Advancing Cancer Diagnostics
Improving Lives

Leica BIOSYSTEMS

Aperio Area Quantification Fluorescent Algorithm

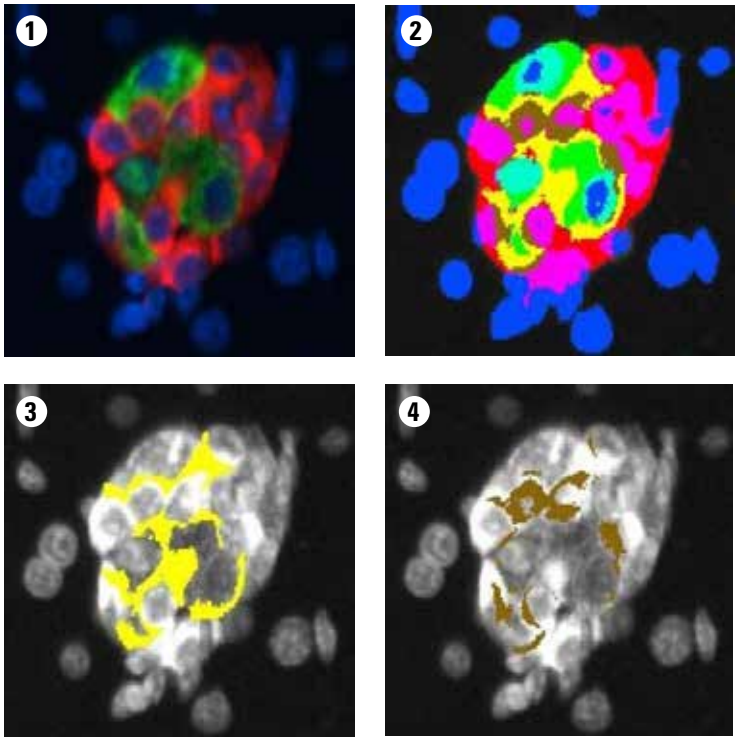
Precision Pixel-Based Analysis of Fluorescent Images

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 STAINS

- Default setting to analyze DAPI, FITC and TRITC fluorochromes
- Easily adapt to other colors using input fluorescent channels
- Independent thresholding for each fluorochrome to remove unwanted background staining
- Generate multiple markups to view pixels containing 0, 1, 2 or all 3 fluochromes: accurate masks of each colocalization group
- Optimized for Aperio scanners & Ariol System
- 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 Area Quantification FL Algorithm is able to threshold and quantify 3 fluorochromes.
(1) Original scanned image with **DAPI**, **FITC** and **Cy3** fluorochromes;
(2) Mask to show all positive pixels containing any combination of the 3 fluorochromes above their threshold settings are color-coded: 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) Mask to show pixels positive for both FITC and Cy3 are highlighted in **yellow** (i.e. colocalized FITC and Cy3) while pixels positive for all other combinations of the 3 fluorochromes are colored white); and
(4) Mask to show pixels positive for DAPI, FITC and Cy3 are highlighted in **gold** (pixels positive for all other combinations of the 3 fluorochromes are colored white).

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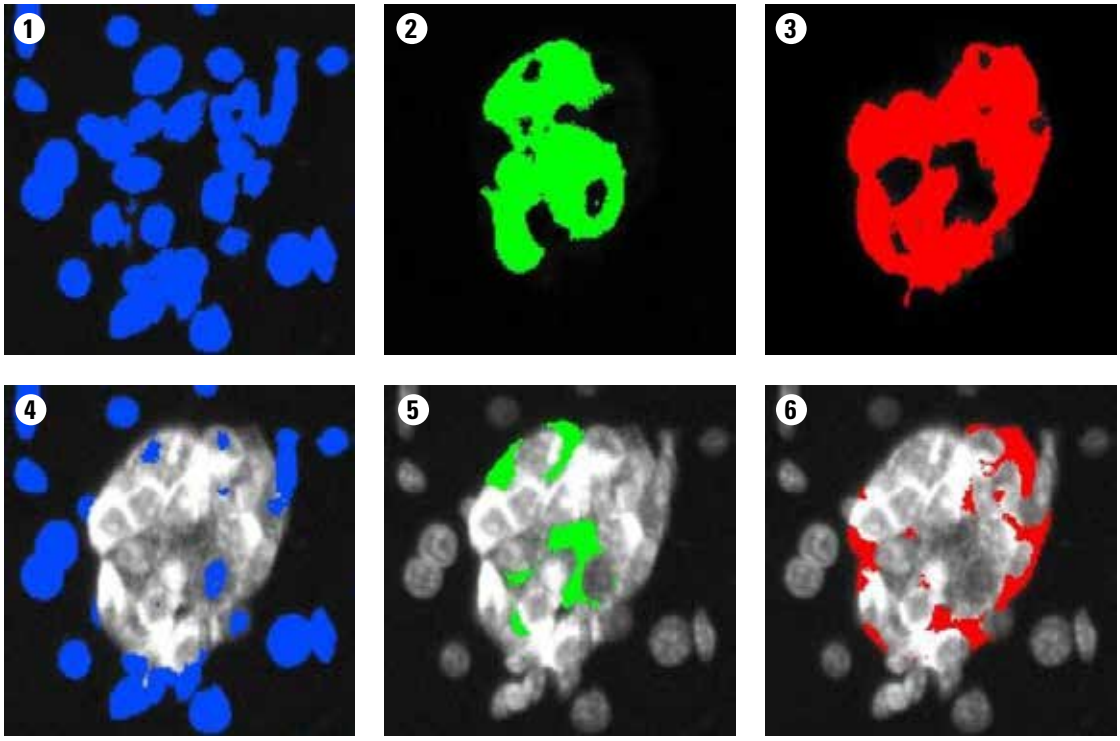
Aperio Area Quantification Fluorescent Algorithm

Simple • Effective • Accurate

ADJUSTABLE ALGORITHM INPUT PARAMETERS

Default parameters enable the Aperio Area Quantification FL Algorithm to be used in a highly automated, one-click mode for analyzing DAPI, FITC and TRITC. In addition, tuneable input parameters enable rapid algorithm optimization, while the intuitive Algorithm Tuning interface provides real-time feedback on adjusted settings.



Masks showing (1) thresholded DAPI signal colored **blue**; (2) thresholded FITC signal colored **green**; and (3) thresholded Cy3 signal colored **red**. Mask showing (4) pixels positive for only DAPI colored **blue**; (5) pixels positive for only FITC colored **green**; and (6) pixels positive for only Cy3 colored **red**; pixels positive for all other combinations of fluorochromes are colored white.

COMPREHENSIVE RESULTS OUTPUT

The Aperio Area Quantification FL Algorithm returns 31 data points, delivering essential information your research needs. Data is color-coded to present pixels containing single-, double- or triple-signal highlighting any real colocalization events. 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

Total Analysis Area (mm^2)	1.318e-002
Total Stained Area (mm^2)	3.598e-003
CO-LOCALIZATION OUTPUTS	
Percent (DAPI)	60.32
Intensity (DAPI)	0.3322
Percent (FITC)	4.923
Intensity (FITC)	0.8284
Percent (CY3)	6.758
Intensity (CY3)	0.4811
Percent (DAPI + FITC)	3.906
Intensity (DAPI, DAPI + FITC)	0.2968
Intensity (FITC, DAPI + FITC)	0.2616
Percent (DAPI + CY3)	11.66
Intensity (DAPI, DAPI + CY3)	0.2885
Intensity (CY3, DAPI + CY3)	0.4205
Percent (FITC + CY3)	7.794
Intensity (FITC, FITC + CY3)	0.268
Intensity (CY3, FITC + CY3)	0.4083
Percent (DAPI, FITC + CY3)	4.645
Intensity (DAPI, DAPI + FITC+ CY3)	0.2265
Intensity (FITC, DAPI + FITC + CY3)	0.1944
Intensity (CY3, DAPI + FITC + CY3)	0.4201
Pearson (DAPI, FITC)	5.758e-002
Overlap (DAPI, FITC)	0.8513

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