Precise Spatial Immune Profiling of PBMC Populations Using a VistaPlex™ ChipCytometry™ Assay Kit

Adam Northcutt, PhD[1]; Karen Kwarta, PhD[1], Tim Sindelar, MS[1]
[1]Canopy Biosciences, St. Louis, MO USA

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Abstract

Blood is a highly valuable clinical analyte and has the potential to inform the treatment of a variety of diseases – from infectious diseases to solid tumors. The gold standard in most cell suspension immunophenotyping is flow cytometry. ChipCytometry, a modified approach to slide-based cytometry, is a novel, highly multiplexed technology that preserves both plex and spatial context to deeply profile immune cell diversity at single-cell resolution. ChipCytometry uses commercially available antibodies and combines iterative immuno-fluorescent staining with high-dynamic range imaging to profile dozens of protein biomarkers in a single tissue specimen. Cellular phenotypes are identified with via flow cytometry-like hierarchical gating from standard multichannel OME-TIFF images, compatible with a variety of computational tools being developed for multiplexed analysis and visualization. Further, ChipCytometry can be applied to both cell suspension and tissue samples. A key requirement for spatial technologies to become a more utilized tool in immunology is reproducibility. Here, we use a broad 16-plex immune profiling assay kit to identify and quantify key immune cell subtypes in PBMCs from multiple donors across multiple storage conditions. The results show precise expression levels for each biomarker in the assay through a reliable and customizable assay kit.

Methods

Highly multiplexed image data was collected via the ChipCytometry™ workflow (Fig. 1) on the CellScape™ instrument using a 16-plex antibody panel (Table 1). After data collection, images were analyzed with built-in CellScape software with hierarchical gating to classify key immune cell types.

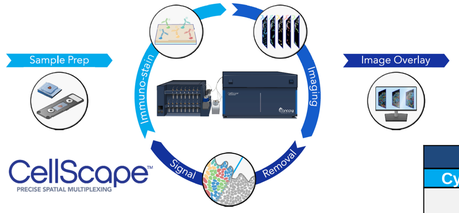

Figure 1. ChipCytometry workflow. After sample preparation, the ChipCytometry workflow consists of successive rounds of immuno-staining, imaging, and signal removal to profile virtually unlimited number of protein targets. An image overlay of each marker in the assay is created by aligning each channel to a reference channel. The process is fully automated with CellScape, the next-generation instrument for precise spatial multiplexing.

Table 1. Antibody Staining

Cycle	Target	Dilution	Fluor
1	CD3	1:300	PerCP-Cy5.5
	CD25	1:60	PE
	CD39	1:30	FITC
	CD8	1:100	BV421
2	CD4	1:30	PerCP-Cy5.5
	CD27	1:300	PE
	CD45RA	1:100	FITC
	CD45	1:300	BV421
3	CD56	1:60	PerCP-Cy5.5
	CD16	1:60	PE
	CD14	1:30	FITC
	CD19	1:100	BV421
4	CD279	1:30	PerCP-Cy5.5
	CD11c	1:300	PE
	HLA-DR	1:100	FITC
	CD123	1:30	BV421

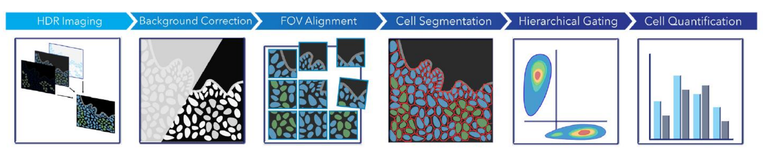

Figure 2. ChipCytometry image processing and analysis. Image process and analysis is managed the CellScape App and includes 6 key steps: (i) multi-exposure HDR image fusion, (ii) background correction, (iii) FOV alignment, (iv) cell segmentation, (v) hierarchical gating, and (vi) cell quantification.

Results

Highly multiplexed image data was collected via the ChipCytometry™ workflow (Fig. 1) on the CellScape™ instrument using a 16-plex antibody panel (Table 1). After data collection, images were analyzed with built-in CellScape software with hierarchical gating to classify key immune cell types.

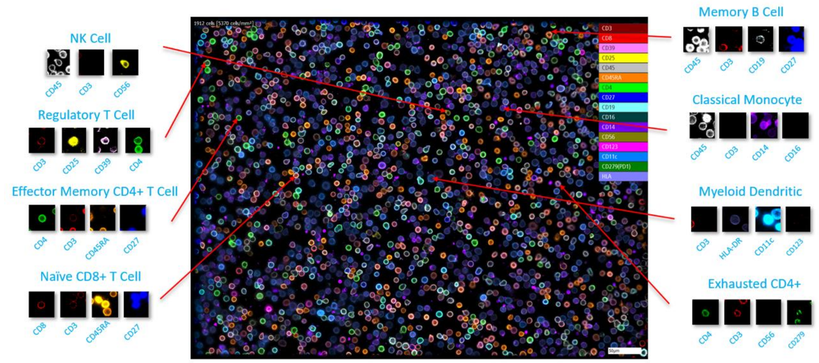

Figure 3a. Highly multiplexed image of healthy PBMCs. A 16-plex antibody stain was applied to healthy PBMC suspension. Here, we show a subset of single marker channel images used to define specific cell phenotypes.

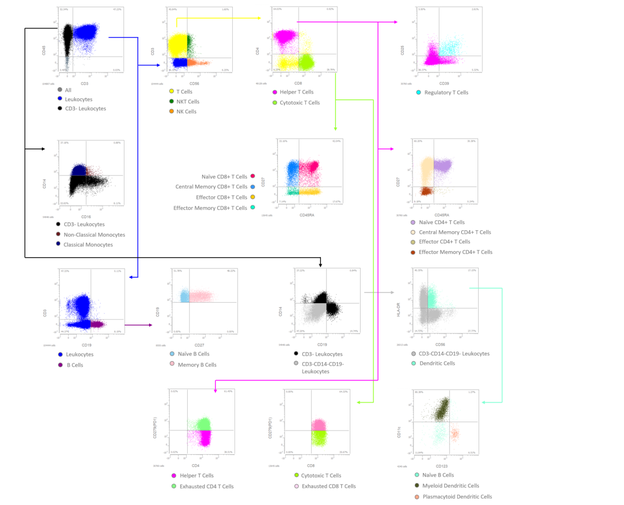

Figure 3b. Hierarchical gating with CellScape app. From image data, each cell is captured by automated segmentation, and the biomarker expression is quantified using HDR imaging to measure fluorescence intensity for every cell. Standard FCS files are generated to enable the identification of cell phenotypes via classic flow cytometry-like hierarchical gating.

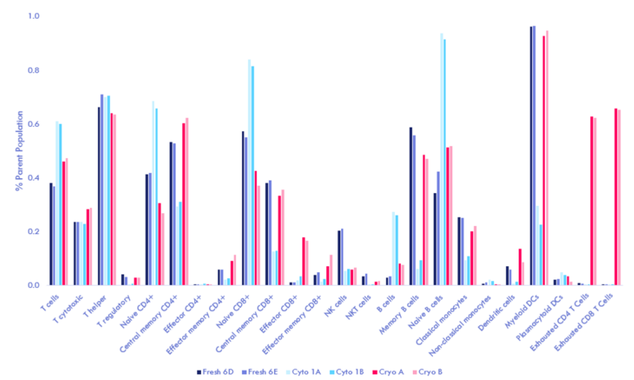

Figure 3c. Immune phenotype quantification. Fresh PMBC samples were used to confirm intra-assay and inter-assay reproducibility. To ensure high quality data can be obtained on a variety of sample types, the assay kit was successfully tested on cryopreserved samples and samples collected with Cyto-Chex BCT tubes.

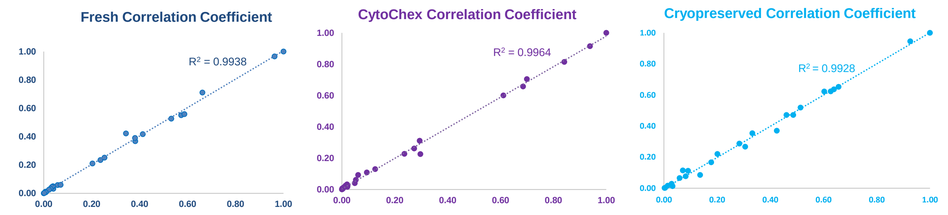

Figure 3d. Correlation plot. Each dot represents a single quantified immune population with one technical replicate on the X-axis, and the other on the Y-axis

Conclusions

- We demonstrate the utility of ChipCytometry to generate highly-multiplexed, spatially resolved protein expression data from multiple samples under multiple storage conditions. We show quantitative measurement of 16 clinically relevant biomarkers at the single-cell level for every cell in the suspension sample
- Strong correlation between replicates in multiple storage conditions demonstrates the ability for ChipCytometry assay kits to capture biological variability, while maintaining technical reproducibility
- Future experiments comparing spatial cell immunophenotypic composition between suspension and tissue, eg lymph and tumor biopsy, from the same patient would be useful as proof-of-concept for larger patient studies

Contact

Tim Sindelar
Product Manager, Spatial Biology
timothy.Sindelar@bruker.com

Selected Publications

1. Hummert, M., Alvermann, S., Gingele, S., Gross, C., Wiendl, H., Mirenska, A., Hennig, C., Stangel, M. Immunophenotyping of Cerebrospinal Fluid Cells by ChipCytometry. (2018). J Neuroinflammation. May 25;15(1). doi: 10.1186/s12974-018-1176-7
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3. FitzPatrick, M., Provine, N., Garner, L., Powell, K., Amini, A., Irwin, S., Ferry, H., Ambrose, T., Friend, T., Vrakas, G., Reddy, S., Soilleux, E., Klenerman, P., Allan, P. (2021). Human Intestinal Tissue-Resident Memory T Cells Comprise Transcriptionally and Functionally Distinct Subsets. Cell Reports.Jan 34;3. doi: 10.1016/j.celrep.2020.108661
4. Leng, T., Akther, H., Hackstein, C.P., Powell, K., King, T., Friedrich, M., Christoforidou, Z., McCuaig, S., Neyazi, M., Arancibia-Carcamo, C., Hagel, J., Powrie, F., Peres, R., Millar, V., Ebner, D., Lamichhane, R., Ussher, J., Hinks, T., Marchi, E., Willberg, C., Klenerman, P. (2019). TCR and Inflammatory Signals Tune Human MAIT Cells to Exert Specific Tissue Repair and Effector Functions. Cell Reports. Sept 28;12:3077-3091. doi: 10.1016/j.celrep.2019.08.050