CHIPCE™: A SILICON MICROSYSTEM FOR MIGRATION, SEPARATION AND DETECTION OF DNA

Ashutosh Shastry and Rakesh Lal,
Department of Electrical Engineering,
Indian Institute of Technology, Bombay, INDIA 400076.
Telephone: 91-22-25721791, Fax: 91-22-5723707, Email: as@ee.iitb.ac.in

ABSTRACT

Hand held point-of-care systems for clinical diagnostics provide an interdisciplinary frontier that has the potential to transform the way diagnosis is done today. Ability to migrate, fractionate and detect charged bio-objects in a completely portable microsystem is a vital component of these hand held point of care systems [1]. These capabilities have been built into a free solution capillary electrophoresis (FSCE) system with on-chip electrical detection. This work reports the principles, fabricated structure, measurement approach and the results obtained for a new schema for on-chip capillary electrophoresis realized in a system that we call the ChipCE™.

INTRODUCTION

On-chip capillary electrophoresis systems have been an area of intense research since the devices provide functional advantages compared to macrosystems. However, the substrates on which these devices have been made have been glass, quartz or plastics, since grown or deposited dielectrics on silicon have not been able to sustain the large voltages required for CE [2].

Dispersive drift has been effected by using gels, through micromachined pillars [3] or synchronized switching to get a moving electric field zone, set up using low voltages [4,5]. Our approach differs from that of Burgraff [4] and Yong Won [5] in the way we set up the moving electric field zone. We use a cascade of low voltage charged balanced unit pulse pairs, referred to as UPP shown in Fig.1, as against pulsed or steady state DC voltage. UPPs allow the drift field to be set up in the electrolyte without the need of electrolysis as is normally done.

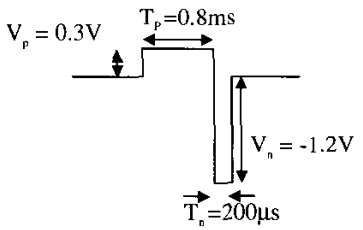

Fig. 1 A Unit Pulse Pair (UPP)

In microCE systems, detection has normally been fluorescence based. These approaches have prevented the development of a monolithic CE system on silicon. On Chip electrical conductivity detection has been reported for ions [6]. The approach we have taken for ChipCE™ differs from Laguere [6] as it detects the presence of charged bio-objects such as DNA. This only-electrical detection scheme also enables monolithic integration of the detector

PRINCIPLE

Operation of the proposed DNA µCE device can be summed up as sample injection, migration, fractionation, and detection. Three ideas that have been successfully demonstrated are:

i. Unit Pulse Pairs (UPP) employed to establish electric field in the electrolyte.
ii. Differential Migration of the DNA
iii. Moving Electric Field Zone that allowed use of low voltages and hence use of silicon substrate.
iv. Pulsed Capacitive Detection enabled a monolithic system that uses only electrical signals.

To establish a time averaged net field in a particular direction, a cascade of such UPPs, is applied across electrodes instead of a DC voltage. The electric field in the bulk of the solution is highest at the onset of the forward pulse. It drops as the electrode gets polarized, that is as ions build up and the double layer grows. Now as the backward pulse is applied, ions accumulated at the interfaces are pushed back into the bulk. Much of the field in this period is screened by the ions and not felt in the bulk. Height of the UPP is kept lower than the voltage required for the onset of electrolysis.

Over the period of the UPP, a net positive average field present in the bulk acts on the DNA and moves the DNA plug closer to an electrode. With suitable choice of pulse height, the objective of causing a net forward drift of the DNA plug, without causing electrolysis is thus achieved

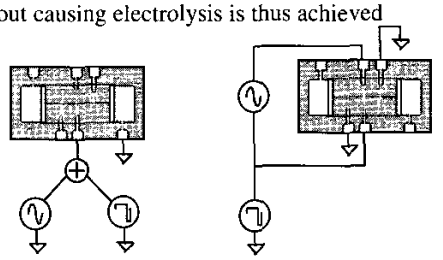

Fig. 2 Two and Four Electrode Detection Schemes

0-7803-7744-3/03/$17.00 ©2003 IEEE.

The device as shown in Fig. 2, is an array of closely spaced electrodes that are used to effect migration and detection. A small voltage is sufficient to cause high fields between these electrodes. Moving electric field zone is set up by applying UPPs such that the pulsed electrodes always bracket the DNA plug. Electrode pairs are switched as the DNA plug moves.

A pulsed capacitive measurement scheme has been employed for detection. UPPs are used to concentrate the DNA in the double layer and electrode capacitance is monitored to detect the DNA plug as it drifts past the detection electrodes. UPPs also enhance the sensitivity by ensuring that a greater distance is probed by the small signal AC. Two electrode and four electrode variations of this technique, schematically represented in Fig. 2 have been implemented.

FABRICATION

Plan and cross sectional elevation of versions three of ChipCE™ are detailed in Fig. 3.

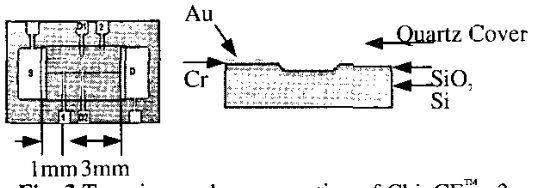

Fig. 3 Top view and cross section of ChipCE™ v3

The ChipCE™ system has been fabricated using a low thermal budget process for patterning microelectrodes in etched microchannels. It could therefore be fabricated using post foundry downstream processes. This process can be employed to make a whole range of MEMS devices and would enable fabrication of systems-on-chip incorporating microfluidic subsystems that have the functionality to migrate-fractionate-detect bio-objects, creating an enabling technology for low cost point-of-care diagnostic systems.

The CE channel is fabricated using shallow anisotropic etching of silicon, which is subsequently oxidized, and chrome-gold electrodes are deposited across the channel. The process outline for fabricating the μCE device is summarized in Table 1.

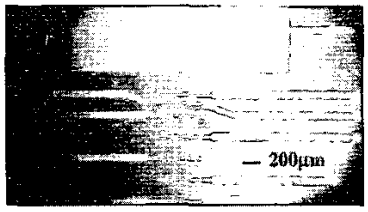

Fig. 4 Successfully fabricated ChipCE™

This device is next mounted on a custom PCB and bond pads indium soldered to the tracks.

Table 1: Process for Device Fabrication

Step No.	Unit Process	Device Cross Section
1	0.3 μm oxide grown by thermal oxidation at 1100 °C.	
2	NPR Lithography + BHF Etch to open windows in oxide	
3	Wet Anisotropic Etching of Si using EDP	
4	0.2 μm thick dry oxide regrown after RCA cleaning	
5	Lift of patterning with PPR and Cr-Au PVD at substrate temperature of 50 °C. Au – 900 A° Cr – 300 A°	
6	Lift of PPR by sonicating in acetone	

Top quartz plate is next sealed on the channel by applying epoxy on the edges of the plate. The mounted device shown in Fig. 5 is ready to be plugged into measurement set up.

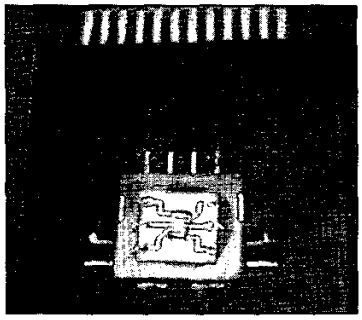

Fig. 5 ChipCE™ version 3 mounted on PCB

MEASUREMENT

CE Setup is shown in Fig. 6. The mounted device is plugged into an edge connector housed in the RH 100 chamber to prevent evaporative loss of water that concentrates the buffer solution and produces detection artifacts. CE Pulser, which is a microcontroller-based hardware, applies the cascade of migration UPPs and switches electrodes to move the field zone. Pulsed capacitive detection is done using the detection circuit and detection UPPs from a programmable pulse generator (HP 81101 A). The controlling C program reads the in phase and out of phase signal values from the lock-in amplifier SR830 and logs the capacitance values.

After the source and sink reservoirs are filled with the buffer, the CE run is started. The CE run summed up in Fig. 7 employs alternate migration and detection cycles.

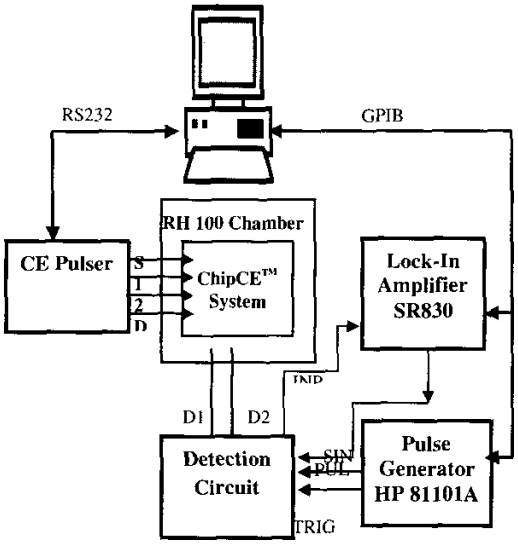

Fig. 6 Schematic of measurement setup

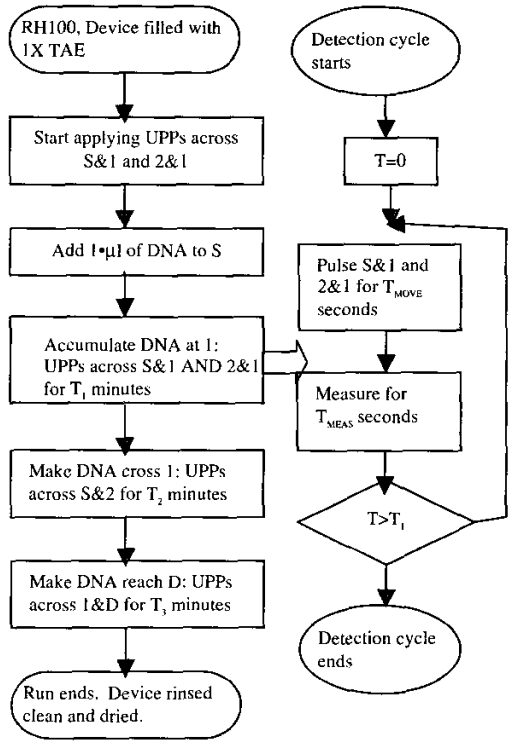

Fig. 7 The CE Run

The UPP parameters were selected so as to maximize the average forward field in the bulk electrolyte. The average polarization time for the gold-TAE 1X buffer electrode-electrolyte interface is approximately 1 ms and that decides the UPP width. This was measured by applying the UPP in a macro cell and monitoring the decay of current. Also the field decay was monitored by measuring the voltage between electrodes 1 & 2 while the UPP was applied across S & D. These plots are summed up in Fig. 8. Considerations of electrolysis and ions penetrating the oxide [7] constrain the pulse height to 1.5 V.

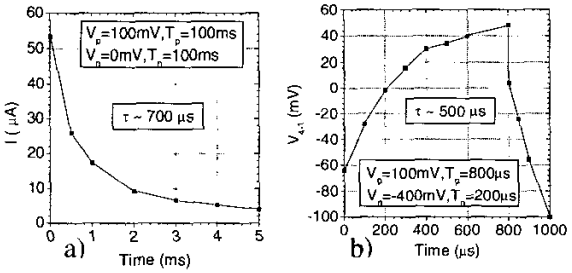

Fig. 8 a) Current vs. time for macro cell **b)** Electric field vs. time in microdevice

RESULTS

Several runs with and without DNA show consistent results that capacitance dips are observed only in presence of DNA. Fig. 9 shows the result of one such CE run. There is an identifiable cluster of dips for each DNA ladder. The location of the cluster is decided by the DNA sample and it moves with change in field. The depth of dips is dependant on the concentration of DNA in the sample.

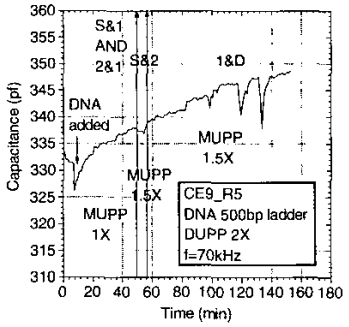

Fig. 9 Capacitance electropherogram using ChipCE™

CONCLUSIONS

This work conclusively establishes that the UPP is successful in establishing electric field in the electrolyte solution. The UPP is also shown to succeed in effecting migration of DNA. This makes it a powerful generic tool for moving charged bio-objects in microfluidic systems. This work also establishes the pulsed capacitance detection for DNA. This again can be used for proteins and other charged bio-objects.

An extremely useful technique is thus developed, one that uses low voltages and is shown to work in a silicon device. Besides migration and detection, the same conceptual framework can be extended to argue tuning the UPP width parameters can effect fractionation. Current work is a proof-of-concept but a really useful device needs to be an order of magnitude smaller for run times to be tens of minutes. Scaling analysis suggests that the times would scale as L^2 and pursuing such a device is a worthwhile pursuit.

REFERENCES

[1] Anna J. Tüdos, Geert A. J. Besselink and Richard B. M. Schasfoort, Lab on a Chip, Vol.1, pp.83–95, 2001

[2] Luc Bousse, C. Cohen, T. Nikiforov, A. Chow, A. R. Kopf-Sill, R. Dubrow, J. W. Parce, Annu. Rev. Biophys. Biomol. Struct., Vol. 29, pp.155-158, 2000

[3] T. Duke and L. Viovy, Phys. Rev. Lett.,Vol.80, pp.1552-1555,1998

[4] N. Burrgraf, A. Manz and N.F. De Rooij, Sensors Actuators B, Vol. 20, Issue 2-3, pp. 103-110, 1994

[5] Y. Jeong, S. Kim, K. Chun, J. Chang and Doo Soo Chung, Lab on a Chip, Issue 1, pp. 143–147, 2001

[6] Laugere F., Lubking G.W., Berthold A., Bastemeijer J., and Vellekoop M.J., Sensors and Actuators A, Vol. 92, pp.109-114, 2001.

[7] A. Topkar and R. Lal, Thin Solid Films, Vol. 232,Issue.2,pp.265-270