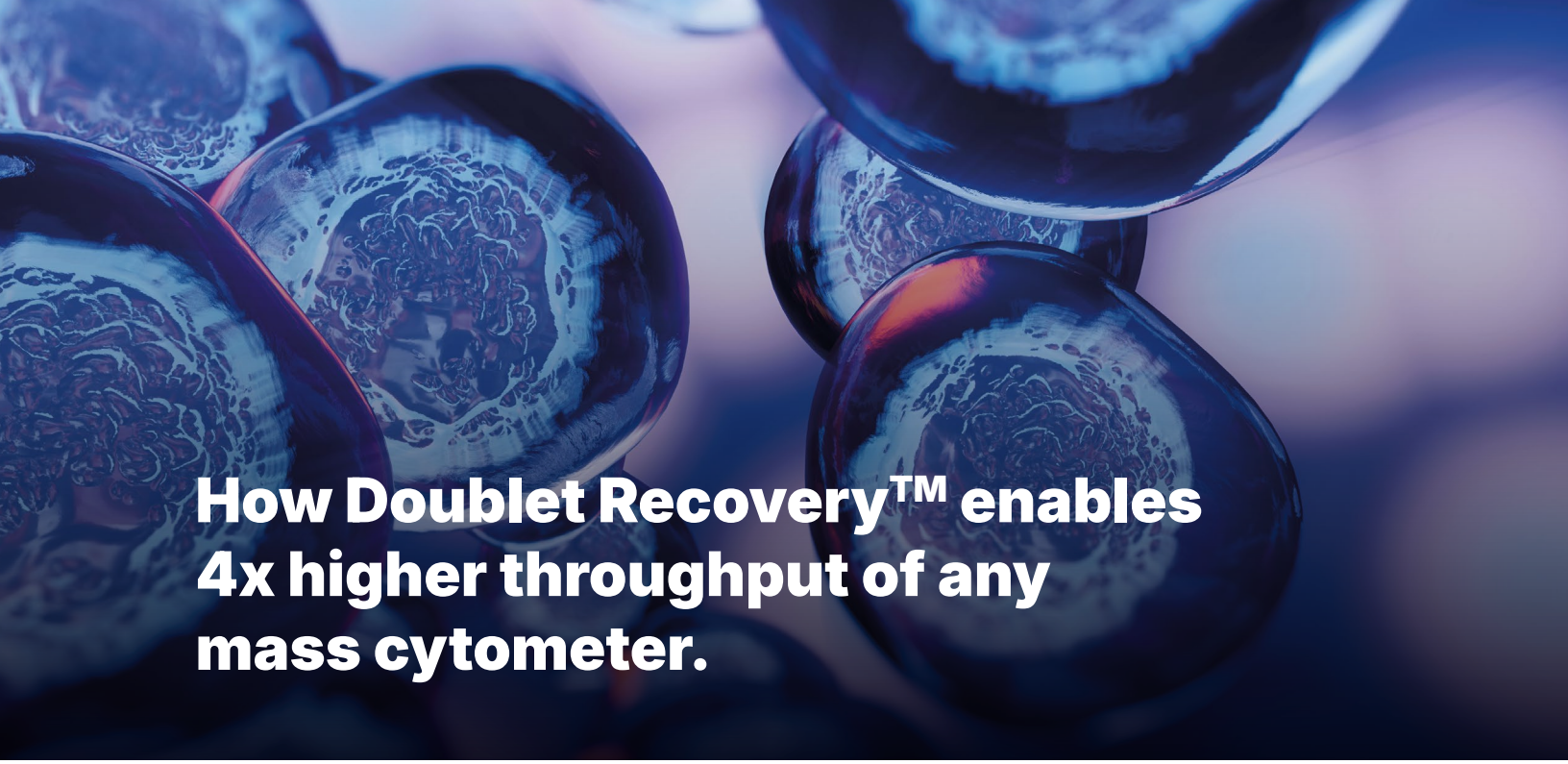

How Doublet Recovery™ enables 4x higher throughput of any mass cytometer.

Abstract

Based on a computational workflow, Doublet Recovery™, we report the throughput increase of up to 4X of any mass cytometer by simply increasing the cell concentration of the acquired sample. By recovering technical doublets, i.e. doublets that arrive at the detector close in time purely by chance, users can increase the event rate up to 1000 events/s without incurring further losses. Doublet Recovery works independently of panel, sample and device type, and enables mass cytometry cores, internal and external CROs to get more out of their team and instrument time while improving the economics on a per sample basis.

Key findings

- Up to 4X higher throughput
- Higher singlet recovery at high cell concentrations
- No significant differences in population distributions

Application Note
Mass Cytometry

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Overview

With Doublet Recovery for mass cytometry data, the throughput of any mass cytometer can be increased up to 4X without further cell loss by recovering technical doublets. This white paper benchmarks the performance of Doublet Recovery on mass cytometry devices running PBMCSs.

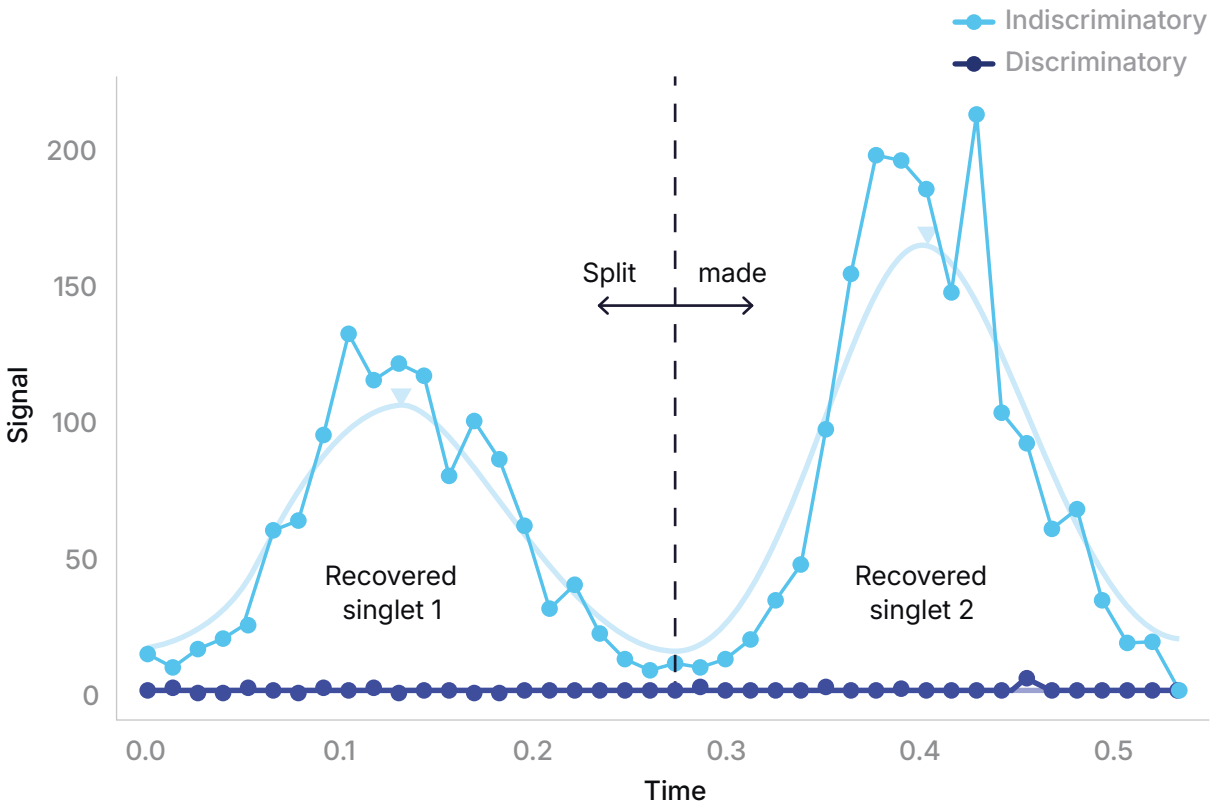

Figure 1. Doublet identifications and recovery process

Figure 1. Doublet Recovery™ identifies doublets at the ion cloud level and splits them into their individual components. The recovered singlets are than added to the fcs file increasing the total useful events.

Introduction

Mass cytometry enables the simultaneous quantification of over 50 biological markers at the single-cell level; comprising both, surface, intracellular structural and cytokine production and signalling. Even though spectral flow has become competitive in terms of panel size and depth, in terms of ease of panel design, mass cytometry still has a significant advantage due to the lack of spill-over effects. One major disadvantage of mass cytometry, though, has been it's low sample throughput. Where spectral devices run routinely at 1500-5000 events/s, mass cytometry has largely been limited to event rates ≤300 events/s. Under comparable conditions, this means that for the same amount of instrument run time, spectral flow users can generate 10-20 times more output than users of mass cytometry. Furthermore, those that have developed proprietary workflows using mass cytometry, can suffer from significant backlogs.

One of the reason for the lower throughput in mass cytometry, is that when users try to increase the event rate by increasing their sample cell concentration, their yield goes down. This is due to the fact that mass cytometry data becomes increasingly contaminated with doublets or multiplets (triplet, quadruplets, etc.), with increasing cell concentration. The higher the cell concentration, the more likely it is that cells arrive at the detector close in time and the higher the chance that they are recorded as doublets. To avoid this, operators typically limit cell concentrations to 0.3 M cells – 0.8 M cells per mL (i.e, event rates ≤300 events/s). Based on a computational workflow, Doublet Recovery™, we report the throughput increase of up to 4X of any mass cytometer by simply increasing the cell concentration of the acquired sample (2-3 M cells/mL). We achieve this by recovering technical doublets, i.e. doublets that arrived at the detector close in time purely by chance (Figure 1). In this way, users can increase the event rate to 1000/s comparable to spectral based workflows without incurring further losses. Doublet Recovery works independently of panel, sample and device type. With Doublet Recovery, mass cytometry cores, internal and external CROs can get more out of their team and instrument time. Moreover, it greatly improves the economics for mass cytometry on a per sample basis.

Experimental

Samples preparation. Human peripheral blood mononuclear cells (PBMCs) were stained with the Maxpar Direct Immune Profiling Assay™ from Standard BioTools Inc., San Francisco, California, USA acquired on a CyTOF XT™ and an inhouse conjugated panel of 22 markers acquired on a Helios system at increasing input concentrations (0.5×10^6, 1.0×10^6, 2.0×10^6 and 4.0×10^6 cells/mL and 1.1×10^6, 2.2×10^6, 3.3×10^6 and 4.4×10^6 cells/mL, respectively). Other than the dilution step before acquisition, no changes to the protocol were made. In all cases, the flow rate was kept at 30 uL/min. Recovery performance was evaluated by comparing singlet yields obtained with Doublet Recovery™ against the standard workflow. Data processing was conducted in FlowJo and CellEngine. Samples originated from the same donor and were acquired on the same instrument on the same day. A minimum of 100'000 events were acquired per concentration.

Results and Discussion

To benchmark the performance of Doublet Recovery vs the standard solution, PBMCs stained with both an in-house conjugated panel as well as using a kit (MDIPA) were acquired on a Helios and CyTOF XT at increasing cell concentrations. Figure 2. shows the event rate vs cell concentration as determined for a Helios mass cytometer. For the standard solution (grey-curve), the singlet rate does not increase with cell concentration and saturates at roughly 300 singlets/s. Instead, with Doublet Recovery, the singlet rate increase linearly with cell concentration up to 4M cells/mL. Further, when comparing the event rate vs cell concentration as determined for a CyTOF XT mass cytometer, the same observation can be made. For the standard solution, the singlet rate does not increase with cell concentration and saturates, and earlier than for the Helios. Instead, with Doublet Recovery, singlet rate increase linearly with cell concentration up to 2M cells/mL.

Concentration vs Event rate Helios

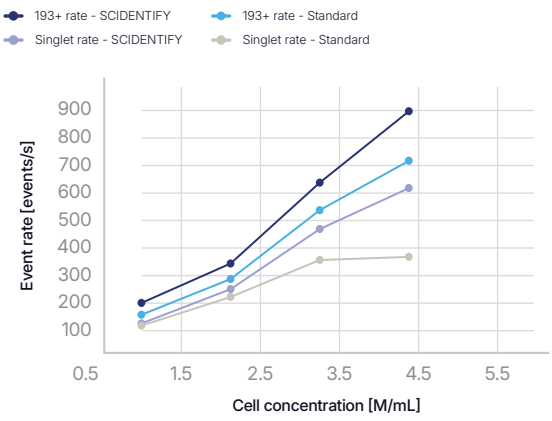

Figure 2. 193+ and singlet event rates as determined by DNA1/CD14 gate. SCIDENTIFY finds more events and extracts more singlets than standard solution. Data generated on Helios from DRFZ.

Concentration vs Event rate CyTOF XT

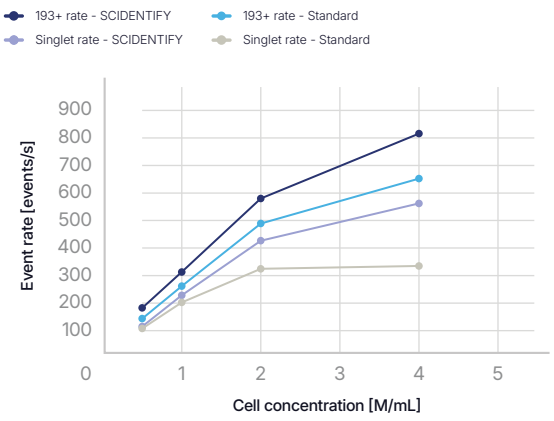

Figure 3. 193+ and singlet event rates as determined by DNA1/CD14 gate. SCIDENTIFY finds more events and extracts more singlets than standard solution. Data generated CyTOF XT from LIH.

Using the cell concentration, the instrument flow rate, the experiment duration and the known found number of useful events, i.e. singlets, it is possible to determine the cell yield as a function of concentration. We performed this experiment for both instruments (Helios and CyTOF XT) and compared the cell yield using both the standard processing and Doublet Recovery. As can be seen in Figure 3, the yield for the standard processing, drops significantly with increasing cell concentration dropping under 20% for the highest concentration.

In case of the CyTOF XT, cell yield drops already significantly for any concentration over 1 million cells/mL. Instead, using Doublet Recovery, the cell yield is always higher than the standard and remains virtually unchanged up to 4X the concentration increase. Using Doublet Recovery, mass cytometry operators can increase their device throughput 4X without further cell loss.

Cell yield vs Concentration Helios

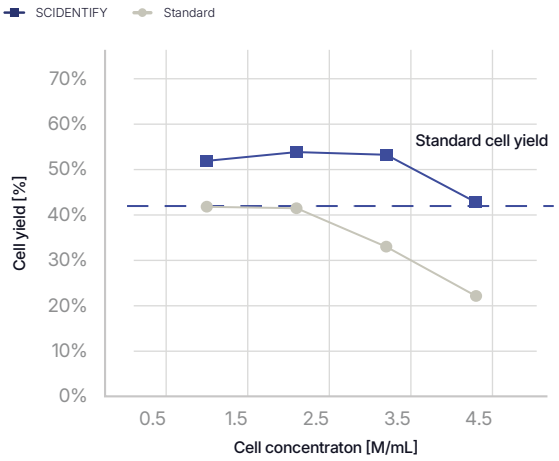

Figure 4. Cell yields as a function of concentration. SCIDENTIFY achieves higher overal cell yield than standard solution. Cell yield = (total singlets found/(flow rate * time * cell concentration)) * 100%. Data generated on Helios from DRFZ.

Cell yield vs Concentration CyTOF XT

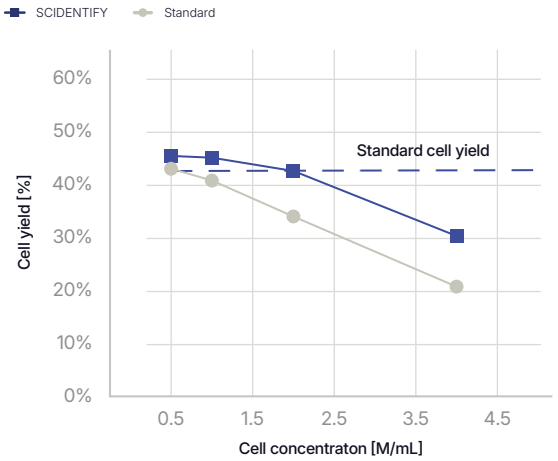

Figure 5. Cell yields as a function of concentration. vvvvvvvSCIDENTIFY achieves higher overal cell yield than standard solution. Cell yield = (total singlets found/(flow rate * time * cell concentration)) * 100%. Data generated on CyTOF XT from LIH.

To validate the performance of Doublet Recovery to recover technical doublets and do not introduce any artifacts during the splitting, 29 subpopulations in human PBMCs where manually gated on both Doublet Recovery generated fcs files and standard fcs files Figure 4. When considering the part of parent percentages of those 29-subpopulations, no significant difference between the standard solution and Doublet Recovery could be observed.

Furthermore, the sample concentration did not influence the part of parent ratios (the concentration analyzed for Doublet Recovery was 2M cells/mL vs 0.5 M cells/mL for the standard solution). Finally, by plotting for all the subpopulations for each of the four concentrations the absolute cell count, it become apparent that Doublet Recovery find more cells for all populations that the standard, as long as the statistics are robust.

No significant variability in percentage of parent between SCIDENTIFY and standard solution

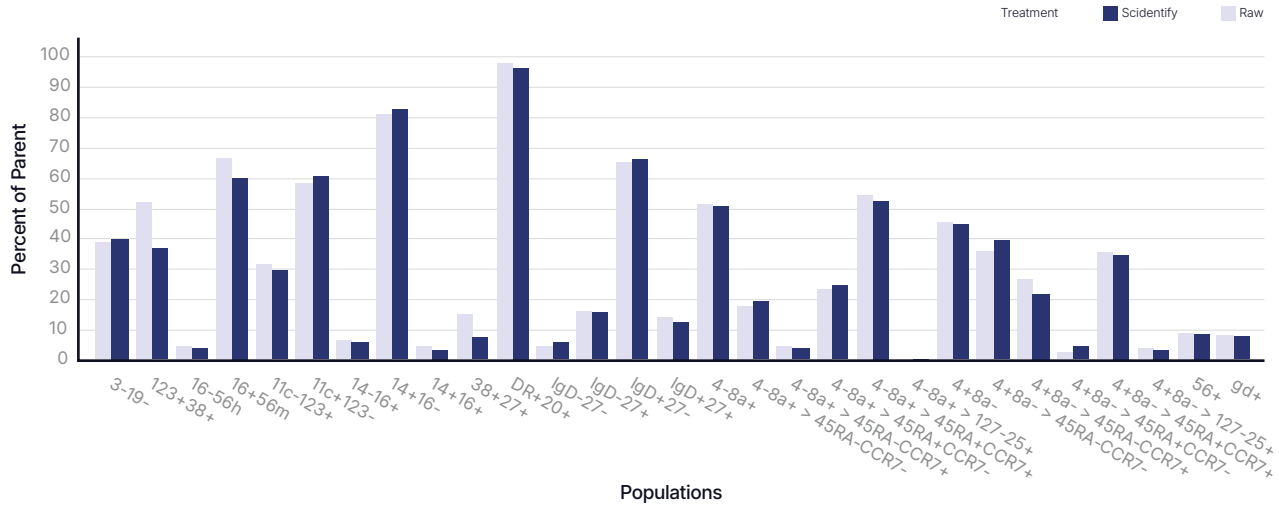

Figure 6. Percentage of parent head-to-head for all 29 subpopulation gated. Part of parent depicted of sample of 3 × 10^6 cell/ml" to " 2 × 10^6 cells/mL. Variability did not differ for lower concentrations. Data generated and analyzed by the LIH.

Doublet Recovery™ yields higher absolute cell count for any subpopulation independent of concentration

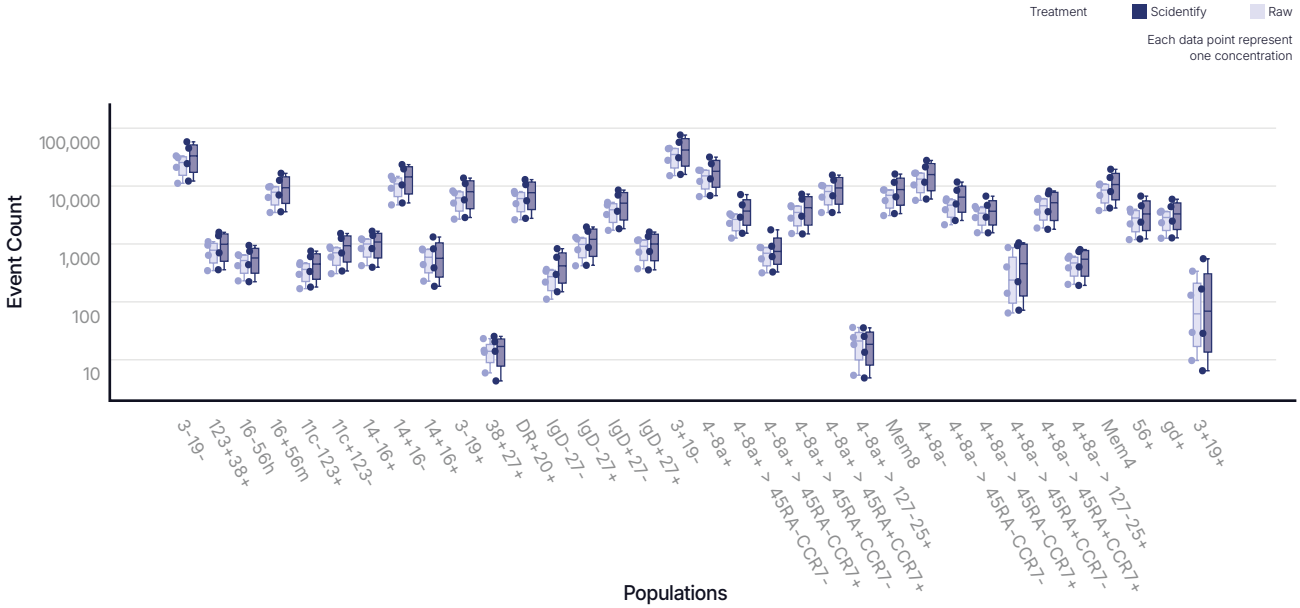

Figure 7. Absolute cell count head-to-head as a function of concentration for all 29 gated subpopulations. For all concentration, SCIDENTIFY finds more events with minimal spread. Data generated and analyzed by the LIH.

Conclusion

Using Doublet Recovery™, the contamination of single-cell data with mutliplet events at higher event rates can be effectively mitigated. It enables the extension of the throughput of a typical mass cytometer by 4 fold without further cell loss. For teams limited or device limited cores and CROs, Doublet Recovery is an excellent solution to expand on their capacity.

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