On-Chip Integrated CMOS Optical Detection Microsystem for Spectrophotometric Analyses in Biological Microfluidic Systems

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***Abstract*—An integrated optical detection microsystem, which includes photodetectors and a light-to-frequency converter for readout, is designed and fabricated in a standard CMOS process without extra masks. This detection microsystem is designed for use in biological microsystems for fluids analysis. The application is in the low-cost concentration measurement of biomolecules in biological fluids, by the optical absorption in a part of the visible spectrum defined by the specific molecule. Signals proportional to the intensity of the light transmitted through the biological fluid are available at the output in the form of bit streams, which allows simple computer interfacing. The quantitative measurement of uric acid in urine is successfully demonstrated. The photodiode responsivity is 224 mA/W at λ = 495 nm (the wavelength at which the uric acid has its absorption maximum). The optical system sensitivity is 1 kHz/Wm^{-2} at λ = 670 nm (using the TLS230 from Texas Instruments as reference).**

I. INTRODUCTION

Spectrophotometric analysis of a sample is one of the most commonly used analytical techniques for biological analysis in clinical diagnostics. This technique is used to determine the concentration and/or amount of a particular component in a biological fluid [1]. Usually, the samples need to be sent to a laboratory for spectrophotometric analysis, and the results become available after several hours, sometimes days. As a consequence a reliable diagnosis cannot be performed within the consultation time. Mistakes in the logistics, such as lost samples and mislabeling, may further delay diagnosis [2]. The need for rapid and on-line measurements with low sample volumes led to the development of microsystems with the fluidic, the detection and the readout systems all integrated in a single module. The advantages associated with shrinking clinical analysis systems include: small sample volume, high degree of system integration, automation of measurement, short response time, improved analytical performance and laboratory safety and reduced cost.

This paper describes a microsystem for application in clinical analysis, especially in the spectrophotometric analysis of biological fluids. It allows the measurement of the concentration of biomolecules in those fluids. 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 molecule with a specific reagent. When the specific molecule reacts with the specific reagent a color is produced. The intensity of that color is directly proportional to the biomolecule concentration.

The microsystem combines in a MCM (Multi Chip Module) the photodetection and readout system and the microfluidic system. The photodetectors measure the transmitted light through the mixture (sample plus reagent). They are placed underneath the microfluidic detection chambers. The readout electronics converts the photodetectors analog signal in a digital one for further computer interfacing. The microfluidic detection chambers contain the chemical reagent, the calibrator and the mixture. It is needed three detection chambers. One is needed to obtain the baseline. The other allows the analysis of the mixed solution. The third is needed to calibrate the biomolecule concentration (it contains a standard with a well-known concentration of the biomolecule that is being analyzed).

II. MICROSYSTEM DESIGN

A. The Photodetectors

The absorption of light in silicon in the visible part of the electromagnetic spectra is wavelength dependent. Radiation with short-wavelengths (high-energy photons) is quickly absorbed near the surface and radiation with long-wavelengths (low-energy photons) penetrates deeper in silicon before being absorbed. This effect is due to the strong wavelength dependence of the silicon absorption coefficient, α, which causes the penetration depth of light in silicon, $d(\lambda)$, to be wavelength dependent and expressed by [3],

$$d(\lambda) = \frac{1}{\alpha(\lambda)}. \quad (1)$$

Selecting the suitable junction depth allows to set a maximum peak at a specific wavelength. An optimal photodetector structure for a particular application might require a special processing of the silicon wafer with several non-standard steps, which usually involves high-costs and the need for easily adaptable and suitable equipped semiconductor facilities. Consequently, most silicon photodetectors structures comply with the possibilities of the standards foundries.

The photodetectors described in this paper are designed using the layers available in a standard CMOS process only, without additional masks or steps. In a standard

n-well CMOS process, three photosensitive structures are possible as vertical junction photodiodes: n-well/p-epilayer, p+/n-well and n+/p-epilayer (see Fig. 1). At a particular wavelength, their quantum efficiency varies according to their junction depth. The quantum efficiency of a photodiode reveals how well it collects the incident light [4]. In the visible spectrum, the blue light (of about 450 nm) is more efficiently collected by a shallower junction (p+/n-well and n+/p-epilayer) and the red light (of about 650 nm) by a deeper junction (n-well/p-epilayer). Moreover, despite the shallower junction of the n+/p-epilayer photodiode, it has the higher quantum efficiency in the wavelengths of about 500 nm, due to the different doping concentration between the *n* and the *p* side, which extends the *p* side depletion area more deeply.

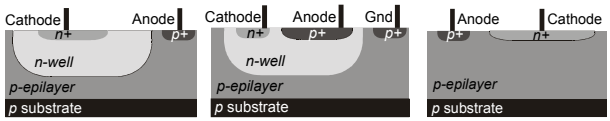

Figure 1. The three junction photodiodes in a standard n-well CMOS process: n-well/p-epilayer, p+/n-well and n+/p-epilayer

In a standard CMOS process the junction depth is fixed. The alternative for improving the quantum efficiency at a certain wavelength is to use a combination of the dielectric layers that may be present on top of the photodiode. They act as a thin-film stack and influence the optical transmittance for each wavelength independently. In addition, the photodiode quantum efficiency can be programmed by design. For that, the thickness and the optical properties of those layers have to be stable and well known for all batches of the standard process [5].

In the standard CMOS process used there are three dielectric layers above the photodiode pn-junction (see Fig. 2). The thickness of the n+ layer is 350 nm and the depth of the epilayer is 12 µm, with a doping concentration of 10^{16} atoms/cm^3. The first oxide (BPSG – Boron Phosphor Silicate Glass) thickness above the photodiode is measured as 650 nm and the second (SiO_2) as 700 nm. The silicon nitride layer (the overlayer), used for scratch protection, is 800 nm thick. Since technology rules from the standard CMOS process have to be met, the design of the optical path is restricted to combinations of those three dielectric layers above the pn-junction. The simulated optical transmissions of those combinations are shown in Fig. 3. The simulations are done with an optics software package TFCalc 3.4, supplied by Software Spectra, Inc., USA.

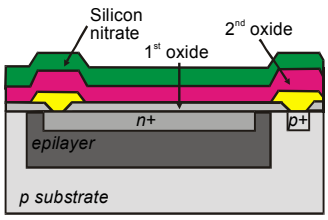

Figure 2. Cross-section of the basic structure of the photodiode fabricated in a standard n-well CMOS process

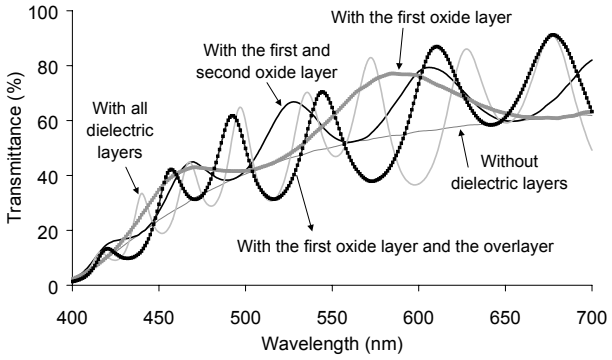

Figure 3. Simulated spectral responses of some typical combinations of the dielectric layers above the photodiode pn-junction

The simulations indicate that when no dielectric layer is present, the air-silicon interface represents a great transmission loss. An oxide layer, with a thickness around 650 nm, can increase the transmittance from 50% to 60%, but also introduces wavelength dependence. A properly designed anti-reflective coating can optimize the transmittance. However, this represents a post-processing step to the standard CMOS, which would damage economically the microsystem. In some cases, the best choice would be the removal of all dielectric layers from the top of the silicon surface. This process is performed at the design level, using the same masks designed for the metal contacts and for the overlayer (without additional masks or steps). However, in the used standard CMOS process, when etching the dielectric layers the metal layers are used as etch stops, and so, the etch time does not need to be very accurately controlled. This might represent a problem if the process steps are kept standard, because the removal of the dielectric layers etches the under adjacent layer (see Fig. 4). Consequently, from the practical point of view, the removal of the first oxide layer is not feasible, because it may damage the pn-junction. A visible effect is the roughness (like bubbles) in the photodiode active area [6]. This random surface-rough interferes, also randomly, in the photocurrent response. Therefore, using the standard CMOS process, the first oxide layer should be left over. Thus, the best photodiode structure for the related application is the one presented in Fig. 5. It is desirable to have the minimal wavelength dependence for suiting this optical detection microsystem to the spectrophotometric analysis of several (bio)molecules and several (bio)chemical fluids.

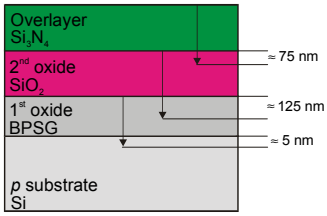

Figure 4. Etched layers when etch masks are applied

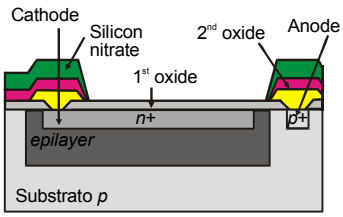

Figure 5. Cross-section of the fabricated CMOS compatible photodiode

B. The Readout Electronics

It is desirable to integrate the analog to digital conversion and the pohotodetectors in the same die. A CMOS light-to-frequency converter is designed (see Fig. 6). It produces a bit stream signal with a frequency proportional to the photodiode current and hence proportional to the intensity of the light transmitted through the biological fluid.

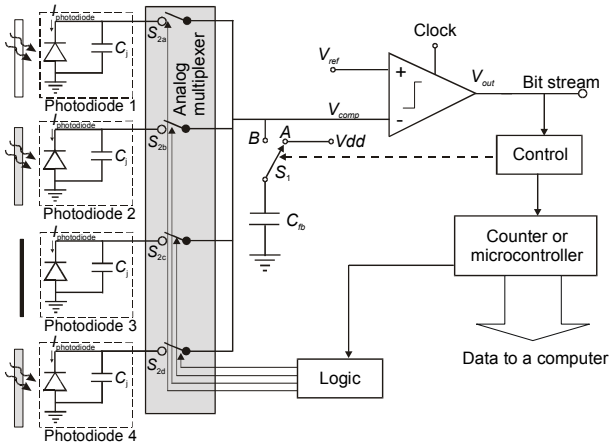

Figure 6. Block diagram of the photodiode readout circuit

The reverse biased junction capacitance of the photodiode C_j and capacitor C_{fb} are used as storage elements. At the voltage V_{comp} lower than V_{ref}, the comparator output V_{out} remains at a high logic level. After synchronization with a clock pulse, the analog switch S_1, is changed for the A position, which forces the capacitor C_{fb} to be quickly charged during one clock period with the voltage Vdd. After that period, this switch is changed again to the B position and the comparator output voltage commutes to the low logic level ($Vdd > V_{ref}$). Thus, the photocurrent discharges the capacitor C_{fb} until the comparator detects $V_{comp} < V_{ref}$, which causes V_{out} to change to the high logic level again and the cycle to repeat (see Fig. 7). The bit stream frequency of the output converter is a function of the charge change in the capacitor, ΔQ, which is directly proportional to the input photocurrent and hence to the biomolecules concentration, with $f_{bitstream}=I_{photodiode}/\Delta Q$.

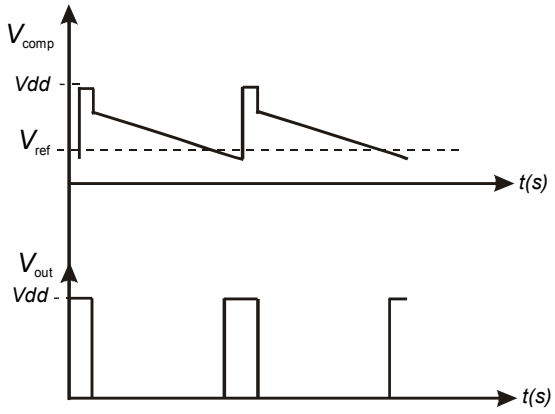

Figure 7. Comparator input and output voltages

Four photodiodes are used in each measurement. The first photodiode is for the reagent (without biomolecules), the second is for the biological fluid that is being analyzed, the third is for measuring the photodiode dark current and the fourth is for the calibrator. The dark current is the current that flows in a photodiode when there is no optical radiation incident on the photodiode. It is usually measured and then subtracted from the flux. As the dark current is temperature dependent, one measurement at the beginning of the experiment is usually not sufficient. Thus, in the reported circuit, a dark current compensation channel is implemented using the photodiode 3. This photodiode is completely covered with metal.

The logic and the analog switches S_{2a} though S_{2d} select the channel to be measured. These switches are identical complementary PN-MOS switches. The comparator is a clocked high-speed regenerative comparator with a rail-to-rail input circuit. For reliable operation a two-phase non-overlapping clock is used for the analog switch S_1 and for the comparator. It is possible to use a digital counter for counting the bit stream output pulses of the comparator during a fixed time period, producing the digital value correspondent to the photocurrent intensity. Alternatively, a microcontroller can substitute the counter, generating all the control logic and performing additional calculations too.

III. MICROSYSTEM FABRICATION

The CMOS compatible photodetectors and readout circuits have been fabricated through a double-metal, single-polysilicon, 1.6 µm n-well CMOS process. The area of each photodetector is 500×500 µm^2. Fig. 8 shows a photograph of the fabricated optical detection microsystem. The photodiode for measuring the dark current is point out with the letter 'B'. A metal layer covered its active area. This microsystem has been packaged in a "Dil 40" package (see Fig. 9).

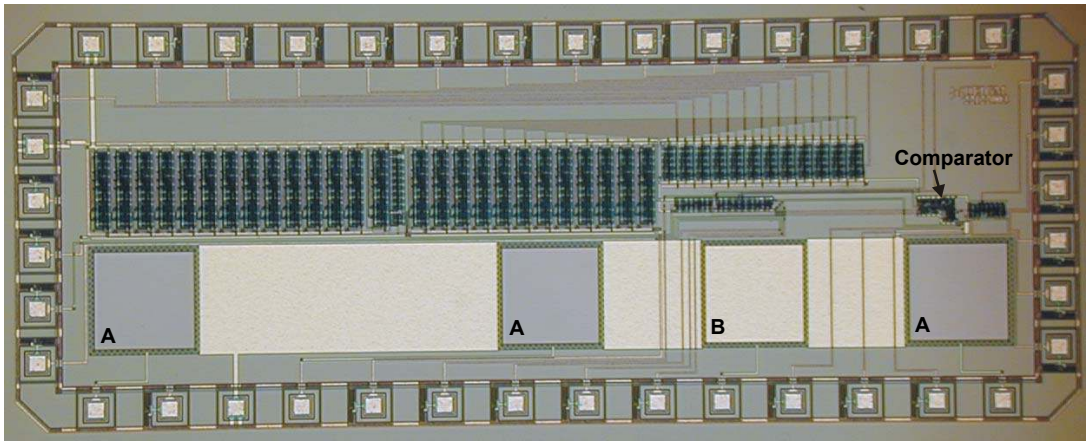

Figure 8. A photograph of the optical detection microsystem

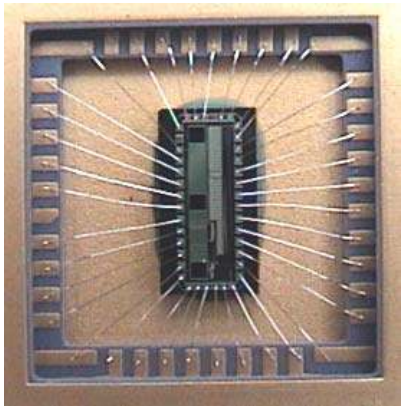

Figure 9. A photograph of the packaged optical detection microsystem

IV. Measurement Setup

The experimental arrangement used in the measurements is shown in Fig. 10. It comprises a 250 W quartz tungsten halogen lamp with a monochromator ORIEL Cornerstone 130^{TM} that is used as light source. An optical fiber is also used to direct the light into the measurement box. A Keithley 487 picoammeter (full-scale range from 10 fA to 2 mA and a resolution of $5^{1/2}$ digit) is used for measuring the photodiodes current. Those photodiodes are calibrated with a calibrated commercial photodiode as reference (Hamamatsu S1336-5BQ).

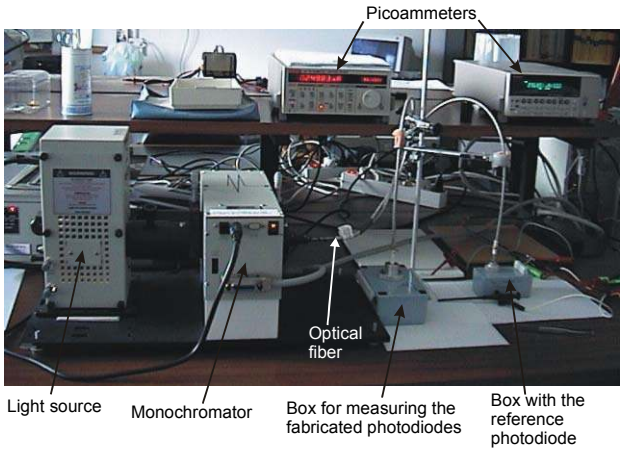

Figure 10. Experimental arrangement used in the measurements

V. Experimental Results

Fig. 11 and Fig. 12 show the spectral response and the quantum efficiency curves, respectively, of the fabricated photodiode, measured using the calibrated Hamamatsu photodiode as reference. The photodiode dark current for several reverse bias voltages is shown in Fig. 13. A dark current of 0.27 pA (1.08×10^{-18} A/μm^2) at 0 V was measured for a photodiode active area 250×10^3 μm^2.

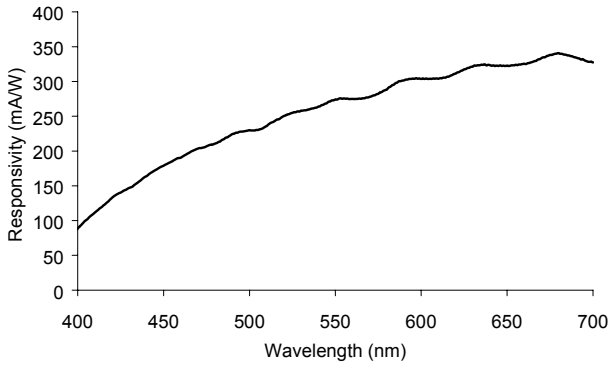

Figure 11. Measured spectral response of the fabricated photodiode

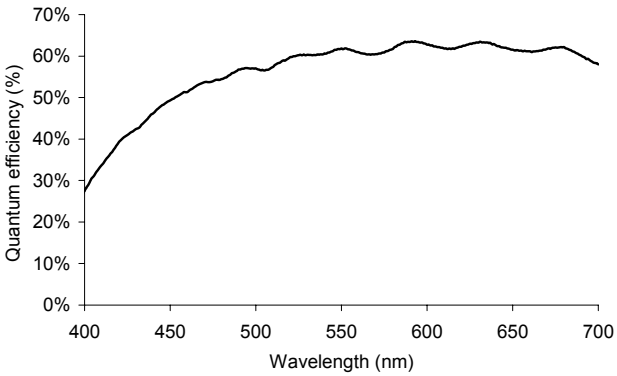

Figure 12. Measured quantum efficiency of the fabricated photodiode

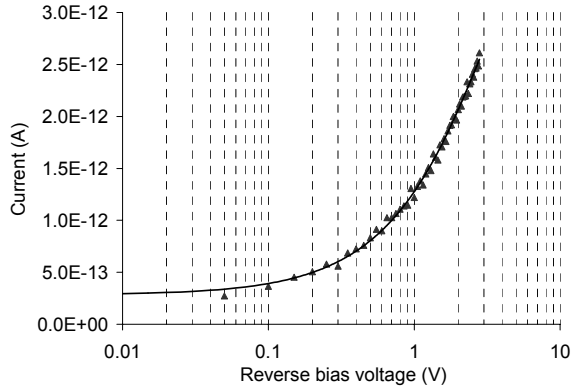

Figure 13. Measured dark current as a function of the reverse bias voltage of the fabricated photodiode

The power supply of the light-to-frequency converter circuit is 5 V. The reference voltage of the comparator (V_{ref}) is 2 V and the clock frequency is 1 MHz. The monochromator light source is used for testing the performance of the converter. This light is approached and removed from the photodiodes. Fig. 14 shows the output frequency of the light-to-frequency converter for different distances of the light source. In Fig. 15 and Fig. 16 samples of the oscilloscope traces, when the light source is at 5 cm and at 50 cm from the photodiode, were photographed, respectively. It can be seen that the output frequency of the comparator is proportional to the light intensity. The optical system sensitivity achieved is 1 kHz/Wm^{-2} at $\lambda = 670$ nm (using the TLS230 from Texas Instruments as reference). Further, the comparator output is used as a clock signal for a counter, which counts pulses during a fixed time period, producing the digital readout correspondent to the light intensity.

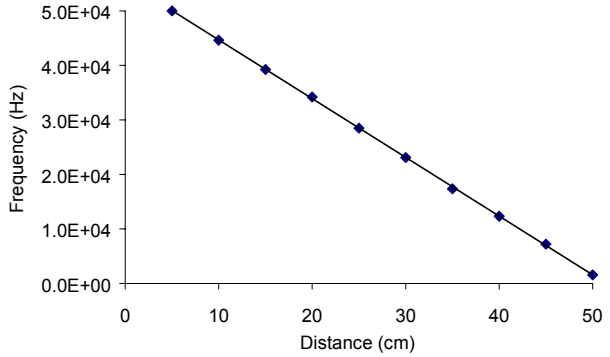

Figure 14. Characteristic signals in the readout circuits

The optical detection microsystem operation is demonstrated in the spectrophotometric analysis of uric acid in urine, especially in the quantitative measurement of its concentration. The reagent used in the measurements is the Infinity^{TM} Uric Acid Reagent from Sigma-Aldrich. This reagent reacts with a sample of urine containing uric acid in a 50:1 ratio, and produces an absorption maximum at a specific wavelength [1]. Fig. 17 shows the measured transmittance response for different uric acid concentrations in urine. Measurement results are done from 5 mg/dl to 120 mg/dl, comprising the range of normal and usually abnormal values in a human being (17 mg/dl to 67 mg/dl). The transmittance is defined as $T = I / I_0$, where I is the measured photodiode current of each solution and I_0 the measured photodiode current of the reagent. These measurements allow to conclude that the intensity of the color produced by the mixture is directly proportional to the uric acid concentration and the absorption spectra shows a maximum peak at the wavelength $\lambda = 495$ nm, with a FWHM (Full-Width--Half-Maximum) of 90 nm.

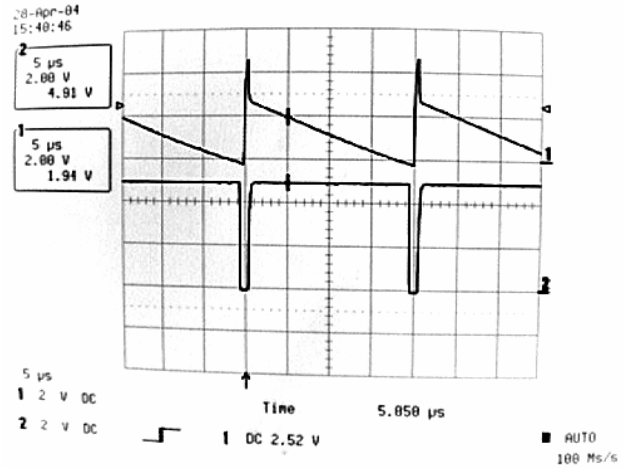

Figure 15. Oscilloscope capacitor voltage (1) and comparator output voltage (2) when the light source is at 5 cm from the photodiode

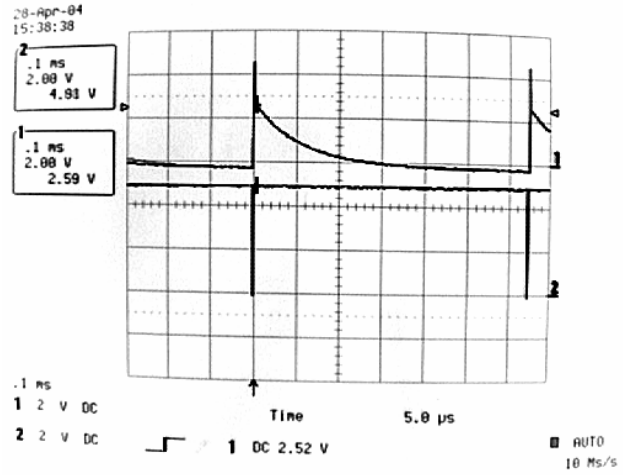

Figure 16. Oscilloscope capacitor voltage (1) and comparator output voltage (2) when the light source is at 50 cm from the photodiode

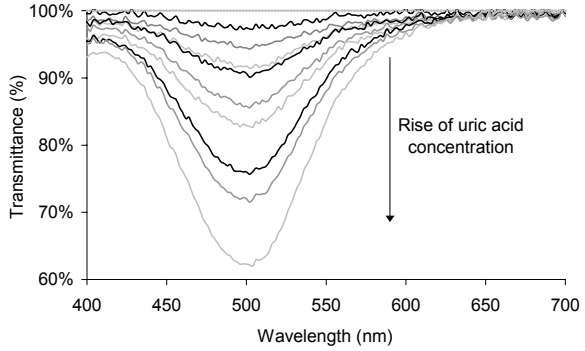

Figure 17. Measured transmittance spectra for different uric acid concentrations. From top to bottom curve: reagent, 5 mg/dl, 10 mg/dl, 15 mg/dl, 20 mg/dl, 30 mg/dl, 40 mg/dl, 60 mg/dl, 80 mg/dl and 120 mg/dl

VI. CONCLUSIONS

The on-chip integrated CMOS optical detection microsystem presented here enables the color and color intensity determination of a fluid. That measurement is based on optical absorption in a well-defined part of the visible spectrum. This microsystem is composed by photodetectors and a light-to-frequency converter for readout. The relatively simple readout circuits and the compliance with a standard CMOS process (without extra masks) allow addition of this optical detection microsystem to an existing CMOS design. The reported microsystem has been developed to be an integrated part of a biological microsystem for measuring the concentration of biomolecules in biological fluids. Its performance has been successfully demonstrated in the quantitative measurement of uric acid in urine. The photodetector responsivity is 224 mA/W at $\lambda = 495$ nm. This is the wavelength at which the uric acid molecule has its absorption maximum. The optical system sensitivity is 1 kHz/Wm^{-2} at $\lambda = 670$ nm (using the TLS230 from Texas Instruments as reference). This microsystem avoids the need of expensive readout optics and opens the door to low-cost disposable devices. Although it is presented for biological fluids analysis, it can also be used for chemical analysis as being the detection and readout system of a micro total analysis system (μTAS).

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