Color Palettes Overview After Thresholding Process with Default Methods of *ImageJ* or FIJI*

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ABSTRACT

Coloring techniques in life sciences continue to progress, which have now used deep learning in the staining stage to create virtual staining. Usually, manual staining produces an image with a more vigorous color intensity than virtual staining. This study aims to obtain an overview of the color palette (310 colors) after processing with the default threshold method in *ImageJ* v1.53s or FIJI (*ImageJ2*) version 2.3.0/1.53q with several variations of the color threshold and color space, which helps determine algorithms in image processing.

The variants of the color threshold were (red, white(W), black(B), and B+W). The color space variants were hue-saturation-brightness (HSB), red-green-blue (RGB), Lab, and YUV. The background variant used in the image processing in this study is dark. Later, after the image is binarized, the object in the image to be analyzed is colored black.

The separation results of 16 descriptions of threshold color and color space variants showed that the combination with HSB color space is the most sensitive to color differences, reducing red color the most. In contrast, the RGB color space is the least susceptible to color differences. Lab and YUV color spaces have almost the same segmentation effect. The results of this study not only can be applied in analyzing all types of manual or digital images in all branches of science and art.

CCS CONCEPTS

• **Image processing**; • **Imaging**; • **Medical Technologies**;

KEYWORDS

Thresholding, Binarization, Staining, Color

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1 INTRODUCTION

Before an image is analyzed, the image must go through image processing stages. The image processing stage aims that later what is analyzed digitally is the object of research. The image is first enriched, segmented at the processing stage, and then enters the binarization process (1).

We will obtain a clearer image at the enrichment end. Image segmentation is essential in several computer vision and image processing applications. In life sciences, segmentation is a crucial tool in detecting and describing particular objects in the image of cells, tissues, and organs of living things by separating the specific object from other parts and the image background.

Thresholding is one of the oldest and most existent image segmentation techniques (2) (3). Segmentation can use thresholding based on gray level, color, intensity, texture, depth, or movement. This study examines the setting of boundaries based on pixel intensity in natural images. In this paper, thresholding is carried out based on color values in natural images.

The threshold value of each image pixel will be binary so that the target object is separate from the non-target part, including the background. We select the more precise thresholding technique in the segmentation stage; after binarization, noise or artifacts in the image are reduced and even lost, and we can separate the target object from other parts of the image (4).

ImageJ is an open-source program which Java-based software that analyzes images. There are hundreds of plugins that operate under ImageJ. We can use these plugins to obtain quantitative data from research in various fields of science. The data are mainly related to two basic quantities: length and luminous intensity and their derivates. Currently, ImageJ has 17 threshold methods. One of them is the default. In *ImageJ*, the threshold process involves three things: the threshold method, threshold color, and color space. FIJI (*ImageJ2*) is *ImageJ* for Mac OS.

In the world of health, most of these images result from conventional or virtual staining (5) (6) (7). The staining involves one or more colorants or color indicators (for digitally generated and even virtual images) (8) (9). Digital pathology is an application of deep learning. Deep learning is part of machine learning. Digital pathology helps pathologists quickly extract the appearance of patterns from specific tissue preparations. The provisions that contain information about colors indicating target, non-target, and background will guide the observers of staining results (10).

Several research results have informed how to segment an image using several thresholding techniques and mathematical calculations (11)(12)(3)(13). Usually, these mathematical calculations are in the corridor of MATLAB applications. The overview of our results

Table 1: Combinations of threshold variants in the default threshold method of *ImageJ* or FIJI

No. of combination	Threshold color	Color space
1.	Red	HSB
2.	Red	RGB
3.	Red	Lab
4.	Red	YUV
5.	White	HSB
6.	White	RGB
7.	White	Lab
8.	White	YUV
9.	Black	HSB
10.	Black	RGB
11.	Black	Lab
12.	Black	YUV
13.	Black and White	HSB
14.	Black and White	RGB
15.	Black and White	Lab
16.	Black and White	YUV

informed about of the results from a binary image of 310 color palettes segmented using a variant of the IsoData thresholding method, namely the default of the thresholding method contained in *ImageJ* or FIJI, in all combinations of threshold color and color space included in the application.

This study aims to obtain an overview of the color palette after processing with the default threshold method in *ImageJ* or FIJI (*ImageJ2*) with several variations of the color threshold and color space. We can use this overview to determine the variance of the threshold color and color space in the thresholding process after obtaining information on the color provisions of the target object in the reading guidelines for each coloring technique (12). Since the overview was not the result of statistically calculating the grayscale value of the neighborhood pixel for each image pixel, if the type of segmentation is classical threshold image segmentation, we can use the summary (14) (15) (13).

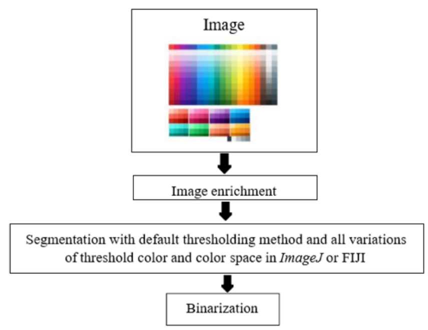


Figure 1: Flowchart of The Color Palettes Image Processing

2 MATERIAL AND METHODS

The color palettes were dummies of the color palettes in https://hexcolorspicker.com/tools/materialui/colors.html (209 colors) (16), and https://afrizatul.com/situs-flat-design/ (101 colors)(17). The shape of each palette is square (Fig. 1). Palettes selected from both sites are placed in a single-page word file converted to TIFF (Temporary Instruction File Format) format. The threshold method used is *ImageJ*'s default threshold method. All variants in the color threshold and color space in *ImageJ* v1.53s or FIJI (*ImageJ2*) version 2.3.0/1.53q are combined. Threshold color variants include red, white, black, and B+W (B&W). Color space variants include hue-saturation-brightness (HSB), red-green-blue (RGB) (11), Lab, and YUV. Sixteen combinations resulted from the threshold color and color space variants, each amounting to 4 (Table). The selected background was dark. According to the purpose of the study, image processing was not set or calibrated in length units.

Image>Adjust>Color Threshold (Fig. 2a.) is the order of the icons clicked before selecting the variant. After selecting the variant (Fig. 2b.), click 'Select' (Fig. 2c.).

After the thresholding process is completed (Fig. 2c.), proceed with binarization (Fig. 3). Binarization is started by clicking 'Process' on the toolbar. After 'Process' is activated, 'Binary,' then 'Make Binary' is clicked (Fig. 3). Figure 1 below shows the flow of all the image processing steps described previously. Calculating the reduced number of color palettes is calculated by summing the reduced number of color palettes contained in an extensive rectangular color palette consisting of 209 color palettes. Several smaller color palette groups below (including a rectangular group composed of 5 color palettes and eight rectangles comprised of 12 color palettes each) make it easier for us to identify the reduction that has occurred quickly. The first threshold combination (Table) process clarified below the thresholding and binarization processes. The whole process of thresholding and binarization used *ImageJ* and FIJI.

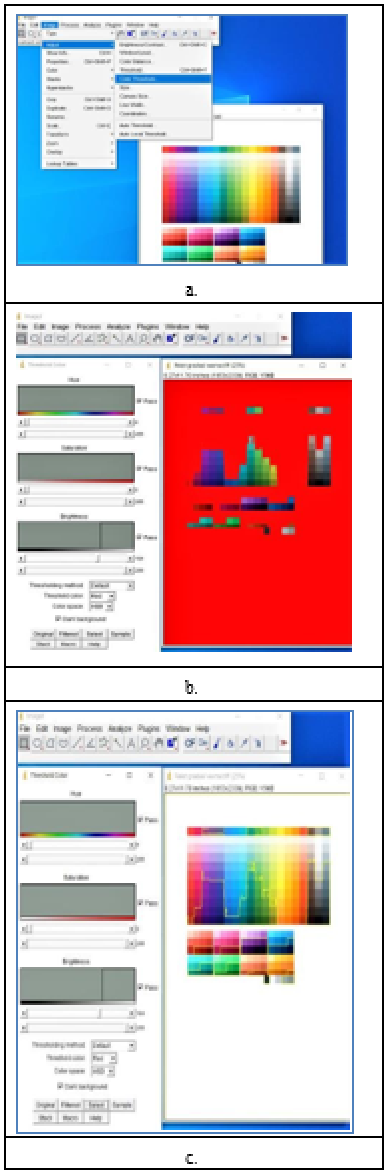


Figure 2: Thresholding Process of The First Threshold Combination

3 RESULTS AND DISCUSSION

The default threshold method of *ImageJ* is a variation of the IsoData algorithm. The IsoData threshold method divides the object and background based on the initial threshold value, then the average pixels at or below the threshold and the pixels above are calculated. The quotient of the sum of the average background pixel values and the average object values produced the threshold value. In the threshold process with the default threshold method of *ImageJ*, there are steps to return the same pixel value by activating the 'Ignore black' and 'Ignore white' functions (18). From all threshold combinations, there were only four variations of results. The difference only occurs between different color spaces. Therefore, the results presented in this article are only four of the combinations with a white threshold.

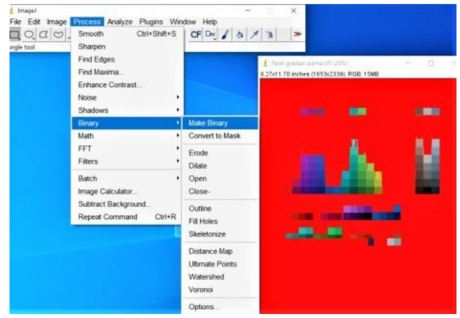


Figure 3: The Beginning of The Binarization Process of The First Threshold Combination

The thresholding results from thresholding combinations with the color space of HSB (Fig. 4a.) and its binarization (Fig. 4b.) showed that the processes reduced the yellow and orange colors by 100%, so the yellow and orange objects will not be presented in the post-binarization process. Red, blue, purple, and brown decreased by 91% (20/22), 79% (26/33), 59% (13/22), and 27% (3/11). The combination group with the RGB color space variant showed the threshold result that reduced the blue color the least, only 27% (5/33) (Fig. 4c.). The groups reduced red, purple, green, yellow, orange, brown, and black colors by 23% (5/22), 23% (5/22), 36% (8/22), 27% (6/ 22), 36% (4/11) and 41% (9/22).

Figure 4.e. showed that the combination of the threshold with Lab color space reduced brown, red, green, purple, orange, and blue by 36% (4/11), 32% (7/22), 32% (7/22), 36% (16/44), and 48% (16/33). This combination group reduces the yellow color ultimately (100%). The reduced colors in combination with the *Lab* and YUV color space were not significantly different (Fig. 4e., and Fig. 4g.) because Lab and YUV color space are both converted from RGB color space. Lab color space consists of brightness (L), component *a* (green-red axis), and component *b* (blue-yellow axis). In the RGB to Lab conversion process, we must convert RGB colors to the XYZ color space with formula (19). There are publications of research results that inform the use of the Lab' color space in image processing of tissue preparations but do not use the default threshold method of *ImageJ* (20). The YUV color space consists of a luminance/brightness component (Y) and two color/chrominance components (chroma, U, and density, V).

The data provide information on the combination of the threshold with the HSB color space variant. We can choose the combination in the threshold process for objects that are colored or colored brown and purple. Still, both colors must not exist in the same image.

The overview of color palettes a, c, e, and g resulted from combining the threshold color white, color space HSB, RGB, Lab, and YUV before the 'Select' toolbar was clicked. Figure b, d, f, and h represent the binarization results of a, c, e, and g.

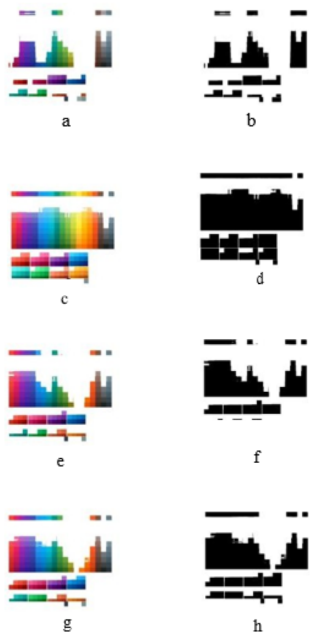


Figure 4: Overview of Color Palettes Before and After Binarization

The whole process of thresholding and binarization used *ImageJ* and FIJI. Figure 4.b., 4.d., 4.f., 4.g., and 5.b. were the example image processing results using FIJI. The results of thresholding with *ImageJ* were the same as the result of thresholding done with FIJI. However, the object color (black) in FIJI binarization results was the background color in the results with *ImageJ*.

We can also perform imaging analysis on various staining results in biomedical sciences, such as Hematoxylin-Eosin (HE), immunohistochemical and Gram stains. We usually stain cells or body tissues with HE and immunohistochemical stains and usually stain bacteria with Gram stain. We can digitally analyze the objects colored purple in the HE and Gram-stained images using the threshold combination groups with the color space of HSB. However, we can not analyze objects colored red in both stains' results by the thresholding combination because the thresholding significantly reduced all things colored red. This thresholding combination distinguishes objects stained with brown from things colored light blue the combinations because their binarization reduces all light blue colors. However, we can not use them to differentiate dark brown objects from dark blue ones.

All combinations can prove the existence of an object in the form of a yellow line between red, blue, green, brown, or black objects that are not after binarization. However, the results of the binarization of all segmentation of all combinations cannot distinguish between objects that are red, purple, blue, green, brown, or black. It caused all threshold combinations not to reduce these colors 100%.

The acquisition of quantitative data regarding collocation will be helpful for the process of analyzing the observed image using a microscope other than a fluorescence or confocal microscope along with the microscope's built-in software. Users of both microscopes can obtain quantitative collocation data by deactivating the fluorescent light channel feature and leaving the other fluorescent light channel feature active and vice versa (21). This research proved that the default threshold method of *ImageJ* is only susceptible to yellow and white colors. To distinguish dark red, purple, dark blue, brown, and black objects, one must use a threshold method that is more sensitive to these colors. HSB is the most sensitive to color intensity among the four color spaces.

After the image is binarized, it can only be analyzed with one of *ImageJ*'s main features, such as 'Analyze,' or can be continued with the appropriate plugin (18). A plugin is a computer program that adds to the functionality of the main program. *ImageJ* has hundreds of plugins. Sometimes in an image processing algorithm, there is more than one binarization step. The next binarization begins with segmentation using a threshold based on things different from the previous threshold, which is more specific according to image processing and analysis.

ImageJ currently has 17 thresholding methods, four threshold colors, and four color spaces, so there are 272 threshold combinations available in ImageJ. This study only studied 5.8% (16/272) of the existing threshold combinations in *ImageJ* or Fiji today. Several research results have informed the use of 16 of the 17 threshold methods available in *ImageJ* (one threshold method, IJ_IsoData, was not examined in these studies). These studies did not try all the available threshold colors and color space variants (4 variants each) (13)(22). In addition, the colors involved in the shade of objects in the two studies did not vary much, only the color of the documented cells. However, the studies showed the sensitivity of each threshold method to the intensity of a specific color and the regularity or irregularity of the object's shape (s).

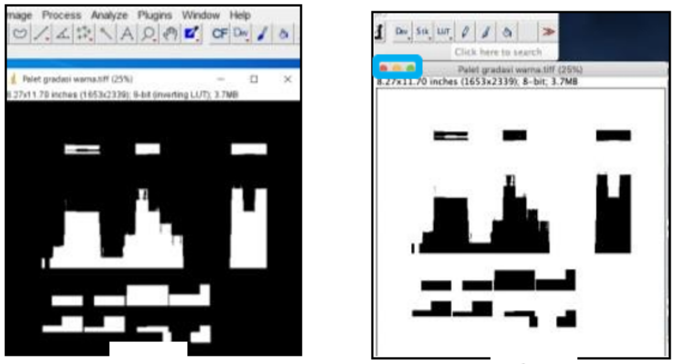
We can use these research results to analyze all digitally generated images in general or in deep learning processes. Although the sensitivity of the threshold method is not as sensitive (to the object's shape), the threshold method is non-default.

Sixteen other thresholding methods (besides the default) have specific mathematical properties and are more detailed than the default. We can use the data of combination information for image processing with a short algorithm or segmentation stages before the first binarization on image launch with relatively long algorithms. We can implement these research results in analyzing all types of all digitally generated images in general or in deep learning processes in all branches of science and art.

4 CONCLUSION

Of the 16 combinations studied, the combination of thresholding with the HSB color space is a threshold that can ideally reduce orange and yellow colors, reducing red color the most. Apart from the various specific purposes of immunohistochemical staining, this combination is quite suitable for observing brown target objects, for example, immunohistochemical staining results. The combination with the RGB color space can reduce the minor colors. The combination of thresholding with *Lab* and YUV color spaces has almost the same segmentation effect.

We need to continue this research to determine the effect of threshold combinations on various colors, essentially represented by pixel intensity.



a. b.

Figure 5: Binarization Results Display. The results of the first combination binarization are in *ImageJ* (a) and FIJI (b).

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