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Grayscale Conversion of Histopathological Slide Images as a Preprocessing Step for Image Segmentation

Jashojit Mukherjee[1], Indra K. Maitra[2], Kashi Nath Dey[1], Samir K. Bandyopadhyay[1], Debnath Bhattacharyya[3] and Tai-Hoon Kim[4*]

[1]*Department of Computer Science and Engineering, University of Calcutta, India*
[2]*B. P. Poddar Institute of Management and Technology, Kolkata, India*
[3]*Department of Information Technology, Bharati Vidyapeeth Deemed University College of Engineering, Pune, India*
[4]*Sungshin Women's University, Dongseon-dong 3-ga, Seoul, Korea*
jashojitmukherjee@gmail.com, ikm.1975@ieee.org, kndey55@gmail.com, skb1@vsnl.com, debnathb@gmail.com, taihoonn@daum.net

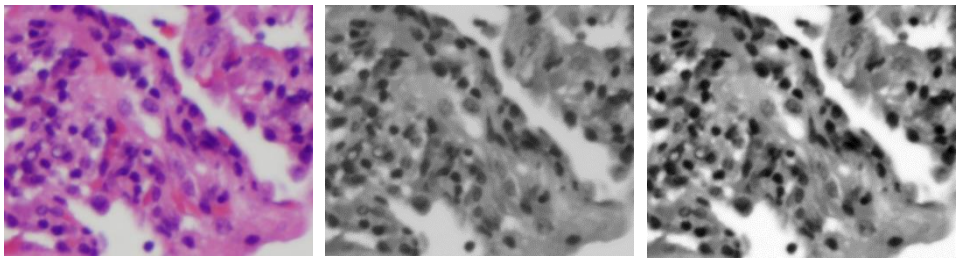
Abstract

This paper presents a method of conversion of color image of histopathological slides to grayscale as a preprocessing step for image segmentation. The method utilizes characteristics of these images to reduce the amount of information lost during the conversion. It is a global mapping scheme and the same color does not map to two different grayscale values. Color information is added to the brightness information to reduce information loss and the weights for the added color are dependent on the distribution of the pixels in a particular image. The method was tested on a large set of histopathological slide images and convincing results were obtained some of which has been published in this paper.

Keywords: *Histopathological slides, Grayscaling, CIE Lab colorspace, Luminance and Hue*

1. Introduction

Histopathology refers to the microscopic examination of tissue in order to study the manifestations of disease. A tissue sample is extracted from the affected area and is placed on a glass slide. Often it is treated with some chemicals and stains to make features more distinguishable. This sample is then observed by a pathologist under a microscope to make a diagnosis.



(a) (b) (c)

Figure 1. (a) A Histopathological Slide Image, (b) Its Grayscale Image when Converted by Standard Method, (c) Result of Conversion by Proposed Method

* Corresponding Author

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In the case of digital histopathology, the tissue sample is digitally photographed and computerized techniques are used on the image to provide a degree of automation to the detection process. Image Processing techniques are widely used for processes like contrast enhancement, edge detection, segmentation, feature extraction, *etc*. Digital histopathology has several significant advantages over manual examination as is discussed later.

The digital image of the slide is usually a high resolution color image. Applying most advanced techniques for processing on these images requires a very high amount of computation. This is because color images are of the order $M * N * 3$ and each pixel is a color triple represented by three values. If this can be reduced to the order $M * N$ with each pixel consisting of a single value within an integer range, the computation for applying the techniques reduces drastically. This dimension reduction problem is in fact conversion of the color image to a grayscale image.

The general problem of conversion of a color image to a grayscale image has been subject to much research. Many such techniques strive to match human perception of the color and grayscale images as much as possible while conversion. The demand in digital histopathology is slightly different. The information loss needs to be minimized for a specific image under consideration. A new method has been proposed in this paper for conversion of color image of digital histopathological slide to its grayscale equivalent. Taking advantage of some observed properties in most histopathological images the technique adds a component calculated from the color information of the pixels to the brightness of the pixels to get the final grayscale value. This color component depends on the distribution of colors available in the specific image and the brightness component is same as the luminosity channel in the CIE Lab colorspace.

Section 2 of the paper discusses the processes involved in analysis of histopathological slide images. It further elaborates on the need of grayscaling and the general purpose and commercial methods available for the same. Section 3 reviews other existing work in grayscale conversion with different objectives. The proposed method in this paper is described in Section 4. The results of testing the method on a large set of histopathological slide images has been discussed in Section 5 and some results have been shown.

2. Background

The task of processing and sorting histopathological slides is a time consuming affair in its traditional form. With the advent of hi resolution cameras and powerful computers the digitization of these processes began in some form in the 1980s. Vast amount of research in the field of image processing has resulted in efficient algorithms and better image compression for storing the slides in a digitized form. As a result the storage of slides has become more cost effective and there has been another greater impact. Image processing techniques have been successfully used to gather a vast amount of information from the slides in an automated fashion thereby making the process faster and requiring much less number of valuable man-hours from doctors. The potential in this area is infinite as automation is being attempted ranging from classification of tumors to detection of diseases from blood samples.

After a tissue sample has been acquired it is mounted on a glass slide and then digitally photographed. This image forms the input for digital histopathology. Complete processing involves five main sub processes or steps. The first step is Image pre-processing. This step deals with preparing the image for further processing including the processes of denoising, removal of background image and finally image registration. Then the image is segmented into parts representing real objects using techniques like thresholding, edge detection and enhancement. The morphological characteristics of image for abnormality or classification of the image for different grades of the disease is done. The properties of

individual cells without considering spatial dependency between these cells are done in this step. Colour and texture information are obtained from the image which is combined in consideration with the distribution of components in cells and tissues which can be further segmented. After segmentation and feature selection the different classifiers are applied to classify images for diagnosing abnormality in them. In this step, a cell or tissue is assigned to one of the classes and then it can also be classified for malignancy level *i.e.*, gradation of disease.

The images of histopathological slides that are obtained are in colour. Usually a stain is used on the extracted tissue samples which show a particular coloration to a particular type of cell. This is then photographed. Obtained colour images are stored in form of pixels in a two dimensional space with three colour values associated with each pixel. So an image of width N pixels and height M pixels would be stored as 3 layers of $M * N$ matrices. Applying any algorithm on these images is a complex affair as many of these algorithms are not linear. So we attempt to convert the colour images to grayscale images. This reduction has some disadvantages but many advantages. There is a tradeoff between the resulting loss of information after conversion and the reduction of algorithmic complexity on conversion.

There are several characteristics of histopathological images which can be exploited to make sure the loss of information is minimized in the process of grayscale conversion. Firstly most histopathalogical slides contain tissues stained by a dye. This usually produces varying intensities centered on a single colour for an overwhelming majority of pixels in the image. Secondly this also implies that a large number of possible colours in the color space are never used in these images. Thirdly for human perception, contrast in terms of intensity plays a much greater role than the contrast in color. So, varying intensities need to be considered more than finely varying colours. In this paper we propose a technique to convert color images into grayscale images exploiting the above characteristics and thereby minimizing loss of meaningful information in the process.

There are several existing algorithms for conversion of colour images to grayscale in general. We consider the RGB and CIE LAB color spaces in our discussion. One common method is to average the RGB intensity values to produce a single gray value. This method is quite inefficient as multiple sets of linear intensities can map to the same gray scale value.

$$G = (R + G + B)/3$$

The triple (20, 60, 40) maps to 40. Similarly (30, 50, 40) also maps to 40. Thus different colors map to exact same colors in the resultant gray image which leads to a massive loss in information. Another method is desaturation. In this method the image is first converted to HSV color space then the saturation value for every pixel is set to 0. This too produces a result that is unsatisfactory. Another method used is the Euclidean distance method which is rather computation intensive. The grayscale value is given by

$$G = \sqrt{(R - G)^2 + (R - B)^2 + (G - B)^2}$$

The most standard method used today uses the following equation to convert RGB to grayscale

$$G = 0.2126 * R + 0.7152 * G + 0.0722 * B$$

The above weights are obtained empirically and are proportional to the sensitivity of the human eye to each of the trichromat colours. The human eye is most sensitive to green while it is least sensitive to blue. Although this method produces excellent results over images in general it is not the most effective method in conversion of histopathological images. One key issue is the fact that if an image contains mainly shades of magenta then the varying colors are majorly the R and B values. The G values might vary much less in comparison. But the low weights assigned to B values and to a lesser extent to R values

result in low importance to fine variations in magenta in the original image. This may be critical in further processing as the diagnosis maybe entirely dependent on magnifying the minute differences in the shades of magenta.

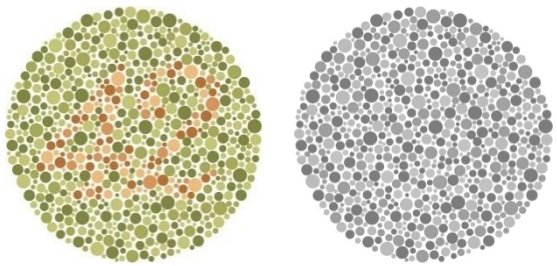


Figure 2. Image with Indistinguishable Luminosity

The resulting luminosity might not even have any distinguishable features following the above methods in some cases. Figure shows an image often used to test colorblindness. The image when converted to grayscale by conventional method shows a complete loss of variation of colors.

Addressing these issues on more general grounds a large amount of research has been conducted. Some of the papers published in this respect are reviewed in this paper.

The original problem of conversion of general color to grayscale is a problem of color theory. Several papers suggesting a solution has been presented on the same both from the color theory point of view as well as the application of the process in image processing and video processing. There have been several global and local mapping schemes. On the other hand there are several linear and nonlinear conversion schemes as well. Many papers have used CIE Lab color space as it is device independent. Some of the earliest works include the incorporation of the Helmholtz-Kohlrausch Effect which states that the perceived lightness of a stimulus increases as the chroma increases. Nayatani [2] proposed a Helmholtz-Kohlrausch Effect lightness predictor in the form of

$$L^{H\text{-}K} = L + L\, f(\theta)\mathrm{S}$$

where Θ is the hue angle and S is the saturation. According to Fairchild and Pirotta [1], a modified lightness measure L** is used to model the H-K effect by the equation

$$L^{**} = L^{*} + 0.143\, C^{*}$$

Among the local mapping schemes, Bala and Eschbach [3] proposed a technique where the high frequency color information is fed back into the system through a high pass filter and added to the converted grayscale values. Since the color information is obtained from a spatial neighborhood local features are enhanced in this technique. However same colored pixels in the image at different locations can map to different grayscale values based on the neighborhood. Neumann *et. al.,* [5] computed a gradient field over the color and luminance spaces. The max of the color and luminance was used which may produce several inconsistencies. A gradient inconsistency removal algorithm was proposed and the modified gradient field is integrated with the source to compute the final image. Smith *et. al*., [10] proposed a method in which the local chromatic contrasts are enhanced to improve the image perceptually. The image was decomposed into several frequency channels and then the weights for these channels are adjusted to compute the final grayscale. Local features are preserved and enhanced using these local mapping methods but some areas of solid single color may get distorted. Also most of the methods are nonlinear in nature. As the schemes are local, same colored pixels in different parts of the images can map to different grayscale values in the final image and also different colored pixels may end up having the same grayscale shade. In the context of medical image processing this cannot be allowed as preservation of relative differences is critical. Perceptual enhancement is not of primary concern.

The other major class of techniques involves global mapping schemes. Bala and Brawn [4] proposed a method to convert input colors into gray levels that are spaced according to their 3D distances in the color space. However this method is only useful for images with a few colors and its application lies in some business graphics, *etc*. It cannot be used in real images containing numerous colors. Gooch *et. al.,* [12] computed the color difference between all pairs of pixels and this relative difference was preserved in the resultant grayscale image. This was done by iteratively adjusting the gray value at each pixel to minimize an objective function. Rasche *et. al.,* [9] used a similar technique except instead of using all pairs of pixels all pairs of color values in the image were used. These methods are constrained multidimensional scaling methods. They are highly computation intensive. But they do produce good results and are technically sound. Rasche *et. al.,* [8] in another proposal first projects the colors on a linear axis preserving the relative distances and a linear mapping technique is used to preserve local features.

Other linear techniques include applying fixed weights to the chrominance and luminance information. If a fixed amount of chrominance is added to the luminance then a uniform contribution can be achieved but this may lead to feature loss. Grundland and Dodgson [6] proposed a technique where this weight for the chrominance component can be constrained to maintain relative ordering of colors. However in some images this technique may produce a large amount of feature loss. Kim *et. al.,* [11] used a non-linear global mapping method that addressed some of the issues present in the previous papers. In this method the difference between the color and grayscale gradients of the image is minimized and a nonlinear mapping is used to optimize the result.

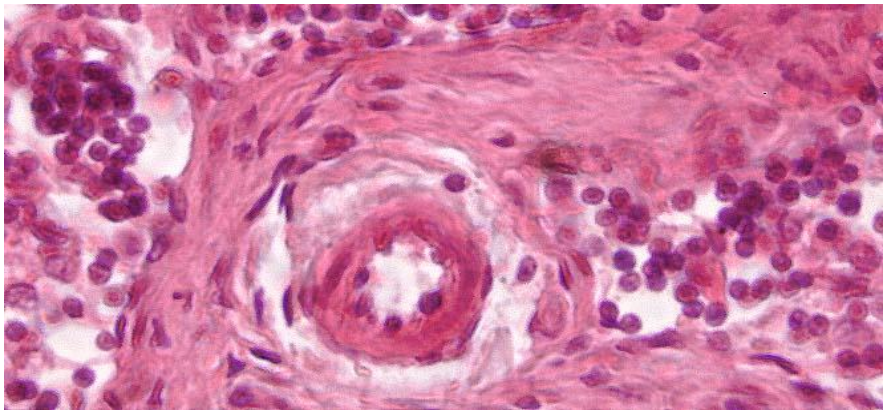
In context with medical image processing it is imperative that a global mapping scheme be used as it is critical that same colors in the color image do not map to different grayscale values as this can result in loss of vital information.

3. Proposed Method

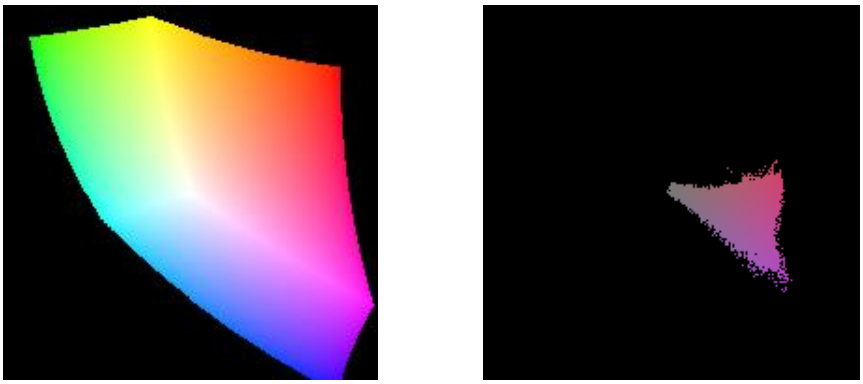
In this paper the CIELab colorspace has been considered to propose a method to convert color image to grayscale. The a and b components of the Lab color space indicate 2D coordinate system. The converted polar coordinate components are given as C and Θ.

$$C = \sqrt{a^2 + b^2} \quad (1)$$

$$\theta = \tan^{-1}(b/a) \quad (2)$$



(a)



(b) (c)

Figure 3. (a) A Histopathological Slide Image, (b) RGB Plot and (c) Plot for Image in (a)

When each color possible RGB color is plotted in a 2D plot of a and b with luminosity component 50 we get the representation of the colorspace given in Figure 3(b). Compared to this, for most histopathological slides, it is found a small cluster area is covered when every color in that image is plotted in a similar method. Here different Θ values indicate different hues and the value of C is a measure of how colorful that pixel is. It is assumed that the difference in hue is the major contributor in terms of color in the final result. The aim is to find the relative contribution of each hue in the given image with respect to the spectrum of hues present in that specific image.

A histogram is plotted where the number of pixels in a given image for every Θ quantized at 0.1 degree is plotted. The histogram for Figure 3. (a) has been given below.

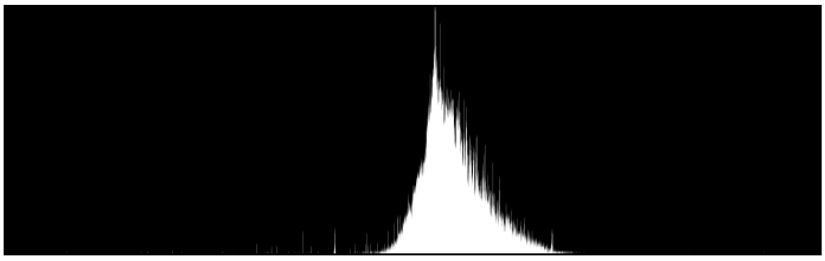


Figure 4. Histogram Hue vs. Number of Pixel

A histogram smoothing is performed by replacing each frequency with the average of itself and its immediate neighbors. This removed irregularities and provides more uniformity in the curves of the histogram.

$$h(\theta_i) = \{h(\theta_{i-1}) + h(\theta_i) + h(\theta_{i+1})\}/3 \quad (3)$$

The upper and lower bounds of each section of the histogram are computed based on the density of pixels. A segment is defined by the portion between two consecutive local minima. In this context this minima is defined as points where the value is lower than either neighbor. $H(\theta_i)$ is a minima if

$$h(\theta_{i-1}) > h(\theta_i) \,\&\, h(\theta_{i+1}) > h(\theta_i)$$

In the histogram given in figure only one such section may be obtained. For each section a value of hue Θ_{med} is found for which the number of pixels with that hue value is highest. In a smoothed histogram this is more likely to be maxima for a larger

neighborhood. The value Θ_{med} is used in the original histogram for further computation. A hue contribution factor $f(\theta)$ is calculated for every Θ where

$$f(\theta) = \Delta\theta/\Delta P \quad (4)$$

where $\Delta\Theta = \Theta_{med} - \Theta$ and ΔP = normalized difference in number of pixels with hue Θ and the number of pixels with hue Θ_{med}.

This difference in hue, ΔΘ is to some degree similar to the color difference used but Gooch *et. al.*, [12]. The ratio $f(\theta)$ is small when ΔΘ is small *i.e.*, the difference in color is small but is also largely dependent on ΔP. If ΔP is large it implies very few pixels are present with a Θ value and hence their contribution is less. A small ΔP implies the number of pixels are comparable and hence the factor $f(\theta)$ increases which is essentially contract enhancement. The sign of $f(\theta)$ is also important to provide a sense of ordering to the variation of hues.

This factor is multiplied to a constant which is computed as a portion of the range of grayscale values. This constant is used to give a fixed weight to the color information as compared to L channel information. For simplicity the constant has been taken as half of the maximum grayscale value.

$$f(\theta)' = f(\theta) * k, where\ k = (256/2) - 1 = 127 \quad (5)$$

Considering the contribution of color to be dependent on the colorfulness of the pixel and the L component of the pixel a proportional value is added. This follows the basic form used by Kim *et. al.*, [11]

We compute the grayscale value, G as

$$G = (L + f(\theta)' * C * L)/(100 * 255) \quad (6)$$

The domain of G is scaled down to a desired 0-255 range to get the final values. In case of multiple segments, if there are N segments in the histogram we compute these G for each segment. The final scale (*e.g.*, 255) is subdivided into N sections the sizes of which are proportional to the number of pixels in each segment. For two segments of size 30% and 70% a scale of 255 is divided into S1 [0 - 77] and S2 [78 - 255]. Then the G values for S1 are scaled to [0, 77] while the G values S2 maps to [78, 255]. The ordering of the segments is preserved as per the original histogram. The final grayscale values provided a measure of color ordering and provide more information than the standard grayscaling methods.

Obtained values for the segments:

$$G(S_1) = \{[0,255]\}$$

$$G(S_2) = \{[0,255]\}$$

The final grayscale is subdivided in sections: G = {[0,77],[78,255]}. If $G_{i,j}$ is the computed grayscale value for all pixels (i,j) in the image then the final values are computed as

$$G = (G_{i,j}/255) * 77 \qquad \forall\ G_{i,j} \in S_1$$

$$G = (G_{i,j}/255) * (255 - 78) + 78 \qquad \forall\ G_{i,j} \in S_2$$

The general formula for each segment is given by

$$G_n = (G / U) * (U_n - L_n) + L_n \quad (7)$$

Where G is the originally computed grayscale value, U is the upper bound for originally computed grayscale values, U_n and L_n are the upper and lower bounds respectively of the subdomain n.

4. Results

The proposed algorithm was implemented using C# and tested with more than 300 histopathological slide samples. Further tests were conducted on images used by other grayscale conversion algorithms and some of the results have been compared. In RGB to CIE Lab conversion D65 reference white was used.

Figure 5 compare the results of the proposed method against the CIE Y channel grayscale commercially used. In Figure 5(a) a color swatch was used where there were variations in hue and saturation but mild variation in luminosity. Figure 5(b) shows the comparative results for a histopathological image of cells. 5(c) is the result obtained when the image in Figure 2 is converted by this method. The color ordering is very evident here as the different number of colors in the image is very low.

Figure 7 shows another set of images from histopathology where (a) is the original image (b) shows the general conversion and (c) is the conversion by the proposed method.

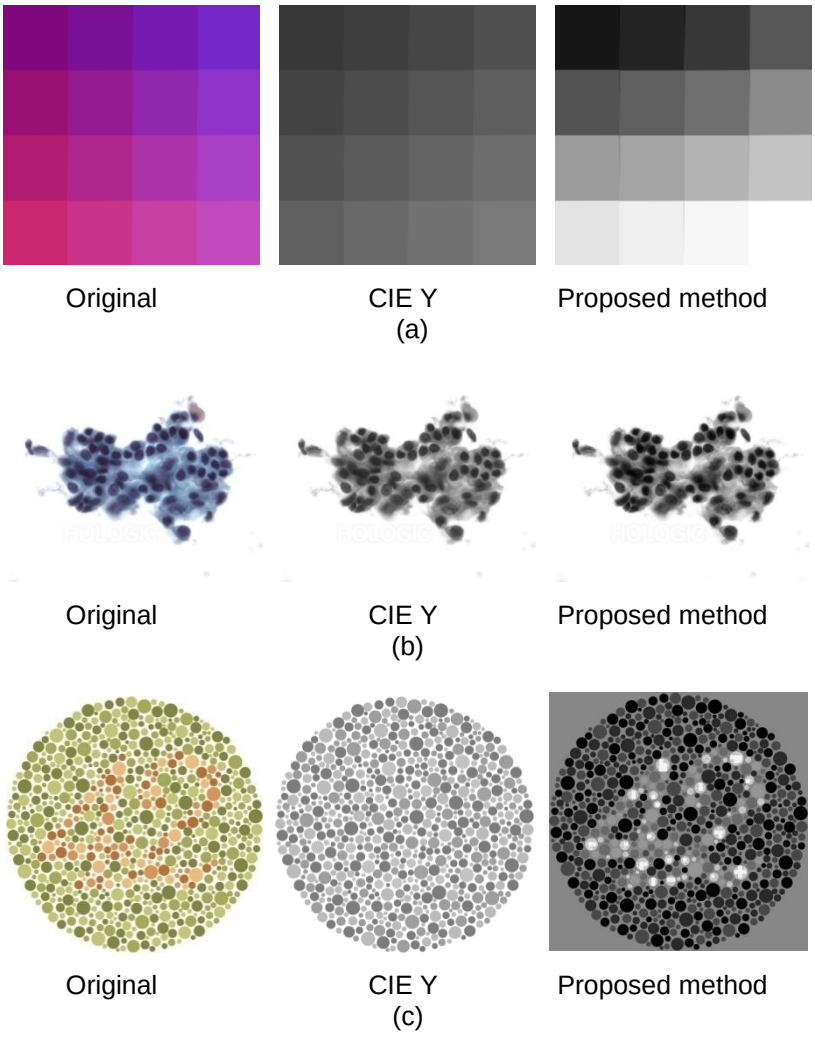


Figure 5. Obtained Results (a) a Color Swatch, (b) a Histopathological Image of Cells and (c) an Image with Indistinguishable Luminosity

Further tests were conducted to calculate other information metrics of the converted grayscale. The following information was obtained pertaining to Entropy of the images.

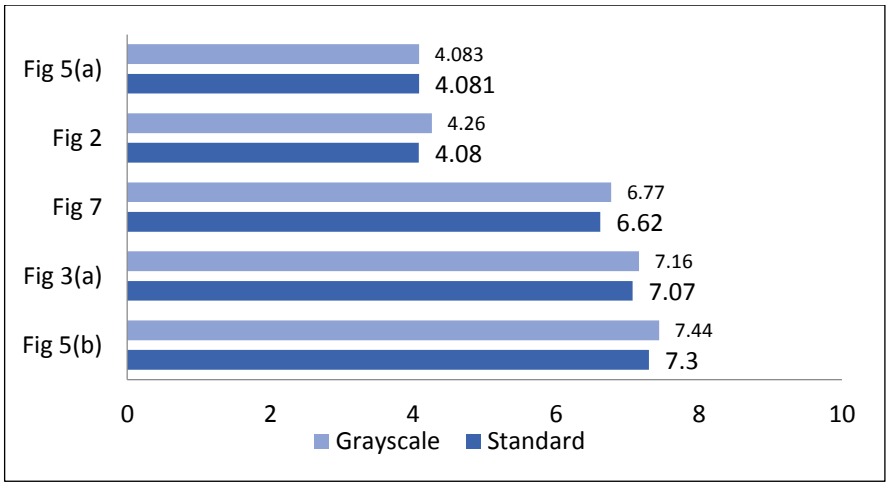


Figure 6. Entropy of Converted Images

5. Discussions

The output of the algorithm is not always perceptually sound. Brighter colors do not necessarily look brighter or relative color brightness is not always preserved. However it does increase the contrast of the image and assigns distinct color levels to wide variation in colors. This can be considered a preprocessing step in terms of the separation of colors before image segmentation. It has been found to be useful as a preprocessing step for histopathological slide images. The contribution of the color and the range of grayscale values is not limited to a 0-255 range and can be expanded to an image with higher range.

There are limitations to this algorithm as it cannot handle images with colors spread over a very large spectrum. It works with an assumption that variation in color in a limited color domain is a decisive factor in isolating objects for the given image. The relative ordering of colors is also not always preserved if two major colors overlap in the histogram segment. However, even in those cases, the increase in contrast is evident. In case of images compressed in a lossy format artifacts may creep into results as nearer colors do not always map to nearby grayscale values. In medical image processing the images required are usually very high resolution and this problem is usually not a very pronounced one.

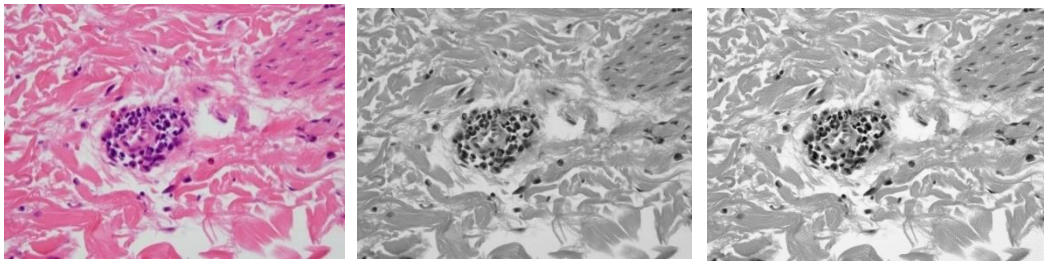


Figure 7. (a) Histopathological Slide Image, (b) CIE Y Channel Converted Value and (c) Proposed Method Result

The algorithm can also become dependent upon a large amount of background color and so further work is possible to not consider those portions while calculating the color component.

6. Conclusion

The conversion from color to grayscale is a dimension reduction problem. Thus loss of information one way or another cannot be avoided. However many algorithms for

processes like edge detection and image segmentations become very complex and time consuming when performed on color images. To reduce this complexity the conversion becomes necessity. So there is a tradeoff between information loss and reduction of complexity. Once it is considered that complexity must be reduced, loss of information becomes inevitable. This paper attempts to minimize that information loss given certain conditions and characteristics and also enhances low contrast areas in the process. Further work is possible in this area and this method is by no means an all-encompassing one.

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Authors

Jashojit Mukherjee, Jashojit Mukherjee is currently pursuing his M.Tech at International Institute of Information Technology, Hyderabad, He passed M.Sc. in Computer and Information Science from University of Calcutta (CU) in 2013 after graduating from St. Xavier's College, Kolkata. He worked under Prof. Kashi Nath Dey, CU in the field of Medical Image Processing, face recognition and stereovision.

Dr. Indra K. Maitra, He is Ph.D. in Computer Science from University of Calcutta and working as Sr. System Analyst at B. P. Poddar Institute of Management and Technology. He obtained Master in Computer Application (MCA) in the year 2002 from St. Xavier's College under IGNOU. He received award in 96th Indian Science Congress, 2009 at Shillong for the Best Poster in Computer Science. He is author of two books and more than 30 publications in National and International Journal and Conference. His working areas of specialization are Image Processing, Network Security, Data Structure, Programming Language, Computer Organization and Architecture, *etc*. He is now doing research in the Govt. of India funded project in the field of Biomedical Image Analysis and CAD.

Prof. Kashi Nath Dey, He is a senior faculty in the Department of Computer Science at the University of Calcutta. He received his Master's degree in applied mathematics and M.Tech from the University of Calcutta. He has also worked as a Systems Analyst in Digisoft, Vadodara; MMC, Kolkata; and DPS (India), Kolkata. His areas of research are optical computing, soft computing approaches in problem solving, and optimization theory.

Prof. Samir K. Bandyopadhyay, He is Professor of Computer Science and Engineering, University of Calcutta, India. He obtained his Ph.D. in Computer Science and Engineering in 1989 from University of Calcutta, M.Tech in Radio-Physics and Electronics in 1979 from University of Calcutta and B.E. in Electronics and Tele-Communication in 1975 from B.E. College, University of Calcutta. He is the Chairman of Science and Engineering Research Support Society (SERSC, Indian Part), Fellow of Computer Society of India, Sectional President of ICT of Indian Science Congress Association, 2008–2009, Senior Member of IEEE, Member of ACM, Fellow of Institution of Engineers (India), Fellow of Institution of Mammographic Density Estimation and Classification Electronics and Tele-Communication Engineering, India, Reviewer of International Journals IEEE Transactions on Neural Networks, ACM and Springer Publications. His fields of specialization are Bio-medical Engineering, Mobile Computing, Pattern Recognition, Graph Theory, Image Processing, Handwritten Signature Verification, Graphical Password Verification, *etc*. He has 25 years of Teaching and Research experience in the Post-graduate and under-graduate studies. He published books like Data structure Using C, Addison Wesley, 2003; C Language, Pearson Publication, 2010. He is author of more than 150 publications in National and International Journals and Conferences.

Prof. Debnath Bhattacharyya, M.tech., Ph.D. Tech., CSE), currently associated with Bharati Vidyapeeth University College of Engineering, Pune. Dr. Bhattacharyya published 170 Research Papers in International Journals and Conferences. He published 6 text books in Computer Science. His Research interests include Data Hiding, Pattern Recognition and Image Processing.

Prof. Tai-hoon Kim, received B.E., and M.E., degrees from Sungkyunkwan University in Korea and and Ph.D. degrees from University of Bristol in UK and University of Tasmania in Australia. Now he is working for Department of Convergence Security, Sungshin W. University, Korea. His main research areas are security engineering for IT products, IT systems, development processes, and operational environments. He published 350 Research Papers in International Journals and Conferences. He also published 12 Text Books in Computer Science and Security Engineering. His Research Interests include Security Engineering, Image Processing, e-Learing and Pattern Recognition.