Numerical Simulation of Particle Dynamics in an Orifice-Electrode System. Application to Counting and Sizing by Impedance Measurement

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This paper describes how to numerically tackle the problem of counting and sizing particles by impedance measurement in an orifice-electrode system. The model allows to simulate the particle dynamics submitted to strong hydrodynamic stresses through a microorifice and to compute the voltage pulses generated by the modification of the inner dielectric medium. This approach gives important information about particles size distribution and allows to quantify the role of trajectory and orientation of particles on the size measurement.

Introduction

Counting and sizing of biological cells are often based on electrical gating through a micro aperture-electrode system. A voltage drop, generated by the particle flowing through the aperture allows to determine the volume of the particle. We expose here a complete numerical approach based on fluidic and electrical cross linked simulations coupled to an equivalent electrical circuit model to Figure a realistic operation of cell impedance measurement of blood cells. The model allows to compute the exact particle size distribution by taking into account trajectory and orientation of the particles through the micro-aperture. For a given particle flow, we also compute full particle size distributions for systems with and without sample hydrodynamic focusing where volume measurement errors can be derived. These numerical results are also compared to experimental measurements.

Impedance Gating and Particle Sizing Principles

Figure 1(a) depicts the considered orifice-electrode system with hydrodynamic focusing for the sample flow. The micro-aperture is a cylindrical aperture of length L and diameter D₀ and is made of electrical insulator material. The two electrodes, placed on each side, are platinum made with anode voltage fixed to V₁ Volt. The sample arrives by an injector of diameter Dᵢ and an external sheath flow is used to focus the sample at the center of the aperture.

Counting and sizing of biological cells are determined by an impedance variation measurement. This is the most popular technology, based on the well-known Coulter Principle, for volume measurement in the hematology field. More precisely, counting is done by an electrical measurement through a micro-aperture. When a particle passes through the orifice, a voltage and/or voltage drop appears in the electrical field. The resistance of the medium is changed and this impedance variation generates an electrical pulse which is directly linked to cell volume (See Figure 1(b)). Unfortunately, for a given particle, volume measurement can be distorted by its trajectory and its orientation and produce overestimations:

- Concerning the impact of particle trajectories, it is obvious that the electrical field experienced by the particle is not the same if the particle flows at the center or close to the edges (respectively streamlines S₁ and S₂ in the Figure 2(a)). Actually, in such a system, the electrical field is non-homogeneous and increases...
dramatically close to the edges of the orifice. Along the central axis, electrical isolines are almost parallel.

- Moreover, the orientation of the particle through the orifice impacts more or less electrical streamlines. More precisely, when an insulating particle passes through the orifice, the resistance of the medium increases proportionally to particle volume. In practice, depending on the particle's orientation, electrical streamline is more or less deflected, and so the particle may appear bigger than it really is.

**Numerical Method to Compute Measured Voltage Pulses**

The aim of this paper is to take advantage of simulation techniques to tackle the problem of counting and sizing particles. This complete numerical approach is fully innovative in diagnostics engineering and allows to understand and optimize physical processes that we don’t experimentally apprehend.

**Framework and Assumptions**

The domain used for simulation is an orifice-electrodes system as in Figure 1 where the orifice aspect ratio \( D_0/L \) corresponds to practical instruments from HORIBA Medical. The model is developed in two dimensions as an approximation of plane flow devices. The particles considered here are shaped like capsules, defined by a central rectangle of length \( nR \) and width \( 2R \) and two half-circles of radius \( R \) at both extremities. At each time step, position of the particle is given by coordinates of its center of gravity \( C_p \) and its orientation is controlled by angle \( \theta \). Also, particles are supposed to be rigid and totally insulating. The size ratio \( 2R/D_0 \) is comprised between 0.05 and 0.15 corresponding to small size particles as compared with the size of the orifice.

**Flow model, particle transport and electrostatic:** To simulate the dynamics of suspended particles, the numerical model solves a fluid-structure interaction problem (the flow affects particle movement and vice versa). Velocity field \( u \) and pressure \( p \) in the system are...
solutions to Navier-Stokes equations (the fluid is assumed to be incompressible and Newtonian and effects of Brownian motion and gravity are ignored). Particle velocity $v_p$ is a sum of a translational velocity $v_{tr}$ and a rotational velocity $v_{rot}$. To compute these two components, we use fluidic parameters $(u, p)$ to determine Hydrodynamic Force $F_{hyd}$ and Torque $T$ applied on the particle boundary. The force and torque exerted by the fluid are found by integrating the stress tensor over the particle surface. \[ E = \nabla \cdot V \] where $V$ is the voltage potential (\( \nabla \) is the gradient operator), solution of a Laplace’s equation for electrostatics. Electric displacement $D$ is defined by $D = \varepsilon E$ where $\varepsilon$ is the absolute permittivity (the particle is supposed to be totally insulating so absolute permittivity of the medium is taken eighty times higher than absolute permittivity of the particle).

**Computation of physical voltage pulses:** The total energy $W(t)$ [J] is given by integrating the energy density on the domain as follows: $W(t) = \int_0^1 (1/2)\varepsilon ||E||^2$. For a small time interval $\Delta t = t_2 - t_1$, the instantaneous power $P$ [J/s] in the system is defined by

$$P = \frac{\Delta W}{\Delta t}$$

On the other hand, the instantaneous electrical power $P$ is given by $P(t) = U(t)I(t)$ where $U(t)$ [V] is the voltage drop and $I(t)$ [A] the current in the system (In our case, the current $I(t)$ is maintained constant thanks to a constant current generator, so $I(t) = I$ for each time $t$). Then, the physical voltage pulse $U_p$ is computed for each time $t$ in the interval $[t_1, t_2]$ by

$$U_p(t) = \frac{W(t_2) - W(t_1)}{(\Delta t)I}$$

where $I = \frac{t_1 + t_2}{2}$

**Equivalent electrical model for the orifice-electrode system and for an RBC-type cell:**

In practice, voltage pulses $U_m$ measured by the electronic card have twice lower amplitude than physical pulses $U_p$, particularly owing to polarization resistance and temperature compensation. To model and compute mathematically the measured voltage pulses, the idea consists in developing an equivalent electrical model for the system and switching between temporal and frequential spaces by Fourier transform in order to filter pulses by transfer function $H$ of the system. More precisely, in the first step, we sample physical pulse $U_p(t)$ and compute its spectrum $F(U_p) = F(U_p)(\nu)$ by Discrete Fourier Transform. Then, we multiply $F(U_p)$ by the transfer function of the sensor $H(\nu)$ and we finally recover measured voltage pulse $U_m$ by Inverse Discrete Fourier Transform such as

$$U_m(t) = F^{-1}[H(\nu)F(U_p)(\nu)](t).$$

To determine the transfer function of the orifice-electrode system, we establish the corresponding equivalent electrical model. In such system, total resistance $R_g$ is concentrated very close to the orifice and we assume that $R_g$ is channeled inside this one (the value $R_g$ is determined by the numerical electrostatic model). In the presence of cell in the orifice, we thus consider the equivalent electrical model described in Figure 3. The incoming voltage is first distributed in the whole space when it penetrates into the orifice; the voltage is then split up in two parts: around the cell and through the cell (the equivalent electrical impedance of this area is called $Z_{eq}$). Finally, the voltage is distributed again in the whole space to the outlet.

Taking into account the parasitic capacity of sensor $C_g$ and insulator resistance $R_{isi}$, the equivalent electrical model for the orifice-electrode system described is given in Figure 4(a). Equivalent electrical model $Z_{eq}$ can be seen as a resistance $R_m$ resistance of the area around the cell, in parallel with impedance $Z_{cel}$, the equivalent electrical model for the cell. In the absence of cell, $Z_{cel}$ can be changed by resistance $R_0$ equal to the resistance of the cell’s volume filled of sheath liquid (see Figure 4(b)) In the presence of cell, $Z_{cell}$ is changed by the equivalent electrical model of an RBC-type cell. In this case, the membrane can be modeled by resistance $R_m$ in parallel with capacity $C_{m}$, and the cellular content by resistance $R_i$. Thus, the cell can be seen as a membrane connected in series with cellular content and connected again in series.
with opposed membrane (see Figure 4(b)). So, the two transfer functions (with or without cell in the system) are then given by the following expressions:

\[
H_{\text{cell}}(\nu) = \frac{2R_mR_p + R_pR_p(1 + 2\pi\nu C_mR_m)}{2R_m + (R_i + R_p)(1 + 2\pi\nu C_mR_m)}
\]

and

\[
H_{\text{no cell}}(\nu) = \frac{R_oR_p}{(R_o + R_p)}
\]

### Computation of Measured Voltage Pulses

Thanks to the above mentioned numerical method, we can exactly compute measured voltage pulses obtained with an orifice-electrode system. We consider two particles passing through the micro-aperture along velocity streamlines S1 and S2 described in Figure 2(a). In Figure 5(a), we expose physical (raw) voltage density \(U_p\) a long a streamline computed by multiphysics numerical simulation (flow model, particle transport and electrostatic). The blue pulse refers to a particle passing at the center of the micro-aperture and the red pulse to a particle passing close to the edges. Figure 5(b) shows spectral magnitude \(F(U_p)\) of these two physical pulses. The spectral magnitude referring to cells passing close the edges is non-null for high frequency. This highlights fast intensity changes in physical pulses due to electrical field peaks at the edges of the gate. The use of band pass filter in the electronic card should smooth the magnitude for high frequencies. Figure 5(c) exposes the corresponding measured filtered voltage pulses.
Effect of Size, Trajectory and Particle Orientation on Voltage Pulses

The numerical method presented in the previous section is used to numerically solve the problem of counting and sizing particles in an orifice-electrode system. The results below quantify the effect of the trajectory, orientation and dimensions of the particle on measured voltage pulse and on particle size distribution.

Effect of particle size: We have seen that the impedance measurement allows to determine the size of the particle. We first simulate the direct effect of particle size on generated voltage pulses. We consider an experimental micro-aperture with an aspect ratio $D_0/L=0.4$. Mean velocity through the orifice is around 7.6 m/s and voltage at the anode is fixed to 6 V. Radius $R$ of the particle is fixed to 1 µm. Size parameter is $nR$ (0.5, 1, 2 and 4 µm). In Figure 6, we display electrical voltage pulses corresponding to 4 particles with different $nR$. Clearly, we see that the bigger the particle, the higher the peak of the resulting voltage pulse. More precisely, the maximum level of voltage pulses is linearly correlated to size parameter $nR$ and particle size can be directly determined by using a peak detection algorithm for the voltage pulse.

Effect of particle trajectory: Here, we study the effect of particle trajectories on resulting voltage pulses. Dimensions of the orifice are $L=65$ µm and $D_0=50$ µm. Mean velocity through the orifice is around 4.25 m/s and voltage is fixed to 7.6 V. Radius $R$ of the particle is fixed to 2.5 µm and $nR=2.15$ µm. The trajectory, and the resulting voltage pulse, of the particle are computed for a particle passing at the center of the orifice and a particle passing close to the edges of the orifice (see Figure 2(a) for corresponding velocity streamlines $S_1$ and $S_2$). Figure 7(a) shows two voltage pulses corresponding to particles passing through the orifice along velocity streamlines $S_1$ and $S_2$. Along the central axis of the orifice (streamline $S_1$), the generated voltage pulse is of the Gaussian type. In this case, peak detection is quite easy and the size of the particle can be precisely determined. On the other hand, a particle passing close to the edges of the orifice (streamline $S_2$) is submitted to high electrical values and the resulting voltage pulse has a distorted shape on its apex. This produces erroneous peak values and computation of particle sizes produces false estimations (until 8% of the maximum of voltage pulse depending on electronic configuration). Figure 7(b) exposes series of experimental voltage pulses obtained with an orifice-electrode prototype developed by HORIBA Medical. It is interesting to see that the two types of voltage pulses are experimentally obtained. For identical configurations, amplitude of pulses obtained numerically is similar to the experimental case.
Effect of particle orientation: To quantify the effect of particle orientation on voltage pulses (and therefore on particle size measurement), we force a particle to pass through the orifice with a fixed orientation of $\theta=90^\circ$ first, and with a fixed orientation $\theta=0^\circ$ secondly. We consider an experimental micro-aperture with an aspect ratio $D_0/L=0.4$. Radius $R$ of the particle is fixed to 1 $\mu$m and $nR=1$ $\mu$m. Results show that, in this mechanical and fluidic configurations, the particle passing perpendicular to the flow ($\theta=0^\circ$) generates a voltage pulse 33% higher than a particle passing parallel to the flow ($\theta=90^\circ$). Voltage pulses are exposed in Figure 8. Controlling this phenomenon allows to avoid erroneous size measurement due to particle orientation through the orifice.

Statistic Particle Size Distribution: Role of Hydrodynamic Focusing

Given the previous results, we can compute the size of particles immerged in a liquid suspension. Dimensions of the orifice are $L=65$ $\mu$m and $D_0=50$ $\mu$m. Mean velocity is approximately 4.25 m/s and voltage is fixed to 7.6 V. We consider a sample flow containing same spherical particles of radius equal to 2 $\mu$m (to take into account manufacturing tolerances for simulation, we consider random radius to be comprised between $R-\epsilon$ and $R+\epsilon$ for a small $\epsilon << 1$ $\mu$m). The mean volume of the particle is 113 $\mu$m$^3$. For each particle, we compute transport through the orifice and detect the peak of generated voltage pulse. Finally, we can construct statistic particle size distribution of the total sample. Here, we study and compare particle size distribution for two fluidic configurations: 1) the classical case where particle sample is evenly distributed in the whole micro-aperture; 2) the hydrodynamic focusing case where sample rate inside the micro-aperture is controlled by a sheath flow in order to direct the sample flow along the central axis of the orifice. This allows to avoid peaks of electrical field due to edge effects. Figure 9 shows sample concentration with hydrodynamic focusing and its distribution in the orifice. Figure 10(b) exposes particle size distribution in the case of sample hydrodynamic focusing. In Figure 10(d), we expose experimental size distribution obtained for the same configuration. Contrary to the classical case, this distribution tends to be a centered Gaussian distribution, and computation of mean particle size value is more precise. More precisely, if we compare numerical and experimental statistical distributions for the two cases, we estimate that hydrofocused flow chamber is twice more accurate than in non-hydrofocused method. In particular,
the variation coefficient for the hydrofocused method is twice smaller than the one obtained without hydrodynamic focusing.

Conclusion and Perspective

A complete numerical approach was developed to tackle the problem of counting and sizing particles by electrical measurement in an orifice-electrode system. The results, in line with experimental tests, highlight the crucial role of trajectory and orientation of particles in generated voltage pulses. This general method can be used to perform particle sizing measurement, in particular by optimizing shape design or fluidic conditions. We are currently working on substantial improvements on the particle transport model, notably including three dimensional processing and deformability of particles under hydrodynamic stresses.

References


[3] European patent EP 0 425 381, Apparatus for counting and determination of at least one leucocyte-subpopulation. - Filing date: 25 octobre 1990; Lefevre D.; Champseix H.; Champseix S.

Figure 10 (UP) Numerical Particle Size Distribution (a) classical case, (b) with sample hydrodynamic focusing; (DOWN) Experimental Particle Size Distribution (c) classical case, (d) with hydrodynamic focusing.
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