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High frequency dielectrophoretic response of microalgae over time

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Abstract

The high frequency dielectrophoresis (>20 MHz) response of microalgae cells with different lipid content was monitored over time. *Chlamydomonas reinhardtii* was cultured in regular medium and under nitrogen-depleted conditions in order to produce populations of cells with low and high lipid content, respectively. The electrical conductivity (EC) of the culture media was also monitored over the same time. The upper crossover frequency (UCOF) decreased for high-lipid cells over time. The single-shell model predicts that the upper crossover frequency is dictated primarily by the dielectric properties of the cytoplasm. The high frequency DEP response of the high-lipid cells' cytoplasm was changed by lipid accumulation. DEP response of the low-lipid cells also varied with the conductivity of the culture media due to nutrient consumption. Relative lipid content was estimated with BODIPY 505/515 dye by calculating the area-weighted intensity average of fluorescent images. Finally, microalgae cells were successfully separated based on lipid content at 41 MHz and DEP media conductivity $106 \pm 1 \mu\text{S/cm}$.

Keywords

dielectrophoresis; lipid content; microalgae; microfluidics; time course

1. Introduction

Dielectrophoresis offers a rapid, noninvasive, label-free method to measure the lipid composition of cells [1]. Polarizability of cells is dependent on the permittivity and conductivity of cellular components, and can provide insight into the cell composition [2, 3]. Briefly, there are two types of DEP motion induced by a non-uniform electric field. A positive DEP (pDEP) occurs when particles move to a region with stronger electric field intensity. Conversely, a negative DEP (nDEP) occurs when particles move to a region with a

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lower electric field intensity [4]. The actual direction of the cell's movement depends on the relative polarizability of the cell and the medium [5]. Previous work has demonstrated the use of DEP at lower frequency based on size [6, 7], viability [8, 9] and cell type [10–13]. Additionally, there have been reports suggesting that the DEP properties of microalgae depend upon lipid content [1, 14–16], which is not surprising as the dielectric properties, namely the permittivity and conductivity, of neutral lipids are significantly different from the aqueous cytoplasm. In particular, Deng et al., [1] showed that by carefully selecting DEP medium conductivity, microalgae could be separated by lipid content.

DEP response can vary based on the properties of cell membranes and/or on the properties of the cytoplasm. In the low frequency regime (10–500 KHz), separation of the cells is often due to shifts in the lower crossover frequency (LCOF) due to changes in membrane properties. This behavior is in agreement with the commonly used single-shell analytical model [4]. This model also predicts that at high frequencies cells should also be able to be separated on the basis of the dielectric properties of the cytoplasm [17, 18]. For the purposes of this work, 'cytoplasm' refers to all parts of the cell contained within, but not including, the cell membrane. This choice of terminology is consistent with the single cell model that lumps the dielectric properties of the cell into membrane, or shell, properties and cytoplasmic, or interior, properties.

Microalgae comprise an attractive model system to test these predictions since the lipid content, and thus the dielectric properties, of their cytoplasm can be altered in a predictable manner. When microalgae are cultured under stressful conditions, e.g., limited nutrients [19–22], suboptimal temperatures [33], or high intensity light [23], they accumulate an energy store of neutral lipids as their growth slows or stops [24]. This lipid content can be estimated by labeling with a fluorescent dye such as BODIPY 505/515 that selectively accumulates in neutral lipids [25–29]. Therefore, it is expected that the cytoplasmic conductivity of the microalgae can be varied in a repeatable fashion by altering culture conditions and inducing lipid accumulation.

The goal of this work is to monitor the dielectrophoretic response at high frequencies (20–80 MHz range) and different DEP media over time. Thus high frequency DEP will be used to explore changes in cytoplasmic properties of microalgae cells in stressed and regular growth conditions. Specifically, this work measures the shift in the upper crossover frequency (UCOF) to determine changes in the DEP response due to lipid accumulation.

2. Theory

The fundamental equations describing DEP are well established and provide a strong foundation for understanding how the DEP response of a cell changes as a function of cell properties [2–4, 18, 30–32]. The DEP force on a spherical particle is given by Equation 1:

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

where F_{DEP} is the DEP force, R is the radius of the particle, $\text{Re}(F_{CM})$ is the real part of the Clausius-Mossotti factor, E_{RMS}^2 is the root mean square of the electric field strength, and ∇

is the gradient. A closer examination of Equation 1 reveals that the positive and negative DEP responses only depend on the Clausius-Mossotti factor as described in Equation 2:

$$F_{CM} = \frac{(\varepsilon_p^* - \varepsilon_m^*)}{(\varepsilon_p^* + 2\varepsilon_m^*)} \quad (2)$$

where ε_p^* and ε_m^* are the complex permittivities of the particle and medium, respectively, and are defined by Equation 3:

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

where j^2 is -1 , 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 since the cell cannot be treated as a homogeneous particle. The most commonly used model is the single-shell model where an effective permittivity can be calculated with Equation 4:

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

where ε_{peff}^* is the effective complex permittivity of the cell, ε_s^* is the complex permittivity of the shell (i.e., membrane), ε_c^* is the complex permittivity of the core (i.e., cell cytoplasm), and δ is the thickness of the shell [33]. In this case, the cell is modeled as a homogeneous sphere surrounded by a thin shell. 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} . There are two mechanisms which are involved in the DEP response: conductive polarization (the physical movement of the free charges, measured by σ) and dielectric polarization (the field-induced disturbance of bound charges, measured by ε). According to the single shell model there can be at most two COFs, a LCOF and an UCOF. At frequencies above the LCOF, the membrane no longer acts as a shield to the imposed electric field as discussed by Gagnon et al., [4, 34]. In cases where the cytoplasm is more conductive than the medium, the cells are more polarizable than the external medium and therefore move toward regions of high field intensity (pDEP). As the frequency further increases, dielectric polarization becomes a more significant effect due to its faster response time. Hence in cases where the medium permittivity is higher than the cytoplasm, this effect is eventually stronger than the conductive polarization and the cells move toward regions of low field intensity (nDEP). The UCOF is the point where these two effects are balanced [4, 34]. Thus, the UCOF explores the dielectric properties of the cell interior [17, 18, 34]. Using a symbolic software package, Broche et al., [18] derived a quantitative estimation of the UCOF which is a function of the cytoplasmic dielectric properties with respect to the medium:

$$UCOF = \frac{1}{2\pi} \sqrt{\frac{\sigma_c^2 - \sigma_c \sigma_m - 2\sigma_m^2}{2\varepsilon_m^2 - \varepsilon_c \varepsilon_m - \varepsilon_c^2}} \quad (5)$$

where σ_c , ε_c and σ_m , ε_m are the real conductivities and permittivities of the cytoplasm and the medium, respectively. This estimate agrees with the previous discussion as it predicts the UCOF does not exist in cases where $\sigma_m > \sigma_{cyto}$ or $\varepsilon_m < \varepsilon_{cyto}$. In these two cases no real root exists, since the radicand is negative. Otherwise, this estimate accurately predicts the upper roots of the F_{CM} curve to within 10% as long as the ε_{cyto} and the σ_m are less than 60 and 50 $\mu\text{S}/\text{cm}$, respectively (analysis provided in supplementary material B). The unlikely case where the numerator and the denominator of the radicand are both negative is not considered here.

3. Materials and Methods

3-1. Sample preparation

Chlamydomonas reinhardtii (Strain 90, UTEX, Austin, TX) was cultured using Minor 70 growth medium [35, 36]. A nitrogen deplete medium was used that consisted of the same formulation except for the substitution of nitrogen-free Ca-Mg stock (2 g/l CaCl_2 , 4 g/l $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) in place of the nitrogen-replete Ca-N-Mg stock solution. The cells were grown at 80°F with continuous air bubbling and an 18/6 hr light/dark cycle. After the cells had grown into a dense culture, they were pelleted and then split into two subcultures, one each using the N^+ -medium and N^- -medium for the first time series study. Each subculture was rinsed three times in their respective media. Conductivities of culture media of both cells were measured every two days with an Ex-tech Exstik II conductivity meter (Nashua, NH) for the three week long experiment. For the second time series study, the N^+ -subculture was prepared using the same procedure, but the conductivity of the culture media was adjusted as follows. Additional N^+ -medium was added to the N^+ -subculture as necessary to maintain the conductivity of the culture medium at a constant level (approximately 1400 $\mu\text{S}/\text{cm}$) over the length of the experiment.

Three different DEP media with conductivities of $10 \pm 2 \mu\text{S}/\text{cm}$, $40 \pm 2 \mu\text{S}/\text{cm}$, and $106 \pm 1 \mu\text{S}/\text{cm}$ were created with potassium chloride in deionized water. Glucose was then added to the three DEP media (85 g/L) to raise the osmotic pressure without affecting its conductivity. Portions (1 mL) of the N^+ - and N^- -subcultures were separately pelleted and then rinsed with a DEP medium three times prior to performing DEP experiments. The cells (1.0 mL) were stained by adding 5 μl of a stock solution containing Bodipy 505/515 (Invitrogen, Grand Island, NY) dissolved in dimethyl sulfoxide at 0.5 mg/ml. Fluorescence was recorded using a Nikon ET-GFP filter (Excitation: 470/40 $\times$, dichromatic mirror: 495, Emission: 525/50m) on a Nikon TE-2000U inverted epifluorescent microscope and with a Nikon DS-Qi1Mc CCD monochrome or Nikon DS-L2 color camera (Melville, NY).

3-2. DEP experiments

The experimental chip was fabricated using a glass slide with a sputtered Cr/Au layer (thickness: 250Å Cr /1000Å Au). The metal layer was patterned into electrodes with

standard photolithographic and wet etching procedures [37, 38]. A needle pattern with 100 μm spacing was used due to the ease of observing positive or negative DEP [5]. Conductive copper tape and alligator clips were used to connect the glass slide to the electronic equipment. Sine waves were generated with a Rigol D64162 signal generator (Oakwood Village, OH). The signal was amplified with either an Ophir 5322 Rev.x1 RF amplifier (Los Angeles, CA) with a Fairview Microwave SA3N511-03 -3dBm attenuator or a Mini-Circuits BNC ZHL-3A+ RF amplifier with a Mini-Circuits BW-N4W5+ +4dBm attenuator. The attenuators were necessary to reduce the reflected power and protect the amplifier output due to the impedance mismatch between the amplifier and chip. This setup was capable of 30V peak-to-peak (Vpp) amplitudes in the given frequency range as monitored by a Rigol 1202 oscilloscope probing the electrodes. The glass slide was placed on the inverted microscope and a camera was used to take pictures of the microalgae (block diagram provided in supplementary material A).

DEP experiments were conducted 3 days per week for up to one month after splitting the culture into N^+ - and N^- - media. Cells were placed on the slide and observed for a period of 3 min for frequencies ranging from 20 to 80 MHz in 5 MHz increments. Finer frequency increments were used in the neighborhood of the UCOF. Due to non-linearities in the electronic setup, the amplitude was adjusted at each individual frequency to maintain a 30 ± 1 Vpp signal across all frequencies. After the 3 min observation, the cells were removed from the slide and replaced with fresh cells to minimize evaporative effects on the small drop of DEP media. This process was repeated until the algae movement had been observed over the entire range of frequencies. Pictures were taken at 1 min intervals with a 40 $\times$ objective. To examine the consistency of the DEP responses, DEP experiments were performed on the same sample of microalgae cells kept in DEP medium for 6–8 hr. The dielectrophoretic response was the same at the beginning and end of this time, indicating that dielectric properties of the cells inside DEP medium do not change within the timeframe of the experiment.

3-3. Image analyses

Lipid contents of algae were estimated by the intensity of BODIPY 505/515 fluorescence as measured on monochrome images taken by a linear CCD grayscale camera (Nikon DS-Qi1Mc) with a Nikon ET-GFP filter. Captured images were analyzed by MATLAB R2011B and Nikon NIS-Element BR software. The goal of the MATLAB image processing was to extract the bright lipid spots from the image background and cell chlorophyll while controlling for varying cell concentrations. To do this, bright regions were separated from the background by defining a lower threshold value, and then the intensity values of the resultant bright pixels were summed and normalized by the number of bright pixels. This calculation gave the area-weighted intensity average for all of the pixels brighter than the lower threshold—referred to as the intensity average in this work.

RGB images were taken using a color camera (Nikon DS-Fi1) and a Nikon UV-2A filter (Excitation: 330–380, dichromatic mirror: 400, Emission: 420 nm cut-on) to define the binary layers and to quantify the separation of high-lipid and low-lipid microalgae cells. Because of the nonlinearities in the color camera response function, binary threshold was

used for RGB images. The low-lipid cells do not absorb the dye and the chlorophyll fluoresces red. The lipid bodies within the high-lipid cells do absorb the dye and the resulting green fluorescence is significantly brighter than the fluorescence from the chlorophyll. As low- and high-lipid cells are red and green, respectively, with the UV filter, a binarized image was defined to segment cells from the background using the red (Figure 4a2, b2 and c2) and green (Figure 4a3, b3 and c3) channels. Total area of high- and low-lipid microalgae cells was measured at defined binaries within the region of interest (ROI) chosen by measuring the maximum separation distance of N^- -subculture from the electrode at the separation frequency. The same ROI was used for all separation image analyses to quantify the area of the red and green channels consistently. The separation fraction was calculated with the ratio of the area of low-lipid (red) microalgae cells to the area of high-lipid (green) microalgae cells within the ROI.

4. Results and Discussion

4-1. Effect of lipid content on UCOF

Microalgal cells grown in N^- -medium exhibited an increase in the intensity average (Figure 1a) due to increasing fluorescence levels that correlate with increasing lipid concentrations. Over the same time period, the UCOF of the N^- -cells decreased (Figure 1b). In contrast, the N^+ -cells in the control culture maintained a relatively constant average intensity. The UCOF of the N^+ -cells also shifted to lower frequencies over time, but to a lesser extent than the N^- -cells. Since the UCOF is primarily a function of the dielectric properties of the cytoplasm [17], it can be inferred that the cytoplasm of the cells cultured in both the N^+ - and N^- -media changed during the cultivation period. The *C. reinhardtii* cells cultured in N^- -medium were observed to have an UCOF approximately 13 MHz lower than N^+ -cells at the end of the experiment. The conductivity of the suspending medium for these DEP experiments was $106 \pm 1 \mu\text{S/cm}$. The increase in the fluorescent intensity indicated that the *C. reinhardtii* cells accumulated non-polar lipids during nitrogen starvation, which is in keeping with previous reports [39–41]. Bright lipid bodies stained by the BODIPY dye were clearly observed after 3 d in the N^- -medium and quantitative measurements agreed with the qualitative observations (data not shown). The reduction in the UCOF of the N^- -cells can be explained by the increased lipid content. The oily nature of the non-polar lipids—as confirmed by the BODIPY staining [27]—would decrease the effective conductivity of the cells' cytoplasm and therefore decrease the UCOF [15]. As was previously discussed in the Theory section, the frequency dependence of the UCOF can be physically explained by polarization mechanics [4] and agrees with Equation 5. In this case, the cells' cytoplasm [16] is still more conductive (on the order of S/m) than the DEP medium (on the order of $\mu\text{S/cm}$), and the DEP response above the UCOF is driven by dielectric polarization mechanics.

As mentioned, the N^+ -cells did not fluoresce when stained with BODIPY, indicating negligible lipid accumulation (Figure 1a). Therefore, another mechanism must be responsible for the reduction in UCOF of the N^+ -cells. One hypothesis is that the properties of the culture medium changed during the cultivation of the N^+ -cells, thus changing the dielectric properties of the cytoplasm [17, 42]. There are two noteworthy observations to be made from examining the conductivity of the culture media over time (Figure 1c). First,

there is a significant difference in the conductivity of the N^+ -medium and the N^- -medium, which can be accounted for by the nitrogen compounds. Second, the conductivity of the culture medium of N^+ -cells decreases linearly over time while it remains constant for N^- -cells. The reduction in the conductivity of the culture medium in the N^+ -culture can be explained by the consumption of nutrients by the growing cells [25]. This observation provides a possible explanation for the decreasing UCOF in the N^+ -culture in the absence of measureable lipid accumulation. It seems that UCOF is influenced by lipid levels in the N^- -culture and by culture medium (indicated by EC) in the N^+ -culture. To investigate this effect, the conductivity of the culture medium was also maintained over time as discussed in the following section.

4-2. Effect of culture media conductivity on UCOF

The electrical conductivity of the culture medium changes during cultivation of microalgae and has been used as a growth parameter [25, 26]. To evaluate the effect of culture conductivity on the UCOF, N^+ -medium was periodically added to a new flask of N^+ -cells to maintain the conductivity of the growing culture approximately constant during the experimental period (Figure 2a). Maintaining the conductivity of the growth medium kept the UCOF response of N^+ -culture relatively constant (Figure 2a). Furthermore, there was no accumulation of lipids in the N^+ -culture, and likewise no shift in the UCOF was observed (Figure 2b). However, there is an initial shift in the UCOF and intensity average that can be explained by replacement of the old medium with fresh N^+ -medium two month after the original inoculation. The results from the N^+ -cultures indicate that lipid accumulation is not the only determining factor and that the culture conditions may also impact the UCOF.

4-3. Effect of DEP media conductivity on UCOF

One of the primary applications of DEP is the label-free separation of bio-particles and cells [6, 9, 24, 43–50]. As discussed, the UCOF shift of *C. reinhardtii* during lipid accumulation can be used as a basis of separation. Dielectrophoretic response depends on the relative dielectric properties of the cells with respect to the external DEP medium [4, 5], which should be optimized to achieve efficient separation. The effect of DEP media conductivity on the high frequency response of N^- - and N^+ -cells is given in Figure 3. The UCOFs of high- and low-lipid microalgae cells increase as the DEP media conductivity increases, which agrees with Deng. et al., [1]. The shift in UCOF of N^- - and N^+ -cells at the end of the experiments is 5 MHz for DEP media with conductivity $10 \pm 2 \mu\text{s/cm}$ (Figure 3a), 5 MHz for DEP media with conductivity $40 \pm 2 \mu\text{s/cm}$ (Figure 3b), and 13 MHz for DEP media with conductivity $106 \pm 1 \mu\text{s/cm}$ (Figure 3c). Therefore $106 \pm 1 \mu\text{s/cm}$ was chosen as the most efficient DEP media conductivity, of those tested, for separation purposes.

4-4. Quantifying separation of microalgae cell mixtures

Results showed that lipid content induced a shift in the UCOF of N^- -cells. Consequently, lipid-enriched microalgae cells can be separated from low-lipid cells via high frequency DEP. To quantify the level of microalgae differentiation based on lipid content, separation efficiency fractions of microalgae mixtures at different frequencies were examined using NIS-Element Image analyzer software. From the time course experiments, there is a 13 MHz

difference between the UCOF of N^+ -cells and N^- -cells when the DEP media conductivity is $106 \pm 1 \mu\text{S/cm}$; this shift is due to the lipid accumulation within the N^- -cells. To demonstrate the ability to separate microalgal cells on the basis of lipid content, portions of the N^+ - and N^- -cultures were mixed together in DEP medium and the high frequency dielectrophoresis response was observed. As expected, at frequencies lower than the UCOF for both cell types, all of the cells experienced pDEP (Figure 4a). Likewise, at frequencies above both UCOFs all of the cells experienced nDEP (Figure 4c). At intermediate frequencies (i.e., frequencies above the N^- -cells crossover and below the N^+ -cells crossover) the N^+ - and N^- -cells could be separated into regions near and far from the electrode, respectively (Figure 4b). To quantify the efficiency of the separation, a ROI was defined around the electrode. Using UV excitation, high- and low-lipid cells were distinguished with BODIPY staining [51]. Image analysis was used to quantify the amount of each type of cell in the ROI (Section 3-3) and the ratio of low- to high-lipid cells was used as a figure of merit for the separation efficiency. The separation efficiency exhibited a peak at 41 MHz (Figure 4).

It is important to note that this separation is due to a fundamentally different mechanism than that showed by Deng, et al., [1]. Based on their discussion, it can be inferred that a DEP medium was found with conductivity (2.06–2.95 mS/cm) in between that of the cytoplasmic conductivity of the high- and low-lipid cells. Thus the nDEP response of their high-lipid cells was because no COF existed (UCOF or LCOF). This approach was taken because the UCOF was beyond their operating range. In contrast, in this report the medium conductivity is lower than that of the cytoplasmic conductivity of the high-lipid cells and the separation is due to a shift in the UCOF. This is made possible by the high frequency capabilities of the experimental set-up reported here.

According to theory (Section 2), reducing either of effective conductivity or permittivity of the cytoplasm could be responsible for reducing UCOF. If the cytoplasmic permittivity of the cells is assumed to be constant (50 [16]), then the cytoplasmic conductivity for the high and low lipid cells shown in Table 1 can be calculated to be 0.095 and 0.2267 S/m, respectively. Conversely if the cytoplasmic conductivity of the cells is assumed to be constant, then no significant change in the cytoplasmic permittivity can account for the entire shift in UCOF (details provided in Supplementary material C). Therefore, these experimental results, coupled with the analytical model, support our hypothesis that lipid accumulation within an algal cell shifts UCOF to lower frequencies by reducing the effective cytoplasmic conductivity of the cell.

5. Conclusions

The high frequency DEP response of microalgae cells grown in N^+ - and N^- -media was measured. It was demonstrated that the UCOF, which is sensitive to changes in the dielectric properties of the cytoplasm, reduced over time indicating change in the cytoplasm of the both low- and high-lipid cells. Time course intensity average of the cells, which is a correlation of the lipid content, increased for the cells grown under stressful conditions (N^- -medium) while it remained nearly constant for the normal growth conditions (N^+ -medium). However, the electrical conductivity of the N^+ -medium decreased as a result of nutrition consumption by the N^+ -cells. Since dielectrophoretic responses are measured in relation to

the DEP medium, the optimum DEP medium conductivity was selected according to the largest shift in the UCOF over the experiment period. The experimental results demonstrate that although lipid content affects the UCOF, it is not the only determining factor and other potential mechanisms that affect the cytoplasm can also have an impact. Despite this, it was found that microalgae cells can be efficiently differentiated based on lipid content using high frequency DEP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

UCOF	upper crossover frequency
LCOF	lower crossover frequency
pDEP	positive dielectrophoresis
nDEP	negative dielectrophoresis
Vpp	Volts peak-to-peak
EC	Electrical Conductivity
N⁺-Medium	nitrogen replete medium
N⁻-Medium	nitrogen deplete medium
N⁺-cells	cells grown in nitrogen replete medium
N⁻-cells	cells grown in nitrogen deplete medium

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Figure 1. Intensity average, UCOF, and culture media conductivity of N^+ - and N^- -cells versus time: (a) Intensity average of $\blacktriangle$ N^+ -cells and $\triangle$ N^- -cells; (b) UCOF of $\bullet$ N^+ -cells and $\circ$ N^- -cells; (c) Culture media conductivity of $\blacksquare$ N^+ -cells and $\square$ N^- -cells (Moving average trendlines are provided as a visual aid).

Figure 2.
UCOF, culture media conductivity, and intensity average versus time for microalgae cells after conductivity adjustments: (a) ■ Culture media conductivity and ● UCOF of N^+ -cells; (b) ▲ Intensity average and ● UCOF of N^+ -cells.

Figure 3.

Effect of DEP media conductivity on UCOF: (a) UCOF of $\blacktriangle$ N^+ -cells and $\triangle$ N^- -cells at $10 \pm 2 \mu\text{S/cm}$ DEP media conductivity; (b) UCOF of $\blacksquare$ N^+ -cells and $\square$ N^- -cells at $40 \pm 2 \mu\text{S/cm}$ DEP media conductivity; (c) UCOF of $\bullet$ N^+ -cells and $\circ$ N^- -cells at $106 \pm 1 \mu\text{S/cm}$ DEP media conductivity (Moving average trendlines are provided as a visual aid).

Figure 4. Separation analysis of high- and low-lipid microalgae cells via Dielectrophoresis: (a1, b1, c1) Original Images taken under UV illumination (Blue channel is filtered), (a,b,c 2–3) Binary layers using threshold for calculation of area of N^+ - and N^- -cells using image analysis at ROI, (d) Fraction of the area of the N^+ - and N^- - microalgae cells at defined binary layers versus frequency.

Table 1

Summary of the values used in conductivity analysis

	UCOF(MHz) ^a	$\epsilon_{\text{cytoplasm}}$ to be fixed ^b	σ_{media} (S/m) ^d	ϵ_{media} ^b	Calculated σ_{media} (S/m) ^c
High lipid cells	20	50	0.0106	80	0.095
Low lipid Cells	50	50	0.0106	80	0.2267

^a Taken from experimental results
^b Taken from Muller et al., [16]
^c Calculated from Broche equation [18]