A Novel Inversion Technique for Imaging Thrombus Volume in Microchannels Fusing Optical and Impedance Data

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The aim of this paper is to present a novel inversion technique to measure the volume of thrombus induced under blood flow conditions in a lab-on-a-chip device. The device is composed by a microscope slide where gold electrodes are sputtered and by a polydimethylsiloxane microchannel placed on the top of the slide. A thrombogenic substance is placed on the slide in such a way that hemostasis is induced when whole blood flows in the microchannel. The novel idea behind the inversion technique is to fuse optical and electrical impedance data to obtain a quasi-real-time reconstruction of thrombus volume. This is not possible with present state-of-the-art optical imaging based on confocal microscopy, which provides the thrombus volume estimation only at the end of the thrombus formation.

Index Terms— **Bioimpedance, biomedical engineering, biomedical imaging, biosensors.**

I. Introduction

In THE perspective of a point-of-care prevention screening system, there is an increasing interest in *in vitro* measurement of thrombus formation under flow conditions to profile the thrombotic process in humans. In particular, the determination of the individual thrombotic profile is of paramount importance for the personalized pharmacological treatment planning.

Current available technologies capture the kinetics of thrombus formation in collagen coated artificial capillary or flow chamber using a video imaging technique and image processing, which include a microscopy device either inverted or confocal. For the kinematic imaging of thrombus formation a fluorescent labeling for platelets is used. In the inverted microscope, the quantification of thrombus formation is performed from a digital bi-dimensional data image of a whole blood sample based on pixel luminance. The 3-D size of the thrombus is therefore mathematically extrapolated only from the pixel luminance and may not reflect its actual size. This problem can be overcame by the use of a confocal microscope [1], but the size of the instrument and its costs are against its usage in a bedside clinical setting. Moreover, confocal microscopy provides the thrombus volume only at the end of the process.

A preliminary electrical characterization of thrombus formation in microchannels under blood flow conditions using electrical impedance spectroscopy [2] has been presented in [3] using an impedance meter and a low cost sensor based on copper printed circuit board technology and improved in [4] using a pair of gold microwires.

In this paper, we present a novel and cheap system that should provide information on the individual thrombotic profile. The proposed technique estimates the volume of thrombus during its formation, by monitoring the electrical impedance of blood flowing inside a microchannel evaluated on a pair of gold microelectrodes together with the luminance extracted from a 2-D optical image captured by the microscope with a CCD camera.

This paper is structured as follows. In Section II, the lab-on-a-chip device is described. Section III gives some detail on the numerical method used to solve the electromagnetic forward problem and its implementation. In Section IV, a novel technique to solve the inverse problem by fusing the impedance and optical data is presented. In Section V, some numerical results are presented, whereas in Section VI, the conclusion is drawn.

II. Lab-on-a-Chip Device

The sensor is composed by a polydimethylsiloxane (PDMS) substrate where the microchannel with microfluidic connections is molded and a quartz slide forming the upper wall of the channel where gold electrodes and electrical connections are sputtered, see Fig. 1(a). The channel dimensions are 400 μm in width, 250 μm in depth, and 25 mm in length. The upper wall of the microchannel is formed by a microscope slide with 200 nm thick sputtered gold electrodes, arranged, as shown in Fig. 1(b). Blood flows inside the sensor at a controlled temperature of 37 °C and at a controlled flow rate to simulate as close as possible the *in vivo* conditions. Namely, blood flows in the microchannel at a flow rate of $Q = 750$ μl/min to obtain a target shear rate of 3000 s^{-1}.

The developed microfluidic lab-on-a-chip is posed under an optical microscope. The image of the microscope is captured by a CCD camera. Collagen is spread on the upper wall of the microchannel to induce the aggregation process of platelets, which are marked with quinacrine; platelets thrombi marked with quinacrine, when excited with blue (488 nm wavelength) fluorescence, re-emit the light in the green zone

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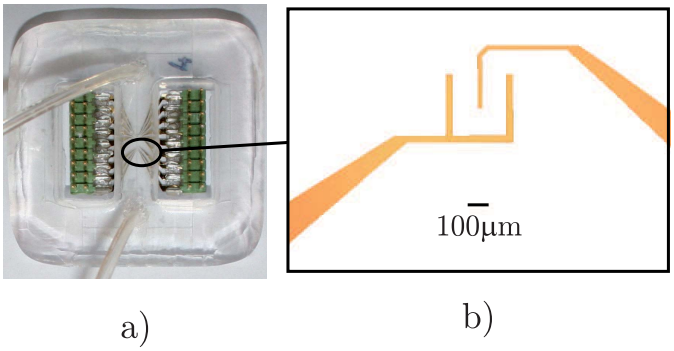

Fig. 1. (a) Assembly of the sensor composed of PDMS microchannel and microelectrodes sputtered on a microscope slide. (b) Microscope image of microelectrodes.

(522 nm wavelength): increasing the thrombus volume, the CCD camera sense a higher light intensity. In particular, we evaluate the light intensity of each pixel p at a given time instant t using the luminance [5]

$$L(p,t) = 0.299R + 0.587G + 0.114B \tag{1}$$

being R (Red), G (Green) and B (Blue) the RGB values of the acquired images.

The sensor is connected also to an LCR meter in such a way that the impedance spectroscopy measurements are performed using the high precision LCR meter Agilent E4980A in the frequency range 1–300 kHz with eight logarithmic spaced steps, a two-wire configuration and a drive voltage of 100 mV. Such drive voltage is by far lower than the standard half cell potential for gold (i.e., 1.5 V) to avoid the artifacts due to redox reactions occurring between the electrodes and salts dissolved in plasma. The LCR meter has been connected to the assembled empty device and an open calibration has been performed to eliminate the capacitive parasitic effects of connections. In these conditions, the impedance measurement accuracy is about 0.1% [6]. Both the LCR meter and the CCD camera communicate with a laptop through the Universal Serial Bus interface and a software control panel simultaneously acquires optical and impedance data.

At every experiment, a collagen is coated on the quartz slide to induce the thrombus formation. Whole blood flows for a specific time interval (typically 3 min) and thrombi grow from the surface of the slide where collagen has been spread.

At low frequencies, the interface electrode-blood (double layer) dominates the impedance behavior masking the effects of thrombus formation. Since it has been already demonstrated that thrombus behaves like an insulator for frequencies below 300 kHz [3], [4], the best frequency interval for the thrombus growth measurement is 100–300 kHz to bypass the double layer effects but not the cells membranes forming the thrombus. In this frequency range, blood behaves mainly like a resistor [7] whose resistance is increased by the thrombus growth.

III. Forward Problem Analysis Tool

A 3-D steady-state current conduction analysis tool expressly developed for this application is based on the discrete geometric approach on a pair of Cartesian interlocked cell complexes where the current through electrodes is assigned [7]–[9].

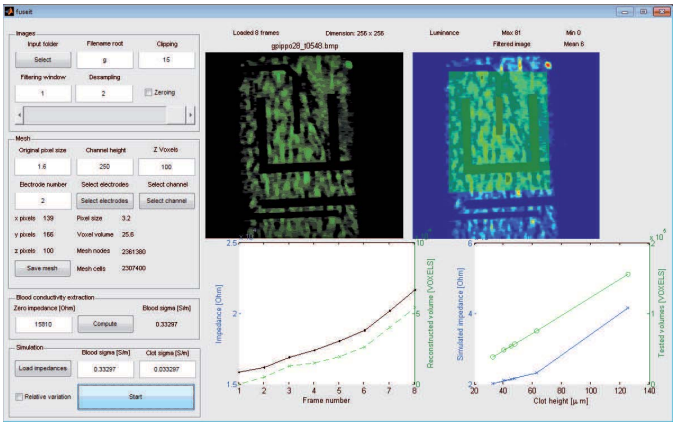

Fig. 2. GUI specifically developed to process the images and to select the shapes of electrodes and the channel. On the left side the raw image is shown, on the right the filtered one (clipped and desampled).

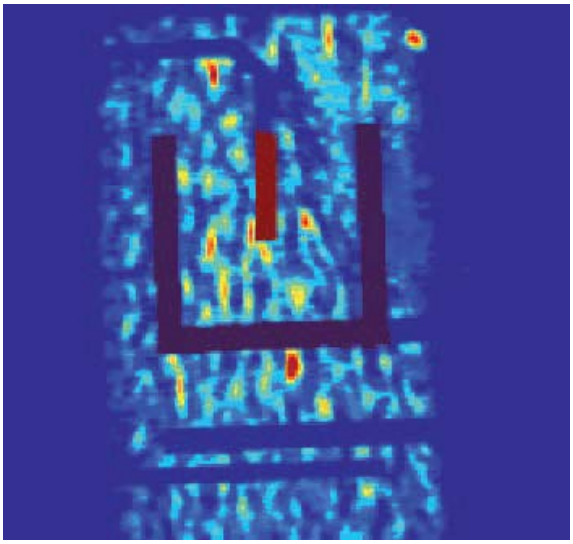

Fig. 3. Microscope image clipped to remove noise and desampled by a factor of two used for generating the cubical Cartesian grid inside the computational domain. The electrodes and the channel are selected with a GUI.

First, each image captured by the CCD camera is clipped to remove noise and desampled by a factor of two, see, for example, Fig. 3. The shapes of the electrodes and the channel are selected by the user with a graphical user interface (GUI). Starting from these information together with the height of the channel, a cubical Cartesian grid is constructed inside the selected computational domain Ω. In the example, the mesh consists of 2.3 millions hexahedra and 2.36 million nodes. The thrombus geometry is easily obtained from the luminance information, as described in the following section. The linear system

$$\mathbf{G}^T\mathbf{S}\mathbf{G}\mathbf{V} = \mathbf{0} \tag{2}$$

is assembled, where $\mathbf{G}$ is the standard edge-node incidence matrix, $\mathbf{S}$ is the ohm's constitutive matrix [10]– [12] constructed by starting from the actual material distribution and $\mathbf{V}$ is the array of potentials attached to the nodes of the primal cell complex. Homogeneous Neumann boundary conditions are imposed on all the boundary of Ω but the electrodes. Each electrode is modeled as an equipotential surface with unknown potential. A nonlocal boundary condition is prescribed on each electrode by imposing the total current flowing through it. The linear system (2) together with the nonlocal equations

[7] is then solved by an iterative linear solver that has been parallelized with OpenMP library for parallel computing. Each simulation takes less than 30 s.

IV. Novel Inversion Technique Based on Impedance and Optical Data Fusion

To reconstruct the thrombus geometry and volume, we present a novel inversion methodology based on fusing optical and impedance data at a given time instant.

First, the geometry of the thrombus is represented by a solid model deduced from the luminance with (1) extracted by a 2-D optical image sampled with the microscope. That is, for each pixel, the height H of the thrombus with respect to the slide surface is assumed to be proportional to the luminance according to a unique scale factor k for all the pixels, such that

$$H(p,t) = k(t)\, L(p,t) \tag{3}$$

where p is the label of a generic pixel. The inverse problem is then related to the determination of this scale factor $k \in \mathbb{R}$ according to the following minimization problem:

$$\min_k |R_c(k,t) - R_m(t)| \tag{4}$$

where $R_m(t)$ is the measured resistance, whereas $R_c(k,t)$ is the resistance computed by using the forward solver and a scale factor k. The considered time interval is from zero to T that usually is assumed equal to 180 s. Therefore, to determine such a scale factor, the idea is to use a number of 3-D steady-state current conduction analyses performed with the developed forward problem solver by modifying the scale factor until the computed resistance matches the measured one; the thrombus volume is then easily evaluated from the thrombus solid model.

Practically, one can implement easily this technique by first normalizing to one the luminance with

$$\bar{L}(p,t) = \frac{L(p,t)}{\max(L(p,t))}. \tag{5}$$

Second, the height H of the thrombus is defined as

$$H(p,t) = \bar{L}(p,t)\, n(t)\, h_p \tag{6}$$

where h_p is the height of each pixel assumed here 2.5 μm and $n \in \mathbb{N}$ is an arbitrary positive integer. Finally, the thrombus height on each image pixel in terms of number of voxels is obtained by applying the floor function to $\bar{L}(p,t)\, n$, which from (6) is proportional to H.

Instead of exhaustively simulating all increasing multiples of the voxel height until the measured resistance is exceeded, we exploit the property of monotonicity [13] of the resistance with respect to changes in conductivity σ. Namely, considering two distributions of conductivity σ_1 and σ_2 in the computational domain Ω such that

$$\sigma_1(p,t) \le \sigma_2(p,t) \quad \forall \text{ pixels } p \tag{7}$$

holds, then the resistance $R(t)$ fulfills

$$R(\sigma_1,t) \ge R(\sigma_2,t). \tag{8}$$

In other words, this means that the resistance monotonically increases when n (proportional to the thrombus volume) grows. Therefore, a simple bisection method may be employed to solve (4) determining the thrombus height that better matches the measurements. That is, the algorithm repeatedly bisects the searching interval (initially $n(t)$ interval is set from 0 to 150 μm/h_p) and selects a subinterval in which the solution must lie for further iterative refinements. Using $h_p = 2.5$ μm, up to six heights have to be simulated to reduce the interval under the geometric tolerance given by h_p.

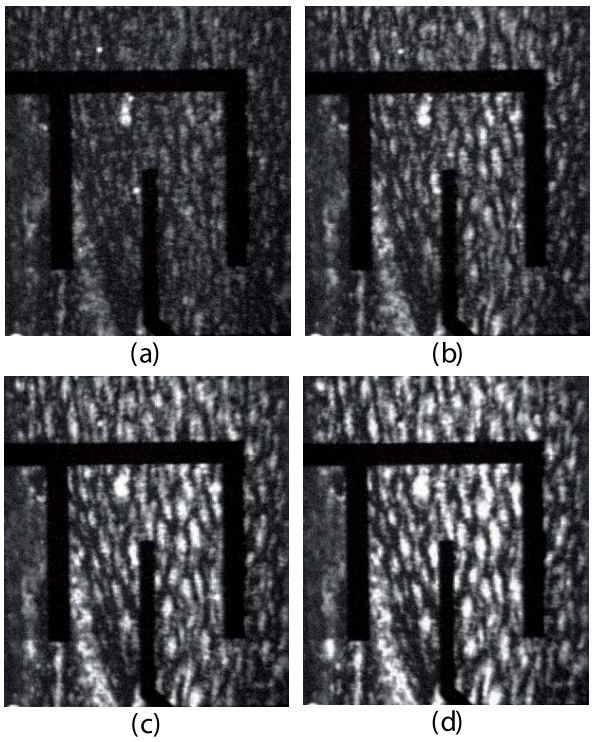

Fig. 4. Acquired frames from microscope during thrombus growth, blood flows from the top to the bottom of each image. Frames acquired (a) after 40 s, (b) after 60 s, (c) after 80 s, and (d) after 100 s from the start of the experiment.

The electrical parameters of blood flowing in microchannels at high shear rates have been experimentally characterized in [7]. The blood conductivity $\sigma_{\text{blood}} = 0.33$ S/m used in the simulations is determined in each experiment from the initial resistance $R(0)$ measured in absence of the thrombus. The thrombus can be considered as a perfect insulator below a few hundreds of kilohertz, as shown in [3] and [4].

V. Experimental Result

The measurements have been performed automatically acquiring data from the LCR meter (5 samples per second) and the CCD camera (10 frames per second). The control panel has been setup to perform a two-wire measurement avoiding redox reactions and compensating the parasitic effects of cables and connections, as described in Section II. The processed frames are the ones acquired by the microscope 40, 60, 80, 100, 120, 140, 160, and 180 s after the start of the experiment, see Figs. 4 and 5. In this picture, the thrombus is represented by the light areas inside the computational domain Ω.

Fig. 6 shows the variation with time of the impedance magnitude at 150 kHz frequency and of the thrombus volume in the considered eight frames. Fig. 7 shows the 3-D image reconstruction of the thrombus geometry computed by the proposed method relative to the last time frame (at $t =$ 180 s). The computed volume is about 1.38 millions of μm^3, whereas the estimation provided by the confocal microscope is

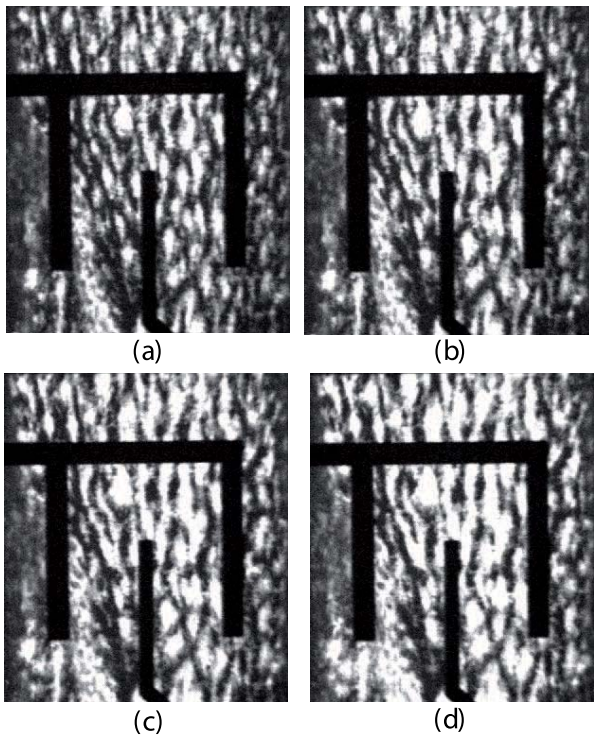

Fig. 5. Acquired frames from microscope during thrombus growth, blood flows from the top to the bottom of each image. Frames acquired (a) after 120 s, (b) after 140 s, (c) after 160 s, and (d) after 180 s from the start of the experiment.

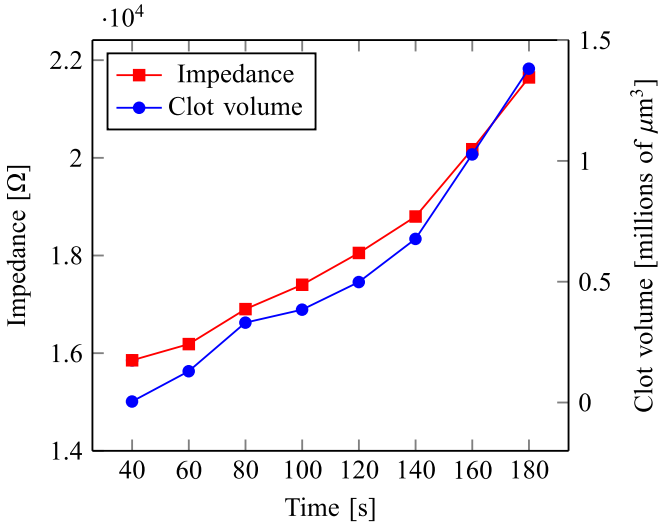

Fig. 6. Measured impedance and reconstructed clot volume.

1.58 millions of μm^3. However, it should be remarked that the clot volume obtained with confocal microscope is sampled several minutes later and during this period the blood is steady. Moreover, the threshold for segmentation of the clot volume adds uncertainty that is difficult to quantify.

VI. Conclusion

We developed a combined methodology coupling light distribution and intensity on a CCD surface from the one hand and impedance measurements from the other. Such a technique allows to estimate the volume of thrombus formation as a function of time along a portion of the microchannel under flow conditions. The experimental results demonstrate how the thrombus volume is well correlated with the amplitude of the measured impedance.

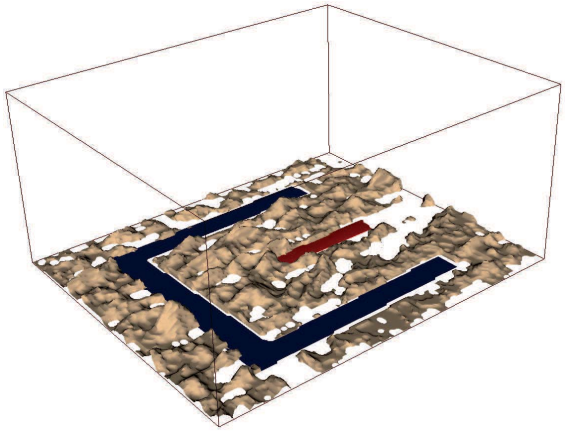

Fig. 7. Reconstructed thrombus geometry at the last time frame (180 s). The thrombus volume is 1.38 millions of μm^3.

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