Michelle M. Meighan
Sarah J. R. Staton
Mark A. Hayes

Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ, USA

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Review

Bioanalytical separations using electric field gradient techniques

The field of separations science will be strongly impacted by new electric-field-gradient-based strategies. Many new capabilities are being developed with analytical targets ranging from particles to small molecules, and soot to living cells. Here we review the emerging area of electric field gradient techniques, dividing the large variety of techniques by the target of separation. In doing so, we have contributions using dielectrophoresis, electric field gradient focusing (including dynamic, true moving bed, and pulsed field), electrocapture and electrophoretic focusing, temperature gradient focusing, and focusing with centrifugal force. We cover the literature from the start of 2007 to June 2008, along with some introductory discussions. Even with the relatively short time frame, this young and dynamic field of inquiry produced some 100 contributions describing new and unique techniques and several new applications.

Keywords:
Bioanalysis / Dielectrophoresis / Electric field gradient / Particles
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1 Introduction

Separations science has made tremendous contributions toward the analysis of biological systems. New and unique capabilities are being developed by exploiting combinations of forces, where the resulting separation modes are unique and commonly non-linear. Here, we review recent contributions within the emerging area of electric field gradient techniques. In addition to traditional separation capabilities, the field gradient techniques are able to manipulate biological molecules for concentration, mixing, and fractionation.

Interest in gradient electric field techniques emerged in the 1950s with Herbert Pohl's pioneering work on dielectrophoresis (DEP) [1, 2]. Although DEP was the dominant electric field gradient technique for some time, in 1971, Giddings broke new ground with the establishment of equilibrium gradient techniques. This work instigated several electric field techniques based on the principles of equilibrium gradients. These gradient techniques continued to flourish in the 1990s, with a renewed surge of research utilizing DEP. Furthermore, in 1996, Koegler and Ivory developed electric field gradient focusing (EFGF), based on Giddings' principles [3]. Since then, a myriad of electric field gradient techniques have been established, including dynamic field gradient focusing (DFGF), temperature gradient focusing (TGF), and electrocapture (EC), among others.

In this review, we present recent work published from 2007 to mid-2008 in the field of electric field gradient techniques. During this time span, the majority of work published in electric field gradients centers on DEP; however, a large variety of these gradient techniques have been investigated, which necessitates the need to compile, compare, and contrast methods. We narrow the field to methods that are applicable to bioanlaytes, specifically cells, protein, and DNA. Additionally, the section on cells is further divided into yeast, bacteria, spores, viruses, blood cells, mammalian cells, and cancer cells. An illustration elucidating the organization of the review is shown in Fig. 1 [4]. Articles for this review were collected using both Web of Science and SciFinder, most recent versions as of the submission of this article (September 2008), searching combinations of the following terms: ''DEP,'' ''cell,'' ''protein,'' ''DNA,'' ''electric field,'' ''gradient,'' ''bioanalyte,'' and ''focusing.'' The review discusses novel and improved techniques and applications of electric field gradients from the original literature and is organized according to bioanalyte. For more information on these techniques, other reviews that encompass the methods discussed here have also been published recently [5–11].

Correspondence: Dr. Mark A. Hayes, Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287-1604, USA
E-mail: Mhayes@asu.edu
Fax: +1-480-965-2747

Abbreviations: **AC,** alternating current; **DEP,** dielectrophoresis; **DFGF,** dynamic field gradient focusing; **DPM,** diesel particulate matter; **EC,** electrocapture; **EFGF,** electric field gradient focusing; **MGE,** microchip gel electrophoresis; **PFSG,** programmed field strength gradients; **TGF,** temperature gradient focusing

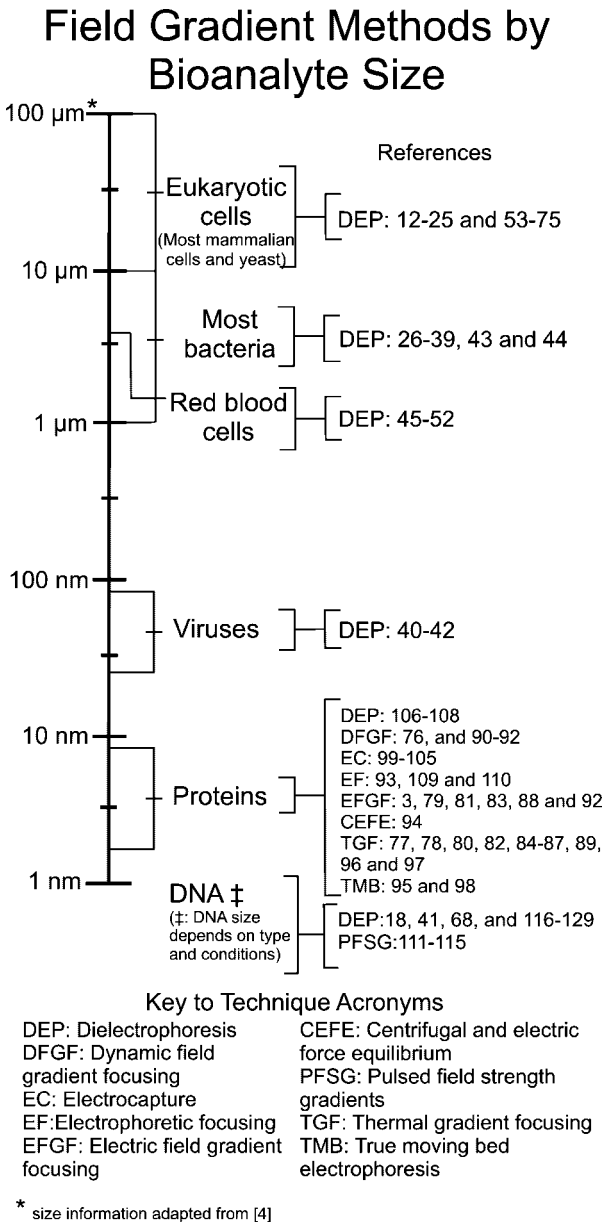

Figure 1. Organizational structure of the review. Electric field gradient techniques are addressed based on the size of the bioanalyte studied.

2 Analysis of cells

2.1 Yeast

Yeast cells have been used as the model system for studying DEP for biological applications. Not surprisingly, much of the novel and innovative technologies are initially applied to yeast cells. For instance, a new method for the rapid determination of dielectric properties of biological cells was created by Fatoyinbo *et al.* [12]. The device used circular apertures in the electrodes and alternating current (AC) voltage, which attract cells from within the radial symmetry of the aperture. The polarizability of the particle could be ascertained by shifts in the light transmitted through the hole or dot. Furthermore, the results enabled the Clausius–Mossotti factor to be determined.

A cell sorting technique that combined equilibrium gradients and continuous flow DEP was presented by Vahey and Voldman [13]. Diagonal electrodes and two solutions with variable conductance were used to create a conductivity gradient capable of separating complex mixtures of different-sized polystyrene particles along with viable from nonviable yeast. Additionally, Li *et al.* [14] presented a proof of principle paper on a device that used a discrete planar electrode to bifurcate living and dead yeast cells using AC voltage.

New methods of cell manipulation were explored by Kua *et al.* [15] where cell movements were achieved *via* moving DEP. This technique moves cells along by turning electrodes ON and OFF sequentially, and it enables the flexibility to control analyte movement after separation. Positive and negative DEP regimes were used to prove controlled movement of yeast.

In addition to new technologies, refinement of existing methods was also investigated. A novel multi-well DEP device that is compatible with standard well plate readers was developed by Hoettges *et al.* [16]. Living and dead yeast could be discriminated, as well as evaluating the effects of chemical stressors on Jurkat cells and *Escherichia coli*. Jurkat cells are derived from T-cell lymphoma and act as a useful model for cell studies. Separations could be performed in parallel by incorporating an electrode into each well, exhibiting a potential high throughput and rapid assay. An integrated circuit technology was combined with DEP-based microfluidic chips to trap and move individual living cells by Hunt *et al.* [17]. The chip was able to control thousands of dielectric objects, including yeast, mammalian cells, and liquid droplets. Not only could cells be moved around rapidly (30 µm/s), but complex designs could also be created.

Scale has been a limitation to DEP applications in the past; however, Abidin *et al.* [18] presented a novel electrode structure for the application of DEP separations on a large scale using textile technology. By weaving 100 µm diameter stainless steel wires and 75 decitex polyester yarns, high surface area electrodes were produced, which could both collect living yeast in addition to separating viable yeast from dead, both executed at large volumes (~14 mL).

A heavily doped Si 3-D electrode was fabricated by Tay *et al.* [19] to create a more uniform DEP field across the cross section of a channel. Modeling that supported the device design was contributed by Yu *et al.* [20]. Yeast could be trapped under both positive and negative DEP conditions, and the 3-D electrodes displayed a higher trapping efficiency than planar electrodes. A 3-D filtering chip was created by Iliescu *et al.* [21] by placing parallel steel mesh electrodes around a dielectric medium consisting of 100 µm diameter silica beads in a buffer solution to generate a DEP field.

The device was able to distinguish between viable and dead yeasts using AC fields.

A new geometrical configuration of metal electrodes was combined with a patterned insulator by Demierre *et al.* [22] to perform insulator DEP. Insulated metal electrodes were layered distantly under a step-patterned insulator, and opposite inhomogeneous AC fields were applied to create two opposing DEP force fields. Modulating the two fields controlled yeast cell movement and enabled separation of yeast and 5 μm polystyrene beads.

As DEP becomes more complex, parasitic traps, or unintended particle traps caused by electric field distortions, becomes more of an issue. Theoretical and experimental work investigated parasitic traps that impede cell manipulation in DEP [23]. The traps were mitigated by placing multiple frequency DEP electrodes on each side of the trap. Additionally, another device capable of controlling yeast movement with negative AC DEP was presented by Christensen *et al.* [24].

Finally, Gupta *et al.* [25] employed a combination of planar gold electrodes as well as four point electrodes to create a chain and a single layer of ''membranes'' of living yeast cells. Single and dual component structures were not only assembled but also manipulated and rearranged using DEP. Through selective staining, yeast were shown to be viable even after exposure to the DEP field. Potential applications of this technique include the creation of biocomposites.

2.2 Pathogens: bacteria, spores, and viruses

Dramatic growth in the area of DEP for the separation, sorting, and identification of pathogens has occurred over the past decade. DEP has the potential to reduce time and cost for rapid detection in bioterrorism, the evaluation of disease causing agents, as well as monitoring water and food supplies for safety. Most of the recent work is focused on the separation of pathogen types from each other or other particle types [26–29], determining viability or life stage of pathogens [30–35], or pathogen separation from environmental samples [36–38]. Additionally, other work builds on fundamental understanding of the effects of DEP fields on pathogens [39–43] and single cell manipulation [44].

Several groups presented work on discriminating bacterial pathogens from either other bacteria or micro- and nanoparticles. A nanopore insulating DEP device was fabricated by Kovarik and Jacobson [28]. Using comparable voltage densities to other papers (10^3–10^4 V/cm), both polystyrene beads (200 nm and 1 μm) and *Caulobacter crescentus* were trapped at the tip of conical nanopores. Additionally, a 3-D DEP gating system capable of continuously filtering, sorting, trapping, and detecting different bioparticles was developed by Cheng *et al.* [27]. Fluorescent latex beads (1–10 μm), *Candida albicans* dyed with Trypan Blue, *E. coli Nissle*, and *Lactobactillus* were analyzed with the device. Once separation frequencies were determined with single bioanalytes, complex mixtures were filtered, focused, and sorted.

In order to improve DEP separations, different techniques were incorporated, such as impedance measurements for identification or electroporation for further sample preparation. A microfluidic system for the sample preparation and detection of the respiratory pathogen *Bordetella pertussis* was developed by de la Rosa *et al.* [39]. Using AC voltage, *B. pertussis* was trapped over the non-uniform field above the electrodes. The cells could be washed with water while trapped and then removed from the system. In another experiment, once the bacteria were trapped, high-voltage AC electroporation was employed to preconcentrate and lyse the cells, leaving DNA intact for PCR and identification.

Using DEP positioning and electrical impedance measurements, Beck *et al.* [26] were able to detect and distinguish between *Bacillus licheniformis* and *Bacillus mycoides*, two different types of bacterial spores. Using patterned gold microelectrodes in a microchannel, the spores were captured and positioned using AC voltage frequencies. Different types of spores were discriminated from a mixture using the impedance measurements in real time. In addition, a model including the effects of electrophoresis, DEP, AC electroosmosis, and Brownian motion was developed by Park and Beskok [43] to better understand AC DEP in microfluidic devices. The model was experimentally validated by testing polystyrene, gold, and *Clostridium sporogenes* bacterial spores.

A new method for particle concentration and separation using traveling-wave DEP in a droplet was described by Zhao *et al.* [29]. Using interdigitated electrodes and a directional traveling AC voltage wave, a single droplet containing a component mixture was split into smaller droplets that were enriched in a single mixture component. Separations were initially tested using mixtures of 5 μm latex beads and 8 μm glass beads, followed by separation of a mixture of ground pine spores and 8 μm glass beads. Results indicated that applied frequency and medium conductivity were key to the method's separation efficiency.

Moving toward virus collection from physiological conditions, Docoslis *et al.* [40] used planar gold electrodes and AC voltages to concentrate viral cells. Vesicular stomatitis virus was studied under physiological conditions of pH 8.0 and a conductivity of 880 mS/m. Under these conditions, passive diffusion of the virus when using titers of vesicular stomatitis virus did not lead to detectable levels of captured virus; however, DEP enabled efficient and quick (less than 2 min) viral capture.

Selective capture of various analytes was attained by Hübner *et al.* [41] using a novel ''zipper'' electrode geometry with combined DEP and AC-electrohydrodynamic flow. Influenza virus, dissolved albumin, and DNA, all fluorescently labeled, were selectively captured along the interior surface of the electrode. A probe array of nanoscale insulating tips within a microfluidic channel was developed by Park *et al.* [42] to concentrate and lyse viral cells. The probe

was placed within the flow of the microfluidic channel, and positive DEP was able to capture Vaccinia viral cells from the flow. After capture, the same probe tip could lyse the cells by applying a high electric field.

While distinguishing pathogens from each other is valuable, so is determining their viability. A reusable method to quantify a viable strain of *E. coli* was reported by Varshney and Li [34]. A double interdigitated array microelectrode-based impedance biosensor was used to measure the impact of ionic concentration of the growth medium by monitoring the growth of the culture over time as well as the bacterial metabolites. Hoettges *et al.* [31] studied bacterial lines that have acquired antibiotic resistance. In the study, *E. coli* were exposed to the antibiotic polmyxin B and the cell membrane interactions with the drug were monitored *via* DEP changes. The method offers greatly reduced analysis time when compared with current culturing techniques.

Cell viability was evaluated utilizing DEP by Guliy *et al.* [30]. DEP was utilized to orient *E. coli* cells for optical analysis. Using phage interaction with the *E. coli* cells, only viable bacterial cells would reproduce the phage, thus enabling assessment of living *versus* dead bacteria. The technique was able to measure the effects of different chemical agents on the viability of populations of *E. coli*.

Germination of spores is also an important parameter to investigate. Impedance measurements were combined with a microfluidic biochip to electrically determine the germination of bacterial spores [32]. A model bacterial spore, *Bacillus anthracis*, was used to establish detection limits for germinating spores in a three-layer microfluidic biochip. As the spores germinated, they released multiple polar and ionic chemicals that could be detected in as little as 2 h.

Pysher and Hayes [33] presented a novel method for separating, concentrating, and sorting bacterial systems using a unique insulating DEP device. Utilizing field strengths on the order of 200 V/cm, opposing electrophoretic and DEP forces were produced that allowed for enhanced separation of analytes based on small variations of internal properties. The study showed the successful separation of living *versus* dead *E. coli* bacteria, as well being able to separate *E. coli* from *Staphylococcus epidermidis* (Fig. 2). Several different channel geometries were explored. The separation allowed for each analyte to be spatially separated and captured. This technique has the potential to separate more complex multi-component systems along with being coupled to a variety of different detection schemes. Additional studies are currently under way to further the fundamental understanding of capture behavior using standardized polystyrene particles and theoretical modeling.

Environmental samples create a large challenge for separation and detection schemes. The following papers work toward applying the current DEP technology directly to environmental samples for the purposes of sample preparation, separation, and detection. Pathogenic bacteria in ground beef samples were detected using a new label-free microfluidic method by Varshney *et al.* [37]. The device

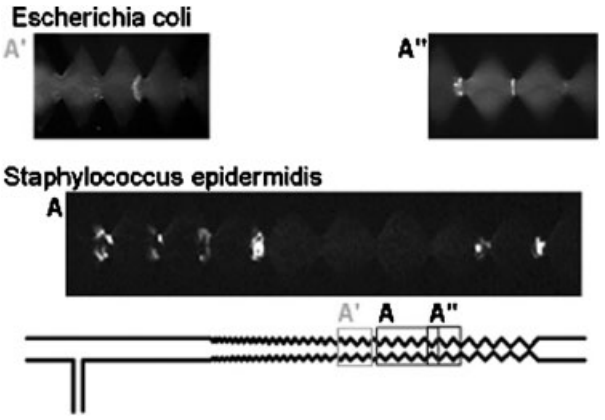

Figure 2. Separation of bacteria using a gradient DEP device. Living and dead *Staphylococcus epidermis* are separated using the device (A), as well as living and dead *E. coli* (A′ and A″).

employed embedded gold interdigitated array microelectrodes that exploit impedance biosensor measurements. Impedance measurements of bacteria samples were able to detect cell counts as low as 1.6×10^2 and 1.2×10^3 cells in pure culture and ground meat sample, respectively. In addition to high sensitivity, the method also boasts short analysis times of 35 min.

DEP filters were employed to directly capture and remove pathogens from water and food by Wu and Wu [38]. AC dielectric fields were used both in a microchip and in a larger-scale filter. The microchip was able to separate both Gram-positive and negative bacteria, as well as bacilli and cocci (*E. coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus*). The DEP filter was able to successfully capture *E. coli* bacteria, and furthermore, with longer circulation times, greater amounts of bacteria were captured. This research may find application in water filtration and food-borne pathogen removal.

The use of DEP to separate bacterial spores from environmental particulates was explored by Fatoyinbo *et al.* [36]. The study examined mixtures of diesel particulate matter (DPM) and variants of *Bacillus globigii*. Interdigitated microelectrodes were used to trap and separate spores from the DPM. The DPM were observed to experience positive DEP while the spores experienced negative DEP at higher frequencies; under continuous flow conditions, the cells were separated based on differences in their dielectrophoretic cross-over frequencies.

Single cell manipulation and control using AC DEP was explored by Arumugam *et al.* [44]. Embedded carbon nanofiber nanoelectrode arrays were used to capture single *E. coli* cells. A highly focused electric field was attained by constructing the carbon nanofibers vertically, allowing for miniaturization of electrode size. The highly focused electric field creates a greatly enhanced field gradient that could sort, separate, and move individual cells. However, current cell movement is not selective or controlled.

Finally, the impact of DEP treatments on the viability, growth profile, and immuno-reactivity of cells treated with

high AC fields was investigated by Yang *et al.* [35]. Using an interdigitated microfluidic device, variations in *L. monocytogenes*, a potentially life-threatening food-borne pathogen, were evaluated after exposure to high AC voltage. After a 1 h treatment, cell viability and growth were largely unchanged; however, after 4 h of DEP treatment, all measures of cell health were negatively affected. This study draws attention to the need to reduce DEP exposure to maintain cell health for improved analysis.

2.3 Blood cells

Blood is a very complex biological fluid; it consists of red blood cells, white blood cells, platelets, and plasma – which is composed of smaller biological molecules such as proteins. In order to effectively analyze blood, it must first be separated into its constituents. The diameter of white and red blood cells is similar, thus making separation difficult. Furthermore, white blood cells are found in low concentrations in blood, which also provides a challenge.

In the study of blood cells, various means of manipulation and separation were investigated. Size-based sorting was explored by several groups. The separation of platelets from diluted whole blood was focused on by both Di Carlo *et al.* and Pommer *et al.* An asymmetrically curved channel that exploited hydrodynamic forces to separate analytes based on differential inertial focusing was developed by Di Carlo *et al.* [45]. Platelets, the smallest cell type in blood, could be separated from whole blood, and separations performed in series increased purity and yield. In work with similar goals, a DEP-activated cell sorter was developed by Pommer *et al.* to fractionate blood based on size [46]. Platelets were separated and enriched to demonstrate label-free separation in a complex sample using DEP in a microfluidic device. Another size-based separation was performed by Kang *et al.* [47]. White blood cells and breast cancer cells could be separated by adjusting the voltage, demonstrating that the method could differentiate cells within a few microns difference in size.

In addition to size-based sorting, several other groups exploited biological properties to organize cells. Multi-frequency continuous cell sorting was performed by Braschler *et al.* to separate viable red blood cells from those infected with *Babesiabovis* [48]. The device opposed two dielectric force fields to focus analytes to arbitrary streamlines in the microchannel; separation occurred when cells with different dielectric properties focused to different streamlines. Furthermore, the research indicated that red blood cells undergo a change in dielectric response when infected. Separation of cells based on starvation age using DEP was explored by Gordon *et al.* [49]. Variables interrogated include working electrolyte solution and suspending particle modification. By exploiting various parameters, bovine red blood cells could be separated based on the starvation age of the cell.

Finally, various means of DEP manipulation using blood cells were investigated. Optoelectronic tweezers were used to manipulate polystyrene microspheres and blood cells by Hwang *et al.* [50]. Both white and red blood cells could be individually manipulated. Additionally, mechanical and DEP force were combined to separate polystyrene particles, red blood cells, and *E. coli* in water by Jung and Kwak [51]. Amplitude and frequency of the applied voltage were determined to be two critical parameters for separation. Furthermore, impedance spectroscopy was combined with DEP by Morgan *et al.* [52]. The method could rapidly interrogate single blood cells to measure dielectric properties.

2.4 Mammalian cells

Both the growth of cellular therapeutics and the advancement of stem cell research warrant improved mammalian cell separation techniques. Bulk filtration methods, such as centrifugation and filtration, often suffer from impure or reduced yield. Using an electric field, however, allows for large amounts of information to be gathered, ranging from separations based on physical properties to single cell manipulations.

The growth, morphology, and alignment of bovine fibroblasts were studied by Yuen *et al.* [53]. Cells were seeded on substrates formed through electric field-directed patterning of multiwall carbon nanotubes on flat surfaces. It was shown that, by utilizing the principle of AC DEP, templates of aligned multiwall carbon nanotubes can be readily and reproducibly formed in microelectrode arrays. Also, the DEP velocities of oocytes were studied with an electrode array chip by Choi *et al.* [54]. Oocytes with higher mobility suggested better developmental potential than the less mobile oocytes. The results demonstrated that this technique has the potential to contribute to assisted reproductive technologies.

In addition to distinguishing between physiologies of the same cell type, different mammalian cells can also be characterized using DEP. The characterization of cells using DEP in a microfluidic device was studied by Flanagan *et al.* [55]. Mouse stem cells and differentiated offspring were analyzed, and it was determined that the stem cells, differentiated neurons, and differentiated astrocytes possessed varying dielectric properties.

Various intricate cell manipulation strategies were also explored. A technique to monitor endothelin-1, a growth factor that indicates cardiac hypertrophy, was explored in two papers by Yang *et al.* and Yang and Zhang [56, 57]. DEP was used to concentrate cardiomyocytes to form a cell monolayer over the electrodes, which was followed by impedance measurements. The cardiomyocytes were then treated with endothelin-1; the treated cells had elevated impedance, which was inferred as a strengthening of the cells' attachment to the substrate surface. By combining DEP with impedance, the overall sensitivity of the system

was increased. In other manipulation work, neurons were accurately positioned onto microelectrodes using a novel technique by Pan *et al.* [58]. The device, termed ''automatic positioning and sensing microelectrode array,'' could both position cells and obtain electrophysiological recordings.

Manipulation of cells was also studied by joining DEP with electroporation. Reversible and irreversible electroporation was investigated as a function of cell position by MacQueen *et al.* [59]. Combining DEP and electroporation in a planar microelectrode device enabled electric field to be correlated to electroporation, thus facilitating the prediction of behavior with the technique.

Additionally, complex structures were controlled with DEP. Hydrogel microstructures were patterned within a bulk phase with by Albrecht *et al.* [60]. Bipotential mouse embryonic liver progenitor cells were encapsulated in a microgel structure and patterned with DEP in a bulk hydrogel. This technique has the potential to aid in the assembly of future inhomogeneous tissue.

Thermal effects in DEP cages were explored theoretically and experimentally with a variety of techniques by Jaeger *et al.* [61], including ohmic resistance measurements, fluorometry, liquid crystal beads, infrared thermometry, and bubble size thermometry. Results indicated that Joule heating could be minimized both by lowering the conductivity of the buffer and through optimizing the cage geometry. Although preliminary studies utilized bubbles and dyes to study Joule heating, the results have applicability for cellular studies.

2.5 Cancer

Chemically induced apoptosis and cell viability studies were prominent in cancer studies. The morphological changes of cells under chemically induced apoptosis were explored by Pethig and Talary [62] with a DEP cell profiler combined with flow activated cell sorting and fluorescence microscopy. The effective capacitance of the plasma membrane of Jurkat cells was obtained to determine the degree of cell viability. Other studies involving early-stage apoptosis detection were performed by Chin *et al.* [63]. Human leukemia cells were exposed to the drug staurosporine, and cytoplasmic conductivity was monitored. Changes in the cytoplasm ion content appeared in as little as 30 min after drug exposure; additionally, considerable changes were observed after 60 min, suggesting that DEP is a rapid, sensitive method for detecting cellular response and apoptosis.

Determining cell viability is also important in cancer-related biological systems. A microfluidic system that employs DEP to separate cells and collect nuclei was reported by Tai *et al.* [64]. Viable and non-viable human lung cancer cells were separated and collected at a throughput of 240 cells/min. Furthermore, the method can also separate nuclei after cell lysis. The viability of cells was also investigated by Hubner *et al.* [65]. Leukemia cells were exposed to varying concentrations of Doxorubicin, and DEP was used to distinguish between living and dead cells. Effects of thedrug were observed in 4 h with DEP, while there was no change in viability when analyzed with Trypan Blue dye in the same time span. In addition to differentiating between living and dead cells, Coley *et al.* [66] used DEP and flow cytometry to study drug resistance of certain cell lines. Parental cell line (MCF-7) and its sublines (MDR derivatives) were analyzed under different anti-cancer drug pressures. Different populations were found to have variable sensitivities, suggesting that DEP is an effective cell separation technique.

Furthermore, other investigations explored the differentiation between cell types or stage in cellular development. In a study by Broche *et al.* [67], two oral squamous cell carcinoma lines were differentiated using DEP. The UP and HPV-16 cell lines were determined to possess considerable distinctions in the dielectric properties of both the membrane and cytoplasm, thus suggesting the potential of DEP as an effective tool for cell differentiation. Cell fractionation based on cell cycle phase was examined by Kim *et al.* [68]. A DEP microfluidic device separated human breast ductal carcinoma cells based on volume, and the volume was associated with its growth phase (G1/S or G2/M). Laminar flow was used to provide a gentle means for efficient separations.

Properties of cell aggregations were also a focus of study by various groups. The shape of Jurkat cell aggregates formed under different positive DEP conditions was analyzed by Sebastian *et al.* [69]. Figure 3 depicts aggregate formation as a function of time. Interparticle forces were found to affect cell aggregation, as well as intensity of the electric field strength. Later work by Sebastian *et al.* demonstrated the DEP immobilization of Jurkat cell aggregates [70]. The application of positive DEP caused cells to adhere to one another, and the effect lingered even after the field was removed. Furthermore, the viability of the cells was not compromised. In other cell aggregate work, the effect of various parameters on the height of cell aggregates was studied by Venkatesh and Markx [71]. Electrode positioning, frequency, conductivity, flow rate, and applied field were investigated with AC DEP. Cell aggregate heights of over 150 μm were obtained for bacteria, yeast, and Jurkat cells.

A ''living cantilever array'' was created by Park *et al.* [72] that could measure the mass of a single, viable cell. Positive DEP was used to capture HeLa cells on the functionalized cantilever in physiological conditions. Mass of cells was determined by measuring the resonant frequency of the cantilever, and the experimental masses were analogous to those calculated from literature.

Negative DEP was employed by Mittal *et al.* [73] to accurately position single cells on a substrate. Non-specific cell adhesion was minimized by integrating interdigitated electrodes in the device. The method could utilize one of two regimes: pressure-driven flow alone or pressure-driven flow combined with electrohydrodynamic convective flow. Although pressure flow alone was quicker, when it was combined with electrohydrodynamic flow, more control was

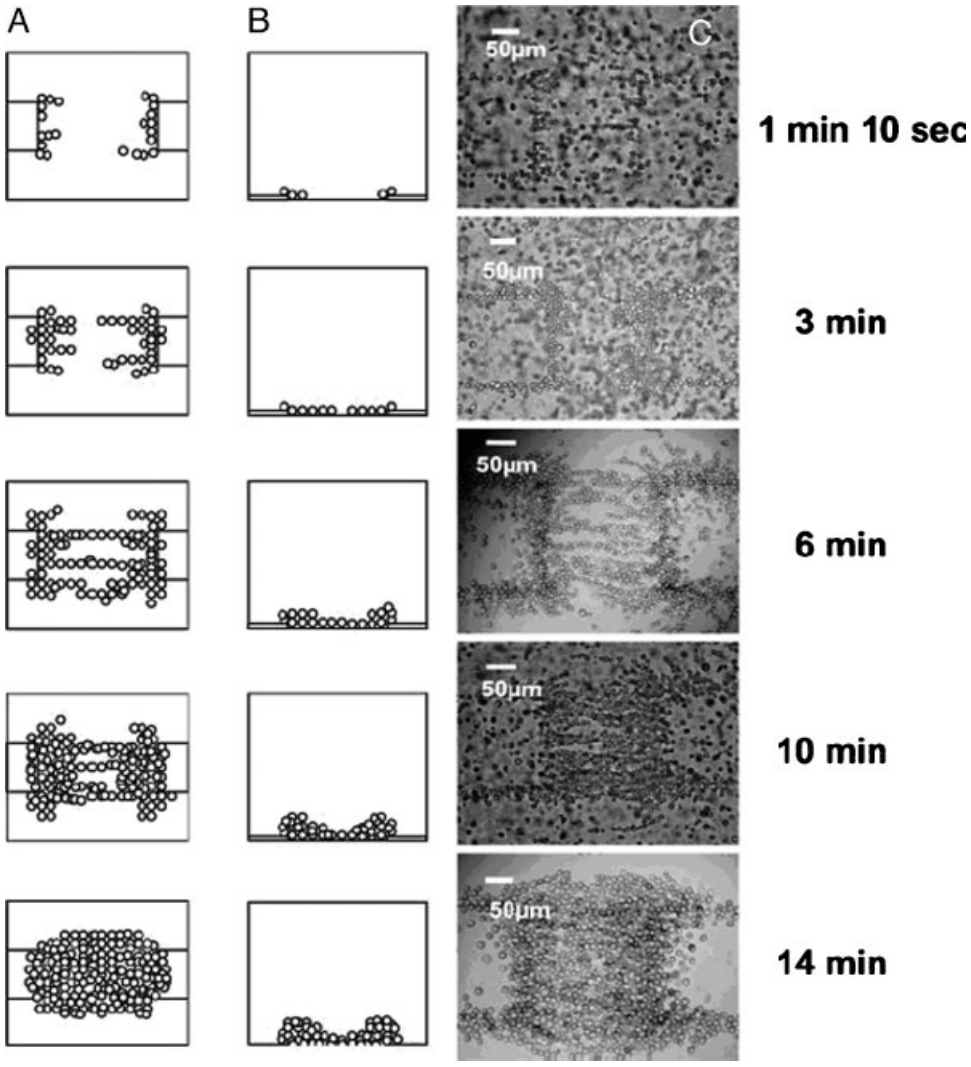

Figure 3. Jurkat cell aggregation as a function of time. Top view (A), side view (B), and photographic images (C).

generated. The method enabled effective patterning of HeLa cells using the device.

DEP modeling and experiments were used to separate electroporated and non-electroporated cells by Oblak *et al.* [74]. Electroporation of cellular membranes was found to cause permittivity changes in the membrane properties. This conclusion was supported by the separation of non-electroporated mouse melanoma cells from the electroporated, confirming that electrical properties between the two are indeed different.

Finally, the ion composition of cytoplasm was studied by Duncan *et al.* [75] using DEP. Ion channel blocking agents were employed to study the effect of various ions (calcium potassium, and chloride) on cytoplasm conductivity. Both leukemia cells and its resistant counterpart were analyzed. Calcium and potassium exhibited no difference between cell lines, whereas chloride concentrations were three times as high in the resistant line. Thus, ion channel function can be interrogated with DEP to determine various cellular properties.

3 Analysis of proteins

There are many analytical challenges in separations science; perhaps one of the most difficult is the capability to separate and/or concentrate proteins and protein mixtures. One of the common targets for analysis is the so-called proteome, where techniques are being developed to provide separations for massively complex samples with each sub-species having extremely similar properties. The proteins in human plasma, for example, span over ten orders of magnitude in abundance and three orders of magnitude in molecular weight, and differences in chirality and isomers are biologically important. Traditional techniques, such as 2-D gel electrophoresis, are unable to effectively characterize such complex samples; therefore, better analytical techniques are needed for more thorough investigation.

The fundamental mechanisms underlying the gradient techniques provide great promise for unique separations, and several strategies are being developed to separate and concentrate proteins. In the relatively short time span that

this review covers, nine different techniques capable of manipulating proteins appeared. The majority of the methods were EFGF techniques or variations thereof [76–92], where TGF was prominent [77, 78, 80, 82, 84–87, 89]. Additionally, several studies focused on modeling to help eliminate performance bottlenecks and improve resolution [76–83, 87, 89, 90, 93–95].

One fundamental electric field gradient technique that has enhanced protein separations is EFGF [3]. In recent years, there have been several new experimental and theoretical advancements in EFGF. Device material for EFGF was studied by Sun *et al.* [88] to limit problems caused by protein adsorption and electroosmotic flow. The group explored the use of poly(ethylene glycol) coated acrylic plastic microfluidic devices, including monolith-filled channels. The device not only separated R-phycoerythrin and green fluorescent protein (Fig. 4), as well as fluorescently labeled β-lactoglobin A and myoglobin, but it was also able to minimize protein adsorption and suppress electroosmotic flow.

Many groups modeled EFGF to improve device performance. Ionic transport properties of an EFGF system were investigated by Humble *et al.* [79]. In initial studies, the experimental resolution was much lower than predicted. An imbalance of cation transport in the system was contributing to the decrease in resolution, and removing acidic impurities from the monomers that compose the hydrogel resolved the problem.

Taylor diffusion, or the combined effects of diffusion in the presence of a flow field, was modeled as a function of electroosmotic flow in EFGF by Maynes *et al.* [83]. Electroosmotic flow in EFGF causes peak broadening, which in turn decreases resolution. The model addressed electroosmotic flow in both rectangular channels and cylindrical capillaries, indicating that the electroosmosis was affected by fluctuations in the electric field, the viscosity of the bulk fluid flow, and variations in the wall zeta potential.

In order to improve resolution, Lin *et al.* [81] modeled a voltage sequence to effectively ''tease apart'' proteins with similar electrophoretic mobilities. Experiments validated the model by demonstrating that lowering the applied voltage in steps could improve resolution. Using this method enables the complete removal of an entire species from the EFGF device while retaining others. The impact of flow rates and capillary diameter on resolution was also studied. Dispersion increased with high bulk flow velocities, but using a smaller separation channel minimized dispersion.

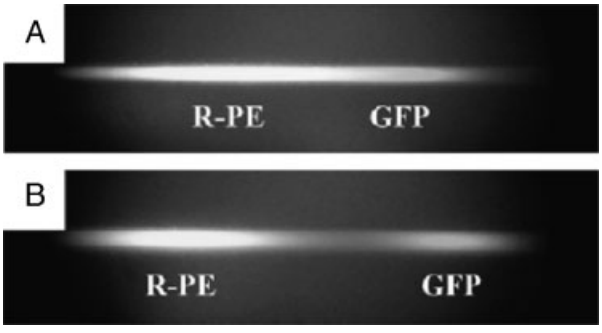

Figure 4. The EFGF separation of R-phycoerythrin and green fluorescent protein at 500 V using a counterflow of 5 nL/min (A) and 8 nL/min (B).

A technique similar to EFGF that also has applicability in protein separations is DFGF. DFGF actively controls the electrode array to manipulate the electric field profile during the course of an experiment [76]. Such command over the electrode array is beneficial because it enables increased peak resolution while allowing individual species to be eluted, even under a constant field gradient.

EFGF and DFGF were compared by Tunon *et al.* [92] for separating myoglobin from bromophenol blue and distinguishing between two oxidation states of myoglobin. Three electrode configurations were used: a fixed 21-electrode system (EFGF), and adjustable 3-electrode and 6-electrode systems (DFGF). The 3-electrode DFGF setup could focus either analyte individually, but not both simultaneously, whereas both the 21-electrode EFGF and the 6-electrode DFGF configurations could perform concurrent separations.

DFGF was scaled to a preparative level by Tracy *et al.* [90, 91]. A simulation was created using the electric field, protein transport, and heat transfer as parameters to separate hemoglobin, bovine serum albumin, and forward motility protein [91]. The theoretical results indicated that dissimilar proteins could be separated in approximately 10 min; however, when the proteins had similar electrophoretic mobilities (less than a 5% difference), much longer time (up to 3 h) was needed for a complete separation. The model was then used to design and construct a preparative-scale DFGF apparatus [90]. With the device, bovine hemoglobin was concentrated from 6.8 to 15 mg/mL, while achieving an 86% recovery of the initially injected protein.

The effects of voltage degradation and electric field distortions on the performance of DFGF were investigated by Burke and Ivory [76] using a 2-D non-linear numerical simulation. Voltage degradation is the drop in electric potential across the space from the electrodes to the separation channel. Electrode placement was determined to be a critical parameter in DFGF. Furthermore, a constant electric field gradient enabled better elution than voltage-controlled elution, which was verified both theoretically and experimentally.

TGF is another technique based off of EFGF, and it was pioneered by Ross and Locascio [96]. In TGF, analytes' electrophoretic mobilities are balanced against a bulk flow that has both hydrodynamic and electroosmotic flow. A temperature gradient applied along the length of the channel causes a gradient in electrophoretic velocity [86]. TGF is addressed in this ''protein'' section, but the technique has also been successful in the separation of DNA.

The simultaneous concentration of small molecules and exclusion of high abundance serum proteins was achieved by Munson *et al.* [85] by exploiting counterflow in TGF. With the method, fluorescein and 5,6-carboxyfluorescein were concentrated while bovine serum albumin was excluded

from the channel. The ability to exclude proteins such as albumin is important in the analysis of many biological samples as it is a high abundance protein, and it often contributes to non-specific adsorption. The group also combined TGF with field amplified continuous sample injection [84]. The method generates a psuedo-stationary interface between a high and low conductivity buffer using counterflow, and analytes focus in the interface before entering into the capillary. A 1000-fold concentration enhancement was observed using fluorescently labeled amino acids.

A PDMS/glass hybrid microfluidic chip was developed by Matsui *et al*. [82] to enhance TGF microfluidic devices. PDMS/glass material was chosen because of its convenience and cost-effectiveness. The authors used Oregon Green 488 carboxylic acid for proof of principle studies and were able to concentrate the dye approximately 30 times the initial concentration in 45 s.

The work focused on TGF is growing significantly and offers very unique capabilities, but the technique currently suffers from some weaknesses, such as limited resolution and specific buffer requirements. These weaknesses are being investigated, and strategies to minimize them are being developed through numerical simulation. Taylor–Aris dispersion and ballistic dispersion were examined by Huber and Santiago [77, 78]. Initial work demonstrated that at low fields, dispersion can be approximated by diffusion [77]. However, applied high fields caused deviation from the basic model, thus requiring auxiliary models incorporating the ballistic dispersion effects. Including both Taylor–Aris dispersion and ballistic dispersion provided an accurate model at both high Peclet numbers and high electric fields [78].

Other modeling, by Lin *et al*. [80], investigated finite sample effect in TGF. A generalized Kohlrausch regulating function was developed for systems where electrophoretic mobilities vary spatially. Additionally, the group formulated an equation capable of predicting non-linear peak distortions.

Joule heating as a means of generating a temperature gradient, as opposed to an external source, was modeled by various groups [87, 89]. Joule heating occurs when an electric field is applied along the length of a conducting medium [97]. Although Joule heating is an appealing option for many TGF devices because of its speed, simplicity, and low power requirements, runaway heating is often a problem. A quasi-1-D numerical model was generated by Sommer *et al*. [87] to account for thermal behavior and species transport in a microchannel. Various channel geometries were also examined to enhance device performance; narrow channels performed faster separations with higher concentrations but were less stable due to runaway heating.

Tang and Yang [89] also studied Joule heating in microfluidic channels *via* numerical modeling. A PDMS microdevice containing both a large chamber and a narrow channel was designed so that the net heat *per* volume was greater in the narrow channel, which results in a sharp temperature gradient at the interface of the channels. Species transport was greatly affected by mass convection and electrophoretic migration but not influenced significantly by mass diffusion. Results suggested that over a 300 times concentration increase is possible with the device.

Other electric field gradient techniques were also studied through modeling. Thome and Ivory modeled the coupling of true moving bed electrophoresis with stepped electric field gradients to separate two bovine proteins [95]. True moving bed electrophoresis utilizes counterflow against analyte electromigration for high resolution [98]. The work demonstrated a 63% increase in throughput of a fluorescein-labeled bovine serum albumin and hemoglobin separation.

Another promising electric field gradient technique is referred to as EC. EC is a microfluidic technique that utilizes electric field and a counteracting hydrodynamic flow to immobilize molecules through a membrane-stacking effect [99–102]. The technique is frequently paired with MALDI mass spectrometry for protein identification [99, 100, 102]. Coupling EC to electrospray ionization instead of MALDI is investigated in Vollmer *et al*. [103]. By using electrospray ionization mass spectrometry, there is no need to manually collect MALDI targets; rather, separated peptides can be analyzed online. Preliminary experiments demonstrated separation of a mixture of myoglobin, bovine serum albumin, and cytochrome *c*. Additionally, this method was utilized to preconcentrate various peptides (from myoglobin, hemoglobin, bovine serum albumin, and cytochrome *c*) [104]. The limit of detection values obtained were in the very low femtomolar region (as low as 3.6 fM for myoglobin). Furthermore, the dynamic range of the system can be increased by a voltage gradient [102].

The EC technique was employed to generate stable zones of differing electric field in a flow stream by Astorga-Wells *et al*. [105]. These zones were able to capture Coomassie and myoglobin in separate regions. Although the technique was shown to capture myoglobin, it also has applicability with other small biological molecules, including DNA and peptides.

A novel separation technique that blends centrifugal force and electric field gradients was modeled by Sideris [94]. The combination of forces allows a sample's constituents to focus at their equilibrium position. Initial modeling revealed that a modest field varying between 0 and 100 V/cm in combination with a rotor spinning at ~130 000 rpm could separate four proteins with masses ranging from 20 to 100 kDa in a radial distance of 20 cm. It was proposed that this method could also be valid for DNA and RNA.

Several novel applications of DEP techniques have also been recently applied to protein separations. Bessette *et al*. [106] successfully mapped antibody epitopes using an AC DEP microfluidic device. The device was able to separate peptide ligands bound to beads from unbound peptides and beads. An additional DEP technique functionalized small particles (880 nm avidin-modified latex) in continuous flow with a ''particle exchanger'' [107]. In the device, AC DEP

force was utilized to extract a particle from a starting medium and expose it to a different reagent. The device controls particle movement using DEP force, thus allowing minimal mixing of the two liquids by briefly exposing them to each other (few tens of milliseconds). Other DEP work entailed the fabrication of 3-D nanopillar electrodes with the goal of capturing biomolecules using DEP [108]. The technique enabled the immobilization of fluorescently tagged bovine serum albumin onto nanopillars. A schematic of the immobilization is depicted in Fig. 5. Conditions for both temporary and permanent immobilization were achieved by adjusting the applied electric field strength.

Another closely related strategy exploits the interfacial region between field and field-free zones in the presence of a second field, for example, flow. A novel technique that utilizes the similar principles of gradient electric fields has been termed ''electrophoretic focusing'' [109]. The principle is similar to other equilibrium gradient techniques as it counters hydrodynamic flow and electric field, but the actual separation occurs at an area exposed to the bulk sample. By controlling the flow and applied potential, charged analytes can be concentrated at the entrance of a capillary, but more importantly, certain species can be selectively excluded from the capillary. The analytes rejected remain a part of the original sample. Initial work by Polson *et al.* demonstrated the ability to preconcentrate small particles (200 nm carboxylate-modified latex spheres). Later modeling of this technique was completed by Pacheco *et al.* [93]. The work quantified the specifications necessary for electrophoretic focusing; basically, defined differential transport at the channel entrance occurs at a particular ratio between the electrophoretic velocity of a species and the bulk fluid flow. A representation of the psuedo-streamlines terminating at the electrode locations is depicted in Fig. 6. A flow-injection regime of this technique is currently under investigation. This setup has demonstrated the ability to concentrate small dye molecules (methyl violet, MW 390) while passing a neutral dye (martius yellow, MW 235) through the capillary. The modeling work combined with recent results shows promise for applicability toward small biomolecules, including proteins.

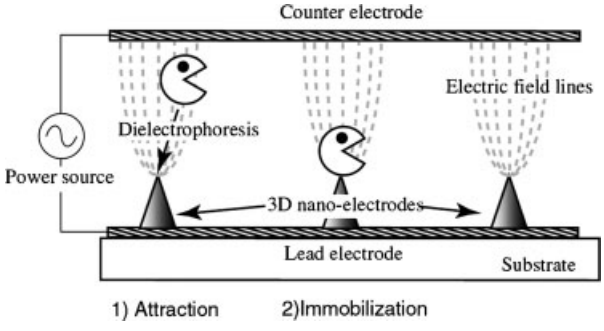

Figure 5. Schematic of the immobilization process. Biomolecules are captured at the tops of 3-D nanoelectrodes.

4 Analysis of DNA

The majority of field gradient DNA separation studies focused on the development or improvement of instrumentation addressing speed, resolution, and cost. Five papers utilized programmed field strength gradients (PFSG) to improve electrophoretic separations [110–114], while the remainder featured a novel or improved DEP device capable of capturing or manipulating DNA [18, 68, 115–128]. The PFSG studies focus on detection techniques of various PCR products, whereas the DEP papers centered on the manipulation, trapping, sorting, or electrostretching of various-sized DNA.

Several groups combined microchip electrophoresis (ME) with PFSG. The technique is analogous to temperature programming in gas chromatography or gradient elution in liquid chromatography; the field strength is actively adjusted to maximize resolution and minimize time for a given target sample separation. The PFSG studies were often compared with slab gel and/or ME. In general, ME gives higher throughput (over 650 000 bases *per* 8 h *versus* under 70 000) and better resolution than slab gels, with a faster analysis time (tens of minutes *versus* hours) [129–131]. The ME-PFSG method can be used in conjunction with other techniques to improve DNA analysis. PCR was coupled to a ME-PFSG to improve diagnosis of canine T-cell lymphoma by Suresh *et al.* [113]. The system was used to separate DNA amplification products from T-cell lymphoma (90 bp DNA) and a positive control (130 bp DNA), and results were compared using both high and low constant fields. In other studies, microchip-based capillary gel electrophoresis was coupled with PFSG to differentiate between two bacterial parasite strains in Korean feral cats [111]. The effects of varying buffer concentrations and electric field strengths on the separation of 202 and 273 bp DNA fragments were analyzed, and the PFSG performed almost seven times faster than microchip-based capillary gel

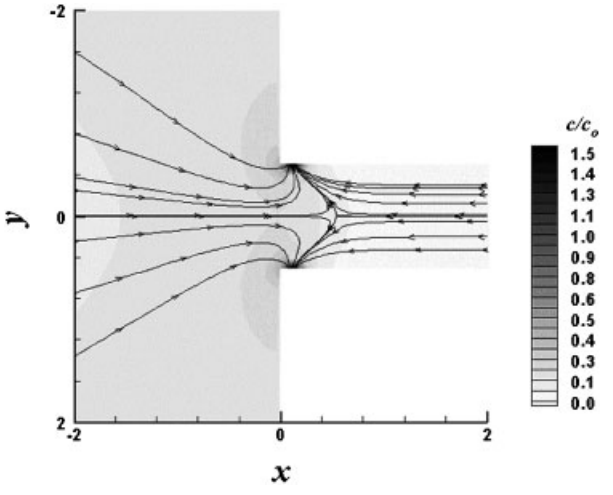

Figure 6. Model of the trajectories of particles captured at the entrance of a channel. Particles are blocked and concentrated at a channel entrance based on electric field and hydrodynamic flow.

electrophoresis alone. Jeon *et al.* [114] investigated PCR products from two *Salmonella* strains (147 and 187 bp DNA) using capillary gel electrophoresis, microchip gel electrophoresis (MGE), and MGE-PFSG. Employing PFSG allowed a 30 s separation of the fragments *versus* an 80 s separation when using MGE alone. Furthermore, the 30 s PFSG analysis was 135-fold faster than their capillary gel electrophoresis analysis and suffered no loss of resolution efficiency.

A few other ME-PFSG studies investigated combining the system with an LIF detector. PCR products of genetically modified and non-genetically modified maize varieties were examined by Kumar and Kang [110]. Results demonstrated that a 30 s run could discriminate between five genetically modified maize varieties; this short analysis time is a vast improvement (up to 60 times) over earlier published data [132]. Additionally, another study employed ME-PFSG-LIF to analyze reverse transcriptase PCR DNA fragments ranging from 50 to 2652 bp from three coenzymes extracted from mice mRNA [112]. The group examined the same fragments using slab gel electrophoresis and ME with a constant field and found that the ME-PFSG could analyze the samples four times faster than the constant field ME and almost 140 times faster than the slab gel.

The other dominant electric field gradient separation technique for DNA is DEP. Several papers explored the impact of electroosmotic flow, humidity, and surface conductivity on dielectophoretic separations. The effect of electroosmotic flow in nanopillar chips was explored both theoretically and experimentally by Kaji *et al.* [117]. The resolution of DNA separations was greatly reduced because of the electroosmotic flow; however, using high buffer strengths was found to suppress electroosmotic flow. In addition, the influence of humidity on charge transport was studied by Yamahata *et al.* [128]. Microelectromechanical system-based nanotweezers were used to trap λ-DNA (48.5 kbp) between the tweezer tips using AC DEP. The backbone of the DNA molecules absorbed water in high relative humidity and, as a result, increased in conductivity. Finally, the influence of surface conductivity on the capture of 20-mer single-stranded DNA, 40-mer double-stranded DNA, and yellow fluorescent protein at the tip of a glass-insulating pipette was explored by Clarke *et al.* [115]. The biomolecules' conductivities were determined by measuring the trapping voltage as a function of salt concentration, and the trapping efficiency of the nanopipette was found to improve with increasing salt concentration.

Electrophoretic and DEP forces were combined to improve separation by Kumemura *et al.* and Regtmeier *et al.* [119, 125]. In the method by Kumemura *et al.*, individual double-stranded DNA migrated through the device *via* electrophoretic force but was captured using DEP [119]. The device captured a single, long strand (10 μm) 48.5 kbp λ-DNA. An insulator-based device was developed by Regtmeier *et al.* that utilized both electrophoretic force and electrodeless DEP to separate and manipulate DNA based on length-dependent DNA polarizabilities [125]. The device separated both linear DNA (48.5 and 164 kbp) as well as plasmids (7 and 14 kbp). Figure 7 shows the capture of a T2-DNA (164 kbp) using the technique.

Two novel devices focus on the manipulation of DNA through electrostretching. Electrostretching is a technique that allows DNA to be oriented and extended using an electric field with DNA that is either anchored or free in solution. A technique that can position individual DNA molecules *via* electrostretching was reported by Kurosawa and Washizu [120]. Using high-intensity electrostatic field, DNA was stretched between an electrode and a solid surface. The stretched position enables the DNA to be mechanically ligated at a known point. Another electrostretching method is used in combination with restriction enzyme assays to determine DNA fragment length [122]. After creating DNA fragments, AC DEP was used to stretch fragments ranging in length from 3 to 60 kbp in the microdevice. For more information, a review article addressing different methods of stretching and immobilizing DNA was published by Kim *et al.* [133]. The review investigated various means of manipulating DNA, including using light, magnets, electric fields, hydrodynamic flow, and DEP forces.

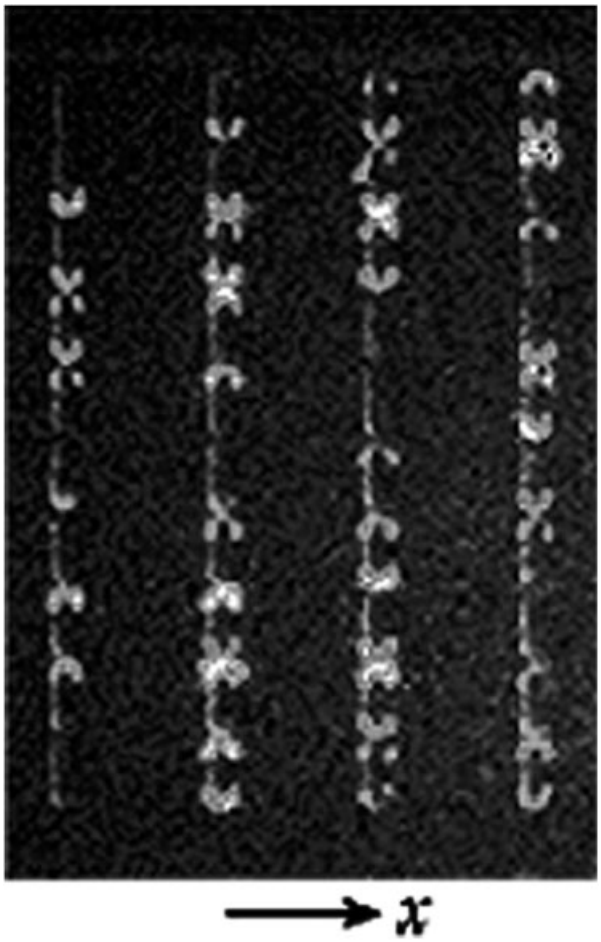

Figure 7. Fluorescence micrograph depicting the DEP entrapment of 164 kbp DNA between posts.

Various groups also explored using DEP for size-dependent sorting. An insulator DEP device designed by Parikesit *et al*. [123] employed sharp corner features and was able to separate λ-DNA (48.5 kbp) and T4GT7 DNA (165.6 kbp) molecules under continuous flow. Furthermore, electric fields as low as 10 V/cm can be used in the separation. A periodic direct current DEP array designed by Petersen *et al*. [124] contained a spatially non-uniform electric field. The technique utilized a combination of different length-scaled traps (1–80 μm) and evaluated migration rates of various-sized λ-DNA (1.5–120 kbp). The results led to the development of a model that can predict the DNA migration patterns.

Another size-dependent separation technique was explored by Krishnan *et al*. [118]. In this method, a DEP microarray was crafted that separated DNA-derivatized nanoparticles from larger polystyrene particles using high conductance and AC electric fields. The device was able to transport smaller particles (60 nm DNA-derivatized nanoparticles and 200 nm nanoparticles) to high-field areas of the array and larger polystyrene particles (10 μm) to low-field regions of the device.

Two studies examined the manipulation and trapping of DNA in gaps between electrodes. The trapping efficiency of nanoscale fingertip electrodes was investigated by Tuukkanen *et al*. [126]. Six different DNA fragments ranging in size from 27 to 8416 bp were trapped in the area between the gaps of the fingertip electrodes using an AC signal. The significant variables affecting capture were DNA length and distance between electrodes, although the frequency and applied voltage influenced results as well. Smaller DNA necessitated higher electric fields for trapping, as the DNA polarizability decreases with length. A balance between efficiency and accuracy was determined to be approximately 1 MHz; higher frequencies manipulated DNA more accurately while lower frequencies captured greater amounts of DNA. Single molecules of λ-DNA (48.5 kbp) were manipulated over an electrode gap using microelectrode technology by Wolff *et al*. [127]. The effects of several parameters influencing DNA separation were investigated, including frequency, gap distance, applied voltage, and field radius. Trapping of DNA occurred at low concentrations of DNA solutions, relatively low voltage (0.5 V), and longer trapping times (20 min), as well as a frequency of 100 kHz and a gap distance of 2 μm.

In addition to the trapping of single molecules, a method capable of capturing complex, self-assembled DNA origami was demonstrated by Kuzyk *et al*. [121]. The two origami shapes used were a rectangular arrangement (71 nm × 98 nm) and a disk-shaped structure (~100 nm diameter). Goals of the experiment included determining the ideal trapping parameters as well as preservation of the complex structures during trapping. Higher frequencies and lower voltages were needed to manipulate the complex DNA as compared with trapping a single strand.

Finally, in new work by Hoeb *et al*. [116], photoaddressable electrodes were created for the dielectric manipulation of 48.5 kbp λ-DNA and polystyrene beads. Analyte movement was tested under three experimental regimes: AC voltage plus laser illumination, laser illumination only, and AC voltage only. When only laser illumination was used, thermal gradients were generated that forced the analytes away from the beam. Furthermore, Brownian particle motion was elicited when only using AC voltages. However, using both AC voltage and laser illumination resulted in successful focusing.

5 Concluding remarks

Overall, electric field gradient techniques offer unique capabilities to the field of separations science. The field is rapidly growing, as evidenced by the large number of papers published in a relatively short time span (less than 2 years). Furthermore, the potential for the methods to be applied to a large variety of biological analytes was demonstrated.

The authors have declared no conflict of interest.

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