UNCERTAINTY IN FLOW IMPEDANCE MEASUREMENTS ARISING FROM SHEAR-INDUCED ROTATION OF PARTICLES IN MICROFLUIDIC CHANNELS

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ABSTRACT

Careful discrimination between subpopulations of cells in different tissues is important for monitoring the state of health or diagnosing health disorders. Among the number of different techniques for detection and sorting of cells, impedance-based cytometry is emerging as a complement to the traditional light scattering- and fluorescence-based methods. However, its incipient applications often assume that cells are spherical. We show explicitly – using anti-CD3/ CD28 antibody activated T-lymphocytes – that the deviations from the spherical symmetry result in a considerable increase in the impedance uncertainty which should be accounted for when interpreting the results of measurements.

KEYWORDS: Impedance Cytometry, Microfluidics, Shear-Induced Rotation, Jeffery Orbits, Measurement Uncertainty, Single-Cell Analysis.

INTRODUCTION

Microfluidic devices allow a possibility of non-destructive cell analysis, especially applicable to single-cell measurements. Detection systems based on flow impedance measurements [1] are particularly interesting because of their benefits over the more traditional, optical methods: they are highly sensitive, easy to set up and control, and allow high throughput screening and markerless detection, as well as analysis of cellular properties and functions using sample volumes on the order of microliters. Even more importantly, they offer a possibility of electronic integration and miniaturization essential for the lab-on-chip applications.

In most such applications, cells are assumed to be spherical. However, deviations from spherical symmetry have an effect on the amplitude of the signal and can possibly result in considerable measurement uncertainty. If the aim is to produce reliable all-electronic devices, it is essential that this kind of uncertainty be properly addressed.

THEORY

Dielectric particles, including biological cells, polarize when they are subjected to the external field. When suspensions of cells are pumped over different configurations of electrodes that provide non-uniform field, polarization induced in the volume occupied by a passing cell results in the transient impedance change registered by an electronic detector. More specifically, in this study, capacitance change is registered. This change in capacitance is directly related to the change in energy of the analysis volume (i.e., volume directly above the electrodes), and therefore proportional to the scalar product of polarization induced in a volume occupied by a particle and the external electric field ($\Delta C \propto \mathbf{P}\cdot\mathbf{E}$) [2]. For a uniform, spherically symmetric particle, polarization **P** is isotropic and linearly related to the external field as $\mathbf{P} = \alpha\mathbf{E}$, so that the energy – and therefore also the signal S -- is proportional to $\mathbf{E}^2$. Any departure from spherical symmetry results in anisotropic polarization, meaning that α becomes a (diagonal) tensor with different eigenvalues α_i in different principal directions; the transient signal due to a non-spherical particle becomes a weighted sum of the squares of components of **E**, i.e., $S \propto \Delta C \propto \Sigma\, \alpha_i E_i^2$. The weight in each direction is proportional to the Clausius-Mossotti factor in that direction, K^i_{CM}, and through this factor it depends on the particle aspect ratio (via depolarization factors, n_i, where $n_i = 1/3$ for sphere), as well as the dielectric contrast between the particle and the medium, $(\tilde{\varepsilon}_p - \tilde{\varepsilon}_m)$, where $\tilde{\varepsilon}_i$ are frequency dependent complex dielectric permittivities. A general expression for Clausius-Mossotti factor is

$$K_{CM}^{i} = \frac{3(\tilde{\varepsilon}_p - \tilde{\varepsilon}_m)}{\tilde{\varepsilon}_m + n_i(\tilde{\varepsilon}_p - \tilde{\varepsilon}_m)} \ . \tag{1}$$

The flow inside the microfluidic channel is laminar, with -- as illustrated in Fig.1 (a) -- a Poiseuille velocity profile and shear-induced particle rotation. Assuming uniform shear, all particles rotate in "Jeffery orbits" [3]: one of their principal axes describes a cone around the axis of rotation. This does not affect the capacitance signature of the isotropically polarized spherical particles, for which $\mathbf{P}\|\mathbf{E}$ always; however, for aspherical particle it modifies the signal profile because the signal depends both on magnitudes of **P** and **E** and on their changing mutual orientation. For example, in case of an axisymmetric particle like a prolate or an oblate spheroid, the Jeffery orbit cone is elliptical since the principal axis changes its initial tilt (which is a stochastic variable) with respect to the axis of rotation as it sweeps around it – see Fig. 1(b). The resulting periodic variations of the angle between the anisotropic polarization **P** and the external electric field **E** ultimately bring about a deviation of the capacitance signature ΔC from the profile expected for a spherically symmetric particle.

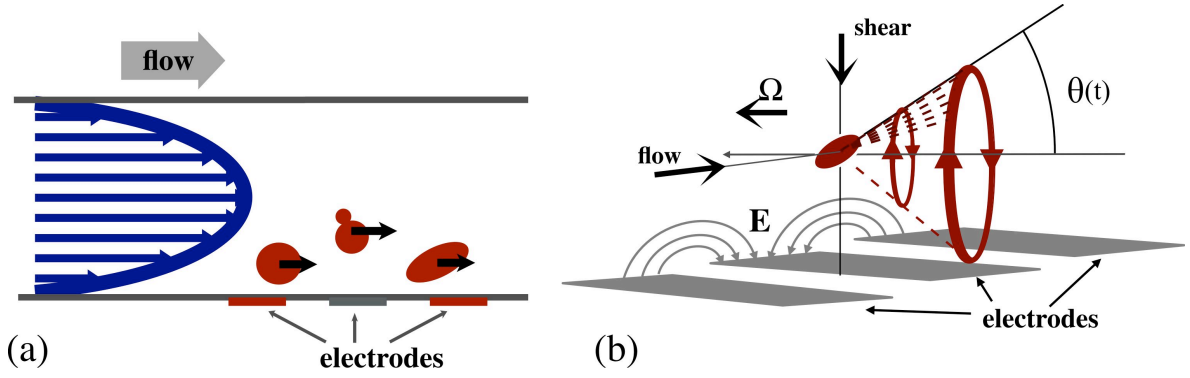

Figure 1: (a) Schematic representation of particles of different symmetry in Poiseuille flow. Velocity profile is parabolic and results in shear in the perpendicular direction to the flow. Under the influence of gravity, cells typically settle in the bottom third of the channel. (b) Jeffery orbit: Under the influence of shear, an axisymmetric particle rotates (with a period of rotation inversely proportional to the shear rate and always comparable to the passage time over the electrodes) in such a way that one of its principal axes describes a cone around the axis of rotation, **Ω;** *this cone is elliptical, indicating that the tilt angle* θ *changes periodically with time.*

EXPERIMENTAL

Figure 2 (a) is a schematic representation of the setup used for measurements presented in this study. Apparatus consists of a microwave interferometer (described in Ref. [2]), coupled to the microfluidic channel with interdigitated two-gap electrode array on the bottom. Characteristic signal for a spherical particle is represented in Fig. 2 (b). It exhibits four prominent peaks corresponding to the four inside edges of the two-gap electrode configuration. Two middle peaks are the most sensitive to variations in orientation between the electric field and particle polarization.

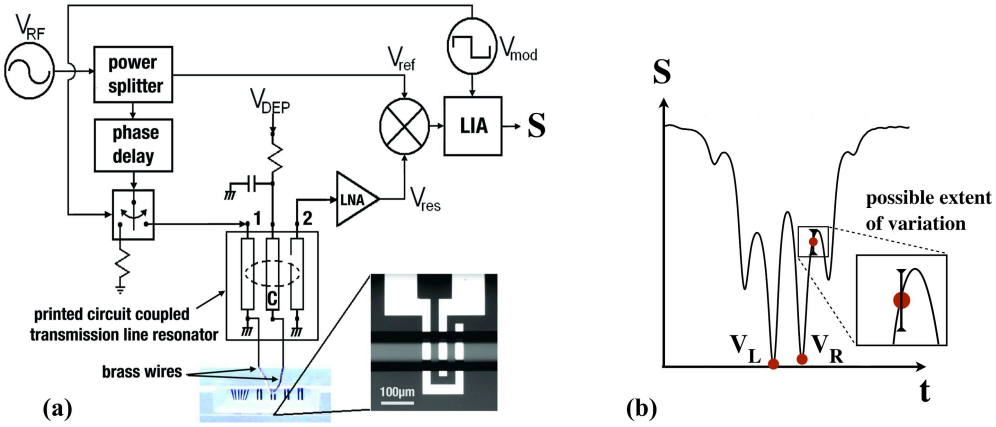

Figure 2: (a) Schematic representation of the microwave interferometer detection apparatus with a two-gap electrode geometry (from Ref. [2]). (b) Typical signal profile for the spherical particle passing above the electrodes. Possible variation from this profile, due to variations in particle orientation, is represented schematically in the figure for a single instance in time.

A fresh culture of anti-CD3/CD28 antibody activated T-lymphocytes (hereafter referred to as "T-cells") obtained from human peripheral blood was prepared according to standard procedures (see [4] for details); they exhibited different non-spherical shapes as confirmed by videos.

The signals were recorded for subsequent analysis. The distribution of differences between the two central peaks, $(V_L - V_R)/V_L$ (normalized, to take care of the differences in particle sizes), calculated for each passing cell, establishes the uncertainty in measurements for a population of particles.

RESULTS AND DISCUSSION

Figure 3 (a) shows the characteristic trace for a spherically symmetric model particle – a 6 μm-diameter polystyrene sphere (PSS), purchased from *Polysciences Inc.*; (b), (c), and (d) show cases of T-cells whose symmetry deviates from spherical. These signals are just examples and in Fig. 3 they are scaled in order to emphasize the profiles resulting from different asymmetries; note that their true amplitudes, proportional to the volumes of the corresponding particles, can differ greatly.

Histograms of the normalized peak difference distribution, produced for a population of model PSS and for a population of T-cells are given in Fig. 4. The observed uncertainty is about 1.5% for PSS, and about 15% for T-cells. Uncertainties of the latter magnitude might also be produced by the asymmetric attachment of beads used for cell assays [5].

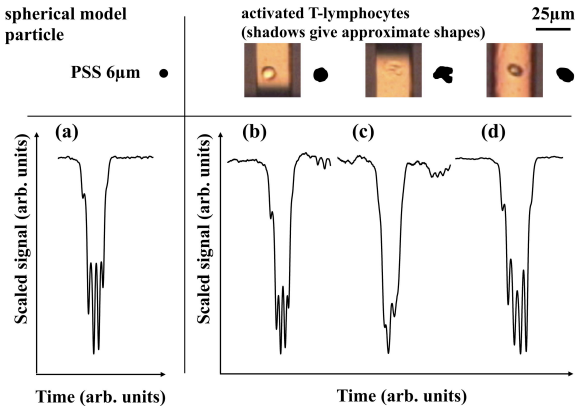

Figure 3: Characteristic signals (scaled, to emphasize the profiles): (a) Signal for a model particle with spherical symmetry shows little difference between the two central peaks; (b) T-cell with a roughly spherical shape is similar to the model particle, but pronounced departures from spherical shape (c) and (d) result in a signal that deviates considerably from the symmetric signature of the sphere.

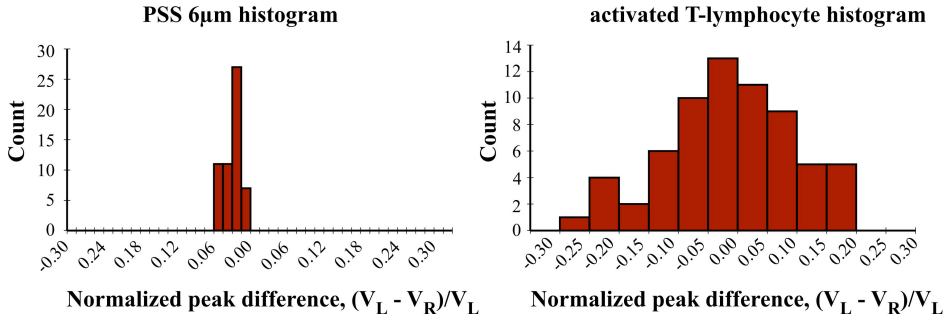

Figure 4: Left: Histogram for a population of 56 PS model spheres (6 µm in diameter) reflects the narrow distribution of normalized differences in peaks, $(V_L - V_R)/V_L$. Slight left skew is the artifact of the electrode fabrication process. Standard deviation is about 1.5%. Right: For a population of 67 T-cells, the distribution of normalized differences between the two central peaks is centered around zero but considerably wider that for the population of spherically symmetric particles. Standard deviation is about 15%.

CONCLUSION

Deviations from spherical symmetry of the particle can result in a considerable increase in the impedance uncertainty; for a population of anti-CD3/CD28 antibody activated T-lymphocytes, the measurement uncertainty was about 15%. The results of this study would apply whenever dielectric particles in a non-uniform field are induced to rotation, e.g., impedance cytometry and dielectrophoresis (DEP) sorting mechanisms like fluid flow fractionation (FFF).

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