PHOTONIC LAB ON A CHIP ON POLYDIMETHYLSILOXANE SEGMENTED WAVEGUIDES FOR LOCAL MEASUREMENT OF OPTICAL DENSITY


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ABSTRACT
In the present work a photonic lab on a chip (LoC) based on poly(dimethylsiloxane) segmented waveguides (SWG) for local monitoring of optical densities is presented. Firstly, to evaluate the performance of SWG in LoC configurations, the limit of detection (LOD) and sensitivity for methylorange and fluorescein were determined, obtaining 5.2 ± 0.1 µM and 0.0055 ± 0.0002 U.A./µM for methylorange and 1.76 ± 0.02 and 0.0191 ± 0.0002 U.A./µM for fluorescein. Once the validity of such configuration was assured, it was used for locally measuring the optical density with a continuous cultivation of Saccharomyces cerevisiae. The presented results are very promising for the application in disposable photonic lab on a chip systems used for online analysis.

KEYWORDS: Optofluidics, bioreactor, segmented waveguide.

INTRODUCTION
Nowadays there is a huge interest in miniaturized systems for analytical, (bio)chemical, medical and environmental applications. These systems should have high sensitivity, fast response time, low amount of reagents needed, low cost, stability and robustness. All these specifications define the well known concept of lab on a chip (LoC). LoC systems have a wide variety of interrogation mechanism such as electrochemical [1], mechanical [2], magnetic [3] and optical [4]. Optical interrogation mechanism is one of the most versatile due to the optical properties that can be used as could be phase [5], wavelength [6], and intensity [7]. Phase measurements present the highest sensitivity, however its daintiness and technological requirements are drawbacks that have not been solved yet. Otherwise, wavelength measurements and concretely, fluorescence detection is largely used in benchtop equipments such as fluorescence-activated cell sorters (FACS) with single cell analysis capabilities. FACS equipments seem to be the best choice for cell analysis. However, cost and size make these equipments limited to important laboratories. Intensity as interrogation mechanism allows a significant reduction of size and cost [8]. In this work, low cost, intensity based, photonic microbioreactors (MBR) are presented. In order to minimize the cost, poly(dimethylsiloxane) (PDMS) is the chosen material to fabricate the final LoC with standard soft-lithographic methods [9].

![Figure 1: Schematic view of the microbioreactor with exact dimensions.](image)

DESIGN AND FABRICATION
To maximize the interaction between light and liquid a segmented waveguide (SWG) has been fabricated. Three different segmented waveguide profiles are proposed, symmetric (s-SWG), asymmetric with normal light injection (a-SWG) and finally asymmetric with reverse light injection (a’-SWG) as shown in figure 1. The PDMS pillars perform a...
double function. From a fluidic point of view, the pillars focus the fluid at the regions between two consecutive pillars. Optically, the PDMS pillars facilitate propagation of a guided beam. The a\textsubscript{c}-SWG and a\textsubscript{r}-SWG can also be used as focusing or expanding waveguide, respectively. A cylindrical PDMS lens is also designed to achieve collimated beams at the SWG start point, reducing losses due to beam divergence.

The actual device has been fabricated using standard soft-lithographic methods. Firstly an SU-8 (SU-8 50 from MicroChem, Corp., Newton, MA, USA) master was done and a PDMS (Sylgard 184 elastomer kit, Dow Corning, Midland, MI, USA) replica was cured. Finally the PDMS replica and a glass substrate were bonded forming the final MBR (figure 2a).

**RESULTS AND DISCUSSION**

Since no SWG were previously used in LoC configurations, it was necessary to evaluate its performance when using analytes with/without photonic reemission. To this effect, methylorange and fluorescein in concentrations from 0.25 µM to 1000 µM were injected into the MBR. The absorbance matches the Beer-Lambert law until a concentration of 125 µM. In table 1 the results are presented for the three different configurations and the two described analytes. As could be seen numerical and experimental losses do not match, this may be due to insertion losses (not calculated in numerical simulations) and also for imperfections on the cylindrical lenses increasing losses due to divergence or a non perfect planarity on the faces of PDMS pillars. Decrease of the total losses is also observed when the Fresnel reflections are reduced due to the low RI difference between the liquid and the pillars. On the other hand all configurations, i.e., s-SWG, a\textsubscript{c}-SWG and a\textsubscript{r}-SWG present similar results, hence the main issue to select a particular configuration is the fluidic behaviour, and in this situation s-SWG is the optimal candidate due to the symmetric fluid focalization observed in the numerical simulations. Once the configuration has been chosen and the SWG tested with fluorescein and methylorange, an application, a cultivation of *Saccharomyces cerevisiae* inside the s-SWG photonic lab on a chip was performed while continuously measuring the local optical density. Before cultivation, the s-SWG device was rinsed for 20 min with 95% ethanol for disinfection purposes and afterwards purged with sterile distilled water. The inoculum culture was cultivated at 30 °C in shake flasks in modified mineral Verduyn medium [10] with 20 g/L glucose at pH 4.5 for 10 h. The s-SWG device was inoculated with 2 mL of harvested yeast cells in exponential growth rate that were diluted in modified Verduyn medium (1 g/L glucose) and set to an optical density at 600 nm of 0.1. After inoculation, fresh Verduyn medium was continuously dosed to the system at a rate of 0.5 mL/h via a syringe pump (TSE Systems GmbH, Germany). A heater set-up allowed constant temperature stabilization of 30±0.2 °C, whereas constant oxygen supply was provided by the gas permeable PDMS membrane. Readout was done through two different SWGs, at positions 4 and 14.

**Table 1: Experimental results of fluorescein and methylorange.**

<table>
<thead>
<tr>
<th>Config.</th>
<th>Sensitivity (x 10\textsuperscript{-3}) [U.A./µM]</th>
<th>R\textsuperscript{2}</th>
<th>LOD [µM]</th>
<th>Numerical Losses [dB] (Water)</th>
<th>Experimental Losses [dB]</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-SWG</td>
<td>Fluorescein</td>
<td>18.8 ± 0.2</td>
<td>0.9995</td>
<td>1.99 ± 0.02</td>
<td>12.3±0.3</td>
</tr>
<tr>
<td></td>
<td>Methylorange</td>
<td>5.5 ± 0.2</td>
<td>0.997</td>
<td>5.2 ± 0.1</td>
<td>8.2±0.2</td>
</tr>
<tr>
<td>a\textsubscript{c}-SWG</td>
<td>Fluorescein</td>
<td>20.2 ± 0.3</td>
<td>0.999</td>
<td>2.94 ± 0.05</td>
<td>11.4±0.2</td>
</tr>
<tr>
<td></td>
<td>Methylorange</td>
<td>6.1 ± 0.1</td>
<td>0.998</td>
<td>6.4 ± 0.1</td>
<td>7.75±0.09</td>
</tr>
<tr>
<td>a\textsubscript{r}-SWG</td>
<td>Fluorescein</td>
<td>19.1 ± 0.2</td>
<td>0.9996</td>
<td>1.76 ± 0.02</td>
<td>12.7±0.3</td>
</tr>
</tbody>
</table>

Figure 2: a) Picture of the microbioreactor. b) Plotted results of absorbance against time of cell cultivation within the MBR on SWGs 4 and 14, inlet and outlet, respectively.
As can be seen in figure 2b a typical transition curve from the inoculation to stationary phase growth can be observed. Within the first six hours cell growth is very slow and unsteady, this may due to the microorganisms adapt themselves to the new habitat and nutrients (the glucose concentrations of 1 g/L has been reduced to one twentieth compared to the pre-culture medium with a glucose concentration of 20 g/L). Then the cell concentration increases and remains the next ten hours: cells multiply themselves. After around 18 hours a stationary phase is reached. This may be associated to the situation where the microbial growth equals the number of cells been transported out of the bioreactor with the effluent, thus stabilizing the number of yeast cells in the system.

CONCLUSIONS
A photonic LoC based on SWGs is presented. Three different configurations (asymmetric SWG with normal or inverse light injection and symmetric SWG) have been studied obtaining LODs of 6.4 and 1.76 µM with methylorange and fluorescein, respectively. Once the linear behaviour has been ensured the best configuration (s-SWG) was chosen to test a biological application for local monitoring of absorbance of growing yeast cells. The cultivation was carried out over a time span of 33 h. By plotting the absorbance at a fixed wavelength versus the cultivation time, a typical transition growth curve from the inoculation to stationary phase – considering the biological point of view – could be locally determined within the reactor. The presented results are very promising and open a wide variety of possible biological applications.

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