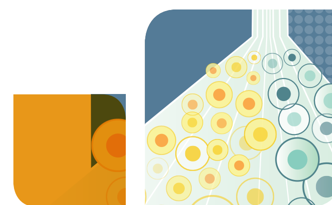
Reliable Single-Cell Sorting with Bio-Rad's S3e™ Cell Sorter



Joyce Lee
Bio-Rad Laboratories, Inc., 6000 James Watson Drive, Hercules, CA 94547

Cell Sorting

Bulletin 6853

Abstract

Accurate isolation of individual cells is essential for single-cell analysis techniques. Here, we demonstrate a simple workflow to verify the reliability of the S3e Cell Sorter in sorting single cells. Using this method, we confirmed sorting accuracies of >94% and 100% for single cells and single beads, respectively.

Introduction

Cellular heterogeneity, originating from stochastic gene expression, leads to difficulties in interpreting cell-to-cell variations in transcription and translation (Raj and van Oudenaarden 2008). Therefore, it is important to examine genomic, epigenomic, and transcriptional variation at the single-cell level. Flow cytometry combined with single-cell sorting allows researchers to identify and separate target cells for cell culture and other downstream analyses such as gene expression, transcriptome sequencing, and cell imaging. In this study, we demonstrate a method to verify the accuracy of single-cell sorting using the ZOE™ Fluorescent Cell Imager as an essential QC step and thus confirm that the S3e Cell Sorter is capable of reliably sorting single cells into 8-well culture strips.

Materials and Methods

Preparation of Jurkat Cells and Beads for Sorting

Jurkat cells (ATCC, catalog #TIB-152) were grown in RPMI media supplemented with 10% FBS and passaged every 2–3 days to ensure viability. Before sorting, the cells were washed twice with sort buffer (3% FBS in PBS), counted with the TC20™ Automated Cell Counter (Bio-Rad Laboratories, #1450102), resuspended to a final concentration of 2×10^6 cells/ml, and split evenly into two 5 ml tubes. The unstained control cells in the first tube were filtered through a 40 µm strainer and used to set up gating and voltage parameters on the S3e Cell Sorter. The Jurkat cells in the second tube were stained with CytoTrack™ Green 511/525 Dye (Bio-Rad, #1351203). The appropriate volume of 500x CytoTrack stock solution was added to the cell sample to make a 1x final concentration and the sample was incubated in the dark at room temperature (RT) for 15 min. The cells were then centrifuged at 1,000 x g for 5 min at RT, the supernatant was discarded, and the cells were washed with 3 ml sort buffer. The cells were then resuspended in 1 ml of fresh sort buffer and filtered through a 40 µm cell strainer prior to loading on the S3e Sorter. For the bead sorting experiment, 30 µl of 15 µm polystyrene bead (Thermo Fisher Scientific, #F13838) were added to 500 µl PBS in a 5 ml tube.

S3e Cell Sorter and Cell Sorting Setup

We used an S3e Cell Sorter equipped with a blue (488 nm) argon laser and a red (640 nm) solid-state laser (Bio-Rad, #1451008). The drop delay was calculated by directly counting the ProLine™ Universal Calibration Bead (Bio-Rad, #1451086) events in the waste stream during the quality control process. In order to ensure optimal sorting conditions into the 8-well flat bottom culture strips (Corning, #9102), deflection values were evaluated and adjusted as necessary. One 8-well strip was placed onto a left-side 8-well flat bottom S3e Collection Adaptor (Bio-Rad, #1441011) and five ProLine Universal Calibration Beads were sorted into each of the 8 wells. The wells were then visually examined to check the position of the sorted beads at the bottom of the wells. Deflection charge settings were adjusted until the sorted droplets landed at the center of each well. The location of the droplet in each well was marked and used as a template for future sorts.

Single-Cell Sorting on the S3e Cell Sorter and Verification with the ZOE Fluorescent Cell Imager

Prior to single-cell sorting, 1 µl of mineral oil (Sigma, #M3516) was added to the center of each well of the 8-well strip. This small amount of mineral oil was used to maintain the sorted cells or beads to a confined area for easy visualization and confirmation of single-cell sorting. (Note: this method is not recommended for other sorting applications or general S3e Sorter use). The cells or beads were then sorted into the 8-well strip. To prevent sample evaporation, the strip containing the sorted sample was placed on a rack and immediately covered with a lid. The number of cells or beads in each

well was verified using the ZOE Fluorescent Cell Imager. Cell images were captured in both brightfield and green fluorescence channels, while only brightfield images were captured for beads.

Results

Optimization of deflection values is crucial for sorting accuracy. Appropriate deflection settings ensure that the sorted cells are directed to the correct position in the 8-well strip in order to optimize recovery. Ideally, sorted cells are directed to land as close as possible to the center of the well. Therefore, test sorts were performed into each well of the 8-well strip. Charge Left and Charge Right deflection values were adjusted by 1% for each side (left and right) and the test sort was repeated until the best deflection values were identified (Figure 1). The ideal sort deflection resulted in all sorted drops landing at the center of the wells in the 8-well strips. For all experiments described, the S3e Cell Sorter consistently sorted cells or beads into each well of the 8-well strip. The S3e Sorter performed sorts to either the left or the right collection points with equal accuracy.

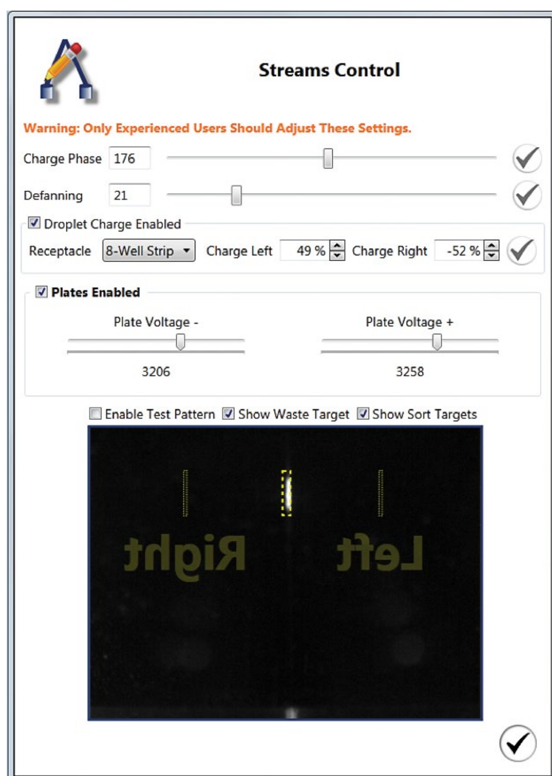


Fig. 1. Deflection value optimization for collection of single cells in 8-well flat bottom culture strips. The Edit Streams Setting (Streams Control window) is found under the Administrator Toolbar. The Charge Left and Charge Right deflection values were adjusted in 1% increments until the test sorts using ProLine Universal Calibration Beads confirmed that the sorted events were directed to the correct position within the 8-well strips.

The accuracy of sorting single cells and single beads was verified using Jurkat cells stained with CytoTrack Green 511/525 Dye and 15 µm polystyrene beads, respectively. CytoTrack Dyes label live cells efficiently and allow easy visualization and verification of single sorts using the ZOE Fluorescent Cell Imager. Images of single sorted cells and beads are shown in Figure 2. Both cells and beads were sorted in groups of 13 strips for a total of 104 wells each. Of these, 98 contained only a single cell. The remaining wells were found to have no fluorescence signal and contained only cellular debris. For the 15 µm bead sorting application, all 104 wells contained only a single bead. Therefore, we calculated the accuracies for single-cell and single-bead sorting applications as >94% and 100%, respectively.

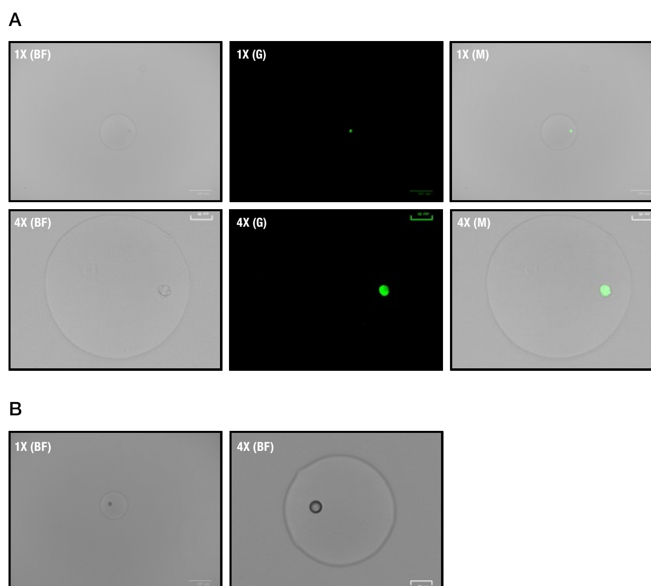


Fig. 2. Verification of single-cell and -bead sorting using the ZOE Fluorescent Cell Imager. Visualization of a single Jurkat cell (**A**) and 15 µm bead (**B**). Magnification power: 1X and 4X. BF, brightfield; G, green fluorescence channel; M, merged brightfield and green channel.

Conclusions

We have demonstrated a simple workflow to verify that the S3e Cell Sorter consistently and reliably sorts single cells into each well of 8-well flat bottom culture strips (Figure 3). The accuracy of sorting single cells and beads was verified using the ZOE Fluorescent Cell Imager. No wells contained more than one cell or bead. While the data presented here illustrate the inherent precision of the S3e Cell Sorter, it is recommended that each lab run this workflow to optimize the system for their cell type or application.

© 2016 Bio-Rad Laboratories, Inc.

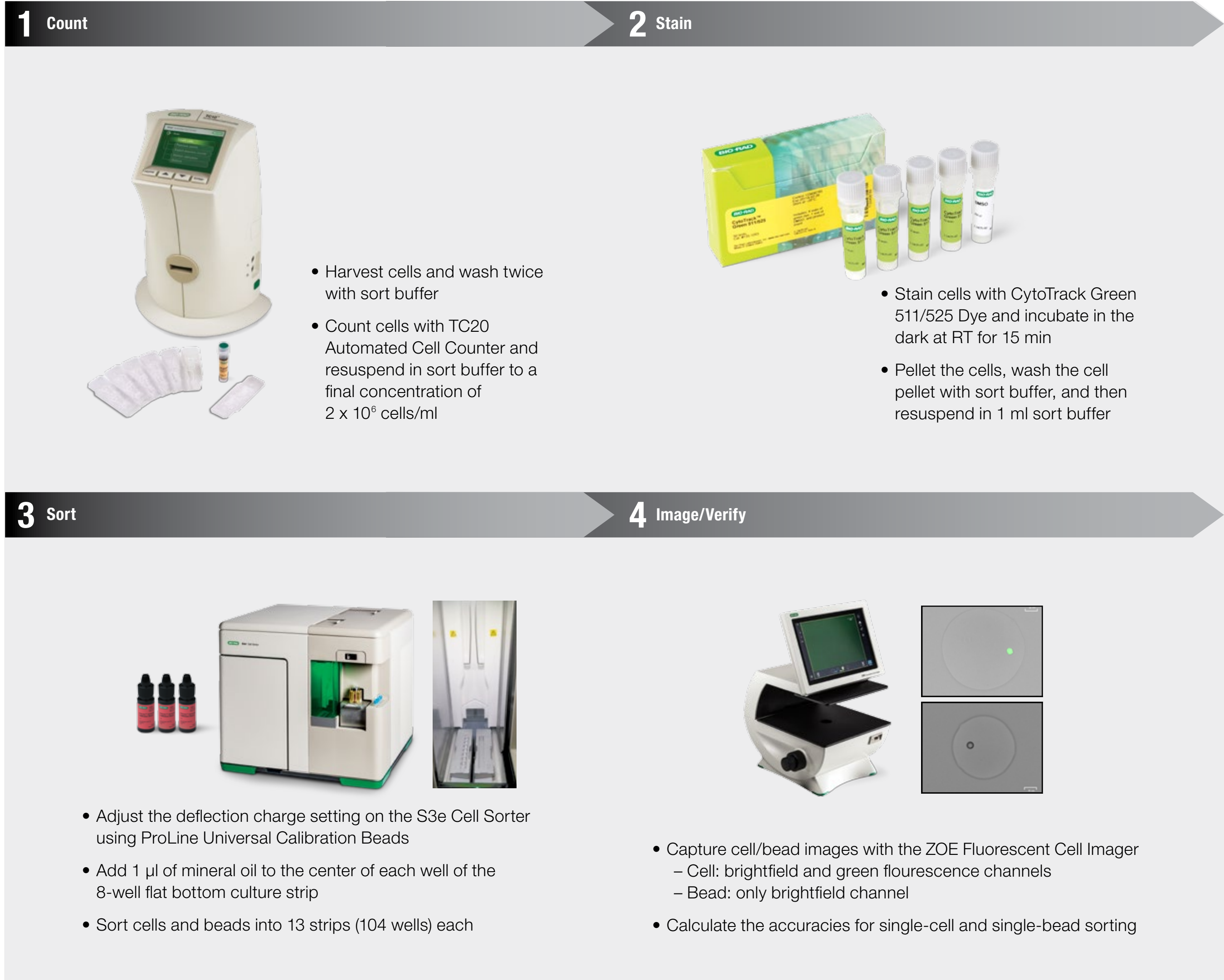


Fig. 3. Single-cell sorting verification workflow. Depiction of a simple workflow to verify the accuracy of single-cell sorting on the S3e Cell Sorter. The ZOE Fluorescent Cell Imager serves as the final QC/verification step.

References

Raj A and van Oudenaarden A (2008). Nature, nurture, or chance: stochastic gene expression and its consequences. Cell 135, 216–226.

© 2016 Bio-Rad Laboratories, Inc.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *43 1 877 89 01 177* ***Belgium*** *32 (0)3 710 53 00* ***Brazil*** *55 11 3065 7550* ***Canada*** *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *45 44 52 10 00* ***Finland*** *358 09 804 22 00* ***France*** *33 01 47 95 69 65* ***Germany*** *49 89 31 884 0* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300* ***Israel*** *972 03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *31 (0)318 540 666* ***New Zealand*** *64 9 415 2280* ***Norway*** *47 23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 (0) 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *46 08 555 12700* ***Switzerland*** *41 026 674 55 05* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 662 651 8311* ***United Arab Emirates*** *971 4 8187300* ***United Kingdom*** *44 020 8328 2000*

Bulletin 6853 Ver A US/EG *16-0782 0916 Sig 1215*