

Quantitative Pathology Imaging and Analysis



Key Features:

- Visualizes, analyzes, quantifies and phenotypes immune and other cells *in situ* in solid tissue
- Pathology Views™ renders immunofluorescence images as H&E, DAB and hematoxylin
- Separates weakly expressing and overlapping markers
- Cellular analysis of H&E, IHC and immunofluorescence in FFPE tissue sections
- Automatic identification of specific cell and tissue compartment types using trainable feature-recognition algorithms

Quantify Biomarkers in Tissue Sections

Introduction

Extracting meaningful information from intact tissue is becoming ever more critical as researchers look for better ways to characterize molecular activity of cells within their native microenvironments. In life science research and pharmaceutical drug development, progress often depends on it. In translational oncology, understanding cancer in its microenvironment is central to identifying disease subtype, validating targets, selecting patients for trials, characterizing efficacy and response, and making correlations with clinical outcome.

inForm® is a patented automated image analysis software package for accurately visualizing and quantifying biomarkers in tissue sections. Two standalone versions of inForm image analysis software are available, inForm Cell Analysis™ and inForm Tissue Finder™. inForm Cell Analysis enables per-cell analysis of immunohistochemistry (IHC) and immunofluorescence samples, allowing the separation and measurement of weak and spectrally overlapping markers in single and multiplex assays. inForm Tissue Finder automates the detection and segmentation of specific tissue types using patented user-trainable algorithms developed to recognize morphological patterns.

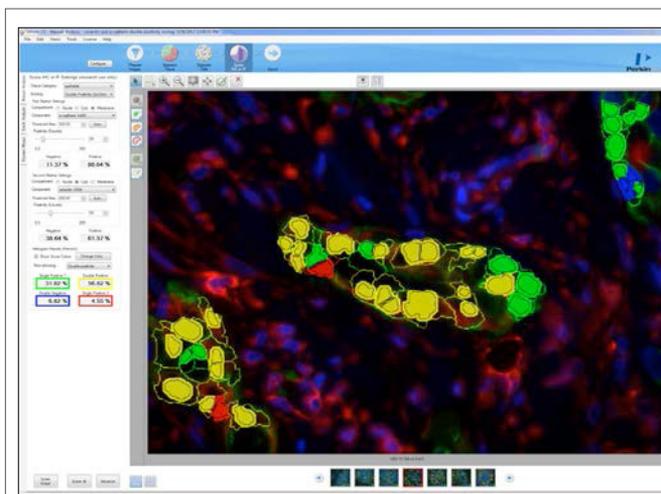
inForm Advanced Analysis Software

inForm Cell Analysis and inForm Tissue Finder are designed to meet the workflow and throughput needs of research pathology, oncology, immunology, enabling researchers to make faster discoveries.

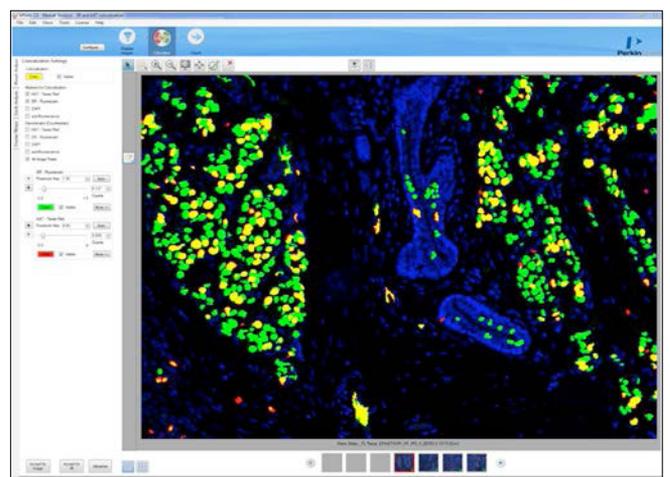
inForm Cell Analysis enables the user to define the biology of interest within a tissue section. It phenotypes cells based on their biomarker expression within cells, nuclei and membranes.

inForm Tissue Finder adds exceptional functionality to inForm Cell Analysis. It automates the detection and segmentation of specific tissue compartments through powerful patented pattern-recognition algorithms. Automation provides consistent reproducible results and enables comparative studies of both multiple markers and multiple specimens. Cell analysis results can then be quantified in each tissue compartment.

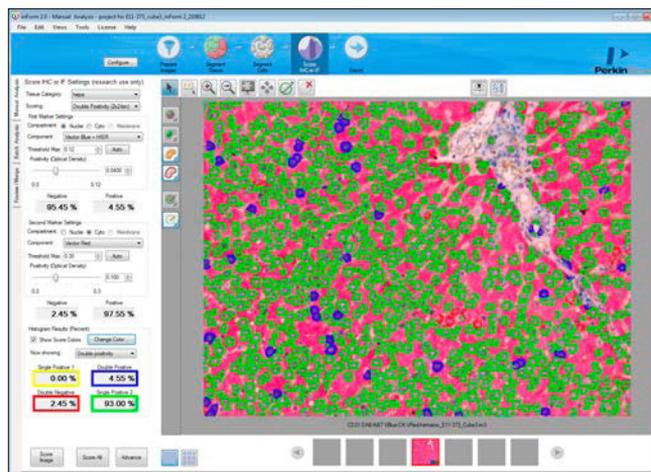
	Cell Analysis	Tissue Finder
Trainable Tissue Segmentation – Learn-by-example interface – Unique 'best-in-class' patented pattern-recognition algorithms		X
Image Review / Data Merge – Interface for rapid review – Improves data reliability		X
Unmix Images	X	X
Manual Tissue Segmentation	X	X
Signal Thresholding	X	X
Colocalization	X	X
Cell Segmentation	X	X
Object Segmentation	X	X
Pathology Views	X	X
Cell Phenotyping	X	X
Batch Processing	X	X
Score (only used with Cell Segmentation)	X	X
Export	X	X



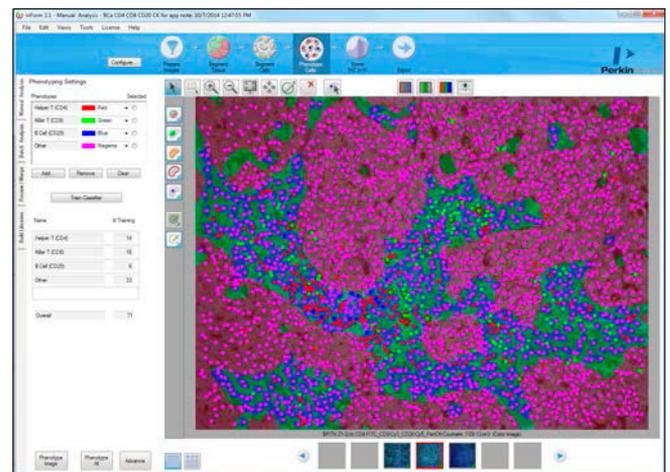
Double-positivity scoring and sub-cellular localization of e-cadherin and vimentin in pancreatic tissue.



Pixel-based colocalization analysis to quantitate the colocalized positivity for two nuclear markers – ER and Ki67 in breast cancer tissue.



Quantitation of Ki67-positive Proliferating Hepatocytes in Rabbit Liver. van der Loos, et. al. J. Histochem Cytochem, 2012.



Phenotyping of CD4, CD8, and CD20 immune cells in breast cancer tumor and the surrounding stroma.

Figure 1. Examples of inForm utilization.

inForm Analysis Software Workflows

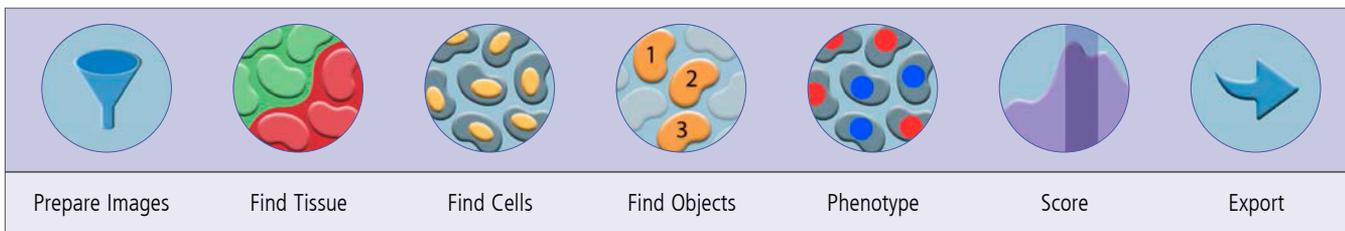


Figure 2. Some of the options available using the Configure Project tool within inForm.

inForm can be tailored to your individual projects using the Configure Project tool. Users select from multiple options depending on the data output being sought. A simple analysis project might involve two steps: Prepare Images and Export, whereas other projects may include additional steps such as those shown in the following examples.

Example: inForm Double-Positivity Scoring

This example illustrates how the inForm Cell Analysis software was used to quantify per-cell expression of EMT (Epithelial-Mesenchymal Transition) markers within pancreatic tumor tissue sections. E-cadherin (Life Technologies Alexa Fluor® 488) was spectrally unmixed from vimentin (Life Technologies Alexa Fluor® 594) and DAPI in the presence of tissue autofluorescence to produce pure images suitable for quantitation. Areas of tumor were then defined using tissue segmentation before the tumor cells were scored by measuring the co-expression of vimentin and E-cadherin using cell segmentation.

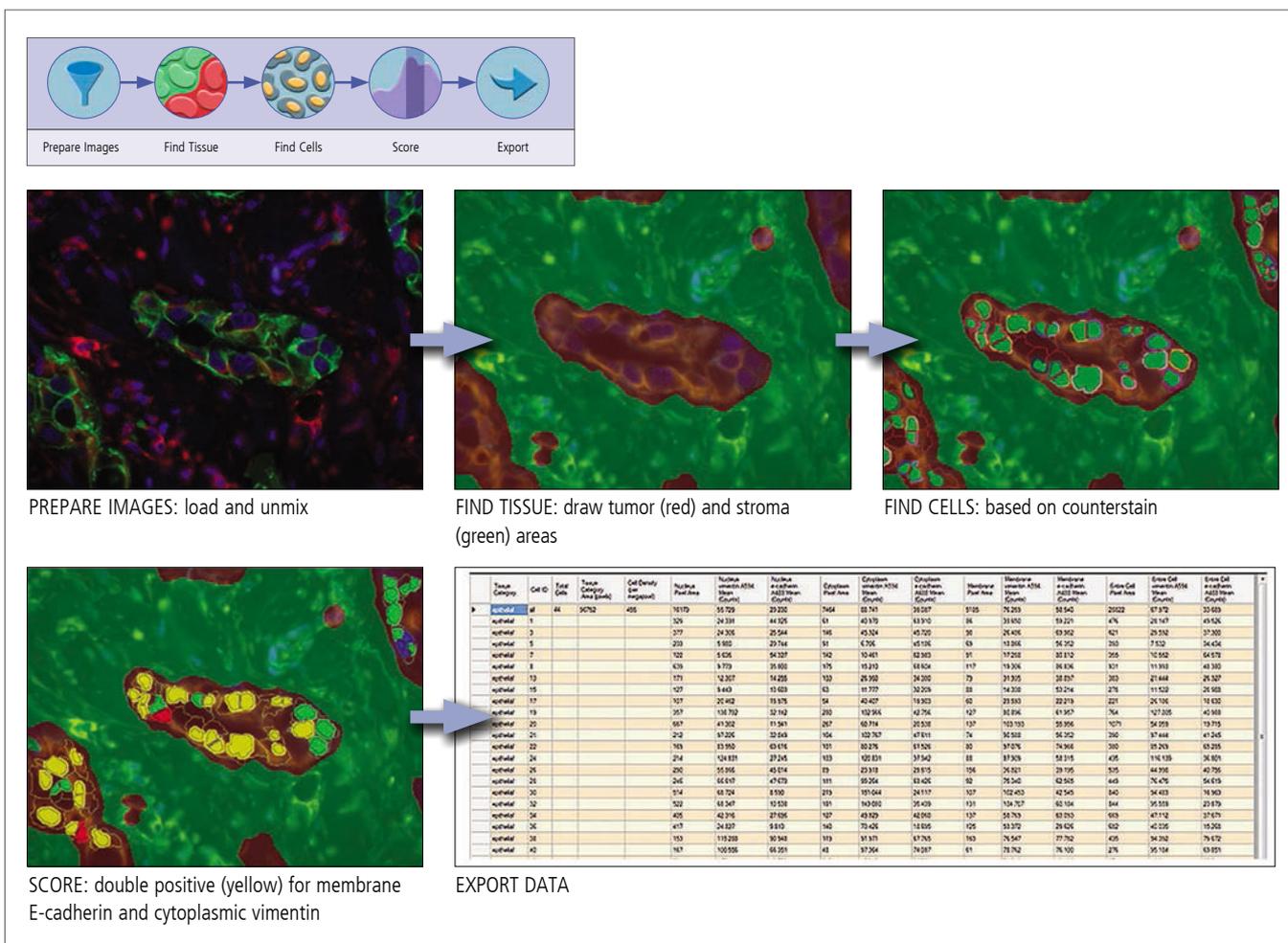


Figure 3. inForm Cell Analysis software demonstrating tissue segmentation, cell segmentation, and scoring options.

Example: inForm Tissue Area Quantitation

This example illustrates how the inForm Tissue Finder analysis software was used to determine whether treatment for fibrosis reduced the amount of damage to the lungs.

Fifty-two tissue sections of rat lung were divided into three groups, each stained with Masson's Trichrome stain, and scanned at 4x using PerkinElmer's Vectra® automated high-throughput quantitative pathology imaging system. The inForm software was user-trained in minutes using just three or four samples from each category to

automatically segment each image into fibrosis (red), normal lung (blue), normal structural tissue (green), and background (yellow). Images from all slides were segmented, reviewed, merged and exported for further analysis using the software's convenient batch-processing review and merge interface, and this data was then merged into a single data set for analysis in Microsoft® Excel®. The percentage area of different tissues and structures was determined, including the amount of fibrosis, enabling an objective comparison between groups.

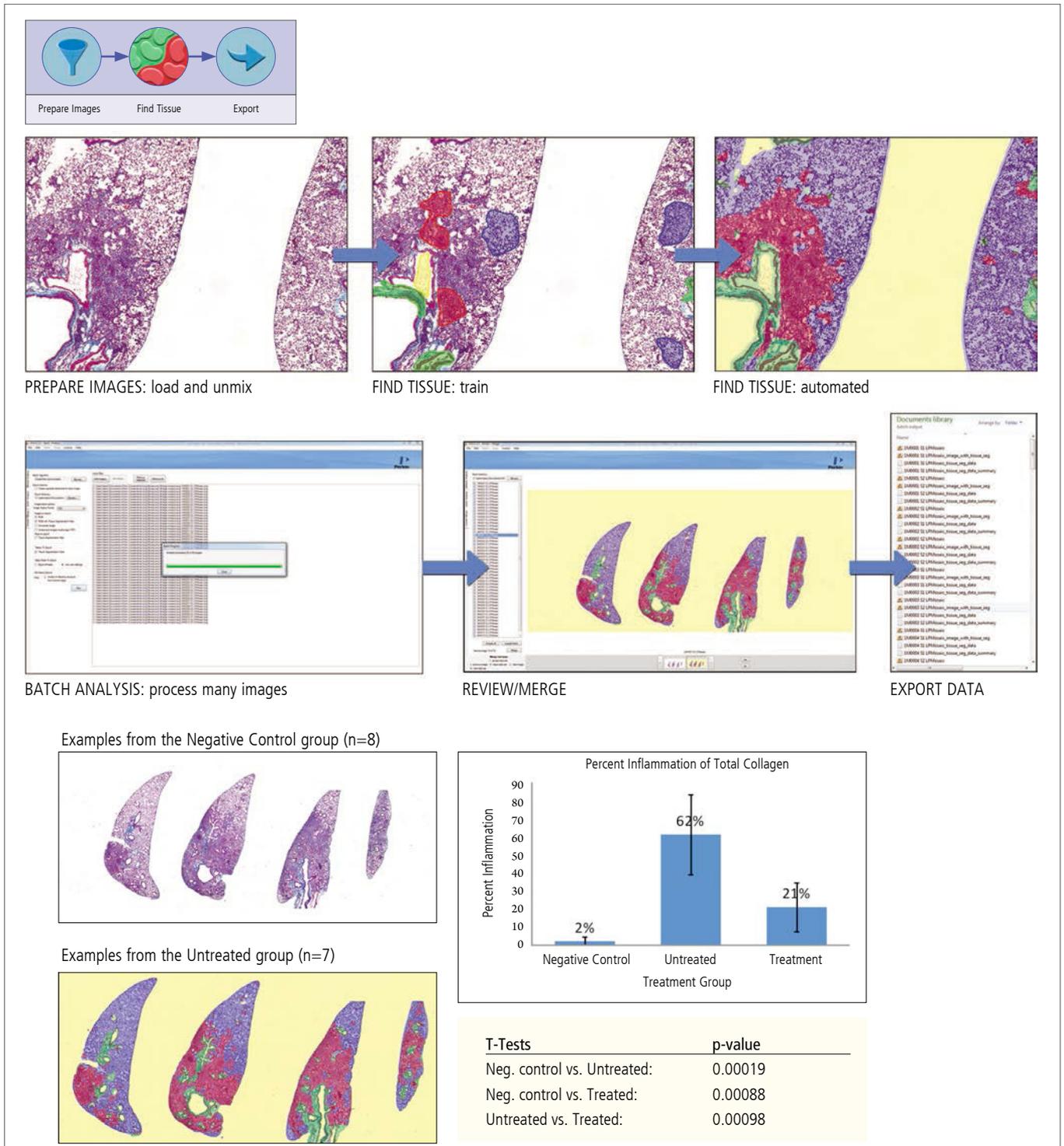


Figure 4. inForm Tissue Finder Analysis: Automated tissue segmentation, batch processing, image review, and merge and data review options. Exported data can be used to produce charts and graphs in third-party software.

Example: inForm Cell Phenotyping

This example illustrates how PerkinElmer's Opal™ multiplex reagent kits and inForm Cell Analysis and Tissue Finder analysis software was used to identify biomarkers that correlate with HPV status, T-cell infiltrate, and patient survival. Previous studies have suggested that CD8 effector T-cell infiltration is associated with prolonged survival in patients with oral, head and neck squamous cell carcinoma (OHNSCC). Multiplexed imaging and relationship analysis may help better delineate the suppressive mechanisms within the tumor microenvironment, which can direct immune interventions that augment tumor-specific immune response.

The images in Figure 5 on the left are from a patient with a good outcome, and on the right from a patient where the disease had

recurred. Each marker was assigned a unique color, and an algorithm was developed to segment tumor (red) from stroma (green) by training the software on a limited number of regions. Cells in the tumor and stroma regions were then phenotyped and counted by training the inForm software to recognize each cell type. PD-L1 expression was measured using inForm's scoring feature. Subsequent analysis can be performed using inForm's exported cell positional data imported into data packages such as TIBCO Spotfire®, MatLab or the R analysis environment.

Positive correlation was found between CD8 and Arg1 immune infiltrate within the tumor and HPV status, as well as positive correlation between CD8 and disease recurrence, consistent with literature.

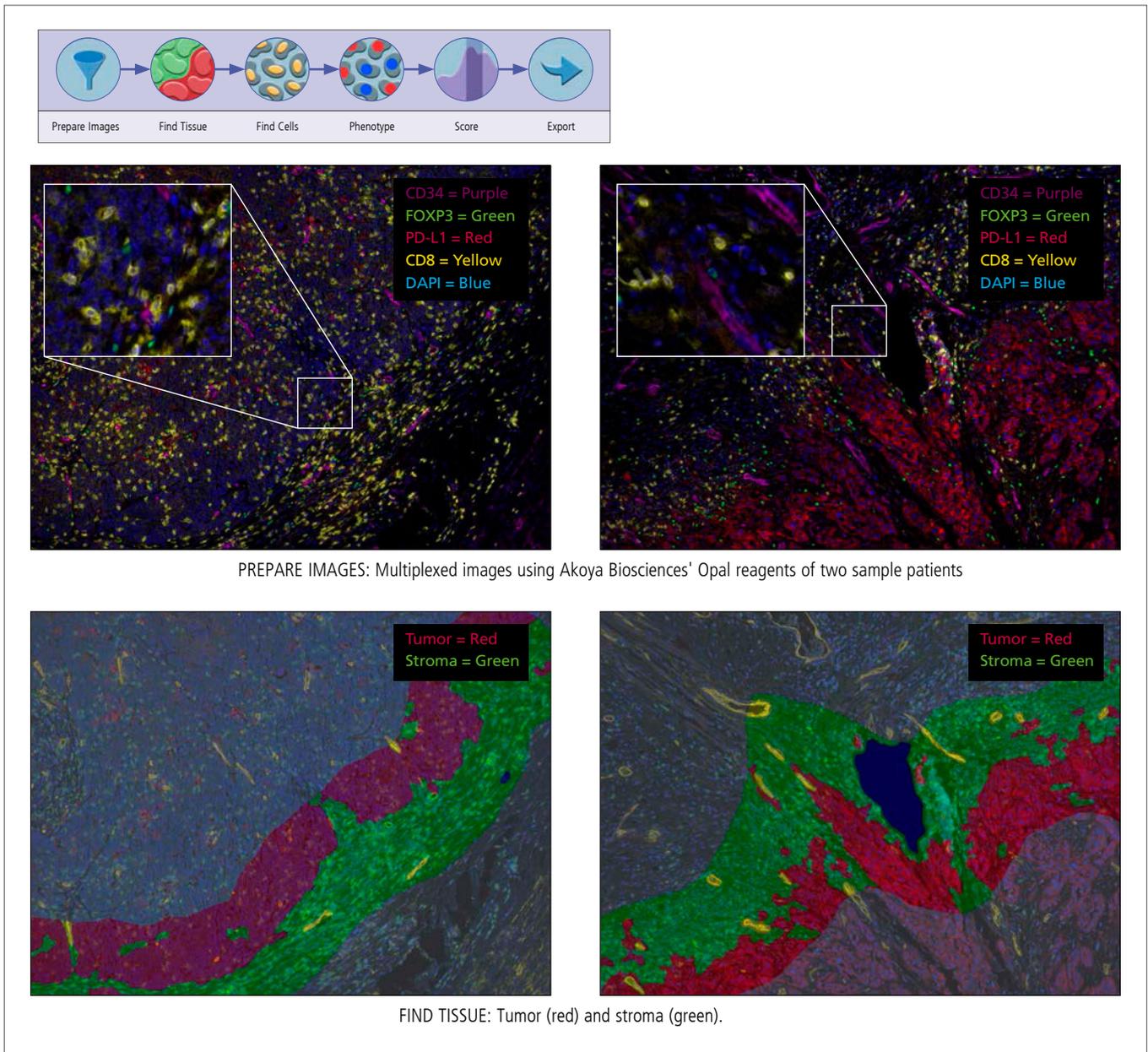
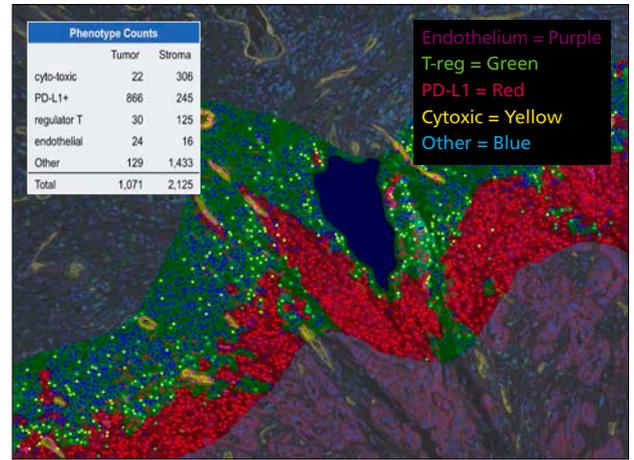
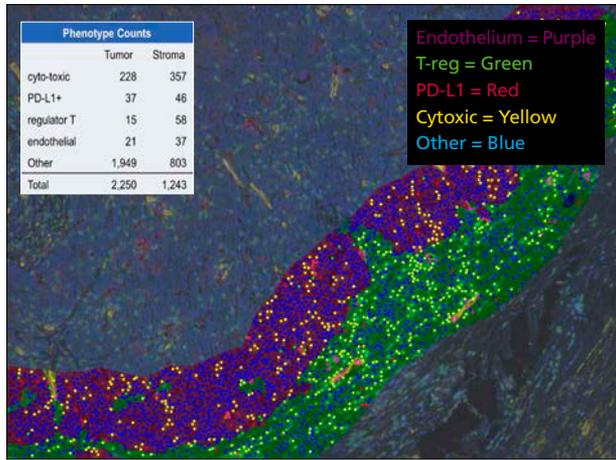
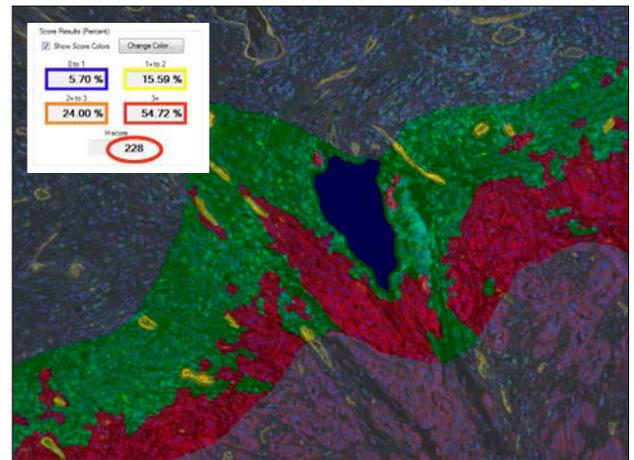
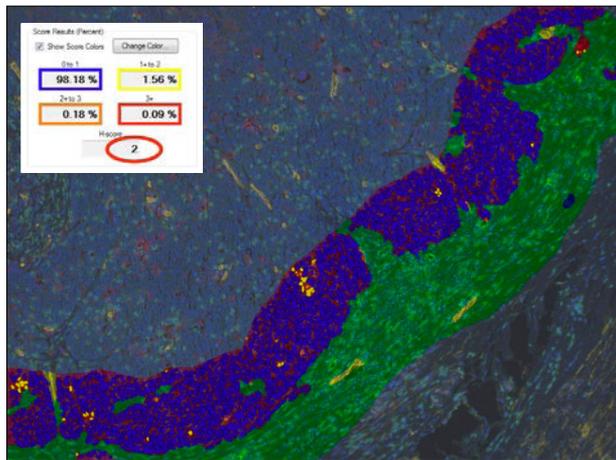


Figure 5. Relationship analysis of tumor microenvironment in head & neck cancer tissue using automated Vectra imaging and inForm cell phenotyping to determine correlation between markers, immune infiltrate, and disease recurrence. Continued on next page...



FIND CELLS & PHENOTYPE: using a learn-by-example method to recognize and identify each phenotype.



SCORE: PD-L1 expression scoring results

Measurement	Sample #1		Sample #2	
	tumor	stroma	tumor	stroma
Ave. No. PD-L1+s within 10 microns of T regs	0.00	0.03	0.37	0.18
Ave. No. PD-L1+s within 25 microns of T regs	0.20	0.52	4.83	1.87
Ave. No. CD8+s within 10 microns of T regs	0.30	0.24	0.10	0.18
Ave. No. CD8+s within 25 microns of T regs	1.20	3.16	0.40	1.49

Measurement	sample #1			sample #2			Delta
	tumor	stroma	ratio	tumor	stroma	ratio	
CD8+ cell density (per sq mm)	855	1406	1.6	94	855	9.0	↑
T reg density (per sq mm)	56	228	4.1	126	349	2.7	↓
Tumor PD-L1 H-score	2			228			↑
% CD8+s with a T reg within 25 microns	7%	40%	5.7	47%	45%	0.95	↓
% CD8+s with a PD-L1+ cell within 25 microns	28%			82%			↑
Ave. No. PD-L1+s within 25 microns of T regs	0.20	0.52	2.6	4.83	1.87	0.39	↓
Ave. No. CD8+s within 25 microns of T regs	1.20	3.16	3.0	0.40	1.49	3.73	↑

EXPORT: positional data used to find the nearest neighbor of cytotoxic CD8+ to PD-L1+ cells in tumor and stroma in two different samples from two patients.

Figure 5. Phenotyping and scoring within the tumor microenvironment in head and neck cancer tissue using automated Vectra imaging and inForm cell phenotyping to determine correlation between markers, immune infiltrate, and disease recurrence.

Tissue prepared with Akoya Biosciences' Opal multiplexed staining kits and analysis performed in collaboration with Bernard A. Fox and Carlo Bilfulco, Earle A. Chiles Research Institute and Providence Cancer Center, Portland, OR.

Pathology Views

One of the challenges with multimarker datasets is visualization of the results after unmixing. With the potential to have up to six markers in a single sample it is clear that simply displaying each in a separate color and merging them all will be insufficient, since all the colors will blend. In addition, fluorescence images do not convey as much morphologic detail to the eye as brightfield chromogenic images. To address this and to enable pathologists to more easily utilize fluorescence-based techniques that are potentially more quantitative than conventional IHC, Pathology Views, a feature of inForm analysis software, can display these fluorescence images in a manner that is familiar

to anyone trained in histopathology, either displaying them as simulated H&E images or as simulated DAB/hematoxylin images, as shown in Figure 6. In both cases, the hematoxylin view is formed by displaying the unmixed DAPI image as the familiar blue-on-white hematoxylin nuclear counterstain as seen in conventional brightfield microscopy, rather than blue on black, as seen in fluorescence. The eosin image is formed by displaying the unmixed autofluorescence image in as pale pink, and the DAB image is formed by inverting a user-selected fluorophore image and displaying it in brown.

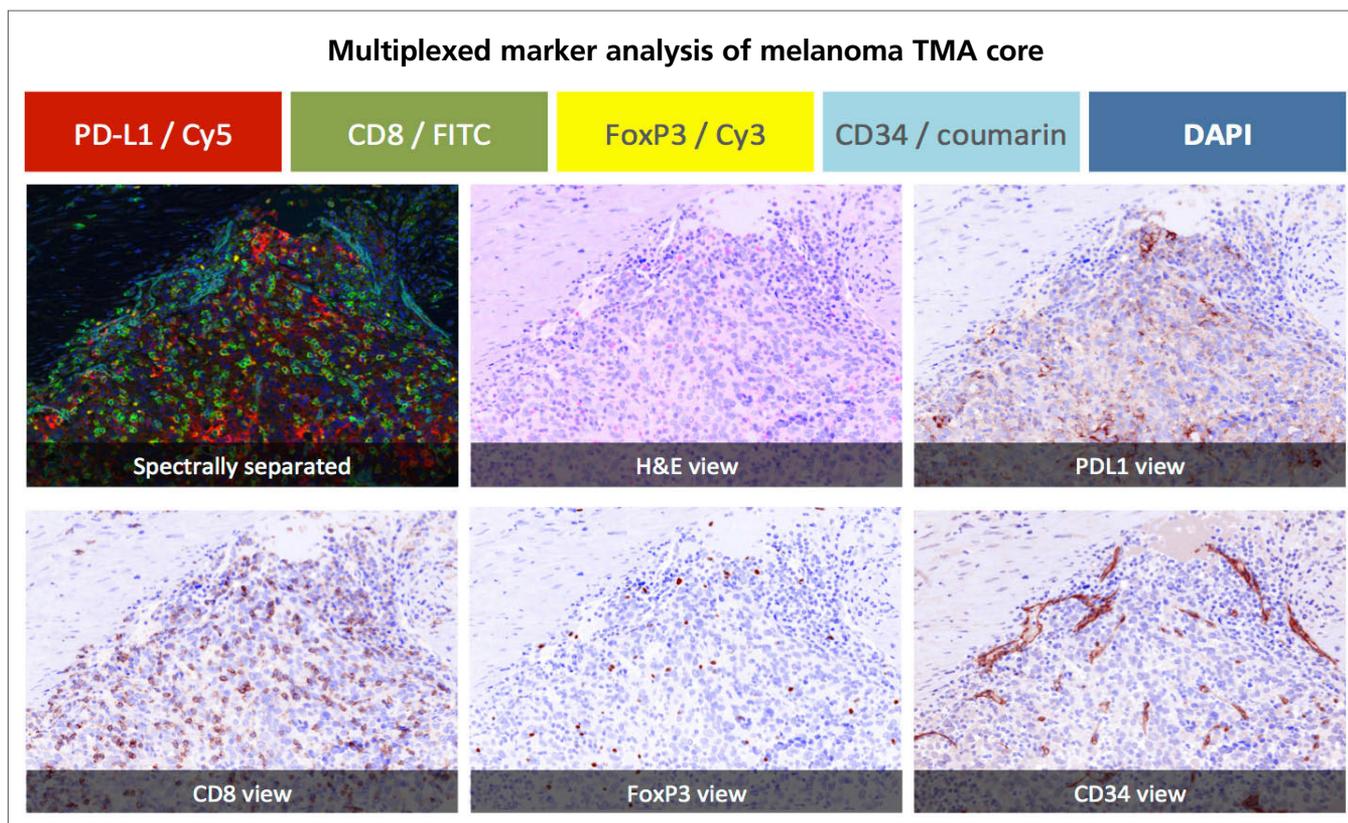
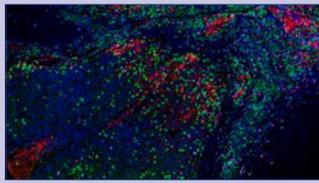


Figure 6. inForm's Pathology Views feature enables viewing of each fluorescent label as if it were seen as DAB on a conventional brightfield IHC slide. Clockwise from upper left, 1) the spectrally separated multi-label fluorescence image, 2) H&E view as if it were a conventionally stained H&E slide in brightfield mode on your microscope, 3) PDL1 view, 4) CD8 view, 5) FoxP3 view, and 6) CD34 view. Each 'view' can be quickly and easily selected using a drop-down menu and saved as a JPEG or TIFF image. Melanoma specimen courtesy Bernard A. Fox, Providence Portland Medical Center, Portland, OR.

inForm Specifications

Image formats	Monochrome or color images (JPEG, single-layer TIFF, BMP, PNG), Akoya Biosciences' Multispectral Images (.im3, .im4)
Maximum number of markers	Extract as many signals per cells as there are spectrally unmixed
Maximum number of images per training set	Typically 20 (for 32 bit) or 400 (for 64 bit)
Maximum number of images per batch set	Unlimited
PC requirements (minimum)	Windows® 7/64 with 2 GB RAM (8 GB RAM recommended)
Training processing speed	Seconds per each image in the training set
Batch processing speed	Multiple images per minute, typical

Akoya's Solutions for Quantitative Pathology Imaging and Analysis



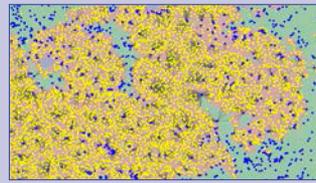
Stain

The Opal method is a practical approach for specific staining of multiple tissue biomarkers up to 6-plex and beyond in a single tissue section.



Image

Vectra automated quantitative pathology imaging system and Mantra workstation for simultaneous quantitative measurement of multiple biomarkers within a single FFPE tissue section.



Analyze and Quantify

Analyze, quantify, visualize and phenotype cells *in situ* using inForm Cell Analysis and Tissue Finder 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 help move your research to the next stage.

Hear What our Customers are Saying



Richard Byers, PhD
*Clinical Senior Lecturer in Pathology,
University of Manchester*

We can now simultaneously analyze several cell populations based upon multiple marker co-expression profiles allowing us to analyze spatial relationships of more accurately phenotyped cells. This function is simple to use and appears to provide, in our experience, near-perfect results.



Christian Slater
*Medical Student,
University of Manchester*

Until now I have been struggling a lot to assess tumor-infiltrating lymphocytes in melanoma. inForm software has multiple functions. With just a few clicks you can extract your spectra and develop your spectral library, segment tissues, segment cells, phenotype, and score them!

Pathology Views allows brightfield display of fluorescence images, which is a great advantage for pathologists and non-pathologists. You can visualize cells in their environment without losing the architecture of the tissue.

For more information, please visit www.akoyabio.com/inForm

For research use only. Not for use in diagnostic procedures.

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