Analysis of FFPE human tumor tissues using CODEX® with signal amplification #387

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Introduction

The spatial relationships between infiltrating immune cells and the remodeling of the cellular matrix is widely recognized as a key component to defining tumor heterogeneity. Current methodologies for studying cells within the context of tissue architecture, like traditional immunofluorescence (IF) and immunohistochemistry (IHC), are limiting—allowing the assessment of only a few parameters at a time. The CODEX® technology has overcome this limitation through a DNA-based labeling strategy, involving adding and removing dye-labeled oligonucleotides (reporters) across multiple cycles to oligonucleotide-labeled antibodies. In this manner, tens of markers can be analyzed on the same tissue. Additionally, Recently, we expanded our workflow to amplify the fluorescent signal intensities of low expressing biomarkers by combining Tyramide Signal Amplification (TSA) with CODEX® , also known as CODEX® Amp. Here, we present the analysis of CODEX® data for a panel of over 20 biomarkers on human FFPE tissue including signal amplification of PD-L1, PD-1 and FOXP3.

CODEX®: CO-Detection by indEXing

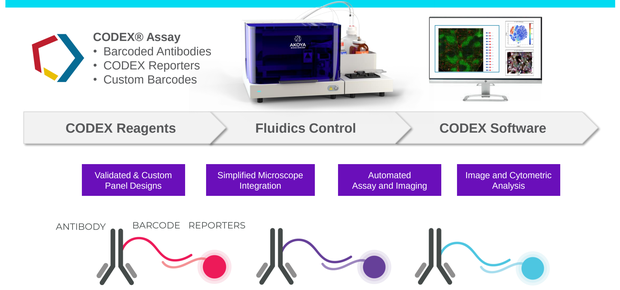


Figure 1: Basic Chemistry: During each imaging cycle, three CODEX® Reporters with fluorophores are assayed to their corresponding barcodes conjugated to antibodies.

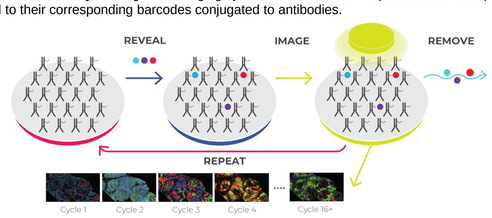


Figure 2: Schematic of cyclical workflow: Iterative cycles of labelling, imaging and removing reporters are performed via a fully automated fluidics system, until all biomarkers of interest are imaged. Images are collected and compiled across cycles to achieve single-cell resolution data.

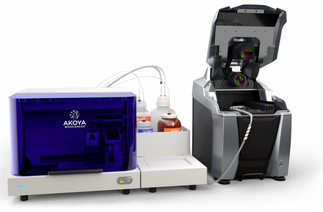


Figure 3: Seamless microscope integration: The CODEX® fluidics device integrates into microscope stages through a custom stage insert. The CODEX Driver Software is compatible with multiple microscope brands/types, including Keyence BZ-X710/800, Leica DMi8, & Zeiss Axio-Observer.

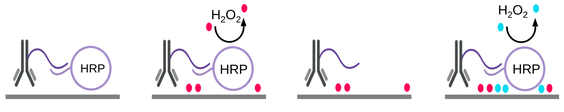


Figure 4: CODEX® Amp workflow: Tissues are stained offline in a single step according to standard CODEX® protocol. After iterative cycles of labelling, imaging, and removing via our standard CODEX® workflow, up to three cycles of HRP oligonucleotides, catalysis, deposition of a single CODEX® Amp dye per cycle, and removal of HRP oligonucleotides can be performed and imaged with our standard microscope filters (Cy3, Cy5, AF750). CODEX® Amp dye deposition is not removable and remains on the tissue after stripping the HRP oligonucleotide.

CODEX® Amp. of low expressing markers

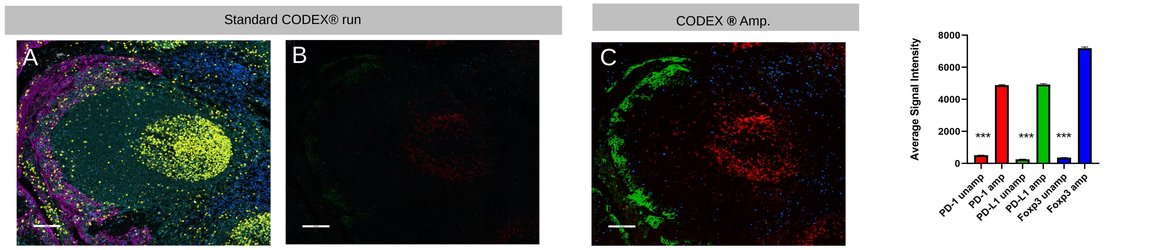


Figure 5: Comparison of standard CODEX® with CODEX® Amp: Standard CODEX® markers (A) CD20-BX007, CD68-BX015, PCK-BX019, CD8-BX026, and Ki67-BX047 and key IO markers (B) PD-L1-BX043, PD-1-BX046, and Foxp3-BX031 were stained and imaged on FFPE human tonsil with standard CODEX® workflow and imaged at 500, 350, and 500 milliseconds respectively. (C) PD-L1-BX043, Foxp3-BX031, and PD-1-BX046 antibodies were then amplified using the HRP scheme described with CODEX ® Amp dyes 570, 670, and 780 and imaged at 2.5, 25, and 50 milliseconds respectively. Zoomed in regions indicate higher signal intensity of amplified signal compared to standard CODEX® run. A one-way ANOVA test was ran and post-hoc comparison indicated statistically significant differences between each group as indicated (*** is p<0.001)

CODEX® Amp. on Non-small cell lung cancer TMA

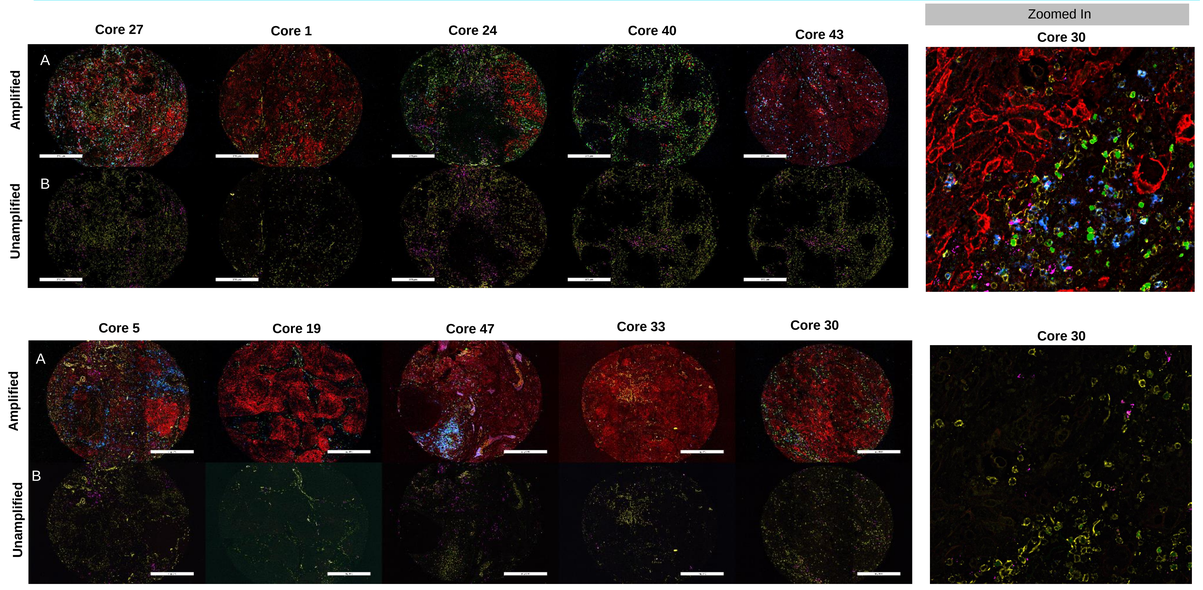
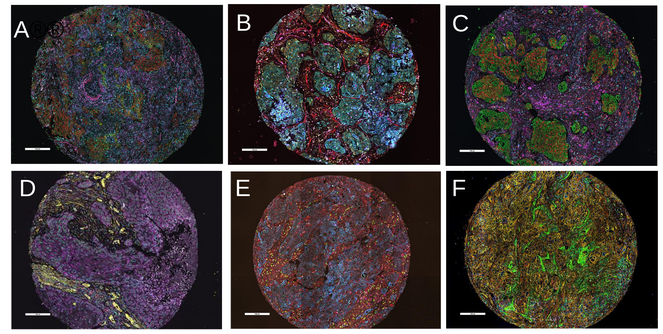


Figure 6: Comparison of Standard CODEX® with CODEX® Amp on TMA cancer samples: A 50 core non-small lung cancer FFPE tissue microarray was stained with 39 CODEX markers. Ten cores are displayed above. PD-L1-BX043, Foxp3-BX031, and PD-1-BX046 antibodies were first run and imaged with standard CODEX® workflow and then amplified using the HRP scheme described above with CODEX ® Amp dyes 570, 670, and 780 respectively. Panel A shows CD68-BX015, CD3-BX045, the amplification of PD-L1-BX043, Foxp3-BX031, and PD-1-BX046. The amplified PD-L1-BX043, Foxp3-BX031, and PD-1-BX043 were imaged at 2.5, 25, and 50 milliseconds respectively. Panel B shows the CD68-BX015, CD3-BX045, PD-L1-BX043, Foxp3-BX031, and PD-1-BX046 imaged using the standard CODEX ® workflow. The unamplified PD-L1-BX043, Foxp3-BX031, and PD-1-BX043 were imaged at 500 and 350 milliseconds respectively. Zoomed in regions indicate higher signal intensity of amplified signal compared to standard CODEX® run. CODEX Multiplex Analysis Viewer (MAV) was used to calculate change in average cell count and signal intensity by gating populations between the unamplified and amplified markers. Panel C and D show an increase in average number of cells and signal intensity between the unamplified and amplified markers, respectively. A one-way ANOVA test was ran and post-hoc comparison indicated statistically significant differences between each group as indicated (*** is p<0.001)

Multiparametric spatial profiling



Panel A: CD4, E-Cadherin, CD66e, Galectin9, CD4, Runx3
Panel B: CD34, CD11b, CD15, Ki67, Keratin-19
Panel C: HLA-DR, PCNA, Pancytokeratin, Collagen IV, CD68, CD20, b-catenin
Panel D: CD8, Lamin 1 ,E2F1, TFAM, IDO1, SMA
Panel E: Perlecan, S100A4,Foxp3 (amp), PD-L1 (amp), PD-1 (amp), Keratin-717
Panel F: b-actin CD31 CD123 Keratin 18 HLA-A

Figure 7: NSCLC Tissue Microarray: This non-small cell lung cancer tissue microarray was stained with 39 CODEX® markers in a single step, revealed via a fully automated fluidics workflow through iterative cycles, and processed using our standalone CODEX® processing software. The CODEX® processor aligns images across cycles, stitches tiles across large regions, subtracts autofluorescence, and segments and integrates marker intensities for each cell. 50 cores were imaged and shown above is one core with six different markers per panel.

Spatial Analysis

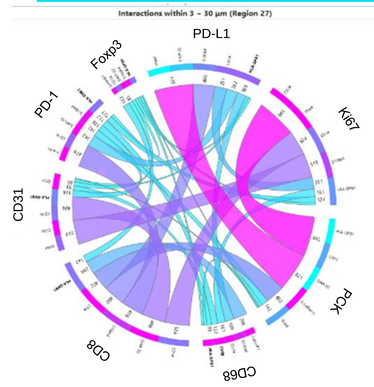


Figure 8: Circos Plot: Circos plot enables simultaneous visualization of spatial interactions and population characteristics. Cells defined as spatially interacting are within a specified spatial proximity. Here we have used 30 microns (approximately one cell diameter), as the proximal distance. The Circos plot displays the population size (arc length is proportional to population size), predominant biomarkers expressed by the cell population (arc components, colored by heatmap), and the number of spatial interactions between populations (ribbon heatmap color and size is determined by the number of interactions). In this example the heatmaps describing biomarker intensity and number of spatial interactions increase from light blue to dark pink. For this specific core, we can see that PD-L1 and PD-1 expressing cells are interacting with each other 242 times.

	PD-L1	PD-1	PCK	Ki67	Foxp3	CD31	CD8	CD68
PD-L1	1026	242	821	251	61	54	460	189
PD-1	242	586	142	127	65	138	478	132
PCK	821	142	3732	845	24	30	402	65
Ki67	251	127	845	1820	52	519	524	161
Foxp3	61	65	24	52	172	85	143	50
CD31	54	138	30	519	85	1142	498	93
CD8	460	478	402	524	143	498	2056	286
CD68	189	132	65	161	50	93	286	464

Conclusions

To learn more about CODEX ® Amp, you can click here or scan the QR code to contact one of our imaging specialists.

The Spatial Biology Company™

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