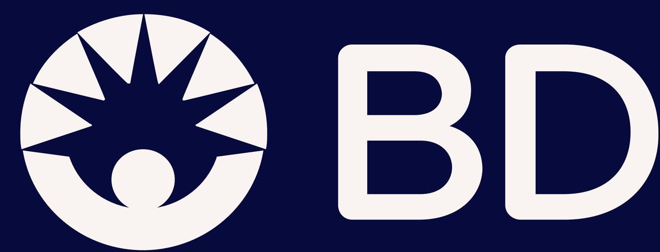

Loader performance and optimal setup for high-throughput data generation using BD FACSDiscover™ A8 Cell Analyzer.

Woodrow Lomas, Tri Le, Henry Lankila, Kyle Dembski, Kory Pennebaker, Tigran Nahapetian, Abhijith Segu, Lauren Tucker and Aaron Middlebrook.

BD Biosciences, Milpitas, CA

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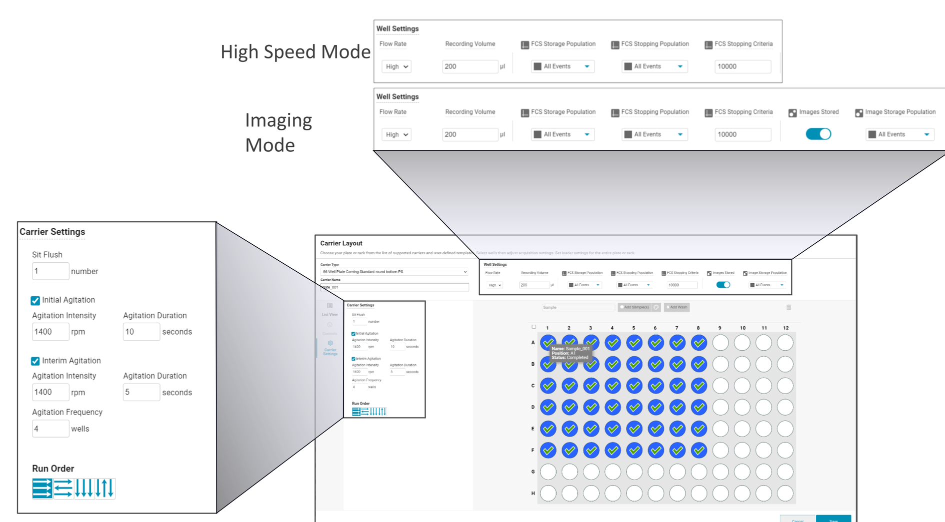
Abstract

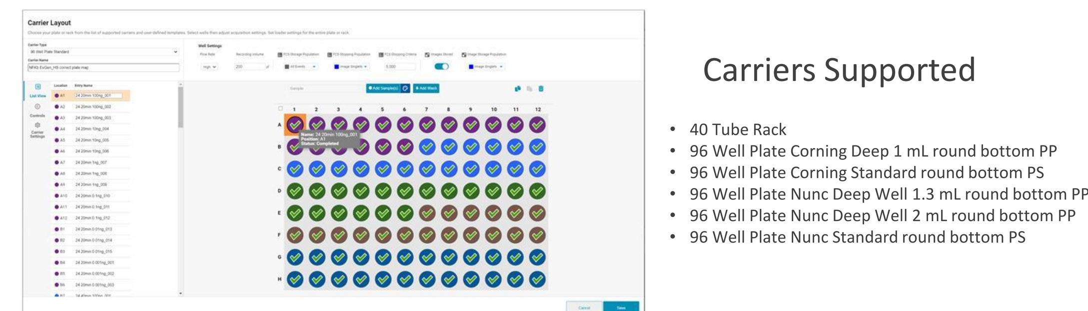
The variables at play during data acquisition through the use of an automated loader can be daunting and can confound an otherwise well-designed experiment. Factors such as cell concentration, resuspension volume, acquisition criteria, mixing frequency and duration can all play significant roles in determining the relative success of the experiment. Using the BD FACSDiscover™ A8 Cell Analyzer with built in automated loader and dual speed functionality, we present a systematic approach to optimizing loader performance to achieve consistent, high-quality data. Mixing efficiency as a function of carrier style, cell concentration, sample volume, agitation and agitation frequency were performed. Output metrics for mixing efficiency are based on the number of events acquired in a fixed volume. Careful tracking of these metrics facilitated the establishment of a range of settings that result in optimized loader performance. Furthermore, we present data to assess the impact of carrier agitation on cell viability and fluorescence stability. Other confounding issues such as acquisition criteria with respect to the minimum number of events needed to achieve statistically robust results in the context of immunophenotyping applications were explored. Based on these data, we present a set of guidelines to achieve maximum throughput without sacrificing the quality of data. In addition, strategies to assess throughput, carryover and dead volume will be presented.

Methods

BD FACSDiscover™ A8 Plate Loader Setup User Interface



BD FACSDiscover™ A8 Plate Annotation Windwo



Carriers Supported

- 40 Tube Rack
- 96 Well Plate Corning Deep 1 mL round bottom PP
- 96 Well Plate Corning Standard round bottom PS
- 96 Well Plate Nunc Deep Well 1.3 mL round bottom PP
- 96 Well Plate Nunc Deep Well 2 mL round bottom PP
- 96 Well Plate Nunc Standard round bottom PS

A series of experiments were performed designed to assess the BD FACSDiscover™ A8 Cell Analyzer's integrated loader. These experiments were designed to identify the performance limits of the loader in the context of mixing efficiency. Mixing efficiency experiments were done with both a cell line (Jurkat T Cells) and peripheral blood mononuclear cells (PBMCs). The consistency of the number of cells acquired in a fixed acquisition volume from samples with fixed cell concentration was used as a measurement of relative mixing efficiency. Deviations from the default agitation settings were explored to determine the validity of these settings and the impact of deviation. Carryover and throughput experiments were performed at default settings in both imaging mode and in high-speed mode (high-speed mode carryover data not shown). Similar sets of experiments were done using the other carriers supported such as 40 tube rack and the deep well, 96 well plate format.

Results: Mixing Efficiency

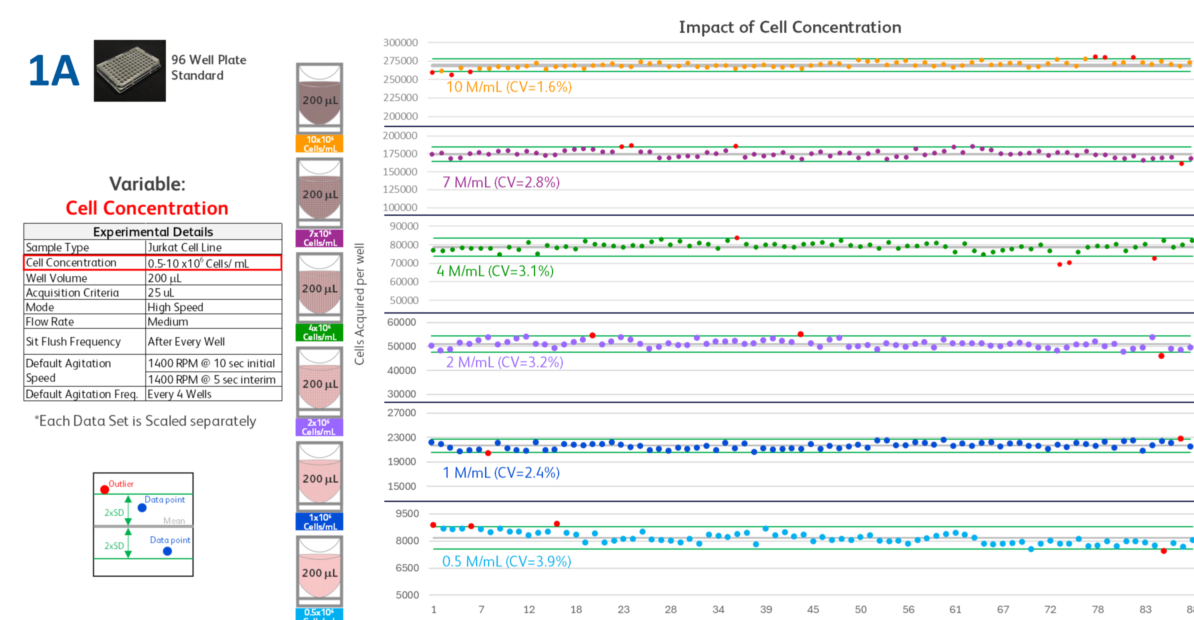


Figure 1A. Impact of Cell Concentration on Mixing Efficiency: The number of cells acquired in a 25 µL acquisition volume is plotted for each well in a 96 well plate. A range of cell concentrations were tested from 0.5 million cells/mL up to 10 million cells per mL. In each case the default loader settings were used with orbital mixing occurring every 4th well for 5 seconds at 1400 rpms. Coefficient of variation (%CV) was calculated for each condition. Data points depicted in red were those that fell out of a range defined as 2 x Standard Deviation above or below the mean.

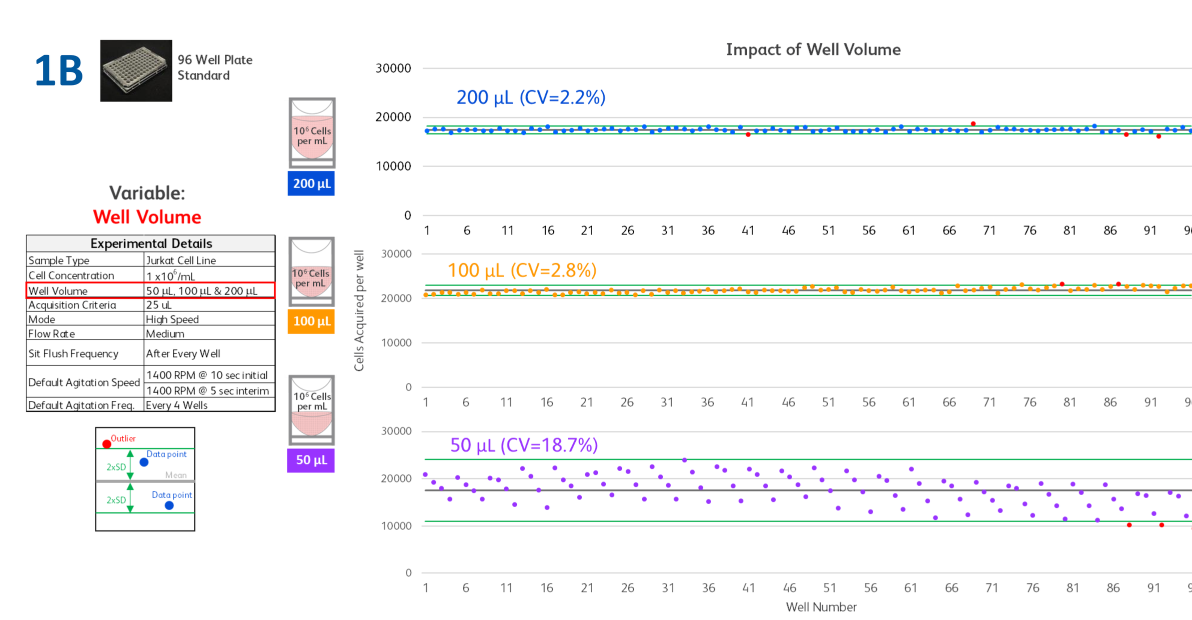


Figure 1B. Impact of Well Volume on Mixing Efficiency: The number of cells acquired in a 25 µL acquisition volume is plotted for each well in a 96 well plate. A range of well volumes were tested from 50 µL up to 200 µL. In each case the default loader settings were used with orbital mixing occurring every 4th well for 5 seconds at 1400 rpms. Coefficient of variation (%CV) was calculated for each condition. Data points depicted in red were those that fell out of a range defined as 2 x Standard Deviation above or below the mean.

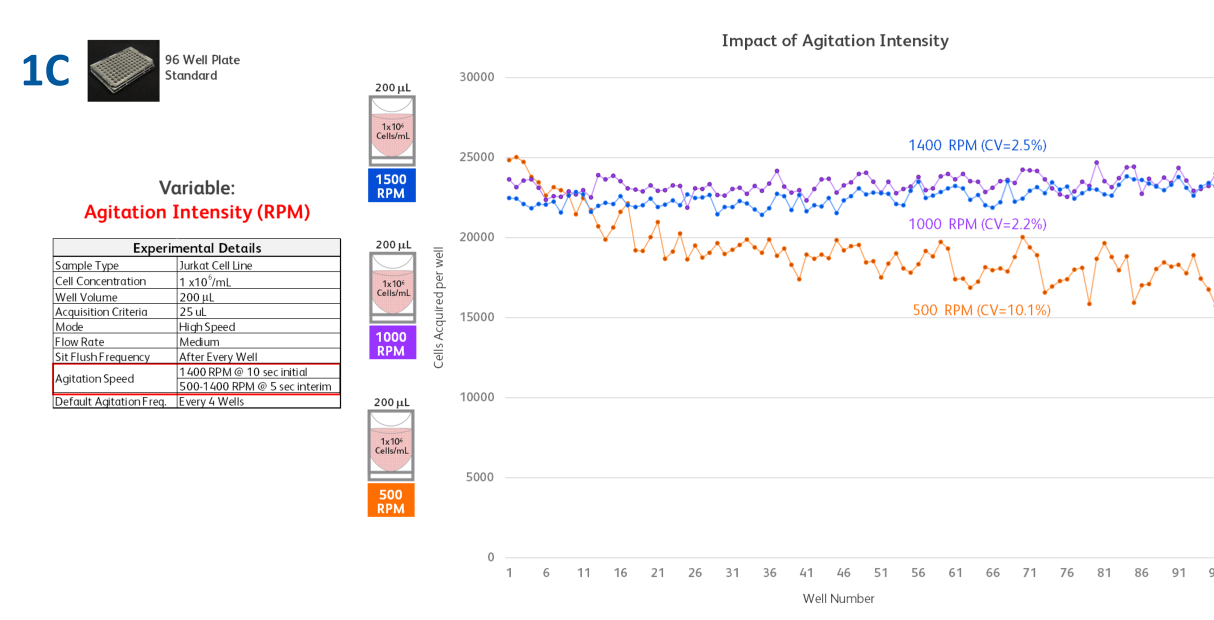


Figure 1C. Impact of Agitation Intensity on Mixing Efficiency: The number of cells acquired in a 25 µL acquisition volume is plotted for each well in a 96 well plate. A range of agitation intensities were tested from 500 RPM up to 1400 RPM. In each case the default loader settings were used with orbital mixing occurring every 4th well for 5 seconds. Coefficient of variation (%CV) was calculated for each condition.

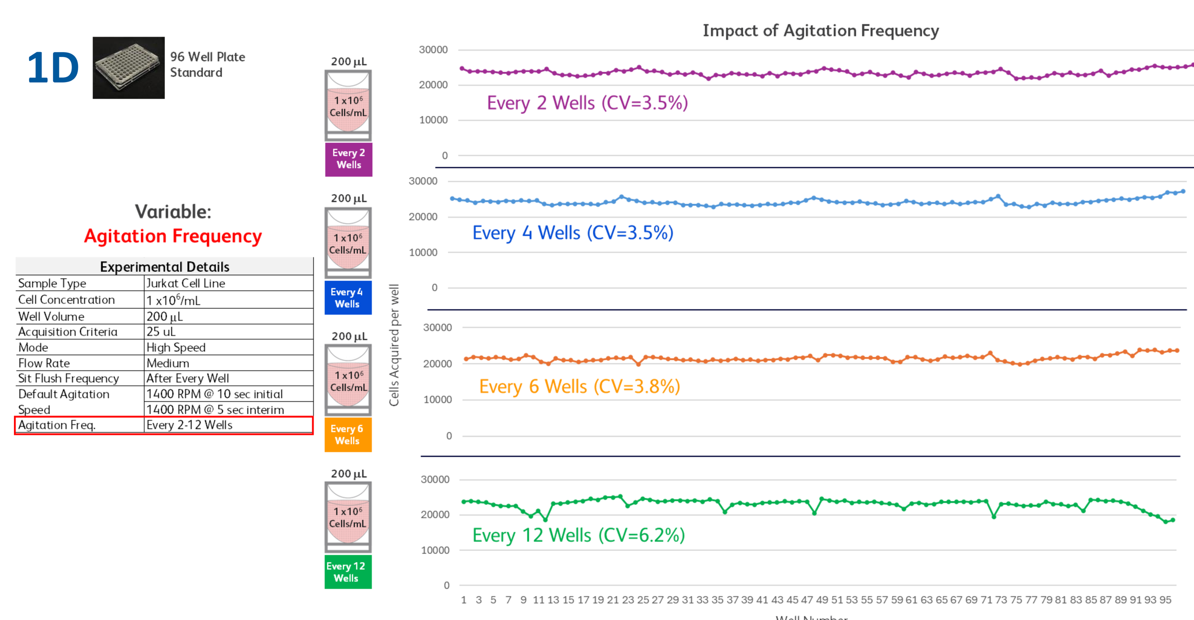


Figure 1D. Impact of Agitation Frequency on Mixing Efficiency: The number of cells acquired in a 25 µL acquisition volume is plotted for each well in a 96 well plate. A range of agitation frequencies were tested from every 2 wells up to every 12 wells. In each case the default loader settings were used with orbital mixing occurring for 5 seconds at 1400 RPM. Coefficient of variation (%CV) was calculated for each condition.

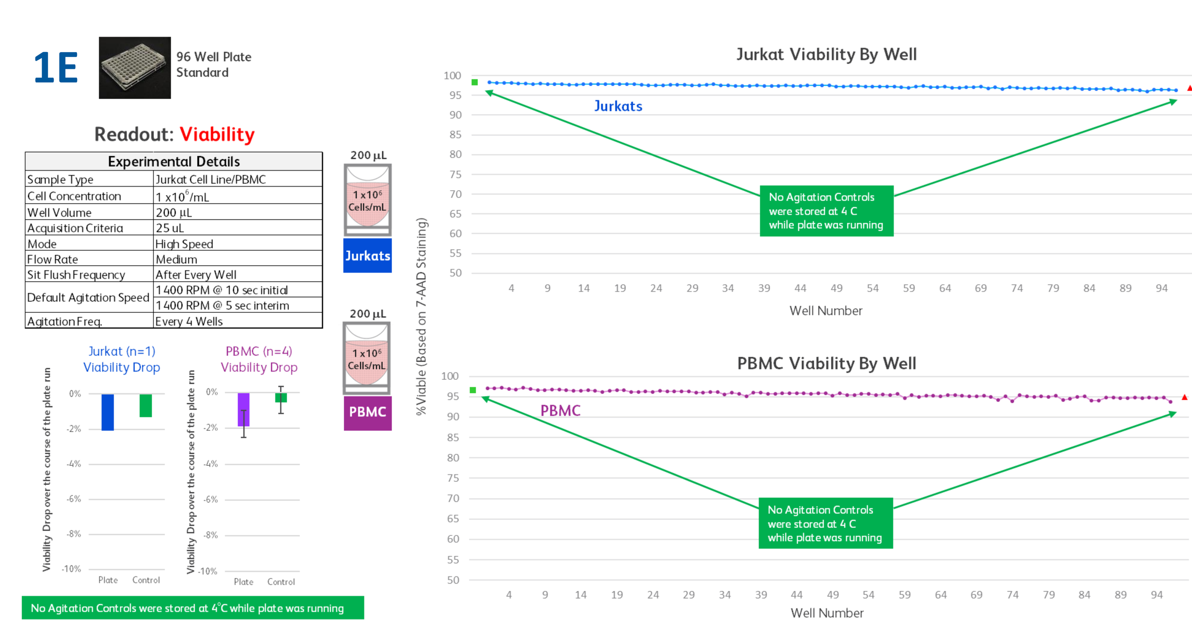


Figure 1E. Impact of Default Agitation Settings on Viability: Using a well volume of 200 µL, and default agitation settings (1400 RPM, every 4th well for 5 seconds), the viability of both Jurkats (upper plot) and PBMC (lower plot) were measured for each of the 96 wells over the course of a plate acquisition. Cell viability was measured by 7-AAD staining. As a reference, a no agitation control was measured- an aliquot of Jurkats or PBMC that was stored at 4°C during plate acquisition. The viability of the no agitation control was tested before and after running the plate.

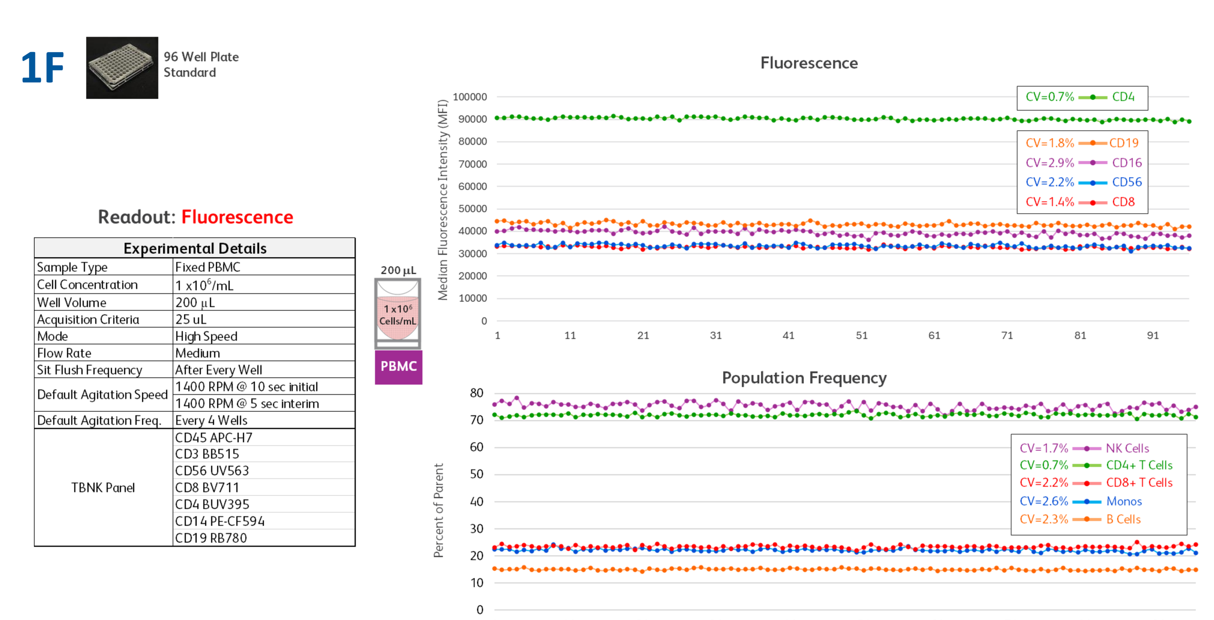


Figure 1E. Impact of Default Agitation Settings on Fluorescence: Using a well volume of 200 µL, and default agitation settings (1400 RPM, every 4th well for 5 seconds), a 7 color TBNK panel was used to investigate the impact of agitation of measured fluorescence. The upper plots shows the median fluorescence intensity (MFI) of several key markers in the panel while the lower plot shows the relative frequencies of populations that can be quantified using the panel.

Results: Carryover and Throughput

Figure 2A & 2B. A8 Carryover: PBMC (2A) and HT-29 Cells (2B). Carryover was tested using a standard protocol in which 1 well of a 96 well plate is loaded with a 10 million cells/mL (2A- PBMC in the plot on the left and2B- HT-29s, an extremely clumpy cell line in the plot on the right). The highly concentrated well is followed by 4 blank wells in which only PBS is loaded. The number of cells still in the system in the blank wells are then measured as 'carryover' events. This was done in both High speed mode (Data not shown) and in imaging mode with similar results. A blank well of PBS was acquired before the first well to establish background noise in the PBS. The bar graph in the bottom left of each figure plots the mean carryover events in 10 replicates of a carryover series (5 wells in total).

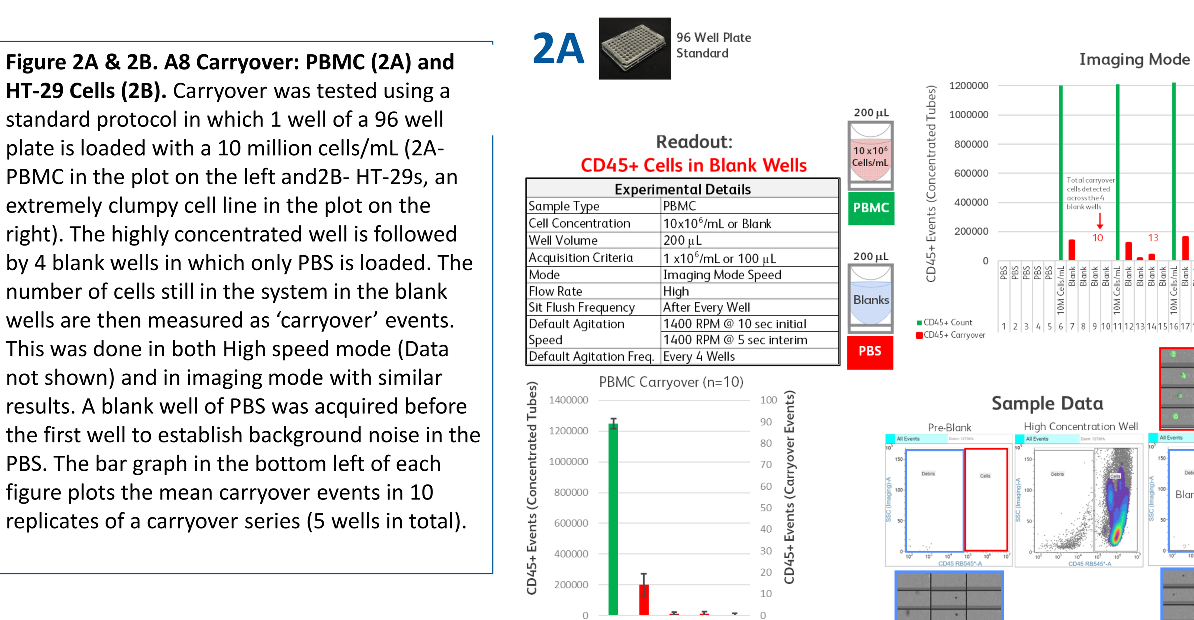
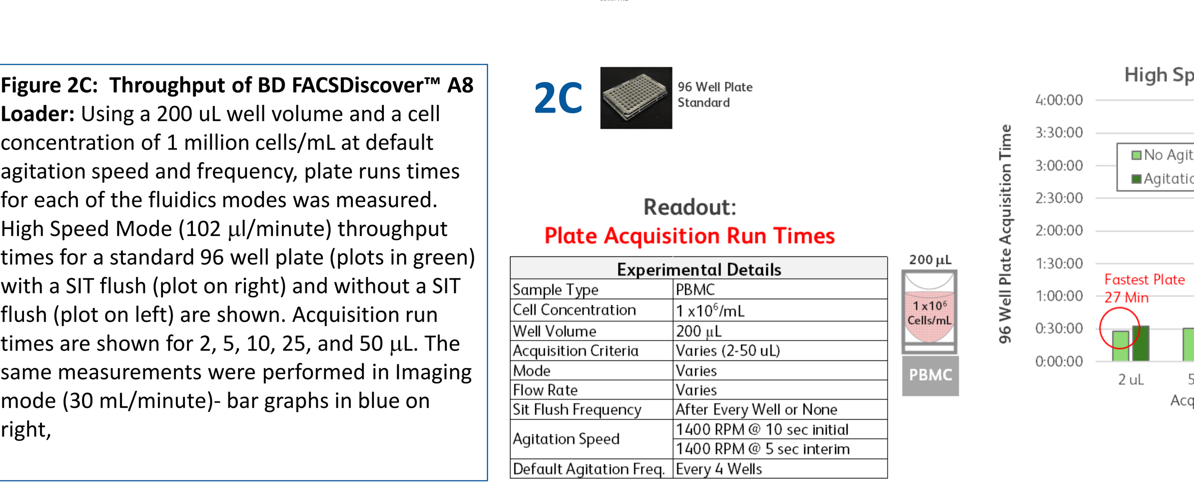


Figure 2C: Throughput of BD FACSDiscover™ A8 Loader: Using a 200 uL well volume and a cell concentration of 1 million cells/mL at default agitation speed and frequency, plate runs times for each of the fluidics modes was measured. High Speed Mode (102 µl/minute) throughput times for a standard 96 well plate (plots in green) with a SIT flush (plot on right) and without a SIT flush (plot on left) are shown. Acquisition run times are shown for 2, 5, 10, 25, and 50 µL. The same measurements were performed in Imaging mode (30 mL/minute)- bar graphs in blue on right,



Mode	Flow Rate	Range (µL/Min)	Nominal Target (µL/Min)
Imaging	Low	8-16	12
	High	23-35	30
High Speed	Low	8-16	12
	High	90-110	102

Conclusions

The integrated Loader on the BD FACSDiscover™ A8 Cell Analyzer is equipped with customizable features that enable the user to achieve maximum mixing efficiency of their samples over the course of a plate acquisition including agitation frequency, duration and intensity (RPMs). These setting should be optimized based on the user's needs. However, the instrument is equipped with default settings that have been tested to produce optimal results.

The objective of this work was to demonstrate how these default values were determined and to provide information on the impact any deviations from these settings might have. We found that the default settings produced the most reliable data and that in most cases, significant deviation from those was required to impact the results. In addition, we explored the loader performance in the context of carryover and throughput in both imaging and high-speed modes.

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