Advancing Cancer Diagnostics
Improving Lives

Leica BIOSYSTEMS

Aperio Colocalization Algorithm

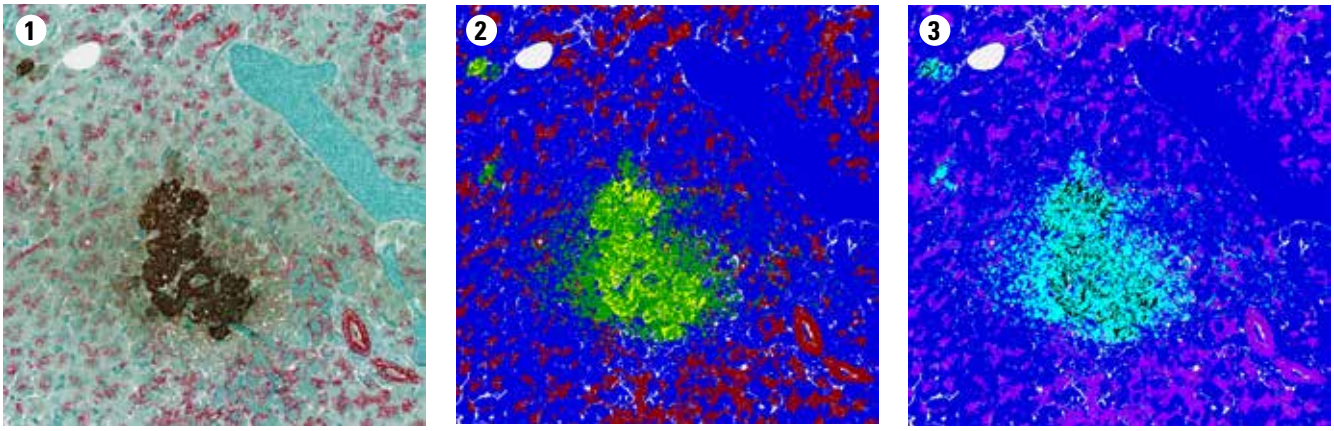
Determine precise colocalization of your chromogen stains

In histology and cytology, 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 deconvolves 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.

FAST AND FLEXIBLE COLOCALIZATION OF YOUR STAINS

- Simple RGB sliders will calibrate the stain color vectors to separate the stains in the image
- Independent thresholding and scoring for deconvolved colors
- Generate alternative outputs and markups of deconvolved colors and colocalization scores:
  - Counterstain with double-label mode, or
  - Triple colocalization mode
- Optimized for Aperio scanners
- Use with 20X or 40X whole slide images and regions of interest (identified by annotations or suitable GENIE classifier)
- Compatible with Aperio eSlide Manager or Aperio Image Analysis Workstation



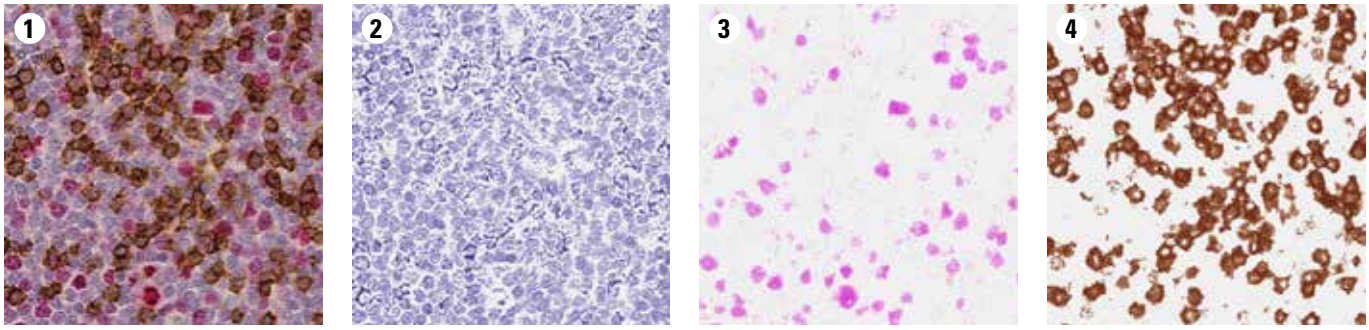
The Aperio Colocalization Algorithm separates the three stains in (1) the original scanned image: Crystal Light Green, Fast Red and DAB; the algorithm then measures and displays the amount of colocalization in 2 modes: either (2) counterstain and double-label mode, or in (3) triple colocalization mode. In markup image (2), pixels only containing Crystal Light Green are **blue**, pixels only containing Fast Red are **red**, pixels only containing DAB are **green**, pixels with both Fast Red and DAB are **yellow**. In markup image (3), pixels only containing Crystal Light Green are **blue**, pixels only containing Fast Red are **red**, pixels only containing DAB are **green**; pixels with both Fast Red and DAB are **yellow**, pixels with both Crystal Light Green and Fast Red are **mauve**, pixels with both Crystal Light Green and DAB are **aqua**; pixels containing all 3 stains are **black**.

Aperio Colocalization Algorithm

Fast and Accurate Pixel-Based Colocalization

ADJUSTABLE ALGORITHM INPUT PARAMETERS

Rigorously tested default parameters enable the Aperio Colocalization Algorithm to be used in a highly automated, one-click mode for the default stains. In addition, tuneable input parameters enable rapid algorithm optimization, while the intuitive Algorithm Tuning interface provides real-time feedback on adjusted settings.



(1) Original scanned image; (2) Deconvolved channel 1 is hematoxylin; (3) Deconvolved channel 2 is Fast Red; (4) Deconvolved channel 3 is DAB. Final colocalization masks are either (5) counterstain and double-label mode, or in (6) triple colocalization mode.

In markup image (5), pixels only containing hematoxylin are **blue**, pixels only containing Fast Red are **red**, pixels only containing DAB are **green**; pixels with both Fast Red and DAB are **yellow**. In markup image (6), pixels only containing hematoxylin are **blue**, pixels only containing Fast Red are **red**, pixels only containing DAB are **green**; pixels with both Fast Red and DAB are **yellow**, pixels with both hematoxylin and Fast Red are **mauve**, pixels with both hematoxylin and DAB are **aqua**; pixels containing all 3 stains are **black**.

COMPREHENSIVE RESULTS OUTPUT

With 27 data points returned for each deconvolved channel, the Aperio Colocalization Algorithm delivers the information your research needs. Data is color-coded to present pixels containing pure deconvolved colors and colocalized stains. Results are easily exported in .csv format for rapid integration into 3rd party statistical or data analysis packages. In addition, analysis masks can be saved for publications and visual representations of the results.

Output parameters	Counterstain and Double-Label mode	Triple-Colocalization mode
Percent (1)	79.6706	79.6706
Intensity (1, 1)	175.699	175.699
Percent (1+2)	0.	9.4827
Intensity (1, 1+2)	0.	182.259
Intensity (2, 1+2)	0.	210.121
Percent (2)	12.7715	3.28879
Intensity (2, 2)	210.034	209.783
Percent (2+3)	3.24242	0.404022
Intensity (2, 2+3)	194.082	191.148
Intensity (3, 2+3)	132.202	129.618
Percent (3)	4.31554	1.09184
Intensity (3, 3)	104.221	108.895
Percent (1+3)	0.	3.2237
Intensity (1, 1+3)	0.	163.899
Intensity (3, 1+3)	0.	102.638
Percent (1+2+3)	0.	2.8384
Intensity (1, 1+2+3)	0.	165.315
Intensity (2, 1+2+3)	0.	194.499
Intensity (3, 1+2+3)	0.	132.569
Overall Intensity (1)	175.643	175.643
Overall Intensity (2)	206.804	206.804
Overall Intensity (3)	116.225	116.225
Total Stained Area (mm^2)	1.61408	1.61408

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1870 Rev A · IMC-1184-REV-A · 01/2018

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