Extraction of dielectric properties from dielectrophoretic collection spectrum data

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Abstract

Introduction

At present, one of the key technologies in high throughput drug assaying is multi-well plates that can perform a large number of experiments in parallel. For cell-based high throughput screening, a model organism is placed in all wells, while a different drug candidate is added to each single well. By means of a marker, such as pH indicators or fluorescence markers, it is established if a candidate interacted with the model organism in the desired way. Since a large number of experiments can be performed and compared simultaneously, well plates allow screening large compound libraries for suitable drug candidates. The dispensing of reagents and candidates and the reading out of the results can easily be automated by using dedicated robots and well plate readers. To further increase throughput and reduce the amount of reagents and candidate compounds used, the number of wells on a single plate has increased over time while the volume of each single well has decreased. Plates currently in use have over 2000 wells with a volume of only a few microlitres each, while plates with over 4000 wells and even smaller sample volumes are currently under development. The trend to smaller assay volumes has made the evaluation more and more challenging. One problem is to find suitable assay markers that do not interact with the model organism and are easy to evaluate in a small sample volume. A method that evades the use of chemical markers is dielectrophoresis (DEP).

DEP is the induced motion of particles in non-uniform electric fields. This technique can be applied to the manipulation, separation and analysis of cellular and viral particles [Jones 1995, Zimmermann and Neil 1996, Hughes 2002]. Particles experiencing such forces can be made to exhibit a variety of motions including

attraction to, and repulsion from, regions of high electric field (termed positive and negative DEP respectively) by changing the frequency of the applied electric field. Since bio-particles such as bacteria, cells or viruses have characteristic dielectric properties, dielectrophoresis can be used to distinguish between different types of bacteria, to detect changes in cell cytoplasmic properties, and to detect whether cells are viable or non-viable. The main factors influencing the dielectric properties of a bioparticle are the surface charge, the membrane capacitance and the conductivity of the cytoplasm. If drugs such as antibiotics change any of these factors, the dielectric properties of the cell change and can be detected. This change can be used to distinguish between cells that are resistant or sensitive to a drug, for example.

By exploiting the fact that different particles may experience forces acting in different directions when all other factors are the same, researchers have been able to use DEP to analyse and separate mixtures of cells on electrode arrays. For example, work has demonstrated that DEP can be used to examine the effect of drugs on cells, such as the effect of nystatin on erythrocytes [Gimsa et al. 1994] or the response of neutrophils to activation by chemotactic factors [Griffith and Cooper 1998]. Since populations of particles may experience forces acting in different directions within specific frequency windows, separation has been demonstrated for mixtures of viable and non-viable yeast cells [Markx et al 1994], and CD34+ cells from bone marrow [Stephens et al 1996], which can be separated from human blood. Other demonstrations of cell separation have been made in separating breast cancer cells from blood, erythrocytes with and without malarial parasite infection, B- and T-lymphocytes, and different types of viruses in solution using this technique have been described [Gascoyne et al 1997a, Gascoyne et al 1997b, Yang et al 1999, Morgan et al

1999]. Studies at Surrey using bacteria (Johari et al., 2003), algae (Hübner et al., submitted), cancer cells (Labeed et al. 2003), and studies in the literature have demonstrated that DEP can be used to detect the manner in which a drug interacts with a bio-particle. This potentially makes DEP a very valuable tool for screening applications.

In order to extract dielectric parameters from dielectrophoretic data, modelling techniques are used to find best-fit parameter sets for given sets of data indicating the time-dependent polarizability of the particles; it has long been held (Jones 1996) that the expansion of the equation linking the dielectrophoretic behaviour to the dielectric properties of the particle is too complex to provide meaningful expression for data analysis. IN this paper we show how simplifying assumptions can be used to extract useful data from the dielectrophoretic collection spectrum, and hence determine the properties of multiple populations of cells within a sample.

The time-averaged force exerted on a spherical particle of radius r suspended in a medium of relative permittivity ϵ_r and exposed to an electric field gradient is given by the equation:

$$\mathbf{F}_{\text{DEP}} = 2\pi\epsilon_0\epsilon_r r^3 \text{Re}[K(\omega)]\nabla E^2 \quad (1)$$

where ∇E^2 is the gradient of the strength of the applied electric field squared and $\text{Re}[K(\omega)]$ is the real part of the Clausius-Mossotti factor given by:

$$K(\omega) = \left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \right) \quad (2)$$

where ε_p and ε_m are the complex permittivities of particle and medium respectively, given by

$$\varepsilon_i^* = \varepsilon_i - j \frac{\sigma_i}{\omega} \quad (3)$$

where ε_i and σ_i refer to the (real) permittivity and conductivity of material i . Where the particle, rather than being homogeneous, consists of a shell surrounding a homogeneous core (as shown schematically in figure 1) we can replace its complex permittivity with an effective value combining the properties of the shell and the core, thus:

$$\varepsilon_{1eff}^* = \varepsilon_2^* \frac{\left(\frac{r_2}{r_1} \right)^3 + 2 \frac{\varepsilon_1^* - \varepsilon_2^*}{\varepsilon_1^* + 2\varepsilon_2^*}}{\left(\frac{r_2}{r_1} \right)^3 - \frac{\varepsilon_1^* - \varepsilon_2^*}{\varepsilon_1^* + 2\varepsilon_2^*}} \quad (4)$$

where subscripts 1 and 2 correspond to the core and shell, and r_1 and r_2 are the radii from the center of the sphere to the inside and outside of the membrane, so that the Clausius-Mossotti factor for the ensemble is given by equation (5).

$$K(\omega) = \frac{\varepsilon_{1eff}^* - \varepsilon_3^*}{\varepsilon_{1eff}^* + 2\varepsilon_3^*} \quad (5)$$

where the subscript 3 refers to the suspending medium. If the full expression for $Re[K(\omega)]$ is expressed analytically, it is sufficiently complex to render direct analysis meaningless; instead, researchers have used numerical methods to determine dielectric properties by analysis of dielectrophoretic spectra. Where analysis of dielectrophoretic data have been analysed (e.g. Gascoyne et al., 1997) it has been by simplifying the solution using assumptions and by examining a number of crossover spectra as a function of medium conductivity. However, such methods are limited, particularly where the sample of cells precludes the repeating of sample analysis or where multiple populations are present.

We have used a combination of experimental data, numerical approximation to that data and the symbolic mathematical tool Maple to examine the key features of dielectrophoretic collection data, and demonstrate here that it can be used to identify a number of cell populations in a single sample by identification of key features of the polarizability spectrum.

Analysis of key spectrum events

For a given shelled sphere, where the shell is of relatively low conductivity compared to the inner compartment and outer medium, we anticipate a polarizability spectrum of the type shown in figure 2. This displays two characteristic dispersions, one rising at lower frequency and one falling at higher frequency. The frequency where the polarizability crosses from negative to positive for a homogenous sphere allows the direct determination of the properties of the sphere from equation (2) by equating it to

zero; however, for a shelled sphere the expression is much more complicated to determine such factors. Some work has been performed in deriving expressions for the lower crossover frequency, but the upper frequency has been largely ignored because of limitations with signal generation equipment.

i. Dispersion transits take approximately one decade

Although the analytical expression for $Re[K(\omega)]$ is too complex to determine the behaviour of the spectrum as it changes between plateaus during interfacial dispersions, empirical analysis of the spectrum for a broad range of values for all variables indicates that the duration of the dispersion takes a frequency multiple of approximately 0.9 to go from 10% to 90% of the total change in $Re[K(\omega)]$, and a 3x multiple to go from 10% change to 50% change.

ii. Lower crossover frequency F_{x1}

iii. Upper crossover frequency F_{x2}

It is known that for the majority of cells, the thickness of the membrane is considerably smaller than the radius of the cell, so that $\left(\frac{r_2}{r_1}\right)^3$ has a value very near 1. If we make the approximation $\left(\frac{r_2}{r_1}\right)^3=1$, we find that the crossover frequency, given by the expression $Re[K(\omega)]=0$, collapses to the following:

$$F_{x2} = \frac{1}{2\pi} \sqrt{-1 \times \frac{\sigma_1^2 + 2\sigma_1\sigma_3 - 2\sigma_3^2}{\varepsilon_1^2 + 2\varepsilon_1\varepsilon_3 - 2\varepsilon_3^2}} \quad (6)$$

Numerical studies of the output of the full expression indicate that this approximation holds for the upper crossover frequency for a wide range of electrical values provided $\left(\frac{r_2}{r_1}\right)^3 < 1.5$ (approx), corresponding to a ratio of cell radius to membrane thickness of 20:1. Within this limit, which represents all cases of biological cells, equation (6) holds. The only case where the equation does not work is where no upper crossover exists, which happens when $\varepsilon_1 > \varepsilon_3$ or $\sigma_1 < \sigma_3$. The former is a highly unlikely event in biology, and the latter can be controlled by the use of low-conductivity media. Where either of these is the case, the equation returns an imaginary value of frequency. Provided these conditions are not met, ε_1 and σ_3 have very little effect on F_{x2} .

Since the upper crossover frequency is independent of size, largely independent (in biological cells) of cytoplasmic permittivity or membrane properties, and of the remaining variables those relating to the medium can be precisely defined, F_{x2} provides a direct measurement of the cytoplasmic conductivity. Furthermore, although the actual crossover frequency is often too high to be observed with conventional function generators, the behaviour of the dielectric dispersion – taking approximately one decade to transit between stable plateaus of $Re[K(\omega)]$ – means that the frequency at which $Re[K(\omega)]$ begins to decline is *also* dependent only on σ_1 . This is useful as this measurement is well within the capabilities of benchtop signal generators.

Experimental applications; determination of multiple populations

As we have discovered, the high-frequency behaviour of the crossover spectrum of a particle is a function only of the cytoplasmic conductivity. This gives us a tremendous advantage in the detection of multiple cell populations, since frequency the transition from a plateau in the value of $Re[K(\omega)]$ to a declining value can only be related to conductivity and therefore populations of cells with different conductivities in the same sample can be quantified.

It is known that the number of cells collected as a function of frequency, which is known to be an indicator of the value of $Re[K(\omega)]$ (e.g. Labeed et al. 2003, Johari et al. 2003). For example, consider the collection spectrum shown in figure 3, which shows the number of cells collected from a population of K562 myelogenous leukaemic cells 24 hours after treatment with apoptosis-inducing agent staurosporine (Labeed et al., submitted). It is evident from the “stepped” nature of the collection spectrum, especially at frequencies above the peak collection, that there are three cell populations present; the difference in collection levels between the plateaus allows the determination of the ratios of cell populations. By treating the net collection spectrum as being a sum of three crossover spectra and then modelling these three populations, a fit-curve such as the one shown in the figure can be produced. Extracting these data, we find that the populations have cytoplasmic conductivities of approximately 500mSm^{-1} , 30mSm^{-1} and 3mSm^{-1} ; corresponding well to expectations for cells in early and late stage apoptosis, and cell necrosis respectively. Furthermore, the cell proportions tally with observations of apoptotic progression measured using Annexin-V. Further details of this work are to be published elsewhere (Labeed et al., submitted).

Conclusion

It has been shown that an analytical treatment of the time-variant polarizability of cells can provide new methods of dielectrophoretic cell analysis. Specifically, the observation of corner frequencies indicating a high-frequency dielectric dispersion allows the identification and enumeration of populations of cells according to cytoplasmic conductivity, with particular relevance to the determination of the action of drugs for high-throughput screening applications. Dielectrophoretic collection methods allow the determination of large numbers of cells simultaneously, and the cytoplasmic conductivity is a significant marker of cell state, particularly with respect to membrane hyperpolarisation, or depolarisation after necrosis. We suggest that the use of automated curve-fitting software based on the principles outlined here would significantly enhance the identification of multiple cell populations.

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Figures

Figure 1. A schematic of a single shell model, showing the shell surrounding a core, and suspended in a dielectric medium. The inner and outer radii of the membrane have values r_1 and r_2 .

Figure 2. A typical spectrum showing the relative polarizability ($Re[K(\omega)]$) of a single-shelled particle as a function of frequency. Note the three plateaus at low frequency (negative DEP), intermediate frequencies (positive DEP) and high frequency (negative DEP). The transit between these plateaus takes approximately 1 decade; the lower and upper crossover frequencies, where $Re[K(\omega)]=0$, are indicated by F_{x1} and F_{x2} respectively.

Figure 3. A collection profile for staurosporine-treated K562 cells, showing the cells collected after 1 minute of exposure to DEP (circles), and the best-fit line to the data for a 3-population heterogeneous mix. Note the presence of three distinct collection plateaus.