The Pathology Company

Leica BIOSYSTEMS

Positive Pixel Count Algorithm

User's Guide

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Positive Pixel Count Algorithm User's Guide

This document applies to eSlide Manager Release 12.3 and later.

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- 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.
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Contents

1 Introduction

This chapter introduces you to the Aperio Positive Pixel Count Algorithm. For general information on using any algorithm, please see the *Aperio Image Analysis User's Guide*.

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).

The 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 Positive Pixel Count Algorithm

The Positive Pixel Count algorithm can be used to quantify the amount of a specific stain present in a scanned slide image. You will specify a color (range of hues and saturation) and three intensity ranges (weak, positive, and strong). For pixels which satisfy the color specification, the algorithm counts the number and intensity-sum in each intensity range, along with three additional quantities: average intensity, ratio of strong/total number, and average intensity of weak+positive pixels.

The algorithm has a set of default input parameters when first selected—these inputs have been pre-configured for Brown color quantification in the three intensity ranges (220-175, 175-100, and 100-0). Pixels which are stained, but do not fall into the positive-color specification, are considered negative stained pixels—these pixels are counted as well, so that the fraction of positive to total stained pixels is determined.

The algorithm is applied to an image by using ImageScope, eSlide Manager or TMALab. These programs allow you to select an image Region of Analysis (set of spots in TMALab), specify the input parameters, run the algorithm, and view/save the algorithm results. When using the ImageScope program, a pseudo-color markup image is also shown as an algorithm result. The markup image allows the user to confirm that specified inputs are measuring the desired color and intensity ranges. Once a set of algorithm inputs has been confirmed, the settings can be saved as an algorithm macro for subsequent repeated use.

Note that the Color Deconvolution algorithm is our professional version of the Positive Pixel Count algorithm that allows automatic and precise training of stain colors (eliminating trial and error), and accurate stain separation, resolving the multi-stain colocalization problem.

Prerequisites

The Positive Pixel Count algorithm requires that you be using Spectrum Release 10 or later or eSlide Manager Release 12 or later.

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

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 appendix "Troubleshooting" in the *Aperio Image Analysis User's Guide* for assistance.

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

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 quick reference to the positive pixel count algorithm input parameters and results, see *"Chapter 2: Tuning Parameters" on page 9*.

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*.

2 Tuning Parameters

This chapter contains information on all positive pixel count algorithm inputs and outputs as well as information on picking hue values.

Tuning 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

The following inputs are accepted by the Positive Pixel Count algorithm:

- **View Width** – Width of processing box.
- **View Height –** Height of processing box.
- **Overlap Size –** Size of the overlap region for each view. The image is processed in blocks (views) and overlap is provided to ensure that regions are processed only once
- **Image Zoom –** 1.0 (recommended) for processing of all pixels. Can be reduced to 0.5 for faster processing; however, the results may not be as accurate.

- **Markup Compression Type –** You can select among "Same as processed image," JPEG, or JPEG2000 for the markup image.
- **Compression Quality –** For the compressed markup image, you can select a compression quality of 0 to 95. Higher quality takes longer and yields larger files.
- **Classifier Definition List –** If you are using this algorithm in conjunction with the Genie image analysis program, you can select Genie classifier definition lists here. See the *Genie User's Guide* for details.
- **Class List –** If you are using this algorithm in conjunction with the Genie image analysis program, you can select Genie class lists here. See the *Genie User's Guide* for details.
- **Hue Value –** This is hue position on the color circle for Positive color, ranging from 0 to 1. It can take on values between 0.0 and 1.0. Red = 0.0, Green = 0.33, Blue = 0.66, Brown = 0.1. For more information on determining this value based on the color you want to detect, see *"Color Concepts" on page 11*.
- **Hue Width –** This value selects the range of hues, centered on the Hue Value, that will satisfy the hue detection process. By increasing this number, you specify that a larger range of hues will be accepted for specifying the Positive color band. By decreasing this number, you "tighten" the range of hues that will be acceptable. The number can range between zero and 1, where zero is a narrow hue width and 1 selects the entire range of hues. A value between 0.33 and 0.5 is usually reasonable.
- **Color Saturation Threshold –** This is the required saturation of the Positive color. RGB values are represented as gray + color. The value can be between 0.0 and 1.0, with 1.0 corresponding to no gray component (fully saturated). Pixels with saturation less than this value are not reported.
- **Iwp (High) –** Upper limit of intensity for weak-positive pixels. Iwp is also used as an intensity threshold for negative stained pixels–pixels which do not meet the hue/saturation limits, but have an intensity less than Iwp, are counted as negative pixels.
- **Iwp (Low) = Ip (High) –** Lower limit of intensity for weak-positive pixels, upper limit of intensity for positive pixels.
- **Ip (Low) = Isp (High)** – Lower limit of intensity for positive pixels, upper limit of intensity for strong-positive pixels.
- **Isp (Low) –** Lower limit of intensity for strong-positive pixels.
- **Intensity = (R+G+B)/3**

  The intensity limits establish three intensity ranges for classifying and summing pixel values. The greater the intensity value, the brighter the pixel. For more information on intensity ranges, see *"Intensity" on page 12*.

Weak-Positive Intensity:	Iwp (High) <= Intensity < Iwp (Low)
Positive Intensity:	Ip (High) <= Intensity < Ip (Low)
Strong-Positive Intensity:	Isp (High) <= Intensity < Isp (Low)

Algorithm Results

The algorithm results appear in the ImageScope Annotations window (go to the ImageScope View menu and select **Annotations**).

The algorithm calculates the following quantities for each region of analysis, as well as the sum of all regions, for each layer that is analyzed. Results are stored in an annotation layer attached to the image and can be viewed in ImageScope.

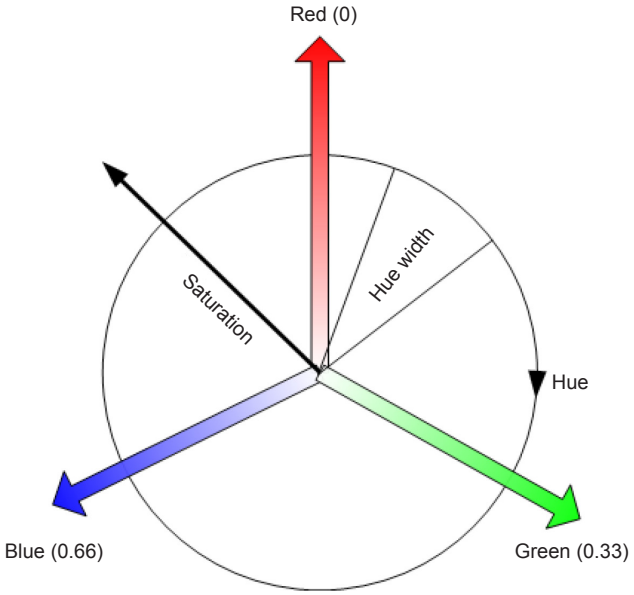
- **Nwp** – Number of Weak-Positive pixels (Yellow in mark-up image)
- **Np** – Number of Positive pixels (Orange in mark-up image)
- **Nsp** – Number of Strong-Positive pixels (Red in mark-up image)
- **Iwp** – Sum of Intensity values for all Weak-Positive pixels
- **Ip** – Sum of Intensity values for all Positive pixels
- **Isp** – Sum of Intensity values for all Strong-Positive pixels
- **Iavg** – Average Intensity of all pixels: Iavg = (Iwp+Ip+Isp)/(Nwp+Np+Nsp)
- **Nsr** – Ratio of Strong-Positive pixels to total pixels: Nsr = Nsp/(Nwp+Np+Nsp)
- **Iwavg** – Average Intensity excluding Strong-Positive pixels: (Iwp+Ip)/(Nwp+Np)
- **Nn** – Number of Negative pixels (Blue in mark-up image)
- **In** – Sum of Intensity values for all Negative pixels
- **NTotal** – Number of Total pixels, Positive+Negative (Nwp+Np+Nsp+Nn)
- **Positivity** – Total number of positive pixels divided by total number of pixels: (NTotal – Nn)/(NTotal)
- **ATotal** – Total area in square millimeters of all pixels counted in the NTotal result.

Color Concepts

The Positive Pixel Count algorithm detects pixels that match the input parameters set for the algorithm. An important group of these parameters relate to color.

This section discusses some of the concepts behind these parameters.

You are probably familiar with the common artist color wheel. The Rare Event Detection algorithm uses a form of it called the HSI (Hue, Saturation, Intensity) wheel that quantifies the RGB (red, blue, green) color space:



In the example above, imagine every color residing on this wheel, with the color red being assigned the value zero. The actual color is called the hue. As you move around the rim of the circle, you move from one hue to another. Each hue has a numeric representation on this wheel. Green is 0.33 (as it is a third of the way around the circle from red, which is 0.00), and Blue is 0.66 (two thirds of the way around the circle). Each hue on the circle has a number assigned to it. Brown, which is almost halfway between Red and Green, has a value of 0.1.

The Hue Value parameter used by this algorithm is the number associated with the hue you want to use based on its position on the wheel.

Saturation represents the "purity" of the color, with the rim of the wheel representing complete saturation. For example, fully saturated Red is the color on the rim of the wheel, a less saturated Red (for example, Pink) resides on the red vector, but closer to the center of the wheel.

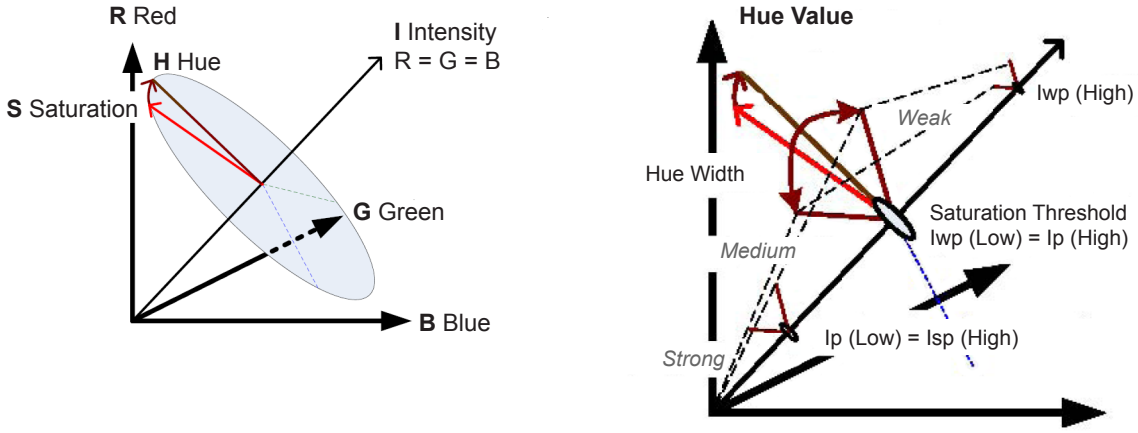
Hue Width is the wedge on the wheel that represents all hues that will satisfy pixel detection based on the Hue Value. The smaller the Hue Width, the more restrictive is the definition of the hues that are acceptable. For example, if you want pixels to be detected only if they are precisely Brown, then you might specify a Hue Width of zero. If slightly reddish brown to slightly greenish brown are all acceptable to identify a pixel, then you might specify a Hue Width of .5. You might think of this as a hue threshold value.

Intensity

Another value that can be used to detect a pixel is the Intensity. Not represented on the wheel shown previously, intensity is the measure of brightness of the pixel and is the average of R+G+B values of the pixel.

Intensity ranges from zero (black) to 255 (bright white), so that a large intensity value means that the pixel is brighter. Intensity is the opposite of density. Intensity is proportional to the amount of light transmitted through the slide, while density is proportional to the amount o f light that is blocked by the stained tissue.

The input parameters allow defining the positive stain color and the weak-positive, positive and strong-positive intensity thresholds for the positive stain using the HSI color model.



The Positive Pixel Count algorithm allows you to specify three ranges of intensity:

- **Iwp** – Weak-positive intensity.

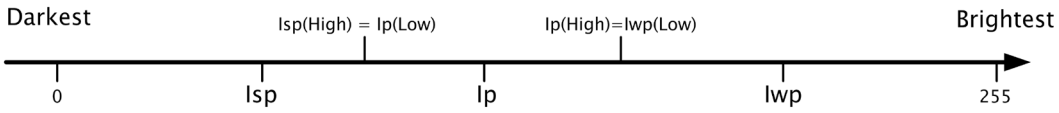
  A high Iwp value is the upper limit of intensity for weak-positive pixels. Pixels which do not meet the hue/saturation limits, but have an intensity less than Iwp, are counted as negative pixels.

A low Iwp value is the lower limit of intensity for weak-positive pixels and the upper limit of intensity for positive pixels—Iwp(Low) = Ip(High).

- **Ip** – Positive intensity.

  Ip is the lower limit of intensity for positive pixels. It is the upper limit of intensity for strong-positive pixels—Ip(Low) = Isp(High).

- **Isp –** Strong-positive intensity. This is the lower limit of intensity for strong-positive pixels.



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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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