## Micro-vessel Segmentation and Tiny Particle Speed Estimation

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#### Abstract

On the basis of the flow feature in the micro blood vessels, a method to track the trace of cell movement was proposed in this paper. The algorithm employed the space domain enhancement, adaptive threshold segmentation techniques. For the sake of calculating blood flow, the active contour-based template matching method was applied to sequential images. The experimental results show that this method can accurately track movement of tiny particles and estimate its moving speed in the micro-blood vessels.

Keywords: image segmentation; micro-blood vessel; particle tacking; noise suppression

#### **1. Introduction**

Accurate segmentation of micro-blood vessels can improve the precise tracking of tiny particles or cells. As the blood vessels are deformable objects, much attention has been paid by researchers to this open-ended question in recent years [1-3].

For instance, a local enhancement technique which used the statistical characteristics of neighboring pixels was proposed [4-6]. The purpose is to increase the contrast between tiny targets and background in microscopic images. Based on neural network and wavelet spectral entropy, a mixed feature presented [7-9] to decide criterion for cluster detection. An optimal filter was employed by several researchers [10-12] under the Fisher criterion to extract texture features for the distinction between malignant and normal tissues. Using support vector machines (SVM) to fix position problems, a continuous enhancement learning scheme was proposed [13-15] to locate small particles in mammograms and to decrease the false detection. Thirty-one traits (mean variance, contrast, shape, *etc.*) [16-18] were listed and a general regressive neural network (GRNN) was used to explore clustered MCs. To achieve similar purposes, an adaptive neighborhood technique was used to classify tiny objects in microscopic images [19].

In this paper, morphological minimum class variance was applied to space domain enhanced images. After that, deformable template matching technique was used to locate the specific tiny particles in the micro-blood vessels and the speed of cell movement was estimated in the final stage. The rest of this paper was organized as follows: the structure of image acquisition system was introduced in the second part as well as some pre-processing methods. In the third part, morphological minimum class variance was employed to segment micro-blood vessels. Cell moving speed was estimated in the fourth part and some conclusions were given in the last part of this article.

## 2. System Description and Image Pre-processing

The real-time acquisition system of micro-blood vessel images consists of MV-VD200SM high resolution digital video camera ( $1600 \times 1200$  pixel, 24-bit true-color image). The maximum data acquisition rate is 10 frames per second under the highest resolution. It is equipped with LED lighting source and AFT-M1024 scenes (Figure 1). The acquired micro-blood vessel image was shown in Figure 2.



Figure 1. Architecture of Image Acquisition System

During the pre-processing of micro-blood vessel images, median filter was employed to reduce the noise corruption to images and improve the quality of images. Presumed the noise signal follows the normal distribution, the variance of median filter output is [9, 10]:

$$\sigma_{med}^2 = \frac{1}{4mf^2(\overline{m})} \approx \frac{\pi \sigma_i^2}{2m + \pi - 1} \tag{1}$$

where  $\sigma_i$  denotes the power of input noise, *m* denotes the mean of input noise. In order to obtain useful information and enhance the interested region, it is a vital step to carry out image enhancement which can be achieved in spatial domain or frequency domain.



Figure 2. Micro-blood Image Acquired by VS-M0910 Camera

Concerning the view of mathematics, spatial domain method can process images immediately in an image space with the help of differentiation and integration, while frequency domain method turns images into its spectra, and then process it in the frequency domain, and finally convert it back to image domain using inverse Fourier transform. By comparing the merits and disadvantages between these two image enhancement methods, space domain image enhancement approach was employed [11]:

$$I_{after}(x, y) = Fun(I_{before}(x, y))$$
<sup>(2)</sup>

where,  $I_{before}(x, y)$  and  $I_{after}(x, y)$  mean images before and after enhancement,  $Fun(\bullet)$  is the image enhancement function.



Figure 3. Flowchart of the Micro-blood Vessel Image Pre-processing

Owing to lighting and other environmental factors in image acquisition, image gray levels between neighbor frames can not be considered as a constant. So it is hard to reach the ideal state of segmenting inner cell mass by the fixed threshold method. So it is necessary to extract erythrocyte mass by setting a threshold adaptively and an adaptive threshold algorithm based on OTSU method was applied to the enhanced image [11, 12]. The flowchart of this preprocessing step is illustrated in Figure 3 and the comparison between several classic preprocessing algorithms is illustrated in Figure 4.



Figure 4. Comparison of Pre-processing Results: Original Gray Image (lefttop), Median Filtered Image (right-top), Neighborhood Filtered Image (leftbottom) and Adaptive Threshold Filtered Image (right-bottom)

# **3.** Micro-blood Vessel Segmentation Based on Morphological Minimum Class Variance

To detect the boundary of micro-blood vessels, gradient operator (the first derivative) was employed. The gradient operator is a vector, not only can it provide size information, but direction as well. The amplitude and direction angle can be calculated on the basis of gradient partial derivatives. The morphological algorithm was combined with the edge detection theory analysis to achieve purpose of segmentation.

The following equations define the background and the object in the micro-blood vessel images:

$$u_B(T) = \frac{1}{w_B(T)} \sum_{0 \le k < T} k \bullet p(k)$$
(3)

$$u_o(T) = \frac{1}{w_o(T)} \sum_{T \le k < m-1} k \bullet p(k)$$
(4)

where p(k) is the probability for corresponding target or background gray value k. According to equations (3) and (4), the total average grey value of the image is:

$$u = w_B(T)u_B(T) + w_o(T)u_o(T)$$
<sup>(5)</sup>

The variance between background and target is defined:

$$G(T) = w_B(T) \bullet [u_B(T) - u]^2 + w_O(T) \bullet [u_O(T) - u]^2$$
(6)

In the procedure of micro-blood vessel segmentation, a derived morphological transform was applied to the pre-processed images. It depicts the basic structure of which all points belong to the medial axis, but due to pixelation and the finite size of the structuring element it is not necessarily connected nor maximally thin. The mathematical equation of this transform is expressed [13]:

$$S(A) = \bigcup_{k=0}^{K} S_k(A)$$
<sup>(7)</sup>

$$S_{k}(A) = (A \Theta \ kB) - (A \Theta \ kB) \circ B$$
(8)

$$K = \max\{k \mid (A \Theta kB) \neq \Phi\}$$
(9)

where A is the set to be morphological operator while B is the structure element. K is the last iterative step before A erodes to an empty set which means to carry out k times successive erosion of the set A.



### Figure 5. Segmentation of the Micro-Blood Vessel Superimposed on the Original Image

Through the morphological operation, the isolated points were removed and the edges became smoother. And Figure 5 demonstrates the result of micro-blood vessel segmentation based on the morphological algorithm.

# 4. Experimental results of tiny particle tracking in the micro-blood vessel images

The cicada frog skin was selected as experimental subjects and the purpose was to estimate the moving speed of interested particles in the image. Three sequential frames of true color images were shown in Figure 6 and it seemed very difficult to identify interested objects. With the aid of the microcirculation blood flow characteristics, a sequential template matching method was applied to track the tiny particle (potential cells) movement in the micro-blood vessel images.



Figure 6. True Color Sequential Images

By observing the color image sequences, it is crystal clear that image quality is not good enough to carry out recognition. So the sequential images were pre-processed by the space domain enhancement and filter techniques introduced in the second part.



Figure 7. Motion of Inner Cell Mass through Consecutive Frames

Due to varying morphology of red blood cells and the resistance of the vessel wall, particles in the blood vessels often collide with each other and belong to deformable objects. Since traditional template matching methods only work effectively for rigid objects, the active contour based template was employed to increase the tracking robustness. In another word, the template was update continuously according to changes between sequential images.



Figure 8. Speed Estimation of Particles in the Micro-blood Vessel

After determining the outline of tiny particles (potential blood cells), centroid-based methodology was used to determine the target group coordinates. Since the time interval for image sequence is a known parameter, the displacement of particular object in the consecutive images can be measured based on the relationship between pixels and the actual physical length. The graphical illustration was shown in Figure 7. And Figure 8 showed the estimated speed of blood flow based on consecutive 12 sequential microblood vessel images (Figure 8).

Owing to the lack of standard speed of tiny particle movement, empirical trials were conducted in different regions. Figure 9 showed the velocity comparison between different regions at the same sampling period. Both of them were calculated using 21 frames of sequential images. More experiments will be carried out to verify the efficacy of the algorithm.



Figure 9. Moving Speed Comparisons of Tiny Particles in the Micro-blood Vessel

### 5. Conclusion

Changes in micro blood vessel features of the conjunctive bulb are warning signals of serious diseases such as heart attack and stroke [20]. Therefore, analysis of conjunctive vascular features can facilitate to detect some potential diseases at the early stage and allow medical staff to carry out further treatment before the illness enters advanced stages.

However, it is a challenging job to conduct deformable blood vessel segmentation and speed estimation of blood cells, not alone automatically. In this paper, an automatic segmentation algorithm was applied to separate the micro-blood vessel from its background. First, the captured images were pre-processed using space domain enhancement and filter techniques. After that, morphological minimum class variance was used to segment the interested micro-blood vessel. In the final step, the speed of tiny particles (potential blood cells) was estimated using the active contour based template matching method.

In the future studies, the accuracy of the speed estimation will be evaluated and the robustness of the micro-blood vessel segmentation will be examined using more experimental data.

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