A Hybrid Thin-Film/CMOS Fluorescence Contact Imager

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Abstract— **A hybrid thin film/CMOS microsystem for fluorescence contact imaging is presented. The microsystem integrates a high-performance optical filter and a 128x128-pixel imager fabricated in a 0.35μm technology. The thin-film filter is fabricated and characterized prior to assembly. Its optical density (OD) is over 6.0 at the wavelength of interest. The performance of the microsystem is experimentally validated by imaging conventional Cy3 fluorophore spots using a low-cost pen-sized laser. The emission intensity as a function of fluorophore concentration is measured with the estimated sensitivity of 20 fluorophore/μm^2.**

I. INTRODUCTION

Applications such as on-site medical, environmental and biothreat monitoring require low-cost, small form factor biochemical sensory systems. Optical techniques such as fluorescence imaging are commonly used in conventional biochemical sensing instruments [1]. In fluorescence-based sensing, a single molecule of interest can emit millions of photons per second which is detected by a photosensor. This makes the technique highly sensitive.

Fluorescence-based sensing involves molecular probes called fluorophores which can chemically bind to a biochemical of interest. These fluorophores emit secondary light when excited by a primary light source. In applications such as DNA detection, fluorophores are chemically bound to single-stranded unknown DNA which is then hybridized with a planar array of single-stranded known DNA [2]. Where the matching known and unknown DNA strands bonded after hybridization, DNA is detected by measuring the secondary light emitted from the fluorophores. Conventional imaging systems involve bulky optics making them unsuitable for use in on-site and point-of-care applications.

Contact imaging is a compact, low-cost imaging technique which involves placing the object to be imaged in close proximity to the photodetector array [3]. It does not require intermediary optics, resulting in significant area and cost savings. Moreover, contact imaging improves the sensitivity by orders of magnitude [4]. These advantages make contact imaging microsystems attractive for on-site deployable, low-cost biosensors which compliment the conventional bulky stationary fluorescence imaging systems.

Fluorescence imaging requires an optical filter that rejects the excitation light but passes the emission light to be detected by a photosensor. Conventional fluorescence dyes have a small difference in the wavelength between the peak excitation and the emission spectrum known as the Stokes shift. Imaging conventional fluorescence dyes requires an optical filter with a steep cut-off to sufficiently block and transmit the excitation and emission light respectively. Specialized fluorophores such as quantum dots have a larger Stokes shift and relax the requirements on the steepness of the filter cut-off [5], but are not conventionally utilized in applications such as DNA detection.

Multi-layer dielectric interference filters are commonly used in fluorescence imaging as they can yield high optical density. The optical rejection of such filters is sensitive to the angle of incidence of the excitation light. This dictates their use primarily together with a collimating excitation light source and a collimating lens.

To implement fluorescence-based contact imaging, an optical filter is placed on or near the surface to the photosensor array. For collimated excitation, a laser light source can be utilized. The lack of a collimating lens further increases the requirements on the optical density of the filter. The use of a low-autofluorescence and low-scattering microarray slide helps to alleviate this problem.

The choices of the photodetector for fluorescence imaging systems have conventionally been the photo multiplier tube (PMT) or the charge-coupled device (CCD). They provide good sensitivity but are not suited for low-cost contact imaging as they are bulky, expensive and do not allow for on-chip signal processing.

Fluorescence imaging with integrated on-chip filters has been performed utilizing costly custom silicon integrated technologies [6], [7] and an aluminum gallium arsenide technology [8] that implement a single photodetector. These results are significant but do not yield a low-cost array-based imager with integrated signal processing.

The CMOS technology has the advantages of the low cost, high integration density, and signal processing versatility. It can be efficiently utilized to implement contact imaging arrays with on-chip signal conditioning capabilities for use in portable, point-of-care applications.

Recently, several proof-of-concept fluorescence contact imaging experiments employing the CMOS technology and high-performance excitation light filters have been performed [9], [10]. A discrete off-chip excitation filter with a
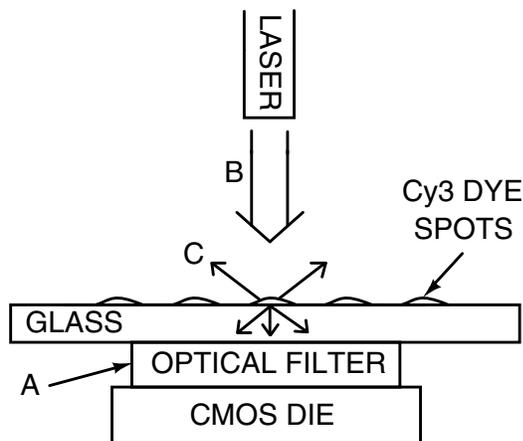
Fig. 1.   Fluorescence contact imaging configuration.

Fig. 2.   Micrograph of the filter placed on the CMOS die.

Fig. 3.   Pixel circuit with its column level biasing.

single-pixel photodetector were reported in [9]. An off-the-shelf CMOS web camera was integrated with an optical filter in [10].

Several research groups have also reported integration of CMOS photosensors with low-cost emission light filters with sub-optimal emission light blocking performance. The applications include brain neural activity monitoring [11], particle and pathogen detection [12], [13], and DNA detection [5]. The filters in these designs are generally fabricated directly on the surface of a CMOS die. In such on-CMOS fabrication methods, the high optical density of the filter is difficult to ensure and its optical characteristics cannot be verified prior to the mycrosystem integration. This results in an increased excitation light interference and reduced sensitivity. The sub-optimal optical density of the filter is inadequate for fluorescence detection application requiring excitation light rejection with a steep cut-off such as DNA detection. This can be remedied by filter-less fluorescence sensing techniques, such as time-resolved fluorescence detection [5], but at the cost of a reduced SNR, a larger pixel and increased design complexity.

We present a high-optical-density, low-cost contact imaging microsystem for accurate detection of fluorophores with a wide range of Stokes shift, as small as a few nanometers. It consists of a thin-film interference filter which is pre-fabricated and optically tested prior to integration with a CMOS die. A conventional fluorescence dye commonly used in clinics for DNA detection is utilized to validate the performance of the microsystem. The components of the assembly and system-level experimental results validating the microsystem performance are presented next.

II. SYSTEM ASSEMBLY

Figure 1 shows a simplified cross-section of the implemented fluorescence contact imaging system. The 100μm thin, long-pass filter (A) was prefabricated, optically tested and diced (Omega Optical), before attaching it to the CMOS die utilizing an epoxy with a matching refractive index (Aspen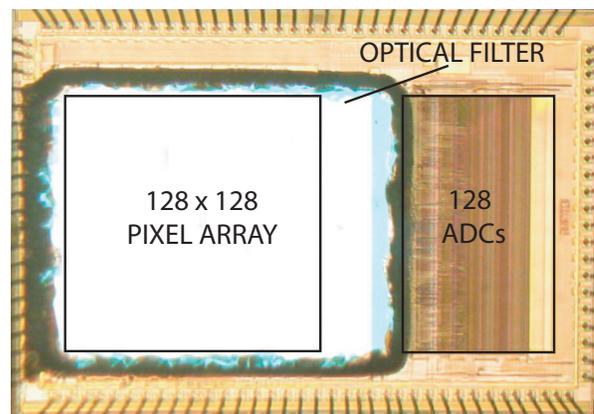 Technologies). The microarray slide with hybridized spots containing fluorophores (C) is placed above the filter. The spots on the microarray slide are excited using a collimated laser source (B).
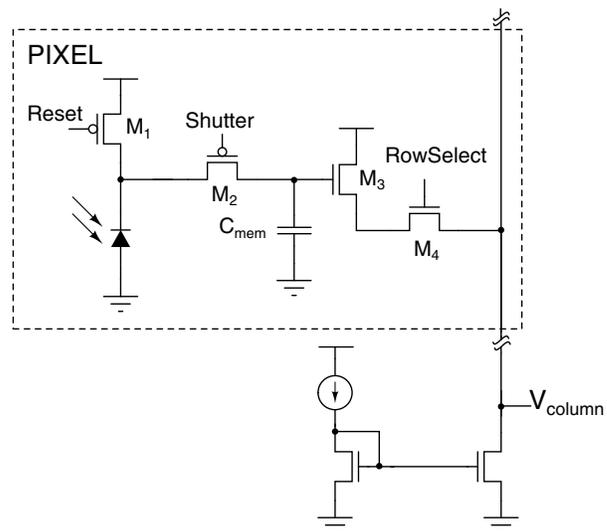
The laser light excites the microarray spots containing the fluorophores. The excited fluorophores emit a secondary light with a higher wavelength. The optical long-pass filter below the microarray slide blocks the lower wavelength excitation light of the laser while allowing the higher wavelength emission light to be sensed by the CMOS pixel array. Figure 2 shows the optical filter attached to the CMOS die.

III. VLSI CIRCUIT IMPLEMENTATION

An array of 128×128 active pixel sensors acquires the optical data. The pixel and its column-parallel biasing circuit are depicted in Figure 3. The pixel comprises an n^+-diffusion–p-substrate photodiode, a reset transistor M_1, an electronic shutter switch M_2, a frame memory C_{mem}, an output source follower M_3, and a readout switch M_4.

The pixel area is chosen to be 15.4μm$\times15.4\mu$m to provide sufficient spatial resolution for imaging the shape of

TABLE I
SUMMARY OF ELECTRICAL CHARACTERISTICS

Technology	0.35μm CMOS
Supply Voltage	3.3V
Die Area	4.4mm x 2.9mm
Array Dimensions	128 x 128 pixels
Pixel Size	15.4μm x 15.4μm
Fill Factor	28%
Dark Current	36 fA/pixel
Frame Rate	30 fps
Output Resolution	8-bit
Total Power	4.0 mW

a microarray spot. The photodiode is implemented as n^+-diffusion–p-substrate owing to its compact layout and hence a higher pixel fill factor. Transistors M_1 and M_2 are of the minimum size as needed to lower channel charge injection and clock feedthrough errors. Using PMOS type reset and shutter switches increases the dynamic range of the pixel output. Channel length of M_3 is selected larger than the minimum size for good matching among pixels and to reduce the source follower flicker noise. The in-pixel frame memory is implemented as a MOS capacitor to achieve a higher integration density. The size of the MOS capacitor was optimally chosen to achieve a small pixel area, lower charge injection and clock feedthrough errors affecting the stored pixel output. In strong inversion, C_{mem} is 15fF. A metal light shield covers the whole pixel except the photodiode area in order to eliminate any photo response from other regions of the pixel.

At the beginning of each frame, the photodiode is reset by the PMOS reset switch, M_1. During the integration period, the pn junction voltage is discharged by an optical current proportional to the incident light intensity. Fixed-pattern noise (FPN) is reduced by taking the difference between the pixel reset and the integrated signal levels. A double sampling of the pixel outputs is performed to suppress FPN and the flicker noise.

Table I summarizes the experimentally measured electrical characteristics of the image sensor chip.

IV. System-Level Experimental Results

The fluorescent contact imaging microsystem has been experimentally validated by imaging spots of conventional cyanine-3(Cy3) fluorescence dye (GE Healthcare) with various concentrations. This dye is commonly utilized for DNA detection. Figure 4 shows the test setup assembly. The optical filter (Omega Optical) is attached to the CMOS die inside the cavity of an open-lid chip package mounted over the PCB. A custom glass slide with different concentrations of the Cy3 dye spotted onto it is placed on the surface of the chip package. An X-Y stage aligns the dye spots over the CMOS pixel array. A 532nm green pen-sized laser with 10mW light intensity is mounted vertically above the CMOS pixel array to excite the fluorescence dye spot aligned over the array. The secondary light emitted by the spot is sensed by the imager while the laser light gets attenuated by the on-chip filter. The laser emits a
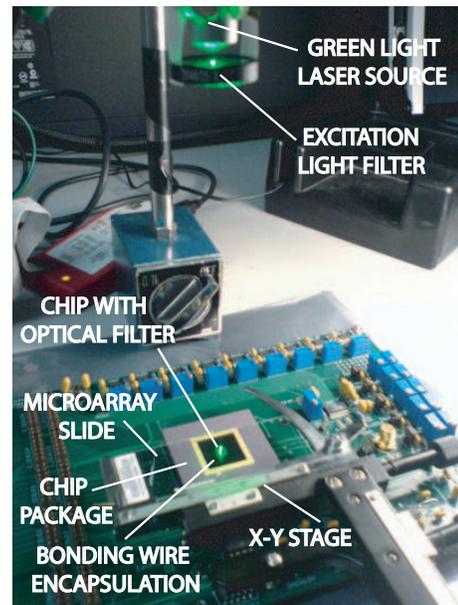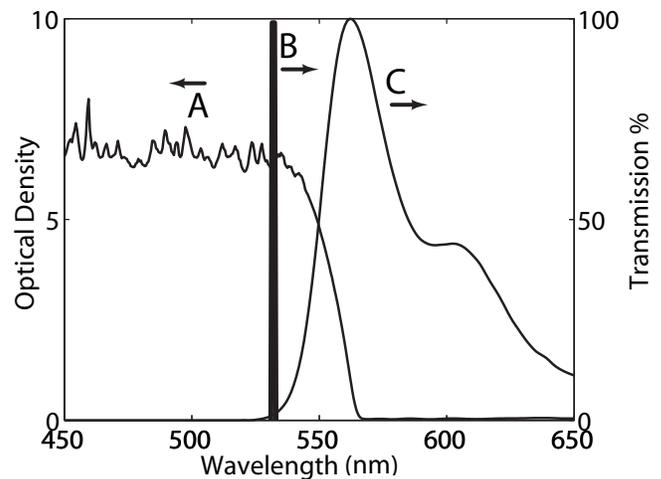

Fig. 4. Imager testing setup.

Fig. 5. Experimentally measured optical density of the filter (A) and the typical excitation and emission spectrum of the laser (B) and the Cy3 fluorescence dye (C) respectively.

parasitic 800nm wavelength which is blocked by an additional excitation parasitics filter.

Figure 5 depicts the experimentally measured characteristics of the optical filter. The optical density of the 100μm-thin filter (A) was measured prior to its dicing. The laser excitation beam (B) is attenuated by more than 60dB (optical density over 6), while the Cy3 dye emission (C) reaches the pixel array almost unattenuated at its peak wavelength.

Figure 6 shows the experimentally measured signal-to-noise ratio (SNR) of the fluorescence imaging microsystem for different concentrations of the Cy3 fluorescence dye. Solutes of different concentrations of the fluorescence dye were spotted on a custom glass slide. The diameter of the dye spots was

TABLE II

CONTACT FLUORESCENCE IMAGERS COMPARISON

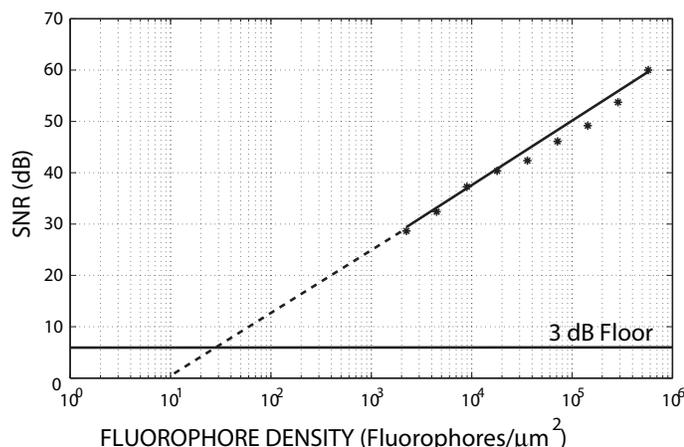|	Fabrication Process	Array Size	Filter Optical Density (OD)	Excitation Source	Fluorescence Dye
[2]	0.25μm CMOS	8x4	5	Laser	Quantum Dots
[3]	0.5μm CMOS	-	5	Monochromator	Fura-2
This work	0.35μm CMOS	128x128	>6.0	Laser	Conventional Cy3 dye

Fig. 6. Experimentally measured SNR of the imaging microsystem.

2mm in order to match the pixel array dimensions. The spot pitch was 2mm to avoid the cross-talk between the adjacent dye spots. The Cy3 fluorescence dye was pre-processed to stabilize it and lengthen its life time (Full Moon Biosystems). Appropriate quantities of the Cy3 dye were measured by micropipettes and mixed into a dilution liquid to create binary-weighted fluorophore concentrations from 10μM down to 0.04μM.

A linear fit was performed on the measured signal for all fluorophore concentrations. As the accuracy of the preparation of smaller concentrations was not well-controlled due to the large difference in the quantities of the dye and the dilution liquid, the plot was extrapolated to lower concentrations. The noise was measured with 16-point averaging and was limited by the resolution of the off-chip ADC. Based on the extrapolation, fluorophore concentrations as small as 20 fluorophores/μm^2 can be measured above the conventional 3dB SNR limit. Table II shows a comparative analysis of existing thin-film filter/CMOS based fluorescence imaging microsystems.

V. CONCLUSION

A hybrid thin-film/CMOS microsystem for fluorescence contact imaging has been presented. The high-performance optical filter was fabricated and characterized prior to its integration with the CMOS die. The microsystem performs fluorescence imaging by exciting microarray spots containing fluorophores using an off-the-shelf laser. The microsystem has been experimentally validated by fluorescence imaging conventional Cy3 fluorescence dye widely employed in DNA detection applications.

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