TECHNICAL NOTE

Rapid and Reproducible Results for Accelerated Insights on the CyTOF XT PRO System

Introduction

Objectives

- To demonstrate increased sample throughput and high data quality with the CyTOF™ XT PRO system
- To demonstrate the robustness and reproducibility of the CyTOF XT PRO instrument

Key findings

- Enhanced throughput: The CyTOF XT PRO system is capable of 2–4x throughput compared with the CyTOF XT system
- Data quality preservation: The CyTOF XT PRO system maintains high data quality, demonstrating low CVs and consistent signal intensities regardless of enhanced throughput
- Multi-instrument reproducibility: Multiple CyTOF XT PRO instruments demonstrate highly consistent population frequencies, marker CVs and marker medians regardless of cell concentration

The fully automated CyTOF XT PRO system is a rapid and robust instrument, offering exceptional data quality with decreased hands-on time, for deep single-cell profiling and advancements in biomarker discovery.

Study design

Whole blood from a healthy donor was stained according to the Maxpar™ Direct Immune Profiling Assay protocol, then frozen and stored at –80 °C until acquisition. Samples were thawed, washed, counted and acquired in triplicate on 3 instruments in either CyTOF XT or CyTOF XT PRO configurations. After acquisition, cell composition and marker expression for each sample was determined.

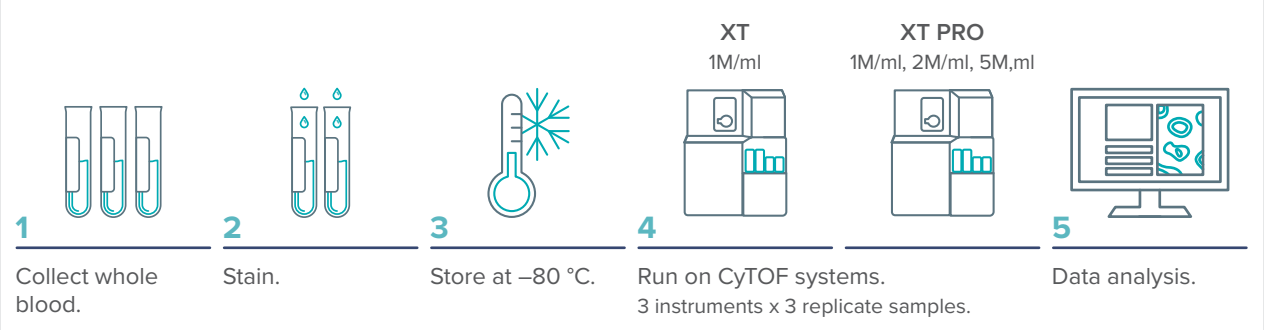


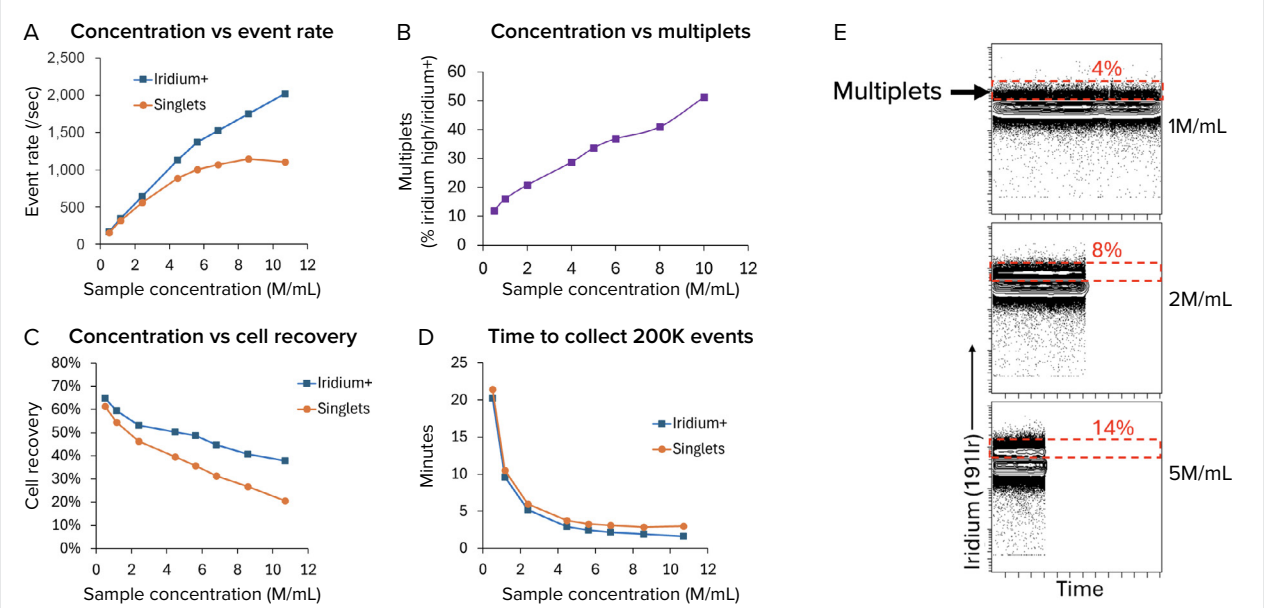
Figure 1. Study workflow using whole blood stained with the Maxpar Direct Immune Profiling Assay and acquired on CyTOF XT and CyTOF XT PRO systems using enhanced throughput

Results

The CyTOF XT PRO system enables enhanced sample throughput without compromising data quality

In both flow and mass cytometry, sampling frequency is a key determinant of data quality. In flow cytometry, high flow rates can broaden the sample core stream such that the cells are not centered on the laser beam, leading to reduced precision and broader peaks in the data. In mass cytometry, the flow rate is typically fixed, and cell concentration is adjusted to reduce clogging, ion cloud fusions and doublets. However, a sufficient sampling rate is desirable to enable rapid data acquisition. With the CyTOF XT system, a cell concentration of 1M/mL leads to an event rate of 250–300 events/sec, which is the recommended event rate for optimal results. With the new CyTOF XT PRO system, a redesigned injector and event-processing improvements allow higher sample throughput without compromising data quality.

The injector redesign enables acquisition of samples at a higher event rate by reducing the inner diameter of the injector and moving the injector tip closer to the plasma, reducing injector tip clogging. Keeping the flow rate constant at 30 µL/min results in a sustained event rate of 500–1,000 events/sec when cell concentration is increased on the CyTOF XT PRO system. As shown in Box 1, when the sample concentration increases, so does the event rate (A) and multiplets (B and E), with the optimal concentrations between 1–4M/mL before seeing a drop-off in singlets. As expected, the time to acquire 200,000 events is appropriately decreased when the sample concentration is increased, even at the optimal range of 2M/mL (Box 1E). This improvement in throughput via concentration adjustment significantly reduces hands-on time, allowing larger sample sets to be run more efficiently.



Box 1. Sample concentration is a key determinant of event rate and singlets rate. Samples were acquired at concentrations from 0.5M/mL to 10M/mL on the CyTOF XT PRO system. A) The event rate correlates with sample concentration for total iridium+ events, while the singlet rate plateaus at above approximately 5M/mL. B) The multiplets rate increases with sample concentration. C) Cell recovery (number of events recorded/number cells input *100) decreases with increased sample concentration, and the decrease is more pronounced for singlet events compared with total iridium+ events. D) A steep drop in the time to collect 200,000 events is observed when going from 0.5M/mL to approximately 2–4M/mL cells, but this plateaus as more cells are lost due to increased multiplets and ion cloud fusions. E) An example showing number of multiplet events from samples acquired at different concentrations.

Unmatched reproducibility and repeatability with the CyTOF XT PRO system and the Maxpar Direct Immune Profiling Assay

Ensuring consistency across instruments is crucial when comparing results from different experiments, collaborators or clinical trials. To demonstrate data quality and consistency of the CyTOF XT PRO system, population frequencies and marker CVs were compared on 3 instruments in CyTOF XT or CyTOF XT PRO configurations. For the CyTOF XT PRO system, a further comparison was made after acquiring samples at different cell concentrations. Figure 2 illustrates that population frequencies were similar across instruments, regardless of instrument type or cell concentration (throughput). Marker expression was similarly very reproducible (Figure 3) and linear regression of each condition yielded r2>0.99, indicating highly reproducible data.

Figure 4 further showcases the consistency and reproducibility of CyTOF XT and CyTOF XT PRO instruments at accelerated throughput. Marker CVs (Figure 4A) and population frequency CVs (Figure 4B) between instruments were examined. Error bars are derived by plotting the average CV of triplicate samples across 3 separate instruments. Notably, the %CVs remain low and similar across all instrument conditions for the majority of markers. The low variability under each condition highlights the robust and reliable performance of CyTOF XT systems even under enhanced throughput.

Figure 4B illustrates the inverse relationship between number of events and variability. As the number of cells increases, the %CV decreases, demonstrating improved precision with a larger number of events. As expected, the highest variability was observed for plasmablasts, which were extremely rare at only 40 events per sample, and considerably lower CVs for 100 events and higher. This holds true across CyTOF XT and CyTOF XT PRO systems at various throughput.

The consistent data quality of the CyTOF XT PRO system at each cell concentration is also shown in Figure 5. The signal intensity was examined for 29 of the 30 markers found in the Maxpar Direct Immune Profiling Assay. Histograms show highly similar signal intensities for each marker regardless of instrument or cell concentration. In addition, t-SNE plots colored by marker are also strikingly similar between conditions, confirming low noise, low instrument variability and low batch effects. This uniformity underscores the ability of the CyTOF XT PRO system to maintain data quality even at higher throughput.

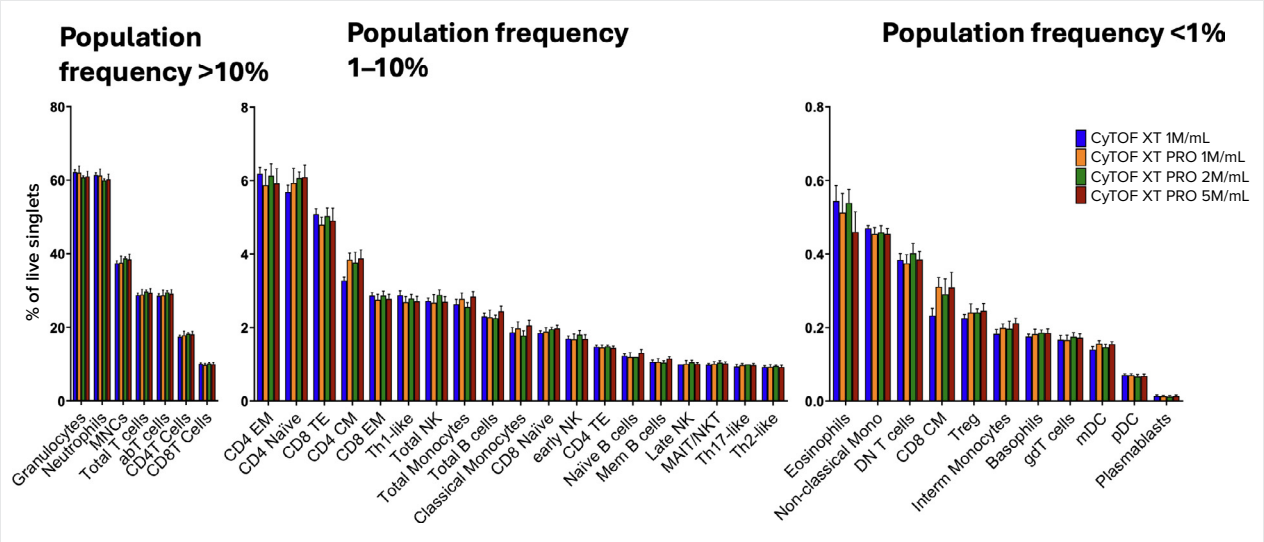


Figure 2. Highly reproducible population frequencies spanning 3 orders of magnitude. There are some differences between the CyTOF XT and CyTOF XT PRO system for some rare cell types (for example, CD8 CM), but variability is low within instruments. MNCs = mononuclear cells, abT cells = αβ T cells, CM = central memory, EM = effector memory, TE = terminal effector, mDC = myeloid dendritic cells, pDC = plasmacytoid dendritic cells, gdT cells = γδ T cells

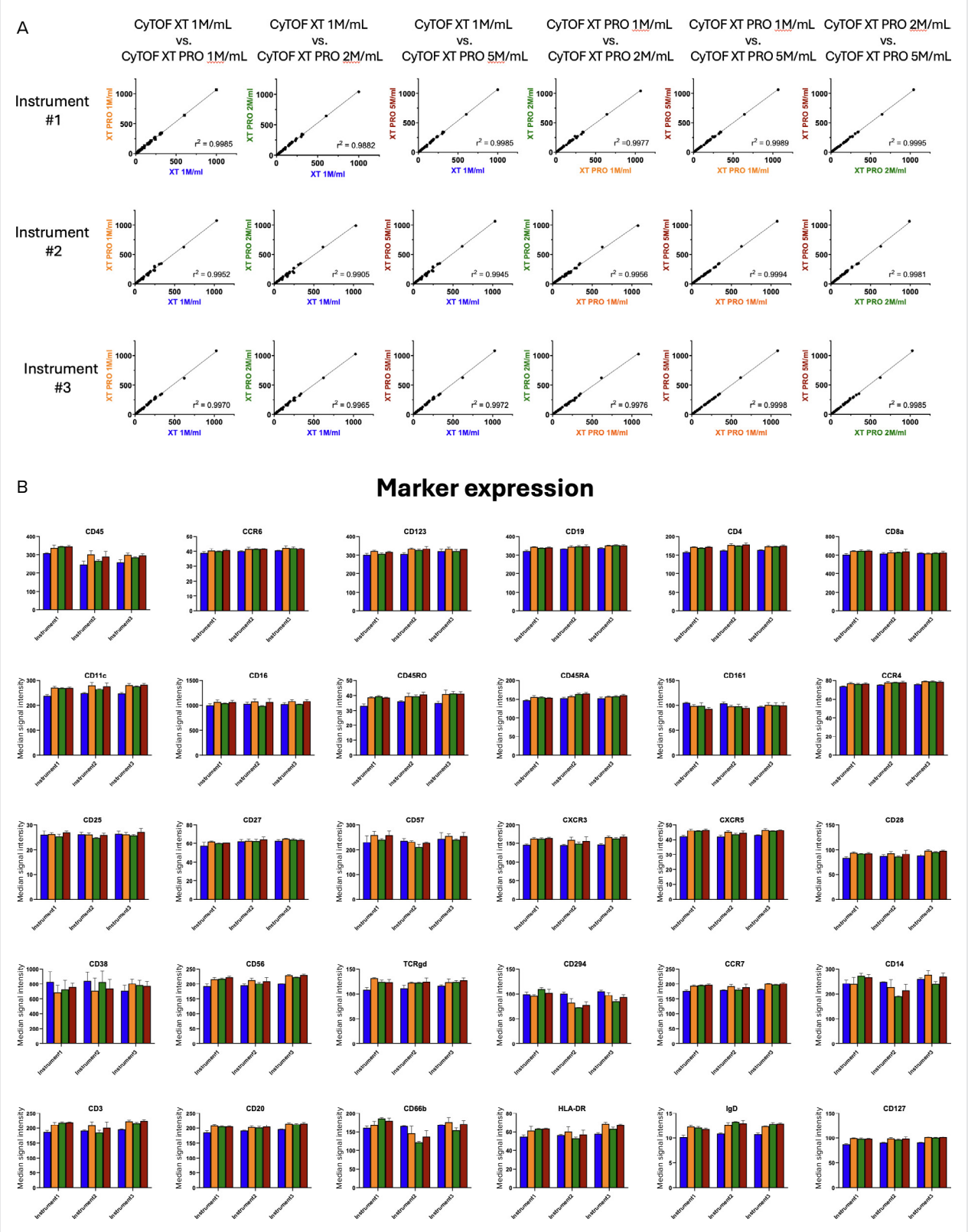


Figure 3. Markers are highly correlated across instruments and sample concentrations. High correlations of marker medians for the 30 markers in the Maxpar Direct Immune Profiling Assay were acquired on different instruments and at different concentrations. A) High correlations (r2>0.99) regardless of instrument configuration or sample concentration. B) Median marker expressions for the 30 markers + SD.

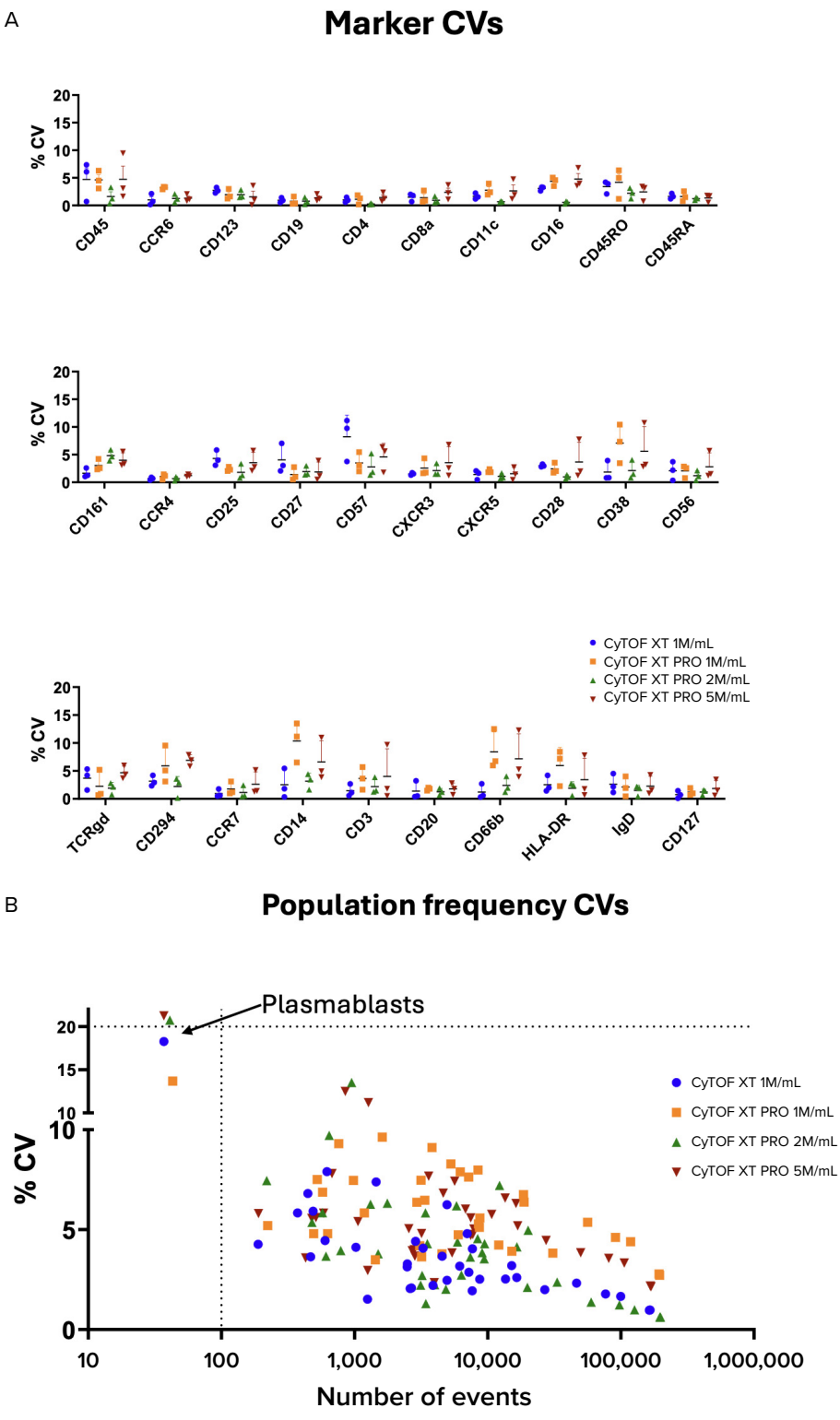


Figure 4. CyTOF XT systems demonstrate robust and reliable performance under enhanced throughput. A) Marker CVs. B) Population frequency CVs were below the 20% threshold for all populations with more than 100 events acquired. Only plasmablasts, sampled at approximately 40 events per sample, had %CVs at or above 20%. CVs decrease with increasing event numbers, as expected.

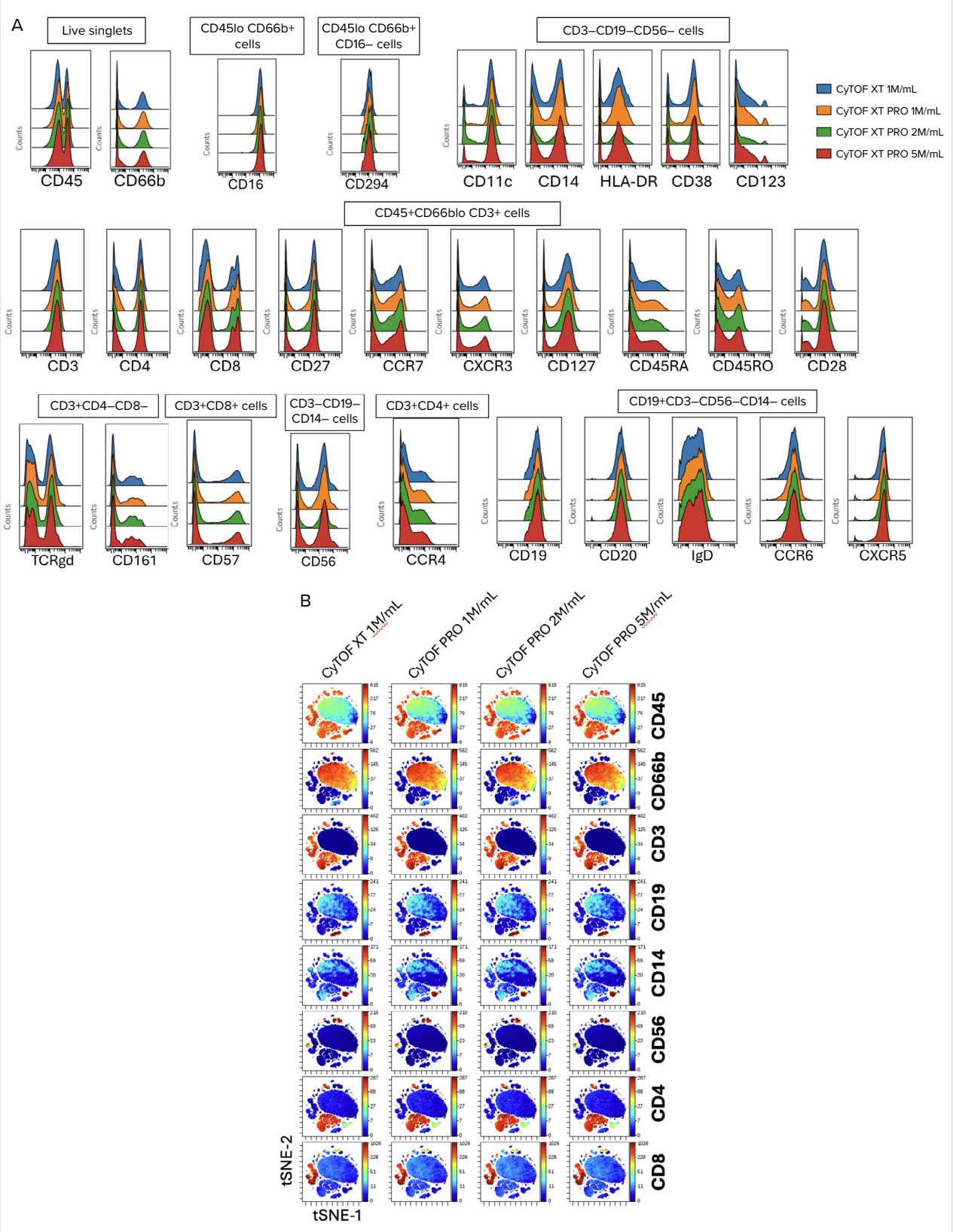


Figure 5. Consistent data quality is shown by highly similar signal intensities for each marker tested. A) Marker histogram overlays and median marker expressions. A) Representative histograms of 29 out of 30 markers in the Maxpar Direct Immune Profiling Assay with greater than 100 cells for the defined cell types. B) Lineage marker expression on t-SNE plots indicates extremely similar results regardless of instrument configuration or sample concentration (up to 5M/mL).

Conclusions

The CyTOF XT PRO system represents a significant advancement in mass cytometry technology, offering enhanced sample throughput with remarkable data quality. The improvements in injector design and event processing have enabled higher event rates without compromising data integrity, making the CyTOF XT PRO system a valuable tool for high-throughput applications. The reproducibility and repeatability of data obtained with the CyTOF XT PRO system further underscore its reliability and robustness. This high level of reproducibility is crucial for reliable data interpretation and downstream analyses essential for making informed decisions in pharmaceutical and clinical research.

These findings suggest that the CyTOF XT PRO system can effectively address the challenges associated with traditional cytometry techniques, providing researchers with a powerful platform for comprehensive immune profiling and other high-dimensional analyses.

Tips for success

- Cell viability, presence of debris and sample integrity can all impact data quality
- Accurate cell counts are important. Cell counting performed just prior to acquisition will yield more accurate estimates of event rate and cell recovery.
- Stickier, clog-prone samples might need to be acquired at lower cell concentrations

References

1. Maxpar Direct Immune Profiling Assay Cell Staining and Data Acquisition User Guide (400286)
2. Impact of Cryopreservation on Performance of the Maxpar Direct Immune Profiling System Application Note (FLDM-00089)
3. Zimmerlin, L. et al. “Rare event detection and analysis in flow cytometry: bone marrow mesenchymal stem cells, breast cancer stem/progenitor cells in malignant effusions, and pericytes in disaggregated adipose tissue.” *Methods in Molecular Biology* 699 (2011): 251–273.

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Rapid and Reproducible Results on the CyTOF XT PRO Technical Note

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