

# Supplemental Material

Saved Pipeline, in file pipeline for blood vessel analysis.txt, Saved on 12-Mar-2007

Pixel Size: 1

Pipeline:

LoadImages  
ColorToGray  
InvertIntensity  
ApplyThreshold  
MeasureImageAreaOccupied

Module #1: LoadImages revision - 1

How do you want to load these files? Text-Exact match

Type the text that one type of image has in common (for TEXT options), or their position in each group (for ORDER option): CA11.jpg

What do you want to call these images within CellProfiler? sample

Type the text that one type of image has in common (for TEXT options), or their position in each group (for ORDER option): /

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If using ORDER, how many images are there in each group (i.e. each field of view)? 3

Are you loading image or movie files? Image

If you are loading a movie, what is the extension? avi

Analyze all subfolders within the selected folder? No

Enter the path name to the folder where the images to be loaded are located. Type period (.) for default image folder.

Module #2: ColorToGray revision - 1

What did you call the image to be converted to Gray? sample

How do you want to convert the color image? Combine

COMBINE options: n/a

What do you want to call the resulting grayscale image? sampleToGray

Enter the relative contribution of the red channel 1

Enter the relative contribution of the green channel 1

Enter the relative contribution of the blue channel 1

SPLIT options: n/a

What do you want to call the image that was red? Type N to ignore red. OrigRed

What do you want to call the image that was green? Type N to ignore green. OrigGreen

What do you want to call the image that was blue? Type N to ignore blue. OrigBlue

Module #3: InvertIntensity revision - 1

What did you call the image to be inverted (made negative)? sampleToGray

What do you want to call the inverted image? sampleInverted

Module #4: ApplyThreshold revision - 4

What did you call the image to be thresholded? sampleInverted

What do you want to call the thresholded image? samplethresholded

Pixels below this value (Range = 0-1) will be set to zero (0 will not threshold any pixels) 0

If your answer was not 0, do you want to shift the remaining pixels' intensities down by that intensity or retain their original values? Retain

Pixels above this value (Range = 0-1) will be set to zero (1 will not threshold any pixels) 0

If your answer was not 1, you can expand the thresholding around those excluded bright pixels by entering the number of pixels to expand here: 0

Binary option: Enter the threshold to use to make the incoming image binary (black and white) where pixels equal to or below this value will be zero and above this value will be 1. If instead you want to use the settings above to preserve grayscale information, enter 0 here. 0.1

Module #5: MeasureImageAreaOccupied revision - 2

What did you call the images you want to process? samplethresholded

What do you want to call the staining measured by this module? sampleVesselArea

Select an automatic thresholding method or enter an absolute threshold in the range [0,1]. Choosing "All" will use the Otsu Global method to calculate a single threshold for the entire image group. The other methods calculate a threshold for each image individually. Set interactively will allow you to manually adjust the threshold to determine what will work well. .5

Threshold correction factor 1

Lower and upper bounds on threshold, in the range [0,1] 0,1

For MoG thresholding, what is the approximate percentage of image covered by objects? 10