High-Selectivity Optical Detection in Microfluidic Systems for Clinical Diagnostics

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Summary. *This paper presents a microfluidic system for determining the protein concentrations in human biological fluids (e.g. urine, blood or serum) with a highly sensitive and selective optical photodetector based on a Fabry-Perot filter. The microsystem consists of two wafers: a Pyrex glass wafer containing the microlaboratory (microchannels to carry chemical reagents and sample solutions) and a silicon wafer that includes the protein detection system and readout electronics. The optical channel operates in the visible spectral range. A FWHM < 3 nm and finesse of 36 for narrow band operation is demonstrated in albumin concentration detection. Detection system compensation techniques are also presented.*

Keywords: lab-on-a-chip, microfluidics, proteins, Fabry-Perot filter

Introduction

The same basic fabrication concepts and materials used in microelectronics enabled the development of biomedical based systems devices, such as a laboratory on a chip (lab-on-a-chip). The implementation of lab-on-a-chip devices presents new and interesting technological challenges and their capabilities to chemical analysis are outstanding. Microscopic versions of liquid-handling devices, including pumps, valves, volume measuring tools, chemical reactors, extractors, filters, mixers and even sophisticated chromatographic techniques, can all be implemented and integrated into a chips' design, thanks to the microsystem technology which enables the fabrication of precise and small structures [1].

The small size and portability of lab-on-a-chip devices result in a reduction of the analytical testing costs and time, and in a significant improvement in laboratory safety. Spills, explosions and other laboratory accidents that can occur with conventional sample preparation techniques are no longer a problem. Since nanoliter quantities of organic solvents and samples are used, the costs associated with buying new reagents and disposing of the used ones are negligible [2]. Moreover, since the lab-on-a-chip rather than a chemist performs the sample preparation, untrained personnel can accurately and precisely perform a complete analysis.

In this paper a microfluidic system designed for protein concentration detection (e. g. albumin) in human biological fluids (e. g. urine or serum) is presented. The detection system consists in color analysis based on optical absorption. The wavelength, which the absorption is maximum or minimum for different proteins, is accurately analyzed.

Microsystem design

The microsystem itself is composed of two wafers: one containing the microlaboratory and other the protein concentration detection system.

Microlaboratory

Fig. 1 presents a schematic top view of the microlaboratory, which is fabricated in a Pyrex glass wafer. Glass was chosen for its transparency and because it is an electrical insulator. Therefore, electrophoretic flow principle can be used to move fluids through the microchannels, which avoids mechanical pumps and valves.

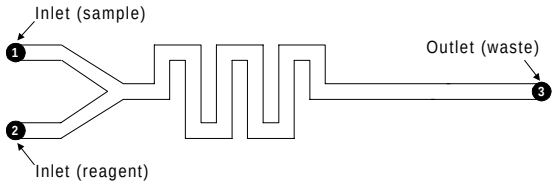

Fig. 1: Microchannel layout of the lab-on-a-chip.

The device comprises two microchannels (from inlets 1 and 2) merging into one (to outlet 3). Sample and reagent solutions are injected in the lab-on-a-chip through inlets and both solutions are mixed in the main microchannel. Low voltages are applied between electrodes (on the silicon wafer) distributed by the flow path. The mixture is analyzed by a photodetector placed in the silicon wafer almost under the outlet. However, some proteins detection requires a capillary electrophoresis separation techniques (e. g. amino acids detection).

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Protein concentration detection system

For the colorimetric detection, a white light is used as incident light into the detection chamber, where some spectral components are absorbed. Once the wavelength region, which the absorption is maximum or minimum for different proteins is very narrow, the optical detector must have a high-spectral selectivity. Therefore, firstly, a commercially high-pass optical filter on the top of the microsystem cuts a wide region of the spectrum. Secondly, a Fabry-Perot filter, that can be used as an effective wavelength-selecting element, selects the specific wavelength which the absorption is a maximum or a minimum. The intensity of the light transmitted through the Fabry-Perot filter is measured in the integrated photodiode and gives information about the proteins concentration.

The Fabry-Perot filter consists of two parallel mirrors with a resonance cavity in the middle (Fig. 2). Two types of high reflective coatings are used in mirrors: dielectric and metallic. The dielectric mirrors, when properly designed and fabricated, features high performance (high reflectivity, low absorption losses). However, metallic mirrors can be attractive in certain applications due to the simplicity of their fabrication (only one layer is deposited). Despite the absorption, two silver mirrors are used due of their high reflectance in the visible spectral range. The resonance cavity is a PECVD SiO_2 film (with a low and almost constant refractive index in the same range).

The equation, $2nd = \lambda q$, shows the operation principle of the Fabry-Perot filter, where n is the refractive index of the cavity medium, d the cavity length, λ the incident wavelength and q the interference order (q = 1, 2, 3,…).

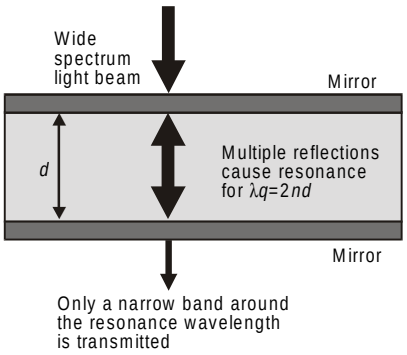

Fig. 2: Fabry-Perot filter.

Device fabrication

The lab-on-a-chip and its cross section are shown in Fig. 3 and 4, respectively.

The microlaboratory consists of a Pyrex glass top wafer with wet-etched microchannels (40 µm deep and 200 µm wide). The silicon wafer has the pn-junction photodetector and respective readout electronics. These functions are done in a CMOS standard process. The Fabry-Perot layers are deposited at the very end of the fabrication sequence. The silver layers are patterned using lift-off.

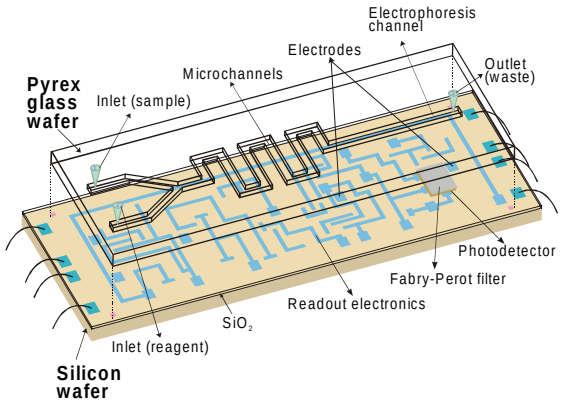

Fig. 3: Lab-on-a-chip. The top wafer shows the microchannels. The bottom wafer shows the detection system and readout electronics.

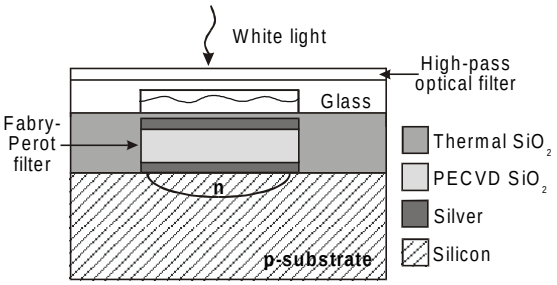

Fig. 4: Cross-section of the microsystem after bonding the two wafers.

Experimental results

Albumin concentration detection is the first target of the lab-on-a-chip. This protein when is bounded with bromcresol green reagent has an absorptance maximum at 628 nm (due to the reagent) and an absorption spectra similar to that one presented in Fig. 5 [3]. The intensity of the color produced by the mixture is directly proportional to the protein concentration.

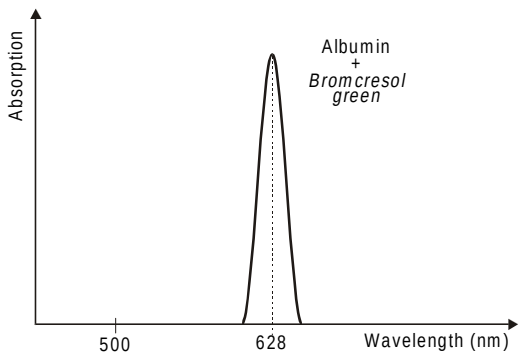

Fig. 5: Absorption spectra shape for albumin.

Optical simulations of the Fabry-Perot filter

A thin-film optics software package (TFCalc 3.3) is used for optimization of the Fabry-Perot filter. The transmittance of a 55 nm-Ag / 1028 nm-SiO_2 / 50 nm-Ag layer stack (Fig. 6) shows a FWHM of 2.9 nm and a finesse of 36. In order to achieve a single peak at 628 nm (albumin detection) a commercially high-pass optical filter is used (with a cut-off wavelength in 550 nm).

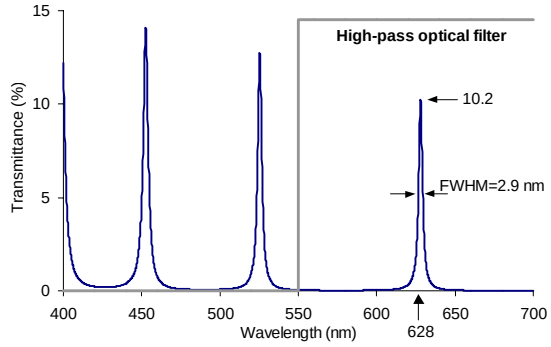

Fig. 6: Simulated transmittance vs. wavelength for a 55 nm-Ag / 1028 nm-SiO_2 / 45 nm-Ag layer stack.

Optical detection system compensation

Any imperfection of the incident light wave in a real Fabry-Perot structure causes increased transmittance outside the narrow band to which the Fabry-Perot filter is tuned (parasitic background signal). The addition of a compensation structure with the cavity optical length below $\lambda/10$ is the possible solution [4]. The transmittance signal is similar to the parasitic background and is subtracted from the real signal.

Albumin concentration detection

In order to calibrate the detection system of the lab-on-a-chip, some measurements need to be performed to find out the real transmitted wavelengths and the real relationship between protein concentration and the intensity of the transmitted light.

Experimental results were obtained in albumin analysis, with a test kit from Sigma-Aldrich. Known protein concentrations were used in order to obtain a calibration curve and several absorption spectra. Fig. 7 shows the absorption spectra when different albumin concentrations react with bromcresol green (0.30 mmol/l, pH = 4.2). It can be seen that as far as the albumin concentration is small, smaller is the spectral peak. Measurements results were done from 50 µg/ml until 50 mg/ml albumin concentration. The normal values of albumin concentrations in human urine are between 10 and 140 µg/ml, but in human serum are higher, from 38 mg/ml to 50 mg/ml [5].

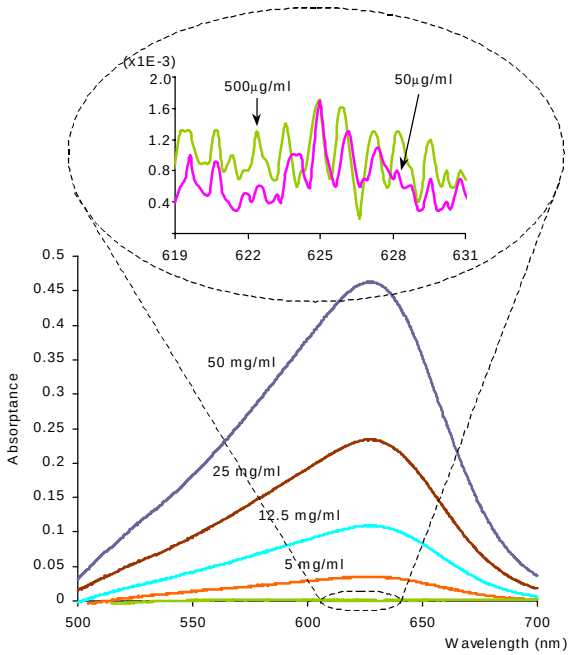

Fig. 7: Absorption spectra for different albumin concentrations, after the binding with bromcresol green.

A disadvantage of those tests is due to manual pipetting. If two different persons pipet the same concentrations solutions, the results are not the same. Furthermore, for lower concentrations, different replicas of the same concentration result in different absorptances, even when the same person does the pipetting. These disadvantages will not occur when the lab-on-a-chip is used to do the tests, once the sample and reagent volume is computed automatically. Meanwhile, others reagents suitable for microassays are being tried (e. g. Bradford reagent, for detecting proteins total concentration).

Although this microsystem was projected for albumin concentration detection in urine or serum analysis, other biological fluids (such as sweat or saliva), or even other proteins are potential candidates for the lab-on-a-chip.

Conclusions

Microtechnology allows not only the realization of microfluidic systems and optical components of reduced size, but also their assembly in stacked forms. The lab-on-chip presents a high level of automation, real-time analysis and in-situ measurements. The optical detection system is highly sensitive to specific wavelengths according the Fabry-Perot filter design. Moreover, tests using the ambient light have been done in order to avoid the use of a known source of light. Therefore, such detection system is extremely suitable for application in microfluidics systems (e.g. µTAS) due to its small size and high-spectral selectivity.

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The lab-on-a-chip for biological fluids analysis can be a powerful tool in hospitals and operating rooms as well as in patient homes due to its rapid, accurate and sophisticated diagnostic tests for numerous critical compounds. Other applications of the lab-on-a-chip are for monitoring the air and the water quality for potential toxins and pesticides, screening foods, and promptly identifying drugs abuse. Lab-on-a-chip devices will probably find their way into forensic, environmental and food testing laboratories in the near future. Moreover, since low quantities of hazardous chemical reagents are needed, the resultant environmental pollution starts to be no longer a problem.

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