33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Digital Staining for Histopathology Multispectral Images by the Combined Application of Spectral Enhancement and Spectral Transformation

Pinky A. Bautista and Yukako Yagi

Abstract—**In this paper we introduced a digital staining method for histopathology images captured with an *n*-band multispectral camera. The method consisted of two major processes: enhancement of the original spectral transmittance and the transformation of the enhanced transmittance to its target spectral configuration. Enhancement is accomplished by shifting the original transmittance with the scaled difference between the original transmittance and the transmittance estimated with *m* dominant principal component (PC) vectors;the *m*- PC vectors were determined from the transmittance samples of the background image. Transformation of the enhanced transmittance to the target spectral configuration was done using an *nxn* transformation matrix, which was derived by applying a least square method to the enhanced and target spectral training data samples of the different tissue components. Experimental results on the digital conversion of a hematoxylin and eosin (H&E) stained multispectral image to its Masson's trichrome stained (MT) equivalent shows the viability of the method.**

I. INTRODUCTION

Multispectral imaging uses $n>3$ narrowband filters in contrast to 3 broadband filters used in RGB color imaging. With such imaging system it is possible to capture salient image features that are indistinguishable with human eyes[1-6]. In [3] the concept of digital staining using multispectral information was introduced. Digital staining was defined as the digital conversion of the original image to an image that reflects the desired staining colors. The authors in [3] investigated the digital conversion of an unstained multispectral tissue-image to its equivalent hematoxylin and eosin (H&E) stained image. Conversion of the original image was performed by utilizing transformation matrices, which were derived from a set of unstained and H&E stained spectra of the different tissue components using least mean square method. Similar concept was utilized in [4] to digitally convert an H&E stained image to its Masson's trichrome (MT) stained image equivalent. The digital staining procedures adapted in [3] and [4] involved the application of spectral classification. Several transformation matrices were defined by the authors, which were activated basing on the spectral classification result. Although the digitally produced Masson's trichrome (MT) stained images in [4] exhibit similar characteristics to the physically stained MT stained images, the delineation between collagen fiber and muscle fiber was not successfully reflected. This can be due to the failure of the spectral classifier to delineate the spectral difference between these structures, and the limitation of the defined transformation matrices themselves.

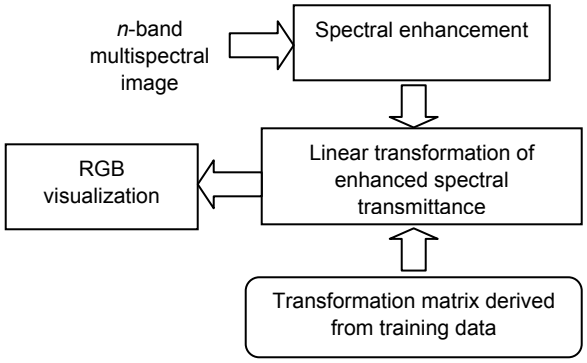

Fig. 1 Schematic diagram of the proposed digital staining method for multispectral images using spectral information.

The spectral enhancement technique introduced in [5] was applied to H&E stained histopathology images to differentiate collagen fiber and muscle fiber[6]. It was shown that with such spectral enhancement method it is possible to modify the spectrum of collagen fiber to distinctly differentiate it from smooth muscle. Hence, the initial digital staining results in [4] can be improved further by using the enhanced spectra rather than the original spectra of the tissue components as features in the digital staining implementation.

In this paper we proposed the combined application of spectral enhancement and linear transformation of spectral transmittance to implement the digital staining. The spectral enhancement performs the differentiation between tissue structures that share close similarity in their colorimetric attributes while the linear transformation converts the enhanced spectra to their target spectral configuration to

Manuscript received March 21, 2011. P.A.B is with the Massachusetts General Hospital, Harvard Medical School Department of Pathology, Boston 02114 MA USA (P.A.B phone: 617-643-7915; fax: 617-643-7901; e-mail: pbautista@ partners.org).

Y. Y. with the Massachusetts General Hospital, Harvard Medical School Department of Pathology, Boston 02114 MA USA (e-mail: yyagi@partners.org).

produce digitally stained images. The schematic diagram of the proposed digital staining procedure is shown in figure 1. The calculated spectral transmittance of the *n*-band multispectral pixel is enhanced by the spectral enhancement method introduced in [5]. The enhanced transmittance is converted to its target spectral configuration and then finally converted to their RGB values for visualization.

II. Digital Staining

A. Spectral enhancement

The spectral enhancement technique we utilized in this work is based on the proposed method of Mitsui et al[5]. In the method the spectral difference between the original transmittance and the spectral transmittance estimated with *m*- dominant principal component (PC) vectors is used as a shifting factor to the original transmittance data. The spectral difference is scaled by a real-valued weighting factor defined in an *nxn* matrix **W**, where *n* is the spectral dimension of the original spectral data. Equation 1 illustrates the mathematical operation of the spectral enhancement.

$$\mathbf{t}_e = \mathbf{t}_o + \mathbf{W}(\mathbf{t}_o - \mathbf{t}') \qquad (1)$$

The notations $\mathbf{t}_o, \mathbf{t}_e$, and $\mathbf{t}'$ are *nx1* column vectors that respectively represents the original spectral transmittance, enhanced transmittance, and the transmittance estimated by *m* dominant PC vectors. Estimation of the *n* dimensional spectral transmittance using *m* principal component vectors is done as follows:

$$\mathbf{t}' = \sum_{i=1}^{m} \alpha_i \mathbf{v}_i + \bar{\mathbf{t}} \quad , \qquad (2)$$

where α_i and $\mathbf{v}_i$ denote the *ith* PC coefficient and PC vector, respectively. It should be noted that the PC vectors were derived from the spectral transmittance of the background image. The notation $\bar{\mathbf{t}}$, on the other hand, represents the average spectral transmittance of the background image, or spectral transmittance of the image objects which were not intended to be enhanced.

B. Linear transformation of spectral transmittance

The enhanced transmittance of a tissue component is transformed to its target spectral configuration. The target spectral configuration corresponds to the spectral response of the tissue when stained with the target stain. Here, we assumed that a linear relation can be established between the two spectral data sets, i.e. enhanced and target spectral transmittance. Let $\mathbf{T}_e$ and $\mathbf{T}_t$ respectively represent the *rxn* matrix containing the training data for the enhanced and the target spectral transmittance of the different tissue components, i.e *n* is the spectral dimension and *r* is the total number of spectral samples. We can the write linear relation between $\mathbf{T}_e$ and $\mathbf{T}_t$ as follows:

$$\mathbf{T}_t = \mathbf{T}_e \mathbf{Q} \qquad (3)$$

The notation **Q** represents the *nxn* transformation matrix. The solution for **Q** using least mean square method can be expressed as:

$$\hat{\mathbf{Q}} = \mathbf{T}_e^{+} \mathbf{T}_t \quad , \qquad (4)$$

where $\mathbf{T}_e^{+}$ is the pseudoinverse of $\mathbf{T}_e$. We will use the result of eqn. 4 to convert the enhanced spectra to the target spectral transmittance:

$$\hat{\mathbf{t}}_t = \mathbf{t}_e \hat{\mathbf{Q}} \quad , \qquad (5)$$

where $\hat{\mathbf{t}}_t$ denotes the estimated target spectral-transmittance of $\mathbf{t}_o$; and $\mathbf{t}_e$ enhanced transmittance of $\mathbf{t}_o$. For instance if the original image is stained with H&E and we would like to digitally convert it to Masson's trichrome (MT) stained image, then $\mathbf{t}_o$ represents the H&E spectral transmittance of a tissue structure and $\hat{\mathbf{t}}_t$ represents the estimated MT stained spectral configuration of $\mathbf{t}_o$.

C. Visualization of the digitally stained multispectral image

The *n*- band transformed spectral transmittance from **eqn. 5** is converted to its equivalent RGB color values for visualization [7]:

$$\begin{bmatrix} \alpha_R \\ \alpha_G \\ \alpha_B \end{bmatrix} = \begin{bmatrix} X_R & X_G & X_B \\ Y_R & Y_G & Y_B \\ Z_R & Z_G & Z_B \end{bmatrix}^{-1} \left(\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} - \begin{bmatrix} X_{black} \\ Y_{black} \\ Z_{black} \end{bmatrix} \right) \qquad (6)$$

where *X,Y,Z* correspond to the CIE 1931/1964 chromacity coordinates:

$$\begin{aligned} X &= \int_{450nm}^{720nm} \bar{x}(\lambda) E(\lambda) \hat{\mathrm{t}}_t(\lambda) d\lambda \\ Y &= \int_{450nm}^{720nm} \bar{y}(\lambda) E(\lambda) \hat{\mathrm{t}}_t(\lambda) d\lambda \\ Z &= \int_{450nm}^{720nm} \bar{z}(\lambda) E(\lambda) \hat{\mathrm{t}}_t(\lambda) d\lambda \end{aligned} \qquad (7)$$

The notations $\bar{x}(\lambda), \bar{y}(\lambda), \bar{z}(\lambda)$ denote the CIE XYZ color matching functions; $E(\lambda)$ denotes the illumination spectrum; X_i, Y_i, Z_i, $i = R, G, B$, are the XYZ chromaticity coordinates of the RGB primary colors; and $X_{black}, Y_{black}, Z_{black}$ are the XYZ chromaticity coordinates of the monitor background.

III. Experiment and Results

A. Tissue images

We used the microscopic multispectral system of Olympus to capture 5 pairs of tissue liver images. Each pair is comprised of an H&E stained and a Masson's trichrome (MT) stained image. The H&E and MT images exhibit similar structural compositions since they were

captured from tissue slides that are of serial sections. From the captured images 8 different tissue components were identified. Each of these tissue components were represented with 200 H&E and MT stained spectral transmittance samples, which serve as the training data.

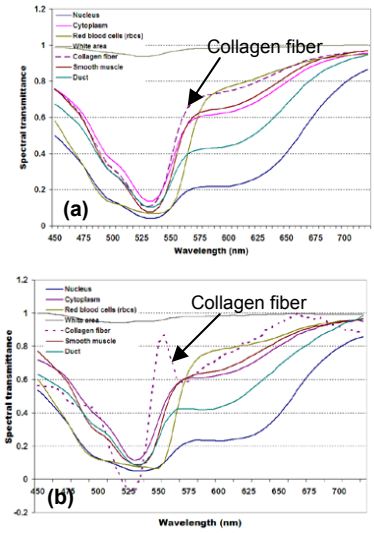

Fig. 2 Each curve corresponds to the average spectral transmittance of each tissue component. (a) Original H&E transmittance; (b) Result of the spectral enhancement.

B. Enhancement of spectral transmittance

The purpose of spectral enhancement is to highlight the difference between tissue structures that are not clearly differentiated in the original image. In our experiment this corresponds to the delineation between muscle fiber and collagen fiber from an H&E stained image. Let us define the matrix $\mathbf{T}_{\mathrm{HE}}$ to contain the H&E spectral transmittance samples of all the different tissue components, and $\mathbf{T}'_{\mathrm{HE}}$ as the matrix that does not contain the spectral transmittance samples of the collagen fiber. To perform the spectral enhancement we first derived the PC vector, $\mathbf{v}_i$, the PC coefficient α_i, i.e. $i=1..m$, and the average spectral transmittance $\bar{\mathbf{t}}$ from $\mathbf{T}'_{\mathrm{HE}}$. Spectral enhancement was performed using **eqn.1** wherein the weighting factor matrix **W** was defined as:

$$[\mathbf{W}]_{ij} = \begin{cases} k & i = j \\ 0 & otherwise \end{cases} \qquad (8)$$

In our experiment we used 5 PC vectors, i.e. $m=5$, to estimate the spectral transmittance, $\mathbf{t}'$, of an n-band multispectral pixel and we set the value of k to 10. Figure 2 shows original and enhanced spectral transmittance configurations of the different tissue components. The original transmittance spectra of the different tissue components are shown in fig.2a and their corresponding enhanced spectra are displayed in fig.2b. Application of the spectral enhancement resulted to a marked difference between the spectral configuration of the collagen fiber and the rest of the tissue components.

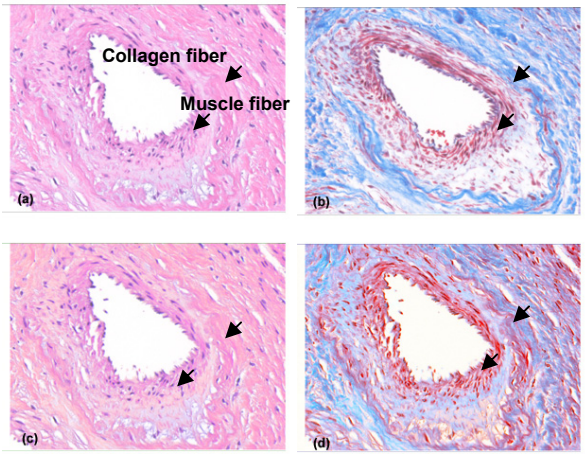

Fig.3 Image visualization.(a) Original H&E stained image; (b) manually stained Masson's trichrome (MT) image; (c) enhanced H&E stained image; (d) digitally produced MT stained version of the H&E stained image, i.e. digitally stained. While the collagen and muscle fiber are distinctly colored in the manually stained Masson's trichrome image in (b), they are hardly differentiated in the original H&E stained image. Clear differentiation between these two structures is not achieved even with spectral enhancement as shown in (c). Application of the proposed digital staining method however, results to the distinctive colors of the structures, which are similar when the tissue is physically stained with Masson's trichrome.

C. Derivation of the transformation matrix $\hat{\mathbf{Q}}$

Let $\mathbf{T}_{\mathrm{e}}$ and $\mathbf{T}_{\mathrm{MT}}$ represent the rxn matrices containing the enhanced H&E and the Masson's trichrome (MT) spectral transmittance samples of all the different tissue components- this includes transmittance spectra of collagen fiber; n is the total number of bands and r refers to the total number of transmittance samples, Let $\overline{\mathbf{T}}_{\mathrm{e}}$ and $\overline{\mathbf{T}}_{\mathrm{MT}}$ represents the cxn matrix containing the average transmittance of the different tissue components; c is the number of spectral transmittance classes, i.e. number of tissue components. Then we derived the transformation matrix $\hat{\mathbf{Q}}$ as follows [3]:

$$\hat{\mathbf{Q}} = \overline{\mathbf{T}}_{\mathrm{e}}^{+}\overline{\mathbf{T}}_{\mathrm{MT}} \qquad (9)$$

The pseudoinverse $\overline{\mathbf{T}}_{\mathrm{e}}^{+}$ was determined using the function in matlab[8].

D. Visualization of the digitally stained images

The input is an n-band, n=55 bands, multispectral image. The spectral transmittance of each multispectral pixel was calculated using the following relation:

$$\mathbf{t}_{\mathrm{o}} = \frac{\mathbf{s}_{\mathrm{o}}}{\mathbf{s}_{\mathrm{g}}} \quad , \qquad (10)$$

where $\mathbf{s}_{\mathrm{o}}$ and $\mathbf{s}_{\mathrm{g}}$ represent the $nx1$ column vectors of the object's and the glass' grey-level signals. Enhancement was performed by applying **eqn.1**, and the spectral transformation was done by applying **eqn.5** wherein the transformation matrix $\hat{\mathbf{Q}}$ was derived from **eqn.9.** For visualization purposes, the transformed spectral

transmittance and the enhanced transmittance were independently converted to their RGB color values using the expressions in eqns. 6 and 7. The PC vectors derived from $\mathbf{T}'_{HE}$ were used to estimate the spectral transmittance of the multispectral pixels. We used $m=5$ PC vectors and we set the diagonal elements of the weighting factor matrix **W** to 5, i.e. $k=5$. Figure 3 shows the physically stained H&E and Masson's trichrome (MT) images, and the results of the spectral enhancement and digital staining. The magnified images shown in fig. 4 emphasize the added advantage of the digital staining method proposed in this paper. While we can observe clearer differentiation between the muscle fiber and collagen fiber areas in the digitally stained image, fig.4d, this is not equally observed in the enhanced image, fig.4c. Also the visualization of collagen fiber is greatly improved in comparison to the original H&E stained image. The digitally stained images, figs. 3d and 4d, also exhibit similar colorimetric attributes with the physically stained MT image, figs. 3b and 4b.

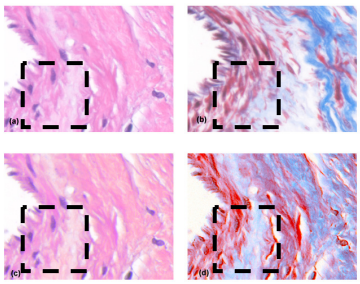

Fig.4 Magnified images of the tissue area containing collagen fiber. Physically stained (a) H&E image, and (b) Masson's trichrome image. Results of (c) enhancement; (d) digital staining.

IV. Discussions

Linear transformation of spectral transmittance has the underlying assumption that the spectral samples included in the training data set exhibit certain degree of separation, especially the original spectral transmittance data. Due to some spectral overlaps attributed by some tissue components multiple transformation matrices were designed in [3,4]. The designs of these transformation matrices defines the color of the resulting digitally stained images. The color of the output image depends on the spectral training set used in the linear mapping. Spectral variations in the original H&E stained transmittance may affect the color of the enhanced image, i.e. a shift in the hue of the output image might be observed. To perform digital staining, spectral classification was utilized to determine the appropriate transformation matrices that have to be used to convert the original spectral transmittance to its target spectral configuration. However, spectral classification has its own limitation, especially when some objects exhibit very close spectral configurations. With the integration of the spectral enhancement to the digital staining procedure we could eliminate the need for spectral classification since only one transformation matrix would be designed. This, in effect, makes the implementation of the digital staining more efficient.

The key to the spectral enhancement is the spectral difference between original transmittance, $\mathbf{t}_o$, and the estimated spectral transmittance, $\mathbf{t}'$. The difference depends on the spectral transmittance data sets from which the PC vectors were calculated, and the number of PC vectors, m, used in the spectral estimation. We have found in our experiment that for the given spectral data set 5 PC vectors produced the desirable spectral error configuration. Figure 5 shows the average spectral error configurations of the different tissue components. Here we see that while the collagen fiber exhibits noticeable peaks at specific wavelengths, the spectral errors of the rest of the tissue components are negligible. This explains why only the spectral transmittance of collagen fiber experienced a marked change after the application of spectral enhancement (refer to fig.2b). The color of the enhanced image in figs.3c and 4c is attributed to the spectral band at which the spectral error is at its maximum peak, which is around 550nm.

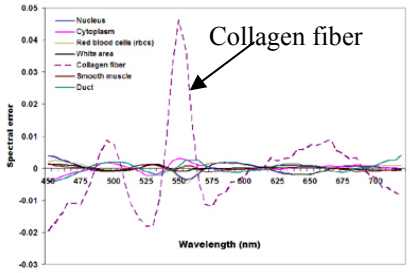

Fig.5 Average spectral difference between the original transmittance and the transmittance estimated with 5-PC vectors.

V. Conclusions

We have introduced a method to digitally convert a stained tissue-image to an image with the desired staining attributes. The combined application of spectral enhancement and linear transformation of spectral transmittance has been shown to be a viable approach to produce digitally stained images that share similar staining attributes to manually stained images.

Acknowledgment

This work is partly supported by Olympus Inc., Japan.

References

[1] W.J. Cukierski and D.J. Foran, "Metamerism in Multispectral Imaging of Histopathology Specimens," Proc. IEEE ISBI 2010, pp.145-148.

[2] W.J. Cukierski, X. Qi, and D.J Foran, " Moving Beyond Color: The case for multispectral imaging in brightfield pathology," Proc. IEEE ISBI 2009 pp.111-114.

[3] P.A. Bautista, t. Abe, M. Yamaguchi, Y. Yagi and N. Ohyama, "Digital Staining of Unstained Pathological Tissue Samples through Spectral Transmittance Classification," Opt. Review, vol.12(1) pp.7-14, 2005

[4] P.A. Bautista, T. Abe, M. Yamaguchi, Y. Yagi and N. Ohyama, " Digital Staining for multispectral images of pathological tissue specimens based on combined classification of spectral transmittance," Comp. Med. Imaging and Graphics, vol. 29, pp.649-657, 2005

[5] M. Mitsui, Y. Murakami, T. Obi, M. Yamaguchi and N. Ohyama, "Color Enhancement inMultispectral Image Using the Karhunen-Loeve Transform, Opt. Review," vol.12(2), pp. 69-75, 2005

[6] P.A. Bautista, T. Abe, M. Yamaguchi, Y. Yagi and N. Ohyama, "Multispectral Image Enhancement for H&E Stained Pathological Specimens," Proc. SPIE Medical Imaging, Vol. 6918, 2008

[7] M. Yamaguchi, T. Teraji, K. Ohsawa et al, "Color image reproduction based on the multispectral and multispectral and multiprimary imaging: experimental evaluation" Proc. SPIE col. 4663, pp. 15-26, 2002

[8] Matlab Technical Computing Version R2008a