

Accudrop for BD Influx

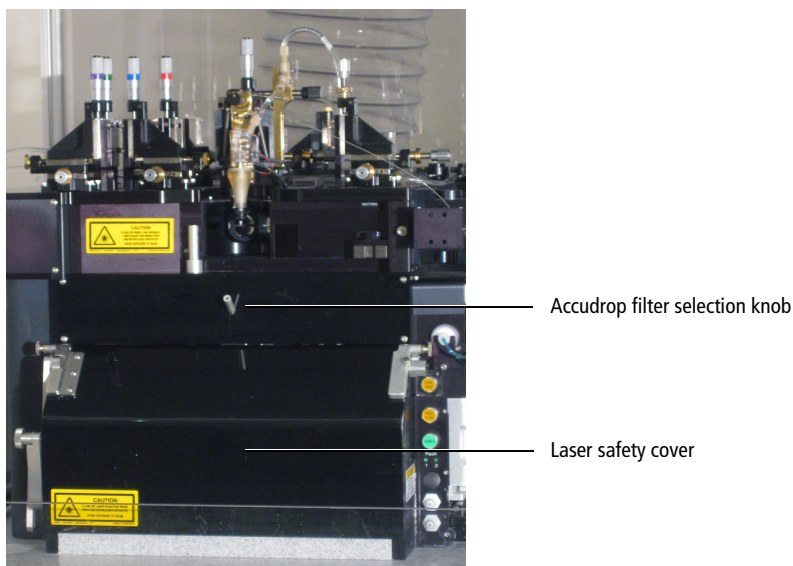
Supplemental Information

After completing this module, you will be able to use BD FACST[™] Accudrop technology to determine a precise drop delay for sorting on the BD Influx[™] cell sorter.

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

Determining the Drop Delay using BD Accudrop

BD FACS Accudrop technology is an alternate method for determining drop delay on the BD Influx cell sorter. To determine the drop delay, the streams are illuminated by the diode laser just below the deflection plates. When the Accudrop optical filter is in place, BD Accudrop beads can be viewed in the center and side streams as the drop delay value is adjusted. The most precise drop delay value yields the most particles in the side streams and the fewest in the center stream.



Setting up the System

- 1 Align the fluidics and optics for the BD Influx.

For more information, see the *BD Influx Operator Course Workbook*, Chapter 3 - Daily Alignment and QC.

NOTICE Pay special attention to placement of the stream in the center of the waste drain to ensure that the streams are properly aligned with the diode laser for drop delay determination.

- 2 Verify that the stream is stable and the side streams have been set up for a sort.

For more information, see the *BD Influx Operator Course Workbook*, Chapter 5 - Sorting.

Determining Drop Delay Using Accudrop

- 1 Use the Drop Delay Calculator to estimate a starting point for drop delay determination via Accudrop.

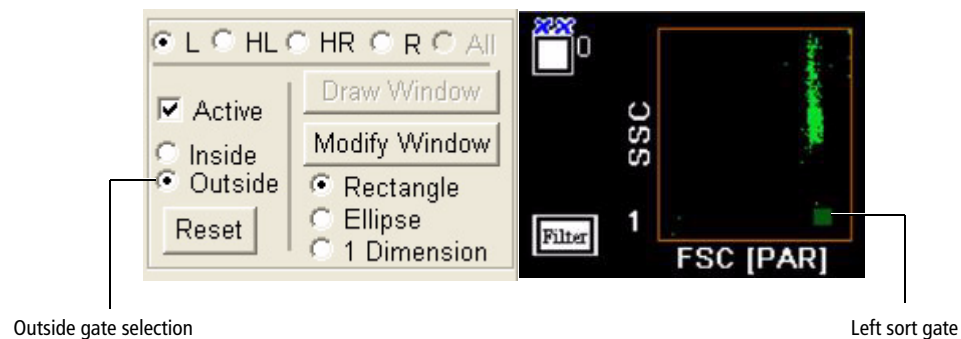
For more information, see the *BD Influx Operator Course Workbook*, Chapter 5 - Sorting.

- 2 Mix approximately one drop of BD FACS Accudrop Beads (BD P/N 345249) in 0.5 mL filtered PBS or sheath fluid.
- 3 Load the tube onto the cytometer.
- 4 Adjust the Sample Offset knob to achieve an event rate between 1,000 and 3,000.

NOTICE You might need to further dilute the beads to reduce coincidence abort rates when setting up the system at lower pressures or when using a large nozzle tip size.

- 5 Create a small left sort gate in the lower right corner of a forward scatter versus side scatter plot and make it an outside gate.

The resulting gate should include all bead populations including aggregates.

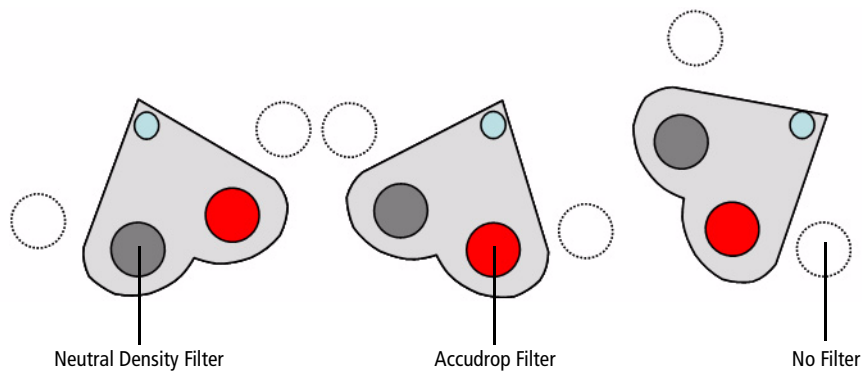


- 6 Select the **Purity - Yield** sort mode in the **Sort Config** window.
- 7 Delete any value in the left sort counter window so that it appears blank.

Leaving the field blank will result in a continuous sort.



- 8 Turn the Accudrop filter selection knob to the Accudrop optical filter position.

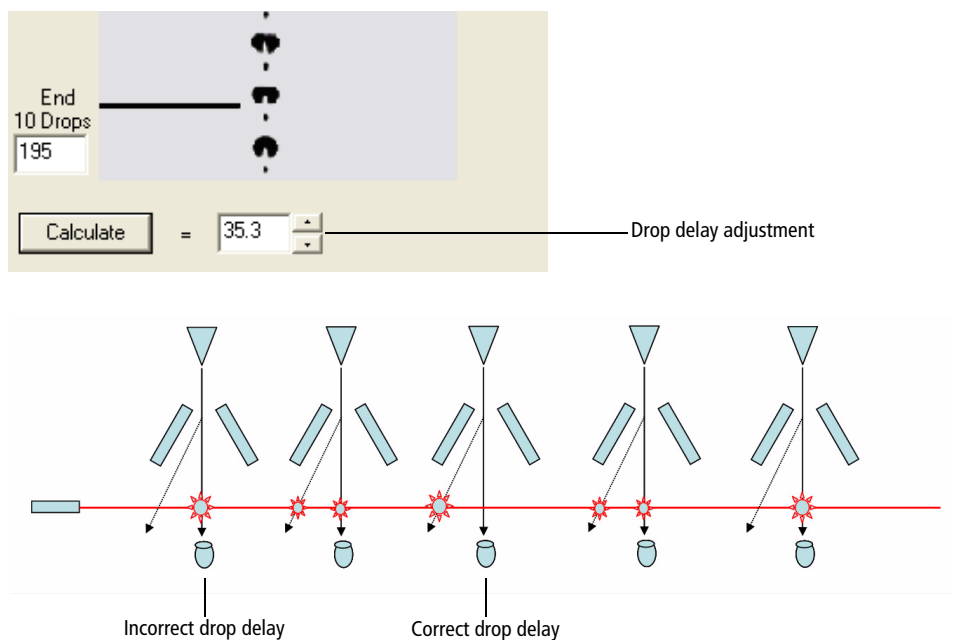


- 9 Start a left sort.
- 10 Adjust the drop delay value next to the **Calculate** button on the **Sort Config** window using the up and down arrows until the left stream is as bright as possible when viewed through the stream camera with the Accudrop filter in place.

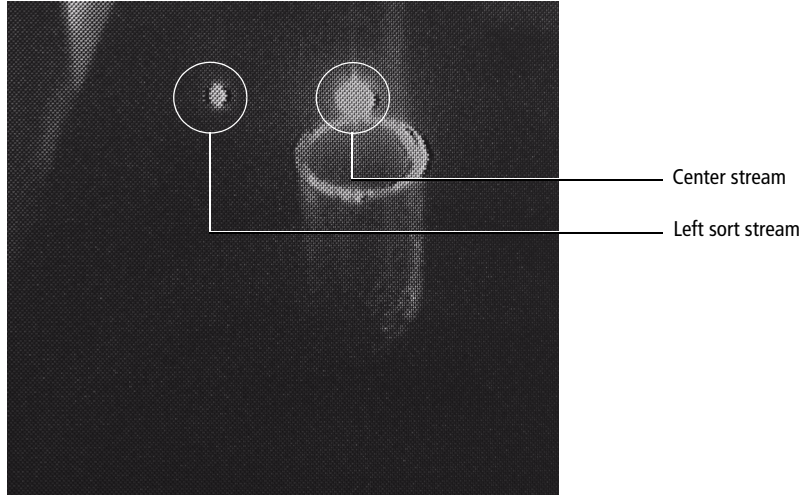
See the following figure and pictures for examples of correct and incorrect drop delays.

Tip It may be easier to look for the depletion of beads from the center stream as you near the correct drop delay.

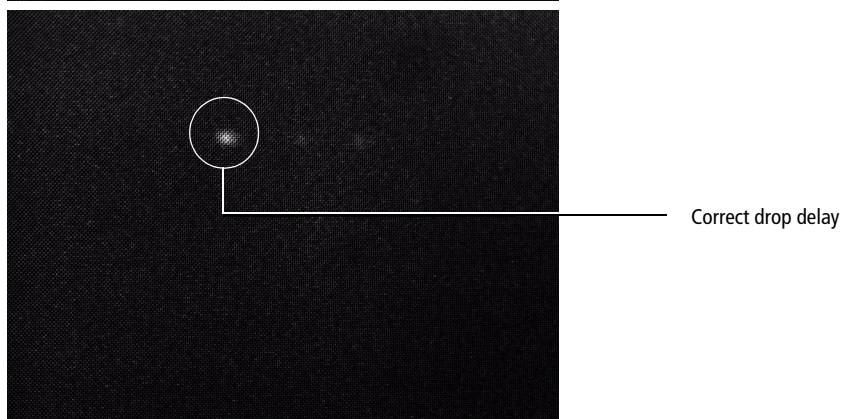
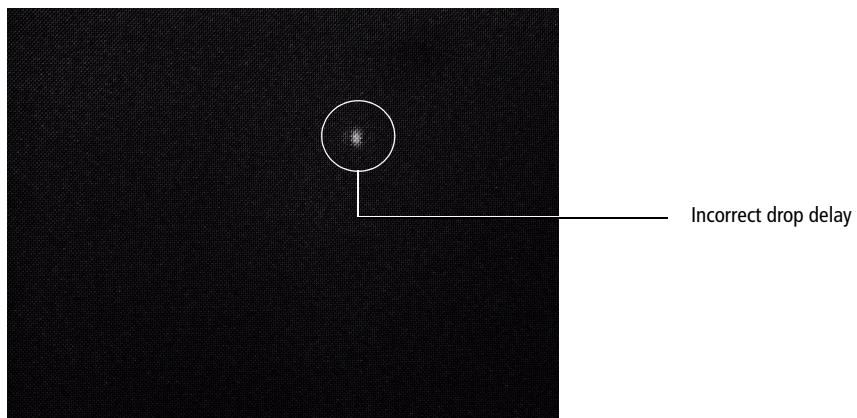
NOTICE The streams will not be visible unless the laser safety cover is closed and the Accudrop beads are running.



Neutral density filter



Accudrop filter



Common Issues and How to Fix Them

- Illumination of streams does not change with alteration of drop delay.
 - Make sure the Accudrop filter is selected.

- Cannot see the fluorescence in either the center or left streams.
 - Verify that the streams are intercepting the Accudrop laser.

NOTICE Recheck optical alignment if you adjust the streams to the Accudrop laser.

- Increase the event rate.

- Beads are visible in the center stream but not in the left sort stream, or, you cannot get all of the beads to populate the left sort stream.

- Check your gating strategy.
- Check your sort mode.
- Check alignment of the left stream to the Accudrop laser.

NOTICE Recheck optical alignment if you adjust the streams to the Accudrop laser.

- Use the **Drop Delay Calculator** to estimate a starting point.
- Slow down the time between adjustments.