Precise Spatial Multiplexing for Immune Profiling in Non-Small Cell Lung Cancer FFPE Samples with ChipCytometry™

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Abstract

Emergent data indicate that highly multiplexed spatial biomarker analysis has the potential to advance precision medicine in immune-oncology and inform the discovery of novel biomarkers. Here we present the analysis of clinical FFPE samples from non-small cell lung cancer patients using ChipCytometry, a novel precise spatial multiplexing technology which combines iterative immuno-fluorescent staining with high-dynamic range imaging to facilitate quantitative phenotyping with single-cell resolution. Standard FCS files are generated from multichannel OME-TIFF images, enabling identification of cellular phenotypes via flow cytometry-like hierarchical gating. In this study, a 26-plex assay was used to identify and quantify more than 30 cellular phenotypes and subtypes in FFPE samples. The results show precise expression levels for each marker in the assay in each individual cell in the sample, maintaining spatial information about each cell. Spatial analysis of the samples reveals quantifiable heterogeneity of immune cell infiltration within the tumor samples, demonstrating the utility of the ChipCytometry platform for the in-depth immune profiling in clinical samples.

Methods
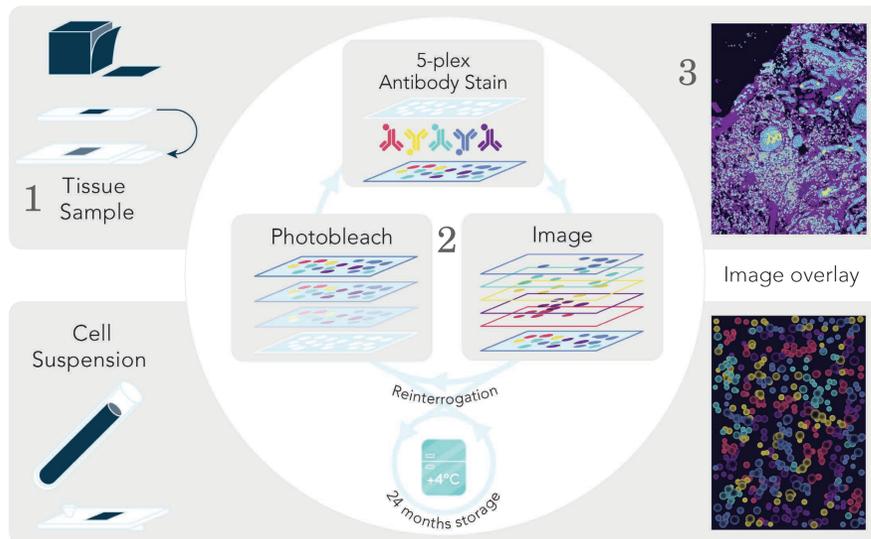
Sample preparation
1. 5μm FFPE tissue sections were mounted onto glass coverslips
2. Sections were deparaffinized and rehydrated by serial immersion in citrisolv and graded ethanol
3. Heat-mediated antigen retrieval (HIER) was performed for 20 min at 92 C
4. Sections were then loaded onto proprietary microfluidic ZellSafe Chips for serial delivery of reagents

Data Collection
1. An initial scan for autofluorescence helped determine ROIs for subsequent imaging
2. Fluorescently-labeled antibodies were applied to tissue sections in rounds, according to Assay Panel
3. Antibody cocktail was incubated for 1 hr at room temperature and excess washed
4. Slides were imaged on the ZellScannerONE™ instrument and then photobleached
5. Steps 2-4 were repeated

Image Analysis
1. Image analysis was performed using custom ZellExplorer™ software
2. Fluorescence intensity images for markers were aligned and overlayed
3. Individual cells from image data were segmented and cell phenotypes were identified and quantified using hierarchical gating
4. Spatial analysis was performed in R to generate quantification plots

Results

A 26-plex antibody panel was designed to identify the major components of the tissue microenvironment including tumor and immune cells, with a particular focus on T-cells and myeloid cells.

26-plex Antibody Panel

DNA	CD27	CTLA-4	HLA-DR
CD3	CD33	LAG-3	Ki-67
CD4	CD45	PD-L1	Pan-CK
CD8	CD45RA	PD-1	SMA
CD11b	CD56	TIM-3	Vimentin
CD14	CD68	FOXP3	
CD20	CD86	Granzyme B	

Individual cells from image data were segmented and standard FCS files were generated, allowing for the identification of cellular phenotypes via flow cytometry-like hierarchical gating. Representative dot plots demonstrate the gating strategy employed to identify and quantify select cell populations.

First Gate	Second Gate	Third Gate	Fourth Gate
CD45 / SMA	CD3 / CD56	CD4 / CD8	Ki67 / Granzyme B

All cells — Leukocytes — Non-leukocytes
T-cells — NKT cells — NK cells
T-cells — CD4+ T-cells — CD8+ T-cells
CD4+ T-cells — Proliferating CD4+ T-cells — CD4+ CTLA+ cells
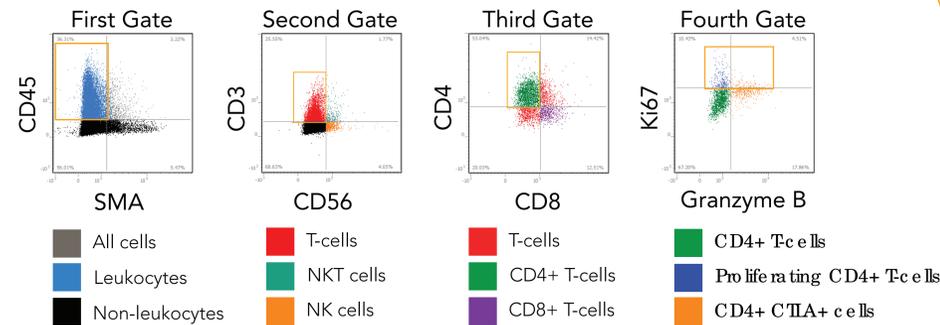
Quantification of cell populations revealed high levels of myeloid cells, which are suspected to play a pro-tumor role in many cancers. Subsequent spatial analyses were conducted to investigate the relationship of T-cell and myeloid cell populations within the tumor microenvironment. A violin plot (left) and spatial distribution pattern plot (right) represent the density of cells found in relation to other cell types in the tissue.
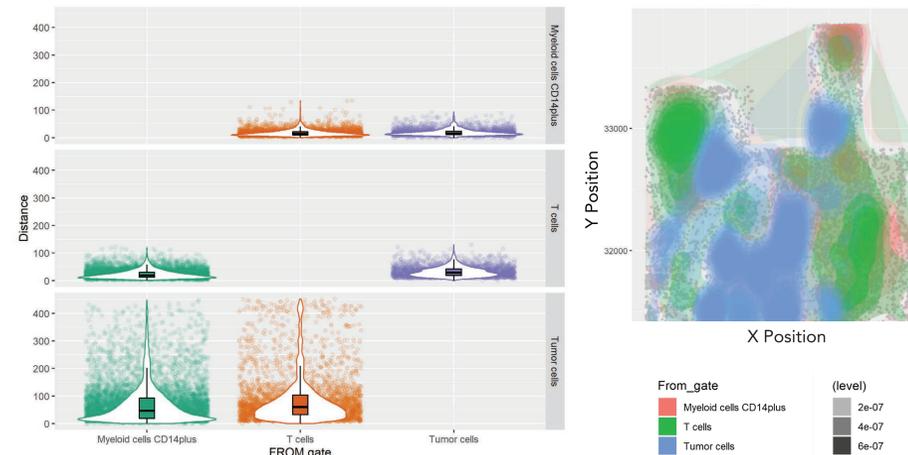
Whole slide autofluorescence scan of unlabeled tissue section. Individual images stitched and registered with ZellExplorer software.
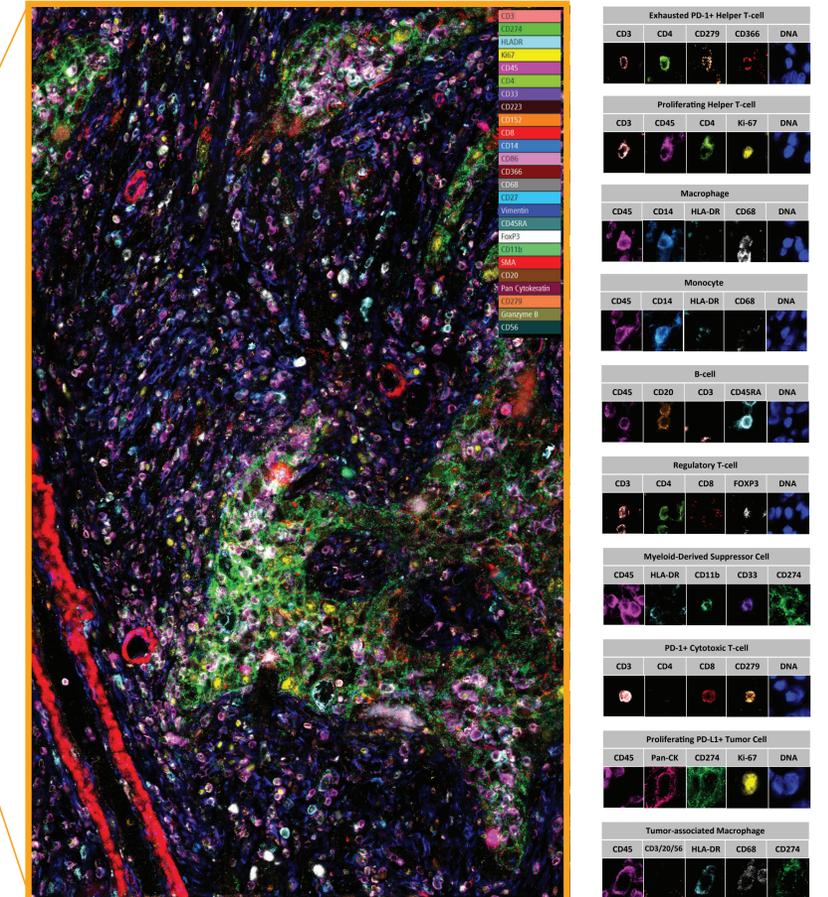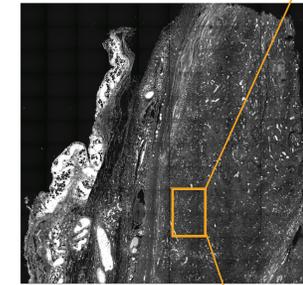
Zoomed in view of lung cancer specimen. Mutliplex immunofluorescence image data demonstrates a prominent myeloid cell infiltration in the tumor stroma and in close proximity to the tumor cells, while T-cells mainly cluster outside tumor regions. Select cell phenotypes are shown on the right.

Conclusions

- The ChipCytometry platform was used for in-depth immune profiling of clinical FFPE samples from a lung cancer specimen

- ChipCytometry enables quantification of precise expression levels for each marker in the assay for each individual cell in the sample

- Thirty cell phenotypes, including myeloid and T-cell subtypes, were identified using the 26-plex antibody panel

- Spatial analysis revealed quantifiable heterogeneity of the tissue microenvironment and the spatial relationship between cell types

ChipCytometry combines high-quality imaging with advanced image analysis software to enable a truly quantitative measure of each marker in your assay for every individual cell in your sample. Scan to learn more about ChipCytometry.