The Pathology Company

Leica BIOSYSTEMS

Rare Event Detection Algorithm

User's Guide

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Rare Event Detection 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.

Patents

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Contents

1 Introduction

This chapter introduces the Rare Event Detection algorithm. For general information on using an 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).

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.

The Rare Event Detection Algorithm

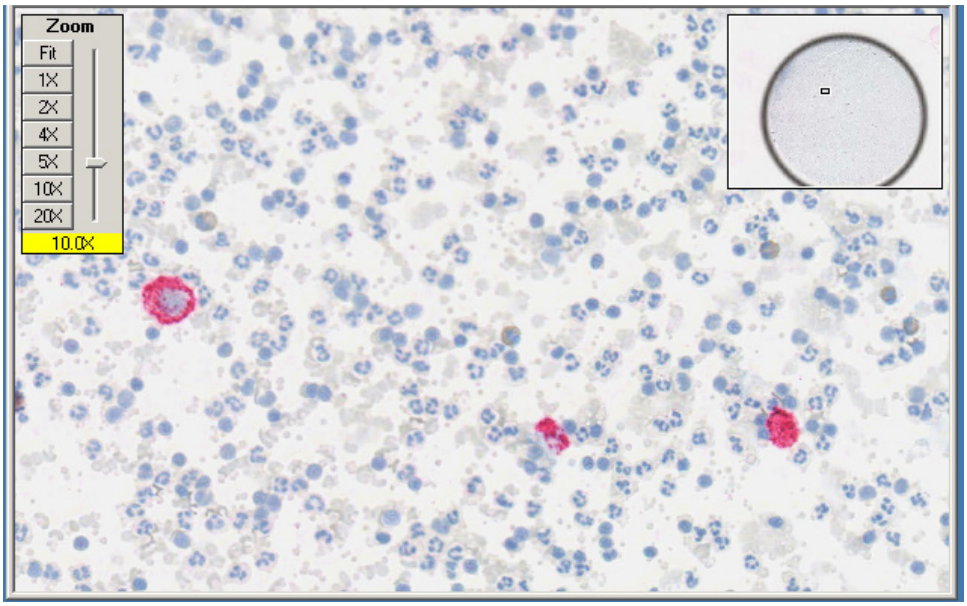
Humans have an incredible vision system, but when it comes to looking for a needle in a hay stack they not only take a long time to process a lot of data, but also fatigue easily and become less reliable. There are many tasks where pathologists have to look for a rare event—for example, detection of micrometastasis of tumor cells in circulating blood. A computer program tuned to find the rare events can process an entire slide quickly and reliably without fatigue. When a pathologist then looks at the slide, the computer program can instantaneously present all the rare events found. This saves the pathologists time as well as providing more reliable test results.

The Rare Event Detection algorithm allows you to define the objects you are looking for by color (defined in the Hue Saturation Intensity color space) and size. The algorithm uses color segmentation and morphological image processing methods to detect and count objects of interest. Size and color-saturation thresholds are used to identify objects such

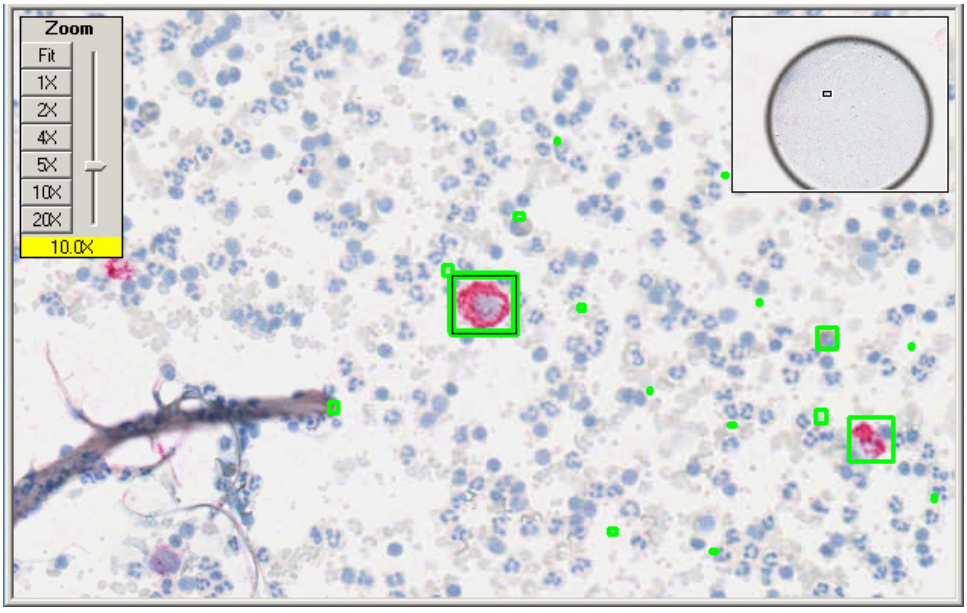
as potential tumor cells and reject background staining artifacts. The roundness threshold is used to count only the most circular objects.

As an example of a rare event detection application, the default parameter set is used to detect micrometastasis of tumor cells in circulating blood.

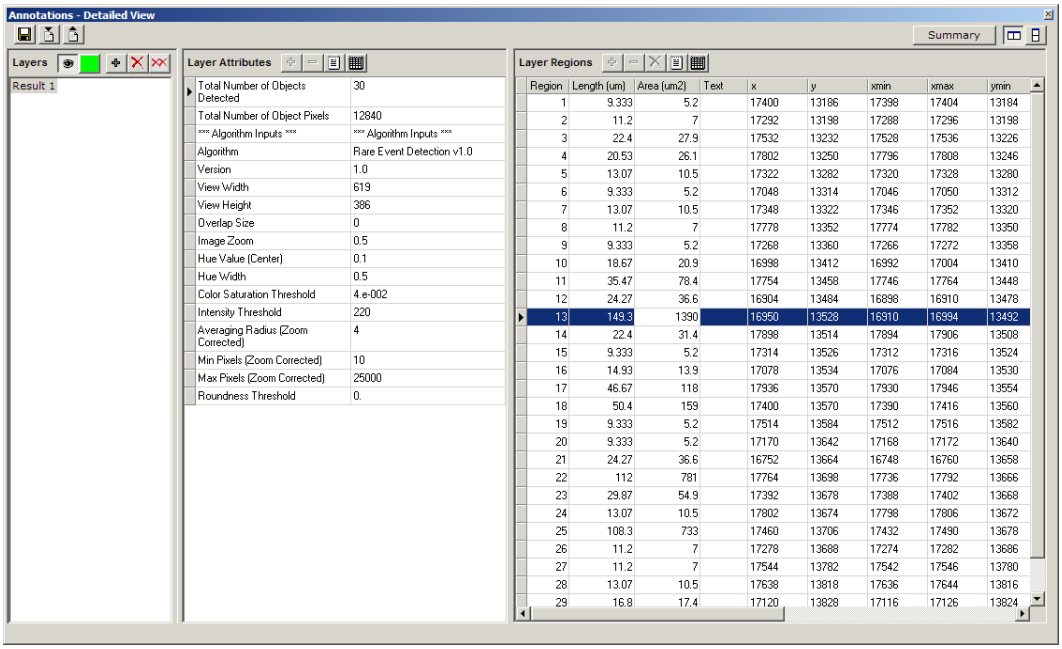
The following illustration below shows an eSlide displayed in the ImageScope main window, with red-stained tumor cells in a background of blue-stained blood cells.



After the analysis is run, the mark-up image shows the objects detected (in green boxes in this example):



The illustration below shows the analysis output for the image. Summary results are shown on the left and measurements for individual tumor cells are shown on the right. Each object can be inspected by scrolling through the list on the right, clicking each object, and viewing the object in the image window for easy verification of the analysis results.



The summary results show 30 objects detected and a total number of 12,840 pixels for those objects in the area analyzed.

Prerequisites

The Rare Event Detection algorithm requires that you use Spectrum Release 9.1 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

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, see the "Troubleshooting" information in the *Aperio 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 when using a local image, refer to the *Aperio Image Analysis User's Guide* for installation and use instructions.

For More Information

For information on the Rare Event Detection 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

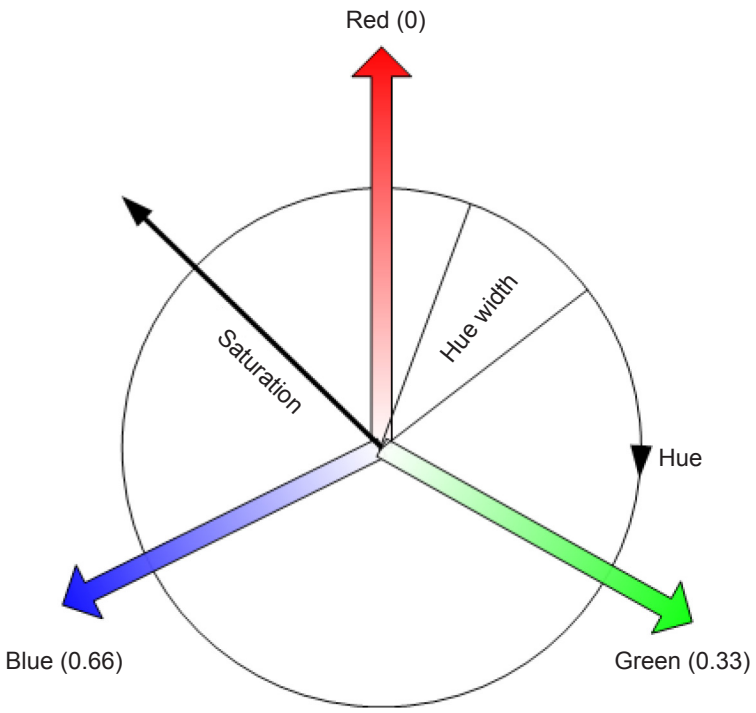
This chapter contains information on Rare Event Detection algorithm inputs and outputs.

Color Concepts

The Rare Event Detection algorithm finds objects by comparing pixels against 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.

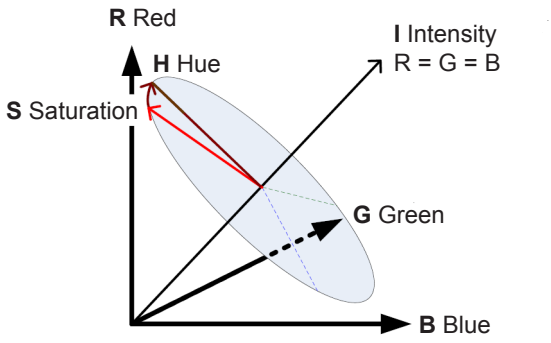
Saturation represents the "purity" of the color, with the rim of the wheel representing complete saturation. For example, fully saturated Red is the color on the rim of the wheel, a less saturated Red (for example, Pink) resides on the red vector, but closer to the center of the wheel.

Hue Width is the wedge on the wheel that represents all hues that will satisfy the object 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 objects 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 an object, then you might specify a Hue Width of .5. You can think of this as a hue threshold value.

Intensity is a measure of brightness and is the average of the R,G,B values of a 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, which is proportional to the amount o f light that is blocked by the stained tissue.

The illustration below shows intensity as it relates to the HSI color space:



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

The following inputs are accepted by the Rare Event Detection algorithm:

- **View Width** – Width of processing box.
- **View Height** – Height of processing box.
- **Overlap Size** – Size of the overlap region for each view. This should be greater than the maximum linear dimension of an object. The image is processed in blocks (views) and overlap is provided to ensure that objects are completely detected and counted 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.
- **Hue Value** – This value is used to select the color of the objects of interest, from zero to 1. Some examples of color values are: 0 for Red, 0.1 for Brown, 0.33 for Green, and 0.66 for Blue. For details on determining this value based on the color you want to detect, see *"Color Concepts" on page 10*.
- **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 determining objects. 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 .33 and .5 is usually reasonable.
- **Color Saturation Threshold** – This is the required saturation of the detected object. RGB values are represented as gray + color. The value can be between 0 and 1, with 1 corresponding to no gray component (fully saturated). Objects with saturation less than this value are not reported.
- **Intensity Threshold** – This value is the intensity threshold of positive pixels; pixels that have an intensity value greater than this value are ignored (the greater the intensity value, the brighter the pixel). For more details, see *"Color Concepts" on page 10*.
- **Averaging Radius** – A smoothing parameter used by the morphological process to remove small-scale noise structure (value > 0).
- **Min Object Pixels** – Objects smaller than this number of pixels are not reported (value > 0)
- **Max Object Pixels** – Objects larger than this number of pixels are not reported (value > 0)

- **Object Roundness Threshold** – Objects with roundness less than this value are not reported (0.0 < Value < 1.0). Circular objects will have a roundness=1, while elongated objects will have a smaller value (a line has roundness=0).

Objects that satisfy all of these hue, saturation, intensity, radius, size, and roundness limits are detected and reported.

Algorithm Results

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

The first section of the annotations window displays the algorithm results; the second portion (labeled "Algorithm Inputs") repeat the algorithm input parameters you specified when you ran the algorithm.

The results give information on all the permutations of the colors detected. And the different colors in the mark-up image reflect those data.

Understanding the Results

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.

- **Total Number of Objects Detected** – This is the number of objects found based on the input parameters.
- **Total Number of Object Pixels** – Total area (in pixels) of the detected objects.
- **Additional Results** – The input parameters are also reported, along with actual algorithm results. This enables you to verify at a future date what parameters were used for processing. The term Zoom Corrected can appear on some of the reported results: If Zoom less than one is used for processing, then certain parameters are adjusted in an effort to provide results that are consistent with Zoom=1 processing. For example, Averaging Radius=8 for Zoom=1 will convert to Averaging Radius=4 for Zoom=0.5.

If the results of the analysis are not what you expect or are otherwise unsatisfactory, see the "Troubleshooting" information in the *Aperio Image Analysis User's Guide* for some tips on identifying problems.

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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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