Cross-technology Comparison of Chipcytometry vs. Flow Cytometry for the Measurement of T Cell Phenotypic and Functional Markers

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Introduction

Chipcytometry is an image-based technology platform that offers advantages over standard flow cytometry. These include immobilization and preservation of cells/tissues on microfluidic chips, allowing for serial analysis and re-analysis of biomarkers, as well as long-term sample storage. A platform cross-comparison between ZellkraftwerkZellScannerONE vs. BD FACSCanto10 was performed to characterize Chipcytometry as a viable alternative to standard flow cytometry; specifically, it's potential utility for the analysis of human T cell subtypes and functional markers. PBMCs from 5 healthy donors were prepared and stained for cell surface markers (CD3, CD4, CD45RA, CCR7, HLA-DR, CD25, PD-1) and intracellular biomarkers (ki67, bcl-2) by standard flow methods or loaded onto ZellSafe Rare cell chips and shipped to Zellkraftwerk for staining and analysis. Stability of several biomarkers was measured up to 6 months. For Chipcytometry evaluations, the results demonstrated that for central memory and effector memory CD4+ cells, the relative error between T = 0 and T = 6 months was less than 20%. Overall, the frequency of naïve and memory T cell populations, as well as expression levels of functional markers, showed good concordance when comparing flow and Chipcytometry methods. Lessons learned include the importance of optimizing 1) clone determination, as observed with PD-1 and 2) staining/fixing conditions with certain antibodies, as observed with CD25. Our results demonstrate that Chipcytometry offers a viable alternative to standard flow cytometry with some methodological considerations, including optimal clone selection and testing of pre- vs. post-fixation antibody binding.

Goal

- Cross-technology validation between flow cytometry and chipcytometry
- Validation of 6 months sample stability on ZellSafe™ chips

Experiment Design

- PBMCs were isolated from blood of five healthy volunteers (IDs 452, 508, 843, 12115 and 12177) at Pfizer site
- PBMCs were applied to 10 ZellSafe™ chips per volunteer, stained with CCR7-PE, fixed and shipped to Zellkraftwerk (Hannover, Germany)
- Remaining PBMCs were immediately analyzed by flow cytometry at Pfizer labs on a FACSCanto™ 10 instrument
- For each volunteer, 10 endpoints were measured with Chipcytometry on one chip upon receipt (T = 0) and on another chip after 6 months storage at 4˚ C (T = 6)
- For cross-platform comparison, chip results at T = 0 were compared with FACS results
- For stability assessment, chip results at T = 6 were compared with those at T = 0
- At T = 0 for chipcytometry, clone MIH4 was used for CD279 (PD-1) but resulted in weak staining
- At T = 6, CD279 clone was changed to clone EH12.1 for chip analysis (same clone used for flow cytometry analysis)

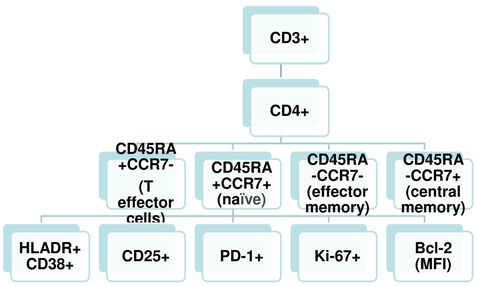
Cell Populations

- The following cell populations were quantified:
  - Effector CD4+ cells as % of CD3+ cells (CD45RA+CCR7-)
  - Naïve CD4+ cells as % of CD3+ cells (CD45RA+CCR7+)
  - Effector memory (EM) CD4+ cells as % of CD3+ cells (CD45RA-CCR7-)
  - Central memory (CM) CD4+ cells as % of CD3+ cells (CD45RA-CCR7+)
  - HLA-DR+CD38+ as % of naïve, EM and CM CD4+CD3+ cells
  - CD25+ as % of naïve, EM and CM CD4+CD3+ cells
  - CD279+ as % of naïve, EM and CM CD4+CD3+ cells
  - Ki-67+ as % of naïve, EM and CM CD4+CD3+ cells
  - Mean Bcl-2 expression of naïve, EM and CM CD4+CD3+ cells divided by mean Bcl-2 expression of all PBMCs in the sample

Endpoints

	Chipcytometry		Flow Cytometry	
Epitope	**Final clone**	**Fluorophore**	**Final clone**	**Fluorophore**
CD3	UCHT1	BUV395	SK7	AF488
CD4	RPA-T4	PerCP-Cy5.5	RPA-T4	PE-Cy7
CD45RA	HI100	BUV395	HI100	PerCP-Cy5.5
CCR7	G043H7	PE	G043H7	PE
CD38	HB7	BUV395	HB7	BV605
HLA-DR	L243	Alexa Fluor 488	G46-6	AF700
CD25	M-A251	PE	M-A251	APC-Cy7
PD-1	MIH4 / EH12.1	PE	EH12.1	BV510
Ki-67	Ki-67	PE	B56	AF647
Bcl-2	100	PE	Bcl-2/100	V450

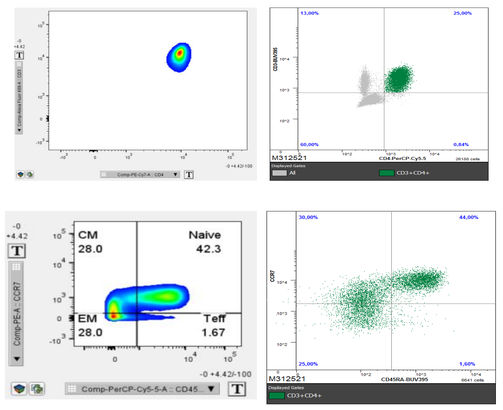
Gating Strategy



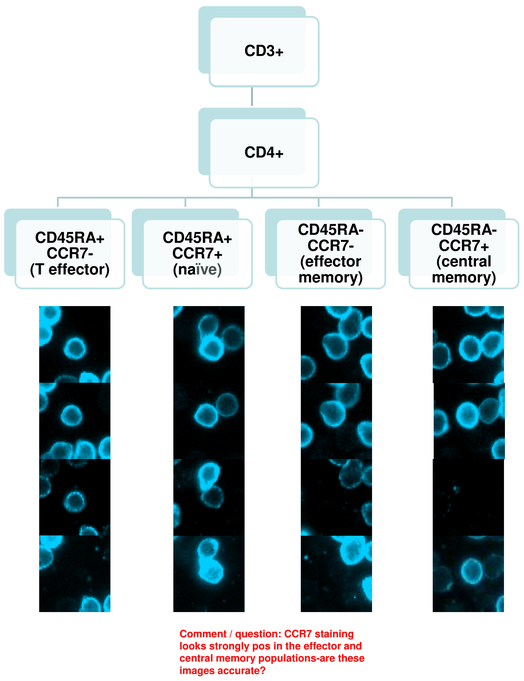
T Cell subtype Gating

Standard flow cytometry gating strategy

Chipcytometry gating strategy



Chipcytometry staining of T cell subtypes



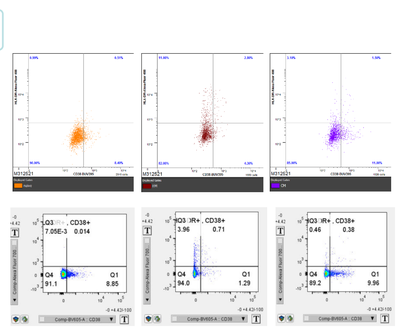
Comment / question: CCR7 staining looks strongly pos in the effector and central memory populations-are these images accurate?

Gating for T cell activation markers (HLA-DR / CD38)

HLADR+CD38+

Chipcytometry gating strategy

Standard flow cytometry gating strategy

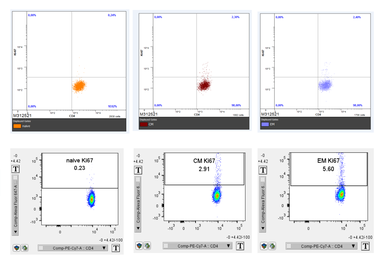


Gating for T cell proliferation marker (ki67)

Ki-67+

Chipcytometry gating strategy

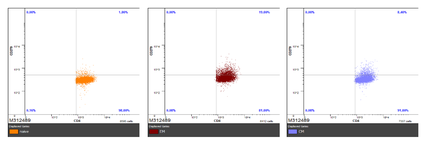
Standard flow cytometry gating strategy



Gating for CD279 (PD-1)

PD-1+

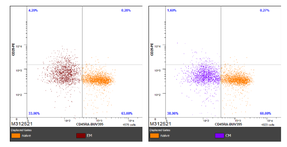
Chipcytometry gating strategy



Gating for T cell CD25 marker

CD25+

Chipcytometry gating strategy



Results – Functional Markers

	Chip 0m	Chip 6m	FACS	%RE Chip 6m vs FACS
naive CD25 %Parent	na	na	na	nd
EM CD25 %Parent	4.1	na	3.5	nd
CM CD25 %Parent	na	na	na	nd
naive Ki67 %Parent	na	na	na	nd
EM Ki67 %Parent	na	2.1	3.4	39.0
CM Ki67 %Parent	na	na	na	nd
naive bcl2 V450-A Median	1.0	1.2	1.3	12.2
EM bcl2 V450-A Median	1.0	1.5	1.3	12.1
CM bcl2 V450-A Median	1.0	1.3	1.4	11.3

Chip 0m	Chip 6m	FACS	%RE Chip 6m vs FACS
na	na	na	nd
11.0	13.0	5.6	130.9
4.0	4.3	na	nd
na	na	na	nd
2.5	1.4	5.3	nd
2.2	0.3	2.5	nd
1.2	1.0	1.7	37.6
1.4	1.0	1.4	28.6
1.3	1.0	1.5	33.6

Chip 0m	Chip 6m	FACS	%RE Chip 6m vs FACS
na	na	na	nd
5.2	na	8.9	nd
na	na	2.2	nd
na	na	na	nd
3.0	4.2	4.3	2.6
na	na	na	nd
1.7	1.3	1.5	15.2
1.7	1.6	1.3	26.2
1.6	1.5	1.5	0.7

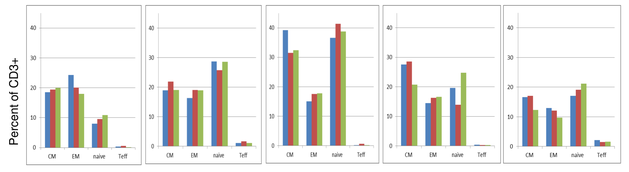
	Chip 0m	Chip 6m	FACS	%RE Chip 6m vs FACS
naive CD25 %Parent	na	na	na	nd
EM CD25 %Parent	4.2	na	9.6	nd
CM CD25 %Parent	4.0	na	1.9	nd
naive Ki67 %Parent	na	na	na	nd
EM Ki67 %Parent	7.2	8.5	8.8	2.9
CM Ki67 %Parent	2.6	3.1	2.2	40.3
naive bcl2 V450-A Median	1.3	1.7	1.6	3.8
EM bcl2 V450-A Median	1.1	1.0	1.2	21.1
CM bcl2 V450-A Median	1.2	1.1	1.7	34.4

Chip 0m	Chip 6m	FACS	%RE Chip 6m vs FACS
na	na	na	nd
6.5	na	5.7	nd
3.0	na	na	nd
na	na	na	nd
4.4	4.7	6.1	23.1
3.3	3.3	3.1	7.5
1.8	1.1	1.3	13.2
0.7	1.3	1.2	8.0
1.1	1.3	1.3	2.9

Average %RE Chip 6m vs FACS
nd
nd
nd
nd
16.9
23.9
16.4
19.2
16.6

- na: values <2 % or not measureable by ChipCytometry. nd: %RE not determined due to insufficient data
- HLA-DR +<CD38+ % parent values not included since all values <2%/na
- PD-1 data not included since different clones used between Chip and FACS.

Results – T Cell Subtypes

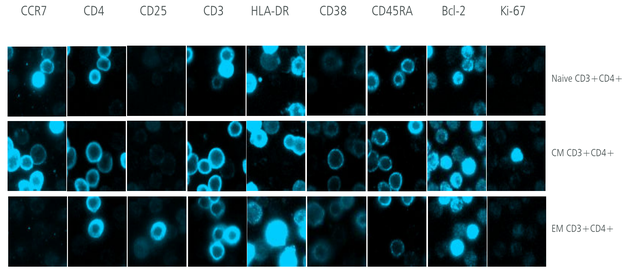


452 508 843 12115 12177

		Chip 0m	Chip 6m	FACS	%RE Chip 6m vs FACS
donor 452	CM	18.5	19.4	20	2.9
	EM	24.3	20.0	18	11.3
	naive	8.0	9.5	10.9	12.8
	Teff	0.4	0.5	0.07	NA
donor 508	CM	18.9	21.8	19	14.8
	EM	16.3	19.0	18.9	0.6
	naive	28.7	25.7	28.6	10.2
	Teff	1.1	1.7	1.13	NA
donor 843	CM	39.2	31.5	32.4	2.8
	EM	15.0	17.6	17.8	1.2
	naive	36.7	41.4	38.8	6.8
	Teff	0.2	0.7	0.17	NA
donor 12115	CM	27.5	28.6	20.8	37.4
	EM	14.5	16.3	16.6	1.6
	naive	19.7	13.9	24.8	43.9
	Teff	0.4	0.4	0.36	NA
donor 12177	CM	16.6	17.0	12.3	38.6
	EM	12.9	12.1	9.67	25.1
	naive	17.0	19.1	21.2	10.1
	Teff	2.2	1.5	1.63	NA

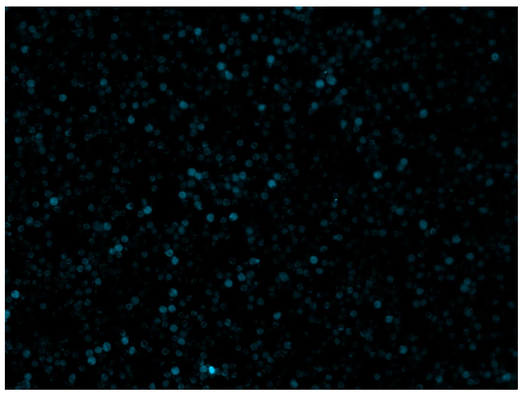
	Average % RE Chip 6m vs FACS
CM	19.3
EM	7.9
naive	16.7
Teff	NA

Representative Chip Staining Across Markers

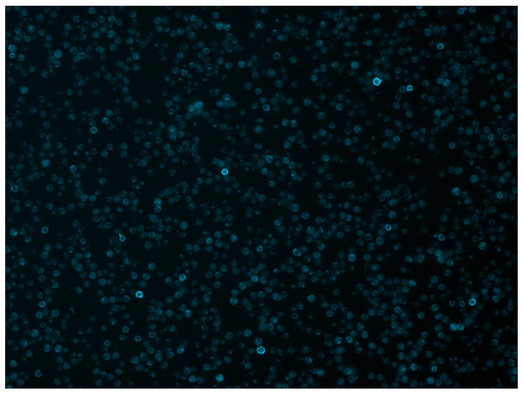


Comparison of CD279 (PD-1) Clones by Chipcytometry

CD279 MIH4 (T = 0)



CD279 EH12.1 (T = 6)



At T=0, PD-1 mAb clone MIH4 was used for chipcytometry, demonstrating weaker staining. At T=6, the EH12.1 clone was used, as was used for the flow cytometry analysis at T=0. This lead to greater staining intensity and more concordant results with flow cytometry.

Conclusions

- For major T cell populations* the relative error (%RE) between T = 0 and T = 6 for Chipcytometry data was less than 20% for all but one measurement. ** The average %RE for T cell subtype and T functional markers, comparing Chipcytometry T=6 data to FACS, was also <20%.
- The initial use of different CD279 clones for flow (EH12.1) and Chipcytometry (MIH4) resulted in large discrepancies at T = 0, but the clones were harmonized at T = 6
- All but one (CD25) biomarker were stable after storage of the chips for 190 days. In the future this can be remedied by staining CD25 along with CCR7 prior to fixation.
- Chipcytometry demonstrated good concordance with flow cytometry for the T cell phenotypic and functional markers tested. This study also highlights the importance of methodological considerations, including optimal clone selection and testing of pre- vs. post-fixation antibody binding.

* naïve, central memory and effector memory CD4+ cells as % of CD3+ cells

** 29% for naïve CD4+CD3+ cells of donor 12115