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Review

A study about color normalization methods for histopathology images

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ABSTRACT

Histopathology images are used for the diagnosis of the cancerous disease by the examination of tissue with the help of Whole Slide Imaging (WSI) scanner. A decision support system works well by the analysis of the histopathology images but a lot of problems arise in its decision. Color variation in the histopathology images is occurring due to use of the different scanner, use of various equipments, different stain coloring and reactivity from a different manufacturer. In this paper, detailed study and performance evaluation of color normalization methods on histopathology image datasets are presented. Color normalization of the source image by transferring the mean color of the target image in the source image and also to separate stain present in the source image. Stain separation and color normalization of the histopathology images can be helped for both pathology and computerized decision support system. Quality performances of different color normalization methods are evaluated and compared in terms of quaternion structure similarity index matrix (QSSIM), structure similarity index matrix (SSIM) and Pearson correlation coefficient (PCC) on various histopathology image datasets. Our experimental analysis suggests that structure-preserving color normalization (SPCN) provides better qualitatively and qualitatively results in comparison to the all the presented methods for breast and colorectal cancer histopathology image datasets.

1. Introduction

Histopathology refers to the pictorial examination of tissue to study the cancerous disease under the microscope and Histology is the visualization of plant and animal tissue under the microscope. Histopathology is defined as the study of change/variation in the tissue caused by the disease. Digital histopathology (Gurcan et al., 2009) is a new field for research where color normalization methods, segmentation methods, feature extraction of histopathology images and classification methods are exploited and make the computer to understand histopathology images for the diagnosis purpose. Histopathology is used in clinical medicine where it involves the examination of tissue removed from the patient for a detailed study. In histology, histologist prepares slide and find out the cell or tissue caused by disease or not. If the cells or tissues are affected by the disease, then the diagnosis of histology is said to be histopathology. Histopathology is a research area. In some area, research was less, and there will be the better opportunity to the work. Usually, in color normalization of H&E histopathology images.

The tissue under the microscope looks like transparent that's why we use stain or dye usually appear in different color. For the analysis of tissue sample, we will treat tissue sample as a stain. The majority of the stains present in the images are absorbed light if there is no stain present in the image then the entire light passes through and appears bright white (McCann, 2015). The area present near stains looks darker in comparison to the area where no stain will present. Color variation in histopathology images causes problems. In histopathology image preparation, the image is stained by stain or dye usually appears in a different color. If we process the images without preprocessing, the result obtained may be undergoing incorrect diagnosis. So, to decrease the outcome of color variations present in the histopathology images. We convert RGB images into the grey scale images. But in gray level, a lot of information is lost, and then we go to another normalization technique. Accordingly, there are various methods present in the color normalization of histopathology images to reduce the effect of color variation by the transformation of RGB color images into grayscale. Cell nuclei are present in the histopathology image looks shadowy under some stains due to the surrounding environment. We use segmentation, feature extraction and classification methods to categorize different types of nuclei present in the histopathology images. However, conversion from RGB to grayscale ignores a lot of information. Commonly, two or three different types of colored stains used for the diagnosis purpose. We use a mixer of stains is hematoxylin and eosin. The intensity of each pixel depends on the concentration of the stain or dye

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present in the pixel.

Color variation in histopathology image is due to the use of different scanner; during slide preparation, different equipment used, different stain coloring, activity from different manufacture and batches of stains. One of the ways to reduced color variation in histopathology image is that convert RGB image into the grayscale, but in grayscale, some of the information is lost. So, we have to go color normalization techniques. Color normalization is the process where we do the mean color transformation from one image to another image. There are the various algorithms for the color normalization of histopathology image like histogram specification, Reinhard method, macenko method, stain color descriptor (SCD), complete color normalization and structure preserving color normalization (SPCN) and other recent color normalization methods are explored in the literature. The details about these color normalization methods have presented in section 2.

The remaining part of this research manuscript is organized as follows: literature survey, regarding the color normalization of histopathology images, is presented in section 2. In Section 3, the material and methods are presented. Section 4 shows simulation results and discussions, concluding remark is given in section 5 and implementation steps of various color normalization methods is given in appendix.

2. Literature Review

In the past, researchers mainly focused on color normalization, and stain separation of histopathology images. There are three types of color normalization method which are (1) Global color normalization (Gurcan et al., 2009; Reinhard, 2001) (2) Color normalization after stain separation by supervised method (Khan et al., 2014) and (3) Color normalization after stain separation by unsupervised methods (Li and Plataniotis, 2015). It is important to notify that color normalization, and stain separation are an entirely different task. But separating stain before color normalization has a significant impact on the experimental results. In general, global color normalization is done by separating color and intensity information in space. But, the second and third types of color normalization method are done after separating the stains by the supervised method and unsupervised method respectively. The supervised method requires some prior information in the form of the training set, and therefore computational complexity is much higher in this method. On the other hand, an unsupervised, supervised method doesn't require any prior information or training set, and it can directly decompose the stain Matrices by some orthogonal linear transformation method. Consequently, its computational complexity is very much lesser than supervised method. A summary of various color normalization methods for histopathology image is presented in Table 1.

3. Materials and methods

This section presents materials and method related to color normalization of histopathology images. In this paper, we have presented the simulation of different color normalization methods on different datasets such as breast, kidney and colorectal cancer which are taken from publically available sources and liver cancer database is provided from the Kasturba Medical College (KMC) hospital, Mangalore, Karnataka, India. These datasets are also used for results comparison of various color normalization methods. The detailed steps for the preparation of histopathology slide is described in McCann and Ozolek (2014).

3.1. Histopathology image datasets

For the conducting experiment, 80 different test histopathology images are considered for results analysis from liver cancer, breast cancer, kidney cancer and colorectal cancer histopathology image datasets. The details about these histopathology image datasets are given below.

1) **Liver Cancer Dataset:** Liver cancer dataset is provided from the Kasturba Medical College (KMC) hospital, Mangalore, Karnataka, India and it is not publically available database of liver cancer. This database consists of 200 numbers of images or slides and each slide is stained by standard dye such as Hematoxyline and Eosin. Each slide consists of several frames at 10x, 20x and 40x magnification inside the tumors of Liver. Each frame consists of 42 numbers of images for 10x frames, 48 numbers of images of 20x frames and 110 numbers of images of 40x frame and these slides are scanned by the Olympus DP 22 scanner. Dimensions of 10x, 20x and 40x are same such as 1920×1440 pixels and resolution for 10x, 20x and 40x are same that is 72dpi horizontal and 72dpi vertical. File format for 10x, 20x and 40x magnification are uncompressed. The frames are in RGB bitmap image in TIFF format.
2) **Breast Cancer Dataset:** MITOS-ATYPIA-14 dataset is accessed from the Pathology Informatics *web-source:* https://mitos-atypia-14.grand-challenge.org/dataset/ and it is a publicly available dataset of breast cancer (Roux and Racoceanue, 2013). This dataset consists of 1302 number of image or slides and each slide are stained by standard dye such as hematoxyline and eosin. These slides are scanned by two scanners which are (1) Aperio Scanscope XT and (2) Hamamatsu Nanozoomer 2.0-HT. This dataset consist of 651 number of slide scanned by Aperio Scanscope XT and another 651 number of slide scanned by Hamamatsu Nanozoomer 2.0-HT. Each slide consists of several frames at 10x, 20x and 40x magnification inside the tumours. 20x and 40x frames are used for the scoring nuclear atypia and to annotate mitosis. 40x frames gave a score related to six nuclear atypia. Dimensions of 10x and 20x are same such as 1539×1376 pixels and resolution for 10x and 20x are also same that is 1dpi horizontal and 1dpi vertical. Dimension of 40x magnification is 1539×1376 pixels but the resolutions are different such as 96dpi for horizontal and 96dpi for vertical. File format for 10x and 20x magnification are compressed but file format for 40x magnifications is uncompressed. The frames are in RGB bitmap image in TIFF format.
3) **Kidney Cancer Dataset:** CC-RCC dataset is accessed from the *web-source:* http://michalkruk.pl/Images.zip*)* and is created by Military Institute of Medicine, Warsaw, Poland (Kruk and Kurek, 2017). The slide is taken from neoplasm cells a part of kidney and it is stained by the standard dye such as Hematoxyline and Eosin. CC-RCC dataset consists of 400x magnification and it is scanned by an Olympus BX-61 microscope and Olympus DP-72 camera. The slides are in RGB bitmap image in TIFF format with dimension 2070×1548 pixels. The Horizontal and Vertical resolution of the slide is 200dpi. The file format of 400x magnification is uncompressed.
4) **Colorectal Cancer Dataset:** Warwick QU Dataset is accessed from the *web-source:* https://warwick.ac.uk/fac/sci/dcs/research/tia/glascontest/download/*)* and it is used for the color normalization method (Sirinukunwattana, 2015). This dataset is suitable for the preprocessing. Warwick QU dataset is scanned by the Zeiss MIRAX MIDI scanner and its file format is bmp. Warwick QU Dataset contains 20x ($0.62005 \mu m$/pixels) magnified H&E stained image with resolution 775×522 of colorectal cancer. There are 165 numbers of images; it contains 85 training dataset and 80 test dataset. In 85 training datasets, 37 are the benign tumor and 48 are the malignant tumor and in 80 test datasets, it contains 37 benign tumors and 43 malignant tumors.

3.2. State-of- the-art color normalization methods

There are three types of color normalization method which are (1) Global color normalization (e.g. Histogram specification, Reinhard method) (2) Color normalization after stain separation by supervised method and (3) Color normalization after stain separation by unsupervised methods.

A. Global Color Normalization

Table 1
Summary of different color normalization methods for histopathology images.

Approach	Method	Advantages	Limitations
Histogram Specification Gonzalez and Woods, 2002	• Convert the image from RGB to $l\alpha\beta$space. • Map source image histogram with target image histogram. • Convert the image from $l\alpha\beta$to RGB space.	• Both brightness and color statistics of processed image are like target image.	• For contrast stretching it follows GHE which is an unnatural process • All source information is not preserved in processed image. • Not applicable if source image and target image have very much dis-similar statistics.
Color transfer algorithm Reinhard, 2001	• Convert the image from RGB to $l\alpha\beta$space. • Transfer the background color from target to source image. • Convert the image from $l\alpha\beta$to RGB space.	• It preserves the structure of source image • Contrast of processed image is approximately same as contrast of target image	• It does the transformation in $l\alpha\beta$space in which stains are not properly separated.
Color Deconvolution method Ruifrok and Johnston, 2001	• Convert the image from RGB to OD space. • Stain color appearance matrix (S) was empirically found by measuring the relative color proportion for R, G and B channel with only single stain. • Stain depth matrix was estimated by taking inverse of S, multiplied with intensity values in OD.	• This was the first attempt to separate stains in a histology image, such that color proportions of H & E stains in each location can be estimated.	• This is a supervised method; its computation complexity is higher. • To estimate color appearance matrix some prior information (single stained slide) is needed. • Once color histogram structure is ruined by non-linear mixing of H & E stains, there is no way we can get back that original color which was before staining.
Spectral decomposition by NMF, ICA By Rabinovich and Agarwal, 2003	• Factorize stained images into color appearance matrix (S) and stain depth matrix (C) by NMF, where all co-efficient of S and C are positive. • Implement Joint Approximate Diagonalization of Eigenmetrices (JADE) to recover independent component for ICA decomposition.	• Unlike supervised method, it doesn't have to estimate all the color appearance co-efficient separately. • Unlike SVD it doesn't have negative co-efficient which has no physical interpretation.	• Solution of NMF is not closed. There is scaling ambiguity. • NMF doesn't say anything about image formation. • ICA assumes that each stain act separately (independently) in the image, which is wrong.
Fringing method By Macenko, 2009	• Convert the image from RGB to OD space. • Find SVD and create plane corresponding to its two largest singular values. • Project data onto that plane and find the corresponding angles. • Minimum and maximum angles are estimated by $\alpha^{th}$and $(100-\alpha)^{th}$percentile for robust estimation. • Those extreme values are converted back to OD space.	• Unlike NMF it doesn't have ambiguity in its solution. • It doesn't have negative co-efficient in color appearance matrix.	• Extreme angle values are evaluated empirically, thus it is not suitable for automatic cancer detection algorithm. • This algorithm doesn't preserve all information of source image which is unacceptable.
Blind color decomposition by Gavrilovic, 2013	• It separates the intensity information from color information by blind color decomposition. • The images are converted from RGB to Maxwellian Color space to know about the color distribution of separate stains. • Identify reference color vectors and by linear decomposition, estimate stain absorption vectors and use them to adjust color variation.	• It is an unsupervised method which implies that it doesn't require any prior information for decomposition or it doesn't need any training data. • It outperforms NMF and other previous supervised stain separation method.	• It doesnot preserves all the source intensity information. • It introduced some artifacts in the processed image.
Color standardization by color correction matrix Bautista et al., 2014	• The image is converted from non-linear RGB to linear RGB. • Color correction matrix is computed by least square error between target image and source image. • Color correction is done in linear RGB space followed by gamma correction. • Convert the image back to original RGB space.	• This algorithm is very easy to compute. Because, it does the transformation globally, not pixel by pixel.	• Least square error estimates all the differences between source image and color image in color space. Thus, it replaces all the color variations of source with target image as well as some new color artifact may appear in the processed image which is in fact color information of target image. • Although it does transformation in a linear RGB space, it doesn't separate the stains.
Wedge finding method by Cann et al., 2014	• It finds a wedge of pixel color values for H & E stained images where maximum and minimum values will be either purely Hematoxylin or purely Eosin. • It follows same stain separation method as Macenko did, except they assume that E-only images are having lower contrast (minimum value) than H-only images (maximum value).	• Assuming E-only images have minimum values and H-only images have maximum values, can reduce the solution space of general fringing method. • It doesn't have negative co-efficient in color appearance matrix.	• Extreme angle values are evaluated empirically. Thus, it is not suitable for CAD. • Its color normalization method doesn't preserve all the source color information. Because combining E only and H only images may not provide the exact H & E stained image.
SCD global method followed by RVM classification Khan et al., 2014	• Deriving Principle Color Histograms from a training set of quantized histograms and compute a global SCD image specific color descriptor. • Learning a supervised classification framework (RVM) to generate stain specific probability maps to find color of each stain separately.	• This is a unique supervised method which. • Works at pixel level and thus achieves a good result for stain separation.	• By employing a non-linear function in color normalization method, we can ruin the structure of original image which is producing undesirable artifacts in the image. • Once color histogram structure is ruined by non-linear mixing of H & E stains, there is

(*continued on next page*)

Table 1 (*continued*)

Approach	Method	Advantages	Limitations
	• A spline based non-linear function is employed to transfer color from target image to source image.		no way to get back the original image structure. • This is a supervised method in which computation complexity is very much higher.
Complete color normalization by Li and Plataniotis, 2015	• Illuminance matching method to adjust the illuminance variation due to imaging. • Spectral normalization method comprises of two steps: First is to employ SW statistics to reduce the solution space of NMF and then by NMF decompose the stains. • 2nd is color normalization in which stain depth matrix is preserved.	• Reduce the solution space of NMF by employing SW statistics which converts the image to saturated image or color appearance matrix is becoming diagonal. • In color normalization method, the structure of original image is preserved.	• Saturated weight (SW) statistics is not a natural process because it depends on the dominant color. • Contrast has not been enhanced for the fed images. • Although in color normalization method, it totally preserves the brightness intensity variation, it doesn't preserve all color information.
Sparse Stain Separation, Structure preserving color normalization by Vahadane, 2016	• Sparseness is added to the optimization problem to reduce the solution space of NMF. It is called Sparse NMF (SNMF) • The non-convex optimization problem is solved by block co-ordinate descent algorithm which is readily available at SPAMS. • A structure preserving color normalization algorithm is employed to preserve all the structure of source image.	• Solution space of NMF is totally reduced by SNMF. • It preserves all the structure of original image. • It outperforms all the previous supervised stain separation method.	• Although solution space of NMF is reduced by SNMF, its computation complexity is higher. • The solution of optimization problem may reach to local minima rather than global minima. • Its color normalization method doesn't preserve all color information of source image.
Centroid alignment, CLAHE by Tam et al., 2016	• It normalizes intensity range of image by centroid alignment. • CLAHE divide the image into blocks and map each block intensity histogram to the target histogram.	• By centroid alignment illuminance variation is corrected.	• Local histogram specification technique for color normalization doesn't work as it depends on the spatial dependency of pixels, thus it can replace the significant source image (color) statistics by the same of target image.
Whole-slide image color standardizer by Bejnordi, 2016	• HSD model transforms RGB data into two chromatic components and a density component. • Obtaining the chromatic and density distribution of hematoxylin, eosin and background. • Weighting the contribution of stain for every pixel. • Transform the HSD color model to RGB color.	• Detection of stain components in WSI has been done perfectly by using unsupervised method. • It preserves the spatial information which makes the algorithm robust.	• The processing time of WSICS is very high. • All the source information is not preserved such as red spot. • It removes the original background color of source image while doing color normalization.
ICA, wavelet decomposition by Alsubaie, 2017	• By wavelet transformation, the images are decomposed into wavelet sub bands where the source stains are becoming kind of independent. • Then by applying ICA decomposition independent components are separated.	• It is an unsupervised method in which computation complexity is very much less.	• The wavelet transform reduces redundancy in image pixels, but it doesn't make the source components really independent. • ICA is not applicable until the stains are exactly independent to each other.
Color normalization using cluster's centroid by Zarella et al., 2017	• Convert RGB color space to the HSV color space. • We perform clustering by first transferring pixel value into the Cartesian coordinate system. • Map each pixel in a cluster to the HSV value of the cluster's Centroid.	• It preserves the tissue structure of the original image. • Color variation is reduced in hue, saturation and value by a factor of 6 to16.	• Dominant color has been transferred from target image to the source image. • Some information is lost because of dominant color such as blue and pink are transferred or preserved. • Color normalization is depending on only two dominant colour blue and pink.
Stain normalization by using Sparse Autoencoders by Janowczyk et al.,2017	• Training of unsupervised deep SAE on randomely sub sampled patches. • Apply the learned filter on both the source and target image. • An unsupervised clustering approach is applied on source image to identify K cluster centers. • Histogram matching across clusters and color channel.	• Color variation is due to different scanner has been reduced. • Global normalization technique (GL and HS) perform poorly but if we combined GL,HS and StaNoSA technique it perform well.	• It scanned the same image multiple number of time so some of the information has been lost. • It reduced the mean and standard deviation by using GL and StaNoSA technique, it may cause wrong color normalization.
Stain normalization using discriminative model by Taieb and Hamarneh, 2017	• This method uses task-specific discriminative model. It include stain normalization component that perform non-linear mapping between the images with different distribution. • Encoder and decoder architecture are used for the stain transfer.	• It preserves all the content present in the original image that is low-level information related to texture. • It also preserves the structural information by using regularization loss.	• Color normalization does not yield expected result. • Normalized image is deviated from the target image. • During color normalization some of the data has been lost.
Stain normalization using generative adversarial network by Zanjani et al., 2018	• Convert the RGB image into the CIE Lab color space. • Generate colorized H&E image by using generator network. • Estimate the element of transformed H&E image noise at its output by using auxiliary network.	• It preserves the source intensity information. • It also preserves the brightness of the source image. • Contrast of the normalized image is increased.	• In CIE Lab color space, stain is not separated properly. • It fully relies on the color of the target image, not the mean color of the target image.

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Table 1 (*continued*)

Approach	Method	Advantages	Limitations
Deep-learning solution inspired by Cycle-Consistent Adversarial Networks by Shaban et al., 2018	• Distinguish the generated color image from the original image by using discriminator network. • Convert the CIE Lab color space into the RGB color space. • It use Unpaired Image-to-Image Translation using Cycle-Consistent Adversarial Networks. • Style-transfer for the classic stain normalization problem.	• It is used for Breast Cancer tumor classification with increase in 12% AUC. • High visual similarity to the target domain. • Removing the need for a manually picked reference template,	• Performance can be improved by Unified representation. • It is not suitable for many to many stain style domains.

Global color normalization is done after separating color and intensity information in $l\alpha\beta$ space by Principal Component Analysis (PCA) (Reinhard, 2001). Global color normalization is very much suitable for histopathology image, because autocorrelation coefficient or spatial dependency of pixel (intensity) values of histopathology images.

Histogram specification (Coltuc and Bolon, 2006; Gurcan et al., 2009) is a global color normalization method, in which source image histogram is mapped with target image histogram such that both brightness and color statistics of source image will be like target image. Histogram specification follows Global Histogram Enhancement method for contrast stretching which is a kind of unnatural process, to the best of our knowledge. Because it forcefully stretches the histogram of source image, until it will be approximately like target image histogram. Due to this unnaturalness, sometimes it may bring artifacts in the processed image. Reinhard (Reinhard, 2001), preferred another global color normalization method which transfers the mean color of the target image to the source image such that all the intensity variations of source image have been preserved and the contrast of the processed image will be approximately equal to contrast of the target image.

B. Stain Separation by Supervised Method

According to Beer's law, the color stains act linearly in Optical Density (OD) space, given in equation (1). Where V is the intensity in OD space, I is the intensity in RGB space, I_0 is the illuminating intensity incident on the sample (Ruifrok and Johnston, 2001). Thus, before stain separation, the source image must be transformed into OD space such that they act linearly.

$$V = \log\left(\frac{I_0}{I}\right) \tag{1}$$

A.C. Ruifrok and D.A. Johnston (Ruifrok and Johnston, 2001) have proposed a novel supervised Color Deconvolution (CD) method, in which stain color appearance matrix (S) was empirically determined by measuring the relative color proportion for R, G and B channel with only single stained (Hematoxylin or Eosin only) histopathology slides. After that stain depth matrix C can be easily evaluated by taking the inverse of S, which is further multiplied with OD space intensity (OD_C), given in equation (2). This is known as color deconvolution method, in which they have tried to get back the original image (before staining) from the H & E stained image.

$$C = (OD_C).\ S^{-1} \tag{2}$$

A.M. Khan et al. (Khan et al., 2014) proposed a novel Stain Color Descriptor (SCD) global method to find overall stain color. Furthermore, a supervised color classification method Relevance Vector Machine (RVM) has been employed to identify the locations where each stain is present. Color appearance matrix and stain depth matrix are then estimated from these set of classified pixels and after that a non-linear spline-based color normalization method is incorporated for transferring color from target image to processed image.

C. Stain Separation by Unsupervised Method

In unsupervised method, computation complexity is very much lesser than that of supervised method, since no training phase is required. Independent Component Analysis (ICA) and Non-negative Matrix Factorization (NMF) methods have been employed in (Alsubaie, 2017; Rabinovich and Agarwal, 2003). The main advantage of NMF is that unlike PCA it doesn't have any negative color co-efficient. NMF is an optimization technique which minimizes the distance between the source image and decomposed matrices (S and C), such that all co-efficient of color appearance matrix must be non-negative (i.e. $S_{i,j} \geq 0$ and $C_{i,j} \geq 0$). NMF method is having some problem with ambiguity and has no closed form of solution. Thus, it has to be computed numerically, which is not compatible in automatic cancer detection algorithm.

M. Macenko (Macenko, 2009) and M. McCann (McCann, 2014) both of them have employed same kind of stain separation method which is based on the fact that color of each pixel in histopathology image is nothing but a linear combination of two stain vectors, whereas the weightage of those vectors is non-negative. Thus, the weightage always lies between those two stain vectors (i.e. only Eosin and only Hematoxylin).

In complete color normalization method, X. Li and Plataniotis (Li and Plataniotis, 2015) have employed both illuminance normalization and spectral normalization. Spectral normalization method comprises of two parts I) NMF based spectral estimation, II) Spectral matching. Before applying NMF, a novel Saturation Weighted (SW) statistics method has been incorporated which smooth out Hue histograms and converted the image to a highly saturated image such that color appearance matrix is converging to a diagonal matrix. SW statistics can significantly reduce the solution space of NMF. However, to the best of our knowledge, SW statistics is not a natural process, which forcefully make the image saturated, thus it can bring some color artifacts in the original image.

Structure preserving color normalization method is recently proposed by A. Vahadane (Vahadane, 2016) which is comprised of two steps. I) Stain separation by Sparse NMF (SNMF), II) Structure Preserving Color Normalization (SPCN). In Sparse NMF, sparseness is added to the optimization equation of NMF to reduce the solution space of NMF. However, it increases the computation complexity significantly.

Moreover, their color normalization method preserves the structure of source image, in the processed image. But, it doesn't preserve all the color variation of the source image.

Andrew Janowczyk et al. (Janowczyk et al., 2017) proposed a novel color normalization method based on neural network. First similar types of tissues (e.g. stromal tissue, nuclei, lymphocytes etc.) of both source image and target image are partitioned using an unsupervised deep learning method, called sparse autoencoder. An iterative learning filters are produced in this method which can optimally reconstruct the original image. Furthermore, color is transferred tissue per tissue from target image to source image, by conventional histogram specification method. This method is more accurate than global color normalization method, since unlike global method it doesn't transfer the same color to all of the pixels in image. However, computation complexity of this method is significantly greater than other global normalization method.

The implementation steps of Histogram specification method (Gurcan et al., 2009; Coltuc and Bolon, 2006), Reinhard method (Reinhard, 2001), Macenko method (Macenko, 2009), Stain color descriptor (Khan et al., 2014), Complete color normalization (Li and Plataniotis, 2015), Structure preserving color normalization (SPCN) (Vahadane, 2016) are presented in appendix.

3.3. Quality evaluation metrics

Image quality is the parameter for the analysis of the different color normalization methods and it decide which is the best method for the color normalization of histopathology images. Image quality metrics such as Structural similarity index metric (SSIM) (Wang and Bovik, 2004), Quaternion structure similarity index metric (QSSIM) (Kolaman and Pecht, 2012) and Pearson correlation coefficient (PCC) (Wang and Bovik, 2002) are the automatic choice for quality analysis of histopathology images. The brief descriptions about these quality metrics are given in the further subsections.

A Structural similarity index metric (SSIM)

The structure of the natural image are highly correlated and there pixels are exhibit strong dependency and these dependencies carry a lot of information about the structure of the image. Structure similarity index metric (SSIM) (Wang and Bovik, 2004) is consisting of the three factors such as luminance, structural and contrast. The luminance is defined as the product of the illumination and the reflectance on the surface of image but the structure of the image is somewhat independent from the illumination. If we extract the structural information then we separate the influence of the illumination from the image. Structure similarity index metric (SSIM) satisfies the three parameters such as symmetry, boundedness and unique maximum.

After visualization, we can say that the human visualization system is highly agreed to the visual information like structural and luminance information from visual scenes. It compares three functions such as luminance, structural and contrasts between the source and processed image which are given in equation (3), (4) and (5) respectively.

a) Luminance can be defined as

$$l(x, y) = \frac{2\mu_x\mu_y + c_1}{\mu_x^2 + \mu_y^2 + c_1} \tag{3}$$

b) Contrast can be defined as

$$c(x, y) = \frac{2\sigma_x\sigma_y + c_2}{\sigma_x^2 + \sigma_y^2 + c_2} \tag{4}$$

c) Structure can be defined as

$$s(x, y) = \frac{\sigma_{xy} + c_3}{\sigma_x\sigma_y + c_3} \tag{5}$$

Where, μ_x and μ_y are the sample means of the source and processed image respectively. σ_x and σ_y are the standard deviations of the source and processed image respectively. σ_{xy} is the correlation coefficient between the source and processed image and c_1, c_2 and c_3 are the constant which is used to stabilize the SSIM when it is approaching to zero. These statistics are calculated within a local window.

Structure similarity index metric equation is derived after combining equations (3), (4) and (5) and is given in equation (6).

$$SSIM(x, y) = \left(\frac{2\mu_x\mu_y + c_1}{\mu_x^2 + \mu_y^2 + c_1}\right)\left(\frac{2\sigma_{xy} + c_2}{\sigma_x^2 + \sigma_y^2 + c_2}\right) \tag{6}$$

SSIM is used to measure the structural, luminance and contrast between the source and processed image and its index denotes the reference metric. In other words, image quality is measured or predicted based on an initial image or source image and the processed image. The numerical value of SSIM lies between 0 to1. The value closer to 1, better is the color normalization method.

B Quaternion Structural similarity index metric (QSSIM)

In SSIM (Wang and Bovik, 2004), we focus on the scalar cross correlation but in quaternion structure similarity index metric (QSSIM) (Kolaman and Pecht, 2012), we focus on both scalar and cross correlation that is basically color cross correlation this is the main reason to go through quaternion. QSSIM is related to vector correlation but SSIM is related to scalar correlation, that why SSIM is failed in measuring combined degradation. But by using quaternion, which shows quality difference between the images. QSSIM measures every type of changes between the color vectors with the help of cross and dot product. It measures luminance, chrominance, and combined degradation while SSIM measures only size but quaternion structure similarity index metric measures both size and direction. QSSIM is basically used to measure the quality of the image degradation by the combination of desaturation or blur and also execute single degradation such as blur, compression and noise. The numerical value of QSSIM lies between 0 to1. The value closer to 1, better is the color normalization method. The parameter QSSIM is mathematical defined in equation (7).

$$QSSIM_{ref,\deg} = \left|\left(\frac{2\mu_{qref}\mu_{q\deg}}{\mu_{qref}^2 + \mu_{q\deg}^2}\right)\left(\frac{\sigma_{qref,q\deg}}{\sigma_{qref}^2 + \sigma_{q\deg}^2}\right)\right| \tag{7}$$

Where, μ_{qref} and $\mu_{q\deg}$ are the sample mean and the normalized image. σ_{qref} and $\sigma_{q\deg}$ are the standard deviation of the source and the normalized image and $\sigma_{qref,q\deg}$ are the correlation coefficient between the two images.

C Pearson correlation coefficient (PCC)

It measures the linear correlation between the two images and its range from 0 to 1. A value of 0 indicates that there is no similarity between the two images (Wang and Bovik, 2002). A value greater than 0 means there is some correlation between the two images. Pearson correlation coefficient is mathematical defined in equation (8).

$$r_1 = \frac{\sum_i (x_i - \mu_x)(y_i - \mu_y)}{\sqrt{\sum_i (x_i - \mu_x)^2}\sqrt{\sum_i (y_i - \mu_y)^2}} \tag{8}$$

Where, x_i and y_i are the source and the processed image. μ_x and μ_y are the sample mean of the source and the processed image.

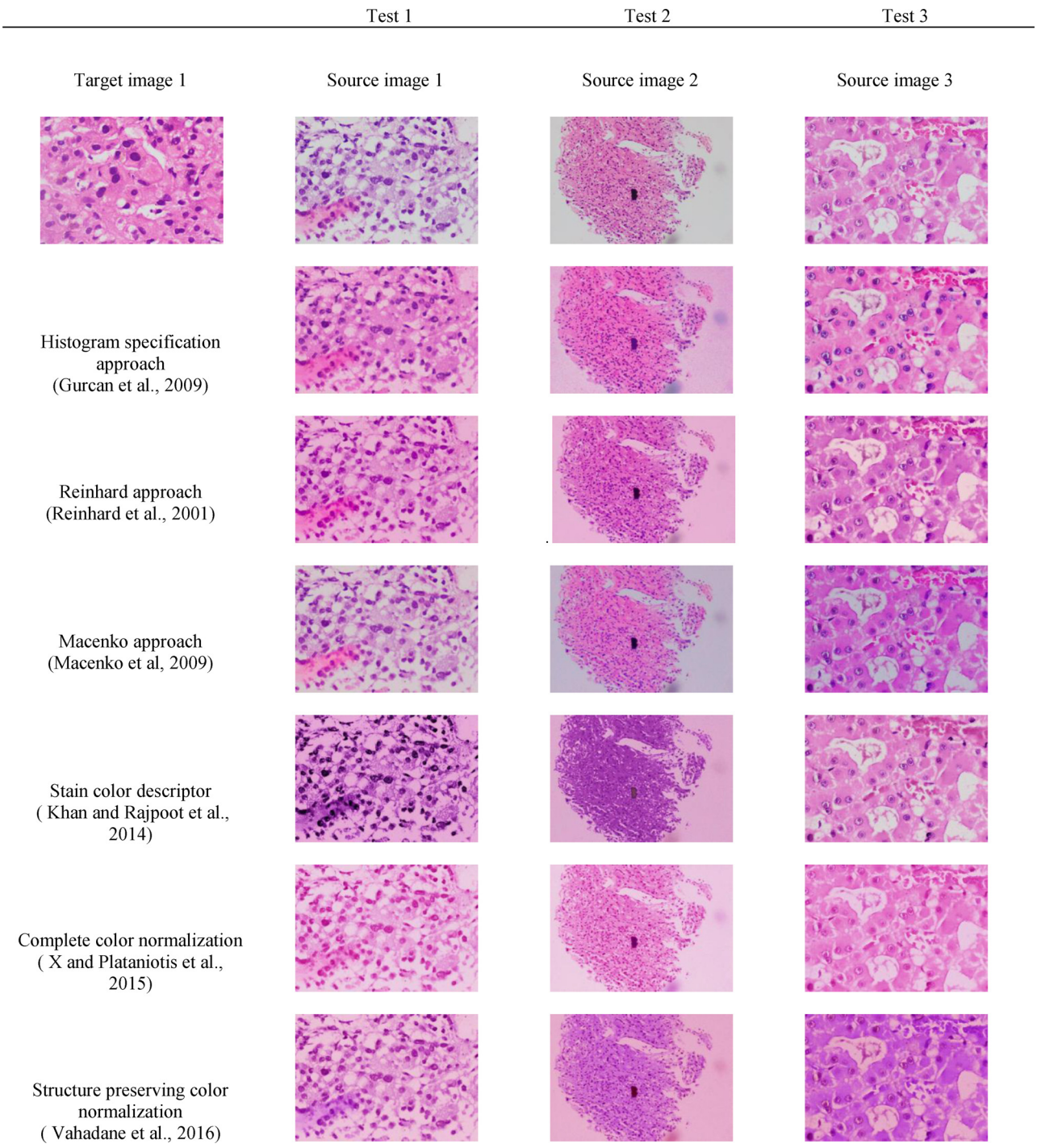

Fig. 1. Comparison of some of the color normalization techniques. The target and the source image are selected from the Liver cancer dataset. Image in the first column represent the normalized image for test 1. The normalized image of the second column represents to test 2 and so on.

4. Simulation results and discussuion

This section presents experimental results and analysis of state-of-the-art color normalization methods on 80 different histopathology images from liver cancer, breast cancer, kidney cancer, and colorectal cancer histopathology image datasets. We have evaluated and compared the qualitatively and quantitatively of results of Histogram specification method (Gurcan et al., 2009; Coltuc and Bolon, 2006), Reinhard method (Reinhard, 2001), Macenko method (Macenko, 2009), Stain color descriptor (Khan et al., 2014), Complete color normalization (Li and Plataniotis, 2015), Structure preserving color normalization (SPCN) (Vahadane, 2016). All the previous mentioned color normalization method were implemented and simulated with MATLAB 2015a running on an Intel® Core™ i3 PC with 2.1 GHz CPU and 8 GB RAM. For experimentation, test breast cancer, kidney cancer, and colorectal cancer histopathology images are procured from publically

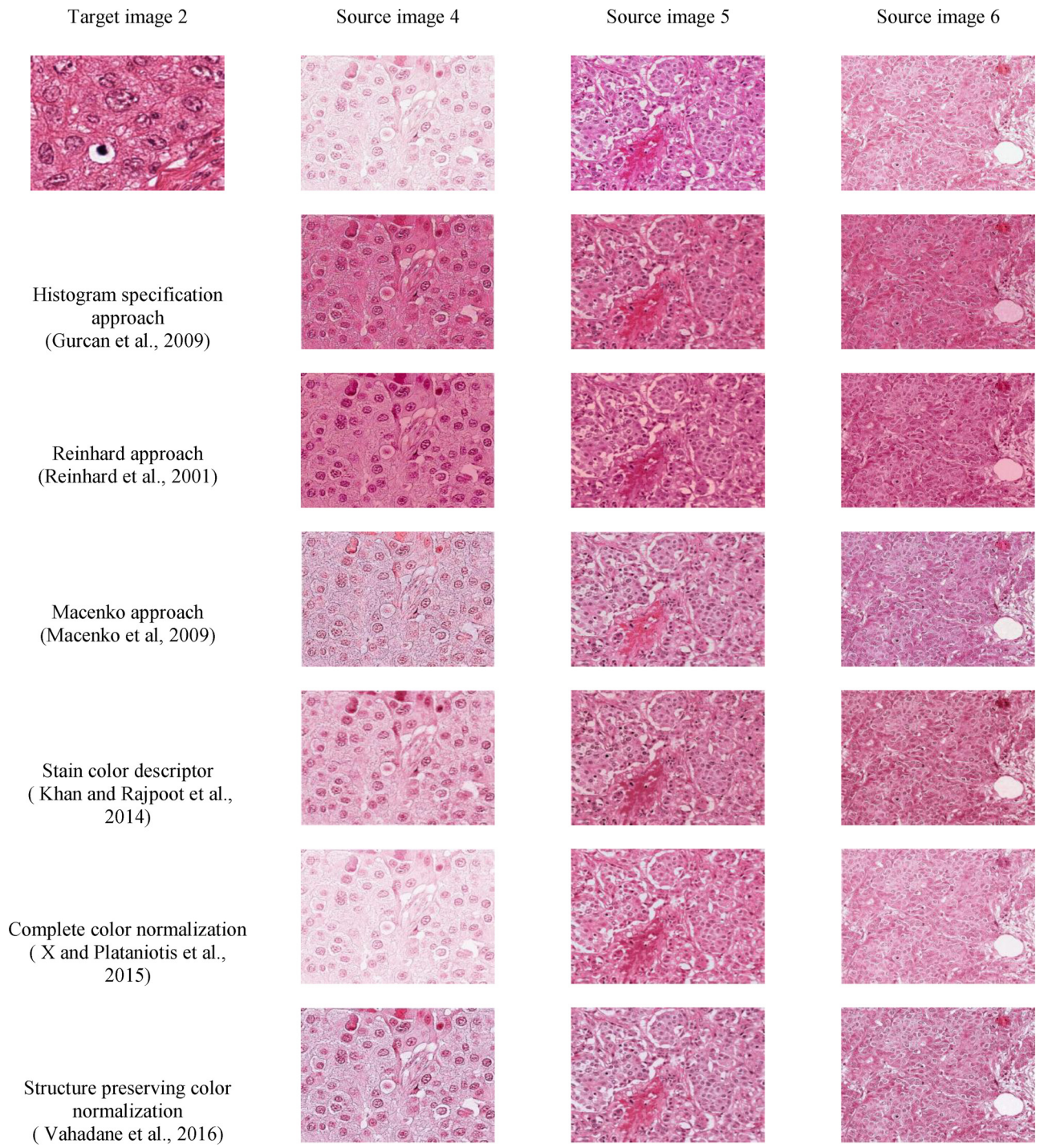

Fig. 2. Comparison of some of the color normalization techniques. The target and the source image are selected from the breast cancer dataset. Image in the first column represent the normalized image for test 1. The normalized image of the second column represents to test 2 and so on.

available databases (Roux and Racoceanue, 2013; Kruk and Kurek, 2017; Sirinukunwattana, 2015) respectively but test liver cancer dataset is not publically available. Qualitative experimental results of the state-of-the-art color normalization methods are given in Figs. 1–4 for liver cancer, breast cancer, kidney cancer, and colorectal cancer histopathology image datasets, respectively. For qualitative experimental results comparison, only 3-3 test histopathology images are presented from each datasets but each color normalization methods is tested on 80 different test histopathology images from liver cancer, breast cancer, kidney cancer, and colorectal cancer datasets.

From the Figs. 1–4, it is clear that in histogram specification method (Gurcan et al., 2009; Coltuc and Bolon, 2006), the processed image contrast is better than the source image. This approach does not perform well if the source and the target image are significantly different from each other. In Reinhard approach (Reinhard, 2001), change of space from RGB color space to $l\alpha\beta$ color space is attractive but due to

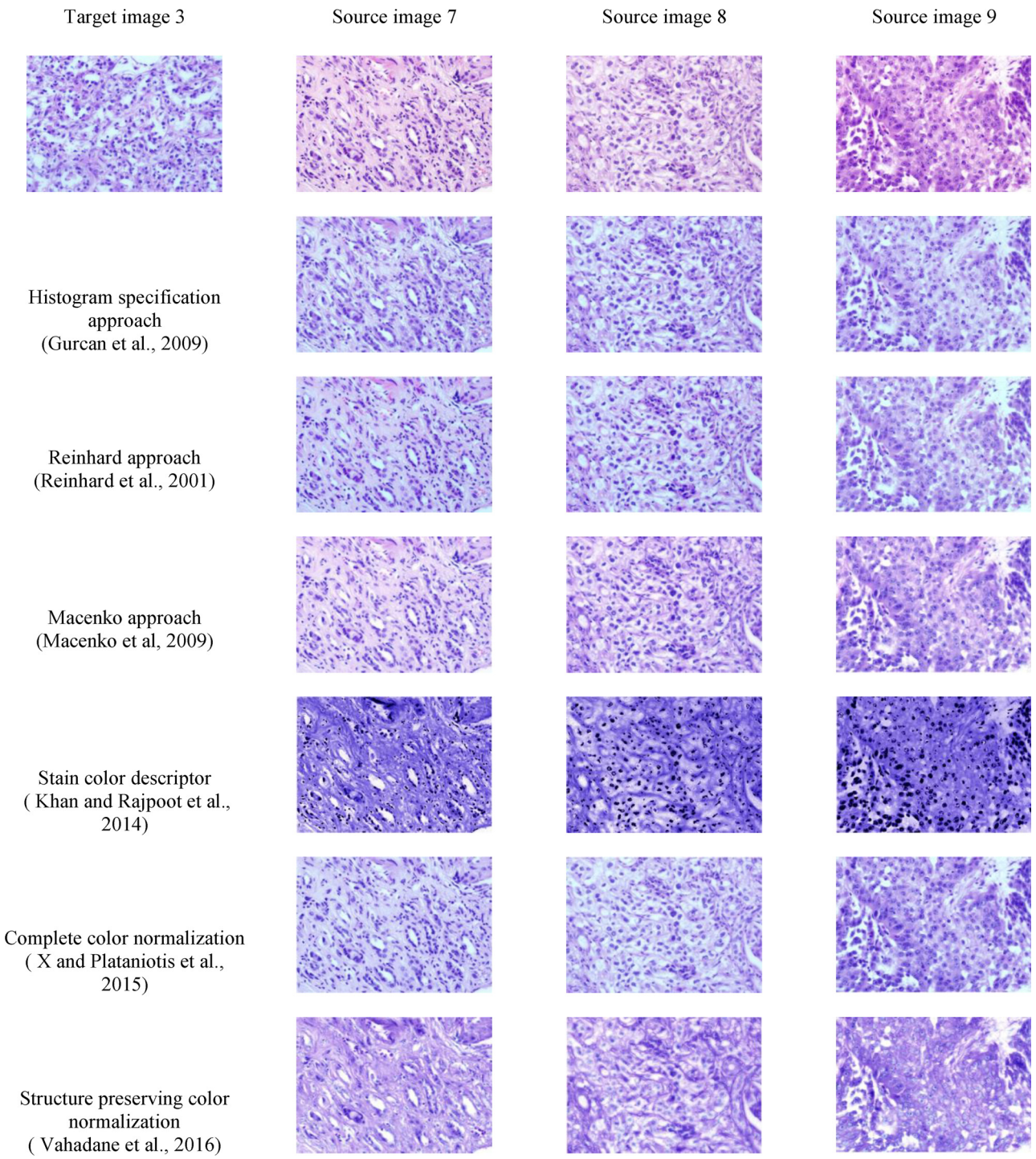

Fig. 3. Comparison of some of the color normalization techniques. The target and the source image are selected from the kidney cancer dataset. Image in the first column represent the normalized image for test 1. The normalized image of the second column represents to test 2 and so on.

the false assumption of uniform distribution of color in each channel. This leads to the poor normalization for the least dominant channel but it preserves all the source intensity variation. In macenko approach (Macenko, 2009), it is very much deviated from the target image.

This method uses color deconvolution approach to find the stain metric but if stain metric estimation fails, this method will also failed. For stain metric estimation, unsupervised method can be used with non-linear mapping. If the concentration of the stain changes non-linearly, the result obtained from macenko approach is more deviated from the target image.

In stain color descriptor approach, they use supervised method with linear mapping and classification approach to find the accurate stain matrix. This approach gave the better result in comparison to the macenko method but if the contrast of the source image is low then this

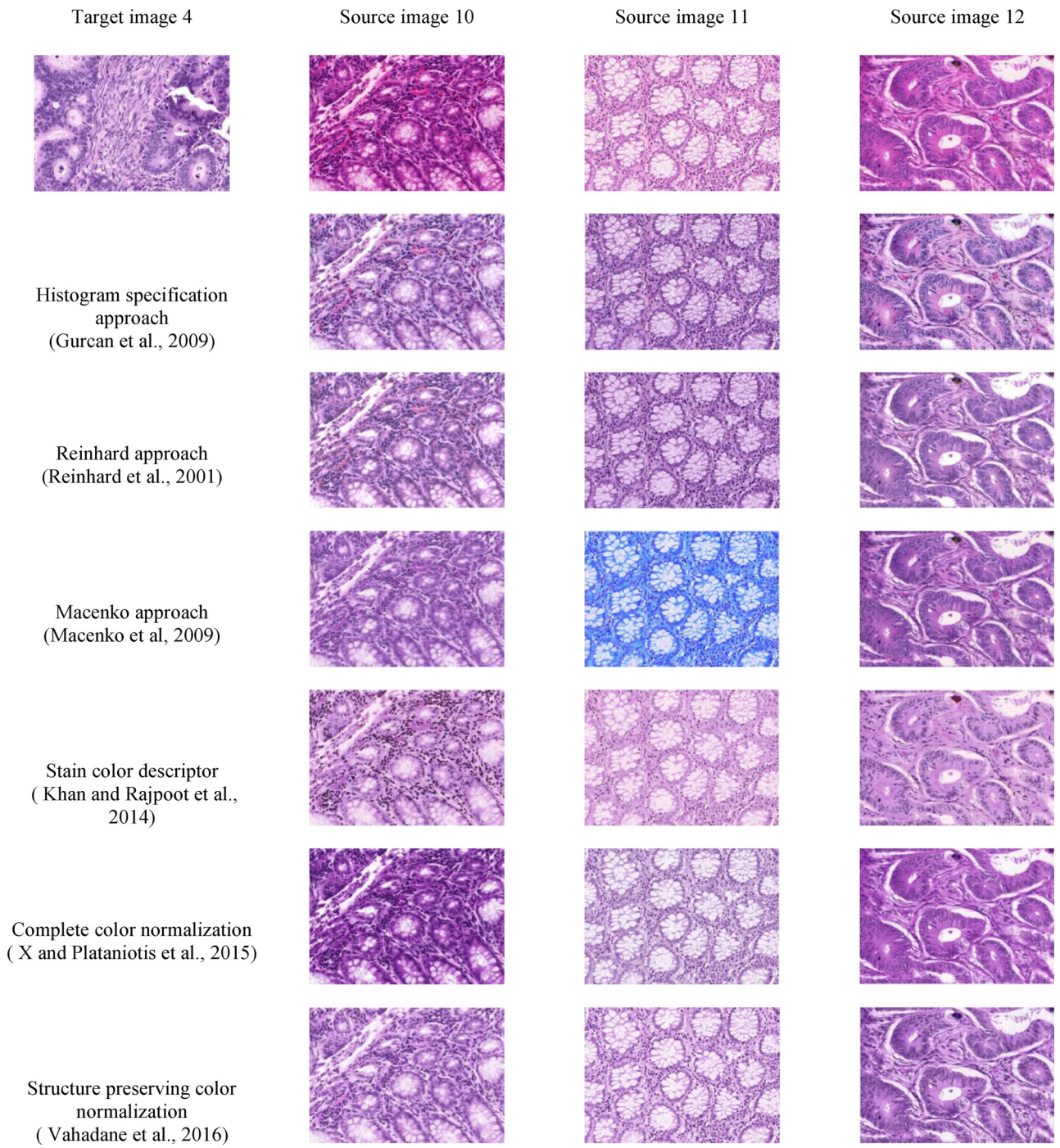

Fig. 4. Comparison of some of the color normalization techniques. The target and the source image are selected from the colorectal cancer dataset. Image in the first column represent the normalized image for test 1. The normalized image of the second column represents to test 2 and so on.

approach is not work well and it is not preserved all the biological information of the source image. In complete color normalization approach, it removes the color bias caused by the illuminant variation in the histopathology image and preserves all the histological information from the source image. This method is more rely on the target image and all the source intensity variation is not preserved. In structure preserving color normalization approach, the mean color of the target image is transfer into the source image while preserving all the histological information. This approach gave better results in comparison to all the presented approach because of the accurate stain separation of both the source and the target image based on the non-matrix factorization (NMF) method.

The simulation experiments have been conducted on different test images from four histopathology images datasets. In the Figs. 1–4, the second column represent normalized image of Test 1, third column represent normalized image of Test 2 and the fourth column represent normalized image of Test 3. Our observation from the simulation results are as follows. In Test 1, Test 2 and Test 3 have some different

Table 2
Quality metrics of various color normalization methods on liver and breast cancer histopathology image datasets.

Color Normalization method	Liver Cancer Dataset			Breast Cancer Dataset		
	QSSIM	SSIM	PCC	QSSIM	SSIM	PCC
Histogram specification approach (Gurcan et al., 2009)	0.8895	0.8605	0.9424	0.8900	0.9306	0.9943
Reinhard approach (Reinhard, 2001)	0.9254	0.9040	0.9630	0.9713	0.9480	0.9798
Macenko approach (Macenko, 2009)	0.9540	0.9411	0.9815	0.8540	0.9470	0.9988
Stain color descriptor (Khan et al., 2014)	0.6534	0.6323	0.7621	0.8534	0.1567	0.8756
Complete color normalization (Li and Plataniotis, 2015)	0.8739	0.8492	0.8957	0.9739	0.9516	0.9728
Structure preserving color normalization (Vahadane, 2016)	0.9603	0.9417	0.9959	0.9763	0.9707	0.9834

information in all the four datasets of three test images. In Test 1 of four source images 1, 4, 7, and10, histogram specification approach preserves all the source intensity variation like red spot and also the source intensity but in Test 2 of four source images 2, 5, 8, and 11, background color transfer from the target image into the source image is perfectly done but source intensity variation is not preserve perfectly. In test 3 of four source images 3, 6, 9, and 12, some information is lost and also color normalization process is not done perfectly. Overall performance by the reinhard approach is significant in all four datasets of three tests sets but stain separation is not done because some of the stains are mixed.

In Test 1 of all the four datasets, macenko approach is worked very well but in source image 7 is not preserved the red spot and it is also not preserve all the source intensity variation. In Test 2 for the Warwick datasets, it is too much deviated from the target image and in Test 3 for all the four datasets; color normalization is not done perfectly. In breast cancer dataset, stain color descriptor method work well. But other three datasets the performance of the stain color descriptor approach is poor because of the data loss and also color normalization is not yield as expected. In all the four datasets, complete color normalization is worked well but it is going to the pure saturated color that is not desirable and it also not preserve source intensity variation. Structure preserving color normalization approach gave good results because it preserves all the source intensity variation, background mean color transfer from the target image into the source image. Color normalization and stain separation is done perfectly but in all other color normalization approach some of the information is lost. So, we can say that the structure preserving color normalization approach gave good results in comparison to all the presented methods.

Quantitative experimental results of the state-of-the-art color normalization methods are evaluated on liver cancer, breast cancer, kidney cancer and colorectal cancer histopathology images presented in terms of SSIM, QSSIM and PCC separately in Tables 2 and 3. In this table, the quality index metrics for average of 20 images from each histopathology image dataset is presented. From the Tables 2 and 3, it is clear that structure preserving color normalization method provided better quantitative results as compared to other existing color normalization methods.

Our observation is that; qualitatively and quantitatively, structures preserving color normalization approach gave better result in comparison to all the presented color normalization methods. Hence, based on experimental results conducted on different histopathology image datasets suggest that structures preserving color normalization approach is best suited for breast and colorectal cancer histopathology image datasets.

5. Conclusions

This paper presented a study and implementation of different color normalization methods for histopathology images. Experimentally, it is clear that histogram specification introduced considerable artifacts in the output images. This method is not applicable if the statistics of target and source images are not same. In reinhard method, it preserves all the source intensity variation but it is too much rely on the target image. This method does not preserve the mean brightness of the original image. In macenko method, if we use more number of stains result obtains from macenko algorithm is sometime inconsistent and it is too much deviated from target image. In stain color descriptor method, structural variation of the source image is not preserved and this method is failed if the contrast of the image is low. In color normalization pipeline method, it is rely on the target image and all the source intensity variation is not preserved. From the visual color normalization results, it is clear that structure-preserving color normalization (SPCN) method was superior in comparison to all the presented color normalization method. In SPCN method, both the brightness and structure of the source image are well preserve and also it introduced fewer artifacts than the other existing color normalization methods. But quantitatively and qualitatively, structure-preserving color normalization method provided better results in comparison to all the presented method shown in table II and III in all the four histopathology datasets

Table 3
Quality metrics of various color normalization methods on kidney and colorectal cancer histopathology image datasets.

Color Normalization method	Kidney Cancer Dataset			Colorectal Cancer Dataset		
	QSSIM	SSIM	PCC	QSSIM	SSIM	PCC
Histogram specification approach (Gurcan et al., 2009)	0.9735	0.9306	0.9943	0.9678	0.9279	0.9931
Reinhard approach (Reinhard, 2001)	0.9623	0.9480	0.9798	0.9560	0.9295	0.9695
Macenko approach (Macenko, 2009)	0.9908	0.9470	0.9988	0.9319	0.8874	0.9879
Stain color descriptor (Khan et al., 2014)	0.5873	0.1567	0.8756	0.8294	0.6318	0.9597
Complete color normalization (Li and Plataniotis, 2015)	0.9668	0.9516	0.9728	0.9775	0.9678	0.9794
Structure preserving color normalization (Vahadane, 2016)	0.9741	0.9707	0.9834	0.9779	0.9695	0.9849

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Appendix A

A.1 Implementation of Histogram specification method

Histogram specification is treated as a problem of optimization which was explored by D. Coltuc and Bolon and used for color normalization of histopathology images (Coltuc and Bolon, 2006). The flow chart of the Histogram specification method is shown in Fig. A1 and implementation steps of Histogram specification method is given below.

Step (1). Read both the input images such as source and target image.

Step (2). Let us consider the intensity of source and target image is a discrete random variable. For this purpose, we resize both the source and target image.

Step (3). Evaluate the probability mass function (PMF) of all the pixels present in the source and target image and then obtained image histogram.

Step (4). Compute the cumulative distribution function (CDF) of all the pixels and then multiply the cumulative distributive function value with Gray levels minus one. Then, we round off the obtained value to the nearest integer.

Step (5). Mapping can be done between the new gray level values into initially present pixels value. This mapping can be done by mapping the source intensity value to the target intensity value with the initially present pixel value.

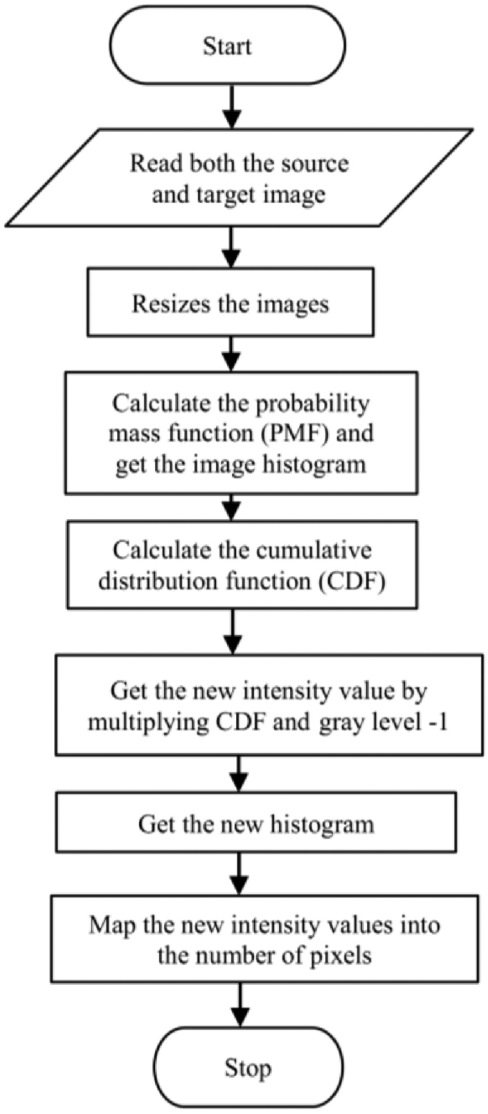

Fig. A1. Flow chart for the histogram specification.

A.2 Implementation of Reinhard method

This method was proposed by Reinhard for color normalization of histopathology images (Reinhard, 2001). The flow chart of the Reinhard method is given in Fig. A2 and implementation steps of Reinhard method are given below.

Step (1). Read both the source and target image which is the input of this color normalization method.

Step (2). Convert the RGB image into *lαβ* color space because it is a transform of LMS cone space. While converting into LMS cone space, we first convert RGB image into XYZ space which is independent and then we convert the independent XYZ space image to LMS cone space. In LMS cone space, the data is in the form of skew which is removed by converting into the logarithmic space. Like RGB image, *lαβ* color space has its own color. *l, α and β* axis represents an achromatic, chromatic blue-yellow, chromatic green-red channels. The information in *lαβ* color space is compact and symmetrical.

Step (3). Initialize the number of channel i = 0 and the number of channel present in the RGB image c = 3 and apply the condition if the number of channel i is less than the number of channel present in the RGB image c, then we do the following transformation given in equation (A.1), (A.2) and (A.3).

$$l_2 = mean(l_1) + (l - mean(l)).*(std(l_1)./std(l)) \tag{A.1}$$

$$\alpha_2 = mean(\alpha_1) + (\alpha - mean(\alpha)).*(std(\alpha_1)./std(\alpha)) \tag{A.2}$$

$$\beta_2 = mean(\beta_1) + (\beta - mean(\beta)).*(std(\beta_1)./std(\beta)) \tag{A.3}$$

where l_2, l_1 and l are the processed image, target image and the source image in l space, α_2, α_1 and α are the processed image, target image and the source image in α space. β_2, β_1 and β are the processed image, target image and the source image in β space.

Step (4). Convert *lαβ* color space to RGB image to display it because there will be no comparison between *lαβ* color space and RGB color space.

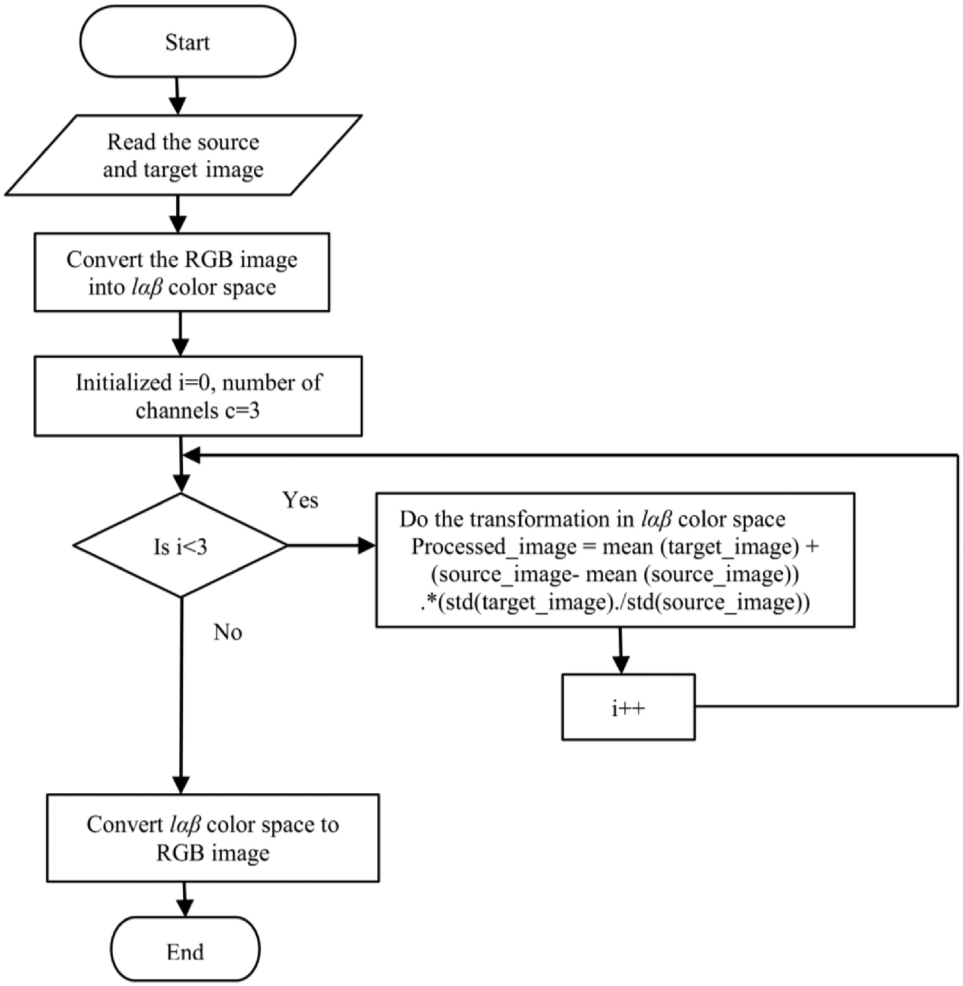

Fig. A2. Flow chart for the Reinhard method.

A.3 Implementation of Macenko method

This method was proposed by Macenko for color normalization of histopathology images (Macenko, 2009). The flow chart of the Macenko method is shown in Fig. A3 and implementation steps of Macenko method are given below.

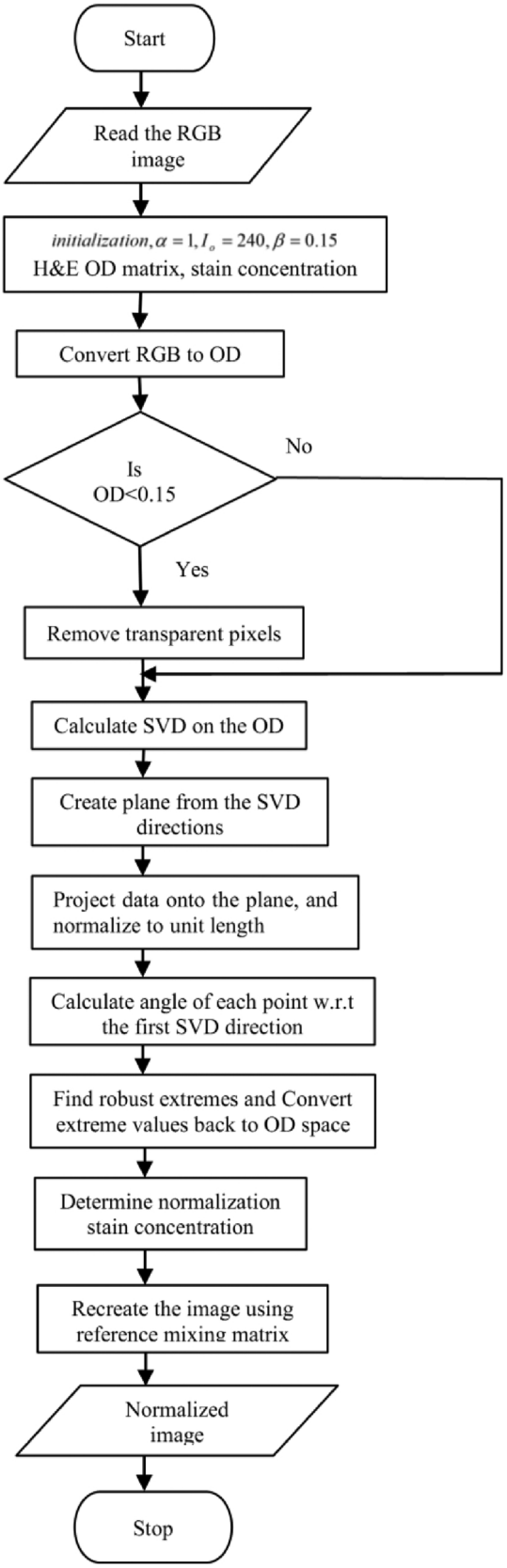

Fig. A3. Flow chart for the Macenko method.

Step (1). Read both the source and target image which is the input of this color normalization method.

Step (2). Convert the RGB image into *lαβ* color space because it is a transform of LMS cone space

Step (3). Initialize the tolerance for the pseudo-minimum α^{th} and pseudo-maximum $(100 - \alpha)^{th}$ percentile. It will give better result at $\alpha = 1$, OD threshold value for transparent pixels $\beta = 0.15$, transmitted light intensity $I_o = 240$, H&E OD matrix and stain concentration for further processing.

Step (4). All the present color in the histopathology image can be converted into the optical density value [9] which is given in equation (A.4).

$$OD = -\log_{10}(I) \tag{A.4}$$

Where, I denotes the source image and each component is normalized in the range of [0 1]. This conversion from RGB image to optical density (OD) values offers a space where a linear combination of stain will give result in a linear combination of optical density values.

Step (5). Apply the condition on the OD threshold value if the OD threshold value $\beta < 0.15$. Then we remove the transparent pixels. For this purpose, the optical density values (OD) is split into two matrixes which are given in equation (A.5) and (A.6) respectively.

$$OD = V^{*}S \tag{A.5}$$

$$S = V'^{*}OD \tag{A.6}$$

Where, OD denotes the optical density values, S is the saturation value of each stain and V is the stain vector matrix. Here, we find the stain vector of each image based on the color. If the OD value is zero, then the corresponding pixel is white that means there are no stain present. For the stability purpose, we assume a threshold value. By experimentally, we found that the threshold value of $\beta = 0.15$ and then defined the geodesic path.

Step (6). Calculate the singular value of the SVD decomposition on the optical density (OD) value. Geodesic path (Bautista et al., 2014) can be used to find the direction where we can project the optical density transformed pixel into the order to find the endpoint of the stain vectors. Geodesic path is the shortest distance between the two color vectors. In the next step, we evaluate the plane that is formed by vectors and this process can be done by creating a plane with the help of two vectors correspond to the two largest singular values of the singular value decomposition of the optical density transformed pixel values.

Step (7). All the optical density values is projected into the plane and normalized to unit length and the projected line is curved.

Step (8). The angle is calculated in every point with respect to the first singular value decomposition direction and thus mapping the direction in the plane. Then, we can obtain the histogram of these angles. Intensity variation is the major problem in histopathology images. The original strength of the stain depends on the intensity of a particular stain.

Step (9). The variation in the slide preparation is due to the different use of equipment, reactivity of the manufacturing, different storage time and different staining producer. Let us consider that two stain present in the image having specific stain vector and their specific color present in optical density (OD) space. The color present at every pixel is a linear combination of stain vectors. Since, it has a positive weight at every pixel. So, we can find the fringes instead of searching for peaks. The minimum and maximum percentile can be used in found direction. For the robust result, we calculate the minimum and maximum α^{th} and $(100 - \alpha)^{th}$ percentile. Numerically, $\alpha = 1$ gives the better result. For each stain, we calculate intensity histogram for all pixels of the stain which is in majority and then we find the 99^{th} percentile and use the robust maximum approximation. Experimentally, it is found that, the above method gives good result by analyzing a patch of slides. All intensity histogram scaled to have the same pseudo-maximum and then compare to each other

Step (10). Determine the concentration of the individual stains by using Hematoxyline and Eosin matrix with respect to the OD values and then normalized the stain concentration.

Step (11). Recreate the image by using reference mixing matrix and it is done by using HE matrix with the normalized stain concentration. After these steps, we got normalized image.

A.4 Implementation of Stain color descriptor method

This method was proposed by A. M. Khan et al. for color normalization of histopathology images (Khan et al., 2014). The flow chart of the Stain color descriptor method is shown in Fig. A4 and implementation steps of stain color descriptor method are given below.

Step (1). Read both the source and target image which is the input of this color normalization method.

Step (2). Determine the image specific stain matrix of target and source image by using a global stain color descriptor (SCD). In image specific SCD, training sets are given for K RGB histopathology images. Then, we calculate the image specific color descriptor (SCD) that is denoted by $\hat{H}$. For this purpose, each image is quantized by using oct-tree quantization (Gervautz and Purgathofer, 1988) and it is used to generate a set of histogram of 255 color prototype. Color to prototype mapping algorithms is very efficient for the Oct-tree quantization (Gervautz and Purgathofer, 1988). Next, we estimate the mean and co-variation of K RGB histopathology image of histogram are calculated to perform linear dimensionality reduction and then we use color classification for probabilistic output.

Step (3). Color deconvolution is used to separate RGB image into three channels. These three channels correspond to the actual color of the stains used. This method can also be use for the estimation of IHC stain (Taylor and Levenson, 2006) and nuclei detection (Khan et al., 2013). Here, we transform the RGB color space V to another color space U because we stained the tissue section. If $I = (P, V)$ is defined as the image with pixel P and associated color space V and each pixels assigning blue, green and red intensities. The relationship between the color space and new color space can be determined by using Beer-Lambert Law (BLT) which is given in equation (A.7).

$$V = e^{SU} \tag{A.7}$$

Where, S represent the stain matrix and it determines the stain vectors (also called absorption factor). The solution (Ruifrok and Johnston, 2001) of the equation (A.7) is determined if the pixel intensity $p \in P$ in another color space is also called new color space U which is defined in equation (A.8).

$$U(p) = D^{*}\varphi(p) \tag{A.8}$$

Where

$$D = S^{-1} \tag{A.9}$$

$$\varphi(p) = -\log(V(p)) \tag{A.10}$$

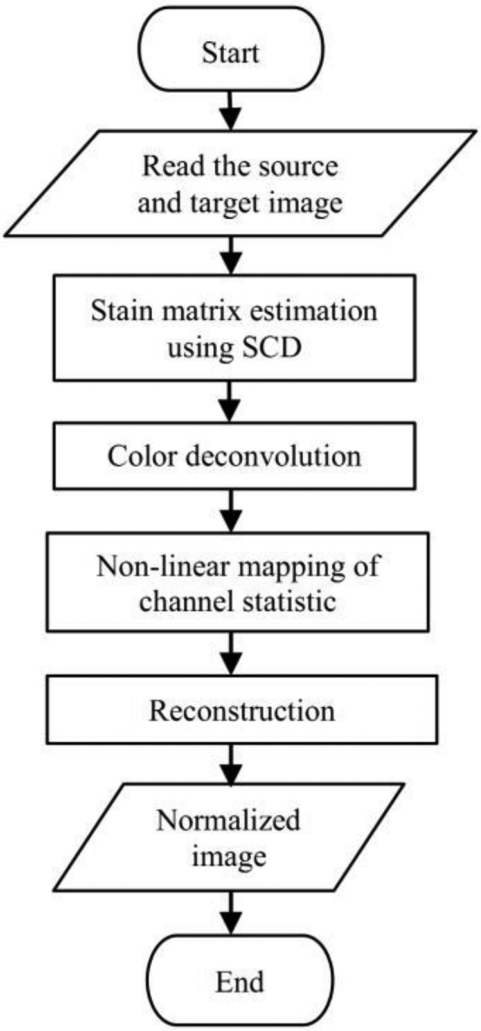

Fig. A4. Flow chart for the stain color descriptor (SCD).

In equation (A.8), D denotes the color deconvolution matrix and it is obtained if we takes the inverse of stain matrix S, φ denotes the optical density space, $U(p)$ represents the concentration of each stain at every pixel. In optical density space, a linear combination of stain value results in a linear combination of optical density value.

Step (4). Finally, we use non-linear mapping of channel statistic to de convolved the source and the target image and then we calculate the set of statistics and these statistics can smoothly map between the statistic of the source image to that of target image by using a spline based non-linear mapping. In non-linear mapping, AB spline is used for mapping purpose between the smooth mapping functions. The AB spline parameters are calculated from the identity pairs plus the input-output pairs to ensure saturated (black) and optically saturated (white) pixels remains unchanged. By solving a linear system using Tikhonov regularization, we are estimated B-spline parameters.

Step (5). Reconstruction can be done by using per-pixel basis.

A.5 Implementation of Complete color normalization

Complete color normalization method was proposed by Li. X for color normalization of histopathology images (Li and Plataniotis, 2015). The flow chart of the Stain color descriptor method is shown in Fig. A5 and implementation steps of Stain color descriptor method is given below.

Step (1). Read both the source and target image which is the input of this color normalization method.

Step (2). Initialize the number of channel c = 3, number of count i = 0, threshold value $th_b = 200$ and the number of satins N = 2.

Step (3). Apply the condition on the number of channel c if the number of count is less than the number of channels then we do the following operation. Calculate the background intensity of source and target image by using equation (A.12) and (A.13). If the above condition is not satisfied then we go for the illuminant normalization.

$$I^b(\lambda_i) = \max[I(p, \lambda_i) \otimes h_N] \tag{A.12}$$

$$I_s^b(\lambda_i) = \max[I(p, \lambda_i) \otimes h_N] \tag{A.13}$$

Step (4). Again, apply the condition on background intensity. If the intensity is greater than the threshold value then, we go for the intensity matching on the source and target image otherwise we directly go for the illuminant normalization. Intensity matching has been done by using the equation (A.14).

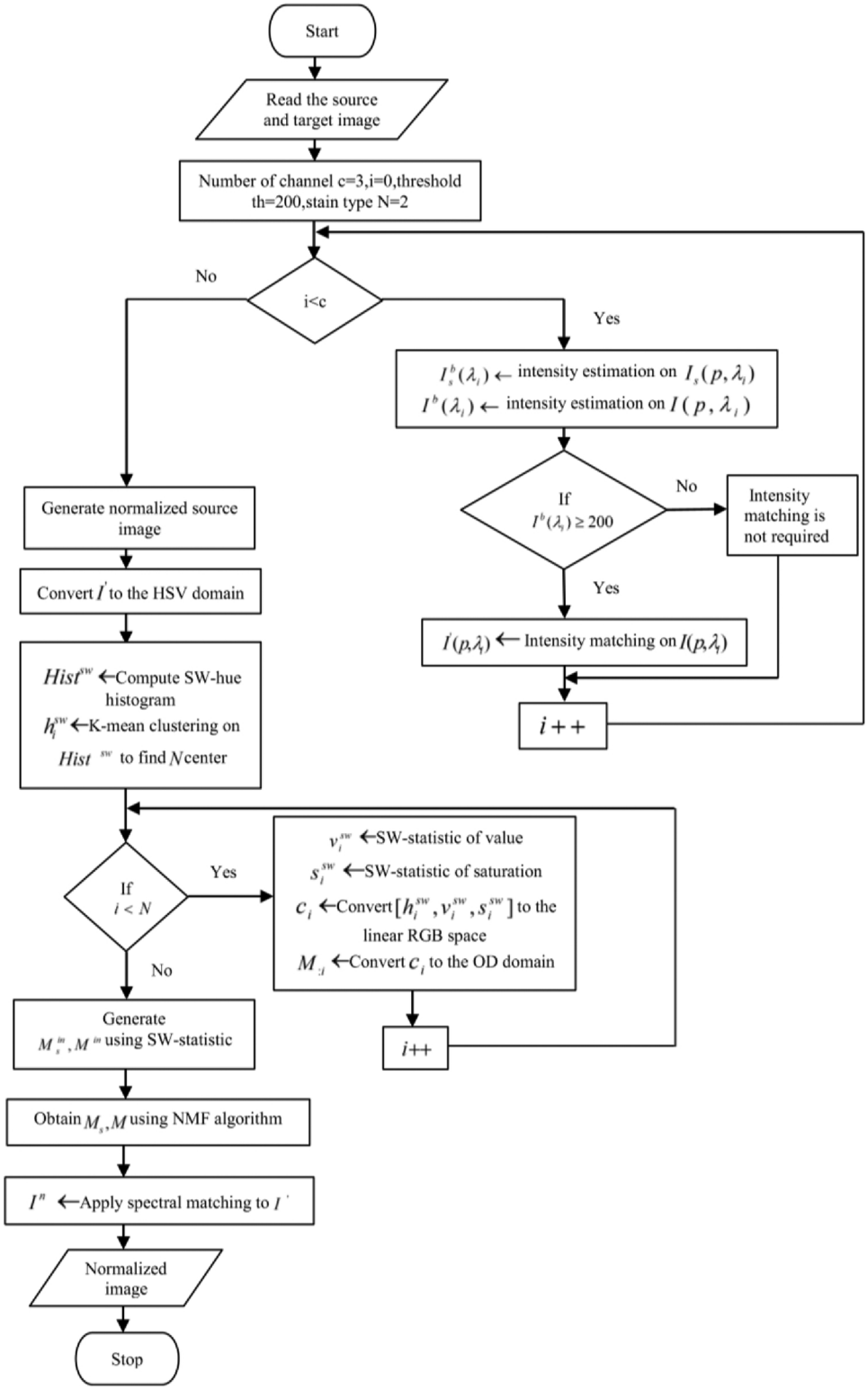

Fig. A5. Flow chart for the color normalization method.

$$\frac{I_s(p,\lambda_i)}{I(p,\lambda_i)} = \frac{I_s^b(\lambda_i)}{I^b(\lambda_i)} \tag{A.14}$$

Step (5). Convert the illuminant normalized image to HSV domain because we want the dominant color or pure saturated color in place of estimation of stain spectra.

Step (6). Spectral estimation is unreliable if we use less saturated color because colors are highly saturated for the estimation. A hue histogram will give the light spectra which are not absorbed. So, this is used to obtained spectral matrix M^{in}. For this purpose, we use SW hue histogram by using equation (A.15).

$$Hist_{\theta}^{SW} = \sum_{p} s_p \delta(\theta, h_p) \tag{A.15}$$

Where, $\delta(\theta, h_p) = \begin{cases} 1, if \theta = h_p \\ 0, otherwise \end{cases}$ $\theta \in [0°, ..., 360°]$ denotes the bin of hue histogram, h_p and s_p represent the hue and saturation at a pixel p in the HSV domain.

Step (7). From the SW-histogram $Hist^{SW}$, it is clear that the light spectra are not absorbed by any stain. So, we go for the estimation of purely saturated color for each stain. For this purpose, we go for the K-mean clustering applied to $Hist^{SW}$ for the estimation of N representative hues h_1^{SW}, ..., h_N^{SW}. Hue value such as pink and purple to blue are used for the cluster centers of hematoxyline and eosin. Then, for each hue h_i^{SW}, we evaluate the value of v_i^{Sw} and s_i^{Sw} in the HSV domain. This is defined in equation (A.16) and (A.17).

$$v_i^{Sw} = \frac{\left[\sum_p s_p v_p \delta(h_i^{SW}, h_p)\right]}{\left[\sum_p s_p \delta(h_i^{SW}, h_p)\right]} \tag{A.16}$$

$$s_i^{SW} = \frac{\left[\sum_p (s_p)^2 \delta(h_i^{m}, h_p)\right]}{\left[\sum_p s_p \delta(h_i^{Sw}, h_p)\right]} \tag{A.17}$$

Step (8). Apply the condition on the N center if the number of count is les than the number of N centers then, we go for the estimation of satuaration s_i^{Sw} and value v_i^{Sw}. After that we convert the hue histogram to the linear RGB color space and form an initial spectrum matrix. if the abve condition is not satisfy then we go for the spectral matrix estimation by using non-negative matrix factorization (NMF) (Cichocki et al., 2009; Lee and Seung, 1999).

Step (9). By using above matrix, we will go for the spectral matching to get the normalized image.

Step (10). Spectal matching can be done by using the equation (A.18).

$$I_s(p, \lambda_i) = I^b(\lambda_i) \times \left[\frac{I(p, \lambda_i)}{I^b(\lambda_i)}\right]^{\frac{M^{si}(\in_4)}{M_i(\in_3)}} \tag{A.18}$$

Where, M_{si} and M_i are denoted as the standard spectra and the estimated spectra.

A.6 Implementation of Structure preserving color normalization (SPCN)

Structure preserving color normalization method was proposed by Vahadane, (2016). The flow chart of the structure preserving color normalization (SPCN) is shown in Fig. A6 and implementation steps of structure preserving color normalization are given below.

Step (1). Read both the source and target image which is the input of this color normalization method.

Step (2). The variation of stained tissue looks like a Beer-Lambert law and it attenuates light spectrum and concentration of stain. Let $I \in R^{m \times n}$ is the matrix of RGB image where $m = 3$, represents three channels of RGB image, n is the number of pixels and let I_o be the illuminant light intensity. Let $w \in R^{m \times r}$ denotes the stain matrix whose column represents color variation of each stain and r denotes the stain number and $H \in R^{r \times n}$ denotes the stain density maps where the rows denotes the stain concentration. Then, mathematically I can be defined as

$$I = I_o e^{-WH} \tag{A.19}$$

Let us consider that V be the optical density maps then,

$$V = \log\left(\frac{I_o}{I}\right) \tag{A.20}$$

By using equation (A.19), we can write

$$V = WH \tag{A.21}$$

Where, V is the observation matrix, H is the stain density map matrix, and W is the stain color appearance matrix.

Step (3). Color deconvolution (Bautista et al., 2014) is mostly used to separate stain. But, another method is used to separate stain is non-negative matrix factorization (NMF) (Lee and Seung, 1999) and it is the unsupervised method (Rabinovich and Agarwal, 2003) to separate stains. The non-negativity is absorbed by the stain optical density and the stain color appearance matrix that can be only absorb but not emits light which makes the stain density and color appearance matrix non-negative. This problem is solved by solving the following problem.

$$\min \frac{1}{2} \| V - WH \|_F^2, \text{ such that } W, H \geq 0 \tag{A.22}$$

The above problem is basically non-convex optimization problem which converge local optimum values in place of global optimum values and give stain vectors (Aharon et al., 2006). Now, they add sparsity constrain on nonnegative matrix factorization (NMF) to preserve the histological information. So, it is called as the sparse nonnegative matrix factorization (SNMF). In SNMF based stain separation, we convert the RGB image into the optical density maps by using the above equation (A.10) on the basis of beer-lambert law. Then, in equation (A.19), they add sparseness constraint. SNMF based stain separation is an improved version of NMF method and it is used for the stain separation with the help of l_1 and H_j. l_1 and H_j are the sparseness and the stain mixing coefficient where, j denotes the index of stains that is $j = 1, 2, ...r, .$

$$\min \frac{1}{2} \| V - WH \|_F^2 + \lambda \sum_{j=1}^{r} \| H(j, :) \|_1, W, H \geq 0 \tag{A.23}$$

$$\| W(:, j) \|_2^2 = 1 \tag{A.24}$$

Where, λ is denotes as the sparsity and regularization parameter. If we include any additional constraint on W and H, it will reduce the solution

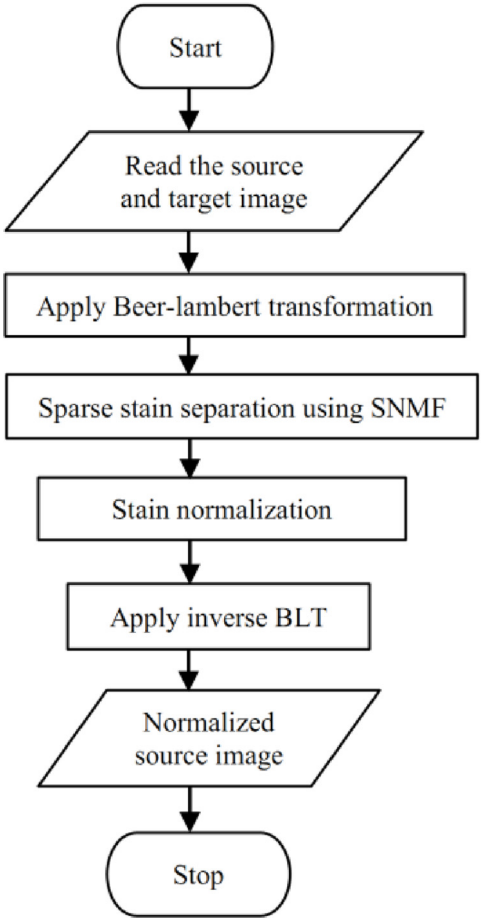

Fig. A6. Flow chart for the structure preserving color normalization.

space of the type W/α and αH, $\alpha > 0$.

The above equation (A.20) is the problem of non-convex optimization and it can be used to solve by alternating between H and W in which we optimize one parameter of H and W while taking other parameter constant. For that purpose, we are going to initialize color appearance matrix W by randomly selected element from the optical density V.

$$\text{For fixed } W,\ \hat{H} = \min \tfrac{1}{2} \| V - \hat{W}H \|_F^2 + \lambda \| H \|_1,\ H \geq 0 \tag{A.25}$$

$$\text{For fixed } H,\ \hat{W} = \min \tfrac{1}{2} \| V - W\hat{H} \|_F^2,\ W \geq 0 \tag{A.26}$$

$$\| W(:,j) \|_2^2 = 1 \tag{A.27}$$

The above equation (A.25) is the problem of dictionary learning (Aharon et al., 2006), but it include additional non-negative constraints on dictionary elements W and stain density maps H. The above two steps is the dictionary learning for W and sparse coding for H. Sparse coding is used to estimate stain density maps H while fixing $\hat{W}$ is an l_1 regularized linear least square optimization problem. This type of problem can be solved by using coordinate descent with the LARS-LASSO algorithm (Efron, 2004) and soft thresholding (Wu and Lange, 2008) algorithm.

Step (4). Color normalization method can be done by transferring the mean color of target image to that of the source image while estimating the color appearance matrix. By using SNMF technique, we are going to factorize the stain density maps V_s into W_sH_s and V_t into W_tH_t. Then, we combine the stain density maps of source H_s to the color appearance matrix of the target W_t in place of the source color appearance matrix W_s to generate the normalized image. Thus, stain density map H preserves the structure and the color appearance matrix W preserves the change in the color appearance.

Step (5). After stain normalization, we apply inverse Beer-Lambert transform (BLT) on the normalized stains to get normalized source image.

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