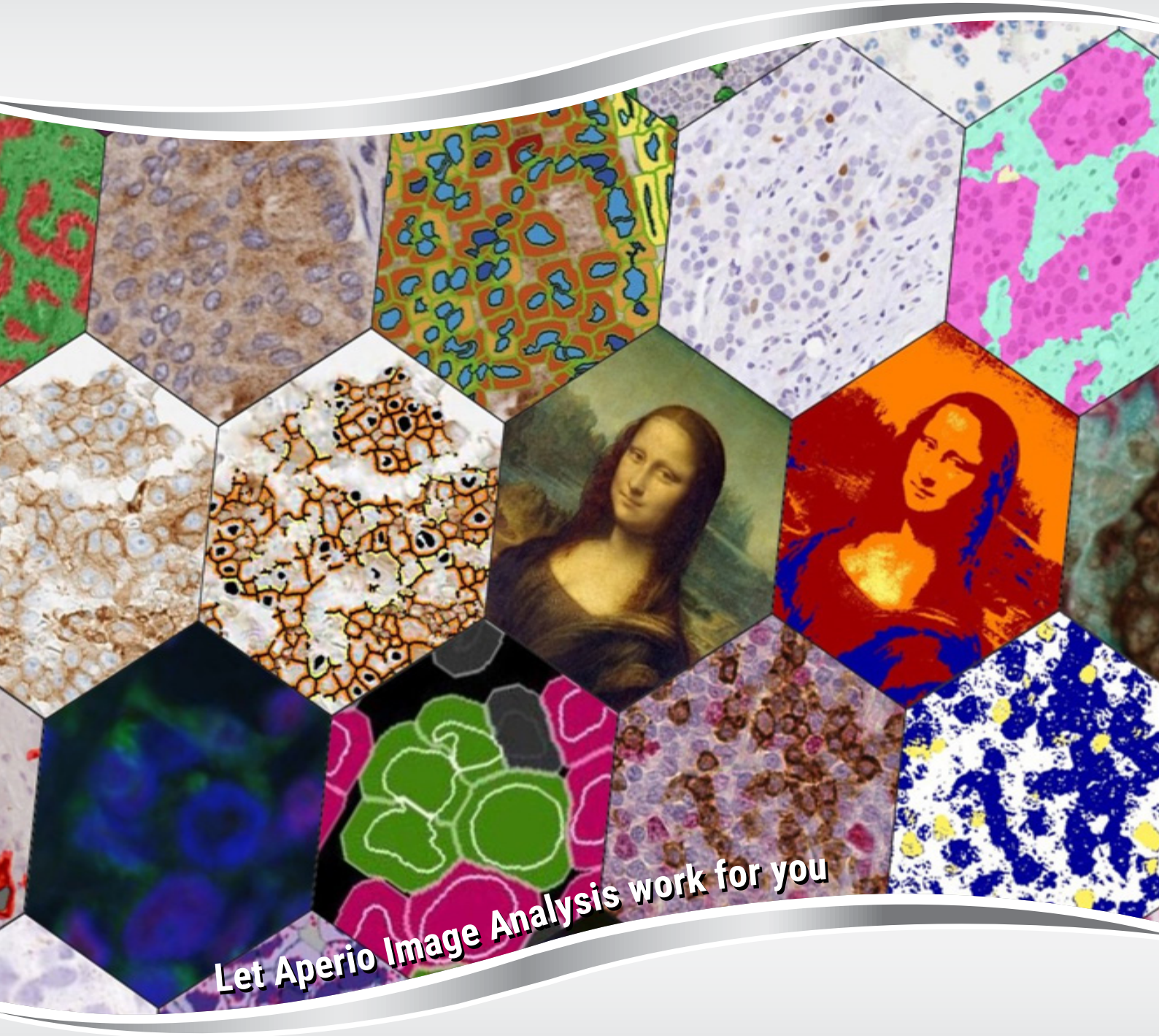
D I G I T A L P A T H O L O G Y

APERIO
IMAGE ANALYSIS

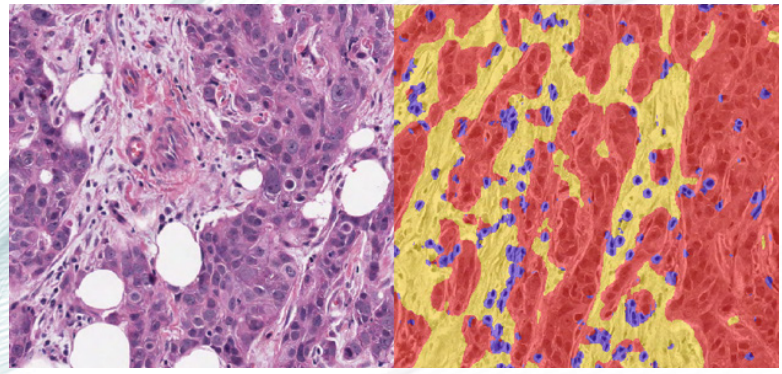


Advancing Cancer Diagnostics
Improving Lives

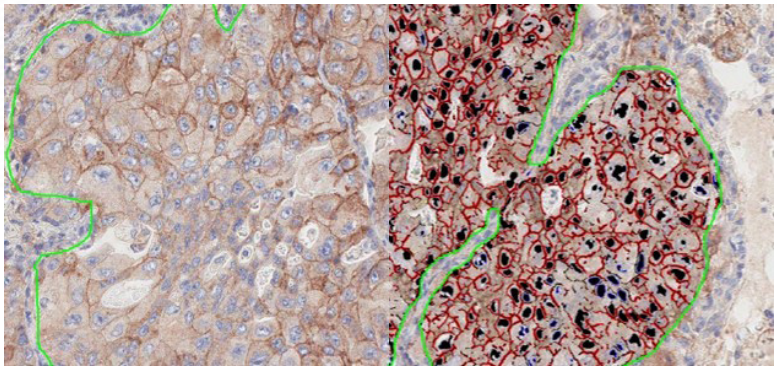
Leica
BIOSYSTEMS

Enhance Your Workflow with Automation

Do you have a digital pathology slide scanner?	✓ Extract more data from your images with quantitative image analysis
Do you have large volumes of slides to score?	✓ Automatic batch image analysis works in the background so you don't have to
Do you worry about the accuracy of slide scoring?	✓ Get quantitative, standardized data for a wide variety of applications
Do you find it time-consuming to maintain records of results?	✓ Results are automatically saved with digital slide records and exported for further use



Tumor and infiltrating lymphocytes in breast H&E stained tissue



PD-L1 membrane stain in lung tumor tissue

Freedom from the Microscope

- ✓ Share and interpret slides digitally where and when it suits you
- ✓ Generate more data from tissue than from manual scoring: limited manual counting assays (hundreds of cells) become automatic whole slide assays (thousands of cells)
- ✓ Optimize the algorithm to align with best practice to then score all slides consistently and reproducibly

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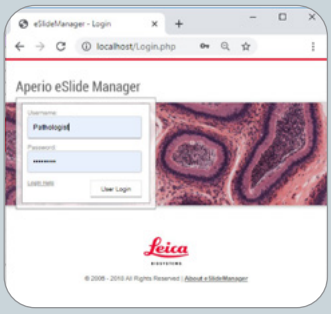
Experience the Full Power of Digital Pathology with Aperio Image Analysis

Unlimited assays provided by each algorithm	✓ Optimize & save flexible parameters to assist in automating multiple assays
Rapid set up & walk away protocol for batch analysis	✓ 5 clicks to analyze a batch of slides using your saved parameters
Seamless workflow within Aperio Digital Pathology platform	✓ Batch analysis of scanned slides supports whole slide images & regions of interest straight from the scanner
Wide range of applications & use cases	✓ Unique & extensive outputs for each algorithm with detailed color overlay

Optimized for Aperio Scanners and Aperio Image Management



DIGITIZED ON APERIO DIGITAL PATHOLOGY PLATFORM

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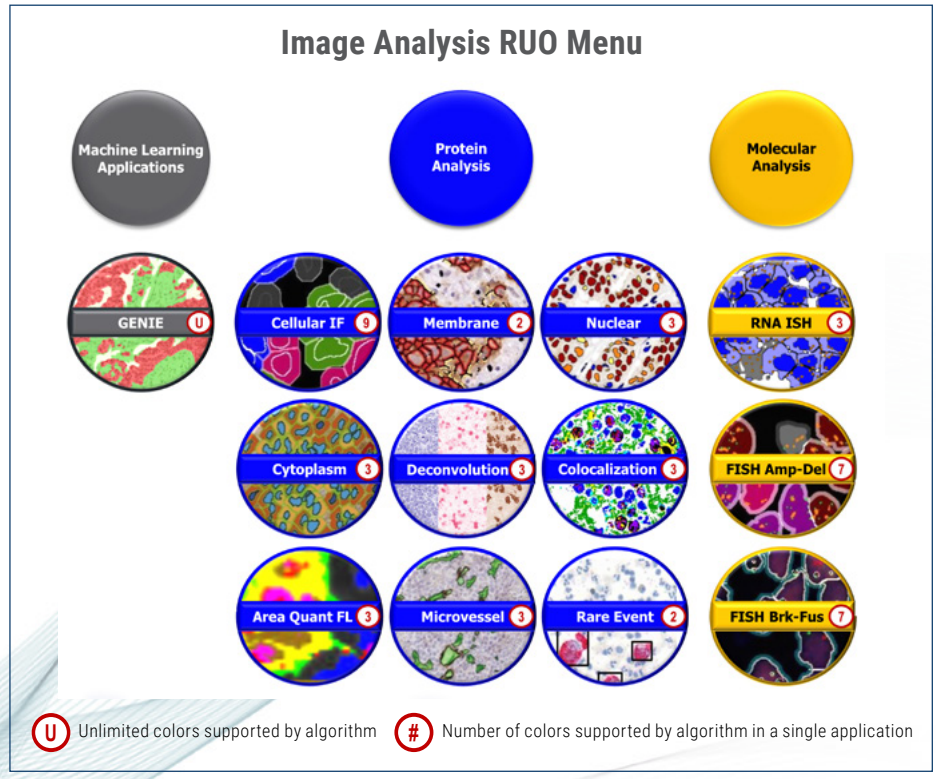
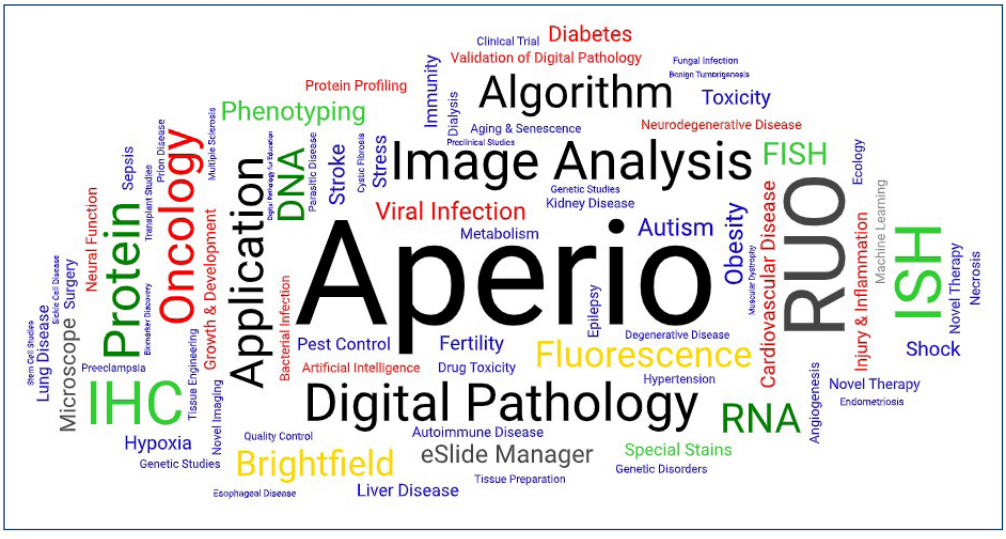
Aperio has a Flexible Menu of Image Analysis Algorithms



1500+

peer-reviewed publications using Aperio Image Analysis Algorithms (PubMed database search for 'Aperio')

A Key Tool for Many Applications in Published Biomedical Research



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Aperio Image Analysis
Algorithms at the Click of a Button

ALGORITHM	TYPE	USE CASES	COMPATIBLE STAINS	PAGE
Aperio GENIE	• BRIGHTFIELD • FLUORESCENCE • Machine Learning	• Tissue pattern recognition software • Train to automatically identify tissue of interest in digital images	• Any chromogen, fluorochrome, counterstain, H&E, special stain • Example: tumor regions of interest; kidney glomeruli; pancreatic islets	6
Aperio Membrane	• BRIGHTFIELD • Antibody Assays	• IHC of membrane antigens • Provides cell count for different intensity classes	• Only Brown membrane staining with Hematoxylin counterstain • Example: HER2, PD-L1, CD3, CD8	7
Aperio Nuclear	• BRIGHTFIELD • Antibody Assays	• IHC of nuclear antigens • Cell count for different intensity classes	• Default: Brown IHC & Hematoxylin counterstain • Train colors to any nuclear counterstain & nuclear stain including red, blue, yellow, purple chromogens • Example: ER, PR, Ki67	8
Aperio Cytoplasmic	• BRIGHTFIELD • Antibody Assays	• IHC of cytoplasmic antigens • Provides cell count for different intensity classes • Also cyto-nuclear translocation assays	• Default: Brown IHC & Hematoxylin counterstain • Train colors to any nuclear counterstain & cytoplasmic stain, including red, blue, yellow, purple chromogens • Example: Bcl-2, CD45	9
Aperio RNA ISH	• BRIGHTFIELD • Molecular Assays	• Quantification of RNA ISH dots and clusters of signal in cells and tissue • Cell count & signal count • Flexible scoring systems	• Red, Brown, Green (Blue), Black signals with Hematoxylin counterstain • Also supports: Red-Green, Red-Brown, Red-Black & Brown-Green duplex assays	10
Aperio Color Deconvolution	• BRIGHTFIELD • Antibody Assays	• IHC of any antigen in any location • Separate 3 brightfield colors • Intensity scores for each color	• Default: Brown IHC with Hematoxylin and Eosin • User adaptable—alter colors to other chromogens and special stains • Example. Duplex IHC, Trichrome, PAS	11
Aperio Colocalization	• BRIGHTFIELD • Antibody Assays	• IHC of any antigen in any location • Separate & compare up to 3 chromogens for pixel colocalization	• Default: Brown IHC with Hematoxylin and Eosin • User adaptable—alter colors to other chromogens and special stains • Example. Duplex IHC, Trichrome, PAS	12
Aperio Rare Event	• BRIGHTFIELD • Molecular Assays	• IHC of Circulating Tumor Cells (CTCs) & other rare events • Cell counts and user can visit each cell to review	• Default: Red chromogen • User adaptable—alter colors to other stains	13
Aperio Microvessel	• BRIGHTFIELD • Antibody Assays	• IHC of epithelial stain that indicates new blood vessel formation • Vessel number & dimensions counted	• Default: Brown IHC with Hematoxylin counterstain • User adaptable to other stains • Example: CD31, CD34, Factor VIII	14
Aperio Cellular Immunofluorescence	• FLUORESCENCE • Antibody Assays	• Immunofluorescent staining of nuclear/ membrane/ cytoplasmic antigens combined into a single assay • User-definable assays with scoring classes and per cell data outputs • Cell counts, phenotypes	• 7 test stains + 1–2 counterstains in multiplex images • User can customize stain names • Example: identification of triple-negative breast cancer specimens • CTC identification & characterization; Immunoncology phenotyping assays	15
Aperio FISH Amplification/Deletion	• FLUORESCENCE • Molecular Assays	• Fluorescent *in situ* hybridization of DNA probes • User-definable assays for multi-probe enumeration and ratio determination with scoring classes • Cell counts, spot counts & intensity	• 7 test stains + 1 counterstain in multiplex images • User can customize probe names • Example: Amplification: HER2; Deletion: Rb1	16
Aperio FISH Breakapart/Fusion	• FLUORESCENCE • Molecular Assays	• Fluorescent *in situ* hybridization of DNA probes • User-definable assays for multi-probe enumeration and fusion-group determination with scoring classes • Cell counts, spot counts & intensity	• 7 test stains + 1 counterstain in multiplex images • User can customize probe names • Example: Break-apart: ALK; Fusion: BCL/ABL	17
Aperio Area Quantification FL	• FLUORESCENCE • Antibody Assays	• Compare any 3 fluorescent channels for pixel colocalization analysis	• Default: DAPI, FITC, TRITC fluorochromes • User adaptable with other fluorescent channels in image • Example: DCX, nestin, tubulin in cultured neurons	18
Chaining Algorithms Together	• BRIGHTFIELD • FLUORESCENCE • Machine Learning	• Fully automated whole slide analysis • GENIE pre-analysis directs the quantification algorithm to analyze only specified tissue type • No need for user annotation to define ROIs	• Any chromogen, fluorochrome, counterstain, H&E, special stain • Example: PD-L1 analysis of viable tumor cells	19

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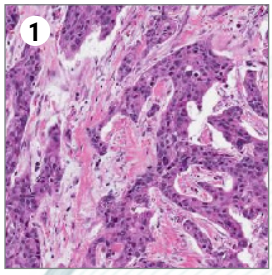
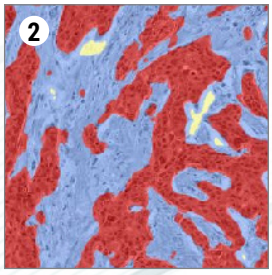
Aperio GENIE Algorithm

Machine Learning Histology Pattern Recognition

Aperio GENIE is an interactive image analysis tool for differentiating tissue subtypes within a digital slide. This Convoluted Neural Network (CNN) can be trained by the user with examples to automatically identify regions of interest for research, e.g. distinguishing tumor from normal tissue, or xenograft from native tissue.

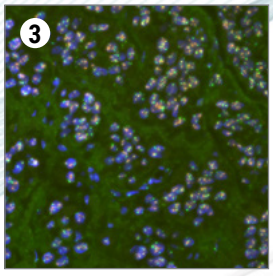
TISSUE CLASSIFICATION FOR BRIGHTFIELD AND FLUORESCENT IMAGES

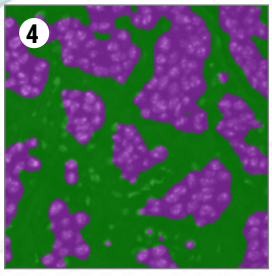




Attribute	Value
Algorithm	Aperio Genie Classifier v1
Tumor (%)	46.9223
Stroma (%)	52.5143
Glass (%)	0.563367
Analysis Area (mm^2)	2.17699

1. Original digital image of H&E stained human breast tissue. **2.** Aperio GENIE mask, with user-defined color-coded classification: **red** represents Tumor, **blue** represents Stroma and **yellow** represents Glass/non-tissue.





Attribute	Value
Algorithm	Aperio Genie Classifier v1
Tumor (%)	43.8389
Stroma (%)	56.1611
Analysis Area (mm^2)	9.06437e-002

3. Original digital image of HER2 FISH of human breast tissue. **4.** Aperio GENIE mask, with user-defined color-coded classification: **purple** represents Tumor and **green** represents Stroma.

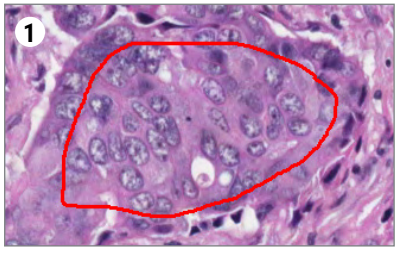
IDENTIFY TISSUE TYPES THAT YOU DEFINE

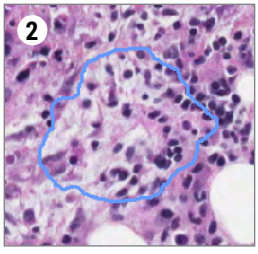
Aperio GENIE provides histology pattern recognition. The user has complete control over the whole training process:

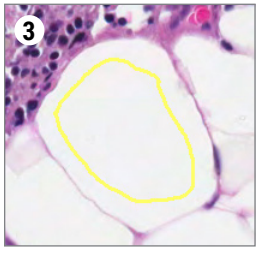
- How many slides to include in the training
- What classes of tissue are to be created
- Identifying representative tissue in each class for training

Aperio GENIE assesses the pixels in the input images to define differences between the classes. Training outputs show success of classifier creation (specificity and sensitivity of pixel detection per class).

The mature Aperio GENIE classifier is specific to the stain and the tissue it has learned to interpret.







Hematoxylin & Eosin stained slide showing training annotations for Aperio GENIE: **1.** Tumor in **red**, **2.** Stroma in **blue**, **3.** Glass in **yellow**.

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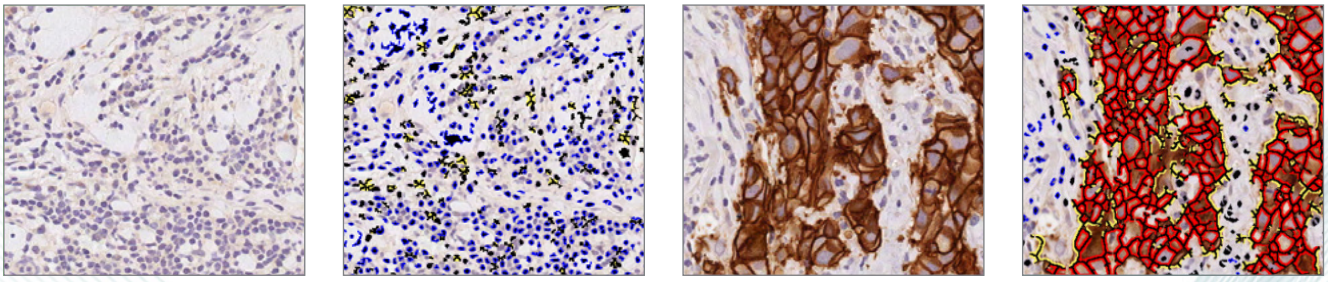
Aperio Membrane Algorithm

Quantitative IHC Cell Membrane Analysis

Cell-by-cell segmentation of membrane staining enables analysis of target membrane proteins. This is fundamental to a number of applications, such as cancer characterization and design of personalized therapies. Manual membrane segmentation is challenging with IHC, as the cellular membrane is visible only in the stained cells. The Aperio Membrane Algorithm uses complex cell modeling techniques to identify both stained and unstained cell membranes, then quantifies the intensity and completeness of the staining with a high level of accuracy and reproducibility.

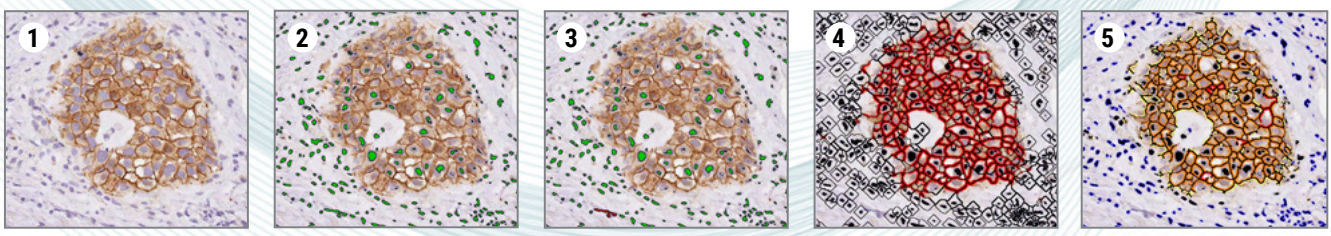
FLEXIBLE CELL SEGMENTATION AND ANALYSIS

ORIGINAL TISSUE | ANALYSIS MASK | ORIGINAL TISSUE | ANALYSIS MASK



NEGATIVE STAINING | POSITIVE STAINING

Original tissue showing negative and positive (Her2) brown chromogen membrane locations plus hematoxylin nuclear counterstain. Masks illustrate Aperio Membrane Algorithm performance. **Black** objects = nuclei identified, Blue-bordered nuclei = negative cells (membrane staining below threshold for positivity), **Black** lines = identified membrane boundaries, Yellow lines = weak positive membrane staining, Orange = moderate positive membrane staining, and Red = strong positive membrane staining.



Tuning steps in Aperio Membrane Algorithm: 1. Original tissue with brown membrane chromogen. **2.** Nuclei identification. **3.** Nuclei exclusion. **4.** Membrane identification. **5.** Complete algorithm mask.

Score	Average intensity score (0 to 3+) for the analyzed region, based on user defined thresholds.
Total Cells	Total number of cells within the analyzed region; percentage of positive cells with completely stained membranes.
Intensity	Overall intensity of staining for the analyzed area.
Positive Cells	Actual number of positively stained cells, and percentage that fall within each scoring category.

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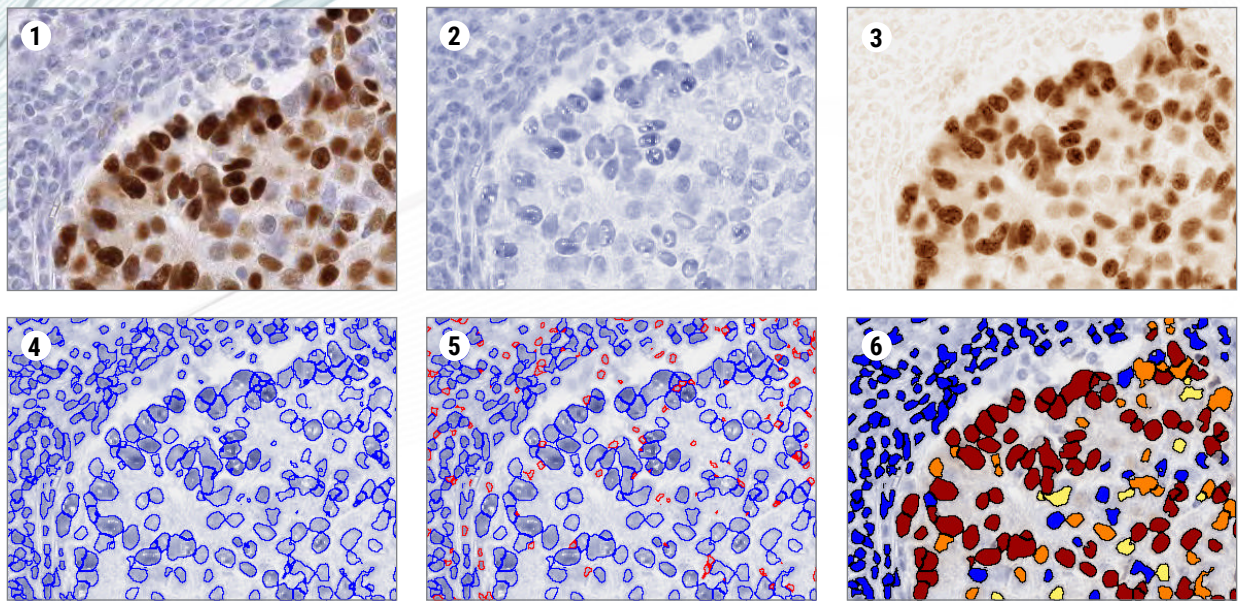
Aperio Nuclear Algorithm

Automatic IHC Nuclear Quantification

Manual nuclear quantification can be time-consuming and subjective, particularly where staining is heterogenous or nuclei are in close proximity. Accurate cell-by-cell nuclear counting allows morphological analysis and quantification of immunohistochemistry (IHC) staining of target proteins.

FLEXIBLE NUCLEAR SEGMENTATION AND ANALYSIS

Original tissue showing negative (Hematoxylin) blue and positive (DAB) brown chromogen nuclear locations. Masks show Aperio Nuclear Algorithm performance where Blue = negative nuclei, Yellow = weak positive nuclei, Orange = moderate positive nuclei and Red = strong positive nuclei.



Tuning steps in Aperio Nuclear Algorithm: 1. Original tissue with blue counterstain & brown positive marker. **2.** Hematoxylin counterstain tuning. **3.** DAB stained biomarker tuning. **4.** Nuclei segmentation. **5.** Nuclear exclusion (red) based on morphology. **6.** Tune thresholds for 0, 1+, 2+ and 3+ staining to generate completed algorithm mask.

Score	Average intensity score (0 to 3+) for the analyzed region, based on user defined thresholds.
Total Nuclei	Total number of cells within the analyzed region, and percent in each intensity scoring group.
Intensity	Overall intensity of staining for positive and negative nuclei.
Nuclear Size	Average size of the nuclei within the analyzed region.

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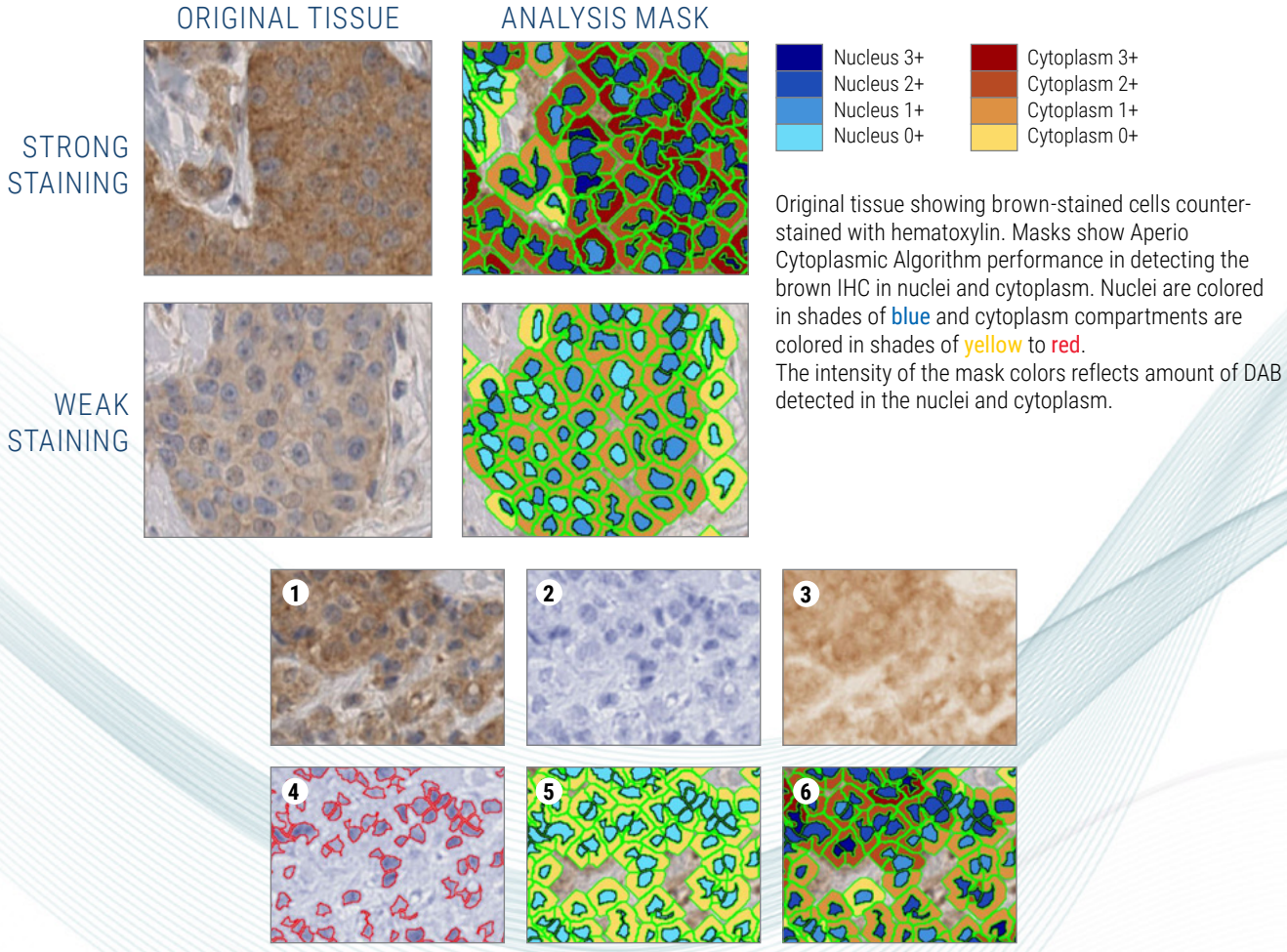
Aperio Cytoplasmic Algorithm

Automatic Positive and Negative Cytoplasm Quantification

Manual analysis of complex IHC staining patterns involving nuclei and cytoplasm can be especially laborious when the nuclei are obscured by strong intensity staining. Automated and quantitative analysis of cellular staining is now possible for any IHC stain with the flexible Aperio Cytoplasmic Algorithm, producing results for both nuclear and cytoplasmic staining for a biomarker.

QUANTITATIVE ANALYSIS OF CYTONUCLEAR STAINING



Original tissue showing brown-stained cells counter-stained with hematoxylin. Masks show Aperio Cytoplasmic Algorithm performance in detecting the brown IHC in nuclei and cytoplasm. Nuclei are colored in shades of blue and cytoplasm compartments are colored in shades of yellow to red.
The intensity of the mask colors reflects amount of DAB detected in the nuclei and cytoplasm.

Tuning steps in Aperio Cytoplasmic Algorithm: **1.** Original tissue dual stained with blue counterstain & brown positive biomarker. **2.** Tuning to identify counterstain. **3.** Tuning to identify IHC. **4.** Nuclei segmentation. **5.** Cytoplasm identification and Cell segmentation. **6.** Complete algorithm mask with 3 scoring bins.

Score	Average intensity score based on number of cells analyzed, their intensity and distribution into the number of scoring bins selected by the user.
Total Nuclei	Total number of nuclei within the analyzed region, and percent in each intensity scoring group.
Intensity	Overall intensity of staining for positive and negative nuclei for both nuclear and cytoplasmic compartments.
Nuclear Size	Average size of the nuclei within the analyzed region.

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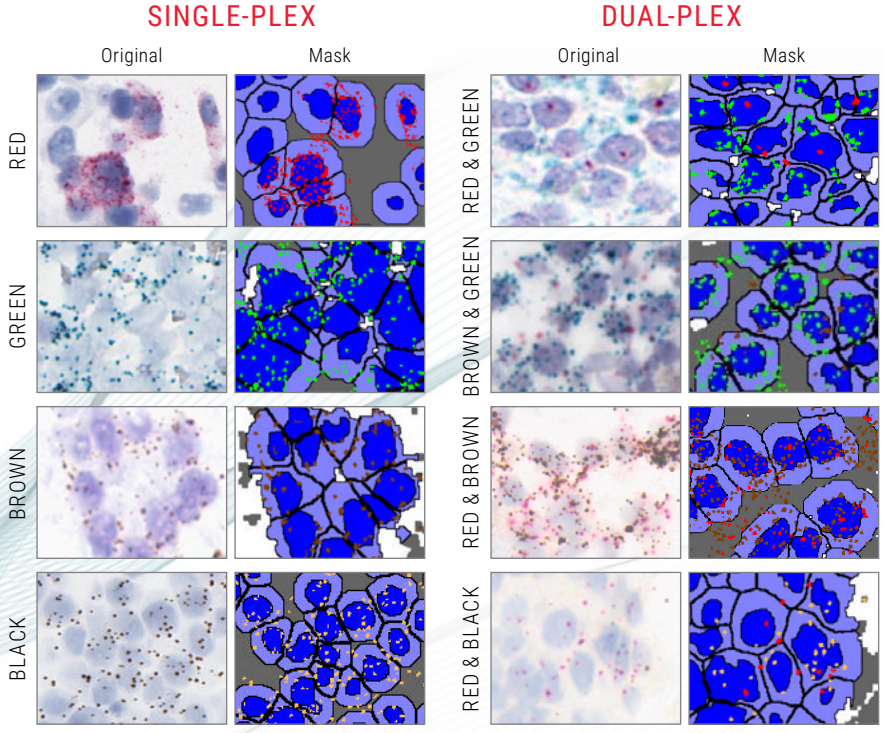
Aperio RNA ISH Algorithm

Automatic RNA *In Situ* Hybridization Quantification

RNA ISH (Ribonucleic acid *in situ* hybridization) enables identification of individual copies of molecular targets within tissue, while maintaining morphology, a feature often lost in other methods such as PCR. Manual RNA ISH interpretation is time-consuming, subject to inter/intra-observer variability and typically employs semi-quantitative reads. The Aperio RNA ISH Algorithm enables accurate counting of individual signals across the tissue, providing standardized, reproducible results, including valuable per-cell data for export.

STANDARDIZATION AND REPRODUCIBILITY THROUGH AUTOMATION

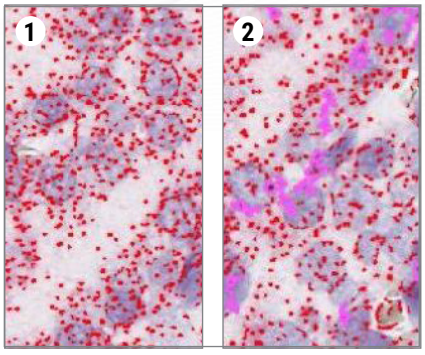


The Aperio RNA ISH algorithm is optimized for specific chromogenic signals:

Single-plex: Red, Green, Brown or Black.
Dual-plex: Red & Green, Brown & Green, Red & Brown, or Red & Black

Masks show Aperio RNA ISH Algorithm performance. Gray is total tissue (non-cellular), light blue is cytoplasm and dark blue indicates nuclei.

Individual RNA signals are also color-coded by chromogen: Red, Green, Brown, and Black.



Signal detection: **1. individual signals**; **2. dots** & **clusters**.

Output parameters	Assay scoring dots only	Assay scoring dots & clusters
Total Cell Count	82	82
Total Cellular Area (um2)	15355.8682	15355.8682
Average Cellular Area (um2)	187.2667	187.2667
Total Nuclear Area (um2)	5306.5289	5306.5289
Average Nuclear Area (um2)	64.7138	64.7138
Total Cytoplasmic Area (um2)	10049.3393	10049.3393
Total Tissue Area (um2)	21413.1895	21413.1895
Number of Cells in '0' for Signal 1	0	0
Number of Cells in '1+' for Signal 1	1	0
Number of Cells in '2+' for Signal 1	32	19
Number of Cells in '3+' for Signal 1	49	63
RNA ISH Score for Signal 1	3+	3+

Table shows a selection of output parameters from the RNA ISH algorithm.

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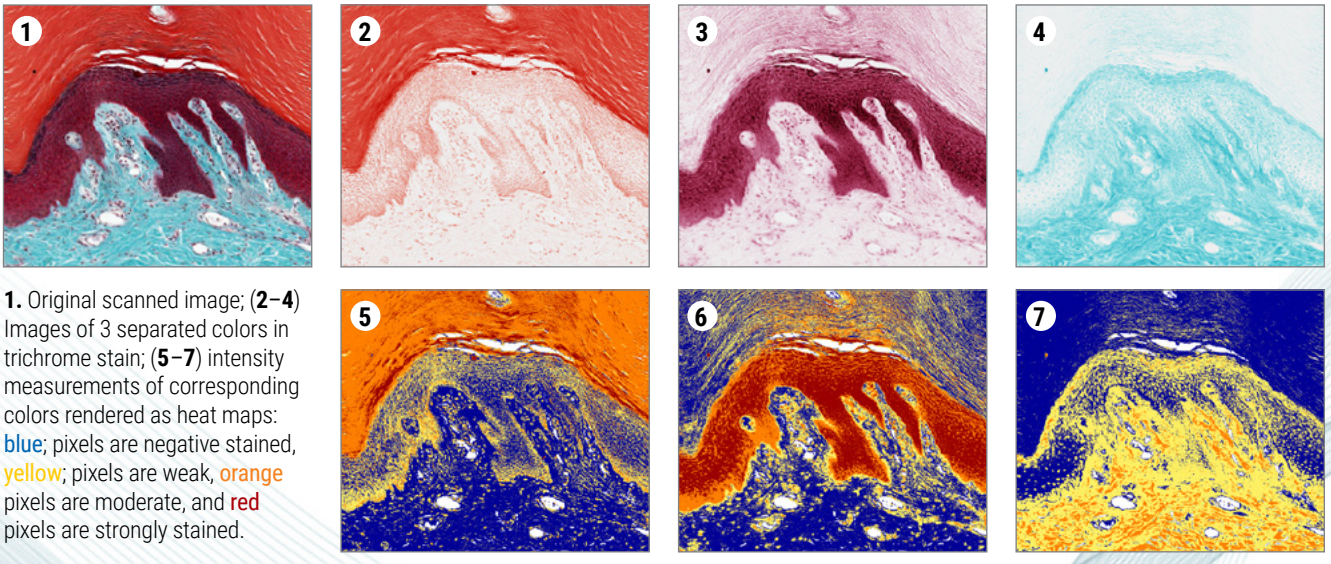
Aperio Color Deconvolution Algorithm

Separate and Analyze Chromogenic Stains

The Aperio Color Deconvolution Algorithm separates a stained tissue image into multiple (up to 3) color channels, corresponding to the actual colors of the stains used. This enables the user to measure both the area and intensity of each stain across the tissue, even when the stains are superimposed at the same location.

Final analysis masks are determined by the user: choose to display any 1 of the 3 separated colors or its corresponding intensity range.

SEPARATE YOUR CHROMOGENS PIXEL BY PIXEL



1. Original scanned image; (**2–4**) Images of 3 separated colors in trichrome stain; (**5–7**) intensity measurements of corresponding colors rendered as heat maps: blue; pixels are negative stained, yellow; pixels are weak, orange pixels are moderate, and red pixels are strongly stained.

The Aperio Color Deconvolution Algorithm gives the user the choice of Analysis mask to display (figures 2-7 above).

When intensity masks are selected, the output parameters are color-coded as shown in the table. This corresponds to the pixel intensities and facilitates data interpretation.

Output parameters	Color 1	Color 2	Color 3
Average Positive Intensity	14.748	155.976	202.397
Percent Weak Positive	23.9508	16.2265	31.5944
Percent Medium Positive	30.7954	12.2239	4.37227
Percent Strong Positive	2.98016	18.6157	0.000975517
Percent Negative	42.2736	52.9339	64.0324
Percent Total Positive	57.7264	47.0661	35.9676
Average Weak Positive Intensity	171.92	207.545	205.681
Average Medium Positive Intensity	133.495	153.909	178.693
Average Strong Positive Intensity	100.761	112.382	104.333
Total Stained Area (mm^2)	0.361364	0.418031	0.418031
Total Analysis Area (mm^2)	0.44504	0.44504	0.44504
Score (0–300)	94.4822	96.5214	40.3418

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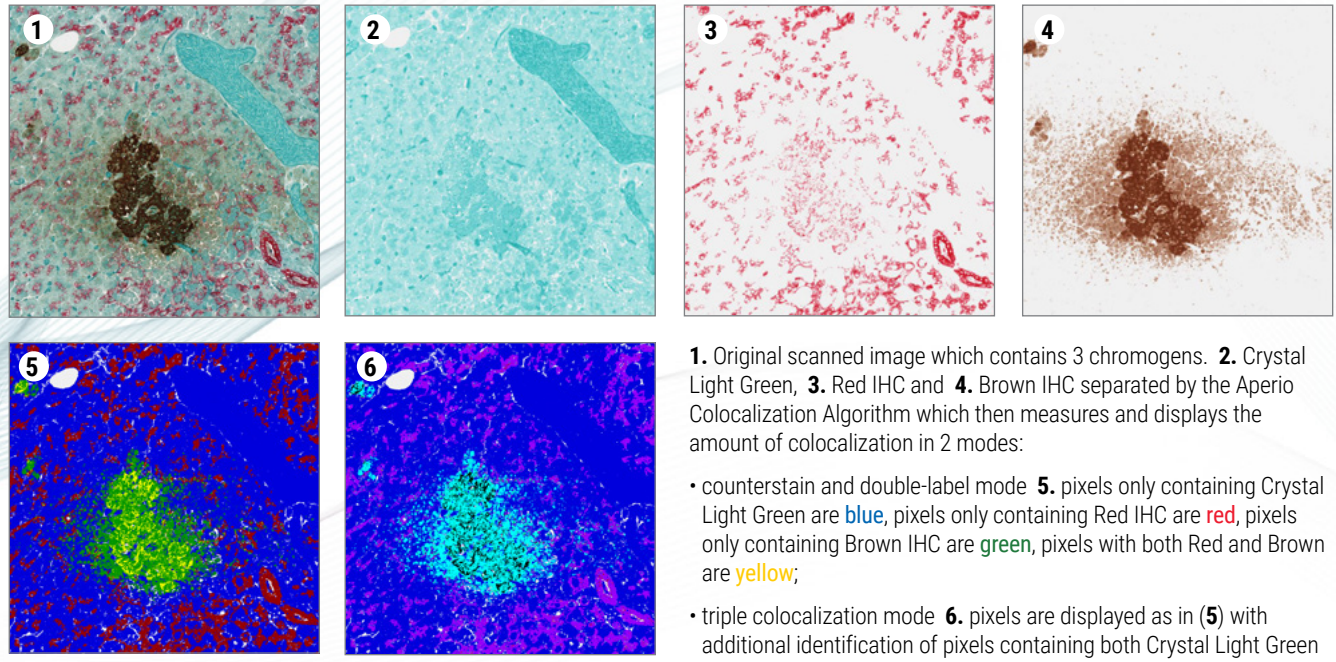
Aperio Colocalization Algorithm

Determine Colocalization of your Chromogen Stains

In histology, a variety of staining methods are used to target different types of tissues, cellular structures and for detection of specific proteins: conventional histochemistry, immunohistochemistry and *in situ* hybridization. Colocalization of multiple antigens is an important part of larger scientific studies, which seek to determine a correlation between the occurrence of these proteins and the outcome of a specific disease treatment.

The Aperio Colocalization Algorithm separates chromogens and classifies each pixel as either a single chromogen or representing a combination of chromogens based on the deconvolution data. The contribution of each stain at every pixel location in the image is then calculated. For IHC, the algorithm determines where specific proteins are present and to what extent the proteins are "colocalized" – that is, whether they occur separately or in combination with each other in the same space.

FLEXIBLE PIXEL-BASED COLOCALIZATION OF YOUR STAINS



1. Original scanned image which contains 3 chromogens. **2.** Crystal Light Green, **3.** Red IHC and **4.** Brown IHC separated by the Aperio Colocalization Algorithm which then measures and displays the amount of colocalization in 2 modes:

- counterstain and double-label mode **5.** pixels only containing Crystal Light Green are blue, pixels only containing Red IHC are red, pixels only containing Brown IHC are green, pixels with both Red and Brown are yellow;
- triple colocalization mode **6.** pixels are displayed as in (**5**) with additional identification of pixels containing both Crystal Light Green and Red shown as mauve, pixels with both Crystal Light Green and Brown are aqua; pixels containing all 3 stains are **black**.

The Aperio Colocalization Algorithm gives the user the choice of Analysis mask to display (figures 2-6 above). The output parameters are color-coded as shown in the table to facilitate data interpretation.

Output parameters	Color 1 (figure 2)	Color 2 (figure 3)	Color 3 (figure 4)	Counterstain & Double Label mode (figure 5)	Counterstain & Double Label mode (figure 6)
Percent (1)	61.41	61.41	61.41	61.41	61.41
Percent (1+2)	14.7354	14.7354	14.7354	0	14.7354
Percent (2)	0.635811	0.635811	0.635811	15.3713	0.635811
Percent (2+3)	0.331755	0.331755	0.331755	2.55495	0.331755
Percent (3)	0.973846	0.973846	0.973846	20.6638	0.973846
Percent (1+3)	19.6899	19.6899	19.6899	0	19.6899
Percent (1+2+3)	2.52177	2.52177	2.52177	0	2.52177
Overall Intensity (1)	195.104	195.104	195.104	195.104	195.104
Overall Intensity (2)	164.539	164.539	164.539	164.539	164.539
Overall Intensity (3)	137.745	137.745	137.745	137.745	137.745
Total Stained Area (mm^2)	0.616941	0.616941	0.616941	0.616941	0.616941
Total Analysis Area (mm^2)	0.651389	0.651389	0.651389	0.651389	0.651389

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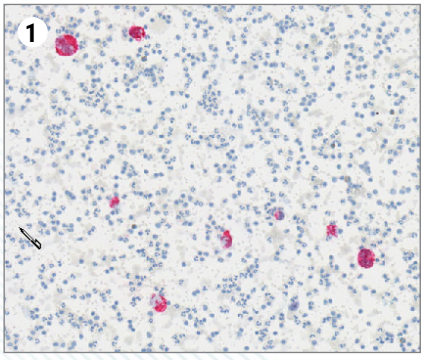
Aperio Rare Event Algorithm

Automatic Micrometastasis Quantification

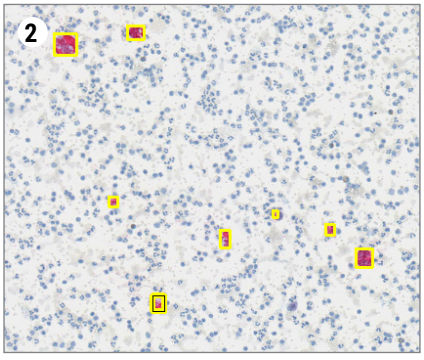
Detection of rare events plays an important role in various biomedical disciplines. In oncology research, it is used to detect and quantify minimal residual disease in tissues or those circulating tumor cells (CTCs) in peripheral blood. In radiation research, the rare number of mutant cells may be counted as a parameter related to the mutagenic effect *in vivo*. Similarly, virus-infected cells circulating in low frequencies in peripheral blood may provide useful research information such as the early detection of cytomegalovirus (CMV) reactivation in transplantation.

Visual inspection of such samples is a laborious task and rare cells can be easily missed, even with the help of antibodies directed to characteristic cellular constituents within the cells of interest. The Aperio Rare Event Algorithm will automatically detect and quantify stained rare cells, and can be tuned to detect the various color, size and forms that micrometastatic structures can assume.

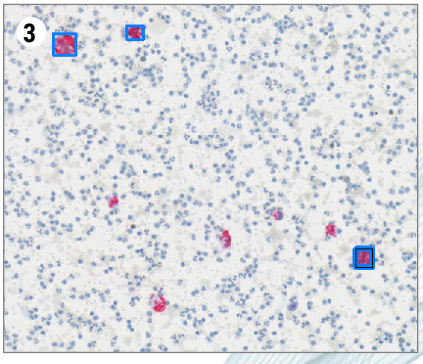
STANDARDIZATION AND REPRODUCIBILITY THROUGH AUTOMATION



1. Original tissue with **blue** counter-stain and **red** positive metastatic stain.

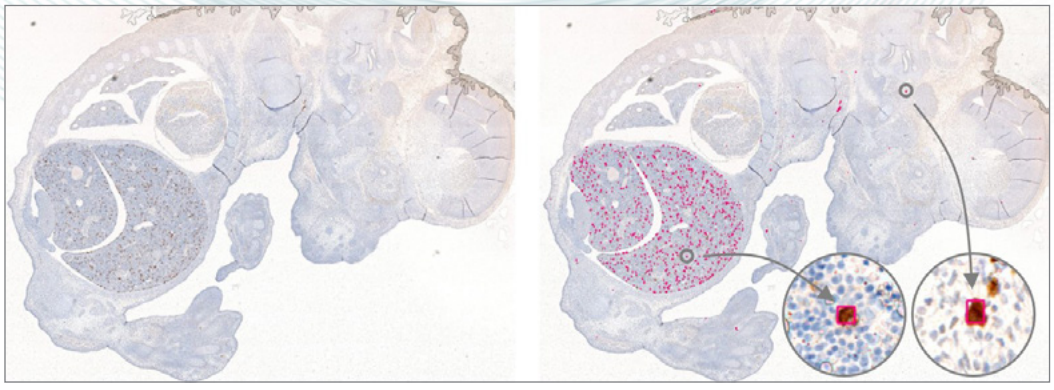


2. Tuning set to include all **red** events, boxed in **yellow**.



3. Tuning set to include only large sized **red** events using the size filter, boxed in **blue**.

The outputs of the Rare Event algorithm, namely, the total number of objects and number of object pixels are the typical information needed for such application(s). Each event is visited as the user clicks through the list of cells found. Results are exported in .csv format for rapid integration into 3rd party statistical or data analysis package. In addition, the algorithm results mask adds a box around positive events detected which aids visualization.



4. Image of rodent embryo with transgene expression detected by Brown IHC.

5. Aperio Rare Event analysis mask displaying 844 objects identified. Two objects are shown at higher power.

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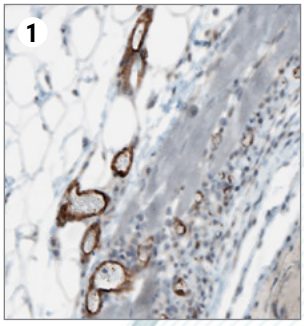
Aperio Microvessel Analysis Algorithm

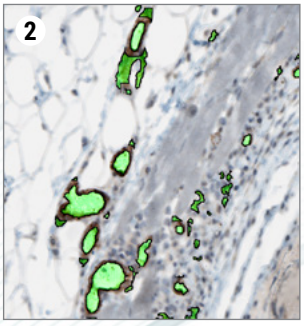
Automatic Angiogenesis Quantification

Virtually all solid tumors require angiogenesis for growth and this process can be tracked by immunostaining for endothelial markers such as CD31 or CD34. Assessing microvessel density and vessel distribution by eye can be challenging. The Aperio Microvessel Analysis Algorithm generates quantitative, standardized data based on user settings, and can be tuned to any endothelial marker stain.

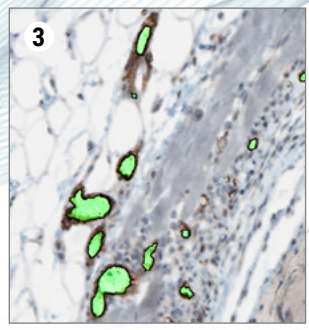
STANDARDIZED MICROVESSEL DETECTION & ANALYSIS



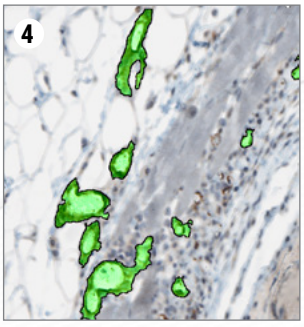
1. Original tissue with blue counter-stain and brown positive endothelial biomarker



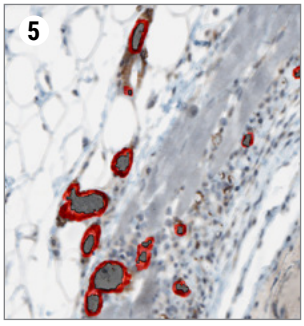
2. Inclusion of incomplete vessels and other stained regions with regular vessels



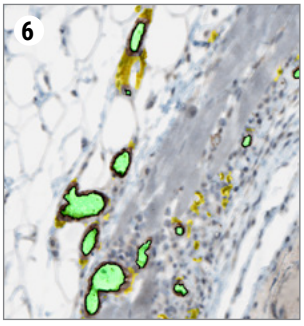
3. Inclusion of irregular long vessels with exclusion of incomplete vessels and other stained regions



4. Inclusion of irregular long vessels with inclusion of incomplete vessels and other stained regions



5. Mode type set to highlight vessel wall and lumen



6. Mode type set to highlight included and excluded vessels

The Aperio Microvessel Analysis Algorithm gives the user the choice of Analysis mask to display (figures 4-6). Alternative masks to highlight different features of microvessels, such as Lumen, Vessel Walls and Excluded Vessels, the output parameters are color-coded as shown in the table. This facilitates data interpretation.

Number of Vessels	23
Total Analysis Area (um2)	469829.9
Total Stain Area (um2)	21939.9
Average Stain Intensity	160.303
Microvessel Density --Number of vessels per unit area (um2)	4.89539e-005
Mean Vessel Area (um2)	1180.22
Median Vessel Area (um2)	773.
Standard Deviation of Vessel Area (um2)	1172.5
Mean Vessel Perimeter (um)	208.913
Median Vessel Perimeter (um)	186.
Standard Deviation of Vessel Perimeter (um)	105.005
Mean Lumen Area (um2)	475.522
Median Lumen Area (um2)	165.
Standard Deviation of Lumen Area (um2)	681.941
Mean Vascular Area (um2)	722.174
Median Vascular Area (um2)	525.
Standard Deviation of Vascular Area (um2)	603.06
Mean Vessel Wall Thickness (um)	3.1052
Median Vessel Wall Thickness (um)	3.13265
Standard Deviation of Vessel Wall Thickness (um)	1.06998
Average Red OD	0.459518
Average Green OD	0.616612
Average Blue OD	0.639244
--Histogram Results --	-- Histogram Results --
Total Number of Vessels	23
Number of Vessels in Histogram Analysis	23
Overall Minimum Vessel Area (um2)	175.
Overall Maximum Vessel Area (um2)	5205.
Vessel Area Bin Centers (um2)	Frequency
130.125	3
390.375	4
650.625	5
910.875	2
1171.13	2
1431.38	1

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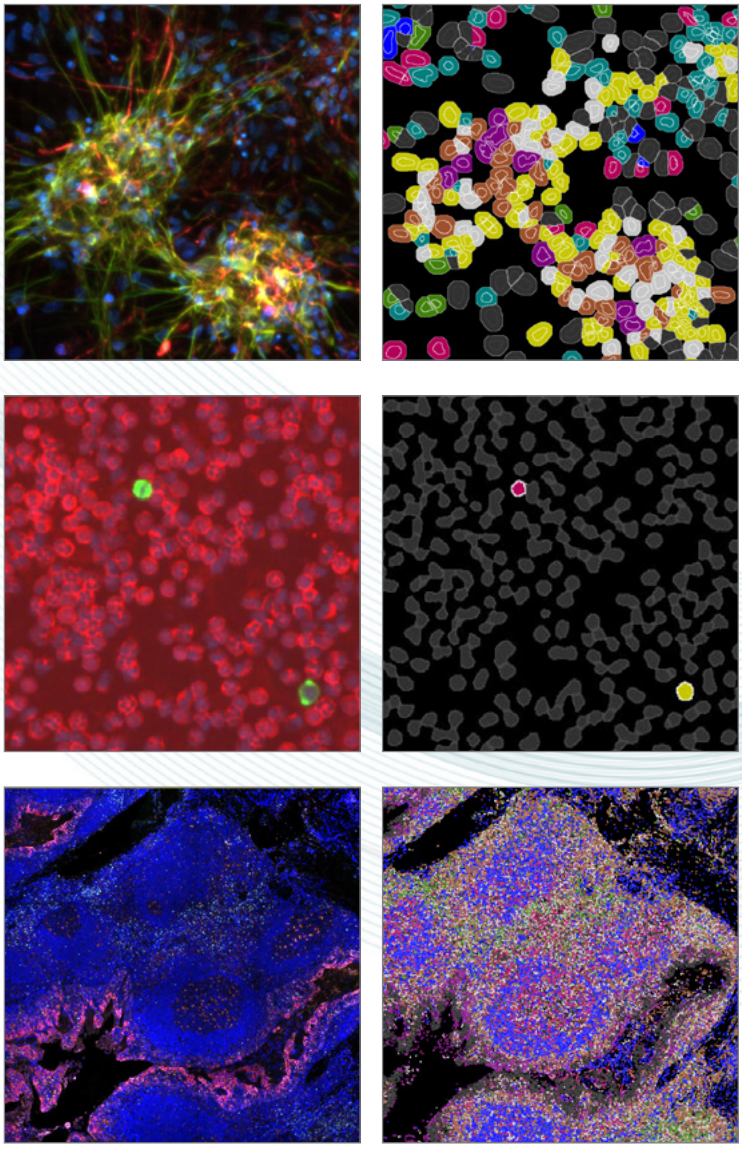
Aperio Cellular Immunofluorescence Algorithm

Automatic Cellular Based Immunofluorescence Phenotyping

Immunofluorescence staining enables application and quantification of multiple fluorescent dyes on tissue. It provides a level of multiplex analysis beyond what is possible with traditional brightfield IHC with chromogens. The complexity of immunofluorescence staining can make it challenging to quantitate accurately by eye. The Aperio Cellular IF Algorithm enables accurate measurement and colocation (present in same cell compartment) of up to 7 fluorescence channels in a single tissue section, localized within membrane, nuclear and/or cytoplasmic cellular compartments. The algorithm results enable interpretation of multiplex staining, to generate cell phenotypes within a tissue for a wide variety of biomedical tissue-based applications including CTC and Immunoncology research.

ACCURATE AND REPRODUCIBLE MULTIPLEX QUANTIFICATION FOR WHOLE SLIDE IMAGES

ORIGINAL IMAGE | ANALYSIS MASK



Neuroscience 7-plex assay

Scoring Class	ROI	Whole Image
Phenotype 1	18	3,125
Phenotype 2	116	3,992
Phenotype 3	51	2,341
Phenotype 4	15	1,204
Phenotype 5	99	5,106
Phenotype 6	5	1,247
Phenotype 7	12	939
Unclassified	82	8,114
Total Cells	349	24,198

Circulating Tumor Cell (CTC) 2-plex assay

Scoring Class	ROI	Whole Image
CTC	1	39
All dual stained cells	1	63
Unclassified	290	89,320
Total Cells	292	89,422

Immunoncology 4-plex assay

Scoring Class	ROI	Whole Image
No staining	24,670	42,860
CD68	24,229	86,232
CD8	8,442	103,909
panCK/SOX10	5,040	205,914
PD-L1	13,438	200,790
Unclassified	2,879	20,153
Total Cells	78,698	553,085

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Aperio FISH Amplification/Deletion Algorithm

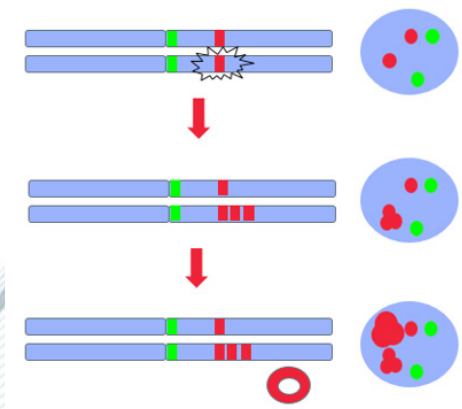
Automatic Fluorescence *In Situ* Hybridization Amplification & Deletion Evaluation

FISH (fluorescence *in situ* hybridization) enables highly-specific and sensitive detection of abnormalities within target DNA regions, including amplification or deletion of gene sequences.

Manual FISH counting is time-consuming and subject to inter/intra-observer variability. It is usually carried out in a dark room for optimal viewing and to reduce fading of the fluorescence probes, which can be fatiguing. The Aperio FISH Amp/Del Algorithm enables amplification or deletion of target DNA sequences to be automatically quantified on digital slides, providing results that are presented in typical FISH scoring class mode.

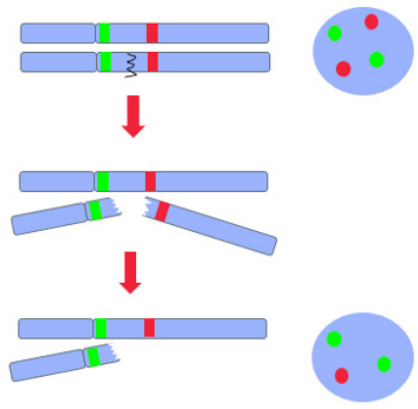
RAPID AND STANDARDIZED SCORING FOR YOUR FISH IMAGES

AMPLIFICATION ASSAY

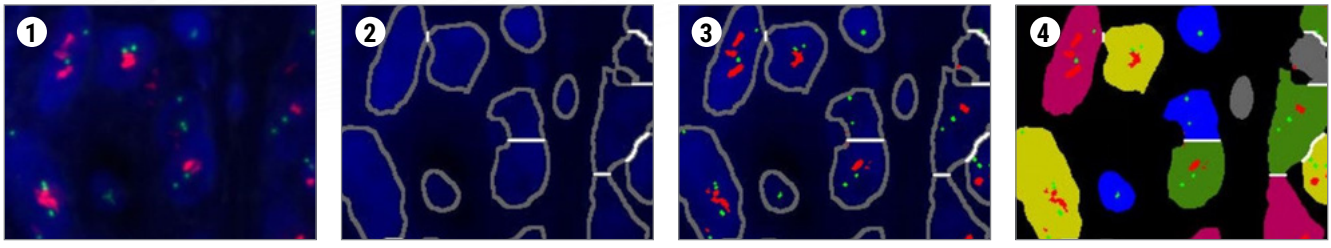


Target gene, e.g. HER2, is in red with a control probe for the same chromosome in green. Normal cells have 2 copies of each. In abnormal cells an event triggers the target gene for amplification, and several copies appear on the chromosome, seen by a cluster of signals. Further copies can be generated in the form of extraneous material (e.g. ring chromosomes) that produce even larger signals in a cell. Aperio FISH Amp/Del Algorithm can automatically detect and quantify these gene amplification events.

DELETION ASSAY



Test gene, e.g. BRCA1, is in red with a control probe for the same chromosome in green. Normal cells have 2 copies of each. In abnormal cells an event targets the red gene for deletion and the chromosome breaks, losing one copy of the red gene. This reduces the number of red signals, but not the number of green signals in the cell. Aperio FISH Amp/Del Algorithm can automatically detect and enumerate these gene deletion events.



Tuning steps in Aperio FISH Amp/Del Algorithm: **1.** Original tissue showing probes labeled with Spectrum Green and Spectrum Orange plus DAPI nuclear counterstain. **2.** Automatic nuclear segmentation. **3.** Detection of control and target signals, marked up in the corresponding color (red/green). **4.** Mark-up showing scoring classification of cells.

Cell Counts	Count of total number of cells, defined as number of nuclei identified.
Signal Counts	Count number and area of each signal within nuclei, and number of nuclei containing each signal.
Ratio	Ratio of target to control signals in nuclei.
Scoring Classification	Number and percentage of cells in each user-defined scoring class.

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Aperio FISH Breakapart/Fusion Algorithm

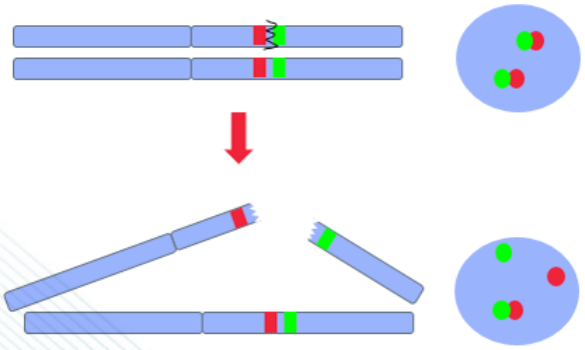
Automatic Fluorescence *In Situ* Hybridization Break-Apart & Fusion Quantification

FISH (fluorescence *in situ* hybridization) can be used to detect DNA sequence translocations within chromosomes (break-apart and fusion) with a high degree of specificity and sensitivity.

The Aperio FISH Brk/Fus Algorithm enables detection and scoring of break-apart and fusion events within whole slide digital images. The algorithm reduces the need to perform time-consuming manual FISH-counts in a dark room. Since the analysis is carried out on digital images, there is no risk of fluorescence signal fading, giving you a permanent copy of your slides, signal and results that are presented in typical FISH score class mode.

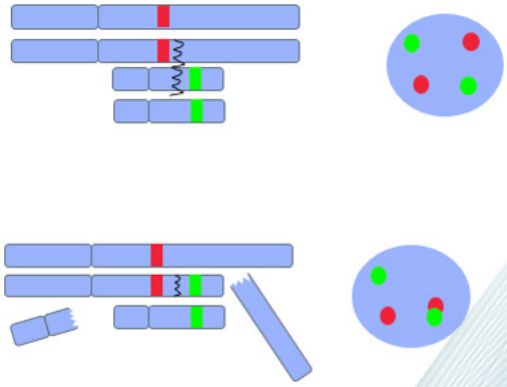
RAPID AND STANDARDIZED SCORING FOR YOUR FISH IMAGES

BREAK-APART ASSAY

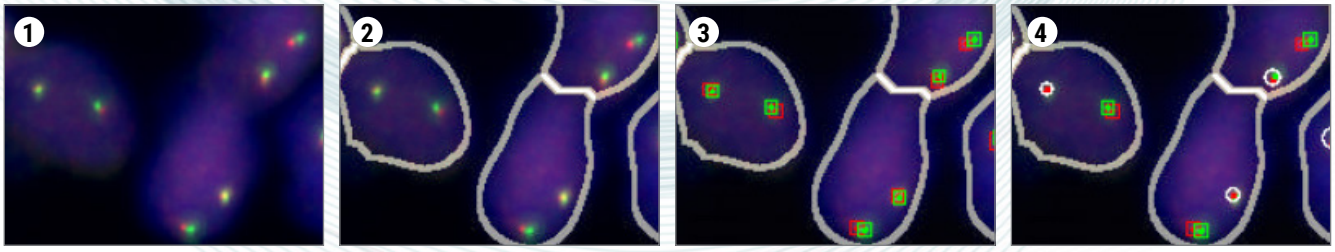


Assay is used to map a known genetic break-point, e.g. ALK. Either side of the break-point is marked by a different colored probe. Normal cells show 'fused' signals where the red and green, for each chromosome, are very close together in the cell. When the breakage event occurs, the chromosome fragments move apart and the red and green signals are separated. Aperio FISH Brk/Fus Algorithm can automatically detect and enumerate these break-apart events.

FUSION ASSAY



Assay is used to map a known genetic break-point, e.g. BCR/ABL. A known translocation of chromosomes is marked with different colored probes, one on each chromosome. Normal cells show clearly separated signals. When the rearrangement event occurs in abnormal cells, the red and green signals come together in the newly-formed chromosome. Aperio FISH Brk/Fus Algorithm can automatically detect and enumerate these fusion events.



Tuning steps in Aperio FISH Brk/Fus Algorithm: **1.** Original tissue showing probes labeled with Spectrum Green and Spectrum Orange plus DAPI nuclear counterstain. **2.** Automatic nuclear segmentation. **3.** Signal of interest detection, marked up in the corresponding color (red/green). **4.** Identification of detected fusion groups, circled in white.

Cell Counts	Count of total number of cells, defined as number of nuclei identified.
Signal Counts	Count number and area of each signal within nuclei, and number of nuclei containing each signal.
Fusion Counts	Count of each fusion type present in nuclei.
Scoring Classification	Number and percentage of cells in each user-defined scoring class. Scoring classes based on probe signal and fusion signal counts within each nucleus.

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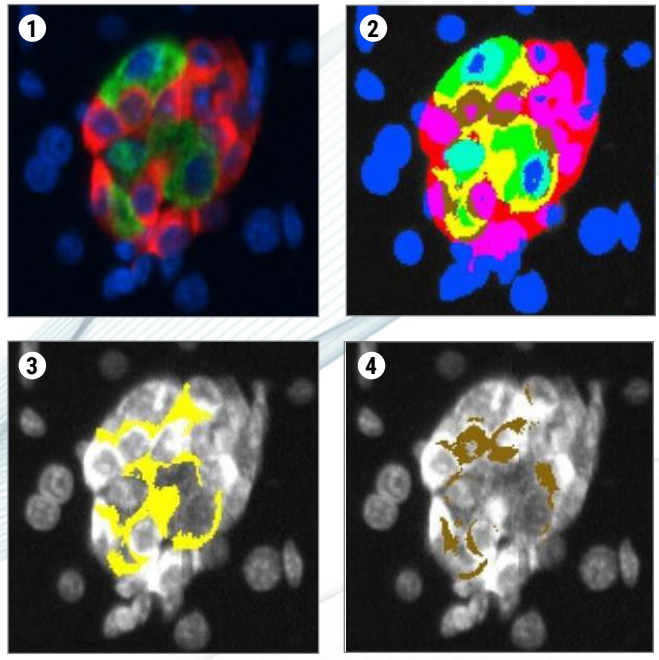
Aperio Area Quantification FL Algorithm

Precision Pixel-Based Analysis of Fluorescent Images

The sensitivity of immunofluorescent techniques often enables researchers to gain more data about their biomarkers of interest than traditional immunohistochemical techniques. In addition, the ability to multiplex fluorescent assays generates more data in a single slide, ideal for limited tissue samples. The frequency with which two fluorochromes occupy the same pixels in an image is an easy first step to determine if the two target antigens directly interact with one another.

The Area Quantification FL algorithm quantifies up to three fluorochromes in an image, providing both individual intensity and colocalization data for all inputs.

FAST AND FLEXIBLE COLOCALIZATION OF YOUR FLUOROCHROMES



1. Original scanned image with DAPI, FITC and Cy3 fluorochromes captured as individual channels/layers. The Aperio Area Quantification FL Algorithm sets thresholds for each and then determines which positive pixels contain 1, 2, or 3 fluorochromes. User can determine which color-coded analysis masks to display, including:

2. All combinations of fluorochromes: DAPI only in blue, FITC only in green, Cy3 only in red, colocalized DAPI and FITC in aqua, colocalized DAPI and Cy3 in magenta, colocalized FITC and Cy3 in yellow, colocalized DAPI, FITC and Cy3 in gold;

3. Those pixels where FITC and Cy3 colocalize, are highlighted in yellow and pixels positive for all other combinations of the 3 fluorochromes are colored white;

4. Those pixels where all 3 fluorochromes colocalize are highlighted in gold and pixels positive for all other combinations of the 3 fluorochromes are colored white.

The Aperio Area Quantification FL Algorithm gives the user the choice of Analysis mask to display (such as figures 2-4 above).

As shown in the table, output parameters are color-coded to correspond to the color-blending of fluorochromes as shown in the table to facilitate data interpretation.

Output parameters	
Total Analysis Area (mm^2)	0.7341
Total Stained Area (mm^2)	0.09338
CO-LOCALIZATION OUTPUTS	
Percent (DAPI)	61.03
Percent (CY3)	11.95
Percent (CY5)	1.669
Percent (DAPI + CY3)	5.357
Percent (DAPI + CY5)	17.51
Percent (CY3 + CY5)	0.6272
Percent (DAPI + CY3+ CY5)	1.856
Pearson (DAPI, CY3)	-0,09718
Overlap (DAPI, CY3)	0.9104
Pearson (DAPI, CY5)	0.1159
Overlap (DAPI, CY5)	0.8856
Pearson (CY3, CY5)	-0.1246
Overlap (CY3, CY5)	0.94

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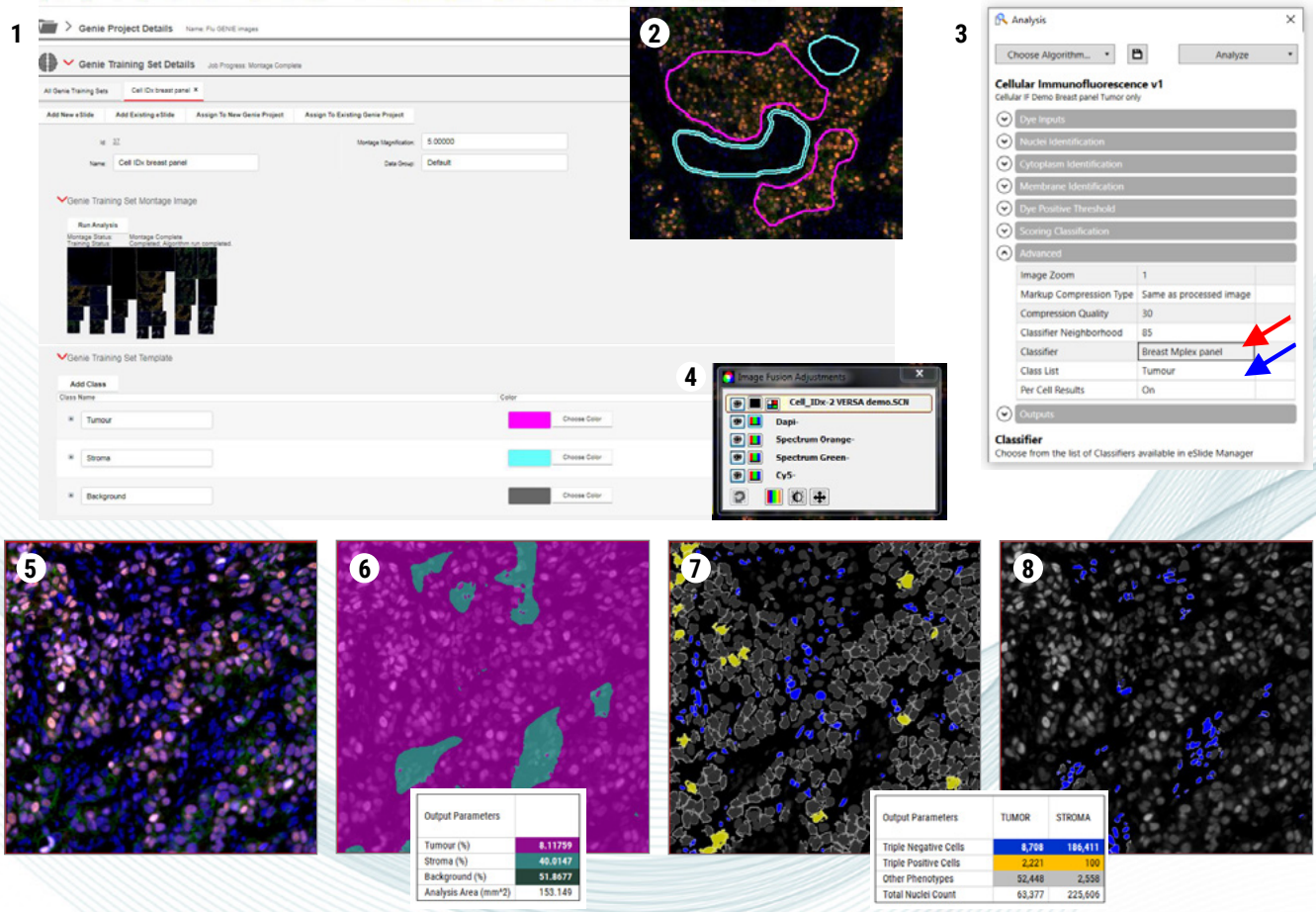
Chaining Aperio Algorithms

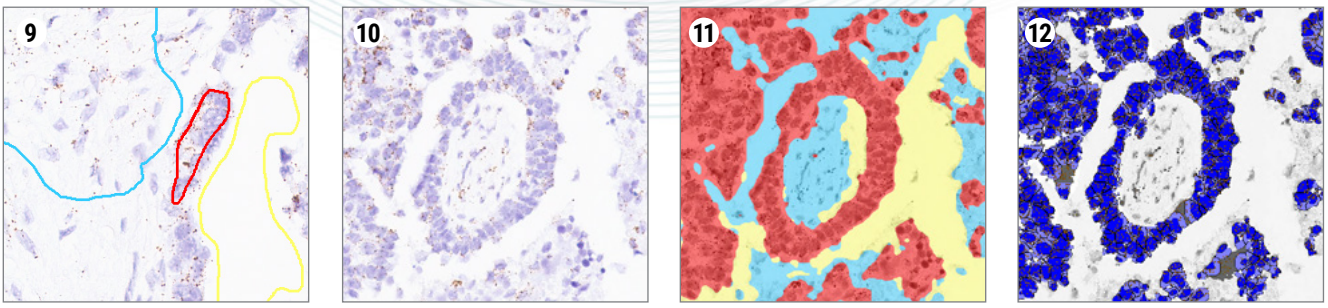
Automate Tissue Finding for Seamless Biomarker Analysis

Aperio GENIE can be used as a pre-classifying step for other Aperio Image Analysis algorithms, directing the algorithm to analyze only specific tissue types in heterogeneous samples. This removes the overhead of manual annotation and automates your analysis.

Aperio Cellular IF algorithm running chained with Aperio GENIE pre-classifier: 1. GENIE training set with 3 classes; **2.** example annotations for classes; **3.** Aperio Cellular IF algorithm (macro) optimized for breast tissue biomarkers with Aperio GENIE classifier chained to macro (red arrow) to direct Cellular IF analysis to only areas of Tumor (blue arrow) in breast tissue; **4.** fluorochromes in; **5.** original 3-plex image analyzed with; **6.** Aperio GENIE 'Breast Mplex panel' classifier only; **7.** Cellular IF macro Tumor tissue only; and **8.** Cellular IF. macro Stroma tissue only



Aperio GENIE pre-classifier chained with Aperio RNA ISH Algorithm: 9. Annotated Aperio GENIE classes: red = Tumor, blue = Stroma, yellow = Glass (N.B. the RNA ISH signals do not form part of the classification); **10.** Original image of RNA ISH probe with brown chromogen on ovarian tissue; **11.** Aperio GENIE analysis of the image; **12.** Aperio RNA ISH Algorithm analyzing tumor, as directed by the Aperio GENIE "tumor" classifier



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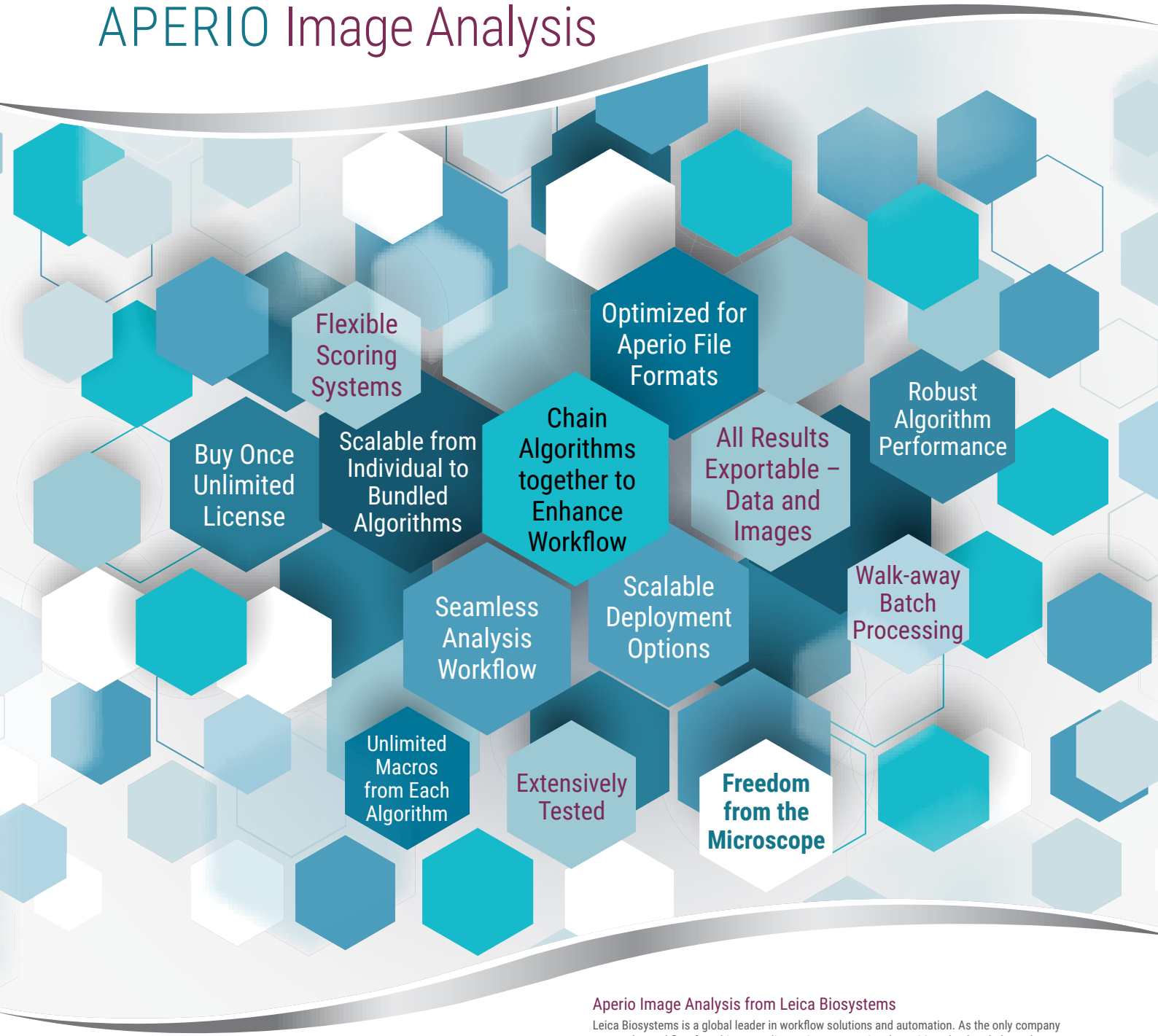
APERIO Image Analysis



Leica Biosystems is an international company with a strong network of worldwide customer services. For detailed contact information on your nearest sales office or distributor please visit our website: LeicaBiosystems.com

Aperio RUO (Research Use Only) Image Analysis Algorithms have been validated by Leica Biosystems for use with .svs images from Aperio AT2, Aperio CS2, and Aperio VERSA RUO scanners. Use of Aperio RUO Algorithms with other available scanners has not been validated, and Leica Biosystems cannot train or support customers in use of Aperio RUO Algorithms with images from these scanners.

Aperio Image Analysis from Leica Biosystems

Leica Biosystems is a global leader in workflow solutions and automation. As the only company to own the workflow from biopsy to diagnosis, we are uniquely positioned to break down the barriers between each of these steps. Our mission of "Advancing Cancer Diagnostics, Improving Lives" is at the heart of our corporate culture. Our easy-to-use and consistently reliable offerings help improve workflow efficiency. The company is represented in over 100 countries. It has manufacturing facilities in 9 countries, sales and service organizations in 19 countries, and an international network of dealers. The company is headquartered in Nussloch, Germany. Visit LeicaBiosystems.com for more information.

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