Flow Cytometry Technologies

Jet in air vs. Cuvette sorting: A modern comparison of post sort viability across diverse cell types

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Abstract

Post-sort viability is a central benchmark for cell sorting performance, particularly as researchers work with increasingly fragile or rare populations. Although cuvette-based instruments are widely adopted, the perception that jet in air sorters facilitate gentler handling persists despite limited direct, matched comparisons. To address this, we evaluated post-sort viability using two jet in air systems (Invitogen™ Bigfoot™ Spectral Cell Sorter and jet in air Competitor B) and a cuvette-based sorter (Competitor A) across a diverse panel of commonly used cell types.

Matched samples—including adherent lines, suspension lines, and stem cell–derived cells—were sorted under comparable operating conditions, with efforts made to align nozzle size, pressure, and collection environments to minimize confounding variables. Viability was assessed immediately post-sort using fluorescence-based assays. Across matched datasets, both jet in air systems demonstrated significantly higher viability compared to the cuvette-based sorter, while no meaningful difference was observed between the two jet in air platforms. Although variability between samples was present, the overall trend consistently favored jet in air sorting.

Taken together, these results provide a data-driven comparison of sorting architectures and their impact on cell viability. The observed advantage of jet in air systems is consistent with their fluidic design, including higher stream velocity and shorter dwell times, which may reduce cellular stress during sorting. These findings support the use of jet in air sorters for applications where maximizing post-sort viability is critical, while also demonstrating comparable performance across modern jet in air platforms.

Materials and methods

Test method: Testing was performed on a 7 laser Bigfoot Spectral Cell Sorter using a standard configuration. Competitor A and B both had standard configurations. All systems used a 100-micron tip and 30 PSI except a few of the cell lines on jet in air Competitor B were sorted at 20 PSI. All data was collected with either Invitrogen™ Attune™ Xenith™ Focusing Fluid or Beckman Coulter IsoFlow™ Sheath Fluid.

Post-sort viability protocol:

1. Setup 100 um tip @ 30 PSI and ensure QC and drop delay are passed before sorting.
2. Prepare cells at desired concentration and add PI at final conc 1.0 ug/mL (no washing)
3. Acquire sample and record pre-sort viability (%)
4. Sort 50,000 to 100,000 cells depending on total amount of cells available. One-way bulk sort into 5 mL tubes (preferably polypropylene) in purity mode. Cells are sorted into PBS buffer with 2% FBS. There should be 500 uL starting fluid in the 5 mL tube. Tubes should be chilled on ice before sorting (if available).
5. Add a viability dye (no washing) to the sorted cells that does not have an emission that overlaps with PI
6. Run through the sorter again. Gate and calculate % viable (PI-negative) cells and calculate percentage of viable cells

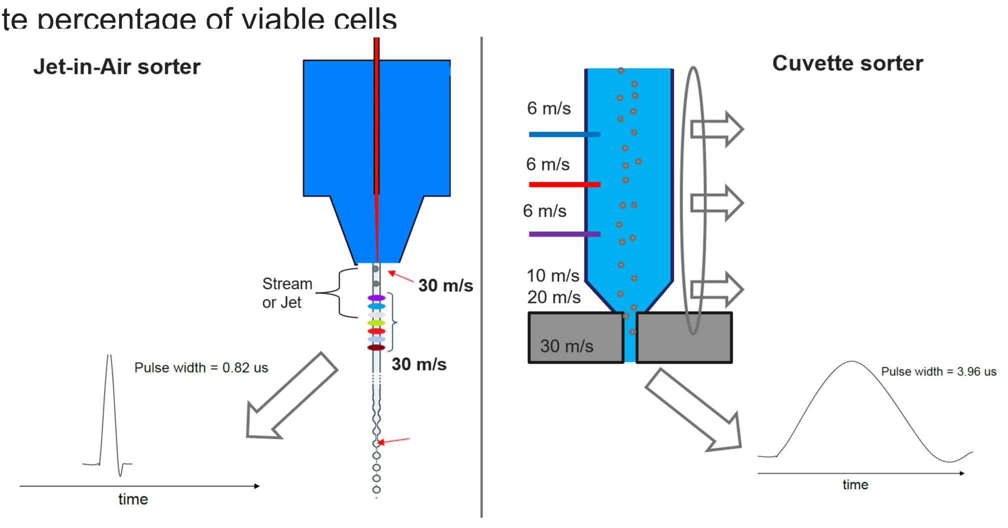

Figure 1: Schematic comparison of jet in air and cuvette-based cell sorting highlights key differences in fluid dynamics and signal timing. In jet in air systems, cells travel in a high-velocity stream (~30 m/s) with rapid droplet formation, resulting in short signal pulse widths (~0.82 µs). In contrast, cuvette-based sorters operate with slower, more variable flow velocities, producing longer pulse widths (~3.96 µs) and increased dwell time in the interrogation region. These differences illustrate how jet in air sorting enables faster transit and reduced exposure time, which may contribute to improved cell viability.

Results

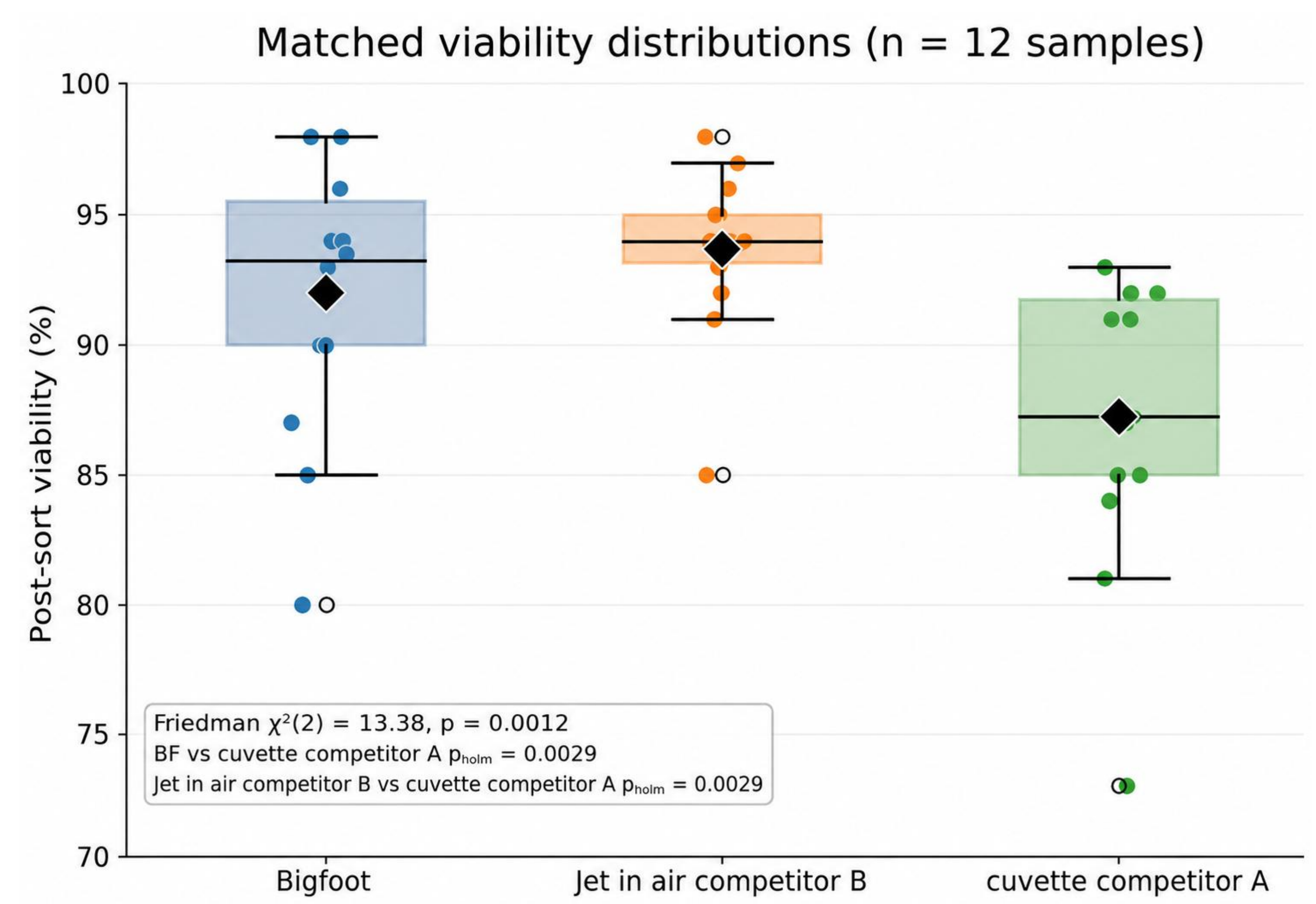

Figure 2: Matched post-sort viability distributions across 12 samples show that both the Bigfoot Spectral Cell Sorter and Competitor A achieve higher viability compared to Competitor B. Boxplots with overlaid individual data points and mean markers (black diamonds) illustrate consistently higher central tendency for jet in air sorters. Statistical analysis using a Friedman test indicates a significant overall difference between instruments ($\chi^2(2) = 13.38$, $p = 0.0012$), with post-hoc comparisons confirming significantly higher viability for both Bigfoot Spectral Cell Sorter and jet in air Competitor B versus Competitor A ($p_{holm} = 0.0029$). These results support a consistent viability advantage of jet in air sorting over cuvette-based sorting across matched samples.

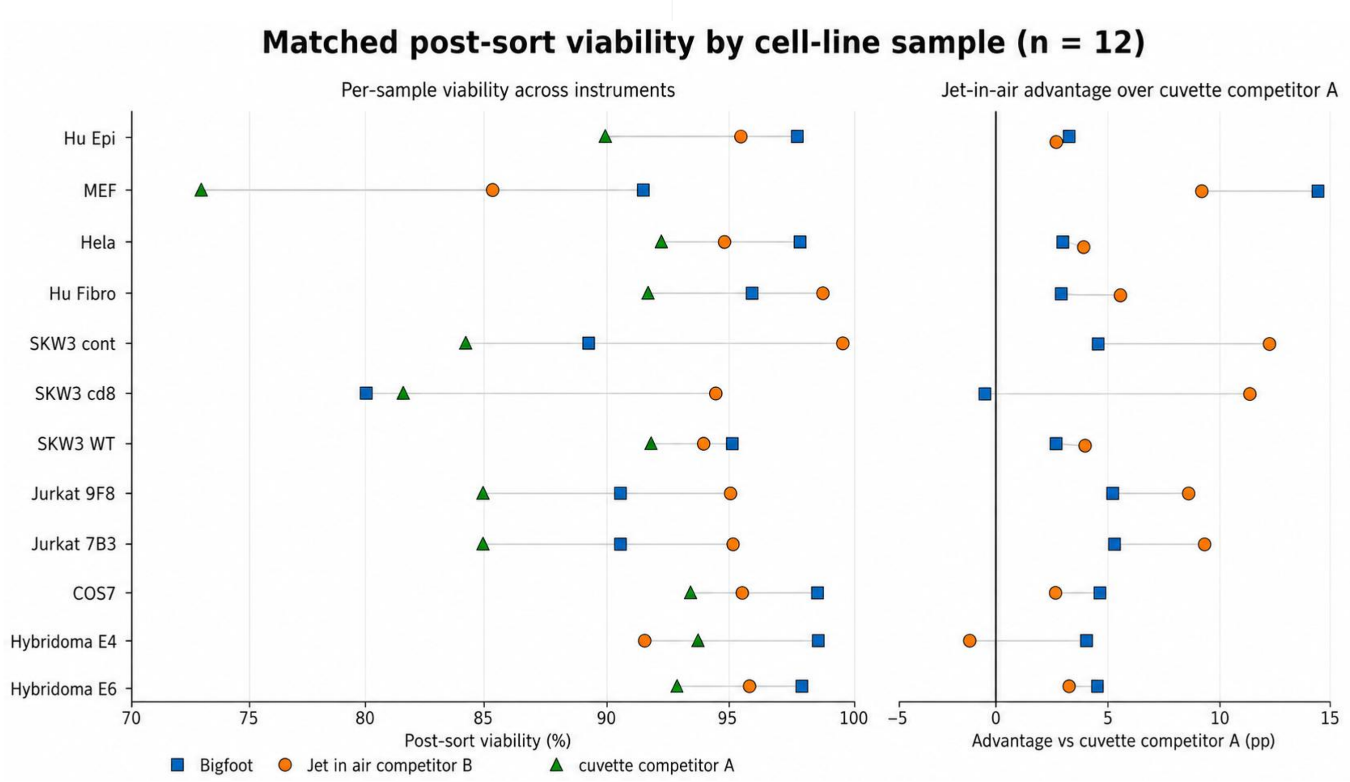

Figure 3: The data shows that the Bigfoot Spectral Cell Sorter and jet in air Competitor B are fairly similar overall, while Cuvette Competitor A tends to sit lower for many of the matched samples. The overall means in this matched set are: Bigfoot Spectral Cell Sorter: 91.9%, jet in air Competitor B: 93.7%, Cuvette Competitor A: 87.2%

Post-sort - Statistics

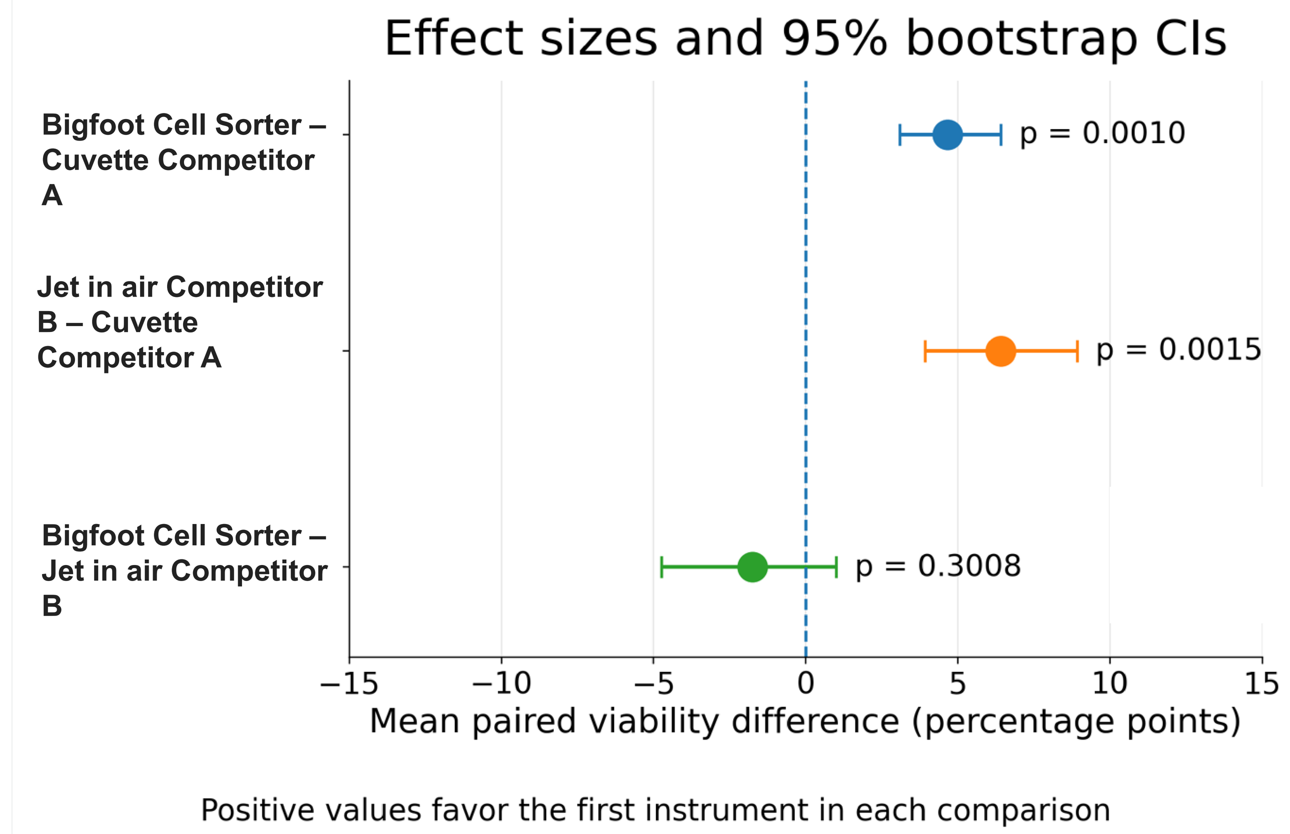

Figure 4: This figure summarizes paired comparisons of post-sort viability across matched samples, showing the mean difference in viability (percentage points) between instruments with 95% confidence intervals and associated statistical significance. Both Bigfoot Spectral Cell Sorter and Competitor A demonstrate significantly higher viability than the Competitor B, with mean increases of approximately +4.7% and +6.4%, respectively ($p < 0.01$ for both), and confidence intervals that do not cross zero, indicating a consistent advantage across samples. In contrast, the comparison between the Bigfoot Spectral Cell Sorter and Competitor A shows no statistically significant difference ($p = 0.30$), with a small mean difference and a confidence interval spanning zero. Overall, the data support that jet in air sorting platforms outperform the cuvette-based Competitor B in preserving cell viability, while performance between jet in air systems is comparable.

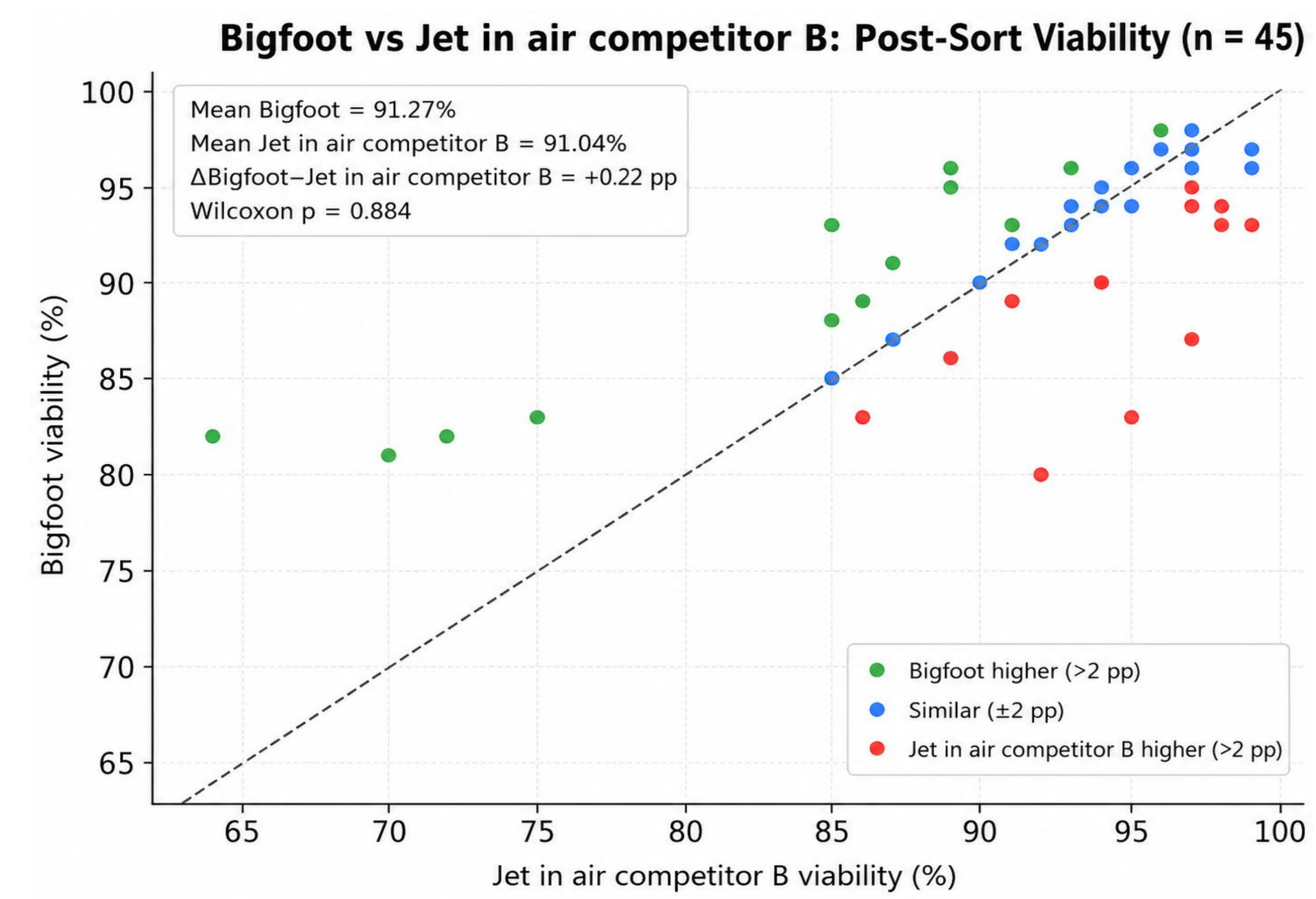

Figure 5: This figure shows a paired, sample-by-sample comparison of post-sort viability between Bigfoot Spectral Cell Sorter and jet in air Competitor B across 45 runs, with each point representing the same sample measured on both instruments. The dashed diagonal line represents equality ($y = x$); points above the line indicate higher viability on Bigfoot Spectral Cell Sorter, while points below indicate higher viability on jet in air Competitor B. The data cluster tightly around this line, indicating strong agreement between the two platforms. The mean viability is nearly identical (Bigfoot Spectral Cell Sorter 91.27% vs Competitor A 91.04%), with a very small average difference of +0.22 percentage points and no statistical significance (Wilcoxon $p = 0.884$). Overall, this figure demonstrates that Bigfoot Spectral Cell Sorter and Competitor A produce comparable post-sort viability across a large number of paired samples, with no meaningful performance difference between the two jet in air sorters.

Murine splenocytes – Post-sort viability analysis

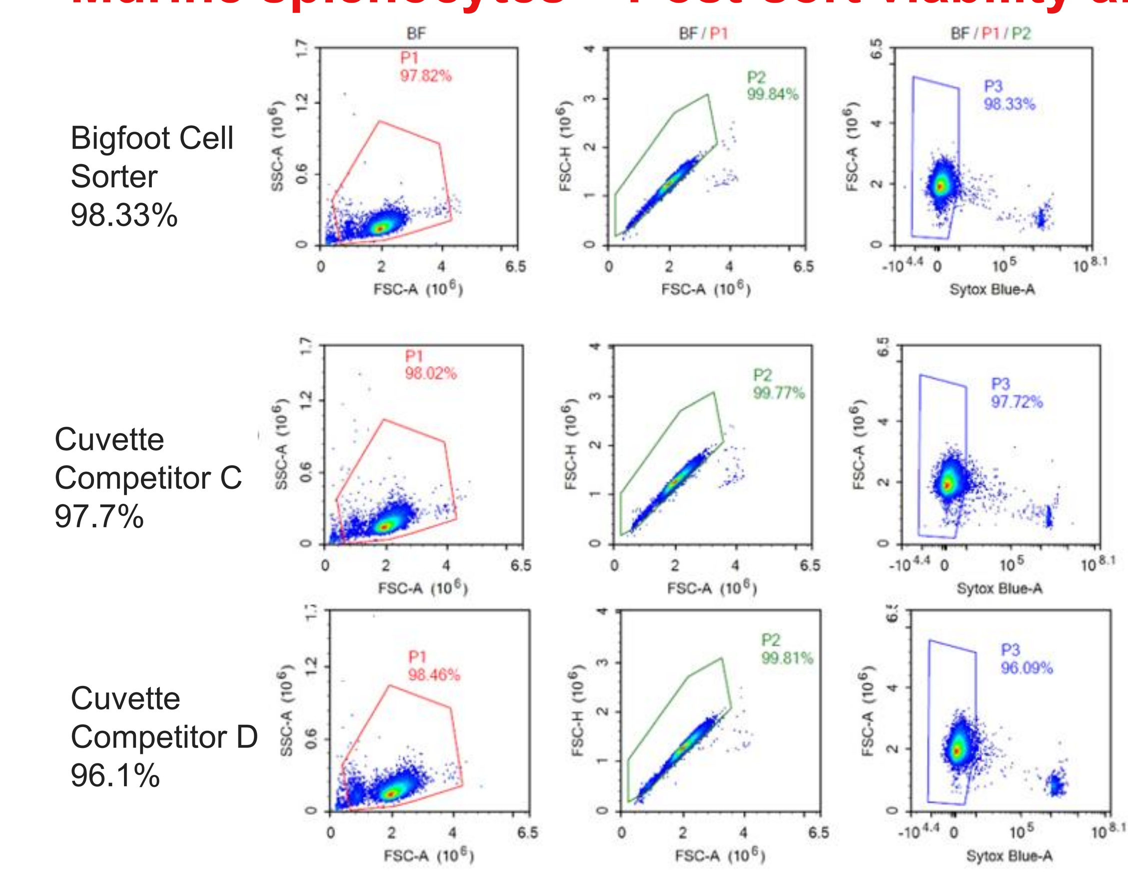

Figure 6. Murine splenocytes were run on the Bigfoot Spectral Cell Sorter, Cuvette Competitor C, and Cuvette Competitor D. Sheath pressure was set to 30 PSI on the Bigfoot Spectral Cell Sorter and 20 PSI on both the competitor instruments. All instruments were run using a 100 µm tip. Viability after sorting was within 2% across all instruments, with no significant difference observed for this sample.

Conclusions

Across multiple matched datasets and analysis approaches, these results consistently demonstrate that jet in air sorting platforms (Bigfoot Spectral Cell Sorter and jet in air Competitor B) provide higher post-sort viability than the cuvette-based Competitor A. Both statistical comparisons and per-sample analyses show a significant and reproducible viability advantage for jet in air sorters, while no meaningful difference is observed between Bigfoot Spectral Cell Sorter and jet in air Competitor B, indicating comparable performance within this class of sorters. These findings align with the underlying fluidic differences, where higher velocity streams and shorter interaction times may reduce cellular stress during sorting. Overall, the data support the conclusion that jet in air sorting is a more cell-friendly approach for preserving viability across diverse cell types and experimental conditions.

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