ABSTRACT
In clinical application, information about various diseases, such as diabetes, myocardial infarction and cerebral infarction, can be obtained from the peripheral blood vessels and their blood flow. Observing peripheral blood vessels and their blood flow yields important information in clinical practice. A novel device for noninvasively observing peripheral blood vessels and their blood flow using high-speed photography of the surface of the sclera was developed in our laboratory. The peripheral vasculature of the sclera was directly observed using our device. Utilising phase-only correlation to detect the displacement of blood vessels, we eliminated the effects of fixational eye movements to stabilise the sclera images. Morphological operations were then applied to segment the data locally and to extract the shape of the blood vessels. High-speed optical flow generation with the steepest descent method was used to detect the velocity of the red cells flowing in the blood vessels. A variety of experiments verified the validity of our novel method for observing capillary blood flow.

KEY WORDS
blood capillary, peripheral blood vessel, blood flow velocity, fixational eye movements, morphological operation, POC, Watershed region tracking, steepest descent method

1. Introduction
In the clinical setting, information about various diseases, such as diabetes, myocardial infarction and cerebral infarction, can be obtained by observing the peripheral blood vessels and their blood flow. Currently, most optical (laser) techniques for monitoring blood flow in both research and clinical settings use either the Doppler effect [7] or the temporal statistics of time-varying laser speckles [8]. These types of blood flow meters are non-invasive, but they cannot be used to acquire additional information, such as blood vessel width. Although these meters can correctly measure blood flow velocity, obtaining accurate information related to parameters such as blood viscosity is impossible. Thus, we developed a novel method for measuring blood flow velocity that uses high-speed photography of the surface of the sclera of the eye. Our method directly images the peripheral vasculature. In addition to facilitating measurements of the blood flow velocity, image processing allows the size and shape of the blood vessels and of red cells to be determined.

This research focus evolved while we were developing a system to measure human fixational eye movements [9][10]. Our method uses two high-speed cameras equipped with high-magnification microscope lenses (Fig. 1). These cameras can capture the distribution of blood vessels on the surface of the sclera. Image processing allows us to detect small movements of the eyeball. In the sclera images, red blood cells and blood flow were clearly observed. Thus, this system not only can measure fixational eye movements but also can be used to observe blood vessels and red blood cells and to measure blood flow velocity. The blood vessels of the sclera of the human eye are the shallowest vessels in the human body. In addition, the sclera is white, a colour that has high light reflectance. These properties make the sclera the best place to observe human blood vessels non-invasively, and it is possible to measure blood flow velocity using sclera...
images. In this study, as an application of the fixational eye movement measurement system, we sought to develop a novel method for measuring blood flow velocity using sclera images. To measure the blood flow velocity on the surface of the sclera, the eyeball should be fixed and immobile while the sclera images are captured. Fixational eye movements made it difficult to fix the eyeball.

Fixational eye movements refer to the continual movement of the eyes while the gaze remains fixed on an object. Three types of eye movement can be detected while a subject gazes at an object: tremor, drift, and microsaccades (Fig. 2) [1]. Previous experiments have demonstrated that microsaccades are the fastest type of fixational eye movement, with amplitudes of 5–120 min (monocular) or 6–120 min (binocular) and potential speeds of approximately 6–120 deg/sec (monocular) or 10–120 deg/sec (binocular) [3].

Although many methods of detecting these features are available, the classic methodologies are unable to directly capture the smallest eye motions because of their significant technological limitations. The accuracy of devices such as an EOG (electrooculogram) [11] or a head-mounted camera [12] is not sufficient to detect the quality of fixational eye movements. Accordingly, Fig. 1 is a schematic diagram rather than an experimental result. Analysing these small movements requires that a measurement technique be able to detect them simultaneously in three rotational degrees of freedom with an accuracy of 0.0001 deg. Moreover, the methodology should be minimally invasive.

We have developed a new device in our laboratory in response to these demanding requirements [10]. In this study, we used our high-accuracy device to demonstrate several new types of fixational eye movements [16]. Our results indicate the need for a review of the conventional thinking about fixational eye movements.

![Fig. 2 Previous experimental results regarding fixational eye movements](image)

2. Method

2.1 System configuration

Table 1 Specifications of the system

<table>
<thead>
<tr>
<th>Component</th>
<th>Manufacturer</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camera</td>
<td>Photron</td>
<td>FASTCAM-PCI R2</td>
</tr>
<tr>
<td>Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scanning area</td>
<td>7.4 µm (H) × 7.4 µm (V)</td>
<td></td>
</tr>
<tr>
<td>Resolution</td>
<td>512 × 480 pix (250 FPS)</td>
<td></td>
</tr>
<tr>
<td>Size of Sensor</td>
<td>842 µm × 793 µm</td>
<td></td>
</tr>
<tr>
<td>Speed</td>
<td>30 ~ 10000 FPS</td>
<td></td>
</tr>
<tr>
<td>Lens</td>
<td>VS Technology</td>
<td>VSZ-M07545</td>
</tr>
<tr>
<td>Magnification</td>
<td>0.75 ~ 4.5 x</td>
<td></td>
</tr>
<tr>
<td>Distortion</td>
<td>0.03% (Telecentric)</td>
<td></td>
</tr>
<tr>
<td>W.D.</td>
<td>95.25 mm</td>
<td></td>
</tr>
<tr>
<td>Focus content</td>
<td>± 3 mm</td>
<td></td>
</tr>
<tr>
<td>Illumination</td>
<td>Sumita Optical Glass</td>
<td>LS-M2105</td>
</tr>
<tr>
<td>Laser</td>
<td>Sakaki Corporation</td>
<td>Z3A-635-lg90</td>
</tr>
</tbody>
</table>

The approach used for observing capillary blood flow was as follows:

First, images of the surface of the sclera were captured using our device. Second, the effects of fixational eye movements on the image of the blood vessel were measured. The movement of the eyelid could be cancelled. A high-speed camera with a microscope lens was used to capture an image of the blood vessel of the white of the eye. With image processing, we could detect displacements of the sclera images. We used a telecentric lens, which produces images of the same size, regardless of the distance between the lens and image sensor. Therefore, we did not need to measure the distance between the eyeball and the camera. We used an optical fibre for even illumination. The display was used to show the experimental target. Laser line marks were used to measure the angle of the camera line. The specifications of the system are shown in Table 1.
movements were eliminated because the sclera images contained vibrations from the fixational eye movements. An algorithm known as POC [4] was used to detect relative displacements among the sclera images. By measuring the displacements of the images, the centre position of each frame could be normalised, producing stable images. The third step was regional segmentation, which was implemented to detect the shape of the blood vessels. The shape of the blood vessels was detected using morphological operations. Additionally, the width of the blood vessels was determined using geometric calculations. Next, optical flow processing was utilised to translate the images into a physical blood flow velocity. Because a telecentric lens was used in our system, the conversion equations were quite simple.

2.2 Capturing sclera images

Fig. 4 illustrates how the images of the sclera surface were captured. The experimental subject sat in front of the target shown in Fig. 3.

The subject’s head was fixed on the stage to prevent facial movement. The camera line made an angle of 45 degrees with the subject’s visual line. The midline of the eyeball was captured. While the experimental subject watched the target on the display, the surface of the sclera was imaged at a frame rate of 250 FPS and with an optical magnification of 4.5. An optical fibre was included in the system to generate even illumination.

2.3 Stabilisation of the sclera images

Phase-only correlation (POC), an image-processing algorithm, was then applied to determine the displacement from the captured sclera images. This algorithm enhanced our ability to detect image displacement with 1/100-pixel accuracy. Fig. 5.a is an example of the displacement of blood vessels extracted by POC.

![Graph showing displacement of blood vessel](image)

Fig. 5.a An example of the displacement of blood vessels. The first frame was set as the benchmark, and the centre of the second frame was shifted to account for the displacements caused by the fixational eye movements. By eliminating the effects of the fixational eye movements, we were able to obtain stable sclera images. The blood vessels and the blood flow were observable in the stable sclera images. Fig. 5.b shows the displacement of the sclera images after horizontal and vertical regulation.

![Graph showing displacement after regulation](image)

Fig. 5.b Displacement of the sclera images after regulation
2.4 Region segmentation

Morphological operations were used to extract the shape of the blood vessels. The regional segmentation proceeded as follows:

To eliminate noise, e.g., noise from the presence of red blood cells, we set the value of each pixel to the minimum value taken by that pixel within a set of 100 frames (2.3.1). The result was Image 1 (Fig. 6.b).

\[
dst_1(x, y) = \min_{i\in[0,N]} src(x_i, y_i)
\]

(2.3.1)

Morphological closing (2.3.2)

\[dst_2 = close(src_1, element) = erode(dilate(src_1, element), element)\]

(2.3.2)

The result was Image 2 (Fig. 6.c).

Black-hat operation (2.3.3)

\[dst_3(x, y) = |src_2(x, y) - src_1(x, y)|\]

(2.3.3)

The result was Image 3 (Fig. 6.d).

Binary images

Two thresholds (high threshold and low threshold) were set in order to produce two binary images.

\[dst_4(x, y) = \begin{cases} src_3(x, y) \leq LowThreshold : 0 \\ src_3(x, y) > LowThreshold : 1 \end{cases}\]

(2.3.4)

\[dst_5(x, y) = \begin{cases} src_3(x, y) \leq HighThreshold : 0 \\ src_3(x, y) > HighThreshold : 1 \end{cases}\]

(2.3.5)

where HighThreshold=20

and LowThreshold=5.

The two resultant binary images were Image 4 and Image 5 (Fig. 6.e and Fig. 6.f).

Image 5 was set as the background, and the regions containing blood vessels were reconstructed through iterative operations (2.3.6). The result was Image 6 (Fig. 6.g).

\[H_{k+1} = (H_k \oplus B) \cap G\]

(2.3.6)

Start while

\[H_0 = src_5, \quad G = src_4, \quad B = \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix}\]

End if

\[dst_6 = H_k = H_{k+1}\]

2.5 Detecting the width of blood vessels

The width of blood vessels could be calculated using geometry. For these calculations, we used a mouse to select two points, \(B(X_B, Y_B)\) and \(C(X_C, Y_C)\), on the same side of the blood vessel. Another point \(A(X_A, Y_A)\) was selected on the other side of the blood vessel. Geometry was used to calculate \(h\), the width of the blood vessel.
According to Heron's formula,
\begin{equation}
\begin{aligned}
    a &= \sqrt{(X_B - X_C)^2 + (Y_B - Y_C)^2} \\
    b &= \sqrt{(X_A - X_C)^2 + (Y_A - Y_C)^2} \\
    c &= \sqrt{(X_A - X_B)^2 + (Y_A - Y_B)^2}
\end{aligned}
\end{equation}

\[ s = \frac{1}{4} \sqrt{(a^2 + b^2 + c^2)^2 - 2(a^4 + b^4 + c^4)} \]

\[ s = \frac{1}{2} a \ast h \]

\[ h = \frac{2s}{a} = \frac{\sqrt{(a^2 + b^2 + c^2)^2 - 2(a^4 + b^4 + c^4)}}{2a} \]

2.6 High-speed optical flow generation using the steepest descent method

In the conventional method, the SFIT feature point detection method \[13]\[14] has often been used to measure the optical flow. Because the computational cost of the method was high, it was difficult to achieve high-speed detection of optical flow from high-resolution images. Using the steepest descent method, we developed a high-speed method for detecting the feature points of an optical flow \[15].

The SDM (steepest descent method) is a method of exploring the minimum energy. Finding the minimum value of a monolithic area is difficult, but the local minimum value can be obtained quickly. The approach for finding the local minimum of the function \( F(x) \), which has a multidimensional vector argument \( x=(x_1,x_2,\ldots,x_n) \), was as follows:

1) The initial value was set as \( x=X(0) \), while \( k=0 \).
2) When the local minimum was found (satisfying equation 2.6.1), the processing was complete.

\[ \frac{\partial f(x^{(k)})}{\partial x_i^{(k)}} = 0 (i = 1, 2, \ldots, n) \]  (2.6.1)

3) The value of \( k \) was reset using equation 2.6.2.

\[ x^{(k+1)} = x^{(k)} - \eta \left[ \frac{\partial f(x^{(k)})}{\partial x_1^{(k)}} \right. \]

\[ \left. \frac{\partial f(x^{(k)})}{\partial x_2^{(k)}} \right. \]

\[ \ldots \]

\[ \left. \frac{\partial f(x^{(k)})}{\partial x_n^{(k)}} \right] \]

4) Set \( k=k+1 \) and return to 2) to start the next exploration.

Determining the optical flow using the SDM was a method to simplify the Watershed region tracking method. Generating optical flow using the SDM has 3 steps: detecting feature points, screening feature points and correlating feature points using the SDM.

2.6.1 Detecting feature points

To eliminate the noise from the images, a Gaussian filter was used to smooth the images. From the smoothed images, the local minima of eight neighbourhoods were found; these were the feature points.

2.6.2 Screening the feature points

The feature points that were on the edge or had a low greyscale difference were deleted because these points were susceptible to noise. Only stable feature points, as measured by their dispersion, were chosen. Using equation 2.6.3, we calculated the dispersion of each feature point. By setting a threshold, feature points that were not required could be removed.

\[ \sigma^2 = \frac{1}{n} \sum_{i=1}^{n} (x_i - \bar{x})^2 \]  (2.6.3)

\( \sigma \) is the dispersion of the feature point, \( x_i \) is the greyscale value of the pixels around the feature point, and \( \bar{x} \) is the average of all the feature points.

2.6.3 Correlating feature points using the SDM

The feature points that had small movements and similar positions between frames were considered to be the same feature point. As shown in Fig. 8, using that relationship, the one-dimensional correlation could be calculated using the steepest descent method.

\[ \frac{\partial f(x^{(k)})}{\partial x_i^{(k)}} = 0 (i = 1, 2, \ldots, n) \]  (2.6.1)

Fig. 8 One-dimensional correlation using the SDM

Next, mutual interframe information was used to match the feature points, analogously to using a two-dimensional correlation to determine the optical flow.
When the relationship between one pair of feature points satisfied with equation 2.6.4, the feature points were deemed to be the same point between frames.

\[
\begin{align*}
  \text{fwd}(P_{t-1}^j) &= P_t^j, \\
  \text{bwd}(P_t^j) &= P_{t-1}^j,
  \end{align*}
\]

if

\[
\begin{align*}
  \text{sdm}(P_{t-1}^j) &= P_t^j, \\
  \text{sdm}(P_t^j) &= P_{t-1}^j.
  \end{align*}
\]

(2.6.4)

\(P_t^j\) and \(P_{t-1}^j\) were the feature points of the time \(t\) frame and the time \(t-1\) frame, respectively. \(\text{fwd}()\) and \(\text{bwd}()\) were the correlation functions between the forward link and the backward link, respectively. \(\text{sdm}()\) was the steepest descent method function. The processing approach is shown in Fig. 9.

**Fig. 9 Interframe correlation using the SDM**

1) Identify feature point \(P_t^j\) from the image at time \(t\).

2) Use the SDM to identify feature point \(P_{t-1}^j\), corresponding to \(P_t^j\) at time \(t-1\).

3) Use the SDM to identify feature point \(P_t^x\), corresponding to \(P_{t-1}^j\) at time \(t-1\).

4) When \(P_t^j = P_t^x\), the feature points had bidirectional correspondence.

4) Using a test video, we compared the SDM with the SFIT and Watershed methods. The results are shown in Table 2.

**Table 2 Performance of the steepest descent method**

<table>
<thead>
<tr>
<th>Method</th>
<th>CPU: 2.66 GHz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QVGA</td>
</tr>
<tr>
<td>SDM</td>
<td>2.5 ms</td>
</tr>
<tr>
<td>SFIT</td>
<td>200 ms</td>
</tr>
<tr>
<td>Watershed</td>
<td>5.7 ms</td>
</tr>
</tbody>
</table>

3. Results

3.1 Width of blood vessels

Using our method, the width of blood vessels in the images was detectable. The width of the blood vessel was approximately 11.49 pix. The height of image was 448 pix. We printed the image and measured the corresponding area with a ruler. The height of the picture was 19.7 cm, and the width of the blood vessel was 0.5 cm.

\[
\bar{d} = \frac{0.5}{19.7} \approx 0.025
\]

\[
\bar{p} = \frac{11.49}{448} \approx 0.025
\]

**Fig. 10 Width of blood vessels**

Because a telecentric lens was used in our system, the distance of one pixel in the image was 1.65[μm]. The width of the blood vessel that we detected was 19.96[μm].

3.2 Blood flow velocity of peripheral blood vessels

Fig. 11 shows the optical flow using the SDM. Optical flow processing was used in the region with blood vessels. The feature points that were continuous for more than 100 frames and in a direction parallel to the blood vessel were chosen.
3.3 Physiological experiments

We asked 4 experimental subjects to perform the physiological experiments. The results are shown in Table 3.

Table 3 Results of the blood flow velocity measurements

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AB</td>
<td>113</td>
<td>1.025</td>
</tr>
<tr>
<td>2</td>
<td>SD</td>
<td>266</td>
<td>1.081</td>
</tr>
<tr>
<td>3</td>
<td>TT</td>
<td>66</td>
<td>3.586</td>
</tr>
<tr>
<td>4</td>
<td>KS</td>
<td>103</td>
<td>1.860</td>
</tr>
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</table>

4. Conclusion

A novel device for observing capillary blood flow using high-speed photography of the surface of the sclera was developed in our laboratory. The method was non-invasive and produced visualisations of the flow. Our experiments demonstrated that the blood vessels under the surface of the sclera are the best place to directly observe blood flow.

Image processing technology enabled measurement of the blood flow velocity on the surface of the sclera. Using POC, we eliminated the effects of fixational eye movements to stabilise the sclera images. Morphological operations were used to extract the shape of the blood vessels. The width of the blood vessels could be accurately measured. The optical flow calculated using the SDM was used to detect the velocity of the red cells flowing in the blood vessels with a precision of 0.42[μm/ms].

A variety of experiments verified the validity of our novel method for measuring blood flow velocity. In future studies, we will attempt to extract the shape of red blood cells using a lens that has sufficient magnification.

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References


