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# Color Deconvolution as a Simple and Rapid Tool in Quantitative Biomedical Research

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Dear Editor,

The quality, efficiency, and speed of a quantitative analysis are critical factors in cytopathology. In this scenario, reliable and reproducible tools are needed to improve results in a shorter period,[1] mainly routine immunohistochemistry (IHC) slides and biomedical research.

Histological experiments rely on visualization of results using staining techniques, due to the ability of light-absorbing dyes to selectively bind to molecules and complexes of interest, which may provide a quantitative analysis when combining computational techniques.[2] Thereby, color deconvolution can be addressed as a versatile tool in quantitative analysis as this method is able to split in channels the different dyes of a staining technique,[3] which allows analysis of the area fraction of the aimed structures.

In ►**Fig. 1**, we can see that the "color deconvolution" tool on ImageJ (National Institutes of Health, United States) allows unmixing brightfield images into channels representing the absorbance of the individual dyes. After splitting the channels, images can be turned into gray with the aid of the "threshold" tool to determine the structure area. Then, we can measure the "area fraction" of the stained structures in contrast with the white background. Using this method, it is possible to quantify the stained area of each field in a semi-automatic manner, which allows a greater flow of analysis.

This simple and rapid technique to analyze the absorbance of different dyes in a quantitative manner has the potential to increase the flow of analysis in biomedical research. To corroborate with our presented toolkit, color deconvolution was previously used by researchers to study, e.g., hepatocellular carcinoma,[4] atherosclerotic lesions,[5] deep neural networks,[6] and skin layers.[7] These studies showed the versatility of this technique in both histochemistry and IHC as the brown color generated by 3,3′-diaminobenzidine from IHC can be separate from the original image and quantitatively analyzed to show the percentage of its stained structure. Additionally, this process of analysis can be addressed in the manuscripts as a supplementary material to show the image after color deconvolution.

The growing availability of image digitization and bioinformatic technologies has driven the search for new ways of analyzing image datasets.[2,8,9] Thus, to have a reliable method to increase the speed of analysis is of paramount importance to conduct cytological studies in larger samples. In this sense, we hope that this toolkit may be a useful method in further histopathological studies.

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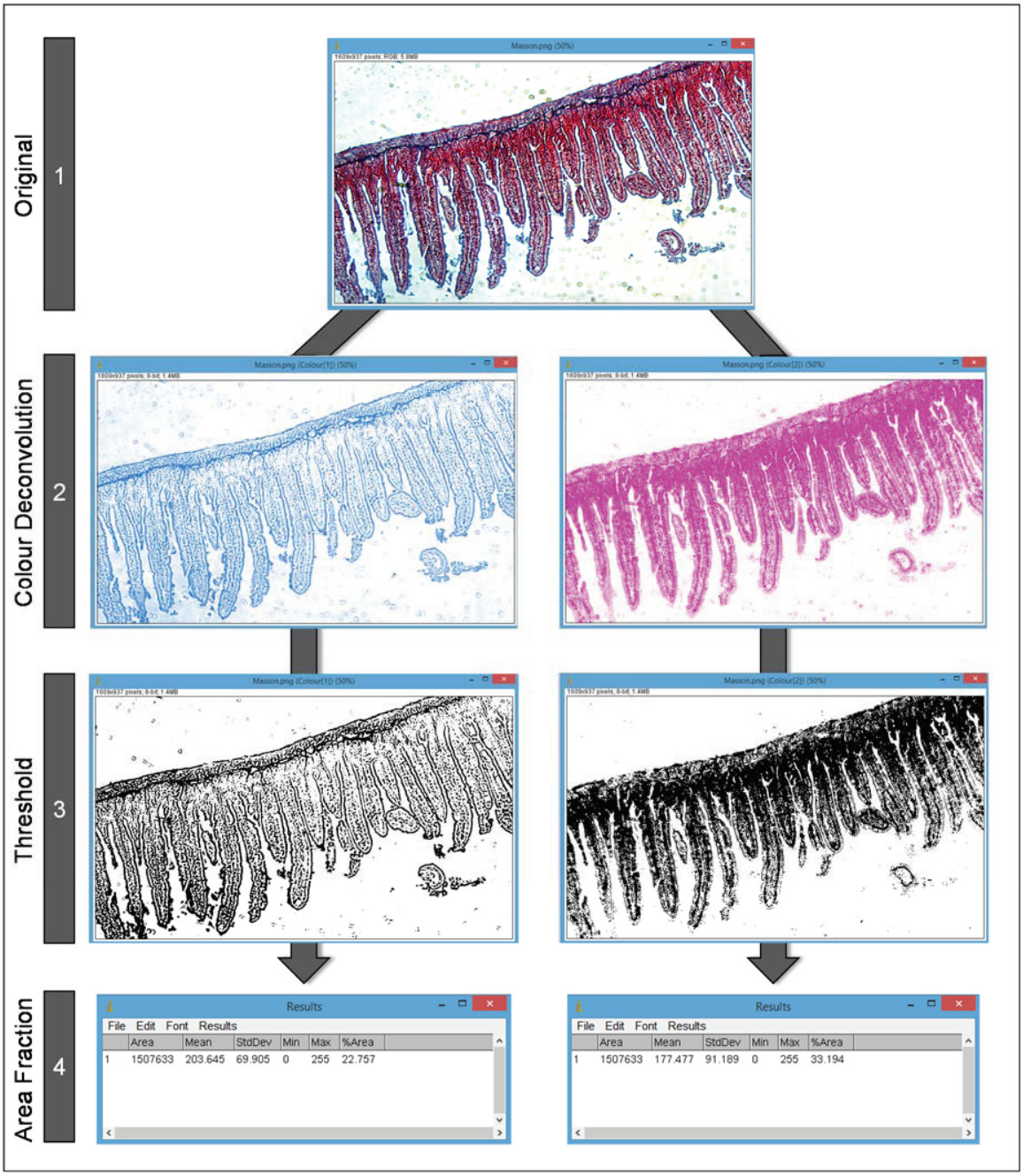


**Fig. 1** **Flowchart.** We choose the Masson's trichrome stain in a small intestine section of an old male Wistar rat, as this technique uses different dyes to elucidate smooth muscle (*red*) and collagen fibers (*blue*). (1) insert the original photomicrograph on ImageJ; (2) then split the channels using color deconvolution (click on plugins > color functions > color deconvolution) by selecting the vectors (i.e., staining technique); (3) then establish the threshold (click on image > adjust > threshold); (4) finally use the area fraction (% area) for your quantitative analysis (click on analyze > measure). The area fraction can be used for quantitative analysis with the aid of a statistical software.

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