

Quantitative comparison of immunohistochemical staining using 2 different antigens.

This document explains how to carry out a comparative analysis of IHC staining on consecutive slides and is intended to illustrate how the Sedeen viewer can be applied to a specific task in cancer research.

In this example we have 2 WSIs showing consecutive sections of liver tissue cut from the same paraffin block. Each section is stained with a different antibody and a brown DAB stain is used to identify the spatial distribution of the antigens. We want to calculate %stain over the same region of tissue on both images.

1. Download the 2 sample images, source.tif and target.tif which can be accessed from here: <https://web.pathcore.com/case/1721?s=uxLuW3TWhG6N>. In order to download an image, open it in the browser by double clicking on the thumbnail. In the web viewer there will be a small ⓘ at the top of the menu pane on the left. Click on that and select download. Ensure that you have enough disk space as the images are approx 1.3 Gb in size.
2. Once the images are downloaded, open them in the sedeen viewer (make sure you have downloaded the latest version) and display them side by side.
3. Define a region of interest (ROI) on the source image using one of the annotation tools (Fig 1.)
4. Switch to the overlay view and manually align the 2 images. Full instructions for this procedure are given here: <https://pathcore.freshdesk.com/support/solutions/articles/16000035806-image-registration-and-overlaying>
5. Close the target image and make sure that you save the transformation if prompted.
6. From the **Analysis Manager** load the ExportTransformedROI plugin, select the region of interest that you want to export and click Run. Select the target.tif image in the file explorer window that will appear. The selected ROI from the source image will now be transformed and saved to the target image. Full instructions for the ExportTransformedROI plugin are provided here: https://github.com/sedeen-piip-plugins/ExportTransformedROI/blob/master/ExportTrandformedROI_UserManual.pdf
7. Reload the target.tif image; the source ROI should have been copied to the target image (fig 3).
8. Now run the StainAnalysis plugin on the source and target images. Detailed instructions for the StainAnalysis plugin can be found here: https://github.com/sedeen-piip-plugins/ExportTransformedROI/blob/master/ExportTrandformedROI_UserManual.pdf
9. It is necessary to select each image in turn and run the plugin twice. For these images the selected stain is set to "Hematoxylin & DAB" and the display option is set to "STAIN2". The threshold was left at the default value. Figs 4 and 5 show the result of running the analysis plugin on each images. The calculation is carried out over the entire source ROI.



Figure 1. The source image is on the left and the target image is on the right. The polygon annotation tool has been used to define an ROI around the tumour on the source image.

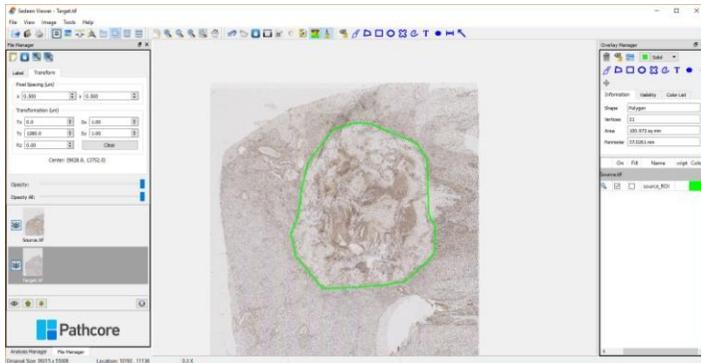


Figure 2. The 2 images are overlaid and the opacity of the source image has been reduced to allow the target image underneath to be seen. The 2 images have been aligned manually.

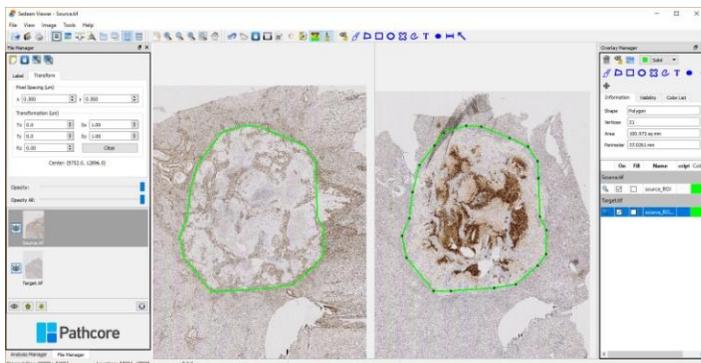


Figure 3. The ROI from the source image has been copied onto the target image.

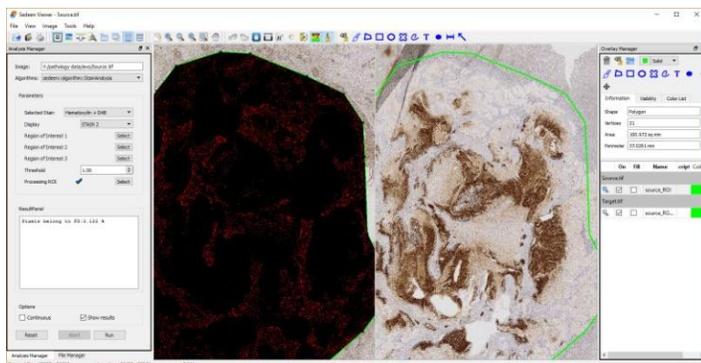


Figure 4. The source image was selected and the StainAnalysis plugin was used to calculate the % ROI area covered by the DAB stain.

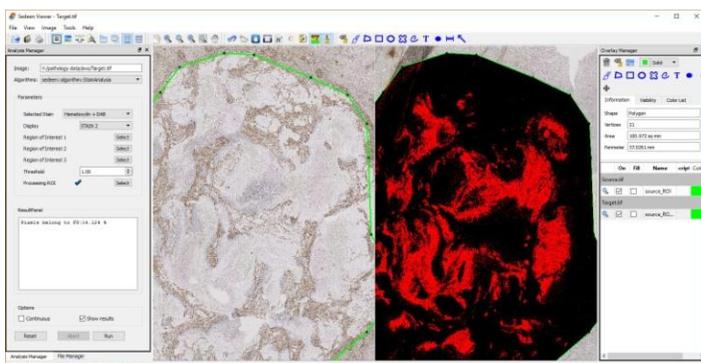


Figure 5. The target image was selected and the StainAnalysis plugin was again used to calculate the % ROI area covered by the DAB stain.