Precise spatial multiplexing in clinical breast tumor specimens with a 60-plex ChipCytometry assay

Thomas Campbell, PhD[*1]; Nancy Stanslowski, PhD[2]; Jennifer Brooks, PhD[2]

[1]Canopy Biosciences, St. Louis, MO USA
[2]Canopy Biosciences, Leipzig, DE

Background

- Understanding the spatial distribution of key immune cell populations is critical in advancing our understanding of cancer to inform the development of novel therapeutics.
- Historically, the spatial analysis of the tumor microenvironment has been limited to relatively low-plex immunohistochemical (IHC) or immunofluorescent (IF) assays, which were inadequate for deep immune cell profiling of the tumor microenvironment.
- ChipCytometry is a novel technology with the ability to profile virtually unlimited number of proteins while preserving the spatial context of cells in the sample.
- ChipCytometry has many applications including immunophenotyping of tissues and cell suspensions, tissue microenvironment (TME) analysis, and rare cell discovery.

Methods

- Human breast carcinoma samples were analyzed using a 60-plex protein panel with the ChipCytometry platform.
- Samples were loaded onto microfluidic chips for serial delivery of reagents on the CellScape Instrument involving cyclic rounds of i) immuno-staining, ii) washing, iii) imaging, and iv) signal removal.
- High-dynamic range imaging facilitated quantitative cell phenotyping at single-cell resolution.
- Standard FCS files were generated from multichannel OME-TIFF images, enabling identification and quantification of cellular phenotypes via flow cytometry-like hierarchical gating.

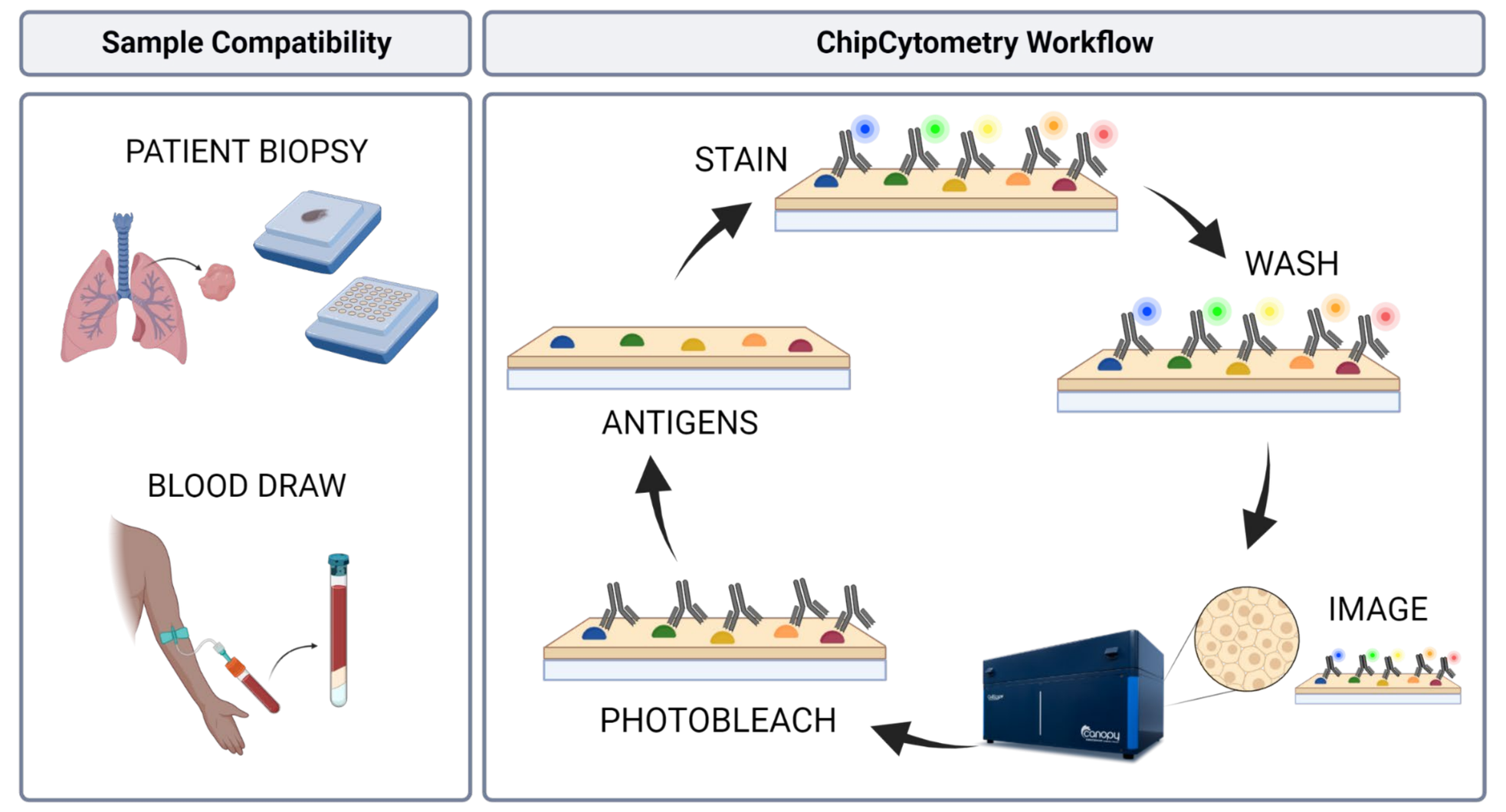

Figure 1. ChipCytometry sample compatibility and workflow. ChipCytometry is compatible with tissue samples from patient biopsies (i.e., FFPE and frozen tissue blocks) and cell suspensions (i.e., PBMCs from clinical blood draw). The CellScape Instrument supports successive rounds of staining with up to 5 fluorescent antibodies in successive rounds of staining, washing, imaging, and photobleaching. (Made with BioRender)

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Learn about Antibody Validation for ChipCytometry

ChipCytometry 60-plex Assay Target List									
Beta-actin	CA-IX	CD11c	CD123	CD134 (Ox40)	CD137	CD138	CD14	CD141	CD15
CD154	CD16	CD161	CD18	CD19	CD195	CD196	CD20	CD207	CD227 (MUC1)
CD25	CD27	CD271 (NGFR)	CD278 (ICOS)	CD279	CD29	CD299	CD3	CD309 (VEGFR2)	CD31
CD326 (EpCAM)	CD335	CD34	CD340 (HER2)	CD366	CD38	CD39	CD4	CD44	CD45
CD45RA	CD45RO	CD56	CD57	CD61	CD68	CD69	CD71	CD8	CD90
CD95	Collagen IV	EGFR	FoxP3	HLA-DR	PDGFRb (CD140b)	Pan-CK	SMA	Vimentin	DNA

Table 1. Target list. A 60-plex antibody panel was applied to a 5 µm tissue specimen resected from a breast cancer patient. Each target corresponds to a monoclonal primary antibody with a fluorescent dye conjugate.

Results

- The results show precise expression levels for each of the 60 markers in the assay in each individual cell in the sample, maintaining spatial information about each cell. Below we show a subset of the markers used to profile tumor cells and key immune cell populations.
- Dozens of immune cell subtypes were identified and quantified based on protein expression profiles including epithelial cells and major immune cell types (B cells, T cells, NKT cells, NK cells, CD4+ T cells, CD8+ T cells, plasma cells, dendritic cells, monocytes, etc.). Below we show a subset of the quantifiable cell types.
- Spatial analysis of the samples reveals quantifiable heterogeneity of immune cell infiltration within the tumor samples, demonstrating the utility of the ChipCytometry platform for in-depth immune profiling in clinical samples.

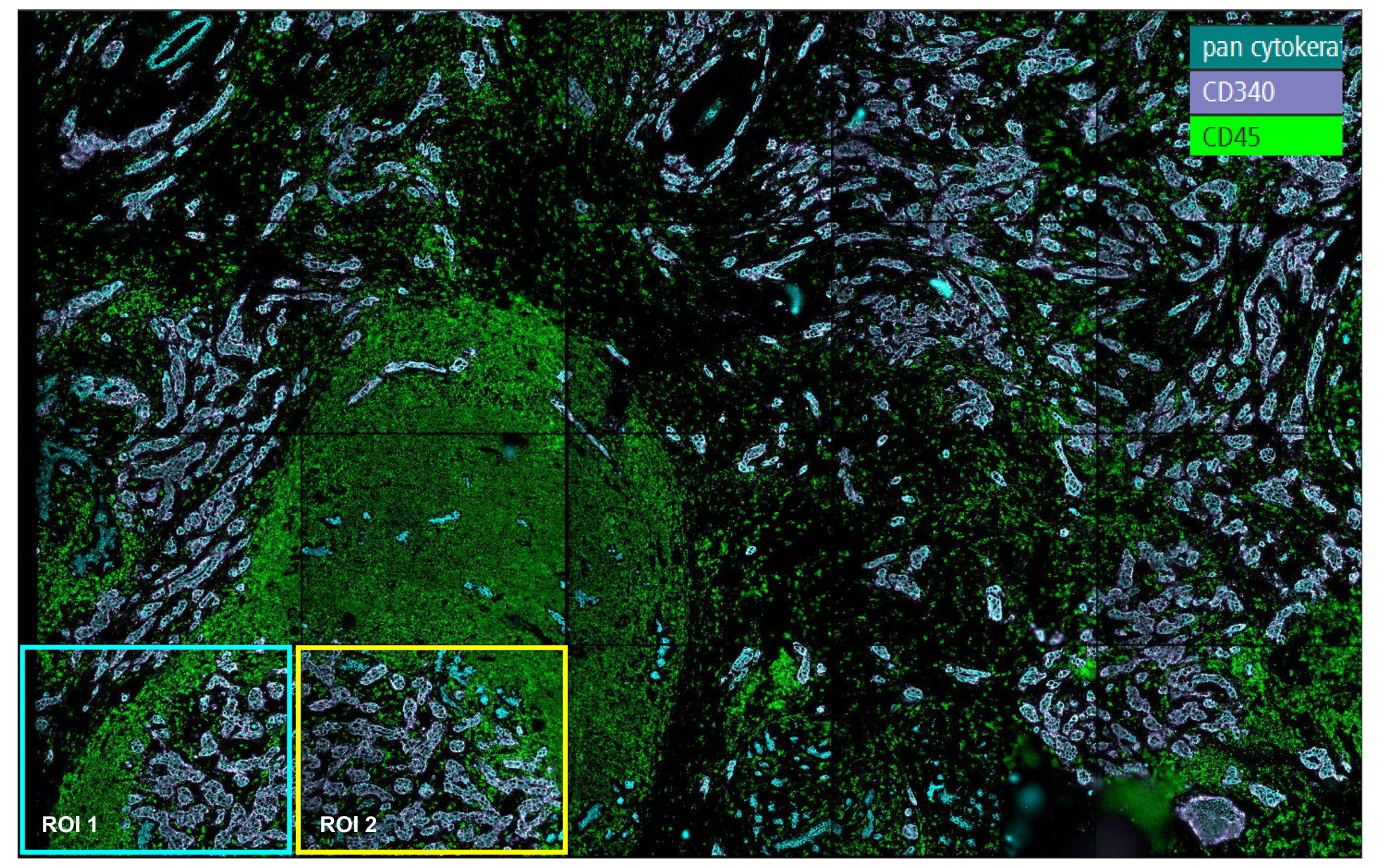

Figure 2. Whole-chip image of breast carcinoma frozen tissue section profiled with ChipCytometry. Pseudo-colored composite images of the marker fluorescence signals for pan-CK, CD340 (HER-2), and CD45 highlight non-tumor epithelium, tumor, and immune cell compartments. ROI 1 and ROI 2 are shown at higher magnification. (Note: A subset of markers shown here.)

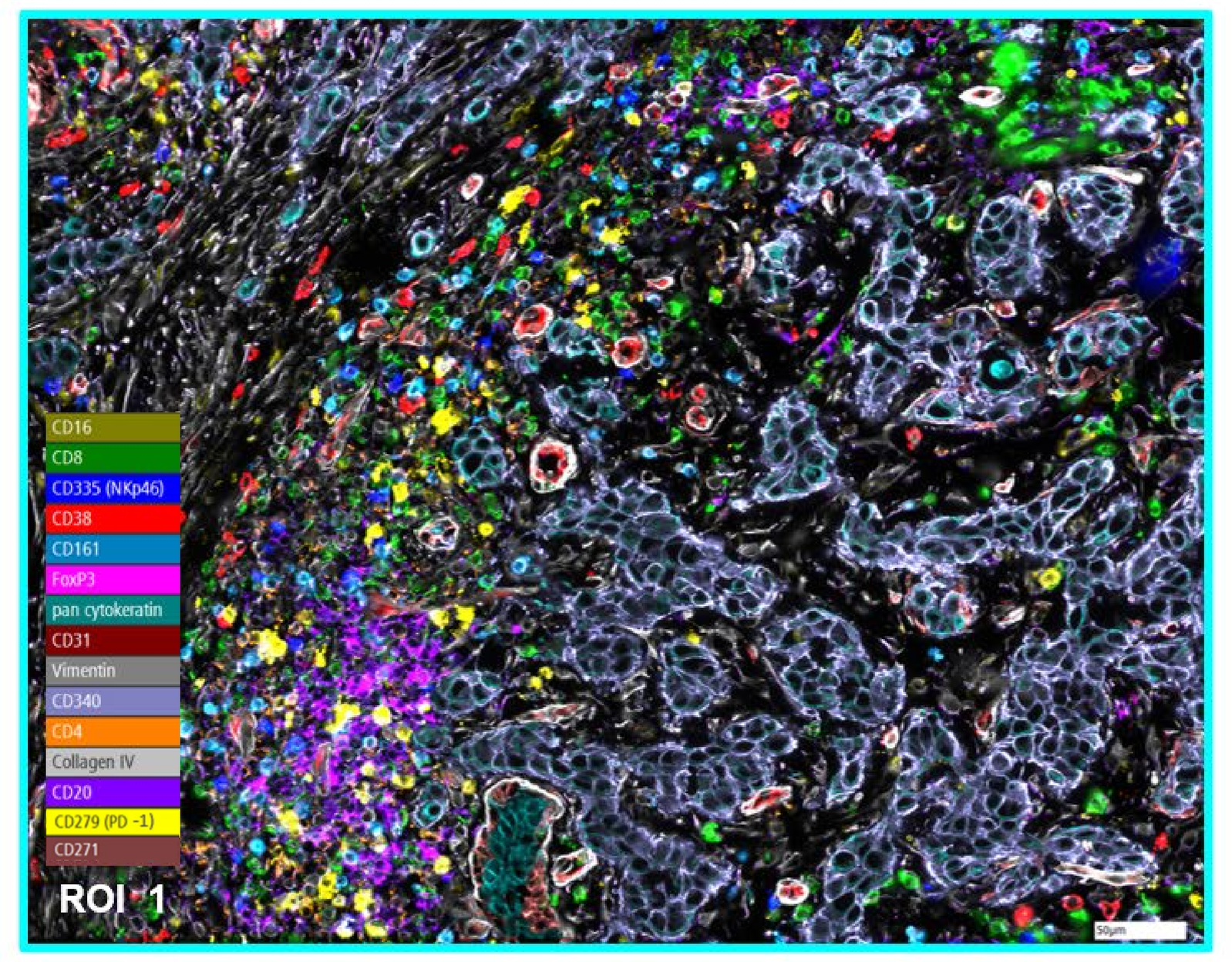

Figure 2a. Zoomed in view of ROI 1. CD8 and CD4 illustrate T cell distribution. CD20 marks B cells and CD56 shows the NK cells in the tissue. Pan-CK in marks epithelial cells and CD340 (HER-2) marks tumor cells.

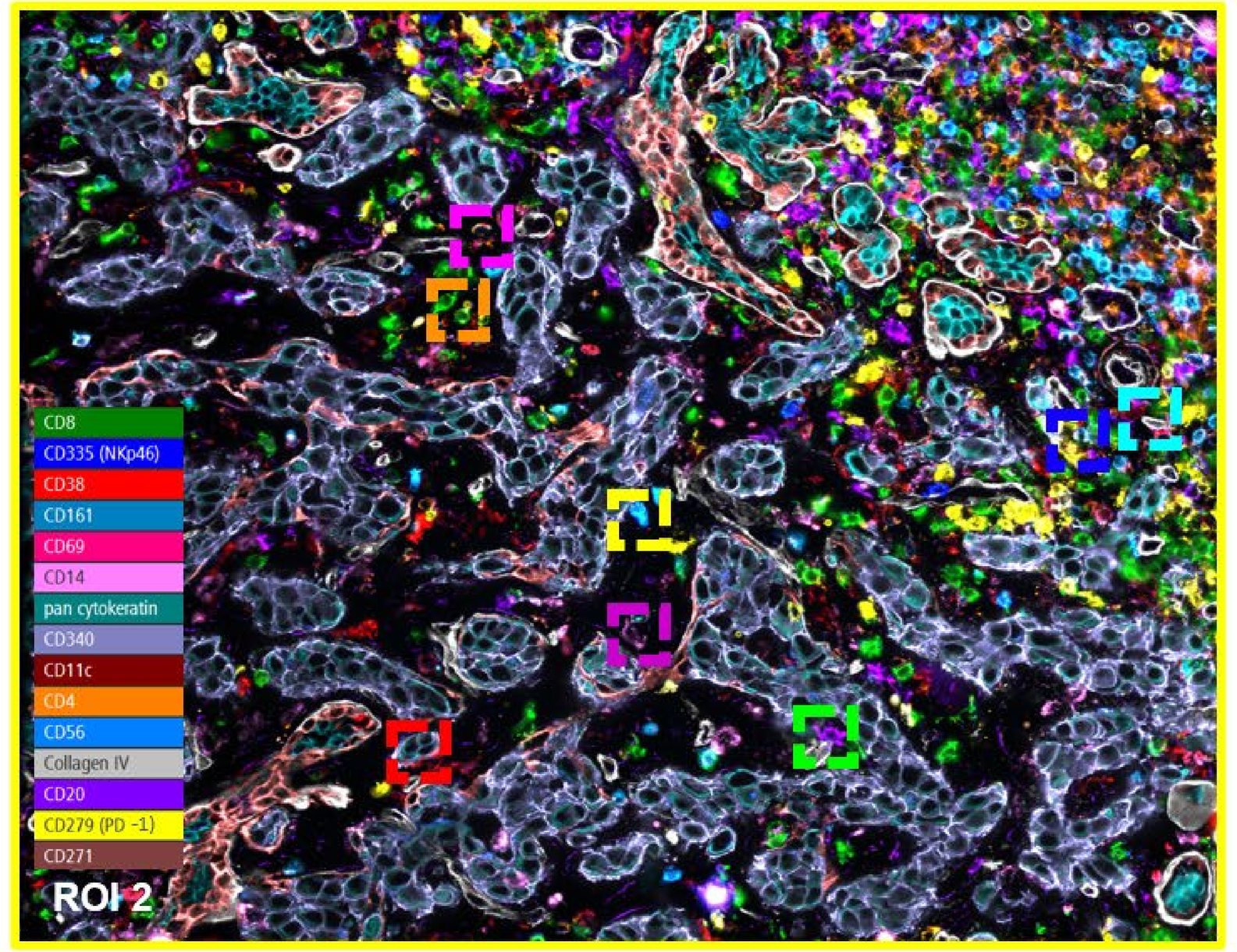

Figure 2b. Zoomed in view of ROI 2. CD8 and CD4 illustrate T cell distribution. CD20 marks B cells and CD56 shows the NK cells in the tissue. Pan-CK marks epithelial cells and CD340 (HER-2) marks tumor cells.

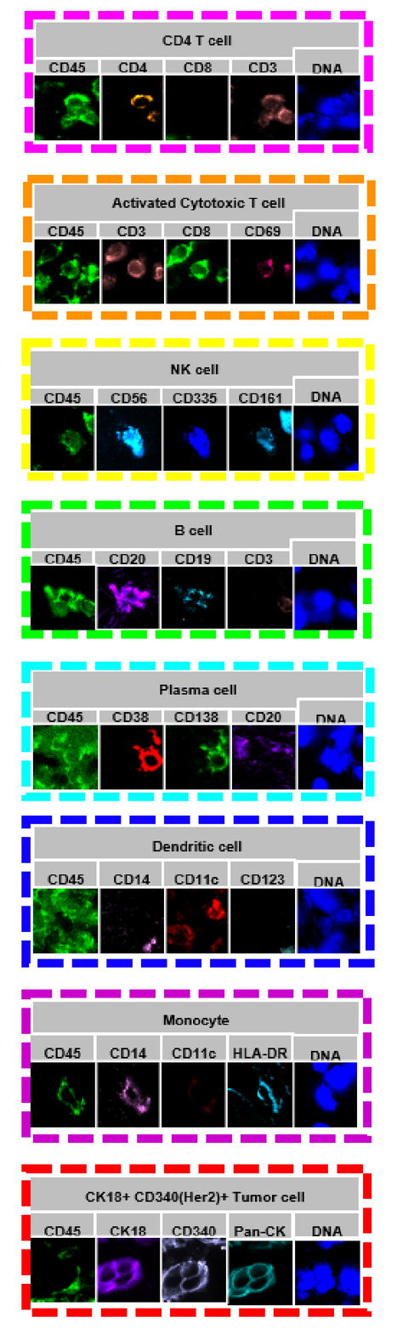

Figure 2c. Expression profile of individual cells from ROI 2. These marker strips show the channels used to identify exemplar cell phenotypes including a CD4+ T cell, activated cytotoxic T cell, NK cell, B cell, plasma cell, dendritic cell, monocyte, and tumor cell.

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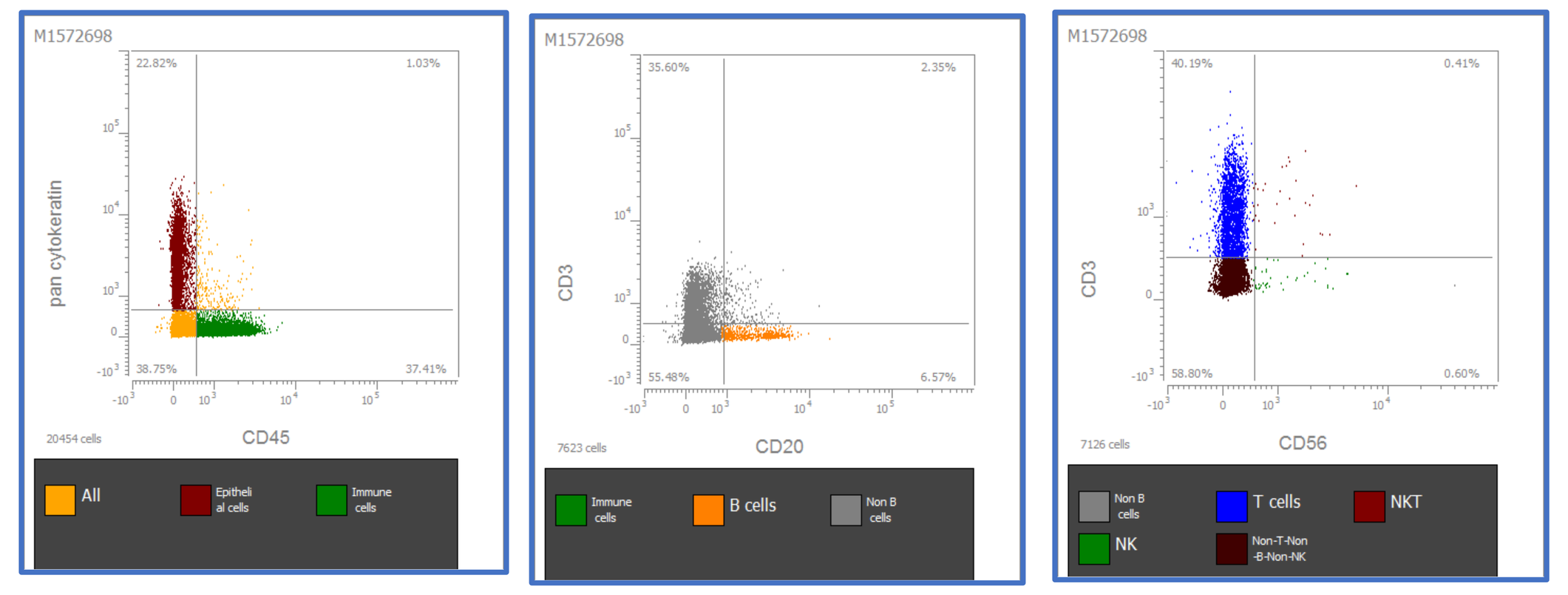

Figure 3. Hierarchical gating strategy for immune cell subtypes. From image data, cells were segmented, and interrogated for quantitative expression of each marker to enable the identification of cell phenotypes via flow cytometry-like hierarchical gating. This series represents a single gating strategy to identify key immune cells including B cells, T cells, and NKT cells.

Population	Absolute cell count	Cells per mm²
All cells	20454	2922
Epithelial cells	4729	676
CK18+Her2+ Tumor cells	4337	620
Immune cells	7623	1089
B cells	537	77
T cells	2918	417
NKT cells	32	5
NK cells	43	6
CD4 T cells	1344	192
CD8 T cells	1036	148
Plasma cells	536	77
Dendritic cells	530	76
Monocytes	591	84

Table 2. Quantification of cell populations. Cell populations were determined via a user-defined hierarchical gating strategy. Absolute cell counts and density of cell populations are represented here.

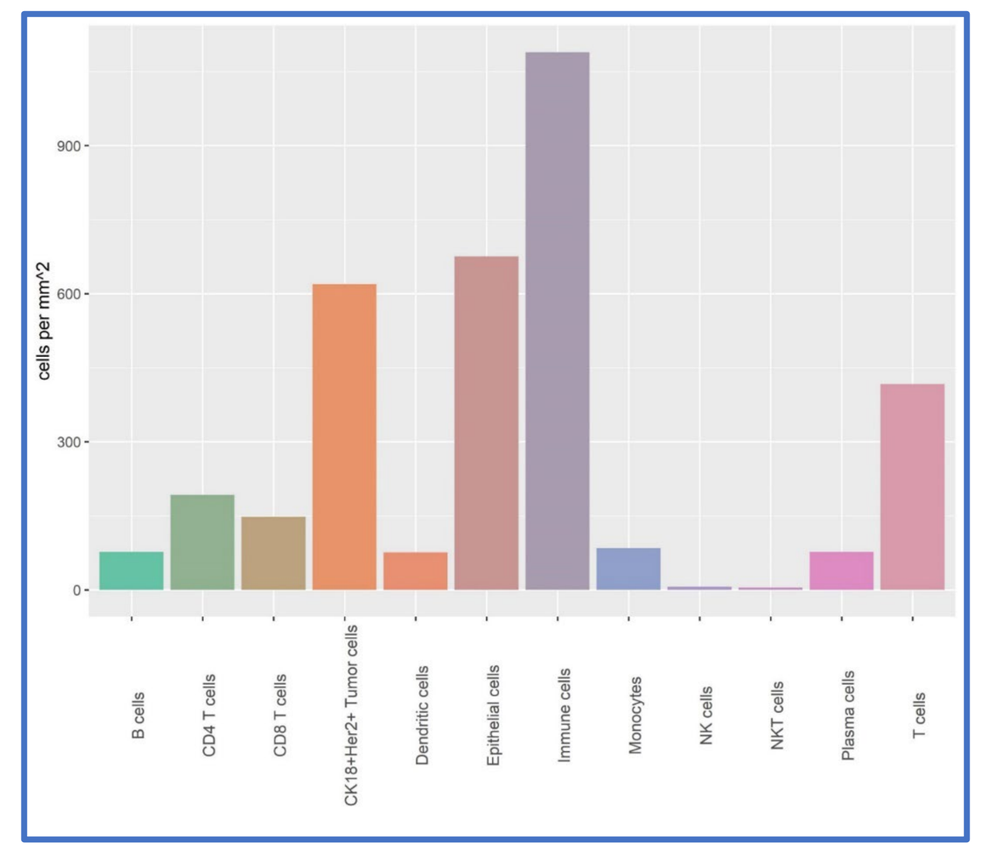

Figure 4. Density of cell populations. Density of key immune cell populations and tumor cell populations are represented here.

Conclusions

1. The ChipCytometry platform enables simultaneous detection of multiple protein markers on a single tissue section for deep immune cell profiling in the tumor microenvironment. Combined with the single-cell spatial information, such data sets provide an opportunity for the discovery of new complex multiplexed biomarker signatures to inform therapeutic development.
2. We demonstrate the utility of ChipCytometry to generate highly-multiplexed, spatially-resolved protein expression data from a clinical sample. We show quantitative measurement of 60 clinically relevant biomarkers at the single-cell level for every cell in this tissue specimen from a patient with HER2+ breast cancer.
3. ChipCytometry is a multiplexed 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.
4. 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 (HDR) imaging.

References

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Contact:
Thomas Campbell, PhD
Thomas.Campbell@Bruker.com