Putting Multiplex IHC To The Challenge

November 16th , 2021

Cerba Research
Your precision medicine partner

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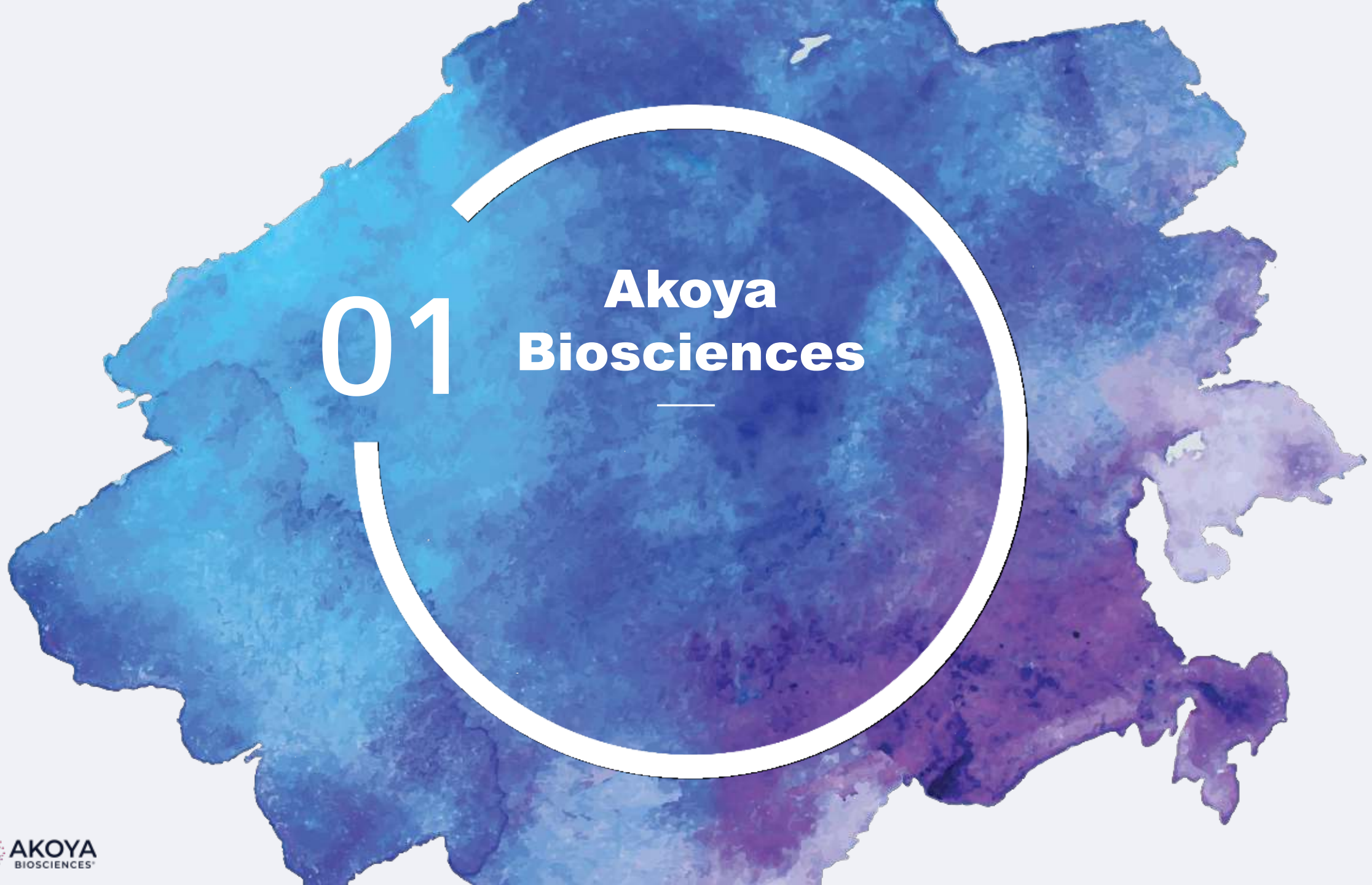


01 Akoya Biosciences

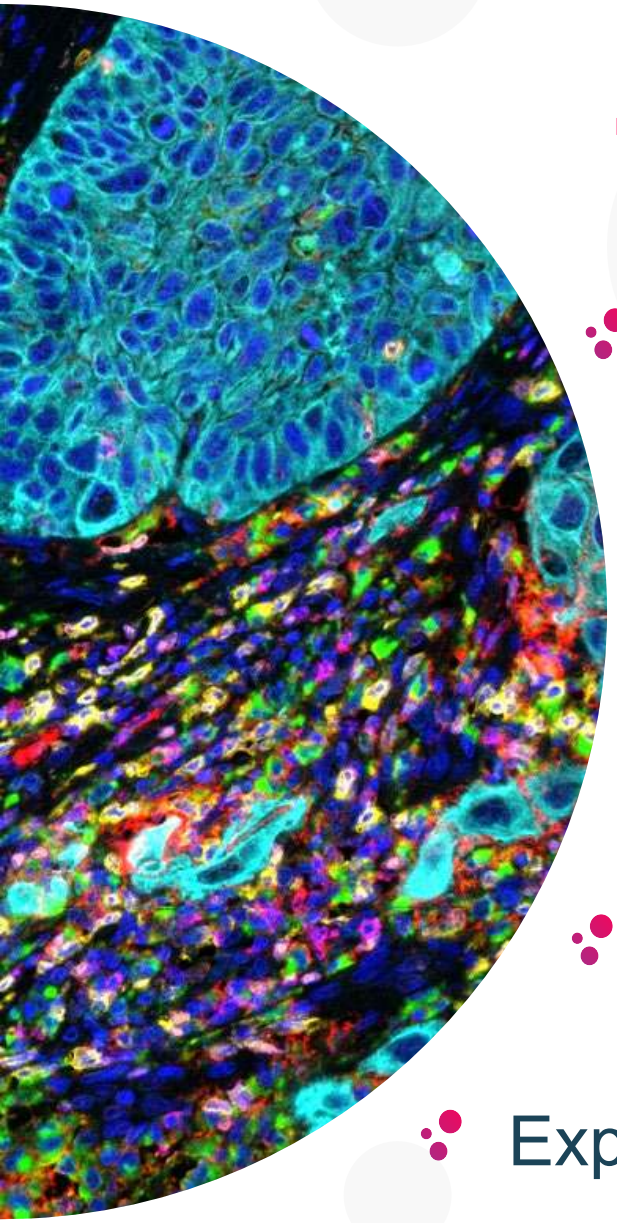
AKOYA BIOSCIENCES®

AKOYA BIOSCIENCES · The Spatial Biology Company

Analyzing multiple biomarkers with IHC: putting a 6 plex kit to the challenge

A deep dive in the tissue microenvironment with multiple IF technology: from ready to use kits to custom validation. Visualize, analyze, quantify and phenotype cells labeled with multiple biomarkers in FFPE tissue

Ivan Masetto
Field Application Scientist

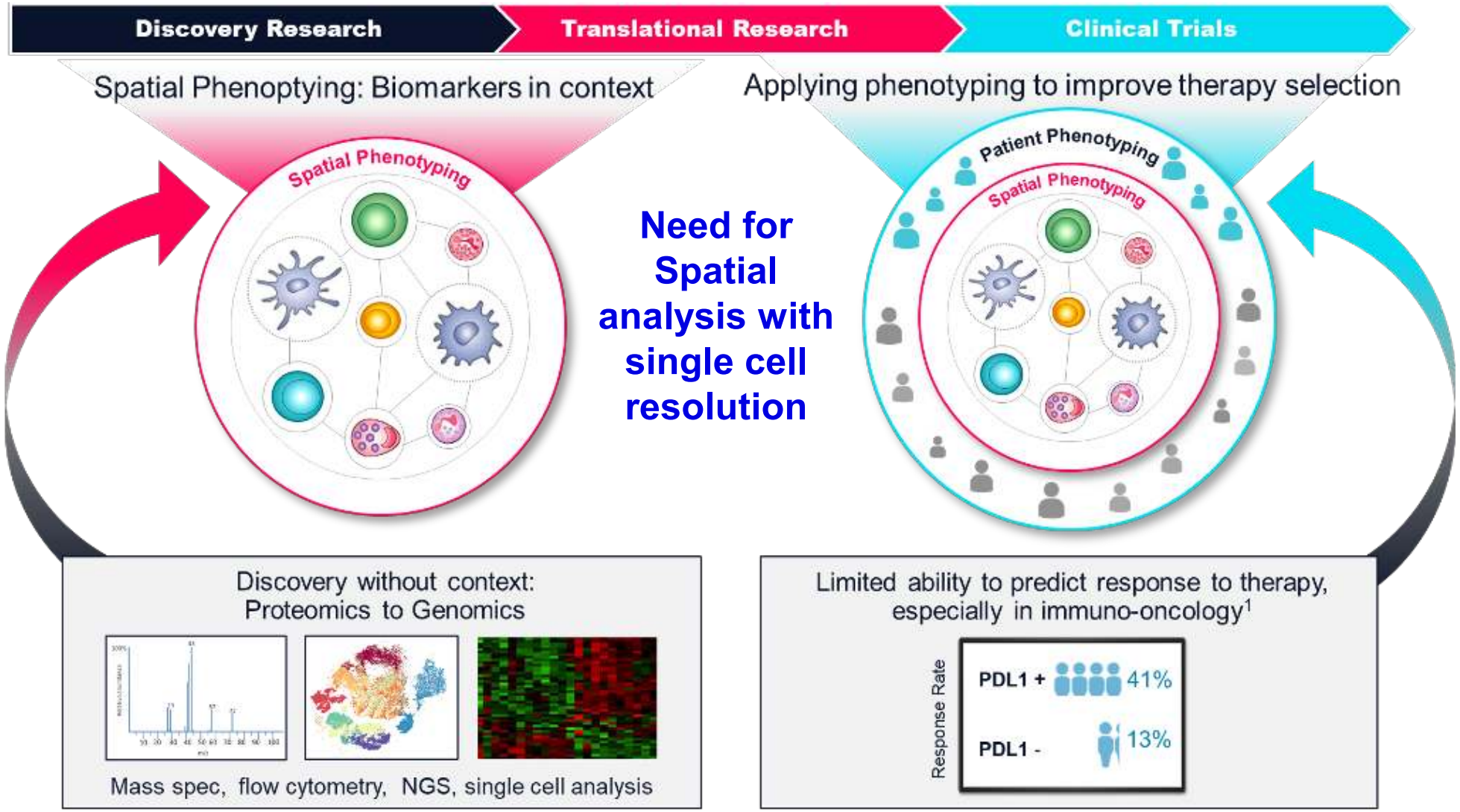


Akoya Biosciences: The Spatial Biology Company

- A leader in the spatial biology revolution, transforming discovery and clinical research
- Groundbreaking high-parameter tissue analysis for spatial phenotyping
- Large global customer base with accelerating publications
- Providing complete solutions: instrument, reagent, software and services
- Experienced senior management team

Spatial Phenotyping: New Biomarker Paradigm

Applicable Across the Research Continuum



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[1] Diggs and Hsueh Biomarker Research (2017) 5:12

Complete Solutions Discovery to Clinical Research

Spatial Phenotyping Cell-by-Cell Across the Whole Tissue Section

Discovery Research | **Translational Research** | **Clinical Trials**

CODEX®

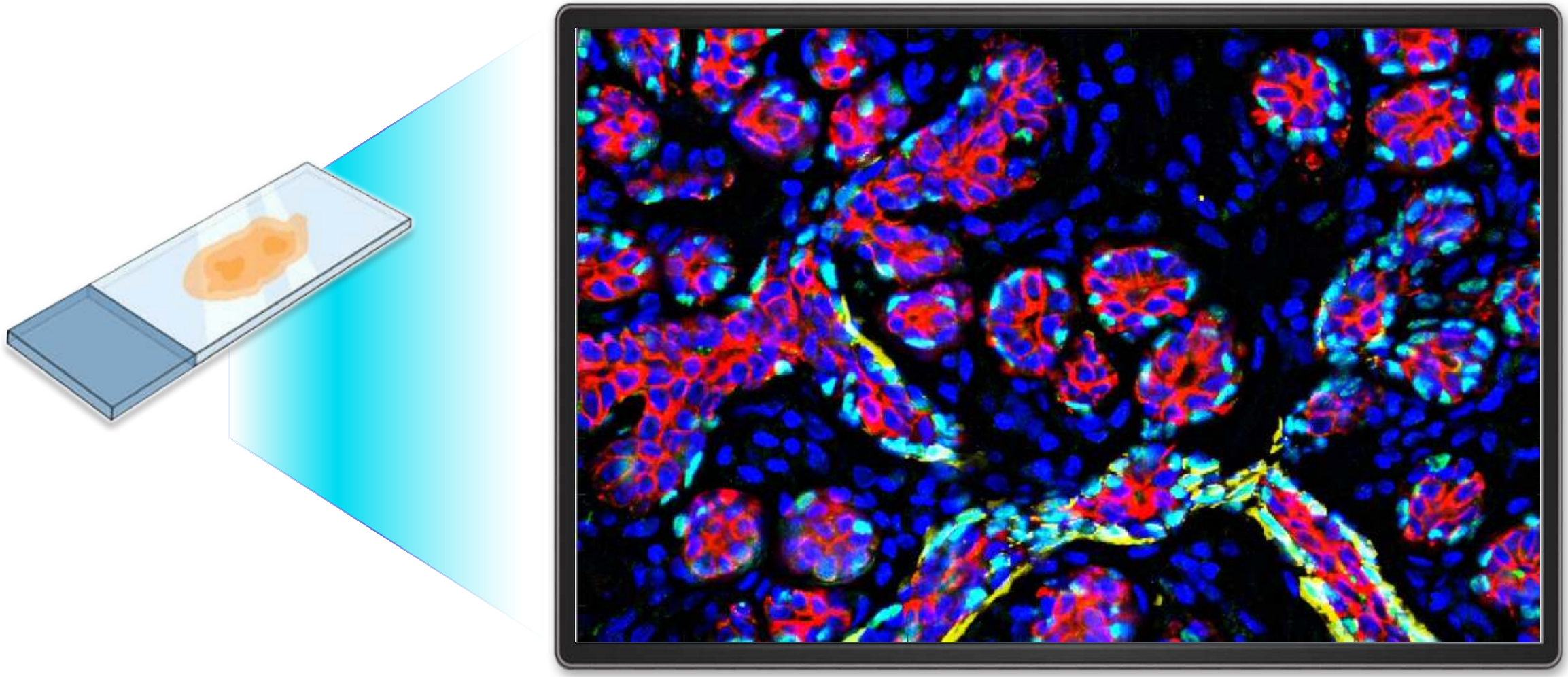
Phenoptics™

# of Markers per run

# of Samples per day

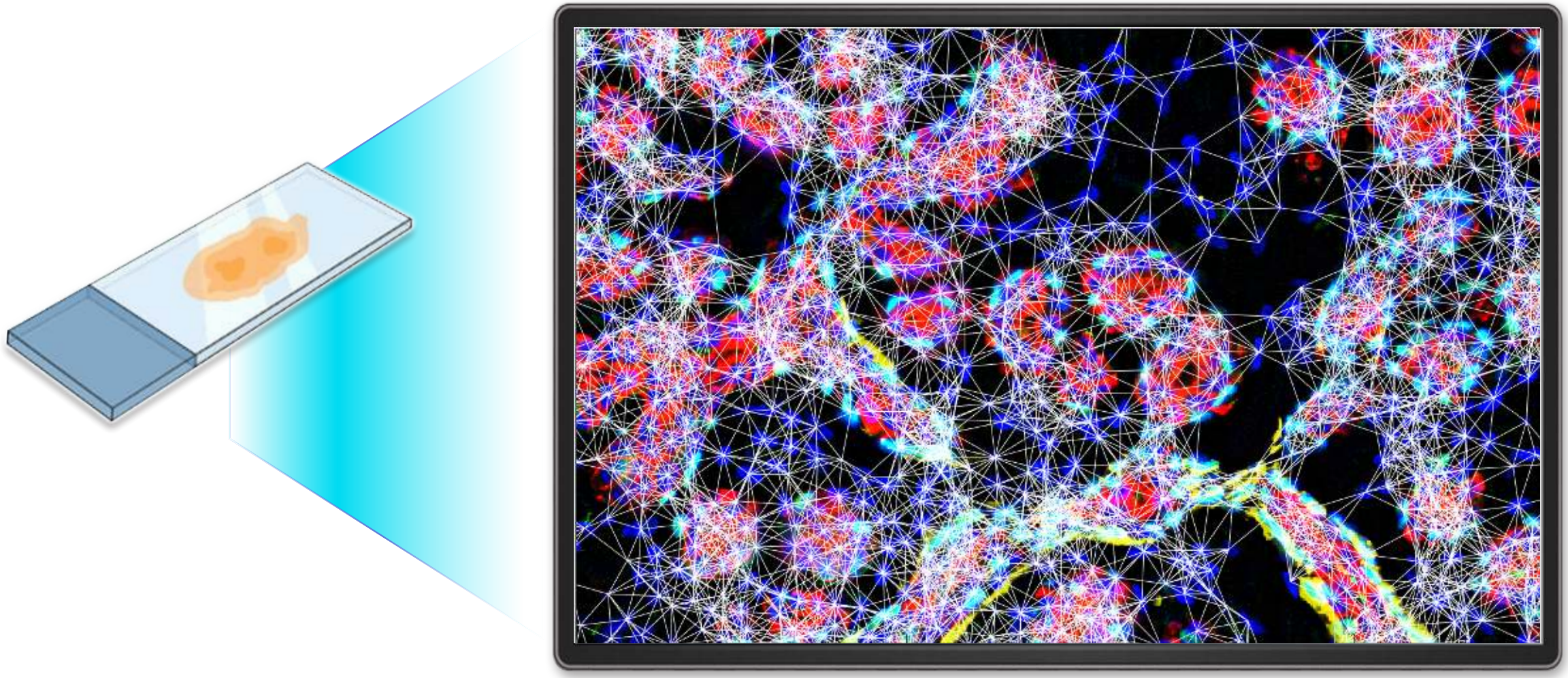
Akoya's Spatial Biology Platform

Spatial Phenotyping Every Cell Across the Entire Tissue



Akoya's Spatial Biology Platform

Spatial Phenotyping Every Cell Across the Entire Tissue



Phenoptics™

Spatial Phenotyping for Translational & Clinical Research

END-TO-END CLINICAL WORKFLOW

High reproducibility and high throughput
Fully automated and built under ISO 13485

ADDRESS LATE-STAGE BIOMARKER NEEDS

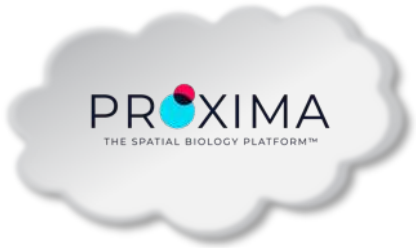
Biomarker validation and use in clinical trials
Understanding & predicting response to therapy

ENABLING CLINICAL STUDIES AT SCALE

Ideal for clinical labs, biopharma, and CRO's
High volume clinical research projects

Phenoptics™ Workflow

Staining → **Imaging** → **Image Storage** → **Image Analysis**



Automated and consistent using Leica Bond Rx autostainer and Opal™ kits and reagents*

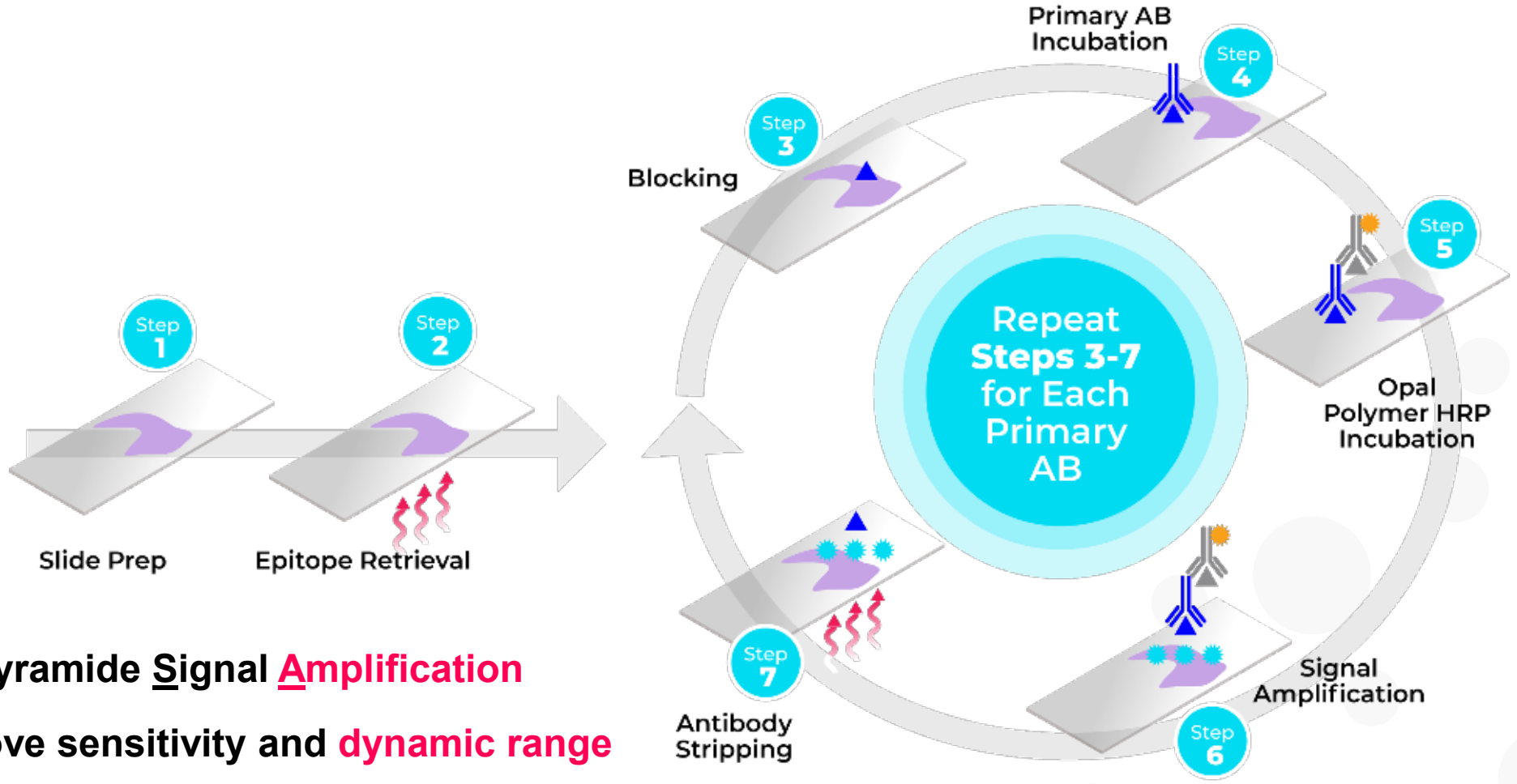
High throughput (up to 60 slides per day) multispectral whole slide image capture (MOTiF™) using Vectra® Polaris™

PROXIMA™ cloud-based HIPAA-compliant platform for data storage and collaboration

Flexible solutions for whole slide analysis including compatibility with third-party software

*Opal protocols compatible with other autostainers.

OPAL™



- TSA Tyramide Signal Amplification
- Improve sensitivity and dynamic range
- Multiplex with same primary Ab species

Easy automated multiplexing with Opal™

Fully Automated 4 or 7 color Kits

Speed

Shorten Workflow from 3 full days to one evening

Flexibility

Open Platform

Consistency

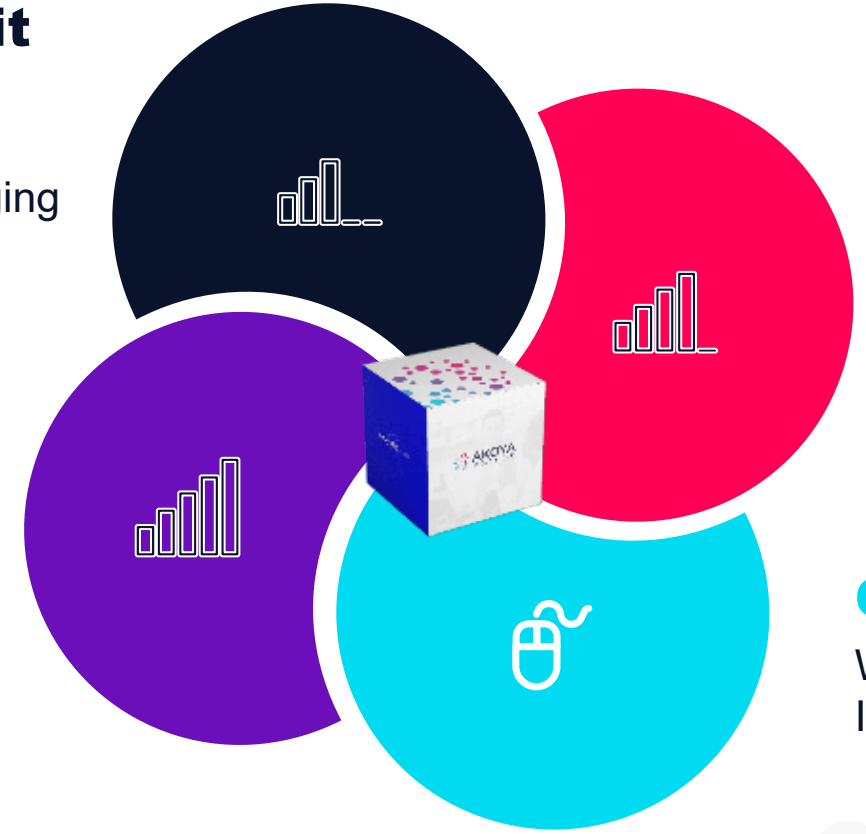
<15% CV

For use with Leica Bond RX
or Roche Discovery Ultra (Ventana)

Opal™ Multiplex kits

Opal 4-color kit

- 3-plex + DAPI assay
- Flexibility and ease
- Std microscope imaging



OPAL 7-color kit

- Manual / Automation
- Optimal Opal dyes and reagents for 6-plex assay
- Use your own primary Abs

Up to 9 Opal dyes

- High flexibility
- high plexing
- **high amount of data**

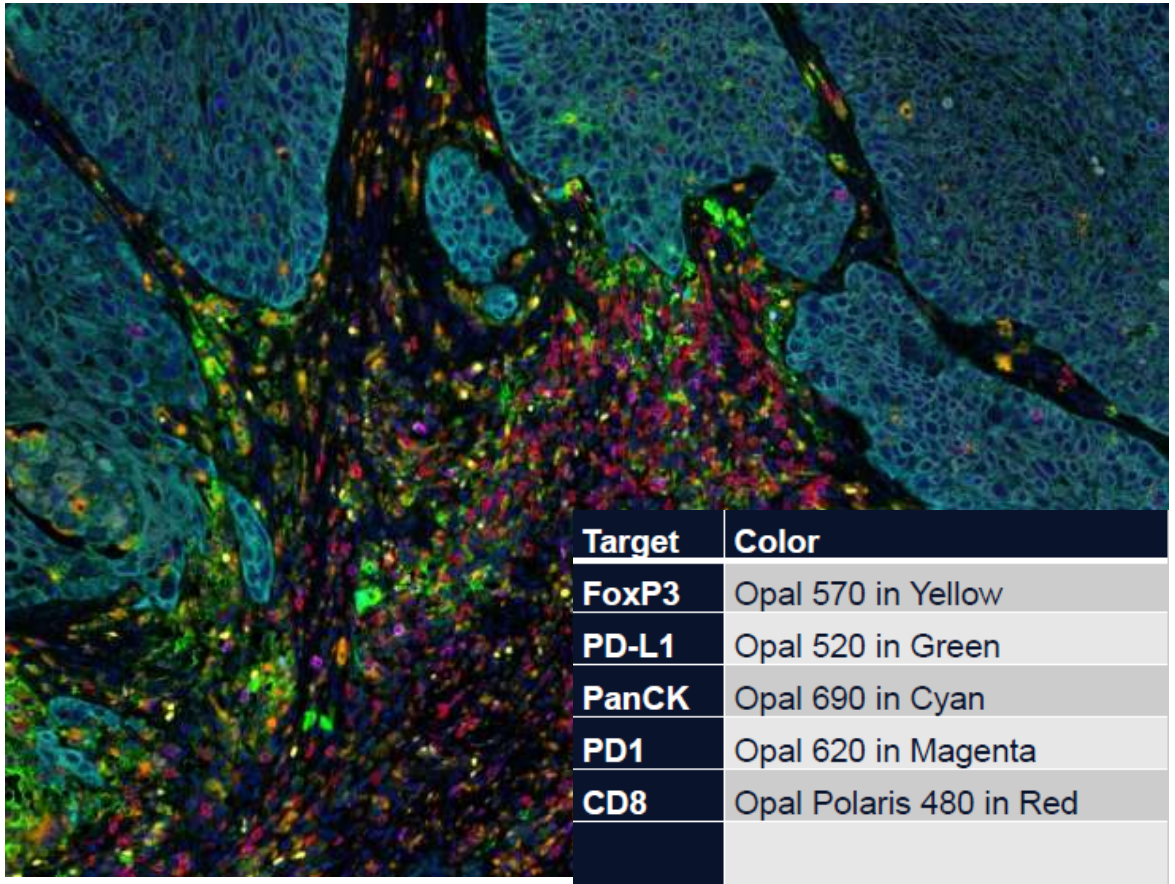
Opal Mouse kit

With optimized anti-Rabbit IgG HRP Secondary Ab

With all reagents you need

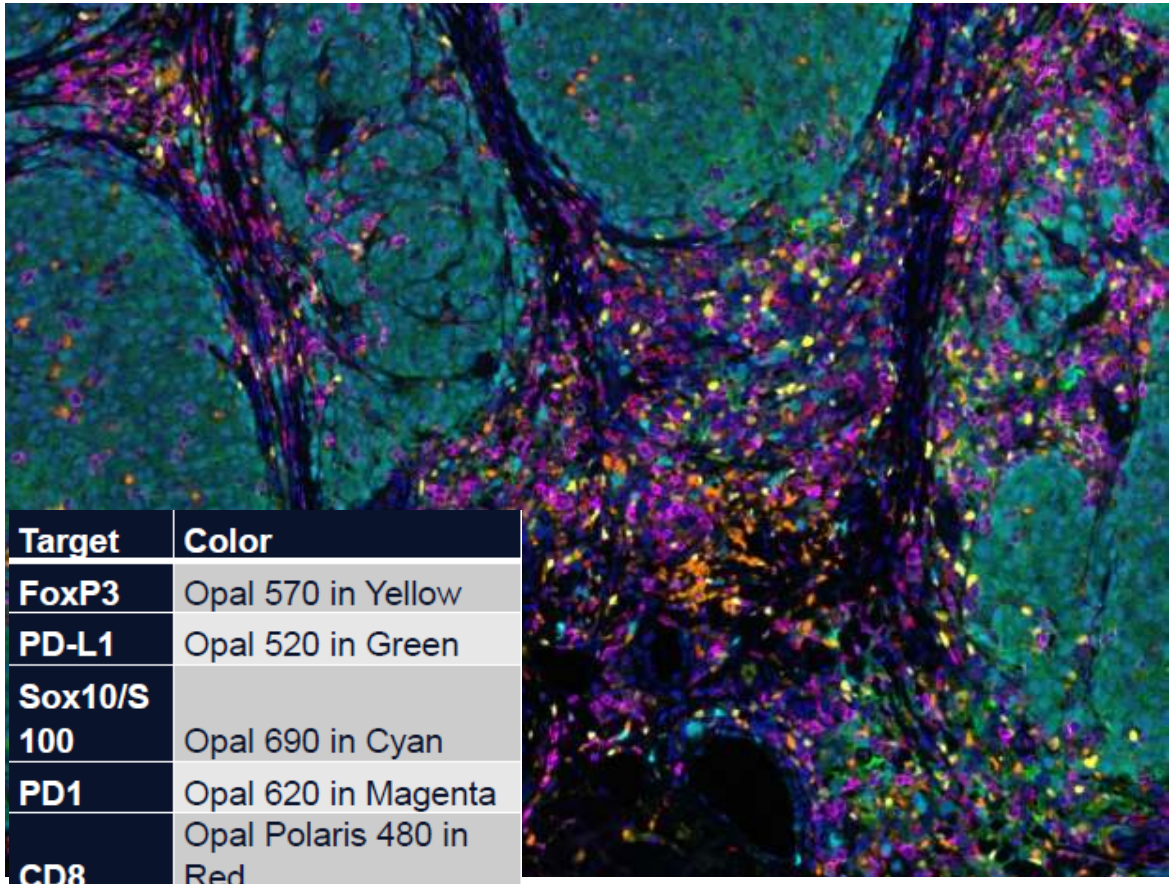
Ready-To-Use panel kits

Pan- Carcinoma Cancer Kit



Target	Color
FoxP3	Opal 570 in Yellow
PD-L1	Opal 520 in Green
PanCK	Opal 690 in Cyan
PD1	Opal 620 in Magenta
CD8	Opal Polaris 480 in Red
CD68	Opal Polaris 780 in Orange
Nucleus	DAPI in Blue

Melanoma Kit



Target	Color
FoxP3	Opal 570 in Yellow
PD-L1	Opal 520 in Green
Sox10/S 100	Opal 690 in Cyan
PD1	Opal 620 in Magenta
CD8	Opal Polaris 480 in Red
CD68	Opal Polaris 780 in Orange
Nucleus	DAPI in Blue

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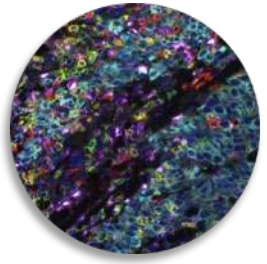
Vectra® Polaris™
The All-in-One Solution for Immuno-Oncology



Supports 9-color multispectral imaging within a single tissue

High speed digital 7-color multispectral whole slide scanning at up to 40x in brightfield or fluorescence

80 slides capacity with touchless automation technology

A new class of tissue imager that provides researchers unparalleled speed, performance, and versatility

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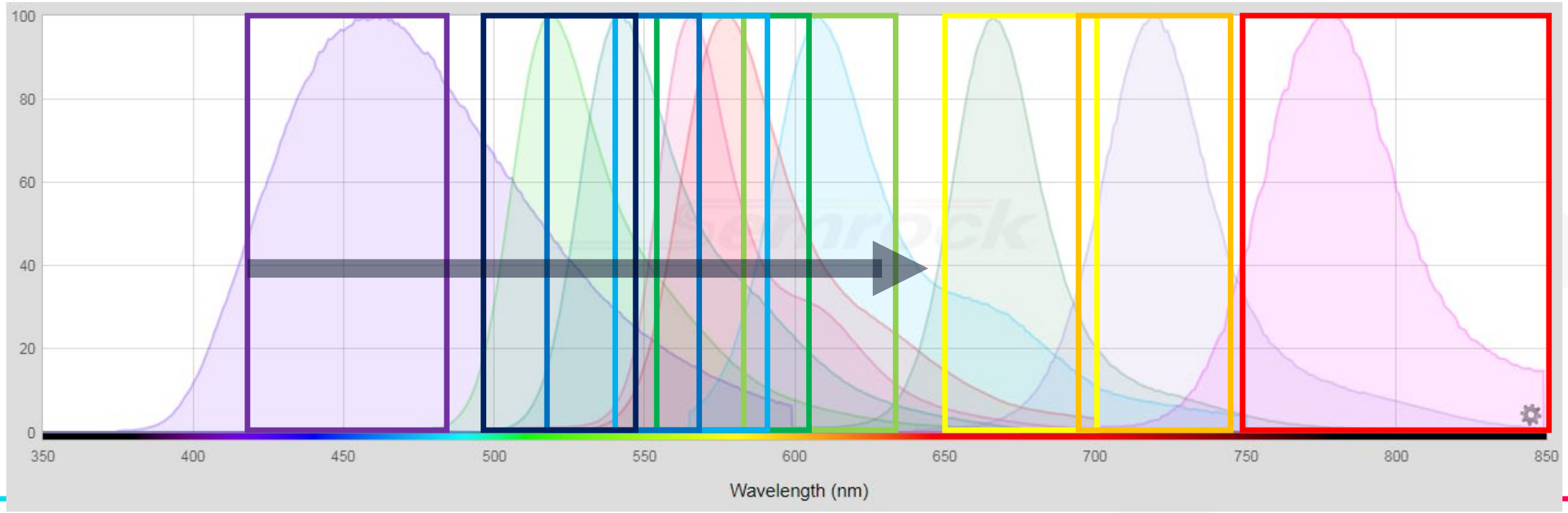
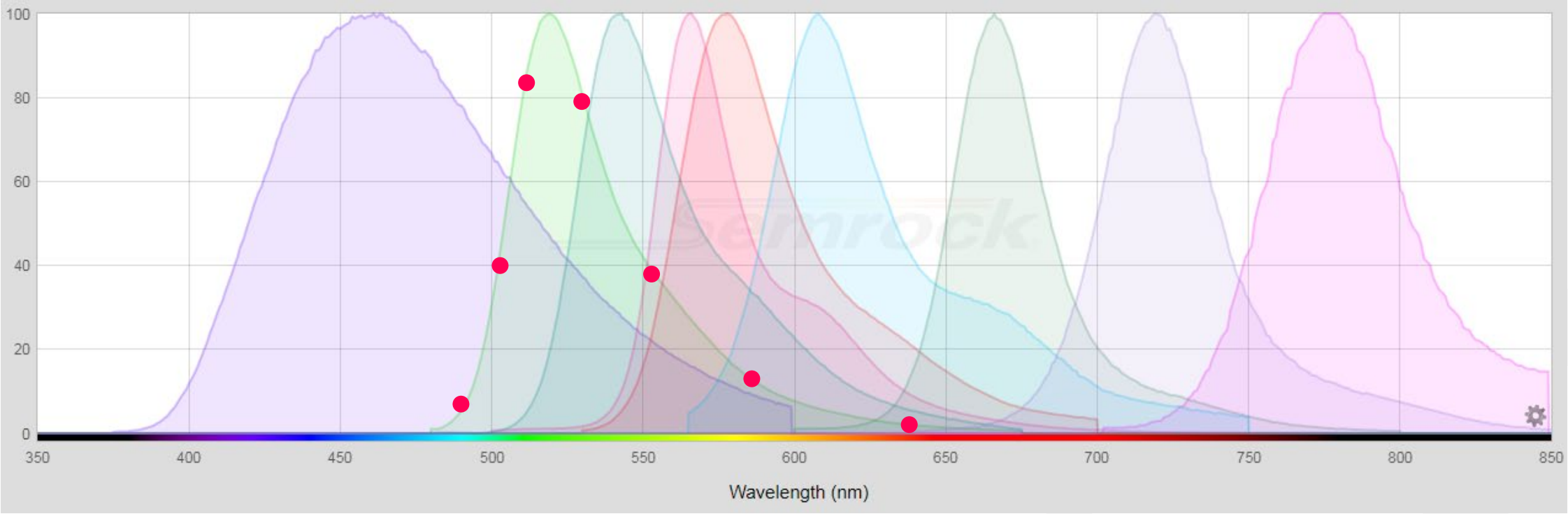
How can we image multiple colours simultaneously?

- Typical strategy is to use fluorophores with emission spectra that are spaced apart, and to use **narrow band-pass emission filters** to try to isolate emission from each fluorophores
- **Multispectral imaging**: Imaging technique used to capture light in multiple fluorescence channels



What do we need?

- **Multispectral imaging => how it works?**



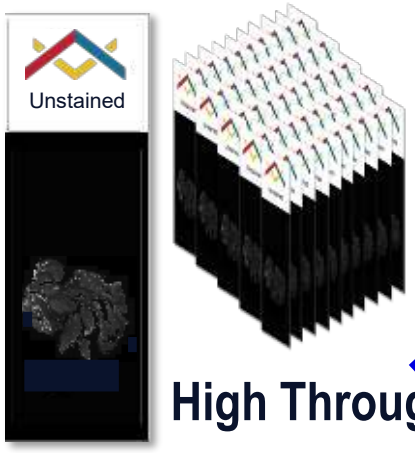
PHENOPTICS™ ADVANTAGE

DESIGNED FOR CLINICAL WORKFLOW
For biomarker validation and integration into clinical studies

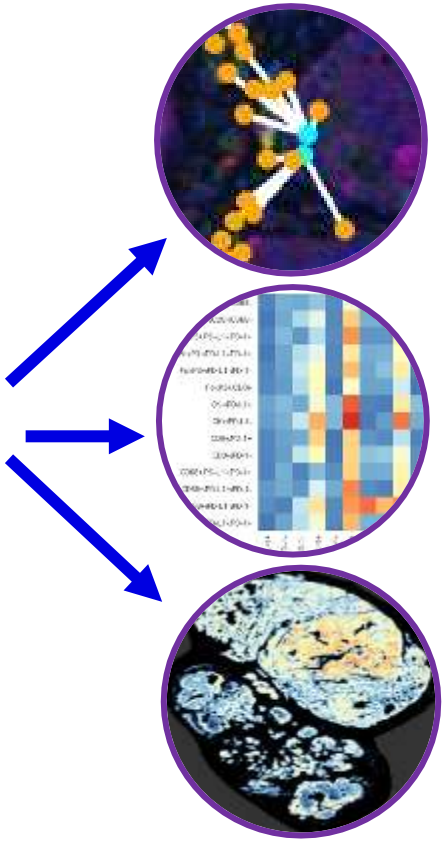
BATTLE TESTED
Only validated platform for translational studies

COMPREHENSIVE
End-to-end solution

Opal™ Reagents



High Throughput



Whole Slide Analysis

02 CERBA RESEARCH

Who is Cerba Research? What do we do?

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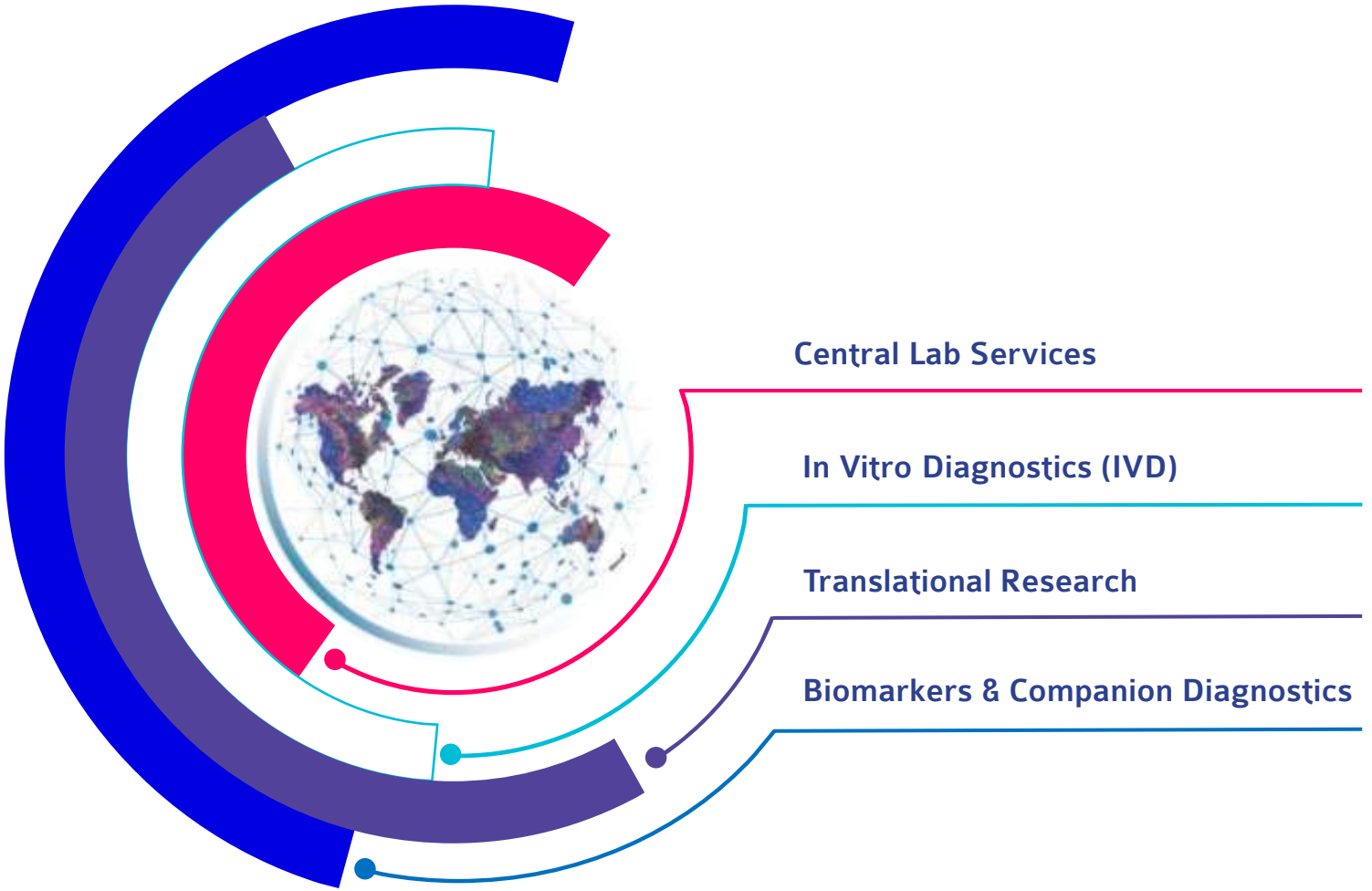
Your Precision Medicine Partner



Full Histology Service

Driven by Scientific Team, Complementary Techniques Available

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Sample Preparation
- Trimming when required
- Tissue processing (dehydration of tissue) + paraffin or OCT embedding
- Sectioning

Staining
- Histology staining (ex. H&E, Masson's Trichrome)
- Simplex and multiplex IHC (chromogenic or fluorescence), pre-clinic to clinically validated
- ISH staining

Digitalization
- Digitalization of fluorescent (up to 9 colors) and chromogenic slides
- Digital slide sharing

Scoring, Diagnosis, Analysis
- Pathologist
- Image analysis (Halo, Visiopharm)

Custom Protocol Development & Validation

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03 MOTiF™ PD-1/PD-L1 Panel

Repeatability, Reproducibility

Lung cancers

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MOTiF™ PD-1/PD-L1 Panel: Pan Carcinoma Cancer Kit

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FFPE Sections

Optimized IF Protocol (MOTiF™ panel kit reageant)

Standardized Imaging Protocol

Pre-configured inForm® algorithm

- Proof of concept on lung cancer samples
- Show utility across other tissue types

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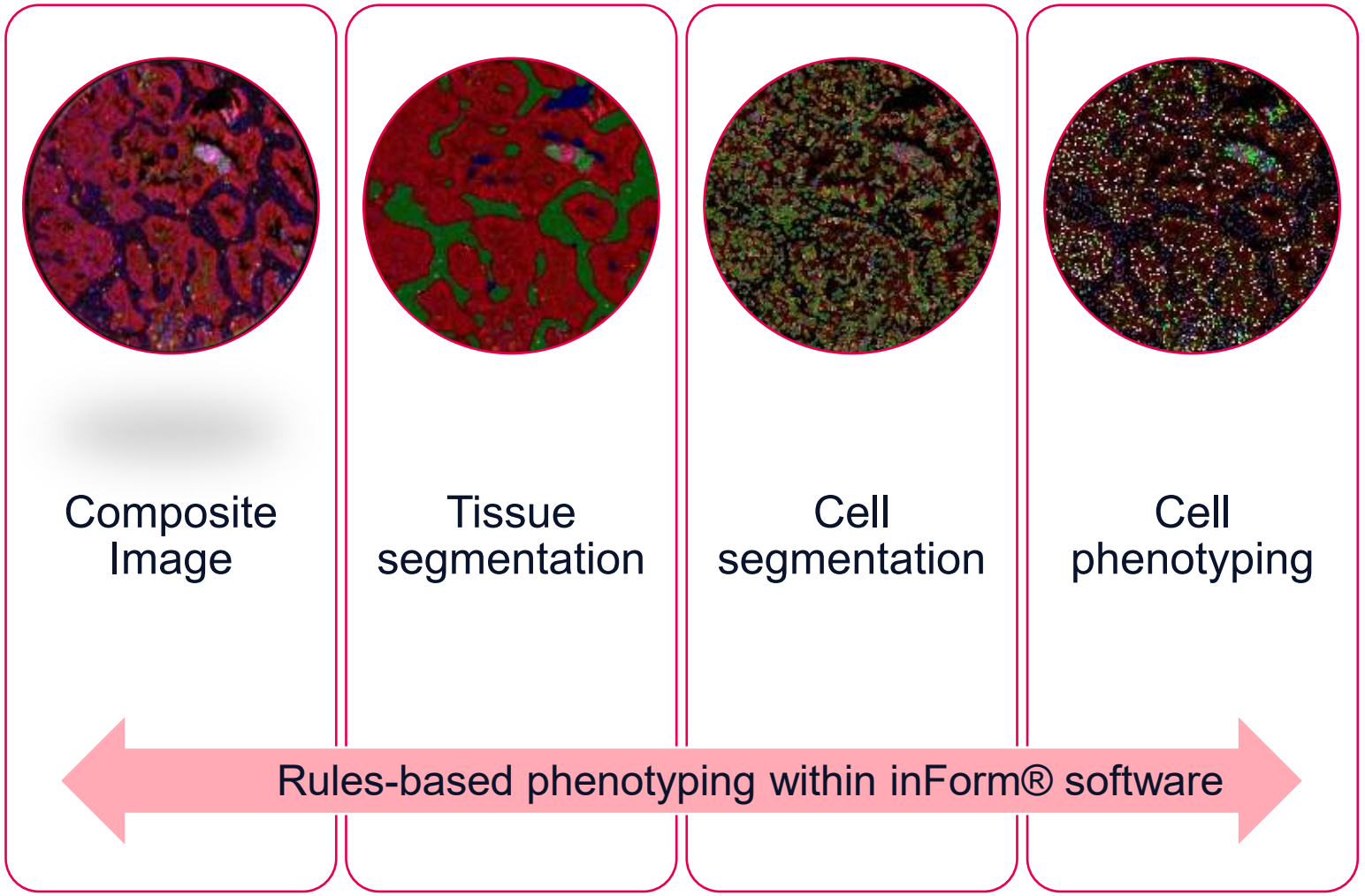
inForm® Workflow

Cerba Research



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inForm®workflow

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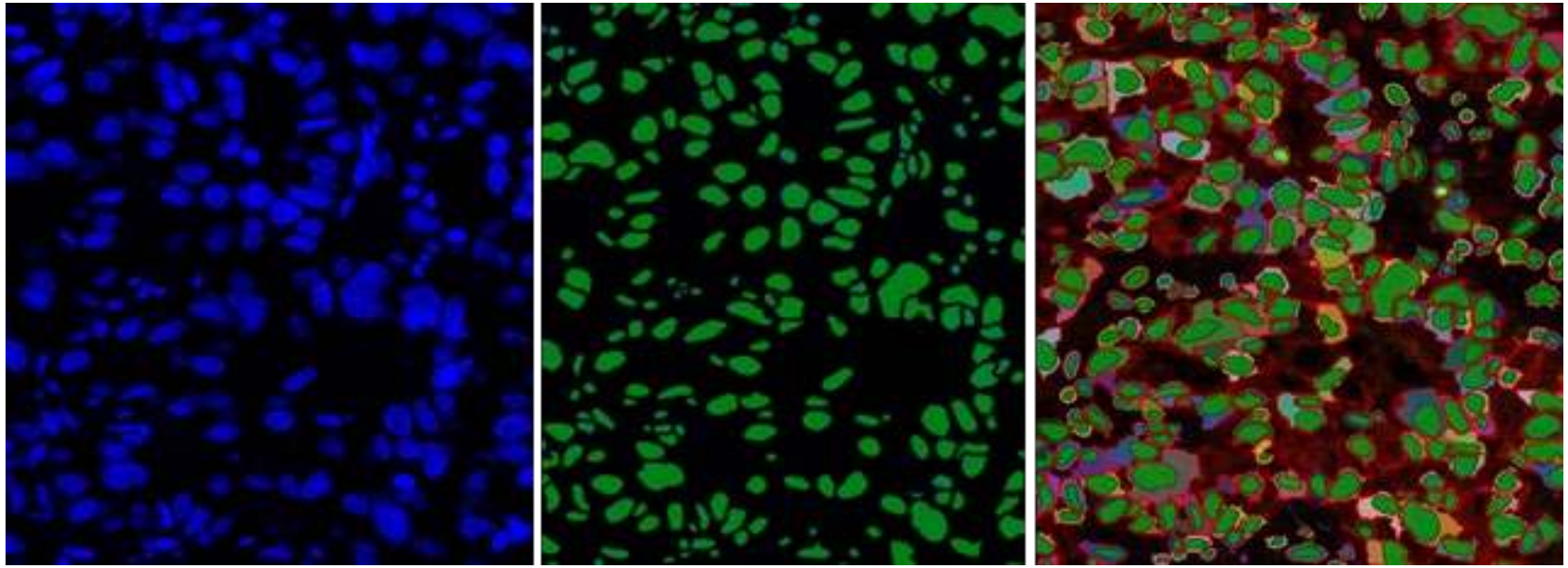
Cell segmentation

Identify the nuclear, cytoplasmic and membrane compartments



Dapi signal

Nuclear segmentation

Cytoplasm & membrane segmentation

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inForm®workflow

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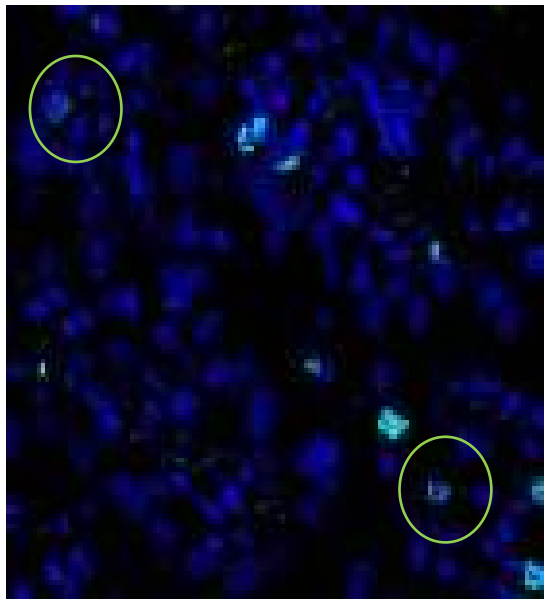
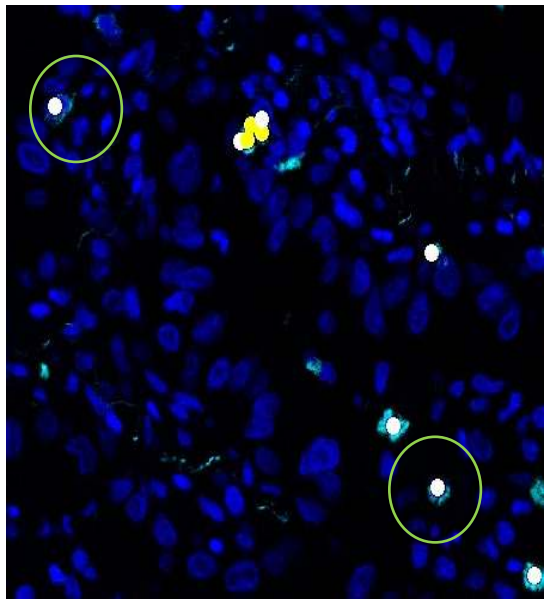
Phenotyping

Single positivity is assigned for each marker (CD8, PD-1, FoxP3, CD68).

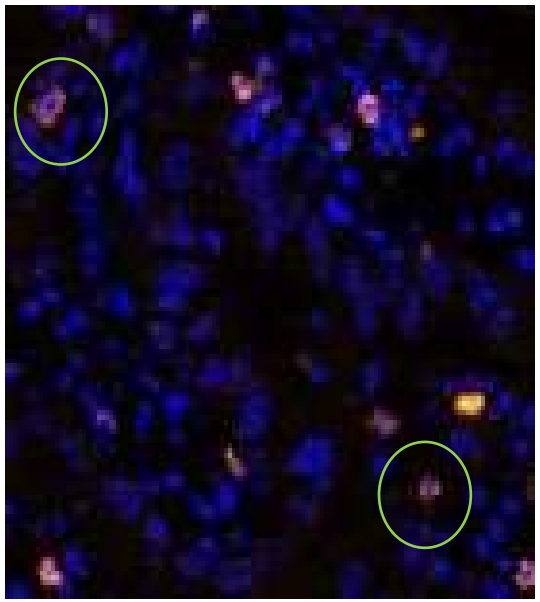
Co-expression assessment.



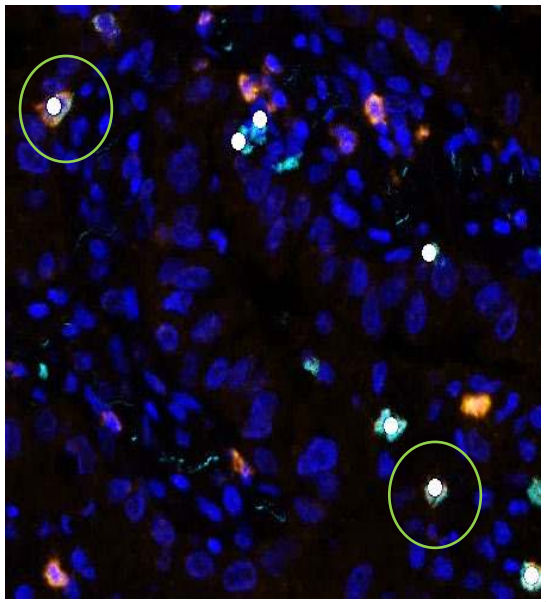
CD8 signal



CD8 positive cells



PD-1 signal



CD8+ PD-1+ positive cells

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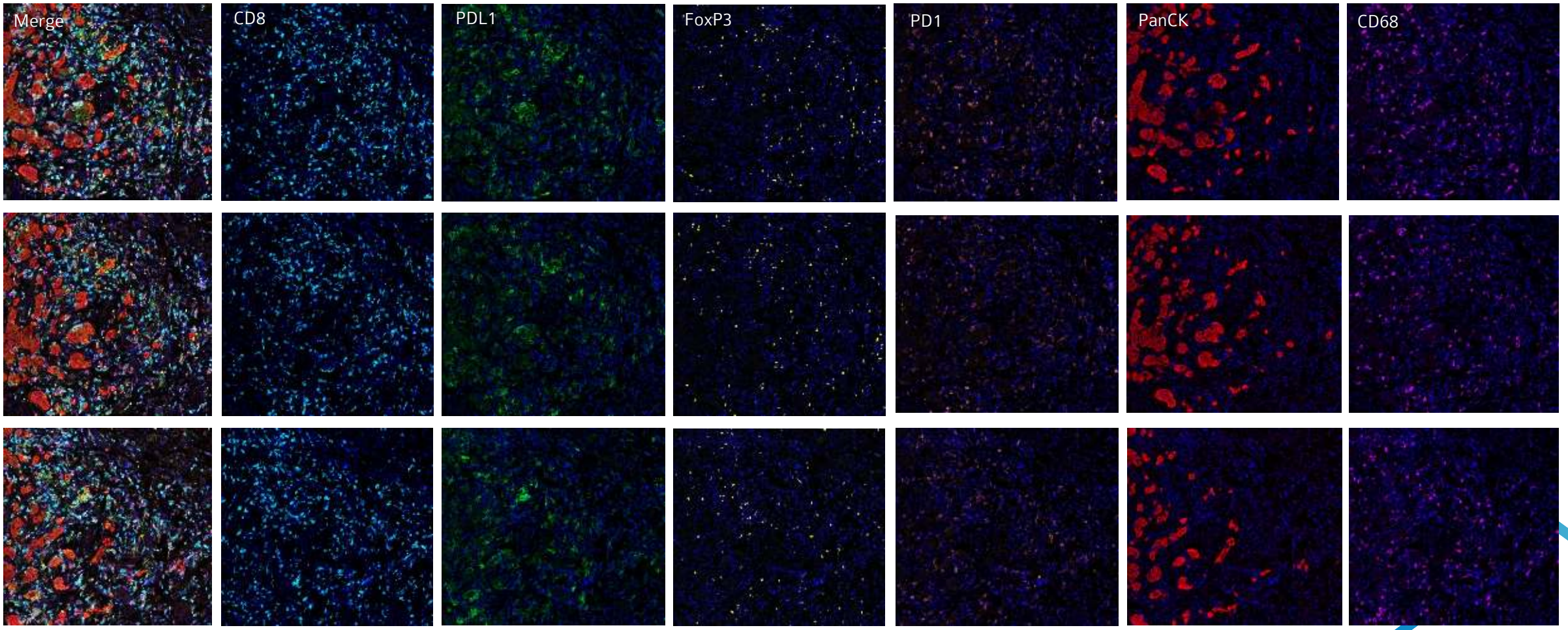
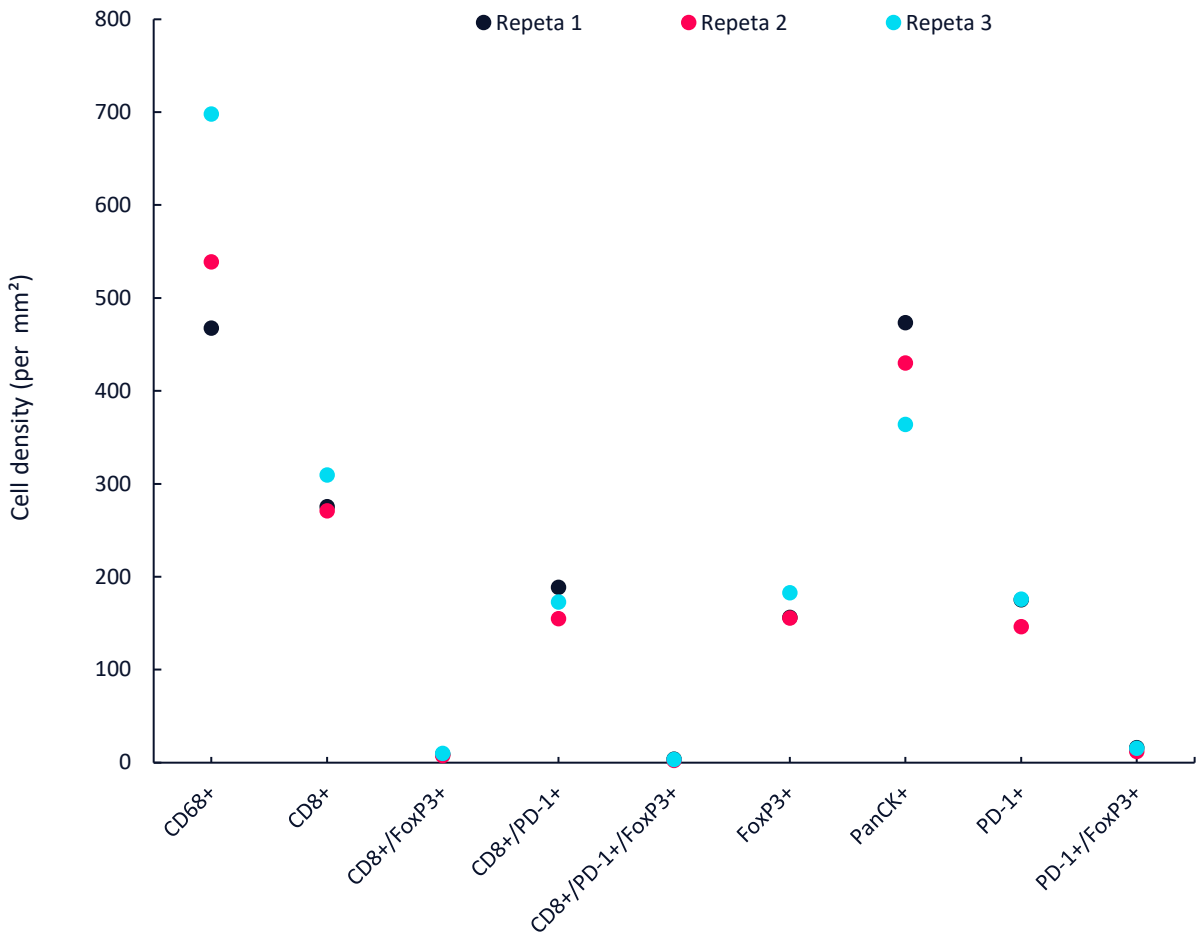
Repeatability

Lung Cancer

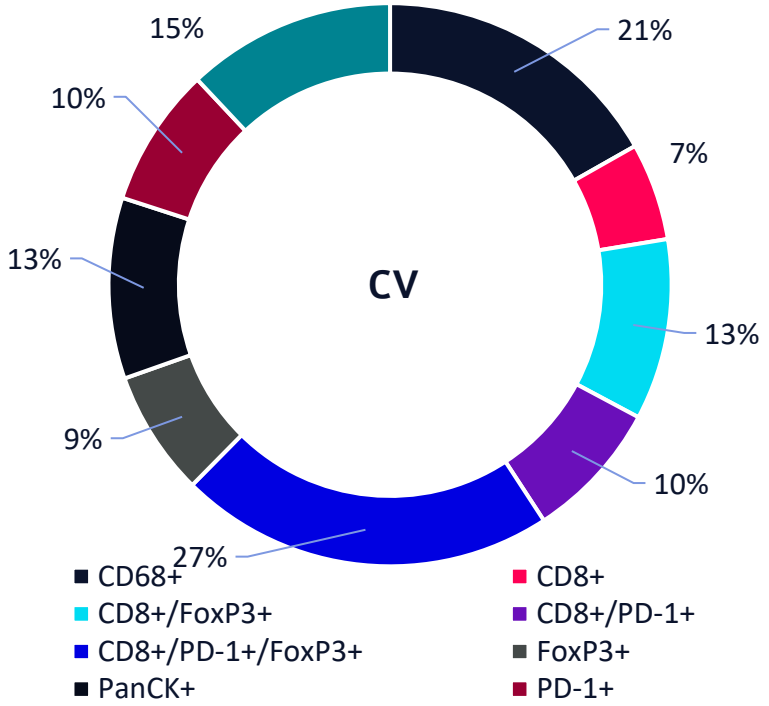


Repeatability

Lung cancer

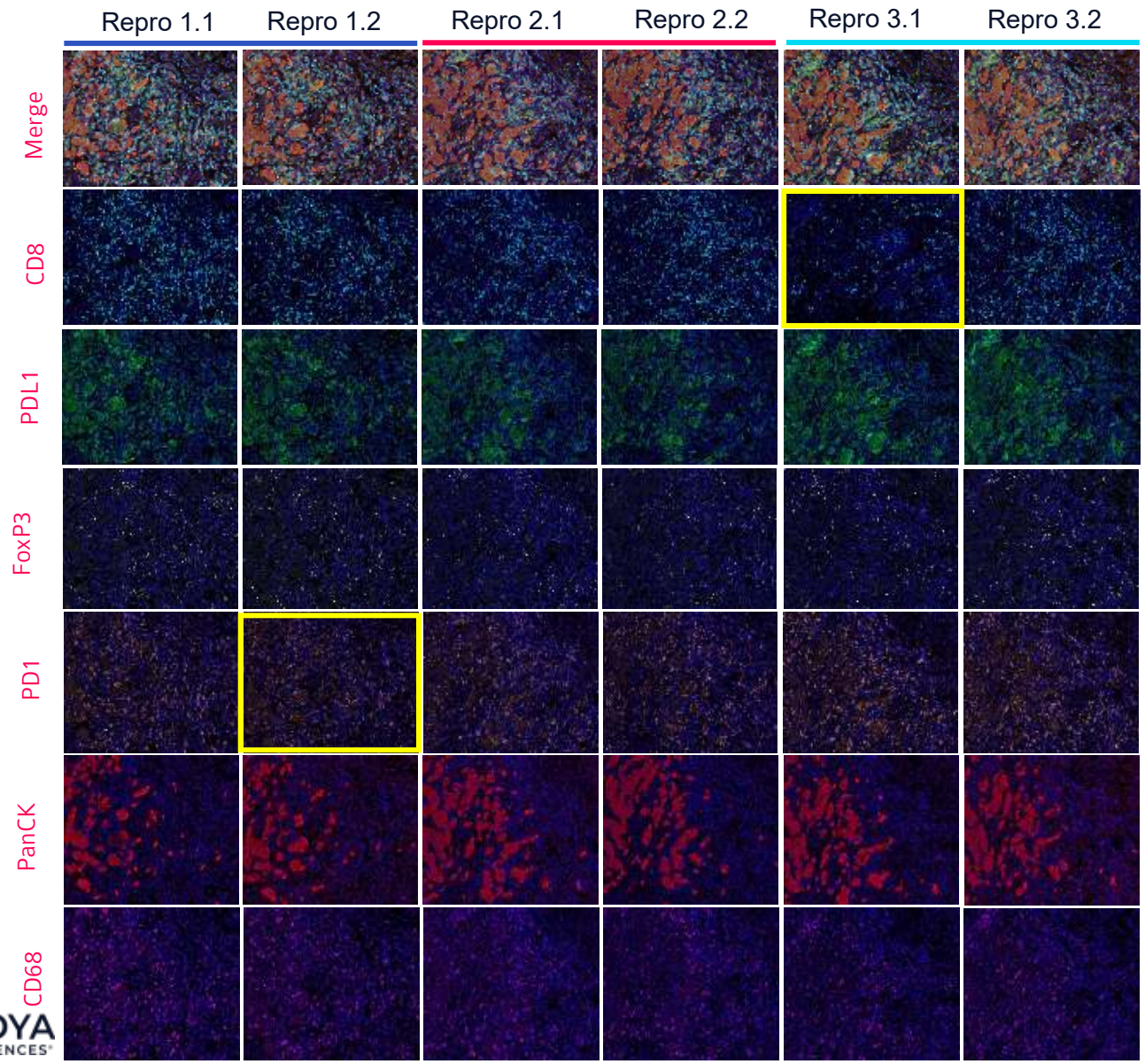


Satisfactory results



Satisfactory if CV is ≤ 20% for strong expression or ≤ 30% for low expression

Reproducibility



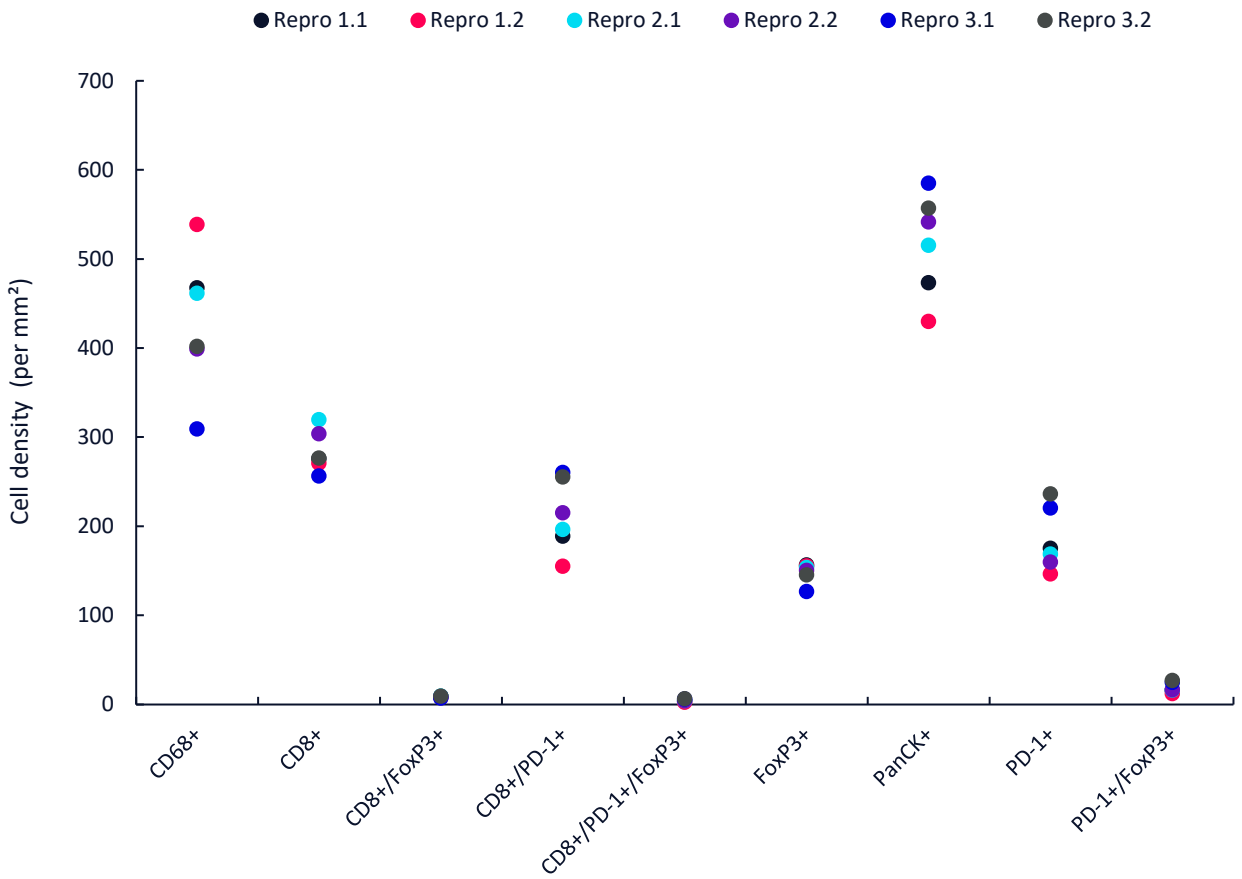
Repro 3.1: CD8 loss

Repro 1.2: PD1 loss

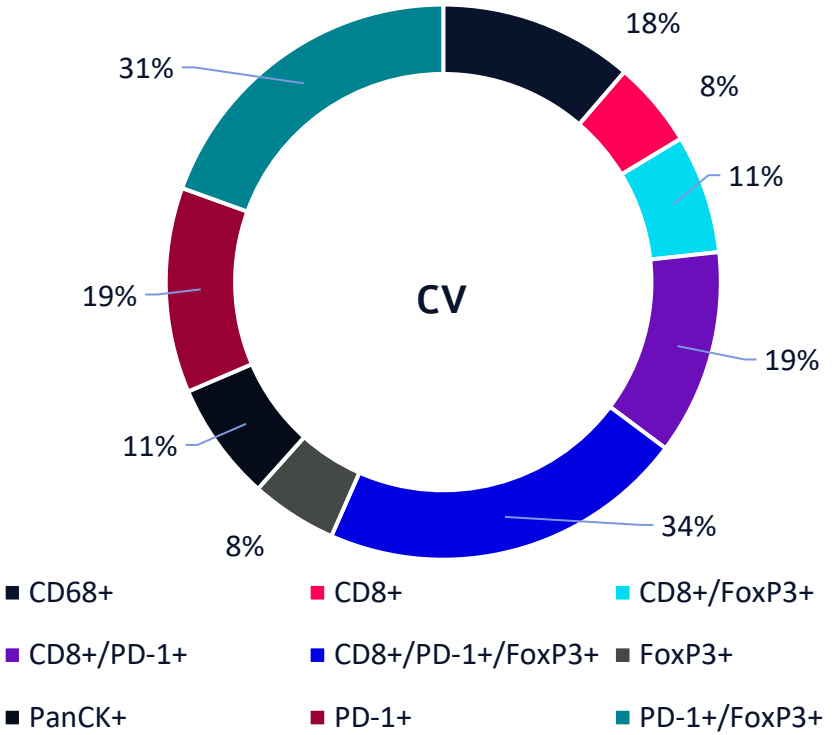
Structural change

Reproducibility

Lung cancer



Satisfactory results



Satisfactory if CV is ≤ 20% for strong expression or ≤ 30% for low expression

03 MOTiF™ PD-1/PD-L1 Panel

Robustness

Breast and Colon cancers

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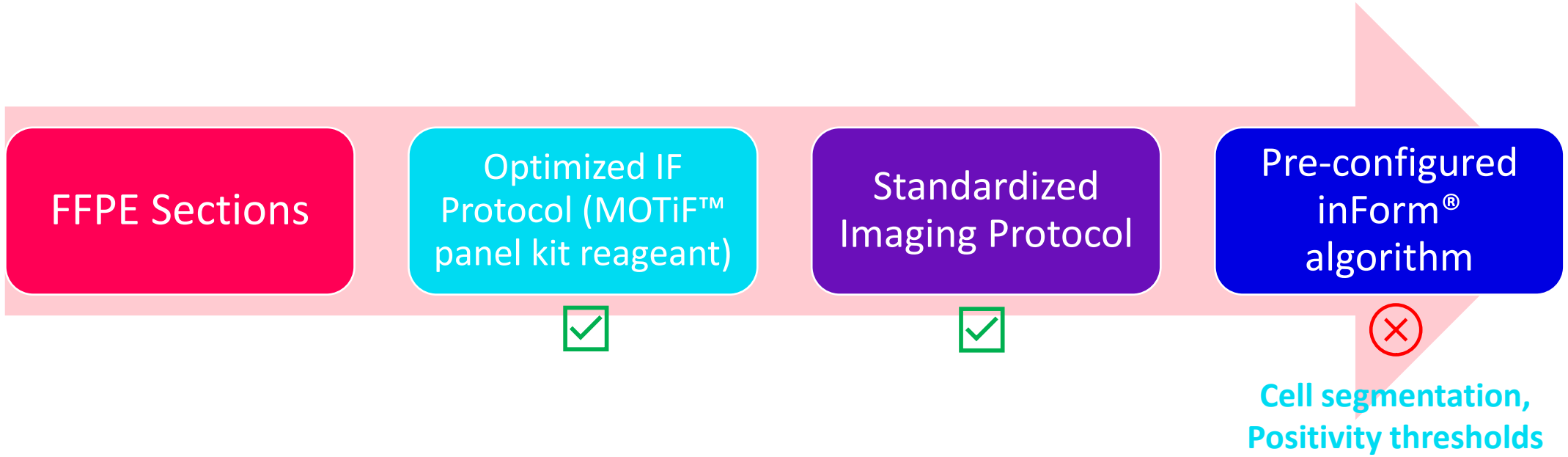
MOTiF™ PD-1/PD-L1 Panel: Robustness

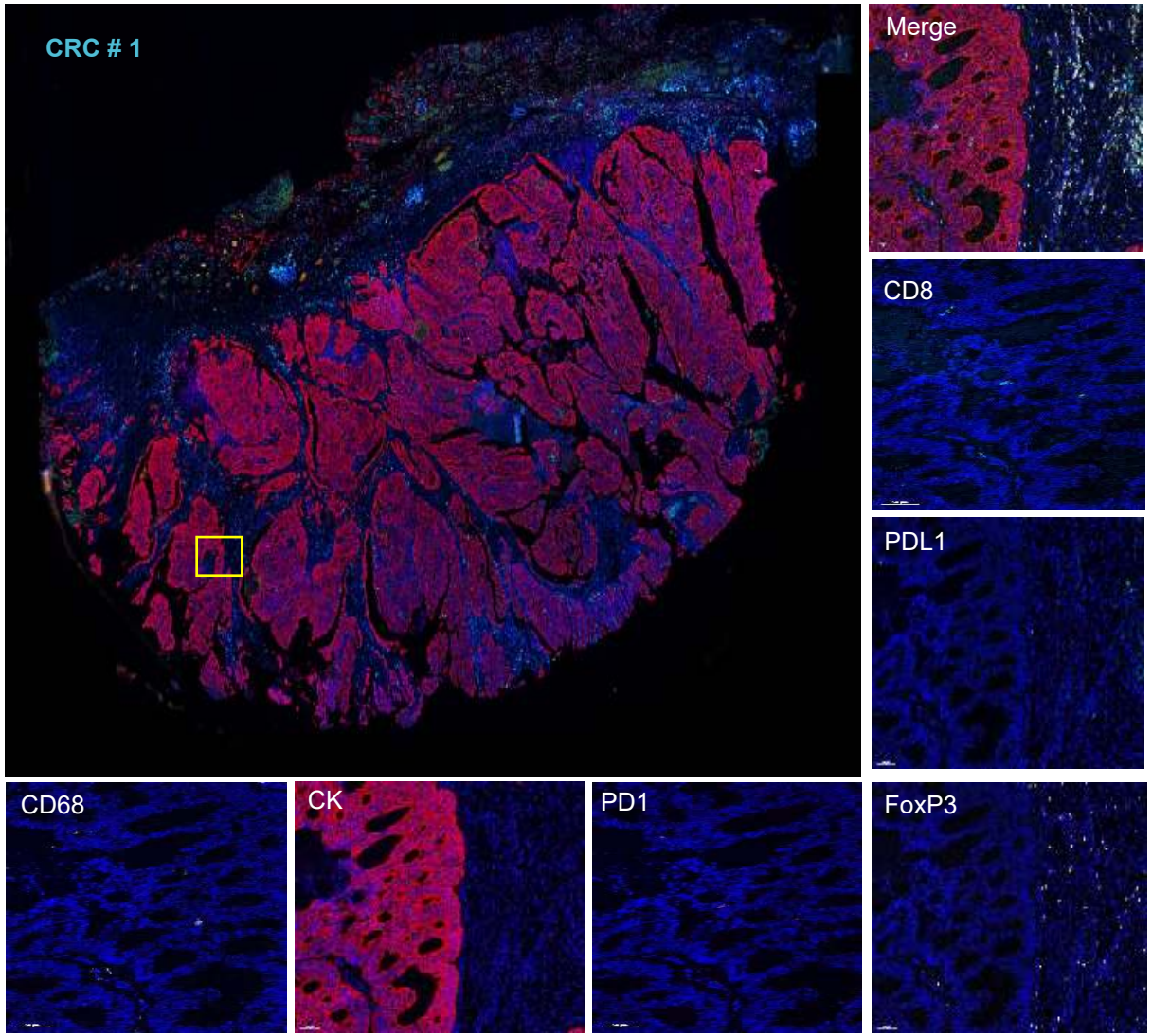
Cerba Research

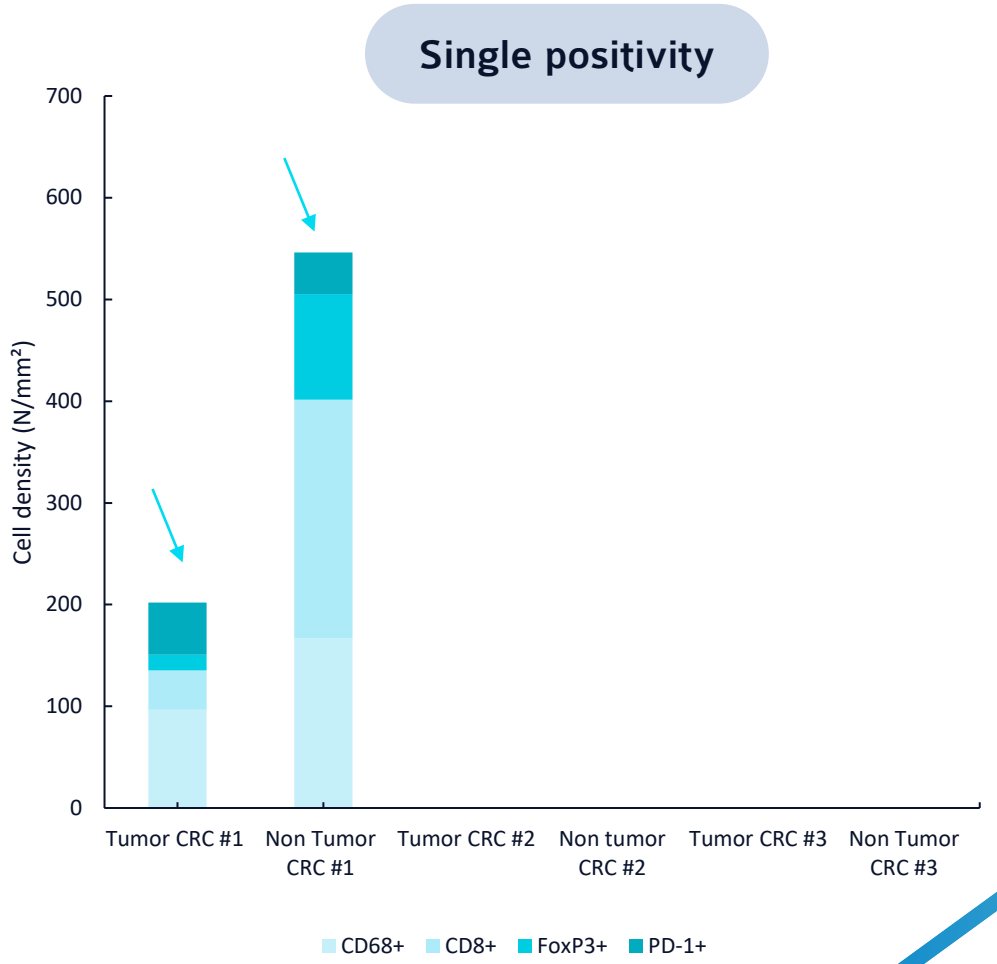


Show utility across other tissue types: Colon, Breast cancers

Colon cancers

Cerba Research





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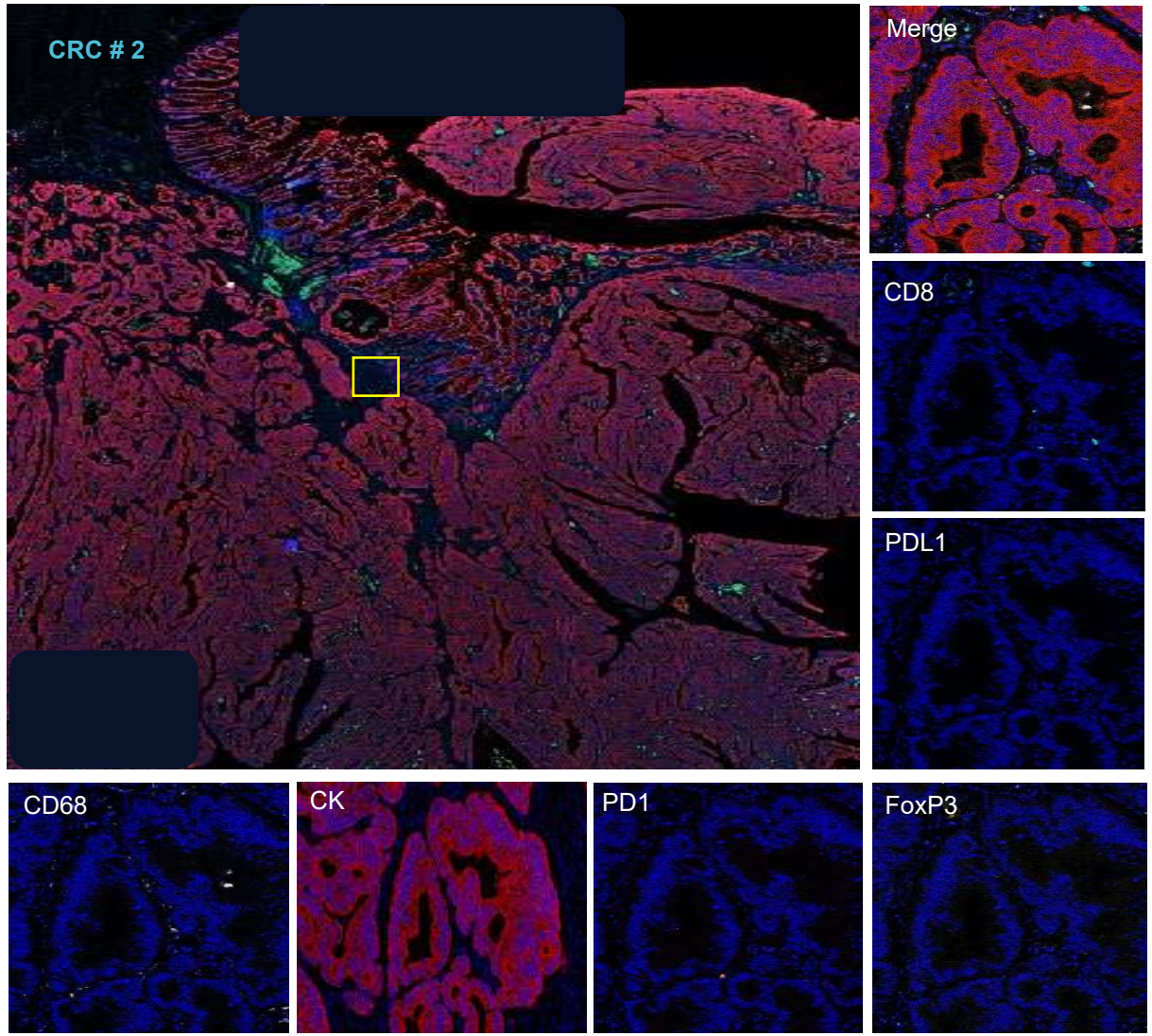
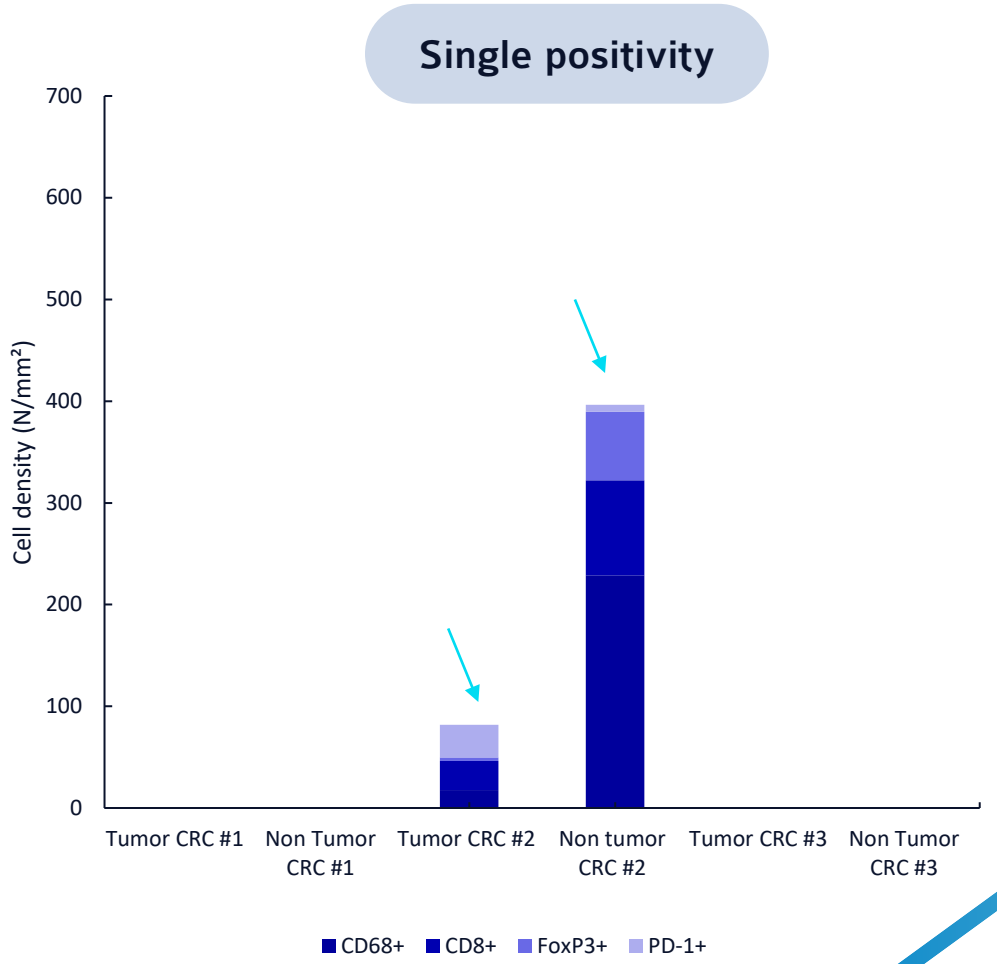
23-nov-21

Colon Cancers

Cerba Research





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Colon Cancers

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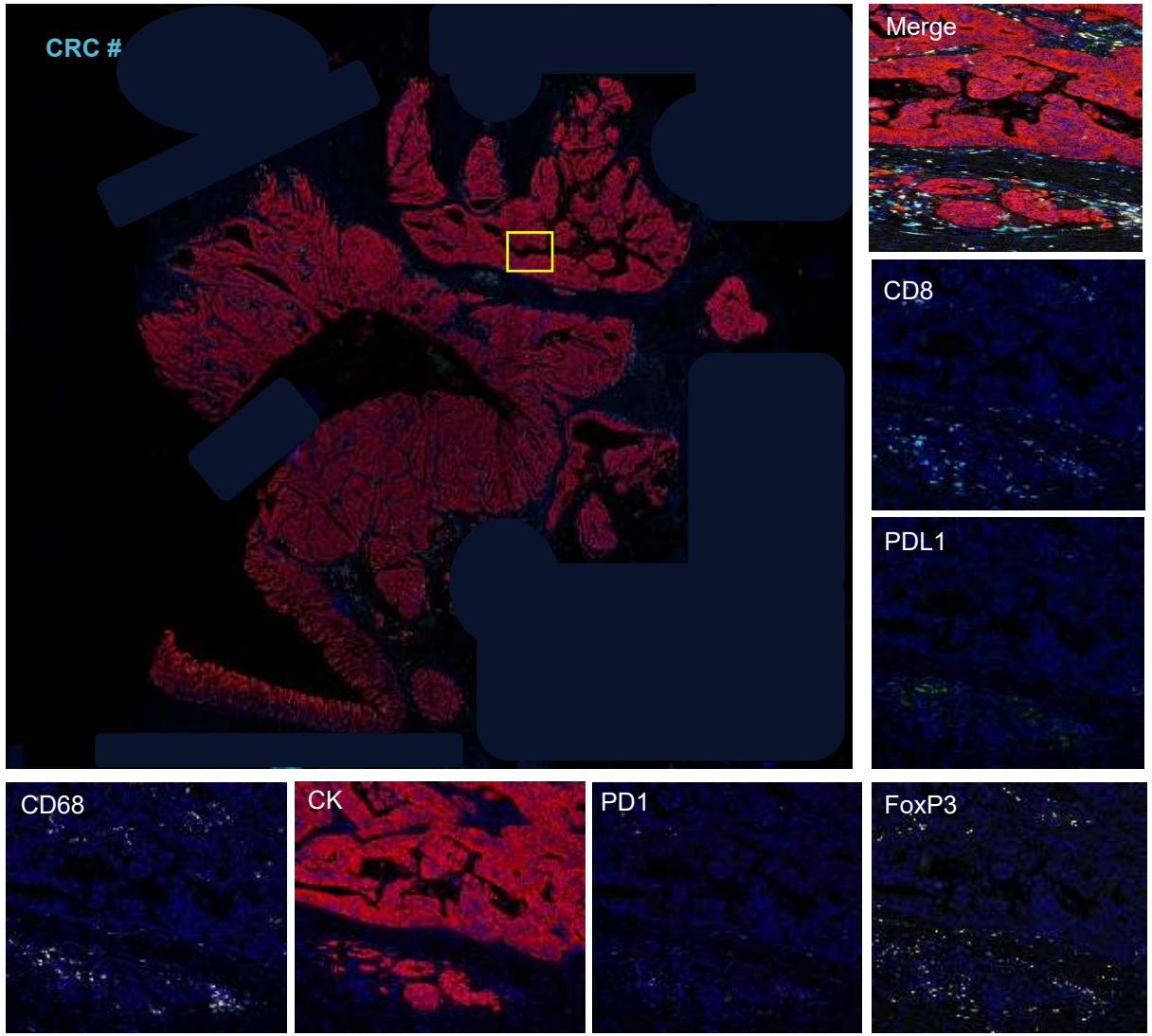

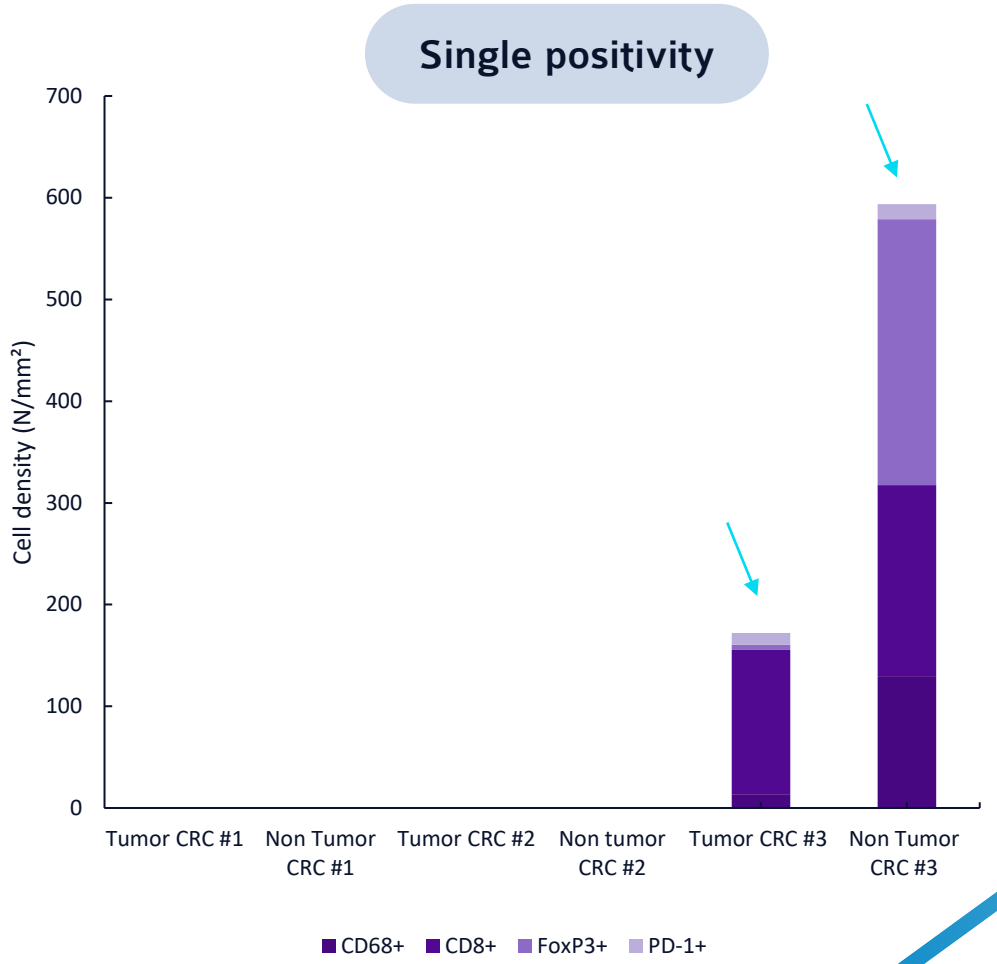

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Colon Cancers

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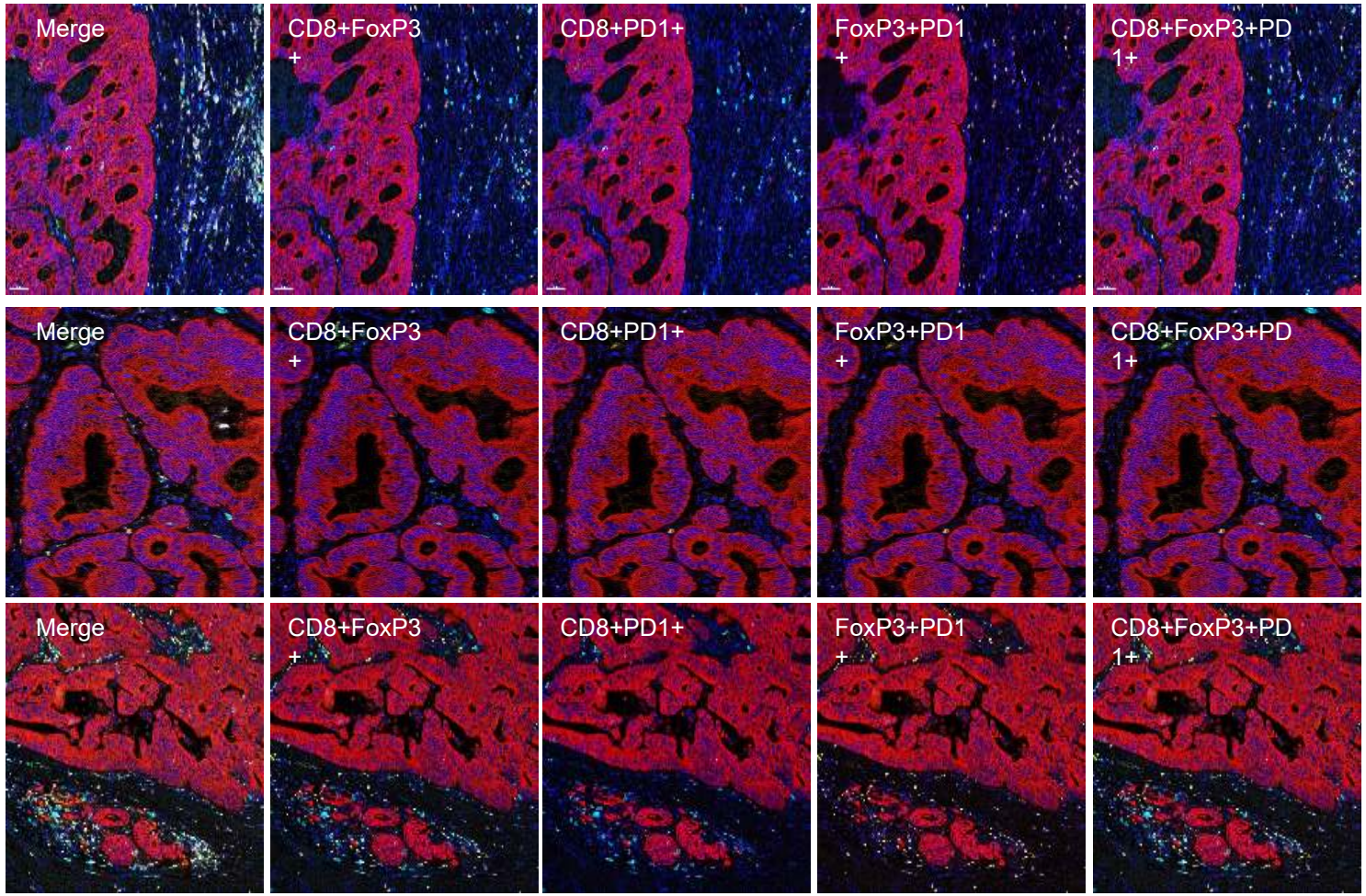

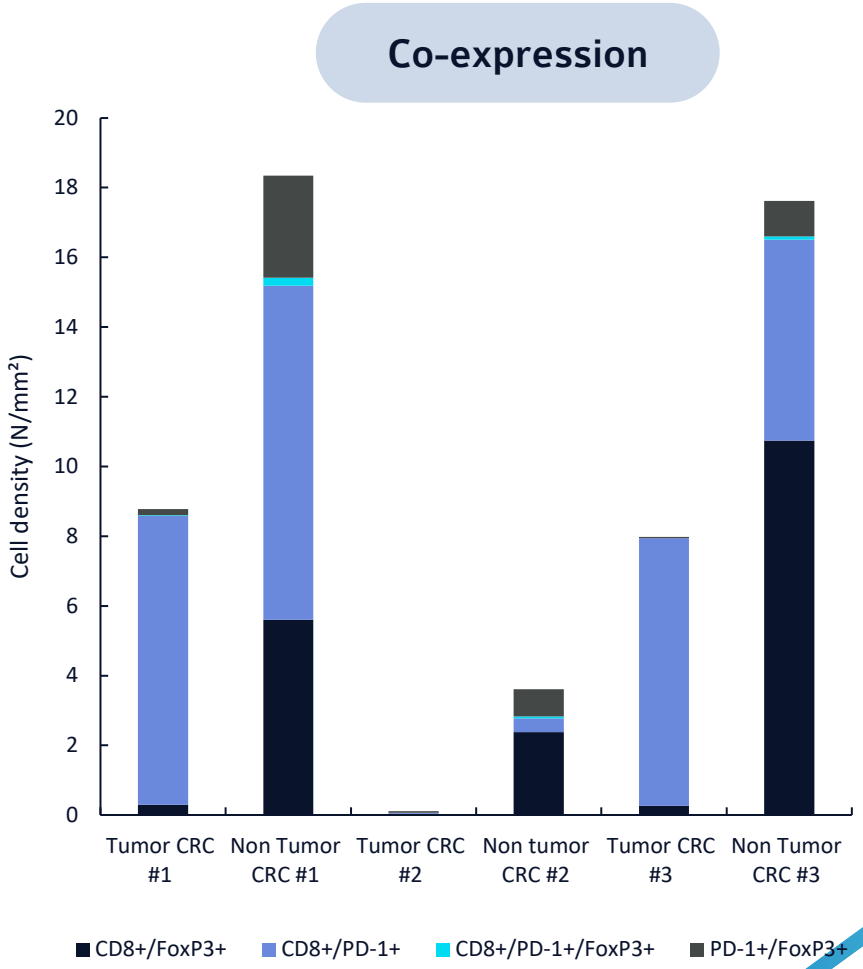

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Breast Cancers

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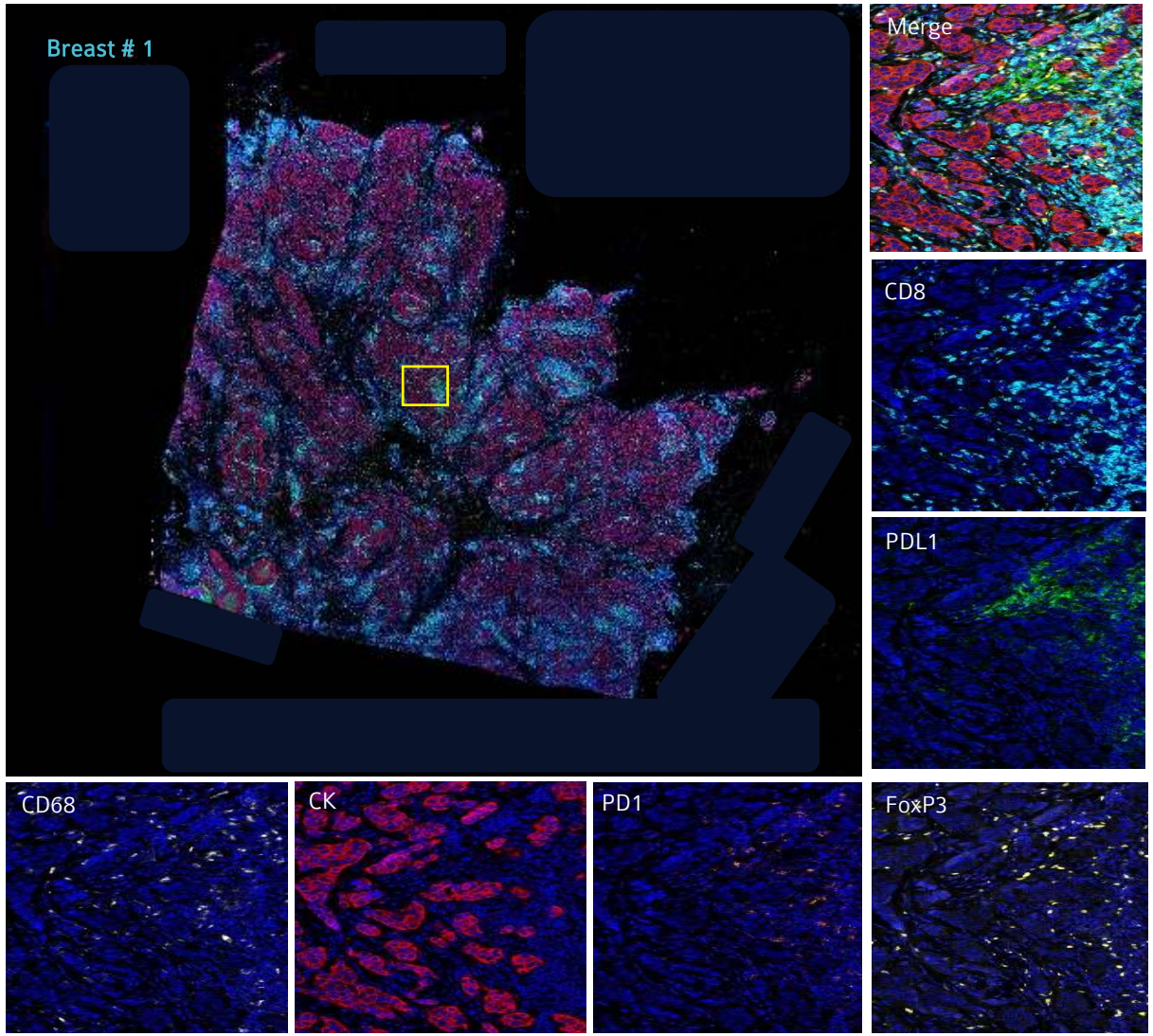

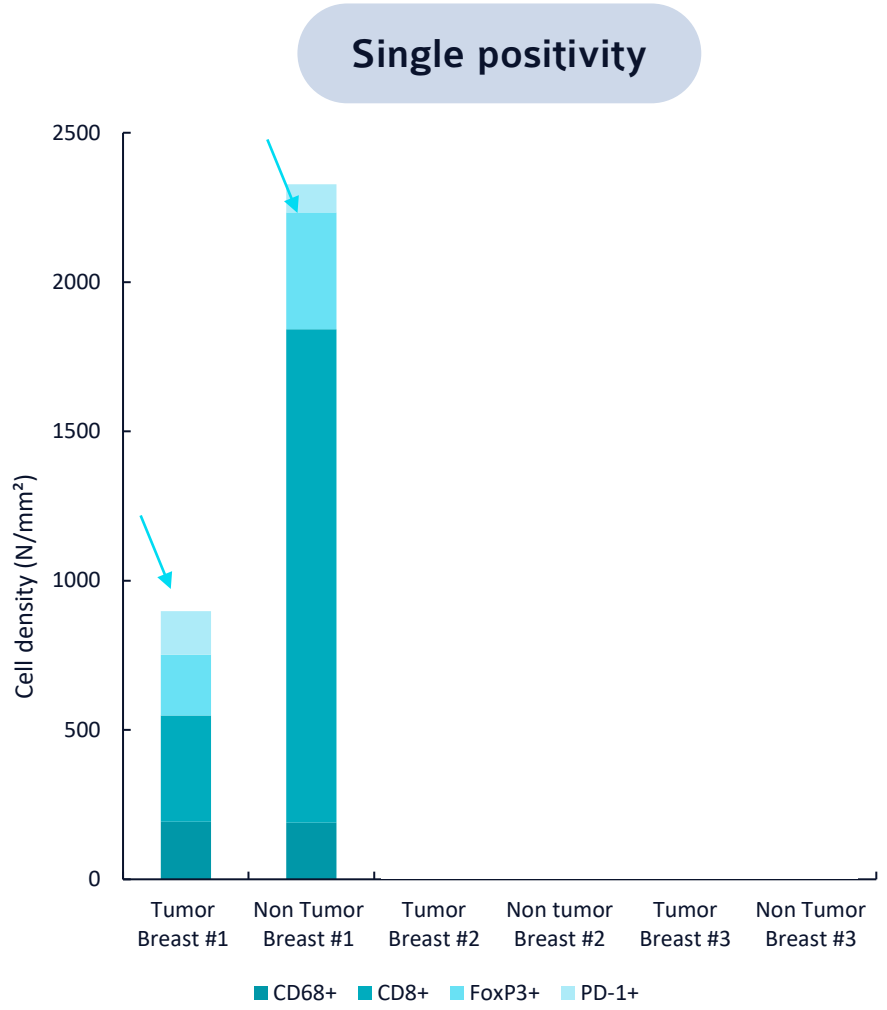

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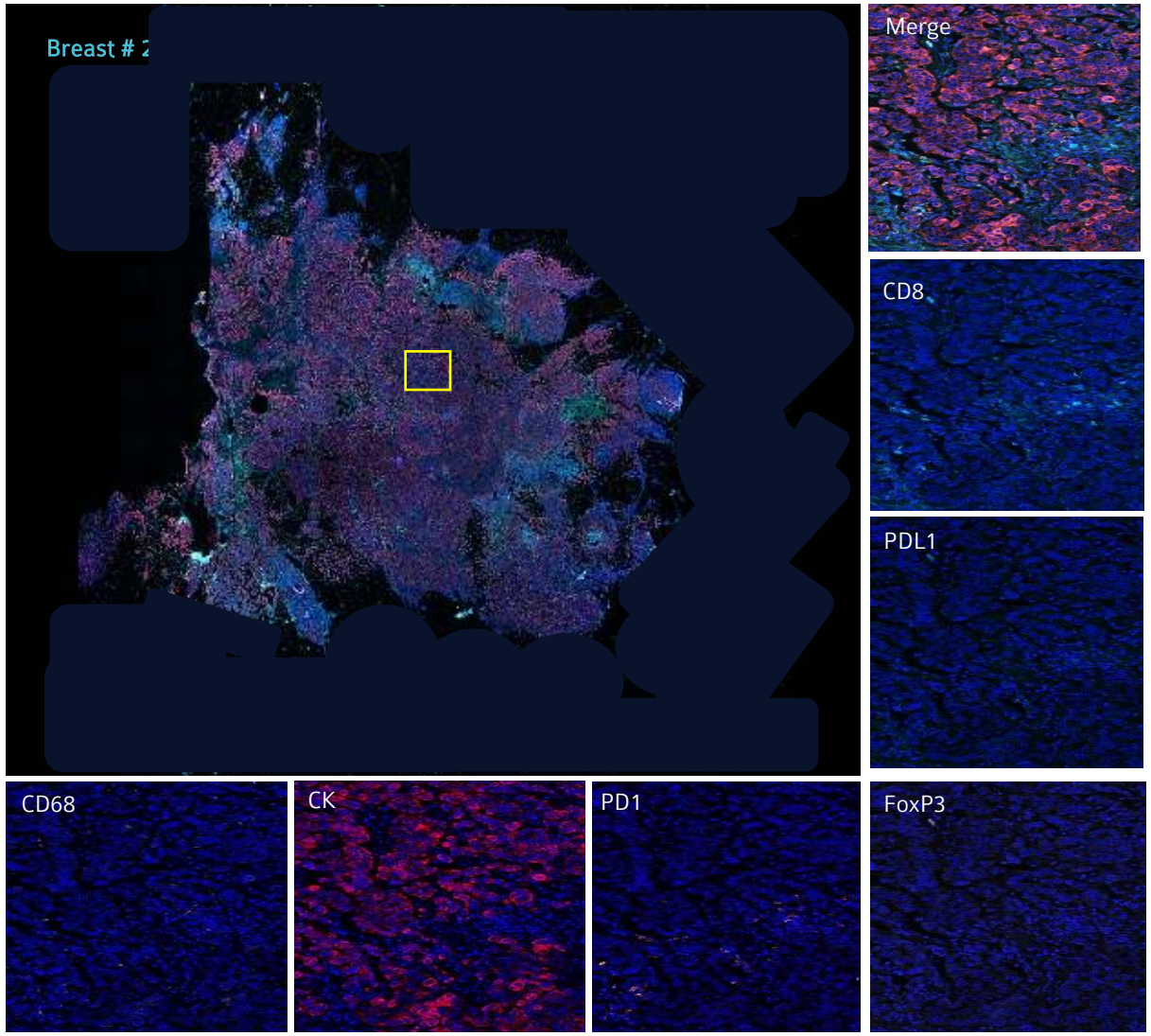
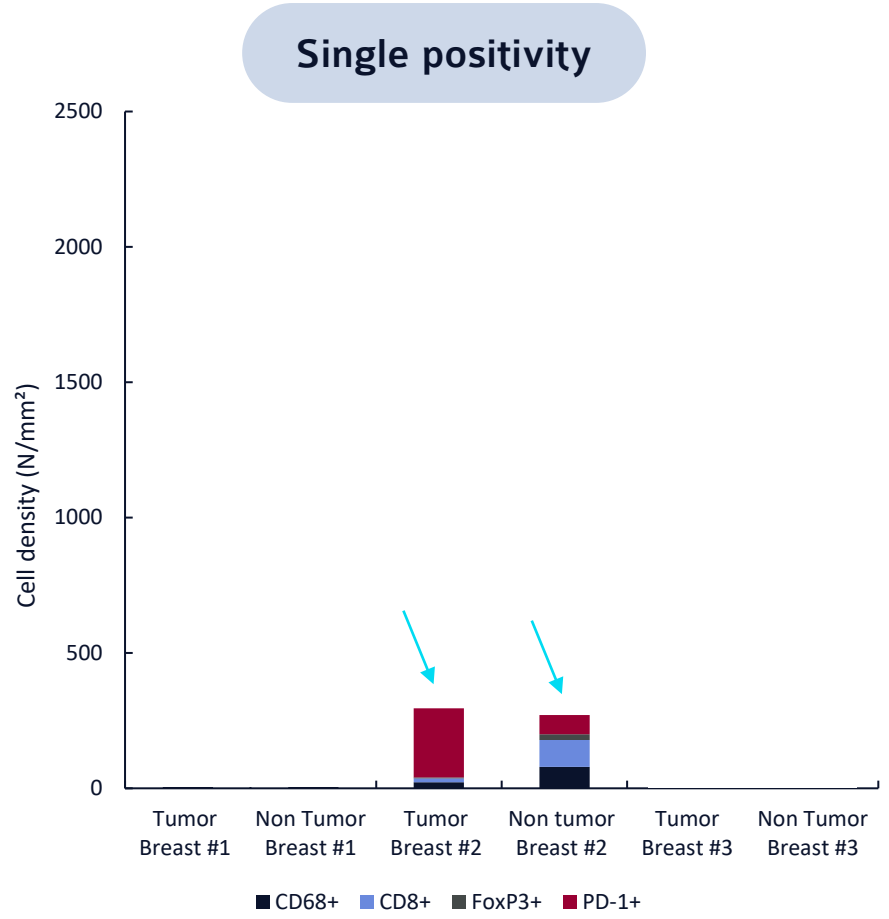
Breast Cancers

Cerba Research





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Breast Cancers

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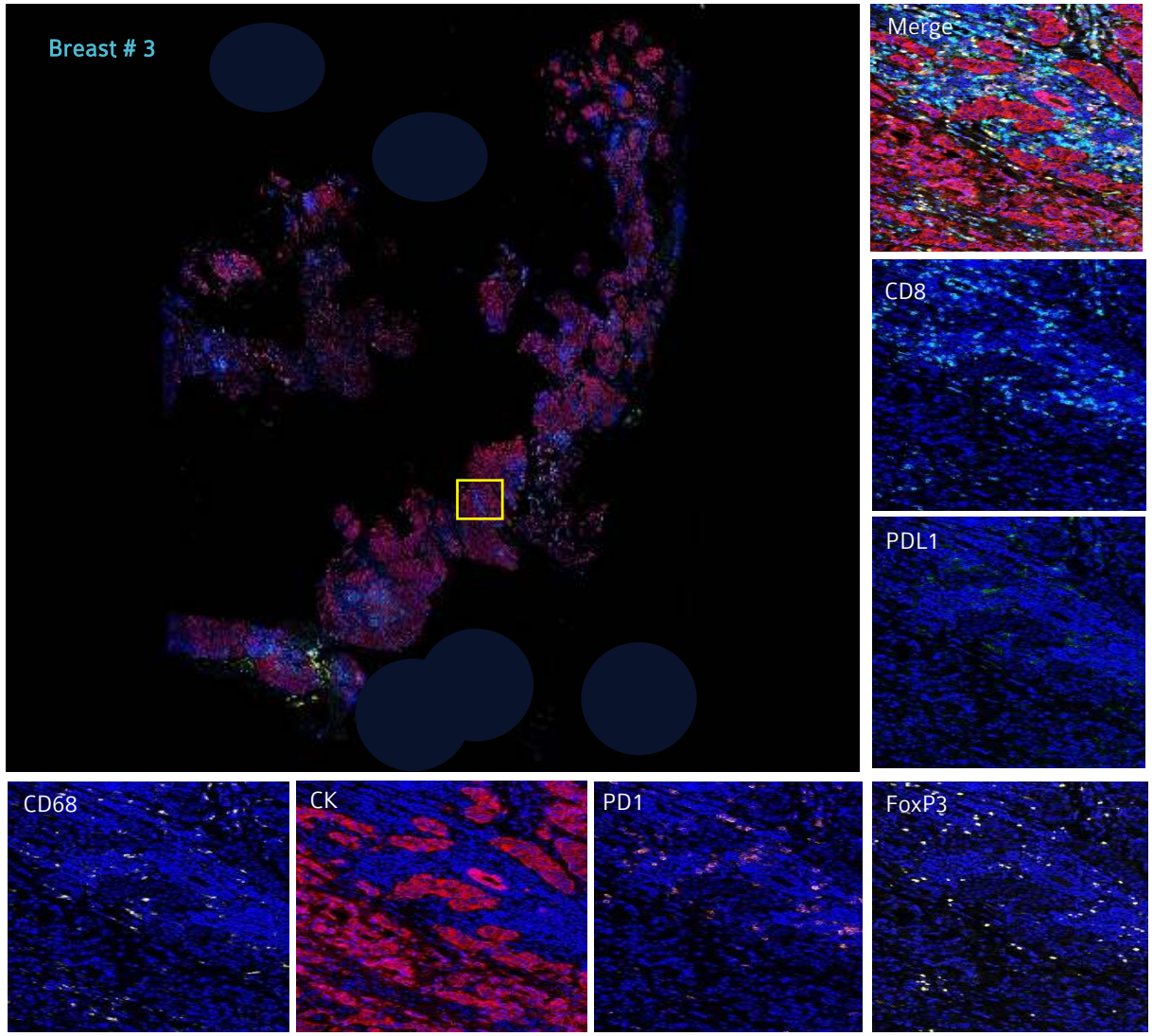

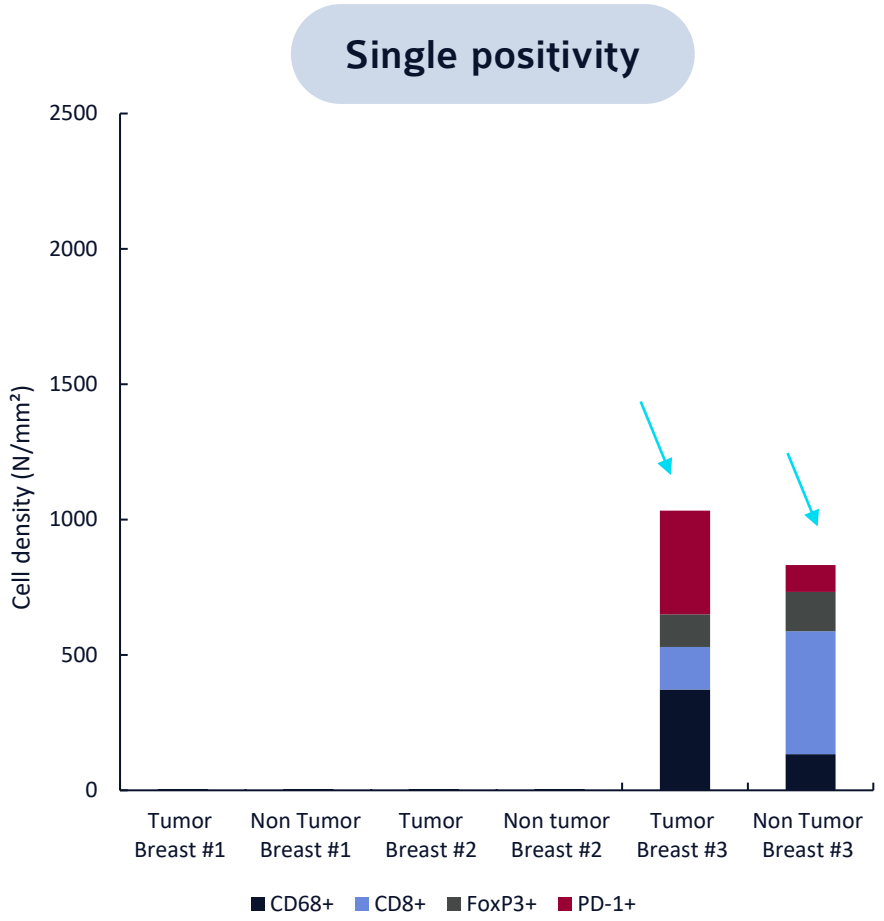

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Breast Cancers

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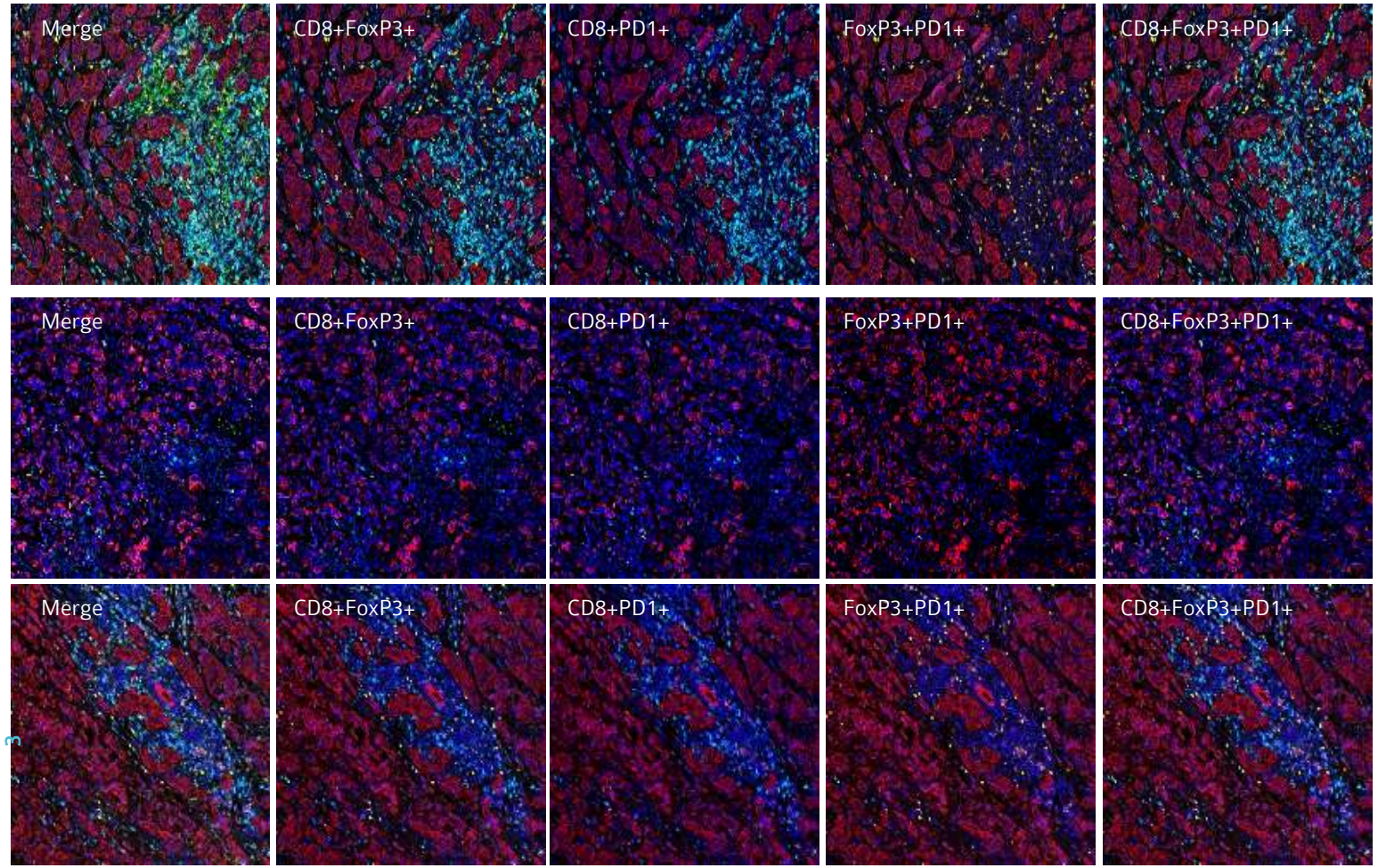

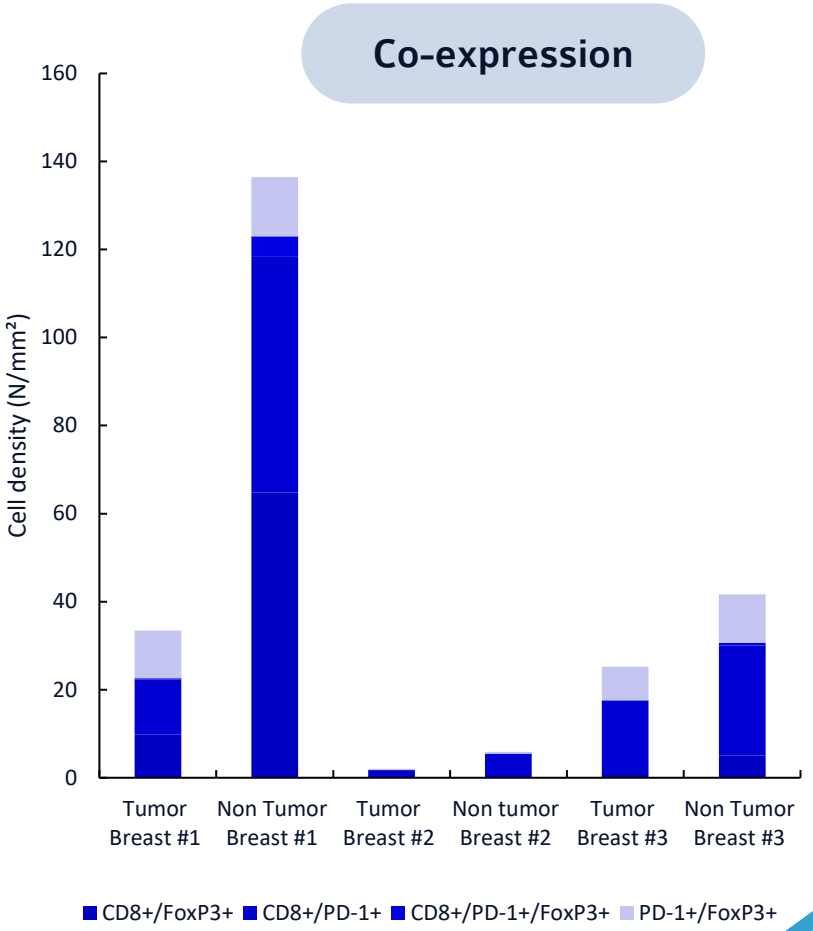

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MOTiF™ PD-1/PD-L1 Panel

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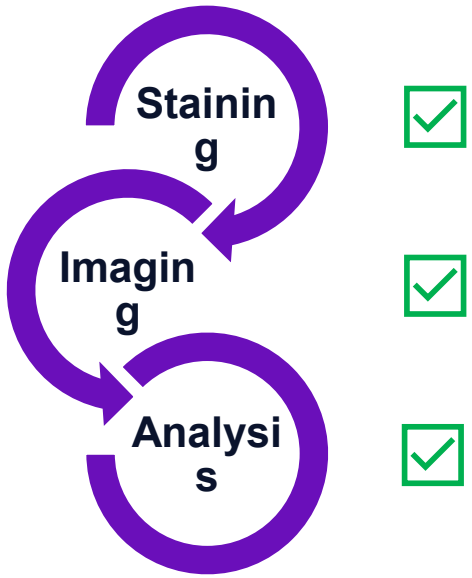
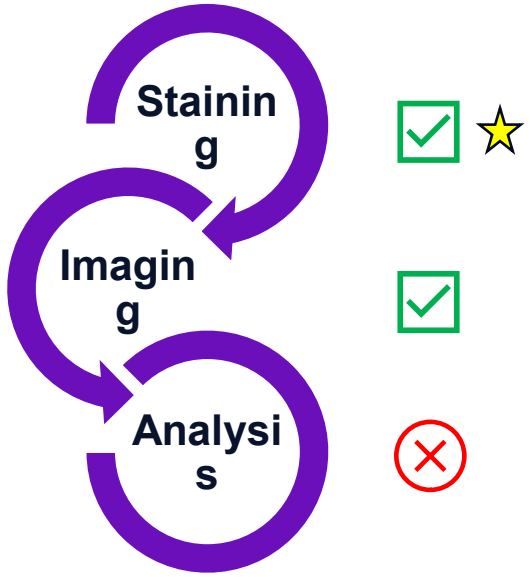
Repeatability, Reproducibility



Proof of concept on lung cancers

Robustness



Cell segmentation,
Positivity thresholds

Show utility across other tissue types

04

Custom multiplex IHC

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Multiplex IHC catalog

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Tumor

Tumor Specific Markers (e.g.)

- Cytokeratin
- Arginase 1
- TTF1
- AMACR
- Vimentin

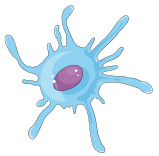
Pro-tumor

T regulatory Cell

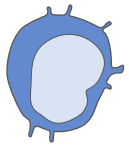
Suppress anticancer immune responses
Stimulate inflammatory cytokine production
Histoprofile®-Treg

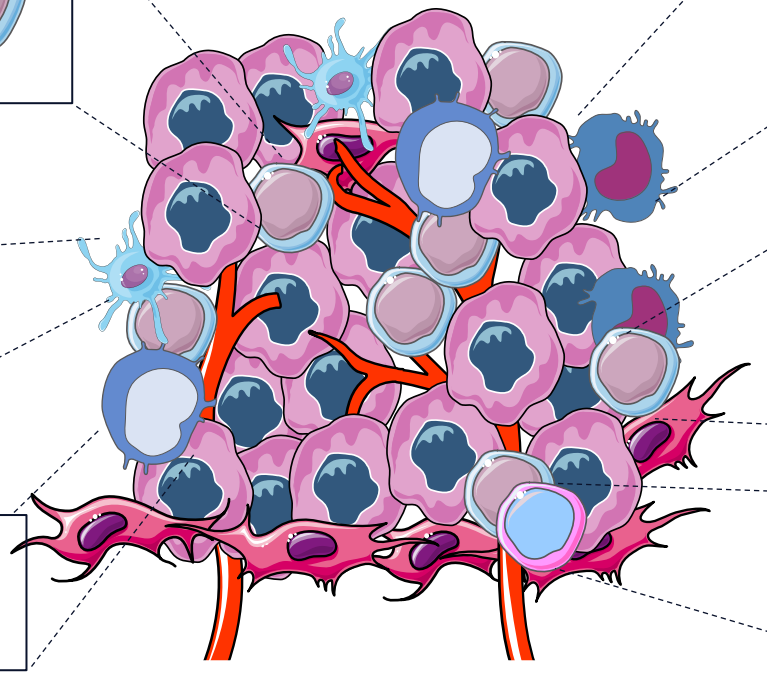
Dendritic Cell

Suppress T cell function
Promote tumor growth

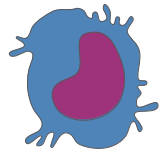


Myeloid Derived Suppressor Cell
Suppress T cell function
Recruit immunosuppressive immune cells
Histoprofile®-MDSC





Macrophage



Promote angiogenesis, tumor infiltration, chemotaxis, and metastasis
Histoprofile®-M1/M2, Histoprofile®-Neuro M1/M2

T cell (CD8/CD4)

Release tumor promoting cytokines
Histoprofile®-Treg, Histoprofile®-CKI
Histoprofile®-Tumor temp, Histoprofile®-Tissue resident T-cells

Natural Killer Cell

Histoprofile®-NK cells

Checkpoint Proteins —

PD-L1, PD-1, TIGIT,
CTLA-4, Lag-3, 4-1BB

Histoprofile®-CKI

Histoprofile®-CD47/SIRPα

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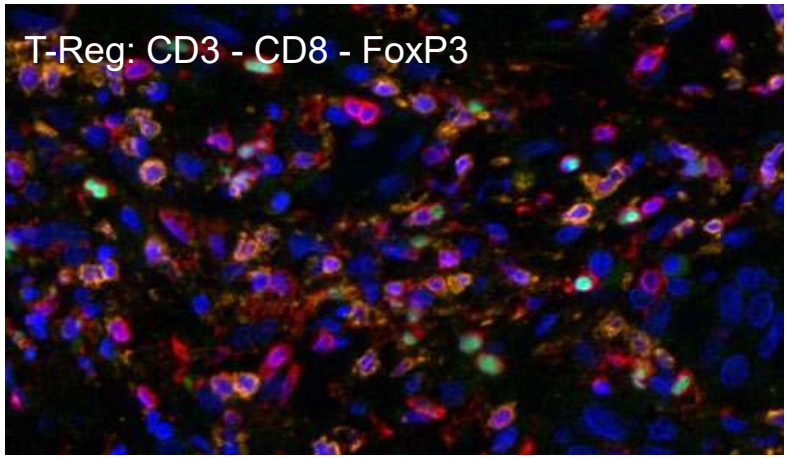
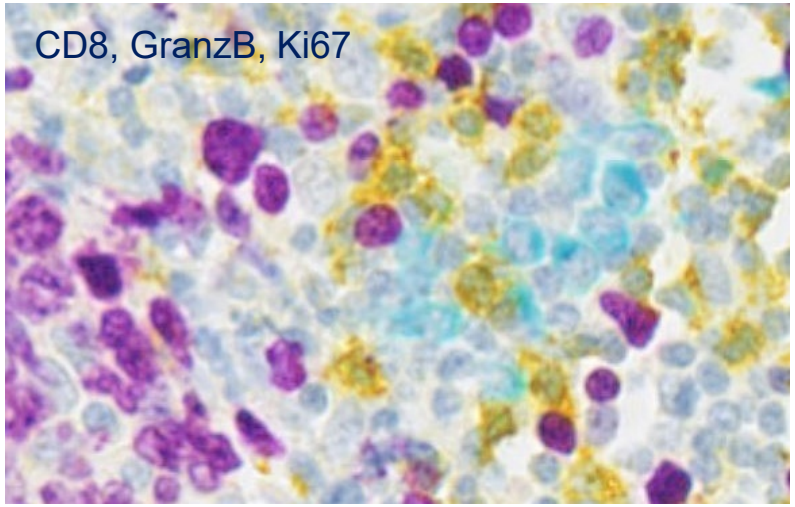
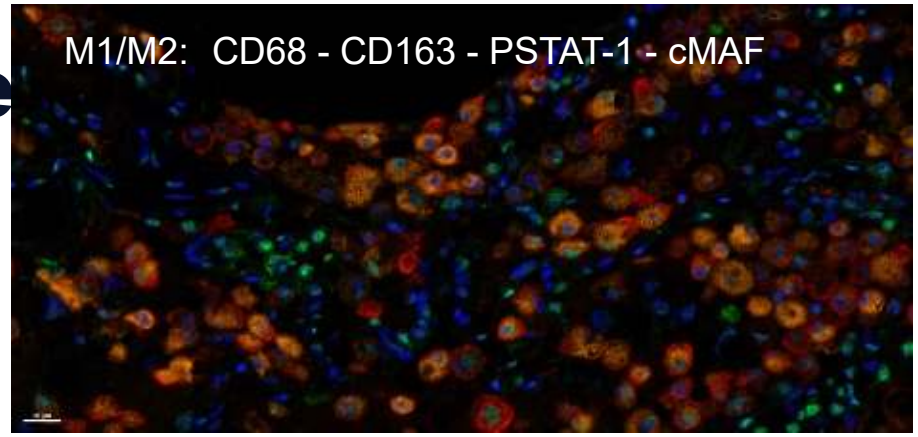
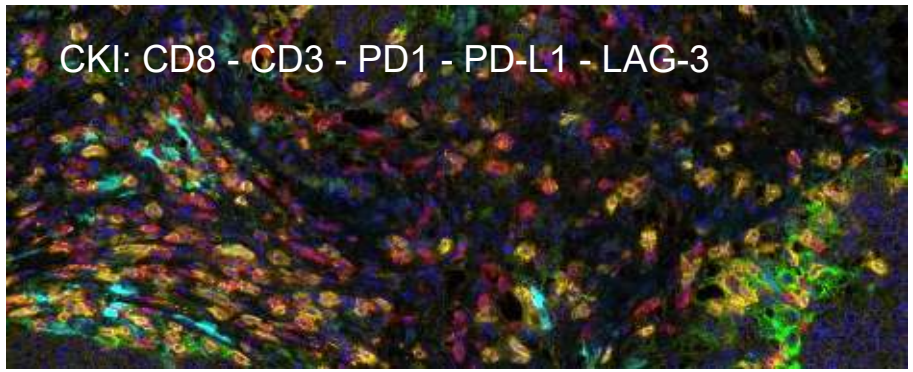
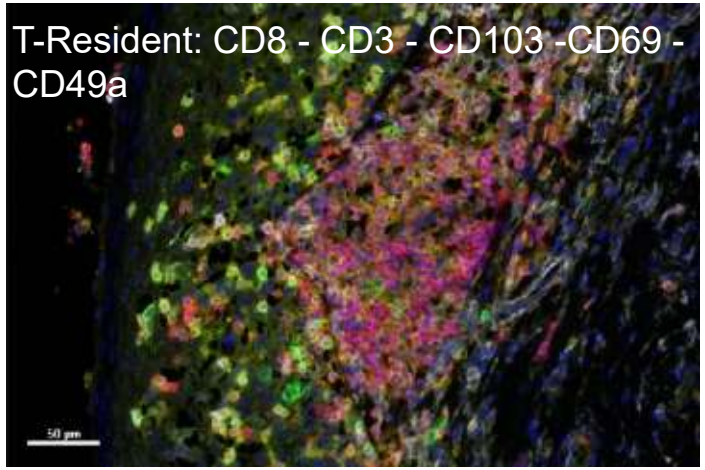
Multiplex IHC catalog











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Custom Multiplex IHC

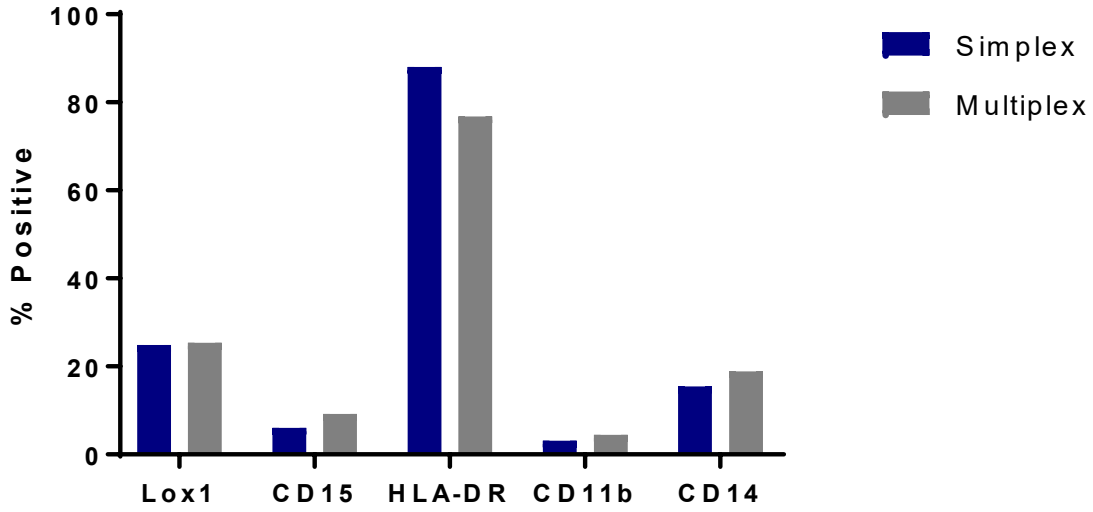
Protocol Development

Simplex Protocols

Multiplex Optimization

Simplex vs Multiplex Analysis

9-12 weeks



Validation

Custom Multiplex IHC

Clinical Validation

	Pre-clinical	Exploratory	Secondary Endpoint	Primary Endpoint
Specificity	X	X	X	X
Sensitivity	X	X	X	X
Repeatability	X	X	X	X
Reproducibility	X	X	X	X
Antigen Stability		Over 3 months	Over 3 months	Over 3 months
Antibody Lot to Lot Variation			X	X
Orthogonal Validation				X
Pathologist/ Image Analysis	X	X	X	X
Timeline	**2 months**	**4-6 months**	**5-7 months**	**6-8 months**

Custom Multiplex IHC

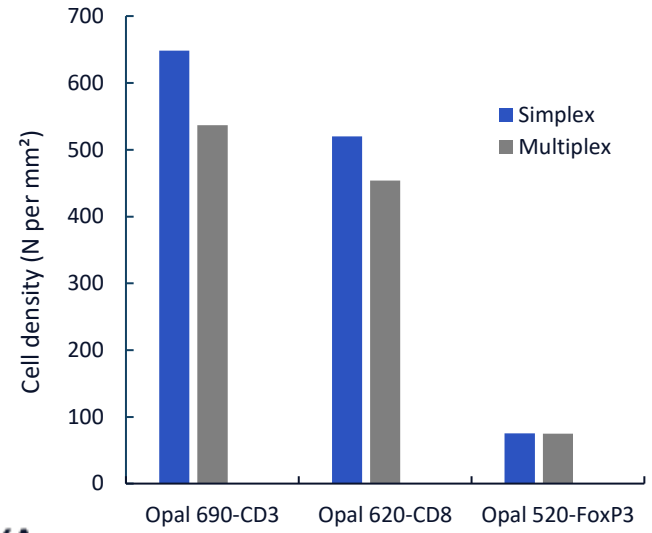
Cerba Research

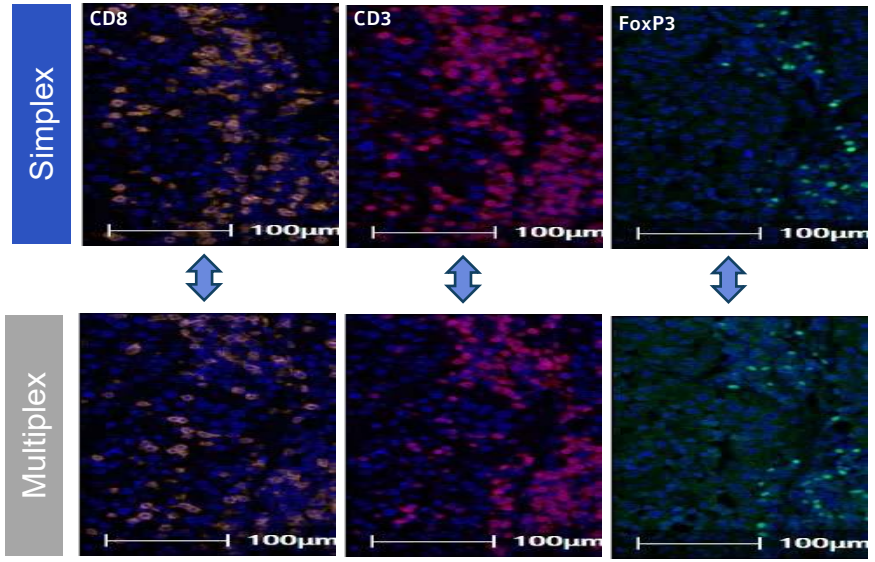
Exploratory validation: 3-plex: CD3/CD8/FoxP3

T-Regs: CD3+CD8+FoxP3+

- Maintain immune tolerance, promote tumor development by suppressing antitumoral CD8 responses.
- High effector CD8 / Tregs cells is predictive of response to treatment in different cancer types.

Simplex Vs Multiplex Analysis





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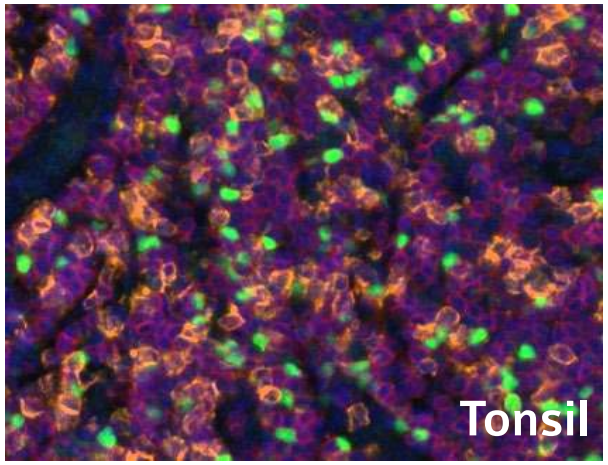
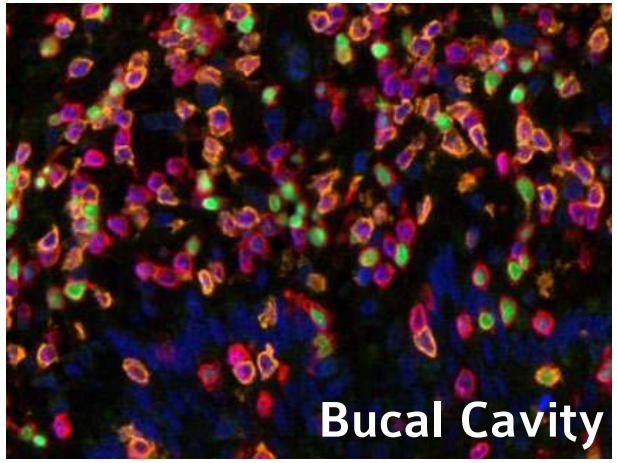
Custom Multiplex IHC

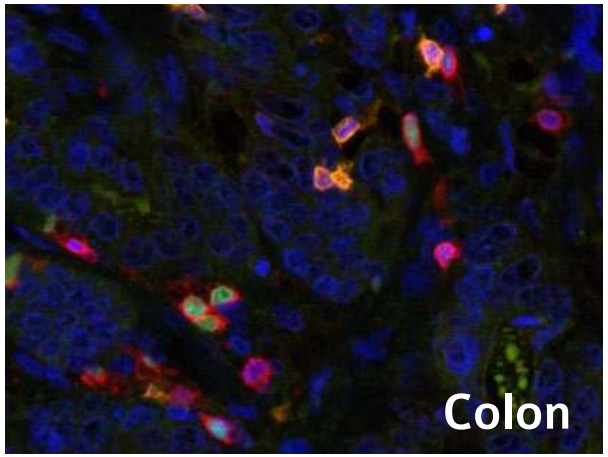
Cerba Research

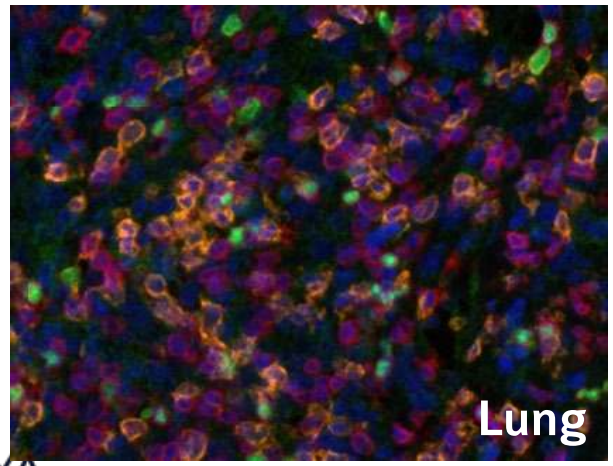
Exploratory validation: 3-plex CD3/CD8/FoxP3

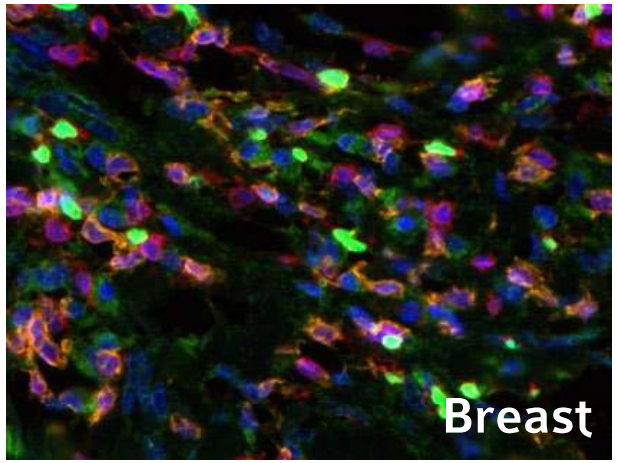
Specificity/Sensitivity











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Custom Multiplex IHC

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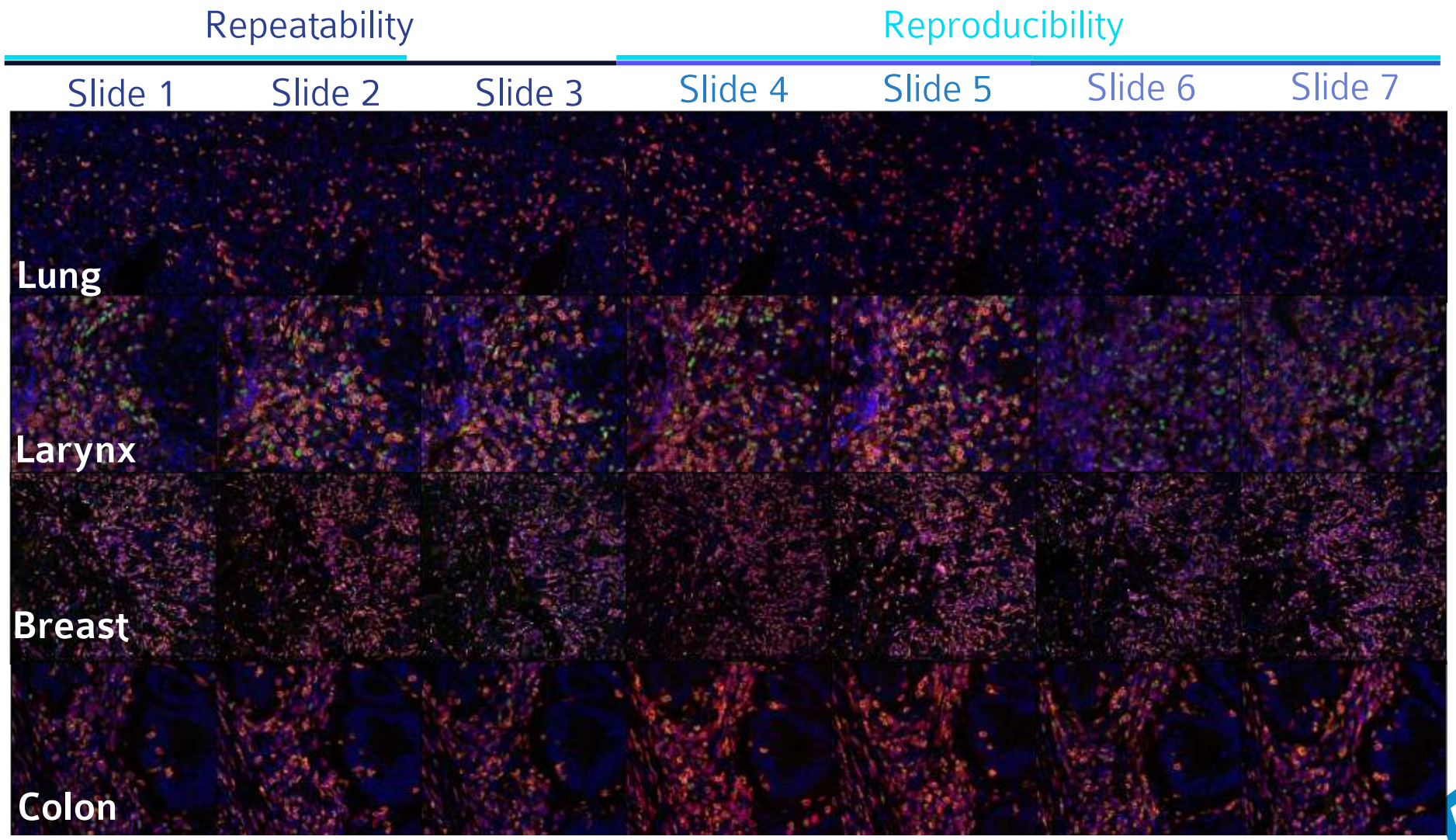
Exploratory Validation 3-plex: CD3/CD8/FoxP3



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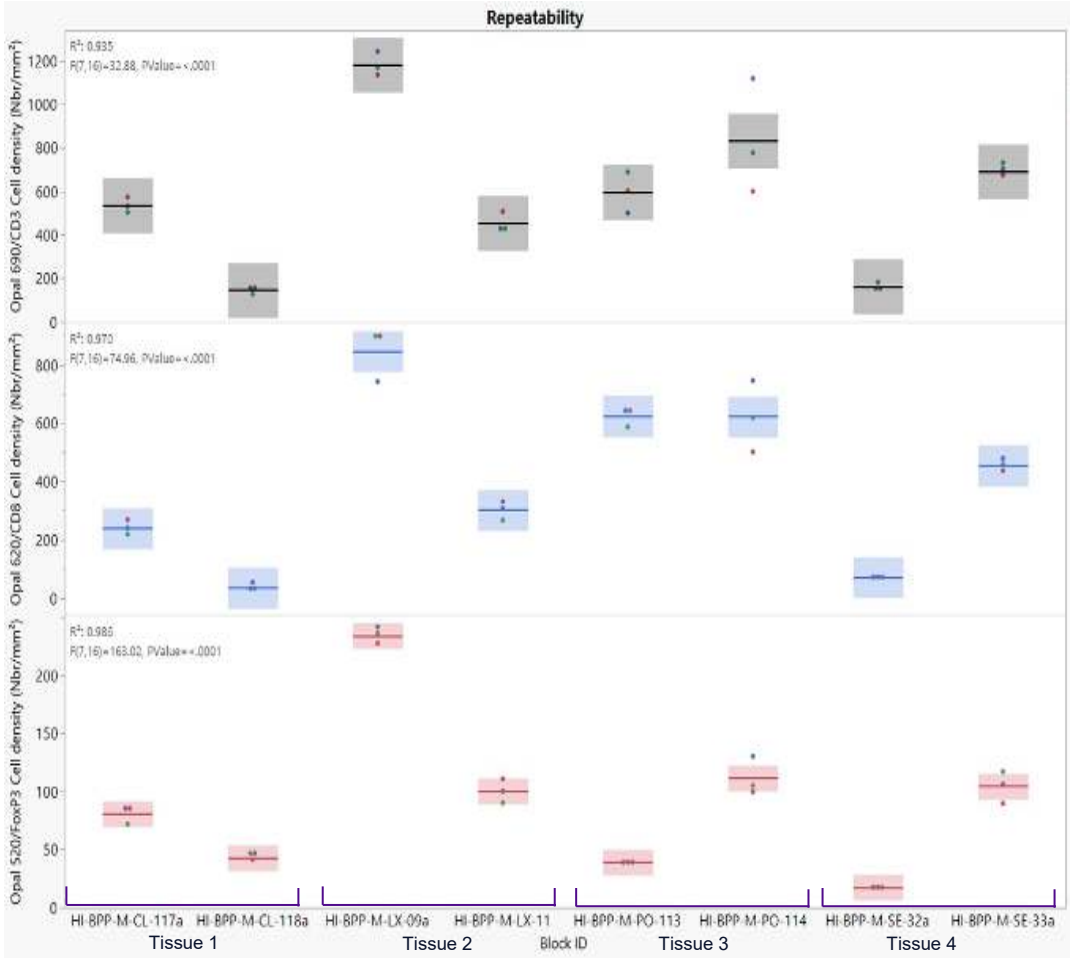
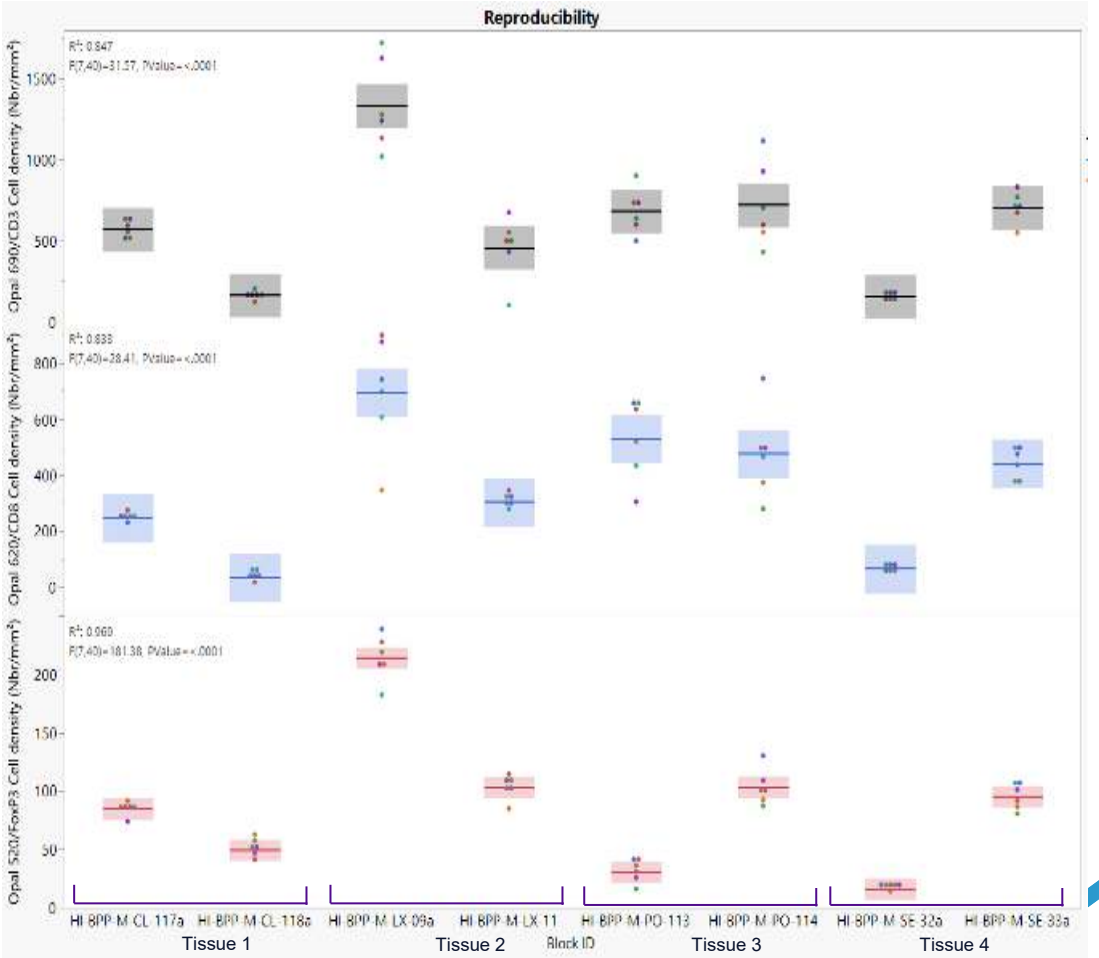
Custom Multiplex IHC

Exploratory validation





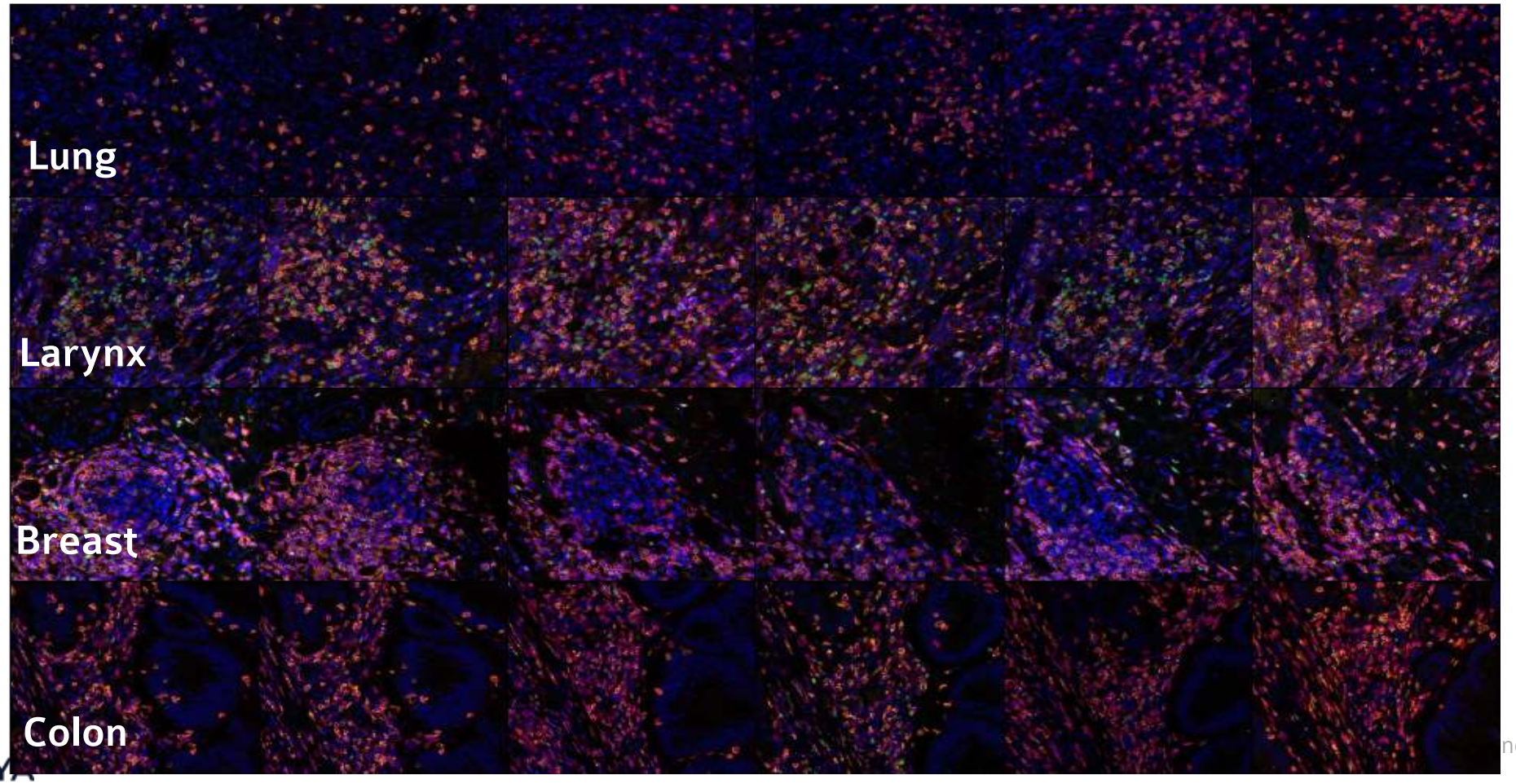
REPEATA ☑ REPRO

Custom Multiplex IHC

Exploratory Validation 3-plex: CD3/CD8/FoxP3

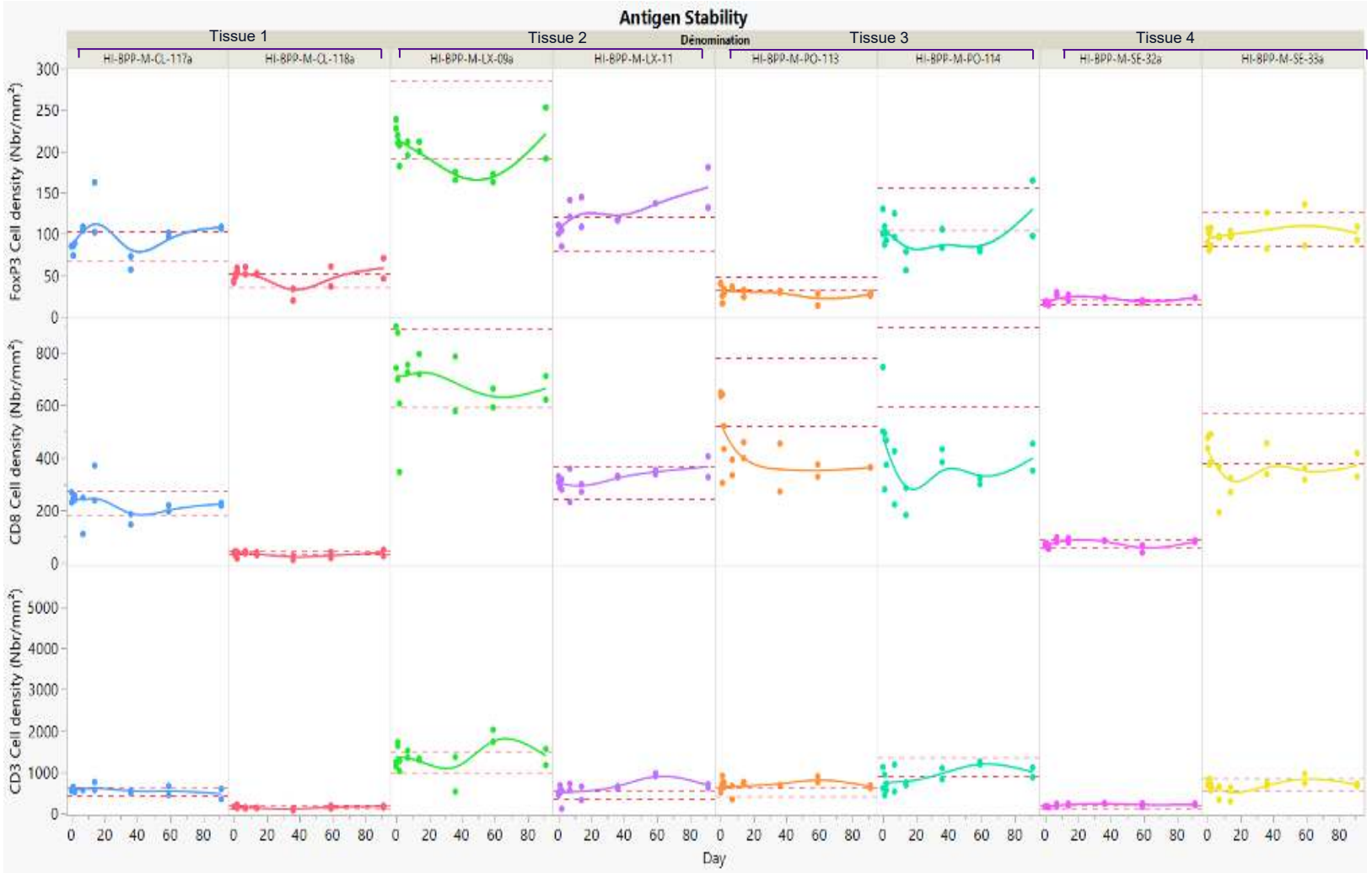
Antigen Stability

Day 0 | Day 30 | Day 90



Custom Multiplex IHC

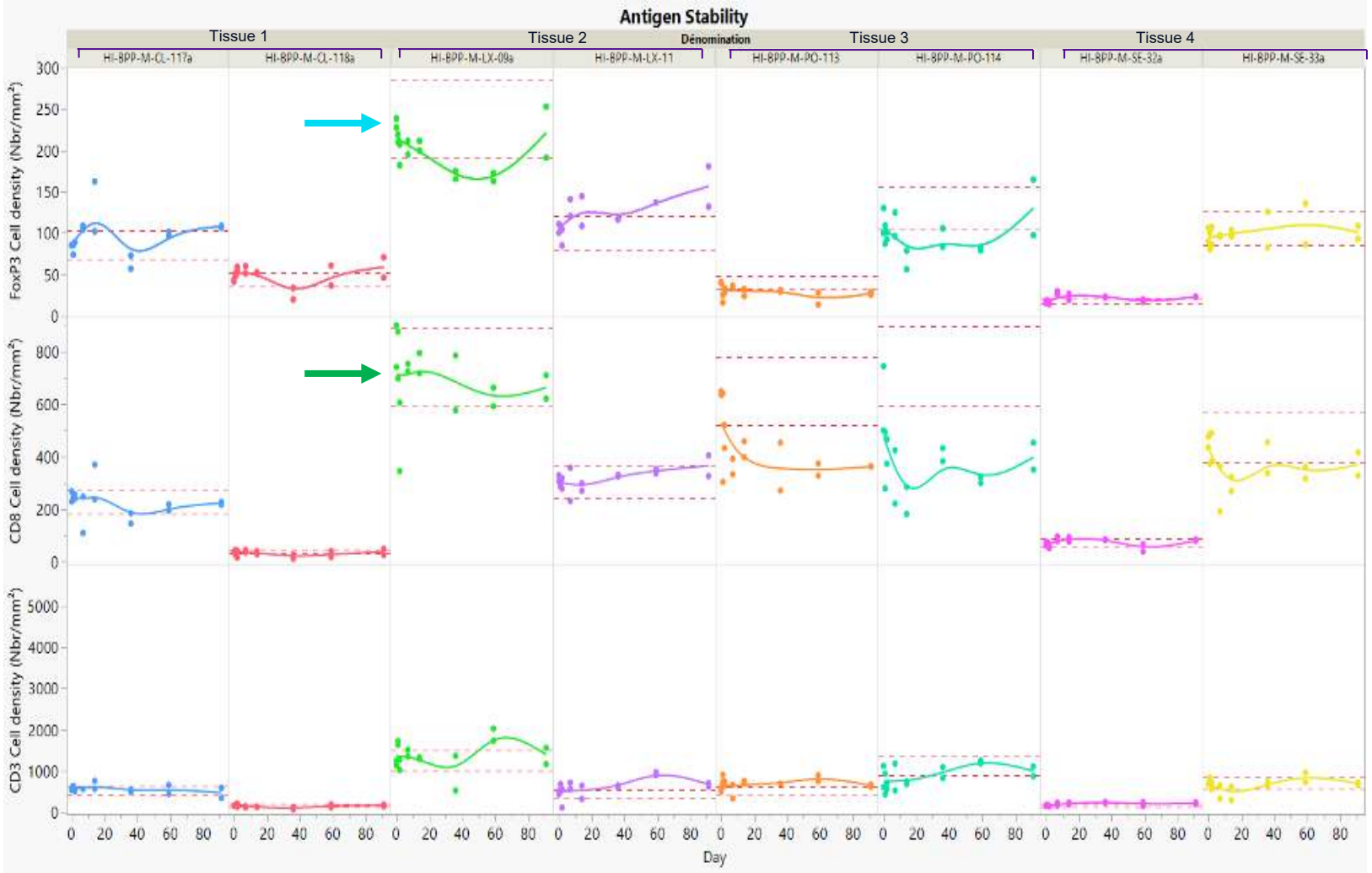
Exploratory Validation 3-plex: CD3/CD8/FoxP3



☑ Antigen stability

Custom Multiplex IHC

Exploratory Validation 3-plex: CD3/CD8/FoxP3



☑ Antigen stability

To Conclude

Cerba Research

MOTiF™ PD-1/PD-L1 Panel : Translational

- Demonstrate a reproducible, easy to use, and standarized workflow for quantitative assessment of biomarkers relationships within TME.
- Fully optimized parameters reduce processing time.

T-Regs custom Panel: Clinical trial

- Validation process at Cerba Research: robust process and reliable data.
- Strong interaction: science team/ customer.
- High flexibility on panel design to fit customer needs.

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Thank you
Cerba Research
Your precision medicine partner

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