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Short communication

Long pathlength, three-dimensional absorbance microchip

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Abstract

A long pathlength, three-dimensional U-type flow cell was microfabricated and evaluated for improved absorbance detection on a glass microdevice. A small diameter hole (75 μm) was laser etched in a thin glass substrate whose thickness (100 μm) defined much of the pathlength of the cell. This substrate was thermally bonded and sandwiched between two different glass substrates. The top substrate contained a typical injection cross and separation microchannel. Projecting out of the plane of the separation device was a 126 μm pathlength flow cell as defined by the laser etched hole and the attached microchannels. The flow cell was connected to a microchannel on the bottom substrate that led to a waste reservoir. The planar, flat windows on the top and bottom of this device made light introduction and collection a simple matter using a light emitting diode (LED) and microscope objective. The experimentally obtained detection limit for rhodamine B was determined to be 0.95 μM, which is nearly identical to the theoretical limit calculated by Beer's Law. A separation of three fluorescent dyes was performed, and direct comparisons were made between the transmittance changes through the narrow pathlength separation microchannel and the adjacent long pathlength, three-dimensional U-type flow cell.

Keywords: Capillary electrophoresis microchip; Absorbance; Long pathlength; Three dimensional

1. Introduction

Despite the wide applicability of absorbance detection and more than a decade of research since the first introduction of the lab on a chip platform, there continues to be few examples of absorbance detection on capillary electrophoresis (CE) microchip devices. The predominant reason for this is their inherently short pathlengths, and the resulting poor sensitivity this engenders. Jindal and Cramer demonstrate, for example, that the trapezoidal-shaped microchannels etched in glass substrates result in an effective pathlength that is approximately 50% that of the etched depth [1]. For a typical microchannel depth of 10 μm, reducing this to an effective pathlength of ∼5 μm becomes a serious sensitivity issue for absorbance detection.

Several researchers have examined methods for increasing the pathlength on a CE microchip. Harrison and co-workers have investigated the application of a microfabricated U-cell within the plane of the glass device (120–140 μm long) [2]. Disadvantages associated with this approach include difficult positioning of optical fibers within the planar device, and the requirement of a laser to prevent stray light effects. Alternatively, a multireflection cell was microfabricated on a microdevice by depositing an aluminum mirror above and below a separation microchannel, adding entrance and exit apertures positioned 200 μm apart in the top mirror [3]. This technique, however, required a laser whose angle of incidence was carefully controlled to define the resulting pathlength. Kutter and co-workers have fabricated optical waveguides into a silicon, planar microchip device containing a 750 μm U-cell, but the multistep fabrication process and applied laser make this approach unpractical for many [4]. Finally, Collins and Lu have examined 100 μm deep microchannels for absorbance detection, although the wide channels necessitate non-aqueous buffer compositions to prevent Joule heating effects [5,6].

The three-dimensional approach applied to absorbance detection in microfluidic systems was an idea first put forth by Caliper Technologies as a patent application in 2002, however, to our knowledge, the viability of this technique has never been verified in the literature [7]. The microfabricated absorbance flow

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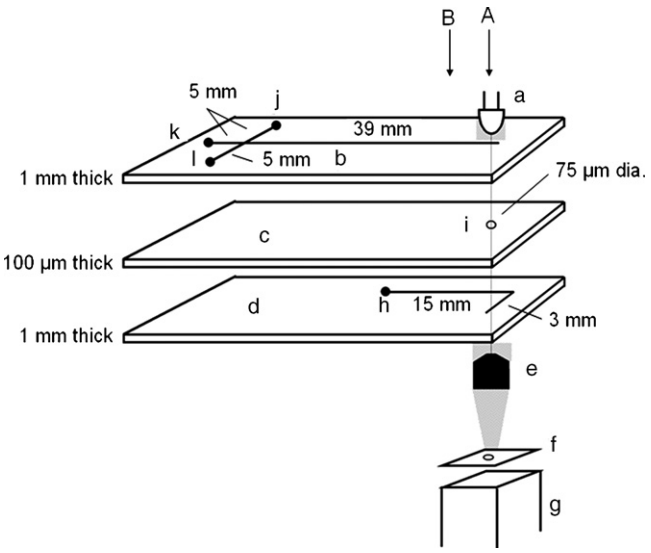

Fig. 1. Design for the long pathlength, three-dimensional absorbance microchip. All three glass substrates are bonded together, creating a sandwich microchip device whose total absorbance pathlength is defined by the thickness of the middle substrate and the etch depth of the channels superpositioned above and below the laser etched hole: (A) long optical pathlength point of detection, (B) short optical pathlength point of detection: (a) light emitting diode, (b) separation microchip substrate, top (c) thin glass substrate, middle, (d) waste microchip substrate, bottom, (e) microscope objective, (f) circular slit, (g) photomultiplier tube, (h) buffer waste reservoir, (i) laser etched hole, (j) sample reservoir, (k) buffer reservoir, (l) sample waste reservoir.

cell discussed here can be considered to be a U-type flow cell that projects out of the plane of the separation microdevice in the third dimension (see Fig. 1). A typical cross injector and separation microchannel pattern was etched into a top glass substrate, and thermally bonded to a thin glass substrate containing a laser etched, small diameter hole. The bottom substrate, containing a microchannel pattern that connected the flow cell to a waste reservoir, was thermally bonded to the middle substrate. The advantages of this type of microchip flow cell are: (1) that the planar top and bottom of the microchip can now be used as easily accessible, flat windows for monitoring the flow cell, for example, with an inexpensive, stable light emitting diode (LED) light source and a microscope objective and (2) that the flow channel does not need to be widened in order to increase the pathlength, e.g. bubble cells, thereby, allowing the separation efficiency to be maintained as resolved bands enter the flow cell.

2. Experimental

2.1. Chemicals

Rhodamine B, sulforhodamine G, eosin Y and *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) were obtained from Sigma (St. Louis, MO) and utilized as received. Stock solutions for each fluorescent dye (100 μM) were prepared in 25 mM HEPES buffer and adjusted to pH 7.0. Subsequent dilutions of the stock were made in an identical buffer composition.

2.2. Device microfabrication

Wet chemical etching of 1 mm thick, soda lime chrome photomask blanks (Telic, Santa Monica, CA) was performed utilizing standard lithographic procedures [8]. Microchannels etched on the top and bottom substrate each had a depth of 13 μm. The top substrate was a standard cross design with dimensions of 5 mm from each reservoir to the intersection point, 39 mm from the injection cross to the absorbance flow cell and 100 μm in width. The bottom substrate had an L-shaped pattern, which was 18 mm in total length from the absorbance flow cell to the buffer waste reservoir and 200 μm in width. The middle substrate consisted of a 100 μm thick soda lime glass (Mark's Optics, San Diego, CA) bearing a 75 μm diameter laser etched hole (Continuum Minilite Q-switched Nd:Yag laser, frequency quadrupled to 266 nm, 100 mJ/cm^2). The beam was focused at the point of ablation using a microscope objective to a diameter of 3 μm, giving a peak fluence of ∼100 mJ/cm^2. A video camera allowed real time visual monitoring of the ablation process. The glass plate was mounted on a two-axis linear translational stage, allowing movement in a plane normal to the incident beam. The hole was drilled by manually moving the plate and repeatedly pulsing the laser on it until the desired shape and size of the hole is achieved. Reservoir access holes (2 mm) were drilled prior to bonding using diamond tipped drill bits. Low temperature bonding of the device was achieved using a high pressure press (Carver Model 3912, Wabash, IN) operated at elevated temperature (200 °C), and performed in a sequential manner, first bonding the bottom substrate to the middle substrate, followed by the top substrate. Glass reservoirs were epoxied to each access hole; the bottom reservoir required a curved length of glass tubing that extended above the plane of the device, ensuring that each reservoir was filled to the same height with buffer and preventing hydrodynamic flow.

2.3. Instrumentation

A green LED (525 nm, Allied Electronics) was oriented directly above the absorbance flow cell, nearly touching the top substrate. Light passing through the microchannel was collected using a microscope objective (Newport, U-27X) and directed through a circular slit (1 mm) positioned at the objective's focal point and onto a miniature, red-shifted photomultiplier tube (Hamamatsu, Model H5784). The photomultiplier had a built in current to voltage convertor, and the voltage signal was monitored using LabView (National Instruments, Austin, TX). The high voltage switching apparatus has been described previously [9]. A floating load mode was utilized for sample injection, wherein a potential of 476 V was applied between the sample and sample waste reservoirs, while both the buffer and buffer waste reservoirs were allowed to float. A field strength of 530 V/cm was applied for separation of the dye mixture.

3. Results and discussion

The design for the three dimensional, long pathlength absorbance microchip is shown in Fig. 1. The key compo-