The motivation for conducting this analysis came from email exchanges with postgraduates commencing projects involving the electrokinetic manipulation of particles, as well as from discussions with business people and venture capitalists. Apart from seeking advice on specific technical issues, opinions were sought by postgrads regarding the ‘hottest’ topics for investigation and the potential for long-term careers in the subject. For the more commercially minded with inbuilt risk aversion, it was appropriate to explain that dielectrophoresis (DEP) was the term coined by Herbert Pohl as far back as 1951 to describe the translational motion of an electrically polarized particle in a non-uniform electric field. Such interested parties also required assurances that the maturity of the technology presents low risk of failure to meet technical objectives. This can be approached by explaining that the phenomenon has been exploited since 1924 (for the separation of minerals), with a theoretical basis to be found in early 19th century text books. Furthermore, various aspects of the subject are treated in textbooks and review articles that take us to the present status of DEP technology. Responses to these intended reassurances are invariably of the form: “If DEP has been around for so long and is considered such an innovative technology, why hasn’t it received significant commercial exploitation?” Although this assessment may be uncomfortable to receive, the so-called Golden Rule (i.e., he who has the gold, rules) should be acknowledged.

At least six commercial products incorporating DEP can be cited. For example, the Panasonic bacteria counter determines the concentration of bacteria through measurement of the change of electrical properties of an induced dipole moment (i.e., the particle assumes the shape of an induced electric dipole in an electric field), through which the particle acquires the electrical polarization of a particular field 

\[
\mathbf{m} = [CM]E
\]

Step 1: This corresponds to the electrical polarization of a particle in an electrical field \(E\), through which the particle assumes the properties of an induced dipole moment \(m\) as depicted in Figure 1a. The magnitude and polarity of \(m\) depends on the polarizability, per unit volume, of the particle. This is usually expressed as the Clausius-Mossotti factor \([CM]\), so that:

\[
\mathbf{m} \propto [CM]E
\]

For spherical particles, values for \([CM]\) lie in the range 1.0 to 0.5. Positive or negative \([CM]\) values correspond to the polarizability of the particle being greater or less, respectively, than that of the surrounding medium. If the dielectric properties of the particle exactly match those of the medium, then \([CM] = 0\). Direct evidence of the electrical polarization of particles in an electric field can be observed, as depicted in Fig. 1b, when the induced moments of neighboring particles electrostatically attract each other to form...
chains of aligned particles, known as “pearl chains”. An example of this is shown in Fig. 2 for the case of polarized mammalian cells.

Step 2: A particle exposed to an electric field gradient experiences a translational force. This is the DEP force $F_{DEP}$, of magnitude and polarity given by

$$F_{DEP} = (m \cdot \nabla)E$$  \[2\]

The symbol $\nabla$ denotes the gradient operator used in vector calculus. The DEP force is proportional to the volume of the particle, so that from 1 and 2 and the simple case of a spherical particle of radius $R$,

$$F_{DEP} = 4\pi R^3/3 [CM] (E \cdot \nabla)E$$  \[3\]

The actions of the two steps described above are shown schematically in Fig. 3 for the case of a particle placed in the field generated by a pin electrode (the grounded microscope stage acted as the counter electrode). Although it may be visible by eye through a microscope, if the particle’s dielectric properties match that of the medium it will be “invisible” from the field’s perspective. The field pattern generated by the electrode, as depicted in Fig. 3a, is not distorted, and the particle is not polarized. For the case of a particle that is more polarizable than the surrounding medium (i.e., $[CM] > 0$) the electric field is drawn into the particle as shown in Fig. 3b. The induced dipole moment is aligned with the field direction. In this situation, work is required to be performed on the particle to withdraw it from the vicinity of the electrode. This is equivalent to saying that the particle has a negative electrostatic energy, which will become more negative with the increase of intensity of the local field $E$. The particle will seek to minimize its electrostatic potential energy and it can achieve this by moving up the field gradient toward the electrode tip. This describes the action of positive DEP. On the other hand, if the particle is less polarizable than the medium (i.e., $[CM] < 0$) the electric field skirts around the particle as shown in Fig. 3c and the induced dipole moment is aligned against the field direction. Work will now be required on the particle to insert it into the medium near the electrode. The particle will have a positive electrostatic energy, and it will move down a field gradient to minimize this potential energy by seeking a field gradient minimum well away from the high field intensity at the electrode tip. This describes negative DEP.

For an electrostatic or quasi-static applied field, it is usually the case that $E$ is irrotational, so that $(E \cdot \nabla)E = 1/2 \nabla E^2$ and from 3 the DEP force is given by the relationship

$$F_{DEP} = 2\pi R^3/3 [CM] \nabla E^2$$  \[4\]

Eq. 4 informs us that both direct (dc) and alternating (ac) voltage signals may be applied to the electrodes. The square-law dependency also indicates that for fixed electrode geometry the magnitude of the DEP force can be quadrupled, for example, by doubling the rms value.
of the applied voltage. An example of the great benefits that arise from the ability to use ac voltage signals is shown in Figs. 4a–4c for the case of a viable mammalian cell subjected to the ac field generated by applying a 10 kHz voltage signal to a pin electrode. In this situation the effective polarizability of the cell is dominated by its plasma membrane, which should exhibit a very high electrical resistance to passive ion flow across it. The $[CM]$ factor in Eqs. 1 and 3 has a negative value. The induced moment of the cell is oriented in the manner shown in Fig. 3c and will be repelled by negative DEP from the high field intensity at the electrode tip. On increasing the frequency of the applied voltage to 1 MHz or higher, the electrical capacitance of the plasma membrane now offers low impedance to passive ion flow. Students who have studied electrical circuit theory will understand this in terms of the high resistance presented by the membrane being electrically short-circuited by a capacitive reactance in parallel with it. The external field penetrates the resistive outer membrane and enters the cytoplasm. In DEP experiments cells are commonly suspended in a medium of conductivity lower than that of their cytoplasm, so that at high electrical frequencies the cells appear as conductive particles and have positive values of $[CM]$. They exhibit positive DEP. This is shown in the images of Figs. 4d–4f when the voltage signal applied to the pin electrode is switched from 10 kHz to 10 MHz.

The effective electrical capacitance and conductance of a cell depend on such features as its size and shape; cell surface topography associated with blebbing or microvilli; the integrity of the cytoplasmic membrane; internal features such as cytoplasm conductivity, and the nucleus-cytoplasm volume ratio. All of these features contribute to the frequency-dependent DEP response of a particular cell type, and can be exploited to selectively manipulate and sort cells according to their viability, their stage of cell cycle, phenotype, differentiation and ability to sustain exposure to toxins and stress, for example. The most common methods currently used to quantitatively characterize or purify cell populations are flow cytometry (FACS) or magnetic bead-coupled cell separation (MACS). These methods depend on the existence of specific cell surface antigens and the formulation or availability of high affinity probes to these antigens. Dielectrophoresis does not require the use of fluorescent or magnetic labels – although using antibody-coated dielectric beads can be used to increase the yield or purity of target cell separation.

Eq. 4 indicates that DEP is a ponderomotive effect – large particles of the same type should (with all other factors remaining fixed) experience a larger DEP force than those of smaller volume. Under the same conditions the DEP force acting on a latex bead of diameter 10 nm, for example, might therefore be expected to be $10^{-3}$-times smaller than one of 10 μm diameter. In this case the DEP force acting on a nanoparticle may not be able to compete against randomizing influences such as Brownian motion. However, as particle size decreases the influence of surface electrical properties can dominate DEP behavior. The effective conductivity $\sigma_p$ of a particle can be expressed as

$$\sigma_p = \sigma_b + K_s/(2R) \tag{5}$$
where $\sigma_b$ is the bulk conductivity and $K_s$ the surface conductance of the particle. For inanimate particles the value of $K_s$ is typically around 2–5 nS and can be dominated by the mobility of counter-ions in its surrounding electrical double-layer. As the radius $R$ decreases, the term $K_s/(2\mu)$ can be dominant in Eq. 5. For example, 10 μm diameter latex beads usually exhibit negative DEP because of their very low bulk conductivity, whereas 10 nm latex beads typically exhibit positive DEP due to the influence of their surface conductance. This effect can be exploited in the design of DEP-based sensors, as for example in the detection and/or collection of antibody-labelled nanoparticles that have captured their target antigen. The influence of counterions associated with electric double layers in increasing the effective polarizability of nanobioparticles was first observed by Washizu et al., who found that protein molecules could be manipulated by DEP at much smaller field strengths than predicted by Eq. 4. This heralded the concept of molecular dielectrophoresis, as well reviewed by Nakano and Ros.

Dielectrophoresis: Where has it Been?

It is helpful, when looking ahead and postulating where DEP might be heading, to examine its record of scientific publications. The yearly totals since 2000 reveal the trend shown in Fig. 5 (not counting conference abstracts or patent publications). After a steadily increasing number of publications over the period 2000–2008, the number of papers has settled down to around 280 per year. Using categories cited by the Web of Science Core Collection, between January 2012 and October 2016 around 76% of the total papers are concerned with the two broad subjects of nanotechnology and analytical chemistry/biochemistry. Electronic engineering and interdisciplinary materials science together contribute the remaining 34% of the peer reviewed papers published in this time period. The subject matter trends since 2000 are shown in Figs. 6 and 7. These statistics suggest that contributions from electronic engineers and materials scientists have maintained their influence, with their work focusing more on developing DEP-based chemical and biochemical analyses that involve nanoparticles.

A good example of DEP operating at the nanoscale is the manipulation and patterning of protein molecules. Contributions to this are shown in Table I, listed chronologically rather than in order of novelty or impact. Nearly a decade spans the time between the demonstration in 1994 by Washizu et al. of their insulator-based DEP device for concentrating proteins by positive DEP, and the use of metal microelectrodes to study the conductivity-dependence of the DEP cross-over of avidin molecules and the collection of actin filaments. From 2008 the field of molecular DEP really takes off, with only some of this activity being encapsulated in Table I. Nakano and Ros fill in other missing gaps and also describe the characterization, separation and focusing of DNA molecules. A striking feature of all this work is the wide range of DEP devices and their modes of operation, aspects of which are summarized in the notes column of Table I. A significant development aiding advances in DEP has been the so-called microfabrication explosion described by Hughes in his article to mark the 50th anniversary of the demonstration by Pohl and Hawk of DEP being used to simultaneously distinguish and separate live from dead cells. The term explosion refers to the increase in DEP publications following the transition from using macroscopic electrode systems (e.g., wires, pins, razor blade edges) to microfabricated ones. To produce the required large values of $(\mathbf{E} \cdot \nabla) \mathbf{E}$ in 3, voltages of the order 1 kVpk or larger were required to be applied to macroelectrodes. The electronics and electromagnetic screening required when generating ac voltages of this magnitude, especially for frequencies above 100 kHz, were cumbersome and expensive. The initial purpose in exploring the use of microelectrodes was driven by the factor $(\mathbf{E} \cdot \nabla) \mathbf{E}$ having dimensions of $(\text{Volt})^2/\text{meter}$. A constant value for $(\mathbf{E} \cdot \nabla) \mathbf{E}$ in 3 can thus be maintained on combining, for example, a 100-fold reduction of the applied voltage with a reduction by a factor of 1000 of the scale of the electrode geometry. This greatly reduced the complexity and cost of the electronics, and reduced experimental problems such as electrolysis and joule heating. Between 1987 and 1991, three groups working independently of each other reported DEP investigations that employed metal microelectrodes fabricated by photolithography. Electrode geometries and configurations were no longer restricted to wires, pins and plates – arbitrary planar shapes of sub-micron definition could be fabricated. For a fixed value of an applied voltage, the value of $(\mathbf{E} \cdot \nabla) \mathbf{E}$ is determined by the electrode geometry. Two examples of the spatial distribution of $(\mathbf{E} \cdot \nabla) \mathbf{E}$ contours generated by two simple forms of electrodes are shown in Fig. 8. A value for $(\mathbf{E} \cdot \nabla) \mathbf{E}$ of $10^{15} \text{ Volt/m}^2$...
Dielectrophoresis: Where is it Going?

Over the past years 20 years or so, DEP devices using planar metal microelectrodes have dominated the subject.9,17,42 In papers describing electrodeless, insulator-based or liquid-electrode based devices, some authors justify their departure from this status quo by stating that metal-based devices have disadvantages related to their cost and complication of fabrication. This is not always a valid justification. For example, the DEP cell manipulator24 and DEP tweezer45 are very simple and inexpensive to construct, and continuous separation of multiple sized particles by DEP has recently been described using two acupuncture needle electrodes embedded in a PDMS ‘hurdle’.43 Another negative aspect often cited for planar metal electrode arrays is that, without extending their geometry to three-dimensions, they limit volumetric throughput. Although this is not perceived as a major limitation for the particular case of cancer cell separators described by Gupta et al.44 and Shim et al.,55 sample throughput can certainly be increased by employing 3D electrodes. An excellent example of where this can be investigated further is given by Wang et al.46 in their development of sheath flow assisted particle separation with a tailored arrangement of cylindrical interdigitated metal electrodes. Other examples of creating 3D electrode structures for DEP is through electroplating,56 by the carbonization of SU-8 photoresist pillars,57 and by high-aspect-ratio ring structures etched in doped silicon.49

In this author’s opinion the major restriction of metal electrodes is that problems can occur at frequencies below ∼5 kHz, associated with electrochemical generation of toxic species, electrolysis effects that lead to the generation of gas bubbles, as well as electro-osmotic induced fluid motion. Also, unless the particle suspension medium has a relatively low conductivity (e.g., <30 mS/m) electrode polarization effects can extend beyond 5 kHz. These various problems can be avoided by shielding metal electrodes from the fluid medium in the main DEP chamber. An example of this includes the liquid-electrode-based DEP device described by Demierre et al.,50 where the term ‘liquid electrode’ refers to the equipotential surfaces located at the junctions of the access and main fluidic channels. Henslee et al.51 describe a device where the fluid electrode channels, containing a conductive solution, are isolated from the sample channel by thin insulating membranes. The geometry of the fluid electrode channels as well as the sample channel, which incorporates insulating barriers, creates the field non-uniformities necessary for DEP. Investigations at frequencies between 120 and 320 kHz and voltages between 20 and 50 Vrms showed that cultured cancer cells could be isolated from a heterogeneous mixture of cells.51 This approach has not been fully explored, and could be beneficial for some niche applications of DEP. An advantage for laboratories without access to photolithography is that liquid-electrode (or contactless) DEP devices can be fabricated using hot embossing and injection molding, which also offers the advantage of relatively inexpensive mass production. Removing the metal electrodes altogether is of course a complete solution to the problems they can bring – and this approach is variously termed as ‘liquid electrode’ or ‘liquid-electrode’ DEP.”

Table I. Examples (in chronological order) of the DEP manipulation of Proteins.

| Protein(s)                          | Investigators                          | Notes                                      |
|------------------------------------|----------------------------------------|--------------------------------------------|
| Avidin; Concanavalin; Chymotrypsinogen; Ribonuclease A. | Washizu et al.25 (1994) | DEP occurred at much lower field than predicted by theory |
| Actin                              | Asokan et al.27 (2003) | Patterned using quadrupole electrodes |
| R-phycoerythrin                    | Hötzl et al.28 (2005) | Trapping of single molecule |
| Kinesin-microtubules               | Uppalapati et al.25 (2008) | Microtubules collected and aligned |
| Bovine serum albumin               | Lapizco-Ecina et al.30 (2008) | 1st protein study using D.C. DEP |
| Amyloid peptide nanotubes          | Castillo et al.31 (2008) | Single nanotubes immobilized |
| Streptavidin                       | Maruyama et al.32 (2008) | Attachment to carbon nanotube |
| Immunoglobulin G, Bovine serum albumin | Nakano et al.33 (2011) | DEP streamline concentration |
| Aβ amyloid                         | Staton et al.34 (2012) | Used DC insulating gradient DEP |
| R-phycoerythrin, IgG antibodies    | Otto et al.35 (2014) | Biological activity after DEP proven |
| Bovine serum albumin               | Laux et al.36 (2015) | Confirmed by atomic force microscopy |

Figure 8. Contours of (E·V)/E values generated by: (a) 1 Vpk signal applied to a pair of 2 μm diameter gold pin electrodes (based on Menachery et al.41), (b) 10 Vrms signal applied to triangular gold electrodes on a silicon substrate (based on Hötzl et al.28).

is readily achieved43 using a pair of wire electrodes of radii 1 μm, spaced ∼5 μm apart with an applied ac voltage of 1 Vpk. This induces a significant DEP force of magnitude ∼5 × 10−12 N on a biological cell of diameter 10 μm. By significant, we mean that this DEP force is considerably larger by a factor of 10-times smaller than the sedimentation force. By reducing the gap between the electrodes to 0.5 μm and applying a 10 Vrms signal, a value of (E·V)/E of 1021 V2/m3 is achieved, as shown in Fig. 8b, enabling Hötzl et al.28 to capture single protein molecules at the electrode tips by positive DEP. These examples should be borne in mind when considering the various electrode materials and geometries adopted by others, as outlined by Hughes47 and Perez-Gonzalez et al.42 in their reviews.
phosphate groups. Counterion fluctuations occur at frequencies that are mostly too low in frequency to be exploited by electrode-based DEP.

For some biological applications of DEP, a suspending medium of conductivity well below that of physiological conditions (∼1.4 S/m) can be inappropriate. This drawback commonly exists with electrode-based DEP, but can be addressed in the form of insulator-based DEP (iDEP) introduced by Cummings and Singh.54 In their initial investigation of this technology, a DC field (80 kV/m) was applied along a shallow channel (7 μm deep) containing 1 mM phosphate solution and uniformly patterned arrays of insulating square or diamond glass posts. With such a chamber geometry fluid flow is generated by electroosmosis, which can be used to move suspended particles along the chamber and through the gaps between the insulating glass posts. These posts distort the DC field and produce high-field regions at their corner edges. The induced electrokinetic mobility of the suspended particles is then the vector sum of its electrophoretic, electroosmotic and DEP mobility, which is a particle separation modality not available to electrode-based DEP (eDEP) devices. These iDEP devices were later shown to be capable of selectively separating and concentrating in a continuous manner different species of live bacteria.55

A simple set of equations can effectively model the performance of iDEP devices.56 The full potential of iDEP has yet to be achieved and exploited, in terms of its ability to handle suspending media of physiological conductivity, as well as the high sample throughput that can be achieved using conventional electrode-based DEP. The ability to handle suspending media of high conductivity is limited by Joule heating effects associated with the requirement of high applied voltages, although some progress has been achieved regarding this aspect.57,58

The high values of the factor (E·∇)E shown in Fig. 8b have yet to be attained with iDEP or any other type of DEP device that does not use metal-based electrodes.

Concluding Comments

So, to summarize this analysis, what top-level advice can be offered regarding where dielectrophoresis might be heading and hence the most promising topics to explore in this subject? If the research is to be application or market led, where the manipulation of small particles (e.g., exosomes, proteins, viruses, carbon-nanotubes) and high values of (E·∇)E are required, then metal-electrode-based DEP would be a sensible path to follow. This would also be the case for the identification or isolation of cells based on differences in membrane properties (e.g., conductance, capacitance) and internal structure (e.g., nucleus-cytoplasm volume ratio, parasitization) or for cell-based drug discovery protocols. For such studies, the ability to explore the DEP properties of the target cells over a wide frequency range (e.g., 10 kHz to 300 MHz) is required. Insulator-based DEP cannot do this. Although the isolation of DNA and RNA has been reported using electrode-based DEP, insulator-based DEP would be a more interesting choice because of its ability to explore different dielectric characteristics associated with surface charge and counterion mobility. DEP has also much to offer in the design of new sensors, and progress in this has been achieved for the detection of bacteria.59,60 viruses, and for label free, sequence specific, DNA detection.61 Its use as a tool for the fabrication of interfaces between cells and carbon-nanotubes for biosensing applications has also been demonstrated.63 Non-biological applications of DEP include its ability to assemble carbon nanotubes according to their electrical properties64 and to form transistors.65,66

However, if the research is curiosity driven, this author would be tempted to recommend one of two paths that have excited his imagination. A laser defraction-induced DEP method for patterning and manipulating individual cells and bioparticles has been reported using an organic photoconductor (titanium oxide phthalocyanine) deposited by a spin-coating process on an indium tin oxide glass surface.67,68 This is certainly breaking new ground that has great potential. Another exciting development has been the identification by Vahey et al.70 of genes whose deletions change the electrical conductivity of a yeast cell. Using their iso-dielectrophoretic separation method they characterized the DEP properties of ∼107 cells that had been pooled from approximately 5000 strains of S. cerevisiae. Through its barcoded DNA the strain-type of each yeast cell was identified and matched to its DEP characteristic at 300 kHz (to probe the electrical properties of the cell envelope) and at 10 MHz (to probe the cell interior). By sorting the deletion collection into fractions with different electrical characteristics and counting the relative abundance of each strain within the different fractions, Vahey et al.70 were thus able to generate for the first time a genome-wide mapping between genotype and DEP phenotype. This mapping revealed that dielectric properties are largely independent of, and thus complimentary to, other phenotypic data including fitness and morphology. This enables the ability to identify specific processes and pathways whose perturbations can be detected through changes in DEP properties. It also demonstrates the feasibility of performing whole-genome screens based on intrinsic properties—a methodology that can be translated to other devices (including hybrid-DEP devices) that sort cells based upon physical properties other than their dielectric ones. How exciting is this?

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