

Integrated Fluorescence Sensing for Lab-on-a-chip Devices

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Abstract—A low noise optical sensor and biocompatible microscale optical filters for integrated fluorescence sensors were developed and tested. The sensor was fabricated in a 0.5 μm CMOS process. The measured reset noise of the sensor is reduced by a factor of 10 compared to conventional active pixel sensors. The transmission ratio in the pass-band and suppression ratio in the stop-band of the optical filters are comparable to that of macroscopic commercial filters for fluorescence microscopy.

I. INTRODUCTION

We are fabricating MEMS devices on CMOS circuits for integrated cell-based sensing. These systems are designed to use biological cells for transducing chemical stimuli to either electrical or optical outputs for applications such as olfactory sensing or pathogen classification. The ability to detect fluorescence without conventional spectroscopic equipment will render these devices more practical by providing an on-chip imaging capability for sorting and characterizing cells.

Microscale fluorescence sensing requires the development of low-noise optical detectors and optical filters capable of rejecting the totality of the excitation light while transmitting the relatively weak fluorescent signal. We have designed and tested a new pixel architecture achieving better noise performance than conventional active pixel sensors (APS). We have also developed ultraviolet-blocking microscale polymeric filters capable of achieving the rejection and transmission performance of macroscale filters, although not their absorption edge sharpness. The new pixel structure and filters were independently characterized.

This paper presents the design of the sensor and of the optical filter. Test results comparing sensor and filter performance with competing technologies are provided.

II. SENSOR DESIGN

Fluorescence from the specimen is expected to be very weak (~ 10 photons/ $(\mu\text{m}^2 \cdot \text{s})$ or less) [1]. It is not feasible to reduce thermal noise by cooling since the integrated fluorescence sensor will directly couple with biological samples.

The stochastic noise voltage for a photodiode active pixel sensor (APS) is often categorized into reset noise, integration noise, and readout noise. Reset noise is due to shot noise from current flowing through the reset transistor during reset. This

noise is characterized as $V_{n_reset} = (kT/2C)^{1/2}$ [2], where k is Boltzmann's constant, T is absolute temperature, and C is the total capacitance at the sensing node. Integration noise arises from the shot noise of photocurrent and dark current during the accumulation of photo-charges. The noise voltage can be expressed as $V_{n_int} = (q(I_{ph} + I_{dc})t_{int})^{1/2}/C$, where I_{ph} is photocurrent, I_{dc} is dark current, and t_{int} is integration time. Input referred readout noise is usually much smaller than reset noise and integration noise due to the high front-end gain. Because the integration noise arises from the stochastic process of photon arrival, it sets the noise limit of the sensor. Therefore, reset noise becomes the most significant noise source for sensor design.

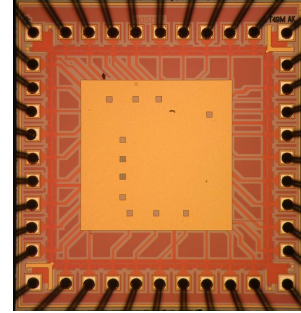


Fig. 1. Photograph of the fabricated sensor chip.

Correlated double sampling (CDS) is often used in the readout of APS arrays to suppress the fixed pattern noise (FPN). Since the two samples used for CDS are obtained from two consecutive integration cycles, reset noise voltage is further increased by $\sqrt{2}$. Given the difficulty of realizing CDS in one cycle for conventional photodiode active pixels, we designed a fully differential sensor to reduce the reset noise. The differential sensor consists of six transistors and one holding capacitor. To increase signal dynamic range, the reset transistor is NMOS. P-type transistors are used for both in-pixel source follower input transistors and row selection switches due to the low reset level. NMOS is used between the capacitor and the photodiode. The photodiode is implemented using a p^+/nwell junction with nwell connected to the power supply. This diode structure offers two advantages. First, p^+/nwell show the highest optical sensitivity among all possible junctions. Second, the nwell is held at a fixed potential to isolate substrate noise and suppress possible blooming effects. The capacitor can be implemented by two poly layers or a p^+/nwell junction inside the same nwell as for the photodiode. In our design, a 20fF capacitor is implemented using two poly layers. A picture of the

We thank the MOSIS service for providing chip fabrication; these chips will be used to teach an undergraduate course in mixed signal VLSI design. This research was supported by the National Science Foundation through awards 0238061 and 0515873 and by the Laboratory for Physical Sciences.

fabricated sensor chip is shown in Figure 1. Several different sensor structures were also designed on the same chip for comparison.

Three control signals are required for operating the differential sensor: reset, row_sel, and i_gate (isolation gate). The timing diagram of these control signals is shown in Figure 2. Since the pixel reaches steady state during reset, the reset noise becomes $V_{n_reset} = (kT/C)^{1/2}$, where C is the total capacitance of photodiode and holding capacitor. By turning off the isolation gate right after reset, the reset noise voltage is held by the capacitor. At the end of the integration, reset noise is subtracted by reading out the differential signal. Two differential signals will be read out before and during the reset. By subtracting these two differential signals, offset due to threshold mismatch, clock feedthrough, and charge injection is eliminated.

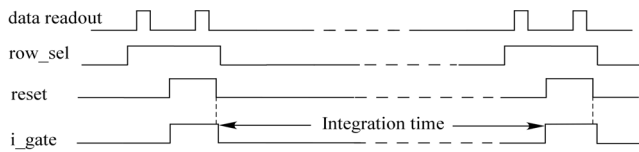


Fig. 2. Timing diagram of control signals.

The optically active area for all sensors is $33.6\mu\text{m} \times 33.6\mu\text{m}$. The sensor chip was fabricated using a two poly, three metal, $0.5\mu\text{m}$ CMOS process. The reset noise of the fluorescence sensor was tested in both differential mode and single-ended mode (by keeping the isolation gate off). The results are summarized in Table 1. The differential sensor has reset noise comparable to that of an APS when operated in single-ended mode. When operated in differential mode, the reset noise of the fluorescence sensor is ten times less than that of an APS. This reset noise was measured at a control frequency of 100Hz. This frequency can be adjusted to provide the integration time appropriate to different intensities.

TABLE I
RESET NOISE OF APS AND DIFFERENTIAL SENSOR

Sensor	In darkness	Under illumination
Conventional APS	2.06 mV	2.08 mV
Diff. sensor (single- end mode)	2.45 mV	2.32 mV
Diff. sensor (diff. mode)	78 μV	0.205 mV

III. OPTICAL FILTER DESIGN

We developed optical filters for use in FURA-2-based fluorescence assays. FURA-2 is a commercially available molecular probe that absorbs light in the ultraviolet range (365 nm) and emits green fluorescence (510 nm). The optical filters that were developed are based on absorption of light by a chromophore embedded in an optically clear polymer matrix. All filters were found to be biocompatible.

A. Filter Fabrication and Characterization

A chromophore, 2-(2'-hydroxy-5'-methylphenyl) benzotriazole (benzotriazole or BTA hereinafter), was obtained in

powder form (Great Lakes Chemical Inc.). The chromophore was physically mixed into two different optically clear polymers (PDMS and Humiseal 1B31). The maximum concentration of benzotriazole required was determined to be 37 vol%. Beyond this concentration, no further decrease in the transmission in the ultraviolet range was observed. It is likely that rejection improved with higher concentrations, but the detection limit of our instrument did not allow measurements beyond the -60dB level. Figure 3 shows the transmission spectra of the polymer filters as compared to a micromachined 39-layer interference filter and a commercial macroscale filter.

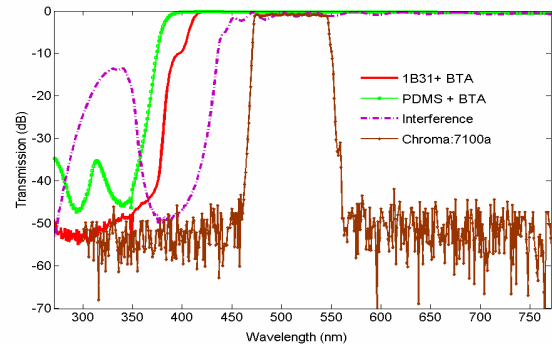


Fig. 3. Transmission spectra of optical filters.

The polymer filters achieved rejection levels from 300 nm to 370 nm comparable to a commercial macroscale bandpass filter for a fluorescence microscope (Chroma: 71000a). Moreover, since they only required one deposition step, the filters proved to be easier to integrate as opposed to micromachined interference filters, which required stringent process control to achieve the desired transition edge and minimized stop-band ripples [3].

IV. CONCLUSIONS

A differential optical sensor was designed and tested. The reset noise is reduced by a factor of 10 compared to a conventional photodiode APS. Chromophore-based biocompatible filters were also developed. In addition to being integrable, the filters achieved performance comparable to a commercial fluorescence filter set. This work demonstrates components that will enable us to develop compact integrated fluorescence sensors.

ACKNOWLEDGMENT

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