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Dependence of the dielectrophoretic upper crossover frequency on the lipid content of microalgal cells

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ABSTRACT

High frequency dielectrophoresis (>20 MHz) was used to demonstrate that the upper crossover frequency of microalgal cells is reduced as lipids accumulate within the cytoplasm. Currently, the majority of AC dielectrophoresis applications differentiate cells by exploiting differences in the lower crossover frequency, typically between 10 and 500 kHz. However, the single shell model also predicts another crossover in the 20–200 MHz range that is dependent upon the dielectric properties of the cytoplasm. We demonstrate this effect with microalgal cells due to the relative ease in which the properties of the cytoplasm can be altered. *Chlamydomonas reinhardtii* cells were cultured in regular media and were observed to have an upper crossover frequency of ~75 MHz. The same cells, when cultured under nitrogen-free conditions, began to accumulate neutral lipids. The lipid content was verified via fluorescence microscopy and the upper crossover frequency was measured to be ~40 MHz. To measure the upper crossover frequency, two needle shaped electrodes were patterned onto a glass slide and the motion of the cells was observed as an AC signal was swept from 10–110 MHz at ~30 voltage peak-to-peak (Vpp). We conclude that the increase in lipid content reduces the effective conductivity of the cytoplasm thus reducing the upper crossover frequency. High frequency dielectrophoresis may be a label free technique to sort algae cells on the basis of lipid content.

1. Introduction

Dielectrophoresis (DEP) [1]is a topic of growing interest for label-free cell characterization, separation, and sorting [1–4]. Briefly, DEP refers to the motion of uncharged particles in a spatially non-uniform electric fields that is induced by differences in the dielectric properties between the particle and the media. Dielectric properties are a function of the frequency of the applied electric field and therefore the DEP response of the particles varies in response to changes in the applied frequency. This effect allows for various particles, including cells, to be differentiated on the basis of differences in dielectric properties. Algae cells in particular have the potential to be separated based on lipid content. Analytical models predict that lipid content separation would require high frequency DEP (20–200 Mhz) before separation would occur due to the cytoplasm changes [5–7]. However, there is little experimental evidence to support the prediction of this upper crossover frequency. One reason for this is that standard bench-top function generators are typically limited to maximum frequency of 20 MHz, and those with higher bandwidths are often unable to generate these high frequency electric fields with sufficient amplitude to induce DEP motion [8]. Despite this, the effect of changes in the conductivity of the cytoplasm has been indirectly observed by measuring changes in the DEP spectra at frequencies up to 20 MHz by Valero et al. [9] and Labeed et al. [10]. While these studies also predict a shift in the upper crossover frequency, the effect was not directly observed at the upper crossover frequency.

Two studies provide preliminary evidence that increases in the cytoplasmic lipid content can affect the dielectric properties of algal cells. Bono et al. [11] measured the dielectric properties of a suspension of algal cells and found that those properties depended on the lipid content of the cells. While this approach would be especially useful for monitoring a commercial algae culture, it is unable to sort cells based on lipid content for further investigation. Of particular interest to the work presented here, Deng et al. [12] demonstrated the ability to separate *Chlorella* cells based on lipid content using DEP, however their separation was highly dependent upon the conductivity of the DEP medium, and an upper crossover frequency was not identified for some DEP media. They theorized that the crossover frequency was higher than the 20 MHz maximum of their equipment.

Abbreviations: F_{x1}, lower crossover frequency; F_{x2}, upper crossover frequency; pDEP, positive dielectrophoresis; nDEP, negative dielectrophoresis; V_{pp}, voltage peak-to-peak.

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[1] Dielectrophoresis (DEP), F_{x1}—Lower crossover frequency, F_{x2}—Upper crossover frequency, pDEP—Positive dielectrophoresis, nDEP—Negative Dielectrophoresis, V_{pp}—Voltage peak-to-peak.

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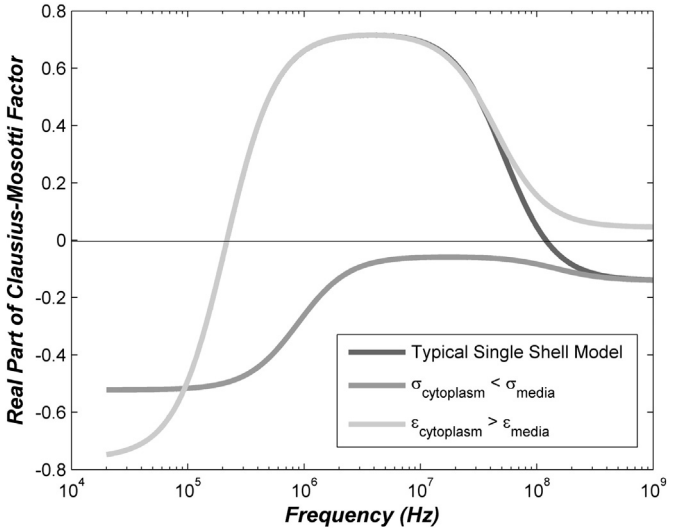

Fig. 1. The typical DEP response predicted by the single shell model has two crossover frequencies. The lower crossover frequency, F_{x1}, is dictated primarily by membrane properties, while the upper crossover frequency, F_{x2}, depends on the properties of the cytoplasm. Modeling parameters are based on Muller et al. [19] and Wu et al. [5]$\sigma_{media} = 0.06$ S/m, $\varepsilon_{media} = 80$, $\sigma_{membrane} = 2 \times 10^{-5}$ S/m, $\varepsilon_{membrane} = 8$, $\sigma_{cytoplasm} = 0.5$ S/m, $\varepsilon_{cytoplasm} = 50$, R = 6 μm, $\delta = 7$ nm. In cases where the media is more conductive than the cytoplasm ($\sigma_{media} = 0.6$ S/m), no crossover exists. In cases where the permittivity of the cytoplasm is higher than the media ($\varepsilon_{cytoplasm} = 90$), no upper crossover frequency exists.

In light of these reports, it is clear that more work is needed to understand the DEP response of algae cells at frequencies above 20 MHz. To explore this high frequency regime, a high bandwidth RF amplifier was used to generate electric field suitable for DEP at frequencies as high as 160 MHz. For these studies, the model species *Chlamydomonas reinhardtii* was used. When the microalgal cells are stressed in a nitrogen free (N-free) environment they accumulate lipids [13–16]; and this lipid accumulation can be quite dramatic [5]. It is hypothesized that increasing the lipid content within the cytoplasm will reduce the cytoplasmic conductivity, and therefore shift the upper crossover frequency to lower frequencies. In this paper, the theory of high frequency DEP will be discussed and it will demonstrate that the upper crossover frequency of microalgal cells does indeed shift as the dielectric properties of the interior of cells changes due to the accumulation of non-polar lipids.

2. Theory

The underlying equations describing dielectrophoresis are well established and provide a strong foundation for understanding how the DEP response of a cell changes as a function of the dielectric properties of the cell and media, and as a function of the frequency of the applied electric field [1,6,17,18]. The DEP force on a homogeneous spherical particle is given by Eq. (1):

$$F_{DEP} = 2\pi\varepsilon_m R^3 Re(F_{CM})\nabla E_{RMS}^2 \tag{1}$$

where F_{DEP} is the DEP force, ε_m is the permittivity of the suspending medium, R is the radius of the particle, $Re(F_{CM})$ is the real part of the Clausius–Mossotti factor, E_{RMS} is the root mean square of the electric field strength, and ∇ is the gradient. A detailed discussion of this derivation of Eq. (1) can be found in [4]. The direction of the DEP force depends only on the Clausius–Mossotti factor given by Eq. (2):

$$F_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \tag{2}$$

where ε_p^* and ε_m^* are the respective complex permittivities of the particle and media, given by Eq. (3):

$$\varepsilon_i^* = \varepsilon_i - j\frac{\sigma_i}{\omega} \tag{3}$$

where ω is the angular frequency, ε_i is the real permittivity, and σ_i is the real conductivity.

In the simple case of a homogeneous sphere, the relative values of the complex permittivities determine the direction of the DEP force. For cells however, the situation is more complex as the cell cannot be treated as a homogeneous particle. The most common model found in the literature is the single shell model where an effective permittivity can be determined using Eq. (4):

$$\varepsilon_{peff}^* = \varepsilon_s^* \frac{\left(\frac{R+\delta}{R}\right)^3 + 2\frac{\varepsilon_c^* - \varepsilon_s^*}{\varepsilon_c^* + 2\varepsilon_s^*}}{\left(\frac{R+\delta}{R}\right)^3 - \frac{\varepsilon_c^* - \varepsilon_s^*}{\varepsilon_c^* + 2\varepsilon_s^*}} \tag{4}$$

where ε_{peff}^* is the new effective complex permittivity of the cell, ε_s^* is the complex permittivity of the shell (i.e. cell membrane), ε_c^* is the complex permittivity of the core (i.e. cell cytoplasm), and δ is the thickness of the shell. In this case the cell is modeled as a homogeneous sphere (the cytoplasm) surrounded by a thin shell (the membrane). At this point two key observations can be made: 1) the DEP force depends linearly on the real part of F_{cm} and 2) the frequency of the electric field only affects the DEP force through F_{cm}. Typically, cells undergo negative DEP (nDEP) at low frequencies. As the frequency is increased, the DEP response passes through a lower crossover frequency, F_{x1}, and the cell experiences positive DEP (pDEP.). At even higher frequencies, an upper crossover frequency, F_{x2}, is observed as the response returns to nDEP.

This work is primarily concerned with shifts in F_{x2} and therefore it is worth examining what parameters affect this frequency. Broche et al. [6] simplified the full Claussius–Mossoti factor using a computer algebra system to find that in cases where the core radius is more than 20 times the shell thickness Eq. (5) can be used to determine F_{x2}:

$$F_{x2} = \frac{1}{2\pi}\sqrt{\frac{\sigma_c^2 - \sigma_c\sigma_m - 2\sigma_m^2}{2\varepsilon_m^2 - \varepsilon_m\varepsilon_c - \varepsilon_c^2}}. \tag{5}$$

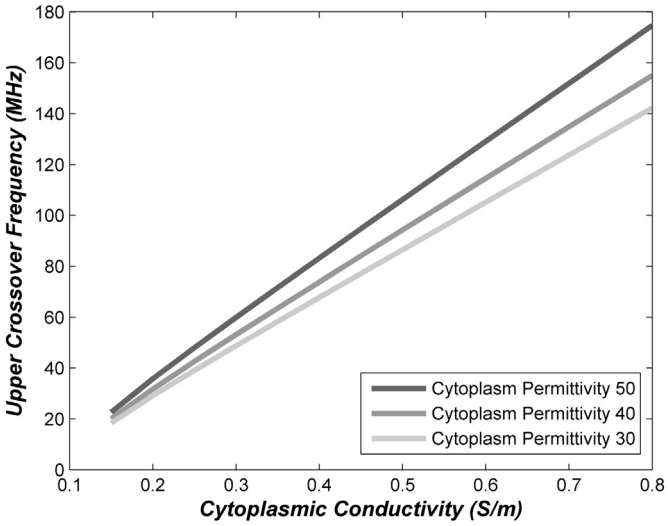

Fig. 2. Using Eq. (5), the effects of cytoplasmic conductivity and permittivity on the upper crossover frequency can be easily seen ($\sigma_{media} = 0.06$ S/m, $\varepsilon_{media} = 80$). At lower cytoplasmic conductivities, the cytoplasmic permittivity has a smaller effect.