Dielectric spectroscopy in a micromachined flow cytometer: theoretical and practical considerations

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We propose a model to determine the influence of different cell properties, such as size, membrane capacitance and cytoplasm conductivity, on the impedance spectrum as measured in a microfabricated cytometer. A dielectric sphere of equivalent complex permittivity is used as a simplified model to describe a biological cell. The measurement takes place between a pair of facing microelectrodes in a microchannel filled with a saline solution. The model incorporates various cell parameters, such as dielectric properties, size and position in the channel. A 3D finite element model is used to evaluate the magnitude of the electric field in the channel and the resultant changes in charge densities at the measurement electrode boundaries as a cell flows past. The charge density is integrated on the electrode surface to determine the displacement current and the channel impedance for the computed frequency range. The complete impedance model combines the finite element model, the electrode-electrolyte interface impedance and stray impedance, which are measured from a real device. The modeled dielectric complex spectra for various cell parameters are discussed and a measurement strategy for cell discrimination with such a system is proposed. Finally we discuss the amount of noise and measurement fluctuations of the sensor.

Introduction

The emerging field of microfabricated flow cytometry devices has recently been a subject of interest for a number of researches using electrical1–3 or optical4,5 detection techniques. Flow impedance measurement of cells in a miniaturized device offers many advantages over conventional techniques, such as the integration of reference measurement electrodes, the use of centering techniques using sheath flow or dielectrophoretic forces, and the implementation of cell sorting or electroporation in situ.6,7 In such a device, the electrode can be directly patterned on the channel walls, using conventional photolithography techniques, and the detection volume can be defined in a channel having an unvarying cross-section. Alternatives to the direct impedance measurement have also raised considerable interest; one example is the use of ponderomotive techniques to measure electrical properties of single cells by dielectrophoresis. Typically, in electrorotation,8,9 the cell is polarized by a rotating AC field. The frequency-dependent torque generates a rotational response from the cell, which is measured optically. This technique is very precise and provides reliable measured values of the dielectric properties of single cells, but the measurement is time consuming, should be carried out by trained personnel, and the interpretation of the spectrum data requires complex models.

Essentially, measurement done with the presented device should give the same dielectric properties found in the literature using cell suspensions, but on a per cell basis. Work presented by Asami and coworkers showed the difference between white and red blood cells in suspensions over a broad spectrum.10 Changes in cell dielectric properties affected by cancer,11 malaria12 or measured through the cell cycle11 have also been demonstrated using different techniques.

Previously, there have been two major approaches to modeling cell impedance: (1) Coulter counter models, which accurately describe the effects of non-uniform electric fields on cell sizing but fail to account for the frequency dependence of the measured impedance, and (2) mixture equations, which are based on a complex dielectric cell model and describe the impedance of cell suspensions placed in an homogeneous electric field of given frequency.

The work presented here uses the finite element method (FEM) to determine the theoretical cell-induced impedance spectrum change in a non-homogeneous electric field and predicts the sensitivity of a modeled microfabricated flow cytometer. In addition, it provides a better understanding of the relative influence of several cell parameters on the measurement.

Thus, we define a complex dielectric model to determine the change in electrical current between a pair of electrodes due to passage of a biological cell. The model’s details are taken from the specific case of a device with a channel of $20 \times 20 \text{mm}^2$ cross-section and two facing electrodes, $20 \times 20 \mu\text{m}^2$ in area, on opposite walls of the channel (Fig. 1). These channel dimensions are chosen in order to determine the sensitivity limit of an ideal sensor, which could accommodate cell diameters up to 15 $\mu\text{m}$ without clogging the channel; the sensitivity decreases quickly for channel dimensions much larger than the cell, as will be discussed later.

Since square electrodes are integrated into the channel walls of this microfabricated cytometer, the resulting electric field is highly non-homogeneous. This type of complicated 3D field is well suited to numerical finite element modeling. The problem thus consists of determining how the impedance changes over a defined frequency range as several cell parameters (cell size, membrane thickness, etc.) vary. For sufficiently high frequencies, the cell membrane will be polarized and cytoplasm currents will be induced; these effects are functions of the cell electrical properties and will affect the cell impedance spectrum.

The results of our finite element model for a single particle will be compared, assuming that the particle is located in a homogeneous electric field, with analytical models used to describe electrical properties of mixtures and cell suspensions of spherical shapes. These mixture equations have been developed by a number of authors,14–16 based on earlier publications from Maxwell17 and Wagner.18 These analytical models have been extended to describe more accurately the dispersion effects observed when studying the dielectric spectrum of real cell suspensions, by including the influence of multiple shells, non-spherical shapes or cell size distribution.19–22 Among the simplifications used by these models, there is the assumption that perfectly shaped particles are placed in an otherwise homogeneous electric field, meaning, in a suspension, that the ratio of inter-particle distance to particle size is large. In our case this translates into having small particles placed in the channel far away from the electrode edges or channel walls. Clearly, these models, which do not take in account the electric field geometry, may not be used to determine the shape of the signal pulse.
generated by a passing cell in a micro flow cytometer or how it will be affected by the cell position in the channel.

This article will first review some developments related to the Coulter counter with regard to the problem of determining the exact change of impedance induced by a passing cell. We will then introduce some aspects of dispersion theory, which are used to determine the equivalent dielectric properties of cell suspensions assuming a homogeneous electric field. Next, to relate the dielectric properties of the measured sample to an effective change in channel impedance we will determine the geometric factor used to compensate for the fringing effect. At this point it will be possible to compare some analytical impedance spectra obtained for a uniform electric field to the values obtained with the proposed finite element model. Then, we will present general considerations on the expected sensitivity spectrum including the effect of the electrical double layer, stray impedances and the related amplification electronics. Finally, we will study the influence of the cell size, position and dielectric parameters at the output of the sensor, devise a measurement strategy and outline the theoretical performance of a microfabricated impedance spectroscopy flow cytometer.

Wall effects and non-homogeneous fields

We shall now review some of the approaches originally used in studying the classical Coulter apparatus, which are of particular interest for the present study even if they do not integrate the complex dielectric cell model jointly with the non-uniform field problem. Generally, in these devices, a constant current is set across a small aperture using a pair of large electrodes and a voltage pulse is recorded as each cell passes through the high current density opening.

De Blois and Bean\textsuperscript{2,3} used an approximation of the Laplace equation solution to determine, in a cylindrical aperture with an axial electric field, the resistance increase due to the passage of a particle that either has finite DC resistance or is an insulator. Their approach assumes that the measurement takes place in an infinitely long channel, and neglects the inhomogeneity of the field due to a short aperture length.

Actually, the authors give an upper bound for the resistive variation $\Delta R$ as a function of the particle diameter $d$ based on an exact solution of the Laplace equation, subjected to insulating boundary conditions, for a slightly bulged cylinder of diameter $D_m$ and length $L$, filled with a liquid of resistivity $\rho$:

$$\Delta R_{D_m/L} = (4\pi \rho D_m^4) F(d/D_m^3)$$

with

$$F(d/D_m^3) \approx 1 + 1.268 d/D_m^3 + 1.17 d/D_m^3$$

For spheres much smaller than the diameter of the tube, this expression reduces to Maxwell’s limit:

$$\Delta R = (4\pi \rho D^4)$$

The authors also mention that for $L$ comparable to $D$, which is particularly true in the case of a microfabricated measurement channel as described in Fig. 1, further corrections for the end effects are required. In practice, the aperture of Coulter counters is made short in order to reduce the thermal noise and increase the sensitivity of the sensor, although this results in a non-uniform current density over the aperture axial length and more complicated signal processing. More recently, Kachel\textsuperscript{24} determined the influence of aperture lengths, particle conductivity, shape and trajectory on the recorded pulse shape and amplitude obtained in a Coulter apparatus. This work emphasized the importance of the particle position inside the channel.

Koch \textit{et al.}\textsuperscript{3} in their early article on micromachined Coulter counter proposed some integral calculus of the resistance change, which assumes a completely insulating particle, neglecting AC polarization effect, and supposing the electric field to be uniformly distributed in channel cross-sections surrounding the particle.

In general, these approaches were developed to understand the difficulties, which arise when determining particle size in standard Coulter counters. They can be used to determine how the impedance change scales at low frequencies as a function of the cell size and channel dimensions, but do not account for the frequency behavior of polarisable dielectric particles.

Dispersion theory and impedance spectrum of a single cell suspension

In this section, we will review the main points of the dispersion theory that are used in modeling the dielectric properties of cell suspensions as a function of frequency.

The complete model of the micromachined flow cytometer is made of several elements as shown in Fig. 2. The channel filled with the conductive solution including the cell can be modeled either analytically or with finite elements. The effects of the stray impedances and of the electrical double layer at the electrode–electrolyte interface, which result in capacitive coupling to the channel bulk are added to the dispersion spectrum in a second phase and will be discussed later. We will now briefly review some theoretical aspects of the model behind the dispersion theory of biological cells.

The polarization of the cell membrane is responsible for the dielectric dispersion measured in cell suspensions, as well as for single cells, with a characteristic frequency $f_c$ in the MHz range.

![Fig. 1 Diagram of flow cytometry measurement in microchannel with integrated microelectrodes. The modeled cell passes between the electrode pair, altering the electric field distribution for different excitation frequencies, thus giving rise to a change in the measured channel impedance spectrum. The electric field density in the channel represented here by isosurfaces, shows typical polarization effects of the Maxwell–Wagner type (both caps on top and bottom of the modeled cell) and the inhomogeneous field due to the square electrode pair. The figure has typical channel dimensions and defines the two subdomains: the channel and the cell used in the model.](image-url)
The mechanism underlying this effect is of the “Maxwell–Wagner” type and is referred as β-dispersion when considering biological material. In the case of a dielectric sphere covered by a shell located in an otherwise homogeneous electric field, Pauly and Schwan reported a set of equations which gives the suspension relaxation characteristics as a function of the number frequency, radius and complex dielectric parameters.

Although we will use the simple single shell model in this article, we would like to refer to previous work done in this field. Asami recently published a review on the subject, including some extended models accounting for double shells and ellipsoidal shapes. Hanai discussed the validity of this model for particles of dimensions below 1 μm. Recently, to underline the fact that the model is dealing with living matter, the membrane potential has also been included in the form of a distribution of charges on the two faces of the shell. The effect of cell surface conductance in the diffuse double layer, which is responsible for the low frequency α-dispersion, was recently reviewed by Morgan and Green. Sukhorukov described a single shell model of biological cell accounting for the dielectric anisotropy of the plasma membrane.

In the single shell model, the outer and inner media frequency dependent complex conductivity are \( \sigma_1 \) and \( \sigma_2 \) respectively and defined as:

\[
\sigma = \sigma_0 + j \omega \varepsilon_\infty \varepsilon_r \\
\sigma_0 = \sigma_\infty (1 - p) / (1 + p) / 2 \\
\sigma_\infty = \frac{1 + 2\rho(\sigma_2 - \sigma_1) / (\sigma_2 + 2\sigma_1)}{1 - \rho(\sigma_2 - \sigma_1) / (\sigma_2 + 2\sigma_1)} \\
\varepsilon_\infty = \frac{(1 + 2\rho)\varepsilon_\infty + 2(1 - p)\varepsilon_1}{(1 - p)\varepsilon_\infty + (2 + p)\varepsilon_1} \\
\varepsilon_0 = \frac{(1 - p)/2}{(1 + p)} + \Delta \varepsilon \text{ with } \Delta \varepsilon = \frac{9\rho}{\varepsilon_1(2 + p)} R \varepsilon_\infty
\]

and

\[
T = \frac{1}{2\pi f_\beta} = R \varepsilon_\infty \left( \frac{1}{\sigma_1} + \frac{1}{\sigma_2(1 - p)} \right)
\]

where \( \varepsilon_\infty \) and \( \sigma_\infty \) are the limit of permittivity and conductivity at low frequency and \( \varepsilon_\infty, \sigma_\infty \) at high frequency, \( T \) is the time constant of the dispersion, and \( p \) the volume fraction of the particle in the detection volume. The simple dispersion behavior resulting from this set of equations is represented in Fig. 3.

Using these equations it is relatively easy to determine cell parameters of interest using the measured equivalent permittivity and conductivity data (\( \varepsilon_\infty, \sigma_\infty, \varepsilon_0, \sigma_0 \) and \( T \)) and the known values of \( \varepsilon_1 \) and \( \sigma_1 \):

\[
p = \frac{\sigma_1 - \sigma_0}{\sigma_1 - \sigma_0/2}
\]

In the case of a single cell measurement, the volume fraction \( p \) can readily be used to determine the cell radius \( R \). The other parameters which can be extracted are:

\[
\varepsilon_0 = \frac{2\varepsilon_\infty - \varepsilon_1}{3R} \left( 1 - \frac{9\rho \varepsilon_0}{\varepsilon_1(2 + p)} \right)
\]

According to this model, complex measurements at two frequencies, one below and one above the dispersion characteristic frequency, are needed in order to determine the four unknown parameters, assuming a single Debye relaxation. Practically, evaluating \( \varepsilon_0 \) precisely at low frequencies is difficult because the out-of-phase current in the sample is very small. As an alternative, it is possible to use eqn. (8) and measurements at a selected intermediate frequency to determine the characteristic frequency \( f_\beta \) of the β-dispersion.

If a complete data set is available as a function of excitation frequency, a better approach to extract those parameters is to use spectrum fitting techniques with an appropriate electrical model.

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![Fig. 2 Scheme of impedance measurement between electrodes in the channel.](image)

**Fig. 2** Scheme of impedance measurement between electrodes in the channel. \( Z_0 \) is the impedance of the solution-filled channel of complex conductivity \( \sigma_1, \varepsilon_1 \) and \( Z_1 \) is the impedance with a cell present. The equivalent frequency dependent conductivity \( \sigma_{eq}^{real} \) and permittivity \( \varepsilon_{eq}^{real} \) of the channel are given by inhomogeneous media dispersion theory and geometric capacitance or using FEM simulations. \( \Delta Z \) is defined as the impedance difference between these two states \( \Delta Z = Z_1 - Z_0 \) taking into account the electrode polarization effect as well as stray impedances.
Geometric factor

The dielectric properties reported in the previous section are related to the channel impedance through a geometrical factor. The electric field determined by the microelectrodes and microchannel geometry is non-uniform and subject to edge effect or fringes. The geometric factor is used to correct for this edge effect. For the theoretical computation a pair of electrodes having a width W equal to the channel height h (Fig. 4) are considered. We can assume that the fringes affect the detection volume in a 2D manner similarly to what is observed with infinitely long stripes, and the contribution to the electric field of the electrode back sides is neglected. The geometric factor is obtained by using the Schwartz–Christoffel mapping to a rectangle. It is a property of conformal mapping that the capacitance and conductance remain invariant. In the case of the microchannel we study, the transformation is carried out using first a $Z$–$T$ plane transformation followed by a second transformation to the $W$-plane using the approximations from Hilberg.\(^{39}\) The transformation of one quarter of the region of interest suffices due to symmetry as the total capacitance of the detection volume is equal to the capacitance of the $Z$ region (twice in parallel and twice in series).

In the final rectangular $W$-plane the geometric factor is given by $K/K'$ and the corrected capacitance per unit of length is given by:

$$C' = C \frac{K(k)}{K'(k)}$$

Here $K$ is the complete elliptic integral with modulus $k$. In our case $k$ is:\(^{40}\)

$$k = \tan(\frac{\pi W}{2h})$$

The corrected admittance and resistance per unit of length are similarly:

$$G' = \frac{G c}{\sigma e} \frac{K(k)}{K'(k)} \quad \text{and} \quad R' = \frac{1}{\sigma e} \frac{K'(k)}{\sigma e} (15)$$

The ratio $K/K'$ can be easily calculated using the approximation given by Hilberg, to an error lower than $3 \times 10^{-6}$:

$$K/K' = 1 + \frac{1}{\pi} \left[ \frac{1 + k^{1/2}}{1 - k^{1/2}} \right]$$

For $W = h$, which corresponds to the case of our micromachined flow cytometer, the value of $k$ is 0.917. The resulting value of $\kappa$ is: $\kappa = 1.441$

In practice, the two parallel facing electrodes are patterned on two glass substrates, which sandwich a polyimide structural layer that defines the channel height (20 $\mu$m) and width (20 $\mu$m). The electrode length $l = 20 \mu$m is actually defined by the channel width. The values of the channel capacitance, resistance and characteristic frequency $F_c$, assuming a value of $78 \epsilon_0$, for the relative permittivity of the saline solution and a conductivity of 1.2 S m\(^{-1}\), are:

- $C_{channel} = C l = 19.9$ F
- $R_{channel} = R_e / l = 28.9$ k$\Omega$
- $F_c = 1/(2\pi R_c C_{closed}) = 276$ MHz

The value of the geometric constant $\kappa$ is useful in many aspects of the present work. First, it is a basis of comparison to estimate the error obtained in determining the electrical properties of the channel without a cell for the finite element simulation and the measured impedance data, as will be shown later.

Second, because of the fringing effect, the detection volume $V_{det}$ used in calculating the particle volume fraction $\rho = V_{par}/V_{det}$, where $V_{par}$ is the particle volume, is difficult to estimate. This parameter was used previously in eqns (4) to (7) to obtain the equivalent complex conductivity of the channel when a cell is present. Using the conformal technique applied to a channel of geometric factor $\kappa$, we can establish a corrected volume fraction, which gives a better estimation of $V_{det}$. This is of course only valid as long as the cell diameter is small compared to the channel dimensions.

The finite element model

The simulation was done with the Femlab (Comsol) finite element toolbox for Matlab (Mathworks) using the 3D electrostatics application mode. Although this model retains the channel geometry, it does not account for the electrode interface impedance or stray capacitance, which will be added later (Fig. 2).

To compute the surface charge density on the electrodes, weak boundary constraints have been used to solve for the Lagrange multipliers permitting much more accurate flux computations. The channel is modeled as a box of $20 \times 20 \times 100 \mu$m\(^3\); we assume here that the electric field is entirely contained in this volume. Two squares of $20 \times 20 \mu$m\(^2\) embedded at the center of the top and bottom walls define the electrodes. All channel surfaces not defined as part of the electrode are given insulating boundary conditions. One electrode is defined as the electrical ground and the other is given a unit potential value. The cell is modeled as a sphere of radius $R$ situated in the modeled channel volume.

In the subdomain 1, which represents the surrounding conducting media, the Laplace equation is solved for the voltage $V$ with the complex permittivity $\varepsilon_0$:

$$-\nabla (\varepsilon_0 \varepsilon_1) \nabla V = 0,$$

where $\varepsilon_1 = \varepsilon_0 + \sigma / j\omega$ \hspace{1cm} (17)

In the subdomain 2, representing the cell, the Laplace equation is solved similarly using the complex equivalent model for a shelled sphere as defined by Jones:\(^8\)

\[ \text{Lab Chip, 2004, 4, 241–251} \]
where $E_{0}$ is the local electric field normal to the surface in subdomain $i$. This expression is derived from the charge continuity condition and thus permits the accumulation of free surface charges at the interface.

The use of the equivalent permittivity $\varepsilon_{e}$ can be avoided by using two separate electrostatic application modes in Femlab, each active in one subdomain. The coupling equation between these two applications is then given by:

$$V_{in}(t) = -i\omega V_{in} e^{-1/R_{0}C_{m}}$$

where $V_{ext}$ and $V_{in}$ define the potential just outside and inside the membrane. This method is more problematic than the equivalent permittivity approach, in terms of convergence for the iterative solver, but has several advantages: the voltage function inside the cell $V_{m}$ and the trans-membrane voltage ($V_{ext} - V_{m}$) can be evaluated, and the cell shape does not need to be spherical.

**Spectral analysis: common mode and sensitivity**

The channel bulk dielectric spectrum in such a device is not measured directly because the chip itself and the amplification electronics represent additional transfer functions in the signal path. An analytical model is used to understand how the signal obtained at the system output is dependent on these effects.

In an ideal trans-impedance amplification scheme, the real and imaginary parts of the measured signal variation at the electronic output to a change in impedance is defined as:

$$\Delta Z_{\text{vout}} = \frac{V_{\text{out}}}{Z_{\text{in}} - \Delta Z_{\text{in}}} = \frac{V_{\text{out}}}{Z_{\text{in}} - Z_{\text{ref}}} = \frac{V_{\text{out}}}{Z_{\text{in}} - \Delta Z_{\text{in}}} = \frac{V_{\text{out}}}{(1 + j\omega R C_{\text{eff}})}$$

This equation can be rewritten to obtain the related transfer function:

$$\Delta Z_{\text{vout}} = \frac{V_{\text{out}}}{Z_{\text{in}} - \Delta Z_{\text{in}}} = \frac{V_{\text{out}}}{(1 + j\omega R C_{\text{eff}})}$$

which, for $\omega > 1/R C_{m}$, i.e. above the lower limit of the resistive window, reduces to:

$$\Delta Z_{\text{vout}} = \frac{V_{\text{out}}}{R C_{m}}$$

The impedance variation maximum $\Delta Z_{\text{max}}$ is obtained when the cell is centered between the measurements electrodes; we assume this is the case for the rest of the present development. When including a trans-impedance amplification stage with a gain of $1/R C_{m}$ followed by a differential gain of $G_{\text{diff}}$ as presented in Fig. 5, the sensitivity at the electronic output to a change in impedance is defined as:

$$\Delta Z_{\text{vout}} = \frac{V_{\text{out}}}{Z_{\text{in}} - \Delta Z_{\text{in}}} = \frac{V_{\text{out}}}{Z_{\text{in}} - \Delta Z_{\text{in}}} = \frac{V_{\text{out}}}{(1 + j\omega R C_{\text{eff}})}$$

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showing a 40 db/decade slope below the frequency associated with the double pole at $1/R C_{m}$ and a constant gain of $V_{\text{out}}/R C_{m}$ for the intermediate frequency range (Fig. 5). In the case of a small difference between the measurement and reference impedance $Z_{\text{m}}$ and $Z_{\text{ref}}$, respectively, two slightly separated poles $1/R C_{m}$ and $1/R C_{\text{ref}}$ will appear instead of the double pole. For frequencies below these two poles, the sensitivity is clearly a function of the interface impedance $Z_{\text{eMax}}$.

To determine the static difference output signal for frequencies below the double poles, we consider that the total impedance difference is dominated by the small disparity between the interface capacitances $C_{m}$ and $C_{\text{ref}}$, thus we have:

$$\Delta Z_{\text{vout}} = \frac{V_{\text{out}}}{Z_{\text{in}} - \Delta Z_{\text{in}}} = \frac{V_{\text{out}}}{Z_{\text{in}} - \Delta Z_{\text{in}}} = \frac{V_{\text{out}}}{(1 + j\omega R C_{\text{eff}})}$$

substituting eqn. (26) in eqn. (25):

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To evaluate cell parameters in this lower frequency range, different compensation techniques have been considered, including calibration or global fitting methods. The electrode material and the effective surface in contact with the electrolyte can also be modified to increase the interface capacitance and reduce the related pole frequency.

In the intermediate to high frequency range, the electrode interface capacitance is considered as shorted and we only consider the channel bulk impedance in parallel with the stray capacitances. The stray capacitances take into account the alternative current and signal obtained is dominated by the channel bulk impedance. This window of interest is located more than a decade below the characteristic frequency $f_{c}$ of our detection device as estimated above. Thus, the current in the channel bulk is essentially due to conduction, and for the purpose of determining the sensitivity of this technique the channel impedance $Z_{c}$ can be approximated by a simple resistance $R$. The interface impedance $Z_{e}$ is considered as purely capacitive. We will now separately consider the low and high frequency limits of this resistive window.

In the lower to intermediate frequency range ($< 1$ MHz), the stray capacitance can be neglected and the current change due to the cell passing through is given by:

$$\Delta I_{\text{cell}} = \frac{V_{\text{in}}}{Z_{\text{in}} - \Delta Z_{\text{in}}} = \frac{V_{\text{in}}}{Z_{\text{in}} - \Delta Z_{\text{in}}} = \frac{V_{\text{in}}}{(1 + j\omega R C_{\text{eff}})}$$

which, for $\omega > 1/R C_{m}$, i.e. above the lower limit of the resistive window, reduces to:

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The impedance variation maximum $\Delta Z_{\text{max}}$ is obtained when the cell is centered between the measurements electrodes; we assume this is the case for the rest of the present development. When including a trans-impedance amplification stage with a gain of $1/R C_{m}$ followed by a differential gain of $G_{\text{diff}}$ as presented in Fig. 5, the sensitivity at the electronic output to a change in impedance is defined as:

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In the intermediate to high frequency range, the electrode interface capacitance is considered as shorted and we only consider the channel bulk impedance in parallel with the stray capacitances. The stray capacitances take into account the alternative current and
paths found in parallel with the detection or reference volume. It is expected that these current lines will not be significantly influenced by the passage of the cell. Symmetric design of the sensor and amplification electronic is used in order to balance those stray currents and cancel, by means of an early differential amplification, the resulting common mode that can potentially saturate the amplification dynamic range.

As before, the channel is considered resistive, meaning that the characteristic frequency $F_c$ is not yet reached. In that case the current change in the measurement channel is:

$$\Delta I = F_n \left[ \frac{1}{Z_1 + \Delta Z} - \frac{1}{Z_1} \right] = -\frac{V}{Z_c} \Delta Z$$  \hspace{1cm} (28)

Again, this results in a flat gain of $V/R_f G_{eff}/R_c^2$ for a change in channel impedance at intermediate frequencies.

In the case of small disparities between the measurement and reference stray capacitance, the transfer function at the output of the differential gain stage is:

$$\frac{\Delta V_{out(SC)}}{V_{in}} = j\omega R_i C_{eff}$$ \hspace{1cm} (29)

This voltage difference is frequency dependent, but, once demodulated, it can easily be DC filtered. The cancellation of any static difference between the reference and measurement volume impedance is possible because cells quickly flow through the detection area and produce a transient voltage peak with a standard profile. Care must of course be taken not to alter the peak amplitude in doing so.

To sum up, in the low frequency range, the transient signal due to the cell passage depends on the capacitive and frequency dependent interface impedance $Z_e$. In the intermediate frequency range, we have a resistive plateau, which permits unbiased impedance measurements of the channel. At high frequencies, the stray currents increase, and if they are not balanced, they could saturate the dynamic range of the amplifiers.

**Measurement of microelectrodes properties in the microfabricated channel**

We will now depart briefly from purely theoretical considerations to discuss some measured impedance spectra, which will permit us to validate the discussed approach.

In practice, the use of a constant phase element (CPE) to model the electronic double layer is commonly favored over the use of a simple capacitor. For small excitation signals below 0.5 V, electrochemical effects can generally be neglected, but only a perfectly smooth and clean electrode such as the mercury drop electrode will present a fully capacitive behavior. On our cytometry chip, the Pt electrodes are patterned on an optically polished glass substrate (Schott) by sputtering (Balzers) and lift-off.

The Bode diagram of Fig. 6 shows a small signal impedance spectrum measured using an HP4284 and an HP4285 LCR meter, both controlled by Labview (National Instruments). The channel has a $20 \times 40 \mu m^2$ cross-section. The $20 \times 40 \mu m^2$ platinum electrodes are on the top and bottom surface of the channel and are $20 \mu m$ apart. These dimensions were chosen to reduce clogging during testing. The channel geometrical factor is unchanged from

**Fig. 6** Bode diagram of measured and fitted impedance spectrum of a typical electrode pair of $20 \times 40 \mu m^2$ micrometers in a rectangular channel. The two electrodes are $20 \mu m$ apart. The measurement was taken using two LCR meters (HP4284, HP4285). The channel is filled with a calibrated KCl solution of conductivity 12 880 mS cm$^{-1}$ (Cole–Parmer). The measurement data was fitted using a simple RC model and with a model using constant phase elements (CPE).

**Fig. 5** Transfer functions of a simplified measurement and reference sensor in an ideal transimpedance difference amplification interface. The two thin lines show the transfer function at the outputs $V_1$ and $V_0$ of the trans-impedance stage assuming a mismatch between $Z_1$ and $Z_0$. The dashed line represents the difference signal amplitude at the output as a function of a difference in the electrode (for low frequencies) and stray (for high frequencies) capacitances. It represents the “non-useful” or static signal, which limits the electronic dynamic range. The thick solid line is the transfer function as measured at the differential output for a change in channel resistance.
our model; while the electrode surface and capacitance are twice as large, the channel bulk resistance is reduced by one half. The channel is filled with a calibrated KCl solution with a conductivity of 12,880 mS cm\(^{-2}\) (Cole–Parmer). The measurement data is first fitted using a simple model defined by \(C_{\text{stray}} \parallel (R_{\text{sol}} + C_{\text{dl}}/2)\) and then with a second model in which the double layer capacitance was defined by a CPE. The fitting was done using the complex nonlinear least-square method. The measured data give a fitted double layer capacitance per surface area of 14.4 \(\mu\)F cm\(^{-2}\) and a stray capacitance of 0.5 pF. The measured impedance value of 17.7 k\(\Omega\) is slightly higher than the expected 14.4 k\(\Omega\) given by a geometric constant of 1.441. The fitted CPE power factor is 0.88.

For frequencies below 100 kHz, the impedance is dominated by the double layer capacitance. The impedance plateau of this sensor extends from 100 kHz to 10 MHz. At higher frequencies, the stray capacitance from the connectors and electronic board shunts the channel impedance.

For small excitation signals, the measured double layer properties show a constant phase of approximately 80°. When an excitation signal over 0.5 V was applied (data not shown here), departure from the capacitive behavior in the lower frequency range was observed, typically with a phase of 45°. This can be attributed to the onset of irreversible electrochemical reactions. It is in that case preferable to use frequencies higher than 500 kHz to avoid gas bubble formation in the channel. Previously published impedance measurements from several devices showed only 5% variation, indicating good chip-to-chip reproducibility. As mentioned previously, the finite element simulation does not include a model for the double layer or stray capacitances. Using the previously measured values for the electrode capacitance per surface area and the stray capacitance in our chip, we can now include these effects on the computed impedance data subsequently. The dashed lines in Fig. 7 demonstrate how they influence the impedance spectra for the two extreme cell dimensions and affect a direct measurement of the dispersion plateaus. Using a differential measurement technique, normalization against the solution-filled channel reference level, which is represented by the lower dashed

**Results and discussion**

As a first step, a validation of the geometric factor obtained using the FEM computation is performed by comparing the solution for an empty channel in 2D and 3D with the value given by conformal mapping. The 2D FEM simulation was accurate within 0.1% of the theoretical value (\(k_{\text{th}} = 1.441\)) when using 2000 elements and weak boundary conditions for the electrodes. The 3D simulation using 60,000 elements yielded a geometric factor within 3% of the theoretical value. Finer meshing could not be achieved due to memory issues.

For the first set of finite element simulations, a modeled cell is centered between the electrodes in the channel as shown in Fig. 1. The default cell dielectric parameters are similar to those used in Fig. 3. The Laplace solution was solved for 15 different cell sizes, from 1 to 15 \(\mu\)m in diameter and for 90 frequency steps ranging from \(10^2\) to \(10^{12}\) Hz. The computed impedance spectra (Fig. 7) show a low frequency amplitude plateau followed by the Maxwell–Wagner dispersion around 1 MHz, then a second plateau, and finally the start of the capacitive behavior due to the characteristic frequency \(F_c\) of the detection channel.

As mentioned previously, the finite element simulation does not include a model for the double layer or stray capacitances. Using the previously measured values for the electrode capacitance per surface area and the stray capacitance in our chip, we can now include these effects on the computed impedance data subsequently. The dashed lines in Fig. 7 demonstrate how they influence the spectra for the two extreme cell dimensions and affect a direct measurement of the dispersion plateaus. Using a differential measurement technique, normalization against the solution-filled channel reference level, which is represented by the lower dashed
curve in the impedance magnitude plot, is achieved. The phase shift induced by the cell presence is small, with a value of about 4° for the largest cell diameter 15 µm. When included in the phase plot, the influence of double layer and stray capacitance on the empty channel result in a minimum phase shift of ~16°.

For each cell, the change in impedance relative to the solution-filled channel reference, at both high and low frequency plateaus of Fig. 7 is plotted as a function of the cell volume in Fig. 8. For the mixture equation curves, the particle volume is calculated from the particle volume fraction \( p \) and the corrected detection volume \( V_{\text{det}} \) given by conformal mapping. For cells smaller than 10 µm there is a good agreement between the analytical and the finite element model.

As cell size increases, however, the increased insulator volume pushes more current lines outside the detection volume, resulting in an overestimation of the particle volume fraction used to calculate the impedance variation. Thus for cells larger than 10 µm, the low frequency FEM differs by a few percent from the mixture equation.

At high frequency, the cell is conducting and once again there is a good agreement between the FEM simulation result and the mixture equation.

A second finite element simulation was performed to determine the influence of a small vertical change of the cell position in the channel. The 10 µm cell was off-centered by 0.5 µm steps from 0 to 2 µm. The simulation gave computed changes in channel impedance on the order of 50 Ω, over a total value of 30 kΩ at 1 MHz, and did not demonstrate clear correlation to the cell position. Using adaptive meshing and weak boundary conditions techniques, the relative resolution we can achieve with 60 000 element is about 0.2%, which seems to be insufficient to predict the influence of small changes in cell position.

Another simulation is performed in a similar manner to predict the pulse shape induced by a passing cell. The cell is moving along the channel axis in 1 µm steps, from an initial position located between the two electrodes. Two different cell trajectories and sizes are computed: a) the first cell is 8 µm in diameter and moves along the central axis, b) the second cell is 4 µm in diameter and moves on a trajectory 7 µm below the center line. The impedance change as a function of the particle position on the trajectory is plotted for both low and high frequency plateaus. Fig. 9(a) shows a typical pulse shape as observed in Coulter counters when the cell is located on the central axis (due to symmetry only half of the pulse is computed and plotted). In Fig. 9(b) the cell is passing just above the bottom electrode, with a 1 µm gap, and the maximum impedance variation is obtained for the cell position close to the electrode edge where the current density is highest. The opacity is a parameter defined as the ratio of the high frequency to low frequency measurement signal. Although the measured signal will depend on the cell size and position, the opacity is remarkably insensitive to these parameters and still carries information on the cell structure and content, as we will demonstrate later. As for the resolution of the simulation, it is important to note that the meshing is different for each particle position and can thus explain part of the observed simulation noise. The adaptive solver does improve the meshing locally until a maximum number of elements is reached; still, a larger noise is observed when a small particle is located close to the edge of the electrodes where the gradient of field is large. In Fig. 9, the impedance variation due to the different cell positions represents only a small percentage of the simulated total impedance of the channel of ~28 kΩ, thus making the simulation noise appear more clearly.

The finite element model also permits us to investigate the influence of cell dielectric parameters, such as \( c_m \), on the channel impedance. For instance, we computed impedance spectra for a 10 µm cell with \( c_m \) ranging from 0.7–2.5 µF cm\(^{-2} \) in a 0.2 µF cm\(^{-2} \) step (other properties similar to Fig. 3). The effect on the impedance curve is a shift toward lower frequencies of the \( \beta \)-dispersion characteristic frequency \( f_c \), from 1.9 MHz down to 530 kHz, which is in good agreement with eqn. (8). In fact, for studies concerning dielectric parameters and in the limit of small cells centered in the channel, the mixture equations provide good results in a fraction of the FEM computation time.

The influence on the impedance spectrum, computed using the mixture equations, of small changes in the cell electrical properties (+10% membrane capacitance and +10% cytoplasm conductivity) is plotted in Fig. 10 for two different cell diameters (10 and 9.5 µm). A higher membrane capacitance shifts the dispersion toward lower frequencies, whereas an increase in cytoplasm conductivity reduces the impedance measured at the high frequency resistive plateau. The impedance curves shown here do not include the stray and

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**Fig. 8** Comparison of FEM (points) and mixture equation (lines) calculated impedance change for different cell sizes (each dot represents a FEM computed cell diameter from 1 to 15 µm). For the FEM, low frequency values are taken below the beginning of the dispersion, high frequency values are taken just after the Maxwell–Wagner dispersion at 10 MHz. The mixture equation values plotted derive from eqn. (2) and (3).

**Fig. 9** (a) Plot of the impedance variation of an 8 µm cell with standard electrical properties moving along the central axis of a channel. (b) An identical plot for a 4 µm cell with same electrical properties moving on a trajectory 7 µm below the center line. Opacity is defined as the ratio of the high frequency to low frequency impedance curves and is essentially independent of the cell position or size.
double layer capacitance effect but demonstrate that cell size effects dominate at frequencies around 100 kHz. These measurements, as will be shown in the next section, can be used to normalize measurements taken simultaneously at high frequencies.

**Discrimination strategy**

From the results obtained using the finite element method or the mixture equations, we can see the change that certain parameter will produce on different parts of the impedance spectrum. The cell size and cell position effects influence the whole spectrum but clearly dominate on other parameters at low frequency. We expect that ratios of signals measured at high or medium frequencies to the signal obtained below the dispersion permit some normalization. To see how this normalization can be used to discriminate different types of cells we will now determine the influence of small changes in 

\[ \text{signal} = \frac{S(F_1)}{S(F_2)} \]

ratios of signals measured at high or medium frequencies to the signal obtained below the dispersion permit some normalization.

The three curves of Fig. 11 illustrate the differences between the amplitude spectra obtained for the 10 μm reference cell and for three possible cells modifications: diameter (−5%), membrane capacitance (+10%) and cytoplasm conductivity (+10%).

A change in cell size influences the whole spectrum. A change in the membrane capacitance has an effect in the intermediate region of the spectrum around 1 MHz, whereas a change in the cytoplasm conductivity will be measurable at frequencies close to 10 MHz. From the differences spectra shown in Fig. 11, we can determine a number of frequencies, which would ideally carry information on a single specific parameter change. We have first selected the frequency \( F_1 \) at 100 kHz to obtain a size dependent measurement, which is only marginally influenced by changes in other cell parameters.

For frequencies below 100 kHz the signal value depends nonlinearly to the cell volume because of the double layer. This is not desirable as it will affect the proposed normalization. In this case, the simulated signal change between the 10 and 9.5 μm cells at 100 kHz is already 13.6% for a 14.2% change in volume. However, as seen in Table 1, the signals obtained at \( F_2 \) are almost independent of changes in cell dielectric parameters. Two other frequencies \( F_2 = 1 \) MHz and \( F_3 = 10 \) MHz are selected to determine the influence of changes in membrane capacitance and cytoplasm conductance, respectively. The ratios \( S(F_2)/S(F_1) \) and \( S(F_3)/S(F_1) \) demonstrate a good sensitivity to the parameter of interest, but are still dependent on the cell size. Defining \( F_1 \) at a frequency of 300 kHz would give a better normalization for size, but it would also introduce some membrane capacitance dependence.

We have determined that although we could discriminate significant changes in cell electrical properties in previous experiments, size scattering in a given cell population remains the parameter to which the impedance sensor is most sensitive. Thus, we present a technique to diminish the influence of size scattering when investigating other cell parameters, taking the signal values and ratios from Table 1. In Fig. 11, the ratios (a) \( S(F_2)/S(F_1) \) and (b) \( S(F_3)/S(F_1) \) at the amplification output vary proportionally to the 14.2% change in cell volume.

![Fig. 10](image-url)  
**Fig. 10** Influence on the impedance spectrum of small changes in membrane capacitance \( C_m \) and cytoplasm conductivity \( \sigma_c \) for two different cell sizes (10 μm and 9.5 μm).

![Fig. 11](image-url)  
**Fig. 11** Summary of the Spice simulated signal amplitude changes in \( mV_{peak} \) at the amplification output due to specific variation in the modeled cell properties (phase information is neglected). Small changes in size will clearly affect the whole spectrum; but, around 100 kHz changes due to other parameters are relatively small. Change in other cell parameters influence the spectrum in different ways at higher frequencies.

**Table 1** Signal amplitudes (phase information is neglected) for different cell properties at three selected frequencies and ratios obtained from the Spice simulation. The bold values represent the ratios of interest for the discrimination strategy.

<table>
<thead>
<tr>
<th>Signal value S (mV)</th>
<th>( S(F_1) )</th>
<th>( S(F_2) )</th>
<th>( S(F_3) )</th>
<th>Ratios</th>
</tr>
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<tr>
<td>( F_1 = 100 ) kHz</td>
<td>286.2</td>
<td>455.6</td>
<td>378.9</td>
<td>1.5919</td>
</tr>
<tr>
<td>( F_2 = 1 ) MHz</td>
<td>286.1</td>
<td>445.6</td>
<td>376.3</td>
<td>1.5575</td>
</tr>
<tr>
<td>( F_3 = 10 ) MHz</td>
<td>286.3</td>
<td>459.9</td>
<td>368.5</td>
<td>1.0666</td>
</tr>
<tr>
<td>10 μm Ref. cell</td>
<td>247.2</td>
<td>400.9</td>
<td>351.6</td>
<td>1.6218</td>
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<tr>
<td>9.5 μm Ref. cell</td>
<td>247.1</td>
<td>392.5</td>
<td>349.2</td>
<td>1.5886</td>
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<tr>
<td>9.5 μm +10% ( C_m )</td>
<td>247.2</td>
<td>404.5</td>
<td>344.0</td>
<td>1.6364</td>
</tr>
<tr>
<td>9.5 μm +10% ( \sigma_c )</td>
<td>247.2</td>
<td>404.5</td>
<td>344.0</td>
<td>1.5913</td>
</tr>
</tbody>
</table>

**Lab Chip, 2004, 4, 241-251**

249
250

$S(F_3)/S(F_1)$ are plotted against the value obtained for $S(F_1)$. The drawn trendlines are characteristic for a scattering due to spreading in cell sizes. Changes in other cell parameters will conversely exhibit spreading along the vertical axis. In Fig. 12(a), a good vertical sensitivity to a change in cell membrane capacitance is illustrated whereas for Fig. 12(b) a change in cytoplasm conductance will prevail. Unfortunately, selecting frequencies that have a good sensitivity for a specific parameter will not produce an independent measurement of that parameter. Some possible solutions would then be to use more measurement frequencies, use the phase information, apply subtractive compensation techniques or calibration with known and homogeneous cell or bead populations.

In order to estimate the resolution of our sensor we need to investigate the different sources of noise in the system. Essentially, electric noise is considered but other parameter like position of the cell can induce some measurement scattering.

From an electrical point of view, a major part of the noise contribution in the frequency range of interest is due to the Johnson noise in the channel and in the resistors around the first stage of amplification. It is relatively easy using the Spice simulation software or a simple analytical noise model to compute the total amplification. The electrical circuit consists of the amplification stages shown in Fig. 5 followed by an RF lock-in amplifier (SR844, Stanford Research Systems). The channel impedance is 28 kΩ, the feedback resistors in the first amplification stage are $R_f = 1.6 \, kΩ$, and the second stage has a gain $G_{2} = 150$ resulting in a total output noise $E_{n} = 2 \, μV/Hz$. To determine the theoretical rms value of the noise at the output we multiply $E_{n}$ by the equivalent noise bandwidth we select for the lock-in amplifier to obtain the total measured rms noise $V_{noise} = 160 \, μV_{rms}$. When the reference and measurement detection volumes are replaced by precision resistors in our measurement setup, the noise as measured by the RF lock-in amplifier at the output of the second amplifier stage is comparable the theoretical value of $V_{noise}$. However, when the actual microfabricated chip is filled with a flowing calibrated KCl 0.1M solution and measured at 1 MHz and with a 100 mV$_{pp}$ applied potential, the rms noise is an order of magnitude higher than predicted. In addition, the noise increases for higher flow speed and larger ($~1 \, V_{pp}$) applied electrical measuring potential. Possible sources of this added noise include charge instability at the electrode surface, heating of the solution or even bubble formation too small or brief to be noticeable. Under normal flow condition and for a 100 mV application potential, the passage of a cell with a diameter of 5 μm gives a 22 mV$_{rms}$ signal at the lock-in output, which represents a 1% change in the channel impedance, while the electrical noise is less than 2 mV$_{rms}$. In comparison, a 2 μm cell gives in theory a 5 mV$_{rms}$ signal for a 0.2% impedance change.

Another source of measurement scattering is the variation of particle position in the channel. Negative dielectrophoresis forces can be used to focus the particle trajectories sufficiently close to the channel centerline and obtain reproducible measurements for polystyrene beads of calibrated size. Using dielectric focusing the fluctuation on the measured signal amplitude is reduced to noise equivalent of about 4 mV$_{rms}$, which we assume to be the remaining position noise. Fig. 13 shows a typically measured speed and signal amplitude scattering for 4.3 and 5.14 μm calibrated beads (molecular probes).

Comparing the signals obtained for the 10 μm cells to the measured these noise levels we can estimate that cells properties dielectric changes in the order of 10% seem to be the resolvable limit of the present device.

It is of course important to consider that the simple cell model, which was used in this article, is far from describing the true complex dielectric behavior of a real biological cell. In addition, extraction of numerical values associated to these model parameters is a difficult task, which will eventually require the use of other simultaneous measurement frequencies.

![Fig. 12](image1.png) (a) Discrimination can be achieved for changes in the cell membrane capacitance independently of a scattering in cell size. The ratio $S(F_3)/S(F_1)$ is plotted against the values for $S(F_1)$. Measurement differences due to cell sizes will be located along the trend lines and changes in the parameter of interest will appear on the vertical axis. (b) Similar graph for the ratio $S(F_3)/S(F_1)$ which is more sensitive to the cytoplasm electrical properties.

![Fig. 13](image2.png) Measurement of 4.3 and 5.14 μm size calibrated beads showing speed and amplitude scattering. The transient time is the time taken by a particle to go through a distance of 60 μm, which separates the two detection volumes.
Conclusion

We have presented the case study of a microfabricated impedance spectroscopy cytomter. We review two previous major approaches to modeling cell impedance and put them into the context of our specific sensor. Both analytical and numerical approaches were used to establish the sensitivity of the device to a number of parameters: cell position, size and dielectric properties. The results of the FEM simulation give the theoretical cell-induced impedance spectrum change depending on the particle position in the non-homogeneous electric field. They also demonstrate the difference to the mixture equations results for relatively large cells. However, the simple mixture equations are much faster and quite accurate in studying how changes in the dielectric properties affect the measurement signal.

Downscaling of the microfluidic channel and the embedding of microelectrodes allow us to define small detection volumes and thus improve the sensor sensitivity. Yet, this has major consequences on the device sensitivity as a function of frequency and requires that high frequencies in the MHz range be used for the measurement. This implies that particular attention should be paid to the detection technique and signal amplification. In this article, we focused our attention on the electrostatic problem and neglected the issues related to microfluidic. Needless to say, precise flow and particle speed control are necessary to achieve reproducible measurement. Overall, considering the presented model, the measured electrode characteristics and device noise as well as results from preliminary experiments, we are confident, that such a chip can differentiate single cells according to changes in dielectric properties much more accurately than has been currently published.

In addition, integration of new on-chip functionality can be envisioned which will open up a number of applications to this technique.

Acknowledgments

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