# Stain Analysis Plugin

The Stain Analysis plug-in applies color deconvolution method described in:  "Ruifrok AC, Johnston DA. Quantification of histochemical staining by color deconvolution. Analytical & Quantitative

Cytology & Histology 2001; 23: 291-299 ". A threshold image is obtained by applying a linear thresholding algorithm on deconvolved image. Then the object pixel percentage is calculated using the threshold image. All the processing is done in the highest resolution.

This Plug-in has been implemented in Visual Studio C++ 2012 and tested with “Sedeen Viewer SDK version V5.2.1.427”.

## User Guide

This guide explains how to use the “Stain Analysis plugin” to analyze histology image. First, the user need to copy the plug-in DLL to plugins folder under the Sedeen Viewer installation folder. This plug-in has no dependencies on other 3rd party library. Once user runs the “Sedeen viewer” and opens an image, the plug-in would be loaded in Sedeen Viewer and appeared in the list of Algorithm in the “Analysis Manager” View (See figure 1). We suggest users to read the original paper to understand how new vectors are determined and how the procedure works before using the plug-in.

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| Figure1. Sedeen Viewer and the Analysis Manager view. The “Stain Analysis” plug-in is appeared in the algorithm pop up menu. |

Now the user should be able to tune the parameters and run the “Stain Analysis” plug-in (See figure 2).

The plug-in parameters are explained as follow:

## Parameters

### Selected Stain:

The plugin provides number of "built in" stain vectors. The built-in vectors are: Hematoxylin and Eosin (H&E), Hematoxylin and DAB (H DAB), and Hematoxylin, Eosin and DAB (H&E DAB). Also, users can determine their own vectors to achieve an accurate stain separation, depending on the stains and methods they use. This option is available in two ways: “From ROI” or “Load From File”. If “Selected Stain” option is set to “From ROI” users also need to choose three regions of interest (stained area) to compute the stain vectors. Select small ROIs areas which are all intensely stained with only one of the dyes. Select fully stained areas, without empty background. Repeat this for each dye. If the staining method uses only 2 colours instead of 3, for the 3rd selection just select small ROI from the background.   
After selecting the three samples, the plug-in will compute the stain vectors based on three regions of interest defined by user. The computed stain vectors will be saved in default directory (the sedeen directory in the same folder as the image): "Path to the image/sedeen/" as “StainsFile.csv” file for future use. The stain vectors can be provided as a “.csv” file by selecting “Load From File” option as well. An example of the “.csv” file will be provided in the plug-in directory. This file needs to be copied in default directory (the sedeen directory in the same folder as the image): “Path to the image /sedeen/”.

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| Figure2. Sedeen Viewer The “Stain Analysis” plug-in parameters. |

### Display:

The plugin takes an RGB image and returns three 8-bit images with a colour look up table that corresponds to the respective vector colours. When the specimen is stained with a 2 colour scheme (such as H&E) the 3rd image represents the complementary of the first two colours (i.e. green). Ideally the 3rd image should be completely white. If not, then either the colour vectors or the background colour correction have not been correctly determined. This option allows the user to see the results of algorithm corresponds to the respective vector colours.

### Region of Interest:

This parameter is related to “Selected Stain” option. As we mentioned before, users can determine their own vectors based on three regions of interest. When the specimen is stained with a 2 colour scheme (such as H&E) the 3rd image represents the complementary of the first two colours (i.e. green).

### Threshold:

This parameter is related to Otsu thresholding algorithm allowing the user to modify the results. The range of threshold value is 0.0 and 50.0.

### Processing ROI:

The algorithm can be applied on whole image or a selected region. The user can select the desired ROI to be process.

## Examples

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| Figure3. Displaying the processed image using built-in **Haematoxylin and DAB** vectors. Based on the “Display” option the result of algorithm corresponds to the respective vector colour is presented. | |