An Automated Approach to High-Plex Cytometric Immunophenotyping with CyTOF XT

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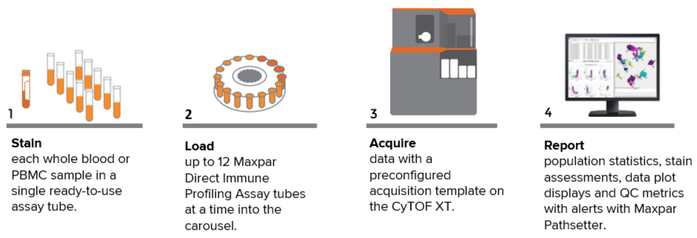
Abstract

CyTOF® technology, based on cytometry by time-of-flight, utilizes metal-tagged antibodies for single-cell detection by mass cytometry. A major advantage of mass cytometry is the ability to conduct comprehensive deep immune profiling studies using highly multiplexed panels comprising over 50 markers[1] without the signal spillover and compensation limitations of flow cytometry.

The Maxpar® Direct™ Immune Profiling Assay™ and Maxpar Pathsetter™ software were developed as a sample-to-answer solution for human immune profiling using mass cytometry. The **Maxpar Direct Immune Profiling Assay** (Cat. No. 201325) utilizes a **ready-to-use dry-format 30-antibody staining panel** for human whole blood and PBMC immunophenotyping by mass cytometry (Figure 1). **Maxpar Pathsetter** is **automated software** that reports population statistics, stain assessments and relevant data plots. The software automatically resolves this core 30-marker panel into **37 immune cell populations** (Figure 2) with highly reproducible results[2]. This assay is ideal for use in longitudinal studies of immune response in the context of immune-mediated diseases and is already in use in COVID-19, CAR T and cancer research studies[3]. The Maxpar Direct Immune Profiling System was originally validated for Helios™ mass cytometers. Now data collection can be simplified using an automated acquisition system on CyTOF XT™. **The objective of this study was to compare CyTOF XT and Helios data using several suspension mass cytometry workflows, including the Maxpar Direct Immune Profiling Assay and Maxpar Pathsetter software.**



Target (clone)	Isotope
CD45 (HI30)	89Y
Cell-ID Intercalator-103Rh	103Rh
Available open channel	**106Cd**
Available open channel	**110Cd**
Available open channel	**111Cd**
Available open channel	**112Cd**
Available open channel	**113Cd**
Available open channel	**114Cd**
Available open channel	**116Cd**
CD196/CCR6 (G034E3)	141Pr
Available open channel	**142Nd**
CD123 (6H6)	143Nd
CD19 (HIB19)	144Nd
CD4 (RPA-T4)	145Nd
CD8a (RPA-T8)	146Nd

Target (clone)	Isotope
CD11c (Bu15)	147Sm
CD16 (3G8)	148Nd
CD45RO (UCHL1)	149Sm
CD45RA (HI100)	150Nd
CD161 (HP-3G10)	151Eu
CD194/CCR4 (L291H4)	152Sm
CD25 (BC96)	153Eu
CD27 (O323)	154Sm
CD57 (HCD57)	155Gd
CD183/CXCR3 (G025H7)	156Gd
CD185/CXCR5 (J252D4)	158Gd
Available open channel	**159Tb**
CD28 (CD28.2)	160Gd
CD38 (HB-7)	161Dy
Available open channel	**162Dy**

Target (clone)	Isotope
CD56/NCAM (NCAM16.2)	163Dy
TCRγδ (B1)	164Dy
Available open channel	**165Ho**
CD294 (BM16)	166Er
CD197/CCR7 (G043H7)	167Er
CD14 (63D3)	168Er
Available open channel	**169Tm**
CD3 (UCHT1)	170Er
CD20 (2H7)	171Yb
CD66b (G10F5)	172Yb
HLA-DR (LN3)	173Yb
IgD (IA6-2)	174Yb
Available open channel	**175Lu**
CD127 (A019D5)	176Yb
Available open channel	**209Bi**

Figure 1. The Maxpar Direct Immune Profiling Assay workflow using CyTOF XT (top), and components of the Maxpar Direct Immune Profiling Assay panel (table, above)

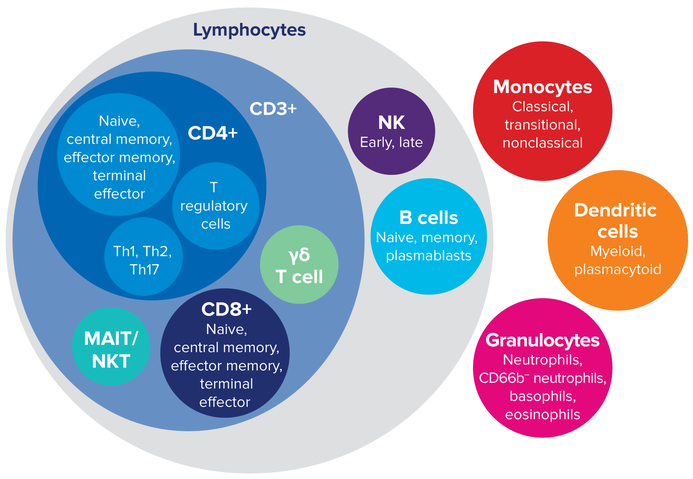


Figure 2. Populations identified by the Maxpar Direct Immune Profiling Assay

Methods and Materials

CyTOF XT: the next generation of mass cytometry

Standard BioTools™ has introduced the next-generation mass cytometer, CyTOF XT (Figure 3). 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. The new Autosampler consists of four major components: the sample probe, a syringe-based pump unit, a bottle station for acquisition and cleaning solutions and a carousel that holds 13 sample tubes chilled at 4–8 °C. The new Autosampler enables automated sample delivery over long acquisitions while maintaining sample integrity.

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

Figure 3. CyTOF XT, featuring a streamlined design and automated sample acquisition

Sample preparation, staining and analysis

- PBMC were stained using suspension mass cytometry protocols including nuclear staining, cytoplasmic staining, phosphostaining, surface staining and staining with the Cell-ID™ 20-Plex Pd Barcoding Kit.
  - 79 Maxpar antibodies were used in various panel combinations. Some antibodies, such as common lineage markers, were tested in multiple protocols.
  - Manual gating was used to identify the median signal and standard deviation of the positive and negative populations for each marker. Resolution index was calculated to assess how well positive and negative populations separated from each other.
- The Maxpar Direct Immune Profiling Assay panel was tested on two frozen human PBMC (STEMCELL™ Technologies) from healthy donors and whole blood from one healthy volunteer donor sourced locally.

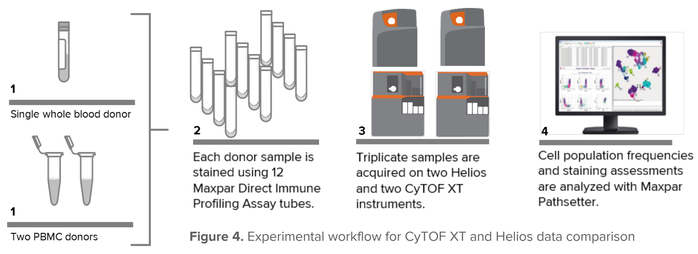


Figure 4. Experimental workflow for CyTOF XT and Helios data comparison

- For each donor, 12 Maxpar Direct Immune Profiling Assay tubes were used for staining. Staining and acquisition proceeded as outlined in the Maxpar Direct Immune Profiling Assay Cell Staining and Data Acquisition User Guide (PN 400286), but with the following exceptions:
  - All samples were washed using Maxpar Cell Acquisition Solution (CAS) Plus for CyTOF XT (Cat. No. 201244). After the first CAS Plus wash, replicate samples were pooled and redistributed in order to control for tube-to-tube variability.
- Samples were acquired in parallel on two Helios instruments and on two CyTOF XT instruments.

Maxpar Direct Immune Profiling Assay (Cat. No. 201325)

Helios, a CyTOF System (PN 107002)

CyTOF XT, a CyTOF System (PN 117002)

Maxpar Pathsetter software (PN 401018)

Results

Increased signal resolution on CyTOF XT compared with Helios

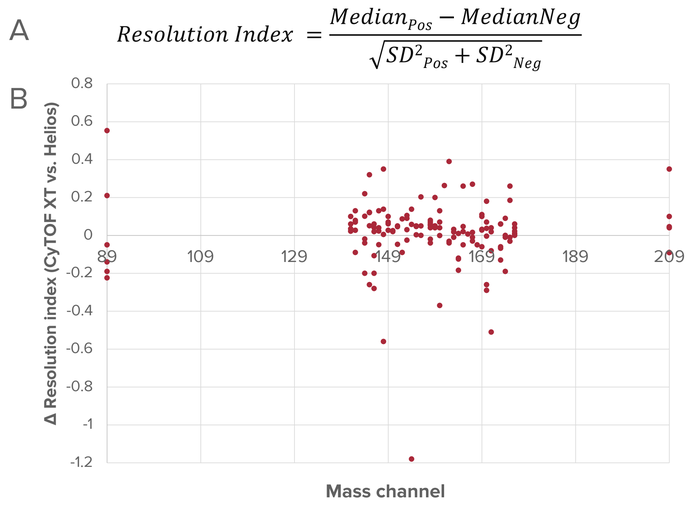


Figure 5. CyTOF XT on average has better signal resolution compared with Helios. **(A)** Resolution index (RI) formula used to determine signal resolution. A higher RI value indicates greater resolution between the positive and negative population. SD: standard deviation, Pos: positive population, Neg: negative population. **(B)** Plot shows Δ resolution index (CyTOF XT vs. Helios) vs. mass channel. Each dot is an individual stain assessment of an antibody used in one of the various panels tested. Several workflows and applications for suspension mass cytometry including sample barcoding with the Cell-ID 20-Plex Pd Barcoding Kit, and surface, cytoplasmic, nuclear and phosphostaining were evaluated on human PBMC. 94 out of 140 antibody tests show better positive and negative signal separation on CyTOF XT (Δ resolution index >0).

Similar population frequencies analyzed by manual gating between CyTOF XT and Helios

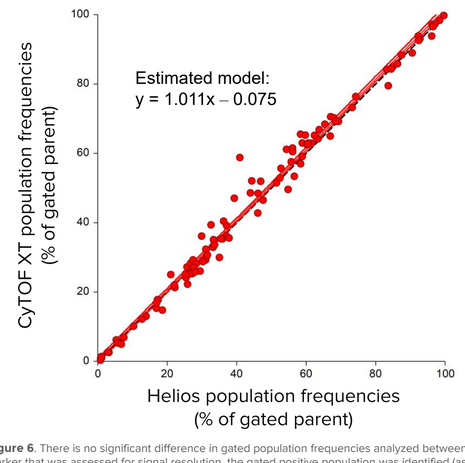


Figure 6. There is no significant difference in gated population frequencies analyzed between CyTOF XT and Helios. For each marker that was assessed for signal resolution, the gated positive population was identified (as a % of the parent gate) and compared between CyTOF XT and Helios. Deming regression was performed to compare the frequencies analyzed from CyTOF XT and Helios acquisitions. The null hypothesis (H0) test that slope = 1 was not rejected, which suggests that there is no statistical difference in the population frequencies analyzed from CyTOF XT and Helios. The shaded area (red) indicates the associated confidence limit bounds. The 95% confidence limits of the slope are shown for the line of best fit. Calculations were performed using NCSS 12.0.

Comparable and repeatable results using the Maxpar Direct Immune Profiling Assay on CyTOF XT and Helios

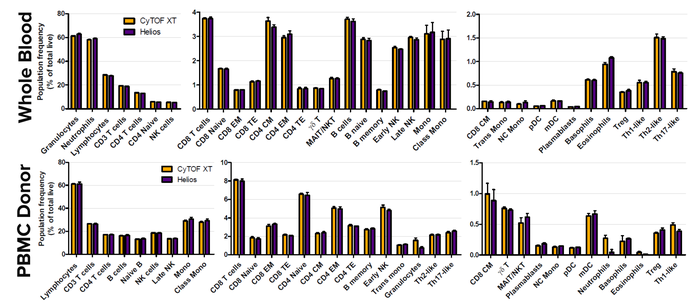
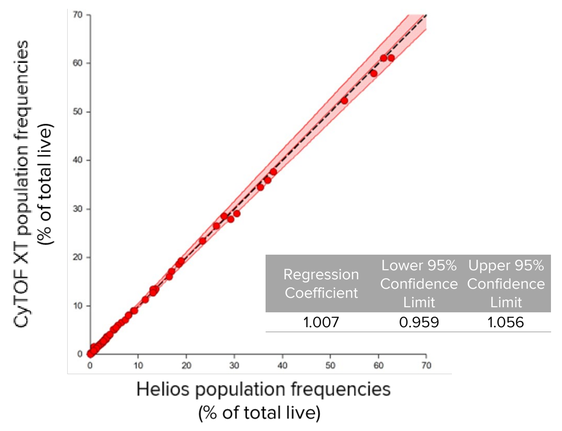


Figure 7. Comparable and repeatable results of the Maxpar Direct Immune Profiling Assay when acquired using CyTOF XT or Helios and analyzed using Maxpar Pathsetter. Triplicate samples were acquired on two CyTOF XT and two Helios instruments for whole blood (top) and PBMC (bottom). One of two representative PBMC donors is shown. Error bars show the standard deviation between the six replicates from CyTOF XT and Helios.

CyTOF XT and Helios provide equivalent population frequencies using the Maxpar Direct Immune Profiling Assay



Regression Coefficient	Lower 95% Confidence Limit	Upper 95% Confidence Limit
1.007	0.959	1.056

Figure 8. There is no statistical difference between the population frequencies analyzed by Maxpar Pathsetter from CyTOF XT and Helios acquired files. The mean population frequencies from whole blood and PBMC samples from CyTOF XT were plotted against Helios (Table 1). Deming regression was performed to compare the population frequencies analyzed between the two instruments. The H0 test that slope = 1 was not rejected, indicating that there is no statistical difference between the population frequencies analyzed from the files acquired using the two different instruments. The shaded area (red) indicates the associated confidence limit bounds. The 95% confidence limits of the slope are shown for the line of best fit. Calculations were performed using NCSS 12.0.

Improved β staining assessment values using CyTOF XT compared with Helios with the Maxpar Direct Immune Profiling Assay

A

$$Beta\ (\beta) = \frac{Median_{Pos} - MedianNeg}{1.4826\sqrt{MAD^2_{Pos} + MAD^2_{Neg}}}$$

B

Regression Coefficient	Lower 95% Confidence Limit	Upper 95% Confidence Limit
1.069	1.026	1.114

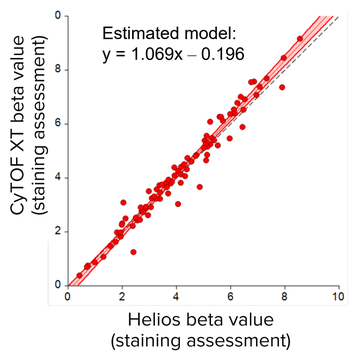


Table 1. Mean beta staining assessment values from whole blood and PBMC donors acquired on Maxpar Pathsetter and acquired on CyTOF XT and Helios.

	Whole Blood		PBMC Donor 1		PBMC Donor 2	
Marker	CyTOF XT	Helios	CyTOF XT	Helios	CyTOF XT	Helios
CCR4	2.14	2.04	2.50	2.12	2.32	2.00
CCR6	1.98	1.81	4.39	3.92	3.58	3.27
CCR7	5.21	5.52	2.43	2.56	4.66	5.09
CD11c	3.86	3.83	6.53	6.47	5.40	5.39
CD123	4.14	4.16	4.85	4.73	3.82	3.61
CD127	2.77	2.72	3.25	3.09	1.83	1.94
CD14	2.91	2.68	6.27	5.59	6.27	5.65
CD16	1.63	1.76	1.47	1.57	1.98	1.94
CD161	9.16	8.55	7.01	6.37	6.09	5.24
CD19	7.58	6.86	6.54	6.12	6.12	5.71
CD20	6.37	5.98	5.39	5.04	5.57	5.11
CD25	2.22	2.39	3.51	2.98	2.74	2.77
CD27	7.38	7.07	3.23	3.39	4.09	3.93
CD28	5.89	6.44	4.32	4.38	4.74	4.41
CD294	3.82	4.16	3.73	3.37	3.94	3.71
CD3	7.36	7.90	5.25	5.25	5.16	5.15
CD38	3.03	4.06	2.62	2.95	3.67	4.86
CD4	7.55	6.76	6.92	6.52	6.79	6.25
CD45	3.46	3.40	0.88	0.98	1.08	1.31
CD45RA	3.24	3.26	2.53	2.50	2.92	3.06
CD45RO	3.31	3.18	2.86	2.86	2.93	2.87
CD56	5.31	5.13	4.82	4.71	3.76	3.46
CD57	3.43	3.69	4.86	5.13	4.07	4.32
CD66b	5.47	5.96	3.09	2.04	1.25	2.41
CD8	8.45	7.96	4.61	4.55	5.25	5.08
CXCR3	0.70	0.70	0.75	0.73	0.39	0.42
CXCR5	7.69	7.33	6.39	6.15	5.12	5.01
HLADR	7.08	6.95	4.26	4.06	4.51	4.28
IgD	4.41	4.17	3.80	3.79	3.66	3.65
TCRγδ	5.49	5.31	2.45	2.65	2.27	1.98

Figure 9. Files acquired using CyTOF XT overall have improved signal resolution compared with Helios. **(A)** Maxpar Pathsetter performs a staining assessment based on a statistical approach called Strictly Standardized Mean Difference (SSMD), represented by a beta value. A higher beta value indicates greater resolution between the positive and negative population. MAD: median absolute deviation, Pos: positive population, Neg: negative population. **(B)** A plot of the average beta values from CyTOF XT acquired files plotted against beta values from Helios files (Table 2). Deming regression was performed to compare the staining assessment between the two instruments. The H0 test that slope = 1 was not rejected and the upper and lower 95% confidence limits are >1.0, indicating that CyTOF XT on average will have a higher beta value compared with Helios. The shaded area (red) indicates the associated confidence limit bounds. The 95% confidence limits of the slope are shown for the line of best fit. Calculations were performed using NCSS 12.0.

Conclusions

- CyTOF XT is a new generation of CyTOF instrument that shares the same reliable level of performance as Helios when using protocols for suspension mass cytometry, including the Maxpar Direct Immune Profiling Assay.
- Files acquired with CyTOF XT and those acquired with Helios showed no statistically significant difference in population frequencies when analyzed in Maxpar Pathsetter or by manual gating.
- CyTOF XT overall resulted in improved staining resolution for whole blood and PBMC samples compared with Helios.
- The hands-free acquisition on CyTOF XT and the automated analysis of the Maxpar Direct Immune Profiling System enable researchers to streamline high-parameter immunophenotyping of human whole blood and PBMC samples.

References

1. Simoni, Y. et al. “Bystander CD8+ T cells are abundant and phenotypically distinct in human tumour infiltrates.” *Nature* 557 (2018): 575–579.
2. Bagwell, C.B. et al. “Multi-site reproducibility of a human immunophenotyping assay in whole blood and peripheral blood mononuclear cells preparations using CyTOF® technology coupled with Maxpar® Pathsetter™, an automated data analysis system.” *Cytometry Part B* 98 (2020): 146–160.
3. “Maxpar Direct Immune Profiling System: Publications and National Clinical Trials, March 2022” .

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