CyTOF XT

The Next Generation of Mass Cytometry

Introduction

CyTOF® Technology. Mass cytometry is a powerful technology that utilizes a time-of-flight mass spectrometer to enable the detection of single cells tagged with isotopically pure metal-labeled reagents. CyTOF overcomes the limitations of fluorescence-based detection systems by separating signals according to differences in isotope mass instead of wavelength. The negligible signal overlap between masses allows for simultaneous detection of over 50 targets in a single sample tube, a panel size that has not been achieved by any flow or spectral cytometer to date. The use of a universal internal standard—multi-element calibration beads—makes it possible to normalize data across instruments and experiments. Mass cytometry thus generates the highest-parameter snapshot of phenotype and function for every cell.

CyTOF Value. Mass cytometers provide impressively high resolution of cytometric profiles, empowering both basic research and practical biomarker-driven clinical research to potentially optimize and personalize disease management. CyTOF instruments have proven to be valuable additions to cytometry core facilities and service labs, and have been adopted for use by key clinical research consortia[1,2,3] and in scores of Clinical Research Trials (see Related documents).

The NeXT Generation. Fluidigm now introduces a new generation of mass cytometer, the CyTOF XT™, (Figure 1). The novel design, fully automated sample acquisition, and easier operational workflows of CyTOF XT simplify the planning and execution of high-parameter cell profiling studies.

Objectives

This application note presents information on key CyTOF XT features demonstrating the following advances in instrument benefits and performance:

- Fully automated acquisition with the new Autosampler to increase productivity and sample throughput with negligible carryover
- Unique system design and software logic to sense and remove clogs
- Automated detector voltage (DV) optimization to maintain signal stability during extended acquisitions
- High degree of agreement between data collected on CyTOF XT and Helios™ instruments
- An array of data management improvements, including on-the-fly normalization and processing, the latest industry file format (FCS 3.1), optimized storage requirements, a range of troubleshooting options, and many more

Figure 1. CyTOF XT, featuring a streamlined design and automatic sample acquisition.

CyTOF XT enables walk-away sample acquisition for increased productivity

Improving on previous CyTOF instruments that required manual loading of each sample before acquisition. CyTOF XT revolutionizes sample delivery with the Autosampler Module and a simplified front end assembly. The new Autosampler consists of 4 major components: the sample probe, a syringe-based pump unit, a tray for acquisition and cleaning solutions, and most important, a carousel that holds 13 sample tubes chilled at 4–8 °C. The Autosampler enables automated sample delivery over long acquisitions while maintaining sample integrity.

The CyTOF XT with the Autosampler Module automates the following processes:

- Tuning the instrument
- Cleaning the sample fluidics
- Acquisition of samples already in suspension
- Resuspension, addition of EQ™ Calibration Beads, and acquisition of pelleted samples
- Detection and removal of clogs

This combination of features greatly reduces the need for operator interaction with the instrument during sample acquisition. Hands-on interaction is required only to load calibration beads and set up samples in the carousel (Figure 2). In addition, CyTOF Software v8.0 for CyTOF XT performs data normalization during sample acquisition, which further frees up operator time while the data file is processed automatically.

Routine workday comparison

Although many workflow steps for Helios and CyTOF XT are similar, there are several key differences. Figure 2 highlights the significant time savings and streamlined workflow of a model workday using CyTOF XT as compared to a workday with Helios. In this example (Figure 2A), 10 whole blood samples stained with the Maxpar® Direct™ Immune Profiling Assay™ (Cat. No. 201325) are acquired in a typical 8-hour workday. Highlighting the instrument's advancement in extended acquisition, Figure 2B demonstrates the ability of CyTOF XT to acquire 42 samples within a 23-hour period. The following sections describe these example workdays on Helios and CyTOF XT in greater detail.

Helios is optimized for single-tube data acquisition.

After the plasma is ignited and the Helios system is warmed up, tuning solution must be loaded manually, followed by instrument tuning, a bead sensitivity test, and conditioning of the plasma with Maxpar Cell Acquisition Solution (CAS, Cat. No. 201240). The first sample may then be loaded into the Sample Loader for acquisition according to the sample criteria selected. In the workday example (Figure 2A, Helios), purple time bars between samples indicate the manual handling steps, such as sample preparation (resuspension and filtering), data normalization, and cleaning between samples with CyTOF Washing Solution (Cat. No. 201071) and CAS. In this example, user intervention is required for routine instrument cleaning and shutdown procedures at the end of a workday. The Helios acquisition of these 10 samples with monitoring requires approximately 7 hr 15 min. (Figure 2A, Helios).

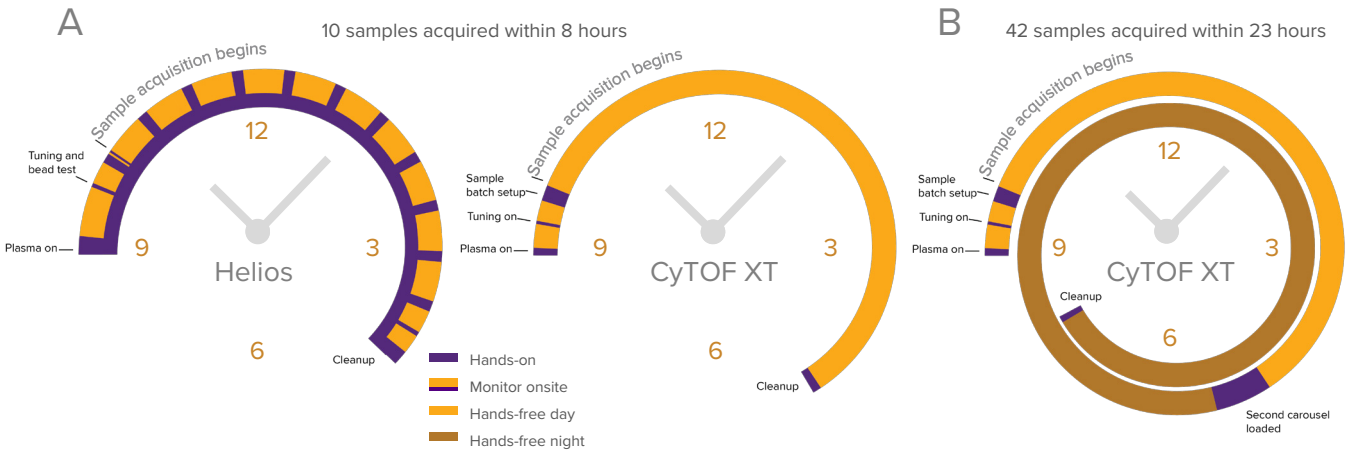


Figure 2. Comparison of a model workday on Helios and CyTOF XT. A) Representation of Helios (left) and CyTOF XT (right) acquisition of 10 tubes of whole blood samples stained with the Maxpar Direct Immune Profiling Assay. Each purple block represents hands-on time when interaction with the instrument is required. Orange blocks represent hands-free automated periods. **B)** Representation of an extended CyTOF XT acquisition when, upon completion of the first 10 samples, the carousel is reloaded with a new batch of 12 samples stained with the Maxpar Direct Immune Profiling Assay and 1 large-volume (12 mL) tube of a 20-plex barcoded sample for unattended acquisition. This model example illustrates how 42 samples could be acquired on the CyTOF XT within a 23-hour period.

CyTOF XT reduces hands-on instrument time and increases sample throughput.

CyTOF XT workflow steps are similar to those for Helios, but most are automated. An operator only needs to load the bottle tray with required solutions (including an improved high-ionic-strength solution, Maxpar Cell Acquisition Solution Plus for CyTOF XT, Cat. No. 201244), ignite and warm up plasma, start the automated tuning protocol, load the carousel with samples, select acquisition criteria, and walk away while the system does the rest (Figure 2A, CyTOF XT).

The CyTOF XT automatically adds EQ beads, resuspends pelleted samples and mixes prior to acquisition, performs washes, notifies the operator upon successful completion of a batch, normalizes and saves files, completes extended cleaning after batch acquisition, and shuts down plasma at the end of a workday. User attention is needed only for nebulizer maintenance cleaning after the instrument shuts itself down. The carousel is accessible during batch acquisition, allowing an operator to add new samples or replace the acquired tubes with a new batch of samples for continuous acquisition. Finally, the Autosampler carousel is cooled and kept at 4–8 °C, maintaining sample integrity for many hours and ensuring high quality data.

In contrast to Helios, the automated sample acquisition on CyTOF XT (Figure 2A, orange time bar) frees up operator time while instrument automation and programming do the work. Acquisition of the 10 assay tubes occurs unattended for 7 hr 50 min on the CyTOF XT, enabling the operator to perform other activities through the day.

CyTOF XT extends the workday with walk-away automation.

CyTOF XT provides an opportunity to go beyond the 8-hour workday with sample acquisition for an overnight run. Figure 2B illustrates how the Autosampler and CyTOF Software v8.0 for CyTOF XT enable increased sample throughput. An additional 12 samples stained with the Maxpar Direct Immune Profiling Assay plus a 20-plex barcoded sample may be run unattended overnight after a standard 8-hour workday on CyTOF XT, bringing the total number of samples run to 42.

Batch sample acquisition is possible because the Autosampler applies stringent washing conditions to ensure consistent data collection and reduce sample carryover, while detecting and resolving clogs during acquisition. Refer to the next sections for more about carryover tests and CyTOF XT unclogging capabilities.

CyTOF XT delivers sample batch acquisitions without sample carryover

The walk-away sample preparation and acquisition enabled by CyTOF XT is achieved through the sample handling advancements of the Autosampler. Sample carryover is a concern for any sample introduction system. This section describes the built-in cleaning of sample probe and Autosampler lines, which minimizes carryover.

CyTOF XT is designed to perform automated washes of the sample line fluidics. Both the inside and outside of the sample probe are washed between samples. Different pre-wash settings can be applied to avoid carryover depending on the sample type: Light, Medium (default setting), and Heavy, which vary in washing time, pulsing, and/or solutions used. For more information, refer to the CyTOF Software v8.0 Help for CyTOF XT (FLDM-00045), also available as the software integrated help guide.

To demonstrate the absence of unwanted sample carryover, the following test was performed.

Sample carryover study design

Two separate sets of human peripheral blood mononuclear cell (PBMC) samples were barcoded using 2 different barcoding workflows: Cell-ID™ 20-Plex Pd Barcoding Kit (Cat. No. 201060) for palladium (Pd) barcoding up to 20 samples, and 7 individual cadmium (Cd)-labeled anti-CD45 antibodies from Fluidigm for 35-plex live-cell barcoding. Barcoding uniquely labels multiple individual samples with a combination of Pd or Cd isotopes, allowing the samples to be pooled together in 1 tube for staining and acquisition. Individual samples can then be identified based on their unique combination of Pd or Cd isotopes. (To learn more about barcoding options, refer to The Benefits of Palladium Barcoding on Data Quality and Workflow (FLDM-00012) and Enabling Live-Cell Barcoding with Anti-CD45 Antibodies in Suspension Mass Cytometry (FLDM-00488) available at fluidigm.com.)

Replicates from each barcoded and pelleted sample were loaded into the CyTOF XT carousel in quadruplicate, in alternating order of Pd- and Cd-barcoded samples. The default pre-wash setting was applied before each sample. The number of all collected events per tube was approximately 2×10^6 for Pd-barcoded samples and 1.5×10^6 for Cd-barcoded samples.

Sample carryover test results and summary

The number of Pd-barcoded events carried over to Cd-barcoded samples was negligible. Fewer than 10 events

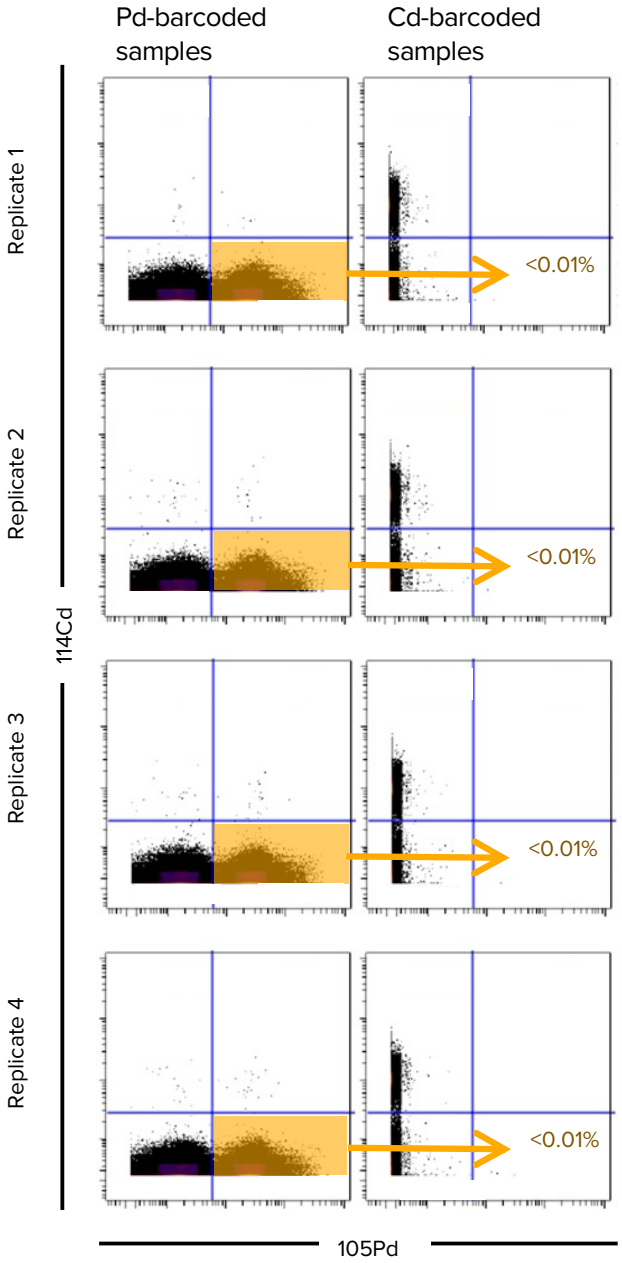


Figure 3. Negligible Pd-barcoded sample carryover observed in subsequent Cd-barcoded samples. A set of 8 Pd- and Cd-barcoded samples was run in quadruplicate in alternating order to assess the carryover of Pd-barcoded cells to the next Cd-barcoded sample. Default pre-wash settings were applied. Biaxial plots of ^{105}Pd vs. ^{114}Cd show Cd-positive and Pd-positive populations. Samples were gated on live singlet cell events identified using the cleanup strategy described in Approach to Bivariate Analysis of Data Acquired Using the Maxpar Direct Immune Profiling Assay (400248). The percentage of Pd-barcoded sample carryover is shown in the adjacent Cd-barcoded sample.

out of all 600,000 Pd+ live events, or <0.01% carryover, were observed (Figure 3). The results demonstrate that the Autosampler has effective washing between samples when default settings are used on the CyTOF XT. The automated pre-wash settings can be customized for more stringent cleaning if required for samples that are anticipated to be challenging to maintain in single-cell suspension. Following the clog prevention recommendations outlined in the next section on challenging sample handling will also minimize carryover.

CyTOF XT is uniquely designed to automatically detect and resolve clogs

Clogging can disrupt and delay cytometry instrument workflow. Helios and CyTOF XT rely on different strategies to manage and unclog the sample fluidics.

Clog management on Helios and CyTOF XT

For challenging samples on the Helios system, a clog becomes apparent when the sample flow rate drops from 30 µL/min to 0 µL/min. The operator must then perform a manual unclogging procedure.

In contrast to Helios, the CyTOF XT Autosampler software detects potential clogs by monitoring whether pressure and flow parameters fluctuate beyond set thresholds. Upon detecting a blockage, the software executes a routine that mimics the manual unclogging procedure typically performed by an operator. To best demonstrate the improvements made to clog management and resolution on CyTOF XT, a dissociated tissue sample test was performed.

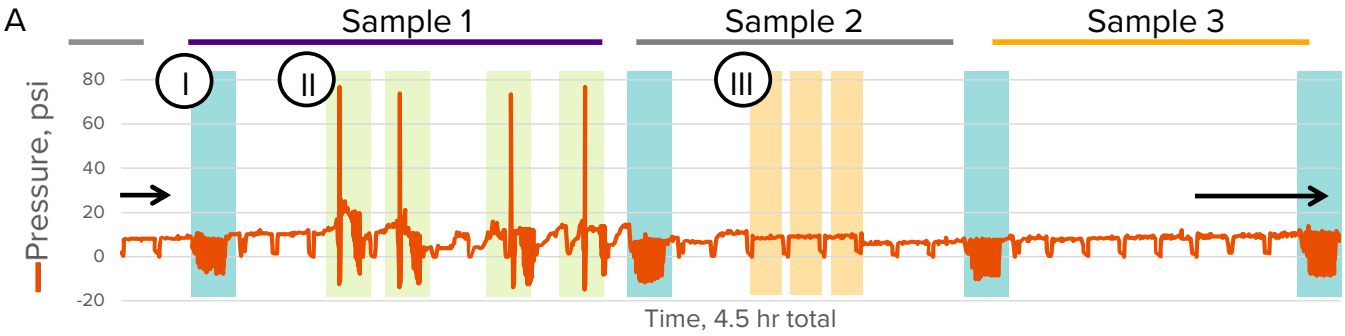
Dissociated tissue sample study design for CyTOF XT

Multiple replicates of cells from dissociated mouse intestinal tissue were run for several hours on a CyTOF XT at the optimized concentration of 0.5 x 10^6 cells/mL. Dissociated cells from solid tissue were chosen because this sample type is known to be more difficult to maintain as a single-cell suspension. Dissociated mouse tissue samples were stained according to Maxpar Cell Surface Staining with Fresh Fix Protocol (400276) and prepared using validated workflows, which include filtering through 35 µm cell strainers before sample acquisition. A batch of 12 dissociated mouse intestinal cell samples in pelleted format was loaded into the carousel and a set volume was acquired. The pressure in the sample introduction line was monitored and recorded during sample acquisition. Sample pressure logs and data quality were analyzed upon completion of the run.

Dissociated tissue sample test results and summary

Figure 4A illustrates the pressure changes during the acquisition of the mouse intestinal cell samples using the default unclogging parameters on CyTOF XT. All samples were acquired successfully. As shown in Figure 4A, Sample 1 triggered the automated unclogging routine 4 times while Samples 2 and 3 were acquired without interruptions. Note that despite the occurrence of multiple clogs during the acquisition, marker signal intensity was not affected by the automated unclogging protocol as demonstrated by comparing Sample 1 to Sample 2 and Sample 3 (Figure 4B). Additionally, only approximately 14% of the expected 700,000 events were lost due to the 4 clogs in Sample 1 (Figure 4C). More important, the automated unclogging routine extended the acquisition by only 30 minutes and completed a batch of samples without need for manual intervention.

CyTOF XT is uniquely designed to detect and automatically resolve clogs. The combination of automated features (sample acquisition, unclogging, and instrument shutdown), makes CyTOF the most productive high-parameter cytometer on the market.



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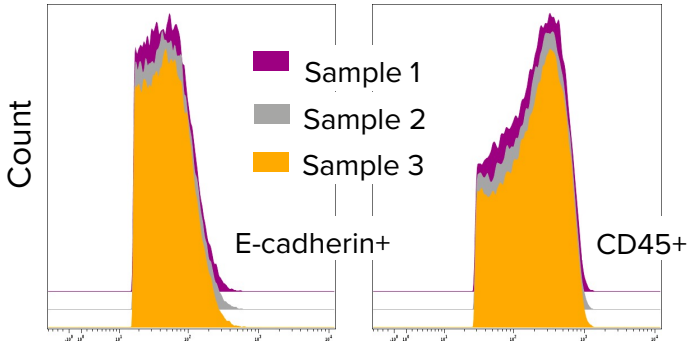

Median signal intensity	E-cadherin+	CD45+
Sample 1	49.068	206.416
Sample 2	49.518	207.846
Sample 3	49.895	210.411

C

	Sample 1	Sample 2	Sample 3
Events found	599,673	693,124	719,634
Bead events found	29,457	31,540	32,538
Time	1 hr 56 min	1 hr 29 min	1 hr 29 min
Clogs found	4	0	0
Clogs resolved	4	n/a	n/a

Figure 4. Automated unclogging by CyTOF XT resolves blockages with no impact on data quality. 12 samples (2.5 mL each) were acquired automatically with several occurrences of high pressure due to multiple clogs in single samples. **A)** Pressure monitoring of the 12 samples identified 3 consecutive samples with varying unclogging routine experiences. Section I (light blue) denotes washing steps between samples. During this time, rapid pressure swings are observed as positive to negative pressure readings, which function to remove the remaining sample in the line. Similarly, pressure during the unclogging routine (Section II, light green) fluctuates to effectively remove the clog and enable the continuation of the acquisition. A rhythmic pattern (Section III, light orange) in the pressure readings is seen in the acquisition of Sample 2 and Sample 3. This pattern is caused by the change in pressure detected between single loops of a sample that are loaded into the Autosampler module. The loop accommodates 250 µL of the sample volume and the entire acquisition happens in intervals, hence the rhythmic pressure pattern. **B)** The automatic unclogging routine did not impact signal intensity, as illustrated by the unchanged signal intensity of 2 major populations between all 3 samples. **C)** Summary of collected events and detected clogs.

Tips for success

Recommendations to reduce clogging:

Sample preparation. Wash samples with Maxpar CAS Plus twice immediately prior to loading the carousel.

Sample filtration. All samples should be filtered through appropriately sized cell strainers to prevent cell aggregates and large particles from entering the fluidics. Best practice is not to force the sample through the mesh with a pipette but instead gently pipette the sample through the mesh cap. Avoid using a pipette to aspirate any remaining sample from the inner side of the straining cap.

Cell concentration. Optimize cell concentration based on sample type. Acquire potentially challenging samples at 0.5 x 10^6 cells/mL or less.

Pre-wash settings. Select a heavy pre-wash cycle for challenging samples.

Acquisition volume. Split large volume samples into smaller volume acquisitions over multiple tubes.

Regular instrument maintenance is key. Clean and maintain parts and fluidics as per Fluidigm recommendation. For more detail, refer to the CyTOF XT User Guide (FLDM-00254) and the CyTOF Software v8.0 Help for CyTOF XT (FLDM-00045), also available as the software integrated help guide.

Detailed recommendations for reducing clogs can also be found in the CyTOF Software v8.0 Help for CyTOF XT (FLDM-00045).

CyTOF XT delivers optimal data quality over extended acquisitions.

In some cases, a typical workday of 8 hours may not be sufficient to fulfill the requirements of the experiment, as when running samples for a large study. Previous sections demonstrated that the CyTOF XT is uniquely designed to accommodate long unattended runs and can successfully resolve clogs and avoid sample carryover. The following section assesses the CyTOF XT detector's potential to maintain a high level of performance during long hours of ion detection.

A new feature of CyTOF XT is the automatic adjustment of the detector voltage. This ensures signal stability regardless of acquisition time.

Extended acquisition study design

The goal of this study was to confirm that all marker signal intensities were maintained at the same level and did not decrease over time due to any loss of sensitivity by the CyTOF XT detector. Twelve tubes containing various samples were loaded into the carousel for long-term unsupervised acquisition. To assess the impact of time on data quality, the first and last tube of the carousel were loaded with the same sample. This sample was prepared by staining PBMC with surface and nuclear markers according to the Maxpar Nuclear Antigen Staining with Fresh Fix Protocol (400277). The time difference between acquisition of the sample in Run 1 and Run 12 was approximately 19 hours. During this time, the chilled carousel maintained the samples in suspension at 4–8 °C. Test results are presented in Figure 5.

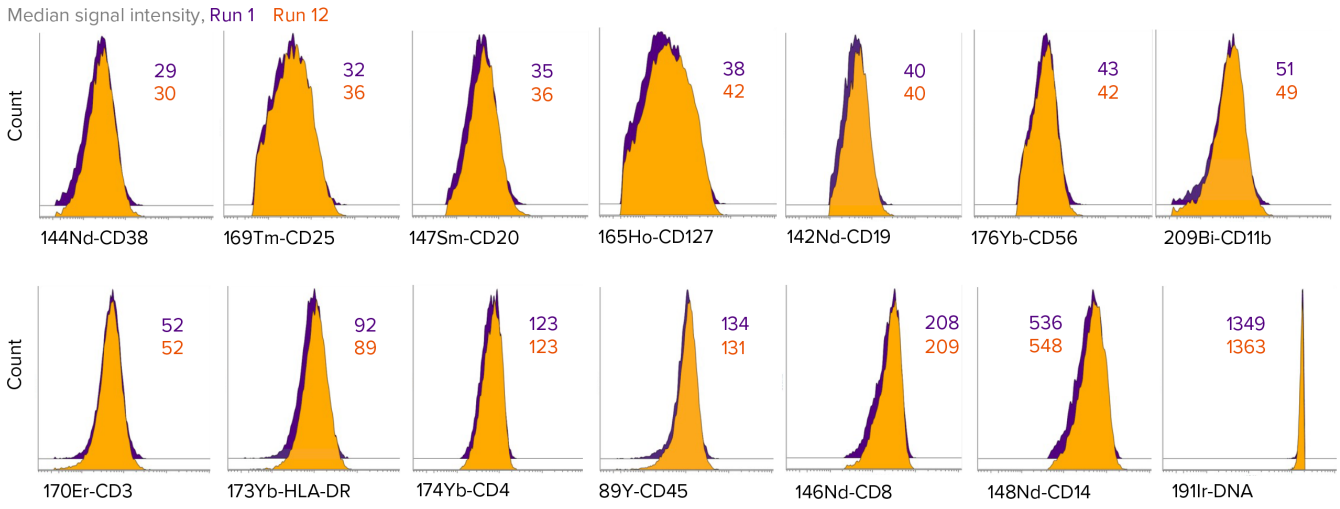

Figure 5. Signal stability over 19 hours of acquisition. 12 tubes of various sample types were continuously collected on CyTOF XT over 19 hours. Run 1 and Run 12 are replicates of the same sample and were acquired first and last, respectively. Run 12 and Run 1 demonstrated the same level of signal. 14 markers are shown as representative data in order of their cell marker signal intensities (low to high). Numbers represent signal intensities (median) over the 19-hour period.

Extended acquisition study results and summary

Fourteen surface markers of low, medium, and high expression range were analyzed. The absolute difference in median signal intensity for each marker was calculated between Run 1 and Run 12. Low expression markers with signal intensity <50 dual counts (CD38, CD25, CD20, CD127, CD19, CD56) showed an average difference of only 4.9%. Medium expression markers (≥50 and 200 dual counts: CD11b, CD3, HLA-DR, CD4, CD45) exhibited an average difference of 1.9%. High expression markers with signal of >200 counts (CD8, CD14, DNA) demonstrated a negligible average difference of 1.2%.

This study demonstrates that the automated detector voltage optimization of CyTOF XT can sustain a constant level of instrument sensitivity during extended runs.

Comparable data performance on Helios and CyTOF XT

Successful completion of extended runs is facilitated by CyTOF Software v8.0 for CyTOF XT, which has been optimized to improve the experience of acquiring samples, data processing, and high-parameter data storage. The enhancements include the following:

- CyTOF XT software automates detector voltage optimization during acquisition, maintaining comparable sensitivity throughout extended acquisitions.
- The software improves upon the built-in sample standardization, using EQ Six Element or EQ Four Element Calibration Beads (Cat. Nos. 201245 and 201078, respectively) to minimize technical variability. The sample normalization algorithm better identifies the EQ calibration beads on the fly, delivering ready-to-analyze data immediately after acquisition.
- CyTOF XT software records a linear mode data (LMD) file, which captures all 135 channels for post-acquisition reprocessing and troubleshooting. Both unprocessed and normalized flow cytometry standard (FCS) files are recorded using FCS 3.1 format. The final output files have been optimized for more efficient use of data storage.

These CyTOF XT software advancements significantly enhance data processing and workflows while maintaining comparable performance between Helios and CyTOF XT. This is illustrated in the section below.

Study design to compare signal intensities and population frequencies on Helios and CyTOF XT

Two independent experiments were performed to demonstrate the comparability of data with regards to signal intensities and cell population frequencies between CyTOF XT and Helios data. Tests were run on CyTOF XT and Helios in parallel using a single donor PBMC sample in Experiment 1 and a single donor whole blood sample in Experiment 2.

Experiment 1: PBMC study design

PBMC from a single donor were stained with the Maxpar Direct Immune Profiling Assay and run in triplicate on both CyTOF XT and Helios in parallel. PBMC sample preparation and acquisition were performed according to recommendations in the Maxpar Direct Immune Profiling Assay Cell Staining and Data Acquisition User Guide (400286), and the Helios User Guide (400250) or the CyTOF Software v8.0 Help for CyTOF XT (FLDM-00045), also available as the software integrated help guide. Data was normalized using the applicable CyTOF Software and analyzed with Maxpar Pathsetter™, a fully automated data analysis solution for samples processed with the Maxpar Direct Immune Profiling Assay. Maxpar Pathsetter generates a report with frequencies of defined populations and their signal intensities. As a comparative analysis, each population was gated manually in Cytobank and compared between the instruments. The manual gating strategies were applied following the technical note: Approach to Bivariate Analysis of Data Acquired Using the Maxpar Direct Immune Profiling Assay (400248).

Experiment 2: Whole blood study design

A test similar to Experiment 1 was performed with whole blood stained with the Maxpar Direct Immune Profiling Assay and run in triplicate on both CyTOF XT and Helios in parallel. Data were normalized and analyzed as described in Experiment 1.

Comparison of signal intensity and population frequency

The automated Maxpar Pathsetter analysis compiled cell classification data from the triplicate PBMC and whole blood datasets acquired on Helios or CyTOF XT. The results of 35 cell populations were graphed in the upper panel of Figures 6 and 7 for PBMC and whole blood samples, respectively. Comparison of population frequencies of data files from Helios and CyTOF XT automatically analyzed in Maxpar Pathsetter demonstrated comparable results for replicate samples between the two instruments (Figures 6 and 7, upper panels). The percent difference was <3.2% and <17.4% for major (>20% of all live events) and minor (<20% of all live events) populations, respectively, in both experiments (Appendix, Tables S1 and S4). The lower panels of Figures 6 and 7 include examples of signal intensities for major populations that were manually gated. The median intensities of positive populations were comparable between Helios and CyTOF XT by manual gating (data not shown).

Using this dataset, an assessment of staining quality for each marker on CyTOF XT and Helios was conducted.

Maxpar Pathsetter performs a staining assessment using a statistical approach called strictly standardized mean difference (SSMD or β). SSMD considers the median and median absolute deviations of both positive and negative populations to assess the resolution of each marker, known as a β value. A higher β value indicates a higher marker resolution that translates to a better separation of positive and negative populations. For the PBMC and whole blood tests, the β value demonstrated a high level of reproducibility across CyTOF XT and Helios data (Appendix, Tables S2 and S6). Furthermore, β values for CyTOF XT data generally exceeded those of Helios.

Deming regression was also used to statistically compare population frequencies, median signal intensities, and β values for both PBMC and whole blood tests performed on Helios and CyTOF XT (Appendix, Figures S1–S4). There was no significant difference between these measurements on each instrument except in the β values of the whole blood tests, which were higher on CyTOF XT. Deming regression analysis found statistically higher B values for samples acquired on CyTOF XT relative to Helios. (Appendix, Figure S4).

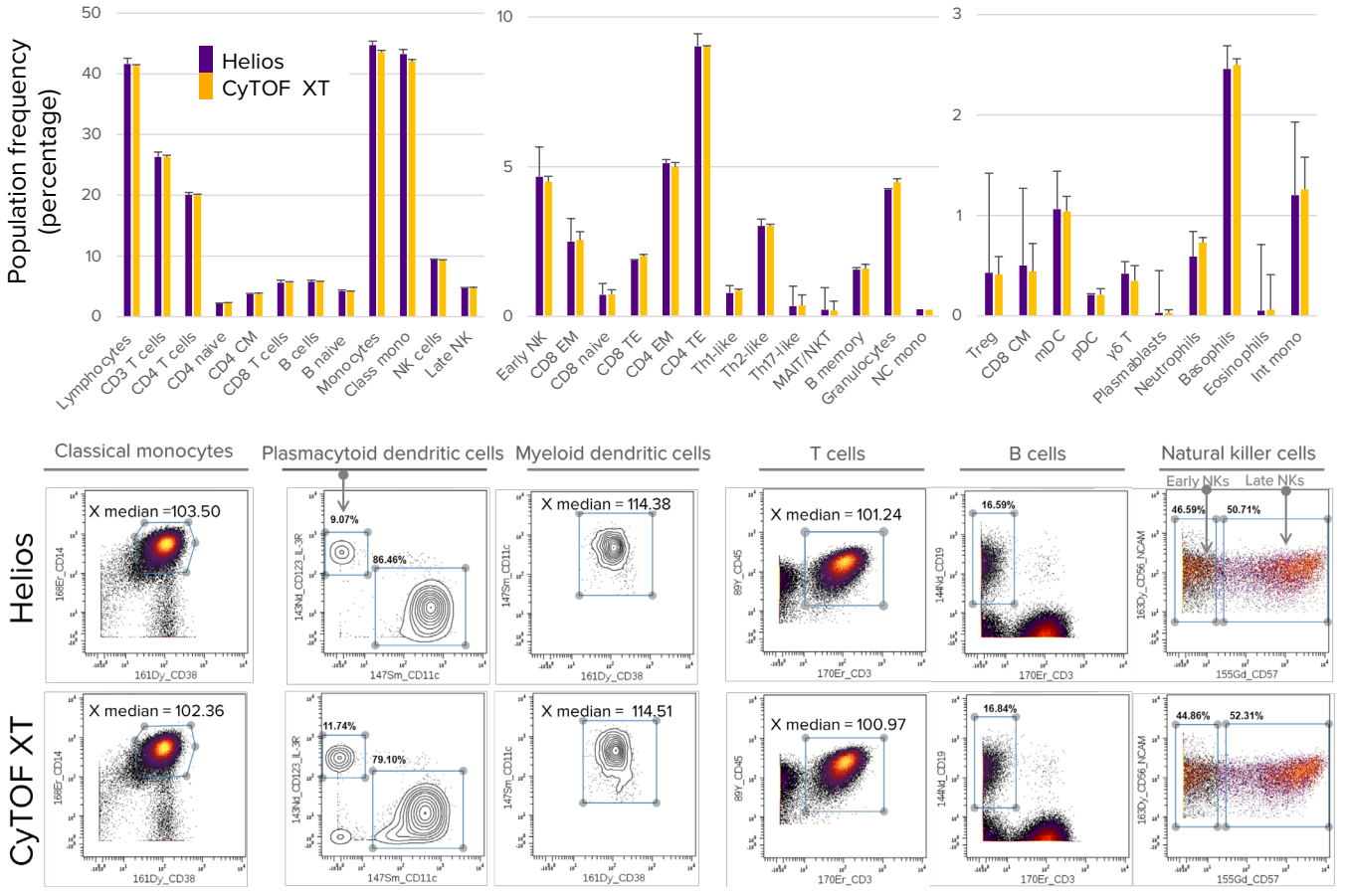

Figure 6. Comparison of PBMC cell population frequencies and signal intensities between Helios and CyTOF XT 6 replicates of PBMC from the same donor were stained with the Maxpar Direct Immune Profiling Assay, pooled together, and split into 6 tubes for acquisition on Helios and CyTOF XT, with 300,000 events acquired per sample. Automated population frequency data obtained from Maxpar Pathsetter analysis is presented in the bar charts (upper panel). Biaxial plots of major populations (lower panel) are used to compare signal intensity (median) or percentage of events as specified in each corresponding plot between Helios and CyTOF XT by manual gating.

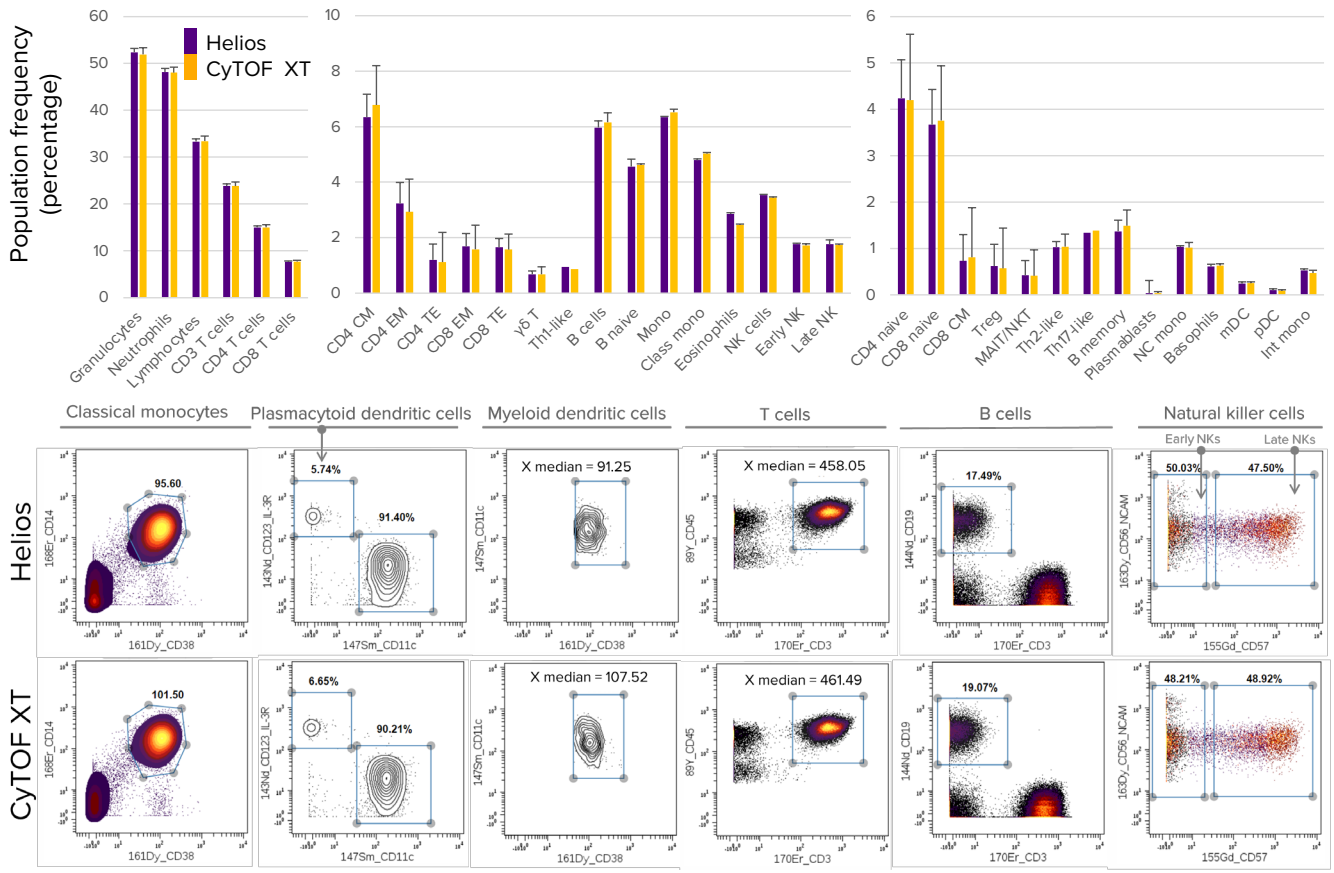

Figure 7. Comparison of stained whole blood cell population frequencies and signal intensities between Helios and CyTOF XT. 6 replicates of whole blood from the same donor were stained with the Maxpar Direct Immune Profiling Assay, pooled together, and split into 6 tubes for acquisition on Helios and CyTOF XT, with 400,000 events acquired per sample. Automated population frequency data obtained from Maxpar Pathsetter analysis is presented in the bar charts (upper panel). Biaxial plots of major populations (lower panel) are used to compare signal intensity (median) and percentage of events as specified in each corresponding plot between Helios and CyTOF XT by manual gating.

CyTOF XT can resolve rare populations.

The high-quality data delivered by CyTOF XT enables the identification of both abundant and rare populations in a sample. An example step-by-step manual gating strategy is shown in Figure 8, demonstrating that 2 replicates of the same sample acquired on both instruments reproducibly resolve markers to identify a desired cell population. The MAIT/NKT CD4– cell population was chosen because it is a rare population that comprises only 0.4% of the total live cell population in whole blood. As shown in the far-right biaxial plot with fixed gates in Figure 8, the same sample acquired on Helios and CyTOF XT yielded a similar number of MAIT/NKT CD4– cells (1,047 and 1,055, respectively) out of 400,000 total events collected.

In this section we showed that comparable performance between Helios and CyTOF XT is demonstrated through the analysis of signal intensities, cell population frequencies, and marker resolution of samples stained with a high parameter-marker panel.

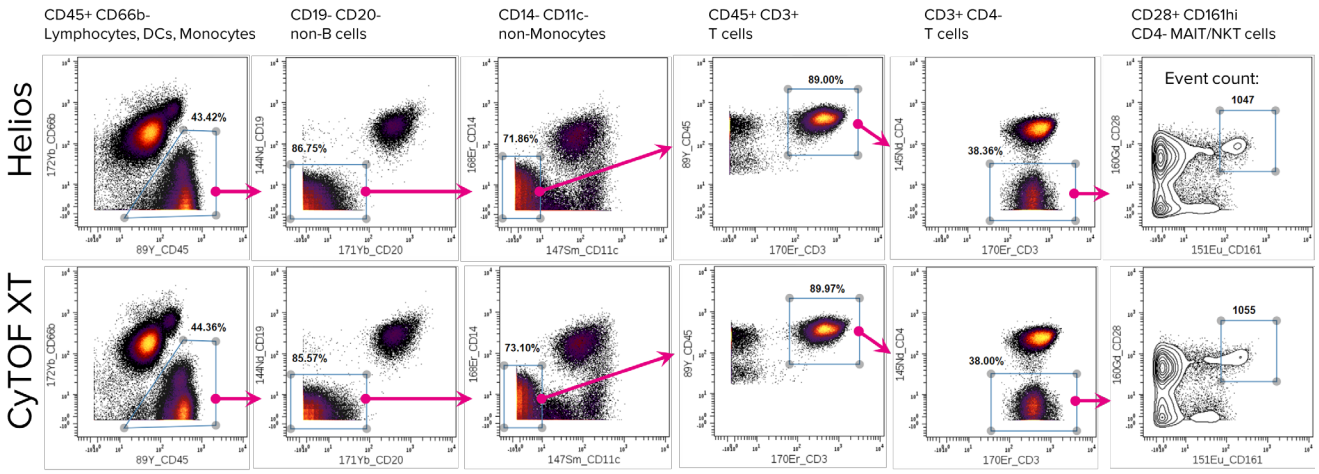

Figure 8. Manual gating strategy of the rare MAIT/NKT CD4– T cell populations, acquired from the same whole blood sample on both Helios and CyTOF XT. An example step-by-step gating strategy for a given rare population after applying the cleanup strategy and gating outlined in Approach to Bivariate Analysis of Data Acquired Using the Maxpar Direct Immune Profiling Assay (400248) is presented. All 35 populations can be identified both manually and automatically using normalized files from both instruments. As shown in the CD28/CD161 gate, the total number of MAIT/NKT CD4– T cells is highly comparable between Helios and CyTOF XT.

Summary

CyTOF XT is a new generation of CyTOF instrument that shares the same reliable level of performance with its predecessor, Helios, but with several key improvements:

- The novel Autosampler design and new CyTOF Software v8.0 for CyTOF XT features automated sample acquisition, EQ bead addition to samples, unclogging, normalization, cleaning, and plasma shutdown. Together, these features improve workflows to reduce hands-on time without impacting data quality.
- Batch acquisition enables up to 13 sample tubes to be loaded into the chilled carousel of the Autosampler module, with the added option to add more samples upon completion of earlier tubes, for added productivity and flexibility.
- An onboard bottle tray and a chilled Autosampler carousel facilitate extended acquisitions.
- Robust automated pre-wash cycles minimize sample carryover.
- Automated detector voltage optimization function helps to maintain consistent signal intensities over the course of extended acquisitions.
- On-the-fly data processing and normalization with the latest FCS format (version FCS 3.1) provides faster time to results with smaller file sizes.

Conclusion

Mass cytometry is widely recognized as a technology that brings a level of multiplexing, precision, and reproducibility to cell analysis not enabled by other single-cell platforms.

CyTOF XT further enhances the capabilities of mass cytometry with workflow automation that refines and simplifies sample processing and data acquisition.

This next generation CyTOF instrument offers a new level of autonomy and reproducibility through streamlined operation, automated system monitoring, and easier system maintenance, making CyTOF XT the superior choice for high-parameter cytometric analysis in clinical and translational research studies.

References

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2 Accelerating Partnership Detailed Research Plan: RA, SLE and Related Autoimmune Disorders Steering Committee. National Institute of Arthritis and Musculoskeletal Diseases.

3 Guo, N., van Unen, V., Ijsselsteijn, M.E. et al. “A 34-marker panel for imaging mass cytometric analysis of human snap-frozen tissue.” *Frontiers in Immunology* 11 (2020): 1466. doi:10.3389/fimmu.2020.01466.

Related documents

Go to fluidigm.com and search for the following related documents.

- Use of CyTOF Technology in Clinical Research Trials Data Sheet
- The Benefits of Palladium Barcoding on Data Quality and Workflow Application Note (FLDM-00012)
- Enabling Live-Cell Barcoding with Anti-CD45 Antibodies in Suspension Mass Cytometry Application Note (FLDM-00488)
- CyTOF XT User Guide (FLDM-00254)
- Helios, a CyTOF System User Guide (400250)
- Maxpar Direct Immune Profiling Assay Cell Staining and Data Acquisition User Guide (400286)
- CyTOF Software v8.0 Help for CyTOF XT (FLDM-00045)
- Approach to Bivariate Analysis of Data Acquired Using the Maxpar Direct Immune Profiling Assay Technical Note (400248)
- Maxpar Cell Surface Staining with Fresh Fix Protocol (400276)
- Maxpar Nuclear Antigen Staining with Fresh Fix Protocol (400277)

Appendix: Supplemental material

Data analysis of population frequencies and signal intensities

The following section details additional analysis of files described in the application note. Two sets of tables and figures are provided for live singlet events of PBMC and whole blood. Samples were acquired in triplicate on both Helios and CyTOF XT. Raw FCS data were normalized in CyTOF Software and then analyzed in Maxpar Pathsetter for summary statistics and calculations of β values. Databases from Maxpar Pathsetter were extracted and further analyzed in NCSS Statistical Analysis and Graphics software program for Deming regression.

Mean, standard deviation (SD), and percent coefficient of variation (%CV) of population frequencies of PBMC samples stained using the Maxpar Direct Immune Profiling Assay from the same donor prepared in individual assay tubes, pooled together, and split into 2 sets of triplicates, 1 triplicate set per instrument

Helios

	Population % Live	Mean	SD	% CV
1	Lymphocytes	41.58	0.99	2.38
2	CD3 T cells	26.35	0.77	2.92
3	CD8 T cells	5.61	0.42	7.46
4	CD8 naive	0.72	0.10	13.26
5	CD8 central memory	0.50	0.07	14.25
6	CD8 effector memory	2.50	0.12	4.93
7	CD8 terminal effector	1.89	0.19	10.01
8	CD4 T cells	20.09	0.38	1.89
9	CD4 naive	2.23	0.01	0.47
10	CD4 central memory	3.72	0.12	3.11
11	CD4 effector memory	5.12	0.22	4.29
12	CD4 terminal effector	9.02	0.26	2.84
13	γδ T cells	0.42	0.02	3.86
14	MAIT/NKT	0.23	0.02	7.58
15	B cells	5.77	0.25	4.30
16	B naive	4.18	0.23	5.57
17	B memory	1.57	0.02	1.32
18	Plasmablasts	0.03	0.01	38.11
19	Natural killer cells	9.45	0.06	0.68
20	Early natural killer	4.67	0.03	0.70
21	Late natural killer	4.78	0.03	0.67
22	Monocytes	44.72	0.66	1.48
23	Classical monocytes	43.28	0.73	1.69
24	Intermediate monocytes	1.20	0.07	6.11
25	Non-classical monocytes	0.24	0.02	6.70
26	Plasmacytoid dendritic cells	0.21	0.00	1.58
27	Myeloid dendritic cells	1.06	0.03	2.70
28	Granulocytes	4.24	0.10	2.37
29	Neutrophils	0.59	0.06	10.46
30	Basophils	2.46	0.04	1.65
31	Eosinophils	0.05	0.00	8.69
32	Treg	0.43	0.01	2.78
33	Th1-like	0.78	0.00	0.30
34	Th2-like	3.02	0.09	3.03
35	Th17-like	0.35	0.04	11.94

CyTOF XT

	Population % Live	Mean	SD	% CV
1	Lymphocytes	41.33	0.18	0.44
2	CD3 T cells	26.31	0.28	1.08
3	CD8 T cells	5.74	0.03	0.47
4	CD8 naive	0.74	0.02	2.66
5	CD8 central memory	0.44	0.04	8.62
6	CD8 effector memory	2.55	0.02	0.73
7	CD8 terminal effector	2.01	0.05	2.65
8	CD4 T cells	20.03	0.15	0.74
9	CD4 naive	2.27	0.06	2.54
10	CD4 central memory	3.76	0.15	4.01
11	CD4 effector memory	4.99	0.25	5.03
12	CD4 terminal effector	9.01	0.11	1.20
13	γδ T cells	0.35	0.12	34.37
14	MAIT/NKT	0.19	0.00	0.20
15	B cells	5.79	0.05	0.87
16	B naive	4.17	0.06	1.33
17	B memory	1.59	0.04	2.47
18	Plasmablasts	0.03	0.00	11.57
19	Natural killer cells	9.23	0.15	1.64
20	Early natural killer	4.50	0.04	0.91
21	Late natural killer	4.73	0.12	2.46
22	Monocytes	43.52	0.35	0.80
23	Classical monocytes	42.04	0.32	0.76
24	Intermediate monocytes	1.26	0.04	3.03
25	Non-classical monocytes	0.22	0.01	6.06
26	Plasmacytoid dendritic cells	0.21	0.02	11.34
27	Myeloid dendritic cells	1.04	0.04	3.95
28	Granulocytes	4.48	0.12	2.64
29	Neutrophils	0.73	0.04	5.94
30	Basophils	2.50	0.03	1.36
31	Eosinophils	0.06	0.01	16.86
32	Treg	0.41	0.02	4.39
33	Th1-like	0.86	0.01	1.71
34	Th2-like	3.02	0.03	0.88
35	Th17-like	0.37	0.04	10.39

Table S1. Descriptive statistics of population frequencies as percentage of total live singlet cells for 1 triplicate from Helios and CyTOF XT

Population frequencies for each PBMC replicate stained with the Maxpar Direct Immune Profiling Assay from the same donor prepared in individual assay tubes, pooled together, and split into 2 sets of triplicates, 1 triplicate set per instrument

% of All Live	Helios Replicate 1	Helios Replicate 2	Helios Replicate 3	CyTOF XT Replicate 1	CyTOF XT Replicate 2	CyTOF XT Replicate 3
Lymphocytes	41.97	40.45	42.31	41.41	41.12	41.46
CD3 T cells	26.71	25.47	26.88	26.24	26.06	26.62
CD8 T cells	5.85	5.12	5.85	5.75	5.71	5.75
CD8 naive	0.82	0.63	0.73	0.72	0.76	0.73
CD8 central memory	0.59	0.45	0.48	0.42	0.41	0.48
CD8 effector memory	2.49	2.38	2.62	2.54	2.57	2.55
CD8 terminal effector	1.96	1.67	2.03	2.07	1.97	2.00
CD4 T cells	20.19	19.67	20.41	19.95	19.94	20.20
CD4 naive	2.23	2.22	2.24	2.31	2.20	2.30
CD4 central memory	3.76	3.81	3.59	3.87	3.81	3.59
CD4 effector memory	5.18	4.87	5.30	4.88	4.82	5.28
CD4 terminal effector	9.01	8.76	9.27	8.89	9.10	9.04
γδ T cells	0.43	0.43	0.40	0.35	0.23	0.47
MAIT/NKT	0.24	0.24	0.21	0.19	0.19	0.19
B cells	5.88	5.49	5.95	5.84	5.74	5.78
B naive	4.31	3.91	4.32	4.19	4.10	4.20
B memory	1.55	1.56	1.59	1.61	1.61	1.54
Plasmablasts	0.02	0.02	0.04	0.04	0.03	0.04
Natural killer cells	9.38	9.49	9.48	9.32	9.32	9.06
Early natural killer	4.63	4.69	4.69	4.54	4.51	4.46
Late natural killer	4.74	4.80	4.80	4.78	4.81	4.60
Monocytes	44.23	45.48	44.46	43.47	43.90	43.20
Classical monocytes	42.71	44.11	43.03	42.02	42.37	41.73
Intermediate mono	1.27	1.12	1.21	1.25	1.31	1.24
Non-classical mono	0.25	0.25	0.22	0.21	0.22	0.23
Plasmacytoid dendritic	0.20	0.20	0.21	0.23	0.22	0.18
Myeloid dendritic	1.05	1.05	1.10	1.07	1.06	1.00
Granulocytes	4.33	4.26	4.13	4.49	4.36	4.60
Neutrophils	0.63	0.52	0.62	0.78	0.73	0.69
Basophils	2.49	2.49	2.42	2.51	2.54	2.47
Eosinophils	0.05	0.06	0.05	0.05	0.07	0.05
Treg	0.43	0.41	0.44	0.40	0.44	0.40
Th1-like	0.78	0.78	0.77	0.85	0.88	0.86
Th2-like	3.10	2.92	3.03	3.01	3.01	3.05
Th17-like	0.30	0.37	0.38	0.33	0.39	0.40

Table S2. Raw values of population frequencies as percentage of total live singlet cells for 2 sets of triplicates from Helios and CyTOF XT. The numbers were extracted from Maxpar Pathsetter analysis results.

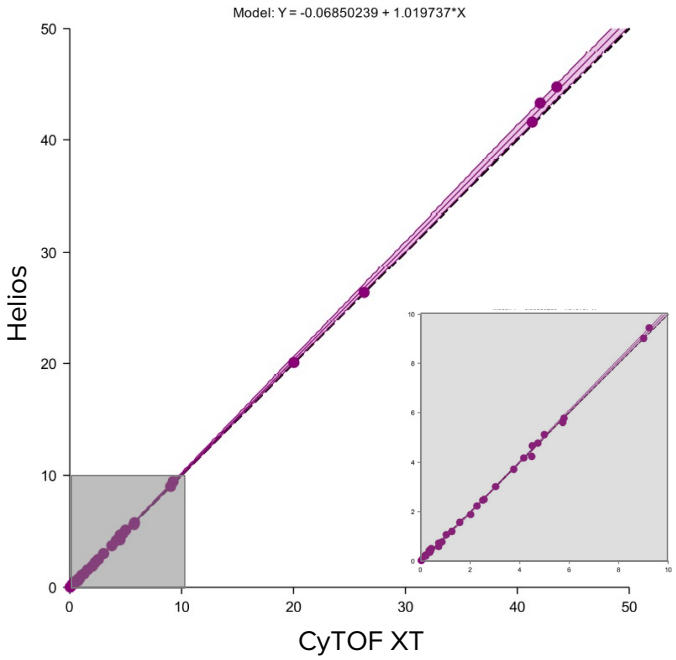

Figure S1. Deming regression of population frequencies between the 2 sets of triplicate data from Table S2. Null hypothesis is accepted at a significance level of 0.05, indicating high similarity between the 2 datasets.

Beta values of marker intensities of each PBMC replicate stained with Maxpar Direct Immune Profiling Assay from the same donor on both instruments

	Helios Replicate 1	Helios Replicate 2	Helios Replicate 3	CyTOF XT Replicate 1	CyTOF XT Replicate 2	CyTOF XT Replicate 3
CD38	4.15	4.45	4.23	3.63	3.52	3.42
CD14	5.39	5.41	5.80	5.29	5.55	5.35
CD19	4.17	4.33	4.27	4.42	4.51	4.39
CD3	4.35	4.43	4.40	4.39	4.49	4.57
CD45RA	2.57	2.43	2.57	2.26	2.68	2.35
CXCR3	0.52	0.53	0.52	0.46	0.49	0.48
CXCR5	1.04	1.02	1.03	1.02	1.03	1.03
CCR4	1.48	1.72	2.04	1.37	1.75	2.40
TCRgd	1.83	1.91	2.21	2.49	2.77	1.76
CD28	3.51	3.52	3.22	3.58	3.38	3.35
CD127	2.97	2.94	2.98	2.89	3.03	2.97
CD56	3.54	3.62	3.56	3.71	3.76	3.68
CD161	3.09	3.09	3.85	3.94	3.70	4.16
CD8	3.27	3.52	3.25	3.13	3.20	3.12
CD4	5.38	5.48	5.36	5.51	5.56	5.55
CCR7	2.41	2.83	2.84	2.72	2.82	2.86
CD25	2.66	2.55	2.57	2.82	2.65	2.68
HLADR	3.15	3.10	3.14	3.33	3.22	3.16
CD20	3.16	3.23	3.14	3.38	3.35	3.17
IgD	2.71	2.80	2.63	2.84	2.83	2.80
CD57	2.84	2.80	2.92	2.81	2.83	2.69
CD66b	3.70	3.61	3.62	3.85	3.78	3.39
CD123	4.01	3.94	4.04	3.97	3.98	4.00
CD11c	6.66	6.98	6.73	7.31	7.00	6.87
CD27	2.61	2.91	2.80	2.91	2.93	2.95
CD45	1.63	1.93	2.08	1.80	1.66	1.92
CCR6	1.65	1.63	1.63	1.63	1.61	1.57
CD294	3.82	3.70	3.78	3.08	2.93	2.75
CD16	1.83	1.79	1.86	1.75	1.85	1.74
CD45RO	1.57	1.54	1.64	1.41	1.58	1.47

Table S3. Beta (β) values of marker intensities of each replicate from Helios and CyTOF XT. The numbers were extracted from Maxpar Pathsetter analysis results.

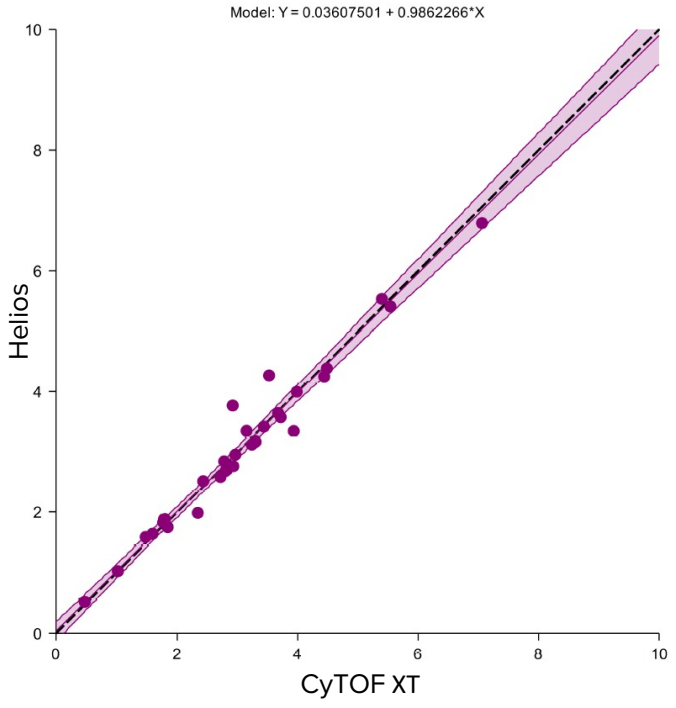

Figure S2. Deming regression of β values of marker intensities from Table S3. Null hypothesis is accepted at a significance level of 0.05, indicating high similarity between the 2 datasets.

Mean, SD, and %CV of population frequencies of whole blood samples stained with the Maxpar Direct Immune Profiling Assay from the same donor prepared in individual assay tubes, pooled together, and split into 2 sets of triplicates, 1 triplicate set per instrument

Helios

	Population % Live	Mean	SD	% CV
1	Lymphocytes	33.34	0.57	1.71
2	CD3 T cells	23.84	0.47	1.96
3	CD8 T cells	7.73	0.12	1.56
4	CD8 naive	3.67	0.07	1.83
5	CD8 central memory	0.73	0.02	3.22
6	CD8 effector memory	1.68	0.05	2.89
7	CD8 terminal effector	1.65	0.04	2.34
8	CD4 T cells	15.02	0.32	2.12
9	CD4 naive	4.24	0.14	3.36
10	CD4 central memory	6.34	0.24	3.72
11	CD4 effector memory	3.23	0.27	8.40
12	CD4 terminal effector	1.20	0.02	1.69
13	γδ T cells	0.68	0.03	3.94
14	MAIT/NKT	0.42	0.01	2.17
15	B cells	5.97	0.16	2.62
16	B naive	4.56	0.13	2.82
17	B memory	1.37	0.04	2.66
18	Plasmablasts	0.04	0.01	15.40
19	Natural killer cells	3.53	0.10	2.97
20	Early natural killer	1.77	0.04	2.49
21	Late natural killer	1.76	0.06	3.47
22	Monocytes	6.35	0.16	2.59
23	Classical monocytes	4.79	0.10	1.99
24	Intermediate monocytes	0.53	0.02	4.55
25	Non-classical monocytes	1.04	0.05	4.72
26	Plasmacytoid dendritic cells	0.10	0.00	2.46
27	Myeloid dendritic cells	0.24	0.01	5.81
28	Granulocytes	52.39	0.83	1.59
29	Neutrophils	48.19	0.76	1.57
30	Basophils	0.61	0.02	3.40
31	Eosinophils	2.86	0.11	3.84
32	Treg	0.62	0.03	5.20
33	Th1-like	0.94	0.03	3.38
34	Th2-like	1.03	0.05	4.90
35	Th17-like	1.34	0.02	1.68

CyTOF XT

	Population % Live	Mean	SD	% CV
1	Lymphocytes	33.44	1.07	3.20
2	CD3 T cells	23.84	0.87	3.65
3	CD8 T cells	7.72	0.27	3.47
4	CD8 naive	3.76	0.14	3.77
5	CD8 central memory	0.81	0.07	8.14
6	CD8 effector memory	1.58	0.04	2.71
7	CD8 terminal effector	1.57	0.03	1.76
8	CD4 T cells	15.03	0.56	3.75
9	CD4 naive	4.20	0.17	4.10
10	CD4 central memory	6.78	0.34	4.98
11	CD4 effector memory	2.93	0.03	0.90
12	CD4 terminal effector	1.12	0.11	9.72
13	γδ T cells	0.68	0.02	2.74
14	MAIT/NKT	0.41	0.03	6.16
15	B cells	6.16	0.04	0.69
16	B naive	4.63	0.02	0.48
17	B memory	1.49	0.04	2.57
18	Plasmablasts	0.04	0.00	9.51
19	Natural killer cells	3.45	0.19	5.48
20	Early natural killer	1.72	0.12	6.80
21	Late natural killer	1.73	0.07	4.28
22	Monocytes	6.52	0.20	3.09
23	Classical monocytes	5.03	0.10	2.04
24	Intermediate monocytes	0.47	0.04	8.89
25	Non-classical monocytes	1.02	0.06	5.80
26	Plasmacytoid dendritic cells	0.09	0.00	5.04
27	Myeloid dendritic cells	0.25	0.02	8.88
28	Granulocytes	51.95	1.42	2.73
29	Neutrophils	48.04	1.18	2.46
30	Basophils	0.63	0.02	3.79
31	Eosinophils	2.46	0.22	8.98
32	Treg	0.57	0.02	4.20
33	Th1-like	0.86	0.06	7.12
34	Th2-like	1.04	0.03	2.98
35	Th17-like	1.39	0.06	4.22

Tables S4. Descriptive statistics of population frequencies as percentage of total live singlet cells for triplicate sample data from Helios and CyTOF XT

Population frequencies of each whole blood sample stained with the Maxpar Direct Immune Profiling Assay from the same donor prepared in individual assay tubes, pooled together, and split into 2 sets of triplicates, 1 triplicate set per instrument

	Helios Replicate 1	Helios Replicate 2	Helios Replicate 3	CyTOF XT Replicate 1	CyTOF XT Replicate 2	CyTOF XT Replicate 3
Lymphocytes	32.90	33.98	33.13	34.66	33.01	32.66
CD3 T cells	23.53	24.37	23.61	24.81	23.57	23.13
CD8 T cells	7.63	7.86	7.69	8.00	7.69	7.47
CD8 naive	3.63	3.74	3.63	3.89	3.78	3.61
CD8 central memory	0.71	0.75	0.74	0.89	0.77	0.78
CD8 effector memory	1.69	1.73	1.63	1.62	1.58	1.53
CD8 TE	1.61	1.64	1.69	1.60	1.56	1.54
CD4 T cells	14.80	15.38	14.86	15.67	14.80	14.61
CD4 naive	4.31	4.34	4.08	4.32	4.27	4.00
CD4 central memory	6.13	6.30	6.59	7.16	6.62	6.55
CD4 effector memory	3.18	3.52	2.99	2.95	2.90	2.95
CD4 terminal effector	1.18	1.23	1.20	1.23	1.01	1.12
γδ T cells	0.68	0.70	0.65	0.70	0.68	0.67
MAIT/NKT	0.41	0.43	0.41	0.44	0.40	0.40
B cells	5.80	6.00	6.11	6.19	6.11	6.18
B naive	4.41	4.59	4.66	4.66	4.63	4.61
B memory	1.35	1.36	1.42	1.49	1.45	1.53
Plasmablasts	0.04	0.05	0.03	0.04	0.03	0.04
Natural killer cells	3.57	3.61	3.41	3.67	3.34	3.34
Early natural killer	1.79	1.80	1.72	1.86	1.64	1.67
Late natural killer	1.78	1.81	1.69	1.81	1.70	1.67
Monocytes	6.41	6.48	6.17	6.74	6.46	6.35
Classical monocytes	4.81	4.88	4.69	5.15	4.99	4.96
Intermediate mono	0.54	0.54	0.50	0.51	0.46	0.43
Non-classical mono	1.07	1.06	0.98	1.08	1.02	0.96
Plasmacytoid dendritic	0.10	0.10	0.10	0.10	0.09	0.09
Myeloid dendritic	0.24	0.25	0.22	0.28	0.23	0.25
Granulocytes	52.88	51.42	52.86	50.32	52.64	52.90
Neutrophils	48.71	47.32	48.54	46.69	48.60	48.84
Basophils	0.59	0.63	0.61	0.65	0.61	0.61
Eosinophils	2.82	2.78	2.99	2.21	2.60	2.58
Treg	0.59	0.65	0.63	0.59	0.56	0.55
Th1-like	0.95	0.96	0.90	0.92	0.86	0.79
Th2-like	1.02	1.09	0.99	1.08	1.04	1.02
Th17-like	1.32	1.37	1.35	1.45	1.33	1.40

Table S5. Raw values of population frequencies as percentage of total live singlet cells of 2 sets of triplicates from Helios and CyTOF XT. The numbers were extracted from Maxpar Pathsetter analysis results.

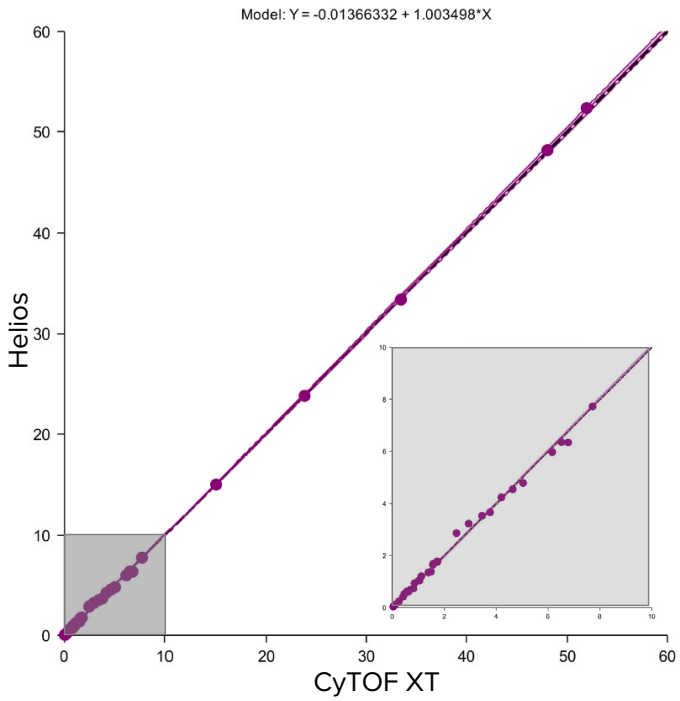

Figure S3. Deming regression of population frequencies between 2 sets of triplicates from Table S5. Null hypothesis is accepted at a significance level of 0.05, indicating high similarity between the 2 datasets.

Beta values of marker intensities of each whole blood sample stained with the Maxpar Direct Immune Profiling Assay from the same donor on both instruments

	Helios Replicate 1	Helios Replicate 2	Helios Replicate 3	CyTOF Replicate 1	CyTOF Replicate 2	CyTOF Replicate 3
CD38	4.28	4.26	4.25	4.55	4.32	4.49
CD14	3.15	3.10	3.14	3.83	3.61	3.80
CD19	6.06	5.96	6.11	6.91	6.80	6.82
CD3	7.38	7.11	7.12	7.00	7.01	7.23
CD45RA	2.57	2.59	2.72	3.08	2.78	3.12
CXCR3	0.71	0.71	0.71	0.72	0.72	0.71
CXCR5	7.14	7.18	7.25	7.43	7.41	7.50
CCR4	2.29	2.26	2.57	2.65	2.51	2.68
TCRgd	5.04	4.88	4.79	4.89	4.95	4.83
CD28	5.07	4.76	4.92	5.20	5.29	5.42
CD127	2.58	2.59	2.51	2.55	2.50	2.60
CD56	5.00	4.92	4.88	5.13	5.16	5.06
CD161	3.68	3.91	3.74	3.71	3.83	3.79
CD8	6.78	6.69	6.64	7.44	7.33	7.40
CD4	6.91	6.69	6.62	7.80	7.30	7.61
CCR7	4.78	4.71	4.61	4.41	4.44	4.70
CD25	2.19	2.15	2.25	2.39	2.36	2.35
HLADR	4.54	4.52	4.46	4.71	4.78	4.81
CD20	7.11	6.89	6.71	7.35	7.32	7.30
IgD	4.85	4.65	4.68	4.61	4.67	4.70
CD57	2.65	2.60	2.77	2.64	2.56	2.66
CD66b	5.57	5.48	5.42	5.73	5.56	5.70
CD123	4.56	4.60	4.77	4.45	5.40	4.50
CD11c	4.20	4.92	5.08	4.23	5.05	4.63
CD27	3.93	3.98	3.70	3.90	3.87	3.94
CD45	3.82	3.81	3.80	4.26	4.15	4.30
CCR6	4.36	4.32	4.41	4.82	4.70	4.85
CD294	4.21	4.14	4.16	4.58	4.44	4.48
CD16	3.20	3.14	3.10	3.31	3.16	3.34
CD45RO	3.19	3.13	3.21	3.59	3.53	3.49

Table S6. Beta (β) values of marker intensities of each replicate from Helios and CyTOF XT. The numbers were extracted from Maxpar Pathsetter analysis results.

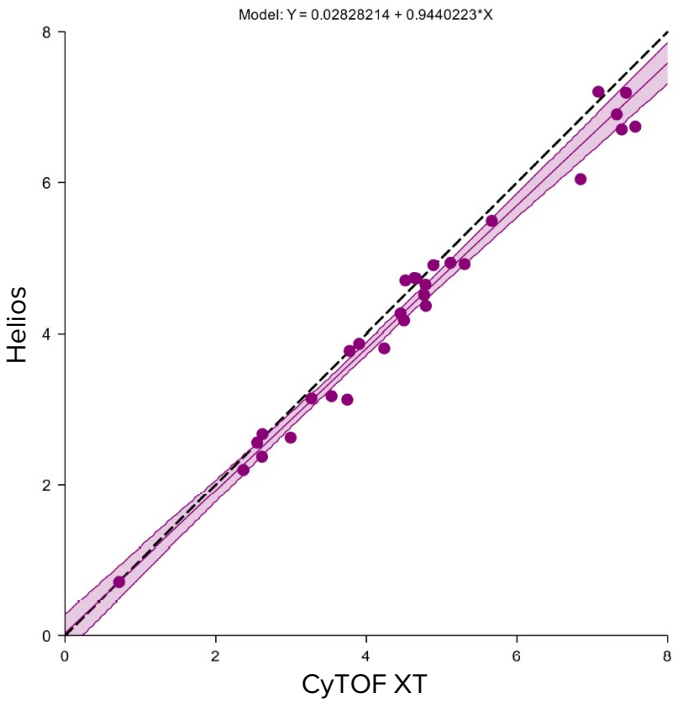

Figure S4. Deming regression of β values of marker intensities from Table S6. Alternative hypothesis is accepted at a significance level of 0.05 because the β values from CyTOF XT are higher than from Helios, indicating better marker resolution. This translates to a better separation of negative and positive populations.

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