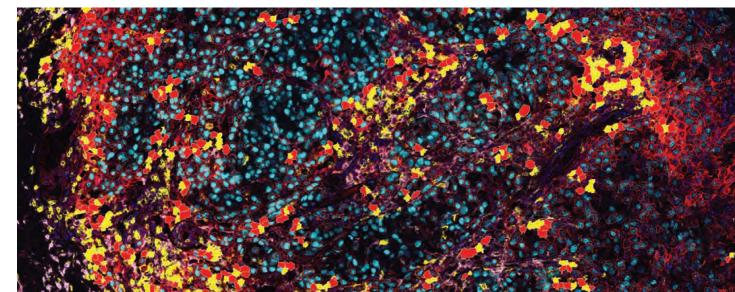
Developing effective cancer immunotherapies - treatments that might harness the body's own immune system to fight cancer - could potentially deliver what the oncology research community has sought for decades - a cure. And to advance this research, it's critical that you see everything a tumor has to show you. This is because better visualization and identification of biomarkers and immune checkpoints lead to improved understanding of the underlying biology that lets cancer thrive.

That's what makes our Phenoptics instrumentation and workflow such a powerful solution. The Phenoptics workflow - multiplex immunohistochemistry staining solutions, multispectral imaging systems, and advanced image-analysis software - enables a more comprehensive and specific view and analysis of the interaction between tumor and the immune system, at a cellular level, in a single tissue section.

Better quantification of cancer-immune interactions may reveal which disease mechanisms are in play, and help researchers discover biomarkers that may eventually lead to better subpopulation stratification methodologies.

Simply put, that means a better understanding of biology that drives disease. And isn't that the goal we're all striving for?

PHENOPTICS RESEARCH SOLUTIONS



Phenotyping of immune cells and cancer cells within the context of the tumor enables advanced analytics like distance mapping.

PHENOPTICS RESEARCH SERVICES

We take understanding to the next level

Want to test the Phenoptics workflow before bringing the capability in house? The Akoya Research Services team can perform multiplexed IHC for up to seven colors (six markers plus DAPI) to help you get results. Our Opal staining method allows all antibodies in a seven-color panel to be selected based on performance and specificity, without consideration of species of origin. Multispectral imaging provides quantitative results regardless of spectral and spatial overlap among fluorescence markers, enabling tissue segmentation, cellular phenotyping, protein expression, and spatial

We follow a detailed staining protocol when working with your precious samples: Antibody specificity is first confirmed in single-plex with positive controls. Then the multiplex panel is tested with the same positive controls, with study samples that you provide - so you're



Phenoptics Research Services Laboratory

sure the protocol works for you. Then we agree on staining performance levels, including confirmation of multiplex staining independence and non-interference. Analysis begins with multispectral imaging, providing quantitative spectral unmixing of each fluorophore signal and tissue autofluorescence, followed by tissue segmentation and cell phenotyping. This complete workflow enables new depths of understanding not achievable with standard chromogenic monoplex or duplex IHC methods.

















PHENOPTICS RESEARCH SOLUTIONS

UNDERSTANDING STARTS RIGHT HERE

Built around Mantra™ and Vectra® imaging instruments, Opal™ Multiplex IHC kits, and inForm® image analysis software, the Phenoptics workflow enables you to quickly visualize and identify cell types in the context of the tumor, measure expression levels of key proteins related to immune evasion, determine cellular functional and activation states, and discover and confirm immune evasion mechanisms. Want a workflow that enables a better understanding of the complex interplay between the immune system and cancer? **Let's get started...**

1. IMMUNOSTAIN

THE SEVEN COLORS OF DISCOVERY

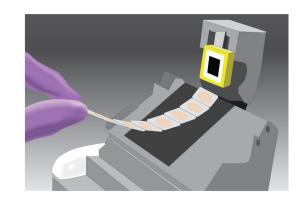
Opal Multiplex IHC kits make multiplex results accessible to anyone who works with standard immunohistochemistry, permitting enhanced visualization and understanding of complex cellular interactions. With Opal, you can select antibodies for simultaneous IHC detection based on performance rather than species. And Opal kits are optimized for reliable spectral unmixing and simultaneous measurement of three to six IHC targets, plus a nuclear stain

Opal enables you to:

- · Measure three to six tissue biomarkers simultaneously
- Use the best primary antibodies, regardless of species with no cross-reactivity
- Identify multiple cell phenotypes while retaining spatial and morphological context that is lost with bulk measurements and flow cytometry
- · Get more information from precious and scarce samples

With our **Opal Automation IHC kits** you can now perform Opal multiplex staining on one of the leading research automated staining platforms – the BOND RX by Leica Biosystems. Automation provides you with the flexibility to support the dynamic demands of translational research.

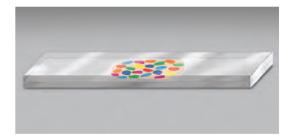
- · Quality, consistency, and reproducibility with every sample
- High throughput protocol perform 7-color immunofluorescence staining on 30-slides in 14 hours
- Simplified, walk-away protocol versus laborius manual process



Opal IHC works with FFPE tissue and is compatible with standard IHC workflows. $\label{eq:compatible} % \begin{center} \end{constraint} % \begin{center} \end{center} % \begin{center} \$



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.

2. IMAGE

REVEAL COMPLEX BIOLOGY IN A SINGLE TISSUE SECTION

 For a deeper understanding of disease, you need faster, better visualization and identification of disease biomarkers. And quickening the pace of that understanding is the whole idea behind **Mantra and Vectra imaging instruments**. These systems enable multiplexed immune-cell and expressionprofiling assays, for tissue-segmentation and immune-cell phenotyping within the tumor and its microenvironment in FFPE tissues and TMAs.

Mantra Quantitative Pathology Workstation

- Compact, easy-to-use workstation with an intuitive manual workflow optimized for the research pathologist
- Detects and measures multiple expressed and overlapping biomarkers within a single IHC or IF tissue section
- Pathology Views[™], feature of inForm image analysis software, displays fluorescence imagery in traditional brightfield mode
- · (H&E, DAB)

Vectra 3 Automated Quantitative Pathology Imaging System

- Fully automated, high-throughput imaging system with six or 200 slide configuration
- Navigate around slides to identify areas of interest for highresolution multispectral acquisition with the Phenochart[™] whole-slide viewer
- Detect and measure multiple weakly expressed and overlapping biomarkers within a single H&E, IHC or IF tissue section and in TMAs
- Automatic identification of specific tissue types using integrated inForm analysis software
- · Vectra Polaris Automated Quantitative Pathology

Imaging System

- Fully automated, high throughput multispectral imaging system combined with true brightfield and fluorescent whole slide scanning functionality
- Fast and efficient digital whole slide scanning speeds, comparable to standalone systems
- On-the-fly, continuous slide loading allows unlimited throughput
- Flexible data analysis, compatible with many image analysis software platforms



Mantra Quantitative Pathology Workstation



Vectra 3 Automated Quantitative Pathology Imaging System



Vectra Polaris Automated Quantitative Pathology Imaging System

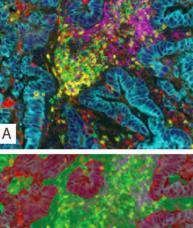
3. ANALYZE AND UNDERSTAND

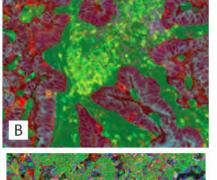
DISCOVERY COMES WITH SEEING CELL-TO-CELL INTERACTIONS

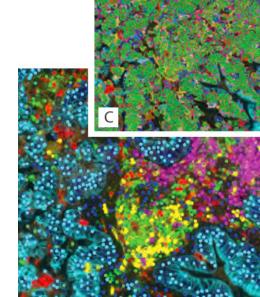
Our patented automated inForm® image analysis software allows you to accurately visualize, analyze, and quantify biomarkers in tissue sections. It enables accurate percell quantification of specific biomarkers defined within multiple tissue contexts, and the phenotyping of immune and other cells in situ in solid tissue. What's more, it enables the quantitation of weakly expressing and overlapping biomarkers within cells and cellular compartments that can't be identified by the naked eye. These sensitive approaches give you the confidence to discover indicators of disease and uncover relationships between specific cell types, and between the immune system and the tumor.

The software:

- Pathology Views™ feature renders immunofluorescence (IF) images as simulated H&E or DAB and hematoxylin, to provide views more familar to you
- · Separates weakly expressing and overlapping markers
- Enables cellular analysis of H&E, IHC, and IF in FFPE tissue sections
- Automatically classifies cell phenotypes using machine learning algorithms
- inForm Tissue Finder™ automates the detection and segmentation of specific tissues through patented pattern recognition algorithms
- inForm Cell Analysis[™] enables quantitative
- · per cell analysis of IHC, IF, and RNA-ISH staining







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

AKOYABIO.COM **3**AKOYABIO.COM **5**