DISCOVERY BEGINS
WHEN YOU SEE EVERYTHING IN CONTEXT

Phenoptics™ Tissue Biomarker Detection Solutions

AKOYA BIOSCIENCES®

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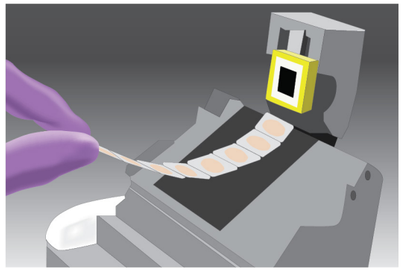
Phenoptics Overview

Shifting the paradigm from visual IHC to quantitative IF

To advance the understanding of disease mechanisms in cancer, it's critical that you see everything the tumor has to show you. With our Phenoptics solutions, you can visualize and measure tumor cells and multiple immune-cell phenotypes simultaneously in FFPE tissue. Phenoptics integrates multiplexed immunohistochemistry and imaging to quantitatively capture systems biology data with cellular detail. It reveals multi-parameter cellular expressions and interactions while retaining spatial context, offering insights into the complexity of immunecancer interactions.

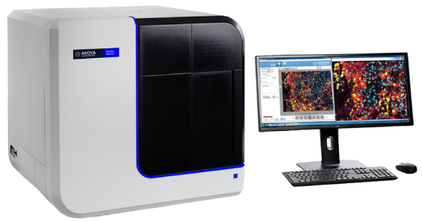
1. IMMUNOSTAIN → **2. IMAGE** → **3. ANALYZE AND UNDERSTAND**



Opal IHC works with FFPE tissue and is compatible with standard IHC workflows.

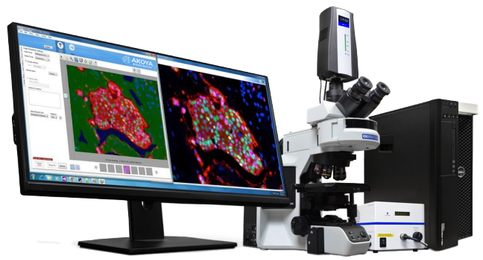
You can use the best primary antibodies together in multiplex panels, with no species-based crosstalk.

Because you retain spatial cellular context, you get more information from your precious samples.

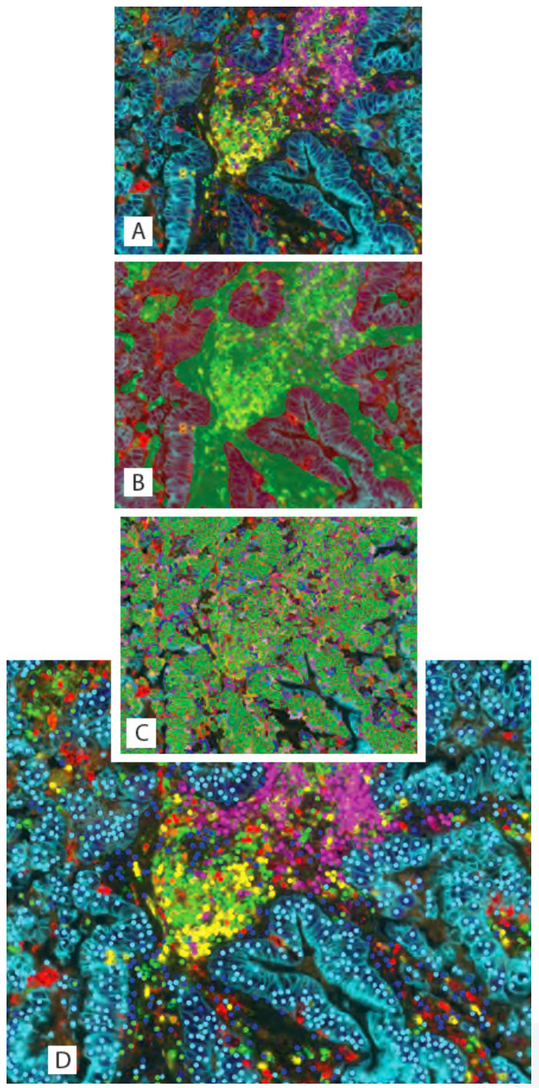


Vectra Polaris™ Automated Quantitative Pathology Imaging System

Vectra® 3 Automated Quantitative Pathology Imaging System



Mantra™ Quantitative Pathology Workstation



A) Spectral Unmixing; B) Tissue Segmentation; C) Cell Segmentation; D) Cell Phenotyping

IMMUNOSTAIN

Multiplexed Immunohistochemistry

The Phenoptics workflow begins with Opal multiplex IHC. Opal enables practical and reliable application of up to six immunohistochemical biomarkers, plus counterstain, onto single FFPE tissue sections, saving valuable tissues and enabling full contextual exploration of multiple cell types and functional states.

Opal multiplex kits are available optimized for both manual and automated workflows.

Opal is a leap forward in the interrogation of cancer-immune interactions.

IMAGE

Multispectral Imaging

Multispectral imaging technology uniquely enables isolation of individual colors to allow independent, noninterfering, and precise measurement of protein expression, while eliminating background.

Phenoptics imaging uses unique multispectral imaging technology and algorithms.

Vectra® Polaris™

Automated Quantitative
Pathology Imaging System

Vectra® 3

Automated Quantitative
Pathology Imaging System

Mantra™

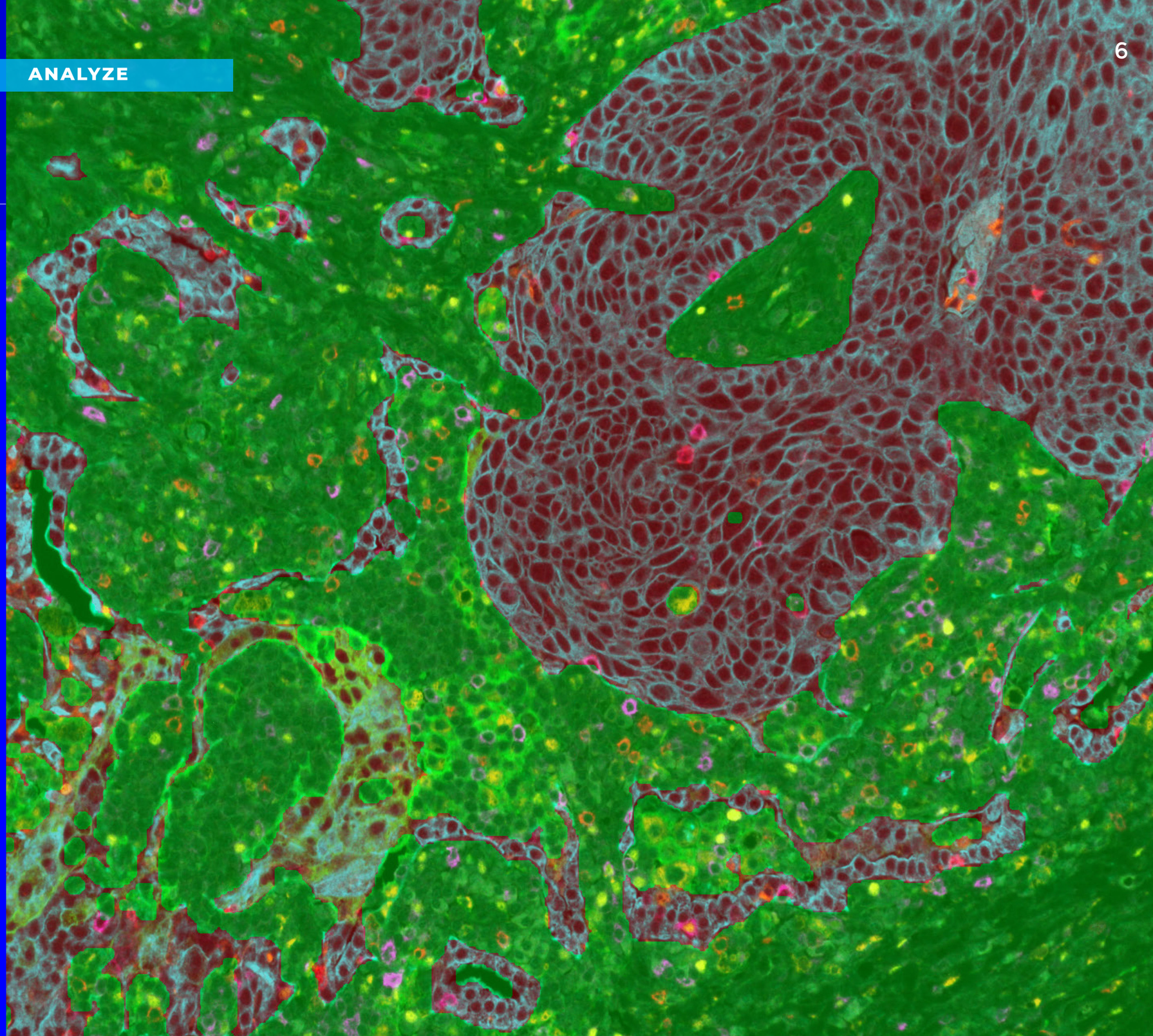
Quantitative Pathology
Workstation

ANALYZE

Identification of Tissue Segmentation

Image analysis starts with automated segmentation of tissue into regions of morphologically distinct architectures, such as tumor and stroma. Trainable pattern recognition makes this possible and avoids often prohibitively laborious manual identification of regions of interest.

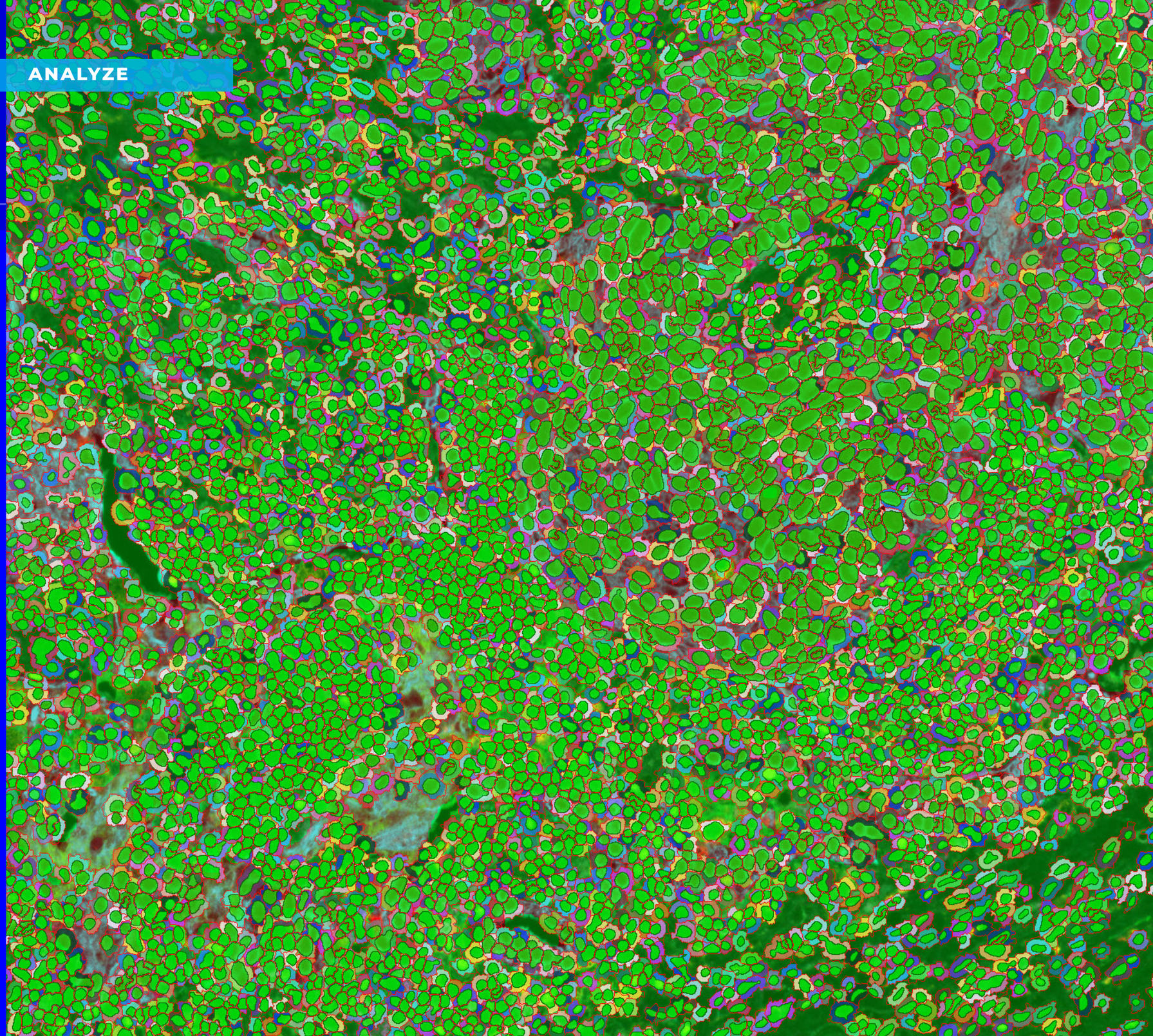
Phenoptics calculates per-cell and per-subcellular compartment intensity values.



Cell Segmentation

The next step is identifying and segmenting individual cells, starting with nuclear segmentation and then membranous and cytoplasmic segmentation. Expressions of markers can then be read out on a per-cell and per-cell-compartment basis.

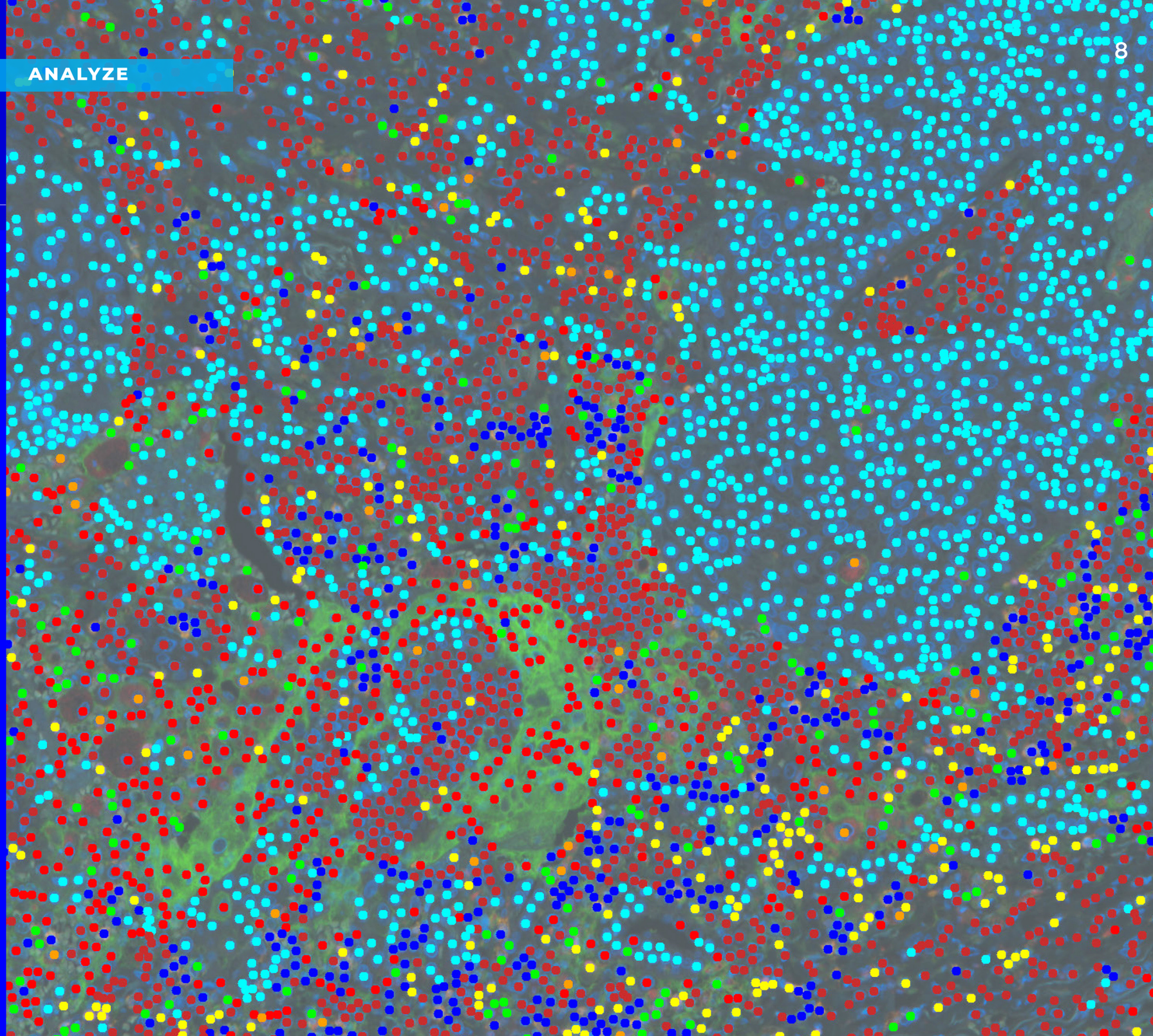
Phenoptics multispectral signal isolation enables unmatched segmentation.



Cell Phenotyping

Once parameters are known for each cell, advanced machine learning approaches can then automatically phenotype cells into user-defined categories. Since x-y coordinates and tissue context are preserved, a wide range of spatial and cellular interaction metrics can be explored.

Phenoptics is the only platform that reliably phenotypes cells in FFPE images.

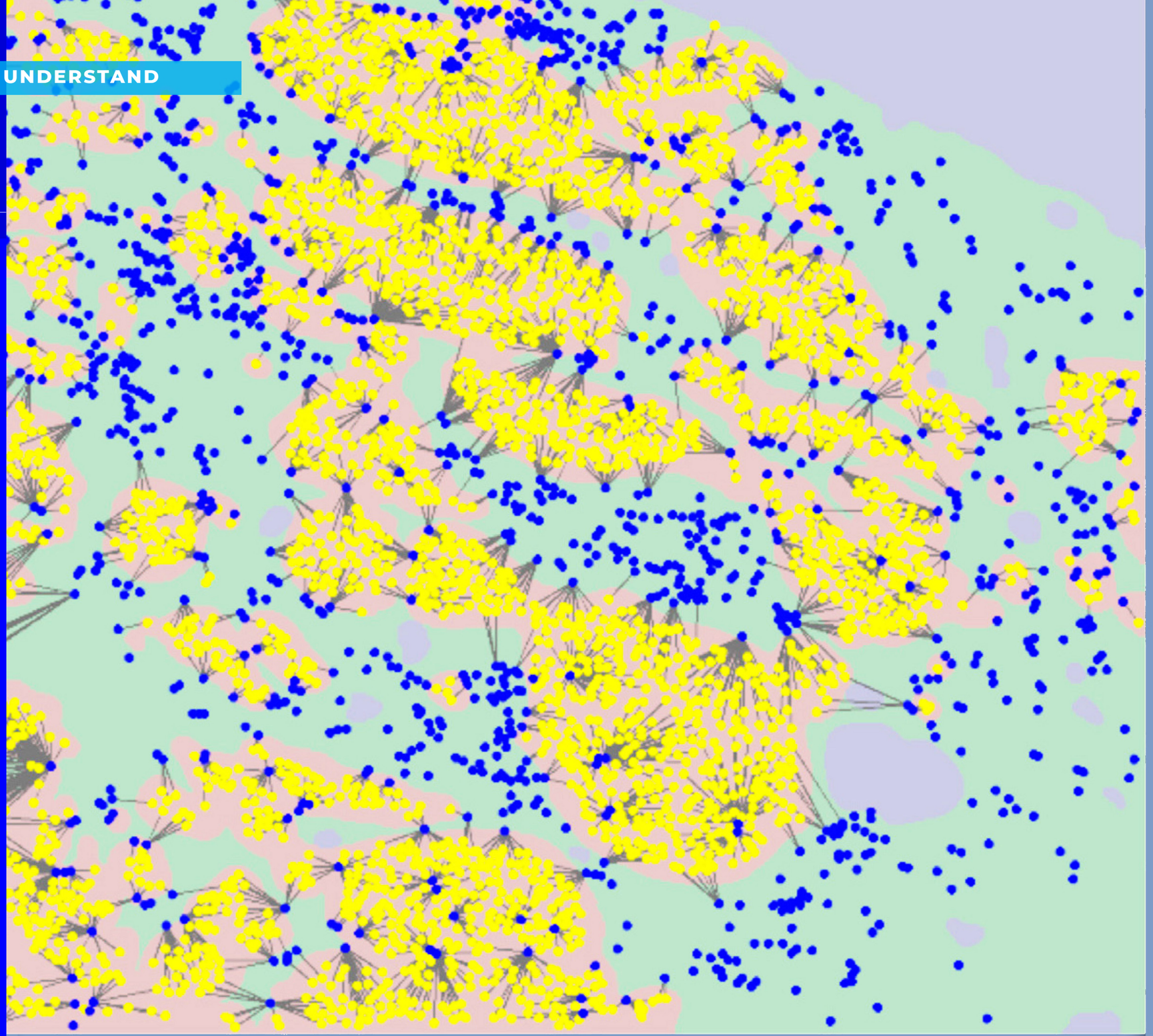


UNDERSTAND

Explore Systems Immuno-biology

To fully explore and capture the value of the resulting dataset—which includes multiparameter per-cell data in spatial contexts—we are prototyping algorithms in R to perform system biology hypothesis-driven experiments to discover predictive biomarkers.

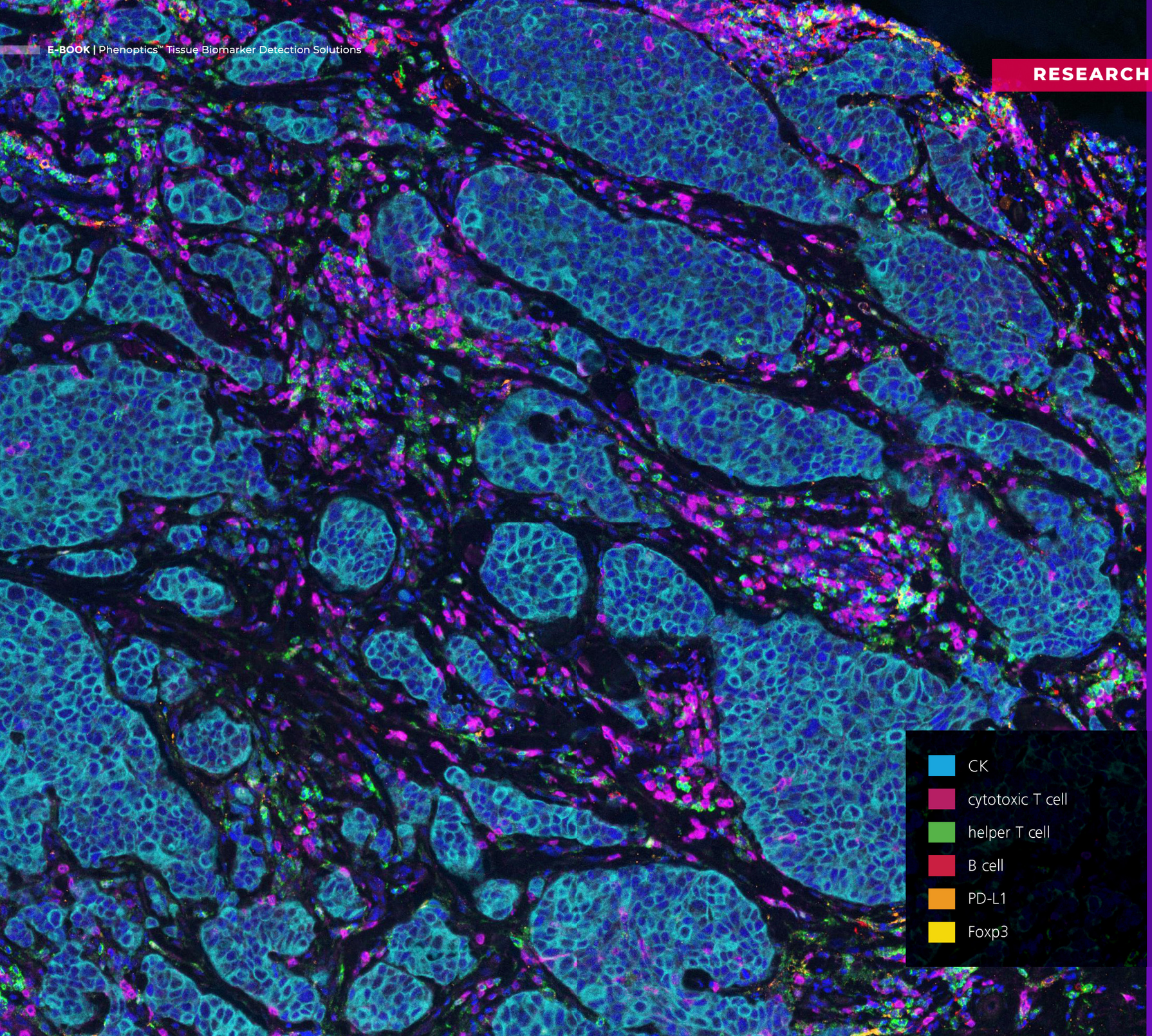
Leverage Phenoptics technology and blaze new trials in immuno-oncology research.



RESEARCH EXAMPLE

Breast Cancer

Dr. Mittendorf is a breast cancer surgeon who, like most surgeons and oncologists, is making critical decisions every day that materially impact the wellbeing of patients. In her research role, she is looking for better ways to capture telltale cancerimmune signatures that will predict how a patient will respond to therapies. Today she is focused on immuno-oncology. Dr. Mittendorf's first study focuses on the role of immune infiltrates in triple negative breast cancer.

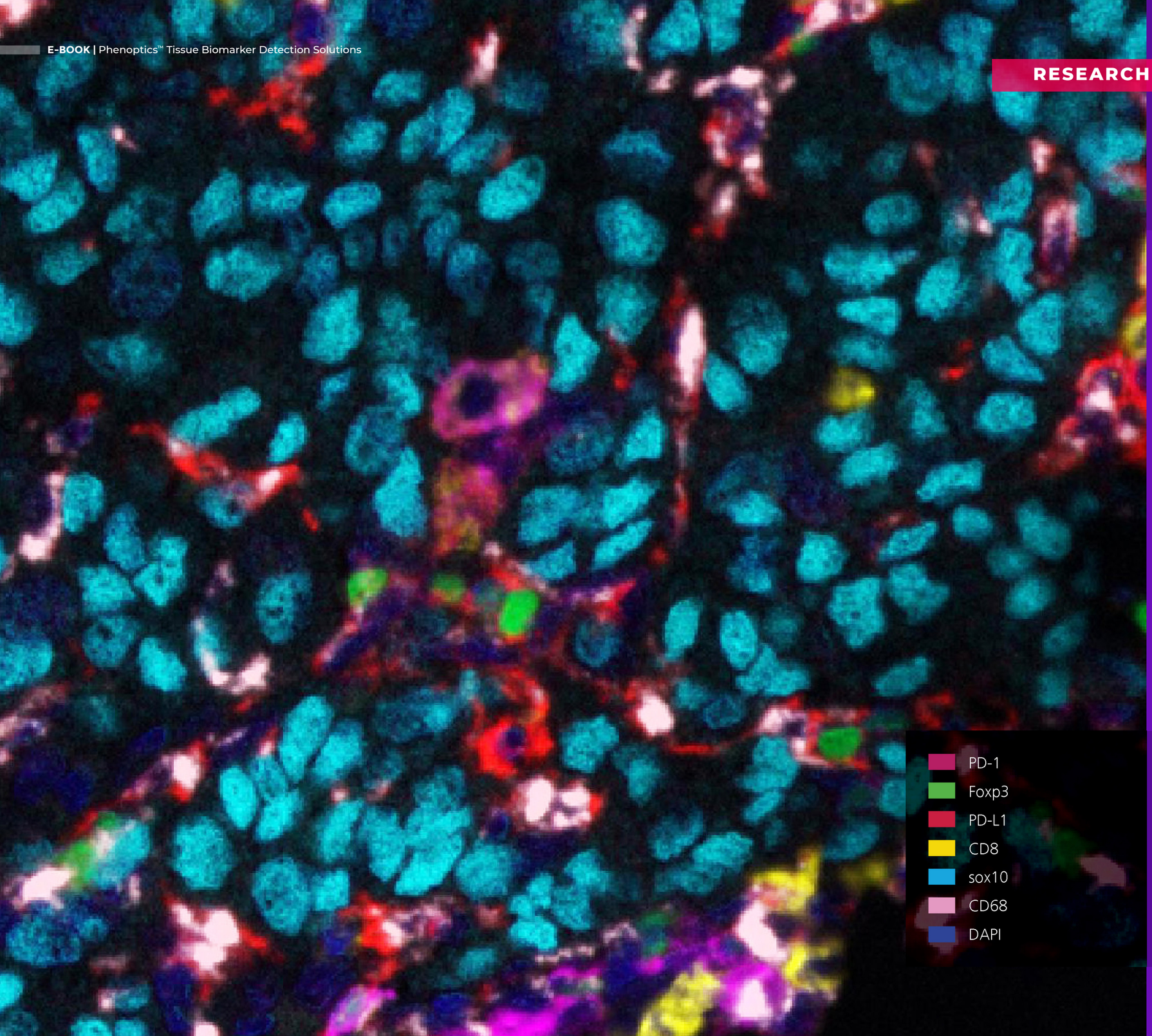


Elizabeth A. Mittendorf, MD, PhD

Associate Professor in the Department of Surgical Oncology

University of Texas MD

Anderson Cancer Center



RESEARCH EXAMPLE

Melanoma

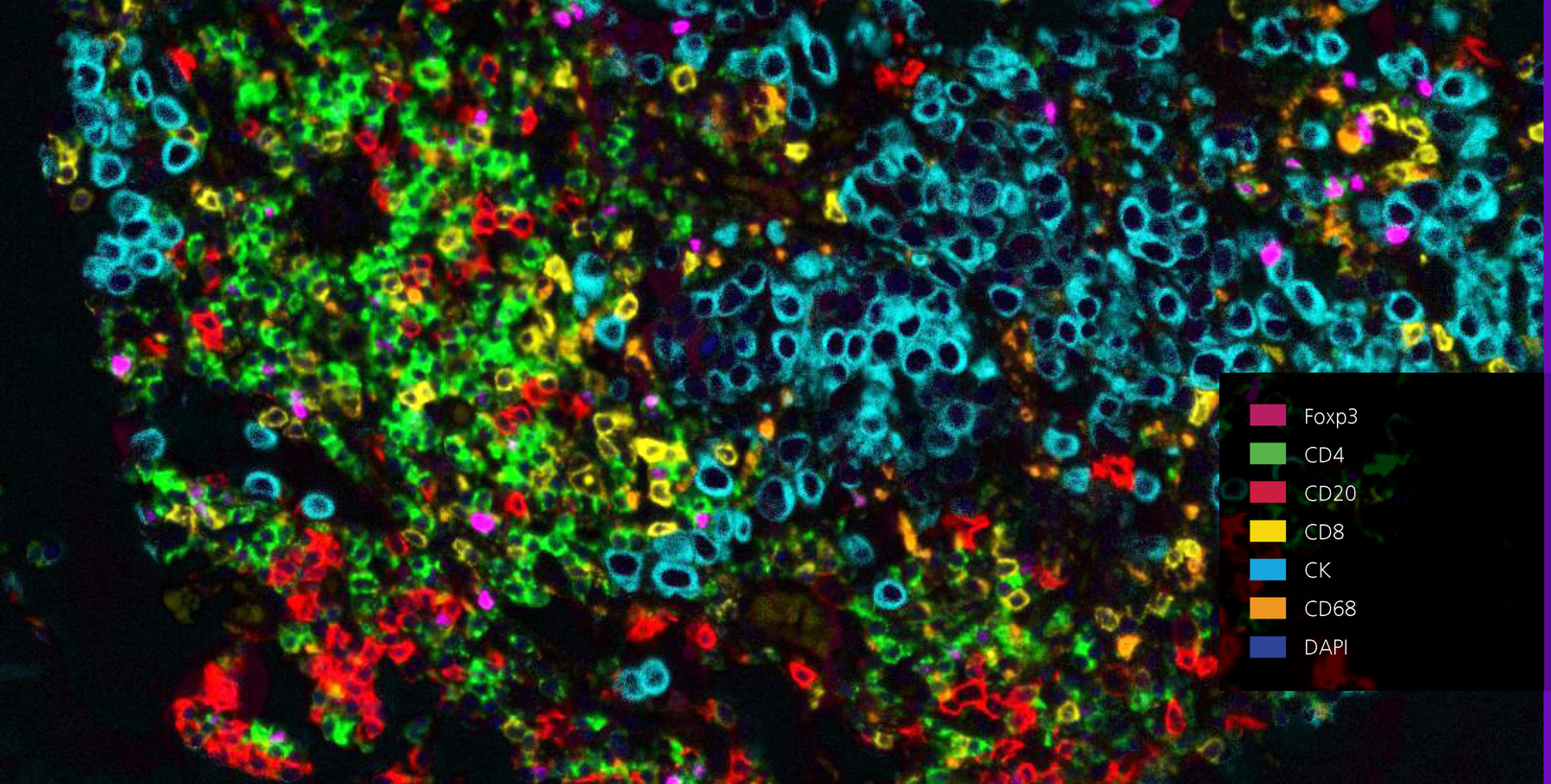
Dr. Tumeh, a rising star in the cancer immunology field, focuses on the identification of immune cell-types that mediate tumor rejections during PD-1 by identifying niches (i.e. discrete cellular microenvironments) within tumors that drive or inhibit response to PD-1/PD-L1- blocking therapies. He is using Akoya Biosciences' Phenoptics platform for research into identifying stratification biomarkers with the potential to indicate likelihood of: a) response, b) response but after the immune system is activated to engage with the tumor thus 'priming' the tumor or c) lack of response even after 'priming'.

Paul Tumeh, MD
Assistant Professor in the Department of Medicine
UCLA

RESEARCH EXAMPLE

Melanoma

Dr. Rimm, pioneer of quantitative immunohistochemistry and inventor of AQUA® technology, focuses on predicting response to therapy in breast cancer and predicting recurrence or metastasis in melanoma and lung cancer. Dr. Rimm is working closely with Akoya Biosciences to bring rigor to the development of Phenoptic research to make it a truly quantitative approach, by helping to establish consistent Opal staining methods and expression measurement controls. He is particularly interested in exploring the per-cell and spatial parameters enabled by Phenoptics. The first project evaluated TIL status in a lung cancer TMA.



David L. Rimm, MD, PhD
Professor of Pathology and of Medicine (Medical Oncology); Director of Pathology Tissue Services; Director of Translational Pathology
Yale University

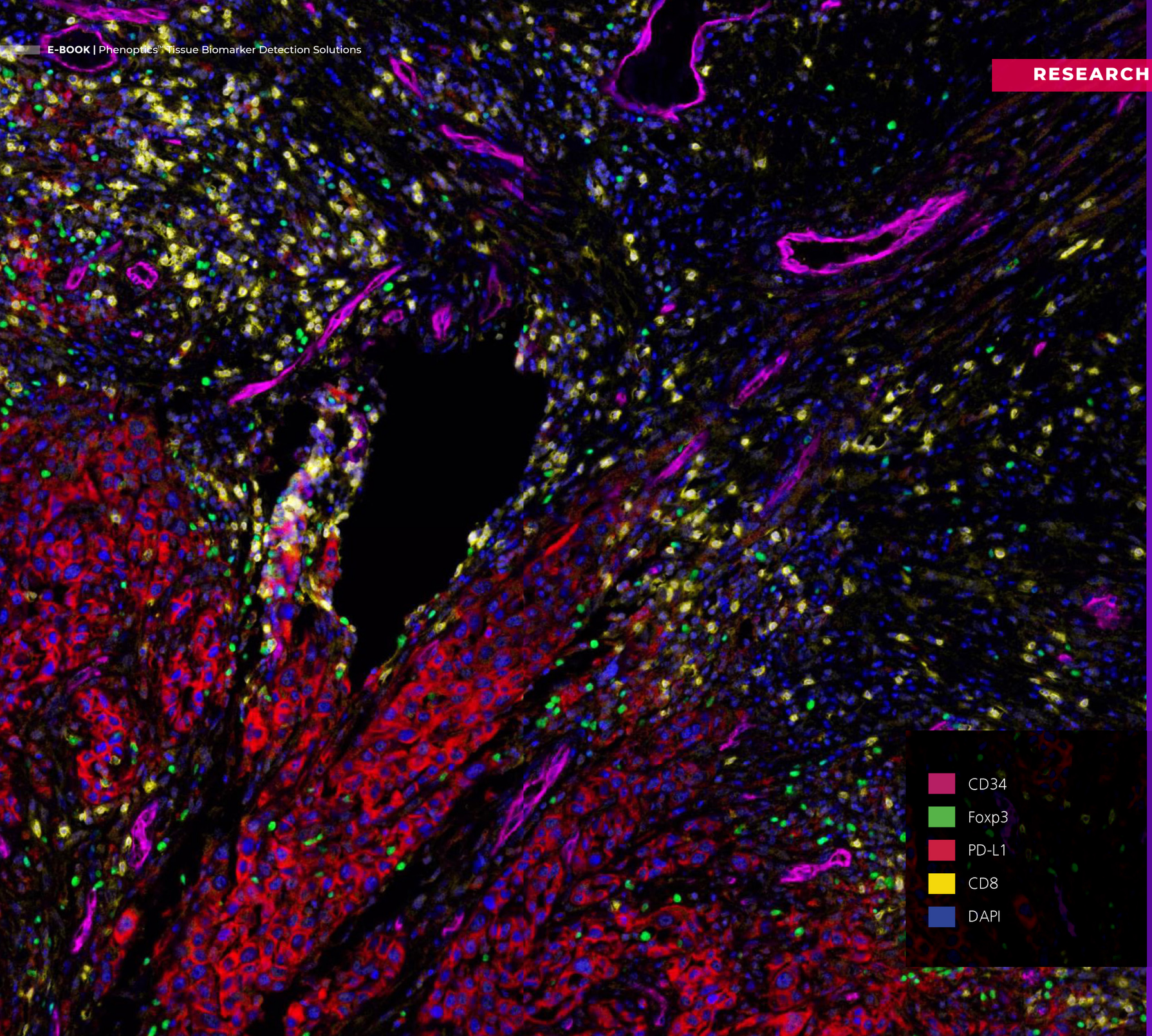
RESEARCH EXAMPLE

Lymphoma

Dr. Rodig has had a long interest in untangling the complex interactions between Hodgkin's tumors and their associated stroma. With the Phenoptics platform, Dr. Rodig is able to pursue a two-pronged approach to his research, gaining insights into tumor pathogenesis while developing novel tissue biomarkers.

- CD30
- PD-L1
- CD68
- pSTAT3
- DAPI

Dr. Scott Rodig, MD
Hematological Pathologist
Brigham and Women's Hospital

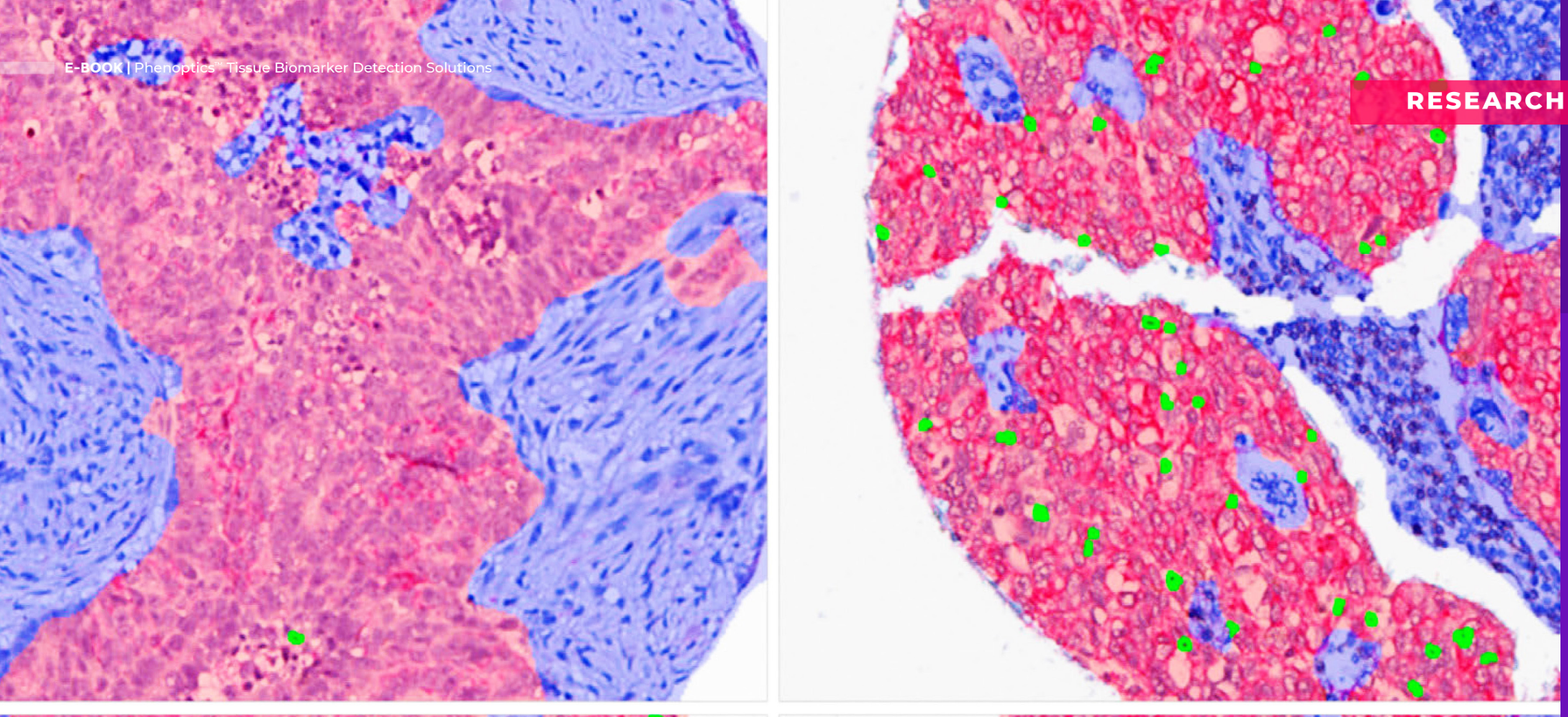


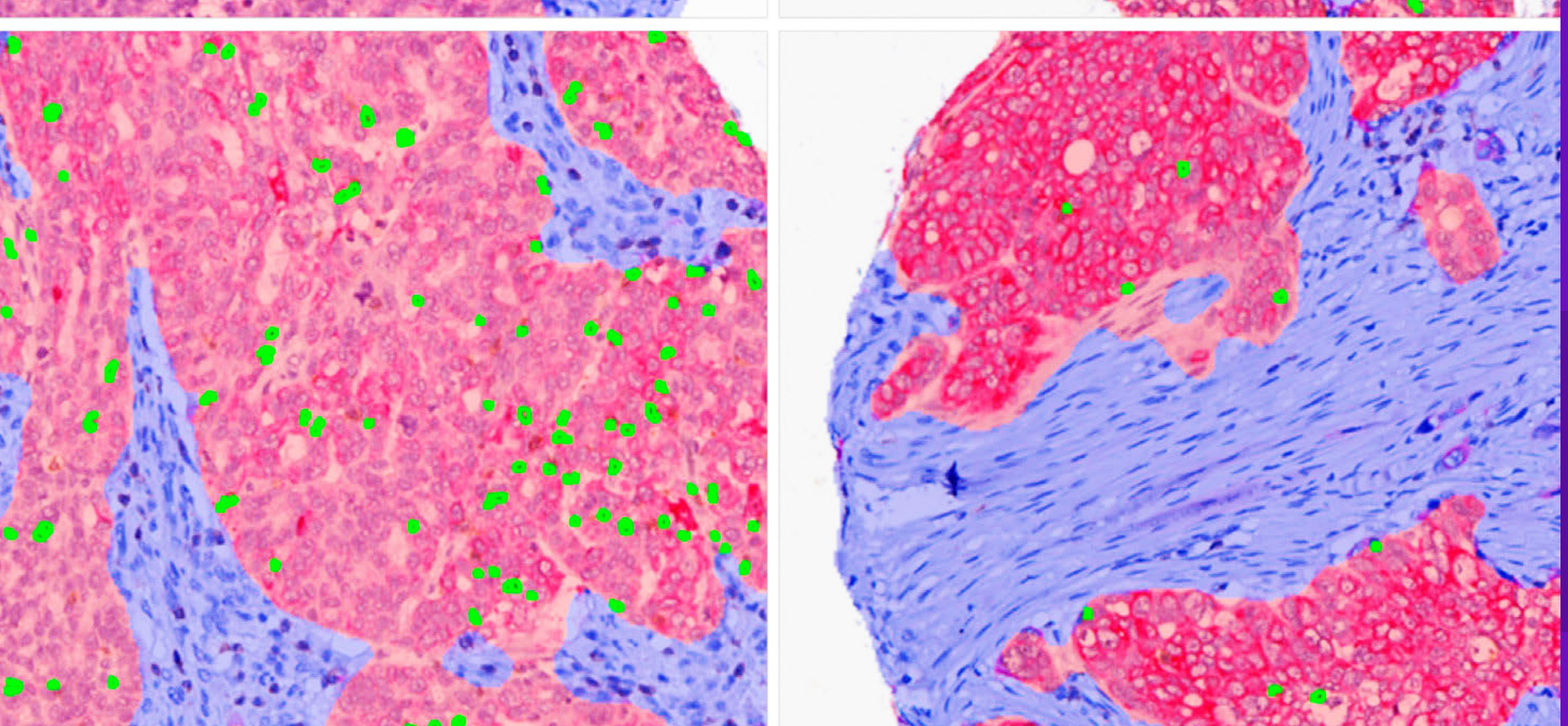
RESEARCH EXAMPLE

Squamous Cell Carcinoma

An acknowledged pioneer and vocal advocate in the rapidly emerging cancer immunotherapy space, Dr. Fox was one of the first to recognize the fit between Akoya Biosciences Phenoptics and analytical needs in cancer immunology research. Today, he is focusing on developing predictive biomarkers for squamous cell carcinoma of the head-and-neck (SCCHN) cancer, and for melanoma. His first study focused on PD-L1 associated down regulation of immune response in SCCHN. He was also the first to publish results of an immune signature biomarker based on a full 6-plex, 7- color Opal assay showing predictive power for identifying melanomas that contained functional tumor-infiltrating lymphocytes (TIL).

Bernie Fox, PhD
Chief, Laboratory of Molecular and Tumor Immunology, Robert W. Franz
Cancer Research Center in the Earle A. Chiles
Research Institute at Providence Cancer Center





RESEARCH EXAMPLE

Ovarian Cancer

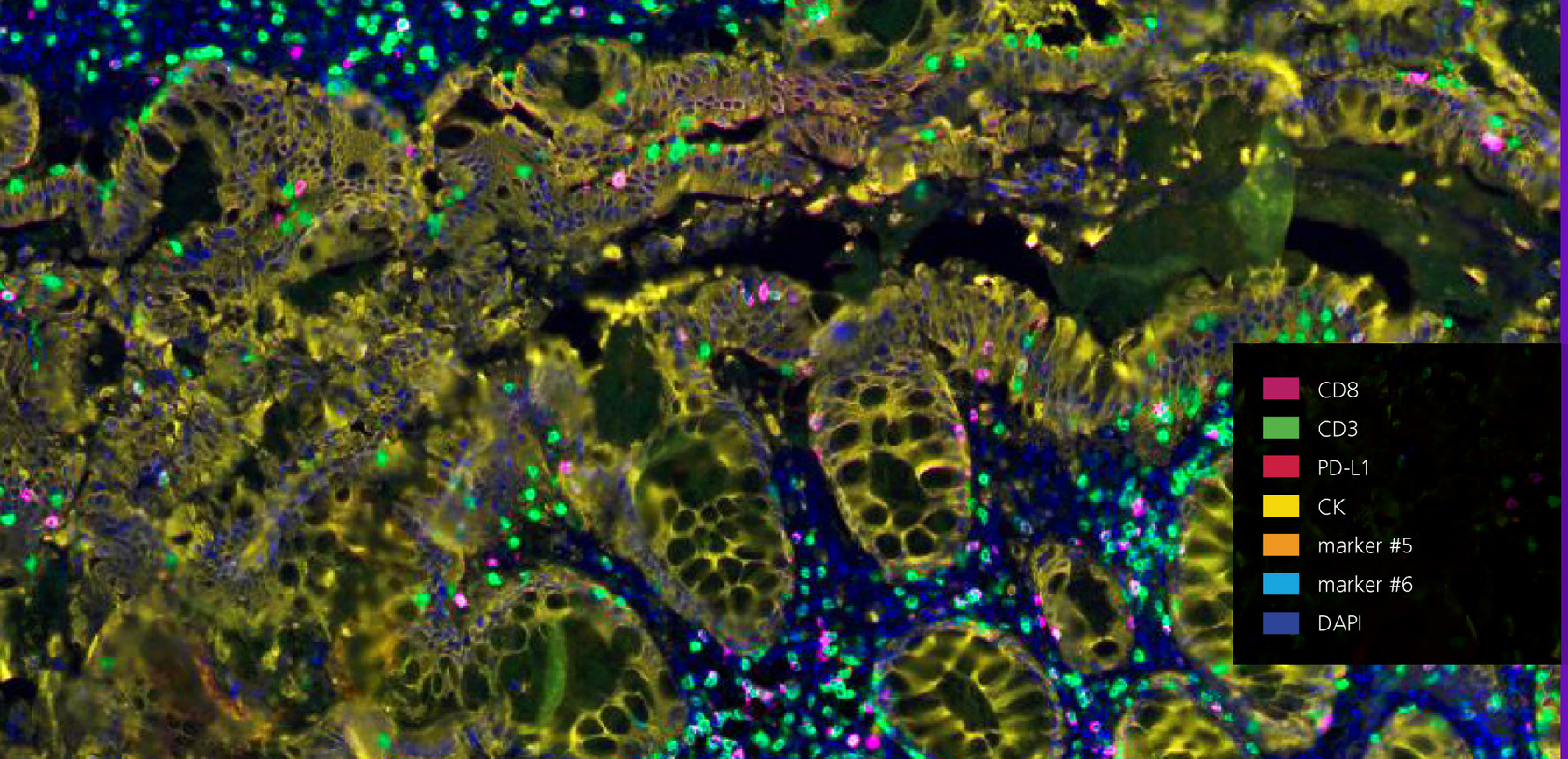
Dr. Feldman, a long-time thought leader in the drive to modernize pathology, has been actively integrating image analysis, algorithms, digital imaging technology and informatics to address the shortcoming of conventional IHC and visual perception. He was an early adopter of Phenoptics technology, and today is focusing on merging quantitative pathology approaches with digital pathology platforms, workflows and laboratory information systems. In this study, Dr. Feldman used Phenoptics imaging capabilities to automate the challenging visual task of assessing TIL counts on conventional 3-color chromogenic IHC.

Michael Feldman, MD, PhD
Associate Professor of Pathology and Laboratory Medicine
Hospital of the University of Pennsylvania

RESEARCH EXAMPLE

Colorectal Cancer

Dr. Galon's research lab aims to understand tumor progression and immune reaction against cancer by using integrative biology and bioinformatics to improve the therapeutic management of cancer patients. He hypothesizes that integrative biology approaches will provide a better knowledge of the local interplay between the immune components and the tumor cells. Dr. Galon is known for having discovered the major importance of the preexisting immunity. He pioneered and developed the 'Immunoscore' for prognostic assessment of colorectal cancer. This was the first clear demonstration of the power of automation and cancerimmunology to stratify patients according to immune status and likelihood of progression. He is now using Phenoptics to go beyond Immunoscore®, to explore higher multiplexed biomarkers and reveal deeper details about how cancer evades the immune system. The expectation is that the richer detail afforded by Phenoptics will lead to new drugable targets and better predictive tests for checkpoint inhibitors.



Jerome Galon, PhD
Director of Research at INSERM
Chief, Laboratory of Integrated Cancer Immunology
Paris, France

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