Highly multiplexed spatial biomarker analysis has demonstrated the potential to advance our current understanding of the immune system and its role in cancer – from tumor initiation to metastatic progression. Previously, a trade-off between spatial and spatial context meant that our understanding of immune cell involvement in cancer was limited by either single-protein technologies or spatial context (e.g., immunohistochemistry) or highly multiplexed technologies without spatial context (e.g., flow cytometry and single-cell RNA sequencing).

ChipCytometry™ is a novel, highly multiplexed technology that preserves both plex and spatial context to deeply profile immune cell subtypes 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 via a flow cytometry-like hierarchical gating strategy 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 a fresh-frozen tissue sample from a patient with breast cancer. 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 reveals quantifiable heterogeneity of immune cell infiltration within the tumor samples, demonstrating the utility of the ChipCytometry platform for the in-depth immune profiling of clinical tissue samples.

**Abstract**

**Methods**

**Sample Preparation:** Five μm FF tissue sections were mounted on glass coverslips. Sections were loaded onto ZetraSafe™ microfluidic chips to preserve sample integrity during serial delivery of reagents.

**Data Collection:** ROIs were selected based on an initial scan of autofluorescence in a single fluorescence channel. Sections were stained with fluorescent antibodies and incubated for 15 min at RT. Sample was imaged in up to 5 channels, then fluorescence was photo-bleached. Sections were stained in cycles until all targets were imaged (Table 1).

**Image Analysis:** Custom software was used to align and overlay scanned ROIs. Cell segmentation was performed using an internal AI algorithm. Cell phenotypes were identified using hierarchical gating strategy.

**Results**

**Conclusions**

- We demonstrate the utility of ChipCytometry to generate highly-multiplexed, spatially-resolved protein expression data from a clinical sample. We show quantitative measurement of 21 clinically relevant biomarkers at the single-cell level for every cell in this tissue specimen from a patient with HER2+ breast cancer.
- ChipCytometry is a multiplex imaging method that uses commercial 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 tumor and immune subpopulations, revealing high relative abundance of key immune cell subtypes in this breast tumor 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 (HIDR) imaging.

**Selected Publications**


