nodexus

APPLICATION NOTE

nx one

Optimizing Single Cell Sorting: Input Concentrations and Ratios

Introduction

The central objective of single-cell sorting is to isolate specific cell types from larger populations to elucidate their function, which is essential for understanding the role they play in disease development and for deriving potential therapeutic approaches. With this perspective, single-cell sorting from a mixed population allows for selective cell isolation and clonogenesis for downstream applications ranging from cell line development to gene editing. Gentle, efficient, and reliable sorting of single cells is thus crucial for the preservation of cell viability and the maximization of quality, throughput, and reproducibility in biological experiments.

This application note demonstrates the ranges of total input and target concentrations required to obtain optimal efficiency of single-cell sorting using the Nodexus NX One benchtop single cell sorting and dispensing system.

Methods

Yellow/Green 15µm fluorescent beads (Thermo Fisher, F8844) were sorted at a variety of input concentrations to determine the optimal range for pure sample deposition accuracy. Under the optimal total concentration thus identified, we then determined the accuracy of sorting and dispensing a specific target of interest (i.e., Orange/Red beads (Thermo Fisher, F8842)) from mixed sample solutions also containing Yellow/Green beads at a range of constituent ratios. For simplicity, Yellow/Green beads will be referred to as 'Green' and Orange/Red beads as 'Red'. The bead sample solutions were sorted using the NX One system into 96-well plates to examine the influence of total bead concentrations and non-target/target sample proportions on the accuracy and runtime of single target deposition.

To ascertain that the trends for deposition accuracy defined using fluorescent beads are reflected in biological applications, a mixed population of COS-7 cells was additionally sorted. This sample was created with 50% unstained cells and 50% cells stained with Cytotell Ultra Green (AAT Bioquest, #22240) at a total input concentration of ~100,000 cells/mL; a sorting gate was applied for the GFP positive population.

All sort runs were performed using Purity mode, and the gating for mixed sample beads was selected for Red beads exclusively. After sorting, the well-plates were imaged using the Cy3 and FITC channels of a Molecular Devices ImageXpress Micro imaging system to identify the presence of Red beads and absence of Green beads, respectively, as visualized in **Figure 1**.

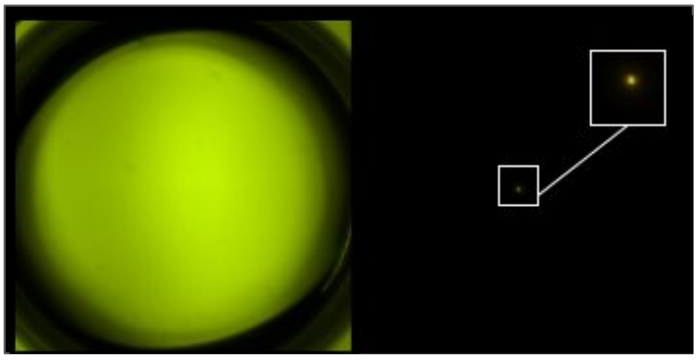

Figure 1: Visualization of target bead deposition. The FITC channel (left) indicates the absence of non-target objects (Green), while the Cy3 channel (right) indicates the presence of a target object (Red). Contrast is different for both images to elucidate well contents.

Results

Based on the input concentrations tested, the optimal input concentration for single-cell purity sorting with a desired output of 1 particle per well lies between 10,000 and 200,000 particles/mL. This concentration range offers high deposition accuracy (96.5% ± 1.7%), as recorded in **Figure 3**, alongside low average run times (5.5 - 8.4 minutes) recorded in **Figure 2**.

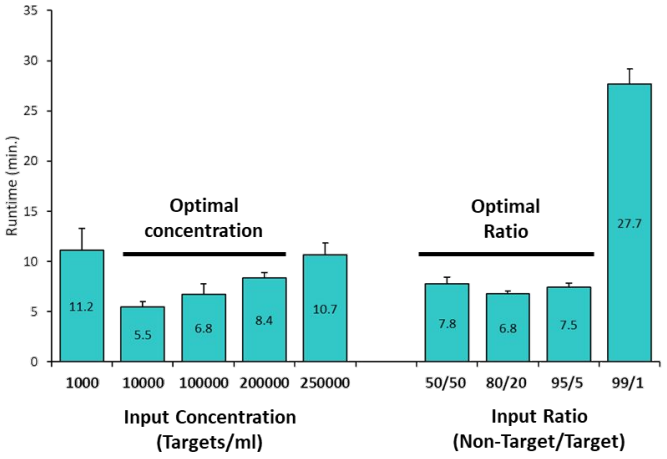

Figure 2: Average sorting runtimes observed for each input concentration (beads per mL of solution) and non-target/target ratio (Green/Red) of beads sorted using the NX One instrument.

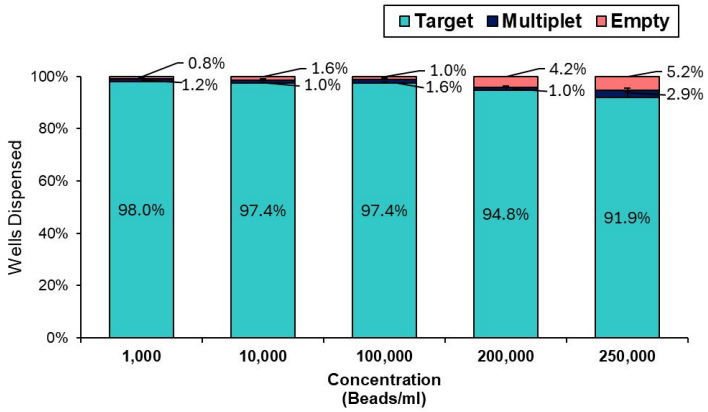

Figure 3: Deposition accuracy of Green bead sorts. Percentages of single target (Green bead), target multiplet, and empty wells are represented with respect to the total number of wells sorted.

When sorting mixed population samples at an optimal input concentration of 100,000 beads/mL, a non-target/target ratio between 50/50 and 95/5 offered the highest average deposition accuracy (91.7% ± 6%) with minimal non-target (2.8% ± 2.2%) and blank/empty (5.0% ± 4.7%) deposition (**Figure 4**). Similarly, a non-target/target input ratio between 50/50 and 95/5 provided the greatest efficiency of sorts by offering the lowest average run-time duration (6.8 - 7.8 minutes) as illustrated in **Figure 2**.

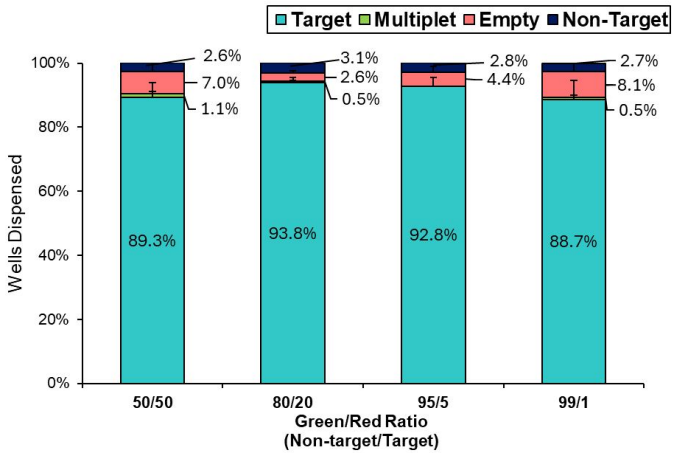

Figure 4: Percent deposition accuracy of mixed sample bead sorts. The percentages of single target (Red bead), target multiplet, non-target (Green bead), and empty wells are visualized with respect to the total wells sorted.

To validate the deposition accuracy of the unstained/stained mixed sample of COS-7 cells, all sorted cells were treated with Hoechst 33342 (Thermo Fisher, H3570), a blue fluorescent nuclear counterstain, and imaged using both FITC and DAPI channels of the ImageXpress imager. The deposition accuracy of single target cells (GFP+, DAPI+) was recorded (**Figure 5**).

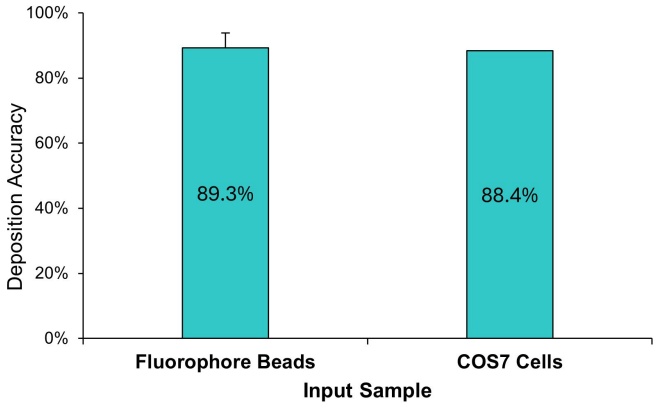

Figure 5: Comparison of the percent deposition accuracy of single target objects (Red beads and GFP+ cells) sorted from 50/50 non-target/target ratio input solutions with concentrations of approximately 100,000 particles/mL.

Overall, the cellular and non-cellular input samples offered similar single-target deposition of 88.4% and 89.3% respectively. By comparing the sorting of a mixed population of fluorescently labeled cells to the sorting of fluorophore-conjugated beads previously outlined, we observe that the optimal total input concentration and target ratio specifications already defined are also consistent in biological samples.

Conclusion

These results indicate that the NX One system offers a reliable process for isolating target objects with varying fluorescent profiles across a range of input concentrations and ratios. The NX One system operates most successfully at a sample input concentration between 10,000 and 200,000 cells per mL and a non-target/target input ratio between 50/50 and 95/5 for mixed sample sorting. These conditions have been shown to provide high deposition accuracy while minimizing sorting run-time, which enables both efficiency and reliability when applied to the sorting of biological samples.

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