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RESEARCH ARTICLE

Estimation of Capillary Blood Flow Velocity using Centroid Displacement and Image Processing Techniques

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Abstract Microvascular blood flow velocity is an important parameter in microcirculation study. Blood flow is often recorded as a digital video format in both in vitro and in vivo research. Commercial software for determining blood flow velocity is expensive for a small laboratory. The aim of this study was to estimate the capillary blood cell velocity using centroid determination in conjunction with image and video processing techniques. Several image processing techniques were applied including video to image frames extraction, image improvement, image binarization and morphological processing. Then the centroids of red blood cell images were determined to be used for capillary blood flow velocity. The results showed that calculated blood flow velocity in an in vitro capillary was in good agreement with the actual velocity value (root mean square error of 0.087 mm/s). The proposed methods can be used to estimate the blood flow velocity in micro vessels. However, further techniques on image enhancement, image segmentation and image recognition should be applied to improve the calculation accuracy of capillary blood flow velocity.

Keywords: Blood flow velocity, Capillary, Microcirculation, Image processing, Centroid.

Introduction

Blood flow is relevant to the occurrence of many diseases such as hypertension, sepsis, diabetes and cardiac diseases. Microcirculation is part of circulation system, it composed of arterioles, capillaries and venules. The vessels have diameters ranging from 5 to 50 μm. The microcirculation is critical to maintain organs and tissue by delivering nutrients, gases, hormones, water and exchanging waste products between blood and cells [1,2]. For both in vitro and in vivo studies, image and video processing is one of popular techniques used in microcirculatory research [3]. These approaches have been validated to offer information of red blood cells (RBCs) velocity and diameter in microcirculation. RBCs is biconcave shape with disk diameter of 6 to 8 μm and thickness of 2 μm. The deformability of RBCs is an essential factor involving an increase in blood viscosity and flow resistance, especially in small vessels [4].

Image and video processing is an alternative and non-invasive approach for blood flow measurement. Previous studies were published in RBCs velocity, flow in capillaries and microvessel diameter by using different techniques such as dynamic video microscopy, spatiotemporal autocorrelation, and confocal laser microscopy [5–7]. Several research studied and focused on the use of cross correlations for evaluation of RBCs velocity [1,8,9]. Cross correlation technique can be distributed into two sections: the temporal correlation and the spatial correlation methods. These methods present an accurate and efficient blood flow velocity estimator. There was another method using fluorescein isothiocyanate (FITC) labelled RBCs and high-speed camera system to determine the mean centerline RBC velocity in rat pial artery [10]. However, to measure blood flow velocity, some techniques need more complex mathematical calculations or a commercial software which comes with an expensive equipment.

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The purpose of this work was to develop a computer program (an inhouse software) to estimate the velocity of blood flow in microvessels from RBC velocity by employing video and image processing. In this study, we proposed video and image processing techniques to determine RBCs velocity in capillaries from videos obtained from in vitro and in vivo studies. Table 1 summarizes the similarities and differences between our study and previous studies.

Table 1. Similarities and differences between our study and previous studies

Reference	Similarity	Difference
Tsukada et.al. (2000) [1]	- Video source from high-speed video camera - Animal blood flow video	- Arteriole - Image correlation - Online automate - Simulated data - Blood flow velocity profile - C language
Bollinger et.al. (1974) [5]	- RBC velocity determination - Velocity determination - Offline	- Nailfold capillaries - Human subject - Television microscopy - Displacement of plasma gaps
Sourice et.al. (2005) [6]	- Animal blood flow video	- Dynamic video microscopy - Spatiotemporal autocorrelation - Blood flow velocity profile
Seylaz et.al. (1999) [7]	- RBC velocity determination - Offline	- Brain microcirculation - Confocal laser microscopy - Labelled FITC RBCs
Huang et.al. (2010) [8]	- RBC velocity determination	- Simulated flow images - Cross correlation - Hough transform - Optical flow
Roman et.al. (2012) [9]	- Video source from high-speed video camera	- In vitro synthetic image sequences - Velocity profile - Dual-slit (temporal correlation)
Ishikawa et.al. (1998) [10]	- Video source from high-speed video camera - Animal blood flow video	- Labelled FITC RBCs

Materials and Methods

Video Images

The capillary blood flow videos were obtained from the in vitro flow experiments performed by Sakai and colleagues at Nara Medical University, Japan (Figure 1) and the hamster's dorsal skin window chamber model was constructed at the Functional Cardiovascular Engineering Laboratory directed by Prof. Pedro Cabrales, University of California at San Diego, USA (Figure 2). The characteristics of video images depend on a qualification of capillary video recording such as image resolution, frame rate, image size and total number of frames. For videos of the in vitro flow model, they were captured with a high-speed digital imaging camera (Phantom Miro 4M) through the microscope (Olympus UPlanApo with an objective lens of x100 magnification). The videos from in vivo study were recorded through the microscope (Olympus BX51W1) with 40x water immersion objective len using a high-speed digital camera (Photron FASTCAM 1024 PCI). The characteristics of video images used in this study were addressed in the Table 2.

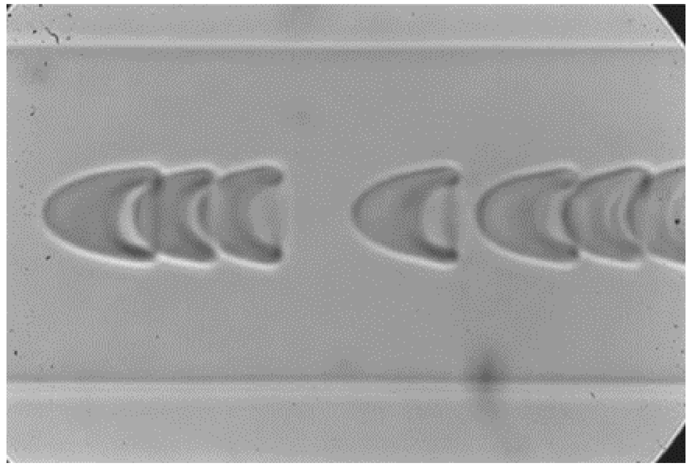


Figure 1. RBCs suspended in viscous fluid and perfused through a narrow tube (25 μm)

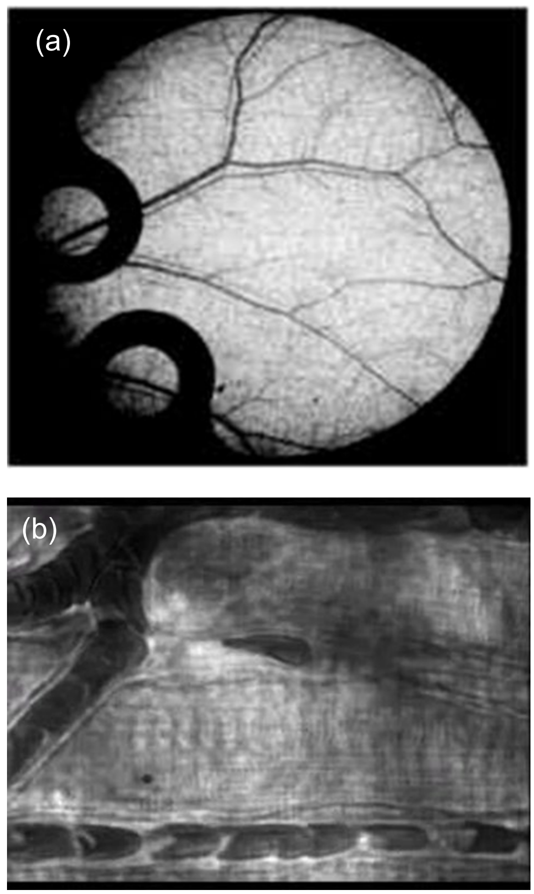
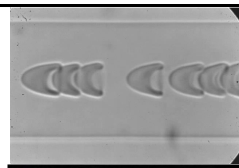
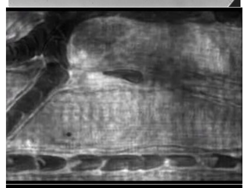
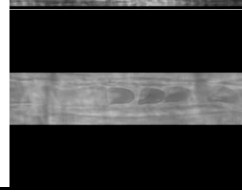


Figure 2. Hamster's dorsal skin window chamber model (a) a vascular network via a camera and (b) capillaries and RBCs under a microscope

Table 2. The characteristics of video images obtained from in vitro and in vivo studies.

Video name	Example of video image	File type	Resolution (pixels)	Frame rate (frame/s)
Video 1 (in vitro)		mov	640x480	1,200
Video 2 (in vivo)		mp4	320x240	10
Video 3 (in vivo)		mp4	320x240	10

Graphical User Interface

To make the developed software user-friendly, a graphical user interface (GUI) was developed. This GUI was based on MATLAB which can provide point-and-click control of the calculation of blood flow velocity in capillary. Figure 3 shows the display of GUI which consists of loading windows of consecutive frames of video images and a slider bar for threshold adjustment. This GUI also has a calibration button to convert a pixel distance into a physical distance.

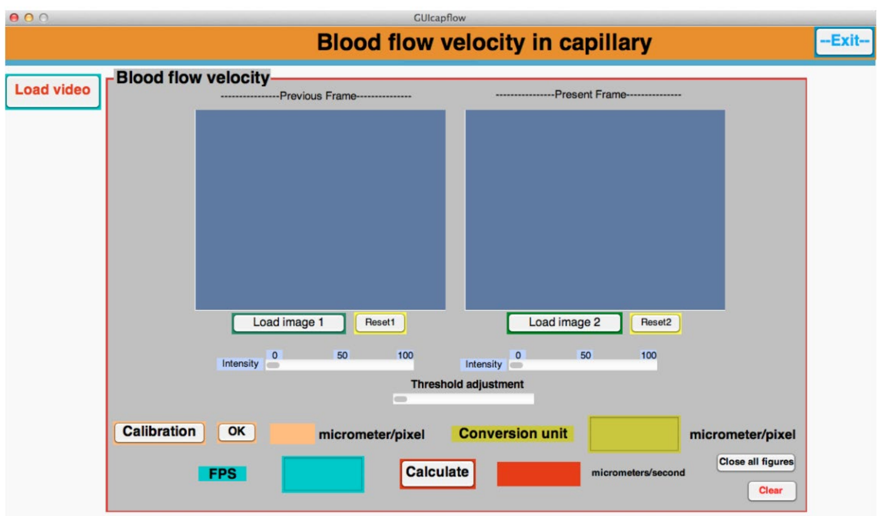


Figure 3. Display of graphical user interface to estimate the blood flow velocity in capillary

Blood Flow Velocity in Capillary Determination

Features of RBCs movement in microcirculation depends on the characteristics of blood vessels that they travel through. Capillaries are the smallest vessels and have an average diameter of 6 μm. When RBCs pass through a capillary, they move in a single pile and their shape becomes a bullet-like pattern as shown in Figure 1 and 2. The RBCs velocity was estimated by using image processing and centroid theory. Steps of image processing were performed as presented in Figure 4. Using MATLAB 2020b, video file was read and extracted to image frames (mmreader() and read()). Table 3 shows key MATLAB commands using for image processing.

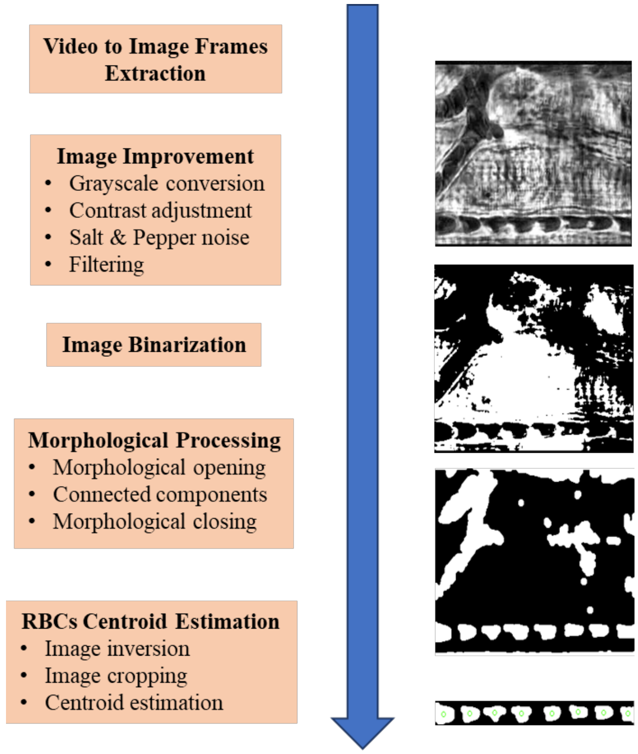


Figure 4. Steps to perform image processing for RBCs centroid determination

Table 3. Commands to use for video and image processing for RBCs centroid determination

Command	Purpose
mmread()	To read a video file
read()	To extract all frames from the video and store
rgb2gray()	To convert RGB image to grayscale image
imadjust()	To adjust contrast and brightness of image
adapthisteq()	To adjust contrast and brightness of image
imnoise(,'salt & pepper',)	To add salt & pepper noise to the image
medfilt2()	To remove Salt and Pepper noise for enhance the image quality
im2bw()	To binarize 2-D grayscale image to black and white image
bwareaopen()	To remove small objects
strel()	To create a flat morphological structuring element
imclose()	To fill holes in binary image
regionprops(,'centroid')	To determine a centroid of the RBCs

Image Improvement

Pre-processing was employed in this procedure to improve image quality and maximize the contrast between the background of the image and objects of interest. Pre-processing of microcirculatory images is mandatory due to the low contrast of microcirculation images. Generally, microcirculation images show the intensity of capillaries and small blood vessels are closed to that of background and tissues [11]. The video images were converted to grayscale images with a range of pixel values from 0 to 255 (rgb2gray()). The grayscale images were adjusted contrast and brightness using adaptive histogram equalization via the commands "imadjust()" and "adapthisteq()" in MATLAB. Salt and Pepper noise was added (imnoise(,'salt & pepper',)) and then removed using a median filtering (medfilt2()) to improve the image quality.

Image Binarization

Image binarization or image thresholding is a technique to determine the difference between RBCs and background [12]. After the images were changed to grayscale images, the intensity of grayscale image was turned to binary numbers-only 0 and 1depending on the set threshold value from the slide bar on the GUI (im2bw()).

Morphological Processing

In this step, each pixel in the image was adjusted based on the value of other pixels in its neighborhood. Morphological operations are commonly applied in image segmentation to extract features and objects from an image, or to clean up the image[13,14]. Morphological operations were performed to complete the structuring element in the image such as small objects removal and hole filling (bwareaopen(), strel(), imclose()).

RBCs Centroid Estimation

A binary image was inverted by flipping its pixel values and only RBCs were cropped to determine a object's centroid. The capillary blood flow could be estimated with centroid position of each RBCs between two frames. After determining the pixel x-y coordination of centroid using the regionprops function (regionprops(,'centroid')), the distance of RBCs movement between two frames was determined by Euclidean distance theory as shown in equation (1).

$$D_c = \sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2} \tag{1}$$

Where Dc is distance between the centroids of red blood cell in different frames; x and y is the coordinates in x and y direction of centroid and I is the frame number of video.

Velocity Calculation

The capillary blood flow was determined by the equation (2).

$$\mathrm{V}_{RBC} = \mathrm{D}_c \times \mathrm{F}_r \tag{2}$$

Where V_{RBC} is the velocity of red blood cell; and F_r is the frame rate.

Data Analysis

Blood flow velocity from the RBCs velocity was compared to know value of blood flow velocity from video file. A simple random sampling was performed to various frames of capillary blood flow velocity. The average blood flow velocity and the root mean square error were calculated using Eq.2 and 3.

Average of blood flow velocity $\overline{V}$calculated by:

$$\overline{V} = \frac{\sum_{i=1}^{N} V_i}{N} \tag{2}$$

where V_i is individual RBC velocity and N is number of observations

Root mean square error (RMSE) expressed as:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(Vc_i - Va_i)^2}{N}} \tag{3}$$

where Vc_i and Va_i are calculated blood flow velocity and actual flow velocity, respectively and N is number of observations.

Results and Discussion

The results presented that microvascular blood flow velocity could be estimated from RBCs velocity. We compared the in vitro blood flow velocity obtained from our proposed methods with the actual value (1mm/s) of the videos obtained from Hiromi Sakai and colleagues [15]. Figure 4 presents six trials of blood flow velocity calculation. The comparison results were presented in Table 4. The error of calculated blood flow velocity ranges between 3% and 13%. The average percentage of mean error and RMSE were 7.67 and 0.087. Therefore, capillary blood flow can be estimated using our suggested methodology, which uses the centroid of each RBC, within an appropriate accepted range.

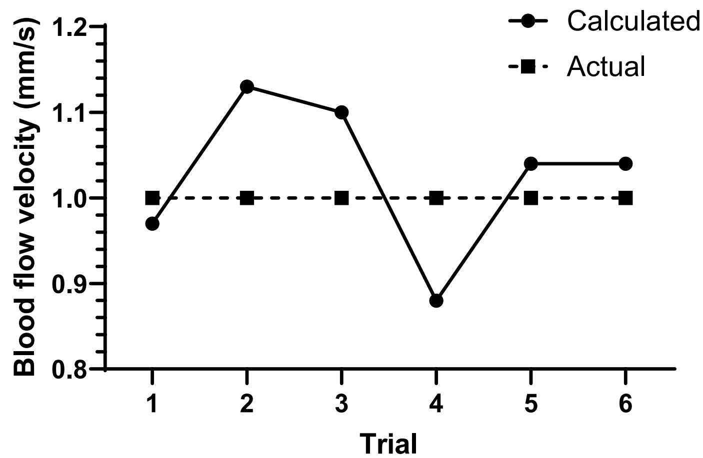


Figure 4 Blood flow velocity from the in vitro study determined by the proposed methods compared to actual velocity set up in six trials

Table 4 The in vitro blood flow velocity comparison: velocity determined from video images vs velocity from experimental set up.

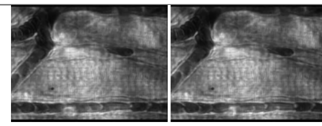
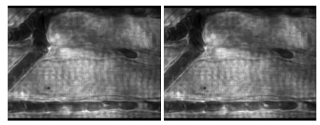
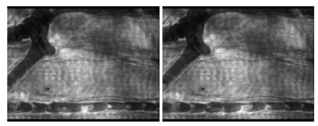
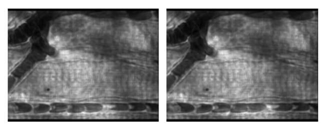
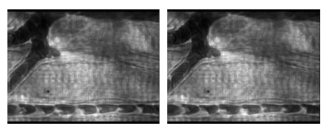
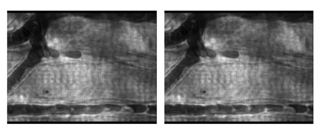
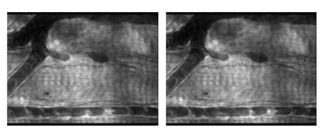
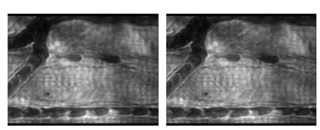
Sequence of frames	Velocity from our methods (mm/s)	Velocity from known value (mm/s)	Percentage of error
1,2	0.97	1	3
10,11	1.13	1	13
21,22	1.1	1	10
26,27	0.88	1	12
61,62	1.04	1	4
64,65	1.04	1	4

Capillary blood flow velocities calculated from RBCs velocity in the in vivo study are shown in Table 5. The results of velocity range between 22.12 μm/s and 32.68 μm/s. The average blood flow velocity of this video is 27.86 μm/s with standard deviation of 4.21 μm/s. From Table 6, we can notice that the shape and distance between each RBC are altered at each time of interest. The calculated RBCs velocities are similar if there is a clear space between RBCs (frames 162 and 163 vs frames 236 and 237). In the case of frames 92 and 93, it is quite difficult to distinguish each RBC which might lead to lower calculated velocity. The capillary blood flow was not similar or constant throughout the study, it is non-uniform flow. It depended on flow resistance in the capillary and driving force from blood pressure. The velocity of RBCs is also affected by the RBC shape and diameter of the capillary [16].

Furthermore, we tested our methods with the video of in vivo blood flow in a single capillary with 6 μm diameter. Table 6 shows the results of capillary blood flow velocity calculated from RBCs velocity. The results of velocity range between 7.79 μm/s and 17.79 μm/s. The average blood flow velocity of this video is 11.10 μm/s with standard deviation of 3.23 μm/s. In this case, the quality of video images is low contrast between RBCs and background which leads to the difficulty of segmentation [17,18].

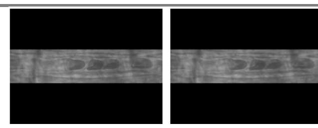
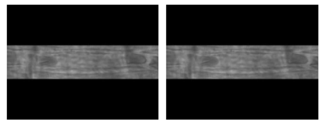
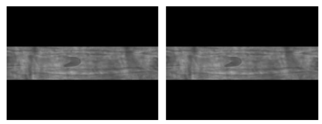
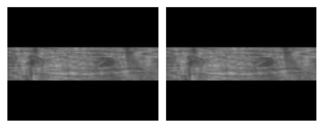
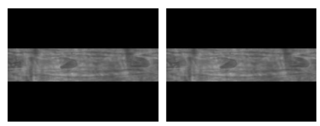
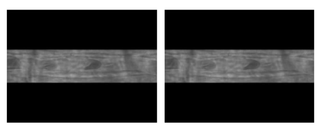
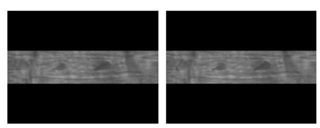
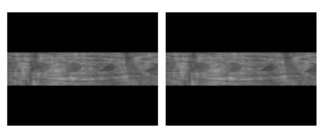
In this study, there is a variation in blood flow velocity or the measurement uncertainty. This uncertainty can be caused by the quality of images after the post-processing that leads to the uncertainty to determine the shape of RBCs which affect the centroid of RBCs and RBCs velocity calculation. Furthermore, the variation might be caused by the nature of actual variation in the in vivo capillary flow. It is better to control the quality of images after the post-processing to reduce the variation.

Table 5. The in vivo blood flow velocity determined from video images

Sequence of frames	Images	Velocity from our methods (μm/s)
92,93		22.12
109,110		30.44
162,163		23.81
167,168		28.57
236,237		24.59
303,304		33.60
353,354		27.10
373,374		32.68

Several limitations of this study had to be addressed. First, the quality of video images obtained from the in vivo study was affected by a surgical preparation when removing each layer of animal's skin. Simple techniques for image segmentation were applied in this study. Some segmented images revealed RBCs shape distortion after thresholding as shown in Figure 5. In our study, it was found that the details of RBCs were missed when converting to binary images. Therefore, it is better to use adaptive thresholding instead of global thresholding. Furthermore, deep learning techniques can be applied to enhance image segmentation [19, 20]. The inability to compare our computed capillary blood flow to an in vivo blood flow velocity measurement causes us to unidentified errors. It is necessary to validate across a range of physiologically relevant velocities to address the accuracy of this developed software.

Table 6. The in vivo blood flow velocity in a single capillary with 6 μm diameter determined from video images

Sequence of frames	Images	Velocity from our methods (μm/s)
9,10		7.79
49,50		8.55
64,65		17.79
89,90		12.85
110,111		8.62
129,130		11.34
135,136		10.10
169,170		11.74

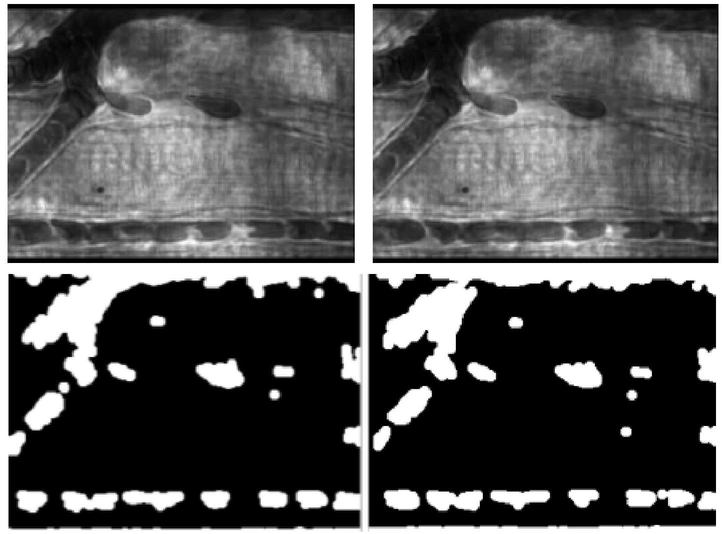


Figure 5. The distortion of RBC shapes after thresholding and segmentation

Conclusions

We developed the inhouse software to determine the blood flow velocity using RBCs velocity obtained from video images. Video and image processing and centroid theory were applied to obtain the RBCs velocity. Our study calculated the blood flow velocity with an average percentage of mean error and root mean square error of 7.67 and 0.087, respectively, when compared to the known value of blood flow velocity from the in vitro experiment. For the in vivo experiment, the RBCs velocity can be estimated but it showed high variation at different time frames. The image quality and segmentation techniques play an important role to the accuracy of the capillary blood flow calculation. To obtain higher accurate calculation, further techniques on image enhancement, image segmentation, and image recognition should be applied.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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