Enabling Sensitivity, Repeatability and Reproducibility Required for Immuno-Oncology Research on the CyTOF XT PRO Mass Cytometer

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CyTOF™ XT PRO

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Introduction

	CyTOF XT PRO	CyTOF XT
21 CFR Part 11 compliance-enabling software to meet relevant requirements	✓	
Enhanced throughput mode Up to 4x faster than CyTOF XT.	✓	
Autosampler Module Automated sample handling with up to 13 tubes that can be loaded into the carousel for unattended acquisition. Additional samples can be added on the fly as space is freed up.	✓	✓
CyTOF Software v9.2 Includes major feature upgrades that increase system automation and data analysis. Key enhancements include support for custom de-barcoding to advance downstream analysis of sample multiplexing and automated sample dilution to improve hands-free operation.	✓	✓
Tissue imager–compatible Can be purchased as a single system or retrofitted later.	✓	✓

CyTOF™ technology is a powerful cytometry platform with proven sensitivity, dynamic range and resolution to capture 50-plus targets simultaneously, including cell surface markers, signaling proteins and cytokines. Powered by mass cytometry, the use of metal-conjugated antibodies overcomes spectral spillover limitations associated with fluorescence flow cytometry – enabling precise detection of phenotypic and functional variation in single cells.

Designed with clinical research in mind, the CyTOF XT PRO system is a next-generation instrument that enables up to 4x enhanced throughput over the CyTOF XT system, has integrated compliance-enabling software, permits increased sample multiplexing and has improved walk-away capabilities.

In this study, human whole blood and PBMC samples were stained with antibody panels containing up to 50 surface and cytoplasmic targets. Stained samples were frozen and acquired on a later date using CyTOF XT PRO and CyTOF XT systems to assess repeatability and reproducibility, and to ensure data quality was not compromised when samples were acquired at 4x the speed with the CyTOF XT PRO system.

Key takeaways

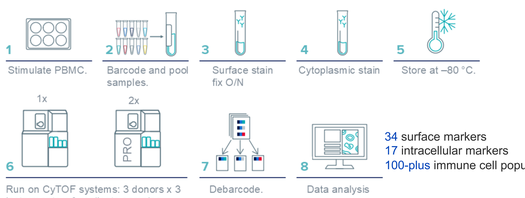
- CyTOF XT and CyTOF XT PRO systems enabled equivalent results and exceptional resolution for accurate and precise interpretation of major immune subsets to unveil striking functional diversity across the immune landscape
- The CyTOF XT PRO system produced high sensitivity and dynamic range for both highly expressed and low-abundance markers at enhanced throughput
- Cell population frequency and staining intensity from both CyTOF systems were repeatable and reproducible across multiple instruments, donors and replicates
- The CyTOF XT PRO system enables enhanced sample throughput without compromising data quality for both deep immunophenotyping and functional profiling

Methods and materials

Deep Immunophenotyping Assay

30 surface markers
37 immune cell populations

Immune Checkpoint and Cytokine Functional Profiling Assay



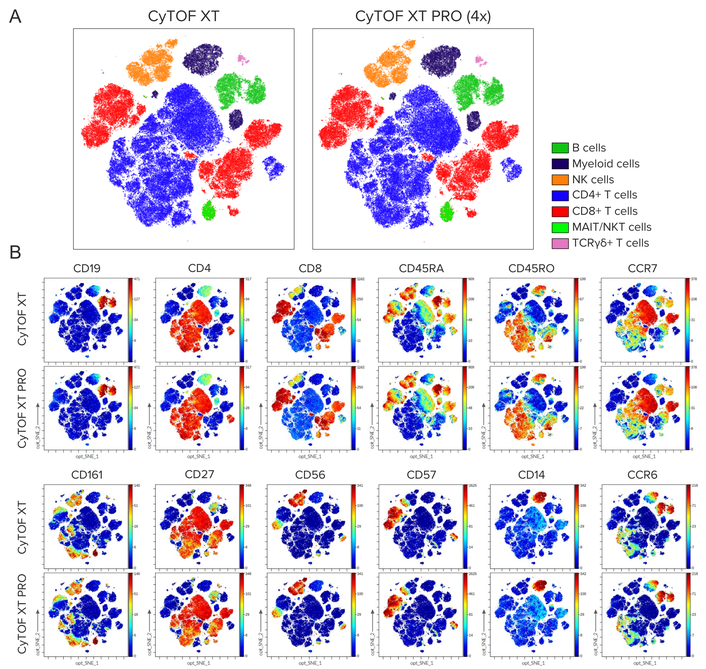
34 surface markers
17 intracellular markers
100-plus immune cell populations

Figure 1. Experimental design. Deep Immunophenotyping Assay: Whole blood from a healthy donor was stained according to the Maxpar™ Direct Immune Profiling Assay Cell Staining and Data Acquisition User Guide (400286), then frozen and stored at –80 °C. Samples were thawed, washed, counted and acquired in triplicate on three instruments in either CyTOF XT or CyTOF XT PRO configurations. Samples were run on the CyTOF XT system at 1x acquisition speed, while samples run on the CyTOF XT PRO system were acquired at 1x, 2x and 4x speeds. **Immune and Cytokine Functional Profiling Assay:** PBMC from three healthy donors were thawed and stimulated overnight with phytohaemagglutinin (PHA) and lipopolysaccharide (LPS) in triplicate. Eighteen hours post stimulation, cells were incubated with phorbol 12-myristate 13-acetate (PMA), ionomycin, brefeldin A and monensin for four hours. Live-cell universal barcoding using a tellurium-based 7-choose-3 strategy was carried out for batch staining. Pooled samples were stained with surface and cytoplasmic antibodies according to the Maxpar Cell Staining with Fresh Fix User Guide (FLDM-01319) and stored at –80 °C. The barcoded sample was thawed, washed, counted and split evenly across the three CyTOF XT and three CyTOF XT PRO instruments for acquisition. Samples were run on the CyTOF XT system at 1x acquisition speed while samples run on the CyTOF XT PRO system were acquired at 2x speeds. Data normalization and debarcoding were performed using CyTOF Software (v9.2). Data analysis was carried out using CytoBank (Beckman Coulter) and CellEngine (CellCarta) for both assays. The full panel list and purchase information can be downloaded using the QR code below.

Results

Deep Immunophenotyping Assay

High-dimensional visualization of data acquired on both CyTOF XT and CyTOF XT PRO instruments revealed equivalent and exceptional resolution of cellular islands in opt-SNE space.



Immune Checkpoint and Cytokine Functional Profiling Assay

Comprehensive cross-lineage immuno-functional diversity captured by CyTOF XT and CyTOF XT PRO instruments

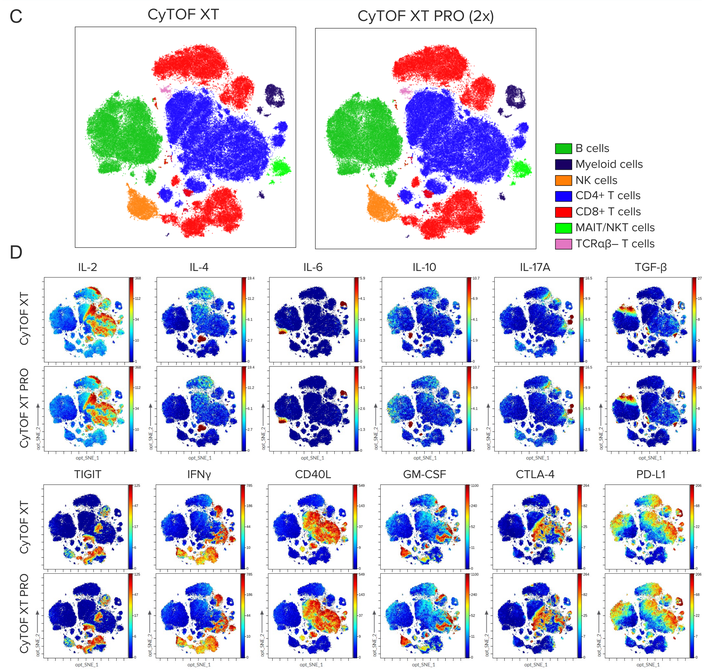


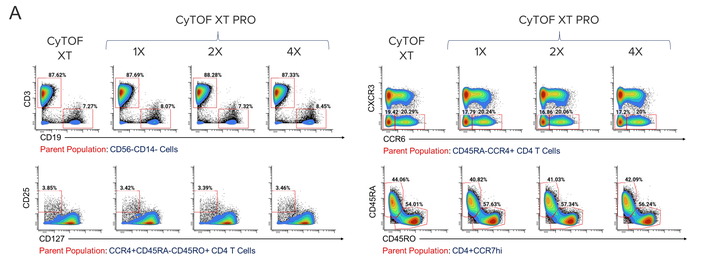
Figure 2. Enhanced throughput of the CyTOF XT PRO system retains clear visualization of major immune subsets and striking functional diversity in high-dimensional space.

A–B) High-dimensional analysis from the Deep Immunophenotyping Assay. An equal number of live singlet lymphocyte events acquired on one representative CyTOF XT system (1x) and one CyTOF XT PRO system at highest throughout (4x) were projected into opt-SNE space. A) Overlay of manually gated cell populations revealed excellent separation of major lineage populations including B cells, myeloid cells, NK cells and T cell subsets. B) Heat maps of signal intensities from select surface markers illustrating relative expression across the immune landscape, highlighting major lineage markers (for example, CD19), memory phenotypes (for example, CD45RA vs. CD45RO) and rare subpopulations (for example, CD161hi MAIT/NKT cells). **C–D) High-dimensional analysis from the Immune Checkpoint and Cytokine Functional Profiling Assay**. An equal number of live singlet lymphocyte events from one representative stimulated donor sample, collected on one representative CyTOF XT system and one CyTOF XT PRO system (2x) were projected into opt-SNE space. C) Overlay of manually gated cell populations including B cells, myeloid cells, NK cells and T cell subsets. D) Visualization of immune cell activation and functional signatures. Heat maps of signal intensities from select functional markers illustrating relative expression of immune checkpoints and cytokines. Both highly expressed (for example, IL-2) and rare cytokines (for example, IL-10) were identified. With over 20 functional markers in this panel, including 17 intracellular targets, an unprecedented level of functional immunology can be explored.

Importantly, dimensionality reduction and clustering of both CyTOF XT and CyTOF XT PRO datasets resulted in equivalent opt-SNE maps, indicating similar results were obtained, regardless of the panel, assay, instrument configuration or sample concentration. Furthermore, comparable results were observed for all instruments and technical replicates across this study, indicating robust reproducibility and repeatability of this high-dimensional dataset.

The improvements in hardware 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 functional immunology.

The CyTOF XT PRO system produces high sensitivity and dynamic range for both highly expressed and low-abundance markers at enhanced throughput.



Increased throughput on the CyTOF XT PRO system retains exceptional resolution of intracellular markers.

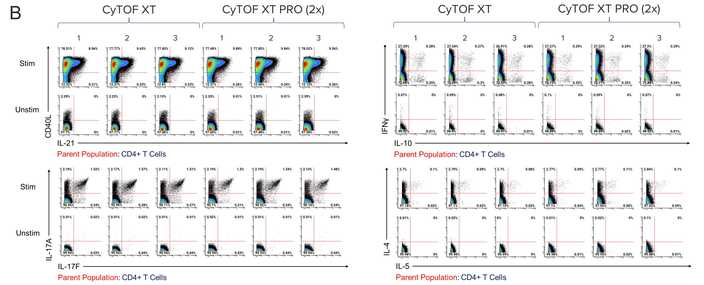


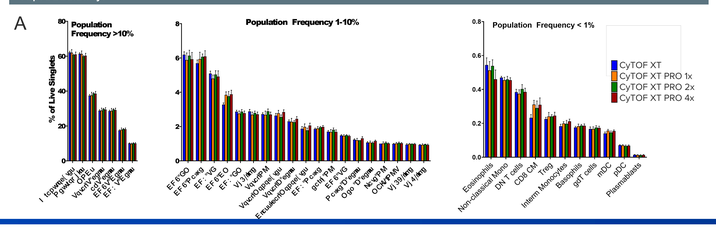
Figure 3. The CyTOF XT PRO system produced high sensitivity and dynamic range for both highly expressed and low-abundance markers at enhanced throughput.

A) Bivariate analysis from the Deep Immunophenotyping Assay. Select bivariate plots were generated from data acquired on one representative CyTOF XT system and one CyTOF XT PRO system at 1x, 2x and 4x speeds. **B) Bivariate analysis from the Immune Checkpoint and Cytokine Functional Profiling Assay.** Select bivariate plots were generated from one representative donor, including matched stimulated and unstimulated samples. Data from all six instruments is shown. Excellent signal to noise enables the detection of polyfunctional T cells, expressing multiple cytokines (double-positive gates).

Gate labels display percent of parent frequency within the drawn gate. Parent populations are defined below each bivariate plot.

Notably, similar frequencies for both high- and low-abundance markers were observed across all samples, instruments and acquisition speeds, further demonstrating comparable and repeatable results for both CyTOF instruments.

CyTOF XT and CyTOF XT PRO instruments generate equivalent population frequencies with high repeatability and reproducibility.



CyTOF XT and CyTOF XT PRO instruments show equivalent frequencies of T cells expressing immune checkpoint markers and intracellular cytokines across multiple instruments.

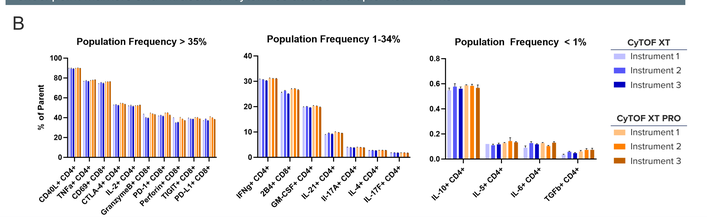


Figure 4. Both CyTOF XT and CyTOF XT PRO instruments produce highly repeatable and reproducible results in either cell phenotyping or functional profiling assays.

A) Population frequencies from 37 immune cell populations identified using the Deep Immunophenotyping Assay. Mean values of population frequencies are depicted in this bar plot, color coded by instrument and sample throughput. Error bars show the standard deviation between the technical replicates collected. **B) Population frequencies from select populations identified using the Immune Checkpoint and Cytokine Functional Profiling Assay, representing 21 functional readouts on CD4+ or CD8+ T cells.** Mean values of population frequencies from one representative stimulated donor are depicted in this bar plot, color coded by instrument. Error bars show standard deviation between the technical replicates collected.

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