Comparison of the Analysis Capabilities of Beckman Coulter MoFlo XDP™ and Becton Dickinson FACSAria™ I and II

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Abstract

The speed at which a flow cytometer can accurately and efficiently measure samples becomes more significant as flow cytometry expands to more demanding applications and experiments. Therefore, a standard, repeatable, singlet bead assay was developed to compare the analytical capabilities of the Beckman Coulter MoFlo XDP™ and the Becton Dickinson FACSAria™ I and II. The Aria II, while capable of bead analysis at 65K events per second (EPS), lost data ranging from 20% of expected events at 20K EPS to 75% of expected events at 65K EPS. MoFlo XDP produced no measurable data loss at rates up to 80K EPS and relatively minimal loss (20%) at 140K EPS. The XDP data was confirmed by third-party analysis. The results of the comparison show that the MoFlo XDP measures more events, and accurately analyzes events four to five times faster than Aria I and II.

Introduction

Scientific and clinical researchers working in flow cytometry today experience increasing demands to perform experiments and applications that involve high throughput, rare-event analysis and detailed immunophenotyping. Analysis of rare populations, such as stem cells, demands that the majority of the sample is analyzed, and that events are not lost due to instrument limitations. Equally important in rare-event analysis is the need for results that provide high confidence in the statistical distribution of data. Furthermore, rare-event analysis and detailed immunophenotyping can be time consuming. Therefore, samples must be run rapidly in order to preserve the quality of time-sensitive material, and to make the most of laboratory resources.

Beckman Coulter (BCI) and Becton Dickinson (BDIS) offer multi-use flow cytometry sorters that, according to specification sheets, can analyze up to 70K EPS with more than nine parameters enabled. The amount of data obtained from a sample varies greatly between instrument platforms. One hypothesis for the cause of the discrepancy in instrument performance is that the platforms have different electronic acquisition structures and sample delivery methods. MoFlo XDP (BCI) uses a narrow pulse width to detect events (Figure 5) and delivers sample through jet-in-air. The Aria (BDIS) uses a wider pulse width as well as a pulse

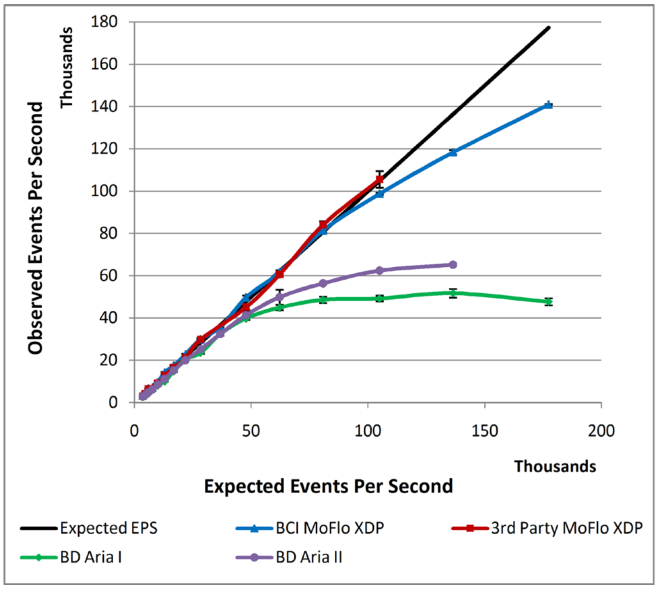

Figure 1. Expected EPS The black line represents the calculated expected EPS values. The discrepancy between the expected and observed events per second is due to the lost events, defined as the events that were expected, but missed and never recorded. Due to the narrower pulse width, the MoFlo XDP missed very few events until speeds reached over 100K EPS, whereas the Aria began to lose events at an acquisition speed of 20K EPS and was unable to reach rates above 65K EPS.

width extension, and delivers sample through a cuvette.

Prior to this study, the theoretical extent to which the Aria pulse width and window extension affect sample measurements had been studied by Dan Fox[1] but not demonstrated empirically. This study was designed to quantify how the differences between XDP and Aria electronics and sample delivery affect speed and sample analysis.

Before examining the data it is important to understand the concepts of pulse width, lost events, and hard aborts.

- Pulse width is defined as time elapsed from the point at which an event signal crosses a set threshold and then falls below the threshold. The wider the pulse width, the longer the interval of elapsed time.
- Events per second (EPS) is defined as the total number of threshold breaches per second, including aborts.
- Lost events are defined as events that were expected, but never detected.
- Hard aborts (or electronic aborts for Aria) are defined as events that were detected, but the electronics could not discern if the signal represented one or more events.

Theoretically, increased pulse width should cause an instrument to detect fewer distinct events. The effect should be amplified as acquisition speed increases because events will arrive at the interrogation point closer together. Considering the theoretical potential of lost and aborted data due to pulse width, it is important to quantify which instrument produces better results when samples are analyzed at top speeds. Therefore, an iterative and reproducible singlet bead assay was created to test the acquisition speed versus sample yield on the XDP and the Aria.

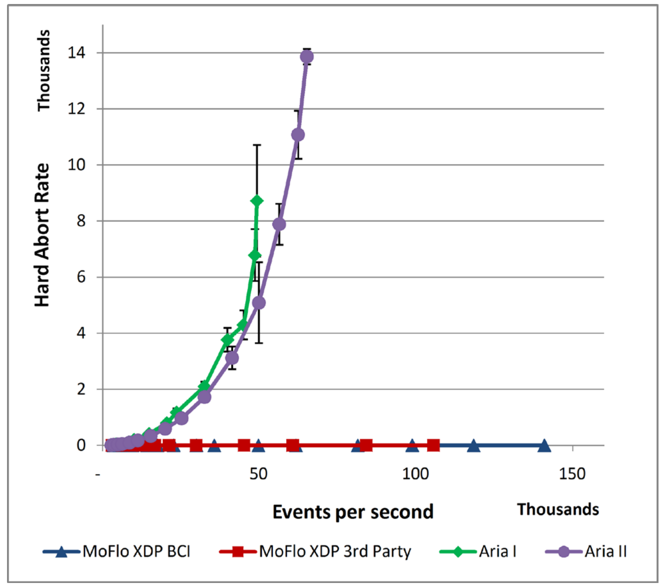

Figure 2. Hard Abort Rate Bead assay samples were run at different speeds while the hard (electronic) abort rate was measured. During analysis, the MoFlo XDP produced no measured hard aborts. The Aria I experienced approximately 9K hard aborts per second at 50K EPS, and the Aria II experienced approximately 14K hard aborts per second at 65K EPS. This translates into a 20% sample loss at the highest Aria speed.

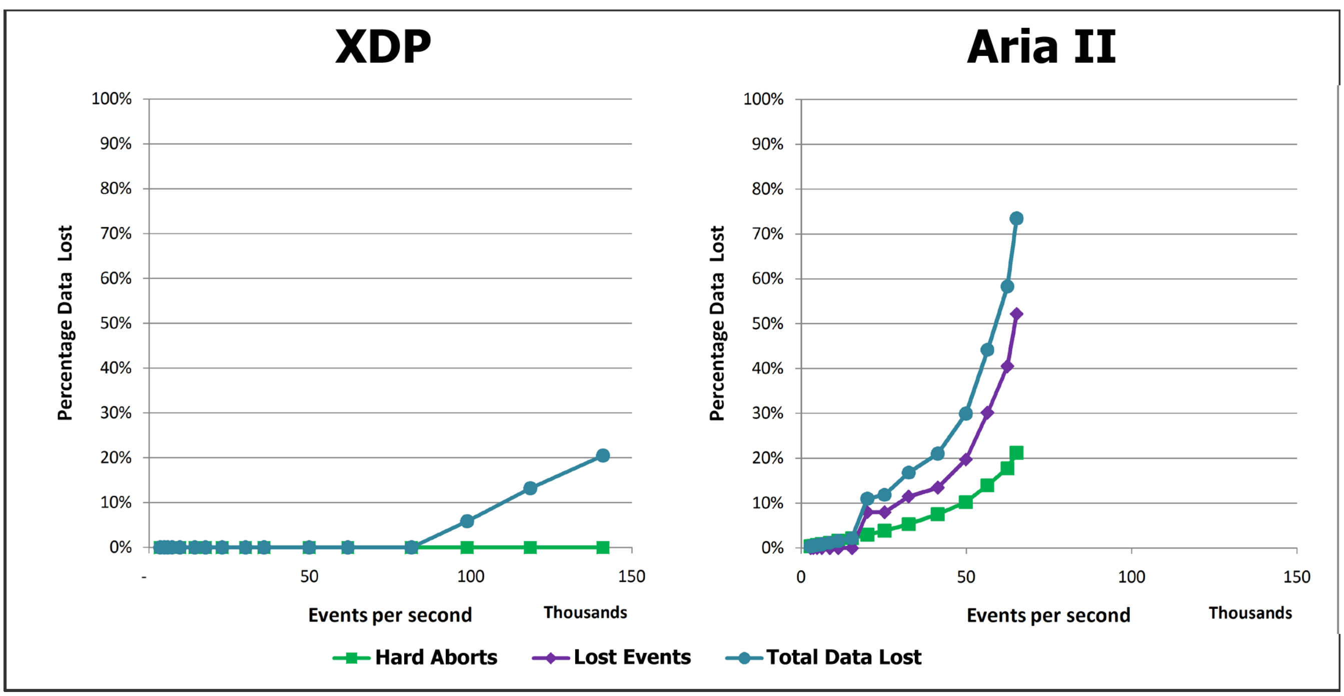

Figure 3 A. MoFlo XDP Data Loss The MoFlo XDP had 0% hard aborts and lost events until acquisition speeds reached above 100K EPS. **B. Aria II Data Loss.** After 20K EPS, the Aria II lost incrementally more data as the speed increased until it reached 75% data loss at 65K EPS.

Results

The comparison of analytical capabilities between the XDP and the Aria demonstrated that the XDP measures more events with fewer aborts, and more accurately analyzes events at rates up to five times faster than the Aria.

A total of 15 sample concentrations were analyzed on the XDP and the Aria. The effect of pulse width and window extension can be seen in the differences between captured events per second (Figure 1) and the hard aborts for each platform (Figure 2).

Speed of acquisition: XDP is faster and obtains more data per sample.

The XDP and the Aria were set at an identical pressure differential to define stream width for both instruments. Theoretically, if the stream width is the same, the two instruments should measure the same number of events per second as the sample concentrations increase. Each experiment was executed in triplicate and the results are displayed with corresponding error bars. Minimal variation in the data points from multiple iterations of the experiment demonstrates the robust nature of the bead assay as a repeatable, dependable measure of analysis.

Aria loses 38% more data than XDP at high speeds and high sample concentrations.

As shown in Figure 1, the comparison between expected and observed EPS on both platforms emphasizes the Aria's loss of event detection. Throughout the bead assay, the EPS recorded by the XDP matched the expected EPS up to approximately 100K EPS. In contrast, the Aria began to lose events at 20K EPS and, regardless of expected events, could detect no more than 65K EPS (Figure 1). Both systems produced lost events. However, the Aria encountered a much earlier loss at 20K EPS, reaching a 50% data loss at 65K EPS.

Hard Aborts: Aria aborts up to 20% of data, XDP aborts 0% of data.

The XDP produced no hard aborts at processing speeds up to 110K EPS (Figure 3A). However, the Aria had an incremental increase in the hard abort rate starting at 3K EPS producing 10 hard aborts per second when a 2 µs window extension was used as recommended in the BD FACSAria User's Guide[2]. When the Aria reached a processing speed of 65K EPS, the hard abort rate increased to 14K, a 20% data loss (Figure 3B).

Total Data Analyzed: Aria loses up to 75% of data, XDP loses 20% of data at highest speeds and sample concentrations.

Total sample recovery was calculated by combining the lost events and hard aborts for the XDP and the Aria. The XDP (Figure 3A) recovered its entire sample until the speed reached 80-100K EPS. The Aria recovered only 25% of the sample at 65K EPS but began losing events at 20K EPS (Figure 3B). Instrument differences can also be seen in Figure 4 where the total data analyzed is plotted for each platform. Again, the XDP analyzed most of its sample, whereas the Aria, at higher speeds, lost up to 75%.

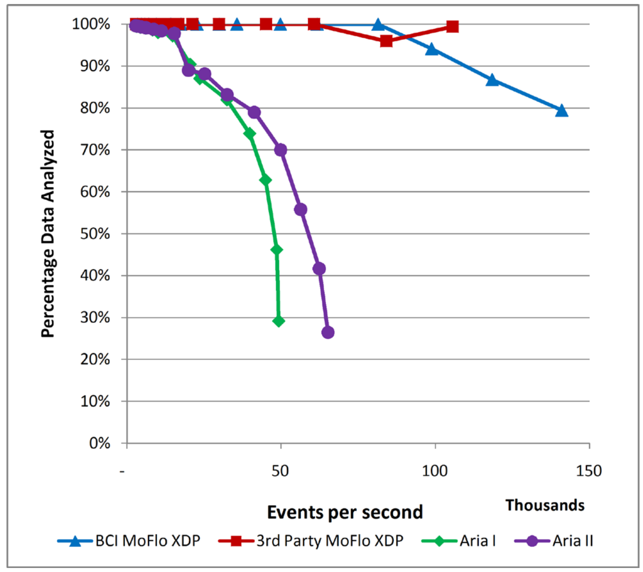

Figure 4. Comparison of the Total Data Analyzed The MoFlo XDP produced few aborts until the acquisition speed reached 100K EPS. The Aria (I and II) lost data beginning at 20K EPS and plateaued at 65K EPS with the total sample analyzed decreasing to 25%.

Discussion

The bead assay was developed with singlet beads as an effective way to measure both the hard aborts and lost events produced by the XDP and the Aria. With the same samples used on each instrument, and the expected events in the samples already established, events lost during analysis could be accurately determined.

Theoretical calculations (Fox[1]) indicate that the pulse width of the Aria is almost five times longer (3.96 µs versus XDP 0.82 µs) for a 13 µm cell. Increased pulse width is detrimental to the determination of singlet versus doublet events. A shorter pulse distinguishes each event separately giving a more accurate measurement of event count and speed. Figure 5 illustrates the calculated theoretical pulse width (Fox[1]) on the XDP and the Aria for random events disregarding hydrodynamic focusing. Events that are well-separated do not exhibit a difference in EPS or hard abort rate during analysis (Figure 5 event A). If the events are closer together (B and C), the size of the pulse width signal changes the data obtained. The XDP analyzes (B and C) events as two discrete measurements. On the other hand, the Aria with the increased pulse width does not distinguish two events, but instead produces one distorted signal (brighter signal, wider scatter) and ignores event C. Event C is not counted by the electronics and therefore is lost.

During multi-laser analysis, adjustments to pulse width must be made to account for changes in particle velocity. The XDP uses a “sliding-window” system to detect events across all three laser detection pinholes. This sliding window maintains pulse width integrity while permitting precise measurements through all laser lines. In contrast, the BD FACSAria User’s Guide[2] recommends that users implement a fixed 2 µs window extension when performing multi-laser analysis. This time added to the threshold is intended to help detect the signal from all laser lines. In theoretical calculations, the resulting pulse width increases from 3.96 µs to 5.96 µs for a 13 µm cell, making the pulse width seven times longer than the XDP pulse width. This difference is noticeable when events (B and C) and (D and E) pass through the stream and result in increased pulse width on the Aria. This causes hard aborts for (D

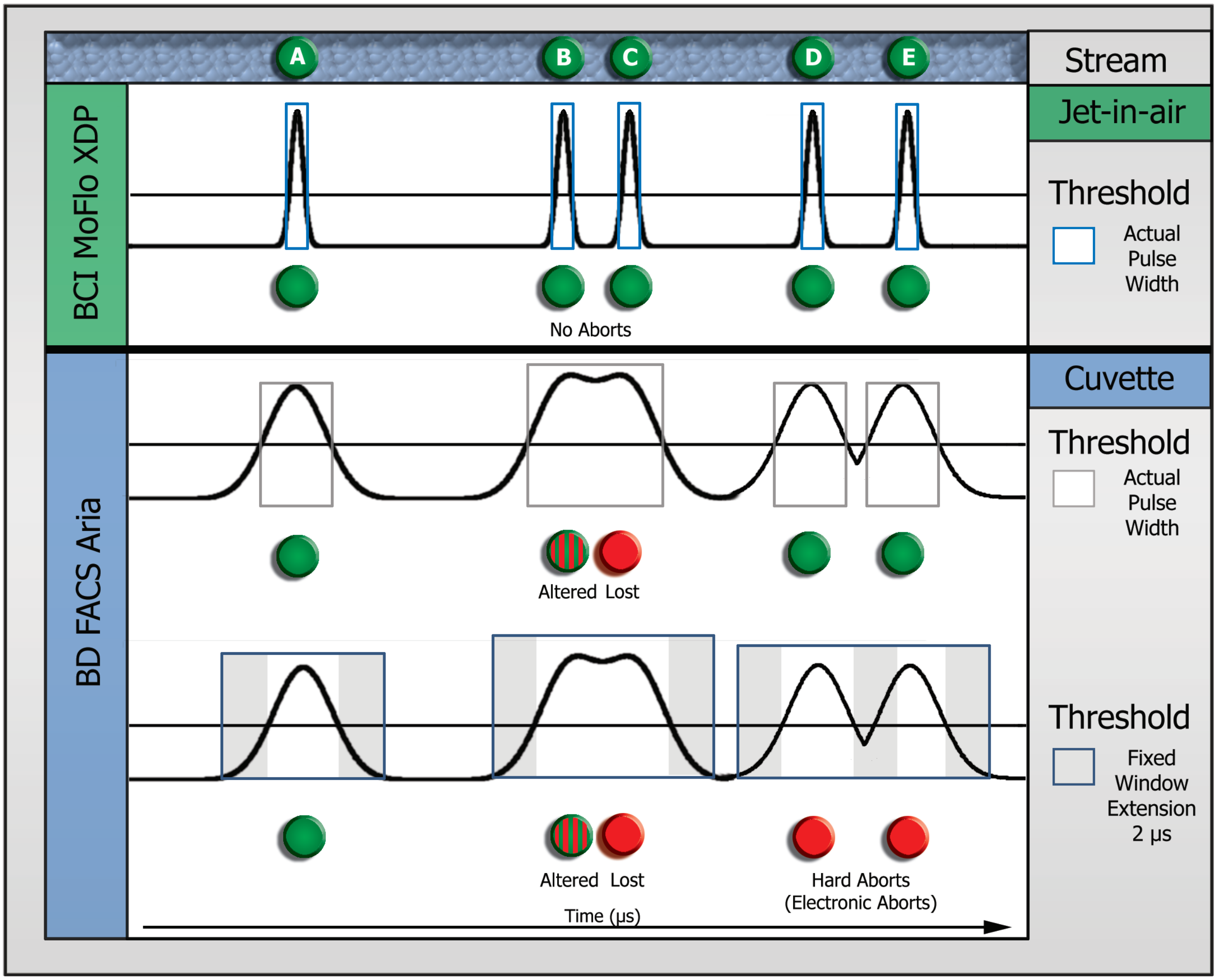

Figure 5. MoFlo XDP vs. Aria Pulse Width Events that are well-separated do not exhibit a difference in EPS or hard abort rate during analysis (Figure 5 event A). If the events are closer together (B and C), the size of the pulse width signal changes the data obtained. The XDP analyzes (B and C) events as two discrete measurements. On the other hand, the Aria with the longer pulse width does not distinguish two events, but instead produces one distorted signal (brighter signal, wider scatter) and ignores event C. Event C is not counted by the electronics and therefore is lost. During multi-laser analysis, adjustments to pulse width must be made to account for changes in particle velocity. The XDP uses a “sliding-window” system to detect events across all three laser detection pinholes. This sliding window maintains pulse width integrity while permitting precise measurements through all laser lines. In contrast, the BD FACSAria User’s Guide[2] recommends that users implement a fixed 2 µs window extension when performing multi-laser analysis. This difference is noticeable when events (B and C) and (D and E) pass through the stream and result in increased pulse width on the Aria. This causes hard aborts for (D and E) events, and is perhaps why the signal from event (B-C) is distorted and event C is lost. The deciding factor in successful multiple laser data acquisition is the pulse width window, sliding or fixed. During multiple laser acquisition the XDP loses 0 events, whereas the Aria possibly aborts 3 and ignores 1 event out of 5.

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Pulse Width: Wider means more data lost.

As previously discussed, the increased pulse width used by the Aria would clearly impact data analyzed. The disparity between expected and observed EPS could be explained by the lost event which occurs when two events are too close together to be measured and are taken as one event (Figure 1, Figure 5). The Aria loses 50% of its sample to lost events at 65K EPS, whereas the XDP only begins to have the same problem at speeds of 80K EPS (4% loss) up to 140K EPS (21% loss). The XDP's narrower pulse width measures more sample and more efficiently resolves doublets.

Aria window extension: Increases pulse width and creates more aborts.

The variation in event acquisition during multi-laser analysis adds additional data loss for the Aria. The Aria's fixed window extension increases the pulse width and therefore causes more aborts (20% hard aborts at 65K EPS). Aria users may adjust the window extension, but sample resolution may suffer. In contrast, the XDP's sliding window keeps the pulse width narrow, thereby producing no aborts.

Conclusion

In conclusion, the Aria, while capable of analyzing beads at 65K EPS, produced data loss ranging from 20% at low speeds to 75% at 65K EPS. However, the MoFlo XDP had 0% data loss at rates up to 80K EPS and relatively minimal loss (20%) at rates twice the specified EPS limit.

Materials and Methods

Sample Preparation

Samples were prepared with 7.06 µm Bangs Laboratory (Fishers, IN) carboxylated beads with a >95% singlet status, 10% w/v beads (4.90 x10^8 beads per mL). The primary sample was created with 2.0 mL of beads and 800 µL of DI H_2O (+0.01% NP40) in a 5 mL polystyrene tube and then 2.0 mL of the dilution was used in succession until the 15th tube was reached in a dilution series. Samples were kept at 4ºC (39°F) until used within a 24-hour period.

Flow Cytometry

Both the MoFlo XDP and the Aria I and II were set up as described in their instrumentation manuals[2,3] with calibration done according to specifications. The threshold setting was also kept constant, and the differential pressure was set to 3000 EPS ± 100 for the lowest dilution sample. This ensured that both instruments had the same stream width.

Acquisition

Beads were analyzed on two parameters, FSC-Height versus SSC-Height with the voltages set within the accepted PMT voltages for maximum signal/minimum noise. All other parameters were removed from capture to reduce computer processor requirements. A minimum of 2 million events per sample were recorded.

Samples were run on both instruments concurrently, using the same bead dilution tube consecutively to remove any variability due to sample preparation. Each experiment was done in triplicate on all instruments. Data was recorded on threshold count, events per second, electronic (hard aborts), and acquisition time (seconds).

Calculations

1. Expected events per second = (Starting EPS/dilution factor)
2. Calculated events per second = (Event count/analysis time)
3. Hard abort rate = (Hard aborts/acquisition time)
4. Percentage of lost events = (Expected EPS - actual EPS)/expected EPS
5. Percentage of hard aborts = Abort count/event count
6. Percentage of total sample recovered = 100% - (100% - Percentage lost events) + (100% - Percentage hard aborts)

References

1. Fox, D. "Analysis of Electronic Yield." available on coulterflow.com, June 2007.
2. BD FACSAria User's Guide, 2003, available on bdbiosciences.com.
3. MoFlo XDP Instructions for Use, 2009, available on coulterflow.com

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Customer Impact

This study demonstrates that hard aborts and lost events, constitute an important difference between the MoFlo XDP and the Aria I and II. By choosing a MoFlo XDP, the user is expected to gain the following benefits:

MoFlo XDP...	Rationale
Obtains more information from each sample during analysis.	Very few events are lost.
Serves more customers in core labs.	Samples can be run faster (4-5X faster) than Aria I and II.
Captures rare events in sample populations quickly.	Reduces potential for missing rare populations because few events are aborted.
Maintains cell viability.	Sample run time is reduced, preventing cell degradation of time sensitive samples.
Heightens customer confidence.	Very little data is lost (even at high speeds) therefore confidence in statistical distribution is high.
Reduces staffing costs.	Shorter sample run time, more samples run on one instrument, equates to fewer people required to operate an instrument.
Decreases reagent requirements.	Fewer events are lost so applications can be run with smaller samples, requiring less reagents.
Expands instrument usage.	Faster sample analysis translates into more customers able to use the instrument.

Table 1. MoFlo XDP Benefits