Precise spatial multiplexing of immune populations in human colorectal cancer FFPE samples with ChipCytometry

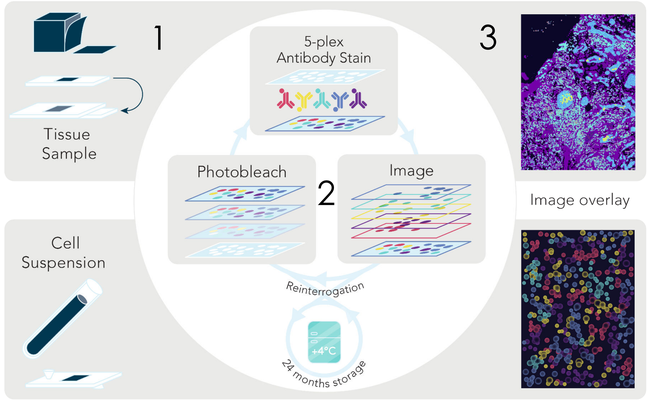
Thomas Campbell, PhD[1]; Adam Northcutt, PhD[1]; Arne Christians, PhD[1]; Nancy Stanslowski, PhD[1]; Spencer Schwarz, MS[1]; Crystal Winkeler, PhD[1]
1. Canopy Biosciences, St. Louis, MO USA

A BRUKER COMPANY
CANOPY BIOSCIENCES

Abstract

Understanding the spatial distribution of key immune cell populations is critical for advancing our understanding of cancer to inform the development of novel therapeutics. Here we present the analysis of clinical FFPE samples from colorectal cancer patients using a novel, precise spatial multiplexing technology called ChipCytometry, which combines iterative immunofluorescence staining with high dynamic range (HDR) 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 16-plex assay was used to identify and quantify 20 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, while maintaining spatial information about each cell. Spatial analysis of the sample reveals quantifiable heterogeneity of multiple tissue regions within the sample, demonstrating the utility of ChipCytometry for the in-depth immune profiling of clinical samples.

Methods

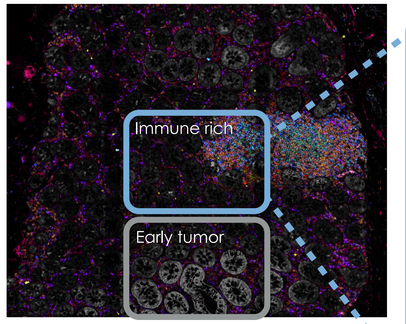


1. **Sample Preparation:** 5µm FFPE tissue sections were mounted onto glass coverslips. Sections were deparaffinized and rehydrated by serial immersion in citrisolv and graded ethanol. Heat-mediated antigen retrieval (HIER) was performed for 20 min at 92 C. Sections were then loaded onto ZellSafe Chips, which preserve sample integrity during serial delivery of reagents.
2. **Data Collection:** Regions of interest were selected based on an initial scan in a single fluorescent channel. Sections were subsequently stained, imaged, and photobleached in rounds, according to the Assay Panel. Antibodies were incubated for 1 hr at room temperature before washing and imaging.
3. **Automated Image Analysis:** Image analysis was performed using an AI-based software. Fluorescence intensity images for markers were aligned and overlayed. Individual cells from image data were segmented and cell phenotypes were identified and quantified using hierarchical gating.

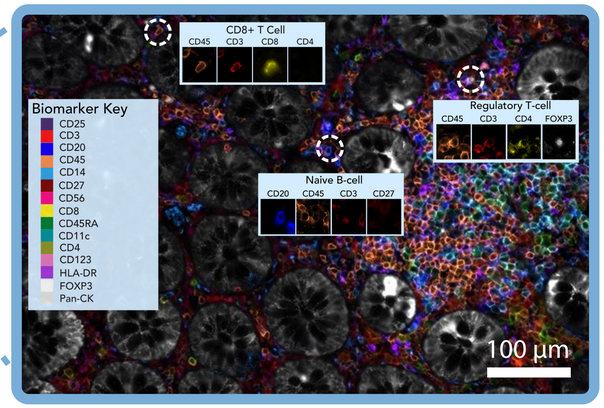
Results

Assay Panel (16 Target Markers)			
CD3	CD14	CD45	FOXP3
CD4	CD20	CD45RA	HLA-DR
CD8	CD25	CD56	Pan-CK
CD11c	CD27	CD123	DNA

The **16-Plex Spatial Immune Cell Phenotyping Panel** was applied to an FFPE sample from a colorectal cancer patient. Data from these markers further enabled identification and quantification of 20 distinct cellular phenotypes.



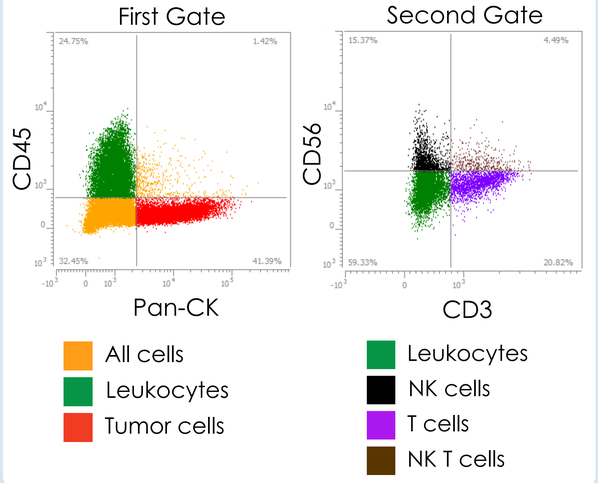
The assay aligns and overlays fluorescence intensity images, providing both precise expression levels for each marker and **spatial information** for each individual cell in the sample.



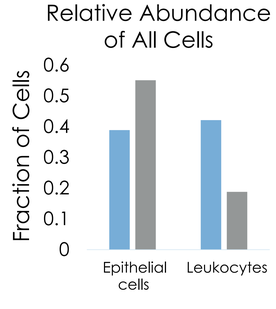
The immune rich region contains a tertiary lymphoid structure, characterized by T cell and B cell phenotypes and subtypes, and highly organized colonic crypts (pan-CK+/CD45-).

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. Dot plots (below) demonstrate the gating strategy employed to identify and quantify populations of:

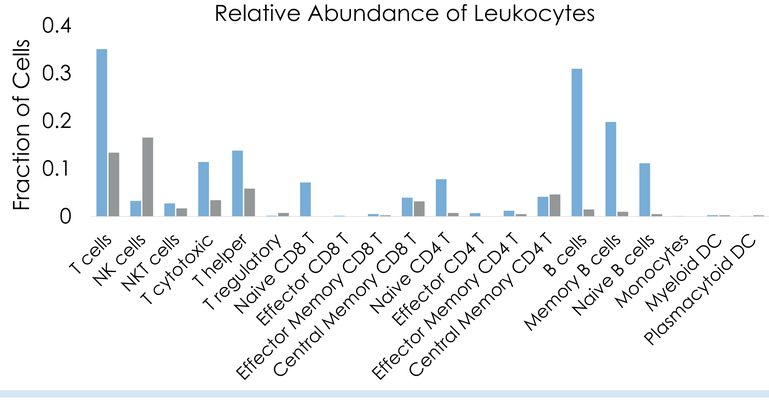
- NK cells (CD45+/CD3-/CD56+)
- T cells (CD45+/CD3+/CD56-) and
- NK T cells (CD45+/CD3+/CD56+) in this sample.



Spatial analysis reveals quantifiable heterogeneity of an immune cell rich region within the sample. **Relative abundance** of immune cell phenotypes (below) is best exemplified by differences in T cell and B cell populations in the immune rich region, as compared to the early tumor region.



Immune rich region
Early tumor region



Conclusions

- The ChipCytometry platform was used for in-depth immune profiling of clinical FFPE samples from a colorectal cancer patient.
- Precise expression levels for each marker in the assay were quantified for each individual cell in the sample.
- Twenty cell phenotypes, including various immune subtypes, were identified using the16-Plex Spatial Immune Cell Phenotyping Panel.
- Spatial analysis revealed quantifiable heterogeneity of immune rich and early tumor regions.
- The relative abundance of T cell and B cell phenotypes and subtypes was higher in the immune rich region, compared to the early tumor region.

More Information

The assay was performed using the ZellScannerONE benchtop imaging system. Scan the QR code to learn more about the ChipCytometry platform.