Performance Verification of NanoCellect's Second Generation Microfluidic Cartridges

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Introduction

NanoCellect has developed a second generation of injection molded microfluidic cartridges for use on the WOLF® Cell Sorter. One major improvement involves changing the microfluidic chip material from polydimethylsiloxane (PDMS) to Cyclic Olefin Copolymer (COC).

Both materials provide excellent injection moldability, low autofluorescence, and high optical clarity but COC is better suited to high volume production methods. COC also improves fluidic properties – priming time is twice as fast, and accuracy is improved through a reduction in piezoelectric transducer (PZT) activation backlash. Here, the performance of these Second Generation (COC material) cartridges was compared to the First Generation (PDMS material) cartridges; bulk and single-cell sorts were evaluated for equivalent efficiency and viability.

Method

Bulk Sort Purity

Second Gen bulk sorting performance was compared to First Gen historical data to confirm equivalent post-sort purity. Two different target population concentrations were prepared; 7.5 µm Dragon Green (DG) beads were mixed with 10 µm Envy Green beads (Bangs Labs, # FSDG007 and #FSEG008) to yield 10% and 30% DG bead stock concentrations. Each stock was diluted to a range of concentrations in PBS and evaluated on the BD Accuri™ for DG and total bead concentrations. Each sample type was sorted multiple times on the WOLF to determine post-sort purity of DG beads.

Bulk Sort Viability

Second Gen bulk sorting performance was compared to First Gen to confirm equivalent post-sort viability. CHO-K1 and peripheral blood mononuclear cells (PBMCs) were evaluated for viability after mock-sorts: cells were passed through the instrument but to avoid variables that might affect viability, cells were unlabeled and only gated to eliminate debris.

800 µL CHO-K1 cells were simultaneously sorted on both cartridge types at 3 x 10^5 cells/mL in HBSS/10% FBS. Sorted cells were stained with Propidium Iodide (PI) and evaluated for viability on the WOLF. This was repeated twice. Similarly, after isolation from whole blood using a Histopaque gradient (Sigma, # 10771), PBMCs were diluted to 3 x 10^5 cells/mL in DPBS/0.02% EDTA/2% FBS and sorted simultaneously with each cartridge type. 50,000 cells were sorted, stained with PI, and evaluated for viability on the Accuri. This was repeated once.

Single-Cell Dispense Efficiency

Second Gen single-cell sorting performance was compared to First Gen historical data to confirm equivalent dispense efficiency. 15.25 µm Dragon Green calibration beads (Bangs Labs, #FSDG009) were diluted in PBS to 1x10^5 beads/mL and sorted into 96- and 384-well plates with the WOLF and N1® Single Cell Dispenser. The plates were imaged with the NyOne® (SYNENTEC) to determine the number of wells containing single beads.

Single-Cell Dispense Outgrowth

Second Gen cell outgrowth was compared to First Gen to confirm equivalent long-term viability. CHO-K1-GFP cells were diluted to 1 x 10^5 cells/mL in HBSS/10% FBS and simultaneously sorted with each cartridge type into three 96-well plates pre-filled with F-12 (K) medium/10% FBS/1% antibiotic-antimycotic. This was repeated twice for each cartridge type. Plates were incubated at 37°C, 5% CO_2 for 14 days and then imaged on the NyOne to detect number of wells with outgrowth.

Conclusion

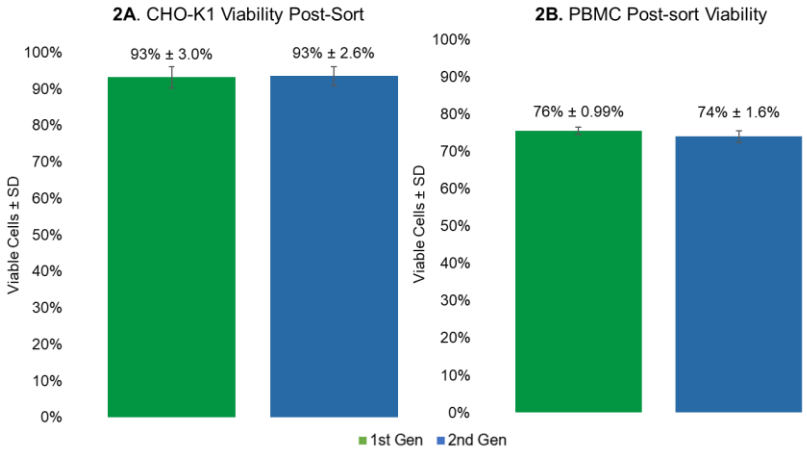
The Second Generation cartridge results were similar for bulk and single-cell sorting efficiency and viability. Therefore, they offer production and fluidic improvements with equivalent performance.

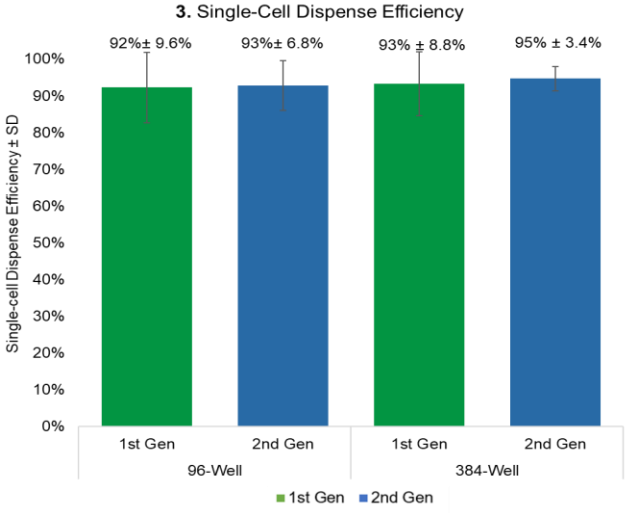
Table 1. 30% Target Population: Average Post-sort Purity and Linear Regression

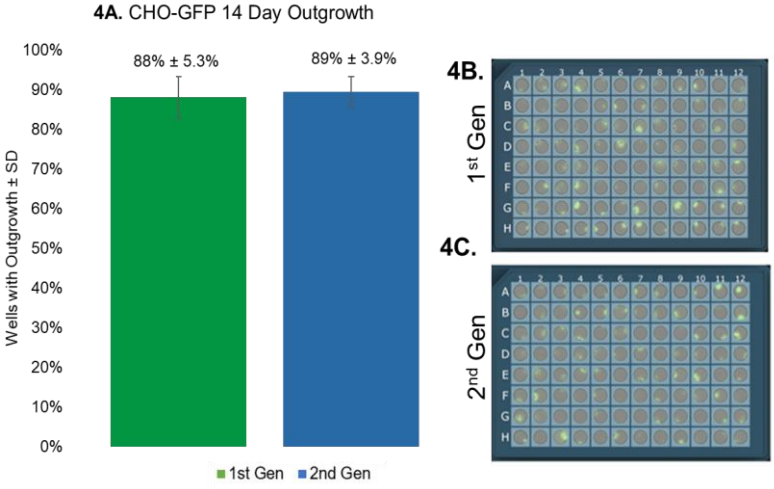
	1st Gen	2nd Gen
Sample conc. (beads/uL)		
100	96% ± 2.2%	96% ± 0.35%
300	94% ± 1.4%	91% ± 2.3%
650	92% ± 1.7%	85% ± 2.5%
1200	78% ± 4.0%	80% ± 3.7%
Linear regression (R^2)	0.92	0.97

Table 2. 10% Target Population: Average Post-sort Purity and Linear Regression

	1st Gen	2nd Gen
Sample conc. (beads/uL)		
100	93% ± 1.9%	92% ± 6.3%
200	87% ± 3.4%	87% ± 1.9%
500	83% ± 2.0%	89% ± 0.61%
1000	77% ± 2.7%	73% ± 2.7%
Linear regression (R^2)	0.96	0.84







Results

Figure 1: Post-sort purity is similar for both cartridge types across multiple target populations and concentrations. (A) The 30% Dragon Green (DG) bead target population was sorted for DG fluorescence at 4 total bead concentrations; each concentration was sorted multiple times per cartridge. n=4, 1, 2, and 3 cartridges for 100, 300, 650, and 1200 beads/uL, respectively. Historical data was similarly tested and displayed for comparison. **(B)** The 10% Dragon Green (DG) bead target population was sorted for DG fluorescence at 4 total bead concentrations; each concentration was sorted multiple times per cartridge. n=2, 2, 1, and 3 cartridges for 100, 200, 500, and 1000 beads/uL, respectively. Historical data was similarly tested and displayed for comparison.

Figure 2: Post-sort viability is similar for both cartridges for multiple cell types. (A) CHO-K1 cells were gated on scatter to mimic a sort. This was completed simultaneously on a First Gen and a Second Gen cartridge and 10,000 sorted cells were evaluated for Propidium Iodide (PI) staining. This was repeated twice. **(B)** The same testing for CHO-K1 cells was applied to PBMCs and 30,000 events were evaluated. This was repeated once.

Figure 3: Single-cell dispense efficiency is similar for both cartridge types across multiple plate types. DG beads were sorted based on FL1+ fluorescence into three 96- or 384-well plates (n=12 or 5 cartridges, respectively). Plates were imaged on the NyOne and counted for wells containing single beads out of total wells (dispense efficiency). Historical data was similarly tested and displayed for comparison.

Figure 4: Post-sort outgrowth is similar for both cartridge types. (A) CHO-K1-GFP cells were sorted based on FL1+ fluorescence by each cartridge type, simultaneously, into three 96-well plates. This was repeated twice, and wells were imaged for outgrowth after 14 days. Representative plate images from the cells sorted with the **(B)** 1st Gen cartridge **(C)** and 2nd Gen cartridges.