Lab-on-a-chip for Biological Fluids Analysis by Spetrophotometry

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Introduction

The healthcare sector is nowadays one of the most dynamic sectors where the novelty is a strategic and operational imperative. The possibility of increasing the quantity and quality of clinical analysis, performed with instantaneous results and outside the clinical laboratories, contributes to a better quality in the healthcare services and to a better efficiency in the clinical and administrative processes [1]. This possibility can be achieved with the presented lab-on-a-chip for spectrophotometric analysis of biological fluids. It allows the selective measurement of the concentration of several biomolecules in biological fluids, with instantaneous results, at any location, with small quantities of reagents and samples and with low-cost. That measurement is based on colorimetric detection by the optical absorption in a part of the visible spectrum defined by the reaction of the specific biomolecule with a specific reagent. The device comprises a highly efficient and selective optical filtering and colorimetric detection system. This system allows the measurement with a regular white light illumination, thus avoiding the use of complex and expensive analysis systems like the ones that comprise spectrophotometers, for example. This feature highly facilitates portability and ensures analysis within consultation time, at a patient house (allowing a first trial) and in clinical laboratories or hospitals. Despite its small dimensions, low energy consumption and the use of small quantities of reagents and samples, it has the same reliability and precision of the biological fluids analysis systems by spectrophotometry available, nowadays, in clinical laboratory analyses.

Lab-on-a-chip Operation

The lab-on-a-chip operation (see Fig. 1) is based on topside illumination with a white light beam that is transmitted through the microchannels containing the samples to analyze. The impinging light is filtered, by the optical filters, to a narrow spectral band centered at the wavelength for which the colored mixture has its absorption maximum.

Table 1. Result of linear fit from absorbance calibration plot measured using a white light as light source (a 200 W halogen lamp).

Analytes (measured conc. range (mg/dl))	∝(abs max) (nm)	Linear conc. range (mg/dl)	Slope (a. u.) dl/mg	Intercept (a.u.)	Correlation coefficient (R^2)
Uric acid (0-120)	495	0 – 30	$(1.9 \pm 0.4)x10^{-3}$	$(3 \pm 2)x10^{-3}$	0.98016
Total protein (0-100)	592	0 – 100	$(1.9 \pm 0.2)x10^{-4}$	$(-0.5 \pm 2)x10^{-4}$	0.99665

The intensity of the selected spectral component transmitted through the fluid is measured using underlying photodetectors. This optical intensity is proportional to the biomolecule concentration.

A light-to-frequency converter is integrated with the photodetectors to convert the analog signal into a digital signal. This digital value is then collected by a microcontroller, which makes the calculations, based on Lambert-Beer's law, to obtain the biomolecule concentration that is being analyzed, and, provides a standardized output format for higher computer levels too.

Lab-on-a-chip Design and Fabrication

The lab-on-a-chip combines in a multichip module the microfluidic system, the optical filtering system and the detection and readout system. The microfluidic system contains the microchannels and the detection chambers. The main channel is 500µm, 70 mm long and 500µm deep, with a liquid volume quantity of 20 µl. Each detection chamber is 2 mm wide, 3 mm long and 500 µm deep. The high depth is crucial for the optical absorption measurements [2]. Three detection chambers are needed. The first, to obtain the baseline reference and to calibrate the light source. The second, for the colored mixture analysis. The third, to calibrate the biomolecule concentration (with a well-known concentration standard) and also to compensate the white light oscillations. The microchannels are fabricated in SU-8 photoresist (Fig. 2). Therefore, they have a deep rectangular vertical profile, which is suitable for optical absorption measurement. Moreover, the SU-8 based fabrication is a low-cost

Fig. 1. Artist impression of the lab-on-a-chip structure.

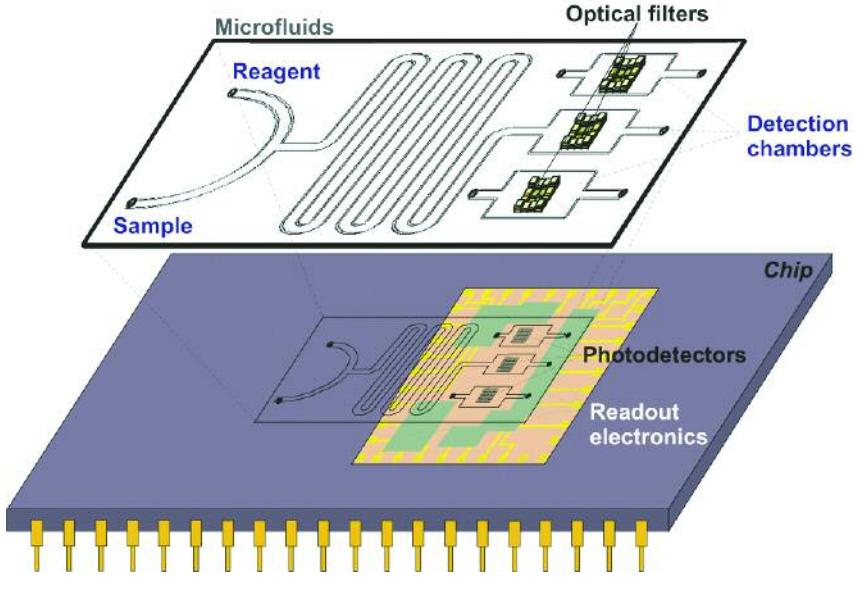
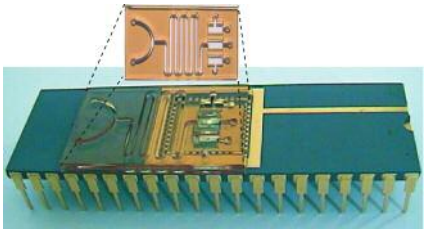


Fig. 2. A photograph of the complete lab-on-a-chip. The microfluidic system is glued to the CMOS chip.



process, UV lithography semiconductor compatible and does not require expensive masks, a regular transparency foil, like the one used in printed circuit boards, is enough [3]. In addition, the microfluidic system can be a disposable die, which minimizes the cost associated with cleaning and avoids the contamination between analyses.

The optical filters are based on highly selective Fabry-Perot thin-films optical resonators fabricated using a stack of dielectric layers (TiO2 and SiO2). These films are deposited on top of the detection chambers by Ion Beam Deposition. They are designed to yield a narrow passband around the wavelength for which the colored mixture has its absorption maximum. This characteristic enables the selective measurement of the light intensity, at the desired wavelength, transmitted through the mixture. Rather than just one optical filter, a 16 optical filter array has been developed. The complete 4 x 4 array allows quantifying the concentration of 16 different biomolecules with the same device. Each optical filter has an active area of 200 x 200 μm^2 and is sensitive in a single wavelength, with a FWHM less than 6 nm and with a peak intensity higher than 86%. The 16 optical filters array fabrication requires only 4 masks, used with different deposition time. They have been designed for analyzing the following biomolecules: 17-ketosteroids, chlorine, uric acid, cholesterol, glucose, magnesium, creatinine, urea, hemoglobin, ß-glucuronidase, bilirubin, leucine aminopeptidase, calcium, oxalate, total protein and albumin, with absorption maximums between 480 nm and 600 nm, spaced 8 nm. However, other biomolecules with absorption maximums at other wavelengths can be analyzed by adjusting the thickness of only one of the dielectric layers, without affecting the lab-on-a-chip layout [4].

The photodetectors array are placed underneath the detection chambers and aligned with the optical filters array. They are n+/p-epilayer junction photodiodes fabricated in a CMOS process. They convert the light intensity that is transmitted through the colored mixture into a photocurrent. Its readout electronics consists of a light-to-frequency converter, integrated with them in the same fabrication process [2]. The filtering and the detection system are the same for several analyses of the 16 biomolecules.

Experimental Results

After testing that the use of small quantities of samples remains compatible with the prescribed procedures that are described in the reagent protocol, which are based on 3 ml of sample volume, proper operation of the lab-on-a-chip is confirmed using a set of experiments that involve the quantitative measurement of uric acid and total protein in urine.

The complete and homogeneous mixing of the urine sample with the specific reagent for the quantitative measurement of the biomolecule concentration was performed in about 40 seconds.

The reagents used are from **Sigma-Aldrich** [5]. They react with a sample of urine in a 50:1 ratio. Measurements were performed comprising the range of normal and typical abnormal biomolecules concentration values in a human being.

The obtained results (Table 1) allow the following conclusions: (1) with correlation coefficients above 0.980 the method presents a linear behavior for the concentration range described in the table (for higher concentrations the sample should be diluted and re-assayed multiplying the result by the dilute factor); (2) the intensity of the color produced by the mixture is directly proportional to the biomolecule concentration, once it obeys to Lambert-Beer's law; (3) the uric acid and the total protein absorption spectra show a maximum peak at the wavelength μ= 495 nm and μ= 592 nm, respectively; (4) the reproducibility mean coefficient variation of 10 replicate analyses for each concentration were less than 10%; (5) the minimum detection of the lab-on-a-chip is 0.5 mg/dl and the achieved sensitivity is 5 mg/dl for both analytes, which corresponds to a relative resolution of 3.3%, enough for human being urine values.

The results confirm the direct proportionality between intensity of the color produced by the mixture and the biomolecule concentration. They agree with macroscopic measurements performed with well known uric acid and total protein standards and using state-of-the-art laboratory equipment. In conclusion, the same performance, precision, reliability and sensibility of the analysis performed, nowadays, in clinical analysis laboratories were obtained. ■

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يعتبر قطاع العناية بالصحة حالياً من أكثر القطاعات ديناميكيةً حيث التجديد هو ضرورة إستراتيجية للعمل. وتساهم إمكانية تحسين النوعية والكمية في الفحوصات السريرية برفع مستوى خدمات العناية بالصحة وتأمين فعالية أفضل في العمليات الكلينيكية. تتمثل هذه الإمكانية بنظام المختبر-على-رقاقة (Lab-on-a-chip) لتحليل حسب المضواء الطيفي للسوائل الحيوية. يؤمن هذا النظام قياساً اختياريا لتركيز الجزيئات الحيوية من هذه السوائل مع نتائج فورية في أي مكان وباستعمال كميات صغيرة من مفاعلات الكشف والعينات وبمصاريف منخفضة يقوم القياس على الكشف اللوني حسب الامتصاص البصري في قسم من الطيف المرئي. يحدده تفاعل الجزيئة مع المفاعل المحدد تحوي آلة Lab-on-a-chip نظام كشف لوني وترشيح بصري انتقائيين ذات فعالية عالية. يسمح هذا النظام بالقياس مع إضاءة نور أبيض عادية فيجنب بالتالي استعمال آلات التحليل المعقدة والباهظة الثمن.
بالرغم من صغر حجمه وضئالة استهلاكه للطاقة واستعماله لكميات صغيرة من المفاعلات يؤمن نظام Lab-on-a-chip الفاعلية والدقة في تحليل السوائل البيولوجية كأي من آلات المضواء الطيفي المستعملة في المختبرات.

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