CODEX®: a novel platform for spatially-resolved deep biomarker profiling of single cells in tissue samples

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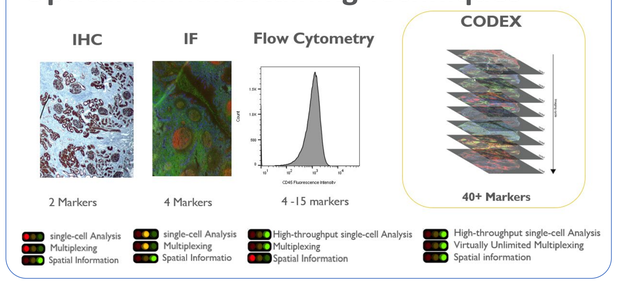
The tumor microenvironment (TME) is comprised of a multitude of cell types that collectively create an immunosuppressive environment, enabling tumor growth. Understanding the spatial organization of these tissues requires a technology that can identify the presence of multiple markers and correlate it to their specific spatial location within the same tissue. Characteristics of the TME including infiltrating immune cells, angiogenesis and the presence of other non-malignant cells influence drug delivery, treatment effectiveness and, ultimately, clinical outcomes. Current methodologies for analyzing the spatial dimension of tissues, e.g. traditional immunofluorescence (IF) and immunohistochemistry (IHC), are limited to measuring a few parameters simultaneously, thereby restricting the number of phenotypes that can be identified. Conversely, single-cell technologies like mass cytometry and Next Generation Sequencing-based tools provide multiplexing capabilities, but at the expense of the associated spatial information.

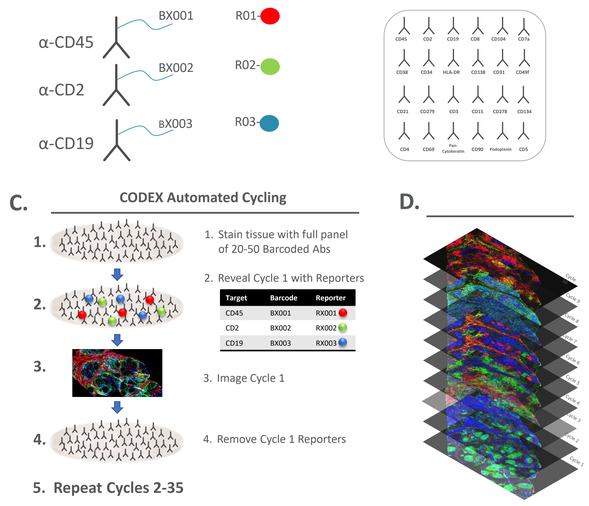
Akoya Biosciences, Inc. is commercializing CODEX™ (CO-Detection by indEXing), a multi-parametric imaging platform that allows the simultaneous detection and quantification of dozens of target epitopes in single cells within a single tissue section. This innovative platform is an end-to-end solution that comprises three components: 1) the CODEX fluidics instrument, 2) a suite of specialized CODEX reagents and 3) an analysis pipeline. The CODEX workflow involves a barcoding system such that each antibody moiety is conjugated to a proprietary tag (the Barcode). Panels of CODEX antibodies are used to stain tissue specimens *en masse* in a single step. Staining data for sets of antibodies are revealed across iterative cycles using corresponding dye-labeled Reporters. The CODEX fluidics instrument integrates with existing microscope units for a fully automated data collection process. CODEX data is processed to achieve noise reduction and analyzed at the single-cell level through a segmentation algorithm based on nuclear staining.

More than 80 antibodies have been validated on the CODEX platform for analysis of human and mouse fresh-frozen and FFPE tissues. Preliminary studies on fresh-frozen tissues with developed antibodies demonstrate an unprecedented capability for revealing the spatial correlation between a variety of target epitopes. These results demonstrate the enormous potential of the CODEX technology to identify spatial correlations in the TME through highly multiplexed detection with single-cell resolution.

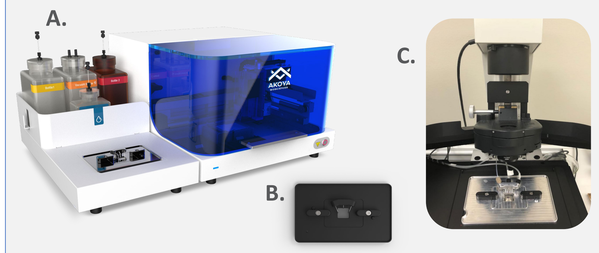
Optical Immunostaining Techniques



CODEX Assay

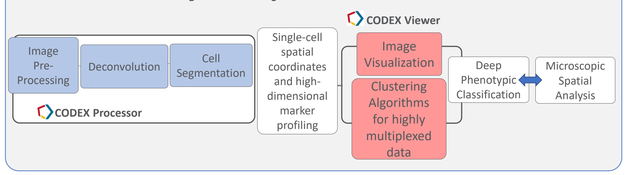


A. Antibodies are conjugated to a CODEX Barcode with a highly specific corresponding dye-labeled CODEX Reporter. **B.** Antibody cocktail made from full panel typically of 20-50 Barcoded antibodies. **C.** CODEX Assay begins with single staining step with antibody cocktail (1.). Subset of targets is revealed by automatic addition of first cycle of Reporters (2.). Imaging of 3 spectrally distinct dyes for first cycle (3). Automatic removal of Cycle 1 Reporters (4.). Repeat process to assay full panel (5.) **D.** In this manner, high quality data is captured for all cycles and targets.

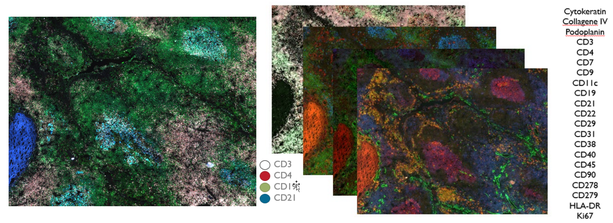


A. The CODEX Instrument integrates with standard inverted fluorescent microscopes for automated reagent dispensation and imaging through cycles. **B.** Tissue samples, mounted on a glass coverslip, reside in a stage insert, which is connected to the instrument through fluidics lines. **C.** Integration has been validated on Keyence, Leica and Zeiss with Nikon systems in progress.

CODEX Analysis Pipeline

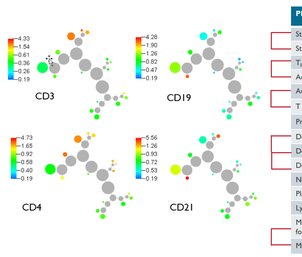


Showcase: CODEX on a fresh-frozen Human Tonsil



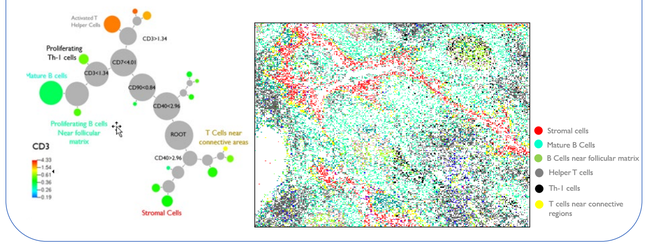
Data Clustering on CODEX data

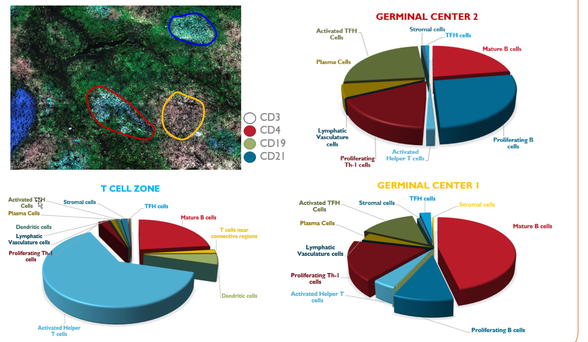
Clustering algorithm Xshift [1] assigned segmented cells to 16 clusters on the basis of expression levels of 21 different biomarkers. We were able to assign 15 clusters to known phenotypes present in tonsils.



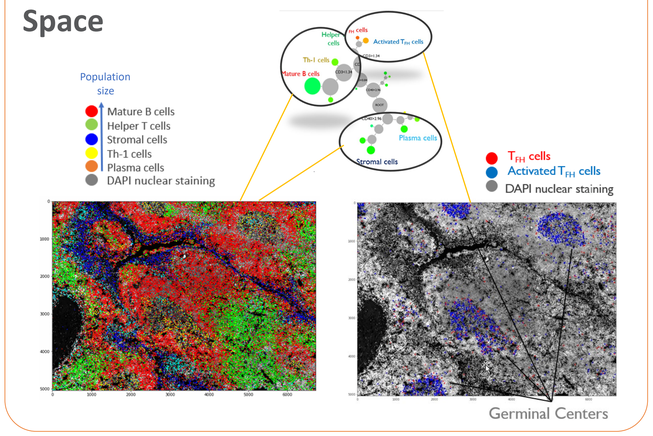
Phenotype Assignation	Population	Positive Markers (All CD45+)
Stromal Cells	3414	CD90+, CD9+, HLA-DR+, Cytokeratin+
Stromal Cells in connective regions	807	CD31+, CD90+, Collagen IV+
T_{FH} Cells	328	CD3+, CD4+, CD7+, CD278+, CD279+
Activated T_{FH} Cells	1459	CD3+, CD4+, CD40+, CD278+, CD279+
Activated Helper T cells	11866	CD3+, CD4+, CD7(+)
T Cells near connective regions	222	CD3+, CD29+, CD90(+), Collagen IV(+)
Proliferating Th-1 cells	2411	CD4+, Ki67+
Dendritic Cells	135	CD4+, HLA-DR+
Dendritic Cells	366	CD4+, CD11c+
Dendritic Cells	429	CD11c+, HLA-DR+
NK cells	126	CD3-, CD7+
Plasma Cells	2186	CD31+, CD38+, CD40+
Lymphatic Vasculature Cells	1336	CD9+, Podoplanin+
Mature, proliferating B cells near follicular matrix	960	CD19+, CD22+, CD21+, CD90+, Ki67+
Mature B cells	32840	CD19+, CD22+

The spatial location of each cell belonging to a specific phenotype (or cluster) is known, making possible to perform proximity studies and spatial analysis.

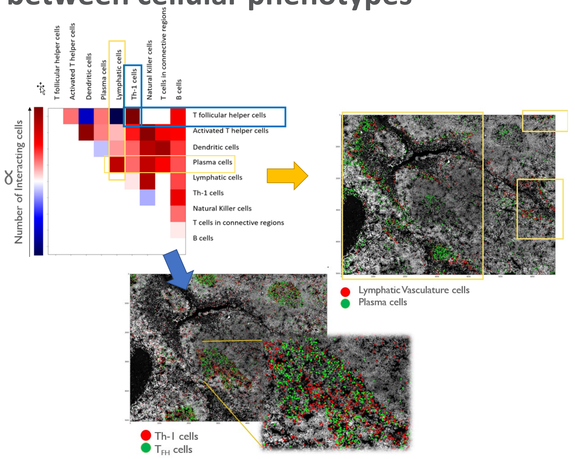




From Clustering to Deep phenotyping in Space



Revealing microscopic interactions between cellular phenotypes



Information derived from CODEX analysis allow to build Interaction Maps, which illustrate the extent of microscopic interactions between phenotypes . Here, interacting cells are defined as cells close to each other 7 µm or less.

Conclusion

- ✓CODEX allows to obtain high throughput single cell information coupled with Virtually Unlimited Multiplexing and Spatial information
- ✓CODEX data can be analyzed through classical flow cytometry analysis platforms and/or by clustering algorithms. Data Clustering allows to identify correlation between multiple markers that are difficult to discern manually in high dimensional dataset.
- ✓ Clusters are manually classified as phenotypes, which can be investigated on the basis of their spatial location in the tissues, allowing us to obtain information on:
- ✓phenotype distribution in space
- ✓Microscopic interactions between single cells

References:
[1] N. Samusik *et al.*, Nature Methods, **2016**, 13, 493-6.