

Highly multiplexed single-cell spatial analysis of tissue specimens using CODEX™

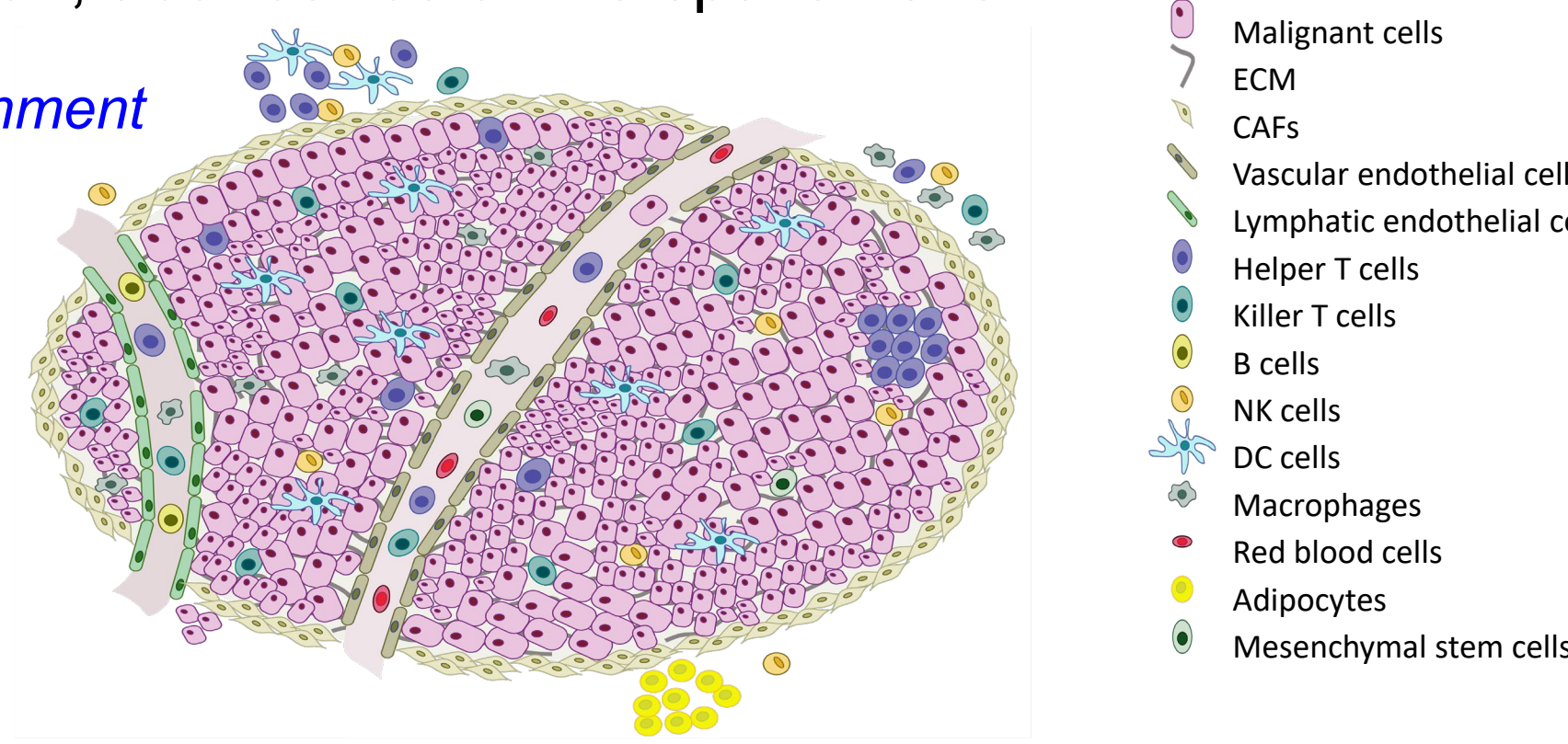


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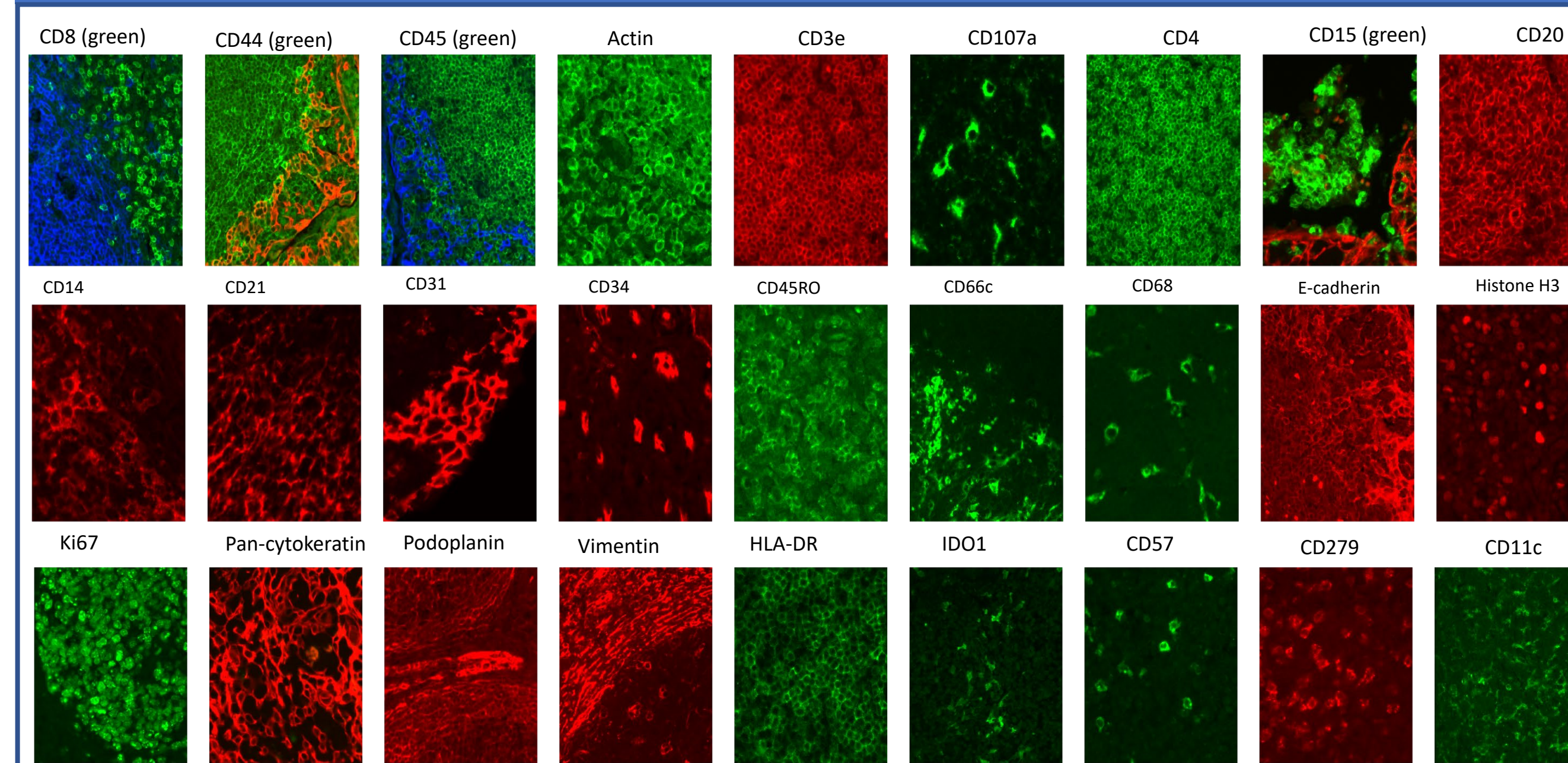
INTRODUCTION

The tumor microenvironment is comprised of numerous infiltrating immune cells, proliferating tumor cells and a variety of associated tissue cells. Immune evasion of tumor cells are collectively contributed by these cells. Hence, the spatial distribution of the associated cell types and tissue features is a critical parameter of tumor biology. Current technologies are limited to either measuring the spatial parameter or measuring high-parameter space but are not able to measure both in the same sample. Here we present a novel multiparametric imaging technology, termed CODEX (CO-Detection by IndEXing), a high-throughput technology to detect more than 50 biomarkers simultaneously in a single tissue, and resolve their relative expression, abundance at the spatial level.

Tumor Microenvironment

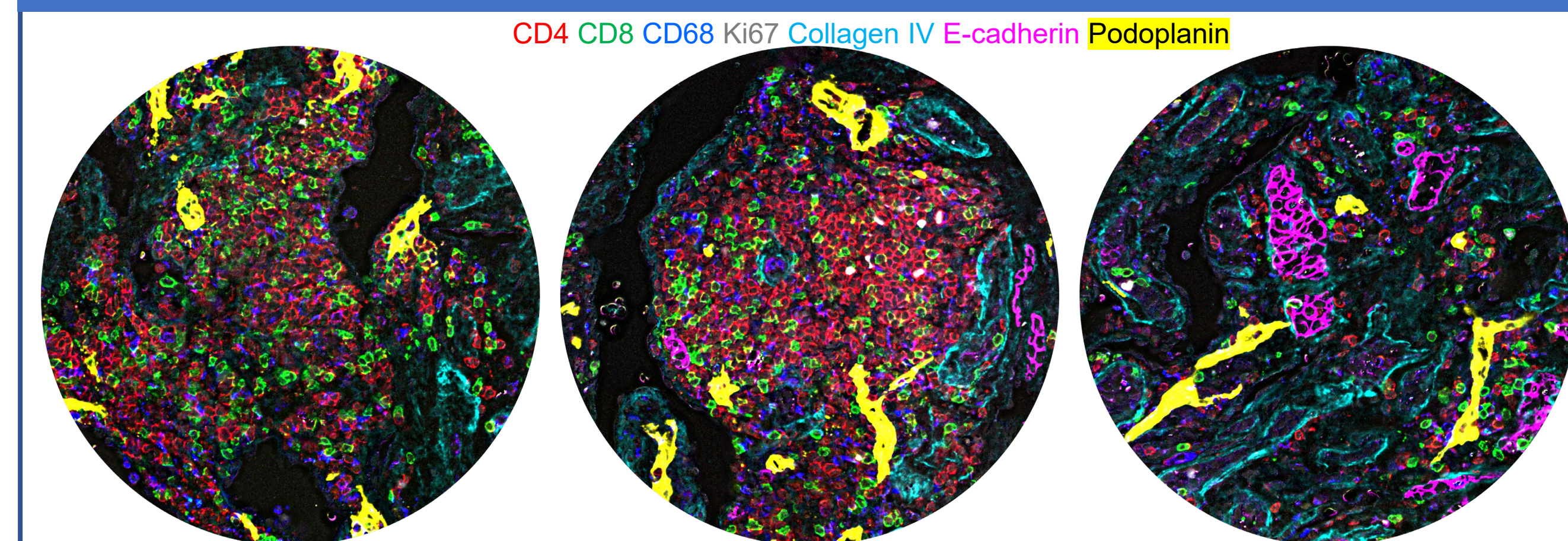


Human FFPE Antibody panel



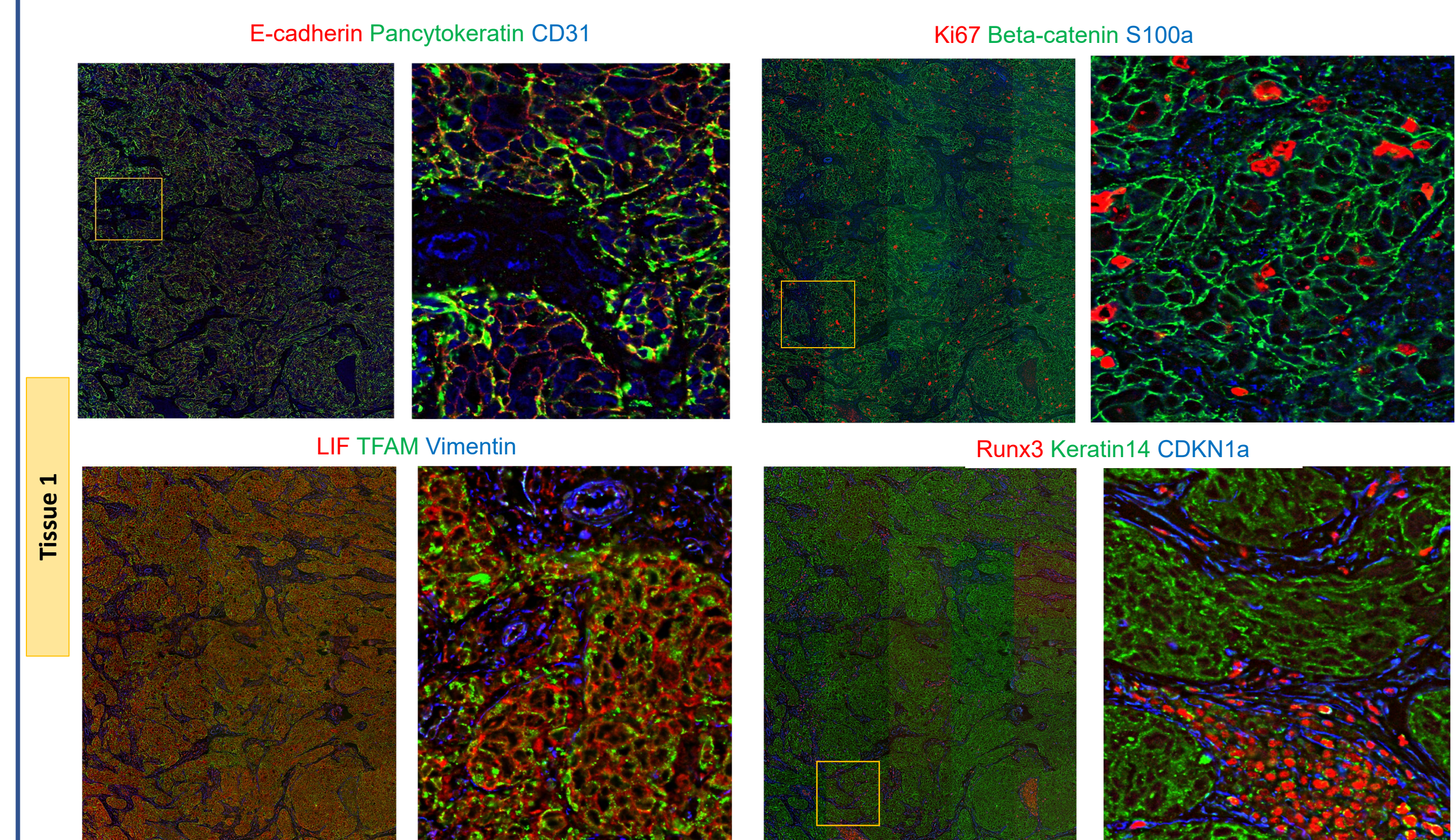
Human FFPE antibody panel: Human FFPE tonsil tissue was stained with 27 markers in a single step and revealed via a full automated fluidics workflow.

Human Renal Cell Carcinoma CODEX Tissue Analysis

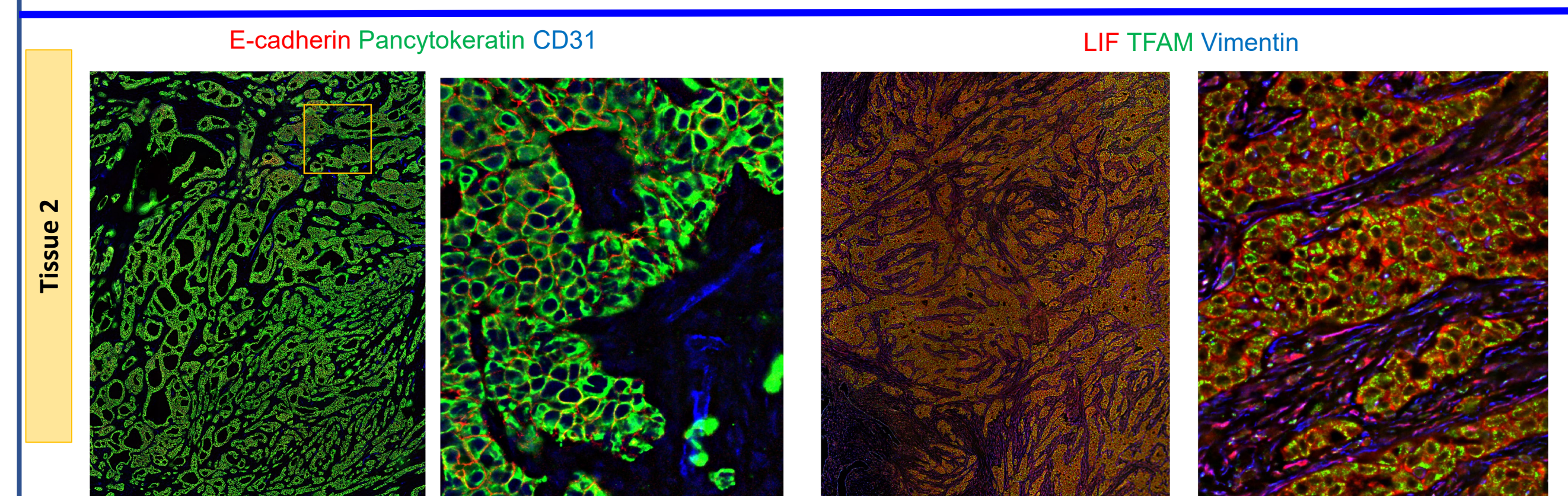


Characterizing Human FFPE Renal Carcinoma: Human FFPE renal carcinoma was stained with 7 markers and imaged using CODEX™ workflow. Interactions of infiltrating immune cells (CD3 and CD8) with proliferating tumor cells (Ki67), epithelial cells (Podoplanin), and cells that may have involved in tumor invasion (E-cadherin) was characterized.

FFPE-Human Breast cancer tissue analysis



Breast carcinoma tissue analysis : Breast carcinoma (Stage IIB: T4d N2a MX with 90% T/ 5% TCS/ 5% N) was stained with different CODEX markers and analyzed using CODEX™ workflow. Image analysis revealed critical markers that are involved in tumorigenesis (LIF, TFAM), Tumor invasion (E-cadherin), Proliferation (Ki67), Prognostic marker for breast carcinoma (Beta-catenin) and cell survival (CDKN1a).



Breast carcinoma tissue analysis : Breast carcinoma (Stage IIB: T3 NX MX with 90% T/ 20% N) was stained with different CODEX™ markers and analyzed using CODEX™ workflow. Image analysis revealed critical markers that are involved in tumorigenesis (LIF, TFAM) and Tumor invasion (E-cadherin).

DEEP MULTIPARAMETRIC ANALYSIS OF METASTATIC LYMPH NODE – BREAST CANCER (FF)

CODEX panel

CD11c	CD279	CD9
CD146	Cd3	collagen
CD152	CD36	Foxp3
CD19	CD38	HLA DR
CD2	CD4	Pan-Cytokeratin
CD205	CD45	perlecan
CD21	CD5	Podoplanin
CD223	CD63	vimentin
CD235a	CD7	vWF
CD278	CD8	

DAPI

Cell segmentation

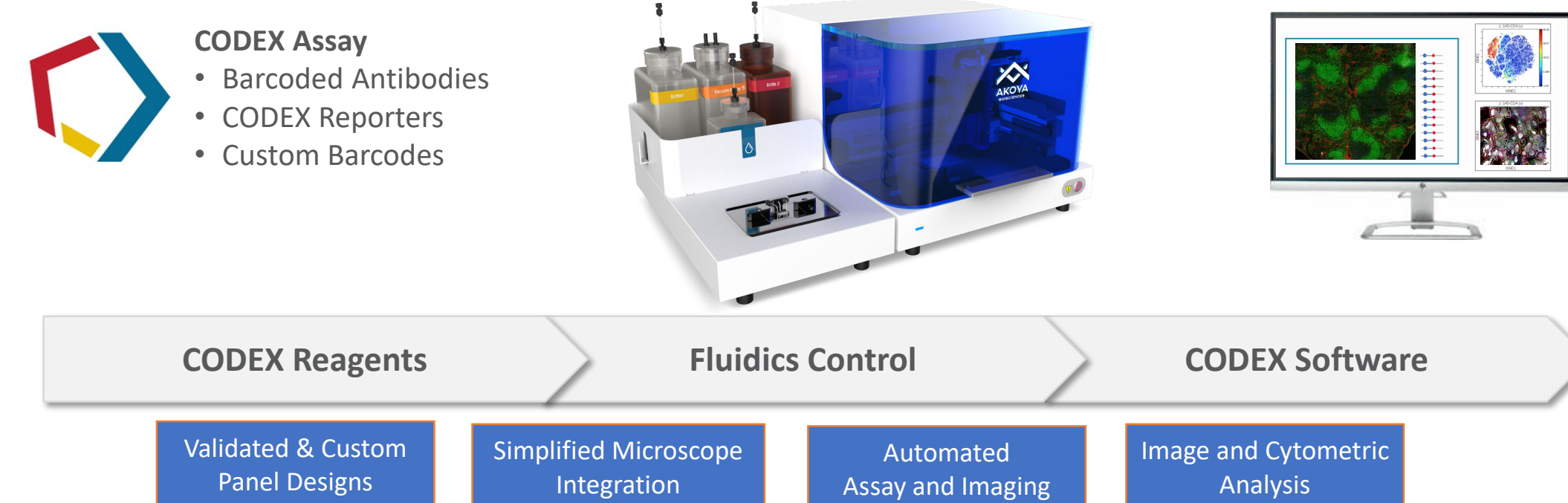
Cell clustering

Annotated cell clusters

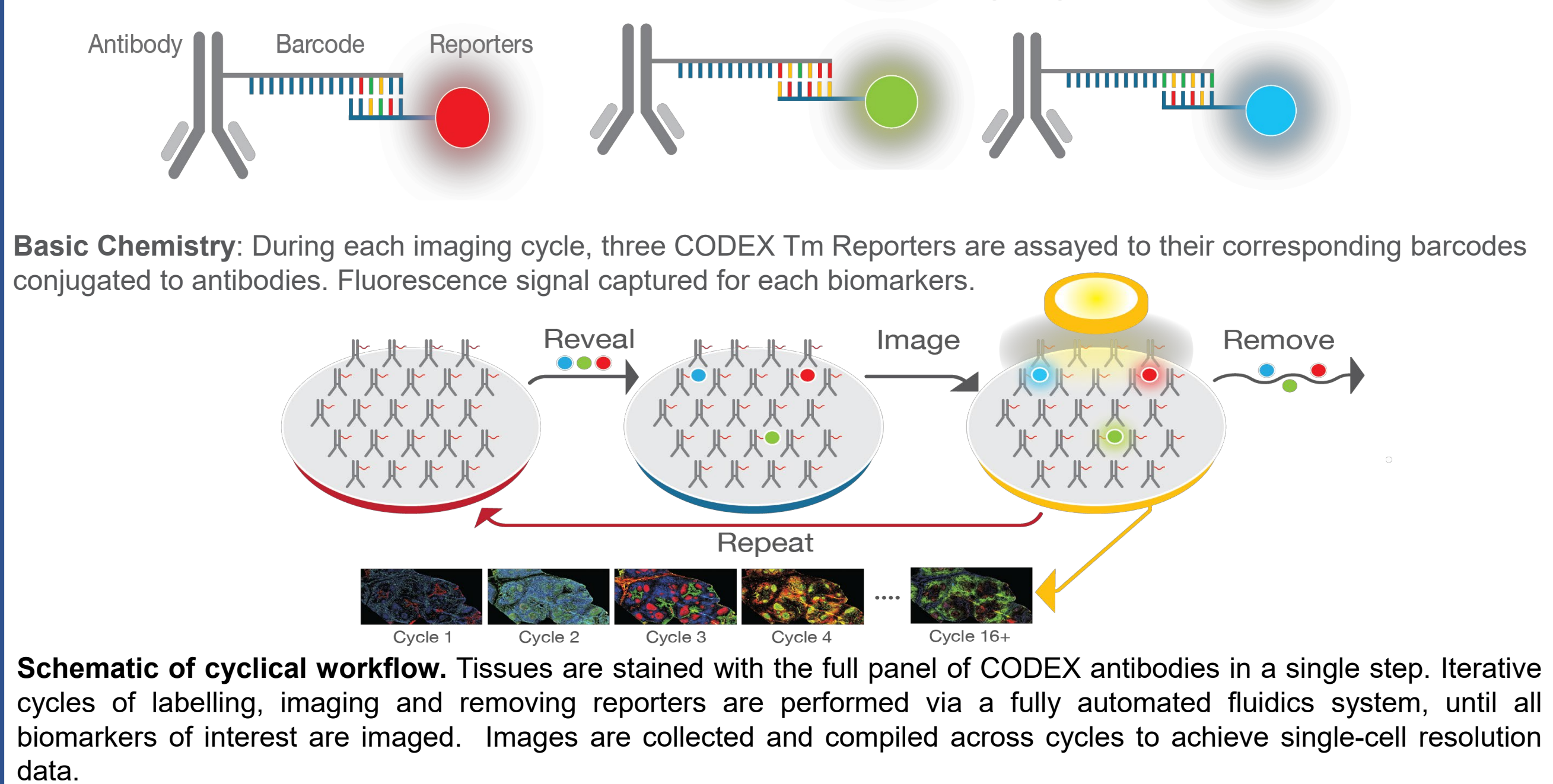
Cell segmentation → **Cell clustering** → **Annotated cell clusters**

Data analysis of Metastatic lymph node breast cancer. Segmented cells were clustered using X- shift clustering based on nearest neighbor estimation and the cell types were annotated by phenotype marker expression.

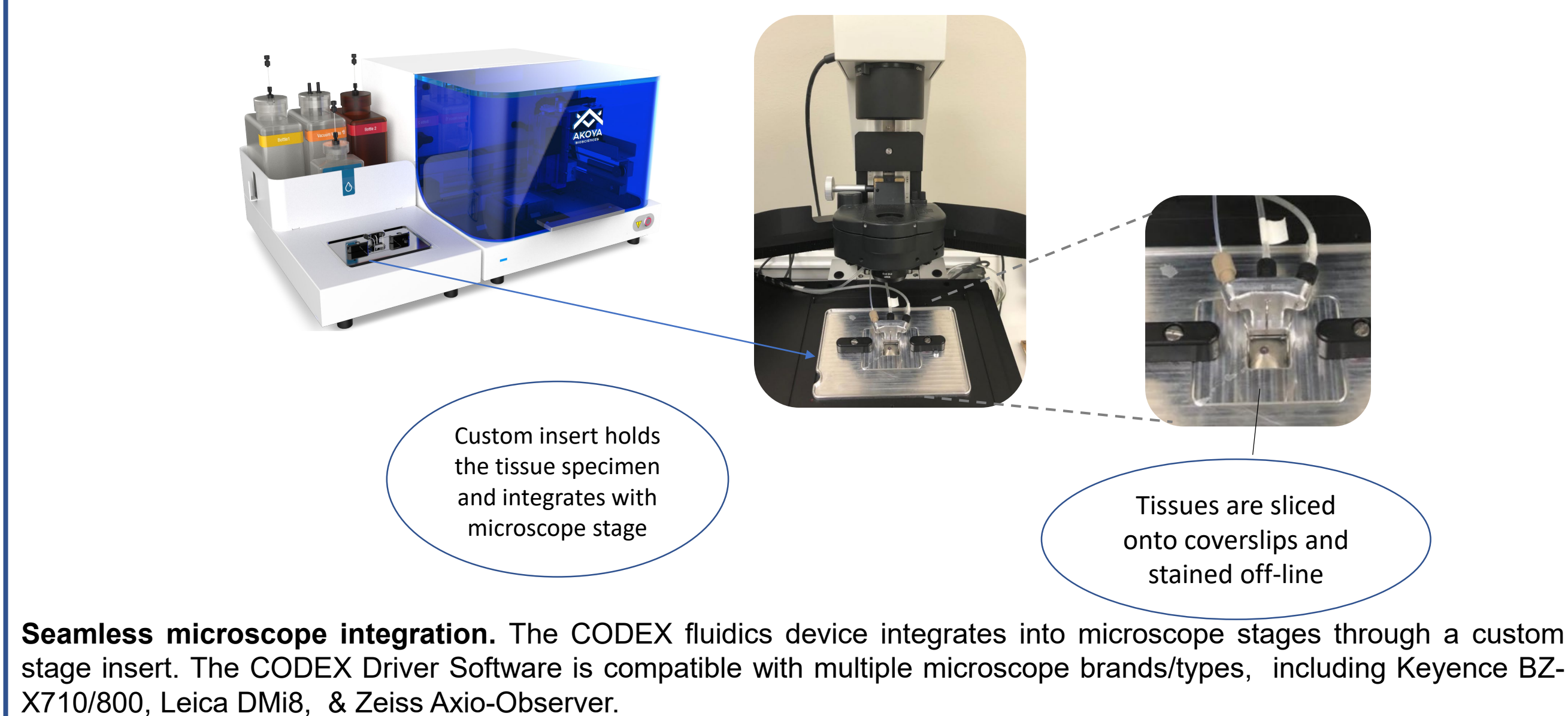
CODEX: CO-Detection by indEXing



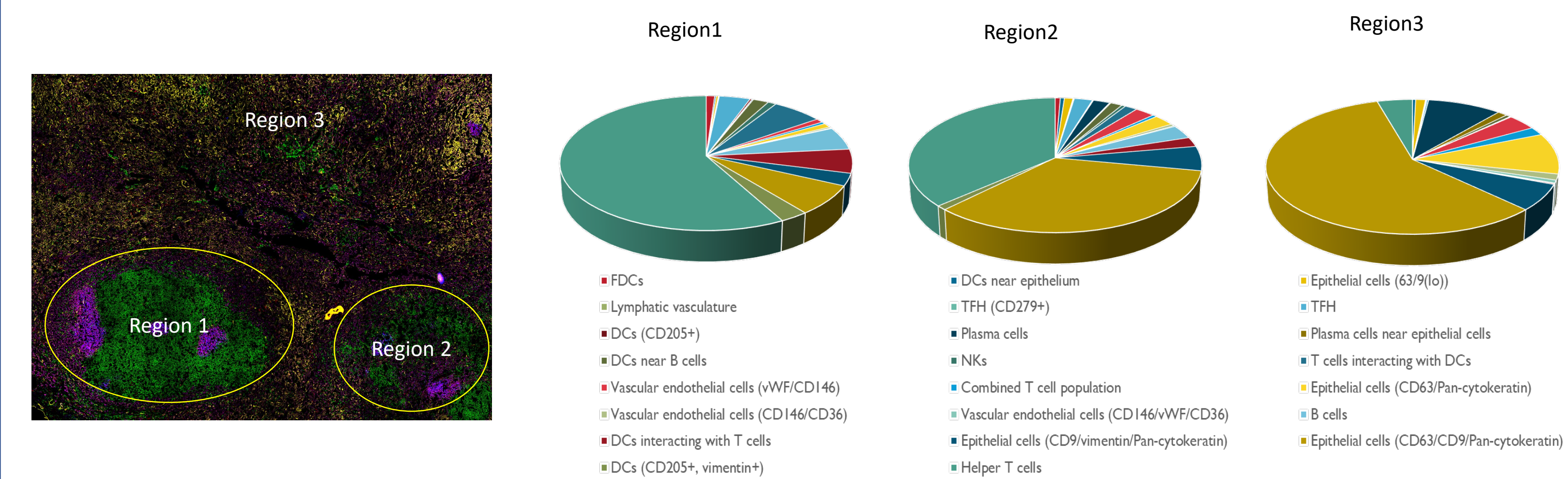
CODEX-Cyclic workflow



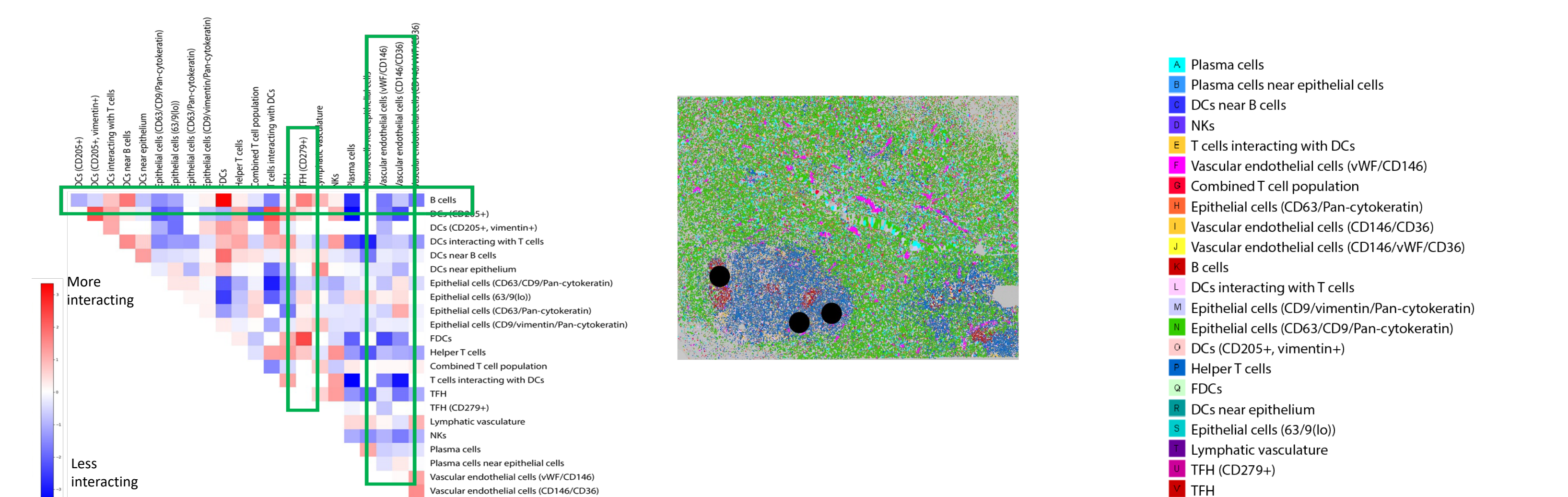
INSTRUMENT / MICROSCOPE INTEGRATION



Mapping tissue architecture



Spatial correlations of multiple cell types were compared among different regions



Conclusion

- CODEX™ enables multiplexed, spatial analysis of tissue specimens in a fully automated workflow.
- CODEX™ is compatible with a variety of tissue specimens, including FF and FFPE formats.
- CODEX™ data can be analyzed using the CODEX analysis tools to characterize cell type, map the tissue architecture and cellular niches