Multiparametric Proteomic Profiling Via Imaging Dozens of Biomarkers Simultaneously

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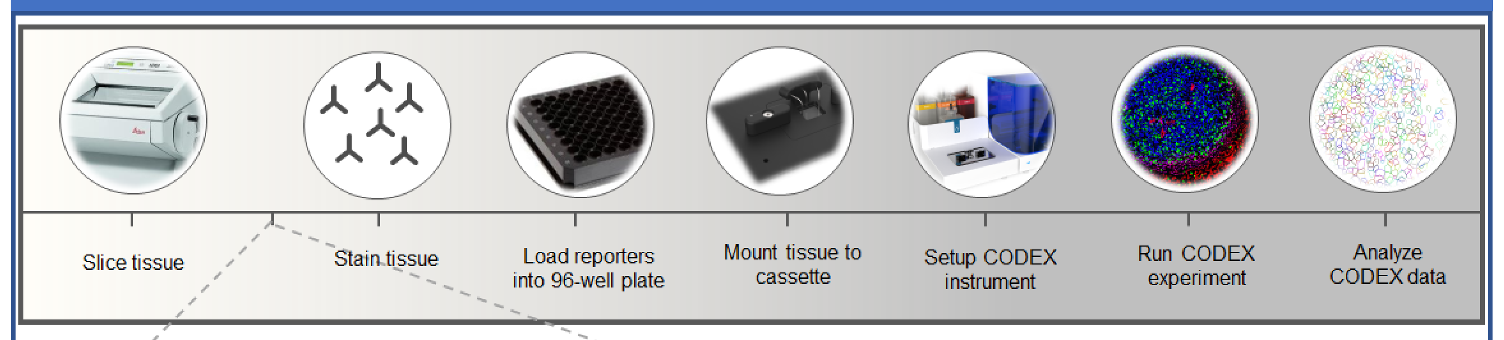
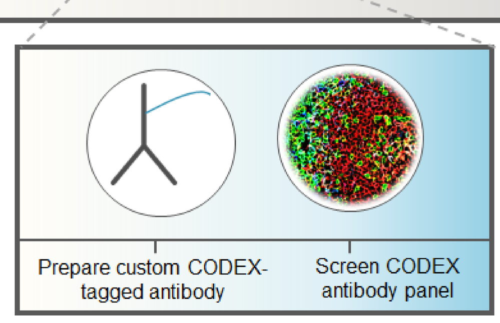
INTRODUCTION

Despite the progress of optical imaging techniques to improve spatial resolution and detection accuracy for biological applications, the number of markers that can be visualized and analyzed simultaneously has largely been restricted by the limited range of the visible light spectrum. Here we present a novel multiparametric imaging technology, termed CODEX (CO-Detection by IndEXing) that combines high-throughput and high-content methodologies to label dozens of biomarkers simultaneously in a single sample, and detect and resolve their relative expression, abundance, and spatial relationships.

EXPERIMENT WORKFLOW OVERVIEW

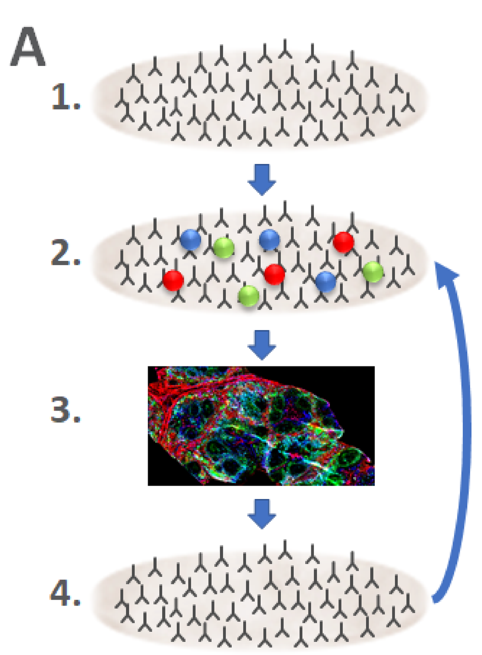




- Off-line preparation of CODEX stained tissue similar to standard IF/IHC protocols
- Sample preparation takes ~1 day
- CODEX data acquisition time dependent on sample size- average 6-12 hours per tissue

Figure 1. CODEX workflow. The CODEX workflow begins with tissue slicing onto glass coverslips. Sections are stained with CODEX antibody panels in a single staining step, where each antibody is labeled with a unique oligonucleotide Barcode. Corresponding reporter sequences are loaded into a 96-well plate with up to three fluorophores per cycle. The stained tissue is mounted one the custom microscope stage insert and placed onto an inverted microscope. CODEX data is collected automatically across numerous cycles. Data is processed and segmented to enable high-parameter spatial analysis.

A



1. Stain tissue with full panel of up to 50 Barcoded Abs
2. Reveal 3 targets w/ Reporters

Target	Barcode	Reporter
CD45	BX001	RX001
CD2	BX002	RX002
CD19	BX003	RX003

3. Image Tissue w/ microscope
4. Remove Reporters
5. **Repeat Steps 2-4 until all targets are revealed**

B

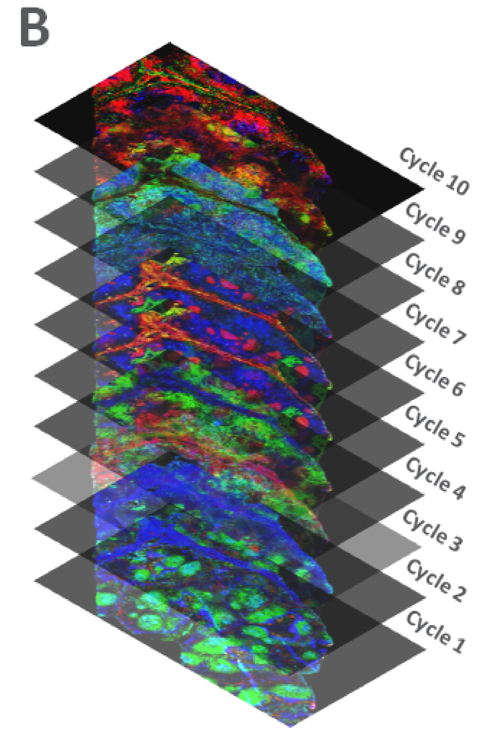


Figure 2. Schematic of cyclical workflow. (A) Tissues are stained with the full panel of CODEX antibodies in a single step. Iterative cycles of labeling, imaging and removing Reporters are performed via a fully automated fluidics system, until all biomarkers of interest are imaged. (B) Images are collected and compiled across cycles to achieve single-cell resolution data.

INSTRUMENT / MICROSCOPE INTEGRATION

Figure 3. Seamless microscope integration. The CODEX fluidics device integrates into microscope stages through a custom stage insert. The CODEX Driver Software is compatible with multiple microscope brands/types, including Keyence BZ-X710/800, Leica DMi8 & Zeiss Axio-Observer.

MULTIPLE MARKERS + DIFFERENT TISSUES

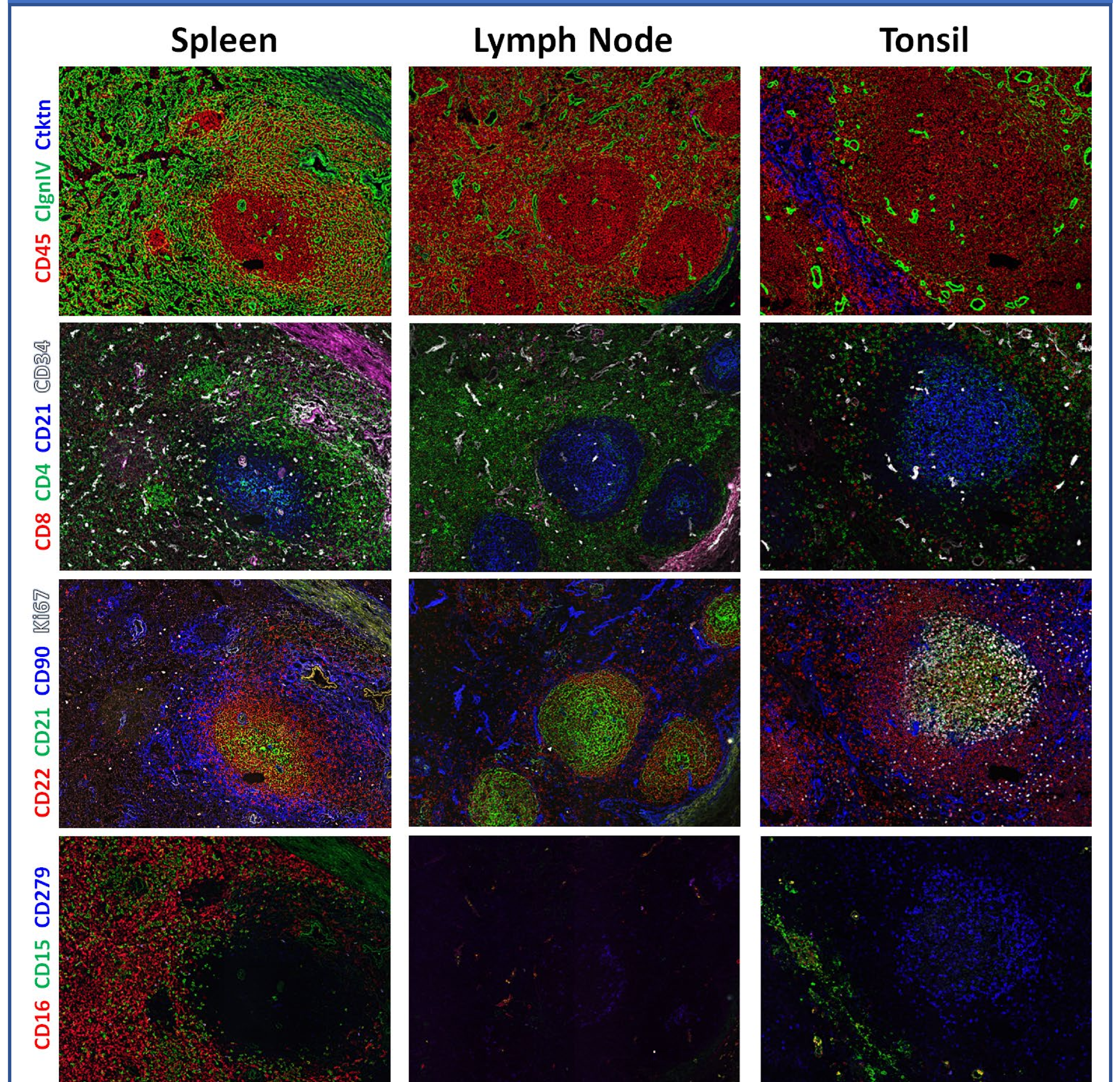


Figure 4. CODEX imaging is compatible with numerous antibodies and tissues. Tissues from different human organs (spleen, lymph node, tonsil) were stained with antibodies for 14 different biomarkers, and these were subsequently revealed via the CODEX fluidics workflow.

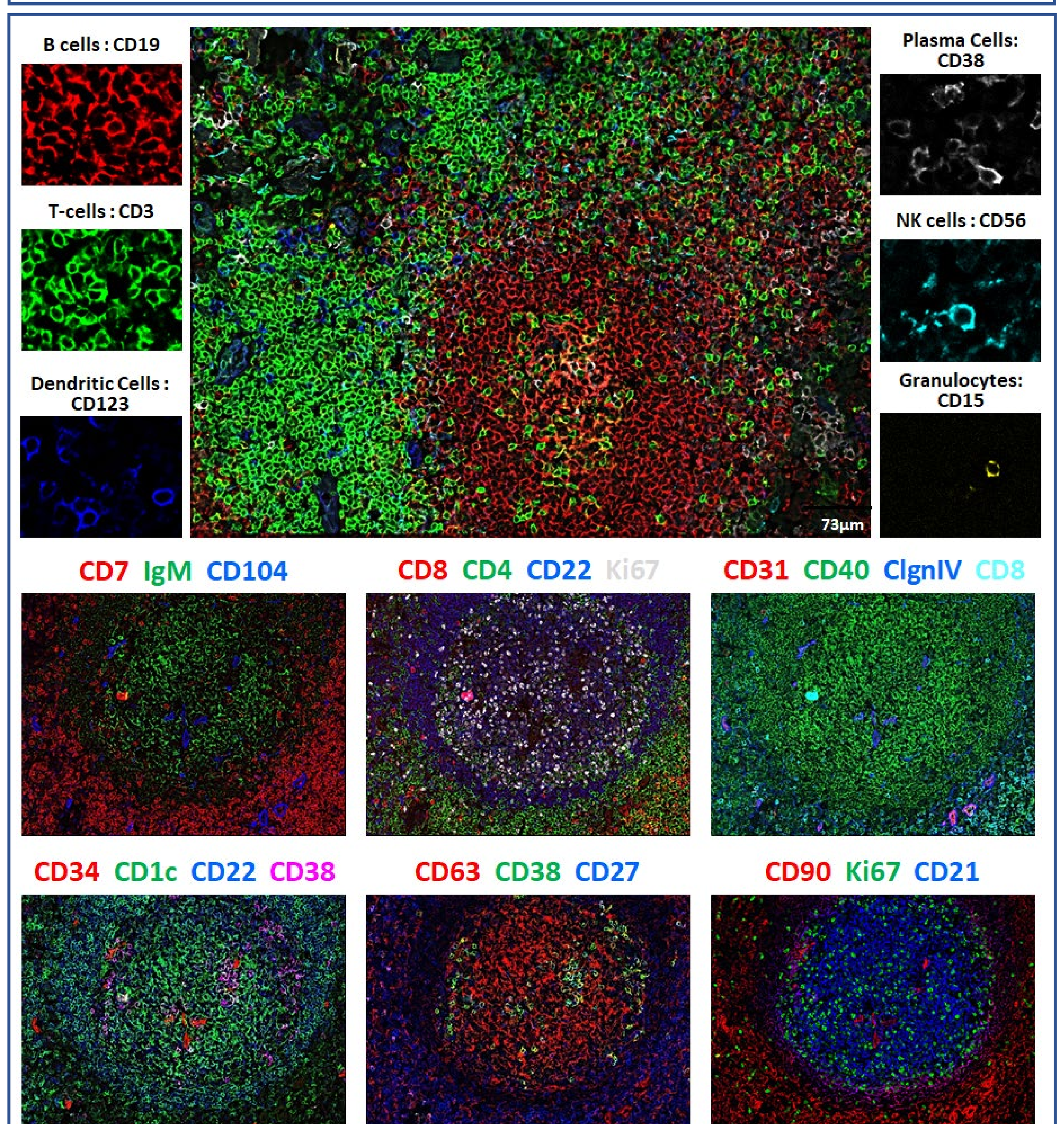


Figure 5. Revealing dozens of biomarkers on the same tissue. A human tonsil tissue was stained with 41 different biomarkers, allowing the visualization and deep analysis of many different cell types and their interactions, including B cells (CD19, CD20), T-cells (CD3), Helper T cells (CD4), Cytotoxic T cells (CD8), Dendritic Cells (CD123), Plasma Cells (CD38), NK cells (CD56), Granulocytes (CD15), and many more.

DEEP MULTIPARAMETRIC ANALYSIS OF MELANOMA SAMPLE

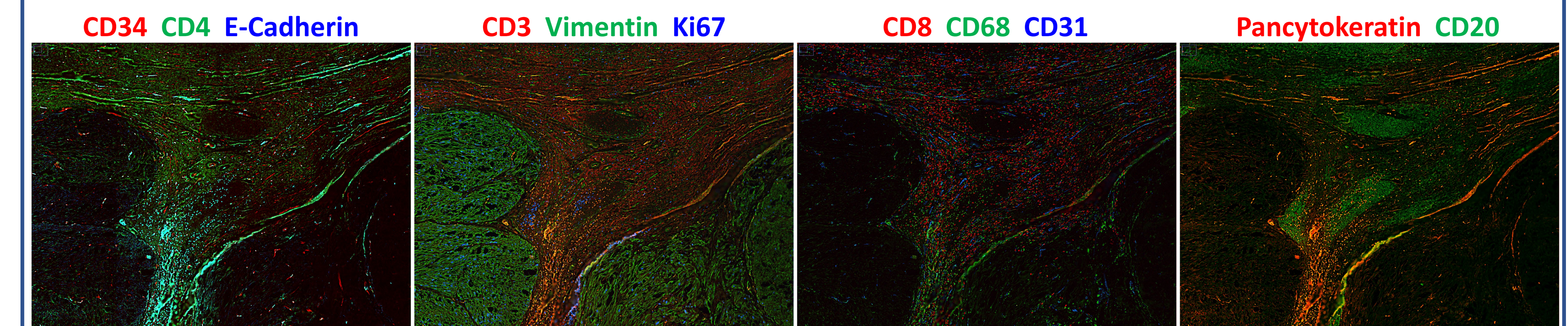


Figure 6. Characterizing the TME. A human FFPE T4bN3cM0 metastatic lymph node melanoma tissue sample was labeled with 11 different antibodies and imaged via the CODEX workflow.

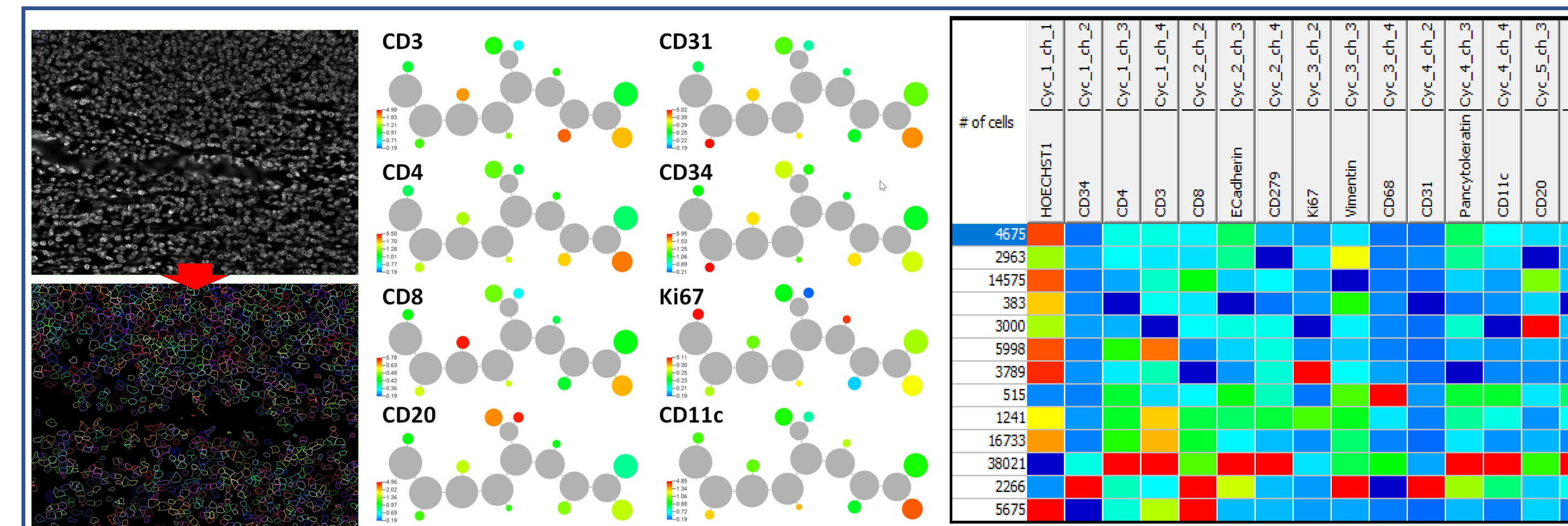


Figure 7. Cluster analysis of image data. Image data was subjected to segmentation to distinguish single cells and generate FCS data. This was subsequently analyzed via decision tree based cluster analysis, which identified 10 distinct clusters corresponding to 10 different cellular phenotypes.

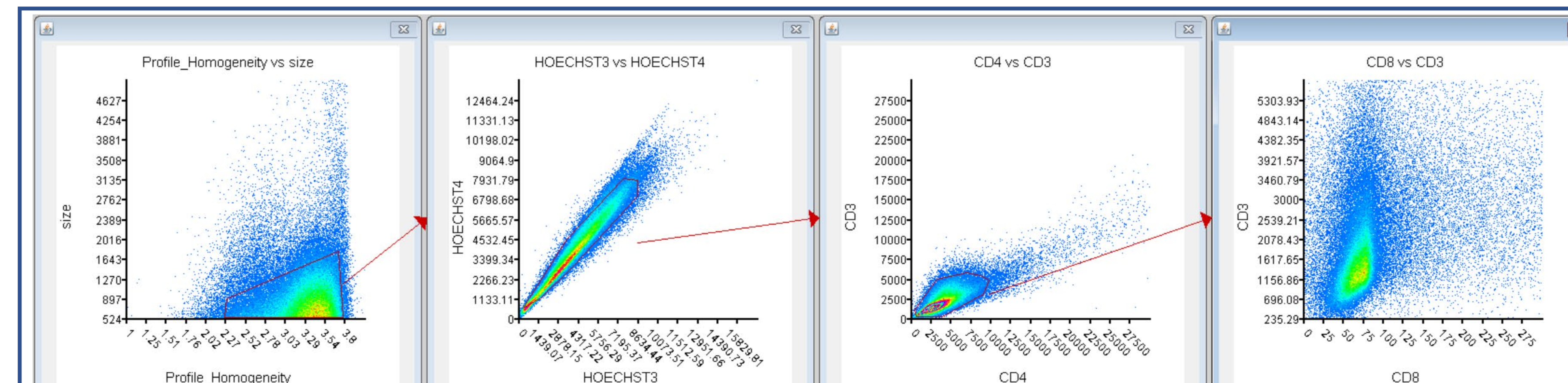


Figure 8. Cell feature based gating. Quantitative FCS data was gated by various cell features, including size, homogeneity, and specific biomarkers. Sequential gating allowed for accurate identification of cytotoxic T-cells (CD3+, CD8+), from which cell counts were determined.

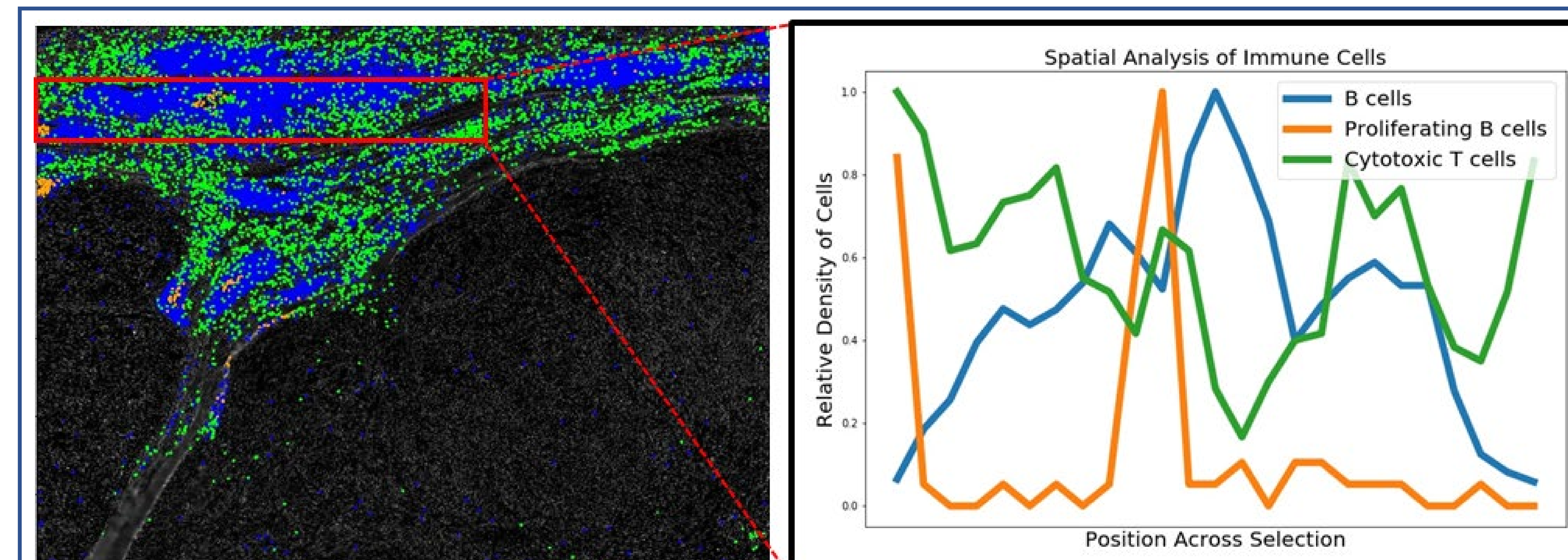


Figure 9. Spatial analysis of immune cells. Relative density of three immune cell types were analyzed across the red rectangular region. The rectangular region was broken up into 25 bins. Abundance of each cell type was counted relative to the maximum abundance of each cell type across bins.

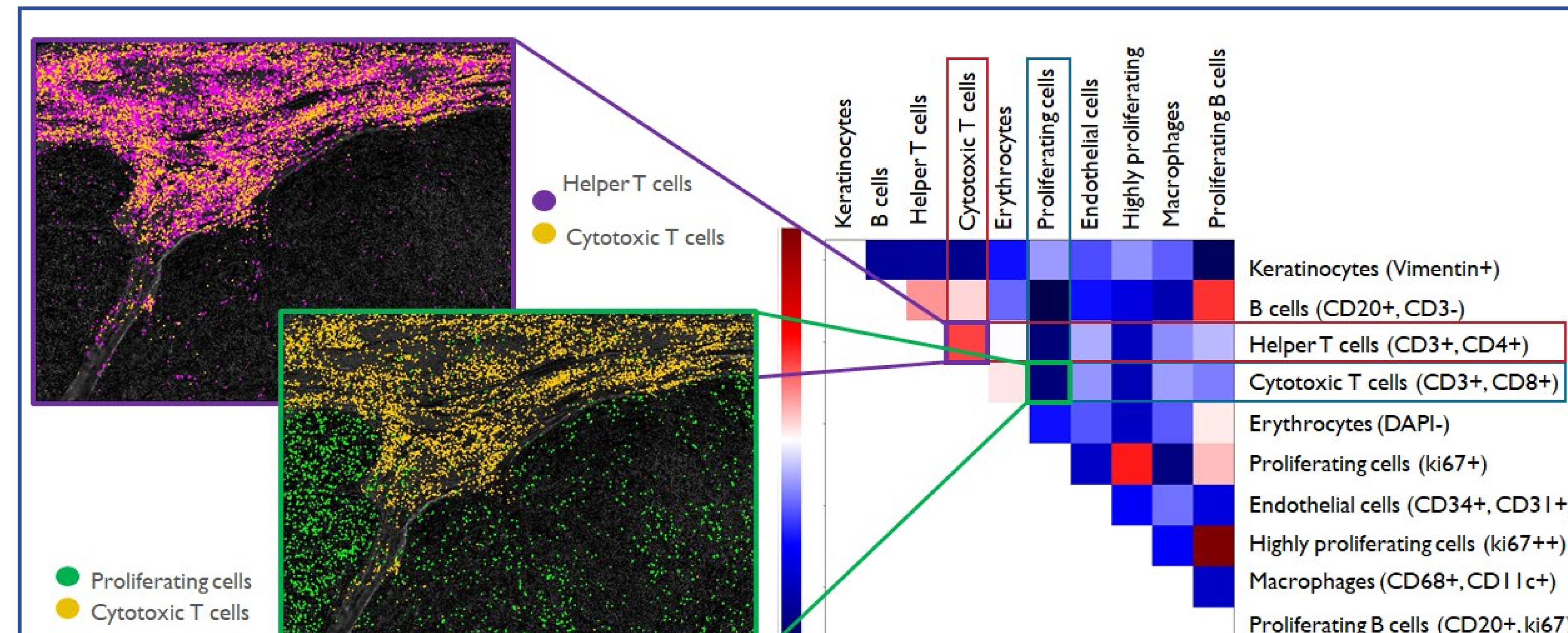


Figure 10. Cell-cell interaction neighborhood analysis. Cell phenotype specific clusters were subjected to cell-cell interaction analysis. Color in heatmap indicates intensity of log-odds ratio of interaction (frequency of cell-cell interactions relative expected frequency of random interactions). One interaction is defined as a less than 7 um distance between cells.