Precise spatial multiplexing of immune cell diversity in clinical tumor samples with ChipCytometry™

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Abstract

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 plex and spatial context meant that our understanding of immune cell involvement in cancer was limited by either single-plex technologies with 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 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 subsets in a fresh frozen tissue sample from a patient with HER2+ 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 specimen, demonstrating the utility of the ChipCytometry platform for the in-depth immune profiling of clinical tissue samples.

Methods

ChipCytometry Protocol:
Sample Preparation
1. 5 μm FF tissue sections were mounted onto glass coverslips
2. Sections were loaded onto ZellSafe™
3. Sample was imaged in up to 5 channels, then fluorescence was photobleached
4. Step 4 and 5 were repeated in rounds until all targets were imaged (Table 1)

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

Figure 2A. Whole sample staining of HER2+ breast carcinoma tissue. A 21-plex antibody staining plan was used to image the entire tissue specimen. Here, we show a subset (T) of those markers and a subset of the area analyzed (8 mm² of 15 mm²) using key markers to highlight tissue architecture including tumor and immune cells.

Figure 2B. Percent of leukocytes. Most identified leukocytes were T helper or T cytotoxic cells.

Table 2: Quantification of cell populations. Cell populations were determined via a hierarchical gating strategy, and each population was quantified. Absolute cell counts and percent of total leukocytes are represented here.

Table 1. Antibody staining plan. A 21-plex antibody staining plan was applied to the breast cancer specimen.

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Selected Publications
1. Carstensen, S., Holz, O., Hohlfeld, J. M., & Müller, M. (2021). Quantitative analysis of endotoxin with high-dynamic range imaging to profile dozens of protein biomarkers in a single tissue 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 subsets in a fresh frozen tissue sample from a patient with HER2+ 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 specimen, demonstrating the utility of the ChipCytometry platform for the in-depth immune profiling of clinical tissue samples.

Abstract

Here we present the analysis of a roughly 8 mm² sample area (Fig. 2A) of 15 mm² scanned area. Most tumor cells (Pan-CR) are also HER2+ (Fig. 2B), which suggests this is a carcinoma – a malignancy of epithelial origin. Yet, we found a single region of normal epithelium (Pan-CK+/HER2-) with relatively normal tissue architecture (Fig. 2C). Each antibody was subjected to rigorous testing during antibody validation (Fig. 4). All 21 markers in this assay were used for cell phenotyping using a hierarchical gating strategy based on expression values (Fig. 3 and 5). The resulting cell populations were quantified (Table 2) and represented in terms of percent of all cells segmented (Fig. 6A) and percent of all leukocytes (Fig. 6B). The resulting cell populations were quantified (Table 2) and represented in terms of percent of all cells segmented (Fig. 6A) and percent of all leukocytes (Fig. 6B).

Results

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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 in this tissue specimen from a patient with HER2+ breast cancer.

• ChipCytometry is a multiplexed imaging method that uses commercial antibodies from any vendor to spatially resolve protein targets 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 subsets in a fresh frozen tissue sample from a patient with HER2+ 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 specimen, demonstrating the utility of the ChipCytometry platform for the in-depth immune profiling of clinical tissue samples.

Figure 3. Highly multiplexed single cell imaging. All 21 markers (note different colors) were used for cell phenotyping. A region of relatively low cell density is highlighted to distinguish individual markers. Here, a single tumor cell expresses pan-cytokeratin, EpCAM, HER2, and DNA markers.

Figure 4. Validation of commercial antibodies for ChipCytometry. Each antibody undergoes clone screening, single-plex development to optimize dilution and evaluate target specificity, and multiplex optimization to evaluate antibody cross-reactivity in a multiplexed assay.

Figure 5. Representative dot plots showing hierarchical gating strategy. From image data, cells were segmented, and interrogated for quantitative expression of each marker to enable the identification of cell phenotypes via flow cytometry-like hierarchical gating. This series represents a single gating strategy to identify some key immune cells including T helper and T cytotoxic cells.

Figure 6B. Percent of leukocytes. Most identified leukocytes were T helper or T cytotoxic cells.