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Checklist for this Paper Submission

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2. Three (3) pages of paper summary included

Detection of Microspheres in Venules for Automated Particle Image Velocimetry

Abstract

In this paper, we propose an automatic approach for detecting particle tracers (microspheres) in microscopic imagery obtained from mouse cremaster venules in vivo. Measurements of the translational speed and radial position of individual microspheres provide the input data needed to extract velocity profiles from steady blood flow in venules. These profiles provide information about local hemodynamics that is critical to a broad range of fields in microvascular physiology, including endothelial-cell mechanotransduction, inflammation, and microvascular resistance. In the preprocessing stage, an active contour method based on dynamic programming is used for vessel region extraction. Each microsphere is then identified using a process of coarse segmentation followed by verification. Segmentation is achieved using a morphological method for microsphere detection while verification is achieved using an analytical model tailored to the microsphere. Experimental results are obtained using the proposed scheme and compared with previously published manually acquired data.

1. Introduction

The interface between blood and the vascular endothelium is of critical importance in microvascular physiology. Recently, salient features of this interface have been discovered using high-resolution near-wall fluorescent micro-partical image velocimetry (μ-PIV) based on m stroboscopic dual-flash epi-illumination of fluorescent microspheres [4]. The translational speed and radial position of individual microspheres can be used to extract velocity profiles in microvessels *in vivo* [4]. These profiles can be used to estimate pressure gradient, volume flow, local and apparent blood viscosity, tube, and even the thickness of the endothelial surface layer (ESL). The raw experimental data yield, in one frame, two fields of the same microsphere displaced a measurable distance over a known time interval (determined by the flash-time interval). To obtain these data, a dual image of a microsphere must be detected, the displaced distance between the two images must be measured (from which the translational speed can be computed), and the shortest distance between the microsphere center and the vessel wall must be measured.

Manual acquisition of these data is tedious and subjective. The goal of this research is to develop a system to identify dual-images of individual microspheres, and to automatically and efficiently collect the necessary parameters with objective precision. As a first step, we describe in this paper an image analysis method that identifies the microspheres. Using *a priori* knowledge regarding the shape, contrast and size of the microspheres, we present an effective model-based approach for pairing dual images and determining radial position and translational speed.

2. Methods

2.1. Extraction of the vessel region

Fig. 1 shows a frame from a microscopic image sequence showing the dual-image of a microsphere and several rolling leukocytes. All microspheres are assumed to be located within the vessel lumen. To initialize the vessel delineation process, an approximate region is estimated by applying a thresholding to the temporal variance image of the sequence. This provides initial boundaries that are close to the vessel wall. A contour extraction method is then implemented to delineate the actual boundary of vessel wall.

Based on the concept of an active contour model [2] [6], the energy function of our contour model consists of an internal energy term and an external energy term. The internal energy term is defined as the summation of squared distances between each vertex on the contour and a neighboring vertex. The internal energy is imposed as a way of achieving robustness to the inherent difficulties of weak contrast and noise. The external energy is expressed as the additive inverse, summed over the entire contour, of the image gradient magnitude computed in the normal direction of the contour. Deriving the active contour essentially amounts to finding the contour

with minimal energy. Using variational calculus, we can produce a set of partial differential equations that update the contour position in an iterative manner.

We note that the combination of noise and multiple edges tend to exist at the vessel wall boundary in our intravital microscopic images. This usually prevents a gradient-descent-based active contour method from converging to a consistent result. To overcome this, we minimize the active contour energy using a novel dynamic programming approach [1]. This technique assures global optimality and avoids impossible edge configurations.

After the vessel is detected in the first frame of a given sequence, automated image registration is applied to locate the vessel in subsequent images.

2.2. Morphological microsphere extraction

Mathematical morphology is a powerful nonlinear image analysis methodology for extracting, modifying and manipulating the features present in an image based on constituent shapes [3] [5]. Attention in this section is focused on the target extraction capabilities of this method.

Consider a grayscale image f, defined on a two-dimensional discrete space E. The *opening by reconstruction* of f given a maker image $m \leq f$ is:

$$\rho^{-}(m \mid f) = \lim_{n\to\infty} g_n, \quad g_n = \delta_C(g_{n-1}) \wedge f \tag{1}$$

where g_n denotes the n-fold composition of the conditional dilation δ_C with itself, and C is a flat structure element. The marker m identifies the portion of the profile of f that needs to be preserved. In this reconstruction process the image is simplified by eliminating small objects inside which the marker cannot fit.

Typically, a dual-image of a microsphere appears as two small peaks in the intensity profile. If the marker demarcates large-scale objects, such as background and leukocytes, then the reconstruction operator ρ^{-} will only reconstruct these objects. A simple subtraction from f will extract these targets. Thresholding based on area is then implemented on the resultant image. Connected components are then located which indicate the positions of image features.

2.3. Circularity model for verification

The results of the morphological operators may yield some spurious objects, including artifacts near the vessel wall. To eliminate these irrelevant features, we propose a circularity model of the microsphere that robustly detects the particles. The model is constructed under the assumption that each microsphere is the only element of our image that is locally uniform in gray value completely described by the following properties: (1) the particles usually maintain an approximately circular shape; (2) the particles fall in a known size range; (3) the intensity profile of the particles is of high contrast.

By the definition of a circular curve, we can define a function

$$F(n_1, n_2) = c_3 - (n_1 - c_1)^2 - (n_2 - c_2)^2 \tag{2}$$

where $F = 0$ corresponds to the microsphere contour, $F > 0$ indicates the interior and $F < 0$ the exterior. Using the regularized version of the Heaviside function H and its derivative δ defined by:

$$H_\varepsilon(x) = \frac{1}{2}(1 + \frac{2}{\pi}\arctan(\frac{x}{\varepsilon})), \quad \delta_\varepsilon(x) = H'_\varepsilon(x) = \frac{1}{\pi}\cdot\frac{\varepsilon}{\varepsilon^2 + x^2}, \tag{3}$$

the circularity model is defined by:

$$\hat{I}_c(n_1, n_2) = c_0 H(f(n_1, n_2)) + c_4 . \tag{4}$$

The cost function (i.e. the difference between the model and image data), is defined as the mean squared error:

$$E(c) = \frac{1}{N_1 N_2}\sum_{n_1=0}^{N_1-1}\sum_{n_2=0}^{N_2-1}[\hat{I}_c(n_1, n_2) - I(n_1, n_2)]^2 . \tag{5}$$

A gradient descent method is used to optimally approximate the image with the defined circularity model by minimizing the cost function. Initial conditions are provided by the morphological technique, which are expected to be close to the global optima. After the convergence of the numerical solution, we use two criteria to verify the identification of a microsphere: the value of the cost function and value of the contrast parameter c_0. We consider only the components that statistically satisfy both criteria.

2.4. Pair Matching

Microsphere pairs are then identified and matched. Ambiguities and false matches can be eliminated using the following two criteria: 1) Given a pair of microspheres observed using interlaced video microscopy, one microsphere should appear in the odd field, the other in the even field. 2) The orientation of the line segment connecting two microspheres forming a pair is congruent with direction of the prevailing flow.

3. Experimental results

A typical result showing an extracted dual-image of a microsphere is given in Fig. 1. Results of manually acquired μ-PIV data and those obtained using the proposed scheme are plotted in Fig. 2 for a microscopic image sequence. The Pearson's correlation coefficient of two series of velocity data is 0.9044, which shows good agreement between the two approaches.

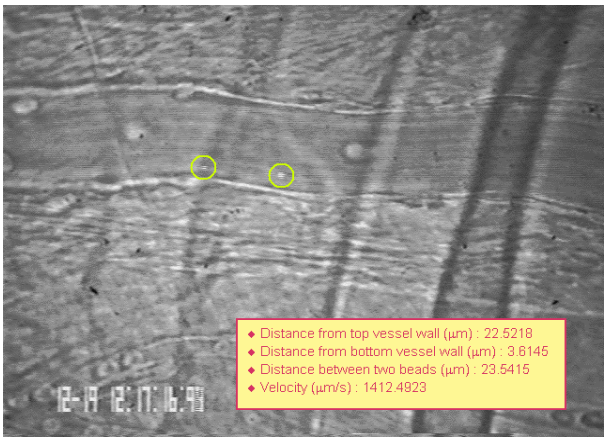

Figure 1. An example frame from the microsphere identification process. Microspheres are located at the center of the circles.

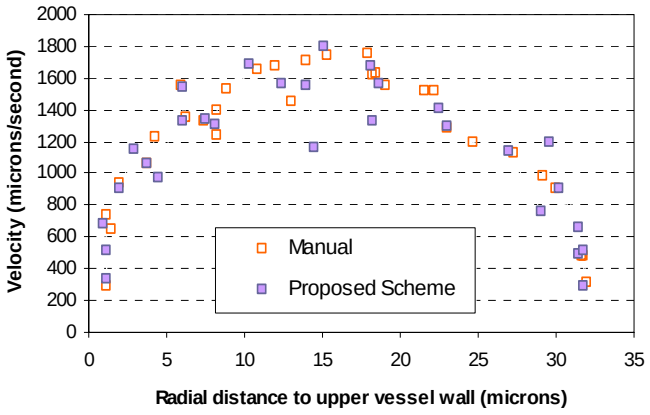

Figure 2. A comparison of microsphere velocities using both the automated data collection method and manual analysis.

4. Conclusion

In this paper, a method based on mathematical morphology and a parametric model is applied to the problem for microsphere detection in microvessels *in vivo*. Preliminary results suggest that automated identification can replace the tedious and subjective process of manual data acquistion.

References

[1] A. Amini, T. E. Weymouth and R. C. Jain, "Using dynamic programming for solving variational problems in vision," *IEEE Trans. Pattern Anal. & Mach. Intell.*, vol. 12, no. 9, 1990.

[2] M. Kass, A. Witkin and D. Terzopolous, "Snakes: active contour models," *Int. Jour. Comput. Vis.*, vol 1, pp. 321-331, 1987.

[3] P. Salembier and J. Serra. "Flat zones filtering, connected operators and filters by reconstruction," *IEEE Trans. Image Processing*, vol. 4, pp. 1153-1160, 1995.

[4] M. L. Smith, D. S. Long, E. R. Damiano, and K. Ley, "Near-wall μ-PIV reveals a hydrodynamically relevant endothelial surface layer in venules in vivo," *Biophysical Journal*, vol. 85, pp. 637-645, 2003.

[5] P. Soille, *Morphological Image Analysis: Principles and Applications*, Berlin, Germany: Springer, 1999.

[6] C. Xu and J. L. Prince, "Snakes, shapes, and gradient vector flow," *IEEE Trans. Image Processing*, vol. 7, pp. 359-369, 1998.