Immune profiling of breast cancer tissue using CellScape™ Precise Spatial Multiplexing and the Enable Medicine Cloud Platform

Highlights

- The CellScape instrument addresses the evolving needs of spatial biology scientists by offering highly multiplexed, high throughput, and high resolution imaging of both tissues and suspension cells.
- By partnering CellScape high dynamic range image generation with Enable Medicine Cloud Platform storage and advanced data analysis capabilities, researchers can explore their tissue samples both visually and quantitatively.
- Here, human breast cancer tissue was analyzed using a 21-plex immuno-oncology antibody panel. Precise biomarker quantification and advanced data analysis facilitated the quantification of immune cell infiltrates as well as the identification of spatial patterns and cell-cell interactions.

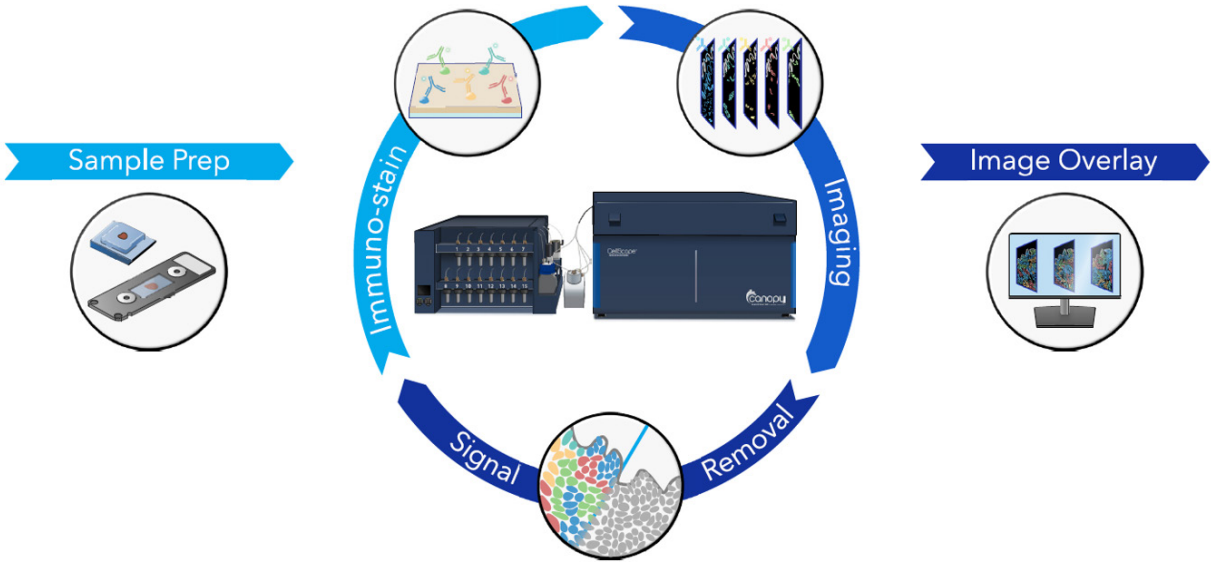

Figure 1. ChipCytometry workflow. After sample preparation, the ChipCytometry workflow consists of successive, automated rounds of immuno-staining, imaging, and photo-inactivation signal removal to profile virtually unlimited number of protein targets. All images are gathered using high dynamic range (HDR) imaging to allow for detection of high- and low-expressing cells and accurate biomarker quantification across more than 6 orders of magnitude. An image overlay of each marker in the assay is then created by aligning each channel to a reference channel.

Introduction

Spatial biology is a groundbreaking field for cancer research and detection and can be implemented for studies ranging from the identification of cancerous tissue and monitoring of immune responses to drug development and personalized medicine[1]. Key innovations in high-resolution imaging technologies and AI-driven data analysis pipelines have made advanced spatial biology research possible. ChipCytometry™ is an imaging-based technology for precise spatial multiplexing that combines iterative immuno-fluorescent staining with high-dynamic range imaging to facilitate quantitative phenotyping using fresh or FFPE tissue samples[2]. Here, the new CellScape platform, featuring an automated ChipCytometry workflow, was used to detect and quantify 21 clinically relevant biomarkers from a patient with HER2+ breast cancer with single-cell resolution. Advanced data analysis revealed key interactions between tumor and immune cells.

Methods

Human breast cancer tissue was loaded into CellScape Microfluidic Tissue Chips per manufacturer's instructions (Canopy Biosciences). Highly multiplexed image data were collected via the ChipCytometry workflow (Figure 1) on the CellScape instrument using a 21-plex antibody panel (Table 1). Specific antibodies chosen were required to have an inactivatable fluorophore but were not limited to specific vendors or conjugation methods. HDR images created by combining multiple exposures allowed for biomarker detection and quantification over 6 orders of magnitude. After data collection, images were analyzed with hierarchical gating to classify key immune cell types. A multi-channel stitched OME-TIFF was generated and uploaded to the Enable Cloud Platform from Enable Medicine for additional analyses, including unsupervised clustering and assessment of cell-cell interactions.

Table 1. 21-plex biomarker panel targets used for multiplex imaging of HER2+ breast carcinoma sample.

Biomarker Targets		
CD3	CD38	PD1
CD4	CD45	EpCAM
CD8	CD45RA	CD340
CD11c	CD45RO	DNA
CD14	CD56	HLA-DR
CD20	CD68	FoxP3
CD31	CD123	Pan-CK

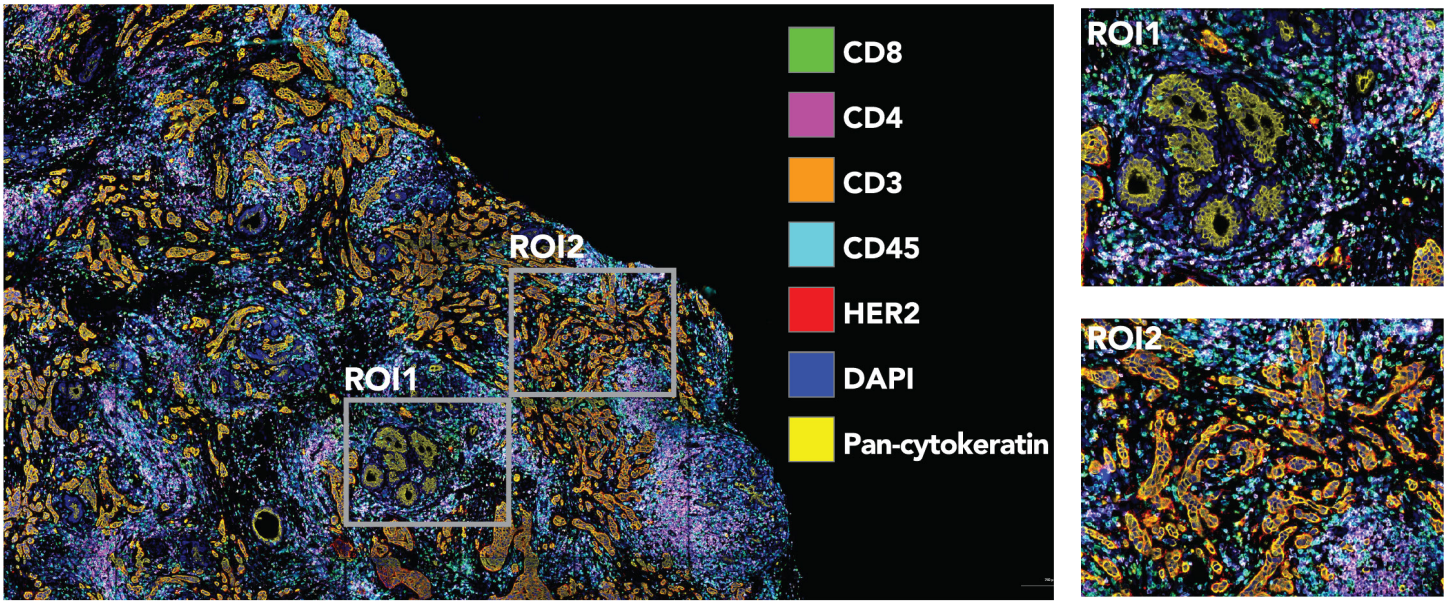

Figure 2. Highly multiplexed image of HER2+ breast carcinoma. The 21-plex antibody panel was applied to a HER2+ breast cancer tissue specimen. A subset of markers is shown to highlight tissue architecture and immune cells across 23 scan positions (18 mm2 total area). ROI 1 demonstrates HER2+ region of epithelial tissue. ROI 2 demonstrates HER2- region of epithelial tissue (Pan-CK+).

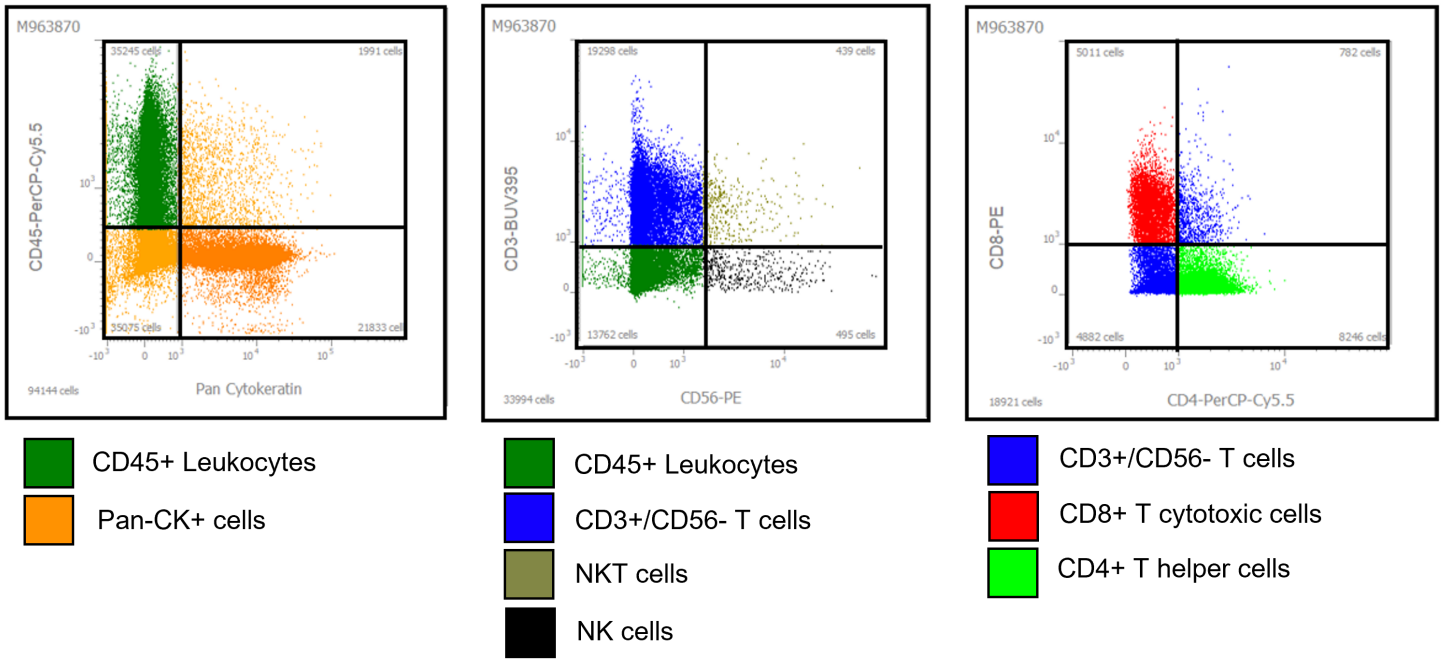

Figure 3. Hierarchical gating with CellScape software. Using the quantitative biomarker data generated from the HDR imaging, cell phenotyping was performed via flow cytometry-like hierarchical gating to identify and count key immune cell populations.

Table 2. Quantification of Immune Cell Populations.

Cell Population	Parent Gate	Gating Strategy	% of Total Leukocytes	Absolute Count
All cells	Hoechst (DNA)	N/A	N/A	94184
Leukocytes	All	CD45+ Pan CK-	100.00%	35245
T cells	CD45+ Leukocytes	CD3+ CD56-	54.75%	19298
NK cells	CD45+ Leukocytes	CD3- CD56+	1.40%	495
NKT cells	CD45+ Leukocytes	CD3+ CD56+	1.24%	439
T cytotoxic	T cells	CD4-CD8+	14.22%	5011
T helper	T cells	CD4+CD8-	23.40%	8246
T regulatory	T helper	FoxP3+	1.33%	468
B cells	CD45+ Leukocytes	CD3-CD20+	1.57%	554
Macrophages	CD45+ Leukocytes	CD68+	9.11%	3210
Dendritic Cells	CD45+ CD3- CD20- CD14-	HLA-DR+ CD56-	18.44%	6499
Myeloid DCs	Dendritic Cells	CD11c+ CD123-	13.24%	4666
Plasmacytoid DCs	Dendritic Cells	CD11c- CD123+	0.21%	74
Tumor cells	All	CD45- Pan-CK+	N/A	21833

Results

An 18 mm^2 area of human breast cancer tissue was imaged using CellScape with cyclic immunohistochemistry. Most tumor cells (Pan-CK+) were also HER2+ (Figure 2), indicating a carcinoma malignancy of epithelial origin. There was also a single region of HER2-/Pan-CK+ epithelium with relatively normal tissue architecture.

Each of the 21 markers in this assay were quantified from HDR images, allowing for discrete cell phenotyping using a hierarchical gating strategy similar to that of flow cytometry (Figure 3). Classification revealed that a majority of the cells were either leukocytes (38%) or tumor cells (23%) (Table 2). Of the leukocytes, the majority identified were helper (23%) or cytotoxic (14%) T cells.

For more in-depth spatial analysis, multichannel OME-TIFF images were exported from CellScape and uploaded to the Enable Cloud Platform from

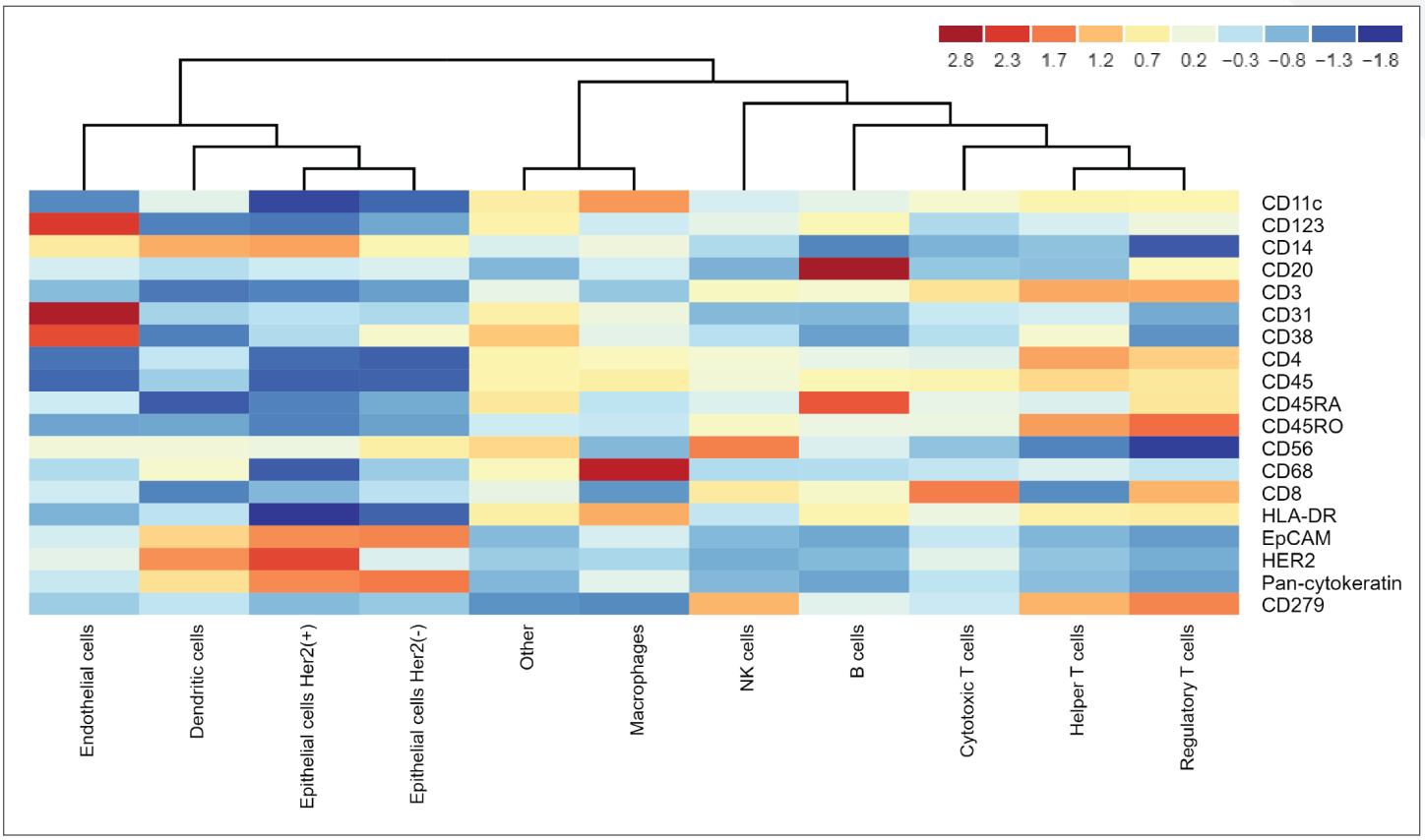

Figure 4. Unsupervised clustering with Enable Cloud Platform. Heatmap showing biomarker expression for cell types identified with AI assistance following unsupervised clustering (Leiden method) using the Enable Cloud Platform.

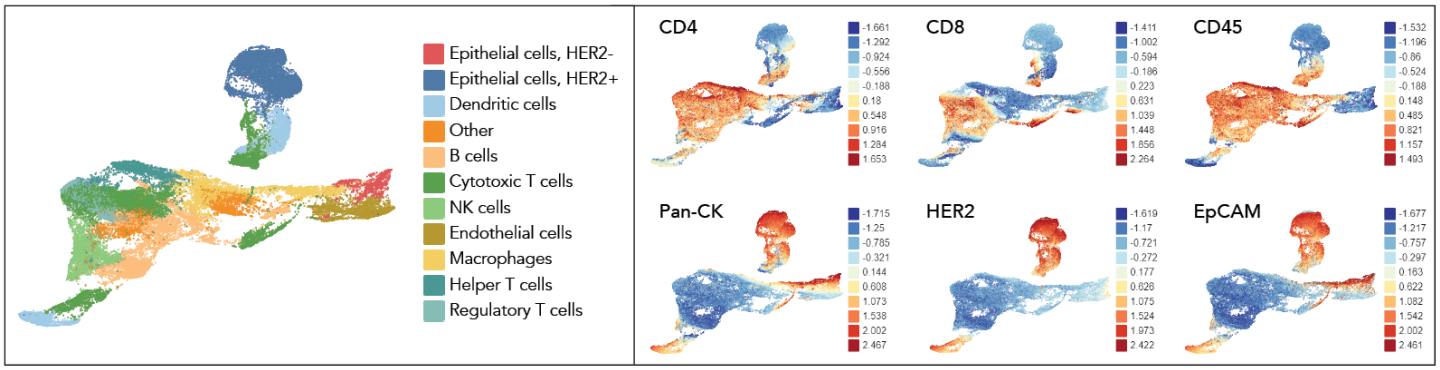

Figure 5. UMAP plots generated with Enable Cloud Platform. The left plot shows the result of UMAP analysis applied to the normalized biomarker expressions of each cell, colored by the identified cell type. The right plots show clustering patterns of individual biomarkers.

Enable Medicine. Unsupervised clustering, a method of annotating and identifying cells with similar attributes, was performed to classify cells based on their biomarker expression profiles (Figure 4). Epithelial cells were first clustered from immune cells, and then additional rounds of subclustering were performed to identify immune subtypes and separate tumor cells from normal epithelial tissues. UMAP plots were created to visualize the relationships between clusters and biomarker expression (Figure 5). Using the Enable Cloud Platform, identified cell types were mapped back onto the original tissue image for spatial visualization (Figure 6). Following unsupervised clustering and neighborhood analysis, chord diagrams demonstrating the strength of interactions between cell types in the tissue sample were produced (Figure 7). These analyses demonstrated a strong association between HER2+ tumor cells and cytotoxic T cells, indicating that an adaptive immune response has been activated to respond to the cancerous tissue.

Summary

Human HER2+ breast cancer tissue was probed with a 21-plex panel using automated cycling immunohistochemistry on a CellScape platform.

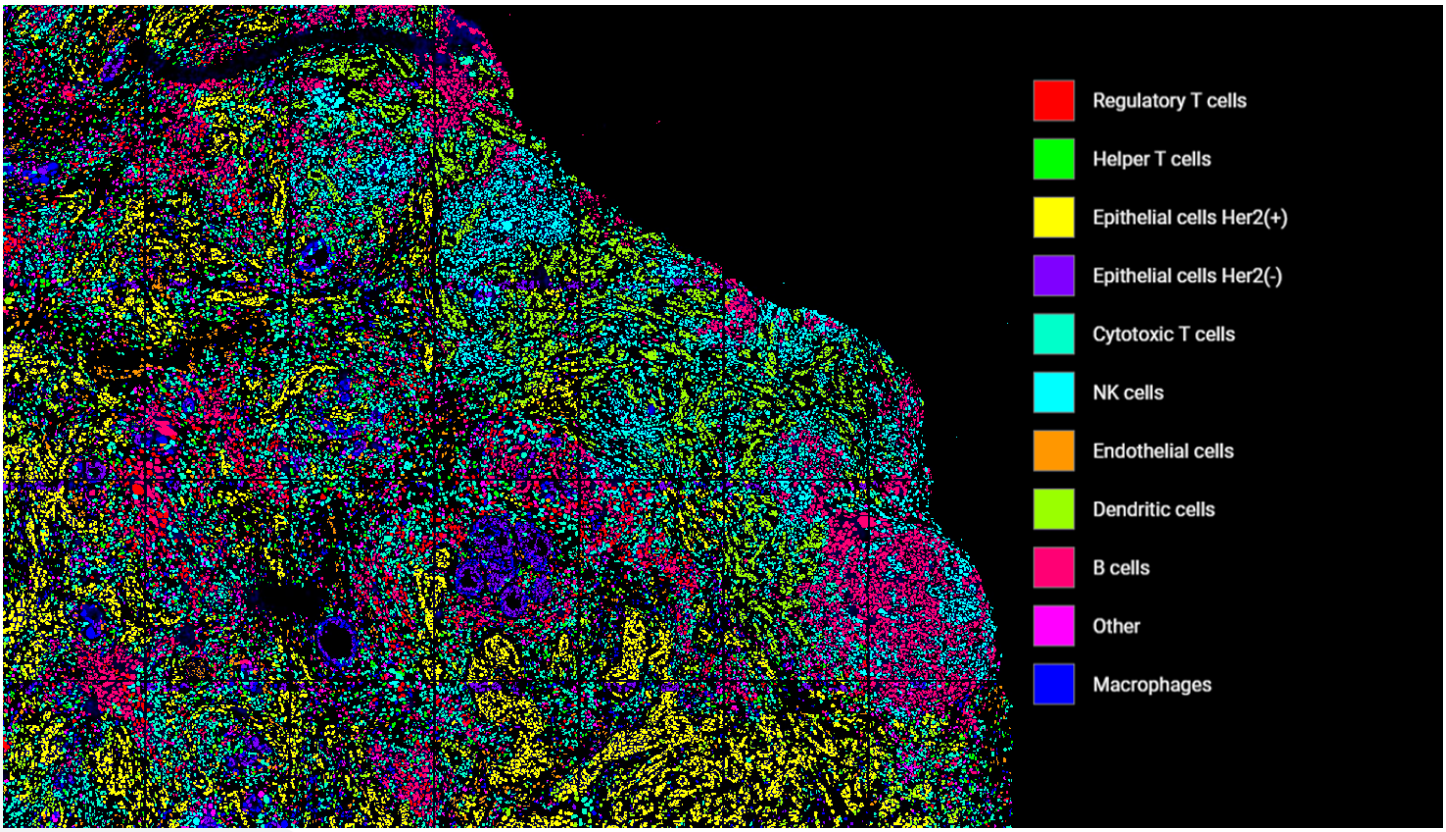

Figure 6. Breast cancer tissue with cell types identified. The quantitative biomarker data facilitates cell type and leukocyte type identification, which can be mapped back to the original tissue to generate spatial insights. Shown above, the same HER2+ breast carcinoma sample as shown in Figure 2, with identified cell types mapped back onto the tissue image.

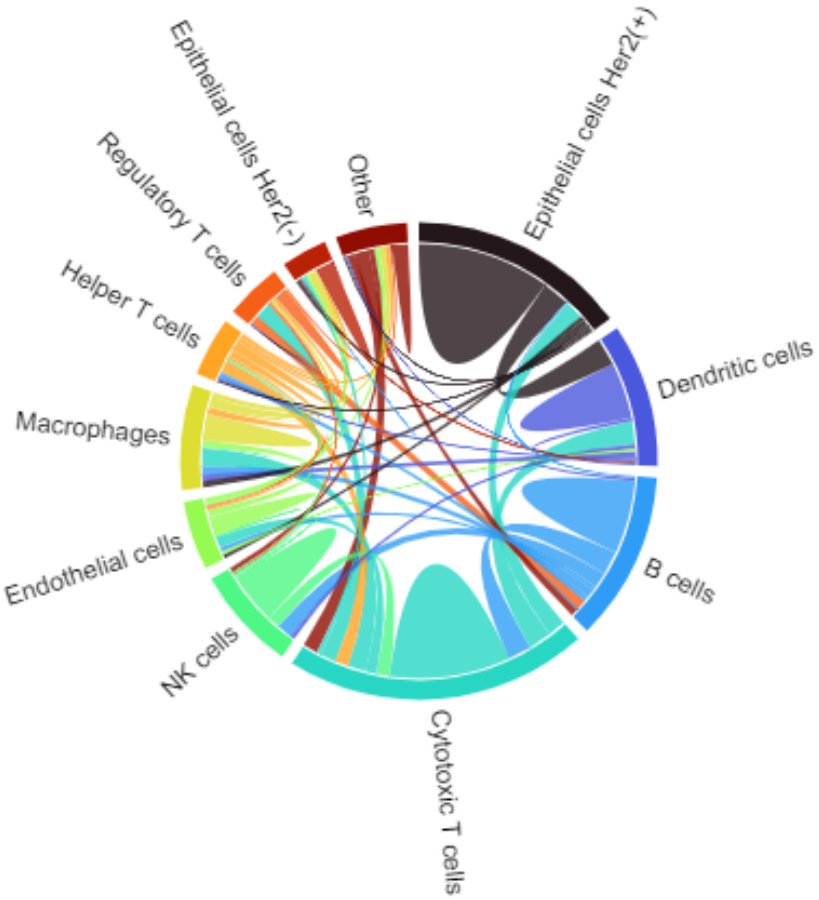

Figure 7. Chord diagram. The strengths of cell-cell interactions are represented by the thickness of chords between cell types. This analysis identified an association between HER2+ epithelial cells (black) and cytotoxic T cells (teal).

Precise biomarker quantification enabled hierarchical gating and classification of tumor and immune cells, revealing high relative abundance of T helper and cytotoxic T cells while maintaining spatial information at single-cell resolution.

Quantification of cell populations expressing very high or low levels of a single biomarker were made possible through the high-dynamic range (HDR) imaging feature of ChipCytometry technology and the CellScape platform, enabling detection of more cells than with standard single-exposure imaging.

The Enable Cloud Platform was used to perform additional image processing and analytics, including unsupervised clustering, to better understand cellular frequencies, interactions, and neighborhoods at single-cell resolution. In a large cohort study, these analyses can be useful to demonstrate treatment efficacy, identify the impact of novel mutations on immune evasion by a malignancy, or explore disease states.

ChipCytometry has also been leveraged for other immunology and immuno-oncology applications,

including detection of rare cell types[3], determining mechanisms of graft vs host disease[4], and microbiome-driven cancer progression[5]. Combining quantitative biomarker data and the single-cell resolution imaging information provides a powerful opportunity for the discovery of new complex multiplexed biomarker signatures to inform therapeutic development and guide personalized medicine.

References

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Canopy Biosciences
4340 Duncan Avenue
Suite 220
Saint Louis, Missouri 63110

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