectral unmixing matrix. After the spectral unmixing matrix is generated, the **Raw Mode** icon no longer displays next to the experiment name. The spectrally unmixed

data can be viewed on the **View Data** page.

**NOTE** The spectral unmixing matrix in a template experiment or a duplicated experiment carries over from the original experiment, but it is recommended that you re-run the controls and the spectral unmixing matrix must be set up again in the new experiment.

After you have confirmed at least one recording for all the controls in your experiment, proceed to set up the spectral unmixing matrix.

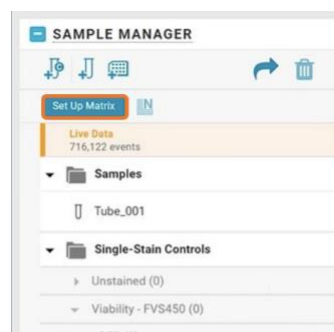
**TIP** Record 2000 events of the fully stained sample before generating the matrix and select it from the **Recording Selection**. This allows a better understanding of how the fully stained sample behaves with the unmixing matrix.

**NOTE** If the spectral unmixing process is not completed or is not successful, the **Raw Mode** icon will continue to display at the top of the Experiment workflow next to the experiment name, and no spectrally unmixed data can be viewed on the **View Data** page.

**NOTE** The **Set Up Matrix** icon is activated only after the system determines that you have at least one confirmed recording for each control, exclusive of the unstained control.

**NOTE** Autofluorescence controls can be assigned only in the **Set Up Matrix** window.

1. Click **Set Up Matrix** in the **Sample Manager**.
2. Select the recording to use for each fluorescent single-stain control.
3. (Optional) Click **Add Autofluorescence** Control in the bottom left and select the unstained control to use for unmixing autofluorescence.
4. Click **Generate Spectral Matrix**.
5. Confirm that a matrix was generated with a timestamp.
6. Close the **Set Up Spectral Matrix** page by clicking the **X** in



Set Up Spectral Matrix		Fluorochromes + AF: 05	
<input checked="" type="checkbox"/>	Single-Stain Control (5)	Recording to Use	Negative
<input checked="" type="checkbox"/>	CD45 - BUV805	CD45_BUV805_001	In-Tube
<input checked="" type="checkbox"/>	CD3 - BB515	CD3_BB515_001	In-Tube
<input checked="" type="checkbox"/>	CD19 - RB780*	CD19_RB780*_002	In-Tube
<input checked="" type="checkbox"/>	CD14 - RB613*	CD14_RB613*_002	In-Tube
<input checked="" type="checkbox"/>	HLA-DR - APC-Cy7	HLA-DR_APC-Cy7_002	In-Tube



the top right of the window.

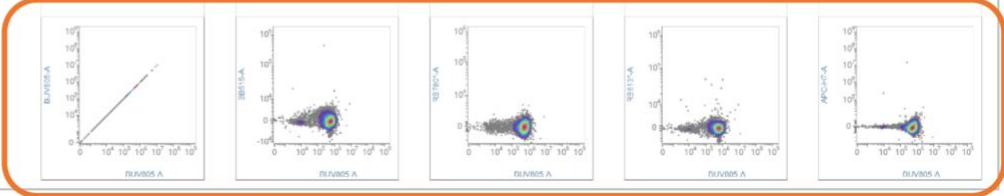
Set Up Spectral Matrix Fluorochromes + AF: 06

**Matrix Preview** Single Stain Complexity: 1.69 Single Stain + AF Complexity: 2.69

[All-by-N](#) [Similarity Matrix](#) [Hotspot Matrix](#)

Matrix: Preview | Recording Selection: BUV805 1 | X-Axis: BUV805 | Plot Scale: Biexponential | R-Value: | Parent Population: All Events | Plot Type: Density

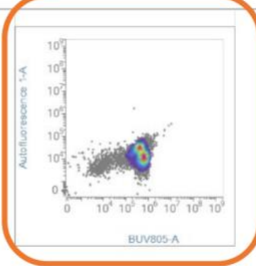
Sync x-axis for all plots



Autofluorescence Control (1) | Recording to Use | Population

Autofluorescence 1 |  Unstained 1 |  All Events

+ Add Autofluorescence to use for Spectral Matrix



The Matrix Preview displays the single-stained controls as All-by-N plots.

An additional Autofluorescence plot displays for each included autofluorescence control.

Successfully generated Latest Matrix 03/20/2025 10:12:58 am

Generate Spectral Matrix

Revert to Raw Mode

Sync x-axis for all plots

R-Value | Parent Population | Plot Type

All Events | Density



# Record and Analyze Data



## Before you begin

- Adjust your scatter and spectral gains and Region of Analysis (ROA) for your sample.
- Perform spectral unmixing by recording data for single-stained controls, if applicable.

## Working with the View Data tab

### Preparing the experiment

1. Click the **View Data** tab.



2. Place a sample onto the loading port. Hit **Load** to load the sample tube.

**TIP** Set the Flow Rate to 1 to conserve sample.

3. Clear the **Doublet Discrimination** checkbox, if needed.



4. Verify that the spectral or imaging detectors are not saturated in the spectral plot.

5. Verify that your population of interest is on scale in the LightLoss (Violet)-H vs. SSC (Imaging)-H plot.

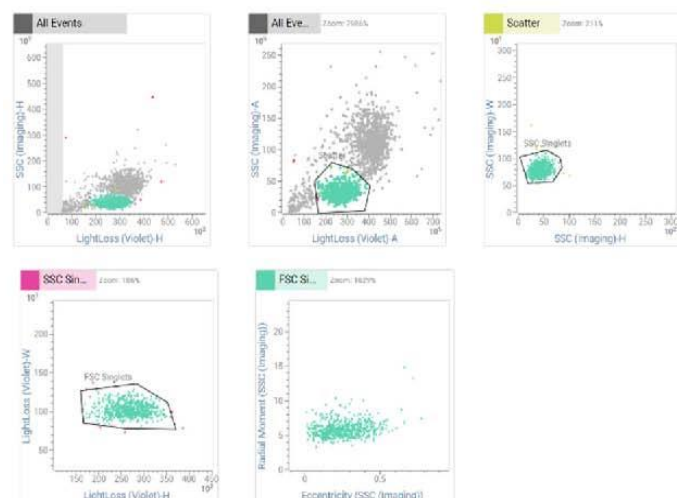
STATISTICS			
Population	Events	% Parent	% Total
All Events	10,000	N/A	100.00 %
Saturated	14	0.14 %	0.14 %
Unsaturated	9,986	99.86 %	99.86 %

6. Adjust the plot zoom, scatter gains, and threshold appropriately. Keep the saturated events to a minimum.

**TIP** In the Statistics panel, verify that most events are Unsaturated.

7. (Optional) To conserve the sample, click **Pause** in the dashboard.

8. In the Plots panel, adjust the Scatter, SSC Singlets, and FSC Singlets gates to encompass the population of interest.



The SSC Singlets and FSC Singlets gates are only

available if the Doublet Discrimination checkbox is selected.

**NOTE** The Eccentricity (LightLoss (Imaging)) vs. Radial Moment (LightLoss (Imaging)) plot can be used for additional doublet discrimination. This plot will be removed when the Doublet Discrimination checkbox is cleared.

9. Adjust the Region of Analysis and Pixel Threshold for the detectors of interest in the Image Wall.

**NOTE** The Region of Analysis (ROA) setting can affect the fluorescent data of your sample. You must set the ROA properly for the current particle type before recording.

- Adjust the Region of Analysis slider until the white area in the images completely encompasses the events of interest while minimizing background pixels.  
**TIP** To adjust the slider in small increments, click the slider then press the arrow keys.
- Adjust the Pixel Threshold slider for the imaging detectors until the white area in the images completely encompasses the area of interest while minimizing background pixels.



10. Set recording criteria:

- a. Under FCS Storage Population, make sure **All Events** is selected.

**NOTE** Selecting children of All Events will only save that population to the FCS file.



- b. Under FCS Stopping Population and FCS Stopping Criteria, select the population and the number of events to record.
- c. Verify that the Images Stored switch is toggled on to save images.

- d. Under Image **Storage Population**, select the population for image storage. It's recommended to select **Scatter** because images for debris are not valuable data.



**NOTE** If images are not needed, turn off the Image Stored to reduce the data set size.

## Recording sample data

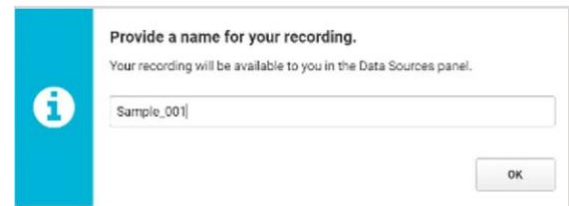
1. Click **Record** in the dashboard.



When the target is reached, acquisition stops.

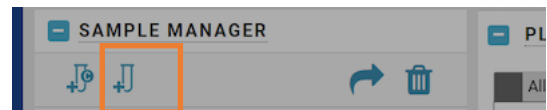
2. Enter a name for your recorded file and click **OK**.

The new data file will appear in the Data Sources panel.

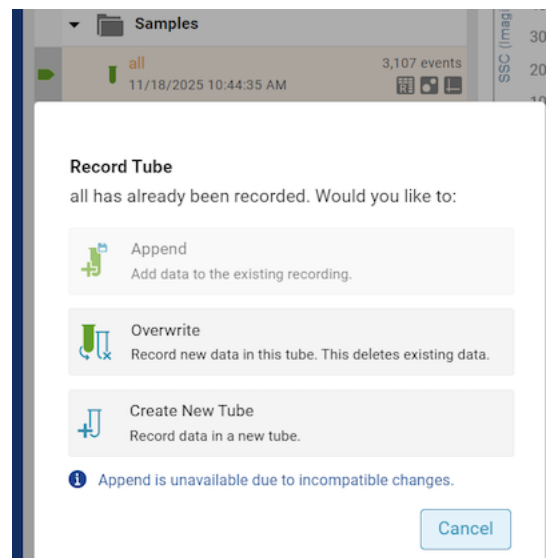


**NOTE** The Data Sources panel displays all previously recorded data files, including Single Stain Control data, if applicable. To view a recorded data file, select it. To view live acquisition, select **Live Data**.

3. To record next sample, click **Add New Tube** icon. Then hit Load, followed by Record.



**NOTE** If you hit Load and Record without adding a new tube, a drop-down will appear and ask whether you want to **Append**, or **Overwrite**, or **Create New Tube**.



## Analyzing sample data

1. Click the **Add Plot** (+) icon and select a plot type from the menu to create any additional plots.
2. Click the plot axes and from the plot parameters dialog, select parameter and scaling options.

**NOTE** Click the **Plot Properties** (gear) icon on the top right of the plot window to delete the plot or change the parent gate or the plot type.

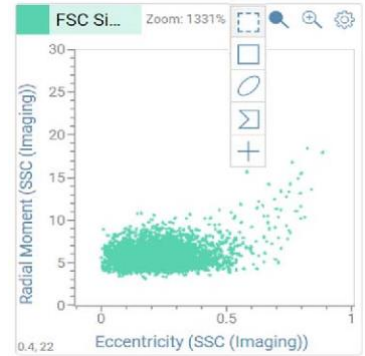
**TIP** Drag plots to reorder them in the Plots panel.

3. Add sort gates.






Hover over a plot, click the **Add New Gate** (  ) icon, and select a gate type.

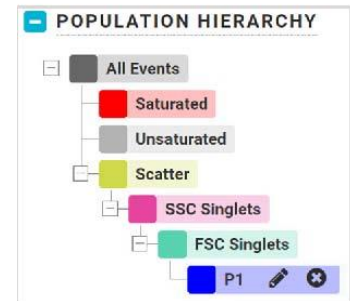
- For rectangular and oval gates, drag an area on the plot to encompass the population of interest.
- For polygon gates, click multiple times to create vertices around the population of interest.
- For quadrant gates, click the plot to place the center of the quadrant gate.



4. In the Population Hierarchy panel:

- Click the **Edit** (  ) icon to rename the population.
- Click the **Delete** (  ) icon to delete the population.
- Click the **Color Swatch** (  ) to change the color of the population.

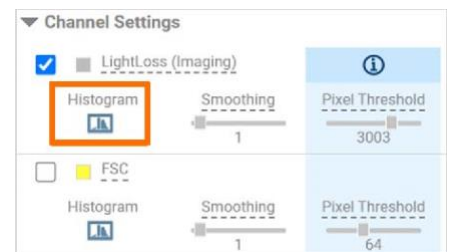
**TIP** Drag gates to other locations in the hierarchy, if needed.




## Analyzing sample images

1. Adjust the channel settings in the image wall for each detector of interest, as needed.

**NOTE** The following adjustments can be made at any time before or after recording data. **Only ROA and Pixel Threshold must be set correctly before recording.**



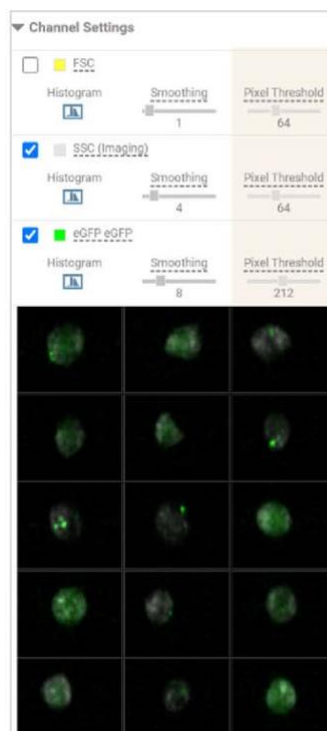
- a. Select the checkbox for the channel of interest and clear other channels.
- b. Click **Histogram** to open the histogram panel.

- i. Adjust the Minimum (red) bar and Maximum (blue) bar around the signal peak to optimize the brightness resolution of the images.
- ii. Adjust the Gamma (  ) as needed to more easily visualize differences in signal intensity between images.



- c. Adjust the **Smoothing** slider to reduce blur in the images.

- d. Select the color swatch to adjust the color.
  - e. Repeat steps **a** through **d** for all other imaging channels of interest.
2. Select the checkboxes for channels of interest to visualize cells appropriately.




## Tips and considerations for working with the View Data tab

### Minimizing saturated events

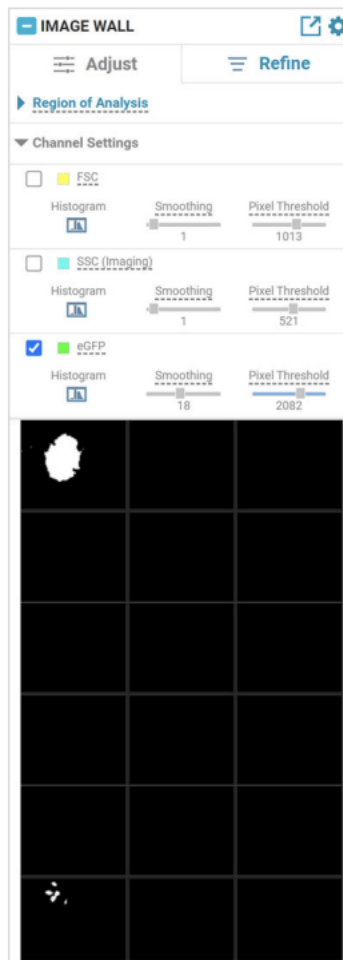
- Use the Statistics panel on the View Data page to determine what percentage of events are saturating. A majority of events should belong to the Unsaturated population.

STATISTICS			
Population	Events <span>×</span>	% Parent <span>×</span>	% Total <span>×</span>
■ All Events	10,000	N/A	100.00 %
◀ ■ Saturated	14	0.14 %	0.14 %
◀ ■ Unsaturated	9,986	99.86 %	99.86 %

- If too many events are Saturated, use the spectral plot to determine which fluorescent channels are saturating. Click the **Plot Properties**  icon to display the Saturated population on the spectral plot.
- Saturated detectors could include scatter parameters, which are not included in the spectral plot. To check the scatter parameters, you need to manually create plots containing FSC-H, SSC (Violet)-H, and LightLoss (Imaging)-H. SSC (Imaging)-H and LightLoss (Violet)-H are automatically displayed in the first default plot.
- If SSC (Imaging) gain is adjusted to minimize saturation, the Region of Analysis setting will be impacted. Verify that Region of Analysis is set correctly after adjusting the SSC (Imaging) gain.

### Adjusting Pixel Threshold for rare populations


- When imaging cells with rare positive populations, it might be easier to adjust the Pixel Threshold for the fluorescent channel of interest if you first display the positive population on the image wall. On the **View Data** tab, create a plot to display data from the channel of interest, and gate the positive population. Select the population in the **Refine** tab of the image wall before adjusting the Pixel Threshold for that channel.



- Both ROA and Pixel Threshold can be adjusted on either paused or live acquisition of the sample.
- Adjust the **Pixel Threshold** on the **View Data** page, after acquiring single-stain controls and before recording experimental data. Pixel Threshold only impacts the **Size** imaging feature, which is not used in the spectral unmixing calculation.

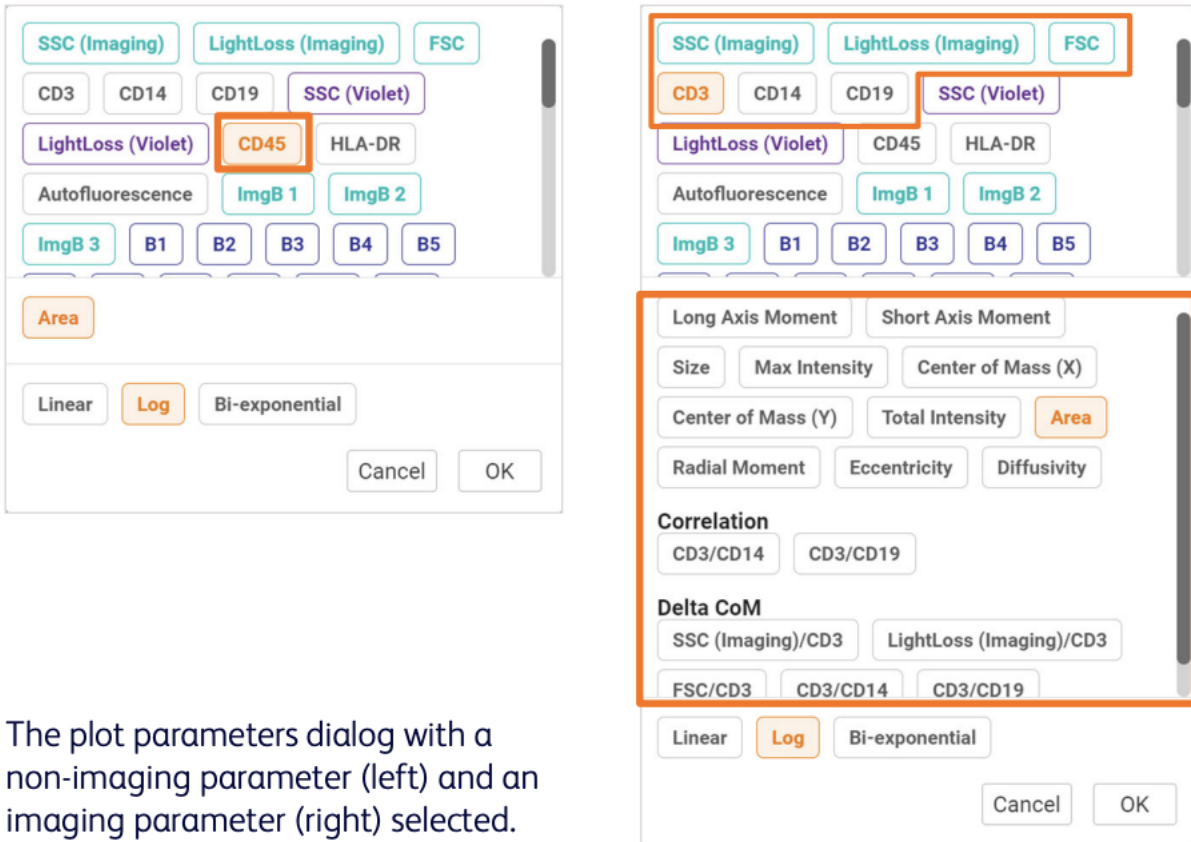
## Data visualization on View Data tab

- For fluorescent parameters, plots display with log scaling by default. If unmixing has been performed, select biexponential scaling in the plot parameters dialog.
- To drill down: Click a gate to select a population of interest, then create a new plot. The plot will automatically display the data from the selected population.
- Change the number of dots to display on dot plots by entering a different number into the **# of Events Displayed** (for Dot Plots) field. Enter -1 to view all recorded events.

# of Events Displayed (for Dot Plots):  

## Using imaging features for analysis

- Imaging features are automatically enabled and calculated for every experiment.
- To generate a list of selectable image features in the plot parameters dialog, you must select an imaging channel parameter. These are displayed at the top of the parameters list, and include SSC (Imaging), LightLoss (Imaging), FSC, and any fluorochromes/labels that have been assigned to imaging channels in the Select Imaging Features tab.



The plot parameters dialog with a non-imaging parameter (left) and an imaging parameter (right) selected.

- Imaging features cannot be displayed in biexponential scaling. Depending on your experiment and the feature(s) used, you might find a linear or log scale to be more helpful in displaying image feature data.
- The Region of Analysis adjustment is necessary to calculate all the imaging features described in the following table, except for the Size parameter, which is calculated by the Pixel Threshold adjustment.
  - The Pixel Threshold is a user-defined brightness threshold above which a pixel will be counted.

- The Region of Analysis is the area of pixels defining a single event, cell, or particle within the image.

Image feature	Definition	Usage example	Representative images (low → high)
Eccentricity	A ratio of the shortest to the longest axis (moment) within the Region of Analysis.	Doublet discrimination, cluster identification, cell morphology	
Radial Moment	The average distance of the pixels from the centroid within the Region of Analysis.	Doublet discrimination, cell-to-cell interactions (cellular synapse)	
Size	The number of pixels in the image which are brighter than a user-defined Pixel Threshold.	Label-free sorting, punctate fluorescence	
Max Intensity	The intensity of the brightest pixel in the image.	Punctate fluorescence, phagocytosis, cell cycle analysis	
Long Moment	The measurement of the longest axis (moment) within the Region of Analysis.	Cell morphology, cell-to-cell interaction, aggregates	
Short Moment	The measurement of the shortest axis (moment) within the Region of Analysis.	Cell morphology, cell-to-cell interaction, aggregates	
Center of Mass (X)	The position of the particle in the horizontal direction within an image.	Image quality control, antigen cellular location, phagocytosis	
Center of Mass (Y)	The position of the particle in the vertical direction within an image.	Image quality control, antigen cellular location, phagocytosis	
Total Intensity	The sum of the intensities of all pixels within the Region of Analysis.	Quantitative fluorescence measurements	
Diffusivity	The ratio of the total intensity to the maximum intensity.	Cell morphology, phagocytosis	
Delta Center of Mass	The distance between two fluorescent signal sources in any two imaging channels within the Region of Analysis.	Cell-to-cell interaction, phagocytosis	
Correlation	The degree to which the location of two imaging channels are the same within the Region of Analysis.	Translocation assay, cell-to-cell interaction	

# Performing Spectral Re-unmixing If Needed

You can record multiple single-stain controls for every fluorochrome in your experiment and then select the confirmed recordings for your fluorochromes to generate the spectral matrix that you want to use for your experiment. This creates a new spectral unmixing matrix that is referred to as the Latest Matrix, and it will be applied to all samples acquired and recorded after it is created.

## Before you begin

- Start up the system and run a daily or extended fluidics startup procedure.
- Add and design an experiment, adjust your scatter and spectral gains, and if imaging, adjust the Region of Analysis (ROA) for your sample.

**NOTE** You can also start with an existing experiment that already contains an unmixing matrix.

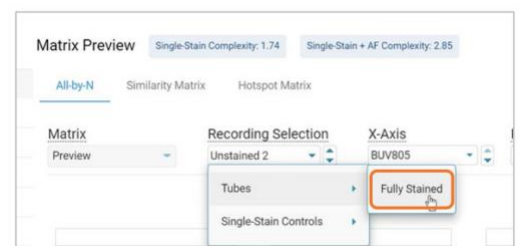
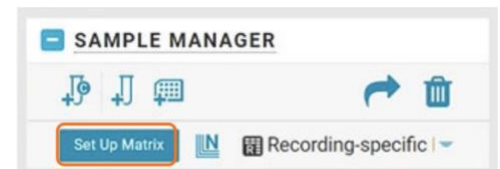
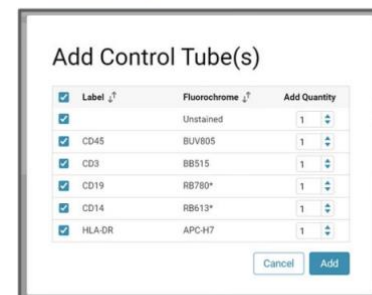
- Run your initial single stain controls, calculate an unmixing matrix, and record unmixed data on a fully stained sample.

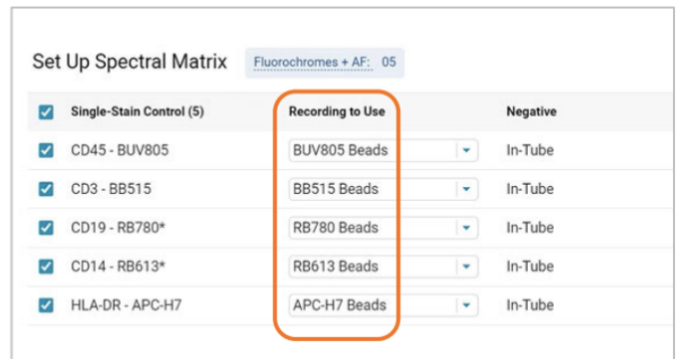
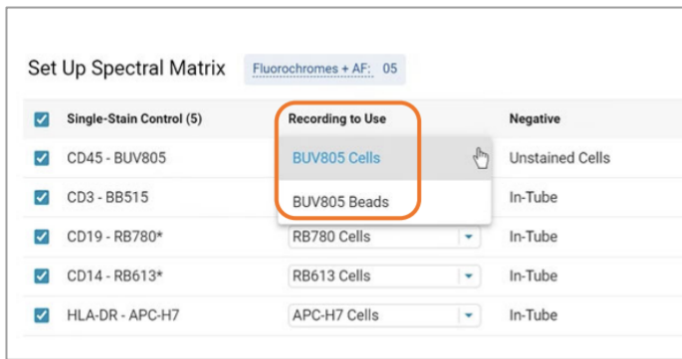
## Generating the new unmixing matrix

1. Set up the re-unmixing controls.
  - a. In the **Sample Manager** panel, click the **Add Control Tube(s)** icon.
  - b. Uncheck all of the markers and then click the controls that need to be re-run.

**NOTE** If you do not uncheck all Labels, a second tube will be created for all fluorochromes.

  - c. Click **Add**.
  - d. Rename the controls by clicking the **pencil icon**.
2. Click **Set up Matrix** in the sample manager.
3. Change the **Recording Selection** to your fully stained sample.
4. Change the **Recording to Use** to the newly recorded single stain controls and preview the changes in the plot(s) by selecting each fluorochrome for the x-axis.



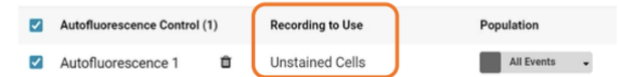


5. (Optional) Add an autofluorescence control(s).

a. In the bottom left of the window, click **+Add Autofluorescence to use for Spectral Matrix**.

b. Check the box for **Autofluorescence 1**.

c. Select an unstained control sample as the recording and population to use.



**TIP** In order to add multiple autofluorescence controls, gate the population(s) in the unstained tube.

d. (Optional) Preview the changes to the unmixing matrix by selecting each fluorochrome for the x-axis display.



6. Click the **Generate Spectral Matrix** button and confirm that a matrix was generated with a timestamp.

### Viewing the data with the new unmixing matrix

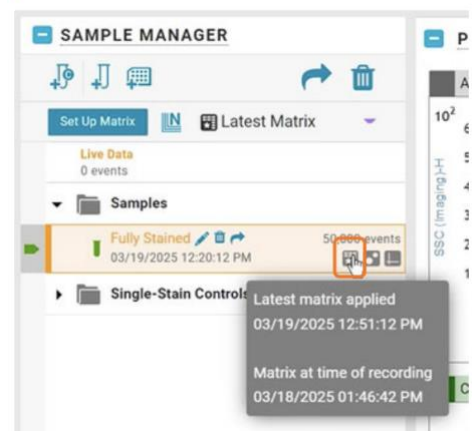
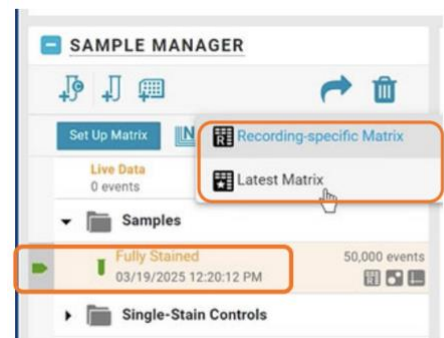
1. Click the **View Data** page.

2. In the **Sample Manager**, click the **Fully Stained** sample to view the data.

3. Click the drop-down by **Recording-specific** and select **Latest Matrix**.

**NOTE** The selected matrix applies to all recorded samples.

4. Verify that the latest matrix has been applied by hovering over the icon in the **Fully Stained** sample.

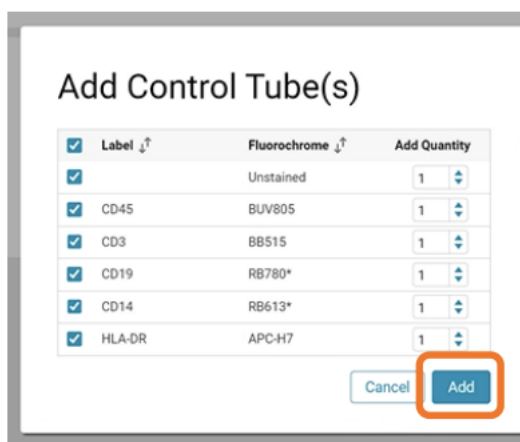


## Tips and troubleshooting

- **CAUTION** The newest spectral unmixing matrix, also known as the Latest Matrix, will be applied to all samples acquired and recorded after it is created.
- Experimental data that has been unmixed can be viewed with either the Recording-specific Matrix (the matrix at the time of recording) or the Latest Matrix.
- Statistics exported from the experiment will be calculated using the matrix that is currently selected.
- The Chorus Experiment File (CEF) will contain only the Latest Matrix.

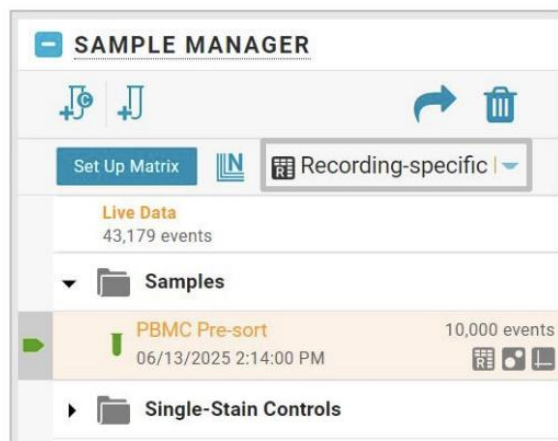
## Re-recording controls

Fluorochrome and autofluorescence controls can be re-recorded as many times as needed. Additional controls can be added by clicking on the add control tubes icon in the sample manager, then selecting the checkboxes and number of tubes of each to add to the experiment.



## Re-unmixing after experimental data is recorded

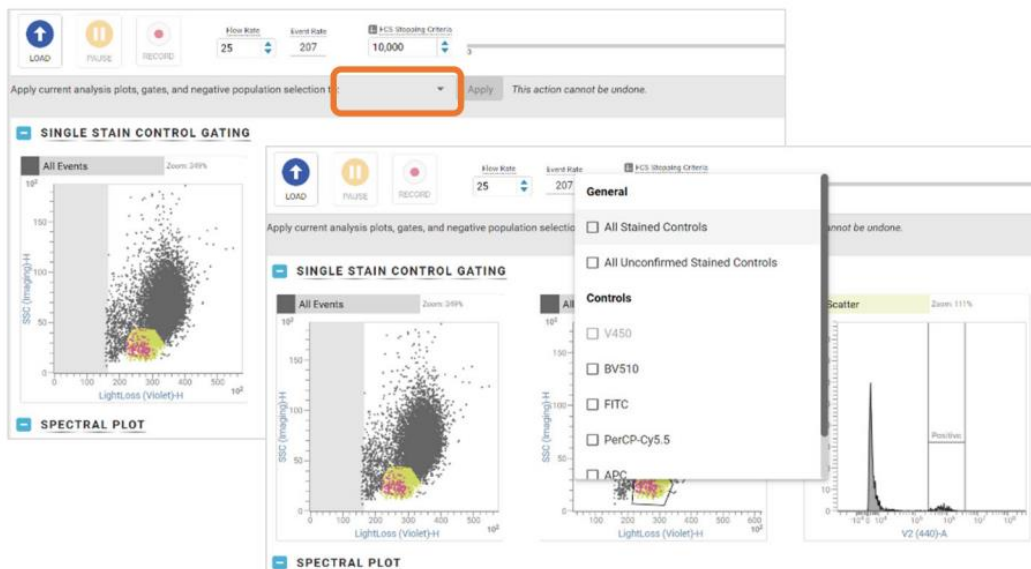
- The spectral unmixing matrix can be recalculated and the new matrix applied to previously recorded data files. To change the spectral unmixing matrix, you must modify the existing fluorochrome controls or record new fluorochrome controls, and then confirm the changes.
- The newest spectral unmixing matrix is referred to as the Latest Matrix, and it will be applied to all samples acquired and recorded after it is created.
- Experimental data that has been unmixed can be viewed with either the Recording-specific Matrix (the matrix at the time of recording) or the Latest Matrix.



- Experimental data that has not been unmixed (acquired in Raw Mode) can be viewed with the latest matrix only.
- Any sorts performed will use the Latest Matrix for unmixing.
- Statistics exported from the experiment will be calculated using the matrix that is currently selected.
- The Chorus Experiment File (CEF) will contain only the Latest Matrix.

## Applying gates to all controls

To apply the current analysis plots, gates, and negative population selection to some or all controls, click the dropdown menu above the scatter plots to select the controls and then click **Apply**.



Apply current analysis plots, gates, and negative population selection to: All Unconfirme... **Apply** This action cannot be undone.

Once overwritten, the default gate positions cannot be restored. However, plots, gates, and negative population selections can be further edited as needed.

## Using the Matrix Preview panel



Preview panel options	Description	Examples																																				
All-by-N	<ul style="list-style-type: none"> <li>Provides a visual overview of unmixing performance.</li> <li>The All-by-N window displays how a single unmixed parameter relates to every other unmixed parameter in your experiment. Each plot in the grid has the same unmixed parameter on the x-axis and a different unmixed parameter on the y-axis.</li> </ul>	<p>The screenshot shows the 'Matrix Preview' panel with 'All-by-N' selected. It displays three scatter plots in a grid. The first plot shows BUV805-A vs BUV805-A, the second shows BB515-A vs BUV805-A, and the third shows RB780-A vs BUV805-A. The plots show the relationship between different unmixed parameters.</p>																																				
Similarity Matrix	<ul style="list-style-type: none"> <li>Provides the similarity scores for each pair of fluorochromes.</li> <li>The similarity score indicates how similar the spectral signatures (SOVs) of two fluorochromes are, ranging from 0 to 1.</li> <li>Nearly identical spectra will have a similarity of 1, while very different spectra will have a similarity of 0.</li> </ul>	<p>The screenshot shows the 'Similarity Matrix' heatmap. The x and y axes are labeled with fluorochrome names: BUV805, BB515, RB780, RB613, and APC-H7. The diagonal elements are 1.00. The off-diagonal elements represent similarity scores between pairs of fluorochromes.</p> <table border="1"> <thead> <tr> <th></th> <th>BUV805</th> <th>BB515</th> <th>RB780</th> <th>RB613</th> <th>APC-H7</th> </tr> </thead> <tbody> <tr> <th>BUV805</th> <td>-</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <th>BB515</th> <td>0.00</td> <td>-</td> <td></td> <td></td> <td></td> </tr> <tr> <th>RB780</th> <td>0.09</td> <td>0.06</td> <td>-</td> <td></td> <td></td> </tr> <tr> <th>RB613</th> <td>0.00</td> <td>0.06</td> <td>0.04</td> <td>-</td> <td></td> </tr> <tr> <th>APC-H7</th> <td>0.25</td> <td>0.00</td> <td>0.14</td> <td>0.00</td> <td>-</td> </tr> </tbody> </table>		BUV805	BB515	RB780	RB613	APC-H7	BUV805	-					BB515	0.00	-				RB780	0.09	0.06	-			RB613	0.00	0.06	0.04	-		APC-H7	0.25	0.00	0.14	0.00	-
	BUV805	BB515	RB780	RB613	APC-H7																																	
BUV805	-																																					
BB515	0.00	-																																				
RB780	0.09	0.06	-																																			
RB613	0.00	0.06	0.04	-																																		
APC-H7	0.25	0.00	0.14	0.00	-																																	
Hotspot Matrix	<ul style="list-style-type: none"> <li>Predicts the impact of unmixing-dependent spread.</li> <li>Summarizes which spectral signatures in a spectral matrix will cause unmixing-dependent spreading (spread in an unmixed parameter that is caused by the unmixing matrix itself).</li> </ul>	<p>The screenshot shows the 'Hotspot Matrix' heatmap. The x and y axes are labeled with fluorochrome names: BUV805, BB515, RB780, RB613, and APC-H7. The diagonal elements are 1.00. The off-diagonal elements represent hotspot values.</p> <table border="1"> <thead> <tr> <th></th> <th>BUV805</th> <th>BB515</th> <th>RB780</th> <th>RB613</th> <th>APC-H7</th> </tr> </thead> <tbody> <tr> <th>BUV805</th> <td>1.03</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <th>BB515</th> <td>0.03</td> <td>1</td> <td></td> <td></td> <td></td> </tr> <tr> <th>RB780</th> <td>0.25</td> <td>0.24</td> <td>1.01</td> <td></td> <td></td> </tr> <tr> <th>RB613</th> <td>0.03</td> <td>0.25</td> <td>0.19</td> <td>1</td> <td></td> </tr> <tr> <th>APC-H7</th> <td>0.51</td> <td>0.09</td> <td>0.35</td> <td>0.04</td> <td>1.04</td> </tr> </tbody> </table>		BUV805	BB515	RB780	RB613	APC-H7	BUV805	1.03					BB515	0.03	1				RB780	0.25	0.24	1.01			RB613	0.03	0.25	0.19	1		APC-H7	0.51	0.09	0.35	0.04	1.04
	BUV805	BB515	RB780	RB613	APC-H7																																	
BUV805	1.03																																					
BB515	0.03	1																																				
RB780	0.25	0.24	1.01																																			
RB613	0.03	0.25	0.19	1																																		
APC-H7	0.51	0.09	0.35	0.04	1.04																																	

# Sort Into Tubes



## Before you begin

- Adjust your scatter and spectral gains and Region of Analysis (ROA) for your sample.
- Perform spectral unmixing by recording data for single-stained controls, if applicable.
- Record pre-sort data and create sort gates on the View Data page.
- Make sure drop delay is run, and temperature control is turned on.

## Working with the Set Up Sort tab

### Preparing the sort

1. Click the **Set Up Sort** tab.



2. In the Collection Setup panel,

- Select the device type needed. Available devices are 2-way tube, 4-tube 15ml combo, 4-tube 50mL combo, 4-way tube, 6-way tube, plate and slide.
- Hit Check Side Stream Deflection to open the window.
- Load empty tubes onto the device to be used.
- Open the front access door and Insert the device into the slot under the sort block chamber.
- Close the front access door.
- Click Start Test Sort and Open Waste Drawer. Wait a few seconds, click Close Waste Drawer and Stop Test Sort.
- Take the device out, check the depositions

in each tube. If they are centered in each tube, click close to close the Check Side Stream Deflection window. If not, reinsert the device, adjust the deflection accordingly by changing the corresponding number under the side stream deflection window until the deposition is centered in the collection tubes.

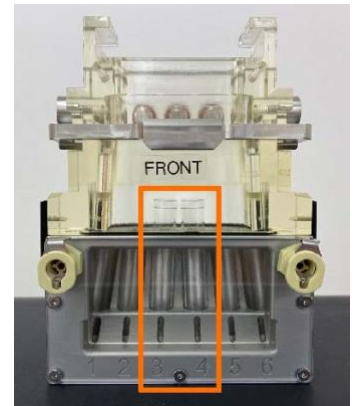
3. In the **Sort Setup** panel, enter the starting buffer volume for each collection tube.
4. Assign populations to tubes by selecting a tube, then clicking the population of interest in the population hierarchy.

**NOTE** A population does not need to be assigned for every tube.

5. Assign the target event count for each tube.

### Loading the collection device

1. Insert your collection tubes into the collection device. Load tubes from the inside out. For example, a 2-way sort will use slots 3 and 4 in the collection device. A 4-way sort will use slots 2 through 5.
2. Install the collection device onto the bottom of the sort block.



Nozzle Size	Sheath Pressure	Maximum Sort Ways	Note
85um	35psi	6	Sorting onto plate is not recommended for the 85um.
100um	20psi	4	6-way sort is not recommended for the 100um.
130um	7psi	2	Sorting more than 3 ways is not recommended for the 130um.

### Working with the Sort tab

#### Sorting

1. Click the **Sort** tab.



2. Load the sample tube and adjust the flow rate, if needed.

**NOTE** Maintain an event rate no more than 1/5 of the stream frequency (no more than 1 cell per 5 drops). If the event rate is too high, merged events occur which leads to fewer singlets. This will lead to worse efficiency and lower overall yield.

Nozzle size	Default stream frequency (KHz)	Recommended max event rate (events/s)
85 um	57.5	11500
100 um	34	6800
130 um	12	3000

3. Click **Start Sort** in the Sort Status panel.

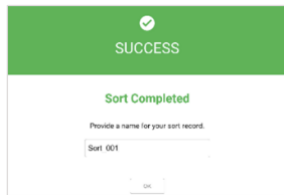
**NOTE** The instrument will take several seconds to initialize the sort before the sort begins.



4. Monitor the sort as it progresses.

- Adjust gates as needed in the Sort Population Plots and Additional Plots panels.
- Monitor the sort count and efficiency of your sorted populations in the Sort Status panel.
- Record additional data while the sort progresses, if needed.
- Adjust the sample tube's temperature and agitation speed in the dashboard.
- Toggle the light switch to help visually monitor the sample volume as the sort progresses.

5. When the sort finishes or is stopped, name the sort report.



6. **Unload** the tube, if needed.

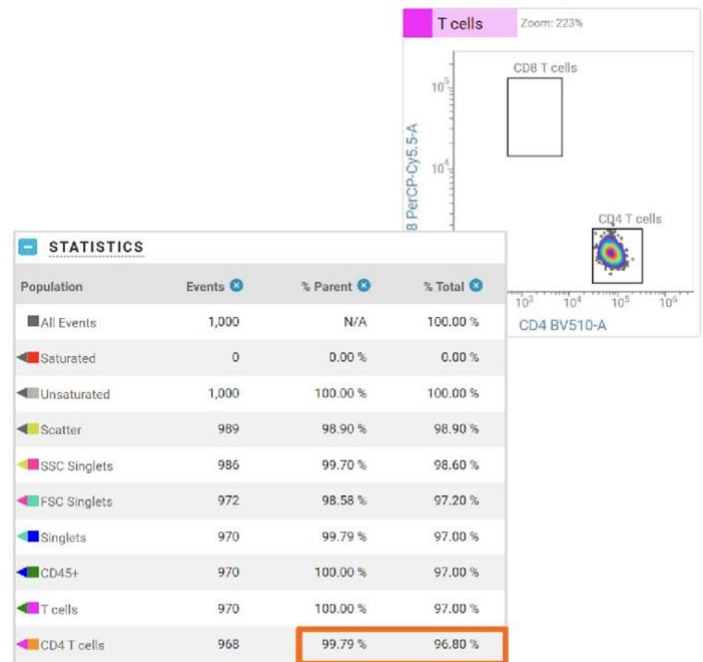
7. Turn the AMS to High, wait 2 minutes, turn back to Low.

8. Open the front access door, take the collection device out.



## (Optional) Checking post-sort purity

1. Click the **View Data** tab.
2. Set the FCS Stopping Criteria to 1,000 events. Toggle off the Images Stored switch if images are not needed.
3. Record the collection tubes.
  - a. Click **Backflush** in the dashboard. Click **OK** to clear the dialog.
  - b. Load a collection tube.
  - c. Click **Record**.
  - d. Name the post-sort data file.
  - e. Repeat steps **a** through **d** for each collection tube.
4. View the Statistics panel to verify post-sort purity.



# Sort Into Plates



## Before you begin

- Adjust your scatter and spectral gains and Region of Analysis (ROA) for your sample.
- Perform spectral unmixing by recording data for single-stained controls, if applicable.
- Record pre-sort data and create sort gates on the View Data page.
- Make sure drop delay is run, and temperature control is turned on.

## Working with the Set Up Sort tab

### Preparing the sort

1. Open the front access door. Install the splash guard below the sort block.

2. Click the **Set Up Sort** tab.



3. Make appropriate selections in the **Collection Setup** panel.

The 'COLLECTION SETUP' panel contains the following settings:

- Format: Plate (dropdown)
- BD-Defined Plate: 96 well (dropdown)
- Plate Name: Default (dropdown)
- Sort Mode: Single Cell (dropdown)
- Enable Index Sort:
- Optimize Plate: (button)

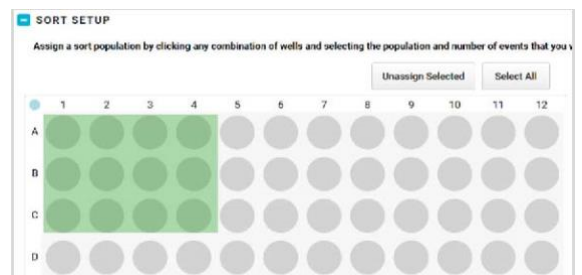
Select the **Enable Index Sort** checkbox, if needed. **For an Index Sort, FCS data is recorded automatically for the entire sort and images are saved for sorted events.**

4. Click **Optimize Plate**, follow the prompts to verify plate alignment.

**NOTE** The splash shield must be installed below the sort block to perform the optimize plate workflow.

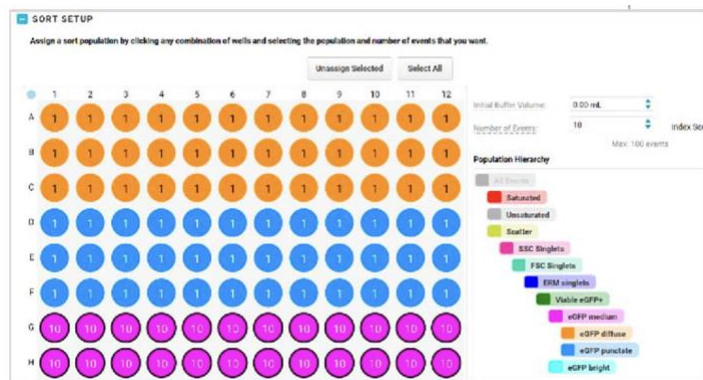
5. In the **Sort Setup** panel, select the well(s) of interest on the plate layout in one of the following ways:

- Drag a region around the wells.
- Select an entire row or column by clicking the letter or number associated with the row or column.
- Select the entire plate by clicking the **Select All** button.



- Assign a population to the selected wells by clicking the population of interest in the population hierarchy.
- Enter the starting buffer volume and number of events to be sorted per well.
- Repeat steps 4 through 6 as needed to set up the remainder of the plate.

**NOTE** A population does not need to be assigned for every well of the plate.



## Loading the collection device

- Load your collection plate onto the stage in the proper orientation, with well **A1** in the front left corner.
- Close the sort chamber door.

## Working with the Sort tab

### Sorting

- Click the **Sort** tab.



- Load the sample tube and adjust the flow rate, if needed. See Page 37 for flow rate recommendation.
- Click **Start Sort** in the Sort Status panel.

**NOTE** The instrument will take several seconds to initialize the sort before the sort begins.

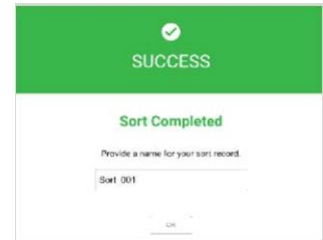
- Monitor the sort as it progresses.

- Adjust gates as needed in the Sort Population Plots and Additional Plots panels.
- Monitor the sort progress in the Sort Status panel.
- Record additional data while the sort progresses, if needed.

**NOTE** Additional data cannot be recorded during an index sort, as the index sort is already recording data and images automatically.

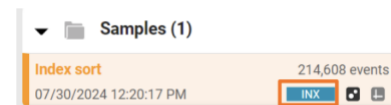


- Adjust the sample tube's temperature and agitation speed in the dashboard.
  - Toggle the light switch to help visually monitor the sample volume as the sort progresses.
5. When the sort finishes or is stopped, name the sort report.
  6. Turn the AMS to High, wait 2 minutes, turn back to Low.
  7. Open the collection chamber door, take the plate out.
  8. To retract the plate stage, click **Set Up Sort** page, change the **Format** to any tube mode. Click the **Sort** page, hit **Retract**.

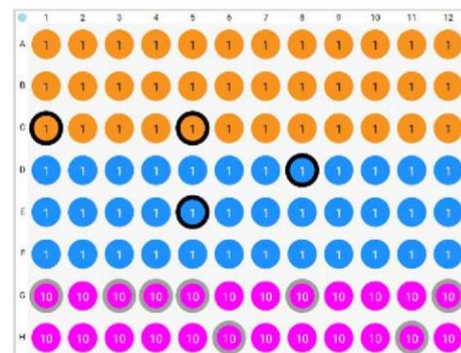
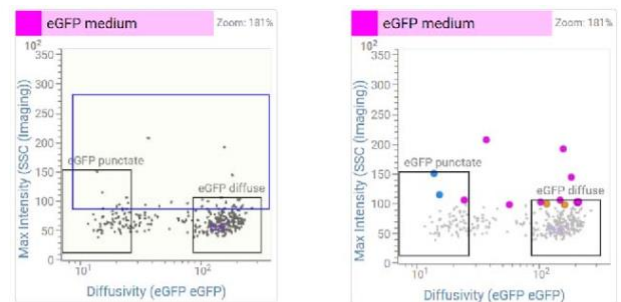


### For index sorts: Reviewing the index sort data

1. Click the **View Data** tab.
2. Click the blue **INX** icon next to the Index Sort file in the Data Sources panel.
3. In the **Index Sort View**, do any of the following:



- Select the well(s) of interest in the Sort Outcome panel. The image wall updates to display the sorted events, and the Sort Population Plots display enlarged dots corresponding to the sorted events.
- Drag a region around events of interest on a plot. Wells in the **Sort Outcome** panel will be outlined in gray or black, indicating that gated events were sorted into those wells.
- Select a sort from the **Select Sort Recording** menu, then click the export (arrow) icon to export index sort data.



4. Click the experiment name to return to the **View Data** page.




# Creating Experiments from Existing Experiments

## Before you begin

You will need:

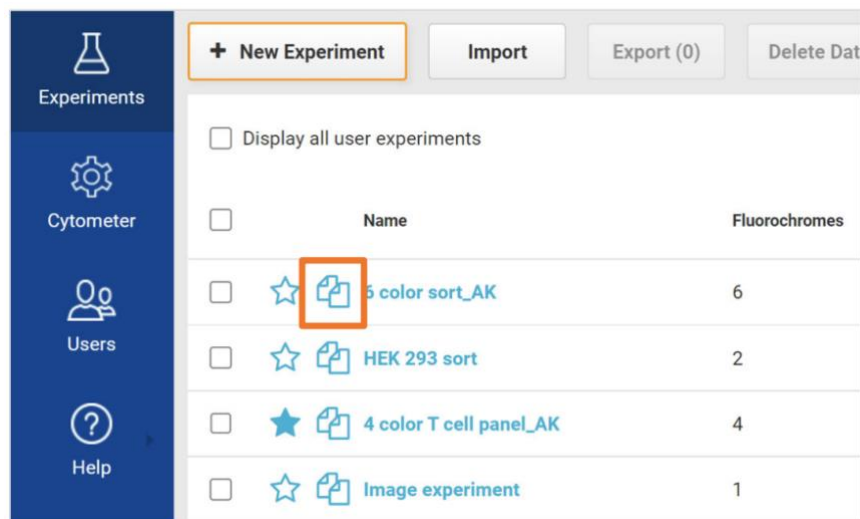
- Experiments and associated data saved in BD FACSCorus Software.
- Exported experiment (CEF) files and associated data.

## Duplicating experiments

1. Navigate to the **Experiments** page.
2. Click the **Duplicate without Data** () icon next to the name of the experiment to be duplicated.

A duplicated experiment is created and opened to the **Design Experiment** tab.



3. (Optional) Edit the experiment name and description.

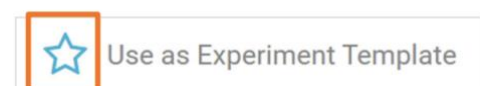
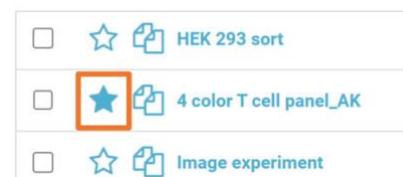


## Creating and using templates

### Creating a template

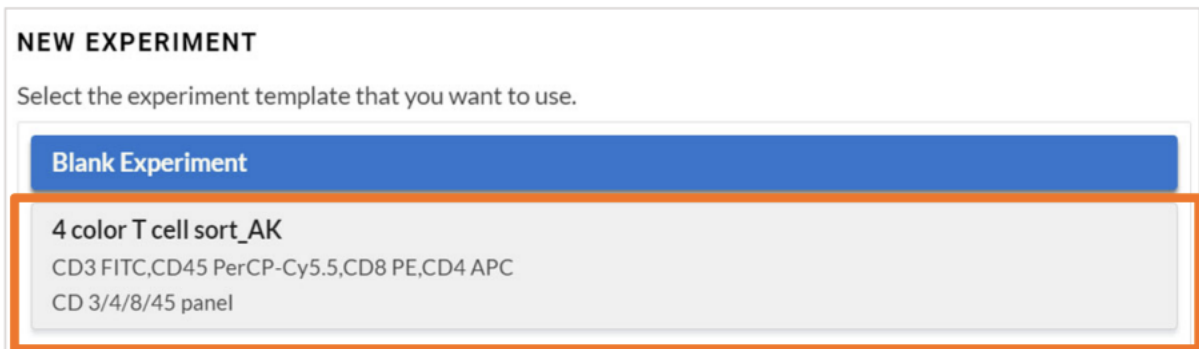
Templates can be created in two ways:

- On the **Experiments** page, click the star () next to the name of the experiment.
- In the **Design Experiment** tab, click the star ()



## Using a template to create a new experiment

1. In the Experiments page, click **+New Experiment**.
2. From the dialog, select the experiment template of interest.
3. Click **Create Experiment**. A duplicated experiment is created and opened to the Design Experiment tab.



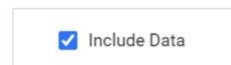
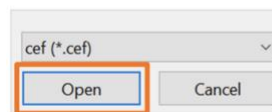
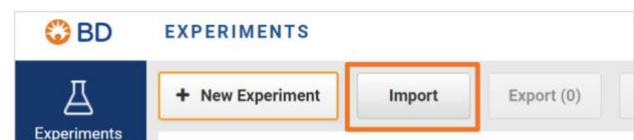
## Importing experiment files



1. In the **Experiments** page, click **Import**.
2. Browse to the folder on the workstation that contains the **Chorus Experiment File (CEF)** of interest.
3. Select the CEF file and click **Open**.
4. (Optional) Clear the **Include Data** checkbox.

If the experiment folder has data, the Include Data checkbox is selected by default.

5. Click **Import**.

The new experiment is added to the list. If a copy of the experiment exists, the name of the imported experiment is modified automatically.



<input type="checkbox"/>	Name
<input type="checkbox"/>	☆  celltoCELL_training_061724_TL 1
<input type="checkbox"/>	☆  celltoCELL_training_061724_TL

## Comparing methods of repeating experiments

### Experiment component information based on experiment type

	Blank experiment	Duplicated experiment	Experiment created from template	Imported CEF without data	Imported CEF with data
Parameters, labels, and imaging detector fluorochrome assignments	User selects	Carries over but can be modified	Carries over but can be modified	Carries over but can be modified	Carries over and cannot be modified <sup>1</sup>
Detector gains	Based on Setup and QC	Updated based on Setup and QC	Updated based on Setup and QC	Updated based on Setup and QC	Updated based on Setup and QC
Threshold	Default	Carries over but can be modified	Carries over but can be modified	Carries over but can be modified	Carries over but can be modified
Image wall settings <sup>2</sup>	Default	Carries over but can be modified	Carries over but can be modified	Carries over but can be modified	Carries over but can be modified
Spectral unmixing	None	Carries over but can be modified	Carries over but can be modified	Carries over but can be modified	Carries over but can be modified
Plots and gates	Default	Carries over but can be modified	Carries over but can be modified	Carries over but can be modified	Carries over but can be modified
Experimental data	None	None	None	None	Yes
Acquisition dashboard settings <sup>3</sup>	Default	Carries over but can be modified	Carries over but can be modified	Carries over but can be modified	Carries over but can be modified
Sort reports	None	None	None	None	Yes

1. Labels can be modified for already selected fluorochromes.
2. Image wall settings include: Region of Analysis setting, Pixel Threshold settings, Channel Settings (Minimum, Maximum, Gamma, Smoothing, and Color selections), and channel checkbox selections.
3. Acquisition dashboard settings include: Recording criteria, flow rate, and agitation and temperature settings.

# Manage the Data



Data Management Responsibilities		
Who	What	How Often
Each user	<ul style="list-style-type: none"> <li>Export the data to D:\UserData\ a folder named as your name.</li> <li>Copy the exported data folder to an external hard drive provided by staff.</li> <li>Delete the old data and experiments from both the Experiment page and D:\UserData\ a folder named as your name.</li> <li><b>Keep only 2 recent experiments in your own account for reference</b></li> </ul>	Daily
Staff	<ul style="list-style-type: none"> <li>Back up the BD Chorus Software database.</li> <li>Transfer to external hard drive.</li> <li>Trim the database to keep it small.</li> </ul>	Monthly

## Managing data from the Experiments page

### Exporting data

1. Navigate to the **Experiments** page.

2. Select the checkboxes for the experiments you need to export.

3. Export the data.

a. Click **Export**.

b. Click **Change** to select **D:\UserData\ a folder named as your name** to save the files.

c. Select the files and data formats to be exported.

d. Click **Export**. When data export is complete, close the dialog.

4. Copy the exported data folder to an external hard drive provided by staff. Then copy this data to your

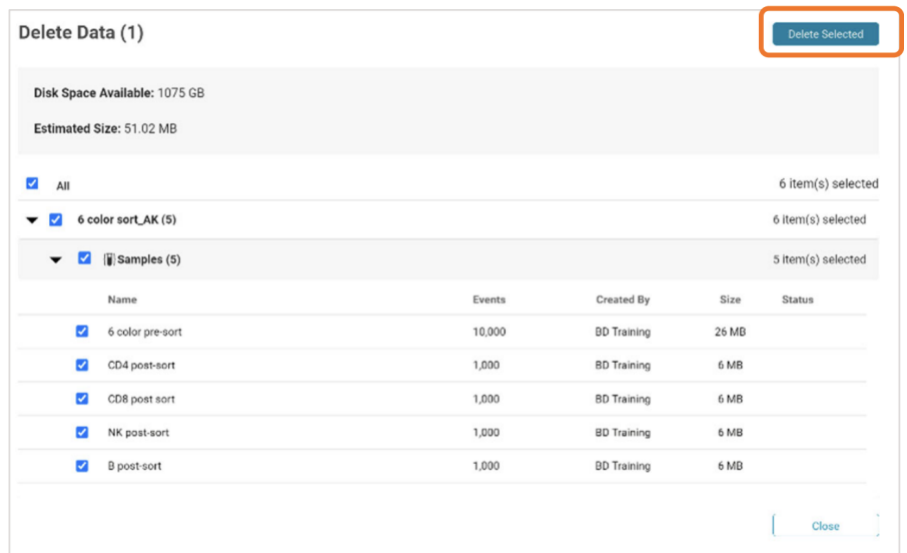
own hard drive on the computer beside the scheduler.

## Deleting old data

**Attention! Chorus Software requires at least 50GB free disk space. A yellow warning indicator will appear on the status menu when the disk is less than 250GB free. To keep the workstation running fluently, the data of an experiment can be kept on the S8 workstation for only 2 weeks. Please delete your old data in a timely manner.**

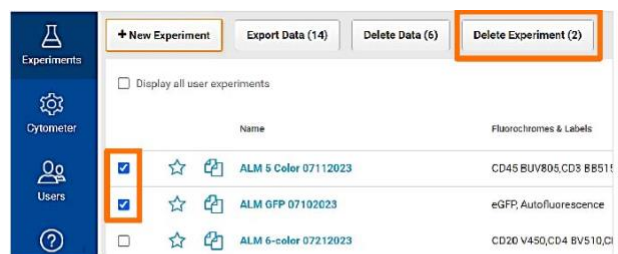
**The experiment without any data can stay longer for repeating purposes. Duplicate without data will carry over the unmixing matrix. Unmixing can be updated automatically when gains are changed.**

1. Navigate to the **Experiments** page.
2. Select the checkboxes for experiments which you want to delete.
3. Delete the data.
  - a. Click **Delete Data**.
  - b. Select the files to be deleted.
  - c. Click **Delete Selected**.
4. Close the dialog.

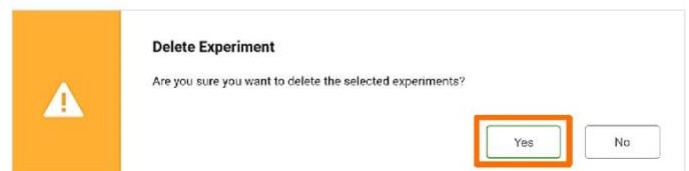


## Deleting experiments

1. Navigate to the **Experiments** page.
2. Select the checkboxes for experiments you want to delete.
3. Click **Delete Experiment**.
4. Click **Yes** in the dialog.



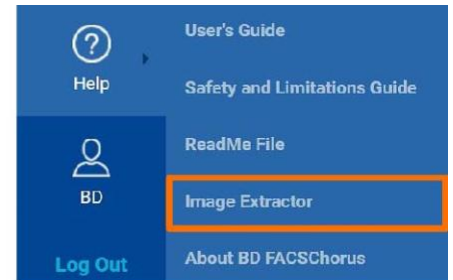
**NOTE** Deleting an experiment that is used as a template will also delete the template.



## Extracting TIFF files from CVW files

1. Plug the data transferring hard drive containing your data onto the analysis computer. It's beside the scheduler computer.
2. Plug onto your own hard drive.
3. Copy all your data in the data transferring hard drive to your own hard drive.

4. When completed, Open the **BD CellView™ Image Extractor** program on the desktop. It is available for download through the BD FACSCorus™ Software Help menu if you want to install it onto your own PC.



5. Click **Select Folder**.

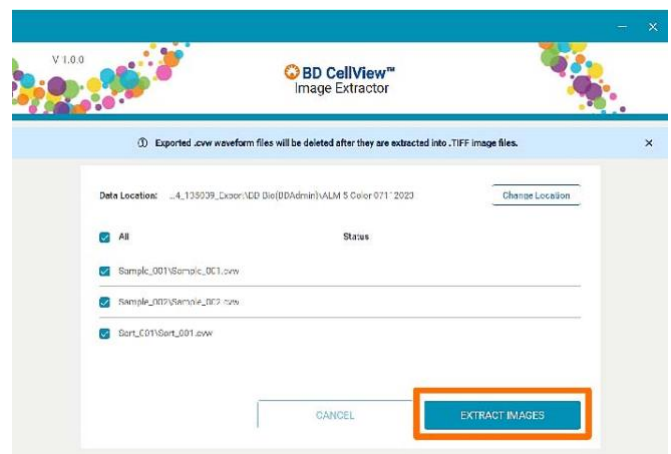
6. Browse to **your experiment folder in your own hard drive** that contains the CVW files of interest and select it, then click Select Folder.

7. Click the checkboxes to select the CVW files to be converted.

8. Click **Extract Images**. The CVW files are automatically deleted after TIFF extraction.

9. (Optional) Click **Change Location** to select another folder containing CVW files.

10. Close the **BD CellView™ Image Extractor** program.



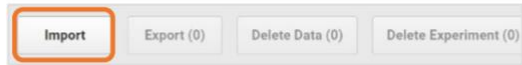
## Importing experiments

1. Navigate to the **Experiments** page.
2. Click **Import**.
3. Browse to the folder on the workstation that contains the Chorus Experiment File (CEF) of interest.
4. Select the CEF file and click **Open**.
5. (Optional) Clear the **Include Data** checkbox.

**NOTE** If the CEF file is in a folder that also contains FCS data, the checkbox is selected by default.

6. Click **Import**.

If a copy of the experiment exists, the name of the imported experiment is modified automatically.



### Import Data

The selected experiment will be imported into Chorus. Select Include Data to import data along with the experiment schema.

Export Location:	C:\Users\BDAdmin\Documents\20250501_123155_Ex...
File Name:	<a href="#">20250501_123155_5 Color PBMC MM.cef</a>
Chorus Version:	6.1.0
Instrument Platform:	FACSDiscover A8
Instrument Name:	
Export Time:	5/1/2025 12:31:55 PM
Number of Recordings:	8
Fluorochromes:	5
Tube:	2
Images:	2
Estimated Size:	3.9 GB
Disk Space Available:	3.6 TB

Include Data

Users	<input type="checkbox"/>	6 color sort_AK 1	6
	<input type="checkbox"/>	6 color sort_AK	6

# Clean Up After Use

## 1. Clean the sample line

**NOTE** Cleaning the sample line at the end of each experiment, between users, and before shutting down is a must.

- 1) Load a tube containing 3 mL of a 10% bleach solution onto the sample loading port.
- 2) From an open experiment, from the **View Data** tab, set the Flow rate to 20, click **Load**.
- 3) After approximately 10 minutes, click **Unload**.
- 4) Load a tube containing 3 mL of FACS Rinse onto the sample loading port. After 1 minutes, unload.
- 5) Load a tube containing 3 mL of sterile DI water onto the sample loading port. After 5 minutes, unload.

## 2. If there is a user after you, and the gap between you and the next user is less than 4 hours, perform Flow Cell Clean with a tube of filtered sterile water as follows.

Flow Cell Clean: Click **Cytometer** navigator > click **Flow Cell Clean** > follow the prompts on the screen through each numbered step.

**Then Log out from BD FACSCorus™ Software.**

## 3. If you are the last user of the day, or the gap between you and the next user is over 4 hours, perform the daily shutdown following the next page. Leaving the machine on all night while not being used is a waste and not acceptable.

## 4. Clean up the workspace.

- 1) Place the biohazard including samples, contaminated tubes, gloves, etc. into biohazard trash bin. Put all other trash into the regular trash bin.
- 2) Decontaminate the sample loading chamber, sort block chamber, sample collection chamber and desktop with Cavicide-wet paper towel.

# Perform A Daily Shutdown



## Before you begin

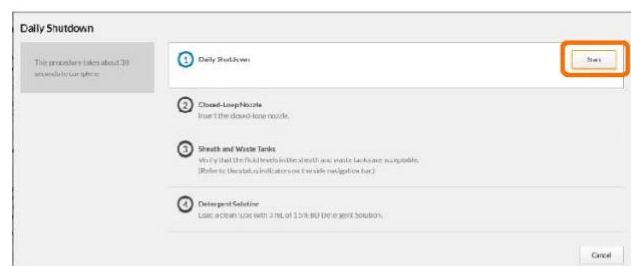
Prepare 3 mL of 1.5% BD® Detergent Solution from the BD® Detergent Solution Concentrate.

**CAUTION** Never mix BD® Detergent Solution and bleach because it can create dangerous fumes.

Make Sure you have cleaned the sample line.

## Performing a daily shutdown

1. In the navigation bar, click **Cytometer**.
2. Click **Daily Shutdown**.
3. Follow the prompts on the screen to complete each numbered step.
  - a. Click **Start** to begin the shutdown.
  - b. At prompt 2, move the sort nozzle out from the Flow Cell, insert the closed loop nozzle with the O-ring facing up, then click **Continue**.
  - c. Verify the fluid levels by checking the status indicators, then click **Continue**.
  - d. Load a clean tube with 3mL of 1.5% BD® Detergent Solution, then click **Continue**.
  - e. Once the procedure has been completed successfully, click **Close**.
4. Log out from BD FACSCorus™ Software.
5. Power off the cytometer unit by pressing the button on the right side of the instrument.
6. Shut down the computer.



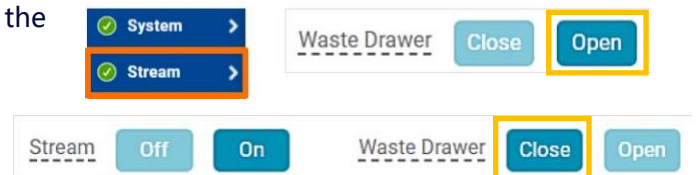
# Responding to A Nozzle Clog During an Experiment

If the nozzle is clogged, the stream will be stopped, an error message will pop out. If sorting, the machine is designed to stop sorting automatically and block the sort tubes. The sort will not restart until you clear the clog. When the nozzle is clogged, follow the procedure below.

1. Keep the front access door of the machine closed.
2. Turn the AMS to High.
3. In the **Stream** window, click **Open** to open the aspirator drawer.



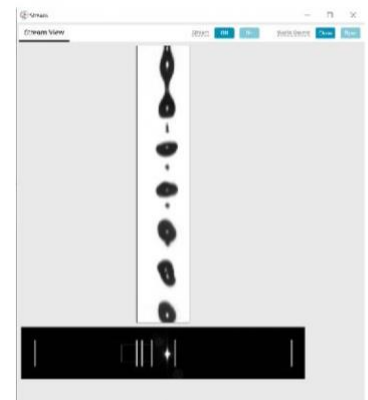
4. Wait 2 minutes. This procedure will clear aerosols from the sort collection chamber.



5. In the **Stream** window, click **Close** to close the aspirator drawer.

6. Recover from the clog:

- a. Open the front access door.
- b. Remove the nozzle, soak it in a tube of 70% Ethanol for 5min~10min to disinfect, then sonicate it in a tube of DI water for 1min to clear the clog.
- c. Open the sort block door, use Cavicide-wet Kimwipe to decontaminate the deflection plates, nozzle lever, and other surfaces, allow the decontaminated area to air dry.
- d. Reinsert the nozzle. Start the stream to verify the clog has been cleared.



**NOTE** If need to continue sorting, re-run the Drop Delay: go to Cytometer navigator > click System Startup > skip until the Run Drop Delay shows > load a tube of Accudrop beads > click Run > when finishes, click Continue.



7. Set the AMS back to Low.
8. Close the sort block door and the front access door.
9. Continue your experiment.