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Utilizing a 200 µm nozzle tip at low pressure to reliably sort large, viable megakaryoblasts on the Invitrogen Bigfoot Spectral Cell Sorter

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Introduction

Sorting very large cells with standard nozzle tip sizes on cell sorting flow cytometers can be challenging due to scarce frequency of large cells and their fragile nature. The required pressures for 70, 100, and even 120 µm nozzle tips may compromise large or delicate cells due to the shearing forces associated with smaller orifices. Megakaryoblasts can be rare within a sample so accurate sorting and preservation of viable cells are of the upmost importance. Since options for large cell sorting are limited, the 200 µm nozzle tip was developed for the Invitrogen™ Bigfoot™ Spectral Cell Sorter. Now very large cells (50 - 100 µm) from complex samples can be sorted for further downstream analyses. Given the large size of megakaryocytes, and the complex samples from which they come, such as bone marrow, or cultured MEG-01 cells, sorting with smaller nozzle tips can be difficult and produce clogs. However, these cells can be sorted accurately, efficiently, and most importantly, remain viable for downstream applications using a 200 µm tip at 6 psi. The transition from a high-pressure nozzle to the 200 µm tip at 6 psi requires minimal user effort and is stable throughout the day during very long sorts, as is found with the smaller nozzle tips. The Bigfoot Spectral Cell Sorter already included the 70 µm, 100 µm, 120 µm, and 150 µm nozzle tip sizes for many cell sorting applications and the recent addition of the 200 µm tip further augments the options available for diverse cell sorting requirements.

Materials and Methods

MEG-01 (CRL-2021) and Jurkat cells (CRL-2899) were cultured with Gibco™ RPMI supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific) and a penicillin/streptomycin antibiotic cocktail at 37° C. Cells were harvested and washed with cold PBS prior to reanalysis on the Bigfoot instrument. Peripheral blood mononuclear cells (PBMCs [STEM Cell]) were rested in RPMI supplemented with 10% FBS for at least one hour at 37° C prior to staining. Cells were stained with a pre-optimized concentration of anti-human antibodies (BUV 395-CD4, BV 421-CD3, BV 750-CD45, PE-Dazzle 594-CD19 [BioLegend], Hoechst 33342 [BioLegend], FITC-CD41α, APC-CD8, PE-Cy7-CD14 [Invitrogen]) and spectrally unmixed prior to sorting. Cells were sorted into cold PBS supplemented with 30% FBS or 100% FBS. Post-sort cell viability was analyzed using SYTOX™ AADvanced™ Dead Cell Stain Kit (Thermo Fisher Scientific) following manufacturer recommendations. MEG-01 and Jurkat cell images were acquired on the Invitrogen™ Attune™ CytPix™ Flow Cytometer. Cell culture images were acquired using an Invitrogen™ EVOS™ M7000 Imaging System using a 4X objective.
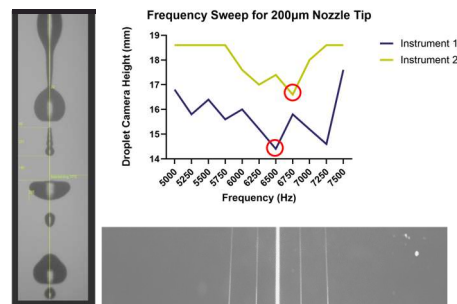
Results

Figure 1. The 200 µm nozzle tip stream and side streams were set up following similar geometric ratios as were the previous nozzle tips. Frequency sweeps were performed on multiple instruments to optimize the camera height and resonate frequency for droplet formation and sort performance. The red circles indicate the chosen frequencies for two instruments. Positions L2, L1, R1, and R2 enable four-way cell sorting in addition to sorting one-way from inner stream positions. Optimized settings allow for the Bigfoot Spectral Cell Sorter equipped with a 200 µm nozzle tip to pass the one-button QC.
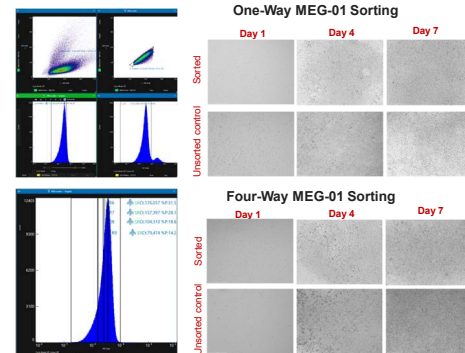

Figure 2. MEG-01 cells were sorted one way from L2, L1, R1, and R2 sort positions or sorted from each sort position simultaneously. Sorted cells and unsorted cell controls were cultured for seven days. Each day, the sorted-cells were imaged via an EVOS M7000 Imaging System to verify proliferation and cell viability. After the cell culture media turned yellow signaling the cells had used the nutrients in the media, the cells were harvested and reanalyzed for viability and growth.
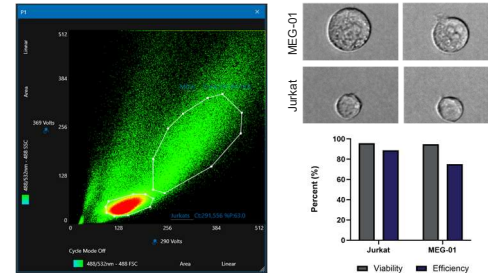

Figure 3. Scatter resolution of MEG-01 cells co-cultured with Jurkat cells were individually sorted and imaged via Invitrogen™ Attune™ CytPix™ Flow Cytometer to show purified, live cells. Post-sort viability of MEG-01 and Jurkat cells was greater than 90%.
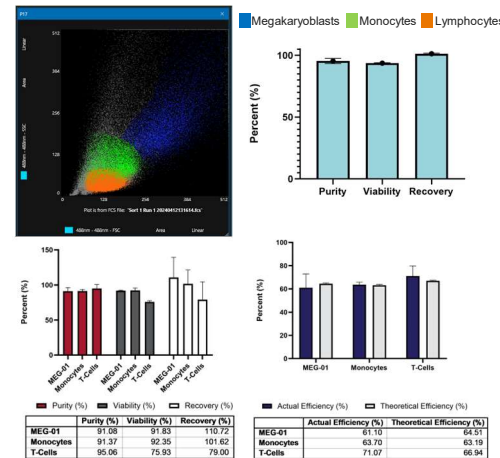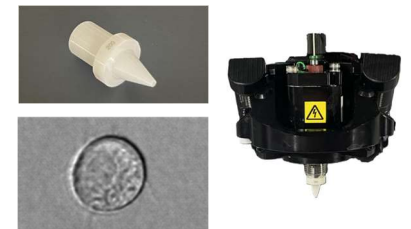

	Purity (%)	Viability (%)	Recovery (%)
MEG-01	91.08	91.83	110.72
Monocytes	91.37	92.35	101.62
T-Cells	95.06	75.93	79.00

	Actual Efficiency (%)	Theoretical Efficiency (%)
MEG-01	61.10	64.51
Monocytes	63.70	63.19
T-Cells	71.07	66.94

Figure 4. Scatter resolution of MEG-01 cells mixed with PBMCs. MEG-01 cells sorted from PBMCs were viable, pure, and accurately sorted. Subsequent experiments were performed where T cells (CD3⁺ CD19⁻) and monocytes (CD14⁺), in addition to MEG-01 cells, were accurately sorted with high viability and purity. Efficiency of each sorted cell type was equal to or greater than the theoretical sorting efficiency at such a low pressure. Data is an average of 10 individual sorts on two Bigfoot Spectral Cell Sorters. The legend shows the scatter profiles of lymphocytes (6 µm - 15 µm), monocytes (12 µm - 22 µm), and MEG-01 cells (50 µm - 100 µm).

Conclusions

- On the Bigfoot Spectral Cell Sorter, the 200 µm nozzle tip at 6 psi is easy to set up and can be used instead of smaller nozzle tips at higher pressures with ease. The 200 µm nozzle tip utilizes daily QC with the click of one button like the other tip sizes offered for the instrument.

- Large cells that may be difficult to sort with smaller nozzle tip sizes can be efficiently sorted and cultured with high viability for downstream applications. Both one-way sorting and four-way sorting can be utilized for downstream needs. Cells sorted from one-way, or four-way sorts can be cultured with high viability.

- Heterogenous samples with a variety of cell sizes and considerations can be resolved via forward and side scatter. Sorting both small cells (Jurkat cells) and large cells (MEG-01 cells) result in purified and viable samples. Images from the Attune CytPix Flow Cytometer confirm cell viability and show the difference in cell sizes after sorting.

- Heterogenous populations with highly variable populations can be visualized through forward and side scatter alone. These cells can be further differentiated through staining with fluorophore antibodies and sorted up to four ways while maintaining high purity and viability. Pure samples with > 95% sorted cells were sorted for downstream applications. Complex samples are sorted with efficiency close to or exceeding the theoretical efficiency.

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