Numerical modeling of transport and accumulation of DNA on electronically active biochips


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Abstract

Transport and accumulation of biomolecules, particularly DNA, in active electronic chips are investigated through numerical modeling and experimental verification. Various geometric and design configurations of electronically active DNA chips are considered. Further, we investigate the effect of electric field distribution on practical design of flow cells and chips. Particular attention is focused on the geometric effects on current and electric field distribution which are well captured by a finite element method-based model. We demonstrate that these geometric effects are observed only in buffers of very low conductivity. We also demonstrate that numerical models which do not include the charge transfer mechanism between electrodes and the buffer solution will fail to predict the reduction of these geometric effects with increased buffer conductivity.

The review of the technology is based on computer simulation using a finite element-based computational model and experimental results of electric field distribution, DNA transport and accumulation. Comparison of theoretical results for electrophoretic DNA accumulation with those obtained from experiments and a simple analytical model is presented.

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1. Introduction

The completion of the physical mapping of the human genome [1] has brought a significant increase in interest in genomics research and development. However, while the completion of the gene sequencing may seem to suggest the end of the genome era and the beginning of the proteomics era, in fact only one significant part of the genome era has been completed. Beyond the sequencing effort lies the development and maturation of DNA molecular diagnostics and eventually gene therapy.

The potential for DNA molecular diagnostics to revolutionize the medical diagnostic market is well recognized by the many commercial companies now developing technologies and assays for diagnostic purposes. Despite this recognition, market development for molecular diagnostic tools has been slow partly due to economical factors as well as technical challenges. Speed, accuracy, sensitivity and reproducibility have been the mantra of technical development of molecular diagnostic tools.

The variety of technologies being developed to overcome the technical challenges are quite clever and intriguing; but it is probably fair to say at this time that no single technology has been able to overcome all the technical hurdles with complete success. Of particular interest to us are electronically active DNA micro-arrays. These arrays have demonstrated very high accuracy and rapid transport [2–4]. There are three assay functions that these electronically active arrays fulfill: transportation and concentration of target DNA at an array site, hybridization and stringency. In this paper, we focus on the concentration of target DNA.

The majority of DNA micro-array technologies depend on diffusion of target DNA to the array sites [5–7]. Diffusion dependent transport scales with the square root of time and often takes several hours to achieve efficient hybridization of DNA molecules to capture probes or fluorescent probes to the DNA amplicons anchored at the detector surface.
is one of the main impeding factors in making the passive DNA sensing faster and more accurate within short period of time. Convection is sometimes used to speed the transport process, but still the assay times can be an hour or longer.

On the other hand, electronically active DNA micro-arrays utilize electrophoresis as the transport mechanism. Electrophoresis has the advantage that transport scales linearly with time and electric field. Consequently, typical transport times are two minutes or less. We have developed micro-electro-optical DNA sensors where transport of biomolecules is achieved through electrophoretic forces [2–4,8–11]. An electrode array covered with a permeation layer with embedded DNA capture probes is used to control the transport of biomolecules and achieve their accumulation and hybridization at the electrodes. The various electrode arrays illustrated in Fig. 1 enable both DC and AC electrokinetic manipulation of biomolecules and particles in the detector cell. The arrays consist of patterned metal electrodes on an insulating substrate and are contained in a plastic housing equipped with fluidic conduits to manipulate sample and other electrolytes. The electrical field applied affects charged analyte biomolecules and particles as well as the ions in the fluid sample.

Aside from a dependence on electric field strength, electrophoretic transport of target DNA is critically dependent on the composition of the transport buffer [8]. In electrophoresis, the total current supplied by the external circuit is carried solely by the charged ions in solution. The portion of current carried by each ionic species is dependent on its charge, mobility and concentration and is known as the transport number. Because it is desirable to maximize the transport number of the target DNA, transport buffers are typically low salt, zwitterionic buffers: 50 mM histidine has functioned well for this purpose [3,8].

In this paper, we discuss the effects of array geometry on the accumulation of DNA during electrophoresis. Accumulation is modeled using an analytical model and finite element analysis (FEA) and the results are compared with experimental data.

The numerical and analytical modeling of such an electrophoretic system has been of some interest to a growing number of researchers in the past few years. The work of Stelzle et al. [12] is among the first in the literature that used analytical modeling to predict electric field in bioships. They have looked at increasing the efficiency of accumulation through “focusing electrodes” and discussed general design principles for the case of a cylindrical shaped flow cell that contains chips with focusing electrodes. Paces et al. [13] were among the first group of researchers to report results in DNA accumulation and hybridization based on numerical modeling. They studied electrokinetic transport of charged species in an electrolyte and reviewed electric potential, electric field distribution and species transport in a chip array that consists of 5 × 5 electrodes. Their model was based on a two-dimensional finite element formulation. Based on their continuum model, they reported geometric effects on electric field with peaks at the edge electrodes. Ozkan [14] has also used the finite element method to study electric field distribution in an electronic chip array. Her work concentrated on investigating the effects of charged species concentration, size, position, type of buffer solution and patterned electrode configuration on the electric field distribution and hence the electrokinetic transport of charged species.

In this work, we investigate various configurations of active DNA chips where experimental results are available for qualitative and quantitative verifications. The quantities of interest for our study are DNA transport, accumulation and electric field distribution. We investigate the effect of electric field distribution on practical design of flow cells and chips. Particular attention is focused on the geometric effects which are well captured by a finite element-based model.

We will further show experimentally that, with increased conductivity of the buffer, such geometric effects are minimized and eventually eliminated. We suggest that the decrease in these geometric effects is caused by the decrease in polarization resistance as solution conductivity is increased. This decrease in polarization resistance in turn results in an increase in current contribution due to polarization (i.e. Faradaic current) that is significant enough to screen the effects of current contribution from solution resistance. Numerical models that do not explicitly include the polarization resistance in their formulation will fail in predicting the decrease and elimination of these effects with increased buffer conductivity.

2. Electronic accumulation of DNA

In this paper, two generations of electronically active micro-arrays were modeled and experimentally tested. The
The computational model in this work is based on the finite element formulations for fluid flow, diffusion and electrophoresis (i.e. movement of charged species relative to the carrier movement under the influence of electric field). Further, the finite element model of the electrokinetic flow problem is based on a combination of Poisson’s equations, diffusion equation and Navier–Stoke’s equations with appropriate electromigratory flux terms to represent the effect of applied electric field on carrier and/or charged species.

The Navier–Stoke’s equations which are defined here to include the equations of mass conservation, momentum conservation, energy conservation and equation of state are given as

\[
\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{v}) = 0 \quad \text{(mass conservation equation)}
\]

\[
\frac{\partial (\rho \mathbf{v})}{\partial t} + \nabla \cdot (\rho \mathbf{v} \mathbf{v} + \frac{2}{3} \rho \mathbf{g}) = -\nabla P + \nabla \cdot \tau + \rho \mathbf{F} \quad \text{(momentum equation)}
\]

\[
\frac{\partial e}{\partial t} + \mathbf{v} \cdot \nabla e = V \cdot (k \nabla T) + H + \Phi_e \quad \text{(energy equation)}
\]

where \( e \) is the internal energy, \( \rho \) the density of the fluid, \( \mathbf{v} \) the local fluid velocity, \( k \) the thermal conductivity of the fluid, \( T \) the temperature, \( P \) the pressure, \( l \) the time, \( \tau \) the shear (viscous) stress on fluid, \( g \) the acceleration due to gravity, \( F \) the body force, \( T \) the temperature, \( P \) the pressure, \( \kappa \) the thermal conductivity of the fluid, \( h \) the heat capacity of the system (given as \( \rho C_p T \) where \( C_p \) is the specific heat of the fluid), \( E \) the electric field, \( \Phi \) the electric potential, \( \mu_e \) the charge density, \( \epsilon \) the permittivity, \( \mu_{ep} \) the electrophoretic mobility of ion in its carrier species, \( V_{ep} \) the electrophoretic velocity of charged species, \( c \) the concentration of species, \( D \) the coefficient of diffusion and \( i \) is the species number.

Generally, the energy equation is coupled to the momentum and continuity equations through the variation of density with temperature and pressure as shown in Eq. (4). For incompressible flows, however, the variation of density is negligible and the Navier–Stoke’s equations are decoupled.

The following assumptions are made in the finite element modeling of the electric field distribution and DNA accumulation in the active DNA micro-array:

2.1. Description of models and experiments

The early generation chips, shown in Fig. 1a and b are the 10 x 10 and 5 x 5 arrays of 80 \( \mu \)m diameter platinum electrodes spaced with 200 \( \mu \)m center-to-center distance. Four large counter-electrodes are located at the corners of the array. External circuitry was used to control the potential or current at the array electrodes, either one electrode at a time or in groups of electrodes. The chip was mounted in a probe station for electrical connection and fluorescent measurements.

The later generation chip, shown in Fig. 1c, is a 16 x 25 array of 50 \( \mu \)m diameter platinum electrodes spaced with 150 \( \mu \)m center-to-center distance. Two pairs of rectangular, individually controlled, counter-electrodes are aligned along the perimeter of the array. Not shown in Fig. 1a–c are off-chip Ag/AgCl quasi-reference electrodes (QRE). The micro-array is flip-chip bonded to a ceramic substrate. The chip was mounted in a probe station housing that contains a flow cell for liquid sample handling and an optical window for fluorescence measurements. An important feature of this latter generation chip is the presence of on-board circuitry that permits the current or potential of each electrode to be individually controlled and measured.

The micro-arrays shown in Fig. 1 are coated with a hydrogel. This coating is about 1 \( \mu \)m thick in dry state and up to 10 \( \mu \)m thick in wet state and serves two main functions. It provides attachment chemistry for anchoring DNA probes and it elevates the anchored probes above the electrodes. The coatings are made of agarose or acrylamide copolymers and contain covalently linked streptavidin to provide attachment sites for biotin labeled oligonucleotides.

During an assay, DNA capture probes, targets or amplification products are addressed to specific array electrodes. Typically, the array electrodes are biased at +2.0 V with respect to the on-board circuitry that permits the current or potential of each electrode to be individually controlled and measured.

The computational model in this work is based on the finite element method (FEM), we consider a simplified model that this simplified electrode system captures the essence of the finite element method (FEM), we consider a simplified model that this simplified electrode system captures the essence of the hydrogel coating.
1. The effect of the charges carried by the DNA species on the electric field is neglected.
2. The effect of permeation layer on the DNA transport and accumulation and electric field distribution is neglected.
3. The sample concentration is assumed to be small compared to the concentration of the buffer solution and the conductivity of the solution is considered to be uniform throughout the liquid volume.
4. Physical parameters such as diffusion coefficients, fluid viscosity, electrokinetic mobilities, and dielectric properties are considered constant.
5. Joule heating in the liquid volume is assumed to be insignificant and the temperature of the solution is considered to be uniform.
6. Charge transfer mechanism between electrodes and the buffer solution is not included in the model. This mechanism has a significance in reducing geometric effects.

2.1.1. Case of single-electrode system

Fig. 2 shows the chip geometry of a single-electrode system used in this study. It consists of a cylindrical flow cell having a depth of 500 μm and a diameter of 1000 μm. The flow cell contains the buffer solution and recessed electrodes. The anode is of 80 μm diameter and has a thickness of 100 nm and is located at the center of the cylindrical cell and has a width of 40 μm and a thickness of 100 nm. This configuration closely mimics the electrode array chip configuration used in experiments.

2.1.1.1. FEA model of single-electrode system. Following are the parameters and constants used in the modeling. These values closely match the experimental conditions used in the modeling. The buffer solution is 50 mM histidine with a conductivity of 60 S/cm. A 5 nM concentration of single strand 20-mer DNA (DNA 20) sample is used. The valency of the DNA sample is −2 and the DNA’s electrophoretic mobility and diffusion constants are assumed to be 15,000 m²/V s and 20 m²/s, respectively. A constant potential of +2 V is applied at the anode and −2 V is applied at the cathode. This corresponds to a constant voltage mode of operation used in the experiments with the active DNA array.

Two modeling approaches are pursued in this study, i.e. a three-dimensional finite element analysis and a simplified analytical model. The finite element analysis (FEA) was carried out using the CoventorWare™ software from Coventor Inc. [15], while the simplified analytical model is based on a simple relationship between the current carried by DNA species and the total current in the chip, as described in Eqs. (10a)-(10c). The finite element simulation consisted of a finite element (FE) mesh for an axisymmetric slice of the flow cell (500 μm deep and 500 μm wide) with 1395 eight-noded parabolic elements and a total number of 6108 nodes, as shown in Fig. 3. This mesh was selected after a convergence study on current density and electric field distribution. The FE mesh, as expected, is much more dense at the location of the electrodes due to the sharp corners in the geometry and also due to the high gradient of electric field and current density at these locations. At locations further from the electrodes, the mesh is noticeably coarser resulting in saving of computational time.

The following boundary conditions are used:

\[ \delta \Phi / \delta n = 0 \quad \text{at the side walls, i.e. side walls and the top wall are insulated} \] (9a)

\[ \Phi = \text{constant} \quad \text{at the anode and cathode} \] (9b)

\[ J_i \cdot n = 0 \quad \text{(no species adhesion on side walls)} \] (9c)

where \( J_i \) is the ionic flux of species \( \text{"i"}, i = 1, \text{number of species types in the flow cell} \).

2.1.1.2. Analytical model for DNA transport. In the micro-arrays described above, the current carried by the DNA, \( J_{\text{DNA}} \), is a fraction of the total current, \( J \), carried by all the ionic species in solution [16]. This is expressed in Eq. (10a) where the conductivity of the solution due to the DNA, \( \sigma_{\text{DNA}} \), is a fraction of the solution conductivity of the buffer, \( \sigma_{\text{buffer}} \).

Fig. 2. Geometry of a ring single-electrode system in a cylindrical flow cell: (a) plan view of a ring electrode chip; (b) section view of the cylindrical flow cell.
conductivity, $\sigma$:

$$J_{\text{DNA}} = \left( \frac{\sigma_{\text{DNA}}}{\sigma} \right) J$$  \hspace{1cm} (10a)

By definition, the conductivity of the solution due to DNA is

$$\sigma_{\text{DNA}} = z_{\text{DNA}} F \mu$$  \hspace{1cm} (10b)

where $z$ is the valence charge of DNA, $c_{\text{DNA}}$ the concentration of DNA (in mol/l), $F$ the Faraday’s constant and $\mu$ is the mobility of DNA. Inserting Eq. (10b) into Eq. (10a) and rearranging we obtain

$$N_{\text{DNA}} = \left( \frac{F \mu}{e} \right) c_{\text{DNA}} J t$$  \hspace{1cm} (10c)

where $e$ is the electronic charge and $N_{\text{DNA}}$ is the number of DNA molecules transported in a given time, $t$, for a given current, $J$. We define the number of DNA molecules accumulated at an array electrode divided by the total number of DNA molecules in the flow cell as the percent accumulation:

$$\text{accumulation} (\%) = \frac{N_{\text{DNA}}}{N_{\text{flow cell}}} \times 100$$  \hspace{1cm} (10d)

where $i$ is time (s).

The parameters used for the analytical model are $\mu = 15,000 \mu m^2/V s$, $\sigma = 60 \mu S/cm$, $c = 5 \text{nM}$ and $J = 400 \text{nA}$.

2.1.1.3. Experimental conditions for single-electrode system.

The sample used for the experimental DNA accumulation studies was a 5 nM solution of a fluorescently labeled 512 oligonucleotide in 50 mM histidine. An amount of 10 µl of this sample was placed on a 25-site micro-array (Fig. 1b) coated with an agarose/streptavidin permeation layer. One of the array sites was addressed at a constant current setting of 400 nA per pad for 60 s. The accumulation of fluorescence over the array site was monitored during the 60 s address and was measured with a Princeton Instrument CCD-based camera system using target DNA containing a Bodipy Texas Red (BTR) fluorescent tag. A 594 nm excitation filter and a 630 nm emission filter was used with the BTR labeled probe. The resulting fluorescence signal was quantified by comparison to fluorescent standards of known concentration.

2.1.2. Case of multi-electrode system

The second set of computer simulations was run on a 400 independently controlled electrode pad system shown in Fig. 1c. The electrodes are configured in a 16 x 25 rectangular distribution and have each a diameter of 50 μm with center-to-center spacing of 150 μm. There are two sets of counter-electrodes placed on the periphery of the chip system. These are a pair of long and short counter-electrodes which are always biased in pairs. The short counter-electrodes, which run in the transverse direction, are 500 μm wide and about 3300 μm long. The long counter-electrodes, which run in the longitudinal direction, are 190 μm wide and about 5500 μm long. The chip’s overall dimension is 7.5 mm x 4.5 mm with a depth of about 0.5 mm.

2.1.2.1. FEA model for multi-electrode system.

A two-dimensional model of the multi-electrode system is used in this study with the finite element mesh and coordinate system as shown in Fig. 4. Different sets of columns of electrodes were biased for the simulations. The finite element model for the case of column 1 electrodes biased has 8650 parabolic elements and 15,450 nodes. The voltage applied at the anodes is a constant voltage of 2 V, whereas a $-2 V$ was applied at the counter-electrodes. In this study, the effect of permeation layer on electric field and accumulation was not modeled, as we were interested only in electric field and current distribution. To be consistent, the chips used in the experiments did not have permeation layer, either.

2.1.2.2. Experimental description for multi-electrode system.

The system was simulated to operate in constant voltage mode. This corresponds to a maximum current of 110 nA at the anodes. The chips used in the experiments did not have a permeation layer. The voltages at the electrodes and counter-electrodes were set through the software interface. Current readings on each of the electrodes were taken automatically by a software-controlled CMOS chip every quarter of a second. Different buffers were used in the experiments and for each reported result, the corresponding buffers and their concentration is given. The counter-electrodes were always biased in pairs.
3. Simulation results

This section presents some of the important numerical results obtained from the simulation of the electrochemical cells. The key quantities of interest covered in this study are: electric field distribution, transport and accumulation of DNA species.

3.1. Single-electrode system

Our numerical simulation indicates that, as soon as the electric field is applied, DNA starts moving towards the anode where it is accumulated. At the cathode, DNA is repelled due to its negative polarity. This onset of transport of DNA species in the flow cell of the active DNA micro-array depends primarily on the electric field distribution. Therefore, a significant part of our modeling efforts was focused on looking at the qualitative and quantitative distribution of electric field in the flow cell. All simulation results shown in Figs. 5–7 apply to the axisymmetric section of the cylindrical flow cell.

In Fig. 5, it can be seen that the electric field distribution depicted by field vectors is most dense at the anode located in the center and to a lesser extent at the cathode located at the periphery. It is in these regions, therefore, where transport of DNA species is highest. Further, note that depth-wise (x-axis), the electric field is very sparse far from the electrodes suggesting that accumulation from these regions will be significantly slower. The radial distribution of the electric field, plotted in Fig. 5b, shows clear peaks at the anode and cathode (at the cathode, the peaks are much lower). Further, a closer look at Fig. 5b reveals that the electric...
field is highest at the edge of the electrodes. Consequently, we expect greater accumulation of DNA at the periphery of the anode. This peak in electric field at the edges of the electrodes is consistent with previous analyses of disk electrodes that showed a higher current density at the periphery [17,18]. Moreover, at the edge of electrodes, the finite element mesh used should be as refined as possible to capture this significant peak at the edges. A significant amount of work in successfully modeling the edge effects in recessed micro-electrodes has been reported in the literature [18].

Fig. 6a and b show the depth (z) and radial (r) components of the electric field distribution for the axisymmetric model. The figures suggest that the depth-wise component of the electric field (i.e. $E_{zz}$ in the axisymmetric model) is much higher than its radial component. We also investigated the effect of flow cell depth on electric field distribution by varying the depth of the cell for a given flow cell diameter and electrode spacing. The microfluidics module of IntelliSuite™ [19] was used for these simulations. The results are summarized in Fig. 7a and b which show that the maximum electric field intensity at the edge of the anode drops only by one-third to one-half when the depth is reduced by 10-fold. The reduction of flow cell depth from 500 to 300 $\mu$m resulted in only about 15% drop of maximum electric field intensity. Moreover, away from the edge of the anode, the drop in electric field intensity is negligible. Fig. 7b shows the electric field distribution along the depth of the cell at the center of the flow cell. The figure shows that the electric field strength drops fast in the first 100 $\mu$m or so of depth. Therefore, from electric field strength and distribution point of view, the flow cell depth could be lowered to as much as 100 $\mu$m with little change in the magnitude of electric field strength and species accumulation rate. However, our experience with active DNA chips shows that considerations of allowing more volume for oxidation, bubble formation and oxygen saturation dictate that a higher flow cell depth of such as 500 $\mu$m be used. Fig. 8 shows the simulation results for the accumulation of DNA at the surface of the anode with respect to time. The DNA accumulation at the anodes was followed for 150 s at which time an onset
Table 1
Comparison of DNA accumulation results from experiments and numerical models

<table>
<thead>
<tr>
<th>Percentage accumulation of DNA</th>
<th>10 s</th>
<th>30 s</th>
<th>60 s</th>
<th>90 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental result</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Analytical model</td>
<td>&lt;1</td>
<td>0.95</td>
<td>1.9</td>
<td>2.8</td>
</tr>
<tr>
<td>FEA model</td>
<td>&lt;1</td>
<td>1.2</td>
<td>2.5</td>
<td>3.5</td>
</tr>
</tbody>
</table>

of saturation was observed. This result compares favorably with the experimental results also shown in Fig. 8.

Quantitative results for the percent accumulation are shown in Table 1. Here, the results for the FEA simulation, the analytical model and the experimental results are compared. While comparing results, it has to be noted that the theoretical models and the experimental observation have limitations. For example, the FEA simulation does not account for the buffering effect of the histidine transport buffer. Similarly, the analytical model does not account for diffusion of DNA as the concentration of DNA increases at the anode. The experimental accumulation results are based on an optical detection system that consists of a camera system that gave a focal depth of field of approximately 10 μm directly above the surface of the chip. The fluorescent data was, therefore, due to the accumulation of fluorescence only in the 10 μm deep column above the electrode. However, despite these limitations, we see that the model results and the experimental results agree reasonably well. Consequently, we believe that the analytical model and the FEA simulation capture the essence of DNA accumulation and that other phenomena not accounted for in the models are of secondary importance. The FEA simulation discussed here, however, does not model the chemistry of the histidine buffer where such effects as the consumption of H+ ions by histidine, and the generation of hydrogen and hydroxyl ions could introduce change in pH, which in turn will affect the analyte, and species transport properties in the flow cell.

The results from finite element analysis, experiment and analytical model confirm that indeed the electrophoretic accumulation of DNA occurs within few seconds and minutes, as compared to passive hybridization which sometimes could take hours. Table 1 also demonstrates that by increasing the accumulation time, significantly higher concentration of DNA is achieved at the anode. This result provides a basis for optimizing the DNA accumulation time with respect to the number of electrodes addressed and the targeted detection limits.

3.2. Results for multi-electrode system

In the following simulations of the 400-site chip (shown in Figs. 1c and 4), the conductivity of the solution is taken as 60 μS/cm, equivalent to that of a 50 mM histidine. The effects of the buffer conductivity are important and will be discussed later. The coordinate system used for the multi-electrode setup is given in Fig. 4b.

Fig. 9a shows the electric potential distribution on the 400-site chip when only the first column of array electrodes (total of 16 electrodes) and the short and long counter-electrodes were biased. It is clear that the potential peaks at the array electrodes and falls off rather quickly.
Fig. 10. Electric field distribution in a 400-site chip with electrodes at the first column biased: (a) distribution of electric field when column 1 biased, (b) distribution of electric field when column 13 biased.

3.2.1. Effect of location of electrodes on current and electric field distributions

To determine the effect of location of the biased electrodes on the electric field distribution, subsequent simulations were run where interior columns of electric pads were biased. For this study, electrodes in columns 1, 13 and 25 were biased independently. As expected, there is always a peak in electric field in the outer electric pads. A comparison of Figs. 10a and b 11a-d shows that as the column of biased array electrodes moves toward the center of the chip, the intensity of the field decreases on the two outer array electrodes and on the inner array electrodes. Again, this is due to the enhanced screening of the inner array electrodes by the two outer array electrodes.

3.2.2. Effect of counter-electrodes on current and electric field distribution

To determine the effect of the counter-electrodes on electric field distribution, two additional simulations were run. The center column of array electrodes (column 13) was positively biased, but in one simulation only the long counter-electrodes were negatively biased, whereas in the other simulation only the short counter-electrodes were biased. The differences in the electric field distributions are shown in Fig. 13. When only the short counter-electrodes are biased, we see that the magnitude of the electric field is considerably reduced compared to the cases where only the long counter-electrodes are biased or where all the counter-electrodes are biased. We also notice that the difference in electric field strength between the two outer array electrodes and the inner array electrodes is less when only the short counter-electrodes are biased.

Once again, the geometry of biased electrodes plays an important role on the electric field strength at the various array electrodes. With only the short counter-electrodes being biased, the field lines between the array electrodes and the short counter-electrodes are screened by the outer array electrodes. More detailed discussion on this important observation is given in Section 3.4.1.

3.3. Accumulation of DNA at anode

Computer simulations based on FEA models were also used to investigate accumulation of DNA on the anodes. Interesting results were obtained that may have a bearing on the design of biochips and, more importantly, assays. In this particular simulation, a bias of $+2$ V and $-2$ V was used at the anode and cathode, respectively. Fig. 13 shows the moving front of DNA as it is transported on a symmetrical half of a 100-site chip (Fig. 1a) with the first column of array electrodes positively biased. The sides of the chip are assumed to be counter-electrodes. The effect of counter-diffusion that may be significant, as discussed before, is neglected here.
In the first few seconds, DNA nearest to the anodes is preferentially accumulated and leaves behind a depletion zone. Later, at $t = 40$ s, we notice that the area surrounding the upper array electrodes has become depleted of DNA and now only the lower array electrodes continue to accumulate DNA. The band of DNA movement that starts around the top left corner then extends to the biased electrodes. DNA material at the top right corner is the last one to be transported to the column of anodes. Further, it needs to be noted that DNA is accumulated first at the biased anodes on the outer edges. The interior anodes will see accumulation of DNA species only slightly at the beginning. However, the DNA species
in the bulk of the chip volume will accumulate at these interior pads as shown in Fig. 13. This is not unexpected, as DNA species travel along the streak lines shown in Fig. 15. The movement of DNA species shown in Fig. 14 closely follows these streak lines. The top left electrode on the chip has stronger electrical fields (cf. Fig. 10) but a smaller region that drains to it as shown by the streak lines that pass through it. On the other hand, the middle electrode shown in the bottom of the symmetrical half of the chip has a relatively weaker electric field strength; but a far larger volume of species draining to it. What happens is, therefore, that the top electrode will first see a fast but decreasing accumulation rate while the bottom electrodes will see a relatively slower albeit higher volume of accumulation (Fig. 14).

Fig. 14 shows the accumulation of DNA species over time at each of the five electrodes being investigated in this model. In the first few fractions of a second of biasing, the outer-edge electrode seems to accumulate the most amount of DNA.
of DNA as shown in the figure. The middle electrode located at the bottom starts with the minimum accumulation in the first few seconds; but nevertheless manages to accumulate the most at the end of the simulation run (i.e. 200 s). The implication of this observation is that for a solution containing a small concentration of species, most of the accumulation in the first few seconds will be at the outer-edge electrodes.

3.4. Experimental results

Numerous experiments were run on the 400-site chip to validate the model predictions previously described. The on-chip electronics in the 400-site chip permit independent control of the potential at each array electrode and independent measurement of the current at each electrode. In the experiments described below the potential on the array electrodes was held constant with respect to the QRE. Current measurements were directly taken from each array electrode.

In the first set of experiments, the fluid chamber containing the chip was filled with 5 mM histidine buffer. The array electrodes on column 1 were biased at $+2\,\text{V}$ while both the long and short counter-electrodes were biased at $-2\,\text{V}$. The resulting currents simultaneously measured at each array electrode are shown in Fig. 16. The qualitative pattern of current distribution is similar to the pattern predicted by simulation for the electric field distribution previously shown in Fig. 10 wherein geometric effects at the outer-edge electrodes are prominent. Note that there is a direct correlation between electric field strength and current. Further, a qualitatively similar pattern is observed when columns 13 (middle) and 25 (right outer-edge array electrodes) are positively biased. Further, Fig. 16 compares the variation in current distribution as the location of biased electrodes is moved from the edges to the center.

In the next set of experiments, we investigated the effect of the short (vertical) and long (longitudinal) counter-electrodes on electric field and current distribution. The solution used was de-ionized (DI) water which was observed to exhibit peaking effects when all pads were turned on. First, the long counter-electrodes were biased along with the electrodes on the first set of columns. As shown in Fig. 17, the geometric effects still existed, even though the magnitude of the peaks had slightly decreased. In the second experiment, only the short pads were biased negatively. Again, as shown in Fig. 17, the peaking effects, in this case, were significantly diminished. In summary, as predicted by the FEA simulation, when a column of electrodes is biased, the long counter-electrodes have a stronger effect than the short ones.
short counter-electrodes on the current peaking at the outer electrodes. Also, as predicted by the FEA simulation, the peaking observed when all counter-electrodes are biased is due mostly to the long counter-electrodes.

In the last set of experiments, we investigated the effect of conductivity of the buffer solution on current distribution. The buffers used along with their measured conductivities are given in Table 2.

As shown in Fig. 18, the current distributions tend to be uniform as the conductivity of the solution increases. For histidine, concentrations of 0.5, 1.0, 2.5, and 5.0 mM show the peaking effect, whereas concentrations above and including 12.5 mM do not show the peaking of electric field and currents at the outer-edge electrodes. For NaNO3, a non-zwitterionic electrolyte, the geometric effect of peaking electric fields at the outer-edge electrodes was eliminated at concentrations as low as 0.001 mM. Generally, above approximately 12.5 mM concentration of histidine, the current distributions are flat. We believe that the flattening of the current distributions is a consequence of the polarization resistance (which is not affected by geometrical arrangement of the electrodes) being the dominant resistance when the solution conductivity is high. This topic is discussed further below.

3.4.1. AC impedance studies

AC impedance study was performed on the 100-site chip array (cf. Fig. 1a) to further corroborate the geometric effect of peaking of electric field and currents at the outer-edge electrodes. No permeation layer was used. Autotolab Eco Chemie potentiostat/frequency response analyzer, Model PGSTAT20 was used to apply a sinusoidal AC signal (10 mV amplitude, frequency range 20–5 Hz) between the array electrodes. The DC potential (E_Dc) was controlled with respect to Ag/AgO QRE surrounding the electrode array. Contact to individual electrodes allowed measurement of geometric effects, i.e. effect of location of the electrodes on the impedance signal.

Fig. 19 shows impedance spectra obtained at E_Dc = 0.0 V between two array electrodes (1.1 and 1.10; first number designates row and second number designates column) as a function of histidine concentration. The spectra exhibit typical Randles equivalent circuit circular shape (cf. Fig. 20). By increasing the concentration of histidine the semicircles become smaller, indicating higher current due to higher concentration of the electroactive species in solution. At zero potential applied, the Faradaic reactions are minimal and the current and/or polarization resistance is determined by the concentration of impurities which are the source of the electroactive species. At increased electrolyte concentrations, Faradaic currents increases and the polarization resistance decreases. This is evident as a decrease in the semicircle diameter in Fig. 19. In this case, the solution resistance is relatively small compared to polarization resistance because the distance between the electrodes in the array is small and no dominant electroactive species such as a redox pair is present in solution. However, solution resistance changed with increased concentration.

When the potential is moved into a region of oxygen evolution, contribution of the Faradaic currents to total current increases compared to the case where E_Dc = 0.0 V. Fig. 21 shows impedance diagrams obtained at E_Dc = 1.3 V where oxygen evolution reaction takes place. The spectra became smaller with increased concentration of the electrolyte. Also, comparing the spectra obtained in Fig. 19 (at E_Dc = 0.0 V) with the spectra obtained at E_Dc = 1.3 V, the polarization resistance decreased in the latter case. Polarization resistance, R_p, and solution resistance, R_s, were calculated for all impedance spectra using a least-square method fit for a semicircle as shown in Fig. 20. Table 3 shows values for R_p/R_s ratios for different concentrations and at the two potentials measured. At low conductivity, i.e. DI water and at low electrolyte concentrations, the R_p/R_s ratio at E_Dc = 0.0 V is up to two orders of magnitude higher compared to

Table 2

Buffers used in experiments on the 400-site chip

<table>
<thead>
<tr>
<th>Buffer type</th>
<th>Conductivity (μS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI water</td>
<td>30000</td>
</tr>
<tr>
<td>0.5 mM histidine</td>
<td>1000</td>
</tr>
<tr>
<td>1.0 mM histidine</td>
<td>500</td>
</tr>
<tr>
<td>2.5 mM histidine</td>
<td>300</td>
</tr>
<tr>
<td>5.0 mM histidine</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3

Variation of the ratio of polarization resistance to solution resistance for different buffers

<table>
<thead>
<tr>
<th>Buffer type</th>
<th>R_p/R_s 0.0 V</th>
<th>R_p/R_s 1.3 V</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI water</td>
<td>30000</td>
<td>1000</td>
</tr>
<tr>
<td>12.5 mM histidine</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>50.0 mM histidine</td>
<td>300</td>
<td>100</td>
</tr>
</tbody>
</table>
higher electrolyte concentrations. This trend is also observable at $E_{DC} = 1.3$ V, although the decrease in the $R_p/R_s$ ratio with concentration is smaller, i.e. up to 10-fold decrease. The results shown in Table 3 clearly demonstrate that at low electrolyte concentrations the total resistance is very high, and is dominated by the polarization resistance. Consequently, the total currents at the electrode array are very small and solution resistance is affected by the geometrical
arrangement of the electrodes. If the electrolyte concentra-
tion is increased, the polarization resistance decreases and
allows much higher Faradaic currents. Total current is domi-
nated by the polarization resistance which screens the effects
of solution resistance, thus the geometrical effects become
negligible. This explanation supports the observation of the
disappearance of current peaks at the edge electrodes at
higher electrolyte concentrations (cf. Figs. 9–11 and 13).
In our DNA chip applications, we utilize 50 mM histidine
as a low-salt buffer to promote the electrophoretic trans-
port of DNA. At this electrolyte concentration, as shown
above, geometric effects are negligible, i.e. current peaks
at edge electrodes are not observed. This assures uniform
electric field distribution throughout the electrode array and
uniform accessibility of the target or probe molecules to all
electrodes.

Impedance data in Figs. 19 and 21 also exhibit a lin-
ear portion of the impedance spectra at lower frequencies.
The phase angle of the linear portion approaches 45° which
indicates a diffusion controlled process in accordance to
Warburg impedance. The Warburg impedance seems to be
observable both at $E_{DC} = 0.0$ and $1.3 \text{ V}$ indicating diffusion
of electro-active impurities and oxygen species, respectively.
A more thorough AC impedance investigation could provide
insight into protonation of histidine and further understand-
ing of diffusion processes in low-salt buffer electrolytes con-
taining amino acids. These studies are in progress.

4. Discussion

Table 1 showed a comparison of two models and experi-
mental data indicating that the amount of accumulated DNA
in 60 s was approximately 1–2% of the total amount ap-
piled to the chip. The conditions of this experiment were
that only one array electrode was biased at a constant cur-
rent of 400 nA for one minute. Although the total amount of
DNA accumulated is small compared to the volume applied,
the 1–2% accumulation on an electrode of 80 $\mu$m representa
concentration increase over the array electrode of two to
three orders of magnitude. For example, in the volume 2 $\mu$m
above the array electrode, the concentration increases from
5 nM to 2.5 $\mu$M, a 1500-fold increase in concentration. This
significant increase of concentration at the electrodes gener-
ates counter-diffusion and repulsion due to similar valency
that resist further accumulation of DNA. For higher concen-
tration of DNA commonly used in these chips, this mag-
nitude of accumulation at a single electrode is found to be
quite adequate. Further, at lower concentrations of the ana-
lYTE, lower and controlled accumulation of DNA per pad may
be desirable to achieve uniform analyte distribution when
multiple electrode addressing is performed.

Attempts have been made to increase the accumulation
by using a series of focusing electrodes [12,20]. Under con-
ditions of extremely low target concentrations, this might
have some benefit. However, without the use of focusing
electrodes we have been able to reproducibly detect labeled,
synthetic targets with as few as $10^6$ molecules. At higher
concentrations typical of the amplicon concentrations from
PCR or SDA amplification reactions, the targets tend to sat-
urate the array electrodes during addressing. This can be
seen in Fig. 8 where the accumulation starts to plateau af-
ter approximately 80 s. Furthermore, the concentration of
accumulated target exceeds the binding capacity of the per-
meation layer covering the electrodes. Because the target
concentrations from PCR and SDA reactions are very high,
we find the use of focusing electrodes unnecessary in most
practical applications.
The FEA simulations showed that the geometry of array of biased electrodes has a significant bearing on the electric field strengths at the array electrode locations. This was corroborated by experiments in low conductivity buffers on the 400-site chip. In low conductivity solutions, the solution resistance is high and represents the predominant resistance in the circuit. Because of the close proximity of the outer array electrodes to the counter-electrodes the solution resistance is lower compared to the inner array electrodes. Furthermore, the outer array electrodes tend to screen the low counter-electrodes such that the inner array electrodes experience a diminished field. Alternatively, this could also be seen as a case where the electric field lines of interior electrodes are screened by the electric field lines of their neighboring electrodes on both sides, whereas the electric field lines of the outer electrodes are affected only in the interior side. The combination of these effects results in the electric field distribution and current distribution shown in Figs. 10a and b, 12a–d and 16, respectively.

In buffers with conductivities approximately above 12.5 mM histidine, the experimental results show little or no effect of the geometry of biased electrodes on the current and electric field distribution, and they are practically uniform. In buffers with higher conductivity, polarization resistance decreases significantly, i.e. Faradaic currents dominate the overall resistance. Thus, the geometric effects on the solution resistance and the overall current distribution at the electrode array are not observed. Because FEA simulations do not account for polarization resistance, they are unable to predict the Faradaic currents and hence the uniform field distribution above 12.5 mM histidine. Earlier studies reported in the literature where FEA was used [13], therefore, will fail in accurately predicting electric field distribution for buffers of higher conductivity.

In diagnostic assays, we typically use 50 mM histidine or similar electrolytes as the transport buffer. At 50 mM concentration of histidine, the charge transfer resistance dominates the circuit resistance. Consequently, the accumulation should be uniform across all the array electrodes. Fig. 8 shows that within experimental error, the accumulation is uniform.

5. Conclusions

This study demonstrates the simulation of the transport and accumulation phenomenon in an active DNA biochip array. Important quantities such as radial and depth-wise electric field distribution, DNA species transport and accumulation are modeled and their results compared with experimental findings and a simplified analytical model. The simulations indicated that understanding the electric field distribution, the driving force for electrophoretic DNA transport is crucial for the cell design and optimizing DNA accumulation at the electrodes. The finite element analysis modeling included the effect of diffusion which cannot be ignored if close correlation with experimental results is intended. Both experimental and modeling results confirmed that electronic addressing of DNA at the electrodes offers substantial advantages compared to passive transport controlled solely by diffusion. DNA accumulation is achieved within seconds at a locus of fluorescence detection. The modeling results were in close correlation with the experimental results, providing a design tool for optimizing the conditions for accumulation of DNA with respect to electric field applied, time of accumulation, targeted detection sensitivity, and the number of electrodes or detection loci.

Further, the following observations were made:

1. In chips with array of electrodes and buffers of low conductivity, geometry effects introduce a current distribution that has peaks on outer-edge electrodes.
2. For buffers with increased conductivity such as histidine (12.5 mM and above) and NaNO_3 (0.001 mM and above), the geometric effects are significantly reduced.
3. Experimental results indicate that the location of the biased electrodes also affects the current distribution and the magnitude of the peaks. Electric pads in the geometric center seem to experience less peak as compared to the edge electrodes.
4. The decrease in these geometric effects is caused by the charge transfer mechanism between electrodes and the buffer (i.e. Faradaic current). Numerical models that do not explicitly include the charge transfer mechanism in their formulation will fail in predicting the decrease and elimination of these effects with increased buffer conductivity.
5. From a consideration of electric field strength, flow cell depths could be kept as small as possible without reducing the electric field strength. However, due to fluidics considerations, oxygen saturation and bubble formation in the chip, the depth of the cell has to be significantly higher.

References

electrophoresis. While working on his fellowship, he also hosted the
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