

MICRO-WELL ARRAY INTERFACE FOR CAPILLARY ARRAY ELECTROPHORESIS

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ABSTRACT

Ultrahigh-throughput denaturing electrophoresis (>5000 channels) can enable rapid, low-cost mutation scans of human genomes. One design challenge for such instruments is successfully interfacing to the capillary channel array ends for loading and independently collecting DNA while enabling electrophoresis, permitting DNA detection, and preventing evaporation. A micro-well array based on tapered thru-holes was developed, both polymer and silicon-based, using unconventional laser drilling and microelectrode discharge machining processes. The devices were used to load and collect 10^8 fluorescently-labelled DNA primers with detection signal-to-noise ratio >7. The devices are easily scaleable to 10,000 micro-wells as required for pangenomic mutational spectrometry.

Keywords: DNA isolation, micro-well, fraction collection, capillary array electrophoresis

1. INTRODUCTION

Ultrahigh-throughput capillary array electrophoresis with 10,000 separation channels will enable pangenomic studies within reasonable time and cost. The potential scope of such pangenomic studies is tremendous, as in the exploration of the correlations between genetic mutation and disease for the 100 most common diseases in 1,000,000 people [1].

The design of this mutational spectrometer involves numerous multi-disciplinary systems to load, separate, and collect DNA mutations. Challenges include DNA manipulation, signal detection, mechanical alignment and assembly, and thermal control. An instrument schematic is shown in Fig. 1. Sensitive fluorescence signal detection which relies on a lenslet array to interrogate the tip of each capillary has been reported elsewhere [2,3]. The instrument design also requires interfacing to the ends of a 100 mm wide capillary array. This paper reports on a micro-well array interface that enables electrokinetic biomolecule loading, detection, and independent mutant fraction collection.

Capillary arrays to date have been limited to hundreds of channels, interfaced to 96-384 well-plates. These plates contribute to channel number limitations, and their large volumes increase consumable costs. Collection of interesting separation fractions from capillaries has been shown for 12-16 channel rows, such as by Minarik *et al.* [4], who devised an agarose gel bed through which the capillary tips were dragged to spread their effluent spatially. Other work [5] transports separation zones to collection vials using sheath flow.

2. DESIGN AND MANUFACTURE

The design involves loading and unloading biomolecules from a micro-well array containing up to 10,000 independent wells. The wells are tapered thru-holes which self-align the capillary tips to the detection system upon insertion. Detection is permitted by a transparent wafer backing. An integrated electrode permits conduction for electrophoresis. An adhesive backed poly-dimethylsiloxane (PDMS) seal mitigates evaporation of a well's

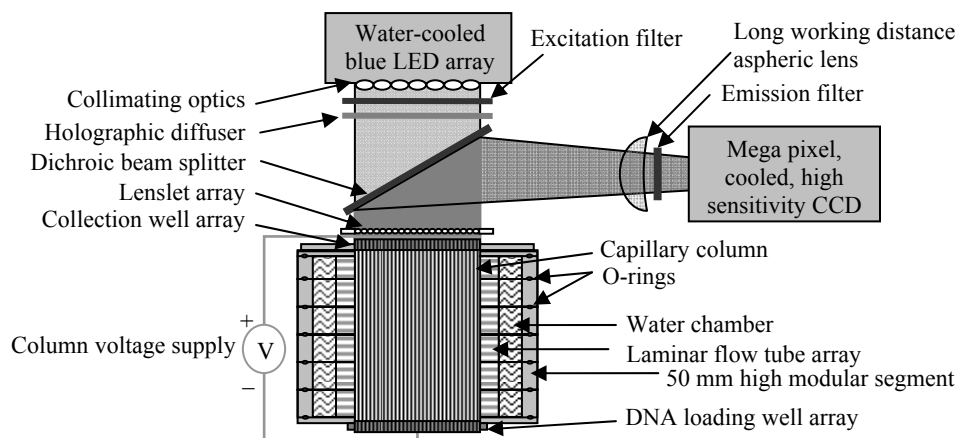


Figure 1. Schematic of the 10,000 capillary mutational spectrometer. Capillaries are spaced 1 mm apart in a rectangular array, under electrophoretic and thermal control. For DNA detection, a lenslet array enables parallel, continuous interrogation of each capillary tip.

250 nL solution for >48 hours and repeatably seals around the shaft of inserted capillaries. The assembly is electrochemically inert, so collected DNA is stable for extended durations. A schematic of the device is shown in Fig. 2a.

Both a polymethyl-methacrylate (PMMA) and a silicon-based version of the micro-fluidic chip have been designed, fabricated, and tested. In the PMMA design, 1 mm thick wafers are laser machined with a high numerical aperture (NA) focusing lens to achieve the tapered sidewalls. Laser machining is accomplished with an 80 W excimer laser with a 100 μm focal spot size directed using an x-y translation stage (Trotec, Speedy 100). The wafer is then metalized with 100 nm of Au by evaporation and bonded to a blank PMMA wafer using dichloromethane. The PDMS seal is then applied.

For the silicon-based chip, hole array fabrication is accomplished using a faster, cheaper set of processes than conventional MEMS etching, as first described for a different application by our laboratory [6]. The array is created by microelectrode discharge machining (microEDM). A plunger is fabricated first using wire EDM (Charmilles Technologies, Robofil 1020SI), and then the hole array is die-sunk through a 500 μm thick, highly doped (0.008-0.02 $\Omega\text{-cm}$) silicon wafer in <1 min using a microsink EDM (Charmilles Technologies, Roboform 30). The holes are spaced 1 mm apart, 700 μm and 400 μm wide at top and base, respectively, with process variation of approximately 10 μm . The plunger can be reused dozens of times with minimal degradation. This process and a

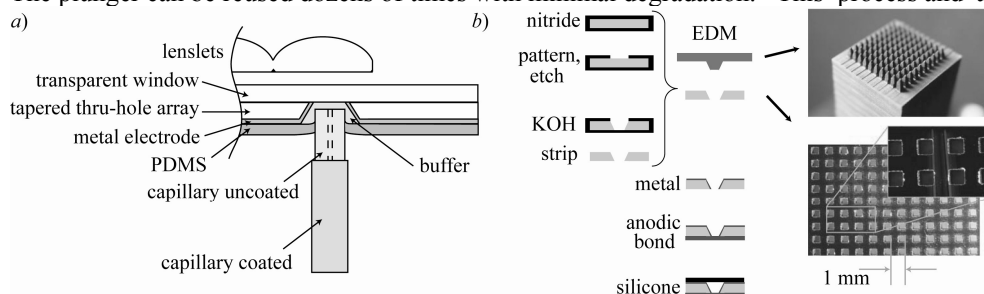


Figure 2. Buffer well array (a) cross-section and (b) process for silicon-based device.

comparison to anisotropic silicon etching are presented in Fig. 2*b*. After plunging, the wafer is cleaned, chemically-mechanically polished (CMP), metalized with Ti/Au (10 nm/100 nm) by evaporation, anodically-bonded to a borosilicate wafer, and PDMS sealed.

2. EXPERIMENTAL

To test DNA loading capability, a 20-base primer fluorescently-labelled with Fluorescein (FITC) at 16.6 nM concentration [7] and water control were electrokinetically injected from both an eppendorf tube and the silicon-based micro-well array into a 125 mm long, 75 μm ID, 360 μm OD capillary (Polymicro Technologies, TSP075375) pre-filled with a fluid polyacrylimide matrix (Spectrummedix). Detection was performed using side-column laser-induced fluorescence with a 100 mW Argon-Ion laser and photo-multiplier tube (PMT) while the DNA was under electrophoresis at 100 V/cm. To inject from the micro-well array, the capillary end was plugged through the PDMS layer into a pre-filled well. A 10 s injection at 2 μA loaded 10^8 molecules [7].

Detection and collection of DNA exiting the capillary into the micro-wells were performed for both the PMMA and silicon-based micro-well arrays. In preparation, the polyimide coating was removed from the tip of the capillary (as shown in Fig. 2*a*), and the capillary was plugged into the micro-well array through the PDMS seal. DNA was again injected electrokinetically, and then the optical detection system shown in Fig. 1 was used to interrogate the contents of each buffer solution-filled micro-well simultaneously and continuously during electrophoresis.

3. RESULTS AND DISCUSSION

For the DNA loading experiments, results in Fig. 3*a* indicate that DNA can be successfully loaded from the 250 nL micro-wells as compared to a traditional eppendorf tube containing 5 μL . Figs. 3*b* and 3*c* show the intensity of the CCD pixels covering the lenslet area versus time during electrophoresis for the PMMA and silicon-based micro-well arrays, respectively. In both cases, a peak with signal-to-noise ratio (SNR) >7 is observed, illustrating successful collection and detection of 10^8 primers using the micro-well.

The silicon-based device was created to avoid PMMA processing challenges. PMMA fabrication variation of approximately 100 μm combined with poor adhesion in the presence of the buffer solution led to unacceptable leakage between wells. The SNR for the micro-well detection could likely be improved with better capillary-lenslet alignment and a reduction in lenslet autofluorescence, both of which are being aggressively pursued to meet the detection threshold requirement of 10^6 molecules.

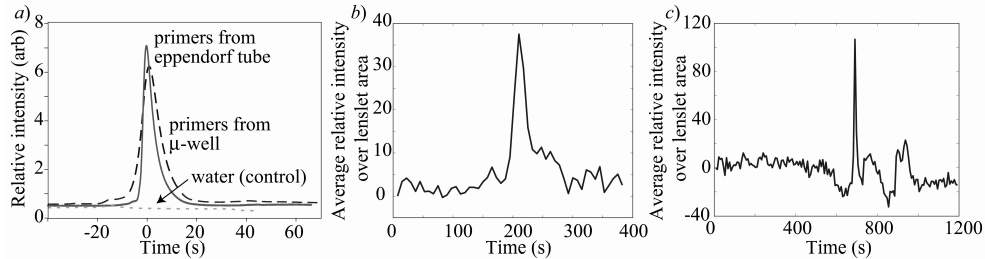


Figure 3. (a) Detection of 10^8 primers loaded from the buffer micro-well array into a capillary compared to conventional loading from an eppendorf tube. Detection of primers collected into buffer well array using (b) PMMA and (c) silicon-based devices.

These results show that an electrode discharge fabricated micro-well array can serve as a platform for interfacing to a tightly packed array of capillaries. DNA injection, detection, and collection—technologies essential for ultra-high throughput electrophoresis—were shown with 10^8 biomolecule manipulation.

4. CONCLUSIONS

To manipulate DNA, we have utilized both a PMMA and silicon-based bioMEMS design platform for interfacing to an array of tightly-packed separation channels, thus simultaneously enabling electrokinetic biomolecule loading, detection, and fraction collection of 10^8 molecules in independent wells. The silicon-based micro-well array is simple and cheap to fabricate, while meeting the design functional requirements. This device is an integral subsystem of the ultra-high throughput mutational spectrometer which will ultimately enable population-wide pangenomic analyses to uncover the genetic causes of common diseases.

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