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An array of Fabry-Perot optical-channels for biological fluids analysis

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Abstract

This paper describes a biosystem (biological system) used to measure the concentration of biochemical substances in urine, serum, plasma or cerebrospinal fluid. Rather than just one channel, it comprises 16 optical-channels that enable the measurement of the concentration of 16 different biochemical substances. An array of 16 optical filters based on Fabry-Perot thin-films optical resonators has been designed. Each optical-channel is sensitive in a single wavelength with a full-width-half-maximum (FWHM) of 7 nm. The filter fabrication requires only four masks, used with different etch time. A commercially available band-pass optical filter with a band-pass wavelength in 450–650 nm is used. The biosystem requires only a white light source for illumination due the use of selective optical filters.

Keywords: Biosystem; Optical filter array; Fabry-Perot

1. Introduction

Spectrophotometric analysis (the study of the interaction of electromagnetic radiation with chemical compounds) is one of the most commonly used analytical techniques for biological fluids analysis in clinical diagnostics. This technique is used to determine the concentration and/or amount of a particular compound in biological fluids samples [1]. Usually, the samples need to be sent to a laboratory for spectrophotometric analysis, and the results become available after several hours or days. The need for rapid and on-line measurements led to the development of biosystems with the fluidic, detection and readout systems integrated in a single-chip [2]. The advantages associated with shrinking clinical analysis systems include improved efficiency with respect to sample size, integration, automation, response times, analytical performance, laboratory safety and costs. Previously developed biosystems on-a-chip with absorbance detection require a wavelength dependent light or waveguides inserted into the biosystem for illumination [3,4]. Illumination using only a white light source requires the use of selective optical filters.

2. Design of the 16 optical-channels array

2.1. Background of the biosystem application

The application of the particular biosystem presented here is the measurement of the concentration, by optical absorption, of 16 different biochemical substances in human's fluids. However, many of the analytes[1] of interest for clinical analysis do not have chromophores that absorb light in a useful part of the visible range. Specific chemical reactions are available (reagents) to transform these analytes into colored products that do have adequate absorbance [1]. In addition, the concentration of biochemical substances is measured by using a mixture of a reagent with an analyte sample. The measurement method has the following characteristics: (1) the intensity of the color produced by the mixture is directly proportional to the concentration of the biochemical substances in analysis; (2) the absorption spectra of the mixture show a maximum peak at a specific wavelength; (3) each mixture presents a linear behavior within the interest concentration range.

A biosystem to measure the concentration of biochemical substances in biological fluids, by optical absorption, was previously implemented [4]. Its operation was successfully

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[1] An analyte is the substance (element, ion, compound or molecule) being analyzed.

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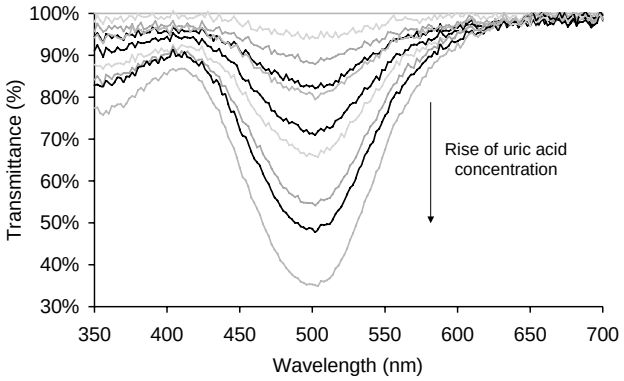

Fig. 1. Measured transmittance spectra for different uric acid concentrations [4].

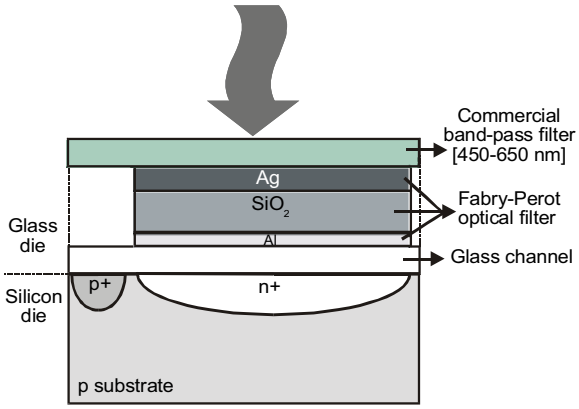

Fig. 2. Schematic structure of the biosystem for an individual optical-channel in cross-section.

demonstrated in uric acid concentration detection (Fig. 1). However, the measurements were carried out with a wavelength dependent light source (monochromatic light).

2.2. Complete structure

An optical filter placed on the top of the biosystem allows the use of only a white light source, giving portability to the biosystem. Fig. 2 shows schematically the cross-section of the biosystem for an individual optical-channel. It is composed of a glass die and a silicon die. The glass die contains the fluidic channels (Fig. 3) and the optical filters. The silicon die contains the photodetectors and readout electronics. A commercially available band-pass optical filter on the top of the biosystem is used to avoid the non-visible spectrum.

Fig. 3. The fluidic channels. It comprises three fluidic channels. Channel A is needed to obtain the baseline reference and to calibrate the light source. Channel B allows the mixed solution analysis (reagent plus sample). Channel C is needed to calibrate the biochemical substance concentration (well-known biochemical calibrator).

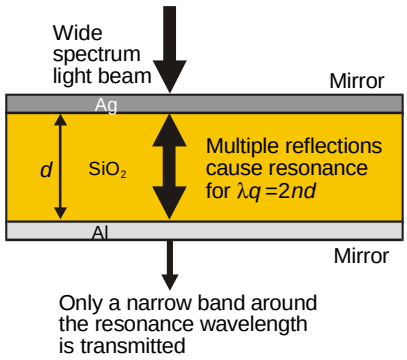

Fig. 4. Fabry-Perot filter.

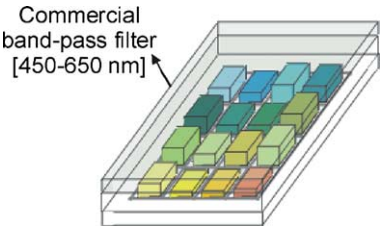

Fig. 5. An artist impression of the 16 optical filters (4 × 4 array) and the commercial band-pass filter. Each of the Fabry-Perot cavities is tuned to transmit in different spectral band.

The device operation is based on optical absorption in a well-defined wavelength of the visible spectrum. The impinging spectrum is filtered by the optical filters to a single wavelength, and the intensity of the selected spectral component transmitted through the fluid is measured using an underlying photodetector. The optical-channel is composed

Table 1
The 16 biochemical substances that can be analyzed in the biosystem [1]

Biochemical substance	Biological fluid	Absorption spectra maximum peak (nm)
Uric acid	U, CSF	495
Cholesterol	S	500
Glucose	S	505
Glutamic oxalacetic/pyruvic transaminase	S, P, CSF	510
Creatinine	U, S, P	515
Magnesium	S	520
Aldolase	S	525
Bile acids	S	530
Blood urea nitrogen	S, P	535
Salicylate	S	540
Hemoglobin	P	545
β-Glucuronidase	S, U	550
Urea nitrogen	U, S, P	555
Bilirubin	S	560
Leucine aminopeptidase	U	565
Calcium	S	570

Note: U: urine, S: serum, P: plasma, CSF: cerebrospinal fluid.

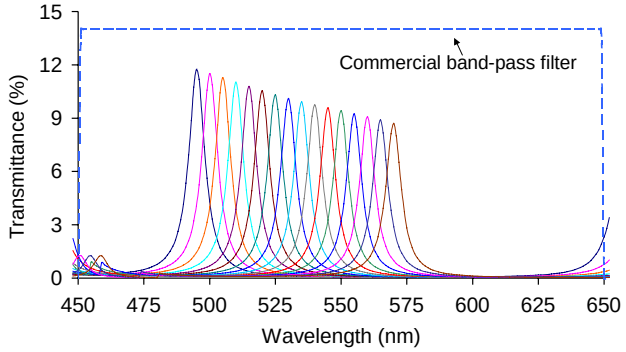

Fig. 6. Simulated transmittance vs. wavelength for the 16 optical filters array. The Fabry-Perot layer stack is 20 nm Al/SiO_2/40 nm Ag. The SiO_2 layer thickness changes from 637 to 742 nm in 7 nm increments.

by a Al/SiO_2/Ag layer stack functioning as a Fabry-Perot optical filter with an optical detector underneath (a CMOS standard photodiode).

The Fabry-Perot filter consists of two parallel mirrors with a resonance cavity in the middle (Fig. 4) [5]. The equation $\lambda q = 2nd$ shows its operation principle, where n is the refractive index of the cavity medium, d the cavity length, λ the incident wavelength and q the interference order ($q = 1, 2, 3, \ldots$). The optical filters use metallic mirrors instead of high-performance dielectric mirrors due to the simplicity of their fabrication: only three layers are deposited and the wavelength selection is performed by changing only the thickness of the SiO_2 layer. Silver and aluminum have been selected due to their high reflectivity at visible wavelengths [6]. Aluminum is the most suitable material in terms of fabrication compatibility (despite its higher absorption losses). Silver exhibits poor long-term stability (tendency to tarnish) [7]. However, that biosystem is sealed, using the commercial band-pass filter, to avoid the oxidation of the silver layer caused by the environment. A special glue is used around the Fabry-Perot optical filter for adhesive bonding the glass commercial band-pass filter to the glass die. It also could be used an intermediate layer of polysilicon to obtain the glass-to-glass anodic bonding.

Rather than just one optical filter it has been developed a 16 optical filters array based on Fabry-Perot thin-films optical resonators. The complete 4 × 4 array, schematically shown in Fig. 5, allows the measurement of the concentration of 16 different biochemical substances in human's fluids. These substances are described in Table 1. Each of the optical filters is tuned for a specific wavelength (third col-

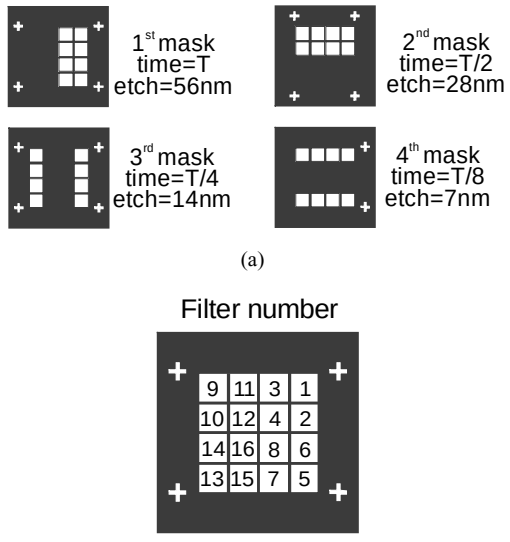

Fig. 7. (a) The four masks used in the SiO_2 etching process. The crosses are alignment marks; (b) the position of each filter in the array. The filter number 1 ($\lambda = 495$ nm) is for uric acid, the filter number 2 ($\lambda = 500$ nm) is for cholesterol, and so one according to Table 1.

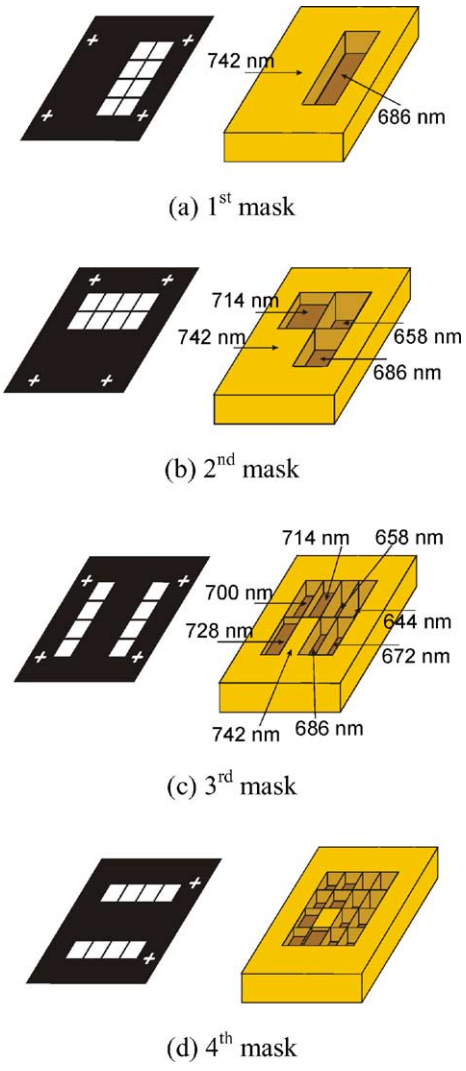

Fig. 8. The SiO_2 etching process. (a) applying the first mask, two different SiO_2 thickness are obtained; (b) applying the second mask, four different SiO_2 thickness are obtained; (c) applying the third mask, eight different SiO_2 thickness are obtained; (d) applying the fourth mask all the 16 SiO_2 thickness are obtained.

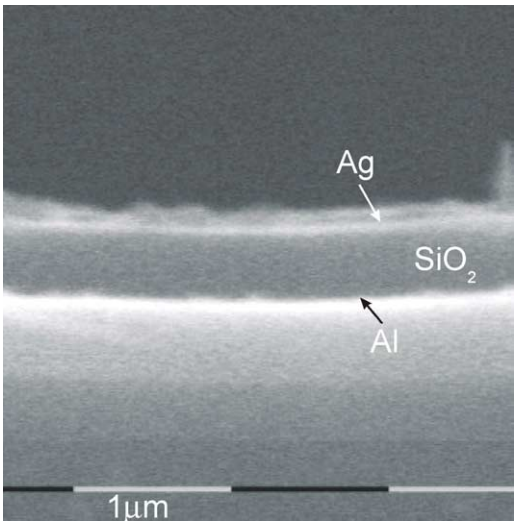

Fig. 9. SEM photograph showing the cross-section of one of the Fabry-Perot filters (SiO_2 thickness = 637 nm).

umn of Table 1). The thickness of the SiO_2 layer determines the tuned wavelength.

2.3. Optical simulations of the Fabry-Perot filters

A thin-film optics software package (TFCalc 3.4) was used for the structural optimization of the optical filters. Simulation results show that a 20 nm Al/SiO_2/40 nm Ag layer stack (Ag on top) is the best option for the optical filters in terms of optical characteristics and feasibility. The SiO_2 layer thickness changes between 637 and 742 nm with 7 nm steps. The simulated transmittances for all the 16 optical filters show that each of the channels is sensitive to a single spectral band, with a FWHM = 7 nm (Fig. 6).

3. Fabrication of the 16 optical-channels array

The filter fabrication starts with the deposition of a 20 nm Al layer by evaporation. Then a 742 nm thick SiO_2 layer is deposited by chemical vapor deposition (equal to the maximum cavity length). In subsequent plasma etching steps, for which a mask is used and each of them with different etch time (see Fig. 7a), the total thickness of the SiO_2 layer is decreased from 742 to 637 nm, in 7 nm steps, forming the filters number 16 ($\lambda = 570$ nm) to 1 ($\lambda = 495$ nm), respectively. Fig. 7b shows the position of each filter in the array. The SiO_2 etching process is visualized in Fig. 8. The fabrication ends with the deposition of a 40 nm Ag layer. A SEM photograph presenting the cross-section of one of the channels (filter number 1) is shown in Fig. 9. The 16-filter fabrication requires only four masks and four etching steps.

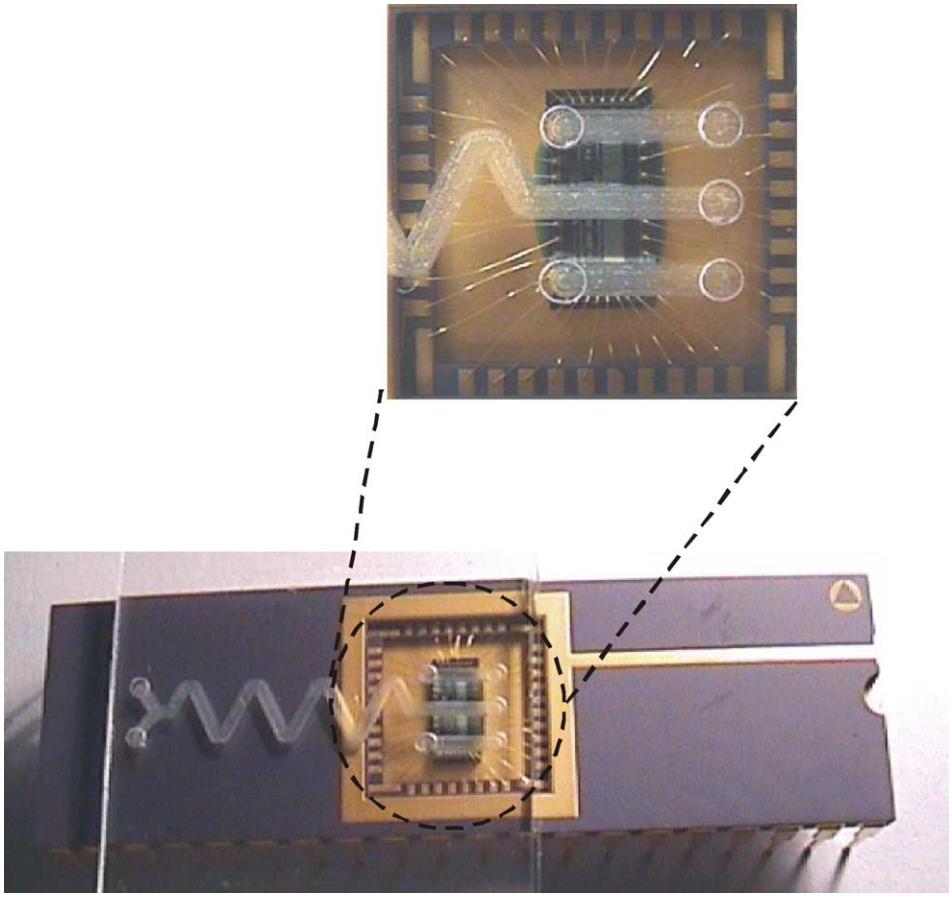

Fig. 10. Photograph of the packaged biosystem.

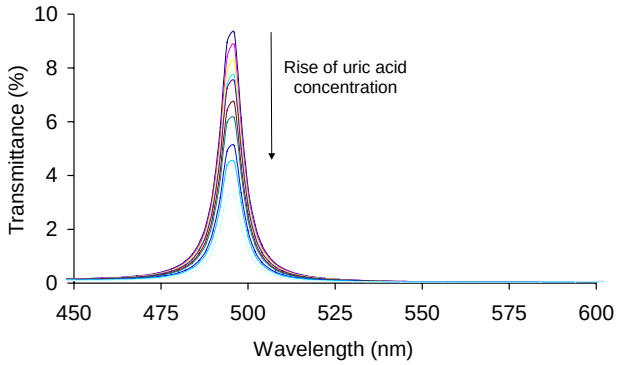

Fig. 11. Measured transmittance for a single channel for the same uric acid concentrations measured in Fig. 1 ($\lambda = 495$ nm).

The filters can be easily tuned to different spectral bands by adjusting only the thickness of the SiO_2 layer without affecting the biosystem layout. Fig. 10 shows a photograph of the complete device. The holes and channels were drilled and milled, respectively, by using a computer numerically controlled (CNC) machine. The glass die is glued to the CMOS chip.

4. Experimental results

The 16 optical filters are now being fabricated. Meanwhile a single channel was previously fabricated and its operation demonstrated in the measurement of uric acid concentration ($\lambda = 495$ nm). The reagent used in those measurements was the infinity^{TM} uric acid reagent from Sigma–Aldrich [8]. A 200 W quartz tungsten halogen lamp was used as the white light source for biosystem illumination. The photodiode current was measured using a Keithley 487 picoammeter. A monochromator is also used in order to obtain the photodiode current versus the wavelength. The optical filter is composed of the 20 nm Al/637 nm SiO_2/40 nm Ag layer stack. Optical spectra measurements on the biosystem show that the single channel is sensitive to its specific wavelength ($\lambda = 495$ nm), with a FWHM of 7 nm (Fig. 11). These measurements, when compared with the measurements without the optical filter (Fig. 1), allow concluding that it can be used only a white light source for the biosystem illumination. However, Fabry-Perot filters using metallic mirrors cannot provide both high-transmittance and low FWHM due to the optical absorption in the metal layers. From Fig. 11 it can be seen that with a FWHM = 7 nm the transmittance of the highest concentration fall off from 35% (see Fig. 1) to 4.5%. This can be avoided using high-performance dielectric mirrors. However, the filters fabrication will be significantly more complex.

5. Conclusions

The reported biosystem offers a new approach for clinical analysis due to the measurements of the concentration of 16 different biochemical substances in human's fluids, with the same device. This performance is obtained with an array of 16 optical filters based on Fabry-Perot thin-films optical resonators. Moreover, the 16 optical-channels array allows the use of only a white light source for illumination. Therefore, the measurements can be performed in any place and the results of those measurements become available immediately.

Acknowledgements

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Biographies

Graça Minas graduated in industrial electronics engineering in 1994 and obtained her MSc degree in 1998 both titles at University of Minho, Portugal. Since 1995 she has been a Lecturer in Department of Industrial Electronics, University of Minho, Portugal and she is involved in biomedical microdevices research. She won the second place for best oral presentation in Eurosensors XVII, presenting the reported work.

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Júlio Sousa Martins graduated in electrical engineering at University of Oporto, Portugal in 1979. He obtained his PhD degree on computer engineering at University of Minho, Portugal in 1993. Since 1998, he has been an associate professor at the Department of Industrial Electronics of the University of Minho and he is involved in electronics and instrumentation research.

Reinoud F. Wolffenbuttel received the MSc and PhD degrees from the Delft University of Technology, Delft, The Netherlands, in 1984 and 1988, respectively. Between 1986 and 1993, he was an assistant professor, and

since 1993, an associate professor, at the Laboratory of Electronic Instrumentation of the Delft University of Technology, where he is involved in instrumentation and measurement in general and on-chip integration of microelectronic circuits and silicon sensor, fabrication compatibility issues, and micromachining in silicon and microsystems, in particular. He was a visitor at the University of Michigan, Ann Arbor, in 1992, 1999, and 2001, at Tohoku University, Sendai, Japan, in 1995, and at EPFL, Lausanne, Switzerland, in 1997. Dr. Wolffenbuttel was the recipient of a 1997 NOW pioneer award. He served as General Chairman of the Dutch National Sensor Conference in 1996 and Eurosensors in 1999.

José Higino Correia graduated in physical engineering from University of Coimbra, Portugal in 1990. He obtained in 1999 a PhD degree at the Laboratory for Electronic Instrumentation, Delft University of Technology, working in the field of microsystems for optical spectral analysis. Presently, he is an associate professor in Department of Industrial Electronics, University of Minho, Portugal. He was the General Chairman of Eurosensors 2003, Guimarães, Portugal. His professional interests are in micromachining and microfabrication technology for mixed-mode systems, solid-state integrated sensors, microactuators and microsystems.