Chipcytometry: Long-term Sample Storage and Re-Interrogation in Clinical Trial Support

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Introduction

Cytometry is a platform for analysing cellular heterogeneity in clinical studies. Flow cytometry samples often have to be run real-time, making careful planning ahead of the study crucial: The staining protocol has to be established, sample stability ensured and data acquisition and analysis standardized across multiple laboratories. Furthermore, samples are discarded post-analysis, making sample reanalysis impossible.

Chipcytometry is an image-based cytometric system that has been designed as an alternative platform to overcome those limitations of flow cytometry. [1] It combines high-plex quantitative phenotyping, cell imaging, sample preservation and long-term biomarker integrity. Logistics is simplified as fixed cells are easily shipped refrigerated to a central laboratory and stored for up to 24 months.

The aim of this study was to investigate the potential benefit of using Chipcytometry for high-dimensional biomarker analysis/re-analysis of the same whole blood-derived sample after long-term storage.

Methods

Peripheral Blood Mononuclear Cells (PBMCs) have been isolated from healthy volunteers blood using BD CPT Tubes. Samples were analyzed using Zellkraftwerk fully-automated Chipcytometry instrument CYTOBOT™ and BD FACSCanto II. Monoclonal antibodies against the proteins listed in **Table 1** were used for the assay. The biomarkers were chosen to represent a typical marker panel ranging from common lineage biomarkers to immune check point proteins. Sequential immune phenotyping was performed according to the Chipcytometry principle **(Figure 1)**. Data analysis was performed either using DIVA software (BD FACSCanto II data) or using Zellkraftwerk ZellExplorer software and the statistical software language R (Chipcytometry data). [3] Firstly 10-plex assay and gating strategy haven been developed, followed by an intra-assay and inter-assay precision assessment for chipcytometry. Next platform cross-comparison was performed. Finally sample and biomarker stability were assessed up to 6 months on the CYTOBOT™ **(Figure 1).**

Table 1: Antibodies used for platform cross-comparison

Epitope	Clone	Vendor
CD3	UCHT1	BD
CD4	RPA-T4	Biolegend
CD8	RPA-T8	BD
CD14	HCD14	Biolegend
CD16	3G8	Biolegend

Epitope	Clone	Vendor
CD19	SJ25C1	BD
CD45	HI30	BD
CD56	AF12-7H3	Miltenyi
CD278 (ICOS)	C398.4A / ISA-3*	Biolegend / Thermo Fisher
CD279 (PD-1)	EH12.1	Thermo Fisher

* Used for Flow Cytometry

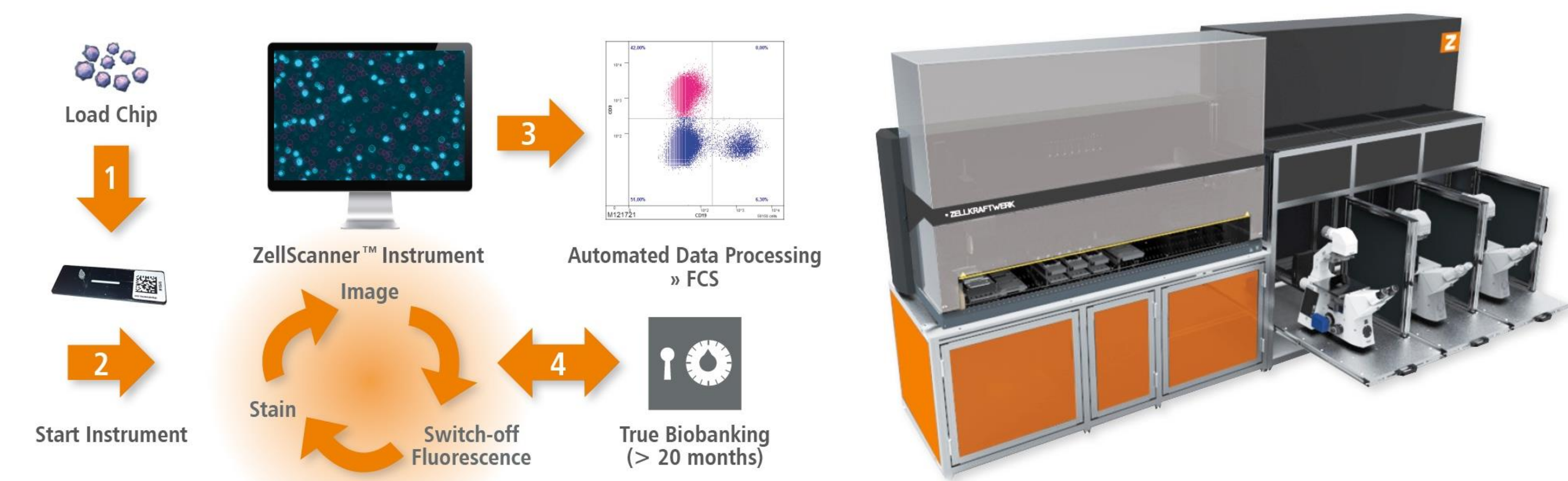

Figure 1: Chipcytometry principle (left) and instrument (right)

Results

Precision assessment in Chipcytometry

For the intra-assay precision assessment, a single sample was split across multiple chips to measure the level of reproducibility. In contrast, the inter-assay precision was defined as the combined level of variability from different sample preparations (PBMC isolation tubes) and intra-assay variation. Both precision measures were assessed for three donors. The mean intra-assay and inter-assay coefficient of variation (CV) was 13.4% and 18.3%, respectively **(Figure 2)**. Both CVs were inversely correlated with the size of the cell population (0.02-65%). With this performance, this assay is within the recommended precision for cytometry testing during drug development.[2]

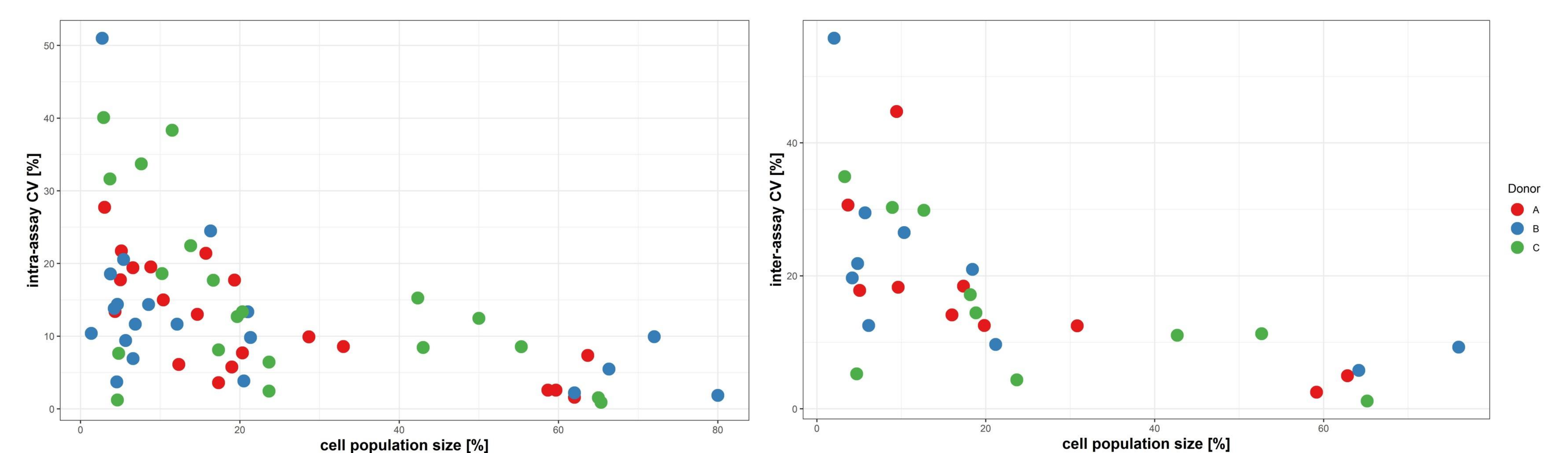

Figure 2: Intra- and inter-assay CV in Chipcytometry

Cross-comparison Flow cytometry vs. Chipcytometry

Cell frequencies as measured in Flow and in Chipcytometry at time point 0 (fresh on day of preparation for Flow cytometry, 1-2 weeks after preparation for Chipcytometry) for three different donors are shown in **Table 2**. For the majority of the analysed cell populations, the results obtained by both technologies are comparable, the CVs ranging from 0.1% to 23.6%. For the ICOS-positive cells, there is a significant discrepancy, with Chipcytometry yielding consistently higher results than Flow cytometry. Investigation of this issue revealed that different clones were used on CYTOBOT and FACSCanto (C398.4A and ISA-3, respectively). When the clones were compared on a single system, ISA-3 stained less cells than C398.4A.

Table 2: Cell frequencies in Flow and Chipcytometry

DonorD	B cells out of CD45+ CD14-	T cells out of CD45+ CD14-	CD4+ out of T cells	CD8+ out of T cells	NK cells out of CD45+ CD14-	CD16+ out of CD45+	Monocytes out of CD45+	PD1pos out of CD4	ICOSpos out of CD4	PD1pos ICOSpos out of CD4
Flow	7.0	64.7	49.6	38.9	24.6	18.3	28.1	49.7	1.6	1.5
Chip	6.6	62.5	47.5	39.5	24.5	19.5	29.0	58.0	4.6	4.1
mean	**6.8**	**63.6**	**48.6**	**39.2**	**24.5**	**18.9**	**28.5**	**53.8**	**3.1**	**2.8**
CV (%)	**3.7**	**2.4**	**3.1**	**1.1**	**0.1**	**4.5**	**2.4**	**10.9**	**66.3**	**67.1**

DonorE	B cells out of CD45+ CD14-	T cells out of CD45+ CD14-	CD4+ out of T cells	CD8+ out of T cells	NK cells out of CD45+ CD14-	CD16+ out of CD45+	Monocytes out of CD45+	PD1pos out of CD4	ICOSpos out of CD4	PD1pos ICOSpos out of CD4
Flow	5.2	58.0	61.4	22.8	28.3	18.8	38.5	44.4	1.3	1.0
Chip	6.2	59.5	61.5	22.5	22.5	17.5	28.0	47.5	4.0	3.4
mean	**5.7**	**58.8**	**61.4**	**22.6**	**25.4**	**18.1**	**33.3**	**46.0**	**2.6**	**2.2**
CV (%)	**12.4**	**1.8**	**0.2**	**0.8**	**16.1**	**4.9**	**22.3**	**4.7**	**73.2**	**75.2**

DonorF	B cells out of CD45+ CD14-	T cells out of CD45+ CD14-	CD4+ out of T cells	CD8+ out of T cells	NK cells out of CD45+ CD14-	CD16+ out of CD45+	Monocytes out of CD45+	PD1pos out of CD4	ICOShi out of CD4	PD1pos ICOShi out of CD4
Flow	3.9	79.0	42.6	53.8	14.1	14.0	22.6	54.5	1.2	1.0
Chip	3.6	78.5	43.5	53.0	12.5	10.0	20.5	49.0	1.9	1.6
mean	**3.8**	**78.8**	**43.0**	**53.4**	**13.3**	**12.0**	**21.5**	**51.8**	**1.5**	**1.3**
CV (%)	**5.7**	**0.4**	**1.6**	**1.1**	**8.5**	**23.6**	**6.7**	**7.5**	**31.8**	**30.0**

Biomarker and sample stability on ZellSafe™ chips

After 6 months storage, replicate chips were analysed with the same marker panel as the chips at time point 0. The mean % deviation between 0 and 6 months was 11.2% **(Figure 3)**. All but 2 cell populations for the three donors were within the 25-30% accepted deviation level as has been recommended for cell-based fluorescence assays.[4] Again, the CV was inversely correlated with the size of the cell population.

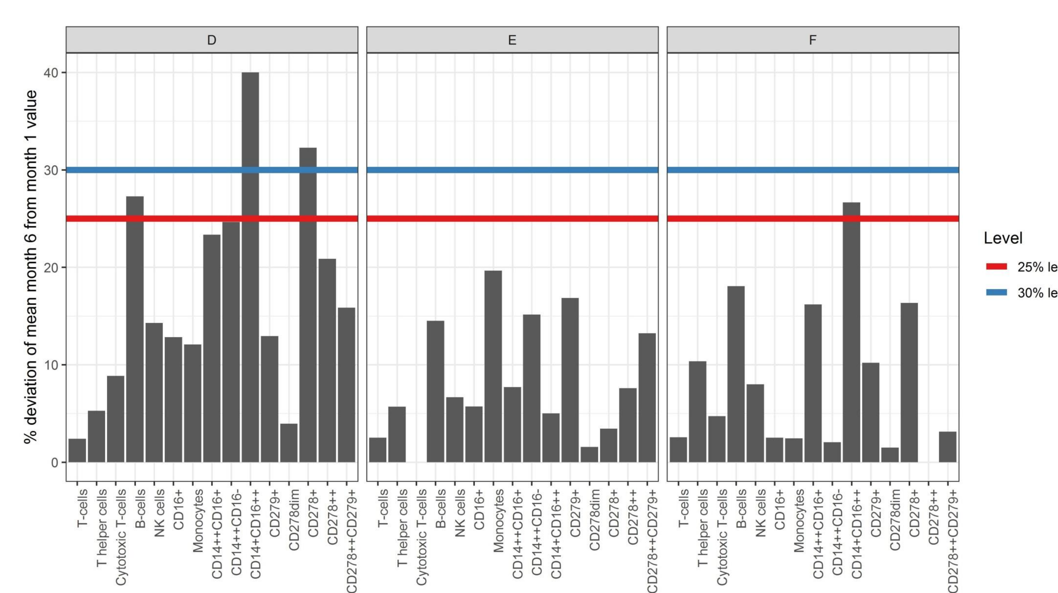

Figure 3: Biomarker stability on ZellSafe™ chip after 6 months for Donors D, E and F

Conclusions

- Cell frequencies as measured with Flow and Chipcytometry are very similar
- Chipcytometry provides an alternative cytometric platform to archive PBMC for batch analysis upon long-term storage
- It also permits retrospective analysis/re-analysis for novel biomarkers that may not have been envisioned at the beginning of a clinical study

References

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