Assembly and constraint technology for large arrays of capillaries

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Abstract

Discovering the genetic causes of common diseases may require pan-genomic mutation scanning of all genes in a million people. An increase in throughput of genetic analysis instrumentation by several orders of magnitude is essential to undertake such an ambitious task. To this end, we report on the design, manufacture, and testing of assembly and constraint technologies for arrays containing as many as 10,000 capillaries packed into 1-D rows or 2-D arrays with 1 mm spacing. Capillaries are sealed around their perimeters against pneumatic and hydrodynamic pressures useful for automated capillary array electrophoresis. We show that both ends of the arrayed capillaries are aligned axially to 11 ± 5 μm accuracy and repeatability. Radially, the capillary tips are aligned well enough for insertion into arrays of sample wells and subsequent end-of-capillary fluorescence detection. We have loaded, electrophoresed, and detected 10^8 to 10^10 fluorescently labeled DNA molecules in a 25 capillary sub-array to validate functionality. Using our semi-automated assembly machine, we demonstrate 100 capillary array assembly in 12 min. This array assembly and constraint technology could be incorporated into commercial capillary instruments, and may enable a new generation of ultrahigh throughput instruments with 2-D arrays of 10,000 or more capillaries.

1. Introduction

To access the massive amount of information contained in complex biological systems in a timely and cost-effective manner, researchers have continually desired to increase the number of simultaneous events measured within a single instrument. Access to 500–10,000 simultaneous events in a single instrument could usher in a new era of scientific understanding of complex biological systems across entire genomes, cells, tissues, for large population samples.

In the search for the genetic causes for common diseases, for example, mutation scanning in several genes from hundreds [1], thousands [2], and ten-thousand [3] people have been undertaken. Morganthaler and Thilly [4] concluded that measurement of the mutation spectra from no less than 10^12 samples from 10^8 people would be required to ferret out statistically significant correlations between mutation and disease. In this study, sets of 10,000 persons/disease for the 100 most common diseases are required to identify genes carrying alleles conferring risk. Identification of the genes that carry multiple alleles, each coding for risk of a common disease, should permit and drive down the cost of genetic screening, increase the effectiveness of medical surveillance, and enable precise targeting for prevention and therapy of particular molecular targets.

In applications for DNA analysis such as sequencing and mutation detection, increased throughput is typically achieved by patterning more channels on a planar substrate using semiconductor manufacturing techniques or manufacturing arrays of multiple capillary tubes in which analyses can be performed. Semiconductor manufacturing techniques have enabled devices with several hundred channels on a glass substrate [5,6]. These devices have proven useful where rapid, portable genetic analysis is required; examples include crime scenes [7] and extraterrestrial rovers [8]. Applying these device designs to ultrahigh throughput tasks requiring 10,000 separation channels becomes increasingly complex when considering interfacing dozens of thermally controlled substrates with macro-scale equipment for loading, separation, detection, and collection.

Capillary array electrophoresis instrumentation containing several hundred capillaries has been commercialized [9–11]. Capillaries are typically 300–3000 mm long, with 363 ± 10 μm outer diameter (o.d.) and 75 ± 3 μm inner diameter (i.d.) (e.g., Polymicro TSP075375). The capillary rows in such instruments are typically purchased as pre-packaged assemblies, with 16–96 capillaries manually bonded in a linear array onto support brackets [J. Macomber, personal communication, January 2007].
ments to the optical properties of these linear arrays have enabled rows containing as many as 550 capillaries [12].

Two-dimensional arrays containing 32 capillaries have been demonstrated by Zhang et al. [13], complete with DNA sequencing data from all capillaries. In their impressive work that could practically be scaled to 1000 capillaries, the capillaries were permanently bonded with epoxy into drilled holes in alignment plates. Capillary alignment was limited to hole clearance, sufficient for the detection method used. Analytes were detected fluorescently in a sheath flow from the capillary tips. Fluorescence was excited with an elliptically shaped laser beam that illuminated all sample streams simultaneously from the side and the emission was captured from the capillary ends by a CCD following a wavelength dispersing prism. From the teachings of Zhang et al., we learned that this end-of-column detection technique could work well for arrays containing as many as 1000 capillaries; arrays with 10,000 capillaries would likely require a 10 W laser, sheath flow volume of approximately 50 L, and bulky and expensive optical elements.

The assembly and constraint of tightly packed 2-D arrays of many thousands of capillaries, as required for the pan genomic mutation discovery study proposed, is impractical using these approaches. Capillary arrays for ultrahigh throughput applications must be densely packed (maximum 1000 μm intercapillary spacing) into a 2-D array with the capillary ends organized regularly to enable access. Specifically, both of the capillaries’ ends should be aligned in a plane with less than 150 μm axial variation for interfacing with planar, microfabricated loading and detecting devices. In contrast with previous work, permanently bonding both aligned ends of a 10,000 capillary array would be extremely difficult. Assembly errors during this process would be permanent and costly. A replaceable constraint method would allow capillary tip alignment axially and radially and also permit capillary replacement for periodic array refurbishment. Radially, the capillary tips should not contact their neighbors and permit isolated immersion in sub-μL wells, channels, and the like, which are particularly useful for collection of eluted fractions of DNA. Isolated wells spaced 1000 μm apart, 900 μm wide would allow 360 μm capillary tip radial variation of 270 μm. The capillaries should be held rigidly in all translational degrees-of-freedom, as forces on the order of 1 N are expected to occur during access to and manipulation of the array. To control the temperature of the capillaries during analyte separation in the range of 25–80 °C, as is commonly required, we require free standing, parallel capillaries rather than a closely packed bundle.

In this paper, we describe the design, manufacture, and testing of a constraint device for arrays of 100–10,000 capillaries and the metrics used to evaluate its performance. We also present the design and evaluation of a semi-automated assembly machine for loading capillaries into the constraint device. Capillary array functionality is demonstrated with detection of electrophoresed DNA molecules in concentrations typically used in DNA sequencing and mutation detection applications.

2. Materials and methods

2.1. Capillary constraint device

2.1.1. Conceptual design

We set out to design a device which could constrain very large arrays of densely packed capillaries. Shown in Fig. 1, the device consists of a sandwich of steel, silicone, and steel that contains an array of holes. Capillaries are inserted through the holes, spaced 1 mm apart, and then the sandwich is clamped. This clamping compresses and deforms the silicone to seal around the capillaries and align them radially. Errors in the initial capillary position are averaged by elastic deformation of the silicone. The design has the additional advantage of high load capacity and stiffness in the x, y plane (see Fig. 1) [14].

This design has a number of advantages over previous methods of assembling capillary arrays as well as semiconductor manufacturing of etched channels in a planar substrate. The device can accommodate a single 1-D row of capillaries, as well as 2-D arrays containing thousands of capillary tubes, while permitting replacement. The capillaries can be aligned axially and clamped en masse. The constraint device seals against pneumatic and hydraulic pressure. Pneumatic pressure application at one end of the array is required for injection of DNA sieving media and/or hydrodynamic loading of analytes. Hydraulic pressure sealing between constraint devices located at either end of the array (see Fig. 1b) can permit flow of a fluid for thermal control. The free-standing capillaries are amenable to useful [15], rapid, uniform thermal cycling in a cross-flow water heat exchanger, due to inherently low thermal capacity and surrounding space for flow. In addition, the constraint devices accurately and repeatedly locates an array of capillaries locally and

![Fig. 1. Schematic of the constraint device (a) and its application to constraint of a 100 capillary array (b). After capillaries are loosely inserted through holes in the device, clamping deforms the silicone layers to locate and seal around the capillaries.](Image)
Fig. 2. Parts for a capillary array constraint device. A tellurium copper pin array (a) is used to etch square holes in the steel plates (b), made using a combination of wire and sink electrical discharge machining, while the silicone parts (c) are laser cut. These parts, with 25–100 holes, are shown for clarity; parts with 2500–10,000 pins/holes have been manufactured (see text).

globally, essential for interfacing to loading, detection, and collection devices.

To design the parts for the constraint device, we performed a mechanics analysis using closed-form equations and Finite Element Analysis (FEA) [16]. When the sandwich is compressed by a bolt pattern, a resulting pressure compresses the silicone layers and causes the steel plates to bulge outward. Our functional requirement for the maximum steel plate non-planarity is 150 μm. We empirically determined that a 4.8 mm thick silicone layer (Durometer 50A, McMasterCarr 5781T17) must be compressed by 1.5 mm (30%) to sufficiently grasp the capillaries, taking into consideration difficult to model effects such as friction between the steel and silicone. Therefore we set a ratio of steel plate to silicone deformation in the sandwich of 150 μm/1.5 mm = 10%. This will ensure that the steel plates remain relatively planar for capillary tip alignment as well as apply sufficient clamping pressure over their entire surfaces. The silicone deformation is calculated from:

\[ \delta_{\text{silicone}} = \frac{Lt}{AE_nXd} \]  

where the silicone properties are thickness \( L \), cross-sectional area \( A \), elastic modulus \( E \), and the compressive force is defined by bolt torque \( t \), number of bolts \( n \), bolt diameter \( d \), with torque coefficient \( X \approx 0.2 \) [17]. For a 10,000 capillary constraint device, four 5 mm diameter bolts with 1 N·m of torque compressing 4.8 mm thick silicone with 0.0025 m² area and modulus of 5 × 10⁶ Pa gives \( \delta_{\text{silicone}} \approx 1.5 \text{ mm} \). A stainless steel type 304 plate of 3 mm thickness only deflects 22 μm under this pressure of 1.6 MPa, so \( \frac{\delta_{\text{steel}}}{\delta_{\text{silicone}}} = 1.4\% \). For a 10,000 capillary constraint device, the same clamping pressure requires a stainless steel plate type 440C of thickness 3.5 mm and nine bolts to reduce the deformation to 0.14 mm, or 9.3%, as determined using FEA. Thus we settled on these bolts, steel plate, and silicone specifications for the designs of our constraint devices.

This constraint technology relies on a compression of 4.8 mm of 50 A Durometer, 5 × 10⁶ Pa modulus silicone with a pressure of 1.6 MPa using a pair of steel plates that deflect less than 10% of the silicone compression, as determined empirically. To generalize this design, the stiffness of the silicone is given by \( k=AE/L \), so as the silicone thickness or modulus is varied, the pressure, \( P=F/A \), where \( F=n/\pi d^2 \), required will change proportionally. For example, half the thickness of silicone should require twice as much clamping force to get the same grasping effect on the capillaries. Effects of capillary size variation are more difficult to generalize. Empirically, we recommend 160 μm of interference fit between the capillaries and silicone holes as a first pass.

2.1.2. Fabrication

Manufacturing is achieved using a combination of micro-electrode discharge machining (EDM) and laser micro-machining processes. These parts are shown in Fig. 2. To fabricate the steel plates, a tellurium copper pin array is first cut on a wire EDM (Charmilles Robofil 1020si) after selective milling to permit clamping bolts. The pins are sized 350 μm × 350 μm, spaced 1 mm apart in a square array. For arrays containing 10,000 holes, the wire EDM job is performed with 0.254 mm wire in 36 h with one part rotation of 90°. The pin array is then used as the tool in a die sink EDM (Charmilles Roboform 30) to etch 10,000 square holes simultaneously in hardened 3.5 mm thick 440C stainless steel. An overburn setting of 90 μm removing an average of 6.7 × 10⁻¹¹ mm³/min of steel required 40 h to etch through the part. The hole array in the silicone layer is pre-drilled with a 75 W CO₂ laser (Trotec Speedy 100). This process yields 200 μm diameter holes located with 25 μm accuracy and diameter variation from one silicone face to the other. A slight taper in the holes due to the laser drilling does not compromise the performance due to the large elastic deformation during use.

2.1.3. Performance evaluation

The accuracy and repeatability of capillary location in the array are critical performance criteria. By accuracy, we desire to know how close the capillaries are to their desired locations after bias error compensation. For repeatability, we ask how close the capillaries are to their previous locations after the constraint device is reclamped (e.g., after capillary replacement). These questions are relevant locally and globally, axially and radially, as functions of the length that the capillary protrudes from the constraint device.

We established an experimental methodology to address these questions. The notation is indicated in Fig. 3. First we measured the positions of the capillary tips radially and axially within the array using a microscope (Zeiss, Stemi SV 11). Our axial measurements were physically limited to single rows of capillaries. Next we performed a least squares fit to position a best-fit grid (radial measurements) or line (axial measurements) with 1 mm period. As shown in Fig. 3, we then calculated the lateral errors,
Fig. 3. Notation for measurement of the accuracy and repeatability of the capillary tip alignment in the constraint device, both radially (a) and axially (b).

Δxi, Δyi, of the actual capillary positions, xi, yi, from the best-fit grid, as well as the axial errors, Δzi, from the best-fit line. This was performed for I × J positions in the x, y plane and K positions in the z direction. This process was repeated for T trials, where each trial t represents a reclamping of the capillary constraint device. The axial accuracy of the capillary tips is then given by

\[ \rho_t = \sqrt{\frac{1}{T} \sum_{t=1}^{T} \left( \frac{1}{I} \sum_{i=1}^{I} \sum_{j=1}^{J} (\Delta x_{ij} - \Delta x_i)^2 \right) + \left( \frac{1}{T} \sum_{t=1}^{T} \left( \frac{1}{J} \sum_{j=1}^{J} \sum_{i=1}^{I} (\Delta y_{ij} - \Delta y_i)^2 \right) \right)^2}. \] (4)

Thus, the lateral accuracies in the Cartesian space are computed by averaging the standard deviation of the capillary tip errors for all positions across all trials. This preserves the spread of Δx, Δy more correctly than converting directly to Δri = \( \sqrt{\Delta x_i^2 + \Delta y_i^2} \) and taking the standard deviation of Δri. To determine the radial repeatability, we switch the order of operations so that we have the average of the standard deviations of the trials across all of the positions. This radial repeatability is

\[ \sigma_r = \sqrt{\frac{1}{T} \sum_{t=1}^{T} \left( \frac{1}{I} \sum_{i=1}^{I} \sum_{j=1}^{J} (\Delta x_{ij} - \Delta x_i)^2 \right) + \left( \frac{1}{T} \sum_{t=1}^{T} \left( \frac{1}{J} \sum_{j=1}^{J} \sum_{i=1}^{I} (\Delta y_{ij} - \Delta y_i)^2 \right) \right)^2}. \] (5)

The radial accuracy, \( \rho_r \), was determined locally for a set of 12 capillaries, as well as globally for a set of 100 capillaries. In all measurements, the tips of the capillaries were aligned in a plane, but the length of the capillary protruding from the constraint device was varied from L = 0–12 mm. The radial repeatability, \( \sigma_r \), was determined from T = 3 trials, on a set of 12 capillaries protruding L = 9 mm. The axial accuracy and repeatability were measured from K = 5 capillary positions reclamped T = 3 times with a fixed protrusion of L = 9 mm. T = 3 was used because it represents a practical number...
of capillary array reclampings during array use for electrophoresis, as well as limiting the time-intensiveness of the measurement process. Additional trials, T, would improve the statistical significance of the results.

Since we intend for the capillary constraint device to be useful for up to 10,000 capillaries, we require satisfactory performance at 3σ accuracy and 3σ repeatability, which statistically accounts for 9973/10,000 capillaries, assuming a normal distribution of errors. Additionally, uncertainty in all of the measurements arises from the resolution of the images captured through the microscope at 2.5–10× magnification. Uncertainty for axial accuracy, local radial accuracy, radial and axial repeatability, is 5μm. For global accuracy, images captured at the lower magnification of 2.5× have a resolution uncertainty of 7μm.

### 2.2. Capillary assembly

To assemble the capillary arrays efficiently, we designed and constructed a semi-automated assembly machine for row-by-row assembly. The procedure for assembling arrays using this machine is depicted in Fig. 4. The key feature of this design is a stainless steel cartridge that contains a row of evenly spaced trapezoidal troughs accurately manufactured by EDM to <5μm dimensional tolerance (see Fig. 4). Each trough pinches a capillary upon application of a preload force, thus constraining it for insertion into the array. This is similar to a technique utilized in the optical fiber industry [18]. Our cartridge is easily filled with capillaries by rolling a bundle of them across the troughs.

The machine is designed for semi-automated assembly of arrays of 100 capillaries, as shown in Fig. 5. In operation, a linear stage is automatically indexed to successive row positions in the array. At each position, the loaded cartridge is repeatably attached to the stage using kinematic couplings and translated to insert the capillary row.

We desired to characterize the performance of the assembly machine by comparing manual to semi-automated assembly times. In addition we measured the cartridge’s ability to align the capillaries for insertion into the constraint device, ρxΔx and ρyΔy from Eq. (4). The Cartesian coordinate system was retained as non-random differences in ρxΔx and ρyΔy were noted. This accuracy was determined for various lengths of protrusion, from 2.9 to 15 mm.

### 2.3. Detection from array

To validate the array assembly and constraint technology beyond the metrics already described, we experimented with detecting fluorescently labeled DNA undergoing electrophoresis in the capillary array using a detection system that can measure fluorescence excited and emitted from the ends of the capillaries in a manner similar to Zhang et al. [13]. We sought to determine that the capillaries were functional and additionally that alignment of all capillaries was sufficient for simultaneous detection, that time-resolved fluorescence emission from the tips of neighboring capillaries could be detected without cross-talk, and that adequate signal-to-noise ratio (S/N) could be achieved for typical analyte concentrations.

In these experiments, sub-arrays of 25 capillaries were first filled with a commercial sieving matrix (Spectrumedix, MPCR-500-004) using a pneumatic loader that mated with the constraint device. The respective ends of the capillaries were pushed through 1 mm thick silicone septums into common buffer reservoirs to enable electrophoresis. We then electrokinetically injected 20 bp AlexaFluor 488-labeled primers (Synthegen) from a 16.6 nM solution into the five capillaries along the diagonal of the sub-array. The capillaries were loaded serially, after every 30 s of electrophoresis. Loading was performed at 2μA for 15 s, interloading electrophoresis was conducted at 8μA for 30 s per capillary, and then the 5 capillaries were electrophoresed in parallel at 8μA×5=40μA for...
3. Results

3.1. Constraint device

We have designed, manufactured, and tested capillary array constraint devices for arrays of 100–10,000 capillaries, and subsequently assembled arrays in them. As shown in Fig. 6, the capillaries are 360 μm outer diameter, 75 μm inner diameter, 300 mm long and are arrayed with 1 mm spacing.

After assembling the arrays, we proceeded to measure the capillary alignment accuracy and repeatability. The local radial accuracy for a neighborhood of 12 capillaries is shown as a function of protrusion length in Fig. 7. At protrusions larger than 10 mm, there is a statistical likelihood that two capillaries will touch each other.

We also measured the radial errors of all of the capillaries in a 100 capillary array at a protrusion of \( L = 7 \) mm, as given by

\[
\Delta r_{ij} = \sqrt{\Delta x_{ij}^2 + \Delta y_{ij}^2}.
\]

These resulting global error map is shown in Fig. 8. The distribution of \( \Delta x_{ij} \) and \( \Delta y_{ij} \) errors is Gaussian (not shown), thus they can be assumed random. The \( 3\sigma_r \) (radial accuracy) over the entire array is 265 ± 7 μm. The largest radial errors in the global map are at the perimeter. Removing these perimeter capillaries reduces \( 3\sigma_r \) to 223 ± 7 μm. Radial repeatability for the array was also measured to be \( 3\sigma_r = 94 ± 5 \) μm (\( T = 3, L = 9 \)). Axial accuracy and repeatability were independent of protrusion length. They were measured and found to be \( 3\sigma_a = 3\sigma_s = 11 ± 5 \) μm (\( L = 9, T = 3 \)).

When the constraint device is clamped, the silicone deformation of 1.5 mm retains the capillaries under and an axial force of 3 ± 0.5 N per capillary. This is 3–15 \times greater than the force required to puncture a thin silicone septum useful for sealing a buffer reservoir. When clamped, the array can readily seal against 700 kPa nitrogen. By unclamping the constraint device, we have demonstrated capillary replacement with the 100 capillary array. Any capillary in the array can be identified by position, removed, and a new one rethreaded.

3.2. Assembly

We have used the assembly machine for arrays containing up to 100 capillaries. Assembly using a the semi-automated row-by-row strategy requires approximately 0.2 h, as compared to 0.5 h for manual assembly, a 56% reduction. Following Taylorism [21], we conducted a time and motion study (see Table 1) and recognized that substantial time is saved in the capillary placement and tip insertion, as it is done en masse for an entire row. With assembly of longer rows of capillaries, challenges were encountered with
Capillary array assembly times for manual and semi-automated assembly.

<table>
<thead>
<tr>
<th>Tasks</th>
<th>Manual assembly (per capillary)</th>
<th>100 capillary array (semi-automated assembly (per row))</th>
<th>1000 capillary array (semi-automated assembly (per row))</th>
<th>10,000 capillary array (semi-automated assembly (per row))</th>
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<tr>
<td>Capillary acquisition (s)</td>
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<td>15</td>
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<td>27</td>
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<td>Accurate placement (s)</td>
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<td>Initial tip insertion (s)</td>
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<tr>
<td>Feed through (s)</td>
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<td>107</td>
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<td>Total (s)</td>
<td>18</td>
<td>80</td>
<td>105</td>
<td>138</td>
</tr>
</tbody>
</table>

Table 1

Square arrays are assumed.

* Estimate.

Fig. 9. The cartridge performance as measured by the accuracy of the capillary tip position vs. length of capillary protruding from the cartridge. The threshold for insertion into the capillary array constraint device is also shown.

retaining the capillaries in the troughs during insertion and aligning the capillaries with the holes in the constraint devices. Thus, though the semi-automated assembly machine was quite useful for assembling 100 capillary arrays, we opted for manual assembly of larger arrays. We expect that additional design improvements will enable a reduction in assembly time of 81% for a 1000 capillary array (from 5 to 0.9 h) and 92% for a 10,000 capillary array (from 50 to 3.8 h). These results are summarized in Table 1.

The alignment of the capillaries in the cartridge for assembly has been measured as well. Shown in Fig. 9, the $\Delta x$ and $\Delta y$ accuracies are measured as a function of capillary protrusion length from the cartridge. Even at protrusions of 15 mm, the capillary tips can glide into the holes in the constraint device without hindrance. The accuracy in the x direction (coordinate system indicated in Fig. 5) is somewhat independent of protrusion length due to the asymmetrical constraint offered by the trapezoidal cross-section. In that direction the capillary rests against the steel face of the cartridge.

3.3. Detection from array

We performed detection of fluorescently labeled DNA molecules undergoing electrophoresis in a 25 capillary sub-array. The capillary tips are imaged continuously by a CCD detector. The results are shown in Fig. 10. Images were collected from a CCD every 3.5 s. Each image was divided into 25 uniformly sized, uniformly spaced regions corresponding to each lenslet. The counts for the pixels in each region were averaged and the background subtracted. These average intensities are shown as a function of time for the five active capillary and one region with two active neighbors. This resembles the common electropherogram for each capillary. Raw CCD images are included in Fig. 10 at several interesting times during the DNA elution.

From the initial 5 µL DNA solution at 16.6 nM, we injection $\sim 10^{10}$ molecules into each capillary [19,20]. Detection results indicate S/N varies from 50 to 800 for the electrophoretic peaks. Elution occurred 10–40 min after injection.

4. Discussion

We have demonstrated a facile means for constraint of hundreds to thousands of capillaries. In a neighborhood of a dozen capillaries protruding less than 6 mm from the constraint device, 99.73% of their tips do not deviate by more than 100 µm radially from their desired positions on a grid with 1 mm period. If protrusion greater than 6 mm is desired, as in fluorescence detection from the 75 µm central lumen of the capillary, a secondary guide with conical holes can be utilized to redirect the capillary tips to within tolerance. In other work [22], we have reported on such guide technology, which has the ability to align the tips to 25 µm radial accuracy over the entire array for loading and detection of analytes and can be used in tandem with the constraint device. For an entire 100 capillary array, the capillary tips have radial accuracy errors of 265 ± 7 µm when protruding 7 mm, which is sufficient for entrance into 900 µm wells spaced 1000 µm apart. This error can be reduced by shortening the protrusion or, again, utilizing a secondary guide if lengthy protrusions are required for access. When the constraint device is reclamped, as in capillary replacement, 99.73% of the capillaries return to their original radial positions within 94 ± 5 µm. Thus, array refurbishment does not hinder performance. Axially, the capillaries are aligned in a plane and stay that way after reclamping, to within 1 ± 5 µm. These repeatability errors are likely due to variations in local stick-slip conditions between the silicone and steel. For loading from a planar array of sample wells [23], or detecting fluorescence through a transparent window enclosing an electrolyte solution, this axial variation is insignificant.

In previous capillary array constraint technologies, replacing an inoperable, relatively inexpensive (~$5) capillary after merely 300 runs or bad fortune typically requires replacement of the entire bundle of 16–96 capillaries (~$2K to $5K), or a substrate with potentially hundreds of channels. In our device, the capillaries are replaceable, which reduces the cost of refurbishing an array by 100–1000x as compared to replacing the array. For the 10,000 capillary array, such refurbishment would prudently be performed after a significant portion of the array is non-functional. This device
Fig. 10. Detection of fluorescence emission from the capillary array. Five capillaries along the diagonal of a 25 capillary sub-array were electrokinetically loaded with fluorescently labeled DNA and electrophoresis was conducted. The capillaries were loaded serially with an effective 2 min phase shift from the upper left to lower right. The lenslet array (upper right) was used to excite and collect fluorescence from the capillary tips. Regions of the CCD images (bottom) were averaged to yield the common electropherogram. A blank sample, “B”, with two active neighbors is also shown.

could contribute to consumable cost and downtime reduction for current capillary array electrophoresis instrumentation, as well as enabling a new generation of ultrahigh throughput instruments with 2-D arrays of many thousands of capillaries.

Capillary array assembly time and cost is reduced by using the semi-automated assembly machine, as compared to manual assembly, since it eliminates time-consuming handling. The simple, accurate, and repeatable procedure for row-by-row assembly makes feasible the rapid manufacture of arrays of hundreds of capillaries. In this assembly machine, the cartridge positions the capillaries more accurately than the 120 µm tolerance required with allowable protrusions from 0 to 15 mm. Applications for thousands of capillaries for ultrahigh throughput electrophoresis instrumentation are probable, but have not been demonstrated in this work. This assembly technology could be readily applied to the capillary array fabrication industry, augmenting the presently entirely manual operations for row assembly of capillary arrays.

Detection results show that the capillary arrays are functional. They can be loaded with sieving matrix hydrodynamically, loaded with DNA electrophoretically and that DNA can be detected at the other end after capillary traversal. From the results in Fig. 10, the capillaries’ inner diameters are aligned sufficiently well with for fluorescence excitation and collection. There is negligible cross-talk between the capillaries, as evidence by the lack of signal in the blank capillary, “B”, with two active neighbors. Furthermore, there appears to be no residual fluorescence after the DNA has exited the capillary. The effective 2 min temporal phase shift between the capillaries is somewhat preserved, but this underscores the importance of a length calibration standard within each capillary. The multiple peaks observed from a single-stranded DNA primer are possibly due to intra- and inter-primer binding, and are not of a concern for this demonstration of array functionality.

Conflicts of interest

None declared.

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