Blood Flow in Microchannels

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Blood flow in large arteries or biomedical devices can be treated as a homogenous fluid where its particulate nature can be ignored. However, in reality, blood is a suspension of deformable cells in a viscous fluid plasma. Hence, in microcirculation and microchannels, it is fundamental to take into account the effects of the multiphase properties of the blood and to study the blood flow behaviour at a cellular level. A clear example of the multiphase nature of the blood is the formation of a plasma layer (or cell-free layer) around the walls of the microchannels.

Keywords: blood flow ; particle tracking ; manual methods ; automatic methods ; image analysis ; biomicrofluidics ; red blood cells ; microcirculation ; microchannels

1. Introduction

Blood flow in microcirculation is crucial for the normal function of tissues and organs. Therefore, a detailed study of blood flow patterns and blood cells flowing in microvessels, microchannels and organs-on-chip is essential to provide a better understanding of the blood rheological properties and disorders in microcirculation [1][2][3][4][5][6][7]. One of the first techniques used for the study of flow patterns was the phase-contrast magnetic resonance imaging (PC-MRI). However, the technique requires long acquisition times and has low resolution [8][9]. Other techniques have been developed and combined to improve the acquisition and image processing. One of the most reliable ways to measure velocity fields in microcirculation is using Eulerian methods, such as the conventional micro-particle image velocimetry (PIV) [1][6][10][11][12] or the confocal micro-PIV [1][2][6][13]. The micro-PIV technique is one of the best suitable methodologies to study blood flow phenomena in microcirculation. Some studies have also combined PIV with ultrasounds (Echo-PIV) [14][15]. However, most in vivo measurements contain physiological fluids with high concentrations of blood cells and as a result, the amount of tracer particles captured within the fluid is often very low [5]. Other approaches for blood flow studies are particle illumination photography, laser doppler velocimetry, fluorescent cytometry [16][17] and computer fluid dynamics [17][18].

In microcirculation, the study of red blood cells (RBCs) flowing in microvessels and microchannels and the study of the cell-free layer (CFL) thickness in different microchannels geometries are very important to get a better understanding of the blood rheological properties and disorders in microvessels in a fast and accurate way. The presence and physiological characteristics of other cell types are also of great clinical relevance [19]. In this kind of study, the image analysis has an important role to obtain crucial information about blood rheology. For blood flow in microvessels, where there is a large number of interacting cells, manual tracking methods have been used to accurately track individual deformable cells flowing through glass capillaries [1][11][20], straight polydimethylsiloxane microchannels [21], stenotic arteries [22][23], hyperbolic contractions [24], and bifurcations [25]. However, the manual data collection is extremely time-consuming to have a statistically representative number of samples and may introduce operators' errors that eventually limit the application of these methods many times at different conditions [26]. Hence, it is crucial to develop versatile and automatic methods able to automatically track and compute multiple cell trajectories and able to measure the cell-free layer thickness in a network of microchannels.

2. An Overview of Image Analysis Methods for Microfluidic Blood Phenomena Quantification

2.1. Image Segmentation and Thresholding

Image analysis processing is a vast area that provides a large number of viable applications that can involve some steps such as image acquisition, image preprocessing, image segmentation, image post-processing and image analysis. Image segmentation is one of the most important and critical elements in automated image analysis, which consists in dividing a digital image into multiple regions, based on a set of pixels or objects, to simplify and/or change the representation of an image [27][28][29]. A variety of techniques can be applied: simple methods such as thresholding, or complex methods such as edge/boundary detection or region growing.

The literature contains hundreds of segmentation techniques [30][31], but there is no single method that can be considered good enough for all kinds of images. The main purpose of segmentation is to divide an image into regions of interest with similar gray-levels and textures in each region [32]. Segmentation methods change according to the imaging modality, application domain, method type—automatic or semi-automatic, depending on the image quality and the image artifacts, such as noise. Some segmentation methods may require image preprocessing prior to the segmentation algorithm [33][34]. Databases with algorithms to compensate for the uncertainties present in real-life datasets were developed [35]. On the other hand, some other methods apply post-processing to overcome the problems arising from over-segmentation. Overall, segmentation methods can be grouped into thresholding, boundary detection, and region growing [27][29][31][36][37]. Those methods vary in the way that the image features are treated and the way the appearance and shape of the target are modeled [38].

Thresholding methods assign pixels with intensities below a certain threshold value into one class and the remaining pixels into another class and form regions by connecting adjacent pixels of the same class, that is, in the thresholding process, each pixel in a grayscale is recognized as either an object or background. The more advanced method creates histograms, oriented to the intensity of grayscale or color, showing the frequency of occurrence of certain intensities in an image so that the regions and objects are recognized from these data [28][29][30]. Thresholding methods work well on simple images where the objects and background have distinctively different intensity distributions. Boundary extraction methods use information about intensity differences between adjacent regions to separate the regions from each other. If the intensities within a region vary gradually but the difference of intensities between adjacent regions remains large, boundary detection methods can successfully delineate the regions [28][29][30][39]. Region growing methods form regions by combining pixels of similar properties [39][40].

2.2. Segmentação e rastreamento de imagens de células sanguíneas

Over the last years, many studies have been conducted in the area of general segmentation methods that can analyze different types of medical images. Most used images are acquired during a diagnostic procedure and useful information is extracted for the medical professional. The development of image analysis in biomedical instrumentation engineering has the purpose of facilitating the acquisition of information useful for diagnosing, monitoring, treating or even investigating certain pathological conditions. It is important to always have in mind that the main purpose of biomedical imaging and image analysis is to provide a certain benefit to the subject or patient [41][42].

In normal human blood microscopic images, a high accumulation of RBCs could be observed, which results in the existence of touch and overlap between these cells [42]. These are two difficult issues in image segmentation where common segmentation algorithms cannot solve this problem [43]. Besides that, staining and illumination inconsistencies also act as uncertainty to the image [44]. This uncertainty makes the blood cell image segmentation a difficult and challenging task [43]. Numerous segmentation methods from peripheral blood or bone marrow smears have been proposed and most of them are region-based or edge-based schemes [42][45].

Jianhua et al. [46] developed an iterative Otsu's approach based on a circular histogram for the leukocyte segmentation. R. Sukesh Kumar et al. [47] developed two methods of color image segmentation using the RGB space as the standard processing space. These techniques might be used in blood cell image segmentation. Color images are a very rich source of information, because they provide a better description of a scene as compared to grayscale images. Hence, color segmentation becomes a very important and valuable issue [42][47]. For instance, Huang et al. [48] investigated a method based on the Otsu's method to segment and then recognize the type of leukocyte based on the characteristics of the nucleus. Willenbrock et al. [49] developed a program for image segmentation to detect both moving and stagnated cells in phase-contrast images. The program contributed to the study of the integrin LFA-1 mediation of lymphocyte arrest.

Khoo Boon et al. [50] performed comparisons between nine image segmentation methods which are gray-level thresholding, pattern matching, morphological operators, filtering operators, gradient-in method, edge detection operators, RGB color thresholding, color matching, HSL (hue, saturation, lightness) and color thresholding techniques on RBC. They concluded that there is no single method that can be considered good for RBC segmentation [42][50]. Meng Wang et al. [51] presented segmentation and online learning algorithms in acquiring, tracking and analyzing cell-cycle behaviors of a population of cells generated by time-lapse microscopy. Kan Jiang et al. [45] combined two techniques for white blood cells (WBCs) segmentation. Two components of WBCs, nucleus and cytoplasm, are extracted respectively using different methods. First, a sub-image containing WBCs is separated from the cell image. Then, scale-space filtering is used to extract the nucleus region from the sub-image. Later, watershed clustering in a 3-D HSV (hue, saturation, value) histogram is processed to extract the cytoplasm region. Finally, morphological operations are performed to obtain the entire connective scheme successfully. Li et al. [52] developed a new method for WBCs identification. The method consists of the combination of an acousto-optic tunable filter (AOTF) adapter and a microscope for the image acquisition and an

algorithm for data treatment. The results showed the high accuracy of the system. Pan et al. [53] trained a support vector machine model to simulate the human visual neuronal system and identify leukocytes from blood and bone marrow smear images.

Farnoosh et al. [54] developed a framework that consists of an integration of several digital image processing techniques, such as active contours, the snake algorithm and Zack thresholding for white blood cells, aiming to separate the nucleus and cytoplasm. Ritter et al. [55] presented an automatic method for segmentation and border identification of all objects that do not overlap the boundary [54]. Ongun et al. [56] did segmentation by morphological preprocessing followed by the snake-balloon algorithm [54]. Jiang et al. [45] proposed a WBC segmentation scheme on color space images using feature space clustering techniques for nucleus extraction [54]. Al-Dulaimi et al. [57] developed a WBC segmentation method using edge-based geometric active contours and the forces curvature, normal direction, and vector field. Maitra et al. [58] presented an approach to automatic segmentation and counting of RBCs in microscopic blood cell images using the Hough transform [54]. Another interesting investigation was carried out by Banik and colleagues [59]. They proposed an automatic WBC nucleus segmentation method, based on the HSI (hue, saturation, intensity), the L × a × b color space, and the k-means algorithm. This increases the generalization capability and evaluation result with a higher score on quality metrics. Then, to classify the localized WBC, they proposed a new convolutional neural network (CNN) model, which is the key factor to reduce the performance dependency between the proposed nucleus segmentation and classification method. In the end, they proved that segmentation performance does not affect the accuracy of the proposed classification method. Kawaguchi et al. [60] presented an image-based analytical method for time-lapse images of RBC and plasma dynamics with automatic segmentation. This method enabled the quantification of the perturbation-induced changes of the RBC and plasma passages in individual vessels and parenchymal microcirculation.

The literature has many more methods, however, most of the techniques presented previously were based in morphological analysis or in the form and constitution of the various blood constituents. Techniques developed for blood flows are still under development because there are many ways and methods for tracking movement. A good summary of object tracking methods can be found in [61] and cell tracking can be found in Miura et al. [62].

Recently other works appeared, for example, Dobbe et al. [63] presented a method applied to the sublingual microcirculation in a healthy volunteer and in a patient during cardiac surgery. Iqbal et al. [64] developed a novel method for the detection of abnormal behavior in cells through real-time images. The method was based in pixel classification using k-means and Bayesian classification. Chang et al. [32] segmented medical images through a charged fluid model. The model is divided in two steps defined by Poisson's equation. Measurements of functional microcirculatory geometry and velocity distributions using image techniques have been made, such as capillaroscopy, orthogonal polarized spectral and a side-stream dark field image [63]. Ashraf et al. [65] said that "cell mobility analysis is an essential process in many biology studies", so they have focused in developing a novel algorithm to image segmentation and tracking system conjugating the advantages of topological alignments and snakes, transforming the output of the topological alignments into the input of the active contour model to begin the analysis in the cells' boundaries and to determine cell mobility [65]. Pan et al. [66] proposed a bacterial foraging-based edge detection (BFED) algorithm for cell image segmentation. The method was compared with the other four edge detector algorithms and showed more accurate and effective results.

In the case of Möller et al. [67], a semi-automatic tracking method with minimal user interaction was proposed. The framework was based on a topology-preserving variational segmentation approach applied to normal velocity components obtained from optical flow computations. Using the advantages of the optical flow, Kirisits et al. [68] introduced variational motion estimation for images that are defined on an evolving surface. Niazi et al. [69] studied an open-source computational method of particle tracking using MATLAB (2014 b, MathWorks, Natick, MA, US). The size and velocity of the particles are acquired from the video sequences from video-microscopic systems. The images are processed by a set of filters, selected by the user, to improve the accuracy. Park et al. [70] developed a deep learning-based super-resolution ultrasound (DL-SRU) for particle tracking. The method is based on a convolutional neural network and deep ultrasound localization microscopy. The DL-SRU was able to identify the positions of the RBCs reconstruct vessel geometry. Carboni et al. [71] used fluorescence to track blood particles flowing through a microfluidic channel. The recordings of the flow were analyzed with an algorithm developed using MATLAB to evaluate the margination parameter at relevant flows. The image processing consisted of three parts: background correction, calculation of the position and size of the particles through a gradient-based method and calculation of the displacements and velocities. Varga et al. [72] trained conventional-, deep- and convolutional neural networks to segment optical coherence tomography images to identify the number of hyperreflective foci. The networks coincide in the majority of the cases with the evaluation performed by different physicians. Chen et al. [73] studied a new approach for the segmentation of erythrocyte (red blood cell) shape. The

technique was called complex local phase based subjective surfaces (CLAPSS) and presented a new variation scheme of stretching factor and was embedded with complex local phase information. The processed images were acquired by differential interference contrast (DIC) microscopy.

Some methods can also be used to track particles for diagnostic or treatments. For instance, Siegmund et al. [74] tested the use of nanoparticle labeling and magnetic resonance imaging (MRI) for in vivo tracking of adipose tissue-derived stromal cells (ASC). The labeling was stable for four months. This method has the disadvantage of not being able to identify the cell since it is an indirect method. Optimization is still required to reduce the amount of nanoparticles. Müller et al. [75] investigated the transport of magnetic particles in vessels of hen's egg models. The flow was subjected to the influence of a magnetic field in dark field reflected light and fluorescence mode. The particles were tracked by single-particle tracking (SPT). Irreversible agglomerates were visualized after stopping the magnetic field. Consequently, further studies of the interaction between cells and particles and of the particle coating are required. Also to support the diagnosis, Kucukal et al. [76] quantified the viscosity of preprocessing-free whole blood samples from the sickle cell disease patient population by using the micro-PIV technique for in vitro assessment of whole blood viscosity and RBC adhesion. More recently, Kucukal et al. [77] have been able to measure the velocity of whole blood flow in a microchannel during coagulation using a simple optical setup and processing the images using PIV and wavelet-based optical flow velocimetry. Both studies demonstrated the viability of image processing methods to obtain data with clinical relevance. Table 1 below shows the chronological progress of the studies and that, recently, the studies have been based on automatic methods with specific algorithms and particle tracking techniques.

Table 1. Summary of image analysis methods used for cell tracking and segmentation.

Reference, Year	Goal	Technical	Conclusion
[45], 2003	White blood cell (WBC) segmentation	Scale-space filtering and watershed clustering	Extracts the WBC region; The HSV space is better than the RGB space due to its low correlation.
[47], 2007	Color image segmentation	Using RGB space as the standard processing space: (1) Non-exclusive RGB segmentation. (2) Exclusive RGB segmentation.	Color images provide a better description of a scene as compared to grayscale images
[54], 2009	WBC segmentation: to separate the nucleus and cytoplasm	It is based on the morphological analysis and the pixel intensity threshold, respectively.	The method is able to yield 92% accuracy for nucleus segmentation and 78% for cytoplasm segmentation.
[60], 2012	To quantify the perturbation-induced changes of the RBC and plasma passages in the individual vessels.	The image-based analytical method for time-lapse images of RBC and plasma dynamics with automatic segmentation	Arterial tones and parenchymal blood flow can be individually coordinated.
[52], 2013	To segment the nuclei and cytoplasm of WBCs	It is based on the pixel-wise ISAM segmentation algorithm	the accuracy of the proposed algorithm is 91.06% (nuclei) and 85.59% (cytoplasm)
[67], 2014	Cell tracking	Topology preservation techniques	The method has good accuracy
[71], 2016	Direct particle tracking	Algorithm developed in MATLAB	Results obtained confirm experimental results
[66], 2017	Optimize traditional edge detection	Edge detection algorithm based on bacterial liner	Identifies boundaries more effectively and provides more accurate image segmentation
[69], 2019	Determine particle velocity and size distributions of large groups of particles by video-microscopic systems.	Open-source computational implementation with MATLAB	It allows the automatic tracking of any fluid with particles, classifies the particles according to their size and calculates the speed.
[70], 2020	Particle tracking	The method is based on a convolutional neural network and deep ultrasound localization microscopy	Its robust, fast and accurate RBC localization, compared with other ULM techniques
[76], 2020	In vitro assessment of whole blood viscosity (WBV) and RBC adhesion	Micro-PIV	WBV and RBC adhesion may serve as clinically relevant biomarkers and endpoints in assessing emerging targeted and curative therapies in SCD.

Reference, Year	Goal	Technical	Conclusion
[77], 2021	Measurements of the velocity of whole blood flow in a microchannel during coagulation	PIV and wavelet-based optical flow velocimetry (wOFV)	The high-resolution wOFV results yield highly detailed information regarding thrombus formation and corresponding flow evolution

For studies based on *in vitro* approaches, there are different automatic algorithms, however, most of them still under development because the results tend to overlap at high hematocrits (Hcts), and most of them are based on images that the researchers have, taking into account their aim. Therefore, to have a good method and take advantage of all its capabilities, it is ideal to develop our own algorithm for the objective that we want to achieve.

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