



# Image Analysis 101

# **Course Objectives**



# Upon conclusion of this training session, learners will be able to complete the following tasks related to their job:

- Describe how Aperio's Image Analysis tools work
- Describe Aperio's Image Analysis tools for Area, Cell, and Event Quantification
- Understand the input parameters for each Image Analysis tool
- Understand the output parameters for each Image Analysis tool
- Complete the steps for running and Aperio Image Analysis tools
- Calibrate stain color using Color Deconvolution
- Create a macro and register it with Spectrum
- Run a macro using Spectrum Plus batch analysis option
- Export Image Analysis results using Spectrum's Export Data tool
- Describe Aperio's Scalable Architecture

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.

# **Terminology Table**



Image Analysis	The extraction of meaningful information from digital images by means of digital image processing techniques.
Deconvolution	Image processing technique that removes the effects of convolution from measured data.
Colocalization	Analysis technique that determines the contribution of each stain at every pixel location.
Intensity	The measure of brightness of the pixel. Intensity ranges from $0 =$ black to 255 = bright white.
Color Vector	The normalized optical density for the Red (R), Green (G), Blue (B) components that represent a specific color (stain).
Immunohistochemistry (IHC)	A method for staining cells; antibodies to specific proteins are used to analyze specimens and identify specific types of cells, especially for certain types of cancer.
Angiogenesis	The formation of new blood vessels.

### **Image Analysis Illustrated**

#### Image Analysis extracts meaningful data:

- Stains are used to highlight features.
  - Tissue and cell components are revealed.
    - Nuclei, membranes and cytoplasm.
  - Specific protein expressions such as, ImmunoHistoChemistry panels.
    - HER2, ER and PR.

#### • Image analysis can help answer questions.

- Where and how much staining is there?
- · Where and how many objects are there?
  - Tumor cells.
- How much staining is there on different objects?
  - Cell nuclei.
  - Cell membranes.



Original

Markup

to highlight analysis results.

Image Analysis tools can present a markup image





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## **Image Analysis Illustrated**



#### How Image Analysis works:

- Analyzing a digital slide by applying an algorithm directly to the digital slide or selected region of the digital slide.
  - Stained slides result in a multicolored image. Image analysis acquires the RGB color of the pixels.



- Image Analysis tools organize the color (RGB pixels) of stains.
  - Classifies pixels by color (stain).
  - Measures pixel (stain) intensities.
  - Indentifies objects by color, shape or size.
  - Measures object (stain) intensities.



Nucleus Membrane





Rare Event

# **Image Analysis Tools**



#### Image Analysis tools and their specific applications:

- Positive Pixel Count\* quantifies the area and intensities of positive and negative staining.
- **Color Deconvolution**\*\* accurately separates up to 3 stains and measures area and intensity separately, resolving multi-stain colocalization problems.
- **Colocalization** quantifies the area and intensity of "colocalized" markers.
- **Nuclear** quantifies nuclear staining and intensity.
- **Membrane** quantifies membrane staining and intensity.
- Microvessel Analysis detects and quantifies microvessels.
- **Rare Event Detection** detects micrometastasis of tumor cells.
- **Digital IHC Analysis** streamlined workflow for IHC analysis.
- **Genie** smart tissue classifier.

\*Positive Pixel Count is licensed without fee with other Aperio Software.

\*\*Color Deconvolution is our professional version of our Positive Pixel Count algorithm.



### **Aperio Image Analysis Capabilities**





### **Image Analysis Applications**



#### **Blood Vessels**





Original

Markup

#### **Stain Separation**



3 colors



#### **Nuclear Staining Quantification**



OriginalMarkupMembrane Staining Quantification



Original

Markup

### **Image Analysis Applications**



#### **Fatty Vacuoles**



Original

Markup

#### **Cytoplasmic Quantification**



Original



Markup

#### **H&E Nuclear Counting**



Original

#### **Rare Event Detection**



Original

Markup



#### Run Analysis several ways.

#### ImageScope Analysis

- Use the Analysis command of ImageScope to analyze either a local digital slide image (that is, an image on your workstation or on the network where your workstation can see it via Microsoft file sharing).
- Use ImageScope to connect an image on an Aperio ImageServer.

#### Spectrum Plus Analysis

• Analyze a single digital slide image that resides on the ImageServer by using Spectrum's **Analyze** command.

#### Batch Analysis

• Analyze a batch of digital slide images that reside on the ImageServer by selecting multiple images and using Spectrum's **Analyze** command.

In most cases, it is expected that a digital slide will be opened in Spectrum. For the purpose of training all images will be opened in Spectrum.



- Analyze entire digital slide or selected areas.
- Use ImageScope drawing tools to select or exclude areas to analyze.
  - Pen draw free-form area of interest.
  - Negative Pen draw free-form area to *exclude* from analysis.
  - Rectangle draw a rectangular area of interest.





#### **Analysis Window**

• When performing analysis via Spectrum the Algorithm Server Job displays registered macros.

Nuclear_macro MicrovesselAnalysis_macro MicrovesselAnalysis_macro COlorDeconvolutionSeperates Colocalization_macro PositivePixelCount_macro Colocalization_SeperateStains Rare Event Detection Aperio Positive Pixel Count Sa	Stains_macro s_macro ample Macro	List of macros
Generate Markup Image	Incremental Processing	

#### **Analysis Buttons**

Test – modify existing macro for selected algorithm and test before saving. Create – creates new macro based on the original algorithm.

Analyze – run analysis.

Cancel – cancel current analysis job.



#### **Incremental Processing**

- Some Aperio algorithms support incremental processing.
- Incremental processing allows the algorithm to analyze only regions added after the initial analysis without re-analyzing the previously analyzed regions.

Macro Name			
IHC HER2 Breast Dako			
Rare Event Macro			
ColorDeconvolution_SeperateStains_macro			
IHC PR Breast Dako Clone			
IHC ER Breast Dako Clone			
PositivePixelLount_macro	LAND THE COLORES	►	List of macro
Nuclear_Stain_ColorDeconv	olution_1 une_color_macro		
Nuclear_Inacio	Quantification macro		
Microvesselánalusis Tupina	_guardineador_macro		
	2 Thresholding macro		
MicrovesselAnalysis_Tuning	2_Thresholding_macro I Color macro		
MicrovesselAnalysis_Tuning MicrovesselAnalysis_Tuning MicrovesselAnalysis_macro	2_Thresholding_macro I_Color_macro		
MicrovesselAnalysis_Tuning MicrovesselAnalysis_Tuning MicrovesselAnalysis_macro MicrovesselAnalysis_Analysis	2_Thresholding_macro I_Color_macro s3_VesselsMode1_macro	~	
MicrovesselAnalysis_Tuning MicrovesselAnalysis_Tuning MicrovesselAnalysis_macro MicrovesselAnalysis_Analysis	2_Thresholding_macro I_Color_macro s3_VesselsMode1_macro	>	
MicrovesselAnalysis_Tuning MicrovesselAnalysis_macro MicrovesselAnalysis_Macro	2_Thresholding_macro I_Color_macro 33 VesselsMode1_macro	>	
MicrovesselAnalysis_Tuning MicrovesselAnalysis_macro MicrovesselAnalysis_macro	2_Thresholding_macro I_Color_macro 3_VesselsMode1_macro	>	Analysis optic
MicrovesselAnalysis_Tuning MicrovesselAnalysis_macro MicrovesselAnalysis_macro	2_Thresholding_macro I_Color_macro 3_VesselsMode1_macro " [	>	Analysis optic
MicrovesselAnalysis_Tuning MicrovesselAnalysis_Tuning MicrovesselAnalysis_macro MicrovesselAnalysis Generate Markup Image	2_Thresholding_macro I_Color_macro 3_VesselsMode1_macro " [	Ssing +	Analysis optic



#### PPC quantifies the area & intensity of two stains.

• Positive Pixel Count (PPC) looks for positive, negative and neutral areas of staining by analyzing the slide staining against user defined values.



Original

Markup

The markup image color-codes the analyzed pixels in the following format: Negative, Weak, Medium, Strong Positive

- Outputs:
  - Negative, Weak, Medium, Strong Positive Staining
    - Number of pixels
  - Intensity sum
  - Average intensity of weak positive pixels
  - Ratio of strong/total number



#### PPC has a set of input parameters.

- Input parameters allow the user to define the positive stain color and intensity thresholds for the positive stain using HSI (Hue, Saturation, Intensity) color model.
  - The user will specify a color (range of hues and saturation) and three intensity ranges (weak, positive, and strong).
  - PPC input parameters are defaulted for brown color quantification.





#### **PPC input parameters:**

- The **Hue Value**, and **Hue Width** can be adjusted to define the color to match the specific stain being used.
  - Hue Value position on the color wheel for positive color.
  - This parameter defines the color of the positive stain.





#### **PPC input parameters:**

- Adjust the **Hue Width** to define the ranges of hues for analysis.
  - Hue Width is the range of hues, centered on the Hue Value that will be analyzed.
  - The larger the Hue Width, a larger range of Hues will be included for positive color.
  - The lower the Hue Width, a smaller range of Hues will be acceptable.





#### **PPC input parameters:**

- PPC classifies each pixel as either negative, weak positive, positive, strong positive or non-tissue (clear glass) based on its color and intensity.
- Positive stain color intensity results are separated into three color coded ranges.
  - Intensity weak positive (lwp) = yellow
  - Intensity positive (lp) = orange
  - Intensity strong positive (Isp) = red







#### **PPC analysis results:**

- Analysis results are viewed from the **Annotations Window** in ImageScope.
- PPC analysis results are area based quantification and intensity values.
  - If running analysis in ImageScope, a false color Markup image can be generated.





#### **PPC** Workshop



Watch Instructor demo the tasks below and perform them using the steps beginning on the next page:

- Open PPC demo file
- Select PPC macro
- Run analysis
- View Results





- 1. Log into Spectrum with a username and password provided by instructor & select the specified **Research Role**.
- 2. Navigate to the digital slide list, open the **Positive Pixel Count Demo** slide as shown below.

Login Required		
Username: Password: User Login		
Digital Slides List all Digital Slides View all Digital Slides as Folders List ScanScope Slides List ScanScope Slides (Past Day) List ScanScope Slides (Past Week)		Positive Pixel Count Demo Data
List ScanScope Slides (Past Month) Search all Digital Slides Add new Digital Slides	Click thumbnail image to open ImageScope	]



- 3. Click View, select Annotations Window (Ctrl N).
- 4. Click **Region 1** in the Annotations Window under the Layer Regions section to select the area for analysis. (For this exercise please use region 1).



**Note:** You can create your own region of analysis by using any of these annotation tools in ImageScope. The negative pen tool will exclude regions for analysis.







5. Click View, Select Analysis (Ctrl G). The Algorithms Window is now displayed.

#### 6. Select PositivePixelCount\_macro & click Test.

The saved input parameters are now displayed.





7. Review the parameters, set Region of Analysis to Selected Annotation Layers, & Generate Markup Image.

Macro on server: APERIO-01959

8. Click Run.

		Select Algorithm Import	Macro	Save Macro	
	7	Positive Pixel Count	/9' Parame	ters	
		View Width	1000		
		View Height	1000		
		Overlap Size	0		
		Image Zoom	1		
		Markup Compression Type	0 - Same as pr	ocessed image	
		Compression Quality	30		
		Classifier Neighborhood	0	Clossifier N	aighborhood Classifier 9
		Classifier	0 - None	Classifier N	eignbornood, Classilier &
The default color for Positive		Class List		Class Lists	are Genie parameters.
The default color for Positive		Hue Value	0.1		•
Pixel Count is brown.		Hue Width	0.5		
		Color Saturation Threshold	0.04		
		lwp(High)	220		
		lwp(Low) = lp (High)	175		
		lp(Low) = lsp(High)	100		
		lsp (Low)	0		
		Inp(High)	-1		
		View Width Width of processing box			Tip: Click on a single parameter value and see the definition below.
		Region of Analysis         ○ Current Screen         ○ Entire Image         ③ Selected Annotation Lay         ☑ Generate Markup Image         Ready	/er	Tune Rut	8



9. View analysis results in the **Annotations window** under **Layer Attributes** section.





10. View **Markup Image**. Click **Show/Hide Layer Icon** in the Annotations window to compare analysis results to the original digital slide image.



#### **Knowledge Check**



- 1. Positive Pixel Count provides outputs for area quantification?
  - a) True
  - b) False
- 2. The user must adjust the color hue of the stain if using anything other than a brown stain?
  - a) True
  - b) False
- 3. What is the default color quantification for Positive Pixel Count?
  - a) Blue
  - b) Red
  - c) Green
  - d) Brown
- 4. What parameter value is defaulted for ScanScope images?
  - a) Hue Value
  - b) lwp
  - c) View Width
  - d) Hue Width

#### Color Deconvolution accurately separates stains, resolving multistain colocalization problems.

- Accurate Stain Separation
  - Separates the image into 3 channels corresponding to the actual colors used.
  - Allows accurate measurement of the area for each stain individually, even when the stains are superimposed at the same location.
- Automatic and precise training of stain colors.
  - Eliminates guesswork and trial and error.



This example is the result of running analysis three times once for each color channel.



Separated Stain Markup Images



#### Also quantifies the area & intensity of separated stains.

• Color Deconvolution does more than just present a visual separation of stains, it also accurately calculates the area and intensity for each individual stain.



*Original Intensity Ranges Markup* The markup image color codes analyzed pixels in the following format: Negative, Weak, Medium, Strong Positive

#### **Outputs:**

- Negative, Weak, Medium, Strong Positive Staining
  - Percentages
  - Average intensity
- Area
- Other statistical measurements

#### **Color Deconvolution input parameters:**

- Color Deconvolution analysis performance is controlled by a set of input user define input parameters
- Default color channels are
  - Color (1) = Hematoxylin
  - Color (2) = Eosin
  - Color (3) = DAB
- **Positive Color Channel**-Particular stain to be analyzed (1, 2, or 3).
- Markup Image Type-
  - Deconvolved Color Channel
  - Intensity Ranges
- Intensity Thresholds-
  - Weak Positive
  - Medium Positive
  - Strong Positive
  - Black Threshold
- **Color (1, 2 or 3)**-Normalized Optical Density values for the Red, Green, Blue components for the corresponding color channel. If only 2 stains are present set color 3 values to 0.
- Clear Area Intensity-Default value for white balance.

lacro on server: APERIO-0	1959
Select Algorithm Import Ma	acro Save Macro
Color Deconvolution v9	)' Parameters
View Width	1000
View Height	1000
Overlap Size	0
Image Zoom	1
Markup Compression Type	0 - Same as processed image
Compression Quality	30
Classifier Neighborhood	0
Classifier	0 - None
Class List	
Positive Color Channel	1
Mark-up Image Type	1 - Deconvolved Color Channel
Weak Positive Threshold	220
Medium Positive Threshold	170
Strong Positive Threshold	100
Black Threshold	0
Color (1) - Red Component	0.9304
Color (1) - Green Component	0.2562
Color (1) - Blue Component	0.2622
Color (2) - Red Component	0.1957
Color (2) - Green Component	0.7678
Color (2) - Blue Component	0.6099
Color (3) - Red Component	0.268
Outra (D) Outra Outra and	0.57

Classifier Neighborhood, Classifier & Class Lists are Genie parameters.

Region of Analysis	Inputs Outputs
Current Screen Entire Image Selected Annotation Layer	
Generate Markup Image	Tune Run
Ready	





#### More on intensity:

- Intensity Raw image data are in RGB format. Intensity is the average of RGB channels in the raw data: (R+G+B)/3.
  - RGB value of (255,255,255) corresponds to bright white.
  - RGB value of (0,0,0) corresponds to black.
  - Large intensity is bright and corresponds to very light staining.
  - Low intensity is very dark and corresponds to dark staining.



Macro on server: APERIO-	01959 Iacro Si	ave Macro		
'Color Deconvolution v	9' Parameters			
Weak Positive Threshold	220	•		
Medium Positive Threshold	170			
Strong Positive Threshold	100			
Black Threshold	0			
<b>Note:</b> Change the intensity input values to determine what intensity will be enumerated.				

Large intensity value

Low intensity value



#### Color Deconvolution Results:

- Color Deconvolution results are area based quantification and intensity values.
  - A positive result applies to pixels that are stained in the Positive Color Channel specified in the analysis inputs.
  - A negative result applies to pixels that are not stained positive.
  - In addition, the Average Red, Green and Blue OD values are given.
    - These results can be used for color (stain) calibration.

Annotations - Detailed View			
		Summary 🖽 🗄	
Layers 🗑 📕 💠 🗙 💌	Layer Attributes 🛛 🕂 😑		
Layer 80	Average Positive Intensity	186.333 🔼	
Result 118	Percent Weak Positive	77.2613	
Layer 81	Percent Medium Positive	18.4567	
	Percent Strong Positive	4.93515e-003	
Total Stained Area is	Percent Negative	4.27701	
the cumulative total	Percent Total Positive	95.723	
negative pixels.	Average Weak Positive Intensity	193.879	
	Average Medium Positive Intensity	154.766	
Total Analysis Area is the total area of	Average Strong Positive Intensity	97.9786	
analysis including	Total Stained Area (mm^2)	0.180148	
any clear glass areas	Total Analysis Area (mm^2)	0.180631	
of the digital slide .	OD (Average Optical Density)	0.109923	
Describe and estan	OD x Percent Total Positive	10.5221	
coded to match the	OD x Total Stained Area (mm^2)	1.98023e-002	
mark up image.	Score (0-300)	114.19	
	Average Red OD	0.460463	
	Average Green OD	0.577601	
	Average Blue OD	0.674055	
1			



#### **Color Deconvolution Workshop**



Watch Instructor demo the tasks below and perform them using the steps beginning on the next page.

- Open Color Deconvolution Demo File
- Select Color Deconvolution Macro
- Run Analysis
- View Results





- 1. Log into Spectrum with a username and password provided by instructor & select the specified **Research Role**.
- 2. Navigate to the digital slide list, open the **Color Deconvolution Demo** slide as shown below.





3. Click View, select Annotations Window (Ctrl N).

The annotations window is now displayed.

4. Click **Region 1** in Annotations Window under the Layer Regions section to select a region for analysis. (For this exercise use Region 1).





5. Click View, select Analysis (Ctrl G).

The Algorithms Server window is now displayed.

#### 6. Select ColorDeconvolutionSeperateStains\_macro & click Test.

The saved input parameters are now displayed.



Algorithm Server Job - Server: APE	RIO-01959 🛛 🛛				
Select Algorithm Macro	6				
Macro Name					
Nuclear_macro					
MicrovesselAnalysis_macro					
Micromet_macro					
COlorDeconvolutionSeperateStains_macro					
PositivePixelCount_macro Colocalization_SeperateStains_m Rare Event Detection Aperio Positive Pixel Count Samp	nacro Ne Macro				
Generate Markup Image	Incremental Processing				
- Region of Analysis					
<ul> <li>Selected Annotation</li> </ul>	🔘 Entire Image				
Test 🥳 Create	Analyze Cancel				


This macro has been saved with the parameter settings for the specific stains in our sample image.

- 7. Verify that **Positive Color Channel** is set to **1**, the **Markup Image Type** is set to **Deconvolved Color Channel**.
- 8. Select Selected Annotation Layer & Generate Markup Image.
- 9. Click Run.

Select Algorithm Import	Macro	Save Macro
'Color Deconvolution	v9' Parameters	
View Width	1000	
View Height	1000	
Overlap Size	0	
Image Zoom	1	
Markup Compression Type	0 - Same as processed image	
Compression Quality	30	=
Classifier Neighborhood	0	
Classifier	0 - None	
Class List		
Positive Color Channel	1	
Mark-up Image Type	1 - Deconvolved Color Channel	
Weak Positive Threshold	220	
Medium Positive Threshold	170	
Strong Positive Threshold	100	
Black Threshold	0	
Color (1) Bod Component	0.0204	•
Width of processing box		
Width of processing box		
C	Innut	Outputs
Region of Analysis		, outputs
O Current Screen	8	
O Entire Image		
<ul> <li>Selected Annotation La</li> </ul>	yer	
	Tune	Duck



10. View the **Color Channel 1** markup image.

The markup image should display automatically if Generate Markup Image was selected.

11. To review numerical results click **View** & select **Annotations**. *Positive results are color coded.* 



Annotations - Detailed View			
	_		
Layers 🗑 📥 🛧 🔀		Layer Attributes 🛛 🖶 📃 🏢	
Layer 80	71	► Average Positive Intensity 186.333	•
Result 120	ш	Percent Weak Positive 77.2613	ſ
Layer 81	- H	Percent Medium Positive 18.4567	
Layer 82	- 1	Percent Strong Positive 4.93515e-003	l
Layer 05	- 1	Percent Negative 4.27701	1
Layer 116	- 1	Percent Total Positive 95.723	1
Layer 117 Layer 119		Average Weak Positive 193.879 Intensity	
		Average Medium Positive 154.766 Intensity	
		Average Strong Positive 97.9786 Intensity	
	- 1	Total Stained Area (mm^2) 0.180148	l
	- 1	Total Analysis Area (mm^2) 0.180631	
		0D (Average Optical 0.109923 Density)	
	- 1	OD x Percent Total Positive 10.5221	
		OD x Total Stained Area 1.98023e-002 (mm <sup>2</sup> )	
	- 1	Score (0-300) 114.19	
	- 1	Average Red OD 0.460463	
	- 1	Average Green OD 0.577601	
	- 1	Average Blue OD 0.674055	
		*** Algorithm Inputs *** Algorithm Inputs ***	
		Algorithm Color Deconvolution v9	
		Version 9.1	ſ



- 12. Select the original **Annotation Layer** in the Annotations Window.
- 13. Change **Positive Color Channel parameter to 2** in the Analysis Window.
- 14. Click Run.





#### 15. View markup image & results.

Now the red color markup image of stain #2 is displayed.



under the appropriate annotation layer.



16. Repeat steps 12 – 15; changing the Positive Color Channel parameter to 3. Now the brown color markup image of stain #3 is displayed.





- 17. Click Original Layer in the Annotations Window.
- 18. Change the **Markup Image Type to Intensity Ranges** in the Analysis Window, leaving everything else the same.

Macro on server: APERTO-01959

19. Click Run.

Annotations - Detailed View	Select Algorithm Import Macro Save Macro
	'Color Deconvolution v9' Parameters
	View Width 1000
	View Height 1000
Layers 🐨 K 🔨	Overlap Size 0
Laver 80	Image Zoom 1
E Result 120	Markup Compression Type 0 - Same as processed image
Deput 121	Compression Quality 30
Besult 122	Classifier Neighborhood 0
Result 122	Classifier 0 - None
III aver 81	Class List
	Positive Color Channel 1
1	3 Mark-up Image Type 0 - Intensity Ranges
	Weak Positive Timeshold 220
	Medium Positive Threshold 170
	Strong Positive Threshold 100
	Black Threshold 0
	Color (1) Dod Component 0.0204
	Mark-up Image Type
	Choose Intensity Ranges or Deconvolved Color Channel
	Region of Analysis
Region of Analysis selections	· O Current Sereen
Selected Annotation Layer	Selected Annotation Laver
Generate Markun Image	
Generale Markup Inaye	Generate Markup Image 19 une Ruk



#### 20. View markup image & results.

Notice that the markup image for intensity for Color Channel 3 (DAB) is displayed.



**Note:** The choice of markup image type does not change the numerical results. Verify this by toggling between the Channel 3 deconvolved results and the intensity ranges (in this example results 4 & 5).

### **Knowledge Check**



- 1. What does Color Deconvolution quantify?
  - a) Cell quantification
  - b) Area and intensity of separated stains
  - c) Area and intensity of colocalized markers
- 2. Color Deconvolution can only separate up to 2 stains.?
  - a) True
  - b) False
- 3. Color Deconvolution uses color channels to determine stain analysis?
  - a) True
  - b) False

### **Color Deconvolution – Color Calibration**



- Color Calibration defines the stain color vector (red, green and blue components) so stained cells will be correctly identified by the analysis tool.
  - Default colors vectors are:
    - Color 1 Hematoxylin
    - Color 2 Eosin
    - Color 3 DAB



- Color vector numbers must be changed if different stains are used.
  - The color for each stain is calibrated separately for each stain that differs from the defaults.
  - Separate control slides for each stain should be used.
  - After calibration is complete, the modified parameter settings can be saved as a macro.

Color (1) - Red Component	0.65	
Color (1) - Green Component	0.704	
Color (1) - Blue Component	0.286	
Color (2) - Red Component	0.072	
Color (2) - Green Component	0.99	
Color (2) - Blue Component	0.105	=
Color (3) - Red Component	0.268	
Color (3) - Green Component	0.57	
Color (3) - Blue Component	0.776	~
		and the second s

## **Color Calibration**



### Calibration Outputs become input parameters.



# **Color Calibration**



### **Calibration Outputs:**

- By using a control slide with one color, you can guarantee the Average Optical Density for the stain's RGB color components will be accurate.
- These results will become the Color Channel inputs (red, blue & green components) for that stain when running Color Deconvolution or Colocalization.

Annotations - Detailed View	
	Summary 🖽 🗄
Layers 😨 📥 🛧 🔀	Layer Attributes 🔄 🗁 🖹 🏢
Result 1	Average Positive Intensity 153.8
	Percent Weak Positive 42.9688
	Percent Medium Positive 33.9023
In this example, the results are	Percent Strong Positive 20.6633
for the DAR stain being used	Percent Negative 2.46562
for the DAB stall being used.	Percent Total Positive 97.5344
	Average Weak Positive 201.026
	Average Medium Positive 141.305 Intensity
	Average Strong Positive 76.097

The analysis outputs define the Average Red, Green & Blue Optical Densities for control stain.

	OD (Average Optical Density)	0.193254
	OD x Percent Total Positive	18.8489
	OD x Total Stained Area (mm^2)	1.23519e-002
į,	Secre (0.200)	172 762
	Average Red OD	0.301777
ĺ	Average Green OD	0.554163
İ	Average Blue OD	0.77578
Ļ	Algorithm Impats	Algorithm impats
t	Algorithm	Color Deconvolution v
		0.4
1	Version	9.1
	Version View Width	739

# **Color Calibration**



### **Calibration Inputs:**

- Performing color calibration on a control slide, results in the Average Red, Green & Blue Optical Density for that stain.
  - The output optical densities are the color vectors for the specific stain being used.
  - These become the Red, Green and Blue Components for the Color Channel inputs for the stain.

Macro on server: APERIO-0	1959			
Select Algorithm Import Ma	Sa	ve Macro		
'Color Deconvolution v9	Parameters			
Class List				~
Positive Color Channel	1			
Mark-up Image Type	1 - Deconvolved	Color Ch	annel	
Weak Positive Threshold	220			
Medium Positive Threshold	170			
Strong Positive Threshold	100			
Black Threshold	0			
Color (1) - Red Component	0.9304			
Color (1) - Green Component	0.2562			
Color (1) - Blue Component	0.2622			
Color (2) - Red Component	0.1957			
Color (2) - Green Component	0.7678			≡
Color (2) - Blue Component	0.6099			
Color (3) - Red Component	0.301777			/
Color (3) - Green Component	0.554163			
Color (3) - Blue Component	0.776			
Clear Area Intensity	240			
Color (3) - Red Componen OD for Color(3) Red: Default i	t s DAB			¥
Region of Analysis		Ing	outs	Outputs
<ul> <li>Current Screen</li> <li>Entire Image</li> <li>Selected Annotation Laye</li> </ul>	r			
Generate Markup Image		Tun	<b>e</b> (	Run
Ready				

Color Calibration takes the guesswork out of the trial and error method of running Color Deconvolution and Colocalization.



### **Automatic Stain Calibration**



Watch Instructor demo the tasks below and perform them using the steps beginning on the next page.

- Open Nuclear Stain demo file
- Select Nuclear Stain Tuning macro
- Run analysis
- View Results





- 1. Log into Spectrum with a username and password provided by instructor & select the specified **Research Role**.
- 2. Navigate to the digital slide list, open the **Nuclear Stain Demo** slide as shown below.





3. Click View and Select Analysis (Ctrl G).

The Algorithms Server window is now displayed.

4. Click Color Deconvolution macro in the Algorithms Server window & click Test.



The algorithm parameters are now displayed.

- 5. Zoom to an area that represents the stain being calibrated. Set the Region of Analysis to **Current Screen** & uncheck the **Generate Markup Image** box.
- 6. Click Run.

Color Deconvolution is run with its erroneous Color settings and the output for the control slide is obtained.





- 8. Click View, select Annotations (Ctrl N).
- 9. View Layer Attributes in Annotations Window.

Color Deconvolution provides the Average, Red, Green and Blue Optical Density (OD) values of the image it analyzes.

View Full Screen	F11					
Scale Axes/Grid Zoom Slider Thumbnail Label Image Magnifier	Ctrl+T	9	Annotations - Detailed View	Layer Attributes 🕂	Summary	
Filmstrip ✔ Status Bar				Average Green OD Average Blue OD	0.553978 0.775964	
Annotations 👫 Analysis Tracker Digital Slide Conferencing TelePath Live	Ctrl+N Ctrl+G Ctrl+D		The Average Red, Gr OD is the RGB color for this stain. Use the	een & Blue component ese values		
Annotation Link Manager Next Annotation Link Previous Annotation Link	F8 Shift+F8 Shift+F7		as inputs when saving for this particular stain	g macros n.		



10. Now that you have the Red, Green & Blue OD values you can use these outputs as parameter inputs every time this stain is used. As an example:

Annotations - Detailed View			×					
		Summary 🔲	B					
Layers 🛞 📥 🕂 🗙 🗱	Layer Attributes 🛛 🕂				10			
Result 1	Average Red OD	0.301644	<b>^</b>					
	Average Green OD	0.553978			Color (3)	- Red Component	0.301644	
	Average Blue OD	0.775964		$\sim$	Color (3)	- Green Component	0.553978	
					Color (3)	- Blue Component	0.776	<b></b>
These outputs become t	he Red, Green	& Blue Compo	onent		Clear Ar	ea Intensity	240	
inputs for the appropriate color channel every time this stain is								
used. This is done by manually adjusting the Red, Gree Blue Components in the Algorithms Window.			een &		OD for 0	color(3) Blue: Default	is DAB	

### **Knowledge Check**



- 1. Stain calibration can be completed using Color Deconvolution.
  - a) True
  - b) False
- 2. Calibration outputs become analysis input parameters.
  - a) True
  - b) False
- 3. What are the important output results for stain calibration?
  - a) Percent Weak Positive
  - b) Percent total Positive
  - c) Average Red, Green and Blue OD

# **Colocalization**



### Quantifies the area & intensity of "colocalized" markers.

- Colocalization Analysis calculates the contribution of each stain at every pixel location in the image as either part of a single stain or representing a combination of staining.
  - Analysis results are based on the separated stain's intensities.
  - For IHC, it determines where specific proteins are present and to that extent the proteins are colocalized.











Original

Hematoxylin

Marker A (Fast Red) Marker B (DAB)

Marker A, Marker A+B, Marker B

- Outputs:
  - A, B, A+B: 3 possible stain combinations for two stains.
  - A, B, C, A+B, B+C, A+C, A+B+C: 7 possible combinations for three stains.
    - Percentages
    - Average Intensities
  - Area

# Colocalization



### Cytoplasmic Quantification by area.

Colocalization can be used for Cytoplasmic analysis by measuring only the • cytoplasmic component of staining.



Original

Hematoxylin (Nuclear)

Markup



DAB (Nuclear + Cytoplasm) Markup



DAB **Cytoplasmic** Staining (Green) Markup

- **Outputs:** 
  - A, B, A+B: 3 possible stain combinations for two stains.
  - A, B, C, A+B, B+C, A+C, A+B+C: 7 possible combinations for three stains.
    - Percentages
    - Average Intensities
  - Area

### **Colocalization**



### **Colocalization input parameters.**

- Colocalization analysis performance is controlled by a set of input parameters allowing users to define:
- Markup Image Type-
  - Colocalization
  - Deconvolved Color Channel 1, 2 or 3
- Mode-
  - Colocalization
  - Counter-stain, Double Label
- Threshold for intensity ranges-
  - Upper and Lower threshold for colors 1, 2 & 3
- **Color (1, 2 or 3)**-Normalized Optical Density values for the Red, Green, Blue components for the corresponding color channel. If only 2 stains are present set color 3 values to 0
- Clear Area Intensity-Default value for white balance

'Colocalization v9' Para	ameters
View Width	1000
View Height	1000
Overlap Size	0
Image Zoom	1
Markup Compression Type	0 - Same as processed image
Compression Quality	30
Classifier Neighborhood	0
Classifier	0 - None
Class List	
Mark-up Image Type	0 - Co-Localization
Mode	0 - Co-Localization mode
Color (1) Threshold	200
Color (1) Lower Threshold	0
Color (2) Threshold	200
Color (2) Lower Threshold	0
Color (3) Threshold	200
Color (3) Lower Threshold	0
Color (1) - Red Component	0.65
Color (1) - Green Component	0.704
Color (1) - Blue Component	0.286
Color (2) - Red Component	0.072
Color (2) - Green Component	0.99
Color (2) - Blue Component	0.105
Color (3) - Red Component	0.268
Color (3) - Green Component	0.57
Color (3) - Blue Component	0.776
Clear Area Intensity	240

Classifier Neighborhood, Classifier & Class Lists are Genie parameters.



### **Colocalization Workshop**



Watch Instructor demo the tasks below and perform them using the steps beginning on the next page.

- Open Colocalization demo file
- Select Colocalization macro
- Run analysis
- View Results





- 1. Log into Spectrum with a username and password provided by instructor & select the specified **Research Role**.
- 2. Navigate to the digital slide list, open the **Colocalization Demo Slide** as shown below.

Login Required 1			
Username: Password: User Login			
Digital Slides     List all Digital Slides     View all Digital Slides     List ScanScope Slides     List ScanScope Slides (Past Day)     List ScanScope Slides (Past Week)     List ScanScope Slides (Past Month)			Colocalization Demo Data
Search all Digital Slides Add new Digital Slides	Click t	humbnail ima	ge to open ImageScope



- 3. Open the Annotations winnow (Ctrl+N) & select the 1<sup>st</sup> Layer.
- 4. Click Region 1 in the Annotations window under the Layer Regions section.
- 5. Open the Analysis window (Ctrl+G) & select the Colocalization macro.
- 6. Click **Test** to display the algorithm parameters.

			Click icon to open annotations window.				
	Annotations - Detailed View		×				
	EBB		Summary D				
3	Layer 84 Layer 85		Layer Attributes + = = + Layer Regions + = × = + + + + + + + + + + + + + + + +				
		5	Algorithm Server Job - Server: APERIO-01959       Image: Comparison of the server server server and the server serve				
			C Generate Markup Image Incremental Processing Region of Analysis				
		6	Selected Annotation     Create     Analyze     Cancel				



7. Click **Run** in the **Algorithms** window.

#### 8. View markup image.

lacro on server: APERIO-01959					
Select Algorithm Import Macro Save Macro					
Colocalization v9' Parameters					
Markup Compression Type	0 - Same as processed image	<ul> <li></li> </ul>			
Compression Quality	30				
Classifier Neighborhood	0				
Classifier	0 - None				
Class List					
Mark-up Image Type	0 - Co-Localization				
Mode	0 - Co-Localization mode				
Color (1) Threshold	100				
Color (1) Lower Threshold	0				
Color (2) Threshold	220				
Color (2) Lower Threshold	0				
Color (3) Threshold	175				
Color (3) Lower Threshold	0				
Color (1) - Red Component	0.65	=			
Color (1) - Green Component	0.704				



Setting the markup image type to **Colocalization** presents color-coded quantification of all stains and their colocalization combinations: stain 1 only, stain 2 only, stain 3 only, 1+2, 2+3, 1+3 & 1+2+3.





Region of Analysis selections: Selected Annotation Layer Generate Markup Image



9. View **results** (Ctrl N will display Annotations window) under **Layer Attributes** in the **Annotations** window.

Annotations - Detailed View					No		
					Summary 🗖 🗄		
Layers 😨 📕 🕂 🗙 📈	a	Layer Attributes 🕀 📼 🔳 🛄			Layer Regions  🖶 🖂 🗙		
Layer 84		Percent (1)	7.26558e-004	~	Region Length (um) Area (u		
Result 86		Intensity (1, 1)	80.5		▶ 1 2866 5		
Layer 85		Percent (1+2)	0.47856				
		Intensity (1, 1+2)	77.2605				
		Intensity (2, 1+2)	61.5174				
		Percent (2)	11.7655				
		Intensity (2, 2)	176.21				
		Percent (2+3)	27.7527				
		Intensity (2, 2+3)	152.955	=			
		Intensity (3, 2+3)	118.666				
		Percent (3)	27.7647				
		Intensity (3, 3)	132.938				
		Percent (1+3)	21.3871				
		Intensity (1, 1+3)	69.8424				
		Intensity (3, 1+3)	58.9168				
		Percent (1+2+3)	10.8507				
		Intensity (1, 1+2+3)	80.2859	_			
		Intensity (2, 1+2+3)	158.422				
		Intensity (3, 1+2+3)	88.8226				
		Overall Intensity (1)	73.4147				
		Overall Intensity (2)	158.642				
		Overall Intensity (3)	104.93				
		Total Stained Area (mm^2)	0.20464				

Colocalization classifies each pixel as either part of a single stain or a combination of stains based on the separated stains' intensities and then provides different statistics for their quantitative assessment in terms of area intensity.

Algorithm	Colocalization v9		-
Version	91	< >	1

**APERIO** UNIVERSITY

Demonstrate that the different stains have been separated properly.

- Hematoxylin
- Marker A (Fast Red)
- Marker B (DAB)
- 10. Reopen the **Analysis** window (Ctrl G) & select the **Colocalization\_SeperateStains\_macro**
- 11. Click **Test** to load parameter values.

10	Algorithm Server Job - Server: APERIO-01959  Select Algorithm Macro  Macro Name Nuclear_macro MicrowesselAnalysis_macro Micromet_macro COlorDeconvolutionSeperateStains_macro Colocalization_macro PositivePixelCount_macro Colocalization_SeperateStains_macro Hare E vent Detection Aperio Positive Pixel Count Sample Macro
	Generate Markup Image     Incremental Processing     Region of Analysis     Selected Annotation     C Entire Image
11	Test Create Analyze Cancel



Macro

utputs

- 12. Change Markup Image Type to 1 Deconvolved Color Channel (1).
- 13. Click **Run** in the **Algorithms window**.
- 14. Repeat steps 12-13 for the **Deconvolved Color Channels 2 & 3**.

acro on server: APERIO-	01929		Macro on server: APER	10-01959	
Select Algorithm Import M	Aacro Save Ma	acro	Select Algorithm Imp	ort Macro	Save M
Colocalization v9' Par	rameters		'Colocalization v9'	Parameters	
View Width	1000 12		View Width	1000	
View Height	1000		View Height	1000	
verlap Size	0		Overlap Size	ight of process	ing how
nage Zoom	1		Image Zoom	1	Ing box
arkup Compression Type	0 - Same as processed image		Markup Compression Ty	pe 0 Same	as processed image
ompression Quality	30		Compression Quality		
lassifier Neighborhood	0	=	Classifier Neighborhood	14	
lassifier	0 - None		Classifier		
lace List			Class List		
/lark-up Image Type	1 - Deconvolved Color Channel (1)	-	Mark-up Image Type	2 - Decon	volved Color Channel (2)
oue	0 - C0-L0Calization mode		mode	0 - 00-20	calization mode
olor (1) Threshold	100		Color (1) Threshold	100	
olor (1) Lower Threshold	0		Color (1) Lower Thresho	old 0	
olor (2) Threshold	220		Color (2) Threshold	220	
olor (2) Lower Threshold	0		Color (2) Lower Thresho	old 0	
olor (3) Threshold	175		Color (3) Threshold	175	
olor (3) Lower Threshold	0		Color (3) Lower Thresho	old 0	
olor (1) - Red Component	0.65	✓	Color (1) - Red Compone	nt 0.65	
Mark-up Image Type Choose Colocalization or De	convolved Color Channel		Mark-up Image Type Choose Colocalization o	r Deconvolved (	Color Channel
Region of Analysis	Inputs Out	tputs	C Region of Analysis		Inputs Or
Current Screen			O Current Screen		
) Entire Image			O Entire Image		
Selected Annotation Lay	er		<ul> <li>Selected Annotation</li> </ul>	Layer	
Generate Markup Image	13 🖻 🗔	11 <mark>. 3</mark>	Generate Markup Imag	e	Tune
Analysis Complete			Ready		

Region of Analysis selections: Selected Annotation Layer Generate Markup Image



15. Show Markup Images & view results in the Annotations window.



### **Knowledge Check**



- 1. Colocalization Analysis determines to what extent proteins occur separately or in combination with each other.
  - a) True
  - b) False
- 2. What does Colocalization Analysis quantify?
  - a) Cell quantification
  - b) Membrane quantification
  - c) Area and intensity
  - d) Nuclear quantification
- 3. When using 3 color channels there are 7 possible output combinations?
  - a) True
  - b) False



### Nuclear analysis quantifies nuclear staining and intensity.

- Markup image highlights the detected nuclei which are color-coded according to their classification.
- Nuclear staining is classified as follows and is based on nuclear staining intensity: Blue = 0, Yellow = 1+, Orange = 2+, Red = 3+



Original



*Markup* 0, 1+, 2+, 3+ Nuclei

Nucleus = 0 when it has no
staining.
Nucleus = 1+ when it has
weak staining.
Nucleus = 2+ when it has
moderate staining.
Nucleus = 3+ when it has
intense staining.

- Outputs:
  - Percentage Positive Nuclei (0.0 100.0)
  - Average Intensities (0, 1+, 2+, 3+)
  - Percentages of 0, 1+, 2+, 3+
  - Other statistical measurements



### Can be tuned for IHC ER and PR stained breast tissue.

- Detects the nuclear staining for target chromogen for individual cells and quantifies intensity.
- Performs same complex analysis as pathologists.
- Detects positive staining for individual nuclei.
- Quantifies nuclei by staining intensity.
- Classifies nuclei as 0, 1+, 2+ and 3+.
- Automatic cytoplasmic stain removal.
- Automatic lab/reagent stain tuning.
- Supports different scoring schemes: Allred, HScore.
- Can be used for other tissue type by changing input parameters.



### Nuclear Analysis input parameters.

- Nuclear Analysis is controlled by a set of input parameters.
- Cell feature detection and scoring scheme parameters are handled separately.
- Avg. Radius-Radius for noise reduction.
- **Curvature Threshold**-determines the level of declustering for touching nuclei.
- Seg Type- method of nuclear segmentation
  - Intensity, Cytoplasmic Reduction.
- **Threshold Type**-Determines method of edge separation (where one cell ends and another begins).
- Min/Max Nuclear Size-Nuclei outside of range will not be counted
- **Min Roundness**-Nuclei with roundness < value will not be reported.
- **Min Compactness**-Nuclei with compactness < value will not be reported.
- **Min Elongation**-Nuclei with elongation < value will not be reported.
- **Remove Light Objects**-Allows the removal of light objects.

Nuclear v9" Parameter	rs	
Averaging Radius (um)	1	1
Curvature Threshold	2.5	
Segmentation Type	2 - Cytoplasmic Rejection	
Threshold Type	1 - Edge Threshold Method	ſ
Edge Trimming	1 - Weighted	
Lower Threshold	0	
Upper Threshold	230	
Min Nuclear Size (um <sup>4</sup> 2)	20	
Max Nuclear Size (um <sup>2</sup> )	1000000	
Min Roundness	0.1	
Min Compactness	0	
Min Elongation	0.1	
Remove Light Objects	0	1



### Nuclear Analysis input parameters.

Stain color parameters can be calculated using Color Deconvolution's stain calibration process.

		Macro on server: APERIO-01959			
		Select Algorithm Import Macr	0	Save Macro	
		'Nuclear v9' Parameters			
		Clear Area Intensity	240		
		Nuclear Stain (Red)	0.696858		
		Nuclear Stain (Green)	0.643073		
		Nuclear Stain (Blue)	0.317563		
	1	Positive Stain (Red)	0.244583		
Stain Color Parameters		Positive Stain (Green)	0.509334		
	•	Positive Stain (Blue)	0.825081		
		3rd Stain (Red)	0		
		3rd Stain (Green)	0		
		3rd Stain (Blue)	0		
		Cytoplasmic Intensity Threshold	230		
	1	Weak(1+) Threshold	210		
Scoring Scheme Parameters		Moderate(2+) Threshold	188	≡	
	•	Strong(3+) Threshold	162		
		Black Threshold	0		
		Use Mode	0 - Analysis/Tuning		
		Mark-up Image Type	1 - Analysis		
		Classifier Type	1 - IHCNuclear		
		Classifier Definition File	IHCNuclearTraining	~	

### Membrane



### Membrane Analysis quantifies membrane staining and intensity.

- Markup image highlights the detected cell features and the membrane staining which is color-coded according to cell classification.
- Membrane staining is classified as follows and is based on membrane staining, intensity and completeness: Blue = 0, Yellow = 1+, Orange = 2+, Red = 3+



Original



*Markup* 0, 1+, 2+, 3+ Cells



Negative = 0 when it has no membrane staining. Tumor Cell = 1+ when it has partial or weak staining. Tumor Cell = 2+ when it has moderate & complete staining. Tumor Cell = 3+ when it has intense & complete staining.

- Outputs:
  - HER2 Score (0, 1+, 2+, 3+)
  - Percentages of 0, 1+, 2+, 3+ cells
  - Other statistical measurements
### Membrane



#### Can be tuned for IHC HER2 stained breast tissue.

- Detects membrane staining for individual tumor cells and quantifies the intensity and completeness of the membrane staining.
- Performs same complex analysis as pathologists.
- Detects positive membrane staining for individual cells.
- Quantifies cells by membrane staining, intensity and completeness.
- Classifies cells as 0, 1+, 2+, 3+.
- Applies HER2 scoring scheme.
- Automatic lab/reagent staining tuning.
- Supports different scoring schemes: HScore.
- Can be used for other tissue types and membrane stains by changing input parameters.

### Membrane



#### Membrane Analysis input parameters.

- Membrane Analysis is controlled by a set of input parameters.
- Cell feature detection and scoring scheme parameters are handled separately.
  - Cell feature parameters specify cell feature detection thresholds and methods as well as size and shape and constraints of nuclei to distinguish tumor cells from normal, lymphocyte and stroma cells.

#### **Cell Feature Parameters**

- **Cytoplasmic Correction**-If set to yes, background staining is weeded out.
- Cell/Nucleus Req.-Include/exclude cells without nucleus.
- **Max Cell radius**-Determines cell size for cells that have a nucleus but no membrane staining.
- Min/Max Cell Size-Cells outside this range will not be counted.
- **Min Roundness**-Cells with roundness less than this value will not be reported.
- **Min Compactness**-Cells with compactness less than this value will not be reported.
- **Min Elongation**-Cells with elongation less than this value will not be reported.

Macro on server: APERIO	-01959	×
Select Algorithm Import	Macro	Save Macro
'Membrane v9' Parar	neters	
Averaging Radius (um)	1	
Blue Curvature Threshold	1	
Threshold Type	0 - Edg	e Threshold Method
Lower Blue Threshold	0	Nuclear staining 9
Upper Blue Threshold	220	
Min Nuclear Size (um <sup>2</sup> )	15	morphological
Max Nuclear Size (um <sup>2</sup> )	400	parameters
Min Nuclear Roundness	0.1	
Min Nuclear Compactness	0.1	
Min Nuclear Elongation	0.1	
Cytoplasmic Correction	1 - Ye	3
Cell/Nucleus Requirement	0 - All	Cells
Max Cell Radius (um)	5	
Min Cell Size (um <sup>2</sup> )	25	Cell Feature Detection
Max Cell Size (um^2)	2000	
Min Cell Roundness	0.1	parameters
Min Cell Compactness	0.1	I
Min Cell Elongation	0.1	

### Membrane



#### Membrane Analysis input parameters.

• The scoring scheme parameters specify the staining intensity and membrane completeness thresholds that determine the individual tumor cell classification.

		Macro on server: APERIO	-01959	<u> </u>
		Select Algorithm Import	Macro	Save Macro
		'Membrane v9' Paran	neters	
		Cytoplasmic Correction	1 - Yes	
		Cell/Nucleus Requirement	0 - All Cells	
		Max Cell Radius (um)	5	
		Min Cell Size (um <sup>2</sup> )	25	
		Max Cell Size (um <sup>2</sup> )	2000	
		Min Cell Roundness	0.1	
		Min Cell Compactness	0.1	
		Min Cell Elongation	0.1	
		Background Threshold	240	
Scoring scheme		Weak(1+) Threshold	200	
		Moderate(2+) Threshold	175	
classification thresholds		Strong(3+) Threshold	140	
	•	Completeness Threshold	60	
		Use Mode	0 - Analysis/Tuning	
		Mark-up Image Type	1 - Analysis	
		Classifier Type	0 - IHCMembrane	
		Classifier Definition File	IHCMembraneTraining	· · · · · · · · · · · · · · · · · · ·
		View Width Width of processing box		
		Region of Analysis		Inputs Outputs
		Current Screen     Entire Image     Selected Annotation La	iyer	
		Generate Markup Image	Tu	ne Run
		Ready		

#### **Nuclear & Membrane Workshop**



#### **Nuclear & Membrane Workshop**



Watch Instructor demo the tasks below and perform them using the steps beginning on the next page.

- Open Nuclear & Membrane demo files
- Select Nuclear & Membrane macros
- Run analysis
- View Results



#### **Nuclear Workshop**



- 1. Log into Spectrum with a username and password provided by instructor & select the specified Research Role.
- 2. Navigate to the digital slide list, open the **Nuclear Demo** slide as shown below.

Login Required 1		
Username:		
Password:		
User Login		
Digital Slides 2		uclear Demo ata
List ScanScope Slides (Past Week) List ScanScope Slides (Past Month) Search all Digital Slides Add new Digital Slides	Click thumbnail image to open ImageScope	

#### **Nuclear Workshop**



- 3. Open the Analysis window (Ctrl G) & select the Nuclear macro.
- 4. Click **Test** to display the parameters.
- 5. Review the parameters & click Run in the Algorithms window.

3	Algorithm Server Job - Server: APERIO-01959				
	Maoro Namo				
	Nuclear_macro				
	MicrovesseiAnalysis_macro Micromet macro				
	Membrane_macro COlorDeconvolutionSeperateStains_macro Colocalization_macro PositivePixelCount_macro Colocalization_SeperateStains_macro Rare Event Detection Aperio Positive Pixel Count Sample Macro				
	Generate Markup Image				
	Region of Analysis				
	Selected Annotation     O Entire Image				
4	Create Analyze Cancel				

lacro on server: APERIO-01959					
Select Algorithm Import Macr	ro Save Macro				
'Nuclear v9' Parameters					
View Width	1000				
View Height	1000				
Overlap Size	100				
Image Zoom	1				
Markup Compression Type	0 - Same as processed image				
Compression Quality	30				
Classifier Neighborhood	0				
Classifier	0 - None				
Class List					
Averaging Radius (um)	1				
Curvature Threshold	2.5				
Segmentation Type	2 - Cytoplasmic Rejection				
Image       Region of Analysis selections:         Selected Annotation Layer         Generate Markup Image					
Region of Analysis     Inputs     Outputs       ○ Current Screen     ○ Entire Image       ③ Selected Annotation Layer       ✓ Generate Markup Image					
Ready					

### **Nuclear Workshop**



#### 6. View Markup Image & results in the Annotations window.

		6
Annotations - Detailed View		
E é		Summary 🗖
Layers 🗑 📕 🕂 🗙 📉	Layer Attributes 🛛 🕂 🖃	
Layer 74	Percent Positive Nuclei	50.4365
Nuclear macro output	Intensity Score	3
Result 110	(3+) Percent Nuclei	33.2438
	(2+) Percent Nuclei	9.33512
	(1+) Percent Nuclei	7.85762
	(0+) Percent Nuclei	49.5635
	Average Positive Intensity	146.233
	Average Negative Intensity	236.48
	(3+) Nuclei	495
	(2+) Nuclei	139
	(1+) Nuclei	117
	(0+) Nuclei	738
	Total Nuclei	1489
	Average Nuclear RGB Intensity	160.097
	Average Nuclear Size (Pixels)	231.398
	Average Nuclear Size (um^2)	58.7789
	Area of Analysis (Pixels)	991681.
	Area of Analysis (mm^2)	0.25190284089600001



### **Membrane Workshop**



- 7. Return to Spectrum and open the **Membrane Demo** slide as shown.
- 8. Open the Annotations window (Ctrl N), and select the first Layer.
- 9. Open the Analysis window (Ctrl G) & select the Membrane macro.
- 10. Click **Test** to display the input parameters.



### Membrane Workshop



#### 11. Review the parameters and click **Run**.

Select Algorithm Import	Macro Save Macro	1
'Membrane v9' Paran	neters	
View Width	1000	
View Height	1000	
Overlap Size	100	
Image Zoom	1	
Markup Compression Type	0 - Same as processed image	
Compression Quality	30	
Classifier Neighborhood	0	
Classifier	0 - None	
Class List		
Averaging Radius (um)	1	
Blue Curvature Threshold	1	
Threshold Type	0 - Edge Threshold Method	
Lower Blue Threshold	0	
Upper Blue Threshold	220	
Min Nuclear Size (um <sup>A</sup> 2)	15 🚩	
View Width Width of processing box Region of Analysis	Inputs Outputs	
Current Screen Entire Image Selected Annotation La	ver	Region of Analysis selection Selected Annotation Layer
Generate Markup Image		Generate Markup Image

#### **Membrane Workshop**



#### 12. View Markup Image & results in the Annotations window (Ctrl+N).

		12	
			A STATE OF A
Annotations - Detailed View			
	Su	mmary 🗖	
Layers 🗑 📕 🕂 🗙	Layer Attributes 🔶 🖃 🗒		
Layer 77	► Her2 Score 2.	<u>^</u>	
Result 78	(3+) Percent Cells 1.934		
	(2+) Percent Cells 32.5	=	
	(1+) Percent Cells 61.8		
	(0+) Percent Cells 3.772		
	Percent Complete 42.46		
	Membrane Intensity 168.6 (Average)		FART SALES
	(3+) Cells 40		The skill be
	(2+) Cells 672		1. A. C.
	(1+) Cells 1278		
	(0+) Cells 78		
	Cells (Total) 2068		
	Complete Cells 878		The second se
	*** Algorithm Inputs *** *** Algorithm In	outs ***	

Based on the percentage of 0, 1+, 2+ & 3+ cells, a score of 0, 1+, 2+, or 3+ is determined according to the HER2 scoring scheme.

### **Knowledge Check**

- 1. Nuclear and Membrane Analysis can be tuned for applications other than breast tissue.
  - a) True
  - b) False
- 2. Nuclear and Membrane markup images use what colors to code analysis results?
  - a) Cyan, Magenta, Yellow
  - b) Red, Green, Blue
  - c) Blue, Red
  - d) Blue, Yellow, Orange, Red
- 3. Curvature threshold parameter is an example of a clustering parameter?
  - a) True
  - b) False
- 4. What is the purpose of the Averaging Radius parameter?
  - a) Establish nuclear or membrane morphology
  - b) Area quantification
  - c) Averages the color intensity across nuclei
  - d) Decluster nuclei

# **Microvessel Analysis**



# Detects and quantifies microvessels on slides stained with endothelial markers.

- Applications include:
  - In vivo angiogenesis assays in cardiovascular research.
  - Vascular morphology changes in oncology xenografts.
  - Microcapillary measurements in rodent models in ophthalmology.
- Analysis results:
  - Number of vessels.
  - Vessel Area.
  - Vessels Density.
  - Stain area and intensity.



Endothelial cells



Grouped to make Vessel regions perime



Vessel perimeter drawn in middle of vessel walls



Vessel Area

Red = vessel wall, vascular area Green = vessel area Black = vessel perimeter Gray = lumen

## **Microvessel Analysis**



#### **Microvessel input parameters.**

- Microvessel has a set of input parameters, which determines the analysis to be performed.
- **Mode**-Choose to include or exclude incomplete vessels.
- Filter/Smoothing Level-Specify the amount of filtering on the deconvolved vessel channel.
- Intensity Thresholds Dark Staining
   Light Staining
- Vessel Completion Thresholds-Specify the distance in microns between pieces of vessel walls and the ends of the vessels walls that are joined to complete the vessel.
- Min & Max Vessel Area Thresholds-Specify internal area size to eliminate vessel from numeric results.
- **Histograms**-Specify histogram details.

Select Algorithm Import Mac	ro Save Mac	ro
Microvessel Analysis v1'	Parameters	
Markup Compression Type	0 - Same as processed image	^
Compression Quality	30	
Classifier Neighborhood	0	
Classifier	0 - None	
Class List		_
Mark-up Image Type	3 - Analysis - Lumen and V	
Mode	0 - Exclude Incomplete Ves	
Filtering / Smoothing Level	2	
Dark Staining threshold	175	
Light Staining threshold	210	
Region Joining Parameter (um)	4	
Vessel Completion Parameter	13	
Minimum Vessel Area Thresh	30	=
Maximum Vessel Area Thres	20000	
Maximum Vessel Wall Thickn	10	
Output Histogram	1 - Vessel Area	
Histogram Start Value (um or	0	
Histogram End Value (um or	1000	
Number of Bins	10	
Endothelial Stain - Red Comp	0.268	
Endothelial Stain - Green Com	0.57	
Endothelial Stain - Blue Comp	0.776	
Background Stain 1 - Red Co	0.68	
Background Stain 1 - Green	0.57	
Background Stain 1 - Blue Co	0.44	~

### **Rare Event Detection**



#### **Rare Event Detection finds specific events on a digital slide.**

- Define the objects you are looking for by color (defined in the Hue Saturation Intensity color space) and size.
- An example:
  - Detect micrometastasis of tumor cells found in circulating blood.
  - Uses color segmentation and morphological image processing methods to detect and count objects of interest.



Original

Markup

### **Rare Event Detection**



#### **Rare Event input parameters.**

- Default parameter set is used to detect micrometastasis of tumor cells in circulating blood.
- Hue Value defines the color of the objects of interest.
- **Hue Width** selects the range of hues, centered on the Hue Value, that will satisfy the hue detection process.
- **Color Saturation Threshold** the required saturation of the detected object.
- **Intensity Threshold** the intensity threshold of positive pixels.
- Averaging Radius A smoothing parameter used by the morphological process to remove small scale noise structure (value > 0).
- Min Object Pixels min pixel size of object.
- Max Object Pixels max pixel size of object.
- **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).

Macro on server: APERIO-0	1959 🗵
Select Algorithm Import Ma	cro Save Macro
'Rare Event Detection v'	1' Parameters
View Width	1000
View Height	1000
Overlap Size	0
Image Zoom	1
Markup Compression Type	0 - Same as processed image
Compression Quality	30
Classifier Neighborhood	0
Classifier	0 - None
Class List	
Hue Value	0
Hue Width	0.4
Color Saturation Threshold	0.07
Intensity Threshold	210
Averaging Radius	8
Min Object Pixels	40
Max Object Pixels	100000
Object Roundness Threshold	0
<u></u>	
View Width Width of processing box	
Region of Analysis	Inputs Outputs
O Current Screen	
O Entire Image	
<ul> <li>Selected Annotation Layer</li> </ul>	r
Generate Markup Image	Tune Run
Ready	

#### **Microvessel & Rare Event Workshop**



#### **Microvessel & Rare Event Workshop**



Watch Instructor demo the tasks below and perform them using the steps beginning on the next page.

- Open Micro Vessel & Rare Event demo files
- Select Micro Vessel & Rare Event macros
- Run analysis
- View Results





- 1. Log into Spectrum with a username and password provided by instructor & select the specified Research Role.
- 2. Navigate to the digital slide list, open the **Microvessel Demo** slide as shown below.

Login Required 1		
Username: Password: User Login		
Digital Slides 2 List all Digital Slides View all Digital Slides List ScanScope Slides List ScanScope Slides (Past Day) List ScanScope Slides (Past Week) List ScanScope Slides (Past Week)		Microvessel Demo Data
List ScanScope Slides (Past Month) Search all Digital Slides Add new Digital Slides	Click thumbnail image to open ImageScope	]



3. Open the Analysis window (Ctrl G) & select the MicrovesselAnalysis\_macro.

Macro on server: APERIO-01959

Entire Image

Ready

Selected Annotation Layer

Generate Markup Image

- Click **Test** to display the parameters. 4.
- 5. Review the parameters and click **Run**.

	_		Select Algorithm Import I	Aacro	Sa	ave Macro
		5	'Microvessel Analysis	/1' Paramet	ters	
		5	Class List			~
Algorithm Server Job - Server: APERIO-01959			Mark-up Image Type	3 - Analysi	s - Lumen a	nd V
3			Mode	0 - Exclude	Incomplete	Ves
C Select Algorithm Macro			Filtering / Smoothing Level	2		
			Dark Staining threshold	175		
Macro Name			Light Staining threshold	210		
Nuclear macro			Region Joining Parameter (u	m) 4		
MicrovesselAnalysis_macro			Vessel Completion Parameter	er 13		
Micromer_macro			Minimum Vessel Area Thres	h 30		
Membrane_macro			Maximum Vessel Area Three	s 20000		
COlorDeconvolutionSeperateStains_macro			Maximum Vessel Wall Thick	n 10		
			Output Histogram	1 - Vessel	Area	
PositivePixelLount_macro			Histogram Start Value (um o	r 0		
Colocalization_seperatestains_macro			Histogram End Value (um or	1000		
Aperio Positive Pixel Count Sample Macro			Number of Bins	10		
Aperior ositiver mer count sample macro			Endothelial Stain - Red Comp	0.268		
			Endothelial Stain - Green Co	m 0.57		=
			Endothelial Stain - Blue Com	p 0.776		
			Background Stain 1 - Red Co	o 0.68		
			Background Stain 1 - Green	0.57		
			Background Stain 1 - Blue C	0 0.44		
Generate Markup Image			Background Stain 2 - Red Co	o 0		
			Background Stain 2 - Green	0		
<ul> <li>Region of Analysis</li> </ul>			Background Stain 2 - Blue C	o 0		
Selected Annotation     O Entire Image			Clear Area Intensity	240		~
4 Test Create Analyze Cancel			View Width Width of processing box			
	1		- Region of Analysis		Inputs	Outputs
			O Current Screen			

Run

Tune



6. View Markup Image & results in the Annotations window (Ctrl+N).

		G	
Annotations - Detailed View		б	
	Summar		
Layers 🛞 📕 🕂 🗙	Layer Attributes 🕂 🖃 🔳		
Layer 73 MicrovesselAnalysis_macro output	Mean Vessel 95.0435 🔨		
	Median Vessel 68. Perimeter (um)		
	Standard Deviation of 80.3076 Vessel Perimeter (um)		CONTRACTOR SALE
	Mean Lumen Area 366.565 (um2)		
	Median Lumen Area 125. (um2)		A STAR MAIN SOME STRAKE
	Standard Deviation of 682,888 Lumen Area (um2)		
	Mean Vascular Area 284.609		COMPANY STREET STREET
	Median Vascular Area 225. (um2)		
	Standard Deviation of 193,229 Vascular Area (um2)		
	Mean Vessel Wall 3.39909 Thickness (um)		
	Median Vessel Wall 3.17778 Thickness (um)		
	Standard Deviation of 1.07686 Vessel Wall Thickness (um)		
	Average Red OD 0.559968		
	Average Green OD 0.635552		
	Average Blue OD 0.531517		



Export results and histogram values into an Excel Spreadsheet.

- 7. Click Export Grid to Excel Spreadsheet button in the Layer Attributes section.
- 8. Save the values, File name: MicrovesselAnalysis\_ExportResults to the desktop.
- 9. Open the file and view the Report.

Annotations - Detailed View				
		7		
Layers 😨 📥 🛧 🔀 Layer 73 MicrovesselAnalysis_macro o	output		vsis 🔨	
	8	File name: M Save as type: E	icrovesselAnalysis_ExportResults xcel Spreadsheet (*.xls)	Save Cancel
9 MicrovesselAn alysis_ExportR esults	Microsoft Excel - MicrovesselAnalysis_Export     Microsoft Excel - MicrovesselAnalysis_Export     Microsoft Excel - MicrovesselAnalysis_Export     Microvessel and the microvessel an	Results		

#### **Rare Event Workshop**



- 10. Log into Spectrum with a username and password provided by instructor & select the specified **Research Role**.
- 11. Navigate to the digital slide list, open the **Rare Event Demo** slide as shown below.

Login Required 10		
Username: Password: User Login		
Digital Slides 11 List all Digital Slides View all Digital Slides List ScanScope Slides List ScanScope Slides (Past Day) List ScanScope Slides (Past Week) List ScanScope Slides (Past Month)		Rare Event Demo Data
Search all Digital Slides Add new Digital Slides	Click thumbnail image to open ImageScope	]

#### **Rare Event Workshop**



- 12. Open the Analysis window (Ctrl G) & select the MicrovesselAnalysis\_macro.
- 13. Click **Test** to display the parameters.
- 14. Review the parameters and click **Run**.

	Algorithm Server Joh - Server Al	EPTO-01959	1		_
12	Algorithm Server 500 - Server. Ar	LK10-01555		Macro on server: APERIO-0	01959
	Select Algorithm Macro			Select Algorithm Import M	acro Save Macro
			14	'Rare Event Detection v	1' Parameters
	Macro Name		14	View Width	1000
	Nuclear_macro MicrovesselApalusis, macro			View Height	1000
	Micromet_macro			Overlap Size	0
	Membrane_macro			Image Zoom	1
	COlorDeconvolutionSeperateSt	ains_macro		Markup Compression Type	0 - Same as processed image
	Colocalization_macro			Compression Quality	30
	PositivePixelCount_macro			Classifier Neighborhood	0
	Bare Event Detection	macro		Classifier	0 - None
	Aperio Positive Pixel Count San	nole Macro		Class List	
		····		Hue Value	0
				Hue Width	0.4
				Color Saturation Threshold	0.07
				Intensity Threshold	210
				Averaging Radius	8
	Generate Markup Image	Incremental Processing		Min Object Pixels	40
	Cenerate Markup Image			Max Object Pixels	100000
	Region of Analysis			Object Roundness Threshold	0
12	Selected Annotation	O Entire Image		View Width Width of processing box	
13	Test 🗫 Create	Analyze Cancel		Region of Analysis	Inputs Outputs
-			_	O Current Screen	
				O Entire Image	
				<ul> <li>Selected Annotation Laye</li> </ul>	er

Tune

Run

Generate Markup Image

Ready

#### **Rare Event Workshop**



#### 15. View Markup Image & results in the Annotations window.

Annotations - Detailed View			1							×
	15						Summa	ry 🔳	B	
Layers 😹 🚽 🗙 💌 🛛 Layer Attributes 🕛 🖃 🏢				Layer Reg	jions 🕂	-  × =				
Layer 75	Total Number of 115		^	Region	Length (um)	Area (um2)	Text	×	у	^
Result 76	Objects Detected	[		86	103.6	670		21161	18435	
	Total Number of 2132	222	ıts ***	87	58.8	164		20349	18611	
		laorithm luonuto ***		88	52.27	115		21455	17780	
				89	178.3	1680		21445	17896	
Ec	or each Event the results	will		90	44.8	120		22468	18308	
				91	81.2	409		21579	18369	
be	e viewed in the Layer Reg	ions		92	102.7	651		21504	18786	
pa	ane of the Annotations win	ndow.								



# **Knowledge Check**



- 1. Rare Event Detection is used for cell quantification.
  - a) True
  - b) False
- 2. Rare Event Detection uses the HIS (Hue, Saturation & Intensity) color model?
  - a) True
  - b) False
- 3. Microvessel has a set of parameters that join incomplete vessels into complete vessels. What are these parameters?
  - a) Filtering/Smoothing
  - b) Minimum Vessel Area Threshold
  - c) Region Joining
  - d) Vessels Completion
  - e) c & d



#### **Creating New Macros**

- Only users with the appropriate Spectrum administrator permissions can create new macros and register them in Spectrum.
  - After opening a digital slide in ImageScope, open the View menu and select Analysis.
  - Click **Create**, the Select an Algorithm window appears.
  - Select the Algorithm you want to create a macro for, the Analysis window appears.

Macro Name Nuclear_macro MicrovesselAnalysis_macro Micromet_macro COlorD econvolutionSeperateS Colocalization_macro PositivePacIount_macro	ains_macro
Colocalization_SeperateStains Rare Event Detection Aperio Positive Pixel Count Sar	macro nple Macro
Colocalization_SeperateStains Rare Event Detection Aperio Positive Pixel Count Sar	macro nple Macro

Select an Algorithm				E
Title	Version	Description	Filename	^
🕂 Positive Pixel Count 2004-08-11	8.100	Counts Positive Pixels into Three Intensity	C:\Program Files\ScanScope	
🚯 Rositive Pixel Count v9	9.1	Counts Positive Pixels into Three Intensity	C:\Program Files\ScanScope	;
🕂 🕂 Color Deconvolution v9	9.1	Measures Intensity of Individual Stains	C:\Program Files\ScanScope	: =
R IHC Membrane Algorithm	8.001	Quantitation of Membrane Staining	C:\Program Files\ScanScope	;
R IHC Nuclear Algorithm	8.001	Quantitation of Nuclear Staining	C:\Program Files\ScanScope	;
🕂 Membrane v9	9.1	Quantitation of Membrane Staining	C:\Program Files\ScanScope	, 💶
R Micromet v9	9.0	Detects Rare Events in MicroMetastasis i	C:\Program Files\ScanScope	;
Nuclear v9	9.1	Quantitation of Nuclear Staining	C:\Program Files\ScanScope	
🕂 Colocalization v9	9.1	Measures Colocalization of Multiple Stains	C:\Program Files\ScanScope	
R Color Deconvolution	8 001	Measures Intensity of Individual Stains	C:\Program Files\ScanScope	<u> </u>
It does not math selected; by de automatically se list. Just ignore	er whi fault Ir elects and c	ch macro is nageScope a macro from the click <b>Create</b> .	Select Cancel	



#### **Creating New Macros**

- Modify the parameters.
- Use the Tuning window to see the effect of the parameter changes made.
- Continue adjusting the inputs until desired results are achieved.

Macro on server: APERIO-0	1959	×.	1		2023				
Select Algorithm Import Ma	acro	Save Macro		Annotati	ons - Detailed View				
'Nuclear v9' Parameter	s				161				
View Width	1000					-			
View Height	1000			Layers	👁 🕂 🛧 📈	1	ayer Attributes		
Overlap Size	100								100
Image Zoom	1			Layer 8	8	1.	Percent Positive	52.2222	^
Markup Compression Type	0 - Same as processed im	age		Tuning	Laver		Nuclei		
Compression Quality	30	and the second sec		0.3	1000 <b>*</b> 0000		Intensitu Score	3	
Classifier Neighborhood	0	Algorithm Tuning		3				0	
Classifier	0 - None			112			(3+) Percent Nuclei	38.8889	
Class List				100			(2+) Percent Nuclei	7.77778	
Averaging Radius (um)	1	0.050					(1.) Deserved Muselei	E EEEEC	-
Segmentation Type	2.0 2 - Cutoplasmic Rejection	0 00					(1+) Fercent Nuclei	0.00006	=
Threshold Type	1 - Edge Threshold Meth		- A Bree	128			(0+) Percent Nuclei	47.7778	
Edge Trimming	1 - Weighted		2 1 2 NO				Average Positive	139.638	
Lower Threshold	0		~ <i>D 2</i> 4 - ,	1000			Intensity	10.00000000	
Upper Threshold	230						A	220.250	_
Min Nuclear Size (um <sup>2</sup> )	20	Pogoo					Average inegative	238.296	
Max Nuclear Size (um <sup>2</sup> )	1000000						Intensity		_
Min Roundness	0.1			14 MA			(3+) Nuclei	70	
Min Compactness	0						(2+) Nuclei	14	
Min Elongation	0.1							1.7	
Remove Light Objects	0						(1+) Nuclei	10	
Clear Area Intensity	240	00000		-			(0+) Nuclei	86	
Nuclear Stain (Red)	0.696858		A REAL PROPERTY AND				T 1 111 1 1	100	
Nuclear Stain (Green)	0.643073	Carl Start (1997) - 1997					I otal Nuclei	180	
Width of processing box						1			
			The outp	ut but	top allowe you to				
·		Inputs Outputs	The outp	սւնսւ	ton allows you to				
Region of Analysis			salact the	rocu	Its that will annear in				
O Current Screen				5 1030	no mai wiii appear in				
Entire Image     Selected Appotation Lave	r		Spectrum	<b>1</b> .					
Concercio Annotation Laye			oposium						
Generate Markup Image	l	Tune Run							
Ready									



#### **Algorithm Tuning Window**

• Quickly see the results of analyzing an area of an image or test changes made to the algorithm parameters.

Select Algorithm Import Ma	cro	Save Macro
Nuclear v9' Parameters	1	
View Width	1000	
View Height	1000	
Overlap Size	100	
Image Zoom	1	
Markup Compression Type	0 - Same as processed image	
Compression Quality	30	
Classifier Neighborhood	0	
Classifier	0 - None	
Class List		=
Averaging Radius (um)	1	
Curvature Threshold	2.5	
Segmentation Type	2 - Cytoplasmic Rejection	
Threshold Type	1 - Edge Threshold Method	
Edge Trimming	1 - Weighted	
Lower Threshold	0	
Upper Threshold	230	
Min Nuclear Size (um <sup>2</sup> )	20	
Max Nuclear Size (um <sup>2</sup> )	1000000	
Min Roundness	0.1	
Min Compactness	0	
Min Elongation	0.1	
Remove Light Objects	0	
Clear Area Intensity	240	
Nuclear Stain (Red)	0.696858	
Nuclear Stain (Green)	0.643073	~
View Width Width of processing box		
Region of Analysis	l II	outputs
<ul> <li>Current Screen</li> <li>Entire Image</li> <li>Selected Annotation Layer</li> </ul>		
Generate Markup Image	Tur	ne Run
Ready		



Every time the Algorithm Tuning window updates the analysis, a new markup image appears in the window and the numeric data in the Annotations window changes to reflect the new analysis.



#### **Algorithm Tuning Window**

- As the parameters are changed, the Tuning window will display the markup image for current parameters.
- The Annotations window will display the numeric results of the analysis in a new **Tuning Layer**.







#### **Saving and Registering Macros with Spectrum**

- After the macro has been created, save it to register it to Spectrum.
  - From the Analysis window click **Save** to register it to Spectrum.
  - Name the macro with a naming convention that helps identify it.

Select Algorithm Import Ma	ICFO	Sa	ve Mac	ro
Nuclear v9' Parameter	s			
View Width	1000			
View Height	1000			Ē
Overlap Size	100			
Image Zoom	1			
Markup Compression Type	0 - Same as processed image			
Compression Quality	30			
Classifier Neighborhood	0			
Classifier	0 - None			
Class List				
Averaging Radius (um)	1			
Curvature Threshold	2.5			
Segmentation Type	2 - Cytoplasmic Rejection			
Threshold Type	1 - Edge Threshold Method			
Edge Trimming	1 - Weighted			
Lower Threshold	0			
Upper Threshold	230			
Min Nuclear Size (um <sup>2</sup> )	20			-
Max Nuclear Size (um <sup>2</sup> )	1000000			
Min Roundness	0.1			
Min Compactness	0			
Min Elongation	0.1			
Remove Light Objects	0			
Clear Area Intensity	240			
Nuclear Stain (Red)	0.696858			
Nuclear Stain (Green) View Width Width of processing box	0.643073			
Region of Analysis		Inputs	Outp	ut
<ul> <li>Current Screen</li> <li>Entire Image</li> <li>Selected Annotation Layer</li> </ul>	r			
Generate Markup Image	Tur	ne (	Rur	1
Ready				-

 Save macro on server: APERIO-01959	$\mathbf{X}$
Enter a name for the macro (50 characters or less),	ОК
then press OK to save the macro or press Cancel to cancel the save	Cancel
Nuclear Breast Dako	

Now, when you bring up the Analysis window, you will be able to select your macro by clicking Select Algorithm because your macro will appear in the list.



#### **Batch Analysis in Spectrum**

- Spectrum provides a convenient tool for batch analysis of slides.
- The Spectrum administrator must first create a macro for each image analysis algorithm used for analysis.
- Select digital slides to be analyzed from Spectrum, click **Analyze**.
- From the Analysis page, determine the analysis details and click **Analyze**.
- Slides chosen will be added to the Job Queue.

Digital Slides						Analy	ze 3 Ima	ages		
View	Image	<u>s   Open Data Ana</u> Thumbnail	alyze <u>Delete   M</u>	<u>ew   Move   Copy   Assign To   Export Data   Annotatic</u> <u>Barcode ID</u>	ns	Select A	nalysis Macro:	Verene	PositivePixelCount_macro	×
•		- all		Select slides for analysis		Select In	put Annotation	Layer:	Most Recent Whole Image	
•	٥		XX			Analyz	e Cancel	age(s)	Select Macro & Markup.	, Annotations
	٥					3 Per	iding Ana	alysis	Jobs	
	-	1 de mai	C 107 9				Job ID 494	Sub 2009	mitted Date 9-02-05 13:42:58	Status In Progress
	Fr	om the job	queue, d	lick <b>F5</b> to check job status or			495	2009	9-02-05 13:42:58	Submitted
	se	lect cance	l jobs.				496	2009	9-02-05 13:42:58	Submitted

### **Scalable Processing Architecture**



#### **Analysis Accelerator**

- Provides the ability to run Whole Slide Analysis at an accelerated speed.
- Provides a multi-user system with a single-user response time.
- Capable of processing up to 7 Image Analysis jobs in parallel.
  - Scalable for 7, 14, 21...job as needed.

![](_page_102_Picture_7.jpeg)

### **Scalable Processing Architecture**

![](_page_103_Picture_1.jpeg)

![](_page_103_Figure_2.jpeg)

# **Course Summary**

![](_page_104_Picture_1.jpeg)

# Upon conclusion of this training session, learners are able to complete the following tasks related to their job:

- Describe how Aperio's Image Analysis tools work
- Describe Aperio's Image Analysis tools for Area, Cell, and Event Quantification
- Understand the input parameters for each Image Analysis tool
- Understand the output parameters for each Image Analysis tool
- Complete the steps for running and Aperio Image Analysis tools
- Calibrate stain color using Color Deconvolution
- Create a macro and register it with Spectrum
- Run a macro using Spectrum Plus batch analysis option
- Export Image Analysis results using Spectrum's Export Data tool
- Describe Aperio's Scalable Architecture

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.