## **Appendix A: Further notes on AC-DEP**

The net dielectric force,  $\vec{F}_{DEP}$  resulting from transient polarization of cells<sup>12</sup> and the electric field<sup>147</sup> is given by

$$\vec{F}_{DEP} = 2\pi r^3 \epsilon_m \alpha \nabla \vec{E^2},\tag{1}$$

where the particle radius is 'r', ' $\varepsilon_m$ ' is the medium permittivity, ' $\vec{E}$ ' is the applied electric field, and ' $\alpha$ ' is the real part of the Clausius-Mossotti factor, which is the effective polarizability of the particle relative to the suspending medium and is frequency dependent.

$$\alpha = Re[K(\omega)] \tag{2}$$

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}$$
(3)

$$\varepsilon^* = \varepsilon - \left(\frac{i\sigma}{\omega}\right) \tag{4}$$

where,  $\varepsilon^*$  denotes complex permittivity and the subscript 'p' refers to a lossless dielectric sphere particle suspended in a medium 'm'. The complex permittivity  $\varepsilon^*$  is given by eqn. 4, which is a function of permittivity,  $\varepsilon$ , medium electrical conductivity,  $\sigma$ , and the angular frequency,  $\omega^{12, 147}$ .

The transient polarization of particles results in their movement in the electric field that scales between two extremes depending on the exciting AC frequency. Herbert Pohl, in his seminal text "*Dielectrophoresis: The behavior of neutral matter in nonuniform electric fields*" defined these two phenomenological extremes as positive dielectrophoresis and negative dielectrophoresis<sup>12</sup>. These two cases arise because of the polarizability of a uniform composition particle being greater or lesser than the polarizability of the medium in which it is suspended. If the real part of the effective polarizability, Re[ $\alpha$ ] of the particle is greater than that of the medium, then the electric field lines pass through the particle causing a polarization, which is slightly skewed due to the spatially varying electric field lines. A resultant force directs the particle to high field density regions and this observed movement is known as 'positive dielectrophoresis' (pDEP). If the effective polarizability, Re[ $\alpha$ ] of the particle is less than that of the medium in which it is suspended, spatially non-uniform electric field lines divert around the outside of the particle causing ion depletion at the particle poles and subsequent polarization. The resulting force directs the particle to the low field density regions and this is termed 'negative dielectrophoresis' (nDEP) <sup>12, 57</sup>.

## **Appendix B: Further notes on DC-DEP**

The observed cell motion in iDEP devices depends on two forces: electrokinetics (EK) and dielectrophoresis (DEP).

$$j \propto u_{EK} + u_{DEP} \tag{5}$$

where  $\vec{J}$  is the particle flux,  $\vec{u}_{EK}$  the electrokinetic velocity (expressed as the sum of electrophoretic  $\vec{u}_{EP}$  and electro-osmotic  $\vec{u}_{EO}$  velocities) and  $\vec{u}_{DEP}$  the dielectrophoretic velocity of the particle. Electrokinetic velocity can be expressed as the sum of electro-osmotic and electrophoretic mobilities:

$$\vec{u}_{EK} = \mu_{EK}\vec{E} = (\mu_{EP} + \mu_{EO})\vec{E}$$
(6)

where  $\mu_{EK}$  is the electrokinetic mobility,  $\mu_{EP}$  electrophoretic mobility,  $\mu_{EO}$  electro-osmotic mobility and  $\vec{E}$  applied electric field to create non-uniformities in the channel. Neglecting the frequency component for strict DC-iDEP, the CM factor in eqn. 3 is modified to:

$$\alpha = \frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m} \tag{7}$$

where  $\sigma_p$  is the conductivity of the particle,  $\sigma_m$  the conductivity of the medium. This simplification is substituted into eqn. 1 yielding<sup>38, 148</sup>

$$\vec{F}_{DEP} = \frac{1}{2} V \frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m} \varepsilon_m \nabla \vec{E}^2$$
(8)

where V the volume of the particle,  $\varepsilon_m$  permittivity of the medium, and  $\vec{E}$  the magnitude of the applied DC electric field.

From eqn. 7, if the conductivity of the particle is greater than the medium, the CM factor gives positive values and the dielectrophoretic force on the particle pushes the particle towards high field density regions thus trapping them i.e. the particle gets attracted towards insulating obstacle region whereas, if the conductivity of the particle is less than that of the medium, the particles are repelled from the high field density regions thus yielding in negative values of CM factor and movement of particles in the fluid streamlines i.e. particles are repelled from the insulating obstacle regions. The conductivity of the particle ( $\sigma_p$ ) is given as a function of surface conductivity and bulk conductivity<sup>149</sup>:

$$\sigma_p = \sigma_b + \frac{2K_s}{r} \tag{9}$$

where  $\sigma_b$ , the bulk conductivity,  $K_s$  is the surface conductance and 'r' the radius of the particle.

Due to the electrodes placed in the large reservoirs at the channel inlet and outlet ports, they often cause re-dilution of the concentrated samples with some Joule heating and bubble formation<sup>45</sup>. To mitigate these effects, a simple, robust device was designed where in the electrodes are not in direct contact with the sample. This technique is referred to as contactless dielectrophoresis (cDEP) wherein the electric field is generated by placing the electrodes in two conductive microchambers separated by thin insulating barriers from the main channel<sup>20</sup>. cDEP is particularly well-suited for manipulating sensitive biological particles<sup>46</sup>.

## Appendix C: Further notes on shell models

Electric polarization is an important factor in DEP operations and has been found to depend inversely on the frequency of the applied field. It is described as a function of dielectric properties of the cell and the suspending medium as given by eqn. 10<sup>51, 55, 56</sup>.

$$\varepsilon_{mix}^{*} = \varepsilon_{m}^{*} \frac{\left(2\varepsilon_{m}^{*} + \varepsilon_{cell}^{*}\right) - 2p(\varepsilon_{m}^{*} - \varepsilon_{cell}^{*})}{\left(2\varepsilon_{m}^{*} + \varepsilon_{cell}^{*}\right) + p(\varepsilon_{m}^{*} - \varepsilon_{cell}^{*})}$$
(10)

where *p* is the cell's volume fraction, and  $\varepsilon^*$  is the complex dielectric permittivity defined by eqn. 4. The indices *mix*, *cell*, and *m* refer to the whole mixture, cell, and the suspending medium. Eqn. 10 holds good until the particle is not perturbed by the neighboring particles<sup>55</sup>

For single-shell model, the dielectric constant  $\varepsilon_p^*$  can be obtained by <sup>51, 58</sup>,

$$\varepsilon_p^* = \varepsilon_{mem}^* \frac{(1+2V_m)\varepsilon_{cp}^* + 2(1-V_m)\varepsilon_{mem}^*}{(1-V_m)\varepsilon_{cp}^* + (2+V_m)\varepsilon_{mem}^*}$$
(11)

where  $V_m = (1 - d_{mem}/R)^3$ , R the outer cell radius and  $d_{mem}$  the shell thickness. This equation allows calculating frequency dependency on conductivity and permittivity of cell from its phase parameters<sup>58</sup>.

In double-shell model, the effective complex permittivity of the whole cell is expressed as

$$\varepsilon_p^* = \varepsilon_{mem}^* \frac{2(1-V_1) + (1+2V_1)E_1}{(2+V_1) + (1-V_1)E_1}$$
(12)

where  $V_1 = (1 - d_{mem}/R)^3$ , R the outer cell radius and  $d_{mem}$  is the plasma membrane thickness and E<sub>1</sub> is given by

$$E_{1} = \frac{\varepsilon_{cp}^{*} 2(1 - V_{2}) + (1 + 2V_{2})E_{2}}{\varepsilon_{mem}^{*} (2 + V_{2}) + (1 - V_{2})E_{2}}$$
(13)

where  $V_2 = (R_n/(R - d_{mem}))^3$ , R<sub>n</sub> the outer radius of nucleus, indices *cp* and *mem* refers to cytoplasm and cell membrane respectively, and E<sub>3</sub> is given by

$$E_{2} = \frac{\varepsilon_{ne}^{*} 2(1 - V_{3}) + (1 + 2V_{3})E_{3}}{\varepsilon_{cp}^{*} (2 + V_{3}) + (1 - V_{3})E_{3}}$$
(14)

where  $V_3 = (1 - d_{ne}/R_n)^3$ ,  $d_{ne}$  is the nuclear envelope thickness, index *ne* refers to nuclear envelope, and  $E_3 = \frac{\varepsilon_{np}^*}{\varepsilon_{ne}^*}$  the ratio of complex permittivities of nucleoplasm and nuclear envelope<sup>51, 55</sup>.

In an ellipsoid cells with a major axis  $a_L$  and minor axis  $a_1$ , the Clausius-Mossotti factor is given bv<sup>62, 150</sup>: \* \*

$$f_{CM,i} = \frac{\varepsilon_p^* - \varepsilon_m^*}{3[\varepsilon_m^* + (\varepsilon_p^* - \varepsilon_m^*)L_i]}$$
(15)

where  $\varepsilon_p^{\tilde{r}}$  is the complex permittivity of the particle and L<sub>i</sub> the depolarization factor.  $L_i$  and  $\varepsilon_p^*$  are further given as,

$$\varepsilon_p^* = \frac{\varepsilon_{mem}\varepsilon_{cp}}{d_m / R^{\varepsilon_{mem}^*}\varepsilon_{cp}^*}$$
(16)

$$L_{i} = \frac{a_{1}a_{L}^{2}}{2} \int_{0}^{\infty} \frac{1}{(l+R^{2})(\sqrt{(l+a_{L}^{2})(l+a_{1}^{2})^{2}}} dl$$
(17)

where l is the integration variable and R represents either major axis  $a_L$  or minor axis,  $a_1$ .