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Generation of Large-area Tunable Uniform Electric Fields in Microfluidic Arrays for Rapid DNA Separation

L. Richard Huang*, Jonas O. Tegenfeldt†‡, Jessica J. Kraeft ‡, James C. Sturm*,
Robert H. Austin† and Edward C. Cox‡

Center for Photonics and Optoelectronic Materials (POEM), *Department of Electrical Engineering, †Department of Physics, ‡Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA.

Correspondence and requests for materials should be addressed to L. Richard Huang (email: lrhuang@ee.princeton.edu).

Abstract

Control of electric fields over large areas is crucial for the accurate delivery and manipulation of biologically important molecules in microfluidic systems. In this paper we present a novel method for generating tunable uniform electric fields over large microfluidic arrays in two dimensions, and its application to a microfabricated device that separates genomic DNA. The device fractionates large DNA molecules over three orders of magnitude faster than conventional methods.

Introduction

Most biologically important molecules, such as proteins and deoxyribonucleic acids (DNA), are electrically charged. A very effective means to manipulate these molecules on microscopic scale is using electric fields. The migration of charged molecules through a fluid under the influence of an applied electric field is known as electrophoresis. In some cases, such as the pulsed-field gel electrophoresis (PFGE) used conventionally to separate DNA of different sizes, one needs to alternate between uniform fields in different directions across a two-dimensional area, which is typically 30cm x 30cm [1, 2]. In principle, one can use two pairs of electrodes to create tunable fields in a two-dimensional area (Fig. 1a), one pair for each field component (vertical or horizontal). However, the resulting field is highly distorted, because the electrodes perturb the field generated by one another. Note that the vertical electrodes which disturbed the field in Fig. 1a cannot be omitted, because they are needed for generating fields in the horizontal direction. In conventional pulsed-field gel electrophoresis equipment, the problem is solved by using many electrodes to clamp the electric potential along a closed contour (Fig. 1b) [2]. Fundamentally, this is equivalent to imposing a Dirichlet boundary condition to the Laplace equation. This “contour-clamped homogeneous electric field (CHEF)” method is inappropriate for microfluidic applications, where the active array is only ~1cm x 1cm, however, because electrodes could interfere with other functions of a device, such as sample loading and extraction. It is also not very effective—even with the 24 electrodes typically used in commercial PFGE apparatuses, the field near the electrodes is not uniform. Furthermore, microelectrodes inside fluidic channels are susceptible to erosion and bubble generation.

We report on a novel electrical current injection method for tunable uniform fields over two-dimensional areas. The goal of our work is to apply uniform alternating fields to a two-

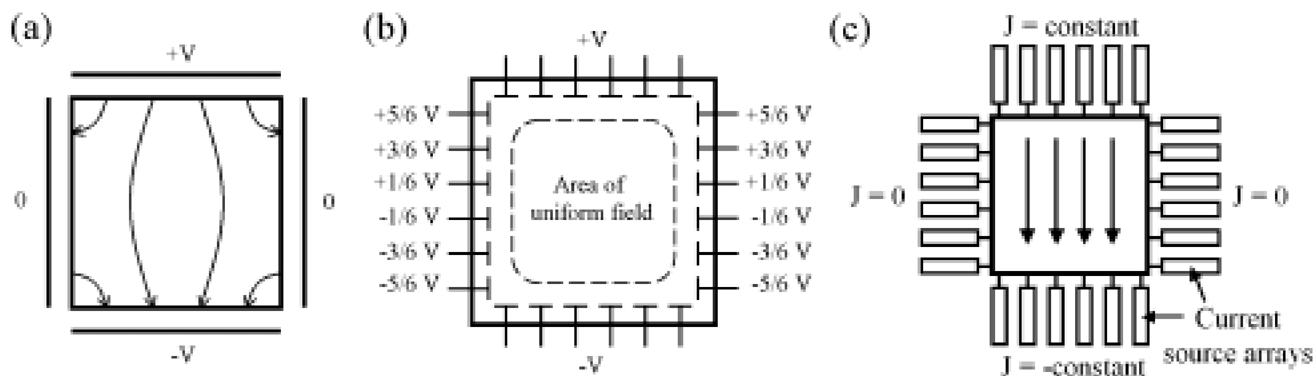

Fig. 1 (a) Highly nonuniform field generated by four electrodes. (b) Contour-clamped homogeneous electric field (CHEF) method, used in conventional PFGE apparatuses. (c) Current injection method.

dimensional array of microposts for genomic DNA separation [3]. The device is microfabricated on fused silica glass, and operates three orders of magnitude faster than conventional methods. The current injection method is extremely useful for this microfluidic application, because it (i) requires no electrode inside the microfluidic channels, (ii) incorporates structures for sample injection and extraction, and (iii) most importantly, generates uniform fields over virtually the entire area of the array.

Current Injection Method

The current injection method uses the electric current injected on a closed contour, instead of the electric potential, to define the electric field (Fig. 1c). Because the medium inside the contour has an electric current that macroscopically is linear with the electric field, defining electric current perpendicular to the boundary is identical to defining the potential gradient (normal component) along the contour. Our use of hundreds of current sources approximates Neumann boundary conditions (instead of Dirichlet boundary conditions), which uniquely determine the fields inside the boundary. In practice, current sources can just be resistors whose resistances are made high compared to that of the central array (Fig. 2a), driven by a common applied voltage. Our device uses microfluidic channels fabricated on fused silica glass as the resistors. The channels connect the central array to a few buffer reservoirs, where voltage is applied through immersed contacts. The electrical resistances of the channels are controlled by their dimensions.

Consider the field distribution in a square area surrounded by identical resistors (Fig. 2a). Because the resistances of the resistors are high, we have to apply a high voltage to drive current through the resistors. The injected vertical current from each resistor on the top is approximately the large voltage drop divided by its resistance, which to the first order is a constant. The current leakage through the resistors on the sides is negligible because the voltage drop across the resistors is small. In the limit where the resistance approaches infinity, the field inside the square area will be perfectly uniform. Since horizontal fields can similarly be generated, fields at any orientation can be created using superposition of conditions for horizontal and vertical fields (Fig. 2b). The thousands of microfluidic channels can be fabricated using one lithographic step. Therefore, this method generates very uniform fields even at regions close to the boundary, without recourse to any electrodes inside the channels. This avoids bubble generation and electrode erosion.

Modeling of Field Nonuniformity

The residual nonuniformity of the field is characterized by the root-mean-square field distortion, defined as

$$\sqrt{\frac{\iint_{\substack{array\\area}} \left|E-E_0\right|^2 dxdy}{\iint_{\substack{array\\area}} \left|E_0\right|^2 dxdy}},$$

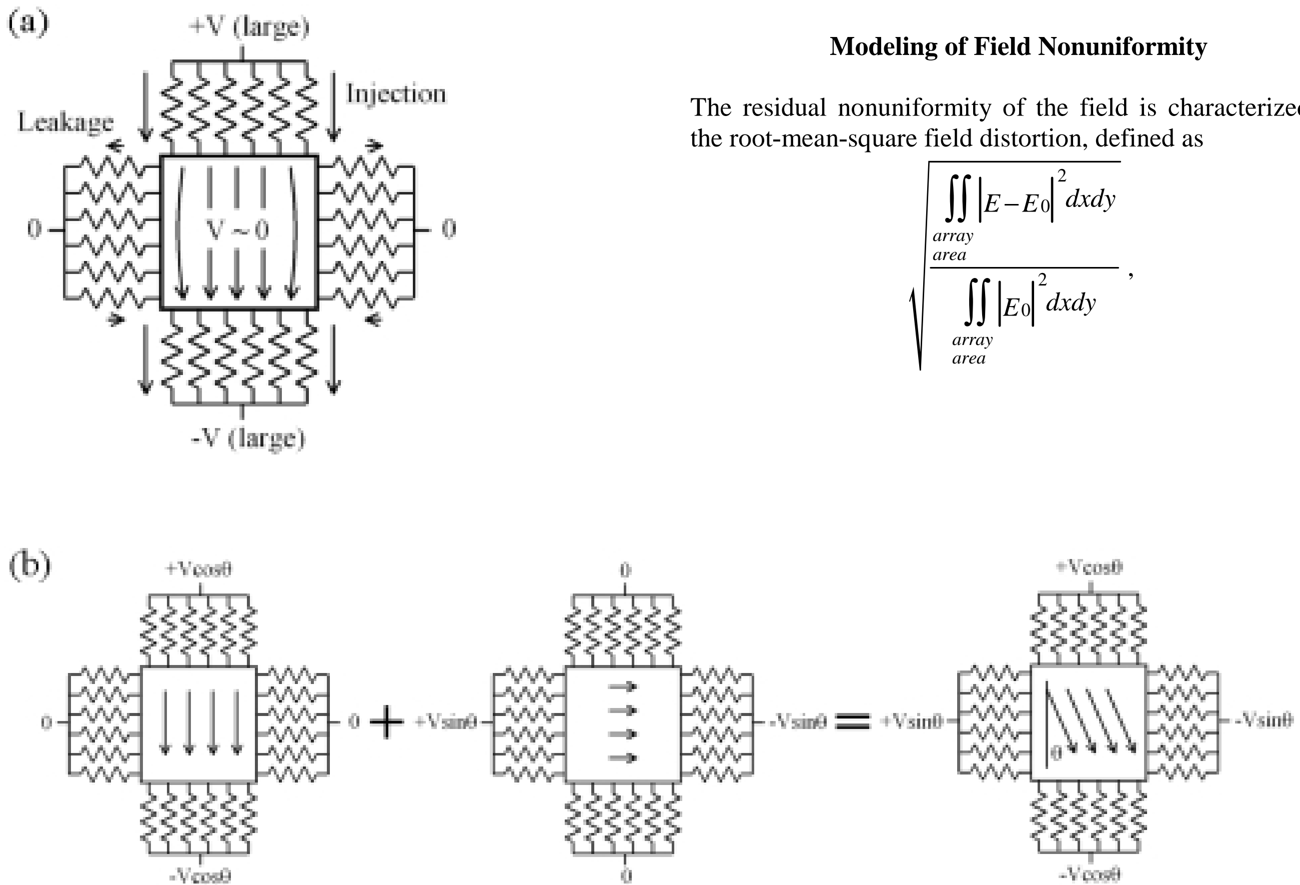

Fig 2 (a) Practical current injection with resistors. (b) Superposition principle for generating uniform fields at arbitrary directions. (θ is angle of field with respect to vertical).

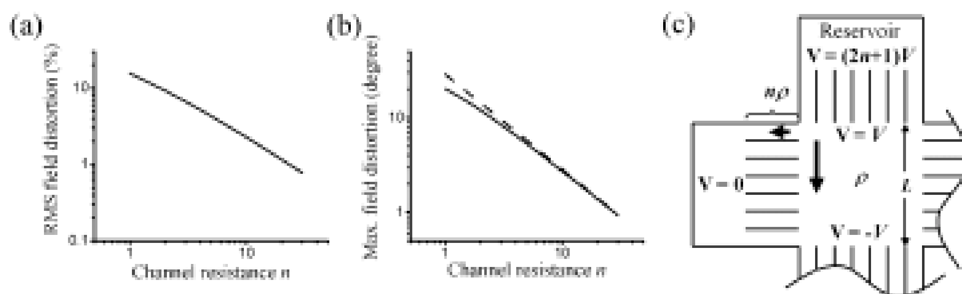

Fig. 3 (a) Computer calculated RMS field distortion as a function of channel resistance. (b) Maximum angle between the actual field and the perfectly homogeneous field. Dashed line: $1/2n$, estimated analytically. Solid line: computer simulated. (c) Microfluidic channels surrounding a square area.

where E is the field to be evaluated, and E_0 is the ideal uniform field. The vertical field generated by a typical 24-electrode CHEF (Fig. 1b) has a RMS distortion of ~9%, according to computer simulation. Most distortions come from the regions near the electrodes. To evaluate the current injection method, assume that the electrical resistance of channels in parallel on each side of the array is $n\rho$, where ρ is the sheet resistance of the central area, and $n > 1$ (Fig. 2a). Computer simulation shows that the field is made uniform as the channel resistance becomes larger (Fig. 3a). In terms of the RMS field distortion, the current injection method outperforms the CHEF method (with 24 electrodes) when $n > 2.1$. The distortion is largest at the four corners of the central area, in the case of vertical fields. The maximum angle of the field with respect to the vertical axis is shown in Fig 3b. Note that the curve approaches $1/2n$ as n becomes larger. This is because at a corner, the horizontal component of the current density is $V/n\rho L$, where V is the electric potential at the corner, and L is the dimension of the central area. Similarly, the vertical component of the current density is $\sim 2V/\rho L$. The angle of the field at the corner with respect to the vertical axis is the ratio of the two components, which is $\sim 1/2n$. Thus the field is made uniform by choosing large n. Because a large fraction of the applied voltages is then dropped over the current source resistors, a uniform field comes at the expense of its strength, for a given set of applied voltages. This is not a serious problem because microfluidic chips are generally small.

Device Design and Fabrication

The heart of the device is a two-dimensional array of microposts (Fig. 4), fabricated on fused silica. The size of the microposts is comparable to that of a DNA molecule in the ~100kbp range as a random coil. Under DC fields, DNA molecules do not interact with the microposts, and migrate at a constant mobility, independent of their molecular weight [4]. It has been shown that when the fields alternate between two directions about 120° apart, the average migration mobility becomes dependent on molecular weight [3], providing a basis on which to separate DNA of different sizes. This is because DNA molecules become stretched, and interact with the microposts. Larger molecules tangle around the posts more than small molecules do, and have lower mobilities.

The channels surrounding the array connect to buffer reservoirs, where voltages are applied. DNA molecules are injected into the array from a single channel connecting to an extra reservoir containing DNA. Eight instead of four buffer reservoirs are used to reduce the resistance needed for a given uniformity goal. The resistance of each bundle of channels in parallel is designed to be 2.2 times as large as the sheet resistance of the array ($n = 2.2$). Computer simulation shows that the field distortion is about 1% around the center section of the array, where DNA is injected and fractionated.

The fabrication process includes only one lithographic step, which defines the posts and the channels. The pattern is transferred anisotropically to fused silica substrate with reactive ion etching (RIE), using CF_4 and H_2. The etch depth is up to 6 μm. Access holes contacting the external reservoirs were mechanically drilled. Finally, the substrate is tightly bonded to a piece of glass cover slip coated with RTV silicone to form enclosed fluidic channels.

Experiments of Uniform Fields

We used the device to generate fields at 0°, 60°, and 90° with

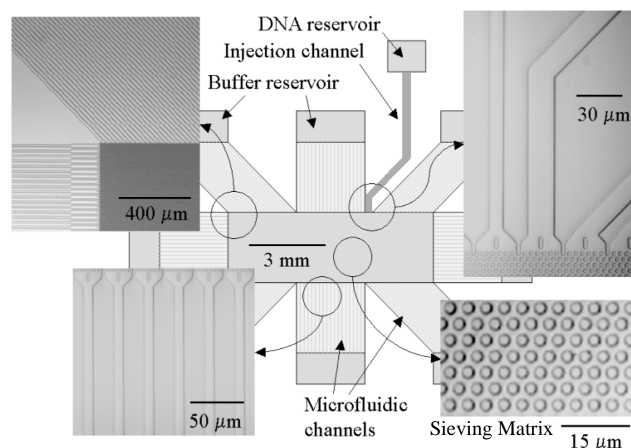

Fig. 4. Structure of the device that produces uniform field with current injection. Insets are micrographs of microfluidic resistive channels connecting arrays to electrolyte buffers reservoirs (for voltage application), and of microfabricated DNA sieving matrix (2-μm microposts with 2-μm spacing). Three electrodes/reservoirs are used on the top and bottom to reduce the required resistor values.

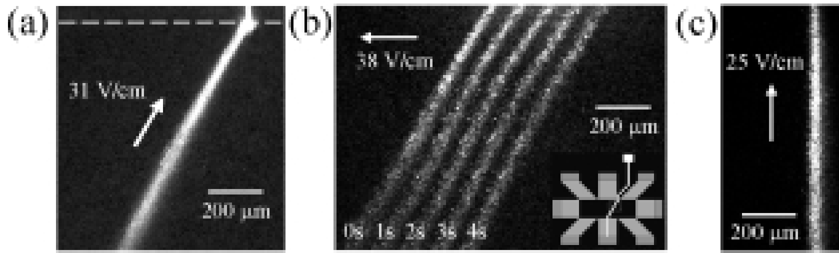

Fig. 5 Visualization of the field by fluorescence microscopy. (a) DNA injection at 60°. The dashed line marks the boundary of the array. (b) Overlay of time sequential fluorescent images of motion of DNA, to show spatial uniformity of electric field through steady motion of band. Band on left (0 s) is after DNA injection using electric field of -30° with respect to vertical (52 V/cm), other four bands are at one second intervals with field of 38 V/cm in horizontal direction. (c) DNA injection at 90°.

respect to the horizontal axis (Fig. 5a, 5b, and 5c). DNA molecules were stained with fluorescent dye, and observed with an optical microscope. First, a 60° field of 31V/cm was applied. The DNA molecules formed a straight band as they travelled along the electric field, with the maximum deviation from the desired angle of ~2°. We then switched the field to horizontal (Fig. 5b). The band moved at a constant speed in the horizontal direction. The trajectories of the molecules revealed that the field is now strictly horizontal. The band of the DNA is also very straight when the field is switched to vertical (Fig. 5c). We conclude that the device generates uniform fields over a large area at multiple angles.

Application to Genomic DNA Separation

The device was used to separate bacterial artificial chromosomes (BAC), a class of recombinant DNA that plays a key role in genome projects. The BACs were isolated and purified from transformed *E. Coli.* strains, using standard miniprep protocols [5]. 61 kbp and 158 kbp of BAC (18µm and 54µm long, respectively) were mixed and injected into the array by a vertical field (Fig. 6a). Note that DNA is negatively charged. The field was then switched alternatively between +60° and – 60° with respect to the horizontal axis to separate DNA (Fig. 6b). The DNA migrated towards the average field direction. In less than 7 seconds, the 61 kbp DNA was cleanly separated from the 158 kbp molecules, well over three orders of magnitude faster than the conventional PFGE method. The resolution, defined as the full width at the half maximum of a band, is ~ 77 kbp at 7 seconds, and ~ 36 kbp at 14 seconds.

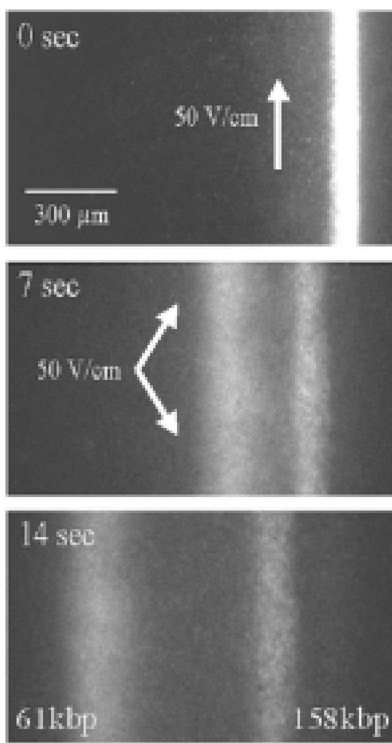

Fig. 6. Progress of separation of DNA mixture in microfabricated device, with loading by 50 V/cm vertical field (0 s), followed by separation after 7 s and 14 s with symmetric pulsing of 50 V/cm fields (± 60° with respect to horizontal, 167 ms pulse duration). Small DNA fragments (61 kbp) move faster than large ones (158 kbp). Such a separation requires uniform fields to keep the bands straight and parallel. DNA is viewed by fluorescent microscopy.

Conclusions

A new method for generating tunable uniform electric fields over entire microfluidic arrays has been demonstrated. The current sources can easily be microfabricated with single lithographic process. Since no electrodes are needed in the microfluidic channels, bubble generation and electrode erosion problems do not exist. The small number of voltage contacts makes the method practical for implementation, and the large number of resistors gives very uniform electric fields. The channels may be used to deliver and extract the DNA to and from the array. The current injection method is compatible with many functions of lab-on-a-chip devices, and should be useful for many practical microfluidic applications.

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