The Pathology Company

Leica BIOSYSTEMS

Color Deconvolution Algorithm

User's Guide

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Color Deconvolution Algorithm User's Guide

This document applies to eSlide Manager Release 12.3 and later.

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Customer Resources

- For the latest information on Leica Biosystems Aperio ePathology products and services, please visit www.LeicaBiosystems.com/ePathology.

Disclaimers

- Use normal care in maintaining and using Aperio ePathology servers. Interrupting network connections or turning off the servers while they are processing data (such as when they are analyzing eSlides or generating an audit report) can result in data loss.
- This manual is not a substitute for the detailed operator training provided by Leica Biosystems Imaging or for other advanced instruction. Leica Biosystems Imaging Field Representatives should be contacted immediately for assistance in the event of any instrument malfunction. Installation of hardware should only be performed by a certified Leica Biosystems Imaging Service Engineer.
- ImageServer is intended for use with eSlides created by scanning glass slides with the scanner. Educators will use Aperio ePathology software to view and modify eSlides in Composite WebSlide (CWS) format.

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Contents

1 Introduction

This chapter introduces the Aperio Color Deconvolution Algorithm.

*The primary source for information on creating, testing, and saving image analysis macros is the **Aperio Image Analysis User's Guide**. That guide also contains details on running image analyses from within eSlide viewers and batch analyses from within eSlide Manager, and viewing and exporting analysis results.*

*The Aperio Image Analysis Workstation provides a streamlined image analysis workflow on your local workstation; if you are using that product, please refer to the **Aperio Image Analysis Workstation User's Guide** for instructions on creating, testing, and saving image analysis macros That guide also contains details on running image analyses from within ImageScope, running batch analyses, and viewing and exporting analysis results.*

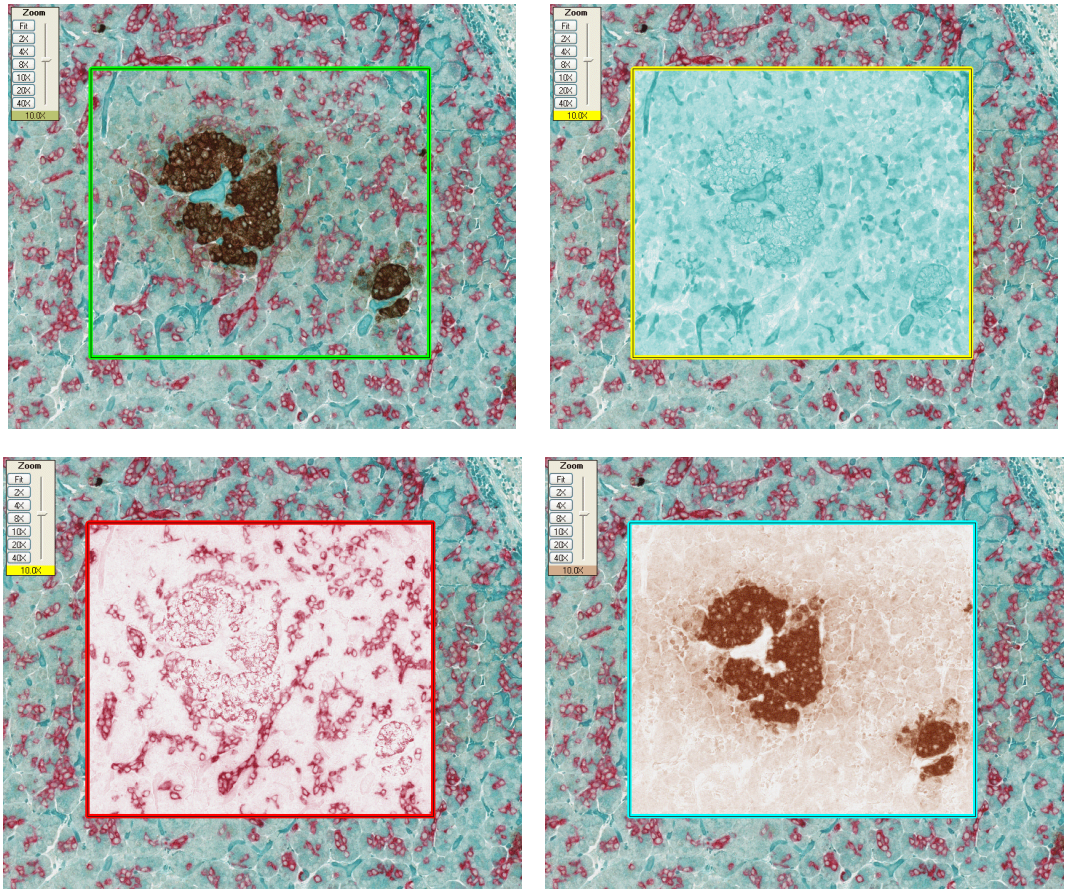
About This Guide

This guide for this image analysis algorithm discusses how to set the algorithm parameters to suit your image analysis needs. After tuning the parameters, you will save the settings as an algorithm macro. The macro can then be used by you and other users to analyze specific eSlides (digital slide images).

This guide works in concert with the *Aperio Image Analysis User's Guide* or the *Aperio Image Analysis Workstation User's Guide* to present the complete picture of Aperio image analysis.

About the Color Deconvolution Algorithm

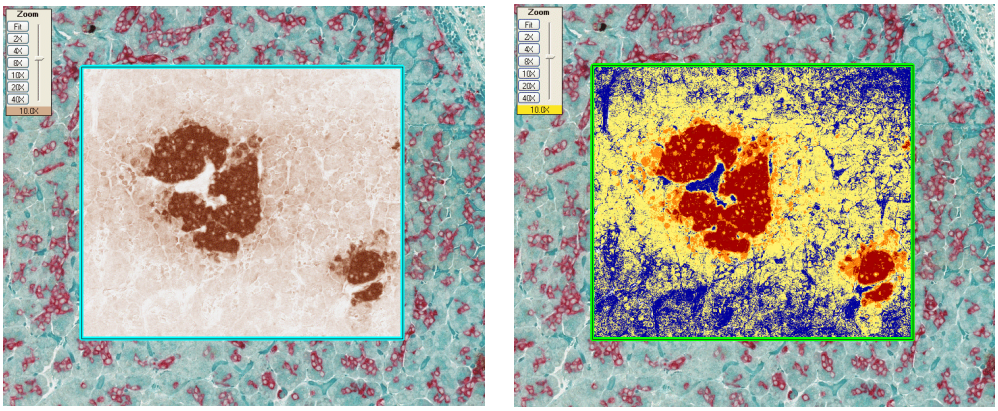
Most image processing algorithms for pathology use color to segment different types of tissue and cellular structures and for detection of specific proteins. The goal of *deconvolution* is to separate the image into three channels, corresponding to the actual colors of the stains used. This allows the pathologist to accurately measure the area for each stain separately, even when the stains are superimposed at the same location. In the image below, three stains can be seen: Crystal Light Green, Fast Red, and DAB. The scanned image, along with the three deconvolved color channels, are shown.



Deconvolution example. Scanned image with rectangular ROA (upper left); Channel 1 , Crystal Light Green (upper right); Channel 2, Fast Red (lower left); Chanel 3, DAB (lower right).

It is clear in the illustration above that Crystal Light Green is present nearly everywhere, while the other two stains are more specific to certain areas. In addition, the Fast Red and DAB have some areas in common. The deconvolution algorithm does more than just present this separation visually, it also accurately calculates the areas for each individual stain as will be shown below.

The example shown in the illustration above is actually a result of running the algorithm three times, once for each color channel and shows the stain for each channel as it would be seen if the other two stains were removed. The image generated in this way is referred to as a markup image. 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 and is shown below along with the Deconvolved Color Channel markup image for DAB.



Deconvolved Color Channel (left) and Intensity Ranges (right) for Channel 3, DAB.

The Intensity Ranges (right) show four colors: Red indicates strong staining, Orange indicates moderate staining, Yellow indicates weak (background) staining in the DAB channel, and Blue indicates staining in one or both of the other two channels. The thresholds that determine the boundaries between weak, medium, and strong staining are set by the pathologist as input parameters to the algorithm.

Algorithm	Color Deconvolution
Version	8.001
Average Positive Intensity	177.988
Percent Weak Positive	53.4537
Percent Medium Positive	7.26969
Percent Strong Positive	10.0327
Percent Negative	29.2439
Percent Total Positive	70.7561
Average Weak Positive Intensity	203.063
Average Medium Positive Intensity	142.122
Average Strong Positive Intensity	70.3819
Total Stained Area (mm^2)	0.193882

Numeric Results for Channel 3, DAB.

The area for each of these four staining categories is also given as numerical output shown in the illustration on the right. The weak positive (Yellow) comprises 53.4% of the total stained area. The medium (Orange) and strong positive (Red) comprise 7.2% and 10.0% respectively. The negative staining (Blue) comprises 29.2% of the total area and corresponds to those pixels that did not receive any positive stain (DAB in this case). The total stained area is 0.19 mm^2, from which the actual area for any of the other categories can be easily calculated. Similar results are obtained for the other two channels.

Prerequisites

Because Aperio eSlides are by design high resolution and information rich, for best results you should use a high quality monitor to view them. Make sure the monitor is at the proper viewing height and in a room with appropriate lighting. We recommend any high quality LCD monitor meeting the requirements recommended in the *Aperio ePathology System Requirements*.

Intended Use

For research use only. Not for use in diagnostic procedures.

Algorithms are intended to be used by trained pathologists who have an understanding of the conditions they are testing for in running the algorithm analysis.

Each algorithm has input parameters that must be adjusted by an expert user who understands the goal of running the analysis and can evaluate the algorithm performance in meeting that goal.

You will adjust (tune) the parameters until the algorithm results are sufficiently accurate for the purpose for which you intend to use the algorithm. You will want to test the algorithm on a variety of images so its performance can be evaluated across the full spectrum of expected imaging conditions. To be successful, it is usually necessary to limit the field of application to a particular tissue type and a specific histological preparation. A more narrowly defined application and consistency in slide preparation generally equates to a higher probability of success in obtaining satisfactory algorithm results.

If you get algorithm analysis results that are not what you expected, please see the troubleshooting appendix in *Aperio ePathology Image Analysis User's Guide* for assistance.

Installing the Algorithm

In most cases you install the algorithm on the eSlide Manager server only. (In fact, Technical Services may install the algorithm for you on your server.) This is because you typically fine-tune and save the algorithm parameters on the eSlide Manager server. For the rare case that you need to fine-tune the algorithm parameters on your local workstation or by using a local image, refer to the *Aperio Image Analysis User's Guide* for installation and use instructions.

For More Information

For a discussion of the algorithm input parameters and results, see *"Chapter 2: Tuning Parameters" on page 10*.

See the *Aperio Image Analysis User's Guide* for information on:

- Installing an algorithm
- Creating a new algorithm macro or modifying an existing one
- Saving or exporting a macro
- Selecting the areas of an eSlide to analyze
- Running an analysis on a single eSlide through the eSlide viewer or running an analysis on one or more eSlides using eSlide Manager batch analysis
- Viewing analysis results quantitatively and visually
- Exporting analysis results

For details on using ImageScope to view eSlides, see the *ImageScope User's Guide*.

If the analysis results are not what you expect, see the *Aperio Image Analysis User's Guide* section on troubleshooting for assistance.

2 Tuning Parameters

This chapter discusses the Color Deconvolution algorithm inputs and outputs.

The Color Deconvolution algorithm performance is controlled by a set of input parameters which determine the thresholds for the intensity ranges, the channel to be analyzed, the type of markup image to be presented, and calibration data that defines the exact colors for the three stains. The default colors are Hematoxylin, Eosin, and DAB. The colors used in the example in the previous chapter were obtained using a calibration procedure described later in this document.

Tuning Algorithm Parameters

To create a macro for the algorithm or to modify an existing macro:

1. In ImageScope, open an eSlide you want to use to tune the algorithm parameters. (Refer to the *ImageScope User's Guide* for instructions.)
2. In ImageScope, open the algorithm and choose to create a new macro or open an existing macro to modify it. (Refer to the *Aperio Image Analysis User's Guide* for instructions.)

   You now see the parameters for the algorithm (these are listed later in this chapter).
3. Now adjust the parameters in the Analysis window as discussed below and move the Algorithm Tuning window on the image to see a mark-up image of the results.

After adjusting the input parameters and selecting the output parameters as discussed in the following sections, move the Algorithm Tuning window to various areas on the eSlide to see an approximation of the analysis results. The results appear in the Annotations window and as a mark-up image in the ImageScope main window.

You can open the ImageScope Annotations window to see the algorithm tuning results displayed numerically.

When saving the algorithm macro by clicking  , you can choose whether to save the macro locally on your workstation or, if connected to eSlide Manager, to save the macro remotely on eSlide Manager. You are asked to supply a name for the macro that will help you identify it in the future. If saving the macro to eSlide Manager, you are also asked to specify which data group the macro will be associated with; only eSlide Manager users who have permission to use that data group will be able to choose that macro for analysis.

Algorithm Parameters

- **Intensity –** The raw image data are in RGB format. An RGB value of (255,255,255) corresponds to bright White, while an RGB value of (0,0,0) corresponds to Black. Intensity is the average of the RGB channels in the raw data: (R+G+B)/3. A large intensity value is very bright and corresponds to very little staining in the histological sample. A low intensity value corresponds to dark staining. Intensity is the opposite of density, in which larger values are darker.

- **Thresholds –** The input thresholds are usually in descending order, since weak intensity is greater than medium intensity which is greater than strong intensity.
- **Positive Color Channel –** Color channel to be analyzed: 1, 2, or 3. The color specification for each channel is given below. Each color channel corresponds to one of up to three possible stains.
- **Markup Image Type –** May be either "Intensity Ranges" or "Deconvolved Color Channel." The choice of markup image does not change the numerical results in any way.
- **Weak Positive Threshold –** Upper intensity limit for the weak positive pixels and may be in the range (0 – 255).
- **Medium Positive Threshold –** Upper intensity limit for medium positive pixels and may be in the range (0 -255).
- **Strong Positive Threshold –** Upper intensity limit for strong positive pixels and may be in the range (0-255).
- **Black Threshold –** Intensity value for Black and is usually set to zero.
- **Color (1) Red, Green, Blue Components –** Normalized optical density values for Channel (1). Default for Channel 1 is Hematoxylin. These values are calculated by a calibration procedure described below.
- **Color (2) Red, Green, Blue Components –** Normalized optical density values for Channel (2). Default for Channel 2 is Eosin. These values are calculated by a calibration procedure described below.
- **Color (3) Red, Green, Blue Components –** Normalized optical density values for Channel (3). Default for Channel 3 is DAB. These values are calculated by a calibration procedure described below.
- **Clear Area Intensity –** This is the intensity for a clear area on the slide. This value is always 240 for ScanScope generated images.

Algorithm Results

The term *positive* in the result name indicates that the result applies to pixels that are stained in the Positive Color Channel specified in the algorithm input. The term *negative* indicates that the result applies to pixels that are not stained positive. The total stained area is then the combined area spanned by the positive and negative pixels.

- **Average Positive Intensity –** Average intensity (R+G+B)/3 for all positive pixels.
- **Percent Weak Positive –** Percent of positive pixels that are weakly stained. Weak is defined by: (Weak Threshold) > Intensity > (Medium Threshold).
- **Percent Medium Positive –** Percent of positive pixels that are moderately stained. Medium is defined by: (Medium Threshold) > Intensity > (Strong Threshold).
- **Percent Strong Positive –** Percent of positive pixels that are strongly stained. Strong is defined by: (Strong Threshold) > Intensity > (Black Threshold).
- **Percent Negative –** Percent of pixels that are not positive, but have intensity defined by: (Weak Threshold) > Intensity > (Black Threshold).
- **Percent Total Positive –** Weak + Medium + Strong positive percentages.
- **Average Weak Positive Intensity –** Average intensity (R+G+B)/3 for all weak positive pixels.
- **Average Medium Positive Intensity –** Average intensity (R+G+B)/3 for all medium positive pixels.
- **Average Strong Positive Intensity –** Average intensity (R+G+B)/3 for all strong positive pixels.

- **Total Stained Area (mm^2) –** Cumulative total area of combined positive and negative pixels in square-millimeters. The area for any other staining category may be obtained by multiplying the relevant percentage times the total area.
- **Score –** The score is calculated by a simple formula involving the positive percentages. Score = 1.0*(%Weak) + 2.0*(%Medium) + 3.0*(%Strong).
- **Average Red OD –** Average OD (optical density) of the Red component for all pixels analyzed. This value is used for calibration of input color specifications.
- **Average Green OD –** Average OD (optical density) of the Green component for all pixels analyzed. This value is used for calibration of input color specifications.
- **Average Blue OD –** Average OD (optical density) of the Blue component for all pixels analyzed. This value is used for calibration of input color specifications.

Color Calibration

Three color components for each of the three channels must be specified in the input parameters to the algorithm—nine numbers total, as shown in the illustration on the right. If only two stains are present, the three numbers for one of the colors can be set to zero. Each channel also has a default color. The default color for channel 1 is Hematoxylin (circled in red on the illustration at the end of this chapter). The default colors for channels 2 and 3 are Eosin and DAB.

These numbers must be changed if different stains are used. The color for each stain is calibrated separately, using a separate image for each stain having only that color present. If three areas within a single image can be identified that are each dominated by a single stain, then these areas may also be used.

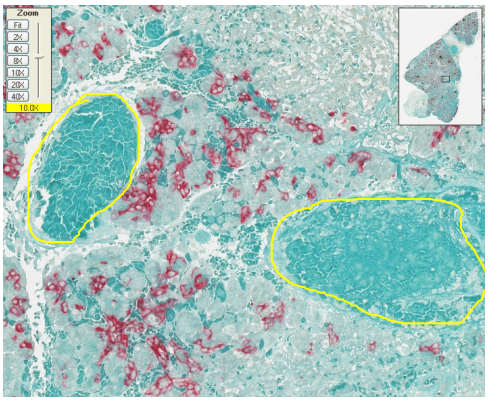
To tune the algorithm:

1. From eSlide Manager, use ImageScope to open an eSlide you want to use to tune the algorithm parameters. (Refer to the *ImageScope User's Guide* for instructions.)
2. In ImageScope, open the Color Deconvolution algorithm and choose to create a new macro or modify an existing macro. (Refer to the *Aperio Image Analysis User's Guide* for instructions.)

   You now see the input parameters for Color Deconvolution (these are listed earlier).
3. Adjust the parameters in the Algorithms window and move the tuning window on the image to see a markup image of the results.

Adjust the algorithm parameters in the Algorithms window as discussed earlier in this document by clicking on a number and typing a new value or using the slider if one is provided for that parameter. In this case, we have changed the values so that the analysis will show the presence of DAB.

To calibrate Color 1 for Crystal Light Green, an area of the image was found (illustration below, left) that has two regions which are stained in only that color (enclosed by yellow lines). The Color Deconvolution algorithm is run with its erroneous Color 1 settings and its output for those regions, shown at the right, is obtained. To adjust the input parameters to detect Crystal Light Green on the Color 1 channel, copy the resulting average OD values back to the Color 1 parameters in the algorithm settings. This procedure must be repeated for each color that differs from the default settings. After calibration is completed, click **Stop Tuning**. Now save the parameter settings as a macro, specifying a macro name that is easily associated with this staining combination.



Algorithm	Color Deconvolution
Version	8.001
Average Positive Intensity	152.492
Percent Weak Positive	25.0493
Percent Medium Positive	74.7004
Percent Strong Positive	0.110772
Percent Negative	0.139525
Percent Total Positive	99.8605
Average Weak Positive Intensity	191.002
Average Medium Positive Intensity	139.66
Average Strong Positive Intensity	97.3206
Total Stained Area (mm^2)	0.0733236
Score (0-300)	174.782
Average Red OD	0.925687
Average Green OD	0.266027
Average Blue OD	0.26895

(Left) Image area containing two regions (yellow lines) with primarily Crystal Light Green stain. (Right) Analysis output with color calibration values (red circle).

What's Next?

After calibrating the stain colors, you can run the algorithm on various eSlides by using the macro you previously saved. For details on running analyses, see the *Aperio Image Analysis User's Guide*.

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Symbols

- The following symbols may appear on your product label or in this user's guide:

	Manufacturer
	Date of manufacture (year - month - day)
	European Union Authorized Representative
	In vitro diagnostic device
	Serial number
	Catalog number
	Relative humidity range
	Storage temperature range
	Electronic and electrical equipment waste disposal
	The exclamation point within an equilateral triangle is intended to alert you to the presence of important operating and maintenance (servicing) instructions. *Le point d'exclamation dans un triangle équilatéral vise à avertir l'utilisateur qu'il s'agit d'instructions d'utilisation et d'entretien importantes.*
	The lightning flash with arrowhead symbol within an equilateral triangle is intended to alert you to the presence of uninsulated "dangerous voltage" within the product's enclosure that may be of sufficient magnitude to constitute a risk of electric shock to persons. *Le symbole de l'éclair avec la pointe de flèche dans un triangle équilatéral vise à avertir l'utilisateur que le boîtier du produit présente une « tension dangereuse » non isolée d'une amplitude suffisante pour constituer un risque d'électrocution.*
	The flat surface with waves symbol within an equilateral triangle is intended to alert you to the presence of hot surfaces which could cause burn damage. *Le symbole d'une surface plane et de vagues dans un triangle équilatéral vise à avertir l'utilisateur de la présence de surfaces chaudes qui peuvent causer des brûlures.*
	The UV lamp within an equilateral triangle is intended to alert you to the presence of UV light within the product's enclosure that may be of sufficient magnitude to constitute a risk to the operator. *La lampe UV dans un triangle équilatéral vise à avertir l'utilisateur de la présence de rayonnement UV dans le boîtier du produit qui peut être d'une amplitude suffisante pour constituer un risque pour l'utilisateur.*

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