Quantitative Pathology Imaging and Analysis

KEY BENEFITS:

- Part of Akoya Biosciences' Phenoptics™ workflow solution for Cancer Immunology Research
- Detect and measure multiple expressed and overlapping biomarkers within a single IHC or IF tissue section or TMA
- Visualize, analyze, quantify and phenotype immune cells in situ insolid tissues
- Obtain context to identify regions of interest within a digital workflow using Phenochart™ whole slide viewer
- Automatic identification of specifictissue types using integrated inForm® analysis software

ACCELERATING THE PACE OF UNDERSTANDING

The fully automated Vectra® 3 6-slide pathology imaging system is a state-of-the-art instrument for extracting proteomic and morphometric information from intact FFPE tissue sections or TMAs. Vectra 3 and inForm software analysis combine the power of multiplexed biomarker imaging and quantitative analysis all within a familiar digital workflow to accelerate cancer immunology research.

The Phenochart whole slide viewer with annotation capability can be combined into the digital workflow, enabling you to navigate around slides and identify areas of interest for high power acquisition. The system features automated slide handling, multispectral imaging technology, and unique pattern-recognition-based image analysis. It accurately measures protein and biomarker expression and morphometric characteristics in distinct tissue regions of interest or on whole slides for translational studies.

Example Applications:

- Phenotyping immune cells for cancer immunology research
- Transduction signaling pathway activity (pERK, pS6,
- P13K/mTOR, MAPK, or EGFR)
- Apoptosis and/or proliferation assays
- Necrosis and fibrosis using conventional stains
- Cell cycle characterization

Phenoptics™ Solutions

- Opal™ Multiplex IHC Kits
- Vectra® Polaris™, Vectra® 3 and Mantra™ Quantitative Pathology Imaging Systems
- inForm® Image Analysis Software
- Phenoptics Research Services

- DNA damage determination
- Inflammation
- Lymph node metastasis

Phenoptics solution for Phenotyping Immune Cells *in situ* using Vectra 3

The Vectra 3 automated quantitative pathology imaging system is a part of Akoya Biosciences' Phenoptics research solution (Figure 1) for cancer immunology and immunotherapy research, which includes multiplexed staining reagents and methodologies, instrumentation with unique channel-separation technology, and advanced image analysis software that can be trained to phenotype cells and measure checkpoint, activation and functional markers between cells and within the tumor microenvironment.

Discover New Phenotypes

It's now possible to phenotype cells and extract per-cell multiparameter data in FFPE leveraging expression and contextual, spatial information, enabling new biomarkers to be discovered and validated. These biomarkers could lead to the development of tests to

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assess safety in pre-clinical studies and potentially to predict efficacy of therapies. Stain Multiplex Biomarker Detection in Intact Tissue Sections can be labeled with conventional stains such as H&E and trichrome, or labeled with either immunohistochemical (IHC) or immunofluorescent (IF) dyes, such as Akoya Biosciences' Opal™ multiplex reagent kits. With IHC or IF, single or multiple proteins can be measured on a per-tissue, per-cell, and per-cell-compartment basis, even if those signals are spectrally similar, are in the same cell compartment, or are obscured by autofluorescence. Using Opal (Figure 2) enables the specific staining of multiple tissue biomarkers, reaching up to 6-plex in a single tissue section. It provides you with a valuable tool to simultaneously interrogate multiple pathways while retaining the context provided by tissue sections. This approach provides information that is not available from alternative techniques such as the analysis of single markers in serial sections. The method is compatible with the standard IHC workflow in your lab and is amenable to automation. Opal reagents are available individually or in 4- and 7-color kits, for automation or manual use with easy-to-follow protocols for FFPE tissue.

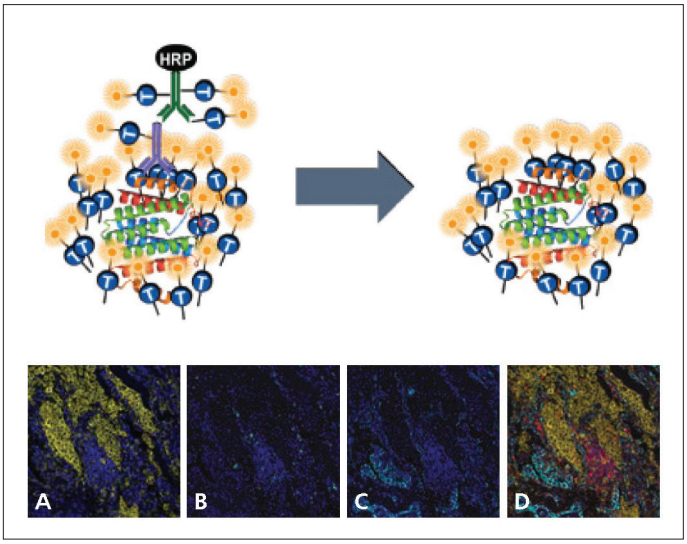


FIGURE 2. Opal schematic (top). Breast cancer tissue stained using Opal reagent kits (bottom). Blockade marker 1 overlaid on DAPI (A). CD8 signal overlaid on DAPI (B). CK signal overlaid on DAPI (C) and composite image of all markers overlaid onto DAPI (D). Additional markers were used, FOXP3, and blockade 2 and 3 (data not shown).

IMAGE AND VISUALIZE

Reveal Multiple Tissue Biomarkers in Context

For cancer immunology researchers who are seeking to understand the role of immune cells within solid tumors and their microenvironment, the Vectra 3 automated multiplexed biomarker imaging system visualizes, analyzes, quantifies and phenotypes immune cells in situ in FFPE tissue sections and TMAs. Unlike alternative instrumentation, which are limited by the number of colors that can be imaged on one slide, Vectra 3 can separate up to 7 colors, allowing the quantification of multiple biomarkers, revealing spatial context, all within a digital workflow.

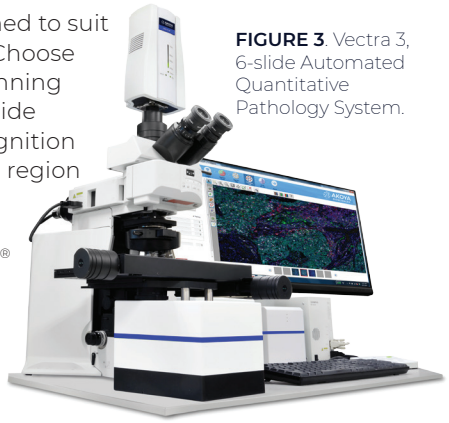


FIGURE 3. Vectra 3, 6-slide Automated Quantitative Pathology System.

Vectra 3 can be programmed to suit your study requirements. Choose from a flexible array of scanning options, including whole slide imaging and pattern-recognition guided multispectral (MSI) region selection and acquisition. Vectra 3 is powered by Akoya Biosciences' inForm® Tissue Finder™ software, which includes tissue segmentation algorithms. The software can be trained through a simple learn-by- example interface, teaching the system to locate tissue regions automatically. Often only a handful of representative images are required for training. Vectra 3 workflows (Figure 5), include scanning a whole slide, viewing and reviewing it in Phenochart, and annotating regions for high power multispectral acquisition (Figure 6).

Additional Features of the Vectra 3 System Include:

- 10x whole slide imaging and scanner-like intuitive workflow in brightfield and fluorescence provides context for higher-powerquantitative multispectral imaging regions
- Phenochart whole slide viewer with annotation capability can be installed on multiple computers – regions can be manually or automatically selected using Vectra 3 software or marked for review by a pathologist
- TMA core detection – select cores for high-resolution MSI acquisition
- inForm® Pathology Views™ for visualization of fluorescence imagery in traditional brightfield mode (H&E, DAB and hematoxylin)
- Two seats of inForm Tissue Finder software for automatically identifying cell phenotypes in different tissue sections
- Reduce auto fluorescence through powerful multispectral imaging
- Export to additional software packages, e.g. Indica Labs HALO™whole slide image analysis software

ANALYZE

Measure and Quantify Multiple Biomarkers *in situ*

Obtain per-cell and per-cell-compartment multiparameter data, and accurately and reliably separate multiple biomarker signals from one another and from autofluorescence in multiplexed assays. Use this rich data to generate tissue-based analysis that retains the architectural context of the intact tissue.

inForm Tissue Finder automates the detection and segmentation of specific tissue types using patented user-trainable algorithms that can recognize morphological patterns. It includes per-cell phenotyping functionality to differentiate marked cell types (e.g. T-cells, macrophages) within a segmented area (e.g. tumor versus stroma) (Figure 7).

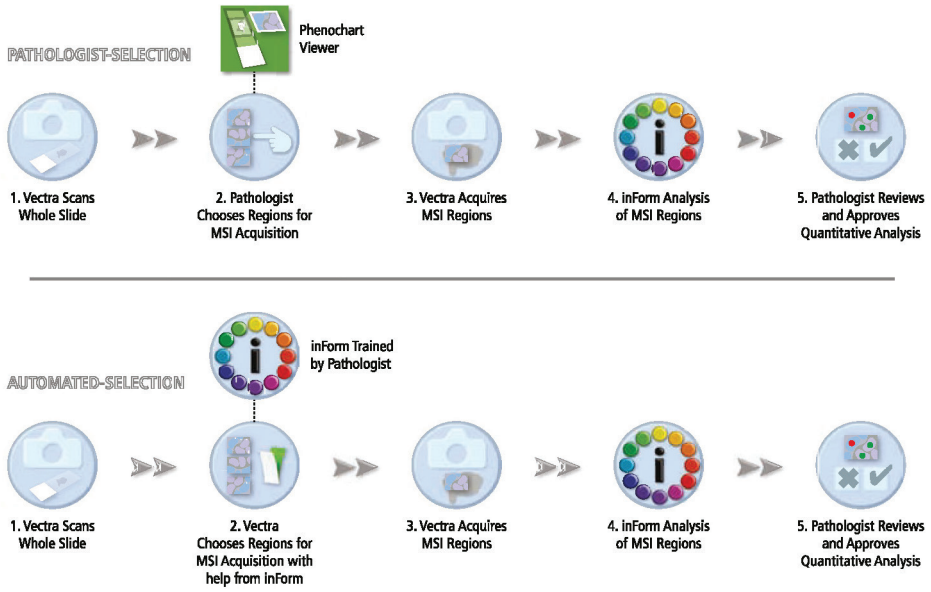


FIGURE 4. Vectra 3 features an intuitive yet flexible workflow that allows the pathologist to view a whole-slide contextual scan and select regions of interest for multispectral acquisition: shown here in the Pathologist-Selection workflow (top). Another option is to enable selection and acquisition of multispectral regions by Vectra 3 with help from inForm: shown here in the Automated-Selection workflow (bottom). inForm can be trained by the pathologist to recognize regions of interest and the algorithm stored for use in selecting regions that meet the desired criteria. In all scenarios, the pathologist can review and ultimately approve the results of the quantitative analysis. The benefit to automated selection is consistency and much greater throughput while retaining the option for review at each step by the pathologist.

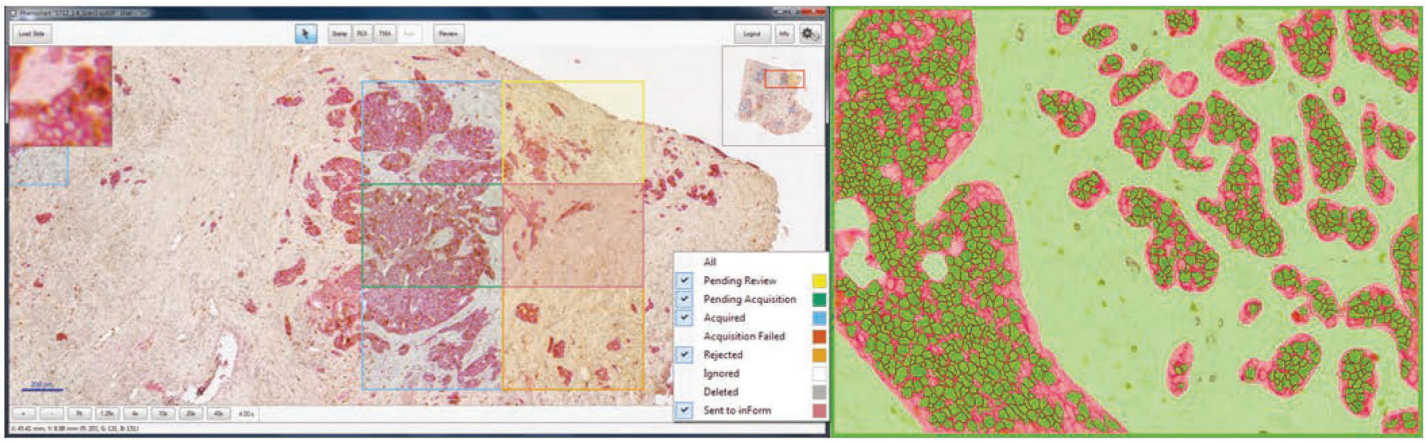


FIGURE 5. Left is the Phenochart whole slide viewer showing annotations pending review by a pathologist (yellow), pending acquisition (green), acquired (blue), rejected (orange), and sent to inForm for processing (purple). Right is an MSI region that was acquired by Vectra 3 and then opened in inForm for cell (green) and tissue segmentation (tumor in red and stroma in light green). The segmentation is overlaid onto the tissue image and can be toggled individually on or off.

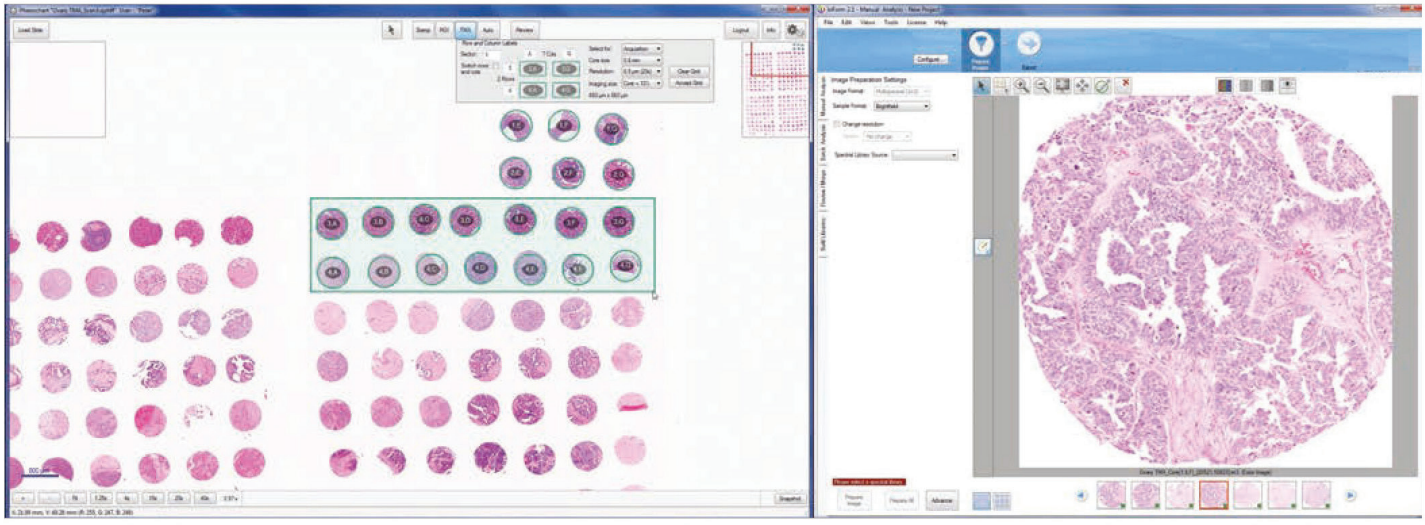


FIGURE 6. The Phenochart whole slide viewer not only enables you to browse through and view whole-slide scans, but also TMAs, H&E brightfield ovary shown here.

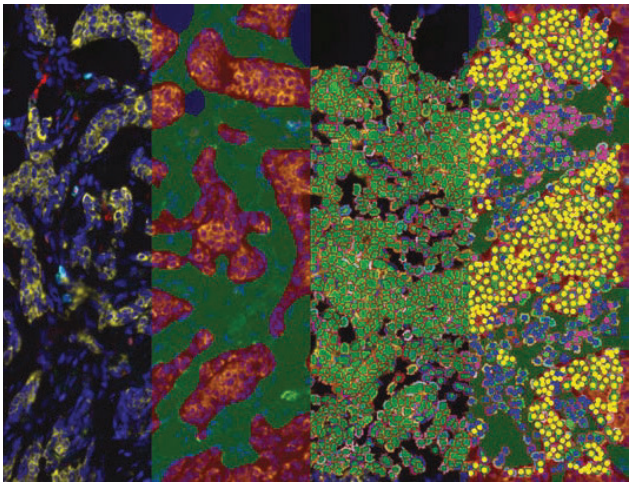


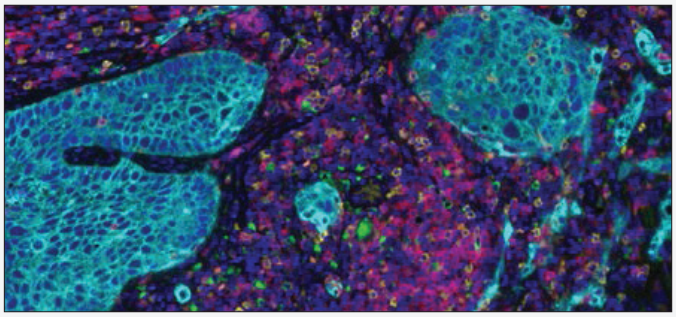
FIGURE 7. Breast cancer tissue labeled with the Opal 7-Color Multiplexed IHC Kit. Left to right: spectrally unmixed composite image with nuclei (Spectral DAPI, blue), PDL1 (Opal 520, green), PD1 (Opal 540, cyan), CD8 (Opal 570, magenta), FoxP3 (Opal 620, orange), CD68 (Opal 650, red), and cytokeratin (Opal 690, yellow); tissue-segmented image with tumor (red) and stroma (green); cell-segmented image (green); and overlaid phenotyping of each cell with CD68+ (red), CD8+/FoxP3- (magenta), CD8+/FoxP3+ (green), FoxP3+/CD8- (orange), CK+ tumor cells (yellow), and other cells (blue).

Features of inForm Tissue Finder Software:

- User-trained feature recognition algorithms enable automatic identification of specific tissue types based on tissue morphology
- Identify individual cell types across an entire tissue section, in the stroma and tumor, for accurate phenotyping and spatial analysis
- Quantitative per-cell analysis of biomarker expression in tissue sections and TMAs
- Separation of weakly expressing and overlapping markers
- Cellular analysis of H&E, IHC and immunofluorescence in FFPE tissues
- Per-cell phenotyping to differentiate cell types
- Pathology Views: creates brightfield pathology (H&E, DAB and hematoxylin) renderings from the original fluorescence image
- Push acquired images from Vectra 3 to inForm in order to develop algorithms for automation

Akoya Biosciences' digital slide data (.qptiff) can also be exported into Indica HALO™ software which enables analysis of whole slide imagery. Users can select analysis modules from an extensive list based on their analysis needs and add additional modules as required at a later date. Indica's image analysis portfolio includes solutions for oncology, neuroscience, metabolism and toxicologic pathology. In addition, inForm unmixed images (inForm 2.2 or later) and unmixed MSI fields (brightfield or fluorescence) can be analyzed using the HALO™ High-Plex analysis module. Individual workstation and group perpetual licenses are available.

Akoya's Phenoptics Solutions for Quantitative Pathology Imaging and Analysis

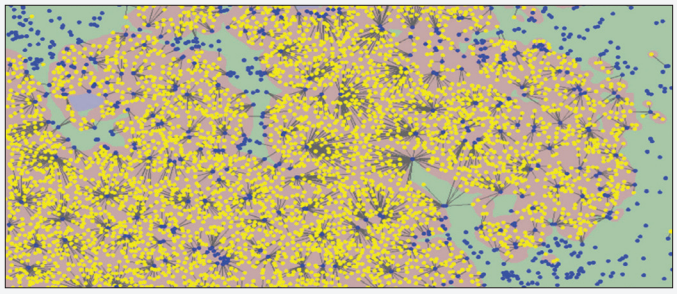


Stain

The Opal™ method is a practical approach for specific staining of multiple tissue biomarkers up to 9 colors in a single tissue section.

Image

The Vectra Polaris, Vectra® 3 and Mantra Quantitative Pathology Imaging Systems enable simultaneous quantitative measurement of multiple biomarkers within a single FFPE tissue section.



Analyze

Analysis, quantification, visualization , phenotyping and spatial analysis of cells *in situ* enabled by inForm and phenoptr tissue analysis software.

Research Services

Don't have the time or resources to do the work yourself? Our in-house experts can perform multiplexed staining, imaging and analysis to move your research to the next stage.

VECTRA 3 SPECIFICATIONS

Tissue formats	Tissue microarrays (TMAs) and tissue sections
Spectral range	440 – 720 nm (tunable)
Modality	Brightfield and Fluorescence (multispectral or color)
Scan time (1.5 cm x 1.5 cm sample)	5-band Fluorescence, 10x 8.0 minutes 5-band Fluorescence, 4x 2.5 minutes Brightfield, 10x 3.5 minutes Brightfield, 4x 0.8 minutes
Throughput – total time (1.5 cm x 1.5 cm sample, scan plus 10 MSI fields at 20x)	5-band Fluorescence, 10x 18 minutes 5-band Fluorescence, 4x 12 minutes Brightfield, 10x 10 minutes
Throughput for TMAs	5 cores/minute (Brightfield, 0.6 mm core +10%)
Multiplexing capability	Separates up to 7 colors even if overlapping
Image Analysis Software (inForm)	Intuitive learn-by-example interface to automatically segment and quantitate tissue structures, cells and sub-cellular signatures
Resolution	4x (2.5 µm/pixel), 10x (1 µm/pixel) and 20x (0.5 µm/pixel)
File format	Akoya Biosciences' whole slide scan image (qptiff), multispectral images (.im3), color images (JPEG, BMP, PNG)
Operating system	Microsoft® Windows® 7 64-bit
Computer	Dual Quad-Core processors with 1 Terabyte for data storage

Note: Product specifications subject to change. Refer to Akoya Biosciences' website for current information.

OPAL MULTIPLEX IHC DETECTION KITS

	Sizes	Product #
Opal 4-color Automation IHC Kit	50 slides	NEL820001KIT
Opal 4-color Manual IHC Kit	50 slides	NEL810001KT
Opal 7-color Automation IHC Kit*	50 slides	NEL821001KT
Opal 7-color Manual IHC Kit*	50 slides	NEL811001KT

The Automation Detection Kits include Opal fluorophores, DAPI, 1X Plus Amplification Diluent, 1X Antibody Diluent, and Opal Polymer HRP Ms+RB detection reagent. The Manual Detection Kits add an additional AR6 buffer. *Opal 7-color Automated and Manual Kits require multispectral imaging.

OPAL CANCER IMMUNOLOGY IHC PANELS

	Sizes	Product #
Opal 7 Tumor Infiltrating Lymphocyte Kit* (CD4, CD8, CD20, FOXP3, CD45RO, panCK)	50 slides	OP7TL3001KT
Opal 7 Solid Tumor Immunology Kit* (CD4, CD8, CD20, FOXP3, CD68, panCK)	50 slides	OP7TL4001KT
Opal 7 Immunology Discovery Kit* (CD4, CD8, CD68, +3 open channels)	50 slides	OP7DS2001KIT
Opal 4 Lymphocyte Kit (CD4, CD8, CD20)	50 slides	OP4LY2001KIT

Opal IHC Panel kits include primary antibodies (human reactivity), Opal Polymer HRP Ms + Rb, Antibody Diluent, Opal fluorophores, DAPI, AR6 and AR9 buffer. *Opal 7-color kits require multispectral imaging.

To learn more visit **AKOYABIO.COM**
or email us at **INFO@AKOYABIO.COM**

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