AN ELECTRICAL MODEL OPTIMIZATION FOR SINGLE CELL FLOW IMPEDANCE SPECTROSCOPY

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Abstract- This paper presents an optimization of a single cell electrical model, based on Maxwell's mixture Theory, applied to flow cytometry coupled to impedance spectroscopy. It is based on the discretization of the measurement area into a square reference volume, centered between micro-electrodes, and fixed impedance areas. The first one represents the sensing area, the one impacted by cell presence during measurement, and the second one, all other areas that contribute to global measured impedance. By removing these last impedances, it is possible to compare and model the electrical response of different electrodes geometries. Simulations, performed for 6 different electrodes geometries using Finite Element Method (FEM), were performed to check our assumptions. Results attest the validity of our model for cells with sizes comprised between 30 and 70% of the channel weigh. Finally, measurements performed with our microfluidic sensor show the same impedance variation distribution during the passage of calibrated beads with an error lower than 5%.

Index terms*: biosensor, microfluidic, modeling, impedance spectroscopy, single cell.*

I. INTRODUCTION

Current researches are focused on MicroElectroMecanical Systems (MEMS) [1], allowing many prospects for new sensor technologies, by reducing sizes, costs and increase sensitivities of sensors. Lab-on-chip devices are one the most promising applications based on one or several classical bio-analysis devices in only one microscopic chip, as electrical and optical characterization [2]. They allow to characterize isolated single cells to increase the sensitivity. A possible method consists to isolate and place the cell in the measurement area using micro-suction system [3]. This technic permits an efficient placement, but increase system complexity. Moreover, it is not suitable for fast characterization of a large amount of cells. Therefore, our interest is centered on cytometry, a technic consisting to concentrate and isolate cells or particles in a restricted area in order to characterize them during they trough in [4]. Several methods can be used as optical or chemical detection but they often need to add markers, and are invasive. BioImpedance spectroscopy (BIS), used for more than one century to characterize materials and biological tissues, is a non-invasive method when using very small energetic signals [5] [6]. Many pathologies, as cancerous cells, induce detectable impedance changes in tissues impedance [7]. This kind of measurement were performed quite a long time with macrometric samples, giving global information about the multitude of cells and particles which compose it, and cannot provide enough sensitivity to detect few percentage of abnormal cells. For this reason, the interest is now focused on single cell measurement.

This paper is based on previous works [8] describing a microfluidic biosensor able to detect and characterize single biological cells at high flow rate. It focuses on the optimization of electric and dielectric analytic model of this living particles, its comparison with results obtained with finite element method (FEM) simulation and experimentation.

The second section describes the theory of single cell BIS measurement and characterization. The global structure of cytometric sensor is described with the classical electrical model for bio-samples. The new optimization, based on discretization of measurement areas, is also described.

Third part concerns FEM simulations providing a first comparison between analytical previous models. Six different channels and electrodes geometries are tested and compared.

In the fourth part, an experimentation with a microfluidic device is performed to validate analytical model and simulation by Finite Elements Method.

II. THEORY

a. General structure

Flow cytometry devices are composed of inlet and outlet tanks for liquid samples deposition and recovery. Measurement area is composed of a microchannel with a section on the same order than cell size, to permit the displacement of cells one by one. In our case, as show on Figure 1, measurements were performed with BIS using microelectrodes of the same size order than living cells and bacteria, placed in the center of the channel,. Two possible electrodes geometries are exposed, namely parallel and coplanar, both in two pairs configuration for single, dual or differential measurement.

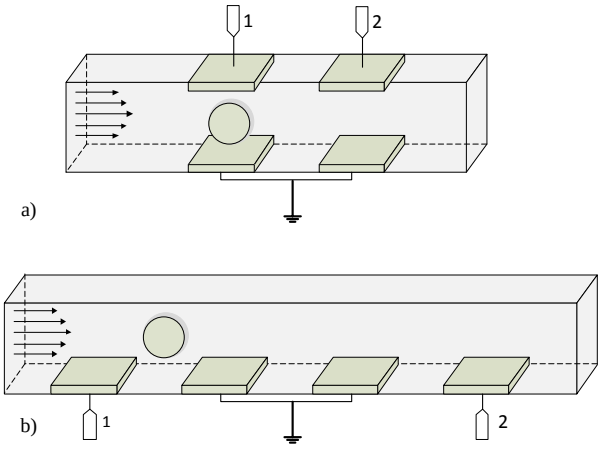


Figure 1. Design of the measurement area for parallel micro-electrodes a) and coplanar microelectrodes b)

b. Modeling

Biological samples can be characterized by their electric and dielectric properties, respectively electric conductivity and dielectric permittivity. Each kind of tissue presents a specific frequency spectrum in function of their constituents, as exposed in C.Gabriel and S.Gabriel works for macroscopic samples [9] [10]. In our case, cells in suspension, external medium and cytoplasm

are of the same nature, namely electrolyte. Cell membrane, composed by an insulating lipid layer of about 10 nm of thickness, can be modeled by a capacitor with high surface capacitance (around 1µF/m²). An important last effect, not intrinsic to cell impedance but related to the microelectrodes interface with living matter is the double layer impedance [11]. It appears during measurement due to the polarization of electrodes in contact with an electrolyte. This effect acts like a barrier in the low frequency range measurement, due to its very high capacitive (in order to 100 times more than membrane) behavior [12]. It is often modeled as a capacitor in series with the sample impedance. Combining the different electrical elements, one obtains Fricke's model [13], shown on Figure 2. Cmed and Rmed represent dielectric and electric properties of medium, Ri electric properties of cytoplasm, Cmem the dielectric properties of cell membrane and Zdl the double layer impedance.

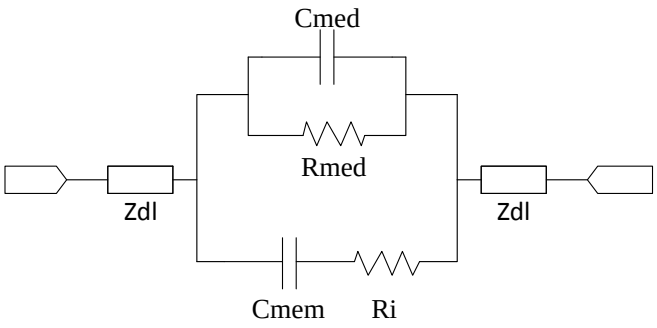


Figure 2. Electric Schematic of Fricke cells suspension model

These electrical elements can be found from impedance measurements however the main difficult is to link it to intrinsic parameters of the biological sample as conductivity or permittivity. For macroscopic sample, it is possible to directly use Maxwell's Mixture Theory, which works well under several conditions. Low particles concentration, good repartition of them and a uniform electric field. These conditions cannot be respected in the case of single cell measurement and must be adapted. For that, Hywell et al. [14] proposes optimization and simplification by using the following relations [(1) to (4)].

$$R_{med} = \frac{1}{\sigma_{med}(1-3\phi/2)k} \quad (1)$$

$$C_{mem} = \frac{9\phi r Cmem,s}{4} k \quad (2)$$

$$R_i = \frac{4\left(\frac{1}{2\sigma_{med}} + \frac{1}{\sigma_i}\right)}{9\phi k} \quad (3)$$

$$C_{med} = \varepsilon_{med}(1-3\phi/2)k \quad (4)$$

σmed and σi are the conductivity of medium and cytoplasm, εmed the electric permittivity of medium, Cmem,s the membrane capacitance per surface units, Φ the volume ratio between cell and measurement area, and k is a cell factor depending on electrodes geometry. It remains one problem due to the parameter Φ. During measurement of large amount of cells in suspension, it is easy to define a volume ratio, which is the same in all sample if it is well mixed. In the case of isolated single cell, Φ represent the cell volume divided by the volume of measurement area. Its volume is difficult to define because of electric field repartition as function of electrodes geometry (see Figure 3). However, it appears two interesting informations: electric field is well uniform in the center of the measurement area and not significantly modified outside it by the presence of a particle. Considering these assumptions, we propose to redefine the measurement area into two parts, as presented in Figure 4. The first, named sensing area, is the biggest square centered in the middle of measurement area that is possible to insert into the channel. Using this very simple geometry, the ratio Φ can be determined easily. The second part, named fixed areas, represent all others areas which participate to the global impedance, but are not impacted by the presence of one particle in the center of the measurement area. The final model integrates the impedance of sensing area, using (1) to (4), in series and/or parallel with fixed areas. Thus, their impedances are also fixed, and depend only of electrolyte properties and geometry. It is possible to define cell factor for them too.

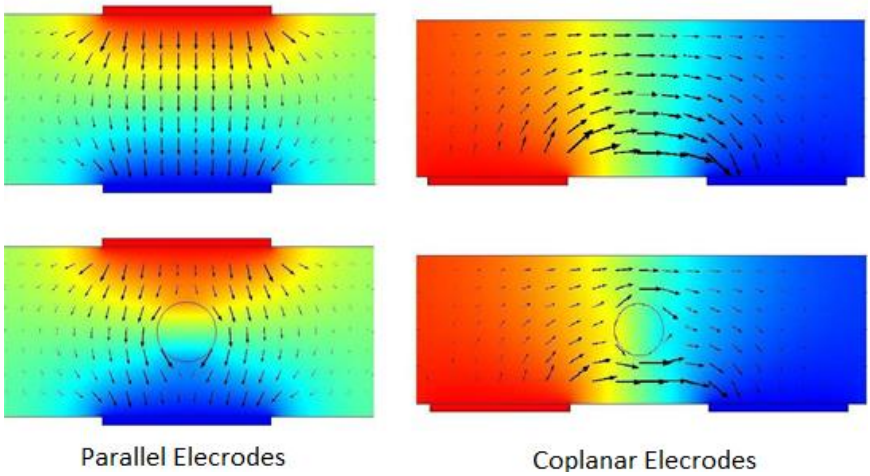


Figure 3. Repartition of electric field for parallel and coplanar electrodes with and without cell

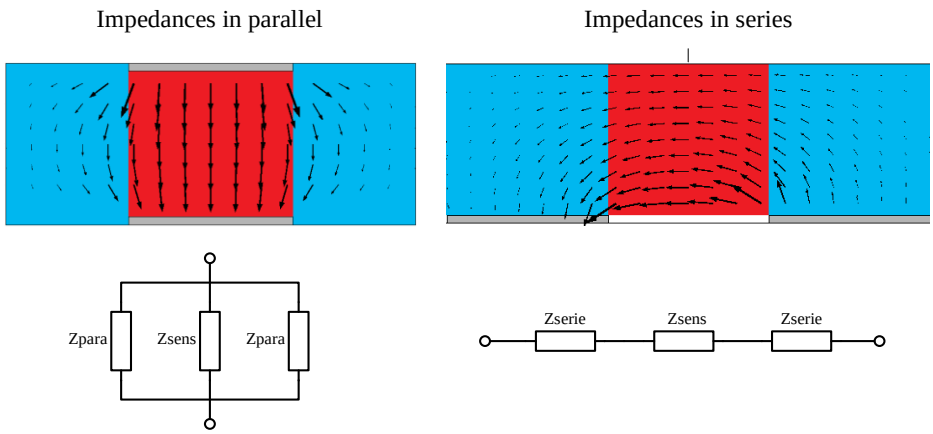
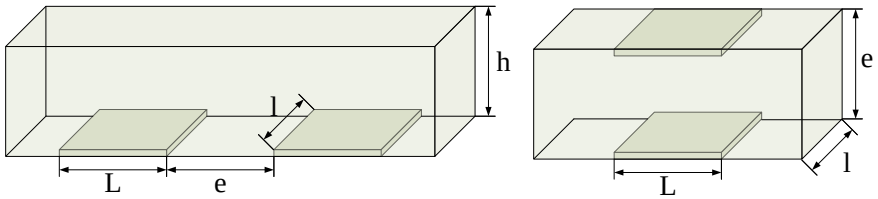


Figure 4. Repartition of electric field for parallel and coplanar electrodes with and without cell, and discrete equivalent models.

III. SIMULATIONS

a. Simulation parameters

To verify as a first step the validity of our assumptions, we have performed FEM simulations with 6 different electrodes geometries having coplanar and parallel configurations. Geometrical parameters are represented in Figure 5 and exposed in Table 1.



a) Coplanar geometry. **b) Parallel geometry.**

Figure 5. Geometrical parameters for coplanar and parallel geometries

Table 1: Geometrical parameters

Electrodes type	**Parameters values (µm)**				
	Name	***L***	***W***	***G***	***H***
Parallel	P-1	20	20		20
	P-2	20	40		10
	P-3	40	20		20
Coplanar	C-1	20	20	20	20
	C-2	20	20	20	10
	C-3	40	40	20	20

For simulations, all parameters of cell must also be defined, as size, conductivity and permittivity of all constitutive elements. The values exposed in this section are considered as reference values for all simulations. Except if mentioned, all parameters are exposed on table I. Relative permittivity of water εm and cytoplasm εi, are fixed to 78, Medium conductivity σm to 1.4 S/m, cytoplasm conductivity σi to 0.7 S/m, 3.5µm for cell radius and membrane permittivity is choose to have 1µF/cm² with 200nm thickness, due to mesh limitations. All simulations were performed with Comsol Multiphysics with fine mesh.

b. Results

First simulations were realized without cell, to determine cell factor of different area in series or parallel with the sensing area. They are represented on Figure 6, where square sensing area is colored in red. Cell factor of a square corresponds to the length of one side. Finding factor are summarized on Table 2.

Table 2: Geometrical cell factors

Type of Cell factor	**Parameters values (µm)**					
	P1	***P2***	***P3***	***C1***	***C2***	***C3***
Entire geometry	29.2	49.59	49.19	10.62	7.02	6.92
Sensing area	20	10	20	20	10	20
Fixed series area				22.64	5.41	10.58
Fixed parallel area	9.2	39.59	29.19		3.51	

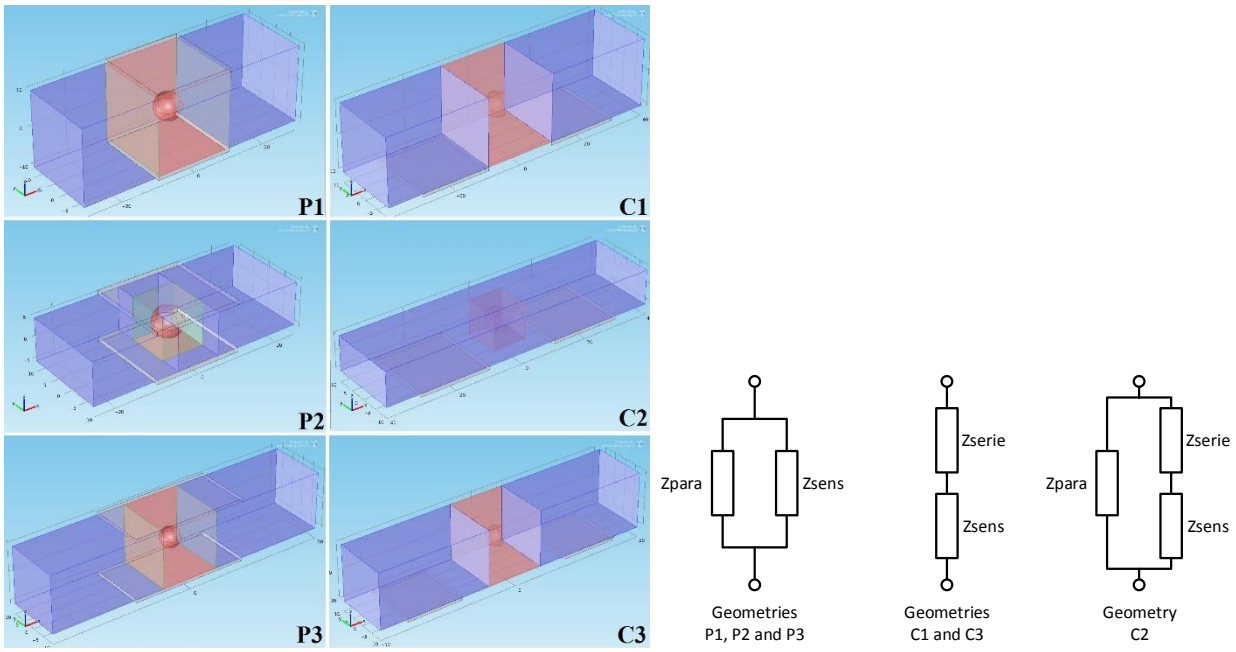


Figure 6. Representation of sensing and fixed areas for different electrodes geometries

Next simulations were performed at 500 kHz and 10 MHz. Two discrete frequencies permit to determinate the effects of cell size, membrane capacitance and cytoplasm conductivity on global measured impedance [15]. These characteristics can be determined from real part of impedance for conductivity and imaginary part for capacitance in the frequency band of interest. One simulation was also performed using just the cubic sensing area as reference. Results are presented on Figure 7.

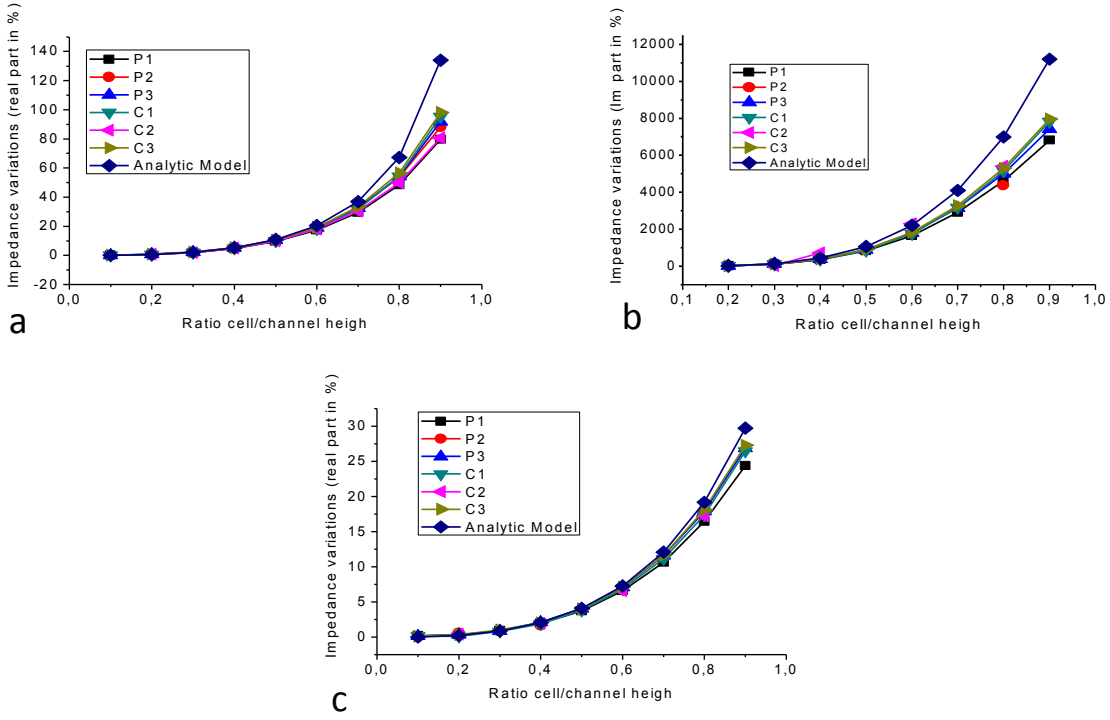


Figure 7. Results in impedance variation obtained respectively for variation of cell diameter at 500 kHz (real part a) and imaginary part b)), and at 10 MHz (real part c))

In order to facilitate comparison between the 6 geometries, the reference geometry and the analytic model, all fixed impedances were removed. The results present the impedance variation as function of the ratio cell size/sensing area size. By this, our assumptions supposing to be true, all the curves may correspond. One can see that it is the case with very small error between all of them, except for particles with dimensions higher than 70% of channel height. This default is due to the approximation of equations (1) to (2), which do not work when ratio Φ is too high and involve high electric field deformation. This problem do not appear for cytoplasm characterization, because in this case, the electric field trough the cell stay well uniform. Globally, for a cell with size between 30%-70% of channel height, maximum errors observed in

worst case between analytical and simulated models were inferior for all geometry to 4% for cytoplasm measurement, 12% for cell size determination and 22% for membrane capacitance.

IV. EXPERIMENTATION

One experimentation was realized to validate previous results obtained with a sensor having C2 coplanar geometry as presented in Figure 6. This sensor, and the choice of its dimensions were already described in a previous work [16]. Validation tests were realized by measuring impedance variation induced by 6µm calibrated beads, and comparing it with FEM and analytical results. Knowing the maximum variation coefficient of 0.1 guaranty by provider, one must combine it with previous results to draw a normalized reparation diagram as function of impedance variations. This is shown on Fig. 8. One can see a good matching of the results obtained by simulation and by analytical model. For measurements performed with our device, the reparation is centered in the same value than the others, with an error lower than 2%, but more concentrated. That can be easily explained because the variation coefficient given by the manufacturer is higher to the real one. These results permit to validate experimentally our model optimization for the geometry C and the validity of simulation perform with finite elements method.

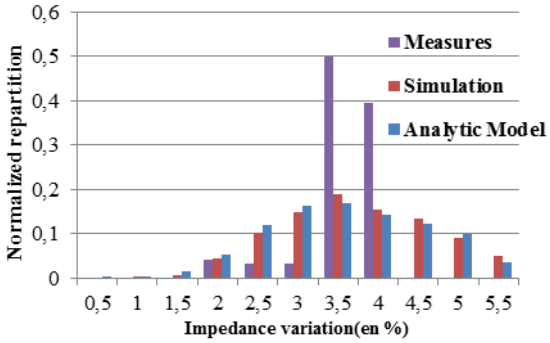


Figure 8. Impedance variation repartition for 6 µm calibrated beads

V. CONCLUSION

A new single cell electrical model, based on the discretization of the measurement area, was described and applied for parallel and coplanar electrodes. It appears that the sensing area of all the geometries can be modeled using the same equations and their measures can be easily

compared. Results obtained by FEM simulations with 6 different coplanar and parallel microelectrodes were compared with our model, proving its validity for single cell impedance model for a biological cell with a size between 30 and 70 % of the channel size.

Results obtained with our microfluidic sensor for 6 µm calibrated beads were compared with results obtained with simulations and the new model. They allow to validate our assumptions with an error lower than 2 % on average repartition.

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