

# Aperio Color Deconvolution Algorithm

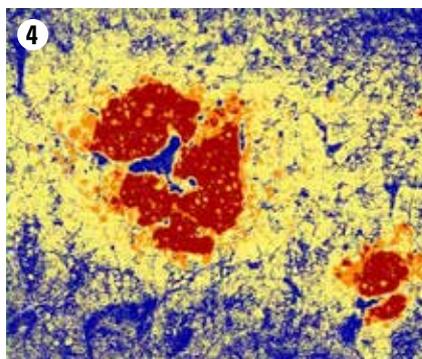
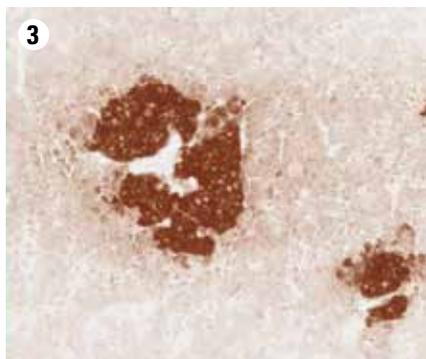
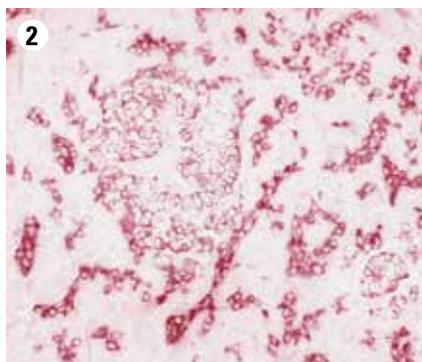
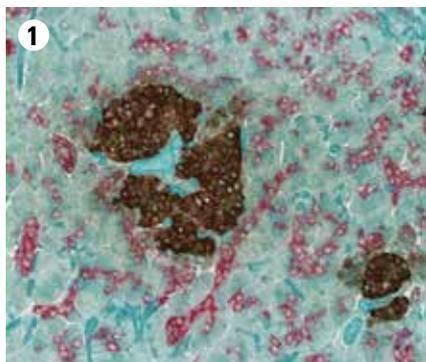
## Separate and Analyze Chromogenic Stains

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 accurately measure both the area and intensity of each stain across the tissue, even when the stains are superimposed at the same location.

Default settings are for Hematoxylin, Eosin and DAB, but the user can tune this flexible algorithm to separate other chromogens.

### FAST AND ACCURATE COLOR SEPARATION

- » One, two or three color separation and analysis
- » Simple RGB sliders will calibrate the stain color vectors to separate the stains in the image
- » Score pixels for stain intensity for each chromogen
- » Generate alternative markups according to user's preferred thresholds
- » 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



Aperio Color Deconvolution Algorithm separates stains:

(1) Original scanned image;

(2) Channel 2, Fast Red and

(3) Channel 3 DAB

(Note: Channel 1, Crystal Light Green is not shown).

Fast Red and DAB have specific locations and have some areas in common.

(4) DAB intensity measurements rendered as a heat map: **blue** pixels are negative stained, **yellow** pixels are weak, **orange** pixels are moderate, and **red** pixels are strongly stained.

These images are a result of running the algorithm three times: Channel 2 deconvolved color, Channel 3 deconvolved color, and Channel 3 intensity.

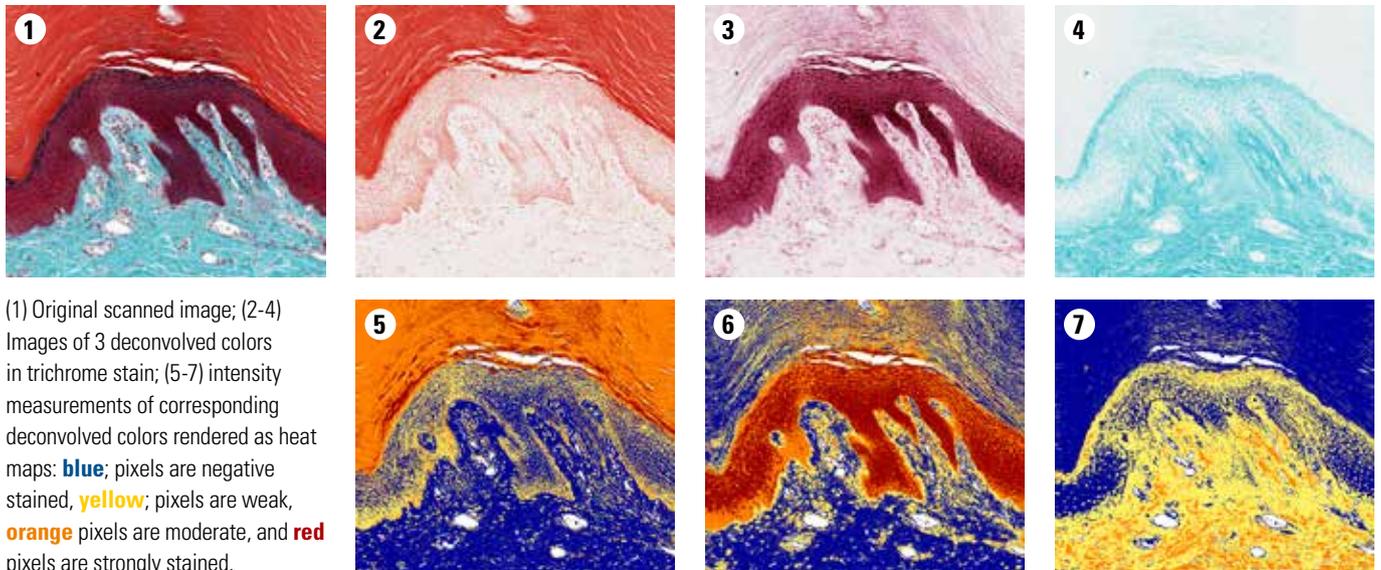
# Aperio Color Deconvolution Algorithm

Fast, Accurate and Informative

## ADJUSTABLE ALGORITHM INPUT PARAMETERS

Rigorously tested default parameters enable the Aperio Color Deconvolution algorithm to be used in a highly automated, one-click mode. In addition, tuneable input parameters enable rapid algorithm optimization, while the algorithm's tuning interface provides real-time feedback on adjusted settings.

The algorithm does more than just present a visual display of the color separation; it also accurately calculates the areas for each individual stain. In addition, there is a second type of markup image that is more useful when measuring areas of different staining density. This image is referred to as the Intensity Ranges markup image.



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

## COMPREHENSIVE RESULTS OUTPUT

With 18 data points returned for each deconvolved channel, the Aperio Color Deconvolution Algorithm delivers the information your research needs. Data is color-coded to match deconvolved color and whether the markup is pure color output or intensity range. 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

### For separated DAB Stain

Output parameters	For separated DAB Stain
Average Positive Intensity	172.549
Percent Weak Positive	49.1116
Percent Medium Positive	20.3885
Percent Strong Positive	9.23534
Percent Negative	21.2645
Percent Total Positive	78.7355
Average Weak Positive Intensity	203.422
Average Medium Positive Intensity	145.162
Average Strong Positive Intensity	68.8372
Total Stained Area (mm <sup>2</sup> )	0.226983
Total Analysis Area (mm <sup>2</sup> )	0.227682
OD (Average Optical Density)	0.143299
OD x Percent Total Positive	11.2827
OD x Total Stained Area (mm <sup>2</sup> )	3.25264e-002
Score (0-300)	117.595
Average Red OD	0.467309
Average Green OD	0.5827
Average Blue OD	0.664893

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