Three-dimensional Vision-based Nail-fold Morphological and Hemodynamic Analysis

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Abstract—In this paper, a Three-dimensional Vision-based Nail-fold Morphological and Hemodynamic Analysis (TVNMHA) is proposed to automatically extract morphological/hemodynamic features from an image sequence, reconstruct the corresponding three-dimensional vessel tubular models, and visualize the dynamic blood flow in the model constructed. The morphological features extracted include number, width/height, density, width of the curved segment, arteriolar limb caliber, curved segment caliber, venular limb caliber, blood color and tortuosity of capillaries. The corresponding pathological information derived has a spatial precision accurate up to 1.6µm. TVNMHA also employs optical flow method (OFM) to measure the velocity of the flowing plasmagaps between two consecutive frames with an error ≈ 10–32µm/sec [1]. The laser Doppler velocimetry is a common tool employed in measuring capillary with larger diameter (≈ 500µm). The average velocity of an area measured comes with an error around 100µm/sec [2], much higher than that of TVNMHA. TVNMHA can separately derive velocities for each capillaries within the whole microscopic range and obtain diversified morphological/hemodynamic features of capillaries with a low-cost equipment setup.

Keywords - microcirculation; finger nail-fold morphological/hemodynamic analysis; three-dimensional capillary reconstruction; 3D visualization.

I. INTRODUCTION

Microcirculation plays an essential role in biological tissues to supply oxygen and nutritive substances and to remove wasted products. Vessel shapes and red blood cell (RBC) dynamics in capillaries have been well understood in relation to Raynaud’s phenomenon [3, 4] and hypertension [5, 6]. A capillary includes three sections, namely, arteriolar limb, curved and venular limb (ref. Fig. 1) [3]. In peripheral microcirculation, blood flows in through the arteriolar limb, passes by the curved segment, and then flows out into venular. If a person suffers microcirculation lesions, vascular anomalies will be the first to reflect this abnormality.

The observation of microcirculation focuses on finger/foot nail-fold, conjunctival, lingual surface and lips. Richardson [7] analyzed the ischemia effect on RBC velocity in nail-fold of human toes. Finger nail-fold capillary microscopy, usually performed on the ring finger, is simple, non-invasive [8, 9] and expedient to observe human's capillaries and RBC dynamics directly. Orthogonal polarization spectral imaging [10, 11] and video capillaroscopy imaging [12, 13] are commercialized and currently used in clinical microcirculatory. Although these approaches have been validated to provide blood flow velocity information, yet limitations and disadvantages remained. For example, the velocity estimation method is limited to measurement of the straight segments of a vessel [14]. Ellis’s [15] velocity estimation method requires the vessel segments to be manually selected first, The laser Doppler velocimetry, proposed by Eiju [2], is a common method employed in measuring capillaries with larger diameter (≈ 500µm) and the average velocity of an area measured comes with an error around 100µm/sec. On the other hand, the velocity error of the optical flow method (OFM) (≈ 10–32µm/sec) is not only much lower than that of laser Doppler velocimetry, but also can separately measure velocities for every capillary located within the observed microscopic area. These desirable features can avoid vital microcirculation information to be ignored. Other than the hemodynamics analysis, a majority of previous studies lack the capability to automatically extract and measure the morphological features of capillaries.

TVNMHA extracts not only hemodynamic but also morphological features of capillaries from an image sequence automatically. Hemodynamic features include velocity and direction of the blood flow measured by OFM with a velocity error ≈ 10–32µm/sec. The morphological features detected include number, width/height, density, width of the curved segment, arteriolar limb caliber, curve segment caliber, venular limb caliber, blood color and

Figure 1. A capillary can be classified into arteriolar limb, curved segment and venular limb.
tortuosity of capillaries. All are significant pathology indicators with a spatial precision accurate up to 1.6 \mu m. To observe capillaries in any orientations, magnifications and viewpoints desirable, three-dimensional description of vessel lumen is provided. The centerlines of vessels derived by thinning method and the radii of the corresponding vessel cross-sections calculated by circular expansion are employed to determine the three-dimensional tubular surface meshes. This information will be applied to reconstruct three-dimensional tubular vessel models. Finally, the source image sequence is mapped to the vessel model constructed to dynamically perform animated blood flow visualization.

II. METHODOLOGY

The Leica MicroFluo™ microscope employed to measure capillary features is shown in Fig. 2, consisting of microscope object lens (Objective 5.0x, magnification = 330), light source (12V, 100W), light filter (58 mm diameter), power supply and camera (Canon 60D) with a video frame rate at 60 frames per second. The dimension of an image frame is 640×480 pixels. Figs. 3a, b show two successive frames acquired.

![Leica MicroFluo™ microscope](image)

Figure 2. Leica MicroFluo™ microscope.

![Two success frames acquired by Leica MicroFluo™ microscope.](image)

Figure 3. Two success frames acquired by Leica MicroFluo™ microscope.

To measure the blood flow velocity and extract the morphological/hemodynamic features from an image sequence, a series of image processing steps were performed.

Fig. 4 illustrates the flowchart of TVNMHA. Image stabilization is first applied to stabilize the target vessels within the region of interest (ROI) by compensating motion between adjacent frames. Then, segmentation of the vessel and non-vessel areas is applied. The skeleton of the vessel area is identified by thinning method to derive the vessel centerline and the radius of the corresponding vessel cross-section. With the above information, morphological features will be extracted by performing geometric analysis on segmented vessel areas and hemodynamic features will be measured by detecting plasmagaps in every frame and calculating their displacements between two consecutive frames. Finally, three-dimensional tubular surface meshes and capillary models can be defined and reconstructed [3]. Each frame of an image sequence will be mapped to the vessel models built to perform three-dimensional blood flow visualization.

A. Stabilization

When measuring the velocity of blood flow, difficulty to register the region of interest is often encountered. This problem becomes even more serious as the magnification of the microscope increases. Stabilization is an indispensable step to eliminate motion due to the involuntary movement of subjects captured under a high magnification ratio. Laplacian of Gaussian (LoG) is the second order derivative of the Gaussian function usually used to detect high frequency blocks, e.g., edges, within an image. To derive the interframe displacement, LoG filter is employed to select center points within high frequency blocks as control points. The blocks between successive frames can be matched through the correspondence of the control points [16, 17].

\[
\text{LoG}(x, y) = \frac{1}{\pi \sigma^4} \left[ 1 - \frac{x^2 + y^2}{2\sigma^2} \right] e^{-\frac{x^2 + y^2}{2\sigma^2}},
\]
where $\sigma$ is the width of the Gaussian kernel. Maximums of the resulting filtered image are selected as the control points, as shown in Fig. 5 (red dots corresponds to pixels with maximum LoG values). A target-block is an $N \times N$ window surrounding a control point. The search space is an $M \times M (M>N)$ window surrounding a target-block. Pel difference classification (PDC) will be performed for all candidate-blocks within the search space on the previous frame. The matching block with the highest degree of similarity will be identified. Thus, the displacement between the current and previous frames can be formulated as [17, 18]:

$$D_t(dx, dy) = D(x_{\text{maxLoG}}, y_{\text{maxLoG}}) + D_{t-1},$$

(2)

where $D_t(dx, dy)$ is displacement between frame $t$ and first frame, $x_{\text{maxLoG}}$ and $y_{\text{maxLoG}}$ are coordinates of the pixel with maximum LoG value in frame $t$, $l$ and $r$ are $X/Y$ coordinates of the center pixel of the most similar candidate-block found in frame $t-1$. All frames are stabilized with the first frame as a reference.

Figure 5. LoG ($\sigma=1.4$) result of the original image in Fig. 3a.

**B. Enhancement and Segmentation**

Due to the fact that green channel usually possesses the highest degree of contrast between background and vessel area in the original color vessel images acquired, as shown in Fig. 6, while the red and blue counterparts tend to be noisy [19], only the green color component will be utilized.

Figure 6. The green channel of the original color image in Fig. 3a.

To enhance the contrast between the background and small-diameter vessels, local histogram equalization is applied to avoid over-enhancement commonly encountered in global equalization techniques. In Fig. 7a, the red rectangle marks the over-enhanced area. On the other hand, the local histogram equalization processes each sub-regions separately. A more satisfactory enhancement result is obtained, as shown in Fig. 7b.

![Figure 7](image)

Figure 7. (a) Global and (b) local histogram equalization of the green channel image in Fig. 6.

Otsu’s method [20, 21] is used to segment the enhanced image into a binary image that contains only 0 (foreground: vessel area) and 255 (background: non-vessel area). Otsu’s method is implemented both on the whole region (Fig. 7a) and sub-regions (Fig. 7b) of the enhanced images. Usually, the major non-vessel area can be accurately presented in the globally thresholded image (Fig. 8a), yet the vessels are often over-segmented. On the contrary, in the locally thresholded image (Fig. 8b), each vessel with different width and depth can be segmented with high degree of precision. However, the noisy regions are often subjected to over-segmentation. Therefore, an “AND” logical operator will be imposed on images obtained by global and local-thresholding operations. Globally thresholded image eliminates noises on the background of a locally thresholded image, while locally thresholded image obtains a more detailed vessel area by improving the over-segmented areas in the globally thresholded counterpart. As shown in Fig. 8c, a more accurately segmented image will be generated.

![Figure 8](image)

Figure 8. (a) Global thresholding, and (b) local thresholding of the equalization image by Otsu’s method, (c) combining global and local thresholded images and (d) removal of noises and shorter branches.

The final step is pixel selection by taking the number of a pixel that is considered as foreground into account as
follows:

\[ R(x, y) = \begin{cases} 
\text{foreground}(0) & \text{if } \text{count}(x, y) \geq \zeta, \\
\text{background}(255) & \text{if } \text{count}(x, y) < \zeta.
\end{cases} \]  

(3)

\[ \text{count}(x, y) = \sum_{t=0}^{N} C(x, y, t), \quad C(x, y, t) = \begin{cases} 
0 & \text{if } I(x, y, t) = 255, \\
1 & \text{if } I(x, y, t) = 0,
\end{cases} \]  

(4)

where \( R(x, y) \) is the pixel selection result image, \((x, y)\) pixel coordinates, \( \zeta \) count threshold, \( I(x, y, t) \) the pixel value in frame \( t \) with coordinate \((x, y)\), \( N \) the frame number of image sequence, and \( \text{count}(x, y) \) the count of a pixel with coordinate \((x, y)\). When \( I(x, y, t) \) is determined as belonging to a vessel area, one will be added to \( \text{count}(x, y) \). If the \( \text{count}(x, y) \) of a pixel exceeds the threshold \( \zeta \), it will be regarded as a vessel area (foreground), as shown in Fig. 8d.

C. Skeleton extraction and radius calculation

To construct three-dimensional tubular vessel model, the centerlines of vessels have to be determined first. Each circular cross-section of a vessel can be defined with a centerline and the corresponding radius. Image thinning techniques will be applied to the segmented binary image to extract skeletons of capillaries [22], as shown in Fig. 9.

![Figure 9. The skeleton of a segmented binary image.](image)

According to the count of neighboring pixels, the skeleton can be classified into three classes, skeleton-twig-pixel (STP, count 1), skeleton-middle-pixel (SMP, count 2), and skeleton-link-pixel (SLP, count 3–8). In Fig. 10, the red and blue pixels represent the SLPs and STPs respectively, while others correspond to SMPs [23].

![Figure 10. The skeleton-twig-pixel (STP), skeleton-middle-pixel (SMP), and skeleton-link-pixel (SLP) of a skeleton](image)

The centerline of a capillary vessel can be derived from the undirected skeleton graph by the following searching procedure. Search the unvisited SMPs from the SLPs until either the STPs or SLPs meet. The positions of the SMPs searched and two endpoints are recorded as a path. All SMPs searched are marked as “visited”. When all SMPs are visited and short paths are removed by comparing to a threshold, the path left is the centerline of a vessel. The radius of a cross-section of a vessel can be determined by taking a point \( N_i \) on the centerline as the center of a circle \( C(N_i, r) \), expanding the radius \( r \) of the circle gradually until touching the periphery of a vessel, as shown in Fig. 11.

![Figure 11. Circular expansion is employed to identify the radius of a vessel cross-section.](image)

The circular expansion algorithm is given as follows:

**Algorithm:** Circular expansion

**Input:** Segmented image \( R \) (Fig. 8d). A set of centerlines \( L \) (Fig. 9)

**Output:** Radius corresponding to every cross-section along the centerline.

**Step 1:** Consider a point \( N_i \) on \( L \) as the center of a circle with a radius \( r \), denoted as \( C(N_i, r) \).

**Step 2:** If \( C(N_i, r) \) covers the vessel area completely, then the radius \( r \) will be expanded with a step size 1. Repeat step 2.

Else, go to step 3. (i.e., at least one pixel on \( C(N_i, r) \) belongs to non-vessel area in the segmented image.)

**Step 3:** Output \( r \) as the radius of the point \( N_i \).

When we reach Step 3 according to the above algorithm, the circle after expansion intersects with the boundary of the vessel. The point located on the centerline and the corresponding radius identified form an accurate description of the vessel cross-section.

D. Feature extraction

The connected-component labeling is an operation to transform a binary image into a symbolic counterpart in that components are assigned a unique label. In Fig. 12, each
connected regions of capillaries are identified and labeled by different colors. Small spurious connected regions are removed. Only areas corresponding to complete capillaries remain.

After identifying the connected regions within the capillaries, the morphological and hemodynamic features can be extracted. The procedures in extracting relevant morphological and hemodynamic features will be proceeded as follows:

1) **Morphological features**
   a) **Number of capillaries**: According to the connected-component labeling, the number of capillaries is equal to the maximum index of labels assigned, e.g., the number of capillaries is 6 in Fig. 12.

b) **Density**: The density of capillaries is calculated by dividing the number of capillaries \( N \) with the area of the microscopic range \( A \) observed:

\[
\text{Density} = \frac{N}{A}.
\]  

\[ (5) \]

Figure 12. The result of applying connected-component to the vessel-segmented binary image in Fig. 8d.

c) **Width/Height of capillary**: The width \( W \) of a capillary is calculated by \( W = P_{\text{rest}}(x) - P_{\text{lest}}(x) \), where \( P_{\text{rest}}(x) \) is the \( x \)-coordinate of the rightmost pixel and \( P_{\text{lest}}(x) \) that of the leftmost one. The height \( H \) is equal to \( P_{\text{rest}}(y) - P_{\text{lest}}(y) \), where \( P_{\text{rest}}(y) \) is the \( y \)-coordinate of bottommost pixel and \( P_{\text{lest}}(y) \) that of the topmost one, as shown in Fig. 13.

\[ \theta_L = \tan \left( \frac{Y_t - Y_l}{X_t - X_l} \right), \quad \{ \text{Leftboundary} = X_t \text{ if } \theta_L > \delta \}, \]

\[ 0 \leq l < t, \]

\[ \theta_L = \tan \left( \frac{Y_t - Y_r}{X_t - X_r} \right), \quad \{ \text{Rightboundary} = X_r \text{ if } \theta_L > \delta \}, \]

\[ t < r \leq L, \quad L: \text{point number of a centerline}. \]  

\[ (6) \]

The topmost node on the centerline of a capillary is selected with index \( t \) and coordinates \( X_t, Y_t \). By computing the angle \( \theta_L \) between the vertical line and \( \overline{P_l P_t} \), where \( P_t \) is topmost node and \( P_l \) are the previous nodes with index \( l \) \((0 \leq l < t)\). When the angle \( \theta_L \) is larger than a threshold \( \delta \), it means the left boundary of the curved segment is encountered and the \( x \)-axis value of \( P_t \), i.e., \( X_t \), will be recorded. Likewise, if the angle \( \theta_R \) between the vertical line and \( \overline{P_t P_r} \) (\( P_t \) is topmost node and \( P_r \) are the following nodes with index \( l \) \(( t < r \leq L \)) is larger than a threshold \( \delta \), it means the right boundary of the curved segment is encountered and the \( x \)-axis value of \( P_r \), i.e., \( X_r \), will be recorded. The width of a curved segment is the distance between \( X_l \) and \( X_r \), as shown in Fig. 14.

\[ \text{Width of a curved segment} \]

\[ \text{Center line} \]

\[ \text{Topmost node } P_t \]

\[ \text{Vertical line} \]

\[ \text{Topmost node } P_t \]

\[ \text{Width of a curved segment} \]

\[ \text{Verticle line} \]

\[ \text{Topmost node } P_t \]

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\[ \text{Verticle line} \]

\[ \text{Topmost node } P_t \]

\[ \text{Width of a curved segment} \]
f) **Blood color of a capillary:** The color of the blood can be divided into three categories, namely, light red, red and deep red. Based on the color saturation of a vessel, the blood color can be differentiated. Fig. 16 shows the detection result of the blood color.

![Image of blood colors](image)

Figure 16. The color of blood: (a) light red, (b) red and (c) deep red.

g) **Tortuosity of a capillary:** The tortuosity of blood vessels, e.g., retinal and cerebral blood vessels, is a vital medical sign. The vessel path is divided into a plural number of constituent parts \( N \), each sharing a constant sign of curvature. The arc-chord ratio for each part is derived and the tortuosity is estimated by:

\[
\tau = N^{-1} \sum_{i=1}^{N} \frac{L_i}{S_i} - 1.
\]

where \( \tau \) is the tortuosity, \( N \) the number of constituent parts segmented for a curved path, \( L_i \) the length of the \( i^{th} \) constituent part, \( S_i \) the Euclidean distance between the two endpoints of \( L_i \). The tortuosity of a straight line is 0.

2) **Hemodynamic features**

a) **Blood Flow Velocity:** The optical flow method (OFM) is applied to calculate the velocity field by detecting and tracking the movement of cells or plasmagaps between two consecutive frames. Thus, the extraction of the cells or plasmagaps of blood flow is essential. The contrast sketching for each capillary is proceeded first to enhance the contrast of blood cells and plasmagaps. Image subtraction between two adjacent frames is followed to segment blood flow plasmagaps.

To find the nearest plasmagaps between adjacent frames, the distances between every matching pair of regions are calculated first. The process is shown in Fig. 17. The blue and red rectangles represent the same plasmagap present in different positions of successive frames. The existing plasmagaps might disappear and new ones might emerge from one frame to another as results of occlusion and circulatory exchange between capillaries and surrounding tissues. The rectangle with dotted line in Fig. 17c corresponds to the existing plasmagap disappeared, while the black color rectangle marks a newly emerging plasmagap.

![Image of plasmagaps](image)

Figure 17. (a)-(d) Detection and tracking of the plasmagaps in successive frames from \( t_0 \) to \( t_3 \).

The estimation of the flood flow velocity is defined as follows:

\[
T_{\text{dis}}(t) = \sum_{i=1}^{M} \sum_{j=1}^{N} \min \{ \text{dist}(P(x_i, y_i), P(x_j, y_j)) \}
\]

\[
v_{\text{velocity}}(t) = \frac{T_{\text{dis}}(t)}{M},
\]

where \( T_{\text{dis}}(t) \) is the summation of displacements in frame \( t \), \( M \) and \( N \) the number of plasmagaps defined in frame \( t \) and \( t+1 \), \( P(x_i, y_i) \) the center point of a plasmagap defined in frame \( t \) with index \( i \) and coordinate \( (x_i, y_i) \), \( \text{dist}(t) \) is the function that returns the distance between two points, \( v_{\text{velocity}}(t) \) represents the average velocity in frame \( t \). In tracking the distance travelled by plasmagaps between successive frames, we start from the plasmagaps defined in frame \( t \), then search the matching plasmagaps with the minimum distances in frame \( t+1 \). If the matching plasmagaps found, the distance will be recorded. When a plasmagap disappears in the next frame, this plasmagap is marked as missing. In the face of newly emerged plasmagap, the procedure describe above will be applied. Finally the velocity of capillaries will be obtained by dividing the distance travelled with the duration of the image sequence.

b) **Blood Flow Direction:** Through the correspondence of plasmagaps between successive frames, the associated directions can also be detected as from arteriolar to venular limb, or vice versa.
E. Three-dimensional vessel model reconstruction

After the derivation of the centerlines and diameters of every cross-sections within vessels, three-dimensional capillary models can be reconstructed, as shown in Fig. 18.

F. Three-dimensional animated blood flow visualization

The image sequence stabilized will be mapped to the three-dimensional vessel models on a frame-by-frame basis to perform animated blood flow visualization, as shown in Fig. 18c and Fig. 18d. The dynamics of the blood flow captured by the camera can be observed at any orientations, magnifications and viewpoints desirable.

III. EXPERIMENTAL RESULTS

Table 1. The features extracted for all capillaries in an image sequence.

<table>
<thead>
<tr>
<th>Features</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of capillaries</td>
<td>6</td>
</tr>
<tr>
<td>Density</td>
<td>0.131 units/mm²</td>
</tr>
<tr>
<td>Average Width</td>
<td>0.1860 mm</td>
</tr>
</tbody>
</table>

Table 2. The features extracted for three capillaries in an image sequence.

<table>
<thead>
<tr>
<th>Capillaries</th>
<th>Features</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary 1</td>
<td>Width: 0.1775 mm</td>
<td>0.5183 mm</td>
</tr>
<tr>
<td></td>
<td>Height: 0.7151 mm</td>
<td>0.6319 mm</td>
</tr>
<tr>
<td>Capillary 2</td>
<td>Width of the segment: 0.0735 mm</td>
<td>0.0911 mm</td>
</tr>
<tr>
<td></td>
<td>Arteriolar limb caliber: 0.0063 mm</td>
<td>0.0063 mm</td>
</tr>
<tr>
<td>Capillary 3</td>
<td>Venular limb caliber: 0.0287 mm</td>
<td>0.0223 mm</td>
</tr>
<tr>
<td></td>
<td>Curve segment caliber: 0.0191 mm</td>
<td>0.0287 mm</td>
</tr>
<tr>
<td></td>
<td>Blood color: Red</td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td>Tortuosity: 2.8399</td>
<td>2.2373</td>
</tr>
<tr>
<td></td>
<td>Blood flow velocity: 0.3395 mm/sec</td>
<td>0.3333 mm/sec</td>
</tr>
<tr>
<td></td>
<td>Blood flow direction: From arteriolar limb to venular limb</td>
<td>From arteriolar limb to venular limb</td>
</tr>
</tbody>
</table>

IV. CONCLUSIONS

The TVNMHA proposed is an automatic morphological and hemodynamic features extraction method. The dynamic three-dimensional vessel model with animated visualization allows practitioners to navigate freely at any desired viewpoint and magnification to observe the capillaries. The vascular anomalies and microcirculation lesions can be recognized more accurately. In addition, the morphological and hemodynamic features extracted also establish a solid pathological basis for proper diagnosis. From the viewpoint of engineering, TVNMHA has a spatial precision accurate up to 1.6µm pending on the ratio of magnification and is suitable for the measurement of capillaries with a wide range of diameters. The velocity error (≈10µm/sec~32µm/sec) of the TVNMHA proposed is lower than that of laser Doppler blood flow velocimetry (≈100µm/sec [2]).

REFERENCES


