

Positive Pixel Count FL Algorithm

User's Guide



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User Resources

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Disclaimers

Use normal care in maintaining and using the Spectrum servers. Interrupting network connections or turning off the Spectrum and DSR servers while they are processing data (such as when they are analyzing digital slides or generating an audit report) can result in data loss.

This manual is not a substitute for the detailed operator training provided by Aperio Technologies, Inc., or for other advanced instruction. Aperio Technologies 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 Aperio Technologies Service Engineer.

ImageServer is intended for use with the SVS file format (the native format for digital slides created by scanning glass slides with the ScanScope scanner). Educators will use Aperio software to view and modify digital slides in Composite WebSlide (CWS) format.

Aperio products are FDA cleared for specific clinical applications, and are intended for research use for other applications. For clearance updates, visit www.aperio.com

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1 Introduction

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

The process of analyzing digital images begins with the ScanScope, which creates digital slides by scanning glass slides. Using Aperio image analysis algorithms to analyze digital slides provides several benefits:

- **Increases productivity** – Computer-based image analysis automates repetitive tasks and answer questions that are beyond the capabilities of manual microscopy.
- Provides unbiased assessment of the distribution and relationship of one or more fluorescent biomarkers through out an entire tissue section, as well as in a user-selected region of interest.
- **Workflow integration** – The Spectrum digital pathology information management software suite integrates image analysis seamlessly into your digital pathology workflow, requiring no additional work by the lab or pathologist. With the click of a button, the algorithm is executed while you review the digital slide.

The Positive Pixel Count FL Algorithm

The Positive Pixel Count FL algorithm quantifies the area of pixels in a single channel of a fluorescence digital slide that is stained with a single fluorescent dye. The analysis can be performed on a multi-channel image, but only on one channel at a time.

This algorithm can be used with fluorescence digital slides created by the Aperio ScanScope FL. It can also be used on fluorescence images from other systems that can be opened by the ImageScope digital slide viewer. The difference is that analysis of non-Aperio images is limited to R, G, and B channels.

The Positive Pixel Count FL algorithm provided for free by Aperio and is a simplified version of the Area Quantification FL algorithm, which quantifies multiple dyes and supports result plots.

Prerequisites

The Positive Pixel Count FL analysis algorithm requires that you be using Aperio Release 11 or later. You will use the algorithm to analyze an Aperio Fused Image (AFI) file, which is a fused image of the multiple dye channels scanned from a fluorescent dye-stained glass slide.

Because Aperio digital slides 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 following requirements:

Display Type:	CRT minimum, LCD (flat panel) recommended
Screen Resolution:	1024(h) x 768(v) pixels minimum, 1920 x 1050 or larger recommended.
Screen Size:	15" minimum, 19" or larger recommended
Color Depth:	24 bit
Brightness:	300 cd/m ² minimum, 500 or higher recommended
Contrast Ratio:	500:1 minimum, 1000:1 or higher recommended

For More Information

For a quick reference to the Positive Pixel Count FL analysis algorithm, refer to Chapter 2, "Quick Reference" on page 9.

For details on using the Positive Pixel Count FL analysis algorithm, go to Chapter 3, "Sample Analysis" on page 11.

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

- Installing an algorithm
- Opening a digital slide to analyze
- Selecting areas of a digital slide to analyze
- Running the analysis
- Exporting analysis results

For details on using the Spectrum digital slide information management system (for example, for information on running batch analyses), see the *Spectrum/Spectrum Plus Operator's Guide*.

For details on using ImageScope to view and analyze digital slides and using annotation tools to select areas of the digital slide to analyze, see the *ImageScope User's Guide*.

FDA Cleared Image Analysis Algorithms

Several Aperio algorithms have been cleared by the FDA for clinical use when used on ScanScope models that are labeled as approved medical devices, and are intended for research use for other applications. Please visit the Aperio website at www.aperio.com for a complete listing of clearances and the associated intended uses. These algorithms have their own user guides. Please see the Intended Use section of the user guides for the specific cleared applications you wish to use for details on in vitro diagnostic use.

Intended Use

Algorithms are intended to be used by trained researchers who have an understanding of the biological characteristics or significance of biomarkers.

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 should 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.

The Positive Pixel Count FL algorithm is not cleared by the FDA for clinical use.

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Quick Reference

This chapter contains a quick reference to all Positive Pixel Count FL analysis algorithm inputs and outputs. See the following chapter for details on using the algorithm.

If you are already familiar with using the Positive Pixel Count FL analysis algorithm, and need just a reminder of the different algorithm input and output parameters, please refer to the sections below. For more information on using the algorithm, see the next chapter.

Algorithm Input Parameters

Positive Pixel Count FL analysis algorithm performance is controlled by a set of input parameters, which determine many different types of analysis.

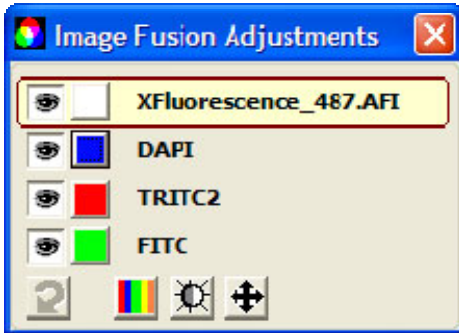
- **Image Zoom** – Zoom level to be used; a higher zoom results in faster algorithm run but less accurate results.

These parameters are used if you are using Genie classifiers to preprocess the image:

- **Classifier Neighborhood** – Size (in microns) of the neighborhood to pad the boundary of each view, as required by the classifier.
- **Classifier** – Choose from the list of classifiers (if none are available, contact the Spectrum Administrator for information on the optional Genie product).
- **Class List** – Pick the classes to retain for further processing.

These parameters are used to specify the dye you want to measure:

- **Dye** – Type the name of the fluorescent dye you want to quantify. (You can only analyze one dye at a time with this algorithm.) This must match one of the dye channel names shown in the AFI Image Fusion Adjustments window in ImageScope:



If you are not using an AFI image, the dye channels names won't be available to you—in this case, type the dye name RED, GREEN, or BLUE, corresponding to the RGB channel in your image you want to analyze.

Setting the Thresholds

The intensity threshold values specify the range of intensities to be included in the analysis. Values that fall outside of these minimum and maximum thresholds will not be identified as containing the specified dye.

The intensity scale is based on the percentage of the image bit depth or gray levels representing intensity. For example, a value of 1 represents the maximum pixel value possible (1024 for an Aperio ScanScope FL image scanned at its default 10-bit setting). A value of 0.2 represents a pixel value of 0.2×1024 , or 205.

- **Dye Min Intensity Threshold** – Minimum intensity threshold for the dye (default value is 0.2).
- **Dye Max Intensity Threshold** – Maximum intensity threshold for the dye (default value is 1).

Algorithm Results

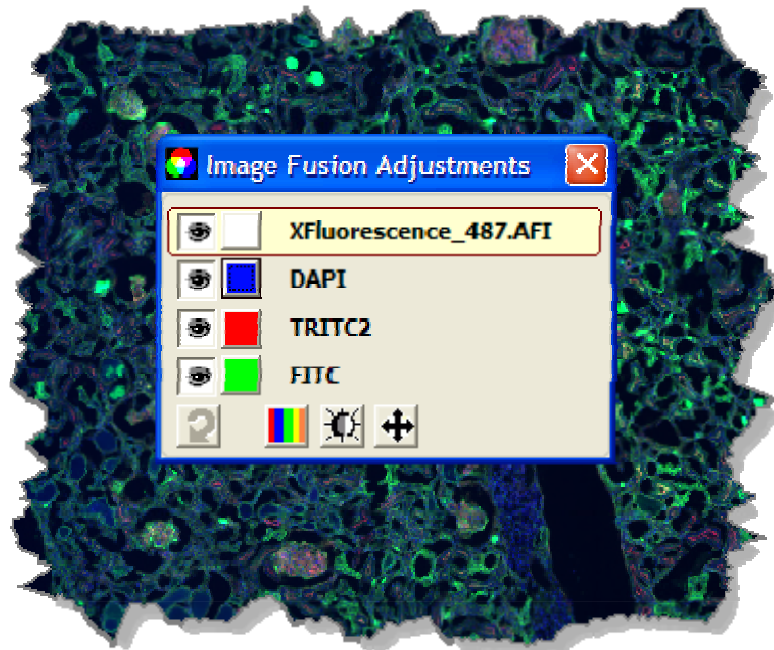
- **Total Analysis Area (mm²)** – Total area analyzed in millimeters squared.
- **Total Area of DYE (mm²)** – Total area in millimeters squared that contains the specified dye within the specified thresholds.
- **DYE Mean Intensity** – Average intensity of all pixels containing this dye within the specified thresholds.

3 Sample Analysis

This chapter shows a sample analysis using Positive Pixel Count FL. For details on installing an algorithm, creating an algorithm macro, registering it on Spectrum, and running an analysis, see the *Aperio Image Analysis User's Guide*.

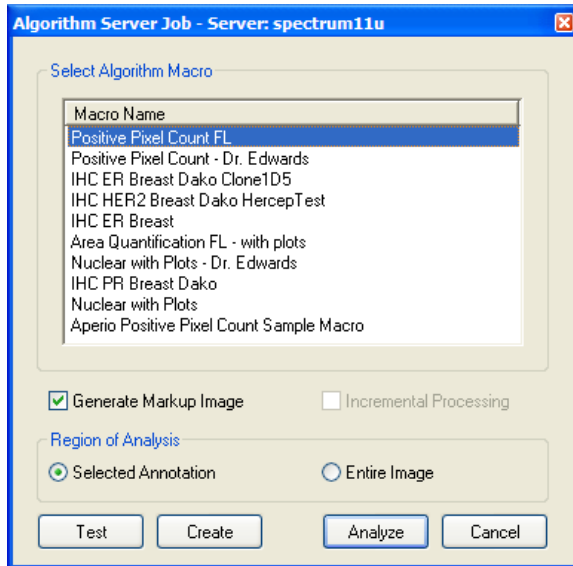
Follow these steps to use the Positive Pixel Count FL algorithm:

1. Install the algorithm (PositivePixelCountFL_v1_v11.0.0.xxx.exe) on both your Spectrum server and the workstation on which you will create the algorithm macro.
2. From Spectrum, open the Aperio Fused Image (AFI) file of the fluorescence image you want to analyze in ImageScope.

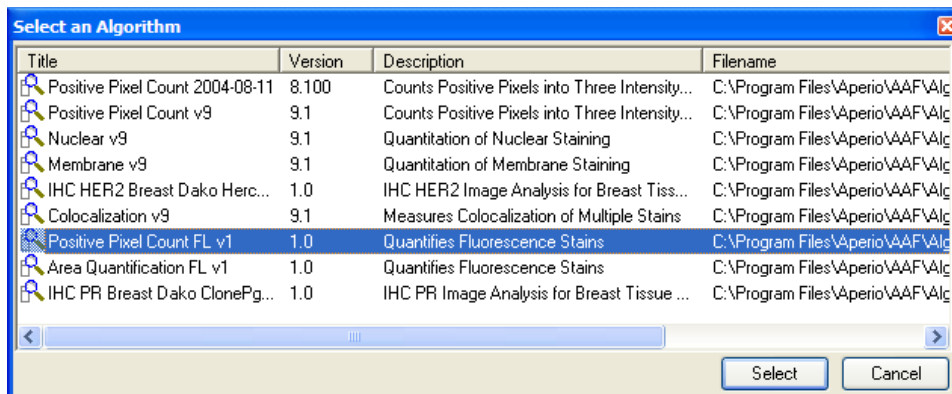


Note the Image Fusion window, which tells you what fluorescent dyes were used on the slide from which the digital slide was created.

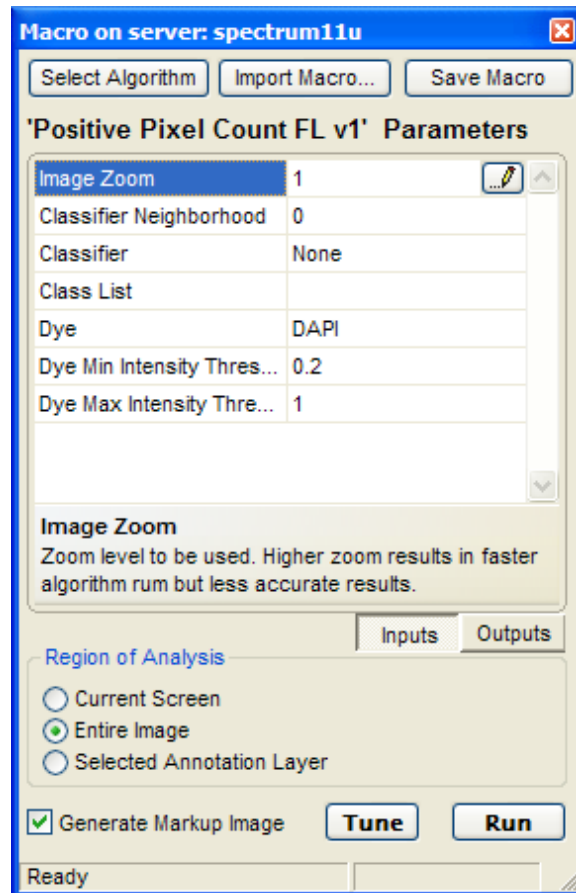
- Go to the View menu and select Analysis. You can either select an existing FL algorithm macro or click **Create** to create a new one. If no macros are listed, none have yet been created.



If you click **Create**, you can then choose to create a macro for the Positive Pixel Count FL algorithm by selecting it from the list:



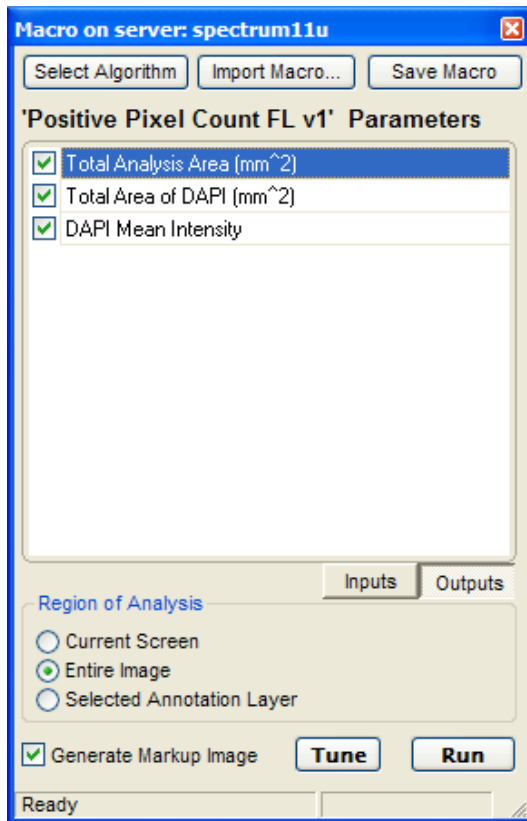
Now you see the input parameters for Positive Pixel Count FL. Note that you only see the Input/Output buttons if you are analyzing a digital slide opened on Spectrum.



Use of this algorithm differs from the similar brightfield Positive Pixel Count algorithm in that you must tell the algorithm which fluorescent dye on the digital slide you want to quantify. You will also define the minimum and maximum intensity threshold of that dye so that the algorithm can correctly separate the dye from background and/or strongly fluorescing artifacts. A good starting point for minimum intensity threshold is 0.2, the default value.

To select a specific dye, type its name in the Dye box (not case specific). Note that if the algorithm cannot find that dye in the AFI file, you will receive an error. If you are not using an AFI image, type RED, GREEN, or BLUE as the dye name, choosing the color that corresponds to the channel you want to analyze.

The Outputs button allows you to select what information you want the algorithm to display in Spectrum.

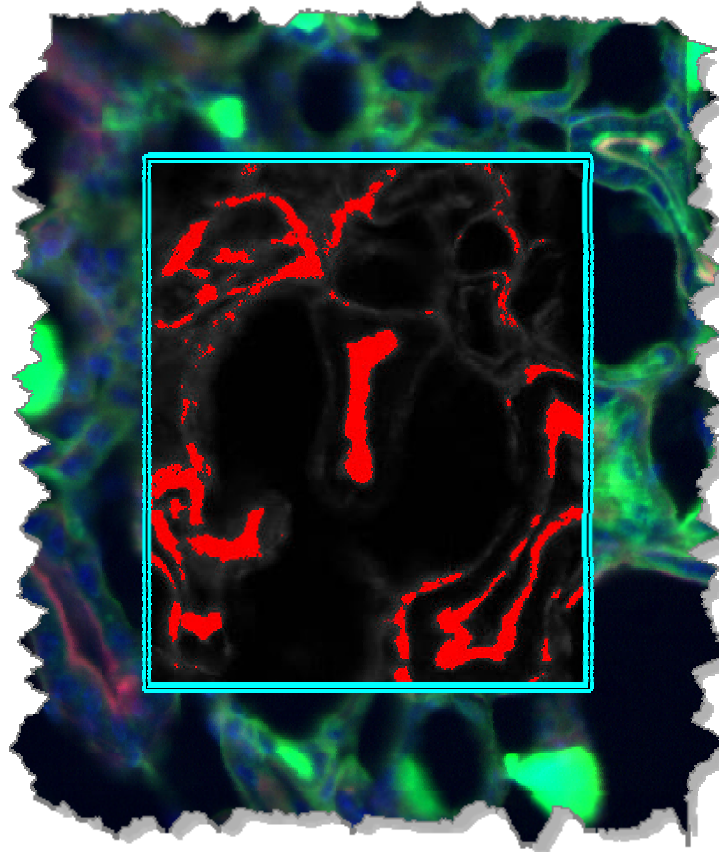


After adjusting the input parameters and selecting the output parameters, you can use the Tune button to see an approximation of the analysis results in a small window or click **Run** to run the algorithm on the selected region of analysis. The results appear in the Annotations window and, optionally, as a mark-up image in the ImageScope main windows. You can now save the algorithm with its parameter settings as a macro by clicking **Save Macro**.

Markup Image

The mark-up image gives you a visual way to see the specified dye as it appears in each individual pixel. The false colors used are based on the dye colors used to display a fluorescence image in ImageScope or WebScope.

Here is the mark-up image in ImageScope for a fluorescence image using DAPI, FITC, and TRITC2 dyes, with the TRITC2 dye selected in the algorithm:



For More Information

For detailed information on the following topics, see the *Aperio Image Analysis User's Guide*:

- Using the analysis tuning window
- Selecting areas to analyze
- Creating an algorithm macro and registering it on Spectrum
- Running an analysis
- Exporting analysis results

Positive Pixel Count FL Algorithm User's Guide

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