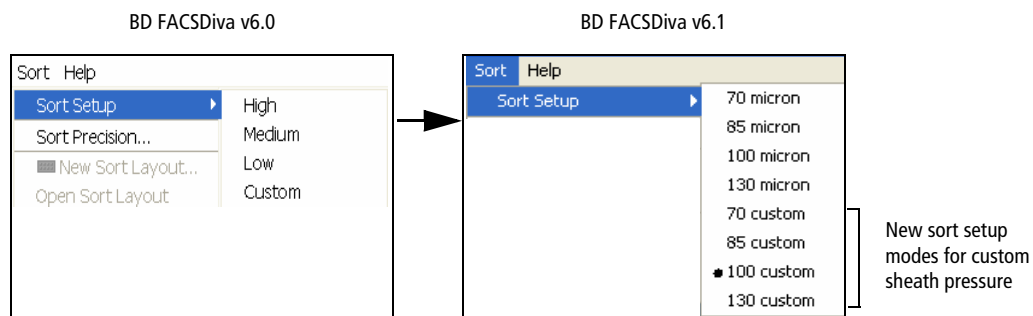


BD FACSDiva 6.1: New Features for the BD FACSAria

BD FACSDiva™ v6.1 software has two new features for the BD FACSAria™ cell sorter. This document contains an overview of those features, as well as an overview of the new Near UV laser option. For more information, refer to the *BD FACSAria User's Guide*.

New Sort Setup Names

When upgrading to BD FACSDiva v6.1 from previous versions, you will notice that the names of the sort setup modes have changed. All optimized or custom sort setup values will be retained. In addition, there will be four new custom sort setup modes, giving you greater flexibility for sorting at different sheath pressures. Refer to the *BD FACSAria User's Guide* for a table of the default values for each sort setup.

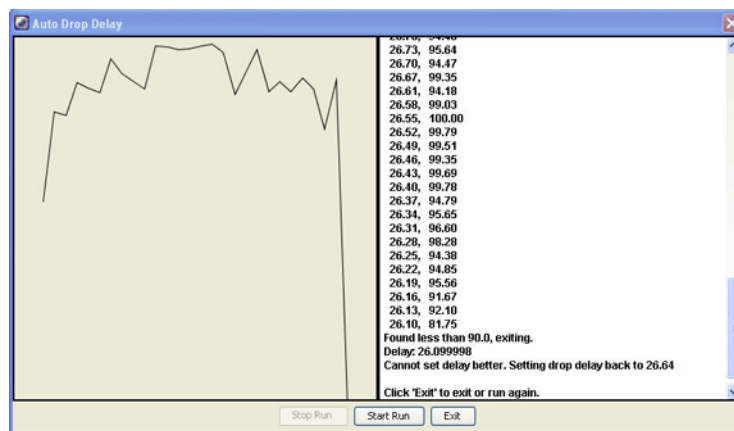
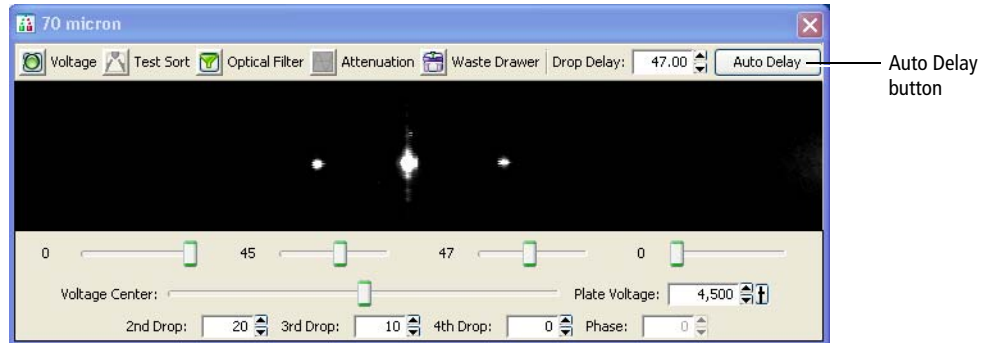


| BD FACSDiva v6.0 Setting | BD FACSDiva v6.1 Setting | Default Sheath Pressure |
|--------------------------|--------------------------|-------------------------|
| High | 70 micron | 70 psi |
| Medium | 85 micron | 45 psi |
| Low | 100 micron | 20 psi |
| Custom | 130 micron | 10 psi |

As a general rule, for optimal results when sorting large or fragile cells, use a larger nozzle size and lower pressure. To increase throughput and yield when sorting smaller or less fragile cells, use a smaller nozzle size and higher pressure.

Auto Drop Delay Feature

The Auto Drop Delay feature automates setting the drop delay to get optimized results from sorting.



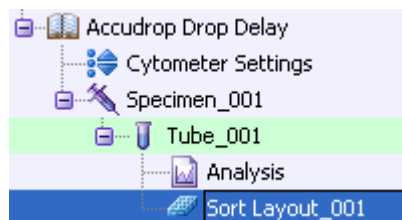
Once a sort experiment is set up, BD FACSDiva 6.1 software can determine the best drop delay value. Auto Drop Delay works best when the sort system is stable and assumes that BD Accudrop beads are used. When the process is started, the auto drop algorithm uses several passes to find the best drop delay possible under the current conditions of the system. Coarse passes are used to find an initial drop delay within two drops of the ideal. Fine tune passes are then used to locate the drop delay value which is ideal under the current conditions.

Using Auto Drop Delay

Setting Up the Workspace

The steps in this section show you how to set the drop delay using the Accudrop template. Because no data is recorded, the experiment can be reused as often as you like.

- 1 Verify that the stream is stable and the Sweet Spot is on.
- 2 Select your experiment folder and select Experiment > New Experiment.
- 3 Select the Accudrop Drop Delay experiment and click OK.
- 4 In the Browser, expand Specimen_001.
- 5 Expand Tube_001.
- 6 Set the current tube pointer to Tube_001.
- 7 Open the Sort Layout by double-clicking it.



Sorting Accudrop Beads

- 1 Load a tube filled with a suspension of BD™ Accudrop beads (approximately 1 drop of beads in 1 mL PBS).
- 2 Adjust the flow rate to achieve the event rates specified in the following table.

| Sort setup | Events per second |
|------------|-------------------|
| 70 micron | 1,000–3,000 |
| 85 micron | 800–2,000 |
| 100 micron | 600–1,500 |
| 130 micron | 400–1,200 |

- Tip** If this cannot be achieved using a flow rate setting between 1 and 5, adjust the bead concentration.

3 Click Sort in the Sort Layout window.

4 Click Cancel in the Confirm dialog.

There is no need to collect the beads. When the drawer is closed, the beads are sorted to waste.

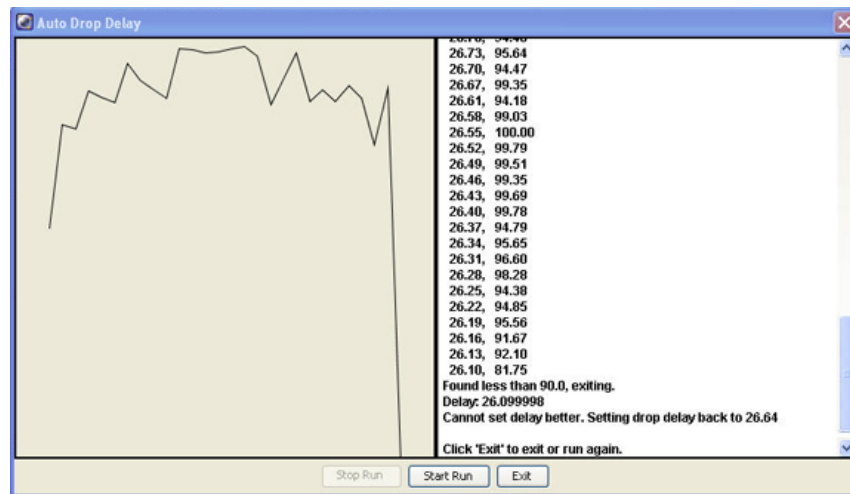
5 Click the Auto Delay button in the sidestream window.

6 Click Start Run in the Auto Delay dialog.

7 Monitor the Auto Drop Delay window for progress. A message appears when the process is completed.

NOTE If the sort is stopped during algorithm execution (either by user action, or because the system detected a failure), the run will be stopped and a message will be displayed. Start the sort again, verify the stream is stable and the sort is not pausing, and re-run the auto drop delay.

Figure 1-1 Auto Drop Delay window



8 Unload the Accudrop tube.

A dialog appears asking if you would like to save the sort report.

9 Click Cancel.

Tip To always make the same decision in the Accudrop experiment, select Save None in the Sort Layout under Save Sort Reports.

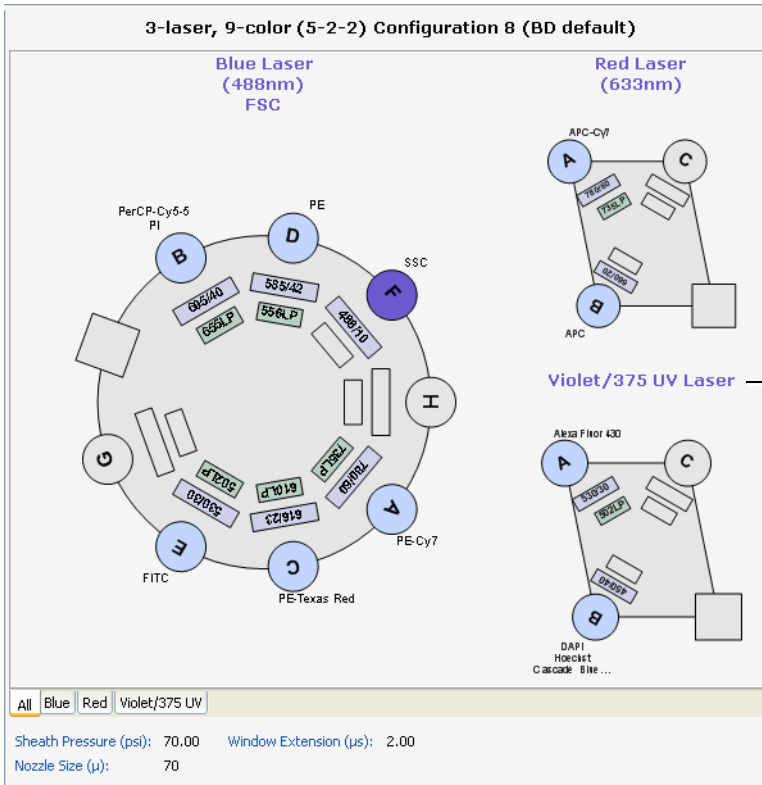
Near UV Laser Option

Specifications

| Laser | Nominal Wavelength (nm) | Power (mW) |
|---------------|-------------------------|------------|
| Near UV laser | 375 | 7 |



Toggle switch for violet and Near UV lasers



Optical Filters for Side Population Studies

When using the Near UV laser for side population studies, you can install a different set of filters in the far-left trigon. The filter set is supplied with the system when the Near UV option is installed. This set is optimized for side population stem cell applications.

| Detector Array (Laser) | PMT | LP Mirror BP Filter | Replacement Part No. | Intended Dye |
|-------------------------------|-----|---------------------|----------------------|--|
| Trigon (375-nm Near UV laser) | A | 610 LP | 640879 | Hoechst-Red, PI |
| | | 670 LP | 642328 | |
| | B | 450/20 | 642329 | Hoechst-Blue, DAPI, Pacific Blue™, Cascade Blue® |

Switching From the Violet to the Near UV Laser

In a four-laser system, the violet and Near UV lasers share the same optical pathway. As a result, you can use either the violet or the Near UV laser, but you cannot use them at the same time.

We recommend that you set up a custom configuration for the Near UV laser in BD FACSDiva software using the CS&T application. See the *BD FACSAria User's Guide* for details on creating custom configurations.

- 1 Place the Near UV/OFF/Violet laser switch in the Near UV position.

This turns on the Near UV laser and turns off the violet laser. Allow the Near UV laser to warm up for 30 minutes before recording data.
- 2 Set the cytometer configuration to a custom configuration that includes parameters for the Near UV laser.

It is a good practice to include Near UV in the configuration name (for example, 70-70 Near UV). This is especially helpful in a four-laser system.
- 3 Ensure that the appropriate optical filters for Near UV operation are installed into the far-left trigon.
- 4 Make sure the cytometer has a valid performance check or adjust the laser delay manually.