Comparison of Human Whole Blood Immunophenotyping by ChipCytometry and Flow Cytometry: Potential Applications for Biomarker Identification and Immunomonitoring in Clinical Studies

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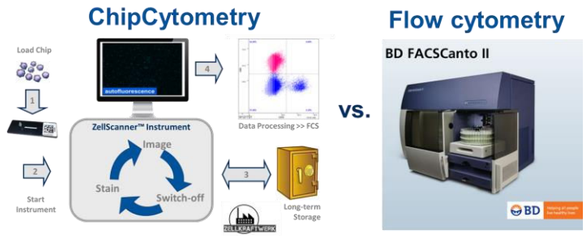
Abstract

Purpose: Flow cytometry is a platform for analyzing cellular heterogeneity and identifying biomarkers in clinical studies. However, whole blood samples for conventional flow cytometry have to be run real-time (within 1-3 days) and discarded post-analysis, making sample reanalysis impossible and sample handling costly for multi-center clinical trials. Chipcytometry is an image-based cytometric system that has been designed as an alternative platform to overcome those limitations of flow cytometry. The aim of this study was to investigate the potential benefit of using chipcytometry for high-dimentional biomarker analysis/re-analysis of the same sample after long-term storage as compared to real-time assessments using flow cytometry.
Methods: Human whole blood from 6 healthy donors was processed for erythrocyte lysis and then loaded to microfluidic chips for storage at 4°C till analysis. Monoclonal antibodies against 21 protein markers were used sequentially to phenotype immune cells immobilized on microfluidic chips. The list-mode data acquired after multiple staining/photobleaching cycles were analyzed with FlowCore/FlowJo to define a variety of cell subpopulations. For comparison, the same whole blood samples were also processed and stained with three 8-color panels of antibodies against the same 21 protein markers for flow cytometry.
Results: The fluorescence signal of all 21 protein markers measured by chipcytometry did not change significantly after 4-week storage. The coefficient of variation (CV) for the nine representative markers (CD3/CD4/CD8 on T cells, CD19/IgD/CD24 on B cells, and CD16/CD11b/CD24 on granulocytes) was 6-22%. Major immune cell populations exhibited comparable cytometric profiles (frequencies) when identified by either chipcytometry or flow cytometry, including CD4+ T cells (60 vs. 59%), CD8+ T cells (27 vs. 24%), naïve B cells (67 vs. 78%), classical monocytes (66 vs. 68%), and mature neutrophils (92 vs. 98%). Moreover, certain low-frequency subpopulations of T cells, B cells, monocytes and neutrophils were also delineated quantitatively using marker sets more flexible by chipcytometry than by flow cytometry due to flow panel restriction.
Conclusion: Chipcytometry provides an alternative cytometric platform to bank whole blood samples 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.



Methods

	ChipCytometry (Chip)	Flow cytometry (Flow)
Procedure	RBC-lysed whole blood cells were loaded/fixed on ZellSafe Chips, shipped to Germany, and analyzed with ZellScannerONE at ZELLKRAFTWERK after 7, 14, and 28 days of storage at 4 °C.	RBC-lysed whole blood cells from the same donor were analyzed at MedImmune with FACS Canto II on Day 0 (< 2 h post-collection)
Protein markers	CD45, CD3, CD4, CD8a, CD45RA, CCR7, CCR6, CXCR3, CD19, CD27, IgD, CD38, CD24, CD14, CD16, CD11b, CD11c, CD123, CD56, CD95, HLA-DR.	**T cell panel :** CD45, CD3, CD4, CD8, CD45RA, CCR7, CCR6, CXCR3. **B cell panel :** CD45, CD3/CD14, CD19, CD20, CD24, CD27, CD38, IgD. **Neutrophil/Monocyte panel :** CD45, CD14, CD16, CD11b, CD11c, HLA-DR, CD123, Lin-1.
Cell collection	~ 30,000 total cells	~200,000 total cells
Compensation	No	Yes
Raw data	Listmode (CSV)	Listmode (compensated FCS)
Data processing	R-language (flowCore) and FlowJo (TreeStar); automated, cutoff-based gating	

Results

Figure 1: The fluorescence signal of all 21 protein markers measured by chipcytometry did not change significantly after 4-week storage

- Arcsinh transformation instead of logarithmic transformation is used to deal with negative values.
- A typical box and whiskers plot is used to show the median of data from 6 donors as a horizontal line in the middle of the box.
- The upper and lower "hinges" correspond to the first and third quartiles (the 25th and 75th percentiles). The upper (lower) whisker extends from the hinge to the highest (lowest) value that is within 1.5 * IQR (inter-quartile range) of the hinge.
- Data beyond the end of the whiskers are outliers and plotted as points.

Figure 2: The mean coefficient of variation (CV) for 9 representative markers was 6 - 22%

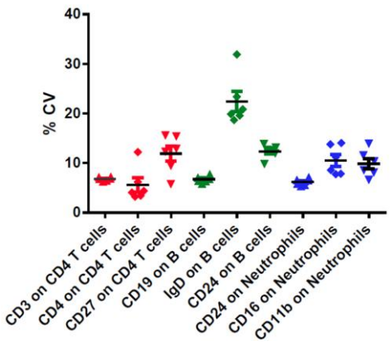
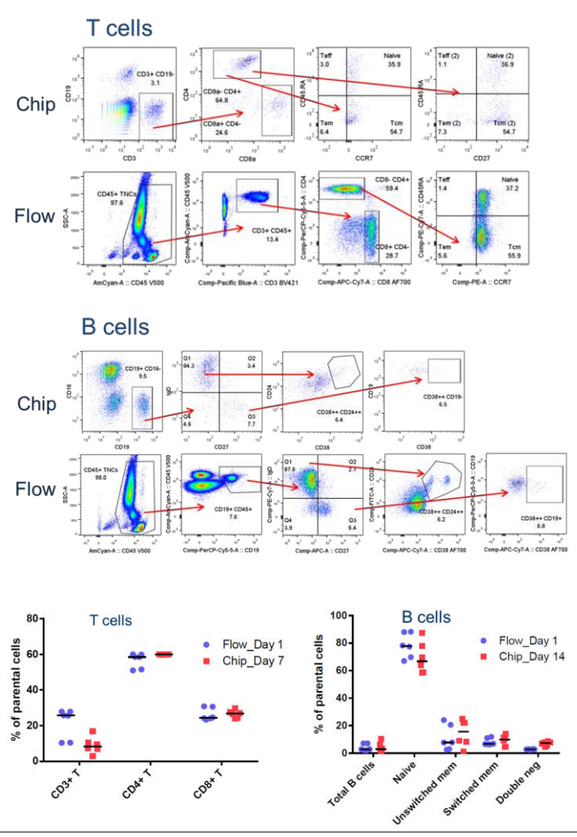
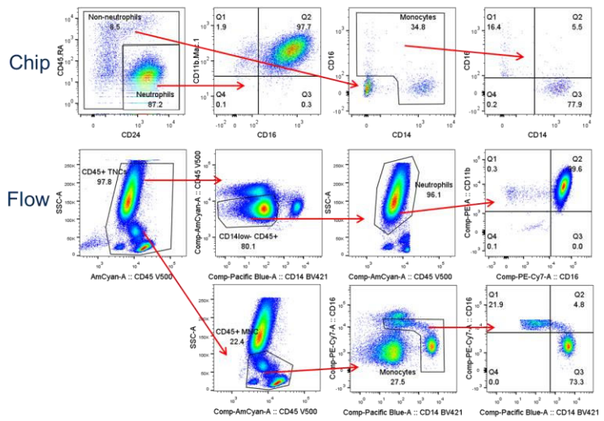
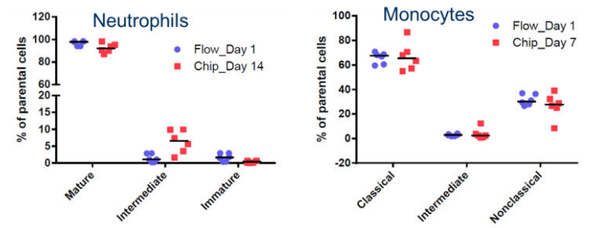


Figure 3: Comparable cytometric profiles of major immune cell populations assessed by Chip vs. Flow



Neutrophils and Monocytes





Conclusions

- Major immune cell populations in whole blood samples exhibited comparable cytometric profiles (frequencies) when assessed by ChipCytometry several weeks post blood collection vs. flow cytometry a couple of hours post blood collection.
- Chipcytometry provides an alternative cytometric platform to bank whole blood samples for high-parameter batch analysis upon long-term storage.
- ChipCytometry permits retrospective analysis/re-analysis for novel biomarkers that may not have been envisioned at the beginning of a clinical trial or sponsored research agreement.
- Chipcytometry-based assay is worth further exploration to implement for biomarker identification and immunomonitoring in the clinical development of immune-based therapeutics for oncology, RIA, and infectious diseases.

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