A Move to Full Spectrum Cell Sorting: How Do the Cytek Aurora CS and BD Influx Compare?

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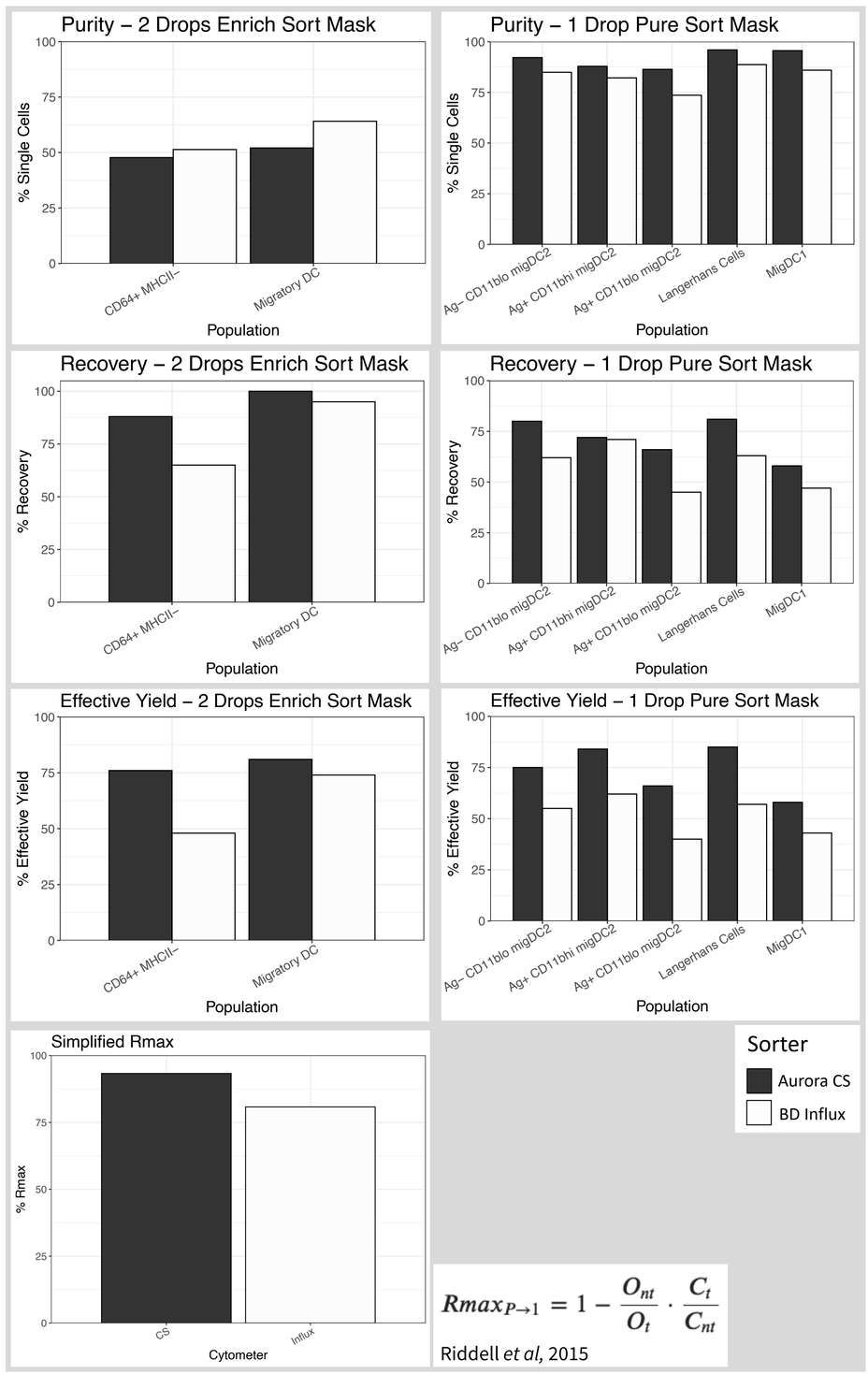
Introduction

The Cytek® Aurora CS (CS) is a cell sorter that provides all the full spectrum profiling benefits of the Cytek® Aurora. Full spectrum flow cytometry has become more widely available in recent years, paving the way for more high-dimensional data to be collected. However, the absolute power of full spectrum profiling was not realized, until the CS was produced. Which is capable of isolating the same unique subsets identified on the Cytek® Aurora, without the need to alter existing panels in any way.

Aim

To compare the recovery, yield, and data resolution of the CS to the well-established BD Influx™ cell sorter, using comparable gating strategies. Also to compare data similarity and experiment transferability from the Cytek® Aurora analyzer to the CS (Figure 3).

FIGURE 1: Comparative histograms showing sort performance of the BD Influx™ and Aurora CS. Mice were treated two days before harvest with Alexa Fluor 488-labelled *Nippostrongylus brasiliensis*, ear draining lymph nodes were collected and stained with a 9-colour monocyte and dendritic cell (DC) panel. Samples were gated as per Figure 2 and enrichment was carried out on two populations at the same level of the population hierarchy. The enriched migratory DC population was sorted again using a purity mask. Purity results and cell counts were obtained using a Cytek® Aurora Analyser. Rmax sorting was performed using BD Calibrite™ PE and APC beads.



$$Rmax_{P\to1} = 1 - \frac{O_{nt}}{O_t} \cdot \frac{C_t}{C_{nt}}$$

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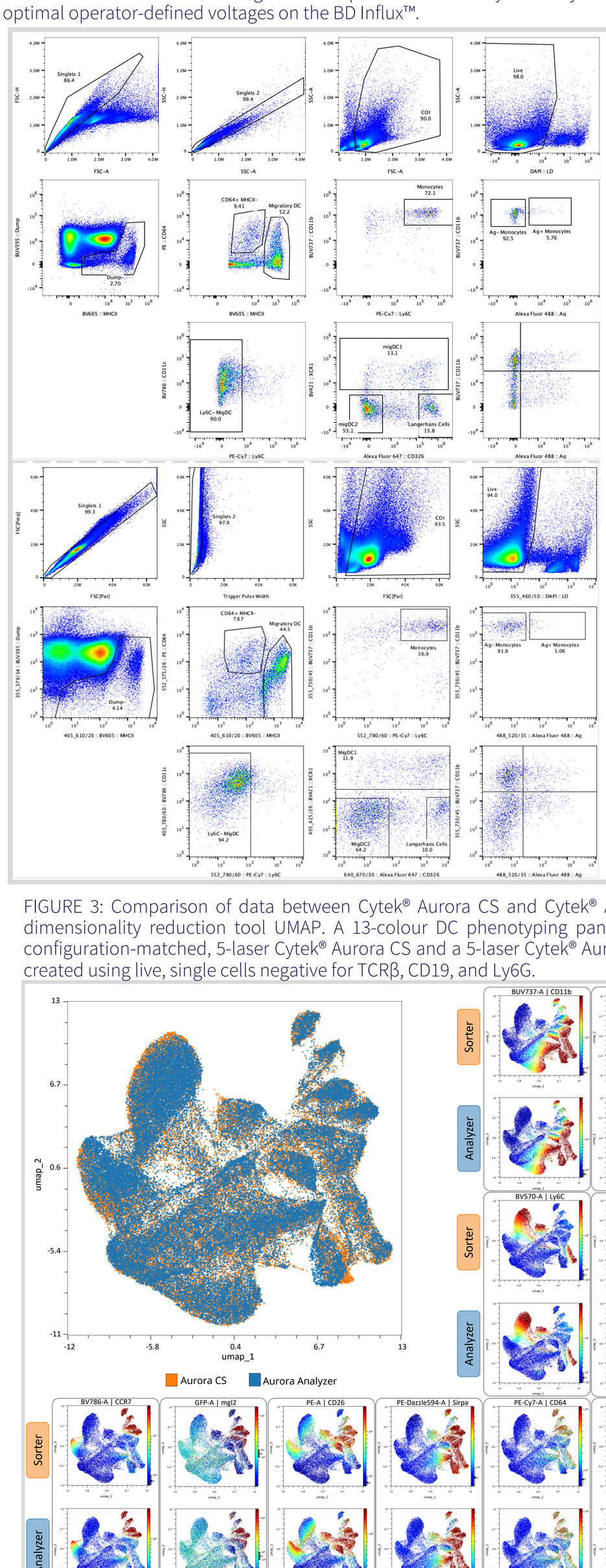
FIGURE 2: Comparable gating strategies between Aurora® CS and BD Influx™. The samples shown are the same as described in Figure 1. Samples were run at Cytek Assay Settings on the CS, and at optimal operator-defined voltages on the BD Influx™.



Population statistics are presented as the percentage of cells of interest due to differences in singlet gating across the two systems.

	% of Cells of Interest	
	Influx	Aurora CS
Live	94	98
Dump-	3.89	2.65
CD64+ MHCII-	0.3	0.25
Monocytes	0.17	0.18
Ag+ Monocytes	0.009	0.01
Ag- Monocytes	0.16	0.17
Migratory DC	1.73	1.38
Ly6C- MigDC	1.63	1.24
Langerhans Cells	0.16	0.2
MigDC1	0.19	0.16
MigDC2	1.05	0.69
Ag+ CD11b-hi MigDC2	0.08	0.075
Ag+ CD11b-lo MigDC2	0.078	0.097
Ag- CD11b-hi MigDC2	0.31	0.26
Ag- CD11b-lo MigDC2	0.58	0.26

FIGURE 3: Comparison of data between Cytek® Aurora CS and Cytek® Aurora analyzer using the dimensionality reduction tool UMAP. A 13-colour DC phenotyping panel was acquired across a configuration-matched, 5-laser Cytek® Aurora CS and a 5-laser Cytek® Aurora analyzer. UMAPs were created using live, single cells negative for TCRβ, CD19, and Ly6G.

Conclusion

Both the BD Influx™ and Cytek® Aurora CS showed similar trends across to all sort metrics measured (Figure 1).

Analysis of a sort panel designed for a conventional flow cytometer showed all populations were able to be easily identified, with similar percentages across the two sorter systems (Figure 2). The Aurora CS has the added benefit of removing the need for user-defined gains, thus ensuring optimal population resolution. The Aurora CS produced highly comparable data to an Aurora analyzer, with near identical data resolution, as shown by the overlaid UMAPs in Figure 3.

The Aurora CS brings a well-performing sort unit to a cytometer that is extremely closely matched to the Aurora analyzer, making transfer of experiments from analyzer to sorter extremely simple.

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REFERENCES

1 Riddell, A., Gardner, R., Perez-Gonzalez, A., Lopes, T., & Martinez, L. (2015). Rmax: A systematic approach to evaluate instrument sort performance using center stream catch. *Methods,* 82, 64-73.