

Microvessel Analysis Algorithm

User's Guide



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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.

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1 Introduction

This chapter introduces the microvessel analysis 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** – Image analysis automates repetitive tasks.
- **Improves healthcare** – Analyzing digital slides helps you to examine slide staining to find patterns that will tell you more about the slide. Using an algorithm to look for these patterns provides precise, quantitative data that is accurate and repeatable.
- **Development of new computer-based methods** – Image analysis helps you answer questions that are beyond the capabilities of manual microscopy, such as “What is the significance of multiple stains at the cell level and colocalization of stains?”
- **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 Microvessel Analysis Algorithm

Analyzing capillary and microvessel infrastructure is critical in many disease areas. From in vivo angiogenesis assays in cardiovascular research to vascular morphology changes in oncology xenografts to microcapillary measurements in rodent models in ophthalmology, researchers need to understand and quantify vascular architecture. With whole slide scanning and the microvessel analysis algorithm, a tedious and subjective manual counting task can be automated and standardized.

The microvessel analysis algorithm detects and quantifies microvessels on slides stained with endothelial markers (for example, CD31, CD34, and Factor VIII).

Microvessel analysis typically starts with a Factor VIII or CD31 stained slide, scanned at 20x or 40x. A dual-level thresholding process screens out non-specific staining. Regions are then assembled into vessels, and individual statistics are

calculated on each vessel. You can choose which statistic to graph, and then can generate a summary report.

Prerequisites

The microvessel analysis algorithm requires that you be using Aperio Release 9 or later.

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 microvessel analysis algorithm, refer to Chapter 2, "Quick Reference" on page 5.

For details on using the microvessel analysis algorithm, begin with Chapter 3, "Microvessel Analysis Process" on page 9. The following chapters give details on using the algorithm.

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*.

Acknowledgments

Aperio thanks the following researchers for their suggestions and for donating images used in this guide:

- Mouse skin containing a subcutaneous matrigel plug immunostained with Factor VIII-related antigen provided by Dr. Reginald Valdez, DVM, MS, PhD, DACVP, Novartis Institutes for BioMedical Research, Inc.
- James Deeds, Novartis NIBR for many helpful suggestions.
- Dr. Maher Albitar, MD, Ph.D. Quest Diagnostics Nichols Institute.
- Dr. Judah Folkman, MD for taking the time to discuss the algorithm, his suggestions on the vessel perimeter measurements for a correlative measure of oxygenation, and for his clear review articles in the field that concisely describe what is required for improved future monitoring of angiogenesis.

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. 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 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.

2

Quick Reference

This chapter contains a quick reference to all microvessel analysis algorithm inputs and outputs. See the following chapters for details on using the algorithm.

If you are already familiar with using the microvessel analysis algorithm, and need just a reminder of the different algorithm input and output parameters, please refer to the sections below. For more detailed information on using the algorithm, see the following chapters.

Algorithm Input Parameters

Microvessel analysis algorithm performance is controlled by a set of input parameters, which determine many different types of analysis.

- **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 at least as big as the largest vessel of interest.
- **Image Zoom** – Zoom level to be used; a higher zoom results in faster algorithm run but less accurate results.
- **Markup Compression Type** – This sets the compression type for the algorithm mark-up image. Choose better compression if you need the image for a special purpose.
- **Compression Quality** – A higher quality takes longer and yields larger files. This selection does not apply to all compression types.
- **Markup Image Type** – There are two types of markup Images – Tuning and Analysis Markups. Tuning markups help in fine-tuning various input parameters before the vessel analysis. Only a few numeric results are valid for these markups and the rest of the results are zero. Analysis markups show different results of the microvessel aAnalysis algorithm. All the numeric results are valid and identical for the analysis markups.
- **Mode** – Choose whether to include/exclude incomplete vessels and other stained objects in the numeric results.
- **Filtering / Smoothing Level** – Specifies amount of filtering on the deconvolved vessel channel. Ranges from 0 – 10 with zero resulting in no filtering and 10 the maximum level of filtering.

- **Dark Staining Threshold** – First of the two parameters in segmenting the deconvolved vessel channel. (0 -255). Vessels don't stain uniformly at all locations depending on the "cut." This intensity corresponds to the darker or more intense regions of the vessels
- **Light Staining Threshold** – Second parameter in segmenting the deconvolved vessel channel. This threshold corresponds to the majority of vessel regions that stain at least as light or intense as this value.
- **Region Joining Parameter** – Distance in microns between pieces of vessel walls that may be joined into a complete vessel.
- **Vessel Completion Parameter** – Distance in microns between the ends of a vessel wall that may be completed into a vessel.
- **Minimum Vessel Area Threshold** – Any vessel which has an internal area (in μm^2) less than this value is eliminated from numeric results.
- **Maximum Vessel Area Threshold** – Any vessel which has an internal area (in μm^2) more than this value is eliminated from numeric results.
- **Maximum Vessel Wall Thickness** – Vessel Walls might seem much thicker than their actual size depending on the way they are cut. A non-orthogonal cut usually result in thicker looking Vessel Walls. This parameter restricts the maximum possible vessel wall thickness.
- **Output Histogram** – Choice of output statistic for histogram analysis. "None" option can be chosen for no histogram analysis.
- **Histogram Start Value** – Specifies the starting point for histogram analysis. Any output statistic value less than the start value (in either μm or μm^2) is not included in the histogram results.
- **Histogram End Value** – Specifies the end point for histogram analysis. Any output statistic value greater than the end value (in either μm or μm^2) is not included in the histogram results. Value of "-1" forces the histogram analysis on all output statistic values.
- **Number of Bins** – Specifies the number of bins in histogram analysis. All the histograms are uniformly spaced.
- **Endothelial Stain Red, Green, Blue Components** – Normalized optical density values for Endothelial Stain. Default for endothelial stain is DAB. These values are calculated by a calibration procedure described earlier in this guide.
- **Background Stain (1) Red, Green, Blue Components** – Normalized optical density values for Background channel (1). Default for background channel (1) is Hematoxylin. These values are calculated by a calibration procedure described earlier in this guide.
- **Background Stain (2) Red, Green, Blue Components** – Normalized optical density values for Background channel (2). Default for background channel

(2) is “None / No Stain”. These values are calculated by a calibration procedure described earlier in this guide.

- **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

Many of the algorithm results are discussed in the following chapters.

- **Number of Vessels** – Total number of vessels detected after microvessel analysis.
- **Total Analysis Area (μm^2)** – Total area that is analyzed in square-microns.
- **Total Stain Area (μm^2)** – Total area that has significant endothelial staining. These regions are segmented by the Dark and Light thresholds.
- **Average Stain Intensity** – Average stain intensity of all the endothelial stained pixels.
- **Microvessel Density (μm^2)** – Number of vessels per unit of analysis area in square-microns.
- **Mean Vessel Area (μm^2)** – Average of all the Vessel Areas in square-microns.
- **Median Vessel Area (μm^2)** – Median values of all the Vessel Areas in square-microns.
- **Standard Deviation of Vessel Area (μm^2)** – Standard deviation of all the Vessel Area in square-microns.
- **Mean, Median and Standard Deviations of Vessel Perimeter (μm), Lumen Area (μm^2), Vascular Area (μm^2) and Vessel Wall Thickness (μm)** – Similar statistics for the parameters as above.
- **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.

Histogram Results

For an example of a histogram of results, see “Generating Histograms” on page 24.

- **Total Number of Vessels** – Total number of vessels detected by the algorithm

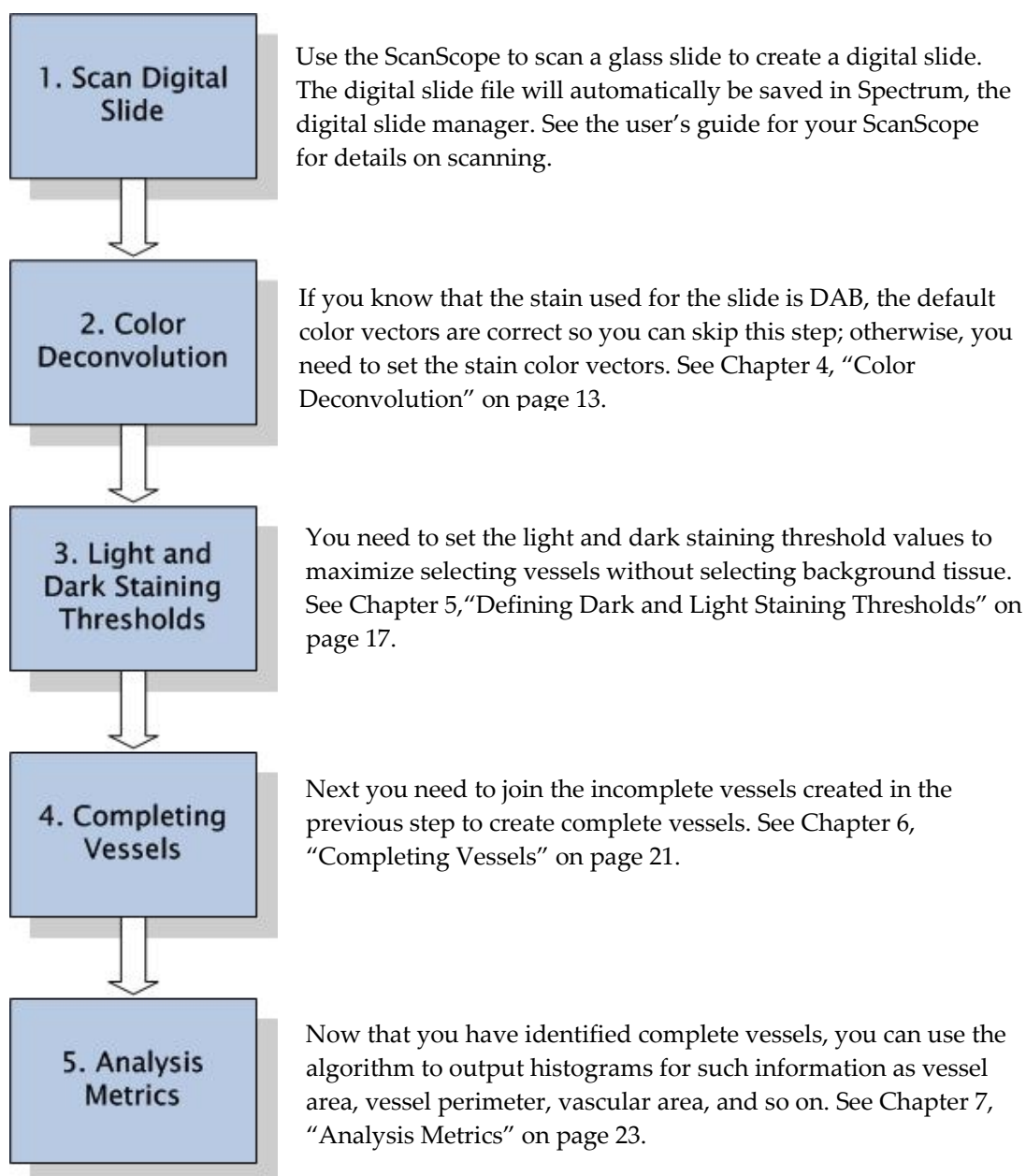
- **Number of vessels in Histogram Analysis** – Number of vessels that are included in the histogram analysis based on the histogram start and end values specified by input parameters.
- **Overall Minimum Value (μm^2)** – Overall minimum value of the statistic which is chosen for histogram analysis. This value is not affected by the histogram start and end values.
- **Overall Maximum Value (μm^2)** – Overall maximum value of the statistic which is chosen for histogram analysis. This value is not affected by the histogram start and end values.
- **Histogram Bin Centers - Frequency** – Histogram Analysis results showing a list of bin centers and their corresponding frequencies.

3

Microvessel Analysis Process

This chapter discusses the steps in microvessel analysis and gets you ready to start using the algorithm.

There are five steps to microvessel analysis:



Microvessel Analysis Definitions

We will be using the following terms in the following chapters. At various steps in using the microvessel algorithm, you will be able to see graphical representations of these elements.

Term	Definition	Example
Endothelial cell	Epithelial cell that lines the circulatory vessel.	See page 18.
Region	The intermediate object the algorithm constructs by putting together endothelial cells.	See page 18.
Vessel	Connection of regions into one object, typically with a lumen	See page 21.
Lumen	Inside area of a vessel.	See page 21.
Vessel wall	Endothelial cells that form the wall of the vessel.	
Vascular area	Sum of all endothelial cells.	
Vessel area	Vascular area/2 + lumen area	

Before You Start

First, be sure that the microvessel analysis algorithm has been installed both on the Spectrum server and on your local workstation. See the *Aperio Image Analysis User's Guide* for details on algorithm installation.

Opening a Digital Slide in Spectrum

Cases, specimens and digital slides are managed using Aperio's Spectrum. A pathologist who wants to access a digital slide first needs to log into Spectrum and navigate to the case and the specimen that shows the list of its associated digital slides and then open the desired digital slide in ImageScope.

As part of using the microvessel analysis algorithm, you will be creating an algorithm *macro* (a file that contains your algorithm settings). In order to do that, you will need to be logged into Spectrum as an administrator.

See the *Aperio Image Analysis User's Guide* for instructions on opening a digital slide and creating an algorithm macro.

Once the digital slide is open in ImageScope, you will be able to use the ImageScope tools to select areas of the digital slide to analyze and use the microvessel analysis algorithm to detect and quantify microvessels.

The instructions in these chapters assume you are opening a digital slide in Spectrum and analyzing it directly in the ImageScope viewer. If this is not the case (that is, you are *not* using Spectrum, but are opening a digital slide that is on your workstation or on a server directly from ImageScope), the appearance of some of the ImageScope windows may differ slightly, although the microvessel analysis algorithm steps will be the same. See the *ImageScope User's Guide* for details on using digital slides that are local or are on a server.

Selecting the Microvessel Analysis Algorithm

Once you have selected a digital slide and opened it in ImageScope (as discussed in the *Aperio Image Analysis User's Guide*), you see the digital slide displayed in the main ImageScope window. (The following is a digital image of mouse skin that contains a subcutaneous matrigel plug which has been immunostained with Factor VIII-related antigen.)

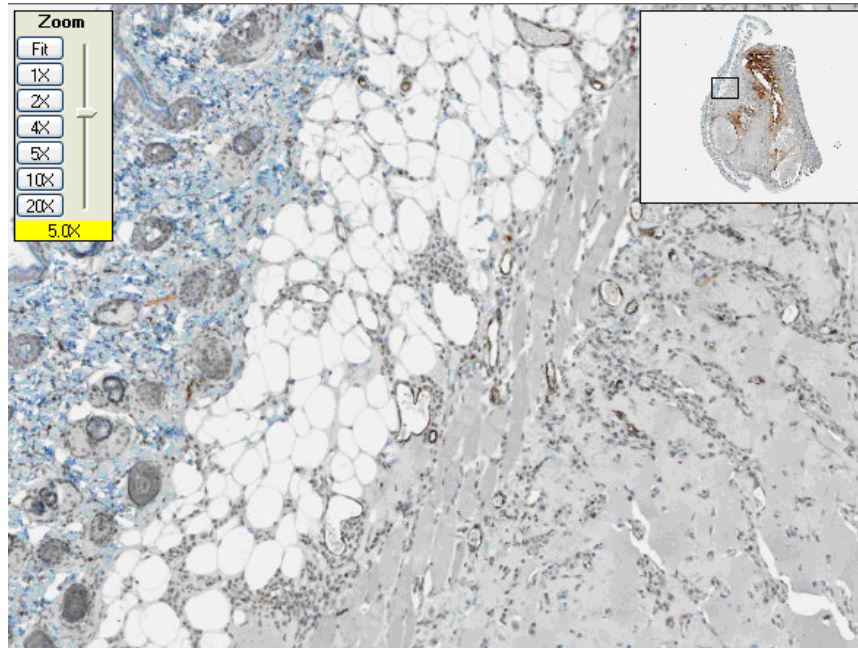


Image of mouse skin containing a subcutaneous matrigel plug immunostained with Factor VIII-related antigen provided by Dr. Reginald Valdez, DVM, MS, PhD, DACVP, Novartis Institutes for BioMedical Research, Inc.

For details on moving around the image, changing its magnification, and using the drawing tools to select areas of the image, see the *ImageScope User's Guide*.

To select the microvessel analysis algorithm:

1. Go to the ImageScope View menu and select **Analysis**. The Algorithm Server Job window displays.
2. If an algorithm macro for the microvessel analysis algorithm has not yet been created, click **Create**. (You must be logged into Spectrum as an Administrator to use the **Create** button.)

The Select an Algorithm window appears, which lists all of the algorithms that have been installed.

3. Select the microvessel analysis algorithm and click **Select**.

The Algorithms window now displays.

4. When the microvessel analysis algorithm is shown in the Algorithms window, you can proceed with the analysis.

Now you are ready to begin using the microvessel analysis algorithm as discussed in the following chapters.

Important: To use the algorithm again, you will need to save the algorithm macro, which consists of all of the settings you have adjusted, so before you close the Algorithm window, save your settings. See “Saving the Algorithm Macro” on page 26.

4 Color Deconvolution

Color Deconvolution defines the stain color vectors so that endothelial cells will be correctly identified by the algorithm. If the slide was stained with DAB, the default color vectors are correct and you can skip this step by going directly to the next chapter.

By defining the stain color vectors, you are identifying to the microvessel analysis algorithm which color identifies the endothelial cells that make up the vessel walls.

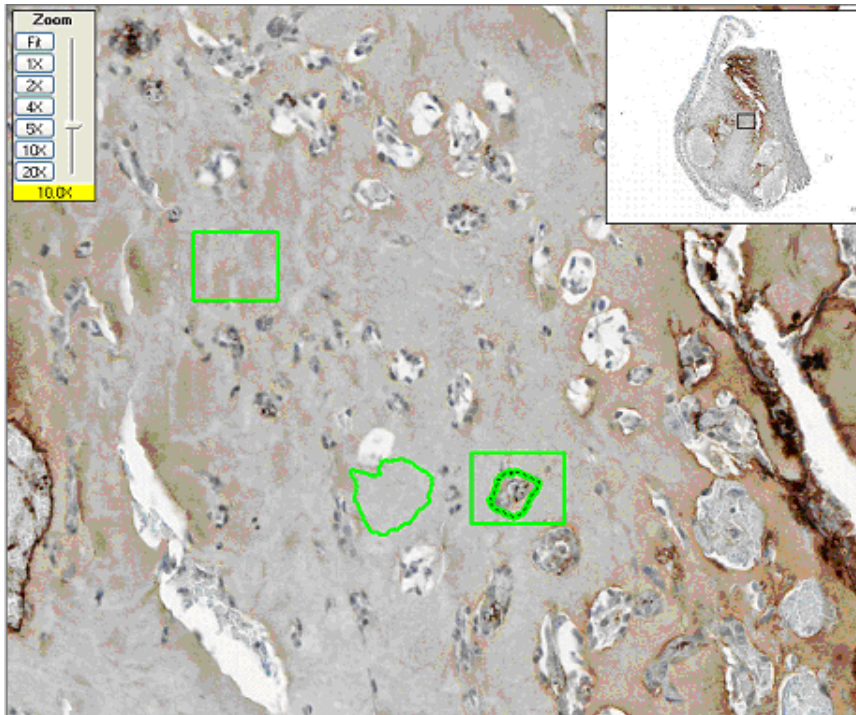
Deconvolution separates the contribution from the endothelial stain (for example, CD31 or Factor VIII) and uses only this color for analysis.

The default color vector settings are for DAB as the endothelial stain and hemotoxylin as the background stain 1 (background stain 2 set to zero; that is, assumed to be nonexistent).

Endothelial Stain - Red Compon...	0.268
Endothelial Stain - Green Comp...	0.57
Endothelial Stain - Blue Compon...	0.776
Background Stain 1 - Red Com...	0.68
Background Stain 1 - Green Co...	0.57
Background Stain 1 - Blue Com...	0.44
Background Stain 2 - Red Com...	0
Background Stain 2 - Green Co...	0
Background Stain 2 - Blue Com...	0

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.

Look for several areas of the digital slide that are mostly stained with endothelial stain and select them by using the ImageScope drawing tools. In other words, pick an area of light staining of only this color. Avoid selecting darker, overstained areas.



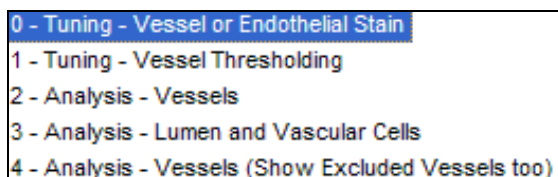
For this example, we selected several light brown areas using the ImageScope drawing tools on the ImageScope toolbar. The tools you can use to select regions are:

	Freehand pen – Use to draw a free-form area of interest.
	Negative freehand pen – Use to draw an area to <i>exclude</i> from the analysis. Note that you can use this in combination with the other drawing tools to first select an area of interest and then exclude areas within the selected area that you do not want to analyze.
	Rectangle tool – Draws a rectangular area. If you want to select a square, hold down the Shift key while drawing.

Setting the Stain Color Vectors

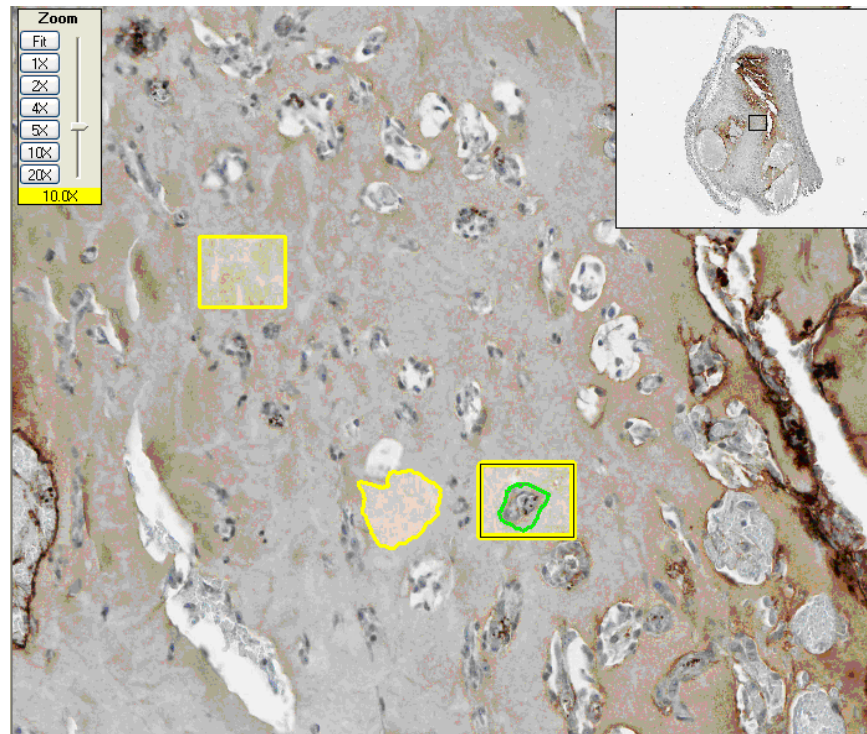
On the Algorithms window:

1. From the **Mark-up Image Type** drop-down list, select **0 – Tuning – Vessel or Endothelial Stain**.



2. Select **Selected Annotation Layer** so that only the selected areas are analyzed.
3. Select the **Generate Markup Image** check box.

- Click **Run**. When the analysis is complete, the selected areas in the image show a visual result of the analysis.



- To see the numeric results of the analysis, go to the View menu and select **Annotations**. The Annotations window displays the color vector information in the OD (optical density) area:

Average Red OD	0.426768
Average Green OD	0.58289
Average Blue OD	0.691454

- Type the values from the **Endothelial Stain – Red OD**, **Endothelial Stain – Green OD**, and **Endothelial Stain – Blue OD** lines into the corresponding areas of the Algorithm window:

Endothelial Stain - Red Component	0.268
Endothelial Stain - Green Component	0.57
Endothelial Stain - Blue Component	0.776

Now perform these same steps for any background staining used to set the color vectors for those stains.

Checking the Deconvolution

After you have set the color vectors, you can double-check your results by drawing a region around a vessel, run the algorithm again in tuning mode, and the vessel walls should be clearly seen.

5

Defining Light and Dark Staining Thresholds

In order to distinguish between endothelial cells and background tissue, you need to define the light and dark staining thresholds.

Defining the light and dark staining thresholds will identify endothelial cells.

- **Dark Staining Threshold** – All vessels must contain some region that stains darker than this threshold.
- **Light Staining Threshold** – All vessels must contain large regions that stain darker than this threshold.

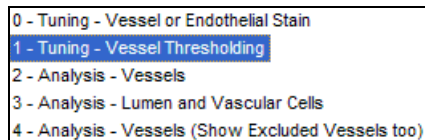
By setting the threshold values correctly, you avoid single-point staining (for example, false stain uptake by non-endothelial cells).

The darkest value equals zero, and the lightest value equals 255.

Vessel Thresholding

With the digital slide open in ImageScope and the microvessel analysis algorithm displayed in the Algorithms window:

1. Select **1 – Tuning – Vessel Thresholding** in the **Mark-up Image Type** drop-down list.



2. Set both threshold values to the same value (try 210).

3. Click **Run** to run the algorithm. Here is a mark-up image when light and dark thresholds are set to 210. As you can see, quite a few non-vessel areas are selected.



4. Now set the dark threshold to a new value to something less than 190. Keep running the algorithm in this tuning mode and inspecting the markup image.
5. Keep reducing the dark threshold value to eliminate non-vessel material, but without eliminating vessels. Here is a mark-up image when light threshold is set to 210 and dark threshold is set to 170:



6. Adjust the light staining threshold value to ensure that nearly all endothelial cells are detected but a minimum of false stained cells are detected.

Your goal for this process is to strike a balance between selecting vessels but not selecting much background tissue. It is okay at this point to have a few false stained cells as the next step can eliminate them.

6

Completing Vessels

In this step, you join incomplete vessels into complete vessels.

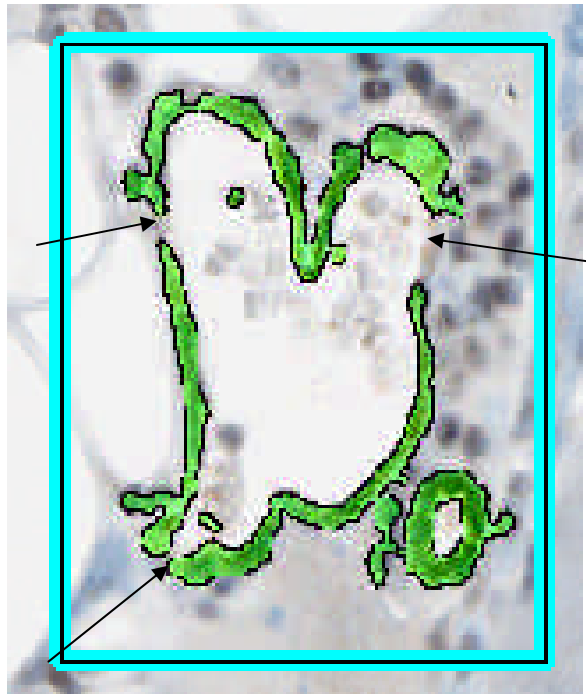
This step looks for lumen and connects endothelial cells around the lumen to identify a vessel.

Two things can be set:

- **Region Joining Parameter** – Connects broken pieces of vessel walls; all pieces within the distance specified will be joined to the same vessel.
- **Vessel Completion Parameter** – Connects incomplete and open vessel walls; incomplete vessel walls with less than this distance of an opening may be completed into one vessel.

Region Joining Parameter

Here is an example of when the Region Joining Parameter can be used to connect cells into a vessel. Notice that this incomplete vessel consists of three regions separated by gaps:



The Region Joining Parameter specifies the maximum distance of the gaps that can be detected and filled in to join two regions when more than one gap exists. Once the regions have been joined so that a single object is created with just one gap, the Vessel Completion Parameter is used to complete the vessel.

Vessel Completion Parameter


Here is an example of when the Vessel Completion Parameter can be used to close an incomplete vessel:

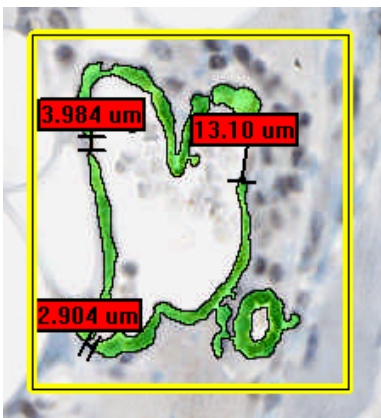


In this example, a complete object exists with a single gap.

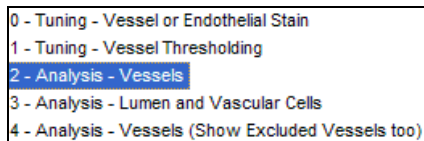
Completing the Vessel

First we will measure the gaps in the vessel:

1. Select the ruler tool on the ImageScope toolbar: .
2. Choose the worst-case incomplete vessel (the gaps are the largest) and drag the ruler across the gaps in the vessel to measure them. The measurements in microns display on the image:



- On the Algorithms window, go to the **Mark-up Image Type** drop-down list and select **2 – Analysis – Vessels**:



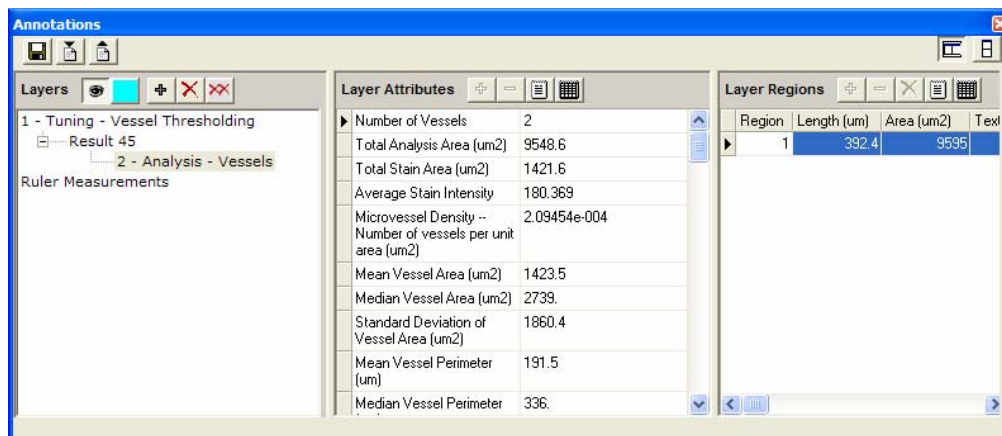
- Type a value for the largest gap measurement into **Vessel Completion Parameter** and the next largest gap measurement into **Region Joining Parameter**.
- Select **Generate Markup Image** and **Selected Annotation Layer**.
- Click **Run**. If the vessel has been completed, you will see an image similar to this, which shows the lumen filling the vessel:



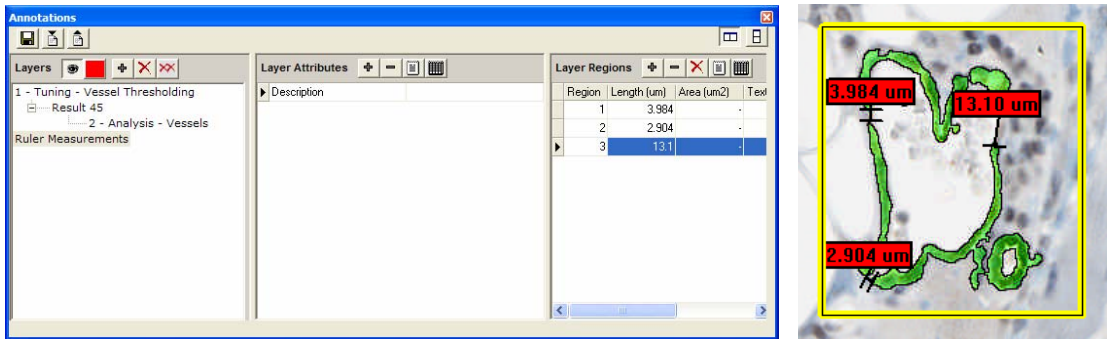
If you supply Vessel Completion Parameter and Region Joining Parameter values that are too large, the algorithm will join multiple vessels together and will also take much longer to run.

To see numeric information from the analysis:

- Go to the ImageScope View menu and select **Annotations**. The Annotations window displays with information on the different tuning and analysis operations we have performed:



- Click on the different annotation layers in the Layers pane to show numeric data for that step (and to show the mark-up image for that step in the ImageScope window). For example, to see the ruler measurements again, click on the annotation layer for those measurements (in the example above, we have renamed that layer to “Ruler Measurements”), and you see both the numeric results (the measurements in the Layer Regions pane) and the mark-up image for that step.



7

Analysis Metrics

Now that complete vessels have been identified, you can use the microvessel algorithm to analyze microvessel distribution to create histograms.

By using the microvessel analysis algorithm parameters on the Algorithms window, you can select many different types of analysis.

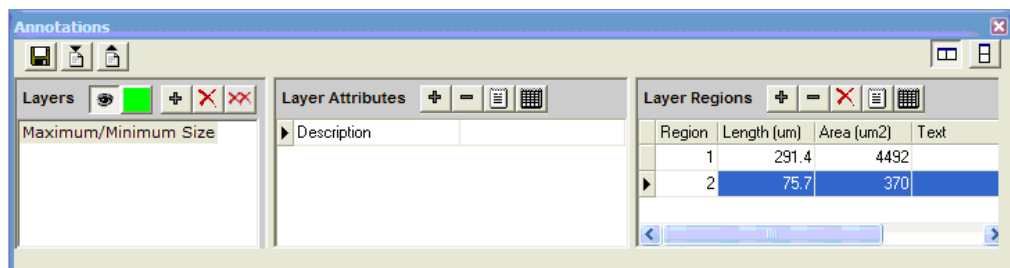
Please see Chapter 2, “Quick Reference” on page 5 for details on each parameter.

By selecting the appropriate algorithm parameters and mark-up image types, you can fine-tune the microvessel selection process (for example, including or excluding microvessels to analyze based on minimum or maximum size), output histograms for vessel area, vessel perimeter, vessel wall thickness, vascular area, and lumen area.

Selecting Maximum/Minimum Vessel Size to Analyze

To fine-tune microvessel detection by selecting the minimum and maximum size vessels to analyze:

1. Draw freehand shapes around maximum and minimum size vessels . You see the area of those selections in the Annotations window:



2. On the Algorithms window, set the maximum and minimum vessel size parameters to reflect your selections. For example, to exclude the smaller vessel selected, enter a minimum size of greater than 370; to include vessels as large or larger than the larger vessel selected, enter a maximum size greater than 4492.

- Now run the algorithm again, and the smaller vessel is excluded from analysis, but the larger one is included as shown by the mark-up images below:

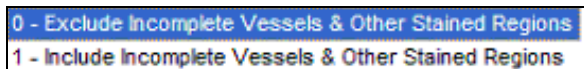


Additional Analysis Modes

The different **Mark-up Image Type** selections will give you many different ways to look at microvessels. For example, choosing **3 – Analysis – Lumen and Vascular Cells** results in a mark-up image like this:



You can also use the **Mode** drop-down list to include/exclude incomplete vessels from analysis:



Generating Histograms

The **Output Histogram** drop-down list on the Algorithm window gives several different choices for the data to output as a histogram:

- 0 – None
- 1 – Vessel Area


- 2 – Vessel Perimeter
- 3 – Lumen Area
- 4 – Vascular Area
- 5 – Vessel Wall Thickness

There are additional parameters that affect how the histogram data is output:

- Histogram Start Value (μm or μm^2)
- Histogram End Value (μm or μm^2)
- Number of bins

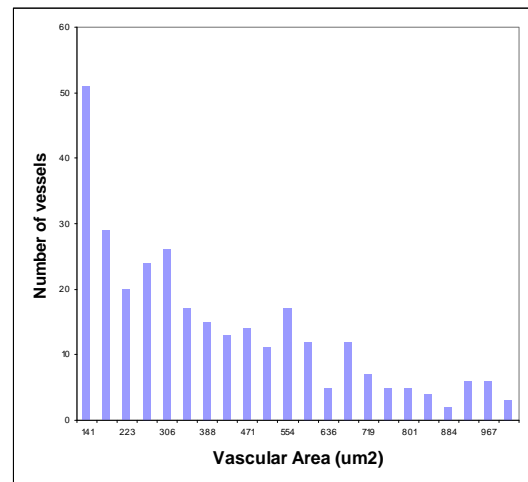
Note that you can adjust the start and end values for the histogram to capture just the data you want. A histogram end value of -1 captures all data, but you might want to set this to the value of the largest vessel you want data for, for example, to fine-tune the range of vessels reported on.

When you have selected a histogram type and run the algorithm, the histogram data is shown in the Layer Attributes pane of the Annotation window.

Now you can use the spreadsheet export button on the Layer Attributes pane, , to export the data into an Excel spreadsheet where you can see the data in graphical form.

For example:

Statistic	Tumor	Normal
Number of Vessels	704	352
Total Analysis Area (μm^2)	12811307	4110861
Total Stain Area (μm^2)	95351.09	62105
Average Stain Intensity	158.667	191
Mean Vessel Area (μm^2)	143.066	213
Median Vessel Area (μm^2)	99.6743	121
Standard Deviation of Vessel Area	135.16	302
Mean Vessel Perimeter (μm)	65.1583	78
Median Vessel Perimeter (μm)	50.17	58
Standard Deviation of Vessel Perimeter (μm)	44.7857	66
Mean Lumen Area (μm^2)	16.9771	46
Median Lumen Area (μm^2)	0	0
Standard Deviation of Lumen Area (μm^2)	65.3677	196
Mean Vascular Area (μm^2)	114.144	157
Median Vascular Area (μm^2)	82.3068	98
Standard Deviation of Vascular Area (μm^2)	105.778	195
Mean Vessel Wall Thickness (μm)	1.5273	1.66
Median Vessel Wall Thickness (μm)	1.47374	1.55
Standard Deviation of Vessel Wall Thickness (μm)	0.402804	0.53



Saving the Algorithm Macro

To use the algorithm again, you will need to save the algorithm macro, which consists of all of the settings you have adjusted.

If you are satisfied with the results based on your parameter changes, click **Save Macro** to save the macro and register it on Spectrum. You are asked to enter a name for the macro; type a name that will help you identify the macro in the future. Because the algorithm has been optimized for a particular specimen, we suggest you use a macro name that will remind you which specimen was analyzed, for example: **Dr. David's Microvessel – Spec 1A**. Click **OK**.

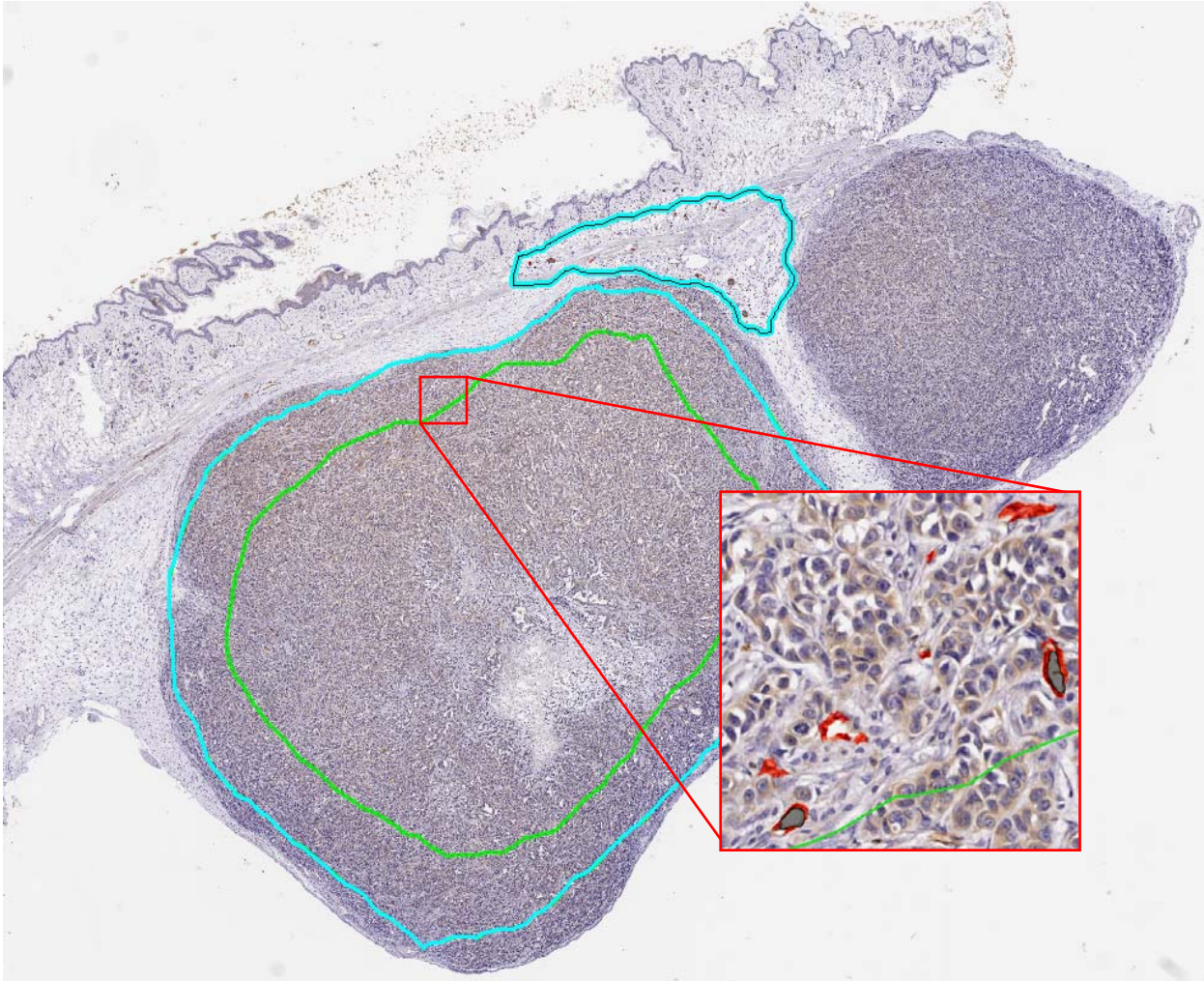
When you see the message that the macro saved successfully, the Analysis window closes.

Now, when you bring up the Analysis window, you will be able select your macro by clicking **Select Algorithm**.

If you have not opened a digital slide through Spectrum, but have directly analyzed a digital slide that resides on your workstation or local network, to save the macro click the **Export Macro** button which appears on the Algorithms window instead of the **Save Macro** button. The macro will be saved only on your workstation, not in the Spectrum database. You can use the Spectrum **Add Macro** command to add the macro to Spectrum—see the *Spectrum/Spectrum Plus Administrator's Guide* for details.

Example – Oncology Research with Xenografts

The following example shows using the microvessel analysis algorithm to quantify vascular changes extending outward from a xenograft, and then comparing with normal tissue vasculature.



For More Information

For details on using the Aperio products mentioned in this guide, see:

- *Spectrum/Spectrum Plus Operator's Guide* and *Spectrum/Spectrum Plus Administrator's Guide* for information on using the Spectrum digital slide management system.
- *ImageScope User's Guide* for details on using the ImageScope viewer.
- *User's Guide* for your ScanScope for information on scanning glass slides.

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