

Estimation of Microcirculatory Blood Flow Velocity Profiles

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Abstract—An approach to estimate blood flow cross-sectional velocity profiles in intravital microscopy videos of post-capillary venules is proposed. Given an image sequence, the cross-section upon which the velocity profile is to be estimated is manually chosen. The velocity of each streamline, assumed perpendicular to the chosen cross-section, is estimated separately from other streamlines, except for a smoothness constraint imposed on the velocity profile. Video instability and undesirable structures transparently superimposed on the flow stream are accounted for in order to diminish their adverse effect on the estimation. The proposed approach is tested on synthetic, *in vitro* and *in vivo* videos.

I. INTRODUCTION

Intravital microscopy is a specialized medical imaging modality for the acquisition of microscopic images *in vivo*. In microcirculation research, the technology plays an important role. So for instance, through the image processing and analysis of video streams acquired with this imaging modality, it is possible to make measurements of microvascular function *in vivo*. The aim of this study is to produce velocity profile estimates that may subsequently be used to derive indexes for signaling vascular pathological disorders in transgenic mice mimicking human pathologies, such as sickle cell anemia and beta-thalassemia.

In intravital microscopy, as well as in other medical imaging modalities, the problem of estimating the velocity at which observed structures move has received considerable attention. Yet, approaches heretofore used to measure cross-sectional blood flow velocities in the microcirculation do not effectively counteract the effects of commonly seen disrupting phenomena such as, for instance, transparency—the mixture of different objects' intensity values at the same image location. Some of the newly proposed approaches seek a description of motion by tracking individual cells in the blood stream [1], [2], [3]. Their resilience to some of the disturbing extraneous phenomena remains untested, however. In addition, these approaches are convoluted in formulation, basically due to the fact that cells must be first identified and then, somehow, followed. Others, rather than attempting to first produce a high-level representation of the image's content,

estimate blood velocity profiles using the intensity values firsthand. These approaches are based notably either on the normalized cross-correlation technique [4] or some form of the standard optical flow constraint [5]. In general, image shaking, illumination changes, blur variations and transparency appear to be the most prevalent disturbing phenomena preventing a reliable estimation of velocity profiles in intravital microscopy. In particular, how to reliably cope with blur variations and transparency has remained an elusive question.

The approach proposed here explicitly accommodates the undesirable effects of video instability (shaking) and transparency so as to diminish their influence in the estimation of the sought blood velocity profiles.

II. HYPOTHESES AND PROPOSED METHODOLOGY

The maximum velocity, the average velocity, or the shear rate can be used to characterize the behavior of the blood flow stream in microvessels. These quantities can be easily obtained if the velocity profile across some sections of the vascular network is known.

It was hypothesized for this study that blood is a fluid whose flow condition can be modeled as laminar. This type of flow is characterized by layers of matter moving in parallel along the vessel, with no transfer of matter from one layer to another. Note that this behavior is readily seen in regions where the vessel resembles a straight pipe, rather than in those regions where the vessel bifurcates, bends or changes diameter.

In the approach proposed here, we manually select a cross-section most perpendicular to the direction of the flow in the chosen vessel. Then, the velocity of each layer (whose direction is taken to be perpendicular to the chosen cross-section) is estimated assuming a constant velocity model (a shift) for all pixels in the layer, and enforcing a smoothness constraint on the overall velocity profile. The estimation is embedded in a multi-resolution scheme, so to be able to estimate large motions reliably.

In the above scheme, there is no account for image patterns superimposed on the flow stream. These patterns (which we refer to as the background) go sometimes unnoticed, but do affect the estimation of the velocity profile. The contribution of the intrusive structures can be cancelled out by constructing a time-wise difference image sequence from a sequence where

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the background has been stabilized. To this end, it is assumed that the motions of the foreground (the image pattern where the blood stream resides) and the background are bounded, in the sense that if the non-flow regions of the foreground (background) move(s) then the background (foreground) also experiences the same motion. Motion of the blood flow can then be estimated, as previously described, from the time-wise difference sequence.

Note that the assumptions on the steady laminar flow behavior of blood and on the admissible deformations of the background-foreground structures upon which the proposed algorithm has been developed represent an idealized picture of the real situation. The transient nature of the blood flow and the pervasiveness of branching points and curved sections along the microvascular network prevent the flow from achieving a steady state. Also, the vascular and surrounding tissues, being elastic, may undergo deformations that may vitiate the background-foreground bounding assumption. Nonetheless, these assumptions have allowed us to propose a formulation for the blood flow velocity estimation problem, which, as shown in the experimental results section, is able to produce reliable velocity profile estimates.

III. FORMULATION

The optical flow constraint equation [5] establishes an instantaneous relationship between the intensity variations of the image and a vector representing the image's motion, at each spatio-temporal location (\mathbf{x}, t) :

$$I_x(\mathbf{x}, t) u(\mathbf{x}, t) + I_y(\mathbf{x}, t) v(\mathbf{x}, t) + I_t(\mathbf{x}, t) = 0, \quad (1)$$

where subscripts stand for partial derivatives of the image sequence $I(\mathbf{x}, t)$ with respect to the coordinate axes direction, and $u(\mathbf{x}, t)$ and $v(\mathbf{x}, t)$ are velocity components expressed in the same coordinate system. Given an image sequence $I(\mathbf{x}, t)$, we wish to estimate the velocity field components $u(\mathbf{x}, t)$ and $v(\mathbf{x}, t)$ across some chosen sections of the image where blood flows.

In a circular tube (our local approximation of the vessel), the laminar velocity profile for fluid shows a single orientation. It is therefore possible to formulate the optical constraint equation in a coordinate system where one of the velocity components (either $u(\mathbf{x}, t)$ or $v(\mathbf{x}, t)$) becomes zero. Suppose that a coordinate system has been identified and that under this system $v(\mathbf{x}, t)$ vanishes. Then, instead of having to solve for two unknown motion components in Equation (1), a single motion function needs to be estimated for each layer, where a layer, denoted as L , is a set of image pixels equidistant from the non-vanishing axis. Under the assumption of steady laminar flow, the velocity across any given layer is a constant quantity, i.e., $u(\mathbf{x}, t)$ is a constant function. The unknown motion can thus be estimated, in the least square sense, as:

$$\min_u \sum_{\mathbf{x} \in L} (I_x(\mathbf{x}) u + I_t(\mathbf{x}))^2, \quad (2)$$

where the estimation is carried out in a specific moment in time (note that the time dependency has been omitted). The

parameter u that minimizes the above function solves the equation:

$$\sum_{\mathbf{x} \in L} I_x^2(\mathbf{x}) u + \sum_{\mathbf{x} \in L} I_x(\mathbf{x}) I_t(\mathbf{x}) = 0, \quad (3)$$

which is the derivative of the argument of Equation (2) with respect to u equated to zero. Clearly, u can be directly solved regardless of neighboring layers. If we consider the equations for all layers, say the set $\{L_i\}_{i=1}^n$, simultaneously, the constraint on the motion parameters, vector $\mathbf{u} = [u_1 \ u_2 \ \dots \ u_n]^T$, across all layers can be expressed in matrix form as:

$$\mathbf{A} \mathbf{u} = -\mathbf{b}, \quad (4)$$

where \mathbf{A} is a $n \times n$ diagonal matrix whose diagonal elements are the coefficients of u in Equation (3) for each profile L_i , and \mathbf{b} is a vector collecting the independent terms of the same equation.

The continuum mechanics formulation of fluid dynamics considers that fluid properties, such as velocity, vary continuously from one point to the next. That is, sudden changes should not happen within the blood vessel. In principle, a velocity profile derived using the optical flow constraint alone (obtained by solving for \mathbf{u} in Equation (4)) should be smooth. However, noise and other artifacts may wreck the continuity of the velocity profile. It is possible, nonetheless, to impose a smoothness constraint between neighboring flow vectors so as to guarantee continuity. Here, the Laplacian of the flow components is adopted as a measure of smoothness. Under this criterion, the smoothest velocity profile minimizes a functional based on the second-order derivative of the profile, written in discrete form as:

$$\min_{u_1, \dots, u_n} \kappa \sum_{i=2}^{n-1} (u_{i-1} - 2u_i + u_{i+1})^2, \quad (5)$$

where κ is the number of pixels in the set L_i , assumed to be the same for all i . The motion parameters, collected in vector \mathbf{u} , minimizing this functional can be found by deriving with respect to \mathbf{u} and equating to zero. In matrix form, the resulting system of equations can be written as:

$$\mathbf{S} \mathbf{u} = \mathbf{0}, \quad (6)$$

where the $n \times n$ matrix \mathbf{S} is band diagonal. Clearly, any velocity profile whose second- and higher-order derivatives vanish minimizes Equation (5).

A velocity profile that is guaranteed to be both smooth and compliant with the optical flow constraint equation can be found from a system of equations that is a combination of the two constraints:

$$(\mathbf{A} + \lambda \mathbf{S}) \mathbf{u} = -\mathbf{b}, \quad (7)$$

where λ is a parameter indicating the relative importance of the individual constraints in the overall system. This is a system of equations with a relatively small number of unknowns, which can be easily solved by using, for instance, Gaussian elimination.

In Equation (2), velocity estimates are produced by pooling information from compatible pixels only, that is, from pixels

that are supposed to be moving at the same velocity. By contrast, in previously proposed motion estimation techniques, either based on cross-correlation or on the optical flow constraint, information is pooled usually from a rectangular region of the image, thus encompassing pixels moving at different velocities. An advantage of our approach is that by considering the motion of each streamline separately it is possible to estimate large motions reliably and to cope satisfactorily with transparently superimposed structures. The details are given in the following subsections.

A. Measuring Large Velocities

In a discrete image, the derivatives of Equation (1) are usually approximated using finite differences. In general, finite differences provide a reasonable approximation; however, these approximations may not yield accurate velocity estimates. This is particularly true when motions are large, for which the temporal derivative becomes less reliable.

Several schemes for motion estimation that effectively cope with large motions have been proposed. These schemes use a pyramidal (multi-resolution) representation of the image to refine velocity vectors calculated at each level of the pyramid [6]. Starting from the coarsest resolution level of the pyramid, velocity vectors at a given level are estimated and then used to warp one of the image frames towards the other. The operation is repeated until the finest level is reached. Here, a multi-resolution Gaussian pyramid is used for the application in hand. At each level, refined velocity estimates are calculated from the sequence:

$$C(\mathbf{x}, t_1) = I(\mathbf{x} + \mathbf{v}(\mathbf{x}), t_1), \quad (8a)$$

$$C(\mathbf{x}, t_2) = I(\mathbf{x}, t_2), \quad (8b)$$

where $\mathbf{v}(\mathbf{x})$ is a vector derived from $\mathbf{u}^c = [u_1^c \ u_2^c \ \dots \ u_n^c]^T$, the total velocity estimate up to the current pyramid level (that is, a vector that cumulates previous velocity estimates), as:

$$\mathbf{v}(\mathbf{x}) = [u_i^c \ 0]^T \quad \text{if } \mathbf{x} \in L_i. \quad (9)$$

At the coarsest level, \mathbf{u}^c is initially set equal to zero.

B. Accounting for Background Structures

A fundamental assumption in Equation (1) is that motion at any point of the image is due to a single pattern. The equation does not account for the motion of any other patterns that may be superimposed. We assume here that the background is a rigid structure and that it is static with respect to the non-flow regions of the image pattern where the blood stream resides (foreground), that is, if the background moves, it does it rigidly and the same motion is also experienced by the foreground. Bergen *et al.* [7] showed that from three consecutive frames of an image sequence made up from the additive combination of two distinctly translating patterns, it is possible to construct a new sequence in which one of the patterns is factored out, and where the motion of the remaining (somehow transformed) pattern is that of its counterpart in the original sequence. We use this result to produce an image sequence where the blood flow stream is the only visible structure. Assuming that the

motion of the background is known, the new sequence is constructed as:

$$D(\mathbf{x}, t_1) = I(\mathbf{x} + \mathbf{s}_2, t_2) - I(\mathbf{x} + \mathbf{s}_1, t_1), \quad (10a)$$

$$D(\mathbf{x}, t_2) = I(\mathbf{x} + \mathbf{s}_3, t_3) - I(\mathbf{x} + \mathbf{s}_2, t_2), \quad (10b)$$

where \mathbf{s}_i is the shift of the background from a given reference time instant to instant t_i . The shifts seek to stabilize the background with respect to a frame given at a certain instant of time. We assume that the original image sequence can be written as $I(\mathbf{x}, t_i) = P(\mathbf{x} - \mathbf{s}_i, t_i) + G(\mathbf{x} - \mathbf{s}_i)$, where $P(\mathbf{x}, t_i)$ is the sequence of interest (foreground) and $G(\mathbf{x})$ is the background. Clearly, subtracting two frames where the background has been stabilized nullifies G . Recall that in our approach it is assumed that particles observed in P move along parallel straight layers. The sequence D is thus the subtraction of layers in P at contiguous time instants moving at the same velocity. It is not difficult to notice then that the motion of any given layer in D is the same as that of its counterpart in P . Observe that, for each layer in P , constant velocity is required in only three consecutive frames.

In practice, the translational shift is not known. Nonetheless, it can be estimated by using a standard image registration technique [8] on the non-flow image regions. We use an approach based on the normalized cross-correlation technique. It is worth noting that the restriction on the rigidity of the background can be relaxed as long as the background-foreground bounding assumption is preserved.

IV. EXPERIMENTAL RESULTS

The proposed approach was first tested on synthetically generated images. With this test, we sought to measure how the estimated velocity profiles would be affected by misplacements (orientation-wise) of the manually selected cross-section. The sequence used in this test was generated from a single image frame of an actual intravital microscopy video. We selected a region in the image where the vessel most resembled a long straight pipe. Consecutive frames were then generated by warping the vessel according to a predefined velocity profile. Finally, a static sinusoidal background (a pattern commonly found in actual videos) was superimposed on the sequence.

The velocity profile was estimated for different orientations of the cross-section within a 10 degree range from the cross-section perpendicular to the actual direction of the blood flow. Cross-sections (line segments of fixed length) were 1 degree apart from one to the next, all of them intersecting in a common point placed at the center of the vessel. From each estimated profile, maximum and average velocities were computed and compared against the maximum and average values of the predefined simulated profile. For a parabolic profile with a maximum velocity of 5 pixels/frame and an average velocity of 2.14 pixels/frame for the considered cross-section, the maximum and the mean of the estimated profiles closely oscillated around these values. For cross-sections within 3 degrees of the actual orientation, the maximum velocity of the estimated profiles was within the [4.8, 5.3] interval; the average, within the [2.10, 2.14] interval.

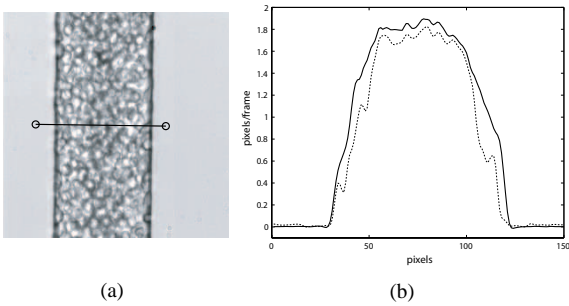


Fig. 1. Test on *in vitro* images. A single frame of the image sequence is shown in (a). The average estimated velocity profiles for the cross-section depicted by the black line in (a) are shown in (b). In (b), the bold plot is the profile estimated with the proposed approach fully enabled. The thin dashed plot is the estimated profile with the background compensation feature disabled.

Using the synthetically generated sequence, we also tested the influence of the background on the maximum and mean values of the velocity profile estimated from a cross-section correctly oriented. To this end, the velocity profile was estimated overriding the background compensation feature of Section III-B. In our experiments, the maximum and mean values of the estimated profile were consistently below the actual values and those obtained by the fully enabled approach. This was also demonstrated in an *in vitro* set-up, where pig blood at a hematocrit of 10% was made to flow through a D-shape microchannel of 50 microns. Image sequences were acquired using a Nikon Eclipse TS100 microscope and a Dalsa digital camera at 955 frames/sec. Figure 1(a) shows the first image frame of the sequence used in the test. In this sequence there are blood cells stuck onto the wall of the microchannel, which produce a marked static background. The estimated profiles are shown in Figure 1(b).

Finally, the approach was tested on a real intravital microscopic video of the mesentery of a normal anesthetized mouse (Figure 2(a)), taken with a Zeiss Axiotech vario microscope fitted with immersion objectives and using the same digital camera as before. Figure 2(b) shows the average estimated velocity profile for 100 consecutive frames of the input sequence. Although the actual velocity profiles are not known, the estimated profiles appeared to be in qualitative agreement with the perceived motions of the blood stream. The maximum velocity of the average profile was 3.42 pixels/frame; the mean velocity of the profile, 1.62 pixels/frame. With the background compensation feature disabled, the maximum velocity of the estimated average profile was 3.20 pixels/frame; the mean, 1.47 pixels/frame.

V. CONCLUSIONS

In this paper, we have presented an original approach for the estimation of cross-sectional blood flow velocities in intravital microscopy videos. The approach was developed by exploiting the assumed laminar character of blood flow across most sections of the microvascular network. The velocity of each streamline was estimated separately from other streamlines, except for a smoothness constraint imposed on the velocity profile. A multiresolution scheme was used to enable the estimation of large motions reliably. Undesirable patterns

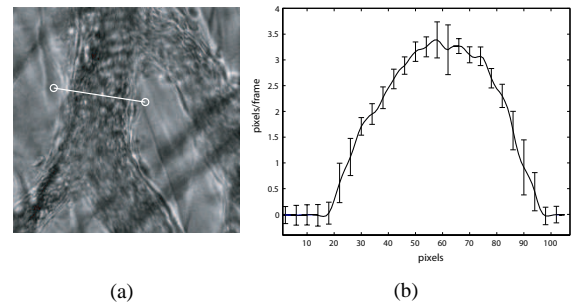


Fig. 2. Test on a real intravital microscopic video. A single frame of the image sequence is shown in (a). For the fully enabled technique, the average velocity and standard deviation of the estimated profile is shown in (b). The standard deviation of the estimated profiles is indicated by the vertical bars.

superimposed on the flow stream were also compensated for to cancel out their influence during the estimation of the velocity profile. The proposed approach has been tested on synthetic sequences and real videos (where the assumptions at the basis of the proposed scheme may not strictly hold). The results have shown accurate or predictable (for the case of the *in vitro* and *in vivo* videos) velocity estimates.

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