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Review: Microbial Analysis in Dielectrophoretic Microfluidic Systems

Renny E. Fernandez¹, Ali Rohani¹, Vahid Farmehini¹, and Nathan S. Swami^{1,*}

¹Department of Electrical and Computer Engineering, University of Virginia, Charlottesville, VA 22904, USA

Abstract

Infections caused by various known and emerging pathogenic microorganisms, including antibiotic-resistant strains, are a major threat to global health and well-being. This highlights the urgent need for detection systems for microbial identification, quantification and characterization towards assessing infections, prescribing therapies and understanding the dynamic cellular modifications. Current state-of-the-art microbial detection systems exhibit a trade-off between sensitivity and assay time, which could be alleviated by selective and label-free microbial capture onto the sensor surface from dilute samples. AC electrokinetic methods, such as dielectrophoresis, enable frequency-selective capture of viable microbial cells and spores due to polarization based on their distinguishing size, shape and sub-cellular compositional characteristics, for downstream coupling to various detection modalities. Following elucidation of the polarization mechanisms that distinguish bacterial cells from each other, as well as from mammalian cells, this review compares the microfluidic platforms for dielectrophoretic manipulation of microbials and their coupling to various detection modalities, including immuno-capture, impedance measurement, Raman spectroscopy and nucleic acid amplification methods, as well as for phenotypic assessment of microbial viability and antibiotic susceptibility. Based on the urgent need within point-of-care diagnostics towards reducing assay times and enhancing capture of the target organism, as well as the emerging interest in isolating intact microbials based on their phenotype and subcellular features, we envision widespread adoption of these label-free and selective electrokinetic techniques.

Keywords

Bacteria; Microfluidics; Sensor; Dielectrophoresis; Phenotype; Antibiotics

*Corresponding Author. Fax: +1-434-924-8818. nswami@virginia.edu.

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1. Introduction

1.1 Relevance of microbial detection

Infectious diseases caused by pathogenic microorganisms (also termed as microbes and bacteria in this review) pose a major challenge to global health and well-being, accounting for 15 million (>25 %) of the ~57 million total annual deaths worldwide¹. A disproportionate portion of this burden is on the people and environment of developing economies, while within developed economies, the infections predominantly affect disadvantaged populations, thereby accentuating disparities. The control and prevention of infections is further impeded by the absence of methods for routine monitoring of the pathogenic microbes in water, air, food, and soil samples². Spores, such as *Salmonella* spp., *Escherichia* spp., *Shigella* spp., *Yersinia* spp., and *Klebsiella* spp., as well as fecal coliform bacteria, such as *Escherichia coli* (*E. coli*) and protozoan parasites, such as *Cryptosporidium parvum* (*C. parvum*) are common pathogens that degrade the safety of drinking water³. Additionally, food-borne sources occurring due to undercooked meat, dairy products, fruits and vegetables^{4,5}, as well as due to exposure of fruits and vegetables to untreated manure^{6,7}, cause the transmission of pathogens such as: *Vibrio*, *Listeria*, *Yersinia*, *Salmonella*, *Shigella*, *Clostridium*, *Campylobacter* and *Hepatitis A*^{8,9}, which can cause infections at low pathogen doses¹⁰. Apart from these established pathogens, an ever-increasing proportion of infections are caused by emerging pathogens, due to the constantly evolving adaptations of pathogenic microorganisms and their dynamic relationships to hosts and the environment. This highlights the urgent need for novel approaches for microbial identification, quantification and characterization, especially within decentralized point-of-care and resource-poor settings for the purpose of assessing infections, prescribing therapies and understanding their dynamic modifications.

1.2. Microfluidic systems for microbial analysis and engineering

The detection needs for identifying bacteria and gauging their likelihood for causing infections can vary widely depending on sample type: water-borne, food-borne, air-borne or hospital-acquired samples. Broadly, there is a need to improve sensitivity for detecting signals from fewer cells within a reasonable assay time, from a typical sample size that can vary considerably for each sample type. Conventional methods rely on agar media to isolate and enumerate viable bacterial cells based on the colony forming unit (CFU) assay, which includes microbiological culturing, followed by confirmation with biochemical tests that can take 5–7 days to confirm for the presence of particular pathogenic organisms^{11,12}. Alternate approaches include the measurement of bacterial metabolites, biomolecular nucleic acid-based tests^{13,14}, and antibody-based immunoassays^{15,16}, whereas some of the emerging methods include those based on impedance and Raman techniques. While sample collection at scales of ~0.1 L remains a major challenge, all of these detection modalities could benefit from microfluidic sample manipulation¹⁷ for enhancing capture of the target microbial cell at the sensing surface under continuous flow^{18,19} and/or selective sample enrichment conditions²⁰. Specifically, through volume confinement at the scale of single cells²¹ to locally enrich secreted analytes in vicinity of the sensor, the laminar flows within microfluidic systems can locally and rapidly modulate pressure differentials to control chemical and physical environments²², create conditions for constant nutrient

replenishment²³ and enable rapid switching of media²⁴. Microfluidics also enables the creation of chemical, pH and thermal gradients at scales relevant to microbes, which is essential for studies on microbial chemotaxis^{25,26,27}. Additionally, by enabling the controlled mixing of materials through diffusion or miscibility differences, microfluidics is capable of controlling the generation of microdroplets, microbubbles or microparticles for encapsulating single microbials^{28,29}, which can then be applied towards highly parallelized detection³⁰, with the capability to manipulate and interrogate the same droplet multiple times after a number of biochemical transformations^{31,32,33}. Finally, advances in microfluidic circuits³⁴ has seen emergence of synthetic genetic circuits based on microbials^{35,36}.

1.3. Electrokinetics for selective and label-free microbial manipulation

In spite of these abilities within microfluidic systems, the direct manipulation of single microbial cells is especially challenging. As the size scale of cells approach down to a few microns, Brownian motion dominates over inertial force fields generated by flow³⁷. In this context, the application of electric field based methods within microfluidic platforms is particularly relevant, due to their ability to confine and spatially pattern fields, for enabling local enhancement of trapping versus other forces^{38,39}. Electrokinetics under DC fields can cause localized flow by electrophoresis and electro-osmosis, due to surface-charge induced particle and channel-wall mobilities, respectively. On the other hand, the net polarization of microbials under a spatially non-uniform field causes dielectrophoresis (DEP), which can enable highly selective enrichment of target cells, especially under AC fields and against an opposing force field. The distinguishing characteristic of electrokinetics as the means to induce sample capture and enrichment is its label-free and selective ability for transporting target particles, as well as its compatibility with the diagnostic needs for miniaturization and portability. Microbials occur over a wide diversity of shapes, with a distinct Gram stain (Gram-positive or Gram-negative) due to their cell wall composed of polysaccharides and S-layer glycoproteins, their cell membrane composed of lipid bilayers, and a characteristic cytoplasmic electrophysiology arising from water within different bound forms. This compositional diversity is reflected, for instance, in the characteristic differences of their net conductivity, as summarized in Table 1. In fact, the prominent cell wall and absence of the outer lipid membrane layer causes Gram-positive bacteria to have higher electrical conductivity than those of Gram negative bacteria. These characteristic differences in shape, composition, and geometry of each sub-cellular layer provide ample opportunity for selective polarization, especially as a function of applied field amplitude, frequency and opposing flow fields.

1.3. Context of this review

We focus this review on non-linear electrokinetic methods that are broadly grouped under dielectrophoresis, carried out on microfluidic platforms for the purpose of enhancing microbial detection through selective and label-free transport, capture, and enrichment of microbial cells. The direct manipulation of microbials by electrophoresis⁴⁹ and isotachophoresis⁵⁰ has been reviewed in prior work. The emerging interest for dielectrophoretic techniques within literature is apparent from the citation analysis in Figure 1. Considering the various types of samples, it seems apparent that while citations for the

keywords of “biomedical” and “dielectrophoresis” dominate, there is steady interest in the application of DEP to “foodborne” and “environmental” samples. Also apparent is the rising interest for the application of DEP techniques towards characterizing the use of antibiotics. Considering prior reviews on topics related to the current one, an early review by Betts and Brown⁵¹ in 1998 was focused on water samples, while much of the later work has been focused on biomedical samples. The next major review on this topic was written by the group of Lapizco-Encinas⁵² in 2011, but its focus was on DEP device platforms for microbial manipulation in environmental applications, similar to a recent one by Qian et al.⁵³, whereas another recent review by Juberi et al.⁵⁴ was focused on dielectrophoretic separation systems. A number of reviews have covered various aspects of microbial sensors, without focusing on their coupling to dielectrophoretic methods. These include reviews of microfluidic systems for pathogen sensing by Mairhofer et al.¹⁷, microfluidic diagnostic systems for infectious diseases by Lee et al.⁵⁵, and on impedance-based detection of foodborne pathogens by Yang et al.⁵⁶. While there has been a brief review of dielectrophoresis for bacterial detection by Yang et al.⁵⁷, its extent is limited, with no coverage of coupling of DEP to Raman spectral methods and towards antibiotic susceptibility. The current review is distinguished by its presentation of the principles of polarization dynamics that are unique to microbials (Section 2), a critical analysis of DEP manipulation platforms (Section 3) and a comparison of the figure of merits for DEP-enhanced detection (Section 4).

2. Microbial Polarization

2.1. Polarization phenomena

The translation of polarized particles under a spatially nonuniform electric field was originally observed by Pohl⁵⁸, and this dielectrophoresis behavior was observed to differ for live versus dead yeast cells^{59,60,61}. The DEP force on a homogeneous spherical particle was solved as^{62,63,64}:

$$F_{\text{DEP}} = 2\pi\epsilon_m R^3 \text{Re}[K_{i(\omega)}] \cdot \nabla(E \cdot E) \dots \quad \text{Eq. (1)}$$

Here, ϵ_m is the permittivity of the medium surrounding the particle, R is the radius of the particle, and E is the applied vector electric field. The term: $K_{i(\omega)}$ or the Clausius-Mossotti factor is the frequency dependent measure of particle polarizability and has the following form for a uniform spherical particle:

$$K_{i(\omega)} = \left[\frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \right] \dots \quad \text{Eq. (2)}$$

Here, ϵ_m^* and ϵ_p^* represent the respective complex permittivity of the medium and the particle, respectively, as function of the radian frequency (ω), is given by:

$$\varepsilon^* = \varepsilon + \sigma / j\omega \dots \quad \text{Ep. (3)}$$

Here, ε represents permittivity and σ represents conductivity (subscript p for particle and subscript m for medium). Based on this, it is clear that while the amplitude of externally applied voltage and the degree of non-uniformity in electric field determine the magnitude of DEP force (Eq. (1)), the frequency-dependent difference of complex permittivity of the particle and its surrounding media determines the direction of particle translation (Eq. (2)). Hence, particles that are more polarizable than the surrounding media are translated towards the high field region by positive DEP (pDEP), while particles possessing a lower polarizability than the surrounding media are translated away from the high field region by negative DEP (nDEP).

2.2. Polarization frequency spectra for microbials

For a composite particle such as the microbial cell (spherical in Fig. 2a and ellipsoidal in Fig. 2b), the less conductive cell wall and the capacitive cell membrane around the conducting cytoplasmic region cause a range of frequency dispersions, as simulated in Fig. 2c. At low frequencies, the field is screened by the cell wall and membrane to cause nDEP behavior, while at successively higher frequencies, the field gradually leaks through the insulating shell to cause cytoplasmic polarization, which reduces nDEP levels down to zero at the so-called first crossover frequency (f_{xo1}). As per Fig. 2c, the low frequency part of the spectra is controlled by membrane conductivity (σ_{mem}) and the cell wall conductivity (σ_{wall}). Beyond f_{xo1} , the pDEP level due to cytoplasmic polarization gradually rises to a maximum level that is dependent on the cytoplasmic conductivity (σ_{cyto}) and which occurs at f_{peak} , wherein the field screening due to ε_{mem} becomes insignificant. At frequencies beyond f_{peak} , the insufficient time available for cytoplasmic polarization causes the pDEP level to gradually fall, whereas the contribution of permittivity terms due to cell ($\varepsilon_{\text{wall}}$ and $\varepsilon_{\text{cyto}}$) and media (ε_m) start to dominate the magnitude and sign of K_{CM} in Eq. (2). The high ε_m due to rapid alignment of water molecules causes an opposing dipole from the media that gradually reduces particle polarization at frequencies beyond f_{peak} to result in nDEP behavior. The frequency in the polarization dispersion for this downward inflection and for its eventual crossover to nDEP (f_{xo2}) is determined by the magnitude and sign of K_{CM} in Eq. (2), which depends on the respective σ and ε terms in Eq. (3). As per Table 1, the distinctly different sizes, shapes and composition of bacterial cells with respect to each other, as well as versus mammalian cells, cause distinct features within their polarization dispersion spectra. In the following sections, we elucidate some of the distinguishing features of microbial polarization, namely: the role of their surface conductance, cell wall and cell shape on the polarization dispersion.

2.3. Microbial surface conductance

The significantly small microbial size (micron scale in vegetative form and sub-micron scale in spore form), which approaches its electrical double layer (EDL) thickness especially within lower conductivity media (σ_m), causes surface conductance effects (K_S). This affects the polarization behavior by increasing the net particle conductivity (σ_p), as per:

$$\sigma_p = \sigma_{\text{core}} + \frac{K_s}{a} \dots \quad \text{Eq. (4)}$$

Hence, for particles of small size (a) and large zeta potential (which sharply increases K_s),

double-layer effects due to: $\frac{K_s}{a}$ can increase the net σ_p over conductivity of their core (σ_{core}). As a result, the low frequency portion of the polarization spectra of smaller bacterial cells show lower levels of nDEP versus larger-sized bacterial and mammalian cells. For nanostructures, such as bacterial spores and virus particles, these higher surface conductance effects can enhance pDEP levels and the pDEP bandwidth, even within media of higher conductivity (σ_m). This feature can be used for the separation of virus, spore and mini-cell particles from microbials in their vegetative form, which usually exhibit nDEP at lower frequencies due to capacitive screening by their cell wall and membrane.

2.3. Effect of microbial cell wall on polarization dispersion

The second distinguishing feature of bacterial versus mammalian cells is the presence of the cell wall, which has significantly higher conductivity than cells that only possess membranes, thereby altering their low-frequency polarization dispersion^{65,66,67}, as presented in Fig. 3a. Gram-positive bacteria (G-P) have a highly charged peptidoglycan structure; with a cell wall (50–70 nm thick) that is densely functionalized with anionic glycopolymers, leading to a relatively conductive cell envelope in comparison to mammalian cells with only a cell membrane. Gram-negative bacteria (G-N) exhibit weaker levels of conductivity due to their thin cell wall (8–10 nm) and a cell surface composed of carboxylic acid and phosphate groups, contributed by phospholipids, membrane proteins and lipopolysaccharides, all which induce a substantial negative charge to the surface. For a composite particle, the ε_p^* term in Eq. (2) is written as net: ε_{p1}^* , due to the respective dielectric properties of the membrane (*mem*) and cytoplasm (*cyto*), based on their volume fraction (ν) that depends on membrane thickness (d_{mem}) and cell radius (R), as per:

$$\varepsilon_{p1}^* = \varepsilon_{\text{mem}}^* \frac{2\varepsilon_{\text{mem}}^* + \varepsilon_{\text{cyto}}^* - 2\nu(\varepsilon_{\text{mem}}^* - \varepsilon_{\text{cyto}}^*)}{2\varepsilon_{\text{mem}}^* + \varepsilon_{\text{cyto}}^* + \nu(\varepsilon_{\text{mem}}^* - \varepsilon_{\text{cyto}}^*)}; \text{where: } \nu = \left(1 - \frac{d_{\text{mem}}}{R}\right)^3 \dots \quad \text{Eq. (5)}$$

The inclusion of the cell wall within the multiple shell model is especially necessary to explain the large rise in conductivity of microbial suspensions within low conductivity media. In this case, ε_p^* is modified as follows from the original ε_{p1}^* term, by including dielectric properties ($\varepsilon_{\text{wall}}^*$) and thickness (d_{wall}) of the wall^{68,69}:

$$\varepsilon_p^* = \varepsilon_{\text{wall}}^* \frac{2\varepsilon_{\text{wall}}^* + \varepsilon_{p1}^* - 2w(\varepsilon_{\text{wall}}^* - \varepsilon_{p1}^*)}{2\varepsilon_{\text{wall}}^* + \varepsilon_{p1}^* + w(\varepsilon_{\text{wall}}^* - \varepsilon_{p1}^*)}; \text{where: } w = \left(1 - \frac{d_{\text{wall}}}{R}\right)^3 \dots \quad \text{Eq. (6)}$$

As per Fig. 3a, comparing bacterial cells with a cell wall and membrane with similar sized mammalian cells that only possess cell membranes, the high σ_{wall} leads to lower levels of nDEP at low frequencies and earlier crossover to pDEP versus cells possessing only the membrane. As indicated, this enables more facile separation of bacterial cells due to their pDEP behavior versus the nDEP behavior of mammalian cells (for example: blood cells). In the mid-frequency range, however, the difference between the two cell types is minimal due to the dominant influence of σ_{cyto} (usually: 0.5–1.5 S/m), which exceeds σ_{wall} (usually: 0.01–0.5 S/m). At even higher frequencies where the respective permittivity terms dominate, the higher permittivity of the wall (ϵ_{wall}) versus that of the membrane (ϵ_{mem}) and/or the cytoplasm (ϵ_{cyto}) can extend the pDEP bandwidth, as indicated by the arrow. Hence, it is essential to consider the cell wall as a separate component of the shell model to differentiate bacterial cells from mammalian cells.

2.4. Effect of microbial shape on polarization dispersion

Unlike the case for spherical particles, ellipsoidal particles do not exhibit equal polarization across all directions under an external electric field. This directional influence of polarizability affects the so-called depolarization factor: A_i , where $i = x, y, z$, which is the ratio of the internal electric field induced by the charges on the surface of a dielectric under an external field. Since the net components of A_i must add to unity, the depolarization factor for a sphere with omnidirectional symmetry has a value of 1/3 in each direction, while for an ellipsoidal particle with a major axis (a) and minor axis (b), the depolarization (A_i) falls with rising eccentricity: $e = \sqrt{1 - (b/a)^2}$. The resulting polarization (K_{CM}) due to the eccentricity-dependent depolarization is given by:

$$K_{CM} = \frac{1}{3} \frac{(\epsilon_p - \epsilon_m)}{(\epsilon_m - A_i(\epsilon_p - \epsilon_m))}; A_i = \frac{1 - e^2}{2e^3} \left[\log \left(\frac{1+e}{1-e} \right) - 2e \right] \dots \text{Eq. (7)}$$

Hence, whereas for shapes of low eccentricities, such as for a spherical or low aspect ratio structure, K_{CM} varies only from –0.5 (polarization antiparallel to field thereby causing nDEP) to +1.0 (polarization parallel to field thereby causing pDEP), its range is enhanced for structures of high eccentricities, such as a prolate ellipsoidal structure of high aspect ratio, wherein the depolarization factor is lowered along the major axis length and K_{CM} can exceed the usual maximum level of +1.0 (at eccentricities > 0.9). As per the simulations in Fig. 3b using a two-shell dielectric model (Eq. 6), the higher eccentricity causes higher pDEP levels at lower f_{peak} values, as well as lower f_{xol} values and lower levels of nDEP levels. Furthermore, it is apparent that the pDEP bandwidth is reduced, thereby causing the permittivity terms due to cell wall (ϵ_{wall}) and cytoplasm (ϵ_{cyto}) to enhance particle polarizability at earlier frequencies in addition to the effect of the cytoplasmic conductivity (σ_{cyto}). While some studies model microbials as spherical particles^{70,71}, the implications from Fig. 3b suggest that microbial shapes that vary from coccal (*Neisseria spp.*) to needle shaped (*Fusobacterium spp.*) should be accounted in the dielectric model. In fact, microbials with ellipsoidal shapes of high eccentricity, such as *Clostridium difficile*⁷² or those with elongated shapes after antibiotic treatment, such as *Klebsiella pneumoniae*⁷³, show strong

pDEP response in spite of their small size. This behavior cannot be attributed to their high cytoplasmic conductivity, but should rather be attributed to their ellipsoidal geometry (high e , due to high: $\frac{b}{a}$).

3. Microfluidic DEP device platforms for microbial separation & isolation

Sample preparation has been widely recognized as a major limitation to the development of integrated microfluidic bioanalytical systems. Methods that reduce sample complexity, such as selective removal of the cells of interest versus other cells and interfering species, can pave the path for more robust downstream determination on a versatile set of detection platforms⁷⁴. Furthermore, molecular identification of bacteria requires nucleic acid amplification, which is often hindered by blood components, thereby requiring the effective separation of bacteria from blood cells and other components. As per Fig. 4a, microfluidics remains the predominant platform for DEP-based separation and isolation, as reviewed within number of articles^{75,54}. Since this review is focused chiefly on microbial detection, we broadly summarize the various platforms of relevance to microbials (Table 2) and highlight some of the key analytical parameters from a sample preparation viewpoint (Table 3). While electrode-based DEP with castellated electrodes has been widely applied conventionally, current separation platforms prefer DEP-FFF (Dielectrophoretic Field Flow Fractionation) based on planar or 3D electrodes; and more recent platforms are based on 3D carbon electrodes that are easy to fabricate. Electrode-less or insulator-based DEP, using either DC fields only, or with AC fields, are another preferred platform since they can be easily constructed using injection molding, embossing or single-layer lithography.

4. DEP enhanced microbial detection systems

Considering state-of-the-art microbial detection systems, it is noteworthy that ELISA (Enzyme-linked immunosorbent assay) methods have assay times of 10–24 hours and detection limits of 10^4 – 10^6 cfu/mL^{113,114}, while miniaturized immunoassays can reduce assay times down to 2–4 hours for detection limits of 10^3 – 10^4 cfu/mL^{115,116,117}. Also, noteworthy is that nucleic acid detection systems based on the PCR (polymerase chain reaction) amplification have assay times of 4–6 hours, with detection limits down to 10–100 cfu/mL. Hence, DEP-enabled microbial detection systems need to be able to improve on these figures of merit, as well be amenable towards multiplexed target detection from samples of reasonable size and complexity, while reducing assay costs and training needs. Given the low infectious doses based on coliform standard of 4 cells/mL of water for *E. coli* and 1 cell per 25 g of food for *Salmonella* (Greenberg et al.¹¹⁸) and the urgency with which these assessments need to be made, there is a significant need to improve sensitivities and lower assay times. Since low cell numbers take a longer detection time, there is usually a trade-off between the detection limit and assay time. In order to address this trade-off, DEP has been widely applied for the purpose of localizing sample enrichment and for the collection of particular strains of live versus dead bacteria. The keyword-based citation analysis in Figure 4b suggests that while DEP enabled impedance measurements, nucleic acid based biomolecular assays and immunoassays stand out as the prominent techniques,

there seems to be a rising stream of citations for DEP enhancement applied to Raman spectral measurements of bacteria.

4.1. DEP enhanced Immunocapture

Surface modification with functional groups (COOH, NH₂) and/or with antibodies can enable the selective capture of microbials on electrode surfaces. Selective capture of *Listeria monocytogenes* has been accomplished by immobilizing a biotinylated BSA–streptavidin–biotinylated monoclonal antibody sandwich structure. Using pDEP enrichment of the bacteria at the edges of interdigitated electrodes, bacterial capture was found to be 3–5 folds higher than for immunocapture without DEP enrichment¹¹⁹, as shown schematically in Fig. 5. Along similar lines, under conditions of dielectrophoresis for 5 min prior to the immunoassay, the capture efficiency of *Listeria monocytogenes* using immobilized Heat shock protein 60 (Hsp60) as receptors was enhanced by 60%¹²⁰. Functionalization of microbial receptors in the area between electrodes, which are regions of low field strength, can enable nDEP-based enrichment of microbials. Based on this, a DEP-enhanced immunosensor for label-free, highly sensitive, detection of *influenza virus H1N1* has been reported¹²¹. COOH-functionalized single wall carbon nanotubes (SWCNTs) were dielectrophoretically deposited on a self-assembled monolayer of polyelectrolyte polydiallyldimethyl-ammonium chloride (PDDA) between two gold electrodes, resulting in a uniformly aligned SWCNT channels (2–10 mm in length). Following Avidin immobilization on PDDA–SWCNT channels, viral antibodies were immobilized *via* Biotin–Avidin coupling. Binding of influenza viruses to the antibodies caused the resistance of the channels to increase linearly from 1 to 10⁴ PFU ml^{−1} within a few minutes. In another recent report¹²², *Salmonella typhimurium* from a sample was bound to RuBpy-doped fluorescent nanoparticles that were conjugated to the respective antibody, followed by pDEP enrichment to capture the antibody-bound particles that were determined by fluorescence. In this manner, detection limits of 56 cfu/mL in deionized water and 110 cfu/mL in mineral water samples were reported, with sample to answer occurring within two hours. To reduce the background fluorescence signal from metal electrodes, an analogous platform used transparent ITO microelectrodes to carry out DEP enrichment, coupled to an array of microcavities to localized immunocapture and determination¹²³. In another study¹²⁴, carboxylate-modified PEG immunobeads were used to isolate select live *E. coli* strains that were present at 500–1000 cfu/mL levels, for downstream capture by DEP from 1 mL samples onto boron-doped ultrananocrystalline diamond electrodes.

4.2. DEP-enhanced impedance measurement

Prior work on monitoring impedance of the media during the growth of bacteria to quantify their cell numbers has been supplemented by recent investigations focused on using impedance to analyze the electrical properties of bacterial cells in contact with electrodes. As summarized in Table 4, the sensitivity of impedance measurements using electrodes within interdigitated arrays (IDAs) is high, since their gaps are on the order of microbial size, thereby enabling detection without an amplification step. Coupling these impedance methods with DEP enrichment is particularly of interest for the purpose of enhancing capture efficiency of bacteria at the electrodes and enabling selectivity towards the capture of viable bacteria of particular strains. This work was pioneered by Milner et al.¹²⁵ and

Suehiro et al.¹²⁶ was termed DEPIM (DEP-based Impedance Measurement), wherein IDAs were used to measure difference in impedance between the cells and the media following application of pDEP to form a chain of cells linking the electrodes (Fig. 6a). The resulting pearl chains caused electrical connection of the cells in parallel to the electrode gap, thereby increasing the conductance and the capacitance between the electrodes. Assuming that the trapped cells possess higher admittance than the media, the total admittance of the microelectrode increases with increasing of the number of trapped cells, thereby allowing estimation of cell concentration based on the increment rate of admittance. To further add selectivity, antibodies have been used. In one case described by Suehiro et al.¹²⁷, following dielectrophoretic trapping of bacteria, antibodies were added to cause agglutination of the target bacteria, so that the pDEP force levels are specifically enhanced due to the size increase, whereas the non-agglutinated bacterial cells were washed out. In another method by Suehiro et al.¹²⁸, anti-*E. coli* antibodies were immobilized onto the electrode surface for antibody-specific microbial binding. In this case, additional wash steps were avoided by maintaining high enough p-DEP levels to bring bacteria to the chip surface, while being just lower than the drag force exerted by fluid flow to enable continuous sampling. To further improve detection sensitivity of *E. coli*, Suehiro et al.¹²⁹ combined their prior impedance work with electroporabilization. Following the capture of *E. coli* cells by positive dielectrophoretic force onto an interdigitated microelectrode array, a high AC electrical field was used for electroporabilization of trapped bacteria, to cause an increase in conductance due to intracellular ion release through the damaged cell membranes to detect down to 10^2 cfu/mL of *E. coli* within 3 hours. In a more recent investigation, separate sets of microelectrodes to induce nDEP and pDEP have been used for simultaneous trapping and characterization in a DEPIM based device by Hamada et al.¹³⁰. Bacteria flowing into the device were repelled under action of nDEP force exerted by the first microelectrode, thereby pushing them toward the second microelectrode, where they were captured by pDEP, for DEPIM measurements. The nDEP concentration step was found to cause two-fold enhancement of DEPIM sensitivity, as a result of increased number of bacteria trapped subsequently by p-DEP at the microelectrodes. Another DEPIM based sensing system was recently developed by Kikutan et al.¹³¹, for detecting aspiration pneumonia among the elderly, by measuring the amount of the infecting oral bacteria within just 20 seconds. The device was benchmarked for reliability and quantitative correlation versus fluorescence-based methods. More recently, Dastider et al.¹³² used two sets of IDAs (schematic in Fig. 6b); the first to focus and concentrate *E. coli* into the center of the microchannel by pDEP (5 V_{pp} at 5.6 MHz) towards the detection zone that is of significantly lower volume than the flow channel and wherein polyclonal anti-*E. coli* antibodies were non-specifically immobilized on the sensing electrode array. As a result, *E. coli* O157:H7 bacterial cells were measured by impedance at a detection limit of: 3×10^2 CFU/mL.

An alternate design for the integration of impedance methods with DEP enrichment of bacteria was described by Gomez et al.⁹⁸ (schematic in Fig. 6b). The broad idea was to cause the confinement of live bacterial cells within a small volume (nL-pL), so that the metabolism of a few live cells in low conductivity buffer can be rapidly detected by impedance measurement at IDAs. To capture and confine cells from a main flow cell into a small volume (~400 pL) measurement chamber, one set of DEP electrodes was used, while an

alternate set was used for monitoring the impedance change due to bacterial cell growth in the chamber. In this manner, detection limit can be reduced to just a few cells. The concentration factor of this chip was 10^4 – 10^5 -fold by concentrating cells from an original sample volume of 40 μ L into the 400 pL chamber, with a collection efficiency of at least 10%. As a result, a significant improvement in detection time was demonstrated by the comparison of the impedance growth curves of *Listeria* cells in their growth media in the chip, with and without DEP concentration. This type of concentration technique eliminates the need for microbial enrichment by long culture steps in conventional cell culture methods, thereby reducing the total assay time. This on-chip impedance microbiology has achieved the detection time of 1 h for a sample with a starting concentration of 10^4 cfu/mL. In another instance by Yang et al.¹¹⁹, DEP enrichment coupled to antibody selective capture has been used to demonstrate selective capture of target cells from a mixture of cells with similar dielectric properties. Using pDEP (20V_{pp} at 1 MHz) to concentrate *Listeria monocytogenes* cells from the fluid flow. The DEP collection efficiency was optimized by varying the channel dimension for each flow rate. In this manner, DEP collection efficiencies of ~ 90% were obtained under continuous flow at 0.2 μ L/min for enrichment into the microchannel, for causing concentration factors of 10^2 – 10^3 -fold within 5–20 μ L sample volumes. At higher flow rates of: 0.6 μ L/min, the DEP capture efficiency reduced to ~ 65%. The enriched cells were bound to monoclonal anti-*Listeria* antibody immobilized to the surface of DEP chamber, through biotin–streptavidin chemistry. The antibody capture efficiency was 20–30% for cell numbers in the range on 10^1 – 10^4 cfu/5 μ L, thereby capturing about 60 cells from this sample size in presence of DEP, whereas only about 16 cells were captured in the absence of DEP. In another example, Ruan et al.¹³³ used $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as the a redox probe to sensitively measure the change in charge transfer resistance upon the attachment of *E.coli* bacterial cells to anti-*E. coli* immobilized on the electrode surface. Additionally, an amplification step based on secondary antibodies labeled with horseradish peroxide to produce insoluble precipitates is used to increase the charge transfer resistance at the electrode, for enhancing sensitivity. This biosensor had a linear response range in the bacterial concentration from: 6.0×10^4 to 6.0×10^7 cells/mL, with a detection limit of: 6.0×10^2 cells/mL. In another example of coupling microbial enrichment to impedance detection, Varshney et al.¹³⁴ used magnetic nanoparticle-antibody conjugates (MNAC) to enrich *E. coli* O157:H7 cells inoculated in ground beef samples under magnetic fields on to the active layer above the impedance microelectrode immobilized with biotin-labeled polyclonal goat anti-*E. coli* antibodies. As a result, *E. coli* O157:H7 was measured at a sensitivity of 1.6×10^2 cells in pure culture and 1.2×10^3 cells in a ground beef sample, within a total detection time from sampling to measurement of 35 min. More recently, Couniot et al.¹³⁵ coupled long-range Joule heating driven electrothermal flow to short-range contactless dielectrophoresis, to direct *Staphylococcus epidermidis* cells towards the center of the impedance sensor array, thereby achieving a detection limit of 10^5 CFU/mL within 20 min of incubation, which is almost two orders of magnitude better than using the impedometric sensor by itself.

Real-time detection of the germination of bacterial spores is of great interest for monitoring systems in healthcare and environmental settings. Current automated systems for this application are based on PCR identification, which requires heavy lab-based equipment.

Furthermore, these methods either perform spore-identification before their germination thereby obviating the ability to distinguish viable versus non-viable spores that is of great interest, or they can only detect growth of the vegetative cells after germination. In this context, Liu et al.¹³⁶ describe an electrical method for real-time detection of spore-germination after the DEP-capture of *Bacillus anthracis* Sterne spores. The rise in electrical conductivity of the medium during spore germination, due to the release polar and ionic chemicals was monitored down to a detection limit of 10^8 spores/mL in 5 mL sample volumes. Using a three-layer device: including a layer with valves constructed on PDMS (polydimethylsiloxane), another layer with micro-fluidic PDMS channels and a third layer with metal electrodes on a pyrex substrate, the spores were captured under flow conditions from the microfluidic channel by pDEP ($20 V_{pp}$ at 100 kHz) onto electrode edges for the measurement. Furthermore, the pDEP forces were used to hold the spores stationary while the carrier fluid was replaced by the germinant solution prior to the measurement. In another example of detection of nanoscale bioparticles, the group of Suehiro applied DEPIM towards virus detection^{137, 138}. Detection of virus capsids is significant, since they cause secondary infections within a short period of time, even at small concentrations. Capsids trapped under pDEP using interdigitated microelectrodes were focused into regions where impedance could be measured. As discussed in Section 2, pDEP of submicron sized virus particles extends within media of higher conductivity and over a wider frequency bandwidth. Based on this, noroviruses (~38 nm) were trapped at 58 MHz within media of $\sigma_m=0.23$ mS/cm, while rotaviruses (~100 nm) were trapped at 5 MHz within media of $\sigma_m = 1.6$ mS/cm. DEPIM responses were measured at various norovirus concentration levels for different flow rates. The whole detection process was completed in 300 s at 2.5 pg/ μ l concentration, which is comparable to conventional immune-chromatography methods. The detection limits were 50 ng/ml for norovirus and 10 ng/ml for rotavirus, since the larger size of the latter virus enables easier pDEP trapping ability for measurements at enhanced sensitivities versus the former. The accuracy of these results was cross verified by fluorescence methods.

4.3. DEP enriched Raman spectroscopy

Raman spectra offer highly selective and sensitive information on the vibrational bands of key functional groups within molecules. Since bacteria are composed of characteristic combination of macromolecules that can serve as taxonomic markers¹⁴⁰, there is great interest in distinguishing bacterial strains¹⁴¹, by following the variations in proportions of their respective vibrational bands^{142,143}. By combining the Raman set-up with optical imaging using so-called confocal micro-Raman spectroscopy, the spatial resolution of this chemical information can be achieved down to 1 μ m, for mapping single bacterial cells. While Raman signals are usually rather weak, the use of excitation wavelengths in the UV region corresponding to electronic absorption bands of particular macromolecular biomarkers can enable signal enhancement by several orders of magnitude to enable specific and highly sensitive detection of these species within complex biological environments. Furthermore, these UV resonance Raman methods avoid the noise from fluorescence emission bands, thereby providing measurement of aromatic amino acids from protein subunits and purine and pyrimidine bases of DNA/RNA nucleic acids for enabling chemical classification of microorganisms. On the other hand, Raman spectra obtained due to visible

or near IR excitation provide phenotypic information on the net biochemical composition of the whole microbial cell. Since Raman spectra provide information on molecular interactions inside the cell, they enable investigations of cellular metabolic changes¹⁴⁴, growth culture conditions¹⁴⁵ and cell interactions with drugs¹⁴⁶. In spite of these features of Raman spectral methods, microbial identification requires their presence at levels of $\sim 10^9$ CFU/mL¹⁴⁷, even after application of surface enhanced Raman spectroscopy (SERS) to enhance signals due to the resonant excitation of surface plasmons on a rough metal surface or at metal nanostructures in close contact to the microbial sample¹⁴⁸. Since many infections, such as sepsis, can occur at microbial levels of 10^3 CFU/mL or lower, there is a need for microfluidic methods such as dielectrophoresis for the specific capture and enrichment of microbial strains to enhance the selectivity and sensitivity of the Raman information. Fig. 7 shows some example device schemes for coupling DEP with Raman spectral methods. Based on the chart of literature citations of DEP-enhanced Raman, it is apparent that interest in this field is apparent steady, since the early work in 2010 reported by Cheng et al.¹⁴⁹. In this work, Cheng et al. used a set of three-dimensional electrodes between two glass slides, with a roughened metal surface placed in front of the DEP concentrator to enhance Raman scattering. In this manner, Gram positive (*Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*) bacteria were distinguished. Separately, *S. aureus* present within a blood sample at 10^6 CFU/mL level was enriched by nDEP (20 V_{pp} at 500 kHz in sucrose media) from the flow cell (1 μ L/min) onto the roughened metal surface to enable SERS-based identification of the strain within 3 minutes. However, the SERS effect is governed by strict surface selection rules for the direction of the molecular dipole and the roughened SERS substrate can cause harmful physiological alterations to the bacterial cell¹⁵⁰. Hence, Schroder et al.¹⁵¹ instead use micro-Raman spectroscopy on glass to characterize pathogens from urine samples (1–3 S/m), by using nDEP (4 V_{pp} at 1 MHz) generated by a set of quadrapole electrodes to focus *Escherichia coli* (Gram negative) and *Enterococcus faecalis* (Gram positive) bacteria at levels of $\sim 10^5$ cells for each species. The short spatial range of DEP forces is a major limitation, thereby requiring its coupling with long range electrohydrodynamics due to electrokinetic and/or electrothermal forces. Cheng et al.¹⁵² describe the coupling of short-range DEP to long-range AC electroosmosis (ACEO) flow using a concentric set of electrodes to selectively concentrate the bacteria of interest over a wider range of the diluted human blood sample for on-chip SERS measurements. In this manner at low frequencies (3 kHz), while blood cells exhibit strong nDEP due to their large size and capacitive field-screening by their membrane, the high conductance of the bacterial cell wall causes pDEP. However, this pDEP behavior is rather weak due to small bacterial cell size, so that ACEO flow dominates over pDEP, thereby driving bacterial particles towards the device center for localizing them for SERS detection. On the other hand, nDEP of blood cells exceeds drag forces under ACEO, thereby transporting blood cells away from bacterial cells, thereby enhancing selectivity. In another approach, Cheng et al.¹⁵³ developed a system to enrich an aggregate of nanoparticles and bacteria as a platform for SERS enhanced microbial detection. However, to address the wide differences in DEP forces for varying sized particles, a mechanism based on the local amplification of fields following microparticle assembly was used to cause the pDEP enrichment of silver nano-colloids (20 nm) as an aggregate with the bacteria, whereas the differing DEP behavior of blood cells versus bacteria enables their separation. In another

recent approach, Madiyar et al.¹⁵⁴, embedded a substrate patterned with Vertically Aligned Carbon Nanofibers (VACNFs) at the bottom of a microfluidic chip for enabling DEP capture and enrichment. To enable SERS enhancement, the nanoparticles were attached specifically attached to *E. coli* DH α 5 cells by immunochemistry. In this manner, *E. coli* was enriched within a 200 $\mu\text{m} \times 200 \mu\text{m}$ area on which the Raman laser probe was focused to enable SERS measurements that were able to detect a single bacterium, whereas using a portable Raman system, a detection limit of 210 cfu/mL was obtained after DEP enrichment for 50 s, without any other sample preparation. It is worthy to compare this study with a similar SERS study¹⁵⁵ to analyze live versus dead bacteria captured using polyclonal antibodies with iron oxide–gold core–shell nanoparticles. While in this study, Zhou et al. were able to accomplish this discrimination purely by SERS, DEP enrichment enabled the measurement to be carried out at higher sensitivities, thereby allowing for detection using equipment with higher portability. Silicon microneedles were used in another study for conducting heterogeneous SERS, wherein bacteria within a solution interact with patterned metallic structures, for DEP aided microbial detection¹⁵⁶. In this study, silicon microneedles with sharp tips enhance trapping as well as detection, since their high electric field gradients generated high DEP forces as well as SERS enhancement (also referred to as tip enhanced Raman spectroscopy, TERS)^{157,158}.

Along the vision of carrying out fluorescence activated sorting in a label-free manner, there has been great interest in Raman-activated cell sorting (RACS). However, given the weak Raman signals (only 1 in about a million incident photons undergo Raman scattering), the acquisition times for the spectra are too high for enabling rapid sorting. For this purpose, immobilization of single cells at the Raman detection point could allow for the acquisition of Raman spectra from samples within a high-speed flow. Laser tweezers, which have been applied towards cell immobilization are unable to selectively capture the cell-type of interest, whereas DEP methods can allow for this within high-speed flows. Using a pause-and-sort RACS microfluidic system that combines positive dielectrophoresis (pDEP) with Raman for single-cell trap-and-release system, Zhang et al.¹⁵⁹ distinguished carotenoid-producing (9%) versus non-carotenoid-producing *Saccharomyces cerevisiae* (91%) cells, so that the former were enriched to 73% levels based on fast Raman-activated cell sorting at the subsecond level. In another application of micro-Raman coupled to DEP, Tomkins et al.¹⁶⁰ captured M13 virus particles using quadrupolar microelectrodes electrodes with a gap spacing of 4 μm with 15 V_{pp} at 1 MHz, within 30 minutes. Surface mapping of dielectrophoretically captured M13 phage particles was conducted using micro-Raman spectroscopy, with NeutrAvidin-Cy3 as labeling molecule.

4.4. Dielectrophoresis-enhanced PCR amplification

PCR is a key tool for the identification and quantification of bacterial strains, especially those that are difficult to culture *in vitro*, such as *Mycobacterium tuberculosis*¹⁶¹. The early identification of these pathogens in bio-fluids is essential for prescribing the appropriate antibiotic therapies. However, a sepsis patient can present just 1–100 CFU/mL of the pathogenic bacteria, while the microliter of blood that is extracted from contains 4–6 billion blood cells, whose components inhibit PCR amplification. Hemoglobin and heparin, which are natural components of blood, have inhibitory effects on PCR^{162,163}. Hence it is

important to isolate microbials from the blood cells, as a prerequisite to PCR. In comparison to Raman detection systems, where detection times are rapid after effective sample enrichment (within 15 minutes), DEP enhanced PCR is considerably more time-consuming. However, PCR can identify and accurately quantify pathogens from complex physiological matrices, such as blood, in a reasonable amount of time (1.5–3 hours). Furthermore, multi-target detection platforms are possible by running multiple PCR assays in parallel in a multi-channel device. An early design for separation of microbials from blood cells by Huang et al.¹⁶⁴ was based on interdigitated electrodes for dielectrophoretic separation of simulants of biological warfare agents, such as *Bacillus cereus*, *Escherichia coli* and *Listeria monocytogenes* from blood, for subsequent PCR amplification. As expected from the polarization dispersion shown in Fig 2a, blood cells experience nDEP at low frequencies (<0.1 MHz), whereas most bacterial cells exhibit weak pDEP levels at this frequency, especially in low conductivity media. A separation efficiency of 97% was reported based on culturing of *E. coli* cells following DEP separation. The number of recovered cells using DEP was found to be influenced by electrode geometry, media conductivity, flow-rate in the device and electric field-strength. Cell collection efficiencies of 90% were observed at media conductivity levels of 50 mS/m or lower. In a similar process described by Perch-Nielsen et al.¹⁶⁵, pDEP was used to hold *Saccharomyces cerevisiae* cells, while haemoglobin and heparin were removed. In another integrated system reported by Bhattacharya et al.¹⁶⁶, one set of electrodes inside the bigger chamber (0.6 μ L volume) was used to divert bacterial cells from a flow stream into a 0.4 nL chamber, wherein a set of interdigitated electrodes were used to specifically capture and enrich the bacterial cells by DEP. In this manner, *Listeria monocytogenes* V7 cells were identified using as few as 60 cells collected from a sample of 10^4 CFU/mL in less than 90 min. In a realization of multi-channel PCR capable of amplifying four independent samples, Cai et al.¹⁶⁷ have reported the ability to identify 20 different pathogens from diluted whole human blood. These pathogens, isolated from the blood *via* DEP, were captured in a parallel array of grooves and were subjected to multiplex array PCR with end-point fluorescence detection. Simultaneous detection of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* O157:H7 were performed within 3 h from dilutions as low as 10^3 CFU mL⁻¹. The limited spatial extent of the DEP field obtained from thin film (~100 nm) interdigitated electrodes in these prior studies has motivated the consideration of carbon-based electrodes (thickness of several microns), since the DEP field can extend several microns into the channel and due to the inertness of carbon electrodes over a wide potential window. In this context, Jaramillo et al.¹⁶⁸ describe a carbon-DEP chip for capturing viable yeast cells present in environmental samples for downstream PCR amplification using off-the shelf equipment. Recently, a PCR-activated cell sorting (PACS) method using DEP-enhanced TaqMan PCR was proposed by Lim et al.¹⁶⁹, wherein microbes were individually encapsulated within aqueous picoliter-volume droplets and maneuvered using ultrahigh-throughput microfluidics, as schematically indicated in Fig. 8. The droplets can withstand harsh high-temperature lysis procedures and screen for millions of cells within hours. Droplets containing the target organism exhibit fluorescence after PCR amplification, which can be used for their downstream recovery using dielectrophoretic (DEP) sorting. This method has the possibility of being extended to the determination of cellular transcriptomes, which can open up opportunities for studying single-cell gene expression in complex microbial ecosystems.

4.5. DEP-enabled phenotypic analysis of viability and antibiotic susceptibility

The differing DEP response between live versus dead bacteria, specifically the increase in cell wall conductance and the steady drop in cytoplasmic conductivity with loss of cell viability has been widely applied towards their selective translation¹⁷⁰ and detection^{171,172}. However, unlike with the case for cells after heat-treatment, cell death is not well-defined after certain antibiotic treatments¹⁷³. The loss of viability is usually associated with the inability of microorganisms to divide and form colonies. This ignores the heterogeneity of bacterial samples, with subpopulations that become dormant and unculturable¹⁷⁴, which is important to detect given the emerging microbial persistence to antibiotics¹⁷⁵. The emergence of global-scale infections due to antibiotic-resistant microbial strains, such as penicillin-resistant *Streptococcus pneumoniae*, multi-resistant *Salmonellae*, multi-resistant *Mycobacterium tuberculosis*, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* (MRSA)^{176,177}, has motivated the need for technologies capable of rapidly screening for novel antibiotics. Current methodologies focus on parallel testing of the multiple antibiotic candidates using high density culture plates to determine which particular combinations suppress growth of the microbial strain of interest. However, this strategy is not rapid (usually takes 24 hours of culture) and does not work for unculturable strains. Additionally, a strategy based purely on suppressing the growth of the pathogenic or antibiotic resistant strain is not sufficient to eliminate the infection, since persistence of a small subpopulation can be sufficient for microbial colonization. Another strategy is based on following the alterations in metabolism of the pathogenic strain in the presence of various antibiotics, but this method requires the presence of significant metabolic markers and is not effective with strains that exhibit slow metabolic rates. An alternate strategy is based on genetic methods detecting particular DNA sequences that are markers for antibiotic resistance. However, this strategy only detects antibiotic resistant strains with known markers and it is prone to easily picking up contaminations¹⁷⁸. In this context, dielectrophoresis can function as an especially effective technique to gauge phenotypic alterations under the influence of the antibiotic, by monitoring electrophysiology variations due to DEP spectral changes within the frequency range of interest. Since cell electrophysiology and shape¹⁷⁹ are rapidly altered post-treatment, the effect of various antibiotics and co-cultured probiotics can be rapidly quantified for enabling the prescription of antibiotics that are targeted to the phenotype of the strain causing the infection, rather than current methods based on broad-spectrum antibiotics. One method is to measure the polarization dispersion to monitor the alterations discussed as part of Figure 3. Another method is to consider the plot of crossover frequency versus media conductivity to determine the optimal conditions for observing alterations in particular subcellular phenotypic features, as shown in Fig. 9a (simulated for dielectric properties of typical bacteria)¹⁸⁰. At low media conductivities ($\sim 10^{-3}$ S/m), the alterations in crossover to pDEP are chiefly attributed to conductivity parameters of the cell wall (σ_{wall}) and membrane (σ_{mem}); whereas at higher media conductivities ($10^{-2} - 10^{-1}$ S/m) the pDEP crossover rises steadily due to alterations in cell envelope capacitance (C_{env}) that occur due to permittivity of the membrane (ϵ_{wall}) and wall (ϵ_{wall}) components. At media conductivities beyond 0.1 S/m, the pDEP crossover gradually approaches the second crossover frequency (f_{x02}), thereby causing a sharp knee. As per the linear scale plot in Fig. 9b, alterations in cell envelope capacitance can be sensitively discerned in the 0.1–0.2 S/m range, whereas beyond

0.2 S/m, the plot is most sensitive to alterations in cytoplasm can be discerned. Early studies on DEP-based phenotypic analysis to study drug and inter-microbial interactions were conducted by the group of Hughes. Based on the dielectrophoretic collection spectra of antibiotic-sensitive and antibiotic-resistant strains of *Staphylococcus epidermidis*, Johari et al.¹⁸¹ demonstrated that alterations in cell wall conductivity occurred solely after antibiotic treatment of the antibiotic-sensitive strain, whereas this parameter was not significantly altered for the antibiotic-resistant strain. Based on this, they suggest the utility of DEP methods for assessment of bacterial resistance to antibiotics. In a follow-up study by Hoetges et al.¹⁸², the use of DEP was shown to significantly speed the assessment of drug resistance of *E. coli* to the antibiotic polymyxin B versus the results obtained based on conventional disk diffusion methods. Jones et al.¹⁸³ observed lower dielectrophoretic mobility under DC fields within a gradient-based dielectrophoresis device for the gentamicin resistant *Staphylococcus epidermidis* strain versus the respective susceptible strain. While they attribute this observation to the presence of antibiotic resistance enzymes in the former strain, it is unclear how this influence correlates to the DC DEP mobility. Since the DEP behavior at DC and low frequency levels is dependent on the cell wall, it is possible that alterations in the cell wall structure (likely thickness) are the cause for the observed DEP mobility differences between the two strain-types. The measurement of antibiotic susceptibility by monitoring microbial metabolism and growth in the presence of antibiotics has recently seen the application of DEP techniques by Peitz et al.¹⁸⁴ Specifically, DEP in a microfluidic channel under laminar flow was used to capture and immobilize live cells in the imaging focal plane, so that the growth rates of single cells could be sensitively monitored for accurately measuring the minimum inhibitory concentration from a dose-response plot of antibiotics on microbial growth. While it is well known that microbial cells undergo shape changes after antibiotic treatment, it has also been shown that cells of *Mycobacterium tuberculosis* in a dormant state, which are likely to be antibiotic-resistant have much thicker cell walls¹⁸⁵, as well as smaller cell size¹⁸⁶ and distinct differences in cell wall composition¹⁸⁷ versus those of the respective viable cells in their active state. In recent work on *Mycobacterium smegmatis* (a close relative of *M. tuberculosis* that is fast-growing and non-pathogenic), Zhu et al.¹¹² used the low frequency DEP spectra where cell wall characteristics dominate, to show distinct differences in conductivity for active (lowest at 56 ± 2 mS/m), versus dormant (81.2 ± 1 mS/m) and non-viable (95 ± 1.5 mS/m) cells that are consistent with their expected cell wall differences. On similar lines, Hawkins et al.¹⁸⁸ used DEP techniques to study the phenotypic changes to *Mycobacterium smegmatis* after treatment with ethambutol, which is known to alter cell wall structure. Based on the steady decrease in permittivity of the cell envelope with antibiotic treatment, they suggest that the ability of DEP to sensitively and selectively monitor phenotypic alteration can be applied to distinguish antibiotic-susceptible versus antibiotic resistant strains. In other work, Elitas et al.¹⁸⁹ used patterned 3D carbon electrodes for high throughput pDEP-based enrichment of intact of persisting cells from cultures of *M. smegmatis* treated with isoniazid to isolate samples for downstream detection using transcriptomic and proteomic determination. The selection purity of the isolated samples, which contain $\sim 3 \times 10^4$ cells, was confirmed to be greater than 99% by flow cytometry. While the application of a label-free technique to achieve cell isolation is remarkable, it also highlights the challenges for DEP in terms of its limited throughput, even in this work that uses 3D carbon electrodes to enhance throughput.

First, the starting sample of this work contained large proportions of intact cell (90%), whereas persistent subpopulations usually occur at the levels of one in a million. Second, in spite of the high starting cell levels, $\sim 10^4$ cells were isolated whereas transcriptomic and proteomic determination techniques usually prefer $>10^5$ cell-levels. The core issue is that DEP microfluidic device volumes are low (usually $<100\ \mu\text{L}$) to retain the ability to create high fields at reasonable voltage levels and the required flow rates are small (few $\mu\text{L}/\text{min}$), since the highly localized DEP forces do not support trapping at higher flow rates. In another study to characterize the emergence of multi-drug resistance within *Mycobacterium tuberculosis* infections, Inoue et al.¹⁸⁰ used DEP on a surrogate strain. The phenotypic changes that are induced by rifampin, which affects the cell interior by binding with the RNA polymerase; versus by isoniazid, which affects the wrinkles on the cell envelope structure were compared. Through analyzing the variations in crossover frequency with respect to media conductivity, the Clausius-Mossotti dispersion was derived. Based on this, it was inferred that isoniazid treatment caused the capacitance of the cell envelope to be reduced by 78%, while treatment with rifampin caused the cytoplasmic conductivity to drop by 27%, consistent with the respective proposed mechanisms for drug action. The shape alterations induced by antibiotics can cause substantial alterations to their polarization dispersion (Fig 3b), since the induced dipole strength of an ellipsoidal particle scales with its length¹⁹⁰. Chung et al.¹⁹¹ track the susceptibility of *E. coli* to antibiotics by using DEP to quantify their elongation rate. Specifically, following treatment with varying levels of the antibiotic Cephalexin (1–64 $\mu\text{g}/\text{mL}$), pDEP is used to fix bacterial cells on the focal plane between electrodes to enable facile and accurate estimation of cell length. Their results show that while a steady rise of cell length is apparent with Cephalexin levels of 8 $\mu\text{g}/\text{mL}$, the DEP crossover frequency shows far greater sensitivity to Cephalexin treatment, with a steady drop apparent from levels of 2 $\mu\text{g}/\text{mL}$ and higher. In follow-up work, Chung et al.⁷³ assessed the influence of β -lactam antibiotics on several Gram-negative bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*, by quantifying their cell elongation and the downshifting of their pDEP crossover frequency. Based on this, the respective minimum inhibitory concentration (MIC) levels were determined and validated versus conventional broth dilution methods. In other recent work, Schroder et al.¹⁹² applied Raman spectroscopy for the detection of DEP trapped *Enterococcus faecalis* and *Enterococcus faecium* bacteria that were cultured in the absence and presence of vancomycin to characterize the molecular response of sensitive versus resistant strains for understanding the mechanisms underlying vancomycin resistant enterococci of induced resistance. By focusing on the Raman spectra within the spectral regions (at $1236\ \text{cm}^{-1}$ and $1450\text{--}1475\ \text{cm}^{-1}$) and using a three-level statistical analysis method to remove strain-specific information from the spectra, comparison of different strains can be conducted to show significant differences within 90 minutes of vacomycin treatment.

C. difficile infection (CDI), which is attributed to colonization by pathogenic bacterial strains following disruption of the intestinal microbiota after administration of broad-spectrum antibiotics within hospital settings, is a major epidemic due to the global emergence of antibiotic-resistant strains. In recent work, Su et al.⁷² showed that phenotypic alterations to the toxigenic strain of *C. difficile* after vancomycin treatment could be monitored based on the sharp drop in its maximum DEP velocity (measured at 1 MHz) that

likely occurs due to a loss in cytoplasmic polarizability. As per Fig. 9f, pDEP velocity of untreated *C. difficile* is $\sim 30 \mu\text{m/s}$, whereas after antibiotic treatment it drops to $5\text{--}7 \mu\text{m/s}$, with no difference between 4 and 24 hours of treatment. On the other hand, the standard microbial technique based on ELISA assay of the toxin produced by *C. difficile* is not very sensitive after antibiotic treatment, thereby requiring various levels of culture and comparison against the requisite control for untreated *C. difficile* at the same culture time. As a result, immunoassay of the secreted toxin requires at least 24 hours for detecting substantial alterations after vancomycin treatment, whereas the same can be done within the first time point (4 hours) based on the pDEP velocity alteration. In related work, Su et al.¹⁹³ show that co-culture of the toxigenic strain of *C. difficile* with a non-toxigenic strain of *C. difficile* caused a sharp drop in the capacitance its cell envelope (to 35% of its original value) as detected by up-shifting of its inflection to pDEP (85 kHz shift), which is correlated with TEM images showing the loss of surface area of cell envelope and the loss of colonization ability of *C. difficile* following co-culture. This is of significance due to the dire need for CDI therapies based on probiotics that alter the colonization ability of *C. difficile*, rather than therapies based on suppressing its growth under antibiotic administration, which usually leaves resistant subpopulations capable of causing infections. As a result, this DEP platform presents a rapid technique for screening probiotic combinations that could be subsequently applied within animal models to reduce CDI.

4.6. Comparison of DEP-enabled detection techniques

Finally, Table 6 compares the figures-of-merit for the discussed DEP-enabled microbial detection platforms. Based on this, it is clear that while DEP-enhanced immuno-capture and impedance platforms achieve high portability, at reasonable levels of sensitivity and assay time, DEP-enhanced PCR methods achieve high levels of sample complexity, even though assay times are longer. Finally, DEP-enhanced Raman methods can achieve good sensitivities at extremely fast assay times for a limited number of microbial detection systems, although the sample complexity is minimal.

5. Challenges and Emerging Needs

While electrokinetic separation modalities are distinguished by their label-free characteristics, with the ability for frequency-selectivity based on subcellular electrophysiology, their application towards the manipulation of a wide variety of colloids requires their integration with various microfluidic platforms. On one hand, the enhanced localized fields that arise due to microfluidic confinement effects enable significant force levels for particle manipulation, while reducing disruptive electrothermal flows, to achieve significant levels of sample enrichment. On the other hand, DEP forces are capable of particle displacement from the laminar flow within microfluidics for enabling effective separation schemes. However, widespread adoption of DEP techniques has been stymied by a few key limitations.

5.1. High-throughput sample manipulation

A key need within sample preparation prior to detection is the selective collection of target cells for enrichment in vicinity of the sensor to enhance sensitivity and reduce assay time. In

this context, DEP techniques enable rapid sample enrichment, since the collection depends on the spatial field non-uniformity (∇E). However, the same ∇E dependence causes DEP forces to be short-range; i.e. they are significant only in the immediate vicinity of field non-uniformity, thereby substantially reducing sample throughput. This is apparent within many of the reports reviewed here, wherein high DEP collection efficiencies can only be obtained at low sample flow rates. While this is not a major problem in situations with small sample volumes, it is a major drawback for particular environmental and biomedical applications, wherein the target cell may be present in low numbers within a large volume of water or blood samples. While the coupling of DEP with other electrokinetic or inertial flow techniques can scale-up the sample throughput to 0.1 mL scales, there is a need for pre-sorting steps using filtration or immune-magnetic separation methods to scale sample throughput to the mL to liter scales.

5.2. Multiplexed separations

Diagnostic platforms for particular infections need to be capable of simultaneous quantification of multiple target cell types, so that the relative levels of each type of pathogenic bacteria can be used to determine the effective therapies. This highlights the need for highly parallelized and multiplexed separations. In this context, a major drawback of the DEP technique is its ability to cause only pDEP and/or nDEP at a given separation stage, thereby separating no more than two cell-types at each stage. Some strategies around this drawback are the balancing of orthogonal flow fields¹⁹⁴, deformability characteristics and sedimentation forces¹⁹⁵ to cause multi-stage DEP separations. Another strategy is the use of multi-frequency¹⁹⁶ and wave sequencing techniques⁸⁸, to simultaneously vary the polarization dynamics on multiple particle types¹⁹⁷, but the adoption of such techniques has not been widespread. While the use of traveling-wave techniques can address this issue¹⁹⁸, it is limited by the overwhelming need to pattern the fields, whereas optical DEP techniques⁸⁹ that eliminate the need for pre-patterning have not made much advancement due to their limited frequency selectivity.

5.3. Manipulation and detection in native culture media

Another major drawback of the DEP technique is the need to adjust the media to low conductivity levels, so that effective contrasts between pDEP and nDEP behavior can be obtained for enabling effective separations. While the re-suspension of microbials from their native culture media does not significantly alter their functionality, it presents some major challenges. First, it is a time consuming and labor intensive step, since buffer changes are not easily integrated within bonded microfluidic devices. Second, the low conductivity media required for effective DEP manipulation may not integrate well with the subsequent detection step. For instance, impedance-based detection of relatively insulating cells requires for the media to be highly conducting, to enhance the impedance contrast upon DEP-capture of target cells. All of this reduces the versatility of the DEP sample manipulation technique and its coupling to various detection modalities.

5.4. Emerging trends

Based on the capabilities that DEP manipulation technologies provide towards rapid microbial detection at enhanced sensitivity levels, as well as its ability for analyzing alterations to subcellular phenotypes, we note the following trends.

(i) 3D electrode platforms—Conventionally, planar electrodes have chiefly been utilized for DEP, since 3D electrodes fabricated on device sidewalls by electrodeposition are rather challenging⁷⁸. However, a number of methodologies have recently emerged¹⁹⁹, including those using conducting polymers²⁰⁰, silver-PDMS²⁰¹, liquid metals²⁰², screen printing²⁰³ and vapor deposition, constructed by techniques of micromolding²⁰⁴, semiconductor fabrication^{205,206,207,208,209}, and low-cost bonding methods on hybrid platforms^{210,203}. The reduced dead volumes of such 3D electrode platforms have been applied to enhance the spatial extents of the field non-uniformity^{211,212} to enable high throughput separations^{213,214}, cytometry^{215,216}, sample enrichment for biomolecular determination^{217,218}, sample transport by traveling-wave dielectrophoresis^{219,220,221}, and dielectric characterization of cells by electro-rotation^{222,223,224}.

(ii) Integrated systems for pathogen determination—While the label-free characteristic of DEP manipulation has traditionally enabled sample preparation for detection of molecular nucleic-acid type biomarkers by enhancing capture efficiency and selectivity for impedance-based detection, current trends are directed towards the application of DEP within miniaturized point-of-care type microbial concentration^{225,226} and sensing devices²²⁷. A prime example of this is the commercialization of the DEPIM technology by Panasonic Healthcare in the form of a microbial counter for dental applications. In this context, the coupling of DEP enrichment methods to Raman detection systems is also increasingly of interest (as per citation analysis in Fig. 4), especially since such enrichment reduce complexity of the instrumentation for Raman detection to enable miniaturization and portability. Another emerging trend is the use of DEP within the so-called slip-chip platform²²⁸, which contains spatially separated wells that are connected by fluidically ducts for loading reagents and samples, after which the top plate can be “slipped” relative to the bottom plate to enable the overlap of complementary patterns of wells in both plates for localizing chemical reactions. In this manner, using a sample of water-diluted blood containing a mixture of pathogens and blood cell ghost cells (BCGs), Dongyang Cai et al²²⁹ used DEP to selectively trap the pathogens within pre-designed grooves, followed by the “slip” function to mix the captured pathogens with preloaded PCR reagents for enabling multiplexed PCR and fluorescence determination. The distinguishing characteristic of this study was the simultaneous capture and detection of 12 separate pathogens from complex sample matrices.

(iii) Aptamer-based microbial recognition—While antibody-based DEP-immuno-capture methods have been widely investigated, their widespread application is limited by antibody cross-reactivity²³⁰, the absence of *in vitro* methods for their synthesis, their denaturation upon long-term storage²³¹ and the low abundance of antigens on microbial surfaces. In contrast, aptamers are recognition molecules that can be synthesized and selected through *in vitro* methods²³², and they possess three-dimensional topologies²³³ that

are designed for binding to a selected molecular²³⁴ or cellular target²³⁵, thereby causing a great deal of interest in the use of aptamers for microbial capture and determination²³⁶. In this context, J. Shanguan et al²³⁷ used aptamer-functionalized silica nanoparticles tagged with fluorophores were bound to *Staphylococcus aureus* within complex samples, so that pDEP methods could be applied to enhance capture of microbials bound to aptamer nanoparticles at the electrode gaps, while washing away other portions of the sample. In this manner, *S. aureus* was determined at detection limits of 93 cfu/mL in deionized water and 270 cfu mL in spiked water samples, within a total assay time of 2 hours.

(iv) Antibiotic susceptibility measurements—The need for alternative antibiotic susceptibility test (AST) methods that reduce detection time and enhance parallelization of the tests for multiple antibiotics is another interesting trend. Within many diseases, there is not only an urgency to detect the bacterial strain, but also to determine the susceptibility of the respective strain to particular antibiotics of interest. Given the global emergence of antibiotic-resistant strains, prescription of the appropriate antibiotic is essential to prevent fatalities in diseases such as sepsis and tuberculosis. Since antibiotic treatments cause characteristic shape changes²³⁸, electric field based polarization methods are uniquely poised to detect these shape alterations (Fig. 2) and manipulate bacteria for immobilizing live cells in the imaging focal plane. In this manner, the surviving cells during antibiotic treatments can be sensitively detected, without the need for time-consuming bacterial cultures. Finally, towards reducing the use of antibiotics^{239,240}, there is the trend of probiotic treatments. In this context, DEP methods offer the ability to quantify the subcellular alterations to the pathogenic bacterial cell resulting from interactions with probiotic strains enable correlations to the observed phenotypic changes in pathogenic colonization and infection. In this manner, DEP-based methods can serve to screen for particular probiotic combinations *in vitro* for subsequent *in vivo* testing of the optimal combinations.

(v) DEP coupled to high-resolution microscopy for live-cell imaging—The examination of dynamical adaptations within live cells, such as the mutations leading to the emergence of antibiotic and multi-drug resistance, require time-dependent imaging of the morphological alterations within live cells, including cell shape, cell shrinkage, loss of membrane asymmetry, mitochondrial membrane depolarization, nuclear fragmentation, and chromosomal DNA fragmentation²⁴¹. Rather than applying microplate imaging and flow cytometry tools towards single cell analysis, there is great interest in applying label-free microfluidics towards sample manipulation²⁴², as well as for trapping single-cells^{243,244}, and model organisms such as zebrafish larvae²⁴⁵, due to the ability for repeated spatial and temporal analysis of single-cells after delivery of precise drug doses. In this context, DEP-based microfluidic platforms are especially powerful for downstream coupling to imaging technologies, due to their ability to enable rapid, non-invasive and selective immobilization of non-adherent cells, without the need for cumbersome chemistries for surface modification. DEP-platforms have been used towards dynamic analysis of hematopoietic stem cells undergoing drug-induced apoptosis, using real-time fluorescent imaging²⁴⁶ and environmental scanning electron microscopy (ESEM)²⁴⁷. This DEP-based ESEM platform has also been used to dynamically image the morphology of selected populations of DEP-immobilized cells²⁴⁸, for comparing the morphological changes of non-budding and budding

yeast cells following drug treatment²⁴⁹. The application of such techniques to microbials, to investigate infectivity based on alterations in cell shape⁷³, S-layer glycoprotein-induced cell wall structure¹⁹³ and lipid-induced cell membrane structure²⁵⁰ can offer dynamic phenotypic information on antibiotic susceptibility.

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Biographies

Renny E. Fernandez: Renny served as a postdoctoral fellow at the University of Virginia, from 2014–2016, specializing in electrochemical detection within microfluidic systems. His contributions include the development of dielectrophoretic platforms utilizing graphene nanomaterials and detection systems based on low-cost materials and instrumentation for application within resource-poor settings. He completed his Ph.D. degree at the Indian Institute of Technology, Madras, India.

Ali Rohani: A final-year Ph.D. student at the University of Virginia, Ali Rohani has completed his dissertation research on signal and image analysis aspects of electrokinetic manipulation of particles within microfluidic systems. His contributions include the development of an electrical tweezer for enabling highly-parallelized electrorotational measurements and the design of nanoslit devices for creating ion conductivity gradients to enhance the spatial extent of negative dielectrophoresis. He has an emerging interest in coupling machine learning techniques with bioanalysis to enable a more integrative understanding of disease models. He completed his MS degree in Electronics at Sharif University of Technology in Iran.

Vahid Farmehini: Vahid is a Ph.D. student at the University of Virginia, with research interests centered on the application of analog electronic circuits within bio-analytical devices. His key contributions have been in the development of wideband amplifiers for frequency-selective particle enrichment by electrode-less dielectrophoresis, circuit designs to enable the coupling of electrical stimulation with electromyography for measuring muscle regeneration and circuits to enable portable systems for forensic analysis in resource-poor settings. He completed his BS and MS degrees in Electronics at Sharif University in Iran.

Nathan Swami: Nathan Swami is an Associate Professor of Electrical & Computer Engineering at the University of Virginia (UVA). His group specializes in label-free microfluidics for sorting, bioanalysis and cytometry of single cells and biological nanostructures, with a focus on impedance and deformability-based methods. He co-directs UVA's Nano/Biosystems Integration Centre that specializes in soft lithography based rapid prototyping. Prior to UVA, he served on the scientific staff of the MEMS group at Motorola Labs and at Clinical Microsensors, Inc. He seeks to impact detection systems within point-of-care and resource-poor settings for enabling precision medicine, personalized healthcare and environmental monitoring systems.

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Figure 1. Citation analysis over the 2002–2016 for: (a) “Dielectrophoresis” (including “Dielectrophoretic”) and “Bacteria”; (b) same as (a), but classified based on sample type.

Figure 2.

Schematic representation of two-shell dielectric model for microbials: (a) Spherical Microbial (example: *Cryptosporidium parvum*); (b) Ellipsoidal microbial (Example: *E.coli*); (c) Example microbial polarization dispersion using a two-shell dielectric model, indicating regions wherein each subcellular phenotype dominates.

Fig. 3.

(a) Comparison of polarization dispersion of bacterial cells with a cell wall (σ_{wall} and ϵ_{wall}) in addition to the membrane (σ_{mem} and ϵ_{mem}) versus that of similar-sized cells with membrane only (mammalian cells). Simulation parameters: $\sigma_{\text{wall}}=6$ mS/m, $\epsilon_{\text{wall}}=60$, $\sigma_{\text{mem}}=26.5$ μ S/m, $\epsilon_{\text{mem}}=10$, $\sigma_{\text{cyto}}=0.5$ S/m, $\epsilon_{\text{cyto}}=60$, $\sigma_{\text{m}}=0.01$ S/m, $\epsilon_{\text{m}}=80$, cell size (a)=1.5 μ m. For bacterial cells, the wall thickness is 50 nm and membrane thickness is 8 nm; while for similar-sized cells with no wall, a membrane thickness of 18 nm is used. (b) Effect of increasing eccentricity on the polarization dispersion. Simulation parameters: $\sigma_{\text{wall}}=6$ mS/m, $\epsilon_{\text{wall}}=60$, $\sigma_{\text{mem}}=2.65$ μ S/m, $\epsilon_{\text{mem}}=10$, $\sigma_{\text{cyto}}=0.5$ S/m, $\epsilon_{\text{cyto}}=60$, $\sigma_{\text{m}}=0.1$ S/m, $\epsilon_{\text{m}}=80$, cell size (a)=1.5 μ m, wall thickness=50 nm, membrane thickness=8 nm

Figure 4.

Citations on application of DEP techniques: (a) within various device platforms; (b) towards various microbial detection modalities: Impedance, Immunoassays, Biomolecular nucleic acid determination and Raman methods. (* indicates keyword used to broaden search).

Figure 5.
Schematic of DEP enhanced immuno-capture assays

Fig. 6.

(a) Schematic of the DEP coupled to impedance measurements; (b) Schematic of DEP-based routing of bacterial cells from a large volume sample flow chamber into a confined small volume channel to enable improved sample enrichment for impedance measurements.

Fig. 7. Schematic set-ups for coupling AC electrokinetic capture to Raman spectral acquisition: (a) quadrupole electrodes used for nDEP enrichment; (b) AC electro-osmosis used to pull bacterial cells towards the center of spiral electrodes versus nDEP on blood cells as per (c). (b) and (c) are reproduced from I.F. Cheng et al.¹⁵².

Fig. 8. Schematic illustration of the application of DEP for fluorescence based sorting of picoliter scale droplets containing the target organism after PCR amplification (adapted from Lim 2015).

Figure 9.

(a) DEP crossover versus media conductivity plot, indicating the respective regions that correspond to electrophysiology of particular sub-cellular region; (b) Non-log plot of the box region from (a) showing three kinds of cells: the Control (blue), altered cell envelope capacitance (red) and altered cytoplasmic conductivity (green). (a) & (b) excerpted from Inoue et al.¹⁸⁰. (c) Co-culture of toxigenic *C. difficile* with non-toxigenic *C. difficile* causes alterations in cell envelope capacitance, as apparent from up-shifting of inflection to pDEP, which corresponds to the altered morphology of the cell envelope and its altered colonization ability (excerpted from Su et al.¹⁹³). The elongation of *E. coli* after cephalixin treatment at indicated $\mu\text{g/mL}$ levels can be sensitively detected by comparing: (d) nDEP for untreated *E. coli* vs. (e) varying degrees of pDEP due to steady elongation after cephalixin treatment (adapted from results of Chung et al.¹⁹¹). (f) A steep drop in DEP velocity of *C. difficile* after vancomycin treatment occurs within the first measurement (4 hours), whereas methods based on ELISA of secreted toxin required 24 hours of culture to enhance signals up to sensitivity level (adapted from Su et al.⁷²).

Table 1

Net particle conductivity (σ_p) of prominent bacteria

Species	Gram stain	σ_p ($\mu\text{S cm}^{-1}$)	Shape	Dimensions (μm)	[Ref]
<i>Acinetobacter calcoaceticus</i>	–	< 20	Cocci, rod	1.5×2.5	[40]
<i>Agrobacterium tumefaciens</i>	–	452 ± 50	Rod	3.0×1.0	[41]
<i>Bacillus megaterium</i>	+	888 ± 39	Rod	4.0×1.5	[42]
<i>Bacillus subtilis</i>	+	935 ± 96	Rod	10.0×1.0	[43]
<i>Enterococcus faecalis</i>	+	230 ± 21	Cocci, diplococci	2.5×2.0	[40]
<i>Erwinia carotovora</i>	–	20 ± 9	Rod	3.0×1.0	[40]
<i>Escherichia coli</i>	–	412 ± 25	Rod	2.0×0.5	[43]
<i>Klebsiella rubiacearum</i>	–	513 ± 102	Rod	3.0×0.7	[44]
<i>Lactobacillus brevis</i>	+	153 ± 18	Rod	10.0×1.2	[40]
<i>Lactococcus lactis</i>	+	416 ± 46	Spherical	1.2×1.0	[45]
<i>Micrococcus lysodeikticus</i>	+	1557 ± 116	Cocci	2.0×2.0	[40]
<i>Pedococcus damnosus</i>	+	1018 ± 68	Rod	2.5×1.0	[40]
<i>Pseudomonas putida</i>	–	195 ± 14	Rod	5.0×1.0	[46]
<i>Rhodobacter sphaeroides</i>	–	967 ± 53	Rod	1.3×0.9	[47]
<i>Saccharomyces cerevisiae</i>	+	16	Spherical (diploid)	Diameter=4	[48]

Table 2

Survey of dielectrophoretic microbial manipulation platforms

Platform	Description	Highlights	Limitations	Ref
Electrode-based	DEP-Field Flow Fractionation	Highly selective particle trapping	Requires significant nDEP behavior of bacteria	76
	Sidewall electrodes for 3D field profiles	Conducting Ag-PDMS or channel sidewall patterned electrodes for stronger lateral DEP forces	Conductivity limitations increases needed voltages for effective DEP	77,78
	DEP coupled mixing for high-throughput (> 100 $\mu\text{L}/\text{min}$)	Localized pDEP coupled to chaotic mixing for microscale enrichment with macroscale collection range	Limited selectivity at higher flow rates (throughput limited by DEP)	79
	Carbon-DEP with 3D electrodes	Facile fabrication of 3D electrodes for DEP over wide spatial extent	Lower conductivity of carbon vs. metal films	80
Insulator-based (DC Fields)	Separation at posts: DEP vs electrokinetic mobility (DC only)	Dead cells have lower DEP mobility vs. live cells, whereas electrokinetic mobilities are indistinguishable	No evidence of species-selective or strain-selective capture	81
	Oil barriers for reconfigurable field-shaping (DC only)	One-pair well for flow focusing and a two-pair well to create spatial traps for particles against crossflows	No demonstration of separation and sample collection method	82
	Gradient-DEP: Increasing localized field across global field gradient	Longitudinal separation of mixtures, rather than two-component bifurcation, produces sequential and spatially resolved separations	In DC fields: Cell wall is the chief phenotype responsible for separations, since cytoplasm polarization requires higher frequency	83
	3D insulator constrictions in depth	Large constriction ratios for particle trapping at 10-fold lower E-fields than 2D (lower Joule heating)		84
Electrode- less (AC fields)	DC-offset AC fields for controlling transverse position	Field gradients enable DEP separation by controlling traverse position of particles	K_{CM} dispersion not very different for bacteria at low frequencies	85
	Wideband single-particle DEP tracking	DEP velocity tracking of single-cells enables detection of strains and subpopulations over wide frequency	Focused chiefly on analysis, with no method for sample collection	86,87,88,72
Optical DEP (photo-conductive)	High-resolution field patterning without need for lithography	Demonstrated parallel manipulation of 15,000 particle traps on a $1.3 \times 1.0 \text{ mm}^2$ area	No evidence of frequency-selectivity and not scalable to smaller particles	89
Droplet (single-cells)	Fluorescence-based droplet sorting at 2000 droplets/s	Very high throughput, easy sample recovery and low error rates (< 1 in 10^4 droplets)	Requires fluorescence labels and droplet compatibility within emulsion	90
Conductivity Gradient	Increase media conductivity to release after pDEP	Separations based on dielectric properties versus flow rate, demonstrated for bacterial mixtures	Low throughput	91

Platform	Description	Highlights	Limitations	Ref
	Spatial nonuniformity in conductivity coupled with force field	Label-free & continuous-flow dielectric focusing: highly parallel separations of multiple populations from a heterogeneous background	Differences in bacterial nDEP dispersions are weak in the high media conductivity range	92,93
Hybrid designs & force fields	High polarizability of carbon nanotubes to enhance capture	Rapid (~ 5 min), High Throughput (~mL), High Capture 10 ⁴ bacteria/mL	Assembles microbial aggregates rather than single cells limiting specificity	94
	Electrokinetic manipulation in high-conductivity media	Couple short range DEP with long range electrothermal flow to enhance capture efficiency	Limited specificity	95,96,39
	Dielectrophoretic-Magnetic Activated Cell Sorter	Use electrical and magnetic force fields for simultaneous isolation of multiple types of bacterial cells	Needs magnetic and fluorescence labels	97

Table 3

Comparison of collection and enrichment on various DEP manipulation platforms

Device Platform	Target Organism	σ_m (mS/m)	Volume/Throughput	Enrichment Factor	Collection Efficiency	Ref
Interdigitated Electrodes	<i>Listeria monocytogenes</i> & <i>L. innocua</i>		40 μ L	10^4 – 10^5	~10%	98
DC-iDEP (insulator posts)	<i>Bacillus subtilis</i> spores, Tobacco Mosaic Virus		20 μ L	~3000	100%	99
iDEP	<i>E. coli</i>	0.5	~1 mL	Unreported	66%	100
Electrodes	<i>E. coli</i> & <i>E. faecalis</i>		60 μ L	Unreported	90%	101
Immuno-mobilized on channel wall	<i>E. coli</i>		1 mL	100 $\times$	71%	102
Nano-immunomagnet particles	<i>E. coli</i> & <i>Salmonella typhimurium</i>		20 μ L	10000 $\times$	Unreported	103
Spiral electrode with tw-DEP	<i>Cryptosporidium parvum</i>		300 μ L	Unreported	90%	104
Interdigitated electrodes with passivation layer	<i>E. coli</i> from blood cells	10	1.5 μ L	Unreported	92%	105
Interdigitated electrodes	<i>E. coli</i> & <i>C. parvum</i>	0.5	50–100 μ L		51.90%	106
Curved gold microelectrodes	<i>Lactobacillus</i> vs. yeast	25	0.3–0.7 μ L/min		89.4%	107
Traveling wave DEP	<i>S. aureus</i> vs. red blood cells	120	10 μ L/min			108
Planar parallel microelectrodes		100	70 μ L			109
Interdigitated electrodes		230			95%	110
Integrated Dielectrophoretic-Magnetic Activated Cell Sorter	<i>E. coli</i> MC1061 from blood	100	100 μ L	900-fold	95%	97
DEP separation of airborne microbes (curved electrodes)	<i>Micrococcus luteus</i> from dust particles	1	100 μ L		90% for <i>Micrococcus luteus</i>	111
Castellated electrodes in a UV cuvette	Dormant vs. nondormant <i>Mycobacterium smegmatis</i>	20	100 μ L			112

Table 4

Survey of DEP-based impedance measurements (LOD is limit of detection)

Target Cell	Sample Type	Device & Method	LOD	Assay Time	Citation
<i>Escherichia coli</i>		Antibodies added after DEP enrichment			Suehiro et al. ¹²⁷
<i>Escherichia coli</i>		Electroporomeabilization after DEP	10 ² cfu/mL	3 hours	Suehiro et al. ¹²⁹
<i>Escherichia coli</i>	Drinking Water	High flow rate (1500 µL/h) pDEP focusing onto sensing electrode	300 cfu/mL	1 minute	Kim et al. ¹³⁹
<i>Listeria</i> cells	Growth Media	DEP-based confinement of bacteria from 40 mL flow cell into 400 pL chamber (enrichment: 10 ⁴ –10 ⁵ -fold for impedance)	10 ⁴ cfu/mL	1 hour	Gomez et al. ⁹⁸
<i>Listeria monocytogenes</i>	Micro liter size in water	10 ² –10 ³ -fold DEP enrichment from 5–20 µL samples for antibody capture based impedance	10 ¹ cfu/5 µl		Yang et al. ¹¹⁹
<i>E. coli</i>	Micro liter size in water	Redox-mediated charge transfer resistance after DEP enrichment and signal amplification on secondary antibody	10 ² cells/mL		Ruan et al. ¹³³
<i>E. coli</i> O157:H7	Ground beef samples	Magnetic nanoparticle-antibody conjugates enrich cells under magnetic fields	10 ³ cells in mg ground beef	Sampling to measurement in 35 min	Varshney et al. ¹³⁴
<i>Bacillus anthracis</i> spores	Spore germination media	Rise in electrical conductivity of media during germination of pDEP captured spores	10 ⁸ spores/mL in 5 mL	~10 minutes	Liu et al. ¹³⁶
<i>Staphylococcus epidermidis</i>	Diluted PBS (mL volume at 1.8 mS/m)	Coupled long-range electrothermal flow to short-range DEP to direct cells on sensor	10 ⁵ CFU/mL in mL volume	20 minutes	Couniot et al. ¹³⁵

Table 5

Phenotypic analysis of drug interactions with target organisms using DEP methods

Drug – Organism interactions	Device Platform	Phenotypic Analysis	Inference	[Ref]
Antibiotic-resistant <i>Staphylococcus epidermidis</i>	Electrode-based DEP	Cell wall conductivity alterations: antibiotic-sensitive vs. resistant	DEP capable of assessing antibiotic resistance	181
Resistance of <i>E. coli</i> to polymyxin B	Electrode-based DEP	Cell wall alterations after treatment based on DEP frequency response	DEP speeds assessment of drug resistance versus disk diffusion methods	182
Gentamicin-resistant <i>Staphylococcus epidermidis</i>	DC DEP mobility measured in gradient DEP device	DEP mobility indicates presence of antibiotic resistance enzymes	Possible separation of resistant versus susceptible strains	183
Antibiotic susceptibility measurements by monitoring microbial metabolism and growth	Live cell DEP capture & immobilization for monitoring growth rate of single cells	Single cells growth rate used to measure response to various antibiotic doses	DEP assisted antibiotic dose-response for minimum inhibitory concentration	184
Antibiotic-resistant <i>Mycobacterium tuberculosis</i>	Frequency response in electrode-based DEP device	Dormant cells have smaller size, thicker wall & altered wall composition	DEP capable of separating dormant subpopulations from majority population	185, 186, 187
<i>Mycobacterium smegmatis</i>	Low frequency DEP (cell wall characteristics)	Wall conductivities of active, dormant and non-viable cells	Conductivity highest for non-viable, then dormant then active	112
Ethambutol treated <i>Mycobacterium smegmatis</i>	Frequency response on electrode-based device	Drop in permittivity of cell envelope with treatment	DEP-based distinction of antibiotic-susceptible versus antibiotic resistant	188
<i>Mycobacterium smegmatis</i> treated with isoniazid	3D carbon electrodes for pDEP enrichment of persisting cells	Cytoplasmic conductivity of persistent cells	Isolation of $\sim 3 \times 10^4$ with a >99% selection purity cells	189
Monitoring multi-drug resistance within <i>Mycobacterium tuberculosis</i> infections	K_{CM} factor by analysis of crossover frequency versus media conductivity	Rifampin affects cytoplasm; isoniazid affects cell envelope capacitance	DEP capable of identifying and quantifying the subcellular component altered	180
Susceptibility of <i>E. coli</i> to antibiotics	DEP frequency response in quadapole device	Elongation rate by pDEP capture of cells on focal plane between electrodes	Steady rise in cell length and drop in crossover frequency with Cephalexin treatment	191
β -lactam antibiotics on <i>Escherichia coli</i> & <i>Klebsiella pneumoniae</i>	Downshifting of pDEP crossover frequency	Cell elongation vs. dose to determine Minimum inhibitory concentration (MIC)	DEP-based MIC determination is validated versus conventional broth dilution methods	73
Vancomycin susceptibility of <i>Enterococcus faecalis</i> and <i>Enterococcus faecium</i>	Raman spectra of DEP trapped antibiotic strains	Spectra at 1236 cm^{-1} and $1450\text{--}1475\text{ cm}^{-1}$	Raman spectra of DEP-captured bacteria for	192

Drug – Organism interactions	Device Platform	Phenotypic Analysis	Inference	[Ref]
		affected by vancomycin treatment	identifying antibiotic resistant strains	
<i>C. difficile</i> after antibiotic treatment and after probiotic co-culture	pDEP velocity after vancomycin treatment and pDEP inflection after probiotic culture	Cytoplasm conductivity after antibiotics and envelope capacitance after probiotic culture	DEP methods can rapidly screen for phenotypic alterations versus conventional methods	72, 193

Table 6

Comparison of DEP-enhanced Microbial Detection Techniques

Technique	Description	Unique Features	Limitations	LOD	Assay Time	Ref
DEP-enhanced Impedance	Specific enrichment microbial on IDAs with gaps equal to microbial size	High-sensitivity information on viability at fast assay times	Identification requires known antibodies	$10^2 - 10^4$ CFU/mL	0.5–1 hr	129
DEP-enhanced Immuno-capture	DEP used to enhance microbial capture onto antibodies	Portable and easy to build platform	No viability information	Not reported	Not reported	119, 120
DEP-enhanced Raman	DEP used to specifically enrich microbials in Raman imaging area	Can identify microbial based on pre-determined fingerprint	Poor quantification in complex matrices	(a) 10^4 CFU/mL (<i>S. aureus</i>); (b) 210 CFU/mL (<i>E. coli</i> DH α 5)	Few Minutes	149, 154
DEP-enhanced PCR	DEP separation of microbials from blood cells and DNA that inhibit PCR amplification	Multi-target identification & quantification in complex matrices	Time consuming (~4 hours) and PCR not portable	10^4 CFU/mL (L. monocytogenes)	4 hours	166