Vessel Box Counting Dimension of Chicken Chorioallantoic Images

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Abstract

Because cancer cells have to rely on oxygen and nutrients in biological vessels to survive, researchers usually need small animals for experimental sample (for example: rabbits, mouses, etc ….), put cancer cells into the small animals' bodies to make them infected, inject the experimental drug and observe the changes in blood vessels to determine whether the experimental drug resist cancers effectively. In recent years, the cost of animal samples becomes increase. Moreover, the animals must be dissected in the experiments, The processing of animal experiments is very troublesome. However, the chicken chorioallantoic membrane grows faster, the price is cheaper, and it is easy to observe the results immediately. The chicken chorioallantoic membrane which has vascular structure is suitable to replace other animal samples. Therefore, we proposed a technology to determine the results of the chicken chorioallantoic membrane. This technology can be divided into two parts: non-yolk and yolk region segmentation and vessel segmentation in yolk region. We use Box Counting Dimension (BCD) to determine the density of blood vessels. Then, for BCD values of Ground Truth, proposed methods and artificial blood vessels judgment methods are calculated. According to the experimental results, Box Counting Dimension (BCD) values of the proposed method are close to those of Ground Truth. The proposed method has better results.

Keywords: chicken chorioallantoic membrane, blood vessels segmentation, box counting dimension

1. Introduction

There are many kinds of tumors causing the death of many people. It has been the leading first of first ten causes of death for humans in the past ten years. As the reason, researchers are urgent to find the solution. In the recent years, researchers found out that when tumors grow over 2~3 centimeter, oxygen and nutrient are necessary to support it to make it grow quickly. If there are not any newborn vessel around the Tumor, it will not be able to grow quickly. There is a fortune for human to inhibit the growth of vessels to control the Tumor's growth and metastasis [5].

There are many substances inhibiting the growth of vessels, which can divided into four parts: (1) A substance that can inhibit tumor to secrete what can make newborn vessels grow quickly, such as Interferon which can inhibit tumor from secreting bFGF. (2) A substance that can inhibit newborn vessels from reaction, such as suramin which can inhibit bFGF to combine with its acceptor. (3) A substance that can inhibit endothelial cells from making newborn vessels react, such as thrombospondin and angiostatin. (4) A metabolite which can interfere the matrix around 錯誤! 找不到參照來源。]錯誤! 找不到參照來源。]錯誤! 找不到參照來源。]錯誤! 找不到參照來源。]錯誤! 找不到參照來源。]錯誤! 找不到參照來源。].

Tumors can make vessels grow, so they start to absorb oxygen and nutrient to make them grow up, such the experiment often applied to rabbits and rats as the samples. Putting the gene which may lead to tumors outburst to the sample for a while, if its vessels start to grow quickly and then shrivel quickly, the experimenter puts the medicine into its body and keeps observe whether its vessels still grow quickly and then shrivel quickly or not. If the sample's vessels still grow quickly and then shrivel quickly, it says that the medicine didn't work on the tumor, otherwise it can work on the tumor.

So we can obtain two results from this experiment : (1) Whether the gene can lead to tumor outburst or not. (2) Whether the medicine can work on the tumor or not.

However, these samples must be brought up carefully to adults, so this can spend a lot of time and patient. Because the cost of these samples is high, the experiment cost is also high too. To observe vessels in samples, dissect samples is necessary. The process of is very complex, and dissected samples are almost dying. The dissection process after the experiments is also complex, and this behavior is protested by Animal Protection Association (APA). To solve the problems above, some experiments may use chicken chorioallantoic [2]. It is very appropriate for this kind of experiment because chicken chorioallantoic also has vessels. The eggs of chicken chorioallantoic only spend 18 days to hatch out since it was born from a

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hen. It is easy to observe vessels after putting gene or medicine because you don't have to dissect it, just cracking the eggshell.

Since the growth of tumors is related to the growth of vessels, the density of vessels become an important index to indicate whether the medicine works on the tumor or not. This paper will segment vessels in chicken chorioallantoic images for evaluation. To calculate the density of vessels, we use Box Counting Dimension (BCD) for evaluation. We will discuss the method in detailed in Chapter 2. We introduce the basic concept of BCD below:

$$BCD = \frac{\log N(E)}{\log\left(\frac{1}{E}\right)} \quad (1)$$

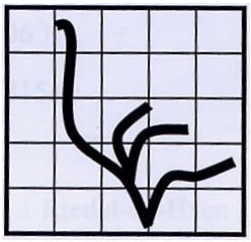
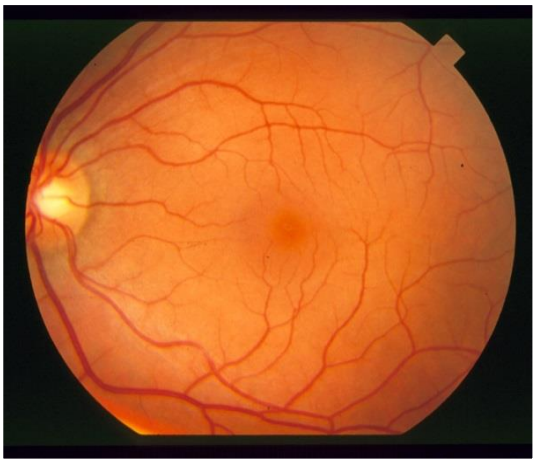


Figure 1: The example for calculating the density of vessels by BCD

BCD is a method to calculate the density of vessels in a matrix; N(E) indicate the quantity of grids which vessels pass through; 1/E indicate the edge size of the matrix. For example, there is a matrix shown in Figure 1, where the edge size is 5, so 1/E is 5. The quantity of grids which vessels pass through is 11, so N(E) is 11. Then we use Equation above to get BCD which is 1.49. BCD value has positive correlation with the density of vessels. If BCD value gets low, it indicates that the vessels density of this image is low. As the result, using BCD to calculate the density of vessels is very appropriate [9][6][11].

Because the quantity of experimental images is too large, and health care workers must calculate BCD values by themselves. It spends too much time and patient, so it is necessary to use computer technology to help them. We propose an image process technology to segment vessels in chicken chorioallantoic images and calculate BCD values. Health care workers just input the image into the system, and it will help them to judge the result of experiment.

Although the technology of image segmentation and recognition are widely implied on medical area, these are not appropriate for chicken chorioallantoic images. For example, in Figure 2 retinopathy images are shown where vessels are less than chicken chorioallantoic images so to use a microscope to take pictures. This can make the photo edges more smoothly, and let image process more easily. However, chicken chorioallantoic images are taken by a digital camera, so it causes shadows, and eggshells, egg membrane taken into the photo. When health care workers cracks the eggshell, it may scratch vessels and let blood flow out. These reasons makes image segmentation more difficult. This paper proposes a better image segment method to separate yolk area, non-yolk area and vessel area as shown in Figure 3.



(a)

(b)

Figure 2: Difference between retinopathy image and chicken chorioallantoic image. (a) retinopathy image(b) chicken chorioallantoic image

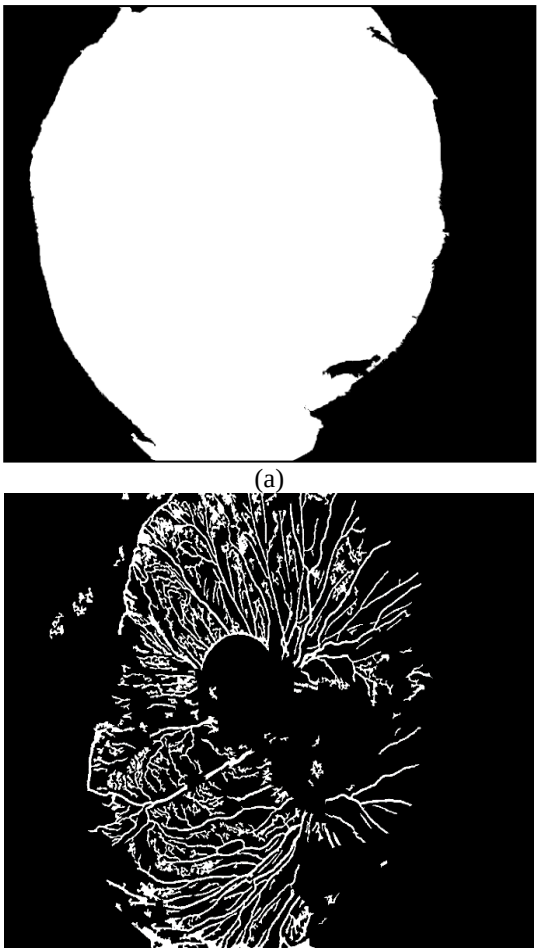


Figure 3: Yolk area, non-yolk area and vessel area. (a) White region is yolk area; black region is non-yolk area (b) White region is vessel area

2. Related Work

In this chapter, we will introduce the technology used.

2.1 Mathematical Morphology

Mathematical Morphology is a way for image processing, and is used to analyze and process the shape of digital images. There are a lot of operation in Mathematical Morphology. In this chapter, we will introduce the operations used in this paper.

2.1.1 Dilation & Erosion

In Dilation and Erosion method, we use structuring elements or kernel to process binary images, meaning that we use a matrix B as a mask to change the shape of regions in an image. Dilation is used to make a region bigger and let slit connect. Erosion is used to make a region smaller to separate the slit which connect two regions, and remove the smaller regions. Dilation operations are as follow:

We use structuring element (SE) B to scan the entire image A to make white area bigger, and Image A is dilated by a 3×3 structuring element B.

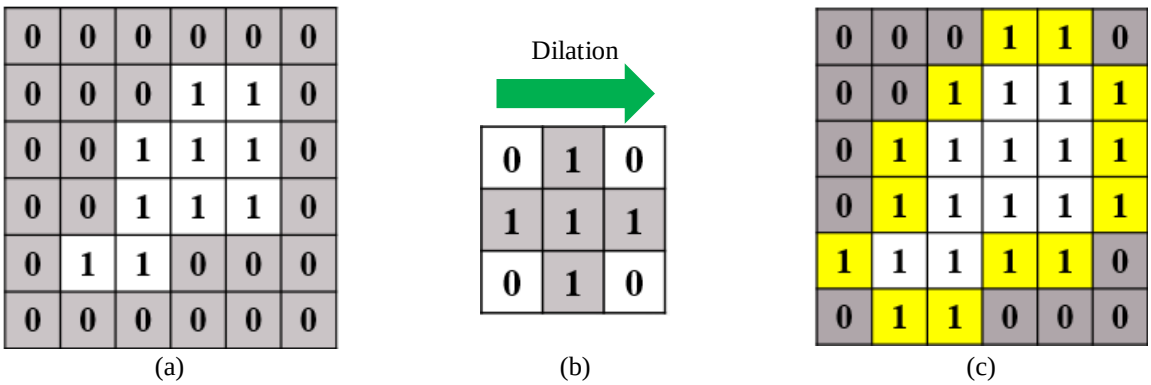


Figure 1: The process of Dilation. (a)Image A (b) structuring element B (c)Result of Dilation

An erosion operation uses a structuring element (SE) B to scan the entire image A to make white areas smaller, and image A is eroded by a 3×3 structuring element B.

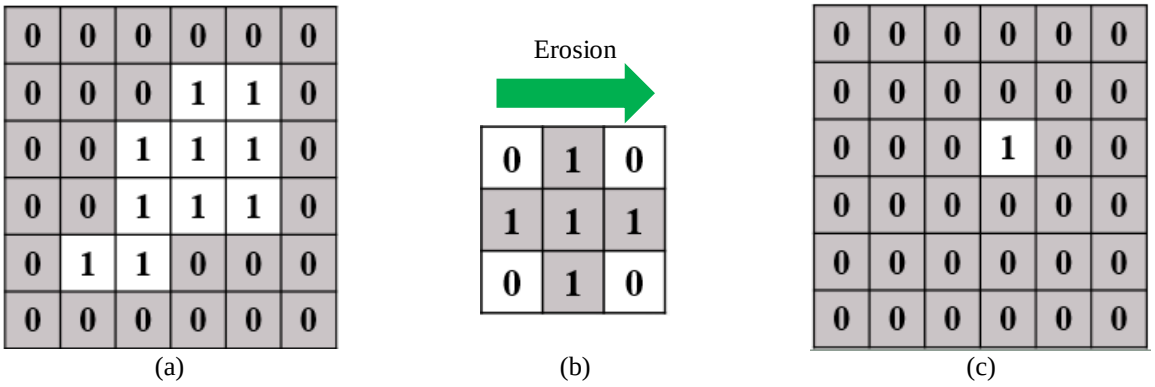


Figure 2: The process of Erosion. (a)Image A (b) structuring element B (c)Result of Erosion

The next operations – Opening and Closing consist of Dilation and Erosion. Opening means erosion image A_O is then dilated. After using Opening, some smaller region will be removed; others will become smaller, and the connection with the white area will be disconnected. Opening operations are as follow:

We use structuring element (SE) B to scan the entire image A_O to make white areas smaller. Image A_O is eroded first and then dilated by a 3×3 structuring element B.

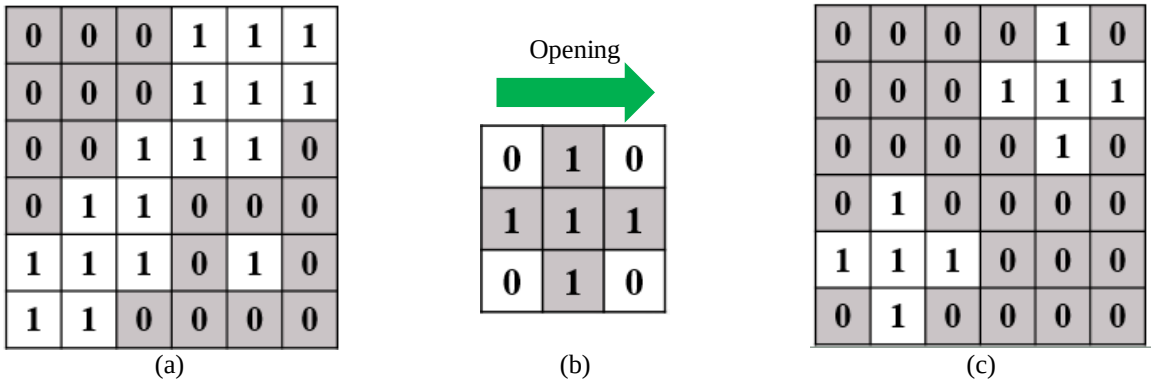


Figure 3: The process of Opening. (a)Image A_O (b)structuring element B (c)Result of Opening

Closing means image A_O is dilated first, and then eroded. After using Closing, the edge will be smooth, make gaps connected, and fill the gap in the white region. Closing operations are as follow:

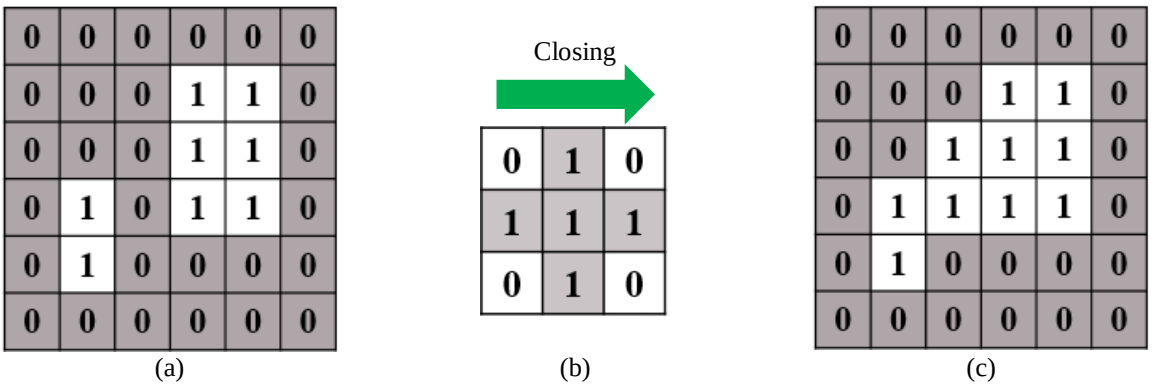


Figure 4: The process of Closing. (a)Image A_C (b)structuring element B (c) Result of closing

2.1.2 Thinning

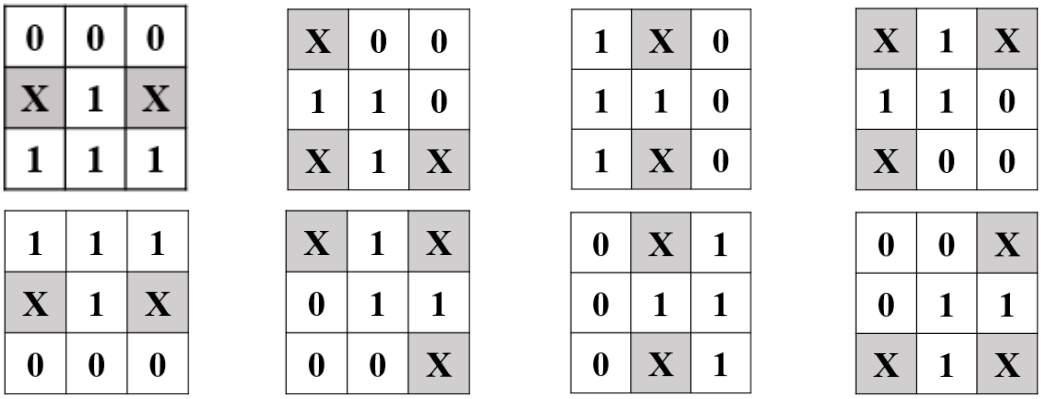


Figure 5: Structuring elements SE1~8 using in Thinning

Thinning can transform the shape of objects in binary image A_{Thin} into one pixel. It uses eight 3×3 structuring elements $SE_{1\sim8}$ to decide every pixel in image A_{Thin} as shown in Figure 10. When the pixel $A_{Thin}(i,j)$ is fit with $SE_{1\sim8}$, we set $A_{Thin}(i,j)$ to 0. Thinning operations will be continued until there are no any pixels fit with $SE_{1\sim8}$ in image A_{Thin}, and then the operation is finished.

2.2. Run-Length

To observe vessels in chicken chorioallantoics which were put with tumors and medicine, we have to separate vessels from yolk area. However, microvessels in chicken chorioallantoics may be too thin, too bending, or its pixel value is similar to the pixel around. It makes us more difficult to segment, so we use Run-Length which can use pixel values from different directions to enhance microvessels in chicken chorioallantoics and also remove noises from images. Run-Length operations are as follows:

For each pixel P(i,j), we set it as a center, and make a window w_r contains L×L pixels. Then lines eight lines $l_0, l_1, \dots, l_7$ are through P(i,j), each angles between two line nearby is 22.5° as shown in Figure 11. If P(i,j) is higher than the average of w_r (Avg w_r), then we set P(i,j) as the maximum of Avg $l_0, l_1, \dots, l_7$; if lower than Avg w_r, then set P(i,j) as the minimum of Avg $l_0, l_1, \dots, l_7$ as shown as Equation (2).

$$P(i,j) = \begin{cases} Max\ (Avg\ l_0, l_1, \dots l_7), if\ P(i,j) > Avg\ w_r \\ Min\ (Avg\ l_0, l_1, \dots l_7), otherwise. \end{cases} \quad (2)$$

Thus, we can enhance the shapes like lines and edges. In this paper, we can use it to enhance vessels in yolk areas.

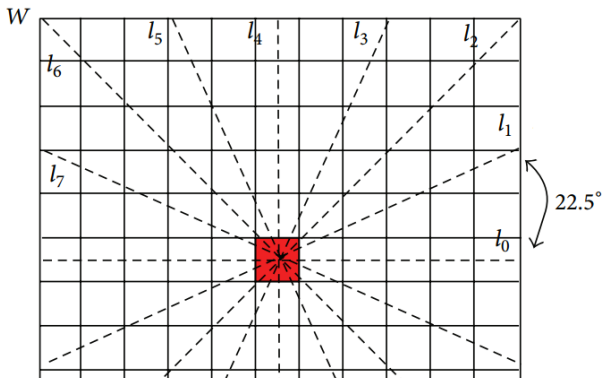


Figure 11: The process of Run-Length

2.3. BCD (Box Counting Dimension)

BCD(Box Counting Dimension) is a method to compute a fractal dimension. In biology, it is used to calculate the quantity of blocks which vessels pass through in a S×S matrix of 1mm×1mm blocks. However, the resolution of samples in this paper are not the same, so we can't use traditional BCD method. Instead, we use the method below:

Shown in Figure 12, use a 5×5 matrix to cover the yolk area, and compute the total quantity of pixels ($P_{total\_central}$) and the quantity of vessels pixels $P_{vessel\_central}$ in the central matrix. Then compute the total quantity of pixels ($P_{retangle}$) and the quantity of yolk pixels (P_{yolk}) in the matrix. Finally, we put the quantity of pixels above into Equation (3):

$$\mathrm{BCD} = \frac{\mathrm{P_{vessel\_central}}}{\mathrm{P_{total\_central}}} \times 25 \times \frac{\mathrm{P_{yolk}}}{\mathrm{P_{retangle}}} \quad (3)$$

If BCD is higher, it indicates the density of vessels in this image is higher, too. Otherwise, the density of vessels in this image is lower.

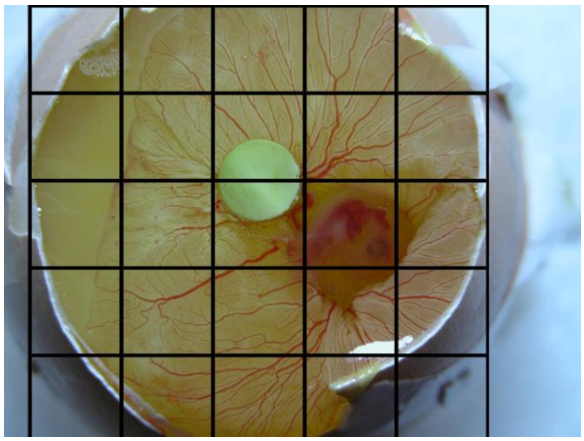


Figure 12: The example of calculation of BCD value

3. Vessel BCD Method

In this chapter, we will introduce the technology and method used in this paper, which are separated into two parts: yolk area and non-yolk area segmentation, and vessel area segmentation. In Section 3.1, we will segment yolk and non-yolk area in eggs, and transform color images into R scale image, G scale image and B scale image in the beginning to contrast yolk and non-yolk area. Then we use Region Labeling, Opening and Thinning to contrast yolk and non-yolk area completely. In Section 3.2, we focus on separating vessels from yolk area. We will use Run-Length and Local Contrast to enhance vessels, and then transform images into binary images. Finally, we use Label Cross Thresholding to separate vessels from yolk area.

3.1 The Segmentation of Yolk Area and Non-Yolk Area

First, we transform color images into R scale image, G scale image, and B scale image, as shown in Figure 13. Because the characteristic of these three kinds of gray scale images are all different, such as vessels in G scale images and the contrast between the yolk and non-yolk area are obvious. Because the color of yolk area is deeper and non-yolk area is lighter in B scale images, we select the maximum pixel value from pixel values which are in the same location of these three images. Then subtract the pixel value which is in the same location of B scale image from the pixel value, and divide by the maximum pixel value, as shown by Equation (4). So we can get a proportion, then we set two different thresholds to classify. If the proportion is lower than the small threshold, we set the pixel value as 0, otherwise set the pixel value as 255. The proportion between these two thresholds will be calculated by Equation (5) below:

$$\mathrm{P(i,j)} = \left|\frac{Max\ - B(\mathrm{i,j})}{Max}\right| . \tag{4}$$

$$\begin{cases} \mathrm{P(i,j)} = 0, if\ \ \mathrm{P(i,j)} < T, \\ \mathrm{P(i,j)} = 255, if\ \mathrm{P(i,j)} > T_2, \\ \mathrm{P(i,j)} = \left(\dfrac{\mathrm{P(i,j)} - T}{Max\_P - T}\right)^{r_c} \times 255. \end{cases} \tag{5}$$

In Equation (4), Max is the maximum value the maximum pixel value from pixel value which are on the same location of three images; B is the pixel value on B scale image, P is the output; Max_P is the maximum proportion ; r_c is the gamma value; the testing value is 0.6; T is the lower threshold; T_2 is the higher threshold; and we get 0.475 and 0.92 from testing as shown in Figure 14 錯誤! 找不到參照來源。]錯誤! 找不到參照來源。].

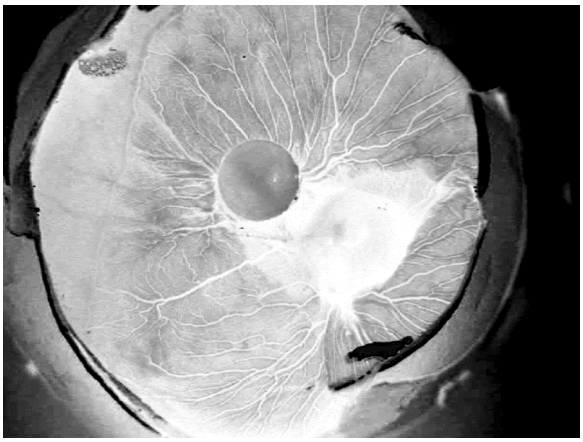
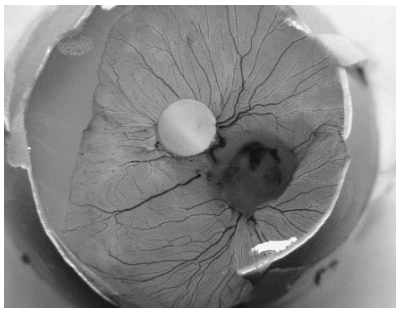


Figure 6: To contrast yolk area and non-yolk area by Equation (4)

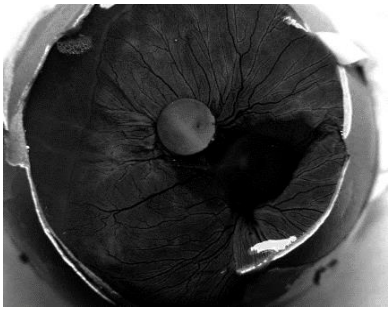
The pixel value of yolk area in R scale images is lower and more smooth, so we use this to enhance the result where we get on the last step. As shown in Equation (6):

$$PR(\mathrm{i,j}) = (1 - x) \times P(\mathrm{i,j}) + x \times R(\mathrm{i,j}) \tag{6}$$

(a)



(b)



(c)

Figure 7: Three kinds of gray scale images. (a) R scale image (b) G scale image (c) B scale image

$$\mathrm{PR_{contrast}(i,j)} = \left(\frac{PR(\mathrm{i,j}) - Min}{Max - Min}\right) \times 255. \tag{7}$$

In Equation (6), P is the result of the last step; R is the pixel value which is on the same location of R scale image; x is the range between 0 to 1; PR is the result of Equation (6). This Equation is to combine P (i,j) and R(i,j) to get a new pixel value called PR(i,j); after testing we get 0.35 to x value as shown in Figure 15(a). After that, we put PR(i,j) into Equation (7). Max is the maximum value of PR; Min is the minimum value of PR; $PR_{contrast}$ is the result of Equation (7). Then we can see the contrast is more obvious after using R scale image to enhance.

The next step is to remove non-yolk area. We use the Equation below:

$$\begin{cases} \mathrm{PR}_{noback}(\mathrm{i,j}) = 0, if\ \ \mathrm{PR}_{contrast}(\mathrm{i,j}) < T_3, \\ \mathrm{PR}_{noback}(\mathrm{i,j}) = 1, if\ \mathrm{PR}_{contrast}(\mathrm{i,j}) > T_4, \\ \mathrm{PR_{noback}(i,j)} = \left(\frac{\mathrm{PR}_{contrast}(\mathrm{i,j}) - T_3}{Max - T_3}\right)^{r_n} \times 255. \end{cases} \tag{8}$$

In Equation (8), T_3 is the lower threshold and T_4 is the higher threshold; the testing results are 150 175, and Max is the maximum value of $PR_{contrast}$, r_n is the gamma value; PR_{noback} is the result of removing non-yolk area. If $PR_{contrast}(i,j)$ is lower than T_3, then we set $PR_{contrast}(i,j)$ as 0; if $PR_{contrast}(i,j)$ is higher than T_4, then we set $PR_{contrast}(i,j)$ as 255. In the pixel between T_3 and T_4, we subtract T_3 from it, then divide by the value that subtracts T_3 from Max. Finally, we use r_n which is 0.3 after testing to contrast, then multiply by 255, and we can get PR_{noback}. Shown in Figure 15(b).

After removing non-yolk area, we will transform the result into a binary image. This step is to let the pixel between 0 and 255 be transformed into 0 and 255. However, we don't use the general method to transform such as Otsu. Instead, we use Local Cross Thresholding to get the threshold.

In the beginning, PR(i,j) is considered as the center to extend a cross K. i is the X axis of the pixel, and j is the Y axis of the pixel. K indicate K_l pixels from PR(i,j) in four direction that means up, down, right and left. K_l means the quantity of pixels of the cross. $T_{(i,j)}$ is the average of the cross, and n is the quantity of pixels of K as shown in Equation (9). If PR(i,j) is higher than T(i,j), then set it as 1, otherwise, set it as 0. Thus, we get the result of binarization – $P_{bw}(i,j)$.

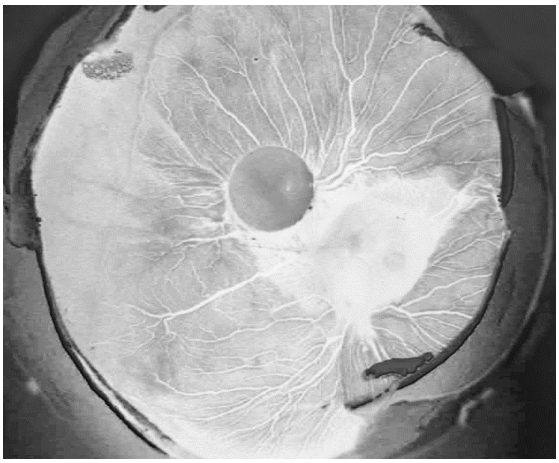
$$K = \begin{Bmatrix} PR_{(i-K_l,j)}, PR_{(i-(K_l-1),j)}, \dots, PR_{(i,j)}, \dots, PR_{(i+(K_l-1),j)}, PR_{(i+K_l,j)}, \\ PR_{(i,j-K_l)}, PR_{(i,j-(K_l-1))}, \dots, PR_{(i,j)}, \dots, PR_{(i,j+(K_l-1))}, PR_{(i,j+K_l)} \end{Bmatrix}$$

$$\mathrm{T(i,j)} = \frac{\sum_{PR \in K} PR}{n} \tag{9}$$

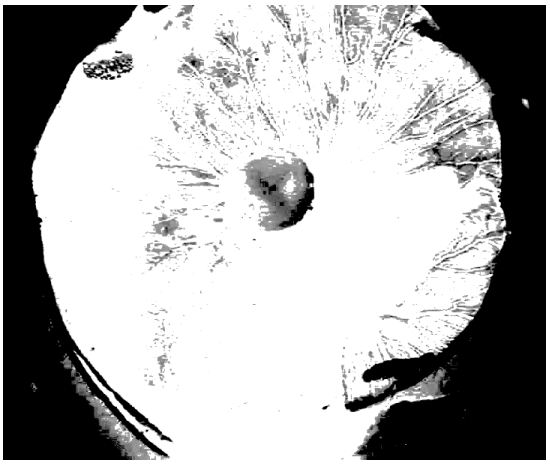
$$P_{BW}(i,j) = \begin{cases} 1, & if\ PR_{(i,j)} > T_{(i,j)}, \\ 0, & otherwise. \end{cases} \tag{10}$$

After the step above, we use Region Labeling and Opening to do the next process. This step is to remove regions which is considered as yolk area in the non-yolk area. Region Labeling is used to label white regions on the binary image. Opening is used to separate the non-yolk area in the yolk area. First, we use Opening to deal with the binary image, executing for two times with the 3×3 structure element. Then, use Region Labeling to label these regions. In the end, we set a threshold T_L; if the quantity of the same label is lower than T_L, we consider the region as non-yolk area and set the pixel value of the region as the pixel value of non-yolk area, which means to delete the region, as shown in Figure 15(c).

After separating yolk and non-yolk area completely, we will separate the vessels in yolk area. In R, G, B scale images, G scale image shows vessels in the yolk area obviously. As the result, we use Figure 15(c) to recover on the G scale image to make the G scale image only shows yolk area, too, as shown in Figure 15(d).

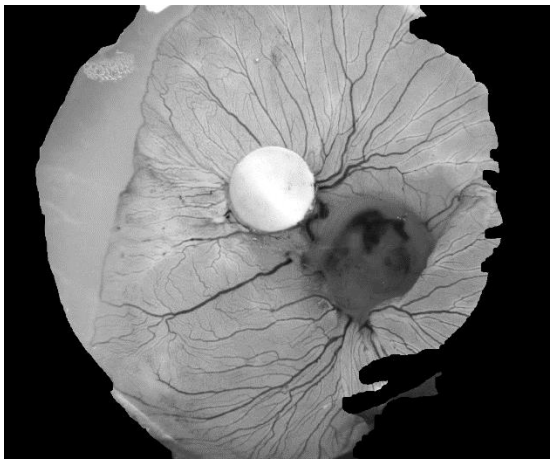


(a)



(b)

(c)



(d)

Figure 15

3.2. Separating Vessels in Yolk Area

In the beginning, for making vessels more obviously and removing noises, we have to use Run-Length to enhance vessels in yolk area. After that, we use Median Filter to make images smoother to avoid noises, and then contrast the whole yolk area.

Samples of chicken chorioallantoic are effected by light and shadow, which lead to not smooth brightness, so we remove the non-yolk area in the last step. Therefore, we use Local Contrast to contrast the image. Consider I(i,j) as the center to extend a 5×5 matrix, and then find out the maximum pixel value Max and the minimum pixel value Min in Equation (11):

$$\mathrm{I}(i,j) = \left(\frac{\mathrm{I}(i,j) - Min}{Max - Min}\right)^{r_b} * 255. \qquad (11)$$

where r_b is set as 2 after testing. As the result, the brightness of the image can be balanced for a while, as shown in Figure 16.

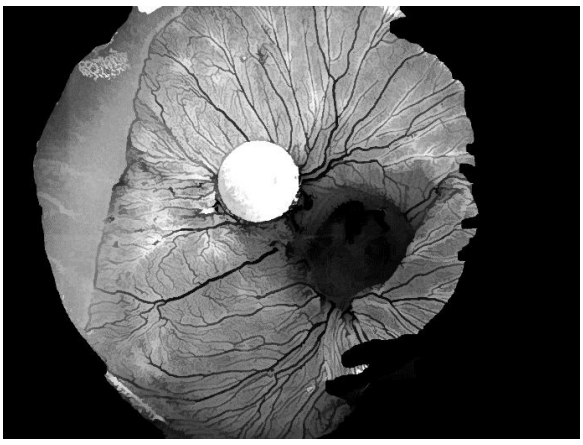


Figure 16: Using Median Filter and Run-Length, then contrasting with Equation (11)

The next step is to transform Figure 16 into a binary image. In this step, we still use Local Cross Thresholding because of the poor brightness balance of yolk area. However, we set two crosses with more quantity and less quantity of pixels of four directions. Consider I(i,j) as the center of these two crosses, then calculate their threshold avg_b and avg_s. After that, we use Equation (12) to classify the pixel value of $I_{bw}(i,j)$:

$$\begin{cases} I_{bw}(i,j) = 255, if\ P(i,j) \geq avg_b\ and\ P(i,j) \geq avg_s, \\ I_{bw}(i,j) = 0, if\ P(i,j) \leq avg_s\ and\ P(i,j) \leq avg_b, \\ I_{bw}(i,j) = P(i,j), otherwise\ . \end{cases} \quad (12)$$

After classifying, there are still some values of pixels between 0 and 255, as shown in Figure 20. To avoid these pixel turning into noise, we use a 5×5 matrix with Equation (13) to classify them.

$$\begin{cases} I'_{bw}(\mathrm{i,j}) = 255, \text{if } \exists a, b \text{ such that } I_{bw}(a,b) = 255 \\ \forall a \in \{i+2, i+1, i-1, i-2\} \\ \forall b \in \{j+2, j+1, j-1, j-2\} \\ I'_{bw}(i,j) = I_{bw}(i,j), \text{otherwise.} \end{cases} \quad (13)$$

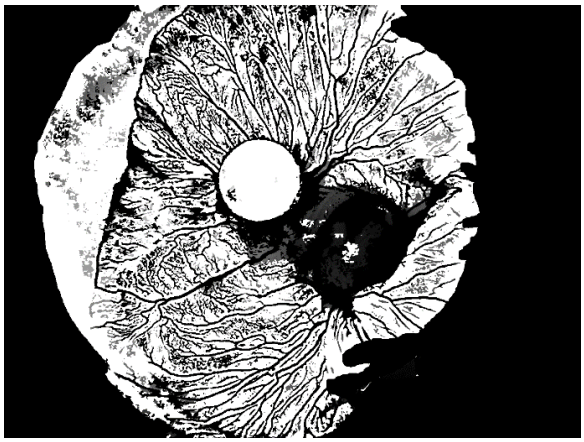


Figure 8: There are still some pixel value between 0and 255

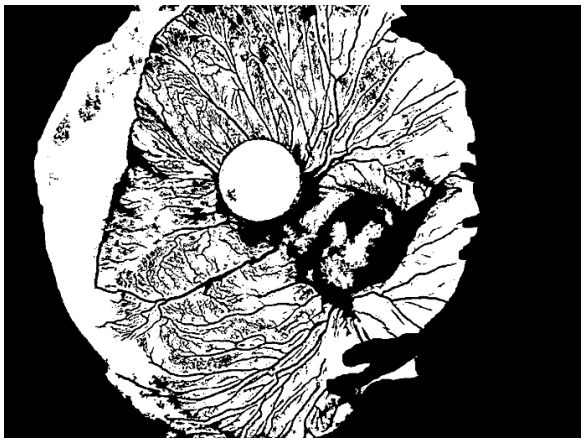


Figure 18: All pixel value were set as 0 or 255.

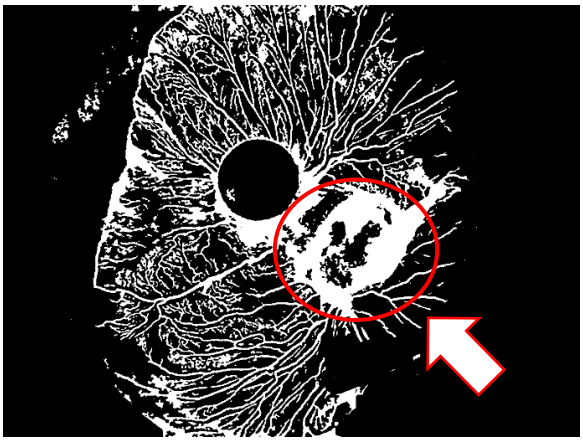


Figure 9: The image only with vessel area, but there is a big region which is the noise

In Equation (13), $I_{bw}(a,b)$ is the pixel value between 0 and 255, and $I'_{bw}(a,b)$ is the pixel value which is classified; (a,b) are the pixels contains a 5×5 matrix besides (i,j). If we detect that there is any pixel whose value is 255, then we set $I'_{bw}(i,j)$ as 255. The process will keep doing until there is no change in the image. If there still have pixels between 0 and 255, then set all of them as 0, as shown in Figure 18. To avoid the impact from non-yolk area, so we use Figure 15(c) and Figure 18 to do exclusive or to let vessels show in whole image only, as shown in Figure 19.

In Figure 19, we can see there is a big region shown from the arrow. Vessels are thin and long, so the big region in the image are considered as noises. It can lead to fatal errors when we calculate BCD value, so they have to be removed from the image. There are many big regions connected with vessels. Therefore, we use Erosion to separate them. In this step, we use a 3×3 matrix and execute three times to four times to leave the big noises; then we can use it to recover on Figure 19, and get the vessels without noises.

The result of using Erosion is shown in Figure 20(a). We can see there are some small noises around the big noise; these might be the noises from vessels after using Erosion or they are noises actually. Therefore, we remove this small noises temporarily. We use the characteristic of vessels that they might be thin and long to detect vessels, Thinning and Region labeling are used to remove this small noise.

(a)

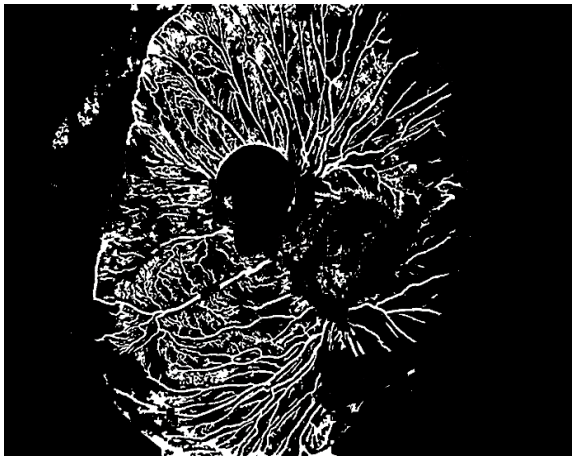
(b)

Figure 10: The result of before using Thinning and after using Thinning. (a) Before using Thinning (b) After using Thinning

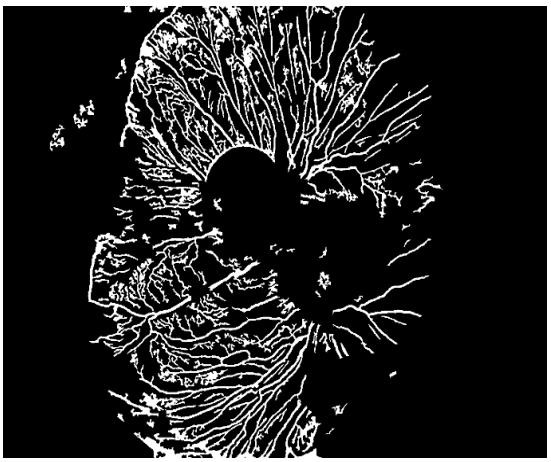
(a)

(b)

Figure 111



(a)



(b)

Figure 22: The result of removing big noise. (a) There are some small region after removing big regions (b) After removing small regions

First, we use Thinning to deal with Figure 20(a) and get the result $Thin_i$, as shown in Figure 20(b). Second, we use Region Labeling to label the regions whose thickness are one pixel. Third, calculate the

quantity of pixel $CountThin_i$ in Figure 20(b). Because the thickness of regions in Figure 20(b) is one pixel, we can be considered $CountThin_i$ as the vessel's length. The quantity of pixels $CountL_i$ in Figure 20(a) can be considered as the vessel's thickness.

We judge the length first. Setting a threshold called $CountThin_T$, its value is 20 after testing, as shown in Equation (14). If $CountThin_i$ is lower than $CountThin_T$, we consider it as the small noise; the same label will be set as 0, which indicate the region is removed, otherwise, set as 1, which indicate the region is left. Then the region which is left corresponds to Figure (a) to remove the small noise, as shown in Figure 21(a).

The next step is to judge the thickness, and we label each white regions and calculate the quantity of pixels $CountL_i$ in Figure 21(a); then use Thinning to thin all the white regions and calculate the quantity of pixels $CountThin_i$. After that, we use $CountL_i$ divided by $CountThin_i$. Thus, we get the proportion W_i, setting a threshold W_T whose value is 20 after testing, as shown in Equation (15). If W_i is lower than W_T, we consider the region as vessels and set its pixel as 0, which indicate the region is removed otherwise, and set as 1, which indicate the region is left. As the result, Figure 21(b) shows the big noise only.

$$Thin_i = \begin{cases} 0, & if\ CountThin_i < CountThin_T\ , \\ 1, & otherwise. \end{cases} \quad (14)$$

$$L_i = \begin{cases} 0, & if\ W_i < W_T\ , \\ 1, & otherwise. \end{cases} \quad (15)$$

However, Figure 21(b) is the result of using Erosion; the white region will be smaller than the white region in Figure 19. Therefore, we have to use Dilation to extend the area, then recover it on Figure 20(a), meaning pixel value is set as 0 at the same location in Figure 19. As the result, the big noise will be removed in Figure 19, and vessels and small noise will be left, as shown in Figure 22(a). Finally, we use Thinning and length of the white region to remove these small noises; then we get the final result of Vessel BCD Method, as shown in Figure 22(b).

4. Experimental Result

In this chapter, we present experimental results of Vessel BCD method. Also, we depict the result of yolk area, non-yolk area and vessels by experts and five people. The results of experts are considered as Ground Truth, and the results of five people are considered as comparators. The different between them is that Ground Truth is the most accurate result, and another is depicted according to that cognition. We use these three results to compute their BCD value, and consider the BCD value of Ground Truth as standard. The method whose BCD value is closer to Ground Truth's BCD value indicates that it is the better method for segmentation.

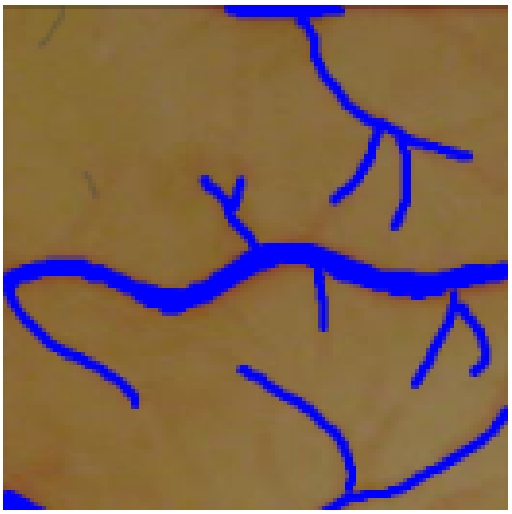
In this paper, we use 23 chicken chorioallantoic images. The size of these images are 1280×960 and 2048×1536, and 8 bits for each RGB channel. All these images of chicken chorioallantoic were captured and collected by biologist and doctor using a digital camera.

4.1 The Result of Segmentation of Vessels in Yolk Area

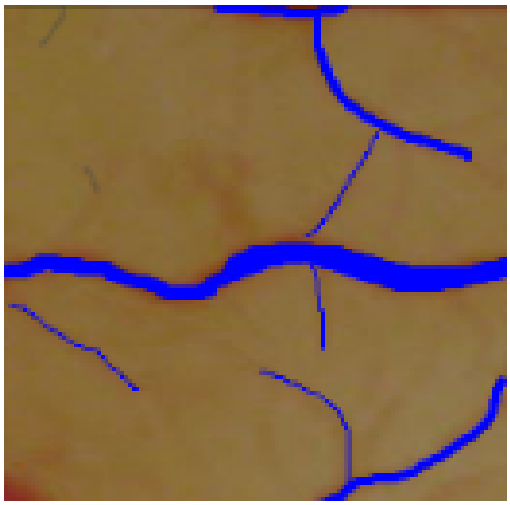
In this section, we explain the segmentation for yolk area and vessels of using Vessel BCD method and depicting by five people. The BCD value of the whole image is estimated by the density of the center of yolk area; therefore, we will use this for comparison. The results of segmentation are shown in Figures 23(a), (b) and (c), and the original image is shown in Figure 23(d).

After comparing, we knew that experts might ignore some microvessels in yolk area because the resolution of these images is not clear. However, Vessel BCD Method could recognize and depict them. In the result of computing by five people, when the images were zoomed in, the microvessels would be too blurred to let these five people to depict them. Also, these five people have a different definition of vessels, so it could cause the degree of thickness not the same.

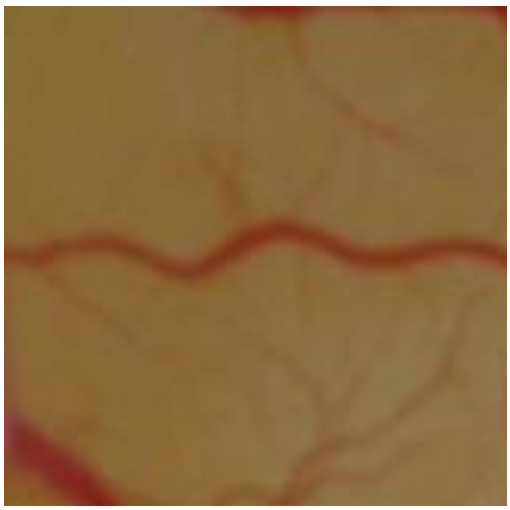
(a)



(b)



(c)



(d)

Figure 23: The segmentation of vessels in the center of yolk area(a)The segmentation of Vessel BCD Method. (b)The segmentation of Ground Truth. (c)The segmentation by one of those five people. (d)The original image.

4.2 The Result and Comparison of BCD Value

In this paper, we use a lot of parameters, as shown in Table 1. These parameters are selected from a lot of parameters of testing. In comparing BCD values of these three methods, we use BCD value of Ground Truth for the standard. If the BCD value of Vessel BCD method or the result is depicted by five people, the closer one is the better method for segmentation, and parameters it used are also considered as the best parameters group, shown in Table 2.

Table 2 shows the BCD values from the better parameters of four groups, and the comparison with the BCD values from Ground Truth. Except for the parameters we use in Table 2, the other parameters we use are shown in Table 1. The Results (W, X, Y, Z) in Table 2 indicates that executing Erosion for W times, and the quantity of pixels of two cross in Local Cross Thresholding are X and Y, and the threshold of removing small noise in the last step. For example, Results (4,151,75,40) are executing Erosion for four times, the quantity of pixels of two cross in Local Cross Thresholding are 151 and 75, and the threshold of removing small noise is 40 in the last step. In Table 2, we can see that if setting X and Y as 151 and 75 in Local Cross Thresholding, we can get the gaps of BCD values, 0.663297787 and 0.92343644, which are compared with the BCD values of Ground Truth. However, setting X and Y as 151 and 45 will get the worse result. The reason is one of their quantity of pixels is too short to remove noise, causing the fatal error on segmentation. In the results where X and Y are set as 151 and 75, if setting Z as 40, we will get the best result.

Table 1: Threshold parameters after testing

T	**T2**	**rc**	***x***	**T3**	**T4**	**rn**
0.475	0.92	0.6	0.35	150	175	0.3
Kl	**rb**	**CountThin$_T$**	**W$_T$**			
50	2	20	20			

Table 1: The BCD value of the result depicted by five people compared with the BCD value of Result (4,151,75,40)

Segment Method	The result of depicting by five pepople	Result (4,151,75,40)
BCD	0.01721025	1.701719546
The gap with Ground Truth	1.021211509	0.663297787

Table 3: The four results of using Vessel BCD Method , compared with Ground Truth

Segment Method	Ground Truth	Result (4,151,75,40)	Result (4,151,75,20)	Result (3,151,45,40)	Result (3,151,45,20)
BCD	1.03842175	1.701719546	1.961858199	2.149035797	2.46311294
The gap with Ground Truth		0.663297787	0.92343644	1.110614038	1.424691181

Table 3 shows the BCD value of the result depicted by five people and Result (4, 151, 75, 40) for comparison. The BCD value of the result depicted by five people are the average of the BCD value of twenty-three images. In Table 3, the BCD value of the result depicted by five people differs by 1.021211509 from Ground Truth. Then comparing with Result (4, 151, 75, 40), we can find that the BCD value of Vessel BCD Method is higher than the result depicted by five people, but it is closer to Ground Truth. The reason of higher BCD value is that there are many bloods, shadow in the image, after transforming into grayscale images and binary images, the pixel value will be similar to vessels' pixel value. It will make us difficult to separate vessels, as shown in Figures 24(a), (b). That experts can't depict vessels is also the reason.

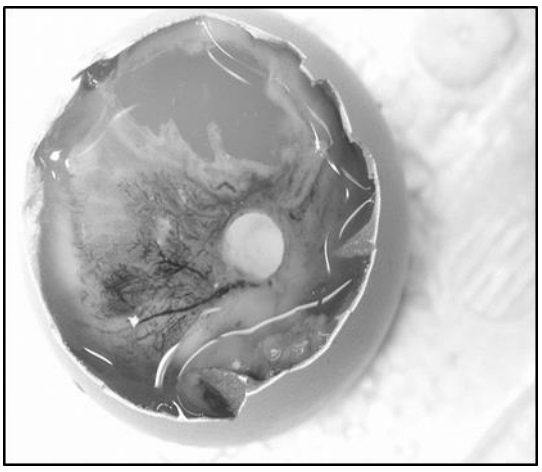
Because images were zoomed in before the five people depict, many microvessels can ignore what leads to the lower BCD value. However, The BCD value of Vessel BCD Method is closer to Ground Truth that indicates Vessel BCD Method is better than the result depicted by five people, as shown in Figures 23(b), (c).

5. Conclusion

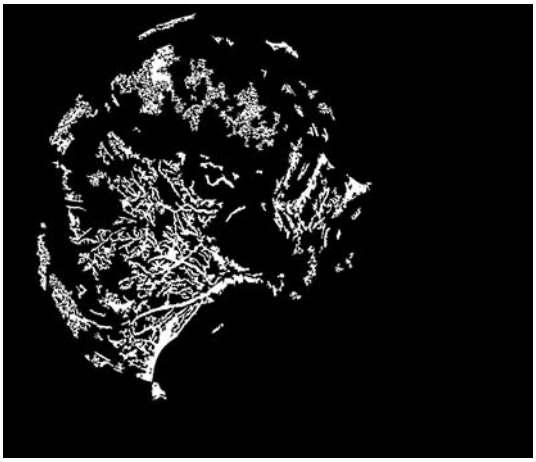
This paper proposed a method to segment chicken chorioallantoic image called Vessel BCD Method which is separated into two parts: one is yolk area and non-yolk area segmentation, and another is area vessel segmentation. First, before segmenting yolk area and non-yolk area, we transform color images into R scale image, G scale image and B scale image. Then we use R scale image and B scale image to enhance these two areas. Second, we use Local Cross Thresholding to get binary images. Because there are many vessels connected with noises in these images, we use Closing and Region Labeling to remove them. At last, we use the result to recover the G scale images to get G scale images with no background.

After segmenting yolk area and non-yolk area, we start to segment vessel area. First, we use Run-Length and Median Filter to make pixels more smooth and let vessels more visible. Then use Local Gamma Correction to enhance vessels. Second, we use Local Cross Thresholding to get binary images, but there is some big noise connected with vessels. Thus, we use Erosion to separate vessels and big noises. Then use Thinning to make vessels and noises thin, and use its total pixels to judge which region is too long. If the region is too long, then we remove it. After that, we use the region to divide the region after using Thinning to get a thickness value. Because vessels are thin and long, the region with too high thickness value will stay. Finally, we remove these high-value thickness regions and noises from binary images; then we get the final output images.

To evaluate whether Vessel BCD Method's output is good or not, this paper use images called Ground Truth which experimenters depict vessels by themselves for standard and comparing with images depicted by five people. Then we calculate BCD value for three outputs above. And BCD value is calculated from the central area of yolk. The BCD value closer to Ground Truth's BCD value will be the better method of segmentation. From the result, we know that Vessel BCD Method's BCD is closer to Ground Truth's BCD, so our method is better than the method depicted by five people.



(a)



(b)

Figure 24: Blood and shadow in grayscale image and binary images. (a) Vessels after transforming into grayscale image (b)Vessels after transforming into binary images

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