AKOYA BIOSCIENCES®
THE SPATIAL BIOLOGY COMPANY®

We've rebranded some of our products. ***Phenoptics™*** *is now* ***PhenoImager™*** *and* ***Vectra® Polaris™*** *is now* ***PhenoImager™ HT****.*

PhenoImager HT Automated Quantitative Pathology Imaging System

Quantitative Pathology Imaging and Analysis

KEY FEATURES:

- State of the art multispectral imaging with spectral unmixing to easily detect and measure multiple overlapping biomarkers within a single tissue section
- High speed digital whole-slide scanning at 10X - 40X in brightfield or fluorescence
- Enclosed system with built-in touchless automation allows users to safely visualize, analyze, quantify, and phenotype immune cells *in situ* with maximum reliability
- Trainable machine-learning algorithms using inForm® software allowing for automatic identification of specific cell and tissue types

MORE ACCURATELY QUANTIFY CELLULAR INTERACTIONS IN TISSUE SAMPLES

The PhenoImager HT™ (formerly Vectra® Polaris™) Automated Quantitative Pathology Imaging System is a new class of tissue imager which provides unparalleled speed, performance, and versatility for visualizing, analyzing, quantifying, and phenotyping immune cells *in situ* in Formalin Fixed Paraffin Embedded (FFPE) tissue sections and TMAs to advance disease research. PhenoImager goes beyond basic functions, like whole-slide, brightfield (BF), and fluorescence (FL) imaging. It integrates the power of multispectral imaging (MSI) and spectral unmixing in a simplified workflow, and is part of our PhenoImager HT translational solution.

With our patented and proven MSI technology with spectral unmixing, you can capture the multiple interactions occurring between cells without the interference of autofluorescence as the signals are unmixed from one another. That means you can have confidence in accurately quantifying the interactions that are really occurring in the biology.

Multispectral imaging on the PhenoImager HT can now be applied across a whole slide for up to 7 colors and unmixed in less than 8 minutes with our state-of-the-art onboard spectral unmixing technology, enabling the biology to be explored at multiple scales, from cell-to-cell interactions to the macroscopic tissue architecture. The whole slide multispectral imaging capability creates a simpler and more robust workflow as fields of view do not need to be selected so there's no selection bias and you have a whole slide record so that you can easily re-analyze imagery as new understanding emerges. With the PhenoImager HT, you can also generate unmixed annotated regions of interests of up to 9 colors using our renowned liquid crystal tunable filter (LCTF) technology.

Combined with a fit-for-need selection of software analysis options and PhenoCode Signature staining panels, you can identify and quantify multiple targets and reveal spatial relationships within a digital workflow for faster, easier and more informative studies.

UNDERSTAND BIOLOGICAL INTERACTIONS ACROSS MANY APPLICATIONS

The PhenoImager HT Automated Quantitative Pathology Imaging System is a cutting-edge tool for revealing the cell-to-cell biology driving tumor progression, immune interactions and response to therapeutic interventions. By combining tissue imaging with multiplexing, the PhenoImager HT system can capture this biology with a workflow that supports the volume and high- throughput demands of clinical research studies.

Example Applications

- Phenotyping immune cells for cancer immunology research
- Transduction signaling pathway activity (pAKT, pERK, pS6, p13K/mTOR, MAPK, or EGFR)
- Apoptosis and/or proliferation assays
- Necrosis and fibrosis using conventional stains
- Cell cycle characterization
- DNA damage determination
- Inflammation
- Lymph node metastasis
- Autoimmune diseases
- Transplant acceptance
- Neurodegenerative diseases
- RNA-ISH
- Tumor microenvironment exploration
- And many more!

DISCOVER NEW TISSUE BIOMARKERS

For researchers seeking to understand cellular interactions within an intact tissue sample, the PhenoImager HT analyzes, quantifies and phenotypes cells *in situ*, in FFPE tissue sections and TMAs. The PhenoImager HT system's multispectral technology with onboard spectral unmixing enables the removal of autofluorescence, improving the signal-to-noise ratio substantially. This allows for a more sensitive and accurate analysis of samples, and enables true cell-to-cell interaction to be revealed, coupled with multicolor IHC reagents designed for best signal isolation. Researchers can see up to 9 colors in a tissue section (Figure 1), revealing cell types, spatial context, quantification of multiple biomarkers and cell-to-cell interactions (Figure 2). PhenoImager HT seamlessly integrates this data into a digital pathology workflow and can provide whole section context, in brightfield (BF) and fluorescence (FL) modes.

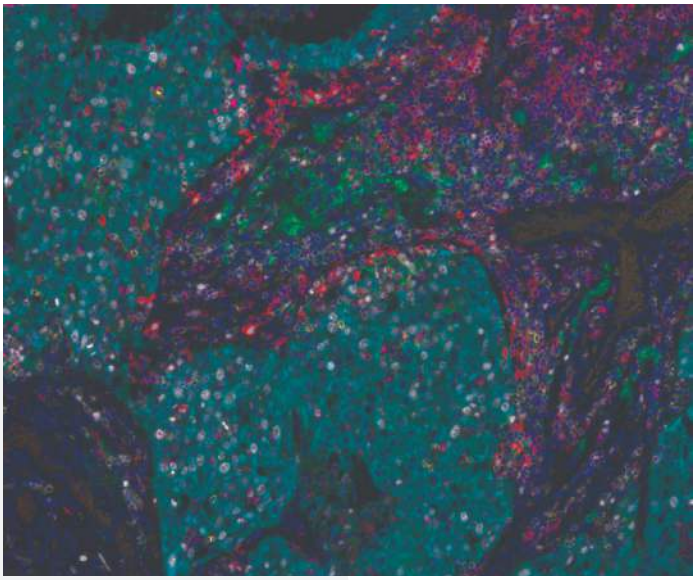


Figure 1. Unmixed 9-color multispectral image of human lung cancer tissue stained against CD20 (Opal 480, red), PD-L1 (Opal 520, green), CD8 (Opal 540, yellow), FoxP3 (Opal 570, orange), CD68 (Opal 620, magenta), PD-1 (Opal 650, pink), Ki67 (Opal 690, white), and PanCK (Opal 780, cyan).

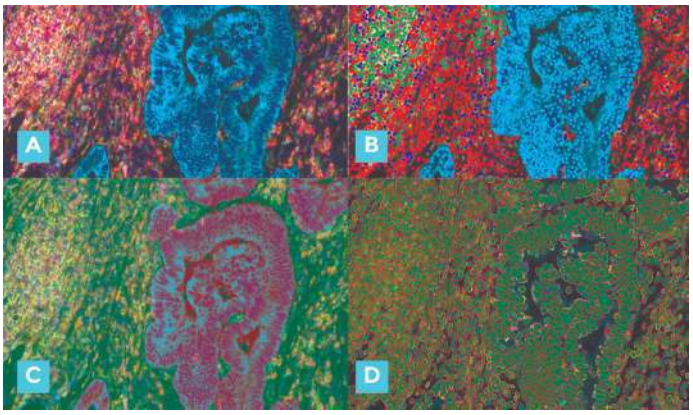


Figure 2. (A) Spectrally unmixed composite image, (B) Cell Phenotyping, (C) Tissue Segmentation, and (D) Cell Segmentation. Colon Cancer stained with the following: Marker (False Color, Opal used), DAPI (Dark Blue), CD68 (Green, Opal 520), CD4 (Yellow, Opal 540), CD8 (Orange, Opal 570), CK (Light Blue, Opal 620), PD-L1 (Purple, Opal 650), and FoxP3 (Red, Opal 690).

GET ACCURATE AND RELIABLE RESULTS, FAST

PhenoImager HT utilizes intelligent automation and patented technology for hands-off, error-free scanning, and an optical configuration designed specifically for accurate signal measurements and unparalleled sample protection. Coupled with advanced machine-learning based approaches to analyze images, this produces quantitative data that accurately captures the biology of interest.

Multispectral Imaging Technologies for Highest Quality Data

- Renowned liquid crystal tunable filter (LCTF) technology generates unmixed, annotated regions of interest of up to 9-colors for deeper interrogation of biology
- Generate whole slide scans of up to 7 colors (formerly called MOTiF™) in less than 12 minutes for analysis of biology across the entire slide in a streamlined workflow without selection bias (See Figure 4)
- Perform whole slide spectral unmixing of up to 7 colors in parallel with imaging to generate spectrally unmixed analysis ready data in less than 8 mins

Integrated, Closed System Design

- No user adjustments, minimizing user error
- Isolated optics minimize environmental interferences
- Manufactured under ISO13485:2016 certification for quality management systems (QMS)

Patent-Pending Technology for Reliable Focusing

- Height sensing optics allow for consistent and reliable image focusing, regardless of slide or sample condition
- Perfect registration from one FL band to another

Unparalleled Slide Protection Technology

- Ultra-bright pulsed LED excitation minimizes photobleaching
- Utilization of touchless automation and unique 4-slide carriers ensures the security and stability of every sample (Figure 3)

Multiple File Formats for a Variety of Applications

- 16-bit extended range data format to reduce saturation rescans
- Unmixed data for simplified 4+ plex workflow
- 8 bit standard range data format with compact file sizes

Figure 3. PhenoImager HT slide carrier and slide hotel.

Meet the Demands of High-Throughput Studies

PhenoImager HT goes beyond conventional brightfield and fluorescence whole-slide scanning. Utilizing the most advanced optics, mechanical design, and imaging sensors; PhenoImager HT delivers both high performance and high-throughput for quantitative digital pathology. Harness the power of whole slide multispectral imaging to accelerate your research and maximize your lab's resources. Choose from a flexible array of scanning options including multispectral brightfield and multiplex fluorescence with whole-slide autofluorescence removal. The human interface is designed for the demands and dynamics of translational labs, with a workflow that optimizes the output from studies, providing permanent unmixed digital slides (Figure 5).

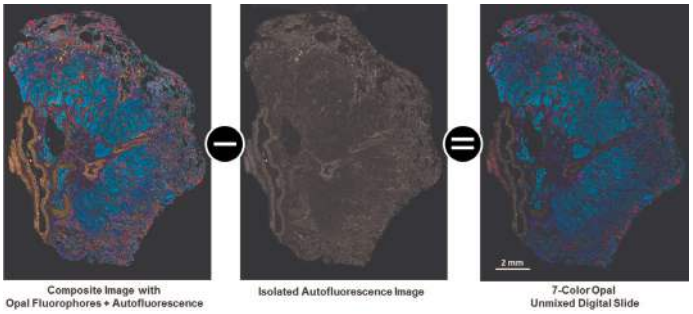


Figure 4. Whole slide imaging using 7-color Opal™ IHC Multiplex staining in lung cancer. Image displays cytotoxic T-cells in yellow (CD8), B-cell lymphocytes in magenta (CD20), programmed death receptor 1 in green (PD-1), programmed death ligand 1 in red (PD-L1), nuclear marker for cellular proliferation in orange (Ki67), epithelial tumor cells in cyan (CK), and DAPI nuclear counterstain. The first image displays the raw composite image. The second image displays the isolated autofluorescence, and the third slide is the unmixed 7-color Opal digital slide.

QUANTIFY BIOMARKERS IN TISSUE SECTIONS

PhenoImager HT utilizes inForm Tissue Finder and phenoptr™ software analysis to combine the power of multiplexed imaging and quantitative analysis, all within a familiar digital workflow to accelerate research. Extract per-cell multiparameter data from one or more cell compartments in order to generate tissue-based analysis that retains the architectural context of the intact tissue.

inForm 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 6). Then use phenoptr to perform downstream spatial analysis.

A **Whole Slide Multispectral Workflow**

B **9-Color, Multispectral Field Workflow**

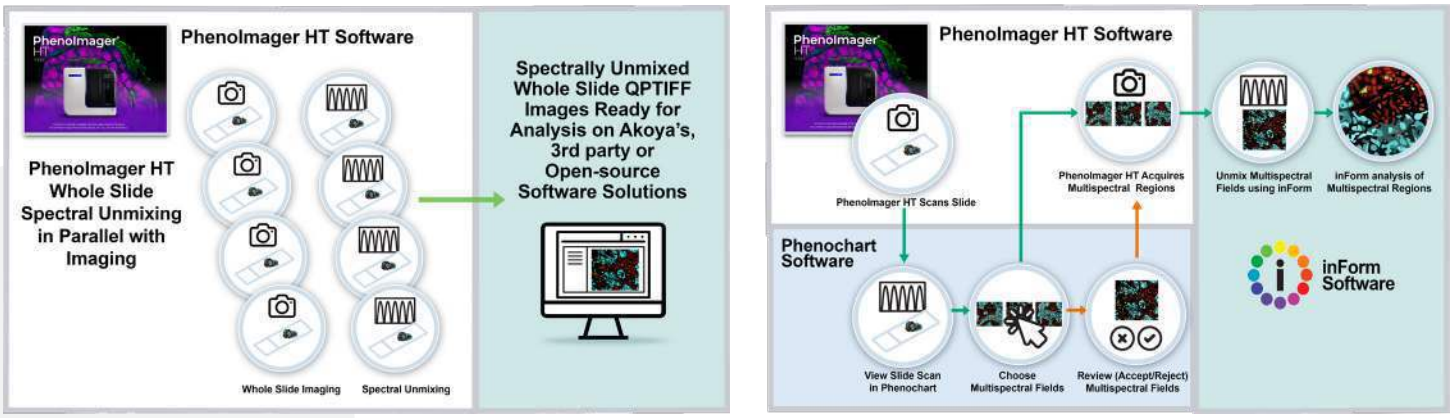


Figure 5a and 5b. The PhenoImager HT features an intuitive, yet flexible, workflow that allows the pathologist to view a whole-slide contextual scan and select regions for analysis using inForm as needed. (a) Using whole slide multispectral imaging with spectral unmixing capabilities and Opal reagents, the entire digital slide is available for analysis without need to select fields for rescan. (b) The PhenoImager HT supports up to 9-color imaging. Here, the pathologist can select any regions of interest for rescan using our LCTF technology for analysis using inForm.

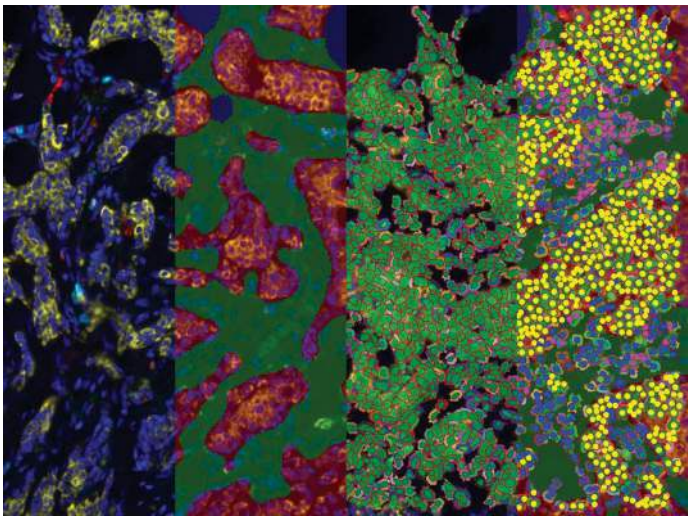


Figure 6. Breast cancer tissue labeled with the Opal 7-Color Multiplexed IHC Kit. Left to right: spectrally unmixed composite image with nuclei (Spectral DAPI, blue), PDL1 (Opal 520, green), PD1 (Opal 540, cyan), CD8 (Opal 570, magenta), FoxP3 (Opal 620, orange), CD68 (Opal 650, red), and cytokeratin (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 Software:

- Deeper integration with Phenochart enables you to push regions of interest, regardless of size, to inForm for algorithm development or analysis
- Powerful unmixing algorithm enables identification and separation of weakly expressing and overlapping signals from background autofluorescence
- User-trained feature recognition algorithms allow automatic identification of specific tissue types based on tissue morphology
- Adaptive Cell Segmentation reliably identifies individual cell types in densely packed, complex morphologies regardless of staining heterogeneity and background levels for accurate phenotyping and downstream spatial analysis in phenoptr
- Allows quantitative per-cell analysis of biomarker expression in tissue sections and TMAs
- Automatically classifies cell phenotypes using machine-learning algorithms to differentiate cell types across a tissue section
- Pathology Views™ renders immunofluorescence (IF) images as simulated H&E or DAB and hematoxylin, to provide views more familiar to you
- Enables cellular analysis of H&E, IHC, and IF in FFPE tissue sections

inForm is designed to meet the workflow and throughput needs of your lab, enabling faster discoveries. 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 (Figure 7).

Whole slide unmixed digital data (unmixed.qptiff) can be exported seamlessly into a variety of analysis software including: Indica® HALO™, Visiopharm® and more. This flexibility enables users to select analysis modules from an extensive list based on their analysis needs and add additional modules as required in the future.

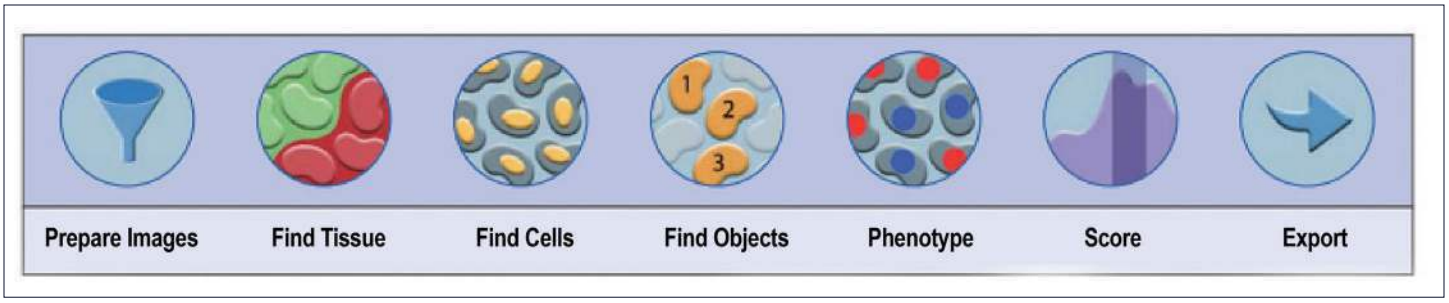


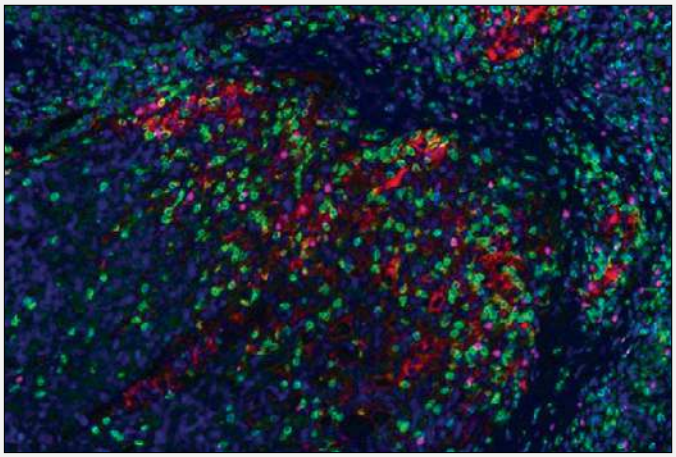
Figure 7. Some of the options available using the Configure Project tool within inForm.

AKOYA'S SOLUTIONS FOR QUANTITATIVE PATHOLOGY IMAGING AND ANALYSIS

The PhenoImager HT Automated Quantitative Pathology Imaging System is a part of Akoya's PhenoImager HT Research Solution. Our complete end-to-end solution includes multiplexed staining reagents and methodologies, instrumentation with industry leading multispectral imaging technology with on-board spectral unmixing and whole slide scanning capabilities (BF and FL), advanced image analysis software that can be trained to phenotype cells as well as for contract research services.

By integrating digital pathology with quantitative immuno-fluorescence, Akoya has developed the PhenoImager HT Workflow solution to meet the needs of today and tomorrow, and to advance translational research.

AKOYA'S PHENOIMAGER HT 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. Incorporating Opal-TSA chemistry, PhenoCode Signature are pre-designed panels that allow for the easy staining of up to 7 colors in a single tissue section.

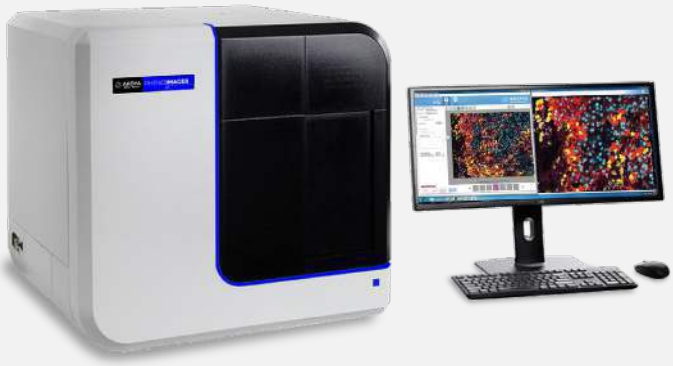
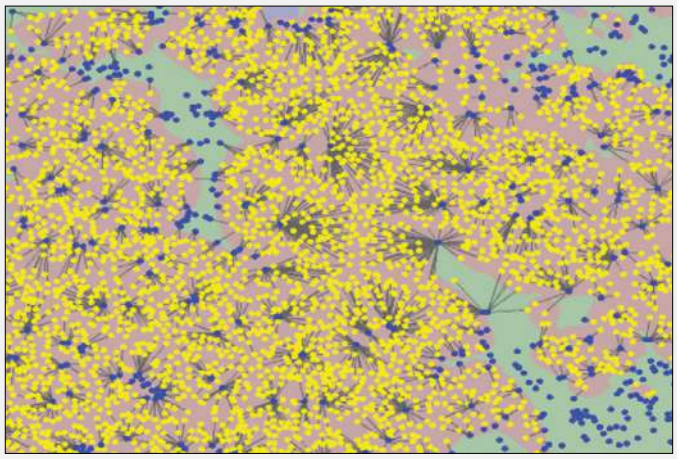


Image
The PhenoImager HT and PhenoImager Fusion Quantitative Pathology Imaging Systems enable simultaneous quantitative measurement of multiple biomarkers within a single FFPE tissue section.



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.

Phenoimager HT Specifications	
Tissue Formats	Tissue microarrays (TMAs) and tissue sections
Multispectral Range	440 – 780 nm
Modality	Brightfield and Fluorescence (multispectral or color)
20X Throughput: Fluorescence (1.5 x 1.5 cm)	5 slides per hour (Whole slide 7 color scan formerly called MOTiF™)
20X Throughput: Brightfield (1.5 x 1.5 cm)	10 slides per hour
Automation	Touchless automation with walk-away image acquisition
Slide Capacity	80 slide capacity with continuous loading technology
Multiplexing Capability	Separates up to 9 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	10X (1.0 um/pixel), 20X (0.5 um/pixel) and 40X (0.25 um/pixel)
File Format	Whole slide digital format (.qptiff), Multispectral Image format (.im3), monochrome or color images (JPEG, single-layer TIFF, BMP, or PNG), Spectrally unmixed whole slide digital format (unmixed.qptiff)

Note: Product specifications subject to change.

PHENOIMAGER HT

CLS143455
(includes instrument, training, one year warranty)

- Virtual microscopy system with automated multispectral imaging camera system
  - Custom designed optics with a 10-position epi-filter wheel
  - Patented active slide surface sensing technology for improved FL focus
  - PL-APO objectives
  - 0.45 NA objective - provides 0.5 um/pixel image resolution (or 1.0 um/pixel via camera binning)
  - 0.75 NA objective - provides 0.25 um/pixel image resolution (or 0.5 um/pixel via camera binning)
- PhenoImager HT 2.0 filter cube configuration, with one available expansion position
- FL reference slide tray with built-in reference slides and protective case
- Agile LED multiband scanning with photobleaching protection
- Slide protocol history to easily recall settings from previous session
- Phenochart viewer
  - View BF and FL unmixed digital slides
  - Isolate and remove autofluorescence across the whole slide
  - Select regions for inForm analysis · inForm Tissue Finder license with one seat

  INCLUDES FUNCTIONALITY FOR:
  - Processing spectral cubes or conventional color images
  - On-board spectral unmixing
  - Phenotyping of individual cell types, with easy training and validation
  - Spectral library development with measured or synthetic spectra
  - Exporting per-region and per-cell data for individual stain components
  - Batch processing of images
  - Pathology views (simulated IHC) of multiplexed fluorescent samples
- 24'' monitor, keyboard and mouse
- Computer (Microsoft® Windows® 10 Pro)

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