

VANDERBILT  UNIVERSITY
MEDICAL CENTER

INNOVATIVE
TRANSLATIONAL
RESEARCH SHARED
RESOURCE (ITR)/
IMMUNOPHENOTYPING
SHARED RESOURCE (IPSR)

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 VANDERBILT-INGRAM CANCER CENTER

CODEx

(PHENOCYCLER)



WHAT IS CODEX?



- ❖ CODEX (CO-Detection by indEXing) is a novel multiplex immunofluorescence system for advanced cell phenotyping developed by Dr. Garry Nolan at Stanford.
- ❖ Highly multiplexed immunofluorescence for spatial characterization of tissue microenvironments at the single cell level.
- ❖ Uses single step staining to preserve tissue integrity and an automated process of imaging biomarkers using the CODEX instrument and the Keyence microscope.
- ❖ To avoid confusion, please note that this system is now called the PhenoCycler after a recent Akoya rebranding effort.



FLEXIBLE

Validated on fresh frozen and FFPE samples



SAMPLE-FRIENDLY

Samples can be used for downstream H&E staining or ROI analysis



SCALABLE

Image 40+ biomarkers per sample



<https://www.akoyabio.com/intro-to-codex/>



Play Akoya
Bioscience
Intro to
CODEX video

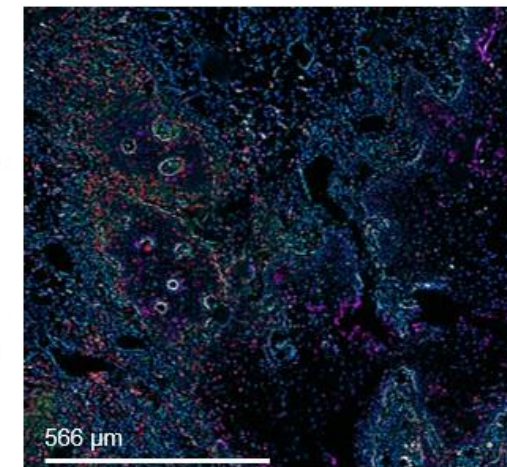
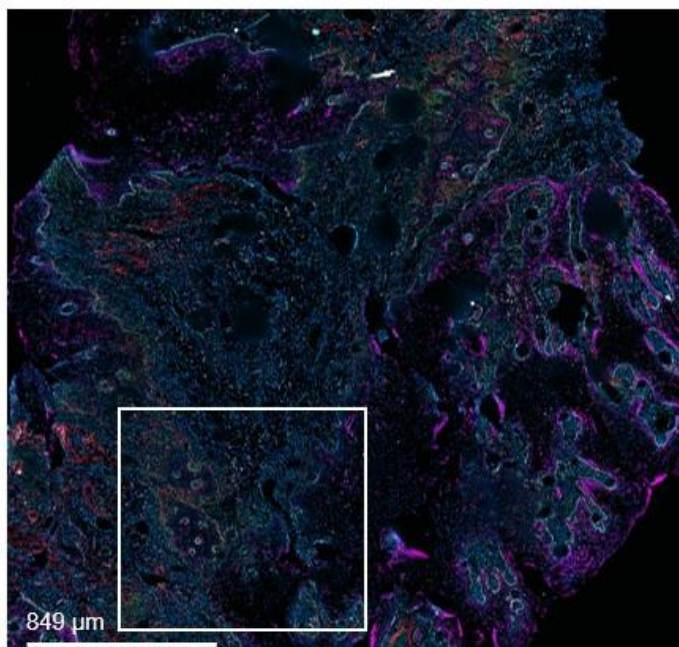


EXAMPLE IMAGES

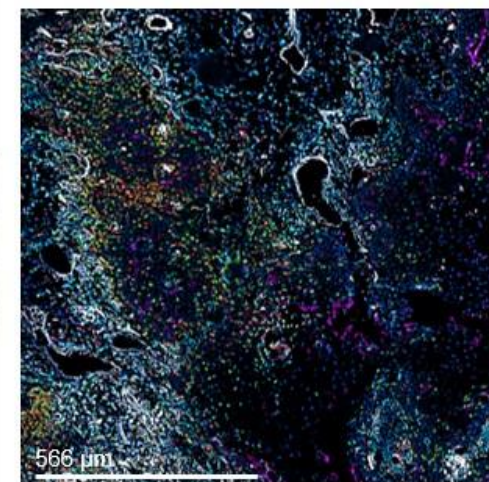
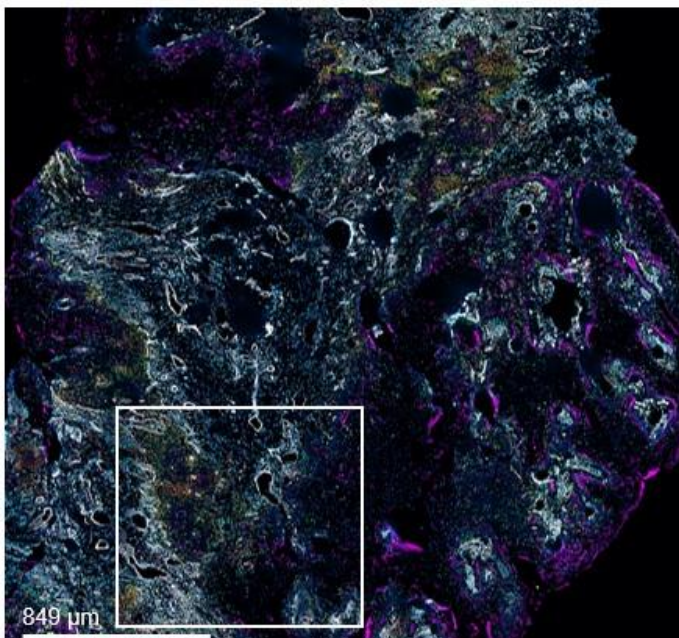
- ❖ 14 antibody panel on **fresh frozen** head and neck squamous cell carcinoma tissue
- ❖ Generated by ITR/IPSR staff on the CODEX instrument
- ❖ Tissue stained with Akoya pre-conjugated antibodies

CD45
CD3
CD4
CD8
CD19
CD69
CD104
HLA-DR
PD-1 (CD279)
Cytokeratin
Ki67
CD31
CD38
CD11c

DAPI PD-1 CD8 CD4 CD104 Cytokeratin



DAPI CD38 CD45 CD3 CD31 Cytokeratin

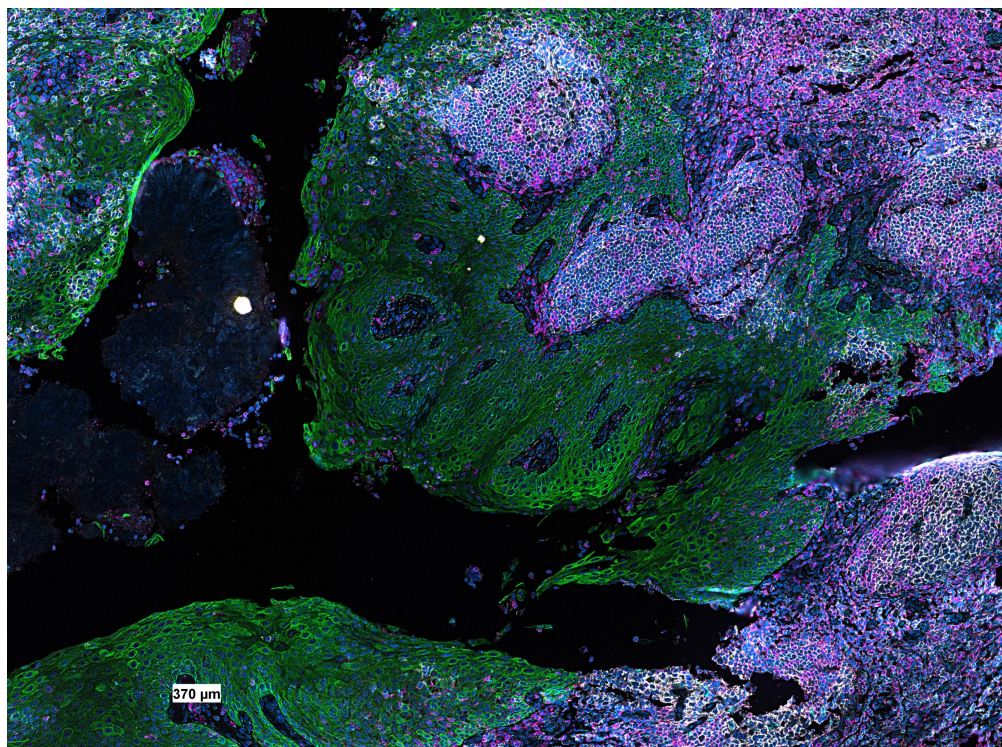


EXAMPLE IMAGES

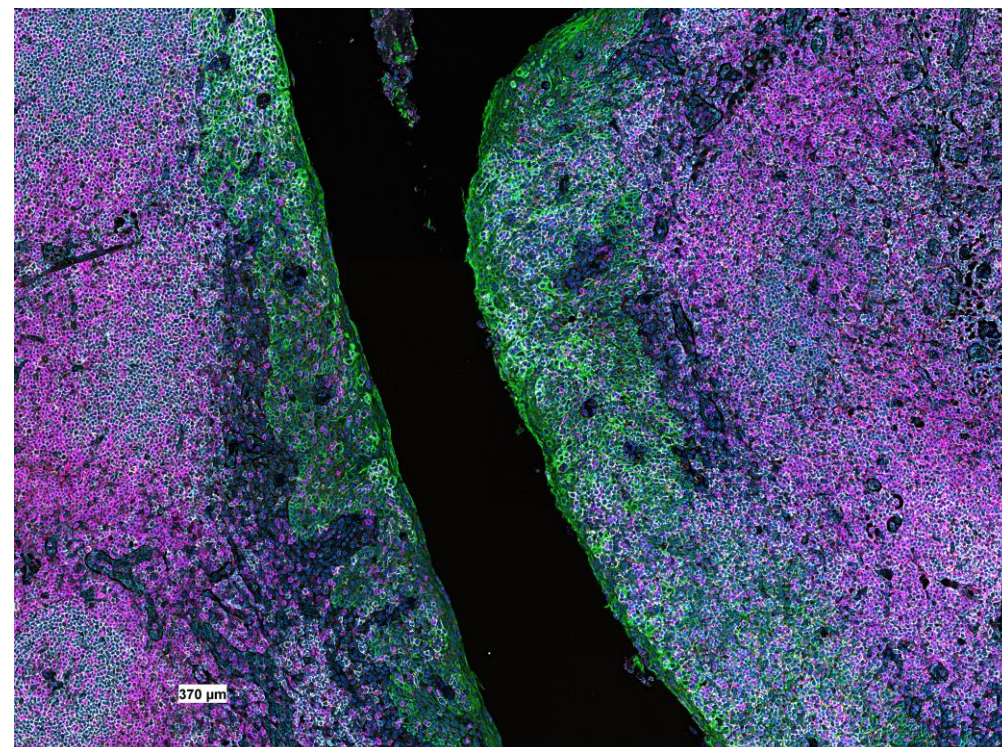
❖ Human **FFPE** tonsil tissue stained with six Akoya pre-conjugated antibodies

❖ Images captured with the CODEX instrument and the ITR/IPSR Keyence BZ-X 810 microscope

❖ Images generated with the CODEX Multiplex Analysis Viewer software

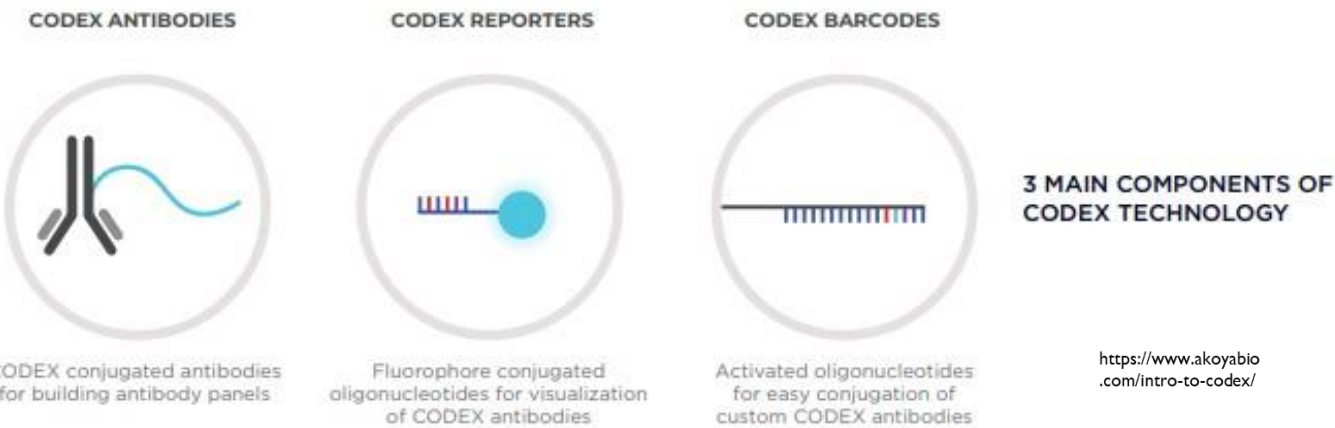


Marker	Color
DAPI	Blue
CD20	White
PanCK	Green
CD45RO	Magenta
MHCII	Yellow
CD3e	Red
CD107a	Cyan



AVAILABLE ANTIBODIES

<https://www.akoyabio.com/phenocycler/assays/>



<https://www.akoyabio.com/intro-to-codex/>

❖ It is important to note that some antibodies come pre-conjugated to CODEX barcodes, while others require custom conjugation using the CODEX conjugation kit.

- ❖ **CODEX Inventoried Antibodies:**
 - ❖ Pre-conjugated antibodies, supplied by Akoya and have passed the full CODEX Antibody Validation Process and manufacturing quality control and supported by Akoya’s field support team. These antibodies still require validation on your tissue of interest.
- ❖ **CODEX Screened Antibodies:**
 - ❖ Not inventoried or supplied by Akoya but have been successfully conjugated to CODEX barcodes and have shown positive staining signal in secondary lymphoid tissue. Conjugation required, but protocols, etc. provided by Akoya.
- ❖ **CODEX Community Antibodies:**
 - ❖ Shared list of CODEX tested clones and protocols among CODEX users. Demonstrated success with the CODEX system but requires conjugation and not sold through Akoya.
- ❖ **Custom Conjugated Antibodies:**
 - ❖ Completely custom-built panels consisting of commercially available antibodies custom conjugated to CODEX barcodes.



Least
Optimization required
Most

ANTIBODY SELECTION

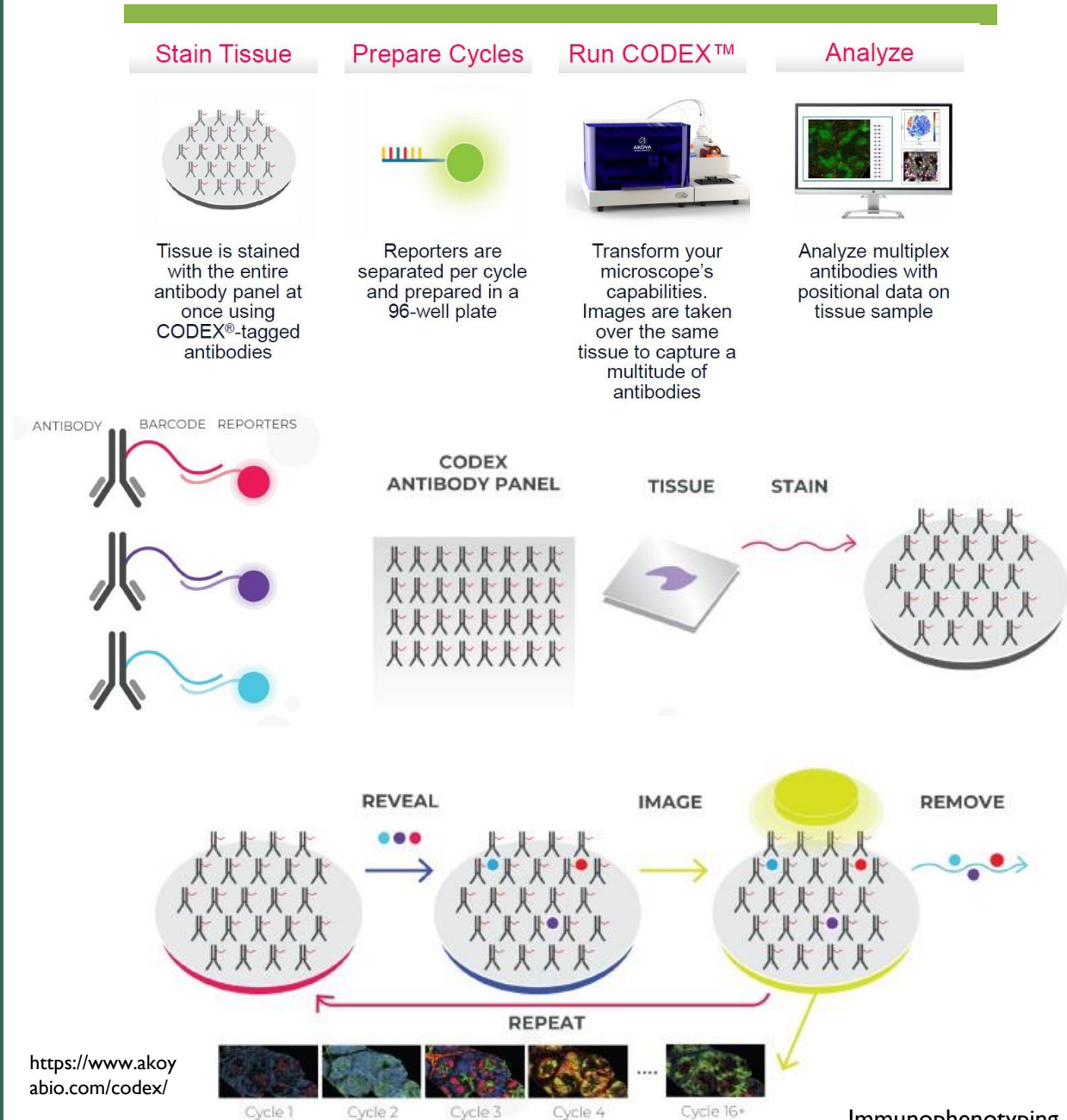
<https://vumc.box.com/s/j46tg76r2tqkl3kd7k0kcs4ygzsksmmka>

- ❖ Antibody selection is **crucial** to a successful CODEX experiment. Please consider the following guidelines when selecting antibodies for your panel:
 - ❖ Purchase antibodies suspended in only PBS. Akoya *strongly* discourages the use of lyophilized antibodies as successful conjugation is not guaranteed.
 - ❖ Many vendors will provide custom formulations with antibodies suspended in only PBS.
 - ❖ Monoclonal antibodies are strongly preferred over polyclonal.
 - ❖ IgG clones have demonstrated a much higher success rate than IgM.
 - ❖ The conjugation reaction requires >50 ug of purified antibody. Please purchase at least 100 ug to account for QC.
 - ❖ When assigning a CODEX barcode and reporter pair to an antibody, consider expression levels and tissue type.
 - ❖ Click the link above to access the **IPSR user guide for CODEX projects** for an in-depth guide to selecting successful antibodies for CODEX experiments.



WORKFLOW

- ❖ Panel design and antibody selection
- ❖ Custom conjugation (if not using Akoya inventoried antibodies) and conjugation validation via protein gel
- ❖ Staining validation
- ❖ Staining and CODEX run
- ❖ Data analysis



<https://www.akoya-bio.com/codex/>

ITR/IPSR CODEX SERVICES

WE CAN PROVIDE ANY COMBINATION OF THE LISTED SERVICES FOR YOUR CODEX PROJECT. PLEASE REACH OUT TO US FOR A COST ESTIMATE FOR YOUR PROJECT.



PANEL DESIGN AND
ANTIBODY
SELECTION



PROTOCOL
DEVELOPMENT AND
OPTIMIZATION



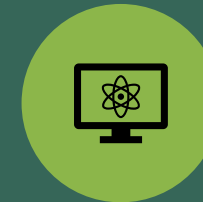
TRAINING AND
CONSULTATION



CUSTOM
CONJUGATION
AND VALIDATION



TISSUE STAINING



CODEX RUNS (NOT
OFFERED AS A
WALK-UP SERVICE)



DATA ANALYSIS
AVAILABLE SOON



QUESTIONS?

Contact the ITR/IPSR Scientific director, Dr. Kim Dahlman:
Kim.Dahlman@vumc.org

Visit our website at:
<https://www.vumc.org/itr-shared-resource/welcome>

Click the link to submit a project request:
<https://redcap.link/ITRIntake>

