Flow Cytometry and Imaging Technologies

Impact of nozzle tip size on side stream stability in cell sorting

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Introduction

Side stream stability plays a pivotal role in cell sorting; significantly affecting the recovery, viability, and purity of the output sample. Several factors contribute to side stream stability, one of which is the nozzle tip size. A wide range of applications demonstrate the flexibility offered by employing a broad spectrum of tip sizes, ranging from 70 µm to 200 µm, to accommodate the sorting of a diverse array of cells sizes on the Invitrogen™ Bigfoot™ Spectral Cell Sorter. We will present data illustrating how sorting parameters, such as sample concentration, plate targeting, and cell size, should be considered to minimize side stream fanning. Practical examples will be provided to demonstrate setup strategies for these nozzle tips to achieve optimal sorting performance through maximizing cell recovery, viability, and purity. Our findings underscore the importance of careful consideration of nozzle tip sizes in enhancing the efficiency and effectiveness of the cell sorting processes.

Results

Nozzle tip guide and side streams visualization

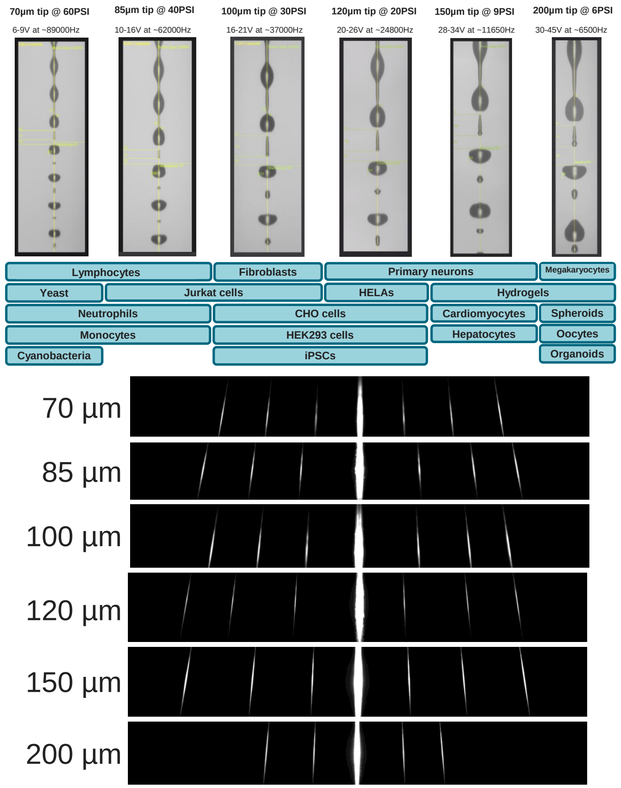
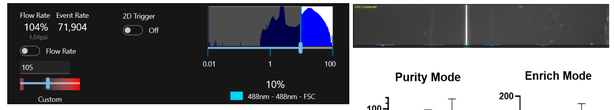
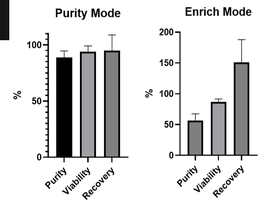


Figure 1. Six nozzle tip sizes available for cell sorting on the Bigfoot. Each nozzle tip size operates at difference pressures with different optimal frequencies for cell sorting applications. Smaller nozzle tip sizes operate at higher pressures allowing for faster sorts while larger nozzle tip sizes are best used for larger or complex cell types at lower pressures. The types of cells and their suggested nozzle tip reflect the considerations required to successfully sort cells. The 70 µm to the 150 µm nozzle tips are capable of sorting six ways while the 200 µm tip can sort up to four ways. Targeting for every nozzle tip is comparable regardless of the pressure or nozzle tip size.

Sorting at 70,000 events per second using the 70 µm nozzle tip

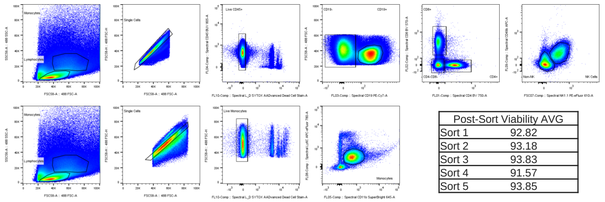




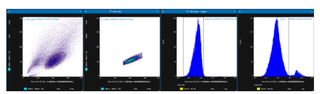
Post-Sort Data (Averages)						
	Efficiency	Max Efficiency	Event Rate	Purity	Viability	Recovery
Purity Mode	28.27	32.31	70337.01	88.84	93.86	94.97%
Enrich Mode	95.39	98.52	72110.93	56.36	87.60	151.06%

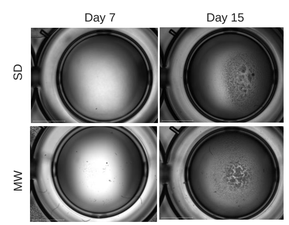
Figure 2. Sorting GFP⁺ Jurkat cells at 70,000 events per second using different sort modes. Purity mode sorts a cell if there are no negative particles in the first 25% of the preceding or succeeding droplets. Enrich mode will sort a droplet if there is a positive particle regardless of the presence of other particles. Sorting on enrich mode and then re-sorting on Purity mode yielded efficient and pure cultures. Multi-way sorting at 70,000 events further allowed for sorting of GFP⁺ and GFP⁻ Jurkat cells very quickly while limiting fanning and spray from side streams.

6-way sorting using the 85 µm nozzle tip



Post-Sort Viability AVG	
Sort 1	92.82
Sort 2	93.18
Sort 3	93.83
Sort 4	91.57
Sort 5	93.85





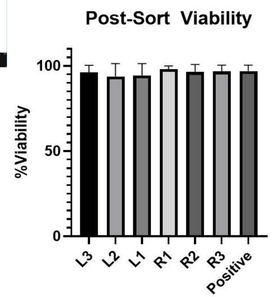


Figure 3. The 85 µm nozzle tip was utilized to sort sub-populations from PBMCs six ways after spectral unmixing. A variety of sub-populations were sorted and subsequently assessed for purity, viability, and recovery. Further showcasing the six-way sorting capability of the 85 µm nozzle tip, CHO cells were sorted into each position (L3 to R3) individually. The post-sort viability for each sample was greater than 90%. Multi-way or straight down sorting of a single CHO cell using the 85 µm nozzle tip into 96-well plates was performed. Cell proliferation was monitored until each well was confluent.

96- 384- and 1,536-well plate sorting using the 100 µm nozzle tip

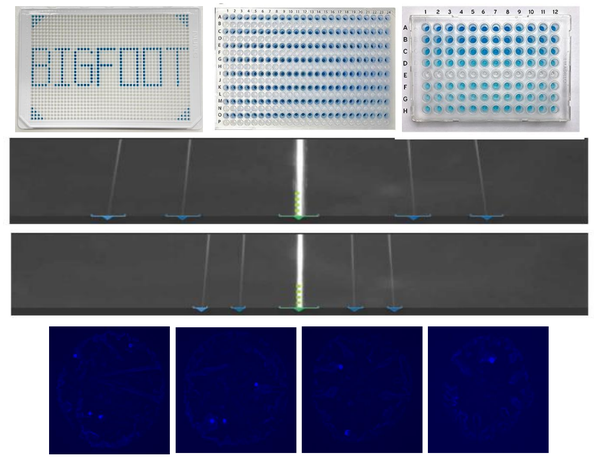


Figure 4. Multi-way and straight down sorting using the 100 µm nozzle tip can be used to successfully sort cells into any size well plate. CHO cells mixed with HRP were sorted into a 1,536 well plate containing TMB. The average time for a full, straight down sort into a 1,536 well plate was 3:14.23 minutes. The same experiment was performed to sort CHO cell mixed with HRP into either 384 well plates or 96 well plates using multi-way sorting. For 96 well plate sorts, cells were sorted as a gradient (4, 3, 2, 1, 0, 1, 1, or 1 per well) in each row as shown by a darker hue of TMB due to the more concentrated number of cells. On average, it took 19.87 second and 7.65 seconds for full 384 and 96 well plate sorts, respectively.

Sorting large cells and spheroids using the 200 µm nozzle tip

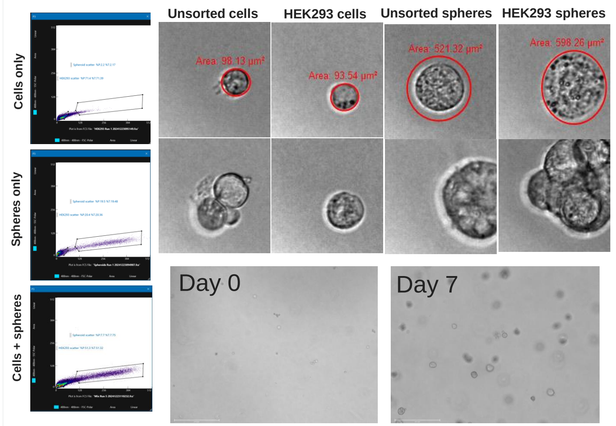


Figure 5. Using larger nozzle tip sizes at lower pressures, larger cells can be sorted into tubes or plates. Using polarized scatter, very large cells, like spheroid cells grown in suspension culture, can be purified from non-spheroid cells. CytPix images detail the larger size and varying complexity that exist within each spheroid. Larger nozzle tip sizes and lower pressures help achieve viable and pure sorts.

Materials and methods

Sample Preparation

Cell lines used include Jurkats, Jurkat constitutively expressing GFP, MEG-01, Chinese Hamster Ovary (CHO), Human Embryonic Kidney (HEK) 293, and peripheral blood mononuclear cells (PBMCs). All cells were cultured in the suggested, fetal bovine serum-supplemented media and passaged when the cells reached 80% confluency. For spheroid generation of HEK293 cells, cells were cultured in a gel matrix to prevent cellular attachment. PBMCs were rested for at least one hour in AIM-V media.

Test Method(s)

Cell concentration and pre-sort viability was determined using a Countess™ 3 Automated Cell Counter and cell cultures were monitored using an EVOS™ M7000 Imaging System. Post-sort viability was determined by reanalyzing the sorted cells on the Bigfoot within five minutes post-sort with 1 µL Sytox™ AADvanced™ per mL of cells. Post-sort purity was determined by measuring the percent of sorted cells that fell within the sort gates. Cell recovery was measured with the Invitrogen™ Attune™ CytPix™ Flow Cytometer to determine the cell concertation and the measured number compared to the desired number of sorted cells.

Colorimetric plate sorting experiments utilized CHO cells incubated with horseradish peroxidase (HRP) and sorted into tetramethylbenzidine (TMB). Cell images were taken with the Attune CytPix. All images are representative of 3 replicates within a given experiment.

Conclusions

- The Bigfoot Spectral Cell Sorter allows for a wide range of nozzle tips for any application. Shear forces and cellular stress during or post-sort can have detrimental effects on downstream applications. Utilizing lower sort pressures for finicky cells with larger nozzle tip orifices will result with healthier sorted cells while smaller, hardier cells can be sorted much quicker with smaller nozzle tips. Successful cell sorting relies on the correct nozzle tip size and is often application-dependent.
- The 70 µm and 85 µm nozzle tips are best suited to sort smaller, homogenous cells and PBMCs very quickly. Optimizing sort setting can allow for the enrichment of cells up to 70,000 events per second or sort six ways with ease.
- Nozzle tip sizes ranging from 70 µm to 120 µm can efficiently and accurately sort a wide variety of cell types in every well plate definition. Multi-way cell sorting in plates saves time and the infini-sort option makes multiple plate sorts easy. Sorting into 1,536 well plates for downstream automated applications is also possible with straight down sorting.
- Larger nozzle tip sizes, like the 150 µm and 200 µm, operate at lower pressures which aid in sorting large or delicate cell types. While slower than smaller nozzle tip sizes, larger orifices reduce the stress and shear cells experience during the sorting process.

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