OPTICAL BEAM GUIDANCE IN MONOLITHIC POLYMER CHIPS FOR MINIATURIZED COLORIMETRIC ASSAYS

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ABSTRACT

For the first time, we present a simple and robust optical concept to enable precise and sensitive read-out of colorimetric assays in flat lab-on-a-chip devices. The optical guidance of the probe beam through an incorporated measurement chamber to the detector is based on the total internal reflection at V-grooves in the polymer chip. This way, the optical path length through the flat measurement chamber and thus the performance of the measurements are massively enhanced compared to direct (perpendicular) beam incidence. This is demonstrated by a chip-based, colorimetric glucose-assay on serum. Outstanding features are an excellent reproducibility ($CV = 1.91$ %), a competitive lower limit of detection ($c_{min} = 124$ µM), and a high degree of linearity ($R^2 = 0.998$) within a working range extending over nearly three orders of magnitude.

1. INTRODUCTION

Colorimetric absorption is, apart from electrochemical detection [1], one of the most commonly used modes of detection for assays on miniaturized lab-on-a-chip systems. This read-out method utilizes the specific absorption of a probe beam as a measure for the initial concentration of a biochemical target. For these miniaturized systems, a major drawback is the relatively short optical path length l_{abs} through the measurement chamber which is limited by the physical dimensions of the system. Several approaches to guide an optical beam on-chip and thus to optimize the sensitivity of the system are related to embedded optical fibers [2], integrated microlenses [3] or waveguides [4,5].

Since these approaches require rather complex designs and manufacturing processes, we present a novel concept to extend the optical path length by total internal reflection (TIR) at integrated V-grooves. This way, the optical path through the measurement chamber becomes independent of the thickness of the chip and is extended to enhance the performance of the sensor. Additionally, the monolithic devices can be manufactured by standard micromachining and do not require on-chip alignment of optical elements.

2. SETUP

We use a micromachined polymer disk (COC) with the size of a common compact disk (CD) as the substrate [6]. It features fluidic and optical elements to perform colorimetric assays. The fluidic elements provide ports for sample and reagent uptake which are connected to a combined mixing and measurement chamber (Fig. 1). The liquid transport is controlled by centrifugal forces F_ω which are generated by spinning of the disk [7].

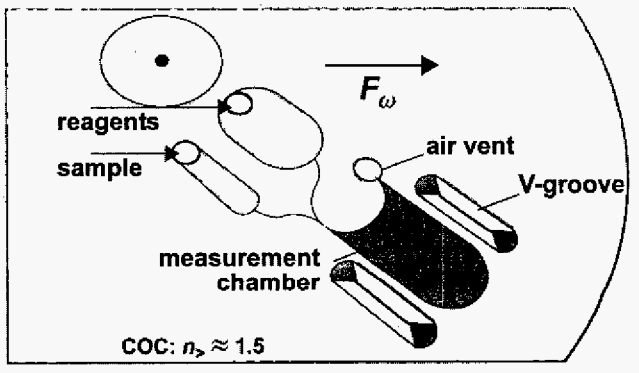

Figure 1. *Schematic of a microfluidic disk which combines fluidic elements to conduct a colorimetric assay with optical elements for on-chip optical beam guidance by total internal reflection (TIR). Inlet ports to load the sample and the reagents are connected to the measurement chamber where mixing of the liquids and the read-out of the assay are conducted. Next to the measurement chamber, V-grooves are embedded to couple the beam into the disk-plane. By spinning the disk, the sample and reagents are transported from their respective inlets into the measurement chamber.*

As optical guidance structure, triangular V-grooves are embedded next to the measurement chamber at the reverse side of the chip. When the beam has entered the polymer chip, it is deflected via total internal reflection (TIR) at the side face of the V-groove. To ensure complete reflection, the angle of incidence α has to exceed the critical angle α_c for TIR according to the law of Snellius (Fig. 2). With $n_> \approx 1.5$ and $n_< = 1$ as the refractive indices of the applied polymer substrate (COC) and the surrounding air, respectively, the critical angle of total internal reflection amounts to $\alpha_c \approx 41°$.

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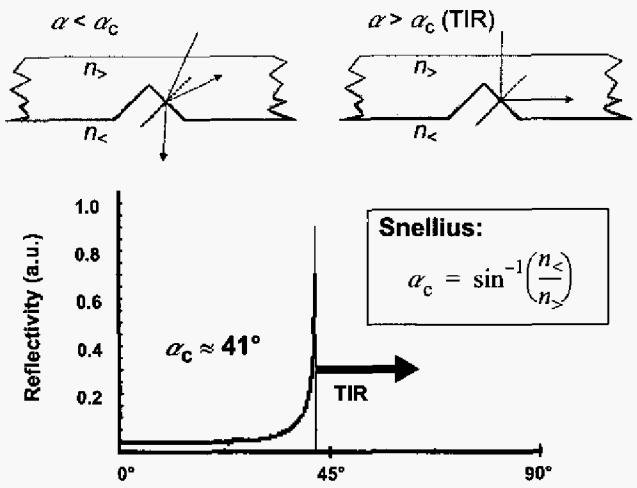

Figure 2. Beam-guidance by total internal reflection (TIR). Governed by the law of Snellius, the critical angle α_c for TIR at the interface of the applied polymer with $n_> \approx 1.5$ and air with $n_< = 1$ is derived to be $\alpha_c \approx 41°$. Thus, an optical beam with an incidence angle $\alpha = 45°$ is totally reflected perpendicular to the incidence.

The optical beam of a standard laser diode [8] is directed at perpendicular incidence on the flat upper side of the chip. After deflection into the chip-plane, the probe beam with an initial intensity I_0 is attenuated in the measurement chamber and then reflected at another V-groove towards a collimation-optics [9] positioned above the chip which focuses the beam onto the aperture of a fiber-probed micro-spectrophotometer [10] (Fig. 3) where the attenuated intensity I is measured. With this setup, an optical path length of $l_{abs} = 10$ mm through the measurement chamber has been realized, even though the height of the chamber amounts to 1 mm, only. Governed by the law of Beer-Lambert

$$A = -\ln\frac{I}{I_0} = \epsilon \cdot c \cdot l_{abs}, \qquad (1)$$

with ϵ as the extinction coefficient and c as the target concentration, the absorbance A increases with l_{abs}, thus the performance of the sensor is enhanced.

3. Colorimetric Glucose-Assay

In clinical diagnostics, a multitude of metabolic parameters are analyzed from whole blood or serum. Most of the methods involve several steps including the detection reaction. To quantify the sensor performance, we present a blood glucose determination which is one of the most important assays in clinical diagnostics. The chosen peroxidase based detection method can be used also for the determination of other metabolic parameters like lactate as well as immuno-assays.

The assay is conducted via a colorimetric, two-step reaction scheme (Fig. 4). As a first step, hydrogen peroxide (H_2O_2) is produced by the catalytic oxidation of glucose with glucose oxidase (GOD).

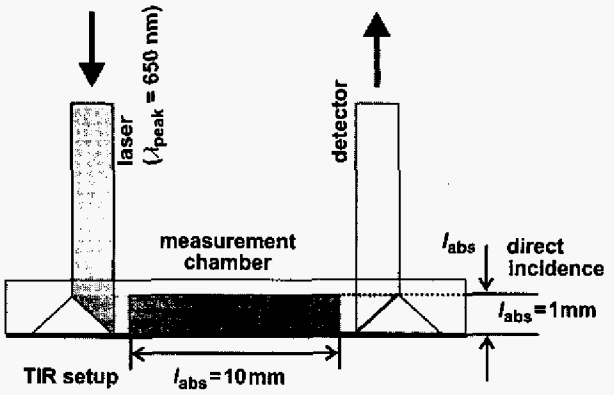

Figure 3. Optical elements to perform TIR on-chip. Next to the measurement chamber, integrated V-grooves deflect the beam into the disk-plane by TIR. After passing the measurement chamber, the attenuated probe beam is measured by a detector positioned above the disk. This concept significantly extends the optical path length of the beam through the flat polymer disk to $l_{abs} = 10$ mm compared to direct incidence ($l_{abs} = 1$ mm), thus sensitive colorimetric assays can be performed.

Subsequently, ABTS(red) is oxidized to ABTS(ox) by H_2O_2 in a POD catalyzed reaction. For the read-out of the assay, a laser diode with an emission wavelength of $\lambda_{peak} = 650$ nm is applied, although the extinction maximum of the ABTS(ox) dye is located at $\lambda = 420$ nm. However, there is sufficient attenuation for sensitive detection at the selected laser wavelength.

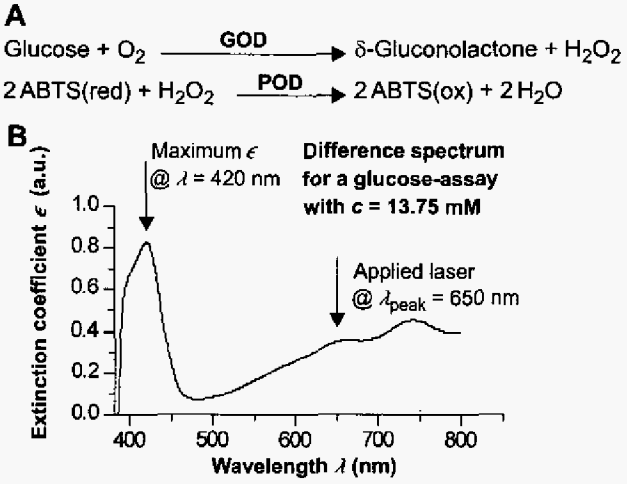

Figure 4. Colorimetric determination of the glucose concentration via a two-step enzymatic reaction. (A) First, hydrogen peroxide (H_2O_2) is generated in the catalyzed oxidation of glucose by glucose oxidase (GOD). Subsequently, the oxidation of the ABTS(red) dye by H_2O_2 to ABTS(ox) is catalyzed by peroxidase (POD). (B) Though the difference spectrum of ABTS(ox) features an extinction maximum at $\lambda = 420$ nm, the applied laser with a wavelength of $\lambda_{peak} = 650$ nm still experiences a sufficient attenuation for a sensitive assay read-out.

To perform the assay, a droplet of sample (500 nl) is dispensed into the chip, diluted with PBS-buffer (30.2 µl) and mixed with the reagents (69.3 µl), namely GOD (40 units/ml), POD (4 units/ml), and ABTS(red) (2 mM), in the measurement chamber.

To enhance the diffusion-limited speed of the reaction, we transferred the assay on our previously developed 'lab-on-a-disk' platform [11,12] (Fig. 5). By frequent reversal of the sense of rotation ('shake-mode') for a period of 2 minutes, inertially induced advection accelerates mixing from 6.5 minutes for mere diffusion to 5.1 seconds [13].

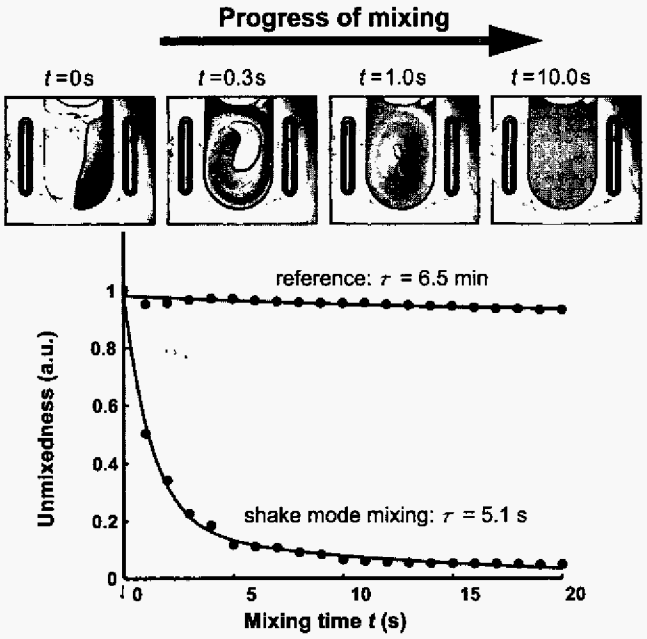

Figure 5. *Accelerated mixing by frequent reversal of the sense of rotation ('shake-mode'). Upon acceleration and deceleration, inertial effects induce advective currents which reduce the diffusion length. This way, the characteristic mixing time t is reduced from 6.5 min for mere diffusion to 5.1 s, only.*

These measurements are calibrated by a series of experiments with horse serum at known glucose concentrations (Fig. 6). The data-points display an absorption characteristics complying with the law of Beer-Lambert (equ. 1). We obtain a CV of 1.91 %, c_{min} = 124 µM as the lower limit of detection, and a highly linear relation between the glucose concentration and the optical signal (R^2 = 0.998). Additionally, the on-chip concept of guiding an optical beam with an optical path length of l_{abs} = 10 mm was compared with a rather conventional alignment (i.e., transmission) with l_{abs} = 1 mm. It turns out that, as predicted by equation 1, the slope $\Delta A/\Delta c$ and thus the resolution follows the ratio of the absorption lengths (Fig. 7) and is improved by one order of magnitude.

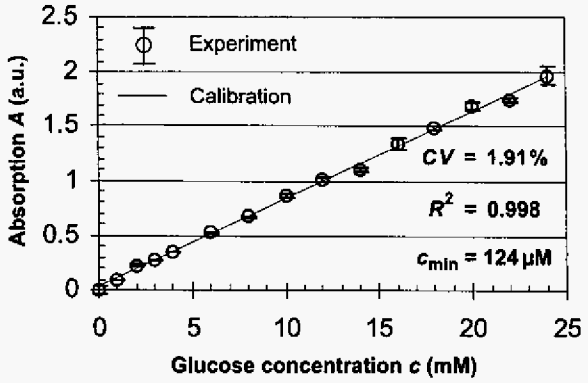

Figure 6. *Results of glucose determination: a sample of horse serum with an initial glucose concentration c = 4.0 mM is spiked to extend the concentration up to 25 mM. With a CV of 1.91 %, a lower detection limit of c_{min} = 124 µM and a linearity of R^2 = 0.998, our setup allows direct measurements at high precision.*

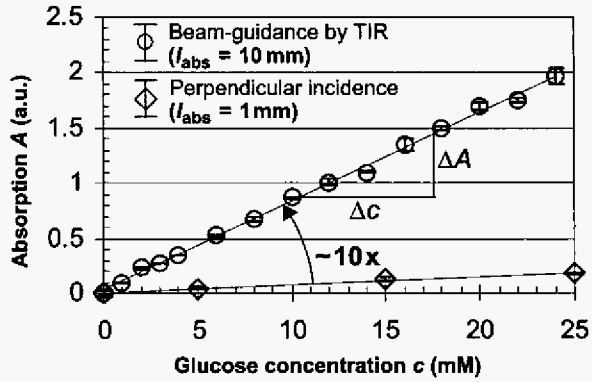

Figure 7. *Improvement of the sensor performance by an enhanced optical path length l_{abs}. The slope of the characteristic absorption curve $\Delta A/\Delta c$, which is a decisive impact factor for the resolution, is improved by a factor of 10 compared to transmission by perpendicular incidence of the beam at the flat side of the device without deflection into the chip-plane.*

4. Conclusion

We realized a versatile optical method for high-performance read-out of colorimetric assays by enhancing the optical path length via total internal reflection. The simple and rugged concept is implemented in a modular setup comprising an optical beam source, a detector, and an exchangeable polymer substrate containing a flat measurement chamber and monolithically integrated optical guidance structures. The microfluidic chip can thus be readily fabricated by standard polymer micromachining techniques and can be recommended for other formats of absorbance assays including ELISAs.

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