A streamlined immune profiling workflow using an automated acquisition system and the Maxpar Direct Immune Profiling System

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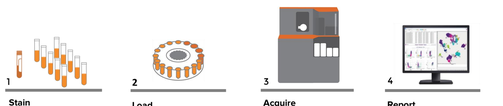
Introduction

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 40 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** (PN 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 research[3-5]. 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 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
TCRgd (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 the CyTOF XT (top) and the Maxpar Direct Immune Profiling Assay panel (bottom)

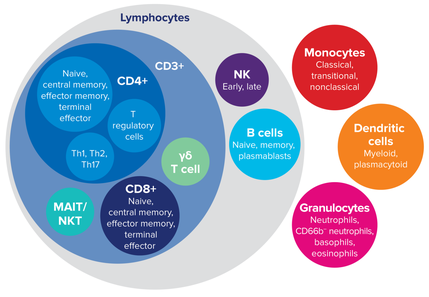


Figure 2. Populations identified by the Maxpar Direct Immune Profiling Assay

Materials and Methods

CyTOF XT: the next generation of mass cytometry

Fluidigm has introduced the new 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 4 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

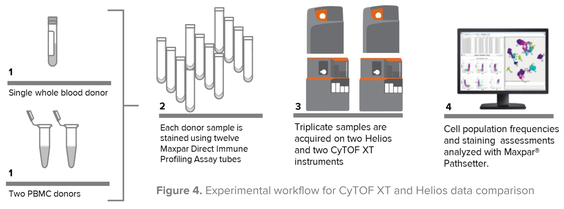


Figure 4. Experimental workflow for CyTOF XT and Helios data comparison

- 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.
- PBMC were thawed using CTL Anti-Aggregate Wash™ 20x Solution (Cellular Technology Limited) according to the manufacturer's instructions.
- For each donor, twelve 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 (PN 201244). After the first CAS Plus wash, replicate samples were pooled and redistributed in order to control for tube-to-tube variability.
- Triplicate samples were acquired in parallel on two Helios instruments running CyTOF Software v7.0.5189 and on two CyTOF XT instruments running CyTOF Software v8.0.12471.
- Normalized FCS files were analyzed using Maxpar Pathsetter software v2.0.45.

Figure 5. Maxpar Direct Immune Profiling Assay stained samples are acquired on Helios or CyTOF XT mass cytometers. Normalized data may be analyzed by Maxpar Pathsetter software for automated analysis.

Results

CyTOF XT and Helios produce comparable and repeatable results

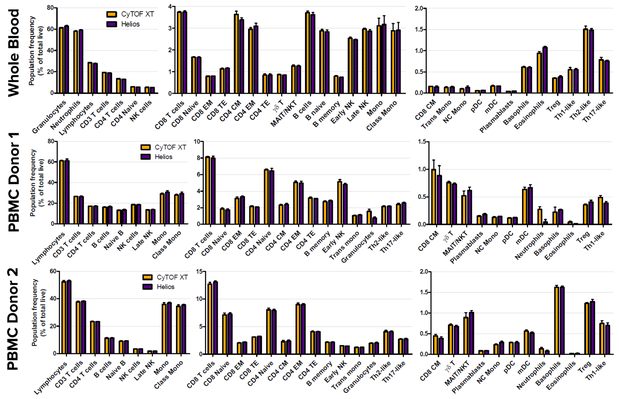


Figure 6. Comparable and repeatable results of the Maxpar Direct Immune Profiling Assay when acquired using CyTOF XT or Helios and analyzed using Maxpar Pathsetter. Triplicate samples were acquired on two CyTOF XT and two Helios instruments for whole blood (top) and PBMC (middle and bottom). Error bars show the standard deviation between the six replicates from CyTOF XT and Helios. Mean values for each sample type and instrument are summarized in Table 1.

Table 1. Mean and percent coefficient of variation (%CV) of population frequencies of whole blood and PBMC samples stained using the Maxpar Direct Immune Profiling Assay, acquired on CyTOF XT and Helios, and analyzed using Maxpar Pathsetter.

	Whole Blood				PBMC Donor 1				PBMC Donor 2			
	CyTOF XT		Helios		CyTOF XT		Helios		CyTOF XT		Helios	
Population	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
Lymphocytes	28.50	0.8	27.86	0.7	61.11	1.0	61.09	2.7	52.33	2.1	52.92	1.2
CD3 T cells	19.29	1.1	18.90	0.8	26.50	1.0	26.25	2.5	37.67	1.8	38.12	1.1
CD8 T cells	3.74	1.1	3.74	1.7	8.12	1.0	7.99	2.9	12.70	2.6	13.06	1.9
CD8 naïve	1.67	1.1	1.65	2.2	1.85	6.3	1.72	8.2	7.12	4.7	7.29	3.6
CD8 central memory	0.15	6.5	0.14	18.0	0.99	17.6	0.89	20.4	0.45	8.0	0.39	10.2
CD8 effector memory	0.79	2.8	0.80	2.6	3.12	5.4	3.31	2.8	2.04	2.7	2.17	2.7
CD8 terminal effector	1.13	3.3	1.16	2.0	2.15	3.8	2.07	2.8	3.09	1.6	3.22	1.4
CD4 T cells	13.41	1.3	13.05	1.0	17.10	1.2	16.91	2.7	23.37	1.4	23.37	0.9
CD4 naïve	5.98	1.4	5.72	1.4	6.57	2.0	6.47	4.5	8.03	4.2	7.92	3.1
CD4 central memory	3.64	4.0	3.39	3.0	2.29	5.3	2.39	5.8	2.27	10.8	2.39	7.1
CD4 effector memory	2.96	2.6	3.09	4.7	5.07	3.0	4.98	4.6	9.00	3.3	8.99	1.9
CD4 terminal effector	0.84	6.0	0.84	6.2	3.17	3.7	3.08	2.6	4.07	3.0	4.07	1.3
γδ T cells	0.87	2.7	0.84	1.6	0.76	3.1	0.73	2.7	0.71	3.6	0.68	3.5
MAIT/NKT	1.27	3.9	1.26	2.5	0.52	16.3	0.62	9.9	0.89	13.5	1.01	4.7
B Cells	3.72	2.1	3.62	2.9	16.01	3.0	16.42	4.2	11.28	4.4	11.43	1.9
B naive	2.88	2.3	2.83	3.7	13.13	3.1	13.42	4.5	9.01	4.8	9.19	1.6
B memory	0.79	2.9	0.74	1.3	2.73	4.2	2.83	3.5	2.18	3.7	2.16	4.3
Plasmablasts	0.04	0.0	0.05	8.4	0.15	6.0	0.18	10.2	0.09	9.3	0.09	8.5
Total NK	5.50	1.2	5.34	1.4	18.60	1.7	18.42	2.6	3.37	1.6	3.37	2.0
Early NK	2.54	2.1	2.47	0.9	5.14	4.8	4.79	3.1	1.53	2.6	1.48	2.2
Late NK	2.96	1.3	2.87	2.3	13.46	2.4	13.63	3.6	1.85	1.3	1.88	2.3
Total monocytes	3.11	11.2	3.17	12.8	29.04	2.9	30.51	5.1	35.91	3.5	36.95	1.2
Classical monocytes	2.89	11.2	2.91	12.6	27.86	2.8	29.24	5.3	34.45	3.5	35.42	1.1
Transitional monocytes	0.13	15.1	0.13	17.5	1.05	6.1	1.13	4.3	1.23	2.6	1.23	6.5
Non-classical monocytes	0.10	12.5	0.13	27.1	0.13	9.1	0.15	5.8	0.24	8.4	0.29	9.9
pDC	0.06	10.0	0.06	16.9	0.12	10.6	0.13	4.4	0.29	4.8	0.28	9.6
mDC	0.16	11.4	0.16	5.6	0.64	6.4	0.67	8.0	0.56	5.7	0.52	4.5
Granulocytes	61.15	0.9	62.67	1.4	1.57	16.3	0.75	18.3	1.99	3.7	2.04	6.9
Neutrophils	57.93	0.8	59.04	0.7	0.27	17.7	0.04	106.9	0.14	25.1	0.08	38.4
Basophils	0.61	3.1	0.60	2.9	0.22	41.5	0.27	4.0	1.63	2.9	1.63	1.9
Eosinophils	0.94	3.6	1.08	2.6	0.04	43.0	0.01	35.0	0.02	22.3	0.03	21.9
Treg	0.35	3.1	0.38	8.3	0.36	3.8	0.41	8.2	1.23	1.5	1.28	4.1
Th1-like	0.55	8.6	0.55	5.1	0.49	7.7	0.39	6.8	0.75	9.2	0.70	9.7
Th2-like	1.51	4.9	1.48	2.9	2.15	4.2	2.16	3.1	4.11	5.4	4.06	4.1
Th17-like	0.78	8.1	0.76	3.0	2.41	4.7	2.58	3.8	2.73	2.2	2.73	5.8

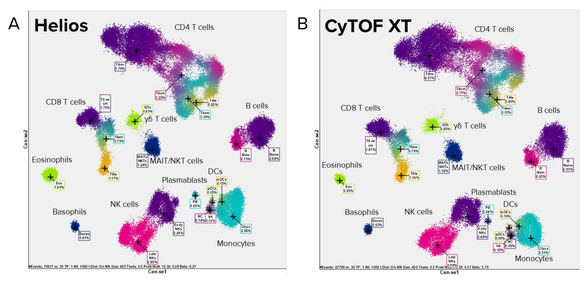


Figure 7. Cen-se[47] (Cauchy enhanced nearest-neighbor stochastic embedding) clustering, a dimensionality reduction tool (GemStone™), performed in Maxpar Pathsetter software shows similar results between CyTOF XT and Helios. Cen-se' clustering on the 30 markers in the Maxpar Direct Immune Profiling Assay stained on whole blood and acquired with **(A)** Helios and **(B)** CyTOF XT. Neutrophils are intentionally excluded in these Cen-se' plots to better visualize the other cell types.

CyTOF XT and Helios files generate equivalent population frequencies

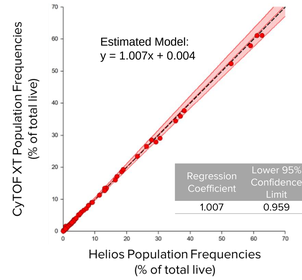


Figure 8. There is no statistical difference between the population frequencies analyzed by Maxpar Pathsetter from CyTOF XT and Helios acquired files. The mean population frequencies from whole blood and PBMC samples from CyTOF XT were plotted against Helios (Table 1). Deming regression was performed 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 for files acquired using CyTOF XT compared to Helios

A

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

B

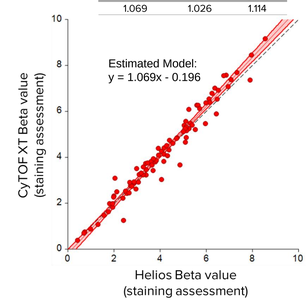


Table 2. Mean Beta staining assessment values from whole blood and PBMC donor samples analyzed using Maxpar Pathsetter and acquired on CyTOF XT and Helios.

	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
TCRgd	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 to 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 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 to 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 the Maxpar Direct Immune Profiling Assay.
- CyTOF XT and Helios acquired files analyzed in Maxpar Pathsetter resulted in no statistically significant difference between the two platforms.
- CyTOF XT overall resulted in improved staining resolution for whole blood and PBMC samples compared to Helios.
- The hands-free acquisition on CyTOF XT and the automated analysis of Maxpar Direct Immune Profiling System enable researchers to streamline high-parameter immunophenotyping of human whole blood and PBMC samples.

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