ProDrop™ Technology — A Novel Approach to Automated Drop Delay Measurement and Monitoring

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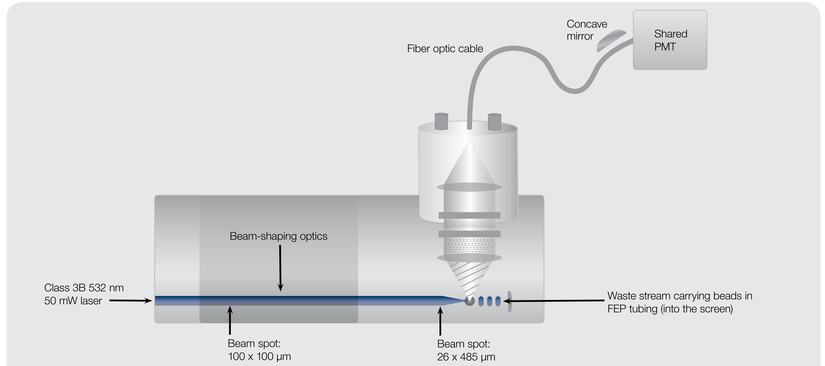
Abstract

Proper determination of the distance between the laser interrogation point and the last connected drop on an electrostatic cell sorter is critical to obtaining optimal sort purity. ProDrop technology measures the drop delay directly and accurately on the S3™ cell sorter using a novel method. The S3 cell sorter uses a laser to interrogate the waste stream while running ProLine™ calibration beads. The fluorescence signal from this laser is routed by a fiber to an existing photomultiplier tube (PMT), which accurately determines the exact number of beads in the waste stream. With deflection enabled, the drop delay setting is automatically adjusted through a set range, first coarsely, then a second time more precisely over a shorter range within the first coarse range for verification. A graph displays the number of beads detected at each setting. When the correct drop delay setting is found, the ProLine calibration beads are no longer detected in the waste stream because they are all being deflected. This is the exact drop delay value. ProDrop technology allows accurate measuring of the drop delay value without requiring user intervention or counting of beads under a microscope for confirmation. The S3 cell sorter continues to monitor the position of the break-off point and the length of the satellite drop and adjusts voltage, if necessary, during the sort to maintain precise sort stability. This ensures optimal sort performance and purity.

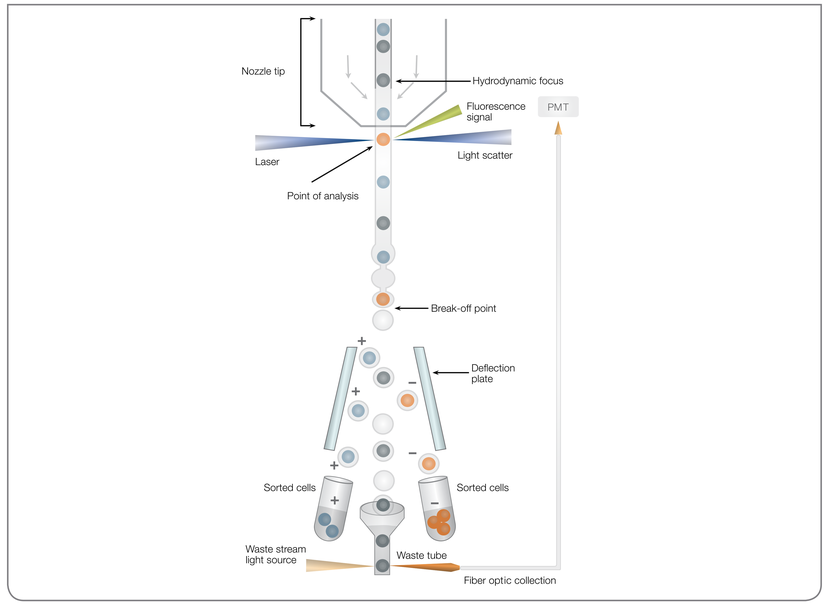
S3 Cell Sorter

Waste Bead Detection Module



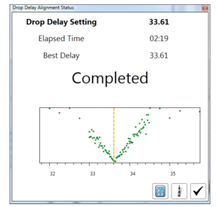
Bio-Rad's novel waste bead detection module serves as a "cytometer within a cytometer." The module uses a 50 mW green laser to illuminate the waste stream. The fluorescence is collected, focused, and directed by a fiber optic cable to one of the existing PMTs for detection. FEP, fluorinated ethylene propylene; PMT, photomultiplier tube.

Sort Stream

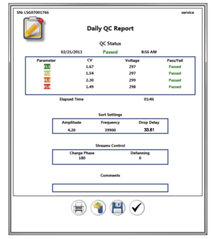


Schematic of sort stream. Schematic depicting the mechanism by which events are sorted. Events are hydrodynamically focused by the sheath fluid before exiting the nozzle tip. With jet-in-air technology, events are interrogated in the stream where the sort decision is made. The stream is then charged at the break-off point prior to electrostatic deflection. Droplets can be deflected in two directions based on predefined sort logic conditions.

Drop Delay and QC Report in ProSort™ Software

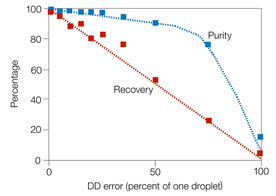


Drop delay is determined during the quality control (QC) process. The Drop Delay Alignment Status window graphically displays the number of ProLine calibration beads detected in the waste stream. The window displays the correct drop delay value and "completed" when finished.



A QC report window appears after QC is performed. Coefficient of variation and PMT values for each channel are displayed along with drop delay value, frequency, and amplitude. All the information is stored in a flow cytometry standard (FCS) file and can be viewed in the Trending Report, which displays trends in values over designated periods of time.

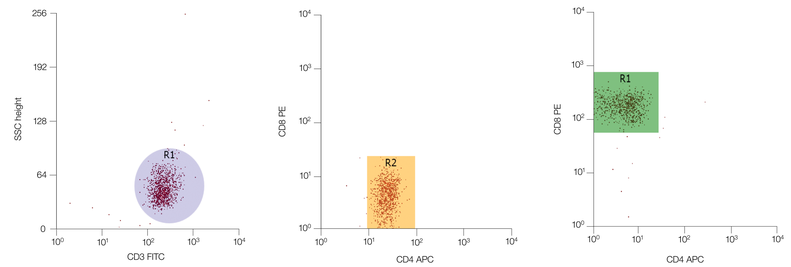
Drop Delay Errors



Drop delay errors reduce recovery more than purity. A mixture containing 5% fluorescent beads in nonfluorescent beads was sorted at an event rate of 11,500/sec on a MoFlo XDP cell sorter using the sort single mode. The drop delay setting was varied by increments of 5–100% of a droplet period to introduce errors into the charge timing of the sorter. At each increment, 5,000 fluorescent beads (Flow Check) were sorted to measure purity in the sorted sample and ten puddles of ten fluorescent beads each were sorted onto a glass slide to measure the recovery of the target beads. The dashed lines represent the mathematical model of the error expected in the purity (■) and the recovery (■) and the markers represent the measured values.

Data courtesy of Karen Helm, University of Colorado.

Peripheral Blood Mononuclear Cell Sorting



Peripheral blood mononuclear cell (PBMC) sorting. Human PBMCs were stained with CD3 FITC, CD4 APC, and CD8 PE and sorted for either double positive CD3+ CD4+ or CD3+ CD8+ cells. Cells were sorted in purity mode and reanalyzed for purity. APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

Summary

ProDrop technology automates and determines an accurate measurement of the drop delay in the S3 cell sorter. With this feature, high purity and recovery of sorted cells can be obtained, providing confidence and reliability in sorting experiments.

Key features and benefits of the automated S3 cell sorter include:

- Automated monitoring of the drop delay setting during the sort with automatic adjustments to any changes during the run
- Automated removal of collection tubes to preserve sorted samples in cases such as clogs, if drop delay is unrecoverable or is not maintained
- Bubble detector in the sample line allows samples to be run completely dry and automatically triggers end of sample in the ProSort software
- Volume tracking avoids costly overflow of sorted samples

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