ELECTROPHORESIS SYSTEM WITH INTEGRATED ON-CHIP FLUORESCENCE DETECTION

J. R. Webster†, M. A. Burns*, D. T. Burke†, and C. H. Mastrangelo†

† Center for Integrated Microsystems
Department of Electrical Engineering and Computer Science

* Department of Chemical Engineering

† Department of Genetics
University of Michigan, Ann Arbor, MI 48109-2122, USA

ABSTRACT

A monolithic capillary electrophoresis system with integrated on-chip fluorescence detection has been microfabricated on a silicon substrate. Photodiodes in the silicon substrate measure fluorescence emitted from eluting molecules. An on-chip thin film interference filter prevents excitation light from inhibiting the fluorescence detection. A transparent conducting ground plane prevents the high electric fields from interfering with the photodiode response. A truly monolithic device has been fabricated using surface micromachining methods eliminating elaborate bonding procedures. Separations of DNA restriction fragments have been performed in these devices with femtogram detection limits using SYBR Green I intercalating dye.

INTRODUCTION

Capillary electrophoresis has benefited greatly from the miniaturization of microfabricated devices. Fast, highly efficient separations have been routinely performed in these devices [1, 2, 3]. Besides the benefits of miniaturization, microfabrication also has the ability to manufacture highly complex devices with a high degree of functionality. The potential for complex DNA microprocessors by microfabrication has been demonstrated [4].

The majority of capillary electrophoresis devices have been fabricated using a bonded, glass substrate technology with off-chip laser induced fluorescence detection. The advantages of miniaturization are reduced, however, when these systems rely on equipment several orders of magnitude larger. The use of on-chip detection techniques makes portable DNA diagnostic instruments practical.

The use of on-chip silicon photodiodes provides an alternative to photomultiplier tubes. Silicon photodiodes have been used for many years in the measurement of electromagnetic radiation from the near UV range to the near infrared [5, 6, 7, 8]. Devices have been reported with noise equivalent powers (NEP) in the femtowatt range [9].

Fluorescence detection methods are attractive for the on-chip detection of DNA samples. Many of the current biochemistry protocols already incorporate fluorescent labels. Reactions such as Sanger sequencing and the polymerase chain reaction (PCR) have been adapted to fluorescent labeling methods [10]. In fact, quantitative PCR amplification reactions are being performed with high efficiency using fluorescent labels [11]. Therefore, chip-based assays can easily be incorporated into existing protocols without changing the biochemistry. Furthermore, fluorescence techniques are chemically decoupled from the analysis steps. Therefore, fluorescence detection can be used in a variety of applications on an analysis chip. Not only can detection be performed anywhere along a separation column but also within reaction chambers to monitor reaction performance.

EXPERIMENTAL

Microfabrication. Figure 1 shows an optical micrograph of a microfabricated electrophoresis device with on-chip fluorescence detection. On a single chip, photodiodes, an interference filter, a transparent ground plane, electrophoresis channels and electrophoresis electrodes are fully integrated into a complete electrophoresis system. Figure 2 shows a cross-sectional schematic of the device.

Figure 2: Schematic of electrophoresis device cross section.

The fabrication process for the electrophoresis device consists of 13 lithography steps. Figure 3 shows a simplified process flow. The starting wafer is a 1000-2000 Ω-cm boron doped, p-type, < 100 > float zone silicon
substrate. The high resistivity substrate is used to increase the depletion region and therefore the efficiency of the photodiode p-n junction. The wafers are first implanted with a field implant of 50 Kev boron with a dose of $1 \times 10^{12} \text{ cm}^{-2}$. The field implant prevents inversion of the substrate below the metal lines near the surface. Using a photoresist mask the silicon substrate is etched with a wet isotropic silicon etchant for 10 minutes. The photodiode is constructed in the substrate by using 2 successive ion implantation steps. For each a photoresist mask is used.

The n-type implantation is done with 50 Kev phosphorus at a dose of $5 \times 10^{14} \text{ cm}^{-2}$. The p-type implantation for the substrate contact is done with 30 Kev boron at a dose of $1 \times 10^{15} \text{ cm}^{-2}$. The photoresist masks from the implantations were stripped using PRS2000 at 110°C followed by a 1:1 clean.

A passivation thermal oxide was next grown on the wafer under wet, trichloroethane (TCA) conditions. The 0.2 μm thick silicon dioxide layer was grown at 900°C. Contacts to the substrate and diodes are made using a wet buffered hydrofluoric acid (BHF) etchant. A chromium/gold metalization was next deposited and patterned to make contact with the photodiodes.

A short, 10 second BHF dip was used to clean the contact areas. The wafer is then immediately placed into a vacuum chamber and 0.05 μm of chromium followed by 0.2 μm of gold were evaporated on the substrate. The Cr/Au metalization was then patterned using a photoresist mask and wet metal etchants. Next, a thin film interference filter was deposited on top of the wafer. The filter layers were designed and deposited by ZC&R Coatings for Optics. The filter consists of alternating layers of SiO$_2$ and TiO$_2$ at nearly quarter wavelength thicknesses. The filter consists of approximately 20 layers and is about 3 μm thick. On top of the interference filter a 2.5 μm thick layer of parylene-C is deposited. Adhesion of the parylene layer to the filter is assisted by a silanation procedure. The immediate deposition of the parylene layer serves as a passivation layer for the filter to maintain the integrity of the optical properties.

After the parylene-C layer, the ground plane is deposited. First a 0.18 μm thick layer of SiO$_2$ is sputtered on top of the parylene-C. Next a 0.15 μm thick layer of aluminum zinc oxide (AZO) is sputtered on top of the SiO$_2$. The AZO layer has a greater than 90% transmission with a sheet resistance of approximately 50 Ω/□. The SiO$_2$ is used as an adhesion layer for the AZO. The AZO is then patterned using photoresist and a wet etchant consisting of 1:4:500 HNO$_3$ : HCl : H$_2$O[12]. The etch rate of the AZO is extremely fast. Contact to the AZO layer is made by depositing a 0.02 μm/0.08 μm Cr/Au layer by liftoff. A second SiO$_2$ layer is sputtered on top.
of the AZO. This layer will provide excellent adhesion to parylene-C and provide additional passivation to the AZO layer underneath. Another parylene-C layer is deposited on top of the SiO₂. This layer is 4.8 μm thick and will be the bottom layer of the electrophoresis channel. Next the contact holes must be etched through the two parylene-C layers to access the photodiode metalization. This etch step is performed in two lithographies using the same mask. The first parylene-C layer is etched in an O₂ plasma RIE using a 20 μm thick photoresist mask. The SiO₂ underneath the parylene is etched using 10% BHF prior to stripping the photoresist. The resist is then stripped in acetone and then the parylene is etched a second time again using a 20 μm thick photoresist mask. This time the filter layers beneath the parylene are etched using a 10% BHF solution for 8 minutes prior to stripping the photoresist. The undercut of the etching was found to be quite large (20 μm), however, fine features were not necessary since only metal pad openings were made. The parylene is etched a third time to open contact to the AZO metalization. Again the oxide is etched in the same way. Next 0.1 μm thick gold electrophoresis electrodes are patterned on the parylene by liftoff. The sacrificial photoresist is then patterned using a 20 μm thick layer of AZ9260 (Clariant). The top of the channel is then formed by depositing a 5.3 μm thick parylene-C layer. The adhesion of this layer is assisted by a short oxygen plasma described earlier. The final parylene layer is etched in a O₂ plasma RIE using a thick photoresist. Channel reservoir openings as well as pad openings to all metal layers are made in this step. Photodefinable silicone rubber rings demonstrated on the plastic electrophoresis devices can be patterned on this device at this point using the same procedure outlined earlier. In the device tested here this step was avoided out of convenience and silicone rubber rings were applied by hand at the time of device testing. The wafer was diced using a diamond tip saw. The final photoresist mask was left in place to protect devices during dicing. The devices were then released in acetone for 20 hours followed by an IPA rinse and N₂ dry.

Separation conditions. Separations were performed in these devices using a sieving matrix consisting of 0.5 % (w/v) hydroxyethylcellulose (HEC) (Polysciences, Inc., Warrington, PA; MW 90000-105000) and 0.1x Tris/Boric Acid/EDTA buffer (Sigma Chemical Co., St. Louis, MO). Approximately 3 μl of the buffer solution is loaded in one reservoir and allowed to fill the entire channel by capillary action. The remaining reservoirs are filled with approximately 3 μl of buffer solution. The channel was preelectrophoresed at 300 V/cm for 10 minutes. This was found to concentrate the HEC in the separation column to more than 0.5% resulting in a higher resolving power. A 0.2 μg/μl sample of DNA was labeled with SYBR Green I (Molecular Probes, Eugene, OR) intercalating dye at an intercalation ratio of 1:5, dye:DNA bp. Approximately 2 μl of the DNA sample was loaded into the injection reservoir. The samples were cross injected using a pinched injection scheme [13]. The electric fields used during separation were 110 V/cm.

Fluorescence Detection. A schematic of the on-chip
fluorescence detection system is shown in figure 4. A

Stanford Research SR830 lock-in amplifier was used to measure the photodiode current. A GaN blue LED was used as an excitation source with a 450±27 nm bandpass filter (Omega Optical). The LED was driven with an in-house built amplifier, 5 V power supply and the TTL output of the lock-in amplifier. The lock-in amplifier output was recorded using an RS232 port and a in-house written data collection program for an IBM compatible PC. Data was recorded at a rate of 64 Hz.

**Instrumentation.** A stereo microscope (Olympus SZX12) equipped with a fluorescence illuminator (100W Mercury lamp illuminator) was used to observe the separations. The filter cube consisted of 470 nm band pass excitation filter, 495 nm long pass dichroic mirror, and a 500 nm long pass emission filter. Separations were recorded to a SVHS video tape using a Dage 68 SIT camera. Optical characterization was performed using a 150 W halogen lamp (Micro lite, Three Rivers, MA), a Monospec 18 monochromator (Thermo Vision, CO), an optical power meter (Ophir Optronics, Israel), and a picoammeter (Keithley).

**RESULTS AND DISCUSSION**

Figure 5 shows the relative efficiency of the photodiode combined with the on-chip interference filter. The filter blocks the excitation light up to approximately 495 nm. The optical density in this region is greater than 2. The photodiode itself is most efficient in the red spectrum due to the finite junction depth (approximately 0.65 μm) of the diode. The absolute efficiency could not be measured since diffusion of carriers dominates the photo response. The maximum efficiency shown is calculated to be between 90-100%. The ripples in the passband are due to the on-chip filter. The photodiode response to fluorescently labeled DNA in a microchannel is shown in figure 6. Various concentrations of DNA labeled with YOYO-1 (Molecular Probes, Eugene, OR) at a ratio of 1:5 dye:DNA bp.

Figure 5: Relative quantum efficiency of photodiode with on-chip filter and transparent ground plane.

Separations of the HaeIII digestion of φX174 RF DNA are shown in figure 7. A video image of fragments is shown in figure 7(a). The separation distance from injection to detection is 0.9 cm. The electrophogram generated by the on-chip detection of fragments is shown in figure 7(b). The baseline has been extracted using a simple curve fit. No other data processing has been done. The 194,234 bp fragments are no longer baseline separated due to the finite width of the detection region. The effective detector width is in fact much larger than the photodiode itself due to diffusion of carriers and the finite distance of the DNA from the diode. The bottom of the electrophoresis channel is 11 μm from the silicon surface. Since the blue LED excitation is a broad area excitation source and the emitted fluorescence is isotropic, the DNA fluorescence can be seen nearly 200 μm from the detector. A simple geometric model predicts an effective detection width of 175 μm. This therefore contributes 2600 μm² to the band variance and significantly alters the resolution of the separation. The use of small area excitation sources would lower the detector width to a more acceptable size. The limits of detection have been estimated from the photocurrent noise and electrophoresis peak heights. The standard deviation of the photocurrent noise was measured to by approximately 0.7 pA. The limit of detection for the 72 bp fragment is 75 fg of DNA (S/N = 2).

**CONCLUSIONS**

With the integration of on-chip detection for microfabricated electrophoresis devices, practical hand held diag-
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References


