Enhancing cell sorting efficiency through optimized forward scatter resolution

Matthew W. Meyer, Susan O'Meara. Thermo Fisher Scientific, 145 East Mountain Avenue, Fort Collins, CO. 80524

Abstract

Purpose: In cell sorting, forward scatter is proportional to cell size and is essential as a gating parameter used to exclude debris and dead cells. The higher the forward scatter resolution, the easier it is to differentiate between debris, live cells, and dead cells.

Methods: In many instances, it is advantageous to evaluate live and dead cell populations without the application of a viability dye, which can impact downstream processes. For sorting, it's crucial to minimize the amount of debris on the trigger channel. Here, we demonstrate with CHO cells how to optimize efficiency on a jet-in-air sorter.

Results: This poster illustrates how optimizing the trigger can contribute to higher efficiency and improved sorting performance. Our findings underscore the importance of optimizing forward scatter resolution in maximizing the efficacy of cell sorting processes.

Introduction

Cell sorting is the process of isolating one or more pure target populations from heterogenous mixtures that have been identified using the principles of flow cytometry. There are many factors that go into defining a successful cell sort. Depending on the downstream application, customers typically gauge the success based on several key-factors, specifically: purity, recovery, efficiency, and viability. A jet-in-air sorter can be optimized in many ways to successfully sort cells.1 The data presented throughout the results section shows that sample preparation and cell conditions play an important role in a successful sort. We will offer concrete data into how to improve cell sorting efficiency through optimizing your sorting parameters.

Materials and methods

Sample Preparation

CHO-K1 and HEK 293T cell lines were used to measure efficiency. The cell lines were cultured in an incubator with a temperature of 37C and 5% CO2. The cells were trypsanzied once they reached confluency and sorted on the same day.

Test Method

Testing was performed on a 9 laser Invitrogen™ Bigfoot™ Spectral Cell Sorter using a standard configuration

Data Analysis

All of the data analysis was performed in SQS v1.17 or using a custom python script wrote in Python 3.9.

Below is an example of the Bigfoot threshold plot in SQS. This plot serves as an important tool for setting the threshold and trigger channel. It is a logarithm scale and ranges from 0.01 to 100 and is based on height. It is important to set this correctly to maintain an acceptable efficiency and FCS files that are not too large. This will look different on other systems and Bigfoot has a very fast pulse processing so it will be more sensitive to debris. Signals lower than the threshold will be invisible to the system when making sort decisions.

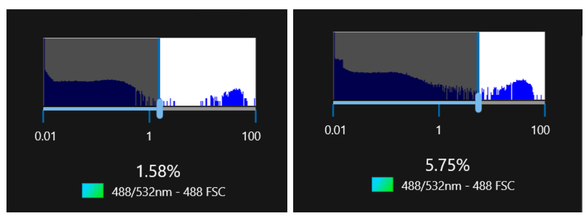


Figure 1: The Bigfoot spectral cell sorter uses SQS which contains a digital trigger threshold plot. The user can select a threshold based on FSC, SSC, Fluorescence and 2D Trigger.

Results

Combining the scatter sensitivity while triggering on forward scatter and correctly setting the threshold can have a substantial impact on sorting efficiency

We show an example here where the same cells are sorted under two different thresholds. We recommend keeping 1% - 5% of the overall events as debris or non-cellular signals. Going above this can drastically reduce sorting efficiency. Below we will describe how to set the threshold and compare how including too much debris can influence the overall sorting efficiency.

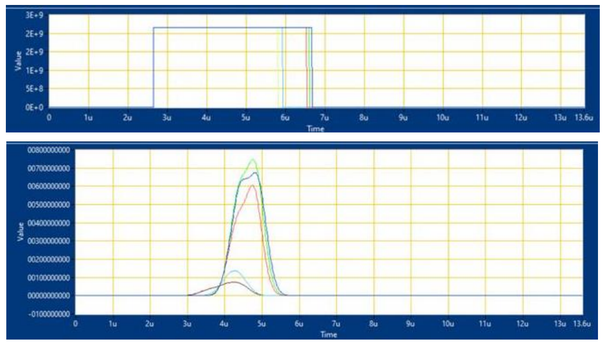


Figure 2: Bigfoot has a built-in service oscilloscope for troubleshooting signals. This panel shows the pulse width durations and signals measured on the system. The x-axis units are in microseconds and the y-axis is arbitrary intensity.

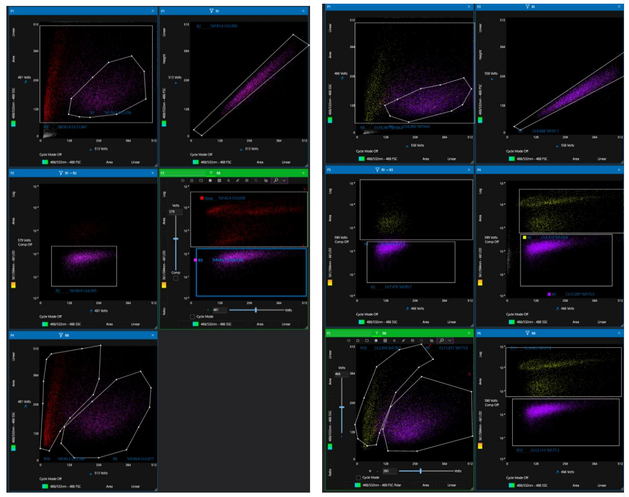
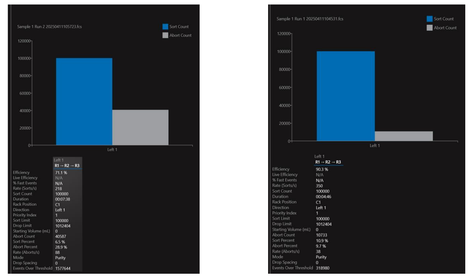


Figure 3: A sort comparison was performed on CHO cells. The cells were the exact same concentrations and sample prep (sorted within an hour of each other). The only difference being how the Threshold was set. The sort on the left was set to have a lower threshold and include more debris compared to sort on the right.

Post Sort - Statistics



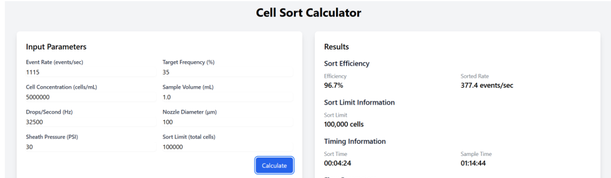


Figure 4: The calculated sort time based on Poisson distribution statistics is 4 mins and 24 seconds with a sort rate of 377 sorts per second. The actual sort time was 4 mins 46 seconds and a sort rate of 350 cells per second. The percent error was 8%.

The sort with a threshold that was not set correctly was 7 mins and 38 seconds with a sort rate of 218 sorts per second. That is a percent error of 53.7%.

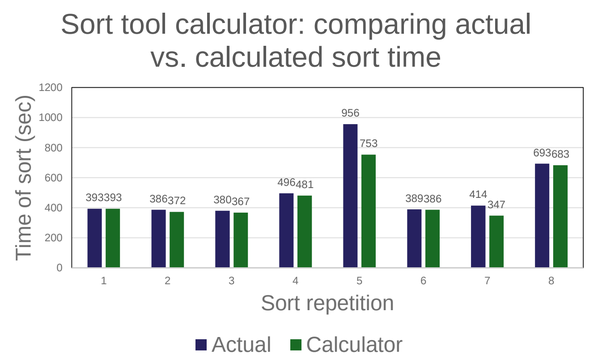
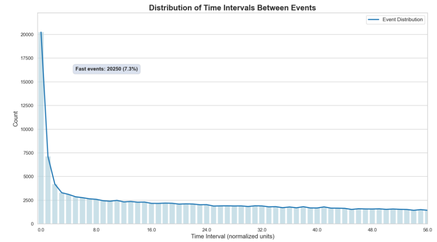


Figure 5: The experiment was repeated with HEK293 cells on a 100-um tip at 30 PSI. The tip frequency was set to 36,900 and an event rate of 3,000 and a sort rate between 200 and 600 sorts per second and a %T of 22%.



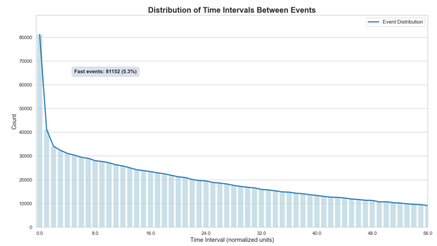


Figure 6: Arrival statistics processed between the two CHO cell sorts. The fast event tracking can be used to quantify sample preparation quality.

Conclusions

The findings presented on this poster show the importance of understanding how to effectively set the sorting threshold.

2D threshold can also be an effective technique for improving efficiency

The Bigfoot spectral cell sorter has a narrow pulse width so it is very sensitive to small particles and debris which can in turn lower efficiency if it is included in the sorting criteria

References

Lindmo, T., Peters, D., & Sweet, R. (1990). Flow sorters for biological cells. In M. Melamed, T. Lindmo, & M. Mendelsohn (Eds.), Flow Cytometry and Sorting (2nd ed., pp. 155–174). Wiley-Liss.

Trademarks/licensing

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.