ABSTRACT

The construction of a total internal reflection-based biochip utilizing an integrated micromirror array is presented. The evanescent field that dramatically increases the signal to noise ratio, is generated through the implementation of a four-layer chip, containing silicon micromirrors and UV curable polymer cavities integrated on top of a microfluidic network. The design enables the compact, vertical integration of a laser diode and a CCD camera, eliminating the need to precisely align the laser beam into the system. The multilayer chip can be used as an optical-microfluidic platform for various multiplexed bioassays or as miniaturized component for an integrated handheld lab-on-a-chip microsystem.

Keywords: Four-layer chip, total internal reflection, silicon micromirrors

1. INTRODUCTION

Total internal reflection fluorescent spectroscopy enables the study of surface molecular dynamics at the single molecule level through the generation of a thin evanescent wave at a glass/liquid interface by total internal reflection (TIR) [1]. Based on TIR, disposable plastic prisms integrated on biochips [2] and various types of evanescent biosensors [3] have been proposed to address the requirements for ultra-sensitive, high-throughput platforms. In all these systems, the excitation laser beam, coming from the side of the chip, must be aligned at a certain angle to achieve efficient light coupling and to create a strong evanescent field. Such optical configuration is unsuitable for the development of a fully integrated miniaturized system, since it requires the precise alignment of tilted optical components into the chip. Moreover, the fabrication of high refractive index waveguides and short-period gratings requires the use of special equipment, which in turn greatly increases the cost of the chip. Gratings and waveguides also suffer from light coupling and propagation losses, these limitations can only be overcome with the use of bulky, high power, expensive lasers. Plastic prisms on the other hand can be inexpensively fabricated, but their use in an array-type format for high throughput processing is still questionable.

In this work, the development of a miniaturized TIR-based chip is presented (figure 1).
It consists of polymer-filled cavities for efficient light coupling, silicon micromirrors for directing the excitation light at a predefined angle and microfluidics for sample delivery. The chip is fabricated using a combination of standard bulk micromachining techniques and PDMS casting. It enables the hybrid vertical integration of all of the optical components, providing great flexibility for future miniaturization. The design can easily incorporate hundreds of detection spots on a single chip.

2. TIR ON THE FOUR-LAYER CHIP

Key element in our design is the use of a silicon micromirror that directs the excitation light at an angle above the critical angle on the glass-liquid interface. The chip consists of four substrates and is fabricated using standard micromachining techniques (figure 2). A silicon KOH etched substrate that contains the micromirror, sits between two thin (~200 μm) glass wafers (index of refraction n$_{glass}$=1.526). The cavity that is formed between the two glass wafers and the micromirror surfaces is filled with UV curable optical cement that couples the exciting light into the system. The top glass wafer serves as the functional substrate where TIR takes place, while the bottom one is used to planarize the polymer cement. The light beam is shown weakly focused on the interface where it is totally internally reflected. The microfluidic network is formed from a PDMS slab patterned on an SU-8 mold. The incident angle can be established by drawing a ray diagram of the light beam as it passes through the chip. The incident angle changes along the interface since the laser beam is not collimated. The inner and outer rays of the beam reach the interface at incident angles of 67.48° and 73.72° respectively for the implemented geometry and materials. Both values are above the critical angle ($\theta_c = \sin^{-1}(n_{water}/n_{glass}) = 64.81°$).
Figure 3a depicts graphically the dependence of the p-polarized $I_p(0)$ and s-polarized $I_s(0)$ (parallel and perpendicular to the plane of incidence respectively) components of the intensity of the evanescent wave at the interface on the incident angle of illumination [1]. The shadowed area represents the operation range of the TIR-based chip. The penetration profiles of the evanescent field for the inner and outer rays are shown in figure 3b.

Figure 3. (a) Intensity of the evanescent wave at the interface versus incident angle, (b) penetration profile of the evanescent field. The inner ray penetrates deeper than the outer one.

3. RESULTS

The ability of our system to detect real time events is experimentally demonstrated by detecting the Brownian motion of fluorescent nanospheres. The experimental setup is shown in figure 4. We observed the movement of 24 nm diameter nile red fluorescent carboxylate-modified nanospheres (emission maximum at 575 nm, absorption maximum
at 535 nm) suspended in DI water at room temperature. Nanospheres that enter and leave the evanescent field due to random Brownian motion are imaged as blinking spots (figure 4).

![Diagram of experimental setup](image)

Figure 4. The experimental setup used for the detection of 24nm fluorescent nanospheres.

5. CONCLUSIONS

An alternative optical configuration design for generating the evanescent field is proposed using a microfabricated silicon micromirror array chip. Efficient optical coupling is achieved through the use of a UV curable polymer that fills the silicon micromirror cavities. Such a design, eliminates the need for precise alignment of the excitation light into the system. Real time detection of the Brownian motion of fluorescent nanospheres is demonstrated using an inexpensive green laser diode and a CCD web camera. We envision the future integration of a laser diode or VSCEL’s array or of an expanded single laser beam directly beneath our chip for a fully miniaturized portable system for high throughput processing and point of care testing.

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REFERENCES