Enhanced Separation Performance in Microfabricated Electrophoresis Devices by Electric Field Induced Collection and Metering of DNA

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ABSTRACT

Microfluidic technology is a key component in the development of microfabricated lab-on-a-chip systems for use in bioanalytical and biosensing applications. These devices continue to be developed to perform a variety of DNA analysis assays, however many of these applications deal with such *minute* amounts of DNA that it must first be pre-concentrated to a *detectable* level. On the macroscale, this pre-concentration is typically performed using centrifugation processes which are difficult to miniaturize and interface with other microfluidic components. In order to address this issue, we have developed microfluidic devices incorporating arrays of on-chip electrodes to locally increase the concentration of DNA in solution. By applying a low voltage (~1-2V) between neighboring microfabricated electrodes positioned inside a microfluidic channel, the intrinsically negatively charged DNA fragments are induced to migrate towards and collect at the anode, thereby allowing the quantity of accumulated DNA to be *precisely* metered. We demonstrate the application of this technique in electrophoresis microchips to inject a well defined *narrow* and concentrated DNA plug into an electrophoresis gel, resulting in enhanced resolution of the separating bands.

Keywords: sample injection, concentration, DNA, microchip, electrode array.

1 ON-CHIP ELECTROPHORESIS

Microfabrication technology and the pertinent economics for producing disposable and portable DNA analysis devices warrant the design of minimal sized devices. Small electrophoresis devices require that separation resolution be achieved in a short separation length. In addition, since such devices use nanoliter sample volumes, detection of the DNA is a challenge, especially if the sample is dilute.

As DNA fragments migrate through a sieving gel during electrophoresis, they exhibit a size dependent mobility such that a sample containing a range of DNA fragment lengths will separate into individual bands incorporating like-sized fragments. Any broadening the migrating bands experience during the electrophoresis run impairs the ability to distinguish neighboring bands (characterized in terms of the separation resolution R), and hence reduces the overall sensitivity of the experiment. Considering the case where dispersion effects and a finite sample injection width are the primary contributors to broadening of the migrating bands, resolution (R) can be expressed in terms of observable parameters as follows [1]:

$$R = \frac{1}{4}\left(\frac{\Delta\mu}{\mu}\right)\frac{L}{\sqrt{\sigma_{inj}^2 + \sigma_{\det}^2 + 2D^E\left(\frac{L}{\mu E}\right)}} \qquad (1)$$

Here, μ is the fragment mobility, $\Delta\mu/\mu$ is the selectivity (it characterizes relative mobilities between fragments of different length), L is the effective separation distance, σ_{inj}^2 is the variance of the injected sample plug, and $\sigma_{\det}^2$ is the variance due to the finite detector size. D^E is the longitudinal dispersion coefficient of the DNA fragments in the gel, where the superscript E indicates the electric field dependence of this parameter which often introduces significant enhancement relative to the zero field diffusion coefficient.

The dependence of the resolution of separating bands on the injected sample plug width and concentration is illustrated in figure (1). As the bands separate, they undergo dispersion and their intensity decreases, till they become undetectable even to sensitive detection equipment.

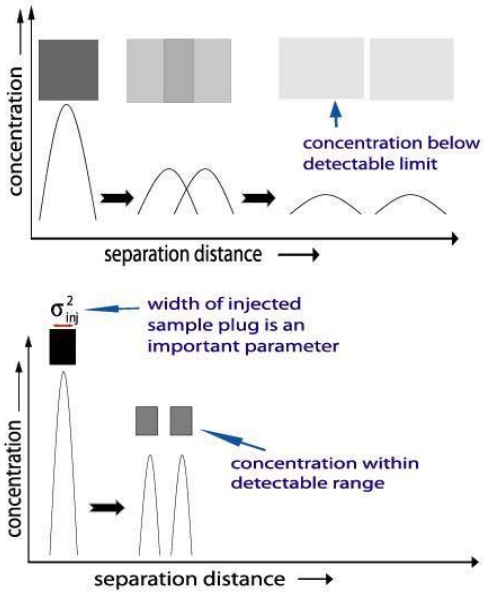


Figure 1. Illustration of the effect of injected plug size and concentration on the ability to resolve neighboring migrating zones during electrophoresis.

It is evident from equation (1) and figure (1) that in order to have a successful electrophoresis run on the microfabricated chip format, it is necessary to both concentrate and focus the injected sample plug.

2 COLLECTION AND METERING

We approach the issue by using an *array of on-chip electrodes* (figure 2) to concentrate and focus the DNA sample. The device is put together as shown in figure 3. Brahmasandra and co-workers [2] used a pair of electrodes to achieve sample compaction using very low voltages (1-2 V). By utilizing a series of such microfabricated electrodes (50µm wide and spaced at 225µm from each other) we induce a 'snowballing effect' to collect additional DNA into the injectable sample plug.

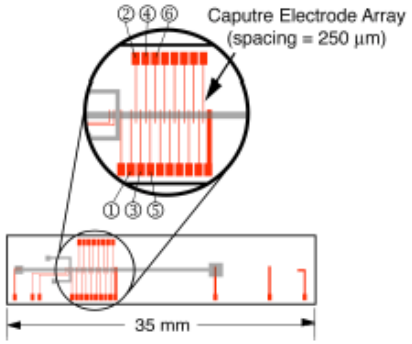


Figure 2: Schematic illustration of the microfabricated electrode array.

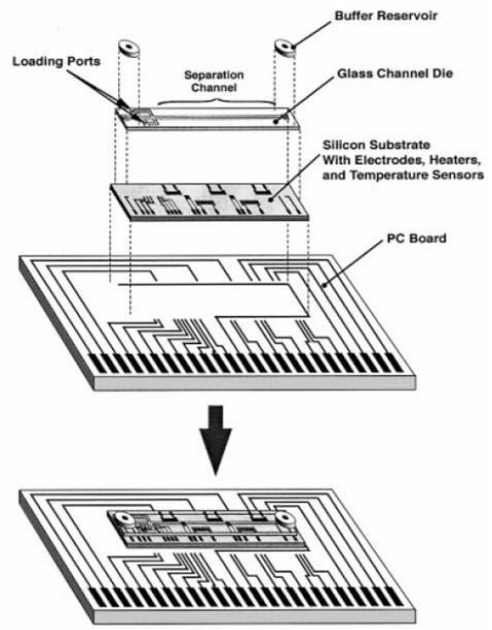


Figure 3: Exploded view of the device showing its components.

In the proposed technique, a small DC voltage (~1 volt) is applied to the first two electrodes out of the series of 'capture-release' electrodes. The DNA present between these two electrodes, being intrinsically a poly-anion, migrates to the anode and is thus 'captured' there (figure 4). The anode is then switched to the next electrode in the array so that DNA captured at the 'old' anode is released and moves toward the 'new' anode. The 'old' cathode is then switched to the 'old' anode. In this 'capture-release' process the DNA acquires a snowballing effect, as the sample gets enriched with incremental amounts of DNA that is present between each pair of electrodes. In addition, the sample plug captured on the anode assumes the shape of the anode. Eventually this leads to a focused and concentrated sample plug that is readily amenable for on-chip electrophoresis.

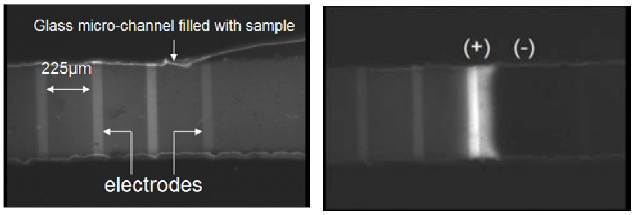


Figure 4: Fluorescently labeled DNA imaged under a microscope. The snapshot on the right shows the DNA being captured onto the anode at a voltage of 1V.

Discrete stepwise increases in the fluorescence intensity at subsequent electrodes of the fluorescently labeled DNA correspond to discrete increases in the DNA concentration occurring during the capture process. This technique presents a simple mechanism for collecting and metering precise quantities of DNA in a low-power format suitable for use in battery operated lab-on-a-chip systems.

3 MATERIALS AND METHODS

Capture-release experiments were performed with double stranded DNA (100bp ladder, Bio-Rad). 1X TBE and 50mM Histidine were used as the buffer and 100bp ladder DNA (Bio-Rad) was used as the sample. The DNA was fluorescently labeled with YOYO-1 (Molecular Probes), an intercalating dye and the sample comprised of 10% of 2-mercaptoethanol (Sigma-Aldrich Co.), which decreases the photobleaching of the dye labeled DNA molecules exposed to high intensity UV light. The UV light is required to induce fluorescence in the dye labeled DNA molecule. Nevertheless, there is some photobleaching and this can be corrected using a photobleaching calibration chart. A sample preparation process is followed to ensure that all DNA solutions tested are exposed to a well-controlled ionic environment, and to maximize consistency between successive experimental runs. First, 10 µl master mixes of fluorescently labeled DNA samples are prepared by adding DNA solution to 1 mM YOYO-1 intercalating dye in a 2:1 v/v ratio. After incubation for approximately 5 minutes at room temperature, the suspension buffer is

extracted using a vacuum centrifuge evaporator. Finally, the dried samples are re-suspended in the desired buffer system with the desired amount of β-mercaptoethanol (BME). This process allows serial dilutions of DNA concentration to be produced while preserving the same ionic environment and fluorescent dye loading conditions. Samples are then loaded into straight microchannels positioned over the interdigitated electrode array, and the access ports are sealed with removable adhesive to prevent evaporation. This setup allows the same microdevice to be used repeatedly by rinsing the channel in between successive samples. The wire bonds making electrical contact between the chip and PC board are encapsulated with epoxy to protect them from damage and prolong the use of the device. Comparison of results between new and re-used devices indicate that little or no electrode fouling (as evidenced through accumulation of fluorescent residue at the electrodes or on the glass channel walls and significant deviations in electrode capture response) occurs as a consequence of repeated use when these cleaning procedures are followed.

A series of customized MATLAB-based image analysis algorithms were used to extract sample concentration using fluorescence measurements. After capture at each successive electrode, a series of images is recorded at a range of camera gating conditions. These images are then digitized as a sequence of still frames. Upon analysis, a detection window is defined and the MATLAB code automatically selects the image whose intensity is best bracketed within the camera's 8-bit intensity range. A correction for photobleaching based on a previous calibration is applied if necessary (although use of a shutter system reduces the samples exposure to excess illumination). Absolute intensities are then computed by scaling by the appropriate gating factor. If desired, these intensity values can then be converted to concentration units using an appropriate calibration curve obtained from serial dilution experiments.

The distance between electrodes and the voltage can be modified to modify the amount of DNA captured on the electrodes. The voltage applied at the electrodes is largely dependant on the rate of gas evolution due to electrolysis at the electrode surface. The gas evolution at the electrodes is counteracted by the rate of its redissolution in the buffer. At voltages higher than 1-1.5 V, the rate of evolution is comparatively higher and leads to the formation of gas-bubbles in the microchannel. Bubble formation is detrimental to the process as it can potentially arrest the run or disperse the existing DNA plug.

4 CONCENTRATION

The results of our initial studies illustrate the robustness of the capture-release technique over different ionic environments (figure 5), initial DNA concentrations and channel widths.

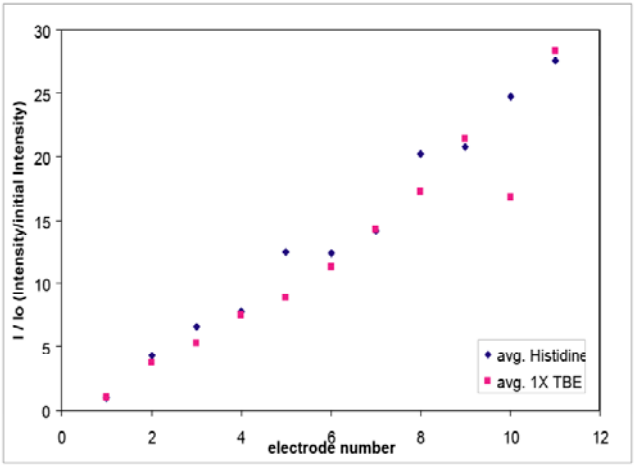


Figure 5: Results of the capture-release in the presence of Histidine and 1X TBE as buffer.

A critical factor influencing performance is the addition of betamercaptoethanol. The original motivation for addition of BME to the sample mixture was to exploit its activity as an anti-photobleaching agent. Subsequent experiments revealed that BME also greatly enhances capture efficiency, possibly creating a more favorable ionic environment through its activity as a free radical scavenger. This activity is largely insensitive to concentration as long as the quantity added to the sample exceeds a threshold value. There is little change observed in capture performance for different electrode materials (platinum and gold) over the range of conditions studied. Some effects may be expected owing to the difference in current density between the materials. Finally, experiments using both single- and double-stranded DNA suggest that the technique is effective for both cases, albeit being more accelerated for double-stranded DNA. Concentrations on the order of 40-folds have been achieved over a span of 8 electrodes (equivalent to ~2 mm in distance) (figure 6).

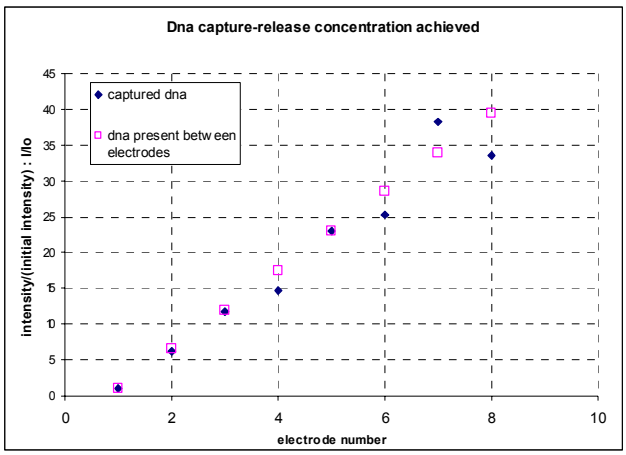
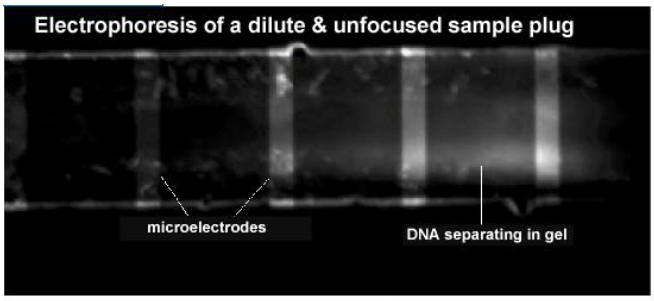


Figure 6: Results of a capture-release run at an applied potential of 1V on a 6μg/ml DNA sample in 50 mM Histidine buffer and 15%v/v BME.



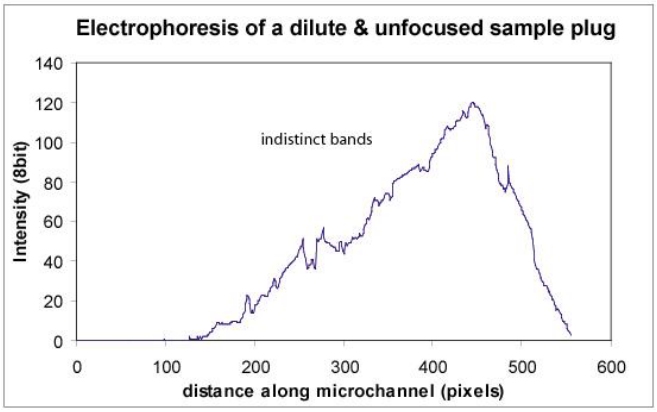


Figure 7: Electrophoresis of a dilute and unfocused sample plug resulting in unresolved bands.

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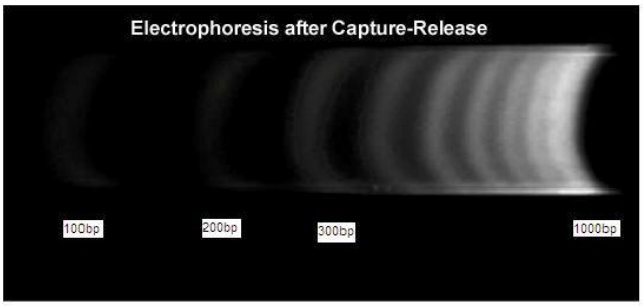
5 A COMPARISON

A comparison is made to gauge the enhancement in separation resolution after capture-release. Figure 7 shows an electrophoresis run on the microfabricated chip without pre-concentrating the sample. The DNA ladder remains unresolved and eventually becomes faint and undetectable along the length of the chip during separation. In contrast, using the same sample, and applying the capture-release procedure, all 10 bands resolve within a distance of 5mm on the chip (figure 8).

6 CONCLUSION

The current microfluidic technology lacks efficient techniques for detecting and analyzing minute samples of biomolecules in a compact and portable format. A miniature DNA analysis device holds little value if it has to be used in conjunction with bench-top scale detection equipment. Consequently, we investigate the use of microfabricated chips with an array of microelectrodes that can be used to collect, concentrate and meter a DNA sample. Considerable enhancement in the DNA electrophoresis separation resolution was demonstrated on application of the capture-release procedure. This will alleviate the challenges originally faced for DNA analysis on the chip and will provide the much needed flexibility in the design of other components, such as detectors, on the chip. Eventually, this research will accelerate the development of a variety of inexpensive, portable and sensitive genomic analysis and biosensing devices.



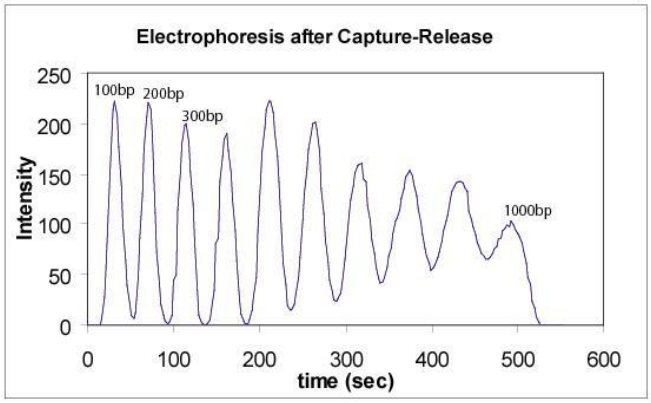


Figure 8: Electrophoresis of a dilute sample after concentrating it via capture-release resulting in all 10 bands getting resolved

7 REFERENCES

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