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Detection of tissue folds in whole slide images

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Abstract—**In whole slide imaging (WSI) the quality of scanned images is an interplay between the hardware specifications of the scanning device and the condition of the tissue slide itself. Tissue artifacts such as folds and bubbles have been known to affect the efficiency of a whole slide scanning system in selecting the focus points wherein the presence of the said artifacts have been found to produce blur or unfocused images. Thus, for a whole slide scanning device to produce the best image quality, even with the presence of tissue artifacts, information on the location of these artifacts should be known such that they can be avoided in the selection of the focus points. In this paper we introduced an enhancement method to emphasize and detect the location of the tissue folds from whole slide images. Results of the experiments that we conducted on various H&E stained images that were scanned using different scanners show the robustness of the method to detect tissue folds.**

I. Introduction

With a microscope pathologists can explore the complex network of a tissue and examine any irregularities in the color and morphology of a tissue component. The introduction of internet leads to the conceptualization of telepathology whereby pathologists from different locations can access and examine the same tissue slide at the same time. The dynamic robotic telepathology which was introduced in the 1980's[1-2] evolves around the utilization of a motorized microscope which can be controlled by a user offsite and that the tissue area imaged by the microscope can be virtually viewed at the user's monitor. This technology, however, was overshadowed by the introduction of whole slide imaging (WSI) in 1990's[3-4]. Although the motivation for the development of both whole slide imaging and robotic pathology is the same their implementations are different. Robotic pathology utilizes a dynamic microscope to image small area of the tissue. On the other hand, WSI does not employ a dynamic microscope and it has the ability to image the whole slide. Aside from this, it is possible to scan several tissue slides sequentially in WSI without user interventions. After the scan is completed a seamless digital slide is produced which can be viewed using the viewer software of the scanner. Whole slide imaging indeed offers a very convenient way for pathologists to review and annotate tissue slides digitally. Today, more and more pathologists, and as more pathologists are getting accustomed to its usage it will soon be used for clinical applications.

One of the main issues in whole slide imaging is the image quality produced by these systems. Faithful reproduction of the image's true physical condition is valued the most i.e. if the image is sharp when viewed under the microscope it should be that the resulting scanned image should also be sharp. There have been studies already conducted on the effect of the presence of slide artifacts such as bubble or tissue folds[5] to image quality. However, the automatic detection of tissue folds was not addressed. In such studies tissue fold areas were manually located and the image sharpness in neighboring areas was investigated.

Generally a high resolution whole slide image has a file size of several hundreds of megabytes. In this study detection of tissue folds from the low pixel resolution version, which has a typical file size of few megabytes, of the high resolution whole-slide image is investigated. Aside from the fact that using the low pixel resolution version speeds up the detection processing, whole slide scanners generally detects tissue areas to be scanned at low resolution i.e thumbnail, and it is aimed that the method proposed in this paper, although with some modifications, will be incorporated to the scanning procedure such that areas occupied by folds are avoided in the selection of focus points.

Image enhancement methodologies facilitate the visualization of objects which are otherwise occluded in ordinary situations. In medical image analysis color enhancement facilitates ease for the segmentation and quantification of objects of interest [6-9]. To improve the visual feel of an image an enhancement of the saturation component of the image is usually employed. In this case a series of two transformation procedures is commonly performed, i.e. RGB to HSV then HSV to RGB, wherein only the saturation component is manipulated by one of several methods [10]. To directly modify the color saturation of an image without necessarily undertaking the transformation procedure, the authors in [11] introduced the shifting of RGB values. However in their method a constant shifting factor was utilized such that the saturation of all the image pixels is modified at the same rate without regard to

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which local structure the pixel belongs. That is, a constant shift factor could not emphasize object of interest and therefore it is not suited for delineating local image structures. For the purpose of emphasizing the areas occupied by tissue folds we introduced an adaptive shifting factor by taking into consideration the saturation and luminance components of each image pixel. Since tissue areas occupied by folds generally have higher saturation compared to its luminance component, it will be shown that pixel areas occupied by folds will be differentiated by utilizing the difference between the saturation and luminance components as shifting metric.

II. MATERIALS AND METHOD

A. Tissue slides

H&E stained tissue slides of different tissue types prepared at the Massachusetts General Hospital under normal condition were used in the experiment to detect tissue folds.

B. Whole slide imaging systems

Two different whole slide imaging systems were used to scan the H&E stained tissue slides. One is the NDP whole slide scanner of Olympus and the other is the dx 40 scanner of Dmetrix Inc. Both of these systems can scan in color (RGB) mode and have features for manual or automatic selection of tissue areas. However, while the Dmetrix system always selects the focus points automatically, the NDP system provides the users the option to select the focus points from the pre-scanned image of the tissue slide. To be consistent with the Dmetrix dx40 setting, the selection of focus points was set in automatic mode for the NDP scanner.

C. Enhancement of tissue folds

Although the colorimetric features of tissue folds vary among slides of differing staining conditions, they generally project higher color saturation compared to non-fold areas. To efficiently magnify the colorimetric difference between tissue folds and the rest of the tissue components, we utilized the shifting of RGB color values[10], but introduced a shifting factor whose magnitude adaptively varies so that larger magnitude is assigned to pixels which most likely belong to tissue folds. For this purpose we utilized the information in the luminance and saturation components of an image pixel:

$$f(x,y,i)_e = f(x,y,i)_o + \alpha\left[S(x,y) - V(x,y)\right], \quad (1)$$

where $f(x,y,i)_e$ denotes the enhanced ith color value at location x,y, of the RGB color components, i.e. i=R=red,G=green or B=blue; $f(x,y,i)_o$ denotes the original color value; $\alpha \in \Re$ is the enhancement coefficient that further enhances the saturation (S) and luminance (V) difference, i.e. $S(x,y) - V(x,y)$ between the saturation and luminance values of an image pixel. Neglecting the x,y location of an image pixel, its saturation S and its luminance V in the HSV system is expressed as follows:

$$S = 1 - \frac{3}{R+G+B}\left[\min(R,G,B)\right] \quad (2)$$

$$V = \frac{1}{3}(R+G+B) \quad (3)$$

The enhancement procedure to detect tissue folds from whole slide images is illustrated in diagram shown in fig.1

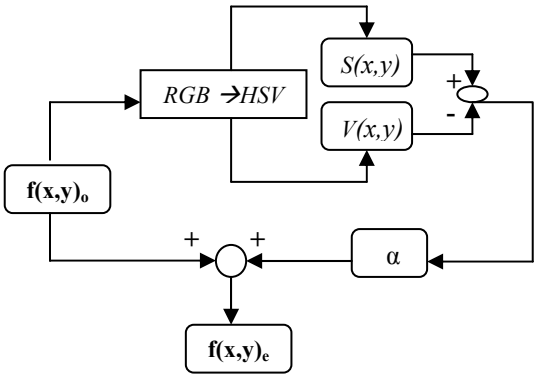

Fig. 1 Enhancement procedure to detect tissue folds

In the diagram, $\mathbf{f(x,y)_o}$ corresponds to a vector representing the original R,G, and B color values of an image pixel at location x,y, and $\mathbf{f(x,y)_e}$ to the enhanced color values; *S(x,y)* and *V(x,y)* are the corresponding saturation and luminance values of the image pixel, respectively. The saturation and luminance components are fed back to the original color values to enhance the area occupied by folds.

Let us investigate how the saturation and luminance of an image pixel are affected by the current shifting metric. Disregarding the x,y location of the pixel to simplify the expression, the new saturation value S' can be derived from (1) and (2):

$$S' = 1 - \left[\frac{3}{R+G+B+3\alpha(S-V)}\right] x \left[\min\left(R+\alpha(S-V), G+\alpha(S-V), B+\alpha(S-V)\right)\right] \quad (4)$$

$$= \begin{cases} 1 - 3\dfrac{R+\alpha(S-V)}{R+G+B+3\alpha(S-V)} & \text{if R is min} \\ 1 - 3\dfrac{G+\alpha(S-V)}{R+G+B+3\alpha(S-V)} & \text{if G is min} \\ 1 - 3\dfrac{B+\alpha(S-V)}{R+G+B+3\alpha(S-V)} & \text{if B is min} \end{cases}$$

The difference between the old and enhance saturation value of the color pixel can be denoted as:

$$\Delta S = S' - S \quad (5)$$

If R is the minimum then it is direct to show that:

$$\Delta S = \left(1 - 3\frac{R + \alpha(S - V)}{R + G + B + 3\alpha(S - V)}\right) - \left(1 - 3\frac{R}{R + G + B}\right) = \frac{3(2R - G - B)\alpha(S - V)}{(R + G + B)(R + G + B + 3\alpha(S - V))} \quad (6)$$

Noting that $(2R - G - B)$ is always a negative in (6) it is clear that the shift in the pixel's saturation depends solely on the shifting parameter, which is the product between α and $(S - V)$. The same derivation can be made when G, or B is the minimum. When S>V, as in the case for tissue fold, and setting $\alpha > 0$, ΔS becomes negative implying a decrease in the saturation of the image pixel. In contrast while the saturation decreases the luminance increases:

$$\Delta V = \alpha(S - V), \quad (7)$$

The change in luminance ΔV becomes positive, i.e. luminance increases, when $\alpha > 0$ and S>V.

III. Experiment and Results

A. Whole slide images

We selected ten (10) H&E stained whole-slide images from the slides scanned by one of the residents at MGH and one of our laboratory's technical staff. Scanning was done in semi-automatic mode which means that we selected the area for the scan while leaving the selection of focus points automatic. Since the aim of our experiment is to detect tissue folds from the low pixel resolution version of the high resolution whole slide images, for the slides that were scanned using the Dmetrix dx40 we used the snapshot function of its viewer software to capture the whole slide image; the captured image was saved in TIFF format and its image file size is about 0.30% of its original size. On the other hand, for the NDP system, we used its software toolkit to convert the original whole slide image format into JPEG format such that the image size is reduced to about 7% of its original size. Figure 2 shows sample of whole slide images whose staining condition varies.

B. Enhancement of tissue folds

The only parameter that the user has control to enhance the tissue fold is the parameter $\alpha \in \Re$. When this is positive the luminance of the tissue folds increases and we found in our experiment that $1 \le \alpha \le 2$ would appropriately enhance the tissue folds. Since it is possible for the enhanced color values to fall beyond the dynamic range of the RGB color, the resulting color values that fall beyond the dynamic range were clipped to the standard minimum or the maximum whichever is appropriate. In fig.3 the enhanced images are displayed. For these images the enhancement coefficient was set to 1.5. Here we can see that the colorimetric difference between tissue folds and the rest of the tissue components is increased and we can also easily detect and locate the tissue folds as their luminance is the highest.

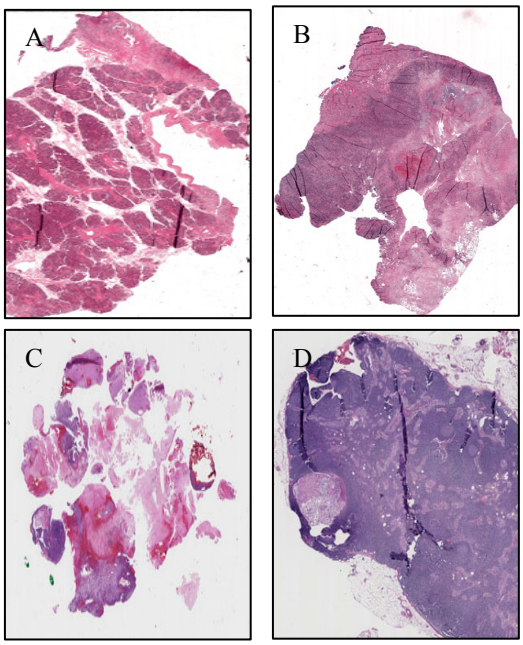

Fig. 2 Whole slide images of tissue slides: A and B were scanned using Dmetrix dx40; C and D were scanned using the Olympus NDP scanner.

IV. DISCUSSIONS

Since H&E staining is the most common among the different types of staining, we used H&E stained tissue images in our experiment. Variation in the staining condition among tissue slides makes it complicated to choose which color channel to use for the detection of tissue folds[12]. However, the utilization of the saturation and luminance information in the enhancement procedure enables the detection of tissue folds independent from the staining condition of tissue images. From the enhanced images shown in fig.3 it is apparent that tissue folds can be localized and segmented directly using the luminance information of the enhanced image.

Shifting the RGB colors by a constant leaves the hue of the image unchanged while changing its saturation [11]. This is true for the current results except for pixels which have high color saturation such as the tissue folds. The color enhancement expressed in (1) therefore only affects the hue of those image structures whose difference between their luminance and saturation are high enough.

The magnified images shown in fig. 4 further assert the effectiveness of the present enhancement scheme to detect tissue folds. We can observe that while folds appear occluded in the original image they are highly emphasized in

the enhanced images.

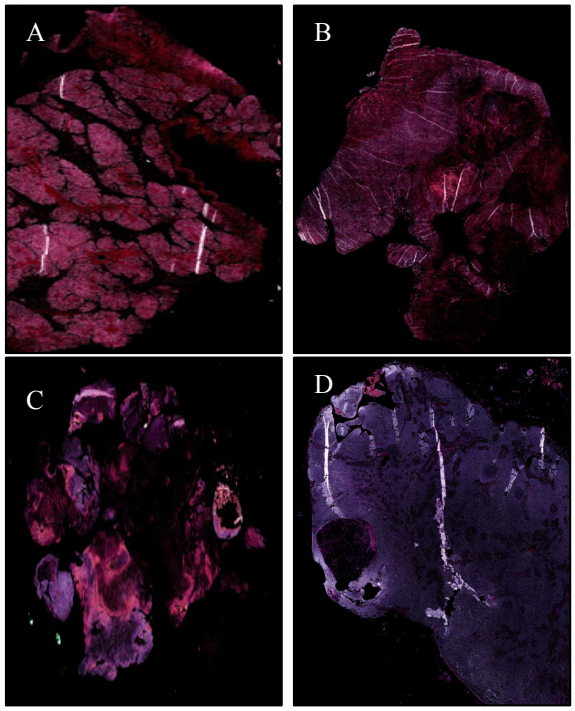

Fig.3 Whole slide images enhanced by setting α=1.5. Scanned using Dmetrix A & B; Scanned using NDP C & D;

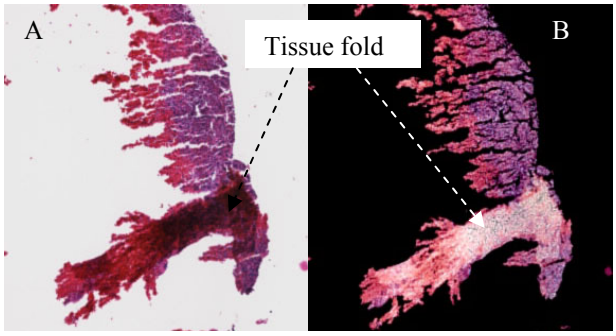

Fig.4 Tissue area containing folds at magnified view. A. Original image; B. after enhancement.

While detecting tissue folds at low resolution has been shown to be possible given the above results, the method may not be totally effective when tissue folds only occupied a very small area such that lowering the resolution of the image will cause these folds to be occluded. In this case, higher resolution version of the image may produce better result. The utilization of the low resolution version of the whole slide image was mainly motivated by the fact that whole slide scanners detect tissue areas and select the focusing points at low pixel resolution, i.e. thumbnail version, and it is usually the larger tissue fold areas that are most likely to affect the quality of scanned images. Moreover, it is part of our future work to incorporate this fold detection methodology to the scanning procedure in whole slide imaging whereby the scanner can detect the presence of tissue folds from the thumbnail image of the whole slide before selecting the focusing points.

IV. Conclusions

Shifting the RGB values to modify the color saturation of an image is an efficient procedure as there would be no need to undertake the forward and reverse transformation processes. A constant color shift however is not effective to enhance specific objects in the image. In this paper we have presented a method to detect a particular image structure, i.e. tissue folds, by adaptively shifting the RGB values based on the difference between the saturation and luminance components of each image pixel. The experiment results showed that the enhancement method presented in this paper can effectively delineate the presence of tissue folds while preserving the hue of other tissue structures.

Detecting and quantifying the presence of tissue folds from whole slide images can be very useful in assessing the image quality of the image, i.e. how much tissue folds the scanner system can tolerate to produce high quality images, and in the implementation of automatic image analysis geared towards quantification of nuclei [12]. Part of our next study is to quantify the amount folds present in the whole slide image and correlate this to the resulting image quality of the scanned image.

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