Spatially-resolved deep biomarker profiling of single cells in FFPE tissue samples through CODEX®

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Spatially resolved, deep biomarker profiling of tissue specimens is crucial for investigating the architecture and cell diversity in complex matrices, such as the tumor microenvironment (TME). The correlation of these two parameters with the progression of a variety of pathologies, ranging from cancer to autoimmune diseases, is still vastly unknown due to the lack of technologies that enable the detection of both high content and spatial, resolution. Formalin-fixed paraffin-embedded (FFPE) tissues constitute the ideal sample for these investigations as they retain tissue morphology at room temperature for long periods of time, which is convenient and cost-effective and are available in vast specimen archives.

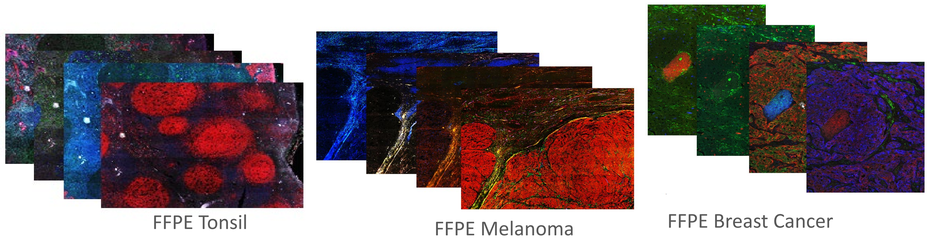
Akoya Biosciences, Inc. is commercializing CODEX™ (CO-Detection by indEXing), a multi-parametric imaging platform that performs the concurrent detection and quantification of dozens of protein targets with single-cell resolution within a tissue specimen. The CODEX platform uses a proprietary Barcodes library to label antibodies and iterative cycles of adding and removing conjugated dye-labeled Reporters to reveal the staining pattern for a subset of the target markers per cycle. The entire data acquisition process is fully automated using the CODEX instrument, which integrates with existing microscopes.

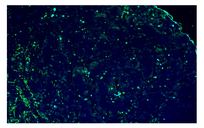
To support high-multiplexing capabilities, more than 45 different antibody clones have been screened and validated for the detection of target epitopes in human FFPE secondary lymphoid tissues for both healthy and cancerous specimens using the CODEX platform. Proof of-concept data have also been obtained for different cancer types, including melanoma and head and neck cancer in both normal and TMA formats.

An analysis software has been developed to extract meaning from CODEX datasets and is included as part of the CODEX platform. The analysis pipeline includes image drift compensation, deconvolution and segmentation to measure integrated fluorescence intensity for each cell across tens of parameters. In this manner, infiltrating lymphocytes and other immune cells can be identified within the TME. With the high multiplexing capabilities, a variety of T cells can be identified to detect the ratios of both immunosuppressive and immune activating subtypes.

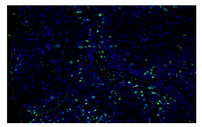
Analysis of FFPE tissues using the CODEX platform demonstrates unparalleled opportunities for simultaneously detecting tens of markers with single-cell resolution and spatial information within a tissue specimen.

CODEX: a Highly Versatile Imaging Platform





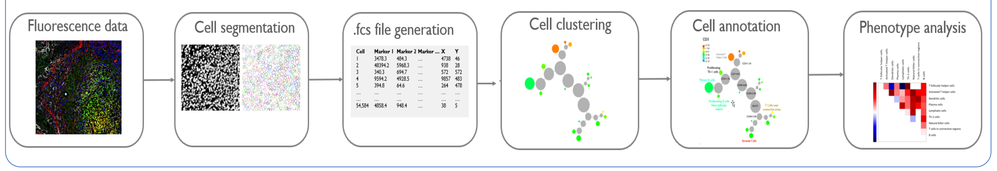
TMA: stage II oral cancer



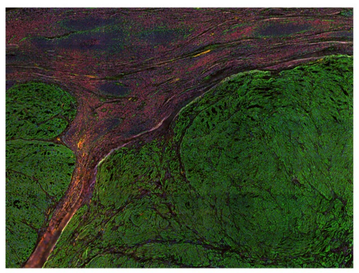
TMA: laryngeal cancer

CODEX assays can be performed on a variety of tissue sections, ranging from FFPE to fresh-frozen samples. CODEX assays are also non-destructive: tissues can be cleared and stored after one or more CODEX runs. Here representative images from CODEX multicycles on FFPE human tonsil, melanoma and breast cancer tissues are reported. CODEX antibody staining is also shown in tissue biopsy cores from tissue microarrays (TMAs) as proof of concept.

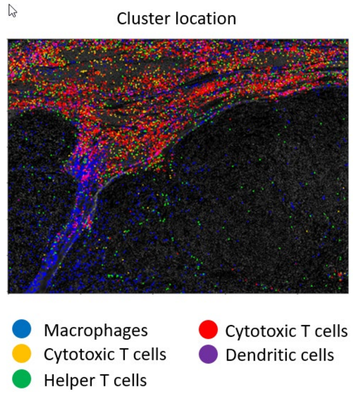
Overview of CODEX workflow



Deep phenotyping with spatial information of FFPE Human Melanoma Tissue

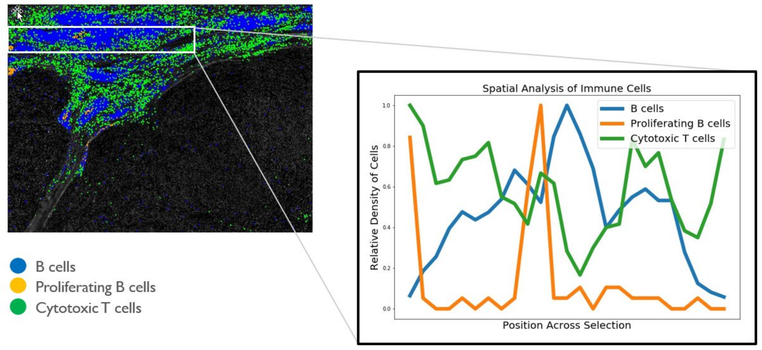


CD34
CD4
CD3
CD8
ECad
ki67
Vimentin
CD68
CD31
Pancytokeratin
CD11c
CD20

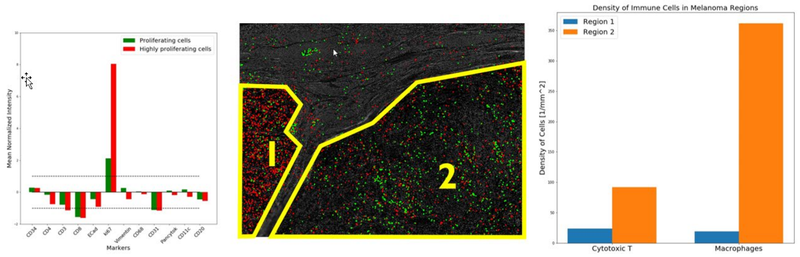


Segmented cells were clustered on the basis of the expression levels of **12 biomarkers** to identify **8 different cellular phenotypes.** The spatial location of different cell types are revealed at single-cell resolution

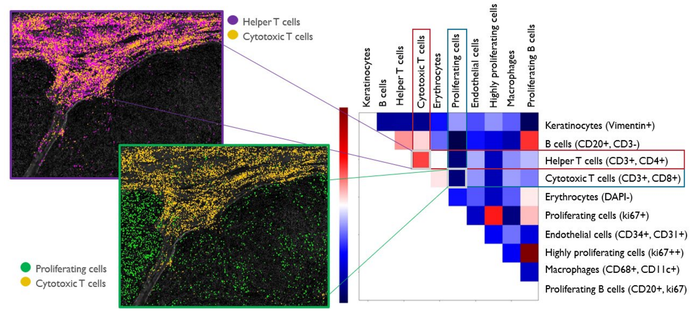
CODEX Analysis: Revealing the Spatial Distribution of relevant cellular phenotypes



Spatial correlations between multiple phenotypes can now be investigated and compared region-to-region



Microscopic interactions between single cells are revealed for a dozen of different phenotypes with a single CODEX run



CODEX technology allows unprecedented possibilities for investigating the microenvironment of FFPE tissue samples by combining deep biomarker profiling with microscopic spatial information.