MICROFLUIDIC GLUCOSE DETECTION WITH AN LASER-INDUCED FLUORESCENCE DETECTION DEVICES

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ABSTRACT

The integrated detection platform comprises a fluorescence-collecting microlens and the annular integrated fluorescence detector where a thick SiO_2/Ta_2O_5 optical interference filter (>6 μm) is monolithically integrated on a hydrogenated amorphous Si (a-Si:H) PIN photodiode, allowing for vertical excitation through the detector. With a microfluidic CE device mounted on the platform, the detection system is demonstrated to separate and detect AMAC labeled glucose (20 μM), using the external excitation source. We are now working towards integration of an excitation source to fabricate fully integrated laser-induced fluorescence detection (LIF) device that would be comprised of an InGaN laser diode (LD), micro-lenses and the integrated a-Si:H fluorescence detector.

KEYWORDS

fluorescence, a-Si:H, laser diode, glucose, microfluidics

INTRODUCTION

Miniaturization and integration of detection devices are imperative to realize point-of-care microfluidic lab-on-a-chip biochemical analysis devices. Even though glucose sensor based on electrochemical detection has been dominant in a biosensor market, fluorescence detection is potentially more advantageous than electrochemical detection in terms of sensitivity, multiplexed detection capability and isolation from analyte. Integration of fluorescence detection, however, is more difficult compared to electrochemical detection, because it requires heterogeneous material and device integration of various optical component such as an excitation source, an optical filter and a detector. Besides most of integrated fluorescence detectors suffer from high limit of detection (LOD) compared to conventional optical system that consists of discrete optical components. In our previous work, we have significantly improved a LOD of an integrated hydrogenated amorphous silicon (a-Si:H) fluorescence detector due to laser light, implying single molecular DNA detection when combined with polymerase chain reaction.[1]

In this work, we have applied the integrated a-Si:H detector combined with a microfluidic capillary electrophoresis (CE) device to detect 2-aminoacridone (AMAC) labeled glucose, providing clinically relevant LOD for glucose. We are now developing wafer level packaging technology of a LD to realize fully integrated LIF device that would be capable of various microfluidic biochemical assays due to visible fluorescence detection.

EXPERIMENT

Figure 1 (A) show cross sectional structure of integrated a-Si:H fluorescence detectors where SiO_2/Ta_2O_5 optical interference filter (511-615 nm) was monolithically integrated on an a-Si:H photodiode. The integrated a-Si:H fluorescence detector fabrication procedure was published elsewhere. An optical micrograph of the top view of the integrated a-Si:H fluorescence detector is shown in Figure 1 (B).

Prior to all electrophoretic operations, the channel walls were charge-neutralized with linear polyacrylamide, following a modified procedure of Hjerten coating. The reductive amination method was used to derivatize D-(+)-glucose by fluorescent 2-aminoacridone (AMAC).[2] 0.1 M AMAC solution was prepared with a mixture of dimethyl sulfoxide (DMSO) and acetic acid (17:3 v/v). A 5 μL of 20 mM glucose solution was mixed with 5 μL of the AMAC and 10 μL of sodium cyanoborohydride, which was incubated for derivatization. 180 μL of 200 mM borate buffer (pH=8.5) was then added to terminate the derivatization. The derivatized glucose solution was further diluted to 20 μM with 200 mM borate buffer for microfluidic separation. 0.5 w/v % methylcellulose (MC) dissolved with 200 mM boric acid (pH=8.5) was used as running buffer for the glucose separation. The diluted glucose sample was placed in the sample reservoir, and 200 mM borate buffer was loaded into the waste, cathode, and anode reservoirs to make electrical contact with the platinum electrodes. The glucose sample were injected 120 s from the sample to waste reservoirs at 375 V/cm while applying a floating potential at the cathode and anode reservoirs. Separation was then performed at 170 V/cm while applying a back-bias electrical field of 110 V/cm between the injection cross point and both the sample and waste reservoirs. The effective separation length was approximately 2.7 cm.

The integrated detection platform comprises a fluorescence-collecting microlens and the annular integrated fluorescence detector, shown in Figure 1. A 476.5 nm line of Ar ion laser was introduced normal to the microfluidic CE device while loosely focused (~30 μm dia.) through a convex lens on the CE channel: note that the external excitation source is used for the separation experiment. The annular a-Si:H photodiode and transparent glass substrate allowed vertical laser excitation while avoiding direct incidence of excitation light on the photodiode. The bottom 200-nm-thick Cr electrode layer of the a-Si:H photodiode acted as an aperture for the incident laser light. Fluorescence was collected and approximately collimated by the microlens and spectrally filtered with the bandpass filter.

For the integrated detectors, a reverse bias voltage of 1 V was applied to the a-Si:H photodiode to optimize

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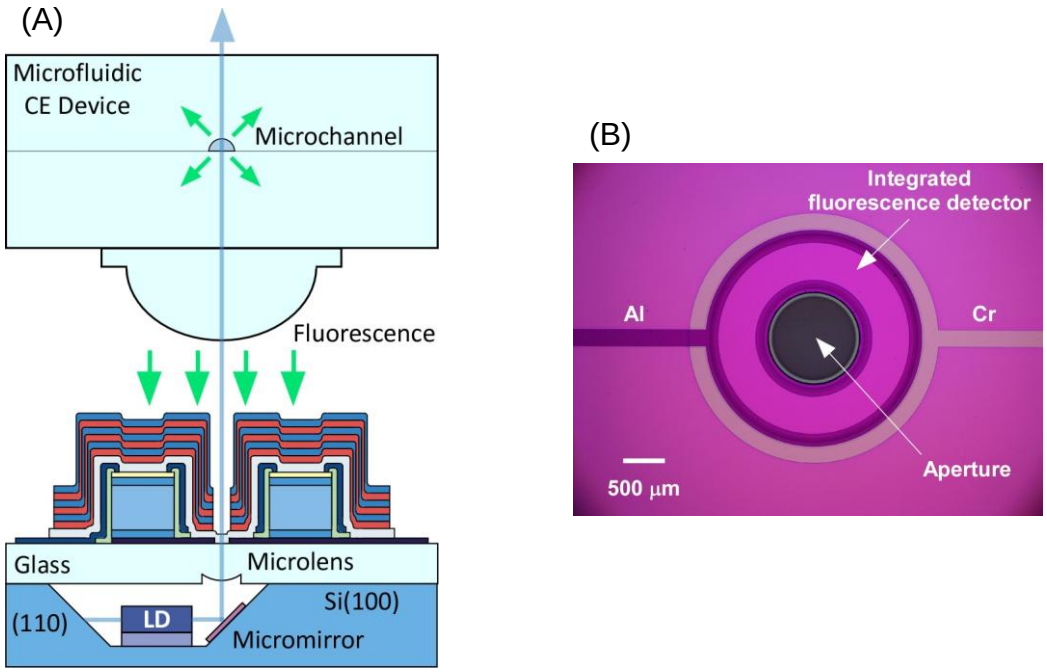

Figure 1. (A) Schematic cross-sectional view of a LIF detection device comprised of a LD, microlens and integrated a-Si:H fluorescence detector for microfluidic biochemical analysis. (B) Optical micrograph of the top view of the integrated fluorescence detector.

carrier collection efficiency. Dark current of the integrated detectors was less than 1 pA at a reverse bias of 1V. The photocurrent was synchronized to chopped laser light at 27 Hz and detected by a lock-in amplifier to reduce background due to environmental light and dark current of the a-Si:H detector. The filtered (300 ms) LIA output was digitized at 20 Hz. Data acquisition and separation control were accomplished using LabView.

RESULTS AND DISCUSSION

Figure 2 presents the microfluidic separation and detection of 20 μM AMAC-labeled glucose. The LOD was 5 μM for the glucose solution. Blood glucose level is normally maintained at 80-120 mg/dL, corresponding to 4-7 mM, so that the integrated detection system presented here could easily monitor the blood glucose level. The current glucose sensor is based on fingertip prick for collecting blood sample, followed by electrochemical detection of glucose, only allowing for intermittent measurement. In order to predict the trend of blood glucose change, however, continuous glucose monitoring is required. Fluorescence-based approach is particularly useful to transdermally monitor the glucose level.[3]

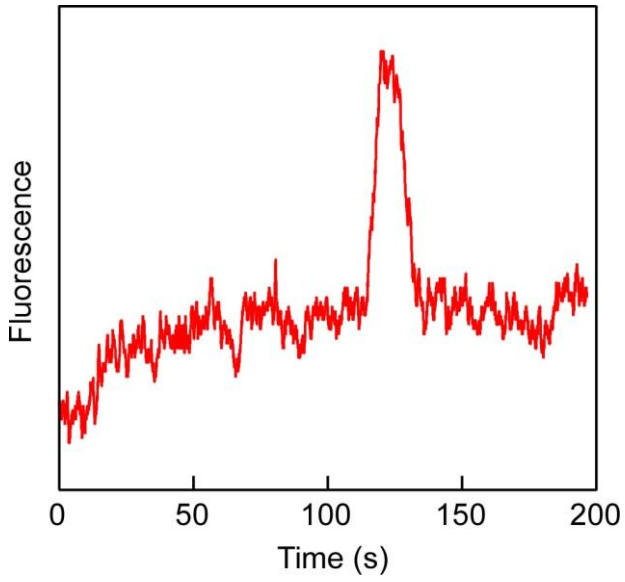

Figure 2. Electropherogram of 20 μM AMAC-labeled glucose solution. 0.5 w/v% of MC dissolved in the 200 mM borate buffer was used as the running buffer.

So far we have concentrated on reducing the LOD using an external laser source. DNA analysis experiments establish clinically-relevant LOD for point-of-care genetic analysis and glucose detection provide sufficient LOD for fluorescence based continuous glucose monitoring. A more complete LIF device integrating a Vertical Cavity Surface Emitting Laser Diode (VCSEL), photodiode and emission filter monolithically on a GaAs substrate has been presented.[4] The monolithic approach should be advantageous for device manufacturing, but this is only possible for GaAs-related materials, limiting to near infrared fluorescence detection. Besides, the LOD was 40 nM for IRDye 800 (Li-COR) in methanol. On the contrary, our approach is to detect visible fluorescence. Currently blue-green InGaN laser diode (LD) would be the only choice to excite practical labeling dyes, naturally requiring heterogeneous integration of an InGaN LD, SiO_2/Ta_2O_5 optical interference filter and the a-Si:H photodiode.

There are two ways to fabricate heterogeneously integrated LIF device. One is to use a InGaN VCSEL. When the a-Si:H detector is combined with VCSEL, a planar integrated LIF device could be realized like MOSFET, suited with heavily multiplexed microfluidic biochemical analysis. The other way is to use an edge-emitting GaN laser diode. In the edge-emitting LD, carrier is injected in a vertical direction while laser is emitted in a horizontal direction, so that 45° mirror should be placed in a path of laser light for vertical excitation. To this end, TMAH wet etching was performed to fabricate a Si cavity. A small amount of addition of certain surfactant to TMAH solution makes Si(110) the slowest etching planes, so that 45° sidewall was obtained for Si (100) wafer (Figure 3(A)). Through Si Vias (TSV) was also fabricated by Cu electroplating for current input to the LD (Figure 3(B)). After the LD is bonded to the bottom of the Si cavity and mirror is formed on the 45° sidewall, the Si cavity will be encapsulated with the a-Si:H detector substrate. This structure will also act as a packaging of the LD.

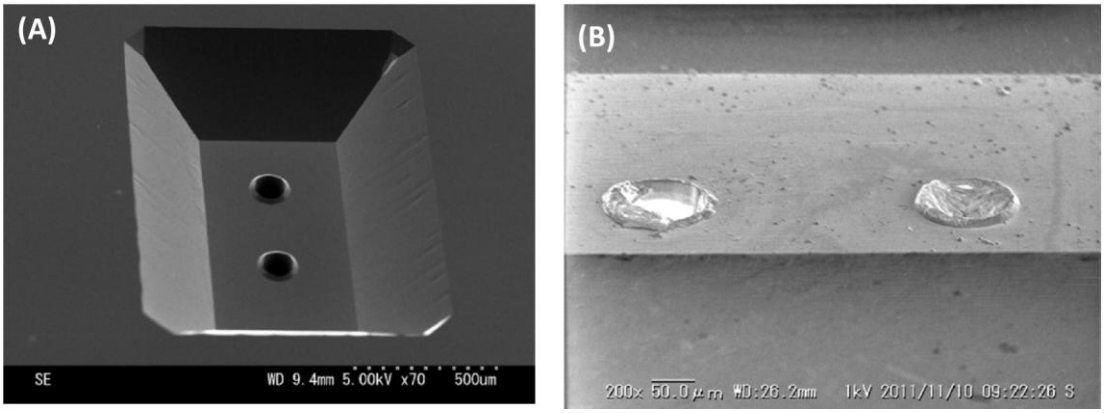

Figure 4. (A) SEM of a Si cavity with 45 sidewall. (B) Cu electroplated TSV's fabricated in the bottom of the Si Cavity.

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