Spatial Immunoprofiling of Active Celiac Disease Tissue with ChipCytometry™

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Poster P962

Abstract

Highly multiplexed spatial biomarker analysis has demonstrated the potential to advance our current understanding of the immune system and its role in immunology, from tumor initiation to immune reaction. Previously, a trade-off between plex and spatial context meant that our understanding of immune cell involvement in immunology was limited to providing deep information on either cell phenotypes or their spatial context, but not both. ChipCytometry 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. Here, we use ChipCytometry to identify and quantify key immune cell subtypes in healthy and active celiac disease (ACD) tissues. The results show precise expression levels for each biomarker in the assay in each individual cell in the sample, while maintaining spatial positioning of each cell. Spatial analysis using the Enable Medicine platform reveals quantifiable immune cell phenotype composition and interaction difference between healthy and active celiac disease samples, demonstrating the utility of the ChipCytometry platform for the in-depth immune profiling in samples.

Methods

Highly multiplexed image data was collected via the ChipCytometry™ workflow (Fig. 1) on the CellScape™ instrument using a 21-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. A multi-channel stitched OME-TIFF was generated and uploaded to Enable Medicine platform as a proof of concept and for additional analyses including unsupervised clustering.

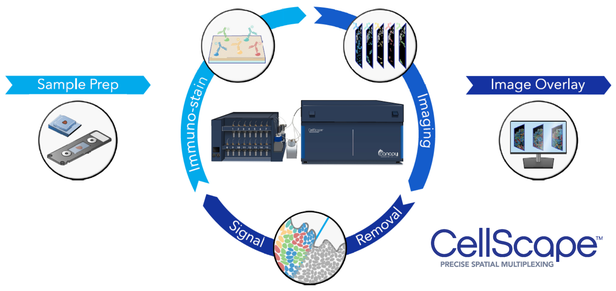

Figure 1. ChipCytometry image acquisition workflow. After sample preparation, the ChipCytometry workflow consists of successive rounds of immuno-staining, imaging, and signal removal to profile virtually unlimited number of protein targets. An image overlay of each marker in the assay is created by aligning 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	
Target	**Dilution**
CD3	1:100
CD4	1:100
CD8	1:200
CD68	1:300
CD11c	1:200
CD45RA	1:1800
CD14	1:100
CD56	1:100
CD20	1:400
CD279 (PD-1)	1:100
CD45	1:500
HLA-DR	1:600
CD45RO	1:900
CD123	1:100
Pan-CK	1:1800
Ki-67	1:100
CD326 (EpCAM)	1:1800
FoxP3	1:100
CD31	1:100
CD38	1:30
DNA (Hoeschst)	1:50000
DNA (PI)	1:20000

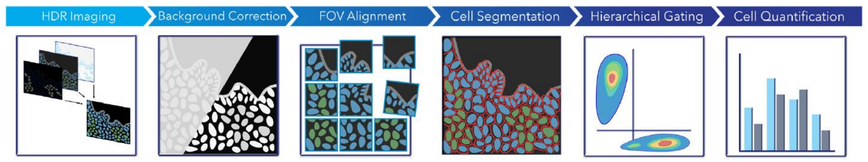

Figure 2. ChipCytometry image processing and analysis. Image process and analysis is managed the CellScape App and includes 6 key steps: (i) multi-exposure HDR image fusion, (ii) background correction, (iii) FOV alignment, (iv) cell segmentation, (v) hierarchical gating, and (vi) cell quantification.

Table 2. ChipCytometry Protocol	
Sample Preparation	
Step 1	5 µm FF tissue sections were mounted onto glass coverslips
Step 2	Sections were loaded onto chips to preserve sample integrity during serial delivery of reagents
Data Collection	
Step 3	An initial autofluorescence scan was performed to identify ROIs in the tissue
Step 4	Tissue sections were stained with up to 5 fluorescent antibodies from commercial vendors and incubated at 15 min at RT
Step 5	Tissue sections were imaged in up to 5 channels using HDR multi-exposure imaging and high-resolution optics
Step 6	Tissue sections were photobleached to remove fluorescence signal
Step 7	Steps 4-6 were repeated in successive rounds until all targets were imaged (Table 1)
Image Analysis	
Step 7	Multi-exposure HDR image fusion and background correction for individual FOVs was performed using CellScape software
Step 8	Cell segmentation, hierarchical gating, and cell quantification were performed using CellScape software
Step 9	A custom ImageJ pipeline was used to stitch FOVs to generate a whole-slide image
Step 10	Cell segmentation, hierarchical gating and clustering were performed using Enable Medicine platform

Results

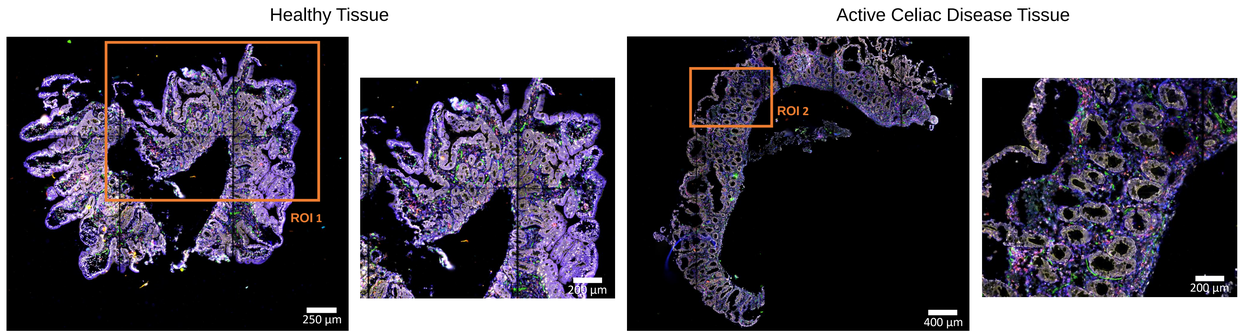

Figure 3a. Highly multiplexed image of active celiac disease tissue. A 21-plex antibody staining panel was applied to healthy and ACD tissue specimens. Here, we show whole tissue scans to highlight tissue architecture, as well as zoomed in regions of interest

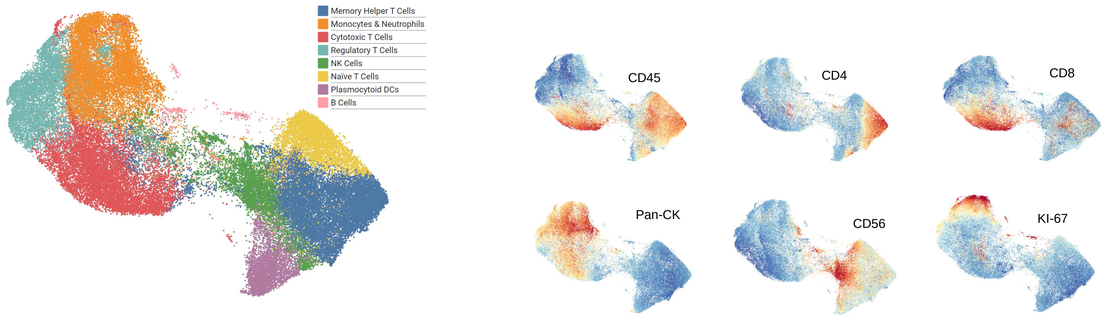

Figure 3b. UMAP unsupervised clustering of active celiac disease tissue. Plot shows the result of UMAP clustering analysis on all channels (minus DNA stain) applied to the normalized biomarker expressions of each cell, colored by the selected feature, using the Enable Medicine platform (UMAP generated using the Leiden method, starting with 50 neighbors, 0.1 UMAP min distance, resolution 0.5)

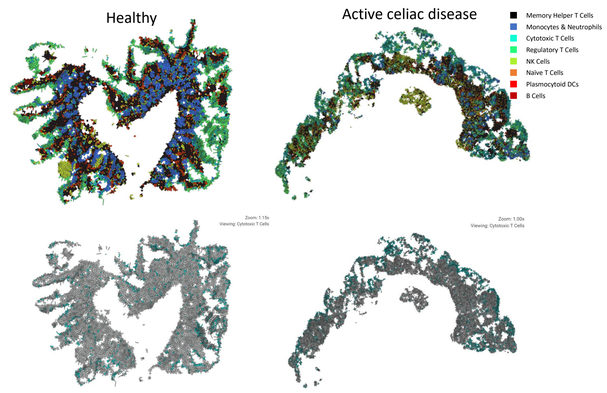

Figure 3c. Cell phenotype mapping analysis. Plot shows the result of cell phenotype mapping analysis comparing spatial cell phenotype locations across healthy and ACD tissue using the Enable Medicine platform.

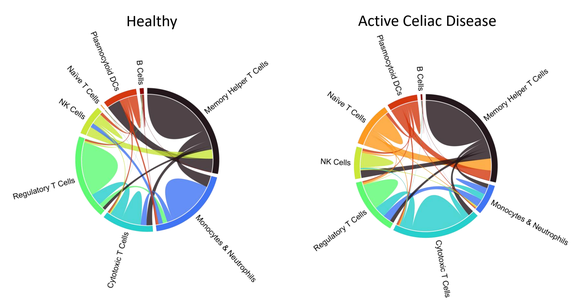

Figure 3d. Dendrogram analysis. Plot shows the result of chord analysis showing frequency of a specific cell type and interaction between cell types using the Enable Medicine platform.

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Conclusions

- We demonstrate the utility of ChipCytometry to generate highly-multiplexed spatially-resolved protein expression data from a patient sample. We show quantitative measurement of 21 clinically relevant biomarkers at the single-cell level for every cell in the tissue specimen from a patient with active celiac disease.
- ChipCytometry is a multiplexed imaging method that uses commercially available antibodies from any vendor to spatially resolve protein targets *in situ.* ChipCytometry does not require any additional abstractions, e.g. oligo-barcoding, which enables a simpler validation workflow and greater target versatility.
- We quantify relevant populations of immune subpopulations, revealing high relative abundance of T cytotoxic cells in this active celiac disease tissue. Quantification of cell populations expressing very high or low levels of a single marker is more challenging and made possible through high-dynamic range (HDR) imaging.
- We demonstrate the compatibility of ChipCytometry datasets with Enable Medicine platform to perform custom image processing and analytics, including unsupervised clustering, to better understand cellular frequencies, interactions, and neighborhoods at single-cell resolution.

Contact

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Selected Publications

1. Hagel, J., Bennet, K., Buffa, F., Klenerman, P., Willberg, C., & Powell, K. (2021). Defining T Cell Subsets in Human Tonsils Using ChipCytometry.J Immunol Jun 15;206(12):3073-3082. doi: 10.4049/jimmunol.2100063
2. 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
3. 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