行政院國家科學委員會補助專題研究計畫成果報告

腎切片影像分析與臨床數據之量測

計畫類別：■個別型計畫　□整合型計畫

計畫編號：NSC 89－2213－E－041－009－

執行期間：89 年 08 月 01 日至 90 年 07 月 31 日

計畫主持人：劉育寰

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中　華　民　國　九十　年　七　月 三十一 日

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腎切片影像分析與臨床數據之量測

The Analysis of Renal Biopsy Images and the Measurement of its Clinical Data

計畫編號：NSC 89－2213－E－041－009－

執行期限：89 年 8 月 1 日至 90 年 7 月 31 日

主持人：劉育裹　嘉南藥理科技大學 資訊管理系

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中文摘要

本計劃延續上年度計劃，針對腎絲球及腎小管做臨床數據的量測。在腎絲球部分，利用 Hough 轉換找出腎絲球內紅血球及細胞核的外廓，再以其周圍環境為條件自動分辨出紅血球及細胞核等物件，進而計算臨床數據供醫師參考。至於腎小管部分，則提出色彩正規化(Color Normalization)理論，成功地解決切片色彩不一致的問題。然後利用色度圖 CIEXYZ 色彩空間分割出腎小管部位，其效果較傳統式的 RGB 色彩空間還好。建置一套系統，其圖形介面提供臨床醫師良好的操作環境，並有數據報表製作功能，有利於醫師診斷之用。

關鍵詞：色彩正規化、Hough 轉換、腎切片分析、色度圖分割。

Abstract

This project, which is the continuation of the previous research, focuses on the clinical measurements of the glomerulus and renal tubular. In the part of glomerulus, the Hough transform is used to extract the boundary of the red blood cells and nucleus. Then, the classification of both objects can be automatically performed based on their environments. The color normalization is proposed and successfully resolves the problem of the inconsistent color of biopsy. The segmentation of the renal tubular is then carried out by using the Chromatic color space. The segmentation results are better than that using the traditional RGB color space. A software system with graphic user interface is developed to provide good operation environment and complete report for clinical application.

Keywords: Color Normalization, Hough Transform, Renal Biopsy

Introduction

Renal disease, such as nephritis or nephropathy, is one of the ten major death diseases in Taiwan. Accordingly, how to achieve early diagnosis and enhance treatment for kidney disorder would be the major lesion of our medical systematically[1],[2]. Nevertheless, the clues from the clinical data, such as biochemistry examination, serological examination and radiological studies are quite indirect and limited. Because of the progress of basic medical science of molecular and cellular biology, pathological reading of renal specimen is not only for staining of tissue structure, length or area of target site. The small and weak signals from the intracellular target gradually become the major roles for disease pathogenesis. However, these always need special tissue staining and quite deftly expert technician.

The major problem of the renal biopsy image analysis is the inconsistence of the hue, which can interfere the criteria of image processing. The neural network method was used to obtain the color patterns for a sequence of processes by training steps[3]. However, the training steps are very time consuming and are not easy to operate for the clinicians.

Under the continuing evolving of instrumentation and technology, tissue pathology has played a decisive role in diagnostic and clinical medicine. Therefore we propose to investigate and analyze the electric microscopic biopsy image of tissue structure of glomerulus in renal and then establish a system to help doctor analyze in clinical. By utilizing the image processing technique to achieve the function of automatic analysis and doing statistics of images, the tedious and time consuming routine image examination procedures expect to be reduced. Therefore it will reduce the human error from the individual diagnosis and obtain more objective analytical data for the diagnostic.

Method

As we know, color can be qualitatively described using the terms hue, saturation (also referred to as chroma), and intensity (also referred as to brightness), which is the HSI color model. The major component of color is hue. Visually, different hue makes the image present different color. Videlicet, if we can make the color of AOIs on different images with the same hue, then we can say that the different images are in the same color. This assumption is very helpful for our study. Therefore, the hue histogram warping technique are applied to make the color of AOIs on different images be the same.

There are two steps in our research; the first is the color normalization by means of HSI histogram warping, and the second is the segmentation based on the chromaticity diagram. They are described in detail as following subsections.

Color Normalization by Means of Warping HSI Histogram

Warping hue histogram means a transformation of the hue histogram. If we want to modify the color of Figure 1 (b) into the color of Figure 1 (a), then the hue of the AOIs have to be obtained. The hue histogram of Figure 1 (a) and Figure 1 (b) are shown in Figure 2 (a) and (b), and the hue of AOI locate at the red '*' mark position. In the hue histogram, the hue value equal to zero represents the red color, and the hue value equal to 1 also represents the red color. The hue histogram is circular repeat.

The simplified case is only one AOI in each image. Let the hue of the AOI in Figure 1 (a) be A_1 and A_2 in Figure 1 (b), respectively. Now, the hue histogram of Figure 1 (b) has to be translated to make A_2 be A_1. The hue component of each pixel in Figure 1 (b) (hue_2) adds the value of $(A_2 - A_1)$, as shown in Eq. (1).

$$hue_2 = hue_2 + (A_2 - A_1) \tag{1}$$

Since the hue histogram is circular repeat, the following step is necessary.

$$hue_2 = \begin{cases} hue_2 - 1 & \text{,if } hue_2 > 1 \\ hue_2 + 1 & \text{,if } hue_2 < 0 \end{cases} \tag{2}$$

The Segmentation Based on the Chromaticity Diagram

The C.I.E. (Commission Internationale de l'Eclairage) has developed a standard artificial primary coordinate system in which all tristimulus values required to match colors are positive[4],[5]. The amounts of red, green, and blue needed to form any particular color are called the tristimulus values and are denoted, X, Y, and Z, respectively. A color is then specified by its trichromatic coefficients, defined as

$$x = \frac{X}{X+Y+Z}$$
$$y = \frac{Y}{X+Y+Z} \tag{3}$$
$$z = \frac{Z}{X+Y+Z}$$

Obviously, from these equations,

$$x + y + z = 1. \tag{4}$$

The C.I.E. chromaticity diagram is used for segmentation in this project. In C.I.E. chromaticity diagram, we find that the colors with little hue deviation locate together, while the RGB color system does not have such behavior. This phenomenon can be used for obtaining better color segmentation results. In addition, hue and saturation taken together are called chromaticity. The segmentation used two parameters (hue and saturation) may be better than that used single parameter[6].

In clinic the tissue anomaly in the biopsy of glomerulus is an important indication to diagnose if there is a disease in renal. Usually the number of cells in glomerulus is a key point to diagnose if there is a disease in renal; by observing the HE color enhanced images, we can find out the location of the nucleus and from this we can tell the number of renal cells.

In glomerulus analysis, we reduce the noises from the original image by Gaussian blur filter and then transform it to a gray level image. In order to locate all the edges of the nucleuses in the image, we utilize the Sobel edge detection as an edge detector. Via the gradient operation in the horizontal and vertical directions in the image, we obtained Gx and Gy two images respectively. The gradient image then can be approximated by $\nabla f = |Gx| + |Gy|$. This gradient image contains the information of the edges of nucleuses. We will use this information to locate all the nucleuses.

Obviously, nucleuses are circular like. Hough proposed a concentric detection method called Hough transform[4],[5]. For the special character of renal biopsy image, we made some changes. In the process of transforming we directly used the gradient image after Sobel transform and selected a threshold value to get rid of the none edge points. In addition, by using the gray level values of the transformed image as accumulative weights, the result we obtained is more precise. Because in the image, the nucleuses are not all the same sizes and the accumulative weights are great difference, the radius parameter is in a range rather than just a fixed value through the whole transforming process. Moreover, we reduce the resolution of the parametric space in the transforming process in order to lower the computation time.

After the Hough transform, we can find out points that add up more times than others in accumulator cells. These points will correspond to centers of circles and with radius they will form specific circles, which will probably be the cells that we are looking for. We adopt small threshold values, but these points have to be the local maximum in order to determine the location and sizes of the nucleuses.

In our system, we provide an easy and friendly user interface (fig-2) in order to reduce the error arising from estranged operation. The system will automatic output the number of blood cells and nucleus (fig-5) after the operator provide a few parameters (fig-3) and circle an active scope (fig-4). This information will provide doctors for pathological reading.

Results

A renal biopsy image is show in Figure 1 (a). The AOIs are the proximal renal tubule with purple color (label as 'A') and the tubular nucleus with dark purple color (label as 'B'). Figure 1 (b) shows the other renal biopsy image. Observably, the colors of these two images are extremely different.

The Color Normalization

The hue histograms of Figure 1 (a) and (b) are shown in Figure 2 (a) and (b), respectively. To simplify the procedure, only the renal tubule is dealt with. The red star mark in Figure 2 (a) and (b) represent the hue of the renal tubule on the position of label 'A', which are interactively selected by GUI interface. Since hue represents the color in term of angle, we use the circular warping to make the both selected hues be the same. Figure 3 shows the hue histogram of Figure

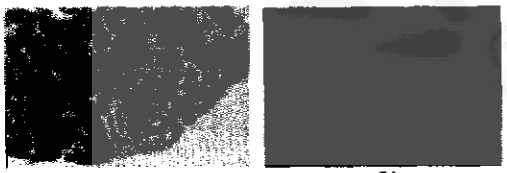
1 (b) after circular warping, and the resulting image is shown in Figure 4. Actually, the colors in Figure 1 (a) and Figure 4 are not exactly the same, but the colors of renal tubule in the two images are very close. Therefore, the results of the color normalization are acceptable.

The segmentation

As we mention before, we use the chromaticity diagram for the segmentation. The chromaticity diagrams of Figure 1 (a), (b), and Figure 4 are shown in Figure 5, Figure 6 (a), and (b), respectively. The '+' mark in Figure 6 (a), and (b) represents the original color of the renal tubule. In Figure 6 (b), the yellow '+' mark represents the color of renal tubule after circular warping of hue histogram, and the cyan '+' mark represents the color of renal tubule in the Figure 1. Observably, they are very close. Therefore, we can use the same color patterns to segment the renal biopsy images, which are applied the hue histogram warping, for obtaining the stable segmentation results.

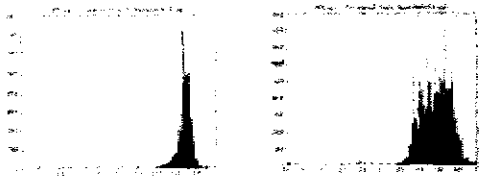
The segmented image of Figure 1 is shown in Figure 7 (a). The renal tubule, tubular nucleus, and the background are represented in green, red, and blue colors, respectively. The resulting image seems to be more reasonable than the conventional method Figure 7 (b).

Figure 8 illustrates the segmentation of the glomerulus. With the original biopsy image that went through the procedures, we got the result image and the scatter of red blood and nucleus. Compare the result and the original image, we can identify the purple part is the nucleus and the redder part is the blood cell. Figure 9 are our experimental results after setting suitable parameters. The report dialogs tell doctors there are 92 and 53 nucleuses respectively. Comparing these outputs with the clinical doctors' results, we can get above 87% accuracy.



(a) (b)

Figure 1: Original renal biopsy images.



(a) (b)

Figure 2: Hue histogram of Figure 1 (a) and (b)

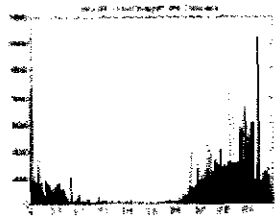


Figure 3: Histogram of hue after circular warping.

Figure 4: Resulting image after circular warping.

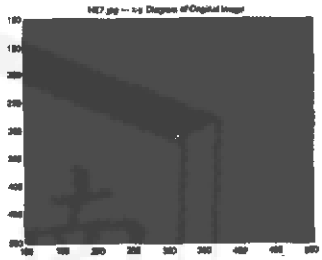
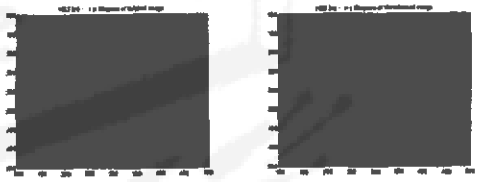


Figure 5: The chromaticity diagram of Figure 1.(a)



(a) (b)

Figure 6: The chromaticity diagram of Figure 1 (b) and Figure 4.

(a) (b)

Figure 7: The segmentation based on the chromaticity diagram (a) and RGB (b).

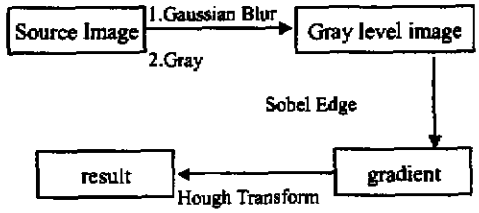


Figure 8 Flowchart of the segmentation of the glomerulus

Figure 9 The segmentation results of the glomerulus

Conclusion

A piece-wise linear warping technique is developed to perform the color normalization. The color normalization can successfully overcome the drawback of the inconsistent colorant during the staining procedures of the renal biopsy. The segmentation based on chromaticity diagram can obtain more reasonable results. The proposed method can reduce the interactive operations during the analysis procedures; therefore, we can setup a almost automatic system for biopsy image analysis. Such a system is very convenience for the clinicians.

We use our proposed method to establish a system to analyze the structure of glomerulus. It can greatly simplify the routine and tedious procedures for examining the renal biopsy images and provide a set of precise data for analysis. With the add of these information we believe that in clinic we can provide doctors a set of quantified data as a reference for diagnosing the renal disease.

In clinical application, not only the information of number of cells can be provided in glomerulus but also like the number of capillaries, the thickness of basement membrane and the number of inflammatory cell are the important significance. With the assistance of these data we believe it might to provide doctor a strong reference in decease reading.

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