Enhancing cell recovery with ultra-recovery mode on the Invitrogen™ Bigfoot™ Spectral Cell Sorter

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Introduction

The jet-in-air sorter provides a consistent cell velocity, reducing cell stress and featuring a faster electronic pulse width, support the detection of more cells and quicker cell sorting. This efficiency necessitates flexibility in various sort modes. The Invitrogen™ Bigfoot™ Spectral Cell Sorter features an ultra-recovery mode, designed to enhance cell recovery by sorting two drops, helping ensure the targeted cell is captured in the post-sort tube. We will present data showcasing the improvement in recovery across a range of samples and specific sorting applications that benefit from this innovative sorting mode. Our findings highlight the potential of ultra-recovery mode to enhance cell sorting efficiency and recovery rates.

Materials and methods

Sample preparation

Jurkat, PBMCs, and HEK293 were sorted in our purity and enrich modes with the Ultra Recovery and comparing to our default mode. Efficiency was measured on the Bigfoot Cell Sorter and compared to our efficiency calculator based on the philosophy from Lindmo et. al., and recovery was measured using the Invitrogen™ Attune™CytPix™ Flow Cytometer.

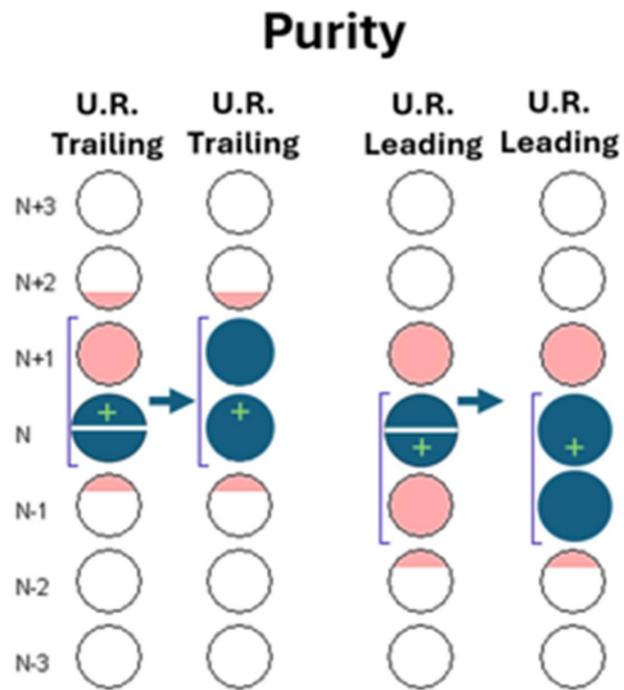### Purity and ultra-recovery diagram

Figure 1: For purity and enrich modes, the ultra-recovery option can be enabled. If the positive particle is in the leading or trailing ½ of the droplet (blue), and there are no particles in the adjacent (red), an additional drop will be sorted to increase the recovery of the positive particles. Note that for purity mode, the adjacent area extends ¼ drop farther to ensure that the purity isn't compromised, while in enrich it uses a smaller scatter adjacent area to maximize recovery.

Ultra-recovery is not available for use in single or custom sort modes

Results

Cell and bead sorting with ultra-recovery in purity and enrich modes

The following figures and table show results of various cell and bead sorts and the comparison between our default and ultra-recovery mode and the impact on sort time, efficiency, and recovery.

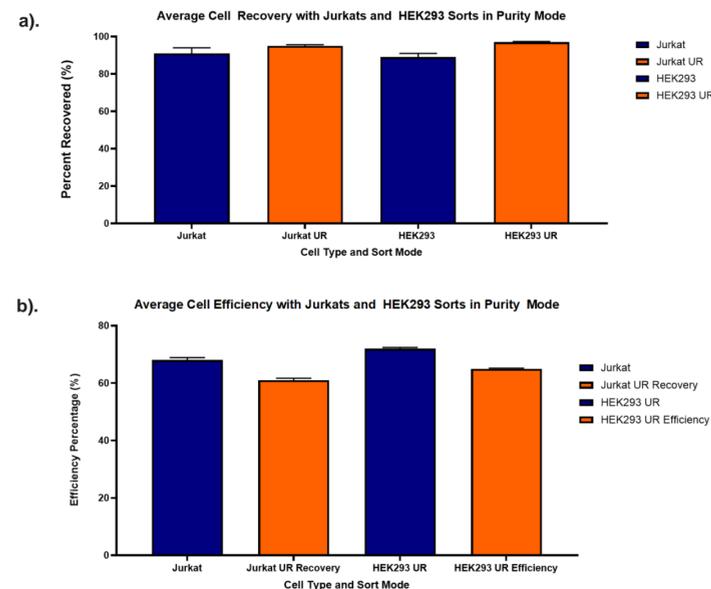### Cell sorting comparison of recovery in purity mode

Figure 2: Jurkat Cells were sorted in triplicates to a sort limit of 100,000 into 5mL tubes coated with FBS and 400µl of HBSS at a sort rate of 3566-3839 and sorted on live using Sytox Advanced (Thermo Fisher Catalog #S34568). HEK293 were sorted in triplicates to a sort limit of 100,000 into 5mL tubes coated with FBS and 400µl of HBSS at a sort rate of 2024–24811 and were sorted using Nuc Green dead viability dye (Thermo Fisher Catalog #R37109). a) Shows the recovery percentage assessed on the Attune CytPix Flow Cytometer. b) Reported efficiency from SQS software.
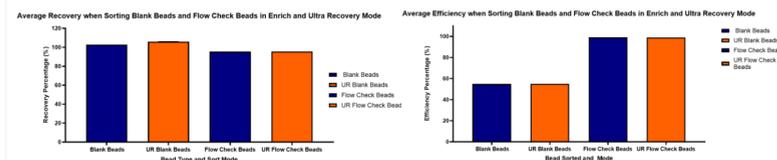
Bead sorting in enrich mode

Figure 3: Flow-Check™ Fluorospheres (Beckam Coulter Cat #: 6605359) and blank beads (Polysciences Inc. Cat #17140-5) were both sorted into 5mL tubes coated with FBS and 400µl of HBSS. The percent target for the Flow Check beads was approximately 0.5%, and for blank beads approximately 90%.
a) Shows the recovery percentage assessed on the Attune CytPix Flow Cytometer.
b) Reported efficiency from Bigfoot SQS platform.

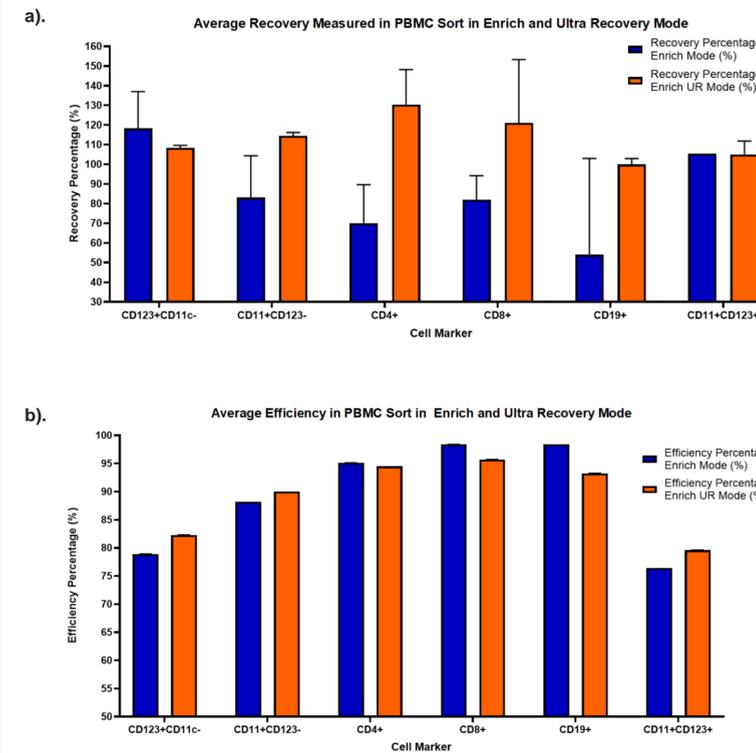### PBMC average recovery and efficiency in enrich mode

Figure 4: a) PBMCs (Discovery Life Science Catalog #DLC-12345) were sorted 6 ways into 5mL tubes coated with FBS and 400µl of HBSS for triplicates. The approximate percent total of the markers is as follows from left to right: 0.1%, 0.6%, 19.3%, 13.5%, 9.8%, and 0.3% .a) Shows recovery of all markers as analyzed on Attune CytPix Flow Cytometer. b) Reported efficiency in SQS software.

Table 1: This table summarizes the actual recovered sorts per second when sorting with cells and beads in purity and enrich modes, with and without ultra-recovery mode.

Sample	Purity Recovered Sorts/sec	Purity UR Recovered Sorts/sec	Enrich Recovered Sorts/sec	Enrich UR Recovered Sorts/sec
Jurkat Cells	3461	3463		
HEK293 Cells	2126	2041		
Flow Check Beads			70.2	74
Blank Beads			245	265
20µm Beads	187	504		

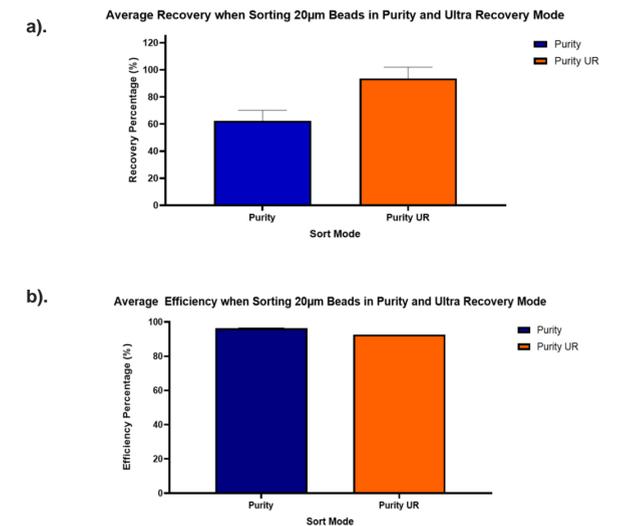### 20µm bead average recovery and efficiency in purity mode

Figure 5:.20µm YG beads (Polysciences Inc, Cat #:19096-2)) were sorted on the 100µm nozzle at 30 psi setting to a sort limit of 100,000 with a sort rate range of 435-599 sorts/sec in purity mode, with and without ultra-recovery.

a) Shows the recovery percentage assessed on the Attune CytPix Flow Cytometer.
b) Reported efficiency from Bigfoot SQS platform.

Conclusions

- Ultra-recovery mode has benefits in recovery over normal modes and a slight decrease in efficiency

- Ultra-recovery results in minor fanning, but sample preparation and output media such as FBS coated tubes support good results in recovery, viability, and efficiency

- Rare cell populations are shown to have increased recovery in enrich ultra-recovery mode compared to our default enrich mode.

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